Angiotensin-Converting Enzyme Inhibitors: Synthesis and Structure–Activity Relationships of Potent *N*-Benzyloxycarbonyl Tripeptide Inhibitors[1)]

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A new series of γ-D-Glu-containing *N*-benzyloxycarbonyl (Z) tripeptide inhibitors of angiotensin-converting enzyme (ACE) was synthesized. The effect of varying the antepenultimate amino acid residue in this series on the biological activity was studied. Introduction of Lys and Orn residues at the P_1 position provided the most potent inhibitors, 25a and 25b (IC_{50}: 3.5 and 4.9 × 10^{-9} M, respectively), which exhibited an oral antihypertensive activity. This result suggests that basic amino acid residues at the P_1 position play an important role in binding with the S_1 subsite of ACE in this series. Oral antihypertensive activity of selected compounds was evaluated.

Keywords angiotensin-converting enzyme; antihypertensive activity; inhibitory activity; S_1 subsite; γ-D-glutamyl residue; tripeptide inhibitor; P_1 substituent

Since captopril **1**,[2)] the first orally active angiotensin-converting enzyme (ACE) inhibitor, was synthesized after rational design based on a consideration of the active site of the enzyme, many efforts to probe the structural requirements for optimal binding to the active site of ACE have been made.[3)] The early study[4)] on snake venom peptide inhibitors represented by pentapeptide **2** suggested that L-Phe at the P_1 position was requisite for tight binding to ACE in the series. The design and development of enalapril **3**[5a)] verified the importance of the interaction of the P_1 substituent with the S_1 subsite of ACE. Studies[5a,b)] with *N*-carboxymethyldipeptide inhibitors and their lactam analogs suggest a large tolerance of the S_1 subsite. On the other hand, systematic studies on the P_1 residue using amino acids or their surrogates resulted in two different conclusions; Natarajan *et al.*[6,7)] demonstrated that an aromatic P_1 substituent (**4a**, **b**) was the most favorable, whereas Rohrbach *et al.*[8)] found the greatest preference for P_1 Arg (**5**) for tight binding to ACE. From these findings, it appears that the nature of the S_1 subsite of ACE remains equivocal and the optimal P_1 substituent depends on the backbone of inhibitors.

We designed and synthesized peptidic ACE inhibitors, *N*-benzyloxycarbonyl(Z)-tripeptides (**7**) containing a penultimate γ-D-Glu residue, based on the assumption that the α-carboxyl group of γ-D-Glu might orient to the catalytic site of ACE as does that of *N*-carboxymethyldipeptides, and in addition, introduction of an optimal P_1 substituent may lead to potent inhibitors. When we initiated this work, no γ-D-Glu-containing inhibitors had been reported. However, Ksander *et al.*[9)] in 1985 described highly potent dipeptidic inhibitors (**6**) containing the γ-D-Glu residue and a systematic study suggested the importance of the P_1 carbamate and a lipophilic P_2' substituent in the series. On the other hand, our approach to find an optimal P_1 substituent was to incorporate various natural amino acid side-chains as P_1 substituents and to examine the influence of the various P_1 amino acids on the biological activity. In this series, the antepenultimate amino acid (P_1) and *C*-terminal imino acid (P_2') were systematically varied with the aims of obtaining potent inhibitors having an orally antihypertensive activity and of achieving a better understanding of the active site, particularly the S_1 subsite. The present paper describes the synthesis and biological activity of these compounds, and the structure–activity relationships.

Synthesis of Tripeptide Inhibitors Chart 2 illustrates the synthesis of several key intermediate dipeptides **13**, **14**, **19**, and **20**. Compounds **13** and **14** were regioselectively derived from *N*-phthaloyl-D-glutamic anhydride **8**. Dipeptide diethyl esters **19** and **20** were obtained by the coupling of α-

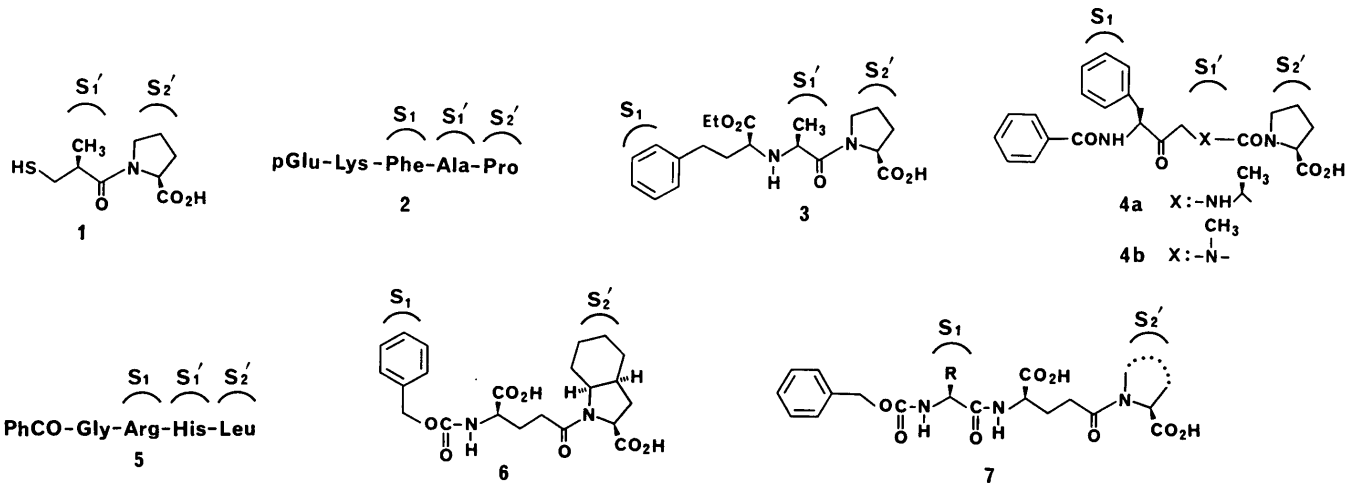


Chart 1

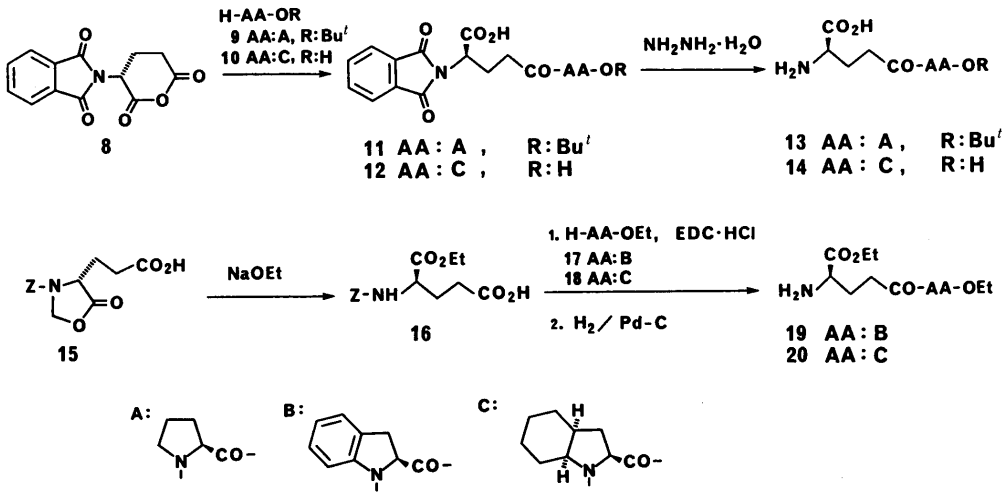


Chart 2

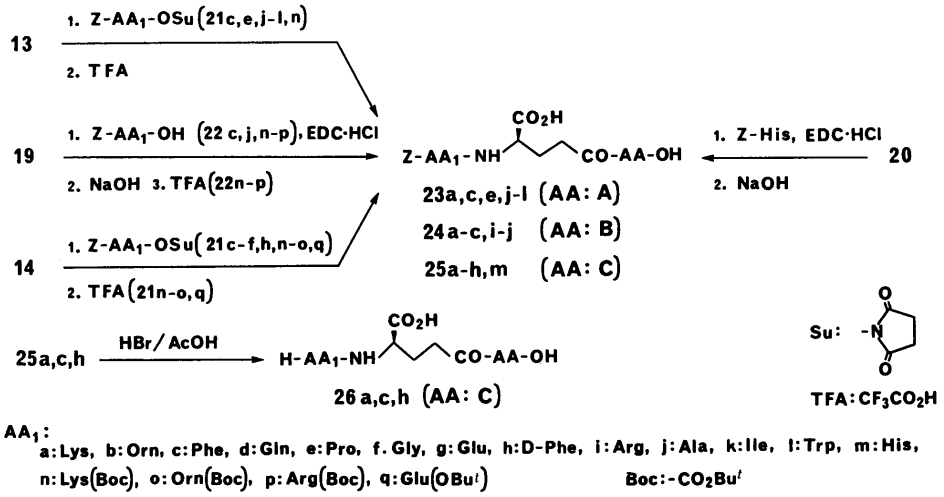


Chart 3

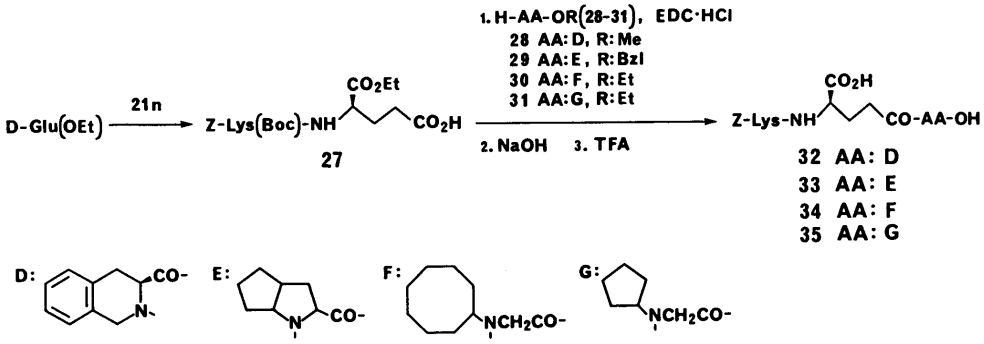


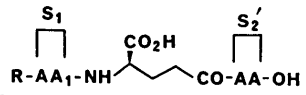
Chart 4

ethyl *N*-Z-D-glutamate **16** with the corresponding amino acid esters and subsequent hydrogenolysis.

The *N*-Z-tripeptide inhibitors **23** and **25** except for **25m** were synthesized by the reaction of the active esters of the corresponding *N*-Z-amino acids with **13** and **14** followed by deprotection of protected carboxyl and/or side-chain amino group(s) (Chart 3). Furthermore, the deprotection of **25** gave the *N*-terminus-free tripeptides **26**. Compounds **24** were prepared by the condensation of the corresponding *N*-Z-amino acids with **19** by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), followed by deprotection. The variation of other *C*-terminal imino acids was achieved by the condensation of compound **27** with the corresponding amino acid esters **28**—**31**, followed by deprotection as shown in Chart 4.

Biological Results and Discussion Table I shows the *in vitro* ACE-inhibitory activities of the tripeptides synthesized. In each series of **23**, **24**, and **25** containing a different P_2' residue, compounds **23a**, **24a** and **25a**, in which a Lys residue was located at the P_1 position, exhibited the most

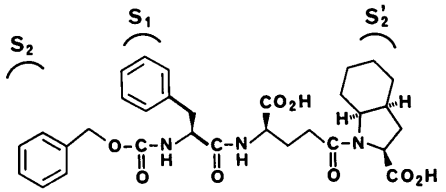
TABLE I. Physicochemical Properties and *in Vitro* ACE Inhibition of Tripeptide Inhibitors



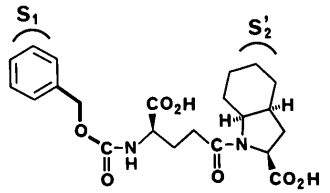
Compd.	R	AA_1	AA[a]	mp (°C)	$[\alpha]_D^{26}$ (°)[b]	Formula[c]	Analysis Calcd (Found) C	H	N	ACE IC_{50} (nM)
23a	Z	Lys	A	am[d]	−45.5° (*c*=0.47, S)	$C_{24}H_{34}N_4O_8 \cdot H_2O$	54.95 (54.87)	6.92 6.74	10.68 10.88)	12
23c	Z	Phe	A	am	−33.8° (*c*=0.20, S)	$C_{27}H_{31}N_3O_8 \cdot 1.25H_2O$	59.17 (59.10)	6.16 6.46	7.67 7.51)	240
23k	Z	Ile	A	am	−48.9° (*c*=0.20, S)	$C_{24}H_{33}N_3O_8 \cdot 0.75H_2O$	57.08 (57.10)	6.89 6.72	8.32 8.16)	290
23l	Z	Trp	A	am	−26.6° (*c*=0.20, S)	$C_{29}H_{32}N_4O_8 \cdot 0.75H_2O$	60.25 (60.33)	5.87 5.81	9.69 9.59)	310
23j	Z	Ala	A	am	−44.9° (*c*=0.20, S)	$C_{21}H_{27}N_3O_8 \cdot 0.1H_2O$	55.89 (55.95)	6.08 6.42	9.31 8.92)	390
23e	Z	Pro	A	am	−78.2° (*c*=0.20, S)	$C_{23}H_{29}N_3O_8 \cdot H_2O$	55.98 (56.10)	6.33 6.16	8.51 8.22)	2500
24a	Z	Lys	B	190—204 (dec.)	−78.5° (*c*=1.0, S)	$C_{28}H_{34}N_4O_8 \cdot 1.25H_2O$	58.27 (58.51)	6.38 6.48	9.71 9.92)	5.6
24b	Z	Orn	B	204—211 (dec.)	−79.0° (*c*=0.68, S)	$C_{27}H_{32}N_4O_8 \cdot 1.25H_2O$	57.59 (57.58)	6.18 6.10	9.95 9.81)	5.6
24i	Z	Arg	B	am	−63.8° (*c*=0.61, S)	$C_{28}H_{33}N_6O_8 \cdot 0.75H_2O$	56.42 (56.52)	6.00 6.02	14.10 14.11)	9.4
24c	Z	Phe	B	137—140 (dec.)	−84.1° (*c*=1.0, S)	$C_{31}H_{31}N_3O_8 \cdot 1.25H_2O$	62.46 (62.41)	5.66 5.55	7.05 7.06)	9.6
24j	Z	Ala	B	125—130 (dec.)	−85.7° (*c*=0.50, S)	$C_{25}H_{27}N_3O_8 \cdot H_2O$	58.25 (58.11)	5.67 5.50	8.15 8.17)	16
25a	Z	Lys	C	am	−40.0° (*c*=1.0, S)	$C_{28}H_{40}N_4O_8 \cdot 2H_2O$	56.36 (56.20)	7.43 7.35	9.39 9.13)	3.5
25b	Z	Orn	C	am	−39.4° (*c*=1.0, S)	$C_{27}H_{38}N_4O_8 \cdot 2H_2O$	55.66 (55.39)	7.27 7.08	9.62 9.49)	4.9
25m	Z	His	C	am	−29.3° (*c*=0.20, S)	$C_{28}H_{35}N_5O_8 \cdot 1.75H_2O$	55.94 (55.91)	6.46 6.35	11.65 11.57)	8.3
25c	Z	Phe	C	am	−37.3° (*c*=1.0, S)	$C_{31}H_{37}N_3O_8 \cdot H_2O$	62.30 (62.38)	6.58 6.47	7.03 6.97)	9.2
25d	Z	Gln	C	am	−36.0° (*c*=0.30, S)	$C_{27}H_{36}N_4O_9 \cdot 0.25H_2O$	57.39 (57.43)	6.51 6.64	9.91 9.94)	11
25e	Z	Pro	C	am	−74.2° (*c*=1.0, S)	$C_{27}H_{35}N_3O_8 \cdot 0.5H_2O$	60.21 (60.42)	6.74 6.81	7.80 7.66)	15
25f	Z	Gly	C	am	−36.4° (*c*=1.0, S)	$C_{24}H_{31}N_3O_8 \cdot 0.5H_2O$	57.82 (58.09)	6.47 6.41	8.43 8.47)	24
25g	Z	Glu	C	am	−36.3° (*c*=0.70, S)	$C_{27}H_{35}N_3O_{10} \cdot 0.5H_2O$	56.83 (56.77)	6.36 6.43	7.36 7.40)	60
25h	Z	D-Phe	C	am	−12.9° (*c*=0.40, S)	$C_{31}H_{37}N_3O_8 \cdot 0.5H_2O$	63.25 (63.39)	6.51 6.53	7.14 7.00)	200
32	Z	Lys	D	am	−11.9° (*c*=0.58, S)	$C_{29}H_{36}N_3O_8 \cdot 1.5H_2O$	58.48 (58.32)	6.60 6.71	9.41 9.03)	6.8
33[e]	Z	Lys	E	am	−10.7° (*c*=0.46, S)	$C_{27}H_{38}N_4O_8 \cdot 0.75H_2O$	57.90 (57.81)	7.11 7.08	10.00 9.90)	11
34	Z	Lys	F	am	−16.0° (*c*=0.58, S)	$C_{29}H_{44}N_4O_8 \cdot 1.25H_2O$	58.13 (57.88)	7.82 7.76	9.35 9.21)	10
35	Z	Lys	G	am	−14.2° (*c*=0.60, S)	$C_{26}H_{38}N_4O_8 \cdot 0.5H_2O$	57.45 (57.33)	7.23 7.24	10.31 10.06)	25
26a	H	Lys	C	am	−5.3° (*c*=0.30, W)	$C_{20}H_{34}N_4O_6 \cdot 1.5H_2O$	52.97 (53.07)	8.22 8.43	12.35 12.44)	12
26c	H	Phe	C	245—250 (dec.)	+2.8° (*c*=0.99, S)	$C_{23}H_{31}N_3O_6$	62.01 (61.91)	7.01 6.91	9.43 9.39)	100
26h	H	D-Phe	C	am	−31.5° (*c*=0.40, S)	$C_{23}H_{31}N_3O_6 \cdot 0.5H_2O$	60.78 (60.69)	7.10 7.23	9.25 9.30)	220
6	Z	—	C							7.4[f]
Captopril (**1**)										8.2[g]
Enalapril (**3**)										5.4[h]

a) See Charts 2 and 4. *b*) Solvent: S, 1N NaOH; W, H_2O. *c*) All compounds exhibited IR and ^{1}H-NMR spectra consistent with the assigned structures. *d*) Amorphous powder. *e*) A mixture of diastereomers. *f*) Lit. (ref. 9), IC_{50}: 2.7 nM. *g*) Lit. (ref. 2), IC_{50}: 23 nM. *h*) Diacid form, Lit. (ref. 5), IC_{50}: 1.2 nM.

potent inhibitory activities. The Orn analogs **24b** and **25b** were nearly equally potent, whereas the Phe analogs **23c**, **24c**, and **25c** were slightly less potent than the corresponding Lys and Orn analogs. The approximate order of preference for the P_1 amino acid residues examined was basic > neutral > acidic > D-amino acid; this result is similar



25c IC_{50}: 9.2 nM



6 IC_{50}: 7.4 nM

Chart 5

TABLE II. Effects of the P_1 Residues on Antihypertensive Activity in Renal Hypertensive Rats

Compd.	P_1 residue	Dose mg/kg *p.o.*	Change of systolic blood pressure (mmHg)	
			Peak response	Response at 24 h
25a	Lys	30	−43 (7—9)[a]	−23
		10	−37 (9)	−19
25b	Orn	30	−35 (9)	−19
		10	−24 (9)	−4
25m	His	10	No effect	
25c	Phe	10	−17 (9)	
25d	Gln	10	−15 (9)	
25e	Pro	10	No effect	
25f	Gly	10	No effect	
26g	Glu	10	−7 (9)	
24a	Lys	30	−45 (9)	−22
		10	−31 (9)	−1
24b	Orn	30	−23 (9)	−2
		10	−14 (9)	−2
24i	Arg	10	−17 (9)	0
24c	Phe	30	No effect	
24j	Ala	30	No effect	
26a	Lys	10	No effect	
Captopril (**1**)		10	−27 (1)	
Enalapril (**3**)		10	−38 (7)	−12

a) Peak time (h).

to that obtained in *N*-benzoyltetrapeptides by Rohrbach *et al.*[8] The 21-fold difference in activity between **25c** and **25h** suggests that the L-Phe side-chain at the P_1 position may orient to the S_1 subsite. In addition, the absence of a significant difference in activity between **25c** and **6** implies that the side-chain phenyl group of **25c** may occupy a location (orienting to the S_1 subsite) similar to that of **6** as depicted in Chart 5; it is conceivable that the Z-NH-moiety of **25c** plays a role in maintaining the favorable orientation of the side-chain, but does not markedly interact with an additional binding site (presumably the S_2 subsite) of the enzyme. The fact that the elimination of the Z group from **25a, c**, resulted in a decrease in activity, therefore, might be due mainly to an unfavorable conformational change arising from the basicity of the newly created α-amino group, not to the lack of the additional interaction of the Z group with the enzyme. Further study will be necessary to explain these findings.

Among various *C*-terminal residues, an octahydroindole-2-carboxylic acid residue was the most potent; increased lipophilicity of the P_2' residue appeared to lead to potent inhibitors, as previously suggested in many other series.[3e,4,9,10]

Interestingly, the effect of the P_1 Lys was modified by the *C*-terminal residue; in the P_2' Pro series (**23**), a 20-fold difference was observed between the activities of **23a** and **23c**, whereas **25a** was only slightly more active than **25c**. Apparently, the P_1 Lys largely compensated for the low affinity of the P_2' Pro to the enzyme in this series.

Table II shows the effects of various P_1 amino acid residues on antihypertensive activity of the peptides in two-kidney renal hypertensive rats after administration by the oral route. The Lys analogs **25a** and **24a** exhibited more potent activities and longer durations of activity. The Orn analogs **24b** and **25b** were slightly less potent. In general, replacement of Lys by other amino acids resulted in a decrease or loss of activity. The *N*-terminus-free **26a** exhibited no effect; this may be due to the lack of lipophilicity required for absorption from the GI tract.

In summary, the present data suggest that a basic amino acid, especially Lys, at the P_1 position in this series plays an important role not only in binding with the S_1 subsite of ACE, but also in lowering blood pressure when these peptides are administrated by the oral route.

Experimental

Melting points were determined with a Yanaco micro melting point apparatus and are uncorrected. Rotations at the Na-D line were observed by using a Jasco DIP-4 digital polarimeter. The proton nuclear magnetic resonance (^{1}H-NMR) and infrared (IR) spectra were recorded on a Varian XL-300 MHz spectrometer and Jasco A-102 spectrophotometer, respectively. *N*-Succinimidyl esters of protected amino acids and (2*S*,3a*S*,7a*S*)-octahydro-1*H*-indole-2-carboxylic acid were purchased from Kokusan Chemical Works, Ltd., Japan and Kawaken Fine Chemicals Co., Ltd., Japan respectively. Solutions were dried over anhydrous sodium sulfate.

***N*-Phthaloyl-D-glutamic Anhydride (8)** This compound was prepared from D-glutamic acid according to the literature[11] procedure for the racemic compound. mp 202—205 °C; $[\alpha]_D$ +44.9° (*c* = 2.2, dioxane). *Anal.* Calcd for $C_{13}H_{19}NO_5$: C, 60.24; H, 3.50; N, 5.40. Found: C, 60.50; H, 3.63; N, 5.38.

***tert*-Butyl 1-(*N*-Phthaloyl-γ-D-glutamyl)prolinate (11)** *tert*-Butyl prolinate **9** (40.4 g, 236 mmol) was added to a solution of **8** (51 g, 189 mmol) and triethylamine (19.9 g, 197 mmol) in ethyl acetate (AcOEt, 500 ml). The mixture was heated under reflux for 2 h, and washed with dilute HCl. The organic layer was dried and evaporated. The residue was recrystallized from AcOEt to give 80 g (94%) of **11**: mp 184—189 °C; $[\alpha]_D^{26}$ −31.3° (*c* = 0.32, EtOH). *Anal.* Calcd for $C_{22}H_{26}N_2O_7$: C, 61.39; H, 6.09; N, 6.51. Found: C, 61.17; H, 6.24; N, 6.42.

***tert*-Butyl 1-(γ-D-Glutamyl)prolinate (13)** A solution of **11** (30 g, 72 mmol) and hydrazine hydrate (7.3 g, 146 mmol) in EtOH (300 ml) was stirred overnight at room temperature, acidified with dilute HCl, cooled, and filtered. The filtrate was concentrated, adjusted to pH 4—5, chromatographed on CHP20P (High Porous Polymer, 75—150 μ, manufactured by Mitsubishi Chemical Ind., Japan), and eluted with a gradient of 10 to 70% acetonitrile (CH_3CN) in H_2O to give 16.7 g (79.7%) of **13**: mp 179—184 °C (dec.); $[\alpha]_D^{26}$ −79.5° (*c* = 1.0, EtOH). *Anal.* Calcd for $C_{14}H_{24}N_2O_5 \cdot 0.5H_2O$: C, 54.36; H, 8.15; N, 9.06. Found: C, 54.16; H, 8.24; N, 9.07.

(2*S*,3a*S*,7a*S*)-1-(*N*-Phthaloyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (12) A mixture of **8** (2.69 g, 10 mmol) and (2*S*,3a*S*,7a*S*)-octahydro-1*H*-indole-2-carboxylic acid (1.69 g, 10 mmol) in pyridine (10 ml) was stirred for 1.5 h at room temperature. The pyridine was evaporated and the residue was partitioned between dilute HCl and AcOEt. The organic layer was washed with water, and extracted with 5% aqueous sodium bicarbonate (5% $NaHCO_3$). The aqueous layer was acidified and

extracted with AcOEt. The organic layer was dried and evaporated. The residue was crystallized from diethyl ether (ether) to give 3.6 g (83%) of **12**: mp 194—198 °C; $[\alpha]_D^{26}$ −7.3° (c=1.0, EtOH). *Anal.* Calcd for $C_{22}H_{24}N_2O_7 \cdot 0.2H_2O$: C, 61.16; H, 5.69; N, 6.48. Found: C, 61.32; H, 5.86; N, 6.12.

(2*S*,3a*S*,7a*S*)-1-(γ-D-Glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (14) This compound was prepared from **12** by the same procedure as described for **11** to give **14** (49%): mp 191—192 °C; $[\alpha]_D^{26}$ −59.0° (c=0.96, H_2O). *Anal.* Calcd for $C_{14}H_{22}N_2O_5 \cdot H_2O$: C, 53.15; H, 7.65; N, 8.86. Found: C, 52.84; H, 7.62; N, 9.14.

(*R*)-3-Benzyloxycarbonyl-5-oxo-4-oxazolinepropionic Acid (15) This compound was prepared from *N*-Z-D-glutamic acid according to the literature[12] procedure for the (*S*)-enantiomer, and purified as the dicyclohexylamine salt of **15**: mp 120—121 °C; $[\alpha]_D^{26}$ −63.0° (c=1.0, AcOEt). *Anal.* Calcd for $C_{26}H_{38}N_2O_6 \cdot 0.25H_2O$: C, 64.57; H, 8.13; N, 5.79. Found: C, 64.39; H, 8.32; N, 5.77.

Ethyl *N*-Benzyloxycarbonyl-D-glutamate (16) This compound was prepared from **15** by the patent[13] procedure for the L-enantiomer, and purified as the dicyclohexylamine salt of **16** (78%): mp 158—160 °C; $[\alpha]_D^{26}$ +11.1° (c=1.0, EtOH). *Anal.* Calcd for $C_{27}H_{42}N_2O_6$: C, 66.10; H, 8.63; N, 5.71. Found: C, 65.80; H, 8.74; N, 5.70. [lit.[9] mp 160—161 °C; $[\alpha]_D$ +12.4° (c=0.5, MeOH)].

The free acid **16** was obtained by the decomposition of the dicyclohexylamine salt in a usual manner. mp 47—48 °C; $[\alpha]_D^{26}$ +22.8° (c=0.5, MeOH). [patent[13] mp 46—48 °C; $[\alpha]_D$ −21.2° (c=8, MeOH)].

Ethyl 1-(O¹-Ethyl-γ-D-glutamyl)indoline-2(*S*)-carboxylate (19) EDC·HCl (26.9 g, 140 mmol) was added to a solution of **16** (44.0 g, 142 mmol), ethyl indoline-2(*S*)-carboxylate hydrochloride[14] (29.0 g, 127 mmol) and triethylamine (12.9 g, 127 mmol) in dichloromethane (CH_2Cl_2, 400 ml). After being stirred for 5 h, the solution was washed with dilute HCl, 5% $NaHCO_3$ and water. The organic layer was dried and evaporated. The residue was chromatographed on silica gel and eluted with $CHCl_3$/MeOH (95:5) to give 42.7 g (70%) of the intermediate diethyl ester: mp 115—121 °C; $[\alpha]_D^{26}$ −54.1° (c=1.0, EtOH). Hydrogen was passed through a stirred mixture of the above diethyl ester (37.7 g, 78 mmol) and 5% palladium carbon (1.5 g) in MeOH (500 ml)/AcOH (100 ml) for 2 h. After removal of the catalyst, the filtrate was concentrated, adjusted to pH 8—9, and then extracted with CH_2Cl_2. The organic layer was washed with 5% $NaHCO_3$ and water, dried, and evaporated. The product was crystallized from petroleum ether to give 22.0 g (81%) of **19**: mp 65—68 °C; $[\alpha]_D^{26}$ −103.9° (c=1.71, EtOH). *Anal.* Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.89; H, 6.75; N, 8.02.

Ethyl (2*S*,3a*S*,7a*S*)-1-(O¹-Ethyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylate (20) EDC·HCl (15.8 g, 82 mmol) was added to a solution of **16** (24.5 g, 79 mmol), ethyl (2*S*,3a*S*,7a*S*)-octahydro-1*H*-indole-2-carboxylate hydrochloride[14] (17.5 g, 75 mmol) and triethylamine (7.58 g, 75 mmol) in CH_2Cl_2 (200 ml). After being stirred overnight, the solution was washed with dilute HCl, H_2O, and 5% $NaHCO_3$. The organic layer was dried and evaporated to give 34.1 g of the intermediate diester as an oil. Hydrogen was passed through a stirred mixture of the above diester and 10% palladium carbon (1.5 g) in AcOH (30 ml)/*tert*-butanol (200 ml) for 4 h. After removal of the catalyst, the filtrate was evaporated. The residue was partitioned between CH_2Cl_2 and 5% $NaHCO_3$. The organic layer was dried and evaporated to give 24.5 g (87%) of **20** as an oil. A portion of the oil was converted to the oxalate of **20**, which was crystallized from 2-propanol/ether: mp 78—84 °C; $[\alpha]_D^{26}$ −51.8° (c=1.0, EtOH). *Anal.* Calcd for $C_{20}H_{32}N_2O_9 \cdot 0.5H_2O$: C, 52.97; H, 7.34; N, 6.18. Found: C, 52.95; H, 7.55; N, 6.13.

1-(N²-Benzyloxycarbonyl-L-lysyl-γ-D-glutamyl)-L-proline (23a) A solution of N^2-Z-N^6-*tert*-butoxycarbonyl-L-lysine *N*-hydroxysuccinimidyl ester **21n** (4.63 g, 9.7 mmol) in tetrahydrofuran (THF) was added to a solution of **13** (3.0 g, 9.7 mmol) in 5% $NaHCO_3$ (30 ml)/THF (10 ml). The solution was stirred for 4 h and washed with AcOEt. The aqueous layer was acidified with 10% citric acid and extracted with CH_2Cl_2. The organic layer was dried and evaporated to give an oil, which was dissolved in trifluoroacetic acid (TFA). After standing for 0.5 h, the TFA was evaporated. The residue was chromatographed on CHP20P and eluted with a gradient of 10 to 80% CH_3CN in H_2O. The product was lyophilized to give 2.7 g (53%) of **23a** as an amorphous powder (Table I).

Compounds **23c**, **23e**, **23j**, **23k**, and **23l** were also prepared by this procedure (Table I).

1-(N²-Benzyloxycarbonyl-L-lysyl-γ-D-glutamyl)indoline-2(*S*)-carboxylic Acid (24a) EDC·HCl (2.34 g, 12 mmol) was added to a solution of N^2-Z-N^6-*tert*-butoxycarbonyl-L-lysine **22n** (2.7 g, 7.18 mmol) and **19** (2.5 g, 7.18 mmol) in CH_2Cl_2 (50 ml). After being stirred overnight, the solution was washed with 10% citric acid, 5% $NaHCO_3$ and water and evaporated to give 4.6 g (90%) of the intermediate diester: mp 139—142 °C. A solution of the diester (2.3 g, 3.2 mmol) in 1 N NaOH (10 ml)/EtOH (50 ml) was stirred for 1.5 h, acidified with 10% citric acid, and extracted with AcOEt. The organic layer was dried and evaporated. The residue was crystallized from petroleum ether/AcOEt to give 1.9 g (90%) of the intermediate diacid: mp 121—9 °C (dec.). The diacid (1.1 g, 1.7 mmol) was dissolved in TFA (20 ml) and the solution was allowed to stand for 0.5 h. After evaporation of the TFA, the residue was chromatographed on CHP20P and eluted with a gradient of 0 to 60% CH_3CN in H_2O to give 0.68 g (70%) of **24a** (Table I).

Compounds **24b** and **24i** were also prepared by this method (Table I).

1-(*N*-Benzyloxycarbonyl-L-phenylalanyl-γ-D-glutamyl)indoline-2(*S*)-carboxylic Acid (24c) EDC·HCl (0.91 g, 4.7 mmol) was added to a solution of *N*-Z-L-phenylalanine (1.42 g, 4.7 mmol) and **19** (1.65 g, 4.7 mmol) in CH_2Cl_2 (20 ml). After being stirred for 5 h, the solution was washed with dilute HCl, 5% $NaHCO_3$ and water, dried, and evaporated. The residue was crystallized from EtOH to give 2.53 g (85%) of the intermediate diester: mp 176—178 °C. A solution of the diester (0.63 g, 1.0 mmol) and 1 N NaOH (3 ml) in EtOH (5 ml) was stirred for 3 h, acidified with dilute HCl, and extracted with CH_2Cl_2. The organic layer was dried and evaporated. The residue was crystallized from CH_2Cl_2 to give 0.57 g (96%) of **24c** (Table I).

Compound **24j** was also prepared by this procedure (Table I).

(2*S*,3a*S*,7a*S*)-1-(N²-Benzyloxycarbonyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (25a) A solution of **21n** (10.0 g, 21 mmol) in THF (200 ml) was added to a solution of **14** (5.97 g, 20 mmol) and sodium carbonate (2.1 g, 20 mmol) in water (100 ml). The solution was stirred for overnight and the THF was evaporated. The residue was acidified with 10% citric acid and extracted with AcOEt. The organic layer was dried and evaporated to give 8.7 g of a crude intermediate diacid. A solution of the diacid (3.46 g) in TFA (35 ml) was allowed to stand for 0.5 h and the TFA was evaporated. The residue was chromatographed on CHP20P and eluted with a gradient of 0 to 60% CH_3CN in H_2O. The product was lyophilized to give 1.78 g (37.6%) of **25a** (Table I).

Compounds **25b** and **25g** were also prepared by this procedure (Table I).

(2*S*,3a*S*,7a*S*)-1-(*N*-Benzyloxycarbonyl-L-phenylalanyl-γ-D-glutamyl)-octahydro-1*H*-indole-2-carboxylic Acid (25c) A solution of *N*-Z-L-phenylalanine *N*-hydroxysuccinimidyl ester **21c** (2.54 g, 6.4 mmol) in THF (10 ml) was added to a solution of **14** (2.0 g, 6.3 mmol) in 5% $NaHCO_3$ (20 ml). The solution was stirred overnight, washed with AcOEt, acidified with dilute HCl, and extracted with AcOEt. The organic layer was dried and evaporated. The residue was chromatographed on CHP20P and eluted with a gradient of 10 to 80% CH_3CN in H_2O to give 2.4 g (64%) of **25c** as an amorphous powder (Table I).

Compounds **25d**, **25e**, **25f** and **25h** were prepared similarly (Table I).

(2*S*,3a*S*,7a*S*)-1-(*N*-Benzyloxycarbonyl-L-histidyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (25m) EDC·HCl (0.54 g, 2.8 mmol) was added to a mixture of **20** (1.0 g, 2.8 mmol) and *N*-Z-L-histidine (0.82 g, 2.8 mmol) in CH_2Cl_2/THF. The mixture was stirred for 6 h, and formed a clear solution. The solution was washed with 5% $NaHCO_3$ and 10% citric acid, and evaporated to give 0.7 g of the intermediate diester as an oil. The above oil was dissolved in dioxane (10 ml), and 1 N NaOH (7.6 ml) was added. After being stirred for 4 h, the solution was adjusted to pH 3, chromatographed on CHP20P and eluted with a gradient of 10 to 30% CH_3CN in H_2O to give 0.185 g (11%) of **25m** as an amorphous powder (Table I).

(2*S*,3a*S*,7a*S*)-1-(L-Phenylalanyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (26c) A solution of **25c** (1.6 g, 2.7 mmol) in 25% HBr/AcOH (10 ml) was stirred at room temperature for 50 min, and then evaporated. The residue was dissolved in water (10 ml) and adjusted to pH 3—4. The solution was chromatographed on CHP20P and eluted with a gradient of 0 to 40% CH_3CN in H_2O to give 0.6 g (50%) of **26c** (Table I).

Compounds **26a** and **26h** were also prepared by the procedure described above (Table I).

Ethyl *N*-(N²-Benzyloxycarbonyl-N⁶-*tert*-butoxycarbonyl-L-lysyl)-D-glutamate (27) A solution of **21n** (8.9 g, 18.7 mmol) in THF (10 ml) was added to a solution of ethyl D-glutamate (2.97 g, 17 mmol) and sodium carbonate (1.8 g, 34 mmol) in water (30 ml). After being stirred overnight, the solution was adjusted to pH 2—3 and extracted with AcOEt. The organic layer was dried and evaporated. The product was crystallized from ether/petroleum ether to give 8.5 g (93%) of **27**: mp 71—73 °C; $[\alpha]_D^{26}$ +2.0° (c=1.0, EtOH). *Anal.* Calcd for $C_{26}H_{39}N_3O_9 \cdot 0.25H_2O$: C, 57.61; H, 7.34; N, 7.75. Found: C, 57.52; H, 7.33; N, 7.92.

2-(N²-Benzyloxycarbonyl-L-lysyl-γ-D-glutamyl)-1,2,3,4-tetrahydroisoquinoline-3(*S*)-carboxylic Acid (32) EDC·HCl (0.42 g, 2.2 mmol) was

added to a mixture of methyl 1,2,3,4-tetrahydroisoquinoline-3(*S*)-carboxylate hydrochloride **28**[15] (0.5 g, 2.2 mmol), *N*-methylmorpholine (0.22 g, 2.2 mmol), and **27** (1.08 g, 2 mmol) in CH_2Cl_2. After being stirred overnight, the mixture was washed with 10% citric acid and 5% $NaHCO_3$. The organic layer was dried and evaporated. The residual oil was chromatographed on CHP20P and eluted with a gradient of 40 to 100% CH_3CN in H_2O to give 1.0 g (70%) of the intermediate diester. The above diester was dissolved in dioxane (20 ml) and 1 N NaOH (4.2 ml) was added. The solution was stirred for 2 h, acidified with 10% citric acid and extracted with AcOEt. The organic layer was dried and evaporated to give 0.9 g (95%) of the intermediate diacid as an oil. A solution of the above diacid in TFA (10 ml) was allowed to stand for 0.5 h and was then evaporated. The residue was chromatographed on CHP20P and eluted with a gradient of 0 to 60% CH_3CN in H_2O to give 0.45 g (58%) of **32** as an amorphous powder (Table I).

(1*R,3*R**,5*R**)-2-(N^2-Benzyloxycarbonyl-L-lysyl-γ-D-glutamyl)-2-azabicyclo[3.3.0]octane-3-carboxylic Acid (33)** Diastereomeric **33** was prepared from **27** and benzyl (1*R**,3*R**,5*R**)-2-azabicyclo[3.3.0]octane-3-carboxylate[16] **29** in the same manner as described for **32** (Table I).

***N*-(N^2-Benzyloxycarbonyl-L-lysyl-γ-D-glutamyl)-*N*-cyclooctylglycine (34)** This compound was prepared from **27** and ethyl *N*-cyclooctylglycinate **30** as described above (Table I).

***N*-(N^2-Benzyloxycarbonyl-L-lysyl-γ-D-glutamyl)-*N*-cyclopentylglycine (35)** This compound was prepared from **27** and ethyl *N*-cyclopentylglycinate **31** as described above (Table I).

***In Vitro* ACE-Inhibitory Activity** ACE was prepared from rabbit lung by the method of Cushman and Cheung[17] and the inhibitory activity of tested compounds was determined by the procedure of Takeyama *et al.*[18] The results are shown in Table I.

Antihypertensive Activity in Renal Hypertensive Rats Male Sprague–Dawley rats (5 weeks old) were subjected to constriction of the left renal artery with a silver clip (internal diameter: 0.22 mm) under light ether anesthesia. The right kidney and renal artery were left intact. About 6—10 weeks after clipping, rats showing a blood pressure above 180 mmHg were used. The blood pressure was measured by a tail-cuff method using a programmed electro-sphygmomanometer (PE-300, Narco Biosystem) after warming at 38 °C for 10 min in a heating box.

The antihypertensive activity of test compounds was evaluated after single oral administration in renal hypertensive rats (3—6 rats/group). The results are shown in Table II.

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