C(10) Halogen 10-Des(carbamoyloxy)porfiromycins: Synthesis, Chemistry, and Biological Activity

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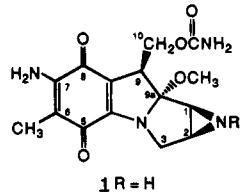
An efficient four-step procedure for the preparation of C(10) halogen 10-des(carbamoyloxy)-porfiromycins **3**–**5** beginning with mitomycin C (**1**) is described. Solvolytic removal (NaOMe, MeOH/benzene) of the C(10) carbamoyl group in **1** followed by N-methylation (dimethyl sulfate (15 equiv), 1,8-bis(dimethylamino)naphthalene (15 equiv) in THF) provided 10-decarbamoylporfiromycin (**7**) in 65% yield. Treatment of **7** with methanesulfonyl chloride in pyridine gave 10-decarbamoyl-10-methanesulfonylporfiromycin (**8**) in 83% yield, which upon heating with metal halides (i.e., LiCl, LiBr, NaI) in either DMF or ethylene glycol dimethyl ether furnished the C(10) halogen 10-des-(carbamoyloxy)porfiromycins **3**–**5** in 68–81% yields. The C(10) halogen 10-des(carbamoyloxy)-porfiromycins served as useful starting materials for C(10)-modified derivatives. Treatment of the C(10) bromo derivative **4** with 1,8-diazabicyclo[5.4.0]undec-7-ene provided the elimination product, 10-des(carbamoyloxy)-9-dehydroporfiromycin (**12**), while addition of AgSCN to the C(10) iodo porfiromycin **5** led to the substituted adducts 10-des(carbamoyloxy)-10-thiocyanatoporfiromycin (**10**) and 10-des(carbamoyloxy)-10-thiocyanato-9-*epi*-mitomycin D (**11**). The C(10) halogen 10-des-(carbamoyloxy)porfiromycins also underwent novel radical and thermal skeletal rearrangements. Treatment of the C(10) iodo derivative **5** with tributyltin hydride and AIBN led to the production of the ring-expanded quinone **14**. Thermolysis of the C(10) bromo (**4**) and the C(10) iodo (**5**) adducts gave the tetracycles **18** and **19**, respectively, in which the C(2) nitrogen bond in the starting porfiromycin had been preferentially cleaved in favor of the C(1) bond. Potential pathways for these rearrangements are briefly outlined. The *in vitro* cytotoxicities of **3**–**5** in human colon carcinoma cell lines were evaluated. All three C(10) halogen 10-des(carbamoyloxy)porfiromycins were noticeably less potent than mitomycin C.

Mitomycin C (**1**) is an antineoplastic agent of major clinical significance.[1] Mechanisms have been advanced which suggest that the drug functions by initial reductive activation followed by covalent bonding of the activated mitomycin species to DNA.[2] Drug attachment is believed to proceed sequentially at carbons 1 and 10 in **1**, leading to the production of DNA cross-linked products. Cytotoxicity and antitumor activity have been associated with the formation of the interstrand, cross-linked adducts.[2a,f]



1 R = H
2 R = CH_3

The ability of mitomycin C to bond to complementary strands of DNA has provided the chemical basis for an active program directed toward the development of semisynthetic mitomycin analogues that display improved activity and decreased toxicity versus **1**.[2b,3] More than 600 mitomycin derivatives have been prepared and tested against experimental tumor and microbial infection models. In most of these, the C(7) substituent has been altered. Changes at this site affect the reduction potential of the quinone ring,[2b,3-5] the partition coefficient of the drug,[5d] and the stability, reactivity, and toxicity of the activated drug.[6,7] By comparison, far fewer structural modifications have been made at the C(10) position. Three factors suggest that modification of this site may lead to improved biological activity. First, the therapeutic basis for mitomycin C has been associated with the C(10) bond formation step leading to the interstrand, cross-linked product.[2a,f] Second, mechanistic studies have demonstrated that under many reductive conditions **1** readily undergoes reaction at the C(1) site, but the C(10) position remains largely unmodified.[8-17] This result indicates that mitomycins with enhanced C(10) reactivity

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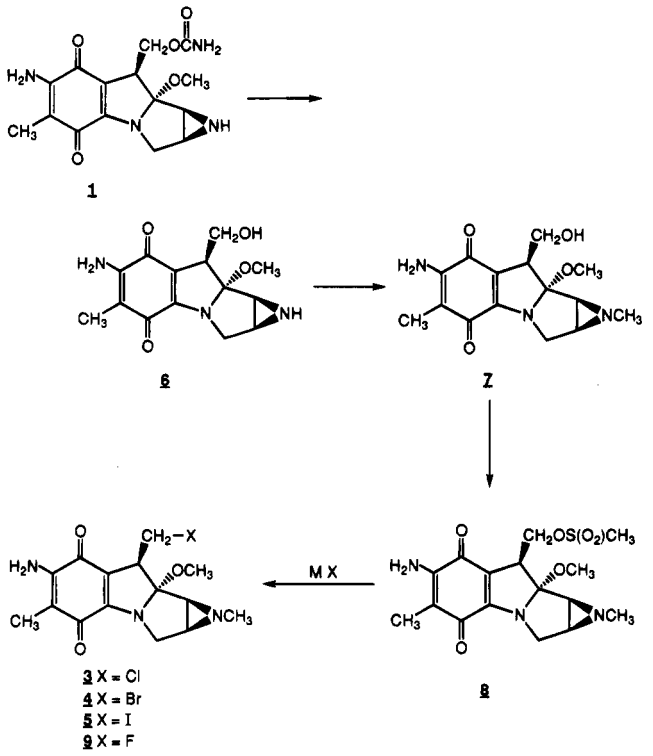
Scheme 1. Preparation of 10-Des(carbamoyloxy)porfiromycins 3–5



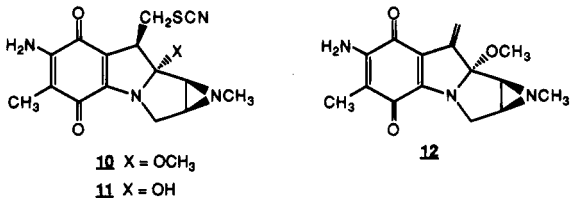
may exhibit improved DNA cross-linking efficiency. Third, evidence has been presented that the C(10) substituent plays a pivotal role in the alignment of the drug in the DNA minor groove, permitting the selective C(1) bonding of the activated drug to specific DNA sequences.[18,19]

In this paper we report the synthesis, selected chemistry, and biological activity of three novel C(10) halogen derivatives within the porfiromycin (*N*-methylmitomycin C) (**2**) series.[20] The synthetic utility of these compounds for the preparation of C(10)-modified derivatives is demonstrated, and two new ring skeleton mitomycin rearrangements are described.

Results and Discussion

A. Synthesis. Efficient procedures were developed for the preparation of C(10) chloro (**3**), C(10) bromo (**4**), and C(10) iodo (**5**) 10-des(carbamoyloxy)porfiromycin beginning with mitomycin C (**1**) (Scheme 1). Since our protocol included the use of methansulfonyl chloride to activate the C(10) position, we first converted the aziridine group in 10-decarbamoylmitomycin C (**6**) to the corresponding *N*-methyl derivative **7**. Minor modification of the previously described two-step synthesis of **7**[21] permitted the rapid preparation of this compound from **1** in 65% overall yield. Addition of methanesulfonyl chloride to a pyridine solution of **7** gave C(10) mesylate **8**[20,22] in 83% yield. Heating **8** with either LiCl, LiBr, or NaI in either DMF or ethylene glycol dimethyl ether[23] furnished the C(10) halogen derivatives **3**, **4**, and **5**, respectively, in 68–81% yields. Attempts to prepare the C(10) fluoro derivative **9** using this methodology (LiF) were unsuccessful. The C(10) halogen 10-des(carbamoyloxy)porfiromycins **3**–**5**, like their synthetic precursor **8**, displayed moderate solubilities in organic solvents (i.e., $CHCl_3$, MeOH, THF) and were stable at room temperature for extended periods of time.

B. Chemistry. The utility of C(10) halogen porfiromycins for the construction of C(10)-modified mitomycins was demonstrated by treatment of **5** with AgSCN and **4** with 1,8-diazabicyclo[5.4.0]undec-7-ene. AgSCN displacement of the C(10) iodo group in **5** provided 10-des(carbamoyloxy)-10-thiocyanatoporfiromycin (**10**) and 10-des(carbamoyloxy)-10-thiocyanato-9-*epi*-mitomycin D (**11**). The proposed structures for **10** and **11** were supported by their NMR and mass spectral data. The pathway for the incorporation of the C(9a) hydroxyl group in **11** has not been elucidated. Addition of 1,8-diazabicyclo[5.4.0]-undec-7-ene to **4** gave the known 10-des(carbamoyloxy)-9-dehydroporfiromycin (mitomycin G)[22] (**12**).



Attempts to reduce 10-des(carbamoyloxy)-10-iodoporfiromycin (**5**) to the corresponding C(10) methyl analogue **13** in ethylene glycol dimethyl ether with tributyltin hydride and a catalytic amount of AIBN (azobisisobutylnitrile) led to the production of the novel ring-expanded quinone **14**.[24] Consistent with the proposed structure, the HMQC[26] spectrum showed the presence of three methylene signals at 17.12 (C(9)), 26.21 (C(10)), and 56.03 (C(3)) ppm. Correspondingly, the COSY spectrum demonstrated the spatial proximity of the C(9) methylene protons (δ 2.66, 2.96) and the C(10) methylene hydrogens (δ 1.89, 2.16), and the coupling of the C(2) methine hydrogen (δ 2.25) with the C_3H_α proton (δ 4.33). The detection of a three-bond connectivity between the C(8) carbon and one of the two C(9) methylene hydrogens in the HMBC[25] NMR experiment (Figure 1) permitted the differentiation of the C(9) resonances from the C(10) signals.

The observed ring conversion of **5** to **14** was reminiscent of comparable radical ring expansion transforma-

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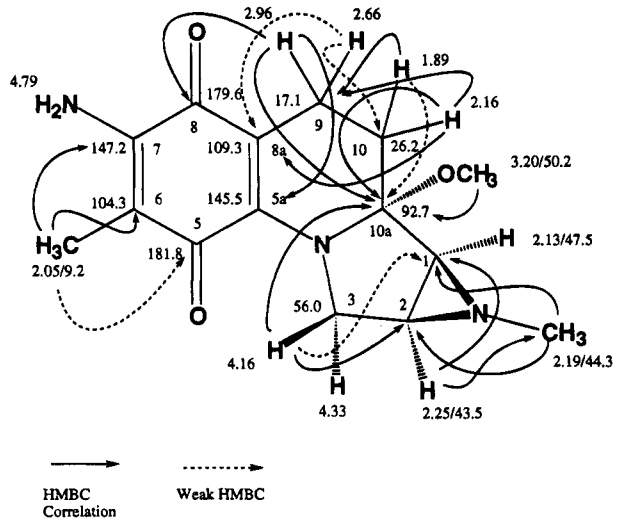
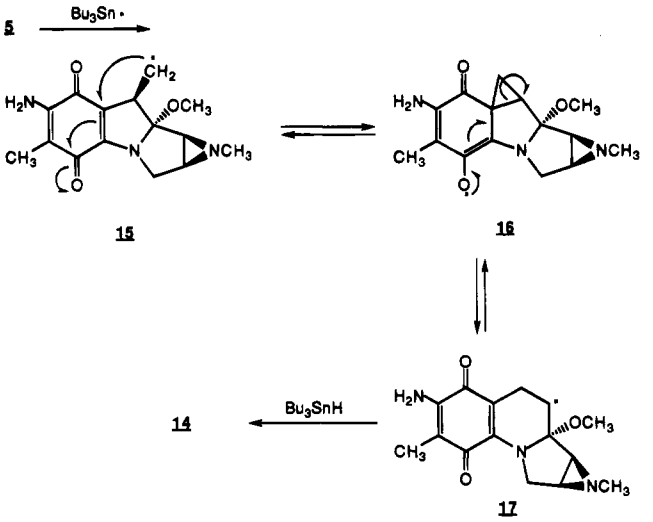
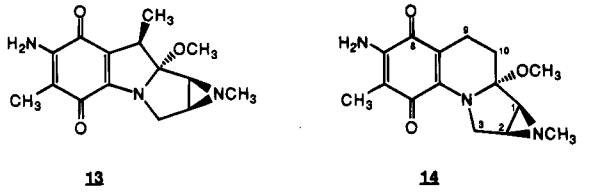


Figure 1. Key NMR responses for compound **14**.

Scheme 2. Proposed Pathway for the Conversion of 5 to 14



tions.[27,28] Accordingly, we suggest that addition of tributyltin hydride to **5** leads to the production of radical **15**,



13 **14**

which then attacks the α,β-unsaturated carbonyl system to give **16** (Scheme 2). Subsequent cleavage of the strained cyclopropane ring in alkoxy radical **16** yields **17**.

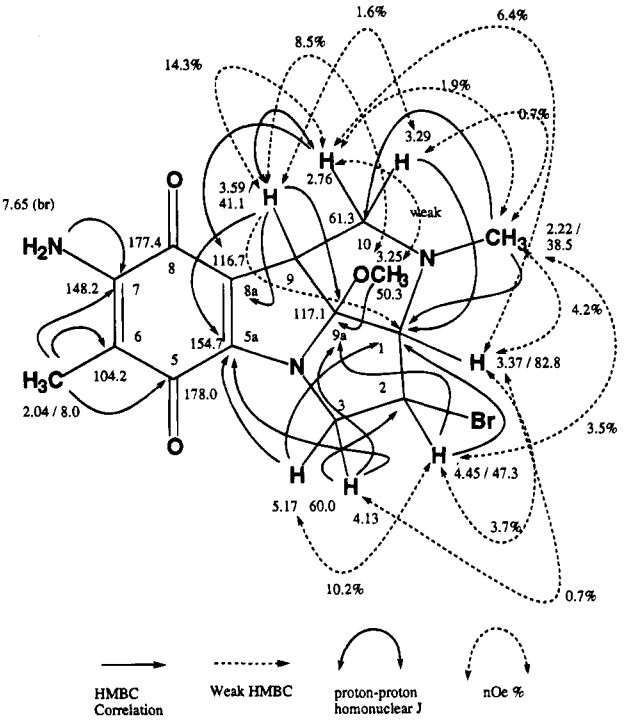
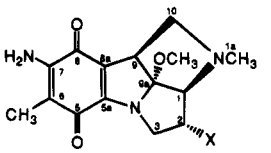


Figure 2. Key NMR responses for compound **18**.

This species is then reduced in the final step by tributyltin hydride to provide **14**.

A second novel rearrangement was observed during the preparation of 10-des(carbamoyloxy)-10-bromoporfiromycin (**4**) and 10-des(carbamoyloxy)-10-iodoporfiromycin (**5**) from **8**. Elevation of the reaction temperature and extension of reaction time led to modest amounts of the tetracycles **18** and **19** prior to workup. HPLC analysis of the NaI reaction indicated the initial rapid production of **5** from mesylate **8** followed by the slower conversion of **5** to **19**. The structure of **18** was assigned on the basis of the observed ^{1}H, ^{13}C, COSY, TOCSY, HMQC, HMBC, and NOE microdetection NMR experiments along with the mass spectral data. Key proton–proton, three-bond heteronuclear connectivities, and NOE responses observed for **18** are shown in Figure 2. The assigned stereochemistry for C(1) and C(2) was consistent with the observed vicinal proton–proton coupling constant and NOE data.



18 X = Br

19 X = I

The most straightforward pathway for the formation of **18** and **19** envisages halide-promoted cleavage of the C(2)–N(CH_3) aziridine bond followed by intramolecular displacement of the C(10) halide group (Scheme 3). Alternatively, the sequence of these two steps may be reversed where aziridinium ion formation precedes C(2)–N(CH_3) nitrogen bond breakage. Significantly, we are unaware of any other examples in which the C(2) nitrogen bond in mitomycin derivatives has been preferentially cleaved in favor of the C(1) nitrogen bond.

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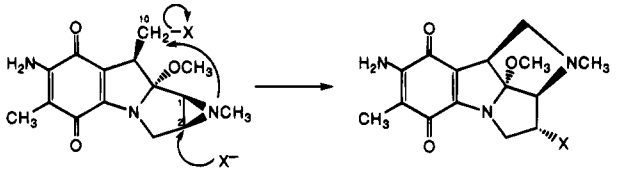
Scheme 3. Proposed Pathway for the Conversion of Porfiromycins 4 and 5 to 18 and 19



4 X = Br
5 X = I
18 X = Br
19 X = I

Table 1. *In Vitro* Cytotoxicity of Porfiromycin Derivatives 3–5 in Human Colon Carcinoma Cell Lines[a]

	IC_{50} (μM)[b]		
no.	HCT116	HCT116(VM)46	HCT116(VP)35
3	6.0	2.6	1.4
4	>5.3	>5.3	>5.3
5	>4.7	>4.7	>4.7
1	0.042	0.114	0.039

[a] The HCT116(VP)35 and HCT116(VM)46 cell lines are resistant to both etoposide and teniposide. The HCT116(VM)46 cells have overexpression of P-glycoprotein, while the HCT116(VP)35 cell line has low topoisomerase II levels. [b] Cytotoxicity assessed by XTT assay after 72 h drug exposure.

C. Biological Activity. The *in vitro* cytotoxicity of mitomycin C (**1**) and the three C(10) halogen 10-des(carbamoyloxy)porfiromycins **3–5** were tested against the human colon carcinoma cell line HCT116 and the two resistant sublines HCT116(VP)35 and HCT116(VM)46 (Table 1).[29] Mitomycin C had an IC_{50} value of 0.042 μM against the sensitive HCT116 cell line, while **3–5** demonstrated IC_{50} values that were >100-fold less potent than **1**.[30] Compounds **3–5** exhibited the same relatively low potency in the two resistant sublines.

Experimental Section

General Methods. Melting points were uncorrected. Chemical ionization mass spectral investigations were conducted at the University of Texas at Austin by Dr. M. Moini. NMR and mass spectral studies of **18** were performed at the Burroughs Wellcome Co. on a Varian Unity 400 spectrometer equipped with either a Nalorac Z-SPEC MID-400-3 or MD-400-3 microprobes and a VG70SQ mass spectrometer using a FAB source, respectively. HPLC analyses were conducted using a C_{18} μBondapak (stainless steel) column (3.9 × 300 mm) and using either one of the two following linear gradient conditions. (a) Program 1: 90% A (aqueous 0.1 M triethylammonium acetate, pH 7.0), 10% B (acetonitrile) isocratic for 5 min, then from 90% A, 10% B to 45% A, 55% B in 30 min. (b) Program 2: 100% A (aqueous 3 mM triethylammonium phosphate, pH 4.7), 0% B (3 mM triethylamine in acetonitrile) to 50% A, 50% B in 25 min. In both cases the flow rate was 1 mL/min, and the eluant was monitored at 280, 313, and 365 nm. Thin-layer chromatography was run on precoated silica gel GHLF slides (20 × 20 cm; Analtech No. 21521). THF was distilled from Na metal and benzophenone. All other solvents and reactants were of the best commercial grade available and were used without further purification unless noted.

Preparation of 10-Decarbamoylmitomycin C (6).[31] To a stirred suspension of **1** (100 mg, 0.30 mmol) in benzene (120 mL) was added a freshly prepared NaOMe solution (prepared from 1.38 g of Na metal and 30 mL of MeOH) all at once. The reaction mixture was stirred at room temperature (12 h) and quenched by the addition of dry ice, and then the reaction mixture was allowed to warm to room temperature and stirred (2 h). The insoluble salts were filtered and washed with acetone (100 mL), and the combined organic layer was removed under reduced pressure. The residue was purified by flash column chromatography on SiO_2 gel using 10% MeOH–$CHCl_3$ as the eluant to give 70 mg (80%) of **6** as a purple solid: HPLC t_R (program 2) 18.1 min; R_f = 0.30 (10% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.75 (s, 3 H), 2.88 (br s, 1 H), 2.91 (br s, 1 H), 3.20 (s, 3 H), 3.37–3.40 (m, 1 H), 3.54 (br d, J = 13.0 Hz, 1 H), 4.01–4.03 (br m, 1 H), 4.21 (br d, J = 13.0 Hz, 1 H), 4.61–4.64 (br m, 1 H), 5.34 (br s, 2 H).

Preparation of 10-Decarbamoylporfiromycin (7).[21] To a dry THF solution (10 mL) containing **6** (64 mg, 0.22 mmol) and 1,8-bis(dimethylamino)naphthalene (710 mg, 3.3 mmol) was added dimethyl sulfate (0.42 mL, 3.3 mmol), and the reaction mixture was stirred at room temperature (12 h). The solvent was removed under reduced pressure, then $CHCl_3$ was added, and the insoluble salts were filtered. The residue was purified by flash column chromatography on SiO_2 using 5% MeOH–$CHCl_3$ as the eluant to give 54 mg (81%) of **7**: HPLC t_R (program 2) 19.8 min; R_f = 0.50 (10% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.74 (s, 3 H), 2.27 (s, 3 H), 2.26–2.27 (m, 1 H, overlapped), 2.32 (dd, J = 2.1, 4.8 Hz, 1 H), 3.17 (s, 3 H), 3.34 (t, J = 5.1 Hz, 1 H), 3.49 (dd, J = 2.1, 13.1 Hz, 1 H), 3.96–3.97 (br m, 1 H), 4.18 (d, J = 13.1 Hz, 1 H), 4.62–4.74 (br m, 1 H), 5.34 (br s, 2 H); ^{13}C NMR ($CDCl_3$) 7.62, 42.88, 43.78, 45.18, 46.24, 49.38, 49.51, 61.38, 104.07, 106.57, 112.58, 149.97, 154.63, 176.20, 177.76 ppm.

Preparation of 10-Decarbamoyl-10-methanesulfonylporfiromycin (8).[20,22] To a stirred solution of **7** (24 mg, 0.079 mmol) in dry pyridine (0.5 mL) was added methanesulfonyl chloride (24.4 μL, 0.315 mmol) through a microsyringe at 0 °C. The reaction solution was maintained at room temperature (10 min). The solvent was removed *in vacuo*, and the residue was purified by flash column chromatography using 5% MeOH–$CHCl_3$ as the eluant to give 25 mg (83%) of **8** as a purple solid: HPLC t_R (program 2) 22.9 min; R_f = 0.57 (10% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.75 (s, 3 H), 2.28 (dd, J = 2.0, 4.6 Hz, 1 H), 2.32 (s, 3 H), 2.45 (d, J = 4.6 Hz, 1 H), 3.10 (s, 3 H), 3.19 (s, 3 H), 3.48 (dd, J = 2.0, 13.0 Hz, 1 H), 3.63 (dd, J = 4.1, 10.9 Hz, 1 H), 4.21 (d, J = 13.0 Hz, 1 H), 4.53 (dd, J = 9.7, 10.9 Hz, 1 H), 4.82 (dd, J = 4.1, 9.7 Hz, 1 H), 5.23 (br s, 2 H); ^{13}C NMR ($CDCl_3$) 7.90, 37.44, 42.60, 43.22, 43.46, 45.51, 49.72, 49.86, 66.43, 105.35, 105.78, 108.64, 147.20, 154.98, 175.56, 178.33 ppm.

Preparation of 10-Des(carbamoyloxy)-10-chloroporfiromycin (3). A dry DMF solution (5 mL) containing **8** (25 mg, 0.065 mmol) and LiCl (55 mg, 2.0 mmol) was heated at 100 °C (1.5 h). The solvent was removed *in vacuo*, and the residue was purified by flash column chromatography using 5% MeOH–$CHCl_3$ as the eluant. The crude product was further purified by preparative TLC using 5% MeOH–$CHCl_3$ as the eluant to give 17 mg (81%) of **3**: mp 157 °C dec; HPLC t_R (program 2) 26.3 min; R_f = 0.61 (5% MeOH–$CHCl_3$); FT-IR (KBr) 1602, 1554, 1446, 1384, 1350, 1282, 1211, 1097 cm^{-1}; UV–vis (MeOH) λ_{max} 355 nm; ^{1}H NMR (CD_3OD) δ 1.74 (s, 3 H), 2.32 (s, 3 H), 2.47 (dd, J = 2.1, 4.8 Hz, 1 H), 2.64 (d, J = 4.8 Hz, 1 H), 3.21 (s, 3 H), 3.48 (dd, J = 2.1, 13.5 Hz, 1 H), 3.49 (dd, J = 3.6, 11.7 Hz, 1 H), 3.79 (dd, J = 10.5, 11.7 Hz, 1 H), 4.13 (dd, J = 3.6, 10.5 Hz, 1 H), 4.14 (d, J = 13.5 Hz, 1 H); ^{13}C NMR (CD_3OD) 8.12, 41.60, 43.24, 43.89, 46.83, 48.39, 50.23, 50.76, 105.04, 107.24, 111.33, 151.14, 156.41, 176.77, 178.73 ppm; MS (+CI) m/e (rel intensity) 326 [M + 1, 39]$^+$, 324 [M + 1, 100]$^+$; M_r (+CI) 324.111 72 (M + 1)$^+$ (calcd for $C_{15}H_{19}ClN_3O_3$, 324.111 49).

Preparation of 10-Des(carbamoyloxy)-10-bromoporfiromycin (4). A dry DMF solution (5 mL) containing **8** (38 mg, 0.1 mmol) and LiBr (174 mg, 2.0 mmol) was heated at 48–50 °C (48 h). The solvent was removed *in vacuo*, and the residue was purified by flash column chromatography using 5% MeOH–$CHCl_3$ as the eluant. The crude product was further purified by preparative TLC using 5% MeOH–$CHCl_3$

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as the eluant to give 25 mg (68%) of **4**: mp 155 °C dec; HPLC t_R (program 2) 26.8 min; R_f = 0.61 (5% MeOH−$CHCl_3$); FT-IR (KBr) 1612, 1562, 1440, 1360, 1259, 1088 cm^{-1}; UV−vis (MeOH) λ_{max} 356 nm; ^1H NMR (acetone-d_6) δ 1.75 (s, 3 H), 2.29 (s, 3 H), 2.43 (dd, J = 2.1, 4.8 Hz, 1 H), 2.63 (d, J = 4.8 Hz, 1 H), 3.20 (s, 3 H), 3.43 (dd, J = 2.1, 12.9 Hz, 1 H), 3.54 (dd, J = 2.7, 11.7 Hz, 1 H), 4.01 (dd, J = 2.7, 9.9 Hz, 1 H), 4.13 (d, J = 12.9 Hz, 1 H), 3.74 (dd, J = 9.9, 11.7 Hz, 1 H), 6.39 (br s, 2 H); ^{13}C NMR (acetone-d_6) 8.16, 29.48, 43.22, 43.89, 45.75, 48.12, 49.96, 50.51, 104.85, 107.07, 112.09, 149.09, 154.81, 176.47, 178.49 ppm; MS (+CI) m/e (rel intensity) 370 [M + 1, 82]$^+$, 368 [M + 1, 100]$^+$; M_r (+CI) 368.060 05 (M + 1)$^+$ (calcd for $C_{15}H_{19}BrN_3O_3$, 368.060 98).

Preparation of 10-Des(carbamoyloxy)-10-iodoporfiromycin (5). An ethylene glycol dimethyl ether suspension (7 mL) containing **8** (29 mg, 0.074 mmol) and NaI (220 mg, 1.48 mmol) was heated at reflux (3 h). The solvent was removed under reduced pressure, and the residue was triturated with $CHCl_3$ (15 mL) and filtered, and then the $CHCl_3$ layer was evaporated *in vacuo*. The residue was purified by preparative TLC using 5% MeOH−$CHCl_3$ as the eluant to give 24 mg (78%) of **5**: mp 165 °C dec; HPLC t_R (program 2) 28.3 min; R_f = 0.61 (5% MeOH−$CHCl_3$); FT-IR (KBr) 1610, 1577, 1560, 1438, 1348, 1332, 1087 cm^{-1}; UV−vis (MeOH) λ_{max} 359 nm; ^1H NMR ($CDCl_3$) δ 1.75 (s, 3 H), 2.27 (dd, J = 2.1, 4.8 Hz, 1 H), 2.36 (s, 3 H), 2.86 (d, J = 4.8 Hz, 1 H), 3.19 (s, 3 H), 3.41 (dd, J = 9.6, 12.0 Hz, 1 H), 3.50 (dd, J = 2.1, 13.0 Hz, 1 H), 3.66 (dd, J = 3.0, 12.0 Hz, 1 H), 3.88 (dd, J = 3.0, 9.6 Hz, 1 H), 4.24 (d, J = 13.0 Hz, 1 H), 5.22 (br s, 2 H); ^{13}C NMR ($CDCl_3$) 0.75, 7.93, 42.68, 43.51, 44.81, 46.90, 49.69, 49.80, 105.28, 106.63, 113.24, 147.41, 153.40, 175.46, 178.52 ppm; MS (+CI) m/e (rel intensity) 416 [M + 1, 100]$^+$; M_r (+CI) 416.047 22 (M + 1)$^+$ (calcd for $C_{15}H_{19}IN_3O_3$, 416.047 12).

Preparation of 10-Des(carbamoyloxy)-10-thiocyanatoporfiromycin (10) and 10-Des(carbamoyloxy)-10-thiocyanato-9-*epi*-mitomycin D (11). To a stirred solution of **5** (5 mg, 0.011 mmol) in anhydrous DMF (0.5 mL) was added AgSCN (9 mg, 0.055 mmol). The reaction mixture was stirred at 45−50 °C (26 h), and then the solid materials were filtered through Celite. The solvent was removed under reduced pressure to give a crude purple solid. Purification by preparative TLC using 10% MeOH−$CHCl_3$ as the eluant afforded **10** (0.5 mg, 13%) and **11** (1.0 mg, 26%) both as purple solids.

Compound **11**: t_R (program 1) 17.1 min; R_f = 0.45 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 360 nm; ^1H NMR ($CDCl_3$) δ 1.66 (s, 3 H), 2.27 (s, 3 H), 2.31−2.33 (m, 1 H), 2.48 (d, J = 4.7 Hz, 1 H), 3.18 (d, J = 12.0 Hz, 1 H, overlapped), 3.26−3.30 (m, 1 H, overlapped), 3.54 (br m, 1 H), 3.87 (dd, J = 2.8, 12.0 Hz, 1 H), 4.09 (d, J = 12.9 Hz, 1 H), 5.25 (br s, 2 H); MS (+CI) m/e (rel intensity) 333 [M + 1, 100]$^+$; M_r (+CI) 333.102 95 (M + 1)$^+$ (calcd for $C_{15}H_{17}N_4O_3S$, 333.102 13).

Compound **10**: t_R (program 1) 24.2 min; R_f = 0.50 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} = 360 nm; ^1H NMR ($CDCl_3$) δ 1.76 (s, 3 H), 2.28 (s, 3 H), 2.31−2.34 (m, 1 H), 2.48 (d, J = 4.8 Hz, 1 H), 3.21 (s, 3 H), 3.23−3.27 (m, 1 H, overlapped), 3.47−3.59 (m, 2 H, overlapped), 3.94 (dd, J = 3.5, 12.6 Hz, 1 H), 4.20 (d, J = 13.1 Hz, 1 H), 5.25 (br, 2 H); ^{13}C NMR ($CDCl_3$) 7.91, 31.77, 43.30, 43.39, 43.73, 45.38, 49.70, 49.96, 105.42, 106.25, 111.51, 111.82, 147.17, 154.15, 175.50, 178.31 ppm; MS (+CI) m/e (rel intensity) 347 [M + 1, 100]$^+$; M_r (+CI) 347.116 68 (M + 1)$^+$ (calcd for $C_{16}H_{19}N_4O_3S$, 347.117 79).

Preparation of 10-Des(carbamoyloxy)-9-dehydroporfiromycin (12)[22] from 10-Des(carbamoyloxy)-10-iodoporfiromycin (5). To a stirred THF solution (20 mL) of **5** (12.6 mg, 0.03 mmol) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (0.49 mL, 3.0 mmol). The reaction mixture was stirred at reflux (24 h). The solvent was removed under reduced pressure, and the residue was purified by preparative TLC using 5% MeOH−$CHCl_3$ as the eluant to give 4 mg (47%) of **12**: HPLC t_R (program 2) 24.6 min; R_f = 0.50 (5% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 367 nm; ^1H NMR (CD_3OD) δ 1.77 (s, 3 H), 2.21 (s, 3 H), 2.43 (dd, J = 1.8, 4.8 Hz, 1 H), 2.47 (d, J = 4.8 Hz, 1 H), 3.05 (s, 3 H), 3.42 (dd, J = 1.8, 13.2 Hz, 1 H), 4.25 (d, J = 13.2 Hz, 1 H), 5.34 (d, J = 0.9 Hz, 1 H), 6.07 (d, J = 0.9 Hz, 1 H).

Preparation of Compound 14. To an ethylene glycol dimethyl ether solution (5 mL) containing **5** (22 mg, 0.053 mmol) were added AIBN (6 mg) and tributyltin hydride (72 μL, 0.26 mmol), and then the reaction solution was heated at reflux (1 h). The solvent was removed under reduced pressure, and the residue was purified by preparative TLC using 10% MeOH−$CHCl_3$ as the eluant to give 8 mg (52%) of **14**: mp 55 °C dec; HPLC t_R (program 2) 22.2 min; R_f = 0.42 (5% MeOH−$CHCl_3$); FT-IR (KBr) 1613, 1551, 1408, 1381, 1352, 1306, 1132, 1064 cm^{-1}; UV−vis (MeOH) λ_{max} 353 nm; ^1H NMR (pyridine-d_5) δ 1.89 (ddd, J = 6.2, 13.3, 14.1 Hz, C(10) *H*H′), 2.05 (s, C(6)CH_3), 2.13 (d, J = 5.0 Hz, C(1)H), 2.16 (dd, J = 6.8, 13.3 Hz, C(10)H*H*′), 2.19 (s, *N*(1a)CH_3), 2.25 (dd, J = 2.7, 5.0 Hz, C(2)H), 2.66 (ddd, J = 6.8, 14.1, 17.0 Hz, C(9)*H*H′), 2.96 (dd, J = 6.2, 17.0 Hz, C(9)H*H*′), 3.20 (s, C(10a)OCH_3), 4.16 (d, J = 13.6 Hz, C(3)H_β), 4.33 (dd, J = 2.7, 13.6 Hz, C(3)H_α), 4.79 (br s, C(7)NH_2); ^{13}C NMR (pyridine-d_5) 9.24 (C(6)*C*H_3), 17.12 (C(9)), 26.21 (C(10)), 43.45 (C(2)), 44.27 (*N*(1a)CH_3), 47.47 (C(1)), 50.21 (OCH_3), 56.03 (C(3)), 92.68 (C(10a)), 104.34 (C(6)), 109.32 (C(8a)), 145.46 (C(5a)), 147.16 (C(7)), 179.58 (C(8)), 181.84 (C(5)) ppm; MS (+CI) m/e (rel intensity) 290 [M + 1, 27]$^+$, 289 [M, 100]$^+$; M_r (+CI) 289.142 88 (M$^+$) (calcd for $C_{15}H_{19}N_3O_3$, 289.142 64).

Preparation of Compound 18. To a DMF solution (1.50 mL) containing **8** (19.2 mg, 0.05 mmol) was added LiBr (217 mg, 2.5 mmol), and then the reaction mixture was heated at 105−112 °C (0.5 h). The insoluble salts were filtered through a Celite pad, and the solvent was removed *in vacuo*. The residue was purified by preparative TLC using 10% MeOH−$CHCl_3$ as the eluant to give 2 mg (11%) of **18**: HPLC t_R (program 2) 29.8 min; R_f = 0.61 (5% MeOH−$CHCl_3$); FT-IR (KBr) 1611, 1561, 1418, 1080 cm^{-1}; UV−vis (MeOH) λ_{max} 356 nm; ^1H NMR (pyridine-d_5) δ 2.04 (s, C(6)CH_3), 2.22 (*N*(1a)-CH_3), 2.76 (dd, J = 6.6, 9.3 Hz, C(10)*H*H′), 3.25 (s, OCH_3), 3.29 (d, J = 9.3 Hz, C(10)H*H*′), 3.37 (br s, C(1)H), 3.59 (d, J = 6.6 Hz, C(9)H), 4.13 (dd, J = 5.4, 13.5 Hz, C(3)H_α), 4.45 (dd, J = 5.4, 7.8 Hz, C(2)H), 5.17 (dd, J = 7.8, 13.5 Hz, C(3)H_β); ^1H NMR (CD_3OD) δ 1.76 (s, C(6)CH_3), 2.32 (s, *N*(1a)CH_3), 2.82 (dd, J = 6.6, 9.6 Hz, C(10)*H*H′), 3.10 (d, J = 9.6 Hz, C(10)-H*H*′), 3.17 (br s, C(1)H), 3.26 (s, C(9a)OCH_3), 3.42 (d, J = 6.6 Hz, C(9)H), 3.84 (dd, J = 5.4, 13.5 Hz, C(3)H_α), 4.23−4.28 (m, C(2)H), 4.84 (dd, J = 8.0, 13.5 Hz, C(3)H_β); ^{13}C NMR (pyridine-d_5) 8.04 (C(6)*C*H_3), 38.45 (*N*(1a)CH_3), 41.10 (C(9)), 47.26 (C(2)), 50.29 (OCH_3), 60.00 (C(3)), 61.29 (C(10)), 82.76 (C(1)), 104.21 (C(6)), 116.65 (C(8a)), 117.12 (C(9a)), 148.21 (C(7)), 154.71 (C(5a)), 177.44 (C(8)), 178.02 (C(5)) ppm; MS (+CI) m/e (rel intensity) 370 [M + 1, 86]$^+$, 368 [M + 1, 100]$^+$; M_r (+CI) 368.059 83 (M + 1)$^+$ (calcd for $C_{15}H_{19}BrN_3O_3$, 368.060 98).

Preparation of Compound 19. A stirred anhydrous ethylene glycol dimethyl ether suspension (1 mL) of **8** (6 mg, 0.016 mmol) and NaI (12 mg, 0.078 mmol) was heated at reflux (45 h). The reaction was monitored by HPLC analysis. After 2 h the major compounds present in the reaction mixture were **8** (4%), **5** (68%), and **19** (2%). After 17 h the reaction mixture consisted (in part) of **8** (0%), **5** (44%), and **19** (41%). After 45 h the reaction mixture included **8** (0%), **5** (0%), and **19** (69%). The solvent was removed under reduced pressure to give a crude purple solid. Purification by preparative TLC using 10% MeOH−$CHCl_3$ as the eluant afforded **19** (1 mg, 15%) as a purple solid: t_R (program 2) 29.4 min; R_f = 0.57 (10% MeOH−$CHCl_3$); UV−vis (MeOH) λ_{max} 360 nm; ^1H NMR ($CDCl_3$) δ 1.75 (s, C(6)CH_3), 2.30 (s, *N*(1a)CH_3), 2.82 (dd, J = 6.9, 9.9 Hz, C(10)*H*H′), 3.14 (d, J = 9.9 Hz, C(10)H*H*′), 3.24 (s, C(9a)OCH_3), 3.35−3.38 (m, C(1)H, C(9)H), 3.92−4.00 (m, C(2)H, C(3)H_α), 4.91 (dd, J = 10.2, 15.9 Hz, C(3)H_β), 5.09 (br s, C(7)NH_2); the assignment was further confirmed by the COSY spectrum; ^{13}C NMR ($CDCl_3$) 7.91, 39.95, 40.80, 51.29, 61.38, 62.08, 84.67, 104.30, 146.53 ppm; the remaining six carbon signals were not detected due to the limited sample size; MS (+CI) m/e (rel intensity) 416 [M + 1, 100]$^+$; M_r (+CI) 415.0396 (M$^+$) (calcd for $C_{15}H_{18}IN_3O_3$, 416.0392).

***In Vitro* Cytotoxicity Assay.** Cytotoxicity was assessed in the human carcinoma cell lines by the XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide) assay.[27b] Cells were plated at 4000 cells/well in 96-well microtiter plates and 24 h later drugs (**1**, **3**−**5**)

were added at concentrations established by serial dilution from a stock solution of 1 mg/mL. The cells were incubated at 37 °C for 72 h at which time a solution containing XTT and phenazine methosulfate was added. A dehydrogenase enzyme in live cells reduces the XTT to a form that absorbs light at 450 nm, which can be quantitated spectrophotometrically. The greater the absorbance the greater the number of live cells. The results are expressed as IC_{50} values, which is the drug concentration required to inhibit cell proliferation (i.e., absorbance at 450 nm) to 50% of that of untreated control cells.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds **3**–**5**, **10**, **11**, **14**, **18**, and **19** (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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