Renin Inhibitors. I. Synthesis and Structure–Activity Relationships of Transition-State Inhibitors Containing Homostatine Analogues at the Scissile Bond

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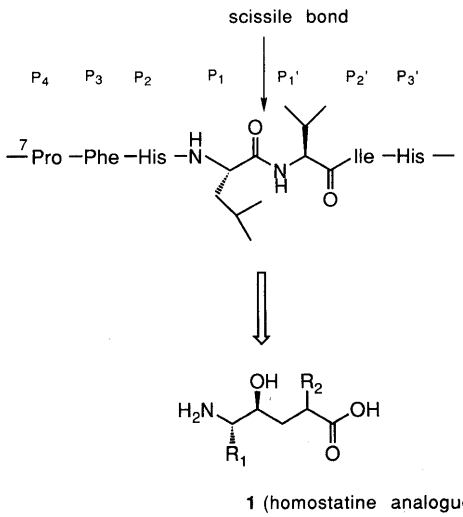
The synthesis and structure–activity relationships of transition-state renin inhibitors containing the homostatine analogues at the scissile bond are described. These inhibitors incorporate the amino acid side chains corresponding to positions 7—12 (P_4–P_2') of angiotensinogen. Ethyl, 2-hydroxyethyl and 3-hydroxypropyl groups at position 2 of the homostatine analogues (P_1') are more effective for increasing potency than the isopropyl group. A combination of residues at P_1, P_3 and P_4 is important for potency and this result suggests that S_1, S_3 and S_4 form a huge hydrophobic core together in renin.

Keywords renin inhibitor; antihypertensive agent; homostatine analogue; transition-state analogue; structure–activity relationship

Renin is an aspartic protease which cleaves the amino terminal decapeptide from angiotensinogen to yield angiotensin I. Angiotensin I is hemodynamically inactive, but it is transformed by angiotensin-converting enzyme (ACE) into the octapeptide angiotensin II, which is a very potent vasoconstrictor. This renin–angiotensin system (RAS) is believed to play a central role in the regulation of blood pressure. Actually, the inhibition of RAS with ACE inhibitors is clinically effective in many hypertensive patients.[1] However, ACE inhibitors have some troubling side effects (*e.g.*, cough and angioneurotic edema) due to the lower specificity of ACE to angiotensin I.[2] Renin catalyzes the first and rate-limiting step in the RAS, and angiotensinogen is the only known substrate of renin. Therefore, renin inhibitors could be more useful therapeutic agents than ACE inhibitors. A large number of renin inhibitors have been investigated as potential agents of antihypertensive therapy.[3] An effective approach to the development of highly potent renin inhibitors has focused on shortened analogues of the natural substrate, angiotensinogen, containing non-proteolytic dipeptide isosteres. The hydroxyethylene dipeptide isostere (homostatine analogue) (**1**) has been used as a transition-state analogue at the scissile bond, Leu[10]–Val[11], of human angiotensinogen.[4] Therefore, most of the homostatine analogues incorporated into potent renin inhibitors have an isopropyl group at position 2 (P_1'), and only a few variations of the 2-alkyl substituent of the homostatine analogues have been reported.[4j–m] We introduced many kinds of alkyl substituents into the homostatine analogue in order to improve the potency and water-solubility. In this paper we report the syntheses and the structure–activity relationships of the potent renin inhibitors containing these homostatine analogues.



1 (homostatine analogue)

Fig. 1

Synthesis The compounds prepared for this study are shown in Table I. Syntheses of **11**—**13** are outlined in Charts 1—3. In a similar manner, we synthesized the compounds shown in Table I. Aldehydes **6a**—**c** were chosen as P_1-containing partners and the P_1' fragment was provided by phosphonate **7** in the modified Horner–Wadsworth–Emmons (HWE) reactions.[5] A similar synthesis of a homostatine analogue has been reported by Wuts and co-workers.[6] The synthetic approach to amide **9a** starting from natural statine followed the method reported previously by us.[7] **9b**, **c** were synthesized as follows. Z-L-Cyclohexylalaninal (**4b**) and Z-L-phenylalaninal (**4c**) were prepared from the corresponding *N*-protected amino acids. Aldehyde **4b** was prepared by reduction of Z-L-cyclohexylalanine 3,5-dimethylpyrazolide with lithium aluminium hydride in tetrahydrofuran (THF) at $-30\,^{\circ}C$.[8] Aldehyde **4c** was prepared by oxidation of Z-L-phenylalaninol, obtained from the ester by $NaBH_4$–LiCl reduction,[9] with SO_3–pyridine complex in dimethyl sulfoxide (DMSO).[10] Treatment of aldehydes **4b**, **c** with vinylmagnesium bromide, followed by protection with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid provided the vinyl compounds **5b**, **c**. The Lemieux–Johnson oxidation[11] of **5b**, **c** yielded a 1 : 2—3 mixture of the *cis* and *trans* aldehydes of **6b** and **6c**. The equilibration of these aldehydes to the desired *trans* isomers with potassium carbonate in methanol gave a 1 : 10—11 *cis*/*trans* ratio of aldehydes **6b**, **c**. An ethyl group at position 2 of the homostatine analogue was introduced by phosphonate **7** obtained from triethylphosphonoacetate by alkylation with NaH–EtBr in dimethylformamide (DMF).[7] The modified HWE reactions of aldehydes **6b**, **c** with phosphonate **7** afforded 1 : 1 *E*/*Z* mixture of esters **8b**, **c**. Esters **8b**, **c** were hydrolyzed with potassium hydroxide

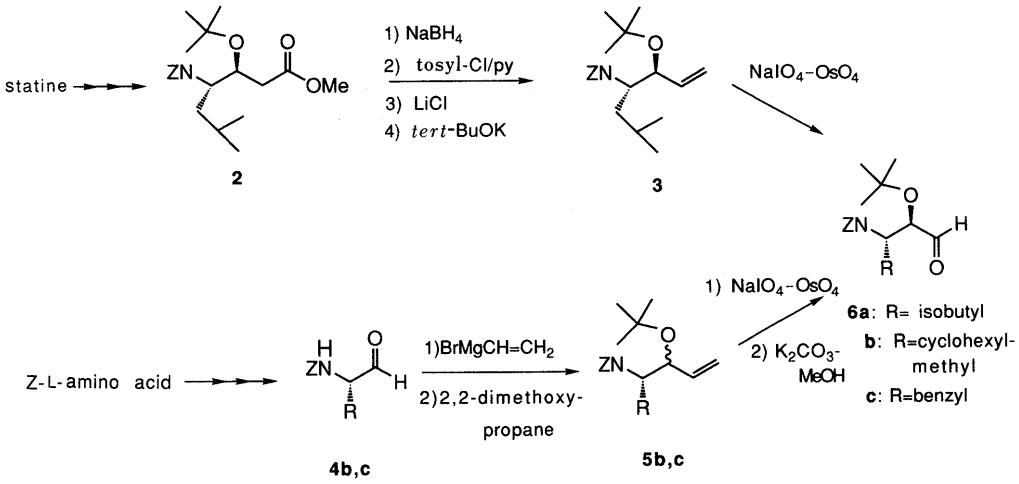


Chart 1

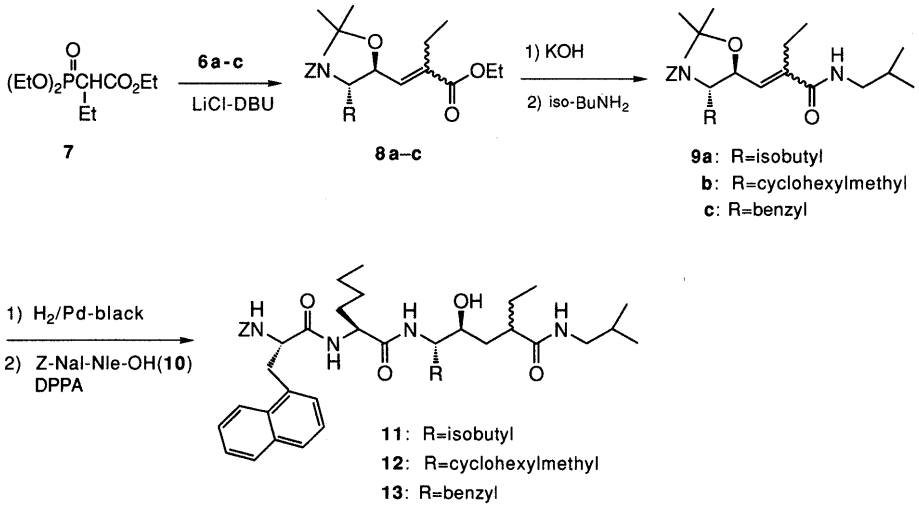


Chart 2

in aqueous ethanol and the resulting acids were condensed with isobutylamine using diphenylphosphorazidate (DPPA)[12] to yield an *E*/*Z* mixture of amides **9b**, **c**. The two desired 4*S* isomers [(2*E*,4*S*) and (2*Z*,4*S*)] were obtained by silica gel chromatography. Catalytic hydrogenation of **9a**—**c**, followed by coupling with Z–3-(1-naphthyl)-L-alanyl-L-norleucine (Z–Nal–Nle–OH, **10**) prepared by the route shown in Chart 5, using DPPA gave the potent renin inhibitors **11**—**13** as diastereomeric mixtures. Separation of the two diastereomers of **11** was performed by reverse phase high performance liquid chromatography (HPLC). Only the faster eluting isomer of **11** had activity against renin. The stereochemistry at position 2 of the homostatine analogue incorporated in the active isomer of **11** was determined by nuclear magnetic resonance (NMR) analysis of the corresponding lactones **14** and **15**[13] prepared by the method shown in Chart 3. Reduction of ester **8a** with $NaBH_4$–$NiCl_2$[14] followed by deprotection–lactonization by treatment with *p*-toluenesulfonic acid in methanol gave lactones **14** and **15**. These diastereomers could be separated by silica gel chromatography. An observed nuclear Overhauser enhancement between H3 and H5 in **15** indicated that the two substituents were present in a *cis* relationship on the lactone ring. *Trans* lactone **14** was readily converted to

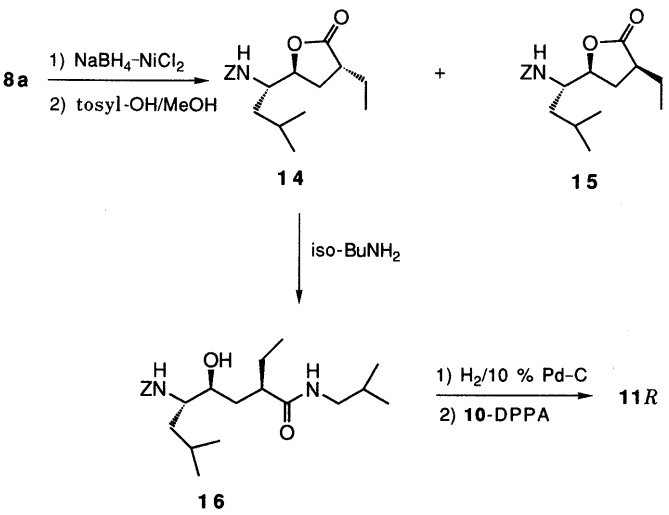


Chart 3

amide **16** by treatment with isobutylamine in good yield. Catalytic hydrogenation of **16** followed by the coupling with **10** gave **11***R*. The stereochemistry at position 2 of the active isomer of **11** was found to be *R*-configuration by comparing the HPLC retention time with that of the

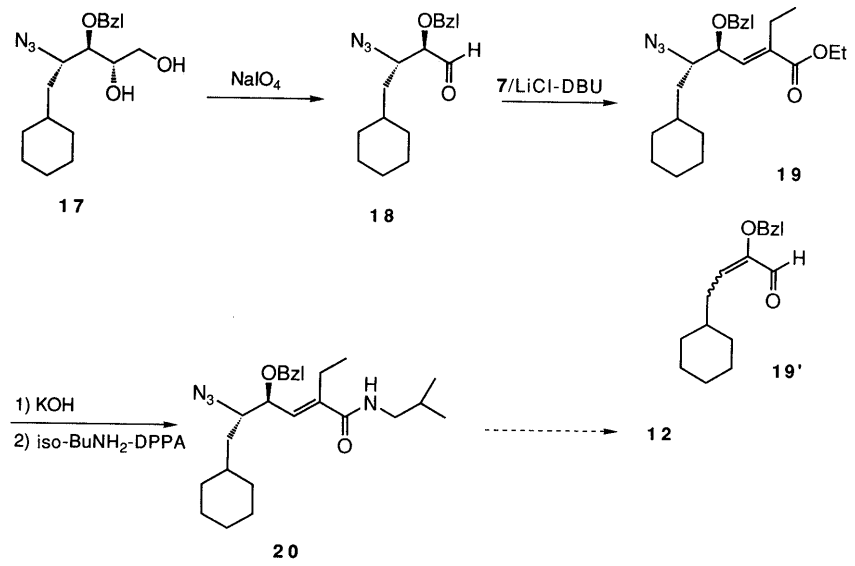


Chart 4

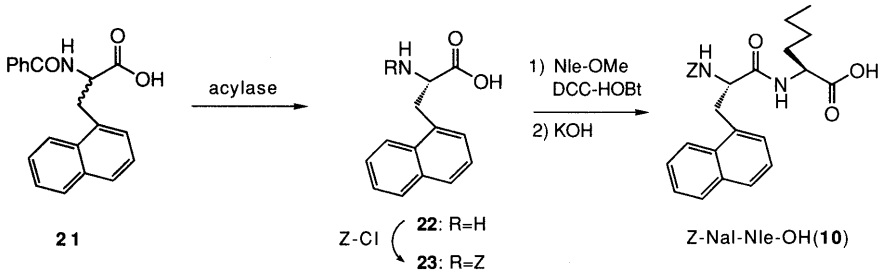


Chart 5

authentic sample derived from **14**. *R*-Configuration at this position corresponds to the absolute configuration of the L-amino acid.

The synthetic route to another precursor of the homostatine analogue is sown in Chart 4. Aldehyde **18** was used as a key intermediate instead of aldehyde **6b**. Aldehyde **18** was prepared from diol **17**, which was the intermediate of the synthesis of a novel dihydroxyethylene isostere,[15] by oxidation with $NaIO_4$. The homostatine analogue was synthesized using a route similar to that described above. The HWE reaction of **18** with phosphonate **7** gave ester **19** as an *E* isomer, but aldehyde **19'** was also obtained as a by-product. **19** was converted to **12** by catalytic hydrogenation followed by coupling with Z–Nal–Nle–OH (**10**).

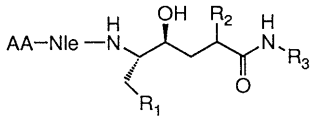
The synthetic route to Z–Nal–Nle–OH (**10**) is shown in Chart 5. Optically pure 3-(1-naphthyl)-L-alanine (Nal, **22**)[16] was prepared by optical resolution of the corresponding racemic *N*-benzoyl acid **21**[16b] using acylase found in our laboratories (originated from *Streptoverticillium rimofaciens*).[17] Acid **21** was obtained from 1-naphthaldehyde and hippuric acid by Erlenmeyer's method. This resolution was not catalyzed by commercially availabe acylases originating from *Aspergillus* sp. and porcine kidney. After benzyloxycarbonylation of amino acid **22**,[18] the coupling of the resulting acid **23** with L-norleucine methyl ester using the DCC–HOBT method, followed by the hydrolysis of the dipeptide ester yielded **10**.

Structure–Activity Relationships The renin inhibitory potencies of the compounds were measured with human plasma renin, and the IC_{50} values are summarized in Table I. Compounds **11** and **24**—**34** explore the variation of the P_1' side chain of the homostatine analogues. Since angiotensinogen, the natural substrate of renin, has an isopropyl group at the P_1' site, most renin inhibitors containing the homostatine analogues have an isopropyl group at this part of the molecule.[4a-i] However, our results indicated that the ethyl group was superior to the isopropyl group (compound **11***R* *vs.* compound **27**). Replacement of the side chain, an ethyl group, of **11***R* with less or more bulky alkyl groups reduced the potency (compounds **26**—**29**). While substitution with hydroxy-containing alkyl groups at position 2 of the homostatine analogue did not decrease potency (compounds **30** and **31**), substitution with an amino-containing alkyl group reduced potency dramatically (compound **34**).

Replacement of the isobutyl group at the P_2' subsite by more polar alkyl groups containing a nitrogen atom did not seriously affect potency (compounds **35** and **36**).

Substitution of the P_1 subsite of **11** with the more hydrophobic groups, cyclohexylmethyl and benzyl, noticeably decreased potency (compounds **12** and **13**). In most renin inhibitor series, the cyclohexylmethyl group is superior to isobutyl as the P_1 subsite.[4f,19] From this discrepancy, we deduced an interaction between P_1 and P_3 subsites. This inference could be confirmed by the fact that replacement of the naphthylmethyl group at the P_3 site in **11** by a benzyl group decreased potency (compound **37**), while the same

TABLE I. Structures and Renin Inhibitory Activities



No.	AA	R_1	R_2	R_3	IC_{50} (nM)
24	Z-Nal	Isopropyl	H	Isobutyl	820
25	Z-Nal	Isopropyl	Methyl(*RS*)	Isobutyl	27
11	Z-Nal	Isopropyl	Ethyl(*RS*)	Isobutyl	9.5
11*R*	Z-Nal	Isopropyl	Ethyl(*R*)	Isobutyl	5.2
11*S*	Z-Nal	Isopropyl	Ethyl(*S*)	Isobutyl	7200
26	Z-Nal	Isopropyl	*n*-Propyl(*R*)	Isobutyl	12
27	Z-Nal	Isopropyl	Isopropyl(*S*)	Isobutyl	13
28	Z-Nal	Isopropyl	Isobutyl(*R*)	Isobutyl	18
29	Z-Nal	Isopropyl	*tert*-Butoxycarbonyl-methyl(*RS*)	Isobutyl	$>1\times10^5$
30	Z-Nal	Isopropyl	2-Hydroxyethyl(*R*)	Isobutyl	4.2
31	Z-Nal	Isopropyl	3-Hydroxypropyl(*R*)	Isobutyl	4.9
32	Z-Nal	Isopropyl	2(*RS*)-Hydroxy-propyl(*RS*)	Isobutyl	15
33	Z-Nal	Isopropyl	(2*RS*)-2,3-Dihydroxy-propyl(*RS*)	Isobutyl	27
34	Z-Nal	Isopropyl	3-Aminopropyl(*RS*)	Isobutyl	4100
35	Z-Nal	Isopropyl	Ethyl(*RS*)	(4-Pyridyl)methyl	7.6
36	Z-Nal	Isopropyl		2-Morpholinoethyl	7.1
12	Z-Nal	Cyclohexyl	Ethyl(*RS*)	Isobutyl	30
13	Z-Nal	Phenyl	Ethyl(*RS*)	Isobutyl	36
37	Z-Phe	Isopropyl	Ethyl(*RS*)	Isobutyl	21
38	Z-Phe	Cyclohexyl	Ethyl(*RS*)	Isobutyl	6.7
39	Nal	Isopropyl	Ethyl(*RS*)	Isobutyl	8.6
40	Nal	Cyclohexyl	Ethyl(*RS*)	Isobutyl	5.4
41	Phe	Isopropyl	Ethyl(*RS*)	Isobutyl	260
42	Phe	Cyclohexyl	Ethyl(*RS*)	Isobutyl	12

TABLE II. Characterization of Renin Inhibitors

No.	t_R (min)[a]	Purity (%)	Formula	FAB-MS[c] Calcd	FAB-MS[c] Found
24	6.11	98	$C_{40}H_{56}N_4O_6$	689.4278	689.4269
25	6.36, 6.50	96	$C_{41}H_{58}N_4O_6$	703.4434	703.4326
11*R*	6.83	98	$C_{42}H_{60}N_4O_6$	717.4591	717.4550
11*S*	7.46	98	$C_{42}H_{60}N_4O_6$	717.4591	717.4567
26	7.26	99	$C_{43}H_{62}N_4O_6$	731.4747	731.4770
27	7.19	99	$C_{43}H_{62}N_4O_6$	731.4747	731.4723
28	9.34	99	$C_{44}H_{64}N_4O_6$	745.4904	745.4923
29	7.97, 8.18	86	$C_{46}H_{66}N_4O_8$	803.4959	803.4836
30	5.37	96	$C_{42}H_{60}N_4O_7$	733.4540	733.4552
31	5.70	98	$C_{43}H_{62}N_4O_7$	747.4697	747.4604
32	5.69, 6.07	87	$C_{43}H_{62}N_4O_6$	747.4697	747.4679
33	5.00, 5.42	86	$C_{43}H_{62}N_4O_8$	763.4646	763.4636
34	9.42, 11.54	84	$C_{43}H_{63}N_5O_6$	746.4857	746.4858
35	5.34	97	$C_{44}H_{57}N_5O_6$	752.4387	752.4396
36	6.81, 7.36	88	$C_{44}H_{63}N_5O_7$	774.4806	774.4841
12	8.69, 9.50	94	$C_{45}H_{64}N_4O_6$	757.4904	757.4929
13	6.76, 7.43	96	$C_{45}H_{58}N_4O_6$	751.4434	751.4429
37	5.44, 5.74	98	$C_{38}H_{58}N_4O_6$	667.4434	667.4412
38	6.80, 7.32	89	$C_{41}H_{62}N_4O_6$	707.4747	—[d]
39	7.04, 7.54[b]	81	$C_{34}H_{54}N_4O_4$	583.4223	583.4286
40	9.61, 10.52[b]	81	$C_{37}H_{58}N_4O_4$	623.4536	623.4522
41	5.41, 5.63[b]	82	$C_{33}H_{56}N_4O_4$	533.4067	533.4095
42	6.99, 7.49[b]	67	$C_{33}H_{56}N_4O_4$	573.4379	573.4406

a) See the experimental section for conditions. *b*) Solvent, MeOH : 10 mM $AcONH_4$ = 7 : 1. *c*) For $[M+H]^+$. *d*) Not determined.

replacement resulted in enhanced potency when the substituent at P_1 was cyclohexylmethyl (compound **12** *vs.* compound **38**). In addition, removal of the benzyloxycarbonyl group from compounds **11**, **12**, **37** and **38** showed an interesting result. The naphthylmethyl–cyclohexylmethyl combination at the P_3–P_1 sites increased potency (compound **12** *vs.* compound **40**), but the benzyl–isobutyl combination gave a 10-fold decrease in potency by removal of the benzyloxycarbonyl group (compound **37** *vs.* compound **41**). These results suggest that the combination of substituents at P_4, P_3 and P_1 subsites is very important for potency, and that the P_4 substituent can be removed from the structure of the inhibitor when the inhibitor has the best combination in the substituents at P_3–P_1. The results further suggest that S_1, S_3 and S_4 form a single huge hydrophobic core in the enzyme.

TABLE III. Enzyme Specificity

No.	Renin (Human)	IC_{50} (nM) Cathepsin D (Bovine)	Pepsin (Porcine)
11*R*	5.2	98	69
35	7.6	18	170
36	7.1	62	360

Enzyme Specificity Enzyme inhibition selectivity is important for clinical utility. High specificity has been reported for inhibitors containing neutral amino acids at the P_2 site,[20] although the contribution of His at the same position as the specificity is reported.[19f,21] However, compounds **11***R*, **35** and **36** inhibit cathepsin D and pepsin as well as renin (Table III).

Experimental

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a HITACHI 270-30 IR spectrophotometer. ^{1}H-NMR (300 MHz) spectra were recorded with a Varian VXR-300 spectrometer in deuteriochloroform ($CDCl_3$). Chemical shifts are reported relative to residual protons of deuterated NMR solvents. Fast atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS-DX 300 mass spectrometer. Optical rotations were determined with a Horiba SEPA-200 high sensitive polarimeter. Elemental analyses were measured by Sumika Chemical Analysis Service, Ltd. Analytical HPLC was carried out on a Hitachi L-6200 system, using packed column Inertsil octadecyl silica (ODS) (5 μm, 4.6 × 250 mm), and MeOH–water (90 : 10) elutions unless otherwise stated (flow rate; 1 ml/min), with ultraviolet (UV) detection at 254 nm (Hitachi L-4000 UV detector). Preparative HPLC was performed on a JASCO 880-PV system with UV detection on a SSC UV detector 3000-A. Thin-layer chromatography was performed on precoated Kieselgel 60F_{254} plates (E. Merck, 0.25 mm). Column chromatography was done on Kieselgel 60 (E. Merck, 70—230 mesh). The organic solutions were dried over $MgSO_4$ before vacuum evaporation.

(4*S*,5*RS*)-3-Benzyloxycarbonyl-4-cyclohexylmethyl-5-ethenyl-2,2-dimethyloxazolidine (5b) A solution of *N*-benzyloxycarbonyl-L-cyclohexylalanine 3,5-dimethyl-pyrazolide (9.4 g, 24.5 mmol) in dry THF (120 ml) was added to a suspension of lithium aluminum hydride (1.9 g, 48.9 mmol) in dry THF (120 ml) over a period of 45 min keeping the temperature at −20 °C. After being stirred at −30 °C for 20 min, 2 N HCl (25 ml) was added slowly at a temperature below −15 °C. After removal of aluminum hydroxide by suction, the solvent was evaporated. The residue was dissolved in diethyl ether (Et_2O), washed with 1 N HCl, water and brine. Drying followed by evaporation gave *N*-benzyloxycarbonyl-L-cyclohexylalaninal (8.1 g) as a colorless oil. This aldehyde (**4b**) was dissolved in dry THF (860 ml) and a 1.0 M solution of vinylmagnesium bromide in THF (86 ml, 86 mmol) was added at −78 °C under an argon atmosphere. The temperature of the mixture was raised to 0 °C for 2 h and the mixture was poured into saturated aqueous NH_4Cl. The resulting mixture was extracted with Et_2O and the organic layer was washed with brine. Drying followed by evaporation and purification by silica gel chromatography [ethylacetate (AcOEt) : toluene = 1 : 8] provided 4.4 g (56.7%) of clear oil. Three grams of this oil was dissolved in dichloromethane (CH_2Cl_2) (15 ml), and then 2,2-dimethoxypropane (12 ml) and *p*-toluenesulfonic acid (160 mg) were

added. The mixture was stirred overnight at room temperature and poured into saturated aqueous $NaHCO_3$. The mixture was extracted with AcOEt and the organic layer was washed with brine. Drying followed by evaporation and purification by silica gel chromatography (AcOEt : hexane = 1 : 20) afforded **5b** (3.1 g, 91.8%) as a pale brown oil. IR (neat): 1712 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.60—1.95 (19H, m), 3.80—4.00 (1H, br), 4.29 (2/3H, dd, J = 3.8, 7.1 Hz), 4.51 (1/3H, t, J = 6.3 Hz), 5.00—5.50 (4H, m), 5.25—6.00 (1H, m), 7.25—7.45 (5H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{22}H_{32}NO_3$ 358.2382. Found: 358.2370.

(4*S*,5*RS*)-4-Benzyl-3-benzyloxycarbonyl-5-ethenyl-2,2-dimethyloxazolidine (5c) A mixture of triethylamine (5.9 ml, 42.1 mmol) and sulfur trioxide–pyridine complex (6.84 g, 42.1 mmol) in DMF (40 ml) was added to a solution of *N*-benzyloxycarbonyl-L-phenylalaninol (4.0 g, 14.0 mmol) in DMF (40 ml) at room temperature. The mixture was stirred for 1 h at room temperature and poured into ice-water. The mixture was extracted with AcOEt and the organic layer was washed with 1 N HCl, saturated aqueous $NaHCO_3$, water and brine. Drying followed by evaporation gave *N*-benzyloxycarbonyl-L-phenylalaninal as a colorless solid. The title compound was prepared by a procedure similar to that described for **5b**, and was chromatographed on silica gel with AcOEt–hexane (1 : 20); 44.2% yield as a pale brown oil. IR (neat): 1707 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.15—1.80 (6H, m), 2.75—3.20 (2H, m), 3.95 (7/10H, m), 4.22—4.40 (1H, m), 4.58 (3/10H, m), 5.00—5.50 (4H, m), 5.65—5.80 (1H, m), 7.00—7.50 (10H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{22}H_{26}NO_3$: 352.1913. Found: 352.1880.

(4*S*,5*R*)-3-Benzyloxycarbonyl-4-cyclohexylmethyl-5-formyl-2,2-dimethyloxazolidine (6b) To a solution of **5b** (1.08 g, 3.00 mmol) in dioxane (8 ml) was added a solution of osmium tetroxide (39.0 mg, 0.15 mmol) in dioxane (6 ml) at room temperature. After being stirred for 10 min in the dark, the mixture was diluted with water (3 ml) and a solution of sodium periodate (1.30 g, 6.10 mmol) in water (9 ml) was added dropwise over a period of 40 min. The mixture was stirred for 2 h and the precipitate was removed by filtration. The filtrate was extracted with AcOEt and the organic layer was washed with 5% sodium sulfide, water and brine. Drying followed by evaporation gave crude **6b** (930 mg) as a mixture of *cis*/*trans* diastereomers (*trans*/*cis* = 2.6). To a solution of this aldehyde in methanol (MeOH, 10 ml) was added powdered anhydrous potassium carbonate (358 mg, 2.59 mmol). After being stirred for 2 h at room temperature, the mixture was treated with acetic acid (0.35 ml, 6.00 mmol) and 1 M phosphate buffer (pH 7.0, 12 ml) at 0 °C. The mixture was stirred for 30 min at room temperature and concentrated. The resulting mixture was extracted with AcOEt and the organic layer was washed with brine. Drying followed by evaporation and purification by silica gel chromatography (AcOEt : hexane = 2 : 5) afforded **6b** (834 mg, 77.0%, *trans*/*cis* = 11) as a colorless oil. $[\alpha]_D^{20}$ −7.8° (c = 0.92, $CHCl_3$). IR (neat): 3430, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.62—2.00 (13H, m), 4.16 (1H, s), 4.25—4.40 (1H, br), 5.05 and 5.16 (2H, ABq, J = 12 Hz), 7.25—7.45 (5H, m), 9.72 (1/12H, d, J = 1.7 Hz), 9.81 (11/12H, s).

(4*S*,5*R*)-4-Benzyl-3-benzyloxycarbonyl-5-formyl-2,2-dimethyloxazolidine (6c) The title compound was prepared from **5c** by a procedure similar to that described for **6b**, and was chromatographed on silica gel with AcOEt–hexane (1 : 20); 95% yield (*trans*/*cis* = 11) as a colorless oil. $[\alpha]_D^{20}$ −18.5° (c = 1.04, $CHCl_3$). IR (neat): 3442, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.95 (6H, m), 2.65—3.45 (2H, m), 4.10—4.28 (1H, m), 4.52 (1H, m), 4.98—5.32 (2H, m), 6.90—7.75 (10H, m), 9.42 (1/12H, br s), 9.65 (11/12H, br s).

(4*S*,5*S*)-3-Benzyloxycarbonyl-4-cyclohexylmethyl-5-(2-ethoxycarbonyl-1-butenyl)-2,2-dimethyloxazolidine (8b) To a stirred suspension of lithium chloride (141 mg, 3.33 mmol) in dry THF (10 ml) were added ethyl 2-(diethoxyphosphinyl)butanoate **7** (671 mg, 2.66 mmol) and a solution of 1,8-diazabicyclo[5.4.0]-7-undecene (507 mg, 3.33 mmol) in benzene (1 ml) at room temperature. After being stirred for 10 min, a solution of **6b** (796 mg, 2.22 mmol) in dry THF (10 ml) was added to the mixture and it was stirred at room temperature overnight. The reaction mixture was acidified to pH 2 with 1 N HCl and extracted with AcOEt, and the organic layer was washed with brine. Drying followed by evaporation and purification by silica gel chromatography (AcOEt : hexane = 1 : 10) afforded **8b** (869 mg, 85.8%) as a colorless oil. $[\alpha]_D^{20}$ −24.9° (c = 1.08, $CHCl_3$). IR (neat): 1713 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.65—1.90 (25H, m), 2.25—2.50 (2H, m), 3.75—4.00 (1H, br), 4.15—4.30 (2H, m), 4.62 (1/2H, dd, J = 2.2, 9.2 Hz), 5.00—5.20 (5/2H, m), 5.85 (1/2H, dt, J = 1.5, 7.8 Hz), 6.69 (1/2H, d, J = 9.3 Hz), 7.25—7.50 (5H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{27}H_{40}NO_5$: 458.2906. Found: 458.2884.

(4*S*,5*S*)-4-Benzyl-3-benzyloxycarbonyl-5-(2-ethoxycarbonyl-1-butenyl)-2,2-dimethyloxazolidine (8c) The title compound was prepared from **6c** by a procedure similar to that described for **8b**, and was chromatographed on silica gel with AcOEt–hexane (1 : 10); 95.8% yield as a colorless oil. $[\alpha]_D^{20}$ −42.5° (c = 1.05, $CHCl_3$). IR (neat): 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.62—0.80 (3/2H, m), 0.96 (3/2H, t, J = 7.4 Hz), 1.10—1.75 (9H, m), 1.80—2.10 (1H, m), 2.15—2.27 (1H, m), 2.70—3.30 (2H, m), 3.90—4.25 (3H, m), 4.69 (1/2H, dd, J = 2.2, 9.0 Hz), 5.00—5.30 (5/2H, m), 5.60—5.75 (1/2H, br), 6.61 (1/2H, d, J = 9.0 Hz), 7.00—7.50 (10H, m). FAB-MS m/z: $[M+H]^+$ calcd for $C_{27}H_{34}NO_5$: 452.2437. Found: 452.2464.

(4*S*,5*S*)-3-Benzyloxycarbonyl-4-cyclohexylmethyl-5-[2-(isobutylcarbamoyl)-1-butenyl]-2,2-dimethyloxazolidine (9b) Compound **8b** (821 mg, 1.80 mmol) was dissolved in 2 M potassium hydroxide (4.5 ml, 9.00 mmol, EtOH : water = 9 : 1) and the solution was stirred at room temperature overnight. The reaction mixture was neutralized with 1 N HCl and concentrated. The resulting mixture was diluted with water and acidified with 1 N HCl. The mixture was extracted with AcOEt and the organic layer was washed with water and brine. Drying followed by evaporation gave a pale yellow oil. The residue was dissolved in DMF (6 ml), and then DPPA (594 mg, 2.16 mmol), triethylamine (218 mg, 2.18 mmol) and isobutylamine (171 mg, 2.34 mmol) were added at −10 °C. The mixture was stirred for 2 h at −10 °C and further stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and washed with 1 N HCl, saturated aqueous $NaHCO_3$, water and brine. Drying followed by evaporation and purification by silica gel chromatography (AcOEt : hexane = 1 : 6 afforded *Z*-isomer (362 mg, 41.6%) and *E*-isomer (383 mg, 44.0%). *Z*-Isomer: colorless oil, $[\alpha]_D^{20}$ −50.1° (c = 0.94, $CHCl_3$). IR (neat): 3352, 1707, 1668 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.90 (14H, m), 0.96 (6H, d, 6.3 Hz), 1.03 (3H, t, 7.5 Hz), 2.15—2.35 (1H, m), 2.35—2.50 (1H, m), 3.10—3.30 (2H, m), 3.75—3.95 (1H, br), 4.50 (1H, dd, J = 2.6, 8.7 Hz), 5.10, 5.17 (2H, ABq, J = 12.3 Hz), 5.60 (1H, d, J = 8.7 Hz), 6.50—6.60 (1H, br), 7.20—7.50 (5H, m), FAB-MS m/z: $[M+H]^+$ Calcd for $C_{29}H_{45}N_2O_4$: 485.3380. Found: 485.3329. *E*-Isomer: Colorless oil, $[\alpha]_D^{20}$ −41.9° (c = 1.09, $CHCl_3$). IR (neat): 3300, 1707, 1632 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.81—1.90 (14H, m), 0.93 (6H, d, J = 6.6 Hz), 1.07 (3H, t, J = 7.4 Hz), 2.25—2.55 (2H, m), 3.05—3.20 (2H, m), 3.80—3.95 (1H, br), 4.60 (1H, dd, J = 3.0, 8.7 Hz), 5.09, 5.17 (2H, ABq, J = 12.0 Hz), 5.65—5.86 (1H, m), 6.08 (1H, d, J = 8.7 Hz), 7.25—7.45 (5H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{29}H_{45}N_2O_4$: 485.3380. Found: 485.3339.

(4*S*,5*S*)-4-Benzyl-3-benzyloxycarbonyl-5-[2-(isobutylcarbamoyl)-1-butenyl]-2,2-dimethyloxazolidine (9c) The title compound was prepared from **8c** by a procedure similar to that described for **9b**, and was chromatographed on silica gel with AcOEt–hexane (1 : 5). *Z*-Isomer: Colorless oil, 30.8% yield. $[\alpha]_D^{20}$ −24.0° (c = 0.99, $CHCl_3$). IR (neat): 3345, 1707, 1644 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.90 (1H, m), 2.00—2.42 (2H, m), 2.80—3.25 (4H, m), 3.96 (1H, m), 4.58 (1H, dd, J = 4.8, 9.0 Hz), 5.18 (2H, br s), 5.43 (1H, d, J = 9.0 Hz), 6.10—6.45 (1H, br), 6.85—7.50 (10H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{29}H_{39}N_2O_4$: 479.2910. Found: 479.2870. *E*-Isomer: colorless oil, 31.4% yield. $[\alpha]_D^{20}$ −59.3° (c = 1.11, $CHCl_3$). IR (neat): 3350, 1707, 1632 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.65—2.20 (3H, m), 2.70—3.25 (4H, m), 3.90—4.10 (1H, br), 4.55—4.72 (1H, m), 5.00—5.25 (2H, m), 5.40—5.65 (1H, br), 5.70—6.05 (1H, br), 6.90—7.55 (10H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{29}H_{39}N_2O_4$ 479.2910. Found: 479.2881.

(2*R*,4*S*,5*S*)- and (2*S*,4*S*,5*S*)-5-(Benzyloxycarbonyl)amino-2-ethyl-4-hydroxy-7-methyloctanoic Acid γ-Lactone (14 and 15) To a solution of **8a** (80 mg, 0.19 mmol) in EtOH (1.5 ml) were added nickel chloride hexahydrate (14 mg, 0.06 mmol) and $NaBH_4$ (22 mg, 0.57 mmol). After being stirred for 30 min at room temperature, the precipitate was removed by filtration. The filtrate was concentrated and the residue was partitioned with water and AcOEt. The organic layer was washed with brine and concentrated. The residue was dissolved in MeOH (1 ml) and *p*-toluenesulfonic acid (15 mg) was added. After being stirred for 3 h at room temperature, the mixture was diluted with AcOEt and washed with saturated aqueous $NaHCO_3$ and brine. Drying followed by evaporation and purification by silica gel chromatography (AcOEt : hexane = 1 : 6) afforded **14** (18.0 mg, 28.2%) and **15** (30.5 mg, 47.7%). Compound **14**: colorless solid, *Rf* 0.23 (AcOEt : hexane = 1 : 5), mp 138.0—139.0 °C. $[\alpha]_D^{20}$ −24.2° (c = 1.00, MeOH). IR (KBr): 3298, 1770, 1698 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (6H, d, J = 6.9 Hz), 0.96 (3H, t, J = 7.5 Hz), 1.25—1.85 (5H, m), 1.90—2.05 (1H, m), 2.20—2.35 (1H, m), 2.40—2.55 (1H, m), 3.85—4.00 (1H, m), 4.42—4.52 (1H, m), 4.60 (1H, d, J = 10.2 Hz), 5.70, 5.14 (2H, ABq, J = 12.0 Hz), 7.25—7.42 (5H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{19}H_{28}NO_4$: 334.2018. Found: 334.2042. Compound **15**: colorless solid, *Rf* 0.18 (AcOEt : hexane = 1 : 5), mp 112.0—113.0 °C. $[\alpha]_D^{20}$ −19.8° (c = 1.00, MeOH), IR (KBr): 3286, 1767, 1695 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.82—1.00 (9H, m), 1.25—1.95 (6H, m), 2.25—2.38 (1H, m),

2.45—2.62 (1H, m), 3.85—3.98 (1H, m), 4.38 (1H, ddd, J=1.8, 6.2, 10.3 Hz), 4.68 (1H, d, J=10.2 Hz), 5.06, 5.16 (2H, ABq, J=12.3 Hz), 7.25—7.42 (5H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{19}H_{28}NO_4$: 334.2018. Found: 334.1989.

(2*R*,4*S*,5*S*)-*N*-Isobutyl-5-(Benzyloxycarbonyl)amino-2-ethyl-4-hydroxy-7-methyloctanamide (16) A mixture of **14** (14 mg, 0.04 mmol) and isobutylamine (0.5 ml) was stirred for 6 h at 100 °C and concentrated. The residue was purified by silica gel chromatography (AcOEt : hexane = 1 : 2) to give **16** (15.1 mg, 88.5%) as a colorless solid. mp 149.0—150.5 °C. $[\alpha]_D^{20}$ −33.0° (c=0.94, $CHCl_3$). IR (KBr): 3330, 1670, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.10 (15H, m), 1.15—1.85 (8H, m), 2.20—2.35 (1H, m), 2.95—3.08 (1H, m), 3.10—3.22 (1H, m), 3.52—3.75 (3H, m), 4.92 (1H, d, J=9.3 Hz), 5.10 (2H, s), 5.70—5.82 (1H, br), 7.25—7.42 (5H, m).

(2*R*,4*S*,5*S*)- and (2*S*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[*N*-Benzyloxycarbonyl-3-(1-naphthyl)-L-alanyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-ethyloctanamide (11*R* and 11*S*) A suspension of **9a** (99.9 mg, 0.23 mmol, a mixture of *Z* and *E*) and Pd-black in EtOH (1 ml) was stirred under hydrogen atmosphere overnight. The catalyst was filtered off and the filtrate was concentrated. The residue was dissolved in DMF (0.5 ml), and then **10** (89.2 mg, 0.19 mmol), DPPA (71.5 mg, 0.26 mmol) and triethylamine (26.3 mg, 0.26 mmol) were added at −10 °C. The mixture was stirred for 2 h at −10 °C and further stirred at room temperature overnight. To this mixture water was added and the precipitate was separated by filtration. The precipitate was purified by silica gel chromatography ($CHCl_3$: MeOH = 40 : 1) to afford **11** (70.2 mg, 50.7%) as a colorless solid. Two diastereomers were separated by HPLC to give **11*R*** and **11*S***. The conditions were as follows: Pre-column, Inertsil ODS (5 μm, 16.7 × 50 mm); column, Inertsil ODS (5 μm, 16.7 × 250 mm); solvent, MeOH : water = 90 : 10; flow rate, 10 ml/min; detection, 254 nm. Compound **11*R***: t_R 10.8 min, mp 234.0—236.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, t, J=7.5 Hz), 0.80—1.85 (29H, m), 2.25—2.38 (1H, m), 2.95—3.05 (1H, m), 3.10—3.22 (1H, m), 3.42—3.68 (3H, m), 3.68—3.90 (2H, m), 4.10—4.22 (1H, m), 4.47—4.58 (1H, m), 5.02, 5.09 (2H, ABq, J=12.0 Hz), 5.36 (1H, d, J=6.0 Hz), 5.80—5.95 (1H, br), 6.03 (1H, d, J=6.9 Hz), 6.27 (1H, br d, J=7.5 Hz), 7.15—7.42 (7H, m), 7.46—7.62 (2H, m), 7.78 (1H, d, J=7.5 Hz), 7.88 (1H, d, J=7.5 Hz), 8.16 (1H, d, J=7.5 Hz). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{42}H_{61}N_4O_6$: 717.4591. Found: 717.4550. Compound **11*S***: t_R 11.6 min, mp 229.0—231.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, t, J=7.3 Hz), 0.91 (12H, d, J=6.6 Hz), 0.95—1.85 (17H, m), 2.13—2.25 (1H, m), 2.95—3.20 (3H, m), 3.45—3.70 (3H, m), 3.85—3.97 (1H, m), 4.12—4.23 (1H, m), 4.48—4.60 (1H, m), 5.03, 5.09 (2H, ABq, J=12.0 Hz), 5.35—5.50 (1H, m), 5.75—5.90 (1H, m), 6.11 (1H, d, J=6.9 Hz), 6.24 (1H, br d, J=7.0 Hz), 7.15—7.42 (7H, m), 7.45—7.64 (2H, m), 7.77 (1H, d, J=8.0 Hz), 7.86 (1H, d, J=8.0 Hz), 8.27 (1H, br d, J=8.7 Hz). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{42}H_{61}N_4O_6$: 717.4591. Found: 717.4567.

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[*N*-Benzyloxycarbonyl-3-(1-naphthyl)-L-alanyl]-L-norleucyl]amino-6-cyclohexyl-2-ethyl-4-hydroxyhexanamide (12) The title compound was prepared from **9a** by a procedure similar to that described for **11**, and was chromatographed on silica gel with $CHCl_3$–MeOH (60 : 1); 52% yield as a colorless solid. mp 214.0—219.0 °C. ^{1}H-NMR ($CDCl_3$: CD_3OD = 3 : 1) δ: 0.50—0.98 (14H, m), 0.98—1.80 (22H, m), 1.97—2.15 (1/2H, m), 2.15—2.32 (1/2H, m), 2.78—2.92 (1H, m), 2.95—3.12 (1H, m), 3.50—3.70 (1H, m), 3.70—3.90 (1H, m), 4.47—4.60 (1H, m), 4.94 (2H, s), 7.10—7.60 (9H, m), 7.61 (1H, d, J=7.8 Hz), 7.70 (1H, d, J=9.0 Hz), 7.99 (1H, d, J=7.8 Hz). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{45}H_{65}N_4O_6$: 757.4904. Found: 757.4929.

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[*N*-Benzyloxycarbonyl-3-(1-naphthyl)-L-alanyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-6-phenylhexanamide (13) The title compound was prepared from **9c** by a procedure similar to that described for **11**, and was chromatographed on silica gel with $CHCl_3$–MeOH (50 : 1); 55.7% yield as colorless needles. mp 205.0—209.0 °C. ^{1}H-NMR ($CDCl_3$: CD_3OD = 3 : 1) δ: 0.65—0.95 (12H, m), 0.95—1.75 (11H, m), 1.94—2.10 (1/2H, m), 2.15—2.30 (1/2H, m), 2.55—3.10 (4H, m), 3.85—4.05 (1H, m), 4.15—4.35 (1H, m), 4.96 (2H, s), 6.95—7.68 (9H, m), 7.71 (1H, d, J=7.5 Hz), 7.81 (1H, d, J=8.1 Hz), 8.10 (1H, br d, J=7.5 Hz). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{45}H_{59}N_4O_6$: 751.4434. Found: 751.4429.

(2*E*,4*S*,5*S*)-5-Azido-4-benzyloxy-6-cyclohexyl-2-ethyl-2-hexenoic Acid Ethyl Ester (19) A mixture of **17** (306 mg, 0.92 mmol) and sodium periodate (393 mg, 1.84 mmol) in dioxane–water (3 : 2, 8 ml) was stirred for 1 h at room temperature. The precipitate was removed by filtration and the filtrate was extracted with AcOEt. The organic layer was washed with brine and was concentrated to give **18** (276 mg) as a pale yellow oil. This residue was used without further purification. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.80 (13H, m), 3.60 (1H, m), 3.71 (1H, m), 4.61, 4.81 (2H, ABq, J=12.0 Hz), 7.20—7.45 (5H, m), 9.72 (1H, d, J=1.5 Hz).

The title compound was prepared from **18** by a procedure similar to that described for **8b**, and was chromatographed on silica gel with AcOEt–hexane (1 : 40). Compound **19**: 46% yield from **17** as a colorless oil. $[\alpha]_D^{20}$ −3.6° (c=1.01, $CHCl_3$). IR (neat): 2104, 1716 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.70—1.85 (16H, m), 1.08 (3H, t, J=7.2 Hz), 2.30—2.42 (2H, m), 3.35—3.45 (1H, m), 4.12—4.22 (2H, m), 4.39 and 4.61 (2H, ABq, J=12.3 Hz), 4.59 (1H, dd, J=4.6, 8.8 Hz), 5.84 (1H, dt, J=1.5, 9.0 Hz), 7.25—7.45 (5H, m), FAB-MS m/z: 400 $[M+H]^+$. Compound **19′**: 31.4% yield from **17** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.80 (11H, m), 2.17 (2H, t, J=7.5 Hz), 5.04 (2H, s), 6.03 (1H, t, J=7.5 Hz), 7.25—7.40 (5H, m), 9.25 (1H, s).

(2*E*,4*S*,5*S*)-*N*-Isobutyl-5-Azido-4-benzyloxy-6-cyclohexyl-2-ethyl-2-hexenamide (20) The title compound was prepared from **19** by a procedure similar to that described for **9b**, and was chromatographed on silica gel with AcOEt–hexane (1 : 15); 62.0% yield as a colorless solid. mp 46.0—47.0 °C. $[\alpha]_D^{20}$ −6.9° (c=1.05, $CHCl_3$). IR (neat): 3310, 2104, 1626 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.05 (1H, m), 0.89 (6H, dd, J=0.9, 6.6 Hz), 1.08 (3H, t, J=7.5 Hz), 1.13—1.35 (3H, m), 1.35—1.50 (3H, m), 1.60—1.82 (7H, m), 2.28—2.40 (2H, m), 2.95—3.15 (2H, m), 3.42—3.52 (1H, m), 4.25 (1H, dd, J=5.1, 9.6 Hz), 4.42, 4.61 (2H, ABq, J=12.3 Hz), 5.46 (1H, dt, J=1.5, 9.6 Hz), 5.60—5.72 (1H, m), 7.23—7.40 (5H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{25}H_{39}N_4O_2$: 427.3073. Found: 427.3102.

3-(1-Naphthyl)-L-alanine (22) DL-*N*-Benzoyl-3-(1-naphthyl)alanine (**21**) (45.0 g, 0.141 mmol) was dissolved in 1 N NaOH (141 ml) and 0.1 M phosphate buffer (pH 7.0, 1000 ml) and acylase solution (350 units/ml, 180 ml) were added. After being stirred for 25 h at 37 °C, the precipitate was collected by filtration to give **22** (13.3 g, 87.6%) as a pale brown powder. mp 228.5—230.0 °C. $[\alpha]_D^{20}$ −14.4° (c=1.00, 1 N HCl) [lit.[16d] mp 230—231 °C, $[\alpha]_D^{20}$ −15.0° (c=0.97, 0.3 N HCl)]. IR (KBr): 3050, 1670 cm^{-1}.

***N*-Benzyloxycarbonyl-3-(1-naphthyl)-L-alanine (23)** To a solution of **22** (1.5 g, 7.7 mmol) in 1 N NaOH (7 ml) were added carbobenzoxy chloride (1.3 g, 7.7 mmol) and 1 N NaOH (7.7 ml) at 0 °C over a period of 10 min. After 40 min, the other portions of carbobenzoxy chloride (1.1 g, 6.3 mmol) and 1 N NaOH (6.3 ml) were added. The mixture was stirred for 1 h at 0 °C and extracted with Et_2O. The aqueous layer was acidified with 6 N HCl and the precipitate was collected by filtration to afford **23** (2.3 g, 94.0%) as a colorless powder. mp 152.0—153.5 °C. $[\alpha]_D^{20}$ −77.7° (c=1.00, MeOH) [lit.[18] mp 143—147 °C, $[\alpha]_D^{20}$ −56.5° (c=1.15, MeOH)]. IR (KBr): 3350, 1795 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.10—4.10 (2H, m), 4.10—4.50 (2H, m), 5.03 (2H, s), 6.60—8.20 (12H, m), 9.90—10.6 (1H, br).

***N*-[*N*-Benzyloxycarbonyl-3-(1-naphthyl)-L-alanyl]-L-norleucine (10)** To a solution of L-norleucine methyl ester hydrochloride (2.74 g, 15.1 mmol), *N*-methylmorpholine (1.52 g, 15.1 mmol) and HOBT (3.05 g, 22.6 mmol) was added DCC (3.9 g, 19.0 mmol) at −5 °C. The mixture was stirred for 3 h at −5 °C and then overnight at room temperature. The precipitate was filtered off and the filtrate was concentrated. The residue was recrystallized from MeOH to afford Z–Nal–Nle–OMe (6.06 g, 84.5%) as colorless needles. mp 136.0—137.0 °C. $[\alpha]_D^{20}$ −7.7° (c=0.977, $CHCl_3$). IR (KBr): 3298, 1749, 1689, 1659 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, t, J=7.9 Hz), 0.95—1.35 (4H, m), 1.40—1.75 (2H, m), 3.30—3.70 (2H, m), 3.60 (3H, s), 4.37 (1H, m), 4.50—4.60 (1H, m), 5.10 (2H, s), 5.52 (1H, d, J=8.0 Hz), 5.72 (1H, d, J=8.0 Hz), 7.28—7.42 (7H, m), 7.50 (2H, m), 7.76 (1H, d, J=8.0 Hz), 7.85 (1H, d, J=8.0 Hz), 8.20 (1H, d, J=8.0 Hz). *Anal.* Calcd for $C_{28}H_{32}N_2O_5$: C, 70.57; H, 6.77; N, 5.88. Found: C, 70.49; H, 6.81; N, 5.87. A solution of methyl ester (5.16 g, 10.8 mmol) in 2 N KOH (27 ml, EtOH : water = 9 : 1) was stirred overnight at room temperature. The mixture was acidified with 1 N HCl and was extracted with AcOEt. The organic layer was washed with water and brine, and concentrated. The residue was recrystallized from AcOEt to afford **10** (4.90 g, 98.0%) as colorless needles. mp 176.0—177.5 °C. $[\alpha]_D^{20}$ +1.6° (c=1.05, $CHCl_3$). IR (KBr): 3310, 1704, 1670 (sh), 1647 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, t, J=7.9 Hz), 1.00—1.35 (4H, m), 1.45—1.65 (1H, m), 1.65—1.82 (1H, m), 3.35—3.68 (2H, m), 4.40 (1H, dd, J=6.6 Hz), 4.50—4.70 (1H, m), 5.09 (2H, s), 5.65 (1H, d, J=8.0 Hz), 6.03 (1H, d, J=8.0 Hz), 7.28—7.42 (7H, m), 7.44—7.59 (2H, m), 7.72 (1H, m), 7.82 (1H, d, J=8.0 Hz), 8.18 (1H, d, J=8.0 Hz). *Anal.* Calcd for $C_{27}H_{30}N_2O_5$: C, 70.11; H, 6.54; N, 6.06. Found: C, 70.11; H, 6.56; N, 6.01.

***In Vitro* Renin Assays** In a total volume of 0.4 ml, a mixture of 0.32 ml of human plasma, 10 mM ethylenediaminetetraacetic acid (EDTA), 3.4 mM 8-hydroxyquinoline and 100 mM Tris–acetate buffer, pH 7.4, was incubated in the presence or absence of 4 different concentrations of inhibitor

dissolved in 0.004 ml DMSO at 37 °C for 60 min. The reaction was stopped by addition of an excess amount of pepstatin A, and the angiotensin I formed was measured by radioimmunoassay using a commercial kit (Renin-Riabead, Dainabot). Percent inhibition was calculated and IC_{50} determined by regression analysis.

Cathepsin D and Pepsin Assays Bovine spleen cathepsin D and porcine stomach mucosa pepsin (both from Sigma) were incubated with bovine hemoglobin at 37 °C, at pH 2.8 for 10 min and pH 1.3 for 30 min, respectively, in the presence or absence of inhibitors. After stopping the reaction by the addition of trichloroacetic acid (TCA), the resulting TCA-soluble peptide was quantified by reacting with the Folin–Ciocalteau reagent.

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