# Novel Renin Inhibitors Containing (2*S*,3*S*,5*S*)-2-Amino-1-cyclohexyl-6-methyl-3,5-heptanediol Fragment as a Transition-state Mimic at the P1–P1′ Cleavage Site[1)]

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**A series of renin inhibitors containing the (2*S*,3*S*,5*S*)-2-amino-1-cyclohexyl-6-methyl-3,5-heptanediol (2-amino-3,5-*anti*-diol) fragment as a novel transition-state mimic was synthesized, and their biological activities were evaluated. All of the synthesized compounds containing the 2-amino-3,5-*anti*-diol fragment at the P1–P1′ position showed high *in vitro* renin-inhibitory activity with $IC_{50}$ values in the $10^{-8}$—$10^{-10}$ M range, and most of them caused a reduction of blood pressure when administered orally to salt-depleted, conscious marmosets. The inhibitor (29) with the 4-hydroxypiperidine residue at the P4 position showed the highest activity in terms of both potency and duration of the blood pressure-lowering effect.**

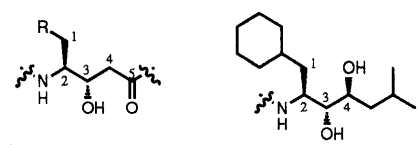
**Key words** renin inhibitor; transition-state mimic; (2*S*,3*S*,5*S*)-2-amino-1-cyclohexyl-6-methyl-3,5-heptanediol; blood pressure-lowering effect

The renin-angiotensin system (RAS) is one of the vasopressor systems in the body and an important blood pressure-humoral electrolyte-adjusting system. Renin, which is an aspartic protease, cleaves angiotensinogen to yield the decapeptide angiotensin I (Ang I). Ang I is then cleaved by angiotensin converting enzyme (ACE) to yield the octapeptide angiotensin II (Ang II), which is a potent vasoconstrictor and stimulant of aldosterone secretion. Although ACE inhibitors are now widely used as therapeutic agents to treat hypertension and congestive heart failure,[2,3)] they have side effects such as dry cough and angioneurotic edema.[4)] These side effects are reported to be attributable to the low specificity of ACE, which also cleaves other substances, such as bradykinin, enkephalins and substance P, besides Ang I.[5)] In contrast, renin catalyzes the production of Ang I at the first and rate-limiting step in the RAS, and angiotensinogen is the only known natural substrate for renin. Renin inhibitors are therefore expected to be antihypertensive agents of a new type, devoid of the side effects reported for ACE inhibitors.

In the past decade, many renin inhibitors designed on the basis of the angiotensinogen sequence have been reported.[6)] Most of them were transition-state mimics based on the tetrahedral intermediate formed during hydrolysis of the peptide bond between Leu-10 and Val-11 of angiotensinogen. Statine (**1a**)[7)] and its cyclohexyl derivative (**1b**)[8)] (Fig. 1) are well-known transition-state mimics, and many compounds containing them at the P1–P1′ position[9)] show potent renin-inhibitory activities. Luly *et al.* also reported a useful transition-state mimic, 2-amino-1-cyclohexyl-6-methyl-3,4-heptanediol (**2**) (Fig. 1).[10)] By utilization of this aminodiol at the P1–P1′ position, they obtained lower-molecular-size inhibitors retaining good inhibitory activity. Molecular modeling studies suggest that the common hydroxyl group at the C3 position with (*S*)-configuration in the above mimics can form hydrogen bonds with the two aspartic acids in the active site of renin.[11,12)] Accordingly, this hydroxyl group is thought to be important for high inhibitory activity. Furthermore, these mimics have additional oxygen-containing groups; C5-carbonyl in **1a**, **b** and C4-hydroxyl in **2**. From the results of molecular modeling studies, these oxygen-containing groups are thought to form hydrogen bonds with Ser-76, enhancing the binding affinity between the inhibitor and renin.[11,12)]

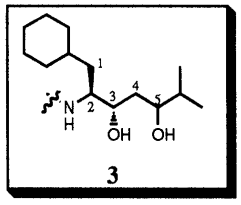
Based on the above information, we hypothesized that the introduction of a C5-hydroxyl group might also enhance the binding affinity to renin through the formation of similar hydrogen bonds. Thus, we designed a new transition-state mimic, 2-amino-1-cyclohexyl-6-methyl-3,5-heptanediol (**3**) (Fig. 1), which was expected to be easily obtainable in a few steps from cyclohexylalaninol.

In this paper, we describe the synthesis and biological activities of novel renin inhibitors containing the 2-amino-1-cyclohexyl-6-methyl-3,5-heptanediol (2-amino-3,5-diol) fragment, and we discuss the structure–activity relationship.




**1a** : R = isopropyl
**1b** : R = cyclohexyl

**2**




**3**

Fig. 1

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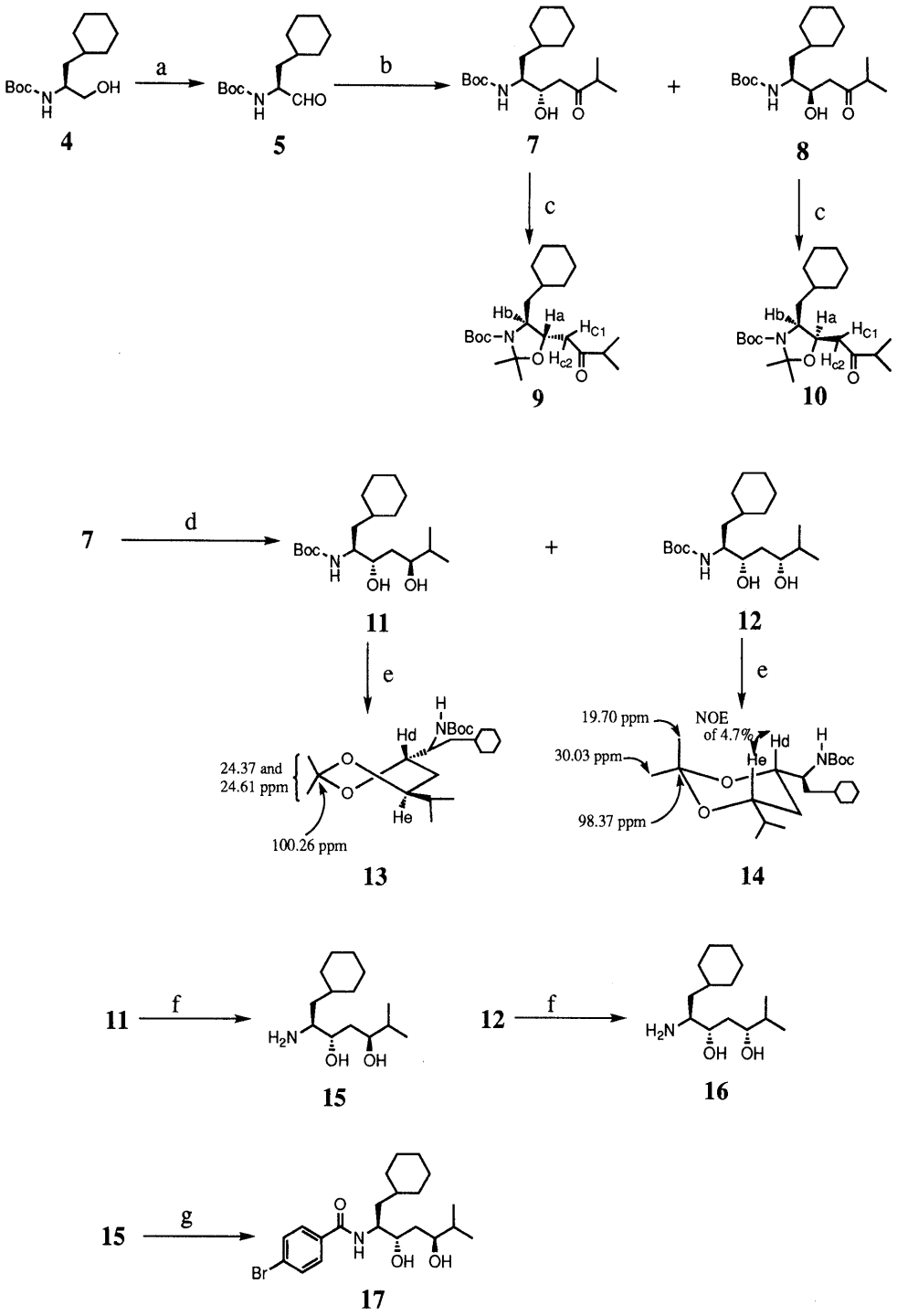
## Results and Discussion

**Synthesis** The synthesis of 2-amino-1-cyclohexyl-6-methyl-3,5-heptanediols is shown in Chart 1. The aldehyde **5** was prepared from *N*-(*tert*-butoxycarbonyl)-L-cyclohexylalaninol (*N*-Boc-L-cyclohexylalaninol) (**4**) according to the known procedure.[13] The aldol reaction of **5** with the lithium enolate **6**, generated from 3-methyl-2-butanone and lithium diisopropylamide (LDA) in tetrahydrofuran (THF) at −70 °C, gave two hydroxyketones **7** and **8** as a diastereo mixture (1 : 1).[14] In order to confirm the stereochemistries of **7** and **8**, each isomer was converted to the corresponding 2,2-dimethyl-1,3-oxazolidine derivative, **9** and **10**, respectively, by treatment with 2-methoxypropene in the presence of pyridinium *p*-toluenesulfonate (PPTS). In $^1$H-NMR analysis, **9** showed an *anti* hydrogen coupling constant of 1.8 Hz between Ha and Hb, while **10** revealed a *syn* hydrogen coupling constant of 4.8 Hz.[15] The nuclear Overhauser enhancement (NOE) between Ha and Hb of **9** was smaller than that of **10**.[16] From these results, it was indicated that the hydroxyketones **7** and **8**




a) $SO_3$·Py, DMSO, $Et_3N$, $C_6H_6$; b) $CH_2=C(OLi)CH(CH_3)_2$ (**6**) THF, −70 °C; c) $CH_2=C(OCH_3)CH_3$, PPTS, $CH_2Cl_2$; d) $NaBH_4$, MeOH; e) $(CH_3)_2C(OCH_3)_2$, *p*-toluenesulfonic acid, THF; f) $CF_3CO_2H$, then $NaHCO_3$; g) 4-bromobenzoyl chloride, $Et_3N$, $CHCl_3$.

Chart 1. Synthesis of 2-Amino-1-cyclohexyl-6-methyl-3,5-heptanediols

have the desired (5*S*,6*S*)- and the undesired (5*R*,6*S*)-configurations, respectively.

Reduction of **7** with $NaBH_4$, afforded the *N*-Boc-aminodiols **11** and **12** in 27% and 59% yields, respectively, after separation by column chromatography. To investigate the configurations of these aminodiols, **11** and **12** were converted to the corresponding acetonide derivatives **13** and **14**, respectively, by treatment with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid. The stereochemistries of the obtained **13** and **14** were studied as follows. In the $^{13}$C-NMR spectra, the chemical shifts of the two methyl carbons in the acetonide segment of **13** were 24.37 and 24.61 ppm, and that of the acetal carbon was 100.26 ppm (Chart 1). These chemical shifts are typical of *anti* (twist boat) 1,3-diol acetonides.[17] On the other hand, the *syn* (chair) conformational resonances were observed at 19.70, 30.03 and 98.37 ppm in the spectrum of **14**, as shown in Chart 1.[17] In the $^1$H-NMR analysis, NOE of 4.7% between Hd and He of **14** was observed, while no NOE between Hd and He of **13** was observed. These data indicated that the two hydrogens (Hd and He) of **13** and **14** are in *anti* and *syn* relationships, respectively. Thus, it was deduced that **11** and **12** have the 3,5-*anti* and 3,5-*syn* configurations, respectively.

By removing the Boc group of **11** and **12** under acidic conditions, the 2-amino-3,5-*anti*-diol **15** and its *syn* isomer **16** were obtained, respectively. To confirm the absolute configurations of the 2-amino-3,5-*anti*-diol **15**, it was acylated with 4-bromobenzoyl chloride to give the *N*-acyl derivative **17**, which was subjected to X-ray crystallographic analysis (Chart 1). The crystal structure, including absolute configurations, of **17** is shown in Fig. 2. Since no epimerization could occur through benzoylation, compound **15** was determined to have the expected (2*S*,3*S*,5*S*)-configuration. The result of this X-ray analysis also established the above deduced absolute stereochemistries of compounds **7**, **8**, **11** and **12**.

The renin-inhibitory compounds **18**—**20** (Table 1) were prepared by coupling of (2*R*)-3-morpholinocarbonyl-2-(1-naphthylmethyl)propionyl-L-histidine hydrazide, which was derived from its ester,[13] with the corresponding aminoalcohols using the standard azide method.

As an example of the synthetic pathways to the renin-inhibitory compounds listed in Tables 2 and 3, the synthesis of compound **21** is shown in Chart 2. Condensation of L-phenylalanine benzyl ester **39** and piperidine using trichloromethyl chloroformate and triethylamine gave the urea derivative **40**. By hydrogenation over Pd–C of **40**, *N*-piperidinocarbonyl-L-phenylalanine (**41**) was obtained. Most of the *N*-cyclic aminocarbonyl-L-phenylalanines corresponding to the P4–P3 residues in the inhibitors listed in Table 2 were prepared by essentially the same method. The $N^{\alpha}$-methylated L-histidine methyl ester derivative **43** was prepared by methylation of $N^{\alpha}$-benzyloxycarbonyl-$N^{im}$-tosyl-L-histidine (**42**)[18] using methyl iodide and NaH.[19] The tosyl (Ts) group of **43** was removed using 1-hydroxybenzotriazole monohydrate (HOBT), followed by treatment with hydrazine monohydrate to give the hydrazide **44**. The coupling of **44** with the 2-amino-3,5-*anti*-diol **15** by the azide method afforded the amide **45**. The benzyloxycarbonyl (Cbz) group in **45** was removed by hydrogenation over Pd–C to give the amine **46**. Compound **21** was obtained by the coupling of **41** with **46** using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and HOBT. In this coupling reaction, racemization of phenylalanine moiety was less than 2%.[20] Most of the inhibitors listed in Tables 2 and 3 were synthesized by similar coupling of the corresponding carboxylic acids with **46**.

Physical data for the renin inhibitory compounds listed in Tables 1—3 are shown in Table 6.

***In Vitro* Renin Inhibition** To evaluate the usefulness of the 2-amino-3,5-diol fragment as a transition-state mimic at the P1–P1′ position for renin inhibition, compounds **18**—**20** were first synthesized and their inhibitory activities against human plasma renin were measured (Table 1). The amino-monoalcohol fragment in compound **18** has already been reported as a transition-state mimic at the P1–P1′

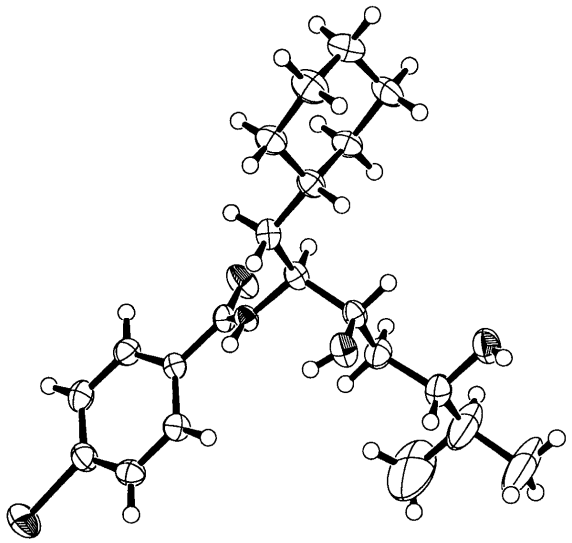
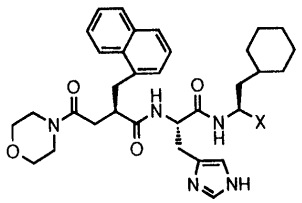
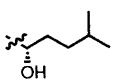
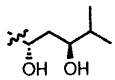
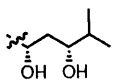


Fig. 2. ORTEP Drawing of the Crystal Structure of **17** with Thermal Ellipsoids at 50% Probability

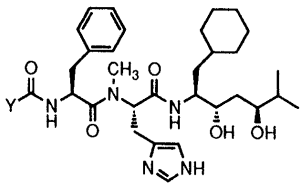
Table 1. *In Vitro* Activity of Renin Inhibitors Containing the 2-Amino-3,5-diol Moiety

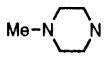
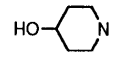
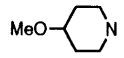
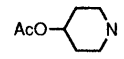
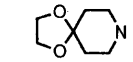


| No. | X | $IC_{50}$ (nM)[a] |
|---|---|---|
| **18** |  | 7.5 |
| **19** |  | 0.53 |
| **20** |  | 19 |

*a*) Inhibitory activity against human plasma renin.

Table 2. P4 Cyclic Type Inhibitors

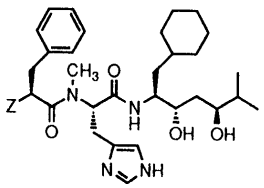


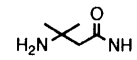
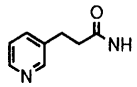
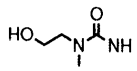
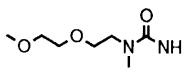
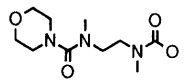
| No. | Y | $IC_{50}$ (nM)[a] |
|---|---|---|
| **21** |  | 0.74 |
| **22** |  | 1.6 |
| **23** |  | 0.33 |
| **24** |  | 1.5 |
| **25** |  | 2.0 |
| **26** |  | 5.7 |
| **27** |  | 23 |
| **28** |  | 12.5 |
| **29** |  | 0.68 |
| **30** |  | 0.52 |
| **31** |  | 0.31 |
| **32** |  | 2.7 |
| **33** |  | 1.0 |

a) Inhibitory activity against human plasma renin.

position in some renin inhibitors such as FK906.[10,21] In the aminodiol fragment, the configurations of C2 and C3 positions were all held as 2*S* and 3*S* on the basis of previously reported structure–activity relationships of related compounds. For the P4–P2 position, the moiety developed by Iizuka and Kiso *et al.* through the study of their renin inhibitors was used.[13,22] As shown in Table 1, compound **19** containing the 2-amino-3,5-*anti*-diol fragment showed 14-fold higher inhibitory activity than the amino-monoalcohol derivative **18**. On the other hand, compound **20** having the *syn*-diol moiety was much less active than the *anti*-diol **19**. Thus, the C5-hydroxyl group with (*S*)-configuration in the aminodiol appeared to play an important role in binding to renin, and the 2-amino-3,5-*anti*-diol fragment was suggested to be a potent transition-state mimic.

Table 3. P4 Acyclic Type Inhibitors



| No. | Z | $IC_{50}$ (nM)[a] |
|---|---|---|
| **34** |  | 49 |
| **35** |  | 0.86 |
| **36** |  | 2.5 |
| **37** |  | 0.86 |
| **38** |  | 0.66 |

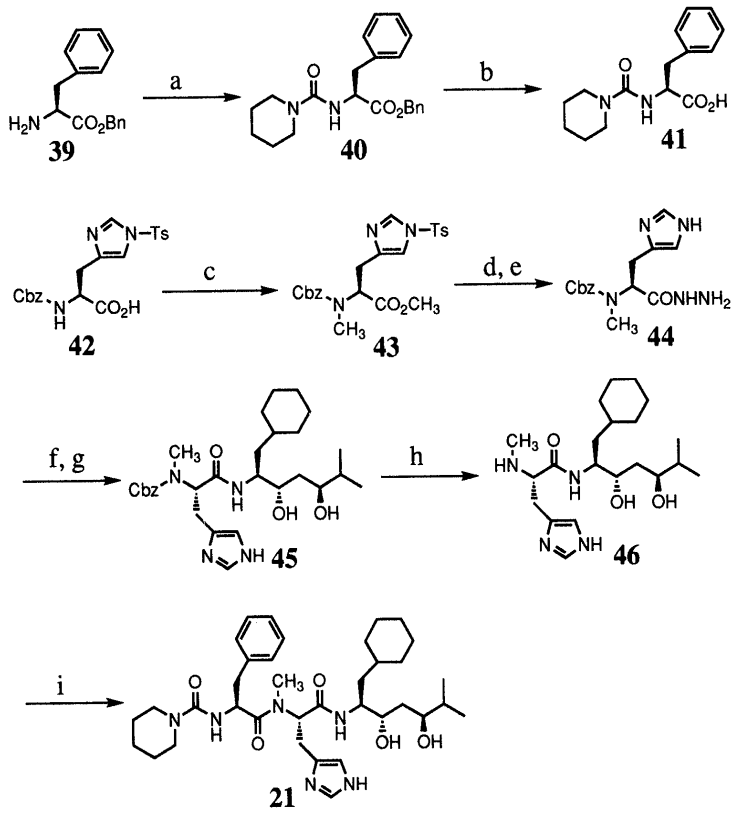
a) Inhibitory activity against human plasma renin.

Then we further prepared various compounds containing the 2-amino-3,5-*anti*-diol fragment at the P1–P1′ position and evaluated their inhibitory activities *in vitro*. In this series, the P3–P2 residue was held as Phe–(Me)His and the P4 position was replaced with various functional groups. The introduction of a methyl group at the $N^{\alpha}$-position of His has been used in other renin inhibitors to increase the stability of the inhibitors to proteolytic enzymes such as chymotrypsin.[21,23] The $IC_{50}$ values of the inhibitors having a cyclic aminocarbonyl moiety at the P4 position are summarized in Table 2. All of the compounds showed high inhibitory activities with $IC_{50}$ values in the range of $10^{-10}$—$10^{-8}$ M. Compounds **27** and **28**, having basic character at the N-terminus, were less active than the other N-terminal neutral compounds.

Besides cyclic aminocarbonyl residues, various acyclic residues have also been utilized as the P4 residue in reported renin inhibitors.[6,21,24,25] Some typical residues were selected, and renin inhibitors containing combinations of these residues and the 2-amino-3,5-*anti*-diol fragment were synthesized; their renin-inhibitory activities are listed in Table 3. Although the inhibitory activity of the N-terminal amino derivative **34** was lower than those of the cyclic amino inhibitors, compounds **35**, **37** and **38** showed high inhibitory activities with $IC_{50}$ values of less than 1 nM.

In the foregoing *in vitro* experiments, all of the prepared compounds containing the 2-amino-3,5-*anti*-diol fragment at the P1–P1′ position showed high renin-inhibitory activities, demonstrating that the 2-amino-3,5-*anti*-diol fragment is a potent transition-state mimic.

**Blood Pressure-Lowering Effect** The blood pressure-lowering effects of selected synthetic inhibitors in marmosets were examined. After oral administration of



a) piperidine, $Cl_3COC(O)Cl$, $Et_3N$, THF; b) $H_2$, Pd–C, MeOH; c) $CH_3I$, NaH, THF, DMF; d) HOBT, THF; e) $NH_2NH_2 \cdot H_2O$, MeOH; f) Isoamyl nitrite, HCl, DMF; g) **15**, $Et_3N$, DMF; h) $H_2$, Pd–C, MeOH; i) **41**, DCC, HOBT, DMF.

Chart 2. Synthesis of Inhibitor **21**

Table 4. Blood Pressure-Lowering Effects of the Inhibitors after Oral Administration to Salt-Depleted, Conscious Marmosets

| No. | Dose (mg/kg) | *N* | ΔSBP (mmHg) | | |
|---|---|---|---|---|---|
| | | | 1 h | 3 h | 5 h |
| **19** | 10 | 3 | −13 | −15 | − 8 |
| **21** | 10 | 4 | − 4 | − 6 | −13 |
| **22** | 10 | 2 | −10 | + 3 | |
| **23** | 10 | 2 | − 4 | − 7 | + 6 |
| **24** | 10 | 1 | − 2 | − 5 | + 8 |
| **26** | 10 | 1 | −15 | −11 | − 2 |
| **29** | 10 | 7 | −15 | −15 | − 9 |
| **29** | 5 | 6 | −13 | − 8 | − 3 |
| **30** | 10 | 2 | − 5 | + 5 | |
| **31** | 10 | 2 | − 8 | − 5 | + 3 |
| **32** | 10 | 2 | −11 | − 4 | + 2 |
| **33** | 10 | 2 | −12 | − 2 | |
| **35** | 5 | 2 | − 6 | ± 0 | + 8 |
| **37** | 5 | 2 | −14 | − 5 | + 1 |
| **38** | 5 | 2 | −14 | + 2 | + 3 |

10 or 5 mg/kg of the inhibitors to salt-depleted, conscious marmosets, the reduction of systolic blood pressure (SBP) was measured. As shown in Table 4, all of the tested inhibitors showed hypotensive effects, although no simple relationship was apparent between *in vivo* hypotensive intensity and *in vitro* renin-inhibitory activity. Among the tested inhibitors, compound **29** with the 4-hydroxypiperidine residue at the P4 position caused the most significant and long-lasting reduction of blood pressure in marmosets at oral doses of 10 and 5 mg/kg. Interestingly,

Table 5. Enzyme Selectivity of **29**

| Enzyme | $IC_{50}$ (nM) |
|---|---|
| Plasma renin | |
| Human | 0.68 |
| Cynomolgus monkey | 1.2 |
| Marmoset | 0.52 |
| Dog | 54 |
| Rat | 3200 |
| Pepsin (porcine) | >100000 |
| Cathepsin D (bovine) | >100000 |
| ACE (human) | >100000 |

compound **21** having piperidine at the P4 position showed a different pattern of blood pressure-lowering effect from those of the other inhibitors. Thus, the maximum drop of blood pressure was observed at 5 h after administration of **21**, in contrast to 1—3 h for the others. Although the replacement of the hydroxyl group in **29** with a methoxy (**30**) or an acetoxy (**31**) group increased the *in vitro* inhibitory activity, the blood pressure-lowering effects of **30** and **31** were weaker than that of **29**, indicating that the oral efficacy of these inhibitor is greatly influenced by slight differences of chemical properties at the P4 position.

**Enzyme Specificity** Inhibitory activities of **29** against other enzymes are shown in Table 5. The inhibitor showed potent inhibitory activity against human, cynomolgus monkey and marmoset plasma renin, but was much less active against dog and rat plasma renin. Although pepsin and cathepsin D are aspartic proteases, like renin, they

Table 6. Physical Data for Renin-Inhibitory Compounds

| No. | $^1$H-NMR ($CDCl_3$) $\delta$ | FAB-MS $m/z$ $(M+H)^+$ | Formula | *Anal.* Calcd (Found) C | H | N |
|---|---|---|---|---|---|---|
| **18** | 0.7—1.8 (18H, m), 0.85 (3H, d, $J$=6.6 Hz, $CH(CH_3)_2$), 0.86 (3H, d, $J$=6.6 Hz, $CH(CH_3)_2$), 2.57 (2H, m, $CH_2CO$), 3.0—3.7 (12H, m), 3.88 (1H, m), 4.59 (1H, m), 6.63 (1H, br d), 6.86 (1H, s, imidazole N-CH=C), 7.30 (1H, d, $J$=6.3 Hz), 7.40 (1H, t, $J$=7.8 Hz), 7.4—7.6 (3H, m), 7.76 (1H, d, $J$=8.1 Hz), 7.87 (1H, d, $J$=7.5 Hz), 8.06 (1H, d, $J$=8.1 Hz) | 674 | $C_{39}H_{55}N_5O_5 \cdot H_2O$ | 67.70 (67.40 | 8.30 8.18 | 10.12 9.90) |
| **19** | 0.6—1.8 (16H, m), 0.86 (3H, d, $J$=6.7 Hz, $CH(CH_3)_2$), 0.91 (3H, d, $J$=6.6 Hz, $CH(CH_3)_2$), 2.45 (1H, m, one of $CH_2CO$), 2.68 (1H, m, one of $CH_2CO$), 3.0—3.3 (6H, m), 3.4—3.7 (8H, m), 3.78 (1H, m), 3.94 (1H, m), 4.59 (1H, m), 6.72 (1H, br d, $J$=9 Hz), 6.89 (1H, s, imidazole N-CH=C), 7.28 (1H, d, $J$=7.1 Hz), 7.38 (1H, t, $J$=7.6 Hz), 7.4—7.6 (3H, m), 7.74 (1H, d, $J$=8.1 Hz), 7.85 (1H, d, $J$=9.2 Hz), 8.06 (1H, d, $J$=7.4 Hz) | 690 | $C_{39}H_{55}N_5O_6 \cdot 0.5H_2O$ | 67.02 (67.06 | 8.07 8.20 | 10.02 10.00) |
| **20** | 0.6—1.9 (16H, m), 0.89 (6H, t, $J$=7.0 Hz, $CH(CH_3)_2$), 2.48 (1H, m, one of $CH_2CO$), 2.68 (1H, m, one of $CH_2CO$), 3.2—3.8 (15H, m), 3.84 (1H, m), 4.57 (1H, m), 6.61 (1H, br d, $J$=9 Hz), 6.87 (1H, s, imidazole N-CH=C), 7.30 (1H, d, $J$=7.3 Hz), 7.39 (1H, t, $J$=7.5 Hz), 7.4—7.6 (3H, m), 7.75 (1H, d, $J$=8.0 Hz), 7.86 (1H, d, $J$=7.6 Hz), 8.06 (1H, d, $J$=8.0 Hz) | 690 | $C_{39}H_{55}N_5O_6 \cdot 2H_2O$ | 64.53 (64.20 | 8.19 7.81 | 9.65 9.44) |
| **21** | 0.7—1.9 (22H, m), 0.84 (3H, d, $J$=6.8 Hz, $CH(CH_3)_2$), 0.88 (3H, d, $J$=6.7 Hz, $CH(CH_3)_2$), 2.5—3.4 (8H, m), 2.77 (3H, s, $NCH_3$), 3.53 (1H, m), 3.73 (1H, m), 4.09 (1H, m), 4.75 (1H, m), 4.90 (1H, m), 5.15 (1H, d, $J$=6.2 Hz), 6.69 (1H, s, imidazole N-CH=C), 7.2—7.4 (5H, m, phenyl H), 7.43 (1H, s, imidazole N-CH=N), 7.94 (1H, d, $J$=10.0 Hz) | 653 | $C_{36}H_{56}N_6O_5 \cdot 0.5H_2O$ | 65.33 (65.18 | 8.68 8.83 | 12.70 12.60) |
| **22** | 0.7—2.0 (20H, m), 0.85 (3H, d, $J$=6.9 Hz, $CH(CH_3)_2$), 0.88 (3H, d, $J$=6.9 Hz, $CH(CH_3)_2$), 2.5—3.4 (8H, m), 2.76 (3H, s, $NCH_3$), 3.54 (1H, m), 3.75 (1H, m), 4.10 (1H, m), 4.77 (2H, m), 4.91 (1H, m), 6.68 (1H, s, imidazole N-CH=C), 7.1—7.4 (5H, m, phenyl H), 7.44 (1H, s, imidazole N-CH=N), 7.88 (1H, d, $J$=9.8 Hz) | 639 | $C_{35}H_{54}N_6O_5 \cdot H_2O$ | 64.00 (63.88 | 8.59 8.60 | 12.79 12.73) |
| **23** | 0.7—1.9 (24H, m), 0.84 (3H, d, $J$=6.8 Hz, $CH(CH_3)_2$), 0.88 (3H, d, $J$=6.7 Hz, $CH(CH_3)_2$), 2.5—3.5 (8H, m), 2.79 (3H, s, $NCH_3$), 3.53 (1H, m), 3.75 (1H, m), 4.10 (1H, m), 4.79 (1H, m), 4.8—5.1 (2H, m), 6.69 (1H, s, imidazole N-CH=C), 7.1—7.4 (5H, m, phenyl H), 7.42 (1H, s, imidazole N-CH=N), 7.93 (1H, d, $J$=9 Hz) | 667 | $C_{37}H_{58}N_6O_5 \cdot H_2O$ | 64.88 64.75 | 8.83 8.86 | 12.27 12.15) |
| **24** | 0.7—1.9 (16H, m), 0.84 (3H, d, $J$=6.7 Hz, $CH(CH_3)_2$), 0.87 (3H, d, $J$=6.7 Hz, $CH(CH_3)_2$), 2.6—3.8 (14H, m), 2.79 (3H, s, $NCH_3$), 4.07 (1H, m), 4.77 (1H, m), 4.93 (1H, m), 5.38 (1H, br d), 6.70 (1H, s, imidazole N-CH=C), 7.1—7.4 (6H, m, phenyl H and imidazole N-CH=N), 7.86 (1H, d, $J$=9.5 Hz) | 655 | $C_{35}H_{54}N_6O_6 \cdot 1.5H_2O$ | 61.65 (61.92 | 8.43 8.51 | 12.32 12.09) |
| **25** | 0.7—1.9 (16H, m), 0.84 (3H, d, $J$=6.8 Hz, $CH(CH_3)_2$), 0.88 (3H, d, $J$=6.7 Hz, $CH(CH_3)_2$), 2.3—3.9 (14H, m), 2.80 (3H, s, $NCH_3$), 4.09 (1H, m), 4.75 (1H, m), 4.94 (1H, m), 5.48 (1H, br d), 6.71 (1H, s, imidazole N-CH=C), 7.1—7.5 (6H, m, phenyl H and imidazole N-CH=N), 7.84 (1H, d, $J$=9.7 Hz) | 671 | $C_{35}H_{54}N_6O_5S \cdot H_2O$ | 61.02 (60.81 | 8.19 8.18 | 12.19 12.13) |
| **26** | 0.7—1.9 (16H, m), 0.85 (3H, d, $J$=6.8 Hz, $CH(CH_3)_2$), 0.88 (3H, d, $J$=6.8 Hz, $CH(CH_3)_2$), 2.5—4.2 (15H, m), 4.6—5.3 (2H, m), 6.22 (1H, m), 6.6—7.8 (8H, m) | 703 | $C_{35}H_{54}N_6O_7S \cdot 2H_2O$ | 56.89 (57.06 | 7.91 7.66 | 11.37 11.37) |
| **27** | 0.7—1.9 (16H, m), 0.84 (3H, d, $J$=6.8 Hz, $CH(CH_3)_2$), 0.87 (3H, d, $J$=6.7 Hz, $CH(CH_3)_2$), 2.5—3.4 (12H, m), 2.78 (3H, s, $NCH_3$), 3.53 (1H, m), 3.73 (1H, m), 4.07 (1H, m), 4.76 (1H, m), 4.91 (1H, m), 5.23 (1H, br d), 6.69 (1H, s, imidazole N-CH=C), 7.1—7.4 (6H, m, phenyl H, imidazole N-CH=N), 7.88 (1H, d, $J$=9.3 Hz) | 654 | $C_{35}H_{55}N_7O_5 \cdot 1.5H_2O$ | 61.74 (61.54 | 8.59 8.59 | 14.40 14.05) |

Table 6. (Continued)

| No. | $^1$H-NMR ($CDCl_3$) $\delta$ | FAB-MS $m/z$ $(M+H)^+$ | Formula | *Anal.* Calcd (Found) C | H | N |
|---|---|---|---|---|---|---|
| **28** | 0.7—1.9 (16H, m), 0.85 (3H, d, $J$=6.7 Hz, $CH(C\underline{H}_3)_2$), 0.88 (3H, d, $J$=6.7 Hz, $CH(C\underline{H}_3)_2$), 2.2—2.4 (4H, m), 2.26 (3H, s, $(CH_2)_2NC\underline{H}_3$), 2.5—3.4 (8H, m), 2.78 (3H, s, $CONC\underline{H}_3$), 3.53 (1H, m), 3.74 (1H, m), 4.09 (1H, m), 4.77 (1H, m), 4.91 (1H, m), 5.12 (1H, m), 6.69 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.4 (5H, m, phenyl H), 7.42 (1H, s, imidazole N-C$\underline{H}$=N), 7.84 (1H, d, $J$=9 Hz) | 668 | $C_{36}H_{57}N_7O_5 \cdot H_2O$ | 63.03 (62.92 | 8.67 8.78 | 14.29 14.27) |
| **29** | 0.7—1.9 (20H, m), 0.83 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 0.86 (3H, d, $J$=7.3 Hz, $CH(C\underline{H}_3)_2$), 2.5—3.9 (11H, m), 2.79 (3H, s, $NC\underline{H}_3$), 4.05 (1H, m), 4.72 (1H, m), 4.91 (1H, m), 5.52 (1H, m), 6.69 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.4 (6H, m, phenyl H and imidazole N-C$\underline{H}$=N), 7.96 (1H, d, $J$=9.0 Hz) | 669 | $C_{36}H_{56}N_6O_6 \cdot 1.7H_2O$ | 61.81 (62.15 | 8.56 8.57 | 12.01 11.64) |
| **30** | 0.7—1.9 (20H, m), 0.83 (3H, d, $J$=6.7 Hz, $CH(C\underline{H}_3)_2$), 0.86 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 2.5—3.6 (10H, m), 2.80 (3H, s, $NC\underline{H}_3$), 3.30 (3H, s, $OC\underline{H}_3$), 3.78 (1H, m), 4.10 (1H, m), 4.94 (1H, m), 5.37 (1H, m), 6.69 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.5 (6H, m, phenyl H and imidazole N-C$\underline{H}$=N), 7.91 (1H, d, $J$=9 Hz) | 683 | $C_{37}H_{58}N_6O_6 \cdot 0.75H_2O$ | 63.81 (63.87 | 8.61 8.72 | 12.07 12.20) |
| **31** | 0.7—1.9 (20H, m), 0.86 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 0.90 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 2.05 (3H, s, $COC\underline{H}_3$), 2.5—4.2 (11H, m), 2.76 (3H, s, $NC\underline{H}_3$), 4.8—5.1 (3H, m), 5.19 (1H, d), 6.76 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.5 (5H, m, phenyl H), 7.65 (1H, s, imidazole N-C$\underline{H}$=N), 8.06 (1H, d, $J$=9 Hz) | 711 | $C_{38}H_{58}N_6O_7 \cdot H_2O$ | 62.61 (62.96 | 8.30 8.56 | 11.53 11.31) |
| **32** | 0.7—1.9 (20H, m), 0.84 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 0.87 (3H, d, $J$=6.7 Hz, $CH(C\underline{H}_3)_2$), 2.5—3.6 (9H, m), 2.79 (3H, s, $NC\underline{H}_3$), 3.75 (1H, m), 3.94 (4H, m), 4.10 (1H, m), 4.76 (1H, m), 4.95 (1H, m), 5.34 (1H, br d), 6.70 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.4 (5H, m, phenyl H), 7.40 (1H, s, imidazole N-C$\underline{H}$=N), 7.88 (1H, d, $J$=9 Hz) | 711 | $C_{38}H_{58}N_6O_7 \cdot 0.75H_2O$ | 63.00 (62.93 | 8.28 8.49 | 11.60 11.67) |
| **33** | 0.7—1.9 (16H, m), 0.85 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 0.88 (3H, d, $J$=6.7 Hz, $CH(C\underline{H}_3)_2$), 2.3—3.8 (14H, m), 2.81 (3H, s, $NC\underline{H}_3$), 4.10 (1H, m), 4.85 (1H, m), 4.99 (1H, m), 5.31 (1H, m), 6.74 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.4 (5H, m, phenyl H), 7.46 (1H, s, N-C$\underline{H}$=N), 7.79 (1H, d, $J$=9 Hz) | 667 | $C_{38}H_{54}N_6O_6 \cdot 1.5H_2O$ | 62.32 (62.72 | 8.28 8.45 | 12.11 11.75) |
| **34** | 0.7—1.9 (22H, m), 0.86 (3H, d, $J$=7.0 Hz, $CH(C\underline{H}_3)_2$), 0.89 (3H, d, $J$=6.9 Hz, $CH(C\underline{H}_3)_2$), 2.11 (2H, m), 2.5—3.4 (7H, m), 3.54 (1H, m), 3.78 (1H, m), 4.08 (1H, m), 4.5—5.5 (2H, m), 6.70 (1H, m), 7.2—7.5 (6H, m), 7.59 (1H, d, $J$=9.6 Hz) | Calcd for $C_{35}H_{57}N_6O_5$: 641.4394 Found: 641.4383 | | | N.D. | |
| **35** | 0.7—1.9 (22H, m), 2.4—3.4 (11H, m), 3.59 (1H, m), 3.82 (1H, m), 4.09 (1H, m), 4.8—5.4 (2H, m), 6.6—7.7 (10H, m), 8.40 (2H, m) | 674 | $C_{38}H_{54}N_6O_6 \cdot 1.25H_2O$ | 65.45 (65.43 | 8.17 8.19 | 12.05 12.12) |
| **36** | 0.7—1.9 (16H, m), 0.82 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 0.86 (3H, d, $J$=6.7 Hz, $CH(C\underline{H}_3)_2$), 2.5—4.0 (10H, m), 2.79 (3H, s, $NC\underline{H}_3$), 2.82 (3H, s, $NC\underline{H}_3$), 4.09 (1H, m), 4.61 (1H, m), 4.91 (1H, m), 6.69 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.5 (6H, m, phenyl H and imidazole N-C$\underline{H}$=N), 7.98 (1H, br d) | Calcd for $C_{34}H_{55}N_6O_6$: 643.4187 Found: 643.4178 | | | N.D. | |
| **37** | 0.7—1.9 (16H, m), 0.85 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 0.88 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 2.5—3.8 (14H, m), 2.77 (3H, s, $NC\underline{H}_3$), 2.83 (3H, s, $NC\underline{H}_3$), 3.38 (3H, s, $OC\underline{H}_3$), 4.11 (1H, m), 4.6—4.8 (4H, m), 4.94 (1H, m), 6.69 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.4 (5H, m, phenyl H), 7.42 (1H, s, imidazole N-C$\underline{H}$=N), 7.94 (1H, d, $J$=9.5 Hz) | Calcd for $C_{38}H_{63}N_6O_8$: 731.4711 Found: 731.4717 | | | N.D. | |
| **38** | 0.7—1.9 (16H, m), 0.85 (3H, d, $J$=6.7 Hz, $CH(C\underline{H}_3)_2$), 0.88 (3H, d, $J$=6.8 Hz, $CH(C\underline{H}_3)_2$), 2.5—3.8 (19H, m), 2.81 (3H, s, $NC\underline{H}_3$), 2.83 (3H, s, $NC\underline{H}_3$), 4.09 (1H, m), 4.93 (1H, m), 5.25 (1H, m), 6.75 (1H, s, imidazole N-C$\underline{H}$=C), 7.1—7.7 (7H, m) | 770 | $C_{40}H_{63}N_7O_8 \cdot 2H_2O$ | 59.61 (59.58 | 8.38 8.42 | 12.16 11.98) |

were not inhibited by **29** even at 100 $\mu$M concentration. Furthermore, compound **29** showed no inhibitory activity against ACE. These results demonstrate that compound **29** has a high degree of specificity for human renin, which is a desirable characteristic for a therapeutic agent.

## Conclusion

(2*S*,3*S*,5*S*)-2-Amino-1-cyclohexyl-6-methyl-3,5-heptanediol (2-amino-3,5-*anti*-diol) was found to be a novel transition-state mimic for use in renin inhibitors. A series of inhibitors containing the 2-amino-3,5-*anti*-diol fragment at the P1–P1′ position showed consistently high inhibitory activities against human plasma renin, and most of them showed blood pressure-lowering effects after oral administration in salt-depleted, conscious marmosets. Among them, compound **29** (JTP-2724) having 4-hydroxypiperidine at the P4 position caused the most significant and long-lasting reduction of blood pressure in marmosets. The oral bioavailability of JTP-2724 is under examination.

### Experimental

Melting points were determined on a Buchi 535 melting point apparatus. Infrared (IR) spectra were recorded on a JASCO IR700 IR spectrometer or on a Perkin-Elmer 1650 FT-IR spectrometer. Optical rotations were measured with Perkin–Elmer 241 polarimeter. Nuclear magnetic resonance (NMR) spectra were measured on Bruker AMX300 (300 MHz), ARX (400 MHz) and JEOL JNMA300 (300 MHz) instruments. Chemical shifts are reported as $\delta$ values (parts per million) relative to tetramethylsilane as an internal standard. Fast atom bombardment mass spectra (FAB-MS) were obtained with a Finnigan MAT TSQ700 mass spectrometer or with a JEOL SX102 mass spectrometer. Elemental analyses were performed by the Analytical Group, Central Pharmaceutical Research Laboratories, Japan Tobacco, Inc. Thin-layer chromatography (TLC) was carried out using Merck precoated Silica gel 60 F-254 plates (thickness 0.25 mm). Preparative TLC was carried out using Merck precoated Silica gel 60 F-254 plates (thickness 0.5—1.0 mm). Column chromatography was carried out using Merck Silica gel 60 (70—230 or 230—400 mesh).

**(5*RS*,6*S*)-6-(*tert*-Butoxycarbonyl)amino-7-cyclohexyl-2-methyl-5-hydroxy-3-heptanone (7, 8)** A stirred solution of diisopropylamine (4.1 ml, 29.3 mmol) in THF (40 ml) was treated with *n*-butyllithiun (19.7 ml of a 1.49 M solution in hexane) at −30 °C. After 1 h the mixture was cooled to −70 °C and a solution of 3-methyl-2-butanone (3.1 ml, 29.3 mmol) in THF (40 ml) was added dropwise. The mixture was warmed to room temperature gradually, and recooled to −70 °C, and then a solution of *N*-Boc-cyclohexylalaninal (**5**) (5.0 g, 19.5 mmol) in THF (75 ml) was added dropwise. After 5 min, the mixture was poured into a combined solution of saturated aqueous $NH_4Cl$ (100 ml) and water (100 ml), then extracted with diethyl ether (100 ml). The organic layer was washed with brine (50 ml × 2), dried over $MgSO_4$, and evaporated *in vacuo*. The residue was separated by silica gel chromatography with a mixture of AcOEt and hexane (1 : 9) to give **7** (2.44 g, 37%) and **8** (2.37 g, 36%).

**7**: Pale yellow oil. *Rf* 0.40 (AcOEt : hexane = 3 : 7). $^1$H-NMR ($CDCl_3$) $\delta$: 0.8—1.9 (13H, m), 1.11 (6H, d, $J$ = 6.9 Hz, –CH(C<u>H</u>$_3$)$_2$), 1.45 (9H, s, –C(C<u>H</u>$_3$)$_3$), 2.5—2.8 (3H, m), 3.40 (1H, d, $J$ = 2.3 Hz), 3.61 (1H, m), 4.02 (1H, m), 4.70 (1H, br d, $J$ = 9.9 Hz, –CONH–). FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{36}NO_4$: 342.2646. Found: 342.2649.

**8**: White solid. *Rf* 0.34 (AcOEt : hexane = 3 : 7). mp 94—96 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 0.7—2.0 (13H, m), 1.10 (6H, d, $J$ = 6.9 Hz, –CH(C<u>H</u>$_3$)$_2$), 1.44 (9H, s, –C(C<u>H</u>$_3$)$_3$), 2.5—2.8 (3H, m), 3.40 (1H, br d), 3.63 (1H, m), 3.97 (1H, m), 4.55 (1H, br d, –CONH–). *Anal.* Calcd for $C_{19}H_{35}NO_4$: C, 66.83; H, 10.33; N, 4.10. Found: C, 66.96; H, 10.49; N, 4.11.

**1-[(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-cyclohexylmethyl-2,2-dimethyl-1,3-oxazolidin-5-yl]-3-methyl-2-butanone (9)** Pyridinium *p*-toluenesulfonate (20 mg, 0.079 mmol) and 2-methoxypropene (0.030 ml, 0.316 mmol) were added to a stirred solution of **7** (54 mg, 0.158 mmol) in $CH_2Cl_2$ (0.5 ml) at room temperature. After 2 h, $CH_2Cl_2$ (2 ml) and saturated aqueous $NaHCO_3$ (2 ml) were added to the mixture. The organic layer was separated, dried over $MgSO_4$, then evaporated *in vacuo*. The residue was separated by preparative TLC with a mixture of AcOEt and hexane (1 : 4) to give **9** (34 mg, 56%) as a colorless oil. $^1$H-NMR ($CDCl_3$) $\delta$: 0.8—1.9 (19H, m), 1.11 (6H, d, $J$ = 6.9 Hz, –CH(C<u>H</u>$_3$)$_2$), 1.48 (9H, s, –C(C<u>H</u>$_3$)$_3$), 1.51 (6H, s, >C(C<u>H</u>$_3$)$_2$), 2.60 (1H, m), 2.63 (1H, dd, $J$ = 6.6, 16.4 Hz), 3.72 (1H, m, –NC<u>H</u><), 4.36 (1H, dt, $J$ = 1.8, 6.6 Hz, –OC<u>H</u><). FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{22}H_{40}NO_4$: 382.2959. Found: 382.2956.

**1-[(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-4-cyclohexylmethyl-2,2-dimethyl-1,3-oxazolidin-5-yl]-3-methyl-2-butanone (10)** This compound was prepared as a white solid in 47% yield from **8** by the same procedure as described for the preparation of **9**. $^1$H-NMR ($CDCl_3$) $\delta$: 0.8—1.9 (19H, m), 1.13 (6H, d, $J$ = 6.9 Hz, –CH(C<u>H</u>$_3$)$_2$), 1.47 (9H, s, –C(C<u>H</u>$_3$)$_3$), 1.51 (6H, s, >C(C<u>H</u>$_3$)$_2$), 2.63 (2H, m), 2.87 (1H, dd, $J$ = 5.8, 17.5 Hz), 4.07 (1H, m, –NC<u>H</u><), 4.40 (1H, m, –OC<u>H</u><). Irradiation of the 2.87 ppm dd resulted in the collapse of the 4.40 ppm resonance to a d, $J$ = 4.8 Hz. FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{22}H_{40}NO_4$: 382.2959. Found: 382.2956.

**(2*S*,3*S*,5*RS*)-2-(*tert*-Butoxycarbonyl)amino-1-cyclohexyl-6-methyl-3,5-heptanediol (11, 12)** A stirred solution of **7** (214 mg, 0.63 mmol) in MeOH (5 ml) was treated with $NaBH_4$ (119 mg, 3.15 mmol) at room temperature. After 1 h, the mixture was evaporated *in vacuo*, and water (10 ml) was added. The resulting mixture was extracted with AcOEt (20 ml). The organic layer was dried over $MgSO_4$, and evaporated *in vacuo*. The residue was separated by preparative TLC with a mixture of AcOEt and hexane (1 : 1) to give **11** (58 mg, 27%) and **12** (128 mg, 59%).

**11**: White solid. *Rf* 0.63 (AcOEt : hexane = 1 : 1). mp 148—150 °C. IR (KBr) cm$^{-1}$: 1716. $[\alpha]_D^{25}$ −54.3° ($c$ = 1.05, MeOH). $^1$H-NMR ($CDCl_3$) $\delta$: 0.8—1.9 (16H, m), 0.90 (3H, d, $J$ = 6.8 Hz, –CH(C<u>H</u>$_3$)$_2$), 0.94 (3H, d, $J$ = 6.7 Hz, –CH(C<u>H</u>$_3$)$_2$), 1.45 (9H, s, –C(C<u>H</u>$_3$)$_3$), 2.47 (1H, br d, –O<u>H</u>), 2.68 (1H, br d, –O<u>H</u>), 3.59 (1H, m), 3.69 (1H, m), 3.83 (1H, m), 4.64 (1H, br d, $J$ = 9.1 Hz, –CONH–). *Anal.* Calcd for $C_{19}H_{37}NO_4$: C, 66.44; H, 10.86; N, 4.08. Found: C, 66.55; H, 11.09; N, 4.31.

**12**: White solid. *Rf* 0.53 (AcOEt : hexane = 1 : 1). mp 108—113 °C. $[\alpha]_D^{25}$ −16.8° ($c$ = 1.10, MeOH). $^1$H-NMR ($CDCl_3$) $\delta$: 0.8—1.9 (16H, m), 0.91 (6H, d, $J$ = 6.9 Hz, –CH(C<u>H</u>$_3$)$_2$), 1.45 (9H, s, –C(C<u>H</u>$_3$)$_3$), 2.60 (1H, br s, –O<u>H</u>), 3.5—3.9 (3H, m), 4.72 (1H, br d, $J$ = 9.7 Hz, –CONH–). FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{38}NO_4$: 344.2802. Found: 344.2780.

**(4*S*,6*S*)-4-[(1*S*)-1-(*tert*-Butoxycarbonyl)amino-2-cyclohexylethyl]-6-isopropyl-2,2-dimethyl-1,3-dioxane (13)** *p*-Toluenesulfonic acid monohydrate (4 mg) and 2,2-dimethoxypropane (0.26 ml, 2.1 mmol) were added successively to a stirred solution of **11** (72 mg, 0.21 mmol) in THF (0.6 ml). The mixture was stirred for 1 h at room temperature, then saturated aqueous $NaHCO_3$ (2 ml) was added and the resulting mixture was extracted with AcOEt (4 ml). The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel preparative chromatography with a mixture of AcOEt and hexane (1 : 3) to give **13** (62 mg, 77%) as a colorless oil. $^1$H-NMR ($CDCl_3$) $\delta$: 0.8—1.9 (16H, m), 0.85 (3H, d, $J$ = 6.8 Hz, –CH(C<u>H</u>$_3$)$_2$), 0.91 (3H, d, $J$ = 6.6 Hz, –CH(C<u>H</u>$_3$)$_2$), 1.30 (6H, s, >C(C<u>H</u>$_3$)$_2$), 1.45 (9H, s, –C(C<u>H</u>$_3$)$_3$), 3.35 (1H, ddd, $J$ = 6.0, 7.5, 9.8 Hz), 3.64 (1H, m), 3.73 (1H, m), 4.66 (br d, $J$ = 9.5 Hz, –CONH–). $^{13}$C-NMR ($CDCl_3$) $\delta$: 17.6, 18.7, 24.4, 24.6, 26.4, 26.5, 26.6, 28.5, 32.9, 33.0, 33.2, 33.8, 34.5, 40.7, 50.6, 68.2, 72.3, 78.9, 100.3, 156.1. FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{22}H_{42}NO_4$: 384.3116. Found: 384.3117.

**(4*S*,6*R*)-4-[(1*S*)-1-(*tert*-Butoxycarbonyl)amino-2-cyclohexylethyl]-6-isopropyl-2,2-dimethyl-1,3-dioxane (14)** This compound was prepared as a colorless oil in 62% yield from **12** by the same procedure as described for the preparation of **13**. $^1$H-NMR ($CDCl_3$) $\delta$: 0.7—1.9 (16H, m), 0.85 (3H, d, $J$ = 7.2 Hz, –CH(C<u>H</u>$_3$)$_2$), 0.90 (3H, d, $J$ = 6.6 Hz, –CH(C<u>H</u>$_3$)$_2$), 1.36 (3H, s, >C(C<u>H</u>$_3$)$_2$), 1.38 (3H, s, >C(C<u>H</u>$_3$)$_2$), 1.45 (9H, s, –C(C<u>H</u>$_3$)$_3$), 3.47 (1H, ddd, $J$ = 3.5, 6.9, 10.4 Hz), 3.63 (1H, m), 3.79 (1H, m), 4.70 (br d, $J$ = 9.9 Hz, –CONH–). $^{13}$C-NMR ($CDCl_3$) $\delta$: 17.7, 18.4, 19.7, 26.3, 26.4, 26.6, 28.4, 30.0, 30.3, 33.1, 33.8, 34.3, 40.3, 51.1, 70.6, 73.9, 78.8, 98.4, 156.1. FAB-MS *m/z*: $[M+H]^+$ Calcd for $C_{22}H_{42}NO_4$: 384.3116. Found: 384.3101.

**(2*S*,3*S*,5*S*)-2-Amino-1-cyclohexyl-6-methyl-3,5-heptanediol (15)** A solution of **11** (2.44 g, 7.1 mmol) in trifluoroacetic acid (50 ml) was stirred for 30 min at room temperature, then concentrated *in vacuo*. Saturated aqueous $NaHCO_3$ (50 ml) was added to the residue, and the mixture was extracted with $CHCl_3$ (50 ml × 3). The combined organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of $CHCl_3$, MeOH and $NH_4OH$

(95:5:0.5) to give **15** (1.59 g, 92%) as white crystals, mp 88—90 °C, $[\alpha]_D^{25}$ −53.3° ($c$=1.02, MeOH). $^1$H-NMR ($CDCl_3$) $\delta$: 0.8—1.9 (16H, m), 0.91 (3H, d, $J$=6.8 Hz, –CH(C<u>H</u>$_3$)$_2$), 0.95 (3H, d, $J$=6.7 Hz, –CH(C<u>H</u>$_3$)$_2$), 2.75 (1H, ddd, $J$=3.3, 6.6, 9.9 Hz, $NH_2$C<u>H</u><), 3.51 (1H, dt, $J$=3.8, 7.0 Hz), 3.61 (1H, ddd, $J$=2.8, 6.1, 8.9 Hz). *Anal.* Calcd for $C_{14}H_{29}NO_2$: C, 69.09; H, 12.01; N, 5.75. Found: C, 69.08; H, 11.99; N, 5.96.

**(2*S*,3*S*,5*R*)-2-Amino-1-cyclohexyl-6-methyl-3,5-heptanediol (16)** The title compound was prepared as a colorless oil in 42% yield from **12** by the same procedure as described for the preparation of **15**. $[\alpha]_D^{25}$ −9.06° ($c$=2.02, MeOH). $^1$H-NMR ($CDCl_3$) $\delta$: 0.8—1.8 (16H, m), 0.93 (6H, d, $J$=6.9 Hz, –CH(C<u>H</u>$_3$)$_2$), 2.72 (1H, m, $NH_2$C<u>H</u><), 3.50 (1H, m), 3.65 (1H, ddd, $J$=1.8, 5.1, 10.2 Hz). FAB-MS $m/z$: $[M+H]^+$ Calcd for $C_{14}H_{30}NO_2$: 244.2278. Found: 244.2290.

**(2*S*,3*S*,5*S*)-2-(4-Bromobenzoyl)amino-1-cyclohexyl-6-methyl-3,5-heptanediol (17)** Triethylamine (0.279 ml, 2.0 mmol) was added to a stirred solution of **15** (243 mg, 1.0 mmol) in $CHCl_3$ (15 ml). A solution of 4-bromobenzoyl chloride (241 mg, 1.1 mmol) in $CHCl_3$ (15 ml) was added dropwise to the mixture at 5—10 °C. The whole was allowed to warm to room temperature, stirred for 1 h, and washed with 1 N NaOH (5 ml × 3), 1 N HCl (5 ml × 3) and brine (5 ml × 2) successively. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to give a white solid. The solid was recrystallized from AcOEt to give the title compound as colorless crystals, mp 150—151 °C, $[\alpha]_D^{25}$ −53.4° ($c$=1.01, MeOH). $^1$H-NMR ($CDCl_3$) $\delta$: 0.87 (3H, d, $J$=6.9 Hz, –CH(C<u>H</u>$_3$)$_2$), 0.93 (3H, d, $J$=6.7 Hz, –CH(C<u>H</u>$_3$)$_2$), 0.9—1.9 (16H, m), 2.38 (1H, br s, –O<u>H</u>), 3.06 (1H, br s, –O<u>H</u>), 3.59 (1H, m), 4.0 (1H, m), 4.25 (1H, m), 6.37 (1H, d, $J$=9.3 Hz, –N<u>H</u>–), 7.5—7.7 (4H, m, phenyl H). *Anal.* Calcd for $C_{21}H_{32}BrNO_3$: C, 59.15; H, 7.56; N, 3.29. Found: C, 59.27; H, 7.77; N, 3.27.

**X-Ray Crystal Structure Determination of 17** $C_{21}H_{32}BrNO_3$, $M_r$=426.39, orthorhombic, space group $P2_12_12_1$, $a$=18.582(2) Å, $b$=22.192(2) Å, $c$=5.204(2) Å, $\alpha=\beta=\gamma=90°$, $V$=2146.1(10) Å$^3$, $Z$=4, $D_{calc.}$=1.320 g cm$^{-3}$, $\mu$=2.757 mm$^{-1}$.

Colorless thin plate crystals were grown from EtOH solution by slow evaporation at room temperature. The measurements were performed with a Rigaku AFC-7R four-circle diffractometer using graphite-monochromatized Cu$K\alpha$ radiation ($\lambda$=1.54178 Å) at 203(1) K. of the total of 2334 reflections measured in the range of $3.10° < \theta < 68.13°$, 2308 were independent and 2009 were observed with $I > 2.0\sigma(I)$. Empirical $\psi$-scan absorption correction[26] was applied. The structure were solved by the direct method (*SAPI-91*)[27] and refined on $wF^2$ by full-matrix least-squares method (*SHELXL*-93)[28] using all independent reflections except for 2 reflections. The isopropyl group was distorted after a few cycles of refinement, and thereafter the group was restrained geometrically. Hydrogen atoms were located at calculated positions. The final $R_1$ and $wR_2$ values were 0.0412 and 0.1266 for 2007 observed reflections, respectively ($R_1 = (\Sigma||F_o|-|F_c||)/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2-F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$). The goodness of fit based on $F_o^2$ was 1.089. The positive and negative maximum peaks of electron density in the final difference Fourier map were 0.649 and −0.505 e Å$^{-3}$, respectively. Flack's absolute configuration parameter $\chi$[29] was 0.01(3), which indicated that the model has the correct hand. Atomic coordinates, bond length, angle and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

***N*-Piperidinocarbonyl-L-phenylalanine Benzyl Ester (40)** Method A: Triethylamine (24.6 ml, 0.176 mol) was dissolved in THF (250 ml), then trichloromethylchloroformate (5.3 ml, 0.049 mol), a solution of L-phenylalanine benzyl ester (15.0 g, 0.059 mol) in THF (125 ml) and a solution of piperidine (11.6 ml, 0.118 mmol) in THF (125 ml) were successively added at 0 °C. The mixture was stirred for 1.5 h at 0 °C, then filtered and the filtrate was evaporated *in vacuo*. The residue was dissolved in AcOEt (400 ml) and the organic solution was washed with 0.1 N HCl (200 ml), saturated aqueous $NaHCO_3$ (200 ml × 2) and brine (200 ml) successively. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of AcOEt and hexane (3:7) to give the title compound (4.45 g, 28%) as white crystals.

Method B: A solution of piperidine (9.37 g, 0.11 mol) in THF (200 ml) was added slowly to a solution of triethylamine (22.3 g, 0.22 mol) and trichloromethylchloroformate (10.9 g, 0.055 mol) in THF (300 ml) at 0 °C. Then L-phenylalanine benzyl ester (28.1 g, 0.11 mol) in THF (100 ml) was added at 0 °C and the mixture was stirred for 25 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was dissolved in AcOEt (500 ml) and the organic solution was washed with 0.1 N HCl (250 ml × 2), saturated aqueous $NaHCO_3$ (250 ml) and brine (250 ml) successively, then dried over $MgSO_4$ and evaporated *in vacuo*. The residue was recrystallized from a mixture of AcOEt and hexane (1:2) to give the title compound (26.4 g, 65%) as white crystals, mp = 89—91 °C. IR (KBr) cm$^{-1}$: 1740, 1620, $[\alpha]_D^{20}$ −22.7° ($c$=1.03, MeOH). $^1$H-NMR ($CDCl_3$) $\delta$: 1.4—1.7 (6H, m, –(C<u>H</u>$_2$)$_3$–), 3.11 (2H, d, $J$=5.3 Hz, >CHC<u>H</u>$_2$Ph), 3.28 (4H, m, –C<u>H</u>$_2$NC<u>H</u>$_2$–), 4.84 (2H, m), 5.14 (2H, AB, –OC<u>H</u>$_2$Ph), 7.02 (2H, m, phenyl H), 7.1—7.4 (8H, m, phenyl H). FAB-MS $m/z$: $[M+H]^+$ 367. *Anal.* Calcd for $C_{22}H_{26}N_2O_3$: C, 72.11; H, 7.15; N, 7.64. Found: C, 72.33; H, 7.25; N, 7.71.

***N*-Piperidinocarbonyl-L-phenylalanine (41)** A suspension of **40** (1.0 g, 2.73 mmol) and 10% Pd on activated carbon (0.1 g) in MeOH (10 ml) was hydrogenated at atmospheric pressure for 1.5 h. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* to give the title compound (0.72 g, 96%) as a colorless amorphous solid, $[\alpha]_D^{20}$ −24.9° ($c$=0.94, MeOH). IR (KBr) cm$^{-1}$: 1725, 1603. $^1$H-NMR ($CDCl_3$) $\delta$: 1.4—1.7 (6H, m, –(C<u>H</u>$_2$)$_3$–), 3.0—3.4 (6H, m, >CHC<u>H</u>$_2$Ph, –C<u>H</u>$_2$NC<u>H</u>$_2$–), 4.54 (1H, m, –NHC<u>H</u><), 4.83 (1H, d, $J$=6.4 Hz, –CON<u>H</u>–), 7.1—7.4 (5H, m, phenyl H).

***N*$^{\alpha}$-Benzyloxycarbonyl-*N*$^{\alpha}$-methyl-*N*$^{im}$-(4-toluenesulfonyl)-L-histidine Methyl Ester (43)** NaH (60% in oil, 25 g, 0.625 mol) was washed with hexane and suspended in THF (700 ml). *N*$^{\alpha}$-Benzyloxycarbonyl-*N*$^{im}$-(4-toluenesulfonyl)-L-histidine (92 g, 0.208 mol) in THF (300 ml) and *N*,*N*-dimethylformamide (DMF) (300 ml) were added to the above suspension at 0 °C, followed by methyl iodide (118 g, 0.832 mol). After having been stirred for 7 h at room temperature, the mixture was poured into 10% aqueous citric acid (1 l) and ice (500 g), then extracted with AcOEt (1 l × 2). The combined organic layer was washed successively with 5% aqueous $Na_2S_2O_3$ (1 l × 2), 1 M aqueous $NaHCO_3$ (1 l × 2) and brine, and dried over $MgSO_4$, then evaporated *in vacuo*. The residue was recrystallized from EtOH (150 ml) to give **43** (58 g, 59%) as pale yellow crystals, mp 108—110 °C. IR (KBr) cm$^{-1}$: 1744, 1697. $[\alpha]_D^{20}$ −38.2° ($c$=1.14, $CH_2Cl_2$). $^1$H-NMR ($CDCl_3$) $\delta$: 2.39 and 2.42 (3H, s, 2 rotamers), 2.78 (3H, s), 2.9—3.3 (2H, m), 3.61 and 3.71 (3H, s, 2 rotamers), 4.78 and 4.87 (1H, dd, $J$=4.9, 10.4 Hz, 2 rotamers), 5.03 (2H, m), 6.94 and 7.06 (1H, s, 2 rotamers), 7.2—7.5 (7H, m), 7.74 (2H, m), 7.86 and 7.89 (1H, s, 2 rotamers). *Anal.* Calcd for $C_{23}H_{25}N_3O_6S$: C, 58.59; H, 5.35; N, 8.91. Found: C, 58.47; H, 5.38; N, 8.81.

***N*$^{\alpha}$-Benzyloxycarbonyl-*N*$^{\alpha}$-methyl-L-histidine Hydrazide (44)** A stirred solution of **43** (15.0 g, 0.032 mol) in THF (150 ml) was treated with HOBT (17.2 g, 0.127 mol). Stirring was continued for 7 h at room temperature, then the mixture was evaporated *in vacuo*. The residue was dissolved in AcOEt (200 ml) and extracted with 1 N aqueous HCl (200 ml). The aqueous layer was adjusted to pH 9 by addition of $K_2CO_3$ and extracted with $CHCl_3$ (200 ml). The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of $CHCl_3$ and MeOH (93:7) to give *N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\alpha}$-methyl-L-histidine methyl ester (9.74 g, 97%) as a pale yellow oil.

Hydrazine monohydrate (10.7 ml, 0.221 mol) was addedd to a stirred solution of this ester (7.0 g, 0.0221 mol) in MeOH (70 ml) at 0 °C. The whole was stirred for 9 h at 0 °C, then evaporated, and saturated aqueous $NaHCO_3$ (75 ml) was added to the residue. The mixture was extracted with $CHCl_3$ (100 ml × 2), and the combined organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of $CHCl_3$, MeOH and $NH_4OH$ (93:7:0.5) to give **44** (4.6 g, 66%) as a colorless amorphous solid. IR (KBr) cm$^{-1}$: 1681. $^1$H-NMR ($CDCl_3$) $\delta$: 2.93 (3H, s, NC<u>H</u>$_3$), 2.98 (1H, m), 3.26 (1H, dd, $J$=7.9, 15.0 Hz, one of –CHC<u>H</u>$_2$–), 4.8—5.2 (3H, m), 6.72 and 6.78 (1H, s, 2 rotamers, imidazole NC<u>H</u>=C), 7.2—7.4 (5H, m), 7.47 (1H, s, imidazole NC<u>H</u>=N), 8.08 and 8.49 (1H, br s). FAB-MS $m/z$: $[M+H]^+$ 318. *Anal.* Calcd for $C_{15}H_{19}N_5O_3 \cdot 1.25H_2O$: C, 53.01; H, 6.38; N, 20.61. Found: C, 52.90; H, 6.09; N, 20.22.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-*N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\alpha}$-methyl-L-histidinamide (45)** A 4 N HCl solution in 1,4-dioxane (18.5 ml, 73.8 mmol) and isoamyl nitrite (3.96 ml, 29.5 mmol) were added successively to a stirred solution of **44** (7.82 g, 24.6 mmol) in DMF (60 ml) at −30 °C. After having been stirred for 40 min at −30 °C, the mixture was cooled to −60 °C and neutralized by addition of triethylamine (10.3 ml, 73.8 mmol). A solution of **15** (4.98 g, 20.5 mmol) in DMF (40 ml) was added to the mixture at −60 °C, and the whole was warmed to 4 °C. It was stirred for 22 h at 4 °C, then

filtered, and the filtrate was evaporated *in vacuo*. The residue was taken up in saturated aqueous $NaHCO_3$ (100 ml), and extracted with AcOEt (150 ml × 2). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (150 ml × 2), dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of $CHCl_3$, MeOH and $NH_4OH$ (97 : 3 : 0.3) to give **45** (5.77 g, 53%) as a colorless amorphous solid. $^1$H-NMR ($CDCl_3$) $\delta$: 0.7—1.8 (16H, m), 0.85 (3H, d, $J$=6.6 Hz, $-CH(C\underline{H}_3)_2$), 0.90 (3H, d, $J$=6.6 Hz, $-CH(C\underline{H}_3)_2$), 2.87 (1H, dd, $J$=5.7, 15.3 Hz), 2.93 (3H, s, $NC\underline{H}_3$), 3.29 (1H, dd, $J$=9.2, 14.3 Hz), 3.55 (1H, m), 3.78 (1H, m), 4.03 (1H, m), 4.95 (1H, m, NC$\underline{H}$CO), 5.11 (2H, AB, $OC\underline{H}_2Ph$), 6.68 (1H, br d, $J$=9.3 Hz), 6.80 (1H, s, imidazole NC$\underline{H}$=C), 7.2—7.4 (5H, m, phenyl H), 7.42 (1H, s, imidazole NC$\underline{H}$=N). FAB-MS $m/z$: $[M+H]^+$ 529. *Anal.* Calcd for $C_{29}H_{44}N_4O_5 \cdot 0.5H_2O$: C, 64.78; H, 8.44; N, 10.42. Found: C, 64.69; H, 8.34; N, 10.33.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-$N^{\alpha}$-methyl-L-histidinamide (46)** A suspension of **45** (1.24 g, 2.35 mmol) and 10% Pd on activated carbon (0.124 g) in MeOH (20 ml) was hydrogenated at atomospheric pressure for 22.5 h. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* to give **46** (0.900 g, 97%) as a colorless amorphous solid. $^1$H-NMR ($CD_3OD$) $\delta$: 0.8—2.0 (16H, m), 0.93 (6H, d, $J$=6.6 Hz, $-CH(C\underline{H}_3)_2$), 2.44 (3H, s, $NC\underline{H}_3$), 2.88 (1H, dd, $J$=7.2, 15.0 Hz), 3.06 (1H, dd, $J$=6.0, 15.0 Hz), 3.46 (1H, dd, $J$=6.0, 7.2 Hz, NC$\underline{H}$CO), 3.57 (1H, m), 3.83 (1H, m), 4.00 (1H, m), 6.97 (1H, s, imidazole NC$\underline{H}$=C), 7.66 (1H, s, imidazole NC$\underline{H}$=N). FAB-MS $m/z$: $[M+H]^+$ 395. *Anal.* Calcd for $C_{21}H_{38}N_4O_3 \cdot H_2O$: C, 61.14; H, 9.77; N, 13.58. Found: C, 61.33; H, 9.54; N, 13.68.

***N*-[(1*S*,2*S*)-1-Cyclohexylmethyl-2-hydroxy-5-methylhexyl]-$N^{\alpha}$-[(2*R*)-3-morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-histidinamide (18)** A stirred solution of (2*R*)-3-morpholinocarbonyl-2-(1-naphthylmethyl)propionyl-L-histidine methyl ester[13] (1.25 g, 2.62 mmol) in methanol (12.5 ml) was treated with hydrazine monohydrate (1.9 g, 38.0 mmol) at room temperature. The mixture was stirred for 4.5 h, then evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of $CHCl_3$, MeOH and $NH_4OH$ (93 : 7 : 1) to give (2*R*)-3-morpholinocarbonyl-2-(1-naphthylmethyl)propionyl-L-histidine hydrazide (1.04 g, 83%) as a colorless amorphous solid. The hydrazide was coupled with (2*S*,3*S*)-2-amino-1-cyclohexyl-3-heptanol[10] by the same procedure as described for the preparation of **45** to give **18** (70%) as a colorless amorphous solid.

Compounds **19** and **20** were prepared from **15** and **16**, respectively, by the same procedure as described for the preparation of **18**.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-$N^{\alpha}$-methyl-$N^{\alpha}$-(*N*-piperidinocarbonyl-L-phenylalanyl)-L-histidinamide (21)** HOBT (89 mg, 0.659 mmol) and DCC (115 mg, 0.558 mmol) were added successively to a stirred solution of *N*-piperidinocarbonyl-L-phenylalanine (**41**) (154 mg, 0.558 mmol) and **46** (200 mg, 0.507 mmol) in DMF (3 ml) at 0 °C. After having been stirred for 2 h at 0 °C, the mixture was warmed to room temperature, further stirred for 20 h, and filtered. The filtrate was evaporated *in vacuo*. The residue was taken up in a mixture of MeOH, water and acetic acid (94 : 3 : 3) and the whole was heated at 60 °C for 30 min. After evaporation *in vacuo*, the residue was dissolved in AcOEt, and the organic solution was washed twice with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of $CHCl_3$ and MeOH (95 : 5) to give **21** (176 mg, 53%) as a colorless amorphous solid, $[\alpha]_D^{25}$ −77.9° ($c$=1.01, MeOH).

Compounds **22**—**25**, **28**—**30** and **35**—**38** were prepared from the corresponding carboxylic acids by the same procedure as described for the preparation of **21**.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-$N^{\alpha}$-[*N*-(perhydro-1,1-dioxo-1,4-thiazin-4-yl)carbonyl-L-phenylalanyl]-$N^{\alpha}$-methyl-L-histidinamide (26)** A stirred solution of *N*-(perhydro-1,4-thiazin-4-yl)carbonyl-L-phenylalanine benzyl ester (500 mg, 1.30 mmol) in $CH_2Cl_2$ (20 ml) was treated with *m*-chloroperbenzoic acid (785 mg, 4.55 mmol) at 0 °C. The mixture was stirred for 1 h, warmed to room temperature, and further stirred for 16 h. Then AcOEt (100 ml) was added and the organic solution was washed with 10% aqueous $Na_2SO_3$ (50 ml × 3), dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of AcOEt and hexane (1 : 1) to give *N*-(perhydro-1,1-dioxo-1,4-thiazin-4-yl)carbonyl-L-phenylalanine benzyl ester (493 mg, 91%) as a white solid. The ester (118 mg) was mixed with 10% Pd on activated carbon (20 mg) and MeOH (5 ml) and the mixture was hydrogenated at atmospheric pressure for 2 h. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was coupled with **46** by the same procedure as described for the synthesis of **21** to give the title compound **26** (45%) as a colorless amorphous solid.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-$N^{\alpha}$-methyl-$N^{\alpha}$-[*N*-(1-piperazinyl)carbonyl-L-phenylalanyl]-L-histidinamide (27)** *N*-(4-Benzyloxycarbonyl-1-piperazinyl)carbonyl-L-phenylalanine was coupled with **46** by the same procedures as described for the synthesis of **21**, followed by hydrogenation over Pd on activated carbon to give the title compound **27** (29%) as a colorless amorphous solid.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-$N^{\alpha}$-[*N*-(4-acetoxypiperidino)carbonyl-L-phenylalanyl]-$N^{\alpha}$-methyl-L-histidinamide (31)** A solution of *N*-(4-hydroxypiperidino)carbonyl-L-phenylalanine benzyl ester (515 mg, 1.35 mmol) and acetic anhydride (0.38 ml, 4.06 mmol) in pyridine (2 ml) was stirred for 16 h at room temperature. After addition of water (2 ml), the mixture was evaporated and the residue was dissolved in AcOEt (25 ml). The organic solution was washed successively with 1 N aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$, and evaporated *in vacuo*. The residue was mixed with 10% Pd on activated carbon (50 mg) and MeOH (8 ml) and the mixture was hydrogenated at atmospheric pressure for 1 h. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was coupled with **46** by the same procedure as described for the synthesis of **21** to give the title compound **31** in 45% yield as a colorless amorphous solid.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-$N^{\alpha}$-[*N*-(1,3-dioxolane-2-spiro-4′-piperidin-1′-yl)carbonyl-L-phenylalanyl]-$N^{\alpha}$-methyl-L-histidinamide (32)** A solution of *N*-(4-piperidinon-1-yl)carbonyl-L-phenylalanine benzyl ester (964 mg, 2.53 mmol), *p*-toluenesulfonic acid (44 mg, 0.253 mmol) and ethylene glycol (188 mg, 3.04 mmol) in benzene (25 ml) was refluxed for 2 h. After having cooled to room temperature, the mixture was poured into water and extracted with ethyl acetate. The organic solution was washed successively with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of ethyl acetate and hexane (1 : 1) to give *N*-(1,3-dioxolane-2-spiro-4′-piperidine-1′-yl)carbonyl-L-phenylalanine benzyl ester (680 mg, 63%) as a white solid. The obtained ester (618 mg) was mixed with 10% Pd on activated carbon (62 mg) and MeOH (20 ml) and the mixture was hydrogenated at atmospheric pressure for 1 h. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was coupled with **46** by the same procedure as described for the synthesis of **21** to give the title compound **32** in 51% yield as a colorless amorphous solid.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-$N^{\alpha}$-methyl-$N^{\alpha}$-[*N*-(4-piperidinon-1-yl)carbonyl-L-phenylalanyl]-L-histidinamide (33)** A solution of **32** (395 mg, 0.556 mmol), *p*-toluenesulufonic acid (862 mg, 5.00 mmol) and water (2 ml) in MeOH (18 ml) was refluxed for 1 d, then evaporated *in vacuo*. The residue was taken up in $CHCl_3$. The organic solution was washed successively with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with a mixture of $CHCl_3$, MeOH and $NH_4OH$ (95 : 5 : 0.5) to give **33** (83 mg, 22%) as a colorless amorphous solid.

***N*-[(1*S*,2*S*,4*S*)-1-Cyclohexylmethyl-2,4-dihydroxy-5-methylhexyl]-$N^{\alpha}$-[*N*-(3-amino-3-methylbutylyl)-L-phenylalanyl]-$N^{\alpha}$-methyl-L-histidinamide (34)** The title compound was prepared as a colorless amorphous solid in 54% yield from 3-benzyloxycarbonylamino-3-methylbutylic acid by the same procedure as described for the preparation of **27**.

***In Vitro* Renin-Inhibitory Assay** The renin-inhibitory effect of compounds was evaluated on plasma renin activity (PRA) of human plasma. The assay mixture consisted of 200 $\mu$l of EDTA-plasma, 20 $\mu$l of citrate buffer, 10 $\mu$l of phenylmethylsulfonyl fluoride (PMSF), and 10 $\mu$l of various concentrations of a test compound in dimethyl sulfoxide (DMSO). The reaction mixture was incubated at 37 °C for 60 min. After incubation, the PRA was estimated by measurement of the produced angiotensin I (Ang I), which was quantified by radioimmunoassay using a commercial kit (RENIN RIABEAD, Dinabot Ltd., Japan). The percentage inhibition was calculated at each concentration point, and the concentration of each renin inhibitor that inhibited PRA by 50% ($IC_{50}$) was estimated. The species specificity of renin inhibitors was evaluated by comparison of the renin-inhibitory effects on human, cynomolgus monkey, marmoset, dog and rat plasmas.

**Enzyme Specificity** The enzyme specificity of renin inhibitors was

evaluated by comparison of the inhibitory effects on renin, pepsin, cathepsin D and Ang I converting enzyme (ACE). Porcine pepsin (Sigma Chemical Co., U.S.A.) or bovine cathepsin D (Sigma Chemical Co.) was incubated at 37 °C for 10 or 30 min with bovine hemoglobin (Sigma Chemical Co.) as a substrate in the presence and absence of inhibitor. After incubation, proteins were precipitated with trichloroacetic acid and the absorbance of the supernatant was measured at the wavelength of 280 nm. Inhibition of human serum ACE was estimated using a commercial assay kit (ACE color, Fujirebio Inc., Japan). Human serum was incubated at 37 °C for 20 min with the substrate mixture of *p*-hydroxybenzoyl-glycyl-L-histidyl-L-leucine and hippuricase in the presence and absence of inhibitor. The reaction mixture was incubated with $NaIO_4$ and the absorbance of the solution was measured at the wavelength of 505 nm.

***In Vivo* Marmoset Model** Conscious marmosets (weighing 305—495 g) raised on a low salt diet (containing 0.02% salt, 1/10 of the normal diet) for a week were orally doesd with the inhibitor (5—10 mg/kg) dissolved in 0.1 M citric acid, in a volume of 2 ml/kg. The blood pressure was measured before and at various times after the administration by the tail-cuff method.

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**References and Notes**

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