Mitomycin Betaines: Synthesis, Structure, and Solvolytic Reactivity†

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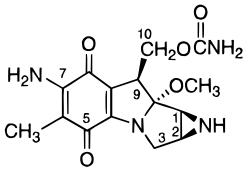
7-*N*-[2-(Methylamino)ethyl]mitomycin C (**2b**) and 7-*N*-[2-(ethylamino)ethyl]mitomycin C (**2c**) underwent rapid C(8) cyclization and oxidation to generate the novel quinoxaline mitomycin betaines **5a**,**b**, respectively. Correspondingly, treatment of mitomycin A with *N*-methyl-1,2-phenylenediamine gave phenazine mitomycin betaine **17**. Similar spectral properties were observed for the three heteroaromatic zwitterionic compounds **5a**,**b**, and **17**. We have learned that the formation of **5a**,**b** proceeds by initial cyclization of the terminal amine group to the mitomycin C(8) quinone carbon to generate the corresponding hemiaminal intermediate. Dissolution of **5a**,**b** in buffered methanolic solutions ("pH" 5.5, 7.4) at 25 °C did not lead to aziridine ring opening. The reactivity of **5a**,**b** is briefly discussed in terms of their electronic structures.

The clinical significance of the bioreductive alkylating agent mitomycin C (**1**) for the treatment of solid tumors[1] has led to the development of analogues that have improved efficacy.[2,3] Among the over 600 semisynthetic analogues evaluated are the 7-*N*-(2-aminoethyl)mitomycin C derivatives **2**.[4] Saito and co-workers were the first to show that the parent compound **2a**[4a] exists as the cyclic C(8) imine adduct **3** and that these compounds possess significant anticancer activities.[5,6]



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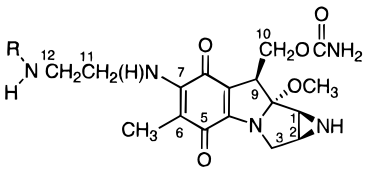
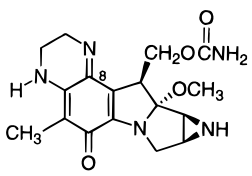


2a R = H

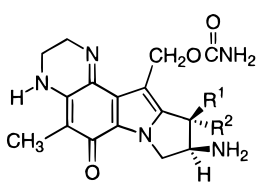
b R = CH_3

c R = CH_2CH_3



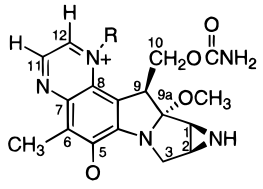
3

Recently, we provided structural information in support of **3** and demonstrated that dissolution of **3** in buffered methanolic solutions ("pH" 7.4, 25 °C) *without* reductants led to the facile ring opening of the aziridine ring to give the C(1) methoxy-substituted mitosenes **4a**,**b**.[7] These findings suggest that **3** can react with DNA *in vivo* without prior reductive activation. Our preliminary results led us to investigate the reactivity of 7-*N*-(2-aminoethyl)mitomycin C derivatives **2b**,**c** where the terminal amine was a secondary amine. We report that these mitomycins undergo rapid intramolecular cyclization at the C(8) carbonyl unit to provide novel quinoxaline betaines **5a**,**b**, respectively.



4a R^1 = OCH_3, R^2 = H

b R^1 = H, R^2 = OCH_3

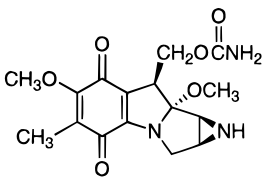


5a R = CH_3

b R = CH_2CH_3

Results and Discussion

Treatment of a pyridine solution of mitomycin A (**6**) with *N*-methylethylenediamine (**7**) and *N*-ethylethylenediamine (**8**) gave **2b**,**c**, respectively, as blue-green solids in yields greater than 98%. Consistent with the proposed structures for **2b**,**c** in pyridine-d_5 were the appearance of two signals in the ^{13}C NMR spectrum between 177.1 and 179.3 ppm for the C(5) and C(8) carbonyl carbon resonances.



6

$RN(H)CH_2CH_2NH_2$

7 R = CH_3

8 R = CH_2CH_3

Dissolution of **2b** in either buffered methanol ("pH" 7.4, 8.9) or dichloromethane led to the rapid formation (<24 h) of orange solid **5a** as the major product. Inspection of the ^1H NMR spectrum revealed the pronounced downfield

† Dedicated to Professor Roy A. Olofson on the occasion of his 60th birthday.

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

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

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

(4) (a) Iyengar, B. S.; Sami, S. M.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 16−20. (b) Iyengar, B. S.; Sami, S. M.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 1453−1457. (c) Sami, S. M.; Iyengar, B. S.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1984**, *27*, 701−708.

(5) 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.

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shifts of the C(6) (δ 3.00) and terminal amine (δ 4.43) methyl resonances and the detection of two downfield vinylic signals (δ 7.26, 8.21) that were strongly coupled in the corresponding COSY spectrum. Noticeably absent in the ^{1}H NMR spectrum were the methylene signals associated with the ethylenediamine unit in **2b**. High-resolution mass spectrometry (FAB(+)) revealed a parent ion ($[M + 1]^+$) at 372, which corresponded to the loss of H_2O and H_2 from the starting mitomycin **2b**.

Repetition of these experiments with **2c** gave similar results. Dissolution of **2c** in buffered methanol ("pH" 7.4, 8.9) or dichloromethane led to orange solid **5b**. Formation of **5b** proceeded more slowly than that of **5a** (HPLC, TLC analyses). The high-resolution mass spectral data (FAB(+)) for **5b** was consistent with the loss of H_2O and H_2 from the starting mitomycin **2c**. In the ^{1}H NMR spectrum for **5b**, the C(6) methyl protons appeared downfield from **2c** (Δ = 0.91 ppm), and the methylene protons of the *N*-ethyl unit appeared as two downfield diastereotopic multiplets at δ 4.34−4.41 and 5.51−5.56. Once again, we observed the presence of two distinctive, downfield signals at δ 7.37 and 8.27 but no methylene protons associated with the ethylenediamine unit. The long-range heteronuclear multiple quantum chemical shift correlation (HMBC) spectrum revealed key three-bond connectivities that allowed the NMR assignment of the quinoxaline heteroaromatic system (Figure 1). Additional evidence in support of **5b** was provided by the NOESY spectra that showed that the *N*-ethyl moiety was in close proximity to the C(9)H−C(10)HH' group. This finding was consistent with molecular modeling experiments.[8]

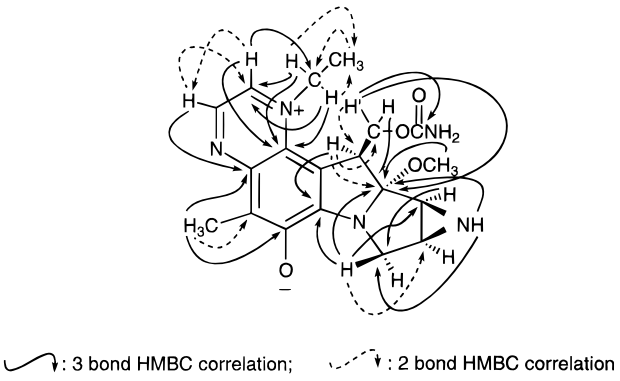
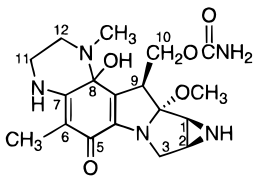


Figure 1. HMBC responses for compound **5b**.

The pathway for the formation of these novel betaines has not been fully established. We have results that show that the reaction proceeds through hemiaminal **9**.



9

Dissolution of **2b** in buffered methanol ("pH" 7.4) at 25 °C led to changes in the UV−vis spectrum over 1 h and a change in the solution from blue to green (Figure 2a). Maintaining this solution at 25.0 °C after 1 h resulted in only small changes. Elevating the temperature of the solution to 36.3 °C initiated a change that took 1 day to complete and turned the solution from green to yellow (Figure 2b). The UV−vis spectrum of the reaction

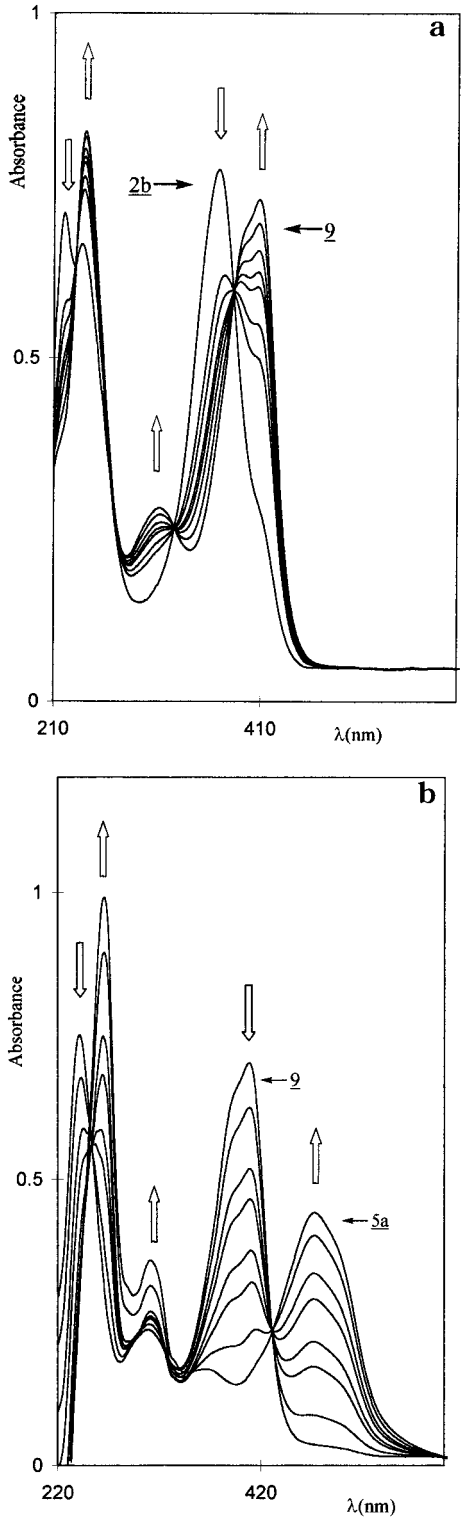


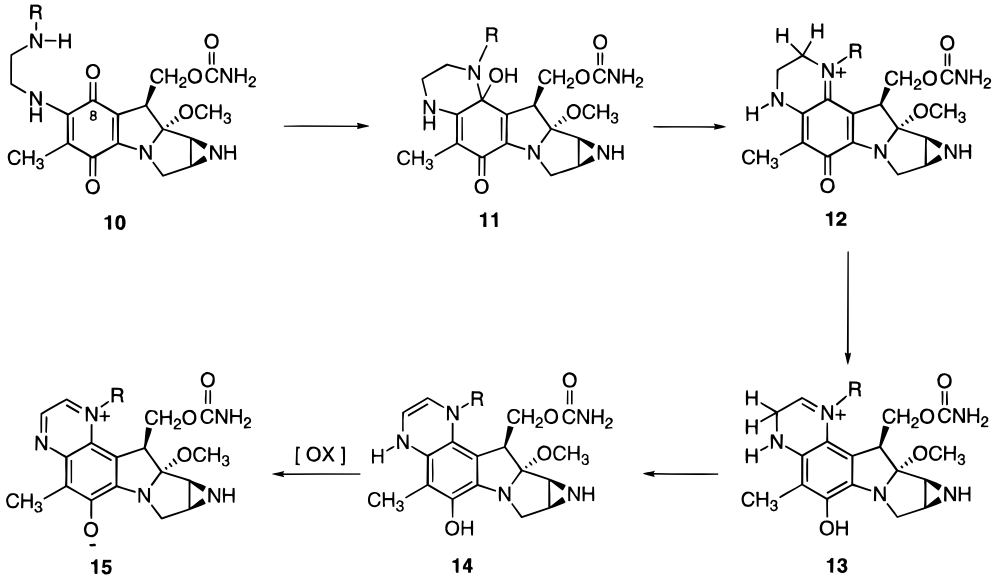
Figure 2. (a) Optical spectra (MeOH, "pH" 7.4, 25.0 °C, 0−60 min) of conversion of **2b** to **9** (isosbestic points: 230, 267, 327, 382 nm). (b) Optical spectra (MeOH, "pH" 7.4, 36.3 °C, 0−24 h) of conversion of **9** to **5a**.

(6) Compound **3** exhibited a LD_{50}/ED_{50} = 1.1 after treatment of ddY mice implanted (ip) with sarcoma 180 cells and increased the life span of CDF_1 mice with lymphocytic leukemia P-388 by 0.43 compared to **1**, while the N(1a) methyl analogue of **3** possessed a LD_{50}/ED_{50} = 1.7 in ddY mice and increased the life span in CDF_1 mice by 0.60 compared to **1**.[5]

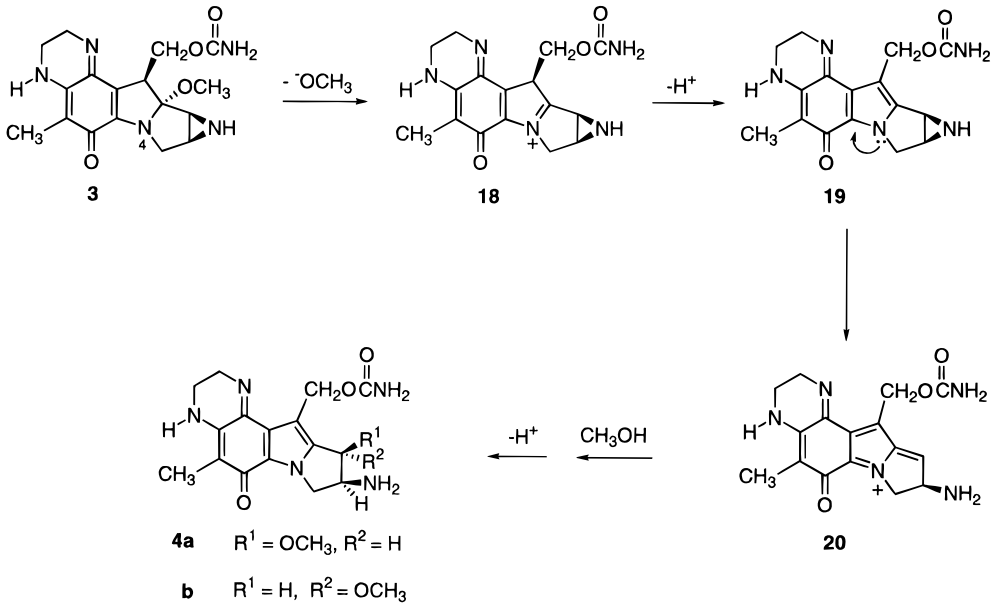
(7) Kohn, H.; Wang, S. *Tetrahedron Lett.* **1996**, *37*, 2337−2340.

(8) The calculations were done using the program PC MODEL version 5.1 from Secena Software, Bloomington, IN.

Scheme 1. Proposed Pathway for Formation of the Quinoxaline Betaines



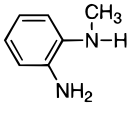
Scheme 2. Proposed Route for Solvolysis of Compound 3

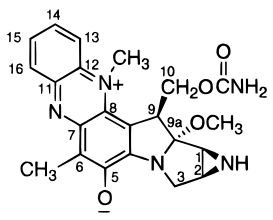


after 1 day at 36.3 °C matched that of **5a**. Repetition of this experiment in CD_3OD permitted the NMR assignment of **9** as the initially formed intermediate generated at 25 °C. In the ^1H NMR spectrum for **9**, the two triplets for the C(11) and C(12) methylene protons in **2b** were replaced by four distinctive multiplets located at δ 3.05−3.59. In the ^{13}C NMR spectrum, we observed the loss of one carbonyl resonance and the appearance of a new peak at 84.8 ppm. We have assigned this signal to C(8). Additional information in support of **9** was obtained from the NOESY spectrum. Significant interactions between the *N*-methyl signal and the C(9) methine and one of the two C(10) methylene protons were detected. The close proximity of these protons was consistent with preliminary molecular modeling experiments of **9**.[8]

Our finding that conversion of **2b** to **5a** likely proceeds through **9** suggests the general pathway in Scheme 1 for *N*-substituted 7-*N*-(aminoethyl)mitomycins **10**. We propose that the reaction proceeds by cyclization of the C(7) *N*-alkylethylenediamine unit in **10** at C(8) to give hemiaminal **11**, which then yields iminium ion **12**. Sequential proton tautomerizations provide dihydroquinoxaline **14**. Disproportionation of **14** with the starting mitomycin **10** or reaction with O_2 present in the solution affords the aromatized quinoxaline betaine **15**.

Additional evidence in favor of **5a**,**b** was provided by the reaction of mitomycin A (**6**) with *N*-methyl-1,2-phenylenediamine (**16**). Treatment of **6** with this amine in either pyridine, methanol, or dichloromethane led to the production of an orange solid. TLC analysis during the pyridine reaction showed the intermediate generation of a blue adduct. The orange solid was identified as phenazine betaine **17** on the basis of the ^1H and ^{13}C NMR,





COSY, APT-^{13}C, HMQC, HMBC, NOESY, TOCSY, in-

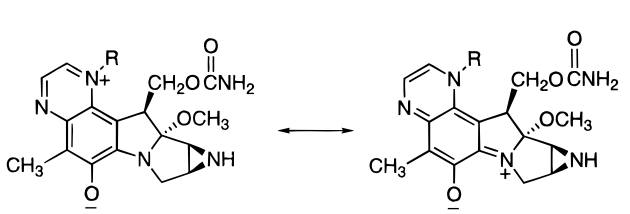
frared, UV−vis, and mass spectrometric data. In particular, the ^{13}C NMR chemical resonances for the mitomycin ring skeleton in **17** mirrored those recorded for **5a**,**b** (Table 1). We propose that the mechanism for the formation of **17** from **6** and **16** is similar to that depicted in Scheme 1. Significantly, no aromatic oxidation step is required to give phenazine betaine **17**.

Table 1. ^{13}C NMR Spectral Data for Mitoymycin Betaines[a]

	5a	5b	17
C(1)	37.7	37.6	37.1
C(2)	33.7	33.9	33.0
C(3)	49.6	49.8	49.8
C(5a)	157.0	157.2	161.5
C(5)	165.7	165.9	164.3
C(7)	147.9	148.2	148.4
C(8)	127.4	125.6	132.5
C(8a)	103.2	102.8	103.7
C(9)	49.5	49.9	48.7
C(9a)	104.0	103.8	104.9
C(10)	63.5	63.4	62.6
C(10a)	158.3	158.3	158.3
C(11)		*b*	139.4
C(12)	120.9	119.6	127.8
C(9a)OCH_3	50.0	50.1	50.3

[a] The number in each entry is the chemical shift value (δ) in ppm relative to the solvent used. The spectra were obtained at either 75 or 151 MHz. The solvent used was pyridine-d_5. [b] The signal was not detected in the ^{13}C NMR spectrum and is believed to be beneath the pyridine peak at 135.5 ppm (HMBC and HMQC data).

The chemical properties of compounds **5a**,**b** were briefly investigated and then compared with those of **3**. Dissolution of either mitomycin **5a** or **5b** in buffered methanolic solutions ("pH" 5.5 (3 days), 7.4 (7 days)) at 25 °C led to no appreciable change (HPLC, TLC analyses). Correspondingly, the half-lives for solvolysis (25 °C) of **3** at "pH" 5.5 and 7.4 were 2.3 and 40 h, respectively.[7] The absence of aziridine ring-opening processes for mitomycins **5a**,**b** compared with **3** was compatible with the predicted electronic structures for mitomycins **15**. We expect that the quaternary nitrogen atom adjacent to C(8) in **15** promotes delocalization of the indoline N(4) electrons with the quinoxaline ring system.



The corresponding resonance interaction of the indoline N(4) electrons with the α,β-unsaturated C(8) imine system in **3** should be diminished. This reduced delocalization in **3** permits the N(4)-assisted expulsion of the C(9a) methoxy group to produce **18** and then **19** (Scheme 2). Formation of **19** permits C(1) nucleophilic substitution to take place by an indole-assisted pathway.[7]

These findings demonstrate the propensity of the *N*-aminoethyl residue in mitomycins **2b**,**c** to react at the C(8) carbonyl site. Studies are underway to develop structural systems that promote the N(4)-assisted expulsion of the C(9a) methoxy unit to give activated mitosenes that modify DNA.

Experimental Section

General Methods. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on 300 and 600 MHz spectrometers. Low- and high-resolution mass spectral investigations were run at the University of Texas at Austin by Dr. M. Moini. HPLC analyses were conducted using a C_{18} μBondapak (stainless steel) column (3.9 × 300 mm) and using the following linear gradient conditions: 90% A (aqueous 0.1 or 0.025 M triethylammonium acetate, "pH" 6.5), 10% B (acetonitrile) isocratic for 5 min, and then from 90% A, 10% B to 45% A, 55% B in 25 min. The flow rate was 1 mL/min, and the eluant was monitored at 313 and 365 nm. Thin-layer chromatography was run on precoated silica gel GHLF slides (20 × 20 cm; Analtech no. 21521). The CH_2Cl_2 was distilled from P_2O_5, and the pyridine was distilled from KOH. All other solvents and reactants were of the best commercial grade available and used without further purification unless noted.

Preparation of 7-*N*-[2-(Methylamino)ethyl]mitomycin C (2b). To a stirred solution of **7** (2.5 μL, 0.029 mmol) in pyridine (1 mL) was added a pyridine solution (1 mL) of **6** (10.0 mg, 0.029 mmol). The solution was stirred at room temperature (1 h), and then the solvent was removed under reduced pressure to give **2b** as a blue-green solid (11.2 mg, 99%): HPLC t_R 16.6 min; R_f 0.08 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3421, 3294, 2934, 1715, 1634, 1555, 1517, 1451, 1329, 1063 cm^{-1}; UV−vis (MeOH) λ_{max} 223, 370 nm; ^1H NMR (pyridine-d_5) δ 2.10−2.15 (C(6)CH_3, C(1)NH), 2.32 (s, NCH_3), 2.67−2.73 (C(2)H, C(12)H_2), 3.13 (dd, J = 4.2, 7.5 Hz, C(1)H), 3.18 (s, C(9a)OCH_3), 3.54−3.60 (C(3)H_α, C(11)H_2), 3.98 (dd, J = 4.2, 10.8 Hz, C(9)H), 4.55 (d, J = 12.6 Hz, C(3)H_β), 5.06 (t, J = 10.8 Hz, C(10)*H*H′), 5.40 (dd, J = 4.2, 10.8 Hz, C(10)H*H*′), 7.38 (t, J = 4.8 Hz, C(7)NH) (^1H NMR assignments were consistent with the COSY spectrum); ^{13}C NMR (pyridine-d_5) 10.4 (C(6)-*C*H_3), 32.9 (C(2)), 36.4 (NCH_3), 37.1 (C(1)), 44.4 (C(11)), 44.7 (C(9)), 50.0 (C(9a)O*C*H_3), 51.0 (C(3)), 51.7 (C(12)), 62.8 (C(10)), 103.7(C(6)), 107.2 (C(9a)), 110.9 (C(8a)), 148.0 (C(7)), 156.7 (C(5a)), 158.5 (C(10a)), 177.1 (C(8)), 179.3 (C(5)) ppm; MS (+CI) *m/e* 391 $[M]^+$; M_r (+CI) 391.187 09 $(M)^+$ (calcd for $C_{18}H_{25}N_5O_5$, 391.185 57).

Preparation of 7-*N*-[2-(Ethylamino)ethyl]mitomycin C (2c). To a stirred solution of **8** (3.0 μL, 0.029 mmol) in pyridine (1 mL) was added a pyridine solution (1 mL) of **6** (10.0 mg, 0.029 mmol). The solution was stirred at room temperature (1.5 h), and then the solvent was removed under reduced pressure to give **2c** as a blue-green solid (11.3 mg, 98%): HPLC t_R 17.4 min; R_f 0.17 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3422, 3294, 1715, 1633, 1555, 1518, 1451, 1331, 1064 cm^{-1}; UV−vis (MeOH) λ_{max} 220, 369 nm; ^1H NMR (pyridine-d_5) δ 1.02 (t, J = 7.2 Hz, NCH_2CH_3), 2.10−2.17 (C(6)CH_3, C(1)NH), 2.55 (q, J = 7.2 Hz, NCH_2CH_3), 2.72−2.75 (C(2)H, C(12)H_2), 3.13 (dd, J = 4.5, 7.8 Hz, C(1)H), 3.18 (s, C(9a)OCH_3), 3.53−3.59 (C(3)-H_α, C(11)H_2), 3.98 (dd, J = 4.2, 10.8 Hz, C(9)H), 4.55 (d, J = 12.9 Hz, C(3)H_β), 5.09 (t, J = 10.8 Hz, C(10)*H*H′), 5.40 (dd, J = 4.2, 10.8 Hz, C(10)H*H*′), 7.45 (t, J = 5.1 Hz, C(7)NH) (^1H NMR assignments were consistent with the COSY spectrum); ^{13}C NMR (pyridine-d_5) 10.4 (C(6)*C*H_3), 15.7 (NCH_2CH_3), 33.00 (C(2)), 37.1 (C(1)), 44.2 (NCH_2CH_3), 44.7 (C(9)), 45.0 (C(11)), 49.5 (C(12)), 50.0 (C(9a)O*C*H_3), 51.0 (C(3)), 62.9 (C(10)), 103.8 (C(6)), 107.2 (C(9a)), 111.0 (C(8a)), 148.1 (C(7)), 156.7 (C(5a)), 158.5 (C(10a)), 177.2 (C(8)), 179.3 (C(5)) ppm; MS (+CI) *m/e* 406 $[M + 1]^+$; M_r (+CI) 406.211 45 $(M + 1)^+$ (calcd for $C_{19}H_{28}N_5O_5$, 406.209 04).

Preparation of 5a. Compound **2b** (15.0 mg, 0.044 mmol) was dissolved in a buffered methanolic solution (0.06 M Tris·HCl, "pH" 7.4, 5 mL) and stirred at room temperature (1 day). The solution was concentrated under reduced pressure and then separated by preparative TLC (50% MeOH−$CHCl_3$) to give **5a** (12.8 mg, 90%) as an orange solid: HPLC t_R 10.5 min; R_f 0.30 (50% MeOH−$CHCl_3$); FT-IR (KBr) 3433, 1716, 1632, 1490, 1457, 1339, 1133, 1105, 1070 cm^{-1}; UV−vis (MeOH) λ_{max} 266, 313, 469 nm; ^1H NMR (pyridine-d_5) δ 1.99 (t, J = 7.2 Hz, C(1)NH), 2.81 (br s, C(2)H), 3.00 (s, C(6)CH_3), 3.22 (br s, C(1)H), 3.38 (s, C(9a)OCH_3), 4.09 (d, J = 12.9 Hz, C(3)H_α), 4.43−4.51 (m, NCH_3, C(9)H), 4.79 (dd, J = 2.4, 11.0 Hz, C(10)-

*H*H′), 4.94 (t, J = 11.0 Hz, C(10)H*H*′), 5.58 (d, J = 12.9 Hz, C(3)H$_\beta$), 7.26 (d, J = 3.0 Hz, C(12)H), 8.21 (d, J = 3.0 Hz, C(11)H) (^{1}H NMR assignments were consistent with the COSY spectrum); ^{13}C NMR (pyridine-d_5) 11.4 (C(6)*C*H$_3$), 33.7 (C(2)), 37.7 (C(1)), 45.5 (NCH$_3$), 49.5 (C(9)), 49.6 (C(3)), 50.0 (C(9a)-O*C*H$_3$), 63.5 (C(10)), 103.2 (C(8a)), 104.0 (C(9a)), 117.4 (C(6)), 120.9 (C(12)), 127.4 (C(8)), 147.9 (C(7)), 157.0 (C(5a)), 158.3 (C(10a)), 165.7 (C(5)) ppm (remaining C(11) signal was not detected); MS (+FAB) *m*/*e* (rel intensity) 372 [M + 1]$^+$; M_r (+FAB) 372.168 45 (M + 1)$^+$ (calcd for $C_{18}H_{22}N_5O_4$, 372.167 18).

Preparation of 5b. Compound **2c** (15.0 mg, 0.044 mmol) was dissolved in a buffered methanolic solution (0.06 M Tris·HCl, "pH" 7.4, 5 mL) and stirred at room temperature (1 day). The solution was concentrated under reduced pressure and then separated by preparative TLC (50% MeOH−CHCl$_3$) to give **5b** (12.8 mg, 90%) as an orange solid: HPLC t_R 16.3 min; R_f 0.35 (50% MeOH−CHCl$_3$); FT-IR (KBr) 3434, 1709, 1634, 1492, 1463, 1412, 1384, 1343, 1073 cm^{-1}; UV−vis (MeOH) λ_{max} 268, 313, 472 nm; ^{1}H NMR (pyridine-d_5) δ 1.31 (t, J = 7.2 Hz, NCH$_2$C*H*$_3$), 2.02 (t, C(1)NH), 2.82 (br s, C(2)H), 3.02 (s, C(6)-CH$_3$), 3.23 (dd, J = 4.8, 7.5 Hz, C(1)H), 3.36 (s, C(9a)OCH$_3$), 4.09 (d, J = 13.2 Hz, C(3)H$_\alpha$), 4.21 (dd, J = 3.0, 10.8 Hz, C(9)H), 4.34−4.41 (m, NC*H*H′CH$_3$), 4.81 (dd, J = 3.0, 10.8 Hz, C(10)-*H*H′), 5.20 (t, J = 10.8 Hz, C(10)H*H*′), 5.51−5.56 (m, NCH-*H*′CH$_3$), 5.59 (d, J = 13.2 Hz, C(3)H$_\beta$), 7.37 (d, J = 3.0 Hz, C(12)H), 8.27 (d, J = 3.0 Hz, C(11)H); ^{13}C NMR (pyridine-d_5) 11.5 (C(6)*C*H$_3$), 15.5 (NCH$_2$*C*H$_3$), 33.9 (C(2)), 37.6 (C(1)), 49.8 (C(3)), 49.9 (C(9)), 50.1 (C(9a)O*C*H$_3$), 52.8 (N*C*H$_2$CH$_3$), 63.4 (C(10)), 102.8 (C(8a)), 103.8 (C(9a)), 117.9 (C(6)), 119.6 (C(12)), 125.6 (C(8)), 148.2 (C(7)), 157.2 (C(5a)), 158.3 (C(10a)), 165.9 (C(5)) ppm (remaining C(11) signal was not detected); the structure was further confirmed by the COSY, NOESY, HMQC, and HMBC spectra; MS (+FAB) *m*/*e* 386 [M + 1]$^+$; M_r (+FAB) 386.183 14 (M + 1)$^+$ (calcd for $C_{19}H_{24}N_5O_4$, 386.182 83).

Preparation of 17. To a stirred methanolic solution (1 mL) of **16** (6.5 μL, 0.057 mmol) was added a methanolic solution (1 mL) of **6** (10.0 mg, 0.029 mmol). The reaction solution was stirred at room temperature (1 day). The solution was concentrated under reduced pressure and then separated by preparative TLC (10% MeOH−CHCl$_3$) to give **17** (11.1 mg, 92%) as an orange solid: HPLC t_R 24.6 min; R_f 0.14 (10% MeOH−CHCl$_3$); FT-IR (KBr) 3433, 2950, 1716, 1633, 1507, 1489, 1382, 1341, 1075 cm^{-1}; UV−vis (MeOH) λ_{max} 225, 293, 450, 480 nm; ^{1}H NMR (600 MHz, pyridine-d_5) δ 2.25 (t, J = 7.3 Hz, C(1)NH), 2.84 (br s, C(2)H), 3.10 (s, C(6)CH$_3$), 3.28 (dd, J = 4.6, 7.3 Hz, C(1)H), 3.42 (s, C(9a)OCH$_3$), 4.10 (d, J = 13.6 Hz, C(3)H$_\alpha$), 4.47 (s, NCH$_3$), 4.59 (dd, J = 4.1, 10.7 Hz, C(9)H), 4.79 (t, J = 10.7 Hz, C(10)*H*H′), 4.84 (dd, J = 4.1, 10.7 Hz, C(10)H*H*′), 5.71 (d, J = 13.6 Hz, C(3)H$_\beta$), 7.37 (t, J = 7.8 Hz, C(14)H), 7.48 (t, J = 7.8 Hz, C(15)H), 7.61 (d, J = 7.8 Hz, C(13)H), 8.17 (d, J = 7.8 Hz, C(16)H); ^{13}C NMR (151 MHz, pyridine-d_5) 11.6 (C(6)*C*H$_3$), 33.0 (C(2)), 37.1 (C(1)), 39.8 (NCH$_3$), 48.7 (C(9)), 49.8 (C(3)), 50.3 (C(9a)O*C*H$_3$), 62.6 (C(10)), 103.7 (C(8a)), 104.9 (C(9a)), 115.3 (C(6)), 115.7 (C(13)), 126.2 (C(15)), 126.8 (C(14)), 127.8 (C(12)), 129.2 (C(16)), 132.5 (C(8)), 139.4 (C(11)), 148.4 (C(7)), 158.3 (C(10a)), 161.5 (C(5a)), 164.3 (C(5)) ppm (assignments were consistent with the COSY, APT, NOESY, TOCSY, HMQC, and HMBC spectra); MS (+FAB) *m*/*e* 422 [M + 1]$^+$; M_r (+FAB) 422.183 42 (M + 1)$^+$ (calcd for $C_{22}H_{24}N_5O_4$, 422.182 83).

Preparation of 9. Compound **2b** (10 mg, 0.029 mmol) was dissolved in CD$_3$OD (0.5 mL) and the solution maintained at room temperature (2 h) to give **9** as a green compound: HPLC t_R 11.6 min; UV−vis (MeOH) λ_{max} 240, 410 nm; ^{1}H NMR (CD$_3$-OD) δ 1.69 (s, C(6)CH$_3$), 2.48 (d, J = 3.1 Hz, C(1)H), 2.69 (d, J = 3.1 Hz, C(2)H), 2.83 (s, NCH$_3$), 3.03 (d, J = 10.7 Hz, C(3)-H$_\alpha$), 3.05−3.14 (m, C(12)*H*H′), 3.20−3.30 (m, C(11)*H*H′, C(12)-H*H*′), 3.42 (s, C(9a)OCH$_3$), 3.54−3.59 (m, C(11)H*H*′), 3.63 (dd, J = 3.3, 9.0 Hz, C(9)H), 4.17 (d, J = 10.7 Hz, C(3)H$_\beta$), 4.27 (dd, J = 9.0, 11.7 Hz, C(10)*H*H′), 4.66 (dd, J = 3.3, 11.7 Hz, C(10)H*H*′) (assignments were in agreement with the COSY, TOCSY, and NOESY experiments); ^{13}C NMR (151 MHz, CD$_3$-OD) 8.2 (C(6)*C*H$_3$), 33.6 (C(2)), 34.0 (C(1)), 38.6 (C(11)), 39.1 (C(9)), 40.0 (NCH$_3$), 50.5 (C(12)), 51.8 (C(3)), 53.0 (C(9a)O*C*H$_3$), 61.4 (C(10)), 84.8 (C(8)), 101.7 (C(6)), 109.4 (C(9a)), 123.3 (C(8a)), 135.4 (C(7)), 155.9 (C(5a)), 159.6 (C(10a)), 188.4 (C(5)) ppm (upfield assignments were in agreement with the HMQC spectrum).

Reactivity of 2b. To a buffered (0.06 M Tris·HCl, "pH" 7.4) MeOH solution (3.5 mL) was added **2b** (50 μg, 0.13 μmol). The solution was transferred to a UV−vis cuvette, sealed, and then maintained at 25.0 ± 0.1 °C. The UV−vis spectrum was monitored with a Cary 3 Bio UV−vis spectrometer for 2.1 h. The temperature was increased to 36.3 °C and then monitored every 30 min for 23 h.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **2b**,**c**, **5a**,**b**, **9**, and **17** and summaries of NMR responses for compounds **5b**, **9**, and **17** (18 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.

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