Comparative Reactivities of Mitomycin C, 7-(*N*-Piperidino)mitomycin, and Mitomycin A. The Role of the C(7) Substituent

Suganthini Subramaniam and Harold Kohn*

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77204-5641

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Abstract: Mitomycin C (**1a**) is considered to be the protypical bioreductive alkylating agent. Enzymatic reduction leads to activation of the two DNA-bonding sites (C(1) and C(10)) in **1a**, permitting the formation of intrahelical interstrand DNA–mitomycin C cross-link adducts. Drug discovery programs have uncovered several C(7) mitomycin analogues that display a superior profile versus **1a** when assayed against a battery of tumor models. The chemical properties of these and related C(7)-substituted mitomycins have not been extensively explored. We have compared the reactivity of mitomycin C (**1a**) with the two C(7)-substituted mitomycins, 7-(*N*-piperidino)mitomycin (**1b**) and mitomycin A (**1c**), under reductive conditions in tetrahydrofuran–water mixtures ("pH" 5.5, 6.5). Key observations included (1) enhanced levels of drug utilization in proceeding from **1a** to **1b** and **1c**, (2) increased percentages in the activation of the C(1) and C(10) bonding sites in **1b,c** versus **1a**, and (3) increased amounts of C(1) nucleophilic and C(10) nucleophilic products for **1b,c** versus **1a**. The results of these findings are discussed in light of previous studies, the role of the C(7) substituent in the mode of mitomycin action, and the design of future mitomycin analogues.

Mitomycin C (**1a**) is an antineoplastic antibiotic isolated in Japan in 1956 from *Streptomyces caespitosus*.[1] Despite its toxicity, this chemotherapeutic agent was introduced in Japan (1960) and later in the United States (1974) for the treatment of several types of neoplasms. A compromise between potency and toxicity established mitomycin C as the best candidate within the naturally occurring mitomycins for clinical use.[2] Mitomycin C has proven to be an effective drug for stomach, breast, and colon cancers and to a lesser extent for pancreatic and lung cancers.[3,4] Biochemical investigations have demonstrated that upon reductive activation the drug reacts first at C(1) and then at C(10) with the nucleophilic 2-amino residue in deoxyguanosines located on complementary DNA strands.[5,6] It has been suggested that the formation of these intrahelical interstrand DNA–mitomycin C adducts inhibits DNA replication, resulting in cessation of cell division.[7]

Surprisingly, recent chemical studies have documented that in the *absence* of added nucleophiles (i.e., RNH_2, RSH, DNA) reductively activated mitomycin C in water functions preferentially as an electrophilic trapping agent, and that only in moderately acidic solutions containing aniline did C(1) nucleophilic products predominate.[8] The mechanism depicted in Scheme I has been advanced to account for the observed product profiles for **1a** in the absence of nucleophiles[8] and is based in part on earlier proposals made by Iyer and Szybalski,[7a] Patrick,[9] Moore and Czerniak,[10] and Tomasz.[11]

Studies on the mode of action of the mitomycins have provided the chemical basis for an active program for the development of semisynthetic mitomycin analogues that display improved activity and decreased toxicity over **1a**.[12] More than 600 mitomycin derivatives have been prepared and tested against experimental tumor and microbial infection models. Many of these compounds displayed enhanced activity versus **1a** in select anticancer screens, and a few possessed superior profiles when assayed against a battery of tumor models.[13] These investigations demonstrated that alteration of the C(7) substituent in mitomycin C influenced the biological activity of the drug candidate. Several factors may have contributed to this phenomenon. First, the C(7) substituent affects the reduction potential of the quinone ring.[12-15] Polarographic half-wave potentials for mitomycins indicated that the more electronegative the C(7) substituent the more easily is the quinone ring reduced. Mitomycins that are readily converted to the hydroquinone (semiquinone) species (i.e., **1** → **2**) should lead to enhanced levels of C(1) and C(10) activation. Second, modification of the C(7) substituent may change the partition coefficient of the drug, and hence affect the cell penetration of the chemotherapeutic agent.[15d] Third, the C(7) substituent can alter the stability, reactivity, and toxicity of the activated drug.[16] Fourth, changes in the steric size and composition of the C(7) substituent may influence the ability of the drug candidate to bind (i.e., van der Waals, hydrogen bond) to DNA.

Mechanistic studies on the chemical reactivity of C(7)-substituted mitomycins other than **1a** have received scant

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(1) Hata, T.; Sano, Y.; Sugawara, R.; Matsumae, A.; Kanamori, K.; Shima, T.; Hoshi, T. *J. Antibiot., Ser. A* **1956**, *9*, 141–146.

(2) (a) Lawley, P. D. *Prog. Nucleic Acid Res. Mol. Biol.* **1966**, 89–97. (b) Kasai, M.; Kono, M. *Synlett.* **1992**, 778–790.

(3) Carter, S. K.; Crooke, S. T. *Mitomycin C. Current Status and New Developments*; Academic Press: New York, 1979.

(4) Crooke, S. T.; Bradner, W. T. *Cancer Treat. Rev.* **1976**, *3*, 121–139.

(5) (a) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science* **1987**, *236*, 1204–1208. (b) Teng, S. P.; Woodson, S. A.; Crothers, D. M. *Biochemistry* **1989**, *28*, 3901–3907. (c) Weidner, M. F.; Millard, J. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 9270–9272 and references therein.

(6) (a) Kohn, H.; Li, V.-S.; Tang, M.-s. *J. Am. Chem. Soc.* **1992**, *114*, 5501–5509. (b) Kumar, S.; Lipman, R.; Tomasz, M. *Biochemistry* **1992**, *31*, 1399–1407 and references therein.

(7) (a) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55–58. (b) Zwelling, L. A.; Anderson, T.; Kohn, K. W. *Cancer Res.* **1979**, *39*, 365–369.

(8) (a) Schiltz, P.; Kohn, H. *J. Am. Chem. Soc.* **1992**, *114*, 7958–7959. (b) Schiltz, P.; Kohn, H. *J. Am. Chem. Soc.* **1992**, preceding publication.

(9) Patrick, J. B.; Williams, R. P.; Meyer, W. E.; Fulmor, W.; Cosulich, D. B.; Broschard, R. W.; Webb, J. S. *J. Am. Chem. Soc.* **1964**, *86*, 1889–1990.

(10) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249–280.

(11) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056–5061.

(12) (a) Remers, W. A.; Dorr, R. T. *Alkaloids; Chemical and Biological Perspective*, Vol. 6; Pelletter, S. W., Ed.; Wiley and Sons: New York, 1988; pp 1–74. (b) Remers, W. A. *The Chemistry of Antitumor Antibiotics*, Vol. 1; Wiley: New York, 1979; pp 221–276.

(13) Bradner, W. T.; Remers, W. A.; Vyas, D. M. *Anticancer Res.* **1989**, *9*, 1095–1100.

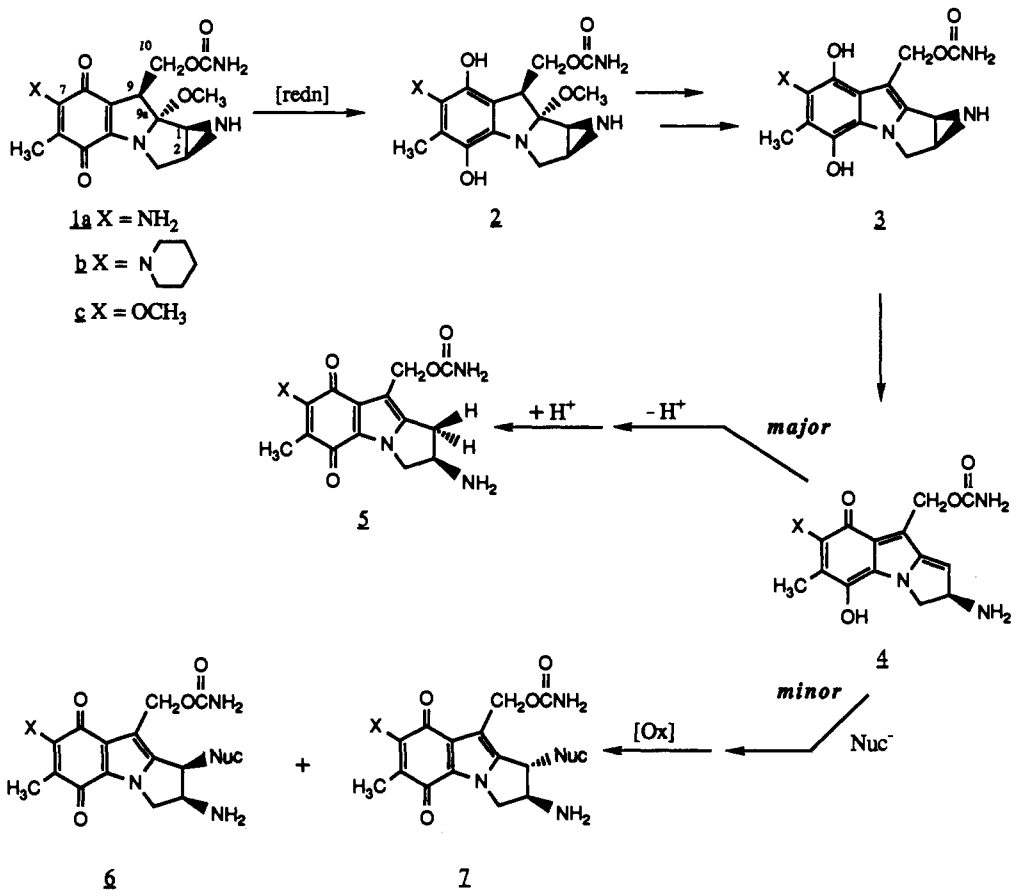
(14) Pan, S.-S.; Gonzalez, H. *Mol. Pharmacol.* **1990**, *37*, 966–970.

(15) (a) Iyengar, B. S.; Sami, S. M.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 1453–1457. (b) Iyengar, B. S.; Lin, H.-J.; Cheng, L.; Remers, W. A. *Ibid.* **1981**, *24*, 975–981. (c) Iyengar, B. S.; Remers, W. A.; Bradner, W. T. *Ibid.* **1986**, *29*, 1864–1868. (d) Sami, S. M.; Iyengar, B. S.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *Ibid.* **1984**, *27*, 701–708.

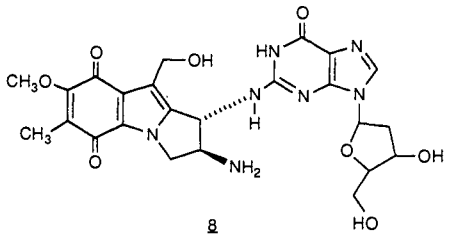
(16) McGuinness, B. F.; Lipman, R.; Goldstein, J.; Nakanishi, K.; Tomasz, M. *Biochemistry* **1991**, *30*, 6444–6453.

0002-7863/93/1515-10519$04.00/0

Scheme I. Proposed Mechanism for the Formation of Mitomycin C(1) Electrophilic and Nucleophilic Products under Reductive Conditions in the Absence of Nucleophiles[8]



attention. Recently, a thoughtful investigation appeared on the *invitro* mechanism for the bonding of mitomycin A (**1c**) to DNA.[16] Key findings reported included that **1a**,**c** displayed comparable specificity for alkylation of deoxyguanosine 2-amino residues and that the sequence selectivity for DNA bonding and the efficiency for cross-link adduct formation were similar for these two mitomycins. The finding that a significant portion of the **1c**-product pool consisted of *C(10)-decarbamoylated* mitosene A–DNA monoadducts (i.e., **8**) led to the proposal that C(10)



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functionalization in reductively activated **1c** was competitive to C(1) modification. This result contrasted to mitomycin C transformations in which reactions proceeded at C(1), and only under select conditions at C(10).[17,18]

The clinical utility of C(7)-substituted mitomycins[12,13] and the recent findings for the bonding of mitomycin A (**1c**) to DNA[16] prompt us to report our studies on the comparative chemical reactivities of mitomycin C (**1a**) and two C(7)-substituted mitomycins, 7-(*N*-piperidino)mitomycin (**1b**)[15a,b] and mitomycin A (**1c**).[19,20] Significant differences in the product profiles for these three mitomycins were observed. Explanations are advanced consistent with the anticipated roles of the C(7) substituent in the reductive activation of the C(1) and C(10) DNA-bonding sites. The results were in agreement with the product profile detected for **1c** in the presence of DNA.[16] An alternative hypothesis for the mitomycin A (**1c**) activation process is offered that does not require the invocation of a new pathway, and is consistent with the established route for mitomycin C.[17,18] The significance of our findings for future drug design is briefly discussed.

Results

(a) Selection of Compounds and Method of Mitomycin Activation. The three mitomycins chosen for study were mitomycin C (**1a**), 7-(*N*-piperidino)mitomycin (**1b**), and mitomycin A (**1c**). Compounds **1b**,**c** were selected because they were more easily reduced than **1a** and because of their ease of preparation from **1a** and their stability in moderate acid for extended periods of time. The polarographic half-wave potentials for **1a**, **1b**, and **1c** are –0.45, –0.27, and –0.21 V versus SCE, respectively.[15a,b] The ^{13}C NMR data for **1a**–**c** are listed in Table I. Proton-coupled ^{13}C NMR spectra were secured for these mitomycins to permit the assignments of the signals. The results obtained for **1a** confirmed a previous study.[21] Prominent downfield shifts (14.6–21.1 ppm) were observed for the C(6) carbon resonance in **1b**,**c** versus **1a**. Smaller downfield shifts (3.5–8.5 ppm) were also noted for the C(5) and C(7) signals. These shifts have been attributed

(17) (a) Hornemann, U.; Iguchi, K.; Keller, P. J.; Vu, H. M.; Kozlowski, J. F.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5026–5033. (b) Bean, M.; Kohn, H. *Ibid.* **1983**, *48*, 5033–5041. (c) Bean, M.; Kohn, H. *Ibid.* **1985**, *50*, 293–298.

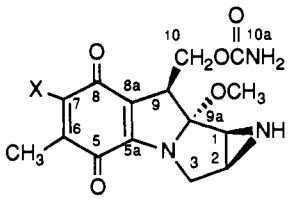
(18) Kohn, H.; Zein, N.; Lin, X. Q.; Ding, J.-Q.; Kadish, K. M. *J. Am. Chem. Soc.* **1987**, *109*, 1833–1840.

(19) Matsui, M.; Yamada, Y.; Uzu, K.; Hirata, T. *J. Antibiot.* **1968**, *21*, 189–198.

(20) Vyas, D. M.; Benigni, D.; Partyka, R. A.; Doyle, T. W. *J. Org. Chem.* **1986**, *51*, 4307–4309.

(21) Keller, P. J.; Hornemann, U. *J. Nat. Prod.* **1983**, *46*, 569–571.

Table I. Proton-Coupled ^{13}C NMR Data for Mitomycin Derivatives[a]



	1a (X = NH_2)[b]	**1b** (X = *N*-piperidino)[b,c]	**1c** (X = OCH_3)[d,e]
C(1)	35.37; d; 184.9	35.79; d; 184.9	36.12; d, d; 182.5, 2.9
C(2)	31.48; d; 181.1	31.67; d; 179.2	32.17; d, d; 182.1, 4.8
C(3)	49.60; d, t; 142.9, 3.8	49.55; t; 151.1	50.23; t; 142.5
C(5)	176.76; q; 3.8	180.27; q; 3.8	182.50; q; 3.8
C(5a)	155.13; br s	151.95; br s	151.79; br s
C(6)	102.61; q; 5.1	117.20; q; 5.7	123.70; q; 5.8
C(6)CH_3	8.33; q; 127.7	12.36; q; 128.3	8.10; q; 128.7
C(7)	148.95; q; 3.8	152.75; q; 3.8	157.47; q; 3.8
C(8)	175.11; br s	178.86; br s	177.64; br s
C(8a)	109.04; d; 7.6	112.68; d; 7.6	113.39; d; 7.6
C(9)	42.79; d; 135.3	43.55; d; 135.3	44.04; d; 135.3
C(9a)	105.53–105.93; m	105.60–105.71; m	106.30–106.34; m
C(10)	60.34; d, t; 150.6, 7.6	61.10; d, t; 149.9, 7.6	61.42; d, t; 150.6, 8.1
C(10a)	156.50; br s	156.44; br s	156.93; br s
C(9a)OCH_3	49.12; q; 142.9	49.01; q; 141.5	*f*

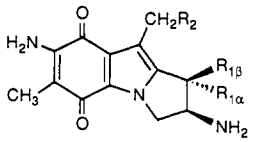
[a] The number in each entry is the chemical shift value (δ) reported in ppm relative to the solvent used, followed by the multiplicity of the signal and coupling constant(s) in Hz. All spectra were obtained at 75 MHz. [b] The solvent used was DMSO-d_6. [c] Additional peaks were located at δ 23.71 (t, 128.2 Hz, C(4')), 26.59 (t, 127.7 Hz, C(3')), and 43.55 (t, 137.2 Hz, C(2')). [d] The solvent used was DMSO-d_6–CD_3OD (9:1). [e] An additional peak was located at δ 49.01 (q, 141.5 Hz, C(7)OCH_3). [f] The C(9a)OCH_3 signal is believed to be beneath the CD_3OD peak.

in part to the same factor(s) responsible for the relative ease in reduction of **1b,c** versus **1a**. Replacement of the C(7) amino group in **1a** by the *N*-piperidino moiety in **1b** should decrease the electron density in the quinone ring. Molecular models of simple C(2),C(5) *N,N*-dipiperidinoquinones indicated that efficient overlap of the nitrogen lone pair with the C(2)–C(3)–C(4)–O(4) α,β-unsaturated carbonyl system is not possible due to the steric interactions imposed by the *N*-piperidino group.[22] No adverse steric repulsions existed in the parent unsubstituted C(2),C(5)-diaminoquinones. Correspondingly, interchange of the C(7) amino group in **1a** by the C(7) methoxy moiety in **1c** is expected to decrease the electron density in the quinone ring due to the relative electron-releasing properties of these two substituents.[23]

Mitomycin activation was accomplished by reducing a deaerated tetrahydrofuran solution (2 mL) containing the test substrate with PtO_2 and H_2 (1 min). During this procedure the intensity of the pink color of the mitomycin C solution, the dark green color of the 7-(*N*-piperidino)mitomycin solution, and the dark red color of mitomycin A solution significantly decreased. Deaerated aqueous buffered solutions (pH 5.5, 6.5; 4 mL) were then transferred *via* a cannula to the tetrahydrofuran reaction mixture, and then the reaction was allowed to remain under Ar (1 min). The reactions were quenched by the admission of oxygen and then analyzed by HPLC and TLC. Each experiment was conducted at least three times at each "pH" value and then averaged.

Several experimental parameters entered into this choice for mitomycin reductive activation. First, a single procedure was required for all three mitomycins. Among the conventional reductants, PtO_2–H_2 proved to be sufficient to activate the quinone ring in mitomycin C, 7-(*N*-piperidino)mitomycin, and mitomycin A in moderately acidic solutions. Moreover, this reducing system unlike $Na_2S_2O_4$[24,25] and the arylhydrazines[26] did not introduce any additional nucleophiles into the reaction system. Second, the poor solubility of 7-(*N*-piperidino)mitomycin and the corresponding mitosenes in water necessitated that tetrahydrofuran be employed as a cosolvent. The effect of this cosolvent on the effective hydrogen ion concentration in solution is not known. However, we observed no pronounced changes in the "pH" of the buffered tetrahydrofuran–water solutions after mixing.[27]

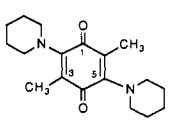
(b) Mitomycin C (1a) Activation. Reduction of mitomycin C (**1a**) led to the production of mitosenes **9**,[11,28] **10**,[29] and **11**[30] along with unreacted **1a** (Table II) (HPLC, TLC analyses). The



9 $R_{1\alpha}$ = H $R_{1\beta}$ = H R_2 = $OC(O)NH_2$
10 $R_{1\alpha}$ = H $R_{1\beta}$ = H R_2 = H
11 $R_{1\alpha}$ = H $R_{1\beta}$ = H R_2 = MMC
12 $R_{1\alpha}$ = H $R_{1\beta}$ = OH R_2 = $OC(O)NH_2$
13 $R_{1\alpha}$ = OH $R_{1\beta}$ = H R_2 = $OC(O)NH_2$

identities of all products in the chromatograms were confirmed

(22) In support of this contention, the dihedral angle between the nitrogen substituent and the quinone ring in I and II for the energy-minimized structures were 53 and 0°, respectively. A comparable result was observed for the fully reduced systems. Energy-minimization calculations were performed using the program PCMODEL V(88.0) from Serena Software, Bloomington, IN.



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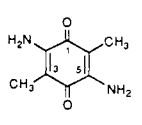

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(23) March, J. *Advanced Organic Chemistry, Reaction, Mechanisms, and Structure*, 4th ed.; Wiley: New York, 1992; Chapter 9, pp 273–292.

(24) Hornemann, U.; Ho, Y.-K.; Mackey, J. K., Jr.; Srivastava, S. C. *J. Am. Chem. Soc.* **1976**, *98*, 7069–7074.
(25) (a) Schiltz, P.; Kohn, H. *Tetrahedron Lett.* **1992**, *33*, 4709–4712. (b) Schiltz, P.; Kohn, H. *J. Am. Chem. Soc.* **1993**, first paper in present series.
(26) Russell, D. J.; Kohn, H. *Heterocycles* **1990**, *31*, 223–227.
(27) Previous investigations have shown that the effective hydrogen concentration of aqueous solutions is largely unaffected by the addition of methanol and ethanol, provided these solvents do not comprise a significant percentage by volume of the reaction system, see: Bates, R. G. *Determination of pH: Theory and Practice*; Wiley: New York, 1974; p 222.
(28) Han, I.; Russell, D. J.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 1799–1807.
(29) Han, I.; Kohn, H. *J. Org. Chem.* **1991**, *56*, 4648–4653.
(30) Han, I. Ph.D. Dissertation, University of Houston, Houston, TX, 1991. For a comparable adduct, see ref 28.

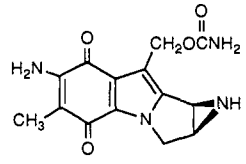
Table II. Product Profiles Observed for the Reductive Activation of Mitomycin C (1a) in Tetrahydrofuran–Water[a]

compd no.	R_1	R_2	"pH" 5.5	"pH" 6.5
9	H	$OC(O)NH_2$	46.7 (91.5)	52.7 (80.1)
10	H	H	1.1 (2.2)	9.4 (14.3)
11	H	MMC		0.6 (0.9)
unknowns			3.2 (6.3)	3.2 (4.8)
1a			49.0	34.1
% C(1) mod[b]			51.0	65.9
% C(1),C(10) mod[c]			1.1	10.0
% C(1) elec[d]			100	100

[a] The reaction was initiated by bubbling H_2 gas through a deaerated tetrahydrofuran mixture containing 1a and PtO_2, and then a deaerated aqueous buffer solution was transferred into the reaction mixture. All reactions were run at least three times 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 parentheses corresponds to the percent of the product after exclusion of unreacted 1a. [b] The percent of compounds modified at C(1) was computed by (%C(1) mod/(% C(1) mod + % C(1),C(10) mod + % 1a + % unknowns)) × 100. [c] The percent of 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 + % 1a + % unknowns)) × 100. [d] C(1) electrophilic compounds corresponded to C(1)-protonated adducts (i.e., 9–11). The percent of C(1) electrophilic compounds was computed by (% C(1) elec/(% C(1) elec + % C(1) nucl)) × 100.

by coinjection and cospotting authentic samples with the reaction mixture. For each reaction, we have determined the percentage of drug remaining, the percentage of the total product mixture in which modification of 1a occurred at only the C(1) or at both the C(1) and C(10) sites, and the percentage of modified products in which electrophilic processes had proceeded at the C(1) position. Reductive activation of mitomycin C at "pH" 5.5 and 6.5 led to C(1) electrophilic products and little C(10) activation.

Mitomycin C (1a) was also activated in aqueous buffered solutions.[30] In these experiments, the solution containing 1a and PtO_2 was deaerated (Ar) and then reduced by passing H_2 through the reaction mixture (5 min). The reactions were exposed to air and analyzed by HPLC and TLC. Table III lists the average percent yields for the observed products for duplicate experiments performed at pH 5.5 and 6.5, along with the tabulation summarizing the extent and type of modification that proceeded at the C(1) site in 1a. The product profile at pH 5.5 mirrored that observed for the tetrahydrofuran–water solution, while at pH 6.5 appreciable amounts of the C(1) nucleophilic adducts 12[11] and 13[11] were detected. Compounds 12 and 13 have been attributed to the hydrolysis of the *oxidized* adduct, 7-aminoazaridinomitosene[29,31] (14), and are *not* believed to be characteristic



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of the reactivity of reductively activated mitomycin C species (i.e., 2–4).[8] In this series of experiments, we once again observed only C(1)-modified products. The overall similarity of the product profiles obtained in tetrahydrofuran–water and in water suggested that comparable pathways were operative in both sets of transformations.

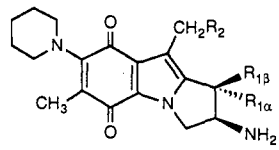
(c) 7-(*N*-Piperidino)mitomycin (1b) Activation. Analytical scale reduction of 7-(*N*-piperidino)mitomycin (1b) at "pH" 5.5 and 6.5 provided mitosenes 15–18 (HPLC, TLC analyses). No starting material was observed at the conclusion of the reactions. HPLC analyses of the analytical scale reactions did not reveal distinct peaks for *cis*- (17) and *trans*-2-amino-10-decarbamoyl-

(31) Rao, G. M.; Beglieter, A.; Lown, J. W.; Plambeck, J. A. *J. Electrochem. Soc.* **1977**, *124*, 199–202.

Table III. Product Profiles Observed for the Reductive Activation of Mitomycin C (1a) in Water[a]

compd no.	R_1	R_2	pH 5.5	pH 6.5
9	H	$OC(O)NH_2$	77.9 (81.1)	35.4 (47.5)
12 + 13[b]	OH	$OC(O)NH_2$	14.4 (15.0)	37.0 (50.0)
unknowns			3.7 (3.9)	2.1 (2.8)
1a			4.0	25.5
% C(1) mod[c]			92.3	72.4
% C(1) elec[d]			84.4	48.9

[a] The reaction was initiated by bubbling H_2 gas through a deaerated aqueous buffered solution containing 1a and PtO_2. All reactions were conducted 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 parentheses corresponds to percent of the product after exclusion of unreacted 1a. Data are taken from ref. 30. [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 + % 1a + % unknowns)) × 100. [d] MC C(1) electrophilic compounds corresponded to the C(1)-protonated adduct (9). The percent of C(1) electrophilic compounds was computed by (% C(1) elec/(% C(1) elec + % C(1) nucl)) × 100.



15 $R_{1\alpha}$ = H $R_{1\beta}$ = H R_2 = $OC(O)NH_2$
16 $R_{1\alpha}$ = H $R_{1\beta}$ = H R_2 = OH
17 $R_{1\alpha}$ = H $R_{1\beta}$ = OH R_2 = OH
18 $R_{1\alpha}$ = OH $R_{1\beta}$ = H R_2 = OH

1-hydroxy-7-(*N*-piperidino)mitosenes (18). Both compounds are believed to have comparable HPLC retention times.[32] Purified samples of 15, 16, and 18 were obtained by rerunning the appropriate reactions on a semipreparative scale. Mitosene 18 was obtained only after repetitive preparative TLC. Tentative evidence for 17 was secured from the ^1H NMR spectrum obtained of the binary reaction mixture containing 17 and 18 prior to further purification. In this spectrum, two new sets of a doublet of doublets were detected at δ 3.89 and 4.38. These signals have been assigned to the C(3β) and C(3α) protons in 17, respectively.[33]

Table IV lists the HPLC percentage yields for the 1b reaction products. The reactions were quantitatively monitored at two different wavelengths (324, 365 nm) and then averaged. Included in this table are calculations summarizing the percentage of modification at C(1) and C(1),C(10) and the extent of C(1) electrophilic and C(10) nucleophilic processes. Inspection of the results revealed several trends. First, complete consumption of 1b was observed at both "pH" values. Second, significant amounts of C(1) nucleophilic products were detected at "pH" 5.5 and 6.5. This pattern was different from mitomycin C (Table II), where C(1) electrophilic adducts were the exclusive products. Third, enhanced levels of C(1),C(10) activation products were observed. Fourth, activation of the C(10) site led to only C(10) nucleophilic substituted compounds.

(d) Mitomycin A (1c) Activation. Catalytic reduction of mitomycin A at "pH" 5.5 and 6.5 provided 19–22. HPLC and TLC analyses indicted that 1c utilization was complete. The identities of 19–22 were established by rerunning the reaction on a semipreparative scale and then identifying the isolated products by spectroscopic methods.

The HPLC chromatograms were monitored at 317 nm since all the compounds absorbed strongly at this wavelength. Table V lists the average percentage yields for the observed 1c products, along with the tabulation summarizing the extent and type of modification that proceeded at both the C(1) and C(10) sites. The results were similar to those observed for 7-(*N*-piperidino)-

(32) A similar result was observed using a second HPLC gradient system.
(33) For a discussion of the NMR chemical shift values in cis- and trans-substituted mitosenes, see ref 17.

Table IV. Product Profiles Observed for the Reductive Activation of 7-(*N*-Piperidino)mitomycin (**1b**) in Tetrahydrofuran–Water[a]

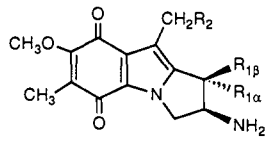
compd no.	R_1	R_2	"pH" 5.5	"pH" 6.5
15	H	$OC(O)NH_2$	5.8	2.8
16	H	OH	55.9	60.8
17 + 18	OH	OH	38.3	36.4
1b			0	0
% C(1) mod[b]			100	100
% C(1),C(10) mod[c]			94.2	97.2
% C(1) elec[d]			61.7	63.6
% C(10) nucl[e]			100	100

[a] The reaction was initiated by bubbling H_2 gas through a deaerated tetrahydrofuran mixture containing **1b** and PtO_2, and then a deaerated aqueous buffered solution was transferred into the reaction mixture. The reactions were run in quadruplicate and averaged. For each entry, the value listed corresponds to the percent of the product in the reaction mixture and is the average of the HPLC chromatograms obtained at 324 and 365 nm. [b] The percent of compounds modified at C(1) was computed by (%C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. [c] The percent of 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. [d] C(1) electrophilic compounds corresponded to C(1)-protonated adducts (i.e., **15, 16**). The percent of C(1) electrophilic compounds was computed by (% C(1) elec/(% C(1) elec + % C(1) nucl)) × 100. [e] The percent of C(10) nucleophilic compounds corresponded to C(10) hydroxy adducts (i.e., **16–18**). The percent of C(10) nucleophilic compounds was computed by (% C(10) nucl/(% C(10) nucl + % C(10) elec)) × 100.

Table V. Product Profiles Observed for the Reductive Activation of Mitomycin A (**1c**) in Tetrahydrofuran–Water[a]

compd. no.	R_1	R_2	"pH" 5.5	"pH" 6.5
19	H	$OC(O)NH_2$	3.4	7.5
20	H	OH	44.1	52.0
21 + 22[b]	OH	OH	52.5	40.5
1c			0	0
% C(1) mod[c]			100	100
% C(1),C(10) mod[d]			96.6	92.5
% C(1) elec[e]			47.5	59.4
% C(10) nucl[f]			100	100

[a] The reaction was initiated by bubbling H_2 gas through a deaerated tetrahydrofuran mixture containing **1c** and PtO_2, and then a deaerated aqueous buffered solution was transferred into the reaction mixture. The reaction were run in quadruplicate and averaged. For each entry, the value listed corresponds to the percent of the product in the reaction mixture. [b] Appreciable quantities of both cis- and trans-adducts were detected. [c] The percent of compounds modified at C(1) was computed by (% C(1) mod/(% C(1) mod + % C(1),C(10) mod)) × 100. [d] The percent of 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] C(1) electrophilic compounds corresponded to C(1)-protonated adducts (i.e., **19, 20**). The percent of C(1) electrophilic compounds was computed by (% C(1) elec/(% C(1) elec + % C(1) nucl)) × 100. [f] The percent of C(10) nucleophilic compounds corresponded to C(10) hydroxy adducts (i.e., **20–22**). The percent of C(10) nucleophilic compounds was computed by (% C(10) nucl/(% C(10) nucl + % C(10) elec)) × 100.



19 $R_{1\alpha}$ = H $R_{1\beta}$ = H R_2 = $OC(O)NH_2$
20 $R_{1\alpha}$ = H $R_{1\beta}$ = H R_2 = OH
21 $R_{1\alpha}$ = H $R_{1\beta}$ = OH R_2 = OH
22 $R_{1\alpha}$ = OH $R_{1\beta}$ = H R_2 = OH

mitomycin (**1b**) (Table IV). Complete consumption of **1c** was observed at "pH" 5.5 and 6.5 leading to significant amounts of C(1) nucleophilic adducts. In addition, high amounts of C(1),C(10) activation products were detected at these "pH" values (>92.5%) in which only C(10) nucleophilic adducts were formed.

Discussion

Analyses of the products furnished after reductive activation of mitomycin C (**1a**), 7-(*N*-piperidino)mitomycin (**1b**), and mitomycin A (**1c**) in tetrahydrofuran–water mixtures permitted us to identify key patterns that can be attributed in part of the C(7) substituent in the starting mitomycin. These trends included the percent of consumption of the mitomycin, the facility of the C(10) modification process, and the relative percentage of nucleophilic versus electrophilic products at each of the two DNA-bonding sites (C(1) and C(10)).

(a) Consumption of Starting Material. 7-(*N*-Piperidino)-mitomycin and mitomycin A were both completely consumed upon catalytic reduction in tetrahydrofuran–water mixtures at "pH" 5.5 and 6.5, while appreciable amounts (34–49%) of mitomycin C remained. This trend corresponded favorably to the reduction potentials reported for these three mitomycins.[15a,b] Accordingly, we have attributed the observed pattern in the efficiency of mitomycin utilization to the ease of reduction of the quinone ring.

(b) C(1) Transformations. Quinone methide **4** serves as a key intermediate in the proposed chemical pathway for reductively activated mitomycin C in water (Scheme I).[8,10,11] In this mechanism, proton-transfer processes yield **9** (**5**), while Michael addition of water to **4** furnishes **12** (**6**) and **13** (**7**). Analysis of the contributing resonance structures and inductive effects operative in **4** suggests that the C(7) substituent and O(5) significantly affect the electron density at C(1) in **4**. We speculate that the greater the electron donation provided by these groups, the more likely C(1) electrophilic reactions will take place (**4** → **5**). Examination of the percentage of C(1) electrophilic adducts for **1a–c** provided support for this notion. At any given "pH", the highest amounts of C(1) electrophilic products were observed for mitomycin C, followed by 7-(*N*-piperidino)mitomycin and mitomycin A. This observation is in agreement with the measured $E_{1/2}$ values for the reduction of the quinone group in these compounds[15a,b] and the ^{13}C NMR chemical shift values for the C(6) resonances in **1a–c** (Table I).

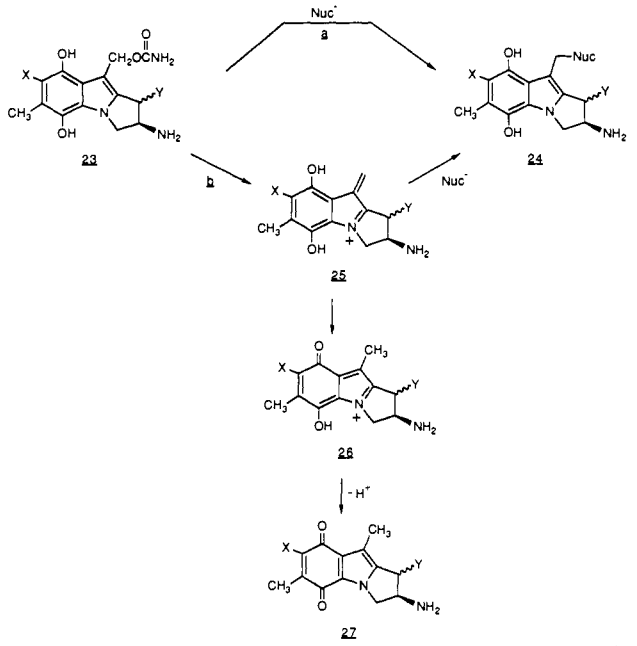
(c) C(10) Activation. Kohn and co-workers[18] provided evidence that two-electron reduction of **1a** in methanol was necessary for the efficient modification of the C(10) site. A similar situation may exist in tetrahydrofuran–water mixtures. Extensive functionalization of the C(10) site (>92.5%) in both 7-(*N*-piperidino)-mitomycin and mitomycin A was observed at "pH" 5.5 and 6.5, while 10% or less of the mitomycin C products were modified at this site. These findings suggested that the quinone rings in **1b** and **1c** were converted to the corresponding hydroquinone species **2b** and **2c**, respectively, permitting the sequential functionalization of the C(1) and C(10) sites, while redox reactions between **2a** and unreacted **1a** minimized functionalization of the recalcitrant C(10) site in this mitomycin.

(d) C(10) Transformations. The observed product profiles (Tables II, IV, and V) indicated that the C(10) modification pathway was dependent upon the C(7) substituent. At "pH" 5.5 and 6.5, little modification of the C(10) site in **1a** was observed (1–10%), and carbamate removal gave principally the C(10) electrophilic product **10**. Correspondingly, reductive activation of **1b,c** led to efficient modification of the C(10) site in which only nucleophilic products were detected.

Several mechanisms have been proposed for mitomycin C(10) transformations. In 1981, Moore and Czerniak[10] suggested that C(10) nucleophilic processes occurred by a S_N2 pathway (Scheme II, route *a*), while in 1987, Kohn and Zein[34] proposed that C(10) nucleophilic and electrophilic reactions could also proceed *via* iminium ion **25** to give **24** and **27**, respectively (Scheme II, route *b*). We suspect that the actual pathway(s) will depend upon the reaction conditions, the mitomycin, and the nucleophiles present in solution. For those reactions run in acid and in the absence of added nucleophiles, the iminium route (Scheme II, route *b*) should be favored. Under these conditions, strongly electron-donating C(7) substituents (i.e., **25**, X = NH_2) are expected to promote electrophilic transformations (i.e., **27**) at the expense of

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

Scheme II. Proposed Mechanisms for Mitomycin C(10) Functionalization Reactions



nucleophilic reactions (i.e., **24**), while increased amounts of C(10) nucleophilic products versus electrophilic adducts are anticipated for less electron-donating C(7) substituents (i.e., X = *N*-piperidino, OCH_3). These predictions were in accord with the product trends observed for **1a–c** (Tables II, IV, and V).

(e) Comparison with Previous Mitomycin A Studies. The high yields of *C(10)-decarbamoylated* mitosene A products **20–22** observed in the **1c** transformations were in agreement with the significant amounts of the 10-decarbamoyl monoadduct, N^2-(2″β-amino-10″-decarbamoyl-7″-methoxymitosen-1″α-yl)-2-deoxyguanosine (**8**), detected in the enzymatic digests of **1c** reactions performed in the presence of DNA.[16] This finding led to the suggestion that mitomycin A C(10) processes were competitive with C(1) transformations.[16] This may be true.[35] However, our studies indicated that production of this mitomycin A–DNA monoadduct was also consistent with a pathway in which functionalization of **1c** proceeded first at C(1) and then at C(10). We have observed that the C(7) methoxy substituent in **1c** permitted the full utilization of this mitomycin upon catalytic reductive activation. Complete conversion of **1c** to leucomitomycin A (**2c**) should allow reaction to take place at the recalcitrant C(10) site by eliminating redox processes with unreacted **1c**.[36] Accordingly, we suggest that in mitomycin A–DNA processes, C(1) functionalization may have occurred first, followed by loss of the C(10) carbamate group by the iminium route depicted in Scheme II. Once formed, the iminium ion would be expected to react rapidly with water to give the C(10) hydroxy adduct or react with a deoxyguanosine unit on the complementary strand to furnish the cross-linked adduct.[37]

This mechanistic pathway was supported by the product profiles determined for mitomycin A at higher "pH" values (data not shown).[38] Elevation of the solution "pH" to 7.5 and 8.5 led to incomplete utilization of **1c** (percent of **1c** remaining = 19.2–32.2). This result was expected because of the increased difficulty in reducing quinones at higher pH values.[31,39] Under these conditions, substantial amounts (96.4–97.2%) of mitosene A products were observed in which only C(1) transformations had occurred. A similar result was observed for 7-(*N*-piperidino)-mitomycin at higher "pH" values (data not shown).[38] We suspect that, in both cases, redox reactions occurred after modification of the C(1) site, thereby quenching subsequent reactions at C(10).

Conclusions

The product profiles for the reductive activation of the three C(7)-substituted mitomycins **1a–c** provided new information concerning the controlling factors which affect C(1)- and C(10)-substitution processes. Several trends were apparent. First, noticeable increases in the amount of drug activation were observed in proceeding from **1a** to **1b** to **1c**. Second, under acidic conditions, the percentage of C(1), C(10) modification was greater for mitomycin A and 7-(*N*-piperidino)mitomycin than for mitomycin C. Third, substantially enhanced levels of C(1) nucleophilic and C(10) nucleophilic products were observed for **1b,c** compared to **1a**. These trends are in agreement with the predicted effects of the C(7) substituent on both the reduction potential of the quinone ring in these compounds and the putative intermediates leading to the modification of the two DNA-bonding sites in the mitomycin.

The beneficial increases in the percentage of drug consumption, the percentage of modification of the mitomycin C(10) site, and the percentage in C(1) and C(10) nucleophilic substitution products observed in proceeding from **1a** to **1b** and **1c**, however, may not necessarily lead to a more efficacious drug. It has been suggested that bioreductive alkylating agents are more readily activated in some tumor cells than in normal tissues, thereby permitting agents like mitomycin C to be site selective. Several hypotheses have been advanced to account for this cellular therapeutic difference. Two of these are the lower O_2 supply[40,41] and the higher concentrations of reductases that exist in malignant versus normal cells.[42] Alteration of this chemical basis for mitomycin site selectivity by promoting quinone reduction by changing the C(7) substituent may lead to decreased cellular differentiation by the drug and hence increased toxicity.[43]

Experimental Section

Instrumentation and Solvents. Proton (^{1}H NMR) and carbon-13 (^{13}C NMR) nuclear magnetic resonance spectra were recorded on either a Nicolet NT-300 or a General Electric QE-300 spectrometer. Proton-coupled ^{13}C NMR spectra were recorded on a Nicolet NT-300 spectrometer by Dr. Ken Hope. Chemical shifts are expressed in parts per million relative to the solvent employed, and coupling constants (*J* values) are given in hertz. Mass spectral data were obtained on a Finnegan TSQ-70 triple quadrupole mass spectrometer under positive and negative conditions by Dr. Mehdi Moini, Department of Chemistry, University of Texas at Austin. IR spectra were recorded on an IBM Model IR/32 spectrometer, and UV spectra were recorded on a Model 330 Perkin-Elmer spectrometer. 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 using the following linear gradient condition: C_{18} μBondapak (SS) column 3.9 × 300 mm, from 100% A (3 mM triethylammonium phosphate, pH 4.7), 0% B (3 mM triethylamine in acetonitrile) to 50% A, 50% B in 25 min. In select cases, a different

(35) Evidence has been recently presented that placement of *equally* good leaving groups at C(1) and C(10) in C(7) methoxymitosene analogues led to the preferential expulsion of the C(10) moiety upon electrochemical reductive activation, see: Maliepaard, M.; de Mol, N. J.; Janssen, L. H. M.; Hoogvliet, J. C.; van der Neut, W.; Verboom, W.; Reinhoudt, D. N. *J. Med. Chem.* **1993**, *36*, 2091–2097.

(36) Further aiding C(10) functionalization in **1c** was the generation of only modest amounts of C(1) electrophilic adducts **19** and **20**. Formation of these compounds leads to the net consumption of electrons, thereby increasing the likelihood of redox transformations.

(37) Mitomycin A C(10) processes may exhibit decreased selectivity (i.e., 2-deoxyguanosine versus water) as compared to mitomycin C due to the expected decreased stability of iminium ion **25** generated from **1c** versus **1a**.

(38) Subramaniam, S. MS Thesis, University of Houston, Houston, TX, 1991.

(39) Bates, R. G. In *Treatise on Analytical Chemistry*; Kolthoff, I. M., Elving, P. J., Eds.; Wiley: New York, 1978; Vol. 1, pp 805–807.

(40) Sartorelli, A. C. *Biochem. Pharmacol.* **1986**, *35*, 67–69.

(41) Kennedy, K. A.; Teicher, B. A.; Rockwell, S.; Sartorelli, A. C. In *Molecular Actions and Targets for Cancer Chemotherapeutic Agents*; Sartorelli, A. C., Lazo, J. S., Bertino, J., Eds.; Academic Press: New York, 1981; Chapter 4, pp 85–100.

(42) Keyes, S. R.; Heimbrook, D. C.; Fracasso, P. M.; Rockwell, S.; Sligar, S. G.; Sartorelli, A. C. *Adv. Enzyme Regul.* **1985**, *23*, 291–307.

(43) For a related discussion, see ref 16. For a discussion on the toxicity of **1a**, see ref 12b and Hodges, J. C.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1981**, *24*, 1184–1191.

linear gradient condition and buffer were used: 90% A (triethylammonium acetate, pH 6.5), 10% B (acetonitrile) for 5 min to 50% A, 50% B in 20 min. The column was fitted with a μBondapak C_{18} guardpak. A flow rate of 1.0 mL/min was used. The organic solvents utilized were HPLC grade and were filtered (Millipore FH, 0.5 μm) and degassed prior to use. The aqueous buffers were prepared from deionized water (Millipore) and filtered (Millipore HA, 0.45 μm) and degassed. 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 solvents and reactions were the best commercial grade available and were used without further purification unless otherwise noted. Tetrahydrofuran was distilled from Na–benzophenone. The water used for all the reactions was HPLC grade. Thin-layer chromatography and thick-layer chromatography were run on precoated silica gel GHLF microscope slides (2.5 × 10 cm, Analtech No. 21521) or silica gel GF plates (20 × 20 cm, Analtech No. 02013). PtO_2 (83% Pt) was purchased from Aldrich Chemical Co., Milwaukee, WI. Solvent systems for TLC chromatographies were A, methanol–chloroform (1:99); B, methanol–chloroform (1:19); C, methanol–chloroform (1:9).

General Procedure for the Analytical Scale Reductive Activation of Mitomycins 1a-c in Tetrahydrofuran–Water. A deaerated (Ar) solution of the mitomycins (**1a-c**) (1 mg) in tetrahydrofuran (2 mL) containing PtO_2 (83% Pt, 0.5 mg) was reduced by bubbling H_2 gas (1 min) through the reaction mixture. A deaerated (Ar) aqueous buffered solution (4 mL) was then transferred via a cannula to the tetrahydrofuran mixture. The buffer employed for the "pH" 5.5 and 6.5 reactions was bis·Tris·HCl (0.05 M). After an additional 1 min, the reaction was quenched by the admission of O_2. The "pH" at the end of the reaction was within ±0.1 "pH" unit of the original aqueous buffered solution. The filtrate was neutralized with solid NH_4HCO_3 and then analyzed by HPLC and TLC. Products were identified by coinjection (cospotting) of an authentic sample with the reaction mixture in the HPLC (TLC).

The HPLC retention time (min) for the mitomycin C-derived compounds were **1a** (19.1), **9** (20.8), **10** (23.3), **11** (28.4), **12** (18.3), **13** (19.6). The integrated area of the product peaks and **1a** in the HPLC chromatograms at 313 nm were adjusted to account for the differences in the absorption coefficients[42,44,45] and the normalized to 100% (Table II). TLC R_f values for the mitomycin C-derived compounds (system C) are **1a** (0.41), **9** (0.15), **10** (0.50), **11** (0.12), **12** and **13** (0.14).

The HPLC retention times (min) for the 7-(*N*-piperidino)mitomycin-derived compounds are **1b** (29.6), **15** (34.5), **16** (31.9), **17** and **18** (28.1). The reactions were monitored at 324 and 365 nm. The percentage values for the reaction products determined at both wavelengths were then averaged (Table IV). The TLC R_f values for the 7-(*N*-piperidino)-mitomycin-derived compounds (system C) are **1b** (0.51), **15** (0.35), **16** (0.48), **17** and **18** (0.34).

The HPLC retention times (min) for the mitomycin A-derived compounds are **1c** (23.8), **19** (23.6), **20** (21.6), **21** (19.9), **22** (20.4). The reaction was monitored by HPLC at 317 and 340 min. The percentage values for the reaction products at 317 nm were averaged (Table V). TLC R_f values for the mitomycin A-derived compounds (system C) are **1c** (0.52), **19** (0.38), **20** (0.45), **21** (0.36), **22** (0.36).

Synthesis of 7-(*N*-Piperidino)mitomycin (1b). 7-(*N*-Piperidino)-mitomycin (**1b**) was prepared from mitomycin A (**1c**) using the method described by Remers and co-workers.[15a] A methanolic (20 mL) solution of **1c** (15 mg, 43 μmol) and piperidine (50 mg, 590 μmol) was stirred under Ar at room temperature (12 h). The volatile materials were removed *in vacuo*, and the green 7-(*N*-piperidino)mitomycin (**1b**) was isolated by preparative TLC (system B): 27 mg (65% yield); HPLC t_R 29.8 min; TLC R_f (system B) 0.51; IR (KBr) 3420, 2125, 1705, 1610, 1575, 1440, 1390, 1205 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.63 (br s, 6 H), 1.84 (s, 3 H), 2.82–2.97 (m, 1 H), 2.89 (d, *J* = 4.6 Hz, 1 H), 3.18 (s, 3 H), 3.30 (br s, 4 H), 3.44 (d, *J* = 12.6 Hz, 1 H), 3.57 (dd, *J* = 4.0, 10.4 Hz, 1 H), 4.11 (d, *J* = 12.6 Hz, 1 H), 4.51 (t, *J* = 10.4 Hz, 1 H), 4.73 (dd, *J* = 4.0, 10.4 Hz, 1 H), 4.91 (br s, 2 H); ^{13}C NMR (CD_3OD) 12.62, 24.23, 26.98, 32.89, 37.00, 43.47, 49.78, 49.79, 53.33, 62.88, 106.00, 113.49, 118.75, 152.44, 153.30, 156.59, 180.18, 181.16 ppm (the signals at 26.98 and 43.47 ppm were approximately twice the intensity of nearby peaks); UV–vis λ_{max} (CH_3OH) 587, 380, 238 nm.

Preparation of 7-(*N*-Piperidino)mitomycin Derivatives. A deaerated (Ar) solution of 7-(*N*-piperidino)mitomycin (**1b**) (10 mg, 25 μmol) in tetrahydrofuran (10 mL) containing PtO_2 (83% Pt, 3 mg) was reduced by bubbling H_2 gas (1 min) through the reaction mixture. A deaerated (Ar) aqueous bis·Tris·HCl (0.05 M, pH 5.5) buffer solution was transferred *via* a cannula into the reduced tetrahydrofuran mixture. The reaction was allowed to proceed for an additional 1 min and then quenched by the admission of O_2, leading to a change in the color of the reaction mixture from green to blue. The "pH" at the end of the reaction was within ±0.1 "pH" unit of the original aqueous buffered solution. The catalyst was filtered and the remaining filtrate neutralized with solid NH_4HCO_3. The reaction mixture was concentrated *in vacuo*, and the residue was purified by preparative TLC (system C) to give **15–18**.

2-Amino-7-(*N*-piperidino)mitosene (15): ^1H NMR (CD_3OD) δ 1.63 (br s, 6 H), 1.90 (s, 3 H), 2.68 (dd, *J* = 4.7, 16.7 Hz, 1 H), 3.20 (dd, *J* = 7.1, 16.7 Hz, 1 H), 3.35 (br s, 4 H), 3.93 (dd, *J* = 4.4, 12.7 Hz, 1 H), 4.15–4.17 (m, 1 H), 4.36 (dd, *J* = 6.7, 12.7 Hz, 1 H), 5.15 (s, 2 H) (all proton-proton connectivities were confirmed by selective decoupling experiments); MS (+Cl) *m/e* (relative intensity) 373 (M + 1, 36), 372 (M, 19), 359 (40), 239 (100); UV–vis λ_{max} (CH_3OH) 554, 324, 220 nm.

2-Amino-10-decarbamoyl-7-(*N*-piperidino)mitosene (16): ^1H NMR (CD_3OD) δ 1.63 (br s, 6 H), 1.90 (s, 3 H), 2.62 (dd, *J* = 4.8, 16.4 Hz, 1 H), 3.15 (dd, *J* = 7.1, 16.4 Hz, 1 H), 3.35 (br s, 4 H), 3.89 (dd, *J* = 4.6, 12.6 Hz, 1 H), 4.12–4.20 (m, 1 H), 4.35 (dd, *J* = 6.6, 12.6 Hz, 1 H), 4.62 (s, 2 H) (all proton–proton connectivities were confirmed by selective decoupling experiments); MS (+Cl) *m/e* (relative intensity) 330 (M + 1, 100), 328 (14), 312 (45), 311 (18), 154 (59); UV–vis λ_{max} (CH_3OH) 554, 324, 220 nm.

***trans*-2-Amino-10-decarbamoyl-1-hydroxy-7-(*N*-piperidino)mitosene (18):** ^1H NMR (CD_3OD) δ 1.63 (br s, 6 H), 1.90 (s, 3 H), 3.35 (br s, 4 H), 3.85–3.91 (m, 1 H), 3.98 (dd, *J* = 4.3, 12.7 Hz, 1 H), 4.52 (dd, *J* = 6.1, 12.7 Hz, 1 H), 4.70 (d, *J* = 13.6 Hz, 1 H), 4.78 (d, *J* = 13.6 Hz, 1 H), 4.87 (d, *J* = 3.1 Hz, 1 H); MS (–Cl) *m/e* (relative intensity) 345 (M, 100), 325 (36), 290 (50); MS (+Cl) *m/e* (relative intensity) 346 (M + 1, 100), 328 (37), 279 (7); UV–vis λ_{max} (CH_3OH) 554, 324, 220 nm.

Preparation of Mitomycin A (1c). Mitomycin A (**1c**) was prepared from mitomycin C (**1a**) using the method of Vyas and co-workers.[20] An aqueous 0.05 M NaOH solution (20 mL) containing **1a** (100 mg, 299 μmol) was stirred under Ar at room temperature (24 h). The pH of the solution was adjusted to pH 4.0 with an aqueous 0.05 M H_2SO_4 solution and extracted with ethyl acetate (3 × 20 mL), and then the combined ethyl acetate extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was dissolved in methylene chloride (15 mL) and stirred with 3-methyl-1-*p*-tolyltriazene (63 mg, 420 μmol) under Ar at room temperature (24 h). The solvent was removed, and the residue was first purified by column chromatography on neutral alumina (system A) and then by preparative TLC (system B) to give **1c**: 36 mg (35% yield); HPLC t_R 23.5 min; R_f (system B) 0.42; IR (KBr) 3390, 3285, 2925, 1690, 1620, 1565, 1435, 1395, 1210 cm^{-1}; ^1H NMR (DMSO-d_6 + CD_3OD) δ 1.65 (s, 3 H), 2.70–2.75 (m, 1 H), 2.82 (d, *J* = 4.4 Hz, 1 H), 3.10 (s, 3 H), 3.38 (d, *J* = 12.5 Hz, 1 H), 3.42 (dd, *J* = 4.4, 11.1 Hz, 1 H), 3.78 (d, *J* = 12.5 Hz, 1 H), 3.92 (s, 3 H), 4.12 (dd, *J* = 10.6, 11.1 Hz), 4.60 (dd, *J* = 4.4, 10.6 Hz, 1 H); ^{13}C NMR (DMSO-d_6 + CD_3OD) 8.23, 32.59, 36.39, 44.53, 50.60, 61.38, 61.90, 106.74, 114.37, 124.09, 152.25, 157.43, 157.94, 178.19, 182.90 ppm (the signal for the C_{9a} (OCH_3) peak was believed to be beneath the solvent peak); UV–vis λ_{max} (CH_3OH) 517, 317, 237 nm.

Preparation of Mitomycin A (1c) Derivatives. A deaerated (Ar) solution of mitomycin A (**1c**) (10 mg, 28.7 μmol) in tetrahydrofuran (10 mL) containing PtO_2 (83% Pt, 3 mg) was reduced by bubbling H_2 gas (1 min) through the reaction mixture. A deaerated (Ar) aqueous bis·Tris·HCl (0.05 M, pH 5.5) buffer solution (20 mL) was transferred *via* a cannula into the reduced tetrahydrofuran mixture. The reaction was allowed to proceed for an additional 1 min and then quenched by the admission of O_2, leading to a change in the color of the reaction mixture from red to yellow. The "pH" at the end of the reaction was within ±0.1 "pH" unit of the original aqueous buffered solution. The catalyst was filtered and the remaining filtrate neutralized with solid NH_4HCO_3. The reaction mixture was concentrated *in vacuo*, and the residue was purified by preparative TLC (system C) to give **19–22**.

2-Amino-7-methoxymitosene (19): ^1H NMR (CD_3OD) δ 1.89 (s, 3 H), 2.68 (dd, *J* = 4.6, 16.6 Hz, 1 H), 3.19 (dd, *J* = 7.2, 16.6 Hz, 1 H), 3.82 (dd, *J* = 4.5, 12.6 Hz, 1 H), 3.91 (s, 3 H), 4.15–4.20 (m, 1 H), 4.37 (dd, *J* = 6.6, 12.6 Hz, 1 H), 5.15 (s, 2 H); MS (–Cl) *m/e* (relative intensity) 245 (100), 244 (10); UV–vis λ_{max} (CH_3OH) 440, 350, 287, 237 nm.

2-Amino-10-decarbamoyl-7-methoxymitosene (20): ^1H NMR (CD_3OD) δ 1.90 (s, 3 H), 2.67 (dd, *J* = 4.6, 16.5 Hz, 1 H), 3.25 (dd, *J* = 7.1, 16.5 Hz, 1 H), 3.89 (dd, *J* = 4.4, 12.6 Hz, 1 H), 3.94 (s, 3 H), 4.15–4.22 (m, 1 H), 4.37 (dd, *J* = 6.6, 12.6 Hz, 1 H), 4.69 (s, 2 H); MS (+Cl) *m/e*

(44) Stevens, C. L.; Taylor, K. G.; Munk, M. E.; Marshall, W. S.; Noll, K.; Shah, G. D.; Shah, L. G.; Uzu, K. *J. Med. Chem.* **1965**, *8*, 1–10.
(45) Tomasz, M.; Lipman, R. *J. Am. Chem. Soc.* **1979**, *101*, 6063–6067.

(relative intensity) 277 (M + 1, 20), 259 (100), 185 (47); UV–vis λ_{max} (CH_3OH) 440, 350, 287, 237 nm.

***cis*-2-Amino-10-decarbamoyl-1-hydroxy-7-methoxymitosene (21)**: ^1H NMR (CD_3OD) δ 1.91 (s, 3 H), 3.80 (dd, J = 7.8, 12.1 Hz, 1 H), 3.90–3.94 (m, 1 H), 3.97 (s, 3 H), 4.49 (dd, J = 7.0, 12.1 Hz, 1 H), 4.74 (d, J = 13.8 Hz, 1 H), 4.83 (d, J = 13.8 Hz, 1 H), 4.85 (d, partially obscured by the H_2O peak); MS (–Cl) m/e (relative intensity) 262 (10), 245 (100); UV–vis λ_{max} (CH_3OH) 440, 350, 287, 237 nm.

***trans*-2-Amino-10-decarbamoyl-1-hydroxy-7-methoxymitosene (22)**: ^1H NMR (CD_3OD) δ 1.91 (s, 3 H), 3.85–3.95 (m, 1 H), 3.92 (dd, J = 4.3 Hz, partially obscured by C(7)OCH_3 peak, 1 H), 3.97 (s, 3 H), 4.52 (dd, J = 5.8, 12.4 Hz, 1 H), 4.82 (d, partially buried beneath the H_2O peak, 1 H), 4.73 (d, J = 13.4 Hz, 1 H), 4.80 (d, J = 13.4 Hz, 1 H); MS (–Cl) m/e (relative intensity) 247 (14), 246 (100); UV–vis λ_{max} (CH_3OH) 440, 350, 287, 237 nm.

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