Angiotensin-Converting Enzyme Inhibitors: Synthesis and Biological Activity of *N*-Substituted Tripeptide Inhibitors[1,2]

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A new series of highly potent angiotensin-converting enzyme (ACE) inhibitors, 1-(N^2-substituted L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic acids, was synthesized; various acyl groups were introduced at the α-amino group of the *N*-terminal P_1 Lys. The effect of the N^2-acyl groups on *in vitro* inhibitory activity and oral antihypertensive effect was examined. All of the synthesized *N*-acyl tripeptides were found to have *in vitro* inhibitory activity at an approximately nanomolar level, and showed antihypertensive potency in renal hypertensive rats at a dose of 10 mg/kg, when administered orally. Among them, compounds 7e, g and 9f, i, m showed potent and long-lasting antihypertensive effects compared with enalapril (2a). Their structure–activity relationships are also discussed.

Keywords angiotensin-converting enzyme; antihypertensive activity; inhibitory activity; S_1 subsite; S_2 subsite; P_1 substituent; P_2 substituent.

In recent years, captopril (**1**)[3] and enalapril (**2a**),[4] which are typical angiotensin-converting enzyme (ACE) inhibitors, have become important drugs in the treatment of hypertension. With the aim of developing more potent drugs, many research groups[5] have explored the structural requirements of the active site of ACE with various structurally different inhibitors.

In a previous paper,[2] we reported highly potent tripeptidic ACE inhibitors; among them, the most potent compound **3** exhibited an *in vitro* inhibitory activity at a nanomolar level (IC_{50}: 3.5 nM), comparable to that of enalaprilat **2b** (IC_{50}: 5.4 nM), and showed a potent oral antihypertensive effect in renal hypertensive rats. In addition, we showed Lys to be most favorable as the P_1 amino acid residue. In addition, the possibility that an additional interaction of the P_2 substituent with the S_2 subsite might lead to an improved activity has been explored in modified *N*-carboxymethyl dipeptides,[6a] *N*-benzamido tripeptidic ketomethylenes[6b] and other inhibitors.[6c,d]

We, therefore, examined the effect of various N^2-substituents (P_2 substituents) and some N^6-substituents of P_1 Lys on the biological activity in the expectation of obtaining more potent inhibitors as potential antihypertensive agents and of better understanding the structure–activity relationships in the present series of inhibitors.

Synthesis

Charts 1 and 2 illustrate the synthetic routes to the present tripeptidic inhibitors. The carbonates **4**, useful for carbamate synthesis, were easily prepared from the corresponding alcohols and disuccinimidyl carbonate (DSC) in the presence of 4-dimethylaminopyridine (DMAP) (Table I). This method has the advantage of using no phosgene.[7] Reaction of the *tert*-butoxycarbonyl (Boc) tripeptide **6** with **4** gave the protected carbamates, which were successively treated with trifluoroacetic acid (TFA) to afford the carbamates **7** (procedure A). The key intermediate N^6-benzyloxycarbonyl (Z) tripeptide **8** was prepared by the

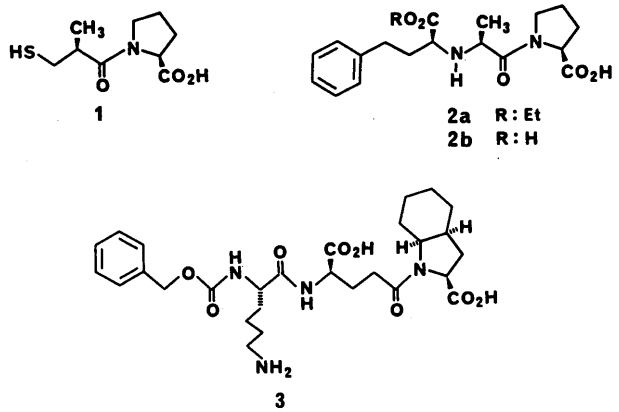


Fig. 1

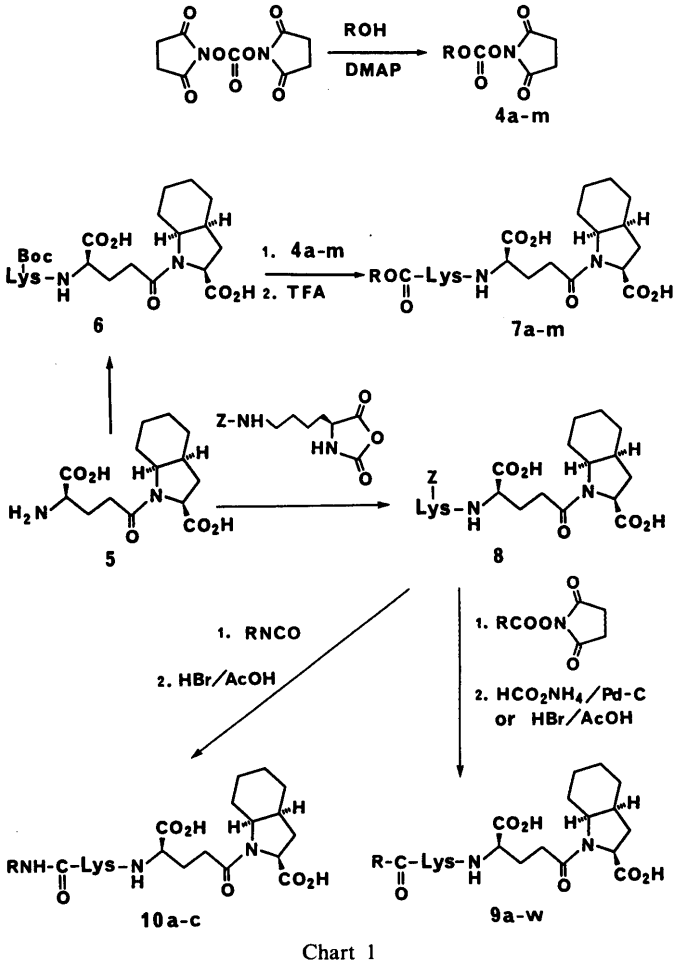


Chart 1

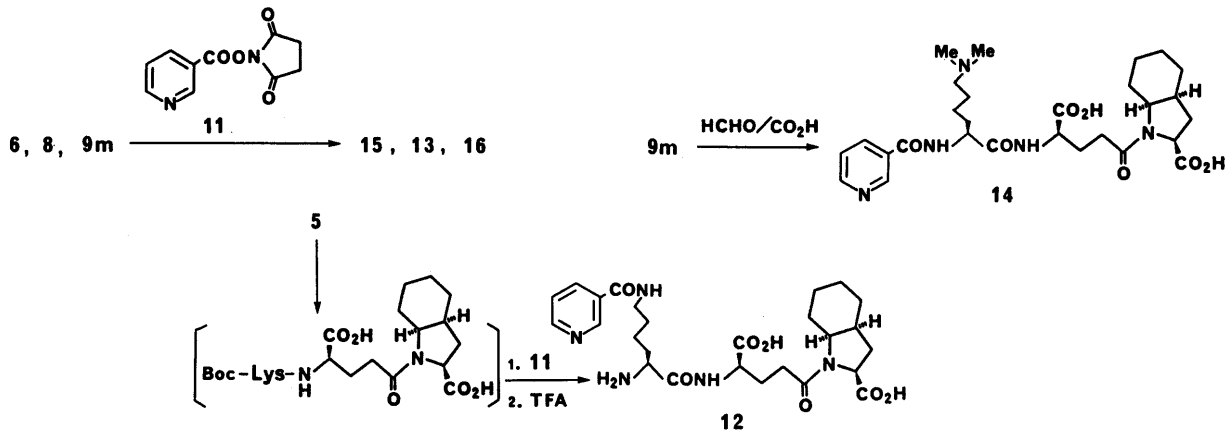
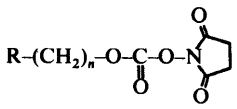


Chart 2

TABLE I. *N*-Succinimidyl Carbonates



Compd.	R	*n*	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd (Found)			
						C	H	N	Halogen
4a	Ph	2	67—72	65	$C_{13}H_{13}NO_5$	59.31 (59.05	4.98 5.11	5.31 5.24)	
4b	2-Me-Ph	1	Wax[a]	27	$C_{13}H_{13}NO_5$				
4c	4-Me-Ph	1	115—117	67	$C_{13}H_{13}NO_5$	59.31 (59.01	4.98 5.05	5.32 5.23)	
4d	2-Br-Ph	1	100—104	61	$C_{12}H_{10}BrNO_5$	43.93 (44.04	3.07 2.85	4.27 4.22	24.35 24.37)
4e[b]	2-Cl-Ph	1	66—70	55	$C_{12}H_{10}ClNO_8$	50.81 (50.60	3.55 3.61	4.94 4.81	12.50 12.22)
4f[c]	4-Cl-Ph	1	120—122	66	$C_{12}H_{10}ClNO_5$	50.81 (50.99	3.55 3.48	4.94 4.80	12.50 12.80)
4g	2-F-Ph	1	107—109	58	$C_{12}H_{10}FNO_5$	53.94 (53.78	3.77 3.97	5.24 5.01	7.11 6.98)
4h	4-Ph-Ph	1	>200	90	$C_{18}H_{15}NO_5$	66.46 (66.92	4.65 4.82	4.31 4.02)	
4i	α-Naphthyl	1	102—105	64	$C_{16}H_{13}NO_5$	64.21 (63.98	4.38 4.41	4.68 4.51)	
4j	α-Naphthyl	2	106—109	62	$C_{17}H_{15}NO_5$	67.17 (66.97	4.82 4.96	4.47 4.23)	
4k	Cyclobutyl	0	65—72	65	$C_9H_{11}NO_5$	50.71 (50.46	5.20 5.41	6.57 6.54)	
4l	Cyclopentyl	0	78—80	54	$C_{10}H_{13}NO_5$	52.86 (52.77	5.77 5.98	6.16 6.08)	
4m	Cyclohexyl	0	94—95	56	$C_{11}H_{15}NO_5$	54.77 (54.67	6.27 6.41	5.81 5.76)	

a) Used without further purification. *b*) Lit.[7] mp 103 °C. *c*) Lit.[7] mp 125 °C.

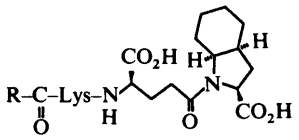
N-carboxyanhydride (NCA) procedure; among several alkaline media examined, the use of potassium carbonate–potassium hydroxide in dioxane–H_2O gave a successful result. Thus, reaction of the dipeptide **5**[2] and N^6-Z-lysine NCA afforded **8** in a good yield. Acylation of **8** with active esters gave the protected amides, which were deprotected by catalytic transfer hydrogenation[8] with ammonium formate–palladium carbon (procedure B) or by the HBr–AcOH procedure (procedure C) to afford the corresponding amides **9**. The ureas **10** were also derived from **8** with the corresponding isocyanates (procedure D). N^6-Dimethylation of **9m** was achieved by the HCHO–HCOOH procedure. N^6-Nicotinoyl compounds **15**, **13** and **16** were prepared by the acylation of **6**, **8** and **9m**, respectively, with the active ester **11**.

Biological Results and Discussion

Tables II and III show the *in vitro* inhibitory activity of the synthesized compounds. In the carbamate series **7**, variation of hydrophobicity, size, and length of the N^2-substituent did not markedly affect the inhibitory activity, compared with the prototype **3**.

The benzoyl analog **9b** is also a highly potent inhibitor, but slightly less active (IC_{50}: 5.4 nM) than **3**. In this amide

TABLE II. Tripeptide Inhibitors with the N^2-Substituted Lys Residue



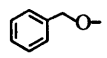
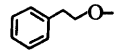
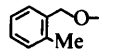
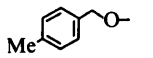
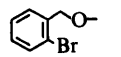
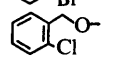
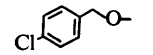
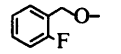
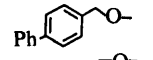
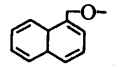
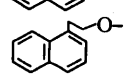
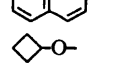
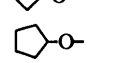
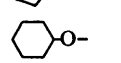
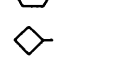
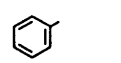
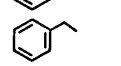
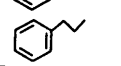
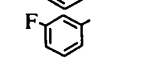
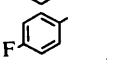
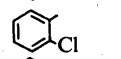
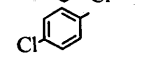
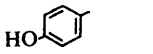
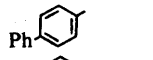
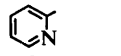
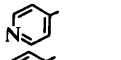
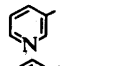
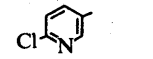
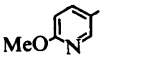
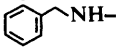
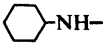
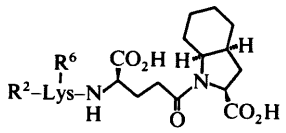
Compd.[a]	R	Procedure[b]	$[\alpha]_D^{c}$ (°) (c, S[d])	Formula	Analysis (%) Calcd (Found) C	H	N	X[e]	ACE IC_{50} (nM)	Change of SBP[f] (mmHg) (10 mg/kg, *p.o.*)
3[g]									3.5	−37 (9 h)[h] −19 (24 h)
7a		A	−36.9 (0.52, N)	$C_{29}H_{42}N_4O_8$ $\cdot 2H_2O \cdot 0.25D$[i]	56.96 (56.92	7.65 7.87	8.85 8.64)		4.6	−15 (9 h)
7b		A	−35.8 (0.33, N)	$C_{29}H_{42}N_4O_8$ $\cdot 1.75H_2O$	57.46 (57.68	7.57 7.63	9.24 9.01)		3.6	−41 (9 h) −26 (24 h)
7c		A	−31.9 (0.32, N)	$C_{29}H_{42}N_4O_8$ $\cdot 1.75H_2O$	57.46 (57.37	7.57 7.51	9.24 9.07)		5.0	−30 (9 h)
7d		A	−38.2 (1.46, N)	$C_{28}H_{39}BrN_4O_8$ $\cdot 0.5H_2O$	52.22 (52.17	6.18 6.39	8.70 8.81	12.41 12.40)	5.0	−33 (9 h)
7e		A	−32.8 (0.31, N)	$C_{28}H_{39}ClN_4O_8$ $\cdot 1.25H_2O$	54.45 (54.55	6.77 6.81	9.07 8.90	5.74 5.60)	5.0	−47 (9 h) −19 (24 h)
7f		A	−30.8 (0.31, N)	$C_{28}H_{39}ClN_4O_8$ $\cdot 1.5H_2O$	54.06 (53.95	6.81 6.65	9.01 8.80	5.70 5.56)	4.7	−39 (9 h) −28 (24 h)
7g		A	−32.5 (0.30, N)	$C_{28}H_{39}FN_4O_8$ $\cdot 1.5H_2O \cdot 0.5D$	55.46 (55.63	7.14 7.08	8.62 8.54	2.92 3.01)	4.7	−53 (9 h) −16 (24 h)
7h		A	−39.1 (0.44, N)	$C_{34}H_{44}N_4O_8$ $\cdot 1.5H_2O \cdot 0.5D$	61.09 (61.23	7.26 7.26	7.92 7.85)		5.2	−14 (7 h)
7i		A	−36.3 (0.30, N)	$C_{32}H_{42}N_4O_8$ $\cdot 1.5H_2O \cdot 0.25D$	60.08 (59.78	7.18 7.41	8.49 8.37)		3.6	−16 (7 h)
7j		A	−38.8 (0.30, N)	$C_{33}H_{44}N_4O_8$ $\cdot 2H_2O \cdot 0.5D$	59.64 (59.87	7.44 7.17	7.95 7.91)		3.9	−14 (7 h)
7k		A	−40.7 (0.49, H)	$C_{25}H_{40}N_4O_8$ $\cdot 2H_2O$	53.56 (53.57	7.91 7.60	9.99 9.93)		4.6	−26 (9 h)
7l		A	−37.1 (0.52, H)	$C_{26}H_{42}N_4O_8$ $\cdot 1.5H_2O$	55.21 (55.05	8.02 7.77	9.90 10.05)		5.8	−38 (9 h)
7m		A	−31.9 (0.50, H)	$C_{27}H_{44}N_4O_8$ $\cdot 1.75H_2O$	55.51 (55.53	8.20 8.42	9.59 9.55)		5.0	−25 (7 h)
9a		B	−46.8 (1.12, N)	$C_{25}H_{40}N_4O_7$ $\cdot 2H_2O \cdot 0.25D$	55.11 (55.16	8.18 7.98	9.89 9.79)		7.5	−42 (9 h) −18 (24 h)
9b		A	−23.1 (0.54, N)	$C_{27}H_{38}N_4O_7$ $\cdot 2.25H_2O$	56.78 (56.91	7.50 7.29	9.81 10.03)		5.4	−42 (9 h) −16 (24 h)
9c		B	−51.5 (1.01, N)	$C_{28}H_{40}N_4O_7$ $\cdot 0.25H_2O$	61.24 (61.25	7.43 7.68	10.20 10.12)		11	−28 (9 h)
9d		C	−67.6 (1.02, N)	$C_{29}H_{42}N_4O_7$ $\cdot 0.5H_2O$	61.76 (61.58	7.64 7.93	9.89 9.71)		10	−27 (9 h)
9e		C	−35.4 (1.19, N)	$C_{27}H_{37}FN_4O_7$ $\cdot 0.25H_2O$	58.63 (58.51	6.83 6.90	10.13 10.00	3.43 3.50)	8.6	−9 (7 h)
9f		C	−31.8 (0.32, N)	$C_{27}N_{37}FN_4O_7$ $\cdot 2H_2O$	55.47 (55.59	7.07 7.36	9.58 9.46	3.25 3.03)	13	−45 (9 h) −27 (24 h)
9g		C	−56.0 (0.40, N)	$C_{27}H_{37}ClN_4O_7$ $\cdot 0.25H_2O$	56.94 (56.78	6.64 6.69	9.84 9.66	6.22 6.18)	11	−24 (9 h)
9h		C	−25.9 (0.20, N)	$C_{27}H_{37}ClN_4O_7$ $\cdot 1.5H_2O$	54.77 (55.07	6.81 7.09	9.46 9.26	5.99 5.79)	6.2	−25 (9 h)
9i		C	−17.4 (0.27, H)	$C_{27}H_{38}N_4O_8$ $\cdot 1.5H_2O$	56.53 (56.71	7.20 7.09	9.77 9.95)		5.2	−51 (9 h) −23 (24 h)
9j		C	−11.6 (0.43, N)	$C_{33}H_{42}N_4O_7$ $\cdot 1.5H_2O \cdot 0.5D$	62.02 (61.98	7.29 7.15	8.27 8.35)		5.6	−12 (7 h)
9k		B	−19.2 (0.49, H)	$C_{26}H_{37}N_5O_7$ $\cdot 1.75H_2O$	55.45 (55.74	7.25 7.05	12.44 12.42)		6.8	−37 (9 h)
9l		A	−29.8 (0.52, H)	$C_{26}H_{37}N_5O_7$ $\cdot 2.5H_2O$	54.16 (54.25	7.34 7.06	12.15 12.23)		7.2	−38 (9 h) −22 (24 h)
9m		B	−27.2 (1.02, H)	$C_{26}H_{37}N_5O_7$ $\cdot 2.25H_2O$	54.58 (54.02	7.31 7.25	12.24 12.20)		6.4	−47 (7 h) −31 (24 h)
9n		C	−21.4 (0.20, H)	$C_{26}H_{36}ClN_5O_7$ $\cdot 2H_2O$	51.87 (51.78	6.70 6.44	11.63 11.86	5.89 6.05)	6.0	−34 (9 h)
9o		C	−18.0 (0.40, H)	$C_{27}H_{39}N_5O_8$ $\cdot 1.5H_2O$	55.09 (55.09	7.19 7.44	11.90 11.77)		7.7	−25 (7—9 h)

TABLE II. (continued)

Compd.[a]	R	Procedure[b]	$[\alpha]_D$[c] (°) (c, S[d])	Formula	Analysis (%) Calcd (Found) C	H	N	X[e]	ACE IC_{50} (nM)	Change of SBP[f] (mmHg) (10 mg/kg, p.o.)
9p		C	−35.0 (1.04, N)	$C_{27}H_{39}N_5O_7 \cdot 0.5H_2O$	58.47 (58.38	7.27 7.41	12.63 12.63)		8.6	−23 (9 h)
9q		C	−13.8 (0.56, H)	$C_{25}H_{36}N_4O_8 \cdot 2H_2O$	53.95 (53.96	7.24 7.39	10.07 9.92)		7.5	−19 (9 h)
9r		C	−23.1 (0.27, H)	$C_{25}H_{36}N_4O_7S \cdot H_2O$	54.14 (54.09	6.91 6.74	10.10 10.14	5.78 5.99)	4.5	−29 (9 h)
9s		C	−14.2 (0.20, N)	$C_{31}H_{40}N_4O_7 \cdot 4H_2O$	57.04 (57.34	7.41 7.27	8.58 8.51)		2.6	−16 (9 h)
9t		C	−43.3 (0.15, N)	$C_{31}H_{40}N_4O_7 \cdot 3H_2O$	58.66 (58.84	7.31 7.14	8.83 8.83)		2.1	−7 (9 h)
9u		C	−25.8 (0.20, N)	$C_{30}H_{39}N_5O_7 \cdot 2H_2O$	58.33 (58.46	7.02 7.30	11.34 11.24)		4.6	−27 (9 h)
9v		C	−51.8 (0.22, N)	$C_{30}H_{39}N_5O_7 \cdot H_2O$	60.09 (59.89	6.89 6.66	11.68 11.61)		7.2	−23 (7 h)
9w		C	−6.8 (0.21, N)	$C_{29}H_{38}N_4O_8 \cdot 1.75H_2O \cdot 0.25D$	57.73 (57.75	7.02 7.23	8.98 8.98)		6.2	−18 (9 h)
10a		D	−42.0 (0.35, N)	$C_{27}H_{39}N_5O_7 \cdot 1.5H_2O$	56.63 (56.80	7.39 7.23	12.23 12.03)		10	−27 (9 h)
10b		D	−32.0 (0.10, N)	$C_{28}H_{41}N_5O_7 \cdot 2H_2O$	56.46 (56.39	7.61 7.32	11.76 11.41)		8.4	−12 (7 h)
10c		D	−31.1 (0.31, N)	$C_{25}H_{45}N_5O_7 \cdot 1.25H_2O \cdot 0.25D$	56.41 (56.23	8.37 8.07	11.75 11.67)		7.3	−25 (9 h)
2b	(Enalaprilat)								5.4[j]	
2a	(Enalapril)									−38 (7 h) −12 (24 h)

a) All compounds are amorphous. b) See the corresponding procedure in Experimental. c) Measured at ambient temperature. d) S, Solvent; N, 1 N NaOH; H, H_2O. e) Halogen. f) Systolic blood pressure (SBP) in two-kidney renal hypertensive rats. Average basal SBP of each group was in the range of 205 to 225 mmHg. g) See ref. 2. h) Maximum response time. i) Dioxane. j) Lit.[4] 1.2 nM.

TABLE III. Tripeptide Inhibitors with the N^6-Substituted Lys Residue



Compd.[a]	R^2	R^6	$[\alpha]_D$[b] (°) (c, S[c])	Formula	Analysis (%) Calcd (Found) C	H	N	ACE IC_{50} (nM)	Change of SBP[d] (mmHg) (10 mg/kg, p.o.)
17[e]	H	H						12	No effect
6	H	Boc	−19.2 (0.50, N)	$C_{25}H_{42}N_4O_8 \cdot 1.75H_2O$	53.80 (53.94	8.22 8.30	10.04 9.82)	170	No effect
8	H	Z	−14.6 (1.01, N)	$C_{28}H_{40}N_4O_8 \cdot 2.5H_2O$	55.53 (55.65	7.49 7.40	9.25 9.14)	39	−9 (7 h)[f]
12	H	Nicotinoyl	−18.8 (0.34, N)	$C_{26}H_{37}N_5O_7 \cdot 1.5H_2O$	55.90 (56.21	7.22 7.32	12.54 12.25)	120	No effect
9m[g]	Nicotinoyl	H						6.4	−47 (7 h)
13	Nicotinoyl	Z	−33.5 (0.65, N)	$C_{34}H_{43}N_5O_9 \cdot 1.5H_2O$	58.95 (58.91	6.69 6.63	10.11 9.85)	14	NT[h]
14	Nicotinoyl	$(Me)_2$	−24.2 (0.24, H)	$C_{34}H_{43}N_5O_9 \cdot 0.5H_2O$	59.14 (59.17	7.44 7.67	12.32 12.29)	14	−11 (7 h)
15	Nicotinoyl	Boc	−35.9 (0.96, N)	$C_{31}H_{45}N_5O_9 \cdot H_2O$	57.31 (57.10	7.29 7.36	10.78 10.86)	16	−8 (7 h)
16	Nicotinoyl	Nicotinoyl	−15.1 (0.53, M)	$C_{32}H_{40}N_6O_8 \cdot 1.5H_2O$	57.91 (57.65	6.53 6.44	12.66 12.42)	25	No effect

a) All compounds are amorphous. b) See Table II. c) S, solvent; N, 1 N NaOH; H, H_2O; M, methanol. d) See Table II. e) See ref. 2. f) See Table II. g) See Table II. h) Not tested.

series **9**, introduction (**9e—j**) of a substituent at the phenyl ring, insertion (**9c, d**) of a methylene group(s), and replacement (**9k—r**) or the phenyl ring by a heteroaromatic ring resulted in an activity slightly inferior or comparable to that of **9b**. The more hydrophobic naphthyl group (**9s,t**), however, slightly improved the activity; **9s** and **9t** exhibited the

most potent inhibitory activities, with IC_{50} values of 2.6 and 2.1 nM, respectively. The urea analogs **10** also exhibited potent activities.

On the whole, these N^2-acylated compounds (**7**, **9**, and **10**) except for **9f** showed a more potent inhibitory activity than the N^2-unsubstituted compound **17** previously reported.[2] In addition, interestingly, introduction (**6**, **8** and **12**) of some substituents (Boc, Z and nicotinoyl) at the ε-amino group of Lys in **17** caused a considerable decrease in activity, whereas in the N^2-nicotinoyl compound **9m**, it only resulted in a relatively small decrease in activity (Table III). These findings indicate that the N^2-substituents in the present inhibitors are favorable for the inhibitory activity. However, variation of the hydrophobic moiety of the N^2-substituent in **7**, **9**, and **10** did not have a remarkable effect on the activity; all these compounds exhibited activity at approximately nanomolar concentrations. From these findings, it does not appear that these hydrophobic groups of the N^2-substituents specifically interact with the S_2 subsite of the enzyme. The N^2-substituents might rather permit the molecules to have a conformation suitable for multiple enzyme-inhibitor interactions, or the –CONH– group of the P_2–P_1 bond of these inhibitors might interact with a minor binding site(s) such as the S_2 hydrogen bond donor and/or the S_1 hydrogen bond acceptor proposed by Petrillo and Ondetti.[5a] The role of the N^2-substituents in the inhibitory activity of the present inhibitors remains to be investigated.

On the other hand, the result of modification of the ε-amino group of the P_1 Lys (Table III), along with our previous result,[2] indicates that the unsubstituted ε-amino group of the P_1 Lys side-chain in the present tripeptide inhibitors best fits the S_1 subsite.

The oral antihypertensive effect of the inhibitors examined in renal hypertensive rats at a dose of 10 mg/kg is shown in Tables II and III. The more lipophilic naphthyl (**7i, j** and **9s, t**) and biphenyl (**7h** and **9j**) groups in both carbamate and amide series led to a considerable decrease in antihypertensive effect, although **9s, t** showed the most potent *in vitro* inhibitory activities. N^6-Modification (**14**, **15**, and **16**) of Lys also markedly diminished the effect. Among the compounds tested, **7e, g** and **9f, i, m** exhibited potent and long-lasting antihypertensive effects compared with **3** and **2a**. The pharmacological evaluations of these promising compounds as antihypertensive agents are in progress.

In conclusion, the present tripeptides were found to be potent ACE inhibitors with an oral antihypertensive potency.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Rotations at the Na-D line were observed at amibient temperature 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 FT-80A spectrometer (with tetramethylsilane as an internal standard) and a Jasco A-102 spectrophotometer, respectively. Solutions were dried over anhydrous sodium sulfate. The highly porous polystyrene resin (CHP20P) was purchased from Mitsubishi Chemical Ind., Japan. Chromatography with CHP20P was carried out by eluting with an appropriate gradient of acetonitrile in water under a medium pressure.

2-Fluorobenzyl *N*-Succinimidyl Carbonate (4g) (General Procedure) A solution of DSC (5.12 g, 20 mmol), 2-fluorobenzyl alcohol (2.5 g, 20 mmol) and DMAP (1.22 g, 10 mmol) in acetonitrile (CH_3CN, 30 ml)–dichloromethane (CH_2Cl_2, 30 ml) was stirred for 6 h at room temperature. The solution was washed with water, dried, and evaporated. The residual crystals were washed with diethyl ether to give 3.5 g (58%) of **4g** (see Table I). ^{1}H-NMR (in $CDCl_3$) δ: 7.00—7.52 (4H, m, arom.), 5.40 (2H, s, $-CH_2-O$), 2.75 (4H, s, $-CH_2CH_2-$). IR (KBr): 1820(CO), 1785(CO), 1730(CO) cm.$^{-1}$

Compounds **4a**—**f**, **h**—**m** were prepared by an analogous procedure.

(2*S*,3a*S*,7a*S*)-1-(N^6-tert-Butoxycarbonyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (6) N^6-Boc-N^2-Z-L-Lysine *N*-succinimidyl ester (7.9 g, 16.5 mmol) was added to a solution of (2*S*,3a*S*,7a*S*)-1-(γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic acid hydrate **5**[2] (5.1 g, 16 mmol) and sodium carbonate (2.0 g, 19 mmol) in H_2O (10 ml)–tetrahydrofuran (THF, 40 ml). The solution was stirred overnight at room temperature, acidified with 10% citric acid, and extracted with ethyl acetate. The organic layer was dried and evaporated to give a crude diprotected tripeptide.

Ammonium formate (2.5 g, 40 mmol) was added to a stirred mixture of an aliquot (5.0 g) of the above tripeptide and 10% palladium carbon (0.8 g) in ethanol (50 ml). The mixture was stirred for 4 h at room temperature. After removal of the catalyst, ethyl acetate was added. The precipitate was collected by filtration and chromatographed on CHP20P to give 3.6 g (90%) of **6** (see Table III).

(2*S*,3a*S*,7a*S*)-1-[N^2-(2-Fluorobenzyloxycarbonyl)-L-lysyl-γ-D-glutamyl]octahydro-1*H*-indole-2-carboxylic Acid (7g) (Procedure A) A solution of **4g** (0.61 g, 2.3 mmol) in THF (10 ml) was added to a solution of **6** (1.0 g, 1.9 mmol) in saturated sodium bicarbonate ($NaHCO_3$, 25 ml). The mixture was stirred overnight at room temperature, acidified with 10% citric acid, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to give 1.95 g of an oil, which was chromatographed on CHP20P to give 0.6 g of an oil. The oil was then dissolved in TFA (5 ml) and the solution was allowed to stand for 0.5 h. After evaporation of TFA, the residue was chromatographed on CHP20P and the product was dissolved in dioxane–H_2O and lyophilized to give 0.35 g (28%) of **7g** (Table II).

Compounds **7a**—**f**, **h**—**m** were prepared in a similar manner (Table II). Compounds **9b** and **9l** were similarly prepared by using *N*-benzoyloxysuccinimide and *N*-isonicotinoyloxysuccinimide, respectively, in place of **4g** (Table II).

(2*S*,3a*S*,7a*S*)-1-(N^6-Benzyloxycarbonyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (8) N^6-Z-L-Lysine NCA (2.3 g, 7.6 mmol) was added to a solution of **5** (1.94 g, 6.1 mmol), potassium hydroxide (0.68 g, 12.2 mmol) and potassium carbonate (0.84 g, 6.1 mmol) in dioxane (30 ml)–H_2O (30 ml). The mixture was vigorously stirred at −5—0 °C for 2 h and then adjusted to pH 1 with concentrated HCl. After evaporation of the dioxane, the residue was chromatographed on CHP20P to give 2.4 g (69%) of **8** as an amorphous powder: $[\alpha]_D$ −14.6° (c = 1.01, 1 N NaOH). *Anal.* Calcd for $C_{28}H_{40}N_4O_8 \cdot 0.25H_2O$: C, 55.53; H, 7.49; N, 9.25. Found: C, 55.65; H, 7.40; N, 9.14.

(2*S*,3a*S*,7a*S*)-1-(N^2-Nicotinoyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (9m) (Procedure B) A mixture of **8** (2.0 g, 3.3 mmol), 1-(nicotinoyloxy)succinimide (**11**) (0.94 g, 4.0 mmol) and $NaHCO_3$ (0.6 g, 7.1 mmol) in H_2O (5 ml)–THF (10 ml) was stirred overnight at room temperature. The mixture was acidified (pH 4—5) with 10% citric acid and extracted with ethyl acetate. The organic layer was dried and evaporated. The residue was chromatographed on CHP20P to give 2.0 g (85%) of (2*S*,3a*S*,7a*S*)-1-(N^6-Z-N^2-nicotinoyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic acid (**13**) as an amorphous powder (see Table III).

Ammonium formate (0.76 g, 10.8 mmol) was added to a mixture of **13** (1.6 g, 2.4 mmol) and 10% palladium carbon (0.3 g) in methanol (30 ml). The mixture was stirred for 3 h at room temperature. After removal of the catalyst by filtration, the filtrate was evaporated. The residue was chromatographed on CHP20P and the product was lyophilized to give 0.88 g (64%) of **9m** as an amorphous powder (Table II).

Compounds **9a**, **k** were prepared in a similar manner (Table II).

(2*S*,3a*S*,7a*S*)-1-(N^2-Phenylacetyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (9c) Phenylacetic acid chloride (5.0 g, 32 mmol) was added to a solution of *N*-hydroxysuccinimide (3.72 g, 32 mmol) and *N*-methylmorpholine (3.27 g, 32 mmol) in CH_2Cl_2 (20 ml). The solution was stirred for 3 h at room temperature and washed successively with saturated $NaHCO_3$, 10% citric acid, and water. The organic layer was dried and evaporated. The residue was recrystallized to give 5.7 g (70%) of the active ester, mp 113—117 °C. By using this active ester, **9c** was prepared by procedure B (Table II).

(2*S*,3a*S*,7a*S*)-1-[N^2-(2-Fluorobenzoyl)-L-lysyl-γ-D-glutamyl]octahydro-1*H*-indole-2-carboxylic Acid (9e) (Procedure C) A solution of 2-fluoro-

benzoic acid (2.53 g, 18 mmol), *N*-hydroxysuccinimide (2.28 g, 19.8 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 3.62 g, 18.9 mmol) in CH_2Cl_2 (50 ml) was stirred overnight at room temperature. The solution was washed successively with saturated $NaHCO_3$, 10% citric acid, and water, then dried, and evaporated. The residue was crystallized from petroleum ether to give 4.0 g (90%) of the active ester, mp 117—121 °C.

The above active ester (0.71 g, 3 mmol) was added to a solution of **8** (1.4 g, 2.5 mmol) and $NaHCO_3$ (0.63 g, 7.5 mmol) in H_2O (15 ml)–THF (30 ml). After being stirred overnight at room temperature, the solution was acidified with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated. The residue was dissolved in 25% HBr–AcOH (20 ml). This solution was stirred for 1 h at room temperature and evaporated. The residue was chromatographed on CHP20P to give 1.0 g (59%) of **9e** as an amorphous powder (Table II).

Compounds **9d**—**j**, **n**—**w** were prepared in a similar manner (Table II).

(2*S*,3a*S*,7a*S*)-1-(N^2-Benzylcarbamoyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (10b) (Procedure D) A solution of **8** (0.56 g, 0.92 mmol) and benzyl isocyanate (0.14 g, 1.05 mmol) in pyridine (5 ml) was stirred overnight at room temperature, then diluted with saturated $NaHCO_3$, and washed with ethyl acetate. The aqueous layer was acidified with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated. The residue was treated in a manner similar to that described in the second paragraph in procedure B to give 0.109 g (18%) of **10b** (Table II).

Compounds **10a**, **c** were prepared in a similar manner (Table II).

(2*S*,3a*S*,7a*S*)-1-(N^6-Nicotinoyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (12) A solution of N^6-*Z*-N^2-Boc-L-lysine *N*-succinimidyl ester (43 g, 90 mmol) in THF (400 ml) was added to a solution of **5** (26.9 g, 90 mmol) and sodium carbonate (14.3 g, 135 mmol) in H_2O (300 ml). The mixture was stirred overnight at room temperature. After evaporation of THF, the solution was acidified with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to give 60 g of the diprotected tripeptide as a crude amorphous powder. An aliquot (10.0 g) of the above tripeptide was treated in a manner similar to that described in the second paragraph in procedure B to give 5.4 g of the Boc tripeptide.

A solution of the above Boc tripeptide (1.0 g) in saturated $NaHCO_3$ (20 ml) was mixed with a solution of **11** (0.5 g, 2.3 mmol) in THF (10 ml). The mixture was stirred overnight at room temperature, acidified with 10% citric acid, and extracted with ethyl acetate. The organic solution was dried and evaporated. The residue was chromatographed on CHP20P to give 0.9 g of an amorphous powder, which was treated in a manner similar to that described in the second paragraph in procedure A to give 0.52 g of **12** (Table III).

(2*S*,3a*S*,7a*S*)-1-(N^6-Dimethyl-N^2-nicotinoyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (14) A solution of **9m** (1.0 g, 1.75 mmol) in formalin (0.5 g) and 90% formic acid (1.0 g) was heated on a boiling water bath for 1 h and then concentrated. The residue was chromatographed on CHP20P to give 0.8 g (80%) of **14** (Table III).

(2*S*,3a*S*,7a*S*)-1-(N^6-*tert*-Butoxycarbonyl-N^2-nicotinoyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (15) A mixture of **6** (3.0 g, 5.4 mmol), **11** (1.5 g, 6.8 mmol), and $NaHCO_3$ (1.5 g, 18 mmol) in H_2O (10 ml)–THF (25 ml) was stirred overnight at room temperature. After evaporation of the THF, the solution was acidified (pH 3—4) with 10% citric acid and extracted with CH_2Cl_2. The organic layer was dried and evaporated. The residue was chromatographed on CHP20P to give 1.4 g (40%) of **15** (Table III).

(2*S*,3a*S*,7a*S*)-1-(N^2,N^6-Dinicotinoyl-L-lysyl-γ-D-glutamyl)octahydro-1*H*-indole-2-carboxylic Acid (16) A solution of **9m** (0.8 g, 1.4 mmol), **11** (0.37 g, 1.7 mmol) and $NaHCO_3$ (0.23 g, 2.8 mmol) in H_2O (5 ml)–THF (10 ml) was stirred overnight at room temperature. The solution was acidified with 10% citric acid and extracted with ethyl acetate. The organic layer was dried and evaporated. The residue was chromatographed on CHP20P to give 0.25 g (28%) of **16** (Table III).

Biological Activity The *in vitro* inhibitory activity was determined by the procedure of Takeyama *et al.*[9] Blood pressure was measured by the same procedure as described previously.[2]

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References and Notes

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