First Synthesis of Mitomycins Completely Labeled at the C-6-Methyl by $^{13}CH_3$ and CD_3

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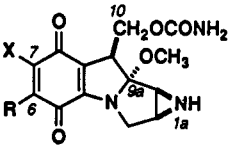
The C-6-methyl group of mitomycins was completely labeled with carbon-13 or deuterium for the first time. The synthesis was accomplished by the C-6-methylation of 6-demethyl-7,7-(ethylenedioxy)-6-(phenylseleno)mitosane 8 that was formed by a novel replacement of the methylene moiety of 6-demethyl-7,7-(ethylenedioxy)-6-methylenemitosane 10 by a phenylseleno group, and followed by conversion to the mitomycins. For the synthesis of 8, we found that selenenamide 11 is an excellent reagent for the replacement. We have also determined that the replacement proceeded via the addition of 11 to the methylene of 10 with a subsequent retro-Mannich reaction.

Introduction

Mitomycins are well-known to be potent antitumor antibiotics,[1] produced by various *Streptomyces* cultures. Among these compounds, mitomycin C (MMC, 1) has been extensively used in cancer chemotherapy against a variety of solid tumors, but its use is limited by side effects, such as severe bone marrow suppression or gastrointestinal damage. Consequently, a number of derivatives targeting less toxicity or more effective activity have been synthesized in our laboratory[2] or by other groups.[3] For evaluation of these new mitomycin derivatives, drug metabolism and pharmacokinetic studies including a comparison with those of 1 need to be performed. In such studies, isotopically labeled drugs with carbon-14 or tritium[4] are effective as a general method. Moreover, they are useful tools to study the detailed mechanism of action of mitomycins or their derivatives using human tumor cells.[5] In the course of our studies, the first synthetic method of effectively labeled mitomycins at a metabolically stable C-6-methyl group was reported in a previous paper.[6] It has allowed reliable studies of metabolism, pharmacokinetics, and mechanism of action of mitomycins. While this method is superior to the conventional methods[4] in many respects, the relatively low incorporation of isotopes and the impossibility of carbon labeling still remains under consideration.

We recently discovered the novel replacement of the methylene moiety by a phenylseleno group and have obtained 6-demethyl-7,7-(ethylenedioxy)-6-(phenylseleno)mitosane 8.[7,8] This compound should enable us to prepare 1 and mitomycin A (MMA, 2) completely labeled at the C-6-methyl position with carbon-14 or tritium through the C-6-methylation of 8 with subsequent conversion to mitomycins. To investigate our synthetic strategy, we tried to synthesize 1 and 2 containing the C-6-methyl group labeled with deuterium or carbon-13 for facility of the handling and structure identifications. In this paper, we report the complete details of our investigation into the synthesis of these specifically and completely labeled compounds 3–6.



1: Mitomycin C (X = NH_2, R = CH_3)
2: Mitomycin A (X = CH_3O, R = CH_3)
3: X = NH_2, R = $^{13}CH_3$
4: X = CH_3O, R = $^{13}CH_3$
5: X = NH_2, R = CD_3
6: X = CH_3O, R = CD_3

Results and Discussion

Our initial finding was made in the course of reactions of 6-demethyl-7,7-(ethylenedioxy)-6-methylenemitosane 10 with secondary amines. We found that 10 prepared in

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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. In *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) (a) Kono, M.; Saitoh, Y.; Kasai, M.; Sato, A.; Shirahata, K.; Morimoto, M.; Ashizawa, T. *Chem. Pharm. Bull.* **1989**, *37*, 1128. (b) 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.

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

(4) In the case of MMC, three methods for labeling were reported to date in addition to our studies. (a) Wiltzbach method: Weissbach, A.; Lisio, A. *Biochemistry* **1965**, *4*, 196. Ørstavik, J. *Acta Path. Microbiol. Scand., Sect. B* **1974**, *82*, 270. (b) Biosynthetic method: Hornemann, U.; Cloyd, J. C. *J. Chem. Soc., Chem. Commun.* **1971**, 301. (c) Chemical method: Haynes, U. J.; Swigor, J. E.; Kaneko, T. *J. Labelled Compd Radiopharm.* **1990**, *28*, 241. Though this method enables us to prepare carbon-14-labeled MMC at the C10-carbamoyloxy group, the carbamoyloxy group of MMC is destined to leave the MMC skeleton during the activating process.

(5) For example, using [1a-CH_3-^{3}H]porfiromycin: Tomasz, M.; Hughes, C. S.; Chowdary, D.; Keyes, S. R.; Lipman, R. L.; Sartorelli, A. C.; Rockwell, S. *Cancer Commun.* **1991**, *3*, 213.

(6) (a) Kanda, Y.; Kasai, M. *J. Org. Chem.* **1990**, *55*, 2515. (b) Kanda, Y.; Akinaga, S.; Kasai, M. *J. Labelled Compd Radiopharm.* **1990**, *28*, 1033.

(7) Kasai, M.; Arai, H.; Kanda, Y. *J. Chem. Soc., Chem. Commun.* **1991**, 600.

(8) To our knowledge, the retro-Mannich reaction of a α-seleno-β-aminocarbonyl moiety has been mentioned only once in the following literature: Hase, T. A.; Kukkola, P. *Synth. Commun.* **1980**, *10*, 451.

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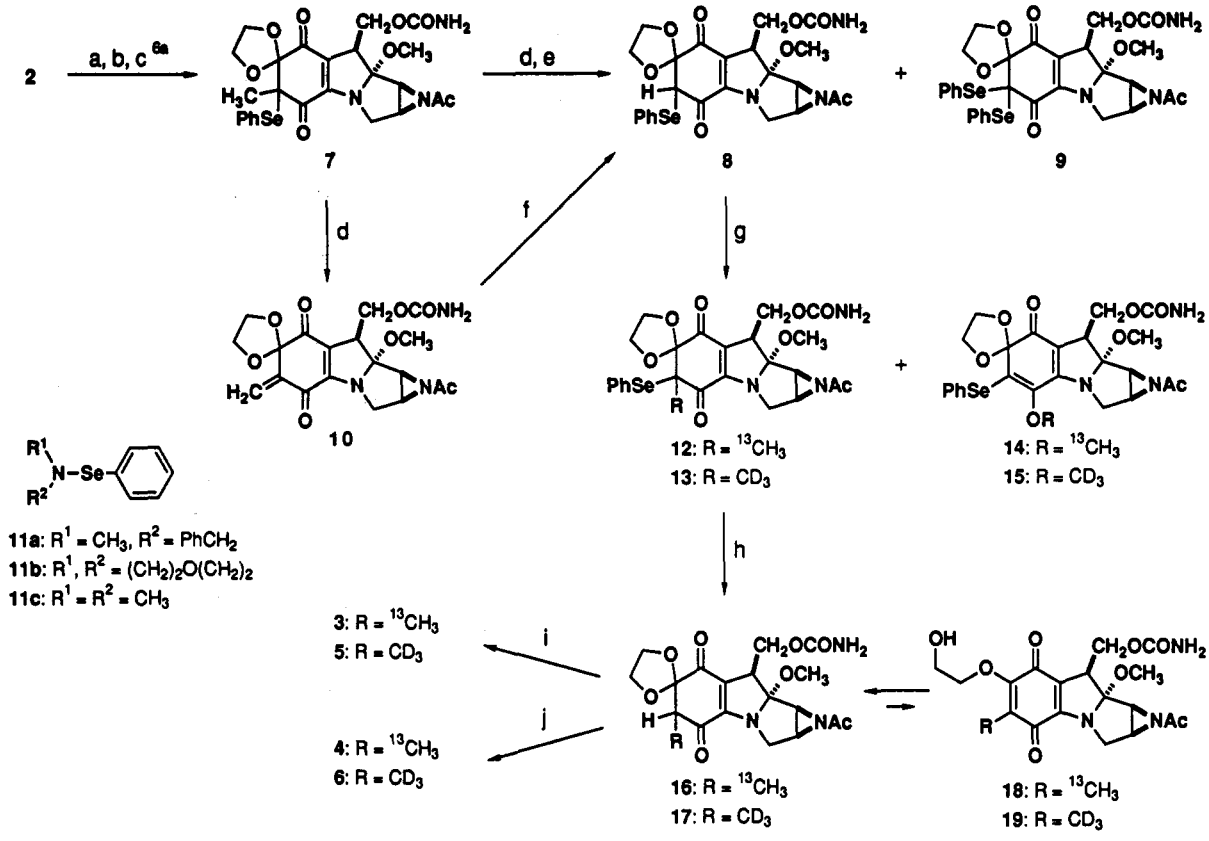
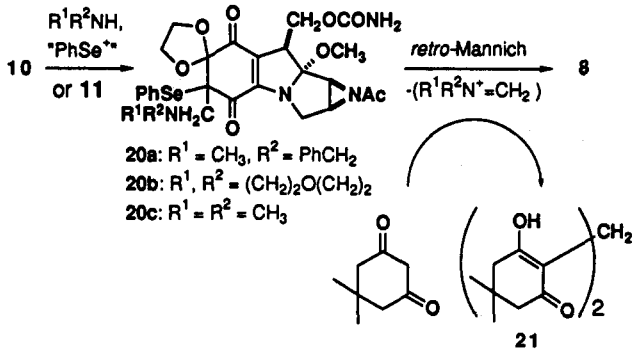
Scheme I[a]



[a] (a) Ac_2O, pyridine, $CHCl_3$; (b) KOH (catalytic), ethylene glycol, THF; (c) PhSeBr, Et_3N, THF; (d) mCPBA, K_2CO_3; (e) $PhCH_2NHMe$; (f) compound 11; (g) RI, K_2CO_3; (h) *n*-Bu_3SnH, Et_3B, benzene, rt; (i) NH_3, MeOH; (j) K_2CO_3, MeOH.

Scheme II



situ from 7,7-(ethylenedioxy)-6-(phenylseleno)mitosane 7 and *m*-chloroperbenzoic acid (mCPBA) reacted with secondary amines to afford 6-demethyl-7,7-(ethylenedioxy)-6-(phenylseleno)mitosane 8 in low yield (about 20%) (Scheme I). The suggested mechanism for this quite new conversion was considered as follows: (1) The formation of the 1,4-adduct **20**, which has a β-amino-α-selenocarbonyl moiety;[8] this was formed in situ from **10** with amines and the cationic phenylseleno species arising from **7**, and (2) the retro-Mannich reaction of **20** (Scheme II). On the basis of this consideration, we attempted to react the selenenamide **11** with the enone **10** to form the adduct **20**.[9]

Actually, by the reaction of the crude enone **10** with **11a** at room temperature for 1.5 h (method A), the 6-(phenylseleno) compound **8** was obtained in 61% yield based on **7** after purification by silica gel chromatography along with a small amount of 6,6-bis(phenylseleno) derivative **9**. Further, we tried to react the isolated enone **10** with **11b**,[10] which is more stable and known to form adducts with reactive Michael acceptors (method B). Similar results were also obtained by this reaction (58% yield based on **10**).

To investigate the mechanism of this reaction, we attempted to trap the formaldehyde generated by hydrolysis of the iminium species formed by the retro-Mannich reaction. As a trapping reagent of formaldehyde, dimedone was chosen for its excellent ability of forming adducts. Dimedone was added as a methanol–water (50:50) solution into the reaction mixture and stirred at room temperature for 15 h and refluxed for an additional 11 h. After purification, methylidenebis(dimethyldihydroresorcin) (**21**)[11] was obtained in 20% yield. In addition, we spectroscopically tried to identify the adduct **20**. First, we investigated by ^1H NMR the mixture of **11c** and **10** labeled at the ethylenedioxy group by deuterium in order to eliminate the complexity of the spectrum caused by multiple proton signals from this group. Immediately after addition of **11c** to the deuterated **10** in $CDCl_3$, the *exo*-methylene signals of **10** disappeared and four doublet signals (J = 13.8 Hz) corresponding to the C-6-methylene of two diastereomers of the newly generated **20c** were observed (2.50 and 3.06 ppm, 2.67 and 3.11 ppm). Subsequent addition of formic acid to the mixture resulted in the formation of **8** in reasonable purity. In the FAB mass spectrum (FABMS) of the **10** and **11b** mixture, *m/z* 661 and 663 as parent peaks ($M^+ + 1$) of the adduct **20b** were observed. This parent peak of **20b** (*m/z* 663) was

(9) Reich, H. J.; Renga, J. M. *J. Org. Chem.* **1975**, *40*, 3313.

(10) Lerouge, P.; Paulmier, C. *Bull. Chim. Soc. Fr.* **1985**, 1219.
(11) Weinberger, W. *Ind. Eng. Chem. Anal. Ed.* **1931**, *3*, 365.

also identified as a desired molecular ion peak with high-resolution FABMS [HRFABMS: calculated for $C_{29}H_{35}N_4O_9Se$ ($M^+ + H$) m/z 663.1570, found 663.1611]. These facts indicated that the formation of the intermediate **20** and a subsequent retro-Mannich reaction had occurred under acidic conditions, namely, in the presence of formic acid or silica gel.

Selenenamide **11** is known to form an adduct with a carbon–carbon double bond of α,β-unsaturated enones,[9,10] but subsequent elimination has not been reported. Therefore, this conversion is the first example of a removal of the *exo*-methylene in α,β-unsaturated enones using selenenamide.

HPLC analysis of this reaction product indicated that the resulting **8** contained a small amount of starting material **7** that was difficult to separate by common chromatographic purification techniques using silica gel. This contamination did not significantly affect further derivation. In this case, however, its presence would affect the labeling percentage of the C-6-methyl position of **1** and **2**. So we repurified it using preparative HPLC and obtained pure **8** with a recovery of 64%. In further derivations, pure **8** was used as the key intermediate for the synthesis of **3–6**.

Using **8**, we next tried to demonstrate the transformation toward C-6-methyl-labeled MMCs **3** and **5**, which would be prepared via the following three steps: (1) C-6-methylation, (2) C-6-deselenenylation, and (3) N-1a-deacetylation and C-7-amination.

The C-6-methylation of **8** was carried out under the usual conditions. A reaction of **8** with ^{13}C- or D-labeled (>99 atom %) methyl iodide and potassium carbonate in acetone at room temperature gave the ^{13}C- and D-labeled 7,7-(ethylenedioxy)-6-(phenylseleno)mitosanes **12** and **13** in 69% and 75% yields, respectively, along with minor products **14** (12%) and **15** (6.9%). Subsequent deselenenylation of **12** and **13** were achieved by the radical reduction using tributyltin hydride. Taking into account the instability of the reactant and the product, we chose triethylborane as a radical initiator that can be used below room temperature.[12] Compounds **12** and **13** reacted with tributyltin hydride in the presence of a catalytic amount of triethylborane at room temperature and afforded 7,7-(ethylenedioxy)mitosanes **16** and **17** in 84% and 88% yields, respectively, as equilibrium mixtures with a small amount of 7-(2-hydroxyethoxy)mitosanes **18** and **19**.[6]

Conversion to labeled MMCs was performed by the N-1a-deacetylation and the C-7-amination of **16** and **17**. These were simultaneously carried out using ammonia in methanol at room temperature[6] to afford [C-6-CH_3-^{13}C]-MMC (**3**) and [C-6-CH_3-$^2H_3$]MMC (**5**) in 75% and 79% yields, respectively. In this manner, the desired **3** and **5** were obtained in 43% and 51% overall yields, respectively, based on **8**.

We next identified the labeled position and the incorporation ratio of these labeled MMCs using ^1H and ^{13}C NMR and mass spectroscopies.

The C-6-methyl group of nonlabeled MMC (**1**) appears as a singlet at 2.02 ppm in the ^1H NMR spectrum,[13] while that of our synthetic [C-6-CH_3-^{13}C]MMC (**3**) appeared as a doublet (J = 127.2 Hz) at 2.02 ppm. This large coupling constant of **3** indicates the existence of coupling between hydrogen and carbon-13 at the C-6-methyl position. Similarly, the C-6-methyl group of [C-6-CH_3-$^2H_3$]MMC (**5**) disappeared in the ^1H NMR spectrum, which showed a complete replacement of hydrogen at the C-6-methyl position by deuterium. In the ^{13}C NMR spectra, the C-6-methyl group of **1** appeared as a singlet at 8.77 ppm,[13] and that of **3** appeared as a singlet with great intensity. Further, a satellite signal of the C-6-methyl at 8.77 ppm and a signal of C-6 at 104.38 ppm of **3** appeared as doublets with a 46.2-Hz coupling constant between them. Also, the signal of C-8 was split (J = 2.5 Hz) by the long-range coupling with carbon-13 at the C-6-methyl position. On the other hand, that of **5** appeared as a septet (J = 19.4 Hz) at 8.01 ppm. This splitting is obviously due to the coupling between carbon and deuterium at the C-6-methyl position. Chemical shifts of carbons at the C-6-methyl and C-6 positions of **5** were observed to shift upfield 0.76 and 0.13 ppm, respectively, when compared to those of **1**.

Comparison of the EI mass spectra (EIMS) of these labeled MMCs **3** and **5** with that of **1**[14] showed appropriate parent peaks (M^+) and fragment ion peaks. Examination of **3** and **5** by high-resolution EIMS (HREIMS) also showed the corresponding fragment ion ($M^+ - CH_3OH$) and molecular ion (M^+) peaks.

Finally, these results represent the exact structures of **3** and **5** and indicate the incorporation of isotopes of those almost reflecting the incorporation (>99 atom %) of the labeled methyl iodide that was used.

In addition, we tried to demonstrate the synthesis of labeled MMAs **4** and **6** that are important as starting materials in the synthesis of various mitomycin derivatives.

Conversion to labeled MMAs was performed by the N-1a-deacetylation and the C-7-transalkoxylation of 7,7-(ethylenedioxy)mitosanes **16** and **17**.[6] These were carried out by the reaction with methanol and potassium carbonate at room temperature to afford [C-6-CH_3-^{13}C]MMA (**4**) and [C-6-CH_3-$^2H_3$]MMA (**6**) in 82% and 83% yields, respectively. Thus, the desired **4** and **6** were obtained in 47% and 54% overall yields based on **8**. Its structure and the incorporation of ^{13}C or D were confirmed by ^1H NMR, EIMS, and HRFABMS spectroscopies in a manner similar to that previously mentioned.

In conclusion, by demonstrating the present synthesis, we established the methodology to prepare C-6-methyl-labeled MMCs and MMAs in excellent yields. It has enabeled us to obtain mitomycins and their derivatives completely labeled at a metabolically stable position by ^{13}C, D and other isotopes.[15] Moreover, the novel 6-demethyl intermediate **8** will serve as a tool for the hitherto impossible modification at the C-6 position of mitomycins.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. Proton (^1H NMR) and carbon-13 (^{13}C NMR) nuclear magnetic resonance spectra were recorded on a Bruker AM 400 and a JEOL JNM-GX270 instruments. Mass spectral (MS) data were obtained on a Hitachi M-80B and a JEOL JMS-D300 mass spectrometers. Infrared spectra (IR) were recorded on a Nihon Bunko IR-810 instrument. Elemental analyses were performed by a Perkin-Elmer 2400 C, H, N analyzer.

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (8) (Method A). To a suspension of **7**[6] (1.143 g, 2.505 mmol) and powdered

(12) Nozaki, K.; Oshima, K.; Utimoto, K. *J. Am. Chem. Soc.* **1987**, *109*, 2547.
(13) Keller, P. J.; Hornemann, U. *J. Nat. Prod.* **1983**, *46*, 569.
(14) Van Lear, G. E. *Tetrahedron* **1970**, *26*, 2587.
(15) Arai, H.; Kasai, M. *J. Labelled Compd Radiopharm.* **1991**, *29*, 903.

potassium carbonate (703 mg, 5.09 mmol, 2.0 equiv) in chloroform (75 mL) was added dropwise *m*-chloroperbenzoic acid (ca. 85% purity, 559 mg, 2.8 mmol, 1.1 equiv) in chloroform (50 mL) over a period of 40 min at −40 °C. The reaction mixture was maintained at −40 °C for 15 min and then allowed to warm slowly to room temperature. After 2 h, a phosphate buffer (0.1 M, pH 7) and aqueous $Na_2S_2O_3$ (0.1 M) were added. The layers were separated, and the aqueous layer was extracted with chloroform. The combined organic layers were washed with brine and dried over Na_2SO_4 to afford the crude solution of **10** (300 mL). This solution of the crude product was used in a subsequent step without purification.

To the above solution of **10** (300 mL) was added dropwise *N*-benzyl-*N*-methylphenylselenenamide (**11a**) in *n*-hexane (0.10 M, 20 mL, 2.0 mmol, 0.80 equiv) over a period of 40 min at 0 °C. The ice bath was removed, and the reaction mixture was allowed to stand at room temperature for 1.5 h. The resulting mixture was concentrated with a rotary evaporator to ca. 100 mL and applied to chromatography (silica gel, 1:5:5 *n*-hexane/acetonitrile/chloroform as an eluent) to afford **8** as a yellow paste along with 6,6-bis(phenylseleno)mitosane **9** (80.3 mg, 0.112 mmol, 4.5%). It was dissolved in a small amount of chloroform and poured into *n*-hexane. The resulting precipitate was filtered off, washed with *n*-hexane, and dried under vacuum to afford **8** (859.9 mg, 1.529 mmol, 61%) as a yellow powder. Since this material was contaminated with several percent of starting material, it was purified with the preparative HPLC (ODS, 45:55 acetonitrile/water as an eluent). After similar treatment as described above, pure **8** was obtained as a yellow powder with a recovery of 64%. The product was a ca. 3.5:1 equilibrium mixture of two diastereomers at C-6 in $CDCl_3$.

8: ^{1}H NMR (400 MHz, $CDCl_3$) (major isomer) δ 2.20 (s, 3 H, 1a-$COCH_3$), 3.21 (s, 3 H, 9a-OCH_3), 3.26 (dd, *J* = 2.0, 4.7 Hz, 1 H, 2-H), 3.41 (dd, *J* = 2.0, 13.0 Hz, 1 H, 3α-H), 3.52 (d, *J* = 4.7 Hz, 1 H, 1-H), 3.73 (dd, *J* = 4.9, 10.8 Hz, 1 H, 9-H), 3.84 (d, *J* = 13.0 Hz, 1 H, 3β-H), 4.02 (s, 1 H, 6-H), 4.19 (t, *J* = 10.8 Hz, 1 H, 10-H_a), 4.01–4.20 (m, 3 H, ethylenedioxy), 4.41 (m, 1 H, ethylenedioxy), 4.91 (br s, 2 H, 10-$OCONH_2$), 4.95 (dd, *J* = 4.9, 11.1 Hz, 1 H, 10-H_b), 7.28–7.38 (m, 3 H, phenyl), 7.60 (m, 2 H, phenyl); (minor isomer) δ 2.10 (s, 3 H, 1a-$COCH_3$), 3.21 (s, 3 H, 9a-OCH_3), 3.23 (dd, *J* = 2.0, 4.4 Hz, 1 H, 2-H), 3.39 (dd, *J* = 2.0, 13.0 Hz, 1 H, 3α-H), 3.48 (d, *J* = 4.4 Hz, 1 H, 1-H), 3.67 (dd, *J* = 4.7, 10.8 Hz, 1 H, 9-H), 4.17 (s, 1 H, 6-H), 4.01–4.20 (m, 4 H, ethylenedioxy + 10-H_a), 4.31 (m, 1 H, ethylenedioxy), 4.40 (d, *J* = 13.0 Hz, 1 H, 3β-H), 4.81 (dd, *J* = 4.7, 10.8 Hz, 1 H, 10-H_b), 4.89 (br s, 2 H, 10-$OCONH_2$), 7.28–7.38 (m, 3 H, phenyl), 7.61 (m, 2 H, phenyl); ^{13}C NMR (67.5 MHz, $CDCl_3$) (major isomer) δ 23.5 (1a-$COCH_3$), 39.3 (C-2), 42.6 (C-9), 43.1 (C-1), 47.8 (C-3), 49.9 (9a-OCH_3), 55.1 (s + d, $^1J[^{13}C^{77}Se]$ = 69.6 Hz, C-6), 61.7 (C-10), 66.2 and 67.6 (ethylenedioxy), 105.3 (C-9a), 105.6 (C-7), 119.1 (C-8a), 127.6 (SePh-1), 128.9 (SePh-4), 129.3 (SePh-3 + -5), 135.7 (SePh-2 + -6), 155.2 (C-4a), 156.4 (10-$OCONH_2$), 180.6 (1a-$COCH_3$), 184.3 (C-5), 186.1 (C-8); (minor isomer) δ 23.5 (1a-$COCH_3$), 39.3 (C-2), 42.2 (C-9), 42.9 (C-1), 47.3 (C-3), 49.8 (9a-OCH_3), 58.0 (s + d, $^1J[^{13}C^{77}Se]$ = 73.9 Hz, C-6), 61.3 (C-10), 66.6 and 67.4 (ethylenedioxy), 105.7 (C-9a), 105.7 (C-7), 119.8 (C-8a), 127.7 (SePh-1), 129.1 (SePh-4), 129.2 (SePh-3 + -5), 135.5 (SePh-2 + -6), 153.0 (C-4a), 156.4 (10-$OCONH_2$), 180.7 (1a-$COCH_3$), 184.5 (C-5), 185.9 (C-8); FABMS *m/z* 562/564 (M^+ + 1); IR (KBr) 3470, 3370, 3060, 2960, 2900, 1700, 1660, 1570, 1460, 1400, 1340, 1250, 1080, 1030 cm^{-1}. Anal. Calcd for $C_{24}H_{25}N_3O_8Se$: C, 51.25; H, 4.48; N, 7.47. Found: C, 51.45; H, 4.53; N, 7.27.

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6,6-bis(phenylseleno)mitomycin A (9): ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.32 (s, 3 H, 1a-$COCH_3$), 3.03 (s, 3 H, 9a-OCH_3), 3.33 (dd, *J* = 1.9, 12.9 Hz, 1 H, 3α-H), 3.45 (dd, *J* = 2.0, 4.6 Hz, 1 H, 2-H), 3.65 (d, *J* = 13.0 Hz, 1 H, 3β-H), 3.82 (d, *J* = 4.4 Hz, 1 H, 1-H), 4.12 (m, 1 H, ethylenedioxy), 4.13 (dd, *J* = 4.6, 11.2 Hz, 1 H, 9-H), 4.21 (m, 1 H, ethylenedioxy), 4.30 (m, 1 H, ethylenedioxy), 4.46 (m, 1 H, ethylenedioxy), 4.63 (t, *J* = 11.1 Hz, 1 H, 10-H_a), 5.75 (dd, *J* = 4.5, 10.7 Hz, 1 H, 10-H_b), 7.24–7.30 (m, 2 H, phenyl), 7.37–7.57 (m, 4 H, phenyl), 7.6–7.9 (br s, 2 H, 10-$OCONH_2$), 7.86–7.98 (m, 4 H, phenyl); FABMS *m/z* 716/718/720/722 (M^+ + 1); HRFABMS calcd for $C_{30}H_{30}N_3O_7{}^{80}Se_2$ (M^+ + H) *m/z* 720.0360, found 720.0399.

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-methylenemitomycin A (10). To a suspension of **7** (5.77 g, 10.0 mmol) and powdered potassium carbonate (2.82 g, 20.4 mmol, 2.0 equiv) in dichloromethane (100 mL) was added dropwise *m*-chloroperbenzoic acid (ca. 80% purity, 2.59 g, 12 mmol, 1.2 equiv) in dichloromethane (50 mL) over a period of 15 min at −40 °C. The reaction mixture was maintained at −40 °C for 40 min and then allowed to warm slowly to room temperature. After 1 h, a phosphate buffer (0.1 M, pH 7) and aqueous $Na_2S_2O_3$ (0.1 M) were added. The layers were separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated with a rotary evaporator to ca. 100 mL below 25 °C. This solution was poured into *n*-hexane (1000 mL). The resulting precipitate was filtered off, washed with *n*-hexane and dried under vacuum to afford crude **10** (3.77 g, 9.00 mmol, 90%) as a yellow powder. The purity of this material was ca. 90% (^{1}H NMR). It was used in a subsequent step without further purification.

10: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.12 (s, 3 H, 1a-$COCH_3$), 3.22 (s, 3 H, 9a-OCH_3), 3.24 (dd, *J* = 2.0, 4.4 Hz, 1 H, 2-H), 3.51 (d, *J* = 4.4 Hz, 1 H, 1-H), 3.52 (dd, *J* = 2.0, 13.3 Hz, 1 H, 3α-H), 3.75 (dd, *J* = 4.9, 10.8 Hz, 1 H, 9-H), 4.15 (t, *J* = 10.8 Hz, 1 H, 10-H_a), 4.08–4.32 (m, 4 H, ethylenedioxy), 4.39 (d, *J* = 13.0 Hz, 1 H, 3β-H), 4.77 (br s, 2 H, 10-$OCONH_2$), 4.92 (dd, *J* = 4.7, 10.8 Hz, 1 H, 10-H_b), 6.11 (d, *J* = 1.2 Hz, 1 H, methylene), 6.39 (d, *J* = 1.2 Hz, 1 H, methylene); SIMS *m/z* 420 (M^+ + 1); IR (KBr) 3470, 3370, 2950, 1700, 1660, 1570, 1460, 1380, 1330, 1270, 1110, 1060, 1030 cm^{-1}; HRFABMS calcd for $C_{19}H_{22}N_3O_8$ (M^+ + H) *m/z* 420.1407, found 420.1406.

1a-Acetyl-7-demethoxy-6-demethyl-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (8) (Method B). To a solution of **10** (3.91 g, 9.33 mmol) in dichloromethane (190 mL) was added dropwise *N*-(phenylseleno)morpholine (**11b**) (2.33 g, 9.62 mmol, 1.0 equiv) over a period of 45 min at 0 °C. The ice bath was removed, and the reaction mixture was allowed to stand at room temperature for 5 h. The resulting mixture was concentrated with a rotary evaporator to ca. 100 mL, and the residue was purified by chromatography (silica gel, 1:5:5 *n*-hexane/acetonitrile/chloroform as an eluent) to afford **8** as a yellow paste. It was dissolved in a small amount of chloroform and poured into *n*-hexane. The resulting precipitate was filtered off, washed with *n*-hexane, and dried under vacuum to afford **8** (3.04 g, 5.41 mmol, 58%) as a yellow powder.

[C-6-CH_3-^{13}C]-1a-Acetyl-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (12). A mixture of **8** (290.4 mg, 0.517 mmol), powdered potassium carbonate (138 mg, 1.00 mmol, 1.9 equiv), and $^{13}CH_3I$ (99 atom %, 325 μL, 5.18 mmol, 10 equiv) in acetone (5.0 mL) was stirred at room temperature for 4 days. After being poured into chloroform, the reaction mixture was washed with a phosphate buffer (pH 7, 0.1 M) and brine and dried over Na_2SO_4. The volatiles were removed with a rotary evaporator, and the residue was purified by chromatography (silica gel, 1:5:5 *n*-hexane/acetonitrile/chloroform as an eluent) to afford **12** and methyl enol ether **14** as yellow pastes. Each product was dissolved in a small amount of chloroform and poured into *n*-hexane. The resulting precipitates were filtered off, washed with *n*-hexane, and dried under vacuum to afford **12** (207.4 mg, 0.359 mmol, 69% based on **8**) and **14** (35.0 mg, 0.0607 mmol, 12% based on **8**) as yellow powders. Compound **12** was a ca. 1:1 mixture of two diastereomers at C-6 in pyridine-d_5.

12: ^{1}H NMR (270 MHz, pyridine-d_5) (isomer A) δ 1.62 (d, J_{CH} = 130.6 Hz, 3 H, 6-$^{13}CH_3$), 2.02 (s, 3 H, 1a-$COCH_3$), 3.31 (s, 3 H, 9a-OCH_3), 3.43 (dd, *J* = 2.0, 12.6 Hz, 1 H, 3α-H), 3.47 (dd, *J* = 2.0, 4.4 Hz, 1 H, 2-H), 3.80 (d, *J* = 4.4 Hz, 1 H, 1-H), 4.01 (d, *J* = 13.2 Hz, 1 H, 3β-H), 3.8–4.3 (m, 4 H, ethylenedioxy + 9-H), 4.54 (m, 1 H, ethylenedioxy), 4.65 (t, *J* = 10.9 Hz, 1 H, 10-H_a), 5.62 (dd, *J* = 4.7, 10.7 Hz, 1 H, 10-H_b), 7.25–7.50 (m, 3 H, phenyl), 7.65–7.80 (m, 2 H, phenyl), 7.5–7.8 (br, 2 H, 10-$OCONH_2$); (isomer B) δ 1.68 (d, J_{CH} = 130.6 Hz, 3 H, 6-$^{13}CH_3$), 2.29 (s, 3 H, 1a-$COCH_3$), 3.12 (s, 3 H, 9a-OCH_3), 3.47 (dd, *J* = 2.0, 13.0 Hz, 1 H, 3α-H), 3.53 (dd, *J* = 1.8, 4.4 Hz, 1 H, 2-H), 3.87 (d, *J* = 4.4 Hz, 1 H, 1-H), 3.8–4.3 (m, 4 H, ethylenedioxy + 9-H), 4.54 (m, 1 H, ethylenedioxy), 4.72 (d, *J* = 12.8 Hz, 1 H, 3β-H), 4.76 (t, *J* = 11.1 Hz, 1 H, 10-H_a), 5.86 (dd, *J* = 4.8, 10.8 Hz, 10-H_b), 7.25–7.50 (m,

3 H, phenyl), 7.65–7.80 (m, 2 H, phenyl), 7.5–7.8 (br, 2 H, 10-$OCONH_2$); FABMS m/z 577/579 ($M^+ + 1$); HRFABMS calcd for $C_{24}{}^{13}CH_{28}N_3O_8{}^{80}Se$ ($M^+ + H$) m/z 579.1076, found 579.1124; IR (KBr) 3450, 3360, 3050, 2920, 1690, 1650, 1570, 1440, 1380, 1330, 1240, 1200, 1060, 1030, 990 cm^{-1}.

Enol methyl ether 14: ^1H NMR (270 MHz, pyridine-d_5) δ 2.03 (s, 3 H, 1a-$COCH_3$), 3.12 (s, 3 H, 9a-OCH_3), 3.38 (dd, J = 1.6, 13.2 Hz, 1 H, 3α-H), 3.40 (dd, J = 2, 5 Hz, 1 H, 2-H), 3.58 (d, J_{CH} = 146.0 Hz, 3 H, 5-$O^{13}CH_3$), 3.76 (d, J = 4.6 Hz, 1 H, 1-H), 3.98 (dd, J = 4.6, 11.2 Hz, 1 H, 9-H), 4.01 (d, J = 12.6 Hz, 1 H, 3β-H), 4.16–4.26 (m, 2 H, ethylenedioxy), 4.50 (t, J = 11.1 Hz, 1 H, 10-H_a), 4.54–4.66 (m, 2 H, ethylenedioxy), 5.70 (dd, J = 4.3, 10.7 Hz, 1 H, 10-H_b), 7.2–7.3 (m, 3 H, phenyl), 7.4–7.7 (br s, 2 H, 10-$OCONH_2$), 7.7–7.8 (m, 2 H, phenyl); FABMS m/z 577/579 ($M^+ + 1$); HRFABMS calcd for $C_{24}{}^{13}CH_{28}N_3O_8{}^{80}Se$ ($M^+ + H$) m/z 579.1076, found 579.1122; IR (KBr) 3470, 3360, 3070, 2930, 1710, 1630, 1550, 1450, 1390, 1340, 1270, 1180, 1080, 1030, 980 cm^{-1}.

[C-6-CH_3-$^2H_3$]-1a-Acetyl-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydro-6-(phenylseleno)mitomycin A (13). The foregoing procedures were employed to convert **8** into **13**, in 75% yield (enol methyl ether **15**, in 6.9% yield) on a 0.5 mmol scale. The product was a ca. 14:11 mixture of two diastereomers at C-6 in pyridine-d_5.

13: ^1H NMR (270 MHz, pyridine-d_5) (major isomer) δ 2.02 (s, 3 H, 1a-$COCH_3$), 3.31 (s, 3 H, 9a-OCH_3), 3.43 (dd, J = 1.8, 12.6 Hz, 1 H, 3α-H), 3.47 (dd, J = 1.8, 4.6 Hz, 1 H, 2-H), 3.80 (d, J = 4.4 Hz, 1 H, 1-H), 4.01 (d, J = 13.2 Hz, 1 H, 3β-H), 3.8–4.3 (m, 4 H, ethylenedioxy + 9-H), 4.54 (m, 1 H, ethylenedioxy), 4.65 (t, J = 10.9 Hz, 1 H, 10-H_a), 5.62 (dd, J = 4.5, 10.7 Hz, 1 H, 10-H_b), 7.25–7.50 (m, 3 H, phenyl), 7.65–7.80 (m, 2 H, phenyl), 7.5–7.8 (br, 2 H, 10-$OCONH_2$); (minor isomer) δ 2.29 (s, 3 H, 1a-$COCH_3$), 3.12 (s, 3 H, 9a-OCH_3), 3.47 (dd, J = 1.8, 13.0 Hz, 1 H, 3α-H), 3.53 (dd, J = 1.8, 4.4 Hz, 1 H, 2-H), 3.87 (d, J = 4.4 Hz, 1 H, 1-H), 3.8–4.3 (m, 4 H, ethylenedioxy + 9-H), 4.54 (m, 1 H, ethylenedioxy), 4.72 (d, J = 12.6 Hz, 1 H, 3β-H), 4.76 (t, J = 11.1 Hz, 1 H, 10-H_a), 5.86 (dd, J = 4.6, 11.0 Hz, 1 H, 10-H_b), 7.25–7.50 (m, 3 H, phenyl), 7.65–7.80 (m, 2 H, phenyl), 7.5–7.8 (br, 2 H, 10-$OCONH_2$); FABMS m/z 579/581 ($M^+ + 1$); HRFABMS calcd for $C_{25}H_{25}D_3N_3O_8{}^{80}Se$ ($M^+ + H$) m/z 581.1230, found 581.1278; IR (KBr) 3450, 3360, 3050, 2950, 1690, 1650, 1570, 1440, 1380, 1330, 1250, 1200, 1090, 1030, 950 cm^{-1}.

Enol methyl ether 15: ^1H NMR (270 MHz, pyridine-d_5) δ 2.04 (s, 3 H, 1a-$COCH_3$), 3.12 (s, 3 H, 9a-OCH_3), 3.38 (dd, J = 2.0, 13.0 Hz, 1 H, 3α-H), 3.40 (dd, J = 1.8, 5 Hz, 1 H, 2-H), 3.76 (d, J = 4.8 Hz, 1 H, 1-H), 3.98 (dd, J = 4.5, 11.2 Hz, 1 H, 9-H), 4.01 (d, J = 12.8 Hz, 1 H, 3β-H), 4.16–4.26 (m, 2 H, ethylenedioxy), 4.50 (t, J = 11.1 Hz, 1 H, 10-H_a), 4.54–4.66 (m, 2 H, ethylenedioxy), 5.70 (dd, J = 4.4, 10.8 Hz, 1 H, 10-H_b), 7.2–7.3 (m, 3 H, phenyl), 7.4–7.7 (br s, 2 H, 10-$OCONH_2$), 7.7–7.8 (m, 2 H, phenyl); FABMS m/z 579/581 ($M^+ + 1$); HRFABMS calcd for $C_{25}H_{25}D_3N_3O_8{}^{80}Se$ ($M^+ + H$) m/z 581.1230, found 581.1203; IR (KBr) 3450, 3370, 3060, 2930, 1710, 1630, 1540, 1450, 1390, 1340, 1270, 1090, 1030, 970 cm^{-1}.

[C-6-CH_3-^{13}C]-1a-Acetyl-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydromitomycin A (16). To a solution of **12** (172 mg, 0.298 mmol) and n-Bu_3SnH (0.40 mL, 1.49 mmol, 5.0 equiv) in dry benzene (6.0 mL) was added Et_3B in n-hexane (1.0 M, 100 μL, 0.100 mmol, 0.34 equiv) under an argon atmosphere at room temperature. After 23 min, Et_3B (1.0 M, 100 μL) was added, and after 2 h and 15 min, Et_3B (1.0 M, 100 μL), n-Bu_3SnH (0.40 mL), and dry benzene (6.0 mL) were added to the reaction mixture. After being stirred for an additional 20 min, the resulting reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with chloroform. The combined organic layers were washed with brine and dried over Na_2SO_4. The mixture was concentrated with a rotary evaporator to ca. 10 mL and poured into n-hexane. The precipitate was filtered off, washed with n-hexane, and purified by chromatography (silica gel, from chloroform to 30:1 chloroform/methanol as eluents) to afford **16** as a reddish brown paste. It was dissolved in a small amount of chloroform and poured into n-hexane. The resulting precipitate was filtered off, washed with n-hexane, and dried under vacuum to afford **16** (106 mg, 0.252 mmol, 84%) as a reddish yellow powder. The product was a ca. 15:5:1 equilibrium mixture of two diastereomers at C-6 and compound **18** in pyridine-d_5.

16: ^1H NMR (270 MHz, pyridine-d_5) (major isomer) δ 1.30 (dd, J = 6.6 Hz, J_{CH} = 129.2 Hz, 3 H, 6-$^{13}CH_3$), 2.13 (s, 3 H, 1a-$COCH_3$), 3.12 (s, 3 H, 9a-OCH_3), 3.48 (br d, J = 4.4 Hz, 1 H, 2-H), 3.53 (dd, J = 1.9, 12.9 Hz, 1 H, 3α-H), 3.58 (m, 1 H, 6-H), 3.82 (d, J = 4.4 Hz, 1 H, 1-H), 4.20 (d, J = 12.8 Hz, 1 H, 3β-H), 3.9–4.6 (m, 5 H, ethylenedioxy + 9-H), 4.61 (t, J = 11.0 Hz, 1 H, 10-H_a), 5.74 (dd, J = 4.6, 10.6 Hz, 1 H, 10-H_b), 7.5–7.8 (br, 2 H, 10-$OCONH_2$); (minor isomer) δ 1.31 (dd, J = 6.6 Hz, J_{CH} = 129.2 Hz, 3 H, 6-$^{13}CH_3$), 2.06 (s, 3 H, 1a-$COCH_3$), 3.15 (s, 3 H, 9a-OCH_3), 3.41 (m, 1 H, 6-H), 3.45 (dd, J = 2, 13 Hz, 1 H, 3α-H), 3.49 (br d, J = 4.4 Hz, 1 H, 2-H), 3.9–4.6 (m, 7 H, ethylenedioxy + 1-H + 3β-H + 9-H), 4.66 (t, J = 11.5 Hz, 1 H, 10-H_a), 5.74 (dd, J = 4.6, 10.6 Hz, 1 H, 10-H_b), 7.5–7.8 (br, 2 H, 10-$OCONH_2$); FABMS m/z 423 ($M^+ + 1$); HRFABMS calcd for $C_{18}{}^{13}CH_{24}N_3O_8$ ($M^+ + H$) m/z 423.1598, found 423.1559; IR (KBr) 3460, 3370, 2920, 2850, 1700, 1660, 1580, 1450, 1390, 1340, 1270, 1190, 1070, 1030, 970, 950 cm^{-1}.

18: ^1H NMR (270 MHz, pyridine-d_5) (major peaks) δ 1.98 (d, J_{CH} = 129.3 Hz, 3 H, 6-$^{13}CH_3$), 2.13 (s, 3 H, 1a-$COCH_3$), 3.21 (s, 3 H, 9a-OCH_3).

[C-6-CH_3-$^2H_3$]-1a-Acetyl-7-demethoxy-7,7-(ethylenedioxy)-6,7-dihydromitomycin A (17). The foregoing procedures were employed to convert **13** into **17**, in 88% yield on a 0.3 mmol scale. The product was a ca. 12:4:1 equilibrium mixture of two diastereomers at C-6 and compound **19** in pyridine-d_5.

17: ^1H NMR (270 MHz, pyridine-d_5) (major isomer) δ 2.13 (s, 3 H, 1a-$COCH_3$), 3.11 (s, 3 H, 9a-OCH_3), 3.47 (d, J = 4.2 Hz, 1 H, 2-H), 3.53 (dd, J = 2.0, 13.0 Hz, 1 H, 3α-H), 3.56 (s, 1 H, 6-H), 3.81 (d, J = 4.4 Hz, 1 H, 1-H), 4.20 (d, J = 13.0 Hz, 1 H, 3β-H), 3.9–4.6 (m, 5 H, ethylenedioxy + 9-H), 4.61 (t, J = 11.0 Hz, 1 H, 10-H_a), 5.74 (dd, J = 4.7, 10.7 Hz, 1 H, 10-H_b), 7.5–7.8 (br, 2 H, 10-$OCONH_2$); (minor isomer) δ 2.05 (s, 3 H, 1a-$COCH_3$), 3.15 (s, 3 H, 9a-OCH_3), 3.39 (s, 1 H, 6-H), 3.45 (dd, J = 2, 13 Hz, 1 H, 3α-H), 3.47 (d, J = 4.4 Hz, 1 H, 2-H), 3.9–4.6 (m, 7 H, 1-H + 3β-H + 9-H + ethylenedioxy), 4.67 (t, J = 11.0 Hz, 1 H, 10-H_a), 5.74 (dd, J = 4.7, 10.7 Hz, 1 H, 10-H_b), 7.5–7.8 (br, 2 H, 10-$OCONH_2$); FABMS m/z 425 ($M^+ + 1$); HRFABMS calcd for $C_{19}H_{21}D_3N_3O_8$ ($M^+ + H$) m/z 425.1752, found 425.1773; IR (KBr) 3460, 3370, 2920, 2850, 1700, 1650, 1570, 1450, 1390, 1350, 1270, 1190, 1170, 1030, 950 cm^{-1}.

19: ^1H NMR (270 MHz, pyridine-d_5) (major peaks) δ 2.13 (s, 3 H, 1a-$COCH_3$), 3.20 (s, 3 H, 9a-OCH_3).

[C-6-CH_3-^{13}C]Mitomycin C (3). A solution of **16** (83.2 mg, 0.197 mmol) in methanol (10 mL) and NH_3 in methanol (6.1 M, 1.0 mL) was allowed to stand at room temperature for 6 h. The mixture was concentrated with a rotary evaporator, and the resulting residue was purified by chromatography (silica gel, from 20:1 to 10:1 chloroform/methanol) to afford **3** (49.8 mg, 0.149 mmol, 75%) as purple crystals.

3: ^1H NMR (400 MHz, pyridine-d_5) δ 2.02 (d, J_{CH} = 127.2 Hz, 3 H, 6-$^{13}CH_3$), 2.08 (br s, 1 H, 1a-H), 2.75 (br s, 1 H, 2-H), 3.16 (br s, 1 H, 1-H), 3.22 (s, 3 H, 9a-OCH_3), 3.60 (br d, J = 12.3 Hz, 1 H, 3α-H), 4.03 (dd, J = 4.2, 11.2 Hz, 1 H, 9-H), 4.55 (d, J = 12.7 Hz, 1 H, 3β-H), 5.11 (br t, J = 10.6 Hz, 1 H, 10-H_a), 5.44 (dd, J = 4.2, 10.4 Hz, 1 H, 10-H_b), 7.3–7.9 (br s, 4 H, 7-NH_2 + 10-$OCONH_2$); ^{13}C NMR (100 MHz, pyridine-d_5) δ 8.77 (d, $^1J_{CC}$ = 46.2 Hz, 6-^{13}C-$^{13}CH_3$), 8.77 (s, 6-^{12}C-$^{13}CH_3$), 32.67 (C-2), 36.77 (C-1), 44.38 (C-9), 49.61 (9a-OCH_3), 50.67 (C-3), 62.59 (C-10), 104.38 (d, $^1J_{CC}$ = 46.2 Hz, C-6), 106.88 (C-9a), 110.88 (C-8a), ca. 149.6 (C-7, overlapped with pyridine), 156.12 (C-4a), 158.14 (10-$OCONH_2$), 176.78 (d, $^3J_{CC}$ = 2.5 Hz, C-8), 178.46 (C-5); EIMS m/z 335 (M^+), 303 ($M^+ - CH_3OH$), 274 ($M^+ - NH_3, CO_2$), 259 ($M^+ - NH_3, CO_2, CH_3$), 243 ($M^+ - NH_3, CO_2, CH_3O$); HREIMS calcd for $C_{13}{}^{13}CH_{14}N_4O_4$ ($M^+ - CH_3OH$) m/z 303.1048, found 303.1072; IR (KBr) 3440, 3310, 3270, 2940, 1730, 1600, 1550, 1450, 1340, 1220, 1060, 960 cm^{-1}. Anal. Calcd for $C_{14}{}^{13}CH_{18}N_4O_5$: C, 53.89; H, 5.43; N, 16.76. Found: C, 53.91; H, 5.46; N, 16.35.

[C-6-CH_3-$^2H_3$]Mitomycin C (5). The foregoing procedures were employed to convert **17** into **5**, in 79% yield on a 0.2 mmol scale: ^1H NMR (400 MHz, pyridine-d_5) δ 2.08 (br s, 1 H, 1a-H), 2.75 (br s, 1 H, 2-H), 3.15 (br s, 1 H, 1-H), 3.22 (s, 3 H, 9a-OCH_3), 3.60 (br d, J = 12.3 Hz, 1 H, 3α-H), 4.03 (dd, J = 4.2, 11.2 Hz, 1 H, 9-H), 4.55 (d, J = 12.7 Hz, 1 H, 3β-H), 5.12 (br t, J = 10.6 Hz, 1 H, 10-H_a), 5.44 (dd, J = 4.2, 10.4 Hz, 1 H, 10-H_b), 7.3–8.0 (br s, 4 H, 7-NH_2 + 10-$OCONH_2$); ^{13}C NMR (100 MHz, pyridine-d_5) δ 8.01 (septet, $^1J_{CD}$ = 19.4 Hz, 6-CD_3), 32.66 (C-2), 36.77 (C-1),

44.38 (C-9), 49.60 (9a-OCH_3), 50.67 (C-3), 62.60 (C-10), 104.26 (C-6), 106.87 (C-9a), 110.90 (C-8a), ca. 149.6 (C-7, overlapped with pyridine), 156.11 (C-4a), 158.12 (10-$OCONH_2$), 176.80 (C-8), 178.52 (C-5); EIMS *m/z* 337 (M^+), 305 ($M^+ - CH_3OH$), 276 ($M^+ - NH_3$, CO_2), 261 ($M^+ - NH_3$, CO_2, CH_3), 245 ($M^+ - NH_3$, CO_2, CH_3O); HREIMS calcd for $C_{15}D_3H_{15}N_4O_5$ *m/z* 337.1465, found 337.1465; IR (KBr) 3440, 3310, 3270, 2950, 1730, 1600, 1550, 1450, 1340, 1220, 1060, 960 cm^{-1}. Anal. Calcd for $C_{15}H_{15}D_3N_4O_5$: C, 53.89; H, 5.43; N, 16.76. Found: C, 53.85; H, 5.51; N, 16.51.

[C-6-CH_3-^{13}C]Mitomycin A (4). A mixture of 16 (22.1 mg, 0.0524 mmol) and powdered potassium carbonate (14 mg) in methanol (2.5 mL) was stirred at room temperature for 6 h and 20 min. The reaction mixture was poured into chloroform and washed with brine. To the yellow aqueous layer was added a small amount of NaOH aqueous solution (0.1 M), and the resulting reddish purple emulsion was reextracted with chloroform. The chloroform layers were combined, dried over Na_2SO_4, and concentrated with a rotary evaporator. The residue was purified by chromatography (silica gel, 20:1 chloroform/methanol as an eluent) to afford 4 as a reddish purple paste. It was dissolved in a small amount of chloroform and poured into *n*-hexane. The resulting precipitate was filtered off, washed with *n*-hexane, and dried under vacuum to afford 4 (15.1 mg, 0.0431 mmol, 82%) as a reddish purple powder.

4: ^{1}H NMR (270 MHz, pyridine-d_5) δ 1.83 (d, $^1J_{CH}$ = 129.3 Hz, 3 H, 6-$^{13}CH_3$), 2.13 (br s, 1 H, 1a-H), 2.77 (br s, 1 H, 2-H), 3.14 (br s, 1 H, 1-H), 3.21 (s, 3 H, 9a-OCH_3), 3.55 (br d, J = 12.3 Hz, 1 H, 3α-H), 3.99 (dd, J = 4.4, 11.0 Hz, 1 H, 9-H), 4.01 (s, 3 H, 7-OCH_3), 4.22 (d, J = 12.5 Hz, 1 H, 3β-H), 5.07 (br t, J = 11 Hz, 1 H, 10-H_a), 5.41 (dd, J = 4.4, 10.4 Hz, 1 H, 10-H_b), 7.4–8.0 (br, 2 H, 10-$OCONH_2$); EIMS *m/z* 350 (M^+), 335 ($M^+ - CH_3$), 318 ($M^+ - CH_3OH$), 303 ($M^+ - CH_3OH$, CH_3), 289 ($M^+ - NH_3$, CO_2); HREIMS calcd for $C_{15}{}^{13}CH_{19}N_3O_6$ *m/z* 350.1308, found 350.1280; IR (KBr) 3420, 3310, 3280, 2950, 2850, 1700, 1630, 1580, 1450, 1410, 1340, 1300, 1220, 1070, 950 cm^{-1}.

[C-6-CH_3-$^2H_3$]Mitomycin A (6). The foregoing procedures were employed to convert 17 into 6, in 83% yield on a 0.05 mmol scale: ^{1}H NMR (270 MHz, pyridine-d_5) δ 2.14 (br s, 1 H, 1a-H), 2.77 (br s, 1 H, 2-H), 3.15 (br s, 1 H, 1-H), 3.24 (s, 3 H, 9a-OCH_3), 3.55 (br d, J = 12 Hz, 1 H, 3α-H), 3.99 (dd, J = 4.2, 11.2 Hz, 1 H, 9-H), 4.01 (s, 3 H, 7-OCH_3), 4.22 (d, J = 12.5 Hz, 1 H, 3β-H), 5.08 (br t, J = 11 Hz, 1 H, 10-H_a), 5.41 (dd, J = 4.2, 10.4 Hz, 1 H, 10-H_b), 7.4–8.0 (br, 2 H, 10-$OCONH_2$); EIMS *m/z* 352 (M^+), 320 ($M^+ - CH_3OH$), 305 ($M^+ - CH_3OH$, CH_3), 291 ($M^+ - NH_3$, CO_2); HREIMS calcd for $C_{16}H_{16}D_3N_3O_6$ *m/z* 352.1463, found 352.1458; IR (KBr) 3420, 3310, 3280, 2950, 2850, 1700, 1630, 1580, 1450, 1410, 1340, 1300, 1220, 1070, 1020 cm^{-1}.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of 3, 5, and 1 (2 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.