MITOMYCIN DERIVATIVES HAVING UNIQUE CONDENSED-RING STRUCTURES

THEIR SYNTHESIS AND ANTITUMOR ACTIVITY

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A series of mitomycin derivatives **1**～**3** having unique condensed-ring structures was synthesized and evaluated for their anticellular and antitumor activity. These compounds were synthesized by the Michael addition of 1,3-dicarbonyl compounds to 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylenemitosanes (**4**～**6**, and **14**) and the subsequent cyclization. For the preparation of **1**, the allyloxycarbonyl (Aloc) group was employable for the protection of the aziridine (1a-*N*-H), since the deprotection proceeded without decomposition of the substrates under the mild conditions with Pd(0) and HCO_2H-NEt_3. Among these structurally unique derivatives, compounds **1a**, **1b**, **1d** and **1e** were quite potent against HeLa S_3 human tumor cells and sarcoma 180 solid tumor in mice.

Mitomycins are well known to be potent antitumor antibiotics produced by various *Streptomyces cultures*.[1～3] Among these compounds, mitomycin C (MMC) has been extensively used in cancer chemotherapy against a variety of solid tumors. However, its use is limited by detrimental side effects such as severe bone marrow suppression and gastrointestinal damage. Consequently, about a thousand derivatives have been synthesized to overcome these disadvantages.[4～6] During the studies of their synthesis and evaluation, several physicochemical factors, *e.g.*, quinone reduction potential, lipophilicity, and the steric influence of the substituents, were found to correlate to biological activity.[7～9] Considering the structure-activity relationship among the derivatives modified at the C-7 and N-1a positions, we have tried to synthesize an alternative series of the derivatives by modification at the C-6-*methyl* position.[10～12] The C-6-*methyl* position is suitable to install *additional* functions because the methyl group does not play an decisive role in the activation processes of mitomycins.[1～3] In the course of our studies, we found that the addition of anionic species of 1,3-dicarbonyl compounds to 6-methylene intermediates **4**～**6**, and **14** afforded mitomycin derivatives **1**～**3** having a unique condensed-ring structure that have not been reported to date. In this paper, we describe the synthesis of these derivatives and their antitumor activity.

Results

6-Methylene intermediate **4**, a key intermediate of the derivatives, was prepared from mitomycin A (MMA).[10] Compound **5** having the allyloxycarbonyl (Aloc) group for protection of the aziridine was prepared according to a similar method[10] from 1a-(allyloxycarbonyl)-7,7-(ethylenedioxy)-6,7-dihydromito-

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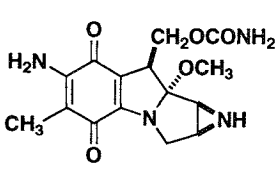
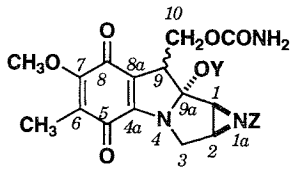
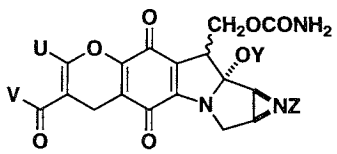
Fig. 1. Structure of mitomycins and their derivatives.



Mitomycin C



Mitomycin A (MMA): Y = CH_3, Z = H, 9-β
Mitomycin B (MMB): Y = H, Z = CH_3, 9-α
Mitomycin F (MMF): Y = Z = CH_3, 9-β



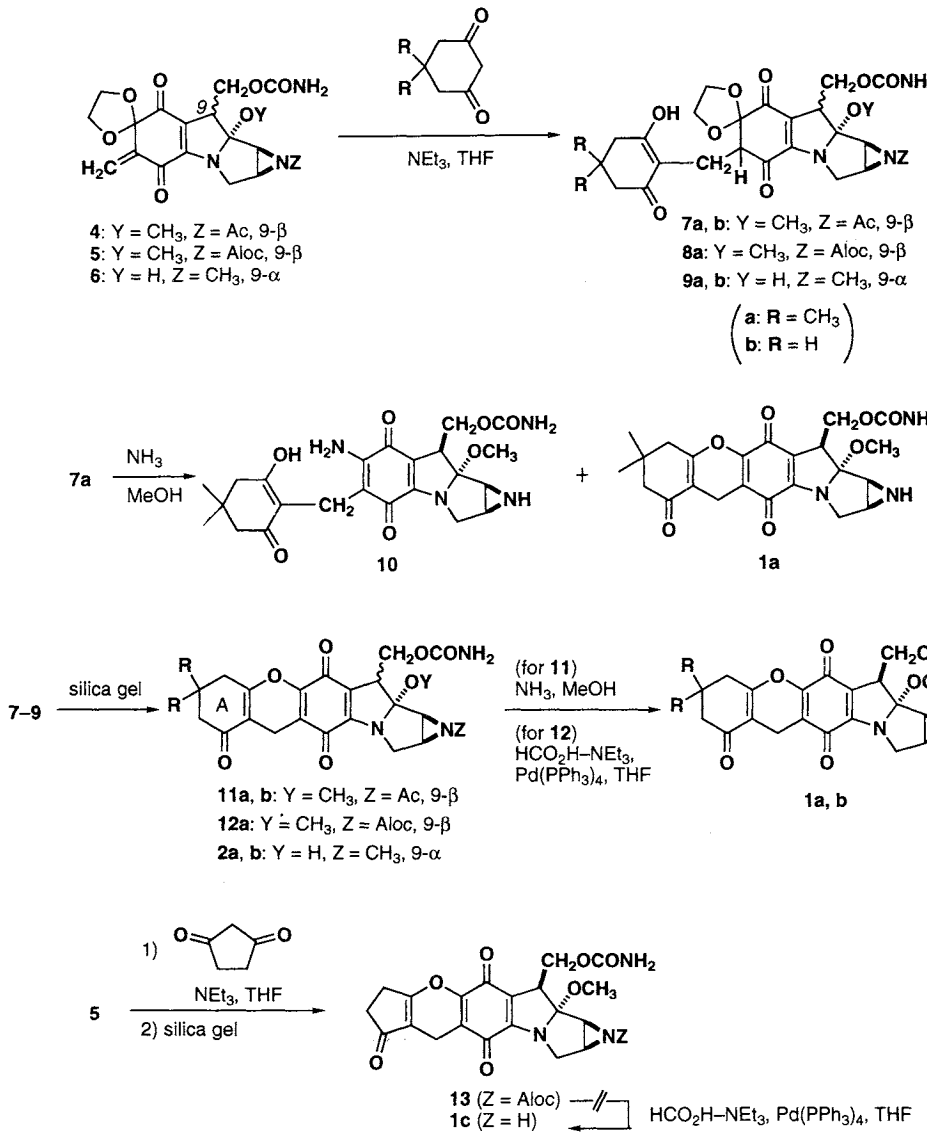
1: MMA type (U, V = Alkyl, Aryl, Alkoxy, Y = CH_3, Z = H, 9-β)
2: MMB type (U, V = Alkyl, Aryl, Alkoxy, Y = H, Z = CH_3, 9-α)
3: MMF type (U, V = Alkyl, Aryl, Alkoxy, Y = Z = CH_3, 9-β)

mycin A.[13] Compound **6** having the mitomycin B (MMB) skeleton were also prepared from MMB.[12] As shown in Scheme 1, compounds **4**~**6** were reacted with dimedone in the presence of NEt_3 at room temperature and afforded the crude Michael adducts **7a**~**9a** in good yields, respectively. Interestingly, treatment of crude **7a** with NH_3 in MeOH[10] afforded **10** (17%) and a small amount of an unexpected product **1a** (4.4%) having a unique condensed-ring structure. From the finding that the adduct **7a** was slowly converted into **11a** on the silica gel TLC plate, intramolecular condensation of the Michael adducts could be performed by using silica gel as a weak acid catalyst. As a result, condensation into **11a**, **12a**, and **2a** was accomplished almost quantitatively by adsorption of **7a**~**9a** onto silica gel, respectively. The removal of the 1a-acetyl group by treatment of **7a** with K_2CO_3 in MeOH, followed by cyclization into **1a** on silica gel was effective (41%),† whereas the Aloc group at the 1a position of **12a** was removed cleanly by a catalytic amount of Pd(0) in the presence of HCO_2H-NEt_3,[13,14] and afforded **1a** in 52% yield. The Michael addition of cyclohexane-1,3-dione to **4** and **6** also proceeded, and after the subsequent conversions, compounds **1b** and **2b** were obtained, respectively. However, compound **1c**, an adduct of **5** and cyclopentane-1,3-dione, was not obtained by the deprotection of **13** due to the decomposition of **13** and **1c**. The decomposition would occur with NEt_3 formed during the reaction since compounds having the condensed-ring structure, especially **13** and **1c**, were unstable in the presence of NEt_3.

We next tried to react **4** and **5** with acyclic 1,3-dicarbonyl compounds (Scheme 2). Michael adducts **15** and **16** were obtained by similar reactions using NEt_3 as a base, but cyclization to **18** and **19** having the condensed-ring structure failed and the substrate was decomposed or recovered even though various reaction conditions were tried. Only in the case of the reaction using silica gel in refluxing MeOH, a trace amount of the cyclization product was obtained. On the other hand, when sodium salts of the 1,3-dicarbonyl compounds were reacted with **4** and **5**, compounds **18** and **19** were formed directly without detection of **15** and **16**. Thus, **1d**~**1f** having the MMA skeleton were prepared from **18d** and **19e**, **19f**, respectively, by the removal of the 1a-protective groups. The 1a-acetyl group of **18d** was also removed by treatment with

† Treatment of **11a** with K_2CO_3 in MeOH caused mainly decomposition of the substrate.

Scheme 1.



NH_4OAc in MeOH to avoid the basic conditions and **1d** was obtained in 41% yield. In the synthesis of **1e**, a small amount of **1f**, a regioisomer of **1e**, was obtained as a byproduct (**1e** : **1f** = *ca.* 5 : 1). The structure of these compounds were confirmed by ^1H NMR and FAB-MS. In particular, compound **1e** showed the fragment peak ($m/z = 105$) of the benzoyl cation, whereas compound **1f** did not show that peak in the FAB-MS analysis. A similar two step conversion of **14**[12] prepared from mitomycin F (MMF) into **3d**, **3e**, and **3g** was also achieved. In contrast to the synthesis of **1f**, compound **3e** was isolated as a sole isomer.

These results are summarized in Table 1.

Compounds **1**~**3** were tested for cytotoxicity against HeLa S_3 cells and antitumor activity against sarcoma 180 solid tumor in mice. As shown in Table 2, all compounds except **2a** showed potent growth-inhibitory activity against HeLa S_3 cells. Among them, compounds **1a**, **1b**, **1d** and **1e** showed

Scheme 2.

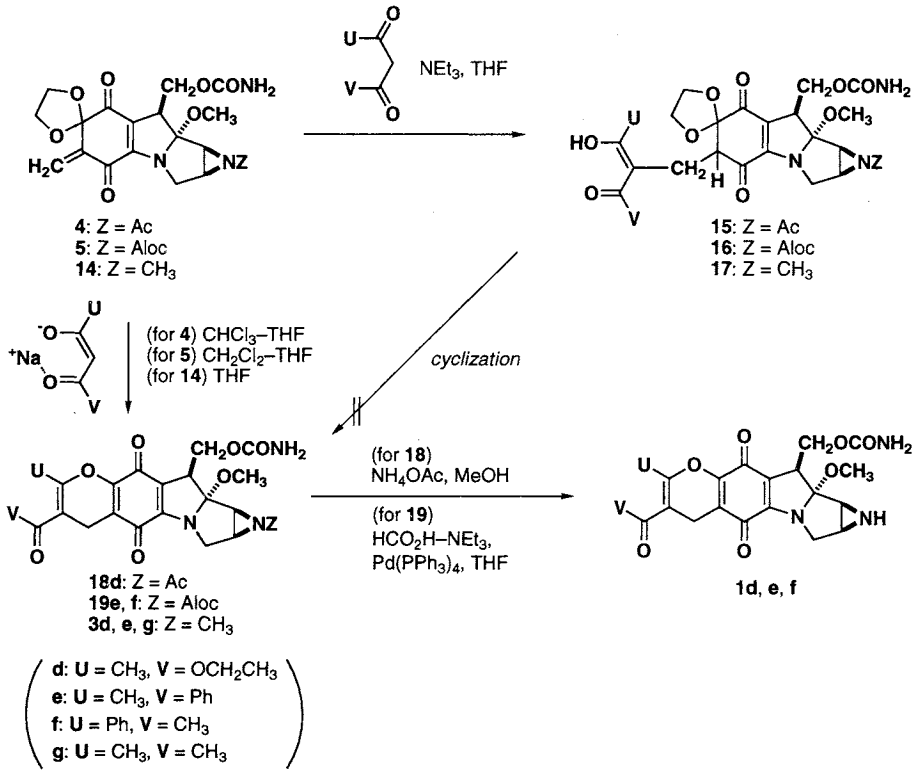


Table 1. Preparations of mitomycin derivatives.

Compound No.	U	V	MM-Skeleton	Substrate	Method[a]		Yield (%)[b]	
					Step 1	Step 2	Step 1	Step 2
1a	$CH_2C(CH_3)_2CH_2$		MMA	**5**	A	A	53	52
1b	$(CH_2)_3$		MMA	**4**	A	B	63	14
1d	CH_3	OCH_2CH_3	MMA	**4**	B	C	42[c]	41
1e	CH_3	Ph	MMA	**5**	B	A	29[c,d]	62[d]
1f	Ph	CH_3	MMA	**5**	B	A	29[c,d]	62[d]
2a	$CH_2C(CH_3)_2CH_2$		MMB	**6**	A	—	31[c]	—
2b	$(CH_2)_3$		MMB	**6**	A	—	25[c]	—
3d	CH_3	OCH_2CH_3	MMF	**14**	B	—	13	—
3e	CH_3	Ph	MMF	**14**	B	—	18	—
3g	CH_3	CH_3	MMF	**14**	B	—	7	—

[a] Reaction conditions. Step 1 (1,4-addition + cyclization); Method A: 1) $UCOCH_2COV$, NEt_3, 2) silica gel, $CHCl_3$; Method B: $UCOCH_2COV$ + NaH. Step 2 (deprotection); Method A: HCO_2H-NEt_3, $Pd(PPh_3)_4$, THF; Method B: NH_3, MeOH; Method C: NH_4OAc, MeOH.

[b] Yield based on the enone used.

[c] Yield based on the corresponding 7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitosanes.[12]

[d] Combined yield of **1e** and **1f**.

significantly stronger activity than MMC. Compounds **1** also showed superior antitumor activity against sarcoma 180 (T/C) *in vivo* at the lower doses, but other compounds (**2** and **3**) were ineffective. These results suggested that the MMA skeleton is necessary to exhibit antitumor activity within the range of compounds studied.

Table 2. Antitumor activities of mitomycin derivatives.

Compound No.	HeLa S_3[a]	Sarcoma-180 (sc-iv)[b]			Compound No.	HeLa S_3[a]	Sarcoma-180 (sc-iv)[b]		
	IC_{50} (μM)	ED_{50} (mg/kg)	OD[c] (mg/kg)	T/C[d] (minimum)		IC_{50} (μM)	ED_{50} (mg/kg)	OD[c] (mg/kg)	T/C[d] (minimum)
1a	0.084	1.3	2.7	0.20	**3d**	0.10	—	9.0	0.55
1b	0.065	0.84	1.8	0.23	**3e**	0.31	—	9.0	0.71
1d	0.016	0.70	2.5	0.26	**3g**	0.36	nt[e]	nt	nt
1e	0.086	5.8	9.0	0.19	**10**	1.6	—	50	0.53
2a	>10	—	14	0.53	MMC	0.59~1.5	2.2~5.0	6.0	0.27~0.43
2b	1.0	—	30	0.52	MMC	0.0024	1.3	1.8	0.20

[a] *In vitro* anticellular activity against HeLa S_3 cells. The cells were cultured in 96-well plates on day 0 and treated with compounds for 1 hour on day 1. The cytotoxicity was determined according to the method described previously (see ref 6).

[b] *In vivo* antitumor activity against sarcoma 180. Sarcoma 180 cells were inoculated sc into the axillary region of ddY mice on day 0. Compounds were administered iv on day 1.

[c] Optimal dose.

[d] Minimum treated *versus* control value of tumor volume. Tumor volume was calculated according to the method described previously (see ref 6).

[e] Not tested.

Several 7-methoxymitosanes have generally higher *in vitro* activity than the corresponding 7-aminomitosane (*e.g.*, MMA *versus* MMC, or MMF *versus* porfiromycin), therefore the substituent effect represented by their electronic effect at the C-7 of the derivatives is important for antitumor activity.[15] On the other hand, the substituent effect at the C-7[16] and C-6[17] positions should be accounted for in terms of steric allowance or lipophilicity as well as their electronic effect. Considering these findings, the excellent *in vitro* and *in vivo* antitumor activity of **1** seems to be attributable to structural features, *e.g.*, the condensed-ring structure, an appropriate lipophilicity, and the presence of an alkoxy function at "the C-7 position" of the mitomycin skeleton.

In conclusion, a series of mitomycin derivatives having unique condensed-ring structures was synthesized and evaluated for their antitumor activity *in vitro* and *in vivo*. Many compounds having the MMA skeleton showed excellent *in vitro* and *in vivo* activity and are interesting with respect to the development of new mitomycin derivatives.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers, except for mitomycins, and were used without purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use. Chromatography and some reactions were performed using Merck 60 70~230 mesh silica gel. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AM 400 and a JEOL JNM-GX270 instruments. Mass spectral (MS) data were obtained from a Hitachi M-80B and a JEOL JMS-D300 mass spectrometers. Infrared spectra (IR) were recorded on a Nihon Bunko IR-810 instrument. Elemental analyses were performed by a Perkin-Elmer 2400 C, H, N analyzer. The purity of the samples was checked by chromatographic methods (HPLC and TLC) and by careful analysis of NMR spectra.

Preparation of 1a-(Allyloxycarbonyl)-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)-mitomycin A

To a solution of 1a-(allyloxycarbonyl)-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydromitomycin A[13] (2.31 g, 4.98 mmol) in MeCN (50 ml) and NEt_3 (2.0 ml) was added dropwise a solution of PhSeBr (1.77 g, 7.48 mmol) in MeCN (20 ml) at 0°C over a period of 10 minutes. After stirring for 30 minutes at 0°C and

for an additional 40 minutes at room temperature, the reaction mixture was poured into an aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was washed successively with an aqueous NH_4Cl solution and brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 40:1~30:1 $CHCl_3$-MeOH as eluents), followed by trituration with $CHCl_3$-*n*-hexane and drying under vacuum to afford the desired product (2.48 g, 81%) as a yellow powder. The product was obtained as a mixture of two diastereomers at C-6 (2.3:1): ^1H NMR (270 MHz, $CDCl_3$) (major isomer) δ 1.55 (3H, s, 6-CH_3), 3.17 (3H, s, 9a-OCH_3), 3.31 (1H, br d, J=5 Hz, 2-H), 3.34 (1H, dd, J=2.0, 13 Hz, 3α-H), 3.45 (1H, d, J=4.5 Hz, 1-H), 3.67 (1H, d, J=12.9 Hz, 3β-H), 3.71 (1H, dd, J=4.8, 10.9 Hz, 9-H), 4.42 (1H, t, J=10.9 Hz, 10-H_a), 4.0~4.7 (6H, m, 1a-CO_2CH_2 and OCH_2CH_2O), 4.79 (2H, br s, 10-$OCONH_2$), 4.98 (1H, dd, J=4.8, 10.9 Hz, 10-H_b), 5.24 (1H, dd, J=1.2, 10.4 Hz, 1a-*E*-$CO_2CH_2CH{=}CH_2$), 5.32 (1H, dd, J=1.2, 18.0 Hz, 1a-Z-$CO_2CH_2CH{=}CH_2$), 5.8~6.0 (1H, m, 1a-CO_2CH_2CH), 7.24~7.44 (3H, m, phenyl), 7.49~7.60 (2H, m, phenyl); (minor isomer) δ 1.39 (3H, s, 6-CH_3), 3.31 (3H, s, 9a-OCH_3), 3.39 (1H, dd, J=2.0, 13 Hz, 3α-H), 3.3~3.4 (1H, 2-H, overlapped with other peaks), 3.43 (1H, d, J=4.5 Hz, 1-H), 3.70 (1H, dd, J=4.6, 11.1 Hz, 9-H), 4.29 (1H, t, J=10.9 Hz, 10-H_a), 4.0~4.7 (7H, m, 1a-CO_2CH_2, 3β-H, and OCH_2CH_2O), 4.76 (1H, dd, J=4.6, 11 Hz, 10-H_b), 4.79 (2H, br s, 10-$OCONH_2$), 5.24 (1H, dd, J=1.2, 10.4 Hz, 1a-*E*-$CO_2CH_2CH{=}CH_2$), 5.29 (1H, dd, J=1.2, 18.0 Hz, 1a-Z-$CO_2CH_2CH{=}CH_2$), 5.8~6.0 (1H, m, 1a-CO_2CH_2CH), 7.24~7.44 (3H, m, phenyl), 7.49~7.60 (2H, m, phenyl); FAB-MS *m/z* 618/620 (2:1) $(M+H)^+$; FAB-HR-MS calcd for $C_{27}H_{30}N_3O_9{}^{80}Se$ $(M+H)^+$ *m/z* 620.1147, found 620.1122; IR (KBr) 3450, 3370, 3200, 3070, 2950, 2900, 1740, 1730, 1720, 1660, 1580, 1450, 1330, 1270, 1200, 1090, 1070 cm^{-1}

Preparation of 1a-(Allyloxycarbonyl)-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylenemitomycin A (**5**)

To a slurry of 1a-(allyloxycarbonyl)-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)-mitomycin A (6.19 g, 10.0 mmol) and powdered K_2CO_3 (3.34 g, 24.2 mmol) in CH_2Cl_2 (100 ml) was added dropwise a solution of mCPBA (80% purity, 3.25 g) in CH_2Cl_2 (50 ml) over a period of 20 minutes at $-40°C$. After stirring for 50 minutes at $-30°C$ and for an additional 40 minutes at room temperature, the mixture was poured into an aqueous $Na_2S_2O_3$-$NaHCO_3$ solution and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to 50 ml on a rotary evaporator. To the solution was added a large amount of *n*-hexane. The obtained powder was collected and dried under vacuum to afford **5** (4.33 g, 94%) as a yellow powder: FAB-MS *m/z* 462 $(M+H)^+$; FAB-HR-MS calcd for $C_{21}H_{23}N_3O_9$ $(M+H)^+$ *m/z* 462.1512, found 462.1489.

Preparation of **1a**

Original method: To a solution of **4**[10] (422 mg, 1.01 mmol) in THF (30 ml) was added dimedone (152 mg, 1.06 mmol) and NEt_3 (100 μl) and the mixture was stirred at room temperature. After 45 minutes, the mixture was poured into brine and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The obtained residue was purified by column chromatography (silica gel, 20:1~10:1 $CHCl_3$-MeOH as eluents), followed by trituration with $CHCl_3$-*n*-hexane and drying under the vacuum to afford crude **11a** (379 mg). The crude **11a** (241 mg) was treated with NH_3 in MeOH (6.1 M, 10 ml) for 3 hours at room temperature. The mixture was diluted with brine and extracted with $CHCl_3$. The combined organic layer was dried over Na_2SO_4 and concentrated on a rotary evaporator. The obtained paste was purified by preparative TLC (silica gel, 9:1 $CHCl_3$-MeOH as a developing solvent), followed by trituration with $CHCl_3$-*n*-hexane and drying under vacuum to afford **1a** (13 mg, 4.4% based on **4**) as a purple powder. In addition, compound **10** (52 mg, 17% based on **4**) was afforded as a green powder.

Improved method: To a solution of **5** (1.03 g, 2.23 mmol) in THF (50 ml) were added dimedone (346 mmol, 2.47 mmol) and NEt_3 (0.20 ml), and the mixture was stirred at room temperature. After 40 minutes, the mixture was poured into brine and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator to afford a paste. Silica gel (50 g) was added to a $CHCl_3$ (100 ml) solution of the paste and the mixture was allowed to stand at room temperature for 15 hours. After the extraction by an eluent (9:1 $CHCl_3$-MeOH), the solution was concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 50:1

$CHCl_3$-MeOH as an eluent), followed by trituration with $CHCl_3$-*n*-hexane and drying under vacuum to afford **12a** (635 mg, 53%) as a purple powder. To a solution of **12a** (252 mg, 0.467 mmol) in THF (20 ml) was added HCO_2H-NEt_3 (0.20 ml) and $Pd(PPh_3)_4$ (30 mg) followed by stirring at room temperature for 20 minutes under an argon atmosphere. The mixture was applied directly to column chromatography (silica gel, 20:1 $CHCl_3$-MeOH as an eluent) to afford a purple fraction, which was concentrated on a rotary evaporator, followed by trituration with $CHCl_3$-*n*-hexane and drying under vacuum to afford **1a** (110 mg, 52%) as a purple powder.

1a: ^{1}H NMR (270 MHz, pyridine-d_5) δ 0.95 (3H, s, CH_3), 0.99 (3H, s, CH_3), 2.12 (1H, br s, 1a-H), 2.33 (4H, br s, $-CH_2-\times 2$), 2.78 (1H, br s, 2-H), 3.08 (1H, d, J=20.6 Hz, 6-CH_2), 3.16 (1H, br s, 1-H), 3.20 (1H, d, J=20.6 Hz, 6-CH_2), 3.27 (3H, s, 9a-OCH_3), 3.53 (1H, br d, J=12 Hz, 3α-H), 4.06 (1H, dd, J=4.4, 11.0 Hz, 9-H), 4.18 (1H, d, J=12.5 Hz, 3β-H), 5.12 (1H, br t, J=10.4 Hz, 10-H_a), 5.42 (1H, dd, J=4.4, 10.4 Hz, 10-H_b), 7.4~7.8 (2H, br s, 10-$OCONH_2$); FAB-MS m/z 456 $(M+H)^+$; FAB-HR-MS calcd for $C_{23}H_{26}N_3O_7$ $(M+H)^+$ m/z 456.1771, found 456.1782; IR (KBr) 3450, 3300, 2950, 2880, 1720, 1710, 1660, 1630, 1570, 1380, 1330, 1200, 1070 cm^{-1}.

Anal Calcd for $C_{23}H_{25}N_3O_7 \cdot 0.4H_2O$: C 59.71, H 5.62, N 9.08.
Found: C 59.51, H 5.35, N 9.33.

10: ^{1}H NMR (270 MHz, pyridine-d_5) δ 0.92 (6H, s, $CH_3\times 2$), 2.11 (1H, br s, 1a-H), 2.33 (4H, br s, $-CH_2-\times 2$), 2.75 (1H, br s, 2-H), 3.12 (1H, br s, 1-H), 3.18 (3H, s, 9a-OCH_3), 3.37 (1H, d, J=15.0 Hz, 6-CH_2), 3.45 (1H, d, J=15.0 Hz, 6-CH_2), 3.58 (1H, br d, J=12.8 Hz, 3α-H), 3.96 (1H, dd, J=4.3, 11.1 Hz, 9-H), 4.45 (1H, d, J=12.8 Hz, 3β-H), 5.02 (1H, br t, J=11 Hz, 10-H_a), 5.33 (1H, dd, J=4.3, 10.4 Hz, 10-H_b), 7.4~7.8 (2H, br, 10-$OCONH_2$), 8.62 (1H, br s, 7-NH_2), 8.95 (1H, br s, 7-NH_2), 14.5 (1H, s, enol-OH); FAB-MS m/z 474 $(M+2H)^+$; IR (KBr) 3400, 3170, 2950, 2880, 1720, 1710, 1610, 1560, 1520, 1450, 1350, 1240, 1220, 1160, 1060 cm^{-1}.

Anal Calcd for $C_{23}H_{28}N_4O_7 \cdot 0.9H_2O$: C 56.53, H 6.15, N 11.46.
Found: C 56.69, H 6.29, N 11.16.

Preparation of **1b**

Enone **4** (840 mg, 2.00 mmol) was treated according to a similar procedure as that described in the synthesis of **12a** (Improved method) with 1,3-cyclohexanedione (232 mg, 2.07 mmol) and NEt_3 (0.20 ml) in THF (50 ml) to afford **11b** (592 mg, 1.26 mmol, 63%) as a purple powder. A similar procedure as that described in the synthesis of **1a** (Original method) was employed to convert **11b** (552 mg, 1.17 mmol) into **1b** (70 mg, 14%) as a purple powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.7~1.9 (2H, m, $-CH_2-$), 2.13 (1H, br s, 1a-H), 2.3~2.5 (4H, m, $-CH_2-\times 2$), 2.79 (1H, br s, 2-H), 3.1~3.3 (3H, m, 6-CH_2 and 1-H), 3.27 (3H, s, 9a-OCH_3), 3.53 (1H, br d, J=*ca.* 13 Hz, 3α-H), 4.04 (1H, dd, J=4.3, 11.1 Hz, 9-H), 4.18 (1H, d, J=12.5 Hz, 3β-H), 5.09 (1H, br t, J=11 Hz, 10-Ha), 5.39 (1H, dd, J=4.3, 10.4 Hz, 10-H_b), 7.4~7.8 (2H, br, 10-$OCONH_2$); FAB-MS m/z 429 $(M+2H)^+$; IR (KBr) 3450, 3350, 3300, 3200, 2950, 2880, 1720, 1660, 1630, 1570, 1450, 1380, 1340, 1200, 1190, 1120, 1070 cm^{-1}.

Anal Calcd for $C_{21}H_{21}N_3O_7 \cdot 0.4H_2O$: C 58.04, H 5.06, N 9.67.
Found: C 58.02, H 4.96, N 9.65.

Preparation of **13**

Enone **5** (1.01 g, 2.19 mmol) was treated according to a similar procedure as that described in the synthesis of **12a** (Improved method) with 1,3-cyclopentanedione (235 mg, 2.40 mmol) and NEt_3 (0.20 ml) in THF (50 ml) to afford **13** (371 mg, 35%) as a purple powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.5~2.6 (2H, m, $-CH_2-$), 2.7~2.8 (2H, m, $-CH_2-$), 3.0~3.5 (5H, m, 1-H, 2-H, 3α-H, and 6-CH_2), 3.22 (3H, s, 9a-OCH_3), 3.78 (1H, dd, J=4.9, 10.9 Hz, 9-H), 4.03 (1H, d, J=13.4 Hz, 3β-H), 4.57 (2H, d, J=5.9 Hz, $CH_2CH=CH_2$), 4.68 (3H, br s, 10-H_a and 10-$OCONH_2$), 4.92 (1H, dd, J=4.9, 10.9 Hz, 10-H_b), 5.25 (1H, br d, J=10.9 Hz, *E*-$CH_2CH=CH_2$), 5.30 (1H, br d, J=17.8 Hz, *Z*-$CH_2CH=CH_2$), 5.8~6.0 (1H, m, $CH_2CH=CH_2$); FAB-MS m/z 499 $(M+2H)^+$; IR (KBr) 3450, 3370, 1730, 1720, 1680, 1660, 1650, 1640, 1580, 1390, 1340, 1330, 1270, 1190, 1090, 1070 cm^{-1}.

Anal Calcd for $C_{24}H_{23}N_3O_9$: C 57.95, H 4.66, N 8.45.
Found: C 58.00, H 4.61, N 8.29.

Preparation of **1d**

To a solution of **4** prepared from 1a-acetyl-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A[10] (300 mg, 0.521 mmol) in $CHCl_3$ (10 ml) was added a sodium salt of ethyl acetoacetate (1.6 mmol) in THF (5.0 ml) at 0°C and the mixture was stirred for 1 hour at that temperature. The resulting mixture was poured into a phosphate buffer (pH 4) and extracted with $CHCl_3$. The organic layer was washed with an aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The obtained paste was purified by column chromatography (silica gel, 97:3 $CHCl_3$-MeOH as an eluent) to afford **18d** (120 mg, 42%). To a solution of **18d** (150 mg, 0.273 mmol) in MeOH (20 ml) was added NH_4OAc (300 mg) and the mixture was stirred at room temperature. After 16 hours, the reaction mixture was poured into brine and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator to afford a paste, which was purified by column chromatography (silica gel, 97:3 $CHCl_3$-MeOH as an eluent) to afford **1d** (50 mg, 41%) as a purple powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.60 (1H, br s, 1a-H), 1.31 (3H, t, J=7.1 Hz, CH_2CH_3), 2.38 (3H, s, CH_3), 2.85 (1H, br s, 2-H), 2.91 (1H, br s, 1-H), 3.09 (1H, d, J=20.4 Hz, 6-CH_2), 3.20 (1H, d, J=20.4 Hz, 6-CH_2), 3.23 (3H, s, 9a-OCH_3), 3.45 (1H, br d, J= 12.3 Hz, 3α-H), 3.67 (1H, dd, J=4.7, 10.1 Hz, 9-H), 4.07 (1H, dd, J=12.8 Hz, 3β-H), 4.23 (2H, q, J=7.1 Hz, CH_2CH_3), 4.60 (1H, br t, J=10.3 Hz, 10-H_a), 4.70 (1H, dd, J=4.7, 10.6 Hz, 10-H_b), 4.72 (2H, br s, 10-$OCONH_2$); SI-MS m/z 447 $(M+2H)^+$; FAB-HR-MS calcd for $C_{21}H_{24}N_3O_8$ $(M+H)^+$ m/z 446.1563, found 446.1528; IR (KBr) 3450, 2910, 1720, 1650, 1630, 1570, 1440, 1390, 1360, 1340, 1310, 1190, 1160, 1090, 1040 cm^{-1}.

Anal Calcd for $C_{21}H_{23}N_3O_8$: C 56.63, H 5.20, N 9.43.
Found: C 58.78, H 5.17, N 9.21.

Preparation of **1e** and **1f**

A solution of **5** prepared from 1a-allyloxycarbonyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (1.249 g, 2.02 mmol) in CH_2Cl_2 (200 ml) was treated according to a similar procedure as that described in the synthesis of **1d** with the sodium salt of benzoylacetone (2.0 mmol) in THF (10 ml) to afford a mixture of **19e** and **19f** (328 mg, 29%). A similar procedure as that described in the synthesis of **1a** (Improved method) was employed to convert a mixture of **19e** and **19f** (328 mg, 0.585 mmol) into a mixture of **1e** and **1f** (173 mg, 62%) as a purple powder. Separation of **1e** and **1f** was performed by preparative HPLC (ODS, 50:50 MeCN-H_2O as an eluent) and afforded isomerically pure **1e** (71 mg) and **1f** (15 mg), respectively.

1e: HPLC Rt 12.90 minutes (ODS, 50:50 MeCN-H_2O); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.66 (1H, br s, 1a-H), 1.87 (3H, s, CH_3), 2.85 (1H, br s, 2-H), 2.93 (1H, br d, J=4.0 Hz, 1-H), 3.15 (1H, d, J=20.3 Hz, 6-CH_2), 3.24 (3H, s, 9a-OCH_3), 3.27 (1H, d, J=20.3 Hz, 6-CH_2), 3.49 (1H, dd, J =1.8, 12.8 Hz, 3α-H), 3.69 (1H, dd, J=4.7, 10.2 Hz, 9-H), 4.07 (1H, d, J=12.8 Hz, 3β-H), 4.61 (1H, t, J=10.6 Hz, 10-H_a), 4.7~4.8 (2H, br, 10-$OCONH_2$), 4.73 (1H, dd, J=4.7, 10.7 Hz, 10-H_b), 7.44~7.51 (2H, m, phenyl), 7.55~7.62 (1H, m, phenyl), 7.78~7.83 (2H, m, phenyl); FAB-MS m/z 478 $(M+H)^+$; IR (KBr) 3400, 3330, 3240, 2900, 1750, 1690, 1660, 1600, 1480, 1390, 1360, 1240, 1230, 1220, 1190, 1100 cm^{-1}.

Anal. Calcd for $C_{25}H_{23}N_3O_7$: C 62.89, H 4.86, N 8.80.
Found: C 63.00, H 4.56, N 8.61.

1f: HPLC Rt 9.20 minutes (ODS, 50:50 MeCN-H_2O); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.8~1.1 (1H, br, 1a-H), 1.81 (3H, s, CH_3), 3.0~3.4 (4H, overlapped with other peaks, 1-H, 2-H, and 6-CH_2), 3.23 (3H, s, 9a-OCH_3), 3.57 (1H, br d, J=12.7 Hz, 3α-H), 3.68 (1H, dd, J=4.6, 9.6 Hz, 9-H), 4.22 (1H, d, J=12.7 Hz, 3β-H), 4.5~4.8 (2H, br, 10-$OCONH_2$), 4.58 (1H, br t, J=10.1 Hz, 10-H_a), 4.66 (1H, dd, J=4.6, 10.9 Hz, 10-H_b), 7.4~7.6 (5H, m, phenyl); FAB-MS m/z 478 $(M+H)^+$; IR (KBr) 3300, 3200, 2950, 1720, 1710, 1660, 1580, 1440, 1360, 1330, 1210, 1110, 1070, 1050 cm^{-1}.

Anal Calcd for $C_{25}H_{23}N_3O_7$: C 62.89, H 4.86, N 8.80.
Found: C 63.11, H 4.71, N 8.65.

Preparation of **2a**

Enone **6**[12] (1.00 g, crude) was treated according to a similar procedure as that described in the synthesis of **12a** (Improved method) with dimedone (372 mg, 2.66 mmol) and NEt_3 (0.20 ml) in THF (50 ml) to

afford **2a** (277 mg, 31% based on 7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin B[12]) as a purple powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ 0.93 (3H, s, CH_3), 0.96 (3H, s, CH_3), 2.17 (3H, s, 1a-CH_3), 2.2~2.4 (5H, m, $-CH_2-$ ×2 and 2-H), 2.50 (1H, d, J=4.6 Hz, 1-H), 3.01 (1H, d, J=20.5 Hz, 6-CH_2), 3.10 (1H, d, J=20.5 Hz, 6-CH_2), 3.57 (1H, dd, J=1.7, 12.7 Hz, 3α-H), 4.13 (1H, d, J=12.7 Hz, 3β-H), 4.26 (1H, dd, J=3.3, 9.1 Hz, 9-H), 5.25 (1H, dd, J=9.1, 10.7 Hz, 10-H_a), 5.43 (1H, dd, J=3.3, 10.7 Hz, 10-H_b), 7.3~7.7 (2H, br, 10-$OCONH_2$), 8.34 (1H, br s, 9a-OH); FAB-MS m/z 458 $(M+3H)^+$; IR (KBr) 3470, 3420, 3300, 2950, 1710, 1660, 1630, 1590, 1390, 1350, 1340, 1200, 1190, 1110 cm^{-1}.

Anal Calcd for $C_{23}H_{25}N_3O_7$: C 60.65, H 5.53, N 9.23.
Found: C 60.88, H 5.50, N 9.08.

Preparation of **2b**

Enone **6**[12] (732 mg, crude) was treated according to a similar method as that described in the synthesis of **12a** (Improved method) with 1,3-cyclohexanedione (218 mg, 1.95 mmol) and NEt_3 (0.20 ml) in THF (50 ml) to afford **2b** (151 mg, 25% based on 7-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)-mitomycin B[12]) as a purple powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.05 (2H, m, $-CH_2-$), 2.26 (3H, s, 1a-CH_3), 2.28 (2H, s, 1-H and 2-H), 2.45 (2H, m, $-CH_2-$), 2.61 (2H, m, $-CH_2-$), 2.95 (1H, d, J =20.8 Hz, 6-CH_2), 3.08 (1H, d, J=20.8 Hz, 6-CH_2), 3.45 (1H, d, J=12.9 Hz, 3α-H), 3.76 (1H, t, J=4.1 Hz, 9-H), 4.00 (1H, d, J=12.9 Hz, 3β-H), 4.62 (1H, br s, 9a-OH), 4.72 (2H, d, J=4.1 Hz, 10-H), 4.77 (2H, br s, 10-$OCONH_2$); FAB-MS m/z 429 $(M+2H)^+$, 430 $(M+3H)^+$; IR (KBr) 3450, 3200, 2950, 1710, 1660, 1620, 1570, 1450, 1380, 1350, 1210, 1190, 1120, 1070, 1060 cm^{-1}.

Anal Calcd for $C_{21}H_{21}N_3O_7 \cdot 0.3H_2O$: C 58.28, H 5.03, N 9.71.
Found: C 58.35, H 4.86, N 9.57.

Preparation of **3d**

A solution of **14**[12] (405 mg, 1.04 mmol) in THF (30 ml) was treated according to a similar method as that described in the synthesis of **1d** with the sodium salt of ethyl acetoacetate (1.02 mmol) in THF (10 ml) to afford **3d** (60 mg, 13%) as a purple powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.32 (3H, t, J=6.9 Hz, CH_2CH_3), 2.27 (3H, s, 1a-CH_3), 2.20~2.35 (2H, m, 1-H and 2-H), 2.39 (3H, s, CH_3), 3.08 (1H, d, J=20.4 Hz, 6-CH_2), 3.20 (1H, d, J=20.4 Hz, 6-CH_2), 3.20 (3H, s, 9a-OCH_3), 3.45 (1H, dd, J=2.0, 12.9 Hz, 3α-H), 3.63 (1H, dd, J=4.6, 10.9 Hz, 9-H), 4.04 (1H, d, J=12.9 Hz, 3β-H), 4.23 (2H, q, CH_2CH_3), 4.41 (1H, br t, J=10.7 Hz, 10-H_a), 4.70 (1H, dd, J=4.6, 10.6 Hz, 10-H_b), 4.76 (2H, br s, 10-$OCONH_2$); FAB-MS m/z 460 $(M+H)^+$; IR (KBr) 3450, 3350, 2950, 1720, 1710, 1700, 1640, 1630, 1580, 1570, 1450, 1360, 1330, 1310, 1210, 1190, 1090 cm^{-1}.

Anal Calcd for $C_{22}H_{25}N_3O_8$: C 57.51, H 5.48, N 9.15.
Found: C 57.66, H 5.45, N 8.93.

Preparation of **3e**

A solution of **14**[12] (400 mg, 1.02 mmol) in THF (30 ml) was treated according to a similar method as that described in the synthesis of **1d** with the sodium salt of benzoylacetone (1.01 mmol) in THF (10 ml) to afford **3e** (89 mg, 18%) as a purple powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.87 (3H, s, CH_3), 2.28 (3H, s, 1a-CH_3), 2.2~2.4 (2H, m, 1-H and 2-H), 3.21 (3H, s, 9a-OCH_3), 3.1~3.3 (2H, m, 6-CH_2), 3.44 (1H, dd, J =1.7, 12.8 Hz, 3α-H), 3.66 (1H, dd, J=4.7, 10.7 Hz, 9-H), 4.04 (1H, d, J=12.8 Hz, 3β-H), 4.42 (1H, t, J=10.7 Hz, 10-H_a), 4.72 (1H, dd, J=4.7, 10.7 Hz, 10-H_b), 4.78 (2H, br s, 10-$OCONH_2$), 7.44~7.53 (2H, m, phenyl), 7.55~7.62 (1H, m, phenyl), 7.78~7.83 (2H, m, phenyl); FAB-MS m/z 492 $(M+H)^+$, 494 $(M+3H)^+$; IR (KBr) 3400, 3300, 2880, 1760, 1740, 1690, 1660, 1610, 1480, 1390 1360, 1240, 1190, 1110, 1040 cm^{-1}.

Anal Calcd for $C_{26}H_{25}N_3O_7 \cdot 0.2H_2O$: C 63.08, H 5.17, N 8.49.
Found: C 63.11, H 4.94, N 7.86.

Preparation of **3g**

A solution of **14**[12] (405 mg, 1.04 mmol) in THF (30 ml) was treated according to a similar method as that described in the synthesis of **1d** with the sodium salt of acetylacetone (1.05 mmol) in THF (10 ml) to afford **3g** (32 mg, 7%) as a purple powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.27 (3H, s, CH_3), 2.30 (3H,

s, CH_3), 2.32 (3H, s, CH_3), 2.2~2.4 (2H, m, 1-H and 2-H), 3.20 (3H, s, 9a-OCH_3), 3.0~3.3 (2H, m, 6-CH_2), 3.45 (1H, dd, J=2.0, 12.9 Hz, 3α-H), 3.64 (1H, dd, J=4.7, 10.6 Hz, 9-H), 4.04 (1H, d, J=12.9 Hz, 3β-H), 4.40 (1H, t, J=10.6 Hz, 10-H_a), 4.70 (1H, dd, J=4.7, 10.6 Hz, 10-H_b), 4.72 (2H, br s, 10-$OCONH_2$); FAB-MS m/z 430 $(M+H)^+$, 432 $(M+3H)^+$; IR (KBr) 3450, 3350, 2950, 2920, 1730, 1710, 1690, 1660, 1630, 1570, 1450, 1350, 1320, 1200, 1190, 1080 cm^{-1}.

Anal Calcd for $C_{21}H_{23}N_3O_7 \cdot 0.2H_2O$: C 58.25, H 5.45, N 9.70.
Found: C 58.40, H 5.32, N 9.32.

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