was purified by column chromatography with 1:1 ethyl ethanoate/hexanes to give 50 mg (44%) of pyrimidine **5a**: mp 274-276 °C; IR (Nujol, cm^{-1}) 3480, 3100, 1620, 1580; UV (CH_3CN) λ_{max} (log ϵ) 230 (4.19), 285 (4.23), 303 (4.21); ^1H NMR (300 MHz, DMSO-d_6) δ 7.11 (m, 1 H), 7.27 (s, 2 H), 7.43 (t, 2 H, J = 7.93 Hz), 7.97 (d, 2 H, J = 7.91 Hz), 8.21 (s, 1 H), 10.64 (s, 1 H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 121.78, 126.02, 132.39, 132.97, 144.36, 155.88, 158.20, 161.18, 161.25; HREIMS 243.0585 (calcd for $C_{11}H_9N_5S$ 243.0579).

7-Amino-2-(phenylamino)thiazolo[5,4-*d*]pyrimidine (5a) was also prepared (75%) from the reaction of thiazole **2c** with triethyl orthoformate (see procedure for the preparation of **5c** below).[17,31] Anal. Calcd for $C_{11}H_9N_5S$: C, 54.31; H, 3.73. Found: C, 54.34; H, 3.60.

7-Amino-2-[(4-methoxyphenyl)amino]thiazolo[5,4-*d*]pyrimidine (5b, 42 mg, 38%, mp 260-262 °C) was prepared as described above using 5-amino-4-cyano-2-[(4-methoxyphenyl)-amino]thiazole (**2b**) and formamidine acetate: IR (Nujol, cm^{-1}) 3100, 1620, 1580, 1520; UV (CH_3CN) λ_{max} (log ϵ) 228 (4.16), 292 (4.23), 300 (4.16); ^1H NMR (300 MHz, DMSO-d_6) δ 3.83 (s, 3 H), 7.00 (d, 2 H, J = 8.95 Hz), 7.19 (s, 2 H), 7.88 (d, 2 H, J = 8.86 Hz), 8.18 (s, 1 H), 10.47 (s, 1 H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 59.17, 118.09, 123.45, 137.82, 155.58, 157.98, 158.51, 161.55, 174.49; HREIMS 273.0673 (calcd for $C_{12}H_{11}N_5OS$ 273.0684). Anal. Calcd for $C_{12}H_{11}N_5OS$: C, 52.73; H, 4.06. Found: C, 52.81; H, 3.89.

7-Amino-5-methyl-2-(phenylamino)thiazolo[5,4-*d*]pyrimidine (5c).[17,31] Ethanoic anhydride (0.23 mL, 25 mg, 0.24 mmol) was added to a round-bottom flask containing thiazole **2c** (100 mg, 0.46 mmol) and triethyl orthoacetate (5 mL, 4.4 g, 30.9 mmol). The mixture was refluxed for 1 h and cooled, and the unreacted triethyl orthoformate was removed under vacuo. To the residue (a red oil) was added 10 mL of 8 M ethanolic ammonia,[32] and the mixture was stirred at 22-24 °C for 24 h and filtered to afford pyrimidine **5c**. Recrystallization from petroleum ether/propanone gave 57 mg (48%) of pyrimidine **5c** (mp 277-278 °C): IR (Nujol, cm^{-1}) 3100, 2980, 1610, 1040; UV (CH_3CN) λ_{max} (log ϵ) 233 (4.08), 290 (4.16); ^1H NMR (300 MHz, DMSO-d_6) δ 2.35 (s, 3 H), 7.06 (s, 2 H), 6.98-7.98 (m, 5 H), 10.42 (s, 1 H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 25.15, 117.66, 121.84, 126.22, 128.96, 132.45, 140.50, 154.00, 156.49, 157.83, 160.46; HREIMS 257.0760 (calcd for $C_{12}H_{11}N_5S$ 257.0760).

Acknowledgment is made to the National Science Foundation (CHE-90-15849) for support of this research and for financial assistance toward the purchase of the nuclear magnetic resonance (NMR) spectrometers. We also thank the Niels Clauson-Kaas Laboratory, Farnum, Denmark, for the generous sample of aminomalononitrile tosylate (AMNT, 1).

Registry No. 1, 5098-14-6; **2a**, 134312-05-3; **2b**, 134312-06-4; **2c**, 134312-07-5; **2d**, 134312-08-6; **2e**, 134312-09-7; **2f**, 134312-10-0; **5a**, 134312-11-1; **5b**, 134312-12-2; **5c**, 134312-13-3; BuNCS, 592-82-5; 4-$CH_3OC_6H_4NCS$, 2284-20-0; PhNCS, 103-72-0; 4-ClC_6H_4NCS, 2131-55-7; 4-O_2NC_6NCS, 2131-61-5; $C_{10}H_7NCS$, 551-06-4; formamidine acetate, 3473-63-0.

Supplementary Material Available: ^{13}C NMR and ^1H NMR spectra of 5-amino-2-[(4-chlorophenyl)amino]-5-cyanothiazole (**2d**), 5-amino-4-cyano-2-[(4-nitrophenyl)amino]thiazole (**2e**), and 7-amino-5-methyl-2-(phenylamino)thiazolo[5,4-*d*]pyrimidine (**5c**) (6 pages). Ordering information is given on any current masthead page.

7-Aminoaziridinomitosenes: Synthesis, Structure, and Chemistry

Insook Han and Harold Kohn*

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

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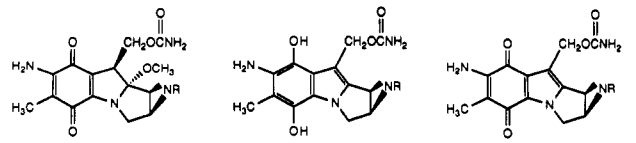
7-Aminoleucoaziridinomitosene (**2a**) has been proposed as a key intermediate in the reductive activation process for the antineoplastic agent, mitomycin C (**1a**). Little is known about **2a** and its oxidized equivalent, 7-aminoaziridinomitosene (**3a**). An expedient electrochemical procedure for **3a** and the corresponding *N*-methyl analogue **3b** has been developed. NMR spectral studies of **3a** in DMF-d_7 and DMSO-d_6 provided important information concerning the solution-state structure for this adduct. Factors controlling the aziridine ring-opening process under reductive and nonreductive conditions have been determined, as well as evidence for the intermediacy of **2a** in the reductive activation cascade of **1a**.

In most commonly accepted proposals pertaining to the mode of action of mitomycin C (**1a**), reductive activation of the antineoplastic agent is believed to generate 7-aminoleucoaziridinomitosene (**2a**), which then undergoes further reaction permitting covalent bonding of the drug to DNA.[1,2] Despite the central importance of this intermediate, few reports have focused on **2a** or its oxidized equivalent **3a**,[3] a situation fostered by the inherent reactivity of this species.[4] In this paper, we describe an expedient synthesis of 7-aminoaziridinomitosenes **3** and their spectral and chemical properties.



1a, R = H; **b**, R = CH_3 **2a**, R = H; **b**, R = CH_3 **3a**, R = H; **b**, R = CH_3

Synthesis. We have reported[5] that electrochemical reduction (−1.0 V, Pt electrode) of 1.5 mM methanolic

(1) (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) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249. (d) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G.; Nakanishi, K. *Science* **1987**, *235*, 1204. (e) Li, V.-S.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 275. (f) Kohn, H.; Hong, Y. P. *Ibid.* **1990**, *112*, 4596. (g) Hong, Y. P.; Kohn, H. *Ibid.* **1991**, *113*, 4634.

(2) For reviews, see: (a) Carter, S. K.; Crooke, S. T. *Mitomycin C. Current Status and New Developments*; Academic Press: New York, 1979. (b) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979; Vol. 1, pp 221-276. (c) 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. (d) Fisher, J. F.; Aristoff, P. A. *Prog. Drug. Res.* **1988**, *32*, 411.

(3) For early descriptions of **3** (**2**), see: ref 1b; Rao, G. M.; Begleiter, A.; Lown, J. W.; Plambeck, J. A. *J. Electrochem. Soc.* **1977**, *124*, 199.

(4) For descriptions of 7-methoxyaziridinomitosenes, see: ref 1b; (a) Cheng, L.; Remers, W. A. *J. Med. Chem.* **1977**, *20*, 767. (b) Danishefsky, S. J.; Egbertson, M. *J. Am. Chem. Soc.* **1986**, *108*, 4648.

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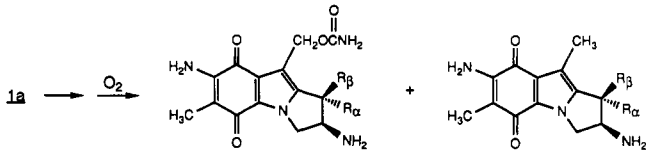
0022-3263/91/1956-4648$02.50/0

solutions of mitomycin C led to the consumption of **1a** and the production of *cis*- (**4a**) and *trans*-1-methoxy-2,7-diaminomitosenes (**4b**)[6] and the corresponding C(10) methyl adducts **5a** and **5b** after oxidative workup.



4a, R_α = H, R_β = OCH_3
b, R_α = OCH_3, R_β = H
6a, R_α = H, R_β = OH
b, R_α = OH, R_β = H

5a, R_α = H, R_β = OCH_3
b, R_α = OCH_3, R_β = H
7, R_α = H, R_β = H

These transformations are projected to proceed through 7-aminoleucoaziridinomitosene (**2a**), which is then sequentially coverted to first reduced **4** and then **5**.[5] Support for the presumptive intermediacy of **2a** is now provided by the periodic quenching (O_2) of the reaction and then analyzing the reaction mixture by HPLC using a binary aqueous acetonitrile solvent system. Reduction (−1.0 V, Pt electrode) of a methanolic solution of **1a** (1.5 mM) for 3 min afforded a product profile containing both *cis*- (**6a**) and *trans*-1-hydroxy-2,7-diaminomitosenes (**6b**)[7] (90%) and the corresponding 1-methoxy adducts **4a** and **4b** (10%), while after 8 min a decrease in **6a,b** (32%), a proportionate increase in **4a,b** (58%), and a small amount of the C(10) methyl products **5a,b** (10%) were observed. Extension of the reduction period to 15 min gave only trace amounts of **6a,b**, noticeable amounts of **4a,b** (61%), and **5a,b** (36%). Prolonged reduction (80 min) of **1a** yielded the fully reduced adduct **7** as the major product (>90%). Significantly, the *cis*- (**6a**) and *trans*-1-hydroxy-2,7-diaminomitosenes (**6b**) detected in the initial stages of the reaction are believed to have been produced by hydrolysis of oxidized 7-aminoaziridinomitosene (**3a**) during the HPLC analysis.

Implementation of this electrochemical procedure to generate **3a** on a semipreparative scale was accomplished using a concentrated 12 mM methanolic solution of mitomycin C. Bulk electrolysis (5 min) of **1a** employing an "H"-type electrochemical cell (−1.0 V, Pt electrode) led to the production of **3a** in greater than 30% yield. Under these conditions, oxidation of **2a** to **3a** occurred during the reaction leading to the precipitation of the desired product from the methanolic solution without the need of further purification.[8]

A similar procedure was employed for the preparation of *N*-methylaziridinomitosene[9] (**3b**) beginning with **1b**. In this case, no precipitate was observed. Accordingly, the reduction was stopped after 3 min and oxidized and then the reaction mixture separated by TLC. *N*-Methylaziridinomitosene (**3b**) was not purified to homogeneity, however, due to the comparable TLC mobilities of **1b** and **3b**.

Structure. Several spectroscopic observations were noted for **3a** that provided important structural information. In the ^1H NMR spectrum for **3a** in DMF-d_7, two upfield, apparent triplets were detected at δ 1.93 (J = 5.84 Hz) and 2.24 (J = 7.85 Hz) (Figure 1a) in a 1:1.7 ratio. COSY analysis indicated that both apparent triplets were

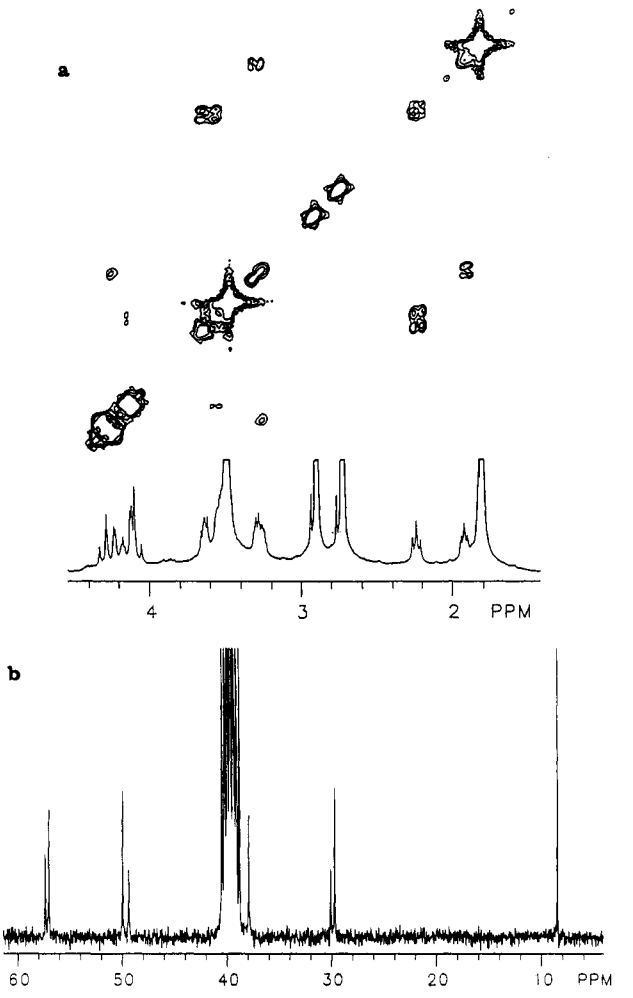
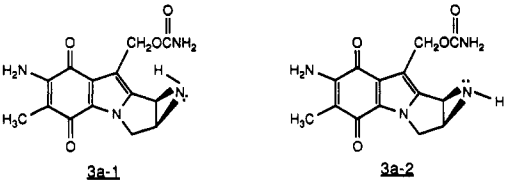


Figure 1. (a) ^1H NMR and COSY spectra of upfield region (δ 1.6–4.4) of compound **3a** in DMF-d_7. (b) ^{13}C NMR spectrum of upfield region (δ 6–60) of compound **3a** in DMSO-d_6.

coupled to signals that have been assigned to the C(1) and C(2) methine hydrogens in **3a** (Figure 1a). A comparable set of observations was noted in DMSO-d_6. Addition of a small amount of D_2O to the DMSO-d_6 NMR sample of **3a** led to a disappearance of both apparent triplets. These findings are consistent with the notion that both the syn (**3a-1**) and anti (**3a-2**) forms of **3a** were present in these solutions.



3a-1 **3a-2**

In agreement with this hypothesis, the upfield region in the proton-decoupled ^{13}C NMR spectrum (DMSO-d_6) of **3a** showed two sets of signals in approximately 1.7:1 ratio for the C(3), C(10), and the C(2) resonances (Figure 1b). Attempts to determine the coalescence temperature (18–72 °C) for the interconversion of the syn and anti forms of **3a** in DMSO-d_6 and DMF-d_7 were unsuccessful. Geometry optimization studies using both semiempirical[10,11] and force field[12] methods suggested that the

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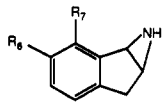
(10) Stewart, J. J. P. MOPAC 6.0, Frank J. Seiler Research Laboratory, United States Air Force Academy, CO, 80840.
(11) Dewar, M. J. S. AMPAC 1.00, QCPE No. 506, Department of Chemistry, Indiana University, Bloomington, IN 47405.

Table I. C(1)-Substitution Patterns Observed for the Ring Opening of Fused Aziridines

entry	compd	solvent or nucleophile (solvent)	products	trans/cis ratio[a]
1	3a	H_2O	6a,b	0.63
2	3a	MeOH	4a,b	0.83
3	3a	EtOH	9a,b	1.10
4[b]	3a	KSC(S)OEt (MeOH)	10b	≥6.00
5	3b	H_2O	11a,b	0.32
6	3b	MeOH	12a,b	0.91

[a] Value determined after HPLC analysis of the reaction mixture prior to workup. [b] HPLC analysis indicated the presence of a minor, unidentified compound that accounted for approximately 13% of the product mixture and that may have corresponded to the cis adduct 10a. Compounds 4a and 4b accounted for approximately 7% of the total product profile (HPLC analysis). The ratio of trans/cis C(1)-methoxy products 4b/4a in this reaction was 1:1.

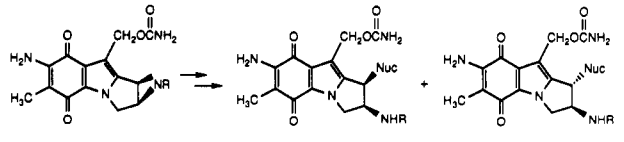
conformers should be of similar stability with a barrier in excess of 20 kcal/mol separating them.[13] Predicted coupling constants of 5.0 and 4.9 Hz for the C(1)H–NH and C(2)H–NH interactions, respectively, were found for 3a-1 using the Karplus relationship, while the corresponding anti isomer is anticipated to have coupling constants of 8.6 and 8.6 Hz, respectively, for these proton–proton interactions. These values suggested that the major conformer present in the DMSO-d_6 and DMF-d_7 solutions was the anti isomer 3a-2. The origin for the unusually high barrier for pyramidal inversion in 3a has not been identified. Similar findings[14,15] were not observed for the related fused indano[1,2-*b*]aziridines 8a[16] and 8c in $CDCl_3$ and CD_3CN, respectively.



8a, R_6 = H, R_7 = H
b, R_6 = OCH_3, R_7 = H
c, R_6 = H, R_7 = OCH_3

The ^1H and ^{13}C NMR chemical shift values for *N*-methyl-7-aminoaziridinomitosene (3b) mirrored those observed for 3a. However, unlike 3a only one set of signals was detected in the ^1H ($CDCl_3$ and pyridine-d_5) and ^{13}C NMR (pyridine-d_5) spectra for 3b.

Chemistry. Select experiments were conducted to determine the reactivity of the aziridine ring in 3a and 3b under various conditions. Dissolution of 3a in H_2O, MeOH, and EtOH led to the production of both *cis*-6a, -4a, and -9a and *trans*-6b, -4b, and -9b C(1)-nucleophilic-substitution products, respectively (Table I, entries 1–3).



3a, R = H
b, R = CH_3

4a, R = H, Nuc = OCH_3
6a, R = H, Nuc = OH
9a, R = H, Nuc = OCH_2CH_3
10a, R = H, Nuc = SC(S)OEt
11a, R = CH_3, Nuc = OH
12a, R = CH_3, Nuc = OCH_3

4b, R = H, Nuc = OCH_3
6b, R = H, Nuc = OH
9b, R = H, Nuc = OCH_2CH_3
10b, R = H, Nuc = SC(S)OEt
11b, R = CH_3, Nuc = OH
12b, R = CH_3, Nuc = OCH_3

Table II. Observed First-Order Rate Constants and Half-Lives for the Methanolysis of 7-Aminoaziridinomitosenes 3[a]

	3a		3b	
"pH"	k_1 (s^{-1})	$t_{1/2}$ (min)	k_1 (s^{-1})	$t_{1/2}$ (min)
7.0[b]	4.1×10^{-3}	3	8×10^{-4}	14
8.5[c]	5.1×10^{-5}	228	3.6×10^{-5}	324

[a] The reactions were monitored by HPLC analysis for at least 2 half-lives and were run in duplicate and averaged. [b] Bis·tris·HCl (0.05 M) + tris·HCl (0.05 M) was used as the buffer in the "pH" 7.0 transformations. [c] Bis·tris·HCl (0.03 M) + tris·HCl (0.07 M) was used as the buffer in the "pH" 8.5 transformations.

A comparable result was observed for 3b in H_2O and MeOH (Table I, entries 5,6) to give 11a,b and 12a,b respectively. Differentiation of the cis and trans adducts was accomplished by use of a Karplussian analysis similar to that described in preceding papers.[17,18] For the cis compounds, the proton–proton coupling interactions of the time-averaged conformation for the constrained pyrrolidine ring led to large proton–proton coupling constants for the C(1)H–C(2)H, C(2)H–C(3)H_α, and C(2)H–C(3)H_β interactions, while in the isomeric trans adducts small couplings between C(1)H–C(2)H and C(2)H–C(3)H_β and a large coupling between C(2)H–C(3)H_α were noted.

The product trends observed for 3a and 3b compared favorably with the profiles observed for the hydrolysis of the three mitomycin C mimics 8a–c.[14,15] These results are consistent with the hypothesis that ring-opening proceeded principally by a S_N1-type pathway. Of interest, the ratio of trans to cis solvolytic products increased as the nucleophilicity[19] of the medium increased. An even more pronounced change in the product distribution was observed upon treatment of 3a with a methanolic solution of KSC(S)OEt (Table I, entry 4). In this case, only the trans adduct 10b was isolated. A small amount of an unidentified compound was detected (HPLC analysis) that accounted for approximately 13% of the product mixture and may have corresponded to the cis isomer 10a. This trend may be a reflection of the steric requirements for the C(1)-substitution process in 3[20] or, alternatively, may indicate an enhanced participation of the nucleophile (solvent) in the ring-opening step as the nucleophilic character of the reagent increased.

The relative reactivity of 3a and 3b and the influence of acid in the ring-opening process was assessed by determining the rate of solvolysis of both 7-aminoaziridinomitosenes in methanol over the "pH" range 7.0–8.5. The averaged value for the first-order rate constants and the calculated half-lives for these transformations are listed in Table II. Several notable patterns were observed. First, placement of a *N*-methyl group on the aziridine ring led to a reduction in the rate of ring-opening to generate the C(1)-methoxy adducts. At "pH" 7.0 and 8.5, 3a underwent solvolysis approximately 5.0–1.4 times faster than the corresponding *N*-methyl analogue 3b. This pattern is consistent with previously observed trends.[21] Second,

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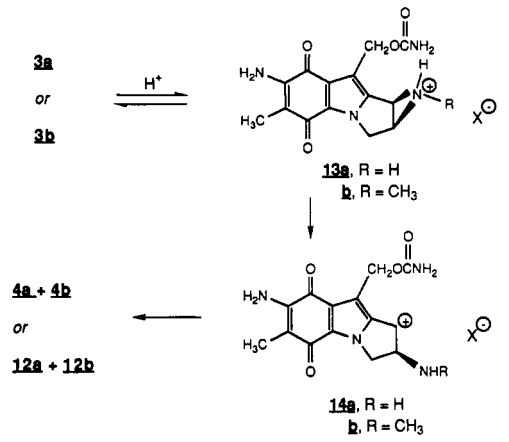
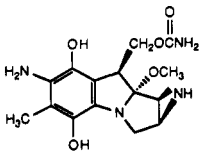
(18) (a) Bean, M.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5033. (b) Bean, M.; Kohn, H. *Ibid.* **1985**, *50*, 293.
(19) (a) Schadt, F. L.; Bentley, T. W.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1976**, *98*, 7667. (b) Bentley, T. W.; Schadt, F. L.; Schleyer, P. v. R. *Ibid.* **1972**, *94*, 992.
(20) Treatment of *reductively* activated mitomycin C with KSC(O)OEt in H_2O led to near-equal amounts of the cis and trans adducts,[18a] while use of KSC(O)Ph gave enhanced levels of the trans products versus the corresponding cis isomers.[18b]
(21) Dermer, O. C.; Ham, G. E. *Ethylenimine and Other Aziridines*; Academic Press: New York, 1969; pp 206–248.

Scheme I. Proposed Pathway for the Nonreductive Ring Opening of 7-Aminoaziridinomitosenes in Methanol



aziridine ring-opening in **3a** and **3b** was acid catalyzed. The estimated half-lives for the consumption of **3a** and **3b** at "pH" 7.0 were 3 and 14 min, respectively, while the corresponding values at "pH" 8.5 were 228 and 324 min, respectively. The observed acid dependency for these transformations was in agreement with a mechanism (Scheme I) in which protonation of the aziridine ring preceded the rate-determining ring-opening step of **13** to generate the benzylic-type carbocation **14**. A similar pathway has been postulated for the hydrolysis of the indano[1,2-*b*]aziridines **8b** and **8c**.[15]

The enhanced stability of **3a** in aqueous basic solutions permitted us to provide additional support for the intermediacy of **2a** in the mitomycin C reductive activation cascade for the generation of C(1)- and C(10)-substituted products. Controlled-potential reduction (−1.0 V, Pt electrode) of **3a** in buffered methanolic solutions ("pH" 10.5) for 3 min followed by HPLC analysis indicated the presence of **6a,b** (54%), **4a,b** (24%), and **5a,b** (22%), while after 8 min the reaction mixture consisted of **6a,b** (15%), **4a,b** (25%), **5a,b** (22%), **7** (18%), and an unidentified compound (20%). HPLC analysis of the reaction solution after reduction for 30 min showed that the fully reduced product **7** was present in 74% yield, along with small amounts of **4a,b** (10%), **5a,b** (7%), and an unidentified compound (9%). The similarity of these results with those observed directly from **1a** suggests that the initial conversion of mitomycin C to **2a** is a necessary step for the activation of the C(1)- and C(10)-bonding centers in the antineoplastic agent. Significantly, compounds **4a** and **4b** were produced more rapidly from **3a** than from **1a**. The decreased reactivity of **1a** versus **3a** has been attributed (in part) to the difficulty encountered in the conversion of leucomitomycin C (**15**) to **2a** under the basic conditions of the electrolysis experiment. Support for this contention stems from mechanistic studies on the hydrolysis of the aziridine ring in the oxidized system **1a**. The rate-limiting step for this transformation is believed to be the acid-catalyzed expulsion of the C(9a)-methoxy group.[22]



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Conclusions

A simple, rapid synthetic method for the preparation of 7-aminoaziridinomitosenes **3** has been developed. NMR analysis of these elusive compounds has furnished new information concerning the solution-state structure of these adducts. Evidence has been provided for the intermediacy of 7-aminoleucoaziridinomitosene (**2a**) in the activation cascade necessary for the rapid functionalization of the C(1)- and C(10)-bonding sites in the antineoplastic agent. Finally, factors controlling the aziridine ring-opening process in **3** under nonreductive conditions have been ascertained by examining the effect of both the nucleophile and the effective "pH" on this transformation.

Experimental Section

General Procedures. Proton (^{1}H NMR) and carbon-13 (^{13}C NMR) nuclear magnetic resonance spectra were recorded on either a Nicolet NT-300 or a General Electric QE-300 spectrometer. Chemical shifts are expressed in parts per million relative to Me_4Si, and coupling constants (J values) are given in hertz. Mass spectral (MS) data were obtained on a Finnegan TSQ-70 triple quadrupole mass spectrometer under negative CI conditions by Drs. David Laude and Mehdi Moini at the University of Texas at Austin. Infrared spectra (FT-IR) were recorded on an IBM FT-IR/32 spectrometer. Ultraviolet–visible absorption spectra were run on a Hitachi Model 100-80 spectrometer. pH measurements were determined with either a Radiometer pHM 26 or a pHM 84 research meter equipped with a Radiometer GK2320C combination glass electrode. The effective "pH" of the MeOH solutions was measured using a Radiometer GK2320C combination glass electrode, which was standardized against aqueous buffer solutions.

HPLC analyses were conducted with the following Waters Associates units: 510 A pump, 510 B pump, Model 680 gradient controller, Model 490 multiwavelength detector, U6K injector. The products were eluted using the following linear gradient conditions: C_{18} μBondapak (SS) column 3.9 mm × 30 cm, from 100% A (3 mM aqueous $Et_3NH{\cdot}H_2PO_4$, pH 4.7), 0% B (3 mM Et_3N in CH_3CN) to 50% A, 50% B in 25 min. The column was fitted with a μBondapak C_{18} guardpak. The organic solvents utilized were HPLC grade, and they were filtered (Millipore FH, 0.50 mm) and degassed prior to use. The aqueous buffers were prepared from deionized water (Milli-Q System, 18+ MΩ·cm) and were filtered (Millipore HA, 0.45 μm) and degassed. The flow rate was 1.0 mL/min, and products were detected at 280, 313, and 365 nm. The HPLC retention times (min) for the mitomycin C and mitosene adducts are as follows: **1a** (19.1), **1b** (20.1), **4a** (21.0), **4b** (20.5), **5a** (26.3), **5b** (24.2), **6a** (19.6), **6b** (18.4), **7** (25.7), **9a** (24.5), **9b** (23.7), **10b** (28.0), **11a** (19.5), **11b** (18.6), **12a** (22.1), and **12b** (21.0). The integrated area of the product peaks and **1a** and **1b** in the HPLC chromatograms at 313 nm were adjusted to account for differences in the adsorption coefficients and then normalized to 100%. All known products were identified by coinjection of an authentic sample with the mixture in the HPLC.

Thin- and thick-layer chromatography were run on precoated silica gel GHLF microscope slides (2.5 × 10 cm; Analtech No. 21521), silica gel GF preparative uniplates (20 × 20 cm; Analtech No. 02013), and hydrocarbon impregnated RPS-F uniplates (10 × 20 cm; Analtech No. 52521). The eluant system employed was 10% MeOH–$CHCl_3$ for normal-phase TLC and 50% aqueous CH_3CN for reversed-phase TLC. The R_f values for the products (normal-phase TLC) are as follows: **1a** (0.38), **1b** (0.44), **3b** (0.48), **4a** (0.35), **4b** (0.29), **5a** (0.70), **5b** (0.65), **6a,b** (0.15), **7** (0.52), **9a** (0.45), **9b** (0.38), **10b** (0.75), **11a** (0.15), **11b** (0.12), and **12a,b** (0.25). All known products were identified by cospotting of an authentic sample with the mixture in the TLC.

Controlled-potential bulk electrolysis of mitomycin C (**1a** and **1b**) was conducted with a SP-2 Synthetic Potentiostat (Bioanalytical Systems Inc., West Lafayette, IN) connected to the electrochemical cell. A conventional "H"-type cell (working area 3 × 2 × 1 cm^3) was utilized in a three-electrode configuration. A

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

Pt wire counter electrode was separated from the Pt gauze (surface area 1 cm^2) working electrode with a sintered glass frit. The working and counter Pt electrode were washed with aqueous HNO_3 (HNO_3/H_2O = 4/1) for 6–8 h after each reaction. The reference electrode was a saturated calomel electrode (SCE). When not in use the SCE was stored in an aqueous saturated KCl solution. Tetrabutylammonium perchlorate (TBAP, 0.1 M) was used as the supporting electrolyte for the analytical scale reductions while $NaClO_4$ (0.1 M) was chosen as the supporting electrolyte for the preparative syntheses of **3**. The TBAP was purified by recrystallization from EtOH, and the $NaClO_4$ was dried in vacuo before use. Deaeration of the reaction was performed by passing O_2-free Ar through the solution (15 min). The solution was agitated with a Teflon-coated magnet and by passing a stream of Ar through the reaction vessel. The controlled-potential reductions were carried out at −1.0 V. MeOH was of the best commercial grade available and used without further purification.

Preparation of 7-Aminoaziridinomitosene (3a). Mitomycin C (**1a**, 20 mg, 60 μmol, final concentration 12 mM) was added to an unbuffered 0.1 M $NaClO_4$ MeOH solution (5 mL) and then deaerated with Ar (15 min). Bulk electrolysis of the solution at −1.0 V (300 s) led to the precipitation of **3a** as a purple solid. The reaction was quenched by the admission of air into the reaction system. The "pH" of the solution changed from 7.6 to 10.8 during the electrolysis. The reaction was cooled (0 °C, 10 min) and filtered, and then the precipitate was washed with cold anhydrous Et_2O and dried in vacuo to give **3a** (8 mg, 30%). HPLC analysis of the precipitate gave two peaks that were identified as **6a** and **6b** after coinjection with authentic samples: mp >240 °C; IR (KBr) 3320, 1695, 1650, 1580, 1480, 1375, 1310, 1190, 890 cm^{-1}; UV–vis (CH_3CN) 248, 312, 348 (sh), 517 nm. The ^1H and ^{13}C NMR analyses of the precipitate indicated the presence of two compounds tentatively identified as *syn*- (**3a-1**) and *anti*-7-aminoaziridinomitosenes (**3a-2**) present in a 1:1.7 ratio. The following spectral properties have been assigned to **3a-1** and **3a-2**: ^1H NMR (DMSO-d_6) **3a-1** δ 1.70 (s, C(6)CH_3), 1.86 (app. t, J = 5.96 Hz, NH), 3.17–3.22 (m, C(1)H, C(2)H), 4.13–4.24 (m, C(3)H_2), 5.06 (s, C(10)H_2); **3a-2** δ 1.70 (s, C(6)CH_3), 2.21 (app. t, J = 6.63 Hz, NH), 3.35–3.44 (m, C(2)H), 3.45–3.50 (m, C(1)H), 3.95–4.07 (m, C(3)H_2), 5.06 (s, C(10)H_2). The signals at δ 1.86 and 2.21 underwent exchange upon addition of D_2O. The ^1H NMR assignments of the two isomers were confirmed by the corresponding COSY spectrum: ^1H NMR (DMF-d_7) **3a-1** δ 1.81 (s, C(6)CH_3), 1.93 (app. t, J = 5.84 Hz, NH), 3.25–3.30 (m, C(1)H, C(2)H), 4.17–4.35 (m, C(3)H_2), 5.18 (s, C(10)H_2); **3a-2** δ 1.81 (s, C(6)CH_3), 2.24 (app. t, J = 7.85 Hz, NH), 3.55–3.60 (m, C(2)H), 3.62–3.66 (m, C(1)H), 4.13–4.24 (m, C(3)H_2), 5.18 (s, C(10)H_2). The ^1H NMR assignments of the two isomers were confirmed by the corresponding COSY spectrum: ^{13}C NMR (DMSO-d_6) δ 8.32 (C(6)*C*H_3), 29.56 [29.94] (C(2)), 37.78 (C(1)), 49.81 [49.23] (C(3)), 56.87 [57.21] (C(10)), 104.25 (C(6)), 111.98 [112.43] (C(8a)), 120.15 (C(9)), 128.76 [128.91] (C(9a)), 140.92 [140.97] (C(7)), 146.99 (C(5a)), 156.64 (C(10)O*C*(O)NH_2), 176.23 (C(8)), 178.33 (C(5)). The signals noted in brackets were approximately 60% in intensity of nearby peaks and have been attributed to **3a-1**.

Preparation of *N*-Methyl-7-aminoaziridinomitosene (3b). *N*-Methylmitomycin C (**1b**, 20 mg, 57 μmol, final concentration 11 mM) was dissolved in a 0.1 M $NaClO_4$ MeOH solution and deaerated with Ar (15 min). Electrolysis of the solution at −1.0 V (300 s) gave **3b** (43%) along with **12a,b** (11%) and unreacted **1b** (46%) (HPLC analysis). The solution was concentrated (~1 mL) in vacuo and then separated by TLC using $CHCl_3$/MeOH/Et_3N (18/2/1) as the eluant. The band containing **3b** was triturated with MeOH containing 1% Et_3N, and then the MeOH solution was evaporated in vacuo to give the desired compound: ^1H NMR ($CDCl_3$) δ 1.78 (s, C(6)CH_3), 2.43 (s, NCH_3), 2.85 (dd, J = 3.93, 4.73 Hz, C(2)H), 3.05 (d, J = 4.73 Hz, C(1)H), 4.10 (dd, J = 3.93, 13.95 Hz, C(3)H_β), 4.32 (d, J = 13.95 Hz, C(3)H_α), 5.23 ($^1/_2$ ABq, J = 12.90 Hz, C(10)*H*H′), 5.30 ($^1/_2$ ABq, J = 12.90 Hz, C(10)H*H*′); ^1H NMR (pyridine-d_5) δ 2.08 (s, C(6)CH_3), 2.20 (s, NCH_3), 2.63 (dd, J = 4.04, 4.63 Hz, C(2)H), 3.20 (d, J = 4.63 Hz, C(1)H), 4.02 (dd, J = 4.04, 13.80 Hz, C(3)H_β), 4.36 (d, J = 13.80 Hz, C(3)H_α), 5.71 ($^1/_2$ ABq, J = 12.09 Hz, C(10)*H*H′), 5.77 ($^1/_2$ ABq, J = 12.09 Hz, C(10)H*H*′); ^{13}C NMR (pyridine-d_5) δ 8.79 (C(6)*C*H_3), 40.14 (C(2)), 44.37 (C(1)), 48.13 (NCH_3), 50.42 (C(3)), 58.25 (C(10)), 106.02 (C(6)), 113.75 (C(9)), 140.46 (C(7)), 147.80 (C(5a)), 158.20 (C(10)O*C*(O)NH_2), 178.40 (C(8)), 179.43 (C(5)). The remaining resonances are believed to be beneath the solvent signals. NMR analysis indicated the presence of a small amount (<20%) of **1b**: UV–vis (CH_3CN) 250, 309, 352 (sh), 520 nm.

Preparation of Compound 7. Compound **7** was prepared with an electrochemical cell (working area 3 × 3 × 10 cm^3) containing a Pt working gauze (surface area 5 cm^2). Mitomycin C (**1a**, 5 mg, 15 μmol, final concentration 1.5 mM) was added to an unbuffered 0.1 M TBAP MeOH solution (50 mL) and then deaerated with Ar (15 min). Bulk electrolysis of the solution at −1.0 V (80 min) led to **7** as the only significant product (HPLC analysis). During the electrolysis the "pH" of the solution changed from 7.6 to 11.2. The solution was neutralized with aqueous 1 M HCl, concentrated in vacuo, and then separated by TLC. Compound **7** was further purified with a G25F Sephadex column (5 × 60 cm) using aqueous NH_4HCO_3 (0.02 M) as the eluant. The fractions containing **7** were collected and lyophilized to yield the desired product (2.5 mg, 60%): ^1H NMR ($CDCl_3$) δ 1.81 (s, C(6)CH_3), 2.21 (s, C(10)H_3), 2.48 (dd, J = 4.45, 15.87 Hz, C(1)H_β), 3.04 (dd, J = 6.64, 15.87 Hz, C(1)H_α), 4.18–4.24 (m, C(2)H), 3.90 (dd, J = 4.45, 12.87 Hz, C(3)H_β), 4.38 (dd, J = 6.64, 12.87 Hz, C(3)H_α); ^{13}C NMR (DMSO-d_6) δ 8.44 C(6)*C*H_3, 10.25 (C(10)), 32.10 (C(1)), 54.88 (C(2) or C(3)), 55.21 (C(2) or C(3)), 104.73 (C(6)), 111.30 (C(8a)), 121.86 (C(9)), 127.42 (C(9a)), 139.35 (C(7)), 146.96 (C(5a)), 176.79 (C(8)), 179.11 (C(5)); MS (-CI) m/e (relative intensity) 245 (M^-, 50), 208 (40), 201 (20), 168 (10), 153 (100); UV–vis (CH_3CN) 240, 312, 520 nm.

General Procedure for the Conversion of 7-Aminoaziridinomitosenes (3a and 3b) to C(1)-Substituted 7-Aminomitosenes (4a,b, 6a,b, 9a,b, 10b, 11a,b, and 12a,b). 7-Aminoaziridinomitosene (**3a** or **3b**) was dissolved in the appropriate solvent (H_2O, MeOH, EtOH) either in the absence or presence of an added nucleophile and then stirred at room temperature (6–18 h). HPLC analysis of the reaction mixture indicated that the reaction was complete and permitted the determination of the ratio of trans to cis adducts (Table I). The solution was concentrated in vacuo and then separated by TLC. The identities of **4a**, **4b**, **6a**, and **6b** were confirmed by coinjection (cospotting) of an authentic sample of each adduct with the reaction mixture in the HPLC (TLC).

***cis*-1-Ethoxy-2,7-diaminomitosene (9a):** ^1H NMR (CD_3OD) δ 1.20 (t, J = 6.97 Hz, OCH_2CH_3), 1.79 (s, C(6)CH_3), 3.61–3.65 (m, OCH_2CH_3), 3.71 (dd, J = 8.90, 11.91 Hz, C(3)H_β), 3.84–3.91 (m, C(2)H), 4.49 (dd, J = 7.15, 11.91 Hz, C(3)H_α), 4.62 (d, J = 4.91 Hz, C(1)H), 5.19 ($^1/_2$ ABq, J = 12.92 Hz, C(10)*H*H′), 5.24 ($^1/_2$ ABq, J = 12.92 Hz, C(10)H*H*′); ^{13}C NMR (DMSO-d_6) δ 8.47 (C(6)CH_3), 15.17 (OCH_2CH_3), 51.92 (C(3)), 57.06 (C(10)), 58.15 (C(2)), 64.01 (OCH_2CH_3), 72.99 (C(1)), 104.49 (C(6)), 113.89 (C(8a)), 120.10 (C(9)), 128.77 (C(9a)), 139.75 (C(7)), 147.21 (C(5a)), 156.53 (C(10)O*C*(O)NH_2), 176.47 (C(8)), 178.49 (C(5)); MS (-CI) m/e (relative intensity) 348 (M^-, 100), 304 (25), 287 (56), 235 (20), 199 (57), 152 (10), 127 (41); UV–vis (CH_3OH) 254, 313, 530 nm.

***trans*-1-Ethoxy-2,7-diaminomitosene (9b):** ^1H NMR (C-D_3OD) δ 1.19 (t, J = 7.00 Hz, OCH_2CH_3), 1.79 (s, C(6)CH_3), 3.62–3.67 (m, OCH_2CH_3), 3.99 (br s, C(2)H), 4.01 (dd, J = 4.99, 12.90 Hz, C(3)H_β), 4.36 (dd, J = 5.40, 12.90 Hz, C(3)H_α), 4.58 (s, C(1)H), 5.19 ($^1/_2$ ABq, J = 13.00 Hz, C(10)*H*H′), 5.24 ($^1/_2$ ABq, J = 13.00 Hz, C(10)H*H*′); ^{13}C NMR (DMSO-d_6) δ 8.45 (C(6)CH_3), 15.28 (OCH_2CH_3), 54.03 (C(3)), 56.69 (C(10)), 60.97 (C(2)), 63.77 (OCH_2CH_3), 80.24 (C(1)), 104.64 (C(6)), 113.66 (C(8a)), 121.17 (C(9)), 128.39 (C(9a)), 139.88 (C(7)), 147.21 (C(5a)), 156.57 (C(10)O*C*(O)NH_2), 176.55 (C(8)), 178.41 (C(5)); MS (-CI) m/e (relative intensity) 348 (M^-, 100), 304 (25), 287 (56), 235 (20), 199 (57), 152 (10), 127 (41); UV–vis (CH_3OH) 254, 313, 530 nm.

***trans*-[(Ethoxythiocarbonyl)thio]-2,7-diaminomitosene (10b).** Dissolution of **3a** (5 mg, 14.5 μmol) in a 0.1 M KSC(S)OEt MeOH solution (5 mL) gave **10b**, **4a**, **4b**, and an unidentified peak in an approximately 24:1:1:4 ratio (HPLC analysis). The reaction sample was concentrated and separated by TLC. The band at R_f 0.75 was further purified by TLC using *i*-PrOH/hexane/ethyl acetate (1/5/5) as the eluant to give **10b**: ^1H NMR ($CDCl_3$) δ 1.45 (t, J = 7.11 Hz, OCH_2CH_3), 1.84 (s, C(6)CH_3), 4.18 (d, J = 13.40 Hz, C(3)H_β), 4.23 (d, J = 5.46 Hz, C(2)H), 4.39 (dd, J = 5.46, 13.40 Hz, C(3)H_α), 4.69 (q, J = 7.11 Hz, OCH_2CH_3), 4.91 (s, C(1)H), 5.22 ($^1/_2$ ABq, J = 13.03 Hz, C(10)*H*H′), 5.28 ($^1/_2$ ABq, J = 13.03

Hz, C(10)H*H'*); UV-vis (CH_3CN) 251, 303, 536 nm.

***cis*-1-Hydroxy-2-(methylamino)-7-aminomitosene (11a)**: ^1H NMR (DMSO-d_6) δ 1.72 (s, C(6)CH_3), 2.35 (s, NCH_3), 3.50–3.55 (m, C(2)H), 3.61 (dd, J = 8.54, 11.65 Hz, C(3)H_β), 4.34 (dd, J = 6.61, 11.65 Hz, C(3)H_α), 4.91 (d, J = 4.60 Hz, C(1)H), 5.01 ($^1/_2$ ABq, J = 12.49 Hz, C(10)*H*H'), 5.06 ($^1/_2$ ABq, J = 12.49 Hz, C(10)H*H'*); ^{13}C NMR (DMSO-d_6) δ 8.30 (C(6)CH_3), 34.00 (NCH_3), 49.68 (C(3)), 56.62 (C(10)), 62.38 (C(2)), 65.59 (C(1)), 104.62 (C(6)), 112.61 (C(8a)), 120.48 (C(9)), 128.07 (C(9a)), 142.40 (C(7)), 146.95 (C(5a)), 156.69 (C(10)O*C*(O)NH_2), 176.47 (C(8)), 178.39 (C(5)); UV-vis (CH_3CN) 248, 312, 516 nm.

***trans*-1-Hydroxy-2-(methylamino)-7-aminomitosene (11b)**: ^1H NMR (CD_3OD) δ 1.79 (s, C(6)CH_3), 2.45 (s, NCH_3), 3.58–3.62 (m, C(2)H), 4.00 (dd, J = 3.20, 13.34 Hz, C(3)H_β), 4.47 (dd, J = 6.32, 13.34 Hz, C(3)H_α), 4.96 (d, J = 3.00 Hz, C(1)H), 5.16 ($^1/_2$ ABq, J = 12.97 Hz, C(10)*H*H'), 5.24 ($^1/_2$ ABq, J = 12.97 Hz, C(10)H*H'*); UV-vis (CH_3CN) 249, 312, 518 nm.

***cis*-1-Methoxy-2-(methylamino)-7-aminomitosene (12a).** Compound **12a** was separated by reversed-phase TLC (R_f = 0.29) using 50% aqueous CH_3CN as the eluant and then purified by passing through a SiO_2 gel column using $CHCl_3$/MeOH (4/1) as the eluant: ^1H NMR (CD_3OD) δ 1.80 (s, C(6)CH_3), 2.49 (s, NCH_3), 3.41 (s, OCH_3), 3.70–3.80 (m, C(2)H, C(3)H_β), 4.56 (dd, J = 4.99, 9.75 Hz, C(3)H_α), 4.76 (d, J = 4.50 Hz, C(1)H), 5.26 (s, C(10)H_2); ^{13}C NMR (DMSO-d_6) δ 8.45 (C(6)CH_3), 34.03 (NCH_3), 49.66 (C(3)), 56.02 (OCH_3), 57.35 (C(10)), 65.96 (C(2)), 71.41 (C(1)), 104.48 (C(6)), 114.55 (C(8a)), 119.98 (C(9)), 128.99 (C(9a)), 138.78 (C(7)), 147.25 (C(5a)), 156.49 (C(10)O*C*(O)NH_2), 176.41 (C(8)), 178.45 (C(5)); UV-vis (CH_3CN) 245, 310, 500 nm.

***trans*-1-Methoxy-2-(methylamino)-7-aminomitosene (12b).** Compound **12b** was separated by reversed-phase TLC (R_f = 0.21) using 50% aqueous CH_3CN as the eluant and then purified by passing through a SiO_2 column using $CHCl_3$/MeOH (4/1) as the eluant: ^1H NMR (CD_3OD) δ 1.80 (s, C(6)CH_3), 2.43 (s, NCH_3), 3.42 (s, OCH_3), 3.71–3.75 (m, C(2)H), 4.13 (d, J = 13.27 Hz, C(3)H_β), 4.34 (dd, J = 5.56, 13.27 Hz, C(3)H_α), 4.65 (s, C(1)H), 5.21 ($^1/_2$ ABq, J = 12.99 Hz, C(10)*H*H'), 5.27 ($^1/_2$ ABq, J = 12.99 Hz, C(10)H*H'*); ^{13}C NMR (DMSO-d_6) δ 8.38 (C(6)CH_3), 33.71 (NCH_3), 51.63 (C(3)), 55.71 (OCH_3), 56.92 (C(10)), 68.71 (C(2)), 78.22 (C(1)), 104.65 (C(6)), 114.08 (C(8a)), 121.01 (C(9)), 128.30 (C(9a)), 139.11 (C(7)), 147.18 (C(5a)), 156.45 (C(10)O*C*(O)NH_2), 176.43 (C(8)), 178.33 (C(5)); UV-vis (CH_3CN) 243, 303, 494 nm.

Solvolysis of 7-Aminoaziridinomitosenes 3 in Buffered MeOH Solutions. A Kinetic Study. 7-Aminoaziridinomitosene (**3a** or **3b**, 2 mg, final concentration 13 mM) was dissolved in DMSO-d_6 (0.5 mL) and deaerated with Ar (15 min). A 50-μL sample of the DMSO-d_6 stock solution was then added to a buffered MeOH solution (0.5 mL) maintained at 20 ± 1 °C. Bis·tris·HCl (0.05 M) + tris·HCl (0.05 M) was utilized in the "pH" 7.0 transformations, and bis·tris·HCl (0.03 M) + tris·HCl (0.07 M) was used in the "pH" 8.5 transformations. Each reaction was carried for at least 2 half-lives and monitored by HPLC. Verification of the product peaks (i.e., **4a**, **4b**, **12a**, **12b**) was conducted by coinjection (cospotting) of an authentic sample with the reaction mixture in the HPLC (TLC). Standard data plots yielded linear slopes from which pseudo-first-order rate constants (k_1, s^{-1}) were calculated. Duplicate kinetic runs were performed and the results averaged (Table II).

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Supplementary Material Available: Copies of the NMR spectra for compounds **3a,b**, **7**, **9a,b**, **10b**, **11a,b**, and **12a,b** (13 pages). Ordering information is given on any current masthead page.

1′,2′-Secothymidines. The Preparation of 2,3′-Anhydro Derivatives and the Formation of Two Unusual Dimeric Products

Anne F. Cichy, Racha Saibaba, Hussein I. El Subbagh, Raymond P. Panzica, and Elie Abushanab*

Departments of Medicinal Chemistry and Chemistry, University of Rhode Island, Kingston, Rhode Island 02881

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2,2′- and 2,3′-anhydropyrimidine nucleosides are rigid compounds that are restricted to the syn conformation. In certain cases, this enforced conformational rigidity has led to biologically active compounds. To capitalize on this rationale, attempts were made to prepare the corresponding anhydro analogues of 1′,2′-secothymidine. Starting with the functionalized butanetetrols and -triols (**1a–c** and **2a–c**), derived from D-isoascorbic acid, the 1′,2′-seco nucleoside tosylates **9a–c** were prepared. While attempts to form the 2,3′-anhydro derivatives (**10a** and **12**) were successful, no 2,2′-anhydro analogues could be obtained even under a variety of reaction conditions. Instead, unusual dimeric compounds were formed whose structures were confirmed by ^{13}C NMR and mass spectrometry. The dimerization reaction does not appear to have been previously reported.

The discovery of acyclovir as an antiviral agent[1] has stimulated the search for other acyclic nucleosides with comparable or broader spectra of activity. Subsequently, 9-(dihydroxypropoxymethyl)guanine (DHPG) was synthesized,[2] shown to have antiherpetic activity, and has recently been approved for clinical use against human cytomegalovirus. In addition, two phosphonic acid acyclic nucleosides, 9-[(*S*)-3-hydroxy-2-(phosphonylmethoxy)propyl]adenine (*S*-HPMPA) and 9-[(phosphonylmethoxy)ethyl]adenine (PMEA) have also demonstrated antiherpetic[3] activity as well as anti-HIV activity.[4]

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