Duocarmycins, Potent Antitumor Antibiotics Produced by *Streptomyces* sp. Structures and Chemistry[1)]

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Seven novel potent antitumor antibiotics, duocarmycins A (1), C1 (2), C2 (3), D (4), B1 (5), B2 (6) and SA (7), were isolated from three independently collected *Streptomyces* sp. The complete structures, including absolute stereochemistry, were determined by spectral and chemical studies of those duocarmycins and several derivatives. Duocarmycins A (1) and SA (7) possess a 1,2,7,7a-tetrahydrocycloprop[1,2-*c*]indol-4-one subunit, a common pharmacophore with that of CC-1065 (10) found from *Streptomyces zelensis*.

Key words *Streptomyces* sp.; antitumor antibiotic; duocarmycin; exciton chirality

In our continuing search for biologically active compounds from microorganisms, we have isolated potent novel antitumor antibiotics, designated duocarmycins, from three different *Streptomyces* species. The producing organism of duocarmycin A (**1**),[1,3,4)] that of duocarmycins C1 (**2**), C2 (**3**) and D (**4**)[1,4-6)] and that of duocarmycin SA (**7**)[7,8)] were isolated from soil collected in Shizuoka, Hyogo and Kyoto, Japan, respectively. During structural studies, **2** and **3** were found to be HCl adducts of **1** and chemically more stable than **1**. It was also ascertained

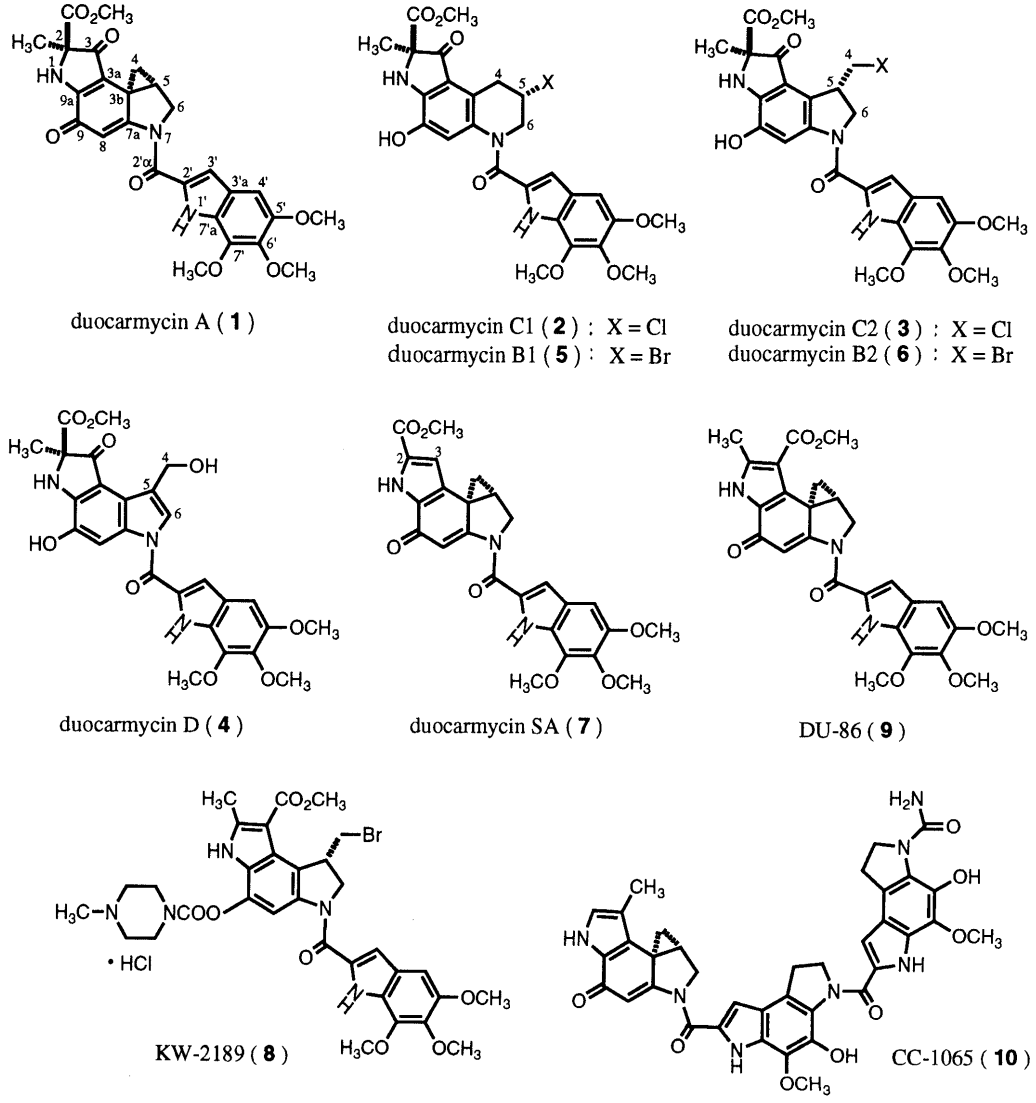

Fig. 1. Structures of Duocarmycins and Related Compounds

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that the organism producing **2** and **3** simultaneously produced **1**. Subsequently, the fermentation conditions were modified to convert **1** into more stable derivatives in the culture medium, and two brominated compounds, duocarmycins B1 (**5**) and B2 (**6**),[9] were isolated. The structures of these duocarmycins were determined to be as shown in Fig. 1, including the absolute stereochemistry.[10] Duocarmycins A (**1**) and SA (**7**) have a cyclopropapyrroloindole ring system, which is also present in CC-1065 (**10**)[11] found in *Streptomyces zelensis*. This ring system is the common pharmacophore of those antibiotics,[12] being responsible for the *in vitro* cytotoxic activity and DNA covalent alkylation properties.[13-17]

Chemical studies of duocarmycins showed that reduction of the 3-keto group of **6** resulted in the rearrangement reaction of the methoxycarbonyl group at C-2. This is the key reaction for the preparation of KW-2189 (**8**), a derivative of duocarmycin B2 (**6**) having improved antitumor activity, water solubility and chemical stability, and currently under phase I clinical trial.[18] KW-2189 (**8**) is thought to be a kind of prodrug that is converted into an active metabolite, DU-86 (**9**), in cells.

Duocarmycins show strong antimicrobial activities against not only gram-positive bacteria (MICs ranged from 0.6 to 80 ng/ml) but also gram-negative bacteria and *Candida albicans* with the following ranking order; SA > A > B1 > B2 > C2 > C1.[19] The cytotoxic activity of duocarmycin SA (**7**) against Balb 3T3/H-ras cells is most potent and its IC_{50} value is >10 times more potent (0.05 nM) than that of CC-1065 (**10**).[19] Duocarmycins also show strong antitumor activities against human tumor cell lines and murine tumors *in vivo*.[18]

We describe here the structure determination and chemical studies of duocarmycins.

Physicochemical Properties of Duocarmycins The physicochemical properties of duocarmycins A (**1**), C1 (**2**), C2 (**3**), D (**4**), B1 (**5**), B2 (**6**) and SA (**7**) are summarized in Table I. Duocarmycin A (**1**) was obtained as an optically active yellow powder, and the high-resolution electron impact mass spectrum (HREIMS) and ^{13}C-NMR spectrum indicated that **1** has the molecular formula $C_{26}H_{25}N_3O_8$. The IR spectrum of **1** showed the presence of hydroxyl and amino groups (3450 and 3300 cm^{-1}), an ester carbonyl group (1740 cm^{-1}), a conjugated ketone group (1684 cm^{-1}), and a tertiary amide group (1630 cm^{-1}). Other duocarmycins were also yellowish, optically active compounds, and showed very similar spectroscopic features to those of **1**. In the mass spectra of duocarmycins, two characteristic fragment ions, *m/z* 234 ($C_{12}H_{12}NO_4$) and the ion which had lost *m/z* 234 from the molecular ion, were observed, indicating that duocarmycins possess a common partial structure. The molecular formulas were determined by HREIMS, suggesting that **2** and **3** are HCl adducts of **1**, while **5** and **6** are HBr adducts of **1**, and **4** is an oxygenated derivative of **1**. These duocarmycins also showed structural similarities in the ^{1}H- and ^{13}C-NMR spectra (Tables II and III) except for the methine and methylene regions, implying structural differences around those aliphatic moieties. Duocarmycin SA (**7**) was different from other duocarmycins in the partial structure I shown in Fig. 2. We began with the structural determination of duocarmycin C1 (**2**) because of its chemical stability and the order of isolation of these compounds.

Structure of Duocarmycin C1(2) In the preceding

TABLE I. Physicochemical Properties of Duocarmycins[a]

	Duocarmycin A (**1**)	Duocarmycin C1 (**2**)	Duocarmycin C2 (**3**)	Duocarmycin D (**4**)
Appearance	Yellow powder	Yellow powder	Yellow crystal	Yellow powder
Molecular formula	$C_{26}H_{25}N_3O_8$	$C_{26}H_{26}ClN_3O_8$	$C_{26}H_{26}ClN_3O_8$	$C_{26}H_{25}N_3O_9$
mp			256—257 °C	
$[\alpha]_D$(MeOH)	+282° (c=0.1)	−126° (c=0.2)	−50° (c=0.2)	−10° (c=0.2)
IR ν, cm^{-1}	3450, 3300, 1740, 1684, 1630	3450, 3300, 1740, 1695, 1620	3450, 3325, 1740, 1700, 1585	3400, 1740, 1725, 1660, 1610
UV λ_{max}^{MeOH} nm (ε)	310 (18000), 358 (28000), 425 (8000)	323 (23000), 415 (4000)	250 (sh, 17000), 300 (19000), 337 (26000), 435 (4000)	250 (sh, 17000), 350 (37000), 425 (5000)
MS *m/z*	507 (M^+), 274, 234	543 (M^+), 310, 234	543 (M^+), 310, 234	523 (M^+), 234, 213
HRMS Calcd :	507.1639	543.1406	543.1406	523.1588
Found:	507.1624	543.1422	543.1424	523.1567

	Duocarmycin B1 (**5**)	Duocarmycin B2 (**6**)	Duocarmycin SA (**7**)
Appearance	Yellow powder	Orange crystal	Yellow powder
Molecular formula	$C_{26}H_{26}BrN_3O_8$	$C_{26}H_{26}BrN_3O_8$	$C_{25}H_{23}N_3O_7$
mp		214—215 °C	
$[\alpha]_D$(MeOH)	−113.5° (c=0.2)	−57.5° (c=0.2)	+118° (c=0.1)
IR ν, cm^{-1}	3373, 1742, 1698, 1625, 1255	3474, 3329, 1742, 1682, 1615, 1236	3460, 1714, 1642, 1619, 1258
UV λ_{max}^{MeOH} nm (ε)	329 (18000), 410 (3200)	248 (sh, 19000), 298 (20000), 337 (32000), 434 (4000)	235 (sh, 21000), 316 (16000), 367 (27000)
MS *m/z*	587 (M^+), 272, 234	587 (M^+), 272, 234	477 (M^+), 234
HRMS Calcd :	587.0903	587.0903	477.1535
Found:	587.0901	587.0893	477.1522
Anal. Calcd :	C 53.07, H 4.45, N 7.14	C 53.07, H 4.45, N 7.14	
Found:	C 52.85, H 4.68, N 6.84	C 53.20, H 4.49, N 7.18	

a) IR spectra of **1**—**4** and **7** were measured in $CHCl_3$, and those of **5** and **6** in KBr. The $[\alpha]_D$ value of **1** was measured at 22°C, those of **2**—**6** at 20°C, and that of **7** at 24°C.

TABLE II. ^{1}H-NMR Data for Duocarmycins (**1**—**7**)[a]

Proton	Duocarmycin A (**1**)	Duocarmycin C1 (**2**)	Duocarmycin C2 (**3**)	Duocaycin D (**4**)
1-NH	6.36 (s)	7.39 (s)	7.33 (br s)	7.56 (br s)
2-CH_3	1.67 (s)	1.47 (s)	1.47 (s)	1.50 (s)
2-$COOCH_3$	3.74 (s)	3.61 (s)	3.61 (s)	3.61 (s)
3-H	—	—	—	—
4-H	2.24 (dd, J=7.6, 4.1 Hz)	3.53 (dd, J=19.2, 5.7 Hz)	4.03 (dd, J=10.0, 3.0 Hz)	4.80 (br d, J=5.7 Hz)
	1.29 (dd, J=4.7, 4.1 Hz)	3.25 (dd, J=19.2, 2.8 Hz)	3.86 (dd, J=10.0, 6.9 Hz)	
4-OH	—	—	—	5.03 (br t, J=5.7 Hz)
5-H	3.05 (m)	4.76 (m)	3.99 (m)	—
6-H	4.45, 4.41 (AB in ABX, J_{AB}=10.3, J_{AX}=7.4, J_{BX}<1 Hz)	4.35 (br dd, J=13.4, 4.6 Hz)	4.62 (br t, J=11.0 Hz)	7.67 (br s)
		3.96 (br d, J=13.4 Hz)	4.32 (dd, J=11.0, 4.0 Hz)	
8-H	7.17 (s)	6.91 (br s)	8.03 (br s)	8.09 (s)
9-OH	—	9.87 (s)	10.20 (s)	*ca.* 10.2 (br)
1′-NH	9.49 (br s)	11.54 (br d, J=1.8 Hz)	11.34 (br d, J=2.0 Hz)	11.99 (br s)
3′-H	6.94 (d, J=2.3 Hz)	6.57 (d, J=1.8 Hz)	6.97 (d, J=2.0 Hz)	7.07 (br s)
4′-H	6.78 (s)	6.87 (s)	6.95 (s)	7.01 (s)
5′-OCH_3	3.88 (s)	3.77 (6H, s)	3.81 (s)[b]	3.82 (6H, s)
6′-OCH_3	3.93 (s)		3.79 (s)[b]	
7′-OCH_3	4.06 (s)	3.92 (s)	3.92 (s)	3.95 (s)

Proton	Duocarmycin B1 (**5**)	Duocarmycin B2 (**6**)	Duocarmycin SA (**7**)
1-NH	7.34 (br s)	7.29 (br s)	10.25 (br s)
2-CH_3	1.47 (s)	1.47 (s)	—
2-$COOCH_3$	3.61 (s)	3.61 (s)	3.88 (s)
3-H	—	—	6.56 (d, J=2.2 Hz)
4-H	3.70 (br dd, J=19.6, 5.8 Hz)	3.93 (br dd, J=9.8, 3.0 Hz)	1.71 (dd, J=7.6, 4.7 Hz)
	3.42 (br dd, J=19.6, 3.6 Hz)	3.77 (br dd, J=9.8, 7.3 Hz)	1.54 (t, J=4.7 Hz)
4-OH	—	—	—
5-H	4.85 (m)	4.04 (m)	2.77 (dt, J=7.6, 4.7 Hz)
6-H	4.37 (br dd, J=13.6, 5.1 Hz)	4.61 (br dd, J=11.2, 9.6 Hz)	4.46 (dd, J=10.6, 4.9 Hz)
	4.03 (br dd, J=13.6, 2.0 Hz)	4.27 (br dd, J=11.2, 4.3 Hz)	4.37 (d, J=10.6 Hz)
8-H	6.92 (s)	8.01 (br s)	7.02 (s)
9-OH	9.58 (s)	10.16 (s)	—
1′-NH	11.47 (br d, J=2.0 Hz)	11.27 (br d, J=1.7 Hz)	9.50 (br s)
3′-H	6.59 (d, J=2.0 Hz)	6.96 (br d, J=*ca.* 2 Hz)	6.92 (d, J=2.2 Hz)
4′-H	6.87 (s)	6.95 (br s)	6.76 (s)
5′-OCH_3	3.78 (6H, s)	3.81 (s)[c]	3.87 (s)
6′-OCH_3		3.80 (s)[c]	3.92 (s)
7′-OCH_3	3.92 (s)	3.93 (s)	4.03 (s)

a) The spectra of **1** and **7** were measured in $CDCl_3$, and the others in DMSO-d_6. *b*, *c*) Exchangeable assignments.

papers, we described spectroscopic analyses of duocarmycin C1 (**2**).[4] The presence of three partial structures, I, II and III, shown in Fig. 2 was proved from the NMR experiments, especially the correlation *via* long-range coupling (COLOC), long-range selective proton decoupling (LSPD) and nuclear Overhauser enhancement and exchange spectroscopy (NOESY) techniques. The partial structure I is composed of methyl, methoxycarbonyl, ketone and amino groups which are bonded to a quaternary carbon. The partial structure II is a tetrahydroquinoline moiety having phenolic hydroxyl and chlorine groups, and the partial structure III is a 5,6,7-trimethoxyindole-2-carbonyl moiety. The connectivities between N-1 and C-9a, between C-3 and C-3a and between C-2′α and N-7 were confirmed by the ^{1}H–^{13}C long-range couplings using COLOC and LSPD, establishing the structure of duocarmycin C1 as **2** shown in Fig. 3.

The structure of duocarmycin C1 (**2**) determined by the above-mentioned spectroscopic analyses was confirmed by the following chemical evidence. The 9-OH group of **2** was acetylated with Ac_2O/pyridine to give a monoacetate **11**, showing a downfield shift (+0.38 ppm) of the *ortho* C-8 proton signal in the ^{1}H-NMR spectrum. Treatment of **2** with CH_2N_2/Et_2O in MeOH gave rise to a monomethyl ether **12**. The ^{1}H-NMR spectrum of **12** showed the disappearance of the 9-OH signal in **2** and the appearance of intense NOE between a newly formed methoxyl group at δ 3.70 (3H, br s) and 8-H, confirming that the phenolic OH group is attached at C-9 (Fig. 3). The presence of the amino groups at the 1- and 1′-position was established by ^{13}C-NMR experiments on the *N*-methyl derivatives **13** and **14**, which were obtained by methylation of **12** with CH_3I/Ag_2O in *N*,*N*-dimethylformamide (DMF). Two additional methyl carbons in **14** resonating at δ 32.1 and δ 33.5 were assigned to *N*-methyl groups on the basis of the chemical shifts, and the corresponding methyl protons at δ 3.24 and δ 4.05 exhibited ^{1}H–^{13}C long-range couplings to C-2 and C-9a through N-1 and to C-2′ and C-7′a through N-1′, respectively, by the COLOC experiment. The presence of the 5-Cl group was confirmed by the dehydrochlorination reaction of **14** by an alkaline treatment (K_2CO_3/MeOH, room tempera-

ture) giving **15**. The ^{1}H-NMR spectrum of **15** showed that two olefinic protons at δ 7.49 and δ 6.07 (4-H and 5-H, respectively) were coupled to the nitrogen-bearing methylene on C-6 (δ 4.61 and δ 4.53), confirming the attachment of the chlorine atom at C-5.

On alkaline hydrolysis of the amide bond, **2** gave an amine moiety and a trimethoxyindole carboxylic acid moiety. These two parts are designated Seg. A and Seg. B, respectively, for all duocarmycins. Heating of **2** in KOH/MeOH gave rise to a methyl ester **16** from Seg. B and two products, **17** (a diastereomeric mixture at C-2) and **18**, from Seg. A (Fig. 4). The ^{1}H-NMR signal pattern of **17** was similar to that of Seg. A of duocarmycin A (**1**) described below, showing the presence of a cyclopropane ring. EIMS of **17** gave the molecular ion at *m/z* 246 and fragment ions at *m/z* 244 (M^+-H_2), 212 (M^+-H_2-MeOH), 184 (M^+-H_2-MeOH$-$CO) and 143 (M^+-H_2-MeOH$-$CO$-CH_3CN$), confirming the attachment of a methoxyl group instead of a methoxycarbonyl group at the C-2 position. The hemi-aminal moiety at the C-2 position in **18** was established by a hydroxyl proton signal at δ 6.17 and EIMS fragment ions at *m/z* 212 (M^+-H_2O), 184 (M^+-H_2O-CO) and 143 (M^+-H_2O-CO$-CH_3CN$).

TABLE III. ^{13}C-NMR Data for Duocarmycins (**1**—**7**)[a]

Carbon	A (1)	C1 (2)	C2 (3)	D (4)	B1 (5)	B2 (6)	SA (7)
C-2	71.3	70.1	71.2	69.9	71.1	71.2	126.9
C-3	194.8	197.8	196.6	197.8	196.8	196.6	107.5
C-3a	112.0	114.8	115.6	190.14	117.1	115.6	130.0
C-3b	30.6	114.7	119.5	116.7	116.6	120.2	31.3
C-4	22.0	32.9	46.4	57.2	33.9	35.6	26.1
C-5	22.3	54.7	42.3	123.5	44.8	42.0	23.6
C-6	55.3	51.2	55.0	124.5	53.0	56.1	54.9
C-7a	165.1	128.4	137.7	129.5	128.9	137.6	161.6
C-8	113.2	117.2	112.5	109.10	118.2	112.5	112.5
C-9	179.7	152.2	150.4	153.7	151.7	150.4	177.9
C-9a	164.4	141.2	144.2	141.7	141.6	144.2	131.6
2-Me	21.1	20.2	22.0	20.7	21.8	22.0	—
2-COOMe	168.0	169.2	169.6	169.3	169.7	169.5	161.0
2-COOMe	53.4	52.6	53.4	52.6	53.4	53.4	52.1
C-2′	128.2	130.7	129.1	128.9	129.1	129.1	128.5
C-2′α	161.2	163.3	160.5	160.2	164.5	160.5	161.2
C-3′	108.2	106.6	107.9	110.4	108.3	107.9	107.8
C-3′a	123.3	122.7	123.5	122.8	123.1	123.5	123.2
C-4′	97.7	97.9	98.0	98.2	97.9	98.0	97.8
C-5′	150.6	149.1	150.1	149.6	150.2	150.1	150.5
C-6′	141.3	139.5	140.9	140.8	140.4	140.9	141.1
C-7′	138.9	139.0	138.7	139.1	138.9	138.7	138.9
C-7′a	126.6	125.4	126.0	127.1	126.1	126.0	126.4
5′-OMe	56.3	55.9	56.4	55.9	56.3	56.4	56.3
6′-OMe	61.5	60.9	61.5	60.9	61.5	61.5	61.4
7′-OMe	61.2	61.0	61.2	61.1	61.2	61.2	61.1

a) The spectra of **2** and **4** were measured in DMSO-d_6, and the others in $CDCl_3$.

On the other hand, a product which retained the methoxycarbonyl group at the C-2 position was obtained by cleavage of the amide bond accompanied with oxidation of Seg. A. This reaction had been found in the course of studies on the conversion of **2** to **1**. Thus, treatment of **2** with triethylamine in MeOH at room temperature in the presence of Ag_2CO_3 produced a quinoline derivative (**19**) and **16** (Fig. 4). This reaction also proceeded in an aprotic solvent to give **19** and two trimethoxyindole-2-carboxylic acid esters, **20** and **21**, obtained from the reaction of the 9-OH groups in **19** and **2**, respectively (Fig. 5). Since this reaction requires both the oxidizing agent (Ag_2CO_3) and base (Et_3N), a mechanism which includes the formation of the enamine intermediate **22** by oxidation and dehydrochlorination of **2** followed by the attack of a nucleophile on the amide bond is proposed. Sodium periodate oxidation of **2** also proceeded in a similar manner, but **20** was selectively produced in this case.

Reduction of the 3-keto group of **19** was performed by methylation of the 9-OH and 1-NH groups followed by borohydride reduction to give **23**. This compound appears to be formed by the successive dehydration and decarboxylation of the 3-hydroxy intermediate of the dimethylate of **19** (Fig. 6).

These chemical reactions described above confirmed the structure of duocarmycin C1 (**2**) derived from the spectroscopic analyses and provided information useful for the syntheses of duocarmycin derivatives.

Structures of Other Duocarmycins Duocarmycin A (**1**) was indicated to be a dehydrochlorinated derivative of duocarmycin C1 (**2**) from the HREIMS. The ^{1}H- and ^{13}C-NMR spectra of both compounds suggested that **1** differed from **2** in the above-mentioned partial structure II. The methylene proton signals at δ 2.24 and 1.29 ($J_{gem.}$ = 4.1 Hz) and the ^{13}C-NMR signals at δ 22.0, 22.3 and 30.6 indicated the presence of a cyclopropane ring system in **1**. Further, the phenolic OH proton signal observed in **2** was absent in **1**, and a conjugated ketone carbon signal (δ 179.7) was observed. Finally, the long-range couplings from the methylene protons (δ 2.24 and 1.29) to C-3a and C-7a, and from a olefinic proton (δ 7.17: 8-H) to C-3b and C-9a confirmed the structure of **1** with the cyclopropane ring conjugated with a dienone moiety (Fig. 7: **1**).

Duocarmycin C2 (**3**) possessed the same molecular formula as that of duocarmycin C1 (**2**), but the structure

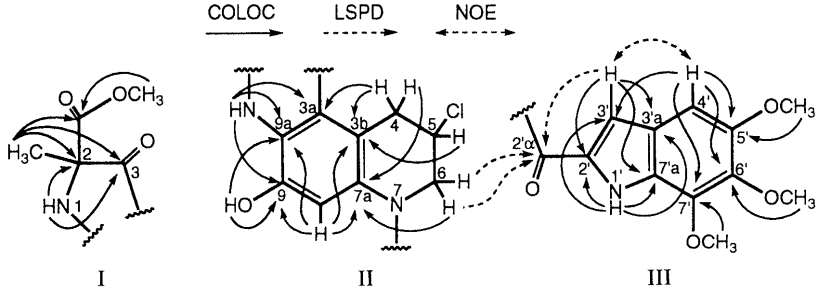

Fig. 2. Partial Structures of Duocarmycin C1 (**2**)

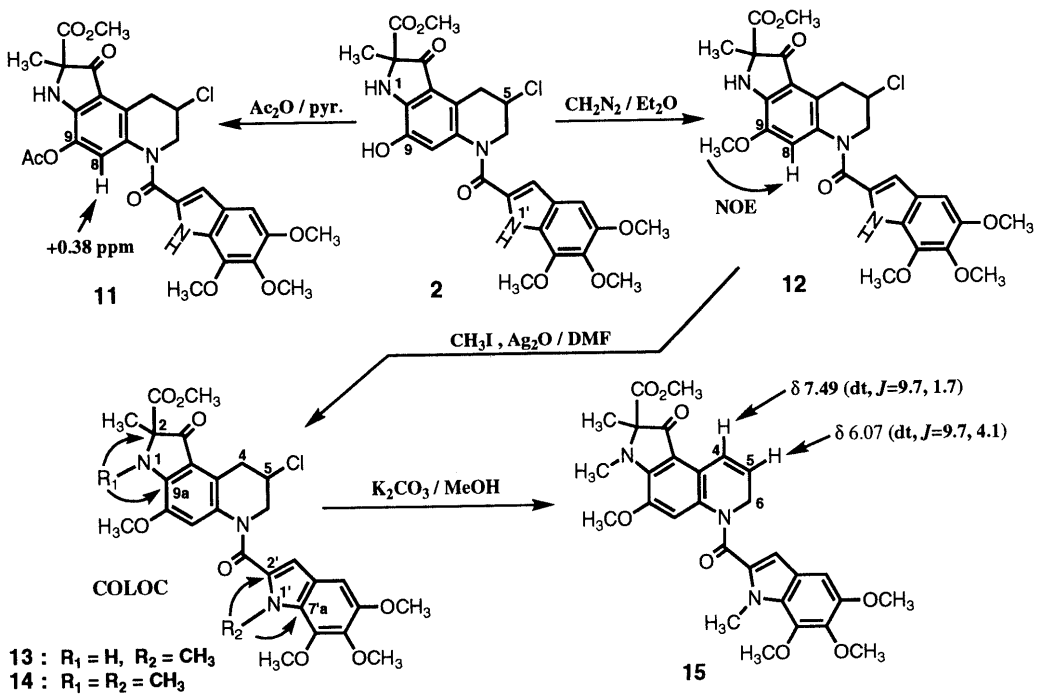

Fig. 3. Reactions of the 9-OH, 1-NH, 1′-NH and 5-Cl Groups in Duocarmycin C1 (**2**)

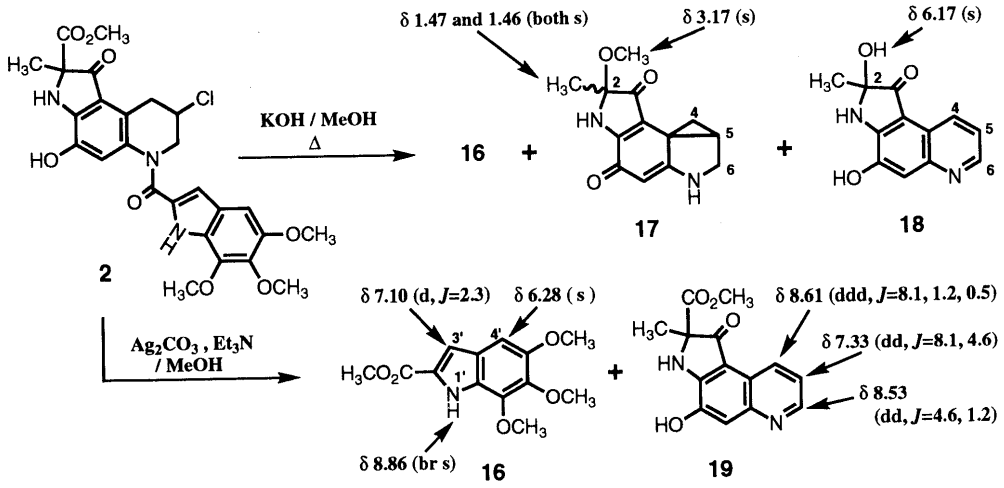

Fig. 4. Cleavage of the Amide Bond of Duocarmycin C1 (**2**)

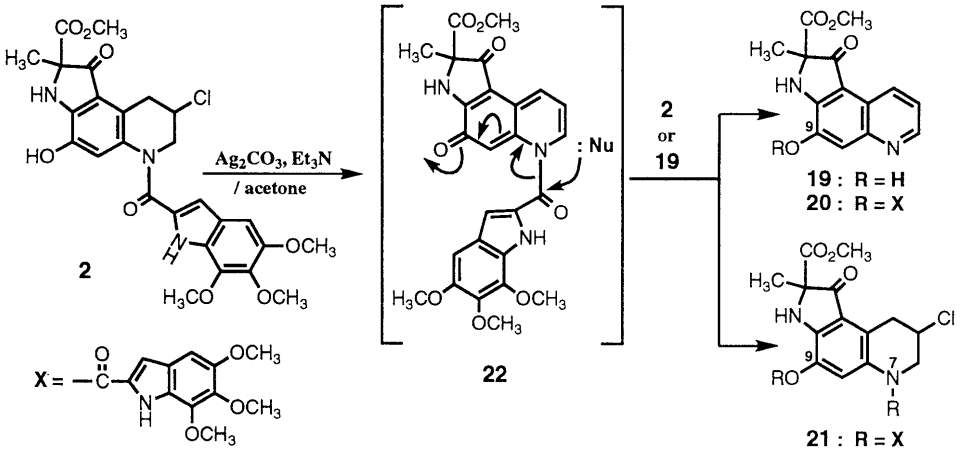

Fig. 5. Presumed Mechanism of Cleavage of the Amide Bond

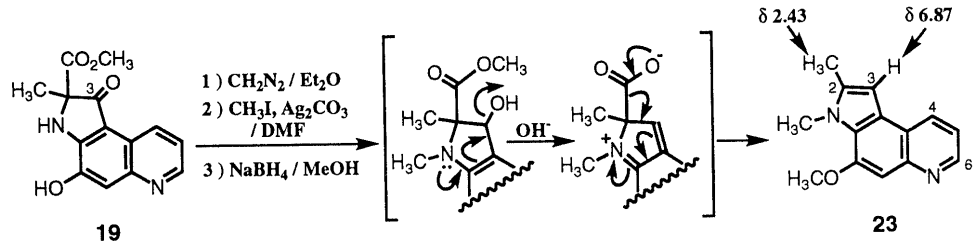

Fig. 6. Reduction of the 3-Keto Group of **19**

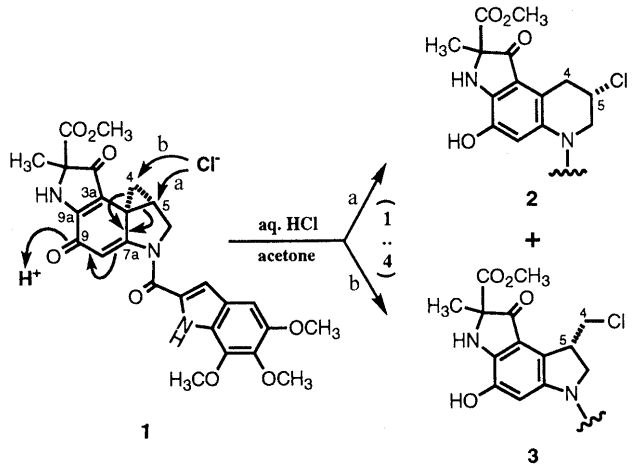

Fig. 7. Chemical Conversion of Duocarmycin A (**1**) to Duocarmycins C1 (**2**) and C2 (**3**)

differed from that of **2** in the chlorine-bearing moiety of II. The ^{1}H-NMR spectrum of **3** showed the presence of a methylene-methine-methylene grouping, though the chemical shift values of the methylene protons on C-4 were lower and that of the methine proton on C-5 was higher than those of **2**. The COLOC spectrum showed long range-couplings from the methine proton (δ 3.99) to C-3a, C-3b and C-7a, confirming that **3** has a dihydroindole skeleton in the corresponding partial structure II of **2** (Fig. 7: **3**).

The structural correlation among **1**, **2** and **3** was evidenced by the following chemical reaction. Treatment of **1** with hydrochloric acid in acetone resulted in the nucleophilic attack of Cl^- at C-5 (path a) and C-4 (path b) to gave **2** and **3**, respectively, in the ratio of *ca.* 1:4 (Fig. 7) . This also revealed the stereochemical correlation among these compounds. If the methylene group in the cyclopropane ring of **1** takes α configuration, the chlorine group in **2** and chloromethyl group in **3** would also take α configuration.

Duocarmycin D (**4**) was indicated to have a molecular formula with one more oxygen atom than that of duocarmycin A (**1**) from its HREIMS. The ^{1}H-NMR spectrum of **4** showed the disappearance of the continuous methylene-methine-methylene signals observed in **1**, **2** and **3**, but one hydroxymethyl, one aromatic and one phenolic OH protons were present. The COLOC spectrum showed long-range couplings from the aromatic proton (δ 7.67) to C-3b, C-5 and C-7a, and from the methylene protons (δ 4.80) to C-3b and C-5, confirming that **4** has a 3-hydroxymethylindole skeleton on the corresponding partial structure II of **2** (Fig. 8: **4**). Duocarmycin D (**4**) was chemically more labile than the other duocarmycins. For example, borohydride reduction of **4** led to complex products, and the amide bond was readily cleaved by refluxing in methanol, though the pyrroloindole moiety from Seg. A was too unstable to isolate. The Seg. A was obtained as **24** by the methylation of 9-OH of **4** followed by alkaline hydrolysis (Fig. 8). Since **24** was still labile to borohydride reduction, **24** was converted to **25** by acetylation of 7-NH and then methylation of 1-NH. Treatment of **25** with $NaBH_4$ in MeOH, however, reduced the C-4 position to give **26**. It was supposed that this reaction proceeded through hydride attack at the C-4 position of the imine intermediate formed by the elimination of the *N*- and *O*-acetyl groups in **25**. Additional support for this mechanism was obtained by the treatment of **25** with K_2CO_3 in MeOH to give a methyl ether **27**. Reduction of **25** under acidic conditions ($NaBH_3CN$/HCl–MeOH) gave rise to **28** and **29**, which have the *N*-acetyl group, but had reacted at the C-4 position as well. These results indicated that the C-4 position of **25** was more reactive to nucleophilic attack than the sterically hindered 3-keto group.

Duocarmycins B1 (**5**) and B2 (**6**) which have the molecular formula $C_{26}H_{26}BrN_3O_8$ were closely analogous to duocarmycins C1 (**2**) and C2 (**3**), respectively. The ^{1}H- and ^{13}C-NMR spectra of **5** were in fair agreement with those of **2**, except for the chemical shifts of the 5-position, and the NMR spectra of **6** were in fair agreement with those of **3**, except for the chemical shifts of the 4-position, confirming that **5** and **6** are bromine-substituted derivatives of **2** and **3** at the 5- and 4-position, respectively (Fig. 9: **5** and **6**).

The structural correlation among **1**, **5** and **6** was also evidenced by the following chemical reactions. Treatment of **1** with 1% aqueous KBr in acetone gave rise to **5** and **6** in the ratio of *ca.* 1:4, and conversely, both **5** and **6** were converted into **1** under basic treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)/CH_3CN. This not only revealed the structural correlation among these compounds, but also provided evidence that these halogenated compounds might be artifacts derived from **1** during the culture or separation process (Fig. 9).

Duocarmycin SA (**7**) which has the molecular formula $C_{25}H_{23}N_3O_7$ showed similar spectroscopic features to those of duocarmycin A (**1**), but the partial structures around C-2 and C-3 in **7** appeared to be different from those in **1** as judged from the ^{1}H- and ^{13}C-NMR spectra (Fig. 10). In the ^{1}H-NMR spectrum of **7**, the 2-CH_3 group in **1** was absent, but one aromatic proton (δ 6.56, d, J=2.2 Hz) having long-range coupling with the 1-NH

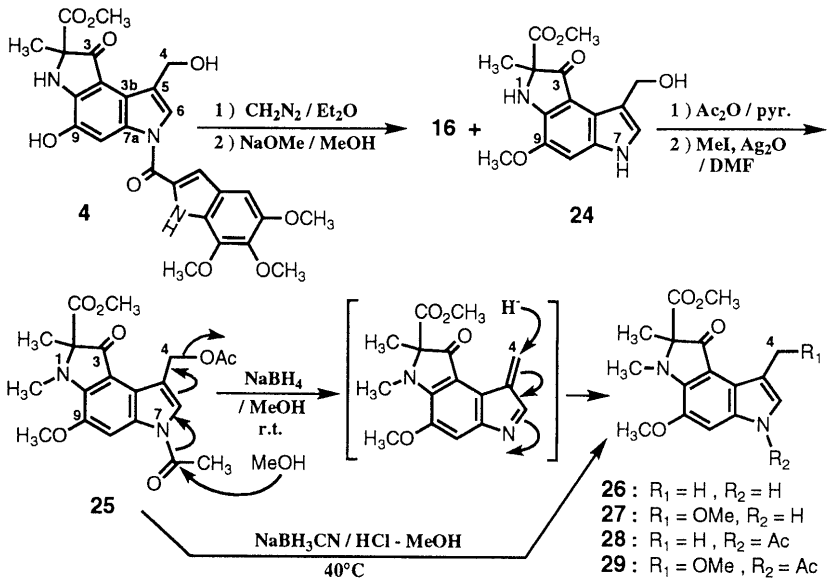

Fig. 8. Reactions of Duocarmycin D (**4**)

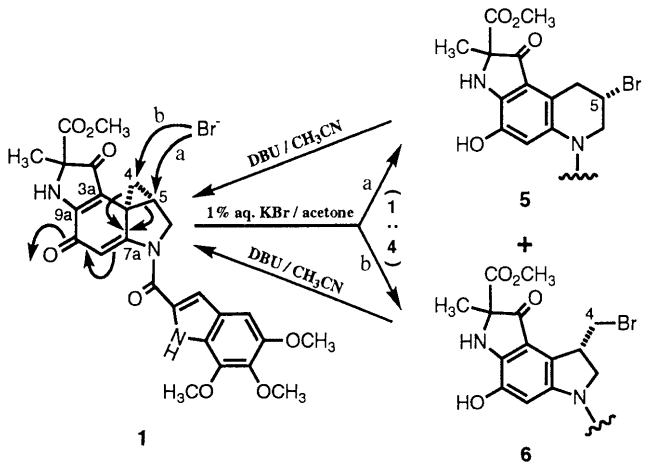

Fig. 9. Interconversion of Duocarmycins A (**1**), B1 (**5**) and B2 (**6**)

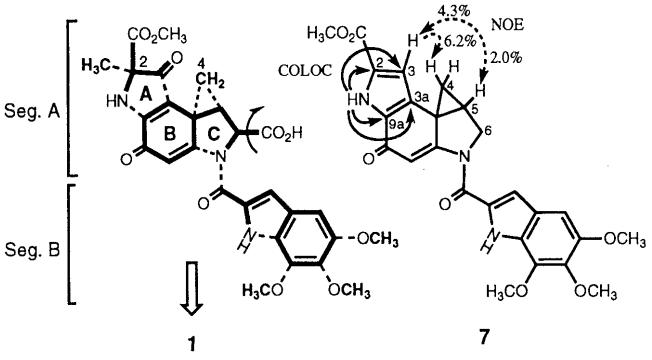

Fig. 10. Structure of Duocarmycin SA (**7**) and Presumed Biosynthetic Origin

proton (δ 10.25, br s) was observed instead. The ^{13}C-NMR spectrum showed the disappearance of the 2-CH_3 and 3-keto groups observed in **1**, and in their place, the presence of one trisubstituted olefinic group (δ 126.9 and 107.5). A COLOC experiment (Fig. 10) revealed long-range couplings from the 1-NH proton (δ 10.25) to the aromatic (δ 107.5) and three quaternary (δ 126.9, 130.0 and 131.6) carbons and from the aromatic proton (δ 6.56) to the latter three carbons, confirming the presence of a pyrrole moiety with a methoxycarbonyl group on ring A of **7**. The location of the methoxycarbonyl group was finally determined to be at the 2-position by the observation of NOEs among 4-H_a, 5-H and the aromatic proton, establishing the whole structure of **7** as shown in Fig. 10.

Duocarmycin A (**1**) was thought to be biosynthetically derived from several amino acids, as proposed for CC-1065[12]: serine (ring A), tyrosine (rings B, C and Seg. B) and methionine (2-CH_3, 4-CH_2 and three OCH_3 groups). Duocarmycin SA (**7**) seems to be a precursor in duocarmycin biosynthesis, because it lacks the 2-CH_3 group in **1** (Fig. 10).

Stereochemistry of Duocarmycins There are three asymmetric carbons in duocarmycin A (**1**), two in C1 (**2**), C2 (**3**), B1 (**5**), B2 (**6**) and SA (**7**), and one in D (**4**) (Fig. 1). The stereochemistry of duocarmycins was examined by application of the following chemical reactions and spectroscopic analyses, employing **1** and **6**.

First, the relative configurations of the C-2 and C-5 substituents in **6** were determined. Since the 2- and 5-positions are far apart in space, NOE experiments on the epimeric 3-hydroxy derivatives **30** and **31** were performed to define the configuration between the 2- and 3-positions and between the 3- and 5-positions. Compound **6** was methylated with diazomethane and then treated with $NaBH_4$ in MeOH to give **30** and **31** (Fig. 11). More intense NOE from 2-CH_3 to 3-H was observed in **31** (14%) than in **30** (4.7%), indicating that the configuration between them is *cis* in **31** and *trans* in **30**. The configuration between 3-H and 5-H was indicated to be *cis* in **30** and *trans* in **31**, because more intense NOE from 5-H to 3-H was observed in **30** (5.2%) than in **31** (2.8%), whereas that from 4-H_2 to 3-H was more intense in **31** (4.2% and 5.6%) than in **30** (2.3% and 4.3%). These results established the *cis* configuration between the methyl group at C-2 and the bromomethyl group at C-5.

During the course of the above experiment, an interesting rearrangement reaction of **31** was found. The hydroxyl group at C-3 was gradually eliminated from **31**

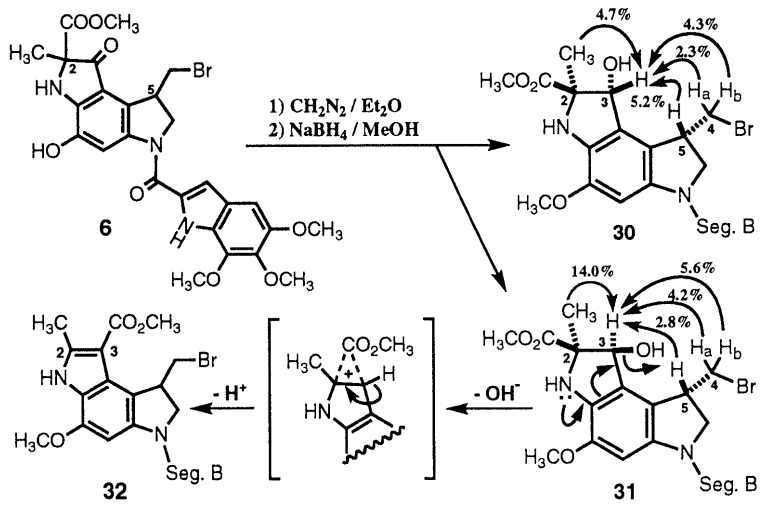

Fig. 11. Relative Configuration between C-2 and C-5 Substituents, and Rearrangement of the Methoxycarbonyl Group in **31**

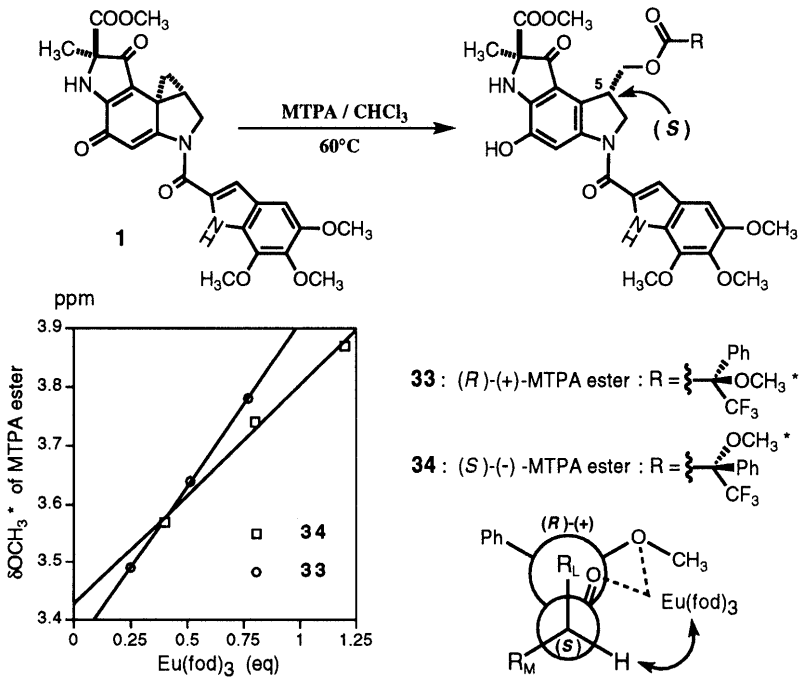

Fig. 12. Absolute Configuration Determined by Using a Shift Reagent with MTPA Esters

at room temperature to form **32** quantitatively, through a 1,2-shift of the methoxycarbonyl group from C-2 to C-3. NOEs from 2-CH_3 to 4-H_2 or 5-H were not observed, but apparent NOE from 2-CH_3 to 1-NH was observed in **32**, confirming the rearrangement of the 2-methoxycarbonyl group to C-3. In **31**, the 3-OH group appears to be *pseudo* axial and well overlapped with π-electrons of the aromatic ring, so that the elimination proceeded more easily than in **30**.

The absolute configurations of the duocarmycins were determined by ^{1}H-NMR lanthanide-induced shift experiments with the α-methoxy-α-trifluoromethylphenylacetic acid [MTPA] esters of **1** and by the exciton chirality method employing dibenzoyl derivatives of **6**. Yasuhara and Yamaguchi reported the determination of the absolute configuration of primary carbinols with the chiral center at the β position by ^{1}H-NMR spectroscopy using a shift reagent with MTPA derivatives.[20] Addition of (*R*)-(+)- and (*S*)-(−)-MTPA to the cyclopropane ring of **1** gave two diastereomers, **33** and **34**, respectively. Figure 12 showed the lanthanide-induced shift of the OCH_3 proton resonance of the MTPA moiety *vs.* molar ratio of tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione) europium [Eu(fod)$_3$] for the diastereomeric MTPA esters. It was found that the slope of **33** was larger than that of **34**, suggesting (*S*)-configuration at the C-5 position in accordance with the preferred conformational model proposed empirically by Yasuhara and Yamaguchi.[20]

Furthermore, the exciton chirality method was applied to **6**. For this purpose, the 3α-hydroxy derivative **35** having known relative configurations among the 2-, 3- and 5-positions was prepared. Reduction of **6** with $NaBH_4$ without protection of the 9-OH group was carried out in tetrahydrofuran (THF) containing a small amount of MeOH at room temperature, giving **35** in 55% yield (Fig. 13). Conversion of ring B in **35** to the corresponding

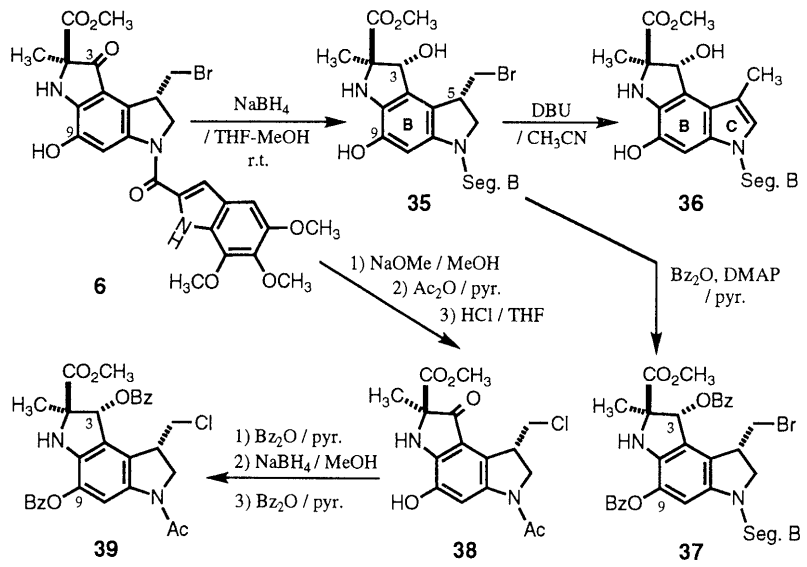

Fig. 13. Synthesis of Dibenzoates from Duocarmycin B2 (**6**)

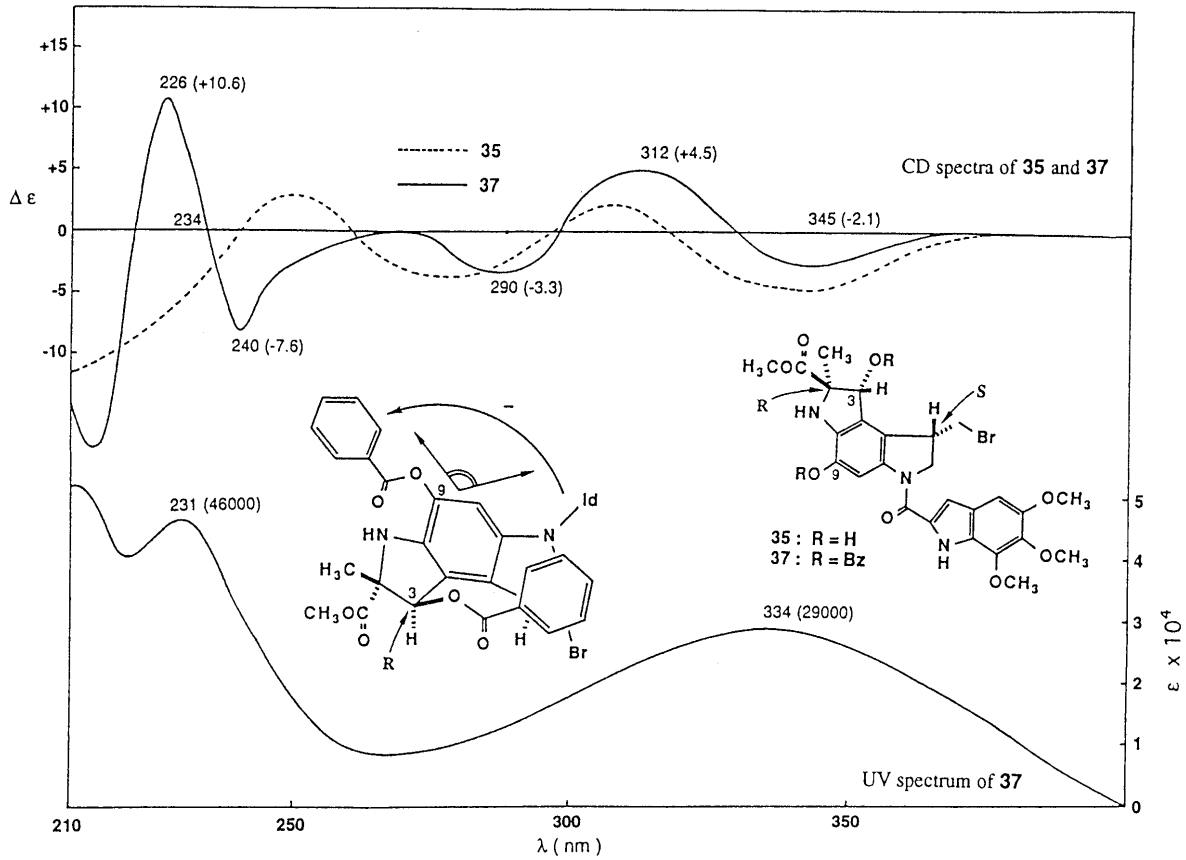

Fig. 14. Absolute Configuration Determined by the Exciton Chirality Method

dienone moiety and subsequent benzoylation of the OH group at C-3 was attempted to utilize the exciton coupling between the two chromophores. Treatment of **35** with DBU in acetonitrile, however, promoted the dehydrobromination at C-5 to give **36**. The indole moiety (B and C ring) of **36** was an unsuitable chromophore for the exciton chirality method because the direction of its transition moment was ambiguous.

Harada and Nakanishi[21] had extended the dibenzoate chirality method to the phenolic compound derived from illudin S and established its absolute configuration, so we next applied this method to **35**, employing the 3- and 9-OH groups. While **35** and its dibenzoate **37** showed complex CD spectra, the obvious Cotton effect centered at 230 nm of the benzoyl chromophore was observed in **37** (Fig. 14). Furthermore, it was found that a similar Cotton effect was observed in the dibenzoate **39**, in which the trimethoxyindole-2-carbonyl moiety was replaced by an acetyl group to eliminate undesirable coupling effects. The observed negative first Cotton effects in **37** and **39**

indicated that each dibenzoate had a negative chirality between the 1,4-dibenzoyloxy groups. This meant that the absolute stereochemistry at C-3 in **35** was (*R*). Since the relative configurations among the 2-, 3- and 5-positions were obvious, the absolute stereochemistry at C-2 and C-5 in **6** was determined to be (*R*) and (*S*), respectively. This result agreed with the above-mentioned lanthanide-induced ^{1}H-NMR shift experiment on **1**. The absolute stereochemistries of **1**, **2**, **3** and **5** were also determined from the structural correlation with **6** (Fig. 1). The absolute configurations of duocarmycins D (**4**) and SA (**7**) presented here are presumed on the basis of the results for duocarmycin A (**1**) and the presumed common biosynthetic pathway of these antibiotics.

Experimental

General Procedures Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured on either a Shimadzu IR-27G infrared spectrometer or a Perkin-Elmer FT-IR spectrometer 1725 X. UV spectra were obtained with a Hitachi 200-20 or U-3500 spectrophotometer. Optical rotations were measured on either a Perkin Elmer 141 polarimeter or a JASCO DIP-370 digital polarimeter. CD spectra were recorded on a JASCO J-500A spectropolarimeter. NMR spectra were recorded on JEOL JNM-FX100, Bruker AM-400 and AM-500 spectrometers. Tetramethylsilane (TMS) was used as an internal standard and chemical shifts are given in δ (ppm) value. MS were determined with either a Hitachi M-80B or JEOL SX-102 spectrometer. Silica gel (Wakogel C-200, Wako) was used for column chromatography, and precoated Silica gel 60 F_{254} plates (Merck, 0.25 mm in thickness) were utilized for thin layer chromatography (TLC). The spots were detected by UV illumination or by spraying 1% cerium(IV) sulfate followed by heating. C-18 reversed-phase (ODS) columns (YMC A-312 ODS, 5 μ, 6 mm × 15 cm or 20 mm × 25 cm) were used for HPLC. Solvents were distilled from glass prior to use or were of spectrophotometric or HPLC grade. Ethereal diazomethane was prepared from bis-(*N*-methyl-*N*-nitroso) terephthalamide (9 g) in ether (40 ml) and distilled.

Isolation of Duocarmycin A (1) The producing organism of duocarmycin A (**1**), *Streptomyces* sp. DO-88 (FERM BP 1002), was isolated from soil collected in Shizuoka, Japan. After incubation of this organism for 48 h at 28 °C, the vegetative seed culture (300 ml) was inoculated into a 30-l jar containing 15 l of the production medium and incubated for 72 h at 28 °C with aeration. The combined culture filtrate and acetone extract of the mycelium (30 l) was adjusted to pH 5.5 with sulfuric acid and subjected to Diaion HP-20 (Mitsubishi Chemical Industries Limited) column chromatography. The column was washed with water and 50% MeOH, and then the antibiotic was eluted with AcOEt. The eluate was concentrated *in vacuo* and extracted with AcOEt. The extract was concentrated and the residue was subjected to silica gel chromatography with a mixture of toluene–acetone. The active fractions were combined and evaporated to dryness. The residue was rechromatographed on silica gel (Lichroprep Si 60, Merck) with toluene–acetone. The crude product was subjected to HPLC (Wako gel-LC-ODS 30 K) and elution with a gradient of 50% aqueous MeOH to MeOH afforded 3 mg of pure duocarmycin A (**1**). The physicochemical properties of **1** are given in Table I and the ^{1}H- and ^{13}C-NMR data in Tables II and III, respectively.

Isolation of Duocarmycins C1 (2), C2 (3) and D (4) The producing organism of duocarmycins C1 (**2**), C2 (**3**) and D (**4**) was isolated from soil collected in Hyogo, Japan and was taxonomically classified as *Streptomyces* sp. DO-89 (FERM BP 988). After incubation of this organism for 48 h at 28 °C, the vegetative seed culture (15 l) was inoculated into a 200-l tank containing 150 l of the production medium and incubated for 57 h at 28 °C with aeration. The culture broth was purified in the manner described above to yield duocarmycins C1 (**2**, 10 mg), C2 (**3**, 45 mg) and D (**4**, 95 mg). Their physicochemical properties are given in Table I and the ^{1}H- and ^{13}C-NMR data in Tables II and III, respectively.

Isolation of Duocarmycins B1 (5) and B2 (6) The producing organism of duocarmycin B1 (**5**) and B2 (**6**) was the same as that of duocarmycin C1 (**2**) and C2 (**3**). The fermentation was carried out in a 2000-l tank under modified conditions with KBr and the obtained culture broth was processed as described above to yield duocarmycins B1 (**5**, 10 g) and B2 (**6**, 50 g). Their physicochemical properties are given in Table I and the ^{1}H- and ^{13}C-NMR data in Tables II and III, respectively.

Isolation of Duocarmycin SA (7) The producing organism of duocarmycin SA (**7**) was isolated from soil collected in Kyoto, Japan and was taxonomically classified as *Streptomyces* sp. DO-113 (FERM BP 2222). The fermentation was carried out in a 2000-l tank under optimum conditions and the obtained culture broth was processed as described above to yield duocarmycin SA (**7**, 16 mg). The physicochemical properties of duocarmycin SA (**7**) are given in Table I and the ^{1}H- and ^{13}C-NMR data in Tables II and III, respectively.

Acetylation of Duocarmycin C1 (2) Duocarmycin C1 (**2**, 10 mg) was treated with acetic anhydride (0.1 ml) and pyridine (0.5 ml) for 15 min at room temperature. Then MeOH (2 ml) was added and the mixture was stirred for 15 min, and evaporated to afford a residue, which was subjected to preparative TLC (toluene–acetone, 7 : 3) to give compound **11** (8 mg, 74%).

11: Yellow powder. $[\alpha]_D^{23}$ −11.3° (c=0.7, MeOH). IR ($CHCl_3$): 3460, 1747, 1707, 1622, 1492, 1312, 1255, 1185, 1108, 828 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 234 (sh 22000), 324 (20000), 410 (3500). EIMS *m/z*: 585 (M^+), 352, 310, 274, 234 (base peak), 215, 179. HREIMS *m/z*: 585.1498 (M^+). Calcd for $C_{28}H_{28}ClN_3O_9$: 585.1512. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.12 (1H, br s, 1′-NH), 7.46 (1H, s, 8-H), 6.76 (1H, s, 4′-H), 6.57 (1H, d, J=2.3 Hz, 3′-H), 5.15 (1H, br s, 1-NH), *ca.* 4.5 (2H, 5-H and 6-H_a), 4.20 (1H, br d, J=10.6 Hz, 6-H_b), 4.07 (3H, s, 7′-OMe), 3.93 (3H, s, 6′-OMe), 3.88 (3H, s, 5′-OMe), 3.79 (3H, s, 2-COOMe), 3.74 and 3.57 (2H, AB in ABX, J_{AB}=19.4 Hz, J_{AX}=5.7 Hz, J_{BX}=5.2 Hz, 4-H_2), 2.30 (3H, s, 9-OAc), 1.69 (3H, s, 2-Me). ^{13}C-NMR (25 MHz, $CDCl_3$) δ: 196.0 (C-3), 169.2 (2-COOMe*), 168.2 (9-OAc*), 163.4 (C-2′α), 152.1 (C-9), 150.3 (C-5′), 140.5 (C-6′), 138.9 (C-7′), 135.1 (C-9a), 130.5 (C-2′), 128.6 (C-7a), 126.6 (C-8), 125.9 (C-7′a), 124.3 (C-3b), 123.1 (C-3′a), 118.7 (C-3a), 107.9 (C-3′), 97.8 (C-4′), 71.1 (C-2), 61.4 (6′-OMe), 61.1 (7′-OMe), 56.3 (5′-OMe), 53.5 (2-COOMe), 52.7 (C-5), 51.7 (C-6), 33.7 (C-4), 22.1 (2-Me**), 20.7 (9-OAc**). Assignments with asterisks may be interchanged.

Methylation of Duocarmycin C1 (2) A solution of duocarmycin C1 (**2**, 25 mg) in MeOH (2 ml) was treated with ethereal diazomethane (1 ml), and the mixture was stirred for 10 min at room temperature, then evaporated to yield a residue, which was purified by silica gel column chromatography (toluene–acetone, 7 : 1) to give compound **12** (25 mg, 97%).

12: Yellow powder. $[\alpha]_D^{23}$ −8.0° (c=0.3, MeOH). IR ($CHCl_3$): 3450, 1705, 1615, 1513, 1440, 1252, 1105, 825 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 236 (sh 18000), 323 (16000), 408 (3000). EIMS *m/z*: 557 (M^+), 380, 324 (base peak), 288, 265, 234, 179, 149. FABMS *m/z*: 558 (MH^+, ^{35}Cl). HRFABMS *m/z*: 558.1635 (MH^+, ^{35}Cl). Calcd for $C_{27}H_{29}{}^{35}ClN_3O_8$: 558.1643. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.10 (1H, br s, 1′-NH), 7.00 (1H, br s, 8-H), 6.75 (1H, s, 4′-H), 6.56 (1H, d, J=1.8 Hz, 3′-H), 5.25 (1H, br s, 1-NH), *ca.* 4.5 (2H, m, 5-H and 6-H_a), 4.17 (1H, br d, J=11.2 Hz, 6-H_b), 4.07 (3H, s, 7′-OMe), 3.92 (3H, s, 6′-OMe), 3.88 (3H, s, 5′-OMe), 3.79 (3H, s, 2-COOMe), 3.70 (3H, br s, 9-OMe), 3.66 and 3.50 (2H, AB in ABX, J_{AB}=19.2 Hz, J_{AX}=5.8 Hz, J_{BX}=5.0 Hz, 4-H_2), 1.69 (3H, s, 2-Me). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 197.5 (C-3), 169.2 (2-COOMe), 163.5 (C-2′α), 155.2 (C-9a), 149.1 (C-5′), 143.2 (C-9), 139.5 (C-6′), 139.0 (C-7′), 130.7 (C-3′a), 128.2 (C-7a), 125.3 (C-7′a), 122.7 (C-2′), 116.0 (C-3b), 114.3 (C-3a), 113.9 (C-8), 106.3 (C-3′), 97.8 (C-4′), 70.1 (C-2), 61.0 (7′-OMe), 60.9 (6′-OMe), 55.9 (5′-OMe), 55.3 (9-OMe), 54.5 (C-5), 52.6 (2-COOMe), 51.1 (C-6), 32.9 (C-4), 20.0 (2-Me).

Compounds 13 and 14 Methyl iodide (20 μl) and silver oxide (15 mg) were added to a solution of **12** (11 mg) in DMF (0.5 ml) and the mixture was stirred for 9 h at room temperature in the dark. It was then diluted with MeOH, filtered *in vacuo*, and evaporated to yield a residue, which was purified by HPLC (ODS, 80% aqueous MeOH) to give compounds **13** (3 mg, 27%) and **14** (5 mg, 43%).

13: Yellow powder. $[\alpha]_D^{23}$ −17.6° (c=0.2, MeOH). IR ($CHCl_3$): 3440, 1742, 1700, 1635, 1615, 1515, 1465, 1444, 1396, 1293, 1252, 1228, 1110 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 238 (sh 20000), 318 (19000), 409 (3600). EIMS *m/z*: 571 (M^+), 535, 512, 323, 287, 248 (base peak), 227, 179. HREIMS *m/z*: 571.1733 (M^+). Calcd for $C_{28}H_{30}{}^{35}ClN_3O$: 571.1719. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.69 (1H, br s, 1-NH), 6.90 (1H, s, 4′-H), 6.90 (1H, br s, 8-H), 6.59 (1H, br s, 3′-H), 4.82 (1H, br s, 5-H), 4.42 (1H, dd, J=13.6, 4.3 Hz, 6-H_a), 3.93 (3H, s, 1′-NMe), 3.90 (3H, s, 7′-OMe), *ca.* 3.9 (6-H_b), 3.792, 3.787 (both 3H, s, 5′- and 6′-OMe),

3.59 (3H, s, 2-COOMe), *ca.* 3.55 (3H, br s, 9-OMe), *ca.* 3.5, 3.3 (both 1H, 4-H_2), 1.45 (3H, s, 2-Me). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 197.4 (C-3), 168.9 (2-COOMe), 163.4 (C-2'α), 152.2 (C-9a), 149.0 (C-5'), 143.1 (C-9), 140.1 (C-6'), 140.0 (C-7'), 133.2 (C-3'a), 127.7 (C-7a), 125.5 (C-7'a), 122.7 (C-2'), 116.0 (C-3b), 114.3 (C-3a), 113.7 (C-8), 105.3 (C-3'), 98.3 (C-4'), 70.0 (C-2), 61.8 (7'-OMe*), 60.8 (6'-OMe*), 55.8 (5'-OMe), 55.2 (9-OMe), 54.6 (C-5), 52.6 (2-COOMe), 51.0 (C-6), 32.9 (C-4 and 1'-NMe), 20.0 (2-Me). Assignments with an asterisk may be interchanged.

14: Yellow powder. $[\alpha]_D^{23}$ $-10.3°$ ($c=0.1$, MeOH). IR ($CHCl_3$): 1740, 1680, 1630, 1609, 1512, 1462, 1392, 1225, 1108 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 245 (16000), 316 (15000), 430 (3200). EIMS *m/z*: 585 (M^+), 526, 337, 282, 248 (base peak), 227, 205, 179. HREIMS *m/z*: 585.1885 (M^+). Calcd for $C_{29}H_{32}{}^{35}ClN_3O_8$: 585.1876. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.04 (1H, br s, 8-H), 6.78 (1H, s, 4'-H), 6.60 (1H, s, 3'-H), 4.55 (1H, dd, $J=13.1$, 4.8 Hz, 6-H_a), 4.49 (1H, m, 5-H), 4.05 (3H, s, 1'-NMe), 3.99 (3H, s, 7'-OMe), 3.93 (3H, s, 6'-OMe), *ca.* 3.9 (1H, 6-H_b), 3.89 (3H, s, 5'-OMe), 3.73 (3H, s, 2-COOMe), 3.67 (3H, br s, 9-OMe), *ca.* 3.6 (2H, AB in ABX, 4-H_2), 3.24 (3H, s, 1-NMe), 1.56 (3H, s, 2-Me). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 196.7 (C-3), 168.3 (2-COOMe), 165.0 (C-2'α), 151.7 (C-9a), 149.7 (C-5'), 145.1 (C-9), 141.0 (C-6'), 140.9 (C-7'), 132.9 (C-3'a), 127.7 (C-7a), 127.0 (C-7'a), 123.2 (C-2'), 117.6 (C-3b), 116.6 (C-3a), 115.3 (C-8), 105.9 (C-3'), 98.0 (C-4'), 75.1 (C-2), 62.0 (7'-OMe*), 61.4 (6'-OMe*), 56.3 (5'-OMe), 55.9 (9-OMe), 53.7 (C-5), 53.1 (2-COOMe), 52.5 (C-6), 33.7 (C-4), 33.5 (1'-NMe), 32.1 (1-NMe), 17.9 (2-Me). Assignments with an asterisk may be interchanged.

Compound 15 A stirred solution of **14** (6 mg) in MeOH (5 ml) was treated with K_2CO_3 (5 mg). The mixture was stirred for 1 h at room temperature, then diluted with water, and extracted with AcOEt. The extract was washed with brine, dried (Na_2SO_4), filtered, and evaporated to give a residue, which was purified by HPLC (80% aqueous methanol) to afford compound **15** (2 mg, 36%).

15: Yellow powder. $[\alpha]_D^{23}$ $-1.2°$ ($c=0.1$, MeOH). EIMS *m/z*: 549 (M^+), 490, 300, 265, 241 (base peak), 221, 198, 179, 149. HREIMS *m/z*: 549.2132 (M^+). Calcd for $C_{29}H_{31}N_3O_8$: 549.2109. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.49 (1H, dt, $J=9.7$, 1.5 Hz, 4-H), 6.77 (1H, s, 4'-H), 6.62 (1H, s, 3'-H), 6.57 (1H, br s, 8-H), 6.07 (1H, m, 5-H), 4.61 and 4.35 (2H, AB in ABMX, $J_{AB}=16.8$ Hz, $J_{AB,M}=4.1$ Hz, $J_{AB,X}=1.8$ Hz, 6-H_2), 3.921, 3.916 and 3.88 (all 3H, s, 1'-NMe, 7'- and 6'-OMe), 3.81 (3H, br s, 5'-OMe), 3.73 (3H, s, 2-COOMe), 3.36 (3H, br s, 9-OMe), 3.17 (3H, s, 1-NMe), 1.57 (3H, s, 2-Me).

Alkaline Hydrolysis of Duocarmycin C1 (2) Duocarmycin C1 (**2**, 20 mg) was treated with 0.5 N KOH in MeOH (1 ml) for 10 min at 80 °C, and the reaction mixture was concentrated and purified in a Chromatotron (Harrison Research, U.S.A.) ($CHCl_3$–MeOH, 10 : 1) to yield compounds **16** (7 mg, 72%), **17** (3 mg, 33%) and **18** (1 mg, 12%).

16: Colorless powder. IR (KBr): 3319, 2945, 2839, 1713, 1228, 998 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 224 (sh 17000), 299 (17000). EIMS *m/z*: 265 (M^+), 250, 233, 218, 204, 190, 175, 71 (base peak). HREIMS *m/z*: 265.0963 (M^+). Calcd for $C_{13}H_{15}NO_5$: 265.0949. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.86 (1H, br s, 1-NH), 7.10 (1H, d, $J=2.3$ Hz, 3-H), 6.82 (1H, s, 4-H), 4.07 (3H, s, 7-OMe), 3.928 and 3.925 (6-OMe and 2-COOMe), 3.90 (5-OMe). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 162.1 (2-COOMe), 150.3 (C-5), 140.6 (C-6), 138.9 (C-7), 126.8, 126.5 (C-2 and C-7a), 123.1 (C-3a), 108.9 (C-3), 97.9 (C-4), 61.4 (7-OMe), 61.1 (6-OMe), 56.3 (5-OMe), 51.9 (2-COOMe).

17: Yellow powder. EIMS *m/z*: 246 (M^+), 244, 231, 212, 184 (base peak), 143, 129, 115. HREIMS *m/z*: 246.1021 (M^+). Calcd for $C_{13}H_{14}N_2O_3$: 246.1004. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.78 and 5.77 (1H, both s, 8-H), 5.63 and 5.60 (1H, both br s, 1-NH*), 5.12 (1H, br s, 7-NH*), 3.83 and 3.70 (2H, AB in ABX, 6-H_2), 3.16 (3H, s, 2-OMe), 3.06 (1H, m, 5-H), 2.03 (1H, m, 4-H_a), 1.05 (1H, m, 4-H_b), 1.47 and 1.46 (3H, both s, 2-Me). Assignments with an asterisk may be interchanged.

18: Yellow powder. EIMS *m/z*: 230 (M^+), 212, 184, 160, 143 (base peak), 115. HREIMS *m/z*: 230.0678 (M^+). Calcd for $C_{12}H_{10}N_2O_3$: 230.0691. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.77 (1H, br s, 9-OH), 8.63 (1H, ddd, $J=8.2$, 1.5, 0.5 Hz, 4-H), 8.50 (1H, dd, $J=4.4$, 1.5 Hz, 6-H), 8.06 (1H, br s, 1-NH), 7.31 (1H, dd, $J=8.2$, 4.4 Hz, 5-H), 7.30 (1H, br s, 8-H), 6.17 (1H, s, 2-OH), 1.36 (3H, s, 2-Me). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 201.1 (C-3), 144.8 (C-6), 128.5 (C-4), 120.9 (C-5), 86.6 (C-2), 22.7 (2-Me). *Not all the carbons were observed.

Degradation of Duocarmycin C1 (2) with Ag_2CO_3 and Triethylamine in MeOH A mixture of a solution of duocarmycin C1 (**2**, 10 mg) in MeOH (2 ml), Ag_2CO_3 (10 mg) and triethylamine (0.1 ml) was stirred for 20 min at room temperature, then filtered and evaporated *in vacuo* to yield a residue. Purification of the residue by HPLC (ODS, 80% aqueous MeOH) gave compounds **19** (4 mg, 80%) and **16** (3 mg, 61%).

19: Yellow powder. $[\alpha]_D^{23}$ $-12.0°$ ($c=0.1$, MeOH). IR (KBr): 3392, 2927, 1739, 1262 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 218 (14000), 246 (15000), 307 (7300), 394 (4600), 410 (sh 4200). EIMS *m/z*: 272 (M^+), 229, 213 (base peak), 185, 143. HREIMS *m/z*: 272.0775 (M^+). Calcd for $C_{14}H_{12}N_2O_4$: 272.0795. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: *ca.* 11.0 (1H, br, 9-OH), 8.61 (1H, ddd, $J=8.1$, 1.5, 0.5 Hz, 4-H), 8.54 (1H, br s, 1-NH), 8.53 (1H, dd, $J=4.5$, 1.5 Hz, 6-H), 7.33 (1H, dd, $J=8.1$, 4.5 Hz, 5-H), 7.31 (1H, br s, 8-H), 3.61 (3H, s, 2-COOMe), 1.53 (3H, s, 2-Me). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 195.9 (C-3), 168.5 (2-COOMe), 158.2 (C-9), 147.1 (C-9a*), 146.4 (C-7a*), 145.1 (C-6), 128.1 (C-4), 120.7 (C-8**), 120.2 (C-5**), 116.5 (C-3b***), 106.8 (C-3a***), 70.6 (C-2), 52.6 (2-COOMe), 19.6 (2-Me). Assignments with the same numbers of asterisks may be interchanged.

Degradation of Duocarmycin C1 (2) with Ag_2CO_3 and Triethylamine in Acetone A mixure of a solution of duocarmycin C1 (**2**, 15 mg) in acetone (5 ml), Ag_2CO_3 (10 mg) and triethylamine (0.1 ml) was stirred for 20 min at room temperature, then filtered and evaporated *in vacuo* to yield a residue. Purification by HPLC (ODS, 80% aqueous MeOH) gave compounds **19** (2 mg, 27%), **20** (3 mg, 22%) and **21** (3 mg, 14%).

20: Yellow powder. $[\alpha]_D^{23}$ $+0.7°$ ($c=0.3$, MeOH). IR ($CHCl_3$): 3450, 1730, 1700, 1622, 1578, 1523, 1210, 1153, 1108 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 250 (34000), 304 (20000), 402 (6500), 423 (sh 5300). EIMS *m/z*: 505 (M^+), 279, 234 (base peak), 213, 167, 149, 113. HREIMS *m/z*: 505.1497 (M^+). Calcd for $C_{26}H_{23}N_3O_8$: 505.1484. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.20 (1H, br s, 1'-NH), 9.01 (1H, dd, $J=8.4$, 1.5 Hz, 4-H), 8.88 (1H, dd, $J=4.4$, 1.5 Hz, 6-H), 8.17 (1H, s, 8-H), 7.49 (1H, dd, $J=8.4$, 4.4 Hz, 5-H), 7.41 (1H, d, $J=2.3$ Hz, 3'-H), 6.84 (1H, s, 4'-H), 5.88 (1H, br s, 1-NH), 4.10 (3H, s, 7'-OMe), 3.96 (3H, s, 6'-OMe), 3.93 (3H, s, 5'-OMe), 3.80 (3H, s, 2-COOMe), 1.75 (3H, s, 2-Me).

21: Yellow powder. $[\alpha]_D^{23}$ $-7.5°$ ($c=0.1$). IR (KBr): 3338, 2938, 1736, 1625, 1498, 1222 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 232 (sh 37000), 312 (36000), 410 (4900). EIMS *m/z*: 777 (M^+), 741, 543, 505, 473, 272, 234 (base peak). HREIMS *m/z*: 777.2194 (M^+). Calcd for $C_{38}H_{37}{}^{35}ClN_4O_{12}$: 777.2172. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.12 and 9.02 (both 1H, br s, 1'- and 1''-NH), 7.62 (1H, s, 8-H), 7.31 (1H, d, $J=2.3$ Hz, 3''-H), 6.82 and 6.78 (both 1H, s, 4'-H and 4''-H), 6.62 (1H, d, $J=2.2$ Hz, 3'-H), 5.34 (1H, s, 1-NH), *ca.* 4.5 (2H, m, 5-H and 6-H_a), 4.24 (1H, br d, $J=9.8$ Hz, 6-H_b), 4.074 and 4.066 (both 3H, s, 7'- and 7''-OMe), 3.94 and 3.92 (both 3H, s, 6'- and 6''-OMe), 3.90 and 3.89 (both 3H, s, 5'- and 5''-OMe), 3.80 (3H, s, 2-COOMe), 3.74 and 3.61 (2H, AB in ABX, $J_{AB}=19.3$ Hz, $J_{AX}=5.7$ Hz, $J_{BX}=4.5$ Hz, 4-H_2), 1.69 (3H, s, 2-Me).

Compound 23 A solution of **19** (4 mg) in MeOH (1 ml) was treated with ethereal diazomethane (0.5 ml), and the mixture was stirred for 30 min at room temperature, then evaporated *in vacuo*. The residue was dissolved in DMF (0.2 ml), and methyl iodide (10 μl) and silver oxide (12 mg) were added. The mixture was stirred for 3 h at room temperature in the dark, then diluted with MeOH, filtered and evaporated *in vacuo* to yield a residue, which was purified by preparative TLC ($CHCl_3$–MeOH, 50 : 1) to give the *N,O*-dimethyl derivative of **19** (2 mg, 45%). A solution of the *N,O*-dimethyl derivative of **19** (2 mg) in MeOH (1 ml) was treated with sodium borohydride (10 mg). The mixture was stirred for 15 min at room temperature, then evaporated to yield a residue, which was purified by preparative TLC ($CHCl_3$–MeOH, 30 : 1) to give compound **23** (1 mg, 66%).

23: Yellow powder. IR (KBr): 3437, 2928, 1526, 1383, 1291 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 230 (30000), 266 (23000), 338 (6000). EIMS *m/z*: 226 (M^+, base peak), 211, 197, 195, 183, 168. HREIMS *m/z*: 226.1104 (M^+). Calcd for $C_{14}H_{14}N_2O$ 226.1105. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.62 (1H, dd, $J=4.3$, 1.7 Hz, 6-H), 8.44 (1H, dd, $J=8.1$, 1.7 Hz, 4-H), 7.30 (1H, dd, $J=8.1$, 4.3 Hz, 5-H), 7.02 (1H, s, 8-H), 6.87 (1H, d, $J=0.7$ Hz, 3-H), 4.04 and 4.00 (both 3H, s, 1-NMe and 9-OMe), 2.43 (3H, d, $J=0.7$ Hz, 2-Me).

Periodate Oxidation of Duocarmycin C1 (2) Sodium periodate (8 mg) was added to a stirred solution of duocarmycin C1 (**2**, 10 mg) in a mixture of MeOH (3 ml) and acetic acid (0.1 ml) at room temperature. After 3 h, the reaction mixture was evaporated to yield a residue. Purification by silica gel column chromatography followed by preparative TLC (toluene–acetone, 7 : 3 in both cases) gave compound **20** (4 mg, 43%).

Addition of HCl to Duocarmycin A (1) A solution of duocarmycin A (**1**, 1 mg) in acetone (1 ml) was treated with 2 N HCl (0.1 ml) and the mixture was allowed to stand for 1 min at room temperature. The

formation of duocarmycins C1 (**2**) and C2 (**3**) was detected by HPLC (YMC A-512 CN, 6.0 mm × 150 mm, 50% MeOH, UV 254 nm) in the ratio of 1 : 4. The mixture was evaporated *in vacuo* to yield a residue, which was purified by HPLC (ODS, 80% aqueous MeOH) to give duocarmycins C1 (*ca.* 0.2 mg) and C2 (*ca.* 0.5 mg). These compounds were identical with authentic samples on the basis of MS and NMR comparisons.

Methylation, Followed by Alkaline Degradation of Duocarmycin D (4) A solution of **4** (21 mg) in a mixture of $CHCl_3$ (1 ml) and MeOH (1 ml) was treated with ethereal diazomethane (1 ml), and the mixture was stirred for 30 min at room temperature, then evaporated *in vacuo* to yield a residue. Purification by silica gel column chromatography ($CHCl_3$–MeOH, 10 : 1) gave compounds **24** (12 mg, 98%) and **16** (10 mg, 94%).

24: Yellow powder. $[\alpha]_D^{23}$ −1.5° (c=0.1, MeOH). EIMS m/z: 304 (M^+), 286, 245, 227 (base peak), 212, 184, 143. HREIMS m/z: 304.1074 (M^+). Calcd for $C_{15}H_{16}N_2O_5$: 304.1058. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.19 (1H, br s, 1-NH), 7.08 (1H, d, J=2.5 Hz, 6-H), 7.07 (1H, s, 8-H), 5.31 (1H, br s, 7-NH), 4.82 and 4.74 (2H, AB, J=13.1 Hz, 4-H_2), 3.94 (3H, s, 9-OMe), 3.78 (3H, s, 2-COOMe).

Compound 25 4-Dimethylaminopyridine (2 mg) was added to a solution of compound **24** (12 mg) in a mixture of acetic anhydride (0.4 ml) and pyridine (1 ml). The mixture was stirred for 1 h at room temperature, then poured into phosphate buffer (pH 7.2), and extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ followed by brine, then dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification by preparative TLC (toluene–acetone, 9 : 1) gave the diacetate of **24** (13 mg, 82%). Methyl iodide (50 μl) and silver oxide (3 mg) were added to a solution of the diacetate of **24** (8 mg) in DMF (0.5 ml), and the mixture was stirred for 30 min at room temperature in the dark. It was then diluted with AcOEt and filtered *in vacuo*. Water was added, and the organic layer was washed with brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue, which was purified by HPLC (ODS, 70% aqueous MeOH) to give compound **25** (7 mg, 91%).

25: Yellow powder. $[\alpha]_D^{23}$ −0.8° (c=0.1, MeOH). IR (KBr): 3436, 1736, 1720, 1240 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 238 (13000), 272 (sh 7000), 324 (7000), 410 (4600). EIMS m/z: 402 (M^+), 359, 343 (base peak), 301, 258, 241. HREIMS m/z: 402.1444 (M^+). Calcd for $C_{20}H_{22}N_2O_7$: 402.1426. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.26 (1H, s, 8-H), 7.35 (1H, s, 6-H), 5.56 (2H, AB, 4-H_2), 3.93 (3H, s, 9-OMe), 3.71 (3H, s, 2-COOMe), 3.30 (3H, s, 1-NMe), 2.61 (3H, s, 7-NAc), 2.06 (3H, s, 4-OAc), 1.60 (3H, s, 2-Me). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 195.5 (C-3), 171.0, 168.9, 168.6 (2-COOMe, 7-NAc, 4-OAc), 152.3 (C-9), 146.3 (C-9a), 129.7 (C-7a), 125.3 (C-6), 119.0 (C-3b*), 118.5 (C-5*), 111.3 (C-3a), 108.1 (C-8), 75.0 (C-2), 60.2 (C-4), 56.2 (9-OMe), 53.0 (2-COOMe), 32.6 (1-NMe), 23.9, 21.2, 17.9 (2-Me, 7-NAc and 4-OAc).

Compound 26 A stirred solution of **25** (3 mg) in MeOH (0.5 ml) at room temperature was treated with sodium borohydride (10 mg). The mixture was stirred for 20 min, then poured into phosphate buffer (pH 7.2), and extracted with AcOEt. The extract was washed with brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification by HPLC (ODS, 70% aqueous MeOH) gave compound **26** (1 mg, 45%).

26: Yellow powder. $[\alpha]_D^{23}$ −11.3° (c=0.1, MeOH). EIMS m/z: 302 (M^+), 275, 243 (base peak), 288, 213, 199, 185, 159, 144. HREIMS m/z: 302.1279 (M^+). Calcd for $C_{16}H_{18}N_2O_4$: 302.1267. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.87 (1H, br s, 7-NH), 7.00 (1H, s, 8-H), 6.87 (1H, q, J=1.0 Hz, 6-H), 3.87 (3H, s, 9-OMe), 3.71 (3H, s, 2-COOMe), 3.26 (3H, s, 1-NMe), 2.60 (3H, d, J=1.0 Hz, 4-H_3), 1.60 (3H, s, 2-Me).

Compound 27 A stirred solution of **25** (3 mg) in MeOH (0.5 ml) at room temperature was treated with K_2CO_3 (1 mg). The mixture was stirred for 4 h, then concentrated *in vacuo*, and purified by silica gel column chromatography (toluene–acetone, 5 : 1) to yield compound **27** (1 mg, 39%).

27: Yellow powder. $[\alpha]_D^{23}$ −14.8° (c=0.1, MeOH). IR (KBr): 3469, 2932, 1742, 1648, 1377, 1257 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 229 (16000), 400 (sh 3000), 463 (5000). FABMS m/z: 333 (MH^+). HRFABMS m/z: 333.1447 (MH^+). Calcd for $C_{17}H_{21}N_2O_5$: 333.1450. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.23 (1H, br s, 7-NH), 7.15 (1H, d, J=2.7 Hz, 6-H), 7.03 (1H, s, 8-H), 5.00 (2H, AB in ABX, J_{AB}=11.1 Hz, $J_{AB,X}$= 0.7 Hz, 4-H_2), 3.87 (3H, s, 9-OMe), 3.71 (3H, s, 2-COOMe), 3.49 (3H, s, 4-OMe), 3.27 (3H, s, 1-NMe), 1.61 (3H, s, 2-Me).

Compounds 28 and 29 Sodium cyanoborohydride (30 mg) and a catalytic amount of methyl orange were added to a stirred solution of **25** (3.5 mg) in MeOH (1 ml), and the solution was acidified with 5% HCl in MeOH. The mixture was stirred for 24 h at 40 °C, then diluted with water, and extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification by preparative TLC (toluene–acetone, 7 : 3) gave compounds **28** (1 mg, 32%) and **29** (1 mg, 30%).

28: Yellow powder. $[\alpha]_D^{23}$ −3.5° (c=0.1, MeOH). EIMS m/z: 344 (M^+), 285 (base peak), 243, 227, 213, 199, 185. HREIMS m/z: 344.1369 (M^+). Calcd for $C_{18}H_{20}N_2O_5$: 344.1370. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.30 (1H, br s, 8-H), 7.05 (1H, br s, 6-H), 3.93 (3H, s, 9-OMe), 3.73 (3H, s, 2-COOMe), 3.30 (3H, s, 1-NMe), 2.58 (3H, s, 7-NAc), 2.55 (3H, d, J=1.2 Hz, 4-H_3), 1.61 (3H, s, 2-Me).

29: Yellow powder. $[\alpha]_D^{23}$ −2.2° (c=0.1, MeOH). IR (KBr): 3436, 2939, 1734, 1701, 1234, 1118 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 238 (14000), 300 (sh 7900), 332 (6500), 438 (5000). EIMS m/z: 374 (M^+), 359, 315 (base peak), 300, 273, 258, 241, 227. HREIMS m/z: 374.1461 (M^+). Calcd for $C_{19}H_{22}N_2O_6$: 374.1475. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.29 (1H, s, 8-H), 7.35 (1H, br s, 6-H), 4.95 (2H, br s, 4-H_2), 3.93 (3H, s, 9-OMe), 3.73 (3H, s, 2-COOMe), 3.54 (3H, s, 4-OMe), 3.30 (3H, s, 1-NMe), 2.61 (3H, s, 7-NAc), 1.61 (3H, s, 2-Me).

Conversion of Duocarmycin A (1) to Duocarmycins B1 (5) and B2 (6) A solution of duocarmycin A (**1**, 5 mg) in acetone (5 ml) was treated with 1% aqueous KBr (5 ml) and the solution was allowed to stand for 12 h at room temperature. The formation of duocarmycins B1 (**5**) and B2 (**6**) was determined by HPLC (ODS, 80% aqueous MeOH, UV 254 nm) and their ratio was 1 : 4. The mixture was evaporated *in vacuo* to yield a residue, which was purified by HPLC (ODS, 80% MeOH) to give duocarmycins B1 (**5**, 1 mg, 17%) and B2 (**6**, 3 mg, 52%). These compounds were identical with authentic samples on the basis of $[\alpha]_D$, MS and NMR comparisons.

Conversion of Duocarmycins B1 (5) and B2 (6) to Duocarmycin A (1) A solution of duocarmycin B2 (**6**, 10 mg) in CH_3CN (1 ml) was treated with 10% DBU in CH_3CN (0.1 ml). The mixture was stirred for 1 h at room temperature, then diluted with water, and extracted with AcOEt. The extract was washed with diluted HCl followed by saturated $NaHCO_3$ and brine, then dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification by HPLC (ODS, 80% aqueous CH_3CN) gave duocarmycin A (**1**, 6 mg, 70%). Duocarmycin B1 (**5**, 10 mg) was also converted to **1** (6 mg, 70%) in a similar manner. These compounds were identical with authentic samples on the basis of $[\alpha]_D$, MS and NMR comparisons.

Compounds 30 and 31 A solution of duocarmycin B2 (**6**, 52 mg) in MeOH (2 ml) and $CHCl_3$ (3 ml) was treated with ethereal diazomethane (1 ml), and the mixture was stirred for 30 min at 0 °C, then evaporated to yield the methylate of **6** (52 mg) quantitatively. A solution of the methylate of **6** (52 mg) in MeOH (3 ml) was treated with sodium borohydride (15 mg). The mixture was stirred for 10 min at room temperature, then diluted with water, and extracted with AcOEt. The extract was washed with diluted HCl followed by saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification with a Chromatotron (Harrison Research, U.S.A.) (toluene–acetone, 5 : 1 then 3 : 1) gave compound **30** (19 mg, 36%) and a fraction containing compound **31**. The latter was further purified by HPLC (ODS, 40% aqueous CH_3CN) to give compound **31** (8 mg, 15%).

30: Yellow powder. $[\alpha]_D^{23}$ −5.6° (c=0.4, MeOH). IR ($CHCl_3$): 3580, 3460, 3380, 1728, 1620, 1593, 1521, 1482, 1440, 1290, 1220, 1104 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 296 (sh 14000), 339 (24000). EIMS m/z: 603 (M^+), 585, 505, 370, 352, 311, 272, 234 (base peak), 201, 179. FABMS m/z: 604 (MH^+, ^{79}Br). HRFABMS m/z: 604.1265 (MH^+, ^{79}Br). Calcd for $C_{27}H_{31}{}^{79}BrN_3O_8$: 604.1294. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.56 (1H, br s, 1′-NH), 7.96 (1H, s, 8-H), 6.91 (1H, d, J=2.3 Hz, 3′-H), 6.86 (1H, s, 4′-H), 5.33 (1H, br s, 3-H), 4.56 and 4.50 (2H, AB in ABX, J_{AB}=10.7, J_{AX}=8.8, J_{BX}=4.0 Hz, 6-H_2), 4.08 (1H, dd, J=10.4, 3.6 Hz, 4-H_a), 4.06 (3H, s, 7′-OMe), 3.93 (3H, s, 6′-OMe), 3.90 (3H, s, 5′-OMe), *ca.* 3.9 (1H, m, 5-H), 3.85 (3H, s, 9-OMe), 3.71 (3H, s, 2-COOMe), 3.45 (1H, t, J=10.4 Hz, 4-H_b), 2.61 (1H, br s, 3-OH), 1.59 (3H, s, 2-Me). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 175.9 (2-COOMe), 159.0 (C-2′α), 149.0 (C-5′), 143.6 (C-9), 139.6 (C-6′), 138.9 (C-7′), 136.6 (C-7a*), 136.1 (C-9a*), 131.2 (C-2′), 125.03, 124.99 (C-7′a, C-3a**), 123.2 (C-3′a), 121.4 (C-3b**), 105.2 (C-3′), 101.9 (C-8), 98.0 (C-4′), 74.8 (C-3), 70.6 (C-2), 61.0 (7′-OMe***), 60.9 (6′-OMe***), 55.9 (5′-OMe****), 55.3 (9-OMe****), 55.4 (C-6), 52.2 (2-COOMe), 41.5 (C-5), 38.0 (C-4), 18.3 (2-Me). Assignments with the same numbers of asterisks may be interchanged.

31: Yellow powder. $[\alpha]_D^{23}$ +0.7° (c=0.1, MeOH). IR (KBr): 3436, 2933, 1736, 1626, 1493, 1446, 1310 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 296 (sh 13000), 338 (20000). EIMS m/z: 603 (M^+), 585, 523, 505, 370 (base peak),

552, 311, 290, 272, 258, 234. FABMS *m/z*: 604 (MH^+, ^{79}Br). HRFABMS *m/z*: 604.1295 (MH^+, ^{79}Br). Calcd for $C_{27}H_{31}{}^{79}BrN_3O_8$: 604.1294. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.43 (1H, br s, 1'-NH), 7.98 (1H, br s, 8-H), 6.90 (1H, d, J=2.3 Hz, 3'-H), 6.86 (1H, s, 4'-H), 5.07 (1H, d, J=8.4 Hz, 3-H), 4.57 (1H, dd, J=10.6, 9.3 Hz, 6-H_a), 4.43 (1H, dd, J=10.6, 5.0 Hz, 6-H_b), 4.40 (1H, br s, 1-NH), 4.06 (3H, s, 7'-OMe), *ca.* 4.1 (1H, m, 5-H), 3.94 (3H, s, 6'-OMe), 3.90 (3H, s, 5'-OMe), 3.87 (3H, s, 9-OMe), 3.81 (3H, s, 2-COOMe), 3.78 (1H, dd, J=10.3, 3.4 Hz, 4-H_a), 3.53 (1H, dd, J=10.3, 9.1 Hz, 4-H_b), 3.19 (1H, d, J=8.5 Hz, 3-OH), 1.59 (3H, s, 2-Me). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 172.8 (2-COOMe), 158.9 (C-2'α), 149.0 (C-5'), 143.4 (C-9), 139.6 (C-6'), 139.0 (C-7'), 136.6 (C-7a*), 136.2 (C-9a*), 131.3 (C-2'), 125.0 (C-7'a), 124.2 (C-3a**), 123.2 (C-3'a), 120.9 (C-3b**), 105.2 (C-3'), 101.8 (C-8), 98.0 (C-4'), 79.9 (C-3), 72.2 (C-2), 61.0 (7'-OMe), 60.9 (6'-OMe), 55.9 (5'-OMe***), 55.24 (9-OMe***), 55.18 (C-6), 51.5 (2-COOMe), 40.3 (C-5), 36.6 (C-4), 23.7 (2-Me). Assignments with the same numbers of asterisks may be interchanged.

Compound 32 Compound **31** (8 mg) in a flask was allowed to stand for 10 d at room temperature to give compound **32** quantitatively.

32: Colorless powder. $[\alpha]_D^{23}$ −5.8° (c=0.1, MeOH). IR (KBr): 3459, 2931, 1698, 1636, 1446, 1414, 1313, 1221, 1113 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 246 (17000), 298 (14000), 336 (15000). EIMS *m/z*: 585 (M^+), 537, 492, 352, 529, 272, 234 (base peak), 227, 179. FABMS *m/z*: 586 (MH^+, ^{79}Br). HRFABMS *m/z*: 586.1192 (MH^+, ^{79}Br). Calcd for $C_{27}H_{29}{}^{79}BrN_3O_7$: 586.1189. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.41 (1H, br s, 1'-NH), 8.67 (1H, br s, 1-NH), 8.02 (1H, s, 8-H), 7.01 (1H, d, J=2.3 Hz, 3'-H), 6.90 (1H, s, 4'-H), 4.74 (1H, m, 6-H_a), *ca.* 4.55 (2H, m, 6-H_b and 5-H), 4.07 (3H, s, 7'-OMe), 4.01 (3H, s, 9-OMe), 3.97 (3H, s, 3-COOMe), 3.95 (3H, s, 6'-OMe), 3.92 (3H, s, 5'-OMe), 3.82 (1H, dt, J=*ca.* 9, 2 Hz, 4-H_a), 3.21 (1H, td, J=*ca.* 9, 2 Hz, 4-H_b), 2.73 (3H, s, 2-Me).

MTPA Esters 33 and 34 A solution of duocarmycin A (**1**, 2.5 mg) in $CHCl_3$ (0.5 ml) was treated with (*R*)-(+)-MTPA (20 mg). The mixture was stirred for 15 h at 60 °C, then evaporated to yield a residue which was purified by HPLC (ODS, 70% MeOH) to give compound **33** (2 mg, 55%). Compound **34** (2 mg, 55%) was also obtained from **1** (2.5 mg) and (*S*)-(−)-MTPA (20 mg) in a similar manner.

33: Yellow powder. $[\alpha]_D^{23}$ +0.1° (c=0.1, MeOH). IR (KBr): 3410, 2954, 1752, 1703, 1619, 1504, 1307, 1114 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 250 (sh 14000), 300 (sh 15000), 337 (23000), 430 (3000). FABMS *m/z*: 742 (MH^+). HRFABMS *m/z*: 742.2212 (MH^+). Calcd for $C_{36}H_{35}F_3N_3O_{11}$: 742.2224. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.40 (1H, br s, 1'-NH), 8.97 (1H, br s, 9-OH), 8.49 (1H, br s, 8-H), 7.4—7.2 (5H, phenyl protons), 6.82 (1H, s, 4'-H), 6.80 (1H, d, J=2.3 Hz, 3'-H), 5.31 (1H, br s, 1-NH), 4.81 and 4.66 (2H, AB in ABX, J_{AB}=11.0 Hz, J_{AX}=3.2 Hz, J_{BX}=6.5 Hz, 4-H_2*), 4.53 and 4.38 (2H, AB in ABX, J_{AB}=10.5 Hz, J_{AX}=10.5 Hz, J_{BX}=4.1 Hz, 6-H_2*), *ca.* 4.2 (1H, 5-H), 4.17 (3H, s, 7'-OMe), 3.98 (3H, s, 6'-OMe), 3.93 (3H, s, 5'-OMe), 3.76 (3H, s, 2-COOMe), 3.40 (3H, s, PhOMe), 1.69 (3H, s, 2-Me). Assignments with an asterisk may be interchanged.

34: Yellow powder. $[\alpha]_D^{23}$ −2.7° (c=0.2, MeOH). IR (KBr): 3405, 2940, 1751, 1702, 1619, 1500, 1307, 1115 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 250 (sh 16000), 300 (sh 16000), 337 (25000), 430 (3300). FABMS *m/z*: 742 (MH^+). HRFABMS *m/z*: 742.2229 (MH^+). Calcd for $C_{36}H_{35}F_3N_3O_{11}$: 742.2224. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.42 (1H, br s, 1'-NH), 8.99 (1H, br s, 9-OH), 8.46 (1H, br s, 8-H), 7.4—7.2 (5H, phenyl protons), 6.82 (1H, s, 4'-H), 6.79 (1H, d, J=2.3 Hz, 3'-H), *ca.* 5.3 (1H, br, 1-NH), 4.79 and 4.66 (2H, AB in ABX, J_{AB}=11.0 Hz, J_{AX}=3.6 Hz, J_{BX}=7.9 Hz, 4-H_2*), 4.52 and 4.32 (2H, AB in ABX, J_{AB}=10.5 Hz, J_{AX}=10.5 Hz, J_{BX}=4.2 Hz, 6-H_2*), *ca.* 4.2 (1H, 5-H), 4.16 (3H, s, 7'-OMe), 3.98 (3H, s, 6'-OMe), 3.94 (3H, s, 5'-OMe), 3.75 (3H, s, 2-COOMe), 3.43 (3H, s, PhOMe), 1.71 (3H, s, 2-Me). Assignments with an asterisk may be interchanged.

Borohydride Reduction of Duocarmycin B2 (6) A solution of duocarmycin B2 (**6**, 140 mg) in MeOH (1 ml) and THF (5 ml) was treated with $NaBH_4$ (20 mg). The mixture was stirred for 1 h at room temperature, then diluted with water, and extracted with AcOEt. The extract was washed with diluted HCl followed by saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification with a Chromatotron (Harrison Research, U.S.A.) ($CHCl_3$–MeOH, 10 : 1) gave compound **35** (77 mg, 55%).

35: Pale yellow powder. $[\alpha]_D^{23}$ −6.2° (c=0.1, MeOH). IR (KBr): 3401, 2938, 1733, 1620, 1490, 1310, 1112 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 300 (sh 13000), 340 (19000). FABMS *m/z*: 590 (MH^+, ^{79}Br). HRFABMS *m/z*: 590.1138 (MH^+, ^{79}Br). Calcd for $C_{26}H_{29}{}^{79}BrN_3O_8$: 590.1138. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.13 (1H, br s, 9-OH), 9.54 (1H, br s, 1'-NH), 8.22 (1H, br s, 8-H), 6.91 (1H, s, 4'-H), 6.85 (1H, d, J=2.1 Hz, 3'-H), 5.17 (1H, br s, 3-H), 4.79 (1H, br s, 1-NH), 4.42 (1H, br t, J=9.8 Hz, 6-H_a), 4.15 (3H, s, 5'-OMe*), *ca.* 4.1 (1H, dd, 6-H_b), 4.00 (3H, s, 7'-OMe*), 3.96 (3H, s, 6'-OMe*), *ca.* 3.9 (1H, dd, 4-H_a), *ca.* 3.8 (1H, m, 5-H), 3.61 (3H, s, 2-COOMe), 2.94 (1H, br t, J=10.5 Hz, 4-H_b), 1.71 (3H, s, 2-Me). Assignments with asterisk may be interchanged.

Compound 36 A solution of **35** (10 mg) in CH_3CN (1 ml) was treated with 10% DBU in CH_3CN (0.1 ml). The mixture was stirred for 10 min at room temperature, then diluted with water, and extracted with AcOEt. The extract was washed with diluted HCl followed by saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification with a Chromatotron (Harrison Research, U.S.A.) ($CHCl_3$–MeOH, 15 : 1) gave compound **36** (6 mg, 70%).

36: Yellow powder. $[\alpha]_D^{23}$ −5.1° (c=0.1, MeOH). IR (KBr): 3376, 2934, 1727, 1615, 1312, 1118 cm^{-1}. EIMS *m/z*: 509 (M^+), 491, 276, 258, 234 (base peak), 198, 179. HREIMS *m/z*: 509.1776 (M^+). Calcd for $C_{26}H_{27}N_3O_8$: 509.1798. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.43 (1H, br s, 1'-NH), 8.06 (1H, br s, 8-H), 7.28 (1H, br s, 6-H), 6.88 (1H, d, J=2.2 Hz, 3'-H), 6.82 (1H, s, 4'-H), 5.57 (1H, s, 3-H), 4.13 (3H, s, 7'-OMe), 3.97 (3H, s, 6'-OMe), 3.90 (3H, s, 5'-OMe), 3.65 (3H, s, 2-COOMe), 2.34 (3H, br s, 4-H_3), 1.73 (3H, br s, 2-Me).

Compound 37 Benzoic anhydride (50 mg) and 4-dimethylaminopyridine (5 mg) were added to a stirred solution of **35** (10 mg) in pyridine (0.5 ml), and the mixture was stirred for 3 h at room temperature. After the reaction, MeOH (0.5 ml) was added and the solution was stirred for 10 min to quench residual anhydride, then diluted with water, and extracted with AcOEt. The extract was washed with diluted HCl followed by saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification by silica gel column chromatography (*n*-hexane–AcOEt, 3 : 1 then 2 : 1) followed by HPLC (ODS, 80% CH_3CN) gave compound **37** (4 mg, 30%).

37: Yellow powder. $[\alpha]_D^{23}$ −3.1° (c=0.2, MeOH). IR (KBr): 3450, 2934, 1736, 1625, 1314, 1247, 1111 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 231 (46000), 334 (29000). FABMS *m/z*: 797 (M^+, ^{79}Br). HRFABMS *m/z*: 797.1601 (M^+, ^{79}Br). Calcd for $C_{40}H_{36}{}^{79}BrN_3O_{10}$: 797.1584. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.30 (1H, br s, 1'-NH), 8.26 (1H, s, 8-H), {8.62 (2H, dd, J=8.4, 1.3 Hz), 8.12 (2H, dd, J=8.4, 1.3 Hz), 7.67 (1H, t, J=7.4 Hz), 7.62 (1H, t, J=7.4 Hz), 7.54 (2H, dd, J=8.4, 7.4 Hz), 7.48 (2H, dd, J=8.4, 7.4 Hz) [phenyl × 2]}, 7.09 (1H, s, 3-H), 6.90 (1H, d, J=2.3 Hz, 3'-H), 6.85 (1H, s, 4'-H), *ca.* 4.6 (2H, AB in ABX, 6-H_2), 4.07 (3H, s, 7'-OMe), 3.93 (3H, s, 6'-OMe), 3.90 (3H, s, 5'-OMe), *ca.* 3.9 (1H, m, 5-H), 2.82 (3H, s, 2-COOMe), 3.45 (1H, dd, J=10.4, 3.2 Hz, 4-H_a), 3.24 (1H, dd, J=10.4, 10.1 Hz, 4-H_b), 1.56 (3H, s, 2-Me).

Compound **38** A solution of duocarmycin B2 (**6**, 230 mg) in MeOH (20 ml) was treated with 28% sodium methoxide in MeOH (75 μl), and the mixture was stirred for 1 h at room temperature. Water was added, and the whole was extracted with AcOEt. The extract was washed with brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification by silica gel column chromatography ($CHCl_3$–MeOH, 20 : 1) gave the Seg. A of **6** (101 mg, 94%). The obtained compound (101 mg) was dissolved in acetic anhydride (1 ml) and pyridine (5 ml), and the solution was stirred for 1 h at room temperature, then MeOH (1 ml) was added. After 15 min, water was added and the mixture was extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to yield an acetate (37 mg, 32%). The obtained acetate (32 mg) was dissolved in THF (10 ml) and 2 N HCl (0.2 ml) was added. The solution was stirred for 10 min at room temperature, then diluted with water, and extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification by silica gel column chromatography ($CHCl_3$–AcOEt, 4 : 1) gave compound **38** (30 mg, 84%).

38: Yellow powder. $[\alpha]_D^{23}$ −4.8° (c=0.1, MeOH). IR (KBr): 3344, 1745, 1698, 1614, 1511, 1254 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 265 (22000), 429 (4000). EIMS *m/z*: 352 (M^+: base peak), 316, 293, 273, 257, 215, 201, 173. FABMS *m/z*: 353 (MH^+, ^{35}Cl). HRFABMS *m/z*: 353.0917 (MH^+, ^{35}Cl). Calcd for $C_{16}H_{18}{}^{35}ClN_2O_5$: 353.0904. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.72 (1H, br s, 9-OH), 8.32 (1H, s, 8-H), 5.52 (1H, br s, 1-NH), 4.19 (1H, dd, J=10.7, 9.3 Hz, 6-H_a), 4.11 (1H, dd, J=10.7, 3.3 Hz, 6-H_b), 4.02 (1H, t, J=10.7 Hz, 4-H_a), *ca.* 4.0 (1H, m, 5-H), 3.80 (3H, s, 2-COOMe), 3.63 (1H, dd, J=10.7, 8.3 Hz, 4-H_b), 2.30 (3H, s, 7-NAc), 1.68 (3H, s, 2-Me).

Compound 39 Benzoic anhydride (50 mg) and 4-dimethylaminopyr-

idine (5 mg) were added to a stirred solution of **38** (25 mg) in pyridine (1.5 ml), and the mixture was stirred for 1 h at room temperature. After the reaction, MeOH (0.5 ml) was added and the solution was stirred for 15 min to quench residual anhydride, and evaporated to yield a residue. Purification by silica gel column chromatography ($CHCl_3$–AcOEt, 5 : 1) gave a monobenzoate (25 mg, 77%). This product (22 mg) was dissolved in MeOH (0.5 ml) and THF (2 ml), and $NaBH_4$ (5 mg) was added. The mixture was stirred for 5 min at 0 °C, then diluted with aqueous HCl, and extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated to yield a residue. Purification by silica gel column chromatography ($CHCl_3$–AcOEt, 10 : 1) gave the 3-ol product (14 mg, 63%). The obtained product (5 mg) was dissolved in pyridine (0.5 ml), then benzoic anhydride (10 mg) and 4-dimethylaminopyridine (2 mg) were added. The mixture was stirred for 3 h at room temperature, then evaporated to yield a residue, which was purified by silica gel column chromatography ($CHCl_3$–AcOEt, 10 : 1) followed by preparative TLC ($CHCl_3$–AcOEt, 5 : 1) to give compound **39** (3.5 mg, 57%).

39: Pale yellow powder. $[\alpha]_D^{23}$ −9.5° (c = 0.2, MeOH). IR (KBr): 3449, 1736, 1655, 1485, 1452, 1250, 1096 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 230 (24000), 273 (12000), 330 (3000). EIMS m/z : 562 (M^+), 503, 440, 404, 391, 349, 317, 257, 225, 105 (base peak). FABMS m/z: 563 (MH^+, ^{35}Cl). HRFABMS m/z: 563.1570 (MH^+, ^{35}Cl). Calcd for $C_{30}H_{28}{}^{35}ClN_2O_7$: 563.1585. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.16 (1H, s, 8-H), {8.24 and 8.10 (both 2H, dd-like), 7.66 and 7.62 (both 1H, t-like), 7.53 and 7.47 (both 2H, t-like) [phenyl × 2]}, 7.04 (1H, s, 3-H), 4.79 (1H, br s, 1-NH), *ca.* 4.1 (2H, AB in ABX, 6-H_2), 3.81 (3H, s, 2-COOMe), 3.75 (1H, m, 5-H), 3.35 (1H, dd, J = 11.1, 3.3 Hz, 4-H_a), 3.35 (1H, dd, J = 11.1, 9.5 Hz, 4-H_b), 2.22 (3H, s, 7-NAc), 1.53 (3H, s, 2-Me). CD λ_{max}^{MeOH} nm ($\Delta\varepsilon$): 375 (0), 332 (−4.5), 296 (0), 274 (+3.3), 247 (0), 239 (−5.8), 233 (0), 222 (+13.0), 215 (0), 210 (−6.4).

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References and Notes

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