MATLYSTATINS, NEW INHIBITORS OF TYPE IV COLLAGENASES FROM *Actinomadura atramentaria*

IV. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF MATLYSTATIN B AND ITS STEREOISOMERS

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The first total synthesis of matlystatin B (**1a**), a low molecular weight inhibitor of type IV collagenases, was accomplished, and its absolute configuration was unambiguously determined. Furthermore, ten stereoisomers of **1a** were synthesized, and the inhibition of the 92 kDa type IV collagenase and of other metalloproteinases by each stereoisomer was investigated.

Several reports to date have suggested a relationship between metastatic potential and type IV collagenase activity[1~3]. The degradation of type IV collagen, a major component of basement membranes, is believed to be requisite for cancer metastatic processes. In fact, experimental metastasis in mice has been shown to be inhibited by a selective type IV collagenase inhibitor, SC-44463[4]. Thus, inhibitors of type IV collagenases are thought to hold promise as antimetastatic agents.

Matlystatins are inhibitors of type IV collagenases and were first isolated from a culture filtrate of *Actinomadura atramentaria* SANK 61488. They comprise 5 congeners designated matlystatins A, B, D, E, and F[5~8]. Spectroscopic methods revealed that matlystatins are pseudopeptide-hydroxamic acids containing a 2-alkyl succinic acid and piperazic acid. As for the stereochemistry, only a single chiral center had been elucidated: the isolation of 2*R*-succinic acid after acid hydrolysis of matlystatin A showed that C-2′ has the *R*-configuration. The absolute configurations at the 3 other asymmetric centers (C-2, C-4″, C-5″) remained to be determined. Recently, we accomplished the total synthesis of matlystatin B (**1a**), and revealed that the 4 asymmetric centers have the 2*S*, 2′*R*, 4″*S*, and 5″*S* configurations as shown in Fig. 1[9].

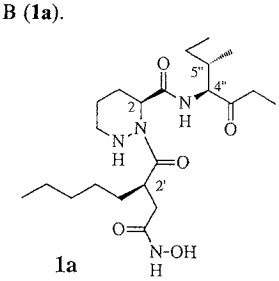
Herein we describe not only the synthesis and the absolute configuration of matlystatin B, but also the synthesis of ten stereoisomers of **1a** to study the relationships between stereochemistry and inhibitory activity.

Chemistry

1a was synthesized as shown in schemes 1~3.

The first segment, *N*-*Z*-ethylketone **4a**, was prepared from *Z*-L-Ile (**2a**). Amidation of starting material was accomplished using DCC with *N*,*O*-dimethyl-hydroxylamine hydrochloride in the presence of $^{i}Pr_2NEt$ and DMAP to give amide **3a** in

Fig. 1. Structure and absolute configuration of matlystatin B (**1a**).



1a

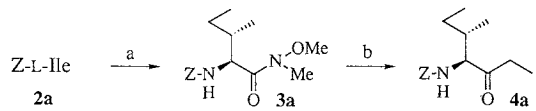
97% yield. The amide **3a** was converted to the ethylketone **4a** by addition of ethylmagnesium bromide according to the Weinreb method[10] in 80% converting yield. After work-up, a small amount of epimerized product was observed, but the desired optically pure ethylketone **4a** was obtained by recrystallization from H_2O-MeOH. (Scheme 1)

The second segment, N^1-*Z*-*S*-piperazic acid *tert*-butyl ester (**12a**), was obtained by esterification of N^1-*Z*-*S*-piperazic acid[11,12] with 2-methylpropene in the presence of sulfuric acid[13].

The last segment, carboxylic acid **10a** was synthesized by applying Evans diastereoselective alkylation method[14] as shown in scheme 2. The lithium enolate derived from *N*-acyl oxazolidinone **5a** and LDA was alkylated with *tert*-butyl bromoacetate to provide **6a** in 91% yield after recrystallization from H_2O-MeOH. Removal of the oxazolidinone using lithium benzyloxide followed by acid hydrolysis of the *tert*-butyl ester with 4 N HCl-1,4-dioxane gave carboxylic acid **8a** in quantitative yield. The carboxyl group of **8a** was esterified with 2,2,2-trichloroethanol using an acid chloride method to give protected compound **9a**. The catalytic hydrogenation of the benzyl ester with 10% Pd-C followed the esterification to provide the desired (2*R*)-2-[(2,2,2-trichloroethoxycarbonyl)methyl]heptanoic acid (**10a**). The overall yield from diester **7a** to carboxylic acid **10a** was 91%. The stereochemistry of the carboxylic acid **10a** was predicted to be *R* based on the previously reported method[14]. The stereochemistry of **10a** was unambiguously determined by the removal[15] of the TCE ester to give *n*-pentylsuccinic acid (**11a**) in 47% yield. By comparing the optical rotation of **11a** with data reported for (*R*)- and (*S*)-*n*-pentylsuccinic acid[16], the stereochemistry of **10a** was unambiguously determined to be *R*.

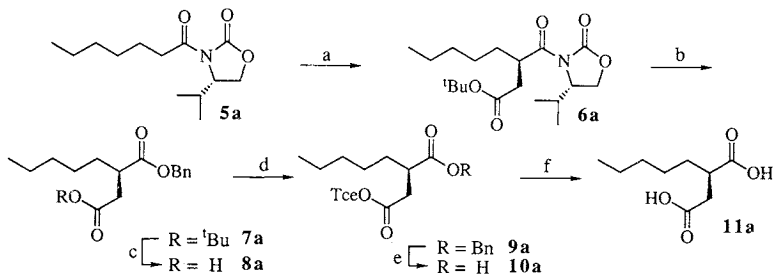
The desired segments that had been prepared as described above were coupled to afford **1a** as shown in scheme 3. The coupling of **12a** and **10a** required a highly activated method; using an acid chloride method, the desired coupling product **13a** was obtained in 90% yield. After removal of undesired diastereomer of **13a** (3.7% measured by HPLC analysis) by silica gel chromatography, the TCE ester was converted to a carboxyl group to give carboxylic acid **14a** in 96% yield. Amidation of this compound with *O*-benzylhydroxylamine using DEPC[17] (77% yield) followed by acid hydrolysis of the *tert*-butyl ester afforded the key intermediate

Scheme 1. Synthesis of segment **4a**.



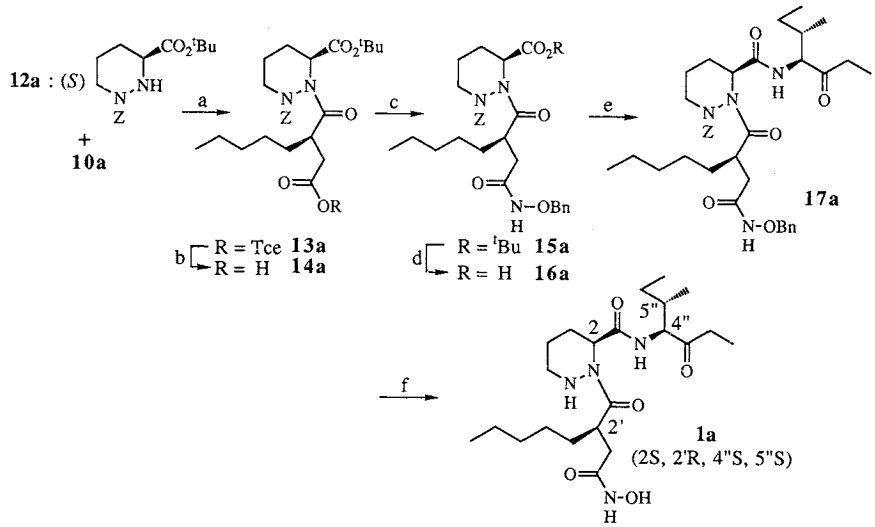
(a) $MeN^+H_2(OMe)Cl^-$, $^iPr_2NEt$, DCC, DMAP, CH_2Cl_2, 0°C, 97%. (b) EtMgBr, THF, −15°C~0°C, 56% (c.y. 80%).

Scheme 2. Synthesis of segment **10a**.



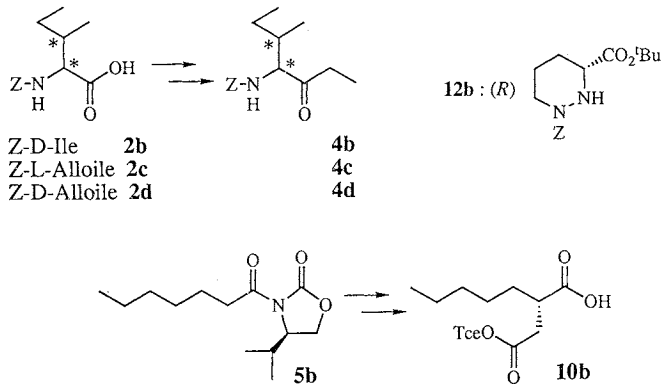
(a) (i) LDA, THF, −78°C (ii) $BrCH_2CO_2{}^tBu$, THF, −78°C, 91%, (b) BnOLi-BnOH, THF, 0°C, quant. (c) 4 N HCl-1,4-dioxane, rt, quant. (d) (i) $(COCl)_2$, benzene, 60°C then Cl_3CCH_2OH, pyridine, THF, −15°C, (e) H_2, 10% Pd-C, MeOH, rt, 91% in 2 steps, (f) Zn, 1 M NH_4OAc aq, THF, rt, 47%.

Scheme 3. Synthesis of matlystatin B (**1a**).



(a) **10a**, $(COCl)_2$, benzene, 50°C then **12a**, *N*-ethylmorpholine, THF, −15°C-rt, 90%, (b) Zn, 1 M NH_4OAc aq, THF, rt, 96%, (c) $H_3N^+OBnCl^-$, DEPC, Et_3N, THF-DMF (10:3), −15°C, 77%, (d) TFA, CH_2Cl_2, rt, 96%, (e) **4a**, H_2, 10% Pd-C, MeOH, rt then **16a**, DEPC, THF, −15°C-rt, 60% (c.y. 80%), (f) H_2, 10% Pd-C, MeOH, 86%.

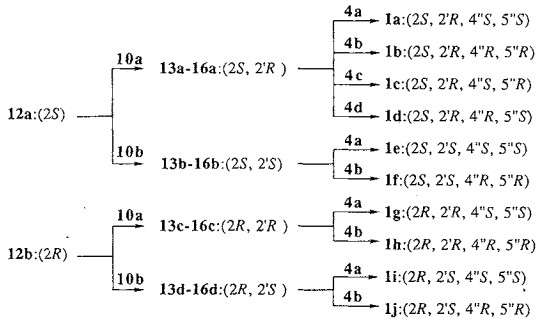
Scheme 4. Synthesis of other segments used for the synthesis of stereoisomers.



carboxylic acid **16a** in 96% yield. Successive coupling of **16a** and the aminoketone, which was prepared by catalytic hydrogenation of **4a**, was accomplished by DEPC coupling to give N^1-*Z*-*O*-benzyl-matlystatin B (**17a**) in 80% converting yield. **1a** was obtained in 86% yield by the usual deprotection method.

The measured spectral properties (^1H NMR, IR, Mass, and $[\alpha]_D$) of synthetic **1a** agreed with those of natural **1a**, demonstrating that the 4

Scheme 5. Synthesis of stereoisomers of **1a**.



asymmetric centers contained in **1a** have 2′*R*, 2*S*, 4″*S*, and 5″*S* configuration as shown in Fig. 1.

Next, in order to reveal the effect of each asymmetric center on the inhibitory activity on target enzymes, we synthesized stereoisomers of **1a**. Desired stereoisomers of **1** were synthesized using the appropriate stereoisomers or enantiomers of segments **4**, **12**, and **10** (Scheme 4). First, to synthesize the 2′*S* stereoisomer of **1**, (2*S*)-2-[(2,2,2-trichloroethoxycarbonyl)methyl]heptanoic acid (**10b**) was prepared from **5b** (enantiomer of **5a**), which was synthesized using (4*R*)-4-isopropyl-2-oxazolidinone as a starting compound (Scheme 4). Second, 2*R*-stereoisomers were prepared from N^1-*Z*-(*R*)-piperazic acid *tert*-butyl ester (**12b**). Finally, ethylketones **4b**～**4d**, which have the desired C-4″ and C-5″ configurations, were prepared using D-ILe (4″*R*,5″*R*), L-Alloile (4″*S*,5″*R*), and D-Alloile (4″*R*,5″*S*), respectively, as starting materials. (Scheme 4)

According to the synthetic route for **1a**, the stereoisomer **1j**, which has 2*R*, 2′*S*, 4″*R*, 5″*R* stereochemistry, was synthesized as follows. The coupling reaction of **10b** and **12b** gave desired product **13d**. The coupling product **13d** was converted to **16d** using the same procedure used for the conversion from **13a** to **16a**. Coupling of **16d** and the amine segment, which was prepared from **4b**, was followed by hydrogenation to give **1j**. All other segments were coupled without difficulty following the same route used for the natural product (Scheme 3). Of sixteen possible stereoisomers ten (**1a**～**1j**) were synthesized as shown in Scheme 5, and the structures of these are depicted in Fig. 2. Then inhibitory activities of **1a**～**1j** against type IV collagenases and other metalloproteinases have been evaluated by the previously reported method[6] and are presented in Table 1.

Results and Discussion

The inhibition of the 92 kDa type IV collagenase by each of ten stereoisomers of matlystatin B (**1a**～**1j**) was examined. The results are shown in Table 1 and can be summarized as follows.

We found that inversion of C-2′ from *R* to *S* had the most drastic effect on inhibitory potency. For example, the 2′*S* compound **1e** was over 250-fold less potent than the 2′*R* compound **1a**. Similar effects were observed between the other 2′*R* compounds (**1b**, **1g**, **1h**) and their corresponding 2′*S* epimers (**1f**, **1i**, **1j**).

C-2 stereochemistry was also found to be important. A comparison between pairs that have identical stereochemistry except at C-2 (**1a**-**1g**, **1b**-**1h**, **1e**-**1i**, **1f**-**1j**) reveals that 2*S* stereoisomers are generally more potent than their corresponding 2*R* epimers.

Further attention should be paid to the C-4″ stereochemistry of the 2′*R* compounds. The 4″*R* compound **1d** was less potent than its 4″*S* counterpart, **1a**. Also, the 4″*R* compound **1b** was approximately 8-fold less potent than the 4″*S* compound **1c**. From these results, 4″*S* stereochemistry seems to enhance the potency of the compound.

Finally, the influence of C-5″ stereochemistry was evaluated. The IC_{50} values of **1a** and **1c** were both approximately 0.5 μM. The stereoisomers **1b** and **1d** were weak inhibitors with IC_{50} values of 4.3 μM and 1.8 μM, respectively. These results indicate that the stereochemistry of the C″-5 position has little effect on inhibitory activity.

Regarding the inhibition of the other matrix metalloproteinases, the relative potencies of the stereoisomers toward the 72 kDa type IV collagenase and stromelysin were similar to those toward the 92 kDa type IV collagenase. Unlike the influence of C-2′ stereochemistry on inhibition of type IV collagenases and stromelysin, C-2′ stereochemistry has little effect on the inhibition of aminopeptidase M. These results

Fig. 2. Structures of **1a**~**1j**.

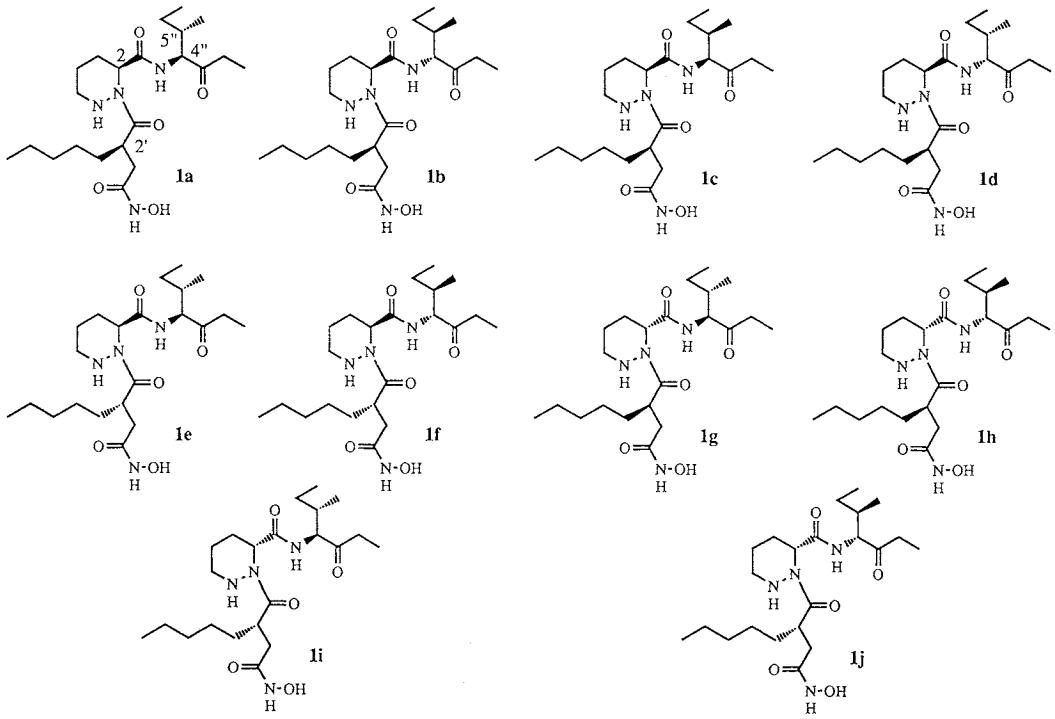


Table 1. Inhibitory activities of **1a**~**1j** against type IV collagenases and other metalloproteinases.

Compound	Inhibitory activity IC_{50} (μM)			
	Type IV collagenase		Stromelysin	Aminopeptidase M
	92 kDa	72 kDa		
1a	0.57	1.7	0.35	3.1
1b	4.3	11	1.4	5.3
1c	0.52	0.61	0.12	0.97
1d	1.8	10	0.25	5.8
1e	22% Inhibition at 150	5% Inhibition at 150	3.1	4.3
1f	75	96	210	4.1
1g	19	8.9	83	24
1h	9.4	4.0	10	17
1i	33% Inhibition at 150	15% Inhibition at 150	6% Inhibition at 150	6.2
1j	28% Inhibition at 150	6% Inhibition at 150	17% Inhibiion at 150	2.5

may provide useful information regarding the structure at the active sites of these metalloproteinases.

Experimental

All melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured on one of the following instruments: JASCO FT-IR 8900, JASCO FT-IR 8300, or JASCO A-102. ^{1}H NMR spectra were recorded on one of the following instruments: JEOL GSX-500, JEOL GSX-400, JEOL GX-270, or JEOL JNM-EX 270. All signals were measured using

tetramethylsilane as an internal standard and are expressed in ppm (δ-value). Mass spectra (MS) and high-resolution mass spectra (HR-MS) were obtained using a JEOL JMS-AX 505H for electron-impact ionization (EI) or using a JEOL JMS-SX/SX 102 A for fast atom bombardment ionization (FAB). Optical rotations were measured with a Perkin-Elmer 241 polarimeter. All reactions were monitored by thin layer chromatography (TLC), which was performed with precoated TLC plates (Merck). Silica gel 60 (230~400 mesh ASTM Merck) was used as an adsorbent for column chromatography. Preparative TLC was performed on Merck $60F_{254}$ (0.5 mm or 2.0 mm) precoated silica gel plates or on Merck $60F_{254}$ (0.25 mm) precoated silanized silica gel plates.

N-Benzyloxycarbonyl-L-isoleucine (*N*-Methyl-*N*-methoxy)amide (**3a**)

To a stirred solution of *Z*-L-ILe (**2a**) (15.0 g, 56.7 mmol) in CH_2Cl_2 (200 ml) was added *N*,*O*-dimethylhydroxylamine hydrochloride (5.80 g, 59.5 mmol), *N*,*N'*-dicyclohexylcarbodiimide (11.7 g, 56.7 mmol), *N*,*N'*-diisopropylethylamine (10.0 ml, 7.42 g, 57.5 mmol) and 4-dimethylaminopyridine (70 mg, 0.62 mmol), and the mixture was stirred at 0°C for 2 hours. The thick mixture was filtered to remove *N*,*N'*-dicyclohexylurea. The filtrate was concentrated under reduced pressure to half the original volume, then poured into 0.5 N aqueous HCl and extracted with EtOAc (×2). The combined organic phase was washed with H_2O and then with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (300 g, hexane - EtOAc, 5 : 2), then recrystallized from H_2O - MeOH to yield **3a** (16.9 g, 97%) as a white crystalline solid: mp 64~66°C; IR (film) 3306, 2965, 1719, 1654 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (3H, t, J=7.3 Hz), 0.93 (3H, d, J=6.8 Hz), 1.12 (1H, m), 1.57 (1H, m), 1.73 (1H, m), 3.22 (3H, s), 3.79 (3H, s), 4.67 (1H, dd, J=9.8, 8.1 Hz), 5.06 (1H, d, J=12.5 Hz), 5.13 (1H, d, J=12.5 Hz), 5.35 (1H, br d, J=9.8 Hz), 7.23~7.41 (5H, complex); MS (EI) *m/z* 309 $(M+H)^+$; HR-MS (EI) *m/z* Calcd for $C_{16}H_{25}N_2O_4$ $(M+H)^+$ 309.1813, Found 309.1804; $[\alpha]_D^{26}$ −4.7° (*c* 2.0, $CHCl_3$).

(4*S*,5*S*)-4-(Benzyloxycarbonylamino)-5-methyl-3-heptanone (**4a**)

To a solution of **3a** (1.71 g, 5.53 mmol) in THF (40 ml) was added ethylmagnesium bromide (16.0 ml of a 0.99 M solution in hexane, 15.8 mmol) while vigorously stirring at −15°C under N_2. After 35 minutes the reaction solution was warmed to 0°C and stirred for another 35 minutes. Then the reaction mixture was quenched with 5% aqueous $KHSO_4$ solution, and extracted with EtOAc (×2). The combined organic phase was washed with H_2O and then with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (40 g, hexane - EtOAc, 4 : 1~2 : 1) to afford **4a** as a pale yellow oil, and **3a** (508 mg, 30%) was recovered as a white solid. The optically pure **4a** (861 mg, 56%, converting yield 80%) was obtained by recrystallization from H_2O - MeOH: a white crystalline solid; mp 57~58°C; IR (film) 3270, 2966, 1710 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (3H, t, J=7.3 Hz), 0.98 (3H, d, J=6.8 Hz), 1.04 (1H, m), 1.08 (3H, t, J=7.3 Hz), 1.27 (1H, m), 1.90 (1H, m), 2.52 (2H, m), 4.36 (1H, dd, J=8.3, 4.6 Hz), 5.09 (2H, S), 5.36 (1H, br d, J=8.3 Hz), 7.24~7.40 (5H, complex); MS (EI) *m/z* 278 $(M+H)^+$; HR-MS (EI) *m/z* Calcd for $C_{16}H_{24}NO_3$ $(M+H)^+$ 278.1756, Found 278.1750; $[\alpha]_D^{26}$ +74.2° (*c* 1.0, $CHCl_3$).

(4*S*)-4-Isopropyl-3-[(2*R*)-2-(*tert*-butoxycarbonylmethyl)-1-oxoheptyl]-2-oxazolidinone (**6a**)

To a stirred solution of **5a** (519 mg, 2.16 mmol) in THF (15 ml) was added LDA (3.90 ml of a 0.59 M solution in THF, 2.30 mmol) at −78°C under N_2. After 10 minutes, *tert*-butyl bromoacetate (1.70 ml, 10.5 mmol) dissolved in THF (5.0 ml) was added dropwise to the reaction mixture at the same temperature over 5 minutes. Stirring was continued for another 5.5 hours. Then 5% aqueous $KHSO_4$ solution and EtOAc were added to the reaction mixture. The aqueous layer was separated and extracted with EtOAc. The combined organic layer was washed with H_2O and then with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (35 g, hexane - EtOAc, 10 : 1), then recrystallized from H_2O - MeOH to yield **6a** (697 mg, 91%) as a white crystalline solid: mp 51~53°C; IR (KBr pellet) 2930, 1763, 1730, 1702 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.86 (3H, t, J=6.4 Hz), 0.91 (3H, d, J=6.3 Hz), 0.93 (3H, d, J=6.3 Hz), 1.14~1.51 (7H, complex), 1.41 (9H, s), 1.62 (1H, m), 2.38 (1H, d hep, J=3.4, 6.3 Hz), 2.43 (1H, dd, J=16.6, 4.9 Hz), 2.74 (1H, dd, J=16.6, 10.3 Hz), 4.15 (1H, m), 4.20 (1H, dd, J=7.9, 3.4 Hz), 4.25 (1H, t, J=7.9 Hz), 4.43 (1H, dt, J=7.9, 3.4 Hz);

MS (EI) m/z 356 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{19}H_{34}NO_5$ $(M+H)^+$ 356.2437, Found 356.2449; $[a]_D^{26}$ +50.8° (c 1.0, $CHCl_3$).

tert-Butyl (3*R*)-3-Benzyloxycarbonyloctanoate (**7a**)

To a stirred solution of **6a** (11.08 g, 31.17 mmol) in THF (80 ml) was added THF solution of lithium benzyloxide-benzyl alcohol (89 ml) (prepared from benzyl alcohol (6.45 ml, 62.3 mmol) and *n*-butyl lithium (2.83 ml of a 1.65 M solution in hexane, 4.67 mmol) in THF (80 ml) at 0°C under N_2, stirred for 20 minutes). After 1 hour, the reaction was quenched with 5% aqueous $KHSO_4$ solution and extracted with EtOAc (×2). The combined organic phase was washed with H_2O and then with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure.The residue was purified by silica gel column chromatography (500 g, hexane-EtOAc, 20:1) to afford **7a** (10.76 g, 100%) as a colorless oil: IR (film) 2931, 1731 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.85 (3H, t, J=6.6 Hz), 1.17~1.32 (6H, complex), 1.49 (9H, s), 1.50 (1H, m), 1.61 (1H, m), 2.36 (1H, dd, J=16.5, 5.3 Hz), 2.65 (1H, dd, J=16.5, 9.2 Hz), 2.83 (1H, m), 5.09 (1H, d, J=12.5 Hz), 5.18 (1H, d, J=12.5 Hz), 7.24~7.42 (5H, complex); MS (EI) m/z 335 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{20}H_{31}O_4$ $(M+H)^+$ 335.2223, Found 335.2230; $[\alpha]_D^{26}$ +0.22° (c 7.9, $CHCl_3$).

(3*R*)-3-Benzyloxycarbonyloctanoic Acid (**8a**)

Compound **7a** (983 mg, 2.94 mmol) was added into 4 N HCl-1,4-dioxane solution (15 ml, 60 mmol) at room temperature. The reaction mixture was stirred overnight, then poured into water, and extracted with EtOAc (×3). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure.The residue was purified by silica gel column chromatography (50 g, $CHCl_3$-MeOH, 30:1) to afford **8a** (838 mg, quant) as a colorless oil: IR (film) 2931, 1735, 1712 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.85 (3H, t, J=6.6 Hz), 1.10~1.38 (6H, complex), 1.42~1.77 (2H, complex), 2.48 (1H, dd, J=16.5, 4.6 Hz), 2.78 (1H, dd, J=16.5, 9.2 Hz), 2.88 (1H, m), 5.14 (2H, s), 7.23~7.48 (5H, complex); MS (EI) m/z 278 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{16}H_{22}O_4$ $(M)^+$ 278.1518, Found 278.1527; $[\alpha]_D^{26}$ +2.4° (c 1.0, EtOH).

(2*R*)-2-[(2,2,2-Trichloroethoxycarbonyl)methyl]heptanoic Acid (**10a**)

To a stirred solution of **8a** (2.37 g, 8.53 mmol) in benzene (20 ml) was added oxalyl chloride (4.5 ml, 51.6 mmol) under N_2 at room temperature. The mixture was warmed to 60°C for 2 hours, then diluted with benzene (30 ml), concentrated under reduced pressure, and dried under high vacuum for 40 minutes to give acid chloride. The prepared acid chloride was dissolved in THF (40 ml). The solution was treated with pyridine (820 μl, 8.27 mmol), and 2,2,2-trichloroethanol (5.5 ml, 57.3 mmol) under N_2 at −15°C. After 1.5 hours, the mixture was poured into 0.2 N HCl and extracted with EtOAc (×3). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on silica gel (50 g, hexane-EtOAc, 13:1) to afford diester **9a** as a colorless oil. To this oil was added MeOH (35 ml) and 10% Pd-C (205 mg). This suspension was stirred at room temperature under H_2 for 2 hours, then the catalyst was removed by celite filtration. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (60 g, $CHCl_3$-MeOH, 50:1) to afford **10a** (2.47 g, 91% in 2 steps) as a colorless oil: IR (film) 2957, 2931, 1758, 1709 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.89 (3H, t, J=6.5 Hz), 1.18~1.47 (6H, complex), 1.48~1.82 (2H, complex), 2.61 (1H, dd, J=15.2, 2.9 Hz), 2.88 (1H, dd, J=15.2, 9.3 Hz), 2.94 (1H, m), 4.72 (1H, d, J=12.0 Hz), 4.79 (1H, d, J=12.0 Hz); MS (EI) m/z 319 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{11}H_{18}O_4{}^{35}Cl_3$ $(M+H)^+$ 319.0271, Found 319.0261; $[\alpha]_D^{26}$ +11.1° (c 4.0, EtOH).

(3*R*)-3-Carboxyoctanoic Acid (**11a**)

To a vigorously stirred solution of **10a** (69 mg, 0.22 mmol) in THF (4.0 ml) was added 1 M aqueous ammonium acetate solution (0.4 ml) and zinc powder (300 mg, 4.58 mmol) at room temperature. After 3 hours, the zinc powder was removed by filtration. The filtrate was poured into 1 N HCl and extracted with EtOAc (×2). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5 g, $CHCl_3$-MeOH, 20:1~4:1) to afford **11a** (21 mg, 52%) as a white solid: IR (KBr pellet) 2929, 1692 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.89 (3H, t, J=6.1 Hz), 1.20~1.45 (6H, complex), 1.53 (1H, m), 1.73 (1H, m), 2.55

(1H, dd, J=16.6, 3.9 Hz), 2.72 (1H, dd, J=16.6, 9.8 Hz), 2.81 (1H, m), 9.40~12.2 (1H, br); MS (EI) m/z 189 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_9H_{17}O_4$ $(M+H)^+$ 189.1127, Found 189.1141; $[\alpha]_D^{26}$ +27.1° (c 1.0, EtOH).

tert-Butyl (3*S*)-1-Benzyloxycarbonyl-2-{(2*R*)-1-oxo-2-[(2,2,2-trichloroethoxycarbonyl)methyl]heptyl}hexahydropyridazine-3-carboxylate (**13a**)

Compound **10a** (573 mg, 1.79 mmol) in benzene (10 ml) was treated with oxalyl chloride (600 μl, 6.88 mmol) under N_2 at 50°C for 2 hours. The mixture was cooled to room temperature, diluted with benzene (20 ml), then concentrated under reduced pressure, and dried under high vacuum for 40 minutes to give acid chloride as a pale yellow oil. Next a solution of the acid chloride in THF (4.0 ml) was prepared and transferred by cannula to a stirred solution of N^1-Z-piperazic acid *tert*-butyl ester (**12a**) (584 mg, 1.83 mmol) and *N*-ethylmorpholine (370 μl, 2.91 mmol) in THF (4.0 ml) under N_2 at −15°C. The mixture was warmed to room temperature gradually, and stirred overnight. The mixture was poured into 0.2 N HCl, and extracted with EtOAc (×2). The combined organic phase was washed with H_2O and then with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (35 g, hexane-EtOAc, 6:1) to afford **13a** (1.00 g, 90%) as a colorless oil: IR (film) 2956, 1739, 1676 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.80 (3H, t, J=6.6 Hz), 0.85~2.12 (12H, complex), 1.43 (9H, s), 2.61 (1H, dd, J=17.2, 3.3 Hz), 2.94 (1H, dd, J=17.2, 10.0 Hz), 3.13 (1H, m), 3.42 (1H, m), 4.28 (1H, br d, J=11.3 Hz), 4.61 (1H, d, J=11.9 Hz), 4.77 (1H, d, J=11.9 Hz), 5.13 (1H, d, J=11.9 Hz), 5.21 (1H, d, J=11.9 Hz), 5.27 (1H, dd, J=4.6, 3.9 Hz), 7.22~7.41 (5H, complex); MS (EI) m/z 620 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{28}H_{39}N_2O_7{}^{35}Cl_3$ $(M)^+$ 620.1822, Found 620.1799; $[\alpha]_D^{26}$ −7.5° (c 2.0, $CHCl_3$).

tert-Butyl (3*S*)-1-Benzyloxycarbonyl-2-[(2*R*)-2-carboxymethyl-1-oxoheptyl]hexahydropyridazine-3-carboxylate (**14a**)

The coupling product **13a** (834 mg, 1.34 mmol) was treated with zinc powder (1.79 g, 27.3 mmol) and 1 M aqueous ammonium acetate solution (1.8 ml) in THF (18 ml) at room temperature while vigorously stirring. After 2.5 hours, the zinc powder was removed by filtration. The filtrate was poured into 1 N HCl and extracted with EtOAc (×2). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (15 g, $CHCl_3$-MeOH, 40:1) to afford **14a** (631 mg, 96%) as a white solid: IR (film) 2952, 1737, 1714, 1675 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.80 (3H, t, J=6.3 Hz), 0.85~2.12 (12H, complex), 1.43 (9H, s), 2.48 (1H, dd, J=17.2, 4.0 Hz), 2.82 (1H, dd, J=17.2, 11.1 Hz), 3.09 (1H, m), 3.42 (1H, m), 4.25 (1H, m), 5.12 (1H, d, J=11.9 Hz), 5.21 (1H, d, J=11.9 Hz), 5.27 (1H, dd, J=4.6, 4.0 Hz), 7.19~7.41 (5H, complex); MS (EI) m/z 491 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{26}H_{39}N_2O_7$ $(M+H)^+$ 491.2756, Found 491.2724; $[\alpha]_D^{26}$ −23.1° (c 1.0, EtOH).

tert-Butyl (3*S*)-1-Benzyloxycarbonyl-2-[(2*R*)-2-benzyloxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxylate (**15a**)

The carboxylic acid **14a** (465 mg, 949 μmol) in THF-DMF (6.5 ml, 10:3) was treated with *O*-benzylhydroxylamine hydrochloride (277 mg, 1.74 mmol), triethylamine (330 μl, 2.37 mmol), and DEPC (190 μl, 1.25 mmol) while stirring under N_2 at −15°C. After 4.5 hours, the mixture was poured into 5% aqueous $KHSO_4$ and extracted with EtOAc (×2). The combined organic phase was washed with H_2O and then with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (30 g, $CHCl_3$-MeOH, 80:1) to afford **15a** (553 mg, 98%) as a colorless oil: IR (film) 3426, 2956, 1734, 1674 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.79 (3H, t, J=6.8 Hz), 0.82~2.10 (12H, complex), 1.42 (9H, s), 2.10~2.46 (2H, complex), 3.19 (1H, m), 3.42 (1H, m), 4.24 (1H, br d, J=11.7 Hz), 4.82 (1H, d, J=11.2 Hz), 4.89 (1H, d, J=11.2 Hz), 5.12 (1H, d, J=12.2 Hz), 5.20 (1H, d, J=12.2 Hz), 5.26 (1H, t, J=3.9 Hz), 7.20~7.48 (10H, complex), 7.99 (1H, m); MS (EI) m/z 596 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{33}H_{46}N_3O_7$ $(M)^+$ 596.3335, Found 596.3328; $[\alpha]_D^{26}$ −37.3° (c 1.0, $CHCl_3$).

(3*S*)-1-Benzyloxycarbonyl-2-[(2*R*)-2-benzyloxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxylic Acid (**16a**)

To a solution of **15a** (707 mg, 1.19 mmol) in CH_2Cl_2 (3 ml) was added trifluoroacetic acid (2.5 ml, 32.7 mmol) at room temperature. The mixture was stirred for 2 hours then diluted with toluene (30 ml), and concentrated under reduced pressure to give a pale yellow oil. The residue was purified by silica gel column chromatography (10 g, $CHCl_3$ - MeOH, 15 : 1) to afford **16a** (620 mg, 96%) as a colorless oil: IR (film) 3224, 2955, 2931, 1719, 1672 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.50~2.60 (14H, complex), 0.88 (3H, t, J=6.6 Hz), 2.91~3.24 (2H, complex), 4.11 (1H, m), 4.70~5.40 (5H, complex), 7.05~7.55 (11H, complex); MS (FAB) m/z 540 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{29}H_{39}N_3O_7$ $(M)^+$ 540.2710, Found 540.2703; $[\alpha]_D^{26}$ −23.3° (*c* 1.0, EtOH).

N-{(1*S*)-1-[(1*S*)-1-Methylpropyl]-2-oxobutyl}-(3*S*)-1-benzyloxycarbonyl-2-[(2*R*)-2-benzyloxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**17a**)

N-*Z*-aminoketone (**4a**) (83 mg, 300 μmol) in MeOH (3.0 ml) was treated with 10% Pd-C (13 mg) while stirring under H_2 at room temperature for 40 minutes. The catalyst was removed by celite filtration. The filtrate was concentrated under reduced pressure to give aminoketone. The aminoketone was dissolved in THF (3.0 ml). The prepared solution and DEPC (120 μl, 791 mmol) were added to a stirred solution of **16a** (115 mg, 213 μmol) in THF (2.0 ml) under N_2 at −15°C, and the mixture was stirred for 6 hours. The mixture was poured into H_2O and extracted with EtOAc (×2). The combined organic phase was washed with H_2O and then with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative thin layer silica gel chromatography (2.0 mm, 20 cm × 20 cm, $CHCl_3$ - MeOH, 10 : 1) to afford **17a** (85 mg, 60%, converting yield 80%) as a colorless oil. Starting material **16a** (30 mg, 26%) was also recovered. Compound **17a**: IR (film) 3300, 2960, 1700, 1670, 1530 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.66~2.12 (22H, complex), 1.02 (3H, t, J=7.3 Hz), 1.34 (3H, t, J=7.3 Hz), 2.28 (1H, m), 2.46 (2H, br q, J=7.3 Hz), 3.12 (1H, m), 3.75 (1H, m), 4.11 (1H, m), 4.21 (1H, t, J=7.0 Hz), 4.82 (1H, d, J=12.2 Hz), 4.87 (1H, d, J=12.2 Hz), 4.92 (1H, m), 5.17 (1H, d, J=11.7 Hz), 5.25 (1H, d, J=11.7 Hz), 7.24~7.48 (10H, complex), 8.12 (1H, m), 8.27 (1H, m); MS (EI) m/z 665 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{37}H_{53}N_4O_7$ $(M)^+$ 665.3913, Found 665.3897; $[\alpha]_D^{26}$ −41.0° (*c* 1.0, $CHCl_3$).

N-{(1*S*)-1-[(1*S*)-1-Methylpropyl]-2-oxobutyl}-(3*S*)-2-[(2*R*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1a**)

Coupling product **17a** (38 mg, 57 μmol) in MeOH (2.0 ml) was treated with 10% Pd-C (9 mg) while stirring under H_2 at room temperature for 2.5 hours. The catalyst was removed by celite filtration. The filtrate was concentrated under reduced pressure to give a pale yellow oil. The residue was purified by preparative thin layer silica gel 60 silanised chromatography (0.25 mm, 20 cm × 20 cm, MeOH - H_2O, 3 : 2) to afford **1a** (22 mg, 86%) as a white solid: mp 58~61°C (recrystallized from hexane - acetone); IR (film) 3303, 2932, 1714, 1667, 1626, 1544 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.85 (3H, d, J=6.7 Hz), 0.87 (3H, t, J=6.1 Hz), 0.92 (3H, t, J=6.7 Hz), 1.00~2.20 (15H, complex), 1.09 (3H, t, J=7.3 Hz), 2.31 (1H, dd, J=12.0, 4.4 Hz), 2.49 (1H, br t, J=12.0 Hz), 2.55 (2H, q, J=7.3 Hz), 2.83 (1H, m), 3.01 (1H, br d, J=12.8 Hz), 3.95 (1H, m), 4.64 (1H, dd, J=8.5, 4.9 Hz), 4.75 (1H, br d, J=12.8 Hz), 5.31 (1H, br s), 7.38 (1H, br s); MS (FAB) m/z 441 $(M+H)^+$; HR-MS (FAB) m/z Calcd for $C_{22}H_{41}N_4O_5$ $(M+H)^+$ 441.3077, Found 441.3055; *Anal.* Calcd for $C_{22}H_{40}N_4O_5 \cdot 1/10H_2O$: C 59.73, H 9.15, N 12.67. Found: C 59.49, H 9.13, N 12.41.; $[\alpha]_D^{25}$ −33.6° (*c* 1.0, EtOH). lit.[5] mp 69~72°C.; $[\alpha]_D^{20}$ −30.7° (*c* 1.0, EtOH).

tert-Butyl (3*R*)-1-Benzyloxycarbonyl-2-{(2*R*)-1-oxo-2-[(2,2,2-trichloroethoxycarbonyl)methyl]heptyl}hexahydropyridazine-3-carboxylate (**13c**)

Compound **13c** (267 mg) was prepared in 56% yield (converting yield 85%) from **10a** (246 mg) and **12b** (246 mg) according to the procedure for preparing **13a**. The product was isolated as a colorless oil: IR (film) 2931, 1735, 1677 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.85 (3H, t, J=6.6 Hz), 0.97~2.14 (12H, complex), 1.43 (9H, s), 2.50 (1H, dd, J=17.5, 4.9 Hz), 2.95 (1H, dd, J=17.5, 9.9 Hz), 2.97 (1H, m), 3.28 (1H, m), 4.40 (1H, m), 4.58 (1H, d, J=12.5 Hz), 4.85 (1H, d, J=12.5 Hz), 5.10 (1H, d, J=12.5 Hz), 5.21 (1H, d, J=12.5 Hz), 5.29 (1H, br d, J=4.3 Hz), 7.22~7.42 (5H, complex); MS (EI) m/z 620 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{28}H_{39}N_2O_7{}^{35}C_{13}$ $(M)^+$ 620.1823, Found 620.1833; $[\alpha]_D^{26}$ +27.3° (*c* 2.0, $CHCl_3$).

tert-Butyl (3*R*)-1-Benzyloxycarbonyl-2-[(2*R*)-2-carboxymethyl-1-oxoheptyl]hexahydropyridazine-3-carboxylate (**14c**)

Compound **14c** (533 mg) was prepared in 97% yield from **13c** (694 mg) according to the procedure used to prepare **14a**. The product was isolated as a colorless oil: IR (film) 3190, 2932, 1735, 1679 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.84 (3H, t, J=6.6 Hz), 0.94~2.17 (12H, complex), 1.42 (9H, s), 2.37 (1H, m), 2.80~3.09 (2H, complex), 3.18 (1H, m), 4.39 (1H, m), 5.00~5.36 (3H, complex), 7.18~7.42 (5H, complex); MS (EI) m/z 491 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{26}H_{37}N_2O_6$ $(M+H-H_2O)^+$ 473.2652, Found 473.2672; $[\alpha]_D^{26}$ +56.2° (c 1.0, EtOH).

tert-Butyl (3*R*)-1-Benzyloxycarbonyl-2-[(2*R*)-2-benzyloxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxylate (**15c**)

Compound **15c** (421 mg) was prepared in 67% yield (converting yield 89%) from **14c** (517 mg) according to the procedure used to prepare **15a**. The product was isolated as a colorless oil: IR (film) 3252, 2931, 1735, 1675 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.87 (3H, t, J=6.6 Hz), 0.93~2.38 (14H, complex), 1.42 (9H, s), 2.81~3.32 (2H, complex), 4.32 (1H, m), 4.75~4.95 (2H, complex), 5.10 (1H, d, J=11.9 Hz), 5.20 (1H, d, J=11.9 Hz), 5.27 (1H, m), 7.22~7.46 (10H, complex); MS (EI) m/z 596 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{33}H_{46}N_3O_7$ $(M)^+$ 596.3335, Found 596.3327; $[\alpha]_D^{26}$ +37.1° (c 1.0, EtOH).

(3*R*)-1-Benzyloxycarbonyl-2-[(2*R*)-2-benzyloxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxylic Acid (**16c**)

Compound **16c** (271 mg) was prepared in 78% yield from **15c** (384 mg) according to the procedure used to prepare **16a**. The product was isolated as a colorless oil: IR (film) 3230, 2940, 1720, 1655 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.83 (3H, t, J=5.8 Hz), 0.97~2.20 (14H, complex), 3.02~3.35 (2H, complex), 4.24 (1H, m), 4.70~4.95 (2H, br s), 5.02~5.33 (3H, complex), 7.18~7.48 (10H, complex); $[\alpha]_D^{26}$ +21.4° (c 1.0, EtOH).

Stereoisomers of matlystatin B (**1b**~**1j**) were prepared from **4a**~**4d** and **16a**~**16d** according to the procedure used to prepare **17a** and **1a** as follows. **1b**~**1j** were purified by preparative thin layer silica gel chromatography ($CHCl_3$ - MeOH, 20 : 1).

N-{(1*R*)-1-[(1*R*)-1-Methylpropyl]-2-oxobutyl}-(3*S*)-2-[(2*R*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1b**)

Compound **1b** (19 mg) was prepared in 25% yield (converting yield 37%) from **4b** (82 mg) and **16a** (95 mg). The product was isolated as a colorless oil: IR (film) 3274, 2933, 1718, 1665, 1628 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.77~0.93 (6H, complex), 0.97 (3H, d, J=6.6 Hz), 1.06 (3H, t, J=7.3 Hz), 1.15~2.63 (17H, complex), 2.52 (2H, q, J=7.3 Hz), 2.70~3.19 (2H, complex), 3.95 (1H, m), 4.47~4.73 (2H, complex), 5.25 (1H, s), 6.81 (1H, d, J=8.6 Hz), 7.70~8.80 (1H, br), 9.54 (1H, br s); MS (EI) m/z 440 $(M)^+$; HR-MS (FAB) m/z Calcd for $C_{22}H_{41}N_4O_5$ $(M+H)^+$ 441.3069, Found 441.3086; $[\alpha]_D^{26}$ +3.5° (c 1.0, EtOH).

N-{(1*S*)-1-[(1*R*)-1-Methylpropyl]-2-oxobutyl}-(3*S*)-2-[(2*R*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1c**)

Compound **1c** (32 mg) was prepared in 80% yield from **4c** (75 mg) and **16a** (49 mg). The product was isolated as a colorless oil: IR (film) 3315, 2933, 1717, 1672, 1627 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.74 (3H, d, J=6.6 Hz), 0.84 (3H, t, J=6.6 Hz), 0.92 (3H, t, J= 6.9 Hz), 1.08~2.10 (15H, complex), 1.10 (3H, t, J=7.3 Hz), 2.15~2.66 (4H, complex), 2.82 (1H, m), 3.00 (1H, m), 3.99 (1H, m), 4.81 (1H, d, J= 4.6 Hz), 4.89 (1H, d, J=11.2 Hz), 5.39 (1H, s), 7.68 (1H, br d, J=6.3 Hz), 9.72~10.31 (1H, br); MS (EI) m/z 440 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{22}H_{40}N_4O_5$ $(M)^+$ 440.2999, Found 440.3012; $[\alpha]_D^{26}$ −14.0° (c 1.0, EtOH).

N-{(1*R*)-1-[(1*S*)-1-Methylpropyl]-2-oxobutyl}-(3*S*)-2-[(2*R*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1d**).

Compound **1d** (36 mg) was prepared in 79% yield from **4d** (116 mg) and **16a** (57 mg). The product was isolated as a colorless oil: IR (film) 3276, 2933, 1718, 1668, 1628 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.73 (3H, d, J=7.3 Hz), 0.85 (3H, t, J=7.3 Hz), 0.97 (3H, t, J=7.3 Hz), 1.07 (3H, t, J=7.3 Hz), 1.08~1.75

(14H, complex), 1.80~2.66 (5H, complex), 2.73~3.19 (2H, complex), 3.98 (1H, m), 4.63 (1H, d, J=11.2 Hz), 4.73 (1H, dd, J=8.6, 3.3 Hz), 5.29 (1H, s), 6.81 (1H, d, J=12.6 Hz), 9.40~9.95 (1H, br); MS (EI) m/z 440 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{22}H_{40}N_4O_5$ $(M)^+$ 440.2988, Found 440.3007; $[\alpha]_D^{26}$ −15.9° (c 1.0, EtOH).

N-{(1*S*)-1-[(1*S*)-1-Methylpropyl]-2-oxobutyl}-(3*S*)-2-[(2*S*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1e**)

Compound **1e** (20 mg) was prepared in 48% yield from **4a** (78 mg) and **16b** (51 mg). The product was recrystallized from hexane-EtOAc: mp 138~139°C; IR (film) 3275, 2945, 1710, 1635 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.78~0.99 (9H, complex), 1.03 (3H, t, J=7.3 Hz), 1.11~1.84 (14H, complex), 2.18 (1H, m), 2.30~2.88 (5H, complex), 3.10 (1H, br d, J=13.2 Hz), 3.82 (1H, d, J= 2.5 Hz), 4.19 (1H, m), 4.64 (1H, dd, J= 8.6, 7.9 Hz), 5.23 (1H, d, J=4.0 Hz), 7.19 (1H, d, J= 8.6 Hz), 7.80 (1H, m), 8.55 (1H, br s); MS (EI) m/z 440 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{22}H_{40}N_4O_5$ $(M)^+$ 440.2998, Found 440.2986; $[\alpha]_D^{26}$ −78.6° (c 0.50, EtOH).

N-{(1*R*)-1-[(1*R*)-1-Methylpropyl]-2-oxobutyl}-(3*S*)-2-[(2*S*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1f**).

Compound **1f** (24 mg) was prepared in 64% yield from **4b** (70 mg) and **16b** (46 mg). The product was isolated as a colorless oil: IR (film) 3270, 2933, 1716, 1650, 1630 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.80~0.92 (6H, complex), 0.95 (3H, d, J=6.6 Hz), 1.06 (3H, t, J=7.3 Hz), 1.10~2.87 (18H, complex), 2.59 (2H, q, J=7.3 Hz), 3.09 (1H, br d, J=13.9 Hz), 4.02 (1H, m), 4.35 (1H, br d, J=12.5 Hz), 4.62 (1H, dd, J=8.2, 5.6 Hz), 5.29 (1H, d, J=3.3 Hz), 7.64 (1H, d, J=17.9 Hz), 9.20~9.62 (1H, br); MS (EI) m/z 440 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{22}H_{40}N_4O_5$ $(M)^+$ 440.2998, Found 440.2994; $[\alpha]_D^{26}$ −48.0° (c 1.0, EtOH).

N-{(1*S*)-1-[(1*S*)-1-Methylpropyl]-2-oxobutyl}-(3*R*)-2-[(2*R*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1g**)

Compound **1g** (41 mg) was prepared in 68% yield from **4a** (61 mg) and **16c** (74 mg). The product was isolated as a colorless oil: Enantiomer of **1f**; MS (EI) m/z 440 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{22}H_{40}N_4O_5$ $(M)^+$ 440.2998, Found 440.3032; $[\alpha]_D^{26}$ +47.2° (c 1.0, EtOH).

N-{(1*R*)-1-[(1*R*)-1-Methylpropyl]-2-oxobutyl}-(3*R*)-2-[(2*R*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1h**)

Compound **1h** (20 mg) was prepared in 63% yield from **4b** (55 mg) and **16c** (72 mg). The product was recrystallized from hexane-acetone: Enantiomer of **1e**; mp 137~139°C; MS (EI) m/z 440 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{22}H_{40}N_4O_5$ $(M)^+$ 440.2998, Found 440.2991; $[\alpha]_D^{26}$ +76.4° (c 0.28, EtOH).

N-{(1*S*)-1-[(1*S*)-1-Methylpropyl]-2-oxobutyl}-(3*R*)-2-[(2*S*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1i**)

Compound **1i** (23 mg) was prepared in 49% yield from **4a** (51 mg) and **16d** (58 mg). The product was isolated as a colorless oil: Enantiomer of **1b**; MS (EI) m/z 441 $(M+H)^+$; HR-MS (EI) m/z Calcd for $C_{22}H_{41}N_4O_5$ $(M+H)^+$ 441.3077, Found 441.3060; $[\alpha]_D^{26}$ −3.7° (c 1.0, EtOH).

N-{(1*R*)-1-[(1*R*)-1-Methylpropyl]-2-oxobutyl}-(3*R*)-2-[(2*S*)-2-hydroxyaminocarbonylmethyl-1-oxoheptyl]hexahydropyridazine-3-carboxamide (**1j**)

Compound **1j** (16 mg) was prepared in 36% yield from **4b** (49 mg) and **16d** (55 mg). The product was isolated as a colorless oil: Enantiomer of **1a**; MS (EI) m/z 440 $(M)^+$; HR-MS (EI) m/z Calcd for $C_{22}H_{41}N_4O_5$ $(M+H)^+$ 441.3061, Found 441.3077; $[\alpha]_D^{26}$ +32.9° (c 1.0, EtOH).

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