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Synthesis and Angiotensin Converting Enzyme-Inhibitory Activity of *N*-[(1*S*)-1-Carboxy-5-(4-piperidyl)pentyl]-L-alanine Derivatives

Masakuni KORI,*[a] Katsumi ITOH,[a] Yoshiyuki INADA,[a] Takeshi KATOH,[b] Yasuhiro SUMINO,[c] Kohei NISHIKAWA,[a] and Hirosada SUGIHARA[a]

Pharmaceutical Research Laboratories,[a] Production Research Laboratories,[b] Technology Development Laboratories,[c] Takeda Chemical Industries, Ltd., 2–17–85, Jusohonmachi, Yodogawaku, Osaka, Japan.

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As part of a search for potent and long-lasting angiotensin converting enzyme (ACE) inhibitors, various types of *N*-[(1*S*)-1-carboxy-5-(4-piperidyl)pentyl]-L-alanine derivatives (7a, 8—11) were prepared. The key synthetic intermediate, *N*-[(1*S*)-5-(1-benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanine (17a), was synthesized by asymmetric reduction of the α-oxoester (13) with *Lactobacillus paracasei* subsp. *paracasei* followed by a substitution reaction with *tert*-butyl L-alaninate (15) and subsequent treatment with hydrogen chloride. Compounds 7a and 8—11 showed potent and long-lasting ACE-inhibitory activity in rats.

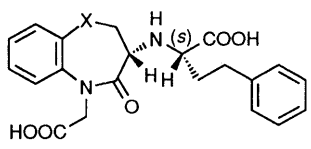
Keywords ACE inhibitor; *N*-[(1*S*)-1-carboxy-5-(4-piperidyl)pentyl]-L-alanine; stereoselective synthesis; activity duration; asymmetric reduction; *Lactobacillus paracasei* subsp. *paracasei*

Since the discovery of captopril[1] and enalapril,[2] a number of analogues have been designed with the aim of obtaining more potent and orally active angiotensin converting enzyme (ACE) inhibitors.[3] In our previous papers,[4] we reported the synthesis of optically active 1,5-benzothiazepine and 1,5-benzoxazepine derivatives (**1**—**4**, Chart 1) and their ACE-inhibitory activities. From the structure–activity relationships of these compounds, replacement of the phenethyl side chain with the ω-(4-piperidyl)alkyl group (**1**, **2**→**3**, **4**) was found to increase markedly the duration of *in vivo* ACE-inhibitory activity. Among the 1-carboxy-ω-(4-piperidyl)alkylamino derivatives (**3**, **4**), compound **5**, which has an (1*S*)-1-carboxy-5-(4-piperidyl)pentyl residue, showed the longest duration of activity *in vivo*.[4c]

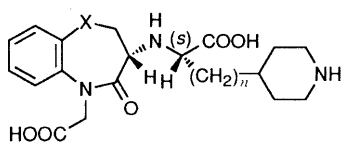
These results prompted us to prepare *N*-[*N*-[(1*S*)-1-carboxy-5-(4-piperidyl)pentyl]-L-alanyl]-*N*-(2-indanyl) glycine (**8**, Chart 2), which is a hybrid compound of **5** and CV-3317-COOH (**6**,[5] Chart 1). Since the biological test[6] results revealed that the indanylglycine derivative (**8**) had inhibitory activity *in vitro*[7] comparable to that of **5**, we synthesized several *N*-[(1*S*)-1-carboxy-5-(4-piperidyl)-pentyl]-L-alanine derivatives **9**—**11** (Chart 2), which contain tetrahydroisoquinoline[8] and 2-azabicyclo[3.3.0]-octane[9] nuclei.[10]

For efficient synthesis of these compounds (**7a**, **8**—**11**), we investigated stereoselective preparation of the key intermediate, *N*-[(1*S*)-5-(1-benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanine (**17a**), and established a route including a chemoenzymatic process using *Lactobacillus paracasei* subsp. *paracasei*. This report describes the synthesis of compounds **7a** and **8**—**11** and their *in vivo* ACE-inhibitory activities.

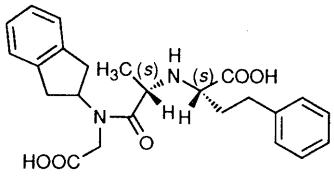
Chemistry Our initial method for the synthesis of 2-indanylglycine derivatives (**7a**, **8**) involved reductive alkylation of the amino ester (**12**)[5] with the α-oxoester **13**[4c] in the presence of sodium cyanoborohydride ($NaBH_3CN$), as illustrated in Chart 3 (method A). This reductive alkylation proceeded without asymmetric induction and gave a diastereomeric mixture (**14a**, **b**) in a ratio of *ca.* 1 : 1. This mixture could be separated by column chromatography on silica gel into the more polar substance **14a** and the less polar substance **14b**. The configuration of the newly formed asymmetric center (C*) of each isomer was predictable from the *Rf* on silica gel thin layer chromatography (TLC, developed with hexane–AcOEt) on the basis of our observation in previous studies on **1**—**6**, that is, the diester derivative of the more active isomer with the (*S*)-configuration at this center (C*) showed a lower *Rf* without exception.[4,5] To ascertain the correctness of our prediction, compounds **14a** and **14b** were deprotected by treatment with a hydrogen bromide–acetic acid solution (HBr–AcOH) to give the monoesters **7a** and **7b**, respectively. In the *in vivo* ACE-inhibitory activity assay[11] in rats using i.v. ad-



1 : X=S
2 : X=O



3 : X=S, *n*=2—6
4 : X=O, *n*=2—6
5 : X=S, *n*=4

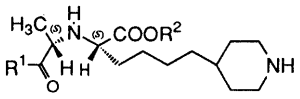


6 : CV-3317-COOH

Chart 1

ministration, **7a** showed more potent activity than **7b**.[12] Therefore, **14a** and **7a** were considered to have the desired (*S*),(*S*)-configurations. Unambiguous chemical assignment of the configurations of **14a** and **7a** was then accomplished. Compound **7a** was hydrolyzed with aqueous sodium hydroxide (NaOH) to give the corresponding diacid **8**.

Next, we investigated stereoselective synthesis of compounds **7a** and **8**. The key intermediate for synthesis was considered to be *N*-[(1*S*)-5-(1-benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanine (**17a**). We initially attempted stereoselective preparation of **17a** by reductive alkylation of *tert*-butyl L-alaninate (**15**) with the α-oxoester **13** (Chart 4). However, asymmetric induction was not observed, and a mixture of two isomers (**16a, b**: *ca.* 1 : 1) was obtained. After chromatographic separation on silica gel, the diesters, **16a** (more polar) and **16b** (less polar), were deprotected to give the monoesters, **17a** and **17b**, respectively, by treatment with a hydrogen chloride–ethyl acetate solution (HCl–AcOEt). The monoesters, **17a** and **17b**, were coupled to *tert*-butyl 2-indanylglycinate **18**[5] to give **14a** (more polar) and **14b** (less polar), respectively (method B). These isomers, **14a** and **14b**, were identical to those prepared by method A. Therefore, the monoester **17a** was considered to have the (*S*),(*S*)-configuration.



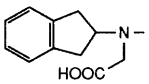
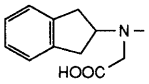
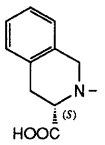
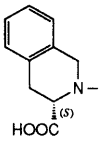
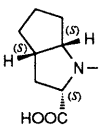
No.	R^1	R^2
7a		C_2H_5
8		H
9		C_2H_5
10		H
11		H

Chart 2

Our second approach for preparing the intermediate **17a** was a route including an *SN*2 reaction of compound **15** and the optically active mesylate **20** (Chart 5). In our previous report[4d] on the synthesis of compound **5**, we described practical synthesis of the (*R*)-α-hydroxyester (**19**), the precursor of the mesylate **20**, by asymmetric reduction of the α-oxoester **13** with baker's yeast and a subsequent *SN*2 reaction of the 3-aminobenzothiazepine derivative (**21**) with the mesylate **20**, affording the diester **22** without racemization.

However, the enantiomeric excess (ee) of **19** upon baker's yeast reduction was *ca.* 60%. Therefore, we searched for other microoganisms which could reduce the α-oxoester **13** to **19** with a higher optical purity. Several enzymes such as lactate dehydrogenase, 2-hydroxy fatty acid dehydrogenase and hydroxyisocaproate dehydrogenase are known to reduce α-oxoacids to α-hydroxyacids.[13] Among them, D-hydroxyisocaproate dehydrogenase seemed to be promising for reduction of the α-oxoester **13** to (*R*)-α-hydroxyester **19** from the viewpoint of substrate specificity.[13b] D-Hydroxyisocaproate dehydrogenase was first isolated from *Lactobacillus paracasei* and subsequently found in certain strains of the genera *Lacto-*

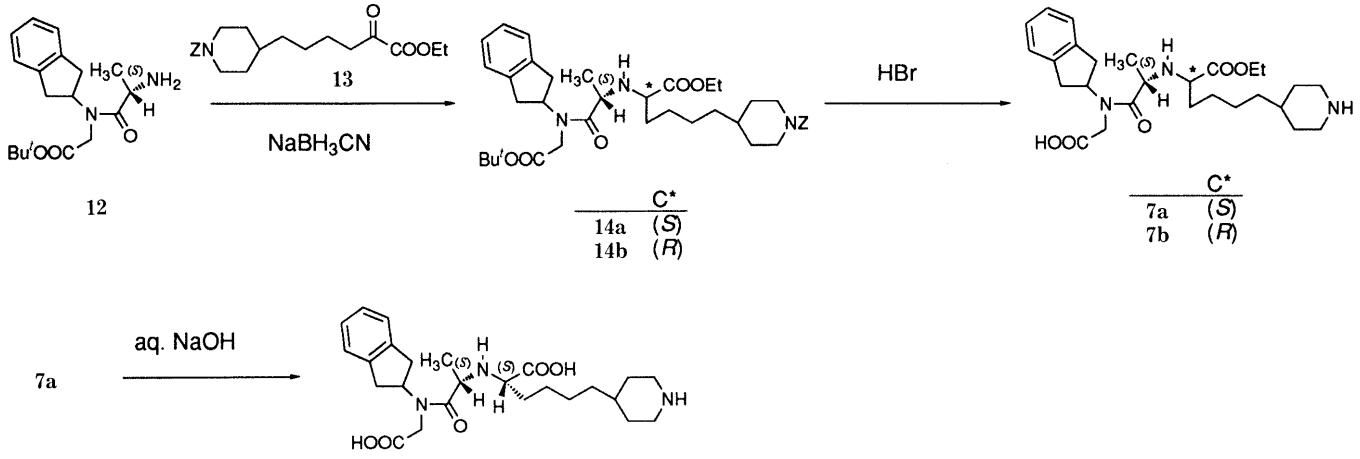
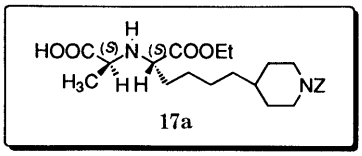
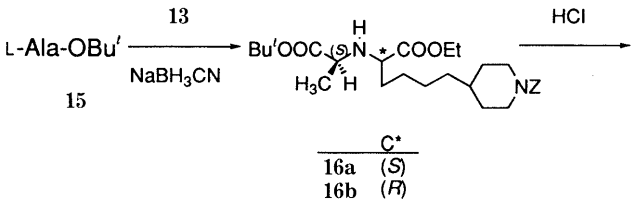


Chart 3



17a



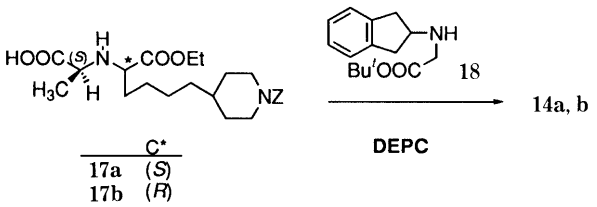


Chart 4

bacillus and *Leuconostoc*,[13b] which are lactic acid bacteria. Thus, we screened a variety of strains of lactic acid bacteria and found that *Lactobacillus paracasei* subsp. *paracasei* could reduce **13** to **19** with high optical purity. The α-oxoester **13** was reduced to (*R*)-α-hydroxyester **19** with a 94% ee.[14,15]

The *SN*-2 reaction of **20** prepared from **19** (94% ee) with **15** afforded the (*S*),(*S*)-diester **16a**, accompanied with a trace amount of the (*S*),(*R*)-diester **16b**, which could be removed by chromatography on a short silica gel column. The diester **16a** was identical to **16a** prepared by reductive alkylation (method B) as described above. From these results, **17a** and **14a** were confirmed unambiguously to have (*S*),(*S*)-configurations.

The (*S*),(*S*)-monoester **17a** was allowed to react with the optically active amino acid esters (**23**,[16] **25**[17]) to yield the diesters (**24**, **26**), which have the favored configurations (Chart 6). Deprotection of the diesters (**24**, **26**) was accomplished by treatment with HBr–AcOH or by catalytic hydrogenolysis and subsequent saponification with aqueous NaOH to obtain the diacids **10** and **11**, respectively. In the case of the synthesis of **10**, the monoester intermediate **9** was isolated.

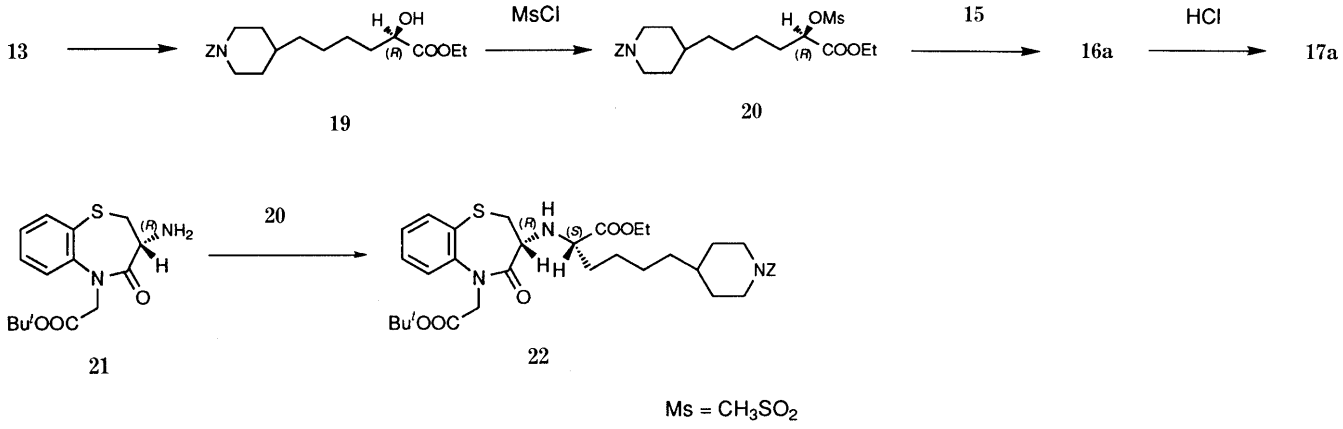


Chart 5

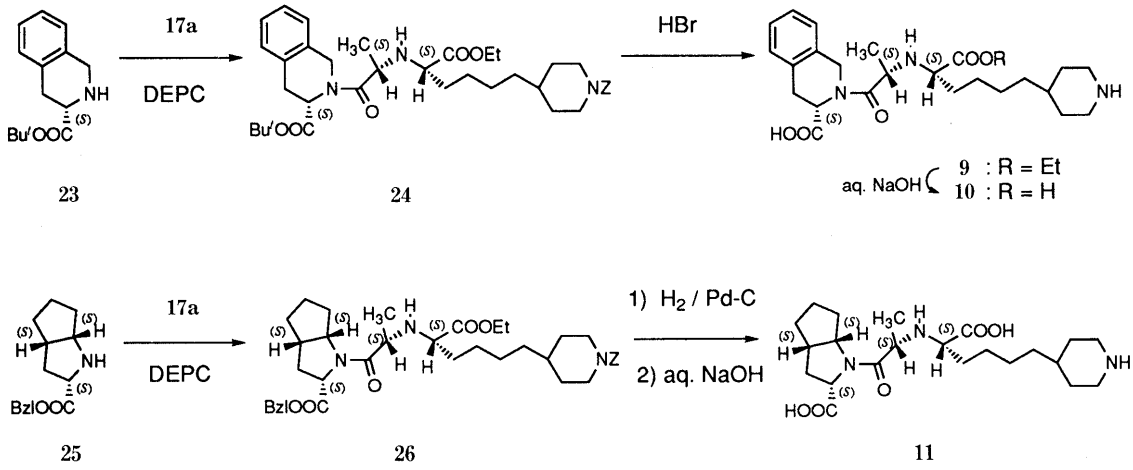


Chart 6

TABLE I. Inhibitory Activities against Angiotensin I-Induced Pressor Response in Rats

No.	Dose (mg/kg)	% inhibition upon *p.o.* administration[a]						
		1/3	1	2	3	5	7	24 (h)
7a	3	21	61	66	72	76	78	63
8	1	2	13	39	52	57	60	43
8	3	83	85	86	89	90	88	80
9	3	58	77	90	86	90	93	79
10	1	5	19	—[b]	50	42	53	36
10	3	80	99	96	100	100	94	91
11	1	3	24	57	54	50	54	49
11	3	55	83	86	91	93	87	85
5	1	1	21	20	43	36	27	14
5	3	70	84	90	91	88	84	43

a) Each value is the average of the results obtained in two or more experiments. *b*) Not determined.

Biological Results

The ACE-inhibitory activities[11] of the derivatives (**7a**, **8**—**11**) upon *p.o.* administration to rats are shown in Table I. All derivatives (**7a**, **8**—**11**) exhibited potent and long-lasting ACE-inhibitory activities, which were comparable to that of compound **5** at doses of 1 and 3 mg/kg. These results indicate that the 1-carboxy-5-(4-piperidyl)pentyl group is effective for both potent ACE-inhibitory activity and long duration of action, even when this group is incorporated into different types of α-amino acids.

The monoesters **7a** and **9** had a somewhat slow onset of action as well as low potency compared with the corresponding diacids **8** and **10**. The monoester **7a** is considered to be hydrolyzed immediately to the diacid **8**, based on the activity after i.v. administration.[12] Therefore, these results are probably due to the relatively low level of absorption of **7a** upon *p.o.* administration. In the case of ACE inhibitors such as enalapril and CV-3317, the monoester form has been shown to be essential for potent *in vivo* activity upon *p.o.* administration because of the low absorbability of the corresponding active-form diacids.[2,5] However, the diacids **8**, **10** and **11** showed potent and long-lasting ACE-inhibitory activity upon *p.o.* administration. Therefore, it is considered unnecessary to convert them to monoester prodrugs.[18]

Experimental

The infrared (IR) spectra were recorded with a Hitachi 260-10 spectrophotometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Varian EM-360, EM-390 and Gemini 200 instruments in the indicated solvents. Chemical shifts are reported as δ-values relative to tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were obtained on a JEOL JMS-01SC mass spectrometer. Secondary ion mass spectra (SIMS) were measured with a Hitachi M-80A spectrometer. The $[\alpha]_D$ values were determined in the indicated solvents on a JASCO DIP 181 4-4822 instrument.

Reactions were run at room temperature unless otherwise noted and followed by TLC on Merck Silica gel F_{254} plates. Standard work-up procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. The organic extract was washed in the indicated order with water, brine, $NaHCO_3$ solution (aqueous $NaHCO_3$), and H_3PO_4 solution (aqueous H_3PO_4), then dried over $MgSO_4$, filtered and evaporated *in vacuo*. Chromatographic separation was done on Merck Silica gel 60 using the indicated eluents.

Asymmetric Reduction of the α-Oxoester (13) with *Lactobacillus paracasei* subsp. *paracasei* A loopful of *L. paracasei* subsp. *paracasei* IFO 12004 cells which had been grown by stab culture on GAM agar medium (Nippon Pharmaceutical Co.) was inoculated into a 200 ml Erlenmeyer flask containing 20 ml of the following seed medium: glucose 2%, meat extract (Erlich) 1%, Polypepton 1%, yeast extract 0.5%, K_2HPO_4 0.2%, Tween 80 0.1%, CH_3COONa 0.5%, ammonium citrate tribasic 0.2%, $MgSO_4 \cdot 7H_2O$ 0.02% and $MnSO_4 \cdot ca.\ 4H_2O$ 0.05%. The cultivation was carried out at 37 °C for 24 h without shaking. Three ml of this culture broth was transferred into each of a series of baffled flasks (200 ml × 83) containing 60 ml of the same seed medium. After incubation for 22 h at 37 °C without shaking, ethyl 6-(1-benzyloxycarbonyl-4-piperidyl)-2-oxohexanoate (**13**[4c]: purity 68.2%) 1%, glucose 3% and $CaCO_3$ 2% were added to the medium and incubation was carried out for 96 h on a rotary shaker (190 rpm). The resulting mixture was extracted with AcOEt (4, 3, 2 l). The organic layers were combined, washed with water, dried over $MgSO_4$ and concentrated *in vacuo* to give the crude product A (50.1 g). The product was dissolved in AcOEt and extracted with aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give ethyl (2*R*)-6-(1-benzyloxycarbonyl-4-piperidyl)-2-hydroxyhexanoate **19** (product B, 23.5 g, 44.2% purity, 29% calculated yield) with 94% ee. The aqueous $NaHCO_3$ layer was acidified with concentrated HCl and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. A mixture of the residue, *p*-TsOH· H_2O (3 g) and EtOH (100 ml) was refluxed for 8 h. The resulting mixture was concentrated *in vacuo* and worked up (AcOEt, aq. $NaHCO_3$) to give **19** (product C, 23.3 g, 81% purity, 54% calculated yield based on **13**) with 50% ee.

Ethyl (2*R*)-6-(1-Benzyloxycarbonyl-4-piperidyl)-2-methanesulfonyloxyhexanoate (20) The crude product B (5 g) obtained above was purified by silica gel column chromatography (hexane : AcOEt = 2 : 1) to give pure **19** (1.8 g) as a colorless oil, $[\alpha]_D^{24}$ −0.85° (*c* = 1.09, MeOH). The (*R*)-α-hydroxyester **19** was allowed to react with methanesulfonyl chloride according to the method described previously[4c] to give **20** (2.1 g) in a 98% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, *J* = 7 Hz, CH_3), 1.0—2.1 (13H, m), 2.4—3.1 (2H, m), 3.15 (3H, s, CH_3SO_2), 4.25 (2H, q, *J* = 7 Hz, OCH_3), 3.9—4.4 (2H, m), 5.01 (1H, t, *J* = 6 Hz, CHOMs), 5.12 (2H, s, CH_2COO), 7.35 (5H, s, Ph).

***tert*-Butyl *N*-[(1*S*)-5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alaninate (16a) and *tert*-Butyl *N*-[(1*R*)-5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alaninate (16b)** A mixture of **15**·$(COOH)_2$ (5 g), **13**[4c] (11 g), AcONa (1.6 g), AcOH (1.2 g) and EtOH (100 ml) was stirred for 1 h. A solution of $NaBH_3CN$ (1.9 g) in EtOH (100 ml) was added dropwise over a period of 4 h to the mixture. After being stirred overnight, the mixture was worked up (AcOEt, water). The residue was purified by silica gel column chromatography (hexane : acetone = 4 : 1) to give firstly **16b** (1.7 g, 17%) as a colorless oil. IR ν_{max}^{neat} cm^{-1}: 3350 (NH), 1730, 1700 (C=O). MS *m/z*: 504 (M^+). $[\alpha]_D^{22}$ −11.4° (*c* = 0.51, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s, *tert*-Bu), 1.0—2.0 (15H, m), 2.6—2.9 (3H, m), 3.1—3.3 (3H, m), 4.0—4.3 (6H, m), 5.13 (2H, s, C$\underline{H}_2$Ph), 7.36 (5H, s, Ph). The second fraction afforded **16a** (1.5 g, 15%) as a colorless oil. IR ν_{max}^{neat} cm^{-1}: 3350 (NH); 1730, 1700 (C=O). MS *m/z*: 504 (M^+). $[\alpha]_D^{22}$ −18.8° (*c* = 0.68, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s, *tert*-Bu), 1.0—1.8 (15H, m), 2.6—2.9 (3H, m), 3.1—3.3 (3H, m), 4.0—4.3 (6H, m), 5.12 (2H, s, C$\underline{H}_2$Ph), 7.35 (5H, s, Ph).

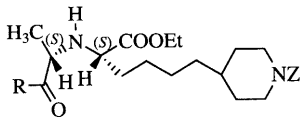
A mixture of **15** (0.48 g) and **20** (0.6 g) prepared from **19** (94.4% ee) was heated for 24 h at 80 °C. The resulting mixture was worked up (AcOEt, water) and purified by silica gel column chromatography (hexane : AcOEt = 3 : 1) to give **16a** (0.50 g, 75%) as a colorless oil, which was identical with **16a** prepared by reductive alkylation described above.

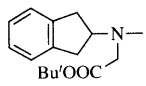
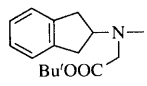
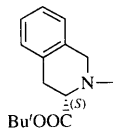
***N*-[(1*S*)-5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanine (17a) and *N*-[(1*R*)-5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanine (17b)** A solution of **16b** (1.7 g) in 5 N HCl/AcOEt (20 ml) was allowed to stand for 4 h. Et_2O (100 ml) was added to the mixture to precipitate **17b**·HCl (1.2 g, 71%). MS *m/z*: 448 (M^+). $[\alpha]_D^{24}$ −9.9° (*c* = 0.5, MeOH). *Anal.* Calcd for $C_{24}H_{36}N_2O_6 \cdot HCl \cdot H_2O$: C, 57.31; H, 7.81; N, 5.57. Found: C, 56.91; H, 7.85; N, 5.92. ^{1}H-NMR (DMSO-d_6–D_2O) δ: 0.8—2.2 (15H, m), 2.6—3.5 (6H, m), 3.8—4.5 (6H, m), 5.15 (2H, s, C$\underline{H}_2$Ph), 7.47 (5H, s, Ph).

Compound **17a**·HCl (1.15 g, 77%) was prepared similarly from **16a**. MS *m/z*: 448 (M^+). $[\alpha]_D^{24}$ +12.7° (*c* = 0.5, MeOH). *Anal.* Calcd for $C_{24}H_{36}N_2O_6 \cdot HCl \cdot H_2O$: C, 57.31; H, 7.81; N, 5.57. Found: C, 57.19; H, 8.06; N, 5.61. ^{1}H-NMR (DMSO-d_6–D_2O) δ: 0.8—2.0 (15H, m), 2.5—3.4 (6H, m), 3.8—4.4 (6H, m), 5.06 (2H, s, C$\underline{H}_2$Ph), 7.37 (5H, s, Ph).

***tert*-Butyl *N*-[*N*-[(1*S*)-5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanyl]-*N*-(2-indanyl)glycinate (14a, Table II) and**

TABLE II. *N*-[5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanine Derivatives



No.	R	Config. C*	Yield (%)	MS M^+ (*m/z*)	IR ν_{max}^{neat} cm^{-1} NH	IR ν_{max}^{neat} cm^{-1} C=O
14a		*S*	12,[a] 51[b]	677	3320	1760, 1690, 1640
14b		*R*	11,[a] 44[b]	677	3320	1730, 1690, 1650
24		*S*	99[b]	663	—	1730, 1690, 1640
26		*S*	39[b]	675	—	1740, 1690, 1640

a) Method A. *b*) Method B.

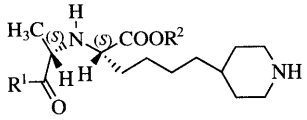
***tert*-Butyl *N*-[*N*-[(1*R*)-5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanyl]-*N*-(2-indanyl)glycinate (14b, Table II)** Method A: A solution of $NaBH_3CN$ (0.6 g) in EtOH (50 ml) was added dropwise to a stirred mixture of **12**·$(COOH)_2$[5] (2 g), AcONa (0.78 g), AcOH (0.58 g), **13** (3.6 g), molecular sieves 3A (10 g) and EtOH (50 ml) over a period of 3 h. The mixture was allowed to stand overnight, then concentrated *in vacuo* and worked up (AcOEt). The residue was purified by silica gel column chromatography (hexane : AcOEt = 1 : 1—2 : 3). The (*S*),(*R*)-isomer **14b** (0.35 g, 11%) was obtained as a colorless oil from the first fraction. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s, *tert*-Bu), 0.8—2.0 (18H, m), 2.3—3.6 (9H, m), 3.6—5.1 (7H, m), 5.13 (2H, s, $C\underline{H}_2Ph$), 7.1—7.4 (9H, m, aromatic). From the second fraction, the (*S*),(*S*)-isomer **14a** (0.4 g, 12%) was obtained as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s, *tert*-Bu), 0.9—1.9 (18H, m), 2.3—3.4 (9H, m), 3.6—4.4 (7H, m), 5.13 (2H, s, $C\underline{H}_2Ph$), 7.1—7.5 (9H, m, aromatic).

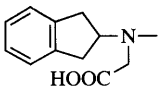
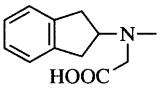
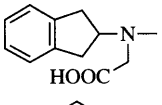
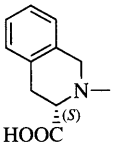
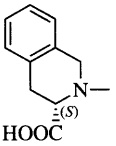
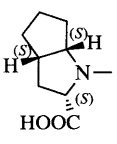
Method B: Diethyl phosphorocyanidate (DEPC) (0.1 ml) was added to an ice-cooled solution of **18**[5] (25 mg) and **17a**·HCl (50 mg) in dimethylformamide (DMF) (2 ml) and the mixure was stirred for 30 min. Et_3N (0.1 ml) was added at ice-bath temperature and the resulting mixture was stirred at 0 °C for 30 min and for a further 30 min at room temperature. Work-up (AcOEt) and purification of the residue by silica gel column chromatography (hexane : AcOEt = 1 : 1) gave **14a** (35 mg, 51%) as a colorless oil.

Compound **14b** was prepared similarly. These compounds **14a, b** were identical with **14a, b**, respectively, prepared by method A.

***tert*-Butyl (3*S*)-2-[*N*-[(1*S*)-5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (24, Table II)** DEPC (0.4 ml) was added to an ice-cooled solution of **23**·HCl[16] (0.4 g) and **17a**·HCl (0.4 g) in DMF (20 ml) and the mixture was stirred for 30 min. Et_3N (0.4 ml) was added at ice

TABLE III. *N*-[1-Carboxy-5-(4-piperidyl)pentyl]-L-alanine Derivatives



No.	R^1	R^2	Config. C*	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	$[\alpha]_D$ (°) Solvent	*c* Temp. (°C)	SIMS *m/z*
7a		Et	*S*	72	$C_{27}H_{41}N_3O_5$ ·2HBr·3H_2O	46.09 (46.19	7.01 6.95	5.97 5.91)	+2.6 MeOH	0.51 (22)	488 (MH^+)
7b		Et	*R*	81	$C_{27}H_{41}N_3O_5$ ·2HBr·4H_2O	44.94 (45.33	7.13 6.97	5.82 5.72)	−7.9 MeOH	0.79 (22)	488 (MH^+)
8		H	*S*	62	$C_{25}H_{37}N_3O_5$ ·2H_2O	60.59 (60.37	8.34 7.99	8.48 8.70)	+6.1 H_2O	0.38 (24)	460 (MH^+)
9		Et	*S*	99	$C_{26}H_{39}N_3O_5$ ·2HBr·H_2O	47.79 (48.00	6.63 6.42	6.43 6.39)	+5.8 MeOH	0.38 (25)	474 (MH^+)
10		H	*S*	68	$C_{24}H_{35}N_3O_5$ ·CH_3CN·3H_2O	58.30 (58.33	8.31 7.88	9.52 9.52)	−7.3 H_2O	0.37 (24)	446 (MH^+)
11		H	*S*	87	$C_{22}H_{37}N_3O_5$ ·3/2H_2O	58.65 (58.46	8.95 8.80	9.33 9.35)	—[a]		424 (MH^+)

a) Not measured.

bath temperature and the resulting mixture was stirred at 0 °C for 1 h and for a further 30 min at room temperature. Work-up (AcOEt, 5% H_3PO_4, water) and purification of the residue by silica gel column chromatography (hexane : acetone = 2 : 1) gave **24** (0.37 g, 99%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (9H, s, *tert*-Bu), 0.8—2.3 (21H, m), 2.3—3.3 (4H, m), 3.3—4.8 (7H, m), 5.07 (2H, s, $C\underline{H}_2Ph$), 7.0—7.3 (9H, m, aromatic).

Benzyl (1*S*,3*S*,5*S*)-2-[*N*-[(1*S*)-5-(1-Benzyloxycarbonyl-4-piperidyl)-1-ethoxycarbonylpentyl]-L-alanyl]-2-azabicyclo[3.3.0]octane-3-carboxylate (26, Table II) Compound **26** was prepared by the same method from benzyl (1*S*,3*S*,5*S*)-2-azabicyclo[3.3.0]octane-3-carboxylate **25**[17] and **17a**·HCl.

***N*-[*N*-[(1*S*)-1-Ethoxycarbonyl-5-(4-piperidyl)pentyl]-L-alanyl]-*N*-(2-indanyl)glycine (7a, Table III) and *N*-[*N*-[(1*R*)-1-Ethoxycarbonyl-5-(4-piperidyl)pentyl]-L-alanyl]-*N*-(2-indanyl)glycine (7b, Table III)** A 30% HBr–AcOH solution (2 ml) was added to a solution of **14a** (0.4 g) in AcOH (2 ml). The resulting solution was allowed to stand for 1 h and then diluted with Et_2O (50 ml). The deposited precipitate was collected by filtration, washed with Et_2O and dried *in vacuo* to give **7a**·2HBr (0.3 g, 72%) as a white powder. ^{1}H-NMR (DMSO-d_6–D_2O) δ: 1.0—2.1 (18H, m), 2.6—3.4 (9H, m), 3.7—5.1 (7H, m), 7.0—7.4 (4H, m, aromatic).

Compound **7b**·2HBr was prepared similarly from **14b**. ^{1}H-NMR (DMSO-d_6–D_2O) δ: 1.0—2.1 (18H, m), 2.6—3.4 (9H, m), 3.7—5.1 (7H, m), 7.0—7.4 (4H, m, arommatic).

(3*S*)-2-[*N*-[(1*S*)-1-Ethoxycarbonyl-5-(4-piperidyl)pentyl]-L-alanyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (9, Table III) Compound **9**·2HBr was prepared from **24** in a manner similar to that described for the synthesis of **7a**. ^{1}H-NMR (DMSO-d_6–D_2O) δ: 1.0—2.2 (22H, m), 2.6—3.5 (4H, m), 3.6—5.3 (7H, m), 7.2—7.4 (4H, m, aromatic).

***N*-[*N*-[(1*S*)-1-Carboxy-5-(4-piperidyl)pentyl]-L-alanyl]-*N*-(2-indanyl)glycine (8, Table III)** A mixture of **7a**·2HBr (0.25 g) and 1 N NaOH (6 ml) was allowed to stand for 30 min, then neutralized with AcOH. The solution was submitted to XAD-2 column chromatography (0.1 M NH_4OH–5% CH_3CN). The eluent was concentrated *in vacuo* and lyophilized to give **8** (0.11 g, 62%) as a white powder. ^{1}H-NMR (DMSO-d_6–D_2O) δ: 1.0—2.0 (15H, m), 2.6—3.8 (11H, m), 4.9—5.4 (2H, m), 7.0—7.3 (4H, m).

(3*S*)-2-[*N*-[(1*S*)-1-Carboxy-5-(4-piperidyl)pentyl]-L-alanyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (10, Table III) Compound **10** was prepared from **9** in a manner similar to that described for the synthesis of **8**. ^{1}H-NMR (DMSO-d_6–D_2O) δ: 1.80 (3H, s, CH_3CN), 1.0—2.2 (19H, m), 2.5—3.5 (4H, m), 3.6—5.0 (5H, m), 7.1—7.3 (4H, m, aromatic).

(1*S*,3*S*,5*S*)-2-[*N*-[(1*S*)-1-Carboxy-5-(4-piperidyl)pentyl]-L-alanyl]-2-azabicylco[3.3.0]octane-3-carboxylic Acid (11, Table III) A solution of **26** (0.26 g) in EtOH (50 ml) was hydrogenated over 10% Pd–C (50% wet, 0.2 g) under atmospheric pressure. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give (1*S*,3*S*,5*S*)-2-[*N*-[(1*S*)-1-ethoxycarbonyl-5-(4-piperidyl)pentyl]-L-alanyl]-2-azabicyclo[3.3.0]octane-3-carboxylic acid as an oil. A solution of this compound in 1 N NaOH (5 ml) was allowed to stand for 1 h, and then neutralized with AcOH. The solution was submitted to XAD-2 column chromatography (0.15 M NH_4OH–5% CH_3CN). The eluent was concentrated *in vacuo* and lypophilized to give **11** (0.15 g, 87%) as a white powder.

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References and Notes

1) D. W. Cushman, H. S. Cheung, E. F. Sabo, M. A. Ondetti, *Biochemistry*, **16**, 5484 (1977).
2) A. A. Patchett, E. Harris, E. W. Tristam, M. J. Wu, D. Taub, E. R. Peterson, T. J. Ikeler, J. ten Broeke, L. G. Payne, D. L. Ondeyka, E. D. Throsett, W. J. Maycock, F. M. Robinson, R. Hirschman, C. S. Sweet, E. H. Ulm, D. M. Gross, T. C. Vassil, C. A. Stone, *Nature* (London), **288**, 280 (1980).
3) *a*) G. Lawton, P. M. Paciorek, J. F. Waterfall, "Advances in Drug Research," ed. by B. Testa, Academic Press, London, 1992, pp. 162—220; *b*) M. A. Ondetti, *Circulation*, **77**, I-74 (1988); *c*) M. J. Wyvratt, A. A. Patchett, *Med. Res. Rev.*, **5**, 483 (1985); *d*) E. W. Petrillo, Jr., M. A. Ondetti, *ibid.*, **2**, 1 (1982).
4) *a*) K. Itoh, M. Kori, Y. Inada, K. Nishikawa, Y. Kawamatsu, H. Sugihara, *Chem. Pharm. Bull.*, **34**, 1128 (1986); *b*) *Idem*, *ibid.*, **34**, 2078 (1986); *c*) *Idem*, *ibid.*, **34**, 3747 (1986); *d*) M. Kori, K. Itoh, H. Sugihara, *ibid.*, **35**, 2319 (1987).
5) A. Miyake, K. Itoh, Y. Oka, *Chem. Pharm. Bull.*, **34**, 2852 (1986). The monoethyl ester of this compound was designated delapril.
6) The ACE-inhibitory activity *in vitro* was measured by the method reported by Cushman *et al.* with a slight modification.[4a] D. W. Cushman, H. S. Cheung, *Biochem. Pharmacol.*, **20**, 1637 (1971).
7) The ACE-inhibitory activities *in vitro* of **5** and **8** were as follows: **5**: 52% (10^{-8} M), 98% (10^{-7} M). **8**: 39% (10^{-9} M), 83% (10^{-8} M), 99% (10^{-7} M).
8) The monoester of the phenethyl derivative, (3*S*)-2-[*N*-[(1*S*)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, was designated quinapril. *a*) H. R. Kaplan, D. M. Cohen, A. D. Essenburg, T. C. Major, T. E. Mertz, M. J. Ryan, *Fed. Proc.*, **43**, 1326 (1984); *b*) M. J. Ryan, D. M. Boucher, D. M. Cohen, B. J. Plszewski, R. M. Singer, R. D. Smith, H. R. Kaplan, *Pharmacologist*, **24**, Abst., 446 (1982); *c*) D. M. Cohen, A. D. Essenburg, R. M. Singer, M. J. Ryan, D. B. Evans, H. R. Kaplan, *ibid.*, **26**, Abst., 266 (1984).
9) The monoester of the phenethyl derivative, (1*S*,3*S*,5*S*)-2-[*N*-[(1*S*)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-2-azabicyclo[3.3.0]-octane-3-carboxylic acid, was designated ramipril. V. Teetz, R. Geiger, H. Gaul, *Arzneim-Forsch./Drug Res.*, **34**, 1386, 1399, 1402 (1984).
10) Quinapril and ramipril are ACE inhibitors of a relatively new type which contain a fused bicyclic amino acid moiety. We chose these nuclei because they seemed to be somewhat more hydrophobic than proline in enalapril. The important role of the hydrophobic character of this amino acid moiety was discussed in connection with our design of delapril.[5]
11) The ACE-inhibitory acitivity *in vivo* was assessed in terms of inhibition (percentage) of the vasopressor response induced by i.v. administration of angiotensin I in conscious rats. Test compounds were administered i.v. or orally (*p.o.*). The method is described in our previous report.[4a]
12) Percentage inhibition at a dose of 0.3 mg/kg i.v. was as follows. **7a**: 100% (6 min), 100% (30 min), 100% (1 h), 98% (2 h). **7b**: 73% (6 min), 68% (30 min), 10% (1 h), 3% (2 h).
13) *a*) H. Schüte, W. Hummel, M.-R. Kula, *Appl. Microbial Biotechnol.*, **19**, 167 (1984); *b*) W. Hummel, H. Schüte, M.-R. Kula, *Appl. Microbial Biotechnol.*, **21**, 7 (1985).
14) The enantiomeric excess (% ee) of **19** was determined by high-performance liquid chromatography (HPLC) after conversion to the corresponding (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester. The method is described in our previous report.[4d]
15) In the course of reduction, a significant amount of the corresponding α-hydroxy acid was obtained. Esterification of this acid gave the (*R*)-α-hydroxyester **19** in a 54% yield (based on **13**) with a 50% ee.
16) S. Klutchko, C. J. Blankley, R. W. Fleming, J. M. Hinkley, A. E. Werner, I. Nordin, A. Holmes, M. L. Hoefle, D. M. Cohen, A. D. Essenburg, H. R. Kaplan, *J. Med. Chem.*, **29**, 1953 (1986).
17) V. Teetz, R. Geiger, H. Gaul, *Tetrahedron Lett.*, **25**, 4479 (1984).
18) The same tendency was observed with compound **5**.