MITOMYCIN DERIVATIVES HAVING UNIQUE CONDENSED-RING STRUCTURES
THEIR SYNTHESIS AND ANTITUMOR ACTIVITY

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(Received for publication March 28, 1994)

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 HCO₂H - NEt₃. Among these structurally unique derivatives, compounds 1a, 1b, 1d and 1e were quite potent against HeLa S₃ human tumor cells and sarcoma 180 solid tumor in mice.

Mitomycins are well known to be potent antitumor antibiotics produced by various Streptomyces cultures. 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. 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. 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. 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. 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). Compound 5 having the allyloxycarbonyl (Aloc) group for protection of the aziridine was prepared according to a similar method from 1a-(allyloxycarbonyl)-7,7-(ethylenedioxy)-6,7-dihydromito-
Compound 6 having the mitomycin B (MMB) skeleton were also prepared from MMB. As shown in Scheme 1, compounds 4~6 were reacted with didecene in the presence of NEt₃ at room temperature and afforded the crude Michael adducts 7a~9a in good yields, respectively. Interestingly, treatment of crude 7a with NH₃ in MeOH 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 K₂CO₃ 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 HCO₂H-NEt₃ 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 NEt₃ formed during the reaction since compounds having the condensed-ring structure, especially 13 and 1c, were unstable in the presence of NEt₃.

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 NEt₃ 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 K₂CO₃ in MeOH caused mainly decomposition of the substrate.
NH₄OAc in MeOH to avoid the basic conditions and 1d was obtained in 41% yield. In the synthesis of 1e, a small amount of If, a regioisomer of 1e, was obtained as a byproduct (1e : If = 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 If did not show that peak in the FAB-MS analysis. A similar two step conversion of 1412) prepared from mitomycin F (MMF) into 3d, 3e, and 3g was also achieved. In contrast to the synthesis of If, compound 3e was isolated as a sole isomer.

These results are summarized in Table 1.

Compounds 1~3 were tested for cytotoxicity against HeLa S₃ 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 S₃ cells. Among them, compounds 1a, 1b, 1d and 1e showed
Table 1. Preparations of mitomycin derivatives.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>U</th>
<th>V</th>
<th>MM-Skeleton</th>
<th>Method</th>
<th>Substrate</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Step 1</th>
<th>Step 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>CH₂C(CH₃)₂CH₂</td>
<td>MMA</td>
<td>5</td>
<td>A</td>
<td>A</td>
<td>53</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>(CH₂)₃</td>
<td>MMA</td>
<td>4</td>
<td>A</td>
<td>B</td>
<td>63</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1d</td>
<td>CH₃</td>
<td>OCH₂CH₃</td>
<td>MMA</td>
<td>4</td>
<td>B</td>
<td>42&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e</td>
<td>CH₃</td>
<td>Ph</td>
<td>MMA</td>
<td>5</td>
<td>B</td>
<td>29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>62&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1f</td>
<td>Ph</td>
<td>CH₃</td>
<td>MMA</td>
<td>5</td>
<td>B</td>
<td>29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>62&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>CH₂C(CH₃)₂CH₂</td>
<td>MMB</td>
<td>6</td>
<td>A</td>
<td>—</td>
<td>31&lt;sup&gt;e&lt;/sup&gt;</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>(CH₂)₃</td>
<td>MMB</td>
<td>6</td>
<td>A</td>
<td>—</td>
<td>25&lt;sup&gt;f&lt;/sup&gt;</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3d</td>
<td>CH₃</td>
<td>OCH₂CH₃</td>
<td>MMF</td>
<td>14</td>
<td>B</td>
<td>13</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3e</td>
<td>CH₃</td>
<td>Ph</td>
<td>MMF</td>
<td>14</td>
<td>B</td>
<td>18</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3g</td>
<td>CH₃</td>
<td>CH₃</td>
<td>MMF</td>
<td>14</td>
<td>B</td>
<td>7</td>
<td>—</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Reaction conditions. Step 1 (1,4-addition + cyclization); Method A: 1) UCOCH₂COV, NEt₃, 2) silica gel, CHCl₃; Method B: UCOCH₂COV + NaH. Step 2 (deprotection); Method A: HCO₂H-NEt₃, Pd(PPh₃)₄, THF; Method B: NH₃, MeOH; Method C: NH₄OAc, MeOH.

<sup>b</sup> Yield based on the enone used.

<sup>c</sup> Yield based on the corresponding 7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitosanes<sup>12</sup>.

<sup>d</sup> 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.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>IC_{50} (µM)</th>
<th>ED_{50} (mg/kg)</th>
<th>ODC (mg/kg)</th>
<th>T/C (minimum)</th>
<th>Compound No.</th>
<th>IC_{50} (µM)</th>
<th>ED_{50} (mg/kg)</th>
<th>ODC (mg/kg)</th>
<th>T/C (minimum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.084</td>
<td>1.3</td>
<td>2.7</td>
<td>0.20</td>
<td>3d</td>
<td>0.10</td>
<td>9.0</td>
<td>0.55</td>
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<tr>
<td>1b</td>
<td>0.065</td>
<td>0.84</td>
<td>1.8</td>
<td>0.23</td>
<td>3e</td>
<td>0.31</td>
<td>9.0</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>1d</td>
<td>0.016</td>
<td>0.70</td>
<td>2.5</td>
<td>0.26</td>
<td>3g</td>
<td>0.36</td>
<td>nt*</td>
<td>nt</td>
<td></td>
</tr>
<tr>
<td>1e</td>
<td>0.086</td>
<td>5.8</td>
<td>9.0</td>
<td>0.19</td>
<td>10</td>
<td>1.6</td>
<td>50</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>&gt; 10</td>
<td></td>
<td>14</td>
<td>0.53</td>
<td>MMC</td>
<td>0.59 ~ 1.5</td>
<td>2.2 ~ 5.0</td>
<td>6.0</td>
<td>0.27 ~ 0.43</td>
</tr>
<tr>
<td>2b</td>
<td>1.0</td>
<td></td>
<td>30</td>
<td>0.52</td>
<td>MMC</td>
<td>0.0024</td>
<td>1.3</td>
<td>1.8</td>
<td>0.20</td>
</tr>
</tbody>
</table>

\[ a \] In vitro anticellular activity against HeLa S3 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. On the other hand, the substituent effect at the C-7 and C-6 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 (1H 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 \[ a \] (2.31 g, 4.98 mmol) in MeCN (50 ml) and NEt3 (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 NaHCO₃ solution and extracted with CHCl₃. The organic layer was washed successively with an aqueous NH₄Cl solution and brine, dried over Na₂SO₄, and concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 40:1~30:1 CHCl₃-MeOH as eluents), followed by trituration with CHCl₃-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, CDCl₃) (major isomer) δ 1.55 (3H, s, 6-CH₃), 3.17 (3H, s, 9α-OCH₃), 3.31 (1H, brd, 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-Ha), 4.47~4.7 (6H, m, la-CO₂CH₂ and OCH₂CH₂O), 4.79 (2H, brs, 10-OCONH₂), 4.98 (1H, dd, J = 4.8, 10.9 Hz, 10-Hb), 5.24 (1H, dd, J = 1.2, 10.4 Hz, 1a-E-CO₂CH₂CH=CH₂), 5.32 (1H, dd, J = 1.2, 18.0 Hz, 1a-Z-CO₂CH₂CH=CH₂), 5.8~6.0 (1H, m, 1a-CO₂CH₂CH), 7.24~7.44 (3H, m, phenyl), 7.49~7.60 (2H, m, phenyl); (minor isomer) δ 1.39 (3H, s, 6-CH₃), 3.31 (3H, s, 9α-OCH₃), 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-Ha), 4.5~4.7 (7H, m, 1a-CO₂CH₂, 3β-H, and OCH₂CH₂O), 4.76 (1H, dd, J = 4.6, 11 Hz, 10-Hb), 4.79 (2H, brs, 10-OCONH₂), 5.24 (1H, dd, J = 1.2, 10.4 Hz, 1a-E-CO₂CH₂CH=CH₂), 5.29 (1H, dd, J = 1.2, 18.0 Hz, 1a-Z-CO₂CH₂CH=CH₂), 5.8~6.0 (1H, m, 1a-CO₂CH₂CH), 7.24~7.44 (2H, m, phenyl); FAB-MS m/z 618/620 (2:1) (M+H)+; FAB-HR-MS calcd for C₂₇H₃₀N₃O₉₈0₈Se (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-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)-mitomycin A (6.19 g, 10.0 mmol) and powdered K₂CO₃ (3.34 g, 24.2 mmol) in CH₂Cl₂ (100 ml) was added dropwise a solution of mCPBA (80% purity, 3.25 g) in CH₂Cl₂ (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 Na₂S₂O₃-NaHCO₃ solution and extracted with CHCl₃. The organic layer was washed with brine, dried over Na₂SO₄, 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 C₂₁H₂₃N₃O₉ (M+H)+ m/z 462.1512, found 462.1489.

Preparation of 1a (Original method: To a solution of 4 (422 mg, 1.01 mmol) in THF (30 ml) was added dimedone (152 mg, 1.06 mmol) and NEt₃ (100 µl) and the mixture was stirred at room temperature. After 45 minutes, the mixture was poured into brine and extracted with CHCl₃. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated on a rotary evaporator. The obtained residue was purified by column chromatography (silica gel, 20:1~10:1 CHCl₃-MeOH as eluents), followed by trituration with CHCl₃-n-hexane and drying under vacuum to afford crude 1a (379 mg). The crude 1a (241 mg) was treated with NH₃ in MeOH (6.1 M, 10 ml) for 3 hours at room temperature. The mixture was diluted with brine and extracted with CHCl₃. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated to 50 ml on a rotary evaporator. The obtained paste was purified by preparative TLC (silica gel, 9:1 CHCl₃-MeOH as a developing solvent), followed by trituration with CHCl₃-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 (433 g, 2.23 mmol) in THF (50 ml) were added dimedone (346 mmol, 2.47 mmol) and NEt₃ (0.20 ml), and the mixture was stirred at room temperature. After 40 minutes, the mixture was poured into brine and extracted with CHCl₃. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated on a rotary evaporator to afford a paste. Silica gel (50 g) was added to a CHCl₃ (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 CHCl₃-MeOH), the solution was concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 50:1
CHCl₃ - MeOH as an eluent), followed by trituration with CHCl₃ - 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 HCO₂H - NEt₃ (0.20 ml) and Pd(PPh₃)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 CHCl₃ - MeOH as an eluent) 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 HCO₂H - NEt₃ (0.20 ml) and Pd(PPh₃)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 CHCl₃ - MeOH as an eluent) 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 HCO₂H - NEt₃ (0.20 ml) and Pd(PPh₃)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 CHCl₃ - MeOH as an eluent) 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 HCO₂H - NEt₃ (0.20 ml) and Pd(PPh₃)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 CHCl₃ - MeOH as an eluent) 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 HCO₂H - NEt₃ (0.20 ml) and Pd(PPh₃)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 CHCl₃ - MeOH as an eluent) 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 HCO₂H - NEt₃ (0.20 ml) and Pd(PPh₃)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 CHCl₃ - MeOH as an eluent) 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 HCO₂H - NEt₃ (0.20 ml) and Pd(PPh₃)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 CHCl₃ - MeOH as an eluent) to afford 12a (635 mg, 53%) as a purple powder.
Preparation of 1d

To a solution of 4 prepared from 1a-acetyl-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (300 mg, 0.521 mmol) in CHCl₃ (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 CHCl₃. The organic layer was washed with an aqueous NaHCO₃ solution and brine, dried over Na₂SO₄, and concentrated on a rotary evaporator. The obtained paste was purified by column chromatography (silica gel, 97:3 CHCl₃ - MeOH as an eluent) to afford 1d (120 mg, 42%). To a solution of 1d (150 mg, 0.273 mmol) in MeOH (20 ml) was added NH₄OAc (300 mg) and the mixture was stirred at room temperature. After 16 hours, the reaction mixture was poured into brine and extracted with CHCl₃. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated on a rotary evaporator to afford a paste, which was purified by column chromatography (silica gel, 97:3 CHCl₃ - MeOH as an eluent) to afford 1d (50 mg, 41%) as a purple powder: ¹H NMR (400 MHz, CDCl₃) δ 0.60 (1H, brs, 1a-H), 1.31 (3H, t, J=7.1 Hz, CH₂CH₃), 2.38 (3H, s, CH₃), 2.85 (1H, brs, 2-H), 2.91 (1H, brs, 1-H), 3.09 (1H, d, J=20.4 Hz, 6-CH₂), 3.20 (1H, d, J=20.4 Hz, 6-CH₂), 3.23 (2H, s, 9a-OCH₃), 3.45 (1H, brd, J=12.3 Hz, 3β-H), 3.67 (1H, dd, J=4.7, 10.1 Hz, 9-H), 4.07 (1H, d, J=12.8 Hz, 3α-H), 4.23 (2H, q, J=7.1 Hz, CH₂CH₃), 4.60 (1H, brt, J=10.3 Hz, 10-Ha), 4.70 (1H, dd, J=4.7, 10.6 Hz, 10-Hb), 4.72 (2H, brs, 10-OCONH₂); SI-MS m/z 447 (M+2H)+; FAB-HR-MS calcd for C₂₁H₂₄N₃O₈ (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 C₂₁H₂₄N₃O₈: C 56.63, H 5.20, N 9.43.

Preparation of 1e and 1f

A solution of 5 prepared from 1a-allyloxy carbonyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (1.249 g, 2.02 mmol) in CH₂Cl₂ (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 - H₂O 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 - H₂O); ¹H NMR (270 MHz, CDCl₃) δ 0.66 (1H, brs, 1a-H), 1.87 (3H, s, CH₃), 2.85 (1H, brs, 2-H), 2.93 (1H, brd, J=4.0 Hz, 1-H), 3.15 (1H, d, J=20.3 Hz, 6-CH₂), 3.24 (3H, s, 9a-OCH₃), 3.27 (1H, d, J=20.3 Hz, 6-CH₂), 3.49 (1H, dd, J=1.8, 12.8 Hz, 3a-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-Ha), 4.7-4.8 (2H, br, 10-OCONH₂), 4.73 (1H, dd, J=4.7, 10.7 Hz, 10-Hb), 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.


Found: C 62.89, H 4.86, N 8.80.

1f: HPLC Rt 9.20 minutes (ODS, 50:50 MeCN - H₂O); ¹H NMR (270 MHz, CDCl₃) δ 0.8~1.1 (1H, br, 1a-H), 1.81 (3H, s, CH₃), 3.0~3.4 (4H, overlapped with other peaks, 1-H, 2-H, and 6-CH₂), 3.23 (3H, s, 9a-OCH₃), 3.57 (1H, brd, J=12.7 Hz, 3a-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-OCONH₂), 4.58 (1H, brt, J=10.1 Hz, 10-Ha), 4.66 (1H, dd, J=4.6, 10.9 Hz, 10-Hb), 7.4~7.6 (5H, 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.


Found: C 63.11, H 4.71, N 8.65.

Preparation of 2a

Enone 6 [1] (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 NEt₃ (0.20 ml) in THF (50 ml) to
afford 2a (277 mg, 31% based on 7-demethoxy-7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin B\(^{13,22}\)) as a purple powder: \(^{1}\)H NMR (270 MHz, pyridine-\(d_5\)) \(\delta\) 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\(_2\)- x 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, 3x-H), 4.13 (1H, d, \(J = 12.7\) Hz, 3\(\beta\)-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, brs, 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\(^{-1}\).

**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 NE\(_t\)\(_3\) (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, CDC\(_3\)) \(\delta\) 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, dd, \(J = 2.0, 12.9\) Hz, 3a-H), 3.76 (1H, t, \(J = 4.1\) Hz, 9-H), 4.62 (1H, brs, 9a-OH), 4.72 (2H, d, \(J = 4.1\) Hz, 10-H), 4.77 (2H, brs, 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\(^{-1}\).

**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 Id 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, CDC\(_3\)) \(\delta\) 1.32 (3H, t, \(J = 6.9\) Hz, CH\(_2\)CH\(_3\)), 2.27 (3H, s, 1a-CH\(_3\)), 2.2~2.35 (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, 3x-H), 3.63 (1H, dd, \(J = 4.6, 10.9\) Hz, 9-H), 4.04 (1H, d, \(J = 12.9\) Hz, 3\(\beta\)-H), 4.23 (2H, q, CH\(_2\)CH\(_3\)), 4.41 (1H, brt, \(J = 10.7\) Hz, 10-H), 4.70 (1H, dd, \(J = 4.6, 10.6\) Hz, 10-H), 4.76 (2H, brs, 10-OCONH\(_2\)); FAB-MS \(m/z\) 460 (M+H\(^+\)), 430 (M+3H\(^+\)); IR (KBr) 3450, 3350, 2950, 1720, 1710, 1700, 1640, 1630, 1580, 1570, 1450, 1360, 1330, 1310, 1210, 1190, 1090 cm\(^{-1}\).

**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 Id 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, CDC\(_3\)) \(\delta\) 1.87 (3H, s, CH\(_3\)), 2.28 (3H, s, 1a-CH\(_3\)), 2.2~2.35 (2H, m, 1-H and 2-H), 3.21 (3H, s, 9a-OCH\(_3\)), 3.1~3.2 (3H, m, 6-CH\(_3\)), 3.44 (1H, dd, \(J = 1.7, 12.8\) Hz, 3x-H), 3.66 (1H, dd, \(J = 4.7, 10.7\) Hz, 9-H), 4.04 (1H, d, \(J = 12.8\) Hz, 3\(\beta\)-H), 4.42 (1H, t, \(J = 10.7\) Hz, 10-H), 4.72 (1H, dd, \(J = 4.7, 10.7\) Hz, 10-H), 4.78 (2H, brs, 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+2H\(^+\)); IR (KBr) 3400, 3300, 2880, 1760, 1740, 1690, 1660, 1610, 1480, 1390 1360, 1240, 1190, 1110, 1040 cm\(^{-1}\).

**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 Id 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, CDC\(_3\)) \(\delta\) 2.27 (3H, s, CH\(_3\)), 2.30 (3H,
s, CH₃), 2.32 (3H, s, CH₃), 2.2~2.4 (2H, m, 1-H and 2-H), 3.20 (3H, s, 9a-OCH₃), 3.0~3.3 (2H, m, 6-CH₂), 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-Ha), 4.70 (1H, dd, J = 4.7, 10.6 Hz, 10-Hb), 4.72 (2H, br s, 10-OCONH₂);
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 C₂₁H₂₃N₃O₇·0.2H₂O: C 58.25, H 5.45, N 9.70.
Found: C 58.40, H 5.32, N 9.32.

Acknowledgment

We would like to thank Drs. Tadashi Hirata and Hiromitsu Saito for their encouragement throughout this work. We also would like to express our gratitude to Miki Haibara, Hiroe Ohno, and Hiroko Takanami for their excellent assistance.

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