SYNTHESIS, STEREOCHEMISTRY, AND BIOLOGICAL PROPERTIES OF THE DEPIGMENTING AGENTS, MELANOSTATIN, FELDAMYCIN AND ANALOGS

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(Received for publication July 14, 1990)

Syntheses of melanostatin and feldamycin have been completed from L-serine and L-threonine, respectively, and the configuration of unknown asymmetric carbons determined. Feldamycin analogs have also been prepared and the L-tryptophyl analog was the most potent in the depigmentation of *Streptomyces bikiniensis* and B16 melanoma cells.

In the course of screening for melanin biosynthesis inhibitors using *Streptomyces bikiniensis* NRRL B-1049, BMY-28565 and BMY-28566 were isolated from *Streptomyces calvus* No. N924-1 and *Streptomyces clavifer* No. N924-2, respectively[1,2]. They inhibited melanin synthesis in the growing cells of *S. bikiniensis* and B16 melanoma, but did not show any inhibitory activity on mushroom tyrosinase.

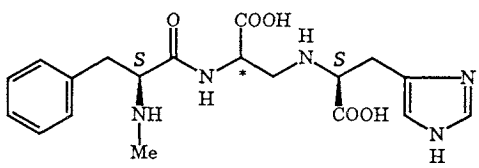
From structural studies BMY-28565 was identical to feldamycin[3,4], reported as an antibacterial antibiotic, whereas BMY-28566 was revealed to be a new compound and named melanostatin[2], which was deduced to have the pseudotripeptide structure **1**. Two of the three asymmetric centers of **1** were determined to have the *S*-configuration, but the stereochemistry of the remaining carbon was still uncertain. The structure of feldamycin (**2**) has been reported[3,4] with no description of the stereochemistry of the four asymmetric carbons in the molecule. Our degradation work indicated that the histidine moiety of **2** has the same *S*-configuration as that of **1**. However, the stereochemistry of the other three carbons remained unknown (Fig. 1).

This report describes the total synthesis and stereochemistry of melanostatin and feldamycin, preparation of the analogs and their inhibitory activity on melanin biosynthesis.

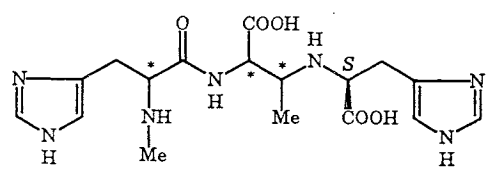
Synthesis of Melanostatin (1) and Feldamycin (2)

The total synthesis of melanostatin has been accomplished from L-serine, according to the procedure shown in Scheme 1.

Fig. 1. Structures of melanostatin (**1**) and feldamycin (**2**).



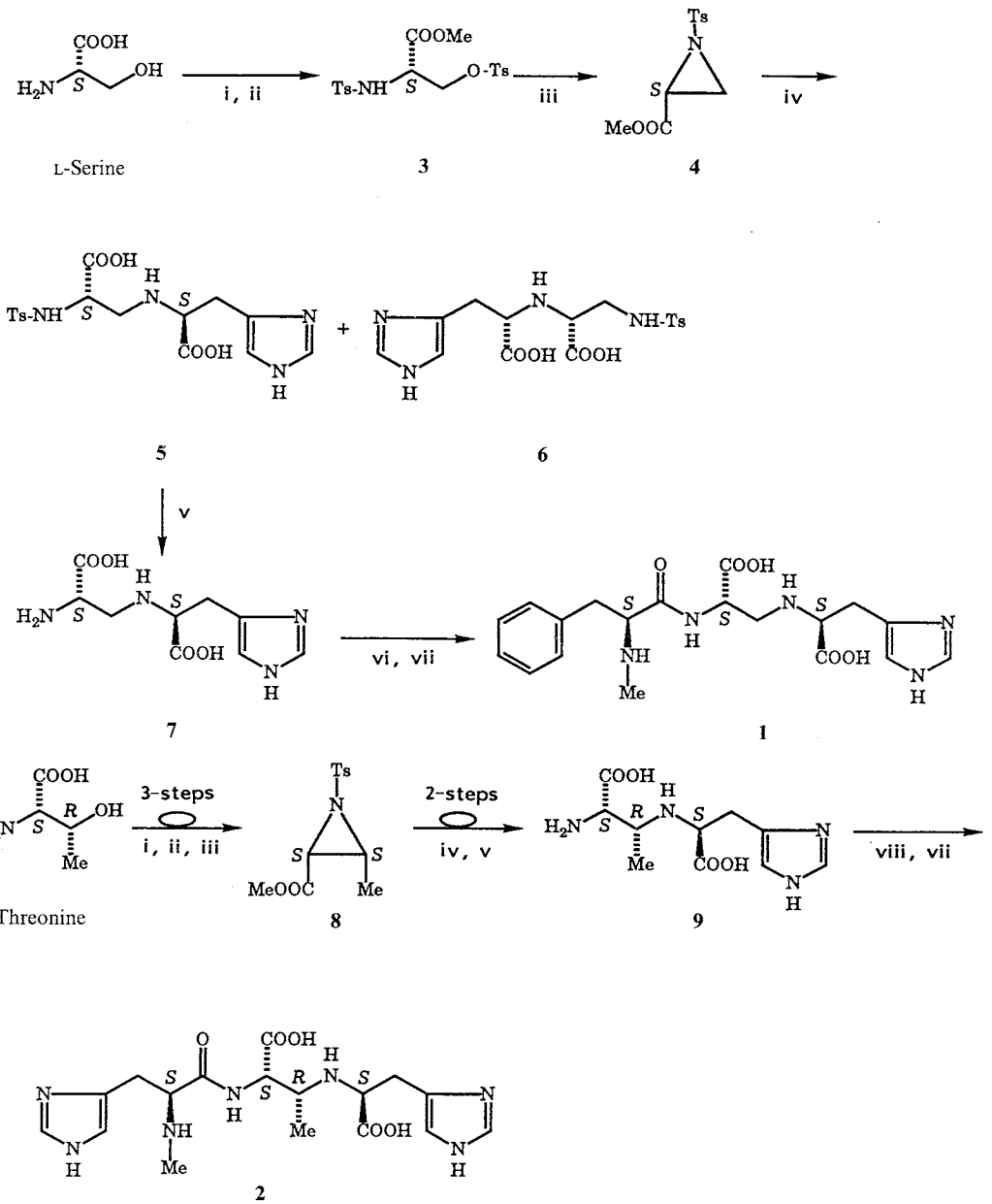
Melanostatin (**1**)



Feldamycin (**2**)

* Stereochemistry unknown.

Scheme 1. Syntheses of melanostatin (**1**) and feldamycin (**2**).



i: MeOH-$SOCl_2$, ii: TsCl-pyridine, iii: Et_3N-MeOH, iv: NaOH-L-His, v: Na-liq NH_3, vi: *N*-BOC-*N*-Me-L-PheOSu-Et_3N, vii: TFA, viii: N^{α},N^{im}-di-BOC-N^{α}-Me-L-His-OSu-Et_3N.

NAKAGAWA *et al.*[5] reported that an attempted cyclization of *N*,*O*-ditosyl-L-serine ethyl ester (**3**, Et ester) to an optically active *N*-tosylaziridine ester (**4**, Et ester) under basic conditions (triethylamine(Et_3N)-THF) resulted in elimination of the *O*-tosyl group to give a dehydroamino ester, although the corresponding *N*-trityl ester[6] gave the *N*-tritylaziridine derivative. NAKAJIMA *et al.*[7] applied this observation to the preparation of optically active *N*-acylaziridine derivatives which were prepared by cyclization of the *N*-trityl derivative followed by detritylation and *N*-acylation. We confirmed that the reaction of **3** with Et_3N in

THF resulted in 1,2-elimination to give a dehydroamino ester as reported in the literature[5], but in the reaction in MeOH, 1,3-elimination of **3** proceeded smoothly to afford the desired *N*-tosylaziridine ester **4** in good yield, although the reason for this observation could not be explained clearly. The nucleophilic ring opening reaction of **4** with L-histidine gave the α,β-diamino acid derivative **5** with a small amount of a regio-isomer (**6**). The desired product **5** was isolated and the *N*-tosyl group removed in the usual manner to give **7**. The acylation of **7** with *N*-methyl-L-phenylalanine active ester followed by deblocking afforded (2*S*)-2-[(2*S*)-2-methylamino-3-phenylpropionyl]amino-3-[(1*S*)-1-carboxy-2-(1*H*-imidazol-4-yl)ethyl]-aminopropionic acid (**1**), which was identical with the natural product[2] (IR, ^{1}H NMR, $[\alpha]_D$ and TLC). This confirmed that the unknown asymmetric carbon of melanostatin was of the *S*-configuration.

The synthesis of feldamycin (**2**) from L-threonine was achieved by a similar sequence. The *N*-tosylaziridine **8**[5] was also obtained by direct cyclization of the corresponding *N*,*O*-ditosyl ester with Et_3N in MeOH and converted to the intermediate **9**[8,9] which retained the configuration of the C-3 asymmetric carbon of L-threonine by a double inversion mechanism[10]. Protected *N*-methyl-L-histidine[11] was coupled with **9** and the acylated product was deblocked to give the pseudotripeptide (**2**), which was then converted to the corresponding hydrochloride (**2**-HCl). The $[\alpha]_D$ values of the synthetic product ($-7.1°$) and its HCl salt ($+11.4°$) were nearly identical to those reported for feldamycin ($-6.6°$ and $+12°$)[3], respectively. Thus, the structure of feldamycin has been established as (2*S*,3*R*)-2-[(2*S*)-2-methylamino-3-(1*H*-imidazol-4-yl)propionyl]amino-3-[(1*S*)-1-carboxy-2-(1*H*-imidazol-4-yl)ethyl]amino-butyric acid (**2**).

Feldamycin Analogs and Their Inhibition of Melanin Biosynthesis

A variety of feldamycin analogs (**10**～**30**) were prepared to characterize the relationship between structure and depigmenting activity. The coupling of **9** with various amino acids was carried out by the active ester method. The products were purified by column chromatography on silica gel and ion exchange resin. The derivatives prepared by this procedure are listed in Table 1 with their depigmenting activity, which was determined by *in vitro* assays using *S. bikiniensis* B-1049 and B16 melanoma cells[1]. The L-asparaginyl derivative (**22**) has been reported[8,9,12] as the immunomodulating agent FR900,490, but its depigmenting activity has never been described.

As shown in Table 1, the inhibitory activity on melanin synthesis was not affected to a large extent by the third amino acid residue (R–CO in Table 1). Little correlation was observed between the activities measured by the two assay methods. The stereochemistry of the amino acid residue (**10** vs. **11**, **16** vs. **17**, **19** vs. **20** and **22** vs. **23**) had little effect on the activity of the diastereoisomers. The L-tryptophan analog (**15**) showed the best activity in both assay systems and was followed by the L- and D-phenylglycine (**16** and **17**), L- and D-asparagine (**22** and **23**), and L-tyrosine (**13**) derivatives.

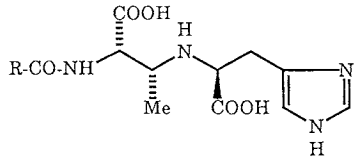
Experimental

IR spectra were measured on an Analect FX-6160 EV spectrometer. ^{1}H NMR spectra were recorded on a Varian FT-80A (80 MHz) or Jeol GX-400 (400 MHz) spectrometer using TMS or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. $[\alpha]_D$ values were recorded on a Jasco DIP-140 digital polarimeter. Mass spectra were obtained with a Jeol JMS-AX 505H mass spectrometer.

(2*S*)-1-Tosyl-2-methoxycarbonylaziridine (**4**)

A mixture of *N*,*O*-bis(tosyl)-L-serine methyl ester (**3**)[7] (12.82 g, 30 mmol) and Et_3N (4.15 ml, 30.5 mmol)

Table 1. Inhibition of melanin biosynthesis by feldamycin analogs (**10**~**30**).



Amino acid residue (R–CO)	Inhibition of melanin synthesis		Amino acid residue (R–CO)	Inhibition of melanin synthesis	
	Streptomyces bikiniensis	B16 melanoma		*Streptomyces bikiniensis*	B16 melanoma
	IC_{50}[a]	MEC[a]		IC_{50}[a]	MEC[a]
Aromatic amino acid:			**22** L-Asn	0.35	12.5~25
10 L-Phe	0.37	100	**23** D-Asn	0.26	25
11 D-Phe	0.84	100	**24** L-Gln	0.33	25
12 *N*-Me-L-Phe	0.23	25~50	Acidic or basic substitution;		
13 L-Tyr	0.35	12.5	**25** L-Asp	1.43	25
14 L-His	0.75	6.3~12.5	**26** L-Lys	0.82	>100
15 L-Try	0.13	6.3	**27** L-Arg	0.58	100
16 L-(Phenyl)Gly	0.21	50	Glycyl derivatives:		
17 D-(Phenyl)Gly	0.28	6.3~12.5	**28** Gly	1.35	25
Non-aromatic amino acid:			**29** Gly-Gly	1.10	25
Lipophilic or neutral substitution;			**30** L-Asn-Gly	1.13	50~100
18 L-Pro	0.72	50	Natural product:		
19 L-Ala	0.54	12.5~50	Melanostatin (**1**)	0.76	50
20 D-Ala	0.96	50	Feldamycin (**2**)	1.29	25
21 L-Thr	0.55	12.5~25			

[a] μg/ml.

in MeOH (60 ml) was heated at reflux for 30 minutes and cooled to room temperature. The resulting mixture was diluted with EtOAc (600 ml) and washed with 10% citric acid, 1 M $NaHCO_3$, and brine. The organic extract was dried and concentrated. The residual oil (8.0 g) was passed through an alumina column (160 g). The eluate with *n*-hexane-EtOAc (4:1~1:1) was concentrated to obtain 3.33 g of **4** as an oily product. Yield 43%. IR ν_{max} (film) cm^{-1} 1745, 1590, 1330, 1230, 1160; ^1H NMR ($CDCl_3$) δ 2.5 (3H, s), 2.6 (2H, m), 3.3 (1H, dd, J=5 and 7 Hz), 3.7 (3H, s), 7.3 (2H, d, J=7 Hz), 7.8 (2H, d, J=7 Hz); $[\alpha]_D^{26}$ −63° (*c* 2.1, MeOH); HRFAB-MS: Calcd for $C_{11}H_{14}NO_4S$ (M+1), *m/z* 256.0644; Found *m/z* 256.0648.

(2*S*)-3-[(1*S*)-1-Carboxy-2-(1*H*-imidazol-4-yl)ethyl]amino-2-tosylaminopropionic Acid (**5**) and Its Regio Isomer (**6**)

A suspension of **4** (3.13 g, 12.3 mmol) in MeOH (12.5 ml) and 1 N NaOH (12.5 ml) was stirred under cooling in an ice bath for 1 hour and to the resulting solution were added L-histidine monohydrochloride hydrate (7.74 g, 36.9 mmol) and NaOH (2.0 g, 50 mmol). The mixture was heated to reflux for 1 hour and then concentrated to dryness. The residual oil was diluted with water (15 ml) and the solution was adjusted to pH 7.0 by adding 2 N H_2SO_4. The resulting crystalline precipitate was filtered off (1.27 g of histidine was recovered) and the filtrate was acidified to pH 4. The acidified solution was adsorbed onto a column of high porous resin Diaion HP-20 (400 ml) which was washed with water and eluted with 50% aq MeOH. The desired fractions were combined and concentrated to obtain 4.7 g of yellow solid, which was subjected to column chromatography (Kieselgel 60, 120 g). Elution with $CHCl_3$-MeOH-conc NH_4OH (5:3:1) was monitored by TLC. The desired fractions were combined and concentrated to afford 2.26 g of **5**. Yield 46%. MP 195~198°C (dec); IR ν_{max} (KBr) cm^{-1} 1640~1610, 1560, 1410, 1370, 1350, 1320, 1160, 1090,

810; ^{1}H NMR (D_2O) δ 2.40 (3H, s), 2.7~2.9 (4H, m), 3.36 (1H, t, J=6.7 Hz), 3.74 (1H, dd, J=4.4 and 8.7 Hz), 6.92 (1H, s), 7.40 (2H, d, J=7.9 Hz), 7.73 (2H, d, J=7.9 Hz), 7.78 (1H, s); ^{13}C NMR (D_2O) δ 21.5 (q), 29.99 (t), 49.8 (t) 58.1 (d), 63.7 (d), 118.6 (d), 127.7 (Ph), 130.8 (Ph), 136.3 (d). HRFAB-MS: Calcd for $C_{16}H_{21}N_4O_6S$ (M+1), m/z 397.1182; Found m/z 397.1180.

The slower moving fractions gave 669 mg of the regio isomer (**6**). Yield 14%. MP 232~239°C (dec); IR ν_{max} (KBr) cm^{-1} 1630, 1505, 1455, 1395, 1355, 1315, 1155, 1090, 835, 810; ^{1}H NMR (D_2O) δ 2.39 (3H, s), 2.85 (1H, dd, J=6.8 and 15.1 Hz), 2.92 (1H, dd, J=5.7 and 15.1 Hz), 3.09 (2H, d, J=5.1 Hz), 3.21 (1H, t, J=5.1 Hz), 3.38 (1H, dd, J=6.8 and 5.7 Hz), 6.96 (1H, s), 7.40 (2H, d, J=8.1 Hz), 7.68 (2H, d, J=8.4 Hz), 7.78 (1H, s); ^{13}C NMR (D_2O) δ 21.5 (q), 29.95 (t), 44.9 (t), 61.5 (d), 62.4 (d), 118.9 (d), 127.6 (Ph), 130.9 (Ph), 136.25 (d). HRFAB-MS: Calcd for $C_{16}H_{21}N_4O_6S$ (M+1), m/z 397.1182; Found m/z 397.1170.

(*S*)-2-Amino-3-[(1*S*)-1-carboxy-2-(1*H*-imidazol-4-yl)ethyl]aminopropionic Acid (**7**)

To a stirred mixture of **5** (1.83 g, 4.62 mmol) in liq ammonia (20 ml) was added Na metal (940 mg, 40.9 m atom) in small pieces. The resulting deep blue mixture was stirred for 1 hour at −78°C and then quenched by the addition of NH_4Cl (2.55 g, 48 mmol). Ammonia was removed on standing under atmospheric pressure for 2 hours. The residual solid was dissolved in water (20 ml), adjusted to pH 4 by the addition of 1 N HCl and was passed through an Amberlite IR-120 column (H^+ type, 100 ml). After being washed with water, the column was eluted with 2.8% NH_4OH. The desired fractions were combined, concentrated to dryness, and the residue was lyophilized to afford 1.01 g of amorphous powder, which was crystallized from aq MeOH to give 654 mg of white powder **7**. Yield 58%. MP 232~235°C (dec); IR ν_{max} (KBr) cm^{-1} 1633~1614, 1582, 1440, 1400, 1367, 840; ^{1}H NMR (D_2O+NaOD) δ 2.55 (1H, dd, J=8.8 and 12.1 Hz), 2.71 (1H, dd, J=4.4 and 11.7 Hz), 2.86 (1H, d, J=6.6 Hz), 2.87 (1H, d, J=7.3 Hz), 3.31 (1H, t, J=6.6 Hz), 3.31 (1H, dd, J=4.4 and 8.4 Hz), 6.88 (1H, s), 7.64 (1H, s).

Anal Calcd for $C_9H_{14}N_4O_4 \cdot \frac{1}{2}H_2O$: C 43.97, H 5.90, N 22.79.
Found: C 43.66, H 5.92, N 23.17.

(2*S*)-2-[(2*S*)-2-Methylamino-3-phenylpropanoyl]amino-3-[(1*S*)-1-carboxy-2-(1*H*-imidazol-4-yl)-ethyl]aminopropionic Acid (Melanostatin) (**1**)

To a stirred solution of **7** (100 mg, 0.41 mmol) and Et_3N (172 μl, 1.24 mmol) in 50% aq dioxane (4 ml) was added *N*-hydroxysuccinimide ester of *N*-*tert*-BOC-*N*-methyl-L-phenylalanine (300 mg, 0.82 mmol). The mixture was stirred overnight, adjusted to pH 5~6 by 1 N HCl, and concentrated to dryness. The residue was subjected to column chromatography (Kieselgel 60, 30 g). The column was eluted with BuOH - AcOH - water (4 : 1 : 2) and the desired fraction was concentrated to obtain an oily residue (*ca.* 600 mg), which was diluted with TFA (4 ml). The solution was stirred for 2.5 hours at room temperature and evaporated. The residue was diluted with a small amount of water and passed through a column of Amberlite IR-120 (H^+ type, *ca.* 50 ml). After washing with water, the column was eluted with 2.8% NH_4OH and the desired fractions were combined and concentrated. The residue was subjected to column chromatography (Kieselgel 60, 50 g). The column was eluted with BuOH - AcOH - water with ratios of 5 : 1 : 2, 4 : 1 : 2, and 3 : 1 : 2, successively. The desired fractions were combined, concentrated, and the residue was further purified on a column of Kieselgel 60 (10 g) eluted with BuOH - AcOH - water (5 : 1 : 2 and 4 : 1 : 2). The desired fractions were combined and concentrated. The residue was diluted with a small amount of water and passed through a column of Amberlite IR-120 (20 ml), which was washed with water and eluted with 2.8% NH_4OH. The desired fractions were concentrated and lyophilized to obtain 58 mg of **1** as an amorphous powder. Yield 35%. MP 165~170°C (dec); HRFAB-MS: Calcd for $C_{19}H_{26}N_5O_5$ (M+1), m/z 404.1934; Found m/z 404.1930. Its physical properties (IR, ^{1}H NMR and $[\alpha]_D$) were identical with those of melanostatin (**1**)[2].

(2*S*,3*S*)-1-Tosyl-2-methoxycarbonyl-3-methylaziridine (**8**)

A mixture of *N*,*O*-ditosyl-L-threonine methyl ester[9] (93 g, 0.21 mol) and Et_3N (29.3 ml, 0.21 mol) in MeOH (450 ml) was refluxed for 1.5 hours and then evaporated to dryness. The residue was diluted with EtOAc and washed with water. The organic extract was dried, concentrated, and the residual oil was diluted with isopropyl ether. The crystalline precipitate was collected by filtration to obtain 37.2 g of **8**,

as colorless crystals. Yield 66%. MP 59~60°C; IR ν_{max} (KBr) cm^{-1} 2880, 1735; ^1H NMR ($CDCl_3$) δ 1.33 (3H, d, J=5.5 Hz), 2.47 (3H, s), 3.20 (2H, m), 3.70 (3H, s), 7.4~8.1 (4H, m). $[\alpha]_D^{26}$ −41.0° (*c* 2.1, MeOH) (literature[5]) $[\alpha]_D^{23}$ −40.2° (*c* 2.0, MeOH)).

(2*R*,3*R*)-2-Amino-3-[(1*S*)-1-carboxy-2-(1*H*-imidazol-4-yl)ethyl]aminobutyric Acid (**9**)

A suspension of **8** (51.15 g, 0.19 mol) in MeOH (200 ml) and 1 N NaOH (190 ml) was stirred at room temperature for 30 minutes and to the resulting solution were added L-histidine monohydrochloride hydrate (145 g, 0.69 mol) and NaOH (64.75 g, 1.62 mol) in MeOH (36.5 ml) and water (50 ml). The mixture was heated to reflux for 2 hours and was concentrated to dryness. The residual oil was diluted with water, and pH of the solution was adjusted to 5.0 by adding 2 N H_2SO_4. The resulting crystalline precipitate was collected by filtration, and washed with water and MeOH to obtain 35.0 g of the ring-opened product (yield 45%). MP 187~190°C (the regio isomer was isolated from the mother liquor).

To a stirred mixture of the above intermediate (34.6 g, 84 mmol) in liq NH_3 (350 ml) was added sodium metal (15.9 g, 0.69 m atom) in small pieces, and the resulting deep blue mixture was stirred for 40 minutes at −78°C. The reaction was quenched by the addition of NH_4Cl, and NH_3 was removed on standing under atmospheric pressure. The residual solid was dissolved in water, adjusted to pH 4 by the addition of 2 N H_2SO_4 and passed through an Amberlite IR-120 column. After being washed with water, the column was eluted with 2.8% NH_4OH. The desired fractions were combined and concentrated to dryness and the residue was crystallized from MeOH to afford 6.71 g of **9** as a colorless powder. Yield 31%. MP 220~225°C. IR ν_{max} (KBr) cm^{-1} 2995, 2850, 1620, 1580. ^1H NMR (D_2O+NaOD) δ 1.13 (3H, d, J=7 Hz), 2.6~4.3 (5H, m), 7.06 (1H, s), 7.81 (1H, d, J=1 Hz).

(2*S*,3*R*)-2-[(2*S*)-2-Methylamino-3-(1*H*-imidazol-4-yl)propionyl]amino-3-[(1*S*)-1-carboxy-2-(1*H*-imidazol-4-yl)ethyl]aminobutyric Acid (Feldamycin) (**2**)

To a stirred solution of **9** (319 mg, 1.24 mmol) and Et_3N (455 mg, 4.5 mmol) in water (4.5 ml) was added a solution of *N*-hydroxysuccinimide ester of *N*,1-bis-BOC-*N*-methyl-L-histidine (1.0 g, 2.14 mmol) in dioxane (9 ml). The mixture was stirred at room temperature overnight and then concentrated to dryness. The residue was dissolved in a small volume of BuOH - H_2O - AcOH (4 : 1 : 2) and purified on a column of Kieselgel 60 (30 g) eluted with the same solvent (500 ml). The eluate was fractionated and desired fractions (Nos. 4~10) combined and concentrated to afford 1.35 g of oily residue, which was diluted with TFA (4 ml). The resulting solution was left at room temperature for 1 hour and then concentrated to dryness. The residue was dissolved with a small volume of water, concentrated again, and diluted with water (20 ml). The solution was passed through a column of Amberlite IR-120 (H^+ type, 5 ml), and after washing with water, the column was eluted with 1 N NH_4OH and the eluate was fractionated. The desired fractions (Nos. 5 and 6) were combined and concentrated to obtain 325 mg of amorphous powder, which was dissolved with BuOH - AcOH - H_2O (4 : 1 : 2), and was subjected to column chromatography on Kieselgel 60 (20 g). The column was successively eluted with BuOH - AcOH - H_2O (4 : 1 : 2, 150 ml; 3 : 1 : 2, 200 ml; and 2 : 1 : 2, 150 ml), and collected in 15 ml fractions. The desired fractions (Nos. 11~16) were combined and concentrated. The residue was dissolved in a small amount of water and passed through a column of Amberlite IR-120 (H^+ type, 5 ml). After washing with water, the column was eluted with 1 N NH_4OH. The desired fraction (No. 6) was concentrated and lyophilized to give 118 mg of **2**, as an amorphous powder. Yield 23%. MP 160~162°C; IR ν_{max} (KBr) cm^{-1} 1623, 1389; $[\alpha]_D^{24}$ −7.1° (*c* 1.0, H_2O), +24° (*c* 1.08, 0.1 N HCl) (literature[3]) $[\alpha]_D^{25}$ −6.6° (*c* 1, H_2O)); ^1H NMR (D_2O) δ 1.10 (3H, d, J=7 Hz), 2.11 (3H, s), 3.17 (2H, m), 3.35 (1H, dq, J=5 and 7 Hz), 3.90 (1H, dd, J=6 and 8 Hz), 3.99 (1H, t-like, J=6 Hz), 4.30 (1H, d, J=5 Hz), 7.05 (2H, d, J=1 Hz), 7.88 (2H, d, J=1 Hz). HRFAB-MS: Calcd for $C_{17}H_{26}N_7O_5$ (M+1), *m/z* 408.1995; Found, *m/z* 408.1989.

Preparation of Feldamycin Hydrochloride (**2**-HCl)

A solution of **2** (30 mg, 0.074 mmol) in 50% MeOH (0.1 ml) was diluted with 4 N HCl in MeOH (0.2 ml, 0.8 mmol). The solution was added dropwise to stirred acetone (20 ml) and the precipitate was collected by filtration, washed with acetone and dried to obtain 31 mg of **2** hydrochloride as a hygroscopic white powder. IR ν_{max} (KBr) cm^{-1} 1687, 1656, 1623, 1556; $[\alpha]_D^{25}$ +11.4° (*c* 1.0, H_2O), (literature[3]) $[\alpha]_D^{25}$ +12° (*c* 1, H_2O)).

Anal Calcd for $C_{17}H_{25}N_7O_5 \cdot 3HCl \cdot 3H_2O$: C 35.77, H 6.00, N 17.18, Cl 18.63.
Found: C 35.68, H 5.62, N 17.68, Cl 18.27.

Preparation of Feldamycin Analogs (**10**~**30**)

Procedure for preparation of **10**~**30** in Table 1 is fundamentally the same as that described for feldamycin above. Analytical data of the compounds are shown below. The yield is indicated as isolated yield from **8**.

L-Phenylalanyl Analog (**10**)

Yield 33%. MP 180°C; IR ν_{max} (KBr) cm^{-1} 1623, 1386; ^1H NMR (D_2O+NaOD) δ 1.02 (3H, d, J=6.5 Hz), 2.9 (5H, m), 3.48 (1H, t-like, J=7 Hz), 3.63 (1H, t-like, J=6 Hz), 3.93 (1H, d, J=8 Hz), 6.90 (1H, s), 7.35 (5H, m), 7.65 (1H, s); HRFAB-MS: Calcd for $C_{19}H_{26}N_5O_5$ (M+1), m/z 404.1934; Found, m/z 404.1926.

Anal Calcd for $C_{19}H_{25}N_5O_5 \cdot 1\frac{1}{2}H_2O$: C 53.02, H 6.56, N 16.27.
Found: C 53.36, H 6.50, N 16.84.

D-Phenylalanyl Analog (**11**)

Yield 43%. MP 174~176°C (dec); IR ν_{max} (KBr) cm^{-1} 1623, 1384; ^1H NMR (D_2O) δ 0.75 (3H, d, J=7 Hz), 2.8~3.5 (5H, m), 3.94 (1H, dd, J=5 and 9 Hz), 4.26 (1H, dd, J=7 and 10 Hz), 4.53 (1H, d, J=4 Hz), 7.21 (1H, s), 7.43 (5H, m), 8.01 (1H, d, J=1 Hz); HRFAB-MS: Calcd for $C_{19}H_{26}N_5O_5$ (M+1), m/z 404.1934; Found, m/z 404.1947.

Anal Calcd for $C_{19}H_{25}N_5O_5 \cdot 1\frac{1}{2}H_2O$: C 53.02, H 6.56, N 16.27.
Found: C 52.68, H 6.40, N 16.32.

N-Methyl-L-phenylalanyl Analog (**12**)

Yield 56%. MP 178~183°C; IR ν_{max} (KBr) cm^{-1} 1630, 1390; ^1H NMR (D_2O+NaOD) δ 1.05 (3H, d, J=7 Hz), 2.40 (3H, s), 7.03 (1H, s), 7.75 (1H, s); HRFAB-MS: Calcd for $C_{20}H_{28}N_5O_5$ (M+1), m/z 418.2091; Found, m/z 418.2097.

L-Tyrosyl Analog (**13**)

Yield 17%. MP 216~218°C; IR ν_{max} (KBr) cm^{-1} 1653, 1623, 1617, 1516, 1399, 1250; ^1H NMR (D_2O) δ 1.04 (3H, d, J=7 Hz), 2.9~3.4 (5H, m), 3.7~4.05 (2H, m), 4.28 (1H, d, J=7 Hz), 6.90 (2H, m) 7.13 (1H, s), 7.18 (2H, m), 7.86 (1H, d, J=1 Hz); HRFAB-MS: Calcd for $C_{19}H_{26}N_5O_6$ (M+1), m/z 420.1883; Found, m/z 420.1893; Calcd for $C_{19}H_{25}N_5O_6Na$ (M+Na), m/z 442.1705; Found, m/z 442.1705.

Anal Calcd for $C_{19}H_{25}N_5O_6 \cdot H_2CO_3 \cdot 1\frac{1}{2}H_2O$: C 47.24, H 5.95, N 13.77.
Found: C 47.46, H 5.69, N 13.62.

L-Histidyl Analog (**14**)

Yield 30%. MP >175°C; IR ν_{max} (KBr) cm^{-1} 1623, 1386; ^1H NMR (D_2O) δ 1.10 (3H, d, J=7 Hz), 3.15 (2H, m), 3.43 (1H, dq, J=5 and 7 Hz), 3.91 (1H, dd, J=6 and 8 Hz), 4.12 (1H, t, J=7 Hz), 4.33 (1H, d, J=5 Hz), 7.10 (2H, s), 7.95 (2H, s); HRFAB-MS: Calcd for $C_{16}H_{24}N_7O_5$ (M+1), m/z 394.1839; Found, m/z 394.1839.

Anal Calcd for $C_{16}H_{23}N_7O_5 \cdot 1\frac{7}{10}H_2O$: C 45.32, H 6.28, N 23.12.
Found: C 45.26, H 6.15, N 23.56.

L-Tryptophyl Analog (**15**)

Yield 31%. MP >184°C (dec); IR ν_{max} (KBr) cm^{-1} 1628, 1384; ^1H NMR (D_2O) δ 1.05 (3H, d, J=7 Hz), 3.25 (2H, m), 3.50 (3H, m), 3.96 (1H, dd, J=6 and 8 Hz), 4.31 (1H, d, J=5 Hz), 4.37 (1H, t-like, J=8 Hz), 7.20 (1H, s), 7.2~7.8 (5H, m), 8.00 (1H, d, J=1 Hz); HRFAB-MS: Calcd for $C_{21}H_{27}N_6O_5$ (M+1), m/z 443.2043; Found, m/z 443.2031.

Anal Calcd for $C_{21}H_{26}N_6O_5 \cdot H_2CO_3$: C 52.38, H 5.94, N 16.66.
Found: C 51.87, H 6.10, N 16.87.

L-Phenylglycyl Analog (**16**)

Yield 58%. MP 182~185°C; IR ν_{max} (KBr) cm^{-1} 1628, 1389; ^1H NMR (D_2O+NaOD) δ 1.05 (3H, d, J=8 Hz), 2.9 (3H, m), 3.5 (1H, t, J=6.5 Hz), 4.0 (1H, d, J=8.5 Hz), 4.65 (1H, s), 6.9 (1H, d, J=<1 Hz), 7.45 (5H, s), 7.65 (1H, d, J=<1 Hz).

Anal Calcd for $C_{18}H_{23}N_5O_5 \cdot 2\frac{1}{2}H_2O$: C 49.76, H 6.50, N 16.12.
Found: C 49.53, H 6.10, N 16.51.

D-Phenylglycyl Analog (**17**)

Yield 51%. MP 198~200°C; IR ν_{max} (KBr) cm^{-1} 1672, 1633, 1499, 1394; ^1H NMR (D_2O+NaOD) δ 0.95 (3H, d, J=8 Hz), 3.0~3.5 (3H, m), 3.95 (1H, dd, J=6 and 9 Hz), 4.6 (1H, d, J=5.5 Hz), 5.25 (1H, s), 7.18 (1H, s), 7.6 (5H, s), 8.00 (1H, s).

Anal Calcd for $C_{18}H_{23}N_5O_5 \cdot 2H_2O$: C 50.81, H 6.40, N 16.46.
Found: C 50.59, H 6.01, N 16.75.

L-Prolyl Analog (**18**)

Yield 36%. MP 173~177°C; IR ν_{max} (KBr) cm^{-1} 3397, 3233, 2883, 1627, 1489, 1385, 1262; ^1H NMR (D_2O) δ 1.15 (3H, d, J=7 Hz), 1.5~2.5 (4H, m), 3.17~3.43 (5H, m), 3.85 (1H, t, J=7 Hz), 4.30 (1H, d, J=7 Hz), 4.35 (1H, m), 7.1 (1H, s), 7.9 (1H, s).

Anal Calcd for $C_{15}H_{23}N_5O_5 \cdot 1\frac{1}{2}H_2O$: C 47.36, H 6.89, N 18.41.
Found: C 47.18, H 6.90, N 18.70.

L-Alanyl Analog (**19**)

Yield 76%. MP 182~188°C; IR ν_{max} (KBr) cm^{-1} 1630, 1385; ^1H NMR (D_2O+NaOD) δ 1.10 (3H, d, J=8 Hz), 1.32 (3H, d, J=8 Hz), 2.9 (3H, s), 3.53 (2H, m), 3.98 (1H, d, J=8 Hz), 6.92 (1H, s), 7.69 (1H, s); HRFAB-MS: Calcd for $C_{13}H_{22}N_5O_5$ (M+1), m/z 328.1621; Found, m/z 328.1637.

D-Alanyl Analog (**20**)

Yield 41%. MP >181°C (dec); IR ν_{max} (KBr) cm^{-1} 1629, 1389; ^1H NMR (D_2O) δ 1.26 (3H, d, J=7 Hz), 1.62 (3H, d, J=7 Hz), 3.3 (2H, m), 3.53 (1H, dq, J=5 and 7 Hz), 4.05 (1H, dd, J=5.5 and 8 Hz), 4.22 (1H, q, J=7 Hz), 4.56 (1H, d, J=5 Hz), 7.21 (1H, s), 8.02 (1H, s); HRFAB-MS: Calcd for $C_{13}H_{22}N_5O_5$ (M+1), m/z 328.1621; Found, m/z 328.1621.

Anal Calcd for $C_{13}H_{21}N_5O_5 \cdot H_2O \cdot \frac{1}{2}H_2CO_3$: C 43.08, H 6.43, N 18.61.
Found: C 43.02, H 6.61, N 18.61.

L-Threonyl Analog (**21**)

Yield 23%. MP 165~170°C; IR ν_{max} (KBr) cm^{-1} 3400, 1628, 1452; ^1H NMR (D_2O) δ 1.20 (3H, d, J=8 Hz), 1.35 (3H, d, J=8 Hz), 3.2 (2H, m), 3.5~4.0 (1H, m), 4.0~4.5 (2H, m), 4.6 (1H, d, J=8 Hz), 7.2 (1H, d, J=<1 Hz), 8.19 (1H, d, J=<1 Hz). HRFAB-MS: Calcd for $C_{14}H_{24}N_5O_6$ (M+1), m/z 358.1727; Found, m/z 358.1712.

L-Asparaginyl Analog (**22**)

Yield 83%. HRFAB-MS: Calcd for $C_{14}H_{23}N_6O_6$ (M+1), m/z 371.1679; Found, m/z 371.1669. The IR spectrum was identical to that of the authentic sample kindly provided by Dr. KOHSAKA of Fujisawa Pharmaceutical Co., Ltd. The ^1H NMR spectrum was also identical with the reported one[12].

D-Asparaginyl Analog (**23**)

Yield 42%. MP 185~190°C; IR ν_{max} cm^{-1} 3366, 1623, 1396; ^1H NMR (D_2O) δ 1.15 (3H, d, J=6.5 Hz), 2.65 (2H, m), 3.0~3.5 (3H, m), 3.5~4.0 (2H, m), 4.25 (1H, d, J=6.5 Hz), 7.1 (1H, s), 7.85 (1H, s). HRFAB-MS: Calcd for $C_{14}H_{23}N_6O_6$ (M+1), m/z 371.1679; Found, m/z 371.1670.

L-Glutaminyl Analog (**24**)

Yield 80%. MP 182~186°C; IR ν_{max} (KBr) cm^{-1} 1675, 1625; ^1H NMR (D_2O+NaOD) δ 1.10 (3H, d, J=6 Hz), 1.7~2.6 (4H, m), 2.93 (3H, m), 3.50 (2H, m), 3.95 (1H, d, J=8 Hz), 6.93 (1H, s), 7.68 (1H, s); HRFAB-MS: Calcd for $C_{15}H_{25}N_6O_6$ (M+1), m/z 385.1836; Found, m/z 385.1839.

Anal Calcd for $C_{15}H_{24}N_6O_6 \cdot \frac{3}{4}H_2O$: C 45.31, H 6.78, N 21.18.
Found: C 45.28, H 6.46, N 21.12.

L-α-Aspartyl Analog (**25**)

Yield 23%. MP 180~185°C; IR ν_{max} (KBr) cm^{-1} 1661, 1651, 1646, 1615, 1558, 1504, 1394; ^1H NMR (D_2O) δ 1.32 (3H, d, J=7 Hz), 2.75~3.20 (2H, m), 3.25~3.50 (2H, m), 3.55~3.90 (1H, m), 4.0~4.50 (1H, m), 4.60 (1H, d, J=5 Hz), 7.38 (1H, s), 8.44 (1H, s); HRFAB-MS: Calcd for $C_{14}H_{22}N_5O_7$ (M+1), *m/z* 372.1519; Found, *m/z* 372.1515.

Anal Calcd for $C_{14}H_{21}N_5O_7 \cdot \frac{1}{2}H_2O \cdot \frac{1}{2}NH_4OH$: C 42.26, H 6.21, N 19.36.
Found: C 42.04, H 6.34, N 19.50.

L-Lysyl Analog (**26**)

Yield 57%. MP 170~175°C; IR ν_{max} (KBr) cm^{-1} 1654, 1636, 1577, 1540, 1458, 1389; ^1H NMR (D_2O) δ 1.10 (3H, d, J=7 Hz), 1.3~2.0 (6H, m), 2.9~3.4 (5H, m), 3.65~4.0 (2H, m), 4.26 (1H, d, J=7 Hz), 7.10 (1H, d, J=1 Hz), 7.83 (1H, d, J=1 Hz).

Anal Calcd for $C_{16}H_{28}N_6O_5 \cdot H_2O$: C 47.75, H 7.51, N 20.88.
Found: C 47.36, H 7.81, N 20.87.

L-Arginyl Analog (**27**)

Yield 43%. MP 206~209°C; IR ν_{max} (KBr) cm^{-1} 1739, 1667, 1654, 1623, 1558, 1404; ^1H NMR (D_2O) δ 1.46 (3H, d, J=7 Hz), 1.6~2.2 (4H, m), 3.33 (2H, t, J=8 Hz), 3.55 (2H, d, J=8 Hz), 3.65~3.90 (1H, m), 4.15~4.5 (2H, m), 4.98 (1H, d, J=5 Hz), 7.51 (1H, d, J=1.5 Hz), 8.76 (1H, d, J=1.5 Hz); HRFAB-MS: Calcd for $C_{16}H_{29}N_8O_5$ (M+1), *m/z* 413.2261; Found, *m/z* 413.2244.

Glycyl Analog (**28**)

Yield 71%. MP >182°C (dec); IR ν_{max} (KBr) cm^{-1} 1628, 1386; ^1H NMR (D_2O) δ 1.25 (3H, d, J=7 Hz), 3.3 (2H, m), 3.58 (1H, dq, J=5 and 7 Hz), 3.98 (2H, s), 4.03 (1H, m), 4.58 (1H, d, J=5 Hz), 7.21 (1H, m), 8.02 (1H, s); HRFAB-MS: Calcd for $C_{12}H_{20}N_5O_5$ (M+1), *m/z* 314.1465; Found, *m/z* 314.1470.

Glycylglycyl Analog (**29**)

Yield 72%. MP >171°C (dec); IR ν_{max} (KBr) cm^{-1} 1628, 1394; ^1H NMR (D_2O) δ 1.26 (3H, d, J=7 Hz), 3.3 (2H, m), 3.59 (1H, dq, J=5 and 7 Hz), 4.01 (2H, s), 4.11 (1H, m), 4.16 (2H, m), 4.53 (1H, d, J=5 Hz), 7.22 (1H, s), 8.04 (1H, s); HRFAB-MS: Calcd for $C_{14}H_{23}N_6O_6$ (M+1), *m/z* 371.1679; Found, *m/z* 371.1659.

L-Asparaginylglycyl Analog (**30**)

Yield 51%. MP >166°C (dec); IR ν_{max} (KBr) cm^{-1} 1628, 1396; ^1H NMR (D_2O) δ 1.26 (3H, d, J=7 Hz), 3.0 (2H, m), 3.33 (2H, m), 3.62 (1H, m), 4.13 (3H, m), 4.45 (1H, d, J=6 Hz), 4.53 (1H, d, J=5 Hz), 7.25 (1H, s), 8.14 (1H, s); HRFAB-MS: Calcd for $C_{16}H_{26}N_7O_7$ (M+1), *m/z* 428.1894; Found, *m/z* 428.1909.

Determination of The Inhibition of Melanin Synthesis

(1) Inhibition of melanin synthesis in *S. bikiniensis* B-1049 is indicated in terms of IC_{50} value, determined as follows: Suspended cell spores of *S. bikiniensis* B-1049 with the vegetative medium (ISP-7 supplemented with 0.2% yeast extract, inoculum size of 1.4×10^6 cfu/ml) were cultured in the presence of 0.4% KNO_3 and varying concentrations of the test compound in a total volume of 1 ml at 28°C for 18 hours. The percent inhibition was estimated by reading the OD at 450 nm of the supernatant of the cultures, and the IC_{50} value was calculated from the % inhibition (of at least 8 serial concentrations of an inhibitor). Data are a mean of duplicate runs.

(2) Inhibitory activity of melanin synthesis in B-16 melanoma is indicated in terms of minimum effective concentration (MEC) value, which was determined as follows: Test compound solution (0.4 ml) and B-16 melanoma cells (3×10^3 cells/ml, 3.6 ml) in EAGLE's minimum essential medium supplemented

with 10% fetal calf serum were incubated for 6 days at 37°C. During the incubation, the culture medium was once renewed with fresh medium containing the same compound solution. After incubation, cells were counted and solubilized with a solution of 1 N NaOH and 10% DMSO (1 : 1). The amount of melanin synthesized was colorimetrically measured at 470 nm, and the inhibition of melanin synthesis was determined from these OD values compared to those of control runs (the vehicle group). The MEC values are expressed as the concentration showing 50% inhibition.

Acknowledgments

The authors thank Dr. M. KOHSAKA of Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd. for the sample of FR-900,490. They also wish to thank Prof. H. KAKISAWA of the Tsukuba University and Dr. H. KAWAGUCHI, the ex-president of this Institute for valuable discussion.

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