CHEMICAL MODIFICATION OF HITACHIMYCIN

SYNTHESIS, ANTIBACTERIAL, CYTOCIDAL AND *IN VIVO* ANTITUMOR ACTIVITIES OF HITACHIMYCIN DERIVATIVES

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Several acyl derivatives of hitachimycin have been synthesized and their activities, including antibacterial, cytocidal against HeLa cells and *in vivo* antitumor against sarcoma 180, evaluated. Some of these derivatives showed higher antitumor activity than hitachimycin. Among the derivatives, 11-*O*-propionyl-15-*O*-butyrylhitachimycin (**12**) and the 11-*O*-acylhitachimycins (**15**~**17**) were most effective in *in vivo* assay.

Hitachimycin (**1**)[†††,2] a novel macrocyclic lactam antibiotic isolated from the culture broth of actinomyces strain KM-4927, shows antitumor[3], antibacterial and antiprotozoal activities. Recently, KOMIYAMA *et al.*, have reported the mode of action of hitachimycin[4,5]. The structure of hitachimycin has been confirmed as a new 19-membered ring lactam including trienamide and 1,3-diketone moieties[2]. The antibiotic was readily distinguishable from the ansamycin antibiotics, herbimycin A[6], geldanamycin[7] and trienomycin[8], in that hitachimycin possesses no aromatic or quinonoid nucleus in the 19-membered ring moiety.

Since hitachimycin is hardly soluble in water and other organic solvents, it is difficult to utilize the drug for *in vivo* evaluation. To obtain highly soluble and highly active derivatives of hitachimycin, modification of the C-11 or/and C-15 hydroxy groups was carried out. In this paper, we describe the synthesis of acyl derivatives of hitachimycin and their *in vivo* antitumor activities against sarcoma 180.

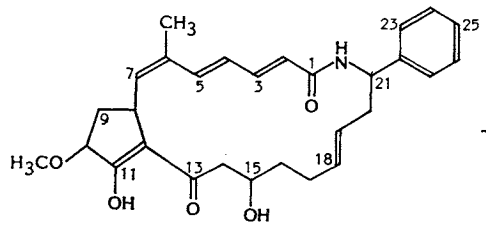
Synthesis

Hitachimycin (**1**) has two hydroxy groups at the C-11 and C-15 positions in its molecule, the 11-hydroxy group being an enol of β-diketone. Treatment of **1** with acid anhydrides such acetic anhydride, propionic anhydride or butyric anhydride in pyridine at room temperature gave the 11,15-di-*O*-acylates (**3**~**5**), respectively. In the ^{13}C NMR spectra of these acylates (**3**~**5**), signals assignable to two acyl groups, and an upfield shift (Δ 29.3~29.6) of the C-11 olefinic carbon, 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

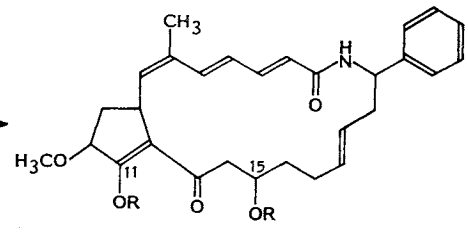
[†††] Hitachimycin was identified with stubomycin by their physico-chemical properties but the production strain of each compound was different[1].

observed, which indicated both hydroxy groups at the C-11 and C-15 positions were acylated.

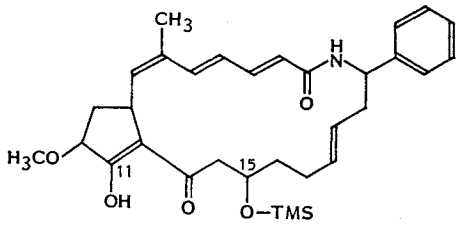
The hydroxy group at the C-15 position is more reactive than that at C-11 on acylation under basic conditions, but the C-11 ester bond of the diacylate is more labile than the C-15 one towards acid hydrolysis. Two alternative processes were considered for synthesis of 15-*O*-acylates of hitachi-



Hitachimycin (**1**)



3 R = $COCH_3$ (Ac)
4 R = $COCH_2CH_3$ (Prl)
5 R = $COCH_2CH_2CH_3$ (Bul)



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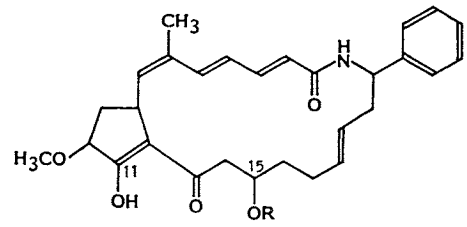
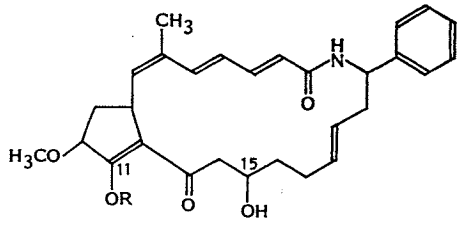
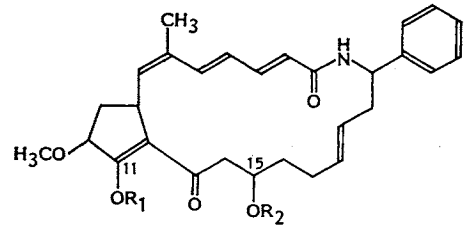


6 R = Ac
7 R = Prl
8 R = Bul



15 R = Ac
16 R = Prl
17 R = Bul



9 R_1 = Ac R_2 = Prl
10 R_1 = Ac R_2 = Bul
11 R_1 = Prl R_2 = Ac
12 R_1 = Prl R_2 = Bul
13 R_1 = Bul R_2 = Ac
14 R_1 = Bul R_2 = Prl

Prl: Propionyl, Bul: butyryl.

Table 1. ^{13}C NMR chemical shifts†

Carbon No.	Hitachimycin	3	4	5	6	7	8
CONH	167.6	166.9	166.7	166.7	166.8	167.2	166.8
C-2	124.5	124.6	124.5	124.6	124.5	124.3	124.7
C-3	141.9	141.4	141.3	141.2	141.4	141.6	141.2
C-4	127.8	127.5	127.4	127.3	127.3	127.1	127.3
C-5	136.9	136.3	136.2	136.3	136.0	136.2	135.9
C-6	131.5	133.0	132.8	132.9	130.7	130.6	130.6
C-7	134.9	134.1	134.2	134.2	135.0	135.2	135.1
C-8	35.1	38.3	38.1	38.1	33.7	33.7	33.7
C-9	35.5	35.9	35.9	36.0	36.7	36.7	36.8
C-10	81.2	81.2	81.1	81.1	80.6	80.7	80.7
C-11	185.5	155.9	156.2	156.2	186.6	186.3	186.2
C-12	112.6	128.6	128.6	128.4	111.8	111.8	111.8
C-13	196.5	195.0	194.8	194.7	193.0	193.5	193.6
C-14	46.5	47.0	47.1	47.2	41.6	41.6	41.5
C-15	68.1	69.2	68.9	68.9	69.2	69.0	69.0
C-16	39.1	35.9	35.8	36.3	34.9	34.9	34.9
C-17	29.4	28.9	28.9	29.0	28.8	28.8	28.9
C-18	134.4	133.5	133.6	133.6	133.2	133.4	133.3
C-19	126.9	126.9	126.8	126.9	127.5	127.4	127.5
C-20	41.6	41.2	41.2	41.2	41.5	41.4	41.5
C-21	52.4	51.9	51.7	51.7	51.7	51.9	51.8
C-22	142.2	141.7	141.6	141.7	141.5	141.3	141.5
C-23	126.9	126.3	126.2	126.3	126.3	126.2	126.2
C-24	128.7	128.6	128.6	128.6	128.6	128.7	128.7
C-25	127.4	127.3	127.3	127.3	127.3	127.4	127.4
C-26	128.7	128.6	128.6	128.6	128.6	128.7	128.7
C-27	126.9	126.3	126.2	126.3	126.3	126.2	126.2
6-CH_3	20.0	19.9	20.0	20.0	20.1	20.1	20.1
10-OCH_3	58.1	56.7	56.8	56.7	57.9	57.9	57.9
11-*O*-acyl group							
C-1		167.3	170.8	170.0			
C-2		19.9	27.5	35.8			
C-3			8.7	18.1			
C-4				13.5			
15-*O*-acyl group							
C-1		170.4	173.8	172.9	170.4	173.9	173.0
C-2		20.9	27.6	36.1	21.0	27.6	36.3
C-3			9.2	18.5		9.1	18.4
C-4				13.7			13.7

† Chemical shifts in ppm are downfield from TMS.

mycin bearing a free hydroxy group at the C-11 position. One was a selective acylation of the 15-hydroxy group and the other a selective removal of the 11-*O*-acyl group of 11,15-di-*O*-acylate. **1** was treated with an equivalent amount of butyric anhydride in pyridine under cooling, but the 15-butyrate was obtained in only 18.4% yield and large amount of starting material was recovered. Accordingly, the acid hydrolysis was examined. Trifluoroacetic acid (TFA) was found to be the most effective acid so far examined. The 11,15-di-*O*-acylates (**3**~**5**) was treated with TFA at room temperature for 5 minutes to obtain the 15-*O*-acylates (**6**~**8**) in quantitative yields. The ^{13}C NMR spectra of these compounds showed signals assignable to one acyl group and the C-11 carbon signal (δ 186.2~186.6) exhibited a downfield shift compared to the corresponding chemical shift of **1**.

for hitachimycin derivatives.

9	10	11	12	13	14	15	16	17
167.1	167.0	166.7	167.0	166.7	166.7	167.0	166.8	166.8
124.7	124.5	124.3	124.4	124.5	124.6	124.4	124.4	124.6
141.4	141.2	141.4	141.2	141.2	141.1	141.2	141.1	141.3
127.3	127.1	127.4	127.1	127.3	127.2	127.3	127.3	127.3
136.3	136.3	136.2	136.1	136.2	136.3	136.2	136.0	136.3
133.0	132.9	132.9	132.9	132.9	133.1	132.9	132.8	132.8
134.0	134.4	134.2	134.2	134.2	134.1	134.0	133.9	134.1
37.9	38.1	38.2	38.1	38.2	38.2	38.0	38.0	38.1
35.9	36.0	35.8	35.9	36.1	36.2	36.0	35.8	35.9
81.1	81.0	80.9	81.2	81.1	80.9	81.1	81.0	81.3
156.0	156.1	156.3	156.2	156.0	156.3	156.4	156.3	156.3
128.7	128.6	128.5	128.6	128.7	128.4	128.6	128.6	128.6
195.0	194.7	194.7	194.8	195.1	194.8	194.7	195.0	195.1
47.1	47.2	47.3	47.2	47.0	47.1	46.9	46.6	46.3
69.1	68.9	68.8	69.0	69.1	68.9	68.7	68.3	68.1
35.6	35.4	35.8	36.1	35.9	36.2	38.1	38.6	39.0
28.9	29.0	29.1	28.8	28.9	29.0	29.0	29.0	29.4
133.5	133.6	133.4	133.5	133.5	133.5	133.5	134.0	134.4
127.3	127.4	127.0	126.9	126.7	126.9	126.9	126.9	126.9
41.3	41.5	41.3	41.3	41.1	41.4	41.5	41.5	41.7
51.9	51.7	51.9	51.9	51.6	51.7	52.0	51.9	52.4
141.7	141.5	141.7	141.6	141.7	141.7	141.8	141.6	142.3
126.3	126.2	126.3	126.3	126.3	126.2	126.3	126.4	126.3
128.6	128.7	128.6	128.6	128.6	128.7	128.6	128.7	128.6
127.3	127.4	127.4	127.3	127.3	127.3	127.3	127.4	127.3
128.6	128.7	128.6	128.6	128.6	128.7	128.6	128.7	128.6
126.3	126.2	126.3	126.3	126.3	126.2	126.3	126.4	126.3
20.0	20.1	20.0	20.0	19.9	19.9	20.0	20.0	20.1
56.7	56.8	56.9	56.7	56.9	56.8	56.8	56.8	56.8
167.4	167.6	170.9	170.6	170.0	170.1	167.4	170.8	170.1
19.8	19.8	27.5	27.4	35.9	35.8	19.9	27.6	36.0
		8.8	9.1	18.2	18.2		8.8	18.2
				13.5	13.4			13.5
173.9	173.0	170.2	172.8	170.1	173.7			
27.5	36.2	20.7	36.3	20.9	27.5			
9.2	18.3		18.6		9.1			
	13.6		13.7					

The 11,15-di-*O*-acylates (**9**~**14**) substituted with two different acyl groups, were synthesized by treatment of **6**~**8** with appropriate acid anhydrides.

In order to modify the C-11 hydroxy group selectively, it is necessary to protect the C-15 hydroxy group which is more reactive as described above. Since the acyl group at the C-11 position is labile in acidic and basic conditions, the removal of the protective group must proceed around neutrality. A trimethylsilyl (TMS) group was found to be suitable for this purpose. Treatment of **1** with trimethylsilyl chloride in pyridine afforded 15-*O*-trimethylsilylhitachimycin (**2**). **2** was reacted with an acid anhydride in pyridine to afford 11-*O*-acyl-15-*O*-trimethylsilylhitachimycins, followed by methanolysis to remove TMS group, giving the 11-*O*-acylates (**15**~**17**).

Chemical shift values for ^{13}C NMR spectra of all synthesized acylates are listed in Table 1. From comparison of the chemical shifts of the acyl groups of these compounds, it was found that there was a correlation between the bonding position of the acyl groups and the chemical shifts of the acyl carbons, as shown in Table 2, which may be useful for determining the position of acyl groups in the other hitachimycin derivatives. A similar correlation between bonding position and chemical shifts is known in the acyl derivatives of spiramycin I[9].

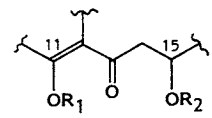
Cytocidal and Antibacterial Activities

Cytocidal activities against HeLa cells measured by IC_{50} values and MIC (μg/ml) against various bacteria of hitachimycin derivatives are given in Table 3. All compounds showed lower antibacterial activities than that of hitachimycin. On the other hand, 11,15-di-*O*-acetyl (**3**) and 11,15-di-*O*-butyrylhitachimycin (**5**) showed high cytocidal activities. Among the derivatives, there were no correlation between antibacterial activities and cytocidal activities.

Antitumor Activity

Antitumor activities (in life span: ILS) at optimal dose of hitachimycin derivatives against sarcoma 180 are given in Table 4. It is notable that 11-*O*-propionyl-15-*O*-butyrylhitachimycin

Table 2. ^{13}C NMR chemical shifts† of acyl groups of hitachimycin derivatives.



Acyl carbon No.	Bonding position	
	C-11 (R_1)	C-15 (R_2)
Acetyl 1	167.3~167.6	170.1~170.4
Acetyl 2	19.8~19.9	20.7~20.9
Propionyl 1	170.8~170.9	173.7~173.9
Propionyl 2	27.5~27.6	27.5~27.6
Propionyl 3	8.7~8.8	9.1~9.2
Butyryl 1	170.0~170.1	172.8~173.0
Butyryl 2	35.8~35.9	36.1~36.3
Butyryl 3	18.1~18.2	18.4~18.6
Butyryl 4	13.4~13.5	13.6~13.7

† Chemical shifts in ppm are downfield from TMS.

Table 3. MIC, cytocidal and hemolysis activities of hitachimycin derivatives.

Compound No.	Acyl group		Cytocidal[a] HeLa cell	Hemolysis[b]	MIC[c] (μg/ml)			
	11	15			SA	BS	ML	MS
1	OH	OH	0.39	6.3	0.2	<0.1	0.2	50
3	Ac	Ac	0.10	>25	3.12	0.78	3.12	>50
4	Prl	Prl	6.25	>25	>50	1.56	>50	>50
5	Bul	Bul	0.10	>25	6.25	0.4	6.25	>50
6	OH	Ac	1.56	>25	50	1.56	12.5	>50
7	OH	Prl	6.25	>25	12.5	1.56	3.12	>50
8	OH	Bul	0.39	>25	3.12	0.4	3.12	>50
9	Ac	Prl	0.39	>25	25	3.12	25	>50
10	Ac	Bul	0.39	>25	12.5	1.56	12.5	>50
11	Prl	Ac	1.56	>25	6.25	1.56	6.25	>50
12	Prl	Bul	1.56	3.9	25	1.56	25	>50
13	Bul	Ac	1.56	>25	6.25	3.12	6.25	>50
14	Bul	Prl	0.39	7.8	12.5	1.56	6.25	>50
15	Ac	OH	1.56	>25	50	3.12	12.5	>50
16	Prl	OH	1.56	3.9	25	3.12	12.5	>50
17	Bul	OH	1.56	0.2	12.5	1.56	12.5	>50

[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).

Prl: Propionyl, Bul: butyryl.

Table 4. Antitumor activity of hitachimycin derivatives against sarcoma 180[a].

Compound No.	Acyl group		Total dose	Dose (mg/kg×days)	ILS (%)	Survivors[b]
	11	15				
1	OH	OH	75	15.0×5	188.6	1/5
3	Ac	Ac	75	15.0×5	94.3	0/5
4	Prl	Prl	150	30.0×5	70.0	0/5
5	Bul	Bul	75	15.0×5	170.0	1/5
6	OH	Ac	150	30.0×5	48.6	0/5
7	OH	Prl	75	15.0×5	21.4	0/5
8	OH	Bul	150	30.0×5	66.7	0/5
9	Ac	Prl	75	15.0×5	178.9	2/5
10	Ac	Bul	150	30.0×5	71.9	0/5
11	Prl	Ac	150	30.0×5	32.9	0/5
12	Prl	Bul	75	15.0×5	539.5	3/5
13	Bul	Ac	150	30.0×5	159.6	1/5
14	Bul	Prl	7.5	1.5×5	64.9	0/5
15	Ac	OH	150	30.0×5	239.4	0/5
16	Prl	OH	75	15.0×5	442.1	2/5
17	Bul	OH	37.5	7.5×5	423.7	1/5

[a] Inoculum size; 2.5×10^6 cells/mouse (ICR, 6-week old female), [b] number of surviving mice at day 60 (survival/total).
Prl: Propionyl, Bul: butyryl.

(**12**), 11-*O*-propionylhitachimycin (**16**) and 11-*O*-butyrylhitachimycin (**17**) showed an increase in life span about twice that of hitachimycin. The solubility of these compounds in organic solvents, *e.g.* methanol, ethanol, was remarkably improved. A relationship between antitumor activity and type of acyl group including of bonding position was not observed. Nor was there observed a correlation between antitumor and antibacterial activities, but compounds of higher antitumor activity show a higher hemolytic activity.

Experimental

NMR spectra were measured with Jeol FX-90Q and Varian PX-400 spectrometers in $CDCl_3$ 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 Kiesel gel 60 F_{254} with $CHCl_3$ - MeOH (50 : 1). Silica gel column chromatography was performed with Merck Kiesel gel 60.

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 increase ILS: $(T/C-1)\times 100\%$ 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*-Trimethylsilylhitachimycin (**2**)

To a solution of **1** (1.20 g) in pyridine, trimethylsilyl chloride (1.0 ml) was added and set for 7 hours at room temp under N_2 gas atmosphere. The reaction mixture was evaporated under reduced pressure, to give a brown solid, which was chromatographed on a silica gel column with benzene - Me_2CO (25 : 1), to give a yellowish powder of **2** (650 mg, 84.1%) together with non-reacted hitachimycin (301 mg): TLC Rf 0.8; $[\alpha]_D^{23}$ +83.2° (*c* 0.5, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 197.6 (C-13), 184.9 (C-11), 112.3 (C-12), 67.8 (C-15), 46.4 (C-14), 38.9 (C-16), 1.94 ($Si(CH_3)_3$).

11,15-Di-*O*-acetylhitachimycin (**3**)

To a solution of **1** (91 mg) in pyridine (2.0 ml), acetic anhydride (0.2 ml) was added and set for 4 hours at room temp. The reaction mixture 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 benzene - Me_2CO (25 : 1), to afford a colorless powder, 81 mg (76.5%): TLC Rf 0.65; $[\alpha]_D^{23}$ +47.1° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (33,700); High resolution (HR)-MS 561.272 (calcd for $C_{33}H_{39}NO_7$: 561.272); ^1H NMR ($CDCl_3$) δ 5.18 (1H, m, 15-H), 4.72 (1H, dd, *J*=5.8 and 5.8 Hz, 10-H), 4.22 (1H, ddd, *J*=9.4, 8.1 and 1.3 Hz, 8-H), 3.35 (3H, s, 10-OCH_3), 1.87 (3H, s, 6-CH_3), 2.22 (11-$OCOCH_3$), 1.96 (15-$OCOCH_3$).

Anal Calcd for $C_{33}H_{39}NO_7$: C 70.59, H 6.95, N 2.50.
Found: C 70.43, H 7.10, N 2.44.

11,15-Di-*O*-propionylhitachimycin (**4**)

4 was prepared from **1** (240 mg) and propionic anhydride (0.5 ml) as described in the preparation of **3**, 210 mg (70.9%): TLC Rf 0.70; $[\alpha]_D^{23}$ +57.6° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (36,500); HR-MS 589.303 (calcd for $C_{35}H_{43}NO_7$: 589.304); ^1H NMR ($CDCl_3$) δ 7.06 (1H, d, *J*=15.0 Hz, 5-H), 6.35 (1H, dd, *J*=15.0 and 11.0 Hz, 4-H), 5.12 (1H, m, 15-H), 4.75 (1H, ddd, *J*=5.8, 5.8 and 1.2 Hz, 10-H), 4.21 (1H, ddd, *J*=9.4, 8.1 and 1.2 Hz, 8-H), 2.53, 1.19 (11-$OCOCH_2CH_3$), 2.23, 1.08 (15-$OCOCH_2CH_3$).

Anal Calcd for $C_{35}H_{43}NO_7$: C 71.31, H 7.30, N 2.38.
Found: C 70.92, H 7.38, N 2.34.

11,15-Di-*O*-butyrylhitachimycin (**5**)

5 was prepared from **1** (400.0 mg) and butyric anhydride (0.8 ml) as described in the preparation of **3**, 425 mg (82.0%): TLC Rf 0.74; $[\alpha]_D^{23}$ +51.0° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (36,200); HR-MS 617.335 (calcd for $C_{37}H_{47}NO_7$: 617.335); ^1H NMR ($CDCl_3$) δ 5.16 (1H, m, 15-H), 4.78 (1H, br t, *J*=6.0 Hz, 10-H), 4.21 (1H, ddd, *J*=9.3, 8.0 and 1.2 Hz, 8-H), 2.47, 1.71, 0.79 (11-$OCOCH_2CH_2CH_3$), 2.20, 1.60, 0.92 (15-$OCOCH_2CH_2CH_3$).

Anal Calcd for $C_{37}H_{47}NO_7$: C 71.96, H 7.62, N 2.27.
Found: C 71.63, H 7.92, N 2.25.

15-*O*-Acetylhitachimycin (**6**)

To a solution of **3** (50 mg) in $CHCl_3$ (2.0 ml), TFA was added and stirred for 5 minutes at room temp. 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 (100 : 1), to give a yellowish powder, 43 mg (93.0%): TLC Rf 0.45; $[\alpha]_D^{23}$ +63.5° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 301 (39,800); HR-MS 519.264 (calcd for $C_{31}H_{37}NO_6$: 519.262); ^1H NMR ($CDCl_3$) δ 5.22 (1H, m, 15-H), 4.34 (1H, dd, *J*= 9.0 and 9.0 Hz, 10-H), 4.03 (1H, ddd, *J*=16.0, 11.0 and 5.0 Hz, 8-H), 1.88 (3H, d, *J*=1.3 Hz, 6-CH_3), 1.99 (15-$OCOCH_3$).

Anal Calcd for $C_{31}H_{37}NO_6$: C 71.68, H 7.13, N 2.70.
Found: C 70.98, H 7.19, N 2.68.

15-*O*-Propionylhitachimycin (**7**)

4 (50 mg) was treated with TFA (0.5 ml) in a similar manner to the preparation of **6**, to give a

yellowish powder of **7**, 39 mg (86.2%): TLC Rf 0.48; $[\alpha]_D^{23}$ +59.2° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (38,000); HR-MS 533.277 (calcd for $C_{32}H_{39}NO_6$: 533.278); ^1H NMR ($CDCl_3$) δ 5.23 (1H, m, 15-H), 4.33 (1H, dd, *J*=9.0 and 9.0 Hz, 10-H), 4.04 (1H, ddd, *J*=15.3, 11.0 and 4.8 Hz, 8-H), 2.26, 1.10 (15-$OCOCH_2CH_3$).

Anal Calcd for $C_{32}H_{39}NO_6$: C 72.05, H 7.32, N 2.63.
Found: C 71.88, H 7.64, N 2.57.

15-*O*-Butyrylhitachimycin (**8**)

5 (50 mg) was treated with TFA (0.5 ml) in a similar manner to the preparation of **6**, to give a yellowish powder of **8**, 41 mg (92.5%): TLC Rf 0.55; $[\alpha]_D^{23}$ +59.3° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 301 (35,900); HR-MS 547.293 (calcd for $C_{33}H_{41}NO_6$: 547.293); ^1H NMR ($CDCl_3$) δ 5.21 (1H, m, 15-H), 4.33 (1H, dd, *J*=9.0 and 8.5 Hz, 10-H), 4.04 (1H, ddd, *J*=15.7, 11.0 and 4.8 Hz, 8-H), 2.59, 1.76, 1.01 (15-$OCOCH_2CH_2CH_3$).

Anal Calcd for $C_{33}H_{41}NO_6$: C 72.39, H 7.50, N 2.56.
Found: C 72.06, H 7.66, N 2.49.

11-*O*-Acetyl-15-*O*-propionylhitachimycin (**9**)

To a solution of **7** (60 mg) in pyridine (1.0 ml), propionic anhydride (0.1 ml) was added and set for 4 hours at room temp. The reaction mixture was diluted with $CHCl_3$ 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 benzene - Me_2CO, to afford a colorless powder, 42 mg (64.9%): TLC Rf 0.68; $[\alpha]_D^{23}$ +43.9° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (39,000); HR-MS 575.287 (calcd for $C_{34}H_{41}NO_7$: 575.278); ^1H NMR ($CDCl_3$) δ 5.12 (1H, m, 15-H), 4.75 (1H, ddd, *J*=6.0, 5.8 and 1.2 Hz, 10-H), 4.20 (1H, ddd, *J*=9.5, 8.0 and 1.2 Hz, 8-H), 2.21 (11-$OCOCH_3$), 2.23, 1.08 (15-$OCOCH_2CH_3$).

Anal Calcd for $C_{34}H_{41}NO_7$: C 70.96, H 7.13, N 2.43.
Found: C 70.61, H 7.20, N 2.22.

11-*O*-Acetyl-15-*O*-butyrylhitachimycin (**10**)

8 (50 mg) was treated with acetic anhydride in a similar manner to the preparation of **9**, to give a colorless powder of **10**, 33 mg (61.3%): TLC Rf 0.71; $[\alpha]_D^{23}$ +41.1° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 303 (35,200); HR-MS 589.303 (calcd for $C_{35}H_{43}NO_7$: 589.304); ^1H NMR ($CDCl_3$) δ 5.15 (1H, m, 15-H), 4.78 (1H, ddd, *J*=6.0, 5.9 and 1.3 Hz, 10-H), 4.21 (1H, ddd, *J*=9.4, 8.1 and 1.3 Hz, 8-H), 2.22 (11-$OCOCH_3$), 2.20, 1.60, 0.92 (15-$OCOCH_2CH_2CH_3$).

Anal Calcd for $C_{35}H_{43}NO_7$: C 71.31, H 7.30, N 2.38.
Found: C 71.09, H 7.41, N 2.29.

11-*O*-Propionyl-15-*O*-acetylhitachimycin (**11**)

To a solution of **6** (50 mg) in pyridine (1.0 ml), propionic anhydride (0.1 ml) was added and set for 24 hours at room temp. The reaction mixture was treated in a similar manner to the preparation of **9**, to give a colorless powder of **11**, 31 mg (56.0%): TLC Rf 0.72; $[\alpha]_D^{23}$ +71.8° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (32,600); HR-MS 575.287 (calcd for $C_{34}H_{41}NO_7$: 575.287); ^1H NMR ($CDCl_3$) δ 5.13 (1H, m, 15-H), 4.72 (1H, br t, *J*=5.8 Hz, 10-H), 4.22 (1H, ddd, *J*=6.0, 6.0 and 1.2 Hz, 8-H), 2.53, 1.20 (11-$OCOCH_2CH_3$), 1.96 (15-$OCOCH_3$).

Anal Calcd for $C_{34}H_{41}NO_7$: C 70.96, H 7.13, N 2.43.
Found: C 70.69, H 7.26, N 2.38.

11-*O*-Propionyl-15-*O*-butyrylhitachimycin (**12**)

8 (51 mg) was treated with propionic anhydride in a similar manner to the preparation of **11**, to give a colorless powder of **12**, 41 mg (72.9%): TLC Rf 0.73; $[\alpha]_D^{23}$ +64.3° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 303 (37,000); HR-MS 603.318 (calcd for $C_{36}H_{45}NO_7$: 603.319); ^1H NMR ($CDCl_3$) δ 5.17 (1H, m, 15-H), 4.77 (1H, ddd, *J*=6.0, 5.8 and 1.2 Hz, 10-H), 4.21 (1H, ddd, *J*=6.0, 6.0 and 1.2 Hz, 8-H), 2.53, 1.19 (11-$OCOCH_2CH_3$), 2.20, 1.60, 0.92 (15-$OCOCH_2CH_2CH_3$).

Anal Calcd for $C_{36}H_{45}NO_7$: C 71.64, H 7.46, N 2.32.
Found: C 71.48, H 7.51, N 2.29.

11-*O*-Butyryl-15-*O*-acetylhitachimycin (**13**)

To a solution of **6** (60 mg) in pyridine (1.0 ml), butyric anhydride (0.1 ml) was added and set for 24 hours at room temp. The reaction mixture was treated in a similar manner to the preparation of **9**, to give a colorless powder of **13**, 34 mg (49.9%): TLC Rf 0.70; $[\alpha]_D^{23}$ +53.6° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 301 (34,600); HR-MS 589.304 (calcd for $C_{35}H_{43}NO_7$: 589.304); ^1H NMR ($CDCl_3$) δ 5.18 (1H, m, 15-H), 4.72 (1H, br t, *J*=6.0 Hz, 10-H), 4.21 (1H, ddd, *J*=9.0, 8.1 and 1.1 Hz, 8-H), 2.47, 1.72, 0.80 (11-$OCOCH_2CH_2CH_3$), 1.96 (15-$OCOCH_3$).

Anal Calcd for $C_{35}H_{43}NO_7$: C 71.31, H 7.30, N 2.38.
Found: C 71.29, H 7.38, N 2.35.

11-*O*-Butyryl-15-*O*-propionylhitachimycin (**14**)

7 (50 mg) was treated with butyric anhydride in a similar manner to the preparation of **13**, to give a colorless powder of **14**, 41 mg (72.5%): TLC Rf 0.74; $[\alpha]_D^{23}$ +72.1° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (37,100); HR-MS 603.319 (calcd for $C_{36}H_{45}NO_7$: 603.319); ^1H NMR ($CDCl_3$) δ 5.12 (1H, m, 15-H), 4.71 (1H, br t, *J*=6.0 Hz, 10-H), 4.20 (1H, br t, *J*=8.1 Hz, 8-H), 2.48, 1.71, 0.80 (11-$OCOCH_2CH_2CH_3$), 2.23, 1.08 (15-$OCOCH_2CH_3$).

Anal Calcd for $C_{36}H_{45}NO_7$: C 71.64, H 7.46, N 2.32.
Found: C 71.39, H 7.51, N 2.29.

11-*O*-Acetylhitachimycin (**15**)

To a solution of **2** (60 mg) in pyridine (1.0 ml), acetic anhydride (0.05 ml) was added and set for 4 hours at room temp. The reaction mixture was diluted with $CHCl_3$ (50 ml) and poured into H_2O. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure, to give a brown solid. The brown solid was dissolved in MeOH and stirred for 12 hours. The solution was evaporated under reduced pressure, to give a brown oil, which was chromatographed on a silica gel column with $CHCl_3$ - MeOH (100:1), to afford as a colorless powder **15**, 23 mg (41.8%) with hitachimycin (15 mg) and compound **3** (10 mg): TLC Rf 0.64; $[\alpha]_D^{23}$ +130.5° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 304 (43,800); HR-MS 519.261 (calcd for $C_{31}H_{37}NO_6$: 519.262); ^1H NMR ($CDCl_3$) δ 4.72 (1H, ddd, *J*=6.1, 5.8 and 1.0 Hz, 10-H), 4.22 (1H, ddd, *J*=9.1, 8.0 and 1.0 Hz, 8-H), 3.90 (1H, br t, *J*=10.2 Hz, 15-H), 2.21 (11-$OCOCH_3$).

Anal Calcd for $C_{31}H_{37}NO_6$: C 71.68, H 7.13, N 2.70.
Found: C 70.98, H 7.33, N 2.49.

11-*O*-Propionylhitachimycin (**16**)

To a solution of **2** (60 mg) in pyridine (1.0 ml), propionic anhydride (0.05 ml) was added and set for 24 hours at room temp. The reaction mixture was treated in a similar manner to the preparation of **15**, to give a colorless powder of **16**, 18 mg (27.2%) with hitachimycin (16 mg) and compound **4** (6 mg): TLC Rf 0.71; $[\alpha]_D^{23}$ +119.3° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (42,000); HR-MS 533.277 (calcd for $C_{32}H_{39}NO_6$: 533.278); ^1H NMR ($CDCl_3$) δ 4.75 (1H, br t, *J*=6.0 Hz, 10-H), 4.22 (1H, ddd, *J*=8.8, 8.1 and 1.0 Hz, 8-H), 3.89 (1H, br t, *J*=9.9 Hz, 15-H), 2.53, 1.19 (11-$OCOCH_2CH_3$).

Anal Calcd for $C_{32}H_{39}NO_6$: C 72.05, H 7.32, N 2.63.
Found: C 71.96, H 7.41, N 2.56.

11-*O*-Butyrylhitachimycin (**17**)

To a solution of **2** (60 mg) in pyridine (1.0 ml), butyric anhydride (0.05 ml) was added and set for 24 hours at room temp. The reaction mixture was treated in a similar manner to the preparation of **15**, to give colorless powder of **17**, 15 mg (24.8%) with hitachimycin (13 mg) and compound **5** (7 mg): TLC Rf 0.73; $[\alpha]_D^{23}$ +98.2° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (45,300); HR-MS 547.293 (calcd for $C_{33}H_{41}NO_6$: 547.293); ^1H NMR ($CDCl_3$) δ 4.78 (1H, br t, *J*=6.1 Hz, 10-H), 4.21 (1H, ddd, *J*=9.0, 8.3 and 1.0 Hz, 8-H), 2.47, 1.71, 0.79 (11-$OCOCH_2CH_2CH_3$).

Anal Calcd for $C_{33}H_{41}NO_6$: C 72.39, H 7.50, N 2.56.
Found: C 72.31, H 7.58, N 2.53.

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