CHEMICAL MODIFICATION OF HITACHIMYCIN

III. SYNTHESIS AND ANTITUMOR ACTIVITIES OF AMINO ACYL DERIVATIVES

KIYOSHI SHIBATA, SADAYOSHI SATSUMABAYASHI, HIROSHI SANO[†], KANKI KOMIYAMA[††], YANG ZHI-BO[††], AKIRA NAKAGAWA[††] and SATOSHI ŌMURA*,[††]

Nippon Dental University,
Chiyoda-ku, Tokyo 102, Japan
[†]Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.,
Machida-shi, Tokyo 194, Japan
[††]The Kitasato Institute, and School of Pharmaceutical Sciences,
Kitasato University,
Minato-ku, Tokyo 108, Japan

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Amino acyl derivatives of hitachimycin have been synthesized and evaluated their activities including antibacterial, cytocidal against HeLa cells and *in vivo* antitumor against sarcoma 180. 15-*O*-(*tert*-Butoxycarbonyl(BOC)-glycyl)hitachimycin (**2**), 15-*O*-(BOC-β-alanyl)hitachimycin (**4**), 15-*O*-(BOC-(*O*-*tert*-Bu)-glutamyl)hitachimycin (**6**) and 15-*O*-L-alanylhitachimycin (**11**) showed comparable *in vivo* antitumor activity with hitachimycin and the solubility of these compounds was improved.

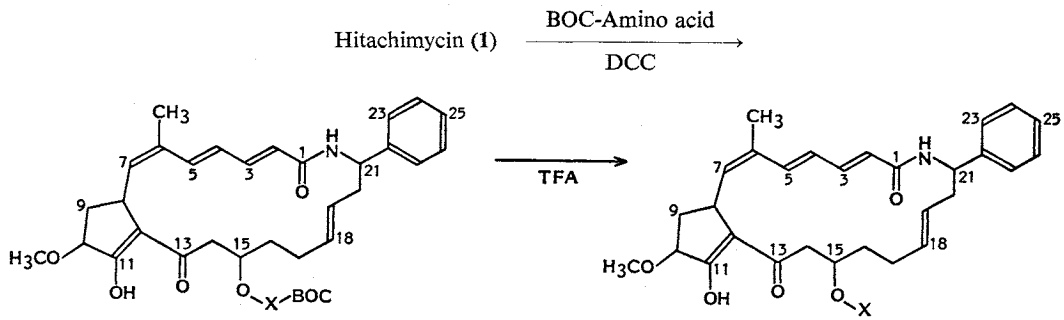
Hitachimycin (**1**)[1],[†††] is a macrocyclic lactam antibiotic, which shows antitumor[3], antibacterial and antiprotozoal activities. Hitachimycin (**1**) acts against a cell wall and is incorporated into the membrane phospholipid bilayer, and increases the permeability of the plasma membrane, ultimately leading to lysis and death of the cells[4]. Furthermore, **1** shows higher antitumor effect in the combination with bleomycin[5]. However, as hitachimycin is hardly soluble in water and the other organic solvents, it is difficult to administer in *in vivo* examination. In the previous paper[6,7], we have reported the synthesis and antitumor activities of 11,15-*O*-acyl and 11,15-carbonate derivatives of hitachimycin, and some of them showed superior antitumor effects in *in vivo* assay and higher solubility in organic solvents. From the facts, we have been interested about a substituted acyl derivatives bearing the hydrophilic functional group, which is expected to contribute to the solubility of the derivatives. In this paper, we describe the synthesis of amino acyl derivatives of hitachimycin and their *in vivo* antitumor activities against sarcoma 180.

Synthesis

The substitution of amino acyl group to hitachimycin (**1**) was carried out through the following step shown in Scheme 1. Treatment of **1** in pyridine with dicyclohexylcarbodiimide (DCC) and *tert*-butoxycarbonyl(BOC)-amino acid[8] such as BOC-L-glycine, BOC-L-alanine, BOC-β-alanine, BOC-(*O*-*tert*-Bu)-L-glutamic acid, N,N^{ε}-di-BOC-L-lysine, BOC-L-aminobutyric acid, BOC-γ-aminobutyric acid and BOC-ε-aminocaproic acid at room temperature gave the 15-BOC-amino acylates (**2**~**9**), re-

[†††] Hitachimycin was identified with stubomycin by their physico-chemical properties but the production strain of each compound was different[2].

Scheme 1.



2 X=L-Gly
3 X=L-Ala
4 X=β-Ala
5 X=L-Lys
6 X=L-Glu(*O-tert*-Bu)
7 X=L-Aba
8 X=γ-Aba
9 X=ε-Aca

10 X=L-Gly
11 X=L-Ala
12 X=β-Ala
13 X=L-Lys
14 X=L-Glu
15 X=L-Aba
16 X=γ-Aba
17 X=ε-Aca

Aba: Aminobutyryl, Aca: aminocaproyl

spectively. In the reaction, a small amount of 11,15-di-BOC-amino acylate was found in the reaction mixture as the minor product, but they disappeared during working up procedure. It was thought that the steric hindrance by the 10-OCH_3 group to the C-11 position prevented the condensation of bulky BOC-amino acid to the C-11 hydroxyl group, and as a result, a small amount of unstable 11-enol ester product was hydrolyzed by the treatment with water. In the ^{13}C NMR spectra of these 15-BOC-amino acylates (**2**~**9**), the signals assignable to BOC group and each acyl group, a downfield shift of the C-15 carbon, and an upfield shift (β-shift) of the C-16 carbon compared with that of **1** were observed, which indicated the hydroxyl group at the C-15 position is substituted.

Trifluoroacetic acid[9] was found to be the most effective acid to remove the BOC group without hydrolysis of ester bond at C-15 position on hitachimycin. The 15-BOC-amino acylates (**2**~**9**) was treated with trifluoroacetic acid at room temperature for 10 minutes to give 15-amino acylates (**10**~**17**), respectively. The ^{13}C NMR spectrum of each compound (**10**~**17**) showed signals assignable to an amino acyl group and its ester carbonyl carbon (δ 167.9~175.6). Furthermore the disappearance of the signal corresponded to BOC group was shown in each spectrum.

Chemical shift values for ^{13}C NMR spectra of all synthesized amino acylates are listed in Table 1.

Cytocidal and Antibacterial Activities and Retention Time (RT) of Reversed Phase HPLC and Solubility in Water

Cytocidal activities against HeLa cells measured by 50% inhibitory concentration (IC_{50}) values, MIC (μg/ml) against various bacteria, hemolytic activity, RT of reversed phase HPLC and solubility in water of hitachimycin derivatives are given in Table 2.

RT was obtained as an indication of hydrophilicity of the derivative. Amino acyl derivatives (**10**~**17**) exhibited rather short RT, indicating the improvement of hydrophilicity of these derivatives. Actually, solubility in water is increased by 5- to 6-fold in the cases of smaller BOC-amino acyl derivatives (**2**~**4**), and 20- to 60-fold in the cases of free amino acyl derivatives (**10**~**17**).

BOC-Amino acyl derivatives bearing smaller groups showed similar antibacterial activities to

Table 1. ^{13}C NMR chemical shift†

Carbon No.	Hitachimycin	2	3	4	5	6	7	8
CONH	167.6	166.8	166.7	166.6	166.9	166.7	166.8	166.8
2	124.5	124.7	124.7	124.3	124.8	124.4	127.4	124.8
3	141.9	141.4	141.3	141.9	141.4	141.8	141.4	141.4
4	127.8	127.5	127.2	127.2	127.3	127.2	127.3	127.3
5	136.9	135.0	134.7	135.0	134.7	134.9	134.8	134.9
6	131.5	130.7	130.7	130.7	130.8	130.6	130.8	130.6
7	134.9	135.9	135.9	136.1	136.0	136.2	136.0	136.0
8	35.1	34.0	33.8	34.1	33.8	33.9	33.8	33.8
9	35.5	35.1	35.0	35.3	35.1	35.1	35.1	35.0
10	81.2	80.6	80.6	80.6	80.7	80.6	80.6	80.6
11	185.5	186.7	186.8	187.1	186.9	187.0	186.8	186.6
12	112.6	112.0	111.9	112.0	112.2	112.0	112.1	112.0
13	196.5	192.7	192.5	192.3	192.8	192.1	192.6	192.9
14	46.5	41.5	41.5	41.5	41.6	41.5	41.6	41.5
15	68.1	70.3	70.3	70.7	70.6	70.7	70.6	69.8
16	39.1	36.5	36.5	36.4	36.5	36.4	36.6	36.6
17	29.4	28.9	28.6	27.9	28.6	27.4	28.6	28.6
18	134.4	133.1	133.0	133.0	133.0	133.0	133.1	133.1
19	126.9	127.4	127.6	127.6	127.7	127.5	127.7	127.7
20	41.6	41.3	41.4	41.1	41.3	41.2	41.3	41.2
21	52.4	51.7	51.8	51.7	51.9	51.7	51.8	51.8
22	142.2	141.8	141.6	141.8	141.7	141.8	141.6	141.7
23	126.9	126.3	126.3	126.3	126.3	126.3	126.3	126.3
24	128.7	128.6	128.6	128.6	128.5	128.6	128.7	128.7
25	127.4	127.3	127.3	127.3	127.3	127.3	127.3	127.3
26	128.7	128.6	128.6	128.6	128.5	128.6	128.7	128.7
27	126.9	126.3	126.3	126.3	126.3	126.3	126.3	126.3
6-CH_3	20.0	20.1	20.1	20.1	20.1	20.1	20.1	20.0
10-OCH_3	58.1	58.1	58.0	58.1	58.0	58.0	58.0	58.0
15-Amino acyl group								
1		169.8	172.9	176.9	172.1	172.1	172.2	172.7
2		42.4	49.2	35.9	53.4	52.9	54.6	32.9
3			18.4	41.6	28.3	28.6	29.7	28.9
4					22.2	31.3	9.5	41.4
5					32.1	171.8		
6					40.7			
Protective group (BOC)					α-Amino			
		156.9	155.1	155.7	155.5	155.4	157.0	156.2
		77.2	78.9	77.9	77.3	77.2	77.3	77.3
		28.3	28.3	28.4	28.3	28.3	28.4	28.5
					ε-Amino	OBu		
					156.3	79.9		
					79.3	28.0		
					28.3			

† Chemical shift in ppm are downfield from TMS.

hitachimycin, but derivatives having bulky groups almost lost antibacterial activities. Amino acyl derivatives exhibited weaker antibacterial activities than hitachimycin. Any derivatives synthesized did not show hemolytic activities at the concentration below 25 μg/ml, and showed lower anti-HeLa activities than hitachimycin. It is interesting that some of BOC-amino acyl derivatives (**5**~**9**) showed considerable anti-HeLa activity in spite of their weak antibacterial activities.

values for hitachimycin derivatives.

9	10	11	12	13	14	15	16	17
166.8	167.3	167.2	167.1	166.9	167.3	167.2	167.1	167.3
124.8	125.0	124.9	125.2	125.0	124.9	124.9	125.0	124.9
141.2	141.7	141.7	141.9	141.7	141.9	142.0	141.6	141.4
127.4	127.9	127.6	127.6	127.5	127.7	127.5	127.6	127.8
135.0	135.1	134.9	134.8	134.8	134.6	134.5	134.5	134.5
130.6	131.6	131.5	131.6	131.7	131.6	131.6	131.4	131.4
135.9	135.5	135.5	135.6	135.9	135.8	135.7	135.7	135.8
33.8	34.1	34.2	34.3	34.2	34.2	34.3	34.2	34.2
35.0	35.3	35.3	35.2	35.3	35.2	35.3	35.5	35.3
80.7	81.0	80.9	81.0	81.0	81.0	80.9	80.7	80.6
186.6	186.9	187.0	187.1	187.3	186.9	187.0	187.0	187.0
111.9	111.7	111.8	111.8	112.0	111.9	111.9	111.8	111.9
193.3	193.2	193.1	193.1	193.6	193.5	193.3	193.3	193.5
41.6	41.9	41.9	41.6	41.9	41.7	41.9	41.9	41.9
69.1	70.6	70.5	70.8	70.8	70.9	70.8	70.7	70.7
36.7	36.5	36.7	36.6	36.6	36.6	36.9	36.9	37.0
28.8	28.5	28.3	28.1	28.4	28.1	28.3	28.5	28.9
133.2	133.1	133.2	133.2	133.2	133.1	133.2	133.2	133.2
127.6	127.3	127.3	127.4	127.6	127.7	127.5	127.5	127.4
41.5	41.5	41.4	41.2	41.5	41.5	41.6	41.6	41.7
51.8	51.9	52.0	52.0	52.0	51.9	52.0	52.1	52.1
141.6	141.9	141.8	141.9	141.9	141.7	141.7	141.9	141.8
126.3	126.4	126.4	126.4	126.5	126.4	126.4	126.5	126.4
128.7	128.6	128.7	128.7	128.6	128.7	128.7	128.7	128.7
127.3	127.3	127.4	127.4	127.3	127.4	127.3	127.4	127.4
128.7	128.6	128.7	128.7	128.6	128.7	128.7	128.7	128.7
126.3	126.4	126.4	126.4	126.5	126.4	126.4	126.5	126.4
20.1	20.0	20.0	20.0	20.0	20.1	20.0	20.0	20.0
57.9	58.2	58.2	58.2	58.2	58.1	58.2	58.2	58.1
172.8	167.9	172.1	175.6	171.6	171.8	171.3	171.9	172.2
33.8	40.1	51.6	36.2	55.3	54.1	57.2	31.6	33.7
24.6		17.2	39.4	27.6	29.7	27.4	28.6	24.9
26.3				22.4	34.5	8.7	41.9	26.6
34.2				30.9	178.9			34.1
40.8				40.0				42.2
156.8								
77.2								
28.5								

Antitumor Activity

Antitumor activities (increase in life span: ILS) at optimal doses of hitachimycin derivatives against sarcoma 180 are given in Table 3. Several BOC-amino acyl derivatives, such as 15-*O*-(BOC-glycyl)-hitachimycin (**2**), 15-*O*-(BOC-*β*-alanyl)hitachimycin (**4**) and 15-*O*-(BOC-(*O*-*tert*-Bu)-L-glutamyl)hitachimycin (**6**) showed an increased ILS as compared with that of hitachimycin. It is noteworthy that 15-

Table 2. Bioactivities of hitachimycin derivatives.

Compound No.	15-Acyl group	Anti-HeLa[a] activity	Hemolysis[b]	MIC[c] (μg/ml)				RT[d] (minutes)	Solubility (mg/ml)
				SA	BS	ML	MS		
1	OH	0.39	6.3	1.56	0.78	3.12	>50	11.76	0.035
BOC-Amino acyl									
2	L-Gly	3.13	>25	3.12	3.12	3.12	>50	32.29	0.260
3	L-Ala	6.25	>25	3.12	<0.1	6.25	>50	22.39	0.210
4	β-Ala	1.56	>25	25	3.12	50	>50	40.98	0.190
5	L-Lys	6.25	>25	>50	>50	>50	>50	75.87	0.005
6	L-Glu(*O-tert*-Bu)	6.25	>25	>50	>50	>50	>50	40.26	0.027
7	L-Aba	3.13	>25	>50	50	>50	>50	59.62	0.017
8	γ-Aba	6.25	>25	>50	>50	>50	>50	43.26	0.011
9	ε-Aca	6.25	>25	>50	>50	>50	>50	28.80	0.018
Amino acyl									
10	L-Gly	25	>25	>50	>50	>50	>50	3.30	2.350
11	L-Ala	3.12	>25	6.25	3.12	6.25	>50	4.04	1.390
12	β-Ala	3.13	>25	12.5	1.56	25	>50	2.49	1.960
13	L-Lys	6.25	25	12.5	6.25	25	>50	7.62	1.260
14	L-Glu	6.25	>25	3.12	1.56	12.5	>50	3.76	1.610
15	L-Aba	12.5	>25	6.25	3.12	12.5	>50	4.26	0.980
16	γ-Aba	12.5	>25	12.5	6.25	12.5	>50	3.83	1.120
17	ε-Aca	25	>25	50	12.5	50	>50	9.25	0.880

[a] IC_{50} (μg/ml), [b] IC_{100} (μg/ml).

[c] SA: *Staphylococcus aureus* KB34 (FDA 209P), BS: *Bacillus subtilis* KB211 (ATCC 6633), ML: *Micrococcus luteus* KB212 (ATCC 9341), MS: *Mycobacterium smegmatis* KB42 (ATCC 607).

[d] RT in HPLC.

Aba: Aminobutyryl, Aca: aminocaproyl.

Table 3. Antitumor activity of hitachimycin derivatives against sarcoma 180[a].

Compound No.	15-Acyl group	Total dose (mg/kg)	Dose (mg/kg×days)	ILS (%)	Survivors[b]
1	OH	150	30.0×5	167.2	0/5
BOC-Amino acyl					
2	L-Gly	150	30.0×5	298.3	3/5
3	L-Ala	75	15.0×5	137.9	1/5
4	β-Ala	37.5	7.5×5	168.9	0/5
5	L-Lys	150	30.0×5	18.9	0/5
6	L-Glu(*O-tert*-Bu)	150	30.0×5	184.4	0/5
7	L-Aba	150	30.0×5	6.8	0/5
8	γ-Aba	75	15.0×5	10.3	0/5
9	ε-Aca	75	15.0×5	91.3	0/5
Amino acyl					
10	L-Gly	150	30.0×5	27.5	0/5
11	L-Ala	75	15.0×5	146.5	0/5
12	β-Ala	37.5	7.5×5	122.4	0/5
13	L-Lys	37.5	7.5×5	5.1	0/5
14	L-Glu	150	30.0×5	43.1	0/5
15	L-Aba	150	30.0×5	10.3	0/5
16	γ-Aba	150	30.0×5	24.1	0/5
17	ε-Aca	150	30.0×5	125.8	0/5

[a] Inoculum size; 2.5×10^6 cells/mouse (ICR, 6-week old female).

[b] Number of surviving mice at day-60 (survival/total).

Aba: Aminobutyryl, Aca: aminocaproyl.

O-L-alanylhitachimycin (**11**), 15-*O*-β-alanylhitachimycin (**12**) and 15-*O*-ε-aminocaproylhitachimycin (**17**) which have a free amine group and a higher solubilities than hitachimycin showed comparable antitumor activity with hitachimycin. This seems to suggest the possibility of the water soluble derivation of hitachimycin having rational antitumor activity.

Experimental

NMR spectra were measured with Jeol FX-90Q and Varian PX-400 spectrometers in $CDCl_3$ or DMSO solution. Mass spectra were obtained with Jeol D-100 and DX-300 spectrometers at 70 eV. Optical rotations were measured with a Jasco DIP-181 polarimeter. TLC was performed on pre-coated plates, Merck Kieselgel 60 F_{254} with $CHCl_3$ - MeOH (50 : 1). Silica gel column chromatography was performed with Merck Kieselgel 60.

RT in HPLC

HPLC was performed on a reversed phase silica gel column (Shiseido Co., Ltd., Capcell pack C18 (SG), 4×250 mm) with CH_3CN - 0.2 M NaH_2PO_4 (1 : 1) as a solvent system[8,10]. RT was recorded at 1 ml/minute of flow rate with a UV monitor (300 nm).

Solubility of Hitachimycin Derivatives to Water

Each derivatives (5.0 mg) was taken in the test tube and added to the distilled water (1.0 ml, pH 7.0). The mixture was stirred for 10 minutes and the unsoluble material was removed with centrifugation (3,000 rpm×2 minutes). Each clear solution (10 μl) was charged on HPLC and analyzed with the same method described above. Concentration of each compound was calculated from the peak area compared with the standard curve of each compound.

MICs

MIC values against various bacteria were determined by the agar dilution method using heart infusion agar (pH 7.0).

Cytocidal Activities

HeLa S3 cells were maintained in monolayers in EAGLE's minimum essential medium supplemented with 10% bovine serum and kanamycin (100 μg/ml) at 37°C. To determine the cytocidal activities of hitachimycin derivatives, HeLa S3 cells (5×10^4) in 1.5 ml of medium were placed in a tissue culture plate (Falcon, 24-well) and incubated for 24 hours at 37°C in a 5% CO_2 - 95% air atmosphere. Each culture well was treated with 0.5 ml of fresh medium containing a different concentration of hitachimycin, and reincubated for 72 hours. The cells were trypsinized to form a single cell suspension, and were counted in a hemocytometer.

Antitumor Activity

Sarcoma 180 cells (1×10^6 cells/mouse) were inoculated ip into ICR mice on day-0. Mice received various doses (<250 mg/kg) of hitachimycin derivatives for 5 successive days from day-1. Antitumor activity was evaluated by the increased ILS: $(T/C-1)\times100\%$ at the optimal dose for each derivatives, where "T" is the median survival days (MSD) of the treated group and "C" is the MSD of the control group.

15-*O*-(BOC-Glycyl)hitachimycin (**2**)

To a solution of **1** (300 mg) and BOC-glycine (150 mg) in pyridine (3.0 ml), DCC (300 mg) was added and stirred for 4 hours at room temperature. After an addition of a drop of H_2O to the reaction mixture, a colorless precipitate was filtered off. The filtrate was diluted with $CHCl_3$ (50 ml) and washed with H_2O. The $CHCl_3$ layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure, to give a brown solid, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH (50 : 1) to afford a colorless powder of **2**, 235 mg (58.9%): MP 182~183°C (dec); $[\alpha]_D^{23}$ +102° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 303 (33,300); high resolution (HR)-MS 634.323 (calcd for $C_{36}H_{46}N_2O_8$: 634.325); ^1H NMR ($CDCl_3$) δ 6.39 (1H, dd, *J*=10.0 and 16.0 Hz, 4-H), 5.98 (1H, d,

J=15.5 Hz, 2-H), 5.45 (1H, br t, 15-H), 4.35 (1H, dd, J=8.4 and 8.4 Hz, 10-H), 3.99 (1H, ddd, J=3.8, 6.0 and 10.0 Hz, 8-H), 3.51 (3H, s, 10-OCH_3), BOC-glycyl moiety; 5.05 (1H, br d, NH), 3.80 (2H, CH_2), 1.42 (9H, s, $C(CH_3)_3$).

Anal Calcd for $C_{38}H_{46}N_2O_8$: C 68.10, H 7.31, N 4.42.
Found: C 68.04, H 7.33, N 4.39.

15-*O*-(BOC-L-Alanyl)hitachimycin (3)

To a solution of **1** (300 mg) and BOC-L-alanine (150 mg) in pyridine (3.0 ml), DCC (300 mg) was added and stirred for 12 hours at room temperature. After an addition of a drop of H_2O to the reaction mixture, a colorless precipitate was filtered off. The filtrate was diluted with $CHCl_3$ (50 ml) and washed with H_2O. The $CHCl_3$ layer was treated in a similar manner to the preparation of **2**, to give a colorless powder of **3**, 250 mg (61.3%): MP 176~178°C (dec); $[\alpha]_D^{23}$ +135° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (34,500); HR-MS 648.341 (calcd for $C_{37}H_{48}N_2O_8$: 648.341); ^1H NMR ($CDCl_3$) δ 6.38 (1H, dd, J=10.0 and 15.5 Hz, 4-H), 5.98 (1H, d, J=16.0 Hz, 2-H), 5.45 (1H, br t, 15-H), 4.34 (1H, dd, J=8.2 and 8.2 Hz, 10-H), 4.00 (1H, ddd, J=4.2, 6.0 and 10.5 Hz, 8-H), 3.51 (3H, s, 10-OCH_3), BOC-L-alanyl moiety; 5.68 (1H, br d, NH), 4.17 (1H, d, CH), 1.42 (9H, s, $C(CH_3)_3$), 1.28 (3H, d, J=7.2 Hz, CH_3).

Anal Calcd for $C_{37}H_{48}N_2O_8$: C 68.48, H 7.46, N 4.32.
Found: C 68.36, H 7.39, N 4.30.

15-*O*-(BOC-β-Alanyl)hitachimycin (4)

Compound **4** was prepared from **1** (300 mg) and BOC-β-alanine (150 mg) with DCC (300 mg) as described in the preparation of **3**, to give a colorless powder of **4**, 261 mg (64.0%): MP 169~170°C (dec); $[\alpha]_D^{23}$ +98° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (31,700); HR-MS 648.342 (calcd for $C_{37}H_{48}N_2O_8$: 648.341); ^1H NMR ($CDCl_3$) δ 6.41 (1H, dd, J=11.0 and 15.0 Hz, 4-H), 5.96 (1H, d, J=15.0 Hz, 2-H), 5.50 (1H, br t, 15-H), 4.37 (1H, dd, J=7.9 and 7.9 Hz, 10-H), 4.03 (1H, ddd, J=3.9, 6.0 and 10.5 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), BOC-β-alanyl moiety; 5.18 (1H, br d, NH), 3.36 (2H, br t, J=6.0 Hz, CH_2), 2.46 (2H, br d, J=6.0 Hz, CH_2), 1.42 (9H, s, $C(CH_3)_3$).

Anal Calcd for $C_{37}H_{48}N_2O_8$: C 68.48, H 7.46, N 4.32.
Found: C 68.41, H 7.42, N 4.31.

15-*O*-(N,N^ε-Di-BOC-L-lysyl)hitachimycin (5)

Compound **5** was prepared from **1** (300 mg) and N,N^ε-di-BOC-L-lysine (150 mg) with DCC (300 mg) as described in the preparation of **3**, to give a colorless powder of **5**, 215 mg (42.5%): MP 136~137°C (dec); $[\alpha]_D^{23}$ +90° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (31,900); HR-MS 805.453 (calcd for $C_{45}H_{63}N_3O_{10}$: 805.451); ^1H NMR ($CDCl_3$) δ 6.39 (1H, dd, J=11.0 and 15.1 Hz, 4-H), 5.98 (1H, d, J=15.0 Hz, 2-H), 5.45 (1H, br t, 15-H), 4.35 (1H, dd, J=8.5 and 8.5 Hz, 10-H), 4.00 (1H, ddd, J=4.0, 6.0 and 10.0 Hz, 8-H), 3.50 (3H, s, 10-OCH_3), N,N^ε-di-BOC-L-lysyl moiety; 5.87 (1H, br d, NH), 5.64 (1H, br d, NH), 4.16 (1H, br t, CH), 3.16 (2H, br t, CH_2), 1.90 and 1.30 (6H, $CH_2\times3$), 1.42 (18H, s, $C(CH_3)_3\times2$).

Anal Calcd for $C_{45}H_{63}N_3O_{10}$: C 67.04, H 7.88, N 5.22.
Found: C 67.01, H 7.75, N 5.18.

15-*O*-(BOC-(*O*-*tert*-Bu)-L-Glutamyl)hitachimycin (6)

Compound **6** was prepared from **1** (400 mg) and BOC-(*O*-*tert*-Bu)-L-glutamic acid (150 mg) with DCC (300 mg) as described in the preparation of **3**, to give a colorless powder of **6**, 321 mg (50.2%): MP 141~143°C (dec); $[\alpha]_D^{23}$ +135° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (35,600); HR-MS 762.405 (calcd for $C_{43}H_{58}N_2O_{10}$: 762.409); ^1H NMR ($CDCl_3$) δ 6.38 (1H, dd, J=11.0 and 15.0 Hz, 4-H), 5.97 (1H, d, J=15.0 Hz, 2-H), 5.46 (1H, br t, 15-H), 4.38 (1H, dd, J=8.0 and 8.0 Hz, 10-H), 3.98 (1H, ddd, J=4.2, 6.0 and 10.0 Hz, 8-H), 3.50 (3H, s, 10-OCH_3), BOC-(*O*-*tert*-Bu)-L-glutamyl moiety; 5.65 (1H, br d, NH), 4.20 (1H, m, CH), 2.24 (2H, dd, CH_2), 1.60 (2H, dd, CH_2), 1.44 (9H, s, $C(CH_3)_3$), 1.42 (9H, s, $C(CH_3)_3$).

Anal Calcd for $C_{43}H_{58}N_2O_{10}$: C 67.68, H 7.67, N 3.67.
Found: C 67.42, H 7.59, N 3.61.

15-*O*-(BOC-L-Aminobutyryl)hitachimycin (**7**)

Compound **7** was prepared from **1** (400 mg) and BOC-L-aminobutyric acid (150 mg) with DCC (300 mg) as described in the preparation of **3**, to give a colorless powder of **7**, 273 mg (49.2%): MP 151~154°C (dec); $[\alpha]_D^{23}$ +79° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (32,100); HR-MS 662.356 (calcd for $C_{38}H_{50}N_2O_8$: 662.356); ^1H NMR ($CDCl_3$) δ 6.40 (1H, dd, *J*=10.2 and 16.0 Hz, 4-H), 5.98 (1H, d, *J*=15.0 Hz, 2-H), 5.47 (1H, br t, 15-H), 4.35 (1H, dd, *J*=8.2 and 8.2 Hz, 10-H), 4.01 (1H, ddd, *J*=4.0, 5.8 and 10.0 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), BOC-L-aminobutyryl moiety; 5.62 (1H, br d, NH), 4.14 (1H, br t, CH), 1.86 (2H, CH_2), 1.42 (9H, s, $C(CH_3)_3$), 0.87 (3H, t, *J*=7.4 Hz, CH_3).

Anal Calcd for $C_{38}H_{50}N_2O_8$: C 68.85, H 7.61, N 4.23.
Found: C 68.82, H 7.57, N 4.19.

15-*O*-(BOC-γ-Aminobutyryl)hitachimycin (**8**)

Compound **8** was prepared from **1** (300 mg) and BOC-γ-aminobutyric acid (150 mg) with DCC (300 mg) as described in the preparation of **3**, to give a colorless powder of **8**, 230 mg (55.2%): MP 163~165°C (dec); $[\alpha]_D^{23}$ +92° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 304 (35,400); HR-MS 662.354 (calcd for $C_{38}H_{50}N_2O_8$: 662.356); ^1H NMR ($CDCl_3$) δ 6.39 (1H, dd, *J*=10.0 and 16.0 Hz, 4-H), 5.98 (1H, d, *J*=15.5 Hz, 2-H), 5.45 (1H, br t, 15-H), 4.37 (1H, dd, *J*=8.2 and 8.2 Hz, 10-H), 4.01 (1H, ddd, *J*=4.0, 6.0 and 10.0 Hz, 8-H), 3.51 (3H, s, 10-OCH_3), BOC-γ-aminobutyryl moiety; 5.60 (1H, br d, NH), 3.08 (2H, CH_2), 2.32 (2H, CH_2), 2.05 (2H, CH_2), 1.42 (9H, s, $C(CH_3)_3$), 0.87 (3H, t, *J*=7.4 Hz, CH_3).

Anal Calcd for $C_{38}H_{50}N_2O_8$: C 68.85, H 7.61, N 4.23.
Found: C 68.79, H 7.55, N 4.17.

15-*O*-(BOC-ε-Aminocaproyl)hitachimycin (**9**)

Compound **9** was prepared from **1** (300 mg) and BOC-ε-aminocaproic acid (150 mg) with DCC (300 mg) as described in the preparation of **3**, to give a colorless powder of **9**, 237 mg (54.6%): MP 140~141°C (dec); $[\alpha]_D^{23}$ +87° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (32,100); HR-MS 690.385 (calcd for $C_{40}H_{54}N_2O_8$: 690.388); ^1H NMR ($CDCl_3$) δ 6.40 (1H, dd, *J*=10.5 and 15.0 Hz, 4-H), 5.98 (1H, d, *J*=15.0 Hz, 2-H), 5.50 (1H, br t, 15-H), 4.36 (1H, dd, *J*=8.0 and 8.0 Hz, 10-H), 4.03 (1H, ddd, *J*=3.8, 5.9 and 10.0 Hz, 8-H), 3.51 (3H, s, 10-OCH_3), BOC-ε-aminocaproyl moiety; 5.65 (1H, br d, NH), 3.10 (2H, CH_2), 2.20 and 1.30 (8H, $CH_2\times4$), 1.42 (9H, s, $C(CH_3)_3$).

Anal Calcd for $C_{40}H_{54}N_2O_8$: C 69.53, H 7.88, N 4.06.
Found: C 69.39, H 7.65, N 4.02.

15-*O*-L-Glycylhitachimycin (**10**)

To a solution of **2** (150 mg) in $CHCl_3$ (2.0 ml), TFA (0.05 ml) was added and stirred for 10 minutes at room temperature. The reaction mixture was evaporated under reduced pressure, to afford a brown oil, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH - AcOH (8 : 1 : 1) to give a colorless powder of **10**, 82 mg (64.9%): MP 191~193°C (dec); $[\alpha]_D^{23}$ +135° (*c* 0.5, DMSO); UV λ_{max}^{MeOH} nm (ε) 303 (37,300); HR-MS 534.272 (calcd for $C_{31}H_{38}N_2O_6$: 534.273); ^1H NMR (DMSO) δ 6.42 (1H, dd, *J*=9.5 and 15.2 Hz, 4-H), 6.10 (1H, d, *J*=15.0 Hz, 2-H), 5.42 (1H, br t, 15-H), 4.46 (1H, dd, *J*=8.0 and 8.0 Hz, 10-H), 4.10 (1H, ddd, *J*=4.0, 5.6 and 9.8 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), glycyl moiety; 5.43 (2H, br s, NH_2), 3.57 (2H, CH_2).

Anal Calcd for $C_{31}H_{38}N_2O_6$: C 69.63, H 7.17, N 5.24.
Found: C 69.50, H 7.11, N 5.20.

15-*O*-L-Alanylhitachimycin (**11**)

To a solution of **3** (150 mg) in $CHCl_3$ (2.0 ml), TFA (0.05 ml) was added and set for 10 minutes at room temperature. The reaction mixture was treated in a similar manner to the preparation of **10**, to give a colorless powder of **11**, 102 mg (80.4%): MP 172~175°C (dec); $[\alpha]_D^{23}$ +129° (*c* 0.5, DMSO); UV λ_{max}^{MeOH} nm (ε) 303 (37,300); HR-MS 548.288 (calcd for $C_{32}H_{40}N_2O_6$: 548.288); ^1H NMR (DMSO) δ 6.41 (1H, dd, *J*=9.6 and 15.3 Hz, 4-H), 6.12 (1H, d, *J*=15.8 Hz, 2-H), 5.43 (1H, br t, 15-H), 4.44 (1H, dd, *J*=8.0 and 8.0 Hz, 10-H), 4.07 (1H, ddd, *J*=4.1, 6.0 and 9.6 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), L-alanyl moiety; 5.48 (2H, br s, NH_2), 3.81 (1H, CH), 1.43 (3H, d, *J*=7.0 Hz, CH_3).

Anal Calcd for $C_{32}H_{40}N_2O_6$: C 70.04, H 7.35, N 5.11.
Found: C 69.99, H 7.32, N 5.08.

15-*O*-β-Alanylhitachimycin (**12**)

To a solution of **4** (150 mg) in $CHCl_3$ (2.0 ml), TFA (0.05 ml) was added and set for 10 minutes at room temperature. The reaction mixture was treated in a similar manner to the preparation of **10**, to give a colorless powder of **12**, 93 mg (73.3%): MP 181~184°C (dec); $[\alpha]_D^{23}$ +106° (*c* 0.5, DMSO); UV λ_{max}^{MeOH} nm (ε) 303 (36,800); HR-MS 548.288 (calcd for $C_{32}H_{40}N_2O_6$: 548.288); ^1H NMR (DMSO) δ 6.45 (1H, dd, *J*=10.6 and 15.0 Hz, 4-H), 5.99 (1H, d, *J*=14.8 Hz, 2-H), 5.48 (1H, br t, 15-H), 4.40 (1H, dd, *J*=7.6 and 7.6 Hz, 10-H), 4.05 (1H, ddd, *J*=4.0, 5.8 and 9.8 Hz, 8-H), 3.53 (3H, s, 10-OCH_3), β-alanyl moiety; 5.65 (2H, br s, NH_2), 3.20 (2H, dd, CH_2), 2.60 (2H, dd, CH_2).

Anal Calcd for $C_{32}H_{40}N_2O_6$: C 70.04, H 7.35, N 5.11.
Found: C 69.95, H 7.31, N 5.06.

15-*O*-L-Lysylhitachimycin (**13**)

5 (150 mg) was treated with TFA in a similar manner to the preparation of **10**, to give a colorless powder of **13**, 72 mg (63.9%): MP 152~154°C (dec); $[\alpha]_D^{23}$ +96° (*c* 0.5, DMSO); UV λ_{max}^{MeOH} nm (ε) 302 (37,800); HR-MS 605.347 (calcd for $C_{35}H_{47}N_3O_6$: 605.346); ^1H NMR (DMSO) δ 6.44 (1H, dd, *J*=10.5 and 15.0 Hz, 4-H), 6.01 (1H, d, *J*=14.5 Hz, 2-H), 5.48 (1H, br t, 15-H), 4.39 (1H, dd, *J*=8.2 and 8.2 Hz, 10-H), 4.03 (1H, ddd, *J*=4.0, 5.8 and 9.8 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), L-lysyl moiety; 5.67 (2H, br s, NH_2), 5.58 (2H, br s, NH_2), 3.42 (1H, br t, CH), 2.96 (2H, CH_2), 1.85 and 1.20 (4H, $CH_2\times2$).

Anal Calcd for $C_{35}H_{47}N_3O_6$: C 69.38, H 6.94, N 6.94.
Found: C 68.98, H 6.88, N 6.89.

15-*O*-L-Glutamylhitachimycin (**14**)

6 (200 mg) was treated with TFA in a similar manner to the preparation of **10**, to give a colorless powder of **14**, 89 mg (56.0%): MP 159~160°C (dec); $[\alpha]_D^{23}$ +143° (*c* 0.5, DMSO); UV λ_{max}^{MeOH} nm (ε) 302 (37,100); HR-MS 607.299 (calcd for $C_{34}H_{43}N_2O_8$: 607.302); ^1H NMR (DMSO) δ 6.42 (1H, dd, *J*=10.5 and 14.8 Hz, 4-H), 6.01 (1H, d, *J*=14.8 Hz, 2-H), 5.48 (1H, br t, 15-H), 4.39 (1H, dd, *J*=8.1 and 8.1 Hz, 10-H), 4.00 (1H, ddd, *J*=4.3, 6.1 and 9.6 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), L-glutamyl moiety; 5.63 (2H, br s, NH_2), 3.23 (1H, br t, CH), 2.40 and 1.60 (6H, $CH_2\times3$).

Anal Calcd for $C_{34}H_{43}N_2O_8$: C 67.29, H 6.98, N 4.62.
Found: C 67.01, H 6.91, N 4.59.

15-*O*-L-Aminobutyrylhitachimycin (**15**)

7 (150 mg) was treated with TFA in a similar manner to the preparation of **10**, to give a colorless powder of **15**, 79 mg (62.0%): MP 191~193°C (dec); $[\alpha]_D^{23}$ +97° (*c* 0.5, DMSO); UV λ_{max}^{MeOH} nm (ε) 302 (37,200); HR-MS 562.304 (calcd for $C_{33}H_{42}N_2O_6$: 562.304); ^1H NMR (DMSO) δ 6.43 (1H, dd, *J*=10.0 and 15.9 Hz, 4-H), 6.00 (1H, d, *J*=15.0 Hz, 2-H), 5.48 (1H, br t, 15-H), 4.42 (1H, dd, *J*=8.1 and 8.1 Hz, 10-H), 4.02 (1H, ddd, *J*=3.8, 5.4 and 9.8 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), L-aminobutyryl moiety; 5.51 (2H, br s, NH_2), 3.78 (1H, br t, CH), 1.86 (2H, m, CH_2), 0.99 (3H, t, *J*=7.0 Hz, CH_3).

Anal Calcd for $C_{33}H_{42}N_2O_6$: C 70.42, H 7.53, N 4.98.
Found: C 70.02, H 7.52, N 4.88.

15-*O*-γ-Aminobutyrylhitachimycin (**16**)

8 (150 mg) was treated with TFA in a similar manner to the preparation of **10**, to give a colorless powder of **16**, 110 mg (86.4%): MP 142~144°C (dec); $[\alpha]_D^{23}$ +84° (*c* 0.5, DMSO); UV λ_{max}^{MeOH} nm (ε) 302 (42,100); HR-MS 562.305 (calcd for $C_{33}H_{42}N_2O_6$: 562.304); ^1H NMR (DMSO) δ 6.42 (1H, dd, *J*=9.8 and 15.6 Hz, 4-H), 6.01 (1H, d, *J*=15.2 Hz, 2-H), 5.48 (1H, br t, 15-H), 4.39 (1H, dd, *J*=8.1 and 8.1 Hz, 10-H), 4.01 (1H, ddd, *J*=4.0, 5.8 and 9.6 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), γ-aminobutyryl moiety; 5.73 (2H, br s, NH_2), 3.14 (2H, br t, CH_2), 2.43 (2H, br t, CH_2), 2.01 (2H, m, CH_2).

Anal Calcd for $C_{33}H_{42}N_2O_6$: C 70.42, H 7.53, N 4.98.
Found: C 69.98, H 7.43, N 4.86.

15-*O*-ε-Aminocaproylhitachimycin (**17**)

9 (200 mg) was treated with TFA in a similar manner to the preparation of **10**, to give a colorless powder of **17**, 92 mg (71.7%): MP 182~185°C (dec); $[\alpha]_D^{23}$ +102° (*c* 0.5, DMSO); UV λ_{max}^{MeOH} nm (ε)

304 (38,600); HR-MS 590.336 (calcd for $C_{35}H_{46}N_2O_6$: 590.335); ^1H NMR (DMSO) δ 6.42 (1H, dd, J=10.0 and 14.7 Hz, 4-H), 6.00 (1H, d, J=14.8 Hz, 2-H), 5.49 (1H, br t, 15-H), 4.42 (1H, dd, J=8.0 and 8.0 Hz, 10-H), 4.05 (1H, ddd, J=4.0, 5.9 and 9.8 Hz, 8-H), 3.52 (3H, s, 10-OCH_3), ε-aminocaproyl moiety; 5.58 (2H, br s, NH_2), 3.10 (2H, br t, CH_2), 2.34 (2H, br t, CH_2), 1.80 and 1.30 (4H, $CH_2 \times 2$).

Anal Calcd for $C_{35}H_{46}N_2O_6$: C 71.15, H 7.85, N 4.74.
Found: C 71.00, H 7.76, N 4.69.

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