Mitomycin C Analogues with a Substituted Hydrazine at Position 7. Synthesis, Spectral Properties, and Biological Activity

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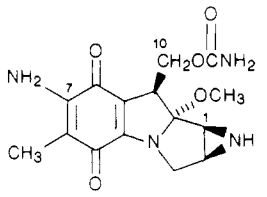
A select number of mitomycin C derivatives with a substituted hydrazine group at position 7 were synthesized and tested for antineoplastic activity by using an in vivo test with murine P388 leukemia. Several of the adducts displayed activity comparable to that of mitomycin C. The X-ray-determined crystal structure of one of the derivatives (**3f**) is reported.

Mitomycin C (1) is a clinically proven antineoplastic agent for the treatment of stomach, breast, colon, and cranial cancer.[1] Extensive studies have provided evidence that reduction of the quinone moiety in 1 activates the carbon-1 and -10 positions in the drug toward nucleophiles.[2-9] The antitumor agent is believed to express its action by mono- and dialkylating (cross-linking) DNA at this sites.[10,11] Consistent with this hypothesis, Sartorelli and co-workers have demonstrated that 1 functions best within hypoxic compartments of tumors.[12] These regions should contain sufficient concentrations of cellular reductases required for the initial activation of the drug and permit the subsequent binding of 1 to the genetic material.



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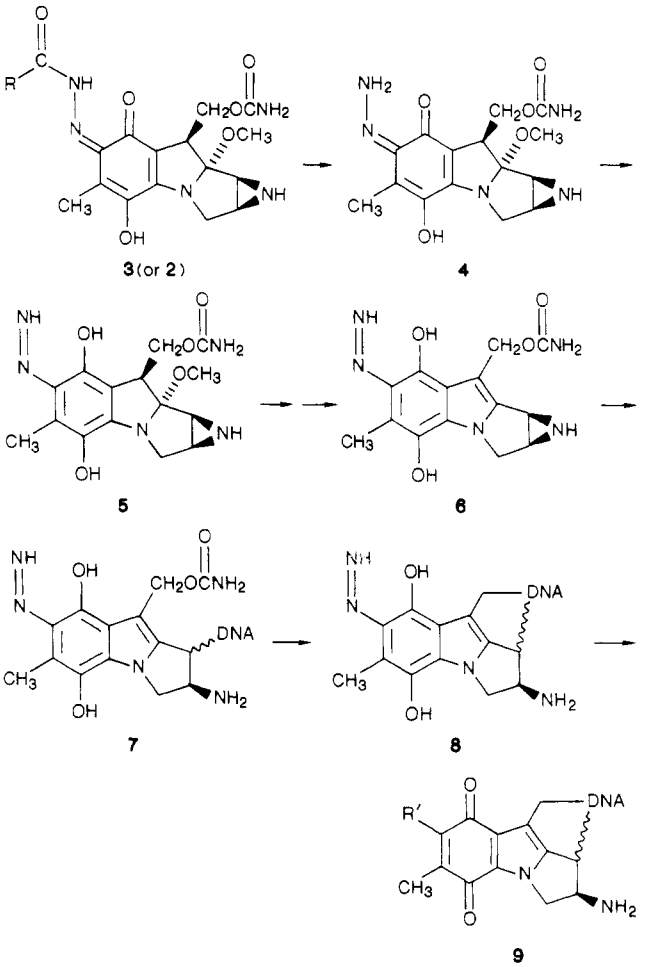
It is well known that tumor growths are heterogeneous, containing regions of varying hypoxia.[13] Since curative therapy requires that all tumor cells capable of indefinite replication be destroyed, a need exists for chemotherapeutic agents that function under a variety of cellular conditions. A recent study in our laboratory demonstrated that hydrazine and alkylhydrazines can serve as suitable chemical reductants for mitomycin C.[14] Subsequent investigations showed that no noticeable activation was observed when acylhydrazines (i.e., acethydrazide, semicarbazide) were employed in the place of hydrazine.[15]

Scheme I



3 (or 2) 4 5 6 7 8 9

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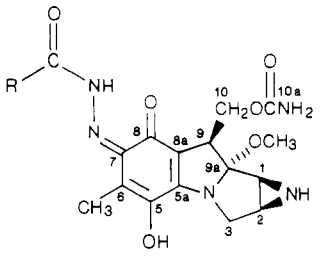
Table I. Characteristic Physical and Spectral Data for Mitomycin C Derivatives **3a–h**[a]

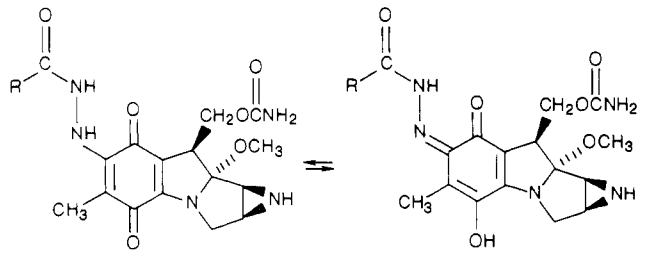


3

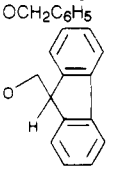
			^{1}H NMR[c]			^{13}C NMR[d]		
no.	% yield	vis[b]	1	2	6-CH_3	5	6-CH_3	8
3a	59	379	3.00 (br s)	2.86 (br s)	2.05 (s)	137.82	10.90	174.86
3b	63	393	2.92 (br s)[e]	2.79 (br s)	2.10 (s)	141.46	11.29	174.97
3c	64	392	3.01 (d, 2.3)	2.88 (d, 2.3)	2.10 (s)	143.33[f]	10.23	175.09
3d	81	396	3.03 (br s)	2.88 (br s)	2.16 (s)	140.28	11.69	174.82
3e	63	376	3.01 (d, 3.9)	2.87 (d, 3.9)	2.08 (s)	139.52	11.16	174.95
3f	72	377	3.00 (d, 4.6)	2.86 (dd, 1.4, 4.6)	2.07 (s)	139.96[f]	10.19	175.00
3g	85	377	2.98 (br s)	2.85 (br s)	2.06 (s)	139.77	11.08	174.87
3h	41	377	3.00 (br s)	2.87 (br s)	2.09 (s)	139.60	10.94	174.66

[a] All the adducts decomposed above 200 °C. [b] Values reported in nm. [c] Value in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si followed by the multiplicity of the signal and the coupling constant in hertz when appropriate. All spectra were recorded at 300 MHz, and the solvent used was CD_3OD unless otherwise specified. [d] The number in each entry is the chemical shift observed in ppm relative to Me_4Si. All spectra were recorded at 75.5 MHz, and the solvent used was pyridine-d_5 unless otherwise specified. [e] Spectrum taken in CD_3CN. [f] Spectrum taken in CD_3OD.

These results suggested the syntheses of a new class of mitomycin C derivatives (**2**) in which an acylated hydrazine moiety is strategically placed within the framework of the drug candidate. The protecting acyl group is projected to serve as a chemical safety latch. Activation of the mitomycin is envisioned to occur by an internal reduction–oxidation process (i.e., **4** → **5**) *only after* rupture (i.e., acid, base, enzymatic) of the *N*-acyl bond (Scheme I). The incorporation of the reductant within the mitomycin may decrease the need for cellular reductases normally required for the activation of the drug and permit the chemotherapeutic agent to function in regions of the tumor growth that are less hypoxic. In this paper, the synthesis, spectral properties, and biological data for this novel series of carbon-7 substituted hydrazine mitomycin C derivatives are presented.



2 **3**

a, R = CH_3 **e**, R = OCH_2CH_3
b, R = C_6H_5 **f**, R = $OC(CH_3)_3$
g, R = $OCH_2C_6H_5$
c, R = 
d, R = 
h, R = 

Chemistry

The synthetic approach adopted for the preparation of **2** (or **3**) was patterned after the procedure utilized by Remers and co-workers for the synthesis of carbon-7 amino-substituted mitomycins.[16] Mitomycin C (**1**) was first converted to mitomycin A and then treated with 2 equiv of the acid hydrazide or alkyl carbazate in methanol. Eight substituted mitomycins (**3a–h**) were produced in this fashion (Table I). Each reaction proceeded in good to excellent yield and gave only a single product (TLC analysis). No evidence for the activation of the mitomycin was observed under these conditions.

The hydrazinomitomycin derivatives were all characterized in the solution state as the *o*-azaquinone tautomer (i.e., **3a–h**) on the basis of several key spectral observations. First, consistent patterns were noted throughout the ultraviolet–visible and ^{1}H and ^{13}C NMR spectra for these eight compounds, suggesting that these adducts contained a common structure. Second, compounds **3a–h** all displayed a pronounced absorption in the visible spectrum between 376 and 395 nm. A comparable band of near equal intensity appeared in mitomycin C at 360 nm^{-1}. Third, the carbon-6 methyl protons in **3a–h** consistently appeared in the ^{1}H NMR spectra at ~δ 2.1. This value was downfield from the value (~δ 1.8) typically observed for mitomycin C adducts.[17] Fourth, the ^{1}H NMR spectrum of **3b** in CD_3CN revealed a peak at δ 17.03 integrating for one proton. Signals in this downfield region are normally associated with acidic hydrogens (i.e., phenolic, carboxylic acid protons).[18] Fifth, the pattern of the signals in the proton-decoupled ^{13}C NMR spectra for the quinone carbons in **3a–h** were atypical for mitomycin C adducts. In particular, only one signal was detected in the range normally observed for quinone carbonyl carbon atoms (i.e., 175–178 ppm),[17,19] while a new resonance appeared be-

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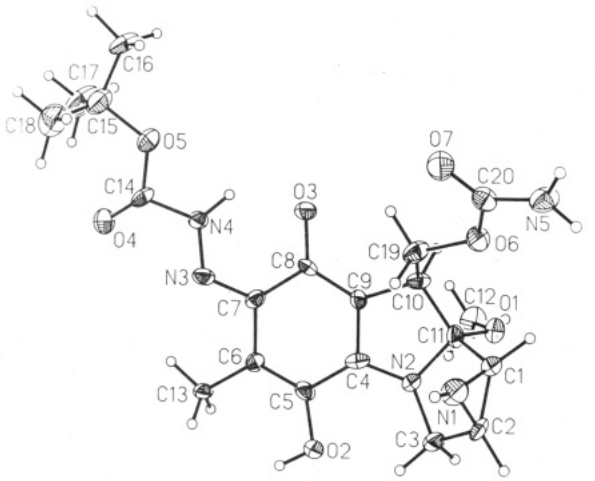

Figure 1. View of **3f** showing the atom labeling. The non-hydrogen atoms are shown as 20% equiprobability ellipsoids and hydrogens as spheres of arbitrary diameter.

tween 137.8 and 143.3 ppm. Confirmation of the proposed assignment was secured from the single-crystal X-ray structure determination of the *tert*-butyl carbazate derivative **3f**. The crystal structure of **3f** clearly showed that the compound existed in the *o*-azaquinone form **3** with an intramolecular hydrogen bond occurring between the hydrazine nitrogen N(4) and the quinone oxygen O(3) (Figure 1). It is of interest to note that analysis of the lengths for the N(2)–C(4)–C(9)–C(8)–O(3) bonds in **3f** indicated that there was substantial bond delocalization within this segment of the molecule. Consistent with this observation, the pyrrolidine nitrogen N(2) did not lie significantly above the plane defined by the quinone-type ring. A larger deviation from planarity was observed for the nitrogen N(2) atom in the single-crystal X-ray structure of *N*-brosylmitomycin A.[20]

Biological Activity

The data for the in vivo tests with mitomycin C derivatives **3a–h** against P388 leukemia are listed in Table II.[21] For the purpose of discussion, the compounds evaluated can be classified as either acylhydrazine (i.e., **3a–d**) or as carbazate (i.e., **3e–h**) mitomycin C analogues. All the compounds were biologically active. Within the carbazate series, adducts **3e**, **3g**, and **3h** displayed the highest efficacy (optimum T/C%) when compared to the concurrently run mitomycin C controls. Of the remaining acylhydrazine mitomycin C derivatives, only the acethydrazide adduct **3a** exhibited pronounced activity (T/C% = 190, 51.2 mg/kg per dose), while the three aroylhydrazide adducts **3b**, **3c**, and **3d** were moderately active.

Conclusions

Select mitomycin C analogues with a substituted hydrazine moiety at position 7 have been prepared and characterized. Evidence has been secured that these adducts existed as the *o*-azaquinone tautomer **3**. Pharmacological evaluation of these compounds documented that many of the mitomycin analogues displayed comparable efficacies to mitomycin C in the P388 test. Significantly, the potencies of several of these compounds were comparable to that observed for mitomycin C. The mode of activation of these drug candidates is currently under investigation.

Table II. In Vivo Test Results for Mitomycin C Derivatives **3a-h**[a]

	P388 screen[b]	
no.	maximum[c] %T/C	MED[d]
3a	190 (51.2) [205 (4.8)]	1.6 [1.6]
3b	150 (25.6) [205 (4.8)]	0.8 [0.8]
3c	150 (25.6) [205 (4.8)]	12.8 [1.6]
3d	140 (6.4) [205 (4.8)]	0.8 [0.8]
3e	180 (25.6) [205 (4.8)]	0.8 [0.8]
3f	160 (25.6) [205 (4.8)]	0.8 [0.8]
3g	185 (6.4) [205 (4.8)]	1.6 [1.6]
3h	195 (25.6) [215 (4.8)]	0.8 [1.6]

[a] Tests conducted at Bristol-Myers Laboratories. [b] P388 lymphocytic leukemia screen: the tumor was ip as was the treatment. [c] The values reported are the median survival time for the test substrate at the optimal dose (mg/kg per dose) listed in parentheses. The corresponding values for the concurrently run mitomycin C sample appear in brackets. %T/C = (median survival time of drug treated animals/median survival time of control animals) × 100. [d] The values reported are minimum effective dose (%T/C ≥ 125) in mg/kg per dose observed for the substrate tested, followed by corresponding values for mitomycin C in brackets. MED = minimum effective dose.

Experimental Section

General Methods. Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. Infrared spectra (IR) were run on a Perkin-Elmer 283 spectrophotometer and calibrated against the 1601 cm^{-1} band of polystyrene. Absorption values are expressed in wave numbers (cm^{-1}). Ultraviolet spectra were recorded on a Hitachi-100 spectrophotometer. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were taken on either a Nicolet NT-300 or General Electric QE-300 NMR instrument. Chemical shifts are in parts per million (δ values) relative to Me_4Si, and coupling constants (J values) are in hertz and are reported using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, complex multiplet; br, broad. Mass spectral data were obtained on either a Finnigan-MAT TSQ-70 tandem mass spectrometer or a VG Analytical ZAB-2E high resolution mass spectrometer by Dr. John Chinn (University of Texas–Austin). CH_4 was used as the ionizing gas for all spectra obtained under negative CI conditions and the spectra were obtained by rapid thermal desorption by direct exposure. Elemental analyses were performed by Spang Microanalysis Laboratory, Eagle Harbor, MI. Ethyl carbazate, *tert*-butyl carbazate, acethydrazide, benzoic acid hydrazide, 2-thiophenecarboxylic acid hydrazide, and nicotinic acid hydrazide were obtained from Aldrich Chemical Company, Milwaukee, WI. Benzyl carbazate was obtained from Lancaster Synthesis Ltd., Windham, NH.

Preparation of Mitomycin C Analogues (General Procedure). A methanolic solution (50 mL) of mitomycin A[22] (100 mg, 0.286 mmol) and 0.573 mmol of alkyl carbazate or acid hydrazide was stirred at room temperature until thin layer chromatographic analysis indicated that there was no further change in the reaction. The solvent was removed under vacuum at room temperature and the crude product was triturated with a 90:10 mixture of $CHCl_3$–MeOH (10 mL). The orange to dark brown pure crystalline material that remained after trituration was filtered. A second crop of the crystalline material was obtained by removal

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of solvent from the filtrate and trituration of the residue with chloroform. The remaining chloroform layer was then concentrated and purified by medium pressure chromatography on a silica gel column (2 cm × 16 cm) using the indicated solvent system for elution. In those cases where trituration of the residue did not yield pure products, the final product was purified by medium pressure chromatography.

Using this procedure, the following compounds were synthesized.[23]

Acetic Acid, [1a*S*-(1aα,8β,8aα,8bα)]-[8-[[(Aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-7-oxoazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-6(7*H*)-ylidene]hydrazide (3a). Acethydrazide (42.4 mg, 0.573 mmol) and mitomycin A gave 140 mg of an orange brown solid, which on purification by trituration yielded 67 mg (59%) of an orange crystalline product: mp >200 °C; R_f 0.30 (20% methanol–chloroform); IR (KBr) 1700, 1620, 1540, 1470, 1400, 1375, 1350, 1320, 1230, 1190, 1165 cm^{-1}; UV (MeOH) λ_{max} 379 nm (ϵ 23 800); ^{1}H NMR (CD_3OD) δ 2.05 (s, 3 H), 2.30 (s, 3 H), 2.86 (br s, 1 H), 3.00 (br s, 1 H), 3.20 (s, 3 H), 3.58 (br dd, J = 4.0, 11.2 Hz, 2 H), 4.31 (br dd, J = 10.4, 11.2 Hz, 2 H), 4.68 (dd, J = 4.0, 10.4 Hz, 1 H); ^{13}C NMR (pyridine-d_5) 10.90, 19.88, 32.46, 37.35, 43.80, 49.51, 50.73, 62.18, 106.82, 110.60, 121.00, 137.82, 142.57, 158.17, 159.81, 173.77, 174.86 ppm; mass spectrum, m/e (relative intensity) (negative CI) 407 (M + 16, 18), 392 (M + 1, 21), 391 (M, 100), 348 (7), 300 (7), 288 (10), 286 (15).

Anal. Calcd for $C_{17}H_{21}N_5O_6$: C, 52.17; H, 5.40; N, 17.89. Found: C, 52.06; H, 5.53; N, 17.77.

Benzoic Acid, [1a*S*-(1aα,8β,8aα,8bα)]-[8-[[(Aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-7-oxoazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-6(7*H*)-ylidene]hydrazide (3b). Benzoic hydrazide (78.0 mg, 0.573 mmol) and mitomycin A yielded 175 mg of a dark brown residue, which on purification by chromatography (10% methanol–chloroform) gave 82 mg (63%) of a dark brown crystalline solid: mp >200 °C; R_f 0.30 (15% methanol–chloroform); IR (KBr) 1690, 1660, 1570, 1480, 1450, 1400, 1325, 1235, 1155 cm^{-1}; UV (MeOH) λ_{max} 393 nm (ϵ 26 800); ^{1}H NMR (CD_3CN) δ 2.10 (s, 3 H), 2.79 (br s, 1 H), 2.92 (br s, 1 H), 3.20 (s, 3 H), 3.52 (br dd, J = 4.4, 10.9 Hz, 2 H), 4.18 (br d, J = 12.7 Hz, 2 H), 4.84 (dd, J = 4.4, 10.5 Hz, 1 H), 7.54–7.64 (m, 3 H), 7.93–7.95 (m, 2 H), 17.03 (s, 1 H); ^{13}C NMR (pyridine-d_5) 11.29, 32.61, 37.28, 43.89, 49.71, 50.73, 62.24, 106.93, 110.61, 121.71, 128.51, 129.24, 132.67, 133.91, 141.47, 143.09, 158.24, 160.01, 165.29, 174.97 ppm.

Anal. Calcd for $C_{22}H_{23}N_5O_6 \cdot 0.9CHCl_3$: C, 49.03; H, 4.29; N, 12.48. Found: C, 48.88; H, 4.85; N, 12.16.

3-Pyridinecarboxylic Acid, [1a*S*-(1aα,8β,8aα,8bα)]-[8-[[(Aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-7-oxoazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-6(7*H*)-ylidene]hydrazide (3c). Nicotinic acid hydrazide (78.6 mg, 0.573 mmol) and mitomycin A gave 175 mg of a dark brown colored residue, which on purification by chromatography (15% methanol–chloroform) furnished 83 mg (64%) of the product as a dark brown crystalline solid: mp >200 °C; R_f 0.17 (15% methanol–chloroform); IR (KBr) 1670, 1590, 1425, 1400, 1380, 1325, 1240, 1160 cm^{-1}; UV (MeOH) λ_{max} 392 nm (ϵ 20 000); ^{1}H NMR (CD_3OD) δ 2.10 (s, 3 H), 2.88 (br s, 1 H), 3.01 (br s, 1 H), 3.25 (s, 3 H), 3.62 (br dd, J = 4.1, 10.9 Hz, 2 H), 4.30 (dd, J = 10.4, 10.9 Hz, 1 H), 4.40 (dd, J = 1.3, 12.5 Hz, 1 H), 4.76 (dd, J = 4.1, 10.4 Hz, 1 H), 7.62 (dd, J = 4.1, 7.8 Hz, 1 H), 8.37 (dd, J = 1.2, 7.8 Hz, 1 H), 8.75 (d, J = 4.1 Hz, 1 H), 9.12 (br s, 1 H); ^{13}C NMR (CD_3OD) 10.23, 33.45, 37.74, 43.80, 50.25, 50.82, 62.63, 107.38, 110.64, 121.77, 125.35, 130.26, 137.51, 143.33, 144.20, 149.97, 153.57, 159.60, 161.09, 165.66, 175.09 ppm.

Anal. Calcd for $C_{21}H_{22}N_6O_6 \cdot 0.7CHCl_3$: C, 48.44; H, 4.25; N, 15.62. Found: C, 48.59; H, 4.46; N, 15.41.

2-Thiophenecarboxylic Acid, [1a*S*-(1aα,8β,8aα,8bα)-[8-[[(Aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-7-oxoazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-6(7*H*)-ylidene]hydrazide (3d). Using mitomycin A and 2-thiophenecarboxylic acid hydrazide (81.5 mg, 0.573 mmol) gave 175 mg of a dark brown residue that was purified by column chromatography (12% methanol–chloroform). The unconsumed mitomycin A and 2-thiophenecarboxylic acid hydrazide that coeluted during the column chromatography were further reacted for another 5 days in methanol (10 mL) and the reaction mixture was purified to give additional product. The desired compound was obtained as dark brown crystalline material in an overall yield of 81% (106 mg): mp >200 °C; R_f 0.27 (15% methanol–chloroform); IR (KBr) 1700, 1630, 1565, 1480, 1410, 1340, 1325, 1225, 1100 cm^{-1}; UV (MeOH) λ_{max} 396 nm (ϵ 24 400); ^{1}H NMR (CD_3OD) δ 2.16 (s, 3 H), 2.88 (br s, 1 H), 3.03 (br s, 1 H), 3.25 (s, 3 H), 3.63 (br dd, J = 4.2, 11.0 Hz, 2 H), 4.30 (br dd, J = 10.5, 11.0 Hz, 2 H), 4.77 (dd, J = 4.2, 10.5 Hz, 1 H), 7.23 (dd, J = 3.7, 5.1 Hz, 1 H), 7.89 (br d, J = 5.1 Hz, 1 H), 7.92 (d, J = 3.7 Hz, 1 H); ^{13}C NMR (pyridine-d_5) 11.69, 32.53, 37.26, 43.78, 49.62, 50.70, 62.16, 106.91, 110.60, 121.34, 128.03, 132.71, 133.64, 136.38, 140.28, 143.11, 158.21, 159.88, 161.43, 174.82 ppm; mass spectrum, m/e (relative intensity) (negative CI) 477 (M + 18, 11), 475 (M + 16, 100), 460 (M + 1, 15), 459 (M, 34), 414 (18), 400 (8), 286 (22).

Anal. Calcd for $C_{20}H_{21}N_5O_6S \cdot 0.6CHCl_3$: C, 46.58; H, 4.09; N, 13.18. Found: C, 46.57; H, 4.35; N, 13.22.

Ethyl [1a*S*-(1aα,8β,8aα,8bα)]-8-[[(Aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-7-oxoazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-6(7*H*)-ylidene]hydrazinecarboxylate (3e). Employing mitomycin A and 2 equiv of ethyl carbazate (59.5 mg, 0.573 mmol) gave 160 mg of an orange brown solid, which after purification by trituration yielded 76 mg (63%) of an orange crystalline solid: mp >200 °C; R_f 0.52 (20% methanol–chloroform); IR (KBr) 1760, 1720, 1690, 1630, 1610, 1560, 1475, 1430, 1405, 1375, 1325, 1230, 1175 cm^{-1}; UV (MeOH) λ_{max} 376 nm (ϵ 26 300); ^{1}H NMR (CD_3OD) δ 1.33 (t, J = 7.0 Hz, 3 H), 2.08 (s, 3 H), 2.87 (d, J = 3.9 Hz, 1 H), 3.01 (d, J = 3.9 Hz, 1 H), 3.23 (s, 3 H), 3.59 (br dd, J = 4.1, 10.7 Hz, 2 H), 4.30 (br q, J = 7.0 Hz, 4 H), 4.70 (dd, J = 4.1, 10.5 Hz, 1 H); ^{13}C NMR (pyridine-d_5) 11.16, 14.52, 32.50, 37.37, 43.89, 49.56, 50.69, 62.05, 62.21, 106.87, 110.58, 121.56, 139.52, 142.24, 154.54, 158.21, 159.85, 174.95 ppm.

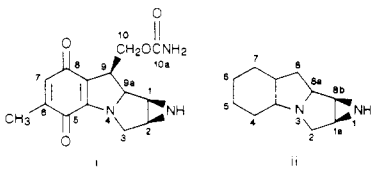
Anal. Calcd for $C_{18}H_{23}N_5O_7$: C, 51.30; H, 5.50; N, 16.62. Found: C, 51.18; H, 5.44; N, 16.45.

1,1-Dimethylethyl [1a*S*-(1aα,8β,8aα,8bα)]-[8-[[(Aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-7-oxoazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-6(7*H*)-ylidene]hydrazinecarboxylate (3f). Using *tert*-butyl carbazate (75.6 mg, 0.573 mmol) and mitomycin A gave 175 mg of an orange brown residue, which after purification by trituration and chromatography (10% methanol–chloroform) furnished 118 mg (72%) of an orange crystalline solid: mp >200 °C; R_f 0.38 (15% methanol–chloroform); IR (KBr) 1720, 1610, 1475, 1330, 1240, 1140 cm^{-1}; UV (MeOH) λ_{max} 377 nm (ϵ 28 700); ^{1}H NMR (CD_3OD) δ 1.54 (s, 9 H), 2.07 (s, 3 H), 2.86 (dd, J = 1.4, 4.6 Hz, 1 H), 3.00 (d, J = 4.6 Hz, 1 H), 3.23 (s, 3 H), 3.58 (br dd, J = 4.1, 11.0 Hz, 2 H), 4.28 (d, J = 12.9 Hz, 1 H), 4.30 (dd, J = 10.4, 11.0 Hz, 1 H), 4.69 (dd, J = 4.1, 10.4 Hz, 1 H); ^{1}H NMR (pyridine-d_5) δ 1.53 (s, 9 H), 2.30 (br d, J = 5.5 Hz, 1 H), 2.41 (s, 3 H), 2.70 (br d, J = 5.5 Hz, 1 H), 3.23 (s, 3 H), 3.69 (br d, J = 12.7 Hz, 1 H), 4.11 (dd, J = 4.0, 10.8 Hz, 1 H), 4.64 (d, J = 12.7 Hz, 1 H), 5.14 (dd, J = 10.3, 10.8 Hz, 1 H), 5.68 (dd, J = 4.0, 10.3 Hz, 1 H); ^{13}C NMR (pyridine-d_5) 10.19, 28.39, 33.42, 37.99, 43.90, 50.09, 50.77, 62.70, 83.37, 107.20, 110.64, 122.79, 139.96, 142.24, 155.00, 159.61, 160.53, 175.00 ppm; mass spectrum, m/e (relative intensity) (CI) 450 (M + 1, 35), 394 (28), 350 (43), 289 (33), 287 (27), 257 (33), 177 (62), 139 (97), 111 (100).

Anal. Calcd for $C_{20}H_{27}N_5O_7 \cdot CHCl_3$: C, 44.34; H, 4.96; N, 12.31. Found: C, 44.43; H, 4.96; N, 12.14.

Phenylmethyl [1a*S*-(1aα,8β,8aα,8bα)]-[8-[[(Aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-

(23) The numbering system used within the paper is based on the conventional system used for the mitomycins and is depicted in i. The names that appear in the Experimental Section are based on the correct Chemical Abstract nomenclature system which employs the numbering system indicated in ii.



8a-methoxy-5-methyl-7-oxoazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-6(7*H*)-ylidene]hydrazinecarboxylate (3g). Using benzyl carbazate (95.0 mg, 0.573 mmol) and mitomycin A furnished approximately 200 mg of an orange brown residue, which was purified by trituration and chromatography (8% methanol–chloroform). The unreacted mitomycin A and benzyl carbazate that coeluted from the chromatographic column were allowed to further react (6 days) in methanol (10 mL) to give an additional amount of the product. The desired product was obtained as orange crystals in 85% yield (117 mg): mp >200 °C; R_f 0.30 (10% methanol–chloroform); IR (KBr) 1715, 1700, 1605, 1560, 1470, 1400, 1325, 1225, 1160 cm^{-1}; UV (MeOH) λ_{max} 377 nm (ϵ 25300); ^{1}H NMR (CD_3OD) δ 2.06 (s, 3 H), 2.85 (br s, 1 H), 2.98 (br s, 1 H), 3.22 (s, 3 H), 3.58 (br dd, J = 4.2, 11.1 Hz, 2 H), 4.30 (br dd, J = 10.6, 11.1 Hz, 2 H), 4.69 (dd, J = 4.2, 10.6 Hz, 1 H), 5.28 (s, 2 H), 7.32–7.43 (m, 5 H); ^{13}C NMR (pyridine-d_5) 11.08, 32.49, 37.32, 43.81, 49.54, 50.65, 62.18, 67.58, 106.86, 110.41, 121.41, 128.52, 128.89, 136.80, 137.69, 139.77, 142.34, 154.53, 158.17, 159.85, 174.87 ppm; mass spectrum, m/e (relative intensity) (negative CI) 501 (M + 18, 7), 499 (M + 16, 100), 483 (M, 38), 467 (7), 439 (7), 438 (22); M_r (negative CI) 499.1696 [M + O]$^-$ (calcd for $C_{23}H_{25}N_5O_8$, 499.1703).

Anal. Calcd for $C_{23}H_{25}N_5O_7 \cdot 0.1CHCl_3$: C, 56.00; H, 5.10; N, 14.13. Found: C, 55.88; H, 5.17; N, 13.76.

9′-Fluorenylmethyl [1a*S*-(1aα,8β,8aα,8bα)]-[8-[[(Aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-7-oxoazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-6(7*H*)-ylidene]hydrazinecarboxylate (3h). Making use of 9-fluorenylmethyl carbazate[24] (145.5 mg, 0.573 mmol) and mitomycin A gave 240 mg of an orange brown residue, which after purification by chromatography (10% methanol–chloroform) furnished 67 mg (41%) of an orange solid: mp >200 °C; R_f 0.14 (10% methanol–chloroform); IR (KBr) 1700, 1610, 1560, 1470, 1445, 1400, 1340, 1320, 1270, 1160 cm^{-1}; UV (MeOH) λ_{max} 263 nm (ϵ 18400), 298 (sh) nm (8000), 377 nm (22900); ^{1}H NMR (CD_3OD) δ 2.09 (s, 3 H), 2.87 (br s, 1 H), 3.00 (br s, 1 H), 3.25 (s, 3 H), 3.61 (dd, J = 3.9, 10.9 Hz, 2 H), 4.29–4.34 (m, 2 H), 4.54–4.61 (m, 3 H), 4.72 (dd, J = 3.9, 10.3 Hz, 1 H), 7.30–7.43 (m, 4 H), 7.69 (d, J = 7.3 Hz, 2 H), 7.82 (d, J = 7.3 Hz, 2 H); ^{13}C NMR (pyridine-d_5) 10.94, 32.14, 37.15, 43.68, 47.20, 49.46, 50.51, 62.05, 68.08, 106.70, 110.34, 120.34, 121.35, 125.66, 127.47, 128.10, 139.60, 141.52, 144.01, 144.10, 154.43, 158.19, 159.82, 174.66 ppm.

Anal. Calcd for $C_{30}H_{29}N_5O_7 \cdot H_2O$: C, 61.11; H, 5.30; N, 11.88. Found: C, 61.43; H, 4.98; N, 11.80.

X-ray Crystal Structure of 3f. Crimson-colored columnar crystals of **3f** were grown by slow evaporation from MeOH, $C_{20}H_{27}N_5O_7 \cdot H_2O$, space group $P2_1$ (monoclinic), with a = 7.381 (4), b = 8.508 (3), and c = 19.865 (5) Å, β = 95.91 (3)°, Z = 2, density = 1.25 g-cm^{-3}. Intensity measurements were made with Mo Kα radiation (λ = 0.71073 Å; graphite monochromator) on a Nicolet R3m/V automatic diffractometer in the ω mode to a limit of 50° <2θ. A total of 1282 unique reflections were corrected for Lorentz and polarization effects. The solution of the structure was obtained from TREF using SHELXTL PLUS direct methods yielding coordinates for all atoms in the asymmetric unit. The usual sequence of isotropic and anisotropic refinement was followed, after which all hydrogens were entered in ideal calculated positions. Only the positions of H21, H22, H24, H25A, and H25B (attached to N1, O2, N4, and N5) were allowed to refine independently. A single isotropic thermal parameter was refined for all of the hydrogen atoms. Additionally, a water molecule of solvation was found. The absolute configuration could not be determined experimentally and so the chirality was adjusted in order to match that determined for the bromobenzoyl derivative of mitomycin C.[20] After all shift/esd ratios were less than 0.1, convergence was reached at R = 0.065 (R_w = 0.064). No unusually high correlations were noted between any of the variables in the last cycle of full-matrix least-squares refinement, and the final difference density map showed no peaks greater than 0.30 e/Å^3. All calculations were made by using Nicolet's SHELXTL PLUS (1987) series of crystallographic programs.

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Registry No. **3a**, 117606-62-9; **3b**, 117606-63-0; **3c**, 117606-64-1; **3d**, 117606-65-2; **3e**, 117606-66-3; **3f**, 117606-67-4; **3g**, 117606-68-5; **3h**, 117606-69-6; mitomycin A, 4055-39-4; acethydrazide, 1068-57-1; benzoic hydrazide, 613-94-5; nicotinic acid hydrazide, 553-53-7; 2-thiophenecarboxylic acid hydrazide, 2361-27-5; ethyl carbazate, 4114-31-2; *tert*-butyl carbazate, 870-46-2; benzyl carbazate, 5331-43-1; 9-fluorenylmethyl carbazate, 35661-51-9.

Supplementary Material Available: ^{1}H (Table 3) and ^{13}C (Table 4) NMR spectral data for mitomycin derivatives **3a–h** and tables listing data collection and processing parameters (Table 5), atomic coordinates and equivalent isotropic displacement parameters (Table 6), bond lengths (Table 7), bond angles (Table 8), and hydrogen-bonding parameters (Table 9) (12 pages); observed and calculated structure factors (Table 10) for compound **3f** (5 pages). Ordering information is given on any current masthead page.

(24) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404–3409.