Synthesis and Antitumor Activity of Various 6-Demethylmitomycins and 6-Demethyl-6-halomitomycins

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A series of 6-demethylmitomycins and 6-demethyl-6-halomitomycins having various mitomycin skeletons were synthesized, taking into account the electronic effect toward the quinone moiety and the partition coefficients. Treatment of enones **15** and **16** with selenenamide or *N*-halosuccinimide–Et_2NH afforded the 6-demethyl intermediates **17**, **18**, and **21–24** via the tandem Michael addition/retro-Mannich reaction sequence. Subsequent conversions into the mitomycin skeletons resulted in the formation of the desired derivatives **7a–c**, **8a–c**, **11a–c**, and **12a,b**. These mitomycin derivatives including **3a–c** and **4a–c** were evaluated for their anticellular activity against HeLa S_3 cells and antitumor activity against Sarcoma 180 in mice. The anticellular activity of **1** and **3a–c** depends on the substituent at the C-6 position and the order of increasing activity is H < CH_3 < Br < Cl. A similar tendency was observed in their antitumor potency (ED_{50}). The activities of **9** and **11a–c** also follow a pattern similar to that of **1** and **3a–c**. Compounds **4b,c**, **8b,c**, and **12b** having both a halogen at the C-6 position and a methoxy group at the C-7 position did not show the activities because of the instability of the compounds. Interestingly, a correlation between the anticellular activity (IC_{50}) and the partition coefficients (log k') determined by HPLC was observed within the compounds studied except the unstable compounds, while their antitumor activity (ED_{50} or T/C) did not correlate with the quinone reduction potential ($E_{1/2}$). These results would indicate the importance of the C-6 substituents and the mitomycin skeletons for exhibiting both anticellular and antitumor activities.

Introduction

Mitomycins are well-known to be potent antitumor antibiotics produced by various *Streptomyces* cultures. Among these compounds, mitomycin C (MMC, **1**) has been used extensively in cancer chemotherapy, but its use is limited by detrimental side effects such as myelosuppression and gastrointestinal damage.[1] MMC is believed to exhibit its antitumor activity through the formation of covalent cross-linking adducts with DNA after the activation caused by the reduction of the quinone moiety.[1c] Therefore, modification of the quinone moiety to modulate the quinone reduction potential with varying the partition coefficient is an attractive strategy for obtaining more effective or less toxic mitomycin derivatives. From this viewpoint, modification at the C-7 position has been frequently used for the derivatization of mitomycins.[2,3] Some effective compounds were found among derivatives modified at the C-7 position.[3] However, few examples of derivatives modified at the C-6 position[4,5] have been reported to date because of the difficulties of modification.

In the previous papers,[6] we have reported methods for modification at the C-6 or C-6-methyl positions. Introduction of several functional groups at the C-6 position has become feasible using the tandem Michael addition/retro-Mannich reaction sequence.[6e] Our study showed a new approach in the modification of mitomycins for making a hitherto unknown modification at the C-6 position.

With the goal of finding new candidates having greater activity than that of conventional mitomycins or their derivatives, various 6-demethylmitomycins and 6-demethyl-6-halomitomycins were synthesized and evaluated for their anticellular and antitumor activities. Bromine and chlorine atoms seemed to be especially suitable for evaluation of the electronic effects because their atomic sizes[7] are similar to that of the methyl group, and they would minimize the conformational change in mitomycins caused by the steric effects of the C-6 substituents. Further, the role of the C-6 substitutent should be revealed by examination of the structure–activity relationships of these derivatives. In this paper, we report our investigation into the synthesis, anticellular and antitumor activities, and structure–activity relationships of these derivatives.

Chemistry

Compounds **3a–c** and **4a–c** were prepared according to the method reported previously.[6d,e] Other derivatives having different mitomycin skeletons were also prepared based on this method (Schemes 1–3).

6-Demethylmitomycins (**7a**, **8a**, **11a**, and **12a**) were prepared as follows (Scheme 1). To obtain the 6-demethyl-6-(phenylseleno) intermediate **17** having the mitomycin B (MMB) skeleton, the tandem Michael addition/retro-Mannich reaction sequence[6b,c] was applied to **15**. Treatment of crude **15** prepared by seleno oxidation of **13**[6f] with *N*-(phenylseleno)morpholine[8] gave **17** in 33% yield based on **13**. Similarly, compound **18** having the mitomycin F (MMF) skeleton was prepared from **14**[6f] in two steps in 45% yield based on **14**. Deselenenylation of **17** and **18** was achieved by a nucleophilic method using dimedone[6d] to afford **19** and **20**, respectively. Sequential treatment of the reaction mixtures containing **19** and **20** with NH_3 in MeOH

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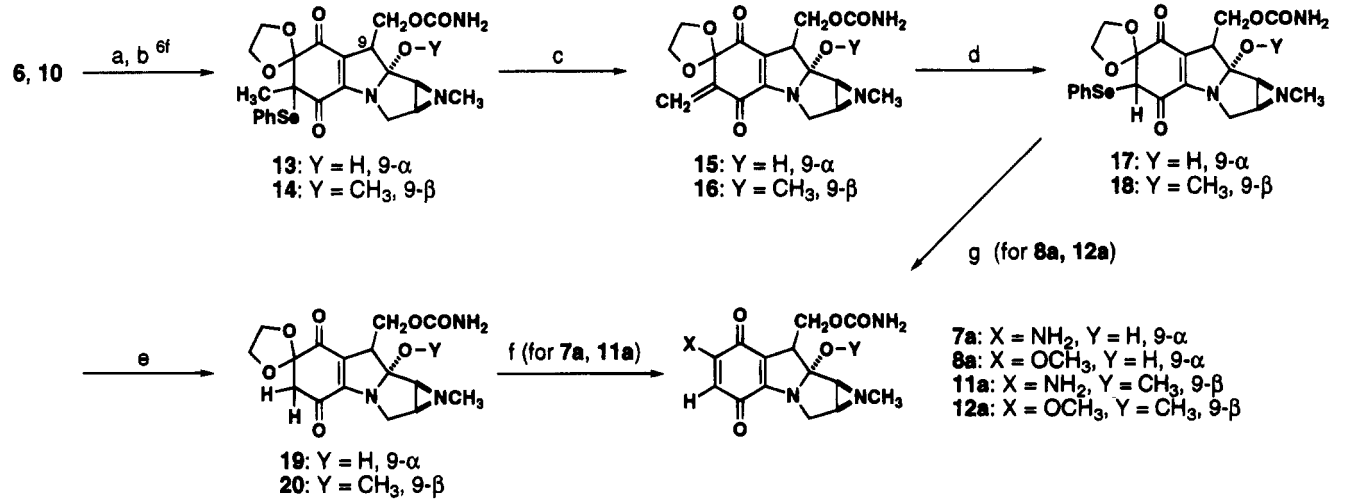
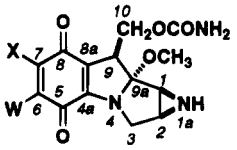
Scheme 1[a]

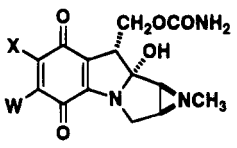


[a] (a) KOH (catalytic), ethylene glycol, THF; (b) PhSeBr, NEt_3, THF; (c) *m*-CPBA, K_2CO_3, CH_2Cl_2; (d) *N*-(phenylseleno)morpholine (e) dimedone, NEt_3, MeCN; (f) NH_3, MeOH; (g) dimedone, K_2CO_3, MeOH.

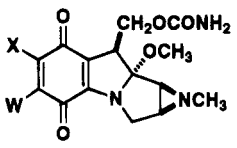
afforded the desired 6-demethylmitomycin D (**7a**) and 6-demethylporfiromycin (**11a**) in 54% and 40% yields, respectively, based on **17** and **18**. For the synthesis of **8a** and **12a** having a methoxy group at the C-7 position, direct conversion was applied to **17** and **18**. Treatment of **17** with K_2CO_3 in MeOH in the presence of dimedone afforded 6-demethylmitomycin B (**8a**) in 54% yield. 6-Demethylmitomycin F (**12a**) was also prepared by the same procedure in 31% yield.



1: Mitomycin C (MMC, X = NH_2, W = CH_3)
2: Mitomycin A (MMA, X = CH_3O, W = CH_3)
3: X = NH_2, W = H (**a**), Br (**b**), Cl (**c**)
4: X = OCH_3, W = H (**a**), Br (**b**), Cl (**c**)



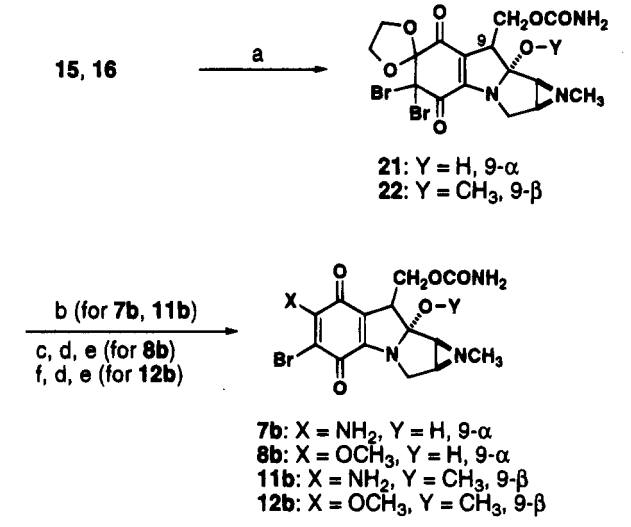
5: Mitomycin D (MMD, X = NH_2, W = CH_3)
6: Mitomycin B (MMB, X = CH_3O, W = CH_3)
7: X = NH_2, W = H (**a**), Br (**b**), Cl (**c**)
8: X = OCH_3, W = H (**a**), Br (**b**), Cl (**c**)



9: Porfiromycin (PFM, X = NH_2, W = CH_3)
10: Mitomycin F (MMF, X = CH_3O, W = CH_3)
11: X = NH_2, W = H (**a**), Br (**b**), Cl (**c**)
12: X = OCH_3, W = H (**a**), Br (**b**)

6-Bromo-6-demethylmitomycins (**7b**, **8b**, **11b**, and **12b**) were prepared as follows (Scheme 2).[6e] The tandem Michael addition/retro-Mannich reaction sequence using Et_2NH and NBS was applied to crude enones **15** and **16**. 6,6-Dibromides **21** and **22** were obtained using this method in 43% and 48% yields, respectively, based on **13** and **14**. Direct conversion of **21** and **22** into 6-bromo-6-demethylmitomycin D (**7b**, 63% yield) and 6-bromo-6-demethylporfiromycin (**11b**, 67% yield) was achieved by treatment with NH_3 in MeOH in the presence of dimedone as a nucleophilic debrominating agent. For the synthesis of **8b** and **12b**, stepwise conversion was necessary.[9] Treatment of **21** with K_2CO_3 in MeOH in the presence of dimedone afforded the 6-monodebrominated compound of **21**. Subsequent treatment with K_2CO_3 in MeOH for the C-7-transalkoxylation followed by contact with silica gel afforded 6-bromo-6-demethylmitomycin B (**8b**) in 40%

Scheme 2[a]



[a] (a) Et_2NH, NBS, THF; (b) dimedone, NH_3, MeOH; (c) dimedone, K_2CO_3, MeOH; (d) K_2CO_3, MeOH; (e) silica gel, $CHCl_3$; (f) dimedone, NEt_3, MeCN.

yield based on **21**. A similar method was employed to convert **22** into 6-bromo-6-demethylmitomycin F (**12b**) in 33% yield based on **22**.

For the preparation of 6-chloro-6-demethylmitomycins (**7c**, **8c**, and **11c**), 6,6-dichlorides **23** and **24** prepared by a method similar to that for dibromide (38% and 84% yields based on **13** and **14**, respectively) were used (Scheme 3). Dechlorination of **23** or **24** was performed by radically initiated dechlorination using the Et_3B–*n*-Bu_3SnH system[10] to afford 6-monochlorides **25** (81%) and **26** (91%), respectively. Subsequent conversion into 6-chloro-6-demethylmitomycin D (**7c**) and 6-chloro-6-demethylporfiromycin (**11c**) was achieved by the same method as that for **7a** and **11a** in 45% and 62% yields, respectively. 6-Chloro-6-demethylmitomycin B (**8c**) was prepared by treatment of **25** with K_2CO_3 in MeOH in 27% yield.

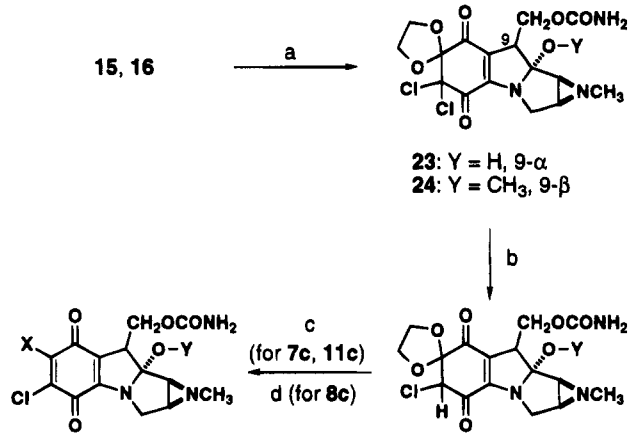
In addition, compounds **11a**, **11b**, and **12a** having the porfiromycin (PFM) and MMF skeletons were also prepared alternatively by methylation at the N-1a position of **3a**, **3b**, and **4a** in 77%, 53%, and 70% yields, respectively (Scheme 4).

Table 1. Anticellular and Antitumor Activities of Various Mitomycin Derivatives

compd	W	HeLa S_3[a] IC_{50}[e] ratio[f]	Sarcoma 180 (sc–iv)[b] ED_{50}[g] (mg/kg)	OD[h] (mg/kg)	T/C (min)[i]	$E_{1/2}$[c]	log k' [d]
1 (MMC)	CH_3	1	1.7–2.3	6.0	0.20–0.28	−0.35	0.321
3a	H	3.2	2.6	5.0	0.25	−0.33	−0.0686
3b	Br	0.49	1.6	3.1	0.27	−0.34	0.454
3c	Cl	0.37	1.4	2.7	0.14	−0.33	0.350
2 (MMA)	CH_3	3.5×10^{-3}	1.3	1.8	0.20	−0.17	1.15
4a	H	0.30	1.6	2.0	0.45	−0.22	0.336
4b	Br	1.9	8.4	14	0.47	nt[j]	nt[j]
4c	Cl	2.5	nt[j]	nt[j]	nt[j]	−0.12	nt
5 (MMD)	CH_3	>20		100	0.59	−0.35	−0.146
7a	H	>71	33	100	0.26	−0.33	−0.528
7b	Br	>35		30	0.55	−0.33	0.0133
7c	Cl	>20	17	20	0.43	−0.33	0.0856
6 (MMB)	CH_3	0.29	2.8	4.0	0.37	−0.17	0.693
8a	H	16	11	20	0.31	−0.21	−0.139
8b	Br	>3.7	22	30	0.44	−0.12	nt
8c	Cl	11		20	0.62	−0.11	nt
9 (PFM)	CH_3	9.5	16	40	0.23	−0.35	0.520
11a	H	16	8.4	20	0.08	−0.33	0.131
11b	Br	1.9		4.0	0.63	−0.33	0.645
11c	Cl	0.76	2.3	4.0	0.11	−0.33	0.658
10 (MMF)	CH_3	<0.031	6.5	8.0	0.32	−0.17	1.34
12a	H	0.29	7.1	10	0.36	−0.22	0.517
12b	Br	>4.8		14	0.97	−0.12	nt

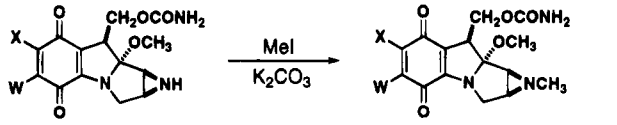
[a] In vitro anticellular activity against HeLa S_3 cells. The cells were cultured in 96-well plates on day 0 and treated with drugs for 1 h on day 1. The anticellular activity was determined according to the method described previously (see ref 3b). [b] In vivo antitumor activity against Sarcoma 180. Sarcoma 180 cells were inoculated sc into the axillary region of ddY mice on day 0. Compounds were administered iv on day 1. [c] Reduction potential ($E_{1/2}$) was determined by differential pulse polarography on a Model Yanako P-1100 polarographic analyzer. Analytical conditions: electrolyte, phosphate buffer (M/30, pH 7.0) containing 1.0 M KCl; sample, 10^{-3} M in the above solution; potential scan rate, 2 mV/s; voltage range, 1.25 V (initial potential: 0 V); modulation amplitude, 50 mV; rate of mercury drops, 60 times/min. [d] Logarithm of chromatographic capacity factors (k') for the estimation of octanol–water partition coefficients (log P). Chromatographic capacity factors (k') were calculated by the formula: $k' = (t_R - t_0)/t_0$. Log k' is the estimated partition coefficient (log P) where t_R is the mitomycin's retention time and t_0 is the retention time of an unretained substance (potassium iodide). Analytical conditions: column, YMC AM-312 S-5 (ODS, 6 mm i.d. × 150 mm); temperature, 37 °C; eluent, MeOH–phosphate buffer (M/30, pH 7.0), 30:70; flow rate, 1.0 mL/min; sample, 1.0 μg/injection (KI: 0.05 mg/injection); detection, UV, 254 nm. [e] Concentration that gave 50% inhibition of cell growth calculated from the concentration–response curve. [f] Ratio of IC_{50} value, treated with the test compound *versus* a reference drug (MMC) (ratio of IC_{50} value) = (IC_{50} value of the test compound)/(IC_{50} value of MMC). [g] Dose that gave 50% inhibition of tumor growth calculated from the dose–response curve. [h] Optimal dose. [i] Treated *versus* control value of tumor volume. Tumor volume was calculated according to the method described previously (see ref 3b). [j] Not tested.

Scheme 3[a]



[a] (a) Et_2NH, NCS, THF; (b) *n*-Bu_3SnH, Et_3B, THF, −40 °C; (c) NH_3, MeOH; (d) K_2CO_3, MeOH.

Scheme 4



Biological Activity and Discussion

Table 1 shows in vitro anticellular activity of mitomycin derivatives against HeLa S_3 cells and in vivo antitumor activity against Sarcoma 180 solid tumor in mice. The anticellular activity of **1** and **3a–c** depends on the substituent at the C-6 position. The order of increasing activity is H < CH_3 < Br < Cl. The same order is also observed in that of **9** and **11a–c**. In the case of compounds having a methoxy group at the C-7 position, the anticellular activity of the natural compounds (**2**, **6**, and **10**) is stronger than that of the corresponding 6-demethyl derivatives (**4a**, **8a**, and **12a**). However, the 6-demethyl-6-halo-7-methoxy derivatives (**4b,c**, **8b,c**, and **12b**) are quite ineffective. To investigate the unusual ineffectiveness of these derivatives, we examined their stability in the cell culture medium (MEM medium). As a result, these 6-demethyl-6-halo-7-methoxy derivatives were easily decomposed in a few minutes after addition to the medium, while other compounds were stable under the same conditions (data are not shown). These results suggest that the ineffectiveness of the 6-demethyl-6-halo-7-methoxy derivatives is due to their instability in the medium.[11] A tendency similar to that observed in the anticellular activity of the C-7-methoxy derivatives was also observed in the antitumor potency (ED_{50}).

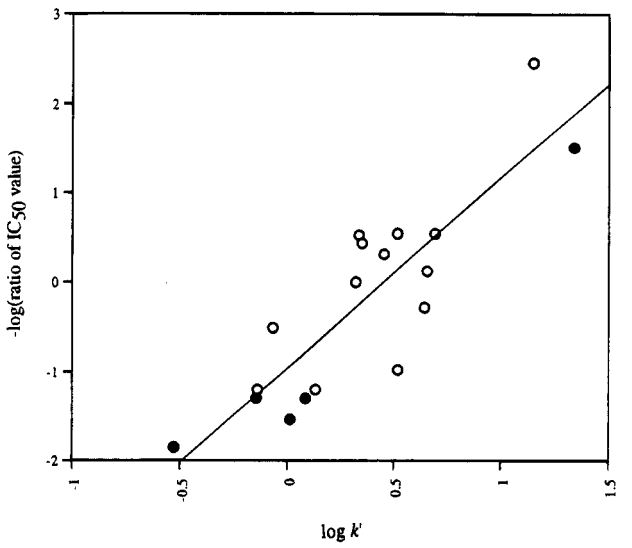


Figure 1. Correlation between the in vitro anticellular activity (ratio of IC_{50} value) and the partition coefficients (log k') of mitomycins. Data from Table 1. (○) Compounds having the ratio of IC_{50} value without sign of inequality. (●) Compounds having the ratio of IC_{50} value with sign of inequality. The correlation coefficient of linear regression (r) is 0.86 (○ + ●) (0.80 for ○ only).

On the other hand, the relationship between the in vivo antitumor activity (T/C) and the C-6 substituents is somewhat different. 7-Amino-6-demethyl derivatives (**3a**, **7a**, and **11a**) showed stronger activity (smaller T/C values) at lower doses compared to the corresponding natural compounds (**1**, **5**, and **9**). 7-Amino-6-demethyl-6-halo derivatives except **11b** (**3b,c**, **7b,c**, and **11c**) are almost as potent as the corresponding natural compounds, whereas the 6-demethyl-6-halo-7-methoxy derivatives (**4b**, **8b,c**, and **12b**) are ineffective. Above all, the in vivo activity of **3b,c** and **11a,c** is outstanding within the derivatives synthesized, although their doses are lower. The substituent effect at the C-6 is remarkably observed in **11c**, which shows a T/C value smaller than that of **9** at a dose of only one-tenth that of **9**.

For further examination of the structure–activity relationship, partition coefficients of each derivative were measured. The partition coefficients of the mitomycins or their derivatives determined by HPLC[12] varied with respect to the C-6 substituent, *i.e.*, the order of increasing lipophilicity is H < CH_3 < halogen. Their anticellular activity also increases in that order. As shown in Figure 1, the anticellular activity (IC_{50}) correlates well with the partition coefficients (log k') except for the unstable 6-demethyl-6-halo-7-methoxy derivatives. This indicates that the partition coefficients are dominant for the anticellular activity of mitomycins within the range of structural variations of the compounds studied exept the unstable compounds, even if the mitomycin skeletons are different.[13] A weak correlation between ED_{50} and the log k' values was also observed, which suggests that the lipophilicity plays some role in the antitumor activity. However, this factor is not sufficient to explain the difference in antitumor potency and activity (ED_{50} and T/C) between compounds having the same mitomycin skeleton (for example, **1** and **3a–c**).

To evaluate the electronic effect of the C-6 substituents toward the quinone moiety, the reduction potential ($E_{1/2}$) of the quinone moiety was measured by polarography. The $E_{1/2}$ values of the C-7-methoxy derivatives vary according to their C-6 substituents; that is, the $E_{1/2}$ values of compounds **4a**, **8a**, and **12a** are higher (more easily reduced) than those of the corresponding natural compounds, whereas those of **4c**, **8b,c**, and **12b** are lower (less easily reduced).[14] These results are acceptable when considering the substituent effect of quinones on their reduction potential.[15] On the contrary, in the case of the 7-amino compounds (**1**, **3a–c**, **5**, **7a–c**, **9**, and **11a–c**), the difference of $E_{1/2}$ values is marginal. These results indicate that the $E_{1/2}$ of the derivatives, which describes the first step in the activation mechanism,[16] seems not to be dominant in their antitumor potency and activity.[17] However, there is a possibility that some electronic effect of halogens contributes to the in vivo antitumor activity, because **3b,c** and **11c** showed superior activity to that of MMC (**1**) and PFM (**9**), respectively, although their log k' values are similar to that of mother compounds (**1** and **9**).

Conclusions

A series of 6-demethylmitomycins and 6-demethyl-6-halomitomycins were prepared and evaluated for their anticellular activity against HeLa S_3 cells and antitumor activity against Sarcoma 180 in mice. Some of these derivatives, for example compounds **3b,c** and **11c**, showed antitumor activity superior to that of MMC even at the lower doses. Modification at the C-6 position was shown to be a promising approach for preparing new candidates for antitumor drugs. Further detailed studies of the structure–activity relationships of these derivatives are in progress.

Experimental Section

General. Unless otherwise noted, materials were obtained from commercial suppliers except for mitomycins and were used without purification. NBS and NCS were recrystallized from water. 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 (^1H) and carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on Bruker AM 400 and JEOL JNM GX270 instruments. Mass spectral (MS) data was obtained from Hitachi M-80B and 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 careful analysis of NMR spectra.

7-Demethoxy-6-demethyl-7,7-(ethylenedioxy)-6-methylene-6,7-dihydromitomycin B (15). To a stirred suspension of **13**[6f] (2.675 g, 5.01 mmol) and K_2CO_3 (1.37 g, 9.93 mmol) in CH_2Cl_2 (50 mL) was added a solution of *m*-CPBA (1.27 g, about 80% purity) in CH_2Cl_2 (20 mL) over a period of 15 min at −40 °C. After an additional 35 min at room temperature, the reaction mixture was filtered through Celite, and the filtrate was concentrated on a rotary evaporator up to about 50 mL. The solution was poured into *n*-hexane to afford a yellow powder, which was filtered off, washed with *n*-hexane, and dried under vacuum to afford crude **15** (2.034 g): FAB-HRMS calcd for $C_{17}H_{20}N_3O_7$ (M^+ + H) *m/z* 378.1300, found 378.1334.

7-Demethoxy-6-demethyl-7,7-(ethylenedioxy)-6-methylene-6,7-dihydromitomycin F (16). To a stirred suspension of **14**[6f] (2.736 g, 4.99 mmol) and K_2CO_3 (2.09 g, 15.1 mmol) in CH_2Cl_2 (100 mL) was added a solution of *m*-CPBA (1.39 g, about 80% purity) in CH_2Cl_2 (50 mL) over a period of 15 min at −40 °C. After stirring for an additional 40 min at −20 °C and for 50 min at room temperature, the reaction mixture was poured into a $NaHCO_3$–$Na_2S_2O_3$ aqueous solution and ex-

tracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator up to about 50 mL. The solution was poured into *n*-hexane to afford a yellow powder, which was filtered off, washed with *n*-hexane, and dried under vacuum to afford **16** (1.731 g, 89%): FAB-HRMS calcd for $C_{18}H_{22}N_3O_7$ ($M^+ + H$) *m/z* 392.1457, found 392.1460.

7-Demethoxy-6-demethyl-7,7-(ethylenedioxy)-6-(phenylseleno)-6,7-dihydromitomycin B (17). To a slurry of **13**[6f] (5.398 g, 10.11 mmol) and K_2CO_3 (2.80 g, 20.3 mmol) in CH_2Cl_2 (200 mL) was added a solution of *m*-CPBA (2.88 g, about 80% purity, 13.3 mmol) in CH_2Cl_2 (100 mL) over a period of 40 min at −40 °C, and the mixture was stirred for an additional 30 min at that temperature. After stirring for an additional 40 min at room temperature, the reaction mixture was filtered through Celite. The filtrate was concentrated to about half volume on a rotary evaporator and poured into *n*-hexane. The precipitate was filtered off, washed with *n*-hexane, and dried under vacuum to afford crude **15** (4.453 g) as a yellow powder. To the solution of crude **15** (1.043 g) in CH_2Cl_2 (50 mL) was added a solution of *N*-(phenylseleno)-morpholine (350 mg, 1.45 mmol) in CH_2Cl_2 (20 mL) over a period of 1.5 h at 0 °C. After stirring for an additional 3 h at room temperature, the reaction mixture was subjected directly to column chromatography (silica gel, 2:1−1:1 $CHCl_3$−MeCN as eluents) to obtain a yellow solution. The solvent was removed on a rotary evaporator, and the residue was triturated with $CHCl_3$−*n*-hexane followed by drying under vacuum to afford **17** (404 mg, 33% based on **13**) as a yellow powder. The product was obtained as an equilibrium mixture of two diastereomers at C-6 (approximately 2:1 in $CDCl_3$). In addition, the 6,6-bis(phenylseleno) derivative (55 mg, 3.4%) was also obtained as a byproduct: ^{1}H NMR (270 MHz, $CDCl_3$) δ (major isomer) 2.23 (1 H, 2-H, overlapped with other peaks), 2.28 (d, J = 5.0 Hz, 1 H, 1-H), 2.34 (s, 3 H, 1a-CH_3), 3.26 (dd, J = 1.5, 12.4 Hz, 1 H, 3α-H), 3.41 (d, J = 12.4 Hz, 1 H, 3β-H), 3.81 (dd, J = 2.6, 4.7 Hz, 1 H, 9-H), 4.12 (s, 1 H, 6-H), 4.0−4.3 (m, 3 H, ethylenedioxy), 4.39 (m, 1 H, ethylenedioxy), 4.70 (dd, J = 2.6, 10.4 Hz, 1 H, 10-H_a), 4.71 (dd, J = 4.7, 10.4 Hz, 1 H, 10-H_b), 4.4−4.8 (br, 3 H, 9a-OH + 10-$OCONH_2$), 7.25−7.36 (m, 3 H, phenyl), 7.63−7.71 (m, 2 H, phenyl); (minor isomer) 2.22 (s, 3 H, 1a-CH_3), 2.2−2.3 (2 H, 1-H + 2-H, overlapped with other peaks), 3.26 (dd, J = 1.5, 12.7 Hz, 1 H, 3α-H), 3.33 (d, J = 12.7 Hz, 1 H, 3β-H), 3.71 (dd, J = 2.0, 5.5 Hz, 1 H, 9-H), 4.27 (s, 1 H, 6-H), 4.0−4.3 (m, 3 H, ethylenedioxy), 4.39 (m, 1 H, ethylenedioxy), 4.4−4.8 (br, 3 H, 9a-OH + 10-$OCONH_2$), 4.6−4.8 (2 H, 10-H_a + 10-H_b, overlapped with other peaks), 7.25−7.36 (m, 3 H, phenyl), 7.63−7.71 (m, 2 H, phenyl); FAB-MS *m/z* 520/522 (2:1) ($M^+ + 1$); FAB-HRMS calcd for $C_{22}H_{24}N_3O_7{}^{80}Se$ ($M^+ + H$) *m/z* 522.0777, found 522.0808; IR (KBr) 3450, 3350, 3050, 2950, 2900, 1720, 1710, 1690, 1660, 1650, 1570, 1560, 1440, 1420, 1340, 1240, 1210, 1110, 1030 cm^{-1}.

7-Demethoxy-6-demethyl-6,7-dihydro-7,7-(ethylenedioxy)-6,6-bis(phenylseleno)mitomycin B: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.19 (br d, J = 4.5 Hz, 1 H, 2-H), 2.26 (d, J = 4.5 Hz, 1 H, 1-H), 2.38 (s, 3 H, 1a-CH_3), 3.08 (d, J = 12.7 Hz, 1 H, 3β-H), 3.14 (dd, J = 2.0, 12.7 Hz, 1 H, 3α-H), 3.89 (dd, J = 2.0, 5.7 Hz, 1 H, 9-H), 3.92 (m, 1 H, ethylenedioxy), 4.04 (m, 1 H, ethylenedioxy), 4.25 (m, 1 H, ethylenedioxy), 4.40 (m, 1 H, ethylenedioxy), 4.5−4.8 (br, 3 H, 9a-OH + 10-$OCONH_2$), 4.60−4.73 (m, 2 H, 10-H_a + 10-H_b), 7.19−7.42 (m, 6 H, phenyl), 7.59−7.80 (m, 4 H, phenyl); FAB-MS *m/z* 674/676/678 (1:2:2) ($M^+ + 1$); FAB-HRMS calcd for $C_{28}H_{28}N_3O_7{}^{80}Se_2$ ($M^+ + H$) *m/z* 678.0256, found 678.0287; IR (KBr) 3450, 3350, 3050, 2950, 2900, 1720, 1710, 1700, 1660, 1650, 1640, 1580, 1570, 1440, 1420, 1340, 1210, 1190, 1110, 1040 cm^{-1}.

7-Demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin F (18). To a stirred solution of **16** (1.292 g, 3.30 mmol) in CH_2Cl_2 (50 mL) was added a solution of *N*-(phenylseleno)morpholine (560 mg, 2.31 mmol) in CH_2Cl_2 (20 mL) over a period of 1.5 h at 0 °C. After stirring for an additional 1 h at room temperature, the reaction mixture was subjected directly to column chromatography (silica gel, 3:1−2:1 $CHCl_3$−MeCN as eluents) to obtain a yellow solution. The solvent was removed on a rotary evaporator, and the residue was triturated with $CHCl_3$−*n*-hexane followed by drying under vacuum to afford **18** (879 mg, 45% based on **14**) as a yellow powder. Compound **18** was obtained as an equilibrium mixture of two diastereomers at C-6 (2.3:1 in $CDCl_3$). In addition, a bis(phenylseleno) derivative (53 mg, 2.9%) was also obtained as a byproduct: ^{1}H NMR (270 MHz, $CDCl_3$) δ (major isomer) 2.21 (dd, J = 2.0, 4.5 Hz, 1 H, 2-H), 2.28 (d, J = 4.5 Hz, 1 H, 1-H), 2.34 (s, 3 H, 1a-CH_3), 3.16 (s, 3 H, 9a-OCH_3), 3.27 (dd, J = 2.0, 12.4 Hz, 1 H, 3α-H), 3.45 (d, J = 12.4 Hz, 1 H, 3β-H), 3.56 (dd, J = 4.7, 10.6 Hz, 1 H, 9-H), 4.12 (s, 1 H, 6-H), 4.46 (t, J = 10.6 Hz, 1 H, 10-H_a), 4.0−4.5 (m, 4H, ethylenedioxy), 4.69 (dd, J = 4.7, 10.6 Hz, 1 H, 10-H_b), 4.76 (br s, 2 H, 10-$OCONH_2$), 7.25−7.40 (m, 3 H, phenyl), 7.60−7.70 (m, 2 H, phenyl); (minor isomer) 2.21 (dd, J = 2.0, 4.5 Hz, 1 H, 2-H), 2.24 (s, 3 H, 1a-CH_3), 2.27 (d, J = 4.5 Hz, 1 H, 1-H), 3.20 (s, 3 H, 9a-OCH_3), 3.32 (dd, J = 2.0, 12.4 Hz, 1 H, 3α-H), 4.0−4.5 (m, 8 H, 3β-H + 9-H + ethylenedioxy + 9-H + 10-H_a), 4.65 (dd, J = 4.7, 10.6 Hz, 1 H, 10-H_b), 4.76 (br s, 2 H, 10-$OCONH_2$), 7.25−7.40 (m, 3 H, phenyl), 7.60−7.70 (m, 2 H, phenyl); FAB-MS *m/z* 534/536 (1:2) ($M^+ + 1$); FAB-HRMS calcd for $C_{23}H_{26}N_3O_7{}^{80}Se$ ($M^+ + H$) *m/z* 536.0934, found 536.0936; IR (KBr) 3450, 3370, 3050, 2950, 2900, 1730, 1710, 1700, 1660, 1650, 1570, 1470, 1450, 1340, 1240, 1210, 1140, 1080, 1030 cm^{-1}.

7-Demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6,6-bis(phenylseleno)mitomycin F: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.16 (br d, J = 4.7 Hz, 1 H, 2-H), 2.26 (d, J = 4.7 Hz, 1 H, 1-H), 2.35 (s, 3 H, 1a-CH_3), 3.13 (s, 3 H, 9a-OCH_3), 3.1−3.2 (2 H, 3-H, overlapped with other peaks), 3.54 (dd, J = 4.5, 10.9 Hz, 1 H, 9-H), 4.0−4.6 (m, 4 H, ethylenedioxy), 4.52 (t, J = 10.6 Hz, 1 H, 10-H_a), 4.72 (dd, J = 4.5, 10.4 Hz, 1 H, 10-H_b), 4.73 (br s, 2 H, 10-$OCONH_2$), 7.20−7.41 (m, 6 H, phenyl), 7.59−7.66 (m, 4 H, phenyl); FAB-MS *m/z* 688/690/692 (1:2:2) ($M^+ + 1$); IR (KBr) 3450, 3350, 3050, 2950, 2900, 1730, 1710, 1660, 1580, 1570, 1450, 1440, 1340, 1190, 1070, 1030 cm^{-1}. Anal. ($C_{23}H_{25}N_3O_7Se{\cdot}0.2CHCl_3$) C, N; H: calcd, 4.55; found: 3.90.

6-Demethylmitomycin D (7a). To a solution of **17** (54 mg, 0.10 mmol) in MeCN (1.0 mL) were added dimedone (28 mg, 0.20 mmol) and NEt_3 (50 μL), and the mixture was allowed to stand at room temperature. After 24 h, consumption of **17** and formation of **19** were checked by TLC. To the resulting mixture containing **19** was added NH_3 in MeOH (6.8 M, 1.0 mL). After 17 h, the volatiles were removed on a rotary evaporator, and the residue 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 the vacuum to afford **7a** (17 mg, 54% based on **17**) as a grayish green powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.12 (s, 3 H, 1a-CH_3), 2.23 (dd, J = 2.0, 4.8 Hz, 1 H, 2-H), 2.48 (d, J = 4.8 Hz, 1 H, 1-H), 3.69 (dd, J = 1.8, 12.9 Hz, 1 H, 3α-H), 4.30 (dd, J = 3.5, 10.8 Hz, 1 H, 9-H), 4.50 (d, J = 12.9 Hz, 1 H, 3β-H), 5.27 (t, J = 10.4 Hz, 1 H, 10-H_a), 5.58 (dd, J = 3.5, 10.5 Hz, 1 H, 10-H_b), 5.77 (s, 1 H, 6-H), 7.3−8.0 (br s, 4 H, 7-NH_2 + 10-$OCONH_2$), 8.3−8.6 (br s, 1 H, 9a-OH); FAB-MS *m/z* 321 ($M^+ + 1$), 343 ($M^+ + Na$); EI-HRMS calcd for $C_{14}H_{16}N_4O_5$ (M^+) *m/z* 320.1121, found 320.1127; IR (KBr) 3420, 3350, 2960, 2920, 1715, 1600, 1550, 1540, 1340, 1080, 1000 cm^{-1}.

7-Demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin B (19). Purification of the above reaction mixture containing **19** using PTLC (silica gel, 9:1 $CHCl_3$−MeOH as a developing solvent) to afford pure **19**, which was triturated with $CHCl_3$−*n*-hexane followed by drying under vacuum to afford **19** as a slight yellow powder. This was used as an analytical sample: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.10 (s, 3 H, 1a-CH_3), 2.20 (dd, J = 1.8, 4.7 Hz, 1 H, 2-H), 2.46 (d, J = 4.7 Hz, 1 H, 1-H), 3.08 (d, J = 16.1 Hz, 1 H, 6-H_a), 3.33 (d, J = 16.1 Hz, 1 H, 6-H_b), 3.53 (dd, J = 1.9, 12.5 Hz, 1 H, 3α-H), 3.8−4.0 (m, 4 H, ethylenedioxy + 3β-H), 4.25−4.35 (m, 1 H, ethylenedioxy), 4.29 (dd, J = 3.4, 9.7 Hz, 1 H, 9-H), 5.21 (br t, J = 10 Hz, 1 H, 10-H_a), 5.44 (dd, J = 3.4, 10.5 Hz, 1 H, 10-H_b), 7.2−7.6 (br s, 2 H, 10-$OCONH_2$), 8.30 (s, 1 H, 9a-OH); FAB-MS *m/z* 366 ($M^+ + 1$); FAB-HRMS calcd for $C_{16}H_{20}N_3O_7$ ($M^+ + H$) *m/z* 366.1301, found 366.1294; IR (KBr) 3480, 3370, 3320, 3200, 3050, 2970, 2900, 1730, 1710, 1640, 1570, 1480, 1460, 1330, 1020 cm^{-1}.

6-Demethylmitomycin B (8a). To a solution of **17** (86 mg, 0.17 mmol) in MeOH (10 mL) were added dimedone (30 mg, 0.21 mmol) and K_2CO_3 (35 mg, 0.25 mmol), and the mixture was stirred at room temperature for 20.5 h. The reaction mixture was diluted with $CHCl_3$ and washed successively with phosphate buffer (pH 7) and brine. The aqueous layer was extracted with $CHCl_3$–*i*-PrOH (5:1), and combined organic layer was dried over Na_2SO_4. After the solvent was removed, the 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 vacuum to afford **8a** (29 mg, 54%) as a purple powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.14 (s, 3 H, 1a-CH_3), 2.24 (dd, J = 1.8, 4.7 Hz, 1 H, 2-H), 2.48 (d, J = 4.7 Hz, 1 H, 1-H), 3.50 (s, 3 H, 7-OCH_3), 3.60 (dd, J = 1.8, 12.6 Hz, 1 H, 3α-H), 4.22 (d, J = 12.6 Hz, 1 H, 3β-H), 4.25 (dd, J = 3.3, 9.3 Hz, 1 H, 9-H), 5.24 (br t, J = 10 Hz, 1 H, 10-H_a), 5.45 (dd, J = 3.3, 10.6 Hz, 1 H, 10-H_b), 5.61 (s, 1 H, 6-H), 7.3–7.8 (br, 2 H, 10-$OCONH_2$), 8.33 (s, 1 H, 9-OH); FAB-MS *m/z* 336 (M^+ + 1); EI-HRMS calcd for $C_{15}H_{17}N_3O_6$ (M^+) *m/z* 335.1117, found 335.1147; IR (KBr) 3460, 3360, 3200, 2950, 1710, 1650, 1570, 1450, 1420, 1340, 1240, 1120, 1070, 1040, 1000 cm^{-1}.

6-Demethylporfiromycin (11a). (1) Methylation of 6-Demethylmitomycin C (3a). To a solution of **3a**[6d] (123 mg, 0.385 mmol) in DMF (10 mL) were added MeI (1.0 mL) and K_2CO_3 (50 mg, 0.36 mmol), and the mixture was stirred at room temperature for 4.5 h. The reaction mixture was diluted with $CHCl_3$, and insoluble salts were removed by filtration. After the solvent was removed on a rotary evaporator, the residue was purified by column chromatography (silica gel, 40:1–20:1 $CHCl_3$–MeOH as eluents) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **11a** (100 mg, 77%) as a purple powder.

(2) Sequential Conversion from 6-Selenide 18. To a solution of **18** (54 mg, 0.10 mmol) in MeCN (1.0 mL) were added dimedone (28 mg, 0.20 mmol) and NEt_3 (50 μL), and the mixture was allowed to stand at room temperature. After 8 h, consumption of **18** and formation of **20** were checked by TLC, and the mixture containing **20** was obtained. Then NH_3 in MeOH (6.8 M, 1.0 mL) was added to the mixture. After 10 h, the volatiles were removed on a rotary evaporator, and the residue was purified by preparative TLC (silica gel, 9:1 $CHCl_3$–MeOH as a developing solvent). The paste obtained was triturated with $CHCl_3$–*n*-hexane followed by drying under vacuum to afford **11a** (14 mg, 40% based on **18**) as a purple powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.15 (dd, J = 2.0, 4.7 Hz, 1 H, 2-H), 2.24 (s, 3 H, 1a-CH_3), 2.54 (d, J = 4.7 Hz, 1 H, 1-H), 3.18 (s, 3 H, 9a-OCH_3), 3.53 (dd, J = 2.0, 12.8 Hz, 1 H, 3α-H), 4.02 (dd, J = 4.3, 11.5 Hz, 1 H, 9-H), 4.53 (d, J = 12.8 Hz, 1 H, 3β-H), 4.82 (br t, J = 11 Hz, 1 H, 10-H_a), 5.40 (dd, J = 4.3, 10.4 Hz, 1 H, 10-H_b), 5.79 (s, 1 H, 6-H), 7.4–8.1 (br s, 4 H, 7-NH_2 + 10-$OCONH_2$); FAB-MS *m/z* 335 (M^+ + 1); EI-HRMS calcd for $C_{14}H_{14}N_4O_4$ (M^+ − MeOH) *m/z* 302.1014, found 302.1036; IR (KBr) 3420, 3320, 3200, 2950, 1710, 1600, 1550, 1450, 1330, 1260, 1080, 950 cm^{-1}. Anal. ($C_{15}H_{18}N_4O_5$·0.4H_2O) C, H, N.

7-Demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin F (20). Purification of the above reaction mixture containing **20** using preparative TLC (silica gel, 5:5:1 $CHCl_3$–MeCN–*n*-hexane as a developing solvent) to afford pure **20**, which was triturated with $CHCl_3$–*n*-hexane followed by drying under vacuum to afford **20** as a slight yellow powder. This was used as an analytical sample: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.24 (dd, J = 2.0, 5.0 Hz, 1 H, 2-H), 2.26 (s, 3 H, 1a-CH_3), 2.30 (d, J = 5.0 Hz, 1 H, 1-H), 2.95 (d, J = 15.8 Hz, 1 H, 6-H_a), 3.19 (s, 3 H, 9a-OCH_3), 3.22 (d, J = 15.8 Hz, 1 H, 6-H_b), 3.39 (dd, J = 2.0, 12.7 Hz, 1 H, 3α-H), 3.58 (dd, J = 4.5, 10.9 Hz, 1 H, 9-H), 3.91 (d, J = 12.7 Hz, 1 H, 3β-H), 4.0–4.1 (m, 3 H, ethylenedioxy), 4.30 (m, 1 H, ethylenedioxy), 4.39 (t, J = 10.9 Hz, 1 H, 10-H_a), 4.76 (dd, J = 4.5, 10.9 Hz, 1 H, 10-H_b), 4.82 (br s, 2 H, 10-$OCONH_2$); FAB-MS *m/z* 380 (M^+ + 1); IR (KBr) 3450, 3200, 2950, 2900, 1710, 1655, 1570, 1450, 1340, 1070, 1030 cm^{-1}. Anal. ($C_{17}H_{21}N_3O_7$) C, H, N.

6-Demethylmitomycin F (12a). (1) Methylation of 6-Demethylmitomycin A (4a). To a solution of **4a**[6d] (152 mg, 0.454 mmol) in acetone (10 mL) were added MeI (1.0 mL) and K_2CO_3 (50 mg, 0.36 mmol), and the mixture was stirred at room temperature for 38.5 h. The reaction mixture was concentrated under the reduced pressure and purified by column chromatography (silica gel, 40:1 $CHCl_3$–MeOH as an eluent). The paste obtained was triturated with $CHCl_3$–*n*-hexane followed by drying under vacuum to afford **12a** (111 mg, 70%) as a reddish purple powder.

(2) Sequential Conversion from 6-Selenide 18. To a stirred solution of **18** (54 mg, 0.10 mmol) in MeOH (3.0 mL) were added dimedone (42 mg, 0.30 mmol) and K_2CO_3 (50 mg, 0.36 mmol) at room temperature. After 24 h, the mixture was diluted with phosphate buffer (pH 5) 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 obtained 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 **12a** (10 mg, 31%) as a red powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.17 (dd, J = 2.2, 4.6 Hz, 1 H, 2-H), 2.25 (s, 3 H, 1a-CH_3), 2.55 (d, J = 4.6 Hz, 1 H, 1-H), 3.20 (s, 3 H, 9a-OCH_3), 3.48 (dd, J = 2.2, 12.6 Hz, 1 H, 3α-H), 3.60 (s, 3 H, 7-OCH_3), 3.99 (dd, J = 4.4, 11.3 Hz, 1 H, 9-H), 4.22 (d, J = 12.6 Hz, 1 H, 3β-H), 4.80 (br t, J = 10.9 Hz, 1 H, 10-H_a), 5.35 (dd, J = 4.4, 10.4 Hz, 1 H, 10-H_b), 5.71 (s, 1 H, 6-H), 7.4–8.0 (br s, 2 H, 10-$OCONH_2$); FAB-MS *m/z* 350 (M^+ + 1); EI-HRMS calcd for $C_{16}H_{19}N_3O_6$ (M^+) *m/z* 349.1274, found 349.1257; IR (KBr) 3450, 3360, 3200, 2950, 1720, 1710, 1660, 1650, 1570, 1450, 1340, 1310, 1080, 1040, 950 cm^{-1}. Anal. ($C_{16}H_{19}N_3O_6$·0.3H_2O) C, H, N.

6,6-Dibromo-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin B (21). To a solution of **15** prepared from **13** (1.32 g, 2.47 mmol) in THF (50 mL) were added Et_2NH (0.50 mL) and NBS (800 mg, 4.49 mmol), and the mixture was stirred for 25 min at room temperature. The reaction mixture was poured into phosphate buffer (pH 4) and extracted with $CHCl_3$. The organic layer was 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, 30:1 $CHCl_3$–MeOH as an eluent). The paste obtained was triturated with $CHCl_3$–*n*-hexane followed by drying under vacuum to afford **21** (561 mg, 43% based on **13**) as a yellow powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.19 (s, 3 H, 1a-CH_3), 2.20 (m, 1 H, 2-H), 2.46 (d, J = 4.6 Hz, 1 H, 1-H), 3.39 (dd, J = 1.4, 12.6 Hz, 1 H, 3α-H), 3.55 (d, J = 12.6 Hz, 1 H, 3β-H), 3.8–4.0 (m, 2 H, ethylenedioxy), 4.1–4.2 (m, 1 H, ethylenedioxy), 4.41 (dd, J = 3.5, 10.0 Hz, 1 H, 9-H), 4.45–4.55 (m, 1 H, ethylenedioxy), 5.22 (br t, J = 10.2 Hz, 1 H, 10-H_a), 5.42 (dd, J = 3.5, 10.5 Hz, 1 H, 10-H_b), 7.2–7.6 (br, 2 H, 10-$OCONH_2$), 8.1–8.4 (br, 1 H, 9-OH); FAB-MS *m/z* 522/524/526 (1:2:1) (M^+ + 1); IR (KBr) 3460, 3340, 3200, 2960, 2900, 1710, 1660, 1580, 1450, 1420, 1340, 1210, 1110, 1070, 1050 cm^{-1}. Anal. ($C_{16}H_{17}Br_2N_3O_7$) C, H, N.

6,6-Dibromo-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin F (22). To a stirred solution of **16** (190 mg, 0.504 mmol) in THF (20 mL) was added Et_2NH (150 μL). After 10 min at room temperature, NBS (268 mg, 1.51 mmol) was added, and the mixture was stirred for an additional 1 h. The reaction was quenched by addition of phosphate buffer (pH 5), and the mixture was stirred for 20 min. The resulting mixture was extracted with $CHCl_3$, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue obtained was purified by column chromatography (silica gel, 30:1 $CHCl_3$–MeOH as an eluent) followed by trituration with $CHCl_3$–*n*-hexane and drying under vacuum to afford **22** (147 mg, 48% based on **14**) as a yellow powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.26 (s, 3 H, 1a-CH_3), 2.2–2.3 (2 H, 1-H + 2-H, overlapped with other peaks), 3.20 (s, 3 H, 9a-OCH_3), 3.43 (dd, J = 2.0, 12.4 Hz, 1 H, 3α-H), 3.65 (dd, J = 4.7, 10.6 Hz, 1 H, 9-H), 3.99 (d, J = 12.4 Hz, 1 H, 3β-H), 4.42 (t, J = 10.6 Hz, 1 H, 10-H_a), 4.1–4.5 (m, 4 H, ethylenedioxy), 4.70 (dd, J = 4.7, 10.6 Hz, 1 H, 10-H_b), 4.76 (br s, 2 H, 10-$OCONH_2$); FAB-MS *m/z* 536/538/540 (1:2:1) (M^+ + 1); FAB-HMRS calcd for $C_{17}H_{20}{}^{79}Br^{81}BrN_3O_7$ (M^+ + H) *m/z* 537.9642, found 537.9666; IR (KBr) 3450, 3400, 3200, 2950, 2900, 1720, 1710, 1660, 1570, 1450, 1340, 1190, 1070, 1030 cm.$^{-1}$

6,6-Dichloro-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin B (23). To a stirred solution of **15** prepared from **13** (2.72 g, 5.09 mmol) in CH_2Cl_2 (300 mL) was added Et_2NH (1.0 mL) at room temperature. After 5 min, NCS (2.04 g, 15.3 mmol) was added, and the mixture was stirred for an additional 35 min at room temperature. The reaction mixture was concentrated on a rotary evaporator followed by purification by column chromatography (silica gel, 30:1−10:1 $CHCl_3$−MeOH as eluents). The paste obtained was triturated with $CHCl_3$−*n*-hexane and dried under vacuum to afford **23** (848 mg, 38% based on **13**) as a yellow powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ (main peaks) 2.08 (s, 3 H, 1a-CH_3), 2.24 (br d, J = 4.7 Hz, 1 H, 2-H), 2.46 (d, J = 4.7 Hz, 1 H, 1-H), 3.56 (dd, J = 2.0, 12.4 Hz, 1 H, 3α-H), 3.93 (d, J = 12.4 Hz, 1 H, 3β-H), 4.1−4.2 (m, 2 H, ethylenedioxy), 4.2−4.4 (m, 2 H, ethylenedioxy), 4.35 (dd, J = 3.7, 9.2 Hz, 1 H, 9-H), 5.22 (br t, J = 9.9 Hz, 1 H, 10-H_a), 5.35 (dd, J = 3.7, 10.6 Hz, 1 H, 10-H_b), 7.52 (br s, 2 H, 10-$OCONH_2$); FAB-MS m/z 434/436 (3:2) (M^+ + 1); IR (KBr) 3450, 3200, 2950, 1720, 1710, 1700, 1650, 1550, 1340, 1200, 1090 cm^{-1}. Anal. ($C_{16}H_{17}Cl_2N_3O_7$) C, H, N.

6,6-Dichloro-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin F (24). To a stirred solution of **16** in CH_2Cl_2 (300 mL, prepared from 2.76 g, 5.04 mmol of **14**) was added Et_2NH (1.7 mL), and the mixture was stirred at room temperature. After 5 min, NCS (3.29 g, 24.6 mmol) was added. After 2 h at room temperature, 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 paste obtained was purified by column chromatography (silica gel, 40:1 $CHCl_3$−MeOH as an eluent) followed by trituration with $CHCl_3$−*n*-hexane and drying under vacuum to afford **24** (1.90 g, 84% based on **14**) as a yellow powder: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.27 (s, 3 H, 1a-CH_3), 2.28 (dd, 1 H, 2-H, overlapped with other peaks), 2.31 (d, J = 4.5 Hz, 1 H, 1-H), 3.20 (s, 3 H, 9a-OCH_3), 3.43 (dd, J = 2.0, 12.7 Hz, 1 H, 3α-H), 3.63 (dd, J = 4.6, 10.6 Hz, 1 H, 9-H), 3.98 (d, J = 12.7 Hz, 1 H, 3β-H), 4.2−4.4 (m, 4 H, ethylenedioxy), 4.42 (t, J = 10.6 Hz, 1 H, 10-H_a), 4.71 (dd, J = 4.6, 10.4 Hz, 1 H, 10-H_b), 4.79 (br s, 2 H, 10-$OCONH_2$); FAB-MS m/z 448/450 (3:2) (M^+ + 1); IR (KBr) 3450, 3350, 3200, 2950, 2900, 1720, 1660, 1570, 1460, 1340, 1200, 1100, 1080, 1030 cm^{-1}. Anal. ($C_{17}H_{19}Cl_2N_3O_7$) C, H, N.

6-Chloro-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin B (25). To a solution of **23** (842 mg, 1.94 mmol) in THF (100 mL) were added *n*-Bu_3SnH (0.60 mL) and Et_3B (1.0 M *n*-hexane solution, 1.0 mL) under an argon atmosphere at −40 °C. After 5 h at that temperature, MeOH (50 mL) was added, and the mixture was concentrated on a rotary evaporator. The paste obtained was triturated with $CHCl_3$−*n*-hexane followed by drying under vacuum to afford **25** (628 mg, 81%) as a yellow powder. The product was obtained as an equilibrium mixture of two diastereomers at C-6 (approximately 4:1 in pyridine-d_5): ^{1}H NMR (270 MHz, pyridine-d_5) δ (major isomer) 2.11 (s, 3 H, 1a-CH_3), 2.18 (br d, J = 4.5 Hz, 1 H, 2-H), 2.46 (d, J = 4.5 Hz, 1 H, 1-H), 3.52 (br d, J = 12.4 Hz, 1 H, 3α-H), 3.78 (d, J = 12.4 Hz, 1 H, 3β-H), 3.9−4.5 (m, 5 H, ethylenedioxy + 9-H), 5.19 (t, J = 10.1 Hz, 1 H, 10-H_a), 5.40 (dd, J = 3.2, 10.6 Hz, 1 H, 10-H_b), 6.02 (s, 1 H, 6-H), 7.3−7.9 (br, 3 H, 10-$OCONH_2$ + 9a-OH); FAB-MS m/z 400/402 (3:1) (M^+ + 1); FAB-HRMS calcd for $C_{16}H_{19}{}^{35}ClN_3O_7$ (M^+ + H) m/z 400.3109, found 400.3132; IR (KBr) 3450, 3350, 3300, 3200, 3000, 2950, 1720, 1700, 1650, 1550, 1400, 1340, 1260, 1130, 1100, 1070 cm^{-1}.

6-Chloro-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydromitomycin F (26). As described in the synthesis of **25**, treatment of **24** (1.90 g, 4.23 mmol) with *n*-Bu_3SnH (1.25 mL) and Et_3B (1.0 M *n*-hexane solution, 1.5 mL) in THF (200 mL) afforded **26** (1.59 g, 91%) as a yellow powder. The product was obtained as an equilibrium mixture of two diastereomers at C-6 (approximately 10:1 in $CDCl_3$): ^{1}H NMR (270 MHz, $CDCl_3$) δ (major isomer (main peaks)) 2.2−2.4 (2 H, 1-H + 2-H, overlapped with other peaks), 2.26 (s, 3 H, 1a-CH_3), 3.20 (s, 3 H, 9a-OCH_3), 3.42 (br d, J = 12.9 Hz, 1 H, 3α-H), 3.63 (dd, J = 4.6, 10.6 Hz, 1 H, 9-H), 3.82 (br d, J = *ca.* 13 Hz, 1 H, 3β-H), 4.0−4.5 (m, 5 H, ethylenedioxy + 10-H_a), 4.69 (dd, J = 4.6, 10.4 Hz, 1 H, 10-H_b), 4.7−4.8 (br, 2 H, 10-$OCONH_2$), 5.10 (s, 1 H, 6-H); FAB-MS m/z 414/416 (3:1) (M^+ + 1); FAB-HRMS calcd for $C_{17}H_{21}{}^{35}ClN_3O_7$ (M^+ + H) m/z 414.3377, found 414.3351; IR (KBr) 3450, 3350, 3200, 2950, 2900, 1710, 1650, 1570, 1460, 1340, 1200, 1120, 1100, 1030 cm^{-1}.

6-Bromo-6-demethylmitomycin D (7b). To a solution of **21** (203 mg, 0.387 mmol) in MeOH (10 mL) were added dimedone (106 mg, 0.756 mmol) and NH_3 in MeOH (6.1 M, 1.0 mL) at room temperature. After stirring for 3.5 h, the reaction mixture was concentrated on a rotary evaporator followed by purification by column chromatography (silica gel, 20:1−10:1 $CHCl_3$−MeOH as eluents). The paste obtained was crystallized from EtOH and dried under vacuum to afford **7b** (84 mg, 54%) as purple crystals. In addition, compound **7b** (14 mg, 9.2%) was also obtained from the filtrate after purification by column chromatogragphy: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.13 (s, 3 H, 1a-CH_3), 2.23 (dd, J = 1.9, 4.8 Hz, 1 H, 2-H), 2.47 (d, J = 4.8 Hz, 1 H, 1-H), 3.65 (dd, J = 1.9, 13.0 Hz, 1 H, 3α-H), 4.22 (dd, J = 3.5, 9.8 Hz, 1 H, 9-H), 4.40 (d, J = 13.0 Hz, 1 H, 3β-H), 5.21 (t, J = 10.3 Hz, 1 H, 10-H_a), 5.41 (dd, J = 3.5, 10.6 Hz, 1 H, 10-H_b), 7.2−7.7 (br, 2 H, 10-$OCONH_2$), 8.3−8.6 (br, 3 H, 7-NH_2 + 9a-OH); EI-MS m/z 398/400 (1:1) (M^+); EI-HRMS calcd for $C_{14}H_{15}{}^{79}BrN_4O_5$ (M^+) m/z 398.0227, found 398.0217; IR (KBr) 3410, 3290, 2950, 1720, 1590, 1560, 1540, 1450, 1420, 1340, 1070 cm^{-1}. Anal. ($C_{14}H_{15}BrN_4O_5$) C, H, N.

6-Bromo-6-demethylmitomycin B (8b). To a solution of **21** (201 mg, 0.383 mmol) in MeOH (20 mL) were added dimedone (52 mg, 0.37 mmol) and K_2CO_3 (104 mg, 0.754 mmol). After stirring for 20 min at room temperature, the reaction mixture was diluted with phosphate buffer (pH 4) and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, concentrated on a rotary evaporator, triturated with $CHCl_3$−*n*-hexane, and dried under vacuum to afford the 6-monobromide (211 mg) as a yellow powder. This bromide (211 mg) was dissolved in MeOH (30 mL) and added to K_2CO_3 (55 mg, 0.40 mmol). After stirring at room temperature for 9 h, the mixture was treated with the same procedure as that described above. The paste obtained 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 **8b** (64 mg, 40%) as a purple powder. In addition, 6-demethylmitomycin B (**8a**) (2.8 mg, 2.2%) was also obtained as a byproduct: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.13 (s, 3 H, 1a-CH_3), 2.24 (dd, J = 2.0, 4.8 Hz, 1 H, 2-H), 2.48 (d, J = 4.8 Hz, 1 H, 1-H), 3.57 (br d, J = 12.4 Hz, 1 H, 3α-H), 4.13 (d, J = 12.4 Hz, 1 H, 3β-H), 4.15 (s, 3 H, 7-OCH_3), 4.20 (dd, J = 3.5, 9.4 Hz, 1 H, 9-H), 5.16 (br t, J = 10.1 Hz, 1 H, 10-H_a), 5.40 (dd, J = 3.5, 10.4 Hz, 1 H, 10-H_b), 7.4−7.8 (br, 3 H, 10-$OCONH_2$ + 9a-OH); FAB-MS m/z 414/416 (4:5) (M^+ + 1), 415/417 (1:1) (M^+ + 2), 416/418 (2:1) (M^+ + 3); FAB-HRMS calcd for $C_{15}H_{19}{}^{79}BrN_3O_6$ (M^+ + 2 H) m/z 415.0379, found 415.0339; IR (KBr) 3460, 3400, 3330, 3200, 2950, 1710, 1660, 1630, 1620, 1560, 1550, 1450, 1410, 1340, 1250, 1070 cm^{-1}.

6-Bromo-6-demethylporfiromycin (11b). (1) Methylation of 6-Bromo-6-demethylmitomycin C (3b). The same procedure as that described in the synthesis of **11a** was employed to convert **3b** (151 mg, 0.379 mmol) into **11b** (83 mg, 53%). In addition, 6-bromo-6-demethyl-7-*N*-methylporfiromycin (47 mg, 29%) was obtained as a byproduct.

(2) Conversion from 6,6-Dibromide 22. Compound **22** (312 mg, 0.580 mmol) and dimedone (120 mg, 0.857 mmol) were dissolved in NH_3 in MeOH (6.8 M, 20 mL), and the reaction mixture was allowed to stand at room temperature for 1 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). The paste obtained was triturated with $CHCl_3$−*n*-hexane followed by drying under vacuum to afford **11b** (161 mg, 67%) as a purple powder: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.15 (dd, J = 2.0, 4.6 Hz, 1 H, 2-H), 2.24 (s, 3 H, 1a-CH_3), 2.51 (d, J = 4.6 Hz, 1 H, 1-H), 3.17 (s, 3 H, 9a-OCH_3), 3.50 (dd, J = 2.0, 12.9 Hz, 1 H, 3α-H), 3.94 (dd, J = 4.3, 11.2 Hz, 1 H, 9-H), 4.41 (d, J = 12.9 Hz, 1 H, 3β-H), 4.75 (t, J = 10.9 Hz, 1 H, 10-H_a), 5.26 (dd, J = 4.3, 10.3

Hz, 1 H, 10-H_b), 7.3–8.0 (br, 2 H, 10-$OCONH_2$), 8.53 (br s, 1 H, 7-NH_2), 8.63 (br s, 1 H, 7-NH_2); FAB-MS m/z 413/415 (4:5) ($M^+ + 1$), 414/416 (1:1) ($M^+ + 2$); IR (KBr) 3450, 3350, 3200, 2950, 1730, 1710, 1660, 1570, 1560, 1450, 1340, 1320, 1290, 1220, 1070 cm^{-1}. Anal. ($C_{15}H_{17}BrN_4O_5$) C, H, N.

6-Bromo-6-demethyl-7-*N*-methylporfiromycin: ^1H NMR (270 MHz, pyridine-d_5) δ 2.16 (dd, J = 2.0, 5.0 Hz, 1 H, 2-H), 2.25 (s, 3 H, 1a-CH_3), 2.51 (d, J = 5.0 Hz, 1 H, 1-H), 3.18 (s, 3 H, 9a-OCH_3), 3.35 (d, J = 5.9 Hz, 3 H, 7-$NHCH_3$), 3.51 (dd, J = 2.0, 12.9 Hz, 1 H, 3α-H), 3.89 (dd, J = 4.3, 11.4 Hz, 1 H, 9-H), 4.43 (d, J = 12.9 Hz, 1 H, 3β-H), 4.71 (t, J = 10.9 Hz, 1 H, 10-H_a), 5.21 (dd, J = 4.3, 10.4 Hz, 1 H, 10-H_b), 7.3–8.0 (br, 2 H, 10-$OCONH_2$), 8.11 (br s, 1 H, 7-NH); FAB-MS m/z 427/429 (3:4) ($M^+ + 1$), 428/430 (1:1) ($M^+ + 2$); FAB-HRMS calcd for $C_{16}H_{20}{}^{79}BrN_4O_5$ ($M^+ + H$) m/z 427.2761, found 427.2775.

6-Bromo-6-demethylmitomycin F (12b). To a solution of **22** (302 mg, 0.562 mmol) in MeCN (30 mL) were added NEt_3 (0.20 mL) and dimedone (80 mg, 0.57 mmol), and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into phosphate buffer (pH 4) and extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator to afford 6-monobromide as a yellow paste. To a stirred solution of the above product in MeOH (50 mL) was added K_2CO_3 (100 mg) at room temperature. After 50 min, additional K_2CO_3 (100 mg) was added, and the reaction mixture was stirred for 20 min at room temperature and for 62 h at 5 °C. The resulting brown reaction mixture was diluted with phosphate buffer (pH 4) and extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated on a rotary evaporator. The paste obtained was dissolved in $CHCl_3$ (100 mL), and silica gel (50 mL) was added to the solution. After 2.5 h at room temperature, the silica gel was eluted with $CHCl_3$–MeOH (9:1) to afford a crude product as a purple paste. This material was purified by preparative HPLC (ODS, 50:50 MeCN–water as an eluent) followed by crystallization with $CHCl_3$–n-hexane and drying under vacuum to afford **12b** (79 mg, 33%) as purple crystals: ^1H NMR (270 MHz, pyridine-d_5) δ 2.10 (dd, J = 2.0, 4.5 Hz, 1 H, 2-H), 2.23 (s, 3 H, 1a-CH_3), 2.52 (d, J = 4.5 Hz, 1 H, 1-H), 3.18 (s, 3 H, 9a-OCH_3), 3.46 (dd, J = 2.0, 12.9 Hz, 1 H, 3α-H), 3.92 (dd, J = 4.5, 11.4 Hz, 1 H, 9-H), 4.11 (J = 12.9 Hz, 1 H, 3β-H), 4.19 (s, 3 H, 7-OCH_3), 4.72 (t, J = 10.7 Hz, 1 H, 10-H_a), 5.26 (dd, J = 4.5, 10.4 Hz, 1 H, 10-H_b), 7.3–8.1 (br, 2 H, 10-$OCONH_2$); FAB-MS m/z 429/431 (1:1) ($M^+ + 2$), 430/432 (1:1) ($M^+ + 3$); IR (KBr) 3450, 3330, 3200, 2950, 1730, 1710, 1670, 1640, 1620, 1570, 1560, 1450, 1410, 1340, 1250, 1220, 1070, 1050, 1030 cm^{-1}. Anal. ($C_{16}H_{18}BrN_3O_6$) C, N; H: calcd, 4.24; found, 3.59.

6-Chloro-6-demethylmitomycin D (7c). To a solution of **25** (103 mg, 0.257 mmol) in MeOH (10 mL) was added NH_3 in MeOH (6.8 M, 2.0 mL), and the mixture was allowed to stand at room temperature for 4.5 h. The volatiles were removed on a rotary evaporator, and the residue was purified by preparative TLC (silica gel, 9:1 $CHCl_3$–MeOH as a developing solvent). The paste obtained was triturated with $CHCl_3$–n-hexane followed by drying under vacuum to afford **7c** (41 mg, 45%) as a purple powder: ^1H NMR (270 MHz, pyridine-d_5) δ 2.13 (s, 3 H, 1a-CH_3), 2.24 (dd, J = 1.9, 4.8 Hz, 1 H, 2-H), 2.46 (d, J = 4.8 Hz, 1 H, 1-H), 3.66 (dd, J = 1.9, 12.9 Hz, 1 H, 3α-H), 4.22 (dd, J = 3.5, 9.9 Hz, 1 H, 9-H), 4.40 (d, J = 12.9 Hz, 1 H, 3β-H), 5.20 (t, J = 10.1 Hz, 1 H, 10-H_a), 5.47 (dd, J = 3.5, 10.4 Hz, 1 H, 10-H_b), 7.2–7.8 (br, 2 H, 10-$OCONH_2$), 8.46 (br s, 2 H, 7-NH_2); FAB-MS m/z 355/357 (3:2) ($M^+ + 1$); FAB-HRMS calcd for $C_{14}H_{16}{}^{35}ClN_4O_5$ ($M^+ + H$) m/z 355.0808, found 355.0823; IR (KBr) 3350, 3250, 3200, 2950, 1720, 1700, 1600, 1540, 1460, 1420, 1340, 1100 cm^{-1}.

6-Chloro-6-demethylmitomycin B (8c). To a solution of **25** (310 mg, 0.776 mmol) in MeOH (50 mL) was added K_2CO_3 (108 mg, 0.783 mmol), and the mixture was stirred for 7 h at room temperature. The reaction mixture was poured into a saturated NH_4Cl solution and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated on a rotary evaporator. The residue obtained 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 **8c** (78 mg, 27%) as a purple powder: ^1H NMR (270 MHz, pyridine-d_5) δ 2.14 (s, 3 H, 1a-CH_3), 2.25 (dd, J = 2.0, 4.5 Hz, 1 H, 2-H), 2.47 (d, J = 4.5 Hz, 1 H, 1-H), 3.57 (dd, J = 2.0, 12.9 Hz, 1 H, 3α-H), 4.12 (d, J = *ca.* 13 Hz, 1 H, 3β-H, overlapped with other peaks), 4.14 (s, 3 H, 7-OCH_3), 4.20 (dd, J = 3.4, 9.6 Hz, 1 H, 9-H), 5.15 (t, J = 9.6 Hz, 1 H, 10-H_a), 5.38 (dd, J = 3.4, 10.4 Hz, 1 H, 10-H_b), 7.2–7.8 (br, 2 H, 10-$OCONH_2$); FAB-MS m/z 370/372 (3:2) ($M^+ + 1$); FAB-HRMS calcd for $C_{15}H_{18}{}^{35}ClN_3O_6$ ($M^+ + 2$ H) m/z 371.0883, found 371.0914; IR (KBr) 3450, 3350, 3200, 2950, 1720, 1710, 1700, 1620, 1560, 1460, 1340, 1250, 1100 cm^{-1}. Anal. ($C_{15}H_{18}ClN_3O_6 \cdot 0.7H_2O$) C, H; N: calcd, 10.99; found, 10.35.

6-Chloro-6-demethylporfiromycin (11c). A similar procedure as that described in the synthesis of **7c** was employed to convert **26** (1.59 g, 3.85 mmol) into **11c**. The paste obtained was crystallized from $CHCl_3$–n-hexane followed by drying under vacuum to afford **11c** (625 mg, 44%) as purple crystals. In addition, compound **11c** (249 mg, 18%) was also obtained from the filtrate after purification by column chromatography: ^1H NMR (270 MHz, pyridine-d_5) δ 2.16 (dd, J = 2.0, 4.8 Hz, 1 H, 2-H), 2.25 (s, 3 H, 1a-CH_3), 2.53 (d, J = 4.8 Hz, 1 H, 1-H), 3.18 (s, 3 H, 9a-OCH_3), 3.52 (dd, J = 2.0, 12.9 Hz, 1 H, 3α-H), 3.96 (dd, J = 4.5, 11.4 Hz, 1 H, 9-H), 4.42 (d, J = 12.9 Hz, 1 H, 3β-H), 4.76 (t, J = 10.9 Hz, 1 H, 10-H_a), 5.29 (dd, J = 4.5, 10.4 Hz, 1 H, 10-H_b), 7.4–8.0 (br, 2 H, 10-$OCONH_2$), 8.57 (br s, 1 H, 7-NH_2), 8.77 (br s, 1 H, 7-NH_2); FAB-MS m/z 369/371 (2:1) ($M^+ + 1$); IR (KBr) 3440, 3400, 3320, 3280, 3200, 2980, 2950, 1730, 1710, 1610, 1560, 1540, 1460, 1410, 1350, 1320, 1300, 1220, 1100, 1080 cm^{-1}. Anal. ($C_{15}H_{17}ClN_4O_5$) N, H; C: calcd, 48.85; found, 49.28.

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Supporting Information Available: ^1H NMR spectra of compounds **7a,c, 8a,b, 17, 18, 19, 22, 25, 26,** and 6-bromo-6-demethyl-7-*N*-methylporfiromycin (11 pages). Ordering information is given on any current masthead page.

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