Studies on the Use of $Cr(ClO_4)_2$ for the Reductive Activation of Mitomycin C†

Y. P. Hong and Harold Kohn*

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77204-5641. *Received November 26, 1990*

Abstract: $Cr(ClO_4)_2$ has been shown to be a highly efficient reductant of the anticancer agent, mitomycin C (**1**). Two different $Cr(ClO_4)_2$-mediated reductive techniques were developed and utilized in buffered water and methanolic solutions. In the first procedure, $Cr(ClO_4)_2$ (1–2 equiv) was directly added to **1** at various "pH" values. Key observations included the following: (1) Consumption of mitomycin C was rapid and generated as the major products *trans*- and *cis*-10-decarbamoyl-1-hydroxy-2,7-diaminomitosenes (**11** and **12**), and *trans*- and *cis*-10-decarbamoyl-1,10-dimethoxymitosenes (**16** and **17**) in acidic-to-neutral aqueous and methanolic solutions, respectively. (2) Between "pH" 6.0 and 7.0, the difunctionalized mitosene adducts accounted for nearly half of the product profile even though noticeable amounts of unreacted **1** remained. (3) Significant amounts of C-1 electrophilic products were not observed under acidic conditions. The product profiles observed with the second $Cr(ClO_4)_2$-mediated reductive procedure were markedly different. Activation of **1** was accomplished by the prior addition of $Cr(ClO_4)_2$ to excess *cis*-10-decarbamoyl-1,10-dimethoxymitosene (**17**) to generate the putative mitosene monochromate **20** and mitosene dichromate **21** species in situ, followed by the addition of **1** (1 equiv per $Cr(ClO_4)_2$). The products obtained by using this protocol were similar to those observed with conventional reductants in which C-1 electrophilic adducts predominated in acid, C-1 nucleophilic products were the major products under neutral and basic conditions, and little modification of the C-10 site was detected throughout the "pH" range examined. The product profiles coupled with select auxiliary experiments have provided information concerning the mechanism of both reductive procedures. The major products furnished by using the direct $Cr(ClO_4)_2$-mediated procedure under acidic and neutral conditions have been attributed to the two one-electron reductions of **1** to give the bis-Cr^{III}-bound species **22**. Complexation of the C-5 and C-8 phenolic-type oxygens in reduced **1** is believed to facilitate the loss of methanol at C-9 and C-9a in **1** and the nucleophilic substitution processes at C-1 and C-10 as well as inhibit the electrophilic transformations at both DNA bonding sites. Explanations and supporting data have also been provided to account for the other products detected in these reactions. Correspondingly, the second procedure is conjectured to occur by an outer-sphere electron-transfer process from **20** and/or **21** to **1** to give the uncomplexed hydroquinone (or semiquinone) mitomycin C species **2**. Subsequent loss of methanol at C-9 and C-9a yields the activated mitosene capable of furnishing the C-1 functionalized adducts **7** and **9** + **10**. The distinctive product profiles observed with the direct addition of $Cr(ClO_4)_2$ to **1** and the remarkable high yields of C-1, C-10 dinucleophilic substitution adducts suggest that similar pathways may be operative in the in vivo process to provide the DNA–mitomycin C cross-link adducts. These notions are discussed in light of the DNA sequence selectivity recently observed for the drug monoalkylation bonding process.

Mitomycin C (**1**) is a member of a class of antibiotics that exhibit potent, specific antitumor activity.[1] The cytotoxic effects of the mitomycins have been attributed to the bioreductive alkylation of DNA.[2] In the most widely accepted mechanism (Scheme I),[2a,b,f] attachment of the drug to the genetic material occurs at C-1 and C-10 in **1**. Special emphasis has been placed on the ability of mitomycin C to cross-link complementary strands of DNA and inhibit DNA replication and subsequent cell division.[2a,g] Accordingly, investigations have been conducted which have focused on discerning the factors which control the C-1 and C-10 modification steps.[3-16] It has been determined that under a variety of reductive conditions (i.e., enzymatic,[3b,4,7a,9] catalytic,[3b,8d,12a] chemical,[4,5b,c] electrochemical,[6,7b,12c] pulse radiolysis[10,13]) activation of the carbon-1 site in **1** proceeds rapidly at moderate pH values (i.e., pH 7–8.5) to give the C-1 cis and trans nucleophilic substituted diastereomeric products, while under slightly acidic conditions (i.e., pH 5–6) C-1 electrophilic transformations predominate. It is also known that C-10 modification processes occur less readily than C-1 reactions due to the diminished reactivity of this site.[5b,c,8a,12c-g,17] Under select conditions both reductive nucleophilic and electrophilic substitution processes have been observed at this site, however. Use of strong nucleophiles[5b,c,8a,12d,e,g] or concentrated solutions[17] have provided access to C-10 nucleophilic substitution adducts, while in base and in the absence of external nucleophiles the corresponding C-10 electrophilic products were obtained.[12e,f]

The notion that mitomycin C cytotoxicity and antitumor activity is associated with the ability of the drug to cross-link DNA has spurred the development of new reductive procedures to efficiently activate **1** toward nucleophilic substitution processes.[12b,f,18] Recently, we reported that stoichiometric amounts of $Cr(ClO_4)_2$ in water at moderate pH values (i.e., pH 6–7) led to the efficient functionalization of *both* DNA bonding sites in mitomycin C.[18] In this paper, a full account of the use of this novel technique in both aqueous and methanolic solutions is provided.[19] Detailed

† Dedicated to Professor Ronald Breslow in honor of his 60th birthday.

(1) Carter, S. K.; Crooke, S. T. *Mitomycin C. Current Status and New Developments*; Academic Press: New York, 1979.
(2) (a) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55. (b) Patrick, J. B.; Williams, R. P.; Meyer, W. E.; Fulmor, W.; Cosulich, D. B.; Broschard, R. W. *J. Am. Chem. Soc.* **1964**, *86*, 1889. (c) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979; Vol. 1, pp 221–276. (d) Franck, R. W.; Tomasz, M. In *The Chemistry of Antitumor Agents*; Wilman, D. E. V., Ed., Blackie and Son, Ltd.: Glasgow, 1990; pp 379–394. (e) Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**, *32*, 411. (f) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249. (g) Zwelling, L. A.; Anderson, T.; Kohn, K. W. *Cancer Res.* **1979**, *39*, 365. (h) Cera, C.; Egbertson, M.; Teng, S. P.; Crothers, D. M.; Danishefsky, S. J. *Biochemistry* **1989**, *28*, 5665.
(3) (a) Tomasz, M.; Mercado, C. M.; Olson, J.; Chatterjie, N. *Biochemistry* **1974**, *13*, 4878. (b) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056.
(4) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science* **1987**, *235*, 1204.
(5) (a) Hornemann, U.; Ho, Y.-K.; Mackey, J. K.; Srivastava, S. C. *J. Am. Chem. Soc.* **1976**, *98*, 7069. (b) Hornemann, U.; Keller, P. J.; Kozlowski, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 7121. (c) Hornemann, U.; Iguchi, K.; Keller, P. J.; Kozlowski, J. F.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5026.
(6) Rao, G. M.; Beglieter, A.; Lown, J. W.; Plambeck, J. A. *Electrochem. Soc.* **1977**, *124*, 199.
(7) (a) Pan, S.-S.; Andrews, P. A.; Glover, C. J.; Bachur, N. R. *J. Biol. Chem.* **1984**, *259*, 959. (b) Andrews, P. A.; Pan, S.-S.; Bachur, N. R. *J. Am. Chem. Soc.* **1986**, *108*, 4158.
(8) (a) Danishefsky, S. J.; Egbertson, M. *J. Am. Chem. Soc.* **1986**, *108*, 4648. (b) Egbertson, M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 2204. (c) Egbertson, M.; Danishefsky, S. J.; Schulte, G. *J. Org. Chem.* **1987**, *52*, 4424. (d) Danishefsky, S.; Ciufolini, M. *J. Am. Chem. Soc.* **1984**, *106*, 6424.
(9) Peterson, D. M.; Fisher, J. *Biochemistry* **1986**, *25*, 4077.
(10) Hoey, B. M.; Butler, J.; Swallow, A. J. *Biochemistry* **1988**, *27*, 2608.
(11) Murakami, H. *J. Theor. Biol.* **1966**, *10*, 236.
(12) (a) Kohn, H.; Zein, N. *J. Am. Chem. Soc.* **1983**, *105*, 4105. (b) Zein, N.; Kohn, H. *J. Am. Chem. Soc.* **1987**, *109*, 1576. (c) Kohn, H.; Zein, N.; Lin, X. Q.; Ding, J.-Q.; Kadish, K. M. *J. Am. Chem. Soc.* **1987**, *109*, 1833. (d) Bean, M.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5033. (e) Bean, M.; Kohn, H. *J. Org. Chem.* **1985**, *50*, 293. (f) Zein, N.; Kohn, H. *J. Am. Chem. Soc.* **1986**, *108*, 296. (g) Russell, D. J.; Kohn, H. *Heterocycles* **1990**, *31*, 233.

0002-7863/91/1513-4634$02.50/0

Scheme I. The Moore (Iyer-Szybalski) Mechanism for the Mode of Action of Mitomycin C

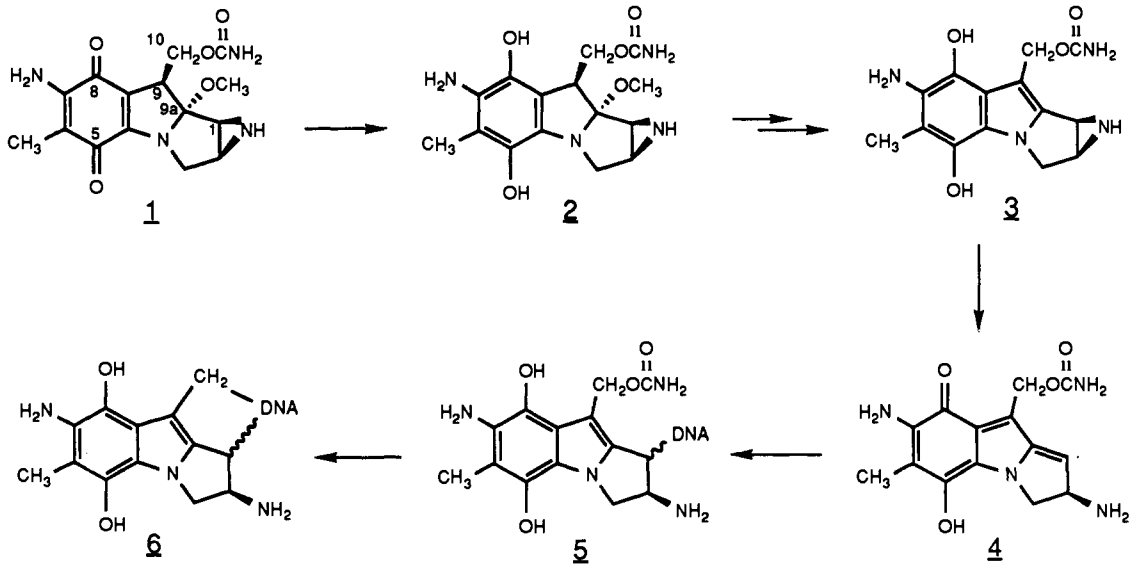
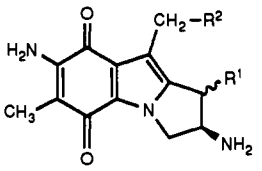


Table I. Product Profile Observed for the Direct Addition of $Cr(ClO_4)_2$ to Mitomycin C (1) in Water[a]



pH[b]	equiv[c]	R^1 = H, R^2 = $OC(O)NH_2$ **7**	R^1 = H, R^2 = OH **8**	R^1 = OH, R^2 = $OC(O)NH_2$ *trans*-**9**	R^1 = OH, R^2 = $OC(O)NH_2$ *cis*-**10**	R^1 = OH, R^2 = OH *trans*-**11**	R^1 = OH, R^2 = OH *cis*-**12**	MMC[d] (1)	%[e] C-1, C-10	%[f] C-1 nucl	%[g] C-10 nucl
5.10	1	5.1 (5.1)	3.3 (3.3)	3.9 (3.9)	5.1 (5.1)	35.5 (35.5)	47.1 (47.1)	0 [100]	85.9	91.6	100
5.00	2	1.8 (1.8)	11.3 (11.3)	0 (0)	0 (0)	35.4 (35.4)	51.5 (51.5)	0 [100]	98.2	86.9	100
6.04	1	26.6 (28.8)	6.3 (6.8)	92 (10.0)	9.6 (10.4)	20.0 (21.7)	20.6 (22.3)	7.7 [92.3]	46.9	69.4	100
6.00	2	2.2 (2.2)	13.3 (13.3)	0 (0)	0 (0)	40.1 (40.1)	44.4 (44.4)	0 [100]	97.8	84.5	100
6.94	1	11.5 (15.4)	11.4 (15.3)	11.5 (15.4)	9.7 (13.0)	16.6 (22.2)	14.0 (18.7)	25.3 [74.7]	42.0	69.3	100
7.00	2	1.6 (1.6)	24.2 (24.2)	3.1 (3.1)	3.5 (3.5)	35.8 (35.8)	31.8 (31.8)	0 [100]	91.8	74.2	100
8.00[h]	1	2.2 (3.8)	0 (0)	26.6 (45.9)	24.5 (42.2)	2.5 (4.3)	2.2 (3.8)	42.0 [58.0]	4.7	96.2	100

[a] Reaction was initiated by the addition of an aqueous solution of $Cr(ClO_4)_2$ to a deaerated, aqueous buffered solution of mitomycin C (MMC) (final concentration: 1.2 mM). The reactions were allowed to proceed for 30 min at room temperature, opened to the air, and analyzed. All reactions were run in duplicate and averaged. The values reported in parentheses are the percentage yields of products after excluding unreacted MMC. [b] Bis·tris·HCl (0.2 M) was employed as the buffer in the pH 5 and 6 reactions, while tris·HCl (0.2 M) was utilized in the pH 7 and 8 transformations. [c] Number of equivalents of $Cr(ClO_4)_2$ per equivalent of mitomycin C. [d] The percent values in brackets corresponds to the total percent C-1 substituted and C-1, C-10-disubstituted MMC-based compounds in the reaction mixture. [e] The percent of MMC compounds modified at both the C-1 and C-10 sites. The percentage calculation includes unreacted MMC. [f] Nucleophilic compounds are defined as all compounds in which reaction has led to an introduction of a hydroxy group at C-1. The percentage calculation does not include unreacted MMC. [g] Nucleophilic compounds are defined as all compounds in which reaction has led to the introduction of a hydroxy group at C-10. The percentage calculation does not include unreacted MMC and only C-1 modified adducts. [h] Use of 2 equiv of $Cr(ClO_4)_2$ at pH 8.00 led to significant decomposition of products.

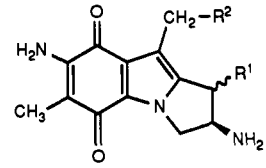
analyses of the product profiles along with supporting experiments have permitted us to propose a mechanism for the formation of the C-1, C-10 dinucleophilic substitution products. It is further suggested that comparable factors which permit activation of these positions in the $Cr(ClO_4)_2$-mediated experiments may be operative in the biological reductive process as well.

Results and Discussion

Two $Cr(ClO_4)_2$-mediated reductive methods[20] were developed. The results obtained by using these two techniques were dramatically different indicating that different pathways for the mitomycin C activation process existed in the two procedures. In the first method, activation of mitomycin C was accomplished by the direct addition of a standardized aqueous solution of $Cr(ClO_4)_2$ (1–2 equiv) to a deaerated buffered aqueous or methanolic solution of the drug. Tables I and II list the average percent yields for the observed products for duplicate experiments as a function of both "pH" and the number of equivalents of $Cr(ClO_4)_2$ employed versus **1** for the aqueous and methanolic studies, respectively. Addition of the reductant led to the rapid decoloration of the blue-violet solution characteristic of **1**. At the higher "pH" values and 2 equiv of $Cr(ClO_4)_2$ the solution remained nearly colorless

(13) Machtalere, G.; Houee-Levin, C.; Gardes-Albert, M.; Ferradini, C.; Hickel, B. *C. R. Acad. Sci. Paris* **1988**, *307* (Serie II), 17.
(14) Weissbach, A.; Lisio, A. *Biochemistry* **1965**, *4*, 196.
(15) Goldberg, I. H.; Friedman, P. A. *Annu. Rev. Biochem.* **1971**, *40*, 775.
(16) Kennedy, K. A.; Rockwell, S.; Sartorelli, A. C. *Cancer Res.* **1980**, *40*, 2356.
(17) (a) Borowy-Borowski, H.; Lipman, R.; Chowdary, D.; Tomasz, M. *Biochemistry* **1990**, *29*, 2992. (b) Borowy-Borowski, H.; Lipman, R.; Tomasz, M. *Biochemistry* **1990**, *29*, 2999.
(18) For a preliminary account of this study, see: Kohn, H.; Hong, Y. P. *J. Am. Chem. Soc.* **1990**, *112*, 4596.
(19) For an earlier report on the use of $Cr(Cl)_2$ for the reduction of benzoquinone and related compounds, see: Hanson, J. R.; Mehta, S. *J. Chem. Soc. C* **1969**, 2349.
(20) (a) Kochi, J. K.; Singleton, D. M.; Andrews, L. J. *Tetrahedron* **1968**, *24*, 3503. (b) Kochi, J. K.; Powers, J. W. *J. Am. Chem. Soc.* **1970**, *92*, 136. (c) House, H. O.; Kinloch, E. F. *J. Org. Chem.* **1974**, *39*, 1173.

Table II. Product Profile Observed for the Direct Addition of $Cr(ClO_4)_2$ to Mitomycin C (**1**) in Methanol[a]



"pH"[b]	equiv[c]	R^1 = H, R^2 = $OC(O)NH_2$ **7**	R^1 = H, R^2 = OCH_3 **13**	R^1 = OCH_3, R^2 = $OC(O)NH_2$ *trans*-**14**, *cis*-**15**[d]	R^1 = OCH_3, R^2 = OCH_3 *trans*-**16**, *cis*-**17**[d]	R^1 = OCH_3, R^2 = H *trans*-**18**, *cis*-**19**[d]	unknowns[e]	MMC[f] (**1**)	%[g] C-1, C-10	%[h] C-1 nucl	%[i] C-10 nucl
5.00	1	20.3 (22.4)	3.4 (3.7)	30.3 (33.5)	36.5 (40.4)			9.5 [90.5]	39.9	73.9	100.0
	2	3.2 (3.2)	11.6 (11.6)	3.2 (3.2)	82.0 (82.0)			0 [100.0]	93.6	85.2	100.0
6.00	1	18.8 (25.7)	3.3 (4.6)	19.9 (23.7)	31.0 (42.4)			27.0 [73.0]	34.3	69.7	100.0
	2	9.9 (10.2)	8.8 (9.1)	6.2 (6.5)	71.6 (74.2)			3.5 [96.5]	80.4	80.7	100.0
7.00	1	15.1 (25.2)	2.9 (4.9)	15.4 (25.6)	26.6 (44.3)			40.0 [60.0]	29.5	69.9	100.0
	2	9.7 (10.2)	8.1 (8.5)	5.7 (6.0)	68.6 (72.2)	2.9 (3.1)		5.0 [95.0]	79.6	81.3	96.3
8.00	1	11.9 (22.8)	2.4 (4.6)	14.0 (26.7)	12.7 (24.1)	2.3 (4.3)	9.2 (17.5)	47.5 [52.5]	17.4	72.6	87.0
	2	15.4 (16.4)	6.4 (6.7)	9.2 (9.7)	31.8 (33.5)	2.7 (2.8)	29.6 (31.1)	4.9 [95.1]	40.9	77.1	93.5
9.00	1	3.7 (7.7)	1.4 (2.8)	20.8 (42.8)	3.5 (7.3)	3.5 (7.3)	15.7 (32.1)	51.4 [48.6]	8.4	89.5	58.0
	2	5.1 (6.4)	3.7 (4.7)	14.1 (17.7)	7.4 (9.2)	3.5 (4.3)	46.1 (57.7)	20.1 [79.9]	14.6	88.9	76.4

[a] Reaction was initiated by the addition of an aqueous solution of $Cr(ClO_4)_2$ to a deaerated, buffered methanol solution (2 mL) of mitomycin C (MMC) (final concentration: 1.2 mM). Water percent by volume in the reaction was 1.2% for the 1 equiv reactions and 2.5% for the 2 equiv reactions. The reactions were allowed to proceed for 1 h at room temperature, opened to the air, and analyzed. All reactions were run in duplicate and averaged. The values reported in parentheses are the percentage yields of products after excluding unreacted MMC. [b] Bis·tris·HCl (0.2 M) was employed as the buffer in the "pH" 5, 6, and 7 reactions, bis·tris·HCl (0.13 M) + tris·HCl (0.07 M) was used in the "pH" 8 transformations, and bis·tris·HCl (0.1 M) + tris·HCl (0.1 M) was utilized in the "pH 9" reactions. [c] Number of equivalents of $Cr(ClO_4)_2$ per equivalent of mitomycin C. [d] The ratio of trans-to-cis C-1 methoxy adducts was approximately 1:1. [e] Four unknown peaks (18.2, 19.8, 20.7, and 20.9 min) were detected by HPLC analysis. [f] The percent values in brackets corresponds to the total percent C-1 substituted and C-1, C-10-disubstituted MMC-based compounds in the reaction mixture. [g] The percent of MMC compounds modified at both the C-1 and C-10 sites. The percentage calculation includes unreacted MMC. [h] Nucleophilic compounds are defined as all compounds in which reaction has led to an introduction of a methoxy group at C-1. The unknowns were included as C-1 nucleophilic products in this calculation. The percentage calculation does not include unreacted MMC. [i] Nucleophilic compounds are defined as all compounds in which reaction has led to the introduction of a methoxy group at C-10. The percentage calculation does not include unreacted MMC and only C-1 modified adducts.

for the entire reaction period, while at the lower pH values and with 1 equiv of reductant the colorless solution rapidly turned blue-red. After 30–60 min, the reactions were exposed to air and analyzed by HPLC and TLC. The identities of all products in the chromatograms were confirmed by coinjection or cospotting of an authentic sample of each adduct with the reaction mixture. Included for each reaction in Tables I and II is the percentage of products in which drug activation occurred at least at C-1 (the percentage of mitomycin C consumption), the percentage of the total reaction mixture in which modification of **1** has occurred at both C-1 and C-10, and the relative percent of *modified products* in which nucleophilic processes have occurred at each of the two DNA bonding sites, C-1 and C-10. In the methanol reactions, at "pH" 8.00 four unidentified peaks were detected in the HPLC. At "pH" 9.00 these adducts comprised a significant fraction of the reaction profile. Attempts to determine the identity of these compounds by performing a comparable experiment on semipreparative scale were unsuccessful.

Analysis of the product profile obtained from the aqueous and methanolic studies revealed several important findings. These include the following: (1) Consumption of **1** in water and methanol was rapid and generated both *trans*- and *cis*-10-decarbamoyl-1-hydroxy-2,7-diaminomitosenes[7b] (**11** and **12**) and *trans*- and *cis*-10-decarbamoyl-1,10-dimethoxy-2,7-diaminomitosenes (**16** and **17**), respectively, as the major products. (2) The reaction efficiency increased at lower pH values. By using 1 equiv of $Cr(ClO_4)_2$ at pH 5.10 in water no unreacted **1** was detected, while only 9.5% of mitomycin C was detected at "pH" 5.00 in methanol.[21] (3) Between "pH" 6.0 and 7.0, the difunctionalized mitosene adducts **8**, **11–13**, and **16–19** accounted for nearly half of the product profile even though noticeable amounts of unreacted **1** remained. (4) The major products in acid were not C-1 electrophilic adducts (i.e., **7**,[3b,12a] **8**, and **13**), while no C-10 electrophilic products were detected at pH 8.00 in water. (5) Elevation of the pH of the reaction led to a marked reduction in the percentage yield of C-1, C-10 difunctionalized products. Significantly, most of these observations are opposite to those reported for the activation of **1** using conventional reductants.[3b,7a,9,10,12a,c,g,13]

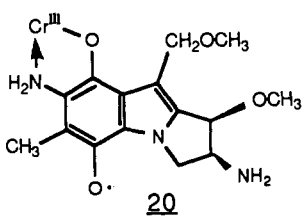
The second $Cr(ClO_4)_2$-mediated mitomycin C activation procedure involved the initial addition of the reductant to a deaerated aqueous or methanolic solution containing an excess of *cis*-10-decarbamoyl-1,10-dimethoxy-2,7-diaminomitosene (**17**) to generate the putative monochromate **20** and dichromate **21** species[22] in situ, followed by the addition of **1** (1 equiv per $Cr(ClO_4)_2$). The product profiles for the water and methanol studies are listed in Tables III and IV, respectively. Significantly, the percentage of C-1 nucleophilic products (i.e., **9–12** and **14–19**) in these experiments was highly dependent upon "pH". In moderately acidic aqueous solutions (Table III), the C-1 electrophilic adducts (i.e., **7** and **8**) accounted for the major products in the reaction,[23] while under neutral to slightly basic conditions the C-1 nucleophilic adducts predominated. Furthermore, unlike the direct $Cr(ClO_4)_2$-mediated procedure little activation of the C-10 site in **1** was detected by using this second method. Similar but less pronounced patterns were observed for the methanol-based experiments (Table IV). The results obtained for this second $Cr(ClO_4)_2$-mediated reductive technique were comparable to the trends previously reported for the activation of **1** by using chemical,[4,5b,c] catalytic,[3b,8d,12a] electrochemical,[6,7b,12c] and enzymatic[3b,4,7a,9] reductive methods.

The composite data permit us to propose the mechanistic hypotheses depicted in Schemes II and III for the two different $Cr(ClO_4)_2$-mediated reductive procedures. Key aspects of the proposed pathway (Scheme II) for the direct $Cr(ClO_4)_2$ addition

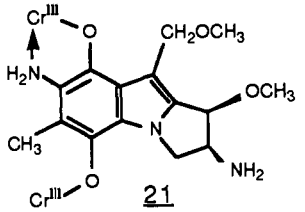
(21) Dissolution of **1** at these "pH" values (30–60 min) in the absence of reductant led to no significant solvolysis (<5%) of the starting material.

(22) For a discussion of ligand substitution reactions of Cr^{II} and Cr^{III} ions, see: Douglas, B. E.; McDaniel, D. H.; Alexander, J. J. *Concepts and Models of Inorganic Chemistry*, 2nd ed.; Wiley: New York, 1983; pp 334–339.

(23) The reduced efficiency of the mitomycin C activation process at the lower pH values by this procedure has been attributed (in part) to the net requirement for the consumption of 2 equiv of $Cr(ClO_4)_2$ per equiv of **7** generated.

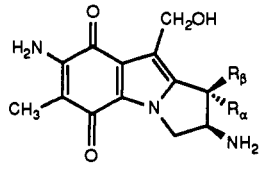


20



21

process are as follows: (1) Two one-electron reductions of the quinone ring in **1** by $Cr(ClO_4)_2$ proceed rapidly at moderate and low "pH" values to give the bis-Cr^{III}-bound complex **22**. (2) Loss of methanol from **22** yields **23** which undergoes preferential C-1 and C-10 nucleophilic substitution processes by an indole-assisted pathway. (3) Complexation by chromium ion of the phenolic-type oxygens at C-5 and C-8 in **23** and **24** inhibits C-1 and C-10 electrophilic transformations. Several key experimental findings supported this scenario. In particular, we noted the approximate correlation of the utilization of 2 equiv of $Cr(ClO_4)_2$ per equiv of **1** for the production of C-1, C-10 *di*substituted adducts at intermediate "pH" values (i.e., water, pH 6–7; methanol, "pH" 6–7). Furthermore, the absence of *trans*- and *cis*-10-decarbamoyl-1-methoxy-2,7-diaminomitosenes (**28** and **29**) in the methanolic studies was consistent with the proposal that formation of **11** and **12** in the water-based experiments did not occur by an acyl bond cleavage pathway of the C-10 carbamate group but rather by a nucleophilic substitution process.

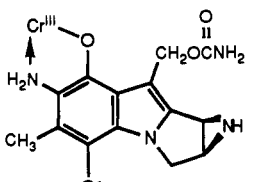


28 $R_\beta = H$, $R_\alpha = OCH_3$
29 $R_\beta = OCH_3$, $R_\alpha = H$

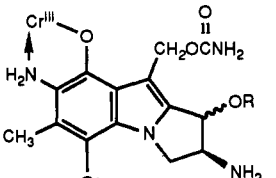
Scheme III outlines the pathway that best explains the majority of results observed in the second $Cr(ClO_4)_2$-mediated reductive procedure. This hypothesis is similar to the mechanism previously proposed for the activation of **1** by conventional reductants.[3b,8a,b] Initial activation of the drug is believed to proceed by an outer-sphere electron-transfer process from **20** and/or **21** to give the *uncomplexed* mitomycin C hydroquinone. Subsequent expulsion of the C-9a methoxy group followed by the loss of the acidic C-9 proton permits aziridine ring opening to give the extended quinone methide **4**. This species can then react with solvent to furnish both **27** (i.e., **9** (**11**), **10** (**12**), **14** (**16** and **18**), **15** (**17** and **19**)) and **7** (**8**). A comparable pathway can be drawn for the activation of the C-1 site in mitomycin C from the semiquinone reduction level in **1** as well.[24]

Inspection of the results in Table I suggests that the pathway outlined in Scheme II does not completely account for the entire data set, however. We noted that under moderate-to-acidic conditions drug consumption exceeded the stoichiometric amounts of $Cr(ClO_4)_2$ utilized. For example, at pH 5.10 in water using 1 equiv of $Cr(ClO_4)_2$ all the mitomycin C was modified and 85.9% of the product mixture consisted of the C-1, C-10 disubstituted products **8**, **11**, and **12**. Furthermore, Scheme II does not provide a rationale for the noticeable amounts (~30%) of C-1 electrophilic adducts produced at intermediate pH values in both water and methanol, the decreased efficiency of the C-10 mediated modification step at pH 8.00 in water and at "pH" 8.0–9.0 in methanol, and the concomitant rise in the percentage C-10 electrophilic adducts generated in the latter solvent at "pH" 8.0–9.0.

These observations suggest that competing processes existed in the $Cr(ClO_4)_2$-mediated reactions involving the direct addition of the reductant to **1**. Several likely candidates exist. One of these is that at moderate to acidic "pH" values a significant fraction of the products arise by an autocatalytic process[9,10] involving the transfer of one or two electrons from a reduced chromium-bound mitomycin C (i.e., **22**) or mitosene (i.e., **23** and **24**) species to **1**. This pathway would be expected to give rise to a product composition similar to that observed by using the second reductive procedure[9] (i.e., Tables III and IV), which was characterized by the preferential formation of C-1 electrophilic products in acid and C-1 nucleophilic adducts in base and the absence of appreciable amounts of C-1, C-10 disubstituted products. Support for this secondary process is provided by previous reports describing the proclivity of mitomycin C to undergo autocatalytic reductive processes.[9,10] Involvement of an autocatalytic pathway in the direct $Cr(ClO_4)_2$ addition processes provides an attractive way to account for the formation of the electrophilic adducts at "pH" 5–6 and the efficiency of these reactions at low "pH" values. This process does not rationalize the consistent formation of C-1 electrophilic adducts at moderate "pH" values and the low yields of C-10 modified adducts at the higher "pH"s employed in these reactions. Both results may stem from a decrease in the amount of the presumed bis-Cr^{III}-bound complexes **22**–**24** at "pH" 7–9.[25] Under these conditions, increased levels of the mono-Cr^{III}-bound species **30** and **31** may exist in solution. We suspect that nucleophilic displacement processes at C-10 from **31** in base will proceed less rapidly than those from **24** at lower "pH" values due to the decreased electron density of the indole ring in **31** versus **24** and the presumed need for acid for the cleavage of the C-10 carbamate group. Correspondingly, formation of **30** in the reaction may be responsible for the noticeable amounts of C-1 electrophilic adducts in the product mixture. Removal of the chromium ion from the O-5 site in reduced **23** may permit electrophilic transformations to proceed at C-1.[26]



30



31

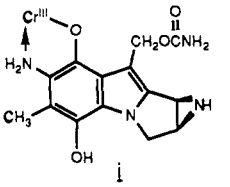
Select experiments were conducted to provide additional information concerning the likelihood of these mechanistic proposals for both the first and the second $Cr(ClO_4)_2$-mediated reductive procedures. First, employment of substoichiometric amounts of $Cr(ClO_4)_2$ in aqueous and methanolic acidic solutions (Table V, entries 1–4) gave qualitative support to the notion that formation of a bis-Cr^{III}-bound species **22** (**23** and **24**) is required for the activation of the C-10 site in **1** toward nucleophilic attack in the direct $Cr(ClO_4)_2$-mediated procedure. Reasonable correlations were observed for the utilizations of 2 equiv of reductant per equiv of **1** for the production of the dinucleophilic substituted adducts **11**, **12**, **16**, and **17**.

Second, replacement of the bis·tris·HCl buffer system (pH 5.0–6.0) in the direct addition of $Cr(ClO_4)_2$ experiments by bis·tris·acetic acid led to product profiles intermediate (data not shown) between that observed for the two different aqueous $Cr(ClO_4)_2$-mediated procedures (i.e., Tables I and III). For

(24) For studies concerning the one-electron pathway for the reductive activation of mitomycins, see refs 3a, 7a, 7b, 8a, 8b, 11, and 12c.

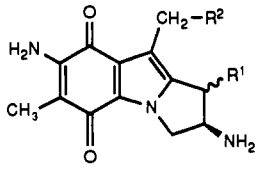
(25) Factors which may contribute to the projected lower amounts of **22**–**24** in solution at these higher "pH" values include the reduction in the conversion efficiency for **1** → **22** and the partial destruction of the reductant. Titration ($Ce(SO_4)_2$) of aqueous $Cr(ClO_4)_2$ solutions maintained for 30 min at pH 5.0 and 8.0 indicated a 5 and 15% loss of activity, respectively.

(26) For example, direct electron transfer from $Cr(ClO_4)_2$ or another reductant to **30**, followed by proton transfer, gives the monochromate hydroquinone species **i** which upon aziridine ring opening would be expected to undergo electrophilic substitution processes at C-1.



i

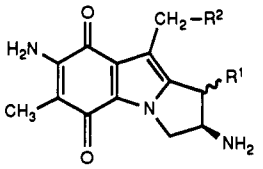
Table III. Product Profile Observed for the Indirect Addition of 1 Equiv of $Cr(ClO_4)_2$ to Mitomycin C (**1**) in Water[a]



pH[b]	$R^1 = H$, $R^2 = OC(O)NH_2$ **7**	$R^1 = H$, $R^2 = OH$ **8**	$R^1 = OH$, $R^2 = OC(O)NH_2$ *trans*-**9**	$R^1 = OH$, $R^2 = OC(O)NH_2$ *cis*-**10**	$R^1 = OH$, $R^2 = OH$ *trans*-**11**	$R^1 = OH$, $R^2 = OH$ *cis*-**12**	MMC[c] (**1**)	%[d] C-1, C-10	%[e] C-1 nucl	%[f] C-10 nucl
4.98	33.7 (62.9)	1.5 (2.8)	4.4 (8.2)	5.3 (9.8)	4.3 (8.0)	4.4 (8.3)	46.5 [53.5]	10.2	34.3	100
5.98	30.6 (64.3)		7.9 (16.5)	9.1 (19.2)			52.5 [47.5]	0	35.7	
7.01	5.5 (7.3)		32.5 (43.4)	37.0 (49.3)			25.1 [74.9]	0	92.7	
8.03	4.5 (4.8)		41.8 (45.4)	45.9 (49.8)			7.9 [92.1]	0	95.2	

[a] *cis*-1,10-Dimethoxymitosene (**17**) (2 equiv, final concentration: 1.3 mM) was dissolved in an aqueous buffered solution (2 mL) and deaerated, and then an aqueous $Cr(ClO_4)_2$ (1 equiv) solution was added and stirred at room temperature (5 min). A deaerated aqueous solution (0.5 mL) of mitomycin C (MMC) (1 equiv) was added, and the reaction was maintained at room temperature (30 min), exposed to air, and analyzed. All reactions were run in duplicate and averaged. The values reported in parentheses are the percentage yields of products after excluding unreacted MMC. [b] Bis·tris·HCl (0.2 M) was employed as the buffer in the pH 5 and 6 reactions, while tris·HCl (0.2 M) was utilized in the pH 7 and 8 transformations. [c] The precent values in brackets corresponds to the total percent C-1 substituted and C-1, C-10-disubstituted MMC-based compounds in the reaction mixture. [d] The percent of MMC compounds modified at both the C-1 and C-10 sites. The percentage calculation includes unreacted MMC. [e] Nucleophilic compounds are defined as all compounds in which reaction has led to an incorporation of a hydroxy group at C-1. The percentage calculation does not include unreacted MMC. [f] Nucleophilic compounds are defined as all compounds in which reaction has led to the introduction of a hydroxy group at C-10. The percentage calculation does not include unreacted MMC and only C-1 modified adducts.

Table IV. Product Profile Observed for the Indirect Addition of 1 Equiv of $Cr(ClO_4)_2$ to Mitomycin C (**1**) in Methanol[a]

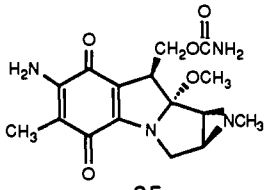


"pH"[b]	$R^1 = H$, $R^2 = OC(O)NH_2$ **7**	$R^1 = OCH_3$, $R^2 = OC(O)NH_2$ *trans*-**14**, *cis*-**15**[c]	$R^1 = OCH_3$, $R^2 = OCH_3$ *trans*-**16**, *cis*-**17**[c]	$R^1 = OCH_3$, $R^2 = H$ *trans*-**18**, *cis*-**19**[c]	MMC[d] (**1**)	%[e] C-1, C-10	%[f] C-1 nucl	%[g] C-10 nucl
6.05	28.1 (57.2)	19.2 (39.1)	1.8 (3.7)		50.9 [49.1]	1.8	42.8	100
8.05	16.3 (41.5)	20.7 (52.7)		2.3 (5.9)	60.7 [39.3]	2.3	58.5	0

[a] *cis*-1,10-Dimethoxymitosene (**17**) (2 equiv, final concentration: 1.3 mM) was dissolved in a buffered methanol solution (2 mL) and deaerated, and then an aqueous $Cr(ClO_4)_2$ (1 equiv) solution was added and stirred at room temperature (5 min). Water percent by volume in the reaction was 0.6%. A deaerated methanol solution (0.5 mL) of mitomycin C (MMC) (1 equiv) was added, and the reaction was maintained at room temperature (30 min), exposed to air, and analyzed. All reactions were run in duplicate and averaged. The values reported in parentheses are the percentage yields of products after excluding unreacted MMC. [b] Bis·tris·HCl (0.2 M) was used as the buffer in the "pH" 6 reactions, while bis·tris·HCl (0.13 M) + tris·HCl (0.07 M) was utilized in the "pH" 8 transformations. [c] The ratio of trans-to-cis C-1 methoxy adducts was approximately 1:1. [d] The percent values in brackets corresponds to the total percent C-1 substituted and C-1, C-10-disubstituted MMC-based compounds in the reaction mixture. [e] The percent of MMC compounds modified at both the C-1 and C-10 sites. The percentage calculation includes unreacted MMC. [f] Nucleophilic compounds are defined as all compounds in which reaction had led to an incorporation of a methoxy group at C-1. The percentage calculation does not include unreacted MMC. [g] Nucleophilic compounds are defined as all compounds in which reaction has led to the introduction of a methoxy group at C-10. The percentage calculation does not include unreacted MMC and only C-1 modified adducts.

example, at pH 5.0 in an aqueous bis·tris·acetic acid (0.2 M) solution all the mitomycin C was consumed using 1 equiv of reductant and 42.6% of the products were modified at C-10. Moreover, the percentage of adducts in which nucleophilic processes had occurred at C-1[27] and C-10 was 52.7 and 100%, respectively. This result was expected based on the previous report that acetic acid catalyzes the cleavage of Cr^{III}–oxygen bonds. Accelerated rupture of the chromium–oxygen bond in **22** (**23**) should promote C-1 electrophilic processes at the expense of nucleophilic transformations and decrease the amount of C-10 nucleophilic adducts formed.

Third, evidence in behalf of the proclivity of the autocatalytic pathway in "pH" 5–7 solutions was provided by introducing *N*-methylmitomycin C (**35**) into an aqueous reaction solution (pH 6.0) 20 min after **1** was activated by the addition of $Cr(ClO_4)_2$ (1 equiv). Analysis of the *N*-methylmitomycin C product profile indicated the significant formation (~35%) of C-1 electrophilic products and the absence of C-1, C-10 modified adducts.[28] A comparable profile was observed when the two mitomycins were reversed in this procedure. This pattern was reminiscent of the trends detected by using the second $Cr(ClO_4)_2$-mediated procedure (i.e., Table III) and have been interpreted that the initially modified chromium-bound mitosenes served as a primary electron source for the second mitomycin derivative.

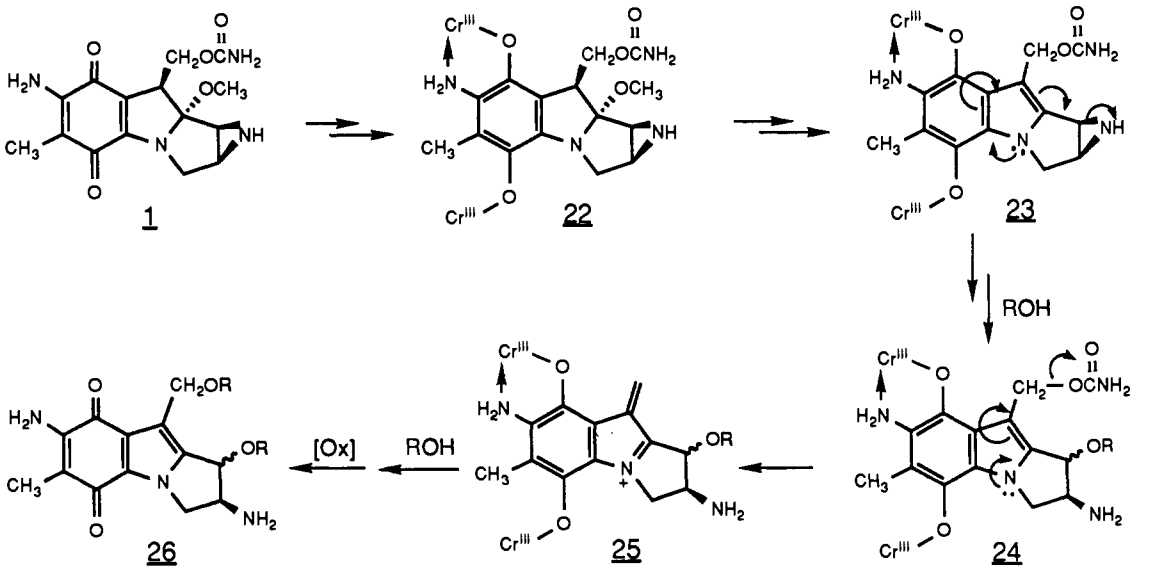


35

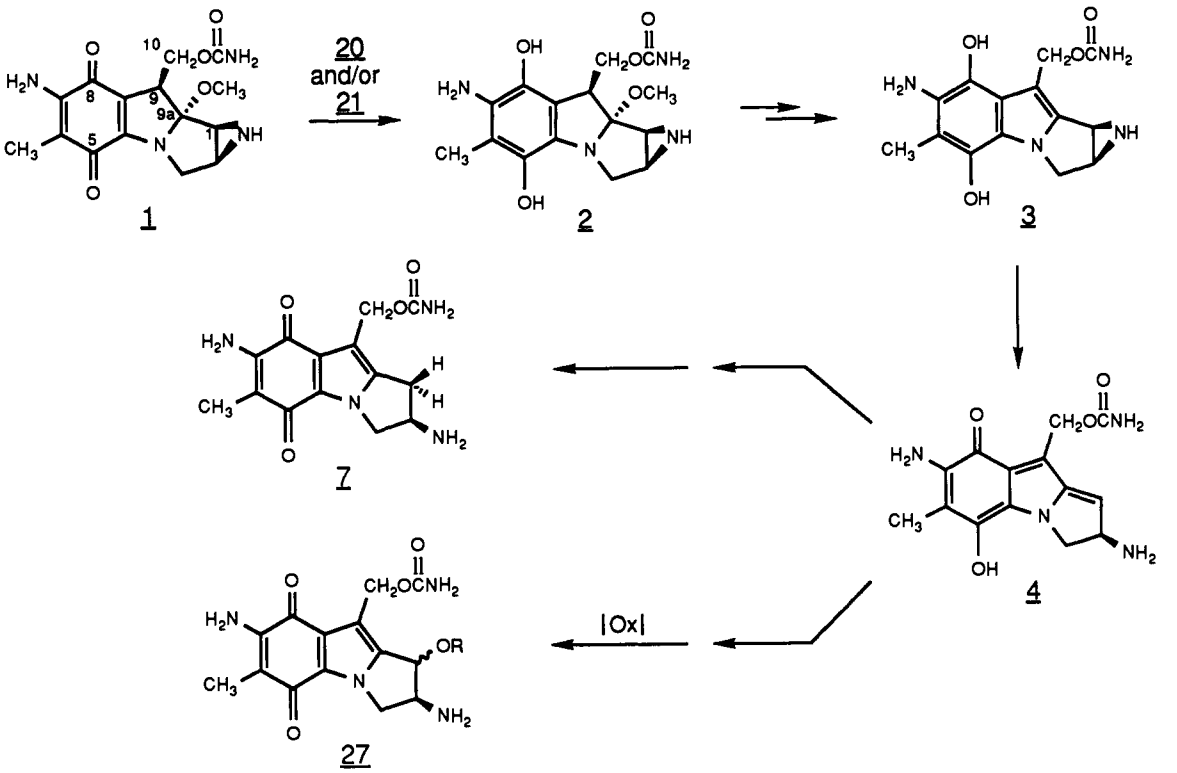
(27) An unusually high C-1 cis-to-trans product ratio (~2.6:1) was noted by using this buffer system versus the corresponding bis·tris·HCl buffer (~1.3:1). This trend has been attributed to the propensity of acetic acid to give preferential cis-substituted compounds, see: Verdine, G. L.; McGuinness, B. F.; Nakanishi, K. *Heterocycles* **1987**, *25*, 577.

(28) The mitomycin C product profile was similar to that reported in Table I, pH 6.00, 1 equiv of $Cr(ClO_4)_2$. The percent **1** consumed was 74.4%, the percent C-1, C-10 modification including **1** was 33.6%, and the relative percent of modified products excluding **1** in which nucleophilic processes occurred at C-1 and C-10 was 64.4% and 100%, respectively.

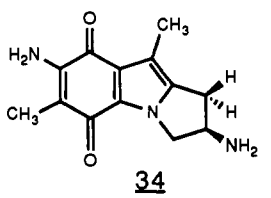
Scheme II. Predominant Pathway for the Reductive Activation of Mitomycin C by $Cr(ClO_4)_2$ in Water and Methanol



Scheme III. Proposed Pathway for Mitomycin C-1 Processes



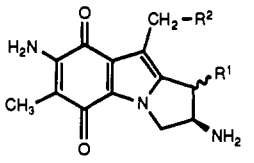
Fourth, experimental support that the C-1 modified adducts were viable precursors for the C-1, C-10 bis-substituted products (Scheme II) was provided by conversion of both **7** and a binary mixture of **9** and **10** to **8** and **11** and **12**, respectively, in water, and **7** and **14** to **13** and **16**, respectively, in methanol using 0.5–2.0 equiv of $Cr(ClO_4)_2$ (Table V, entries 5–13). Surprisingly, in these transformations noticeable amounts of the corresponding C-10 electrophilic products[12c,f] (i.e., **18**, **19**, **32–34**) were observed. Moreover, formation of the C-10 electrophilic products was found to increase with increasing amounts of $Cr(ClO_4)_2$. Information on the source of these C-10 electrophilic products was derived from the treatment of **1** in water and methanol and **7** in methanol with excess $Cr(ClO_4)_2$ (3–8.0 equiv) (Table V, entries 14–18). In these cases considerable amounts of the C-10 electrophilic adducts were also detected. Replacement of the water and methanol by deuterium oxide and methanol-d_1 in the reduction of **1** and **14** led to the production of **34**-d_2 and **18**-d_1, respectively, in which a single deuterium was incorporated in the C-10 methyl group of these adducts. We have attributed these products (in part) to the rapid, direct reduction of the C-10 carbamate group in both complexed and uncomplexed mitosenes by the excess $Cr(ClO_4)_2$ present in solution.[29]



34

(29) Attempts to reduce 2-naphthalenemethanol-*O*-carbamate by a similar procedure (CH_3OH, "pH" 9.0, $Cr(ClO_4)_2$ (4 equiv), 25 °C, 18 h) were unsuccessful.

Table V. Product Profiles Observed for Auxiliary Experiments Involving the Direct Addition of $Cr(ClO_4)_2$ to Mitomycin C (1) or Select Mitosenes in Either Water or Methanol[a]



entry	sm[b]	solvent	"pH"[c]	equiv[d]	R^1 = H R^2 = $OC(O)NH_2$ **7**	R^1 = H R^2 = $OH(OCH_3)$ **8, 13**	R^1 = $OH(OCH_3)$ R^2 = $OC(O)NH_2$ **9, 10, 14, 15**	R^1 = $OH(OCH_3)$ R^2 = $OH(OCH_3)$ **11, 12, 16, 17**	R^1 = $OH(OCH_3)$ R^2 = H **32, 33, 18, 19**	R^1 = H R^2 = H **34**	MMC[e] (1)	%/ C-1, C-10	%[g] C-1 nucl	%[h] C-10 nucl
1	**1**	H_2O	5.5	0.2	8.5 (29.6)	1.9 (6.6)	6.7 (23.3)	11.6 (40.4)			71.3 [28.7]	13.5	63.7	100
2	**1**	H_2O	5.5	0.5	19.4 (31.2)	3.8 (6.1)	11.9 (19.1)	27.1 (43.6)			37.8 [62.2]	30.9	62.7	100
3	**1**	H_2O	6.0	0.5	11.0 (27.9)	1.5 (3.8)	13.6 (34.5)	13.3 (33.8)			60.6 [39.4]	14.8	68.3	100
4	**1**	CH_3OH	6.0	0.5	12.0 (26.3)	1.1 (2.4)	18.6 (40.8)	13.9 (30.5)			54.4 [45.6]	15.0	71.3	100
5	**7**	H_2O	6.0	0.5	84.1	11.1				4.8		15.9		69.8
6	**7**	H_2O	6.0	1.0	59.7	27.6				12.7		40.3		68.5
7	**7**	H_2O	6.0	2.0	2.1	57.1				40.8		97.9		58.3
8	**9 + 10**	H_2O	6.0	1.0			43.5	37.9	18.6			56.5		67.1
9	**9 + 10**	H_2O	6.0	2.0			0	62.8	37.2			100		62.8
10	**7**	CH_3OH	6.0	1.0	56.3	38.5				5.2		43.7		88.1
11	**7**	CH_3OH	9.0	1.0	79.3	7.7				13.0		20.7		37.2
12	**14**	CH_3OH	6.0	1.0			9.6	75.2	15.2			90.4		83.2
13	**14**	CH_3OH	9.0	1.0			57.1	6.1	36.9			43.0		14.2
14	**1**	H_2O	6.0	4.0		30.7		46.4		22.9	0 [100]	100	46.4	77.1
15	**1**	H_2O	6.0	8.0		12.8		18.1	13.4	55.7	0 [100]	100	31.5	30.9
16	**1**	CH_3OH	6.0	4.0	0	21.6	0	68.3	5.9	4.2	0 [100]	100	74.2	89.9
17	**7**	CH_3OH	6.0	3.0	0	58.6				41.4		100		58.6
18	**7**	CH_3OH	9.0	3.0	0	40.6				59.4		100		40.6

[a] Reaction was initiated by the addition of an aqueous solution of $Cr(ClO_4)_2$ to a deaerated, aqueous, or methanolic buffered solution of mitomycin C (MMC) or mitosene (final concentration: 1.2 mM). The reactions were allowed to proceed at room temperature (30–60 min), opened to the air, and analyzed. All reactions were run in duplicate and averaged. The values reported in parentheses are the percentage yields of products after excluding unreacted MMC where appropriate. [b] sm = starting material. [c] Bis·tris·HCl (0.2 M) was employed as the buffer in the aqueous pH 5–6 reactions, while bis·tris·HCl (0.2 M) was employed as the buffer in the methanolic "pH" 6 reactions, and bis·tris·HCl (0.1 M) + tris·HCl (0.1 M) was utilized in the "pH" 9 reactions. [d] Number of equivalents of $Cr(ClO_4)_2$ per equivalent of mitomycin C or mitosene. [e] The percent values in brackets corresponds to the total percent C-1 substituted and C-1, C-10-disubstituted MMC-based compounds in the reaction mixture. [f] The percent of MMC compounds modified at both the C-1 and C-10 sites. The percentage calculation includes unreacted MMC. [g] Nucleophilic compounds are defined as all compounds in which reaction has led to an introduction of a hydroxy (methoxy) group at C-1. The percentage calculation does not include unreacted MMC. [h] Nucleophilic compounds are defined as all compounds in which reaction has led to the introduction of a hydroxy (methoxy) group at C-10. The percentage calculation does not include unreacted MMC and only C-1 modified adducts.

Table VI. Results Obtained from the Reduction of Mitomycin C (**1**) in Deuterated Solvents

entry	reductant	equiv	solvent	pD[a] ("pD")	product	site of C-1 D (%)[b]
1	**16**·$Cr(ClO_4)_2$[c]	2.0	D_2O	6.05	**7**-d_1	α (86)
2	$Cr(ClO_4)_2$[d]	2.0	D_2O	7.05	**8**-d_1	α (80)
3	$Cr(ClO_4)_2$[e]	2.0	CH_3OD	8.46	**7**-d_1	α (90)
					13-d_1	α (90)
4	$Cr(ClO_4)_2$[d]	8.0	D_2O	7.20	**34**-d_2[f]	α (57)
						β (43)
5	$Cr(ClO_4)_2$[e]	5.0	CH_3OD	9.41	**34**-d_2[f]	α (60)
						β (40)

[a] The pD of the solution was determined by using the following relationship: pD = pH meter reading + 0.45.[42] [b] Percent deuterium incorporation was determined by integration of the C-1α and C-1β proton signals in the ^1H NMR spectrum versus nearby resonances. [c] See Table III, footnote *a* for reductive activation procedure. [d] See Table I, footnote *a* for the reductive activation procedure. [e] See Table II, footnote *a* for the reductive activation procedure. [f] Analysis of the ^1H NMR spectrum indicated that a single deuterium was incorporated in the C-10 methyl group of **34**-d_2.

Fifth, comparable increases in the percentage of C-1 electrophilic products were observed in water and methanol with increasing amounts of reductant. For example, employing the direct addition of $Cr(ClO_4)_2$ protocol at pH 6.0 in water, the C-1 electrophilic adducts comprised 15.5 (Table I), 53.6 (Table V, entry 14), and 68.5% (Table V, entry 15) of the product mixture when 2, 4, and 8 equiv of reductant were employed, respectively,[30] while a more modest increase from 18.7 (Table II) to 25.8% (Table V, entry 16) was noted upon increasing the number of $Cr(ClO_4)_2$ equiv from 2 to 4 in methanol at "pH" 6.0. The analogous pattern was detected by using the second $Cr(ClO_4)_2$ procedure proceeding through the putative intermediates **20** and **21**. In water at pH 6.0, the percentage of C-1 electrophilic adducts (excluding unreacted **1**) increased from 64.3 (Table III) to 83.2% (data not shown) as the number of equiv of $Cr(ClO_4)_2$ utilized increased from 1.0 to 5.0. We have tentatively attributed these increases in the C-1 electrophilic products to the direct reductive cleavage of the aziridine ring in the activated mitosene species (i.e., **23**, **3**, and **30**) by the excess reductant present in solution. In support of this notion, treatment of the mitosene mimic, indano[1,2-*b*]-aziridine[31,32] (**36**), in methanol ("pH" 9.0) with $Cr(ClO_4)_2$ (2 equiv) led to the production of 2-aminoindane[33] (**37**).

36 **37**

Sixth, information concerning the factors which govern the C-1 electrophilic transformations was ascertained by performing the $Cr(ClO_4)_2$-mediated reductive activation procedures in deuterium oxide and methanol-d_1 with varying amounts of reductant versus **1** (Table VI). Use of 2 equiv of $Cr(ClO_4)_2$ and excess *trans*-

(30) No significant changes in the product profile were noted with the stronger reductant[20b] $Cr(en)_2(ClO_4)_2$ (0.5–2.0 equiv) at pH 6.0.

(31) Chiu, I.-C.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 2857.
(32) (a) Horwell, D. C.; Rees, C. W. *J. Chem. Soc., Chem. Commun.* **1969**, 1428. (b) Schmitz, E.; Janisch, K. *Khim. Geterot. Soed.* **1974**, *12*, 1629. (c) Hassner, A.; Heathcock, C. *Tetrahedron* **1964**, *20*, 1037.
(33) Aldrich Chemical Co.

Table VII. Comparison of the Product Profiles for the Direct Addition of 1 Equiv of $Cr(ClO_4)_2$ to Mitomycin C (**1**) in Water, Methanol, and Water–Methanol Mixtures at "pH" 6.0 and 8.0[a]

	"pH" 6.0			"pH" 8.0		
	H_2O[b]	CH_3OH–H_2O[c] (1/1.22)	CH_3OH[d]	H_2O[b]	CH_3OH–H_2O[e] (1/1.22)	CH_3OH[d]
%MMC[f]	7.7 (92.3)	11.4 (88.6)	27.0 (73.0)	42.0 (58.0)	42.9 (57.1)	47.5 (52.5)
%C-1, C-10[g]	46.9	44.8	34.3	4.7	14.9	17.4
%C-1 nucl[h]	64.3	67.4	69.7	96.2	82.7	73.0
ratio C-1 OCH_3/OH[i]		5.5 [6.7]			4.2 [5.1]	
%C-10 nucl[j]	100	100	100	100	100	87.0
ratio C-10 OCH_3/OH[i]		1.8 [2.2]			1.4 [1.7]	

[a] See Tables I and II, footnote *a* for the reduction activation procedure. [b] Data taken from Table I. [c] Bis·tris·HCl (0.2 M) was employed as the buffer. The molar ratio of methanol/water was 1:1.22. [d] Data taken from Table II. [e] Tris·HCl (0.2 M) was utilized as the buffer. The molar ratio of methanol/water was 1:1.22. [f] MMC = mitomycin C. The percent values in parentheses corresponds to the total percent C-1 substituted and C-1, C-10 disubstituted MMC-based compounds in the reaction mixture. [g] The percent of MMC compounds modified at both the C-1 and C-10 sites. The percentage calculation includes unreacted MMC. [h] Nucleophilic compounds are defined as all compounds in which reaction has led to an introduction of a hydroxy (methoxy) group at C-1. The percentage calculations does not include unreacted MMC. [i] The numbers in brackets are the values after statistical correction for the molar excess of water in solution. [j] Nucleophilic compounds are defined as all compounds in which reaction has led to the introduction of a hydroxy (methoxy) group at C-10. The percentage calculation does not include unreacted MMC and only C-1 modified adducts.

1,10-dimethoxy-10-decarbamoylmitosene (**16**) to activate **1** led to the stereoselective incorporation of deuterium at C-1 to give **7**-d_1[12a] (Table VI, entry 1). The site of incorporation has been tentatively assigned as the C-1 α-site based on the ^1H NMR chemical shift and coupling constant data. A comparable result was secured for the conversion of **1** to **7**-d_1 by using catalytic (PtO_2, H_2),[12a] electrochemical (Hg electrode, −1.2 V; Pt electrode, −1.0 V),[12c] and chemical ((4-methoxyphenyl)hydrazine)[34] methods, and a mechanism similar to that depicted in Scheme III has been proposed to account for this result. High stereospecific deuterium incorporation was also observed for the direct reduction of **1** in deuterium oxide and methanol-d_1 when 2 equiv of $Cr(ClO_4)_2$ were employed (Table VI, entries 2 and 3). Formation of **8**-d_1, **7**-d_1, and **13**-d_1 in these transformations is consistent with our suggestion that these electrophilic adducts are produced by an autocatalytic pathway similar to that depicted in Scheme III in which electron transfer occurs from a chromium-bound mitomycin derivative (i.e., **22–24**) to **1**. Of interest, the direct addition of excess $Cr(ClO_4)_2$ (5–8 equiv) to deuterium oxide and methanol-d_1 solutions of **1** led to a significant decrease in the stereospecificity of the deuterium incorporation process at C-1 (Table VI, entries 4 and 5). This result may be indicative that alternative pathways (i.e., direct aziridine reduction) are competitive with the route depicted in Scheme III for the formation of C-1 electrophilic adducts.

Seventh, insights into the role of the solvent in the direct Cr-$(ClO_4)_2$-mediated reductive activation of **1** and the mechanism of the C-1 and C-10 nucleophilic substitution processes were provided by comparison of the product profiles for the aqueous (Table I) and methanolic (Table II) studies performed at pH ("pH") 6.0 and 8.0 using 1 equiv of reductant with those obtained in a 1:1.22 molar mixture of methanol to water (Table VII). A decrease in the consumption of **1** was noted at both pH ("pH") values in proceeding from water to methanol–water mixtures to methanol. This result is consistent with the increased potentials needed to reduce **1** in methanol compared to water[12c,35] and the decreased ionizing power[36] and proton content of methanol versus water. These factors should act in concert to reduce the efficiency of the quinone reduction process, the loss of methanol at C-9 and C-9a in **22**, and the opening of the aziridine ring in **23**. Analysis of the C-1 and C-10 nucleophilic products for the methanol–water mixture reaction at "pH" 6.0 and 8.0 indicated that preferential substitution by methanol versus water had occurred at both sites. Furthermore, the ratio of the cis-to-trans C-1 hydroxy adducts was approximately 1.3 at "pH" 6.0, while the corresponding ratio for the two C-1 methoxy diastereomers was 1.3 and 1.1 at "pH" 6.0 and 8.0, respectively. The detection of near equal quantities of both pairs of isomers provided strong support that the C-1 substitution process proceeded by a S_N1-type mechanism. The similarity of the C-10 and C-1 methoxy/hydroxy ratios suggested that substitution at the C-10 position may have also occurred by a S_N1-type pathway. In both substitution processes, we have attributed the enhanced levels of C-1 and C-10 methoxy adducts versus the corresponding C-1 and C-10 hydroxy products to the increased nucleophilicity of methanol versus water.[37] Significantly, the methanol-to-water selectivity for the C-1 site (i.e., 5.1–6.7) was higher than that observed for the C-10 position (i.e., 1.7–2.2). This finding provides preliminary evidence that the resonance-stabilized carbocation at C-1 is more stable than the corresponding species generated at C-10.[38] This notion implies that the proclivity for reduced mitosenes to undergo substitution processes at C-1 versus C-10 is due not only to the ease of cleavage of the aziridine ring versus the C-10 carbamate group but also to the differences in stability of the two carbocations.

Conclusions

Analysis of the product profiles from both $Cr(ClO_4)_2$-mediated mitomycin C reductive procedures has provided new information concerning the factors which control the C-1 and C-10 substitution processes in the anticancer agent. Addition of 2 equiv of $Cr(ClO_4)_2$ to aqueous and methanolic solutions of **1** led to the efficient activation of both sites at moderate "pH" values to give exceptionally high levels of nucleophilic substitution products. In contrast, the second reductive process entailing the initial introduction of $Cr(ClO_4)_2$ to a *cis*-1,10-dimethoxy-10-decarbamoylmitosene (**17**) solution followed by the addition of **1** furnished principally C-1 electrophilic adducts in aqueous acid, C-1 nucleophilic products under neutral and basic conditions, and little or no modification of the C-10 site. The latter set of results mirrored those previously reported by using conventional reductants suggesting that a common pathway existed for these transformations which was distinct from the processes occurring in the direct addition of $Cr(ClO_4)_2$ to **1**.

The distinguishing feature of the proposed direct Cr-$(ClO_4)_2$-mediated reductive activation pathway for mitomycin C (Scheme II) is the conversion of the quinone ring in **1** to the

(34) Russell, D. J.; Kohn, H. Unpublished results.
(35) Rao, G. M.; Begleiter, A.; Lown, J. W.; Plambeck, J. A. *J. Electrochem. Soc.* **1977**, *124*, 199.
(36) (a) Grunwald, E.; Winstein, S. *J. Am. Chem. Soc.* **1948**, *70*, 846. (b) Fainberg, A. H.; Winstein, S. *J. Am. Chem. Soc.* **1956**, *78*, 2770.
(37) (a) Schadt, F. L.; Bentley, T. W.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1976**, *98*, 7667. (b) Bentley, T. W.; Schadt, F. L.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1972**, *94*, 993.
(38) This inference is only speculative in light of the known deviations to the reactivity–selectivity principle for the capture of carbocations by nucleophilic species. For a critical discussion, see: (a) Ta-Shma, R.; Rappoport, Z. *J. Am. Chem. Soc.* **1983**, *105*, 6082. (b) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 4689. (c) Pross, A. *Adv. Phys. Org. Chem.* **1977**, *14*, 69. (d) Johnson, C. D. *Chem. Rev.* **1975**, *75*, 755 and references therein.

bis-Cr^{III} bound species **22**. We expect that complexation of the C-5 and C-8 phenolic oxygens in **22** will allow the formal loss of methanol at C-9 and C-9a to yield **23**, facilitate the indole-assisted cleavage of the aziridine ring and expulsion of the C-10 carbamate group, and inhibit electrophilic transformations from proceeding at C-1 and C-10.

The striking product profile observed for the direct $Cr(ClO_4)_2$-mediated reduction of mitomycin C suggests that similar factors may be necessary for the efficient and selective utilization of the drug in in vivo processes. We suspect that formation of a *hydrogen bond* between the carbon-8 hydroxyl group in the activated mitomycin species with DNA bases within the minor groove will both align the C-1 and C-10 bonding sites in **1** in close proximity to nucleophilic residues present in DNA[39] and inhibit "self-destructive" C-1 and C-10 electrophilic and nucleophilic transformations from occurring with water. This scenario is compatible with recent information which has demonstrated that the mitomycin C-DNA monoalkylation event is sequence specific.[39] On the basis of the present study, DNA sequence selectivity for mitomycin C bonding is then projected to result from *both* the productive formation of a hydrogen-bonded mitomycin–DNA complex capable of undergoing nucleophilic substitution processes at C-1 and C-10 as well as the destruction of the activated mitomycin species by electrophilic and nucleophilic substitution pathways with the solvent when this drug-receptor site structural requirement is not met.[40] A ramification of this hypothesis is that 2,7-diaminomitosene (**7**) and *trans*- and *cis*-1-hydroxy-2,7-diaminomitosenes (**9** and **10**) should be significant metabolic products upon clinical administration of mitomycin C. We are unaware of any definitive studies directed toward identifying the various in vivo mitomycin C metabolites.[41] Finally, the proposed pathway for drug bonding suggests that C-10 transformations (i.e., cross-linking of complementary DNA strands) in **1** proceed best at the hydroquinone reduction level.

Experimental Section

Instrumentation and Solvents. Proton (^1H NMR) and carbon-13 (^{13}C NMR) nuclear magnetic resonance spectra were recorded on either a Nicolet NT-300 or General Electric QE-300 spectrometer. Chemical shifts are expressed in parts per million relative to Me_4Si, and coupling constants (J values) are given in hertz. Mass spectral data were obtained on a Finnegan TSQ-70 triple quadrupole mass spectrometer under negative Cl conditions by Dr. David Laude at University of Texas at Austin. High-resolution mass spectral studies were conducted by Dr. Marshall M. Siegel at Lederle Laboratories, Pearl River, NY. 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 peak areas in the HPLC were determined with Waters Associates 740 and 745 and Hewlett Packard 3392A integrators. The products were eluted by using the following linear gradient conditions: C_{18} μBondapak (SS) column 3.9 mm × 30 cm, 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. The column was fitted with a μBondapak C_{18} guardpak. A flow rate of 1.0 mL/min was used, and products were detected at 313 and 365 nm. The organic solvents utilized were HPLC grade, and they were filtered (Millipore FH, 0.5 μm) and degassed prior to use. The aqueous buffers were prepared from deionized water (Millipore) and were filtered (Millipore HA, 0.45 μm) and degassed. pH measurements were determined with either a Radiometer pHM 26 meter or pHM 84 research meter equipped with a Radiometer GK2320C combination glass electrode. The effective "pH" of the buffered methanolic solutions was determined by using a glass electrode, which was standardized against aqueous buffer solutions. The pD of solution for deuterated solvents was determined from the observed pH meter reading by using the relationship: pD = pH meter reading + 0.45.[42]

The solvents and reactions were of the best commercial grade available and used without further purification. All water used for the mitomycin C or mitosene 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 (20 × 20 cm; Analtech No. 02013). Solvent systems for chromatographies were A, methanol/chloroform (1:5); B, methanol/chloroform (1:9); C, methanol/chloroform (1:3); and D, acetone/methanol/chloroform (3:1:7).

Preparation and Titration of $Cr(ClO_4)_2$. The procedure of House and Kinloch[20c] was employed for the preparation of aqueous solutions of $Cr(ClO_4)_2$. A deaerated (Ar) aqueous solution (100 mL) including Cr (0.57 g, 11 mmol) and an aqueous 60% $HClO_4$ solution (1.1 mL, 10 mmol) was stirred at room temperature (1 day) under Ar atmosphere. The clear blue $Cr(ClO_4)_2$ solution prepared in this manner was stored at 5 °C and was found to be stable for several weeks. The aqueous $Cr(ClO_4)_2$ solution was assayed before use by adding a known aliquot to an aqueous solution of excess $FeCl_3$. The $FeCl_2$ generated was titrated with a standard $Ce(SO_4)_2$ solution (0.02 N) by using 1,10-phenanthroline as an indicator.

General Procedure for the Direct $Cr(ClO_4)_2$-Mediated Reductive Activation of Mitomycin C (1) in Water and Methanol. Mitomycin C (**1**) (1 mg, 3.0 μmol, final concentration: 1.2 mM) was added to a buffered aqueous or methanolic solution (2.5 mL) and then deaerated by passing a stream of Ar through the solution (10 min). The reaction was then initiated by the addition of an aqueous solution of $Cr(ClO_4)_2$ (1–2 equiv). The reaction was maintained under a positive pressure of Ar at room temperature (water: 30 min, methanol: 60 min), opened to the air, and analyzed. The water percent by volume in the methanolic solutions was 1.2% for the one equivalent reactions and 2.5% for the two equivalent transformations. In the aqueous reactions, bis·tris·HCl (0.2 M) was employed as the buffer in the pH 5 and 6 reactions, and tris·HCl (0.2 M) was used in the pH 7 and 8 transformations. In the methanolic reactions, bis·tris·HCl (0.2 M) was employed as the buffer in the "pH" 5, 6, and 7 reactions, bis·tris·HCl (0.13 M) + tris·HCl (0.07 M) was used in the "pH" 8 transformations, and bis·tris·HCl (0.1 M) + tris·HCl (0.1 M) was utilized in the "pH" 9 reaction. The pH of the solution was determined at the conclusion of the reaction and was shown to be within ±0.03 pH units of the original solution. Analysis of the product mixtures was conducted by HPLC and TLC. All products were identified by coinjection (cospotting) of an authentic sample with the reaction mixture in the HPLC (TLC). The HPLC retention times (min) for the mitomycin C derived adducts are as follows: **1** (19.1), **7** (19.7), **8** (18.2), **9** (17.5), **10** (18.5), **11** (16.4), **12** (17.1), **13** (21.0), **14** (20.0), **15** (20.3), **16** (22.5), **17** (23.0), **18** (22.8), **19** (24.0), **32** (18.0), **33** (20.5), and **34** (23.5). The peak area of the products and **1** in the HPLC chromatograms at 313 nm were adjusted to account for differences in the absorption coefficients of the mitosane and mitosene[3b] products by multiplying the observed area of the mitosane adducts by 3.6, and then the values were normalized to 100%. The R_f values for the products (system A) are as follows: **1** (0.41), **7** (0.15), **8** (0.25), **9**, **10**, **11**, **12** (0.14), **13** (0.47), **14** (0.27), **15** (0.34), **16** (0.59), **17** (0.65), **18** (0.65), **19** (0.71), **32** (0.29), **33** (0.32), and **34** (0.50).

Preparation of *trans*- and *cis*-10-Decarbamoyl-1,10-dimethoxy-2,7-diaminomitosenes (16 and 17). To a deaerated (Ar) methanol solution (50 mL, "pH" 5.0) of **1** (20 mg, 60 μmol) was added an aqueous solution of $Cr(ClO_4)_2$ (0.1 M, 1.2 mL, 2 equiv). The reaction solution was stirred at room temperature (1 h) under Ar, opened to the air, and neutralized with a saturated aqueous $NaHCO_3$ solution. The reaction mixture was concentrated in vacuo, and then water (20 mL) was added to the residue and extracted with methylene chloride (4 × 30 mL). The organic layers were combined, dried (Na_2SO_4), and evaporated to dryness. The residue was separated by preparative TLC (system B) to give pure **16** and **17**.

***trans*-10-Decarbamoyl-1,10-dimethoxy-2,7-diaminomitosene (16)**: ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 1.70 (s, 3 H), 3.28 (s, 3 H), 3.31 (s, 3 H), 3.96 (dd, J = 6.4, 13.3 Hz, 1 H), 3.94–3.99 (m, 1 H), 4.28 (dd, J = 5.3, 13.3 Hz, 1 H), 4.32 (s, 1 H), 4.50 (1/2 ABq, J = 12.6 Hz, 1 H), 4.54 (1/2 ABq, J = 12.6 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 8.36, 53.41, 55.99,

(39) Li, V.-S.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 275.
(40) For a related discussion, see ref 10.
(41) For earlier studies and related in vitro investigations, see: refs 3b, 7a, 9 and the following references: (a) Reich, S. D. In *Mitomycin C. Current Status and New Developments*; Carter, S. K.; Crooke, S. T., Eds.; Academic Press: New York, 1979; p 243. (b) Remers, W. A.; Dorr, R. In *Alkaloids: Chemical and Biological Perspective*; Vol. 6, Pelleter, S. W., Ed.; Wiley: New York, 1988; p 2. (c) van Hazel, G.; Kovach, J. S. *Cancer Chemother. Pharmacol.* **1982**, *8*, 189. (d) Schwartz, H.-S.; Philips, F. S. *J. Pharmac. Exp. Ther.* **1961**, *133*, 335. (e) Fujita, H. *Jpn. J. Clin. Oncol.* **1971**, *12*, 151. (f) Fujiwara, K.; Saikusa, H.; Yasuno, M.; Kitagawa, T. *Cancer Res.* **1982**, *42*, 1487. (g) Marshall, R. S.; Erlichman, C.; Rauth, A. M. *Cancer Res.* **1985**, *45*, 5939. (h) Kennedy, K. A.; Sligar, S. G.; Polomski, L.; Sartorelli, A. C. *Biochem. Pharmac.* **1982**, *31*, 2011.
(42) Kolthoff, I. M.; Elving, P. J. *Treatise on Analytical Chemistry. Part I. Theory and Practice*; Wiley: New York, 1978; pp 859–861, and references therein.
(43) 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.
(44) For a similar procedure for the preparation of compounds **14** and **15**, see: Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307.

57.46, 60.35, 65.03, 81.30, 104.60, 115.84, 121.03, 128.42, 138.70, 147.21, 176.51, 178.39 ppm; MS, *m/e* (rel intensity) 305 (25), 263 (100), 203 (13); M_r (EI) 305.1381 (calcd for $C_{15}H_{19}N_3O_4$ 305.1376).

***cis*-10-Decarbamoyl-1,10-dimethoxy-2,7-diaminomitosene (17)**: ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.71 (s, 3 H), 3.31 (s, 3 H), 3.32 (s, 3 H), 3.64 (dd, J = 9.1, 12.2 Hz, 1 H), 3.80–3.87 (m, 1 H), 4.33 (d, J = 4.9 Hz, 1 H), 4.44 (dd, J = 7.4, 12.2 Hz, 1 H), 4.49 (1/2 ABq, J = 12.6 Hz, 1 H), 4.58 (1/2 ABq, J = 12.6 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 8.39, 51.57, 56.30, 57.58, 58.31, 65.24, 74.68, 104.66, 115.93, 119.95, 128.75, 138.85, 147.19, 176.45, 178.44 ppm; MS, *m/e* (rel intensity) 305 (31), 263 (100), 203 (11); M_r (EI) 305.1370 (calcd for $C_{15}H_{19}N_3O_4$ 305.1376).

General Procedure for the $Cr(ClO_4)_2$-Mediated Reductive Activation of Mitomycin C (1) in Water and Methanol Using *cis*-10-Decarbamoyl-1,10-dimethoxy-2,7-diaminomitosene (17) and $Cr(ClO_4)_2$. *cis*-10-Decarbamoyl-1,10-dimethoxy-2,7-diaminomitosene (**17**) (2 mg, 6.5 μmol, 2 equiv, final concentration: 2.6 mM) was dissolved in an aqueous buffered solution (2 mL) and deaerated with Ar, and then an aqueous $Cr(ClO_4)_2$ (1 equiv) solution was added and stirred at room temperature (5 min). The water by volume in the methanolic solutions was 0.6%. A deaerated aqueous solution (0.5 mL) of **1** (1 mg, 3.0 μmol, 1 equiv, final concentration: 1.2 mM) was added, and the reaction was maintained at room temperature (30 min), exposed to air, and analyzed. The pH of the solution was taken at the conclusion of the reaction and was shown to be within ±0.03 pH units of the original solution. In the aqueous reactions, bis·tris·HCl (0.2 M) was employed as the buffer in the pH 5 and 6 reactions, and tris·HCl (0.2 M) was utilized in the pH 7 and 8 reactions. In the methanolic reactions, bis·tris·HCl (0.2 M) was employed as the buffer in the "pH" 6 reaction, and bis·tris·HCl (0.13 M) + tris·HCl (0.07 M) was utilized in the "pH" 8 reaction. The reactions were monitored by HPLC and TLC. All products were identified by coinjection (cospotting) of an authentic sample with the reaction mixture in the HPLC (TLC).

Preparation of Mitosenes. a. 2,7-Diaminomitosene (7).[3b,12a] H_2 gas was bubbled through a deaerated (Ar) mixture of **1** (50 mg, 0.15 mmol) in tetrahydrofuran (50 mL) and PtO_2 (20 mg, 0.09 mmol) at room temperature (70 s). The reduced mixture was transferred by cannula to a deaerated, aqueous buffered (bis·tris·HCl (0.05 M), pH 5.0) solution (20 mL) and stirred (5 min). The mixture was exposed to air and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography by using SiO_2 (system C) to give pure **7** (60% yield, R_f 0.15, system A): ^{1}H NMR (DMSO-d_6) δ 1.70 (s, 3 H), 2.24 (dd, J = 5.0, 16.2 Hz, 1 H), 2.95 (dd, J = 7.0, 16.2 Hz, 1 H), 3.69 (dd, J = 5.0, 12.4 Hz, 1 H), 3.98–4.01 (m, 1 H), 4.19 (dd, J = 6.5, 12.4 Hz, 1 H), 4.99 (s 2 H).

b. *trans*- and *cis*-1-Hydroxy-2,7-diaminomitosenes (9 and 10).[43] Mitomycin C (**1**) (50 mg, 0.15 mmol) was dissolved in an aqueous 0.05 N HCl solution (50 mL) and stirred at room temperature (30 min). The mixture was neutralized with a saturated aqueous $NaHCO_3$ solution, concentrated in vacuo, and dissolved in methanol (2 mL). This mixture was purified by preparative TLC (system C) to give **9** and **10** as a diastereomeric mixture.

c. *trans*- and *cis*-10-Decarbamoyl-1,10-dihydroxy-2,7-diaminomitosenes (11 and 12). To a deaerated (Ar) aqueous solution (100 mL, pH 5.0) of **1** (40 mg, 120 μmol) was added an aqueous solution of $Cr(ClO_4)_2$ (0.1 M, 2.4 mL, 2 equiv). The reaction solution was stirred at room temperature (30 min) under Ar, opened to the air, and neutralized with a saturated aqueous $NaHCO_3$ solution. The reaction solution was then passed through a Sephadex G-25 column by using aqueous 0.02 M NH_4HCO_3 as the eluent to remove the buffer and the chromium salts, and then the aqueous solution containing the products was concentrated in vacuo, and the residue was separated by column chromatography (SiO_2, system D) to yield pure **11** (R_f 0.16) and **12** (R_f 0.39).

***trans*-10-Decarbamoyl-1,10-dihydroxy-2,7-diaminomitosene (11)**: ^{1}H NMR (CD_3OD) δ 1.80 (s, 3 H), 3.84–3.89 (m, 1 H), 3.94 (dd, J = 4.0, 13.0 Hz, 1 H), 4.51 (dd, J = 6.2, 13.0 Hz, 1 H), 4.72 (1/2 ABq, J = 13.5 Hz, 1 H), 4.79 (1/2 ABq, J = 13.5 Hz, 1 H), 4.83 (d, J = 3.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) 8.08, 52.83, 55.24, 63.17, 73.39, 104.30, 118.72, 124.37, 127.58, 140.06, 146.57, 176.57, 178.60 ppm.

***cis*-10-Decarbamoyl-1,10-dihydroxy-2,7-diaminomitosene (12)**: ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.71 (s, 3 H), 3.58 (dd, J = 8.0, 12.2 Hz, 1 H), 3.73–3.80 (m, 1 H), 4.30 (dd, J = 7.1, 12.2 Hz, 1 H), 4.60 (s, 2 H), 4.66 (d, J = 5.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) 8.11, 51.50, 55.17, 57.76, 65.31, 104.76, 119.09, 120.03, 127.88, 140.54, 146.64, 176.57, 178.64 ppm; MS (–CI), *m/e* 277 (M^-).

d. *trans*- and *cis*-1-Methoxy-2,7-diaminomitosenes (14 and 15).[44] Compound **1** (50 mg, 0.15 mmol) was dissolved in methanol (50 mL) containing concentrated aqueous HCl (0.21 mL, 2.5 mmol) and stirred at room temperature (30 min). The solution was neutralized with a saturated aqueous $NaHCO_3$ solution, concentrated in vacuo, and dissolved in methanol (2 mL). This mixture was separated by preparative TLC (system A) to give pure **14** and **15**.

***trans*-1-Methoxy-2,7-diaminomitosene (14)**: ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 3 H), 3.29 (s, 3 H), 3.87 (dd, J = 6.6, 12.5 Hz, 1 H), 3.83–3.89 (m, 1 H), 4.19 (dd, J = 4.8, 12.5 Hz, 1 H), 4.36 (s, 1 H), 5.04 (s, 2 H).

***cis*-1-Methoxy-2,7-diaminomitosene (15)**: ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 3 H), 3.32 (s, 3 H), 3.54 (dd, J = 9.0, 11.9 Hz, 1 H), 3.80–3.87 (m, 1 H), 4.42 (d, J = 4.9 Hz, 1 H), 4.34 (dd, J = 7.3, 11.9 Hz, 1 H), 5.09 (s, 2 H).

General Procedure for the $Cr(ClO_4)_2$-Mediated Reductive Activation of Mitosenes in Water and Methanol. To a deaerated aqueous or methanolic, buffered solution (2.5 mL) containing the mitosene (i.e., **9** + **10**, **7**, **14**, and **15**) (final concentration: 1.3 mM), an aqueous solution of $Cr(ClO_4)_2$ was added. The reactions were allowed to proceed at room temperature (water: 30 min, methanol: 60 min), opened to the air, and analyzed. The pH of the reaction was determined at the conclusion of the reaction and shown to be within ±0.04 pH units of the original solution. The reactions were monitored by HPLC. All products were identified by coinjection (cospotting) of an authentic sample with the reaction mixture in the HPLC (TLC). In the aqueous reactions, bis·tris·HCl (0.2 M) was employed as the buffer in the pH 6.0 reactions, and tris·HCl (0.2 M) was used as the buffer in the pH 8.0 reactions. In the methanolic reactions, bis·tris·HCl (0.2 M) was employed as the buffer in the "pH" 6 reactions, and bis·tris·HCl (0.1 M) + tris·HCl (0.1 M) was used as the buffer for the "pH" 9 reactions.

General Procedure for the $Cr(ClO_4)_2$-Mediated Sequential Reductive Activation of Mitomycin C (1) (*N*-Methylmitomycin C (35)) and *N*-Methylmitomycin C (35) (Mitomycin C (1)) in Water. Mitomycin C (**1**) (*N*-methylmitomycin C (**35**)) (1 equiv, final concentration: 1.3 mM) was dissolved in an aqueous (bis·tris·HCl (0.2 M), pH 6.0) buffered solution (2 mL) and deaerated (Ar), and then an aqueous $Cr(ClO_4)_2$ (1 equiv) solution was added and stirred at room temperature (20 min). A deaerated aqueous solution (0.5 mL) of **35** (**1**) (1 equiv) was added, and the reaction was maintained at room temperature (10–25 min), exposed to air, and analyzed. The pH of the solutions was determined at the conclusion of the reaction and shown to be within ±0.05 pH units of the original solution. The reactions were monitored by HPLC and TLC. All products were identified by coinjection (cospotting) of an authentic sample with the reaction mixture in the HPLC (TLC).

Preparation of *N*-Methylmitomycin C[45] (35). Mitomycin C (**1**) (50 mg, 0.15 mmol) and 1,8-bis(dimethylamino)naphthalene (128 mg, 0.6 mmol) were dissolved in dry tetrahydrofuran (60 mL). Dimethyl sulfate (190 mg, 0.14 mL, 1.5 mmol) was added to the reaction mixture and stirred (2 days). The reaction was then quenched with an aqueous 10% $NaHCO_3$ solution (10 mL) and concentrated in vacuo. The residue was diluted with water (20 mL) and extracted with methylene chloride (4 × 30 mL). The organic layers were combined, dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC (system B) to give pure **35** (R_f 0.51, system A): ^{1}H NMR (pyridine-d_5) δ 2.02 (s, 3 H), 2.12 (dd, J = 1.9, 4.6 Hz, 1 H), 2.22 (s, 3 H), 2.52 (d, J = 4.6 Hz, 1 H), 3.16 (s, 3 H), 3.51 (dd, J = 2.0, 12.9 Hz, 1 H), 3.99 (dd, J = 4.3, 11.2 Hz, 1 H), 4.47 (d, J = 12.9 Hz, 1 H), 4.81 (dd, J = 10.6, 11.2 Hz, 1 H), 5.38 (dd, J = 4.3, 10.6 Hz, 1 H).

Preparation of Deuterated Mitosenes. a. 1α-Deuterio-2,7-diaminomitosene (7-d_1) (Table VI, Entry 1). *trans*-10-Decarbamoyl-1,10-dimethoxy-2,7-diaminomitosene (**16**) (33 mg, 0.11 mmol, 2 equiv) was dissolved in a buffered (deuterated bis·tris·DCl, 0.2 M) deuterium oxide solution (100 mL, pD 6.05) and deaerated (Ar), and then a $Cr(ClO_4)_2$ (1 equiv) solution was added and stirred at room temperature (5 min). A deaerated deuterium oxide solution (6 mL) containing **1** (18 mg, 0.054 mmol, 1 equiv) was added, and the reaction was maintained at room temperature (30 min) and then exposed to air. The reaction solution was neutralized with an aqueous saturated $NaHCO_3$ solution and lyophilized. The residue was dissolved in methanol (5 mL) and purified by preparative TLC (system A) to give **7-d_1**: ^{1}H NMR (pyridine-d_5) δ 2.13 (s, 3 H), 2.72 (d, J = 4.7 Hz, 1 H), 3.98 (dd, J = 5.0, 11.9 Hz, 1 H), 3.97–4.05 (m, 1 H), 4.38 (dd, J = 6.0, 11.9 Hz, 1 H), 5.65 (s, 2 H). The percent deuterium incorporation at the C-1α position was estimated to be 86% by integration of the residual signal at δ 3.14–3.25 and then comparison of this value to the integrals for nearby methine and methyl resonances.

b. 10-Decarbamoyl-1α-deuterio-2,7-diaminomitosene (8-d_1) (Table VI, Entry 2). To a deaerated (Ar), buffered (deuterated bis·tris·DCl, 0.2 M) deuterium oxide solution (100 mL, pD 7.05) containing **1** (50 mg, 0.15 mmol) was added $Cr(ClO_4)_2$ (2 equiv). The reaction was allowed to proceed at room temperature (30 min), opened to the air, and lyophilized.

(45) For a similar procedure for the preparation of **35**, see: Kinoshita, S.; Uzu, K.; Nakano, K.; Shimizu, M.; Takahashi, T. *J. Med. Chem.* **1971**, *14*, 103.

The residue was purified by preparative TLC (system A) to give **8-d_1**: ^{1}H NMR (pyridine-d_5) δ 2.14 (s, 3 H), 2.72 (d, J = 5.3 Hz, 1 H), 4.04 (dd, J = 4.7, 11.9 Hz, 1 H), 4.00–4.15 (m, 1 H), 4.41 (dd, J = 5.8, 11.9 Hz, 1 H), 5.18 (s, 2 H). The percent deuterium incorporation at the C-1α position was estimated to be 80% by integration of the residual signal at δ 3.12–3.20 and then comparison of this value to the integrals for nearby methine and methyl resonances.

c. 1α-Deuterio-2,7-diaminomitosene (7-d_1) and 10-Decarbamoyl-1α-deuterio-10-methoxy-2,7-diaminomitosene (13-d_1) (Table VI, Entry 3). To a deaerated (Ar), buffered (deuterated bis·tris·DCl, 0.2 M) methanol-d_1 solution (50 mL, "pD" 8.46) containing **1** (20 mg, 0.06 mmol) was added $Cr(ClO_4)_2$ (2 equiv). The reaction was allowed to proceed at room temperature (30 min), opened to the air, and concentrated in vacuo. The residue was separated by preparative TLC (system B) to give **7-d_1** and **13-d_1**.

1α-Deuterio-2,7-diaminomitosene (7-d_1): ^{1}H NMR (CD_3OD) δ 1.79 (s, 3 H), 2.67 (br s, 1 H), 3.94 (dd, J = 3.9, 12.7 Hz, 1 H), 4.16–4.20 (m, 1 H), 4.38 (dd, J = 6.9, 12.7 Hz, 1 H), 5.15 (s, 2 H). The percent deuterium incorporation at the C-1α position was estimated to be 90% by integration of the residual signal at δ 3.15–3.22 and then comparison of this value to the integrals for nearby methine and methyl resonances.

10-Decarbamoyl-1α-deuterio-10-methoxy-2,7-diaminomitosene (13-d_1): ^{1}H NMR ($CDCl_3$) δ 1.76 (s, 3 H), 2.52 (br s, 1 H), 3.34 (s, 3 H), 3.85 (dd, J = 2.8, 12.5 Hz, 1 H), 4.15–4.20 (m, 1 H), 4.33 (dd, J = 6.6, 12.5 Hz, 1 H), 4.51 (s, 2 H). The percent deuterium incorporation at the C-1α position was estimated to be 90% by integration of the residual signal at δ 3.00–3.12 and then comparison of this value to the integrals for nearby methine and methyl resonances.

d. Compound (34-d_2) (Table VI, Entry 4). To a deaerated (Ar), buffered (deuterated bis·tris·DCl, 0.2 M) deuterium oxide solution (120 mL, pD 7.20) containing **1** (31 mg, 0.093 mmol) was added $Cr(ClO_4)_2$ (8 equiv). The reaction was allowed to proceed at room temperature (30 min), opened to the air, and lyophilized. The residue was purified by preparative TLC (system B) to give **34-d_2**: ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3 H), 2.20 (br s, 2 H), 2.42–2.49 (m, 0.57 H), 2.97–3.06 (m, 0.43 H), 3.91 (dd, J = 4.0, 12.8 Hz, 1 H), 4.15–4.25 (m, 1 H), 4.39 (dd, J = 6.4, 12.8 Hz, 1 H); MS (–CI), m/e 247 (M^-). The percent deuterium incorporation at the C-1α, C-1β, and C-10 sites was estimated to be 57%, 43%, and 100% (for a single D), respectively, by integration of the residual signals at these sites and then comparison of these values to the integrals for nearby methine and methyl resonances.

e. Compound (34-d_2) (Table VI, Entry 5). To a deaerated (Ar), buffered (deuterated bis·tris·DCl (0.1 M) + tris·DCl (0.1 M)) methanol-d_1 solution (50 mL, "pD" 9.41) containing **1** (20 mg, 0.06 mmol) was added $Cr(ClO_4)_2$ (5 equiv). The reaction was allowed to proceed at room temperature (1 h), opened to the air, and concentrated in vacuo. The residue was separated by preparative TLC (system B) to give **34-d_2**: ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3 H), 2.20 (br s, 2 H), 2.43–2.51 (m, 0.60 H), 2.98–3.07 (m, 0.40 H), 3.91 (dd, J = 3.9, 12.8 Hz, 1 H), 4.15–4.25 (m, 1 H), 4.37 (dd, J = 6.4, 12.8 Hz, 1 H). The percent deuterium incorporation at the C-1α, C-1β, and C-10 sites was estimated to be 60%, 40%, and 100% (for a single D), respectively, by integration of the residual signals at these sites and then comparison of these values to the integrals for nearby methine and methyl resonances.

f. Compound (18-d_1). To a deaerated, buffered (deuterated bis·tris·DCl (0.1 M) + tris·DCl (0.1 M)) methanol-d_1 solution (30 mL, "pD" 8.93) containing *trans*-1-methoxy-2,7-diaminomitosene (**14**) (15 mg, 0.045 mmol) was added $Cr(ClO_4)_2$ (2 equiv). The reaction was allowed to proceed at room temperature (1 h), opened to the air, and concentrated in vacuo. The residue was separated by preparative TLC (system B) to give **18-d_1**: ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 3 H), 2.35 (br s, 2 H), 3.38 (s, 3 H), 4.07 (dd, J = 5.5, 13.0 Hz, 1 H), 4.02–4.10 (m, 1 H), 4.30 (s, 1 H), 4.44 (dd, J = 5.0, 13.0 Hz, 1 H); MS (–CI), m/e 276 (M^-). The percent deuterium incorporation at C-10 was estimated to be 93% for one deuterium by integration of the residual signal for the C-10 methyl group (δ 2.35) and then comparison of this value to the integrals for nearby methine and methyl resonances.

Reduction of Indano[1,2-*b*]aziridine (36) with $Cr(ClO_4)_2$. Indano[1,2-*b*]aziridine[31] (**36**) (90 mg, 0.69 mmol) was added to a buffered methanol solution (500 mL, "pH" 9.0, bis·tris·HCl (0.1 M) + tris·HCl (0.1 M)) and deaerated by passing a stream of Ar through the solution (1 h), and then an aqueous solution (27.5 mL, 4 equiv) of $Cr(ClO_4)_2$ was added at 0 °C. The reaction was stirred under a positive pressure of Ar at room temperature (12 h), opened to the air, and then concentrated to 20 mL. Water (150 mL) was added, and the solution was made basic to "pH" 11.5 with aqueous KOH. The solution was extracted with CH_2Cl_2 (3 × 100 mL), dried (K_2CO_3), and evaporated to dryness. TLC analysis of the crude product showed one major spot (R_f 0.26, system A) and a large amount of unreacted starting material (R_f 0.77, system A). The major product was separated by column chromatography (SiO_2, system B) and converted to the hydrochloride salt with ethereal HCl to give pure 2-aminoindane hydrochloride (**37**·HCl) (6.9 mg, 6% yield): ^{1}H NMR (CD_3OD) δ 3.01 (dd, J = 4.3, 16.6 Hz, 2 H), 3.40 (dd, J = 7.3, 16.6 Hz, 2 H), 4.04–4.13 (m, 1 H), 7.20–7.35 (m, 4 H); ^{13}C NMR (CD_3OD) 38.75, 52.72, 125.87, 128.54, 140.21 ppm. Addition of an authentic sample[33] of **37**·HCl to the NMR sample led to no new additional peaks in the ^{1}H and ^{13}C NMR spectra.

Acknowledgment. We thank the National Institutes of Health (RO1CA29756) and the Robert. A. Welch Foundation (E607) for their generous support of our work. Grateful acknowledgment is made to Dr. A. M. Casazza and Bristol-Myers-Squibb Laboratories, Wallingford, CT, for a generous gift of mitomycin C. Special thanks are given to Professor Thomas Albright for many helpful discussions.