New Anticancer Antibiotics Pelagiomicins, Produced by a New Marine Bacterium *Pelagiobacter variabilis*

NOBUTAKA IMAMURA, MIYUKI NISHIJIMA, TAKAHIDE TAKADERA, KYOKO ADACHI, MIHO SAKAI and HIROSHI SANO

Marine Biotechnology Institute, Shimizu Laboratories,
1900 Sodeshi, Shimizu, Shizuoka 424, Japan

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In the course of our screening for new anticancer compounds produced by marine bacteria, we found that a new genus marine bacterium *Pelagiobacter variabilis* produced new phenazine antibiotics, pelagiomicins A, B and C. Those compounds were labile in water and alcohols. The absolute structure of the main component, pelagiomicin A, and the structures of the minor ones were determined from the spectroscopic data and by synthesis. Pelagiomicin A exhibits activity against Gram-positive and -negative bacteria and antitumor activity *in vitro* and *in vivo*.

There are a few studies of products of marine microorganisms compared with terrestrial ones. Marine microorganisms live in a quite different environment from terrestrial ones, and would be expected to produce compounds which possess unique structures and activities. The investigation of metabolites of marine bacteria ought to be one of the important fields of natural products studies, and should draw increasing attention[1].

In the course of our screening for new anticancer compounds from marine bacteria, we found new antibiotics produced by a new marine bacterium. In this paper, we will describe the fermentation of the producer, isolation, and structural determination and the total syntheses of the new antibiotics, the pelagiomicins.

Fermentation and Isolation of Pelagiomicins

The producer, a halophilic Gram-negative bacterium *Pelagiobacter variabilis* was isolated from a macro alga *Pocockiella variegata* collected at Palau. The taxonomy of this strain was investigated and the results indicated that the strain should be classified as a new genus[2] and was named *Pelagiobacter variabilis*.

The fermentation was carried out in the medium of Marine broth (Diffco) at 30°C for 24 hours with rotary shaking. The filtrate (2 liters) was concentrated *in vacuo* below 30°C, washed with EtOAc and then extracted with $CHCl_3$. The extract was concentrated to dryness, and the residue was dissolved in phosphate buffer (pH 7.0, 0.01 M). The solution was applied to a preparative HPLC (Shisei-do, Capcellpack-CN, i.d. 15 mm × 250 mm) with a gradient elution with CH_3CN in phosphate buffer (pH 7). Four active fractions were obtained. Each active fraction was separately extracted with $CHCl_3$. The extracts were evaporated *in vacuo* to give fine red needles (**1**; 10 mg), reddish-orange needles (**2**; 10 mg), and reddish-orange amorphous solids (**3**; 1 mg and **4**; 1 mg).

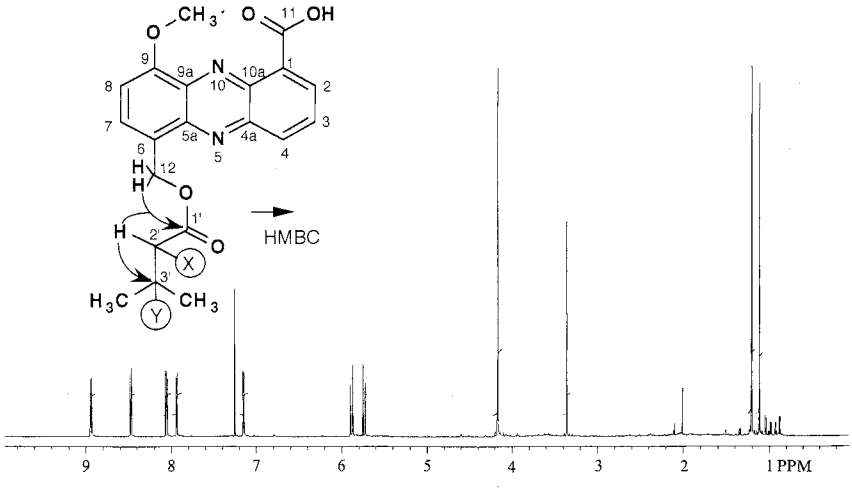
Structural Determination of Pelagiomicin A (2)

The compound **1** was identified to be griseoluteic acid[3] by ^1H NMR, ^{13}C NMR, FAB-MS and UV data. The physico-chemical properties of **2** are summerized in Table 1. The compound **2** was unstable in water and alcohols. The ^{13}C NMR and HR-FAB-MS data, indicated a molecular formula of $C_{20}H_{21}N_3O_6$. Since the UV spectrum of **2** could be superimposed with that of **1**, the existence of the same chromophore in **2** was suggested. In the ^1H NMR spectrum of **2** (Fig. 1), the signals attributed to the griseoluteic acid part as well as extra signals for two methyls (1.12 (s, 3H) and 1.21 (s, 3H) ppm) and a methine (3.36 (s, 3H) ppm) group were observed. The signals of the hydroxymethyl group (H-12; 5.74 (d, 1H), 5.89 (d, 1H) ppm) of the griseoluteic acid

Table 1. The physico-chemical properties of pelagiomicin A (**2**).

Appearance	Reddish orange needles
MP	130°C (dec)
Molecular formula	$C_{20}H_{21}N_3O_6$
HR-FAB-MS *m/z*	
Calcd for $C_{20}H_{22}N_3O_6$ $[M+H]^+$	400.1509
Found	400.1524
UV λ_{max}^{EtOH} nm (log ε)	209 (4.59), 265 (4.62), 368 (3.96)
IR ν_{max}^{KBr} cm^{-1}	3432, 2928, 1740, 1545, 1460, 1130, 1100
$[\alpha]_D^{20}$	+19.8° (*c* 1.0, $CHCl_3$)

Fig. 1. ^{1}H NMR spectrum of **2** and summarized results of HMBC.



portion of **2** were shifted down-field compared with those in **1**, and split by a geminal coupling ($J=12.5$ Hz). These observations suggested an acyl group containing asymmetric carbon bonded to the oxygen atom of the hydroxymethyl group. In the ^{13}C NMR spectrum of **2**, the signals of griseoluteic acid part as well as an additional five signals, *i.e.*, two methyl carbons (25.1 and 26.2 ppm), a methine carbon (62.6 ppm), a non-protonated sp^3 carbon (70.9 ppm) and a carbonyl carbon (174.1 ppm), were detected. From the results of HMBC data shown in Fig. 1 and the molecular formula of **2**, the acyl group could be either 2-amino-3-hydroxyisovaleryl or 3-amino-2-hydroxyisovaleryl (X, Y = NH_2, OH in Fig. 1).

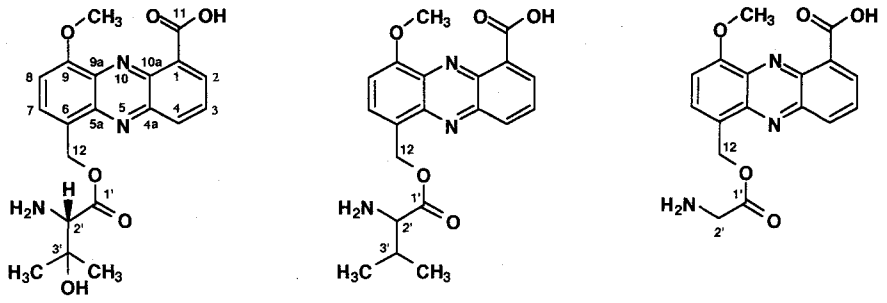
To confirm the structure of the disubstituted isovalerate, the hydrolysis of **2** was carried out (0.1 N HCl, 60°C, 1 hour). The precipitate was collected by filtration and was identified to be **1** by ^{1}H NMR data. The filtrate was evaporated to dryness and gave an amino acid (**5**). The ^{1}H NMR spectrum of **5** in D_2O showed only three singlet signals (1.31 (3H), 1.52 (3H), 3.68 (1H) ppm) and the ^{13}C NMR spectrum revealed signals due to two methyl groups (25.7, 29.8 ppm), a methine (69.9 ppm), a non-protonated sp^3 carbon (73.3 ppm) and a carbonyl carbon (174.5 ppm). The ^{13}C NMR chemical shifts of the methine and the non-protonated sp^3 carbon, suggested that **5** was 3-hydroxyvaline. The measured optical rotation value did not allow us to decide on the absolute structure of **5**, because of the small amout of the sample that was available and because the reported optical rotation of 3-hydroxyvaline was numerically small. To confirm the structure and establish the stereochemistry of this molecule, D- and L-3-hydroxyvaline were synthesized from the L- and D-amino acids, respectively[4]. The ^{1}H and ^{13}C NMR spectra of synthetic 3-hydroxyvaline were identical with those of **5**. Furthermore, the synthesized D- and L-forms of 3-hydroxyvaline were compared with **5** by pre-labeled HPLC analysis using a chiral reagent, FLEC[5] and the derivative of **5** overlapped with that of the L-isomer. Thus, the structure including absolute chemistry of **2** was determined as shown in Fig. 2, and the total assignment of signals of the ^{13}C NMR spectrum is given in Table 2.

Presumed Structures of Pelagiomicins B (3) and C (4)

The compounds **3** and **4** are also unstable in water and alcohols. Although the samples of **3** and **4** contained impurities, the existence of griseoluteic acid in both compounds was indicated by the UV and the ^{1}H NMR spectra. The signals for H-12 were shifted downfield in **3** and **4** compared with that in **1**, and were observed to show geminal coupling (5.82 and 5.89 ppm, $J=12.7$ Hz) and a singlet (5.77 ppm, 2H) in **3** and **4**, respectively.

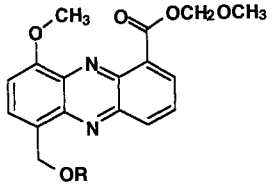
The ^{1}H NMR spectrum of **3**, showed the characteristic signals for the griseoluteic acid part as well as extra doublets due to two methyl groups (0.89 and 0.95 ppm, $J=6.7$ Hz). These signals were correlated to a methine signal (2.02 ppm) which coupled with a doublet methine proton (3.35 ppm, $J=4.9$ Hz) by the COSY spectra. The chemical shift of the latter methine proton was attributed to the α-proton of the amino acid. From the results of HR-FAB-MS (found *m/z* 384.1592 calcd. *m/z* 384.1560 $[M+H]^+$), the molecular formula of **3** was established

Fig. 2. Structures of pelagiomicins A (**2**), B (**3**) and C (**4**).



Pelagiomicin A (**2**) Pelagiomicin B (**3**) Pelagiomicin C (**4**)

Fig. 3. Structures of synthetic intermediates **6**, **7** and **8**.



6 R=H
7 R=BOCVal
8 R=BOCGly

Table 2. The assignments of ^{13}C NMR signals of pelagiomicins A (**2**), B (**3**) and C (**4**).

Position	2	3	4
1	124.9	126.8	125.7
2	137.0	135.1	134.8
3	131.0	131.4	130.8
4	134.6	133.7	133.5
4a	142.6	142.0	141.4
5a	142.7	142.0	141.3
6	125.1	124.2	124.0
7	132.9	133.7	132.2
8	107.6	108.6	108.1
9	154.4	154.3	153.4
9-OCH_3	56.6	56.8	56.3
9a	132.8	133.1	132.3
10a	138.4	138.2	137.5
11	165.9	165.6	165.0
12	62.8	63.1	62.0
1′	174.1	168.9	167.2
2′	62.6	57.3	39.5
3′	70.9	29.4	—
3′-CH_3	25.1	18.0	—
3′-CH_3	26.2	17.4	—
	$CDCl_3$	DMSO-d_6	DMSO-d_6

as $C_{20}H_{21}N_3O_5$, and that of the structurally unknown part was $C_5H_{10}NO$. Thus, the structure of **3** was speculated to be valylgriseoluteic acid.

The ^1H NMR spectrum of **4**, showed the characteristic signals for the griseoluteic acid part as well as additional methylene singlet signal (3.89 ppm). This together with the HR-FAB-MS data (HR-negative FAB found *m/z* 340.0917, calcd. *m/z* 340.0933 $[M-H]^-$) established the molecular formula to be $C_{17}H_{15}N_3O_5$ and suggested that the structure of **4** was glycylgriseoluteic acid.

Table 3. Antimicrobial activities of pelagiomicin A (**2**).

	MIC (μg/ml)
Staphylococcus aureus ATCC 6538P	2.6
Enterococcus hirae ATCC 10541	0.16
Bacillus subtilis #10707	0.16
Klebisiella pneumoniae ATCC 10031	0.16
Escherichia coli ATCC 26	1.3
Pseudomonas aeruginosa BinH#1	5.2
Salmonella choleraesuis ATCC 9992	10
Proteus vulgaris ATCC 6897	<0.04
Shigella sonnei ATCC 9290	1.3
Candida albicans ATCC 10231	>83

Synthesis of Pelagiomicins B (3) and C (4)

To confirm the structures of **3** and **4**, the total syntheses were carried out. Compound **1** was synthesized from 4-methoxy-3-nitrobenzoic acid in low yield according to the method of CHALLAND *et al.*[6]. The carboxyl group of **1** was protected as the methoxymethyl ester (**6**), and then reacted with the appropriate *N-t*-butoxycarbonyl-amino acids in the presence of dicyclohexylcarbodiimide to afford **7** and **8**. The protecting groups of **7** and **8** were removed with TFA under dry conditions to obtain valylgriseoluteic acid and glycylgriseoluteic acid, respectively. These synthesized compounds were identical to natural **3** and **4** by HPLC and NMR analyses. The assignments of the ^{13}C NMR signals for the synthetic **3** and **4** are shown in Table 2.

Biological Activities of Pelagiomicin A (2)

The antimicrobial activity (MIC values) of **2** are shown in Table 3. The antibiotic exhibits strong activities against Gram-positive and -negative bacteria, and is not active against yeast (*Candida albicans*) at a concentration of

87 μg/ml. It also shows cytotoxic activity against cultured cells *in vivo*, *i.e.*, IC_{50} values were 0.04, 0.2 and 0.07 μg/ml against HeLa, BALB3T3 and BALB3T3/H-ras, respectively, and weak antitumor activity against murine P388 leukemia *in vivo*.

Discussion

Although many phenazine antibiotics have been reported from microorganisms, the pelagiomicins are only the third compounds to contain griseoluteic acid. The other two were griseoluteins[3] and senacarcin A[7], and these were produced by terrestrial *Streptomyces* sp. Pelagiomicins were produced by a new marine halophilic Gram-negative bacterium, *Pelagiobacter variabilis*. It is interesting to consider the propagation of genes of secondary metabolites.

Experimental

General Methods

UV-Visible spectra were obtained on a Shimadzu UV-2100S spectrometer. High performance liquid chromatography was carried out with Tosoh CCP & 8010 series and a photo diode array detector, Shimadzu PSD-M6A. ^1H and ^{13}C NMR spectra were measured with a Varian UNITY500 NMR spectrometer. Mass spectra were recorded with a JEOL JMS-SX102 mass spectrometer. Optical rotation was determined with a Horiba SEPA-300 polarimeter.

Spectroscopical Data of **1**

FAB-MS *m/z* 285 $[M+H]^+$; ^1H NMR (δ ppm, in DMSO-d_6) 4.11 (3H, s), 5.18 (2H, s), 7.45 (1H, d, $J=7.8$ Hz), 7.96 (1H, d, $J=7.8$ Hz), 8.12 (1H, dd, $J=8.8$ and 7.1 Hz), 8.52 (1H, d, $J=8.8$ Hz), 8.66 (1H, br d, $J=7.1$ Hz); ^{13}C NMR (δ ppm, in DMSO-d_6) 56.5 (q), 58.5 (t), 109.1 (s), 126.1 (s), 128.6 (d), 130.8 (d), 131.9 (s), 132.7 (s), 134.0 (d), 135.0 (d), 137.8 (s), 141.6 (s), 141.7 (s), 152.1 (s), 165.6 (s); UV λ_{max} nm in EtOH 209, 265, 368.

HPLC Analysis of 3-Hydroxyvaline

L- and D-3-hydroxyvaline were synthesized from D- and L-valine, respectively, according to literature of SCHOLLKOPF *et al.*[4]. To the solution of amino acid (0.5 mg) in water (400 μl) were added saturated aqueous $NaHCO_3$ (100 μl) and (+)-1-(9-fluorenyl)ethyl chloroformate (100 μl : FLEC : 18 mM/acetone : purchased from Aldrich) and stirred for 5 minutes at room temperature. The reaction mixture was washed with hexane (500 μl, three times), and the water layer was applied on HPLC analysis. That was carried out on Capcellpack CN (Shisei-do, i.d. 4.6 mm × 250 mm) column with the following conditions, *i.e.*, eluate; CH_3CN-H_2O-AcONa buffer (0.3 M acetic acid was adjusted to pH 3.8 with 1 N-NaOH) = 25 : 65 : 10, flow rate; 1.2 ml/minute, detection; 269 nm. Retention times of FLEC derivatives of L- and D-3-hydroxyvaline were 20.9 and 19.6 minutes, respectively.

Synthesis of Valylgriseoluteic Acid and Glycylgriseoluteic Acid

Methoxymethyl Griseoluteic Acid (**6**)

From 4-methoxy-3-nitrobenzoic acid, **1** was synthesized in three steps according to the method of CHALLAND *et al.*[6]. To the suspension of **1** (50 mg) in dry DMF (4 ml) was added Et_3N (150 μl) and cooled to −10°C. Methoxymethyl chloride (250 μl) was added and stirred for 30 minutes at −10°C. The reaction mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4. After evaporation, the residue was recrystallized from EtOAc-hexane to afford **6** (45 mg, 76%) as fine yellow needles; $C_{17}H_{16}N_2O_5$; mp 157~159°C (dec.); HR-FAB-MS found 329.1146, calcd. for $C_{17}H_{17}N_2O_5$ 329.1137; ^1H NMR (δ ppm, acetone-d_6) 3.79 (3H, s, MOM-CH_3), 4.10 (3H, s, 9-OCH_3), 4.31 (1H, d, $J=5.9$ Hz, 12-OH), 5.30 (2H, dd, $J=5.9$ and 1.2 Hz, H-12), 5.61 (2H, s, MOM-CH_2), 7.27 (1H, d, $J=7.8$ Hz, H-8), 7.94 (1H, dd, $J=7.8$ and 1.2 Hz, H-7), 8.02 (1H, dd, $J=8.6$ and 6.8 Hz, H-3), 8.22 (1H, dd, $J=6.8$ and 1.5 Hz, H-2), 8.41 (1H, dd, $J=8.6$ and 1.5 Hz, H-4); ^{13}C NMR (δ ppm, acetone-d_6) 55.2 (q, 9-OCH_3), 56.4 (q, MOM-CH_3), 59.8 (t, C-12), 90.6 (t, MOM-CH_2), 107.0 (d, C-8), 127.9 (d, C-7), 129.1 (d, C-3), 130.2 (d, C-2), 131.4 (s, C-6), 132.1 (d, C-4), 132.7 (s, C-1), 136.4 (s, C-9a), 138.4 (s, C-10a), 140.9 (s, C-4a), 141.5 (s, C-5a), 154.1 (s, C-9), 166.1 (s, C-11); UV λ_{max} nm (log ε) 209 (4.20), 270 (4.28), 363 (3.65) in EtOH; IR ν_{max} cm^{-1} KBr 3492, 2926, 1719, 1543, 1460, 1278, 1151.

Methoxymethyl *N*-*t*-Butoxycarbonyl-L-valylgriseoluteic acid (**7**)

To a solution of **6** (25 mg), *N*-*t*-butoxycarbonyl-L-valine (25 mg), and 4-dimethylaminopyridine (15 mg) in dry DMF (1 ml) was added dicyclohexylcarbodiimide (50 mg), and the mixture was stirred for 24 hours at 70°C. The reaction mixture was poured into water (20 ml), and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash silica-gel column chromatography (hexane-acetone, 3 : 1) to obtain **7** (17 mg, 42%); $C_{27}H_{31}N_3O_8$; HR-FAB-MS found 528.2358, calcd. for $C_{27}H_{32}N_3O_8$ 528.2346; ^1H NMR (δ ppm, acetone-d_6) 0.92 (3H, d, $J=6.8$ Hz, 3′-CH_3), 0.94 (3H, d, $J=7.1$ Hz, 3′-CH_3), 1.37 (9H, s, BOC-CH_3 × 3), 2.14 (1H, m, H-3′), 3.79 (3H, s, MOM-CH_3), 4.12 (3H, s, 9-OCH_3), 4.15 (1H, dd, $J=9.0$ and 5.5 Hz, H-2′), 5.61 (2H, s, MOM-CH_2), 5.79 (1H, d, $J=12.5$ Hz, H-12), 5.89 (1H, dd, $J=12.5$ and 0.7 Hz, H-12), 6.08 (1H, d, $J=9.0$ Hz, 2′-NH), 7.29 (1H, d , $J=7.8$ Hz, H-8), 8.01 (1H, d, $J=7.8$ Hz, H-7), 8.04 (1H, dd, $J=8.5$ and 7.1 Hz, H-3), 8.24 (1H, dd, $J=8.5$ and 1.2 Hz, H-2), 8.40 (1H, dd, $J=7.1$ and 1.2 Hz, H-4); ^{13}C NMR (δ ppm, acetone-d_6) 18.0 (q, 3′-CH_3 × 2), 27.1 (q,

BOC-CH_3 × 3), 30.2 (d, C-3'), 55.3 (q, 9-OCH_3), 56.5 (q, MOM-CH_3), 58.8 (d, C-2'), 61.7 (t, C-12), 77.8 (s, BOC-C), 90.6 (t, MOM-CH_2), 106.6 (d, C-8), 125.2 (s, C-6), 129.5 (d, C-3), 130.5 (d, C-2), 131.2 (d, C-7), 132.2 (d, C-4), 132.7 (s, C-1), 136.3 (s, C-9a), 138.6 (s, C-10a), 141.3 (s, C-4a), 141.6 (s, C-5a), 155.2 (s, C-9), 155.4 (s, BOC-C=O), 166.0 (s, C-11), 171.4 (s, C-1'); UV ν_{max} nm (log ε) 210 (4.61), 267 (4.66), 362 (4.05) in EtOH; IR ν_{max} cm^{-1} KBr 3448, 2970, 1717, 1543, 1458, 1280, 1158; $[\alpha]_D^{25}$ −12.8° (*c* 0.5, acetone).

Valylgriseoluteic Acid (**3**)

To a mixed solvent of dry CH_2Cl_2 (1 ml) and dry TFA (1 ml) was added **7** (10 mg). The mixture was stirred for 30 minutes and evaporated *in vacuo* to give **3**; $C_{20}H_{21}N_3O_5$; HR-FAB-MS found 384.1576, calcd. for $C_{20}H_{22}N_3O_5$ 384.1560; ^1H NMR (δ ppm, DMSO-d_6) 0.87 (3H, d, *J*=6.8 Hz, 3'-CH_3), 0.90 (3H, d, *J*=6.8 Hz, 3'-CH_3), 2.09 (1H, dd, *J*=6.8 and 4.2 Hz, H-3'), 3.97 (1H, d, *J*=4.2 Hz, H-2'), 4.15 (3H, s, 9-OCH_3), 5.80 (1H, d, *J*=12.5 Hz, H-12), 5.95 (1H, d, *J*=12.5 Hz, H-12), 7.48 (1H, d , *J*=8.1 Hz, H-8), 8.15 (1H, d, *J*=8.1 Hz, H-7), 8.17 (1H, dd, *J*=8.5 and 6.8 Hz, H-3), 8.47 (1H, dd, *J*=8.5 and 1.2 Hz, H-4), 8.67 (1H, dd, *J*=6.8 and 1.2 Hz, H-2); ^{13}C NMR (δ ppm, DMSO-d_6) 17.4 (q, 3'-CH_3), 18.0 (q, 3'-CH_3), 29.4 (d, C-3'), 56.8 (q, 9-OCH_3), 57.3 (d, C-2'), 63.1 (t, C-12), 108.6 (d, C-8), 124.2 (s, C-6), 126.8 (s, C-1), 131.4 (d, C-3), 133.1 (s, C-9a), 133.7 (d, C-7), 133.7 (d, C-4), 135.1 (d, C-2), 138.2 (s, C-10a), 142.0 (s, C-4a), 142.0 (s, C-5a), 154.3 (s, C-9), 165.6 (s, C-11), 168.9 (s, C-1'); UV λ_{max} nm (log ε) 210 (4.70), 267 (4.66), 369 (4.10) in EtOH; IR ν_{max} cm^{-1} KBr 3452, 2928, 1686, 1545, 1460, 1207, 1135; $[\alpha]_D^{24}$ +15.5° (*c* 0.1, CH_3CN).

Methoxymethyl *N*-*t*-Butoxycarbonylglycylgriseoluteic Acid (**8**)

To a solution of **6** (25 mg), *N*-*t*-butoxycarbonylglycine (25 mg), and 4-dimethylaminopyridine (15 mg) in dry DMF (1 ml) was added dicyclohexylcarbodiimide (50 mg), and the mixture was stirred for 24 hours at 70°C. The reaction mixture was poured into water (20 ml), and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash silica-gel column chromatography (hexane-acetone, 3:1) to obtain **8** (20 mg, 77%); $C_{24}H_{27}N_3O_8$; HR-FAB-MS found 486.1858, calcd. for $C_{24}H_{28}N_3O_8$ 486.1877; ^1H NMR (δ ppm, acetone-d_6) 1.40 (9H, s, BOC-CH_3 × 3), 3.79 (3H, s, MOM-CH_3), 3.92 (2H, d, *J*=5.9 Hz, H-2'), 4.10 (3H, s, 9-OCH_3), 5.83 (2H, s, H-12), 6.30 (1H, d, *J*=5.9 Hz, 2'-NH), 7.23 (1H, d , *J*=7.8 Hz, H-8), 7.91 (1H, d, *J*=7.8 Hz, H-7), 8.02 (1H, dd, *J*=8.8 and 7.1 Hz, H-3), 8.23 (1H, dd, *J*=7.1 and 1.5 Hz, H-2), 8.39 (1H, dd, *J*=8.8 and 1.5 Hz, H-4); ^{13}C NMR (δ ppm, acetone-d_6) 27.2 (q, BOC-CH_3 × 3), 41.7 (t, C-2'), 55.3 (q, 9-OCH_3), 56.5 (q, MOM-CH_3), 61.4 (t, C-12), 77.9 (s, BOC-C), 90.6 (t, MOM-CH_2), 106.6 (d, C-8), 125.2 (s, C-6), 129.4 (d, C-3), 130.5 (d, C-7), 130.6 (d, C-2), 132.3 (d, C-4), 132.3 (s, C-1), 136.2 (s, C-9a), 138.5 (s, C-10a), 141.2 (s, C-4a), 141.4 (s, C-5a), 155.1 (s, C-9), 155.4 (s, BOC-C=O), 166.0 (s, C-11), 169.7 (s, C-1'); UV λ_{max} nm (log ε) 210 (4.47), 267 (4.53), 363 (3.92) in EtOH; IR ν_{max} cm^{-1} KBr 3434, 2928, 1708, 1543, 1460, 1286, 1160.

Glycylgriseoluteic Acid (**4**)

To a mixed solvent of dry CH_2Cl_2 (1 ml) and dry TFA (1 ml) was added **8** (10 mg). The mixture was stirred for 30 minutes and evaporated *in vacuo* to give **4**; $C_{17}H_{15}N_3O_5$; HR-FAB-MS found 342.1079, calcd. for $C_{17}H_{16}N_3O_5$ 342.1090; ^1H NMR (δ ppm, DMSO-d_6) 3.89 (2H, s, H-2'), 4.12 (3H, s, 9-OCH_3), 5.85 (2H, s, H-12), 7.40 (1H, d , *J*=8.1 Hz, H-8), 8.05 (1H, d, *J*=8.1 Hz, H-7), 8.12 (1H, dd, *J*=8.6 and 7.1 Hz, H-3), 8.47 (1H, dd, *J*=8.6 and 1.5 Hz, H-4), 8.64 (1H, dd, *J*=7.1 and 1.5 Hz, H-2); ^{13}C NMR (δ ppm, DMSO-d_6) 39.5 (t, C-2'), 56.3 (q, 9-OCH_3), 62.0 (t, C-12), 108.1 (d, C-8), 124.0 (s, C-6), 125.7 (s, C-1), 130.8 (d, C-3), 132.2 (d, C-7), 132.3 (s, C-9a), 133.5 (d, C-4), 134.8 (d, C-2), 137.5 (s, C-10a), 141.3 (s, C-5a), 141.4 (s, C-4a), 153.4 (s, C-9), 165.0 (s, C-11), 167.2 (s, C-1'); UV λ_{max} nm (log ε) 210 (4.67), 267 (4.65), 369 (4.09) in EtOH; IR ν_{max} cm^{-1} KBr 3450, 2926, 1686, 1543, 1460, 1207, 1137.

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