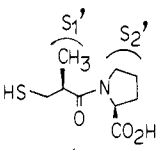
Angiotensin-Converting Enzyme Inhibitors: Perhydro-1,4-thiazepin-5-one Derivatives

Hiroaki Yanagisawa,* Sadao Ishihara, Akiko Ando, Takuro Kanazaki, Shuichi Miyamoto, Hiroyuki Koike,† Yasuteru Iijima,† Kiyoshi Oizumi,† Yoichi Matsushita,† and Tadashi Hata‡

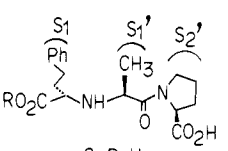
Chemical Research Laboratory, Sankyo Co., Ltd., Hiromachi, Shinagawa-ku, Tokyo 140, Japan. *Received April 13, 1987*

α-[6-[[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-5-oxoperhydro-1,4-thiazepin-4-yl]acetic acids (monoester monoacids) and their dicarboxylic acids having the hydrophobic substituents at the 2- or 3-position of the thiazepinone ring were prepared and assayed for angiotensin-converting enzyme (ACE) inhibitory activity. The dicarboxylic acids having the pseudoequatorial amino groups at the 6-position and the pseudoequatorial hydrophobic substituents at the 2- or 3-position of the chair conformation of the thiazepinone ring had potent in vitro inhibitory activity. The monoester monoacids having the hydrophobic substituents at the 2-position suppressed pressor response to angiotensin I for a longer duration than those having the substituents at the 3-position when administered orally. The structure–activity relationship was studied by conformational energy calculations of the thiazepinone ring.

The renin–angiotensin–aldosterone pressor system plays an important role in the control of blood pressure and electrolyte homeostasis.[1] The octapeptide hormone angiotensin II (AII) produced from angiotensin I (AI) by the action of angiotensin-converting enzyme (ACE) constricts blood vessels and stimulates the release of aldosterone to raise blood pressure. ACE is also known as kininase II,[2] which cleaves the C-terminal dipeptide of the vasodepressor nonapeptide bradykinin to give an inactive peptide. Thus, inhibition of ACE had been expected to lower blood pressure in high-renin hypertensive patients. The first orally active ACE inhibitor, captopril[3] (1), has proven



1

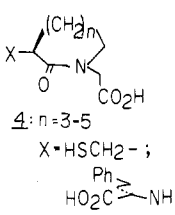


2: R=H
3: R=Et

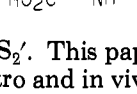
effective not only in high-renin hypertension but also in the essential hypertension where plasma renin activity (PRA) is not elevated. Captopril (1) has now become one of the most important agents in the treatment of hypertension. After the success of 1, many medicinal chemists have been interested in ACE inhibitors and synthesized new potent orally active inhibitors[4] such as enalapril[5] (3), the prodrug of enalaprilat (2).

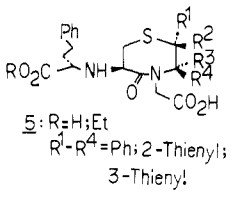
Although the three-dimensional structure of the ACE active site has not yet been elucidated, it seems necessary for the inhibitor to have zinc ligands such as the sulfhydryl or carboxyl group, the terminal carboxyl group binding to the arginine moiety in ACE and the amide group forming a hydrogen bond with ACE.[4a] Hydrophobic groups binding to the enzyme subsites S_1, S_1', and S_2' enhance the inhibitory potency.[4a] Recently, the conformation of the common structure propionyl-L-proline in 1 and 2 was suggested to be restricted during binding to ACE and propionyl-L-proline was replaced by the lactams 4.[6] Further modifications of 4 such as benzofused lactams,[7] 1,5-benzothiazepines,[8,9] 1,5-benzoxazepines,[9] and pyridazino[1,2-*a*][1,2]diazepines[10] were reported to have potent inhibitory activity.

We intended to synthesize the perhydro-1,4-thiazepin-5-ones 5 having the hydrophobic substituents R^1–R^4, phenyl or thienyl groups, at the 2- or 3-position and evaluate their inhibitory activities in order to clarify the spatial orientation of hydrophobicity required for binding



4: n=3-5
X=HSCH2-;





5: R=H;Et
R^1-R^4=Ph; 2-Thienyl; 3-Thienyl

to subsite S_2'. This paper describes the preparation of 5, their in vitro and in vivo activities, and the discussion of

(1) Ondetti, M. A.; Cushman, D. W. *J. Med. Chem.* **1981**, *24*, 355.
(2) Yang, H. Y. T.; Erdos, E. G.; Levin, Y. *Biochim. Biophys. Acta* **1970**, *214*, 374.
(3) (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.
(4) Reviews: (a) Petrillo, E. W., Jr.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*, 1. (b) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483.
(5) 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.; Hirshmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature (London)* **1980**, *288*, 280.
(6) (a) Thorsett, E. D.; Harris, E. E.; Aster, S.; Peterson, E. R.; Taub, D.; Patchett, A. A. *Biochem. Biophys. Res. Commun.* **1983**, *111*, 166. (b) Thorsett, E. D.; Harris, E. E.; Aster, S. D.; Peterson, E. R.; Tristram, E. W.; Snyder, J. P.; Springer, J. P.; Patchett, A. A. *Pept.: Struct. Funct., Proc. Am. Pept. Symp., 8th, 1983* **1983**, 555. (c) Wyvratt, M. J.; Tischler, M. H.; Ikeler, T. J.; Springer, J. P.; Tristram, E. W.; Patchett, A. A. *Pept.: Struct. Funct., Proc. Am. Pept. Symp., 8th, 1983* **1983**, 551. (d) Thorsett, E. D.; Harris, E. E.; Aster, S. D.; Peterson, E. R.; Snyder, J. P.; Springer, J. P.; Hirshfield, J.; Tristram, E. W.; Patchett, A. A.; Ulm, E. H.; Vassil, T. C. *J. Med. Chem.* **1986**, *29*, 251.
(7) (a) Parson, 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. (b) Watthey, J. W. H.; Gavin, T.; Desai, M. *J. Med. Chem.* **1984**, *27*, 816. (c) Watthey, J. W. H.; Stanton, J. L.; Desai, M.; Babiarz, J. E.; Finn, B. M. *J. Med. Chem.* **1985**, *28*, 1511. (d) 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.
(8) Slade, J.; Stanton, J. L.; Ben-David, D.; Mazzenga, G. C. *J. Med. Chem.* **1985**, *28*, 1517.
(9) (a) Itoh, K.; Kori, M.; Inada, Y.; Nishikawa, K.; Kawamatsu, Y.; Sugihara, H. *Chem. Pharm. Bull.* **1986**, *34*, 1128. (b) Itoh, K.; Kori, M.; Inada, Y.; Nishikawa, K.; Kawamatsu, Y.; Sugihara, H. *Chem. Pharm. Bull.* **1986**, *34*, 2078.

† Biological Research Laboratory.
‡ Analytical and Metabolic Research Laboratories.

0022-2623/87/1830-1984$01.50/0

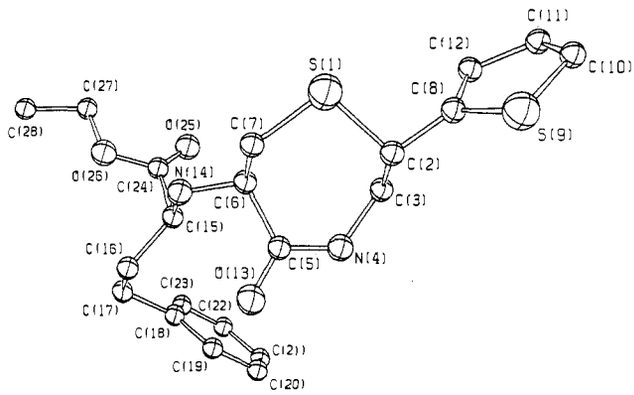
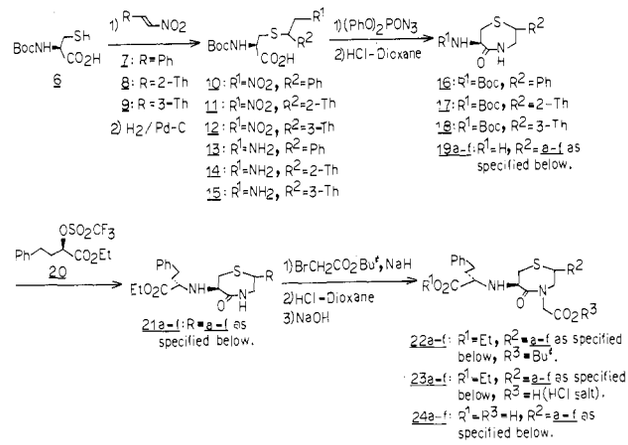


Figure 1. X-ray determined structure of **21c**.

Scheme I[a]

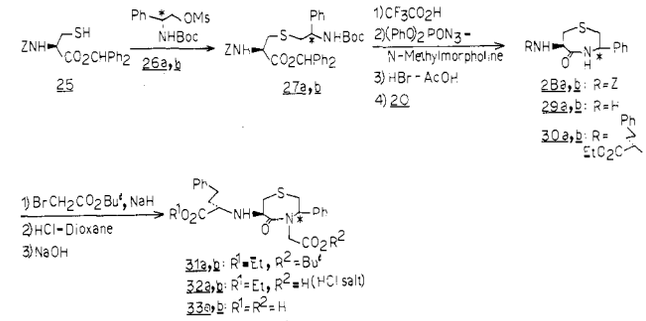


[a] **a**, ---Ph; **b**, ◄Ph; **c**, ---2-Th; **d**, ◄2-Th; **e**, ---3-Th; **f**, ◄3-Th; Th = thienyl; Boc = *t*-BuOCO.

structure–activity relationship with the aid of computer graphics.

Chemistry. A series of the perhydrothiazepinones **24a–f** having the substituents at the 2-position were prepared from Boc-L-cysteine (**6**) as shown in Scheme I. A Michael addition of β-nitro olefins **7–9** with **6** gave the nitro acids **10–12**, respectively, which were mixtures of the diastereoisomers derived from the new asymmetric carbon at the S-substituent. Hydrogenation of **10–12** in the presence of Pd–C in AcOH gave the amino acids **13–15**, respectively, which were converted to the perhydrothiazepinones **16–18**, respectively, by intramolecular condensation with diphenyl phosphorazidate. The products were a mixture of 2*R*,6*R* and 2*S*,6*R* isomers. Treatment of **16–18** with HCl–dioxane followed by fractional recrystallization afforded the optically pure 6-aminoperhydrothiazepinones **19a–f**. The isomers **19a,c,e** were less polar on TLC (1-BuOH–AcOH–H_2O, 4:1:1) and crystallized more easily than the other isomers, **19b,d,f**, respectively. The chiralities at the 2-positions of **19a–f** were confirmed by X-ray analysis of the compound **21c**, whose preparation is described below, and their NMR spectra. The X-ray determined structure of **21c** is shown in Figure 1. The perhydrothiazepinone ring of **21c** has a chair conformation, and both substituents at the 2- and 6-positions are situated in pseudoequatorial orientation, i.e., the carbon at the 2-position is assigned to *S* configuration. The less polar isomers, **19a,c,e**, are distinguished from the others, **19b,d,f**, by their NMR spectra. There are large couplings (9 Hz) between 2-H and one of two 3-H in **19c** and between 6-H and one of two 7-H in **19a,c,e**. These results show that 2-H and 6-H of **19a,c,e** should be located in a pseudoaxial orientation, which is consistent with the above-mentioned X-ray analysis. On the other hand, the more polar isomers, **19b,d,f**, show distinctive signals of 2-H or 6-H at δ 4.13–4.41 with two small coupling constants (J = 3–5 Hz), which suggests that 2-H or 6-H might not be located in a pseudoaxial orientation of the chair conformation. Alkylation of the amino group of **19a–f** with the triflate **20**[11] gave crystalline **21a–f**, respectively. The preparation of **20** is shown in Scheme IV and elucidated later. The subsequent alkylation of the amide nitrogen of **21a–f** with *tert*-butyl bromoacetate afforded the diesters **22a–f**, respectively. Removal of the *tert*-butyl groups in **22a–f** was carried out with HCl–dioxane to give the hydrochlorides of the monoester monoacids **23a–f**, respectively. Alkaline hydrolysis of **23a–f** afforded the diacids **24a–f**, respectively.

Scheme II[a]



[a] **a**, * = *S* configuration; **b**, * = *R* configuration; Z = $PhCH_2OCO$.

The preparations of the 3-phenylperhydrothiazepinones **33a,b** are illustrated in Scheme II. The protected cysteine **25** was reacted with the *S* and *R* mesylates **26a,b** to give the S-alkylated cysteines **27a,b**, respectively. Removal of the protecting groups with trifluoroacetic acid followed by intramolecular condensation gave **28a,b**. Treatment of **28a,b** with HBr–AcOH afforded the 6-amino compounds **29a,b**, respectively, which were converted to the diacids **33a,b** via **30a,b**, **31a,b**, and **32a,b** in the same procedures described in the preparations of **24a–f**.

In the synthesis of the 3-(2-thienyl)perhydrothiazepinone **43** shown in Scheme III, the amino group of L-cysteine was protected by the phthaloyl group instead of the benzyloxycarbonyl group because of the instability of the thienyl moiety with HBr–AcOH. The conversion of *N*-phthaloylcysteine **34** to the perhydrothiazepinone **38** was carried out in the same manner as described in the preparations of **28a,b**. Considerable racemization at the carbon having the phthalimino group occurred during the intramolecular condensation of **37**. The desired **38** was separated as crystals from the solution of the products in EtOAc–CH_2Cl_2 during concentration. Alkylation of **38** with *tert*-butyl bromoacetate followed by removal of the phthaloyl group with hydrazine and then alkylation with **20** gave the diester **41**, which was converted to the diacid **43** via the acid ester **42** in the same manner as described in the preparations of **24a–f**.

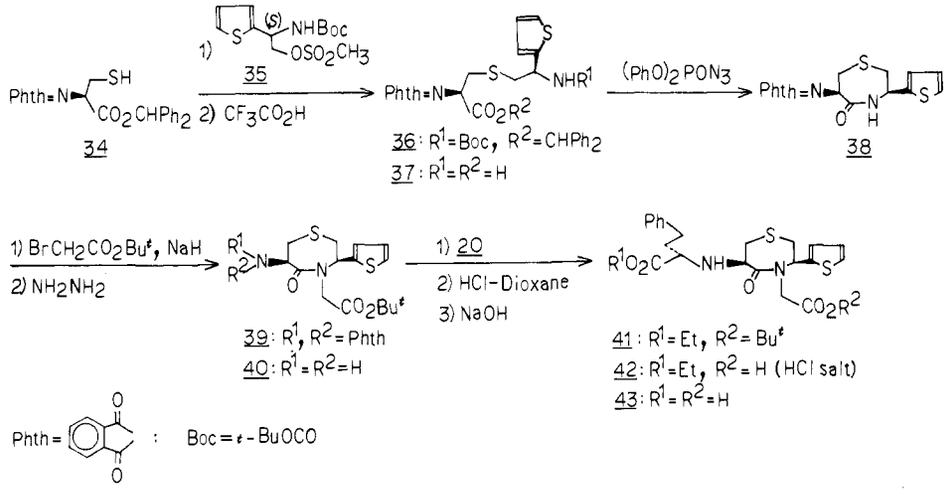
The preparation of the triflate **20**[11] is illustrated in Scheme IV. Esterification of the carboxylic acid **44**[12] with

(10) (a) Attwood, M. R.; Francis, R. J.; Hassall, C. H.; Kröhn, A.; Lawton, G.; Natoff, I. L.; Nixon, J. S.; Redshaw, S.; Thomas, W. A. *FEBS Lett.* **1984**, *165*, 201. (b) Thomas, W. A.; Whitcombe, W. A. *J. Chem. Soc., Perkin Trans. 2* **1986**, 747. (c) Attwood, M. R.; Hasall, C. H.; Kröhn, A.; Lawton, G. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1011.

(11) Compound **20** has recently been reported in ref 10c.

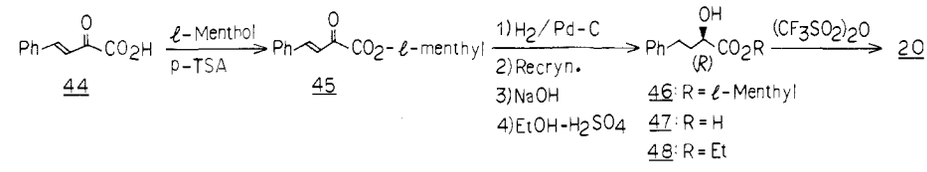
(12) Stecher, E. D.; Ryder, H. F. *J. Am. Chem. Soc.* **1952**, *74*, 4392.

Scheme III



34 36: R1 = Boc, R2 = CHPh2; 37: R1 = R2 = H 38 39: R1, R2 = Phth; 40: R1 = R2 = H 41: R1 = Et, R2 = But; 42: R1 = Et, R2 = H (HCl salt); 43: R1 = R2 = H

Scheme IV



44 45 46: R = l-Menthyl; 47: R = H; 48: R = Et 20

Table I. ACE Inhibitory Activity of **24a–f**, **33a,b**, **43**, and **2**

	IC_{50},[a] nM		IC_{50},[a] nM
24a	3.7	**24f**	86.9
24b	35.5	**33a**	78.2
24c	3.6	**33b**	3.4
24d	64.7	**43**	2.8
24e	4.1	**2**	5.7

[a] The concentration required for 50% inhibition of rabbit lung ACE with 5 mM hippurylhistidylleucine as substrate. Assays were done in duplicate, and the values were determined by linear regression of logit against log concentration over a 20–80% inhibition range.

equimolar *l*-menthol in the presence of *p*-toluenesulfonic acid gave the keto ester **45** quantitatively, which was hydrogenated with Pd–C to give the α-hydroxy ester. HPLC analysis of a product showed the mixture of the 2*R* and 2*S* isomers in a ratio of 55:45. Recrystallization from petroleum ether gave the pure *R* isomer **46**.[13] Alkaline hydrolysis of **46** followed by esterification with EtOH and then treatment with trifluoromethanesulfonic anhydride gave the desired compound **20** as a syrup.

Biological Activity. The diacids **24a–f**, **33a,b**, and **43** were evaluated in vitro for inhibition of rabbit lung ACE. The results are shown in Table I, in comparison with **2**. Compounds **24a,c,e** and **33b** were 10–20 times as potent as the corresponding epimers **24b,d,f** and **33a**. The 2-thienyl derivative **43** also possessed potent inhibitory activity. These active compounds **24a,c,e**, **33b**, and **43** were more potent than **2**.

Since oral availability of diacid compounds is poor,[14] we employed the monoethyl esters for in vivo evaluations. Monoester monoacids **23a,c,e**, **32b**, and **42** of the respective

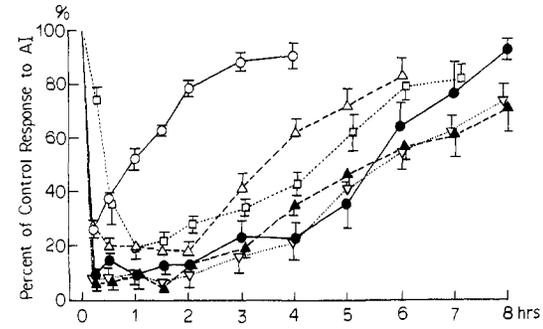


Figure 2. Time course for inhibition of pressor response to AI after a single oral administration of test compounds in conscious rats. Test compounds were administered at a dose of 1 mg/kg po in conscious normotensive rats. The pressor responses to AI, 0.3 μg/kg iv, were measured and plotted as percents of the predrug response. Each plot represents mean ± SE from four to seven experiments. Compounds and symbols: **3** (□), **23a** (●), **23c** (▲), **23e** (▽), **32b** (○), and **42** (△).

potent active diacids **24a,c,e**, **33b**, and **43** were administered orally at a 1 mg/kg dose to conscious normotensive rats, and inhibition of the pressor response to AI was measured. The results are shown in Figure 2, in comparison with **3**. Compounds **23a,c,e**, which have the phenyl, 2-thienyl, and 3-thienyl substituents at the 2-position, respectively, showed potent inhibitory activity with long duration. Compounds **32b** and **42**, having the phenyl and 2-thienyl groups at the 3-position, respectively, also showed potent inhibition, but less potent and with a shorter duration than that of the corresponding **23a,c**, respectively. All these thiazepinone compounds showed faster onset than **3**.

Discussion

In compound **5**, the diacids **24a,c,e** and **33b** possessing the hydrophobic substituents R^1 and R^4 have more potent in vitro inhibitory activity than the diacids **24b,d,f** and **33a** having the substituents R^2 and R^3. In order to elucidate the spatial situation of the substituents R^1–R^4 and the carboxyl group of **5**, conformational energy calculations

(13) Biquard, D. *Ann. chim.* **1933**, *20*, 97. The isomer **46** was easily crystallized. The stereochemistry of the corresponding carboxylic acid **47** was confirmed by Prelog and Wilhelm: Prelog, V.; Wilhelm, M. *Helv. Chim. Acta* **1954**, *37*, 1634.

(14) Gross, D. M.; Sweet, C. S.; Ulm, E. H.; Borklund, E. P.; Morris, A. A.; Weitz, D.; Bohn, D. L.; Wenger, H. C.; Vassil, T. C.; Stone, C. A. *J. Pharmacol. Exp. Ther.* **1981**, *216*, 552.

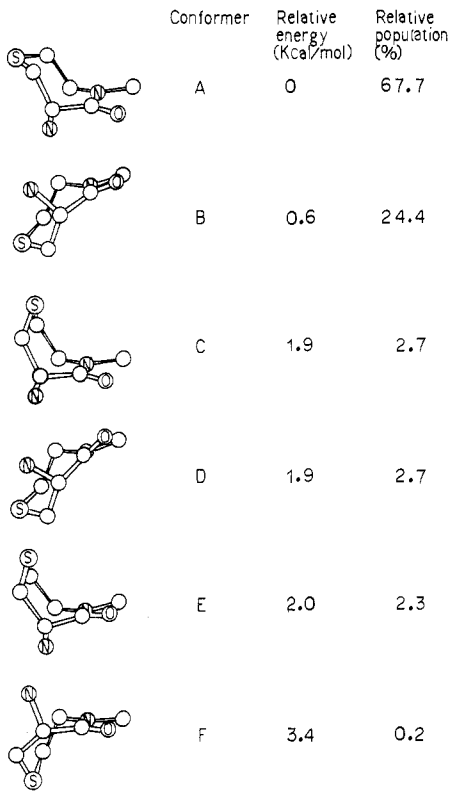
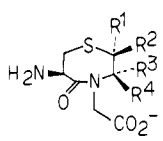
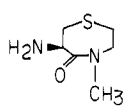


Figure 3. Stable conformations of 3-aminothiazepin-2-one derivative **50**.

were performed on the simplified model compounds **49a–d** by using the MNDO program.[15] First, geometry optimi-



49a: R^1=Ph, 49b: R^2=Ph, 49c: R^3=Ph, 49d: R^4=Ph



50

zations were carried out on **50** to find stable conformations of the thiazepinone ring. The six stable structures A–F shown in Figure 3 were obtained. While the energy difference between the most stable conformer (A) and the second one (B) was only 0.6 kcal/mol, the third one (C) was 1.9 kcal/mol higher in energy than A. Conformers A and B were calculated to comprise 92% of the equilibrium mixture at 25 °C from a Boltzmann distribution based on the relative energies. Therefore, the conformers A and B, with chair conformations, were employed to calculate the conformational energies of **49a–d**.

Energy minimizations were then done on **49a–d** in the form of a carboxylate anion by fixing the ring structures. Several stable conformations were found for each model compound. The lowest energy conformations of the molecules are shown in Figure 4 and compared with captopril (1).[16] They all have the phenyl group in a pseudoequatorial orientation. In the three-dimensional structure of **21c** determined by X-ray analysis, the thienyl group (R^1 in **5**) was also found to be located in the pseudoequatorial position.

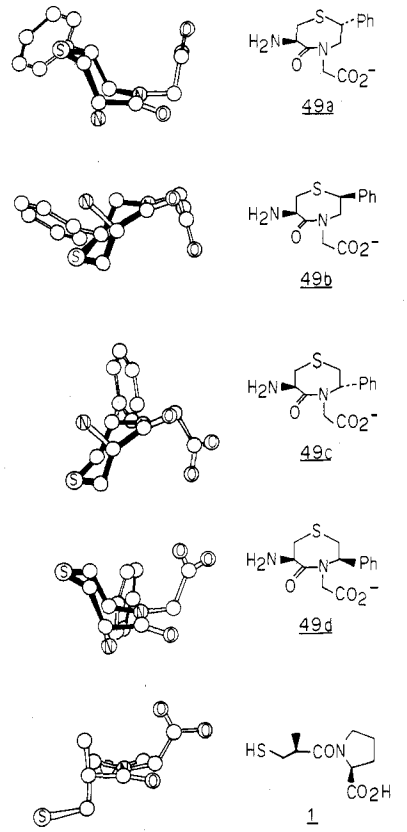


Figure 4. The most stable conformers of **49a–d** and 1. Thiazepinone ring is represented by solid bonds.

As seen in Figure 4, the carboxyl groups and the amide groups of **49a,d** corresponding to the potent inhibitors **24a** and **33b**, respectively, are well-superposed upon those of captopril (1). In contrast, **49b,c** corresponding to the weak inhibitors **24b** and **33a**, respectively, have carboxyl groups in places different from 1. It seems that the carboxyl group of the latter does not bind easily to the arginine moiety of ACE. Compounds **24a** and **33b** are more potent inhibitors than enalaprilat (2). This may be explained by increased binding of **24a** and **33b** due to interaction of the phenyl group at subsite S_2' of ACE and/or an effect of the phenyl substituent on the conformation of the thiazepinone ring that places a hydrophobic moiety in a favorable position[6] for binding to subsite S_1' of ACE. The amino groups of **49a,d** are in a pseudoequatorial orientation while those of **49b,c** are pseudoaxial. From a comparison with enalaprilat (2),[17] a pseudoequatorial amino group is considered to be preferable to a pseudoaxial one. The monoester monoacids **23a,c,e** possessing the substituents at the 2-position of the thiazepinone ring are superior to **32b** and **42** having substituents at the 3-position when administered orally. Such a superiority might be due to the better fit of the substituent at the 2-position to ACE subsite S_2'.

Experimental Section

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Recrystallization solvents for analytical samples are described in parentheses after melting points. Optical rotations were measured at 25 °C with a Perkin-Elmer 241 polarimeter at a 1% solution in DMF except where noted otherwise. Proton NMR spectra were obtained on a Varian EM390 or EM360L spectrometer. IR spectra were taken on a

(15) Dewar, M. J. S.; Mckee, M. L.; Rzepa, H. S. *J. Am. Chem. Soc.* **1978**, *100*, 3607.

(16) Fujinaga, M.; James, M. N. G. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1980**, *B36*, 3196.

(17) 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.

JASCO A-102 infrared spectrophotometer. Elemental analyses were performed by Analytical and Metabolic Research Laboratories of Sankyo Co., and results obtained were within ±0.4% of the theoretical values unless indicated otherwise. TLC analyses were performed on precoated plates of Merck silica gel 60 F_{254}, and spots were detected by UV irradiation or iodine vapor. Flash chromatography[18] was done on Merck silica gel 60 (230–400 mesh). The following solvent systems were used in TLC and flash chromatography: A (1-butanol–AcOH–H_2O, 4:1:1); B (EtOAc–cyclohexane, 1:1); C (EtOAc–cyclohexane, 1:3); D (EtOAc–cyclohexane, 1:4); E (EtOAc–CH_2Cl_2–cyclohexane, 1:6:3); F (EtOAc–CH_2Cl_2, 1:9); G (EtOAc–CH_2Cl_2, 1:20); H (MeOH–CH_2Cl_2 1:9); and I (MeOH–CH_2Cl_2, 1:20). HPLC analyses were performed on a System 8000 of Toyo Soda Manufacturing Co. Ltd. equipped with a UV detector (230 nm). A reversed-phase column, Unisil Pack 5c18 (4.6 × 250 mm; Gasukuro Kogyo Co., Ltd.), was used. Isopropyl alcohol and isopropyl ether are abbreviated as IPA and IPE, respectively.

***N*-(*tert*-Butoxycarbonyl)-*S*-[(*RS*)-2-nitro-1-phenylethyl]-L-cysteine (10).** A mixture of L-cysteine (18.15 g, 0.15 mol), di-*tert*-butyl dicarbonate (32.7 g, 0.15 mol), and $NaHCO_3$ (31.5 g, 0.375 mol) in THF (75 mL) and water (180 mL) was stirred under N_2 at room temperature for 5 h. Ice and EtOAc were added, and the solution was adjusted to pH 2.9 with concentrated HCl. The organic phase was separated, washed with brine, dried over $MgSO_4$, and evaporated in vacuo to give an oil of Boc-L-cysteine (**6**). To a solution of **6** in toluene (375 mL) were added β-nitrostyrene (**7**) (22.4 g, 0.15 mol) and *N*-methylmorpholine (16.5 mL, 0.15 mol) dropwise in an ice bath. The mixture was allowed to stand at room temperature for 16 h. The product was extracted with water (0.3 L) and 5% aqueous solution of *N*-methylmorpholine (5 × 0.1 L). The combined aqueous phases were mixed with ice and EtOAc and adjusted to pH 3 with concentrated HCl. The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo to give **10** (49.0 g, 88%) as a syrup: $[\alpha]_D$ −22.9°; NMR ($CDCl_3$) δ 1.45 (9 H, s), 2.8–3.0 (2 H, m), 4.3–4.8 (4 H, m), 5.2–5.5 (1 H, br d), 7.35 (5 H, s), 9.43 (1 H, s); IR (neat) 3400, 3300, 1710, 1660 (sh), 1555 cm^{-1}.

The following compounds were prepared in a similar manner. Compound **11**: syrup; 84%; $[\alpha]_D$ −21.5°. Compound **12**: syrup; 93%; $[\alpha]_D$ −33.0°.

***S*-[(*RS*)-2-Amino-1-phenylethyl]-*N*-(*tert*-butoxycarbonyl)-L-cysteine (13).** A mixture of **10** (49 g, 0.13 mol) and 5% Pd–C (24 g) in AcOH (600 mL) was stirred under 3 kg/cm^2 of H_2 at 70 °C for 5 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residual syrup was dissolved in MeOH (55 mL) and H_2O (55 mL), subjected to column chromatography of HP-20 (high porous resin of Mitsubishi Chemical Ind.), and eluted successively with H_2O and 20% and 50% aqueous acetone. The fractions containing **13** were concentrated in vacuo to give **13** as a white solid (22.6 g, 51%): mp 172–182 °C dec; TLC R_f 0.58 (solvent A); $[\alpha]_D$ −29.2° (*c* 1, 0.1 N NaOH); NMR (NaOD) δ 1.87 and 1.92 (9 H, s), 3.2–3.67 (4 H, m), 4.28–4.70 (2 H, m), 7.94 (5 H, s); IR (Nujol) 3400, 1715, 1690, 1640, 1590 cm^{-1}. Anal. ($C_{16}H_{24}N_2O_4S \cdot {}^1/_2H_2O$) C, H, N, S.

The following compounds were prepared in a similar manner. Compound **14**: 64%; mp 127–130 °C; TLC R_f 0.58 (solvent A); $[\alpha]_D$ −19.9°. Anal. ($C_{14}H_{22}N_2O_4S_2 \cdot {}^1/_2H_2O$) C, H, N, S. Compound **15**: 61%; mp 116–118 °C; TLC R_f 0.58 (solvent A); $[\alpha]_D$ +61.9°. Anal. ($C_{14}H_{22}N_2O_4S_2 \cdot {}^1/_2H_2O$) C, H, N, S.

(2*RS*,6*R*)-6-[(*tert*-Butoxycarbonyl)amino]-2-phenylperhydro-1,4-thiazepin-5-one (16). To a solution of **13** (21.6 g, 0.063 mol) and diphenyl phosphorazidate (16.4 mL, 0.075 mol) in DMF (220 mL) was added *N*-methylmorpholine (16.7 mL, 0.152 mol) dropwise in an ice bath, and the mixture was stirred at room temperature for 16 h. The reaction mixture was partitioned between EtOAc (0.5 L) and H_2O (0.5 L). The organic phase was separated, and the aqueous phase was extracted with EtOAc (2 × 0.25 L). The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give crystals, which were triturated in Et_2O: yield 15.9 g (78%); mp 201–213 °C (EtOAc–Et_2O); $[\alpha]_D$ +5.8°; NMR (DMSO-d_6) δ 1.41 (9 H, s), 2.65–3.1 (2 H, m), 3.6–4.35 (3 H, m), 4.55–4.9 (1 H, m), 6.4–6.8 (1 H, m), 7.25–7.5 (5 H, m), 7.8–8.1 (1 H, m); IR (Nujol) 3380, 3310, 3250, 1710, 1690, 1670 cm^{-1}. Anal. ($C_{16}H_{22}N_2O_3S$) C, H, N, S.

The following compounds were prepared in a similar manner. Compound **17**: 69%; mp 180–195 °C (EtOH–Et_2O); $[\alpha]_D$ −15.0°. Anal. ($C_{14}H_{20}N_2O_3S_2$) C, H, N, S. Compound **18**: 62%; mp 200–217 °C dec (EtOH–Et_2O); $[\alpha]_D$ −16.1°. Anal. ($C_{14}H_{20}N_2O_3S_2$) C, H, N, S.

(2*R*,6*R*)-6-Amino-2-phenylperhydro-1,4-thiazepin-5-one (19a) and Its 2*S* Isomer 19b. A solution of **16** (9.5 g, 29.5 mmol) in 4 N HCl–dioxane (24 mL) was stirred at room temperature for 4 h. Addition of Et_2O gave the precipitates (7.85 g) of the HCl salts of **19a,b**, which were collected by filtration. To a suspension of the hydrochlorides in MeOH (15 mL) and CH_2Cl_2 (150 mL) was added a solution of K_2CO_3 (10.4 g, 75.4 mmol) in H_2O (30 mL), and the mixture was stirred at room temperature until the hydrochlorides were dissolved. The organic phase was separated, and the aqueous phase was extracted with MeOH–CH_2Cl_2 (1:9) (3 × 30 mL). The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to give a mixture of **19a,b**. TLC (solvent A) showed two spots of R_f 0.38 and 0.45. Fractional recrystallization from MeOH–EtOAc afforded **19a** (2.2 g, 33%): mp 222–229 °C (dec started at 205 °C); TLC R_f 0.45 (solvent A); $[\alpha]_D$ +19.5°; NMR (DMSO-d_6) δ 2.58 (1 H, d, d, *J* = 3, 14 Hz), 2.89 (1 H, d, d, *J* = 9, 14 Hz), 3.2–4.0 (3 H, m), 4.09 (1 H, d, d, *J* = 3, 9 Hz), 7.39 (5 H, s); IR (Nujol) 3380, 3340, 3250 (sh), 3200, 1680, 1620 cm^{-1}. Anal. ($C_{11}H_{14}N_2OS$) C, H, N, S. The mother solution was concentrated, and fractional recrystallization was repeated to give further **19a** (1.2 g, 18%) containing small amounts of **19b**. The mother solution was evaporated, and the residue was recrystallized from aqueous acetone to give **19b** (2.1 g, 32%): mp 176–180 °C; TLC R_f 0.38 (solvent A); $[\alpha]_D$ +20.9°; NMR (DMSO-d_6) δ 2.59 (1 H, d, d, *J* = 8, 14 Hz), 2.93 (1 H, d, d, *J* = 5, 14 Hz), 3.67–3.83 (2 H, m), 3.95 (1 H, d, d, *J* = 5, 8 Hz), 4.13 (1 H, t, *J* = 4 Hz), 7.25–7.5 (5 H, m), 7.64 (1 H, m); IR (Nujol) 3380, 3340, 3250 (sh), 3200, 1680, 1620 cm^{-1}. Anal. ($C_{11}H_{14}N_2OS$) C, H, N, S.

The following compounds were prepared in a similar manner. Compound **19c**: 35%; mp 163–165 °C (EtOH); TLC R_f 0.46 (solvent A); $[\alpha]_D$ +54.4°. Anal. ($C_9H_{12}N_2OS_2$) C, H, N, S. Compound **19d**: 31%; mp 117–119 °C (EtOH); TLC R_f 0.38 (solvent A); $[\alpha]_D$ −128.6°. Anal. ($C_9H_{12}N_2OS_2$) C, H, N, S. Compound **19e**: 32%; mp 194–197 °C (dec started from 173 °C) (EtOH); TLC R_f 0.42 (solvent A); $[\alpha]_D$ +57.2°. Anal. ($C_9H_{12}N_2OS_2$) C, H, N, S. Compound **19f**: 37%; mp 153–156 °C (dec started from 142 °C) (EtOH–EtOAc); TLC R_f 0.36 (solvent A); $[\alpha]_D$ −111.0°. Anal. ($C_9H_{12}N_2OS_2$) C, H, N, S.

(2*R*,6*R*)-6-[[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2-phenylperhydro-1,4-thiazepin-5-one (21a). To a suspension of **19a** (1.66 g, 7.47 mmol) in CH_2Cl_2 (32 mL) were added Et_3N (1.2 mL, 10.6 mmol) and then a solution of **20** (3.6 g, 10.6 mmol) in CH_2Cl_2 (10 mL). After being stirred at room temperature for 16 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in EtOAc, washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The crystalline residue was triturated in IPE: yield 2.75 g (89%). The mother solution was concentrated, and the residue was subjected to flash chromatography (solvent B) to give further **21a** (0.25 g, 8%): mp 118–119.5 °C (EtOAc–cyclohexane); TLC R_f 0.35 (solvent B); $[\alpha]_D$ +3.5°; NMR ($CDCl_3$) δ 1.28 (3 H, t, *J* = 7 Hz), 1.8–2.2 (2 H, m), 2.6–3.15 (5 H, m), 3.3–4.05 (5 H, m), 4.18 (2 H, q, *J* = 7 Hz), 6.4–6.7 (1 H, m), 7.24 (5 H, s), 7.33 (5 H, s); IR (Nujol) 3290, 3250, 1720, 1665, 1630 cm^{-1}. Anal. ($C_{23}H_{28}N_2O_3S$) C, H, N, S.

The following compounds were prepared in a similar manner. Compound **21b**: 97%; mp 79–81 °C (EtOAc–cyclohexane); TLC R_f 0.33 (solvent B); $[\alpha]_D$ +7.7°. Anal. ($C_{23}H_{28}N_2O_3S$) C, H, N, S. Compound **21c**: 80%; mp 104–105 °C (EtOH); TLC R_f 0.37 (solvent B); $[\alpha]_D$ +33.0°. Anal. ($C_{21}H_{26}N_2O_3S_2$) C, H, N, S. Compound **21d**: 79%; mp 80–82 °C (IPE); TLC R_f 0.41 (solvent B); $[\alpha]_D$ −80.4°. Anal. ($C_{21}H_{26}N_2O_3S_2$) C, H, N, S. Compound **21e**: 93%; mp 120.5–121 °C (EtOAc–cyclohexane); TLC R_f 0.31 (solvent B); $[\alpha]_D$ +31.1°. Anal. ($C_{21}H_{26}N_2O_3S_2$) C, H, N, S. Compound **21f**: 97%; mp 70–72 °C (EtOAc–cyclohexane); TLC R_f 0.35 (solvent B); $[\alpha]_D$ −61.1°. Anal. ($C_{21}H_{26}N_2O_3S_2$) C, H, N, S. Compound **30a**: 87%; mp 105 °C (EtOAc–IPE); TLC R_f 0.41 (solvent B); $[\alpha]_D$ +29.9°. Anal. ($C_{23}H_{28}N_2O_3S$) C, H, N, S.

(18) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

Compound **30b**: syrup; quantitative; TLC R_f 0.53 (solvent B); the analytical sample was obtained as the HCl salt; mp 164–168 °C (EtOH–EtOAc); $[\alpha]_D$ +24.5°. Anal. ($C_{23}H_{29}ClN_2O_3S$) H, Cl, N, S; C: calcd, 6.99; found, 6.44.

***tert*-Butyl α-[(2*R*,6*R*)-6-[[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-5-oxo-2-phenylperhydro-1,4-thiazepin-4-yl]acetate (22a).** To a solution of **21a** (2.7 g, 6.54 mmol) in DMF (27 mL) were added *tert*-butyl bromoacetate (1.16 mL, 7.19 mmol) and NaH (55% dispersion in mineral oil; 314 mg, 7.19 mmol) at 5 °C under N_2. After being stirred at room temperature for 5 h, the reaction mixture was partitioned between EtOAc and H_2O. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to flash chromatography (solvent C) to give a syrup: yield 3.35 g (97%); TLC *Rf* 0.35 (solvent C); $[\alpha]_D$ +21.7°; NMR ($CDCl_3$) δ 1.28 (3 H, t, *J* = 7 Hz), 1.47 (9 H, s), 1.8–2.3 (2 H, m), 2.5–4.5 (8 H, m), 4.07 (2 H, AB q, Δδ = 0.46 ppm, *J* = 17 Hz), 4.18 (2 H, q, *J* = 7 Hz), 7.23 (5 H, s), 7.32 (5 H, s); IR (neat) 3330, 1740, 1660 cm^{-1}.

The following compounds were prepared in a similar manner. Compound **22b**: syrup; 99%; TLC R_f 0.34 (solvent C); $[\alpha]_D$ −15.4°. Compound **22c**: syrup; 94%; TLC R_f 0.35 (solvent C); $[\alpha]_D$ +39.3°. Compound **22d**: syrup; 97%; TLC R_f 0.34 (solvent C); $[\alpha]_D$ −46.6°. Compound **22e**: syrup; 99%; TLC R_f 0.34 (solvent C); $[\alpha]_D$ +43.9°. Compound **22f**: syrup; 94%; TLC R_f 0.33 (solvent C); $[\alpha]_D$ −45.3°. Compound **31a**: syrup; quantitative; TLC R_f 0.38 (solvent C); $[\alpha]_D$ +58.3°. Compound **31b**: syrup; quantitative; TLC R_f 0.38 (solvent C); $[\alpha]_D$ +8.5°.

α-[(2*R*,6*R*)-6-[[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-5-oxo-2-phenylperhydro-1,4-thiazepin-4-yl]acetic Acid Hydrochloride (23a). A solution of **22a** (3.1 g, 5.89 mmol) in 4 N HCl–dioxane (16 mL) was allowed to stand at room temperature for 16 h. The solvent was evaporated in vacuo, and the residual syrup was powdered in ether. The powder was collected by filtration: yield 2.82 g (94%); mp 112–115 °C (softened from 96 °C); $[\alpha]_D$ +25.9°; NMR (DMSO-d_6) δ 1.28 (3 H, t, *J* = 7 Hz), 2.0–2.35 (2 H, m), 2.5–4.7 (10 H, m), 4.25 (2 H, q, *J* = 7 Hz), 4.9–5.15 (1 H, m), 7.30 (5 H, s), 7.40 (5 H, s); IR (Nujol) 2680, 1740, 1665 cm^{-1}. Anal. ($C_{25}H_{31}ClN_2O_5S\cdot{}^1/_2H_2O$) C, H, Cl, N, S.

The following compounds were prepared in a similar manner. Compound **23b**: 94%; mp 142–144 °C; $[\alpha]_D$ +50.0°. Anal. ($C_{25}H_{31}ClN_2O_5S$) C, H, Cl, N, S. Compound **23c**: 78%; mp 187 °C dec (EtOH–EtOAc); $[\alpha]_D$ +47.7°. Anal. ($C_{23}H_{29}ClN_2O_5S_2$) C, H, Cl, N, S. Compound **23d**: 81%; mp 174–175 °C dec (EtOH); $[\alpha]_D$ +7.5°. Anal. ($C_{23}H_{29}ClN_2O_5S_2$) C, H, Cl, N, S. Compound **23e**: 90%; mp 145–148 °C (colored at 140 °C); $[\alpha]_D$ +48.9°. Anal. ($C_{23}H_{29}ClN_2O_5S_2$) C, H, Cl, N, S. Compound **23f**: 88%; mp 147–149°C (EtOH–EtOAc); $[\alpha]_D$ +8.1°. Anal. ($C_{23}H_{29}ClN_2O_5S_2$) C, H, Cl, N, S. Compound **32a**: 94%; mp 100 °C (softened) (EtOAc–Et_2O); $[\alpha]_D$ +42.1°. Anal. ($C_{25}H_{31}ClN_2O_5S$) C, H, N, S; Cl: calcd, 6.99; found, 6.44. Compound **32b**: 81%; mp 130 °C (EtOH–EtOAc); $[\alpha]_D$ +25.5°. Anal. ($C_{25}H_{31}ClN_2O_5S\cdot{}^1/_2H_2O$) C, H, Cl, N, S. Compound **42**: 98%; mp 97 °C (softened) (EtOAc–Et_2O); $[\alpha]_D$ +31.5°. Anal. ($C_{23}H_{29}ClN_2O_5S_2$) C, H, N, S; Cl: calcd, 6.91; found, 6.18.

α-[(2*R*,6*R*)-6-[[(*S*)-1-Carboxy-3-phenylpropyl]amino]-5-oxo-2-phenylperhydro-1,4-thiazepin-4-yl]acetic Acid (24a). A suspension of **23a** (0.5 g, 0.98 mmol) in 1 N aqueous NaOH (12 mL) was stirred at room temperature for 16 h. The reaction solution was adjusted to pH 3 with 1 N HCl. The precipitates were collected by filtration: yield 0.42 g (96%); mp 239–241 °C (dec started at 235 °C); $[\alpha]_D$ +36.6°; NMR (NaOD) δ 2.3–2.6 (2 H, m), 3.0–5.0 (11 H, m), 7.85 (5 H, s), 7.90 (5 H, s); IR (Nujol) 3150, 2650, 1735, 1680, 1610 cm^{-1}. Anal. ($C_{23}H_{26}N_2O_5S$) C, H, N, S.

The following compounds were prepared in a similar manner. Compound **24b**: 96%; mp 201 °C dec (softened from 190 °C); $[\alpha]_D$ −6.9°. Anal. ($C_{23}H_{26}N_2O_5S\cdot{}^1/_2H_2O$) C, H, N, S. Compound **24c**: 96%; mp 246 °C dec (EtOH); $[\alpha]_D$ +63.4°. Anal. ($C_{21}H_{24}N_2O_5S_2$) C, H, N, S. Compound **24d**: 97%; mp 125–130 °C (softened) (EtOH); $[\alpha]_D$ −45.1°. Anal. ($C_{21}H_{24}N_2O_5S_2\cdot{}^1/_2H_2O$) C, H, N, S. Compound **24e**: 98%; mp 252–254 °C dec; $[\alpha]_D$ +64.3°. Anal. ($C_{21}H_{24}N_2O_5S_2$) C, H, N, S. Compound **24f**: quantitative; mp 137–140 °C (softened); $[\alpha]_D$ −33.3°. Anal. ($C_{21}H_{24}N_2O_5S_2\cdot 2H_2O$) C, H, N, S. Compound **33a**: 95%; mp 145 °C (softened); $[\alpha]_D$ +72.5°. Anal. ($C_{23}H_{26}N_2O_5S\cdot H_2O$) C, H, N, S. Compound **33b**: 88%; mp 216 °C dec; $[\alpha]_D$ +29.2°. Anal. ($C_{23}H_{26}N_2O_5S\cdot{}^1/_2H_2O$) C, H, N, S. Compound **43**: 97%; mp 210–212 °C (colored at 165 °C); $[\alpha]_D$ +30.3°. Anal. ($C_{21}H_{24}N_2O_5S_2\cdot H_2O$) C, H, N, S.

***N*-(Benzyloxycarbonyl)-*S*-[(*R*)-2-[(*tert*-butoxycarbonyl)amino]-2-phenylethyl]-L-cysteine Diphenylmethyl Ester (27b).** To a solution of L-cysteine (6.1 g, 0.05 mol) and $NaHCO_3$ (10 g, 0.12 mol) in H_2O (150 mL) was added a solution of benzyloxycarbonyl azide (10 g, 0.056 mol) in DMF (100 mL). The mixture was stirred at room temperature for 0.5 h and then at 50 °C for 1.5 h under N_2. IPE and H_2O were added. The aqueous phase was separated, mixed with EtOAc, and adjusted to pH 2 with concentrated HCl. The organic phase was separated, washed with brine, dried over $MgSO_4$, and treated with diphenyldiazomethane (10 g, 0.051 mol). After evaporation of the solvent in vacuo, the residue was purified by flash chromatography (solvent D) to provide *N*-(benzyloxycarbonyl)-L-cysteine diphenylmethyl ester (18 g, 85%) as a syrup. To a solution of this ester (12.7 g, 30.1 mmol) in DMF (140 mL) were added [(*R*)-1-[(butoxycarbonyl)amino]-2-[(methylsulfonyl)oxy]ethyl]benzene (**26b**) (9.5 g, 30.1 mmol), prepared from D-2-phenylgycinol, and spray-dried powder of KF (Morita Chemical Industry Co., Ltd.) (25 g, 0.43 mol). The mixture was stirred at 70 °C for 18 h and then partitioned between EtOAc and H_2O. The organic phase was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give the crystalline residue, which was triturated in IPE–cyclohexane: yield 9.5 g (49%). The filtrate was concentrated in vacuo, and the residue was subjected to flash chromatography (solvent D) to give further **27b** (2.2 g, 11%): mp 140–142 °C (EtOAc–IPE); TLC R_f 0.48 (solvent C); $[\alpha]_D$ −49.0°; NMR ($CDCl_3$) δ 1.38 (9 H, s), 2.6–2.9 (4 H, m), 4.4–5.4 (3 H, m), 5.05 (2 H, s), 5.80 (1 H, br d, *J* = 8 Hz), 6.88 (1 H, s), 7.20 (5 H, s), 7.29 (10 H, s); IR (Nujol) 3360, 1745, 1690 cm^{-1}. Anal. ($C_{37}H_{40}N_2O_6S$) C, H, N, S.

Compound **27a** was prepared in a similar manner: 45%; mp 130–132 °C (EtOAc–IPE); TLC R_f 0.42 (solvent C); $[\alpha]_D$ +28.5°. Anal. ($C_{37}H_{40}N_2O_5S$) C, H, N, S.

(3*R*,6*R*)-6-[(Benzyloxycarbonyl)amino]-3-phenylperhydro-1,4-thiazepin-5-one (28b). A solution of **27b** (9.0 g, 14 mmol) in anisole (40 mL) and trifluoroacetic acid (60 mL) was allowed to stand at room temperature for 2 h and then concentrated in vacuo. The residual syrup was solidified in IPE, and the solid was collected by filtration to give the crude trifluoroacetate of *S*-[(*R*)-2-amino-2-phenylethyl]-*N*-(benzyloxycarbonyl)-L-cysteine (8.5 g, quantitative). To a solution of this salt in DMF (85 mL) was added diphenyl phosphorazidate (6.24 g, 22.7 mmol) and then *N*-methylmorpholine (6.32 mL, 57.1 mmol) in an ice bath, and the mixture was allowed to stand at room temperature for 16 h. The reaction mixture was partitioned between EtOAc and H_2O. The organic phase was separated, washed with brine, aqueous $KHSO_4$, and aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to flash chromatography (solvent E and then solvent G) to give **28b** (3.93 g, 74%) as a solid: mp 150 °C (EtOAc–IPE); TLC R_f 0.42 (solvent E); $[\alpha]_D$ +29.4°; NMR ($CDCl_3$) δ 2.6–3.2 (4 H, m), 4.75–5.0 (2 H, m), 5.15 (2 H, s), 6.1–6.4 (2 H, m), 7.38 (10 H, m); IR (Nujol) 3400, 3260, 3210, 1725, 1675, 1660 cm^{-1}. Anal. ($C_{19}H_{20}N_2O_3S$) C, H, N, S.

Compound **28a** was prepared in a similar manner: 43%; mp 128 °C (EtOAc–IPE); TLC R_f 0.08 (solvent E); $[\alpha]_D$ +98.9°. Anal. ($C_{19}H_{20}N_2O_3S$) C, H, N, S.

(3*R*,6*R*)-6-Amino-3-phenylperhydro-1,4-thiazepin-5-one (29b). A solution of **28b** (3.0 g, 8.43 mmol) in 25% HBr–AcOH (15 mL) was allowed to stand at room temperature for 3 h. Ether was added to give a precipitate of the HBr salt of **29b**, which was added to a mixture of 10% EtOH–CH_2Cl_2 (150 mL) and 25% aqueous K_2CO_3 (30 mL). The mixture was stirred for 0.5 h. The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo to give the crystalline **29b** (1.86 g, 99%); mp 123–125 °C (EtOAC); $[\alpha]_D$ +71.7°; NMR ($CDCl_3$) δ 2.13 (2 H, s), 2.5–3.25 (4 H, m), 4.14 (1 H, d, d, *J* = 3, 10 Hz), 4.83 (1 H, m), 6.03 (1 H, m), 7.38 (5 H, s); IR (Nujol) 3390, 3240, 1680, 1645 cm^{-1}. Anal. ($C_{11}H_{14}N_2OS\cdot{}^1/_4H_2O$) C, H, N, S.

Compound **29a** was prepared in a similar manner: gelatinous substance; quantitative; the analytical sample was obtained as the crystalline HCl salt; mp 223–225 °C dec (EtOAc–IPA); $[\alpha]_D$ +145.8°. Anal. ($C_{11}H_{15}ClN_2OS$) C, H, Cl, N, S.

***S*-[(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-2-(2-thienyl)ethyl]-*N*-phthaloyl-L-cysteine Diphenylmethyl Ester (36).** A mixture of L-cysteine (12.1 g, 0.1 mol), *N*-carbethoxyphthalimide (21.9 g, 0.1 mol), and $NaHCO_3$ (20 g, 0.24 mol) in H_2O (200 mL) and DMF (200 mL) was stirred at 80 °C for 2 h under N_2. EtOAc and H_2O were added. The solution was adjusted to pH 2 with concentrated HCl, and the organic phase was separated, washed with brine, and dried over $MgSO_4$. To the dried solution was added diphenyldiazomethane (20 g, 0.1 mol), and the solution was stirred at room temperature for 1.5 h. The solvent was evaporated in vacuo, and the residue was subjected to flash chromatography (solvent D) to give *N*-phthaloyl-L-cysteine diphenylmethyl ester (24.4 g, 58%) as a syrup: TLC R_f 0.49 (solvent C). This ester was S-alkylated with 2-[(*S*)-1-[(*tert*-butoxycarbonyl)amino]-2-[(methylsulfonyl)oxy]ethyl]thiophene (**35**) (18.5 g, 0.064 mol) by the same procedure as described in the preparation of **27b** to give **36** (28.2 g, 77%) as a semisolid; mp 50 °C (softened); TLC R_f 0.42 (solvent C); $[\alpha]_D$ −29.9°; NMR ($CDCl_3$) δ 1.42 (9 H, s), 2.85–3.4 (4 H, m), 4.85–5.3 (3 H, m), 6.8–7.2 (4 H, m), 7.28 (5 H, s), 7.32 (5 H, s), 7.55–8.05 (4 H, m); IR (Nujol) 3370, 1775 (wk), 1745 (wk), 1715 cm^{-1}. Anal. ($C_{35}H_{34}N_2O_6S_2$) C, H, N, S.

(3*S*,6*R*)-6-Phthalimido-3-(2-thienyl)perhydro-1,4-thiazepin-5-one (38). Conversion of **36** (26.2 g, 40.8 mmol) to **38** was carried out by the same procedure as described in the preparation of **28b**. The product showed two spots of R_f 0.6 and 0.3 on TLC (solvent F). The less polar **38** was separated as crystals during concentration of the extract of the products and collected by filtration: yield 3.3 g (22%). The filtrate was subjected to flash chromatography (solvent F) to give further **38** (0.35 g, 2%) and the more polar 3*S*,6*S* isomer (6.9 g, 46%) as an amorphous solid. Compound **38**: mp 264 °C; $[\alpha]_D$ +51.8°; NMR (DMSO-d_6) δ 2.8–3.6 (4 H, m), 5.30 (1 H, d, t, J = 3, 9 Hz), 5.53 (1 H, t, J = 6.5 Hz), 7.02 (1 H, d, d, J = 4, 6 Hz), 7.28 (1 H, d, J = 4 Hz), 7.52 (1 H, d, J = 6 Hz), 7.94 (4, H, s), 7.98 (1 H, d, J = 6.5 Hz); IR (Nujol) 3330, 1775, 1725, 1675 cm^{-1}. Anal. ($C_{17}H_{14}N_2O_3S_2$) C, H, N, S.

***tert*-Butyl [(3*S*,6*R*)-5-Oxo-6-phthalimido-3-(2-thienyl)perhydro-1,4-thiazepin-4-yl]acetate (39).** By the same procedure as described in the preparation of **22a**, **38** (3.25 g, 9.1 mmol) was alkylated with *tert*-butyl bromoacetate to give **39**: yield 2.6 g (61%); mp 212 °C dec (EtOAc); TLC R_f 0.28 (solvent C); $[\alpha]_D$ +66.9°; NMR ($CDCl_3$) δ 1.39 (9 H, s), 2.9–4.0 (4 H, m), 3.78 (2 H, AB q, $\Delta\delta$ = 0.48 ppm, J = 17 Hz), 5.60 (1 H, d, d, J = 1.5, 10 Hz), 5.76 (1 H, d, d, J = 4, 8 Hz), 6.98–7.3 (2 H, m), 7.39 (1 H, d, d, J = 2, 5 Hz), 7.65–7.95 (4 H, m); IR (Nujol) 1775, 1735, 1720, 1660 cm^{-1}. Anal. ($C_{23}H_{24}N_2O_5S_2$) C, H, N, S.

***tert*-Butyl [(3*S*,6*R*)-6-Amino-5-oxo-3-(2-thienyl)perhydro-1,4-thiazepin-4-yl]acetate (40).** A mixture of **39** (2.2 g, 4.66 mmol) and hydrazine monohydrate (1.1 mL, 22.7 mmol) in MeOH (20 mL) was stirred at room temperature for 40 h. The precipitates were filtered off, and the filtrate was concentrated in vacuo. The residue was subjected to flash chromatography (solvent I) to give **40** (1.50 g, quantitative) as a syrup: TLC R_f 0.71 (solvent H). The analytical sample was obtained as the maleate: mp 90 °C (softened) (EtOAc–Et_2O); $[\alpha]_D$ +60.9°; NMR (DMSO-d_6) δ 1.30 (9 H, s), 2.7–4.0 (4 H, m), 3.74 (2 H, AB q, $\Delta\delta$ = 0.24 ppm, J = 17 Hz), 5.12 (1 H, br t, J = 5 Hz), 5.55 (1 H, d, d, J = 4.5, 10 Hz), 6.05 (2 H, s), 7.08 (1 H, d, d, J = 3, 5 Hz), 7.29 (1 H, d, J = 3 Hz), 7.64 (1 H, d, J = 5 Hz); IR (Nujol) 3450, 2600, 1730, 1660 cm^{-1}. Anal. ($C_{19}H_{26}N_2O_7S_2$) C, H, N, S.

***tert*-Butyl α-[(3*S*,6*R*)-6-[[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-5-oxo-3-(2-thienyl)perhydro-1,4-thiazepin-4-yl]acetate (41).** By the same procedure as described in the preparation of **21a**, **40** (1.14 g, 4.54 mmol) was alkylated with **20** to give **41** (1.98 g, 82%) as a syrup: TLC R_f 0.36 (solvent C); $[\alpha]_D$ +26.3°; NMR ($CDCl_3$) δ 1.28 (3 H, t, J = 7.5 Hz), 1.39 (9 H, s), 1.8–2.2 (2 H, m), 2.55–4.2 (12 H, m), 4.16 (2 H, q, J = 7.5 Hz), 5.45 (1 H, d, d, J = 2.5, 10 Hz), 6.95–7.1 (2 H, m), 7.25 (5 H, s), 7.35 (1 H, d, d, J = 3, 5.5 Hz); IR (neat) 3320, 1735, 1655 cm^{-1}.

Ethyl (*R*)-4-Phenyl-2-[[(trifluoromethyl)sulfonyl]oxy]butyrate (20).[11] A mixture of benzylidenepyruvic acid[12] (62 g, 0.35 mol), *l*-menthol (49.45 g, 0.316 mol), and *p*-toluenesulfonic acid monohydrate (6.2 g, 0.033 mol) in benzene (300 mL) was stirred under reflux for 5 h in a flask fitted with a Dean–Stark dehydrator. The solution was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to give *l*-menthyl benzylidenepyruvate (**45**) (105.8 g, quantitative) as an oil. A mixture of **45** (33.9 g, 0.108 mol) and 5% Pd–C (3.3 g) in IPA (250 mL) was stirred under 3 kg/cm^2 of H_2 at 50 °C for 5 h. HPLC of the reduction product showed the mixture of **46**[13] (t_R 22.5 min) and its diastereoisomer (t_R 24.0 min) in the ratio 55:45. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residual syrup was dissolved in petroleum ether (30 mL) and seeded with crystals of **46**. After refrigeration, the crystals were collected by filtration to give **46** (11.6 g, 34%). Recrystallization from petroleum ether provided pure **46** (9.8 g, 29%): mp 85–86 °C; $[\alpha]_D$ −67.0° (*c* 1, $CHCl_3$).

A mixture of **46** (132.8 g, 0.418 mol) and KOH (46.8 g, 0.834 mol) in EtOH (320 mL) was stirred at room temperature for 16 h. The solution was concentrated in vacuo, and the residue was partitioned between H_2O and petroleum ether. The aqueous phase was separated and acidified with concentrated HCl, and **47** was extracted with EtOAc. The extract was dried over $MgSO_4$ and concentrated in vacuo to give crystals, which were recrysallized from toluene to give pure **47**[13] (65 g, 86%): mp 114–116 °C; $[\alpha]_D$ −8.6° (*c* 1, EtOH).

A solution of **47** (60 g, 0.33 mol) and concentrated H_2SO_4 (1.7 mL) in EtOH (1.8 L) was allowed to stand at room temperature for 16 h. The solvent was evaporated in vacuo, and the residue was partitioned between EtOAc and H_2O. The organic phase was separated, washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to give **48** (58.1 g, 98%) as an oil: $[\alpha]_D$ −20.1° (*c* 1, $CHCl_3$).

To a solution of **48** (16 g, 0.077 mol) and pyridine (6.2 mL, 0.077 mol) in CH_2Cl_2 (160 mL) was added dropwise trifluoromethanesulfonic anhydride (14.9 mL, 0.089 mol) over a period of 1.3 h in an ice bath. The mixture was stirred for 0.5 h in an ice bath and concentrated in vacuo. The residue was mixed with EtOAc–cyclohexane (1:1, 150 mL), the precipitates were filtered off, and the filtrate was subjected to flash chromatography (solvent B) to give **20** (24.4 g, quantitative) as an oil: TLC R_f 0.62 (solvent C); NMR ($CDCl_3$) δ 1.28 (3 H, t, J = 7 Hz), 2.0–2.95 (4 H, m), 4.25 (2 H, q, J = 7 Hz), 5.14 (1 H, t, J = 6 Hz), 7.27 (5 H, s).

Enzyme Assay. The ACE activity was assayed as described by Cushman and Cheung[19] by using hippurylhistidylleucine as substrate. The ACE used was solubilized from particulate fraction of rabbit lung with Nonidet-P40 and fractionated with DEAE-cellulose (DE-52, Whatman) according to the method of Manjuski and Soffer.[20]

The test solutions of diacids were prepared by dissolving 1 mg of test compound in 1 mL of 0.5% $NaHCO_3$ and diluting to the desired concentration with distilled water and added to the assay mixture. IC_{50} values are expressed as the concentrations of test compounds required for 50% inhibition of the ACE activity.

In Vivo Efficacy Test in Conscious Rats. The time course for the inhibition of ACE following a single oral administration of a test compound was obtained in the following manner. Male Wistar–Imamichi rats weighing 300–350 g were anesthetized with sodium pentobarbital (30 mg/kg ip), and two cannulae were placed: one in the femoral artery for measuring blood pressure and the other in the femoral vein for injecting drugs. The other ends of the cannulae were led under the skin and exteriorized at the back of the neck. The animals were used for the experiments in the conscious state on the day following surgery. The arterial cannula was connected to a pressure transducer, and mean blood pressure and heart rate were continuously recorded. After blood pressure and heart rate were stabilized, AI was administered at a dose of 0.3 μg/kg iv via the venous cannula. Intravenous administration of AI was repeated until a constant pressor response was obtained, and a test compound was orally administered at a dose of 1 mg/kg. AI was again administered at different time intervals after administration of the test compound. The pressor

(19) Cushman, D. W.; Cheung, H. S. *Biochem. Pharmacol.* **1971**, *20*, 1637.
(20) Manjuski, D.; Soffer, R. L. *J. Biol. Chem.* **1975**, *250*, 6762.

response after administration of the test compound was expressed as a percent of the predrug response.

Crystal Structure of 21c. Colorless prism crystals were grown by slow evaporation of a solution in EtOH and mounted on a fully automated Rigaku AFC-5 X-ray diffractometer using Cu Kα radiation. The unit cell parameters were a = 11.785 (1) Å, b = 7.4047 (4) Å, c = 12.687 (1) Å, and β = 103.13 (1)° in space group $P2_1$ (Z = 2). Of the 2158 reflections measured with $2\theta \leq 130°$ employing a $2\theta/\omega$ scan, 1732 were independently observed at a level $F \geq 3\sigma(F)$. A partial solution was found by MULTAN78[21] and expanded into a complete structure by a series of Fourier and difference electron density syntheses. Refinement was carried out with block-diagonal least-squares with anisotropic temperature factors for nonhydrogen atoms. Hydrogen atoms were assigned equivalent isotropic temperature factors for the atoms to which they were bound and refined for positional parameter variation only. The final residual index (R factor) was 0.059. Calculations were carried out with the DIRECT-SEARCH program system.[22]

Acknowledgment. We thank Drs. Y. Tsujita and T. Kohama for preparing the rabbit lung ACE used. Our appreciation is also extended to Dr. M. Yoshimoto for helpful discussions on structure–activity relationship.

Registry No. **6**, 20887-95-0; **7**, 102-96-5; **8**, 34312-77-1; **9**, 110143-52-7; **10** (isomer 1), 72150-56-2; **10** (isomer 2), 72150-54-0; **11** (isomer 1), 110221-19-7; **11** (isomer 2), 110221-65-3; **12** (isomer 1), 110221-20-0; **12** (isomer 2), 110221-66-4; **13** (isomer 1), 110143-53-8; **13** (isomer 2), 110143-71-0; **14** (isomer 1), 110221-21-1; **14** (isomer 2), 110221-22-2; **15** (isomer 1), 110221-22-2; **15** (isomer 2), 110221-68-6; **16** (isomer 1), 110143-54-9; **16** (isomer 2), 110221-69-7; **17** (isomer 1), 110221-23-3; **17** (isomer 2), 110221-70-0; **18** (isomer 1), 110221-24-4; **18** (isomer 2), 110221-71-1; **19a**, 110143-55-0; **19a**·HCl, 110221-30-2; **19b**, 110221-25-5; **19b**·HCl, 110267-53-3; **19c**, 110221-26-6; **19c**·HCl, 110267-54-4; **19d**, 110221-27-7; **19d**·HCl, 110267-55-5; **19e**, 110221-28-8; **19e**·HCl, 110267-56-6; **19f**, 110221-29-9; **19f**·HCl, 110267-57-7; **20**, 88767-98-0; **21a**, 110143-56-1; **21b**, 110221-31-3; **21c**, 110143-57-2; **21d**, 110221-32-4; **21e**, 110143-58-3; **21f**, 110221-33-5; **22a**, 110221-35-7; **22b**, 110221-36-8; **22c**, 110221-37-9; **22d**, 110221-38-0; **22e**, 110221-39-1; **22f**, 110221-40-4; **23a**·HCl, 110221-42-6; **23b**·HCl, 110221-43-7; **23d**·HCl, 110221-45-9; **23e**·HCl, 110221-46-0; **23f**·HCl, 110221-47-1; **24a**, 110221-51-7; **24b**, 110221-52-8; **24c**, 110221-53-9; **24d**, 110221-54-0; **24e**, 110221-55-1; **24f**, 110221-56-2; **25**, 110143-61-8; **26a**, 110143-62-9; **26b**, 102089-75-8; **27a**, 110173-68-7; **27b**, 110143-60-7; **28a**, 110221-58-4; **28b**, 110143-63-0; **29a**, 110221-59-5; **29a**·HCl, 110267-59-9; **29a**·HBr, 110267-60-2; **29b**, 110143-64-1; **29b**·HBr, 110221-60-8; **30a**, 110143-59-4; **30b**, 110267-58-8; **30b**·HCl, 110221-34-6; **31a**, 110221-41-5; **31b**, 102208-40-2; **32a**·HCl, 110221-48-2; **32b**·HCl, 110221-49-3; **33a**, 110221-57-3; **33b**, 102208-42-4; **34**, 102089-87-2; **35**, 102089-97-4; **36**, 102089-98-5; **37**, 102089-99-6; (3*S*,6*R*)-**38**, 102090-01-7; (3*S*,6*S*)-**38**, 110221-61-9; **39**, 102090-02-8; **40**, 102090-03-9; **40** maleate, 110221-62-0; **41**, 102208-46-8; **42**·HCl, 110221-50-6; **43**, 102090-06-2; **45**, 110143-65-2; (*R*)-**46**, 110143-66-3; (*S*)-**46**, 110143-67-4; **47**, 29678-81-7; **48**, 90315-82-5; **49a**, 110143-68-5; **49b**, 110221-63-1; **49c**, 110143-69-6; **49d**, 110221-64-2; **50**, 110143-70-9; L-cysteine, 52-90-4; diphenyl phosphorazidate, 26386-88-9; *N*-carbethoxynaphthalimide, 22509-74-6; *S*-[(*R*)-2-amino-2-phenylethyl]-*N*-[(benzyloxy)carbonyl]-L-cysteine-trifluoroacetic acid salt, 110173-70-1; *S*-[(*S*)-2-amino-2-phenylethyl]-*N*-[(benzyloxy)carbonyl]-L-cysteine-trifluoroacetic acid salt, 110173-72-3; *tert*-butyl bromoacetate, 5292-43-3; (*E*)-benzylidenepyruvic acid, 1914-59-6; *l*-menthol, 2216-51-5; angiotensin-converting enzyme, 9015-82-1.

Supplementary Material Available: Tables listing X-ray diffraction study data of **21c** (5 pages). Ordering information is given on any current masthead page.

(21) Main, P.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M. M. *MULTAN78, A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*; University of York, England and Louvain, Belgium, 1978.
(22) Koyama, Y.; Okada, K. *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.* **1975**, *A31*, S18.

Hybrid Bivalent Ligands with Opiate and Enkephalin Pharmacophores

P. S. Portoghese,*† D. L. Larson,† G. Ronsisvalle,† P. W. Schiller,§ T. M.-D. Nguyen,§ C. Lemieux,§ and A. E. Takemori‡

Departments of Medicinal Chemistry and Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455, and Clinical Research Institute of Montreal, Montreal, Quebec H2W1R7. *Received May 15, 1987*

Bivalent ligands consisting of oxymorphamine and [D-Glu^2]enkephalin pharmacophores linked through a spacer attached to the 6-amino group of the former and D-Glu of the latter were synthesized in an effort to investigate the possible coexistence of μ and δ recognition sites in the same opioid receptor complex. Of the two bivalent ligands (**1**, **2**) synthesized, only **1** had substantially greater antinociceptive potency in mice than its monovalent analogues (**1a**, **1b**). Testing of **1**, **1a**, and **1b** in the guinea pig ileum preparation (GPI) revealed a potency profile similar to that found in vivo, whereas no correlation was observed in the mouse vas deferens (MVD). Binding data indicated the same rank-order affinities at δ receptors as the opioid activities in the GPI and in mice. However, μ binding exhibited no relationship with activity. These results are consistent with the simultaneous occupation of μ and δ by a single bivalent ligand **1**, but they are also in harmony with the interaction of **1** with an opioid receptor and an accessory binding site.

Several lines of evidence suggest that μ and δ opioid receptors coexist as distinct recognition sites on an opioid receptor complex in the brain.[1-3] Moreover, it has been proposed that the observed potentiation of morphine analgesia by leucine enkephalin occurs through a coupling mechanism that links the μ receptor to the effector system.[3]

If μ and δ receptors are indeed located within the same complex, it is conceivable that a "bivalent ligand"[4] containing a μ-selective opiate and a δ-selective enkephalin pharmacophore could possess analgesic activity that is

†Department of Medicinal Chemistry, University of Minnesota.
‡Department of Pharmacology, University of Minnesota.
§Clinical Research Institute of Montreal.

(1) Lee, N. M.; Smith, A. P. *Life Sci.* **1980**, *26*, 1459.
(2) Rothman, R. B.; Westfall, T. C. *Eur. J. Pharmacol.* **1981**, *72*, 365.
(3) Vaught, J. L.; Rothman, R. B.; Westfall, T. C. *Life Sci.* **1982**, *30*, 1443.
(4) Erez, M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847.

0022-2623/87/1830-1991$01.50/0