C(7)-Substituted Diaminomitomycins: Synthesis, Structure, and Chemical Reactivity

Shuang Wang and Harold Kohn*

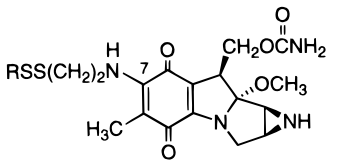
Department of Chemistry, University of Houston, Houston, Texas 77204-5641

Received April 15, 1997[⊗]

Five mitomycins (**23**−**27**) were prepared to determine if an appended C(7) diamine substituent would promote C(1) nucleophilic substitution transformations by covalent modification of the C(8) quinone group. The appended C(7) diamine units varied in the basicity of the terminal amine and in the type and length of the carbon backbone. The mitomycins were prepared in high yield by treatment of mitomycin A (**28**) with selected diamines. Mitomycin **25**, which contained a C(7) 3-amino-2,2-dimethylpropylamine unit, underwent rapid conversion to the corresponding albomitomycin-type adduct **34** in which the C(8) quinone group was converted to the corresponding C(8) imine. Dissolution of each mitomycin (**23**, **24**, **26**, **27**, and **34**) in methanol ("pH" 5.5, 25 °C) led to the production of the *cis*- and *trans*-C(1) methoxymitosenes. The rates of solvolysis were monitored by HPLC and followed pseudo-first-order kinetics. Modest rate enhancements (5.1−15.2-fold), compared with mitomycin C (**1**), were observed for 7-*N*-(2-aminobenzyl)mitomycin C (**23**) and 7-*N*-(2-anilinoethyl)mitomycin C (**24**), the two mitomycins containing terminal aniline groups. Solvolysis of **23** gave the C(1) methoxymitosenes **37** and **38**, in which the C(8) site was converted to the cyclized C(8) imine; solvolysis of **24** gave C(1) methoxy products **39** and **40**, in which the C(8) quinone unit was not modified. No appreciable rate enhancements over **1** were observed for **26** and **27**, the two mitomycins containing terminal-substituted aliphatic amine groups. Albomitomycin **34** solvolyzed 6.9 times faster than **1**. The observed rate data indicated that the aniline units in **23** and **24** promoted solvolysis by modifying the C(8) quinone group to give either the C(8) hemiaminal or the C(8) imine adduct. Formation of these adducts disrupted the delocalization of the indoline N(4) electrons with the C(5a)−C(8a)−C(8)−O conjugated system, permitting the sequential activation of the C(1) site toward nucleophilic substitution. The significance of these findings for the mode of action of KW-2149 and BMS-181174 is briefly discussed.

Mitomycin C (**1**) is a commercially available antitumor antibiotic that undergoes bioreduction prior to action.[1] Reductive activation leads to the selective alkylation of guanine residues within 5′CG sequences to give both mono-(**4**) and disubstituted (**5**) adducts[2−5] (Scheme 1). Surprisingly, when solvolytic studies were conducted under reductive conditions in the absence of DNA, **1** was seen as an inefficient alkylating agent that gave as the predominant product the C(1) electrophilic substitution adduct **8** rather than the C(1) nucleophilic substitution adducts **6** and **7** (Scheme 2).[6] We attributed the facile formation of **8** to the ease in which **3** undergoes proton transfer to give **8**.

Recently, the mitomycins KW-2149[7] (**9**) and BMS-181174[8] (formerly BMY-25067) (**10**) have been advanced to clinical trials[9] because of their improved pharmacological profile over **1**.[10,11]



9 R = $HO_2CCH(CH_2)_2CN(CH_2)_2$ (KW-2149)

10 R = *p*-nitrophenyl (BMS-181174)

Both mitomycins contain C(7) aminoethylene disulfide groups in place of the C(7) amino substituent in **1**. Two mechanisms have been proposed for their enhanced activity. In one, an external thiol cleavage (*e.g.*, glu-

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.

(1) (a) Szybalski, W.; Iyer, V. N. In *Antibiotics I. Mechanism of Action;* Gottlieb, D., Shaw, P. D., Eds.; Springer: New York, 1967; pp 211−245. (b) Keyes, S. R.; Heimbrook, D. C.; Fracasso, P. M.; Rockwell, S.; Sligar, S. G.; Sartorelli, A. C. *Adv. Enzyme Regul.* **1985**, *23*, 291−307. (c) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249−280.

(2) (a) Tomasz, M.; Lipman, R.; Verdine, G. C.; Nakanishi, K. *Biochemistry* **1986**, *25*, 4337−4344. (b) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science* **1987**, *235*, 1204−1208. (c) Bizanek, R.; McGuinness, B. F.; Nakanishi, K.; Tomasz, M. *Biochemistry* **1992**, *31*, 3084−3091.

(3) Teng, S. P.; Woodson, S. A.; Crothers, D. M. *Biochemistry* **1989**, *28*, 3901−3907.

(4) Millard, J. T.; Weidner, M. F.; Raucher, S.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 3637−3641.

(5) (a) Li, V.-S.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 275−283. (b) Kohn, H.; Li, V.-S.; Tang, M.-s. *J. Am. Chem. Soc.*, **1992**, *114*, 5501−5509.

(6) (a) Schiltz, P.; Kohn, H. *J. Am. Chem. Soc.* **1992**, *114*, 7958−7959. (b) Schiltz, P.; Kohn, H. *Ibid.* **1993**, *115*, 10510−10518.

(7) Kono, M.; Saitoh, Y.; Kasai, M.; Saito, A.; Shirahata, K.; Morimoto, M.; Ashizawa, T. *Chem. Pharm. Bull.* **1989**, *37*, 1128−1130.

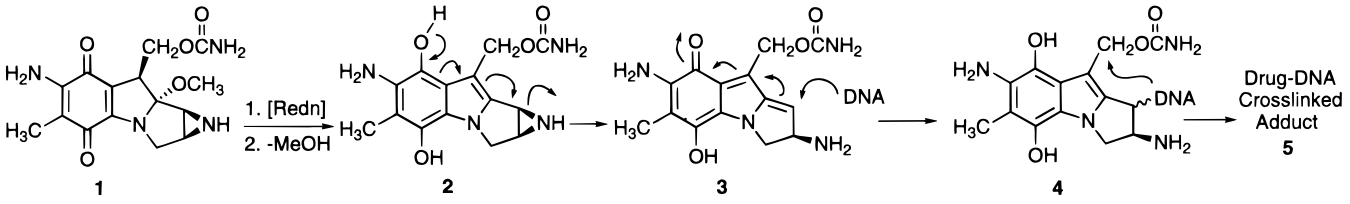
(8) Vyas, D. M.; Chiang, Y.; Benigni, D.; Rose, W. C.; Bradner, W. T. In *Recent Advances in Chemotherapy. Anticancer Section*; Tshigami, J., Ed.; University of Tokyo Press: Tokyo, 1985; pp 485−486.

(9) (a) Dirix, L.; Catimel, G.; Koier, I.; Prove, A.; Schrijvers, D.; Joossens, E.; de Bruijn, E.; Ardiet, C.; Evene, E.; Dumortier, A.; Clavel, M.; van Oosterom, A. *Anti-Cancer Drugs* **1995**, *6*, 53−63. (b) Doyle, T. W.; Vyas, D. M. *Cancer Treat. Rev.* **1990**, *17*, 127−131.

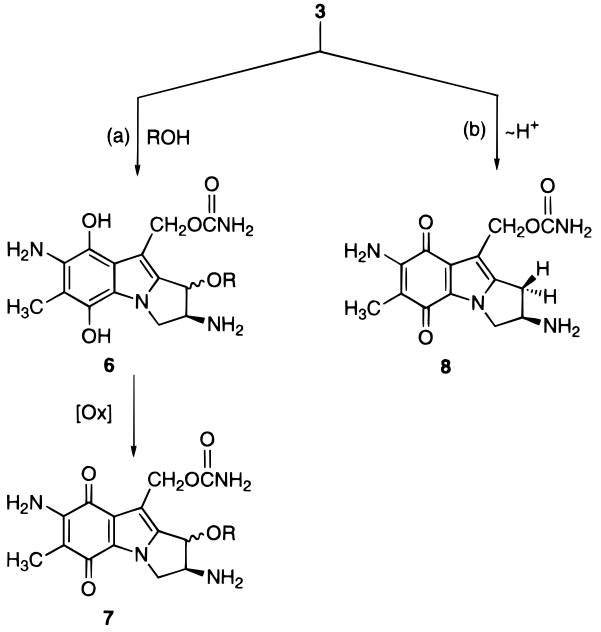
(10) Compound **9** (KW-2149): (a) Ashizawa, T.; Okabe, M.; Gomi, K.; Hirata, T. *Anti-Cancer Drugs* **1993**, *4*, 181−188. (b) Ohe, Y.; Nakagawa, K.; Fujiwara, Y.; Sasaki, Y.; Minato, K.; Bungo, M.; Niimi, S.; Horichi, N.; Fukuda, M.; Saijo, N. *Cancer Res.* **1989**, *49*, 4098−4102. (c) Morimoto, M.; Ashizawa, T.; Ohno, H.; Azuma, M.; Kobayashi, E.; Okabe, M.; Gomi, K.; Kono, M.; Saitoh, Y.; Kanda, Y.; Arai, H.; Sato, A.; Kasai, M.; Tsuruo, T. *Cancer Res.* **1991**, *51*, 110−115. (d) Tsuruo, T.; Sudo, Y.; Asami, N.; Inaba, M.; Morimoto, M. *Cancer Chemother. Pharmacol.* **1990**, *27*, 89−93. (e) Dirix, L.; Gheuens, E. E. O.; van der Heyden, S.; van Oosterom, A. T.; De Bruijn, E. A. *Anti-Cancer Drugs* **1994**, *5*, 343−354.

S0022-3263(97)00675-0 CCC: $14.00

Scheme 1. Proposed Pathway for Mitomycin C Activation under Reductive Conditions



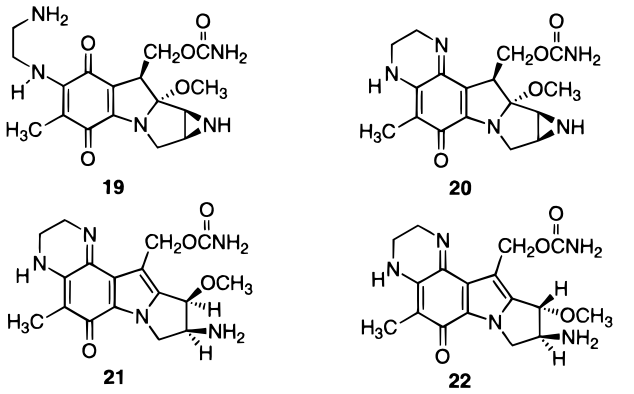
Scheme 2. Proposed Pathway for Mitomycin C C(1) Nucleophilic and Electrophilic Processes



tathione, cysteine) of **11** provides thiol **12**, which undergoes C(6) cyclization to give **13**[12] (Scheme 3). Subsequent C(6)−S homolytic cleavage and external thiol reduction led to fully reduced hydroquinone **14**. Loss of methanol at C(9) and C(9a) generated the activated mitosene **15**. Species **15** is projected to have reactivity comparable to reductively activated mitosene **2**. If this pathway is operative, C(1) electrophilic transformations may exceed C(1) nucleophilic substitution processes (Scheme 2) and may lead to inefficient DNA alkylation.

In the second hypothetical mechanism, an external thiol attack is projected to provide thiol **12**, which undergoes C(8) carbonyl addition to give **16** and the disruption of the N(4)−C(5a)−C(8a)−C(8)−O conjugated system[13] (Scheme 4). This process permits the facile loss of methanol at C(9) and C(9a) to generate pyrrole **17**. The subsequent aziridine ring opening gives the resonance-stabilized carbocation **18**, permitting C(1) and then C(10) substitution reactions. In this pathway only nucleophilic transformations can proceed at C(1).

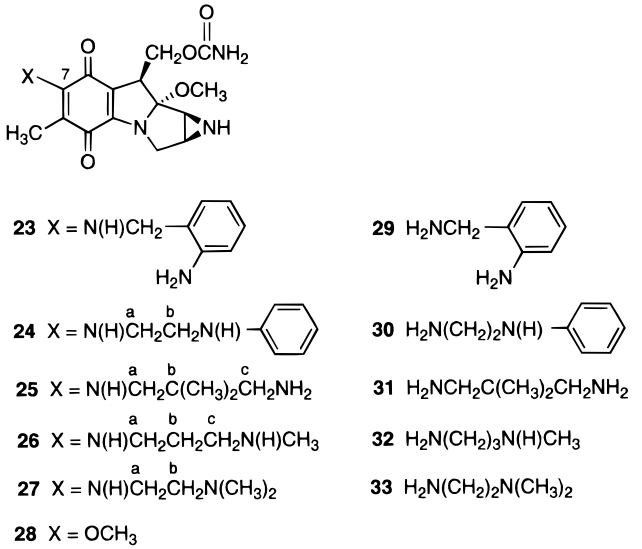
We found preliminary chemical support for the ionic pathway depicted in Scheme 4 as we attempted to prepare 7-*N*-(aminoethyl)mitomycin C[14] (**19**).[13] In this compound the terminal amino group was expected to serve as a surrogate for the thiol moiety in **12**. Our attempts to synthesize **19** yielded the C(8) *imino*mitomycin **20**.[13,15] Furthermore, dissolution of **20** in methanol furnished only the C(1) nucleophilic substitution adducts **21** and **22**, and these compounds were produced at accelerated rates, compared with the corresponding compounds from **1** under similar conditions.[13]



In this study, additional evidence is provided in support of the activation pathway depicted in Scheme 4. We asked whether an appended C(7) diamine unit in *quinone*-containing mitomycins can promote C(1) nucleophilic processes.

Results and Discussion

A. Choice of Substrates and Synthesis. Mitomycins **23**−**27**[14] were used to explore the feasibility of the mechanistic pathway outlined in Scheme 4. They varied in the basicity of the C(7)-substituted terminal amine and in the type and length of the carbon backbone bridging the C(7) diamine unit. Mitomycin C (**1**) was selected as the control substrate because it cannot undergo C(7)-assisted mitomycin activation.



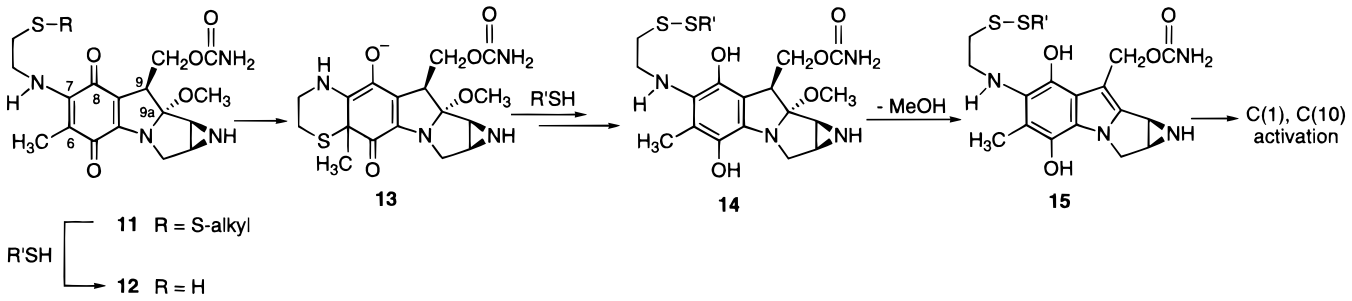
We used the methods of Remers and co-workers[14] to synthesize compounds **23**−**27**. Treatment of mitomycin

(11) Compound **10** (BMS-181174): (a) Bradner, W. T.; Rose, W. C.; Schurig, J. E.; Florczyk, A. P. *Invest. New Drugs* **1990**, *8*, S1−S7. (b) Dusre, L.; Rajagopalan, S.; Eliot, H. M.; Covey, J. M.; Sinha, B. K. *Cancer Res.* **1990**, *50*, 648−652. (c) Xu, B. H.; Singh, S. V. *Cancer Res.* **1992**, *52*, 6666−6670. (d) Rockwell, S.; Kemple, B.; Kelley, M. *Biochem. Pharamcol.* **1995**, *50*, 1239−1243. (e) Xu, B. H.; Gupta, V.; Singh, S. V. *Br. J. Cancer* **1994**, *69*, 242−246.

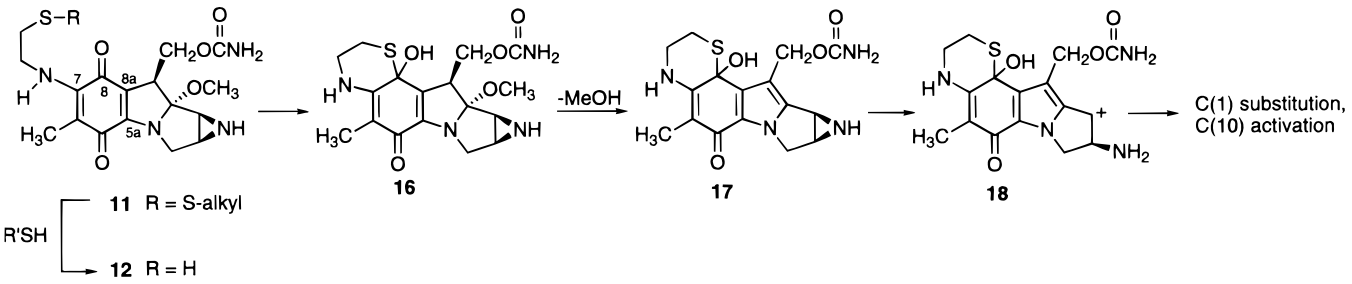
(12) He, Q.-Y.; Maruenda, H.; Tomasz, M. *J. Am. Chem. Soc.* **1994**, *116*, 9349−9350.

(13) Kohn, H.; Wang, S. *Tetrahedron Lett.* **1996**, *37*, 2337−2340.

Scheme 3. Proposed Pathway for Mitomycins 9 and 10 Activation



Scheme 4. Proposed Pathway for Mitomycins 9 and 10 Activation



A (**28**) with diamines **29**−**33** gave mitomycins **23**−**27**, respectively, in high yields. We observed satisfactory spectroscopic properties, consistent with previous literature values,[16] for all new mitomycins (**23**−**26**).[17] Mitomycin **25** proved to be unstable in MeOH and CH_2Cl_2, rearranging to the albomitomycin derivative **34** presumably through C(8) mitomycin imine **35**. This N(1a)−C(5a) intramolecular cyclization process was originally reported to occur with mitomycin A (**28**) to give **36**.[18] Conversion of **25** and **28** to **34** and **36**, respectively, is accompanied by distinctive changes in the NMR spectra (Table 1). In particular, we observed in the ^{1}H NMR spectrum for **34** the appearance of the C(8a) methine hydrogen (δ 3.92), the loss of the N(1a)H resonance, and the upfield shifts of the C(1) methine (0.52 ppm) and the C(3)H_β (1.58 ppm) hydrogens compared to **25**. In addition, the C(c)H_2 methylene protons in **34** appeared as two diastereotopic multiplets centered at δ 3.16 and 3.72, consistent with C(8) ring cyclization. Interestingly, the proton signal that gave rise to the doublet of doublets at δ 3.16 was coupled (^5J)[19] to the C(8a) methine hydrogen (COSY analysis). In the ^{13}C NMR spectrum for **34**, the C(5a) and C(8a) resonances appeared at significantly higher fields (59.2−67.9 ppm) than those observed in **25**. Further evidence in favor of **34** was obtained from the COSY, HMQC (heteronuclear multiple quantum correlated spectroscopy), and HMBC (heteronuclear multiple bond correlated spectroscopy) NMR experiments. Diagnostic three-bond heteronuclear connectivities were observed for the C(1) and C(2) methine hydrogens and C(5a) and for the C(c)H_2 methylene imine protons and C(8) (Figure 1). The factors that contributed to the facile rearrangement of **25** to **34** were not determined. A comparable reaction for **20** was not detected.[13] The rapid interconversion of **25** to **34** at "pH" 5.5 in MeOH led us to substitute albomitomycin **34** for **25** in our solvolytic studies.

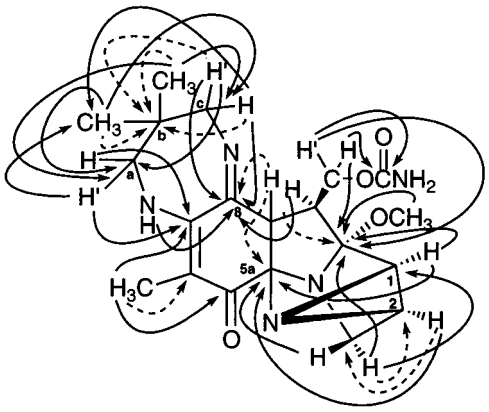
Table 1. Key NMR Properties of 25, 28, 34, and 36

	25[a]	**34**[a]	**28**[b,c]	**36**[b,c]
		^{1}H NMR[d]		
N(1a)H	*e*		0.79	
C(1)H	3.15−3.18	2.65	2.93	2.36
C(2)H	2.67−2.74	2.72	2.83	2.72
C(3)H_α	3.60	3.83	3.48	2.81
C(3)H_β	4.61	3.03	4.03	2.93
C(8a)H		3.92		3.21
C(9)H	4.04	4.46	3.61	3.34
		^{13}C NMR[f]		
C(1)	36.5	33.3	36.8	32.5
C(2)	35.6	33.0	32.8	32.8
C(3)	50.4	50.3	49.9	49.3
C(5a)	156.1	88.2	151.8	87.5
C(8a)	110.3	51.1	114.1	52.2
C(8)	176.8	163.5	178.3	191.8
C(9)	44.4	34.0	43.5	36.5

[a] The solvent used was pyridine-d_5. [b] Values reported in reference 18. [c] The solvent used was $CDCl_3$. [d] The number in each entry is the chemical shift value (δ) in ppm relative to the solvent used. The spectra were obtained at 300 MHz (**25**, **34**) and 400 MHz (**28**, **36**). [e] The signal was beneath the peak at δ 2.17 (C(6)CH_3). [f] The number in each entry is the chemical shift value (δ) in ppm relative to the solvent used. The spectra were obtained at 75 MHz (**25**), 101 MHz (**28**, **36**), and 151 MHz (**34**).

B. Solvolytic Studies. The rates of mitomycins **23**, **24**, **26**, **27**, and **34** solvolysis in buffered methanol solutions were monitored to determine if the appended C(7) diamine moiety promoted C(1) nucleophilic substitution processes compared with **1** (Table 2). All reactions were conducted at "pH" 5.5 (25 °C). Use of more basic solutions ("pH" 6.5, 7.4) led to appreciably slower rates (data not shown). Mitomycins **23**, **24**, **26**, **27**, and **34** underwent clean solvolysis in methanol to give the corresponding *cis*- and *trans*-1-methoxymitosenes **37**−**46** (HPLC and TLC analyses). The *cis*- and *trans*-isomers were formed in near equal yields. The reactions were

(14) Iyengar, B. S.; Sami, S. M.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 16−20.
(15) Saito, Y.; Kasai, M.; Shirahata, K.; Kono, M.; Morimoto, M.; Ashizawa, T. (Kyowa Hakko Kogyo Co., Ltd.) U. S. Patent 4,853,385, Aug. 1, 1989; Eur. Pat. Appl. EP 287,855, Oct. 26, 1988; JP Appl. 63246379, March 31, 1987; *Chem. Abstr.* **1989**, *111*, 7145v.
(16) (a) Fishbein, P. L.; Kohn, H. *J. Med. Chem.* **1987**, *30*, 1767−1773. (b) Subramaniam, S.; Kohn, H. *J. Am. Chem. Soc.* **1993**, *115*, 10519−10526. (c) Choi, D.; Yoo, B.; Colson, K. L.; Martin, G. E.; Kohn, H. *J. Org. Chem.* **1995**, *60*, 3391−3396.
(17) Compound **27**: Cosulich, D. B.; Patrick, J. B.; Williams, R. P. (American Cyanamid Co.) U.S. Patent 3,332,944, July 25, 1967; *Chem. Abstr.* **1968**, *68*, 49581h.
(18) Kono, M.; Saitoh, Y.; Shirahata, K; Arai, Y.; Ishii, S. *J. Am. Chem. Soc.* **1987**, *109*, 7224−7225.
(19) (a) Günther, H. *NMR-Spektroskopie. Eine Einführung in die Protonenresonanz und ihre Anwendungen in der Chemie*, 2nd ed., Georg Thieme, Stuttgart, 1983. (b) Friebolin, H. *Ein- und Zweidimensionale NMR-Spektroskopie. Eine Einführung*; VCH: Weinheimm, 1988.



34

: 3 bond HMBC correlation;

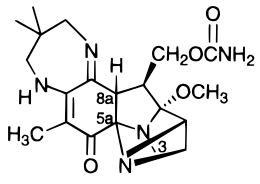
: 2 bond HMBC correlation.

Figure 1. HMBC correlations observed for **34**.

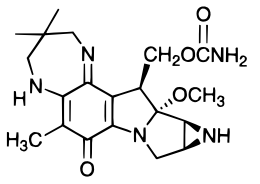
Table 2. Methanolysis of Mitomycins 23, 24, 26, 27, and 34[a]

compd	k_{obs} (d^{-1})	$t_{1/2}$ (d)
23	0.77	0.9
24	0.26	2.7
26	0.05	13.7
27	0.06	11.3
34	0.35	2.0
20	7.2	0.1
1	0.05	13.7

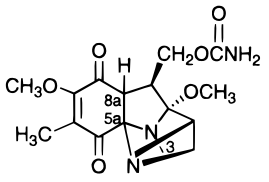
[a] Reactions were run in buffered methanol (bis-Tris-HCl) solutions ("pH" 5.5) at 25 °C. All reactions were monitored for two half-lives (HPLC analyses) and were run in duplicate. The rates were calculated from the HPLC data collected at 313 nm.



34



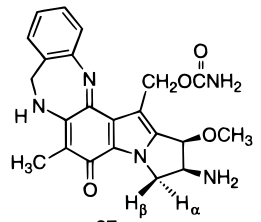
35



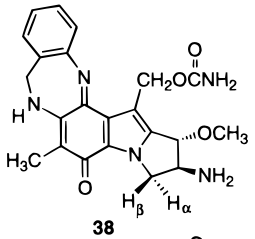
36

monitored by HPLC at 313 and 365 nm for at least two half-lives. The pseudo-first-order kinetic plots gave linear responses, and the corresponding k_{obs} and $t_{1/2}$ values are listed in Table 2.

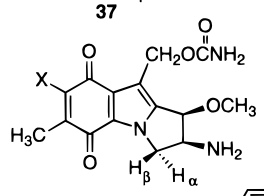
Authentic samples of the mitomycin *cis*- and *trans*-C(1) methoxy products **37**−**48**[20] were prepared by dissolving the corresponding mitomycin in "pH" 3−5.5 methanolic solutions. The solvolytic products were identified by their spectroscopic properties. For mitosenes **37**, **38**, **41**, and **42**, the products were assigned as the cyclized C(8) imine derivatives on the basis of the upfield resonance for the C(8) signal in the ^{13}C NMR spectra (155.3−158.7 ppm), on their distinctive UV-visible spectra, and on the finding that the mass spectral molecular ion peaks for **41** and **42** corresponded to 18 mass units (− H_2O) less than that

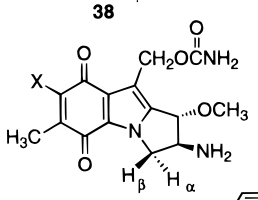


37



38





39 X = N(H)$(CH_2)_2$N(H)–Ph

40 X = N(H)$(CH_2)_2$N(H)–Ph

43 X = N(H)$(CH_2)_3$N(H)CH_3

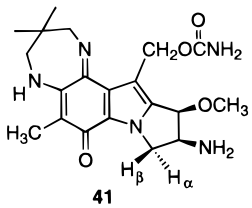
44 X = N(H)$(CH_2)_3$N(H)CH_3

45 X = N(H)$(CH_2)_2$N$(CH_3)_2$

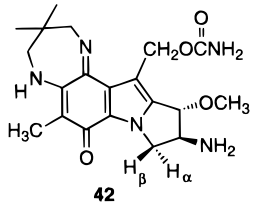
46 X = N(H)$(CH_2)_2$N$(CH_3)_2$

47 X = NH_2

48 X = NH_2



41



42

anticipated for the corresponding noncyclized *quinone* adducts. Furthermore, for mitosenes **41** and **42**, the C(c) methylene protons appeared as an AB quartet centered at δ 3.76. Both the downfield shift and the multiplicity of these diastereotopic protons, compared with the corresponding resonance in **25** (δ 2.61, singlet), were in agreement with the cyclized C(8) structure.[13] Distinctive trends within the NMR data set permitted us to identify the *cis*- and *trans*-1-methoxymitosenes within each pair of isomers. In the ^1H NMR spectra for the *cis*-adducts **37**, **39**, **41**, **43**, **45**, and **47**, we observed that the C(1)H−C(2)H and C(2)H−C(3)H_β vicinal coupling constants were moderate, and the C(2)H−C(3)H_α coupling interaction was large, whereas for the *trans*-adducts **38**, **40**, **42**, **44**, **46**, and **48**, the C(1)H−C(2)H and C(2)H−C(3)H_β vicinal coupling constants were small, and the C(2)H−C(3)H_α coupling interaction was large.[21] In the ^{13}C NMR spectra, we consistently found that the C(1) and C(2) signals for the *trans*-adducts **38**, **40**, **42**, **44**, **46**, and **48** appeared downfield from their corresponding *cis*-isomers **37**, **39**, **41**, **43**, **45**, and **47**.[20b] Similar NMR trends have been previously observed.[20b,21]

The kinetic experiments showed that mitomycins **20**, **23**, **24**, and **34** underwent solvolysis at rates faster than **1**, whereas **26** and **27** exhibited comparable reactivity to **1**. The rate enhancement of **23** was only 15.2-fold over **1** and that of **24** and **34** was 5.1−6.9-fold.

The mechanism of mitomycin C hydrolysis under *nonreductive* conditions has been investigated by several

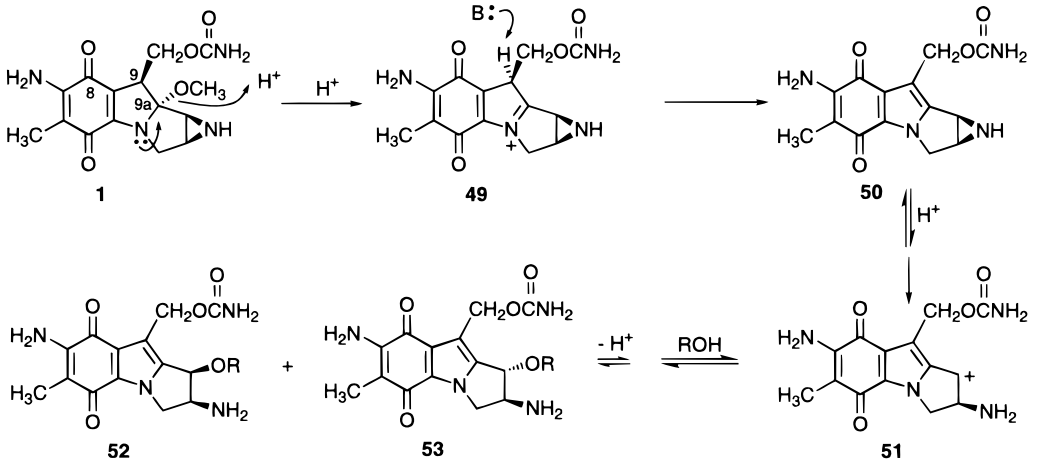
(20) *cis*- and *trans*-1-Methoxy-2,7-diaminomitosenes: (a) Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307−311. (b) Hong, Y. P.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 4634−4644.

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

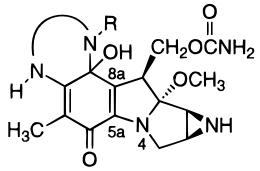
(22) Stevens, C. L.; Taylor, K. G.; Munk, M. E.; Marshall, W. S.; Noll, K.; Shah, G. D.; Shah, L. G.; Uzu, K *J. Med. Chem.* **1964**, *8*, 1−10.

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

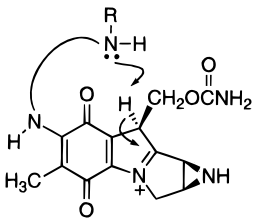
Scheme 5. Proposed Pathway for Mitomycin C Solvolysis under Nonreductive Conditions



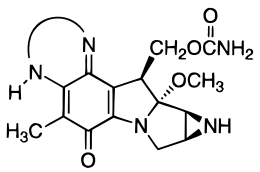
research groups.[22−28] Solvolysis is believed to proceed by aromatization of the dihydropyrrole ring (**1** → **49** → **50**) followed by aziridine ring opening to give the resonance-stabilized carbocation **51** (Scheme 5). Subsequent attack by water and proton loss gives the *cis*-**52** (R = H) and the *trans*-**53** (R = H) adducts. Boruah and Skibo have provided evidence that the loss of the C(9a) methoxy group is specific-acid catalyzed.[28] Our finding that mitomycins **23**, **24**, **26**, **27**, and **34** gave near equal amounts of the corresponding aziridine ring-opened *cis*- and *trans*-1-methoxymitosenes indicates that solvolysis proceeded by an S_N1-type pathway. This study also showed that compared with mitomycin C select C(7) substituents promoted C(1) solvolysis and that the C(7) activation process was finely tuned to the nucleophilicity, basicity, and the structure of the appended C(7) substituent (Table 2). Two different pathways may account for these observed rate enhancements. First, the C(7)-appended amine can add to the C(8) carbonyl unit to give either the C(8) hemiaminal **54** or the C(8) imine **55** adducts. Formation of either **54** or **55** disrupts the resonance interaction of the starting mitomycin's indoline N(4) electrons with the C(5a)−C(8a)−C(8)−O conjugated system. This disruption facilitates the N(4)-assisted expulsion of the C(9a) methoxy group. Of the two, we anticipate that **54** will undergo solvolysis more rapidly than **55** since the N(4) electrons are delocalized to a lesser extent. Second, the appended C(7) amine may assist mitomycin solvolysis by abstracting the C(9) proton in **56** to give the corresponding mitosene adduct.



54

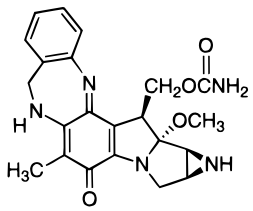


56

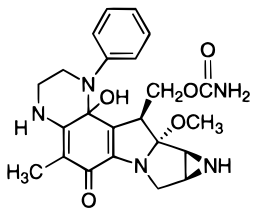


55

Our findings were consistent with the notion that the C(7) substituent promoted C(1) solvolysis by directly interacting with the C(8) carbonyl unit in the mitomycin. Several observations supported this conclusion even though extensive kinetic studies were not conducted to sort out the details of each reaction pathway. First, for **23** the solvolysis products **37** and **38** were identified as the cyclic C(8)-*imine* adducts, demonstrating that C(8) functionalization occurred during the reaction. Second, molecular models indicated that intramolecular amine abstraction of the C(9) proton in **23**, **24**, **26** and **27** is difficult and that the proposed transition states for these processes are either equal to or exceed nine atoms. Third, for the two aniline derivatives **23** and **24**, **23** underwent methanolysis three times faster than **24**. We suspect that solvolysis of **23** proceeded through the seven-membered C(8) iminomitomycin **57** and for **24** through the sterically congested six-membered hemiaminal **58** and that the relative solvolysis rates observed for **23** and **24** reflect, in part, the ease of formation of these intermediates. Fourth, no solvolytic rate enhancements were observed for the two C(7)-substituted mitomycins **26** and **27** compared with **1**. These two mitomycins contained distal-substituted aliphatic amine moieties that unlike **25** could not generate a neutral C(8) imine product. We expect that these substituted terminal amine groups are largely protonated at "pH" 5.5, thereby diminishing their ability to activate mitomycin C(1) transformations by C(8) quinone cyclization.



57



58

Finally, the quinone group in albomitomycin **34** was already modified. Compound **34** underwent solvolysis 6.9 times faster than **1** but 20 times slower than **20**. We attributed the enhanced rate of **34** over **1** to the modification of the C(8) quinone carbonyl group and the decreased

(24) Iyengar, B. S.; Remers, W. A. *J. Med. Chem.* **1985**, *28*, 963−967.
(25) Hornemann, U.; Keller, P. J.; Takeda, K. *J. Med. Chem.* **1985**, *28*, 31−36.
(26) Rebek, J.; Shaber, S. H.; Shue, Y.-K.; Gehret, J.-C.; Zimmerman, S. *J. Org. Chem.* **1984**, *49*, 5164−5174.
(27) McClelland, R. A.; Lam, K. *J. Am. Chem. Soc.* **1985**, *107*, 5182−5186.
(28) Boruah, R. C.; Skibo, E. B. *J. Org. Chem.* **1995**, *60*, 2232−2243.

rate of **34** compared with **20** to the need for albomitomycin **34** to rearrange to the corresponding mitomycin C(8) imine **35** prior to solvolysis. We suspect that this step is difficult compared with **35** solvolysis. Support for this notion comes from monitoring the reactivity of **25** at "pH" 7.4 (data not shown). Under these more basic conditions, the rate of cyclization of **25** to **34** and the subsequent solvolysis of **34** to **41** and **42** can be independently measured. We observed that conversion of **25** to **34** proceeded at an appreciably faster rate ($t_{1/2}$ = 1.0 d) than the generation of the C(1) methoxy adducts **41** and **42** from **34** ($t_{1/2}$ = 26.3 d). Moreover, HPLC analysis showed no evidence for **35** during the generation or the solvolysis of **34**, indicating that **35** is a relatively high energy intermediate in both transformations.

Conclusions

Select C(7)-substituted diaminomitomycins undergo solvolysis at enhanced rates compared with mitomycin C and give only C(1) nucleophilic-substituted products. The rate enhancements were modest and depended on the nucleophilicity, basicity, and structure of the appended C(7) substituent. In the case of mitomycin **23**, solvolysis proceeded 15.2 times faster than **1**, and the solvolytic products were determined to be the *cis*-**37** and *trans*-**38** C(1) methoxy *C(8)-imino* mitosenes, demonstrating that C(8) functionalization proceeded during the reaction. Correspondingly, dissolution of mitomycin **24** in methanol yielded the corresponding *cis*-**39** and *trans*-**40** C(1) methoxymitosenes *without* C(8) functionalization. The 5.1-fold rate increase of **24** over **1** has been attributed to the transient production of the C(8) hemiaminal mitomycin **58** leading to C(1) solvolysis. These cumulative findings demonstrate that C(8) functionalization can influence both the rate of mitomycin C(1)-mediated processes and the product type. The modest rate enhancements observed for C(7)-substituted diaminomitomycins demonstrate that if a similar process occurs with **9** or **10** (Scheme 4) then a greater degree of involvement of the intermediate thiol **12** with the C(8) quinone group is required.

Experimental Section[29]

General Procedure for the Preparation of C(7)-Substituted Diaminomitomycins 23−27. To an anhydrous CH_2Cl_2 solution (1 mL) of **28** (1 equiv) was added an anhydrous CH_2Cl_2 solution (1 mL) of the appropriate diamine (**29**−**33**) (1 equiv). The reaction solution was stirred at room temperature (4−24 h). The solution was then concentrated under reduced pressure, and the residue was purified by preparative TLC (10−50% MeOH−$CHCl_3$) to afford the desired products. Solvent systems for chromatographies were A, 10% MeOH−$CHCl_3$; B, 20% MeOH−$CHCl_3$; C, 50% MeOH−$CHCl_3$.

By using this procedure, the following compounds were prepared.

Preparation of 7-*N*-(2-Aminobenzyl)mitomycin C (23). Using **28** (10.0 mg, 0.029 mmol) and **29** (7.0 mg, 0.058 mmol) gave **23** (1 d) as a blue solid after TLC purification (solvent system A): yield, 12 mg (98%); HPLC t_R 27.6 min; R_f 0.47 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3425, 3360, 3288, 2934, 1716, 1634, 1555 cm^{-1}; UV-vis (MeOH) λ_{max} 366 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 2.13 (s, C(6)CH_3), 2.16 (br s, N(1a)H), 2.74 (d, J = 4.1 Hz, C(2)H), 3.14 (d, J = 4.1 Hz, C(1)H), 3.22 (s, C(9a)OCH_3), 3.58−3.74 (m, C(3)H_α), 4.01 (dd, J = 4.2, 10.7 Hz, C(9)H), 4.53 (d, J = 12.6 Hz, C(3)H_β), 4.94 (d, J = 6.0 Hz, C(a)H_2), 5.10 (t, J = 10.7 Hz, C(10)*H*H′), 5.40 (dd, J = 4.2, 10.7 Hz, C(10)H*H′*), 6.82 (t, J = 7.5 Hz, C(4′)H), 7.06 (d, J = 7.5 Hz, C(6′)H), 7.23 (t, J = 7.5 Hz, C(5′)H), 7.33 (d, J = 7.5 Hz, C(3′)H), 7.54 (t, J = 6.0 Hz, C(7)NH), the C(10)OC(O)NH_2 protons were not detected, the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 9.6, 32.6, 36.7, 44.4, 46.1, 49.6, 50.6, 62.5, 104.0, 106.8, 110.7, 116.1, 117.8, 122.6, 128.8, 129.1, 146.4, 147.7, 156.0, 158.1, 176.7, 179.2 ppm; MS (+CI, methane) *m/e* 439 $[M]^+$; M_r (+CI, methane) 439.185 59 $(M)^+$ (calcd for $C_{22}H_{25}N_5O_5$, 439.185 57).

Preparation of 7-*N*-(2-Anilinoethyl)mitomycin C (24). Using **28** (10.0 mg, 0.029 mmol) and **30** (7.5 μL, 0.058 mmol) gave **24** (5 h) as a green solid after TLC purification (solvent system A): yield, 13 mg (99%); HPLC t_R 30.6 min; R_f 0.59 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3420, 3293, 2931, 1717, 1634, 1603, 1556 cm^{-1}; UV-vis (MeOH) λ_{max} 220, 245, 366 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 2.08 (s, C(6)CH_3), 2.75 (d, J = 2.4 Hz, C(2)H), 3.14 (d, J = 2.4 Hz, C(1)H), 3.22 (s, C(9a)OCH_3), 3.47 (q, J = 5.7 Hz, C(b)H_2), 3.63 (d, J = 12.6 Hz, C(3)H_α), 3.83 (q, J = 5.7 Hz, C(a)H_2), 3.98 (dd, J = 4.2, 10.8 Hz, C(9)H), 4.54 (d, J = 12.6 Hz, C(3)H_β), 5.39 (dd, J = 4.2, 10.8 Hz, C(10)H*H′*), 6.45 (t, J = 5.7 Hz, C(1′)NH), 6.81 (t, J = 7.9 Hz, C(4′)H), 6.90 (d, J = 7.9 Hz, C(2′)H, C(6′)H), 7.22−7.32 (m, C(3′)H, C(5′)H, C(7)NH), the signal for the C(10)*H*H′ peak is beneath the water signal, the N(1a)H and C(10)OC(O)NH_2 protons were not detected, the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 9.9 (C(6)*C*H_3), 32.6 (C(2)), 36.7 (C(1)), 44.1 (C(a) or C(b)), 44.3 (C(9)), 44.4 (C(b) or C(a)), 49.6 (C(9a)O*C*H_3), 50.6 (C(3)), 62.4 (C(10)), 103.7 (C(6)), 106.8 (C(9a)), 110.6 (C(8a)), 113.1 (C(2′), C(6′)), 117.2 (C(4′)), 129.7 (C(3′), C(5′)), 147.4 (C(7)), 149.3 (C(1′)), 156.0 (C(5a)), 158.1 (C(10a)), 176.6 (C(8)), 179.0 (C(5)) ppm, the assignments were in agreement with the APT spectrum; MS (+CI, methane) *m/e* 454 $[M + 1]^+$; M_r (+CI, methane) 454.207 78 $(M + 1)^+$ (calcd for $C_{23}H_{28}N_5O_5$, 454.209 04).

Preparation of 7-*N*-(3-Amino-2,2-dimethylpropyl)mitomycin C (25). Using **28** (10.0 mg, 0.029 mmol) and **31** (7.0 mL, 0.058 mmol) gave **25** (5 h) as a blue solid after TLC purification (solvent system B): yield, 11 mg (90%); HPLC t_R 20.0 min; R_f 0.27 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3427, 3294, 2956, 1717, 1636, 1558, 1521 cm^{-1}; UV-vis (MeOH) λ_{max} 221, 370 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 0.88 (s, 2 CH_3), 2.03−2.17 (m, N(1a)H), 2.17 (s, C(6)CH_3), 2.61 (s, C(c)H_2), 2.67−2.74 (m, C(2)H), 3.15−3.18 (m, C(1)H), 3.22 (s, C(9a)OCH_3), 3.58−3.62 (m, C(a)H_2, C(3)H_α), 4.04 (dd, J = 4.2, 10.7 Hz, C(9)H), 4.61 (d, J = 12.6 Hz, C(3)H_β), 5.10 (t, J = 10.7 Hz, C(10)*H*H′), 5.50 (dd, J = 4.2, 10.7 Hz, C(10)H*H′*), the C(7)NH and C(10)OC(O)NH_2 protons were not detected, the ^1H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.2 (C(6)*C*H_3), 23.3 (2 CH_3), 32.6 (C(2)), 35.9 (C(b)), 36.5 (C(1)), 44.4 (C(9)), 49.3 (C(9a)O*C*H_3), 50.4 (C(3)), 51.3 (C(a) or C(c)), 54.8 (C(c) or C(a)), 62.2 (C(10)), 103.1 (C(6)), 106.7 (C(9a)), 110.3 (C(8a)), 148.1 (C(7)), 156.1 (C(5a)), 157.9 (C(10a)), 176.8 (C(8)), 178.5 (C(5)) ppm, the assignments were in agreement with the APT spectrum; MS (+CI, methane) *m/e* 420 $[M + 1]^+$; M_r (+CI, methane) 420.222 91 $(M + 1)^+$ (calcd for $C_{20}H_{30}N_5O_5$, 420.224 69).

Preparation of 7-*N*-[3-(*N*′-Methylamino)propyl]mitomycin C (26). Using **28** (10.0 mg, 0.029 mmol) and **32** (3.0 μL, 0.029 mmol) gave **26** (4 h) as a blue solid after TLC purification (solvent system C): yield, 10 mg (90%); HPLC t_R 21.0 min; R_f 0.08 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3430, 3293, 2938, 1717, 1634, 1556 cm^{-1}; UV-vis (MeOH) λ_{max} 245, 366 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 1.67 (quintet, J = 6.6 Hz, C(b)H_2), 2.13−2.19 (m, N(1a)H), 2.13 (s, C(6)CH_3), 2.35 (s, NCH_3), 2.60 (t, J = 6.6 Hz, C(c)H_2), 2.75 (br s, C(2)H), 3.15 (br s, C(1)H), 3.22 (s, C(9a)OCH_3), 3.59−3.68 (m, C(a)H_2, C(3)H_α), 4.02 (dd, J = 4.2, 10.9 Hz, C(9)H), 4.59 (d, J = 12.6 Hz, C(3)H_β), 5.12 (t, J = 10.9 Hz, C(10)*H*H′), 5.43 (dd, J = 4.2, 10.9 Hz, C(10)H*H′*), 7.61 (br s, C(7)NH), the C(10)OC(O)NH_2 protons were not detected, the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.0 (C(6)*C*H_3), 30.4 (C(b)), 32.5 (C(2)), 36.5 (NCH_3), 36.7 (C(1)), 44.3 (C(a), C(9)), 49.5 (C(9a)O*C*H_3), 50.0 (C(c)), 50.6 (C(3)), 62.4 (C(10)), 103.3 (C(6)), 106.8 (C(9a)), 110.5 (C(8a)), 147.8 (C(7)), 156.2 (C(5a)), 158.1 (C(10a)), 176.9 (C(8)), 178.7 (C(5)) ppm,

(29) For the general experimental procedures employed, see: Wang, S.; Kohn, H. *J. Org. Chem.* **1996**, *61*, 9202−9206.

the assignments were in agreement with the APT spectrum; MS (+CI, methane) *m/e* 406 $[M + 1]^+$; M_r (+CI, methane) 406.207 08 $(M + 1)^+$ (calcd for $C_{19}H_{28}N_5O_5$, 406.209 04).

Preparation of 7-*N*-[2-(*N*′,*N*′-Dimethylamino)ethyl]mitomycin C[17] (27). Using **28** (10.0 mg, 0.029 mmol) and **33** (3.0 μL, 0.029 mmol) gave **27** (4 h) as a blue solid after TLC purification (solvent system C): yield, 11 mg (98%); HPLC t_R 19.7 min; R_f 0.23 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3427, 3289, 2948, 1715, 1634, 1556, 1515 cm^{-1}; UV-vis (MeOH) λ_{max} 220, 365 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 2.13 (s, NCH_3), 2.15 (s, C(6)CH_3), 2.29 (t, J = 6.0 Hz, N(1a)H), 2.38 (t, J = 6.0 Hz, C(b)H_2), 2.77 (t, J = 5.1 Hz, C(2)H), 3.17 (dd, J = 5.1, 7.2 Hz, C(1)H), 3.22 (s, C(9a)OCH_3), 3.53−3.63 (m, C(a)H_2, C(3)H_α), 4.02 (dd, J = 4.5, 10.9 Hz, C(9)H), 4.59 (d, J = 12.9 Hz, C(3)H_β), 5.12 (t, J = 10.9 Hz, C(10)*H*H′), 5.45 (dd, J = 4.5, 10.9 Hz, C(10)H*H*′), 7.31 (t, J = 5.1 Hz, C(7)NH), the C(10)OC(O)NH_2 protons were not detected, the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.0, 32.6, 36.7, 42.2, 44.3, 44.9, 49.6, 50.7, 58.3, 62.5, 103.5, 106.9, 110.6, 147.5, 156.3, 158.1, 176.7, 179.3 ppm; MS (+CI, methane) *m/e* 406 $[M + 1]^+$; M_r (+CI, methane) 406.209 48 $(M + 1)^+$ (calcd for $C_{19}H_{28}N_5O_5$, 406.209 04).

Preparation of 34. Compound **25** (14.3 mg, 0.034 mmol) was dissolved in a buffered methanolic solution (0.06 M Tris-HCl, "pH" 7.4, 5 mL) and stirred at room temperature (1 d). The solution was concentrated under reduced pressure and then separated by preparative TLC using solvent system B to give **34** (10 mg, 70%) as an orange compound, along with an unidentified green adduct (3 mg, 21%) and recovered starting material **25** (1 mg, 7%): HPLC t_R 17.1 min; R_f 0.64 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3432, 2956, 2868, 1715, 1632, 1561 cm^{-1}; UV-vis (MeOH) λ_{max} 220, 262, 338 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 0.84 (s, CH_3), 0.97 (s, CH_3), 2.00 (s, C(6)CH_3), 2.65 (d, J = 3.0 Hz, C(1)H), 2.72 (d, J = 3.0 Hz, C(2)H), 2.79 (dd, J = 4.4, 13.8 Hz, C(a)*H*H′), 2.89 (dd, J = 4.4, 13.8 Hz, C(a)H*H*′), 3.03 (d, J = 10.7 Hz, C(3)H_β), 3.16 (dd, J = 1.2, 10.2 Hz, C(c)*H*H′), 3.52 (s, C(9a)OCH_3), 3.72 (d, J = 10.2 Hz, C(c)H*H*′), 3.83 (d, J = 10.7 Hz, C(3)H_α), 3.92 (dd, J = 1.2, 6.1 Hz, C(8a)H), 4.46 (q, J = 6.1 Hz, C(9)H), 4.68 (dd, J = 6.1, 11.6 Hz, C(10)*H*H′), 4.89 (dd, J = 6.1, 11.6 Hz, C(10)H*H*′), 8.09 (t, J = 4.4 Hz, C(7)NH), the C(10)OC(O)NH_2 protons were not detected, the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 151 Hz) 9.1 (C(6)*C*H_3), 24.7 (CH_3), 25.6 (CH_3), 33.0 (C(2)), 33.3 (C(1)), 34.0 (C(9)), 40.3 (C(b)), 50.3 (C(3)), 51.1 (C(8a)), 52.4 (C(9a)O*C*H_3), 52.8 (C(a)), 60.7 (C(c)), 64.0 (C(10)), 88.2 (C(5a)), 108.9 (C(9a)), 109.7 (C(6)), 156.4 (C(7)), 158.3 (C(10a)), 163.5 (C(8)), 187.6 (C(5)) ppm, the assignments were in agreement with the APT, HMQC, and HMBC spectra; MS (−CI, methane) *m/e* 401 $[M]^-$; M_r (−CI, methane) 401.208 45 $(M)^-$ (calcd for $C_{20}H_{27}N_5O_4$, 401.206 31).

General Procedure for the Preparation of *cis*- and *trans*-C(1) Methoxymitosenes 37, 38, 41, and 42. The mitomycin (**23**, **25**) was dissolved in a buffered methanolic solution (0.06 M Tris-HCl, "pH" 5.5, 5 mL) and stirred at room temperature (2−5 d). The solution was concentrated under reduced pressure and then separated by preparative TLC (20% MeOH−$CHCl_3$) to afford the desired products.

By using this procedure, the following compounds were prepared.

Preparation of 37 and 38. Using **23** (30 mg, 0.068 mmol) gave **37** and **38** after 2 d. The orange products were purified by two repetitive TLCs.

Compound **37**: yield, 12 mg (40%); HPLC t_R 30.6 min; R_f 0.22 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3444, 2936, 1617, 1559 cm^{-1}; UV-vis (MeOH) λ_{max} 248, 275, 330, 485 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 2.39 (s, C(6)CH_3), 3.55 (s, C(1)OCH_3), 3.91 (dd, J = 4.8, 7.2 Hz, C(2)H), 4.00 (d, J = 11.3 Hz, C(3)H_β), 4.71 (d, J = 4.8 Hz, C(1)H), 4.81 (dd, J = 7.2, 11.3 Hz, C(3)H_α), 5.86 (1/2ABq, J = 13.1 Hz, C(10)*H*H′), 5.96 (1/2ABq, J = 13.1 Hz, C(10)H*H*′), 7.10 (t, J = 7.4 Hz, ArH), 7.22−7.25 (m, ArH), 7.38 (d, J = 7.4 Hz, ArH), 7.45 (d, J = 7.4 Hz, ArH), 7.62 (br s, C(7)NH), the $ArCH_2$ signal is beneath the water peak, the C(10)OC(O)NH_2 protons were not detected, the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 151 MHz) 9.6, 52.3, 54.5, 56.3, 59.6, 60.0, 75.7, 110.7, 113.8, 121.6, 125.0, 126.2, 128.2, 128.4, 132.6, 139.6, 141.7, 143.8, 155.3, 158.4, 175.7 ppm, the remaining signal (C(5a)) is believed to be under the NMR solvent peak.

Compound 38: yield, 12 mg (40%); HPLC t_R 29.7 min; R_f 0.18 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3427, 2929, 1604, 1555 cm^{-1}; UV-vis (MeOH) λ_{max} 248, 275, 330, 485 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 2.37 (s, C(6)CH_3), 3.52 (s, C(1)OCH_3), 4.18 (br d, J = 5.2 Hz, C(2)H), 4.38 (dd, J = 1.5, 12.3 Hz, C(3)H_β), 4.69 (dd, J = 5.2, 12.3 Hz, C(3)H_α), 4.88 (s, C(1)H), 5.90 (s, C(10)H_2), 7.09 (t, J = 7.3 Hz, ArH), 7.22−7.26 (m, ArH), 7.37 (d, J = 7.3 Hz, ArH), 7.44−7.66 (m, C(7)NH, ArH), the $ArCH_2$ signal is beneath the water peak, the C(10)OC(O)NH_2 protons were not detected, the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 151 MHz) 9.5, 54.4, 54.9, 56.3, 59.9, 61.8, 83.5, 110.6, 114.0, 121.6, 125.0, 127.4, 128.2, 128.4, 132.6, 139.5, 141.7, 143.7, 155.3, 158.5, 175.7 ppm, the remaining signal (C(5a)) is believed to be under the NMR solvent peak.

Preparation of 41 and 42. Using **25** (30 mg, 0.075 mmol) gave **41** and **42** after 5 d. The orange products were purified by two repetitive TLCs.

Compound 41: yield, 12 mg (40%): HPLC t_R 25.6 min; R_f 0.49 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3434, 2957, 2929, 1708, 1596, 1560 cm^{-1}; UV-vis (MeOH) λ_{max} 220, 260, 325, 481 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 1.02 (s, 2 CH_3), 2.19 (s, C(6)CH_3), 2.87 (d, J = 4.2 Hz, C(a)H_2), 3.53 (s, C(1)OCH_3), 3.73 (1/2ABq, J = 9.8 Hz, C(c)*H*H′), 3.81 (1/2ABq, J = 9.8 Hz, C(c)H*H*′), 3.93−4.10 (m, C(2)H, C(3)H_β), 4.67 (d, J = 4.8 Hz, C(1)H), 4.86 (dd, J = 6.9, 11.1 Hz, C(3)H_α), 5.91 (1/2ABq, J = 12.6 Hz, C(10)*H*H′), 5.99 (1/2ABq, J = 12.6 Hz, C(10)H*H*′), 7.28 (br s, C(7)NH), 7.65 (br s, C(10)OC(O)NH_2), the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 8.6 (C(6)*C*H_3), 25.0 (CH_3), 25.1 (CH_3), 45.9 (C(b)), 52.5 (C(3)), 54.4 (C(a)), 56.5 (C(1)O*C*H_3), 59.7 (C(2)), 59.7 (C(10)), 62.3 (C(c)), 75.8 (C(1)), 108.2 (C(6)), 113.3 (C(8a)), 125.6 (C(9)), 126.4 (C(9a)), 139.2 (C(7)), 152.7 (C(5a)), 158.4 (C(8)), 158.7 (C(10a)), 176.1 (C(5)) ppm, the assignments were in agreement with the APT spectrum; MS (+CI, methane) *m/e* 402 $[M + 1]^+$; M_r (+CI, methane) 402.213 59 $(M + 1)^+$ (calcd for $C_{20}H_{28}N_5O_4$, 402.214 13).

Compound 42: yield, 12 mg (40%): HPLC t_R 25.0 min; R_f 0.43 (20% MeOH−$CHCl_3$); FT-IR (KBr) 3434, 2955, 2923, 1709, 1591, 1560 cm^{-1}; UV-vis (MeOH) λ_{max} 220, 260, 325, 481 nm; ^1H NMR (pyridine-d_5, 300 MHz) δ 1.01 (s, CH_3), 1.02 (s, CH_3), 2.17 (s, C(6)CH_3), 2.86 (d, J = 4.8 Hz, C(a)H_2), 3.51 (s, C(1)OCH_3), 3.72 (1/2ABq, J = 9.9 Hz, C(c)*H*H′), 3.79 (1/2ABq, J = 9.9 Hz, C(c)H*H*′), 4.20 (br d, J = 5.3 Hz, C(2)H), 4.41 (dd, J = 0.9, 12.5 Hz, C(3)H_β), 4.71 (dd, J = 5.3, 12.5 Hz, C(3)H_α), 4.85 (s, C(1)H), 5.94 (s, C(10)H_2), 7.22 (br s, C(7)NH), the C(10)OC(O)NH_2 protons were not detected, the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 8.6 (C(6)*C*H_3), 25.0 (CH_3), 25.1 (CH_3), 45.8 (C(b)), 54.4 (C(3) or C(a)), 55.0 (C(a) or C(3)), 56.4 (C(1)O*C*H_3), 59.5 (C(10)), 62.0 (C(2)), 62.3 (C(c)), 83.7 (C(1)), 108.2 (C(6)), 113.3 (C(8a)), 126.1 (C(9)), 126.8 (C(9a)), 139.1 (C(7)), 152.6 (C(5a)), 158.5 (C(8) or C(10a)), 158.7 (C(10a) or C(8)), 176.1 (C(5)) ppm, the assignments were in agreement with the APT spectrum; MS (+CI, methane) *m/e* 402 $[M + 1]^+$; M_r (+CI, methane) 402.214 21 $(M + 1)^+$ (calcd for $C_{20}H_{28}N_5O_4$, 402.214 13).

General Procedure for the Preparation of *cis*- and *trans*-C(1) Methoxymitosenes 39, 40, and 43−46. The mitomycin (**23**, **26**, **27**) was dissolved in methanol and the "pH" was adjusted with HCl to "pH" ∼3−5. The solution was stirred at room temperature (5−8 d) and then concentrated under reduced pressure. The products were purified by flash column chromatography (basic alumina) or by preparative TLC. Solvent systems for chromatographies were A, MeOH/$CHCl_3$ (1:4); B, MeOH/$CHCl_3$ (5:95); C, MeOH/$CHCl_3$ (2:1).

By using this procedure, the following compounds were prepared.

Preparation of 39 and 40. Using **24** (60 mg, 0.132 mmol) gave enriched mixtures of **39** and **40** after 5 d. The purple products were purified by TLC (solvent system A).

***cis*-2-Amino-1-methoxy-7-[(2-anilinoethyl)amino]mitosene (39):** yield, 12 mg (4:1 mixture of **39** to **40**, respectively, combined yield 20%); HPLC t_R 32.7 min; R_f 0.28 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3428, 2937, 1715, 1609, 1556 cm^{-1}; UV-

vis (MeOH) λ_{max} 255, 313, 538 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 2.20 (s, C(6)CH_3), 3.48−3.54 (m, C(b)H_2), 3.52 (s, C(1)OCH_3), 3.82−3.93 (m, C(a)H_2, C(2)H, C(3)H_β), 4.64 (d, J = 4.8 Hz, C(1)H), 4.63−4.70 (m, C(3)H_α), 5.73 (s, C(10)H_2), 6.36 (t, J = 5.7 Hz, ArNH), 6.78−6.83 (m, C(4′)ArH, C(7)NH), 6.91 (d, J = 7.7 Hz, C(2′)ArH), 7.30 (t, J = 7.7 Hz, C(3′)ArH), 7.66 (br s, C(10)OC(O)NH_2), the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.1 (C(6)*C*H_3), 44.5 (C(a), C(b)), 52.5 (C(3)), 56.7 (C(1)O*C*H_3), 58.5 (C(10)), 59.5 (C(2)), 75.5 (C(1)), 107.0 (C(6)), 113.1 (Ar), 115.8 (C(8a)), 117.2 (Ar), 129.7 (Ar), 139.8 (C(7)), 147.0 (C(5a)), 149.2 (Ar), 158.1 (C(10a)), 178.1 (C(8)), 178.7 (C(5)) ppm, the signals for C(9) and C(9a) were not detected, the assignments were in agreement with the APT spectrum; MS (+FAB) *m/e* 454 $[M + 1]^+$; M_r (+FAB) 454.209 48 $(M + 1)^+$ (calcd for $C_{23}H_{28}N_5O_5$ 454.209 04).

***trans*-2-Amino-1-methoxy-7-[(2-anilinoethyl)amino]mitosene (40):** yield, 15 mg (2:1 mixture of **40** to **39**, respectively; combined yield 25%); HPLC t_R 31.6 min; R_f 0.24 (10% MeOH−$CHCl_3$); FT-IR (KBr) 3433, 2930, 1714, 1611, 1556 cm^{-1}; UV-vis (MeOH) λ_{max} 255, 313, 538 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 2.18 (s, C(6)CH_3), 3.48−3.54 (m, C(b)H_2), 3.51 (s, C(1)OCH_3), 3.83 (q, J = 5.7 Hz, C(a)H_2), 4.19 (br dd, J = 1.5, 5.4 Hz, C(2)H), 4.28 (dd, J = 1.5, 12.9 Hz, C(3)H_β), 4.55 (dd, J = 5.4, 12.9 Hz, C(3)H_α), 4.81 (br s, C(1)H), 5.70 (1/2ABq, J = 12.9 Hz, C(10)*H*H′), 5.77 (1/2ABq, J = 12.9 Hz, C(10)H*H*′), 6.36 (t, J = 5.7 Hz, ArNH), 6.73 (br s, C(7)NH), 6.81 (t, J = 7.7 Hz, C(4′)ArH), 6.91 (d, J = 7.7 Hz, C(2′)ArH), 7.30 (t, J = 7.7 Hz, C(3′)ArH), 7.64 (br s, C(10)OC(O)NH_2), the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.1 (C(6)*C*H_3), 44.4 (C(a), C(b)), 55.0 (C(3)), 56.7 (C(1)O*C*H_3), 58.3 (C(10)), 61.8 (C(2)), 83.1 (C(1)), 106.9 (C(6)), 113.1 (Ar), 115.9 (C(8a)), 117.2 (Ar), 129.7 (Ar), 139.9 (C(7)), 147.0 (C(5a)), 149.4 (Ar), 158.1 (C(10a)), 178.1 (C(8)), 178.7 (C(5)) ppm, the signals for C(9) and C(9a) were not detected, the assignments were in agreement with the APT spectrum; MS (+FAB) *m/e* 454 $[M + 1]^+$; M_r (+FAB) 454.209 60 $(M + 1)^+$ (calcd for $C_{23}H_{28}N_5O_5$ 454.209 04).

Preparation of 43 and 44. Using **26** (30 mg, 0.074 mmol) gave enriched mixtures of **43** and **44** after 5 d. The purple products were purified by flash column chromatography using solvent system C.

***cis*-2-Amino-1-methoxy-7-[[2-(*N*′-methylamino)propyl]amino]mitosene (43):** yield, 11 mg (5:1 mixture of **43** and **44**, respectively; combined 37% yield); HPLC t_R 27.2 min; R_f 0.03 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3441, 2931, 1716, 1654, 1596, 1507 cm^{-1}; UV-vis (MeOH) λ_{max} 215, 260, 313, 530 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.70 (quintet, J = 6.3 Hz, C(b)H_2), 2.24 (s, C(6)CH_3), 2.37 (s, NCH_3), 2.64 (t, J = 6.3 Hz, C(c)H_2), 3.51 (s, C(1)OCH_3), 3.61−3.68 (m, C(a)H_2), 3.90−3.92 (m, C(2)H, C(3)H_β), 4.64 (d, J = 4.0 Hz, C(1)H), 4.69−4.71 (m, C(3)H_α), 5.77 (s, C(10)H_2), 7.11 (t, J = 5.3 Hz, C(7)NH), 7.73 (br s, C(10)OC(O)NH_2), the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.1 (C(6)*C*H_3), 30.6 (C(b)), 36.6 (NCH_3), 44.6 (C(a)), 50.1 (C(c)), 52.6 (C(3)), 56.7 (C(1)O*C*H_3), 58.5 (C(10)), 59.5 (C(2)), 75.5 (C(1)), 106.2 (C(6)), 115.7 (C(8a)), 120.9 (C(9)), 130.2 (C(9a)), 139.7 (C(7)), 147.4 (C(5a)), 158.1 (C(10a)), 178.0 (C(8)), 180.0 (C(5)) ppm, the assignments were in agreement with the APT spectrum; MS (−CI, methane) *m/e* 405 $[M]^-$; M_r (−CI, methane) 405.201 50 $(M)^-$ (calcd for $C_{19}H_{27}N_5O_5$ 405.201 22).

***trans*-2-Amino-1-methoxy-7-[[2-(*N*′-methylamino)propyl]amino]mitosene (44):** yield, 13 mg (2:1 mixture of **44** and **43**, respectively; combined 44% yield); HPLC t_R 25.4 min; R_f 0.03 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3433, 2936, 1708, 1659, 1594, 1507 cm^{-1}; UV-vis (MeOH) λ_{max} 215, 260, 313, 530 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.72 (quintet, J = 6.3 Hz, C(b)H_2), 2.22 (s, C(6)CH_3), 2.37 (s, NCH_3), 2.63 (t, J = 6.3 Hz, C(c)H_2), 3.50 (s, C(1)OCH_3), 3.62−3.70 (m, C(a)H_2), 4.19 (d, J = 5.0 Hz, C(2)H), 4.31 (d, J = 13.1 Hz, C(3)H_β), 4.57 (dd, J = 5.0, 13.1 Hz, C(3)H_α), 4.81 (s, C(1)H), 5.74 (1/2ABq, J = 13.5 Hz, C(10)*H*H′), 5.80 (1/2ABq, J = 13.5 Hz, C(10)H*H*′), 7.03 (t, J = 5.4 Hz, C(7)NH), 7.76 (br s, C(10)OC(O)NH_2), the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.1 (C(6)*C*H_3), 30.6 (C(b)), 36.6 (NCH_3), 44.5 (C(a)), 50.1 (C(c)), 55.1 (C(3)), 56.6 (C(1)O*C*H_3), 58.4 (C(10)), 61.9 (C(2)), 83.2 (C(1)), 106.1 (C(6)), 115.8 (C(8a)), 122.1 (C(9)), 129.8 (C(9a)), 139.7 (C(7)), 147.3 (C(5a)), 158.1 (C(10a)), 178.1 (C(8)), 179.9 (C(5)) ppm, the assignments were in agreement with the APT spectrum; MS (−CI, methane) *m/e* 405 $[M]^-$; M_r (−CI, methane) 405.198 96 $(M)^-$ (calcd for $C_{19}H_{27}N_5O_5$ 405.201 22).

Table 3. UV-Visible ϵ-Values for Mitomycins 1, 20, 23−26, 34 and Mitosenes 21, 22, 37−44 in MeOH

compd	ϵ (313 nm)	ϵ (365 nm)
1	2 780	17 900
20	2 850	10 000
22	8 320	1 870
23	2 340	17 200
24	2 920	19 400
25	1 370	16 800
26	1 750	13 500
34	8 390	5 950
37	7 920	4 400
38	8 280	4 340
40[a]	6 650	2 710
41	7 180	2 720
42	9 040	2 440
43+**44**	7 930	2 720

[a] Sample **40** is a 2:1 mixture of **40** and **39**, respectively.

Preparation of 45 and 46. Using **27** (40 mg, 0.099 mmol) gave enriched mixtures of **45** and **46** after 8 d. The purple products were purified by three repetitive flash column chromatographies using solvent system B.

***cis*-2-Amino-1-methoxy-7-[[2-(*N*′,*N*′-dimethylamino)ethyl]amino]mitosene (45):** yield, 20 mg (3:1 mixture of **45** and **46**, respectively; combined 50% yield); HPLC t_R 24.3 min; R_f 0.31 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3428, 3200, 2947, 1716, 1661, 1596, 1505 cm^{-1}; UV-vis (MeOH) λ_{max} 255, 313, 325, 525 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 2.16 (s, N$(CH_3)_2$), 2.25 (s, C(6)CH_3), 2.41−2.45 (m, C(b)H_2), 3.51 (s, C(1)OCH_3), 3.52−3.58 (m, C(a)H_2), 3.87−3.98 (m, C(2)H, C(3)H_β), 4.64 (d, J = 4.8 Hz, C(1)H), 4.70 (dd, J = 5.6, 10.1 Hz, C(3)H_α), 5.74 (s, C(10)H_2), 6.91 (t, J = 4.8 Hz, C(7)NH), 7.73 (br s, C(10)OC(O)NH_2), the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.0 (C(6)*C*H_3), 42.6 (C(a)), 44.9 (NCH_3), 52.5 (C(3)), 56.7 (C(1)O*C*H_3), 58.4 (C(b)), 58.5 (C(10)), 59.5 (C(2)), 75.4 (C(1)), 106.3 (C(6)), 115.7 (C(8a)), 120.9 (C(9)), 130.2 (C(9a)), 139.8 (C(7)), 147.1 (C(5a)), 158.1 (C(10a)), 178.3 (C(8)), 179.7 (C(5)) ppm, the assignments were in agreement with the APT spectrum; MS (+CI, methane) *m/e* 406 $[M + 1]^+$; M_r (+CI, methane) 406.207 98 $(M + 1)^+$ (calcd for $C_{19}H_{28}N_5O_5$ 406.209 04).

***trans*-2-Amino-1-methoxy-7-[[2-(*N*′,*N*-dimethylamino)ethyl]amino]mitosene (46):** yield, 15 mg (3:1 mixture of **46** and **45**, respectively; combined 38% yield); HPLC t_R 22.7 min; R_f 0.31 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3438, 3204, 2955, 1718, 1655, 1595, 1499 cm^{-1}; UV-vis (MeOH) λ_{max} 255, 313, 325, 525 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 2.17 (s, N$(CH_3)_2$), 2.23 (s, C(6)CH_3), 2.42 (t, J = 5.7 Hz, C(b)H_2), 3.50 (s, C(1)OCH_3), 3.55 (q, J = 5.7 Hz, C(a)H_2), 4.19 (br d, J = 5.3 Hz, C(2)H), 4.30 (dd, J =1.2, 13.1 Hz, C(3)H_β), 4.57 (dd, J = 5.3, 13.1 Hz, C(3)H_α), 4.81 (s, C(1)H), 5.71 (1/2ABq, J = 12.8 Hz, C(10)*H*H′), 5.78 (1/2ABq, J = 12.8 Hz, C(10)H*H*′), 6.86 (t, J = 5.7 Hz, C(7)NH), 7.65 (br s, C(10)OC(O)NH_2), the assignments were in agreement with the COSY spectrum; ^{13}C NMR (pyridine-d_5, 75 MHz) 10.0 (C(6)*C*H_3), 42.6 (C(a)), 44.9 (NCH_3), 55.0 (C(3)), 56.5 (C(1)O*C*H_3), 58.2 (C(b)), 58.5 (C(10)), 61.9 (C(2)), 83.1 (C(1)), 106.3 (C(6)), 115.8 (C(8a)), 122.1 (C(9)), 129.8 (C(9a)), 139.8 (C(7)), 147.1 (C(5a)), 158.2 (C(10a)), 178.3 (C(8)), 179.6 (C(5)) ppm, the assignments were in agreement with the APT spectrum; MS (+CI, methane) *m/e* 406 $[M + 1]^+$; M_r (+CI, methane) 406.208 58 $(M + 1)^+$ (calcd for $C_{19}H_{28}N_5O_5$ 406.209 04).

Determination of the Absorption Coefficients for Mitomycins 1, 20, 23−26, 34 and Mitosenes 21, 22, 37−44. The UV absorptions at 313 and 365 nm of methanolic solutions were determined in duplicate over a 10−20−fold range in concentrations. The ϵ-values were determined by graphical methods ($r \geq 0.98$) and are listed in Table 3. The ϵ-values for **21**, **27**, **39**, **45** and **46** were assumed to be the same as **22**, **26**, **40**, **43** and **44**, respectively.

General Procedure for the Solvolysis of Mitomycins 20, 23, 24, 26, 27, and 34. A Kinetic Study. Mitomycins **20**, **23**, **24**, **26**, **27**, **34** (1 mg, final concentration ~2 mM) were added to methanolic buffered (0.06 M bis-Tris-HCl, "pH" 5.5) solutions maintained at 25.0 ± 0.1 °C. Each reaction was monitored (HPLC (313, 365 nm), TLC) for at least two half-lives. The "pH" of the solution was determined at the conclusion of the reaction and found to be within 0.1 pH units of the original solution for mitomycins **1**, **20**, **23**, and **24**, within 0.2 "pH" units for **26**, and within 0.3 "pH" units for **27**. The products were identified by coinjection (cospotting) of authentic samples with the reaction mixture in the HPLC (TLC). The peak areas of the products and starting materials in the HPLC chromatograms at 313 nm were adjusted to account for the differences in their relative absorption coefficients (Table 3). Standard data plots yielded linear plots from which pseudo-first-order rate constants (k_{obs}, d^{-1}) and half-lives (d) were calculated. Duplicate kinetic runs were performed and the results averaged (Table 2).

Acknowledgment. This work was funded by NIH grant R01CA29756 and the Robert A. Welch Foundation (E-607). We gratefully acknowledge Dr. M. Kasai (Osaka) and H. Arai (Shizuoka) (Kyowa Hakko Kogyo Co., Ltd., Japan) and Dr. W. Rose (Bristol−Myers Squibb Co., Princeton, NJ) for their gifts of mitomycin A and C, respectively.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for all new compounds (32 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO970675T