

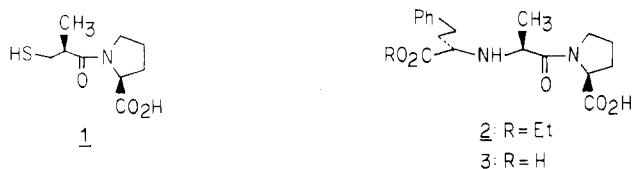
Angiotensin-Converting Enzyme Inhibitors. 2. Perhydroazepin-2-one Derivatives[†]

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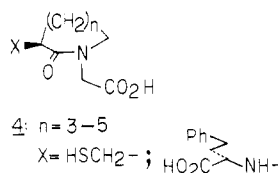
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α -[(3*S*)-3-[[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-6 or 7-phenylperhydroazepin-1-yl]acetic acids (monoester monoacids) and their dicarboxylic acids were synthesized, and their angiotensin-converting enzyme (ACE) inhibitory activities 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

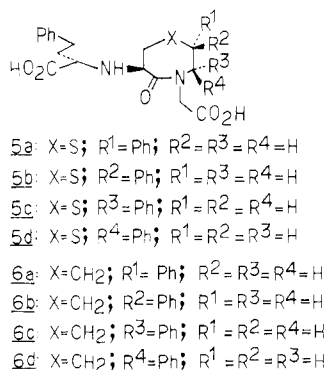


The seven- to nine-membered lactams 4,² which are considered to be the conformationally restricted analogues of 1 and 2, have been shown to have potent inhibitory activities. Recently we have synthesized the thiazepinones



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.



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. Bromination⁴ 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).

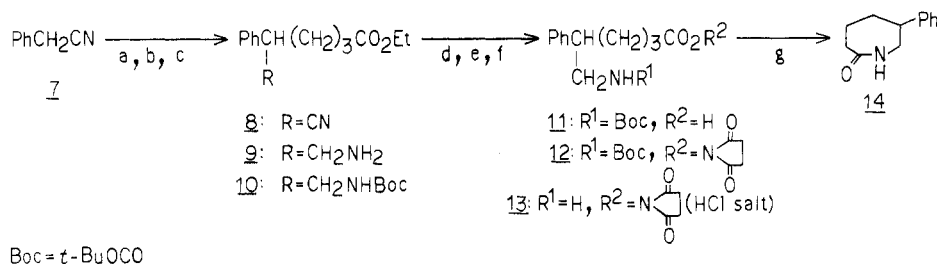
Aalkylation of the amide nitrogen of 25a-d with *tert*-butyl bromoacetate followed by catalytic hydrogenation with palladium on charcoal or Raney Ni gave the amines 27a-d, respectively. Amino alkylation of the racemate 27a with ethyl (*R*)-4-phenyl-2-[[[(trifluoromethyl)sulfonyl]-oxy]butyrate³ in the presence of triethylamine gave the

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- (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.
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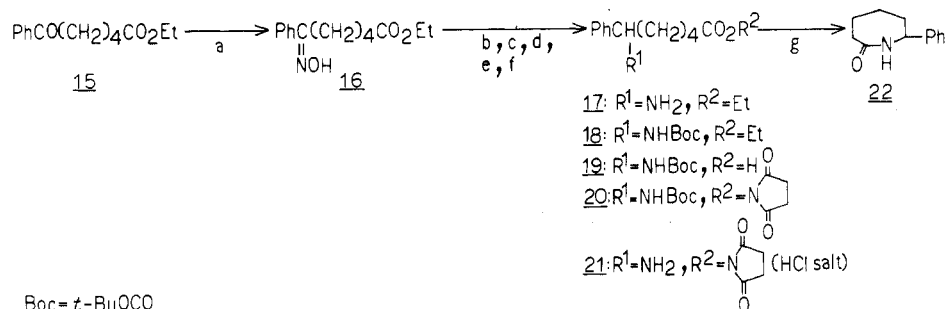
[†] Dedicated to Prof. Edward C. Taylor on the occasion of his 65th birthday.

[‡] Biological Research Laboratories.

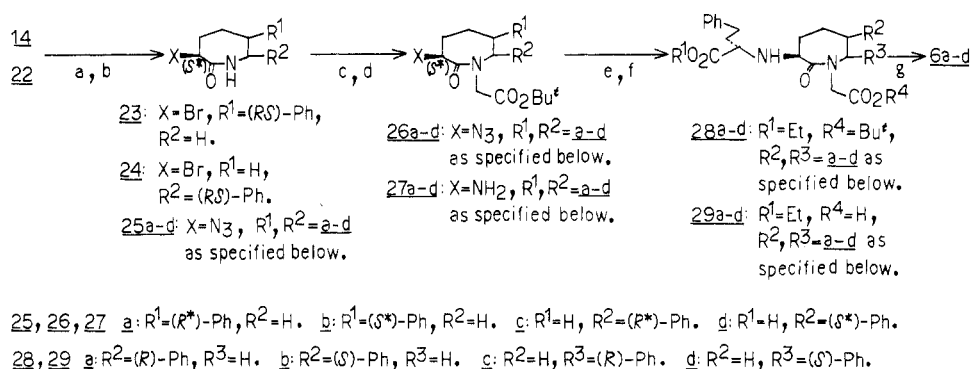
[§] Analytical and Metabolic Research Laboratories.

Scheme I^a

^a Reagents: (a) NaH, Br(CH₂)₃CO₂Et; (b) H₂, Raney Ni; (c) Boc₂O, Et₃N; (d) NaOH; (e) *N*-hydroxysuccinimide, DCC; (f) 4 N HCl-dioxane; (g) Et₃N.

Scheme II^a

^a Reagents: (a) NH₂OH·HCl; (b) H₂, Raney Ni; (c) Boc₂O, Et₃N; (d) NaOH; (e) *N*-hydroxysuccinimide, DCC; (f) 4 N HCl-dioxane; (g) Et₃N.

Scheme III^a

^a Reagents: (a) Br₂, PCl₅, I₂ (cat.); (b) NaN₃; (c) BrCH₂CO₂-*t*-Bu, NaH; (d) H₂, Pd/C or Raney Ni; (e) (*R*)-Ph(CH₂)₂CH(OSO₂CF₃)CO₂Et, Et₃N; (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 10²–10³ 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 (IC₅₀ = 3.1 nM) derived from the more polar diester was 100 times as potent as one (IC₅₀ = 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 series⁶ 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.

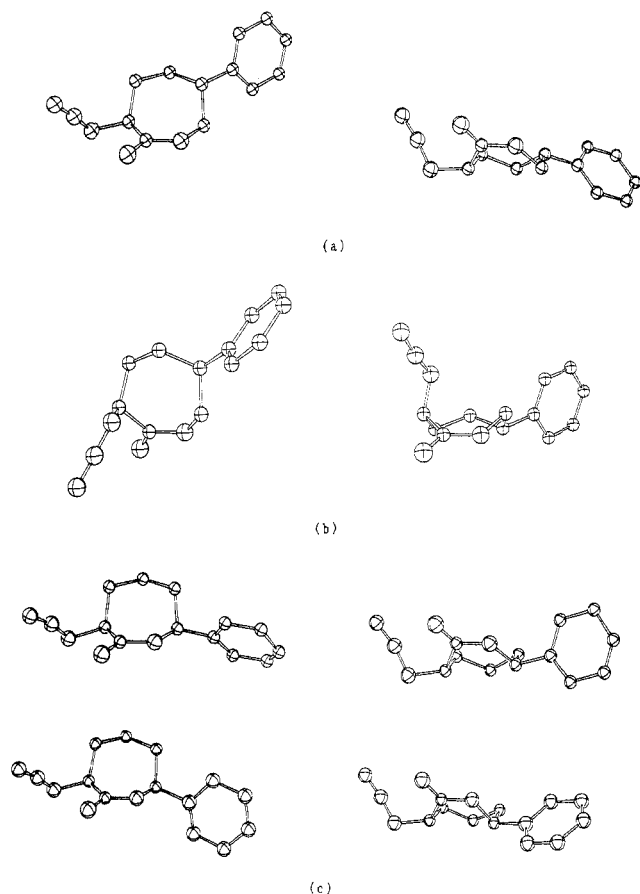


Figure 1. Perspective view of molecules of (a) **25a**, (b) **25b**, and (c) **25d** (two independent molecules).

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.

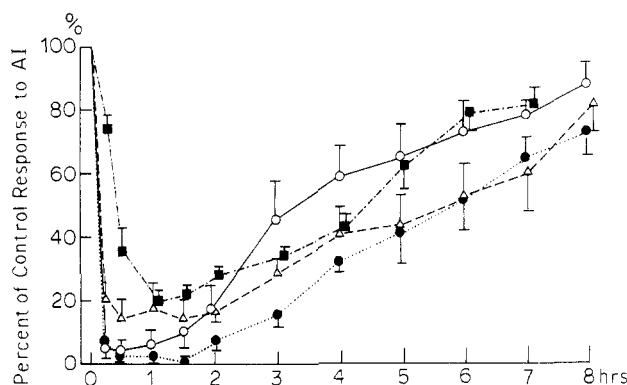
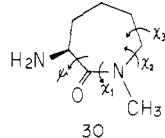


Figure 2. Time course for inhibition of pressor response to AI after a single oral administration of test compounds in conscious rats. Test compounds were administered at a dose of 1 mg/kg po in conscious normotensive rats. The pressor responses to AI, 0.3 μ g/kg iv, were measured and plotted as percents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

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



conformation	ΔE , kcal/mol	torsional angles, deg			
		ψ^a	χ_1^b	χ_2^c	χ_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 : $\text{NH}_2\text{CHC}(=\text{O})\text{N}(\text{CH}_3)$. ^b χ_1 : $\text{CHC}(=\text{O})\text{N}(\text{CH}_3)\text{CH}_2$. ^c χ_2 : $\text{C}(=\text{O})\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_2$. ^d χ_3 : $\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2$.

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 semi-empirical 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.

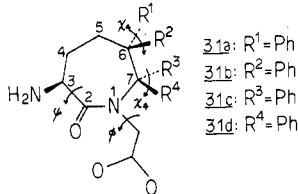
The favorable three-dimensional arrays of the pharmacophoric groups in the ACE inhibitors have been studied.^{2a,d,8} The angle ψ from 130° to 170° for the peptide backbone of L-alanyl-L-proline in enalaprilat (**3**) was shown to be important for the inhibitors to bind with ACE. Furthermore it was suggested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

(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	χ_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 : $\text{NH}_2\text{-C3-C2-N1}$. ^b ϕ : $\text{C2-N1-CH}_2\text{-CO}_2$. ^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 $\psi = 168^\circ$ and $\phi = -108^\circ$, 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 $\psi = 168^\circ$ and $\phi = -84^\circ$ 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 $\pm 0.4\%$ of the theoretical values unless indicated otherwise. TLC analyses were performed on precoated plates of Merck silica gel 60 F₂₅₄, and the spots were detected by UV irradiation or iodine vapor. Flash chromatography¹⁰ 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 KH_2SO_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 H₂O (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 H₂O, dried over MgSO₄, 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 CH₂Cl₂ (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 (Et₂O–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 Et₂O (100 mL) and stirred for 1.5 h. After further addition of Et₂O (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 CH₂Cl₂ (213 mL) was added dropwise a solution of Et₃N (18 mL, 0.13 mol) in CH₂Cl₂ (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 H₂O, and the organic phase was separated, washed with water, dried over MgSO₄, 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 H₂O. The organic phase was separated, washed with H₂O, dried over MgSO₄, 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/cm² of H₂ 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 H₂O (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 H₂O (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 H₂O, dried over MgSO₄, 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. ($C_{16}H_{21}ClN_2O_4 \cdot 1/2 H_2O$) C, H, Cl, N. Compound 22: 71%; mp 135–136 °C (EtOAc–IPE); NMR (CDCl₃) 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 CH₂Cl₂ (150 mL) was added PCl₅ (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 CH₂Cl₂ (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 H₂O, aqueous Na₂S₂O₃, and brine, dried over MgSO₄, 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 (CDCl₃) 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 NaN₃ (23 g, 0.35 mol) in DMF (160 mL) was stirred at 60 °C for 4 h. EtOAc and H₂O were added. The organic phase was separated, washed with H₂O, dried over MgSO₄, 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 (CDCl₃) 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 (CDCl₃) 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 N₂. After being stirred in an ice bath for 1 h, the mixture was mixed with EtOAc and H₂O. The organic phase was separated, washed with H₂O, dried over MgSO₄, 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 (CDCl₃) 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 (CDCl₃) 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 (CDCl₃) 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 (CDCl₃) 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.

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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 H₂ 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–Et₂O: mp 112 °C; NMR (CDCl₃) 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. (C₁₈H₂₆N₂O₃) C, H, N.

Similarly 26b afforded 27b: quantitative; syrup; the analytical sample was obtained as the maleate; mp 138–140 °C (EtOAc–Et₂O); 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, $\Delta\delta$ = 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. (C₂₂H₃₀N₂O₇) 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–Et₂O); 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, $\Delta\delta$ = 0.60 ppm, J = 17 Hz), 5.02 (1 H, br t, J = 5 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) 3200–2400, 1740, 1670, 1655 (sh) cm⁻¹. Anal. (C₂₂H₃₀N₂O₇) C, H, N. Compound 27d: 97%; syrup; the analytical sample was obtained as the maleate; mp 160–161 °C (EtOAc–Et₂O); NMR (DMSO-*d*₆) δ 1.29 (9 H, s), 1.7–2.1 (6 H, m), 3.58 (2 H, AB q, $\Delta\delta$ = 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. (C₂₂H₃₀N₂O₇) 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 CH₂Cl₂ (20 mL) was added Et₃N (0.73 mL, 5.3 mmol) and ethyl (*R*)-4-phenyl-2-[(trifluoromethyl)sulfonyl]oxy]butyrate³ (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 H₂O. The organic phase was separated, washed with H₂O, dried over MgSO₄, 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); [α]_D +28.1°; NMR (CDCl₃) 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, $\Delta\delta$ = 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); [α]_D -45.3°; NMR (CDCl₃) 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, $\Delta\delta$ = 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); [α]_D -11.8°; NMR (CDCl₃) 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, $\Delta\delta$ = 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); [α]_D -29.2°; NMR (CDCl₃) 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, $\Delta\delta$ = 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); [α]_D -8.9°; NMR (CDCl₃) 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, $\Delta\delta$ = 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); [α]_D -1.1°; NMR (CDCl₃) 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, $\Delta\delta$ = 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); [α]_D +2.7°; NMR (CDCl₃) 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, $\Delta\delta$ = 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); [α]_D -7.4°; NMR (CDCl₃) 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, $\Delta\delta$ = 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); [α]_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. (C₂₆H₃₃ClN₂O₅) C, H, Cl, N.

Similarly the following compounds were prepared. Compound 29b: 75%; mp 185–186 °C (EtOH–EtOAc); [α]_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. (C₂₆H₃₃ClN₂O₅·1/4 H₂O) C, H, Cl, N. Compound 29c: quantitative; mp 100–105 °C (softened) (EtOAc–IPE); [α]_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. (C₂₆H₃₃ClN₂O₅·1/4 H₂O) C, H, Cl, N. Compound 29d: 98%; mp 125–127 °C (softened) (EtOAc–IPE); [α]_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. (C₂₆H₃₃ClN₂O₅) 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; [α]_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. (C₂₄H₂₈N₂O₅) C, H, N.

Similarly the following compounds were prepared. Compound 6b: 95%; mp 245 °C dec; [α]_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. (C₂₄H₂₈N₂O₅·1/2 H₂O) C, H, N. Compound 6c: 96%; mp 218–220 °C (softened at 201–203 °C); [α]_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, $\Delta\delta$ = 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. (C₂₄H₂₈N₂O₅·H₂O) C, H, N. Compound 6d: 95%; mp 161–163 °C (softened); [α]_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, $\Delta\delta$ = 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. (C₂₄H₂₈N₂O₅·3/4 H₂O) C, H, N.

Biological Methods. The in vitro inhibitory activities (IC₅₀) 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 K α 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 MULTAN78¹² and refined by using the full-matrix least-squares method with anisotropic

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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 Et₂O 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 $\beta = 91.57$ (1)° in space group $P2_1/a$ ($Z = 4$). Of the 2058 reflections measured with $2\theta \leq 130$ using Cu K α , 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, $\alpha = 110.95$ (2)°, $\beta = 70.92$ (2)°, and $\gamma = 118.95$ (2)° for $Z = 4$. Of the 3203 reflections measured with $2\theta \leq 115$ using Cu K α , 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; (\pm)-8, 111742-86-0; (\pm)-10, 111742-88-2; (\pm)-11, 111742-89-3; (\pm)-12, 111742-90-6; (\pm)-13-HCl, 111742-91-7; (\pm)-14, 111742-92-8; 15, 4248-25-3; 16, 111742-93-9; (\pm)-19, 111742-96-2; (\pm)-20, 111742-97-3; (\pm)-21-HCl, 111742-98-4; (\pm)-22, 111742-99-5; (\pm)-cis-23, 111743-00-1; (\pm)-trans-23, 111742-87-1; (\pm)-cis-24, 111743-01-2; (γ)-trans-24, 111742-94-0; (\pm)-25a, 111743-02-3; (\pm)-25b, 111742-95-1; (\pm)-25c, 111743-07-8; (\pm)-25d, 111743-08-9; (\pm)-26a, 111743-03-4; (\pm)-26b, 111743-10-3; (\pm)-26c, 111743-10-3; (\pm)-26d, 111743-11-4; (\pm)-27a, 111743-04-5; (\pm)-27b, 111743-12-5; (\pm)-27b-maleate, 111743-15-8; (\pm)-27c, 111743-13-6; (\pm)-27c-maleate, 111743-16-9; (\pm)-27d, 111743-14-7; (\pm)-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; (\pm)-30, 91417-30-0; ACE, 9015-82-1; Br(CH₂)₃CO₂Et, 2969-81-5; BrCH₂CO₂Bu-*t*, 5292-43-3; (*R*)-Ph(CH₂)₂CH(OSO₂(F₃))CO₂Et, 88767-98-0; *N*-hydroxy-succinimide, 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 (M₄PO), 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. M₄PO 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-pyrrolidinyloxy (DMPO-OOH), make data interpretation more complex. For example, DMPO-OOH is unstable and

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