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Efficient Synthetic Method for Ethyl (+)-(2*S*,3*S*)-3-[(*S*)-3-Methyl-1-(3-methylbutylcarbamoyl)butylcarbamoyl]-2-oxiranecarboxylate (EST), a New Inhibitor of Cysteine Proteinases

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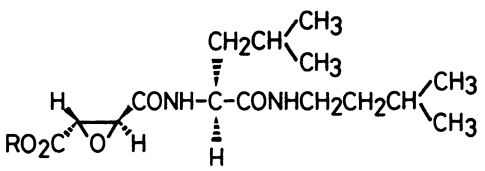
Ethyl (+)-(2*S*,3*S*)-3-[(*S*)-3-methyl-1-(3-methylbutylcarbamoyl)butylcarbamoyl]-2-oxiranecarboxylate (EST; **1a**) is expected to be useful as an oral therapeutic agent for muscular dystrophy on the basis of its potent inhibitory activities against the cysteine proteinases involved in the myofibrillar protein degradation that occurs in the disease. Through extensive investigations aimed at developing a new synthetic method for **1a** that would be suitable for industrial application, it has been found that L-arginine can be used as a new, efficient resolving agent to obtain optically pure L-*trans*-epoxysuccinic acid (**3a**), and the active ester method using *p*-nitrophenol is very effective in the coupling reaction of ethyl L-*trans*-epoxysuccinate (**7a**) and L-leucine isoamylamide (**8a**) because of the extremely low formation of by-products.

To examine the contribution of the stereochemistry of the *trans*-epoxysuccinic acid and leucine moieties to the inhibitory activity against cysteine proteinases, the diastereomers (**1b**—**d**) of **1a** were synthesized by a similar method and the rate constants of inactivation of papain by **1a**—**d** were measured. Compound **1a**, having L-*trans*-epoxysuccinic acid and L-leucine moieties, showed the most potent activity among them.

Keywords——synthesis; L-*trans*-epoxysuccinic acid; L-leucine isoamylamide; optical resolution; arginine; active ester method; inhibitor; cysteine proteinase; muscular dystrophy

Ethyl (+)-(2*S*,3*S*)-3-[(*S*)-3-methyl-1-(3-methylbutylcarbamoyl)butylcarbamoyl]-2-oxiranecarboxylate (EST; **1a**) is an ester derivative of (+)-(2*S*,3*S*)-3-[(*S*)-3-methyl-1-(3-methylbutylcarbamoyl)butylcarbamoyl]-2-oxiranecarboxylic acid (E-64-c; **2**),[1] which is a potent inhibitor of cysteine proteinases.[2,3]

Compound **2** is expected to be a new type of therapeutic agent for muscular dystrophy on the basis of recent data suggesting that the progressive loss of muscle proteins in this disease arises because of a marked increase of intracellular cysteine proteinases such as calcium-activated neutral protease and lysosomal cathepsins.[4,5] However, the low absorbability of **2** from the intestine was one of the problems to be solved for practical use, since an oral drug is desirable for long-term use in chronic diseases, such as muscular dystrophy. Compound **1a**



EST (**1a**): R = C_2H_5
E-64-c (**2**): R = H

Fig. 1

has been demonstrated to show potent inhibitory activity against cysteine proteinases in tissues when given orally.[6] Further, the long-term administration of **1a** to inherited muscular dystrophic hamsters prevented the development of necrotic changes in the myocardium, accompanied with a marked decrease in calcium deposition and prolonged life span.[7] These effects were presumed to be due to the potent inhibitory activity against cysteine proteinases. Therefore, there has been a growing expectation that this drug may be practically useful as a therapeutic agent for muscular dystrophy.

On the other hand, the development of a method suitable for industrial production of **1a** is necessary. We report herein efficient synthetic methods for **1a** and its diastereomers (**1b—d**), and the effect of the stereochemistry on the inhibitory activity.

As shown in Fig. 1, **1a** consists of L-*trans*-epoxysuccinic acid (**3a**; 2*S*, 3*S*), L-leucine and isoamylamine. Although there are some reports on the preparation of D-*trans*-epoxysuccinic acid (**3b**; 2*R*, 3*R*) by stereoselective synthesis[8] or fermentation,[9] little work has been done on the preparation of the L-isomer (**3a**). Ohashi and Harada[10] reported the optical resolution of DL-*trans*-epoxysuccinic acid (**4**) with (−)-ephedrine by diastereomeric salt formation. Mori and Iwasawa,[11] and Seebach and Wasmuth[12] have reported a stereoselective synthesis of the diethyl ester of **3a** from D-tartaric acid and (−)-malic acid, respectively. However, the resolution seems to be available only on a laboratory scale, and the stereoselective syntheses were considered to be expensive because of the complex processes involved. Therefore, we tried to find a new resolving agent (for **4**) available for industrial use. Compound **4** was prepared by epoxidation of fumaric acid according to the method of Payne and Williams.[13]

As a result of studies with various optically active amines, five compounds were found to be useful for the resolution of **4**, as shown in Table I.

D-α-Phenylglycine amide and dehydroabiethylamine gave **3a** in low yield. Though (−)-ephedrine[10] was superior to the above two reagents, it was inferior to arginine in terms of optical purity and yield. When L-phenylalanine amide was compared with L-arginine, the former was inferior in optical purity though the former was superior in yield. The optical purity of **3a** obtained by using L-phenylalaninol was at the same level as that obtained by using L-arginine, though the yield was much lower. In terms of economy, L-arginine is preferable to L-phenylalaninol. Consequently, L-arginine was selected for the practical resolution of **4**. The D-isomer (**3b**) was also obtained in high optical purity by the same procedure using D-arginine. As shown in Chart 1, diethyl L-*trans*-epoxysuccinate (**5a**) was synthesized from **3a** in good yield.

However, the extraction of **3a** from the aqueous solution was so troublesome that the direct esterification of the diastereomeric salt (**6a**) was tried by the use of 3.0 eq of sulfuric acid without isolation of **3a**. This method turned out to be much more efficient than the two-step method.

TABLE I. Resolution of DL-*trans*-Epoxysuccinic Acid (**4**) by Using Various Optically Active Amines

Resolving agent	L-*trans*-Epoxysuccinic acid (**3a**)	
	$[\alpha]_D$	Yield (%)
L-Phenylalaninol	+121.0°	40.0
L-Phenylalanine amide	+109.8°	68.0
D-α-Phenylglycine amide	+110.8°	32.2
Dehydroabiethylamine	+116.0°	26.2
L-Arginine	+122.2°	54.7
(−)-Ephedrine[10]	+117.8°	48.0

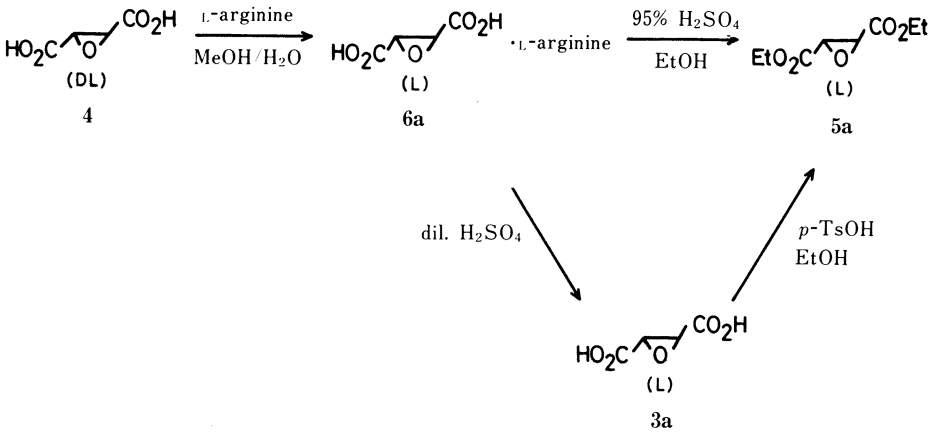


Chart 1

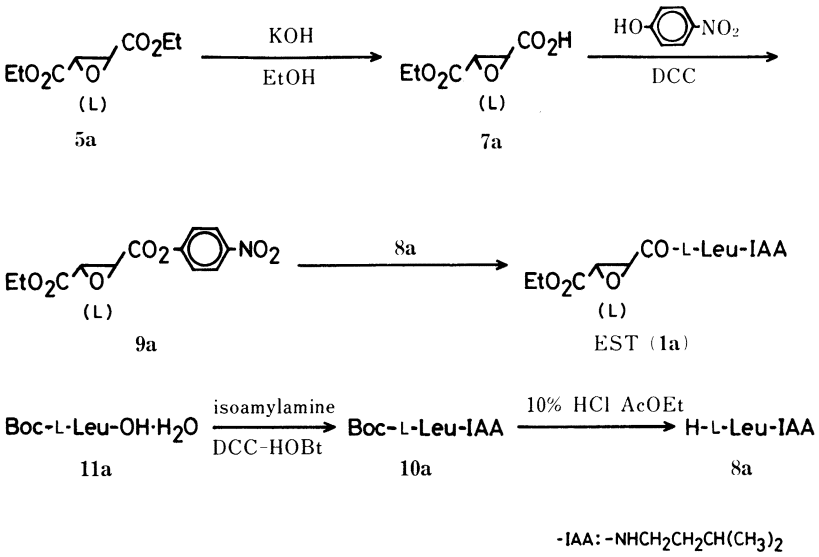


Chart 2

Compound **1a** was prepared from the diethyl ester (**5a**) and L-leucine according to the scheme shown in Chart 2. The diethyl ester (**5a**) was hydrolyzed to the half ester (**7a**) and then coupled with L-leucine isoamylamide (H-L-Leu-IAA) (**8a**). Among many possible methods, the active ester method using *p*-nitrophenol (HONp) was finally selected for this reaction because of the extremely low formation of by-products. The active ester (**9a**) was easily prepared from the half ester (**7a**) and HONp by using *N*,*N'*-dicyclohexylcarbodiimide (DCC) in good yield. Compound **8a** was prepared from *N*-(*tert*-butoxycarbonyl)-L-leucine isoamylamide (Boc-L-Leu-IAA) (**10a**)[14] by using a conventional method of peptide chemistry.

This synthetic route (shown in Chart 2) was expected to be suitable for industrial production since no special purification step, such as column chromatography, was necessary in any reaction step.

The physical properties of **1a** and its isomers (**1b**—**d**) prepared by similar procedures are summarized in Table II. The chemical shifts of the epoxy ring protons, the melting point and

TABLE II. Physical Data for EST (**1a**) and Its Diastereomers (**1b—d**)

Compound	Configuration		$[\alpha]_D^{20}$ [a]	mp (°C)	Chemical shift of epoxy ring protons (ppm)	
	t-ES[b]	Leu[c]				
1a	L	L	+51.7°	126.2	3.48	3.69
1b	L	D	+93.4°	133.5	3.55	3.69
1c	D	L	−92.6°	133.4	3.55	3.69
1d	D	D	−50.0°	123.8	3.48	3.69

a) (c=1.00, EtOH). *b*) *trans*-Epoxysuccinic acid moiety. *c*) Leucine moiety.

TABLE III. Rate Constant for Inactivation of Papain

Compound	Configuration		Rate constant[c] (M^{-1}, min^{-1})
	t-ES[a]	Leu[b]	
1a	L	L	277
1b	L	D	58
1c	D	L	102
1d	D	D	25

a) *trans*-Epoxysuccinic acid moiety. *b*) Leucine moiety. *c*) The rate constants were measured according to the method of Barrett *et al.*[17]

the specific rotation showed that **1d** and **1b** were the antipodes of **1a** and **1c**, respectively.

To examine the contribution of the stereochemistry of the *trans*-epoxysuccinic acid and leucine moieties to the inhibitory activity against cysteine proteinases, we measured the rate constants of **1a** and its isomers (**1b—d**) for the inactivation of papain. As shown in Table III, the stereochemistry strongly influenced the inhibitory activity. Compound **1a** reacted with papain faster than the other three isomers. The reactivity of the D–D isomer (**1d**), which showed the lowest rate, was approximately one-tenth of that of **1a** (L–L isomer). The configuration of the leucine moiety seems to play an important role in the binding of **1a** to papain rather than that of the epoxysuccinic acid group, based on a comparison of the rate constants of **1b**, **1c** and **1a**. It was suggested that the L–L isomer (**1a**) has the best conformation for approach to the active site of papain, and its epoxy ring may be located closer to the active thiol group as compared with the epoxy rings of the other three isomers. The *in vitro* and *in vivo* inhibitory activities of **1a** for cysteine proteinases were reported in the previous paper.[6]

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a JASCO DS-701G spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Varian XL-200 spectrometer using tetramethylsilane or sodium trimethylsilyl propionate-d_4 as an internal standard. Chemical shifts are given on the δ scale. Optical rotations were obtained with a JASCO DIP-140 or JASCO DIP-360 digital polarimeter. Mass spectra (MS) were recorded on a Shimadzu LKB-9000 spectrometer.

Examples of Optical Resolution of DL-*trans*-Epoxysuccinic Acid (4)——a) Optical Resolution with L-Phenylalanine Amide: A mixture of **4** (0.396 g, 3.0 mmol) in 95% MeOH (1.0 ml) and L-phenylalanine amide[15] (0.492 g, 3.0 mmol) in 95% MeOH (2.0 ml) was kept at room temperature overnight. The separated crystals were collected by suction. Yield, 0.372 g of acidic salt (83.8%). mp 176—177 °C. $[\alpha]_D^{17}$ +64.6° (c=0.90, MeOH). *Anal.* Calcd for $C_{13}H_{16}N_2O_6$: C, 73.75; H, 9.69; N, 3.74. Found: C, 73.95; H, 9.54; N, 3.65.

A solution of the above salt (0.354 g, 1.54 mmol) in 5 ml of water was applied to a column of Dowex 50 × 8 (H-

form, 100—200 mesh, 0.5 × 7 cm) and the column was washed with water until the effluent become neutral. The effluent was evaporated to dryness under reduced pressure to give the crude (+)epoxy acid, 0.154 g. Recrystallization from dioxane and *n*-hexane gave **3a** as colorless prisms of mp 176—177 °C (lit.[10] mp 188 °C). Yield, 0.128 g (81.1%). $[\alpha]_D^{13}$ +109.8° (*c* = 0.82, EtOH). The overall yield of optical resolution was 68.0%.

b) Optical Resolution with L-Phenylalaninol: L-Phenylalaninol[16] (7.55 g, 50 mmol) in 20 ml of EtOH was added to a solution of **4** (6.60 g, 50 mmol) in 20 ml of hot EtOH (80 °C) at 80 °C. The mixture was allowed to cool to room temperature and then kept at 5 °C overnight. The crystals were collected by suction and washed with cold EtOH. Recrystallization of this salt from MeOH (43 ml) gave 4.61 g of neutral salt (42.5%), mp 167—169.5 °C. $[\alpha]_D^{18}$ +39.8° (*c* = 0.6, MeOH). *Anal.* Calcd for $C_{22}H_{30}N_2O_7$: C, 60.81; H, 6.96; N, 6.45. Found: C, 60.79; H, 6.99; N, 6.43.

The above salt (4.61 g, 11 mmol) was treated with an ion-exchange column by the same procedure as in a). Colorless prisms 1.32 g of **3a** (94.3%). mp 171—172 °C. $[\alpha]_D^{20}$ +121° (*c* = 1.20, EtOH). The overall yield of optical resolution was 40.0%.

c) Optical Resolution with L-Arginine: L-Arginine (234.9 g, 1.35 mol) in warm water (650 ml) was added gradually to a stirred solution of **4** (178 g, 1.35 mol) in MeOH (2600 ml), and the mixture was allowed to stand overnight at room temperature. The precipitate formed was filtered by suction and washed with MeOH–water (4 : 1, 1000 ml) to give crude **6a** (201.2 g). Recrystallization of **6a** from MeOH–water (2 : 1, 3000 ml) gave pure **6a** as colorless prisms. Yield, 170.2 g (82.5%). mp 172 °C (dec.). $[\alpha]_D^{22}$ +54.7° (*c* = 1.00, H_2O). *Anal.* Calcd for $C_{10}H_{18}N_4O_7$: C, 39.21; H, 5.92; N, 18.29. Found: C, 39.17; H, 5.90; N, 18.46. IR (KBr): 3340, 3140, 1650, 1490, 1390, 1330, 1260, 1170 cm^{-1}. ^{1}H-NMR (D_2O) δ: 1.54—2.02 (4H, m, $-CH_2CH_2-$), 3.22 (2H, t, *J* = 6 Hz, $-NHCH_2-$), 3.48 (2H, s, epoxy ring), 3.84 (1H, t, *J* = 6 Hz, $-CH<$).

The corresponding D,D-isomer (**6b**) was obtained as colorless prisms by the same procedure as described above. Yield, (84.0%). $[\alpha]_D^{26}$ −54.0° (*c* = 1.00, H_2O).

Compound **6a** (73.1 g) was added to a stirred solution of dilute H_2SO_4 (95% H_2SO_4–H_2O, 1 : 10, 330 ml) under ice-cooling. The mixture was saturated with sodium chloride, and then extracted with acetone–AcOEt (7 : 3, 400 ml × 3). The combined extract was washed with brine (40 ml × 3), dried over $MgSO_4$, and then concentrated *in vacuo*. Crude **3a** obtained was crystallized from dioxane–*n*-hexane (95 : 5) to give colorless needles. Yield, 20.9 g (66.3%). mp 178—180 °C. $[\alpha]_D^{26}$ +122.2° (*c* = 1.01, EtOH). IR (KBr): 3080, 1685, 1405, 1295, 1235, 1080 cm^{-1}. ^{1}H-NMR (D_2O) δ: 3.77 (2H, s, epoxy ring). *Anal.* Calcd for $C_4H_4O_5$: C, 36.37; H, 3.06. Found: C, 36.52; H, 3.27.

By the same procedure as described above, the D-isomer (**3b**) was obtained as colorless needles. Yield, (67.0%). $[\alpha]_D^{26}$ −121.6° (*c* = 1.00, EtOH).

Diethyl L-*trans*-Epoxysuccinate (5a)——Method A: A stirred suspension of **6a** (107.1 g, 0.35 mol) in EtOH (1050 ml) was treated dropwise with 95% H_2SO_4 (102.9 g, 1.05 mol) at room temperature, and then the mixture was stirred under reflux for 4.5 h. The solvent was evaporated off *in vacuo* and the residue was poured into ice-water (200 ml), then extracted with AcOEt (300 ml × 3). The extract was washed with saturated aqueous $NaHCO_3$ (200 ml × 2) and brine (200 ml × 2) successively, then dried over $MgSO_4$ and filtered by suction. After evaporation of the solvent, the residue was purified by fractional distillation to provide **5a** as a colorless oil. Yield, 50.4 g (76.6%). bp 78—80 °C/0.9 mmHg (lit.[11] bp 98—99 °C/3 mmHg). $[\alpha]_D^{26}$ +110.5° (*c* = 1.12, EtOH). IR (neat): 2980, 1740, 1370, 1325, 1275, 1195, 1025 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (6H, t, *J* = 7 Hz, $-CH_3$ × 2), 3.66 (2H, s, epoxy ring), 4.28 (4H, dq, *J* = 7, 2 Hz, $-CH_2-$ × 2).

By the same procedure as described above, the D-isomer (**5b**) was obtained as a colorless oil. Yield, (72.3%). $[\alpha]_D^{26}$ −109.4° (*c* = 1.00, EtOH).

Method B: *p*-Toluenesulfonic acid monohydrate (1.73 g, 0.009 mol) was added to a stirred suspension of **3a** (40 g, 0.3 mol) in EtOH (350 ml), and the mixture was refluxed for 7 h, then the solvent was evaporated off *in vacuo*. The resulting residue was dissolved in benzene (500 ml) and washed with saturated aqueous $NaHCO_3$ (250 ml), water (250 ml) and brine (250 ml) successively, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* and the residue was purified by fractional distillation to give **5a** as a colorless oil. Yield, 44 g (78%). bp 77—79 °C/0.9 mmHg. $[\alpha]_D^{25}$ +109.3° (*c* = 1.00, EtOH).

Ethyl *p*-Nitrophenyl L-*trans*-Epoxysuccinate (9a)——A solution of 85% KOH (6.72 g, 0.1 mol) in EtOH (67 ml) was added dropwise to a stirred solution of **5a** (18.8 g, 0.1 mol) in EtOH (150 ml) at 4—6 °C. The reaction mixture was stirred for 1 h under ice-cooling and then for 4 h at room temperature. After evaporation of the solvent *in vacuo* below 50 °C, water (50 ml) was added to the residue and the solution was washed with AcOEt (50 ml × 2). The aqueous layer was acidified (pH = 2) by using 6 N HCl under ice-cooling and extracted with AcOEt (70 ml × 3). The extract was washed with brine (70 ml × 2), dried over $MgSO_4$ and filtered. Evaporation of the filtrate *in vacuo* gave the crude half ester (**7a**) (13.1 g) as a colorless oil.

N,N'-Dicyclohexylcarbodiimide (12.9 g, 0.0625 mol) in AcOEt (26 ml) was added dropwise to a stirred solution of the crude **7a** (10.0 g) and *p*-nitrophenol (8.69 g, 0.0625 mol) in AcOEt (55 ml) at 4—5 °C, and the mixture was stirred for 3 h under ice-cooling and for 1 h at room temperature.

The precipitate of *N,N'*-dicyclohexylurea formed was filtered off and washed with AcOEt (20 ml). The combined filtrate and washings were concentrated *in vacuo* and the residue was crystallized from AcOEt–*n*-hexane to give pure **9a** as pale yellow needles. Yield, 14.1 g (65.8% from **5a**). mp 86—87 °C. $[\alpha]_D^{20}$ +114.8° (*c* = 1.00, AcOEt). *Anal.* Calcd

for $C_{12}H_{11}NO_7$: C, 51.24; H, 3.95; N, 4.98. Found: C, 51.38; H, 3.95; N, 5.05. IR (KBr): 1755, 1735, 1515, 1340, 1305, 1195, 1170, 1020, 860, 855 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, t, J=8 Hz, $-OCH_2C\underline{H}_3$), 4.06 (1H, d, J=2 Hz, epoxy ring), 4.08 (1H, d, J=2 Hz, epoxy ring), 4.24 (2H, q, J=8 Hz, $-OC\underline{H}_2CH_3$), 7.58 (2H, d, J=8 Hz, aromatic), 8.36 (2H, d, J=8 Hz, aromatic).

By the same procedure as described above, the D-isomer (**9b**) was obtained as pale yellow needles. Yield, (67.1% from **5b**). $[\alpha]_D^{26}$ −114.1° (c=1.01, AcOEt).

EST (1a)——L-Leucine isoamylamide (**8a**) (7.12 g, 0.035 mol) in AcOEt (13 ml) was added dropwise to a stirred solution of the active ester (**9a**) obtained by the above procedure (10.0 g, 0.035 mol) in AcOEt (100 ml) at room temperature, and stirring was continued for 4 h at the same temperature. The precipitate formed was filtered off and the filtrate was washed with 2% aqueous NaOH (30 ml × 5), brine (40 ml), 5% HCl (40 ml) and brine (40 ml × 4) successively, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* and the residue was crystallized from EtOH to give pure **1a** as colorless fine needles. Yield, 9.08 g (74.6%). mp 126.2 °C. $[\alpha]_D^{20}$ +51.7° (c=1.00, EtOH). *Anal.* Calcd for $C_{17}H_{30}N_2O_5$: C, 59.63; H, 8.83; N, 8.18. Found: C, 59.78; H, 8.60; N, 7.95. IR (KBr): 3290, 2960, 1755, 1642, 1555, 900 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.85—1.04 (12H, m, $-CH_3$ × 4), 1.32 (3H, t, J=7 Hz, $-OCH_2C\underline{H}_3$), 1.41 (2H, q, J=7 Hz, $-NHCH_2C\underline{H}_2-$), 1.47—1.76 (4H, m, $-CH<$ × 2, $\underset{HN}{\overset{CO}{>}}CHC\underline{H}_2-$), 3.11—3.41 (2H, m, $-NHC\underline{H}_2-$), 3.48 (1H, d, J=2 Hz, epoxy ring), 3.69 (1H, d, J=2 Hz, epoxy ring), 4.27 (2H, dq, J=7, 2 Hz, $-OC\underline{H}_2CH_3$), 4.35—4.50 (1H, m, $-NHC\underline{H}<$), 6.16—6.30 (1H, m, $-CONHCH_2-$), 6.80 (1H, d, J=8 Hz, $-CONHCH<$).

By the same procedures as described above, **1b**, **1c** and **1d** were obtained as colorless fine needles by using the active ester (**9a** or **9b**) and leucine isoamylamide (**8a** or **8b**).

1b: Yield, (69.8%). mp 133.5 °C. $[\alpha]_D^{20}$ +93.4° (c=1.00, EtOH). IR (KBr): 3300, 3240, 2950, 1760, 1640, 1563, 900 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86—1.08 (12H, m, $-CH_3$ × 4), 1.32 (3H, t, J=7 Hz, $-OCH_2C\underline{H}_3$), 1.39 (2H, q, J=7 Hz, $-NHCH_2C\underline{H}_2-$), 1.48—1.76 (4H, m, $-CH<$ × 2, $\underset{HN}{\overset{CO}{>}}CHC\underline{H}_2-$), 3.13—3.40 (2H, m, $-NHC\underline{H}_2-$), 3.55 (1H, d, J=2 Hz, epoxy ring), 3.69 (1H, d, J=2 Hz, epoxy ring), 4.27 (2H, dq, J=7, 2 Hz, $-OC\underline{H}_2CH_3$), 4.32—4.48 (1H, m, $-NHC\underline{H}<$), 5.94—6.12 (1H, m, $-CONHCH_2-$), 6.68 (1H, d, J=8 Hz, $-CONHCH<$).

1c: Yield, (70.5%). mp 133.4 °C. $[\alpha]_D^{20}$ −92.6° (c=1.00, EtOH).

1d: Yield, (73.6%). mp 123.8 °C. $[\alpha]_D^{20}$ −50.0° (c=1.00, EtOH).

Boc-L-Leucine Isoamylamide (10a)——*N*,*N*′-Dicyclohexylcarbodiimide (10.32 g, 0.05 mol) in AcOEt (20 ml) was added dropwise to a stirred solution of Boc-L-leucine monohydrate (**11a**) (12.47 g, 0.05 mol), isoamylamine (4.36 g, 0.05 mol) and 1-hydroxybenzotriazole (6.76 g, 0.05 mol) in AcOEt (35 ml) while keeping the temperature at 3—8 °C, and the mixture was stirred for 1.5 h at the same temperature and then for 2.5 h at room temperature. The precipitate of *N*,*N*′-dicyclohexylurea formed was filtered off and washed with AcOEt (40 ml). The combined filtrate and washings were washed with 5% HCl (100 ml), brine (100 ml), saturated aqueous $NaHCO_3$ (100 ml) and brine (100 ml) successively.

The organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated *in vacuo*. The residue was dissolved in *n*-hexane (50 ml) and insoluble materials were filtered off and washed with small amounts of *n*-hexane. The filtrate and washings were concentrated *in vacuo* to give **10a** as a colorless solid, which could be used for the next step without further purification. Yield, 14.45 g (96.1%). An analytical sample was obtained by recrystallization from MeOH–water (2 : 1) after purification by silica gel column chromatography (Wako gel C-200, AcOEt–*n*-hexane, 1 : 3). mp 88—90 °C. $[\alpha]_D^{24}$ −24.6° (c=1.00 EtOH). *Anal.* Calcd for $C_{16}H_{32}N_2O_3$: C, 63.95; H, 10.76; N, 9.32. Found: C, 64.28; H, 10.37; N, 9.40. MS *m*/*z*: 300 (M^+). IR (KBr): 3240, 2960, 1690, 1640, 1555, 1520, 1310, 1240, 1165 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (12H, m, $-CH_3$ × 4), 1.30—1.80 (15H, m, *tert*-Bu, $-C\underline{H}_2C\underline{H}<$ × 2), 3.27 (2H, m, $-CONHC\underline{H}_2-$), 4.07 (1H, m, $-CONHC\underline{H}<$), 4.96 (1H, d, J=8 Hz, $-N\underline{H}$Boc), 6.22 (1H, m, $-CON\underline{H}CH_2-$).

By the same procedure as described above, the D-isomer (**10b**) was obtained from Boc-D-leucine monohydrate (**11b**) as a colorless solid. Yield, (97.0%). mp 87—89 °C. $[\alpha]_D^{24}$ +24.0° (c=1.02, EtOH).

L-Leucine Isoamylamide (8a)——Compound **10a** (18.5 g, 0.062 mol) was dissolved in 10% HCl–AcOEt (65 ml) and the mixture was stirred for 2.5 h at room temperature. After evaporation of the solvent, water (50 ml) was added to the residue and the solution was washed with AcOEt (50 ml). The aqueous layer was alkalified (pH > 10) with 25% aqueous NaOH and extracted with AcOEt (50 ml × 1, 25 ml × 2). The extract was washed with brine, dried over $MgSO_4$ and filtered by suction. The filtrate was evaporated *in vacuo* to give crude **8a** as a pale yellow oil (12.3 g, 99.6%), which could be used for the next step without further purification. IR (neat): 3280—2940, 1640, 1530, 1465, 1365 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88—1.05 (12H, m, $-CH_3$ × 4), 1.22—1.86 (8H, m, $-C\underline{H}_2C\underline{H}<$ × 2, $-NH_2$), 3.22—3.46 (3H, m, $-CONHC\underline{H}_2-$, $H_2NC\underline{H}<$), 7.12—7.36 (1H, m, $-CON\underline{H}-$).

The corresponding D-isomer (**8b**) was obtained from **10b** by the same procedure as described above, as a pale yellow oil. Yield, (99.3%).

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