CHEMICAL MODIFICATION OF HITACHIMYCIN

II. SYNTHESIS AND ANTITUMOR ACTIVITIES OF CARBONATE 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

(Received for publication October 27, 1988)

Several carbonate derivatives of hitachimycin have been synthesized and evaluated their activities including antibacterial, cytocidal against HeLa cells and *in vivo* antitumor against Sarcoma 180. Some of these derivatives showed higher antitumor activity than hitachimycin. Among the derivatives, 11,15-di-*O*-methoxycarbonylhitachimycin (**2**), 11,15-di-*O*-ethoxycarbonylhitachimycin (**3**) and 15-*O*-methoxycarbonylhitachimycin (**9**) were most effective in *in vivo* assay.

Hitachimycin (**1**)[1),†††] is a macrocyclic lactam antibiotic isolated from the culture broth of actinomyces strain KM-4927, which shows antitumor[3)], antibacterial and antiprotozoal activities. Hitachimycin has an unique 19-membered ring lactam structure including trienamide and 1,3-diketone moieties[1)]. The mode of action of hitachimycin and combined effect with bleomycin has been reported by KOMIYAMA *et al.*[4,5)].

Since hitachimycin is hardly soluble in water and other organic solvents, it is difficult to utilize the drug for *in vivo* evaluation. In the course of the chemical modification of hitachimycin to obtain highly soluble and highly active derivatives, 11 and 15-*O*-acyl derivatives have been synthesized and some of them showed superior antitumor effect *in vivo*, as reported in a previous paper[6)]. In this paper, we describe the synthesis of carbonate derivatives of hitachimycin and their *in vivo* antitumor activities against Sarcoma 180.

Synthesis

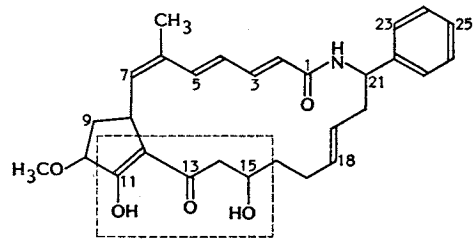
Hitachimycin (**1**) has two hydroxy groups at the C-11 and C-15 positions in its molecule, the 11-hydroxy group being an enol of β-diketone. Treatment of **1** with several alkyl chloroformate such as methyl[7)], ethyl, propyl, *n*-butyl, *iso*-butyl, 2,2,2-trichloroethyl[8)] and vinyl chloroformate[9)], in pyridine at room temperature gave the 11,15-dicarbonates (**2**~**8**), respectively. In the ^{13}C NMR spectra of these carbonates (**2**~**8**), a paired signals assignable to carbonyl carbon of each carbonate group (δ 154.9~156.2) and corresponded alkyl carbons were observed. Furthermore, upfield shift (Δ 29.3~

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

29.6) of the C-11 olefinic carbon and the C-16 carbon, and a downfield shift of the C-15 carbon compared with that of **1** were observed, which indicated both hydroxy groups at the C-11 and C-15 positions were substituted.

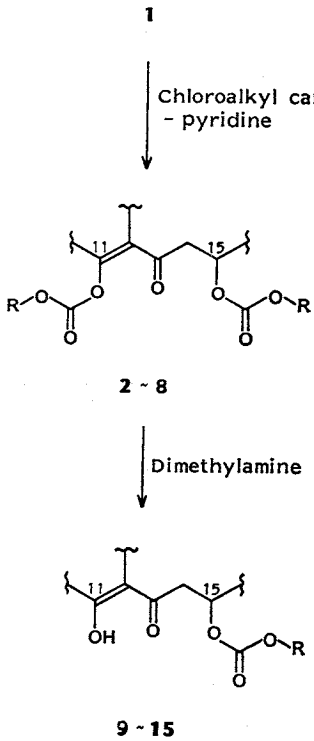
In a previous report[6], we showed that the hydroxy group at the C-15 position is more reactive than that at C-11 on acylation under basic conditions, but the C-11 ester bond of the diacylate is more labile than the C-15 one against hydrolysis under acidic or basic conditions. Two alternative processes were considered for synthesis of 15-monocarbonates of hitachimycin bearing a free hydroxy group at the C-11 position. One was a selective carbonylation of the 15-hydroxy group of hitachimycin and the other a selective removal of the 11-carbonate group of 11,15-dicarbonate. When **1** was treated with an equivalent amount of alkyl chloroformate in pyridine under cooling, the yield of the 15-carbonate was low and large amount of starting material was recovered. Accordingly, the solvolysis of 11,15-dicarbonate under acidic or basic condition was examined, and base solvolysis with dimethylamine was found to be the most effective so far examined.

The 11,15-dicarbonates (**2**~**8**) was treated with dimethylamine in chloroform at room temperature for 10 minutes to obtain the 15-monocarbonates (**9**~**15**) in high yields. The ^{13}C NMR spectra of these compounds (**9**~**15**) showed signals assignable to one carbonate group and the C-11 carbon signal (δ 186.2~186.6) exhibited a downfield shift compared to the corresponding



Hitachimycin (**1**)

Scheme 1.



2, **9** R=CH_3
3, **10** R=CH_2CH_3
4, **11** R=$CH_2CH_2CH_3$
5, **12** R=$CH_2CH_2CH_2CH_3$
6, **13** R=$CH_2CH(CH_3)_2$
7, **14** R=CH_2CCl_3
8, **15** R=$CH{=}CH_2$

Table 1. ^{13}C NMR chemical shift†

Carbon No.	Hitachimycin	2	3	4	5	6	7	8
CONH	167.6	166.5	166.7	166.5	166.8	167.0	166.8	166.5
2	124.5	124.4	124.5	124.5	124.6	124.7	124.6	124.4
3	141.9	141.4	141.8	141.5	141.7	141.4	141.7	141.6
4	127.8	126.9	127.0	126.8	127.0	127.2	127.1	126.9
5	136.9	136.3	136.5	136.3	136.7	136.1	136.3	136.3
6	131.5	133.2	132.9	133.0	133.1	133.2	133.1	133.3
7	134.9	133.5	133.4	133.7	133.2	133.6	133.6	133.7
8	35.1	37.9	38.0	38.1	38.4	38.6	37.9	37.8
9	35.5	36.1	36.1	35.8	36.4	35.9	35.9	36.2
10	81.2	80.8	81.0	81.2	80.9	81.0	81.2	80.2
11	185.5	151.5	151.6	151.8	151.3	152.1	152.3	151.4
12	112.6	125.2	125.3	125.2	125.0	125.7	125.7	125.3
13	196.5	194.3	195.0	195.1	194.8	195.1	194.8	195.0
14	46.5	46.9	46.8	46.9	47.0	47.0	47.2	46.8
15	68.1	73.3	73.6	73.5	73.6	73.7	74.1	73.2
16	39.1	35.7	35.9	36.1	35.3	35.7	35.9	35.6
17	29.4	28.6	28.6	28.4	28.4	28.6	28.9	28.7
18	134.4	133.3	133.8	133.6	133.8	133.6	133.5	133.4
19	126.9	127.3	127.3	127.4	127.4	127.4	127.4	127.4
20	41.6	41.0	41.2	41.3	41.2	41.1	41.3	41.0
21	52.4	51.7	51.8	51.7	51.7	51.7	51.9	51.7
22	142.2	141.6	141.5	141.6	141.6	141.8	141.7	141.5
23	126.9	126.2	126.3	126.3	126.2	126.4	126.2	126.2
24	128.7	128.6	128.7	128.7	128.6	128.6	128.7	128.6
25	127.4	127.3	127.3	127.2	127.3	127.3	127.3	127.3
26	128.7	128.6	128.7	128.7	128.6	128.6	128.7	128.6
27	126.9	126.2	126.3	126.3	126.2	126.4	126.2	126.2
6-CH_3	20.0	19.9	20.0	20.1	20.0	20.1	20.1	19.9
10-OCH_3	58.1	57.0	57.1	57.2	57.6	57.3	57.6	57.1
11-Carbonate group								
CO		155.2	155.0	154.9	154.9	155.6	156.3	155.9
1		55.8	65.1	65.0	65.2	70.8	67.3	142.3
2			14.2	23.6	31.5	31.4	78.5	112.2
3				10.0	20.6	19.3		
4					13.9			
15-Carbonate group								
CO		155.7	155.6	155.7	155.8	155.8	156.9	156.2
1		54.7	64.5	64.7	65.1	71.3	66.1	142.0
2			14.1	23.0	31.2	31.1	78.2	111.7
3				9.8	20.1	19.0		
4					13.8			

† Chemical shifts in ppm are downfield from TMS.

chemical shift of **1**. On the other hand, if the excess amount of base was employed in this reaction, an elimination of 15-hydroxy group was occurred with a solvolysis of 11-carbonate group, to give 15-deoxo-14-enohitachimycin (**16**). A similar elimination of 15-hydroxy group was observed in the solvolysis reaction of 15-acyl compounds.

In the previous paper[6], we have reported the synthesis and antitumor activity of 11,15-*O*-diacyl and 11 or 15-*O*-monoacyl derivatives of hitachimycin and shown that 11-*O*-monoacyl derivatives were superior in antitumor assay. So we have interested to synthesize the 11-monocarbonate derivatives of hitachimycin which were expected to have higher potency. But every examination failed,

values for hitachimycin derivatives.

9	10	11	12	13	14	15	16
166.6	166.5	166.5	166.4	166.5	165.9	166.3	167.6
124.3	124.3	124.3	124.4	124.4	124.2	124.5	123.6
141.6	141.6	141.5	141.6	141.8	141.7	141.2	141.6
127.1	127.4	127.3	127.1	127.4	127.2	126.9	126.8
136.4	136.3	136.3	136.4	136.8	136.4	136.1	136.4
131.0	130.8	130.5	131.1	130.8	132.4	133.6	129.6
135.1	135.6	135.7	135.2	135.4	136.0	134.9	134.4
34.7	34.1	34.3	34.9	34.2	34.9	34.7	32.2
36.5	36.8	36.8	36.4	35.9	35.8	36.4	34.8
80.5	80.7	80.9	80.5	80.1	80.2	80.9	80.8
188.6	188.1	187.9	188.7	187.8	188.1	188.7	169.5
111.9	111.8	110.8	111.6	112.0	112.6	111.7	110.4
190.6	190.8	191.1	190.3	190.6	191.2	190.6	203.4
42.7	42.2	42.5	42.6	42.5	43.1	42.9	123.0
72.9	73.3	73.4	73.6	72.8	73.9	73.1	144.7
34.9	35.1	35.0	34.9	35.1	35.0	34.8	34.2
28.6	28.6	28.6	28.5	28.6	28.9	28.6	31.2
133.3	133.1	133.1	133.8	133.2	133.5	132.9	132.8
127.5	127.4	127.3	127.3	127.5	127.4	127.6	126.8
41.2	41.3	41.2	41.1	41.0	41.5	41.0	41.3
51.7	51.7	52.0	51.8	51.7	51.7	51.9	51.8
141.5	141.6	141.6	141.5	141.5	141.8	141.5	141.3
126.2	126.3	126.2	126.3	126.3	126.2	126.2	125.9
128.6	128.6	128.6	128.7	128.6	128.6	128.6	128.1
127.3	127.2	127.3	127.3	127.3	127.3	127.2	127.3
128.6	128.6	128.6	128.7	128.6	128.6	128.6	128.1
126.2	126.3	126.3	126.3	126.3	126.2	126.2	125.9
20.0	20.0	19.9	20.0	20.1	20.1	19.9	19.3
57.6	57.9	58.1	57.5	57.7	57.8	57.4	57.4
154.7	154.7	154.5	154.7	154.9	155.6	155.4	
54.9	64.2	64.3	65.1	68.9	66.2	143.1	
	14.2	22.8	31.0	29.9	78.6	113.1	
		9.8	19.9	19.1			
			13.8				

because 11-*O*-carbonate bondage is more labile than 11-*O*-acyl bondage and we could not find out a suitable blocking group for 15-hydroxy group to be removed without suffering 11-*O*-carbonate group.

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

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 2. The 15- and 11,15-di-*O*-methoxy, ethoxy, trichloroethoxy and vinyloxycarbonyl derivatives (**2**, **3**, **7**~**10**, **14** and **15**) showed considerable anti-

Table 2. Bioactivities of hitachimycin derivatives.

Compound No.	Acyl group 11	Acyl group 15	Anti-HeLa[a] activity	Hemolysis[b]	MIC[c] (μg/ml) SA	BS	ML	MS
1	OH	OH	0.39	6.3	1.56	0.78	3.12	>50
16	OH	—	6.25	>25	3.12	1.56	6.25	>50
Carbonate								
2	Me	Me	0.39	>25	0.78	1.56	6.25	>50
3	Et	Et	1.56	25	3.12	3.12	6.25	>50
4	Pr	Pr	6.25	>25	>50	>50	>50	>50
5	Bu	Bu	25	>25	>50	>50	>50	>50
6	*iso*-Bu	*iso*-Bu	25	>25	>50	>50	>50	>50
7	TCEt	TCEt	0.39	1.56	3.12	3.12	25	>50
8	Vinyl	Vinyl	1.56	1.56	0.4	0.4	0.78	>50
9	OH	Me	1.56	>25	1.56	3.12	6.25	>50
10	OH	Et	3.12	>25	3.12	3.12	25	>50
11	OH	Pr	6.25	>25	>50	>50	>50	>50
12	OH	Bu	6.25	>25	>50	>50	>50	>50
13	OH	*iso*-Bu	6.25	>25	>50	>50	>50	>50
14	OH	TCEt	6.25	25	3.12	3.12	>50	>50
15	OH	Vinyl	0.39	1.56	3.12	1.56	6.25	>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).

TCEt: Trichloroethyl.

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

Compound No.	Acyl group 11	Acyl group 15	Total dose (mg/kg)	Dose (mg/kg × days)	ILS (%)	Survivors[b]
1	OH	OH	150	30.0×5	144.2	0/5
16	OH	—	150	30.0×5	51.0	0/5
Carbonate						
2	Me	Me	75	15.0×5	184.6	0/5
3	Et	Et	150	30.0×5	323.1	2/5
4	Pr	Pr	150	30.0×5	46.9	0/5
5	Bu	Bu	150	30.0×5	55.1	0/5
6	*iso*-Bu	*iso*-Bu	150	30.0×5	46.9	0/5
7	TCEt	TCEt	150	30.0×5	238.5	1/5
8	Vinyl	Vinyl	37.5	7.5×5	226.9	1/5
9	OH	Me	150	30.0×5	280.8	1/5
10	OH	Et	150	30.0×5	201.9	1/5
11	OH	Pr	150	30.0×5	82.7	0/5
12	OH	Bu	150	30.0×5	92.3	0/5
13	OH	*iso*-Bu	150	30.0×5	65.4	0/5
14	OH	TCEt	150	30.0×5	248.7	1/5
15	OH	Vinyl	75	15.0×5	259.6	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).

TCEt: Trichloroethyl.

HeLa and antibacterial activities. Some of them, **2**, **7** and **15** were comparable in cytocidal activities, and **2** and **8** were superior in antibacterial activities. There were a quantitative tendency between antibacterial activities and cytocidal activities.

Antitumor Activity

Antitumor activities (increase in life span: ILS) at optimal doses of hitachimycin derivatives against Sarcoma 180 are given in Table 3. It is notable that 11,15-di-*O*-ethoxycarbonyl (**3**) and 15-*O*-methoxycarbonylhitachimycin (**9**) showed an increase ILS about twice, and 11,15-di-*O*-trichloroethoxycarbonyl (**7**), 11,15-di-*O*-vinyloxycarbonyl (**8**), 15-*O*-ethoxycarbonyl (**10**), 15-*O*-trichloroethoxycarbonyl (**14**) and 15-*O*-vinyloxycarbonylhitachimycin (**15**) also showed considerable improvement in ILS compared with that of hitachimycin. It is interesting that length of alkyl chain affects antitumor activities and the smaller ones exhibited higher activities. In acyl derivatives, we found that 11-acylates are more effective than 15-acylates, but in this case a relationship between antitumor activity and bonding position of carbonate group was not observed. The compounds possessed higher antitumor activity show a higher cytocidal and antibacterial activity. In spite of their higher antitumor activity *in vitro* and *in vivo*, **2**, **3**, **9**, **10** and **14** did not show significant hemolysis activities. The solubility of these compounds in organic solvents, *e.g.*, MeOH, EtOH, was remarkably improved.

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 Kieselgel 60 F_{254} with $CHCl_3$ - MeOH (50 : 1). Silica gel column chromatography was performed with Merck Kieselgel 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 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.

11,15-Di-*O*-methoxycarbonylhitachimycin (2)

To a solution of **1** (320 mg) in pyridine (1.0 ml), methyl chloroformate (0.5 ml) was added and set for 4 hours at room temperature. 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 $CHCl_3$ - MeOH (50 : 1) to afford a colorless powder, 275 mg (69.1%): MP 162~164°C (dec); $[\alpha]_D^{23}$ +73° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 301 (29,300); high resolution (HR)-MS 593.260 (calcd for $C_{33}H_{39}NO_9$: 593.262); ^1H NMR ($CDCl_3$) δ 5.05 (1H, m, 15-H), 4.77 (1H, ddd, J=6.5, 6.5 and 2.1 Hz, 10-H), 4.19 (1H, m, 8-H), 3.37 (3H, s, 10-OCH_3), 1.87 (3H, s, 6-CH_3), 3.88 (11-$OCOOCH_3$), 3.76 (15-$OCOOCH_3$).

Anal Calcd for $C_{33}H_{39}NO_9$: C 66.75, H 6.63, N 2.36.
Found: C 66.89, H 6.87, N 2.34.

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

To a solution of **1** (320 mg) in pyridine (1.0 ml), ethyl chloroformate (0.5 ml) was added and set for 6 hours at room temperature in a similar manner to the preparation of **2**, to give a colorless powder of **3**, 239 mg (52.5%): MP 137~139°C (dec); $[\alpha]_D^{23}$ +48° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 300 (27,500); HR-MS 621.294 (calcd for $C_{35}H_{43}NO_9$: 621.294); ^1H NMR ($CDCl_3$) δ 5.05 (1H, m, 15-H), 4.78 (1H, ddd, *J*=6.6, 6.6 and 2.0 Hz, 10-H), 4.20 (1H, m, 8-H), 3.38 (3H, s, 10-OCH_3), 1.89 (3H, s, 6-CH_3), 4.27, 1.36 (11-$OCOOCH_2CH_3$), 4.20, 1.29 (15-$OCOOCH_2CH_3$).

Anal Calcd for $C_{35}H_{43}NO_9$: C 67.60, H 6.98, N 2.25.
Found: C 67.56, H 6.95, N 2.27.

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

4 was prepared from **1** (300 mg) and propyl chloroformate (0.5 ml) as described in the preparation of **3**, 265 mg (64.9%): MP 151~154°C (dec); $[\alpha]_D^{23}$ +49° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (26,800); HR-MS 649.324 (calcd for $C_{37}H_{47}NO_9$: 649.325); ^1H NMR ($CDCl_3$) δ 5.06 (1H, m, 15-H), 4.78 (1H, ddd, *J*=6.5, 6.5 and 2.0 Hz, 10-H), 4.19 (1H, m, 8-H), 3.37 (3H, s, 10-OCH_3), 1.87 (3H, s, 6-CH_3), 4.08, 1.07, 0.96 (11-$OCOOCH_2CH_2CH_3$), 4.03, 1.65, 0.95 (15-$OCOOCH_2CH_2CH_3$).

Anal Calcd for $C_{37}H_{47}NO_9$: C 68.38, H 7.29, N 2.16.
Found: C 68.46, H 7.38, N 2.19.

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

5 was prepared from **1** (320 mg) and butyl chloroformate (0.5 ml) as described in the preparation of **3**, 221 mg (48.7%): MP 140~142°C (dec); $[\alpha]_D^{23}$ +92° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 301 (24,900); HR-MS 677.356 (calcd for $C_{39}H_{51}NO_9$: 677.356); ^1H NMR ($CDCl_3$) δ 5.05 (1H, m, 15-H), 4.77 (1H, ddd, *J*=6.5, 6.5 and 2.1 Hz, 10-H), 4.19 (1H, m, 8-H), 3.39 (3H, s, 10-OCH_3), 1.88 (3H, s, 6-CH_3), 4.23, 1.70, 1.41, 0.96 (11-$OCOOCH_2CH_2CH_2CH_3$), 4.20, 1.66, 1.38, 0.95 (15-$OCOOCH_2$-$CH_2CH_2CH_3$).

Anal Calcd for $C_{39}H_{51}NO_9$: C 74.85, H 7.59, N 2.07.
Found: C 75.01, H 7.70, N 2.03.

11,15-Di-*O*-*iso*-butoxycarbonylhitachimycin (**6**)

6 was prepared from **1** (350 mg) and *iso*-butyl chloroformate (0.5 ml) as described in the preparation of **3**, 279 mg (56.1%): MP 163~164°C (dec); $[\alpha]_D^{23}$ +59° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 303 (30,900); HR-MS 677.354 (calcd for $C_{39}H_{51}NO_9$: 677.356); ^1H NMR ($CDCl_3$) δ 5.08 (1H, m, 15-H), 4.76 (1H, ddd, *J*=6.7, 6.7 and 2.0 Hz, 10-H), 4.20 (1H, m, 8-H), 3.37 (3H, s, 10-OCH_3), 1.90 (3H, s, 6-CH_3), 3.88, 1.92, 0.95 (11-$OCOOCH_2CH(CH_3)_2$), 3.85, 1.88, 0.93 (15-$OCOOCH_2CH(CH_3)_2$).

Anal Calcd for $C_{39}H_{51}NO_9$: C 74.85, H 7.59, N 2.07.
Found: C 74.68, H 7.69, N 2.01.

11,15-Di-*O*-(2′,2′,2′)-trichloroethoxycarbonylhitachimycin (**7**)

7 was prepared from **1** (320 mg) and 2,2,2-trichloroethyl chloroformate (0.5 ml) as described in the preparation of **3**, 215 mg (38.8%): MP 204~206°C (dec); $[\alpha]_D^{23}$ +105° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 304 (36,000); HR-MS 825.062 (calcd for $C_{35}H_{37}NO_9Cl_6$: 825.060); ^1H NMR ($CDCl_3$) δ 5.09 (1H, m, 15-H), 4.81 (1H, ddd, *J*=6.8, 6.8 and 2.0 Hz, 10-H), 4.22 (1H, m, 8-H), 3.41 (3H, s, 10-OCH_3), 1.88 (3H, s, 6-CH_3), 4.27 (11-$OCOOCH_2CCl_3$), 4.28 (15-$OCOOCH_2CCl_3$).

Anal Calcd for $C_{35}H_{37}NO_9Cl_6$: C 50.91, H 4.48, N 1.70, Cl 25.81.
Found: C 49.89, H 4.51, N 1.66, Cl 26.23.

11,15-Di-*O*-vinyloxycarbonylhitachimycin (**8**)

8 was prepared from **1** (300 mg) and vinyl chloroformate (0.5 ml) as described in the preparation of **3**, 261 mg (67.3%): MP 157~158°C (dec); $[\alpha]_D^{23}$ +102° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 303 (31,500); HR-MS 617.261 (calcd for $C_{35}H_{39}NO_9$: 617.262); ^1H NMR ($CDCl_3$) δ 5.07 (1H, m, 15-H), 4.69 (1H, ddd, *J*=6.6, 6.6 and 1.9 Hz, 10-H), 4.20 (1H, m, 8-H), 3.37 (3H, s, 10-OCH_3), 1.87 (3H, s, 6-CH_3), 7.28, 4.88, 4.61 (11-$OCOOCH{=}CH_2$), 7.27, 4.88, 4.57 (15-$OCOOCH{=}CH_2$).

Anal Calcd for $C_{35}H_{39}NO_9$: C 68.04, H 6.37, N 2.27.
Found: C 68.00, H 6.48, N 2.40.

15-*O*-Methoxycarbonylhitachimycin (**9**)

To a solution of **2** (165 mg) in $CHCl_3$ (2.0 ml), dimethylamine (0.05 ml) was added and stirred for 5 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 (50 : 1) to give a colorless powder, 123 mg (82.6%): MP 188~189°C (dec); $[\alpha]_D^{23}$ +127° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 301 (35,900); HR-MS 535.257 (calcd for $C_{31}H_{37}NO_7$: 535.257); ^1H NMR ($CDCl_3$) δ 5.07 (1H, m, 15-H), 4.37 (1H, dd, *J*=8.0 and 8.0 Hz, 10-H), 4.01 (1H, ddd, *J*=5.5, 5.5 and 4.8 Hz, 8-H), 3.54 (3H, s, 10-OCH_3), 1.88 (3H, s, 6-CH_3), 3.78 (15-$OCOOCH_3$).

Anal Calcd for $C_{31}H_{37}NO_7$: C 69.50, H 6.97, N 2.62.
Found: C 69.50, H 6.95, N 2.60.

15-*O*-Ethoxycarbonylhitachimycin (**10**)

To a solution of **3** (150 mg) in $CHCl_3$ (2.0 ml), dimethylamine (0.05 ml) was added and set for 5 minutes at room temperature. The reaction mixture was treated in a similar manner to the preparation of **9**, to give a colorless powder of **10**, 113 mg (85.2%): MP 162~165°C (dec); $[\alpha]_D^{23}$ +117° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 304 (31,000); HR-MS 549.271 (calcd for $C_{32}H_{39}NO_7$: 549.272); ^1H NMR ($CDCl_3$) δ 5.05 (1H, m, 15-H), 4.38 (1H, dd, *J*=7.6 and 7.6 Hz, 10-H), 4.03 (1H, ddd, *J*=5.8, 5.5 and 5.5 Hz, 8-H), 3.53 (3H, s, 10-OCH_3), 1.88 (3H, s, 6-CH_3), 4.18 ,1.28 (15-$OCOOCH_2CH_3$).

Anal Calcd for $C_{32}H_{39}NO_7$: C 69.91, H 7.16, N 2.55.
Found: C 69.85, H 7.30, N 2.50.

15-*O*-Propoxycarbonylhitachimycin (**11**)

To a solution of **4** (120 mg) in $CHCl_3$ (2.0 ml), dimethylamine (0.05 ml) was added and set for 5 minutes at room temperature. The reaction mixture was treated in a similar manner to the preparation of **9**, to give a colorless powder of **11**, 89 mg (85.5%): MP 157~159°C (dec); $[\alpha]_D^{23}$ +131° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 304 (32,300); HR-MS 563.286 (calcd for $C_{33}H_{41}NO_7$: 563.288); ^1H NMR ($CDCl_3$) δ 5.04 (1H, m, 15-H), 4.36 (1H, dd, *J*=7.9 and 7.9 Hz, 10-H), 4.01 (1H, ddd, *J*=5.5, 5.5 and 4.9 Hz, 8-H), 3.56 (3H, s, 10-OCH_3), 1.86 (3H, s, 6-CH_3), 4.03, 1.65, 0.95 (15-$OCOOCH_2CH_2CH_3$).

Anal Calcd for $C_{33}H_{41}NO_7$: C 70.30, H 7.34, N 2.49.
Found: C 69.98, H 7.41, N 2.48.

15-*O*-Butoxycarbonylhitachimycin (**12**)

5 (120 mg) was treated with dimethylamine in a similar manner to the preparation of **9**, to give a colorless powder of **12**, 86 mg (84.1%): MP 172~173°C (dec); $[\alpha]_D^{23}$ +98° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 302 (35,100); HR-MS 577.305 (calcd for $C_{34}H_{43}NO_7$: 577.304); ^1H NMR ($CDCl_3$) δ 5.02 (1H, m, 15-H), 4.36 (1H, dd, *J*=8.0 and 8.0 Hz, 10-H), 4.02 (1H, ddd, *J*=5.5, 5.5 and 4.7 Hz, 8-H), 3.57 (3H, s, 10-OCH_3), 1.86 (3H, s, 6-CH_3), 4.18, 1.62, 1.35, 0.96 (15-$OCOOCH_2CH_2CH_2CH_3$).

Anal Calcd for $C_{34}H_{43}NO_7$: C 70.67, H 7.51, N 2.43.
Found: C 70.41, H 7.63, N 2.46.

15-*O*-*iso*-Butoxycarbonylhitachimycin (**13**)

6 (120 mg) was treated with dimethylamine in a similar manner to the preparation of **9**, to give a colorless powder of **13**, 73 mg (71.4%): MP 143~145°C (dec); $[\alpha]_D^{23}$ +127° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 301 (37,100); HR-MS 577.304 (calcd for $C_{34}H_{43}NO_7$: 577.304); ^1H NMR ($CDCl_3$) δ 5.09 (1H, m, 15-H), 4.37 (1H, dd, *J*=7.8 and 7.8 Hz, 10-H), 4.01 (1H, ddd, *J*=5.5, 5.5 and 4.8 Hz, 8-H), 3.54 (3H, s, 10-OCH_3), 1.88 (3H, s, 6-CH_3), 3.86, 1.89, 0.94 (15-$OCOOCH_2CH(CH_3)_2$).

Anal Calcd for $C_{34}H_{43}NO_7$: C 70.67, H 7.51, N 2.43.
Found: C 70.51, H 7.56, N 2.45.

15-*O*-(2′,2′,2′)-Trichloroethoxycarbonylhitachimycin (**14**)

7 (120 mg) was treated with dimethylamine in a similar manner to the preparation of **9**, to give a colorless powder of **14**, 73 mg (71.4%): MP 209~212°C (dec); $[\alpha]_D^{23}$ +143° (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 305 (39,300); HR-MS 651.115 (calcd for $C_{32}H_{36}NO_7Cl_3$: 651.116); ^1H NMR ($CDCl_3$) δ 5.12

(1H, m, 15-H), 4.39 (1H, dd, J=8.0 and 8.0 Hz, 10-H), 4.03 (1H, ddd, J=5.7, 5.7 and 4.8 Hz, 8-H), 3.53 (3H, s, 10-OCH_3), 1.88 (3H, s, 6-CH_3), 4.24 (15-$OCOOCH_2CCl_3$).

Anal Calcd for $C_{32}H_{36}NO_7Cl_3$: C 58.99, H 5.53, N 2.15, Cl 16.4.
Found: C 58.65, H 5.49, N 2.11, Cl 17.2.

15-*O*-Vinyloxycarbonylhitachimycin (**15**)

8 (100 mg) was treated with dimethylamine in a similar manner to the preparation of **9**, to give a colorless powder of **15**, 65 mg (73.3%): MP 161~163°C (dec); $[\alpha]_D^{23}$ +104° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 301 (40,100); HR-MS 547.257 (calcd for $C_{32}H_{37}NO_7$: 547.257); ^{1}H NMR ($CDCl_3$) δ 5.06 (1H, m, 15-H), 4.37 (1H, dd, J=8.0 and 8.0 Hz, 10-H), 4.02 (1H, ddd, J=5.7, 5.7 and 4.8 Hz, 8-H), 3.54 (3H, s, 10-OCH_3), 1.87 (3H, s, 6-CH_3), 7.25, 4.86, 4.59 (15-$OCOOCH{=}CH_2$).

Anal Calcd for $C_{32}H_{37}NO_7$: C 70.17, H 6.81, N 2.56.
Found: C 70.20, H 6.95, N 2.53.

15-Deoxo-14-enohitachimycin (**16**)

To a solution of **2** (60 mg) in $CHCl_3$ (1.0 ml), dimethylamine (0.1 ml) was added and set for 1 hour at room temperature. 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, which was chromatographed on a silica gel column with $CHCl_3$-MeOH (100:1) to afford as a colorless powder of **16**, 23 mg (41.8%): MP 213~215°C (dec); $[\alpha]_D^{23}$ +122° (c 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 298 (27,200); HR-MS 459.240 (calcd for $C_{29}H_{33}NO_4$: 459.239); ^{1}H NMR ($CDCl_3$) δ 6.74 (1H, ddd, J=14.5, 9.6 and 4.5 Hz, 15-H), 6.49 (1H, dd, J=15.0 and 11.0 Hz, 4-H), 6.13 (1H, d, J=15.0 Hz, 14-H), 5.97 (1H, d, J=15.2 Hz, 2-H), 5.32 (1H, dd, J=12.1 and 3.6 Hz, 7-H), 4.33 (1H, dd, J=10.1 and 8.0 Hz, 10-H), 4.13 (1H, dd, J=11.3 and 8.2 Hz, 8-H), 1.91 (3H, s, 6-CH_3).

Anal Calcd for $C_{29}H_{33}NO_4$: C 75.81, H 7.19, N 3.05.
Found: C 75.79, H 7.22, N 3.09.

Acknowledgment

The authors wish to thank Mr. R. MASUMA, The Kitasato Institute, for the MIC assays. The authors also thank to Mr. Y. EBARA for his technical assistance. This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan and funds from the Japan Keirin Association.

References

1) ŌMURA, S.; A. NAKAGAWA, K. SHIBATA & H. SANO: The structure of hitachimycin, a novel macrocyclic lactam involving β-phenylalanine. Tetrahedron Lett. 23: 4713~4716, 1982
2) UMEZAWA, I.; H. TAKESHIMA, K. KOMIYAMA, Y. KOH, H. YAMAMOTO & M. KAWAGUCHI: A new antitumor antibiotic, stubomycin. J. Antibiotics 34: 259~265, 1981
3) KOMIYAMA, K.; K. EDANAMI, H. YAMAMOTO & I. UMEZAWA: Antitumor activity of a new antitumor antibiotic, stubomycin. J. Antibiotics 35: 703~706, 1982
4) KOMIYAMA, K.; K. EDANAMI, A. TANOH, H. YAMAMOTO & I. UMEZAWA: Studies on the biological activity of stubomycin. J. Antibiotics 36: 301~311, 1983
5) KOMIYAMA, K.; K. IWASAKI, M. MIURA, H. YAMAMOTO, Y. NOZAWA & I. UMEZAWA: Mechanism of action of antitumor antibiotic, stubomycin. J. Antibiotics 38: 1614~1616, 1985
6) SHIBATA, K.; S. SATSUMABAYASHI, H. SANO, K. KOMIYAMA, A. NAKAGAWA & S. ŌMURA: Chemical modification of hitachimycin. Synthesis, antibacterial, cytocidal and *in vivo* antitumor activities of hitachimycin derivatives. J. Antibiotics 41: 614~623, 1988
7) MARSH, J. P., Jr.; R. H. IWAMOTO & L. GOODOMAN: Synthesis and characterisation of compounds related to daunomycin. J. Chem. Soc. Chem. Commun. 1968: 589~590, 1968
8) MEYERS, A. I.; K. TOMIOKA, D. M. ROLAND & D. COMINS: Progress toward the total synthesis of maytansinoids. An efficient route to two major precursors (Western-Southern zone). Tetrahedron Lett. 1978: 1375~1378, 1978
9) WINDHOLZ, T. B. & D. B. R. JOHNSTON: Trichloroethoxycarbonyl, a generally applicable protecting group. Tetrahedron Lett. 1967: 2555~2557, 1967