

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 3 α -hydroxy compound (**4a**) in 74% yield, as shown in Chart 2.¹³⁾ The configuration at the C₃ 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-3 α -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 (⁸¹Br), confirming the dehydration of **4a–c**, respectively. The ¹H-NMR spectra of **5a–c** showed downfield shifts of the C₂-methyl proton signal (about +1.0 ppm) and of the N₁-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 C₂-methyl group and the NH proton in the NMR spectra, but NOEs from the C₂-methyl group to 4-H or 9-H₂ were not observed.⁹⁾ As a result, a 1,2-shift of the methoxycarbonyl group from C₂ to C₃ 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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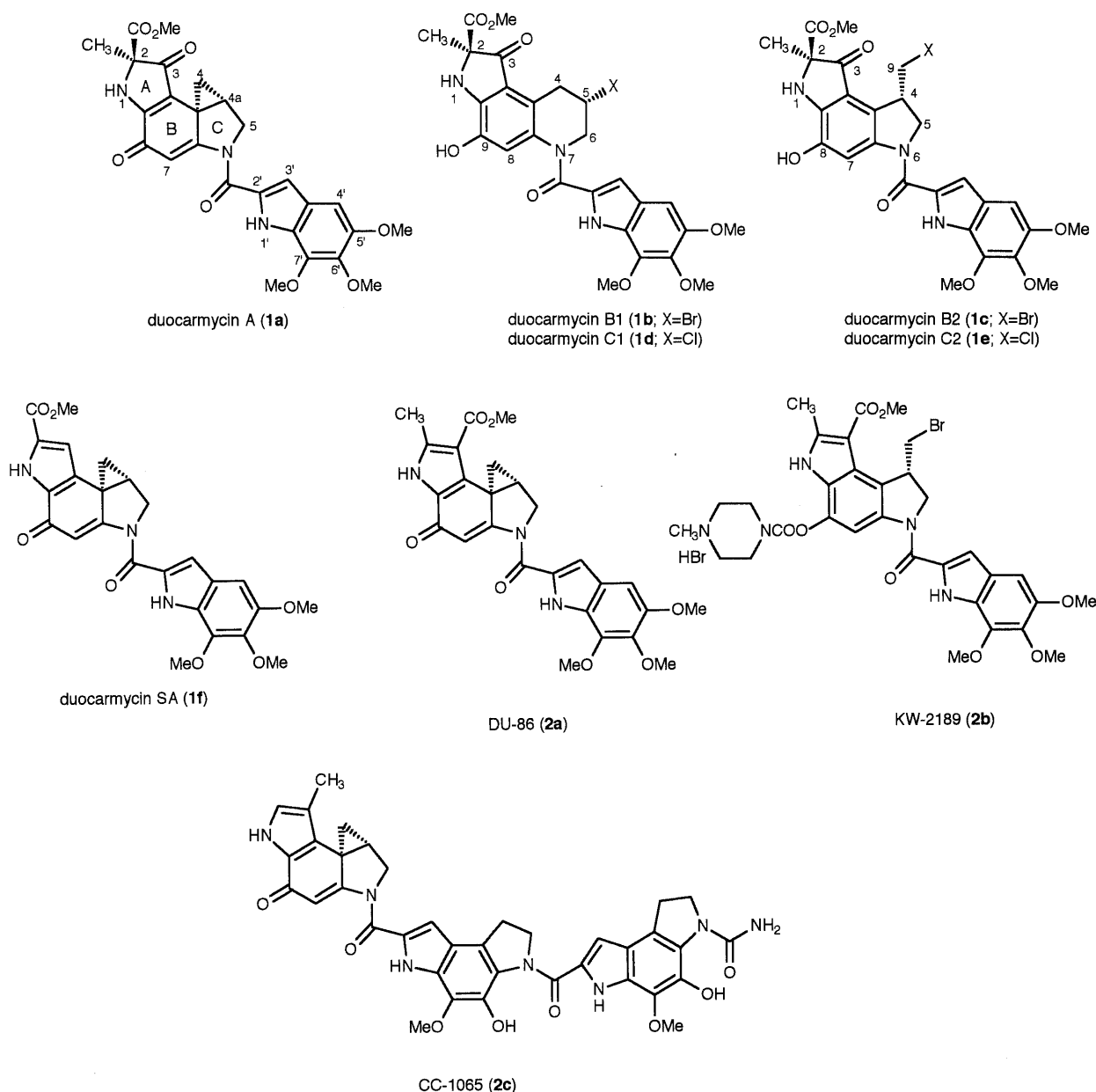


Chart 1

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 NaBH₄ in allyl alcohol to afford the corresponding 3 α -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 (C₁₂H₁₂NO₄; 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%,

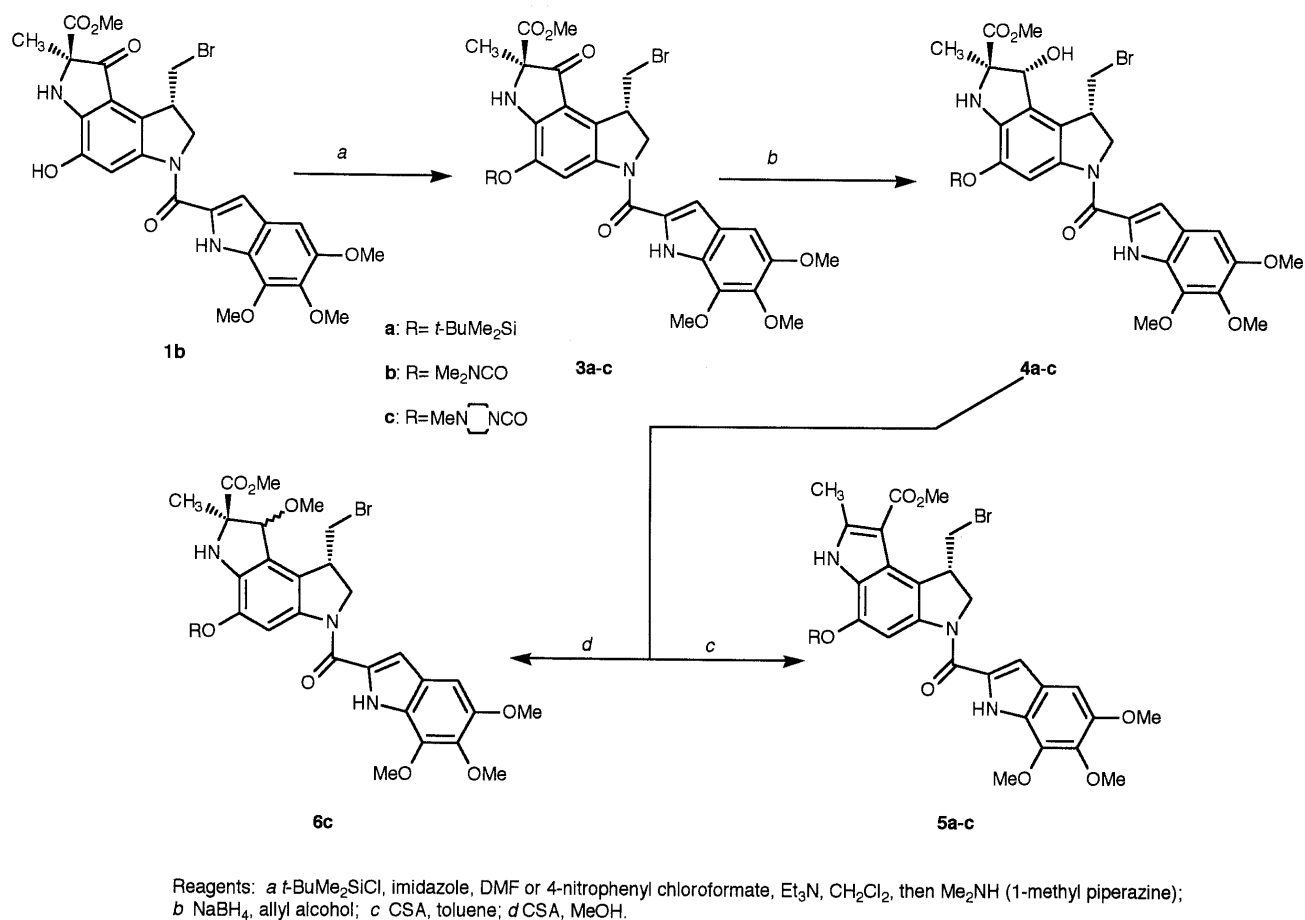
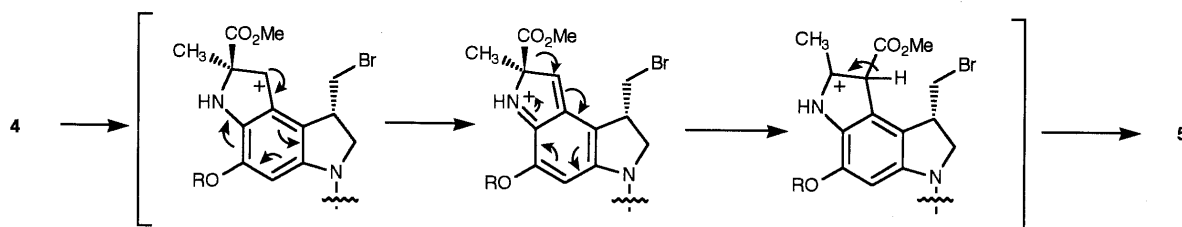


Chart 2

Fig. 1. Proposed Mechanism of Wagner-Meerwein Rearrangement for Production of **5**Table 1. The Acid-Catalyzed Rearrangement from **4a** to **5a**

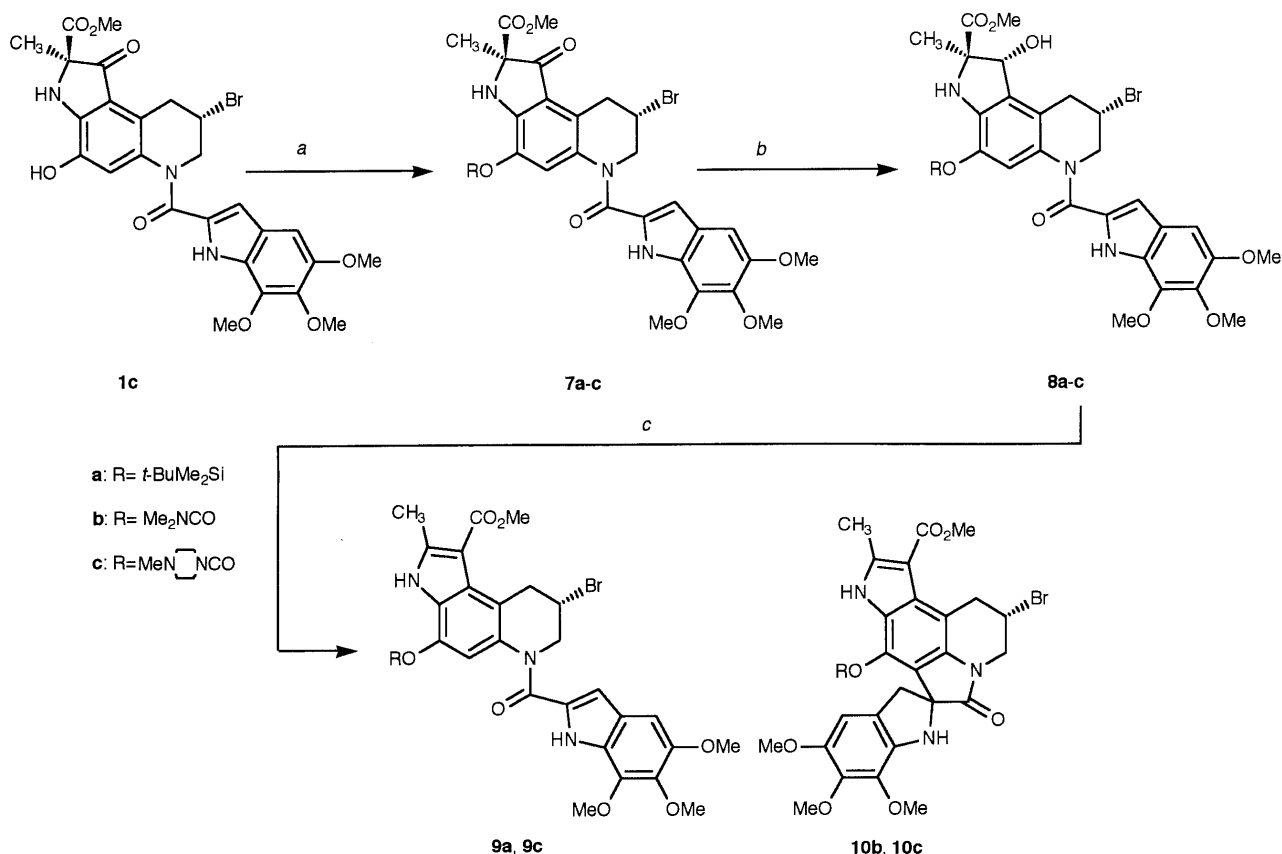
Entry	Acid	Solvent	Time (h)	Temp. (°C)	Isolated yield (%)
1	CSA	Toluene	6	25	26
2	CSA	Toluene	1	50	65
3	MsOH	CHCl_3	6	25	29
4	$p\text{-TsOH}$	CHCl_3	6	25	23
5	PPA ^{a)}	CHCl_3	6	25	14
6	AlCl_3	CHCl_3	2	25	86
7	$\text{BF}_3 \cdot \text{OEt}_2$	CHCl_3	4	25	80
8	ZnCl_2	CHCl_3	6	25	29
9	SnCl_4	CHCl_3	6	25	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 S₃ 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 $t\text{-BuMe}_2\text{SiCl}$, 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. ^1H -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 ₂ CH 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; ^1H -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 F₂₅₄

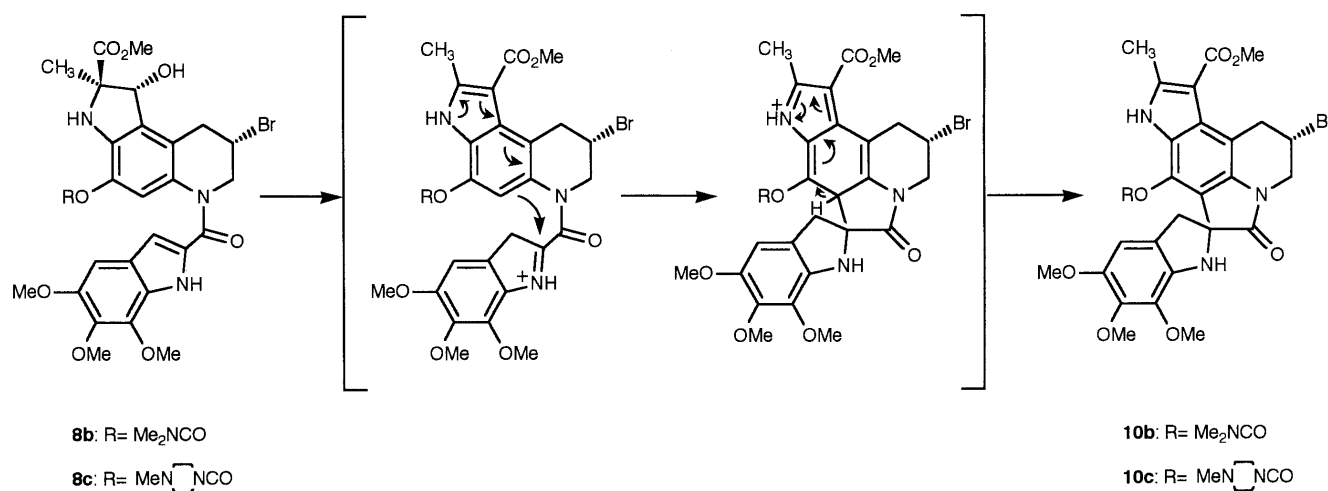


Fig. 2. Proposed Mechanism for Production of 10b and 10c

Table 3. Anticellular Activities against HeLa S₃ 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 NaHCO₃ and brine. The organic layer was dried over Na₂SO₄ 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.). ¹H-NMR (400 MHz CDCl₃) δ: 0.35 (3H, s, CH₃), 0.36 (3H, s, CH₃), 1.06 (9H, s, C(CH₃)₃), 1.69 (3H, s, CH₃), 3.57 (1H, dd, *J* = 10.3, 9.1 Hz, 9-H), 3.78 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 4.06 (1H, dd, *J* = 10.3, 3.0 Hz, 9-H), 4.06 (3H, s, OCH₃), 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, brs, 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, brs, NH). IR (KBr): 1745, 1700, 1618, 1497, 1293, 837 cm⁻¹. SIMS *m/z*: 704 702 (M + H)⁺, 470 468, 234. Anal. Calcd for C₃₂H₄₀BrN₃O₈Si: C, 54.70; H, 5.74; N, 5.98. Found: C, 54.82; H, 5.93; N, 5.75.

8-*O*-*tert*-Butyldimethylsilyl-3 α -hydroxy-DUMB2 (4a) NaBH₄ (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 CHCl₃. The combined extracts were washed with aqueous NaHCO₃ and brine. The organic layer was dried over Na₂SO₄ 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. ¹H-NMR (400 MHz, CDCl₃) δ: 0.30 (3H, s, CH₃), 0.32 (3H, s, CH₃), 1.04 (9H, s, C(CH₃)₃), 1.60 (3H, s, CH₃), 2.09 (1H, brs, OH), 3.49 (1H, dd, *J* = 10.3, 9.8 Hz, 9-H), 3.72 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 3.92 (1H, m, 4-H), 3.93 (3H, s, OCH₃), 4.05 (3H, s, OCH₃), 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, brs, 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, brs, NH). IR (KBr): 3406, 1734, 1621, 1485, 1111, 838 cm⁻¹.

SIMS *m/z*: 706 704 (M + H)⁺, 234. Anal. Calcd for C₃₂H₄₂BrN₃O₈Si: C, 54.54; H, 6.01; N, 5.96. Found: C, 54.47; H, 6.19; N, 5.71.

8-*O*-Dimethylcarbamoyl-3 α -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. ¹H-NMR (400 MHz, CDCl₃) δ: 1.66 (3H, s, CH₃), 1.70 (1H, brs, OH), 3.04 (3H, s, N-CH₃), 3.13 (3H, s, N-CH₃), 3.49 (2H, dd, *J* = 10.2, 10.2 Hz, 9-H × 2), 3.77 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 3.95 (1H, m, 4-H), 3.94 (3H, s, OCH₃), 4.08 (3H, s, OCH₃), 4.13 (1H, dd, *J* = 10.1, 3.2 Hz, 5-H), 4.58 (2H, m, 3-H, 5-H), 5.41 (1H, brs, 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, brs, 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 C₂₉H₃₃BrN₄O₉: C, 52.66; H, 5.03; N, 8.47. Found: C, 52.55; H, 4.95; N, 8.44.

8-*O*-(4-Methyl-1-piperazincarbonyl)-3 α -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.). ¹H-NMR (400 MHz, DMSO-*d*₆) δ: 1.41 (3H, s, CH₃), 2.24 (3H, s, N-CH₃), 2.49 (4H, brs, N-CH₂), 3.42 (1H, m, OH), 3.45 (2H, brs, N-CH₂), 3.60 (2H, brs, N-CH₂), 3.64 (3H, s, OCH₃), 3.65 (1H, m, 4-H), 3.79 (3H, s, OCH₃), 3.81 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 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, brd, *J* = 7.8 Hz, 5-H), 5.89 (1H, s, NH), 5.96 (1H, brd, *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, brs, 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 C₃₂H₃₈BrN₅O₉ · 2.0 H₂O: 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 NaHCO₃ and the whole was extracted with EtOAc. The extract was washed with brine. The organic layer was dried over Na₂SO₄ 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. ¹H-NMR (400 MHz, CDCl₃) δ: 0.37 (3H, s, CH₃), 0.39 (3H, s, CH₃), 1.07 (9H, s, C(CH₃)₃), 2.76 (3H, s, CH₃), 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, OCH₃), 3.95 (3H, s, OCH₃), 3.98 (3H, s, OCH₃), 4.07 (3H, s, OCH₃), 4.52 (1H, m, 4-H), 4.54 (1H, brd, *J* = 8.5 Hz, 5-H), 4.73 (1H, brd, *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, brs, NH), 9.40 (1H, brs, 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 C₃₂H₄₀BrN₃O₇Si: 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. ¹H-NMR (400 MHz, CDCl₃) δ: 2.59 (3H, s, CH₃), 3.07 (3H, s, N-CH₃), 3.20 (3H, s, N-CH₃), 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, OCH₃), 3.95 (3H, s, OCH₃), 3.97 (3H, s, OCH₃), 4.08 (3H, s, OCH₃), 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, brs, NH), 9.37 (1H, brs, 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 C₂₉H₃₁BrN₄O₈·1.5 CH₃OH: C, 52.97; H, 5.39; N, 8.10. Found: C, 53.15; H, 5.46; N, 7.70.

Methyl (1S)-1-Bromomethyl-7-methyl-5-(4-methylpiperazinylcarbonyloxy)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3H-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. ¹H-NMR (400 MHz, CDCl₃) δ: 2.37 (3H, s, N-CH₃), 2.50 (4H, brs, N-CH₂ × 2), 2.70 (3H, s, CH₃), 3.23 (1H, dd, *J* = 10.0, 10.0 Hz, 9-H), 3.64 (2H, brs, N-CH₂), 3.78 (2H, brs, N-CH₂), 3.82 (1H, dd, *J* = 10.0, 2.2 Hz, 9-H), 3.92 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 3.97 (3H, s, OCH₃), 4.08 (3H, s, OCH₃), 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, brs, NH), 9.34 (1H, brs, 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 C₃₂H₃₆BrN₅O₈·1.0 H₂O: C, 53.64; H, 5.34; N, 9.77. Found: C, 53.31; H, 5.30; N, 9.45.

Methyl (1S)-1-Bromomethyl-8-methoxy-7-methyl-5-(4-methylpiperazinylcarbonyloxy)-3-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-1,2-dihydro-3H-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 NaHCO₃ and the whole was extracted with EtOAc. The extract was washed with brine. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was chromatographed on silica gel with CHCl₃-MeOH (30:1) to give 30.2 mg (59%) of **6c** as a white powder, mp 122–125 °C. ¹H-NMR (400 MHz, DMSO-*d*₆) δ: 1.41 and 1.42 (3H, s, CH₃), 2.24 and 2.25 (3H, s, N-CH₃), 2.49–2.52 (4H, brs, N-CH₂ × 2), 3.17 and 3.18 (3H, s, OCH₃), 3.45–3.47 (2H, brs, N-CH₂), 3.60–3.63 (2H, brs, N-CH₂), 3.64 (3H, s, OCH₃), 3.65 (1H, m, 4-H), 3.79 and 3.80 (3H, s, OCH₃), 3.81 and 3.83 (3H, s, OCH₃), 3.95 and 3.97 (3H, s, OCH₃), 4.12–4.32 (3H, m, 3-H, 9-H × 2), 5.24–5.30 (2H, m, 5-H × 2), 5.82 (1H, brs, 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, brs, NH). IR (KBr): 1700, 1623, 1438, 1321, 1237, 1151 cm⁻¹. SIMS *m/z*: 732 730 (M+H)⁺, 234. *Anal.* Calcd for C₃₃H₄₀BrN₅O₉·1.0 CH₃OH: 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 NaHCO₃ and brine. The organic layer was dried over Na₂SO₄ 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. ¹H-NMR (400 MHz, CDCl₃) δ: 0.15 (6H, s, CH₃ × 2), 0.91 (9H, s, (CH₃)₃), 1.69 (3H, s, CH₃), 3.65 (1H, dd, *J* = 19.3, 5.2 Hz, 4-H), 3.78 (3H, s, OCH₃), 3.83 (1H, dd, *J* = 19.3, 6.2 Hz, 4-H), 3.85 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 4.06 (3H, s, OCH₃), 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 C₃₂H₄₀BrN₃O₈Si·2.0 H₂O: 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 CHCl₃ and washed with aqueous NaHCO₃ and brine. The organic layer was dried and concentrated under reduced pressure. The residue was chromatographed on silica gel with CHCl₃-MeOH (50:1) to give 55 mg (98%) of **7b** as a light-tan powder, mp 145–150 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 1.68 (3H, s, CH₃), 3.00 (3H, s, N-CH₃), 3.07 (3H, s, N-CH₃), 3.71 (1H, dd, *J* = 19.4, 5.3 Hz, 4-H), 3.79 (3H, s, OCH₃), 3.88 (1H, dd, *J* = 19.4, 6.0 Hz, 4-H), 3.88 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 4.08 (3H, s, OCH₃), 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, brs, 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 C₂₉H₃₁BrN₄O₈·1.5 H₂O: C, 50.74; H, 4.99; N, 8.16. Found: C, 50.65; H, 4.66; N, 7.77.

9-O-(4-Methyl-1-piperazinylcarbonyl)-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 CHCl₃. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated on a rotary evaporator. The residue was chromatographed on silica gel with CHCl₃-MeOH (50:1) to give 96 mg (79%) of **7c** as a pale yellow powder, mp 180–183 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 1.68 (3H, s, CH₃), 2.43 (3H, s, N-CH₃), 2.58 (4H, brs, N-CH₂ × 2), 3.60 (2H, brs, N-CH₂), 3.80 (2H, brs, N-CH₂), 3.71 (1H, dd, *J* = 19.8, 5.3 Hz, 4-H), 3.79 (3H, s, OCH₃), 3.86 (1H, dd, *J* = 19.8, 5.9 Hz, 4-H), 3.88 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 4.08 (3H, s, OCH₃), 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, brs, NH). IR (KBr): 1715, 1623, 1506, 1388, 1312, 1245, 1161 cm⁻¹. SIMS *m/z*: 716 714 (M+H)⁺, 234. *Anal.* Calcd for C₃₂H₃₆BrN₅O₉·1.0 H₂O: C, 52.47; H, 5.23; N, 9.56. Found: C, 52.22; H, 5.07; N, 9.37.

9-O-tert-Butyldimethylsilyl-3 α -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. ¹H-NMR (400 MHz, CDCl₃) δ: -0.51 (3H, s, CH₃), -0.23 (3H, s, CH₃), 0.89 (9H, s, (CH₃)₃), 1.61 (3H, s, CH₃), 1.74 (1H, brs, 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 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.05 (3H, s, OCH₃), 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, brs, 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, brs, 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 C₃₂H₄₂BrN₃O₈Si: C, 54.54; H, 6.01; N, 5.96. Found: C, 54.85; H, 6.35; N, 5.52.

9-O-Dimethylcarbamoyl-3 α -hydroxy-DUMB1 (8b) The procedure was the same as that of **4a** except **7b** was used instead (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. ¹H-NMR (400 MHz, CDCl₃) δ: 1.61 (3H, s, CH₃), 2.02 (1H, brs, OH), 2.96 (3H, s, N-CH₃), 3.02 (3H, s, N-CH₃), 3.49 (2H, br d, *J* = 6.4 Hz, 4-H × 2), 3.78 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 4.06 (3H, s, OCH₃), 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, brs, 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, brs, 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 C₂₉H₃₃BrN₄O₈·0.5 H₂O: C, 51.95; H, 5.11; N, 8.36. Found: C, 52.00; H, 5.11; N, 8.22.

9-O-(4-Methyl-1-piperazinylcarbonyl)-3 α -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. ¹H-NMR (400 MHz, CDCl₃) δ: 1.61 (3H, s, CH₃), 2.31 (3H, s, N-CH₃), 2.41 (4H, brs, N-CH₂ × 2), 3.48 (2H, m, 4-H × 2), 3.54 (2H, brs, N-CH₂), 3.61 (2H, brs, N-CH₂), 3.78 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 4.06 (3H, s, OCH₃), 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 (8S)-8-Bromo-4-(*tert*-butyldimethylsilyloxy)-2-methyl-6-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-6,7,8,9-tetrahydro-3H-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. ¹H-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 $C_{32}H_{40}BrN_5O_9 \cdot Si$: C, 55.97; H, 5.87; N, 6.12. Found: C, 55.96; H, 5.88; N, 6.12.

Methyl (8S)-8-Bromo-4-(dimethylaminocarbonyloxy)-2-methyl-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 (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.). ¹H-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 (8S)-8-Bromo-2-methyl-4-[(4-methylpiperazinyl)carbonyloxy]-6-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-6,7,8,9-tetrahydro-3H-pyrrolo[3,2-*f*]-quinoline-1-carboxylate (9c) and Methyl (8S)-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. ¹H-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.). ¹H-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 S₃ 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 IC₅₀ value (drug concentration required for 50 % inhibition of the cell growth) was determined.

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References and Notes

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- 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.
- Details of the synthetic study of KW-2189 will be published elsewhere by M. Kinugawa *et al.*
- 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.