Studies on the Mechanism of Mitomycin C(1) Electrophilic Transformations: Structure–Reactivity Relationships

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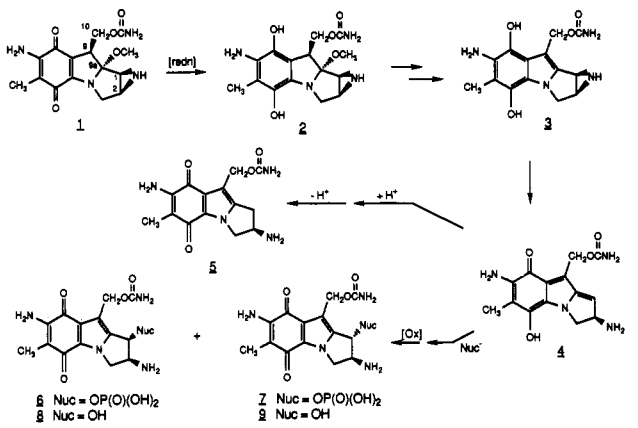
Previous studies have demonstrated that reductive activation of mitomycin C (**1**) under acidic conditions furnished high yields of the C(1) electrophilic product 2,7-diaminomitosene (**5**). This adduct was also the major metabolite produced upon administration of **1** to HT-29 cytosol, purified HT-29 colon carcinoma cells, and rat hepatic DT-diaphorase. Proton capture at C(1) in **1** is known to proceed with high stereoselectivity. Information concerning the mechanism and the controlling factors that govern this transformation have been determined by examining the structure–reactivity relationship for mitomycin C (**1**), 10-decarbamoylmitomycin C (**10**), *N*(1a)-methyl-10-decarbamoyl-10-acetoxymitomycin C (**11**), mitomycin D (**12**), 10-decarbamoylmitomycin D (**13**), 7-amino-aziridinomitosene (**14**), *N*(1a)-(methanesulfonyl)mitomycin C (**15**), and *N*(1a)-(toluenesulfonyl)mitomycin C (**16**). The combined results obtained were consistent with the hypothesis that mitomycin C C(1) electrophilic reactions funneled through quinone methide **4**. The high stereoselectivity of this process has been attributed (in part) to the protonated C(2) amino group in **4**. In this scenario, proton capture occurred preferentially from the site opposite to the C(2) ammonium group in order to minimize adverse coulombic interactions.

In 1981, Tomasz and Lipman reported that reductive activation of aqueous buffered solutions containing mitomycin C (**1**) generated the novel C(1) *electrophilic* adduct **5** in high yields in acid, while the C(1) *nucleophilic* substitution products **6–9** predominated in base (Scheme I).[1] Since this discovery, numerous investigations have confirmed the propensity of mitomycin C to undergo C(1) electrophilic transformations in acid using a variety of reductive techniques.[2–6] Furthermore, Ross and coworkers have shown that 2,7-diaminomitosene (**5**) was the major metabolite produced upon administration of **1** to HT-29 cytosol, purified HT-29 colon carcinoma cells, and rat hepatic DT-diaphorase.[7]

Formation of **5** has been proposed by Tomasz and Lipman to occur by an internal redox reaction proceeding through quinone methide **4**.[1] Information concerning this transformation was also provided by Kohn and Zein who demonstrated that the C(1) electrophilic substitution reaction occurred stereoselectively.[2] Catalytic or electrochemical reduction of D_2O (pD 5.0–5.1) solutions containing **1** led to the selective incorporation of deuterium (>80%) at one of the two methylene positions in **5**.[2,5]

The bifunctional reactivity displayed by mitomycin C is an intriguing property shared by several clinically important anticancer agents.[8] Moreover, it is an unusual feature for a drug such as **1**, which ostensibly has evolved as a DNA alkylating agent.[9] Recently, we have suggested[10] that mitomycin C(1) electrophilic and nucleophilic transformations with solvent may contribute to the remarkable DNA sequence selectivity exhibited by **1**[11–14] by permitting the drug to "self-destruct" when suitable binding opportunities with DNA do not exist.

Scheme I. Proposed Mechanism for the Formation of Mitomycin C Electrophilic and Nucleophilic Products under Reductive Conditions



In view of the importance of mitomycin C C(1) electrophilic processes we have undertaken a general study of this transformation. In this paper information is provided on the pathway for the conversion of **1** to **5** and the structure–reactivity relationships for this process.

Results

A. The Role of Mitomycin Structure in C(1) Electrophilic Transformations. (1) Selection and Synthesis of Mitomycin Substrates. Seven structurally modified mitomycin C derivatives were employed in this study. In two the C(10) substituent in mitomycin C was altered. These compounds were 10-decarbamoylmitomycin C[15] (**10**) and *N*(1a)-methyl-10-decarbamoyl-10-acetoxymitomycin C (**11**). The C(9) and C(9a) substituents

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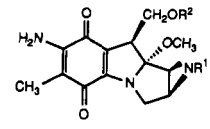
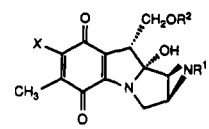
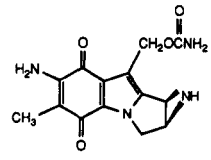




1 $R^1 = H, R^2 = C(O)NH_2$
10 $R^1 = H, R^2 = H$
11 $R^1 = CH_3, R^2 = C(O)CH_3$
15 $R^1 = S(O_2)CH_3, R^2 = C(O)NH_2$
16 $R^1 = S(O_2)C_6H_4p\text{-}CH_3, R^2 = C(O)NH_2$
18 $R^1 = CH_3, R^2 = H$

12 $X = NH_2, R^1 = CH_3, R^2 = C(O)NH_2$
13 $X = NH_2, R^1 = CH_3, R^2 = H$
17 $X = OCH_3, R^1 = CH_3, R^2 = C(O)NH_2$



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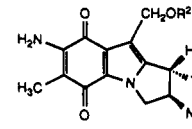
in mitomycin C were modified in three analogues. Compounds in this subset were mitomycin D[16] (12), 10-decarbamoylmitomycin D[16] (13), and 7-aminoaziridinomitosene[17,18] (14). In the two remaining compounds, *N*(1a)-(methanesulfonyl)mitomycin C[19] (15) and N(1a)-(toluenesulfonyl)mitomycin C[19] (16), an electron-withdrawing group was incorporated at the aziridine N(1a) position in 1.

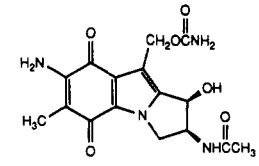
Compounds 10, 14, 15, and 16 were synthesized from 1 according to literature methods. Of the two rare mitomycin D compounds, 13 was generously provided by Kyowa Hakko Kogyo Co., Ltd., and 12 was prepared by treatment of 17 (Kyowa Hakko Kogyo Co., Ltd.) with NH_3. The remaining substrate 11 was synthesized from 10-decarbamoylmitomycin C (10) in two steps. Reaction of 10 with 1,8-bis(dimethylamino)naphthalene and excess dimethyl sulfate gave 18. Treatment of 18 with acetic anhydride and a catalytic amount of (dimethylamino)-pyridine in pyridine afforded 11.

(2) The C(10) Substituent. The role of the C(10) substituent in mitomycin C C(1) electrophilic transformations has been determined by comparing the reactivity of 1 with 10 and 11 under reductive conditions in aqueous buffered pH 5.50 solutions. Treatment of 1 with the chemical reductant 4-methoxyphenylhydrazine[20] (1.2 equiv) furnished the C(1) electrophilic product 5 in 63% yield along with the known solvolytic products 8, 9, and 19[1,3-5,21,22] (combined yield: 19%), an unidentified adduct (4%), and unreacted starting material (14%) (HPLC analysis). Repetition of this experiment on a semipreparative scale permitted the isolation and identification of the major product 5. Key signals observed in the ^1H NMR spectrum (DMSO-d_6) for the C(1) electrophilic adduct were the two doublets of doublets at δ 2.44 (J = 5.0, 16.2 Hz) and 2.95 (J = 7.0, 16.2 Hz) for the C(1) methylene protons (Table I). Replacement of H_2O by D_2O in this experiment furnished the corresponding monodeuterated adduct 5-d_1,[2,5] in which the deuterium atom was incorporated stereoselectively trans to the C(2) amino group. In the ^1H NMR spectrum (DMSO-d_6) of 5-d_1 no noticeable signals were observed at $\sim\delta$ 2.95 ($\leq$0.06 protons) and the resonances centered at δ 2.44 appeared as a doublet (J = 5.0 Hz).



5 Y = H, R^1 = H, R^2 = C(O)NH_2
5-d_1 Y = D, R^1 = H, R^2 = C(O)NH_2
20 Y = H, R^1 = H, R^2 = H
20-d_1 Y = D, R^1 = H, R^2 = H
21 Y = H, R^1 = CH_3, R^2 = C(O)CH_3
21-d_1 Y = D, R^1 = CH_3, R^2 = C(O)CH_3

22 Y = H, R^1 = CH_3, R^2 = C(O)NH_2
22-d_1 Y = D, R^1 = CH_3, R^2 = C(O)NH_2
25 Y = H, R^1 = CH_3, R^2 = H
25-d_1 Y = D, R^1 = CH_3, R^2 = H
32 Y = H, R^1 = $S(O_2)CH_3$, R^2 = C(O)NH_2
36 Y = H, R^1 = $S(O_2)C_6H_4p\text{-}CH_3$, R^2 = C(O)NH_2



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The structural assignment of 5-d_1 is based on the assumption that the upfield (δ 2.44) and downfield (δ 2.95) C(1) methylene resonances in 5 corresponded to the C(1)H_β and C(1)H_α protons, respectively. Early support for this contention was derived from the chemical shift values observed for the corresponding C(3)H_β and C(3)H_α protons in a series of mitosene compounds.[2] Comparison of the relative position of these signals revealed that the resonance corresponding to the C(3)H_β proton appeared upfield (0.25–0.47 ppm) from the C(3)H_α peak. The C(3) methylene assignment stemmed from a detailed Karplus analysis of the homonuclear coupling constants for these protons.[23,24] Our assignment was also in agreement with the pattern observed for the C(1) chemical shift values in isomeric C(1) substituted mitosenes. In most cases, the C(1)H_α resonance in the cis-substituted mitosene compounds appeared downfield ($\sim$0.15 ppm) from the C(1)H_β proton in the corresponding trans-substituted isomer. A comparable trend in chemical shift values existed for protons adjacent to an α-substituted electron-withdrawing group in constrained five-membered and three-membered ring compounds and other rigid bicyclic systems.[25] In general, the proton trans to the electron-withdrawing group resonated downfield from the corresponding cis proton. Additional data in support of this structural assignment is provided in Section B.

Deletion or alteration of the C(10) carbamate group in 1 did not appreciably alter the course of the C(1) electrophilic substitution reaction. Reductive activation (4-methoxyphenylhydrazine) of 10 in aqueous (pH 5.50) and D_2O (pD 5.50) solutions afforded 20[5,26] and 20-d_1,[5] respectively, as the only major product along with a small amount of starting material. Consistent with the proposed structural assignments, the ^1H NMR resonances (CD_3OD) for the C(1) methylene hydrogens in 20 were located at δ 2.62 (J = 4.9, 16.1 Hz) and 3.15 (J = 7.5, 16.1 Hz), while in 20-d_1 no significant signals were observed at δ 3.15 ($\leq$10%) and the upfield proton resonance at δ 2.68 appeared as a doublet (Table I). A similar result was observed for the N(H),C(10)-modified mitomycin C analogue,

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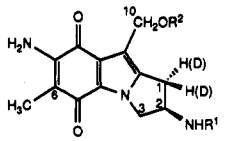
Table I. ^{1}H NMR Assignments for Mitomycin C(1) Electrophilic Substituted Products[a]



compd	solvent	R^1	R^2	C(1)H_β	C(1)H_α	C(2)H	C(3)H_β	C(3)H_α	C(10)HH′	C(6)CH_3	NR^1
5	CD_3OD	H	$C(O)NH_2$	2.63; d, d; 4.6, 16.6	3.16; d, d; 7.2, 16.6	4.10–4.22; m	3.88; d, d; 4.6, 12.5	4.35; d, d; 6.5, 12.5	5.13; s	1.80; s	
	pyridine-d_5			2.77; d, d; 4.8, 16.1	3.20; d, d; 6.8, 16.1	3.98–4.09; m	3.98–4.09; m	4.37; d, d; 5.7, 11.7	5.64; s	2.14; s	
	DMSO-d_6			2.44; d, d; 5.0, 16.2	2.95; d, d; 7.0, 16.2	3.98–4.01; m	3.69; d, d; 5.0, 12.4	4.19; d, d; 6.5, 12.4	4.97; s	1.70; s	
5-d_1	CD_3OD	H	$C(O)NH_2$	2.62; d; 4.2	3.10[b]	4.12–4.18; m	3.89; d, d; 4.0, 12.5	4.36; d, d; 6.5, 12.5	5.14; s	1.79; s	
	pyridine-d_5			2.71; d; 4.6	3.15[b]	3.95–4.05; m	3.95–4.05; m	4.37; d, d; 5.3, 11.1	5.65; s	2.14; s	
	DMSO-d_6	H		2.44; d; 5.0		4.01–4.17; m	3.70; d, d; 5.0, 12.4	4.20; d, d; 6.6, 12.4	4.99; s	1.68; s	
20	CD_3OD	H	H	2.62; d, d; 4.9, 16.1	3.15; d, d; 7.5, 16.1	4.10–4.19; m	3.90; d, d; 4.7, 12.9	4.35; d, d; 7.2, 12.9	4.66; s	1.78; s	
20-d_1	CD_3OD	H	H	2.68; d; 3.6		4.17–4.27; m	3.97; d, d; 4.2, 13.0	4.38; d, d; 6.7, 13.0	4.66; s	1.77; s	
21[c]	$CDCl_3$	CH_3	$C(O)CH_3$	2.68; d, d; 4.6, 16.4	3.13; d, d; 7.0, 16.4	3.90–3.96; m	4.05; d, d; 4.5, 12.9	4.39; d, d; 6.7, 12.9	5.19; s	1.82; s	2.48; s
21-d_1[c]	$CDCl_3$	CH_3	$C(O)CH_3$	2.68; d; 9.5	3.10[b]	3.89–3.95; m	4.05; d, d; 8.3, 13.0	4.38; d, d; 6.6, 13.0	5.19; s	1.82; s	2.48; s
22	pyridine-d_5	CH_3	$C(O)NH_2$	2.79; d, d; 4.9, 14.6	3.15; d, d; 7.7, 14.6	3.59–3.70; m	4.06; d, d; 4.9, 12.9	4.36; d, d; 6.8, 12.9	5.65; s	2.14; s	2.26; s
22-d_1	pyridine-d_5	CH_3	$C(O)NH_2$	2.78; d; 4.3	3.15[b]	3.59–3.70; m	4.07; d, d; 4.7, 12.8	4.35; d, d; 6.7, 12.8	5.65; s	2.14; s	2.27; s
25	pyridine-d_5	CH_3	H	2.82; d, d; 4.7, 16.5	3.13; d, d; 7.6, 16.5	3.68–3.73; m	4.12; d, d; 4.5, 12.7	4.38; d, d; 6.6, 12.7	5.20; s	2.15; s	2.29; s
25-d_1	pyridine-d_5	CH_3	H	2.79; d; 4.7	3.12[b]	3.68–3.73; m	4.11; d, d; 4.9, 12.8	4.39; d, d; 6.6, 12.8	5.20; s	2.14; s	2.29; s
32	pyridine-d_5	SO_2CH_3	$C(O)NH_2$	3.25; d, d; 5.2, 16.5	3.51; d, d; 7.5, 16.5	4.84–4.91; m	4.44; d, d; 5.0, 12.9	4.69; d, d; 7.0, 12.9	5.59, 5.64; ABq, 13.2	2.12; s	3.16; s
	DMSO-d_6			2.72; d, d; 5.9, 16.2	3.21; d, d; 7.9, 16.2	4.50–4.60; m	3.91; d, d; 5.2, 12.2	4.45, d, d; 6.1, 12.2	4.99; s	1.72; s	3.00; s
32-d_1	pyridine-d_5	SO_2CH_3	$C(O)NH_2$	3.24; d;[d] 5.0	3.49; d;[e] 7.2	4.85–4.90; m	4.44; d, d; 5.0, 12.8	4.70; d, d; 7.1, 12.8	5.58, 5.64; ABq, 13.1	2.12; s	3.16; s
	DMSO-d_6			2.70; d;[f] 4.3	3.19; d;[g] 6.0	4.49–4.55; m	3.91; d, d; 5.5, 12.0	4.46; d, d; 7.9, 12.0	4.99; s	1.72; s	3.00; s
36[h]	pyridine-d_5	$SO_2C_6H_4$-*p*-CH_3	$C(O)NH_2$	3.12; d, d; 5.8, 16.4	3.40; d, d; 7.0, 16.4	4.74–4.84; m	4.39; d, d; 5.5, 12.9	4.59; d, d; 6.6, 12.9	5.57, 5.63; ABq, 12.8	2.14; s	
36-d_1[i]	pyridine-d_5	$SO_2C_6H_4$-*p*-CH_3	$C(O)NH_2$	3.16; d;[j] 4.8	3.35; d;[k] 6.9	4.69–4.86; m	4.32; d, d; 4.7, 12.9	4.53; d, d; 6.9, 12.9	5.53, 5.60; ABq, 11.4	2.08; s	

[a] Each entry is the chemical shift (δ) reported in ppm relative to the solvent employed, followed by the multiplicity of the signal and the coupling constant(s) in hertz. All spectra were recorded at 300 MHz. [b] A trace signal (≤0.20 protons) was observed and has been tentatively attributed to the C(1)H_α. [c] The signal for the C(10) acetyl protons appeared at δ 2.06. [d] The signal at δ 3.24 integrated for 0.55 protons. [e] The signal at δ 3.49 integrated for 0.45 protons. [f] The signal at δ 2.70 integrated for 0.55 protons. [g] The signal at δ 3.19 integrated for 0.45 protons. [h] Signals were observed at δ 2.22 (s, 3 H), and δ 7.25 (d, J = 8.0 Hz, 2 ArH), 8.29 (d, J = 8.0 Hz, 2 ArH) for the *p*-tolyl group. [i] Signals were observed at δ 2.19 and 7.00–7.30 (m, 2 ArH), 8.10 (d, J = 8.0 Hz, 2 ArH) for the *p*-tolyl group. [j] The signal at δ 3.16 integrated for 0.60 protons. [k] The signal at δ 3.35 integrated for 0.40 protons.

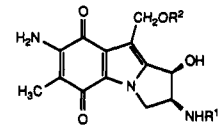
N(1a)-methyl-10-decarbamoyl-10-acetoxymitomycin C (**11**). Treatment of **11** with 4-methoxyphenylhydrazine at pH 5.50 (Tris·HOAc) afforded **21** in high yield, while the corresponding reaction in D_2O gave **21**-d_1. Once again in the ^{1}H NMR spectrum ($CDCl_3$) for the monodeuterated product a trace signal for the downfield C(1) methylene proton (i.e., ~δ 3.10) was observed (≤20%), while the upfield resonance at δ 2.68 appeared as a doublet (Table I).

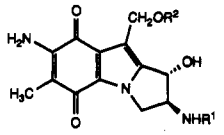
The efficiency of both the 10-decarbamoylmitomycin C (**10**) and the *N*(1a)-methyl-10-decarbamoyl-10-acetoxymitomycin C (**11**) reactions at pH 5.50 was comparable to that observed for mitomycin C. In the corresponding D_2O reactions, ^{1}H NMR analyses indicated that deuterium incorporation occurred with high stereoselectivity and at the same site as in mitomycin C transformations. Accordingly, we have assigned the structures of the monodeuterated adducts generated in the **10**- and **11**-mediated reactions as **20**-d_1 and **21**-d_1, respectively, in which the deuterium at C(1) was trans to the C(2) amino group. The results secured from **10** and **11** indicated that neither alteration of the C(10) substituent in mitomycin C nor replacement of the aziridine N(1a) proton by a methyl group influenced the stereoselective incorporation of a proton (deuterium) in mitomycin C(1) electrophilic transformations.

(3) The C(9) and C(9a) Substituents. The effect of structural modifications of the C(9) and C(9a) positions in mitomycin C on C(1) electrophilic transformations was determined by comparing the reactivities of **1** with the two mitomycin D substrates **12** and **13** and 7-aminoaziridinomitosene (**14**). In **12** and **13** the relative configuration of the C(9) substituent is reversed from that of **1**, and the C(9a) methoxy group has been replaced by a hydroxy moiety, while in **14** the two stereochemical centers at C(9) and C(9a) have been removed. Catalytic reduction (PtO_2, H_2) of an aqueous bis-Tris·HCl (pH 5.50) solution of **12** led to the high yield production of **22** (58%), along with *cis*- (**23**) and *trans*-1-hydroxy-2-(methylamino)-7-aminomitosenes[27] (**24**) (6%), two unidentified compounds (3%), and unreacted starting material (33%) (HPLC

(27) Tomasz, M.; Jung, M.; Verdine, G.; Nakanishi, K. *J. Am. Chem. Soc.* **1984**, *106*, 7367.

analysis). A comparable result was observed in D_2O at





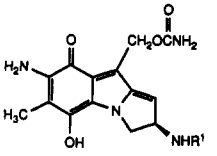
23 $R^1 = CH_3$, $R^2 = C(O)NH_2$ 24 $R^1 = CH_3$, $R^2 = C(O)NH_2$
26 $R^1 = CH_3$, $R^2 = H$ 27 $R^1 = CH_3$, $R^2 = H$
28 $R^1 = H$, $R^2 = H$ 29 $R^1 = H$, $R^2 = H$
33 $R^1 = S(O_2)CH_3$, $R^2 = C(O)NH_2$ 34 $R^1 = S(O_2)CH_3$, $R^2 = C(O)NH_2$

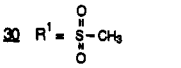
pD 5.50 both on analytical and semipreparative scale. Analysis of the ^1H NMR spectrum for **22**-d_1 indicated that the characteristic downfield doublet of doublets (J = 7.7, 14.6 Hz) at δ 3.15 (pyridine-d_5) in **22** was nearly absent (≤20%), and the corresponding upfield doublet of doublets (J = 4.9, 14.6 Hz) at δ 2.79 in **22** appeared at δ 2.78 as a doublet (Table I). Catalytic reduction of 10-decarbamoylmitomycin D (**13**) in H_2O (pH 5.50) and D_2O (pD 5.50) gave similar results. In both reactions, the major compound produced (71–77%) was the C(1) electrophilic adduct **25** (**25**-d_1) along with small amounts (12%) of *cis*- (**26**) and *trans*-10-decarbamoyl-1,10-dihydroxy-2-(methylamino)-7-aminomitosenes (**27**) (12%). Comparison of the ^1H NMR spectra (pyridine-d_5) for these products provided evidence that proton (deuterium) incorporation had proceeded in a stereoselective manner. In **25**-d_1 only a trace signal (≤20%) was observed for the downfield C(1) methylene proton at δ 3.12, while the upfield resonance appeared as a doublet at δ 2.79 (Table I). The similarity of the ^1H NMR spectra for **22**-d_1 and **25**-d_1 with those obtained for **5**-d_1, **20**-d_1, and **21**-d_1 suggested that deuterium incorporation occurred preferentially at the C(1)α site, trans to the C(2) amino group.

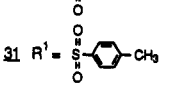
Evaluation of the stereochemical course for the C(1) electrophilic substitution process in reduced 7-aminoaziridinomitosene (**14**) proved difficult due to the heightened reactivity of this substrate. We have previously shown that **14** undergoes rapid hydrolysis under *nonreductive* conditions to give *cis*- (**8**) and *trans*-1-hydroxy-7-aminomitosenes (**9**).[18] This solvolytic reaction required us to first reduce (PtO_2, H_2) **14** in a deaerated THF solution and then transfer the solution via a cannula to a deaerated aqueous buffered solution. At "pH" 6.40, HPLC analysis indicated that the C(1) electrophilic product **5** accounted for 17% of the product mixture. In addition to **5** significant amounts of **8** and **9** (52%) were detected along with **28** and **29**[10,28] (3%) and several unknown compounds (28%). Repetition of this experiment in D_2O at "pD" 7.40 provided **5**-d_1 (19% yield). ^1H NMR analysis (CD_3OD) of **5**-d_1 indicated that deuterium incorporation at C(1) had occurred stereoselectively. Only a trace signal (≤20%) for the downfield C(1) methylene proton at δ 3.10 was noted, while the upfield proton at δ 2.62 appeared as a doublet. Similar findings were observed for the catalytic reductive activation of **1** directly in THF–H_2O and THF–D_2O mixtures. ^1H NMR analysis of **5**-d_1 generated in THF–D_2O mixtures indicated that deuterium incorporation occurred stereoselectively (≥80%) and was comparable to that observed for **1** in D_2O. Under these conditions moderate yields (31–57%) of the C(1) electrophilic product **5** were observed between "pH" 5.50–7.50.[29] A similar "pH"-product profile was also observed for **1** using the two-stage THF–H_2O reductive procedure employed for the catalytic reductive activation of **14**. These combined results indicated that the use of a binary solvent system did not appreciably effect the stereochemical course of the C(1) electrophilic substitution process.

The results obtained from **12–14** mirrored those secured from **1**, thereby removing any notion that the C(9) and C(9a) chiral centers in **1** are responsible for the stereoselective incorporation of a proton (deuterium) at C(1). Moreover, our findings that the reductive activation of 7-aminoaziridinomitosene (**14**) in both aqueous–THF and D_2O–THF mixtures gave results comparable to that observed for **1** provided suggestive evidence that mitomycin electrophilic transformations proceeded through **3**.

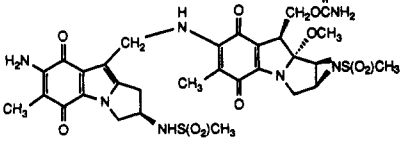
(4) The N(1a) Substituent. The two remaining compounds evaluated were *N*(1a)-(methanesulfonyl)mitomycin C (**15**) and *N*(1a)-(toluenesulfonyl)mitomycin C (**16**). These substrates were examined to determine the importance of the C(2) amino group in **4** in the stereoselective incorporation of a proton (deuterium) at the C(1) site in mitomycin electrophilic transformations. We reasoned that the electron-withdrawing sulfonyl groups in **15** and **16** should decrease the likelihood that the functionalized C(2) amino group in putative intermediates **30** and **31**, respectively, would be protonated under the acidic conditions of these reactions.







Catalytic reduction (PtO_2–H_2) of **15** in THF–H_2O mixtures ("pH" 5.50) gave 2-[(methanesulfonyl)amino]-7-aminomitosene (**32**) (24%), *cis*- (**33**) and *trans*-1-hydroxy-2-[(methanesufonyl)amino]-7-aminomitosenes (**34**) (20%), dimeric adduct **35** (23%), an unidentified product (5%), along with unreacted **15** (28%) (HPLC analysis). Repetitive experiments on a semipreparative scale permitted the isolation and identification of compounds **32–35**. Examination of the ^1H NMR spectrum



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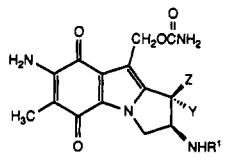
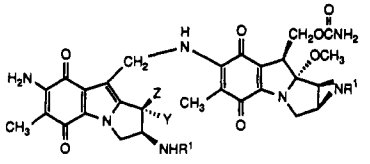
for **32** (pyridine-d_5) revealed a characteristic pair of doublets of doublets at δ 3.25 (J = 5.2, 16.5 Hz) and δ 3.51 (J = 7.5, 16.5 Hz) which have been assigned to the C(1) methylene protons (Table I). The corresponding ^1H NMR spectrum (DMSO-d_6) of **35** was composed of two sets of resonances. The first set was comparable to the C(1) electrophilic compound **32** except for the mitosene C(10) methylene protons (Table I). The C(10) hydrogens in **35** appeared as a multiplet (δ 4.74–4.78) and resonated significantly upfield (Δ 0.24 ppm) from the corresponding signals in **32**. The other set of resonances was similar to the peaks observed for **15**. Significantly, a triplet (J = 5.9 Hz) was observed at δ 7.39, which integrated for one proton. This signal was absent in the corresponding ^1H NMR spectrum recorded in CD_3OD. The UV–vis spectrum for **35** indicated that this compound was composed of equal parts of a mitomycin and a mitosene chromophore. The

(28) Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307.
(29) Han, I.; Kohn, H. Unpublished results.

combined ^{1}H NMR and UV–vis spectral data have led us to assign this compound as dimeric adduct **35** in which the C(7) amino group of mitomycin **15** is covalently attached to the C(10) position in 10-decarbamoyl-2-[(methanesulfonyl)amino]-7-aminomitosene.

Repetition of the catalytic reduction of **15** in THF–D_2O ("pD" 5.50, 0.1 M deuterated bis-Tris·DCl) mixtures led to the formation of the corresponding monodeuterated adducts **32**-d_1 and **35**-d_1. Analysis of the ^{1}H NMR data





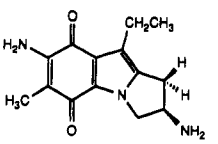
32a-d_1 Y = D, Z = H, R^1 = $S(O_2)CH_3$
32b-d_1 Y = H, Z = D, R^1 = $S(O_2)CH_3$
36a-d_1 Y = D, Z = H, R^1 = $S(O_2)C_6H_4p$-CH_3
36b-d_1 Y = H, Z = D, R^1 = $S(O_2)C_6H_4p$-CH_3

35a-d_1 Y = D, Z = H, R^1 = $S(O_2)CH_3$
35b-d_1 Y = H, Z = D, R^1 = $S(O_2)CH_3$

for **32**-d_1 indicated that substantial deuterium incorporation had occurred at both the C(1)H_α and the C(1)H_β sites. Resonances for both C(1) methylene protons were clearly observed at δ 3.24 and 3.49. The doublet at δ 3.24 integrated for 0.55 protons, while the doublet at δ 3.49 integrated for 0.45 protons (Table I). A similar result was observed for the dimeric adduct **35**-d_1. The only significant difference in the ^{1}H NMR spectra of **35** and **35**-d_1 was that the doublets of doublets at δ 2.61 and 3.07 in **35** appeared as doublets in **35**-d_1 at δ 2.59 and 3.02 and integrated for approximately 0.65 and 0.35 protons, respectively. These findings indicated that deuterium incorporation in **15**-mediated reactions proceeded with little stereoselectivity to give substantial amounts of both **32a**-d_1 and **32b**-d_1, as well as **35a**-d_1 and **35b**-d_1.

The catalytic reduction (PtO_2-H_2) of **16** in THF–H_2O ("pH" 5.50) and THF–D_2O ("pD" 5.50) mixtures gave results similar to that observed for **15**. The C(1) electrophilic-substituted products **36** and **36**-d_1 were observed to be the major compounds and were isolated and identified by ^{1}H NMR spectroscopy. The only significant difference in ^{1}H NMR spectra (pyridine-d_5) for these two compounds was that the doublets of doublets at δ 3.12 (J = 5.8, 16.4 Hz) and 3.40 (J = 7.0, 16.4 Hz) in **36** appeared as doublets in **36**-d_1 at δ 3.16 and 3.35, respectively (Table I). Integration of the ^{1}H NMR spectrum of **36**-d_1 indicated that the extent of the deuterium incorporation at the C(1)α and C(1)β sites was approximately 60 and 40%, respectively, and is consistent with the presence of substantial amounts of both **36a**-d_1 and **36b**-d_1 in the product mixture.

B. ^{1}H NMR Assignments of C(1) Methylene Protons in Mitomycin Electrophilic Products. Critical to our understanding of the factor(s) responsible for the marked stereoselectivity of mitomycin C C(1) electrophilic reactions is the determination of the specific site for deuterium incorporation in **5**. In an earlier study, we tentatively concluded that the upfield and downfield C(1) methylene resonances in **5** corresponded to the C(1)H_β and C(1)H_α protons, respectively.[2] These assignments are now supported by the determination of the predicted coupling constants for the C(1)H_β and C(1)H_α protons using the Karplus relationship[30] for the energy-minimized structure of **37**.[31] This computational study indicated that the



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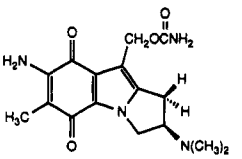
coupling constant for the C(1)H_α–C(2)H proton–proton interaction (J = 8.2 Hz) is larger than the corresponding coupling interaction (J = 5.2 Hz) for the C(1)H_β–C(2)H protons. In agreement with this prediction, the downfield C(1) signal displayed a larger vicinal coupling interaction than the corresponding upfield peak (Table I). A similar finding for the predicted and observed coupling interactions was observed for the C(2)H–C(3)H_α and C(2)H–C(3)H_β proton–proton interactions. Ancillary support for these stereochemical conclusions was derived from the COSY spectrum of **5**. The two-dimensional COSY cross peak intensities are known to be approximately proportional to the magnitudes of the coupling constants.[33] In the COSY spectrum for **5**, stronger through-bond scalar coupling for the C(1)H_α and C(2)H protons were observed than between the C(1)H_β and C(2)H protons. Similarly, a stronger scalar coupling was noted for the C(2)H–C(3)H_α protons than for the C(2)H–C(3)H_β protons.

Attempts to confirm the proposed C(1) assignments in mitomycin C(1) electrophilic products using either one-dimensional or two-dimensional NOE spectroscopy were unsuccessful. No significant proton–proton interactions were noted for either the C(1) or the C(3) methylene protons with the C(2) methine hydrogen in **5**. Similarly, no interactions were noted in the NOE spectra for **22**, **32**, and **38** for the C(2) amino substituent and the C(1) and C(3) methylene protons. Compound **38** was prepared by methylation of 2-(methylamino)-7-aminomitosene (**22**) with dimethyl sulfate in the presence of 2,5-di-*tert*-butylpyridine.



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Discussion

Our studies indicated that most structural changes at the C(10), C(9), C(9a), and N(1a) sites in mitomycin C did not diminish the efficiency of the C(1) electrophilic transformation in acid. Moreover, the demonstration that **14** furnished **5**, albeit in low yields, suggested that the corresponding 7-aminoleucoaziridinomitosene (**3**) is an intermediate in the reaction pathway leading to 2,7-diaminomitosene.

The results obtained for the corresponding experiments performed in D_2O or THF–D_2O mixtures provided information concerning the principal factor responsible for the remarkable stereoselectivity of the proton (deuterium) incorporation process. The ^{1}H NMR, COSY, and energy-minimized molecular calculation data indicated that protonation (deuteration) occurred selectively at the C(1)α site. The demonstration that C(1) electrophilic processes proceeded with high stereoselectivity with both 10-decarbamoylmitomycin C (**10**) and *N*(1a)-methyl-10-decarbamoyl-10-acetoxymitomycin C (**11**) ruled out any

(30) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.
(31) Calculations were performed by Drs. Thomas Albright and James Briggs using the MOPAC 6.0 program.[32]
(32) Stewart, J. J. P. MOPAC 6.0, Frank J. Seiler Research Laboratory, United States Air Force Academy, CO, 80840.
(33) Saunders, J. K. M.; Hunter, B. K. *Modern NMR Spectroscopy, A Guide for Chemists*; Oxford University Press: New York, 1989; pp 108–112.

potential effects provided by the C(10) substituent in the delivery of a proton (deuterium) to the C(1) site. Correspondingly, the similar results obtained with **12**, **13**, and **14** eliminated the involvement of the C(9) and C(9a) substituents in the C(1) electrophilic substitution process. These collective findings suggested that proton capture at C(1) occurred from quinone methide **4**. We suggest that proton (deuterium) incorporation occurs preferentially from the face opposite the *protonated* C(2) amino group in **4** in order to minimize the repulsive interactions between these two charged groups (Scheme II, pathway a).[34] Consistent with this hypothesis, electrophilic transformations proceeding from the *N*-methylmitomycin analogue **11** exhibited similar stereoselectivity to **1**, while reduced stereoselectivity versus **1** was observed for the two *N*-sulfonyl adducts **15** and **16**. In **15** and **16** protonation (deuteration) of the C(2) amino substituent in the putative intermediates **30** and **31**, respectively, is unlikely due to the electron-withdrawing properties of the sulfonyl moiety. Accordingly, we suspect that C(1) protonation (deuteration) of these quinone methides can occur from both faces of the molecule.

Conclusions

Detailed structural information has been secured on the controlling factors for mitomycin C(1) electrophilic transformations. The combined results are consistent with the hypothesis that these reactions proceeded through quinone methide **4**.[1] The high stereoselectivity observed for this transformation has been attributed (in part) to the protonated C(2) amino group in **4**. In this hypothesis, protonation (deuteration) occurred preferentially from the site opposite to the C(2) ammonium group in order to minimize adverse coulombic interactions.

Experimental Section

General Procedures. The experimental procedures used in this study were identical to those employed in previous investigations.[5,10,18] Generous supplies of mitomycin C (**1**) were obtained from the Bristol-Myers Squibb Laboratories, Wallingford, CT. Mitomycin B (**17**) and 10-decarbamoylmitomycin D (**13**) were graciously provided by the Kyowa Hakko Kogyo Company, Ltd., Japan. *N*(1a)-(Methanesulfonyl)mitomycin C (**15**) and *N*(1a)-(toluenesulfonyl)mitomycin C (**16**) were synthesized by a published procedure[19] and purified by preparative TLC using 10% MeOH–$CHCl_3$ as the eluant. TLC R_f values were calculated using 10% MeOH–$CHCl_3$ as the eluant unless otherwise noted. All H_2O used for the reactions was HPLC grade. THF was distilled from Na^0 and benzophenone. The deuterated buffers were prepared by repetitive (2×) dissolution of the buffer components in D_2O and then removal of solvent in vacuo, while the exchangeable protons in 4-methoxyphenylhydrazine·HCl were replaced by repetitive dissolution of the hydrazine in CH_3OD and drying the sample in vacuo. The pD values for the reaction solutions were determined using the relationship pD = pH(meter) + 0.40.[35]

Preparation of *N*(1a)-Methyl-10-decarbamoylmitomycin C (18). Decarbamoylmitomycin C[15] (**10**) (50 mg, 0.2 mmol) and 1,8-bis(dimethylamino)naphthalene (200 mg, 0.9 mmol) were dissolved in dry THF (60 mL), and then Me_2SO_4 (400 mg, 0.30 mL, 3.2 mmol) was added. HPLC analysis indicated that the reaction was complete after 2 d. A 10% aqueous $NaHCO_3$ solution (15 mL) was added to the reaction and the product extracted with $CHCl_3$ (5 × 25 mL). The organic layers were combined and concentrated in vacuo to yield a brown oil. The crude reaction product was dissolved in a minimum amount of MeOH and purified by semipreparative TLC using 5% MeOH–$CHCl_3$ as the eluant to give **18**: HPLC retention time 19.8 min; TLC R_f 0.50; ^1H NMR (CD_3OD) δ 1.74 (s, C(6)CH_3), 2.28 (s, N(1a)CH_3), 2.47 (dd, J = 2.0, 4.9 Hz, C(2)H), 2.55 (d, J = 4.9 Hz, C(1)H), 3.18 (s, C(9a)OCH_3), 3.34 (dd, J = 5.1, 8.0 Hz, C(9)H), 3.56 (dd, J = 2.0, 13.2 Hz, C(3)H_β), 3.79 (dd, J = 8.0, 10.7 Hz, C(10)*H*H′), 4.08 (dd, J = 5.1, 10.7 Hz, C(10)H*H*′), 4.15 (d, J = 13.2 Hz, C(3)H_α). All proton–proton connectivities were confirmed by the COSY spectrum. M_r (EI) 305.138 26 (calcd for $C_{15}H_{19}N_3O_4$, 305.137 56); MS (+CI) m/e 306 (MH^+); UV–vis (MeOH) 223, 359 nm.

Preparation of *N*(1a)-Methyl-10-decarbamoyl-10-acetoxymitomycin C (11). *N*(1a)-Methyl-10-decarbamoylmitomycin C (**18**) (40 mg, 0.13 mmol) was dissolved in pyridine (30 mL) and Ac_2O (1.5 mL), and then a single crystal of (dimethylamino)-pyridine was added and the reaction was allowed to stand overnight at ~4 °C. HPLC analysis of the reaction after 12 h showed the reaction was complete. The reaction was then poured into a cold 10% aqueous $NaHCO_3$ solution (15 mL), stirred (30 min), and then extracted with $CHCl_3$ (2 × 35 mL). The organic layers were combined and concentrated in vacuo. Compound **11** was purified by semipreparative TLC using 94:5:1 $CHCl_3$/MeOH/Et_3N as the eluant: TLC R_f 0.72 (94:5:1 $CHCl_3$/MeOH/Et_3N); ^1H NMR ($CDCl_3$) δ 1.95 (s, C(6)CH_3), 2.34 (s, N(1a)CH_3), 2.43 (s, C(10)OC(O)CH_3), 2.50–2.60 (m, C(1)H, C(2)H), 3.39 (s, C(9a)OCH_3), 3.68 (dd, J = 2.8, 13.6 Hz, C(3)H_β), 3.82 (dd, J = 6.8, 10.3 Hz, C(9)H), 4.42 (d, J = 13.6 Hz, C(3)H_α), 4.55 (dd, J = 10.3, 10.4 Hz, C(10)*H*H′), 4.99 (dd, J = 6.8, 10.4 Hz, C(10)-H*H*′); all proton–proton connectivities were confirmed by the COSY spectrum; M_r (EI) 347.148 42 (calcd for $C_{17}H_{21}N_3O_5$, 347.148 12); MS (EI) m/e (rel intensity) 347 (M^+, 18), 315 (100), 287 (45), 256 (95); UV–vis (MeOH) 256 (sh), 353, 525 nm.

Preparation of Mitomycin D (12).[16] Mitomycin B (**17**) (50 mg, 0.14 mmol) was dissolved in anhydrous MeOH (5 mL). A saturated solution of NH_3 in MeOH (2 mL) was added, and the solution was stirred at room temperature (6 h). TLC analysis indicated the complete conversion of **17** to **12**. The solvent was removed in vacuo to give **12** (48 mg) as a blue solid.

Mitomycin D (12): ^1H NMR (CD_3OD) δ 1.80 (s, C(6)CH_3), 2.29 (s, N(1a)CH_3), 2.42 (d, J = 4.8 Hz, C(1)H), 2.48 (dd, J = 1.8, 4.8 Hz, C(2)H), 3.46 (dd, J = 1.8, 13.1 Hz, C(3)H_β), 3.68 (dd, J = 3.4, 9.0 Hz, C(9)H), 3.88 (d, J = 13.1 Hz, C(3)H_α), 4.41 (dd, J = 9.0, 10.2 Hz, C(10)*H*H′), 4.63 (dd, J = 3.4, 10.2 Hz, C(10)H*H*′); ^{13}C NMR (CD_3OD) 7.74 (C(6)*C*H_3), 43.30 (C(2)), 45.15 (C(1)), 45.44 (C(9)), 61.67 (C(10)), 101.15 (C(6)), 104.19 (C(9a)), 110.19 (C(8a)), 151.59 (C(7)), 156.28 (C(10)O*C*(O)NH_2), 159.59 (C(5a)), 176.04 (C(8)), 178.98 (C(5)) ppm. Signals for the C(3) and the N(1a)CH_3 resonances were not observed and are believed to be beneath the solvent peak.

Preparation of *N*(1a)-(Methanesulfonyl)mitomycin C (15) and *N*(1a)-(Toluenesulfonyl)mitomycin C (16).[19] To a cooled (0 °C) pyridine solution (30 mL) containing **1** (60 mg, 0.18 mmol) and Et_3N (25 mg, 0.24 mmol) was added methanesulfonyl chloride (20 mg, 0.18 mmol) or toluenesulfonyl chloride (35 mg, 0.18 mmol) dropwise with stirring. The reaction mixture was stirred (20 min), filtered, and concentrated (1 mL) in vacuo. Ligroin (2 × 20 mL) was added to the remaining residue, and the precipitate formed was isolated. The precipitate was dissolved in MeOH (1 mL), separated by preparative TLC using 10% MeOH–$CHCl_3$ as the eluant, and further purified by passing through a silica gel column (5 cm × 30 cm, 10% MeOH–$CHCl_3$) to give **15** and **16** in 80% and 70% yields, respectively.

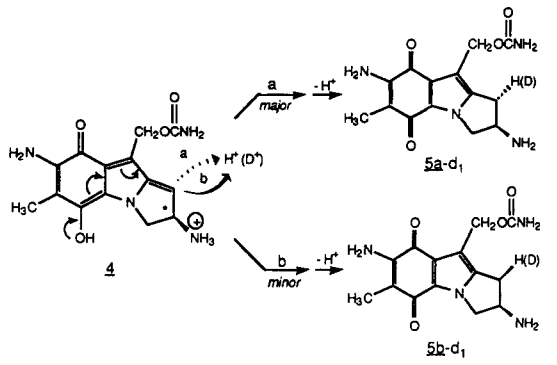
***N*(1a)-(Methanesulfonyl)mitomycin C (15):** HPLC retention time 22.2 min; TLC R_f 0.51; ^1H NMR (pyridine-d_5) δ 2.00 (s, C(6)CH_3), 3.14 (s, SO_2CH_3), 3.40 (s, OCH_3), 3.70 (dd, J = 1.8, 13.7 Hz, C(3)H_β), 3.77 (dd, J = 1.8, 5.1 Hz, C(2)H), 4.11 (d, J = 5.1 Hz, C(1)H), 4.13 (dd, J = 4.7, 11.0 Hz, C(9)H), 4.67 (dd, J = 11.0, 11.4 Hz, C(10)*H*H′), 4.75 (d, J = 13.7 Hz, C(3)H_α), 5.74 (dd, J = 4.7, 11.4 Hz, C(10)H*H*′); ^{13}C NMR (pyridine-d_5) 8.80 (C(6)-*C*H_3), 39.70 (SO_2CH_3), 42.16 (C(2)), 43.87 (C(1)), 45.87 (C(9)), 49.41 (OCH_3), 49.62 (C(3)), 61.72 (C(10)), 102.93 (C(6)), 105.58 (C(9a)), 109.76 (C(8a)), 148.73 (C(7)), 153.81 (C(5a)), 156.17 (C(10)O*C*-(O)NH_2), 175.84 (C(8)), 176.56 (C(5)) ppm; UV–vis (MeOH) 360 nm.

***N*(1a)-(Toluenesulfonyl)mitomycin C (16):** ^1H NMR ($CDCl_3$) δ 1.75 (s, C(6)CH_3), 2.43 (s, $SO_2C_6H_4CH_3$), 3.18 (s, OCH_3), 3.48 (dd, J = 1.7, 5.1 Hz, C(2)H), 3.54 (dd, J = 1.7, 13.6 Hz,

(34) An additional factor that may contribute to the observed stereoselective proton (deuterium) incorporation at C(1) in **5** is the conformation of the reactive dihydropyrrole ring in **4**.

(35) (a) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188. (b) Bates, R. G. *Determination of pH. Theory and Practice*; John Wiley & Sons: New York, 1965; Chapter 8, pp 201–229.

Scheme II. Possible Pathways for Proton (Deuterium) Incorporation at the C(1) Site in Quinone Methide 4



C(3)H_β), 3.62 (dd, J = 4.3, 10.7 Hz, C(9)H), 3.72 (dd, J = 10.7, 11.0 Hz, C(10)HH'), 3.77 (d, J = 5.1 Hz, C(1)H), 4.28 (d, J = 13.6 Hz, C(3)H_α), 4.84 (dd, J = 4.3, 11.0 Hz, C(10)HH'), 7.31 (d, J = 8.2 Hz, 2 ArH), 7.76 (d, J = 8.2, Hz, 2 ArH); ^{13}C NMR ($CDCl_3$) 7.23 (C(6)CH_3), 21.05 ($SO_2C_6H_4CH_3$), 41.89 (C(2)), 42.11 (C(1)), 45.21 (C(9)), 48.14 (OCH_3), 49.25 (C(3)), 60.94 (C(10)), 104.90 (C(6)), 105.40 (C(9a)), 110.48 (C(8a)), 127.39 (2C(3')), 129.18 (2C(2')), 133.70 (C(4')), 144.90 (C(1')), 146.70 (C(7)), 153.17 (C(5a)), 155.75 (C(10)OC(O)NH_2), 175.53 (C(8)), 177.85 (C(5)) ppm; UV–vis (MeOH) 362 nm.

General Procedures for the Reduction of Mitomycin C (1) and Mitomycin Derivatives 10 and 11 with 4-Methoxyphenylhydrazine. The mitomycin was dissolved in an aqueous buffered (0.1 M Tris·HOAc, pH 5.50) or D_2O-buffered (0.1 M deuterated Tris·DOAc, pD 5.50) solution, and the reaction vial was sealed with a Teflon–silicone rubber septum. Ar was bubbled through the stirred solution (20 min), and then a deaerated aqueous (pH 5.50) or D_2O (pD 5.50) buffered solution of 4-methoxyphenylhydrazine·HCl (1–1.25 equiv) was added. The reaction was maintained at room temperature (2 d) under a positive blanket of Ar. At the conclusion of the reaction the pH (pD) was remeasured to ensure that no significant change (±0.2) in the pH (pD) of the solution had occurred. The reaction mixture was neutralized with a 10% aqueous $NaHCO_3$ solution and lyophilized. The crude product was dissolved in MeOH and purified by semipreparative TLC.

2,7-Diaminomitosene (5).[1-6] Treatment of **1** (25 mg, 0.075 mmol) with 4-methoxyphenylhydrazine·HCl (16.2 mg, 0.093 mmol) in an aqueous buffered solution (2 mL) afforded **5** (63%), **8** + **9** + **19** (19%), **1** (14%), and an unidentified adduct (4%) (HPLC analysis). The identities of **8**, **9**, and **19** were verified by coinjection of authentic samples of these compounds with the reaction mixture in the HPLC. Compound **5** was isolated by preparative TLC using 8% MeOH–$CHCl_3$ as the eluant: ^{1}H NMR (CD_3OD) δ 1.80 (s, C(6)CH_3), 2.63 (dd, J = 4.6, 16.6 Hz, C(1)H_β), 3.16 (dd, J = 7.2, 16.6 Hz, C(1)H_α), 3.88 (dd, J = 4.6, 12.7 Hz, C(3)H_β), 4.10–4.22 (m, C(2)H), 4.35 (dd, J = 6.5, 12.7 Hz, C(3)H_α), 5.13 (s, C(10)H_2). The ^{1}H NMR assignments were confirmed from the corresponding COSY spectrum. UV–vis (MeOH) 245 (1.00), 313 (0.60), 545 (0.06) nm.

1α-Deuterio-2,7-diaminomitosene (5-d_1).[2,5] Using the preceding reaction and workup conditions **1** (25 mg, 0.075 mmol) was treated with 4-methoxyphenylhydrazine·HCl (1.24 equiv) in a buffered D_2O (pD 5.50) solution (48 mL) to afford **5-d_1**: ^{1}H NMR (DMSO-d_6) δ 1.68 (s, C(6)CH_3), 2.44 (d, J = 5.0 Hz, C(1)H_β), 3.70 (dd, J = 5.0, 12.4 Hz, C(3)H_β), 4.01–4.17 (m, C(2)H), 4.20 (dd, J = 6.6, 12.4 Hz, C(3)H_α), 4.99 (s, C(10)H_2). An unidentified weak signal was observed at ~δ 3.10 integrating for ≤0.06 protons. The ^{1}H NMR assignments were confirmed from the corresponding COSY spectrum. UV–vis (MeOH) 243 (1.00), 313 (0.60), 545 (0.06) nm.

2,7-Diamino-10-decarbamoylmitosene (20).[5,26] Treatment of an aqueous buffered solution (pH 5.50, 2.5 mL) of **10** (1.15 mg, 0.0039 mmol) with 4-methoxyphenylhydrazine·HCl (0.81 mg, 0.0046 mmol) afforded **20** in near-quantitative yield (HPLC analysis). The identity of **20** was verified by coinjection of an authentic sample of **20** with the reaction mixture in the HPLC.

1α-Deuterio-2,7-diamino-10-decarbamoylmitosene (20-d_1).[5] Treatment of **10** (28.6 mg, 0.098 mmol) with 4-methoxyphenylhydrazine·HCl (1.06 equiv) in a deuterated buffer solution (pD 5.60, 80 mL) afforded **20-d_1**. The crude product was purified by repetitive preparative TLC using 10% MeOH–$CHCl_3$ as the eluant to give **20-d_1**: ^{1}H NMR (CD_3OD) δ 1.77 (s, C(6)CH_3), 2.68 (br d, J = 3.6 Hz, C(1)H_β), 3.97 (dd, J = 4.2, 13.0 Hz, C(3)H_α), 4.17–4.27 (m, C(2)H), 4.38 (dd, J = 6.7, 13.0 Hz, C(3)H_α), 4.66 (s, C(10)H_2). All proton–proton connectivities were confirmed by the COSY spectrum. No evidence of the C(1)H_α resonance was detected. UV–vis (MeOH) 218, 240 (sh), 350 nm.

2-(Methylamino)-7-amino-10-decarbamoyl-10-acetoxymitosene (21). Compound **11** (15 mg, 0.043 mmol) was treated with 4-methoxyphenylhydrazine·HCl (7.45 mg, 0.043 mmol) in a buffered aqueous solution (pH 5.50, 30 mL). HPLC analysis at the conclusion of the reaction revealed that **21** had been generated in high yield. Purification of the product mixture by semipreparative TLC using 9% MeOH–$CHCl_3$ as the eluant afforded **21** as the major product and a small amount of 2-(methylamino)-7-amino-10-decarbamoylmitosene.

Compound 21: TLC R_f 0.62 (9% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.82 (s, C(6)CH_3), 2.06 (s, C(10)OC(O)CH_3), 2.48 (s, NCH_3), 2.68 (dd, J = 4.6, 16.4 Hz, C(1)H_β), 3.13 (dd, J = 7.0, 16.4 Hz, C(1)H_α), 3.90–3.96 (m, C(2)H), 4.05 (dd, J = 4.5, 12.9 Hz, C(3)H_β), 4.39 (dd, J = 6.7, 12.9 Hz, C(3)H_α), 5.19 (s, C(10)H_2). All proton–proton connectivities were confirmed by the COSY spectrum. MS (+CI) m/e (rel intensity) 317 (M^+, 18), 258 (100); UV–vis (MeOH) 244, 310, 355 (sh), 535 nm.

10-Decarbamoyl-2-(methylamino)-7-aminomitosene (25): TLC R_f 0.41 (9% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.80 (s, C(6)CH_3), 2.44 (s, NCH_3), 2.59 (dd, J = 5.0, 15.7 Hz, C(1)H_β), 3.06 (dd, J = 7.0, 15.7 Hz, C(1)H_α), 3.85–3.90 (m, C(2)H), 3.98 (dd, J = 5.0, 12.7 Hz, C(3)H_β), 4.33 (dd, J = 6.5, 12.7 Hz, C(3)H_α), 4.55 (s, C(10)H_2); UV–vis (MeOH) 234, 304, 348, 575 nm.

1α-Deuterio-2-(methylamino)-7-amino-10-decarbamoyl-10-acetoxymitosene (21-d_1). The preceeding reaction in a buffered D_2O solution was repeated on the same scale to give **21-d_1**: ^{1}H NMR ($CDCl_3$) δ 1.82 (s, C(6)CH_3), 2.06 (s, C(10)OC(O)CH_3), 2.48 (s, NCH_3), 2.68 (d, J = 9.5 Hz, C(1)H_β), 3.89–3.95 (m, C(2)H), 4.05 (dd, J = 8.3, 13.0 Hz, C(3)H_β), 4.38 (dd, J = 6.6, 13.0 Hz, C(3)H_α), 5.19 (s, C(10)H_2). All proton–proton connectivities were confirmed by the COSY spectrum. A small signal integrating for ≤0.20 protons was observed at approximately δ 3.10 and may have been the response from the C(1)H_α proton. MS (+CI) m/e (rel intensity) 318 (M^+, 22), 259 (100); UV–vis (MeOH) 240, 300, 350 (sh) nm.

General Procedure for the Catalytic Reduction of Mitomycin D (12) and 10-Decarbamoylmitomycin D (13). Mitomycin **12** (10 mg, 0.03 mmol) or **13** (5 mg, 0.02 mmol) was dissolved in an aqueous buffered mixture (pH 5.50, 0.1 M bis-Tris·HCl, 3 mL) containing PtO_2 (5 mg) and then deaerated with Ar (15 min). H_2 gas was bubbled through the solution (300 s) at room temperature, and then the reaction was exposed to air. The solution was filtered, neutralized with Et_3N and concentrated in vacuo. The residue was separated by TLC (10% MeOH–$CHCl_3$) and reversed-phase TLC (50% aqueous CH_3CN) and then further purified by passing through a silica gel column (2 cm × 30 cm, 30% MeOH–$CHCl_3$). Deuterated bis-Tris·DCl (0.1 M) was used in the pD 5.50 reactions.

2-(Methylamino)-7-aminomitosene (22): HPLC retention time 20.7 min; TLC R_f 0.19; ^{1}H NMR (pyridine-d_5) δ 2.14 (s, C(6)CH_3), 2.26 (s, NCH_3), 2.79 (dd, J = 4.9, 14.6 Hz, C(1)H_β), 3.15 (dd, J = 7.7, 14.6 Hz, C(1)H_α), 3.59–3.70 (m, C(2)H), 4.06 (dd, J = 4.9, 12.9 Hz, C(3)H_β), 4.36 (dd, J = 6.8, 12.9 Hz, C(3)H_α), 5.65 (s, C(10)H_2); MS (–CI) m/e (rel intensity) 277 (30), 261 (20), 232 (10), 214 (5), 153 (100). Structural assignment is based on the observed ^{1}H NMR spectra and mass spectral fragmentation patterns. HPLC analysis (313 nm) indicated that the isolated adduct was >90% pure.

1α-Deuterio-2-(methylamino)-7-aminomitosene (22-d_1): HPLC retention time 20.7 min; TLC R_f 0.19; ^{1}H NMR (pyridine-d_5) δ 2.14 (s, C(6)CH_3), 2.27 (s, NCH_3), 2.78 (d, J = 4.3 Hz, C(1)H_β), 3.59–3.70 (m, C(2)H), 4.07 (dd, J = 4.8, 12.8 Hz, C(3)H_β), 4.35 (dd, J = 6.7, 12.8 Hz, C(3)H_α), 5.65 (s, C(10)H_2). A small signal (≤0.20 protons) was observed at δ 3.15 and may be attributed to the C(1)H_α proton. MS (–CI) m/e (rel intensity) 278 (20), 262 (12), 232 (5), 214 (10), 153 (100). Structural assignment

is based on the observed ^1H NMR spectra and mass spectral fragmentation patterns. HPLC analysis (313 nm) indicated that the isolated adduct was >90% pure.

10-Decarbamoyl-2-(methylamino)-7-aminomitosene (25): HPLC retention time 18.0 min; TLC R_f 0.16; ^1H NMR (pyridine-d_5) δ 2.15 (s, C(6)CH_3), 2.29 (s, NCH_3), 2.82 (dd, J = 4.7, 16.5 Hz, C(1)H_β), 3.13 (dd, J = 7.6, 16.5 Hz, C(1)H_α), 3.68–3.73 (m, C(2)H), 4.12 (dd, J = 4.5, 12.7 Hz, C(3)H_β), 4.38 (dd, J = 6.6, 12.7 Hz, C(3)H_α), 5.20 (s, C(10)H_2); MS (+CI) m/e (rel intensity) 275 (M^+, 18), 258 (23), 239 (73), 183 (100), 155 (63), 127 (71). Structural assignment is based on the observed ^1H NMR spectra and mass spectral fragmentation patterns. HPLC analysis (313 nm) indicated that the isolated adduct was >90% pure.

10-Decarbamoyl-1α-deuterio-2-(methylamino)-7-aminomitosene (25-d_1): HPLC retention time 18.0 min; TLC R_f 0.16; ^1H NMR (pyridine-d_5) δ 2.14 (s, C(6)CH_3), 2.29 (s, NCH_3), 2.79 (d, J = 4.7 Hz, C(1)H_β), 3.68–3.73 (m, C(2)H), 4.11 (dd, J = 4.9, 12.8 Hz, C(3)H_β), 4.39 (dd, J = 6.6, 12.8 Hz, C(3)H_α), 5.20 (s, C(10)H_2). A trace signal (≤0.20 protons) was observed at δ 3.12 and may be attributed to the C(1)H_α proton. MS (+CI) m/e (rel intensity) 276 (M^+, 10), 231 (5), 153 (100), 121 (11).

Hydrolysis of 10-Decarbamoyl-*N*(1a)-methylmitomycin C (18). Preparation of *cis*- (26) and *trans*-10-Decarbamoyl-1,10-dihydroxy-2-(methylamino)-7-aminomitosenes (27). 10-Decarbamoyl-*N*(1a)-methylmitomycin C (18) (10 mg, 0.34 mmol) was dissolved in an aqueous 0.2 N HCl solution (2 mL) and allowed to stand for 30 min. HPLC analysis indicated the complete hydrolysis of 18 to *cis*- (26) and *trans*-10-decarbamoyl-1,10-dihydroxy-2-(methylamino)-7-aminomitosenes (27). The reaction was neutralized with solid $NaHCO_3$, separated by TLC using 10% MeOH–$CHCl_3$ as the eluant, and further purified by silica gel column chromatography (2 cm × 30 cm, 30% MeOH–$CHCl_3$) to give compounds 26 and 27.

***cis*-10-Decarbamoyl-1,10-dihydroxy-2-(methylamino)-7-aminomitosene (26):** HPLC retention time 17.0 min; TLC R_f 0.25; ^1H NMR (CD_3OD) δ 1.79 (s, C(6)CH_3), 2.49 (s, NCH_3), 3.60–3.67 (m, C(2)H), 3.77 (dd, J = 8.5, 12.3 Hz, C(3)H_β), 4.50 (dd, J = 7.1, 12.3 Hz, C(3)H_α), 4.72 ($^1/_2$ ABq, J = 13.6 Hz, C(10)HH'), 4.79 ($^1/_2$ ABq, J = 13.6 Hz, C(10)HH'), 5.07 (d, J = 5.1 Hz, C(1)H_α); UV–vis (MeOH) 244 (1.00), 312 (0.56), 548 (0.06) nm. Structural assignment is based on the observed ^1H NMR spectra. HPLC analysis (313 nm) indicated that the isolated adduct was >90% pure.

***trans*-10-Decarbamoyl-1,10-dihydroxy-2-(methylamino)-7-aminomitosene (27):** HPLC retention time 16.7 min; TLC R_f 0.23; ^1H NMR (CD_3OD) δ 1.80 (s, C(6)CH_3), 2.47 (s, NCH_3), 3.58–3.66 (m, C(2)H), 3.94 (dd, J = 4.2, 13.2 Hz, C(3)H_β), 4.51 (dd, J = 6.6, 13.2 Hz, C(3)H_α), 4.72 ($^1/_2$ ABq, J = 13.6 Hz, C(10)HH'), 4.78 ($^1/_2$ ABq, J = 13.6 Hz, C(10)HH'), 4.91 (d, J = 3.7 Hz, C(1)H_β); UV–vis (MeOH) 245 (1.00), 312 (0.54), 548 (0.06) nm. Structural assignment is based on the observed ^1H NMR spectra. HPLC analysis (313 nm) indicated that the isolated adduct was >90% pure.

General Procedure for the Catalytic Reduction of Mitomycin C (1) and 7-Aminoaziridinomitosene[18] (14) in THF–H_2O and THF–D_2O Mixtures. H_2 gas (300 s) was bubbled through a deaerated (Ar) THF mixture containing 1 or 14 and PtO_2. During this time interval the color of the solution turned from blue-violet to colorless. The reduced 1 or 14 solution was transferred via a cannula to a deaerated (Ar) buffered aqueous or D_2O solution. The reaction mixture was then exposed to air, neutralized if necessary with Et_3N, filtered, and analyzed by HPLC. In select experiments the product was isolated by TLC and/or column chromatography.

2,7-Diaminomitosene (5) from 1. Use of 1 (10 mg, 0.03 mmol) and PtO_2 (8 mg) and a final THF (20 mL)–aqueous 0.1 M bis-Tris·HCl (pH 5.50, 10 mL) mixture afforded 5 as the major product (HPLC analysis). The "pH" at the conclusion of the reaction was 5.30. The reaction mixture was purified first by TLC (10% MeOH–$CHCl_3$) and then by silica gel chromatography (2 cm × 30 cm, 30% MeOH–$CHCl_3$) to afford 5: HPLC retention time 20.0 min; TLC R_f 0.20; ^1H NMR (CD_3OD) δ 1.80 (s, C(6)CH_3), 2.63 (dd, J = 4.6, 16.6 Hz, C(1)H_β), 3.16 (dd, J = 7.2, 16.6 Hz, C(1)H_α), 3.88 (dd, J = 4.6, 12.5 Hz, C(3)H_β), 4.10–4.22 (m, C(2)H), 4.35 (dd, J = 6.5, 12.5 Hz, C(3)H_α), 5.13 (s, C(10)H_2); ^1H NMR (pyridine-d_5) δ 2.14 (s, C(6)CH_3), 2.77 (dd, J = 4.8, 16.1 Hz, C(1)H_β), 3.20 (dd, J = 6.8, 16.1 Hz, C(1)H_α), 3.98–4.09 (m, C(2)H, C(3)H_β), 4.37 (dd, J = 5.7, 11.7 Hz, C(3)H_α), 5.64 (s, C(10)H_2); MS (+CI) m/e (rel intensity) 304 (M^+, 10), 261 (100), 244 (86), 219 (68), 203 (57), 149 (35); UV–vis (MeOH) 245 (1.00), 313 (0.60), 545 (0.06) nm.

1α-Deuterio-2,7-diaminomitosene (5-d_1) from 1. The preceding procedure was employed using 1 (10 mg, 0.03 mmol) and PtO_2 (8 mg) in a final THF (20 mL)–D_2O (10 mL, pD 5.50, 0.1 M deuterated bis-Tris·DCl) mixture to afford 5-d_1 as the major product (80%, HPLC analysis). The "pD" at the conclusion of the reaction was 5.40: HPLC retention time 20.0 min; TLC R_f 0.20; ^1H NMR (pyridine-d_5) δ 2.13 (s, C(6)CH_3), 2.72 (d, J = 4.5 Hz, C(1)H_β), 3.95–4.07 (m, C(2)H, C(3)H_β), 4.38 (dd, J = 5.9, 11.8 Hz, C(3)H_α), 5.65 (s, C(10)H_2); a trace signal (≤0.20 protons) was observed at δ 3.18 and has been attributed to the C(1)H_α peak; UV–vis (MeOH) 245 (1.00), 313 (0.61), 547 (0.06) nm.

2,7-Diaminomitosene (5) from 14. Use of 14 (2 mg, 0.007 mmol) and PtO_2 (1 mg) in a final THF (2 mL)–aqueous 0.1 M bis-Tris·HCl (pH 6.50, 1 mL) mixture gave 5 (17%), 8 + 9 (52%), 28 + 29 (3%), along with several unknown compounds (28%) (HPLC analysis). The identity of all known compounds was verified by coinjection of an authentic sample with the reaction mixture in the HPLC. The "pH" at the conclusion of the reaction was 6.40.

1α-Deuterio-2,7-diaminomitosene (5-d_1) from 14. The preceding procedure was employed using 14 (2 mg, 0.007 mmol) and PtO_2 (1 mg) in a final THF (2 mL)–D_2O (1 mL, pD 7.50, 0.1 M deuterated Tris·DCl) mixture. HPLC analysis indicated the presence of 5-d_1 (19%), 8 + 9 (45%), along with several unknown compounds (36%). The "pD" at the conclusion of the reaction was 7.40. Twenty repetitive experiments were conducted to provide a sufficient sample of 5-d_1 for ^1H NMR analysis: HPLC retention time 20.0 min; TLC R_f 0.20; ^1H NMR (CD_3OD) δ 1.79 (s, C(6)CH_3), 2.62 (d, J = 4.1 Hz, C(1)H_β), 3.89 (dd, J = 4.0, 12.5 Hz, C(3)H_β), 4.12–4.18 (m, C(2)H), 4.36 (dd, J = 6.5, 12.5 Hz, C(3)H_α), 5.14 (s, C(10)H_2). A trace signal (≤0.20 protons) was observed at δ 3.10 and may be attributed to the C(1)H_α proton: ^1H NMR (pyridine-d_5) δ 2.14 (s, C(6)CH_3), 2.71 (d, J = 4.6 Hz, C(1)H_β), 3.95–4.05 (m, C(2)H, C(3)H_β), 4.37 (dd, J = 5.3, 11.1 Hz, C(3)H_α), 5.65 (s, C(10)H_2). A trace signal (≤0.20 protons) was observed at δ 3.15 and has been attributed to the C(1)H_α peak. MS (+CI) m/e (rel intensity) 305 (M^+, 13), 279 (29), 262 (18), 245 (52), 167 (33), 149 (100).

General Procedure for the Catalytic Reductive Activation of *N*(1a)-(Methanesulfonyl)mitomycin C (15) in THF–H_2O and THF–D_2O Mixtures. H_2 gas was bubbled (300 s) through a deaerated (Ar) buffered THF(2)–H_2O(1) mixture (3 mL, "pH" 5.50, 0.1 M bis-Tris·HCl) or a THF(2)–D_2O(1) mixture (3 mL, "pD" 5.50, 0.1 M deuterated bis-Tris·DCl) containing 15 (20 mg) and PtO_2 (10 mg). The reaction mixture was exposed to air, filtered, and analyzed. HPLC analysis of the reaction solution indicated the presence of 32 (32-d_1) (18–24%), 33, 34 (20%), 35 (35-d_1) (15–23%), and unreacted 15 (28–42%), respectively. The reaction solution was directly separated by TLC (10% MeOH–$CHCl_3$) and further purified by passing through a silica gel column (2 cm × 30 cm, 30% MeOH–$CHCl_3$) to give the desired products.

2-[(Methanesulfonyl)amino]-7-aminomitosene (32): HPLC retention time 23.2 min; TLC R_f 0.30; ^1H NMR (pyridine-d_5) δ 2.12 (s, C(6)CH_3), 3.16 (s, SO_2CH_3), 3.25 (dd, J = 5.2, 16.5 Hz, C(1)H_β), 3.51 (dd, J = 7.5, 16.5 Hz, C(1)H_α), 4.44 (dd, J = 5.0, 12.9 Hz, C(3)H_β), 4.69 (dd, J = 7.0, 12.9 Hz, C(3)H_α), 4.84–4.91 (m, C(2)H), 5.59 ($^1/_2$ ABq, J = 13.2 Hz, C(10)HH'), 5.64 ($^1/_2$ ABq, J = 13.2 Hz, C(10)HH'); UV–vis (CH_3CN) 244 (1.00), 312 (0.62), 532 (0.05) nm. Structural assignment is based on the observed ^1H NMR spectra. Attempts to obtain satisfactory mass spectral data using CI (±) mass spectroscopy proved unsuccessful. HPLC analysis (313 nm) indicated that the isolated adduct was >88% pure.

1α-Deuterio-2-[(methanesulfonyl)amino]-7-aminomitosene (32-d_1): HPLC retention time 23.2 min; TLC R_f 0.30; ^1H NMR (pyridine-d_5) δ 2.12 (s, C(6)CH_3), 3.16 (s, SO_2CH_3), 3.24 (d, J = 5.0 Hz, C(1)H_β, 0.55 H), 3.49 (d, J = 7.2 Hz, C(1)H_α, 0.45 H), 4.44 (dd, J = 5.0, 12.8 Hz, C(3)H_β), 4.70 (dd, J = 7.1, 12.8 Hz, C(3)H_α), 4.85–4.90 (m, C(2)H), 5.58 ($^1/_2$ ABq, J = 13.1 Hz, C(10)HH'), 5.64 ($^1/_2$ ABq, J = 13.1 Hz, C(10)HH'); UV–vis (CH_3CN) 242 (1.00), 312 (0.61), 525 (0.05) nm. Attempts to obtain satisfactory mass

spectral data using CI (±) mass spectroscopy proved unsuccessful.

***cis*-1-Hydroxy-2-[(methanesulfonyl)amino]-7-aminomitosene (33)**: HPLC retention time 20.6 min; TLC R_f 0.27; ^{1}H NMR (CD_3OD) δ 1.79 (s, C(6)CH_3), 3.06 (s, SO_2CH_3), 3.89 (dd, J = 8.5, 12.0 Hz, C(3)H_β), 4.44–4.51 (m, C(2)H), 4.62 (dd, J = 7.9, 12.0 Hz, C(3)H_α), 5.04 (d, J = 5.3 Hz, C(1)H_α), 5.17 ($^1/_2$ ABq, J = 13.0 Hz, C(10)*H*H′), 5.24 ($^1/_2$ ABq, J = 13.0 Hz, C(10)H*H*′); ^{13}C NMR (DMSO-d_6) 8.37 (C(6)*C*H_3), 40.83 (SO_2CH_3), 48.95 (C(3)), 56.74 (C(10)), 57.59 (C(2)), 64.03 (C(1)), 104.63 C(6)), 112.87 (C(8a)), 120.50 (C(9)), 128.35 (C(9a)), 140.84 (C(7)), 147.13 (C(5a)), 156.28 (C(10)O*C*(O)NH_2), 176.34 (C(8)), 178.41 (C(5)) ppm; MS (–CI) m/e (rel intensity) 398 (M^-, 9), 385 (9), 355 (88), 339 (100), 275 (20); UV–vis (CH_3CN) 254 (1.00), 313 (0.60), 535 (0.05) nm. Structural assignment is based on the observed ^{1}H NMR, ^{13}C NMR, and mass spectra. HPLC analysis (313 nm) indicated that the isolated adduct was >92% pure.

***trans*-1-Hydroxy-2-[(methanesulfonyl)amino]-7-aminomitosene (34)**: HPLC retention time 19.5 min; TLC R_f 0.24; ^{1}H NMR (CD_3OD) δ 1.80 (s, C(6)CH_3), 3.01 (s, SO_2CH_3), 4.04 (dd, J = 4.8, 13.0 Hz, C(3)H_β), 4.30–4.36 (m, C(2)H), 4.62 (dd, J = 7.0, 13.0 Hz, C(3)H_α), 5.03 (d, J = 3.9 Hz, C(1)H_β), 5.17 ($^1/_2$ ABq, J = 12.6 Hz, C(10)*H*H′), 5.23 ($^1/_2$ ABq, J = 12.6 Hz, C(10)H*H*′); ^{13}C NMR (DMSO-d_6) 8.44 (C(6)*C*H_3), 40.77 (SO_2CH_3), 50.94 (C(3)), 56.18 (C(10)), 63.95 (C(2)), 71.50 (C(1)), 104.48 (C(6)), 112.41 (C(8a)), 121.43 (C(9)), 128.11 (C(9a)), 140.51 (C(7)), 147.08 (C(5a)), 156.17 (C(10)O*C*(O)NH_2), 176.49 (C(8)), 178.47 (C(5)) ppm; MS (–CI) m/e (rel intensity) 355 (88), 339 (100), 275 (21); UV–vis (CH_3CN) 254 (1.00), 316 (0.61), 549 (0.06) nm. Structural assignment is based on the observed ^{1}H NMR and ^{13}C NMR spectra and mass spectral fragmentation patterns. HPLC analysis (313 nm) indicated that the isolated adduct was >94% pure.

Compound 35: HPLC retention time 28.0 min; TLC R_f 0.32; ^{1}H NMR (DMSO-d_6) δ 1.72 (s, *C(6)CH*$_3$), 1.88 (s, C(6)CH_3), 2.61 (dd, J = 5.6, 16.1 Hz, C(1)H_β), 2.96 (s, SO_2CH_3), 3.07 (dd, J = 8.7, 16.1 Hz, C(1)H_α), 3.13 (s, *SO*$_2$*CH*$_3$), 3.16 (s, *OCH*$_3$), 3.51 (dd, J = 4.7, 9.6 Hz, *C(9)H*), 3.56 (d, J = 13.6 Hz, *C(3)H*$_\beta$), 3.64–3.67 (m, *C(2)H*), 3.77 (d, J = 4.9 Hz, *C(1)H*), 3.83 (dd, J = 9.6, 10.7 Hz, *C(10)*$\underline{H}$*H*′), 3.90 (dd, J = 5.3, 12.4 Hz, C(3)H_β), 4.42 (dd, J = 7.0, 12.4 Hz, C(3)H_α), 4.27 (d, J = 13.6 Hz, *C(3)H*$_\alpha$), 4.48–4.56 (m, C(2)H), 4.74–4.78 (m, C(10)H_2), 4.81 (dd, J = 4.7, 10.7 Hz, *C(10)H*$\underline{H}$′), 6.43 (br s, NH_2), 7.39 (t, J = 6.4 Hz, *C(7)NH*). The proton–proton connectivities were confirmed by the COSY spectrum. UV–vis (CH_3CN) 317 (0.70), 370 (1.00), 538 (0.03) nm. Structural assignment is based on the observed ^{1}H NMR spectra and UV–vis spectra. Attempts to obtain satisfactory mass spectral data using EI, CI, and FAB mass spectroscopy proved unsuccessful. HPLC analysis (313 nm) indicated that the isolated adduct was >90% pure.

Compound 35-d_1: HPLC retention time 28.0 min; TLC R_f 0.32; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, *C(6)CH*$_3$), 1.85 (s, C(6)CH_3), 2.59 (d, J = 4.6 Hz, C(1)H_β, 0.65 H), 2.90 (s, SO_2CH_3), 3.02 (d, J = 6.7 Hz, C(1)H_α, 0.35 H), 3.14 (s, *SO*$_2$*CH*$_3$), 3.19 (s, *OCH*$_3$), 3.50 (dd, J = 4.6, 9.9 Hz, *C(9)H*), 3.53 (dd, J = 2.4, 12.7 Hz, *C(3)H*$_\beta$), 3.67 (dd, J = 2.4, 5.0 Hz, *C(2)H*), 3.77 (d, J = 5.0 Hz, *C(1)H*), 3.80 (dd, J = 9.9, 10.6 Hz, *C(10)*$\underline{H}$*H*′), 3.90 (dd, J = 4.9, 12.1 Hz, C(3)H_β), 4.28 (d, J = 12.7 Hz, *C(3)H*$_\alpha$), 4.42 (dd, J = 6.9, 12.1 Hz, C(3)H_α), 4.46–4.55 (m, C(2)H), 4.74–4.78 (m, C(10)H_2), 4.80 (dd, J = 4.6, 10.6 Hz, *C(10)H*$\underline{H}$′), 6.60 (br s, NH_2), 7.40 (t, J = 5.7 Hz, *C(7)NH*); UV–vis (CH_3CN) 315 (0.70), 368 (1.00), 543 (0.03) nm.

General Procedure for the Catalytic Reductive Activation of *N*(1a)-(Toluenesulfonyl)mitomycin C (16) in THF–H_2O and THF–D_2O Mixtures. H_2 gas was bubbled (300 s) through a deaerated (Ar) buffered THF(2)–H_2O(1) mixture (3 mL, "pH" 5.50, 0.1 M bis-Tris·HCl) or a THF(2)–D_2O(1) (3 mL, "pD" 5.50, 0.1 M deuterated bis-Tris·DCl) mixture containing **16** (20 mg) and PtO_2 (10 mg). The reaction mixture was exposed to air, filtered, and analyzed. HPLC analysis of the reaction solution indicated the presence of **36** (**36**-d_1) (35–41%), several unidentified products (15–20%), and unreacted **16** (39–50%). The reaction mixture was exposed to air, directly separated by TLC (10% MeOH–$CHCl_3$), and further purified by passing through a silica gel column (2 cm × 30 cm, 30% MeOH–$CHCl_3$) to give **36** (**36**-d_1).

2-[(Toluenesulfonyl)amino]-7-aminomitosene (36): HPLC retention time 30.3 min; TLC R_f 0.31 (5% MeOH–$CHCl_3$); ^{1}H NMR (pyridine-d_5) δ 2.14 (s, C(6)CH_3), 2.22 (s, $SO_2C_6H_4CH_3$), 3.12 (dd, J = 5.8, 16.4 Hz, C(1)H_β), 3.44 (dd, J = 7.0, 16.4 Hz, C(1)H_α), 4.39 (dd, J = 5.5, 12.9 Hz, C(3)H_β), 4.59 (dd, J = 6.6, 12.9 Hz, C(3)H_α), 4.74–4.84 (m, C(2)H), 5.57 ($^1/_2$ ABq, J = 12.8 Hz, C-(10)*H*H′), 5.63 ($^1/_2$ ABq, J = 12.8 Hz, C(10)H*H*′), 7.25 (d, J = 8.0 Hz, 2 ArH), 8.29 (d, J = 8.0 Hz, 2 ArH); UV–vis (CH_3CN) 244 (1.00), 314 (0.70), 550 (0.05) nm. Structural assignment is based on the observed ^{1}H NMR spectra. Attempts to obtain satisfactory mass spectral data using CI (±) mass spectroscopy proved unsuccessful. HPLC analysis (313 nm) indicated that the isolated adduct was >78% pure.

1α-Deuterio-2-[(toluenesulfonyl)amino]-7-aminomitosene (36-d_1): HPLC retention time 30.3 min; TLC R_f 0.31 (5% MeOH–$CHCl_3$); ^{1}H NMR (pyridine-d_5) δ 2.08 (s, C(6)CH_3), 2.19 (s, $SO_2C_6H_4CH_3$), 3.16 (d, J = 4.8 Hz, C(1)H_β, 0.60 H), 3.35 (d, J = 6.9 Hz, C(1)H_α, 0.40 H), 4.32 (dd, J = 4.7, 12.9 Hz, C(3)H_β), 4.53 (dd, J = 6.9, 12.9 Hz, C(3)H_α), 4.69–4.86 (m, C(2)H), 5.53 ($^1/_2$ ABq, J = 11.4 Hz, C(10)*H*H′), 5.60 ($^1/_2$ ABq, J = 11.4 Hz, C(10)H*H*′), 7.00–7.30 (m, 2 ArH), 8.10 (d, J = 8.0 Hz, 2 ArH); UV–vis (CH_3CN) 245 (1.00), 312 (0.71), 548 (0.05) nm.

Procedure for the Preparation of 2-(Dimethylamino)-7-aminomitosene (38) from 2-(Methylamino)-7-aminomitosene (22). To a deaerated MeOH solution (3 mL) containing **22** (10 mg, 0.03 mmol, final concentration: 10 mM) were added 2,6-di-*tert*-butylpyridine (50 mg, 0.26 mmol) and dimethyl sulfate (20 mg, 0.16 mmol). The reaction solution was stirred (30 min) at room temperature and exposed to air, and then the reaction mixture was directly separated by TLC using 10% MeOH–$CHCl_3$ as the eluant and further purified by passing through a silica gel column (2 cm × 30 cm, 30% MeOH–$CHCl_3$).

2-(Dimethylamino)-7-aminomitosene (38): HPLC retention time 23.8 min; TLC R_f 0.35; ^{1}H NMR (CD_3OD) δ 1.78 (s, C(6)CH_3), 2.28 (s, N$(CH_3)_2$), 2.81 (dd, J = 6.4, 16.4 Hz, C(1)H_β), 3.12 (dd, J = 7.8, 16.4 Hz, C(1)H_α), 3.65–3.73 (m, C(2)H), 4.01 (dd, J = 6.2, 13.0 Hz, C(3)H_β), 4.38 (dd, J = 7.5, 13.0 Hz, C(3)H_α), 5.14 (s, C(10)CH_2); MS (+CI) m/e (rel intensity) 333 (MH^+, 31), 308 (20), 272 (14), 154 (100); UV–vis (CH_3CN) 245 (1.00), 313 (0.60), 547 (0.06) nm.

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Registry No. **1**, 50-07-7; **5**, 92695-32-4; **5**-d_1, 106471-85-6; **8**, 98462-75-0; **9**, 99745-88-7; **10**, 26909-37-5; **11**, 26909-40-0; **12**, 10169-34-3; **13**, 78879-23-9; **14**, 103422-25-9; **15**, 5091-31-6; **16**, 5091-32-7; **17**, 4055-40-7; **18**, 26909-38-6; **19**, 54911-22-7; **20**, 102587-27-9; **20**-d_1, 106471-86-7; **21**, 138724-38-6; **21**-d_1, 138724-39-7; **22**, 138724-40-0; **22**-d_1, 138724-41-1; **25**, 138724-42-2; **25**-d_1, 138724-43-3; **26**, 138724-44-4; **27**, 138810-15-8; **32**, 138724-45-5; **32a**-d_1, 138724-51-3; **32b**-d_1, 138810-18-1; **33**, 138724-46-6; **34**, 138810-16-9; **35**, 138724-47-7; **35a**-d_1, 138724-48-8; **35b**-d_1, 138810-17-0; **36**, 138724-50-2; **36a**-d_1, 138724-49-9; **36b**-d_1, 138810-19-2.

Supplementary Material Available: ^{1}H NMR spectra for compounds **5**, **5**-d_1, **20**, **21**, **21**-d_1, **22**, **22**-d_1, **25**, **25**-d_1, **26**, **27**, **32**, **32**-d_1, **33**, **34**, **35**, **35**-d_1, **36**, and **38** and HPLC traces for compounds **32–36** (28 pages). Ordering information is given on any current masthead page.