6-Demethyl-6-halo- and 6-Demethyl-6-thiomitomycins: Synthesis of Novel Mitomycin Derivatives Involving a Tandem Michael Addition/ Retro-Mannich Reaction Sequence

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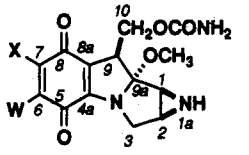
Synthesis of novel 6-demethyl-6-halomitomycins (**3**, **4**, **6**, and **7**) and 6-demethyl-6-thiomitomycins (**5** and **8**) is described from key intermediates, namely, 6-demethyl-7,7-(ethylenedioxy)-6,6-dihalo-6,7-dihydromitosanes (**18** and **19**) and 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-thiomitosane (**20**), respectively. Compounds **18–20** were prepared through a tandem Michael addition and a retro-Mannich reaction sequence (*N*-halosuccinimide or cationic arylsulfenyl species in the presence of Et_2NH) on **10**.

Introduction

Mitomycin C (MMC, **1**) is one of the most potent antitumor antibiotics and has been widely used in cancer chemotherapy, but it also has detrimental side effects such as myelosuppression and gastrointestinal damage.[1] To overcome these disadvantages, hundreds of derivatives targeting less toxicity and more efficacy have been synthesized in our laboratories[2] and by other groups.[3] However these transformations have been limited except for a few examples[3a,4] to the C-7, N-1a, and C-10 positions due to the difficulty of modification at other positions. The reductive activation of the quinone is believed to be essential for the antitumor activity of mitomycins.[1b] Recent investigations have indicated that there is a relationship between antitumor activity and reduction potential[3b,5] or spatial charge distribution in the molecule.[6] Therefore, modification at the C-6 position of the quinone moiety is attractive for developing novel mitomycin derivatives which may show different chemical and biological characteristics compared to conventional mitomycins or their derivatives.

During the course of our investigations of mitomycin chemistry, we have discovered a novel replacement of the methylidene moiety of 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylidenemitosane (**10**)[7] by a phenylseleno group *via* the retro-Mannich reaction. It enables us to obtain 6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitosane (**11**).[8] This unusual replacement has prompted us to introduce other functional groups at the C-6 position of mitomycins for controlling the electronic character of the quinone moiety. In a previous paper,[9] we had reported the synthesis of 6-demethylmitomycins. Our study showed a new approach in the modification of mitomycins for making a hitherto unknown modification at the C-6 position. Taking into account the provision of stronger electronic inductive effects toward the quinone moiety than the methyl group or hydrogen, introduction of electron-withdrawing groups such as halogen and arylthio were studied.[10] Bromine or chlorine atoms seemed to be especially suitable for the evaluation of the electronic effects because their atomic sizes[11] are similar to that of the methyl group which would minimize the conformational change in mitomycins arising from steric effects of the C-6 substituents. Now we describe the synthesis of 6-demethyl-6-halomitomycins (**3**, **4**, **6**, and **7**) and 6-demethyl-6-thiomitomycins (**5** and **8**) *via* a novel process involving a unique retro-Mannich reaction.



1: Mitomycin C (X = NH_2, W = CH_3)
2: Mitomycin A (X = CH_3O, W = CH_3)
3: X = NH_2, W = Br
4: X = NH_2, W = Cl
5: X = NH_2, W = RS
6: X = CH_3O, W = Br
7: X = CH_3O, W = Cl
8: X = CH_3O, W = PhS

Results and Discussion

Our initial strategy for the introduction of halogen at the C-6 position instead of the methyl group involved halogenation of 6-demethyl-7,7-(ethylenedioxy)-6,7-dihy-

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(1) For key reviews: (a) Carter, S. K.; Crooke, S. T. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1979. (b) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979. (c) Franck, R. W.; Tomasz, M. *The Chemistry of Antitumor Agents*; Wilman, D. F. V., Ed.; Blackie and Sons, Ltd.: Scotland, 1989. (d) *Mitomycin C in Cancer Chemotherapy Today*; Taguchi, T., Aigner, K. R., Eds.; Selected Proceedings of the 15th International Cancer Congress, Hamburg, Germany, Aug 16–22, 1990, Excerpta Medica, 1991.

(2) For recent examples: (a) Morimoto, M.; Ashizawa, T.; Ohno, H.; Azuma, M.; Kobayashi, E.; Okabe, M.; Gomi, K.; Kono, M.; Saitoh, Y.; Kanda, Y.; Arai, H.; Sato, A.; Kasai, M.; Tsuruo, T. *Cancer Res.* **1991**, *51*, 110. (b) Kono, M.; Saitoh, Y.; Kasai, M.; Sato, A.; Shirahata, K.; Morimoto, M.; Ashizawa, T. *Chem. Pharm. Bull.* **1989**, *37*, 1128.

(3) For recent examples: (a) Takayanagi, H.; Oshima, J.; Furuhata, K.; Takeda, K.; Ogura, H.; Komiyama, K.; Hata, T. *J. Antibiot.* **1992**, *45*, 1815. (b) Kunz, K. R.; Iyengar, B. S.; Dorr, R. T.; Alberts, D. S.; Remers, W. A. *J. Med. Chem.* **1991**, *34*, 2281. (c) Kaneko, T.; Wong, H.; Rose, W. C.; Bradner, W. T.; Doyle, T. W. *J. Antibiot.* **1990**, *43*, 122. (d) Sawhney, K. N.; Kohn, H. *J. Med. Chem.* **1989**, *32*, 248. (e) Vyas, D. M.; Benigni, D.; Rose, W. C.; Bradner, W. T.; Doyle, T. W. *J. Antibiot.* **1989**, *42*, 1199.

(4) Kasai, M.; Kono, M.; Shirahata, K. *J. Antibiot.* **1991**, *44*, 301.

(5) Pan, S.-S.; Gonzalez, H. *Mol. Pharmacol.* **1990**, *37*, 966.

(6) Yoda, N.; Hirayama, N. *J. Med. Chem.* **1993**, *36*, 1461.

(7) Kanda, Y.; Kasai, M. *J. Org. Chem.* **1990**, *55*, 2515.

(8) (a) Kasai, M.; Arai, H.; Kanda, Y. *J. Chem. Soc., Chem. Commun.* **1991**, 600. (b) Arai, H.; Kanda, Y.; Kasai, M. *J. Org. Chem.* **1993**, *58*, 892.

(9) Arai, H.; Kasai, M. *J. Org. Chem.* **1993**, *58*, 4151.

(10) An examination using substituted *p*-benzoquinone shows that electron-donating substituents lower the reduction potential with respect to the unsubstituted *p*-benzoquinone, whereas electron-withdrawing substituents increase it: Berger St.; Rieker A. In *The Chemistry of the Quinonoid Compounds. Part 1*; Patai, S., Ed.; Wiley: London, 1974; pp 204–207.

(11) The bond length of C(sp^2)–X (Å): X = Br, 1.89; X = Cl, 1.74; X = C(sp^3), 1.51. Trotter J. In *The Chemistry of the Carbon-Halogen Bond. Part 1*; Patai, S., Ed.; Wiley: London, 1973; pp 49–53.

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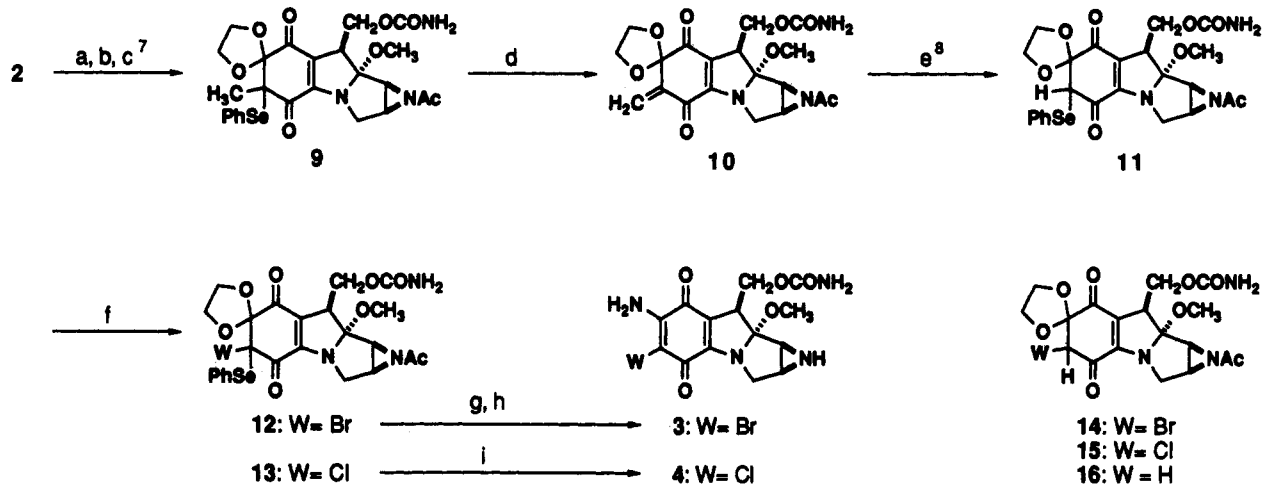
Scheme 1[a]



[a] (a) Ac_2O, pyridine, $CHCl_3$; (b) KOH (catalytic), ethylene glycol, THF; (c) PhSeBr, Et_3N, THF; (d) *m*-CPBA, K_2CO_3; (e) *N*-(phenylseleno)morpholine; (f) *N*-halosuccinimide; (g) *n*-Bu_3SnH, Δ; (h) NH_3, MeOH; (i) NH_3, MeOH, dimedone.

dro-6-(phenylseleno)mitosane (11) (Scheme 1). As previously described, 11 was prepared readily from MMA (2) in ca. 45% yield by five sequential steps.[8] Electrophilic bromination at the C-6 position of 11 with *N*-bromosuccinimide (NBS) at room temperature proceeded easily to afford the bromide 12 in 70% yield. In the case of chlorination, although a similar reaction with *N*-chlorosuccinimide (NCS) did not proceed smoothly, the chloride 13 was obtained in 29% yield by an addition of silica gel as a weak acid catalyst into the reaction mixture.

Selective deselenenylation of 12 and 13 to 3 and 4, respectively, was considered feasible by reaction with NH_3 in the presence of dimedone.[9] Accordingly, the chloride 13 when treated with a methanolic solution of NH_3 in the presence of dimedone at room temperature afforded the desired product 4 in 57% yield. However, when bromide 12 was treated under the same conditions, the yield of 3 was low because the selective deselenenylation did not proceed successfully and 6-demethyl-6-(phenylseleno)-MMC[12] was obtained as a byproduct. Thus for the selective deselenenylation of 12, we tried some conditions and found that reduction of 12 with *n*-Bu_3SnH upon heating at 50 °C provided the deselenenylated product 14 in 50% yield along with small amounts of 11 and 16.[9] Conversion to the MMC skeleton was also accomplished by treatment of 14 with NH_3 in methanol at room temperature[7] and afforded the desired 3 in 86% yield.

Although our first goal in the synthesis of 6-demethyl-6-halomitomycin derivatives was met, the overall yields of 3 and 4 were quite low, mainly due to the inefficiency of the halogenation of 11 (especially for 4) and the deselenenylation of 12 (for 3). It seemed from this observation that the main problem involved the nature of the phenylseleno group at the C-6 position, *i.e.*, the lability and the bulkiness of the phenylseleno group of 11, and the difficulty of selective deselenenylation between the seleno atom and the halogen under the above conditions. We therefore turned our attention to alternative routes for the synthesis of 3 and 4 without using the intermediate 11. Earlier observations in our laboratory regarding the conversion of 10 to 11 had demonstrated the requirement of the formation of an adduct with selenenamide.[8b] This prompted us to replace the methylidene group of 10 by halogen through a similar retro-Mannich reaction, *i.e.*, the Michael addition of amines to 10 followed by halogenation at the C-6 position would afford an intermediate, which then lost iminium through the use of secondary amines and cationic halogen species. As described in Scheme 2, addition of diethylamine to the yellow solution of 10 at room temperature immediately resulted in a reddish brown solution, which indicated that the 1,4-addition of the amine to the enone moiety might occur readily to afford intermediate 17 (yellow) along with a small amount of 17′ having the quinone skeleton (purple).[13] By adding 2 equiv of NBS to this solution, the reaction mixture turned yellow within a few minutes, and after the purification, 6,6-dibromo-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitosane (18) was obtained (58% from 10). A similar reaction occurred using NCS (4 equiv) instead of NBS and afforded 6,6-dichloro-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitosane (19) (61% from 10).[14,15] Since enone 10 was highly reactive and decomposed easily in the isolation process from the reaction mixture, the yields of 18 and 19 from 9 without isolation of 10 were considerably improved (62% and 74%, respectively, based on 9) in the sequential treatment with *m*-CPBA followed by treatment with Et_2NH and *N*-halosuccinimides.

Conversion of 6,6-dibromide 18 into the MMC skeleton proceeded simultaneously on treatment with NH_3 in methanol at room temperature in the presence of dimedone for the *in situ* mono-debromination and afforded 3 in 55% yield, whereas 6,6-dichloride 19 was inert under the same conditions. Thus in the case of 19, we applied the two-step process for its conversion to 4, *i.e.*, treatment with

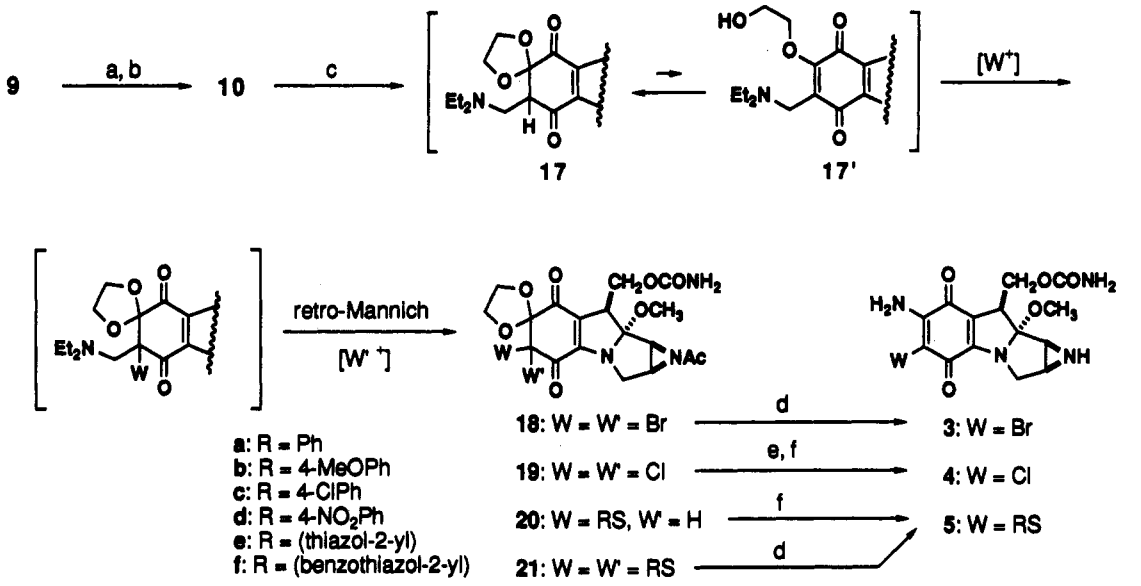
(12) ^1H NMR (270 MHz, pyridine-d_5) δ 2.2 (br s, 1 H), 2.72 (dd, *J* = 2.0, 4.4 Hz, 1 H), 3.11 (d, *J* = 4.4 Hz, 1 H), 3.19 (s, 3 H), 3.58 (dd, *J* = 2.0, 12.8 Hz, 1 H), 4.03 (dd, *J* = 4.2, 11.1 Hz, 1 H), 4.53 (d, *J* = 12.8 Hz, 1 H), 5.03 (br t, *J* = ca. 11 Hz, 1 H), 5.35 (dd, *J* = 4.2, 10.3 Hz, 1 H), 7.05–7.15 (m, 3 H), 7.40–7.45 (m, 2 H), 7.1–7.9 (br, 2 H), 8.28 (br s, 1 H), 8.72 (br s, 1 H); EI-HRMS calcd for $C_{20}H_{20}N_4O_6{}^{78}Se$ (M^+) *m/z* 476.0597, found 476.0598; IR (KBr) 3450, 3300, 1720, 1590, 1560 cm^{-1}.

(13) Isolation of the adduct was not successful since the elimination of amines occurred on contact with the silica gel (ref 7). However, the formation of adduct 17 or 17′ was confirmed by FAB-HRMS: calcd for $C_{23}H_{33}N_4O_8$ (M^+ + H) *m/z* 493.2299, found 493.2329.

(14) A similar reaction sequence containing a retro-Mannich reaction was reported as an unexpected result: Dickens, J. P.; Ellames, G. J.; Hare, N. J.; Lawson, K. R.; McKay, W. R.; Metters, A. P.; Myers, P. L.; Pope, A. M. S.; Upton, R. M. *J. Med. Chem.* **1991**, *34*, 2356.

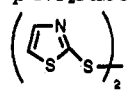
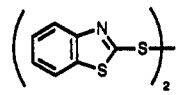
(15) In the case of *N*-iodosuccinimide, the corresponding 6,6-diiodide was not isolated due to the instability of the product on silica gel.

Scheme 2[a]



[a] (a) *m*-CPBA, K_2CO_3; (b) isolation; (c) Et_2NH, then *N*-halosuccinimide (2–4 equiv) or RSX (X = Cl or RS); (d) NH_3, MeOH, dimedone; (e) *n*-Bu_3SnH, Et_3B, −40 °C; (f) NH_3, MeOH.

Table 1. Preparation of Various 6-(Arylthio)-6-demethylmitomycin C

entry	sulfenylating reagent	10 → 20 and/or 21				20 and/or 21 → 5	
		product	yield, %	product	yield, %	product	yield, %
1	PhSCl	**20a**	44	**21a**	6	**5a**	58[a]
2	*p*-MeOPhSCl	**20b**	36	*b*	*b*	**5b**	57[a]
3	*p*-ClPhSCl	**20c**	25	**21c**	19	**5c**	44[a]
4	*p*-NO_2PhSCl	*b*	*b*	**21d**	30	**5d**	27[c]
5		**20e**	*d*	**21e**	*d*	**5e**	23[e]
6		**20f**	*d*	**21f**	*d*	**5f**	36[e]

[a] Yield based on compound **20**. [b] Not isolated. [c] Yield based on compound **21**. [d] It was used for further reaction without purification. [e] Yield based on compound **10**.

NH_3 after mono-dechlorination employing the *n*-Bu_3SnH–Et_3B system[16] in a radical-initiated reaction. Reduction with *n*-Bu_3SnH in the presence of Et_3B at −40 °C afforded the crude monochloride **15**. After the treatment with NH_3 in methanol, the desired **4** was consequently obtained in 60% yield based on **19**.

We next tried to introduce other functional groups such as a thio group at the C-6 position using a novel process involving a tandem Michael addition and a retro-Mannich reaction. A similar reaction proceeded by the addition of diethylamine and cationic arylthio species to the solution of **10** to form 6-(arylthio)-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitosane (**20**) and 6,6-bis(arylthio)-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitosane (**21**).[17] Benzenesulfenyl chlorides were effective for the introduction of the phenylthio groups, but bis(*p*-nitrophenyl) disulfide and *N*-(phenylthio)succinimide were inert in the reaction. The introduction of (thiazol-2-yl)thio or (benzothiazol-2-yl)thio groups was achieved by use of the respective disulfides as reactants. These results are summarized in Table I. When benzenesulfenyl or *p*-methoxybenzenesulfenyl chloride was used as a reactant, compounds **20a** and **20b** were the major products (entries 1 and 2). However, reactions using *p*-chloro- or *p*-nitrobenzenesulfenyl chlorides afforded large amounts of **21c** and **21d** (entries 3 and 4). These facts suggest that the formation of **21** is mainly due to the electron-withdrawing effect of the substituent on the phenyl group. In other words, an increase of the electrophilic character of sulfur in the sulfenyl chloride accelerates the sulfenylation of **20**.

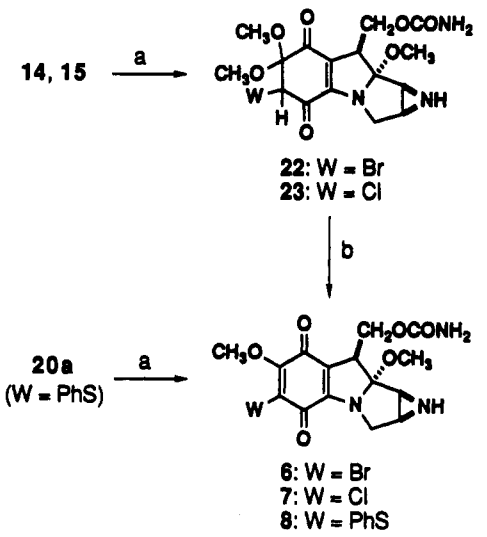
Conversion of **20** into **5** having the MMC skeleton was achieved by the treatment with NH_3 in methanol at room temperature. Since bis(arylthio)-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitosane **21** could also be converted to **5** with the above condition by an addition of dimedone for capturing the arylthio group (entry 4), the mixture of **20** and **21** was used as a substrate for this treatment to obtain **5** without separation of each compound (entries 5 and 6).

Further, we tried to synthesize MMA derivatives **6**, **7**, and **8** from **14**,[18] **15**, and **20a**, respectively, by treatment with potassium carbonate in methanol at room temper-

(16) Miura, K.; Ichinose, Y.; Nozaki, K.; Fugami, K.; Oshima, K.; Utimoto, K. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 143.

(17) A two-step conversion of enone into α-thiocarbonyl via retro-Aldol reaction of β-hydroxy-α-phenylsulfenyl ketone prepared by the ring opening reaction of the corresponding epoxide with thiophenoxide was reported: Caine, D.; Crews, E.; Salvino, J. M. *Tetrahedron Lett.* **1983**, 2083.

Scheme 3[a]



[a] (a) MeOH, K_2CO_3; (b) silica gel.

ature (Scheme 3).[7,19] Interestingly 7,7-bis(methoxy) intermediates **22** and **23** were isolated by silica gel flash chromatography after treatment of **14** and **15** with potassium carbonate in methanol.[20] Compounds **22** and **23** were slowly converted quantitatively to **6** and **7**, respectively, by prolonged contact with silica gel. In the case of the reaction of **20a**, a similar stable intermediate was not isolated, and the desired **8** was obtained as the sole product (27%).

In conclusion, we have described the syntheses of 6-demethyl-6-halo- and 6-demethyl-6-thiomitomycins (**3**–**8**) that were hitherto unaccessible.[21] The novel tandem Michael addition and retro-Mannich reaction sequence plays a critical role in these syntheses and makes it possible to substitute the C-6 methyl group by a variety of substituents. We think that this sequence has provided a new approach to the synthesis of novel mitomycin derivatives, which may display unique biological properties.

Experimental Section

General. Unless otherwise noted, materials were obtained from commercial suppliers except for the mitomycins and were used without purification. NBS and NCS were recrystallized from water. THF was distilled from sodium/benzophenone immediately prior to use. Benzene was distilled at atmospheric pressure and stored over 4-Å molecular sieves. Chromatography and some reactions were performed using Merck 60 70–230-mesh silica gel.

1a-Acetyl-6-bromo-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (12). To a stirred solution of **11** (28 mg, 0.050 mmol) in CH_2Cl_2 (3.0 mL) was added NBS (14 mg, 0.079 mmol). After 3 h at rt, the reaction mixture was washed with brine and dried over Na_2SO_4. The solvent was removed on a rotary evaporator and the residue was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent) to afford a yellow paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, compound **12** (21 mg, 70%) was obtained as a yellow powder. This material was a mixture of two diastereomers at the C-6 position (about 3:1): ^{1}H NMR (270 MHz, pyridine-d_5) (major isomer) δ 2.35 (s, 3 H), 3.09 (s, 3 H), 3.39 (dd, J = 2.0, 13.0 Hz, 1 H), 3.51 (dd, J = 1.8, 4.6 Hz, 1 H), 3.67 (d, J = 13.2 Hz, 1 H), 3.85 (d, J = 4.6 Hz, 1 H), 4.0–4.7 (m, 4 H), 4.22 (dd, J = 4.5, 11.1 Hz, 1 H), 4.71 (t, J = 11.1 Hz, 1 H), 5.81 (dd, J = 4.7, 10.9 Hz, 1 H), 7.3–7.9 (m, 5 H), 7.5–8.0 (br s, 2 H); (minor isomer) δ 1.99 (s, 3 H), 3.15 (d, J = 13.4 Hz, 1 H), 3.31 (s, 3 H), 3.33 (dd, J = 1.8, 12.6 Hz, 1 H), 3.46 (dd, J = 1.8, 4.6 Hz, 1 H), 3.79 (d, J = 4.6 Hz, 1 H), 4.0–4.7 (m, 5 H), 4.64 (t, J = 10.8 Hz, 1 H), 5.56 (dd, J = 4.6, 10.6 Hz, 1 H), 7.3–7.9 (m, 5 H), 7.5–8.0 (br s, 2 H); FAB-MS m/z 640/642/644 (2:4:3) (M^+ + 1); FAB-HRMS calcd for $C_{24}H_{25}{}^{81}BrN_3O_8{}^{80}Se$ (M^+ + H) m/z 641.9988, found 642.0014; IR (KBr) 3450, 3370, 3060, 1710, 1665, 1580 cm^{-1}.

1a-Acetyl-6-chloro-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (13). To a stirred solution of **11** (606 mg, 1.08 mmol) in CH_2Cl_2 (30 mL) were added silica gel (5.0 g) and NCS (215 mg). After 30 min at rt, the reaction mixture was directly subjected to column chromatography (silica gel, 2:1 $CHCl_3$/MeCN as an eluent) to afford a yellow paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, the desired **13** (186 mg, 29%) was obtained as a yellow powder. This material was a mixture of two diastereomers at the C-6 position (about 2:1): ^{1}H NMR (270 MHz, pyridine-d_5) (major isomer) δ 2.34 (s, 3 H), 3.10 (s, 3 H), 3.3–3.5 (m, 2 H), 3.66 (br d, J = 13 Hz, 1 H), 3.75–4.8 (m, 7 H), 5.81 (dd, J = 4.7, 11.1 Hz, 1 H), 7.2–8.0 (m, 7 H); (minor isomer, main peaks) δ 2.00 (s, 3 H), 3.33 (s, 3 H), 5.56 (dd, J = 4.6, 10.6 Hz, 1 H); FAB-MS m/z 596/598/600 (1:2:1) (M^+ + 1); FAB-HRMS calcd for $C_{24}H_{25}{}^{35}ClN_3O_8{}^{80}Se$ (M^+ + H) m/z 598.0490, found 598.0458; IR (KBr) 3450, 3050, 1710, 1660, 1570 cm^{-1}. Anal. Calcd for $C_{24}H_{24}ClN_3O_8Se$: C, 48.30; H, 4.05; N, 7.04. Found: C, 48.45; H, 4.20; N, 7.32.

1a-Acetyl-6-bromo-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin A (14). To a stirred solution of **12** (19 mg, 0.029 mmol) in benzene (3.0 mL) was added *n*-Bu_3SnH (8.5 μL, 0.032 mmol) under an argon atmosphere. The reaction mixture was stirred for 100 min at rt and heated at 50 °C for an additional 190 min. *n*-Bu_3SnH (8.5 μL) was added and the mixture was maintained at 50 °C for an additional 2 h. The resulting mixture was diluted with $CHCl_3$, washed with brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator, and the residue was purified by preparative TLC (silica gel, 5:5:1 $CHCl_3$/MeCN/*n*-hexane as a developing solvent) to afford a yellow paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, the desired **14** (7.2 mg, 50%) was obtained as a yellow powder. The material was an equilibrium mixture of two diastereomers (about 1.5:1 by ^{1}H NMR in pyridine-d_5) at the C-6 position. In addition, compounds **16**[9] (1.2 mg, 10%) and **11**[8] (1.5 mg, 9.1%) were obtained as byproducts: ^{1}H NMR (270 MHz, pyridine-d_5) (major isomer) δ 2.07 (s, 3 H), 3.13 (s, 3 H), 3.4–3.6 (m, 2 H), 3.8–4.5 (m, 7 H), 4.55 (br t, J = 11 Hz, 1 H), 5.65 (dd, J = 4.6, 10.8 Hz, 1 H), 6.34 (s, 1 H), 7.4–7.9 (br s, 2 H); (minor isomer) δ 2.08 (s, 3 H), 3.14 (s, 3 H), 3.4–3.6 (m, 2 H), 3.8–4.5 (m, 7 H), 4.73 (br t, J = 11 Hz, 1 H), 5.54 (s, 1 H), 5.78 (dd, J = 4.7, 10.7 Hz, 1 H), 7.4–7.9 (br s, 2 H); FAB-MS m/z 486/488 (1:1) (M^+ + 1); FAB-HRMS calcd for $C_{18}H_{21}{}^{79}BrN_3O_8$ (M^+ + H) m/z 486.0511, found 486.0537; IR (KBr) 3460, 3340, 1710, 1600, 1570 cm^{-1}. Anal. Calcd for $C_{18}H_{20}BrN_3O_8$: C, 44.46; H, 4.15; N, 8.64. Found: C, 44.29; H, 4.28; N, 8.37.

1a-Acetyl-6-chloro-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin A (15). To a stirred solution of **13** (36 mg, about 50% purity, 0.030 mmol) in benzene (5.0 mL) was added *n*-Bu_3SnH (15 μL, 0.056 mmol) under an argon

(18) Monobromide **14** was also prepared in good yield from **18** by debromination using dimedone in the presence of potassium carbonate.

(19) Direct conversion of 6,6-dibromide **18** into 6-bromo-6-demethyl-MMA (**6**) using potassium carbonate in methanol in the presence of dimedone was unsuccessful because overdebromination occurred.

(20) The related 7,7-bis(methoxy) derivative was not isolated under the same reaction conditions when W was methyl or hydrogen: refs 7, 8, and 9. The formation of **22** and **23** was confirmed by ^{1}H NMR and FAB-MS. See Experimental Section.

(21) Reduction potential ($E_{1/2}$, V) values of mitomycins and their derivatives. 1 (MMC), −0.35; 2 (MMA), −0.17; **3**, −0.34; **4**, −0.33; **5a**, −0.35, **6**, −0.12; **7**, −0.12. The values were determined by differential pulse polarography. The electrolyte was a phosphate buffer (M/30, pH 7) containing 1.0 M of KCl. Compounds were measured at 10^{-3} M in the above solution. The biological activities of these compounds and derivatives with other mitomycin skeletons will be published elsewhere.

atmosphere. The reaction mixture was stirred at rt for 75 min and heated at reflux for an additional 105 min. *n*-Bu_3SnH (15 μL) was added to the mixture and the mixture was refluxed for an additional 1 h. The resulting mixture was diluted with $CHCl_3$, washed with brine, and dried over Na_2SO_4. The solvent was removed on a rotary evaporator, and the residue was purified by preparative TLC (silica gel, 5:5:1 $CHCl_3$/MeCN/*n*-hexane as a developing solvent) to afford a yellow paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, the desired **15** (4.8 mg, about 40%) was obtained as a yellow powder. The material was an equilibrium mixture of two diastereomers (about 2:1 by ^1H NMR in pyridine-d_5) at the C-6 position: ^1H NMR (270 MHz, pyridine-d_5) (major isomer) δ 2.12 (s, 3 H), 3.12 (s, 3 H), 3.4–3.6 (m, 1 H), 3.54 (br d, J = 13 Hz, 1 H), 3.8–4.6 (m, 7 H), 4.53 (br t, J = 11 Hz, 1 H), 5.63 (dd, J = 4.6, 10.8 Hz, 1 H), 6.28 (s, 1 H), 7.4–7.8 (br s, 2 H); (minor isomer) δ 2.07 (s, 3 H), 3.12 (s, 3 H), 3.4–3.6 (m, 2 H), 3.8–4.6 (m, 7 H), 4.67 (br t, J = 11 Hz, 1 H), 5.70 (dd, J = 5.0, 11.0 Hz, 1 H), 5.77 (s, 1 H), 7.4–7.8 (br s, 2 H); FAB-MS *m/z* 442/444 (2:1) (M^+ + 1); FAB-HRMS calcd for $C_{16}H_{21}{}^{35}ClN_3O_8$ (M^+ + H) *m/z* 442.1016, found 442.1058; IR (KBr) 3460, 3340, 1710, 1660, 1570 cm^{-1}. Anal. Calcd for $C_{18}H_{20}ClN_3O_8$: C, 48.93; H, 4.56; N, 9.51. Found: C, 49.28; H, 4.86; N, 9.23.

1a-Acetyl-6,6-dibromo-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin A (18). (1) Conversion from Enone 10. To a stirred solution of **10**[8b] (210 mg, 0.501 mmol) in THF (20 mL) was added Et_2NH (0.21 mL). The yellow solution turned brown immediately after addition of the amine. After 2 min, NBS (177 mg, 0.994 mmol) was added and stirring of the mixture was continued for 40 min. The resulting yellow mixture was quenched by the addition of phosphate buffer (pH 4). The aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with brine and dried over Na_2SO_4. After the solvent was removed, the residue was purified by column chromatography (silica gel, 30:1 $CHCl_3$/MeOH as an eluent) to afford a yellow paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, compound **18** (165 mg, 58%) was obtained as a yellow powder.

(2) Improved Method. To a slurry of **9**[7] (1.15 g, 2.00 mmol) and K_2CO_3 (828 mg, 6.00 mmol) in CH_2Cl_2 (40 mL) was added a solution of *m*-CPBA (655 mg, about 80% purity, 3.04 mmol) in CH_2Cl_2 (40 mL) over a period of 15 min at −40 °C. Stirring was continued for 1 h at −40 to −20 °C and an additional 90 min at 20 °C. The reaction mixture was quenched by the addition of saturated $NaHCO_3$ aqueous solution–10% $Na_2S_2O_3$ aqueous solution. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine and dried over Na_2SO_4 to afford a yellow solution of CH_2Cl_2 (about 100 mL) containing the crude **10**. To a stirred solution of **10** (about 100 mL in CH_2Cl_2) was added Et_2NH (620 μL). After 5 min at rt, NBS (715 mg, 4.02 mmol) was added, and stirring was continued at rt for an additional 30 min. The reaction mixture was quenched by the addition of phosphate buffer (pH 4). The aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed with brine and dried over Na_2SO_4. After the solvent was removed, the residue was purified by column chromatography (silica gel, 30:1 $CHCl_3$/MeOH as an eluent) to afford a yellow paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, compound **18** (702 mg, 62%) was obtained as a yellow powder: ^1H NMR (270 MHz, pyridine-d_5) δ 2.11 (s, 3 H), 3.24 (s, 3 H), 3.27 (dd, J = 2.0, 4.5 Hz, 1 H), 3.50 (dd, J = 2.0, 12.9 Hz, 1 H), 3.54 (d, J = 4.5 Hz, 1 H), 3.84 (dd, J = 4.8, 10.6 Hz, 1 H), 4.20 (t, J = 10.9 Hz, 1 H), 4.22 (d, J = 12.9 Hz, 1 H), 4.2–4.5 (m, 4 H), 4.82 (br s, 2 H), 4.92 (dd, J = 4.8, 10.9 Hz, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 23.6, 39.2, 42.2, 42.9, 48.0, 50.1, 61.6, 68.2, 68.4, 68.7, 106.1, 107.4, 120.3, 150.3, 156.4, 177.1, 180.4, 182.5; FAB-MS *m/z* 564/566/568 (1:2:1) (M^+ + 1); IR (KBr) 3450, 3360, 1710, 1670, 1575 cm^{-1}. Anal. Calcd for $C_{18}H_{19}Br_2N_3O_8$: C, 38.25; H, 3.39; N, 7.43. Found: C, 38.24; H, 3.18; 7.18.

1a-Acetyl-6,6-dichloro-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin A (19). (1) Conversion from Enone 10. To a stirred solution of **10** (211 mg, 0.504 mmol) in THF (20 mL) was added Et_2NH (0.21 mL) at rt. The yellow solution turned brown immediately after addition of the amine. After 2 min, NCS (267 mg, 2.00 mmol) was added and the mixture was stirred at rt. After 1.5 h, phosphate buffer (0.1 M, pH 4, 10 mL) was added to the resulting yellow solution, and the mixture was stirred for an additional 10 min. The resulting 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, 30:1 $CHCl_3$/MeOH as an eluent) to afford a yellow paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. The precipitate was filtered off and dried under vacuum to afford **19** (146 mg, 61%) as a yellow powder.

(2) Improved Method. To a solution of **10** (about 100 mL in CH_2Cl_2) prepared from **9** (1.17 g, 2.02 mmol) using the same method as that described in the synthesis of **19** was added Et_2NH (0.62 mL), and the mixture was stirred at rt for 5 min. NCS (1.07 g, 8.00 mmol) was added to the mixture by portions, and the mixture was stirred at rt. After an additional 1.5 h, the resulting yellow solution was poured into phosphate buffer (pH 4) and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed with brine and dried over Na_2SO_4. After the solvent was removed on a rotary evaporator, the residue was purified by column chromatography (silica gel, 20:1 $CHCl_3$/MeOH as an eluent) to afford a yellow paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. The resulting precipitate was filtered off and dried under vacuum to afford **19** (716 mg, 74%) as a yellow powder: ^1H NMR (270 MHz, $CDCl_3$) δ 2.11 (s, 3 H), 3.24 (s, 3 H), 3.27 (dd, J = 2.0, 4.5 Hz, 1 H), 3.50 (dd, J = 2.0, 12.7 Hz, 1 H), 3.54 (d, J = 4.5 Hz, 1 H), 3.81 (dd, J = 4.7, 10.6 Hz, 1 H), 4.20 (d, J = 12.7 Hz, 1 H), 4.21 (t, J = 10.9 Hz, 1 H), 4.1–4.5 (m, 4 H), 4.80 (br s, 2 H), 4.92 (dd, J = 4.7, 11.1 Hz, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 23.5, 39.2, 42.3, 42.9, 48.0, 50.0, 61.5, 68.2, 68.7, 87.7, 106.1, 107.2, 121.3, 151.7, 156.5, 176.9, 180.5, 183.0; FAB-MS *m/z* 476/478 (4:3) (M^+ + 1); FAB-HRMS calcd for $C_{18}H_{20}{}^{35}Cl_2N_3O_8$ (M^+ + H) *m/z* 476.0627, found 476.0661; IR (KBr) 3470, 3360, 1715, 1670, 1575 cm^{-1}. Anal. Calcd for $C_{18}H_{19}Cl_2N_3O_8$: C, 45.39; H, 4.02; N, 8.82. Found: C, 45.56; H, 4.06; N, 8.50.

6-Bromo-6-demethylmitomycin C (3). (1) Conversion from 6-Bromide 14. Compound **14** (11 mg, 0.022 mmol) was dissolved in a solution of NH_3 in MeOH (6.8 M, 1.0 mL), and the mixture was allowed to stand at rt for 2.5 h. After the volatiles were removed on a rotary evaporator, the residue was purified by preparative TLC (silica gel, 9:1 $CHCl_3$/MeOH as a developing solvent) to afford a purple paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, the desired **3** (7.5 mg, 86%) was obtained as a purple powder.

(2) Conversion from 6,6-Dibromide 18. Compound **18** (119 mg, 0.210 mmol) was dissolved in a solution of NH_3 in MeOH (6.1 M, 10 mL). Dimedone (90 mg, 0.64 mmol) was added to the solution, and the mixture was allowed to stand at rt for 3.5 h. After the solvent was removed on a rotary evaporator, the residue was purified by column chromatography (silica gel, 20:1–10:1 $CHCl_3$/MeOH as eluents) to afford a purple paste, which was dissolved in a small amount of CH_2Cl_2. The separated crystals were filtered off and dried under vacuum to afford **3** (46 mg, 55%) as fine needle, purple crystals: ^1H NMR (270 MHz, pyridine-d_5) δ 2.18 (br s, 1 H), 2.75 (br s, 1 H), 3.14 (br s, 1 H), 3.22 (s, 3 H), 3.59 (br d, J = 12 Hz, 1 H), 4.01 (dd, J = 4.2, 11.2 Hz, 1 H), 4.51 (d, J = 12.8 Hz, 1 H), 5.09 (br t, J = 11 Hz, 1 H), 5.37 (dd, J = 4.3, 10.5 Hz, 1 H), 7.4–7.9 (br s, 2 H), 8.5–8.8 (br s, 2 H); ^{13}C NMR (67.5 MHz, pyridine-d_5) δ 32.6 (C-2), 36.6 (C-1), 44.1 (C-9), 49.8 (9a-OCH_3), 50.7 (C-3), 62.3 (C-10), 94.0 (C-6), 107.1 (C-9a), 111.0 (C-8a), 151.5 (C-7), 156.0 (C-4a), 158.1 (10-$OCONH_2$), 172.0 (C-8), 173.3 (C-5); FAB-MS *m/z* 399/401 (1:1) (M^+ + 1); EI-HRMS calcd for $C_{14}H_{15}{}^{79}BrN_4O_5$ (M^+) *m/z* 398.0226, found 398.0255; IR (KBr) 3450, 3280, 1710, 1600, 1550 cm^{-1}. Anal. Calcd for $C_{14}H_{15}BrN_4O_5$: C, 42.12; H, 3.79; N, 14.03. Found: C, 42.25; H, 3.84; N, 13.54.

6-Chloro-6-demethylmitomycin C (4). (1) Conversion from 6-Chloride 13. Compound **13** (100 mg, 0.168 mmol) was dissolved in a solution of NH_3 in MeOH (6.1 M, 10 mL).

Dimedone (45 mg) was added to the solution and the mixture was allowed to stand at rt for 3.5 h. After the volatiles were removed on a rotary evaporator, the residue was purified by preparative TLC (silica gel, 9:1 $CHCl_3$/MeOH as a developing solvent) to afford a purple paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, the desired **4** (35 mg, 57%) was obtained as a purple powder.

(2) Conversion from 6,6-Dichloride 19. To a solution of **19** (1.88 g, 3.94 mmol) in THF (200 mL) were added *n*-Bu_3SnH (1.2 mL, 1.1 equiv) and Et_3B (1.0 M in *n*-hexane solution, 2.0 mL, 2.0 mmol) under an argon atmosphere, and the mixture was stirred at −40 °C for 4.5 h. The reaction was quenched by the addition of MeOH (50 mL), and the volatiles were removed on a rotary evaporator. The obtained residue was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane to afford a yellow precipitate, which was filtered off, washed with *n*-hexane, and dried under vacuum to afford crude **15** (1.46 g, 84%) as a yellow powder. To a solution of **15** (1.45 g, 3.30 mmol) in MeOH (50 mL) was added NH_3 in MeOH (6.8 M, 30 mL), and the mixture was allowed to stand at rt for 2.5 h. The volatiles were removed on a rotary evaporator, and the residue was purified by column chromatography (silica gel, 20:1–6:1 $CHCl_3$/MeOH as eluents) to afford a purple paste, which was crystallized from $CHCl_3$. The resulting fine needle, purple crystals were filtered off, washed with $CHCl_3$, and dried under vacuum to afford **4** (645 mg, 46% based on **19**). In addition, compound **4** (195 mg, 14% based on **19**) was also obtained as a purple powder from the filtrate: ^1H NMR (270 MHz, pyridine-d_5) δ 2.12 (br s, 1 H), 2.76 (br s, 1 H), 3.13 (br s, 1 H), 3.22 (s, 3 H), 3.59 (br d, J = 12.7 Hz, 1 H), 4.00 (dd, J = 4.2, 11.1 Hz, 1 H), 4.50 (d, J = 12.7 Hz, 1 H), 5.06 (br t, J = 10.6 Hz, 1 H), 5.34 (dd, J = 4.2, 10.2 Hz, 1 H), 7.3–7.8 (br s, 2 H), 8.48 (br s, 1 H), 8.67 (br s, 1 H); ^{13}C NMR (67.5 MHz, pyridine-d_5) δ 32.6 (C-2), 36.6 (C-1), 44.1 (C-9), 49.8 (9a-OCH_3), 50.7 (C-3), 62.3 (C-10), 103.0 (C-6), 107.1 (C-9a), 111.0 (C-8a), 149.8 (C-7), 156.0 (C-4a), 158.1 (10-$OCONH_2$), 171.9 (C-8), 173.7 (C-5); FAB-MS m/z 355/357 (2:1) (M^+ + 1); EI-HRMS calcd for $C_{14}H_{15}{}^{35}ClN_4O_5$ (M^+) m/z 354.0732, found 354.0720; IR (KBr) 3430, 3300, 1715, 1605, 1550 cm^{-1}. Anal. Calcd for $C_{14}H_{15}ClN_4O_5 \cdot 0.5H_2O$: C, 46.23; H, 4.43; N, 15.40. Found: C, 46.20; H, 4.39; N, 15.16.

6-Bromo-6-demethylmitomycin A (6). To a stirred solution of **18** (112 mg, 0.197 mmol) in MeOH (10 mL) were added dimedone (28 mg, 0.20 mmol) and K_2CO_3 (41 mg, 0.297 mmol). After 20 min at rt, the reaction mixture was poured into phosphate buffer (pH 4) 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 crude **14** (89 mg). To the obtained crude **14** (82 mg) in MeOH (10 mL) was added K_2CO_3 (520 mg), and the mixture was stirred for 24 h at rt. The reaction mixture was poured into phosphate buffer (pH 4) 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 preparative TLC (silica gel, 9:1 $CHCl_3$/MeOH as a developing solvent) to afford **22** as a brown paste, which was dissolved in a small amount of $CHCl_3$, and silica gel was added to the solution. After 140 min at rt, the reddish purple product was extracted with $CHCl_3$–MeOH (9:1). The solvent was removed and the residue was dissolved in a small amount of $CHCl_3$, which was poured into *n*-hexane, and the solvent was removed on a rotary evaporator. After drying under vacuum, the desired **6** (30 mg, 40% based on **18**) was obtained as a purple powder: ^1H NMR (270 MHz, pyridine-d_5) δ 2.20 (br s, 1 H), 2.76 (br s, 1 H), 3.13 (br s, 1 H), 3.22 (s, 3 H), 3.54 (br d, J = 12 Hz, 1 H), 3.98 (dd, J = 4.4, 10.8 Hz, 1 H), 4.19 (s, 3 H), 4.21 (d, J = 12.4 Hz, 1 H), 5.12 (br t, J = ca. 10 Hz, 1 H), 5.34 (dd, J = 4.5, 10.4 Hz, 1 H), 7.3–7.9 (br, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 32.7, 36.7, 43.2, 49.9, 49.9, 62.2, 62.4, 106.5, 112.1, 114.0, 151.4, 156.5, 158.8, 175.0, 175.4; FAB-MS m/z 414/416 (2:3) (M^+ + 1), 415/417 (1:1) (M^+ + 2), 416/418 (2:1) (M^+ + 3); FAB-HRMS calcd for $C_{15}H_{18}{}^{79}BrN_3O_6$ (M^+ + 2 H) m/z 415.0378, found 415.0348; IR (KBr) 3450, 1720, 1660, 1630, 1565 cm^{-1}.

Dimethyl acetal 22 (a mixture of two diastereomers, about 2:1): ^1H NMR (270 MHz, pyridine-d_5) (major isomer) δ 2.22 (br s, 1 H), 2.77 (br s, 1 H), 3.15 (br s, 1 H), 3.21 (s, 3 H), 3.24 (s, 3 H), 3.40 (s, 3 H), 3.51 (br d, J = ca. 12 Hz, 1 H), 3.92 (d, J = 12.4 Hz, 1 H), 4.11 (dd, J = 4.5, 11.4 Hz, 1 H), 4.9–5.2 (1 H, overlapped with H_2O), 5.34 (s, 1 H), 5.52 (dd, J = 4.5, 9.9 Hz, 1 H), 7.3–8.0 (br, 2 H); (minor isomer) δ 2.22 (s, 1 H), 2.77 (s, 1 H), 3.15 (s, 1 H), 3.19 (s, 3 H), 3.25 (s, 3 H), 3.37 (s, 3 H), 3.47 (br d, J = ca. 12 Hz, 1 H), 4.11 (dd, J = 4.5, 11.4 Hz, 1 H), 4.26 (d, J = 12.4 Hz, 1 H), 4.9–5.2 (1 H, overlapped with H_2O), 5.31 (dd, J = 4.5, 9.9 Hz, 1 H), 5.63 (s, 1 H), 7.3–8.0 (br, 2 H); FAB-MS m/z 446/448 (1:1) (M^+ + 1), $C_{16}H_{20}{}^{79}BrN_3O_7$ = 445.

6-Chloro-6-demethylmitomycin A (7). Crude **15** (1.18 g) was prepared from **19** (1.35 g, 2.84 mmol) using the same method as that described in the synthesis of **4**. To the crude **15** (677 mg) in MeOH (100 mL) was added K_2CO_3 (442 mg), and the mixture was stirred for 28.5 h at rt. The reaction mixture was poured into phosphate buffer (pH 4) 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 containing crude **23** was dissolved in a small amount of $CHCl_3$, and silica gel was added to the solution. After 2 h at rt, the reddish purple product was extracted with $CHCl_3$–MeOH (9:1). The solvent was removed and the residue was purified by column chromatography (silica gel, 30:1–20:1 $CHCl_3$/MeOH as eluents). The purple paste was dissolved in a small amount of $CHCl_3$, which was poured into *n*-hexane, and the solvent was removed on a rotary evaporator. After drying under vacuum, the desired **7** (168 mg, 28% based on **19**) was obtained as a purple powder: ^1H NMR (270 MHz, pyridine-d_5) δ 2.0–2.5 (br, 1 H), 2.78 (br s, 1 H), 3.14 (br s, 1 H), 3.23 (s, 3 H), 3.55 (br d, J = 12.4 Hz, 1 H), 3.98 (dd, J = 4.5, 10.9 Hz, 1 H), 4.18 (s, 3 H), 4.20 (d, J = 12.4 Hz, 1 H), 4.9–5.2 (1 H, overlapped with H_2O), 5.34 (dd, J = 4.5, 10.4 Hz, 1 H), 7.3–8.0 (br, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 32.7, 36.6, 43.2, 49.8, 49.8, 62.0, 62.3, 106.5, 113.9, 114.6, 151.4, 156.3, 156.5, 175.0, 175.7; FAB-MS m/z 370/372 (5:4) (M^+ + 1), 371/373 (1:1) (M^+ + 2); FAB-HRMS calcd for $C_{15}H_{17}{}^{35}ClN_3O_6$ (M^+ + H) m/z 370.0808, found 370.0814; IR (KBr) 3500, 1720, 1670, 1630, 1565 cm^{-1}.

Dimethyl acetal 23 (a mixture of two diastereomers, about 5:4): ^1H NMR (270 MHz, pyridine-d_5) (major isomer) δ 2.22 (br s, 1 H), 2.77 (br s, 1 H), 3.15 (br s, 1 H), 3.21 (s, 3 H), 3.25 (s, 3 H), 3.39 (s, 3 H), 3.50 (dd, J = 2.0, 12.4 Hz, 1 H), 3.94 (d, J = 12.4 Hz, 1 H), 4.08 (dd, J = 4.5, 10.9 Hz, 1 H), 4.9–5.2 (1 H, overlapped with H_2O), 5.29 (s, 1 H), 5.50 (dd, J = 4.5, 10.6 Hz, 1 H), 7.3–8.0 (br, 2 H); (minor isomer) δ 2.22 (br s, 1 H), 2.77 (br s, 1 H), 3.15 (br s, 1 H), 3.18 (s, 3 H), 3.32 (s, 3 H), 3.37 (s, 3 H), 3.45 (dd, J = 2.0, 12.4 Hz, 1 H), 4.08 (dd, J = 4.5, 10.9 Hz, 1 H), 4.15 (d, J = 12.4 Hz, 1 H), 4.9–5.2 (1 H, overlapped with H_2O), 5.33 (dd, J = 4.5, 10.4 Hz, 1 H), 5.68 (s, 1 H), 7.3–8.0 (br, 2 H); FAB-MS m/z 402/404 (3:1) (M^+ + 1), $C_{16}H_{20}{}^{35}ClN_3O_7$ = 401.

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylthio)mitomycin A (20a). To a stirred solution of **10** (1.27 g, 3.03 mmol) in CH_2Cl_2 (90 mL) were added Et_2NH (315 μL) and a solution of benzenesulfenyl chloride (prepared from 450 μL of thiophenol and 640 mg of NCS) in CH_2Cl_2 (3.0 mL) in three portions. After 5 h at rt, the reaction mixture was washed with brine and dried over Na_2SO_4. The solvent was removed, and the residue was purified by column chromatography (silica gel, 2:1–1:1 $CHCl_3$/MeCN as eluents) to afford a brown paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed and the residue was dried under vacuum, the desired **20a** (678 mg, 44%) was obtained as a brown powder. The material was an equilibrium mixture of diastereomers (about 2:1 in $CDCl_3$) at the C-6 position. In addition, 6,6-bis(phenylthio) derivative **21a** (111 mg, 6%) was obtained as a byproduct: ^1H NMR (270 MHz, $CDCl_3$) (major isomer) δ 2.18 (s, 3 H), 3.21 (s, 3 H), 3.25 (dd, J = 1.9, 4.5 Hz, 1 H), 3.42 (dd, J = 1.9, 13.0 Hz, 1 H), 3.51 (d, J = 4.5 Hz, 1 H), 3.74 (dd, J = 4.9, 11.1 Hz, 1 H), 3.91 (d, J = 13.0 Hz, 1 H), 3.96 (s, 1 H), 4.0–4.4 (m, 4 H), 4.20 (t, J = 10.9 Hz, 1 H), 4.77 (br s, 2 H), 4.94 (dd, J = 4.9, 10.9 Hz, 1 H), 7.1–7.4 (m, 3 H), 7.4–7.6 (m, 2 H); (minor isomer) δ 2.11 (s, 3 H), 3.15–3.2 (1 H, overlapped with other peaks), 3.21 (s, 3 H), 3.4–3.5 (1 H, overlapped with other peaks), 3.48 (d, J = 4.5 Hz, 1 H), 3.71 (dd, J = 11.9 Hz, 1 H, overlapped with other peaks), 4.0–4.4 (m, 7 H), 4.77 (br s, 2 H), 4.85 (dd, J = 4.7, 10.6 Hz, 1 H), 7.1–7.4 (m, 3 H), 7.4–7.6 (m, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) (major isomer) δ 23.6 (1a-$COCH_3$), 39.3 (C-2), 42.7 (C-9), 43.1 (C-1), 47.9 (C-3),

50.0 (9a-OCH_3), 61.8 (C-10), 62.4 (C-6), 66.3 (ethylenedioxy), 67.7 (ethylenedioxy), 105.6 (C-7 or C-9a), 105.7 (C-7 or C-9a), 120.1 (C-8a), 129.0 (phenyl), 129.3 (phenyl), 132.2 (phenyl), 133.7 (phenyl), 155.1 (C-4a), 156.5 (10-$OCONH_2$), 180.7 (1a-$COCH_3$), 184.4 (C-5), 186.0 (C-8); (minor isomer) δ 23.6 (1a-$COCH_3$), 39.3 (C-2), 42.4 (C-9), 43.1 (C-1), 47.6 (C-3), 49.9 (9a-OCH_3), 61.5 (C-10), 65.4 (C-6), 67.2 (ethylenedioxy), 67.2 (ethylenedioxy), 105.4 (C-7 or C-9a), 105.9 (C-7 or C-9a), 120.6 (C-8a), 128.3 (phenyl), 129.2 (phenyl), 132.8 (phenyl), 133.5 (phenyl), 153.9 (C-4a), 156.5 (10-$OCONH_2$), 180.9 (1a-$COCH_3$), 184.6 (C-5), 186.2 (C-8); FAB-MS *m/z* 516 (M^+ + 1); FAB-HRMS calcd for $C_{24}H_{26}N_3O_8S$ (M^+ + H) *m/z* 516.1442, found 516.1461; IR (KBr) 3450, 3050, 1700, 1650, 1570 cm^{-1}.

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6,6-bis(phenylthio)mitomycin A (21a): ^1H NMR (270 MHz, $CDCl_3$) δ 2.21 (s, 3 H), 3.16 (s, 3 H), 3.21 (dd, *J* = 2.0, 4.7 Hz, 1 H), 3.30 (dd, *J* = 2.0, 13.4 Hz, 1 H), 3.45 (d, *J* = 4.7 Hz, 1 H), 3.52 (d, *J* = 13.4 Hz, 1 H), 3.68 (dd, *J* = 4.7, 11.1 Hz, 1 H), 4.0–4.4 (m, 4 H), 4.23 (t, *J* = 10.9 Hz, 1 H), 4.80 (br s, 2 H), 4.94 (dd, *J* = 4.7, 11.1 Hz, 1 H), 7.2–7.7 (m, 10 H); FAB-MS *m/z* 624 (M^+ + 1); FAB-HRMS calcd for $C_{30}H_{30}N_3O_8S_2$ (M^+ + H) *m/z* 624.1475, found 624.1499; IR (KBr) 3450, 3350, 3050, 1700, 1655, 1575 cm^{-1}.

6-Demethyl-6-(phenylthio)mitomycin C (5a). To a solution of **20a** (226 mg, 0.440 mmol) in MeOH (10 mL) was added a solution of NH_3 in MeOH (6.8 M, 4.0 mL), and the mixture was allowed to stand at rt for 2.5 h. The volatiles were removed on a rotary evaporator, and the residue was purified by column chromatography (silica gel, 30:1 $CHCl_3$/MeOH as an eluent) to afford a brown paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed and the residue was dried under vacuum, the desired **5a** (108 mg, 58%) was obtained as a brown powder: ^1H NMR (270 MHz, pyridine-d_5) δ 2.18 (br s, 1 H), 2.74 (br s, 1 H), 3.15 (br s, 1 H), 3.21 (s, 3 H), 3.59 (br d, *J* = ca. 13 Hz, 1 H), 4.10 (dd, *J* = 4.5, 11.4 Hz, 1 H), 4.56 (d, *J* = 12.9 Hz, 1 H), 5.03 (br t, *J* = ca. 11 Hz, 1 H), 5.44 (dd, *J* = 4.5, 10.4 Hz, 1 H), 7.0–7.4 (m, 5 H), 7.3–7.9 (br, 2 H), 8.91 (br s, 1 H), 8.98 (br s, 1 H); FAB-MS *m/z* 429 (M^+ + 1); IR (KBr) 3430, 3300, 3070, 1720, 1590, 1560 cm^{-1}. Anal. Calcd for $C_{20}H_{20}N_4O_5S \cdot 0.8H_2O$: C, 54.24; H, 4.92; N, 12.65. Found: C, 54.24; H, 4.95; N, 12.46.

6-Demethyl-6-(phenylthio)mitomycin A (8). To a stirred solution of **20a** (277 mg, 0.537 mmol) in MeOH (40 mL) was added K_2CO_3 (235 mg, 1.70 mmol). After 23 h at rt, the reaction mixture was poured into phosphate buffer (pH 4) and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by column chromatography (silica gel, 40:1–30:1 $CHCl_3$/MeOH as eluents) to afford a dark brown paste, which was dissolved in a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed on a rotary evaporator and the residue was dried under vacuum, the desired **8** (65 mg, 27%) was obtained as a brown powder: ^1H NMR (270 MHz, pyridine-d_5) δ 2.18 (br s, 1 H), 2.73 (br s, 1 H), 3.15 (br s, 1 H), 3.20 (s, 3 H), 3.48 (br d, *J* = 12.4 Hz, 1 H), 4.02 (dd, *J* = 4.3, 11.4 Hz, 1 H), 4.14 (s, 3 H), 4.17 (d, 1 H, overlapped with 7-OCH_3), 5.06 (br t, *J* = ca. 11 Hz, 1 H), 5.38 (dd, *J* = 4.3, 10.4 Hz, 1 H), 7.1–7.3 (m, 3 H), 7.2–8.0 (br, 2 H), 7.4–7.5 (m, 2 H); FAB-MS *m/z* 445 (M^+ + 2); IR (KBr) 3400, 3000, 1750, 1655, 1630, 1575 cm^{-1}. Anal. Calcd for $C_{21}H_{21}N_3O_6S$: C, 56.88; H, 4.77; N, 9.48. Found: C, 56.53; H, 5.07; N, 8.89.

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-((*p*-methoxyphenyl)thio)mitomycin A (20b). As described in the synthesis of **20a**, compound **10** (430 mg, 1.03 mmol) was treated with *p*-methoxybenzenesulfenyl chloride (prepared from 130 μL of *p*-methoxythiophenol and 145 mg of NCS) and Et_2NH (105 μL) to afford **20b** (202 mg, 36%) as a dark yellow powder. The product was an equilibrium mixture of two diastereomers (about 2:1 in $CDCl_3$) at the C-6 position: ^1H NMR (270 MHz, $CDCl_3$) (major isomer) δ 2.20 (s, 3 H), 3.20 (s, 3 H), 3.27 (dd, *J* = 2.0, 4.8 Hz, 1 H), 3.44 (dd, *J* = 2.0, 13.2 Hz, 1 H), 3.50 (d, *J* = 4.8 Hz, 1 H), 3.70 (dd, *J* = 4.8, 10.9 Hz, 1 H), 3.80 or 3.82 (s, 1 H), 3.81 (s, 3 H), 3.9–4.5 (m, 4 H), 3.92 (d, *J* = 13.2 Hz, 1 H), 4.14 (t, *J* = 11.1 Hz, 1 H), 4.81 (br s, 2 H), 4.90 (dd, *J* = 4.8, 10.9 Hz, 1 H), 6.8–6.9 (m, 2 H), 7.4–7.5 (m, 2 H); (minor isomer) δ 2.10 (s, 3 H), 3.21 (s, 3 H), 3.23 (dd, *J* = 2.0 Hz, 1 H), 3.48 (d, 1 H, overlapped with other peaks), 3.68 (dd, *J* = 11.4 Hz, 1 H, overlapped with other peaks), 3.80 or 3.82 (s, 3 H), 3.9–4.5 (m, 7 H), 4.33 (d, *J* = 12.9 Hz, 1 H), 4.81 (br s, 2 H), 4.82 (dd, 1 H, overlapped with other peaks), 6.8–6.9 (m, 2 H), 7.4–7.5 (m, 2 H); FAB-MS *m/z* 546 (M^+ + 1); FAB-HRMS calcd for $C_{25}H_{28}N_3O_9S$ (M^+ + H) *m/z* 546.1547, found 546.1576; IR (KBr) 3450, 3100, 1700, 1650, 1570 cm^{-1}.

6-Demethyl-6-((*p*-methoxyphenyl)thio)mitomycin C (5b). A similar procedure as that described in the synthesis of **5a** was employed to convert **20b** (181 mg, 0.332 mmol) into the desired **5b** (87 mg, 57%): ^1H NMR (270 MHz, pyridine-d_5) δ 2.15 (br s, 1 H), 2.73 (br s, 1 H), 3.14 (br s, 1 H), 3.20 (s, 3 H), 3.57 (s, 3 H), 3.59 (br d, *J* = ca. 13 Hz, 1 H), 4.06 (dd, *J* = 4.2, 11.1 Hz, 1 H), 4.57 (d, *J* = 12.9 Hz, 1 H), 5.10 (br t, *J* = ca. 10 Hz, 1 H), 5.40 (dd, *J* = 4.5, 10.4 Hz, 1 H), 6.79 (d, *J* = 8.9 Hz, 2 H), 7.2–7.9 (br, 2H), 7.41 (d, *J* = 8.9 Hz, 2 H), 8.78 (br s, 1 H), 8.87 (br s, 1 H); FAB-MS *m/z* 460 (M^+ + 2); IR (KBr) 3450, 3300, 1720, 1590, 1550 cm^{-1}. Anal. Calcd for $C_{21}H_{22}N_4O_6S \cdot 0.5H_2O$: C, 53.95; H, 4.96; N, 11.98. Found: C, 53.92; H, 4.70; N, 11.81.

1a-Acetyl-6-((*p*-chlorophenyl)thio)-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin A (20c). As described in the synthesis of **20a**, compound **10** (208 mg, 0.496 mmol) was treated with *p*-chlorobenzenesulfenyl chloride (prepared from 126 mg of *p*-chlorothiophenol and 117 mg of NCS) and Et_2NH (150 μL) to afford **20c** (67 mg, 25%) as a dark yellow powder. The product was an equilibrium mixture of two diastereomers (about 2:1 in $CDCl_3$) at the C-6 position. In addition, 6,6-bis((*p*-chlorophenyl)thio) derivative **21c** (64 mg, 19%) was also obtained as a byproduct: ^1H NMR (270 MHz, $CDCl_3$) (major isomer) δ 2.18 (s, 3 H), 3.22 (s, 3 H), 3.27 (dd, 1 H, overlapped with other peaks), 3.43 (dd, *J* = 2.0, 13.2 Hz, 1 H), 3.52 (d, *J* = 4.5 Hz, 1 H), 3.75 (dd, *J* = 4.5, 10.9 Hz, 1 H), 3.92 (s, 1 H), 3.95 (d, *J* = 13.2 Hz, 1 H), 4.0–4.4 (m, 4 H), 4.22 (t, *J* = 10.9 Hz, 1 H), 4.77 (br s, 2 H), 4.96 (dd, *J* = 5.0, 10.9 Hz, 1 H), 7.1–7.4 (m, 2 H), 7.4–7.6 (m, 2 H); (minor isomer, main peaks) δ 2.11 (s, 3 H), 3.2–3.3 (1 H, overlapped with other peaks), 3.4–3.5 (1 H, overlapped with other peaks), 3.48 (d, *J* = 4.5 Hz, 1 H), 3.49 (s, 3 H), 3.71 (dd, *J* = 4.5, 10.9 Hz, 1 H), 4.0–4.4 (m, 7 H), 4.77 (br s, 2 H), 4.86 (dd, *J* = 5.0, 10.9 Hz, 1 H), 7.1–7.4 (m, 2 H), 7.4–7.6 (m, 2 H); FAB-MS *m/z* 550/552 (2:1) (M^+ + 1); FAB-HRMS calcd for $C_{24}H_{25}{}^{35}ClN_3O_8S$ (M^+ + H) *m/z* 550.1053, found 550.1073; IR (KBr) 3450, 3350, 3050, 1700, 1660, 1570 cm^{-1}.

1a-Acetyl-6,6-bis((*p*-chlorophenyl)thio)-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin A (21c): ^1H NMR (270 MHz, $CDCl_3$) δ 2.20 (s, 3 H), 3.17 (s, 3 H), 3.27 (dd, *J* = 1.9, 4.7 Hz, 1 H), 3.34 (dd, *J* = 1.9, 12.9 Hz, 1 H), 3.50 (d, *J* = 4.5 Hz, 1 H), 3.62 (d, *J* = 12.9 Hz, 1 H), 3.70 (dd, *J* = 5.0, 10.9 Hz, 1 H), 4.0–4.4 (m, 4 H), 4.21 (t, *J* = 10.9 Hz, 1 H), 4.89 (br s, 2 H), 4.96 (dd, *J* = 5.0, 10.9 Hz, 1 H), 7.21–7.32 (m, 4 H), 7.42–7.51 (m, 4 H); FAB-MS *m/z* 692/694/696 (4:3:1) (M^+ + 1); FAB-HRMS calcd for $C_{30}H_{28}{}^{35}Cl_2N_3O_8S_2$ (M^+ + H) *m/z* 692.0699, found 692.0679; IR (KBr) 3450, 3350, 3050, 1700, 1650, 1570 cm^{-1}.

6-((*p*-Chlorophenyl)thio)-6-demethylmitomycin C (5c). A similar procedure as that described in the synthesis of **5a** was employed to convert **20c** (180 mg, 0.327 mmol) into the desired **5c** (67 mg, 44%): ^1H NMR (270 MHz, pyridine-d_5) δ 2.17 (s, 1 H), 2.75 (br s, 1 H), 3.14 (br s, 1 H), 3.22 (s, 3 H), 3.59 (br d, *J* = ca. 13 Hz, 1 H), 4.07 (dd, *J* = 4.2, 11.1 Hz, 1 H), 4.55 (d, *J* = 12.9 Hz, 1 H), 5.09 (br t, *J* = ca. 11 Hz, 1 H), 5.40 (dd, *J* = 4.0, 10.4 Hz, 1 H), 7.10–7.23 (m, 4H), 7.6 (br s, 2H), 9.03 (br s, 2H); FAB-MS *m/z* 464 (M^+ + 2); IR (KBr) 3420, 3300, 1710, 1590, 1550 cm^{-1}. Anal. Calcd for $C_{20}H_{19}ClN_4O_5S \cdot 0.6H_2O$: C, 50.60; H, 4.50; N, 11.80. Found: C, 50.65; H, 4.11; N, 11.49.

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6,6-bis((*p*-nitrophenyl)thio)mitomycin A (21d). As described in the synthesis of **20a**, compound **10** (210 mg, 0.501 mmol) was treated with *p*-nitrobenzenesulfenyl chloride (600 mg, prepared from bis(*p*-nitrophenyl) disulfide and chlorine) and Et_2NH (400 μL) to afford 6,6-bis((*p*-nitrophenyl)thio) derivative **21d** (107 mg, 30%): ^1H NMR (270 MHz, $CDCl_3$) δ 2.21 (s, 3 H), 3.23 (s, 3 H), 3.31 (dd, 1 H, overlapped with other peaks), 3.34 (dd, *J* = 2.0, 13 Hz, 1 H), 3.55 (d, *J* = 13 Hz, 1 H), 3.58 (d, *J* = 4.6 Hz, 1 H), 3.78 (dd, *J* = 4.9, 10.9 Hz, 1 H), 4.04–4.43 (m, 4 H), 4.22 (t, *J* = 10.9 Hz, 1 H), 5.01 (dd, *J* = 4.9, 11.1 Hz, 1 H), 5.13 (br s, 2 H), 7.60–7.78 (m, 4 H), 8.06–8.20 (m, 4 H); FAB-MS *m/z*

714 ($M^+ + 1$); FAB-HRMS calcd for $C_{30}H_{28}N_5O_{12}S_2$ ($M^+ + H$) m/z 714.1178, found 714.1182; IR (KBr) 3450, 3350, 3050, 1700, 1660, 1570, 1370 cm^{-1}. Anal. Calcd for $C_{30}H_{27}N_5O_{12}S_2$: C, 50.49; H, 3.81; N, 9.81. Found: C, 50.27; H, 3.98; N, 9.59.

6-Demethyl-6-((*p*-nitrophenyl)thio)mitomycin C (5d). To a stirred solution of **21d** (55 mg, 0.0774 mmol) was added NH_3 in MeOH (6.8 M, 10 mL). Dimedone (23 mg, 0.164 mmol) was added to the solution. After 75 min at rt, the reaction mixture was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by preparative TLC (silica gel, 9:1 $CHCl_3$/MeOH as a developing solvent) to afford a brown paste, which was dissolved into a small amount of $CHCl_3$ and poured into *n*-hexane. After the solvent was removed and the residue was dried under vacuum, the desired **5d** (9.9 mg, 27%) was obtained as a brown powder: ^1H NMR (270 MHz, pyridine-d_5) δ 2.25 (br s, 1 H), 2.79 (br s, 1 H), 3.20 (br s, 1 H), 3.26 (s, 3 H), 3.63 (br d, J = ca. 13 Hz, 1 H), 4.13 (dd, J = 4.0, 10.9 Hz, 1 H), 4.59 (d, J = 12.9 Hz, 1 H), 5.14 (br t, J = 10.6 Hz, 1 H), 5.45 (dd, J = 4.0, 11.4 Hz, 1 H), 7.25 (d, J = 8.9 Hz, 2 H), 7.4–7.9 (br, 2 H), 7.94 (d, J = 8.9 Hz, 2 H), 9.28 (br s, 1 H), 9.36 (br s, 1 H); FAB-MS m/z 475 ($M^+ + 2$); IR (KBr) 3370, 3260, 3150, 1750, 1625, 1595, 1545, 1370 cm^{-1}. Anal. Calcd for $C_{20}H_{19}N_5O_7S \cdot 1.1CHCl_3$: C, 41.91; H, 3.34; N, 11.58. Found: C, 41.92; H, 3.16; N, 11.19.

6-Demethyl-6-[(thiazol-2-yl)thio]mitomycin C (5e). To a stirred solution of **10** (426 mg, 1.02 mmol) in CH_2Cl_2 (40 mL) was added Et_2NH (110 μL) and bis(benzothiazol-2-yl) disulfide (335 mg, 1.01 mmol). After 2 h at rt, the reaction mixture was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator to afford the crude **20e** as a brown paste. A similar procedure as that described in the synthesis of **5a** was employed to convert this crude **20e** into the desired **5e** (100 mg, 23% based on **10**): ^1H NMR (270 MHz, pyridine-d_5) δ 2.21 (br s, 1 H), 2.76 (br s, 1 H), 3.17 (br s, 1 H), 3.23 (s, 3 H), 3.60 (br d, J = 13.4 Hz, 1 H), 4.09 (dd, J = 4.2, 11.1 Hz, 1 H), 4.57 (d, J = 12.9 Hz, 1 H), 5.05 (br t, J = ca. 11 Hz, 1 H), 5.42 (dd, J = 4.2, 10.6 Hz, 1 H), 7.21 (1 H, overlapped with pyridine), 7.64 (d, J = 3.0 Hz, 1 H), 7.3–8.0 (br, 2 H), 9.33 (br s, 1 H), 9.67 (br s, 1 H); FAB-MS m/z 437 ($M^+ + 2$); IR (KBr) 3400, 3340, 1750, 1625, 1580 cm^{-1}. Anal. Calcd for $C_{17}H_{17}N_5O_5S_2 \cdot 0.6H_2O$: C, 45.75; H, 4.11; N, 15.69. Found: C, 45.88; H, 3.87; N, 14.89.

6-[(Benzothiazol-2-yl)thio]-6-demethylmitomycin C (5f). To a stirred solution of **10** (421 mg, 1.00 mmol) in CH_2Cl_2 (40 mL) were added Et_2NH (105 μL) and bis(benzothiazol-2-yl) disulfide (335 mg, 1.01 mmol). After 30 min at rt, the reaction mixture was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator to afford the crude **20f** as a brown paste. A similar procedure as that described in the synthesis of **5a** was employed to convert this crude **20f** into the desired **5f** (172 mg, 36% based on **10**): ^1H NMR (270 MHz, pyridine-d_5) δ 2.28 (br s, 1 H), 2.80 (br s, 1 H), 3.20 (br s, 1 H), 3.28 (s, 3 H), 3.64 (br d, J = 12.6 Hz, 1 H), 4.17 (dd, J = 4.1, 11.1 Hz, 1 H), 4.61 (d, J = 12.8 Hz, 1 H), 5.19 (br t, J = 10.5 Hz, 1 H), 5.49 (dd, J = 4.2, 10.4 Hz, 1 H), 7.20 (m, 1 H), 7.33 (m, 1 H), 7.4–8.0 (br, 2 H), 7.73 (m, 1 H), 7.85 (m, 1 H), 9.89 (br s, 1 H), 9.56 (br s, 1 H); FAB-MS m/z 486 ($M^+ + 1$); FAB-HRMS calcd for $C_{21}H_{20}N_5O_5S_2$ ($M^+ + H$) m/z 486.0907, found 486.0929; IR (KBr) 3470, 3370, 1720, 1600, 1570 cm^{-1}. Anal. Calcd for $C_{21}H_{19}N_5O_5S_2 \cdot 0.1CHCl_3$: C, 50.94; H, 3.87; N, 14.08. Found: C, 51.36; H, 4.17; N, 13.70.

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Supplementary Material Available: ^1H NMR spectra of **5f, 6, 7, 12, 20a, 20b,** and **21a** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.