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# Studies on the Mechanism of Activation of C(7) Ethylenediamine Substituted Mitomycins. Relevance to the Proposed Mode of Action of BMY-25067 and KW-2149

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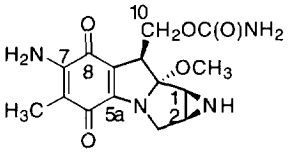
***Abstract:*** Select C(7) ethylenediamine-substituted mitomycins were prepared, and their reactivity toward nonreductive, nucleophilic aziridine ring opening transformations assessed.


Mitomycin C (1) is the most notable of a series of pharmacologically active compounds known as the mitomycins.[1] It is the prototype of a major class of antitumor antibiotics, termed bioreductive alkylating agents. Mitomycin C has found wide use in combination with other chemotherapeutic agents in the management of advanced breast cancer and, to a lesser extent, advanced cervical and ovarian cancers.[1] Despite the therapeutic value of 1, adverse toxicity prohibits its prolonged use. This has led to an intense effort to develop semisynthetic variants with improved efficacy and decreased toxicity.[1b,2] Two compounds have emerged from these studies, BMY-25067[3] (2) and KW-2419[4] (3). Both are currently undergoing clinical trials.

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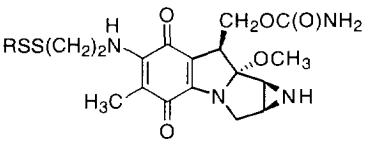
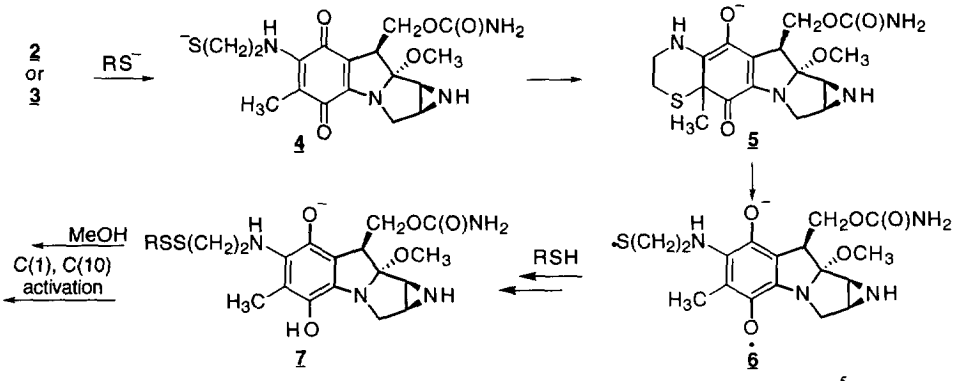



2 R = *p*-nitrophenyl (BMY-25067)

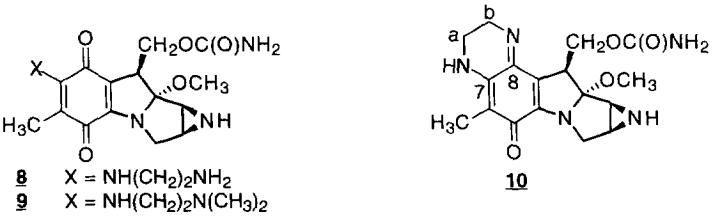
3 R = $HO_2CCH(NH_2)(CH_2)_7C(=O)N(H)(CH_2)_2$ (KW-2149)

The mode of action of 2 and 3 is the subject of current debate.[5] It is known that both compounds cross-link DNA. A mechanism has been proposed for 2 and 3 (Scheme 1).[5] Key aspects include thiol (e.g., glutathione)-initiated disulfide exchange to give 4,[6] generation of cyclic intermediate 5[7] and the formal intramolecular transfer of electrons from the disulfide group to the quinone moiety to produce 7. Generating 7 is expected to permit activation of the C(1) and C(10) mitomycin alkylation sites.



**Scheme 1**. Proposed Mechanism of Tomasz and Coworkers for 2 and 3[5]

In this paper, we report on the structure and chemistry of the related C(7)-substituted ethylenediamine mitomycins 8,[8a] 9,[8a] and 10. Our findings require that we consider a novel *ionic, nonreductive* activation pathway for these mitomycins, as well as for 2 and 3.

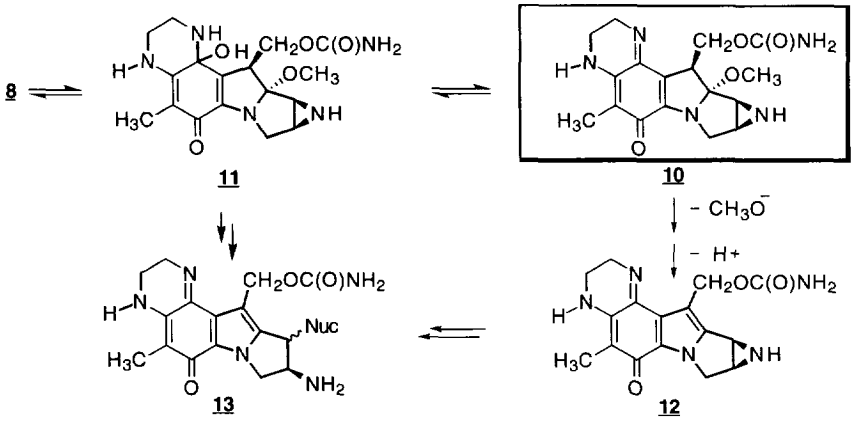


**8** X = $NH(CH_2)_2NH_2$
**9** X = $NH(CH_2)_2N(CH_3)_2$

**10**

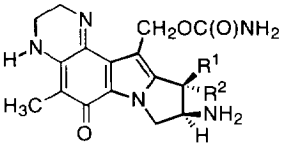
The pioneering SAR studies of Remers and associates demonstrated that excellent *in vivo* antitumor activities were observed for specifically substituted C(7) ethylenediamine derivatives of mitomycin C.[8] Two compounds reported were 8 and 9.[8a] We have prepared both compounds.[9] The spectroscopic properties (IR, UV, $^1$H NMR, COSY $^{13}$C NMR, high-resolution MS) for 9 were consistent with the proposed structure, but they were not for 8. In the infrared spectrum of 8, we observed a prominent absorption at 1694 cm$^{-1}$, and in the $^{13}$C NMR spectrum, we detected a single carbonyl resonance at 179.2 ppm and an unique signal at 154.0 ppm. The high-resolution mass spectrum displayed a peak at 360.167 22, consistent with the formula for 8 ([M+1]$^+$) minus water. These findings were compatible with cyclic imine 10. Confirmation of this reassigned structure was obtained from the DQF-COSY, HMQC, HMBC, and $^{15}$N NMR experiments. In particular, the DQF-COSY spectrum showed a $^1$H-$^1$H interaction between the C(7) N-H proton and the upfield $C_aH_2$ multiplet ($\delta$ 3.03), and the HMBC experiment exhibited a three-bond connectivity between the downfield $C_bH_2$ multiplet ($\delta$ 3.67, 3.83) and the C(8) imine carbon signal (154.0 ppm).[10] Synthesis of 10 with $^{15}$N-enriched ethylenediamine led to the detection of two signals at 83.6 and 320.2 ppm in the proton-decoupled $^{15}$N NMR spectrum. These resonances are consistent with the proposed C(7) amide and C(8) imine functionalities, respectively, for 10,[11] and were further supported by the proton-coupled[12] and HMQC $^{15}$N NMR spectra.

The spectroscopic studies for 10 demonstrated that an appended C(7) ethylenediamine mitomycin residue can react at the C(8) carbonyl site. Formation of 10 diminishes the delocalization of the indoline N(4) electrons with the adjacent $\alpha$, $\beta$, $\gamma$, $\delta$-unsaturated system. This process may permit the N(4)-assisted expulsion of the C(9a) methoxy group to produce 12 and the subsequent activation of the C(1) (and C(10)) bonding sites by an indole-assisted pathway (Scheme 2).



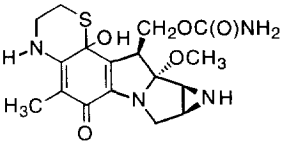
**Scheme 2.** Proposed Nonreductive, Ionic Pathway for Activation of **10**

This possibility was tested by monitoring the reactivities of 9 and 10 in buffered methanolic solutions (Tris•HCl, "pH" 7.4, 25 °C). No reaction was observed for 9 for 10 days (HPLC analyses), while compound 10 reacted ($t_{1/2}$ = 40 h) to give the C(1) substituted mitosenes 14 and 15.[13]



**14** $R^1 = OCH_3$, $R^2 = H$

**15** $R^1 = H$, $R^2 = OCH_3$



**16**

These investigations demonstrated that mitomycin 10 underwent facile ring activation and nucleophilic reaction at C(1) through a nonreductive, ionic pathway. Of particular importance was the absence of C(1) electrophilic adducts in the product profile. We have shown that at near neutral pH values reductively activated mitomycin C undergoes preferential C(1) electrophilic substitution,[14] thereby minimizing its therapeutic value. The facility of the C(7) ethylenediamine-assisted mitomycin activation process suggests that a similar ionic pathway may be operative for 2 and 3. Generating 4 may lead to hemithioketal 16 and activation of the C(1) and C(10) DNA alkylation sites in these agents. Studies are underway to further document the mechanism of these C(7) ethylenediaminemitomycin transformations and to determine whether a similar route exists for disulfides 2 and 3.

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7. Although the intramolecular addition in Scheme 1 was depicted to proceed at C(6), other sites (C(8), C(7)) were not excluded.[5]
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9. Compound 10: HPLC $t_R$ 14.8 min; IR (KBr) 3408, 3288, 1694, 1608, 1567, 1537, 1418, 1324, 1058 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 225, 258 (sh), 365 nm; $^1H$ NMR (300 MHz, pyridine-$d_5$) $\delta$ 1.89-1.94 (m, 1 H, C(1)NH), 1.94 (s, 3 H, C(6)$CH_3$), 2.71 (t, 1 H, $J$ = 4.5 Hz, C(2)H), 2.95-3.15 (m, 2 H, C(a)$H_2$), 3.19 (dd, 1 H, $J$ = 4.5, 8.1 Hz, C(1)H), 3.28 (s, 3 H, C(9a)$OCH_3$), 3.62 (d, 1 H, $J$ = 12.5 Hz, C(3)$H_\alpha$), 3.61-3.75 (m, 1 H, C(b)*H*H'), 3.76-3.90 (m, C(b)H*H'*), 4.10 (dd, $J$ = 4.2, 10.8 Hz, C(9)H), 4.46 (d, $J$ = 12.5 Hz, C(3)$H_\beta$), 5.29 (t, $J$ = 10.8 Hz, C(10)*H*H'), 5.62 (dd, $J$ = 4.2, 10.8 Hz, C(10)H*H'*), 7.39 (s, C(7)NH); $^{13}C$ NMR (75.5 MHz, pyridine-$d_5$) 8.4, 33.8, 38.2, 38.6, 46.8, 48.5, 49.6, 51.8, 63.9, 106.0, 107.0, 116.1, 141.4, 148.8, 154.0, 158.8, 179.2 ppm; MS (+Cl) m/e (rel intensity) 360 $[M+1]^+$; $M_r$ (+Cl) 360.167 22 $(M+1)^+$ (calcd for $C_{17}H_{22}N_5O_4$, 360.167 18).
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13. Compound 14: HPLC $t_R$ 21.6 min; IR (KBr) 3434, 2929, 1719, 1620, 1588, 1560, 1384, 1339, 1090 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 258, 313, 322, 483 nm; $^1H$ NMR (300 MHz, pyridine-$d_5$) $\delta$ 2.14 (s, 3 H, C(6)$CH_3$), 3.21 (dd, 2 H, $J$ = 4.2, 6.6 Hz, C(a)$H_2$), 3.55 (s, 3 H, C(1)$OCH_3$), 3.89-4.05 (m, 4 H, C(2)H, C(b)$H_2$, C(3)$H_\beta$), 4.72 (d, $J$ = 5.1 Hz, C(1)H), 4.85 (dd, $J$ = 7.1, 11.3 Hz, C(3)$H_\alpha$), 5.89 (1/2ABq, 1 H, $J$ = 12.9 Hz, C(10)*H*H'), 6.05 (1/2ABq, 1 H, $J$ = 12.9 Hz, C(10)H*H'*), 7.24 (s, 1 H, C(7)NH), 7.68 (s, 2 H, C(10)OC(O)$NH_2$); $^{13}C$ NMR (75.5 MHz, pyridine-$d_5$) 8.1, 38.1, 48.6, 52.7, 56.6, 59.7, 60.1, 75.9, 106.2, 113.8, 126.7, 139.1, 141.2, 149.2, 153.9, 158.4, 176.8 ppm; MS (+Cl) m/e (rel intensity) 360 $[M+1]^+$; $M_r$ (+Cl) 360.169 11 $(M+1)^+$ (calcd for $C_{17}H_{22}N_5O_4$), 360.167 18). Compound 15: HPLC $t_R$ 21.0 min; IR (KBr) 3434, 2930, 1706, 1634, 1565, 1384, 1340, 1081 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 258, 313, 322, 483 nm; $^1H$ NMR (300 MHz, pyridine-$d_5$) $\delta$ 2.11 (s, 3 H, C(6)$CH_3$), 3.18-3.23 (m, 2 H, C(a)$H_2$), 3.53 (s, 3 H, C(1)$OCH_3$), 3.93 (t, 2 H, $J$ = 6.3 Hz, C(b)$H_2$), 4.19 (dd, 1 H, $J$ = 1.2, 5.3 Hz, C(2)H), 4.40 (dd, $J$ = 1.2, 12.5 Hz, C(3)$H_\beta$), 4.69 (dd, 1 H, $J$ = 5.3, 12.5 Hz, C(3)$H_\alpha$), 4.90 (s, 1 H, C(1)H), 5.93 (1/2ABq, $J$ = 12.8 Hz, C(10)*H*H'), 6.00 (1/2ABq, $J$ = 12.8 Hz, C(10)H*H'*), 7.14 (s, 1 H, C(7)NH); $^{13}C$ NMR (75.5 MHz, pyridine-$d_5$) 8.1, 38.0, 48.5, 55.1, 56.4, 59.8, 61.9, 83.7, 106.0, 113.8, 126.2, 139.0, 141.2, 150.0, 153.8, 158.5, 176.7 ppm; MS (+Cl) m/e (rel intensity) 360 $[M+1]^+$; $M_r$ (+Cl) 360.167 26 $(M+1)^+$ (calcd for $C_{17}H_{22}N_5O_4$, 360.167 18).
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