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First total synthesis of Mer-N5075A and a diastereomeric mixture of α and β-MAPI, new HIV-I protease inhibitors from a species of *Streptomyces*

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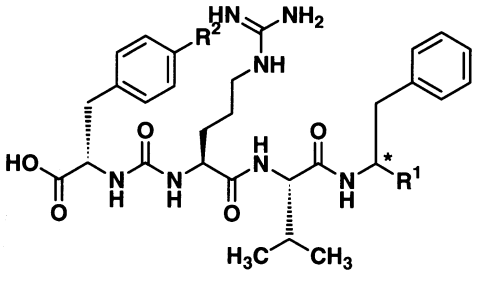
Abstract—Mer-N5075A (**1**) and α-MAPI and β-MAPI (**2** and **3**) produced from a species of *Streptomyces* are new anomalous tetrapeptides having potential HIV-I protease inhibitory activity. The first total synthesis of **1** and a diastereomeric mixture of **2** and **3** was achieved simply by a route connecting two dipeptides. The synthetic method is applicable for synthesis of Mer-N5075A analogues, such as GE20372 A and B (**4** and **5**) and other chemically modified compounds. In addition, the inhibition of HIV-1 Protease and anti HIV activity by the compounds **1**, a mixture of **2** and **3**, and **24**, **25**, and **26** were described. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Recently, development of therapeutic approaches for AIDs has progressed rapidly. In particular, the combined use of two or more drugs having different actions, such as reverse transcriptase inhibitors[1] and HIV (Human Immunodeficiency Virus) protease inhibitors,[2] has been shown to be highly effective in the treatment of AIDs. The development of HIV protease inhibitors is comparatively slow relative to that of reverse transcriptase inhibitors, though several peptide-based protease inhibitors have been synthesized and shown to be highly effective in AIDS therapy, which are exemplified by Indinavir[2a] and Saquinavir.[2b] Hence, discovery of a new HIV protease inhibitor is anticipated.

Mer-N5075A (**1**) is an anomalous tetrapeptide having potent human immunodeficiency virus type I (HIV-I) protease inhibition. It is isolated from *Streptomyces chromofuscus* Mer-N5075, which was collected in Okinawa Prefecture, Japan.[3] Mer-N5075A belongs to the MAPI group[4] of compounds **2** and **3** having microbial alkaline protease inhibition isolated from *Streptomyces nigrescens*. Recently, Stefanelli et al. isolated the novel HIV-I protease inhibitors, GE20372 A (**4**) and B (**5**),[5] which are structurally related to **1**, **2**, and **3**. These tetrapeptides (**1**–**5**) show the structural characteristics of a carboxyl group in one terminal, and an alcohol or aldehyde group in the other terminal. They also have a characteristic carbonyl linked between two amino acid which form ureido. In spite of their interesting



	Compound	R^1	R^2	Config. at *
1	Mer-N5075A	CH_2OH	H	*S*
2	α-MAPI	CHO	H	*S*
3	β-MAPI	CHO	H	*R*
4	GE20372A	CHO	OH	*S*
5	GE20372B	CHO	OH	*R*

Figure 1. Mer-N5075A analogies having HIV-1 protease inhibitory activity.

Keywords: HIV-I protease inhibitor; syntheses; tetrapeptide; *Streptomyces* species; ureido moiety.

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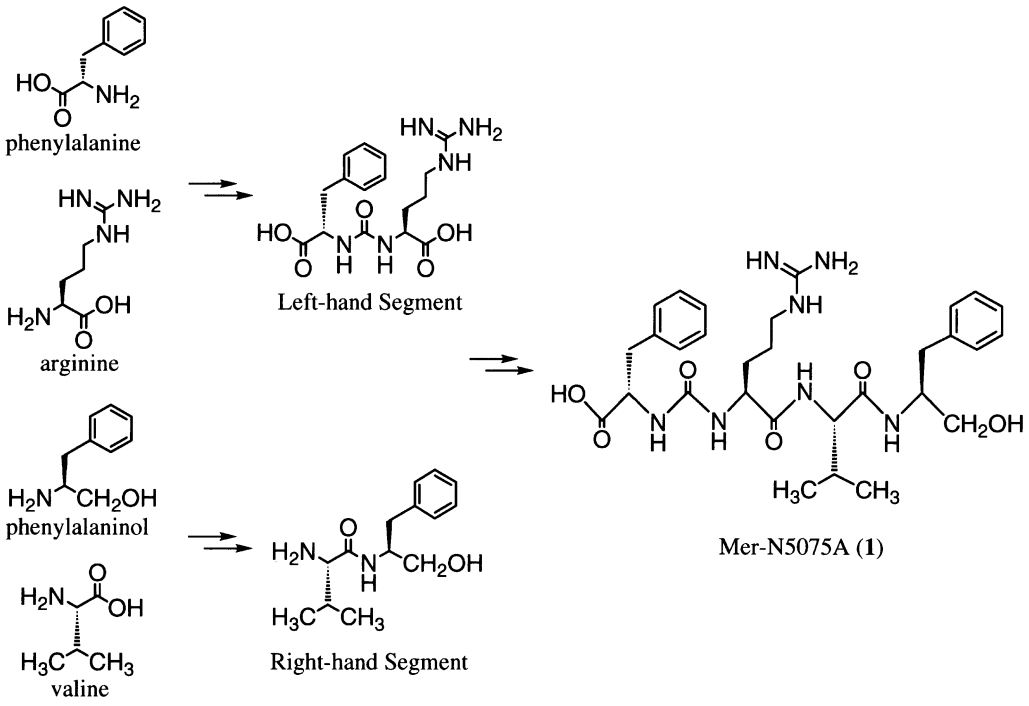


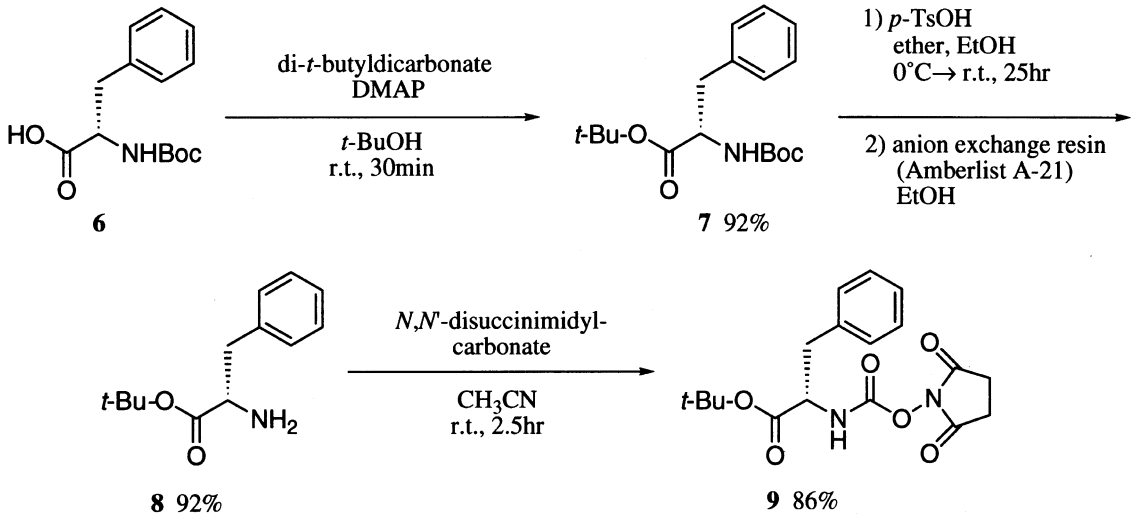
Scheme 1. Synthetic strategy for Mer-N5075A.

structural features and biological activity, their total syntheses have not been reported. As a part of our synthetic and biological studies on naturally occurring compounds which inhibit HIV-protease, we focused our attention on **1** and its analogues. HIV-I protease inhibition of α-MAPI (**2**) is reportedly ten times that of Mer-N5075A[3] (**1**). The terminal hydroxymethyl group of **1** is thought to be converted to a compound having more potent activity, such as α-MAPI and other analogous compounds, thus leading to the development of effective therapeutic agents for the treatment of AIDs. This background prompted us to synthesize **1** and its analogues **2** and **3**.

Recently, we reported the total synthesis of **1** in a preliminary study.[6] In this paper, we describe in detail the first synthesis of **1** and α and β-MAPI (**2** and **3**) (Fig. 1).

2. Results and discussion

Compound **1** consists of three ordinal amino acids, L-phenylalanine (Phe), L-arginine (Arg), L-valine (Val) and (*S*)-phenylalaninol (Pheol). Our synthetic strategy for **1** was to connect two dipeptides **16** as a left-hand segment and **22** as a right-hand segment composed of protected Arg and Phe, and of Val and Pheol, respectively. This yields protected tetrapeptide **24**. We then remove the protective groups at the same time to provide **1**. The introduction of the ureido group is an interesting key reaction for the synthesis of **1**. Several methods have been previously investigated and reported[7] concerning the introduction of a ureido group. We effectively used N,N′-disuccinimidylcarbonate (DSC),[8] which was developed by us as an activating reagent to introduce the ureido group (Scheme 1).



Scheme 2. Synthesis of active carbamate ester (**9**).

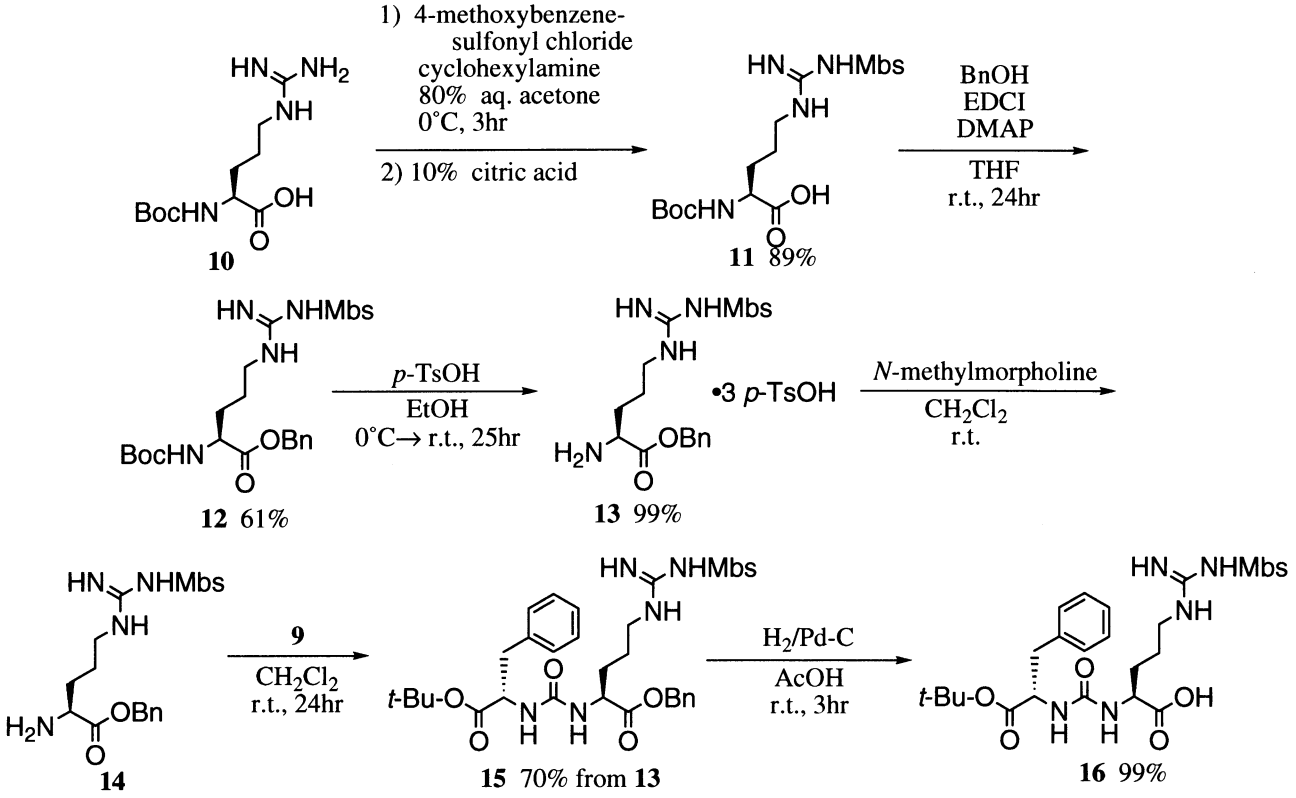
3. Synthesis of Mer-N5075A

Dipeptide **16** was synthesized as follows. Compound **6** was esterified by treatment with di-*tert*-butyl dicarbonate[9] in the presence of DMAP (dimethylaminopyridine) in *tert*-BuOH, thereby producing a *tert*-butyl ester **7** in 92% yield, followed by the selective deprotection of the Boc group of **7** in the presence of a *tert*-butyl ester according to the procedure described by Goodacre et al.[10] Thus, treatment of **7** with *p*-toluenesulfonic acid (*p*-TsOH) gave the *p*-TsOH salt. The salt was then passed through anion-exchange resin (Amberlyst A-21) giving amine **8** in 92% yield from **7**. This was converted to the activated ester **9** by treatment with DSC in 86% yield (Scheme 2).

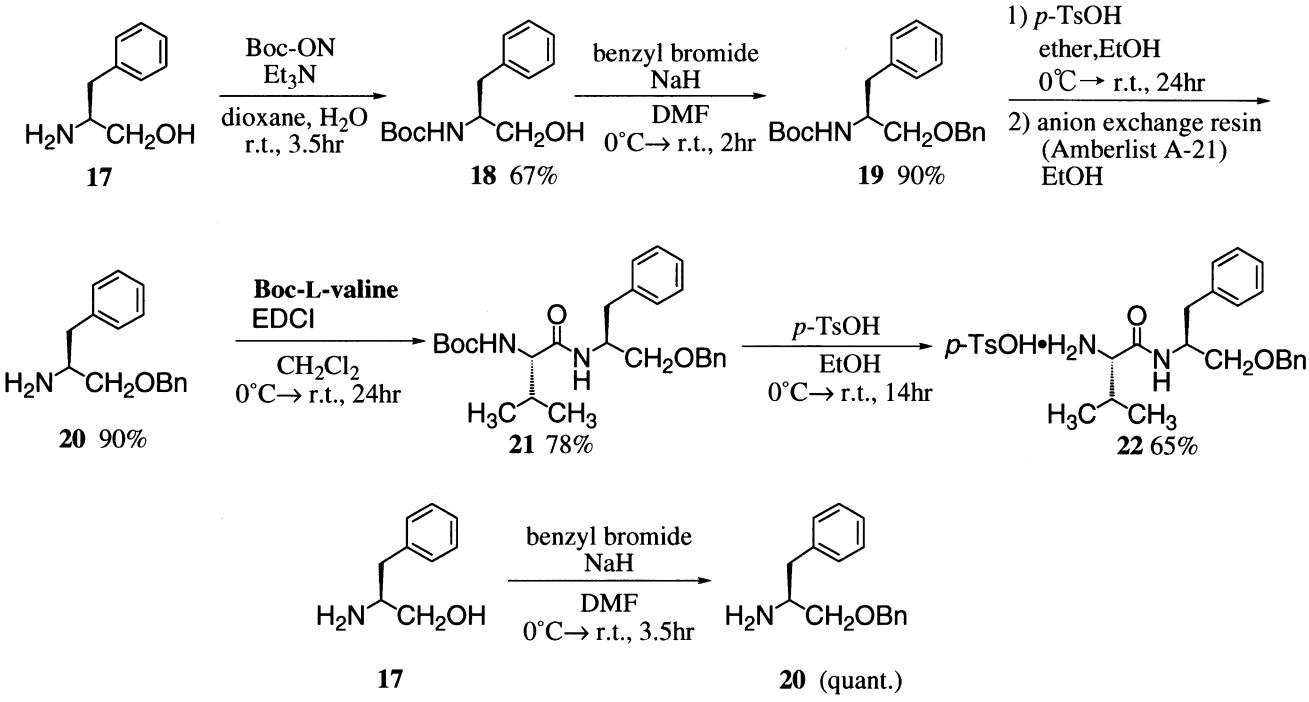
The primary amino group of Boc-L-arginine (**10**) was protected by the 4-methoxybenzenesulfonyl (Mbs) group using Mbs chloride followed by purification as a cyclohexylamine salt; then, neutralization by citric acid afforded the acid **11** in total yield of 89% from **10**. Esterification of **11** by benzyl alcohol in the presence of 1-ethyl-3-[3- (dimethylamino)propyl] carbodiimide (EDCI) and DMAP gave the ester **12** in 61% yield. Deprotection of the Boc group of **12** with *p*-TsOH gave the salt **13** in 99% yield. The salt **13** was then neutralized with N-methylmorpholine (NMM)[11] to give amine **14** and then connected with the active ester **9** to afford the dipeptide **15** in total yield of 70% from **13**. Debenzylation of **15** by catalytic hydrogenation with Pd-C in ethanol gave the acid **16** in 99% yield (Scheme 3).

Dipeptide **22** was synthesized as follows. Protection of the amino group in phenylalaninol (**17**) by treatment with Boc-ON in the presence of triethylamine afforded alcohol **18** in 67% yield. The alcohol **18** was etherified with benzyl bromide and sodium hydride in 90% yield. The Boc group of **19** was removed by *p*-TsOH and then passed through anion exchange resin to afford amine **20** in 90% yield. The amine **20** was coupled with Boc-L-valine in the presence of EDCI to give protected dipeptide **21** in 78% yield. The amine **20** was alternatively synthesized from **17** in only one step as follows. The primary hydroxyl group of **17** was selectively etherified by benzyl bromide and sodium hydride to give the amine **20** quantitatively, which was sufficiently pure and thus used without further purification. Deprotection of the Boc group of **21** by treatment with *p*-TsOH afforded the dipeptide salt **22** in 65% yield (Scheme 4).

The salt **22** was neutralized with NMM to afford dipeptide amine **23** quantitatively, which was condensed with the dipeptide **16** in the presence of EDCI, hydroxybenzotriazole (HOBt), and NMM to afford successfully the protected tetrapeptide **24** in 90% yield from **22**. The Mbs, *tert*-butyl, and benzyl groups in **24** were simultaneously removed by treatment with methanesulfonic acid[12] and passed through Amberlite IRA-410 to remove the acid, then purified by Sephadex LH-20 to afford Mer-N5075 A (**1**) in 60% yield. The ^{1}H and ^{13}C NMR spectra of synthetic Mer-N5075A were almost identical to those of the natural compound described in the literature.[3] Morever, the ^{1}H and ^{13}C NMR spectra of synthetic Mer-N5075A perfectly coincided with the spectra of the natural compound supplied by Mercian Co., Ltd. Compound **1** showed the optical rotation value of $[\alpha]_D^{27}$ −24.4° (*c*=0.32, AcOH), which is close to the reported value ($[\alpha]_D^{28}$ −27.6°, *c*=0.11, AcOH) (Scheme 5).



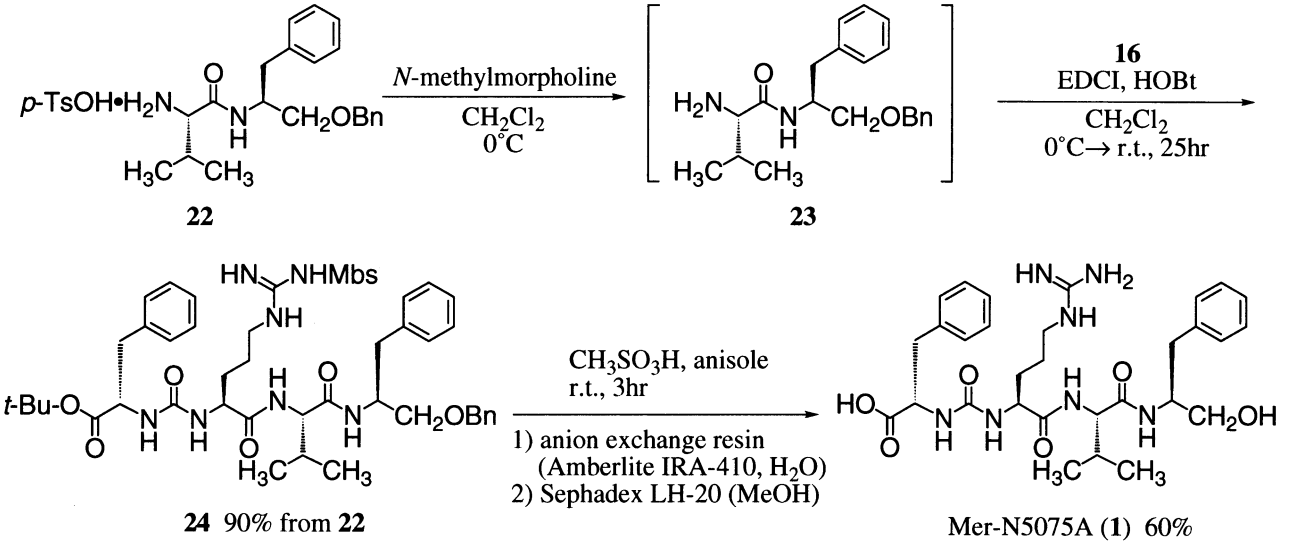
Scheme 3. Synthesis of left-hand segment (**16**).



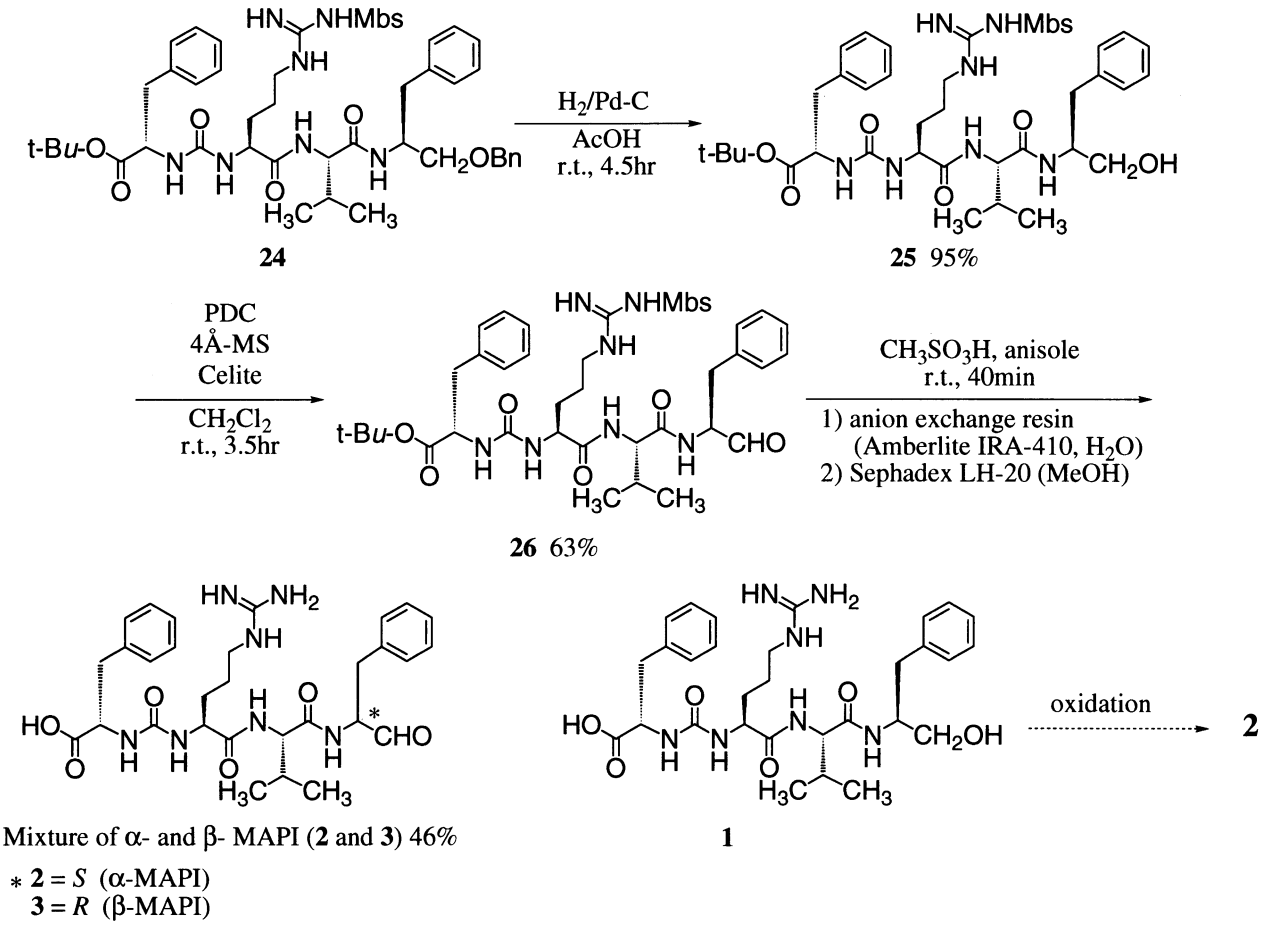
Scheme 4. Synthesis of right-hand segment (**22**).

4. Synthesis of the mixture of α and β-MAPI

In order to synthesize α-MAPI (**2**), debenzylation of the tetrapeptide **24** was examined. Debenzylation did not occur under either the conditions of catalytic hydrogenation over Pd-C in EtOH, or those of hydrogen transfer using Pd-black and Pd-C in EtOH. However, debenzylation of **24** by catalytic hydrogenation with Pd-C in AcOH afforded the alcohol **25** successfully in 92% yield. Oxidation of the primary hydroxyl group of **25** by treatment with PDC in the presence of molecular sieves and celite gave the aldehyde **26** in 60% yield as a single product which was confirmed from ^{1}H and ^{13}C-NMR spectra. Deprotection of the *t*-butyl and Mbs groups was performed by treatment with methanesulfonic acid and passage through Amberlite IRA-410 and Sephadex LH-20, which is the same method used for **1**. This gave the diastereomeric mixture of α and β-MAPI (**2** and **3**) in 46% yield. These results show that after the formation of α-MAPI (**2**), enolization of the terminal aldehyde occurred under these acidic conditions and **2** was epimerized to β-MAPI (**3**). We are planning to analytically separate these diastereomers by HPLC or preparative TLC to gain **2** and **3** as a single product. Furthermore, we will chemically synthesize **2** by converting MerN-5075A (**1**) directly to **2** by Swern oxidation or Dessmartin oxidation shown in Scheme 6.



Scheme 5. Synthesis of **1**.



Scheme 6. Synthesis of **2** and **3**.

5. The inhibition of the HIV-1 protease and anti HIV activity of 1, a mixture of 2 and 3 and related compounds

Measurement of the inhibitory activity of HIV-1 protease was carried out by our co-workers at Mercian Co., Ltd. in the same manner as used in the case of Mer-N5075A.[3] The enzyme reaction was achieved using a substrate solution, which was monitored by HPLC as described in the Experimental Section. The IC_{50} values against the HIV-1 protease of compound **1**, a mixture of **2** and **3**, **24**, **25**, and **26** are summarized in Table 1. Mer-N5075A and a mixture of α-MAPI and β-MAPI (**2** and **3**) showed the HIV-1 protease inhibitory activity, although the values shown were relatively low when compared with those in the literature (lit.3 IC_{50} values (μM): Mer-N5075A 17.8; α-MAPI 1.3; β-MAPI 18.3). One past study showed[3] that the aldehyde function at the terminal is more attributable to HIV-1 protease inhibitory action than the alcoholic function. It is interesting to note that the inhibitory activity of compound **25**, which has an alcohol group is superior to that of compound **26**, which has an aldehyde group at the terminal position. The details of the experimental procedures will be reported in *Biol. Pharm. Bull* in which the results of the screening of HIV-1 protease inhibition of nine synthetic compounds related to Mer-N5075A, α and β-MAPI, and GE20372-A will be shown. We will also discuss the structural biological activity relationship in future studies.

Table 1. The HIV-1 protease inhibitory activity of **1**, a mixture of **2** and **3**, **24**, **25**, **26**

Compound	IC_{50} (μM)
1	64.3
a mixture of **2** and **3**	8.95
24	>100
25	68.2
26	>100

In 1993, Kaneto et al. reported that no anti HIV-1 activity was detected when natural products **1** and **2** were subjected to an in vitro system using MT-4 cell and HIV-1. We are particulary interested in our anti HIV-1 activity in vitro system. Thus, we subjected synthetic compounds **1**, a mixture of **2** and **3**, and **24** and **26** to an in vitro system using MT-4 cell and HIV-1 developed by our collaborator, Dr. Baba. The system uses as control reagents nelfinavir, an HIV-1 porotease inhibitor, and lamiudine, a reverse transcriptase. The 50% effective concentration (EC_{50}) and 50% cytotoxicity concentration (CC_{50}) values are summarized in Table 2.

Although compound **1**, **2** and **3** showed HIV-1 protease inhibitory action, they did not show anti HIV-1 activity, as shown in Table 2. We believe the above result is due to the fact that the samples are so hydrophilic that they are miscible with the CD4 cell membrane, and thus they were

Table 2. The anti HIV activity of **1**, a mixture of **2** and **3**, **24**, **26** in vitro system using MT-4 cell and HIV-1

Compound	EC_{50} (μM)	CC_{50} (μM)
1	>100	>100
24	>100	>100
a mixture of **2** and **3**	>41	41
26	<1.7	1.7
nelfinavir	<0.032	6.8
lamivudine	0.35	>20

unable to invade the cell. Alternatively, their HIV-1 protease inhibitory activity is so weak that they do not effectively inhibit anti HIV-1 activity. It is noteworthy that a mixture of **2** and **3**, and **26** showed cytotoxicity. Details of the experimental procedure for measuring the bioactivities will be reported in *Biol. Pharm. Bull* in the near future (Tables 1 and 2).

6. Conclusion

The first synthesis of a novel HIV-I protease inhibitor, Mer-N5075 A and a mixture of α-MAPI and β-MAPI was achieved. Each reaction involved in this procedure is employable in a large scale preparation and applicable to the synthesis of its analogues. Furthermore, Mer-N5075A, its related compound **25**, and a mixture of α and β-MAPI showed the HIV-1 protease inhibitory activity. We are currently investigating synthesis of **4** and **5** and other analogues to obtain more potent HIV-I protease inhibitors. Notably, in the present study, a mixture of **2** and **3**, **26** having an aldehyde group at the terminal showed cytotoxicity.

7. Experimental

7.1. General

Melting points were taken on a Yanagimoto hot-stage and are uncorrected. ^1H and ^{13}C NMR were recorded on a Varian VXR-300, XL-400 spectrometers. The signals were assigned by ^1H-^1H COSY, DEPT, HMQC, HMBC experiments. Mass spectra were obtained on a JEOL-JMX-DX 300 mass spectrometer (low resolution mass spectrometry) and JEOL-JMS-AX505 HA mass spectrometer (high resolution mass spectrometry). Routine monitoring of reactions was carried out using Merck 60 GF254 silica gel, glass-supported TLC plates. Flash column chromatography was performed on silica gel 60 H (Merck). Thin-layer chromatography (TLC) was done on silica gel 60 PF254 (Merck). Ethyl ether, tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) was each distilled from sodium, potassium hydride. Dimethylformaldehyde (DMF) was dried by molecular sieves 3A.

7.1.1. N-*t*-Boc-L-phenylalanine *t*-butyl ester (7). Di-*tert*-butyldicarbonate (1.65 g, 3.54 μmol), dimethylaminopyridine (138 mg, 1.13 μmol) were added to a stirred solution of N-*t*-boc-L-phenylalanine (**6**) (1.00 g, 3.77 μmol) in *tert*-buthanol (8 ml) at room temperature under argon. After 30 min, the mixture was concentrated in vacuo and the residue was purified by flash column chromatography (n-hexane: AcOEt=2:1) to give **7** (1.11 g, 91.6%) as yellow oil. *Rf*=0.53 (hexane:AcOEt=2:1). $[\alpha]_D^{24}$+32.04° (*c*=1.08, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1490 (arom), 1700 (-NHCOO-), 1720 (COO-), 3430 (-NH-)HRFABMS *m/z*: 322.2017$[M+H]^+$, Calcd for $C_{18}H_{28}NO_4$: 322.2018. *Anal*. Calcd for $C_{18}H_{28}NO_4$: C, 67.26; H, 8.47; N,4.36. Found: C, 67.12; H, 8.39; N, 4.58. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.39, 1.42 (each 9H, s, *t*-Bu, Boc), 3.05 (2H, d, *J*=6.0, Phe3-H_2), 4.45 (1H, dt, *J*=7.0, 6.0, Phe2-H), 4.95 (1H, brd, *J*=7.0, Phe2-NH), 7.12–7.35 (5H, m, Phe-arom.-H).

7.1.2. L-Phenylalanine *t*-butyl ester (8). *p*-Toluenesulfonic acid (2.06 g, 12.0 μmol) in EtOH (10 ml) was added to a solution of **7** (3.85 g, 12.0 μmol) in ethyl ether (3 ml) and stirred for 30 min at 0°C. The reaction was continued for further 24 h at room temperature. The mixture was concentrated in vacuo to give the *p*-toluenesulfonic acid salt (4.59 g). mp 134°C (EtOH). *Rf*=0.50 (hexane/AcOEt=2:1). $[\alpha]_D^{26}$+37.69° (*c*=1.04, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1735 (-COO-), 2800–3300 (-SO_3H), 3450 (-NH_2). HRFABMS *m/z*: 222.1514 $[M+H]^+$, Calcd for $C_{13}H_{20}N_{O2}$: 222.1494. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.42 (9H, s, *t*-Bu), 2.84 (1H, dd, *J*=13.0, 8.0, Phe3-Ha), 3.04 (1H, dd, *J*=13.0, 6.0, Phe3-Hb), 3.60 (1H, dd, *J*=8.0, 6.0, Phe2-H), 7.17–7.34 (5H, m, Phe-arom.-5H). Ethanolic solution (15 ml) of the salt (4.59 g, 11.7 μmol) was passed through a column of anion exchange resin (Amberlist A-21, 100 g, EtOH) to give **8** (2.43 g, 91.5% from *7*). *Rf*=0.13 (hexane:AcOEt=2:1). $[\alpha]_D^{27}$+10.22° (*c*=0.90, $CHCl_3$). IR ($CHCl_3$) $\upsilon_{max}cm^{-1}$: 1720 (-COO-), 3400 (-NH_2). HRFABMS *m/z*: 222.1514 $[M+H]^+$, Calcd for $C_{13}H_{20}NO_2$.: 222.1494. ^1H NMR (300 MHz, $CDCl_3$) δ_H: 1.42 (9H, s, *t*-butyl-H), 2.84 (1H, dd, *J*=13.0, 8.0, HRFABMS *m/z*: 222.1514 $[M+H]^+$, Calcd for $C_{13}H_20NO2$.: 222.1494. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.42 (9H, s, *t*-Bu), 2.84 (1H, dd, *J*=13.0, 8.0, Phe3-Ha), 3.04 (1H, dd, *J*=13.0, 6.0, Phe3-Hb), 3.60 (1H, dd, *J*=8.0, 6.0, Phe2-H), 7.17–7.34 (5H, m, Phe-arom.-H).

7.1.3. L-Phenylalanine *t*-butyl ester N-hydroxysuccinimide carboxamide (9). Compound **8** (61.2 mg, 0.28 μmol) in acetonitrile (1 ml) was added to a stirred solution of N, N′-disuccinimidyl carbonate (70.0 mg, 0.27 μmol) in acetonitrile (4 ml) at room temperature under argon. After 2.5 h, the mixture was concentrated in vacuo. The residue was diluted with AcOEt (30 ml). The organic layer was washed with 10% citric acid (7 ml×2), saturated NaCl (5 ml×2), dried over Na_2SO_4, concentrated in vacuo to give **9** as a crude product. (85.9 mg, 85.9%). *Rf*=0.42 (hexane:AcOEt=1:3). mp 126°C (AcOEt). *Rf*=0.42 (hexane/AcOEt=1:3). $[\alpha]_D^{28}$+88.79° (*c*=1.07, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1730 (-COO-), 1780 (cyclic imide), 3400 (-NH-). HRFABMS *m/z*: 385.1390 [M+Na]+, Calcd for $C_{18}H_{22}N_2O_6Na$: 385.1376. *Anal*. Calcd for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12; N,7.73. Found: C, 59.57; H, 7.68; N, 7.68. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.41(9H, s, *t*-Bu), 2.82 (4H, brs, NSu-CH_2×2), 3.15 (2H, d, *J*=5.5, Phe3-H_2), 4.46 (1H, dt, *J*=7.0, 5.5, Phe2-H), 5.82 (1H, d, *J*=7.0, Phe2-NH), 7.15–7.35 (5H, m, Phe-arom.-5H).

7.1.4. N-Boc-N^G-methoxybenzenesulfonyl-L-arginine (11) cyclohexylamine salt. 4-Methoxybenzenesulfonyl chloride (1.7 g, 8.0 μmol) was added to a stirred solution of

N-Boc-L-arginine **10** (1.0 g, 3.2 μmol) in 80% aqueous acetone (20 ml) at 0°C maintained at pH=11.0–11.5 with 4N NaOH through the addition. After 3 h, 4-methoxybenzenesulfonyl chloride (664.9 mg, 3.2 μmol) was again added. After 18 h, the mixture was neutralized with 10% aqueous citric acid and acetone was removed in vacuo. The aqueous residue was washed with ether (6 ml×3), acidified at pH=3.0 with 10% aqueous citric acid, saturate with NaCl, extracted with AcOEt (7 ml×3). The organic layer was washed with 10% citric acid (7 ml), saturated NaCl (6 ml×3), dried over Na_2SO_4, evaporated in vacuo to give light yellow oil (1.7 g). To the oil in MeOH (3 ml), cyclohexylamine (371 mg, 3.7 μmol) was added, evaporated in vacuo to give the **11** cyclohexylamine salt, quantitatively as light yellow crystals. mp 50°C (crude). HRFABMS *m/z*: 445.1757 $[M+H]^+$, Calcd for $C_{18}H_{29}N_4O_7S$: 445.1754. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.00–1.60 (11H, m, CHA-11H), 1.41 (9H, s, *t*-Bu), 1.72 (2H, brd, *J*=10.0, Arg4-H_2), 1.99 (2H, brd, *J*=10.0, Arg3-H_2), 2.95 (1H, br, Arg5-Ha), 3.16 (1H, br, Arg5-Hb), 3.81 (3H, s, Mbs-OCH_3), 3.89 (1H, br, Arg2-H), 5.00–6.20 (3H, br, CHA-NH_3^+), 5.74 (1H, d, *J*=6.0, Arg2-NH), 6.89 (2H, d, *J*=9.0, Mbs-arom.-Ha), 7.76 (2H, d, *J*=9.0, Mbs-arom.-Hb).

7.1.5. N-*t*-Boc-N^G-methoxybenzenesulfonyl-L-arginine (11). After the solution of **11** cyclohexylamine salt described above in AcOEt (40 ml) was acidified with 10% citric acid (10 ml), the aqueous layer was extracted with AcOEt (10 ml×2). The organic layer was combined, washed with saturated NaCl, dried over Na_2SO_4, evaporated in vacuo to give **11** as light yellow crystals (1.8 g, 89% from **10**). *Rf*=0.22 ($CHCl_3$/MeOH=3:1). $[\alpha]_D^{23}$+1.92° (*c*=0.52, MeOH). HRFABMS *m/z*: 445.1765 $[M+H]^+$, Calcd for $C_{18}H_{29}N_4O_7S$: 445.1754. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.41 (9H, s, *t*-Bu), 1.59 (2H, br, Arg4-H_2), 1.69 (1H, m, Arg3a-H), 1.81 (1H, m, Arg3b-H), 3.19 (2H, brs, Arg5-H_2), 3.81 (3H, s, Mbs-OCH_3), 4.21 (1H, br, Arg1-H), 5.66 (1H, d, *J*=8.0, Arg2-NH), 6.26 (1H, br, guanidine-NH), 6.52 (2H, br, guanidine-NH×2), 6.90 (2H, d, *J*=9.0, Mbs-arom.-Ha), 7.76 (2H, d, *J*=9.0, Mbs-arom.-Hb).

7.1.6. N-*t*-Boc-N^G-methoxybenzenesulfonyl-L-arginine benzyl ester (12). Benzyl alcohol (77.8 mg, 0.72 μmol), EDCI (207 mg, 1.08 μmol), DMAP (7.3 mg, 0.06 μmol) were added to a solution of **11** (1.33 mg, 0.30 μmol) in THF (4 ml) and stirred for 24 h at room temperature under argon. The mixture was diluted with AcOEt (25 ml). The organic layer was washed with saturated NaCl (15 ml×2), saturated $NaHCO_3$ (15 ml×2), saturated aqueous NaCl (15 ml×2) and then, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by preparative TLC (SiO_2, hexane: AcOEt=1:3) to give **12** as light yellow crystals (98.1 mg, 61%). mp 48–52°C. *Rf*=0.28 (hexane:AcOEt=1:3). $[\alpha]_D^{25}$−7.89° (*c*=0.71, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1500, 1560, 1590 (arom), 1620 (-NHCOO-), 1680 (-C=NH), 1740 (-COO-), 3350 (-C=NH), 3450 (-NH-). HRFABMS *m/z*: 535.2260 $[M+H]^+$, Calcd for $C_{25}H_{35}N_4O_7S$: 535.2226. ^1H NMR (300 MHz, $CDCl_3$)δ: 1.41 (9H, s, *t*-Bu), 1.57 (2H, m, Arg4-H_2), 1.72 (2H, m, Arg3-H_2), 3.14 (1H, m, Arg5-Ha), 3.25 (1H, m, Arg5-Hb), 3.82 (3H, s, Mbs-OCH_3), 4.26 (1H, m, Arg2-H), 5.12, 5.16 (each 1H, d, *J*=12.0, benzyl-CH_2), 5.31 (1H, d, *J*=8.0, Arg2-NH), 6.27 (3H, brs, guanidine-NH×3), 6.88 (2H, d, *J*=9.0, Mbs-arom.-Ha), 7.28-7.40 (5H, m, benzyl), 7.76 (2H, d, *J*=9.0, Mbs-arom.-Hb).

7.1.7. N^G-methoxybenzenesulfonyl-L-arginine benzyl ester *p*-toluenesulfonic acid salt (13). *p*-Toluenesulfonic acid (1.0 g, 5.6 μmol) in THF (10 ml) was added to a solution of **12** (1.0 g, 1.9 μmol)in THF (25 ml). After the solution was stirred for 30 min at 0°C, then stirred for further 17 h at room temperature. The mixture was evaporated in vacuo to give the salt **13** (1.82 g, 99%) as colorless crystals. *Rf*=0.69 (hexane:AcOEt=1:3). $[\alpha]_D^{24}$−3.80° (*c*=1.00, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1500, 1570, 1590 (arom), 1680 (-C=NH), 1740 (-COO-), 2800–3300 (-SO_3H), 3400 (-NH_2). HRFABMS *m/z*: 435.1735 $[M+H]^+$, Calcd for $C_{20}H_{27}N_4O_5S$: 435.1702. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.70 (2H, br, Arg4-H_2), 1.95 (2H, br, Arg3-H_2), 2.32 (9H, s, *p*-TsOH-CH_3× 3), 3.18 (2H, br, Arg5-H_2), 3.77 (3H, s, Mbs-OCH_3), 4.26 (1H, m, Arg2-H), 5.08, 5.10 (each 1H, d, *J*=12.0, benzyl-CH_2), 5.90 (6H, br, NH_3^+, guanidine-NH×3), 6.85 (2H, d, *J*=9.0, Mbs-arom.-H_2), 7.16 (6H, d, *J*=7.0, *p*-TsOH-arom.-Ha×3), 7.24 (5H, m, benzyl-arom.-H), 7.68 (6H, d, *J*=7.0, *p*-TsOH-arom-×3), 7.75 (2H, d, *J*=9.0, Mbs-arom.-H).

7.1.8. Dipeptide 15. A solution of the salt **13** (1.82 g, 1.87 μmol) in CH_2Cl_2 (25 ml) was neutralized with N-methylmorpholine (568.3 mg, 5.6 μmol) and then, carboxamide **9** (814.8 mg, 2.2 μmol) in CH_2Cl_2 (15 ml) was added to the reaction mixture under argon and stirred for 21 h at room temperature. A solution of **9** (339.5 mg, 0.9 μmol)was added to the reaction mixture and stirred further 3 h at room temperature. The mixture was diluted in $CHCl_3$ (500 ml). The organic layer was washed with saturated $NaHCO_3$ (20 ml×2), 10% citric acid (20 ml×2), saturated NaCl (20 ml×2), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative TLC (SiO_2, $CHCl_3$: MeOH=10:1) to give **15** (889.5 mg 70% from 13) as yellow crystals. mp 59°C. *Rf*=0.59 ($CHCl_3$: MeOH=10: 1). $[\alpha]_D^{28}$+9.72° (*c*=1.07, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1500 (arom), 1680 (-NHCONH-), 1720 (-COO-), 3430 (-NH-).HRFABMS *m/z*: 682.2921-$[M+H]^+$, Calcd for $C_{34}H_{44}O_8N_5S$: 682.2911. Anal. Calcd for $C_{34}H_{43}N_{5O}8S$: C, 59.90; H, 6.36; N,10.27. Found: C, 59.80; H, 6.48; N, 9.98. ^1H NMR (400 MHz, pyridine) δ: 1.35 (9H, s, *t*-Bu), 1.54 (1H, m, Arg4-Ha), 1.70 (1H, m, Arg4-Hb), 1.90 (2H, m, Arg3-H_2), 2.88 (1H, dd, *J*=13.0, 6.5, Phe3-Ha), 2.93 (1H, dd, *J*=13.0, 6.5, Phe3-Hb), 3.13 (2H, br, Arg5-H_2), 3.80 (3H, s, Mbs-OCH_3), 4.31 (1H, br, Arg2-H), 4.51 (1H, dt, *J*=6.5, 6.0, Phe2-H), 5.05, 5.12 (each, 1H, d, *J*=12.0, Bn-CH_2), 5.86 (1H, br, Phe2-NH), 5.92 (1H, br, Arg2-NH), 6.86 (2H, d, *J*=9.0, Mbs-arom.-H_2), 7.13–7.40 (10H, m, phenyl, benzyl arom.-H), 7.77 (2H, d, *J*=9.0, Mbs-arom.-H_2).

7.1.9. Debenzylated dipetide 16. 10% Palladium carbon (1.09 g) was added to the solution of the dipeptide **15** (2.01 g, 2.93 μmol) in acetic acid (30 ml), and mixture was stirred under hydrogen gas for 2.5 h at room temperature and then, filtered. The filtrate was evaporated in vacuo to give the acid **16** (1.74 g, 99%) as a light yellow oil. *Rf*=0.07 ($CHCl_3$: MeOH=5: 1). mp 79–88°C (MeOH). $[\alpha]_D^{28}$+9.36° (*c*=1.09, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}:

1500, 1570, 1600 (arom), 1630 (-NHCONH-), 1730 (-COO-), 3350 (-OH). HRFABMS *m/z*: 592.2436 $[M+H]^+$, Calcd for $C_{27}H_{38}N_5O_8S$: 592.2441. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.28 (9H, s, *t*-Bu), 1.51 (2H, m, Arg4-H_2), 1.58 (1H, m, Arg4-Hb), 1.90 (2H, m, Arg3-H_2), 2.88 (1H, dd, *J*=13.0, 6.5, Phe3-Ha), 2.93 (1H, dd, *J*=13.0, 6.5, Phe3-Hb), 3.13 (2H, br, Arg5-H_2), 3.80 (3H, s, Mbs-OCH_3), 4.31 (1H, br, Arg2-H), 4.51 (1H, dt, *J*=6.5, 6.0, Phe2-H), 5.05, 5.12 (each 1H, d, *J*=12.0, Bn-CH_2), 5.12 (1H, d, *J*=12.0, Bn-CH_2), 5.86 (1H, br, Phe2-NH), 5.92 (1H, br, Arg2-NH), 6.86 (2H, d, *J*=9.0, Mbs-arom.-H_2), 7.13–7.40 (10H, m, phenyl, benzyl arom.-H), 7.77 (2H, d, *J*=9.0, Mbs-arom.-H_2).

7.1.10. N-(*t*-Butoxycarbonyl)-L-phenylalaninol (18). A solution of Boc-ON (1.6 g, 6.6 μmol) in dioxane (3 ml) was added to a mixture of L-phenylalaninol (17) (1 g, 6.6 μmol), triethylamine (1 ml) and water (3 ml). The mixture was stirred at room temperature for 3.5 h, then concentrated in vacuo. The residue was purified by preparative TLC (Silica gel, *n*-hexane: AcOEt=2:1) to give 18 (1.11 g, 66.9%) as colourless crystals. mp 93–95°C. *Rf*=0.47 ($CHCl_3$: MeOH=10:1). $[\alpha]_D^{26}$ −19.15°C (*c*=1.07, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1500 (arom), 1700 (-NHCOO-), 3450 (-NH-), 3650 (-OH)HRFABMS *m/z*: 252.1605 $[M+H]^+$, Calcd for $C_{14}H_{22}NO_3$: 252.1600. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.42 (9H, s, *t*-Bu), 1.86 (1H, brs, PheOH1-OH), 2.84 (2H, d, *J*=8.0, PheOH3-H_2), 3.58 (1H, dd, *J*=10.0, 5.0, PheOH1-Ha), 3.70 (1H, brd, *J*=10.0, PheOH1-Hb), 3.90 (1H, br, PheOH_2-H), 4.87 (1H, br, PheOH 2-NH), 7.18–7.32 (5H, m, arom.-H).

7.1.11. N-(*t*-Butoxycarbonyl)-L-phenylalaninol benzyl ether (19). 60% NaH (320 mg, 8.0 μmol) was added to a stirred solution of **18** in DMF (20 ml) at 0°C under argon. After 30 min, benzyl bromide (752 mg, 4.40 μmol) was added to the reaction mixture. The mixture was stirred for further 1.5 h at room temperature and evaporated in vacuo. The residue was poured into ice water, extracted with $CHCl_3$ (50 ml×4). The organic layer was washed with saturated NaCl (25 ml×3), dried over Na_2SO_4 and concentrated in vacuo. Residue was purified by preparative TLC (Silica gel, $CHCl_3$: MeOH=10: 1) to give 19 (1.22 g, 90.0%) as colourless oil. *Rf*=0.77 ($CHCl_3$: MeOH=10: 1). $[\alpha]_D^{28}$ −4.89° (*c*=0.45, $CHCl_3$)IR ($CHCl_3$) $\upsilon_{max}cm^{-1}$: 1500 (arom), 1690 (-NHCOO-), 3450 (-NH-). HRFABMS *m/z*: 364.1907 $[M+Na]^+$, Calcd for $C_{21}H_27NO_3Na$: 364.1889. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.44 (9H, s, *t*-Bu), 2.90 (2H, m, PheOH 3-H_2), 3.40 (1H, dd, *J*=9.0, 3.5, PheOH 1-Ha), 3.41 (1H, dd, *J*=9.0, 4.0, PheOH 1-Hb), 3.88 (1H, br, PheOH 2-H), 4.44, 4.52 (each, 1H, d, *J*=12.0, benzyl-CH_2), 4.52, (each 1H, d, *J*=12.0, benzyl-CH_2), 4.88 (1H, br, PheOH 2-NH), 7.10–7.50 (10H, m, PheOH, benzyl-arom-H).

7.1.12. L-Phenylalaninol benzyl ether (20).

1. *p*-Toluenesulfonic acid (50 mg, 0.30 mol) in EtOH (0.3 ml) was dropped to a stirred solution of **19** (100 mg, 0.30 μmol) in ether (1.5 ml) during 1 h at 0°C. After 30 min, the mixture was stirred for further 24 h at room temperature. The solvent was removed, and the residue was crystallized with AcOEt to give *p*-toluenesulfonic salt of **2**0 (118 mg, 95.3%) as colourless crystals.
   The salt of **20** (118 mg, 0.30 μmol) in EtOH (5 ml) was passed through a column of anion exchange resin (Amberlist A-21) to give the amine **20** (65 mg, 90% from **19**) as colourless oil. *Rf*=0.50 ($CHCl_3$: MeOH=10:1). mp 110–117°C (EtOH). $[\alpha]_D^{23}$ +29.07° (*c*=0.97, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1600 (arom), 2800–3200 (-SO3H). HRFABMS *m/z*: 242.1566 $[M+H]^+$, Calcd for $C_{16}H_{20}N_O$: 242.1545. ^1H NMR (300 MHz, $CDCl_3$) δ: 2.00 (2H, s, PheOH-NH_2), 2.61 (1H, dd, *J*=13.5, 7.7, PheOH 3-Ha), 2.82 (1H, dd, *J*=13.5, 5.3, PheOH 3-Hb), 3.29 (1H, m, PheOH 2-H), 3.36 (1H, dd, *J*=8.7, 7.0, PheOH 1-Ha), 3.50 (1H, dd, *J*=8.7, 3.5, PheOH 1-Hb), 4.52 (1H, d, *J*=12.0, benzyl-CH_2), 4.55 (1H, d, *J*=12.0, phenyl-CH_2), 7.15–7.40 (10H, m, phenyl, benzyl-arom.-H).
2. NaH (1.59 g, 39.7 μmol) was added to a solution of L-phenylalaninol (**17**) (3.00 g, 19.8 μmol) in DMF (40 ml) at 0°C and stirred for 2 h. After the evolution of hydrogen gas deceased, benzyl bromide (2.60 ml, 21.8 μmol) was added at room temperature and stirred 1.5 h. DMF was removed in vacuo and the residue was diluted with water (150 ml), extracted with $CHCl_3$ (300 ml×3). The combined organic layer was washed with saturated NaCl (150 ml), dried over Na_2SO_4, concentrated in vacuo to give the crude product **20**, quantitatively (4.80 g) which was pure enough and used in the next reaction without purification. Physico-chemical data (*Rf*, HRFABMS) and the ^1H NMR spectrum agreed with those of **20** obtained from **19**.

7.1.13. N-(*tert*-Butoxycarbonyl)-L-valyl-L-phenylalaninol benzyl ether (21). N-(*tert*-Butoxycarbonyl)-L-valine (110 mg, 0.51 μmol) and EDCI (98 mg, 0.51 mol) were added to a solution of compound **20** in CH_2Cl_2 (6 ml) at 0°C under argon, and the mixture was stirried for 1 h at 0°C, and then for 24 h at room temperature. The mixture was diluted with $CHCl_3$ (68 ml), washed with saturated $NaHCO_3$ (13 ml×3), saturated NaCl (13×3), 1 M HCl (12.5 ml×3), saturated NaCl (13 ml×3), dried over Na_2SO_4, evaporated in vacuo. The residue was purified by preparative TLC (Silica gel, n-hexane: AcOEt=2:1) to give 21 (0.18 g, 78.0%) as light yellow crystalls. mp 110–113°C (AcOEt). *Rf*=0.66 ($CHCl_3$/MeOH=10:1). $[\alpha]_D^{24}$ −36.50° (*c*=0.40, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1450, 1490 (arom), 1660 (-NHCO-), 1700 (-NHCOO-). HRFABMS *m/z*: 441.2735 $[M+H]^+$, Calcd for $C_{26}H_{37}N_2O_4$: 441.2753 [M+H]. Anal. Calcd for $C_{26}H_{36}N_2O_4$: C, 70.88; H, 8.24; N,6.36. Found: C, 70.64; H, 8.28; N, 6.33. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.84 (3H, brd, *J*=6.0, Val4-H3), 0.91 (3H, d, *J*=7.0, Val4′-H3), 1.44 (9H, s, *t*-Bu), 2.09 (1H, m, Val3-H), 2.87 (1H, dd, *J*=14.0, 8.0, PheOH3-Ha), 2.90 (1H, dd, *J*=14.0, 7.5, PheOH3-Hb), 3.37 (1H, dd, *J*=10.0, 3.5, PheOH1-Ha), 3.39 (1H, dd, *J*=10.0, 3.5, PheOH1-Hb), 3.86 (1H, dd, *J*=8.0, 6.0, Val2-H), 4.30 (1H, m, PheOH_2-H), 4.46, 4.50 (each 1H, d, *J*=12.0, benzyl-CH_2), 5.00 (1H, *J*=8.0, Val2-NH), 6.24 (1H, *J*=9.0, PheOH-NH), 7.15–7.40 (10H, m, phenyl, benzyl-arom.-H).

7.1.14. L-Valyl-L-phenylalaninol benzyl ether *p*-toluenesurfonic acid salt (22). Anhydrous *p*-toluenesulfonic acid

(69 mg, 0.40 μmol) in EtOH (0.4 ml) was added to a stirred solution of **21** (176 mg, 0.40 μmol) in ether (2.0 ml) dropwise during 1 h at 0°C and stirred for 30 min. After stirring for 24 h at room temperature, anhydrous *p*-toluenesulfonic acid (69 mg, 0.40 μmol) was again added to the reaction mixture and stirred at 50°C for 24 h. The mixture was concentrated to give a crude product which was recrystallized from AcOEt to give crude *p*-toluenesulfonic acid salt **22** (205 mg) which was recrystallized from AcOEt to give colorless needles (133 mg, 65.0%). mp. 177-179°C. *Rf*=0.47 ($CHCl_3$: MeOH=10:1). $[\alpha]_D^{25}$ −20.6° (*c*=0.53, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1500, 1520, 1600 (arom), 1670 (-NHCO-), 2800–3200 (-SO3H), 3250 (-NH_2). HRFABMS *m/z*: 341.2253$[M+H]^+$, Calcd for $C2^1H_{29}N_2O_2$: 341.2229. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.81 (3H, d, *J*=7.0, Val4-H_3), 0.89 (3H, d, *J*=7.0, Val4′-H3), 2.08 (1H, sextet, Val3-H), 2.23 (3H, s, *p*-TsOH-CH_3), 2.87 (1H, dd, *J*=14.0, 8.0, PheOH3-Ha), 2.90 (1H, dd, *J*=14.0, 7.5, PheOH3-Hb), 3.37 (1H, dd, *J*=10.0, 3.5, PheOH1-Ha), 3.39 (1H, dd, *J*=10.0, 3.5, PheOH1-Hb), 3.86 (1H, dd, *J*=8.0, 6.0, Val2-H), 4.30 (1H, m, PheOH2-H), 4.46 (1H, d, *J*=12.0, Bn-CH_2-H), 4.50 (1H, d, *J*=12.0, Bn-CH_2-H), 5.00 (1H, *J*=8.0, Val2-NH), 6.24 (1H, *J*=9.0, PheOH-NH), 7.15–7.40 (10H, m, phenyl, benzyl-arom.-H).

7.1.15. Tetrapeptide (24). The solution of the salt **22** (685 mg, 1.31 μmol) in THF (12 ml) was neutralized with N-methylmorphorine (278 mg, 2.75 μmol) at 0°C under argon to give a solution of amine **23**; then a solution of **16** (774 mg, 1.31 μmol) in CH_2Cl_2 (10 ml), EDCI (276 mg, 1.44 μmol), HOBt (353 mg, 2.62 μmol) was added and stirred for 1 h under argon at 0°C. After that the solution was stirred further for 17 h at room temperature. The reaction mixture was diluted with AcOEt (300 ml), washed with saturated NaCl (15 ml×2), saturated $NaHCO_3$ (15 ml), 10% citric acid, dried over Na_2SO_4 and concentrated in vacuo to give a light yellow oil (1.32 g), which was purified by preparative TLC (Silica gel, AcOEt) to give colourless granules **24** (1.08 g, 90%) from 22.mp 87°C (AcOEt). *Rf*=0.46 ($CHCl_3$/MeOH=10:1). $[\alpha]_D^{21}$ −12.6° (*c*=1.03, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1500, 1540, 1590 (arom), 1630 (-NHCO-), 1650 (-NHCONH-), 1710 (-COO-). HRFABMS *m/z*: 914.4496 $[M+H]^+$, Calcd for $C_{48}H_{64}N_7O_9S$: 914.4486. Anal. Calcd for $C_{48}H_{63}N_7O_9S\cdot 1/2H_2O$: C, 62.45; H, 6.99; N,10.62. Found: C, 62.62; H, 7.02; N, 10.62. ^{1}H NMR (400 MHz, pyridine-d_5) δ: 0.96, 0.99 (each 3H, d, *J*=7.0, Val3-CH_3×2), 1.38 (9H, s, *t*-Bu), 1.69 (2H, br, Arg4-H_2), 1.82, 1.99 (each 1H, dt, *J*=13.5, 6.5, Arg3-H_2), 2.27 (1H, octet, *J*=7.0, Val3-H), 2.99, 3.07 (each 1H, dd, *J*=13.5, 7.0, PheOH3-H_2), 3.20, 3.25 (each 1H, dd, *J*=13.5, 6.0, Phe3-H_2), 3.28 (2H, m, Arg5-H_2), 3.58, 3.68 (each 1H, dd, *J*=9.5, 4.5, PheOH1-H_2), 3.64 (3H, s, Mbs-OCH_3), 4.49, 4.53 (each 1H, d, *J*=12.0, benzyl-CH_2), 4.75 (1H, m, PheOH2-H), 4.84 (1H, dd, *J*=8.5, 7.0, Val2-H), 5.00 (hidden, Arg2-H), 5.10 (1H, dt, *J*=8.0, 6.0, Phe2-H), 7.02 (2H, d, *J*=9.0, Mbs-arom-H), 7.11 (1H, brd, *J*=8.0, Phe2-NH), 7.15–7.48 (15H, m, Phe-, PheOH-, benzyl-arom.-H), 7.40 (1H, d, *J*=8.0, Arg2-NH), 7.48 (hidden, Val2-NH), 8.29 (2H, d, *J*=9.0, Mbs-arom-H), 9.22 (1H, brd, *J*=8.0, PheOH2-NH). ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ_c: 18.96, 19.84 (each q, Val3-CH_3×2), 25.97 (t, Arg C-4), 28.04 (q, *t*-Bu-CH_3), 31.04 (t, Arg C-3), 31.57 (d, Val C-3), 37.82 (t, PheOH C-3), 39.13 (t, Phe C-3), 41.22 (t, Arg C-5), 51.24 (d, PheOH C-2), 53.86 (d, Arg C-2), 55.55 (q, Mbs-OCH_3), 55.64 (d, Phe C-2), 59.40 (d, Val C-2), 71.64 (t, PheOH C-1), 73.43 (t, benzyl-CH_2), 81.45 (s, *t*-butyl-$OC(CH_3)_3$), 114.26 (d, Mbs-arom C-3′, 5′), 126.70, 127.06, 128.11 (each d, Phe-, PheOH-, benzyl-arom. C-4′), 128.43 (each d, benzyl-arom.- C-2′, 6′), 128.67, 128.85, 128.87, 129.48 (each d, Phe-, benzyl-, PheOH-arom.- C3′, 5′), 128.72 (d, Mbs-arom.- C-2′, 6′), 129.91 (d, PheOH-arom.- C2′, 6′), 130.17 (d, Phe-arom.- C2′, 6′), 137.74 (s, Phe-arom.- C-1′), 138.30 (s, Mbs-arom C-1′), 139.02 (s, benzyl-arom C-1′), 139.14 (s, PheOH-arom C-1′), 158.16 (s, Arg C-7), 158.67 (s, ureido-C), 162.15 (s, Mbs-arom.- C-4′), 172.27 (s, Val C-1), 172.35 (s, Phe C-1), 173.46 (s, Arg C-1).

7.1.16. Mer-N5075A (1). Compound **24** (69.2 mg, 0.076 μmol) was dissolved in methanesulfonic acid (0.38 ml, 5.83 μmol) and anisole (19 μl, 0.174 μmol) was added and stirred for 3 h at room temperature. Ether (6 ml×3) was added to the reaction mixture and decanted to remove ether soluble portions to give the ether insoluble portion as a light yellow oil (89.9 mg), which was purified by column chromatography (Amberlite IRA-410 acetate form, H_2O, Sephadex LH-20, MeOH) to provide **1** (27.0 mg, 60%) as colourless granulars. *Rf*=0.15 (BuOH/AcOH=5/1, Silica gel), 0.56 (MeOH/AcOH=100/1, Silica gel). mp 182°C. HRFABMS: *m/z*: 598.3387 $[M+H]^+$, Calcd for $C_{30}H_{44}N_7O_6$ 598.3353. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.74 (3H, d, *J*=7.0, Val3-CH_3), 0.75 (3H, d, *J*=6.5, Val3-CH_3), 1.42 (3H, m, Arg3-Ha, 4-H_2), 1.65 (1H, br, Arg3-Hb), 1.88 (1H, m, Val3-H), 2.62 (1H, dd, *J*=14.0, 8.0, PheOH3-Ha). 2.62 (1H, dd, *J*=14.0, 8.0, PheOH3-Ha), 2.82 (1H, 1H, m, Phe3-Ha), 2.82 (1H, dd, *J*=14.0, 5.5, PheOH3-Hb), 2.95 (2H, dd, *J*=13.0, 5.5, Arg5-Ha, Phe3-Hb), 3.04 (1H, br, Arg5-Hb), 3.24 (1H, dd, *J*=10.0, 6.0, Phe-OH1-Ha), 3.31 (1H, dd, *J*=10.0, 5.0, PheOH1-Hb), 3.88 (1H, m, PheOH2-H), 3.97 (1H, m, Phe2-H), 4.04 (1H, dd, *J*=9.0, 7.0, Val2-H), 4.09 (1H, m, Arg2-H), 4.95 (1H, br, PheOH1-OH), 6.05 (1H, brd, *J*=8.0, Phe2-NH), 6.68 (1H, brd, *J*=8.0, Arg2-NH), 7.05-7.28 (10H, m, Phe, PheOH-arom-H), 7.50-8.10 (3H, br, -C(=NH)-NH_2), 7.73 (1H, br, Val2-NH), 7.82 (1H, brd, *J*=8.0, $PheOH_2$-NH), 7.82 (1H, brd, *J*=8.0, $PheOH_2$-NH), 8.90 (1H, br, Arg6-NH). ^{13}C-NMR (100.6 MHz, DMSO-d_5) δ_c: 18.89, 19.89 (each q, Val3-CH_3×2), 24.77 (t, Arg C-4), 29.17 (t, Arg C-3), 30.56 (d, Val C-3), 36.32 (t, PheOH C-3), 38.36 (t, Phe C-3), 40.20 (t, Arg C-5), 52.31 (d, PheOH C-2), 52.73 (d, Arg C-2), 56.60 (d, Phe C-2), 57.96 (d, Val C-2), 62.287 (t, PheOH C-1), 125.42, 125.82 (each d, Phe-, PheOH-arom C-4′), 127.61, 128.02, 129.02, 129.48 (each dx2, Phe-, PheOH-arom C2′, 3′, 5′, 6′), 139.03 (s, Phe-arom C-1′), 139.46 (s, PheOH-arom C-1′), 157.45 (s, Arg C-7), 157.99 (s, ureido-C), 170.36 (s, Val C-1), 172.17 (s, Arg C-1). 175.44 (s, Phe C-1).

7.1.17. Debenzylation of 24. 10% palladium carbon (384 mg) was added to a solution of **24** (767 mg, 0.84 μmol) in AcOH (15 ml) and stirred under a stream of hydrogen at room temperature for 4.5 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo to give 25 (635 mg, 92%) as colorless plates. mp 90–110°C (MeOH). *Rf*=0.30 ($CHCl_3$/MeOH=3:1). $[\alpha]_D^{25}$ −11.07° (*c*=1.03, $CHCl_3$). IR ($CHCl_3$) υ_{max} cm^{-1}: 1590 (arom), 1650 (-NHCO-), 1670 (-NHCONH-), 1720

(-COO-), 3350 (-OH). HRFABMS *m/z*: 846.3832 $[M+Na]^+$, Calcd for $C_{41}H_{57}N_7O_9SNa$: 846.3836.^{1}H NMR (400 MHz, pyridine-d5) δ: 0.97, 1.00 (each 3H, d, *J*=7.0, Val3-CH_3×2), 1.38 (9H, s, *t*-Bu-H), 1.71(2H, br, Arg4-H_2), 1.82, 1.99 (each 1H, m, Arg3-H_2), 2.32 (1H, m, Val3-H), 3.12, 3.23 (each 1H, dd, *J*=13.0, 7.0, PheOH3-H_2), 3.22 (2H, br, Phe3-H_2), 3.31 (2H, br, Arg5-H_2), 3.64 (3H, s, Mbs-OCH_3), 3.97, 4.00 (each 1H, dd, *J*=11.0, 5.0, PheOH1-H_2), 4.72 (1H, m, PheOH2-H), 4.85 (1H, t, *J*=8.0, Val2-H), 4.97 (1H, br, Arg2-H), 5.08 (1H, dt, *J*=8.0, 6.0, Phe2-H), 7.02 (2H, d, *J*=8.5, Mbs-arom-H), 7.12–7.44 (10H, m, Phe, PheOH-arom-H), 7.16 (1H, d, *J*=8.0, Phe2-NH), 7.47 (1H, d, *J*=8.0, Arg2-NH), 8.27 (2H, d, *J*=8.5, Mbs-arom-H), 9.02 (1H, d,*J*=8.0, PheOH2-NH). ^{13}C-NMR (100.6 MHz, pyridine-d5) δ_c: 18.89, 19.89 (each q, Val3-CH3×2), 26.00 (t, Arg C-4), 28.06 (q, *t*-Bu-CH_3), 30.67 (t, Arg C-3), 31.42 (d, Val C-3), 37.76 (t, PheOH C-3), 39.15 (t, Phe C-3), 41.18 (t, Arg C-5), 53.83 (d, PheOH C-2), 54.04 (d, Arg C-2), 55.58 (q, Mbs-OCH_3), 55.71 (d, Phe C-2), 59.68 (d, Val C-2), 63.57 (t, PheOH C-1), 81.47 (s, *t*-Bu-$OC(CH_3)_3$), 114.29 (d, Mbs-arom C-3′, 5′), 126.57, 127.0 (each d, Phe-, PheOH–arom C-4′), 128.69 (d, Mbs-arom C-2′, 6′), 128.76, 128.81 (each d, Phe-, PheOH-arom C3′, 5′), 130.01, 130.18 (each d, Phe-, PheOH-arom C2′, 6′), 137.67 (s, Phe-arom C-1′), 138.35 (s, Mbs-arom C-1′), 139.68 (s, PheOH-arom C-1′), 158.22 (s, Arg C-7), 158.81 (s, ureido-C), 162.19 (s, Mbs-arom C-4′), 172.37 (s, Phe C-1), 172.54 (s, Val C-1), 173.64 (s, Arg C-1).

7.1.18. Oxidation of tetrapeptide 25. PDC (51 mg, 0.13 μmol), 4A-MS (300 mg), celite 545 (300 mg) were added to the solution of **25** (100 mg, 0.12 μmol) in CH_2Cl_2 (12 ml) and stirred for 3.5 h at room temperature under argon. The reaction mixture was purified by flash column chromatography (Silica gel, $CHCl_3$/MeOH=20:1) to afford colorless plates **26** (63 mg, 63%). mp 107–114°C (MeOH). *Rf*=0.36 ($CHCl_3$/MeOH=20:1). $[\alpha]_D^{27}$ −22.13° (*c*=0.47, $CHCl_3$)IR ($CHCl_3$) υ_{max} cm^{-1}: 1590 (arom), 1630 (-NHCONH-), 1650 (-NHCO-), 1710 (-CHO), 1730 (-COO-).HRFABMS *m/z*: 822.3828 $[M+H]^+$, Calcd for $C_{41}H_{56}N_7O_9S$: 822.3860 [M+H]. ^{1}H NMR (400 MHz, acetone-d_6) δ: 0.83, 0.86 (each 3H, d, *J*=7.0, Val3-CH_3×2), 1.39 (9H, s, *t*-butyl-H), 1.57 (2H, br, Arg4-H_2), 1.58 (1H, br, Arg3-Ha), 1.77 (1H, m, Arg3-Hb), 2.08 (1H, m, Val3-H), 2.97 (1H, dd, *J*=15.0, 9.0, Phe3-Ha), 2.99, 3.03 (each 1H, dd, *J*=14.0, 6.0, PheCHO3-H_2), 3.24 (1H, dd, *J*=15.0, 5.0, Phe3-Hb), 3.25 (2H, br, Arg5-H_2), 3.86 (3H, s, Mbs-OCH_3), 4.29 (1H, dd, *J*=9.0, 6.0, Val2-H), 4.33 (1H, br, Arg2-H), 4.52 (1H, m, Phe2-H), 4.55 (1H, m, PheCHO2-H), 6.19 (1H, brd, *J*=8.0, Phe2-NH), 6.38 (1H, brd, *J*=8.0, Arg2-NH), 6.67 (2H, br, Arg-HN-C(=NH)-NHMbs), 7.00 (2H, d, *J*=9.0, Mbs-arom-H), 7.18–7.31 (10H, m, Phe-arom, PheCHO-arom-H), 7.43 (1H, brd, *J*=9.0, Val2-NH), 7.82 (2H, d, *J*=9.0, Mbs-arom-H), 7.80 (1H, br, Arg-HN-C(=NH)-NHMbs), 7.86 (1H, br, PheCHO2-NH), 9.58 (1H, br, PheCHO1-H). ^{13}C-NMR (100.6MHz, DMSO-d_6) δ: 18.10, 19.70 (each q, Val3-CH_3×2), 26.44 (t, Arg C-4), 28.13 (q, $C(CH_3)_3$), 29.80 (d, Val C-3), 30.20 (t, Arg C-3), 34.89 (t, Phe C-3), 39.08 (t, PheCHO C-3), 41.01 (t, Arg C-5), 54.39 (d, Arg C-2), 55.81 (d, Phe C-2), 55.87 (q, Mbs-OCH_3), 59.23 (d, Val C-2), 60.80 (d, PheCHO C-2), 81.73 (s, $C(CH_3)_3$), 114.50 (d, Mbs-arom C-3′, 5′), 127.27, 127.35, 128.71 (each d, Phe-, PheCHO-, Bn-arom C-4′), 129.00 (d, Mbs-arom C-2′, 6′), 130.09 (d, PheCHO-arom C-2′, 6′), 130.36 (d, Phe-arom C-2′, 6′), 138.06 (s, Phe-arom C-1′), 138.30 (s, Mbs-arom C-1′), 157.95, 157.99 (s, Arg C-7), 158.56, 158.59 (s, ureido-C), 162.64 (s, Mbs-arom C-4′), 172.17 (s, Val C-1), 172.47 (s, Phe C-1), 173.54 (s, Arg C-1), 200.16 (d, PheCHO C-1).

7.1.19. Mixture of α and β-MAPI (2 and 3). The compound **26** (30.0 mg, 36.5 μmol) was dissolved in methanesulfonic acid (184 ml, 2.80 μmol) and anisole (9 ml, 84 μmol) was added. The mixture was stirred for 40 min. Ether (6 ml×3) was added to the reaction mixture and decanted. Concentration of ether insoluble portion gave light yellow oil (38.2 mg) which was purified by column chromatography (Amberlite IRA-410 acetone form, eluated with water), followed by Sephadex LH-20 column chromatography (eluated with MeOH) to afford colorless grannules (**2** and **3**)(10.0 mg, 46%). **2** and **3**: *Rf*=0.72 (MeOH/AcOH=100: 1), mp 189–198° (lit., 2: 204–205[4c]; **3**: 221–212[4e]); $[\alpha]_D^{25}$ −6.3° (*c*=0.32, AcOH) (lit., 2: −18, *c*=1.0, AcOH[12c]; 3: 0, *c*=1, AcOH[12e]). HRFABMS *m/z*: 596.3188 $[M+H]^+$, Calcd for $C_{30}H_{42}N_7O_6$: 596.3197. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.65 (3H, d, *J*=7.0, Val3-CH_3), 0.69 (3H, d, *J*=6.8, Val3-CH_3), 0.75 (3H, dd, *J*=7.0, 1.3, Val3′*-CH_3), 0.78 (3H, dd, *J*=7.0, 2.0, Val3′-CH_3), 1.36 (2H, m, Arg3-Ha, Arg3′-Ha), 1.42 (4H, m, Arg4-H_2, Arg4′-H_2), 1.66 (2H, m, Arg3-Hb, Arg3′-Hb), 1.82 (1H, quint, Val3-H), 1.92 (1H, quint, Val3′-H), 2.72 (2H, dd, *J*=14.0, 10.0, PheCHO3-Ha, PheCHO3′-Ha), 2.80 (2H, dd, *J*=10.5, 5.0, Phe3-Ha, Phe3′-Ha), 2.94 (2H, m, Phe3-Hb, Phe3′-Hb), 2.97, 3.05 (each 2H, m, Arg5-H_2, Arg5′-H_2), 3.14 (2H, dd, *J*=14.0, 6.0, PheCHO3-Hb, PheCHO3′-Hb), 3.96 (2H, m, Phe2-H, Phe2′-H), 4.14 (2H, m, Val2-H, Val2′-H), 4.16 (2H, m, Arg2-H, Arg2′-H), 4.32 (2H, m, PheCHO2-H, PheCHO2′-H), 6.05 (2H, d, *J*=7.0, Phe2-NH, Phe2′-NH), 6.58, 6.60 (each 1H, d, *J*=9.0, Arg2-NH, Arg2′-NH), 7.04-7.31 (10H, m, Phe, PheCHO-arom-H), 7.28-7.86 (6H, br, -C(=NH)-NH_2), 7.66, 7.71 (each 1H, d, *J*=9.0, Val2-NH, Val2′-NH), 8.50 (2H, d, *J*=7.5, PheCHO2-NH, PheCHO2′-NH), 8.80 (2H, br, Arg6-NH, Arg6′-NH), 9.42, 9.47 (each 1H, brs, PheCHO1-H, PheCHO1′-H). ^{13}C-NMR (100.6MHz, DMSO-d_6) δ: 17.37, 19.14 (each q, Val3-CH_3×2), 17.52, 19.04 (each q, Val3′-CH_3×2), 24.60, 24.64 (each t, Arg C-4, Arg C′-4), 29.15, 29.19 (each t, Arg C-3, Arg C′-3), 30.47 (d, Val C-3, Val C′-3), 33.25, 33.33 (each t, PheCHO C-3, PheCHO C′-3), 38.34, 38.36 (each t, Phe C-3, Phe C′-3), 40.20 (t, Arg C-5, Arg C′-5), 52.46 (d, Arg C-2, Arg C′-2), 56.53, 56.58 (each d, Phe C-2, Phe C′-2), 57.21, 57.30 (each d, Val C-2, Val C′-2), 59.46, 59.72 (each d, PheCHO C-2, PheCHO C′-2), 125.43, 126.19, 127.59, 128.12, 128.15, 128.99, 129.11, 129.39 (each d, Phe, PheOH-arom C-2′, 3′, 4′, 5′, 6′, C′-2′, 3′, 4′, 5′, 6′), 137.44, 137.59 (each s, Phe-arom C-1′, C′-1′), 139.36, 139.39 (each s, PheCHO-arom C-1′, C′-1′), 157.27 (s, ureido-C, C′), 157.90, 157.95 (each s, Arg C-7, Arg C′-7), 171.30, 171.36 (each s, Val C-1, Val C′-1), 172.19 (s, Arg C-1, Arg C′-1), 175.65 (s, Phe C-1, Phe C′-1), 200.01, 200.07 (s, PheCHO C-1, PheCHO C′-1). *The sign of H′ and C′ shows one of the diastereoisomer in accordance with the other isomer which is described as H and C.

7.1.20. HIV-1 protease inhibition assay. Inhibition assay of HIV-1 protease was carried out as follows. A mixture of 4 μL of substrate solution (VSQNYPIV) (1 mg/mL in 2MNaCl), 500 mM MES Buffer (pH 6.0), 3M NaCl, 10mM EDTA, 1% Triton X-100, 28 μL of enzyme solution, 8 μL of HIV protease (2 mg/ml), 4 μL of sample solution was incubated for 2h at 37°C. After that, 80 μL of D.W. was added to the reaction mixture and heated for 2.5 h at 100°C to stop the reaction. Then the mixture was centrifugated for 5 min at 12,000 rpm. The HPLC analysis was carried out with 80 μL of the solution above under following condition. Column: TSK gel ODS-120T; Eluent: 21% CH_3CN/0.1% TFA; Injection volume: 50 μL, Detection: 210 nm.

References

1. (a) Fink, D. M.; Bores, G. M.; Effiand, R. C.; Huger, F. P.; Kurys, B. E.; Rush, D. K.; Selk, D. E. *J. Med. Chem.* **1995**, *38*, 3645. (b) Sugimoto, H.; Iimura, Y.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1995**, *38*, 4821. (c) Omura, S.; Kuno, F.; Otoguro, K.; Sunazuka, T.; Shiomi, K.; Masuma, R.; Iwai, Y. *J. Antibiot.* **1995**, *48*, 745.
2. (a) Cohen, J. *Science* **1996**, *272*, 1182. (b) Caring, J. C.; Duncan, I. B.; Hockely, D.; Grief, C.; Roberts, N. A.; Mills, J. S. *Antiviral. Res.* **1991**, *16*, 295. (c) Danner, S. A.; Carr, A.; Leonard, J. M.; Lehman, L. M.; Gudiol, F.; Gonzales, J.; Raventos, A.; Rubio, R.; Bouza, E.; Pintado, V.; Aguado, A. G.; de Lomas, J. G.; Delgado, R.; Borleffs, J. C. C.; Hsu, A.; Valdes, J. M.; Boucher, C. A. B.; Cooper, D. A. *N. Eng. J. Med.* **1995**, *33*, 1528.
3. Kaneto, R.; Chiba, H.; Dobashi, K.; Kojima, I.; Sasaki, K.; Shibamoto, N.; Nishida, H.; Okamoto, R.; Akagawa, H.; Mizuno, S. J. *J. Antibiotics* **1993**, *46*, 1622.
4. (a) Murano, S.; Watanabe, T. *Agri. Biol. Chem.* **1977**, *41*, 1313. (b) Murano, S.; Watanabe, T. *Agri. Biol. Chem.* **1978**, *42*, 2209. (c) Watanabe, T.; Fukuhara, K.; Murano, S. *Tetrahedron Lett.* **1979**, *46*, 625. (d) Watanabe, T.; Murano, S. *Agri. Biol. Chem.* **1979**, *43*, 243. (e) Shin-Watanabe, T.; Fukuhara, K.; Murano, S. *Tetrahedron* **1982**, *38*, 1775.
5. Stefanelli, S.; Cavaletti, E.; Sarubbi, E.; Ragg, L.; Colombo, L.; Selva, E. J. *Antibiotics* **1995**, *48*, 332.
6. Konda, Y.; Takahashi, Y.; Mita, H.; Takeda, K.; Harigaya, Y. *Chem. Lett.* **1997**, 345.
7. (a) Majer, P.; Randad, R. S. *J. Org. Chem.* **1994**, *59*, 1937. (b) Leung, M. K.; Lai, J. L.; Lau, K. H.; Yu, H. H.; Hsiao, H. J. *J. Org. Chem.* **1996**, *61*, 4175. (c) Zhang, X.; Rodrigues, J.; Evans, L.; Hinkle, B.; Ballantyne, L.; Pena, M. *J. Org. Chem.* **1997**, *62*, 6420. (d) Eckert, H.; Foster, B. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 894.
8. Ogura, H.; Kobaya, T.; Shimizu, K.; Kawabe, K.; Takeda, K. *Tetrahedron Lett.* **1979**, *20*, 4745.
9. Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.; Mizuno, Y.; Takayanagi, H.; Harigata, Y. *Synthesis* **1994**, 1063.
10. Goodacre, J.; Ponsford, R. J.; Stirling, I. *Tetrahedron Lett.* **1975**, *16*, 3609.
11. Koenig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
12. Nishimura, O.; Fujimo, M. *Chem. Pharm. Bull.* **1976**, *24*, 1568.