Monamidocin, a Novel Fibrinogen Receptor Antagonist

II. Biological Activity and Structure-activity Relationships

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Monamidocin, a fibrinogen receptor binding inhibitor produced by *Streptomyces* sp. NR 0637, inhibits the binding of fibrinogen to its receptor with an IC_{50} of 0.21 μM. It also inhibits ADP-, collagen- and thrombin-induced aggregation of human platelet with IC_{50}s of 46, 30 and 77 μM, respectively. To obtain more potent inhibitors, twenty analogs have been synthesized, among which *N*-[(*R*)-5-guanidino-2-hydroxypentanoyl]-L-tyrosine is the most potent. It inhibits the binding of fibrinogen to its receptors with an IC_{50} of 0.022 μM and is about ten times more potent than monamidocin.

A novel fibrinogen receptor antagonist, monamidocin (**1**), was isolated from the culture broth of *Streptomyces* sp. NR 0637[1]. The structure of **1** was determined to be *N*-[(*S*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine. Many peptidic fibrinogen receptor antagonists[2~4] containing a RGD (Arg-Gly-Asp) peptide sequence have been synthesized, because the RGD peptide sequence was found to be the minimal sequence involved in the binding of fibrinogen to its receptor during aggregation. Since monamidocin has a much simpler structure than those reported for the arginine-containing peptides or that of our novel fibrinogen receptor antagonist, tetrafibricin, isolated from *Streptomyces neyagawaensis* NR 0577[5~8], we prepared twenty analogs to obtain more active compounds.

In the course of our synthetic study, one analog, *N*-[(*R*)-5-guanidino-2-hydroxypentanoyl]-L-tyrosine (**17**), was found to be ten times more potent than monamidocin. Compound **17** also inhibited the aggregation of human platelets induced by ADP, collagen and thrombin with IC_{50}s of 24, 9 and 22 μM, respectively.

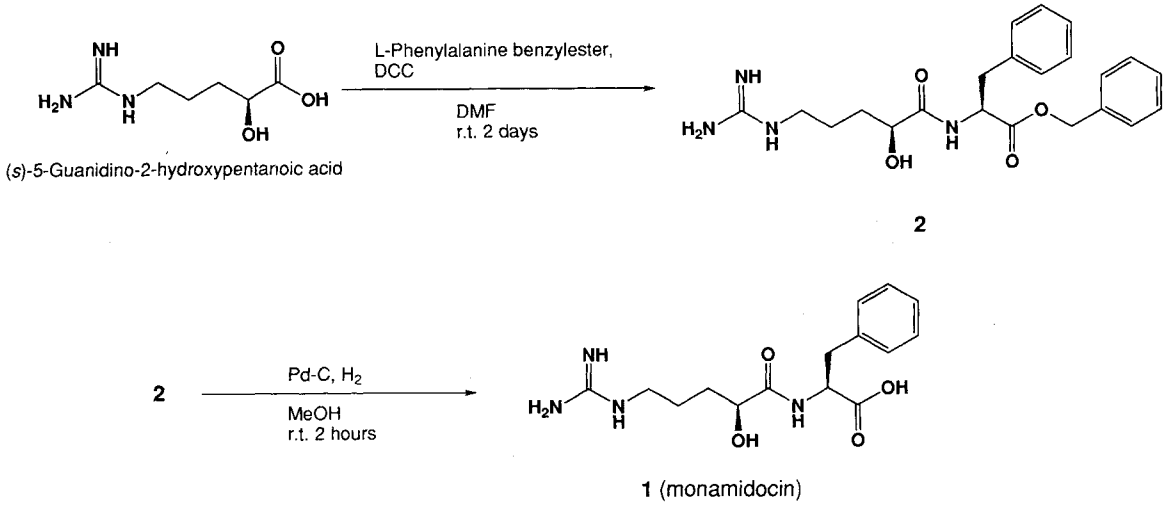
Results and Discussion

Chemistry

Synthesis of 1

The scheme for synthesizing monamidocin (**1**) is shown in Fig. 1. First, (*S*)-5-guanidino-2-hydroxypentanoic acid was treated with L-phenylalanine benzylester p-toluenesulfonate and dicyclohexylcarbodiimide (DCC) to give *N*-[(*S*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine benzylester p-toluenesulfonate (**2**). Then the

Fig. 1. Preparation of monamidocin (**1**)



benzylester in **2** was hydrogenolyzed over Pd-charcoal (Pd-C) to give *N*-[(*S*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine p-toluenesulfonate (**1**).

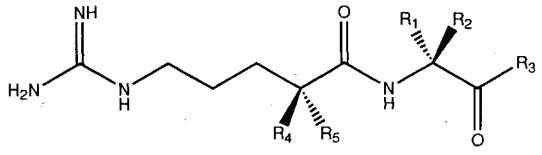
Synthesis of Analogs 3~20

Various monamidocin analogs (shown in Fig. 2) were synthesized as described below. Treatment of (*S*)-5-guanidino-2-hydroxypentanoic acid with D-phenylalanine benzylester gave **3**, which was converted into **4** by the hydrogenolysis over Pd-C. Acetylation of **2** with acetic anhydride in pyridine gave the acetyl ester (**5**), which was converted into **6** by the hydrogenolysis. Treatment of (*S*)-5-guanidino-2-hydroxypentanoic acid with L-phenylalaninamide hydrochloride gave **7**.

To evaluate the role of the 2-hydroxy group in the 5-guanidino-2-hydroxypentanoic acid moiety, a dehydroxy analog and *R*-isomer of the 5-guanidino-2-hydroxypentanoic acid moiety were prepared. Treatment of 5-guanidinopentanoic acid with L-phenylalanine benzylester gave **12**, which was converted into the dehydroxy compound (**13**) by the hydrogenolysis. Treatment of (*R*)-5-guanidino-2-hydroxypentanoic acid with L- and D-phenylalanine benzylester gave **8** and **10**, respectively. Compounds **8** and **10** were also converted into **9** and **11**, respectively, by the hydrogenolysis in procedures similar to that for **4**.

L-Tyrosine analogs in place of phenylalanine were also prepared. Treatment of (*S*)- and (*R*)-5-guanidino-2-hydroxypentanoic acid with 3-(4-benzyloxyphenyl)-L-alanine benzylester gave **14** and **16**, respectively. By the hydrogenolysis, **14** and **16** were converted into **15** and **17**, respectively. Treatment of (*R*)-5-guanidino-

Fig. 2. Structures of monamidocin (**1**) and its analogs.



Compounds	R_1	R_2	R_3	R_4	R_5
1	H	CH_2-Ph	OH	OH	H
2	H	CH_2-Ph	OCH_2-Ph	OH	H
3	CH_2-Ph	H	OCH_2-Ph	OH	H
4	CH_2-Ph	H	OH	OH	H
5	H	CH_2-Ph	OCH_2-Ph	OAc	H
6	H	CH_2-Ph	OH	OAc	H
7	H	CH_2-Ph	NH2	OH	H
8	H	CH_2-Ph	OCH_2-Ph	H	OH
9	H	CH_2-Ph	OH	H	OH
10	CH_2-Ph	H	OCH_2-Ph	H	OH
11	CH_2-Ph	H	OH	H	OH
12	H	CH_2-Ph	OCH_2-Ph	H	H
13	H	CH_2-Ph	OH	H	H
14	H	CH_2-Ph-OCH_2-Ph	OCH_2-Ph	OH	H
15	H	CH_2-Ph-OH	OH	OH	H
16	H	CH_2-Ph-OCH_2-Ph	OCH_2-Ph	H	OH
17	H	CH_2-Ph-OH	OH	H	OH
18	H	CH_2-Ph-OCH_2-Ph	OEt	H	OH
19	H	CH_2-Ph-OH	OEt	H	OH
20	H	CH_2-Ph-OAc	OEt	H	OAc

2-hydroxypentanoic acid with 3-(4-benzyloxyphenyl)-L-alanine ethyl ester gave **18**, which was converted into **19** by the hydrogenolysis. Acetylation of **19** with acetic anhydride in pyridine gave the acetyl ester (**20**).

Biological Activity

Inhibition of Fibrinogen Binding to GP IIb/IIIa Receptors

The inhibitory activitiy of monamidocin (**1**) and its synthesized analogs against the binding between fibrinogen and solid phase GP IIb/IIIa were examined (Table 1). From the results of these binding experiments on the analogs, the following preliminary structure-activity relationships were obtained.

1. From the comparison of the inhibitory activity of **1** with those of L-arginyl-L-phenylalanine (purchased from Kokusan Chemical Co. Ltd.), the 2-acetoxy analog (**6**) and the 2-dehydroxy analog (**13**), the 2-hydroxy group in **1** was shown to be essential for the inhibitory activity.
2. As regard to the stereochemistry of the 2-hydroxy group in the 5-guanidino-2-hydroxypentanoic acid moiety, the inhibitory activity of the 2-(*R*)-isomer (**9**) was ten times more potent than that of the 2-(*S*)-isomer (**1**). Therefore, the *R* configuration is preferable for the position.
3. Ester or amide derivatives of the C-terminal end of the amino acid moiety decreased the inhibitory activity by more than one order of magnitude.
4. As regard to the stereochemistry of the phenylalanine residue, the inhibitory activity of the D-phenylalanine analog was twenty times less potent than that of **1**.
5. Substitution of the L-phenylalanine moiety with the L-tyrosine moiety increased the inhibitory activity.

Inhibition of Platelet Aggregation

Among the synthesized compounds (**1**～**20**), five of them were examined for their activity to inhibit the platelet aggregation in human platelet-rich plasma induced by ADP, collagen or thrombin (Table 2). Although the inhibitory activity of **1** is ten times less

Table 1. Effect of monamidocin (**1**) and its analogs on bindings between fibrinogen and solid phase GP IIb/IIIa.

Compound	IC_{50} values in μM	Compound	IC_{50} values in μM
1	0.21	14	2.4
2	1.9	15	0.068
3	85	16	>145
4	5.0	17	0.022
5	>16	18	>100
6	6.1	19	14
7	>28	20	>100
8	2.8	L-Arg-L-Phe	16
9	0.026	L-Arg-L-Tyr	7.4
10	>17	L-Phe-L-Arg	>100
11	1.8	RGDS	0.12
12	>18	Tetrafibricin	0.046
13	2.5		

Table 2. Inhibitory activities of monamidocin (**1**) and its analogs against ADP-, collagen- and thrombin-induced platelet aggregation.

	IC_{50} values in μM		
Compound	ADP	Collagen	Thrombin
1	46	30	77
9	21	19	18
13	>300	209	>300
15	44	14	46
17	24	9	22
RGDS	134	65	119
Tetrafibricin	5.6	11	7.6

potent than that of **17** against the binding between fibrinogen and solid phase GP IIb/IIIa, it is almost equal to that of **17** against the inhibition of platelet aggregation. To explain the difference in the effects between solid phase binding and platelet aggregation assays, we considered the following reasons: 1) because the solid phase binding assay is more sensitive than the platelet aggregation assay, the concentrations of ligand and GP IIb/IIIa receptors for the solid phase assay are lower than those for platelet aggregation; 2) the microenvironment of the purified GP IIb/IIIa receptors coated on the plate is different from that of the natural GP IIb/IIIa receptors on platelets, because the receptors on the platelets are surrounded by phospholipids and cytoskeletal proteins.

On the other hand, since the IC_{50} values for the tested compounds were in the same range independent of the agonist used for platelet activation, it was also shown that the active compounds, including monamidocin, inhibited a step that was common to all these agonistic pathways in platelet aggregation.

Antimicrobial Activity and Cytotoxicity of **1**

At a concentration of 100 μg/ml, **1** didn't show antibacterial or antifungal activity against the following strains; *Staphylococcus aureus* NIHJ JC1, *Bacillus subtilis* ATCC 6633, *Streptococcus faecalis* IFO 3826, *Micrococcus flavus* BP 1202, *Escherichia coli* NIHJ JC2, *Klebsiella pneumoniae* ATCC 27736, *Enterobacter cloacae* ATCC 13047, *Serratia marcescens* ATCC 12648, *Shigella sonnei* ATCC 11060, *Salmonella typhimurium* ATCC 13311, *Proteus rettgeri* ATCC 14505, *Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 by broth dilution method using a 96-well microtiter plate. Also, **1** did not show significant cytotoxicity against HeLa cells at a concentration of 300 μM.

Experimental

General Procedures

IR spectra were recorded on a Hitachi 270-30 or a Perkin Elmer 1600 IR spectrometer. FAB-MS and HRFAB-MS were measured on a JEOL JMS-DX303 mass spectrometer. ^{1}H and ^{13}C NMR were recorded on a JMN-GSX-400 NMR spectrometer at 400 and 100 MHz, respectively, using TMS as an internal standard. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. The solid phase binding was monitored by measuring the absorbance using a MTP-32 microplate photometer (Corona electric Co.). Platelet aggregation was observed by a aggregometer (Hema-tracer (Niko science, Japan)).

Fibrinogen Binding to Immobilized GP IIb/IIIa (Solid Phase Assay)

Biotinylated human fibrinogen and an assay sample were added to micro titer wells coated with purified GP IIb/IIIa. After washing the free fibrinogen, peroxidase-conjugated streptavidin (avidin-POD) was added to each well. After a second wash, 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid was added to each well. The binding was assessed by measuring the absorbance at 415 nm using a microplate photometer. Detailed assay methods were described by MORI *et al.*[9].

Inhibition of Platelet Aggregation in Human Plasma

Human platelet-rich plasma was prepared from whole blood anticoagulated with acid-citrate-dextrose (ACD) and the aggregation of platelets was measured by recording the velocity of change in light transmission with an aggregometer at 37°C. Serial dilutions of the inhibitors were added, and aggregation was induced in human plasma by ADP, collagen or thrombin at a final concentration of 10 μM, 2.0 μg/ml or 0.2 unit/ml, respectively. Detailed assay methods have been reported[8].

Preparation of *N*-[(*S*)-5-Guanidino-2-hydroxypentanoyl]-L-phenylalanine Benzyl Ester p-Toluenesulfonate (**2**)

A solution of (*S*)-5-guanidino-2-hydroxypentanoic acid (875 mg) in DMF (15 ml) were added L-phenylalanine benzylester p-tuluenesulfonate (2.1 g) and DCC (1.1 g). After stirring for 2 days, the reaction mixture was concentrated to remove DMF, diluted with EtOAc and washed with water. The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was triturated with diethyl ether (Et_2O). Crystallization of the triturated oil from MeOH/Et_2O yielded **2** (807 mg) as colorless needles. **2**: mp: 118~119°C; HRFAB-MS *m/z*: 413.2210 $(M+H)^+$ (calcd for $C_{22}H_{29}N_4O_4$ 413.2189); IR ν_{max} (KBr) cm^{-1}: 3330, 3200, 1740, 1670, 1630, 1510, 1280, 1210, 1130, 1030, 1010, 690; ^{1}H NMR (DMSO-d_6): δ 1.38 (3H, m), 1.53 (1H, m), 2.29 (3H, s), 3.02 (2H, m), 3.06 (2H, m), 3.86 (1H, m), 4.65 (1H, dt, J=5.0, 8.0 Hz), 5.12 (2H, s), 5.69 (1H, d, J=5.2 Hz), 7.12 (2H, d, J=8.0 Hz), 7.15~7.40 (11H), 7.48 (2H, d, J=8.0 Hz), 7.94 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-hydroxypentanoyl]-L-phenylalanine p-Toluenesulfonate (**1**)

To a solution of **2** (200 mg) in MeOH (100 ml) was added 10% Pd-charcoal. The reaction mixture was hydrogenated at room temperature for 2 hours. Thereafter, the catalyst was filtered off, washed with MeOH and the filtrate was concentrated. The residue was purified by chromatography on Bond elute (C_{18}) (Varian) using 60% MeOH as an eluent to yield **1**

(103 mg) as a colorless amorphous material. **1**: HRFAB-MS *m/z*: 323.1723 $(M+H)^+$ (calcd for $C_{15}H_{23}N_4O_4$ 323.1719); IR ν_{max} (KBr) cm^{-1}: 3360, 1730, 1660, 1530, 1170, 1120, 1030, 1010, 690; ^1H NMR (DMSO-d_6): δ 1.37 (3H, m), 1.51 (1H, m), 2.28 (3H, s), 3.01 (4H, m), 3.84 (1H, m), 4.51 (1H, dt, J=5.2, 8.0 Hz), 5.71 (1H, d, J=5.6 Hz), 7.10 (2H, d, J=8.0 Hz), 7.12~7.27 (5H), 7.39 (1H, m), 7.47 (2H, d, J=8.0 Hz), 7.67 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-hydroxypentanoyl]-D-phenylalanine Benzyl Ester p-Toluensulfonate (**3**)

D-Phenylalanine benzylester p-tuluenesulfonate (450 mg) and (*S*)-5-guanidino-2-hydroxypentanoic acid (175 mg) were subjected to a procedure to similar that for preparing **2**. Crystallization from MeOH/Et_2O gave **3** (85.6 mg) as colorless needles. **3**: mp: 114~115°C; HRFAB-MS *m/z*: 413.2186 $(M+H)^+$ (calcd for $C_{22}H_{29}N_4O_4$ 413.2189); IR ν_{max} (KBr) cm^{-1}: 3330, 3190, 1730, 1670, 1630, 1570, 1240, 1200, 1120, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.43 (3H, m), 1.59 (1H, m), 2.28 (3H, s), 3.06 (4H, m), 3.84 (1H, m), 4.59 (1H, dt, J=5.9, 8.0 Hz), 5.10 (2H, s), 5.63 (1H, d, J=5.2 Hz), 7.10 (2H, d, J=8.0 Hz), 7.17~7.39 (11H), 7.48 (2H, d, J=8.0 Hz), 7.96 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-hydroxypentanoyl]-D-phenylalanine p-Toluenesulfonate (**4**)

A solution of **3** (20 mg) in MeOH (10 ml) was subjected to a procedure similar to that for preparing **1**. Chromatography on a column of bond elute (C_{18}) using 60% MeOH as an eluent provided **4** (11 mg) as a colorless amorphous material. **4**: HRFAB-MS *m/z*: 323.1719 $(M+H)^+$ (calcd for $C_{15}H_{23}N_4O_4$ 323.1719); IR ν_{max} (KBr) cm^{-1}: 3350, 1730, 1650, 1530, 1170, 1120, 1030, 1010, 690; ^1H NMR (DMSO-d_6): δ 1.41 (3H, m), 1.59 (1H, m), 2.25 (3H, s), 3.06 (4H, m), 3.86 (1H, m), 4.48 (1H, dt, J=5.2, 8.0 Hz), 5.61 (1H, d, J=5.2 Hz), 7.11 (2H, d, J=8.0 Hz), 7.19~7.29 (5H), 7.38 (1H, br t, J=5.2 Hz), 7.47 (2H, d, J=8.0 Hz), 7.73 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-acetoxypentanoyl]-L-phenylalanine Benzyl Ester p-Toluenesulfonate (**5**)

A solution of **2** (30 mg) and acetic anhydride (5.5 μl) in pyridine (3 ml) was left to stand at room temperature for 3 hours. There was obtained, after *n*-BuOH extraction and preparative TLC on silica gel using $CHCl_3$ - MeOH (3 : 1) as an eluent, **5** (14 mg) as a colorless amorphous material. **5**: HRFAB-MS *m/z*: 455.2277 $(M+H)^+$ (calcd for $C_{24}H_{31}N_4O_5$ 455.2295); IR ν_{max} (KBr) cm^{-1}: 3420, 1740, 1660, 1630, 1540, 1200, 1120, 1030, 1010, 680; ^1H NMR ($CDCl_3$): δ 1.57 (2H, m), 1.77 (1H, m), 1.85 (1H, m), 2.02 (3H, s), 2.35 (3H, s), 3.09 (4H, m), 4.79 (1H, dd, J=12.0, 6.8 Hz), 5.05 (1H, m), 5.06 (1H, d, J=12.0 Hz), 5.12 (1H, d, J=12.0 Hz), 7.01~7.33 (13H), 7.68 (1H, m), 7.71 (2H, d, J=6.8 Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-acetoxypentanoyl]-L-phenylalanine p-Toluenesulfonate (**6**)

A solution of **5** (10 mg) in MeOH (10 ml) was subjected to a procedure similar to that for preparing **1**. Chromatography on a column of bond elute (C_{18}) using 60% MeOH as an eluent provided **6** (5.5 mg) as a colorless amorphous material. **6**: HRFAB-MS *m/z*: 365.1819 $(M+H)^+$ (calcd for $C_{17}H_{25}N_4O_5$ 365.1825); IR ν_{max} (KBr) cm^{-1}: 3380, 1740, 1650, 1530, 1170, 1120, 1030, 1010, 690; ^1H NMR (DMSO-d_6): δ 1.40 (2H, m), 1.65 (2H, m), 2.05 (3H, s), 2.29 (3H, s), 2.94 (1H, dd, J=13.2, 8.8 Hz), 3.06 (3H, m), 4.41 (1H, dt, J=10.8, 5.2 Hz), 4.94 (1H, m), 7.12 (2H, d, J=8.0 Hz), 7.16~7.34 (5H), 7.48 (2H, d, J=8.0 Hz), 7.54 (1H, br t, J=5.2 Hz), 8.14 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-hydroxypentanoyl]-L-phenylalanineamide Hydrochloride (**7**)

To a solution of (*S*)-5-guanidino-2-hydroxypentanoic acid (175 mg) in DMF (3 ml) were added L-phenylalaninamide hydrochloride (220 mg) and DCC (230 mg). The reaction mixture was stirred for 2 days, concentrated to remove DMF, diluted with *n*-BuOH and washed with water. The organic layer was evaporated under reduced pressure. The residue was triturated with acetonitrile. The triturated oil was purified by silica gel column chromatography using $CHCl_3$ - MeOH (3 : 1) as an eluent to yield **7** (59.8 mg) as a colorless amorphous material. **7**: HRFAB-MS *m/z*: 322.1879 $(M+H)^+$ (calcd for $C_{15}H_{24}N_5O_3$ 322.1879); IR ν_{max} (KBr) cm^{-1}: 3340, 1660, 1520, 1120, 700; ^1H NMR (DMSO-d_6): δ 1.37 (3H, m), 1.48 (1H, m), 2.88 (1H, dd, J=5.6, 13.2 Hz), 3.05 (3H, m), 3.82 (1H, m), 4.50 (1H, dt, J=8.8, 5.2 Hz), 5.72 (1H, d, J=4.8 Hz), 7.17~7.27 (5H), 7.48 (1H, br t, J=5.2 Hz), 7.63 (1H, d, J=8.8 Hz).

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl]-L-phenylalanine Benzyl Ester p-Toluensulfonate (**8**)

L-Phenylalanine benzylester p-tuluenesulfonate (470 mg) and (*R*)-5-guanidino-2-hydroxypentanoic acid (175 mg) obtained from D-arginine as described[10] were subjected to a procedure similar to that for preparing **2**. Crystallization from MeOH/Et_2O gave **8** (117 mg) as colorless needles. **8**: mp: 113.5~114.5°C; HRFAB-MS *m/z*: 413.2202 $(M+H)^+$ (calcd for $C_{22}H_{29}N_4O_4$ 413.2189); IR ν_{max} (KBr) cm^{-1}: 3330, 3190, 1730, 1670, 1630, 1570, 1240, 1200, 1120, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.43 (3H, m), 1.51 (1H, m), 2.29 (3H, s), 3.05 (4H, m), 3.85 (1H, m), 4.60 (1H, dt, J=6.0, 8.0 Hz), 5.11 (2H, s), 5.65 (1H, d, J=5.6 Hz), 7.11 (2H, d, J=8.0 Hz), 7.17~7.40 (11H), 7.47 (2H, d, J=8.0 Hz), 7.96 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl]-L-phenylalanine p-Toluenesulfonate (**9**)

A solution of **8** (60 mg) in a MeOH (30 ml) was subjected to a procedure similar to that for preparing **1**. Chromatography on a column of bond elute (C_{18}) using

60% MeOH as an eluent provided **9** (11 mg) as a colorless amorphous material. **9**: HRFAB-MS *m/z*: 323.1727 $(M+H)^+$ (calcd for $C_{15}H_{23}N_4O_4$ 323.1719); IR ν_{max} (KBr) cm^{-1}: 3350, 1730, 1650, 1530, 1170, 1120, 1030, 1010, 690; ^1H NMR (DMSO-d_6): δ 1.43 (3H, m), 1.61 (1H, m), 2.29 (3H, s), 3.06 (4H, m), 3.84 (1H, m), 4.46 (1H, dt, J=4.8, 8.0 Hz), 5.62 (1H, d, J=4.8 Hz), 7.11 (2H, d, J=8.0 Hz), 7.19~7.29 (5H), 7.38 (1H, m), 7.47 (2H, d, J=8.0 Hz), 7.74 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl]-D-phenylalanine Benzyl Ester p-Toluensulfonate (**10**)

D-Phenylalanine benzylester p-tuluenesulfonate (470 mg) and (*R*)-5-guanidino-2-hydroxypentanoic acid (175 mg) were subjected to a procedure similar to that for preparing **2**. Crystallization from MeOH/Et_2O gave **10** (140 mg) as a colorless needles. **10**: mp: 103~104°C; HRFAB-MS *m/z*: 413.2207 $(M+H)^+$ (calcd for $C_{22}H_{29}N_4O_4$ 413.2189); IR ν_{max} (KBr) cm^{-1}: 3330, 3200, 1740, 1670, 1630, 1520, 1240, 1190, 1120, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.39 (3H, m), 1.59 (1H, m), 2.29 (3H, s), 3.06 (4H, m), 3.87 (1H, m), 4.63 (1H, dt, J=8.8, 10.4 Hz), 5.12 (2H, s), 5.69 (1H, d, J=5.2 Hz), 7.11 (2H, d, J=8.0 Hz), 7.12~7.40 (11H), 7.47 (2H, d, J=8.0 Hz), 7.94 (1H, d, J=8.8 Hz).

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl]-D-phenylalanine p-Toluenesulfonate (**11**)

A solution of **10** (60 mg) in MeOH (30 ml) was subjected to a procedure similar to that for preparing **1**. Chromatography on a column of bond elute (C_{18}) using 60% MeOH as an eluent provided **11** (28.3 mg) as a colorless amorphous material. **11**: HRFAB-MS *m/z*: 323.1729 $(M+H)^+$ (calcd for $C_{15}H_{23}N_4O_4$ 323.1719); IR ν_{max} (KBr) cm^{-1}: 3360, 1730, 1660, 1530, 1170, 1120, 1030, 1010, 690; ^1H NMR (DMSO-d_6): δ 1.38 (3H, m), 1.53 (1H, m), 2.29 (3H, s), 3.06 (4H, m), 3.86 (1H, m), 4.53 (1H, m), 5.72 (1H, d, J=6.0 Hz), 7.11 (2H, d, J=8.0 Hz), 7.17~7.29 (5H), 7.35 (1H, br t, J=5.6 Hz), 7.47 (2H, d, J=8.0 Hz), 7.75 (1H, d, J=8.0 Hz).

Preparation of *N*-(5-Guanidinopentanoyl)-L-phenylalanine Benzylester p-Toluenesulfonate (**12**)

A solution of 3,5-dimethylpyrazole-1-carboxamidine nitrate (1.03 g) and 5-aminopentanoic acid (585 mg) in pH 10.4 of aqueous solution adjusted with 1 N NaOH was stirred at room temperature for 5 days as described[11]. The reaction mixture was washed with EtOAc and *n*-BuOH, and adjusted to pH 6 with 1 N HCl. There was obtained, after evapolation of water, washing with MeOH and chrystallization from hot water, 420 mg of 5-guanidinopentanoic acid as colorless needles, mp: >220°C, FAB-MS *m/z*: 154 $(M+H)^+$, ^1H NMR (D_2O): δ 1.61 (4H, m), 2.40 (2H, t, J=7.4 Hz), 3.16 (2H, t, J=6.6 Hz).

L-Phenylalanine benzylester p-tuluenesulfonate (470 mg) and 5-guanidinopentanoic acid (160 mg) were subjected to a procedure similar to that for preparing **2**. The reaction mixture was applied to a silica gel column chromatography using $CHCl_3$-MeOH (10:1) as an eluent to yield **12** (306.3 mg) as a colorless amorphous material. **12**: HRFAB-MS *m/z*: 397.2231 $(M+H)^+$ (calcd for $C_{22}H_{29}N_4O_3$ 397.2240); IR ν_{max} (KBr) cm^{-1}: 3400, 1740, 1650, 1540, 1200, 1120, 1030, 1010, 680; ^1H NMR (CD_3OD): δ 1.47~1.53 (3H, m), 1.69 (1H, m), 2.10 (2H, m), 2.29 (3H, s), 2.94 (4H, m), 4.71 (1H, t, J=6.4 Hz), 5.02 (1H, d, J=11.6 Hz), 5.06 (1H, d, J=11.6 Hz), 6.99~7.27 (12H), 7.65 (2H, d, J=8.8 Hz).

Preparation of *N*-(5-Guanidinopentanoyl)-L-phenylalanine p-Toluenesulfonate (**13**)

A solution of **12** (50 mg) in MeOH (20 ml) was subjected to a similar procedure for **1**. Chromatography on a column of bond elute (C_{18}) using 70% MeOH as an eluent provided **13** (24 mg) as a colorless amorphous material. **13**: HRFAB-MS *m/z*: 307.1767 $(M+H)^+$ (calcd for $C_{15}H_{23}N_3O_4$ 307.1770); IR ν_{max} (KBr) cm^{-1}: 3370, 1700, 1650, 1530, 1180, 1120, 1030, 1010, 690; ^1H NMR (DMSO-d_6): δ 1.33 (2H, m), 1.43 (2H, m), 2.07 (2H, t, J=4.4 Hz), 2.29 (3H, s), 2.79 (1H, dd, J=9.2, 13.4 Hz), 3.04 (2H, m), 3.08 (1H, dd, J=13.4, 4.4 Hz), 4.33 (1H, m), 7.11 (2H, d, J=8.0 Hz), 7.14~7.30 (5H), 7.47 (2H, d, J=8.0 Hz), 7.87 (1H, m), 7.95 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-hydroxypentanoyl]-3-(4-benzyloxyphenyl)-L-alanine Benzyl Ester p-Toluensulfonate (**14**)

3-(4-benzyloxyphenyl)-L-alanine benzyl ester p-toluenesulfonate (540 mg) and (*S*)-5-guanidino-2-hydroxypentanoic acid (175 mg) were subjected to a procedure similar to that for preparing **2**. Crystallization from MeOH/Et_2O gave **14** (199.6 mg) as colorless needles. **14**: mp: 122~123°C; HRFAB-MS *m/z*: 519.2617 $(M+H)^+$ (calcd for $C_{29}H_{35}N_4O_5$ 519.2607); IR ν_{max} (KBr) cm^{-1}: 3370, 3200, 1740, 1670, 1630, 1510, 1240, 1220, 1120, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.44 (3H, m), 1.57 (1H, m), 2.29 (3H, s), 3.01 (2H, m), 3.04 (2H, m), 3.88 (1H, m), 4.58 (1H, dt, J=7.0, 8.2 Hz), 5.04 (2H, s), 5.11 (2H, s), 5.69 (1H, d, J=5.6 Hz), 6.68 (2H, d, J=8.8 Hz), 7.06 (2H, d, J=8.8 Hz), 7.14 (2H, d, J=8.0 Hz), 7.30~7.44 (11H), 7.48 (2H, d, J=8.0 Hz), 7.89 (1H, d, J=8.2 Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-hydroxypentanoyl]-L-tyrosine p-Toluenesulfonate (**15**)

A solution of **14** (20 mg) in MeOH (10 ml) was subjected to a procedure similar to that for preparing **1**. Chromatography on a column of bond elute (C_{18}) using 60% MeOH as an eluent provided **15** (7 mg) as a colorless amorphous material. **15**: HRFAB-MS *m/z*: 339.1686 $(M+H)^+$ (calcd for $C_{15}H_{23}N_4O_5$ 339.1669); IR ν_{max} (KBr) cm^{-1}: 3400, 1740, 1650, 1520, 1240, 1200, 1120, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.46 (3H, m), 1.59 (1H, m), 2.29 (3H, s), 2.91 (2H, m), 3.05 (2H, m), 3.86 (1H, m), 4.45 (1H, dt, J=7.0, 8.2 Hz), 5.72 (1H, d,

J=6.0 Hz), 6.65 (2H, d, J=8.8 Hz), 6.90 (2H, d, J=8.8 Hz), 7.11 (2H, d, J=8.0 Hz), 7.39 (1H, br t, J=5.2 Hz), 7.47 (2H, d, J=8.0 Hz), 7.58 (1H, d, J=8.2 Hz), 9.21 (1H, s).

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl]-3-(4-benzyloxyphenyl)-L-alanine Benzyl Ester p-Toluenesulfonate (**16**)

3-(4-benzyloxyphenyl)-L-alanine benzyl ester p-toluenesulfonate (500 mg), (*R*)-5-guanidino-2-hydroxypentanoic acid (150 mg) and *N*-hydroxysuccinimide (Su-OH) (130 mg) were subjected to a procedure similar to that for preparing **2**. The reaction mixture was applied to a silica gel column chromatography developed with $CHCl_3$ - MeOH (80 : 20) to yield **16** (413 mg) as a colorless amorphous material. **16**: HRFAB-MS *m/z*: 519.2618 $(M+H)^+$ (calcd for $C_{29}H_{35}N_4O_5$ 519.2607); IR ν_{max} (KBr) cm^{-1}: 3360, 3190, 1740, 1660, 1630, 1510, 1180, 1120, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.47 (3H, m), 1.60 (1H, m), 2.29 (3H, s), 3.00~3.10 (4H, m), 3.87 (1H, m), 4.55 (1H, m), 5.05 (2H, s), 5.10 (2H, s), 5.67 (1H, d, J=6.0 Hz), 6.88 (2H, d, J=8.8 Hz), 7.08 (2H, d, J=8.8 Hz), 7.11 (2H, d, J=8.0 Hz), 7.29~7.44 (11H), 7.47 (2H, d, J=8.0 Hz), 7.90 (1H, d, J=8.0 Hz).

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl]-L-tyrosine p-Toluenesulfonate (**17**)

A solution of **16** (400 mg) in MeOH (100 ml) was subjected to a procedure similar to that for preparing **1**. Chromatography on a column of mega-bond elute (C_{18}) using 60% MeOH as an eluent provided **17** (284.1 mg) as a colorless amorphous material. **17**: HRFAB-MS *m/z*: 339.1686 $(M+H)^+$ (calcd for $C_{15}H_{23}N_4O_5$ 339.1692); IR ν_{max} (KBr) cm^{-1}: 3360, 1740, 1650, 1520, 1240, 1200, 1120, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.47 (3H, m), 1.63 (1H, m), 2.29 (3H, s), 2.88 (1H, dd, J=14.0, 8.0 Hz), 2.95 (1H, dd, J=14.0, 4.8 Hz), 3.09 (2H, m), 3.85 (1H, m), 4.39 (1H, m), 5.66 (1H, d, J=6.0 Hz), 6.64 (2H, d, J=8.0 Hz), 6.97 (2H, d, J=8.0 Hz), 7.11 (2H, d, J=8.0 Hz), 7.41 (1H, m), 7.47 (2H, d, J=8.0 Hz), 7.61 (1H, d, J=8.0 Hz), 9.22 (1H, s).

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl]-3-(4-benzyloxyphenyl)-L-alanine Ethyl Ester p-Toluenesulfonate (**18**)

A solution of 9-Fluorenylmethoxycarbonyl(Fmoc)-3-(4-benzyloxyphenyl)-L-alanine (2.5 g) (purchased from Kokusan chemistry works, Tokyo), ethyl iodide (0.8 g) and $NaHCO_3$ (1 g) in DMF (10 ml) was stirred at room temperature overnight and subsequently filtered. After evaporation of the organic extracts (EtOAc) and crystallization from EtOAc/hexane, there was obtained Fmoc-3-(4-benzyloxyphenyl)-L-alanine ethyl ester (1.99 g) as colorless needle, mp: 120~121°C, EI-MS *m/z*: 521 (M^+).

A solution of Fmoc-3-(4-benzyloxyphenyl)-L-alanine ethyl ester (800 mg) in 20% piperidine was left to stand at room temperature for 7 hours. After evaporation of the organic extracts ($CHCl_3$) and chromatography of a column of silica gel using $CHCl_3$ - MeOH (20 : 1), crude 3-(4-benzyloxyphenyl)-L-alanine ethyl ester was obtained. The addition of p-toluenesulfonic acid (260 mg) and crystallization from water/MeOH provided the p-toluenesulfonate of 3-(4-benzyloxyphenyl)-L-alanine ethyl ester (600.3 mg) as colorless needles, mp: 161°C, FAB-MS *m/z*: 300 $(M+H)^+$.

The p-toluenesulfonate of 3-(4-benzyloxyphenyl)-L-alanine ethyl ester (700 mg), (*R*)-5-guanidino-2-hydroxypentanoic acid (260 mg) and Su-OH (200 mg) were subjected to a procedure similar to that for preparing **16**. Chromatography on a column of silica gel using $CHCl_3$ - MeOH (10 : 1) as an eluent provided 647 mg of **18** as an oilish material. **18**: HRFAB-MS *m/z*: 457.2454 $(M+H)^+$ (calcd for $C_{24}H_{33}N_4O_5$ 457.2451); IR ν_{max} (KBr) cm^{-1}: 3360, 1740, 1660, 1510, 1240, 1180, 1120, 1030, 1010, 680; ^1H NMR ($CDCl_3$): δ 1.12 (3H, t, J=6.4 Hz), 1.66 (3H, m), 1.85 (1H, m), 2.31 (3H, s), 2.94 (2H, m), 3.08 (2H, m), 4.03 (2H, q, J=6.4 Hz), 4.10 (1H, m), 4.63 (1H, q, J=6.8 Hz), 4.92 (2H, s), 5.18 (1H, br), 6.80 (2H, d, J=8.4 Hz), 7.00 (2H, d, J=8.4 Hz), 7.14 (2H, d, J=8.0 Hz), 7.25~7.39 (6H), 7.52 (1H, br d, J=8.0 Hz), 7.68 (2H, d, J=8.0 Hz).

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl]-L-tyrosine Ethyl Ester p-Toluensulfonate (**19**)

A solution of **18** (310 mg) in MeOH (100 ml) was subjected to a procedure similar to that for **2**. Crystallization from MeOH/Et_2O gave **19** (195 mg) as colorless needles. **19**: mp: 153~154°C; HRFAB-MS *m/z*: 367.1973 $(M+H)^+$ (calcd for $C_{17}H_{27}N_4O_5$ 367.1982); IR ν_{max} (KBr) cm^{-1}: 3330, 1740, 1680, 1650, 1620, 1520, 1270, 1190, 1120, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.14 (3H, t, J=7.2 Hz), 1.47 (3H, m), 1.58 (1H, m), 2.29 (3H, s), 2.92 (2H, d, J=6.8 Hz), 3.07 (2H, m), 3.87 (1H, m), 4.06 (2H, m), 4.42 (1H, dt, J=6.8, 8.0 Hz), 5.69 (1H, d, J=7.2 Hz), 6.66 (2H, d, J=8.0 Hz), 6.92 (2H, d, J=8.0 Hz), 7.11 (2H, d, J=8.0 Hz), 7.36 (1H, m), 7.47 (2H, d, J=8.0 Hz), 7.75 (1H, d, J=8.0 Hz), 9.25 (1H, s).

Preparation of *N*-[(*R*)-5-Guanidino-2-acetoxypentanoyl]-3-(4-acetoxyphenyl)-L-alanine Ethyl Ester p-Toluenesulfonate (**20**)

A solution of **19** (180 mg) in acetic anhydride (0.1 ml) and pyridine (10 ml) was subjected to a procedure similar to that for **5**. Crystallization from hexane - EtOH - dichloromethane gave **20** (135 mg) as colorless needles. **20**: mp: 119~121°C; HRFAB-MS *m/z*: 451.2185 $(M+H)^+$ (calcd for $C_{21}H_{31}N_4O_7$ 451.2193); IR ν_{max} (KBr) cm^{-1}: 3330, 3180, 1760, 1740, 1670, 1630, 1560, 1240, 1190, 1130, 1030, 1010, 680; ^1H NMR (DMSO-d_6): δ 1.15 (3H, t, J=6.8 Hz), 1.41 (2H, m), 1.58 (2H, m), 2.06 (3H, s), 2.25 (3H, s), 2.29 (3H, s), 2.94 (1H, dd, J=14.0, 5.6 Hz), 3.05 (3H, m), 4.07 (2H, q, J=6.8 Hz), 4.44 (1H, m), 4.85 (1H, br t, J=6.0 Hz), 7.02 (2H, d, J=8.8 Hz), 7.11 (2H, d, J=8.0 Hz), 7.23 (2H, d, J=8.8 Hz), 7.37 (1H, br t, J=5.6 Hz), 7.47 (2H, d,

$J=8.0$ Hz), 8.46 (1H, d, $J=8.0$ Hz).

Preparation of *N*-[(*S*)-5-Guanidino-2-hydroxypentanoyl)-L-phenylalanine Hydrochloride for the Structural Elucidation of Monamidocin

A solution of **1** (20 mg) in H_2O (5 ml) was adjusted to pH 2.0 with 1 N HCl and applied to a SP-Toyopearl (Na^+ form, course grade) column developed stepwise with water and 0.1 M aq NaCl. The fractions containing **1** were combined and extracted with *n*-BuOH at pH 2.0 adjusted with 1 N HCl. The *n*-BuOH extracts were concentrated to dryness. The concentrate was applied to a LH-20 column developed with MeOH. The MeOH fractions were collected and concentrated to dryness. The crude *N*-[(*S*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine hydrochloride was purified by preparative HPLC over a C_{18} reversed phase column of YMC pack D-ODS-10-A (20 mm i.d. × 250 mm) using 20% MeOH at flow rate of 25 ml/minute to give *N*-[(*S*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine hydrochloride (7.2 mg) as a white powder. *N*-[(*S*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine hydrochloride: $[\alpha]_D^{24}$: 24° (*c* 0.5, MeOH); HPLC retention time 9.0 minutes. Conditions: column Capcell pak C_{18} SG 4.6 mm i.d. × 250 mm, solvent: 7.5% aq acetonitrile, flow rate: 1.0 ml/minute, detection UV 210 nm.

Preparation of *N*-[(*R*)-5-Guanidino-2-hydroxypentanoyl)-L-phenylalanine Hydrochloride for the Structural Elucidation of Monamidocin

N-[(*R*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine hydrochloride (5.2 mg) was also obtained by a procedure similar to that for preparing *N*-[(*S*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine hydrochloride from **9** (20 mg). *N*-[(*R*)-5-guanidino-2-hydroxypentanoyl]-L-phenylalanine hydrochloride: $[\alpha]_D^{24}$: 31° (*c* 0.5, MeOH); HPLC retention time 8.4 minutes. Conditions: see the description for (*S*)-isomer.

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