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ANTITUMOR ANTIBIOTICS: DUOCARMYCINS

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INTRODUCTION

In our continuing search for biologically active compounds from microorganisms to develop new alkylating agents and other compounds that interfere with the functioning of DNA, novel potent antitumor antibiotics designated duocarmycins were isolated from three different *Streptomyces* species. The producing organisms of duocarmycin A (**1**) [1-5], that of duocarmycin B1 (**2**) [6], B2 (**3**) [7], C1 (**4**) [8], and C2 (**5**) [9, 10], and that of duocarmycin SA (**6**) [11, 12] were isolated from soil collected in Shizuoka, Hyogo, and Kyoto, Japan.* These duocarmycins showed strong antimicrobial activity, mainly against Gram-positive bacteria. The MICs of duocarmycin A, C1, and SA against *Staphylococcus aureus* are 0.0064, 0.03 and 0.0013 μg/ml, respectively. Duocarmycins also showed antitumor activity against murine lymphocytic leukemia P388 transplanted in CDF1 mice and murine sarcoma 180 in *ddY* mice. The structural determinations of these compounds (Chart 1) were accomplished by spectroscopic and chemical analyses [13]. Duocarmycin A and SA possess a unique structure having dienone segment conjugated with the cyclopropane ring, which is also in CC-1065 (**7**) found in *Streptomyces zelenis* [14-18].

The mode of action of **1** has been reported to derive cytotoxicity through a sequence-selective minor groove alkylation of double-stranded DNA which mediates, as in the case of CC-1065, N(3) adenine covalent adduct formation, although some differences were detected in their sequence specificity [19-25]. In the course of our investigation of the thermal depurination products of DNA covalently bonded to **1**, we obtained not only the duocarmycin A-N(3) adenine adduct but also the duocarmycin A-N(3) guanine adduct (Chart 2) [26]. In addition, it was observed that **1** can be released from its covalent DNA adducts under the biological conditions; namely the reversibility of formation of duocarmycins—DNA adducts was demonstrated [27, 28]. Therefore, duocarmycins have generated considerable interest as a consequence of the potent cytotoxic activity exhibited by these agents [see reviews 29-32].

The excellent *in vivo* antitumor activities of CC-1065 derivatives prompted intense research aimed at understanding the influence of structural modifications of the duocarmycin A molecule on biological activity [33-41]. Therefore, we modified the A-ring part of **1** to have significant biological activity and good stability of an active form. The structural difference between **1** (as an active form) and CC-1065 in electrophilic DNA alkylating segment (segment A) is that they are pyrrolidone and pyrrole derivatives, respectively. Compound **1** has several advantages and opportunities afforded by this novel structure: (i) the presence of the C(2) methoxycarbonyl group serves as a site for reduction or transesterification; (ii) the presence of the C(3) carbonyl group serves as an additional site for reduction, followed by modifications to obtain more suitable analogs; (iii) amine N(1) atom serves as an alkylation or acylation site. After conducting a number of exploratory experiments, we happened to find that the A-ring pyrrole compound could be produced in good yield by acid-catalyzed rearrangement of the 3-hydroxy derivatives of **3**.

In this paper, we describe the synthesis of the A-ring pyrrole derivatives of duocarmycins, and the evaluation of their antitumor activity and the relationship between the physical properties and biological potency.

*Pyrindamycin A and B were found by workers at Meiji Seika Co., Ltd. to be identical to duocarmycin C2 and C1, respectively.

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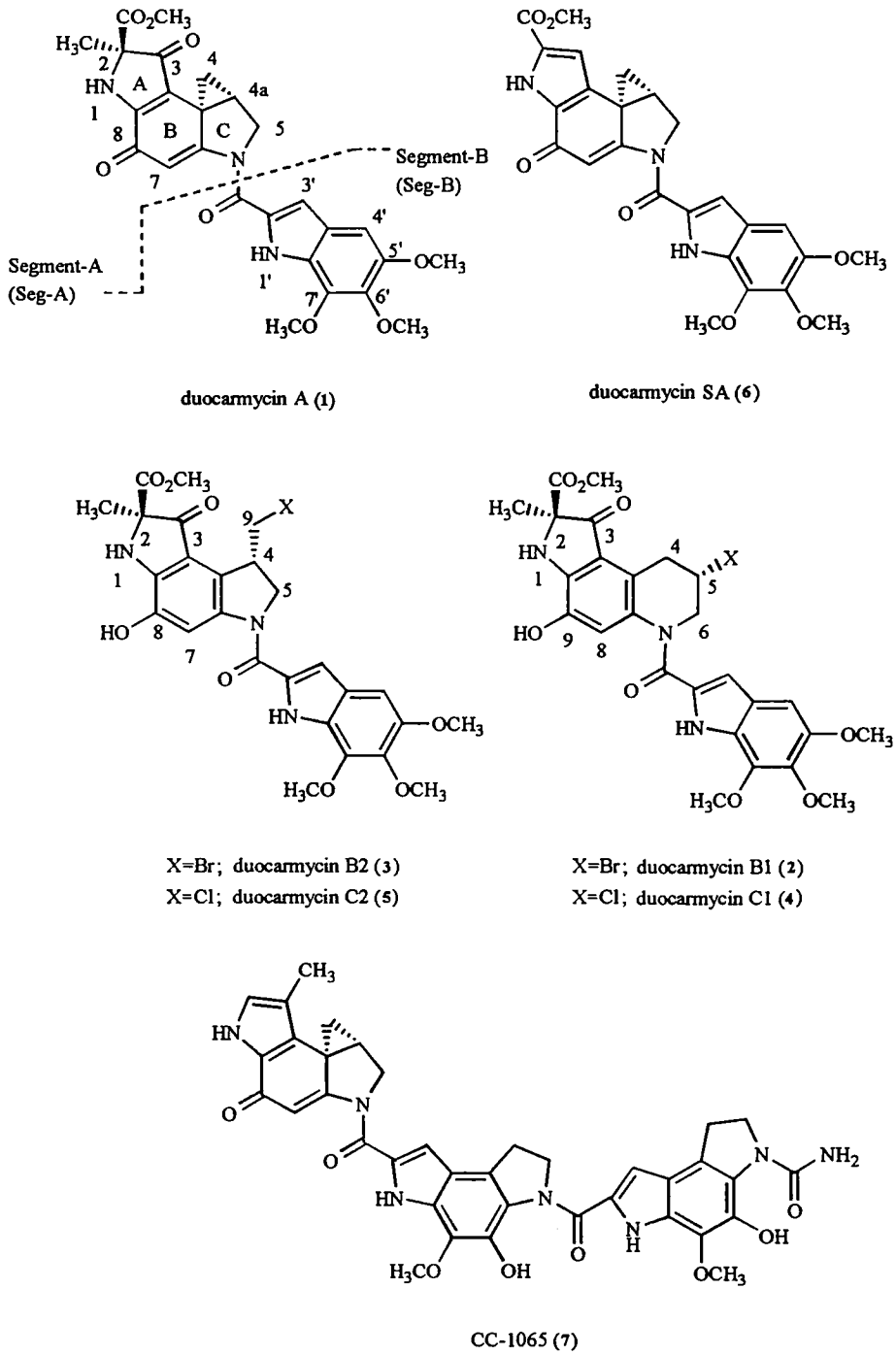
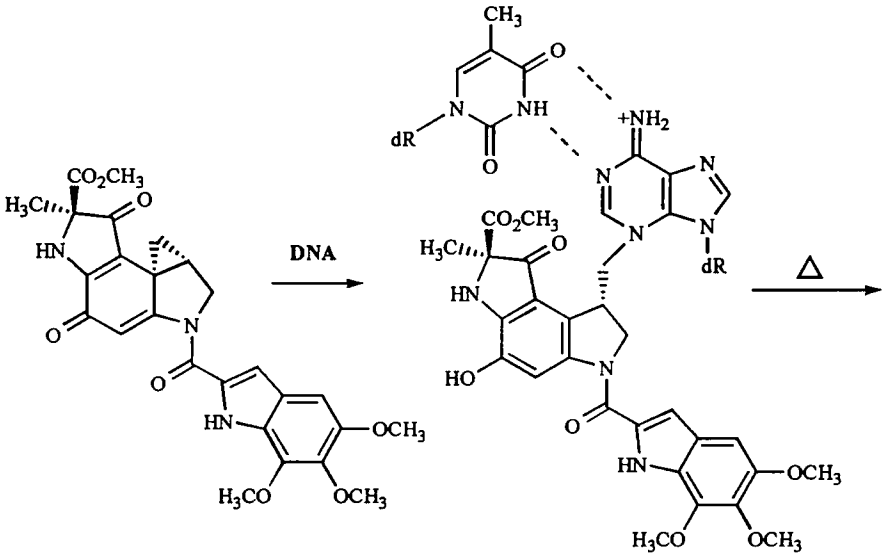


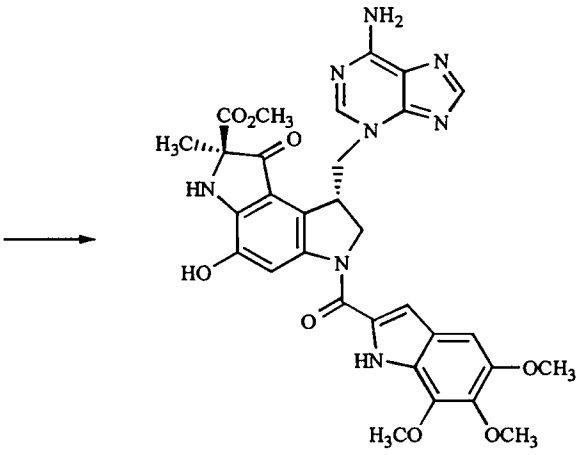
Chart 1. Structures of Duocarmycins and CC-1065

CHEMISTRY

The A-ring pyrrole analogs of **1** were synthesized according to Scheme 1. The phenolic hydroxyl group of **3** was protected by *tert*-butyldimethylchlorosilane in DMF to give **8** quantitatively. The protection facilitated further chemical modification since **3** is easily transformed to **1**, followed by decomposition, under basic conditions. Compound **8** was reduced with sodium borohydride in methanol or allyl alcohol to afford the 3α- or 3β-hydroxy com-



duocarmycin A (1)



duocarmycin A - N3 adenine adduct

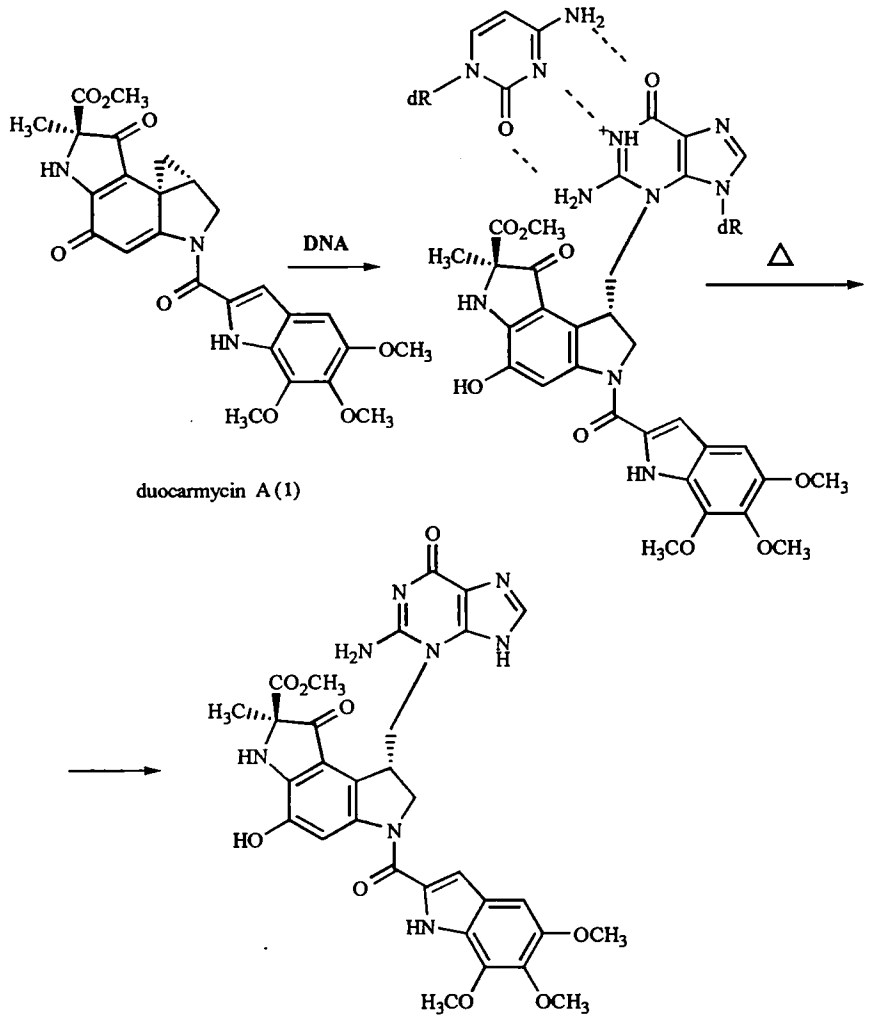
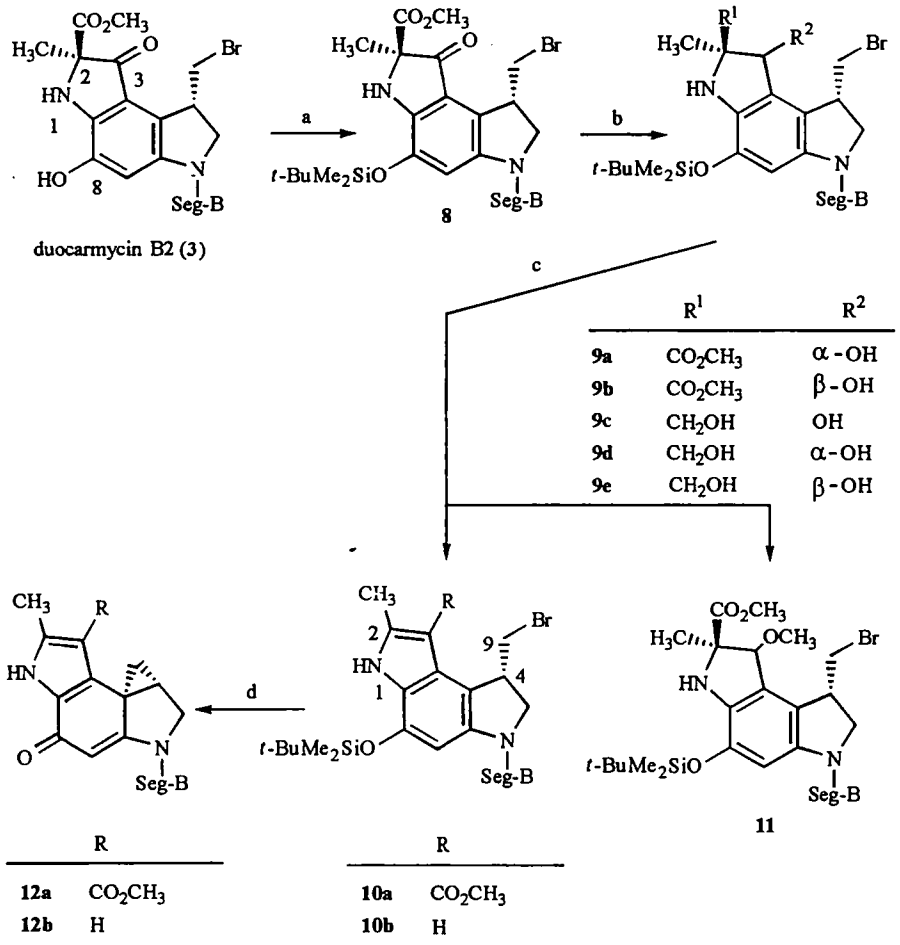


Chart 2. Duocarmycin A—DNA Adducts

pounds (**9a** and **9b**), the 2-decarbomethoxy-3-hydroxy compound (**9c**), and the diols (**9d** and **9e**). It is speculated that compound **9c** is formed by hydrolysis, decarboxylation and reduction under the reaction conditions. The production ratio of **9a-e** was greatly affected by solvent and temperature. The reduction of **8** was carried out in methanol to afford many overreductive compounds. We found that the reduction was best carried out in allyl alcohol as a solvent at 0°C to give **9a** as the major product in 74% yield. Herein, the configuration at the $C_{(3)}$ center was confirmed by NMR.

The obtained **9a** or **9b** was treated with camphorsulfonic acid (CSA) in toluene. In this reaction, an interesting rearrangement of the methoxycarbonyl group occurred to afford the A-ring pyrrole analog **10a** in reasonable yield. The structure of **10a** was elucidated on the basis of NMR and mass spectrometry [13]. The mass spectrometry of **10a** gave molecular ions at *m/z* 688 (^{81}Br), confirming the dehydration of **9a**. The ^1H-NMR spectra of **10a** showed downfield shifts of the 2-CH_3 proton signal (~1.0 ppm) and of the 1-NH proton signal (~3.0 ppm), confirming the structure of A-ring pyrrole compound. Nuclear Overhauser effects (NOEs) from 2-CH_3 to 9-H_2 or 4-H were not observed in the NMR spectra, but apparent NOE and long range coupling from 2-CH_3 to 1-NH were observed in **10a**. As NOEs from 3-H to 9-H_2 or 4-H are observed in **9a-e**, a 1,2-shift of the methoxycarbonyl group from $C_{(2)}$ to $C_{(3)}$ was established. The mechanism was considered to be a Wagner-Meerwein type rearrangement which was reported by D. Berner *et al.* [42]. When this rearrangement reaction of **9a** was achieved in methanol as a reaction

Scheme 1



(a) *tert*-BuMe2SiCl, imidazole, DMF, (b) $NaBH_4$, allyl alcohol or MeOH, (c) CSA, toluene MeOH, (d) *n*-Bu_4NF, THF

solvent, compound **11** as a 1:1 mixture of the two diastereomers was predominantly produced. Therefore, it is indicated that this reaction has occurred through a carbocation intermediate, as depicted in Chart 3, and this result also supports that this reaction is a Wagner-Meerwein type rearrangement. The 2-decarbomethoxy-3-hydroxy compound **9c** was also treated with CSA to afford the dehydration product **10b** in 77% yield. In contrast, the diols (**9d** and **9e**) gave no rearrangement products under the same conditions. The desilylation of **10a** and **10b** was carried out with *n*-Bu_4NF in THF to give **12a** and **12b**, respectively.

We found that treatment of the 8-O-*tert*-butyldimethylsilyl-3-hydroxy derivatives of **3** with CSA in toluene gave the A-ring pyrrole analogs of **1** in good yields. As an extension of that research, we investigated this acid-catalyzed rearrangement of various 3-hydroxy-derivatives of duocarmycin B1 (**2**) (Scheme 2). Compound **2** was converted to the 9-O-silyl (**13a**) and the 9-O-N,N-dialkylcarbamoyl compounds (**13b,c**) in 99%, 98%, and 79% yields, respectively. The obtained **13a-c** were reduced by $NaBH_4$ in allyl alcohol to afford the corresponding 3α-hydroxy compounds (**14a-c**) in reasonable yields. The rearrangement of **14a** was performed by CSA in toluene to afford **15a** in 79% yield. However, treatment of **14b** with CSA did not give the desired product, but exclusively afforded the spiro compound **16b** as a single isomer, which was derived from a 1,2-shift of the methoxycarbonyl group and a bonding between the C(8) position and the C(2′) position (72% yield). This structure was elucidated by NMR and mass spectrometry (Table 1). The mass spectrometry of **16b** showed an ion corresponding to the entire molecule at *m/z*

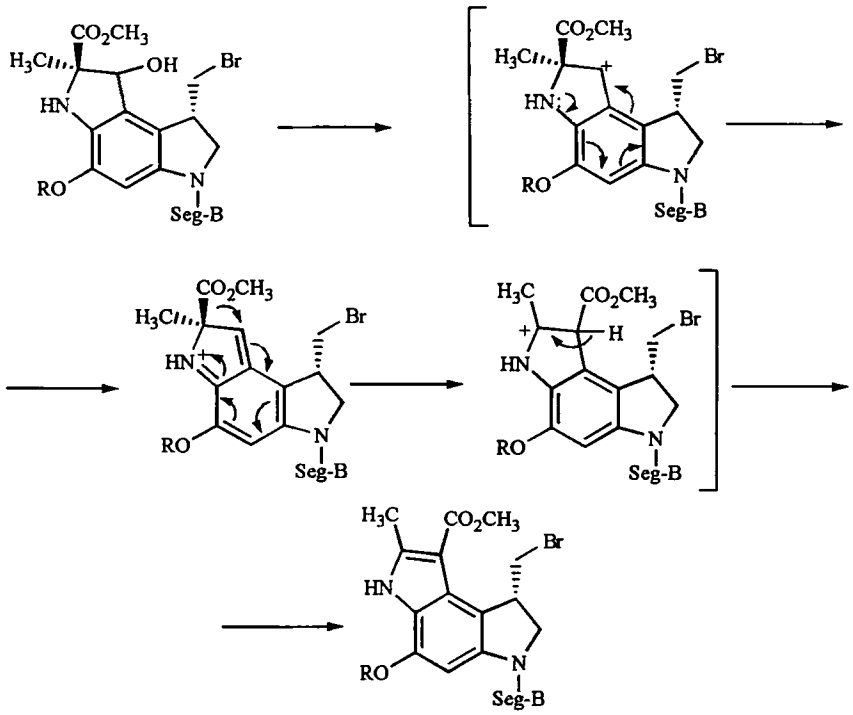


Chart 3. Proposed Mechanism of Wagner-Meerwein Rearrangement for Production of the A-Ring Pyrrole Derivatives

645 [^{81}Br; (M + H)$^+$], but the characteristic fragment ion *m/z* 234 ($C_{12}H_{12}NO_4$, trimethoxyindol-2-yl-carbonyl) of duocarmycin derivatives was not observed. These results confirmed the structure of **16b**.

On the other hand, the same treatment of **14c**, having a protecting group of medium size between those in **14a** and **14b** at the C(9) position, gave both **15c** and the spiro compound **16c** as a single isomer in 79% and 11% yields, respectively. When the reaction time of **14c** was prolonged from 2 h to 5 h at the same temperature, the yield of spiro compound **16c** was increased to 19%, and that of **15c** was decreased to 70%. On the basis of these results, a plausible mechanism for the formation of **16b** and **16c** is as follows: a 1,2-shift of the methoxycarbonyl group has occurred initially, then the spiro compound is produced as shown in Chart 4. Herein, the effect of the substituents at the C(9) position that involve an increase in bulk is important. In contrast, none of the spiro compounds in this rearrangement of duocarmycin B2 derivatives was observed; it is considered that steric straining in a 5-membered system of the C-rings is presumably responsible for this inertness [44].*

In conjunction with these studies, we synthesized the derivatives of **12a**. Compound **12a** was dissolved in $CHCl_3$, and stirred at room temperature for 10 days under fluorescent lamp. Under this condition, an interesting cyclization occurred to afford compound **17**, which was derived from a bonding between the C(7) position and the C(3′) position (45% yield) as shown in Scheme 3. Compound **17** is less polar than **12a**, and the structure was elucidated on the basis of mass spectrometry and NMR, especially two-dimensional heteronuclear multiple bond connectivity spectroscopy (HMBC). The mechanism was considered to be a photochemical cyclization between the enamide and the indole, as reported by Hutchins *et al.* [45]. When this reaction was conducted without light, the cyclized product was not obtained. This result also supports that this reaction is a photochemical cyclization. Compound **12a** was also treated by slightly excess iodomethane in the presence of K_2CO_3 in DMF to afford the compound **18** methylated at the N(1) position selectively. The structure of **18** was proved by the observation of the coupling between the N(1′) proton and the C(3′) proton in the NMR spectrum. When this reaction was achieved with 3 eq. of iodomethane, compound **19** methylated both at the N(1) and at the N(1′) positions was produced.

*Treatment of **9a** with CSA in toluene at 50°C afforded **10a** in 65% yield accompanied with **12a** in 30% yield. It is probably considered that the silyl moiety of **10a** was removed under acidic conditions to produce **12a** during the purification process. Therefore, it is indicated that a 1,2-shift of the methoxycarbonyl group has proceeded quantitatively.

TABLE 1. ^1H NMR Data for Compounds **15a**, **15c**, **16b**, and **16c** at 400 MHz (in $CDCl_3$)

Proton	15a	15c	16b	16c
1-NH	9.02 (br. s)	9.13 (br. s)	8.91 (br. s)	8.92 (br. s)
2-CH_3	2.69 (s)	2.66 (s)	2.33 (s)	2.65 (s)
3-CO_2CH_3	3.78 (s)	3.81 (s)	3.80 (s)	3.81 (s)
4-H	3.74 (br. d, J = 6.5 Hz) 4.07 (br. d, J = 5.7 Hz)	3.74 (m) 3.79 (br. d, J = 7.4 Hz)	3.77 (dd, J = 18.0, 6.0 Hz) 3.83 (dd, J = 18.0, 3.5 Hz)	3.75 (m) 3.76 (m)
5-H	4.58 (m)	4.56 (m)	4.63 (m)	4.64 (m)
6-H	4.19 (dd, J = 11.0, 6.1 Hz) 4.24 (dd, J = 11.0, 5.7 Hz)	4.32 (m) 4.44 (m)	4.03 (dd, J = 13.6, 3.6 Hz) 4.07 (dd, J = 13.6, 5.8 Hz)	4.04 (m) 4.20 (m)
8-H	6.55 (s)	6.94 (s)	—	—
1'-NH	8.21 (br. s)	8.88 (br. s)	3.85 (br. s)	4.03 (br. s)
3'-H	6.05 (d, J = 2.3 Hz)	6.15 (br. s)	3.43 (d, J = 16.4 Hz) 3.62 (d, J = 16.4 Hz)	3.45 (d, J = 16.8 Hz) 3.59 (d, J = 16.8 Hz)
4'-H	6.45 (s)	6.64 (s)	6.52 (s)	6.52 (s)
5'-OCH_3[a]	3.90 (s)	3.90 (s)	3.86 (s)	3.88 (s)
6'-OCH_3[a]	3.93 (s)	3.92 (s)	3.88 (s)	3.88 (s)
7'-OCH_3[a]	4.03 (s)	4.05 (s)	3.89 (s)	3.89 (s)
	−0.08 (s, CH_3×2)	2.37 (s, N—CH_3)	2.51 (s, N—CH_3)	2.38 (s, N—CH_3)
	0.88 (s, $(CH_3)_3$)	2.50 (br. s, N—CH_2×2)	2.76 (s, N—CH_3)	2.49 (br. s, N—CH_2×2)
		3.61 (br. s, N—CH_2)		3.56 (br. s, N—CH_2)
		3.72 (br. s, N—CH_2)		3.63 (br. s, N—CH_2)

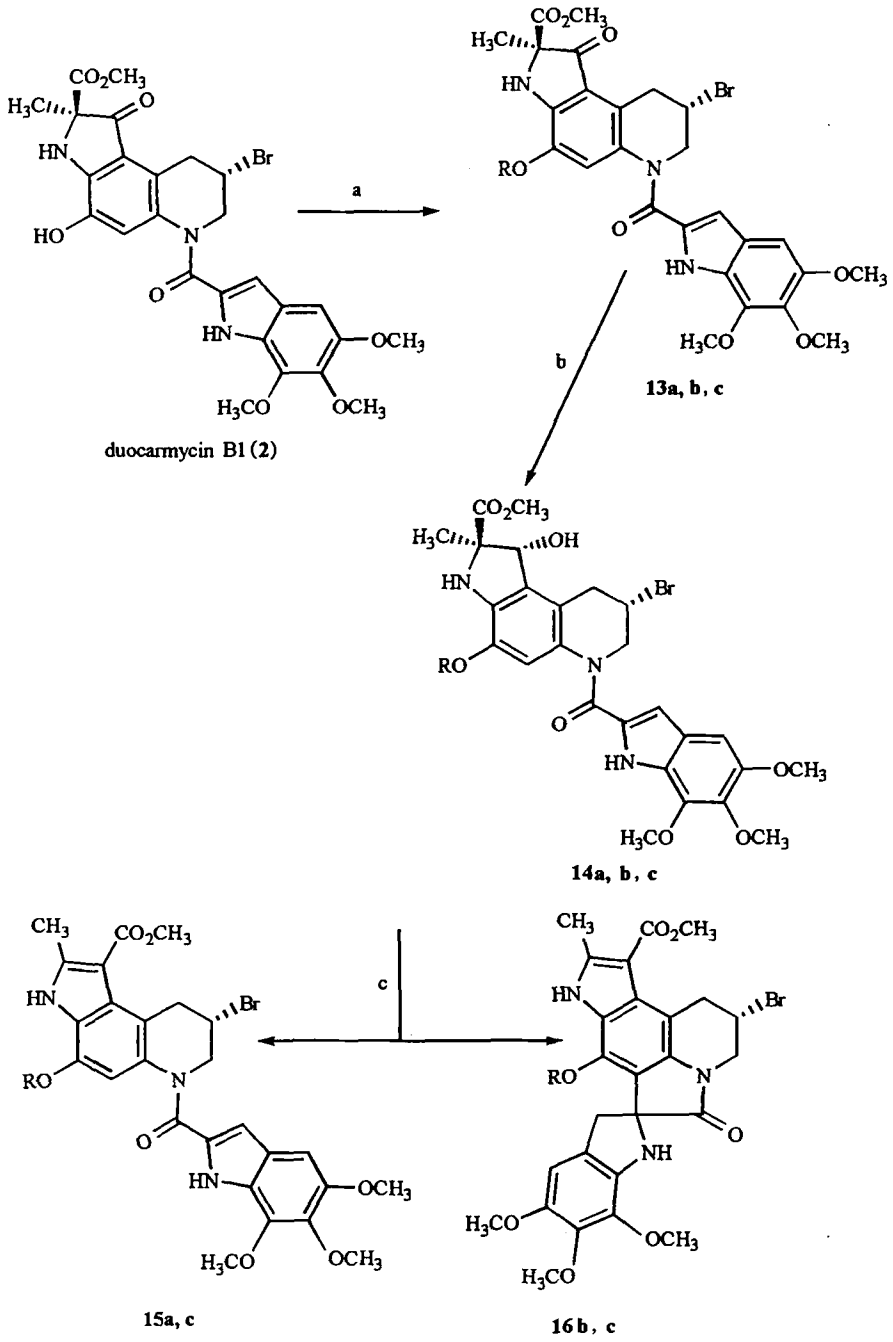
[a] Exchangeable assignments.

As will be mentioned later, the A-ring pyrrole compound **12a** exhibited good stability superior to **1** or **3**, and exceptionally potent *in vitro* anticellular activity equal to that of **1** or **3**. However, the solubility of **12a** was very poor, and it did not show attractive *in vivo* antitumor activity against sarcoma 180; the effective range of dose was extremely narrow. We have already acquired the information that the introduction of a N,N-dialkylcarbamoyl moiety at the 8-O-phenolic hydroxy group of duocarmycins showed excellent *in vivo* antitumor activity, superior to that of the parent compounds [46]. Therefore, in order to improve the *in vivo* antitumor activity and solubility, compound **12a** was converted to the 8-O-N,N-dialkylcarbamoyl derivatives. Compound **12a** was treated with 48% HBr, followed by the addition of *p*-nitrophenyl chloroformate in the presence of triethylamine in methylene chloride at −78°C to give a carbonate **20** as an intermediate (Scheme 3). Various secondary amines were added to **20** to give the 8-O-N,N-dialkylcarbamoyl derivatives **21a-d** in good yields. The HBr adducts of **12a** could not be isolated due to chemical instability. They immediately reverted to the cyclopropane compounds during purification. Compound **21b** was transformed to hydrobromide upon treatment with 48% hydrobromic acid in acetone–ethanol. The solubility of this salt **21e** in water was found to be above 10 mg/mL.

BIOLOGICAL RESULTS AND DISCUSSION

The antitumor activity of some representative derivatives was evaluated primarily by assays of growth inhibition against HeLa S_3 cells (*in vitro*), and antitumor activity against murine sarcoma 180 (*in vivo*), as shown in Table 2.

Scheme 2



(a) *tert*-$BuMe_2SiCl$, imidazole, DMF or *p*-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, then dimethylamine or 4-methylpiperazine; (b) $NaBH_4$, allyl alcohol; (c) CSA, toluene

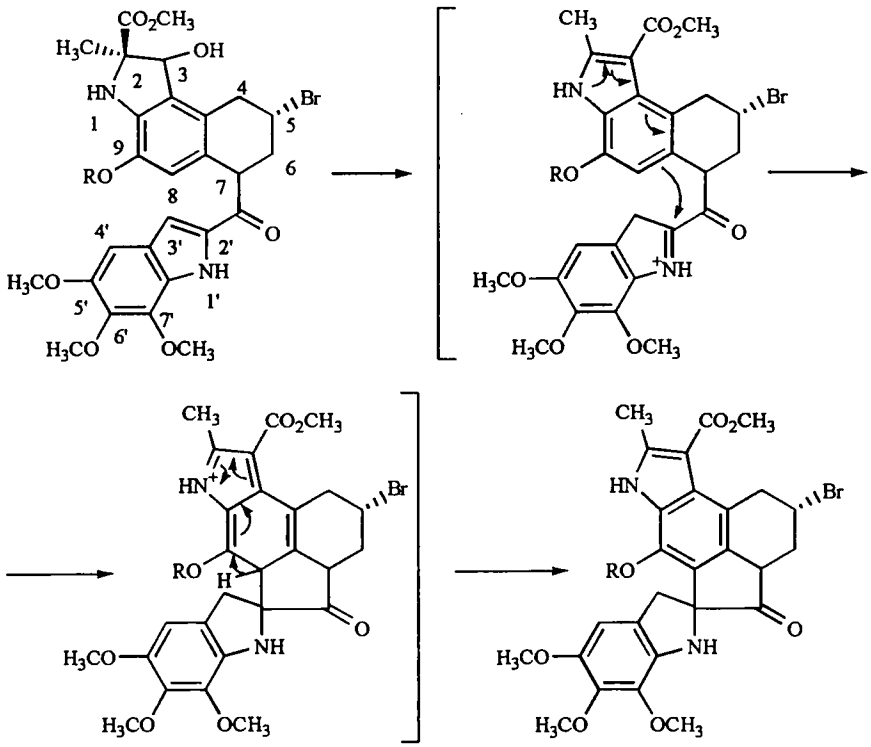


Chart 4. Proposed Mechanism for Production of Spiro Derivatives of Duocarmycin B1

The *in vitro* anticellular activity of the synthetic intermediate **10a** protected by a silyl group increased with increasing exposure time. The IC_{50} value of 72 h exposure was approximately 100-fold smaller than that of 1 h exposure, and was similar to that of **12a** (IC_{50} = 0.002 nM). Compound **10a** was readily converted to **12a** in aqueous buffer solution and in calf serum. Compounds **12a** and **12b**, which were considered as active forms of A-ring pyrrole analogs, exhibited exceptionally potent anticellular activity almost equal to that of **1**. The IC_{50} values of **12a** and **12b** at 72 h exposure were 0.0052 and 0.0004 nM, respectively. The *in vivo* antitumor activity of **12a** against sarcoma 180 was sufficient at MTD (maximum tolerated dose). But, the efficacy at a half dose of MTD was not revealed. Accordingly, its effective range of doses was very narrow. In addition, **12a** did not have enough aqueous solubility to further development.

The cyclized compound **17** showed decreased *in vitro* anticellular activity about 1×10^3 times inferior to **12a**. The IC_{50} value at 72 h exposure was 6.6 nM. This result suggests that the shape of compound **17** is not complementary enough to the DNA minor groove to permit and promote the association with DNA. Moreover, the methylation of the aromatic NH group seems to not contribute to an increase of the biological activity. Compounds **18** and **19** exhibited **10** and 40,000 times less potent *in vitro* anticellular activity than **12a**, respectively. They did not exhibit superior *in vivo* antitumor activity to **12a**. The dimethyl derivative (**19**) showed no *in vivo* activity. These results exhibit that the free NH moiety in the aromatic ring is essential for *in vitro* anticellular and *in vivo* antitumor activity (Table 3).

On the other hand, the 8-O-N,N-dialkylcarbamoyl derivatives **21a-e** showed decreased *in vitro* anticellular activity, about 1000 times inferior to that of **12a** (72 h exposure), but they exhibited promising *in vivo* antitumor activity against murine sarcoma 180 (*T/C* = 0.055-0.24). As the results of promising *in vivo* antitumor activity against murine sarcoma 180 and sufficient aqueous water solubility, the 8-O-N,N-dialkylcarbamoyl derivatives **21a** and **21e** were selected for further evaluation against several murine solid tumors (M5076 sarcoma, B-16 melanoma and Colon 26 adenocarcinoma) and human solid tumors (St-4, Co-3 and LC-6). As shown in Table 4, they showed statistically significant antitumor activity against murine solid tumors with *T/C* values less than 0.2, and possessed high activity against human solid tumors that were insensitive to most chemotherapeutic drugs (*T/C* = 0.04-0.27). Furthermore, tumor regression was also observed in mice bearing St-4 and LC-6 carcinomas [47], and they do not cause delayed irreversible toxicity, which is induced by CC-1065 (7) [48, 49]. Consequently, **21e** among these analogs was selected

TABLE 2. Results of Stability Tests, and Anticellular and Antitumor Activities

Compound	Stability $T_{1/2}$ (h)[a]	HeLa S_3 IC_{50} (nM)[b]		Sarcoma 180 (sc-iv)[c]	
		1 h	72 h	mg/kg	T/C[d]
10a	2	0.17	0.002	0.5	0.15
21a	19	55	7.3	1.0	0.055
21b	16	210	1.3	0.5	0.24
21c	26	22	2.6	1.0	0.098
21d	20	2.6	0.3	1.0	0.088
21e	16	53	1.6	0.5	0.14
12a	130	0.045	0.0052	0.25	0.21
12b	324	0.0088	0.0004	0.063	0.40
1	1	0.0055	0.0058	0.075	0.26
3	<1	0.033	0.028	0.25	0.24

[a] A Half-life in aqueous buffer solution (pH 7) containing 20% CH_3CN at 35 °C.
[b] Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%.
[c] Mice (five mice/group) were implanted subcutaneously (sc) with tumor cells, and the drug was dosed (mg/kg) intravenously (iv).

for clinical trial as KW-2189, based on its improved *in vivo* antitumor activity, suitable stability, and water solubility in excess of 10 mg/ml [50-55].

The stability of the A-ring pyrrole analogs was measured in aqueous buffer solution (pH 7) containing CH_3CN. As shown in Tables 2 and 3, compound **12a** and **12b**, having the electrophilic cyclopropane ring, were very much more stable than **1**. It is speculated that this unusual stability is a consequence of overlap of the π-system in the A-ring part with the cyclohexadienone π-system. Moreover, the N-methyl compounds demonstrated significantly increased stability greater than the N-H compounds in aqueous buffer solution (**18** and **19** vs. **12a**).

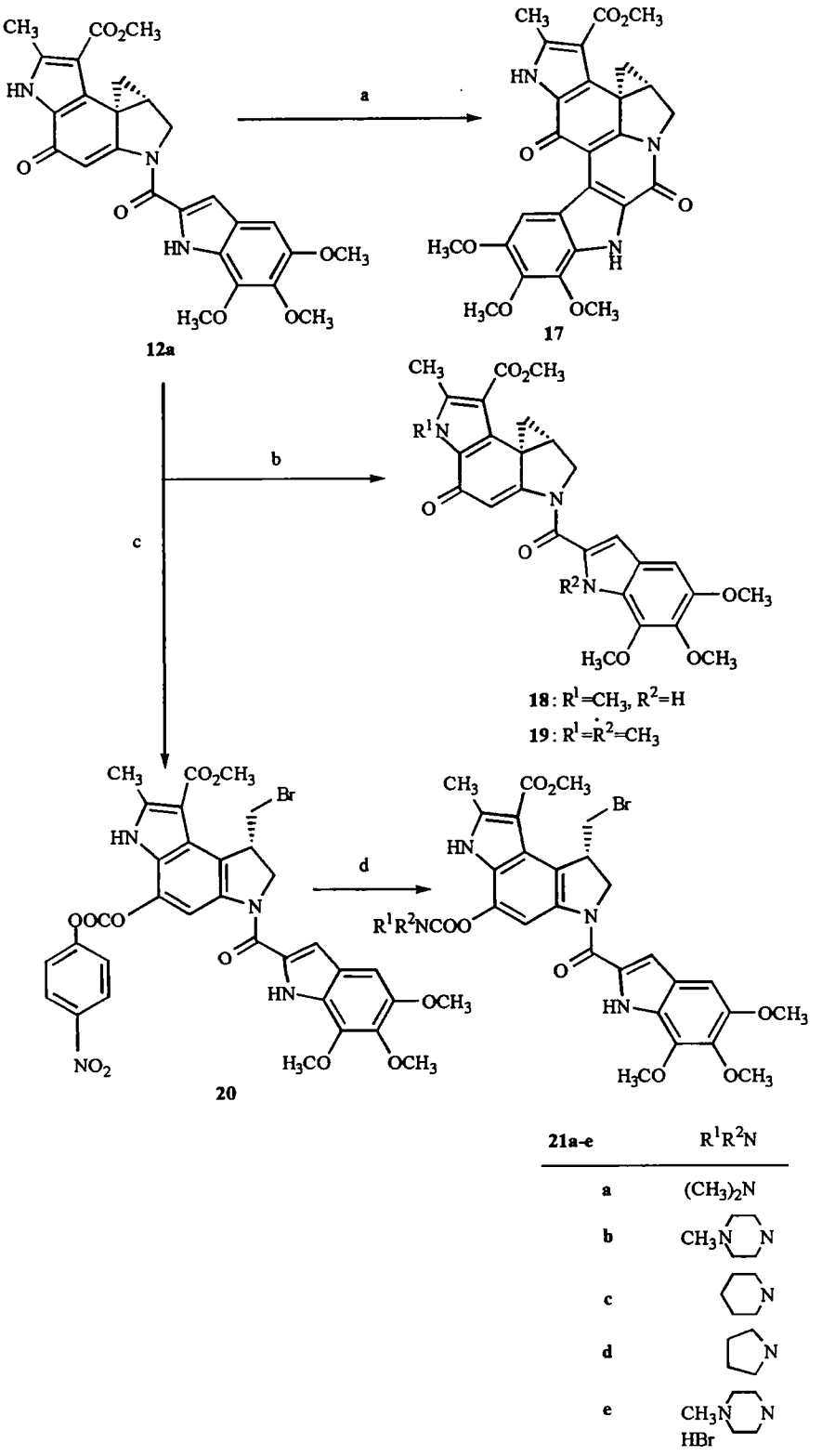
The structure-activity relationship in the related compounds of **12a** are studied by many groups. Among them, the fundamental correlation between solvolytic chemical stability and *in vitro* biological potency of the agents had already been reported; in the examination of the simple derivatives of tetrahydrocyclopropa[1,2-*c*]benz[1,2-*e*]indole (CBI), the solvolytically more stable derivatives of CBI proved to be the most potent in assay systems *in vitro* (Chart 5) [56-61]. In our test of the stability (pH 7), however, a correlation between solvolytic chemical stability and biological potency among **12a**, **17**, **18**, and **19** was not observed. These findings may prove useful in creating the next generation of duocarmycin derivatives, especially in the modification of the trimethoxy indole part [62-65].

TABLE 3. Results of Stability Tests, and Anticellular and Antitumor Activities

Compound	Stability $T_{1/2}$ (h)[a]	HeLa S_3 IC_{50} (nM)[b]		Sarcoma 180 (sc-iv)[c]	
		1 h	72 h	mg/kg	T/C[d]
17	880	36	6.6	N. T.	
18	990	1.4	0.069	1.0	0.22
19	580	890	230	8.0	0.96
12a	340	0.045	0.0052	0.25	0.21
6	>1000	0.0045	0.0069	0.10	0.21

[a] A Half-life in aqueous buffer solution (pH 7) containing 50% CH_3CN at 35 °C.
[b] Drug concentration required to inhibit the growth of HeLa S_3 cells by 50%.
[c] Mice (five mice/group) were implanted subcutaneously (sc) with tumor cells, and the drug was dosed (mg/kg) intravenously (iv).
[d] T and C are the values of mean tumor volume of treated and control mice, respectively.

Scheme 3



21a-e	R^1R^2N
a	$(CH_3)_2N$
b	
c	
d	
e	HBr

(a) *hν*, $CHCl_3$; (b) CH_3I (1 eq. or 3 eq.), K_2CO_3, DMF; (c) HBr, CH_3CN, then *p*-nitrophenyl chloroformate, Et_3N, CH_2Cl_2; (d) R^1R^2NH, then HBr, $CH_3CO—CH_3OH$

TABLE 4. Antitumor Activity of **21a** and **21e** Against Murine Tumors and Human Xenografted Carcinomas

	T/C			
	21a	**21e**	**3**	**Cisplatin**
	Dose (mg/kg)			
	0.64	0.63	0.25	11.0
M5076 sarcoma	0.02[a]	0.03	0.23	0.05
B16 melanoma	0.10[a]	0.07	0.36	0.03
Colon 26 adenocarcinoma	0.08[b]	0.19	0.54	0.22
St-4 (stomach)	0.15 (1)[c]	0.12	0.53	0.62
Co-3 (colon)	0.25	0.27	N. T.	0.50
LC-6 (lung)	0.024	0.04	0.70	0.20

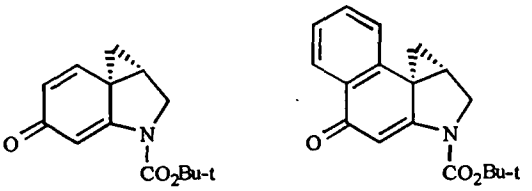
[a] The dose was 0.41 mg/kg.
[b] The dose was 1.0 mg/kg.
[c] Motality (5 mice in a group).

EXPERIMENTAL

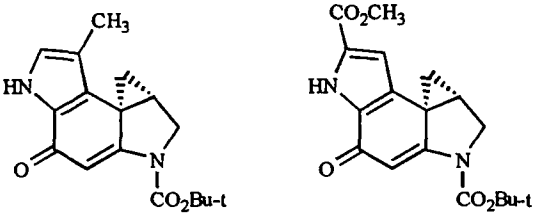
All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a JASCO IR-810; ^{1}H NMR spectra were measured on a JEOL FX-100 (100 MHz), a JEOL JNM-GX270 (270 MHz), a Varian EM-390 (390 MHz), or a Bruker AM-400 (400 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Elemental analyses were performed with a Perkin-Elmer 2400 C, H, N analyzer. Mass spectra were measured with a Hitachi B-80 and a Shimadzu QP-1000 instruments. CD spectra were measured on a JASCO 500 spectrometer. For column chromatography, silica gel (SiO_2, Wako C-200) was used. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck). All solvents were purified and dried prior to use according to standard procedures. Organic solvent extracts were dried over anhydrous sodium sulfate.

8-O-*tert*-Butyldimethylsilylduocarmycin B2 (8). *tert*-Butyldimethylchlorosilane (50 mg, 0.33 mmol) was added to a solution of **3** (123 mg, 0.21 mmol) and imidazole (43 mg, 0.63 mmol) in DMF (3 ml), and the mixture was stirred at room temperature for 4.5 h. Then 2 N HCl was added to the reaction mixture and the mixture was extracted with EtOAc twice. The combined extracts were washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to column chromatography (*n*-hexane—EtOAc, 3:1) to give 140 mg (95%) of **8** as a light-tan powder. mp 120-130°C dec.; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.35 (3 H, s); 0.36 (3 H, s); 1.06 (9 H, s); 1.69 (3 H, s); 3.57 (1 H, dd, J = 10.3, 9.1 Hz); 3.78 (3 H, s); 3.91 (3 H, s); 3.94 (3 H, s); 4.06 (1 H, dd, J = 10.3, 3.0 Hz); 4.06 (3 H, s); 4.17 (1 H, m); 4.54 (1 H, dd, J = 10.6, 4.4 Hz); 4.62 (1 H, dd, J = 10.6, 9.1 Hz); 5.04 (1 H, br s); 6.87 (1 H, s); 6.95 (1 H, d, J = 2.2 Hz); 7.91 (1 H, s); 9.38 (1 H, br s). IR (KBr) 1745, 1700, 1618, 1497, 1293, 837 cm^{-1}. SI-MS *m/z* 704 702 $(M + H)^+$, 470, 468, 234. Found: C 54.82; H 5.93; N 5.75%. $C_{32}H_{40}BrN_3O_8Si$. Calculated: C 54.70; H 5.74; N 5.98%.

8-O-*tert*-Butyldimethylsilyl-3a-hydroxyduocarmycin B2 (9a). $NaBH_4$ (25 mg, 0.66 mmol) was added to a solution of **8** (155 mg, 0.22 mmol) in allyl alcohol (7 ml), and the mixture was stirred at 0°C for 2.5 h. Then, 2 N HCl was added, and the resulting mixture was extracted with $CHCl_3$. The combined extracts were washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to column chromatography (*n*-hexane—EtOAc, 1 : 1) to give 115 mg (74%) of **9a** as a white powder, mp 124-125°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.30 (3 H, s); 0.32 (3 H, s); 1.04 (9 H, s); 1.60 (3 H, s); 2.09 (1 H, br s); 3.49 (1 H, dd, J = 10.3, 9.8 Hz); 3.72 (3 H, s); 3.91 (3 H, s); 3.92 (1 H, m); 3.93 (3 H, s); 4.05 (3 H, s); 4.07 (1 H, dd, J = 10.3, 3.2 Hz); 4.50 (1 H, dd, J = 10.6, 3.9 Hz); 4.57 (1 H, dd, J = 10.6, 8.9 Hz); 5.31 (1 H, br. s); 6.86 (1 H, s); 6.91 (1 H, d, J = 2.2 Hz); 7.91 (1 H, s); 9.43 (1 H, br. s). IR (KBr) 3406, 1734, 1621, 1485, 1111, 838 cm^{-1}. SI-MS *m/z* 706 704 $(M + H)^+$, 234. Found: C 54.47; H 6.19; N 5.71%. $C_{32}H_{42}BrN_3O_8Si$. Calculated: C 54.54; H 6.01; N 5.96%.



$T_{1/2}$(pH3)	0.01 h	2.1 h
IC_{50}(L1210)	18000 nM	2000 nM



$T_{1/2}$(pH3)	37 h	177 h
IC_{50}(L1210)	330 nM	6 nM

R	CONHMe	CO_2Me	COEt	SO_2Et
$T_{1/2}$(pH3)	36 h	57 h	96 h	383 h
IC_{50}(L1210)	200 nM	140 nM	110 nM	24 nM

Chart 5. CBI(1,2,9,9a-tetrahydrocyclopropa[1,2-c]benz[1,2-e]indole-4-one) derivatives

Reduction of **8** by $NaBH_4$ in MeOH afforded **9a** (40%), **9b** (22%), **9c** (7%), **9d** (6%), and **9e** (7%). The whole was chromatographed on silica gel with *n*-hexane—EtOAc (1:1).

8-O-*tert*-Butyldimethylsilyl-3b-hydroxyduocarmycin B2 (9b). Yield: 22% (a white powder). mp 129-130°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.29 (3 H, s); 0.30 (3 H, s); 1.02 (9 H, s); 1.61 (3 H, s); 3.24 (1 H, br. s); 3.54 (1 H, dd, *J* = 10.1, 9.4 Hz); 3.79 (3 H, s); 3.82 (1 H, dd, *J* = 10.1, 3.4 Hz); 3.91 (3 H, s); 3.93 (3 H, s); 4.06 (3 H, s); 4.08 (1 H, m); 4.44 (1 H, dd, *J* = 10.6, 4.9 Hz); 4.61 (1 H, dd, *J* = 10.6, 9.4 Hz); 5.08 (1 H, br. s); 6.86 (1 H, s); 6.91 (1 H, d, *J* = 2.2 Hz); 7.88 (1 H, s); 9.40 (1 H, br s). IR (KBr) 1732, 1600, 1485, 1111 cm^{-1}. EI-MS *m/z* 705 703 $(M)^+$. Found: C 54.57; H 6.20; N 5.81%. $C_{32}H_{42}BrN_3O_8Si$. Calculated: C 54.54; H 6.01; N 5.96%.

8-O-*tert*-Butyldimethylsilyl-2-decarbomethoxy-3-hydroxyduocarmycin B2 (9c). Yield: 7% (a white solid), mp 140-142°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.29 (3 H, s); 0.30 (3 H, s); 1.01 (9 H, s); 1.35 (3 H, s); 1.37 (1 H, d, *J* = 6.4 Hz); 3.49 (1 H, dd, *J* = 10.1, 9.6 Hz); 3.71 (1 H, m); 3.81 (1 H, dd, *J* = 10.1, 3.4 Hz); 3.89 (3 H, s); 3.93 (3 H, s); 4.05 (3 H, s); 4.05 (1 H, m); 4.40 (1 H, dd, *J* = 10.8, 5.2 Hz); 4.55 (1 H, dd, *J* = 10.8, 9.3 Hz); 4.90 (1 H, d, *J* = 6.4 Hz); 5.11 (1 H, br. s); 6.85 (1 H, s); 6.88 (1 H, d, *J* = 2.2 Hz); 7.87 (1 H, br. s); 9.43 (1 H, br. s). IR (KBr) 3450, 2934, 1618, 1486, 1309, 840 cm^{-1}. EI-MS *m/z* 629 627 $(M - H_2O)^+$. Found: C 55.92; H 6.63; N 6.82%. $C_{30}H_{40}BrN_3O_6Si$. Calculated: C 55.72; H 6.23; N 6.50%.

8-O-*tert*-Butyldimethylsilyl-3α-hydroxy-2-hydroxymethylduocarmycin B2 (9d). Yield: 6% (a white solid), mp 135-138°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.28 (3 H, s); 0.30 (3 H, s); 1.01 (9 H, s); 1.34 (3 H, s); 2.01 (1 H, br. s); 2.08 (1 H, d, *J* = 8.9 Hz), 3.47 (1 H, dd, *J* = 10.1, 10.1 Hz); 3.48 (2 H, br. s); 3.62 (1 H, br. s), 3.86 (1 H, m);

3.91 (3 H, s); 3.94 (3 H, s); 4.06 (3 H, s); 4.14 (1 H, dd, J = 10.1, 3.0 Hz); 4.53 (1 H, dd, J = 10.8, 8.4 Hz), 4.58 (1 H, dd, J = 10.8, 3.9 Hz); 5.04 (1 H, d, J = 8.9 Hz); 6.87 (1 H, s); 6.92 (1 H, d, J = 2.2 Hz); 7.87 (1 H, br. s); 9.41 (1 H, br. s). IR (KBr) 3400, 2934, 1619, 1473, 1313, 839 cm^{-1}. SI-MS *m/z* 678 676 $(M + H)^+$, 234. Found: C 55.13; H 6.16; N 6.01%. $C_{31}H_{42}BrN_3O_7Si$. Calculated: C 55.02; H 6.26; N 6.21%.

8-O-*tert*-Butyldimethylsilyl-3β-hydroxy-2-hydroxymethylduocarmycin B2 (9e). Yield: 7% (a white solid), mp 135-138°C; ^1H NMR (270 MHz, $CDCl_3$) δ: 0.30 (6 H, s); 0.95 (9 H, s); 1.30 (3 H, s); 3.20-3.80 (3 H, m); 3.85 (3 H, s); 3.90 (3 H, s); 4.05 (3 H, s); 4.00-5.10 (3 H, m); 6.84 (1 H, s); 6.90 (1 H, d, J = 2.0 Hz); 7.94 (1 H, s); 9.40 (1 H, br. s). SI-MS *m/z* 678 676 $(M + H)^+$, 234. Found: C 55.33; H 6.19; N 5.70%. $C_{31}H_{42}BrN_3O_7Si$. Calculated: C 55.02; H 6.26; N 6.21%.

8-O-*tert*-Butyldimethylsilyl-3-methoxycarbonyl-2-methylduocarmycin B2 (10a). Camphorsulfonic acid (1.6 g, 6.81 mmol) was added to a solution of **9a** (1.6 g, 2.27 mmol) in dry toluene (30 ml), and the reaction mixture was stirred for 1 h at 50°C. Then the mixture was poured into aqueous $NaHCO_3$ and the whole was extracted with EtOAc. The extract was washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with *n*-hexane—EtOAc (4:1) to give 0.95 g (61%) of 10a as a white powder, mp 140-142°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.37 (3 H, s); 0.39 (3 H, s); 1.07 (9 H, s); 2.76 (3 H, s); 3.21 (1 H, dd, J = 9.9, 9.9 Hz); 3.80 (1 H, dd, J = 9.9, 2.1 Hz); 3.92 (3 H, s); 3.95 (3 H, s); 3.98 (3 H, s); 4.07 (3 H, s); 4.52 (1 H, m); 4.54 (1 H, br. d, J = 8.5 Hz); 4.73 (1 H, br. d, J = 8.9 Hz); 6.89 (1 H, s); 6.99 (1 H, d, J = 2.3 Hz); 7.98 (1 H, s); 8.30 (1 H, br. s); 9.40 (1 H, br. s). IR (KBr) 2934, 1696, 1628, 1493, 1412, 1305, 1213, 1112, 837 cm^{-1}. SI-MS *m/z* 688 686 $(M + H)^+$, 454 452, 359, 234. Found: C 55.86; H 6.03; N 5.91%. $C_{32}H_{40}BrN_3O_7Si$. Calculated: C 55.97; H 5.87; N 6.12%.

8-O-*tert*-Butyldimethylsilyl-2-methylduocarmycin B2 (10b). Camphorsulfonic acid (32.7 mg, 0.141 mmol) was added to a solution of **9c** (30.5 mg, 0.047 mmol) in dry toluene (5 ml), and the reaction mixture was treated as in the case of compound **10a** to give 22.9 mg (77%) of **10b** as a white powder, mp 110-115°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.36 (6 H, s); 1.07 (9 H, s); 2.48 (3 H, s); 3.38 (1 H, dd, J = 10.3, 10.3 Hz); 3.90 (1 H, m); 3.91 (3 H, s); 3.94 (3 H, s); 4.06 (3 H, s); 4.50 (2 H, m); 4.85 (1 H, m); 6.14 (1 H, q, J = 1.1 Hz); 6.88 (1 H, s); 6.95 (1 H, d, J = 2.4 Hz); 7.83 (1 H, s); 7.90 (1 H, br. s); 9.44 (1 H, br. s). IR (KBr) 2934, 1631, 1609, 1493, 1413, 1307, 839 cm^{-1}. EI-MS *m/z* 629 627 $(M)^+$, 396 394, 234. Found: C 56.65; H 6.27; N 6.56%. $C_{30}H_{38}BrN_3O_5Si \cdot 0.5H_2O$. Calculated: C 56.51; H 6.16; N 6.59%.

8-O-*tert*-Butyldimethylsilyl-3-methoxyduocarmycin B2 (11). Boron trifluoride diethyl etherate (26 ml, 0.21 mmol) was added to a solution of **9a** (50 mg, 0.07 mmol) in MeOH (5 ml), and the reaction mixture was stirred for 1 h at room temperature. Then, the mixture was poured into aqueous $NaHCO_3$ and the whole was extracted with EtOAc. The extract was washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$—MeOH (30:1) to give 30.2 mg (59%) of **11** as a white powder, mp 122-125°C; ^1H NMR (400 MHz, DMSO-d_6) δ: 1.41 and 1.42 (3 H, s); 2.24 and 2.25 (3 H, s); 2.49-2.52 (4 H, br. s); 3.17 and 3.18 (3 H, s); 3.45-3.47 (2 H, br. s); 3.60-3.63 (2 H, br. s); 3.64 (3 H, s); 3.65 (1 H, m); 3.79 and 3.80 (3 H, s); 3.81 and 3.83 (3 H, s); 3.95 and 3.97 (3 H, s); 4.12-4.32 (3 H, m); 5.24-5.30 (2 H, m); 5.82 (1 H, br. s); 6.94 and 6.96 (1 H, d, J = 2.2 Hz); 6.96 and 6.98 (1 H, s); 7.73 and 7.75 (1 H, s); 11.28 (1 H, br s). IR (KBr) 1700, 1623, 1438, 1321, 1237, 1151 cm^{-1}. SI-MS *m/z* 732 730 $(M + H)^+$, 234. Found: C 53.46; H 5.88; N 9.00%. $C_{33}H_{40}BrN_5O_9 \cdot 1.0CH_3OH$. Calculated: C 53.55; H 5.81; N 9.18%.

3-Methoxycarbonyl-2-methylduocarmycin A (12a). A solution of **10a** (500 mg, 0.73 mmol) in dry THF (50 ml) was stirred at room temperature. A 1.0 M solution in THF of tetrabutylammonium fluoride (1.1 ml, 1.1 mmol) was added, and the mixture was stirred for 1 h. Then, 0.01 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$—MeOH (50:1) to give 290 mg (81%) of **12a** as a white powder, mp 185-188°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.37 (1 H, br. d, J = 5.4 Hz); 2.38 (1 H, dd, J = 7.5, 3.3 Hz); 2.63 (3 H, s); 3.67 (1 H, m); 3.82 (3 H, s); 3.90 (3 H, s); 3.94 (3 H, s); 4.08 (3 H, s); 4.45 (2 H, m); 6.81 (1 H, s); 6.95 (1 H, d, J = 2.3 Hz); 7.12 (1 H, s); 9.40 (1 H, br. s); 11.58 (1 H, br. s). IR (KBr) 2934, 1700, 1637, 1525, 1487, 1459, 1385, 1295, 1264, 1106 cm^{-1}. SI-MS *m/z* 492 $(M + H)^+$, 234. Found: C 61.01; H 5.20; N 7.93%. $C_{26}H_{25}N_3O_7 \cdot 1.0H_2O$. Calculated: C 61.29; H 5.34; N 8.25%.

2-Methylduocarmycin A (12b). The procedure was the same as that of **12a** except for the use of **10b** (20 mg, 0.032 mmol). The crude product was purified by silica gel chromatography to afford 12.4 mg (90%) of **12b** as a white powder, mp 176-182°C dec.; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.53 (1 H, t, J= 4.6 Hz); 1.70 (1 H, m); 2.36 (3 H, s); 2.69 (1 H, m); 3.89 (3 H, s); 3.93 (3 H, s); 4.07 (3 H, s); 4.36 (1 H, dd, J = 10.4, 10.4 Hz); 4.40 (1 H, dd,

J = 10.4, 4.5 Hz); 5.67 (1 H, m); 6.78 (1 H, s); 6.92 (1 H, s); 6.94 (1 H, d, J = 2.3 Hz); 9.31 (1 H, br. s); 9.99 (1 H, br. s). IR (KBr) 3455, 3495, 2938, 1636, 1477, 1388, 1305, 1265 cm^{-1}. SI-MS m/z 434 $(M + H)^+$, 234. Found: C 63.97; H 5.69; N 9.29%. $C_{24}H_{23}N_3O_5 \cdot 1.0H_2O$. Calculated: C 63.85; H 5.58; N 9.31%.

9-O-*tert*-Butyldimethylsilylduocarmycin B1 (13a). *tert*-Butyldimethylchlorosilane (90 mg, 0.56 mmol) was added to a solution of **2** (100 mg, 0.17 mmol) and imidazole (41 mg, 0.56 mmol) in DMF (5 ml), and the mixture was stirred at 0°C for 3 h. Then, 1 N HCl was added to the reaction mixture, and the mixture was extracted with EtOAc twice. The combined extracts were washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to column chromatography (*n*-hexane—EtOAc, 3:1) to give 122 mg (99%) of **13a** as a light-tan powder, mp 119-113°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.15 (6 H, s); 0.91 (9 H, s); 1.69 (3 H, s); 3.65 (1 H, dd, J = 19.3, 5.2 Hz); 3.78 (3 H, s); 3.83 (1 H, dd, J = 19.3, 6.2 Hz); 3.85 (3 H, s); 3.92 (3 H, s); 4.06 (3 H, s); 4.20 (1 H, br. d, J = 13.3 Hz); 4.46 (1 H, dd, J = 13.3, 6.2 Hz); 4.57 (1 H, m); 5.02 (1 H, s); 6.52 (1 H, d, J = 2.2 Hz); 6.72 (1 H, s); 6.90 (1 H, s); 9.03 (1 H, s). IR (KBr) 2936, 2860, 1747, 1701, 1612, 1508, 1394, 1301, 1254, 1109, 828 cm^{-1}. SI-MS m/z 704 702 (M + H)+, 470 468, 234. Found: C 52.00; H 6.12; N 5.55%. $C_{32}H_{40}BrN_3O_8Si \cdot 2.0H_2O$. Calculated: C 52.03; H 6.00; N 5.69%.

9-O-Dimethylcarbamoylduocarmycin B1 (13b). *p*-Nitrophenyl chloroformate (51 mg, 0.255 mmol) and triethylamine (0.029 ml, 0.255 mmol) were added to a solution of **2** (50 mg, 0.085 mmol) in dry CH_2Cl_2 (5 ml) under cooling at 0°C. The mixture was stirred at the same temperature for 1 h. Then dimethylamine solution (40 wt. %, 0.09 ml, 0.85 mmol) was added, and stirring was continued at 0°C for 2 h. The mixture was diluted with $CHCl_3$ and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried and concentrated under reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$—MeOH (50:1) to give 55 mg (98%) of **13b** as a light-tan powder, mp 145-150°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.68 (3 H, s); 3.00 (3 H, s); 3.07 (3 H, s); 3.71 (1 H, dd, J = 19.4, 5.3 Hz); 3.79 (3 H, s); 3.88 (1 H, dd, J = 19.4, 6.0 Hz); 3.88 (3 H, s); 3.93 (3H, s); 4.08 (3 H, s); 4.32 (1 H, dd, J = 12.8, 2.6 Hz); 4.48 (1 H, dd, J = 12.8, 6.4 Hz); 4.55 (1 H, m); 5.46 (1 H, s); 6.60 (1 H, d, J = 2.3 Hz); 6.78 (1 H, s); 7.46 (1 H, s); 9.09 (1 H, br. s). IR (KBr) 3332, 2938, 1715, 1623, 1506, 1388, 1312, 1245, 1161 cm^{-1}. SI-MS m/z 661 659 $(M + H)^+$, 427 425, 234. Found: C 50.65; H 4.66; N 7.77%. $C_{29}H_{31}BrN_4O_9 \cdot 1.5H_2O$. Calculated: C 50.74; H 4.99; N 8.16%.

9-O-(4-Methyl-1-piperazinylcarbonyl)duocarmycin B1 (13c). *p*-Nitrophenyl chloroformate (103 mg, 0.51 mmol) and triethylamine (0.071 ml, 0.51 mmol) were added to a stirred solution of **2** (100 mg, 0.17 mmol) in dry CH_2Cl_2 (4 ml) at 0°C. Then, the resulting mixture was stirred at the same temperature for 0.5 h. 4-Methylpiperazine (0.066 ml, 0.60 mmol) was added to the solution, and the mixture was stirred at 0°C for 1 h. The resulting reaction was quenched by the addition of 0.01 M phosphate buffer (pH 7), and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was chromatographed on silica gel with $CHCl_3$—MeOH (50:1) to give 96 mg (79%) of **13c** as a pale yellow powder, mp 180-183°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.68 (3 H, s); 2.43 (3 H, s); 2.58 (4 H, br. s); 3.60 (2 H, br. s); 3.80 (2 H, br. s); 3.71 (1 H, dd, J= 19.8, 5.3 Hz); 3.79 (3 H, s); 3.86 (1 H, dd, J = 19.8, 5.9 Hz); 3.88 (3 H, s); 3.93 (3 H, s); 4.08 (3 H, s); 4.32 (1 H, dd, J = 12.4, 1.6 Hz); 4.49 (1 H, dd, J = 12.4, 6.5 Hz); 4.54 (1 H, m); 5.44 (1 H, s); 6.61 (1 H, d, J = 2.3 Hz); 6.78 (1 H, s); 7.47 (1 H, s); 9.09 (1 H, br. s). IR (KBr) 1715, 1623, 1506, 1388, 1312, 1245, 1161 cm^{-1}. SI-MS m/z 716 714 $(M + H)^+$, 234. Found: C 52.22; H 5.07; N 9.37%. $C_{32}H_{36}BrN_5O_9 \cdot 1.0H_2O$. Calculated: C 52.47; H 5.23; N 9.56%.

9-O-*tert*-Butyldimethylsilyl-3a-hydroxyduocarmycin B1 (14a). The procedure was the same as that for the preparation of **9a** except **13a** (347 mg, 0.49 mmol) was used as starting compound. The crude product was purified by silica gel chromatography to afford 73 mg (21%) of **14a** as a white powder, mp 109-114°C; ^1H NMR (400 MHz, $CDCl_3$) δ: -0.51 (3 H, s); -0.23 (3 H, s); 0.89 (9 H, s); 1.61 (3 H, s); 1.74 (1 H, br. s); 3.42 (1 H, br. d, J = 3.4 Hz); 3.44 (1 H, br. d, J = 2.9 Hz); 3.76 (3 H, s); 3.84 (3 H, s); 3.91 (3 H, s); 4.05 (3 H, s); 4.16 (1 H, dd, J = 13.9, 6.7 Hz); 4.52 (3 H, m); 5.34 (1 H, br. s); 6.35 (1 H, d, J = 2.1 Hz); 6.63 (1 H, s); 6.67 (1 H, s); 9.00 (1 H, br. s). IR (KBr) 2934, 2858, 1734, 1616, 1495, 1389, 1255, 1108, 1047, 838 cm^{-1}. SI-MS m/z 706 704 $(M + H)^+$, 472 470, 234. Found: C 54.85; H 6.35; N 5.52%. $C_{32}H_{42}BrN_3O_8Si$. Calculated: C 54.54; H 6.01; N 5.96%.

9-O-Dimethylcarbamoyl-3a-hydroxyduocarmycin B1 (14b). The procedure was the same as that of **9a** except **13b** (100 mg, 0.151 mmol) was used instead. The crude product was purified by silica gel chromatography to afford 30.0 mg (30%) of **14b** as a white powder, mp 130-35°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.61 (3 H, s); 2.02 (1 H, br. s); 2.96 (3 H, s); 3.02 (3 H, s); 3.49 (2 H, br. d, J = 6.4 Hz); 3.78 (3 H, s); 3.88 (3 H, s); 3.96 (3 H, s); 4.06 (3 H, s); 4.18 (1 H, dd, J = 13.4, 7.2 Hz); 4.50 (1 H, m); 4.55 (1 H, dd, J = 13.4, 4.1 Hz); 4.82 (1 H, m); 5.40 (1 H, br. s); 6.45 (1 H, d, J = 2.2 Hz); 6.75 (1 H, s); 7.09 (1 H, s); 9.08 (1 H, br. s). IR (KBr) 2940, 1714, 1621, 1492,

1385, 1309, 1245, 1218, 1169, 1110, 1044 cm^{-1}. SI-MS *m/z* 663 661 $(M + H)^+$, 429 427, 234. Found: C 52.00; H 5.11; N 8.22%. $C_{29}H_{33}BrN_4O_9 \cdot 0.5H_2O$. Calculated: C 51.95; H 5.11; N 8.36%.

3α-Hydroxy-9-O-(4-methyl-1-piperazinylcarbonyl)duocarmycin B1 (14c). The procedure was the same as that of **9a** except for the use of **13c** (20 mg, 0.028 mmol) as starting compound. The crude product was purified by silica gel chromatography to afford 14.1 mg (70%) of **14c** as a white powder, mp 192-194°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.61 (3 H, s); 2.31 (3 H, s); 2.41 (4 H, br. s); 3.48 (2 H, m); 3.54 (2 H, br. s); 3.61 (2 H, br. s); 3.78 (3 H, s); 3.87 (3 H, s); 3.92 (3 H, s); 4.06 (3 H, s); 4.20 (1 H, dd, J = 12.2, 6.6 Hz); 4.54 (2 H, m); 4.81 (1 H, m); 5.38 (1 H, s); 6.47 (1 H, d, J = 2.0 Hz); 6.75 (1 H, s); 7.11 (1 H, s); 9.13 (1H, br. s). IR (KBr) 1716, 1497, 1255, 1238, 1221, 1153, 1049 cm^{-1}. SI-MS *m/z* 718 716 $(M + H)^+$, 234. Found: C 52.90; H 5.55; N 9.66%. $C_{32}H_{38}BrN_5O_9 \cdot 0.5H_2O$. Calculated: C 52.97; H 5.42; N 9.65%.

9-O-*tert*-Butyldimethylsilyl-3-methoxycarbonyl-2-methylduocarmycin B1 (15a). The procedure was the same as that of **10a** except **14a** (73 mg, 0.10 mmol) was used instead. The crude product was purified by silica gel chromatography to afford 54 mg (79%) of **15a** as a white powder, mp 114-118°C; ^1H NMR: see Table 3. IR (KBr) 3468, 3306, 2936, 2860, 1703, 1615, 1586, 1528, 1496, 1443, 1311, 1256, 1214, 1124, 1088, 997 cm^{-1}. SI-MS *m/z* 688, 686 $(M + H)^+$, 454, 452, 234. Found: C 55.96; H 5.88; N 6.12%. $C_{32}H_{40}BrN_3O_7Si$. Calculated: C 55.97; H 5.87; N 6.12%.

Methyl (8*S*)-8-Bromo-4-(dimethylaminocarbonyloxy)-2-methyl-5,6,6a,7,8,9-hexahydro-3H-pyrrolo[3,2-*f*]-6-a-azaacenaphthylene-6-one-5-spiro-2′-(5′,6′,7′-trimethoxy)indoline-1-carboxylate (16b). Camforsulfonic acid (14 mg, 0.06 mmol) was added to a solution of **14b** (20 mg, 0.03 mmol) in dry toluene (2 ml), and the reaction mixture was stirred for 2 h at 50°C. Then the mixture was poured into aqueous $NaHCO_3$ and the whole was extracted with $CHCl_3$. The extract was washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$—MeOH (80:1) to give 14 mg (72%) of **16b** as a white powder, mp 172-177°C dec.; ^1H NMR: see Table 3. IR (KBr) 3238, 2938, 1699, 1646, 1540, 1443, 1348, 1242, 1166, 1121, 1090 cm^{-1}. SI-MS *m/z* 645 643 $(M + H)^+$. Found: C 53.01; H 5.17; N 8.17%. $C_{29}H_{31}BrN_4O_8 \cdot 1.0H_2O$. Calculated: C 52.66; H 5.03; N 8.47%.

9-O-(4-Methyl-1-piperazinylcarbonyl)-2-methyl-3-methoxycarbonylduocarmycin B1 (15c) and **Methyl (8*S*)-8-Bromo-2-methyl-4-[(4-methylpiperazinyl)carbonyloxy]-5,6,6a,7,8,9-hexahydro-3*H*-pyrrolo[3,2-*f*]-6a-azaacenaphthylene-6-one-5-spiro-2′-(5′,6′,7-trimethoxy)-indoline-1-carboxylate (16c).** To a solution of **14c** (68 mg, 0.094 mmol) in dry toluene (6 ml) CSA (65 mg, 0.282 mmol) was added, and the reaction mixture was stirred for 2 h at 50°C. Then the mixture was poured into aqueous $NaHCO_3$, and the whole was extracted with $CHCl_3$. The extract was washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$—MeOH (30:1) to give 52 mg (79%) of **15c** as a white powder and 7 mg (11%) of **16c** as a white powder. For **15c** mp 192-194°C; ^1H NMR: see Table 3. IR (KBr) 1699, 1653, 1505, 1456, 1439, 1314, 1210, 1125, 1091 cm^{-1}. SI-MS *m/z* 700 698 $(M + H)^+$, 234. Found: C 54.50; H 5.33; N 9.66%. $C_{32}H_{36}BrN_5O_8 \cdot 0.5H_2O$. Calculated: C 54.32; H 5.27; N 9.90%.

For **16c** mp 198-203°C dec.; ^1H NMR: see Table 3. IR (KBr) 1700, 1647, 1457, 1237, 1122, 1092 cm^{-1}. EI-MS *m/z* 699 697 $(M)^+$. Found: C 52.47; H 5.38; N 9.03%. $C_{32}H_{36}BrN_5O_8 \cdot 2.0H_2O$. Calculated: C 52.32; H 5.49; N 9.53%.

2-Methyl-3-methoxycarbonylsegment-B Cyclized Duocarmycin A (17). A solution of **12a** (20 mg, 0.041 mmol) in $CHCl_3$ (10 ml) was stirred at room temperature for 10 days. The reaction mixture was concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$—MeOH (90:1) as an eluent to afford 9 mg (45%) of **17** as a pale yellow powder, mp 135-140°C; ^1H NMR (400 MHz, DMSO-d_6) δ: 12.67 (1 H, br s); 12.12 (1 H, s); 8.85 (1 H, s); 4.49 (1 H, dd, J = 12.8, 12.8 Hz); 4.34 (1 H, dd, J = 12.8, 5.7 Hz); 3.92 (3 H, s); 3.91 (3 H, s); 3.86 (3 H, s); 3.80 (1 H, m); 3.77 (3 H, s); 2.53 (3 H, s); 2.19 (1 H, dd, J = 7.7, 3.4 Hz); 1.43 (1 H, dd, J = 4.5, 3.4 Hz).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.3, 164.1, 154.3, 153.9, 147.8, 142.0, 141.4, 138.6, 129.5, 129.5, 129.1, 126.7, 121.4, 117.9, 108.4, 106.8, 103.5, 61.2, 60.8, 52.1, 55.7, 50.7, 32.6, 26.4, 25.2, 13.7. SI-MS *m/z* 489 $(M)^+$. Found: C 63.99; H 4.55; N 8.71%. $C_{26}H_{23}N_3O_7$. Calculated: C 63.80; H 4.74; N 8.58%.

3-Methoxycarbonyl-1,2-dimethylduocarmycin A (18). Iodomethane (19 ml, 0.31 mmol) and potassium carbonate (35 mg, 0.25 mmol) were added to a solution of **12a** (105 mg, 0.21 mmol) in anhydrous DMF (2 ml), and the mixture was stirred at room temperature for 2 h. Then, 0.01 M phosphate buffer (pH 7) was added to the resulting mixture, and the whole was extracted with EtOAc. The combined organic extracts were washed with brine, and concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$—MeOH (100:1) as an eluent to give 52 mg (49%) of **18** as a white powder, mp 205-210°C dec.; ^1H NMR (270 MHz, $CDCl_3$) δ: 1.25 (1 H, dd, J = 4.7, 3.8 Hz); 2.18 (1 H, dd, J = 7.6, 3.8 Hz); 2.55 (3 H, s); 3.64 (1 H, m); 3.83 (3 H, s); 3.89 (3 H, s); 3.93 (3 H, s); 4.07 (3 H, s); 4.07 (3 H, s); 4.38 (2 H, br d, J = 2.3 Hz); 6.80 (1 H, s); 6.93 (1 H, d, J = 2.3 Hz); 6.98

(1 H, s); 9.25 (1 H, br. s). IR (KBr) 1699, 1639, 1610, 1458, 1400, 1304, 1292, 1265, 1105 cm^{-1}. FAB-MS *m/z* 506 $(M + H)^+$, 234. Found: C 63.41; H 5.46; N 8.14%. $C_{27}H_{27}N_3O_7 \cdot 0.5H_2O$. Calculated: C 63.03; H 5.48; N 8.17%.

3-Methoxycarbonyl-1,1'-2-trimethylduocarmycin A (19). The procedure was the same as that employed for the preparation of **18**. Iodomethane (39 ml, 0.63 mmol) and potassium carbonate (87 mg, 0.63 mmol) and **12a** (105 mg, 0.21 mmol) were subjected to the reaction to afford 87 mg (80%) of **19** as a white powder, mp 120-125°C dec.; ^1H NMR (270 MHz, $CDCl_3$) δ: 6.73 (1 H, s); 6.69 (1 H, s); 6.17 (1 H, s); 4.15 (2 H, m); 4.09 (3 H, s); 4.03 (3 H, s); 4.01 (3 H, s); 3.93 (3 H, s); 3.86 (3 H, s); 3.82 (3 H, s); 3.56 (1 H, m); 2.53 (3 H, s); 2.34 (1 H, dd, *J* = 7.6, 3.6 Hz); 1.41 (1 H, dd, *J* = 4.9, 3.7 Hz). IR (KBr) 1697, 1654, 1621, 1467, 1446, 1390, 1261, 1234, 1110 cm^{-1}. FAB-MS *m/z* 520 $(M + H)^+$. Found: C 62.40; H 5.92; N 7.44%. $C_{28}H_{29}N_3O_7 \cdot 1.0H_2O$. Calculated: C 62.56; H 5.81; N 7.82%.

3-Methoxycarbonyl-2-methyl-8-O-N,N-dimethylcarbamoylduocarmycin B2 (21a). Hydrobromic acid (48%, 5 ml) was added to a solution of **12a** (285 mg, 0.58 mmol) in CH_3CN (15 ml), and the mixture was stirred for 2 h at room temperature. It was poured into 1 N HBr and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. *p*-Nitrophenyl chloroformate (235 mg, 1.17 mmol) and triethylamine (0.16 ml, 1.17 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (10 ml) at -78°C, and the resulting mixture was stirred at the same temperature for 0.5 h. Then an aqueous solution of 50% dimethylamine (0.52 ml, 5.8 mmol) was added to the solution, and the mixture was stirred at 0°C for 1 h. The mixture was diluted with $CHCl_3$ and the whole was washed with 0.01 M phosphate buffer (pH 7) and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$—MeOH (50:1) to give 303 mg (81%) of **21a** as a white powder, mp 180-182°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.59 (3 H, s); 3.07 (3 H, s); 3.20 (3 H, s); 3.22 (1 H, dd, *J* = 9.9, 9.9 Hz); 3.81 (1 H, dd, *J* = 9.9, 2.3 Hz); 3.92 (3 H, s); 3.95 (3 H, s); 3.97 (3 H, s); 4.08 (3 H, s); 4.58 (2 H, m); 4.73 (1 H, br. d, *J* = 9.7 Hz); 6.90 (1 H, s); 7.00 (1 H, d, *J* = 2.3 Hz); 8.14 (1 H, s); 9.09 (1 H, br. s); 9.37 (1 H, br. s). IR (KBr) 3470, 3300, 2946, 1701, 1411, 1313, 1217, 1167, 1109 cm^{-1}. SI-MS *m/z* 645 643 $(M + H)^+$, 565, 411 409, 234. Found: C 53.15; H 5.46; N 7.70%. $C_{29}H_{31}BrN_4O_8 \cdot 1.5CH_3OH$. Calculated: C 52.97; H 5.39; N 8.10%.

3-Methoxycarbonyl-2-methyl-8-O-(4-methyl-1-piperazinylcarbonyl)duocarmycin B2 (21b). Hydrobromic acid (48%, 7.5 ml) was added to a solution of **12a** (900 mg, 1.83 mmol) in CH_3CN (20 ml), and the mixture was stirred for 2 h at room temperature. The resulting mixture was poured into 1 N HBr and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. *p*-Nitrophenyl chloroformate (740 mg, 3.67 mmol) and triethylamine (0.51 ml, 3.67 mmol) were added to a stirred solution of the residue in dry CH_2Cl_2 (15 ml) at -78°C, then the resulting mixture was stirred at the same temperature for 0.5 h. 4-Methylpiperazine (0.51 ml, 4.61 mmol) was added, and the whole was stirred at 0°C for 1 h. It was diluted with $CHCl_3$ and washed with 0.01 M phosphate buffer (pH 7) and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$—MeOH (10:1) to give 1.03 g (81%) of **21b** as a white powder, mp 160-163°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 2.37 (3 H, s); 2.50 (4 H, br. s); 2.70 (3 H, s); 3.23 (1 H, dd, *J* = 10.0, 10.0 Hz); 3.64 (2 H, br. s); 3.78 (2 H, br. s); 3.82 (1 H, dd, *J* = 10.0, 2.2 Hz); 3.92 (3 H, s); 3.95 (3 H, s); 3.97 (3 H, s); 4.08 (3 H, s); 4.54 (1 H, m); 4.63 (1 H, m); 4.74 (1 H, dd, *J* = 10.2, 1.2 Hz); 6.90 (1 H, s); 6.99 (1 H, d, *J* = 2.3 Hz); 8.15 (1 H, s); 8.81 (1 H, br. s); 9.34 (1 H, br. s). IR (KBr) 3475, 3232, 2944, 1698, 1491, 1410, 1313, 1217, 1110 cm^{-1}. SI-MS *m/z* 700 698 $(M + H)^+$, 466 464, 339, 234. Found: C 53.31; H 5.30; N 9.45%. $C_{32}H_{36}BrN_5O_8 \cdot 1.0H_2O$. Calculated: C 53.64; H 5.34; N 9.77%.

3-Methoxycarbonyl-2-methyl-8-O-piperidinylcarbonyl-2-methylduocarmycin B2 (21c). The procedure was the same as that for **21a** except that piperidine was used. The crude product was purified by silica gel chromatography to afford **21c** (65%) as a white solid, mp 134-137°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.68 (6 H, br. s); 2.59 (3 H, s); 3.22 (1 H, dd, *J* = 10.1, 10.1 Hz); 3.54 (2 H, br. s); 3.69 (2 H, br. s); 3.81 (1 H, dd, *J* = 10.1, 2.1 Hz); 3.92 (3 H, s); 3.95 (3 H, s); 3.96 (3 H, s); 4.07 (3 H, s); 4.61 (2 H, m); 4.74 (1 H, dd, *J* = 10.3, 1.0 Hz); 6.90 (1 H, s); 7.00 (1 H, d, *J* = 2.4 Hz); 8.14 (1 H, s); 9.09 (1 H, br. s); 9.38 (1 H, br. s, NH). IR (KBr) 3470, 3250, 2940, 2858, 1698, 1491, 1410, 1312, 1255, 1214, 1165, 1109 cm^{-1}. SI-MS *m/z* 685 683 $(M + H)^+$, 234. Found: C 55.78; H 5.30; N 7.90%. $C_{32}H_{35}BrN_4O_8 \cdot 0.5H_2O$. Calculated: C 55.50; H 5.24; N 8.09%.

3-Methoxycarbonyl-2-methyl-8-O-pyrrolidinylcarbonylduocarmycin B2 (21d). The procedure was the same as that for **21a** except that pyrrolidine was used. The crude product was purified by silica gel chromatography to afford **21d** (65%) as a white powder, mp 152-160°C; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.99 (4 H, m); 2.65 (3 H, s); 3.22 (1 H, dd, *J* = 10.1, 10.1 Hz); 3.52 (2 H, t, *J* = 6.6 Hz); 3.67 (2 H, t, *J* = 6.6 Hz); 3.81 (1 H, dd, *J* = 10.1, 2.1 Hz); 3.92 (3 H, s); 3.95 (3 H, s); 3.97 (3 H, s); 4.08 (3 H, s); 4.55 (1 H, dd, *J* = 10.2, 2.4 Hz); 4.63 (1 H, m); 4.74 (1 H, dd, *J* = 10.2, 1.0 Hz); 6.90 (1 H, s); 7.00 (1 H, d, *J* = 2.3 Hz); 8.16 (1 H, s); 9.06 (1 H, br. s); 9.36 (1 H,

br. s). IR (KBr) 3230, 2942, 1699, 1490, 1415, 1312, 1216, 1109 cm^{-1}. SI-MS *m/z* 671 669 $(M + H)^+$, 591, 234. Found: C 52.63; H 5.20; N 7.31%. $C_{31}H_{33}BrN_4O_8 \cdot 1.5H_2O \cdot 1.0CH_3OH$. Calculated: C 52.75; H 5.53; N 7.69%.

3-Methoxycarbonyl-2-methyl-8-O-(4-methyl-1-piperazinylcarbonyl)duocarmycin B2 Hydrobromide (21e). A solution of **21b** (6.69 g, 9.57 mmol) in Me_2CO (60 ml) and MeOH (270 ml) was treated with 48% hydrobromic acid (1.7 ml, 9.95 mmol) at room temperature for 4 h. The resulting mixture was evaporated *in vacuo* to give 6.42 g (86%) of **21e** as a white crystalline compound, mp 207-213°C dec.; ^1H NMR (400 MHz, DMSO-d_6) δ: 2.69 (3 H, s); 2.89 (3 H, s); 3.26 (4 H, br. s); 3.41 (1 H, dd, J = 9.0, 9.0 Hz); 3.53 (3 H, m); 3.80 (3 H, s); 3.82 (3 H, s); 3.85 (3 H, s); 3.94 (3 H, s); 4.20 (1 H, m); 4.47 (3 H, m); 4.65 (1 H, dd, J = 10.5, 8.5 Hz); 6.97 (1 H, s); 7.00 (1 H, d, J = 2.1 Hz); 7.94 (1 H, s); 9.81 (1 H, br. s); 11.30 (1 H, d, J = 2.1 Hz); 11.97 (1 H, s). IR (KBr) 1717, 1692, 1608, 1525, 1490, 1409, 1310, 1218, 1167, 1108 cm^{-1}. SI-MS *m/z* 700 698 $(M + H)^+$, 466, 464, 339, 234. Found: C 47.92; H 5.10; N 8.49%. $C_{32}H_{36}BrN_5O_8 \cdot 1.0HBr \cdot 1.0H_2O$. Calculated: C 48.19; H 4.93; N 8.78%.

STABILITY OF DRUG IN AQUEOUS SOLUTION

The stability of the duocarmycin B2 derivatives under aqueous conditions was examined by chromatography on a UNISIL pack 5C18 reversed-phase HPLC column (GL Science, Co., Ltd., Tokyo, Japan). A test compound (1 mg) was dissolved in acetonitrile (10 ml). This solution (2 ml) was diluted with aqueous solution or culture medium or calf serum (each 8 ml). Aqueous solution and culture medium were composed of 0.01 M phosphate buffer (pH 7) and Eagle's minimum essential medium (MEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% fetal bovine serum (Grand Island Biological Co.), respectively. The resulting solution was incubated at 35°C. Samples were removed at intervals and injected directly into the HPLC injection port. The compound was eluted with 0.05 M phosphate buffer (pH 5.9)—acetonitrile (30:70) and detected by measuring the absorbance at 330 nm.

ANTICELLULAR AND ANTITUMOR ACTIVITIES

Human uterine cervix carcinoma HeLa S_3 cells were obtained from American Type Culture Collection through Dainippon Pharmaceutical Co. (Osaka, Japan). The cells (2×10^4/well) were precultured in the culture medium in 24-well multidishes (Nunc, Roskilde, Denmark) for 24 h at 37°C in a humidified atmosphere of 5% CO_2. For the pulse exposure experiment, cells were treated with each compound for 1 h, washed with Dulbecco's phosphate-buffered saline [Ca^{2+}-, Mg^{2+}-free; PBS(−)] and further incubated in fresh medium for 71 h. For the continuous exposure experiment, cells were treated with each compound for 72 h. Then, the cells were treated with PBS(−) containing 0.05% trypsin (Difco Laboratories, Detroit, MI) and 0.02% EDTA (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan) and counted by using a Microcell Counter (Toa Medical Electronics Co., Ltd., Kobe, Japan). The IC_{50} values (drug concentration required for 50% inhibition of the cell growth) were determined.

Sarcoma 180, St-4 (poorly differentiated stomach adenocarcinoma), Co-3 (well-differentiated colon adenocarcinoma), and LC-6 (large cell lung adenocarcinoma) were kindly supplied by the National Cancer Center (Tokyo, Japan). M5076 reticulum cell sarcoma, B-16 melanoma, and Colon 26 adenocarcinoma were supplied by the Japanese Foundation for Cancer Research (Tokyo, Japan). Sarcoma 180 cells were passaged and used for the experiment in adult male *ddY* mice. B-16 melanoma and M5076 reticulum cell sarcoma cells were passaged and used in adult male C57BL/6 mice. Colon 26 adenocarcinoma cells were passaged and used in adult male BALB/c mice. Human xenografts were passaged and used in adult male BALB/c-*nu/nu* mice. All murine solid tumors were inoculated subcutaneously at the axillary region of mice. Human xenografts were inoculated subcutaneously in the flank of nude mice. Drugs were administered intravenously, beginning 1 day after tumor inoculation. Antitumor efficacy was expressed as *T/C*, where *T* and *C* are the values of mean tumor volume of treated and control mice. The length and width of the tumors were measured, and tumor volume was calculated as

$$\text{tumor volume } (mm^3) = \text{length (mm)} \times [\text{width (mm)}]^2/2$$

according to the method of the National Cancer Institute [66].

The criteria for effectiveness against murine solid tumors were a *T/C* value of 42% or less, and statistical significance determined by the Mann—Whitney *U* test ($P < 0.05$). Drug efficacy against human xenografts was expressed as the percentage of mean V/V_0 value against that of the control group, where V is the tumor volume at the day of evaluation and V_0 is the tumor volume at the day of initial drug treatment. The criteria for effectiveness were a *T/C* value of 50% or less, and statistical significance determined by the Mann—Whitney *U* test ($P < 0.01$, one-sided) [67].

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