Angiotensin-Converting Enzyme Inhibitors. 2. Perhydroazepin-2-one Derivatives†

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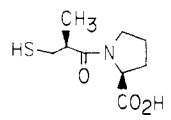
α-[(3*S*)-3-[[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-6 or 7-phenylperhydroazepin-1-yl]acetic acids (monoester monoacids) and their dicarboxylic acids were synthesized, and their angiotensin-converting enzyme (ACE) inhibitory activities were evaluated. The dicarboxylic acids having phenyl substituents at the 6*R*, 6*S*, and 7*S* positions on the azepinone ring showed potent inhibition in vitro. The corresponding monoester monoacids, when administered orally, suppressed the pressor response to angiotensin I administered intravenously. The monoester monoacids having the phenyl substituent at the 6-position showed a longer duration of action than one having the substituent at the 7-position. The structure–activity relationship was studied on the basis of the conformational energy calculation.

The angiotensin-converting enzyme (ACE) inhibitors captopril (**1**) and enalapril (**2**) (prodrug of enalaprilat (**3**)) have become very useful agents for the treatment of hypertension and congestive heart failure.[1]



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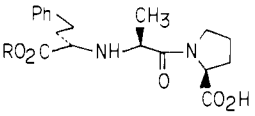
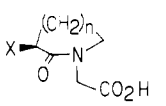


2: R = Et
3: R = H

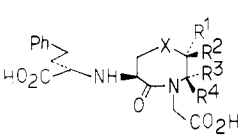
The seven- to nine-membered lactams **4**,[2] which are considered to be the conformationally restricted analogues of **1** and **2**, have been shown to have potent inhibitory activities. Recently we have synthesized the thiazepinones



4: n = 3–5
X = $HSCH_2$–;

5a–d having a hydrophobic moiety at the 2- or 3-position and examined their inhibitory potency against ACE.[3] We found that the orientation of the hydrophobic moiety dramatically influenced the potency, i.e., the compounds **5a,d** have more potent activity than the compounds **5b,c**, respectively, and furthermore, the monoethyl ester of **5a** suppressed pressor response to angiotensin I (AI) for a longer period of time than the monoethyl ester of **5d** when administered orally.

The present paper describes the syntheses of the azepin-2-ones **6a–d** corresponding to **5a–d** and their biological activities.



5a: X = S; R^1 = Ph; R^2 = R^3 = R^4 = H
5b: X = S; R^2 = Ph; R^1 = R^3 = R^4 = H
5c: X = S; R^3 = Ph; R^1 = R^2 = R^4 = H
5d: X = S; R^4 = Ph; R^1 = R^2 = R^3 = H
6a: X = CH_2; R^1 = Ph; R^2 = R^3 = R^4 = H
6b: X = CH_2; R^2 = Ph; R^1 = R^3 = R^4 = H
6c: X = CH_2; R^3 = Ph; R^1 = R^2 = R^4 = H
6d: X = CH_2; R^4 = Ph; R^1 = R^2 = R^3 = H

Chemistry. The synthesis of 6-phenylazepin-2-one **14**, the key intermediate of **6a,b**, is illustrated in Scheme I. Condensation of phenylacetonitrile (**7**) with ethyl 4-bromobutyrate provided the cyano ester **8**. Catalytic hydrogenation of **8** with Raney Ni followed by N-*tert*-butoxycarbonylation gave crystalline **10**. After alkaline hydrolysis of **10**, the resulting carboxylic acid **11** was esterified with *N*-hydroxysuccinimide and DCC to furnish the active ester **12**. Conversion of **12** to **14** was carried out by removal of the amino-protecting group with HCl–dioxane followed by neutralization with triethylamine.

7-Phenylazepin-2-one **22**, the key intermediate of **6c,d**, was obtained as depicted in Scheme II. Condensation of the δ-benzoyl ester **15** with hydroxylamine in ethanol afforded the oxime ester **16**. Catalytic hydrogenation of **16** with Raney Ni followed by protection of the amino group with di-*tert*-butyl dicarbonate and then alkaline hydrolysis gave the crystalline carboxylic acid **19**. The carboxylic acid **19** was converted to **22** by the same procedure as described in the preparation of **14** from **11**.

Preparation of the desired compounds **6a–d** from **14** and **22** is shown in Scheme III. Bromination[4] of **14** with phosphorus pentachloride and bromine in the presence of a catalytic amount of iodine followed by treatment of the resultant bromide **23** with sodium azide in DMF gave the mixture of the racemic azido compounds **25a** and **25b**. These isomers were separated by fractional recrystallization and chromatography.

Similarly **25c** and **25d** were prepared from **22**. The stereochemistries of **25a**, **25b**, and **25d** were determined by X-ray analyses, which showed that **25a** is (3*S**,6*R**)-3-azido-6-phenylperhydroazepin-2-one, **25b** is the 3*S**,6*S** isomer, and **25d** is the 3*S**,7*S** isomer (Figure 1).

Alkylation of the amide nitrogen of **25a–d** with *tert*-butyl bromoacetate followed by catalytic hydrogenation with palladium on charcoal or Raney Ni gave the amines **27a–d**, respectively. Amino alkylation of the racemate **27a** with ethyl (*R*)-4-phenyl-2-[[(trifluoromethyl)sulfonyl]-oxy]butyrate[3] in the presence of triethylamine gave the

† Dedicated to Prof. Edward C. Taylor on the occasion of his 65th birthday.
‡ Biological Research Laboratories.
§ Analytical and Metabolic Research Laboratories.

(1) (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.
(2) (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.; 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.
(3) Yanagisawa, H.; Ishihara, S.; Ando, A.; Kanazaki, T.; Miyamoto, S.; Koike, H.; Iijima, Y.; Oizumi, K.; Matsushita, Y.; Hata, T. *J. Med. Chem.* **1987**, *30*, 1984.
(4) Nagasawa, H. T.; Elberling, J. A.; Fraser, P. S.; Mizuno, N. S. *J. Med. Chem.* **1971**, *14*, 501.

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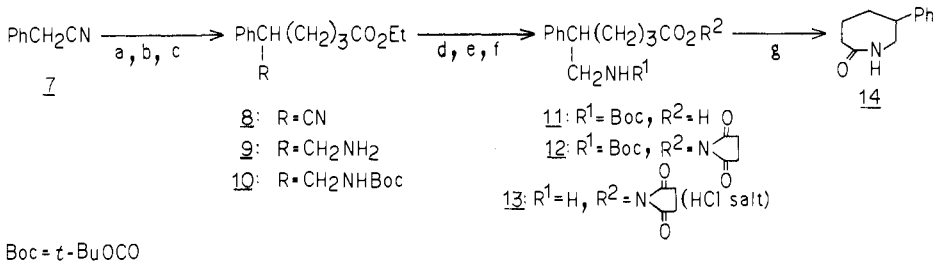
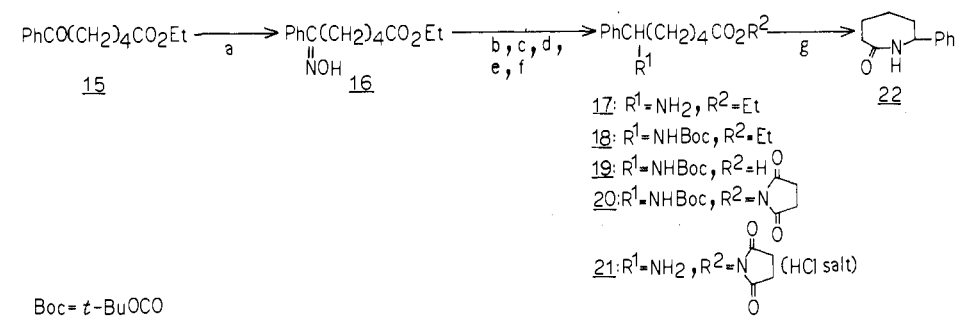
Scheme I[a]



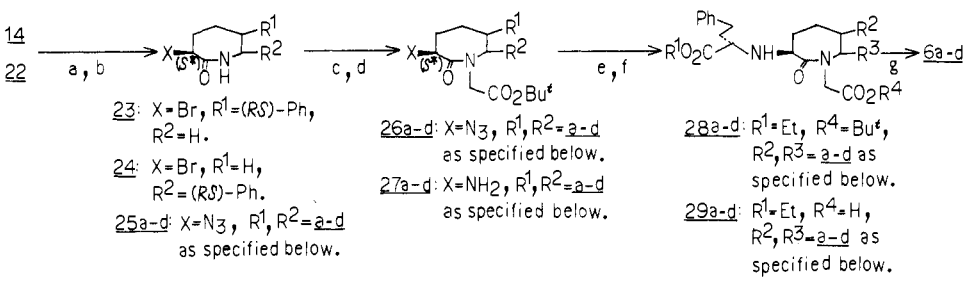
[a] Reagents: (a) NaH, $Br(CH_2)_3CO_2Et$; (b) H_2, Raney Ni; (c) Boc_2O, Et_3N; (d) NaOH; (e) *N*-hydroxysuccinimide, DCC; (f) 4 N HCl–dioxane; (g) Et_3N.

Scheme II[a]



[a] Reagents: (a) NH_2OH·HCl; (b) H_2, Raney Ni; (c) Boc_2O, Et_3N; (d) NaOH; (e) *N*-hydroxysuccinimide, DCC; (f) 4 N HCl–dioxane; (g) Et_3N.

Scheme III[a]



25, 26, 27 a: $R^1=(R^*)$-Ph, $R^2=H$. b: $R^1=(S^*)$-Ph, $R^2=H$. c: $R^1=H$, $R^2=(R^*)$-Ph. d: $R^1=H$, $R^2=(S^*)$-Ph.

28, 29 a: $R^2=(R)$-Ph, $R^3=H$. b: $R^2=(S)$-Ph, $R^3=H$. c: $R^2=H$, $R^3=(R)$-Ph. d: $R^2=H$, $R^3=(S)$-Ph.

[a] Reagents: (a) Br_2, PCl_5, I_2 (cat.); (b) NaN_3; (c) $BrCH_2CO_2$-*t*-Bu, NaH; (d) H_2, Pd/C or Raney Ni; (e) (R)-$Ph(CH_2)_2CH(OSO_2CF_3)CO_2Et$, Et_3N; (f) 4 N HCl–dioxane; (g) NaOH.

Table I. ACE Inhibitory Activity of **6a–d**, **55a–d**, and **3**

no.	IC_{50},[a] nM	no.	IC_{50},[a] nM
6a	3.1	**5b**	35.5
6b	4.3	**5c**	78.2
6c	16.2	**5d**	3.4
6d	3.6	**3**	5.7
5a	3.7		

[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.

diesters as a mixture of the two diastereoisomers, the (3*S*,6*R*)-3-amino-6-phenylazepinone having the (*S*)-1-(ethoxycarbonyl)-3-phenylpropyl group at the 3-position and the 3*R*,6*S* isomer having the same substituent at the 3-position, which were separated by flash chromatography (EtOAc–cyclohexane, 1:3). The 3-position of these isomers corresponds to the α position of the (*S*)-3-mercapto-2-methylpropionyl group of **1** and that of the L-alanyl moiety of **3**. The stereochemistry of this α position has been known to remarkably influence the inhibitory activity,[1] that is, the isomer having the same configuration as the L-amino acid is 10^2–10^3 times as potent as the other.[5] The absolute configuration at the 3-position of the azepinone ring in the diesters was assigned on the basis of the biological activity of the corresponding diacids. The diacid (IC_{50} = 3.1 nM) derived from the more polar diester was 100 times as potent as one (IC_{50} = 320 nM) derived from the less polar diester, and consequently, the configuration at the 3-position of the more active diacid and its diester was assigned to be *S* as shown in **6a** and **28a**, respectively. This assignment is consistent with the observation in the 1,5-benzothiazepine and 1,5-benzoxazepine series[6] in which

(5) (a) Captopril series: Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484. (b) Enalapril series: 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.; Paine, 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. (c) Pyridazino[1,2-*a*][1,2]diazepine derivatives: Attwood, M. R.; Hassall, C. H.; Kröhn, A.; Lawton, G. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1011.

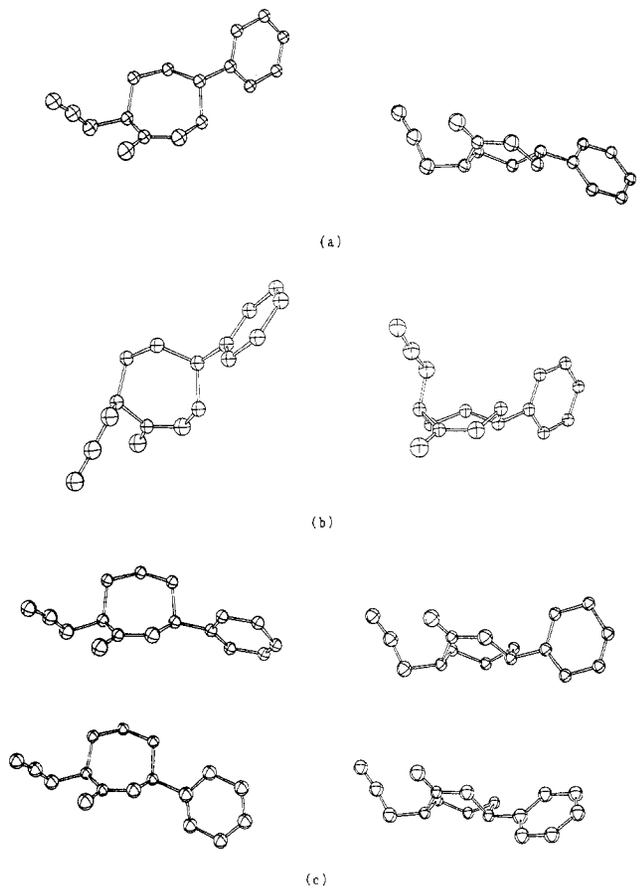


Figure 1. Perspective view of molecules of (a) **25a**, (b) **25b**, and (c) **25d** (two independent molecules).

the authors mentioned that the more active diastereoisomer showed lower R_f on TLC.

The other compounds **28b–d** were prepared by the same procedure as described above. Treatment of the diesters **28a–d** with 4 N HCl–dioxane provided the monoethyl ester hydrochlorides **29a–d**, respectively. Alkaline hydrolysis of **29a–d** gave the dicarboxylic acids **6a–d**, respectively.

Biological Activity and Discussion. The in vitro inhibitory activities (IC_{50}) of the dicarboxylic acids **6a–d** are shown in Table I in comparison with **5a–d** and enalaprilat (**3**). Compounds **6a,d** are more potent than **3** and as potent as the corresponding thiazepinones **5a,d**. Compounds **6b,c** are less potent than **6a,d**, respectively, as observed in the thiazepinone series **5a–d**; however, they still show considerable activities unlike **5b,c**, especially **6b**, which is more active than **3**.

The inhibition of the AI pressor action following a single oral administration of prodrugs was measured in conscious rats. Figure 2 shows the time course of the inhibition for **29a,b,d**, which are the prodrugs of the potent diacids **6a,b,d**, respectively, in comparison with **2**. Compound **29a**, having the 6(*R*)-phenyl group, exhibited the most potent and long lasting suppression of pressor response to AI in the compounds examined. Compound **29b**, the 6(*S*)-phenyl isomer, suppressed the AI pressor response less markedly than **29a** but showed a long duration which was comparable with that of **29a**. The 7(*S*)-phenyl derivative **29d** had more potent activity but shorter duration than **29b**. These results indicate that 6(*R*)- and 7(*S*)-phenyl groups enhance the potency and that the substitution at the 6-position is more suitable for inhibitory duration.

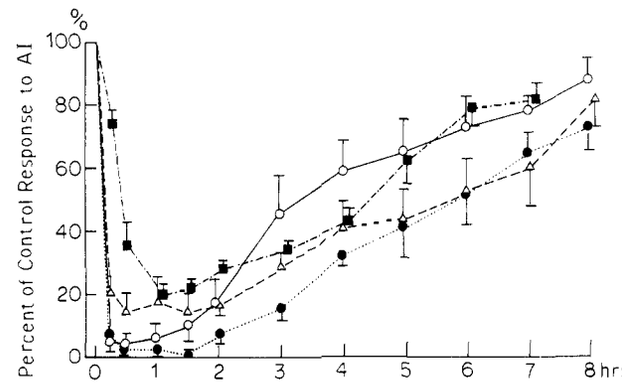
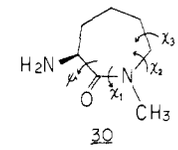


Figure 2. Time course for inhibition of pressor response to AI after a single oral administration of test compounds in conscious rats. Test compounds were administered at a dose of 1 mg/kg po in conscious normotensive rats. The pressor responses to AI, 0.3 μg/kg iv, were measured and plotted as percents of the predrug response. Each plot represents mean ± SE from four to seven experiments. Compounds and symbols: **29a** (●), **29b** (△), **29d** (○), and **2** (■).

Table II. Relative Energies and Torsional Angles of **30** with the Minimum Energies



30

conformation	ΔE, kcal/mol	torsional angles, deg			
		ψ^a	$\chi_1{}^b$	$\chi_2{}^c$	$\chi_3{}^d$
A (chair)	0	168	1	66	-78
B (chair)	0.9	−75	10	−70	−28
C (boat)	1.9	167	−1	66	77
D (boat)	3.4	−79	−2	−69	45

[a] ψ: $NH_2CHC(=O)N(CH_3)$. [b] χ_1: $CHC(=O)N(CH_3)CH_2$. [c] χ_2: $C(=O)N(CH_3)CH_2CH_2$. [d] χ_3: $N(CH_3)CH_2CH_2CH_2$.

These tendencies are consistent with the results of the thiazepinone series.[3] However, **6b** and **29b** are more potent than expected from the studies on the thiazepinone series.

In order to explore reasons for the unexpected activity of **6b** compared with **5b**, we performed conformational analyses of the azepinone compounds by using the semiempirical molecular orbital method called MNDO.[7] First the three-dimensional structure of 3-amino-1-methylperhydroazepin-2-one (**30**) was examined to find the stable conformations of the azepinone ring. The four low-energy conformations **30**-A,B,C,D were found and are shown in Table II. The most stable one is almost the same as that elucidated by the molecular mechanics calculations.[2d] On the basis of the above conformations of the azepinone ring, energy minimizations were then done on **31a–d** with the ring structures fixed. Geometrical features of the stable conformations thus obtained are shown in Table III.

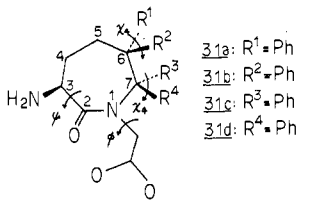
The favorable three-dimensional arrays of the pharmacophoric groups in the ACE inhibitors have been studied.[2a,d,8] The angle ψ from 130° to 170° for the peptide backbone of L-alanyl-L-proline in enalaprilat (**3**) was shown to be important for the inhibitors to bind with ACE. Furthermore it was suggested that the preferred angle ϕ which restricts the direction of the terminal carboxylate group is in the large of −45 ± 45°.

(6) Itoh, K.; Kori, M.; Inada, Y.; Nishikawa, K.; Kawamatsu, Y.; Sugihara, H. *Chem. Pharm. Bull.* **1986**, *34*, 2078; ref 4.

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

(8) Andrews, R. P.; Carson, J. M.; Caselli, A.; Spark, M. J.; Woods, R. *J. Med. Chem.* **1985**, *28*, 393.

Table III. Relative Energies and Torsional Angles of **31a–d** with the Minimum Energies



no.	conformation	ΔE, kcal/mol	7-membered ring	torsional angles, deg		
				ψ^a	ϕ^b	$\chi_4{}^c$
31a	A	0	chair	168	87	59
	B	1.1	chair	168	−108	62
	C	4.1	boat	167	89	53
	D	4.9	boat	167	−121	55
	E	5.8	boat	−79	−92	73
31b	A	0	boat	167	82	114
	B	1.2	chair	−75	103	118
	C	1.6	chair	−75	−95	122
	D	4.5	boat	167	−126	101
	E	5.5	boat	167	−77	96
31c	A	0	chair	−75	81	100
	B	2.1	chair	−75	−78	74
	C	3.0	boat	−79	−80	77
31d	A	0	chair	168	74	112
	B	0.8	chair	168	−84	92
	C	2.6	boat	167	74	115

[a] ψ: NH_2–C3–C2–N1. [b] ϕ: C2–N1–CH_2–CO_2. [c] χ_4: C7–C6–Ph for **31a,b**; N1–C7–Ph for **31c,d**.

Among the conformations of **31a**, the second stable conformation B has angles of ψ = 168° and ϕ = −108°, which are suitable for binding to ACE, and is only 1.1 kcal/mol higher in energy than the most stable conformation A. Also **31d** can easily adopt the conformation favorable for binding with the enzyme. In other words, conformation **31d**-B with ψ = 168° and ϕ = −84° has only 0.8 kcal/mol higher energy than the global minimum. These results are consistent with the biological activities, i.e., the inhibitors **6a,d** corresponding to **31a,d** have very potent inhibitory activities. The three-dimensional structure (Figure 1) of **25a,d** elucidated by the X-ray analyses showed the same chair conformation of the azepinone ring as those of more stable conformations of **31a,d**; that is, the angle ψ for **25a** is 170° and those for **25d** are 169° and 165°.[9] Compound **31c** does not have a stable conformation suitable for binding to the enzyme. In fact, the corresponding inhibitor **6c** was the weakest one in **6a–d**. These results about **31a,c,d** agree with those about the thiazepinones.[3]

Compound **31b**, which corresponds to the inhibitor **6b**, has the five conformations **31b**-A,B,C,D,E. The conformation **31d**-D, 4.5 kcal/mol higher in energy than the most stable conformation **31b**-A, is suitable for binding with ACE. The conformation of the thiazepinone corresponding to **31b**-D has 13.0 kcal/mol relative energy compared to the lowest energy conformation, and therefore the probability of existence of this conformation is extremely low. The results of these conformational energy calculations suggest that the azepinone inhibitor **6b** would bind to ACE more easily than the thiazepinone **5b**. This may be the reason that **6b** is more potent than we imagined from the biological activities of the thiazepinone series.

Although the relative energy, 4.5 kcal/mol, of **31b**-D seems to be too large to explain the potent inhibitory activity of **6b**, the binding energy between the inhibitor and ACE might compensate for that energy disadvantage. We speculate that the hydrophobic interaction between the 6(*R*)-phenyl group and subsite S_2' in ACE is stronger than that of the other molecules. This might also account for the long duration which is observed in compounds **29a,b** having the phenyl group at the 6-position.

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 JASCO A-102 infrared spectrophotometer. Elemental analyses were performed by Analytical and Metabolic Research Laboratories of Sankyo Co., and the 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 the spots were detected by UV irradiation or iodine vapor. Flash chromatography[10] was done on Merck silica gel 60 (230–400 mesh). The following EtOAc–cyclohexane solvent systems were used in TLC and flash chromatography: A (1:1); B (1:2); C (1:3); D (1:4); and E (1:5). Isopropyl ether is abbreviated as IPE.

6-Phenylperhydroazepin-2-one (14). To a solution of phenylacetonitrile (11.7 g, 0.1 mol) and ethyl 4-bromobutyrate (19.5 g, 0.1 mol) in DMF (150 mL) was added portionwise NaH (55% dispersion in mineral oil; 5 g, 0.11 mol) in an ice salt bath. The mixture was stirred at room temperature for 4 h. EtOAc and H_2O were added. The organic phase was separated, washed with aqueous $KHSO_4$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to flash chromatography (solvent D) to give **8** (13.5 g, 58%) as an oil: TLC R_f 0.39 (solvent D). A solution of **8** (58 g, 0.25 mol) in EtOH (400 mL) was hydrogenated over Raney Ni (10 mL) under 3 kg/cm^2 of H_2 at 40 °C for 2.5 h. The catalyst was filtered off, and the filtrate was concentrated to give **9** as an oil. To a solution of **9** and Et_3N (40 mL, 0.29 mol) in CH_2Cl_2 (300 mL) was added portionwise di-*tert*-butyl dicarbonate (55 g, 0.25 mol) in an ice bath. The mixture was stirred at room temperature for 1 h and then concentrated in vacuo. The residue was dissolved in EtOAc and H_2O. The organic phase was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual oil was dissolved in cyclohexane and allowed to stand. The Boc-amino ester **10** was separated as crystals, which were collected by filtration: yield

(9) Two independent molecules exist in the crystal of **25d**.

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

25 g (30%). The filtrate was concentrated, and the residue was subjected to flash chromatography (solvent E) to give further **10** (20.5 g, 24%): mp 82–84 °C (hexane); TLC R_f 0.38 (solvent D). Anal. ($C_{19}H_{29}NO_4$) C, H, N.

To a suspension of **10** (37 g, 0.11 mol) in EtOH (370 mL) was added a solution of NaOH (8.8 g, 0.22 mol) in H_2O (79 mL). The mixture was stirred at room temperature for 1 h, and EtOH was distilled off in vacuo. The residual aqueous solution was washed with IPE, mixed with ice and EtOAc, and adjusted to pH 2.5 with concentrated HCl. The organic phase was separated, washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo to give **11** (34 g, quantitative) as an oil, which crystallized on standing: mp 79 °C (60% aqueous EtOH). Anal. ($C_{17}H_{25}NO_4$) C, H, N.

To a solution of **11** (23.1 g, 0.075 mol) in CH_2Cl_2 (200 mL) were added *N*-hydroxysuccinimide (9.5 g, 0.083 mol) and then DCC (15.5 g, 0.075 mol) in an ice bath. The mixture was stirred for 2 h in an ice bath and then allowed to stand overnight at room temperature. The precipitate was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography (solvent A) to give **12** (26.5 g, 87%): mp 100–102 °C (Et_2O–hexane); TLC R_f 0.37 (solvent A). Anal. ($C_{21}H_{28}N_2O_6$) C, H, N.

A solution of **12** (26.5 g, 0.0655 mol) in 4 N HCl–dioxane (100 mL) was stirred at room temperature for 1.5 h. The slurry obtained was mixed with Et_2O (100 mL) and stirred for 1.5 h. After further addition of Et_2O (0.5 L), the hydrochloride **13** was collected by filtration: yield 21.3 g, 95%; mp 167 °C. Anal. ($C_{16}H_{21}ClN_2O_4$) C, H, Cl, N.

To a suspension of **13** (21.3 g, 0.0625 mol) in CH_2Cl_2 (213 mL) was added dropwise a solution of Et_3N (18 mL, 0.13 mol) in CH_2Cl_2 (50 mL) in an ice bath. The mixture was stirred for 2 h in an ice bath, allowed to stand overnight at room temperature, and then concentrated in vacuo. The residue was dissolved in EtOAc and H_2O, and the organic phase was separated, washed with water, dried over $MgSO_4$, and concentrated in vacuo to give **14** (9.2 g, 78%): mp 150 °C (EtOH); NMR (DMSO-d_6) δ 1.4–2.3 (4 H, m), 2.4–2.8 (2 H, m), 2.8–3.7 (3 H, m), 7.1–7.6 (6 H, m); IR (Nujol) 3290, 3210, 3060, 1660, 1650 (sh) cm^{-1}. Anal. ($C_{12}H_{15}NO$) C, H, N.

7-Phenylperhydroazepin-2-one (22). A mixture of ethyl δ-benzoylvalerate (**15**)[11] (39.0 g, 0.167 mol) and *N*-hydroxylamine hydrochloride (39.0 g, 0.56 mol) in EtOH (500 mL) was stirred at 50 °C for 6 h. After evaporation of EtOH in vacuo, the residue was dissolved in EtOAc and H_2O. The organic phase was separated, washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo to give the oxime **16** (41.6 g, quantitative) as an oil. A solution of **16** (41.6 g, 0.167 mol) in EtOH (420 mL) was hydrogenated over Raney Ni (20 mL) at 50 °C under 3 kg/cm^2 of H_2 for 5.5 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo to give the amine **17** as a syrup. This was mixed with a solution of NaOH (14 g, 0.35 mol) in H_2O (250 mL) and MeOH (70 mL), and the mixture was stirred at room temperature for 1.5 h. MeOH was distilled off in vacuo. The residual aqueous solution was washed with IPE. To the aqueous solution were added H_2O (100 mL), MeOH (100 mL), and di-*tert*-butyl dicarbonate (40 g, 0.183 mol) portionwise at room temperature. The mixture was stirred overnight at room temperature. After evaporation of MeOH in vacuo, the aqueous solution was washed with IPE, mixed with ice and EtOAc, and adjusted to pH 2.5 with concentrated HCl. The organic phase was separated, washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The resulting syrup was dissolved in IPE and kept in a refrigerator to give crystalline **19**, which was collected by filtration: yield 31.5 g (61% from **16**); mp 111 °C (EtOAc). Anal. ($C_{17}H_{25}NO_4$) C, H, N.

Compound **19** (31.5 g, 0.113 mol) was converted to **22** (13.8 g, 71%) via **20** and **21** by the same procedure as described in the preparation of **14** from **11**. Compound **20**: quantitative; mp 106.5 °C (EtOAc–IPE); TLC R_f 0.38 (solvent A). Anal. ($C_{21}H_{28}N_2O_6$) C, H, N. Compound **21**: quantitative; mp 126–128 °C. Anal. ($C_{16}H_{21}ClN_2O_4 \cdot {}^1/_2H_2O$) C, H, Cl, N. Compound **22**: 71%; mp 135–136 °C (EtOAc–IPE); NMR ($CDCl_3$) δ 1.5–2.2 (6 H, m), 2.46–2.67 (2 H, m), 4.46 (1 H, m), 5.70 (1 H, br m), 7.36 (5 H, s); IR (Nujol) 3300, 3210, 1660 cm^{-1}. Anal. ($C_{12}H_{15}NO$) C, H, N.

3-Bromo-6-phenylperhydroazepin-2-one (23). Bromination of **14** was carried out by the method of Nagasawa et al.[4] To a solution of **14** (9.2 g, 48.7 mmol) in CH_2Cl_2 (150 mL) was added PCl_5 (10.2 g, 48.7 mmol) at 3 °C, and the mixture was stirred at 0–5 °C for 50 min. Iodine (0.11 g, 0.44 mmol) was added, and stirring was continued for 5 min. Then a solution of bromine (2.5 mL, 48.7 mmol) in CH_2Cl_2 (30 mL) was added dropwise at −5 °C, and the mixture was stirred at room temperature for 1.5 h. Ice–water (60 mL) was added, and stirring was continued for 30 min. The precipitates of **23** were collected by filtration: yield 8.2 g (63%). The organic phase of the filtrate was separated, washed with H_2O, aqueous $Na_2S_2O_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo. The crystalline residue was pulverized in a small amount of EtOAc and IPE, and the crystals were collected by filtration: 3.6 g (28%). The analytical sample was obtained by recrystallization of the first crop: mp 229 °C dec (EtOAc); NMR (DMSO-d_6) δ 1.7–2.25 (4 H, m), 2.5–3.9 (3 H, m), 4.72 (1 H, m), 7.26 (5 H, s), 7.9 (1 H, br m); IR (Nujol) 3200, 3050, 1680, 1660 (sh) cm^{-1}. Anal. ($C_{12}H_{14}BrNO$) C, H, Br, N.

Similarly compound **24** was prepared: 72%; mp 80–100 °C (EtOAc–IPE); NMR ($CDCl_3$) δ 1.6–2.6 (6 H, m), 4.44 (1 H, m), 4.90 (12 H, d, d, J = 2.5, 9.5 Hz), 5.85 (1 H, br m), 7.37 (5 H, s); IR (Nujol) 3200, 3080, 1665 cm^{-1}. Anal. ($C_{12}H_{14}BrNO$) C, H, Br, N.

(3*S,6*R**)-3-Azido-6-phenylperhydroazepin-2-one (25a) and Its 3*S**,6*S** Isomer 25b.** A mixture of **23** (11.8 g, 44.0 mmol) and NaN_3 (23 g, 0.35 mol) in DMF (160 mL) was stirred at 60 °C for 4 h. EtOAc and H_2O were added. The organic phase was separated, washed with H_2O, dried over $MgSO_4$, and concentrated to 50 mL. After standing overnight, the crystals of **25a** separated from the solution were collected by filtration: yield 5.4 g (53%). The filtrate was concentrated in vacuo, and the residue was subjected to flash chromatography (solvent A) to give **25a** (0.87 g, 9%) and **25b** (2.50 g, 25%). Compound **25a**: mp 149 °C (EtOAc); TLC R_f 0.44 (solvent A); NMR (DMSO-d_6) δ 1.7–2.2 (4 H, m), 2.5–3.7 (3 H, m), 4.47 (1 H, m), 7.1–7.4 (5 H, m), 7.96 (1 H, m); IR (Nujol) 3300, 3200, 3070, 2100, 1675, 1670 cm^{-1}. Anal. ($C_{12}H_{14}N_4O$) C, H, N. Compound **25b**: mp 124.5 °C (EtOAc); TLC R_f 0.36 (solvent A); NMR (DMSO-d_6) δ 1.7–2.15 (4 H, m), 2.86–2.95 (1 H, m), 3.2–3.5 (2 H, m), 4.46 (1 H, br t, J = 3.5 Hz), 7.31 (5 H, s), 7.90 (1 H, m); IR (Nujol) 3300, 3200, 3060, 2100, 1670, 1665 cm^{-1}. Anal. ($C_{12}H_{14}N_4O$) C, H, N.

Similarly **24** afforded **25c,d**. Compound **25c**: syrup (31%); TLC R_f 0.40 (solvent A); NMR ($CDCl_3$) δ 1.7–2.4 (6 H, m), 4.27 (1 H, m), 4.77 (1 H, m), 6.12 (1 H, m), 7.36 (5 H, s); IR (neat) 3240, 3100, 3070, 2120, 1665 cm^{-1}. Compound **25d**: 66%; mp 117–118 °C (EtOAc); TLC R_f 0.60 (solvent A); NMR ($CDCl_3$) δ 1.6–2.3 (6 H, m), 4.0–4.5 (2 H, m), 6.05 (1 H, m), 7.36 (5 H, s); IR (Nujol) 3220, 3100, 2110, 1655 cm^{-1}. Anal. ($C_{12}H_{14}N_4O$) C, H, N.

***tert*-Butyl α-[(3*S**,6*R**)-3-Azido-2-oxo-6-phenylperhydroazepin-1-yl]acetate (26a).** To a solution of **25a** (6.4 g, 27.8 mmol) in DMF (64 mL) were added *tert*-butyl bromoacetate (4.71 mL, 29.2 mmol) and NaH (55% dispersion in mineral oil; 1.33 g, 30 mmol) in an ice bath under N_2. After being stirred in an ice bath for 1 h, the mixture was mixed with EtOAc and H_2O. The organic phase was separated, washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The resulting syrup was crystallized in cyclohexane, and the crystals were collected by filtration: yield 9.0 g (94%); mp 115–116 °C (EtOAc–IPE); NMR ($CDCl_3$) δ 1.47 (9 H, s), 1.8–2.3 (4 H, m), 2.8–4.6 (4 H, m), 4.11 (2 H, AB q, $\Delta\delta$ = 0.33 ppm, J = 17 Hz), 7.0–7.5 (5 H, m); IR (Nujol) 2110, 1750, 1655 cm^{-1}. Anal. ($C_{18}H_{24}N_4O_3$) C, H, N.

Similarly the following compounds were prepared. Compound **26b**: quantitative; syrup; NMR ($CDCl_3$) δ 1.47 (9 H, s), 1.9–2.3 (4 H, m), 2.9–3.3 (2 H, m), 3.96 (2 H, AB q, $\Delta\delta$ = 0.51 ppm, J = 17 Hz), 3.65–4.6 (2 H, m), 7.1–7.4 (5 H, m); IR (neat) 2120, 1740, 1660 cm^{-1}. Compound **26c**: 60%; syrup; NMR ($CDCl_3$) δ 1.51 (9 H, s), 1.7–2.6 (6 H, m), 3.72 (1 H, m), 4.14 (2 H, AB q, $\Delta\delta$ = 1.19 ppm, J = 17.5 Hz), 4.75 (1 H, t, J = 5.5 Hz), 7.1–7.5 (5 H, m); IR (neat) 2110, 1740, 1655 cm^{-1}. Compound **26d**: 69%; mp 131–133 °C (EtOAc); NMR ($CDCl_3$) δ 1.37 (9 H, s), 1.8–2.5 (6 H, m), 3.64 (2 H, AB q, $\Delta\delta$ = 0.67 ppm, J = 17 Hz), 4.41 (1 H, m), 4.82 (1 H, br d, J = 9 Hz), 7.34 (5 H, s); IR (Nujol) 2100, 1735, 1655 cm^{-1}. Anal. ($C_{18}H_{24}N_4O_3$) C, H, N.

(11) Papa, D.; Schwenk, E.; Hankin, H. *J. Am. Chem. Soc.* **1947**, *69*, 3018.

***tert*-Butyl α-[(3*S**,6*R**)-3-Amino-2-oxo-6-phenylperhydroazepin-1-yl]acetate (27a).** A mixture of **26a** (1.40 g, 4.07 mmol) and 5% Pd/C (0.2 g) in EtOH (30 mL) was stirred at 40 °C under an atmosphere of H_2 for 4 h. The catalyst was filtered off, and the solvent was evaporated in vacuo to give **27a** (1.56 g, quantitative) as a gummy substance, which was crystallized from EtOAc–Et_2O: mp 112 °C; NMR ($CDCl_3$) δ 1.47 (9 H, s), 1.5–2.3 (6 H, m), 2.7–4.2 (4 H, m), 4.10 (2 H, s), 7.0–7.5 (5 H, m); IR (Nujol) 3640, 3380, 1735, 1655 cm^{-1}. Anal. ($C_{18}H_{26}N_2O_3$) C, H, N.

Similarly **26b** afforded **27b**: quantitative; syrup; the analytical sample was obtained as the maleate; mp 138–140 °C (EtOAc–Et_2O); NMR (DMSO-d_6) δ 1.39 (9 H, s), 1.85–2.3 (4 H, m), 3.0–4.35 (3 H, m), 3.66 (2 H, AB q, Δδ = 0.83 ppm, J = 17 Hz), 4.56 (1 H, br t, J = 6 Hz), 6.07 (2 H, s), 7.33 (5 H, s); IR (Nujol) 2800–2400, 1760, 1740, 1710, 1670, 1660 (sh), 1630 cm^{-1}. Anal. ($C_{22}H_{30}N_2O_7$) C, H, N.

Compounds **27c,d** were prepared from **26c,d**, respectively, by the same procedure as described above except for the use of Raney Ni instead of 5% Pd/C. Compound **27c**: 80%; syrup; the analytical sample was obtained as the maleate; mp 149–150.5 °C (EtOAc–Et_2O); NMR (DMSO-d_6) δ 1.44 (9 H, s), 1.5–2.5 (6 H, m), 3.52 (1 H, br d), 4.19 (2 H, AB q, Δδ = 0.60 ppm, J = 17 Hz), 5.02 (1 H, br t, J = 5 Hz), 6.03 (2 H, s), 7.2–7.5 (5 H, m); IR (Nujol) 3200–2400, 1740, 1670, 1655 (sh) cm^{-1}. Anal. ($C_{22}H_{30}N_2O_7$) C, H, N. Compound **27d**: 97%; syrup; the analytical sample was obtained as the maleate; mp 160–161 °C (EtOAc–Et_2O); NMR (DMSO-d_6) δ 1.29 (9 H, s), 1.7–2.1 (6 H, m), 3.58 (2 H, AB q, Δδ = 0.50 ppm, J = 17 Hz), 4.78 (1 H, dd, J = 3, 8 Hz), 5.18 (1 H, d, J = 9 Hz), 6.04 (2 H, s), 7.40 (5 H, s); IR (Nujol) 3220–2400, 1750, 1740, 1670, 1610 cm^{-1}. Anal. ($C_{22}H_{30}N_2O_7$) C, H, N.

***tert*-Butyl α-[(3*S*,6*R*)-3-[[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-6-phenylperhydroazepin-1-yl]-acetate (28a) and Its 3*R*,6*S* Isomer.** To a solution of **27a** (1.56 g, 4.9 mmol) in CH_2Cl_2 (20 mL) was added Et_3N (0.73 mL, 5.3 mmol) and ethyl (*R*)-4-phenyl-2-[[(trifluoromethyl)sulfonyl]-oxy]butyrate[3] (1.80 g, 5.3 mmol) in an ice bath, and the mixture was allowed to stand at room temperature for 16 h. After concentration in vacuo, the residue was dissolved in EtOAc and H_2O. The organic phase was separated, washed with H_2O, dried over $MgSO_4$, and then concentrated in vacuo. The residue was subjected to flash chromatography (solvent C) to separate the two diastereoisomers, **28a** and its 3*R*,6*S* isomer. Compound **28a**: syrup (0.95 g, 38%); TLC R_f 0.40 (solvent C); $[\alpha]_D$ +28.1°; NMR ($CDCl_3$) δ 1.25 (3 H, t, J = 7 Hz), 1.46 (9 H, s), 1.7–2.3 (7 H, m), 2.5–4.1 (7 H, m), 4.05 (2 H, AB q, Δ δ = 0.37 ppm, J = 18 Hz), 4.16 (2 H, q, J = 7 Hz), 7.0–7.4 (10 H, m); IR (neat) 3310, 1745, 1645 cm^{-1}. The 3*R*,6*S* isomer: syrup (0.84 g, 34%); TLC R_f 0.49 (solvent C); $[\alpha]_D$ −45.3°; NMR ($CDCl_3$) δ 1.24 (3 H, t, J = 7 Hz), 1.46 (9 H, s), 1.6–2.3 (7 H, m), 2.6–4.0 (7 H, m), 4.04 (2 H, AB q, Δδ = 0.40 ppm, J = 18 Hz), 4.14 (2 H, q, J = 7 Hz), 7.0–7.4 (10 H, m); IR (neat) 3310, 1740, 1645 cm^{-1}.

Similarly the following compounds **28b–d** and their diastereoisomers were prepared. Compound **28b**: syrup (40%); TLC R_f 0.40 (solvent C); $[\alpha]_D$ −11.8°; NMR ($CDCl_3$) δ 1.25 (3 H, t, J = 7 Hz), 1.46 (9 H, s), 1.7–2.3 (7 H, m), 2.6–4.2 (7 H, m), 4.07 (2 H, AB q, Δδ = 0.34 ppm, J = 18 Hz), 4.16 (2 H, q, J = 7 Hz), 7.24 (10 H, s); IR (neat) 3320, 1745, 1645 cm^{-1}. The 3*R*,6*R* isomer of **28b**: syrup (34%); TLC R_f 0.50 (solvent C); $[\alpha]_D$ −29.2°; NMR ($CDCl_3$) δ 1.25 (3 H, t, J = 7 Hz), 1.44 (9 H, s), 1.7–2.2 (7 H, m), 2.5–4.1 (7 H, m), 4.07 (2 H, AB q, Δδ = 0.43 ppm, J = 18 Hz), 4.16 (2 H, q, J = 7 Hz), 7.24 (10 H, s); IR (neat) 3320, 1745, 1645 cm^{-1}. Compound **28c**: syrup (44%); TLC R_f 0.24 (solvent B); $[\alpha]_D$ −8.9°; NMR ($CDCl_3$) δ 1.28 (3 H, t, J = 7 Hz), 1.37 (9 H, s), 1.6–2.1 (8 H, m), 2.2–3.25 (4 H, m), 4.04 (2 H, q, J = 7 Hz), 4.11 (2 H, AB q, Δδ = 1.32 ppm, J = 17 Hz), 4.67 (1 H, br t, J = 6 Hz), 7.16 (5 H, s), 7.0–7.4 (5 H, m); IR (neat) 3300, 1735, 1640 cm^{-1}. The 3*R*,7*S* isomer of **28c**: syrup (43%); TLC R_f 0.37 (solvent B); $[\alpha]_D$ −1.1°; NMR ($CDCl_3$) δ 1.12 (3 H, t, J = 7 Hz), 1.47 (9 H, s), 1.6–2.15 (8 H, m), 2.31 (1 H, br s), 2.55–2.9 (2 H, m), 3.1–3.4 (1 H, m), 3.99 (2 H, q, J = 7 Hz), 4.05 (2 H, AB q, Δδ = 1.17 ppm, J = 17.5 Hz), 4.74 (1 H, br t, J = 7 Hz), 7.23 (5 H, s), 7.1–7.4 (5 H, m); IR (neat) 3300, 1730, 1640 cm^{-1}. Compound **28d**: syrup (35%); TLC R_f 0.24 (solvent B); $[\alpha]_D$ +2.7°; NMR ($CDCl_3$) δ 1.29 (3 H, t, J = 7 Hz), 1.36 (9 H, s), 1.7–2.3 (8 H, m), 2.4–2.95 (3 H, m), 3.62 (2 H, AB q, Δδ = 0.65 ppm, J = 17 Hz), 3.65–4.0 (1 H, m), 4.21 (2 H, q, J = 7 Hz), 4.90 (1 H, br d, J = 9 Hz), 7.23 (5 H, s), 7.34 (5 H, s); IR (neat) 3300, 1735, 1645 cm^{-1}. The 3*R*,7*R* isomer of **28d**: syrup (35%); TLC R_f 0.35 (solvent B); $[\alpha]_D$ −7.4°; NMR ($CDCl_3$) δ 1.28 (3 H, t, J = 7 Hz), 1.37 (9 H, s), 1.65–2.2 (8 H, m), 2.6–3.4 (3 H, m), 3.62 (2 H, AB q, Δδ = 0.70 ppm, J = 17 Hz), 3.55–3.85 (1 H, m), 4.18 (2 H, q, J = 7 Hz), 4.83 (1 H, br d, J = 9 Hz), 7.25 (5 H, s), 7.33 (5 H, s); IR (neat) 3320, 1730, 1650 cm^{-1}.

α-[(3*S*,6*R*)-3-[[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-amino]-2-oxo-6-phenylperhydroazepin-1-yl]acetic Acid Hydrochloride (29a). A solution of **28a** (0.95 g, 1.87 mmol) in 4 N HCl–dioxane (4 mL) was allowed to stand at room temperature for 16 h. The solvent was distilled off in vacuo, and the residue was crystallized from EtOAc and IPE: yield 0.80 g (87%); mp 200–202 °C (EtOH–EtOAc); $[\alpha]_D$ +27.3°; NMR (DMSO-d_6) δ 1.27 (3 H, t, J = 7 Hz), 1.7–4.9 (15 H, m), 4.24 (2 H, q, J = 7 Hz), 7.31 (10 H, s); IR (Nujol) 1760, 1730, 1675, 1660 (sh) cm^{-1}. Anal. ($C_{26}H_{33}ClN_2O_5$) C, H, Cl, N.

Similarly the following compounds were prepared. Compound **29b**: 75%; mp 185–186 °C (EtOH–EtOAc); $[\alpha]_D$ +48.6°; NMR (DMSO-d_6) δ 1.29 (3 H, t, J = 7 Hz), 1.6–4.9 (15 H, m), 4.26 (2 H, q, J = 7 Hz), 7.29 (10 H, s); IR (Nujol) 1760 (sh), 1745, 1730 (sh), 1670, 1655 (sh) cm^{-1}. Anal. ($C_{26}H_{33}ClN_2O_5 \cdot {}^1/_4H_2O$) C, H, Cl, N. Compound **29c**: quantitative; mp 100–105 °C (softened) (EtOAc–IPE); $[\alpha]_D$ +36.1°; NMR (DMSO-d_6) δ 1.13 (3 H, t, J = 7 Hz), 1.45–4.2 (13 H, m), 4.06 (2 H, q, J = 7 Hz), 4.60 (1 H, d, J = 17 Hz), 5.04 (1 H, m), 7.38 (5 H, s), 7.1–7.45 (5 H, m); IR (Nujol) 1740, 1660 cm^{-1}. Anal. ($C_{26}H_{33}ClN_2O_5 \cdot {}^1/_4H_2O$) C, H, Cl, N. Compound **29d**: 98%; mp 125–127 °C (softened) (EtOAc–IPE); $[\alpha]_D$ +11.5°; NMR (DMSO-d_6) δ 1.31 (3 H, t, J = 7 Hz), 1.7–2.9 (10 H, m), 3.24 (1 H, d, J = 17 Hz), 3.8–4.2 (2 H, m), 4.30 (2 H, q, J = 7 Hz), 4.87 (1 H, m), 5.23 (1 H, br d, J = 10 Hz), 7.32 (5 H, s), 7.45 (5 H, s); IR (Nujol) 1745, 1720 (sh), 1660 (sh), 1650 cm^{-1}. Anal. ($C_{26}H_{33}ClN_2O_5$) C, H, Cl, N.

α-[(3*S*,6*R*)-3-[[(*S*)-1-Carboxy-3-phenylpropyl]amino]-2-oxo-6-phenylperhydroazepin-1-yl]acetic Acid (6a). A solution of **29a** (400 mg, 0.82 mmol) in 1 N aqueous NaOH (3.3 mL) was stirred in an ice bath for 7 h. The solution was adjusted to pH 2.9 with 1 N HCl, and the precipitates were collected by filtration: yield 338 mg (97%); mp 266 °C dec; $[\alpha]_D$ +37.1° (*c* 1.0, 0.1 N aqueous NaOH); NMR (DMSO-d_6) δ 1.6–2.1 (6 H, m), 2.5–4.3 (9 H, m), 7.29 (10 H, s); IR (Nujol) 1740 (w), 1665 cm^{-1}. Anal. ($C_{24}H_{28}N_2O_5$) C, H, N.

Similarly the following compounds were prepared. Compound **6b**: 95%; mp 245 °C dec; $[\alpha]_D$ −106.0° (*c* 1, 0.1 N aqueous NaOH); NMR (DMSO-d_6) δ 1.6–2.1 (6 H, m), 2.5–4.3 (9 H, m), 7.29 (10 H, s); IR (Nujol) 1735 (w), 1680 (sh), 1665 cm^{-1}. Anal. ($C_{24}H_{28}N_2O_5 \cdot {}^1/_2H_2O$) C, H, N. Compound **6c**: 96%; mp 218–220 °C (softened at 201–203 °C); $[\alpha]_D$ +21.5°; NMR (DMSO-d_6) δ 1.3–1.95 (6 H, m), 2.1–2.4 (2 H, m), 2.5–2.65 (2 H, m), 3.15 (1 H, t, J = 6 Hz), 3.32 (2 H, m), 4.07 (2 H, AB q, Δδ = 0.72 ppm, J = 17 Hz), 4.95 (1 H, br t, J = 7 Hz), 7.0–7.4 (5 H, m), 7.31 (5 H, s); IR (Nujol) 1705, 1660 cm^{-1}. Anal. ($C_{24}H_{28}N_2O_5 \cdot H_2O$) C, H, N. Compound **6d**: 95%; mp 161–163 °C (softened); $[\alpha]_D$ −20.0°; NMR (DMSO-d_6) δ 1.5–2.35 (8 H, m), 2.6–2.85 (2 H, m), 3.37 (1 H, t, J = 6 Hz), 3.52 (2 H, AB q, Δδ = 0.71 ppm, J = 17 Hz), 4.45 (1 H, m), 5.15 (1 H, br d, J = 9 Hz), 7.28 (5 H, s), 7.40 (5 H, s); IR (Nujol) 1725, 1655 cm^{-1}. Anal. ($C_{24}H_{28}N_2O_5 \cdot {}^2/_3H_2O$) C, H, N.

Biological Methods. The in vitro inhibitory activities (IC_{50}) of **6a–d** and the in vivo efficacy tests of **29a,b,d** in conscious rats against the pressor response of AI were determined by the same procedure as reported previously.[3]

Crystal Structure of 25a. 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 Mo Kα radiation. The unit cell parameters are a = 12.469 (1) Å, b = 7.7194 (8) Å, c = 13.181 (1) Å, and β = 115.46 (1)° in space group $P2_1/a$ (Z = 4). Of the 2029 reflections measured with $2\theta \leq 50$ employing a $2\theta/\omega$ scan, 1308 were independently observed at level $F \geq 3\sigma(F)$. The structure was solved by MULTAN78[12] and refined by using the full-matrix least-squares method with anisotropic

(12) Main, P.; Hull, S. E.; Lessinger, L.; Germin, G.; Declercq, J.-P.; Woolfson, M. M. *MULTAN-78, 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.

temperature factors for non-hydrogen atoms. All hydrogen atoms were located from the difference Fourier map and refined with isotropic temperature factors. The final residual index (R factor) was 0.054. Calculations were carried out with the DIRECT-SEARCH program system.[13] Three tables consisting of atomic fractional coordinates, bond lengths, and bond angles have been deposited as supplementary material.

Crystal Structure of 25b. Recrystallization from Et_2O led to colorless prism crystals; the unit cell constants are a = 6.359 (1) Å, b = 10.145 (1) Å, c = 18.713 (1) Å, and β = 91.57 (1)° in space group $P2_1/a$ (Z = 4). Of the 2058 reflections measured with $2\theta \leq 130$ using Cu Kα, 1620 were independently observed at level $F \geq 3\sigma(F)$. Data reduction, least squares, electron-density synthesis, and related calculations were performed by using the methods and procedures described for **25a**. The final discrepancy factor was 0.076.

Crystal Structure of 25d. Crystals were formed in space group $P\bar{1}$ with a = 11.182 (3) Å, b = 11.733 (3) Å, c = 11.364 (3) Å, α = 110.95 (2)°, β = 70.92 (2)°, and γ = 118.95 (2)° for Z = 4. Of the 3203 reflections measured with $2\theta \leq 115$ using Cu Kα, 2190 were independently observed at level $F \geq 3\sigma(F)$. Refinement was carried out with block-diagonal least squares, and other calculations were performed by using the methods and procedures described for **25a**. The final R factor was 0.053.

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Registry No. **6a**, 111742-85-9; **6b**, 111820-50-9; **6c**, 111820-51-0; **6d**, 81938-91-2; **7**, 140-29-4; (±)-**8**, 111742-86-0; (±)-**10**, 111742-88-2; (±)-**11**, 111742-89-3; (±)-**12**, 111742-90-6; (±)-**13**·HCl, 111742-91-7; (±)-**14**, 111742-92-8; **15**, 4248-25-3; **16**, 111742-93-9; (±)-**19**, 111742-96-2; (±)-**20**, 111742-97-3; (±)-**21**·HCl, 111742-98-4; (±)-**22**, 111742-99-5; (±)-*cis*-**23**, 111743-00-1; (±)-***trans*-23**, 111742-87-1; (±)-*cis*-**24**, 111743-01-2; (γ)-*trans*-**24**, 111742-94-0; (±)-**25a**, 111743-02-3; (±)-**25b**, 111742-95-1; (±)-**25c**, 111743-07-8; (±)-**25d**, 111743-08-9; (±)-**26a**, 111743-03-4; (±)-**26b**, 111743-10-3; (±)-**26c**, 111743-10-3; (±)-**26d**, 111743-11-4; (±)-**27a**, 111743-04-5; (±)-**27b**, 111743-12-5; (±)-**27b**·maleate, 111743-15-8; (±)-**27c**, 111743-13-6; (±)-**27c**·maleate, 111743-16-9; (±)-**27d**, 111743-14-7; (±)-**27d**·maleate, 111743-17-0; **28a**, 111743-05-6; (3*R*,6*S*)-**28a**, 111820-57-6; **28b**, 111821-30-8; (3*R*,6*R*)-**28b**, 111820-58-7; **28c**, 111820-52-1; (3*R*,7*S*)-**28c**, 111820-59-8; **28d**, 111820-53-2; (3*R*,7*R*)-**28d**, 111820-60-1; **29a**·HCl, 111743-06-7; **29b**·HCl, 111820-54-3; **29c**·HCl, 111820-55-4; **29d**·HCl, 111820-56-5; (±)-**30**, 91417-30-0; ACE, 9015-82-1; $Br(CH_2)_3CO_2Et$, 2969-81-5; $BrCH_2CO_2Bu$-*t*, 5292-43-3; (*R*)-$Ph(CH_2)_2CH(OSO_2(F_3)CO_2Et$, 88767-98-0; *N*-hydroxysuccinimide, 6066-82-6.

Supplementary Material Available: Tables listing X-ray diffraction study data of **25a,b,d** (14 pages). Ordering information is given on any current masthead page.

Synthesis of Spin Traps Specific for Hydroxyl Radical

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Two nitrones, 3,3-diethyl-5,5-dimethylpyrroline 1-oxide (DEDMPO) and 3,3,5,5-tetramethylpyrroline 1-oxide (M_4PO), were synthesized by the zinc/ammonium chloride reduction of appropriately substituted γ-nitrocarbonyl compounds, followed by addition of methylmagnesium bromide to the resulting intermediate nitrones. The lipophilicities of these nitrones were estimated by determining their partition coefficients in an 1-octanol/water system. They were found to be considerably more lipophilic than 5,5-dimethylpyrroline 1-oxide (DMPO). The spin trapping of hydroxyl and superoxide radicals with these nitrones was investigated, and the hyperfine coupling constants were determined. M_4PO was found to spin trap both free radicals, while DEDMPO spin trapped only hydroxyl radical. DEDMPO was used to determine if hydroxyl radical was produced during the metabolism of menadione or nitrazepam by porcine thoracic aorta endothelial cells. Our results indicate, in conjunction with spin-trapping studies utilizing DMPO, that only superoxide is generated during cellular metabolism of quinones and aromatic nitro-containing compounds by endothelial cells.

Free radicals have been proposed to initiate a variety of pathological conditions, including ischemia/reperfusion injury.[1] Yet, verification of the role that these reactive intermediates play in mediating cellular injury is limited, in part, to our ability to be able to monitor free-radical reactions in vivo or at least in an in vitro cell model. With the application of spin-trapping techniques to this problem, great advances have been made in understanding the mechanism by which free radicals induce cellular injury.[2] From these studies, superoxide and hydroxyl radicals appear to be the most important biologically generated free radicals. Because of this, nitrones have emerged as the principal spin traps due to the rapid decomposition of the corresponding nitroxides derived from the reaction of nitroso compounds (e.g., 2-methyl-2-nitrosopropane) with these free radicals.[3] Among the several nitrones used as spin traps,[2] 5,5-dimethylpyrroline 1-oxide (DMPO) has received the most attention. Reaction of this spin trap with either superoxide or hydroxyl radical produces spin-trapped adducts with characteristic ESR spectra.[4] When both free radicals are generated in the presence of DMPO, the resulting ESR signal is a composite of the individual spectrum of the two spin-trapped adducts. In theory, differences in the hyperfine splitting pattern of each spin-trapped adduct should provide a means to distinguish among these free radicals. However, the kinetics of superoxide spin trapping and ongoing side reactions involving the spin-trapped adduct, 2-hydroperoxy-5,5-dimethyl-1-pyrrolidinyloxyl (DMPO-OOH), make data interpretation more complex. For example, DMPO-OOH is unstable and

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