An Efficient Chemical Conversion of Mitomycin A to Isomitomycin A[1]

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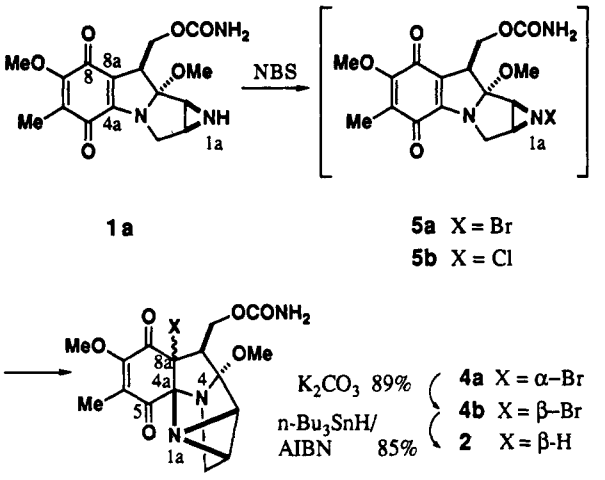
After inspection of the X-ray crystallographic analyses of mitomycin A (**1a**) and albomitomycin A (**2**), an efficient chemical conversion of **1a** to isomitomycin A (**3**) via **2** was accomplished in 68% overall yield. First, **1a** was converted to (8a*S*)-8a-bromoalbomitomycin A (**4b**) in 89% yield, which was reduced with tributyltin hydride and AIBN to give **2** in 85% yield. Second, the C4a–N4 bond of **2** was selectively cleaved under hydrogenolysis conditions, and subsequent air oxidation afforded **3** in 90% yield from **2**. Treatment of **2** or **3** with NH_3 in methanol afforded mitomycin C (**1b**) in good yield.

Introduction

Mitomycin C (**1b**, Figure 1) has been used extensively in cancer chemotherapy against a variety of solid tumors,[2] but its use is limited by side effects, such as severe bone marrow suppression and gastrointestinal damage. A number of derivatives targeting less toxicity and more effective activity have been synthesized in our laboratories[3] and by other groups.[4] We have screened the minor constituents from the fermentation broth of *Streptomyces caespitous*[5] and have found two novel isomers of mitomycin A (**1a**), designated as albomitomycin A (**2**) and isomitomycin A (**3**).[6] These novel compounds have engendered much interest in their biological activities, molecular structures, and chemical reactivities and have provided a new synthetic pathway for mitomycins[7] and novel mitomycin analogs that could never have been derived from conventional mitomycins. These two new isomers are related to **1a** through Michael and retro-Michael reactions, and the interconversions among them have been designated as the "mitomycin rearrangement."[6] Unfortunately, the equilibrium of the "mitomycin rearrangement" overwhelmingly favors **1a**. Therefore, the search for an efficient preparation method for **2** and **3** was undertaken in order to probe new aspects of their chemistry and biology.

Scheme I



Using information from the detailed X-ray crystallographic analyses of **1a** and **2**, we have accomplished the synthetic conversion of **1a** to **3** via **2**. Additionally, the versatility of **2** and **3** has been demonstrated by their direct conversion to **1b**. Herein, we describe our successful efforts to develop this new mitomycin chemistry.

Albomitomycin A (2) from Mitomycin A (1a)

The X-ray crystallographic analysis of **1a**[8] suggests two reasons that the cyclization between N1a and C4a should occur. First, the aziridine ring is bent over the quinone ring, and the distance between N1a and C4a is 3.32 Å, which is almost equal to the sum of the van der Waals radii of nitrogen and carbon atoms. Second, the quinone ring of **1a** is composed of two separate α,β-unsaturated enones. The C4a–C5 and the C7–C8 bond lengths are longer than the C8–C8a and the C5–C6 bond lengths,[8] respectively. The separation of the two enone systems suggests that the 4a-position of **1a** could be an electrophilic center conjugated with the 8a-carbonyl function.

On the basis of the above considerations, we attempted the halogen-initiated amino cyclization of **1a**. Treatment of **1a** with *N*-bromosuccinimide in wet ethyl acetate at

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(2) (a) Carter, S. K.; Crooke, S. T. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1987. (b) Taguchi, T.; Aigner, K. R. *Mitomycin C in Cancer Chemotherapy Today*; Excerpta Medica, Ltd.: Tokyo, 1991.

(3) (a) Recently, a clinical study of a mitomycin derivative KW-2149 has been developed. The synthesis of KW-2149 has been described in the following literature: Kono, M.; Saitoh, Y.; Kasai, M.; Sato, A.; Shirahata, K.; Morimoto, M.; Ashizawa, T. *Chem. Pharm. Bull.* **1989**, *37*, 1128. (b) A mitomycin derivative KW-2083 once was evaluated in the clinical study. Okabe, M.; Imai, R.; Morimoto, M. *J. Antibiot.* **1982**, *35*, 1055.

(4) (a) Rockwell, S.; Keyes, S. R.; Loomis, R.; Kelley, M.; Vyas, D. M.; Wong, H.; Doyle, T. W.; Sartorelli, A. C. *Cancer Commun.* **1991**, *3*, 191. (b) Furuhata, K.; Komiyama, K.; Ogura, H.; Hata, T. *Chem. Pharm. Bull.* **1991**, *39*, 255. (c) Ghiorghis, A.; Clarke, R.; Talebian. A. *Proc. Am. Assoc. Cancer Res.* **1991**, 431. (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. (f) Sami, S. M.; Iyenger, B. S.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schuring, J. E. *J. Med. Chem.* **1984**, *27*, 701.

(5) (a) Shirahata, K.; Kono, M.; Matsubara, I.; Kasai, M. *Abstracts of 23rd Symposium on The Chemistry of Natural Products*; 1980, p 608. (b) Shirahata, K.; Morimoto, M.; Ashizawa, T.; Mineura, K.; Kono, M.; Saito, Y.; Kasai, M. *Program and Abstracts of 21st Interscience Conference on Antimicrobial Agents and Chemotherapy*; No. 421; Chicago, Nov. 1981. (c) Urakawa, C.; Tsuchiya, H.; Nakano, K. *J. Antibiot.* **1981**, *34*, 243.

(6) Kono, M.; Saitoh, Y.; Shirahata, K.; Arai, Y.; Ishii, S. *J. Am. Chem. Soc.* **1987**, *109*, 7224.

(7) Fukuyama and co-worker utilized **3** as a chemical equivalent of **1a** for the synthesis of **1b**, see: Fukuyama, T.; Yang, L.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8303.

(8) Hirayama, N.; Shirahata, K. *Acta Crystallogr.* **1989**, *C45*, 1780.

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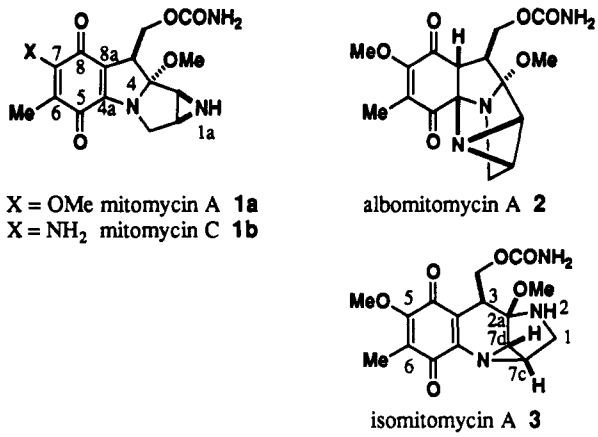


Figure 1.

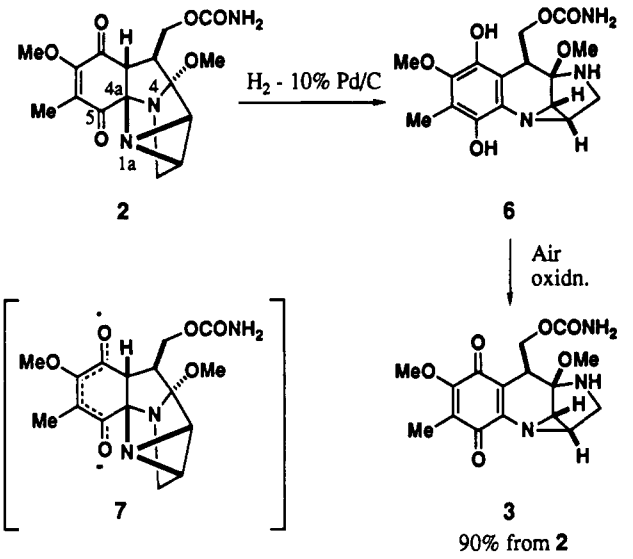
room temperature afforded a mixture of the cyclization products, (8a*R*)-8a-bromoalbomitomycin A (**4a**) and its 8a-epimer (**4b**),[9] quantitatively (Scheme I). The ratio of **4a** and **4b** depended on the solvent used in the cyclization reaction (**4a**:**4b** = 2.8:1 in wet ethyl acetate and 1:1 in chloroform).

This cyclization reaction was initiated by 1a-*N*-bromination of **1a** to afford an intermediate, 1a-*N*-bromomitomycin A (**5a**, Scheme I),[11] that was too unstable to isolate. However, ^{1}H NMR of the reaction mixture clearly indicated that **5a** was generated, and the structure of **5a** was confirmed by the comparison of the chemical shifts and coupling patterns of the ^{1}H NMR with those of the stable 1a-*N*-chloride (**5b**).[12] Intermediate **5a** was gradually converted to **4a** and **4b**.

Hydrogenolyses (H_2, 5% Pd on $BaSO_4$, $NaHCO_3$ in aqueous methanol) of **4a** and **4b** afforded **2** in 56 and 54% yields, respectively, accompanied by **1a** (ca. 20% yield in each case).

The mitomycin A (**1a**) generated under the above reaction conditions could arise from the "mitomycin rearrangement" of **2**. To minimize the formation of **1a** and optimize the yield of **2**, methanol was replaced with an aprotic solvent (acetonitrile), powdered K_2CO_3 was used instead of aqueous $NaHCO_3$, and 10% Pd on carbon was used as a catalyst. Although the yield of **2** did not improve when the hydrogenolysis of **4a** was carried out under these reaction conditions, we found that **4a** was isomerized to afford the more soluble **4b** accompanied by **1a**. Moreover, this isomerization at C8a occurred only on treatment of **4a** with powdered K_2CO_3 in acetonitrile to afford **4b** in 89% yield (Scheme I).[13] In contrast, the fact that isomerization from **4b** to **4a** was not observed showed that **4b** was thermodynamically more stable than **4a**. We then tried the debromination of **4b** under radical-initiated reduction conditions. First, the reduction of **4b** with tributyltin hydride and triethylborane[14] at room temperature mainly afforded **1a**. Since we believed that **1a** was generated through the "mitomycin rearrangement" accelerated by triethylborane as a Lewis acid, triethylborane was replaced with AIBN. However, when the reaction with AIBN was carried out under the usual conditions, the thermal instability of **2** led to a low yield. Prolonged reaction times at high temperature caused degradation of **2** and the "mitomycin rearrangement" of **2** to **1a**. A search for the optimum reaction conditions resulted in the treatment of **4b** with excess tributyltin hydride (15 equiv) and AIBN (1.5 equiv) in toluene at 65 °C for 1.5 h to afford **2** in 85% yield (Scheme I).

Scheme II



Isomitomycin A (3) from Albomitomycin A (2)

Albomitomycin A (**2**) contains a reactive imidazolidine ring system. The "mitomycin rearrangement" of **2** could proceed by C–N bond scission in this ring. C4a–N4 bond cleavage would produce **3**, and C4a–N1a bond cleavage would produce **1a**. The fact that the equilibrium of the "mitomycin rearrangement" favors **1a**[6] implied that **1a** should be thermodynamically more stable than **2** and **3** and that mild reaction conditions should be required for the conversion of **2** to **3**.

Because it is well-known that one of the two C–N bonds in the N–C–N bond system is easily cleaved under hydrogenolysis conditions,[15] we attempted hydrogenolysis of the C–N bonds in **2**. Fortuitously, the C4a–N4 bond of **2** was selectively cleaved under these conditions (H_2, 10% Pd on carbon) to afford unstable intermediate **6**,[16] which was air-oxidized to afford **3** in 90% yield from **2** (Scheme II).

Next we tried to determine the mechanism of the C–N bond scission. The X-ray crystallographic analysis of **2**[17] revealed that the dihedral angle between O5–C5–C4a–N1a is -28.5°, and that between O5–C5–C4a–N4 is 86.4°. The C4a–N4 bond of **2** is almost parallel to the C5–O5 carbonyl π orbital, suggesting that the C4a–N4 bond should be kinetically more scissile than the C4a–N1a bond because

(9) The stereochemistries at C8a of **4a** and **4b** were evaluated by ^{1}H NMR. The comparison of the chemical shift of the 9a-methoxy group and the methylene protons at C3 and C10 of **4a** and **4b** with those of albomitomycin A (**2a**) revealed that **4a** was the (8a*R*)-bromide and **4b** was the (8a*S*)-bromide. The structure of **4a** was finally confirmed by X-ray crystallographic analysis.[10]

(10) Full details of the X-ray crystallographic analysis of **4a** will be published elsewhere.

(11) Corey and co-workers reported a similar halogen-initiated amino cyclization via *N*-bromide, see: Corey, E. J.; Chen, C.-P.; Reichard, G. A. *Tetrahedron Lett.* **1989**, *30*, 5547.

(12) Treatment of mitomycin A (**1a**) with *tert*-butyl hypochlorite in dichloromethane at 0 °C afforded **5b**, which was stable enough to isolate; see the Experimental Section.

(13) The isomerization of α-bromo ketone in a steroidal skeleton under acidic conditions is known, but the mechanism of this isomerization under basic reaction conditions is not known; see: House, H. O. *Modern Synthetic Reactions*, 2nd ed., W. A. Benjamin, Inc.: Menlo Park, CA, 1972; p 466 and references cited therein.

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

(15) Korte, F.; Bocz, A. K.; Buchel, K. H. *Chem. Ber.* **1966**, *99*, 737.

(16) Although unstable in the presence of oxygen, **6** was isolated under a nitrogen atmosphere by radial chromatography in a moderate yield; see the Experimental Section.

(17) Hirayama, N.; Shirahata, K. *Acta Crystallogr.* **1991**, *C47*, 409.

of π orbital overlap. However, in this transformation, the question of whether hydrogenolysis or hydrogenation would first occur arises. Compound **2** has two functional groups where hydrogenolysis or hydrogenation can readily occur. It is also known that the 2-butene-1,4-dione system can be readily hydrogenated under similar conditions.[18]

Since no intermediate leading to **6** was observed, we employed the semiempirical MO calculation method MNDO-PM3[19] to estimate each orbital in **2**, hoping to determine which bond would be more readily reduced. The resultant LUMO (π-like orbital, −1.004 eV) unambiguously supported the idea that hydrogenation of the 2-butene-1,4-dione should be dominant over a direct hydrogenolysis of the C–N bond in **2**. Further calculations on a putative radical anion (**7**) were performed to determine why aromatization led to **6** in a specific manner. The dihedral angles between O5–C5–C4a–N1a and O5–C5–C4a–N4 were calculated to be −33.5° and 78.2°, respectively, predicting a trans E_2 elimination of the C4a–N4 bond. Thus, on the basis of MNDO-PM3 calculations, we concluded that the mechanism of the hydrogenolysis was a combination of an initial hydrogenation of the C6–C7 double bond and subsequent aromatization of the resulting intermediate to yield **6**.

To the best of our knowledge, no other selective C–N bond cleavage in imidazolidine ring system has been observed to date. As discussed above, the direction of the bond plays an important role in the reactivity of C4a–N4 bond.[20]

We then tried direct conversions of **2** and **3** to **1b** to examine their usefulness for the synthesis of new mitomycin derivatives. Treatment of **2** and **3** with NH_3 in methanol afforded **1b** in 99% and 75% yield, respectively.

Conclusion

Using structural features observed by means of X-ray crystallography, we have developed new mitomycin chemistry for the preparation of **2** and **3** in large quantities. Crystal structures implied the structure–reactivity relationships. The versatility of **2** and **3** as semisynthetic equivalents for mitomycin derivatives with structures similar to **1b** has been successfully exemplified by direct conversions of **2** and **3** to **1b**. Compounds **2** and **3** may be used for the synthesis of *new mitomycin derivatives*[21] that could never be synthesized from conventional mitomycin precursors.

Experimental Section

Materials other than mitomycins were obtained from commercial suppliers and used without purification. Nuclear magnetic resonance spectra were determined on a Bruker AM400, a JNM-GX270 or a JEOL FX-100 spectrometer. Mass spectra were determined on a Hitachi M-80B or a JMS-D300 mass spectrometer. Infrared spectra were determined on a JASCO IR-810 spectrometer. Analytical thin-layer chromatography was carried out on E. Merck Reagents 0.25-mm 60-F_{254} plates. Molecular orbital calculations were carried out on a IBM RS6000/550 workstation.

Molecular Orbital Calculations. The calculations for **2** and **7** were carried out by using the MNDO-PM3 Hamiltonian[19] within the MOPAC Ver. 6.01 program.[22] A starting structure of **2** was built up on the basis of X-ray crystallographic data,[17] and the structure was optimized using the eigenvector following method.[23] A starting structure of **7** was built up on the basis of the optimized structure of **2**, and the geometry of **7** was similarly optimized.

(8a*S*)-8a-Bromoalbomitomycin A (4b). To a solution of **1a** (5.00 g, 14.3 mmol) in wet ethyl acetate (28 mL, saturated with water) at 25 °C was added *N*-bromosuccinimide (2.55 g). The solution was stirred for 0.5 h. The precipitate that appeared in the reaction medium was filtered to afford (8a*R*)-8a-bromoalbomitomycin A (**4a**, 3.37 g, 55% yield), and the filtrate was washed with 5% aqueous $NaHCO_3$ and brine. The organic layer was then dried over $MgSO_4$ and evaporated under reduced pressure to afford an approximately 1:1 mixture of **4a** and (8a*S*)-8a-bromoalbomitomycin A (**4b**) (2.80 g, 45% yield). The isolated **4a** and the mixture of **4a** and **4b** were combined and suspended in acetonitrile (125 mL) in the presence of K_2CO_3 (1.98 g) and stirred at 25 °C for 24 h. The reaction mixture was filtered, and the residue was resuspended in acetonitrile (125 mL) at 25 °C for 24 h. This procedure was repeated three times, and the combined filtrate was evaporated under reduced pressure. The residue was suspended in acetonitrile (50 mL) and filtered to afford **4b** (4.83 g). The filtrate was evaporated under reduced pressure and purified by silica gel chromatography with 10% acetone in chloroform to afford **4b** (0.43 g, the combined yield of **4b** was 89% from **1a**). **4a**: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 1.95 (s, 3 H, 6-CH_3), 2.69 (ddd, J = 3.2, 1.2, 1.1 Hz, 1 H, 1-H), 2.82 (d, J = 3.2 Hz, 1 H, 2-H), 3.12 (d, J = 11.5 Hz, 1 H, 3-Ha), 3.53 (s, 3 H, 9a-OCH_3), 3.65 (dd, J = 11.5, 1.1 Hz, 1 H, 3-Hb), 3.92 (s, 3 H, 7-OCH_3), 4.12 (ddd, J = 8.2, 6.9, 1.2 Hz, 1 H, 9-H), 4.99 (dd, J = 11.8, 8.2 Hz, 1 H, 10-Ha), 5.41 (dd, J = 11.8, 6.9 Hz, 1 H, 10-Hb), 7.68 (br s, 2 H, $CONH_2$); IR (KBr disk) 3450, 1730, 1712, 1686, 1605, 1593, 1446, 1353, 1332, 1299, 1257, 1065, 959, 940 cm^{-1}; SIMS m/z 430/428 (M^+ + 1); R_f = 0.46 (chloroform/acetone = 7:3). Anal. Calcd for $C_{16}H_{18}BrN_3O_6$: C, 44.87; H, 4.24; N, 9.81. Found: C, 44.45; H, 4.23; N, 9.51. **4b**: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 1.92 (s, 3 H, 6-CH_3), 2.74 (d, J = 3.2 Hz, 1 H, 2-H), 2.83 (ddd, J = 3.2, 1.1, 1.0 Hz, 1 H, 1-H), 2.86 (dd, J = 11.6, 1.1 Hz, 1 H, 3-Ha), 3.08 (d, J = 11.6 Hz, 1 H, 3-Hb), 3.45 (s, 1 H, 9a-OCH_3), 3.95 (s, 3 H, 7-OCH_3), 4.12 (ddd, J = 7.9, 5.9, 1.0 Hz, 1 H, 9-H), 4.68 (dd, J = 11.5, 7.9 Hz, 1 H, 10-Ha), 5.01 (dd, J = 11.5, 5.9 Hz, 1 H, 10-Hb), 7.74 (br s, 2 H, $CONH_2$); IR (KBr disk) 3440, 3300, 3210, 2950, 1730, 1700, 1660, 1610, 1442, 1440, 1330, 1247, 1229, 1208, 1191, 1169, 1134, 1080, 1038 cm^{-1}; SIMS m/z 430/428 (M^+ + 1); R_f = 0.40 (chloroform/acetone = 7:3). Anal. Calcd for $C_{16}H_{18}BrN_3O_6$: C, 44.87; H, 4.24; N, 9.81. Found: C, 44.81; H, 4.27; N, 9.44.

1a-*N*-Bromomitomycin A (5a). To a solution of **1a** (100 mg, 0.286 mmol) in $CDCl_3$ (5.6 mL) was added *N*-bromosuccinimide (51 mg) at 25 °C. The ^{1}H NMR of the reaction mixture was measured. **5a**: ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.87 (s, 3 H, 6-CH_3), 3.18 (s, 3 H, 9a-OCH_3), 3.18 (dd, J = 5.1, 2.3 Hz, 1 H, 2-H), 3.25 (d, J = 5.1 Hz, 1 H, 1-H), 3.45 (dd, J = 13.6, 2.3 Hz, 1 H, 3α-H), 3.66 (dd, J = 10.9, 4.7 Hz, 1 H, 9-H), 4.05 (s, 3 H, 7-OCH_3), 4.23 (d, J = 13.6 Hz, 1 H, 3β-H), 4.24 (t, J = 10.9 Hz, 1 H, 10-Ha), 4.90 (dd, J = 10.9, 4.7 Hz, 1 H, 10-Hb), 5.03 (br s, 2 H, $CONH_2$).

1a-*N*-Chloromitomycin A (5b). To a solution of **1a** (94.8 mg, 0.264 mmol) in dichloromethane (1 mL) was added *tert*-butyl hypochlorite (30 μL) at 0 °C, and the reaction mixture was stirred for 30 min. The reaction mixture was diluted with chloroform and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography with 20% acetone in chloroform to afford **5b** (63.2 mg, 61% yield). **5b**: ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.85 (s, 3 H, 6-CH_3), 3.16 (s, 3 H, 9a-OCH_3), 3.16 (dd, J = 5.5, 2.3 Hz, 1 H, 2-H), 3.30 (d, J = 5.5 Hz, 1 H, 1-H), 3.53 (dd, J = 13.4, 2.3 Hz, 1 H, 3α-H), 3.67 (dd, J = 10.9, 4.6 Hz, 1 H, 9-H), 4.05 (s, 3 H, 7-OCH_3), 4.25 (d, J = 13.4 Hz, 1 H, 3β-H), 4.31 (t, J = 10.9 Hz, 1 H, 10-Ha), 4.80 (br s, 2 H, $CONH_2$), 4.92 (dd, J = 10.9, 4.6 Hz, 1 H, 10-Hb); IR (KBr disk) 3362, 2924, 1722,

(18) (a) Anderson, N. H.; Ollis, W. D.; Thorpe, J. E.; Ward, A. D. *J. Chem. Soc. Perkin Trans. 1*, **1975**, 825. (b) Miki, T.; Hiraga, K.; Asako, T.; Masuya, H. *Chem. Pharm. Bull.* **1967**, *15*, 670.

(19) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209.

(20) This selective hydrogenolysis could not be explained by the bond length comparison of C4a–N4 and C4a–N1a in **2** and **7**, because their lengths are equal in **2**[17] and **7** (C4a–N4 = 1.543 Å and C4a–N1a = 1.541 Å).

(21) The above-mentioned "new mitomycin derivatives" are exemplified by 9a-*O*-demethylmitomycins A and C; see: (a) Kono, M.; Kasai, M. *J. Syn. Org. Chem. Jpn.* **1990**, *48*, 824. (b) Kasai, M.; Kono, M. *Synlett*, in press.

(22) MOPAC Ver. 6, Stewart, J. J. P. *QCPE Bull.* **1989**, *9*, 10. Revised as Ver. 6.01, Hirano, T. *JCPE Newlett.* **1991**, *3*, 27.

(23) Baker, J. *J. Comput. Chem.* **1986**, *7*, 385.

1640, 1579, 1441, 1338, 1296, 1206, 1067 cm^{-1}; FABMS m/z 384/386 ($M^+ + 1$). Exact mass calcd for $C_{16}H_{19}{}^{35}ClN_3O_6$ ($M^+ + 1$) 384.0999, found 384.0981.

Albomitomycin A (2). (1) By Hydrogenolysis of 4a or 4b. To a solution of **4b** (43.0 mg, 1.00 mmol) and 5% Pd on $BaSO_4$ (40 mg) in methanol (3 mL) was added $NaHCO_3$ (12.6 mg) in water (1 mL), and the reaction mixture was hydrogenated at 25 °C for 10 min. The reaction mixture was poured into chloroform (50 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure, and the residue was purified by silica gel chromatography with 5% methanol in chloroform to afford **2** (18.8 mg, 54% yield) and **1a** (7.1 mg, 20% yield). By the same procedure, **4a** (44.2 mg, 0.103 mmol) afforded **2** (20.2 mg, 56% yield) and **1a** (7.2 mg, 20% yield). **2**: ^1H NMR (pyridine-d_5, 100 MHz) δ 1.90 (s, 3 H, 6-CH_3), 2.63 (br d, J = 3.0 Hz, 1 H, 1-H), 2.71 (br d, J = 3.0 Hz, 1 H, 2-H), 2.93 (d, J = 11.5 Hz, 1 H, 3-Ha), 2.98 (br d, J = 11.5 Hz, 1 H, 3-Hb), 3.40 (s, 3 H, 9a-OCH_3), 3.62 (d, J = 5.4 Hz, 1 H, 8a-H), 3.76 (ddd, J = 6.6, 6.1, 5.4 Hz, 1 H, 9-H), 3.94 (s, 3 H, 7-OCH_3), 4.57 (dd, J = 11.4, 6.1 Hz, 1 H, 10-Ha), 4.83 (dd, J = 11.4, 6.6 Hz, 1 H, 10-Hb), 7.62 (br s, 2 H, $CONH_2$); IR (KBr disk) 3500, 3370, 2970, 1740, 1700, 1660, 1600, 1130 cm^{-1}; EI-MS m/z 349 (M^+). Anal. Calcd for $C_{16}H_{19}N_3O_6$: C, 55.01; H, 5.48; N, 12.03. Found: C, 55.08; H, 5.74; N, 11.55.

(2) By Radical Reduction of 4b. To a solution of **4b** (10.7 g, 25.0 mmol) in toluene (1 L) at 60 °C were added tributyltin hydride (100.8 mL) and AIBN (2.40 g) in small portions over 1 h, and the mixture was stirred at the same temperature for 40 min. The reaction mixture was cooled to 25 °C and purified by silica gel flash chromatography first with chloroform and then with 3% methanol in chloroform. The residue was suspended in ethyl acetate (25 mL) to remove the small amount of **1a** and filtered to afford **2** (7.42 g, 85% yield).

Isomitomycin A (3). Albomitomycin A (**2**, 1.0 g, 2.78 mmol) and 10% Pd on carbon (500 mg) were stirred in acetonitrile (100 mL) under a hydrogen atmosphere at 25 °C for 2 h and then stirred under air at 25 °C for 2 h. The catalyst was filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel flash chromatography with 10% acetone in ethyl acetate to afford **3** (898 mg, 90% yield). **3**: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.88 (br s, 1 H, 2-NH), 1.95 (s, 3 H, 6-CH_3), 2.85 (br d, J = 12.1 Hz, 1 H, 1-Ha), 3.14 (t, J = 3.6 Hz, 1 H, 3-H), 3.17 (dd, J = 3.4, 0.7 Hz, 1 H, 7d-H), 3.33 (dd, J = 12.1, 2.7 Hz, 1 H, 1-Hb), 3.38 (s, 3 H, 2a-OCH_3), 3.57 (dd, J = 3.4, 2.7 Hz, 1 H, 7c-H), 4.05 (s, 3 H, 5-OCH_3), 4.30 (dd, J = 11.1, 3.6 Hz, 1 H, 3-CHa), 4.47 (dd, J = 11.1, 3.6 Hz, 1 H, 3-CHb), 4.59 (br s, 2 H, $CONH_2$); IR (KBr disk) 3440, 3350, 2920, 1710, 1641, 1591, 1076 cm^{-1}; EI-MS m/z 349 (M^+). Anal. Calcd for $C_{16}H_{19}N_3O_6$: C, 55.01; H, 5.48; N, 12.03. Found: C, 55.35; H, 5.91; N, 11.97.

Dihydroisomitomycin A (6). Albomitomycin A (**2**, 175 mg, 0.501 mmol) and 10% Pd on carbon (100 mg) were stirred in ethyl acetate (10 mL) under a hydrogen atmosphere at 25 °C for 5 h. The catalyst was filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by radial chromatography on a 2-mm-layer silica gel plate with 10% methanol in chloroform under a nitrogen atmosphere to afford **6** (82.7 mg, 47% yield). **6**: ^1H NMR ($CDCl_3$, 400 MHz) δ 0.9 (br s, 1 H, 2-NH), 2.20 (s, 3 H, 6-CH_3), 2.59 (br d, J = 11.7 Hz, 1 H, 1-Ha), 3.18 (dd, J = 3.6, 2.8 Hz, 1 H, 7c-H), 3.24 (dd, J = 3.6, 0.8 Hz, 1 H, 7d-H), 3.26 (dd, J = 11.7, 2.8 Hz, 1 H, 1-Hb), 3.37 (t, J = 4.6 Hz, 1 H, 3-H), 3.41 (s, 3 H, 2a-OCH_3), 3.77 (s, 3 H, 5-OCH_3), 4.29 (dd, J = 10.9, 4.7 Hz, 1 H, 3-CHa), 4.51 (dd, J = 10.9, 4.5 Hz, 1 H, 3-CHb), 4.66 (br s, 2 H, $CONH_2$), 5.63 (br s, 1 H, one of OH), 5.77 (br s, 1 H, one of OH); IR (KBr disk) 3380, 1710, 1600, 1461, 1423, 1331, 1063 cm^{-1}; EI-MS m/z 351 (M^+).

Mitomycin C (1b) from Isomitomycin A (3). Isomitomycin A (**3**, 62.4 mg, 0.18 mmol) was dissolved in 6 M NH_3 in methanol (20 mL) at 25 °C and stirred for 5 h. The solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography with 5% methanol in chloroform to afford **1b** (44.7 mg, 75% yield). **1b**: ^1H NMR (pyridine-d_5, 400 MHz) δ 2.02 (s, 3 H, 6-CH_3), 2.07 (br t, J = 7.4 Hz, 1 H, 1a-NH), 2.75 (br s, 1 H, 2-H), 3.15 (dd, J = 7.4, 4.4 Hz, 1 H, 1-H), 3.22 (s, 3 H, 9a-OCH_3), 3.60 (br d, J = 12.6 Hz, 1 H, 3α-H), 4.03 (dd, J = 11.0, 4.2 Hz, 1 H, 9-H), 4.55 (d, J = 12.6 Hz, 1 H, 3β-H), 5.11 (dd, J = 11.0, 10.5 Hz, 1 H, 10-Ha), 5.43 (dd, J = 10.5, 4.2 Hz, 1 H, 10-Hb), 7.58 (br s, 4 H, 7-NH_2 and $CONH_2$); IR (KBr disk) 3440, 3310, 3260, 1722, 1592, 1544, 1440 cm^{-1}; EI-MS m/z 334 (M^+).

Mitomycin C (1b) from Albomitomycin A (2). Albomitomycin A (**2**, 53.0 mg, 0.15 mmol) was dissolved in 6 M NH_3 in methanol (20 mL) at 25 °C and stirred for 30 min. The solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography with 5% methanol in chloroform to afford **1b** (50.7 mg, 99% yield).