Wagner–Meerwein Rearrangement of Duocarmycins

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We found that treatment of the 8-*O*-protected-3-hydroxy derivatives of duocarmycin B2 (DUMB2, 1c) with camphorsulfonic acid (CSA) in toluene interestingly gave A-ring pyrrole analogs of DUMB2 (1c) in good yields. Their structures were unambiguously elucidated on the basis of NMR and mass spectrometry, and the mechanism was considered to be a Wagner–Meerwein type rearrangement. On the other hand, treatment of the 9-*O*-protected-3-hydroxy derivatives of duocarmycin B1 (1b) with CSA afforded different rearrangement products. In the case of bulky groups at the 9-O position, such as a *tert*-butyldimethylsilyl group, normal A-ring pyrrole analogs were obtained. Under the same condition, however, the 9-*O*-*N*,*N*-dimethylcarbamoyl-3-hydroxy compound of 1b gave a spiro compound, 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. Compounds having a protective group of medium size gave a mixture of the normal rearrangement and the spiro derivative.

Key words duocarmycin; Wagner–Meerwein rearrangement; carbamoyl derivative; spiro compound; anticellular activity

Duocarmycin (DUM)s (A, B1, B2, C1, C2, SA) are novel antitumor antibiotics isolated from the culture broth of Streptomyces sp. (Chart 1).[2)] The structures of DUMs were confirmed by spectroscopic and chemical analysis.[3)] DUMA (**1a**),[2a)] which is considered to be an active form among these DUMs, possesses a unique cyclopropane ring with the alkylating ability of DNA, which has been reported in the "left hand segment" of CC-1065 (**2c**).[4)] In addition, it was indicated that **1a** could also alkylate DNA by a mechanism similar to that of **2c**.[5)] The mechanism of action of **2c** was reported to involve binding to the DNA minor groove, followed by alkylation of the N-3 position of adenine.[6)] We have been interested in synthesizing analogs in order to enhance and broaden the spectrum of their antitumor activity, and to improve their stability or solubility.[7)] Recently, we found that an A-ring pyrrole analog (**2a**) was produced by acid-catalyzed rearrangement from DUMB2 (**1c**) derivatives in good yield.[8)] The structure of **2a** was elucidated on the basis of NMR and mass spectrometry.[9)] The mechanism was considered to be a Wagner–Meerwein type rearrangement which was reported by D. Berner *et al.*[10)] Compound **2a** was converted to KW-2189 (**2b**), a novel derivative of **1c**, which demonstrated excellent *in vivo* antitumor activity, good stability in the culture medium and aqueous solubility greater than 10 mg/ml.[8,11)] Therefore, this rearrangement is a key reaction to obtain novel derivatives of DUMs. KW-2189 was designed as a prodrug requiring enzymatic hydrolysis, followed by regeneration of DU-86 (**2a**), which was an active metabolite.[8,11,12)] It is currently under phase I clinical trial. As an extension of that research, we investigated this acid-catalyzed rearrangement of various 3-hydroxy-derivatives of **1c** and DUMB1 (**1b**).

In this paper, we report on a Wagner–Meerwein type rearrangement of DUM derivatives and evaluate their antitumor activity.

Results and Discussion

The phenolic hydroxyl group of **1c** was protected by *tert*-butyldimethylsilyl chloride in *N*,*N*-dimethylformamide (DMF) to give **3a** quantitatively, and compound **3a** was reduced by sodium borohydride in allyl alcohol to afford the 3α-hydroxy compound (**4a**) in 74% yield, as shown in Chart 2.[13)] The configuration at the C_3 center was confirmed by NMR. In the carbamoyl series, **1c** was treated with 4-nitrophenyl chloroformate in the presence of triethylamine in methylene chloride at 0 °C to give a carbonate as an intermediate, followed by the addition of dimethylamine or 1-methylpiperazine to afford the 8-*O*-carbamoyl derivatives (**3b** and **3c**) in good yields.[7a)] **3b** and **3c** thus obtained were reduced using the same method to give **4b** and **4c** in reasonable yield, respectively. These obtained 8-*O*-protected-3α-hydroxy derivatives (**4a—c**) were treated by camphorsulfonic acid (CSA) in toluene, separately. In these reactions, an interesting rearrangement of the methoxycarbonyl group occurred to afford the corresponding A-ring pyrrole analogs (**5a-c**) in good yields.[8,14)]

Their structures were elucidated on the basis of NMR and mass spectrometry. SIMS of **5a—c** gave molecular ions at *m/z* 688, 645 and 700 (^{81}Br), confirming the dehydration of **4a—c**, respectively. The ^{1}H-NMR spectra of **5a—c** showed downfield shifts of the C_2-methyl proton signal (about +1.0 ppm) and of the N_1-NH proton signal (about +3.0 ppm), confirming the structure of A-ring pyrrole compounds. Nuclear overhauser effect (NOE) and long range coupling were observed between the C_2-methyl group and the NH proton in the NMR spectra, but NOEs from the C_2-methyl group to 4-H or 9-H_2 were not observed.[9)] As a result, 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.*[10)] When this rearrangement reaction of **4c** was achieved in methanol as a reaction solvent, compound **6c** 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 Fig. 1,

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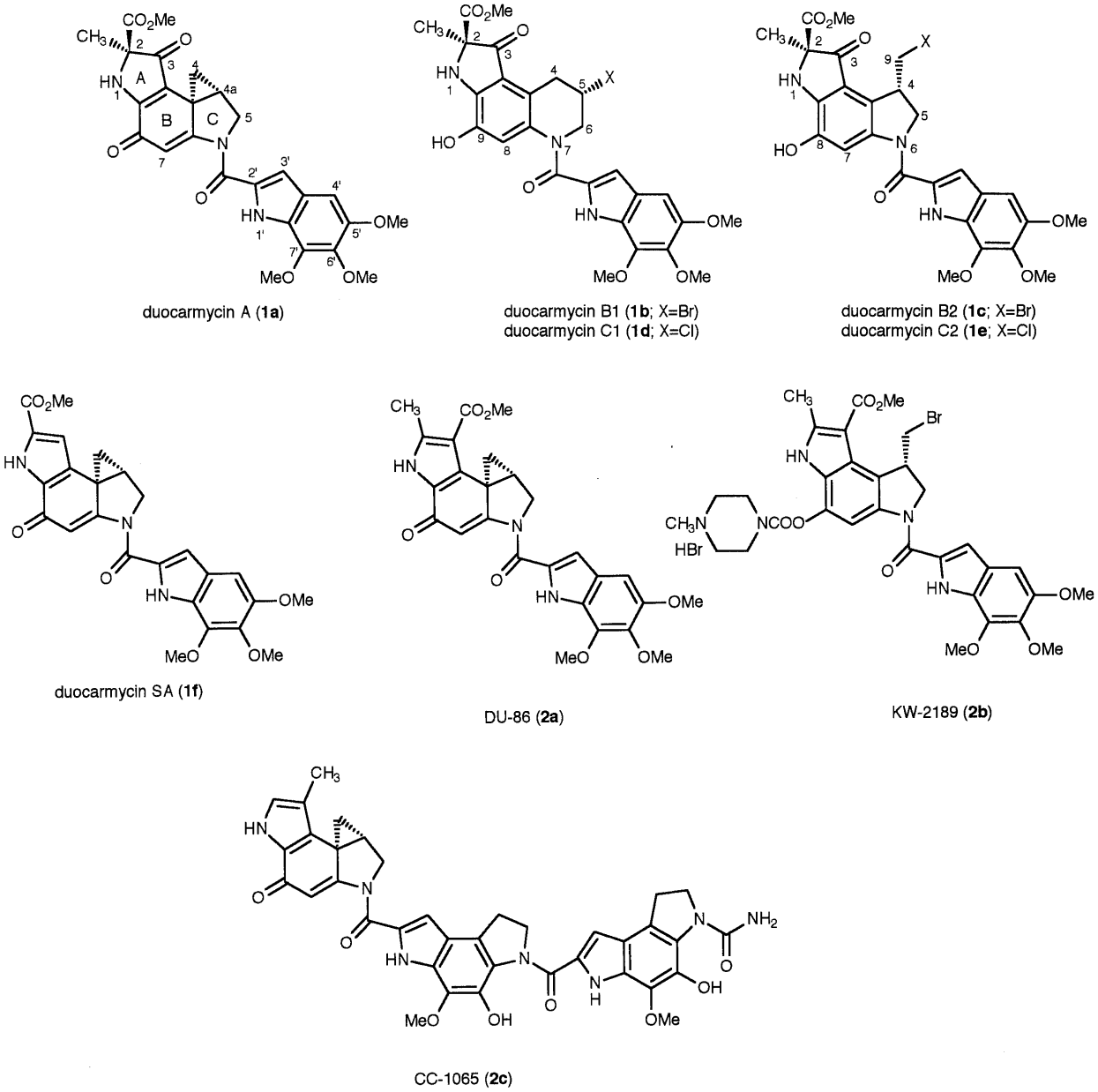


Chart 1

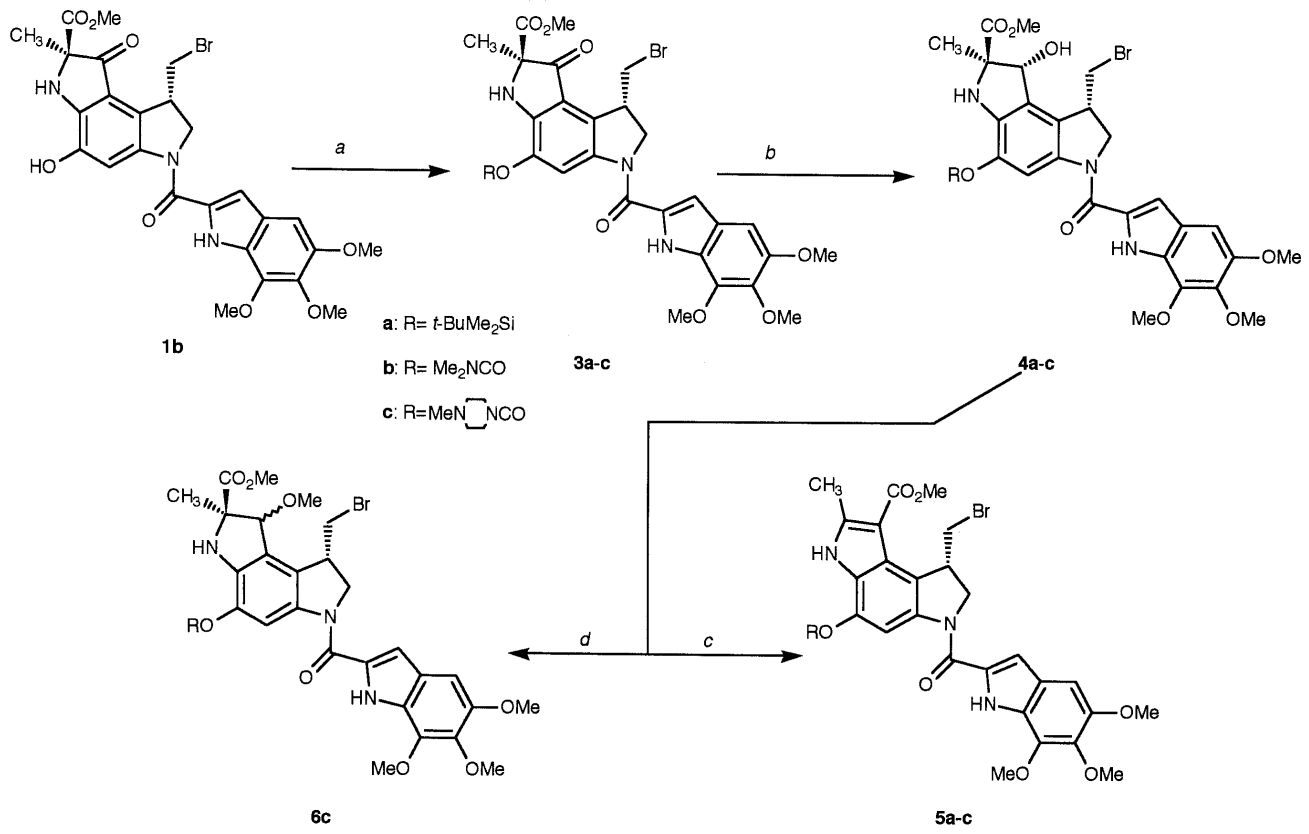
and this result also supports that this reaction is a Wagner–Meerwein type rearrangement.

Optimal conditions for the reaction from **4a** to **5a** are characterized in Table 1. Both *p*-toluenesulfonic acid (*p*-TsOH) and methanesulfonic acid (MsOH) were effective for the rearrangement, as well as CSA, but the yields were not sufficient. At 50 °C this reaction proceeded more effectively, resulting in 65% yield.[15] In addition, aluminum chloride and boron trifluoride etherate were more effective than CSA, giving 86% and 80% yield at room temperature, respectively.

In conjunction with these studies, we also examined the rearrangement of the DUMB1 (**1b**) derivatives (Chart 3). Compound **1b** was converted to the 9-*O*-silyl and the 9-*O*-carbamoyl compounds (**7a**—**c**) by the same method as that for **3a**—**c** in 99%, 98% and 79% yields, respectively. Obtained **7a**—**c** were reduced by $NaBH_4$ in allyl alcohol to afford the corresponding 3α-hydroxy compounds (**8a**—**c**) in reasonable yields. The rearrangement of **8a** was performed by CSA in toluene to afford **9a** in 79% yield.

Treatment of **8b** with CSA, however, did not give the desirable product, but exclusively afforded the spiro compound **10b**, 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 2). The mass spectrometry of **10b** showed an ion corresponding to the entire molecule at *m/z* 645 643 $(M+H)^+$, similar to **5b**, 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 **10b**.

On the other hand, the same treatment of **8c**, having a protecting group of medium size between **8a** and **8b** at the C-9 position, gave both **9c** and the spiro compound **10c** in 79% and 11% yields, respectively. When the reaction time of **8c** was prolonged from 2 to 5 h at the same temperature, the yield of spiro compound **10c** was increased to 19%,



Reagents: *a* t-BuMe$_2$SiCl, imidazole, DMF or 4-nitrophenyl chloroformate, Et$_3$N, CH$_2$Cl$_2$, then Me$_2$NH (1-methyl piperazine); *b* NaBH$_4$, allyl alcohol; *c* CSA, toluene; *d* CSA, MeOH.

Chart 2

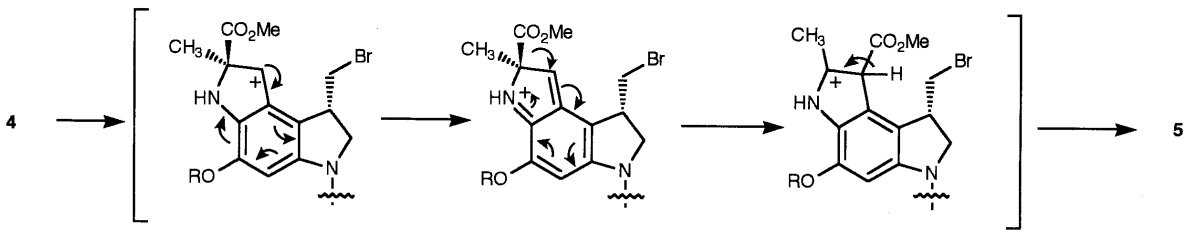


Fig. 1. Proposed Mechanism of Wagner–Meerwein Rearrangement for Production of **5**

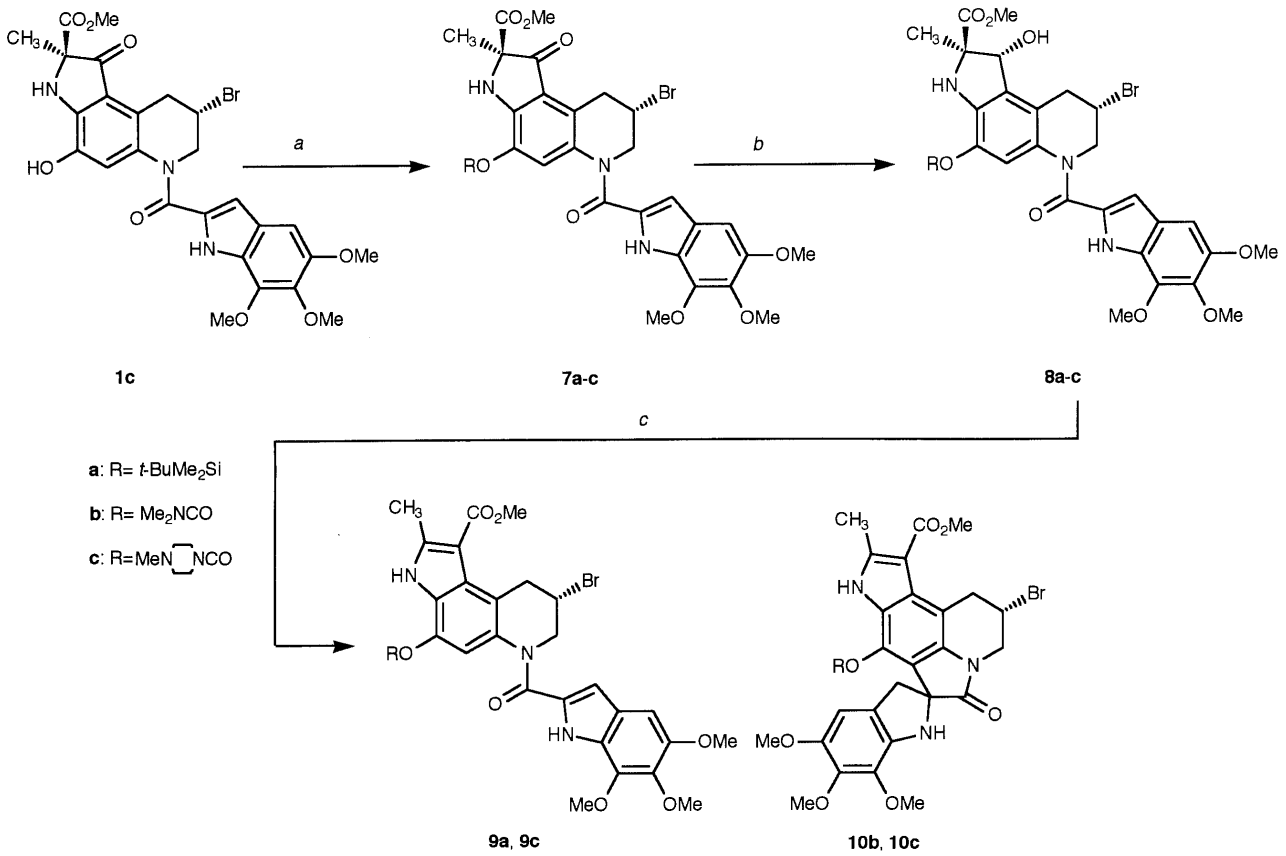
Table 1. The Acid-Catalyzed Rearrangement from **4a** to **5a**

Entry	Acid	Solvent	Time (h)	Temp. (°C)	Isolated yield (%)
1	CSA	Toluene	6	25	26
2	CSA	Toluene	1	50	65
3	MsOH	$CHCl_3$	6	25	29
4	*p*-TsOH	$CHCl_3$	6	25	23
5	PPA[a)]	$CHCl_3$	6	25	14
6	$AlCl_3$	$CHCl_3$	2	25	86
7	$BF_3 \cdot OEt_2$	$CHCl_3$	4	25	80
8	$ZnCl_2$	$CHCl_3$	6	25	29
9	$SnCl_4$	$CHCl_3$	6	25	40

a) Polyphosphoric acid.

and that of **9c** was decreased to 70%. On the basis of these results, a plausible mechanism for the formation of **10b** and **10c** is as follows: a 1, 2-shift of the methoxycarbonyl group has occurred initially, then the spiro compound is produced as shown in Fig. 2. Herein, the effect of the substituents at the C-9 position which involve an increase in bulk is important. In contrast, none of the spiro compounds in this rearrangement of DUMB2 derivatives (**4a—c**) was observed; it is considered that steric straining in a 5-membered system of the C-rings is presumably responsible for this inertness.

The anticellular activity of these A-ring pyrrole derivatives was evaluated by assays of growth inhibition against HeLa S_3 cells. As shown in Table 3, the anticellular activity of the derivatives of DUMB2 type (**5**) was superior to that of the derivatives of DUMB1 type (**9**) (**5a** *vs.* **9a**, **5c** *vs.* **9c**). Also, the activity of the silyl derivatives (**5a** and **9a**) was as potent as that of **2a**. It is considered that these silyl derivatives readily released **2a** under aqueous conditions, and indeed, their anticellular activity was similar to that of **2a**.[8)] In contrast, the carbamoyl derivatives (**5b—c, 9c**) showed decreased anticellular activity, about 1×10^2—1×10^3 times inferior to the corresponding silyl derivatives (**5a** and **9a**) (72 h exposure).[8)] Moreover, the



Reagents: *a* *t*-$BuMe_2SiCl$, imidazole, DMF or 4-nitrophenyl chloroformate, Et_3N, CH_2Cl_2, then Me_2NH (1-methyl piperazine); *b* $NaBH_4$, allyl alcohol; *c* CSA, toluene.

Chart 3

Table 2. ^{1}H-NMR Data for **9a**, **9c**, **10b** and **10c** at 400 MHz (in $CDCl_3$)

Proton	9a	9c	10b	10c
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)	3.74 (m)	3.77 (dd, J=18.0, 6.0 Hz)	3.75 (m)
	4.07 (br d, J=5.7 Hz)	3.79 (br d, J=7.4 Hz)	3.83 (dd, J=18.0, 3.5 Hz)	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)	4.32 (m)	4.03 (dd, J=13.6, 3.6 Hz)	4.04 (m)
	4.24 (dd, J=11.0, 5.7 Hz)	4.44 (m)	4.07 (dd, J=13.6, 5.8 Hz)	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)	3.45 (d, J=16.8 Hz)
			3.62 (d, J=16.4 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.

spiro compounds exhibited weak anticellular activity compared with the normal rearrangement compounds (**10c** *vs.* **9c**). The IC_{50} values of **10b** and **10c** at 72 h exposure were 1100 and 99 nM, respectively.

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 Bruker AM-400 (400 MHz) spectrometer. Chemical shifts were 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. 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}

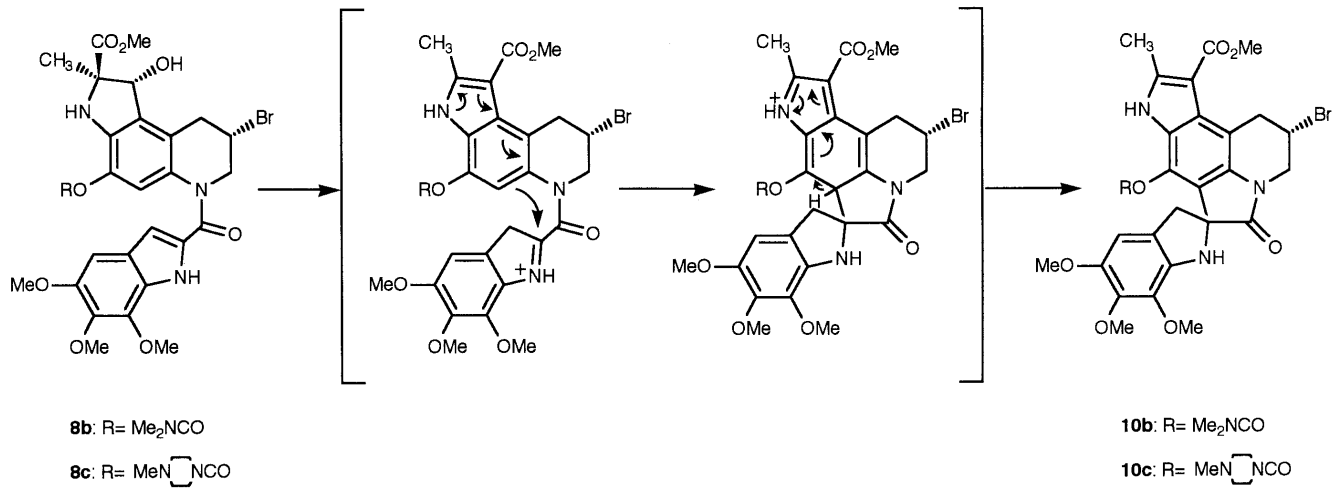


Fig. 2. Proposed Mechanism for Production of **10b** and **10c**

Table 3. Anticellular Activities against HeLa S_3 Cells

Compound	HeLa S_3 IC_{50} (nM)	
	1 h	72 h
5a	0.17	0.002
5b	55	7.3
5c	210	1.3
9a	0.53	0.065
9c	190	3.4
10b	6000	1100
10c	2500	99
2a	0.045	0.0052

plates (Merck).

8-*O*-*tert*-Butyldimethylsilyl-DUMB2 (3a) *tert*-Butyldimethylsilyl chloride (50 mg, 0.33 mmol) was added to a solution of DUMB2 (**1c**) (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 (hexane–EtOAc, 3 : 1) to give 140 mg (95%) of **3a** as a light-tan powder, mp 120—130 °C (dec.) ^{1}H-NMR (400 MHz $CDCl_3$) δ: 0.35 (3H, s, CH_3), 0.36 (3H, s, CH_3), 1.06 (9H, s, $C(CH_3)_3$), 1.69 (3H, s, CH_3), 3.57 (1H, dd, J=10.3, 9.1 Hz, 9-H), 3.78 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.06 (1H, dd, J=10.3, 3.0 Hz, 9-H), 4.06 (3H, s, OCH_3), 4.17 (1H, m, 4-H), 4.54 (1H, dd, J=10.6, 4.4 Hz, 5-H), 4.62 (1H, dd, J=10.6, 9.1 Hz, 5-H), 5.04 (1H, br s, NH), 6.87 (1H, s, 4′-H), 6.95 (1H, d, J=2.2 Hz, 3′-H), 7.91 (1H, s, 7-H), 9.38 (1H, br s, NH). IR (KBr): 1745, 1700, 1618, 1497, 1293, 837 cm^{-1}. SIMS *m/z*: 704 702 $(M+H)^+$, 470 468, 234. *Anal.* Calcd for $C_{32}H_{40}BrN_3O_8Si$: C, 54.70; H, 5.74; N, 5.98. Found: C, 54.82; H, 5.93; N, 5.75.

8-*O*-*tert*-Butyldimethylsilyl-3α-hydroxy-DUMB2 (4a) $NaBH_4$ (25 mg, 0.66 mmol) was added to a solution of **3a** (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 to the resulting mixture, and the 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 (hexane–EtOAc, 1 : 1) to give 115 mg (74%) of **4a** as a white powder, mp 124—125 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.30 (3H, s, CH_3), 0.32 (3H, s, CH_3), 1.04 (9H, s, $C(CH_3)_3$), 1.60 (3H, s, CH_3), 2.09 (1H, br s, OH), 3.49 (1H, dd, J=10.3, 9.8 Hz, 9-H), 3.72 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.92 (1H, m, 4-H), 3.93 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.07 (1H, dd, J=10.3, 3.2 Hz, 9-H), 4.50 (1H, dd, J=10.6, 3.9 Hz, 5-H), 4.56 (1H, m, 3-H), 4.57 (1H, dd, J=10.6, 8.9 Hz, 5-H), 5.31 (1H, br s, NH), 6.86 (1H, s, 4′-H), 6.91 (1H, d, J=2.2 Hz, 3′-H), 7.91 (1H, s, 7-H), 9.43 (1H, br s, NH). IR (KBr): 3406, 1734, 1621, 1485, 1111, 838 cm^{-1}. SIMS *m/z*: 706 704 $(M+H)^+$, 234. *Anal.* Calcd for $C_{32}H_{42}BrN_3O_8Si$: C, 54.54; H, 6.01; N, 5.96. Found: C, 54.47; H, 6.19; N, 5.71.

8-*O*-Dimethylcarbamoyl-3α-hydroxy-DUMB2 (4b) The procedure was the same as that of **4a** except for the use of **3b** (40 mg, 0.06 mmol). The crude product was purified by silica gel chromatography to afford 23.4 mg (59%) of **4b** as a white powder, mp 136—141 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.66 (3H, s, CH_3), 1.70 (1H, br s, OH), 3.04 (3H, s, N–CH_3), 3.13 (3H, s, N–CH_3), 3.49 (2H, dd, J=10.2, 10.2 Hz, 9-H ×2), 3.77 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.95 (1H, m, 4-H), 3.94 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.13 (1H, dd, J=10.1, 3.2 Hz, 5-H), 4.58 (2H, m, 3-H, 5-H), 5.41 (1H, br s, NH), 6.87 (1H, s, 4′-H), 6.93 (1H, d, J=2.3 Hz, 3′-H), 8.08 (1H, s, 7-H), 9.30 (1H, br s, NH). IR (KBr): 2936, 1718, 1619, 1521, 1448, 1383, 1311, 1164, 1111, 1050 cm^{-1}. SIMS *m/z*: 663 661 $(M+H)^+$, 429 427, 234. *Anal.* Calcd for $C_{29}H_{33}BrN_4O_9$: C, 52.66; H, 5.03; N, 8.47. Found: C, 52.55; H, 4.95; N, 8.44.

8-*O*-(4-Methyl-1-piperazinycarbony)-3α-hydroxy-DUMB2 (4c) The procedure was the same as that of **4a** except for the use of **3c** (50 mg, 0.070 mmol). The crude product was purified by silica gel chromatography to afford 28.6 mg (57%) of **4c** as a white powder, mp 200—205 °C (dec.) ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.41 (3H, s, CH_3), 2.24 (3H, s, N–CH_3), 2.49 (4H, br s, N–CH_2), 3.42 (1H, m, OH), 3.45 (2H, br s, N–CH_2), 3.60 (2H, br s, N–CH_2), 3.64 (3H, s, OCH_3), 3.65 (1H, m, 4-H), 3.79 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 4.12 (1H, dd, J=11.0, 3.2 Hz, 9-H), 4.32 (1H, dd, J=11.0, 2.9 Hz, 9-H), 4.56 (1H, m, 3-H), 5.24 (1H, br d, J=7.8 Hz, 5-H), 5.89 (1H, s, NH), 5.96 (1H, br d, J=7.8 Hz, 5-H), 6.94 (1H, d, J=2.2 Hz, 3′-H), 6.96 (1H, s, 4′-H), 7.73 (1H, s, 7-H), 11.28 (1H, br s, NH). IR (KBr): 1709, 1623, 1428, 1311, 1227, 1151, 1114 cm^{-1}. SIMS *m/z*: 718 716 $(M+H)^+$, 638, 484 482, 234. *Anal.* Calcd for $C_{32}H_{38}BrN_5O_9 \cdot 2.0\ H_2O$: C, 51.07; H, 5.62; N, 9.31. Found: C, 51.10; H, 5.88; N, 9.00.

Methyl (1*S*)-1-Bromomethyl-5-(*tert*-butyldimethylsilyloxy)-7-methyl-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]-indole-8-carboxylate (5a) CSA (1.6 g, 6.81 mmol) was added to a solution of **4a** (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 hexane–EtOAc (4 : 1) to give 1.01 g (65%) of **5a** as a white powder, mp 140—142 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.37 (3H, s, CH_3), 0.39 (3H, s, CH_3), 1.07 (9H, s, $C(CH_3)_3$), 2.76 (3H, s, CH_3), 3.21 (1H, dd, J=9.9, 9.9 Hz, 9-H), 3.80 (1H, dd, J=9.9, 2.1 Hz, 9-H), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 4.52 (1H, m, 4-H), 4.54 (1H, br d, J=8.5 Hz, 5-H), 4.73 (1H, br d, J=8.9 Hz, 5-H), 6.89 (1H, s, 4′-H), 6.99 (1H, d, J=2.3 Hz, 3′-H), 7.98 (1H, s, 7-H), 8.30 (1H, br s, NH), 9.40 (1H, br s, NH). IR (KBr): 2934, 1696, 1628, 1493, 1412, 1305, 1213, 1112, 837 cm^{-1}. SIMS *m/z*: 688 686 $(M+H)^+$, 454 452, 234. *Anal.* Calcd for $C_{32}H_{40}BrN_3O_7Si$: C, 55.97; H, 5.87; N, 6.12. Found: C, 55.86; H, 6.03; N, 5.91.

Methyl (1*S*)-1-Bromomethyl-5-(dimethylaminocarbonyloxy)-7-methyl-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]-

indole-8-carboxylate (5b) The procedure was the same as that of **5a** except **4b** was used instead (20 mg, 0.030 mmol). The crude product was purified by silica gel chromatography to afford 15.8 mg (81%) of **5b** as a white powder, mp 180—182 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.59 (3H, s, CH_3), 3.07 (3H, s, N–CH_3), 3.20 (3H, s, N–CH_3), 3.22 (1H, dd, J=9.9, 9.9 Hz, 9-H), 3.81 (1H, dd, J=9.9, 2.3 Hz, 9-H), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.58 (2H, m, 4-H, 5-H), 4.73 (1H, br d, J=9.7 Hz, 5-H), 6.90 (1H, s, 4′-H), 7.00 (1H, d, J=2.3 Hz, 3′-H), 8.14 (1H, s, 7-H), 9.09 (1H, br s, NH), 9.37 (1H, br s, NH). IR (KBr): 3470, 3300, 2946, 1701, 1411, 1313, 1217, 1167, 1109 cm^{-1}. SIMS m/z: 645 643 $(M+H)^+$, 411 409, 234. *Anal.* Calcd for $C_{29}H_{31}BrN_4O_8 \cdot 1.5\ CH_3OH$: C, 52.97; H, 5.39; N, 8.10. Found: C, 53.15; H, 5.46; N, 7.70.

Methyl (1*S*)-1-Bromomethyl-7-methyl-5-(4-methylpiperazinylcarbonyloxy)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo-[3,2-*e*]indole-8-carboxylate (5c) The procedure was the same as that of **5a** except for the use of **4c** (20 mg, 0.028 mmol). The crude product was purified by silica gel chromatography to afford 15.9 mg (81%) of **5c** as a white powder, mp 160—163 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.37 (3H, s, N–CH_3), 2.50 (4H, br s, N–$CH_2 \times 2$), 2.70 (3H, s, CH_3), 3.23 (1H, dd, J=10.0, 10.0 Hz, 9-H), 3.64 (2H, br s, N–CH_2), 3.78 (2H, br s, N–CH_2), 3.82 (1H, dd, J=10.0, 2.2 Hz, 9-H), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.54 (1H, m, 4-H), 4.63 (1H, m, 5-H), 4.74 (1H, dd, J=10.2, 1.2 Hz, 5-H), 6.90 (1H, s, 4′-H), 6.99 (1H, d, J=2.3 Hz, 3′-H), 8.15 (1H, s, 7-H), 8.81 (1H, br s, NH), 9.34 (1H, br s, NH). IR (KBr): 3475, 3232, 2944, 1698, 1491, 1410, 1313, 1217, 1110 cm^{-1}. SIMS m/z: 700 698 $(M+H)^+$, 466 464, 234. *Anal.* Calcd for $C_{32}H_{36}BrN_5O_8 \cdot 1.0\ H_2O$: C, 53.64; H, 5.34; N, 9.77. Found: C, 53.31; H, 5.30; N, 9.45.

Methyl (1*S*)-1-Bromomethyl-8-methoxy-7-methyl-5-(4-methylpiperazinylcarbonyloxy)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indoline-7-carboxylate (6c) Boron trifluoride diethyl etherate (26 μl, 0.21 mmol) was added to a solution of **4c** (50 mg, 0.07 mmol) in methanol (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 **6c** as a white powder, mp 122—125 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.41 and 1.42 (3H, s, CH_3), 2.24 and 2.25 (3H, s, N–CH_3), 2.49—2.52 (4H, br s, N–$CH_2 \times 2$), 3.17 and 3.18 (3H, s, OCH_3), 3.45—3.47 (2H, br s, N–CH_2), 3.60—3.63 (2H, br s, N–CH_2), 3.64 (3H, s, OCH_3), 3.65 (1H, m, 4-H), 3.79 and 3.80 (3H, s, OCH_3), 3.81 and 3.83 (3H, s, OCH_3), 3.95 and 3.97 (3H, s, OCH_3), 4.12—4.32 (3H, m, 3-H, 9-H $\times$ 2), 5.24—5.30 (2H, m, 5-H $\times$ 2), 5.82 (1H, br s, NH), 6.94 and 6.96 (1H, d, J=2.2 Hz, 3′-H), 6.96 and 6.98 (1H, s, 4′-H), 7.73 and 7.75 (1H, s, 8-H), 11.28 (1H, br s, NH). IR (KBr): 1700, 1623, 1438, 1321, 1237, 1151 cm^{-1}. SIMS m/z: 732 730 $(M+H)^+$, 234. *Anal.* Calcd for $C_{33}H_{40}BrN_5O_9 \cdot 1.0\ CH_3OH$: C, 53.55; H, 5.81; N, 9.18. Found: C, 53.46; H, 5.88; N, 9.00.

9-*O*-*tert*-Butyldimethylsilyl-DUMB1 (7a) *tert*-Butyldimethylsilyl chloride (90 mg, 0.56 mmol) was added to a solution of **1b** (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 (hexane–EtOAc, 3 : 1) to give 122 mg (99%) of **7a** as a light-tan powder, mp 119—113 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.15 (6H, s, $CH_3 \times 2$), 0.91 (9H, s, $(CH_3)_3$), 1.69 (3H, s, CH_3), 3.65 (1H, dd, J=19.3, 5.2 Hz, 4-H), 3.78 (3H, s, OCH_3), 3.83 (1H, dd, J=19.3, 6.2 Hz, 4-H), 3.85 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.20 (1H, br d, J=13.3 Hz, 6-H), 4.46 (1H, dd, J=13.3, 6.2 Hz, 6-H), 4.57 (1H, m, 5-H), 5.02 (1H, s, NH), 6.52 (1H, d, J=2.2 Hz, 3′-H), 6.72 (1H, s, 4′-H), 6.90 (1H, s, 8-H), 9.03 (1H, s, NH). IR (KBr): 2936, 2860, 1747, 1701, 1612, 1508, 1394, 1301, 1254, 1109, 828 cm^{-1}. SIMS m/z: 704 702 $(M+H)^+$, 470 468, 234. *Anal.* Calcd for $C_{32}H_{40}BrN_3O_8Si \cdot 2.0\ H_2O$: C, 52.03; H, 6.00; N, 5.69. Found: C, 52.00; H, 6.12; N, 5.55.

9-*O*-Dimethylcarbamoyl-DUMB1 (7b) 4-Nitrophenyl chloroformate (51 mg, 0.255 mmol) and triethylamine (0.029 ml, 0.255 mmol) were added to a solution of **1b** (50 mg, 0.085 mmol) in dry methylene chloride (5 ml) under cooling at 0 °C. The mixture was stirred at the same temperature for 1 h. Then, dimethylamine solution (40 w.t.%, 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 **7b** as a light-tan powder, mp 145—150 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.68 (3H, s, CH_3), 3.00 (3H, s, N–CH_3), 3.07 (3H, s, N–CH_3), 3.71 (1H, dd, J=19.4, 5.3 Hz, 4-H), 3.79 (3H, s, OCH_3), 3.88 (1H, dd, J=19.4, 6.0 Hz, 4-H), 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.32 (1H, dd, J=12.8, 2.6 Hz, 6-H), 4.48 (1H, dd, J=12.8, 6.4 Hz, 6-H), 4.55 (1H, m, 5-H), 5.46 (1H, s, NH), 6.60 (1H, d, J=2.3 Hz, 3′-H), 6.78 (1H, s, 4′-H),7.46 (1H, s, 8-H), 9.09 (1H, br s, NH). IR (KBr): 3332, 2938, 1715, 1623, 1506, 1388, 1312, 1245, 1161 cm^{-1}. SIMS m/z: 661 659 $(M+H)^+$, 427 425, 234. *Anal.* Calcd for $C_{29}H_{31}BrN_4O_9 \cdot 1.5\ H_2O$: C, 50.74; H, 4.99; N, 8.16. Found: C, 50.65; H, 4.66; N, 7.77.

9-*O*-(4-Methyl-1-piperazinycarbony)-DUMB1 (7c) 4-Nitrophenyl chloroformate (103 mg, 0.51 mmol) and triethylamine (0.071 ml, 0.51 mmol) were added to a stirred solution of **1b** (100 mg, 0.17 mmol) in dry methylene chloride (4 ml) at 0 °C, then the resulting mixture was stirred at the same temperature for 0.5 h. 1-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 **7c** as a pale yellow powder, mp 180—183 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.68 (3H, s, CH_3), 2.43 (3H, s, N–CH_3), 2.58 (4H, br s, N–$CH_2 \times 2$), 3.60 (2H, br s, N–CH_2), 3.80 (2H, br s, N–CH_2), 3.71 (1H, dd, J=19.8, 5.3 Hz, 4-H), 3.79 (3 H, s, OCH_3), 3.86 (1 H, dd, J=19.8, 5.9 Hz, 4-H), 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.32 (1H, dd, J=12.4, 1.6 Hz, 6-H), 4.49 (1H, dd, J=12.4, 6.5 Hz, 6-H), 4.54 (1H, m, 5-H), 5.44 (1H, s, NH), 6.61 (1H, d, J=2.3 Hz, 3′-H), 6. 78 (1H, s, 4′-H), 7.47 (1H, s, 8-H), 9.09 (1H, br s, NH). IR (KBr): 1715, 1623, 1506, 1388, 1312, 1245, 1161 cm^{-1}. SIMS m/z: 716 714 $(M+H)^+$, 234. *Anal.* Calcd for $C_{32}H_{36}BrN_5O_9 \cdot 1.0\ H_2O$: C, 52.47; H, 5.23; N, 9.56. Found: C, 52.22; H, 5.07; N, 9.37.

9-*O*-*tert*-Butyldimethylsilyl-3α-hydroxy-DUMB1 (8a) The procedures was the same as that of **4a** except **7a** was used instead (347 mg, 0.49 mmol). The crude product was purified by silica gel chromatography to afford 73 mg (21%) of **8a** as a white powder, mp 109—114 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: −0.51 (3H, s, CH_3), −0.23 (3H, s, CH_3), 0.89 (9H, s, $(CH_3)_3$), 1.61 (3H, s, CH_3), 1.74 (1H, br s, OH), 3.42 (1H, br d, J=3.4 Hz, 4-H), 3.44 (1H, br d, J=2.9 Hz, 4-H), 3.76 (3 H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.16 (1H, dd, J=13.9, 6.7 Hz, 6-H), 4.52 (3H, m, 3-H, 5-H, 6-H), 5.34 (1H, br s, NH), 6.35 (1H, d, J=2.1 Hz, 3′-H), 6.63 (1H, s, 4′-H), 6,67 (1H, s, 8-H), 9.00 (1H, br s, NH). IR (KBr): 2934, 2858, 1734, 1616, 1495, 1389, 1255, 1108, 1047, 838 cm^{-1}. SIMS m/z: 706 704 $(M+H)^+$, 472 470, 234. *Anal.* Calcd for $C_{32}H_{42}BrN_3O_8Si$: C, 54.54; H, 6.01; N, 5.96. Found: C, 54.85; H, 6.35; N, 5.52.

9-*O*-Dimethylcarbamoyl-3α-hydroxy-DUMB1 (8b) The procedure was the same as that of **4a** except **7b** was used insted (100 mg, 0.151 mmol). The crude product was purified by silica gel chromatography to afford 30.0 mg (30%) of **8b** as a white powder, mp 130—135 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.61 (3H, s, CH_3), 2.02 (1H, br s, OH), 2.96 (3H, s, N–CH_3), 3.02 (3H, s, N–CH_3), 3.49 (2H, br d, J=6.4 Hz, 4-H $\times$ 2), 3.78 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.18 (1H, dd, J=13.4, 7.2 Hz, 6-H), 4.50 (1H, m, 3-H), 4.55 (1H, dd, J=13.4, 4.1 Hz, 6-H), 4.82 (1H, m, 5-H), 5.40 (1H, br s, NH), 6.45 (1H, d, J=2.2 Hz, 3′-H), 6.75 (1H, s, 4′-H), 7.09 (1H, s, 8-H), 9.08 (1H, br s, NH). IR (KBr): 2940, 1714, 1621, 1492, 1385, 1309, 1245, 1218, 1169, 1110, 1044 cm^{-1}. SIMS m/z: 663 661 $(M+H)^+$, 429 427, 234. *Anal.* Calcd for $C_{29}H_{33}BrN_4O_9 \cdot 0.5\ H_2O$: C, 51.95; H, 5.11; N, 8.36. Found: C, 52.00; H, 5.11; N, 8.22.

9-*O*-(4-Methyl-1-piperazinycarbony)-3α-hydroxy-DUMB1 (8c) The procedure was the same as that of **4a** except for the use of **7c** (20 mg, 0.028 mmol). The crude product was purified by silica gel chromatography to afford 14.1 mg (70%) of 8c as a white powder, mp 192—194 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.61 (3H, s, CH_3), 2.31 (3H, s, N–CH_3), 2.41 (4H, br s, N–$CH_2 \times 2$), 3.48 (2H, m, 4-H $\times$ 2), 3.54 (2H, br s, N–CH_2), 3.61 (2H, br s, N–CH_2), 3.78 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.20 (1H, dd, J=12.2, 6.6 Hz, 6-H), 4.54 (2H, m, 3-H, 6-H), 4.81 (1H, m, 5-H), 5.38 (1H, s, NH), 6.47 (1H,

d, $J=2.0$ Hz, 3′-H), 6.75 (1H, s, 4′-H), 7.11 (1H, s, 8-H), 9.13 (1H, br s, NH). IR (KBr): 1716, 1497, 1255, 1238, 1221, 1153, 1049 cm^{-1}. SIMS *m/z*: 718 716 $(M+H)^+$, 234. *Anal.* Calcd for $C_{32}H_{38}BrN_5O_9 \cdot 0.5\ H_2O$: C, 52.97; H, 5.42; N, 9.65. Found: C, 52.90; H, 5.55; N, 9.66.

Methyl (8*S*)-8-Bromo-4-(*tert*-butyldimethylsilyloxy)-2-methyl-6-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-6,7,8,9-tetrahydro-3*H*-pyrrolo[3,2-*f*]-quinoline-1-carboxylate (9a) The procedure was the same as that of **5a** except **8a** was used instead (73 mg, 0.10 mmol). The crude product was purified by silica gel chromatography to afford 54 mg (79%) of **9a** as a white powder, mp 114—118 °C. ^{1}H-NMR: see Table 2. IR (KBr): 3468, 3306, 2936, 2860, 1703, 1615, 1586, 1528, 1496, 1443, 1311, 1256, 1214, 1124, 1088, 997 cm^{-1}. SIMS *m/z*: 688 686 $(M+H)^+$, 454 452, 234. *Anal.* Calcd for $C_{32}H_{40}BrN_3O_7Si$: C, 55.97; H, 5.87; N, 6.12. Found: C, 55.96; H, 5.88; N, 6.12.

Methyl (8*S*)-8-Bromo-4-(dimethylaminocarbonyloxy)-2-methyl-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 (10b) CSA (14 mg, 0.06 mmol) was added to a solution of **8b** (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 **10b** as a white powder, mp 172—177 °C (dec.). ^{1}H-NMR: see Table 2. IR (KBr): 3238, 2938, 1699, 1646, 1540, 1443, 1348, 1242, 1166, 1121, 1090 cm^{-1}. SIMS *m/z*: 645 643 $(M+H)^+$. *Anal.* Calcd for $C_{29}H_{31}BrN_4O_8 \cdot 1.0\ H_2O$: C, 52.66; H, 5.03; N, 8.47. Found: C, 53.01; H, 5.17; N, 8.17.

Methyl (8*S*)-8-Bromo-2methyl-4-[(4-methylpiperazinyl)carbonyloxy]-6-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-6,7,8,9-tetrahydro-3*H*-pyrrolo-[3,2-*f*]quinoline-1-carboxylate (9c) and Methyl (8*S*)-8-bromo-2-methyl-4-[(4-methylpiperazinyl)carbonyloxy]-5,6,6a,7,8,9-hexahydro-3H-pyrrolo-[3,2-*f*]-6a-azaacenaphthylene-6-one-5-spiro-2′-(5′,6′,7′-trimethoxy)-indoline-1-carboxylate (10c) CSA (65 mg, 0.282 mmol) was added to a solution of **8c** (68 mg, 0.094 mmol) in dry toluene (6 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 (30 : 1) to give 52 mg (79%) of **9c** as a white powder and 7 mg (11%) of **10c** as a white powder. For **9c** mp 192—194 °C. ^{1}H-NMR: see Table 2. IR (KBr): 1699, 1653, 1505, 1456, 1439, 1314, 1210, 1125, 1091 cm^{-1}. SIMS *m/z*: 700 698 $(M+H)^+$, 234. *Anal.* Calcd for $C_{32}H_{36}BrN_5O_8 \cdot 0.5\ H_2O$: C, 54.32; H, 5.27; N, 9.90. Found: C, 54.50; H, 5.33; N, 9.66. For **10c** mp 198—203 °C (dec.). ^{1}H-NMR: see Table 2. IR (KBr): 1700, 1647, 1457, 1237, 1122, 1092 cm^{-1}. EIMS *m/z*: 699 697 $(M)^+$. *Anal.* Calcd for $C_{32}H_{36}BrN_5O_8 \cdot 2.0\ H_2O$: C, 52.32; H, 5.49; N, 9.53. Found: C, 52.47; H, 5.38; N, 9.03.

Biological Studies HeLa S_3 cells (5×10^4 were seeded in Eagle's minimum essential medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10 % fetal bovine serum (Grand Island Biological Co.) and 0.06 mg/ml of kanamycin. Graded concentrations of drugs, appropriately diluted with growth medium, were added 24 h after the cells were seeded. The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. After 72 h of drug exposure, the monolayer cells were washed with a phosphate-buffered salt solution (Flow Laboratories) and incubated with 0.05% trypsin (Difco Laboratories, Detroit, MI) and 0.02% EDTA (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan). The cells were counted with a Toa Micro-Cell counter (Toa Medical Electronics Co., Ltd., Kobe, Japan) and the IC_{50} value (drug concentration required for 50 % inhibition of the cell growth) was determined.

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References and Notes

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13) The reduction of **3a** was carried out in methanol to afford a lot of over reductive compounds. When allyl alcohol was used as a reaction solvent, the desirable hydroxy compounds at the C-3 position were predominantly obtained.
14) Details of the synthetic study of KW-2189 will be published elsewhere by M. Kinugawa *et al.*
15) Treatment of **4a** with CSA in toluene at 50 °C afforded **5a** in 65% yield accompanied with **2a** in 30% yield. It is probably considered that the silyl moiety of **5a** was removed under acidic conditions to produce **2a** during the purification process. Therefore, it is indicated that a 1, 2-shift of the methoxycarbonyl group has proceeded quantitatively.