Synthesis of Human Renin Inhibitory Peptides, Angiotensinogen Transition-State Analogs Containing a *Retro-Inverso* Amide Bond[1)]

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The experimental details for the synthesis of human renin inhibitors are described. In order to aviod metabolic degradation of the Phe–His (P_3–P_2) amide bond in transition-state analogs, structurally modified acyl residues (P_4–P_3) were incorporated into the inhibitors. Compound 1a, which contained 2-(1-naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionyl residue (P_4–P_3) with a *retro-inverso* amide bond, L-histidine, and norstatine isoamylamide residue (P_1–P_1') as a transition-state mimic, had potent human renin inhibitory activity, and it lowered blood pressure when administered orally to common marmosets.

Keywords renin inhibitor; antihypertensive drug; angiotensinogen; β-carbamoyl propionyl residue; *retro-inverso* amide bond; norstatine; transition-state analog; bioisostere

Renin catalyzes the first and rate-limiting step in the renin–angiotensin system, and the action of renin on angiotensinogen is highly specific. Thus, a large number of renin inhibitors have been investigated as targets of antihypertensive drugs.[2)] Peptide inhibitors[3)] have been considered to be unsuitable as drugs for oral administration due to degradation by proteases such as chymotrypsin, especially at the Phe–His amide bond (P_3–P_2)[4)] of the inhibitors. As shown in Fig. 1, compound **12a**[5)] was hydrolyzed by chymotrypsin, which was specific to aromatic L-amino acids, *e.g.*, tryptophan, tyrosine, and phenylalanine. The hydrolysis apparently occurs at the 1-naphthylalanyl-His amide bond. Peptide bonds, which are metabolized easily by proteases, should be modified to avoid metabolic degradation. Further, orally active renin inhibitors for clinical use should be compact to achieve efficient intestinal absorption, and should have high potency and high specificity.

Recently,[1)] we reported an orally potent human renin inhibitor **1a** (Chart 3), which was stabilized against chymotrypsin (Fig. 1) by incorporating β-carbamoylpropionyl residue (**2**) with a *retro-inverso* amide bond as the P_4–P_3 moiety. Compound **1a** was designed from the angiotensinogen transition state based on a three-dimensional structure of complex of human renin[6)] and the scissile site Pro–Phe–His–Leu–Val (P_4 to P_1') of angiotensinogen. The oral administration of 30 mg/kg of **1a** resulted in a lowering of approximately 20 mmHg of mean blood pressure for about 7 h in common marmosets.[7)] Herein we describe the experimental details for the synthesis of human renin inhibitors containing structurally modified residues as P_4–P_3 and P_1–P_1' moiety and inhibitory activities.

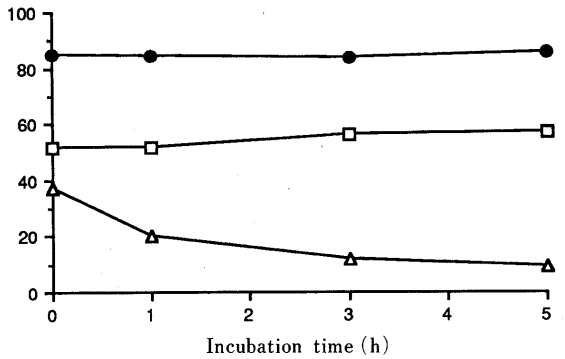


Fig. 1. Stability of Renin Inhibitors to Chymotrypsin
●, **1b** 5×10^{-6} M; □, **1a** 1×10^{-5} M; △, **12a** 1×10^{-4} M.

Results and Discussion

Synthesis Synthesis of the inhibitors listed in Table I is outlined in Charts 1—3. The modified acyl groups at P_4—P_3 were prepared as shown in Chart 1. The ethyl 2-cyano-3-(1-naphthyl)propionate (**4**) was prepared by condensation of 1-naphthaldehyde (**3**) with ethyl cyanoacetate in the presence of piperidine followed by hydrogenation with Pd/C (85% yield from **3**). After hydrogenation of the cyano group in **4** with PtO_2, the product was coupled with phenylacetyl chloride, followed by hydrolysis, to give 2-(1-naphthylmethyl)-3-(phenylacetylamino)propionic acid (**5**) (24.5% yield from **4**). 2-Cyano-3-(1-naphthyl)propionic acid (**6**), which was prepared from **4** by hydrolysis, was coupled with phenethylamine using *N*,*N*′-carbonyldiimidazole (CDI) followed by hydrolysis to give 3-(1-naphthyl)-2-(*N*-phenethylcarbamoyl)propionic acid (**7**) (26% yield from **6**).

2-(1-Naphthylmethylidene)succinic acid (**8**) was prepared by condensation of **3** with diethyl succinate in the presence of NaOMe, followed by hydrolysis as reported previously.[8)] The acid **8** was treated with $SOCl_2$ and then coupled with benzylamine or phenethylamine. The resulting amides were hydrogenated to give a racemic 3-(*N*-benzylcarbamoyl)-2-(1-naphthylmethyl)propionic acid **9a** (50% yield from **8**) and 2-(1-naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionic acid **9b** (65.7% yield from **8**).

Chart 2 shows the syntheses of leucinol derivatives **12a**—**e**. *p*-Methoxybenzyloxycarbonyl-L-histidine hydrazide (PMZ–His–$NHNH_2$) (**10**)[9)] was coupled with L-leucinol by the azide method using isoamyl nitrite followed by hydrogenolysis to give **11**·2*p*-toluenesulfonic acid (TsOH) salt (79% yield). The above acid (**5**, **7**, **9a**, or **9b**) and **11** were coupled using diphenylphosphoryl azide (DPPA)[10)] followed by separations with silica gel chromatography to afford leucinol derivatives **12a**—**e**, respectively.

The phenethylcarbamoyl propionyl derivatives contain-

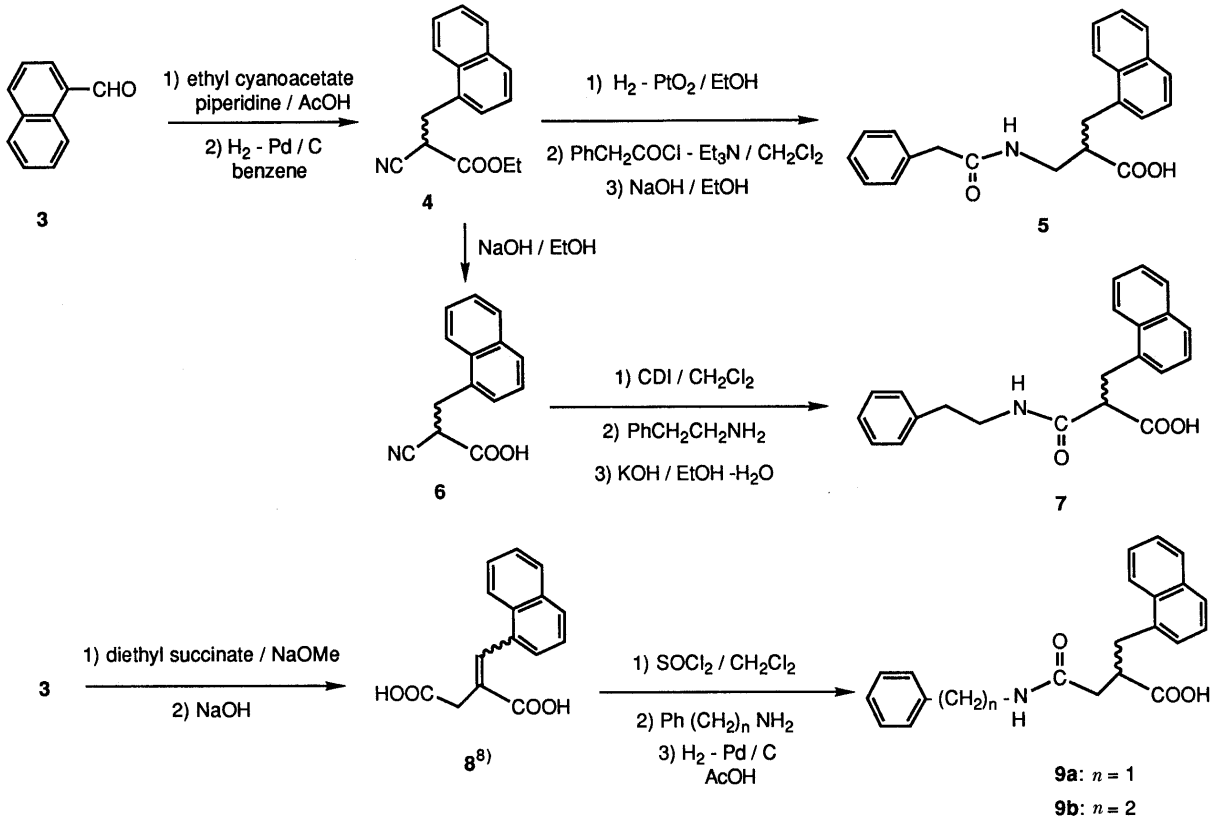


Chart 1

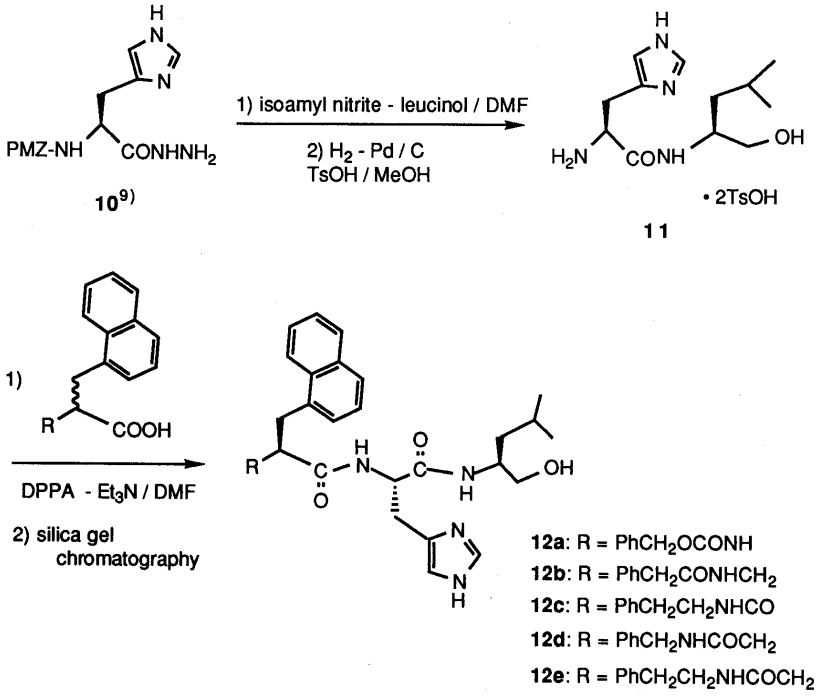


Chart 2

ing norstatine or statine residue were repared as shown in Chart 3. A stereoisomeric mixture **9b** was separated by optical resolution with (*R*)-α-methylbenzylamine from ethyl acetate to give **2** with *R* configuration.[8] The coupling of **2** and His–OMe·2HCl with DPPA, followed by treatment of the product with $NH_2NH_2 \cdot H_2O$, gave the hydrazide **13** in an 89% yield. The hydrazide **13** was coupled with norstatine isoamylamide[11] (**14a**), norstatine methyl ester[11] (**14b**), or statine methyl ester[12] (**14c**) by the azide method to obtain inhibitors (**1a—c**), respectively.

Renin Inhibitory Activity The β-amino acid derivative (**12b**) and the malonylamide derivative (**12c**) showed very weak inhibition. However, the β-carbamoyl-3-(1-naphthyl)-propionyl derivatives (**12d** and **12e**) with a carbonyl group

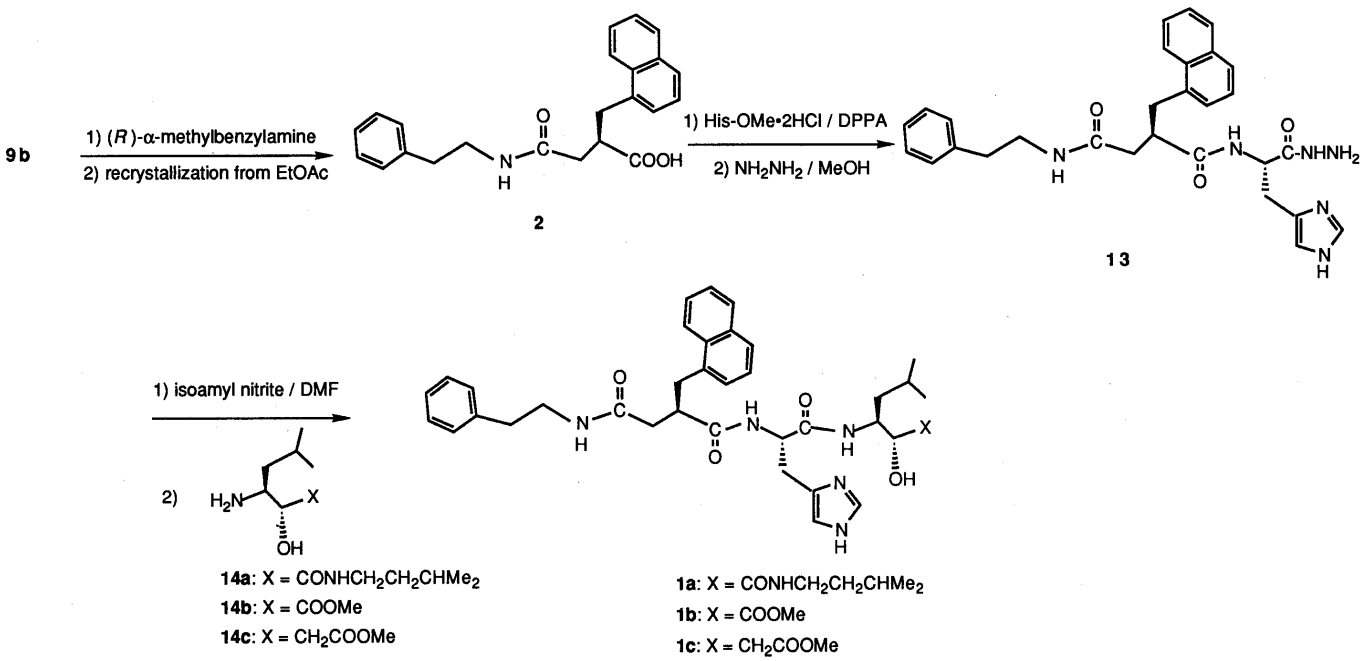
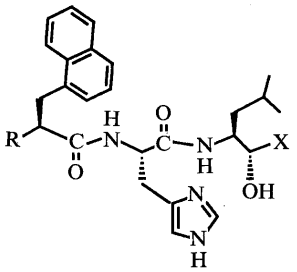


Chart 3

TABLE I. *In Vitro* Activity for Inhibitors



No.	R	X	IC_{50} (M)	
			Human renin	Human plasma renin
12a	$PhCH_2OCONH$	H	4.3×10^{-6}	3.6×10^{-6}
12b	$PhCH_2CONHCH_2$	H	$>10^{-4}$	N.D.[b]
12c	$PhCH_2CH_2NHCO$	H	$>10^{-4}$	N.D.
12d	$PhCH_2NHCOCH_2$	H	1.8×10^{-5}	N.D.
12e	$PhCH_2CH_2$-$NHCOCH_2$	H	7.4×10^{-6}	8.3×10^{-6}
1a	$PhCH_2CH_2$-$NHCOCH_2$	$CONHCH_2CH_2$-$CHMe_2$[a]	2.5×10^{-7}	7.9×10^{-7}
1b	$PhCH_2CH_2$-$NHCOCH_2$	COOMe	3.1×10^{-8}	7.7×10^{-8}
1c	$PhCH_2CH_2$-$NHCOCH_2$	CH_2COOMe	3.1×10^{-7}	1.7×10^{-6}

a) A mixture (2*R*,3*S*/2*S*,3*S*=13/7) of diastereoisomers of norstatine. *b*) N.D.= not determined.

at the same position as that of P_4 Pro showed inhibitory activities comparable to compound **12a**. It was presumed that the β-carbonyl group of **2** in **12d** or **12e** was located at a suitable position to accept a hydrogen bond from the side chain OH of Ser-230 in human renin, and the naphthyl group was accommodated in the hydrophobic subsite S_3 of renin.[13] The phenethylamino group-containing compound (**12e**) was more active than the corresponding benzylamino derivative (**12d**). We deduced that the phenethyl group fitted more favorably to renin than the benzyl group because the former was more flexible than the latter.

The replacement of leucinol residue with the statine residue (**1c**) enhanced the potency 10-fold and with norstatine (**1b**) as a transition-state mimic enhanced it 100-fold. The norstatine isoamylamide derivative (**1a**) was less active than the ester derivative (**1b**). This is attributed to the decreased fitness to renin because of the rigidity of **1a**. These compounds also exhibited very specific inhibition for human renin over other related aspartic proteases, *e.g.* cathepsin D and pepsin.[14]

Intravenous administration of 5 mg/kg of **1b** to a Japanese monkey led to a rapid drop in mean blood pressure.[14] The oral administration of **1a** resulted in a lowering of approximately 20 mmHg of mean blood pressure in common marmosets, but **1b** showed no significant oral activity.[7] One cause may be attributed to the hydrolysis of methyl ester in compound **1b** *in vivo*.

Conclusion

The P_4–P_3 modification, with the β-carbamoyl-1-naphthylmethylpropionyl residue having a *retro-inverso* amide bond, stabilized the P_3–P_2 bond against chymotrypsin. Replacement of the Leu–Val (P_1–P_1') scissile site with a norstatine isoamylamide residue enhanced renin inhibitory potency as well as stability to proteases. Thus, the hydroxylmethylcarboxamide bioisostere was effective in both potency and stability. Compound **1a** had potent human renin inhibitory activity and exhibited lowering of blood pressure when administered orally to common marmosets.

Experimental

Proton nuclear magnetic resonance spectra (^{1}H-NMR) were measured on a JEOL JMX-GX270 (270 MHz) instrument. Chemical shifts are reported as δ values (parts per million) relative to Me_4Si or $(CH_3)_3Si$-$(CH_2)_3$-SO_3Na as an internal standard. Mass spectra (MS) were obtained with a JEOL JMX-DX300 (FAB) spectrometer having JMA-DA5000 (data processor). Infrared spectra (IR) were measured on a JASCO IR-810 infrared spectrophotometer. High performance liquid chromatography

(HPLC) analyses were performed on a Shimadzu LC-6A liquid chromatograph instrument, YMC-Packed Column R-ODS-5, and 0.05 M aq. NH_4OAc–CH_3CN elutions, with ultraviolet (UV) detection at 223 nm (JASCO UVIDEC-100-V). Optical rotations were measured with a Horiba SEPA-200 high sensitive polarimeter. Melting points were measured on a Yamato micro melting point apparatus and are uncorrected. Preparative thin layer chromatography (TLC) was carried out using Merck precoated Silica gel 60 F-254 plates (thickness 0.5 mm). Flash column chromatography was carried out using Merck Silica gel 60 Art 9385 (230—400 mesh). *Rf* values of products refer to the following v/v solvent system: $CHCl_3$–methanol (5 : 1). Elemental analyses were performed by the Analytical Research Department, Central Research Laboratories, Kissei Pharmaceutical Co., Ltd.

2-(1-Naphthylmethyl)-3-(phenylacetylamino)propionic Acid (5) To a solution of 1-naphthaldehyde (**3**) (10.0 g, 64 mmol) and ethyl cyanoacetate (7.8 g, 64 mmol) in benzene (150 ml) were added piperidine (2 ml) and acetic acid (2 ml). After reflux for 3 h, the solution was washed with 2 N HCl, 5% $NaHCO_3$, and brine. The solution was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was recrystallized from benzene and hexane to give ethyl 2-(1-naphthylmethylidene)cyanoacetate (13.8 g, 85.9%). A suspension of the cyanoacetate (13.6 g, 54 mmol) and 10% Pd on activated carbon (1.3 g) in benzene (300 ml) was hydrogenated at atmospheric pressure. After Pd on activated carbon was filtered out, the filtrate was concentrated to afford ethyl 2-cyano-3-(1-naphthyl)propionate (**4**) (13.75 g, quant.). A suspension of **4** (2.53 g, 10 mmol), PtO_2 (250 mg) and 2 N HCl (10 ml) in ethanol (90 ml) was hydrogenated at atmospheric pressure. After PtO_2 was filtered out, the filtrate was concentrated and then washed with ether. The aqueous solution was alkalified with 5% $NaHCO_3$ and extracted with ethyl acetate. Ethanolic HCl was added to the organic solution and evaporated to give a viscous material as a crude product. The crude material was crystallized from ether, and ethyl 3-amino-2-(1-naphthylmethyl)propionate·HCl (1.14 g, 38.8%) was collected by filtration. To a stirred 0 °C solution of the propionate (500 mg, 1.7 mmol) and Et_3N (1 ml, 5 mmol) in CH_2Cl_2 (30 ml) was added phenylacetyl chloride (264 mg, 1.7 mmol). After 1 h at 0°C, ethyl acetate and 2 N HCl were added and then the organic layer was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with $CHCl_3$ and benzene (1 : 1) for eluent to afford ethyl 2-(1-naphthylmethyl)-3-(phenylacetylamino)propionate (428 mg, 67.1%). To a solution of the ester (426 mg, 1.13 mmol) in ethanol (10 ml) was added 1 N NaOH (1.25 ml, 1.25 mmol) and stirred at room temperature overnight. The reaction mixture was evaporated and washed with ether. The aqueous solution was acidified with conc. HCl and extracted with $CHCl_3$. The combined layers were washed with brine, dried over $MgSO_4$, and evaporated to give **5** (370 mg, 94.4%) as a white solid: mp 130—133 °C. IR (KBr): 1700, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.7—1.85 (m, 1H), 2.6—3.2 (m, 4H), 3.4—3.6 (m, 3H), 5.9—6.05 (m, 1H), 6.85—7.05 (m, 1H), 7.1—7.6 (m, 8H), 7.73 (d, 1H, J=7.3 Hz), 7.84 (d, 1H, J=8.8 Hz), 7.96 (d, 1H, J=8.8 Hz). *Anal.* Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C. 76.33; H, 6.01; N, 3.75.

3-(1-Naphthyl)-2-(*N*-phenethylcarbamoyl)propionic Acid (7) To a solution of **4** (4.5 g, 17.8 mmol) in ethanol (30 ml) was added 2 N NaOH (10 ml, 20.0 mmol) and then stirred overnight. After removal of the solvent, water and ether were added to the residue. The aqueous layer was acidified with 2 N HCl and extracted with ether. The ether solution was washed with brine, dried over $MgSO_4$, and evaporated to afford 2-cyano-3-(1-naphthyl)propionic acid (**6**) (4.0 g, quant.). To a solution of **6** (2.25 g, 10.0 mmol) in CH_2Cl_2 (20 ml) was added *N*,*N*′-carbonyldiimidazole (1.95 g, 12.0 mmol), and after stirring for 20 min, phenethylamine (1.25 ml, 10.0 mmol) was added. After stirring overnight, the reaction mixture was evaporated and ethyl acetate was added to the residue. The ethyl acetate solution was washed wtih 2N HCl, 5% $NaHCO_3$, and brine, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography with benzene and ethyl acetate (100 : 1 to 10 : 1) for eluent to give *N*-phenethyl 3-(1-naphthyl)cyanopropionamide (2.3 g, 70.0%). A solution of the amide (2.2 g, 9.1 mmol) and KOH (18 g, 0.32 mol) in ethanol (30 ml) and water (60 ml) was refluxed overnight. After the removal of the solvent, water and ether were added to the residue. The aqueous layer was acidified with 2 N HCl and extracted with ether. The ether solution was washed with brine, dried over $MgSO_4$, and evaporated to afford **7** (1.18 g, 37%) as a white solid: mp 144—147 °C. IR (KBr): 1750, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.0—3.7 (m, 7H), 7.02 (d, 2H, J=7.1 Hz), 7.1—7.65 (m, 7H). 7.80 (d, 1H, J=8.2 Hz), 7.93 (d, 1H, J=7.1 Hz), 8.0—8.15 (m, 2H). *Anal.* Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 75.79; H, 5.95; N, 3.82.

3-(*N*-Benzylcarbamoyl)-2-(1-naphthylmethyl)propionic Acid (9a) To a suspension of **8**[8] (5.1 g, 19.9 mmol) in CH_2Cl_2 (50 ml) was added thionyl chloride (14.5 ml, 2 mol), followed by reflux until the suspension was homogenated (2—3 h). The solution was concentrated under reduced pressure and benzene (12.5 ml) and hexane (37.5 ml) were added to the residue to afford 2-(1-naphthylmethylidene)succinic anhydride (4.2 g, 88%) as orange crystals. To a suspension of the anhydride (500 mg, 2.1 mmol) in CH_2Cl_2 (20 ml) was added benzylamine (225 mg, 2.1 mmol), followed by stirring overnight to give 3-(benzylcarbamonyl)-2-(1-naphthylmethylidene)propionic acid (510 mg, 70.3%) as white crystals. A suspension of the acid (495 mg, 1.4 mmol) and 10% Pd on activated carbon (50 mg) in acetic acid (50 ml) was hydrogenated at atmospheric pressure overnight. After Pd on activated carbon was filtered out, the filtrate was concentrated *in vacuo* and hexane was added to the residue to afford **9a** (395 mg, 81.2%) as a white powder: mp 134—138 °C. IR (KBr): 1705, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.25—2.65 (m, 2H), 3.05—3.4 (m, 3H), 4.25 (d, 2H, J=5.5 Hz), 7.15—8.2 (m, 12H), 8.35—8.55 (br, 1H). *Anal.* Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 76.29; H, 6.15; N, 3.82.

2-(1-Naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionic Acid (9b) The synthesis of **9b** was carried out with phenethylamine as described above for **9a** to afford **9b** as a white solid: mp 131—135 °C. IR (KBr) 1720, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.15—2.55 (m, 2H), 2.68 (t, 2H, J=7.1 Hz), 3.0—3.5 (m, 5H), 7.1—8.2 (m, 13H). *Anal.* Calcd for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.74; H, 6.52; N, 3.76.

L-Histidyl-L-leucinol·2*p*-toluenesulfonic Acid (11) The azide [prepared from PMZ–His–$NHNH_2$ **10** (4.1 g, 12.3 mmol)] in dimethylformamide (DMF) (30 ml) was added to a −30 °C stirred solution of L-leucinol·HCl (1.57 g, 10.2 mmol) and Et_3N (1.67 ml, 12.0 mmol) in DMF (38 ml). After 16 h at 0°C, the solvent was removed *in vacuo* and 5% $NaHCO_3$ was added to the residue. The aqueous solution was extracted with ethyl acetate and the organic solution was washed with water, dried over $MgSO_4$, and evaporated to obtain PMZ–His–L-leucinol (3.70 g, 93%). A suspension of the PMZ–His-L-leucinol (3.60 g, 9.0 mmol), *p*-toluenesulfonic acid (3.1 g, 18.0 mmol), and 10% Pd (0.36 g) in methanol (100 ml) was hydrogenated at atmospheric pressure. After the removal of 10% Pd, the solution was evaporated to give **11** (4.6 g, 85%) as a white solid: mp 201—209 °C $[\alpha]_D^{16}$ −3.69 (c=0.65, MeOH). IR (KBr): 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (t, 6H, J=6.1 Hz), 1.27 (t, 2H, J=7.1 Hz), 1.55—1.65 (m, 1H), 2.29 (s, 6H), 3.17 (d, 2H, J=5.6 Hz), 3.25 (d, 2H, J=5.5 Hz), 3.8—3.9 (m, 1H), 4.05—4.15 (m, 1H), 7.12 (d, 4H, J=8.2 Hz), 7.50 (d, 4H, J=8.2 Hz), 8.16 (d, 1H, J=8.2 Hz), 9.00 (s, 1H). *Anal.* Calcd for $C_{12}H_{22}N_4O_2 \cdot 2C_7H_8O_3S$: C, 52.16; H, 6.40; N, 9.36. Found: C, 51.71; H, 6.62; N, 9.07.

2-(1-Naphthylmethyl)-3-(phenylacetylamino)propionyl-L-histidyl-L-leucinol (12b) To a stirred 0 °C solution of **5** (140 mg, 0.4 mmol) and **11** (241 mg, 0.5 mmol) in DMF (5 ml) were added Et_3N (0.19 ml, 1.4 mmol) and DPPA (0.108 ml, 0.5 mmol). After stirring overnight, the mixture was dissolved in ethyl acetate and the solution was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with $CHCl_3$ and methanol (10 : 1) for eluent (Rf=0.42) to afford **12b** (83 mg, 36%) as a white powder: mp 98—101 °C. $[\alpha]_D^{24}$ −11.3° (c=1.13, MeOH). IR (KBr): 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.78 (d, 3H, J=6.1 Hz), 0.82 (d, 3H, J=6.6 Hz), 1.1—1.6 (m, 3H), 2.7—3.3 (m, 7H), 3.40 (s, 2H), 3.65—3.85 (m, 1H), 4.35—4.5 (m, 1H), 6.80 (s, 1H), 7.1—7.6 (m, 9H), 7.75 (d, 1H, J=8.2 Hz), 7.85—8.1 (m, 2H), 8.31 (s, 1H). HPLC 95% purity (column, YMC-Packed Column R-ODS-5, 4.6 × 250 mm; solvent, acetonitrile/0.05 M NH_4Ac (11/9); flow rate, 1 ml/min; time 5.1 min); fast atom bombardment mass spectrum (FABMS) m/z: 584 (M+1). *Anal.* Calcd for $C_{34}H_{41}N_5O_4 \cdot 1/4CHCl_3$: C, 67.05; H, 6.78; N, 11.41. Found C, 66.91; H, 6.85; N, 11.12.

12a—e were prepared by essentially the same procedure for preparing **12b**.

Benzyloxycarbonyl-L-(1-naphthyl)alanyl-L-histidyl-L-leucinol (12a) (Yield 50.5%) as a white solid: mp 102—105 °C. $[\alpha]_D^{24}$ −23.8° (c=0.58, MeOH). Rf=0.55. IR (KBr): 1705, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.83 (d, 6H, J=6.7 Hz), 1.1—1.4 (m, 3H), 2.9—4.0 (m, 8H), 4.4—4.55 (m, 2H), 5.02 (d, 1H, J=11.5 Hz), 5.4—5.5 (m, 1H), 6.2—6.8 (m, 2H), 7.2—7.6 (m, 9H), 7.77 (d, 1H, J=7.7 Hz), 7.86 (d, 1H, J=6.6 Hz). HPLC 94% purity (time 7.1 min). FABMS m/z: 586 (M+1). *Anal.* Calcd for $C_{33}H_{39}N_5O_5 \cdot 1/5CHCl_3$: 65.42; H, 6.48; N, 11.49. Found: C, 65.18; H, 6.36; N, 11.21.

2-(1-Naphthylmethyl)-2-(*N*-phenethylcarbamoyl)acetyl-L-histidyl-L-leucinol (12c) (Yield 47%) as a white solid: mp 177—181 °C. $[\alpha]_D^{24}$ −34.0° (c=0.84, MeOH). Rf=0.61. IR (KBr): 1655 cm^{-1}. ^{1}H-NMR (DMSO-d_6)

δ: 0.7—0.9 (m, 6H), 1.15—1.6 (m, 3H), 2.3—3.9 (m, 12H), 4.35—4.55 (m, 1H), 6.55—6.8 (m, 1H), 7.01 (d, 1H, J=6.6 Hz), 7.06 (d, 1H, J=6.6 Hz), 7.1—7.7 (m, 7H), 7.79 (d, 1H, J=8.2 Hz), 7.93 (d, 1H, J=8.2 Hz), 8.0—8.15 (m, 2H). HPLC 98% purity (time 6.5 min). FABMS m/z: 584 (M+1). *Anal.* Calcd for $C_{34}H_{41}N_5O_4 \cdot 1/5CHCl_3$: C, 67.61; H, 6.83; N, 11.53. Found: C, 67.43; H, 6.79: N, 11.41.

3-(*N*-Benzylcarbamoyl)-2-(1-naphthylmethyl)propionyl-L-histidyl-L-leucinol (12d) (Yield 22%) as a white solid: mp 105—110 °C. $[\alpha]_D^{24}$ −10.6° (c=0.51, MeOH). Rf= 0.38. IR (KBr): 1650 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.77 (t, 6H, J=7.4 Hz), 1.2—1.55 (m, 3H), 2.3—3.6 (m, 8H), 3.7—3.9 (m, 1H), 4.1—4.4 (m, 3H), 6.61 (s, 1H), 7.1—7.65 (m, 8H), 7.78 (d, 1H, J=8.2 Hz), 7.91 (d, 1H, J=7.7 Hz), 8.11 (d, 1H, J=8.2 Hz), 8.18 (d, 1H, J=7.7 Hz), 8.31 (s, 1H), 8.4—8.55 (m, 1H). HPLC 92% purity (time 5.9 min); FABMS m/z: 584 (M+1). *Anal.* Calcd for $C_{34}H_{41}N_5O_4 \cdot 1/4CHCl_3$: C, 67.05; H, 6.78; N, 11.41. Found: C, 66.83; H, 6.81; N, 11.19.

2-(1-Naphthylmethyl)-3-(*N*-phenethylcarbamoyl)-propionyl-L-histidyl-L-leucinol (12e) (Yield 22%) as a white solid: mp 96—99 °C. $[\alpha]_D^{24}$ −12.3° (c=0.91, MeOH). Rf=0.42; IR (KBr): 1650 cm^{-1} ^{1}H-NMR ($CDCl_3$) δ: 0.84 (d, 3H, J=4.4 Hz), 0.87 (d, 3H, J=5.0 Hz), 1.15—1.55 (m, 3H), 2.3—3.7 (m, 14H), 3.8—4.0 (m, 1H), 4.45—4.55 (m, 1H), 5.85—6.0 (m, 1H), 6.54 (d, 1H, J=8.2 Hz), 6.89 (s, 1H), 7.1—7.6 (m, 9H), 7.71 (d, 1H, J=7.7 Hz), 7.83 (d, 1H, J=7.7 Hz) 8.02 (d, 1H, J=9.3 Hz). HPLC 96% purity (time 6.3 min). FABMS m/z: 598 (M+1). *Anal.* Calcd for $C_{35}H_{43}N_5O_4 \cdot 1/4CHCl_3$: C, 67.46; H, 6.95; N, 11.16. Found: C, 67.21; H, 7.03; N, 11.01.

(*R*)-2-(1-Naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionic Acid (2) Recrystallization of **9b** (500 mg, 1.4 mmol) and (*R*)-α-methylbenzylamine (168 mg, 1.4 mmol) from ethyl acetate gave **2**·(*R*)-α-methylbenzylamine salt. To the salt was added 1 N HCl, and the aqueous solution was extracted with ethyl acetate. The organic solution was washed with brine, dried over $MgSO_4$, and evaporated to give **2** (110 mg). $[\alpha]_D^{22}$ +7.3° (c=1.0, MeOH). Other physical and spectral characteristics were identical to those of **9b**.

***N*-[(2*R*)-2-(1-Naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionyl]-L-histidine Hydrazide (13)** To a 0 °C stirred solution of **2** (3.0 g, 8.3 mmol) and His–OMe·2HCl (2.01 g, 8.3 mmol) in DMF (24 ml) were added DPPA (2.16 ml, 10.0 mmol) and Et_3N (3.81 ml, 27.4 mmol). After 16 h, the solvent was removed and ethyl acetate was added to the residue. The organic layer was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated *in vacuo* to give *N*-[(2*R*)-2-(1-naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionyl]-L-histidine methyl ester (4.08 g, 96%). To a solution of the ester (4.0 g, 7.8 mmol) in methanol (25 ml) was added $NH_2NH_2 \cdot H_2O$ (2.75 g, 54.9 mmol). After standing overnight, **13** (3.72 g, 93%) was obtained by filtration as a white solid: mp 214—218°C. $[\alpha]_D^{10}$ +20.6° (c=0.19, MeOH). IR (KBr): 1620 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.1—3.5 (m, 11H), 3.9—4.5 (m, 3H), 6.75 (s, 1H), 7.05—7.3 (m, 4H), 7.38 (t, 1H, J=7.7 Hz), 7.45—7.7 (m, 2H), 7.76 (d, 2H, J=8.2 Hz), 7.91 (d, 2H, J=7.2 Hz), 7.95—8.1 (m, 2H), 8.17 (d, 1H, J=7.7 Hz), 8.75 (s, 1H). FABMS m/z: 513 (M+1). *Anal.* Calcd for $C_{29}H_{32}N_6O_3$: C, 67.95; H, 6.29; N, 16.39. Found: C, 68.29; H, 6.35; N, 16.21.

Methyl (2*RS*,3*S*)-3-Amino-2-hydroxy-5-methylhexanoate·HCl (14b) The synthesis of (2*RS*, 3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid (2*R*:2*S*∼7:3) was carried out as described[11] using Boc-L-leucinol as a starting material. Then, the acid (2*R*:2*S*∼7:3) was esterified to give **14b** as a viscous oil. IR (neat): 1740 cm^{-1}. ^{1}H-NMR (D_2O) δ: 0.85—1.0 (m, 6H), 1.4—1.9 (m, 3H), 3.65—3.8 (m, 1H), 3.83 (s, 1H), 4.45—4.7 (m, 1H), 4.49 (d, 0.7H, J=3.8 Hz), 4.62 (d, 0.3H, J=3.8 Hz). *Anal.* Calcd for $C_8H_{17}NO_3 \cdot HCl$: C, 45.39; H, 8.57; N, 6.62. Found: C, 45.01; H, 8.31; N, 6.33.

***N*-Isoamyl (2*RS*,3*S*)-3-Amino-2-hydroxy-5-methylhexanamide·HCl (14a)** The synthesis of **14a** was carried out as described[11] using (2*RS*, 3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid (2*R*:2*S*∼7:3) as a staring material. IR (KBr): 1640 cm^{-1}. ^{1}H-NMR (D_2O) δ: 0.8—1.0 (m, 12H), 1.2—1.8 (m, 6H), 3.1—3.8 (m, 4H), 4.21 (d, 0.7H, J=4.4 Hz), 4.40 (d, 0.3H, J=3.1 Hz). *Anal.* Calcd for $C_{12}H_{26}N_2O_2 \cdot HCl$: C, 54.02; H, 10.20; N, 10.50. Found: C, 53.67; H, 10.31; N, 10.33.

Methyl (2*R*,3*S*)-3-{*N*-[(2*R*)-2-(1-Naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionyl]-L-histidyl}amino-2-hydroxy-5-methylhexanoate (1b) The azide [prepared from **13** (154 mg, 0.3 mmol)] in DMF (4 ml) was added to a −30 °C stirred solution of **14b** (64 mg, 0.3 mmol) and Et_3N (90 μl, 0.6 mmol) in DMF (2 ml). After 16 h at 0 °C, the solvent was removed *in vacuo* and 5% $NaHCO_3$ was added to the residue. The aqueous solution was extracted with ethyl acetate and the organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was separated by silica gel chromatography with $CHCl_3$ and methanol (1:1) for eluent (Rf=0.58) to afford **1b** (66 mg, 34%) as a white solid: mp 91—95 °C. $[\alpha]_D^{24}$ −24.2° (c=0.19, MeOH). IR (KBr): 1745, 1650 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.81 (t, 6H, J=6.0 Hz), 1.1—1.6 (m, 3H), 2.1—3.5 (m, 11H), 3.53 (s, 3H), 4.0—4.2 (m, 2H), 4.3—4.5 (m, 1H), 4.8—5.0 (m, 1H), 6.6—6.8 (m, 1H), 7.1—7.3 (m, 4H), 7.35 (t, 1H, J=7.7 Hz), 7.4—7.65 (m, 3H), 7.75 (d, 1H, J=6.0 Hz), 7.90 (d, 1H, J=7.1 Hz), 7.97 (d, 1H, J=4.4 Hz), 8.1—8.3 (m, 2H). HPLC 86% purity (time 7.0 min). FABMS m/z: 656 (M+1). *Anal.* Calcd for $C_{37}H_{45}N_5O_6 \cdot 1/5CHCl_3$: C, 65.74; H, 6.70; N, 10.30. Found: C, 65.41; H, 6.69; N, 10.03.

1a and **1c** were prepared by essentially the same procedure for preparing **1b**.

***N*-Isoamyl (2*RS*,3*S*)-3-{*N*-[(2*R*)-2-(1-Naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionyl]-L-histidyl}amino-2-hydroxy-5-methylhexanamide (1a)** (Yield 11%) as a white solid: mp 103—107 °C. IR (KBr): 1650 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.65—0.9 (m, 12H), 1.2—1.6 (m, 6H), 2.1—3.6 (m, 12H), 3.7—4.5 (m, 3H), 6.5—6.7 (m, 1H), 7.1—8.25 (m, 13H). HPLC 91 [58 (2*R*), 33 (2*S*)]% purity (time 10.4, 13.7 min). FABMS m/z: 711 (M+1). *Anal.* Calcd for $C_{41}H_{54}N_6O_5 \cdot 1/4CHCl_3$: C, 66.88; H, 7.38; N, 11.35. Found: C, 66.61; H, 7.29; N, 11.17.

Methyl (3*S*,4*S*)-4-{*N*-[(2*R*)-2-(1-Naphthylmethyl)-3-(*N*-phenethylcarbamoyl)propionyl]-L-histidyl}amino-3-hydroxy-6-methylpentanoate (1c) (Yield 16%) as a white solid: mp 90—94 °C. $[\alpha]_D^{24}$ −46.7° (c=0.30, MeOH), Rf=0.67. IR (KBr): 1735, 1640 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.80 (t, 6H, J=6.6 Hz), 1.1—1.6 (m, 3H), 2.05—3.4 (m, 13H), 3.54 (s, 3H), 3.7—3.9 (m, 2H), 4.3—4.45 (m, 1H), 5.0—5.15 (m, 1H), 6.80 (s, 1H), 7.05—7.6 (m, 8H), 7.76 (d, 1H, J=7.7 Hz), 7.91 (d, 1H, J=7.2), 8.0—8.1 (m, 1H), 8.14 (d, 1H, J=7.7 Hz), 8.20 (d, 1H, J=7.7 Hz). HPLC 86% purity (time 7.1 min). FABMS m/z: 670 (M+1). *Anal.* Calcd for $C_{38}H_{47}N_5O_6 \cdot 1/4CHCl_3$: C, 65.66; H, 6.80; N, 10.01. Found: C, 65.31; H, 6.61; N, 9.83.

Biological Methods *In Vitro* Renin Assay: A 25 μl aqueous solution of human renin (20—30 ng of angiotensin I/ml per hour) was incubated at 37 °C with a mixture of sheep angiotensinogen (2000 ng of angiotensin I/ml, 50 μl), Phe–Ala–Pro (25 μl of 20 mM aqueous solution), a DMSO solution of the inhibitor (50 μl), water (150 μl), and 125 mM of pyrophosphate buffer (pH 7.4, 200 μl). The angiotensin I that formed after 15 min of incubation was measured by radioimmunoassay. A human plasma (500 μl) containing ethylenediaminetetraacetic acid (EDTA)·2Na (14 mM) and neomycin sulfate (0.3%) was added to a mixture of 0.5 M phosphate buffer (pH 7.0, 350 μl), Phe–Ala–Pro (50 μl of 20 mM aqueous solution) and a DMSO solution of the inhibitor (100 μl). After incubation (37 °C, 60 min), the angiotensin I produced was measured by radioimmunoassay.

In Vitro Stability Assay: The degradation of the inhibitors with 0.1 mg/ml of bovine chymotrypsin (Sigma) in a 0.1 M borate buffer (pH 8.0) at 37°C was assayed by measuring the decrease of renin inhibition of the human renin-sheep substrate.

References and Notes

1) Preliminary report: K. Iizuka, T. Kamijo, H. Harada, K. Akahane, T. Kubota, I. Shimaoka, H. Umeyama, and Y. Kiso, *Chem. Pharm. Bull.*, **36**, 2278 (1988).
2) *a*) W. J. Greenlee, *Pharmaceutical Research*, **4**, 364 (1987); *b*) J. Boger, *Trends. Pharmacol. Sci.*, **8**, 370 (1987).
3) *a*) M. Szelke, B. Leckie, A. Hallett, D. M. Jones, J. Sueiras, B. Atrash, and A. F. Lever, *Nature* (London) **299**, 555 (1982); *b*) M. Szelke, D. M. Jones, B. Atrash, A. Hallett, and B. Leckie, "Peptides: Structure and Function", Proc. 8th Amer. Peptide Symp., ed by V. J. Hruby and D. H. Rich, Pierce Chem. Co., Rockford, Ill, 1983, p. 579; *c*) J. Boger, N. S. Lohr, E. H. Ulm, M. Poe, E. H. Blaine, G. M. Fanelli, T.-Y. Lin, L. S. Payne, T. W. Schorn, B. I. LaMont, T. C. Vassil, I. I. Stabilito, D. F. Veber, D. H. Rich, and A. Bopari, *Nature* (London), **303**, 81 (1983).
4) The positions (P) and subsites (S) are indicated according to the scheme of Schechter and Berger: I. Schechter and A. Berger, *Biochem. Biophys. Res. Commun.*, **27**, 157 (1967).
5) T. Kokubu, K. Hiwada, Y. Sato, T. Iwata, Y. Imamura, R. Matsueda, Y. Yabe, H. Kogen, M. Yamazaki, Y. Iijima, and Y. Baba, *Biochem. Biophys. Res. Commun.*, **118**, 929 (1984).
6) K. Akahane, H. Umeyama, S. Nakagawa, I. Moriguchi, S. Hirose, K. Iizuka, and K. Murakami, *Hypertension*, **7**, 3 (1985).
7) T. Kubota, S. Maruyama, M. Murakami, I. Shimaoka, Y. Etoh, K. Iizuka, H. Koizumi, and Y. Tanioka, In Abstracts of 37th Meeting of North Branch, The Japanese Pharmacological Society, Hokkaido, 1986, p. 33.
8) H. Harada, T. Yamaguchi, A. Iyobe, A. Tsubaki, T. Kamijo, K.

Iizuka, K. Ogura, and Y. Kiso, *J. Org. Chem.*, **55**, 1679 (1990).
9) H. Yajima, F. Tamura, Y. Kiso, and M. Kurobe, *Chem. Pharm. Bull.*, **21**, 1380 (1973).
10) T. Shioiri, K. Ninomiya, and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972).
11) K. Iizuka, T. Kamijo, H. Harada, K. Akahane, T. Kubota, Y. Etoh, I. Shimaoka, A. Tsubaki, M. Murakami, T. Yamaguchi, A. Iyobe, H. Umeyama, and Y. Kiso, *Chem. Pharm. Bull.*, **38**, 2487 (1990).
12) Statine methyl ester (**15c**) was prepared from commercially available statine with methanolic HCl.
13) *a*) K. Iizuka, T. Kamijo, H. Harada, K. Akahane, T. Kubota, H. Umeyama, and Y. Kiso, *J. Chem. Soc., Chem. Commun.*, **1989**, 1678; *b*) K. Iizuka, T. Kamijo, H. Harada, K. Akahane, T. Kubata, H. Umeyama, T. Ishida, and Y. Kiso, *J. Med. Chem.*, **33**, 2707 (1990).
14) N. Toda, M. Miyazaki, Y. Etoh, T. Kubota, and K. Iizuka, *Eur. J. Pharmacol.*, **129**, 393 (1986).