Studies on the Use of $Na_2S_2O_4$ for the Reductive Activation of Mitomycin C

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Abstract: Mitomycin C (**1a**) is considered to be the prototypical bioreductive alkylating agent. Among the numerous reductive procedures employed for the *in vitro* activation of mitomycin C, incremental addition of $Na_2S_2O_4$ has emerged as the method of choice for generating high yields of mitomycin C–DNA adducts. The major products and distinguishing features of the incremental addition $Na_2S_2O_4$–mitomycin C reductive processes in water (pH 7.4) in the absence of DNA are reported. Key observations included (1) rapid and efficient consumption of mitomycin C, (2) production of high amounts of 7-aminomitosane-9a-sulfonate (**1b**) in the early stages of the reaction, and (3) generation of significant amounts of C(1) and C(10) sulfonato adducts. The complexity of this transformation has been attributed in part to HSO_3^-, a byproduct of the $Na_2S_2O_4$ reduction process. Use of buffered methanol solutions ("pH" 7.4) in place of water simplified the product profile. The poor solubility of $Na_2S_2O_4$ and $NaHSO_3$ in methanol produced only trace amounts of mitosene sulfonato adducts. There were significant differences between product profiles for the incremental addition $Na_2S_2O_4$ procedure versus a protocol in which the equivalent amount of $Na_2S_2O_4$ was added in a single shot. First, higher amounts of C(1) electrophilic versus C(1) nucleophilic products were observed using the single shot technique. Second, C(1) sulfonato adducts composed a larger amount of the C(1) nucleophilic product pool when the $Na_2S_2O_4$ was added using the single shot protocol than with the incremental addition method. Third, higher amounts of **1a** were converted to C(1), C(10) fully functionalized mitosene adducts using the incremental procedure. Select auxiliary experiments provided additional information concerning the $Na_2S_2O_4$-mediated mitomycin C reductive process. Examination of the reactivity of key C(1), C(9a), and C(10) mitomycin sulfonato products demonstrated that 7-aminomitosane-9a-sulfonate (**1b**) was efficiently converted to C(1)- and C(10)-functionalized mitosenes under reductive conditions, whereas mitosene C(1) and C(10) sulfonates did not undergo displacement reactions and hence did not function as viable alkylating agents. On the basis of these cumulative studies, we suggest the likely mechanism for the $Na_2S_2O_4$-mediated mitomycin C reductive process and the beneficial properties accrued by the use of the incremental addition technique. These notions are discussed in light of the pathway that may be operative in *in vitro* mitomycin C–DNA bonding transformations.

Mitomycin C (**1a**) is an antineoplastic antibiotic with proven clinical efficacy for the treatment of breast, colon, and stomach cancer.[1] Studies indicate that drug activation proceeds by reduction of the quinone moiety in **1a**, followed by the stepwise loss of methanol at carbons 9 and 9a, to give leucoaziridinomitosene **3** (Scheme I).[2] Generation of **3** permits the covalent modification of nucleophilic residues on DNA to take place at carbons 1 and 10 in the activated drug.[3,4] Initial DNA bonding is believed to occur at carbon 1 through quinone methide **4**. Both the drug bonding to generate DNA-monoalkylated adducts (i.e., **5**) and the production of interstrand cross-linked products (i.e., **6**) occurred preferentially at 5′CG sequences.[3,4] It has been conjectured that the cytotoxicity of mitomycin C is associated with the formation of specific DNA lesions that prevent the subsequent synthesis of gene products vital for tumor growth.[2a,5]

The significance of individual mitomycin C–DNA adducts in arresting the disease process has prompted researchers to determine which products are generated in these transformations. These investigations have shown $Na_2S_2O_4$ as the reductant of choice for the *in vitro* functionalization of DNA by mitomycin C and, especially, for the activation of the recalcitrant carbon 10 bonding site in the drug.[2a,3a,c,4a,6] It has also been reported that *incremental* addition of substoichiometric amounts of $Na_2S_2O_4$ to an aqueous solution of **1a** and DNA (incremental addition method) gave higher mitomycin C–DNA bonding ratios than when the same amount of reductant was added all at once (single shot method).[7] Significantly, the benefits of using $Na_2S_2O_4$ for the production of mitomycin C–DNA adducts over other reductants contrasted with the corresponding results in the *absence* of DNA.[8] Without DNA, the product profiles were far more complex with $Na_2S_2O_4$[8] than with catalytic,[9] enzymatic,[3a,10]

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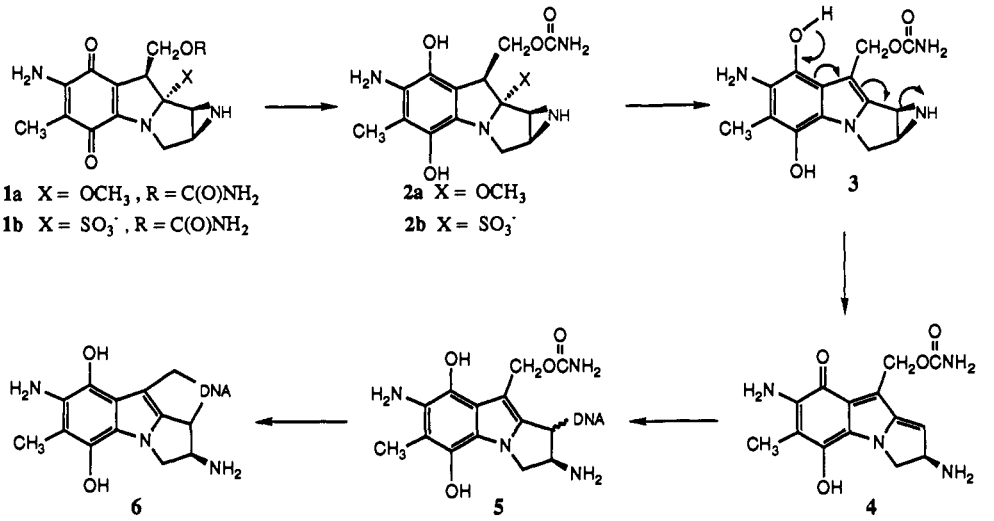
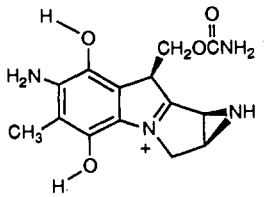
Scheme I. The Moore and Czerniak Mechanism for the Mode of Action of Mitomycin C



electrochemical,[11] and other[12] chemical reductive-based methods. The increase in the number of mitomycin products with $Na_2S_2O_4$ has been attributed in part to HSO_3^-, a byproduct in the reductive activation process and a contaminant in commercial $Na_2S_2O_4$.[8,9a] One HSO_3^--derived mitomycin adduct has been identified. Hornemann and co-workers observed that $Na_2S_2O_4$ reduction of aqueous solutions of **1a** at 0 °C followed by O_2 quenching furnished 7-aminomitosane-9a-sulfonate (**1b**).[8] Isolation of **1b** provided preliminary evidence of the intermediacy of iminium ion **7** in the



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drug activation process (i.e., **2** → **7** → **3**)[8] and subsequently led to speculation that that reduced **1b** may have facilitated the drug–DNA bonding process.[6b]

Surprisingly, despite the wide use of $Na_2S_2O_4$ in DNA–mitomycin C studies, little is known concerning the pathway of this reductive activation process and the reasons for its efficiency in the generation of mitomycin–DNA adducts.[13] In this paper, we provide information on the $Na_2S_2O_4$-mediated pathway for the activation of mitomycin C in both water and methanol. Key products have been identified and their roles in the functionalization of the mitomycin C–DNA bonding sites established. The product profiles of the incremental addition (method A) versus the single shot (method B) $Na_2S_2O_4$ reductive methods are also compared. The collective information yielded insights into the contributing factors that led to high yields of mitomycin C–DNA adducts obtained with this reductant and the beneficial factors gained with the use of the incremental addition versus the single shot method.

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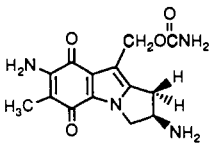
Results

Our investigation began with the determination of the product profiles for the incremental addition of substoichiometric amounts of $Na_2S_2O_4$ to both aqueous and methanolic solutions of mitomycin C and the single shot addition of $Na_2S_2O_4$ to aqueous solutions of **1a**. Information was also secured on the reactivity of the principal products generated in these transformations. These results serve as the basis for the proposed pathways in the Discussion for the $Na_2S_2O_4$-mediated reduction of mitomycin C.

a. Incremental Addition of $Na_2S_2O_4$ to Aqueous Mitomycin C Solutions (Method A). The $Na_2S_2O_4$-based protocols adopted were similar to procedures first described by Tomasz and co-workers.[7] Accordingly, substoichiometric amounts of a deaerated, aqueous buffered (pH 7.4) solution of $Na_2S_2O_4$ were sequentially added to a deaerated, aqueous buffered solution of mitomycin C. Five increments containing 0.2 equiv of $Na_2S_2O_4$ were added in equally spaced 12-min intervals, permitting the administration of 1 equiv of $Na_2S_2O_4$ over 48 min. Upon addition of $Na_2S_2O_4$, the characteristic blue of aqueous mitomycin C solutions turned to purple. Complete decoloration of the solution was observed after injection of 0.6 equiv of $Na_2S_2O_4$. The corresponding HPLC profiles were complex. Ten major and one minor peaks, including unreacted **1a**, were observed in the HPLC chromatograms after $Na_2S_2O_4$ addition. Use of other reductants (i.e., catalytic, electrochemical, and enzymatic)[9a,c,10b,c,11a,c,12] in place of $Na_2S_2O_4$ gave 2,7-diaminomitosene[9a,9c,14] (**8**) and *trans*- (**9**) and *cis*-1-hydroxy-2,7-diaminomitosene[11c,12a,15] (**10**) as the only principal products along with unreacted mitomycin C.



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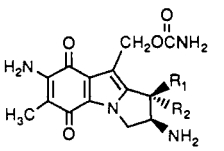

9 R_1 = H, R_2 = OH; 18 R_1 = H, R_2 = OCH_3
10 R_1 = OH, R_2 = H; 19 R_1 = OCH_3, R_2 = H
11 R_1 = H, R_2 = SO_3^-
12 R_1 = SO_3^-, R_2 = H

HPLC traces at 313 and 365 nm obtained after each substoichiometric addition of $Na_2S_2O_4$ provided information on the course of the reaction. The HPLC chromatograms corre-

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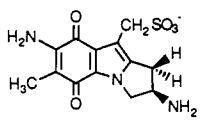
sponding to the administration of 0.2 equiv of reductant indicated the presence of significant amounts of mitosane **1b**,[8] along with **8–10**, two unidentified mitosene compounds **11** and **12**, and unreacted **1a**. Mitosane **1b** was the major product in the reaction mixture. These compounds were termed the "primary" reaction products. Subsequent introduction of additional amounts of $Na_2S_2O_4$ to the reaction solution resulted in the gradual decrease in the five mitosene peaks (i.e., **8–12**) and the concomitant appearance of four new major (i.e., **13–16**) and one new minor



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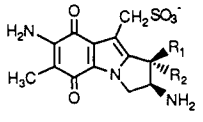

14 $R_1 = H, R_2 = OH$ **22** $R_1 = H, R_2 = OCH_3$
15 $R_1 = OH, R_2 = H$ **23** $R_1 = OCH_3, R_2 = H$
16 $R_1 = H, R_2 = SO_3^-$
17 $R_1 = SO_3^-, R_2 = H$

(i.e., **17**) mitosene signals that have been termed the "secondary" reaction products. Furthermore, we noted a drop in the relative amounts of the mitosane adduct **1b**, while the amounts of **1a** remained relatively unchanged. Significantly, the secondary adducts exhibited shorter HPLC retention times than the primary compounds, suggesting that these mitosenes were more polar compounds than the initial products generated in the reaction. Characteristic of the later HPLC measurements (i.e., 0.8–1.0 equiv of $Na_2S_2O_4$) was the presence of a broad, undefined pattern of products located between 17.5 and 23.5 min (approximately 10–15% of the reaction mixture) that was not fully resolved using one of the two HPLC programs (program 1) employed in this study. Use of the second HPLC condition (program 2) gave a similar product profile, except no appreciable number of unidentified peaks were detected during the later stages of the reaction. The absence of these peaks in this chromatogram led us to not include in our analyses the unidentified peaks observed in the 17.5–23.5-min region of the HPLC chromatograms using program 1.[16]

The new primary and secondary products were synthesized on a preparative scale for structural identification beginning with either **1a** or mitosene **8**, **9**, or **10**. The reaction conditions were altered by including Na_2SO_3 in the reaction and varying the amount of $Na_2S_2O_4$ used to increase the yields of individual products.

The compounds were identified from the ^1H and ^{13}C NMR, UV–visible, and mass spectral data. The ^1H and ^{13}C NMR spectral data obtained for mitosane adduct **1b** ($M^+ = NH_4^+$) corresponded to the values reported for sodium 7-aminomitosane-9a-sulfonate (**1b**, $M^+ = Na^+$).[8] We noted that the C(9a)–OCH_3 signal observed in both the ^1H and ^{13}C NMR spectra of **1a** was absent in **1b**, and the C(9) methine peak in the ^1H NMR spectrum of **1b** (δ 3.83, DMSO-d_6) was deshielded versus that of **1a**[17] (δ 3.38, DMSO-d_6). Key resonances observed in the ^1H and ^{13}C NMR spectra for the mitosene products are listed in Table I. We have assigned the two new primary adducts **11** and **12** as *trans*- and *cis*-2,7-diaminomitosene-1-sulfonate, respectively, whereas three of the secondary products were identified as 10-(decarbamoyloxy)-2,7-diaminomitosene-10-sulfonate (**13**), *trans*-10-(decarbamoyloxy)-1-hydroxy-2,7-diaminomitosene-10-sulfonate (**14**), and *cis*-10-(decarbamoyloxy)-1-hydroxy-2,7-diaminomitosene-10-sulfonate (**15**). Several trends in the NMR spectral data aided these assignments. First, replacement of either a C(1) hydroxy or a C(10) carbamate moiety by a sulfonate group led to an upfield shift (0.7–1.1 ppm) for the corresponding C(1) methine or C(10) methylene protons, respectively. A comparable shielding phenomenon (9–10 ppm) was observed in the ^{13}C NMR spectra for the C(1) and C(10) resonances. Second, in the trans adducts **11**, **14**, and **16**, the proton–proton coupling interactions observed for the time-average conformation for the constrained mitosene pyrrolidine ring gave rise to relatively small couplings between C(1)H–C(2)H and C(2)H–C(3)H_β and a large coupling between C(2)H–C(3)H_α, whereas in the cis compounds **12** and **15**, large proton–proton coupling constants were typically observed for the C(1)H–C(2)H, C(2)H–C(3)H_α, and C(2)H–C(3)H_β interactions. These patterns agree with previously reported trends.[18] Third, a downfield shift (2.22–9.64 ppm) was observed in the ^{13}C NMR spectra for the C(1), C(2), and C(3) carbon resonances in the trans adducts **11** and **14** versus the cis isomers **12** and **15**.

Conclusive structural identification of the remaining two secondary mitosene products **16** and **17** proved difficult. These compounds eluted at 4.5 and 5.0 min, respectively, in an approximate 4:1 ratio. The early retention times of these peaks suggested that the signals corresponded to disulfonato mitosenes. This contention was supported by the treatment of **11** with $Na_2S_2O_4$. HPLC analysis showed only one mitosene product corresponding to the 4.5-min adduct. In the same way, adding $Na_2S_2O_4$ to aqueous buffered solutions of **12** produced two peaks at 4.5 and 5.0 min in a 4:1 ratio. These findings supported the notion that the major and minor peaks in the $Na_2S_2O_4$-mediated **1a** reactions were *trans*- (**16**) and *cis*-10-(decarbamoyloxy)-2,7-diaminomitosene-1,10-disulfonate (**17**) and that partial isomerization of **17** to **16** occurred during either the reaction or the HPLC analysis. Efforts to isolate purified samples of both isomeric disulfonato adducts **16** and **17** were only partially successful. Treatment of **1a** with 50 equiv of Na_2SO_3 and 0.5 equiv of $Na_2S_2O_4$ led to enhanced yields of **16** and **17** (HPLC analysis). Unfortunately, only **16** was isolated after Sephadex chromatography. FAB mass spectrometry indicated that this compound was a disulfonato adduct, while the ^1H NMR chemical shift and proton–proton coupling data were consistent with the proposed trans stereochemical assignment. The corresponding ^{13}C NMR spectrum did not indicate the structure. The C(1), C(2), and C(3) ^{13}C NMR resonances were between those detected for **11** and **12**.

Identification of the key peaks in the HPLC chromatograms for the incremental $Na_2S_2O_4$ reduction of mitomycin C in H_2O permitted us to quantitatively monitor the $Na_2S_2O_4$-mediated mitomycin C reductive activation processes. Table II records the average percentage yields for the observed products for aliquots removed from the reaction solution at specific time intervals. For the sake of space, the data observed after 30 min (0.6-equiv $Na_2S_2O_4$ addition) and 42 min (0.8-equiv $Na_2S_2O_4$ addition) have not been included here. (These data are contained in Table VII, supplementary material.) For each analysis, we have included the percentage of the drug remaining, the percentage of the total reaction mixture in which modification of **1a** occurred at only the C(1) or at both the C(1) and C(10) sites, the percentage of modified products in which nucleophilic processes had proceeded at the C(1) position, and the percentage of the C(1) nucleophilic products in which nucleophilic sulfonation processes had occurred. Analysis of the product profiles obtained from the aqueous studies revealed the following findings. (1) High amounts of mitomycin C were converted to products in the early stages (i.e., 0.2–0.4 equiv of $Na_2S_2O_4$) of the reduction process. For example, addition of 0.2 equiv of reductant led to the modification of 40.5% of **1a** after 1 min and 50.4% after 6 min. Significantly, in the second half of the reaction (i.e., 0.6–1.0 equiv), the percent consumption of mitomycin C per substoichiometric addition of

(16) Program 2 was not employed for the calculation of the products in the reaction because of the near identical HPLC retention times of **8**, **10**, and **1a**.

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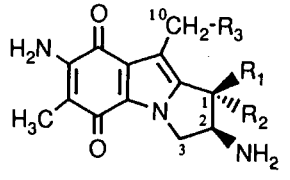
Table I. Selected ^1H and ^{13}C NMR Assignments for Mitosene Derivatives[a]



compd no.	R_1	R_2	R_3	^1H NMR[b] C(1)–H_α	C(1)–H_β	C(2)–H	C(3)–H_α	C(3)–H_β	C(10)–H,H′	^{13}C NMR[c] C(1)	C(2)	C(3)	C(10)
8	H_β	H_α	$OC(O)NH_2$	2.95; d, d;[d] 7.0, 16.2	2.44; d, d; 5.0, 16.2	3.98–4.01; m	4.19; d, d; 6.5, 12.4	3.69; d, d; 5.0, 12.4	4.99; s	33.05	55.39	55.21	57.02
13	H_β	H_α	SO_3^-	3.13; d, d; 7.5, 16.8	2.65; d, d; 4.9, 16.8	4.12–4.14; m	4.29; d, d; 7.0, 12.9	3.85; d, d; 4.6, 12.5	3.82, 3.91; ABq; 13.2	33.05[e]	53.53	53.32	47.25
11	H_β	SO_3^-	$OC(O)NH_2$		3.75; s	4.23; d; 5.6	4.17; d, d; 5.6, 12.2	3.78; d; 12.2	4.99, 5.10; ABq; 11.3	65.48	58.29	54.89	56.25
12	SO_3^-	H_α	$OC(O)NH_2$	3.98; d; 7.1		4.03–4.08; m	4.25; d, d; 7.8, 11.4	3.75; d, d; 9.9, 11.4	4.93, 5.05; ABq; 11.5	58.26	56.07[f]	50.90	56.05[f]
9	H_β	OH	$OC(O)NH_2$		4.59; s	3.65–3.73; m	4.25; d, d; 5.8, 12.5	3.75; d, d; 3.6, 12.5	5.00, 5.05; ABq; 12.2	74.78[d,g]	64.33	54.43	58.77
10	OH	H_α	$OC(O)NH_2$	4.66; d; 5.1		3.75–3.80; m	4.31; d, d; 7.8, 12.0	3.55; d, d; 8.4, 12.0	5.00, 5.05; ABq; 12.2	65.14[d,g]	57.85	51.78	56.67
14	H_β	OH	SO_3^-		4.54; d; 1.6	3.70–3.71; m	4.20; d, d; 5.5, 12.9	4.04; d, d; 2.0, 12.9	3.80, 4.28; ABq; 13.5	71.90	61.24	53.53	46.79
15	OH	H_α	SO_3^-	4.74; d; 5.4		3.86–3.87; m	4.38; d, d; 7.7, 12.3	3.62; d, d; 8.5, 12.3	3.84, 4.25; ABq; 13.4	63.70	56.60	50.90	46.90
16	H_β	SO_3^-	SO_3^-		4.40; s	4.34; d; 6.1	4.29; d, d; 6.1, 13.1	4.04; d; 13.1	4.13, 4.16; ABq; 12.6	61.90	57.20	51.54	46.42
18	H_β	OMe	$OC(O)NH_2$		4.36; s	3.84–3.89; m	4.18; d, d; 5.6, 12.5	3.87; d, d; 6.6, 12.5	5.04; s	81.59	60.48	53.99	56.80
19	OMe	H_α	$OC(O)NH_2$	4.42; d; 4.9		3.78–3.87; m	4.38; d, d; 7.7, 12.0	3.59; d, d; 8.5, 12.0	5.09; s	74.39	58.24	51.63	57.20
22	H_β	OMe	SO_3^-		5.03; s	4.03; br s	4.39; d, d; 5.3, 12.6	4.01; d; 12.6	3.62, 4.40; ABq; 13.2	79.24	58.57	50.57	46.71
23	OMe	H_α	SO_3^-	5.03; d; 5.5		4.12–4.18; m	4.41; d, d; 7.6, 12.3	3.90; d, d; 7.6, 12.3	3.71, 4.47; ABq; 13.3	73.11	57.21	48.45	46.93

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to DMSO-d_6, followed by the multiplicity of the signal and the coupling constant(s) expressed in hertz (Hz). The solvent used was DMSO-d_6. [b] The spectra were obtained at 300 or 500 MHz. [c] The spectra were obtained at 75 or 125 MHz. [d] Reference 11c. [e] The intensity of this peak was weak. [f] The assignment of these two peaks is tentative and may be interchanged. [g] The solvent used was CD_3OD.

Table II. Product Profile Observed for *Incremental Addition* of $Na_2S_2O_4$ to Mitomycin C(**1a**) in Water (Method A)[a]

compd no.	1 min (0.2 equiv)	6 min (0.2 equiv)	18 min (0.4 equiv)	54 min (1.0 equiv)
8	2.3 [5.7]	3.4 [6.8]	6.4 [10.6]	3.7 [5.3]
9 + 10[b]	11.1 [26.6]	19.5 [38.5]	20.3 [34.0]	2.1 [3.3]
11 + 12[b]	1.6 [3.9]	2.5 [5.0]	4.5 [7.3]	0.7 [1.1]
13	1.3 [3.2]	1.2 [2.5]	3.0 [5.0]	15.2 [22.5]
14 + 15[b]	0.2 [0.5]	0.8 [1.5]	4.2 [6.8]	28.9 [43.0]
16 + 17[c]	0.1 [0.3]	0.3 [0.4]	1.3 [2.1]	12.5 [18.2]
1b	23.9 [59.8]	22.7 [45.3]	20.3 [34.2]	4.5 [6.6]
1a	59.5	49.6	40.0	32.4
% C(1) mod[d,e]	90.4 (37.0)	91.7 (50.4)	78.6 (52.0)	10.3 (9.6)
% C(1),C(10) mod[e,f]	9.6 (4.0)	8.3 (4.6)	21.4 (14.2)	89.7 (83.7)
% C(1) nucl[g]	78.3	83.4	76.3	70.0
% C(1) SO_3^- nucl[h]	13.1	12.1	19.1	29.9

[a] Reductive activation of a deaerated aqueous solution (pH 7.4) of **1a** was conducted by the incremental addition of a deaerated aqueous solution (pH 7.4) of $Na_2S_2O_4$ (five incremental additions over 48 min, 0.2 equiv every 12 min) at room temperature. Aliquots were withdrawn from the reaction at the specified time intervals and directly injected into the HPLC. The reactions were run in triplicate and averaged. For each entry, the first value listed corresponds to the percent of the product in the reaction mixture, while the value reported in brackets corresponds to the percent of the product after exclusion of unreacted **1a**. [b] Appreciable quantities of both cis and trans adducts were detected. [c] Significantly greater amounts of **16** versus **17** were detected. [d] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. [e] The value in parentheses included the percent of **1b** in the denominator. [f] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. [g] MC C(1) nucleophilic compounds corresponded to C(1) hydroxy and C(1) sulfonato adducts. Unreacted **1a,b** was not included in this calculation. [h] The percent of MC C(1) sulfonato products over all C(1) nucleophilic products.

$Na_2S_2O_4$ tapered off, leaving substantial amounts (i.e., 32.4%) of **1a** at the conclusion of the reaction. (2) 7-Aminomitosane-9a-sulfonate (**1b**) was the major product generated in the early stages of reaction. The percentage of **1b** in the solution, however, steadily decreased during the course of the reaction. (3) Nucleophilic transformations at C(1) providing the C(1) hydroxy adducts **9**, **10**, **14**, and **15** and the C(1) sulfonato mitosenes **11**, **12**, **16**, and **17** predominated over C(1) electrophilic reactions yielding **8** and **13**. Both cis and trans C(1) hydroxy and C(1) sulfonato derivatives were observed. The relative percentage of C(1) sulfonato versus C(1) hydroxy adducts in the total C(1) nucleophilic pool increased from 13.1% to 29.9% during the reduction process. (4) Only C(10) nucleophilic adducts were detected. In all these cases, carbamate displacement took place with sulfonate substitution.[19] The C(10) sulfonato compounds have been classified as the secondary reaction adducts **13–17**. Plots are provided in Figure 1 of the percentage of the C(1)-modified adducts **8–12** and the C(1),C(10)-modified compounds **13–17** in the reaction mixture as a function of the amount of $Na_2S_2O_4$ added (time). The conversion of the primary products **8–12** to the secondary adducts **13–17** provided support for the sequential nature of the mitomycin modification process. Further evidence in behalf of this contention was obtained by independently treating aqueous, buffered (pH 7.4) solutions containing **8**, **9**, and **10** with $Na_2S_2O_4$. HPLC analysis of these transformations showed only one peak in each case corresponding to **13**, **14**, and **15**, respectively (data not shown).

b. Incremental Addition of $Na_2S_2O_4$ to Methanolic Mitomycin C Solutions. Information concerning the $Na_2S_2O_4$-mediated mitomycin C activation pathway was obtained by a complementary study conducted in buffered ("pH" 7.4) methanolic solutions. The experimental protocol employed in this investigation was

(19) For a recent report of a C(10) sulfonato adduct, see: McGuinness, B. F.; Lipman, R.; Nakanishi, K.; Tomasz, M. *J. Org. Chem.* **1991**, *56*, 4826–4829.

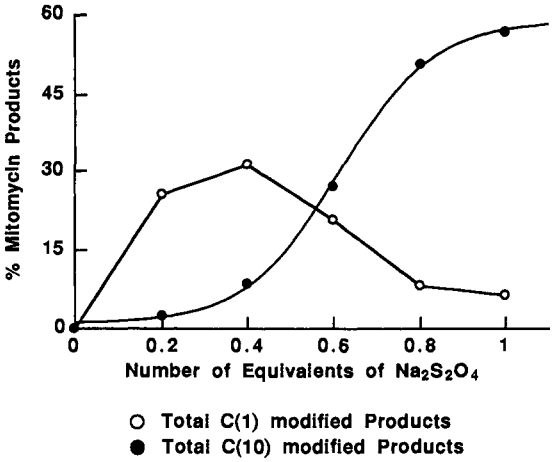
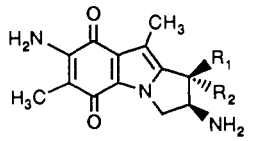


Figure 1. Percentage of primary (**8–12**) and secondary (**13–17**) mitosene products in the reaction mixture for $Na_2S_2O_4$-mediated mitomycin C reductions using method A.

comparable to the one used in the aqueous studies. Aqueous buffered (pH 7.4) solutions of $Na_2S_2O_4$ were used because of the insolubility of the reductant in methanol.[20] Accordingly, the percentage of H_2O by volume in the reaction solution increased from 1% (0.2 equiv of $Na_2S_2O_4$) to 5% (1 equiv of $Na_2S_2O_4$). Use of methanol in place of water simplified the product profile. We attribute the production of trace amounts of mitosene sulfonato adducts to the poor solubility of $Na_2S_2O_4$ and $NaHSO_3$[20] in methanol.

The HPLC chromatograms indicated the presence of the known mitomycin C products 2,7-diaminomitosene (**8**), *trans*- (**18**) and *cis*-1-methoxy-2,7-diaminomitosene (**19**),[11c,21] and *trans*- (**20**) and



20 $R_1 = H$, $R_2 = OCH_3$ **24** $R_1 = H$, $R_2 = OH$
21 $R_1 = OCH_3$, $R_2 = H$ **25** $R_1 = OH$, $R_2 = H$

cis-10-(decarbamoyloxy)-1-methoxy-2,7-diaminomitosene (**21**),[12a,b] along with unreacted **1a** and trace amounts of the sulfonato compounds **1b** and **13–15**. Two unknown peaks were also detected in the HPLC chromatogram at 15.6 and 16.5 min (program 1). The identity of these two unknown compounds was deduced from the treatment of separate aqueous solutions of **18** and **19** with $Na_2S_2O_4$ in the presence of Na_2SO_3 on a semipreparative scale. Under these conditions, **18** furnished the 15.6-min adduct, while **19** yielded a compound that eluted at 16.5 min. Subsequent isolation and spectral characterization (Table I) of the 15.6- and 16.5-min adducts permitted the assignment of these compounds as *trans*- (**22**) and *cis*-10-(decarbamoyloxy)-1-methoxy-2,7-diaminomitosene-10-sulfonate (**23**), respectively.

Table III lists the average percentage yields for the observed HPLC product compositions of aliquots removed from the methanolic reaction solution at select time intervals along with a summary of the extent and type of modification that proceeded at the C(1) and C(10) sites in **1a**. (The full data for each $Na_2S_2O_4$ incremental addition are contained in Table VIII, supplementary material.) Our key findings were as follows: (1) Each incremental addition of $Na_2S_2O_4$ led to appreciable usage of the drug. After the first addition of $Na_2S_2O_4$, 21.5% of mitomycin C was

(20) For information concerning the solubility of $Na_2S_2O_4$, $NaHSO_3$, and Na_2SO_3 in methanol, see: *The Merck Index*, 11th ed.; Merck and Co.: Rahway, NJ, 1989, pp 8582, 8532, 8649.

(21) Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307–311.

Table III. Product Profile Observed for *Incremental Addition* of $Na_2S_2O_4$ to Mitomycin C(**1a**) in Methanol[a]

compd no.	1 min (0.2 equiv)	6 min (0.2 equiv)	18 min (0.4 equiv)	54 min (1.0 equiv)
8	10.6 [50.2]	11.5 [53.1]	22.6 [53.5]	33.1 [45.4]
11 + 12[b]	0.3 [1.4]	0.3 [1.4]	1.2 [3.0]	1.4 [1.6]
13	0.3 [1.7]	0.1 [0.6]	0.3 [0.8]	2.7 [3.7]
14 + 15[b]	0.1 [0.4]	0.1 [0.4]	0.1 [0.1]	2.1 [2.7]
18 + 19[b]	4.4 [22.0]	4.7 [23.0]	8.2 [24.1]	14.1 [21.3]
20 + 21[b]	3.1 [14.0]	3.3 [14.5]	5.4 [12.4]	7.5 [10.4]
22 + 23[b]	1.4 [6.5]	1.5 [7.0]	3.4 [8.0]	9.2 [12.4]
1b	0.7 [3.8]		0.4 [1.1]	1.6 [2.5]
1a	79.1	78.5	58.4	28.3
% C(1) mod[c,d]	75.7 (73.2)	76.7 (76.7)	77.7 (76.9)	69.3 (67.8)
% C(1),C(10) mod[d,e]	24.3 (23.4)	23.3 (23.3)	22.3 (22.1)	30.7 (30.0)
% C(1) nucl[f]	46.0	46.0	44.4	48.9
% C(10) nucl[g]	36.7	34.0	41.3	65.1
% C(1) OMe nucl[h]	95.7	96.0	92.9	89.8

[a] Reductive activation of a deaerated, methanolic solution ("pH" 7.4) of **1a** was conducted by the incremental addition of a deaerated, aqueous solution (pH 7.4) of $Na_2S_2O_4$ (five incremental additions over 48 min, 0.2 equiv every 12 min) at room temperature. Aliquots were withdrawn from the solution at the specified time intervals and directly injected into the HPLC. The reactions were run in duplicate and averaged. For each entry, the first value listed corresponds to the percentage yield of the product in the reaction mixture, while the value reported in brackets corresponds to the percentage yield of product after exclusion of unreacted **1a**. [b] Appreciable quantities of both cis and trans adducts were detected. [c] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. [d] The value in parentheses included the percent of **1b** in the denominator. [e] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. [f] MC C(1) nucleophilic compounds corresponded to C(1) methoxy, sulfonato, or hydroxy adducts. Unreacted **1a,b** was not included in this calculation. [g] MC C(10) nucleophilic compounds corresponded to C(10) sulfonato adducts. Unreacted **1a,b** and only C(1)-modified MC compounds were not included in this calculation. [h] Percent of MC C(1) OMe products over all C(1) nucleophilic products.

consumed, whereas the next three additions of reductant consumed 20.1%, 11.6%, and 12.6% of **1a**. Only in the last addition of $Na_2S_2O_4$ did we observe a noticeable decline (5.8%) in **1a** activation. This pattern differed from the aqueous studies where mitomycin C consumption appreciably declined after the second incremental addition of $Na_2S_2O_4$. (2) The ratio of C(1) nucleophilic versus electrophilic adducts was nearly 1. This value was lower than that observed in the aqueous studies in which nucleophilic products dominated by approximately 3:1. Among the C(1) nucleophilic products, the C(1) methoxy mitosene derivatives accounted for the vast majority of the adducts generated (95.7% after 0.2 equiv of $Na_2S_2O_4$, 89.8% after 1.0 equiv of $Na_2S_2O_4$). (3) The C(10) electrophilic mitosene adducts **20** and **21** were observed. The corresponding products **24** and **25**[22] were not detected in the aqueous studies. The ratio of C(10) electrophilic versus C(10) nucleophilic adducts decreased during the course of the reaction from 2 to 0.5. Finally, only C(10) sulfonato nucleophilic products were generated. (4) Appreciable quantities of C(1), C(10) difunctionalized compounds in the total *product* mixture were observed in the early stages of the reaction (0.2 equiv $Na_2S_2O_4$, 23.4%), and this percentage remained constant throughout the reaction. This pattern differed from the product profiles observed in the aqueous study where significant amounts of C(1) and C(10) difunctionalized adducts were detected only in the latter stages of the reaction (Table II, Figure 1).

c. Single Shot Addition of $Na_2S_2O_4$ to Aqueous Mitomycin C Solutions (Method B). Previous studies have demonstrated that the incremental addition of $Na_2S_2O_4$ to mitomycin C–DNA solutions led to higher drug–DNA bonding ratios than a comparable procedure when an equivalent amount of reductant was added in a single step.[7] In order to provide information concerning the factors responsible for this finding, we have determined the percentages of the various products generated by the single shot $Na_2S_2O_4$ addition protocol in the absence of DNA as the molar equivalents of $Na_2S_2O_4$ were successively increased. The procedure used in this series of experiments was similar to method A developed for the incremental addition of $Na_2S_2O_4$ to aqueous solutions of mitomycin C (Table II). Deaerated, aqueous buffered (pH 7.4) solutions of **1a** were treated with deaerated, aqueous buffered (pH 7.4) solutions of $Na_2S_2O_4$ that contained 0.2, 0.4, 0.6, 0.8, and 1.0 equiv of $Na_2S_2O_4$. Using method B, the $Na_2S_2O_4$ was added to the **1a** solution in a *single* shot. To facilitate our comparison with the results obtained with method A, each reaction was monitored by HPLC at the same time point used in method A when an equivalent amount of $Na_2S_2O_4$ was added. For example, for 0.4 equiv of $Na_2S_2O_4$, the HPLC aliquot was removed after 18 min. This time matched that recorded in Table II for the second 0.2-equiv incremental addition of $Na_2S_2O_4$. Correspondingly, the times of HPLC analysis for single shot addition of 0.6, 0.8, and 1.0 equiv of $Na_2S_2O_4$ were 30, 42, and 54 min, respectively.

Table IV. Product Profile Observed for *Single Shot Addition* of $Na_2S_2O_4$ to Mitomycin C(**1a**) in Water (Method B)[a]

compd no.	6 min (0.2 equiv)	18 min (0.4 equiv)	54 min (1.0 equiv)
8	3.4 [6.8]	6.6 [8.2]	12.5 [15.9]
9 + 10[b]	19.5 [38.5]	27.9 [34.7]	
11 + 12[b]	2.5 [5.0]	11.4 [14.3]	1.3 [1.6]
13	1.2 [2.5]	6.9 [8.6]	30.9 [39.3]
14 + 15[b]	0.8 [1.5]	7.6 [9.4]	12.6 [16.0]
16 + 17[c]	0.3 [0.4]	3.0 [3.6]	14.6 [15.4]
1b	22.7 [45.3]	17.1 [21.2]	9.3 [11.8]
1a	49.6	19.5	18.8
% C(1) mod[d,e]	91.7 (50.4)	72.4 (57.0)	19.2 (17.0)
% C(1),C(10) mod[e,f]	8.3 (4.6)	27.6 (21.7)	80.8 (71.6)
% C(1) nucl[g]	83.4	78.7	39.6
% C(1) SO_3^- nucl[h]	12.1	28.9	55.8

[a] Reductive activation of a deaerated aqueous buffered solution (pH 7.4) of **1a** was conducted by the addition of a deaerated aqueous buffered solution (pH 7.4) of $Na_2S_2O_4$ in a single shot at room temperature. Aliquots were withdrawn from the reaction at the specified time intervals and directly injected into the HPLC. The reactions were run in duplicate and averaged. For each entry, the first value listed corresponds to the percentage yield of the product in the reaction mixture, while the value reported in brackets corresponds to the percent of the product after exclusion of unreacted **1a**. [b] Appreciable quantities of both cis and trans adducts were detected. [c] Significantly greater amounts of **16** versus **17** were detected. [d] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. [e] The value in parentheses included the percent of **1b** in the denominator. [f] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. [g] MC C(1) nucleophilic compounds corresponded to C(1) hydroxy and C(1) sulfonato adducts. Unreacted **1a,b** was not included in this calculation. [h] The percent of MC C(1) sulfonato products over all C(1) nucleophilic products.

The HPLC peak patterns observed for both $Na_2S_2O_4$ addition protocols were similar. The same mitomycin C products (i.e., **1b** and **8–17**) detected with method A were observed with method B. Furthermore, the HPLC chromatograms after the addition of 0.2 and 0.4 equiv of $Na_2S_2O_4$ using method B revealed the predominance of primary products, **1b** and **8–12**, while the secondary products, **13–17**, were the major adducts after 0.6–1.0 equiv of $Na_2S_2O_4$ had been added.

The average percentage yields for the observed products as a function of the number of equivalents of $Na_2S_2O_4$ added are compiled in Table IV along with a summary of the reaction processes that occurred at both C(1) and C(10) in **1a**. (The full listing of the results for each incremental $Na_2S_2O_4$ addition is given in Table IX, supplementary material.) Analysis of Table IV revealed the following trends: (1) There were high levels of drug activation (80.5%) after 0.4 equiv of $Na_2S_2O_4$ was added.

(22) Zein, N.; Kohn, H. *J. Am. Chem. Soc.* **1986**, *108*, 296–297.

Subsequent addition of $Na_2S_2O_4$ did not lead to further appreciable drug consumption. This curtailment in mitomycin C consumption was similar to that detected with method A. (2) The percentage of **1b** in solution decreased as the amount of $Na_2S_2O_4$ used increased. (3) The proportion of C(1),C(10)-modified products versus C(1)-monofunctionalized adducts regularly increased as the amount of $Na_2S_2O_4$ was increased from 0.2 equiv (8.3%) to 1.0 equiv (80.8%). A comparable pattern was observed for the incremental addition of $Na_2S_2O_4$ protocol (Table II, Figure 1). (4) Nucleophilic transformations predominated over electrophilic reactions at the C(1) position when 0.2–0.6 equiv of $Na_2S_2O_4$ was added in a single shot (% C(1) nucl = 83.4–77.0% for 0.2–0.6 equiv), but this balance in favor of C(1) nucleophilic adducts markedly decreased when 0.8 and 1.0 equiv of $Na_2S_2O_4$ (% C(1) nucl = 55.6% for 0.8 equiv; 39.6% for 1.0 equiv) was added. These latter values were substantially lower than those detected with method A. (5) Both cis and trans C(1) hydroxy and C(1) sulfonato mitosene derivatives were observed. Significantly, the relative percentage of C(1) hydroxy versus C(1) sulfonato adducts depended on how much $Na_2S_2O_4$ was added. The percentage of C(1) sulfonato derivatives in the total C(1) nucleophilic pool was 55.8% when 1.0 equiv of $Na_2S_2O_4$ was added and only 11.6% when 0.2 equiv of the reductant had been added. Moreover, we noted that C(1) nucleophilic sulfonation adducts represented a greater fraction of the C(1) nucleophilic product pool in the single shot method than in the incremental addition method. For example, after the addition of 1 equiv of $Na_2S_2O_4$, this percentage was 55.8% with method B (Table IV) and 29.9% with method A (Table II). (6) Only C(10) mitosene-modified products were observed in which nucleophilic sulfonation processes had occurred.

d. Reactivity of Mitomycin C Sulfonato Compounds. Both the aqueous incremental addition and the single shot $Na_2S_2O_4$ protocols furnished high amounts of C(1), C(9a), and C(10) mitomycin sulfonato products. This finding prompted us to determine whether these compounds or their reduced analogues could serve as intermediates in mitomycin C transformations.

1. 7-Aminomitosane-9a-sulfonate (1b). Chief among the observed mitomycin sulfonato adducts was 7-aminomitosane-9a-sulfonate. Compound **1b** accounted for 45.3% of the total *product* mixture during the early stages of the $Na_2S_2O_4$-mediated **1a** reduction (Tables II and IV). Several experiments were performed to analyze the reactivity of **1a,b**. Compound **1b** was first treated with substoichiometric amounts of $Na_2S_2O_4$ using the incremental addition protocol. The HPLC profiles observed for this transformation were similar to those obtained for **1a**. Accordingly, the primary products **8–12** were the principal adducts generated in the early stages of the reaction (0.2–0.4 equiv of $Na_2S_2O_4$), whereas the secondary products **13–16** constituted the major component of the reaction mixture after the addition of 0.6–1.0 equiv of $Na_2S_2O_4$. No peak corresponding to **17** was detected.

The average percentage yields for the observed products after select incremental additions of $Na_2S_2O_4$, along with the tabulation summarizing the extent and type of modification that proceeded at the C(1) and C(10) sites in **1b**, are listed in Table V. (Table X in the supplementary material provides the data for all the incremental $Na_2S_2O_4$ additions.) Analysis of this compilation for **1b** along with the corresponding data for **1a** (Table II) provided the following information. (1) Appreciable amounts of **1b** were converted to products with the first two incremental additions of $Na_2S_2O_4$ (~32%/addition) and then diminished for the last three additions of the reductant (~8%/addition). (2) At the conclusion of the reaction, **1b** was more efficiently converted (87.9%) to products than **1a** (67.6%). (3) The amount of C(1),C(10)-modified adducts constituted 18.4% of the product mixture after 1 min of reaction. This value increased to 92.3% at the end of the reaction. We have attributed the higher levels of C(1),C(10)-modified products observed in the early stages of the **1b** reaction versus the **1a** transformation to the increased amounts of HSO_3^- present in solution. (4) The proportion of C(1) nucleophilic to C(1) electrophilic products was approximately 1.9:1. Moreover, both C(1) hydroxy and sulfonato adducts were detected. (5) Modification of the C(10) site furnished only nucleophilic sulfonation products.

Table V. Product Profile Observed for *Incremental Addition* of $Na_2S_2O_4$ to Ammonium 7-Aminomitosane-9a-sulfonate (**1b**, $M^+ = NH_4^+$) in Water[a]

compd no.	1 min (0.2 equiv)	6 min (0.2 equiv)	18 min (0.4 equiv)	54 min (1.0 equiv)
8	4.9 [21.5]	6.7 [19.4]	8.3 [13.2]	3.8 [4.4]
9 + 10[b]	11.1 [48.1]	16.7 [48.3]	23.8 [37.4]	0.5 [0.7]
11 + 12[b]	3.0 [13.4]	5.1 [14.8]	12.2 [19.6]	2.5 [3.0]
13	2.5 [10.1]	3.8 [10.4]	9.4 [14.3]	26.9 [29.1]
14 + 15[b]	1.0 [3.6]	1.8 [5.0]	7.4 [11.4]	33.4 [38.3]
16	0.8 [3.4]	0.8 [2.2]	2.6 [4.2]	20.8 [24.5]
1b	76.7	65.1	36.3	12.1
% C(1) mod[c]	81.6	81.4	69.5	7.7
% C(1),C(10) mod[d]	18.4	18.6	30.5	92.3
% C(1) nucl[e]	68.2	69.9	72.2	65.1
% C(1) SO_3^- nucl[f]	23.9	24.2	32.2	40.7

[a] Reductive activation of a deaerated aqueous solution (pH 7.4) of **1b** (3 mM) was conducted by the incremental addition of a deaerated aqueous solution (pH 7.4) of $Na_2S_2O_4$ (five incremental additions over 48 min, 0.2 equiv every 12 min) at room temperature. Aliquots were withdrawn from the reaction at the specified time intervals and directly injected into the HPLC. The reactions were run in duplicate and averaged. For each entry, the first value listed corresponds to the percent of the product in the reaction mixture, while the value reported in brackets corresponds to the percent of the product after exclusion of unreacted **1b**. [b] Appreciable quantities of both cis and trans adducts were detected. [c] The percent of MC compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. [d] The percent of MC compounds modified at both C(1) and C(10) was computed by (% C(1),C(10) mod/(% C(1),C(10) mod + % C(1) mod)) × 100. [e] MC C(1) nucleophilic compounds corresponds to C(1) hydroxy and C(1) sulfonato adducts. Unreacted **1b** was not included in this calculation. [f] The percent of MC C(1) sulfonato products over all C(1) nucleophilic products.

Use of other reductive methods to activate **1a,b** provided additional information concerning the roles of both $Na_2S_2O_4$ and HSO_3^- in $Na_2S_2O_4$-mediated mitomycin C transformations. Catalytic reduction (PtO_2/H_2, 5 min) of deaerated, aqueous buffered (pH 7.0) solutions of **1b** afforded mitosenes **8–16** as the major products. HPLC analysis of the reaction mixture after 3 min indicated that the primary products **8–12** were the major adducts, whereas the secondary products **13–16** were the predominant compounds observed after 5 min had elapsed. Comparable product profiles with time were noted for the catalytic reduction (PtO_2/H_2) of a deaerated, buffered solution (pH 7.0) containing **1a** and 1 equiv of K_2SO_3. Enzymatic reduction of **1b** using xanthine oxidase (1.25 units) and NADH[23] furnished **8–15** and unreacted **1b** (32.5%).

Comparison of the product profiles for the $Na_2S_2O_4$-mediated reduction of **1a** (Table II) and **1b** (Table V), the catalytic reduction of **1a**[9] and **1b**, and the enzymatic reduction of **1a**[10] and **1b** indicated that (1) the $Na_2S_2O_4$ protocol was the most efficient procedure of the three methods for mitomycin activation, (2) the reactions appeared to proceed through common intermediates independent of the reductive method, and (3) HSO_3^- played a significant role in the product-determining steps but not in the initial activation of the mitomycin.

The higher level of **1b** versus **1a** consumption detected with method A (Tables II and V) was consistent with our observation that mitomycin C utilization tapered off after the addition of 0.4

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Table VI. pH Dependence on the Competitive $Na_2S_2O_4$ Reduction of **1a**,b[a]

entry no.	pH	trapping agent	% **1b** mod	% **1a** mod	% **1b** mod/ % **1a** mod
1	5.5	*b*	9.8	13.4	0.7
2	5.5	formaldehyde (10 equiv)	8.2	9.9	0.8
3	5.5	formaldehyde (20 equiv)	6.7	12.8	0.5
4	7.4	*b*	12.1	7.0	1.7
5	7.4	formaldehyde (10 equiv)	25.0	11.0	2.3
6	7.4	formaldehyde (20 equiv)	21.8	2.0	10.9
7	7.4	Eschenmoser's salt (20 equiv)	28.2	1.8	15.7
8	8.5	*b*	46.4	7.9	5.9
9	8.5	formaldehyde (10 equiv)	42.0	5.5	7.6
10	8.5	formaldehyde (20 equiv)	65.6	6.6	9.9

[a] Reductive activation of a deaerated (Ar), aqueous buffered (pH 7.4) solution containing equimolar amounts (0.3 mM) of mitomycin C (**1a**) and ammonium 7-aminomitosane-9a-sulfonate (**1b**, $M^+ = NH_4^+$) was conducted by the addition of a deaerated (Ar), aqueous buffered (pH 7.4) solution (10 μL) of $Na_2S_2O_4$ (0.1 equiv) at room temperature. The reaction was allowed to proceed at room temperature (1 min) and then analyzed by HPLC. The reactions were run in duplicate and averaged. [b] No trapping agent was used.

equiv of $Na_2S_2O_4$ to **1a**, while the **1b** generated from **1a** in this transformation steadily diminished with each successive addition of reductant (Table II). These findings led us to determine the differential reactivity of **1a**,**b** under reductive conditions. Treatment of a deaerated, aqueous buffered solution (pH 7.4) containing equimolar amounts (3 mM) of **1a** and **1b** with limiting amounts of $Na_2S_2O_4$ (0.1 equiv) led to the apparent higher consumption of **1a** versus **1b** (i.e., **1a** consumed = 24.0%, **1b** consumed = 5.2%) (data not shown). Unfortunately, no conclusions concerning the differential reactivity of **1a** versus **1b** could be derived from this experiment, since our previous studies (Table II) suggested that the HSO_3^- liberated in the **1b** reductive process should have yielded additional **1b** from **1a** through **7**. Accordingly, several experimental modifications were implemented to minimize the conversion of **1a** to **1b**. A 10-fold dilution of the reaction led to a reversal in the **1b**:**1a** consumption from 0.2 to 1.7 (Table VI, entry 4). Further increases in the reactivity of **1b** versus **1a** were observed after the inclusion of the HSO_3^--trapping agents,[24] formaldehyde and *N*,*N*-dimethylmethyleneammonium iodide (Eschenmoser's salt), in the reaction (Table VI, entries 5–7). Use of Eschenmoser's salt (20 equiv) provided a **1b**:**1a** consumption ratio of 15.7 to 1. Significantly, reductive activation of **1a** alone with $Na_2S_2O_4$ (0.2 equiv) in the presence of Eschenmoser's salt (20 equiv) gave only **8**–**10** (HPLC analysis). No detectable amounts of the sulfonato adducts **1b** and **11**–**17** were observed.

The effect of pH on the differential reactivity of **1a** versus **1b** in the presence of the HSO_3^--trapping agent, formaldehyde, was also determined. Comparable values were observed for the percentage of **1b** versus **1a** modification at pH 8.5 and 7.4 (**1b**:**1a** consumption = 9.9–10.9; Table VI, entries 6 and 10). At pH 5.5, a reversal in the reactivity of **1b** versus **1a** was observed (**1b**:**1a** consumption = 0.5; Table VI, entry 3). We have tentatively attributed this change to an increase in the reactivity of **1a** versus **1b** due to the need for acid for the expulsion of the C(9a) OCH_3 group in **2a** in proceeding to leucoaziridinomitosene **3**.[25]

2. Mitosene C(1) and C(10) Sulfonato Adducts. Experiments were conducted to determine whether C(1) or C(10) sulfonato adducts could alkylate nucleophiles present in the reaction. Select mitosene sulfonato compounds were treated with nucleophiles (H_2O, ROH, RNH_2, and RSH) under reductive conditions ($Na_2S_2O_4$, 1 equiv; PtO_2/H_2, 3 min). Compounds **11** and **12** were chosen as representative examples of mitosene C(1) sulfonato compounds and **16** and **22** as mitosene C(10) sulfonato adducts. The reactions were conducted at three different "pH" ranges ("pH" 5.5, 7.0–7.4, and 8.2–8.5) in both water and methanol, and an excess (10 equiv) of the added nucleophile was used. At pH 5.5, aniline was employed; at pH 7.0–7.4, both aniline and thiophenol were used; and at pH 8.2–8.5, thiophenol was employed. HPLC analyses of these experiments showed no displacement of the sulfonate moiety, demonstrating that these substrates and their reduced counterparts did not function as alkylating agents.

Discussion

The results obtained from the $Na_2S_2O_4$-mediated mitomycin C product profiles in water and methanol (Tables II–IV), along with the results of the reactivity of C(1), C(9a), and C(10) mitomycin-based sulfonates (Tables V and VI), have permitted us to propose the likely pathways for these transformations (Schemes II and III) and to suggest the causative factors for the enhanced DNA–drug bonding ratios observed after the incremental addition of $Na_2S_2O_4$ to aqueous solutions containing DNA and **1a** compared with conventional reductants.[2a,3a,4a,6] We propose that, at near neutral pH values, reduction of **1a** by $Na_2S_2O_4$ proceeds rapidly to give leucomitomycin C (**2a**) (Scheme II). Subsequent expulsion of the C(9a) OCH_3 group, then, by an acid-catalyzed process yields iminium ion **7**.[25] Species **7** can either react with HSO_3^- ($S_2O_4^{2-}$ [26]) in a reversible process to give **2b**[27] or undergo loss of the C(9) methine proton to furnish leucoaziridinomitosene **3**. Two different product-determining steps that are dependent upon pH and the amount of reductant present in solution proceed from **3**. At pH 7.4 and with limiting amounts of $Na_2S_2O_4$ (0.2 equiv) reoxidation of **3** by the excess **1a** present can occur to give **26** and **2a** along with aziridine ring opening of **3** to yield quinone methide **4**. The proposed redox reaction suggests that, overall, mitomycin C consumption proceeded in part by autocatalytic transformations. Similar processes have been documented.[10c,28] Quinone methide **4**, once generated, then furnishes **8** by proton transfer and **27** by nucleophilic substitution.[9a,c,14,29] Correspondingly, **26** yields C(1) nucleophilic products by a S_N1-type pathway. We have recently provided information on the facility of the latter substitution process at near neutral pH values.[30] Additional evidence in support of this duality of pathways from **3** (**3** + **1a** ⇌ **26** + **2a**, **3** → **4**) is provided in the accompanying paper.[31] Conversion of the primary products **28** (**8** and **27**) to the secondary adducts **30** proceeds by quinone reduction[11c,12,32] and then S_N2 displacement of the C(10) carbamate group by HSO_3^- ($S_2O_4^{2-}$ [26]) present in solution (Scheme III).

The experimental data did not provide information on whether C(1) and C(10) modification processes in mitomycin C occurred at the one- or two-electron reduction level.[9b,11b,11c,28] The reaction pathway in Scheme II for the aqueous studies has been drawn at the hydroquinone stage on the basis of the pulse radiolysis studies of the Swallow[28a] and Hickel[28b] groups. These investigators demonstrated that reduction of **1a** in water proceeded by a one-electron route to give the corresponding semiquinone species, but that subsequent loss of methanol at C(9) and C(9a) and opening of the aziridine ring did not proceed until dismutation occurred to give leucomitomycin C (**2a**). Our results, however, do support

(24) Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 1228–1235.

(25) McClelland, R. A.; Lam, K. *J. Am. Chem. Soc.* **1985**, *107*, 5182–5186.

(26) Our results do not permit us to distinguish whether HSO_3^- and/or $S_2O_4^{2-}$ served as the attacking nucleophile. $S_2O_4^{2-}$ addition followed by oxidation is expected to lead to the observed products. For a related discussion, see: Gaudiano, G.; Frigerio, M.; Sangsurasak, C.; Bravo, P.; Koch, T. H. *J. Am. Chem. Soc.* **1992**, *114*, 5546–5553.

(27) The $E_{1/2}$ values as determined by cyclic voltammetry (scan rate = 100 mV/s) for the reversible one-electron reduction of **1a** and **1b** in dimethyl sulfoxide were comparable: **1a**, $E_{1/2} = -0.89$ V; **1b**, $E_{1/2} = -0.99$ V (Drs. K. Kadish and D. Dubois, University of Houston, unpublished results).

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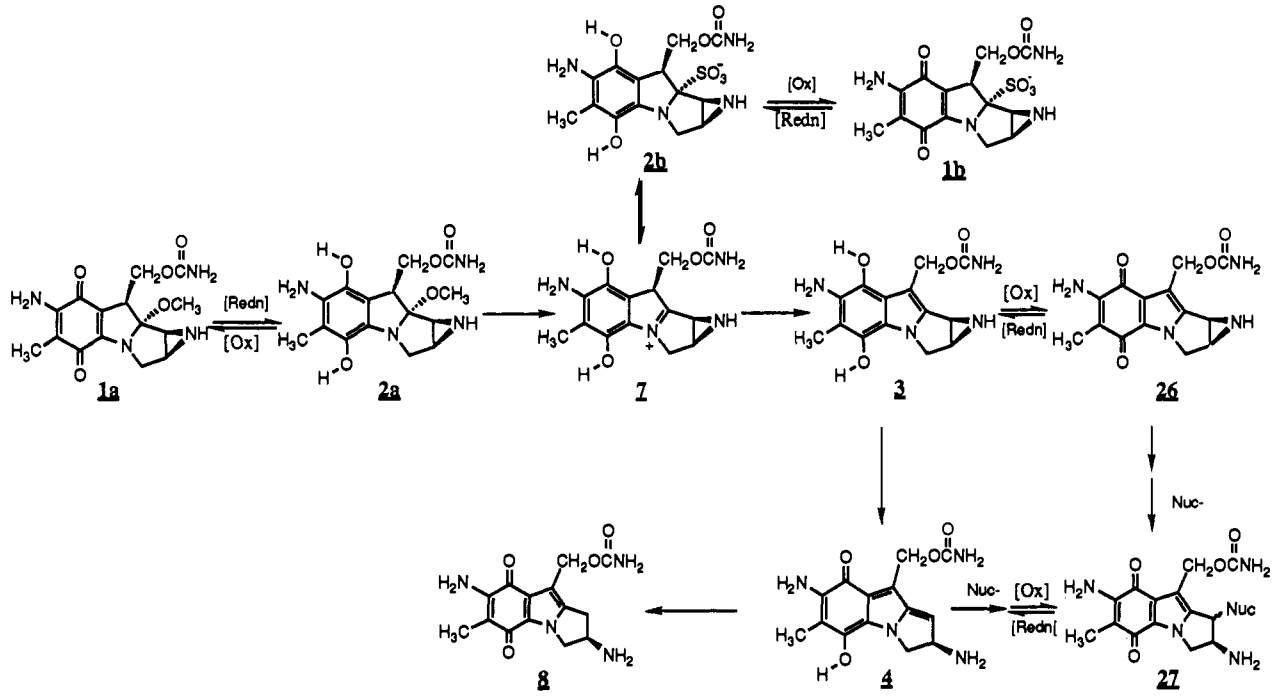
(29) Schiltz, P.; Kohn, H. *J. Am. Chem. Soc.* **1992**, *114*, 7958–7959.

(30) Han, I.; Kohn, H. *J. Org. Chem.* **1991**, *56*, 4648–4653.

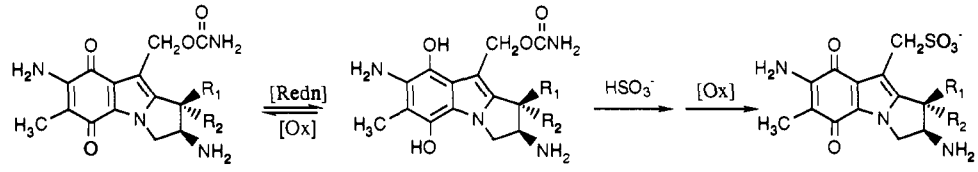
(31) Schiltz, P.; Kohn, H., the following paper in this issue.

(32) Egbertson, M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 2204–2205.

Scheme II. Proposed Pathway for the Generation of the Primary Products in the $Na_2S_2O_4$-Mediated Mitomycin C Transformations



Scheme III. Proposed Pathway for the Generation of the Secondary Products in the $Na_2S_2O_4$-Mediated Mitomycin C Transformations



earlier reports that electron-transfer reactions are an important component of the mitomycin reductive activation process.[10c,28] For example, we noted that **1a** utilization increased from 40.5% (after 1 min) to 50.4% (after 6 min) upon addition of 0.2 equiv of $Na_2S_2O_4$ (Table II). These findings suggested that complete consumption of **1a** should have been observed after substoichiometric additions of $Na_2S_2O_4$, since only the production of **8** and **13** led to the net utilization of electrons. We suspect that HSO_3^--induced decomposition of $Na_2S_2O_4$[33] accounted in part for the observed decreased consumption of mitomycin C under these conditions. Mitomycin C autocatalytic reductive processes were not apparent in the buffered methanolic studies. Unlike in water, little change in the product profile was noted with time after the addition of 0.2 equiv of $Na_2S_2O_4$ (1 min, 20.9%; 6 min, 21.5%) (Table III). Moreover, we noted a higher proportion of C(1),C(10) mitomycin-modified products in the methanolic studies in the early stages of the reaction (0.2 equiv, 24.3%) (Table III) than in water (0.2 equiv, 9.6%) (Table II), and this value remained fairly constant throughout the reaction (1.0 equiv, 30.7%) (Table III). We have attributed this decrease in the autocatalytic process in part to the higher yields of C(1) electrophilic products observed. Generation of **8** and **13** led to the net consumption of electrons, thereby quenching subsequent autocatalytic transformations.

Several additional observations provided support for the individual steps drawn in Schemes II and III.

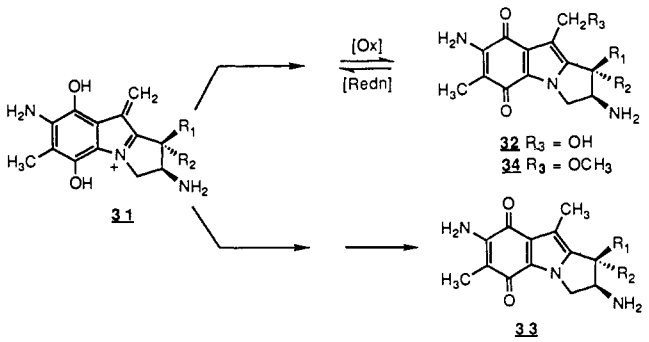
First, the detection of substantial amounts of 7-aminomitosane-9a-sulfonate (**1b**) in the aqueous studies indicated that reductive activation of mitomycin C proceeded through iminium ion **7**. This finding is in agreement with the earlier report of Hornemann and co-workers.[8] Moreover, our demonstration that **1b** is more efficiently converted to mitosene products than **1a** (Table VI) at near neutral pH values provided evidence that **2b** can serve as a viable substrate for generating mitomycin C–DNA products in $Na_2S_2O_4$-mediated transformations.[27] The increased reactivity of **1b** (**2b**) over **1a** (**2a**) has been attributed to the apparent need for acid for the removal of the C(9a) OCH_3 group in **2a** and the enhanced leaving group ability of the sulfonate group over methoxide ion. Consistent with this notion, the relative reactivity of **2a** (**1a**) versus **2b** (**1b**) increased over 20-fold in proceeding from pH 7.4 to 5.5 (Table VI). Significantly, McClelland and co-workers have previously shown that, in the solvolysis of **1a**, expulsion of the C(9a) OCH_3 group proceeded by a general acid-catalyzed pathway.[25] A similar process may be operative under reductive conditions.

Secondly, we detected appreciable amounts of both cis and trans C(1) hydroxy and sulfonato adducts in water (Tables II and IV) and C(1) methoxy products in methanol (Table III), which indicated that C(1) functionalization occurred by a S_N1-type pathway. We suspect, at pH 7.4, these C(1) nucleophilic processes occur at both the quinone[29] and hydroquinone (semiquinone) reduction levels. The detection of appreciable amounts of **8** and **13** provided evidence that the reaction funnels in part through **4**.

Finally, we observed that activation of the C(10) site in mitomycin C occurred only after quinone ring reduction. Modification of this site proceeded by one of two pathways that

(33) Burlamacchi, L.; Guarini, G.; Tiezzi, E. *Trans. Faraday Soc.* **1969**, *65*, 496–502.

depended upon the solvent and the nucleophilic composition of the medium. In water at near neutral pH values, the reaction proceeded by S_N2 displacement of the carbamate group by HSO_3^- ($S_2O_4^{2-}$ [26]) in the reductively activated mitosene. No evidence was obtained for the competing substitution pathway through iminium ion **31**.[22] We suspect that generation of **31** would have



led to the formation of **32** and **33**. In the methanol-based studies, appreciable amounts of the C(10) methyl adducts **20** and **21** were observed, consistent with the prior generation of iminium **31**. The percentage of these C(10)-modified adducts in the C(10) product profile decreased from 63.3% to 34.9%, whereas the percentage of C(10) sulfonato adducts (**22** and **23**) rose from 36.7% to 65.1% as the amount of $Na_2S_2O_4$ added increased from 0.2 to 1.0 equiv (Table III). The observed shift in product distribution has been attributed to a change in the predominant pathway for C(10) modification from a S_N1-type process through iminium ion **31** to a S_N2 transformation and correlated with the increased H_2O and HSO_3^- ($S_2O_4^{2-}$ [26]) concentrations in solution. The detection of significant amounts of C(10) methyl adducts in the methanol studies was consistent with earlier findings. We reported that catalytic reduction of **9** and **10** in methanol furnished high yields of **24** and **25**, respectively.[22] Finally, we observed no displacement of the C(10) carbamate group in **8–11** in the presence of excess $NaHSO_3$ and the absence of reductant (data not shown), demonstrating the need for prior reduction of the mitosene quinone ring for activation of the C(10) site.

Comparison of the product profiles obtained from the incremental and single shot $Na_2S_2O_4$ addition protocols provided information on why $Na_2S_2O_4$ has emerged as the reducing agent of choice for the modification of DNA by mitomycin C and the causative factors that contributed to the high DNA-bonding ratios observed upon incremental addition of this reductant. First, $Na_2S_2O_4$-induced activation of mitomycin C was rapid.[34] Extensive drug modification using either method A or B was detected within the first 1 min of reaction, although appreciable amounts (18.8–32.4%) of **1a** remained unreacted after the addition of 1 equiv of $Na_2S_2O_4$ with both methods A and B at pH 7.4. Second, the percentage of C(1) nucleophilic products in the total C(1)-modified mitosene pool markedly decreased as the amount of $Na_2S_2O_4$ added in a single shot was increased (Table IV; 0.2 equiv, 83.4%; 1.0 equiv, 39.6%). By comparison, only a slight decline was noted using the incremental addition method (Table II; 0.2 equiv, 83.4%; 1.0 equiv, 70.0%). Third, addition of a full equivalent of $Na_2S_2O_4$ in a single shot led to a higher percentage of C(1) sulfonato adducts in the C(1) mitosene nucleophilic pool (Table IV, 55.8%) than after the incremental addition of 1 equiv of $Na_2S_2O_4$ (Table II, 29.9%). Fourth, lower amounts of C(1)-monofunctionalized (% C(1) mod) and higher levels of C(1), C(10) difunctionalized (% C(1),C(10) mod) mitosene adducts were observed with method A (Table II) than with method B (Table IV) using comparable amounts of $Na_2S_2O_4$ (0.2–0.6 equiv).

(34) For discussions on the $Na_2S_2O_4$ reductive process, see ref 26, 33, and Lambeth, D. O.; Palmer, G. *J. Biol. Chem.* **1973**, *248*, 6095–6103 and references therein.

Conclusions

We found that the incremental addition protocol provided both higher amounts of C(1) nucleophilic products and lower levels of C(1) sulfonato adducts with 1 equiv of $Na_2S_2O_4$ than the corresponding single shot method. This finding suggested that the use of the former technique in the *presence* of *DNA* should promote the bonding of the activated mitomycin C species to DNA rather than the reaction with solvent and $NaHSO_3$.[7] These studies also indicated that, during the initial stages of the incremental addition protocol, autocatalytic processes occurred (**2b + 1a ⇌ 1b + 2a, 1a + 3 ⇌ 2a + 26**). We expected these transformations to decrease the percentage of C(1) and C(10) sulfonato products generated in the reaction, thereby leading to an increase in the yield of DNA–mitosene adducts. The observed product profiles further indicated that mitomycin C utilization and production of C(1)-monosubstituted mitosene adducts in DNA-bonding studies were not likely to increase appreciably after the first two substoichiometric (0.2 equiv) additions of $Na_2S_2O_4$, while the introduction of further amounts of $Na_2S_2O_4$ should promote the generation of C(1) and C(10) DNA-difunctionalized mitosenes (DNA–**1a** cross-linked adducts).

The analyses of these transformations prompted us to alert researchers that initial C(1) bonding of mitomycin C to DNA in $Na_2S_2O_4$-mediated processes and related reactions may proceed in part through 7-aminoaziridinomitosene (**26**).[29,30] We have previously shown that this species preferentially bonded DNA at 5′CG sequences and that the bonding patterns were virtually identical to those observed for mitomycin C under reductive conditions.[4a] Subsequent reduction of the quinone group in the C(1)-modified mitosene–DNA adduct by $Na_2S_2O_4$ would permit functionalization of the C(10) site and the generation of the drug–DNA interstrand cross-linked adduct.

Experimental Section

Instrumentation, Materials, and Solvents. Proton (^1H NMR) and carbon-13 (^{13}C NMR) nuclear magnetic resonance spectra were recorded on either a Nicolet NT-300, a General Electric QE-300, or a Bruker AMX-500 MHz NMR spectrometer. The 500-MHz spectra were recorded at Rice University by Dr. Garry King. Chemical shifts are expressed in parts per million relative to the solvent employed, and coupling constants (*J* values) are given in hertz (Hz). Proton–proton connectivities were confirmed by correlated spectroscopy (COSY). Mass spectrometric (MS) analyses were performed at the Baylor College of Medicine by Drs. S. J. Gaskell and Odile Burlet using a VG ZAB SEQ hybrid mass spectrometer of BEqQ geometry. Fast atom bombardment (FAB, 8-keV xenon atom beam) ionization was used in the negative mode. The liquid matrix used was either glycerol or 3-nitrobenzyl alcohol. UV–visible spectra were recorded using a Hitachi 100-80 spectrophotometer. pH measurements were determined with either a Radiometer pHM 26 meter or a pHM 84 research meter equipped with a Radiometer GK2320C combination glass electrode. The effective "pH" of the buffered methanolic solutions was determined using a Radiometer GK2320C combination glass electrode which was standardized against aqueous buffer solutions. HPLC analyses were conducted with the following Waters Associates units: 510 A pump, 510 B pump, Model 680 gradient controller, Model 490 multiwavelength detector, U6K injector. The products were eluted from a C_{18} μBondapak (stainless steel) column (3.9 × 300 mm) using either one of the two following linear gradient conditions. (a) Program 1: 90% A (0.1 M triethylammonium acetate, pH 6.5) and 10% B (acetonitrile) isocratic for 5 min, then from 90% A and 10% B to 50% A and 50% B in 20 min. (b) Program 2: 100% A (3 mM triethylammonium phosphate, pH 4.7) and 0% B (3 mM triethylamine in acetonitrile) to 50% A and 50% B in 25 min. In both cases, the column was fitted with a μBondapak guardpak precolumn and a flow rate of 1 mL/min was used. The eluent was monitored at 280, 313, and 365 nm. The HPLC solvents were filtered (aqueous solution with Millipore HA, 0.45 μm; acetonitrile with Millipore FH, 0.5 μm) and degassed before utilization. Product mixtures separated by Sephadex chromatography were conducted using G-25 fine Sephadex (bead size 20–80 mesh) and a 2.5- × 45-cm column. The eluent was an aqueous buffered NH_4HCO_3 (0.02 M, pH 8.0) solution. The flow rate was 24 mL/h. The eluting solvent was

monitored at 313 nm with a LKB UV monitor and recorder system. Precoated silica gel GHLF microscope slides (2.5 × 10 cm, Analtech No. 21521) were utilized for products purified by thin-layer chromatography, while G-25 fine Sephadex and a 1- × 12-cm column was employed using the conditions previously described for compounds purified by Sephadex chromatography.

The mitomycin C used in this study was kindly provided by Drs. M. Casazza and W. Rose from the Bristol-Myers Squibb Laboratories (Wallingford, CT). The $Na_2S_2O_4$ utilized was the best commercial grade available from Fisher ("purified" sodium hydrosulfite, Catalog No. S300-100, estimated purity by manufacturer = 85–88%). The $Na_2S_2O_4$ powder was stored under Ar in a dessicator in the dark. Freshly prepared $Na_2S_2O_4$ solutions were always used and utilized within 1 min. All experiments in a given series were run with the same $Na_2S_2O_4$ within a 2-week period. The amount of $Na_2S_2O_4$ added was not adjusted to account for impurities (i.e., Na_2CO_3, NaCl, and $NaHSO_3$) present. All solvents and other reactants were of the best commercial grade available and were used without further purification unless otherwise noted. Deionized water was obtained with a Milli-Q (18 MΩ) water system (Millipore). The Ar routinely used was O_2-free (O_2 < 5 ppm).

Reduction of Aqueous Solutions of Mitomycin C (1a) by Incremental Addition of $Na_2S_2O_4$ (Method A). Reductive activation of a deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (1 mL) of **1a** (1 mg, 3.0 μmol, 3 mM) was conducted by the incremental addition of a freshly prepared, deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL/addition) of $Na_2S_2O_4$ (0.06 M) (five incremental additions over 48 min, 0.2 equiv every 12 min). The reaction was maintained under a positive pressure of Ar. Aliquots (10 μL) were withdrawn from the reaction at specific time intervals and directly injected into the HPLC. The pH of the solution did not change by more than 0.15 pH unit. The integrated areas of the product peaks and **1a** in the HPLC chromatogram at 313 nm were adjusted to account for the differences in the absorption coefficients of **1a,b** and the mitosene products and normalized to 100%. All products were identified by coinjection of an authentic sample with the reaction mixture using two sets of HPLC conditions (programs 1 and 2). The reaction was run in triplicate, and the data were averaged. The observed experimental results are listed in Tables II and VII.

Preparation of Ammonium 7-Aminomitosane-9a-sulfonate (1b, $M^+ = NH_4^+$). To a deaerated (Ar), aqueous solution (25 mL) of **1a** (25 mg, 75 μmol, 3 mM) and Na_2SO_3 (9.4 mg, 0.75 mmol) was added a freshly prepared, deaerated (Ar), aqueous solution (100 μL) of $Na_2S_2O_4$ (3.3 mg, 19.0 μmol). The reaction was stirred at room temperature (2 min), during which time the color of the solution changed from intense blue to dark purple. The reaction mixture was then rapidly quenched by filtration, leading to the reappearance of the blue color, and lyophilized. HPLC analysis prior to lyophilization indicated that **1b** was produced in 65% yield. Separation of the reaction mixture was accomplished by Sephadex chromatography. Fractions containing the desired material were combined and lyophilized. Purification by Sephadex chromatography afforded **1b** ($M^+ = NH_4^+$).

Ammonium 7-Aminomitosane-9a-sulfonate (1b, $M^+ = NH_4^+$): HPLC t_R program 1 5.2 min, program 2 12.9 min; ^{1}H NMR (DMSO-d_6) δ 1.63 (s, C(6)CH_3), 2.59 (d, J = 4.5 Hz, C(2)H), 2.90 (d, J = 4.5 Hz, C(1)H), 3.46 (d, J = 11.6 Hz, C(3)H_α), 3.73 (dd, J = 4.2, 10.1 Hz, C(9)H), 3.84 (d, J = 11.6 Hz, C(3)H_β), 4.16 (t, J = 10.1 Hz, C(10)*H*H′), 4.41 (dd, J = 4.2, 10.1 Hz, C(10)H*H*′); ^{1}H NMR (D_2O + dioxane) δ 1.68 (s, C(6)CH_3), 3.14 (d, J = 4.3 Hz, C(2)H), 3.23 (d, J = 4.3 Hz, C(1)H), 3.72 (d, J = 13.0 Hz, C(3)H_α), 3.99 (dd, J = 5.2, 10.8 Hz, C(9)H), 4.09 (d, J = 13.0 Hz, C(3)H_β), 4.30 (t, J = 10.8 Hz, C(10)*H*H′), 4.53 (dd, J = 5.2, 10.8 Hz, C(10)H*H*′); ^{13}C NMR (DMSO-d_6) 8.12 (C(6)*C*H_3), 35.48 (C(2)), 35.82 (C(1)), 42.67 (C(9)), 50.68 (C(3)), 61.22 (C(10)), 93.98 (C(9a)), 101.83 (C(6)), 104.38 (C(8a)), 149.30 (C(7)), 154.87 (C(5a)), 156.64 (C(10a)), 177.23 ppm (C(8) or C(5)) (the second peak attributed to C(5) or C(8) was not observed); MS (–FAB) m/z 385 [M + 2H]$^-$, 384 [M + H]$^-$, 383 [M]$^-$; UV–visible (H_2O) λ_{max} 313, 371 nm.

Determination of the Absorption Coefficient of Ammonium 7-Aminomitosane-9a-sulfonate (1b, $M^+ = NH_4^+$) at 313 nm and Comparison with Other Mitomycin C Compounds. The UV absorption at 313 nm of aqueous solutions of ammonium 7-aminomitosane-9a-sulfonate (**1b**, $M^+ = NH_4^+$) was determined in the range of concentrations 0.03–0.5 mM and plotted as a function of concentration. The ε value determined by graphical methods was 1840 L mol^{-1} cm^{-1}. Previously reported[9a,15,35] ε values for mitomycin C (**1a**) and 2,7-diaminomitosene (**8**) at 313 nm were used to calculate the following relative ε ratios: ϵ_{313}(**8**)/ϵ_{313}(**1a**) = 3.6; ϵ_{313}(**8**)/ϵ_{313}(**1b**) = 5.5; ϵ_{313}(**1a**)/ϵ_{313}(**1b**) = 1.5. The ϵ_{313}(**1a**)/ϵ_{313}(**1b**) ratio was confirmed by injecting in the HPLC a solution containing equimolar amounts of **1a** and **1b** (the ratio of the concentrations was determined by integration of the ^{1}H NMR signals for the C(9a) OCH_3 resonance of **1a** and the C(6) CH_3 resonances of **1a,b**). The HPLC integral for **1a,b** at 313 nm was 1.5:1.

Preparation of Mitosenes Obtained in the Aqueous Studies. a. *trans*-(11) and *cis*-2,7-Diaminomitosene-1-sulfonate (12). To a deaerated (Ar), aqueous solution (25 mL) of **1a** (25 mg, 75 μmol) and Na_2SO_3 (9.4 mg, 0.75 mmol, 30 mM) was added a freshly prepared, deaerated (Ar), aqueous solution (100 μL) of $Na_2S_2O_4$ (6.5 mg, 3.7 μmol). The reaction was stirred at room temperature (5 min), rapidly quenched by filtration, and lyophilized. Separation of **11** and **12** was accomplished by Sephadex chromatography. Fractions containing one of the two isomers were combined and lyophilized. Further purification of **11** and **12** was conducted by passage of these compounds through a smaller Sephadex column.

***trans*-2,7-Diaminomitosene-1-sulfonate (11):** HPLC t_R program 1 6.6 min, program 2 13.7 min; ^{1}H NMR (DMSO-d_6) δ 1.68 (s, C(6)CH_3), 3.75 (s, C(1)H_β), 3.78 (d, J = 12.2 Hz, C(3)H_β), 4.17 (dd, J = 5.6, 12.2 Hz, C(3)H_α), 4.23 (d, J = 5.6 Hz, C(2)H), 4.99 (^{1}/$_2$ABq, J = 11.3 Hz, C(10)*H*H′), 5.10 (^{1}/$_2$ABq, J = 11.3 Hz, C(10)H*H*′); ^{13}C NMR (DMSO-d_6) 8.90 (C(6)*C*H_3), 54.89 (C(3)), 56.25 (C(10)), 58.29 (C(2)), 65.48 (C(1)) ppm (the concentration of the NMR sample did not permit the detection of the remaining signals); MS (–FAB) m/z 384 [M + 2H]$^-$, 383 [M + H]$^-$, 382 [M]$^-$; UV–visible (H_2O) λ_{max} 255, 316, 365 (sh), 544 nm.

***cis*-2,7-Diaminomitosene-1-sulfonate (12):** HPLC t_R program 1 13.2 min, program 2 16.6 min; ^{1}H NMR (DMSO-d_6) δ 1.68 (s, C(6)CH_3), 3.75 (dd, J = 9.9, 11.4 Hz, C(3)H_β), 3.98 (d, J = 7.1 Hz, C(1)H_α), 4.03–4.08 (m, C(2)H), 4.25 (dd, J = 7.8, 11.4 Hz, C(3)H_α), 4.93 (^{1}/$_2$ABq, J = 11.5 Hz, C(10)*H*H′), 5.05 (^{1}/$_2$ABq, J = 11.5 Hz, C(10)-H*H*′); ^{13}C NMR (DMSO-d_6) 8.94 (C(6)*C*H_3), 50.90 (C(3)), 56.06 (C(10)), 56.07 (C(2)), 58.26 (C(1)) ppm (the concentration of the NMR sample did not permit the detection of the remaining signals); MS (–FAB) m/z 384 [M + 2H]$^-$, 383 [M + H]$^-$, 382 [M]$^-$; UV–visible (H_2O) λ_{max} 255, 314, 364 (sh), 544 nm.

b. Preparation of *trans*-10-(Decarbamoyloxy)-2,7-diaminomitosene-1,10-disulfonate (16). To a deaerated (Ar), aqueous solution (25 mL) of **1a** (25 mg, 75 μmol) and K_2SO_3 (594 mg, 3.8 mmol) was added a freshly prepared, deaerated (Ar), aqueous solution (100 μL) of $Na_2S_2O_4$ (6.5 mg, 37 μmol). The reaction was stirred at room temperature (30 min), quenched by filtration, lyophilized, and purified by Sephadex chromatography. Fractions containing the desired product were combined and lyophilized. Subsequent use of a smaller Sephadex chromatography column gave pure **16**.

***trans*-10-(Decarbamoyloxy)-2,7-diaminomitosene-1,10-disulfonate (16):** HPLC t_R program 1 4.5 min, program 2 11.8 min; ^{1}H NMR (DMSO-d_6) δ 1.72 (s, C(6)CH_3), 4.04 (d, J = 13.1 Hz, C(3)H_β), 4.13 (^{1}/$_2$ABq, J = 12.6 Hz, C(10)*H*H′), 4.16 (^{1}/$_2$ABq, J = 12.6 Hz, C(10)-H*H*′), 4.29 (dd, J = 6.1, 13.1 Hz, C(3)H_α), 4.34 (d, J = 6.1 Hz, C(2)H), 4.40 (s, C(1)H_β); ^{13}C NMR (DMSO-d_6) 8.85 (C(6)*C*H_3), 46.42 (C(10)), 51.54 (C(3)), 57.20 (C(2)), 61.90 (C(1)) ppm (the concentration of the NMR sample did not permit the detection of the remaining signals); MS (–FAB) m/z 404 [M + H]$^-$, 403 [M]$^-$; UV–visible (H_2O) λ_{max} 254, 317, 366 (sh), 550 nm.

c. Preparation of *trans*-10-(Decarbamoyloxy)-1-hydroxy-2,7-diaminomitosene-10-sulfonate (14). To a deaerated (Ar), aqueous solution (10 mL) of **9**[15] (10 mg, 31 μmol) and K_2SO_3 (247 mg, 1.5 mmol) was added a freshly prepared, deaerated (Ar), aqueous solution (100 μL) of $Na_2S_2O_4$ (2.7 mg, 15 μmol). The reaction was stirred at room temperature (10 min), quenched by filtration, lyophilized, and purified by Sephadex chromatography. Compound **14** was obtained as the only detectable product of the reaction. Further purification of **14** was accomplished by passage through a small Sephadex column.

***trans*-10-(Decarbamoyloxy)-1-hydroxy-2,7-diaminomitosene-10-sulfonate (14):** HPLC t_R program 1 7.3 min, program 2 14.8 min; ^{1}H NMR (DMSO-d_6) δ 1.72 (s, C(6)CH_3), 3.70–3.71 (m, C(2)H), 3.80 (^{1}/$_2$ABq, J = 13.5 Hz, C(10)*H*H′), 4.04 (dd, J = 2.0, 12.9 Hz, C(3)H_β), 4.20 (dd, J = 5.5, 12.9 Hz, C(3)H_α), 4.28 (^{1}/$_2$ABq, J = 13.5 Hz, C(10)H*H*′), 4.54 (d, J = 1.6 Hz, C(1)H_β); ^{13}C NMR (DMSO-d_6) 8.71 (C(6)*C*H_3), 46.79 (C(10)), 53.53 (C(3)), 61.24 (C(2)), 71.90 (C(1)) ppm (the concentration of the NMR sample did not permit the detection of the remaining signals); MS (–FAB) m/z 342 [M + 2H]$^-$, 341 [M + H]$^-$, 340 [M]$^-$; UV–visible (H_2O) λ_{max} 254, 315, 363 (sh), 544 nm.

(35) Tomasz, M.; Lipman, R. *J. Am. Chem. Soc.* **1979**, *101*, 6063–6067.

d. Preparation of *cis*-10-(Decarbamoyloxy)-1-hydroxy-2,7-diaminomitosene-10-sulfonate (15). The preceding protocol was repeated for the preparation of **15** beginning with a deaerated (Ar), aqueous solution (10 mL) of **10**[15] (10 mg, 31 μmol), K_2SO_3 (248 mg, 1.6 mmol), and a freshly prepared, deaerated (Ar), aqueous solution (100 μL) of $Na_2S_2O_4$ (2.7 mg, 15 μmol). The reaction mixture was sonicated during the deaeration period and the early stages of the reaction due to the poor solubility of **10** in H_2O. Complete solubilization was observed after the addition of $Na_2S_2O_4$, and then the sonication was stopped. The solution was quenched by filtration, lyophilized, and purified by passage through a Sephadex column. Compound **15** was obtained as the only product of the reaction and was further purified with a small Sephadex column.

***cis*-10-(Decarbamoyloxy)-1-hydroxy-2,7-diaminomitosene-10-sulfonate (15)**: HPLC t_R program 1 8.3 min, program 2 15.4 min; ^1H NMR (DMSO-d_6) δ 1.71 (s, C(6)CH_3), 3.62 (dd, J = 8.5, 12.3 Hz, C(3)H_β), 3.84 ($^1/_2$ABq, J = 13.4 Hz, C(10)HH'), 3.86–3.87 (m, C(2)H), 4.38 (dd, J = 7.7, 12.3 Hz, C(3)H_α), 4.25 ($^1/_2$ABq, J = 13.4 Hz, C(10)HH'), 4.74 (d, J = 5.4 Hz, C(1)H_α); ^{13}C NMR (DMSO-d_6) 8.80 (C(6)CH_3), 46.90 (C(10)), 50.90 (C(3)), 56.60 (C(2)), 63.70 (C(1)) ppm (the concentration of the NMR sample did not permit the detection of the remaining signals); MS (–FAB) m/z 342 $[M + 2H]^-$, 341 $[M + H]^-$, 340 $[M]^-$; UV–visible (H_2O) λ_{max} 255, 314, 363 (sh), 544 nm.

e. Preparation of 10-(Decarbamoyloxy)-2,7-diaminomitosene-10-sulfonate (13). To a deaerated (Ar), aqueous solution (10 mL) of 2,7-diaminomitosene[9a] (**8**) (10 mg, 33 μmol) and K_2SO_3 (265 mg, 1.65 mmol) was added a freshly prepared, deaerated (Ar), aqueous solution (100 μL) of $Na_2S_2O_4$ (2.7 mg, 15 μmol). The solution was stirred at room temperature (30 min) and then quenched by filtration. HPLC analysis indicated only the presence of 10-(decarbamoyloxy)-2,7-diaminomitosene-10-sulfonate (**13**). The desired compound was purified by Sephadex chromatography.

10-(Decarbamoyloxy)-2,7-diaminomitosene-10-sulfonate (13): HPLC t_R program 1 9.6 min, program 2 16.5 min; ^1H NMR (DMSO-d_6) δ 1.72 (s, C(6)CH_3), 2.65 (dd, J = 4.9, 16.8 Hz, C(1)H_β), 3.13 (dd, J = 7.5, 16.8 Hz, C(1)H_α), 3.82 ($^1/_2$ABq, J = 13.2 Hz, C(10)HH'), 3.85 (dd, J = 4.6, 12.9 Hz, C(3)H_β), 3.91 ($^1/_2$ABq, J = 13.2 Hz, C(10)HH'), 4.12–4.14 (m, C(2)H), 4.29 (dd, J = 7.0, 12.9 Hz, C(3)H_α); ^{13}C NMR (DMSO-d_6) 8.45 (C(6)CH_3), 33.05 (C(1)), 47.25 (C(10)), 53.32 (C(3)), 55.39 (C(2)) ppm (the concentration of the NMR sample did not permit the detection of the remaining signals); MS (–FAB) m/z 326 $[M + 2H]^-$, 325 $[M + H]^-$, 324 $[M]^-$; UV–visible (H_2O) λ_{max} 250, 313, 361 (sh), 542 nm.

Reduction of *trans*-2,7-Diaminomitosene-1-sulfonate (11) in H_2O. To a stirred deaerated (Ar), aqueous solution of *trans*-2,7-diaminomitosene-1-sulfonate (**11**) (1 mg, 2.6 μmol, 2.6 mM) was added a freshly prepared, deaerated, aqueous solution (10 μL) of $Na_2S_2O_4$ (0.23 mg, 1.3 μmol). HPLC analysis of the reaction indicated the presence of only one new mitosene (HPLC t_R program 1 4.5 min, program 2, 11.8 min). The new mitosene was identified as **16** by coinjection with an authentic sample under two sets of HPLC conditions (programs 1 and 2).

Reduction of *cis*-2,7-Diaminomitosene-1-sulfonate (12) in H_2O. The preceding reaction was repeated using *cis*-2,7-diaminomitosene-1-sulfonate (**12**). To a stirred deaerated (Ar), aqueous solution of **12** (1 mg, 2.6 μmol, 2.6 mM) was added a freshly prepared, deaerated aqueous solution (10 μL) of $Na_2S_2O_4$ (0.23 mg, 1.3 mmol). Two peaks (HPLC t_R: program 1 4.5, 5.0 min; program 2 11.8, 12.2 min) were observed along with that of the starting material. The peaks at 4.5 min (program 1) and 11.8 min (program 2) were determined to be **16** by coinjection of an authentic sample.

Reduction of Methanolic Solutions of Mitomycin C (1a) by Incremental Addition of $Na_2S_2O_4$ (Method A). The protocol previously described for the aqueous $Na_2S_2O_4$-mediated (method A) reduction of mitomycin C was adapted for the methanolic studies. Accordingly, reductive activation of a deaerated (Ar), methanolic buffered (0.1 M Tris·HCl, "pH" 7.4) solution (1 mL) of **1a** (1 mg, 3.0 mmol, 3 mM) was initiated by the incremental addition of a freshly prepared, deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL/addition) of $Na_2S_2O_4$ (0.06 M) (five incremental additions over 48 min, 0.2 equiv every 12 min). The percent of water by volume in the methanolic solutions increased from 1% (0.2 equiv of $Na_2S_2O_4$) to 5% (1.0 equiv of $Na_2S_2O_4$). The "pH" of the solution did not change by more than 0.15 pH unit. The reaction was run in duplicate, and the data were averaged. The observed experimental results are listed in Tables III and VIII.

Preparation of Mitosenes Obtained in the Methanolic Studies. a. Preparation of *trans*-10-(Decarbamoyloxy)-1-methoxy-2,7-diaminomitosene-10-sulfonate (22). To a deaerated (Ar), aqueous solution (25 mL) of **18**[11c,21] (25 mg, 75 μmol) and K_2SO_3 (595 mg, 3.8 mmol) was added a freshly prepared, deaerated aqueous solution (100 μL) of $Na_2S_2O_4$ (7.8 mg, 45 μmol). The solution was stirred at room temperature (30 min), quenched by filtration, lyophilized, and purified using Sephadex chromatography.

***trans*-10-(Decarbamoyloxy)-1-methoxy-2,7-diaminomitosene-10-sulfonate (22)**: HPLC t_R program 1 15.6 min, program 2 20.3 min; ^1H NMR (DMSO-d_6) δ 1.72 (s, C(6)CH_3), 3.35 (s, C(1)OCH_3), 3.62 ($^1/_2$ABq, J = 13.2 Hz, C(10)HH'), 4.01 (d, J = 12.6 Hz, C(3)H_β), 4.03 (br s, C(2)H), 4.39 (dd, J = 5.3, 12.6 Hz, C(3)H_α), 4.40 ($^1/_2$ABq, J = 13.2 Hz, C(10)HH'), 5.03 (s, C(1)H_β); ^{13}C NMR (DMSO-d_6) 8.23 (C(6)CH_3), 46.71 (C(10)), 50.57 (C(3)), 56.52 (C(1)OCH_3), 58.57 (C(2)), 79.24 (C(1)) ppm (the concentration of the NMR sample did not permit the detection of the remaining signals); MS (–FAB) m/z 356 $[M + 2H]^-$, 355 $[M + H]^-$, 354 $[M]^-$; UV–visible (H_2O) λ_{max} 255, 317, 367 (sh), 550 nm.

b. Preparation of *cis*-10-(Decarbamoyloxy)-1-methoxy-2,7-diaminomitosene-10-sulfonate (23). The preceding reaction protocol was employed using a deaerated (Ar), aqueous solution (25 mL) of **19**[11c,21] (25 mg, 75 μmol), K_2SO_3 (595 mg, 3.8 mmol), and a freshly prepared, deaerated aqueous solution (100 μL) of $Na_2S_2O_4$ (7.8 mg, 45 μmol). The reaction mixture was sonicated during the deaeration period and the early stages of the reaction due to the poor solubility of **19** in H_2O. After the addition of the reducing agent was completed, the reaction was observed to be homogeneous and the sonication was stopped.

***cis*-10-(Decarbamoyloxy)-1-methoxy-2,7-diaminomitosene-10-sulfonate (23)**: HPLC t_R program 1 15.6 min, program 2 20.6 min; ^1H NMR (DMSO-d_6) δ 1.72 (s, C(6)CH_3), 3.38 (s, C(1)OCH_3), 3.71 ($^1/_2$ABq, J = 13.3 Hz, C(10)HH'), 3.90 (dd, J = 7.6, 12.3 Hz, C(3)H_β), 4.14–4.18 (m, C(2)H), 4.41 (dd, J = 7.6, 12.3 Hz, C(3)H_α), 4.47 ($^1/_2$ABq, J = 13.3 Hz, C(10)HH'), 5.03 (d, J = 5.5 Hz, C(1)H_α); ^{13}C NMR (DMSO-d_6) 8.28 (C(6)CH_3), 46.93 (C(10)), 48.45 (C(3)), 54.30 (C(1)OCH_3), 57.21 (C(2)), 73.11 (C(1)) ppm (the concentration of the NMR sample did not permit the detection of the remaining signals); MS (–FAB) m/z 356 $[M + 2H]^-$, 355 $[M + H]^-$, 354 $[M]^-$; UV–visible (H_2O) λ_{max} 255, 317, 367 (sh), 550 nm.

Reduction of Aqueous Solutions of Ammonium 7-Aminomitosane-9a-sulfonate (1b, $M^+ = NH_4^+$) by Incremental Addition of $Na_2S_2O_4$ (Method A). The protocol previously described for the aqueous $Na_2S_2O_4$-mediated (method A) reduction of mitomycin C was adapted for the reduction of **1b**. Accordingly, reductive activation of a deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (1 mL) of **1b** (1.25 mg, 3.0 μmol) was conducted by the incremental addition of a freshly prepared, deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL/addition) of $Na_2S_2O_4$ (0.06 M) (five incremental additions over 48 min, 0.2 equiv every 12 min). The pH of the solution did not decrease by more than 0.15 pH unit. The reaction was run in duplicate and averaged. The results listed in Tables V and X were calculated as previously described.

Reduction of Aqueous Solutions of Mitomycin C (1a) by Addition of $Na_2S_2O_4$ in a Single Shot (Method B). Reductive activation of a deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (1 mL) of mitomycin C (1 mg, 3.0 μmol) was conducted by the addition of specified amounts of a freshly prepared, deaerated (Ar), aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL) of $Na_2S_2O_4$ (0.06–0.3 M) in a single step at room temperature. The reaction was maintained under a positive pressure of Ar. Aliquots (10 μL) were withdrawn from the reaction at specific time intervals and directly injected into the HPLC. The pH of the solution did not change by more than 0.1 pH unit. All products were identified by coinjection of an authentic sample with the reaction mixture using two sets of HPLC conditions (programs 1 and 2). The reaction was run in duplicate, and the data were averaged. The results are listed in Tables IV and IX and were calculated as previously described for method A.

Catalytic Reduction of Ammonium 7-Aminomitosane-9a-sulfonate (1b, $M^+ = NH_4^+$). An aqueous buffered (0.1 M Tris·HCl, pH 7.0) solution (1 mL) containing **1b** (1.25 mg, 3.0 μmol) and PtO_2 (0.3 mg) was deaerated at room temperature (15 min). H_2 gas was then bubbled through the reaction mixture (5 min). Aliquots were removed from the reaction solution after 3 min and, at the conclusion of the reaction, filtered through a 0.45-μm filter (Millipore) and analyzed by HPLC. All products were identified by coinjection of an authentic sample with the reaction mixture using two sets of HPLC conditions (programs 1 and 2). The reaction was run in duplicate, the data were averaged, and the percentages given in parentheses for the observed products were calculated as previously described: at 3 min, **1b** (78.1), **8** (7.1), **13** (3.8), **9 + 10** (8.2), **11 + 12**

(0.7), **14 + 15** (1.2), **16** (0.9); at 5 min, **1b** (23.3), **8** (8.9), **13** (35.0), **9 + 10** (16.8), **14 + 15** (10.4), **16** (5.6).

Catalytic Reduction of Mitomycin C (1a) in the Presence of K_2SO_3. The preceding experimental protocol was utilized with an aqueous buffered (0.1 M Tris·HCl, pH 7.0) solution (1 mL) containing **1a** (1 mg, 3.0 μmol), K_2SO_3 (0.5 mg, 3.0 μmol), and PtO_2 (0.3 mg). The reaction was run in duplicate, the data were averaged, and the percentages given in parentheses for the observed products were calculated as previously described: at 3 min, **1a** (19.8), **1b** (25.5), **8** (15.5), **13** (9.2), **9 + 10** (18.9), **11 + 12** (5.4), **14 + 15** (4.8), **16** (0.9); at 5 min, **1a** (18.1), **1b** (21.4), **8** (7.7), **13** (20.4), **9 + 10** (7.8), **11 + 12** (1.9), **14 + 15** (14.2), **16** (8.5).

Enzymatic Activation of Mitomycin C (1a) and Ammonium 7-Aminomitosane-9a-sulfonate (1b, $M^+ = NH_4^+$). An aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (2.5 mL) containing either **1a** (0.85 mg, 2.6 mmol) or **1b** (1.05 mg, 2.6 mmol) and NADH (4 mg, 5.12 mmol) was deaerated (Ar) (37 °C, 15 min). Xanthine oxidase (1.25 units from bovine milk, Sigma) was added to the solution. The reaction was maintained at 37 °C (20 min) during which time decoloration of the solution was observed. An aliquot of the solution was then filtered through an "ultra-free-MC" (molecular cut = 10 000 MW, Millipore) by centrifugation prior to HPLC analysis. All products were identified by coinjection of an authentic sample with the reaction mixture using two sets of HPLC conditions (programs 1 and 2). The reaction was run in duplicate, the data were averaged, and the percentages given in the parentheses for the observed products were calculated as previously described: for compound **1a**, **1a** (38.6), **8** (17.7), **9 + 10** (43.7); for compound **1b**, **1b** (32.5), **8** (12.1), **9 + 10** (29.1), **13** (8.2), **11 + 12** (8.6), **14 + 15** (9.5).

Reduction of Aqueous Solutions of Mitomycin C (1a) in the Presence of HSO_3^--Trapping Reagents. To a deaerated (Ar), aqueous buffered solution (10 mL) of **1a** (1.0 mg, 3 μmol) and formaldehyde (60 μmol, 20 equiv) was added a deaerated, aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL) of $Na_2S_2O_4$ (0.052 mg, 0.3 μmol). The solution was stirred at room temperature (1 min), filtered through a 0.45-μm filter (Millipore), and analyzed by HPLC. The reaction was run at pH 5.5, 7.4, and 8.5. The buffer utilized for the pH 5.5 reaction was 0.1 M bis·Tris·HCl, while 0.1 M Tris·HCl was used for the pH 7.4 and 8.5 reactions. The pH of the solution at the conclusion of the reaction was unchanged. The percentages given in the parentheses for the observed products in the HPLC profiles were calculated as previously described: at pH 5.50, **1a** (81.7), **1b** (3.5), **8 – 10** (14.8); at pH 7.40, **1a** (77.1), **1b** (3.5), **8–10** (19.3), C(1) and/or C(10) sulfonato adducts (0.1); at pH 8.50, **1a** (53.5), **1b** (2.0), **8-10** (32.8), **39** (11.7).

The preceding protocol was used with *N,N*-dimethylmethylene ammonium iodide (Eschenmoser's salt) (11.1 mg, 60 μmol, 20 equiv) in place of formaldehyde, and the reaction was performed at pH 7.4. The percentages given in the parentheses for the observed products in the HPLC profile were calculated as previously described: at pH 7.4, **1a** (75.5), **8–10** (24.5).

General Procedure for the Competitive Reactivity of Mitomycin C (1a) and Ammonium 7-Aminomitosane-9a-sulfonate (1b, $M^+ = NH_4^+$) in H_2O. To a deaerated (Ar), aqueous buffered solution (10 mL) containing equimolar amounts of **1a** (1.0 mg, 3 μmol)) and **1b** (1.25 mg, 3 μmol) and the HSO_3^--trapping reagent (formaldehyde or Eschenmoser's salt) was added in a single shot a deaerated, aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL) of $Na_2S_2O_4$ (0.052 mg, 0.3 μmol). The reactions were performed using either formaldehyde (10 equiv), formaldehyde (20 equiv), or Eschenmoser's salt (20 equiv). The solution was stirred at room temperature (1 min), the reaction quenched by filtration through a 0.45-μm filter (Millipore), and the solution analyzed by HPLC. The reaction was run at pH 5.5, 7.4, and 8.5. The buffer utilized for the pH 5.5 reaction was 0.1 M bis·Tris·HCl, while 0.1 M Tris·HCl was used for the pH 7.4 and 8.5 reactions. The pH of the solution at the conclusion of the reaction did not change by more than 0.15 pH unit. The reaction was run in duplicate, and the data were averaged. The results listed in Table VI were calculated as previously described.

Studies on the Activation of Aqueous and Methanolic Solutions of Mitosenes 11, 12, and 16. Separate DMSO stock solutions containing the mitosene (*trans*- (**11**), *cis*-2,7-diaminomitosene-1-sulfonate (**12**) (1 mg in 175 μL of DMSO, 15 mM), or *trans*-10-(decarbamoyloxy)-2,7-diaminomitosene-1,10-disulfonate (**16**) (1 mg in 166 μL of DMSO, 15 mM) and the nucleophile (thiophenol (26 μL in 1 mL of DMSO, 150 mM) or aniline (23 μL in 1 mL of DMSO, 150 mM)) were prepared. To a deaerated aqueous buffered solution (100 μL) containing the mitosene (10 μL of the stock solution, final concentration = 1.5 mM) and the nucleophile (10 μL of the stock solution, final concentration = 0.15 M, 10 equiv) was added in a single shot a deaerated, aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (10 μL) of $Na_2S_2O_4$ (0.15 M, 1 equiv). The solution was stirred at room temperature (45 min). The reaction was run at pH 5.5, 7.0, and 8.2. The buffer utilized for the pH 5.5 reaction was 0.1 M bis·Tris·HCl, while 0.1 M Tris·HCl was used for the pH 7.0 and 8.2 reactions. The pH of the solution at the conclusion of the reaction did not change by more than 0.1 pH unit. Similar reactions were performed in buffered methanolic solutions ("pH" 5.5, 7.0, and 8.2) and in the absence of the nucleophile in both water and methanol. In select cases, a comparable procedure using PtO_2/H_2 (5 min) was employed in place of $Na_2S_2O_4$. Each reaction was run in duplicate and analyzed by HPLC.

Studies on the Activation of Aqueous and Methanolic Solutions of Mitosene 22. DMSO stock solutions containing mitosene **22** (1 mg in 196 μL, 15 mM) and the nucleophile (thiophenol (26 μL in 1 mL of DMSO, 150 mM) or aniline (23 μL in 1 mL of DMSO, 150 mM)) were prepared. Reductive activation of a deaerated aqueous buffered solution (100 μL) containing **22** (10 μL of the stock solution, final concentration = 1.5 mM) and the nucleophile (10 μL of the stock solution, final concentration = 15 mM, 10 equiv) was accomplished by the incremental addition (three increments) of a deaerated, aqueous buffered (0.1 M Tris·HCl, pH 7.4) solution (5 μL/addition) of $Na_2S_2O_4$ (0.1 M, 1 equiv). The solution was stirred at room temperature (45 min). The reaction was run at pH 5.5, 7.4, and 8.5. The buffer utilized for the pH 5.5 reaction was 0.1 M bis·Tris·HCl, while 0.1 M Tris·HCl was used for the pH 7.4 and 8.5 reactions. The pH of the solution at the conclusion of the reaction did not change by more than 0.1 pH unit. Similar reactions were performed in buffered methanolic solutions ("pH" 5.5, 7.0, and 8.2) and in the absence of the nucleophile in both water and methanol. In select cases, a comparable procedure using PtO_2/H_2 (5 min) was employed in place of $Na_2S_2O_4$. Each reaction was run in duplicate and analyzed by HPLC.

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Supplementary Material Available: Tables VII–X, listing observed product profiles for the addition of $Na_2S_2O_4$ to aqueous and methanolic solutions of **1a,b** (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS. Ordering information is given on any current masthead page.