A Practical and Diastereoselective Synthesis of Angiotensin Converting Enzyme Inhibitors

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The diastereoselective synthesis of a series of potent angiotensin converting enzyme (ACE) inhibitors is described. The optically active intermediate, *N*-carbamyl (*R*)-2-amino-4-phenylbutyric acid (2) leading to (*R*)-2-halogeno or (*R*)-2-hydroxy-4-phenylbutyric acid was prepared by asymmetric hydrolysis of DL-5-phenethylhydantoin (1) by microbial hydantoinase. The halogenoester was subjected to S_N2 reaction with L-amino acid derivatives to afford N-substituted amino acids, which were easily converted to ACE inhibitors or intermediates by deprotection.

Keywords ACE inhibitor; enalapril; lisinopril; enzymatic hydrolysis; hydantoinase; *N*-carbamyl (*R*)-2-amino-4-phenylbutyric acid; (*R*)-2-halogeno-4-phenylbutyric acid

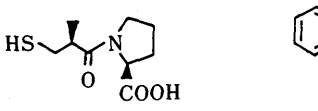
Current attention in the renin–angiotensin system has been focused on the pharmacological use of enzyme inhibitor for this system to control clinical hypertension. Angiotensin-converting enzyme (ACE) inhibitors which intervene in the conversion of the precursor decapeptide angiotensin I to the powerful vasoconstrictor substance angiotensin II have been demonstrated to be potent antihypertension drugs. In 1977, Ondetti and his co-workers showed the first orally active ACE inhibitor, captopril, to be effective in the treatment of hypertension.[2] More recently, Patchett and his co-workers have developed a new class of potent inhibitors of this enzyme, that is, enalapril (MK 421), enalaprilat (MK 422),[3] and its lysine analogue (MK 521, lisinopril),[4] which are non-sulfhydyl inhibitors and have higher inhibitory potency than captopril (Fig. 1). Hitherto, these novel ACE inhibitors have been prepared by the reductive amination of 2-oxo-4-phenylbutyric acid or its ester with suitably protected dipeptides in the presence of sodium cyanoborohydride or by utilizing palladium-on-carbon. However, in these synthetic methods, excess α-keto acid or ester was required since the reaction proceeded with the simultaneous formation of the α-hydroxy ester as a side product. Furthermore, this reductive amination usually results in the formation of a diastereomeric mixture (*S,S,S* and *R,S,S*) in a ratio of *ca.* 1 : 1. Based on structure–activity relationships, it has been confirmed that in these ACE inhibitors, the (*S,S,S*) diastereoisomer is the biologically active one.[3a] Thus, by the above-mentioned method, the desired diastereomeric product should be separated and purified at the final stage. As another method, the substitution reaction of a racemic α-halogeno ester with a suitable dipeptide has been employed for preparation of similar ACE inhibitors.[5] In this case again, however, the resulting diastereomixture should be separated at the final stage.

Now, we wish to report a more efficient and stereoselective route to ACE inhibitors which possess the desired stereochemistry. If the optically active α-halogeno esters could be prepared, they would react with the amino terminus of protected dipeptides by an S_N2 process to give the desired diastereoisomer exclusively. In this work, enalapril and lisinopril were taken as representative ACE inhibitors.

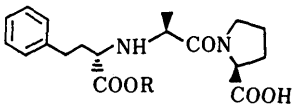
As the first step leading to an optically active α-halogeno ester, we examined an enzymatic hydrolysis of suitably substituted hydantoin[6] followed by halogenation as described below.

In generally, hydantoinase is well known to catalyze the hydrolytic reaction of various 5-substituted hydantoins to afford the corresponding D-isomers of *N*-carbamyl α-amino acids exclusively.[7] This fact prompted us to examine the asymmetric hydrolysis of DL-5-phenethyl hydantoin leading to *N*-carbamyl (*R*)-2-amino-4-phenylbutyric acid (**2**). The hydrolysis was carried out with vigorous mixing of the substrate and the acetone-dried cells of *Pseudomonas putida* (IFO 12996) in an alkaline medium (pH 9.0) at 37 °C for 2 d to give **2** in high yield. After conversion to the corresponding methyl ester with diazomethane, the optical purity of **2** was determined to be >95% by 400 MHz nuclear magnetic resonance (NMR) analysis using a chiral shift reagent [Eu(HFC)$_3$].

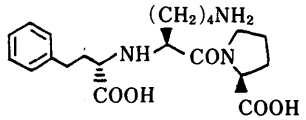
Next, transformation of **2** into (*R*)-2-halogeno-4-phenylbutyric acid was done with $NaNO_2$ and halogenating reagents (KBr or KCl) in aqueous acidic medium. In this transformation step, the reaction proceeded with retention



captopril (SQ 14225)



enalapril (MK 421) : R=Et
enalaprilat (MK 422) : R=H



lisinopril (MK 521)

Fig. 1

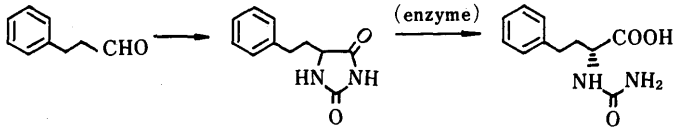


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Chart 1

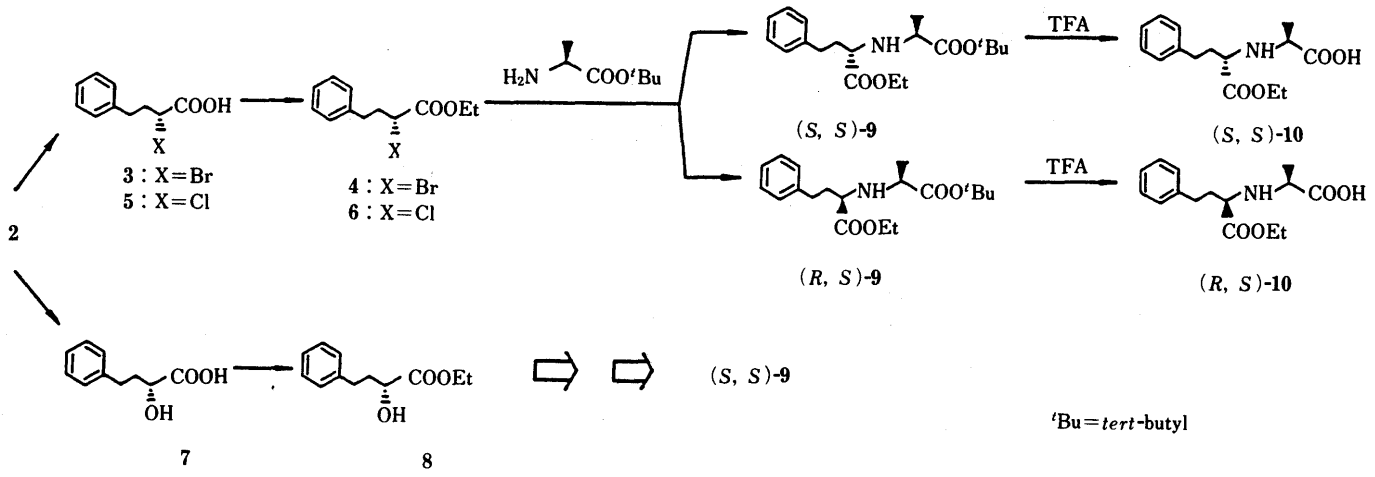
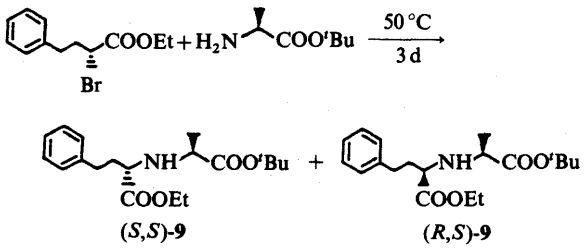


Chart 2

TABLE I. The Reaction[a] of (*R*)-Ethyl 2-Bromo-4-phenylbutyrate with L-Alanine *tert*-Butylester



Run	Solvent	Base	Total yield (%)	Ratio[c] (*S,S*:*R,S*)
1	CH_3CN	sat. $NaHCO_3$	59	2.9:1
2	CH_3CN	sat. K_2CO_3	71	4.2:1
3	CH_3CN	$(NH_4)_2CO_3$	24	7.5:1
4	CH_3NO_2	sat. K_2CO_3	63	4.2:1
5	CH_3NO_2:H_2O (4:1)	$(NH_4)_2CO_3$	79	7.5:1
6	CH_3NO_2:H_2O (1:4)	$(NH_4)_2CO_3$	88	8.5:1
7	CH_3OH	$(NH_4)_2CO_3$	33	14:1
8	CH_2OH CH_2OH	$(NH_4)_2CO_3$	17	30:1
9[b]	CH_3NO_2:H_2O (1:4)	$(NH_4)_2CO_3$	70	15:1
10	DMSO:H_2O (1:4)	$(NH_4)_2CO_3$	33	9.2:1
11	DMF:H_2O (1:4)	$(NH_4)_2CO_3$	38	4.3:1
12	HMPA:H_2O (1:4)	$(NH_4)_2CO_3$	46	3.4:1

a) Four equivalents of amino ester was employed with respect to α-bromo-ester. *b*) The reaction was carried out at 70 °C. *c*) After isolation of each isomer by silica gel column chromatography, the ratio was determined.

of the configuration. Furthermore, when the reaction was carried out without halogenating reagents under the same conditions, (*R*)-2-hydroxy-4-phenylbutyric acid (**7**) was obtained in good yield. These optically active halogeno and hydroxy acids were converted smoothly to the corresponding esters in a usual manner (*i.e.* $SOCl_2$–EtOH system) (Chart 2).

Usually, **7** has been prepared by resolution of the racemic α-hydroxy acid using optically active amines or after esterification with an optically active alcohol.[8] Urbach and Henning[9] have reported a convenient method to prepare N-substituted amino acids utilizing the triflate of the hydroxy ester.[10]

In a preliminary investigation to prepare the desired (*S,S,S*) diastereoisomer of ACE inhibitors, the reaction of (*R*)-ethyl 2-bromo-4-phenylbutyrate (**4**) with L-alanine *tert*-butylester was examined in various solvent–base systems. These results are summarized in Table I.

As shown in Table I, when the reaction was carried out in the presence of $NaHCO_3$ or K_2CO_3, it proceeded with the racemization of **4** to give a low selectivity (runs 1, 2 and 4). However, further investigations showed that the racemization was suppressed efficiently by employment of a weak base, that is, $(NH_4)_2\,CO_3$ (run 3). In order to obtain further confirmation, a series of reactions using $(NH_4)_2CO_3$ as the base was examined in various solvent systems. Thus, in the case of protic solvents, such as MeOH or ethyleneglycol, the desired diastereoisomer (*S,S*)-**9** was obtained predominantly, but in low yield (runs 7 and 8). Furthermore, it was found that use of aprotic solvents, such as dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and hexamethylphosphoramide (HMPA), was not effective for this reaction (runs 10, 11 and 12). Among the conditions so far examined, excellent results were obtained only in the case of the CH_3NO_2–H_2O system (runs 5, 6 and 9). The utilization of mixed solvent (CH_3NO_2 : H_2O = 1 : 4) turned out to be favorable for this S_N2 reaction.

Finally, as an extension of this reaction, we intended to apply it to the synthesis of representative ACE inhibitors, such as enalapril, enalaprilat, and lisinopril. According to the above preliminary investigations, the reaction of **4** with L-alanyl-L-proline *tert*-butylester under the same conditions was examined. It was found that the reaction proceeded in a diastereoselective fashion to give the desired (*S,S,S*) diastereoisomer exclusively with a ratio of 9.8:1 in 81.5% yield. The desired diastereoisomer (*S,S,S*)-**11** was converted smoothly to enalaprilat (MK 422) after stepwise removal of protecting groups with trifluoroacetic acid (TFA) and 1 N NaOH. The similar treatment of **4** with *N*-(N^6-*tert*-butoxycarbonyl-L-lysyl)-L-proline *tert*-butylester also gave predominantly one isomer, (*S,S,S*)-**12**, in 70% yield. This (*S,S,S*) isomer was also converted to lisinopril (MK

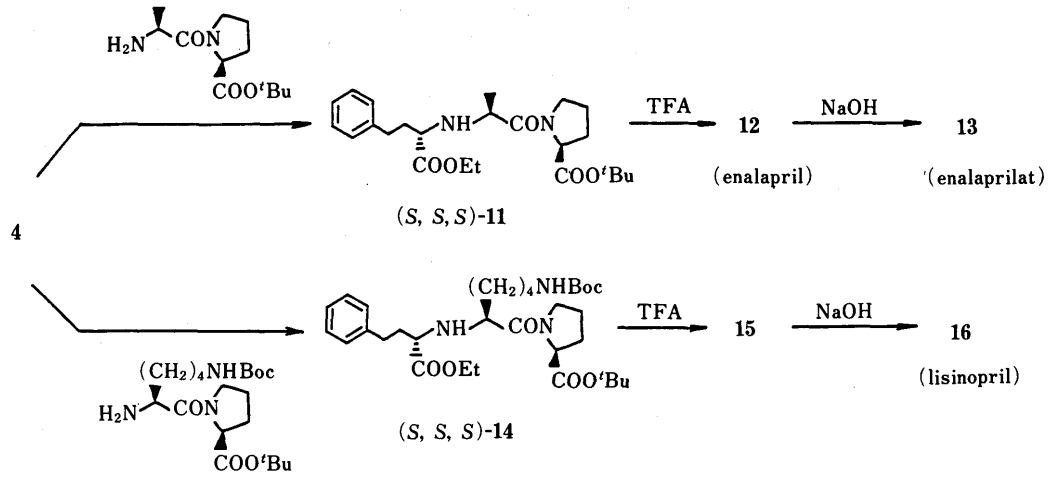


Chart 3

521) after similar successive deprotection (Chart 3).

In conclusion, we have established a practical and efficient route for the stereoselective synthesis of a variety of ACE inhibitors. In this method using optically active α-halogenoester, the final coupling reaction proceeds in a highly diastereoselective fashion and the diastereomeric mixture thus obtained can be separated easily.

Experimental

All melting points were determined on a Yanagimoto micromelting apparatus and are uncorrected. The infared (IR) spectra were recorded on a JASCO A 202 diffraction grating infrared spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian EM-390 NMR spectrometer or a Hitachi R-90H Fourier-transform NMR spectrometer or a Bruker AN-400 spectrometer with tetramethylsilane as an internal standard. Low-resolution mass spectra (MS) were obtained with a Hitachi RMU-6MG mass spectrometer. The $[\alpha]_D$ values were determined in the indicated solvents on a Horiba SEPA-200 high-sensitivity polarimeter. Chromatographic separation was done on Wako gel C-200 or C-300. Preparative thin layer chromatography (TLC) was carried out on precoated plates of Merck silicagel 60 F_{254}.

DL-5-Phenethyl Hydantoin (1) A solution of β-phenylpropionaldehyde (30.8 g, 0.23 mol) in 70 ml of 50% MeOH was added to a solution of KCN (29.3 g, 0.45 mol) and $(NH_4)_2CO_3$ (86.4 g, 0.9 mol) in 200 ml of 50% MeOH, and the mixture was stirred for 8 h at 70 °C. After cooling, the precipitate was collected and rinsed with water and ether to give **1** (21.1 g, 45%). The product was recrystallized from MeOH. mp 167—169 °C. IR (KBr): 3300, 2950, 1780, 1720 cm^{-1}. ^{1}H-NMR ($(CD_3)_2CO$) δ: 1.13—2.22 (2H, m), 2.83 (1H, d, J=7 Hz), 2.77 (2H, t, J=9 Hz), 4.08 (1H, dd, J=6, 9 Hz), 7.24 (5H, s), 9.50 (1H, br s). *Anal.* Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.69; H, 5.93; N, 13.75.

Enzymatic Hydrolysis of 1 Acetone-dried cells of *Pseudomonas putida* (1.97 g) were added to a mixture of **1** (1 g, 4.9 mmol) and 100 ml of 1 M NH_4Cl–NH_4OH buffer), and then the mixture was diluted with water to 1 l, adjusted to pH 9.0 with 4 N NaOH, and shaken for 50 h at 37 °C. Then MeOH was added to the mixture and the cells were removed by centrifugation.

The supernatant solution was concentrated *in vacuo*, and the residue was filtered off after addition of MeOH. The filtrate was concentrated *in vacuo*, adjusted to pH 9.0 with 4 N NaOH, and then adjusted to pH 3.0 with 3 N HCl at 0 °C to give **2** (1.08 g, 99.3%) as colorless needles. mp 196—198 °C, $[\alpha]_D^{25}$ −16.8° (c=2, 1 N NH_4OH). IR (KBr): 3500, 3300, 1690, 1570 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.78—2.20 (2H, m), 2.70 (2H, t, J=8.4 Hz), 4.23 (1H, dd, J=5.1, 8.1 Hz), 7.23 (5H, s). *Anal.* Calcd for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.68, H, 6.44, N, 12.61.

(*R*)-2-Bromo-4-phenylbutyric Acid (3) A solution of **2** (100 mg, 0.45 mmol) in 1.5 ml of 2 N H_2SO_4 in 50% CH_3COOH, was treated with 37 mg of $NaNO_2$ (0.54 mmol) in small portions at 0—5 °C. The mixture was stirred for 24 h at room temperature, then 374 mg of KBr (3.15 mmol), 1.5 ml of 3 N H_2SO_4, and 99 mg of $NaNO_2$ (1.44 mmol) were added successively to the reaction mixture at 0—5 °C. The mixture was stirred for 1 h at 0—5 °C, and for a further 3 h at room temperature. The reaction mixture was extracted with ether and the extracts were washed successively with saturated $Na_2S_2O_3$, water and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give **3** (78 mg, 72%) as a colorless oil. $[\alpha]_D^{20}$ +65.5° (c=1, $CHCl_3$). IR (neat): 3000, 1710, 1610, 1500, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.18—2.48 (2H, m), 4.18 (1H, t, J=7.5 Hz), 7.27 (5H, s), 9.38 (1H, br s). *Anal.* Calcd for $C_{11}H_{11}BrO_2$: C, 49.40; H, 4.56; Br, 32.87. Found: C, 49.20; H, 4.73; Br, 33.04.

(*R*)-Ethyl 2-Bromo-4-phenylbutyrate (4) A solution of **3** (101 mg, 0.41 mmol) in 1 ml of EtOH was treated with $SOCl_2$ (59 mg, 0.49 mmol) at −5 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. After removal of the solvents, the residue was dissolved in AcOEt. The AcOEt layer was washed successively with saturated $NaHCO_3$, water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by preparative TLC (hexane : AcOEt = 5 : 1) afforded **4** (107 mg, 95%) as a colorless oil. $[\alpha]_D^{20}$ +55° (c=1, $CHCl_3$). IR (neat): 3000, 2950, 1740, 1610, 1500, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.5 Hz), 2.20—2.55 (2H, m), 2.60—2.93 (2H, m), 4.18 (3H, q, J=7.5 Hz), 7.27 (5H, s). *Anal.* Calcd for $C_{12}H_{15}BrO_2$: C, 53.16; H, 5.58; Br, 29.47. Found: C, 53.45; H, 5.68; Br, 29.42.

(*R*)-2-Chloro-4-phenylbutyric Acid (5) Conversion of **2** to **5** was carried out by the same procedure as described for **3**. The reaction of **2** (100 mg, 0.45 mmol) with KCl (291 mg, 3.15 mmol) afforded **5** (59 mg, 66%) as a colorless oil. $[\alpha]_D^{20}$ +30.7° (c=1, $CHCl_3$). IR (neat): 2900, 1720, 1600, 1500, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.13—2.46 (2H, m), 2.73—2.97 (2H, m), 4.27 (1H, dd, J=6.0, 7.5 Hz), 7.27 (5H, s).

(*R*)-Ethyl 2-Chloro-4-phenylbutyrate (6) By the same procedure as described for the preparation of **4**, **5** (105 mg, 0.53 mmol) was esterified with $SOCl_2$ (76 mg, 0.63 mmol) in 1 ml of EtOH to give **6** (113 mg, 94%). $[\alpha]_D^{20}$ +26.3° (c=1, $CHCl_3$). IR (neat): 2900, 1750, 1610, 1500, 1470 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.5 Hz), 2.10—2.43 (2H, m), 2.60—2.90 (2H, m), 4.0—4.36 (3H, m), 7.27 (5H, s). *Anal.* Calcd for $C_{12}H_{15}ClO$: C, 63.58; H, 6.67; Cl, 15.64. Found: C, 63.35; H, 6.72; Cl, 15.36.

(*R*)-2-Hydroxy-4-phenylbutyric Acid (7) A solution of **2** (200 mg, 0.9 mmol) in 8 ml of 50% AcOH was mixed with 5 ml of 2 N H_2SO_4 then by $NaNO_2$ (75 mg, 1.08 mmol) was added in small portions at 0—5 °C. The mixture was stirred for 24 h at room temperature, then 5 ml of 4 N H_2SO_4 was added and the whole mixture was heated at 100 °C. After repeated addition of $NaNO_2$ (199 mg, 2.88 mmol) in small portions, the mixture was stirred for a further 3 h at the same temperature. After cooling, the mixture was extracted with ether, and the extracts were dried over $MgSO_4$, then concentrated *in vacuo* to give **7** (144 mg, 89%) as colorless needles. IR (KBr): 3460, 2950, 1730, 1600, 1500, 1450, 1290, 1270, 1240 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.90—2.30 (2H, m), 2.67—2.97 (2H, t, J=7.5 Hz), 4.40 (1H, dd, J=4.5, 7.5 Hz), 5.40 (2H, br s), 7.27 (5H, s). MS m/z: 180 (M^+), 117, 105, 92, 91. *Anal.* Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.48; H, 6.70.

(*R*)-Ethyl 2-Hydroxy-4-phenylbutyrate (8) By the same procedure as described for the preparation of **4**, **7** (130 mg, 0.72 mmol) was esterified with $SOCl_2$ (103 mg, 0.86 mmol) in 1 ml of EtOH to give **8** (97 mg, 65%) as a colorless oil. $[\alpha]_D^{20}$ −20° (c=1, $CHCl_3$) (lit.[9]) $[\alpha]_D^{20}$ −22.1° (c=1, $CHCl_3$). IR (neat): 3450, 1730, 1600, 1500, 1450, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.5 Hz), 1.77—2.27 (2H, m), 2.76 (2H, t, J=8.0 Hz), 2.93

(1H, br s), 4.20 (3H, q, J=7.5 Hz), 7.26 (5H, s). MS m/z: 208 (M^+), 149, 117, 105, 104, 92, 91.

***N*-[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanine *tert*-Butylester ((*S,S*)-9)** Compound **4** (150 mg, 0.55 mmol) in 0.5 ml of CH_3NO_2 was added to a mixture of L-alanine *tert*-butylester hydrochloride (302 mg, 1.66 mmol) and $(NH_4)_2CO_3$ (169 mg, 1.76 mmol) in 2 ml of H_2O. The mixture was stirred for 24 h at 50 °C. After repeated addition of L-alanine *tert*-butylester hydrochloride (100 mg, 0.55 mmol) and $(NH_4)_2CO_3$ (53 mg, 0.55 mmol), stirring was continued for a further 24 h at 50 °C. Furthermore, the same procedure was repeated twice more. The reaction mixture was extracted with AcOEt, and the extracts were dried over $MgSO_4$, then concentrated *in vacuo*. The residue was subjected to preparative TLC (hexane : AcOEt = 5 : 1) to give (*S,S*)-**9** (145 mg, 78%) and the isomer (*R,S*)-**9** (17 mg, 9.2%) as colorless oils. $[\alpha]_D^{20}$ +21.5° (c=1, MeOH). IR (neat): 3350, 3000, 1730, 1600, 1450, 1370, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, d, J=7.5 Hz), 1.33 (3H, t, J=7.5 Hz), 1.46 (9H, s), 1.70—1.83 (1H, br s), 1.87—2.13 (2H, m), 2.60—2.90 (2H, m), 3.14—3.49 (2H, m), 4.23 (2H, q, J=7.5 Hz), 7.25 (5H, s). MS m/z: 336 (M^+ + 1). (*R,S*)-**9**: $[\alpha]_D^{20}$ −22.5° (c=1, MeOH). IR (neat): 3350, 2990, 1750, 1605, 1450, 1370, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, d, J=7.5 Hz), 1.30 (3H, t, J=7.5 Hz), 1.46 (9H, s), 1.76—2.10 (3H, m), 2.60—2.86 (2H, m), 3.10—3.38 (2H, m) 4.18 (2H, q, J=7.5 Hz), 7.25 (5H, s). MS m/z: 336 (M^+ + 1).

***N*-[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanine ((*S,S*)-10) and *N*-[(*R*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanine ((*R,S*)-10)** A solution of (*S,S*)-**9** (85 mg, 0.25 mmol) in 3.5 ml of CF_3COOH was stirred at room temperature for 2 h, and then concentrated *in vacuo*. The residue was applied to a Dowex 50wX8 ion-exchange column. The column was washed with water until the eluate was neutral. Then the product was eluted from the column with 2 N NH_4OH. Concentration of appropriate fractions gave **10** (64 mg, 90%) as a colorless powder. mp 153—154 °C. $[\alpha]_D^{20}$ +30° (c=1, MeOH). (lit.[9] mp 148—150 °C. $[\alpha]_D^{20}$ +28.2° (c=1, MeOH)). ^{1}H-NMR (CD_3OD) δ: 1.40 (3H, t, J=7.5 Hz), 1.59 (2H, d, J=7.5 Hz), 2.16—2.50 (2H, m), 2.76—3.03 (2H, m), 3.67 (1H, q, J=7.5 Hz), 4.09 (1H, t, J=6.9 Hz), 4.40 (2H, q, J=7.5 Hz), 7.46 (5H, s).

The (*R,S*)-diastereoisomer ((*R,S*)-**9**) was subjected to acid hydrolysis as described for the preparation of (*S,S*)-**10** to give (*R,S*)-**10** as a colorless powder. mp 129—130 °C. $[\alpha]_D^{20}$ −26.3° (c=1, MeOH) (lit.[9] mp 128—130 °C. $[\alpha]_D^{20}$ −27.9° (c=1, MeOH)). ^{1}H-NMR (CD_3OD) δ: 1.36 (3H, t, J=7.5 Hz), 1.45 (3H, d, J=7.5 Hz), 2.07—2.33 (2H, m), 2.63—2.90 (2H, m), 3.60 (1H, q, J=7.5 Hz), 3.93 (1H, t, J=6.0 Hz), 4.36 (2H, q, J=7.5 Hz), 7.28 (5H, s).

***N*-[(*S*)-1-Ethoxycarbonyl-3-phenylpropyl]-L-alanyl-L-proline *tert*-Butylester (11)** A mixture of L-alanyl-L-proline *tert*-butylester (268 mg, 1.107 mmol) and $(NH_4)_2CO_3$ (35 mg, 0.369 mmol) in 2 ml of H_2O was added to a solution of **4** (100 mg, 0.369 mmol) in 0.5 ml of CH_3NO_2, and the mixture was stirred for 4 d at 50 °C. Then, the mixture was extracted with AcOEt, and the extracts were dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by preparative TLC (hexane : AcOEt = 1 : 1) gave **11** (117 mg, 74%) and its (*R,S,S*)isomer (12 mg, 7.5%) as colorless oils. $[\alpha]_D^{20}$ −78° (c=1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=6.0 Hz), 1.33 (3H, d, J=6.0 Hz), 1.45 (9H, s), 1.65—2.06 (6H, m), 2.20 (1H, br s), 2.69 (2H, t, J=9 Hz), 3.22 (1H, t, J=6.0 Hz), 3.35—3.64 (3H, m), 4.15 (2H, q, J=7.0 Hz), 4.32—4.50 (1H, m), 7.15 (5H, s).

***N*-[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl-L-proline (12)** A solution of **11** (273 mg, 0.63 mmol) in 7 ml of TFA was stirred for 3 h at room temperature, and then the mixture was concentrated *in vacuo*, and extracted with AcOEt. The extracts were dried over $MgSO_4$, and concentrated *in vacuo* to give **12** (195 mg, 85%) as a colorless oil. ^{1}H-NMR (CD_3OD) δ: 1.40 (3H, t, J=7.5 Hz), 1.58 (3H, d, J=6.0 Hz), 1.86—2.50 (6H, m), 2.82 (2H, m), 3.63 (2H, m), 4.02 (1H, q, J=6.0 Hz), 4.32 (2H, q, J=7.5 Hz), 4.53 (2H, m), 7.26 (5H, s).

***N*-[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl-L-proline (13)** A solution of **12** (195 mg, 0.519 mmol) in 1.29 ml of 1 N NaOH was stirred for 24 h at room temperature. The reaction mixture was added to a Dowex 50wX8 ion-exchange column. The column was washed with water until the eluate was neutral. Then, the product was removed from the column with aqueous 2% pyridine. After concentration of the appropriate fractions, the resulting residue was crystallized with acetone, followed by filtration to give **13** (136 mg, 75%) as a colorless powder. mp 147—158 °C. $[\alpha]_D^{25}$ −52° (c=1, MeOH) (lit.[3b] mp 149—151 °C, $[\alpha]_D^{25}$ −53.5° (c=1, MeOH). ^{1}H-NMR (CD_3OD) δ: 1.67 (3H, d, J=7.0 Hz), 1.88—2.46 (6H, m), 2.82 (2H, m), 3.44—3.88 (3H, m), 4.32 (1H, q, J=7.0 Hz), 4.62—4.45 (1H, m), 7.27 (5H, m).

***N*^{6}-(*tert*-Butoxycarbonyl)-*N*^{2}-[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-lysyl-L-proline *tert*-Butylester (14)** A solution of *N*^{6}-*tert*-butoxycarbonyl-L-lysyl-L-proline *tert*-butylester (442 mg, 1.11 mmol) and $(NH_4)_2CO_3$ (71 mg, 0.738 mmol) in 3.2 ml of H_2O was added to a solution of **4** (200 mg, 0.738 mmol) in 0.8 ml of CH_3NO_2, and the mixture was stirred for 4 d at 50 °C. The mixture was extracted with AcOEt, and the extracts were dried over $MgSO_4$, then concentrated *in vacuo*. Purification of the residue by preparative TLC gave **14** (300 mg, 70%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=6.6 Hz), 1.43 (9H, s), 1.46—1.70 (16H, m), 1.80—2.20 (6H, m), 2.35 (1H, s), 2.68 (2H, m), 2.98—3.34 (3H, m), 3.38—3.65 (2H, m), 4.13 (2H, q, J=6.6 Hz), 4.40 (1H, m), 4.95 (1H, br s), 7.20 (5H, s).

***N*^{2}-[(*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-lysyl-L-proline (15)** A solution of **14** (168 mg, 0.29 mmol) in 5 ml of TFA was stirred for 24 h at room temperature. After removal of the solvent, the residue was applied to a Dowex 50wX8 ion-exchange column. The column was washed with water, and then the product was eluted with aqueous 2% pyridine. Concentration of the appropriate fractions gave **15** (110 mg, 89%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, t, J=7.5 Hz), 1.43—2.21 (15H, m), 2.73 (2H, m), 4.27 (2H, q, J=7.5 Hz), 4.43 (1H, m), 6.0 (2H, br s), 7.28 (5H, s).

***N*^{2}-[(*S*)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline (16)** Conversion of **15** to **16** was carried out by the same procedure as described for the preparation of **13**. The reaction of **15** (110 mg, 0.25 mmol) in 0.5 ml of 1 N NaOH afforded **16** (78 mg, 75.8%) as a colorless powder. The product was recrystallized from MeOH–AcOEt. $[\alpha]_D^{25}$ −23.5° (c=0.596, MeOH). (lit.[4] $[\alpha]_D^{25}$ −23.3° (c=1, MeOH)). ^{1}H-NMR (CD_3OD) δ: 1.46—2.40 (12H, m), 2.62—3.12 (4H, m), 3.50—3.90 (3H, m), 4.0—4.59 (2H, m), 7.30 (5H, s).

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References and Notes

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