progress of the reaction was monitored at 720 nm (appearance of Os(II), see Figure 1).

In the flow experiment, the appearance kinetics can be modeled by consecutive mixing and ET processes. (Microscopic re-solvation is viewed, in this case, as faster than either.[9]) Following Margerum,[8] the appropriate transient absorbance expression is

$$M_{exp} = \left(\frac{A - A_\infty}{A_0 - A_\infty}\right) = \frac{1 - e^{-Y}}{Y} \quad (2)$$

where

$$Y = \left(\frac{1}{b}\right)\left(\frac{1}{k_{mixing}} + \frac{\nu}{k_{ET}}\right) \quad (3)$$

In the expression, b is the reaction path length, ν is the flow velocity, and A_0, A, and A_∞ are initial, intermediate, and final absorbances. For 1.4×10^{-5} M **1**, measurements of M_{exp} at each of 250 separate velocities (per push) between 3.5 and 12.5 meter s^{-1} yielded $k_{ET} = 136 \pm 18$ s^{-1}.[10,11] Follow-up experiments with a 9-fold variation in reactant concentration overall yielded nearly identical ET kinetics,[12] confirming the intramolecular (i.e., first-order) nature of the reaction. Finally, it should be noted that the observed ET rate falls well below the upper rate measurement limit of the current instrument (ca. 2×10^5 s^{-1}).[8]

A detailed comparison of this rate with the predictions of contemporary theory is clearly of interest, but is necessarily beyond the scope of the current paper. It is worth noting, however, that simple computer models suggest a metal-to-metal separation distance of ca. 16 Å (fully extended bridged) and that thermal charge tranfer over a similar distance in an isoproline-linked Os/Ru complex yields much faster kinetics ($k_{ET} \approx 3 \times 10^5$ s^{-1}).[2] For the isoproline case both the solvent and the Os coordination environment differ. The ligand environments for the ruthenium centers, however, are similar. For the two systems, driving-force effects should account for about a factor of 5 in reactivity difference. The balance may be due to a combination of (1) unique barrier effects associated with microscopic re-solvation,[6,13] and (2) enhanced nonadiabaticity effects associated with (formal) π/σ orthogonality effects along the length of the TMB bridging ligand.

In addition to the theory comparisons, current work focuses on bridge modifications and on systematic driving-force variations. Indeed, the ability to employ solvent to obtain a continuously adjustable range of driving forces (and rates) may be the most promising feature of the new method.

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Supplementary Material Available: Figure S1 showing representative M_{exp} vs velocity data for a single PAF push (1 page). Ordering information is given on any current masthead page.

(9) This assumption was confirmed by examining the DMSO-induced solvatochromism of $Ru(NH_3)_5(4\text{-methylpyridine})^{2+}$ with the PAF instrument; microscopic re-solvation was too fast to measure. We note further that photophysical (time-resolved luminescence) studies with $(NH_3)_2Ru(bpy)_2^{2+}$ in mixed solvents have previously shown that charge-transfer-induced re-solvation (by DMSO) is complete in less than 5 ns (Doorn, S. K.; Kosmoski, J.; Hupp, J. T. Unpublished results).

(10) Standard deviation is based on an average of 6 runs of 3–6 pushes each. Single-push signal-to-noise was typically 10–20. In some instances, M_{exp} values greater than unity (a nonphysical result) were observed at high velocities, because of base-line drift. Consequently, M_{exp} vs velocity plots were translated slightly (0–0.1) along the M_{exp} axis to achieve optimal kinetic fits (250 points, eqs 2 and 3).

(11) k_{mixing} was measured separately as $(2.7 \pm 0.3) \times 10^3$ m^{-1}.

(12) Five concentrations between 6.6×10^{-6} and 6.2×10^{-5} M were examined. The average rate constant was 118 ± 30 s^{-1}, with no reactant concentration pattern to the variations, except that the single largest k_{ET} value (160 s^{-1}) was recorded at the highest concentration. Attempted fits instead to second-order (bimolecular) kinetics yielded very low correlation coefficients and enormous variations in apparent rate constant with initial reactant concentration.

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Reductively Activated Mitomycin C: An Efficient Trapping Reagent for Electrophiles

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Mitomycin C, a clinically significant antineoplastic antibiotic, is considered to be the prototype of the bioreductive alkylating agents.[1] Studies with nucleosides, oligonucleotides, and DNA restriction fragments documented that **1** selectively bonds to the nucleophilic 2-amino site of specific guanines.[2-5] It is accepted, however, that mitomycin C undergoes both C(1)-nucleophilic and electrophilic substitution transformations under aqueous reductive conditions in the absence of external nucleophiles (Scheme I).[6] Investigations have shown that when the pH was above 7, the C(1)-nucleophilic adducts *cis*- and *trans*-1-hydroxy-2,7-diaminomitosenes[6] (**4**) were produced almost exclusively, and when conditions were moderately acidic, the electrophilic adduct 2,7-diaminomitosene[6,7] (**5**) predominated. It has been suggested that quinone methide **3**[1c] served as the central precursor to both **4** and **5**.[6a] In this communication, we provide evidence that *reductively activated mitomycin C functions primarily as a trapping agent for electrophiles in water at all operational pH values* and that this pattern is altered only when nucleophiles are added under select conditions. The origin for previous misconceptions[6] concerning the reactivity of reduced **1** has been identified.

Plots are provided in Figure 1 for the percentage of C(1)-electrophilic mitosene products generated as a function of pH using two different $Na_2S_2O_4$-mediated reductive conditions[8] (HPLC analysis,[9] protocol 1[10a]). In method A, only 0.2 equiv of $Na_2S_2O_4$ was used, thereby ensuring substantial levels (>64%) of unreacted **1**. In method B, we employed excess $Na_2S_2O_4$ (1.2–2.0 equiv). Under these conditions, **1** accounted for less than 11% of the

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(9) All products were identified by coinjection of an authentic sample with the reaction mixture in the HPLC using two different sets of HPLC conditions.[10]

(10) HPLC conditions using C_{18} μBondapak (SS) column 3.9 mm × 30 cm. (a) Protocol **1**: linear gradient from 100% A (3 mM triethylammonium phosphate, pH 4.7), 0% B (3 mM triethylamine in acetonitrile) to 50% A, 50% B in 25 min. (b) Protocol **2**: isocratic for 5 min 90% A (0.1 M triethylammonium acetate, pH 6.5), 10% B (acetonitrile) and then linear gradient from 90% A, 10% B to 50% A, 50% B in 20 min.

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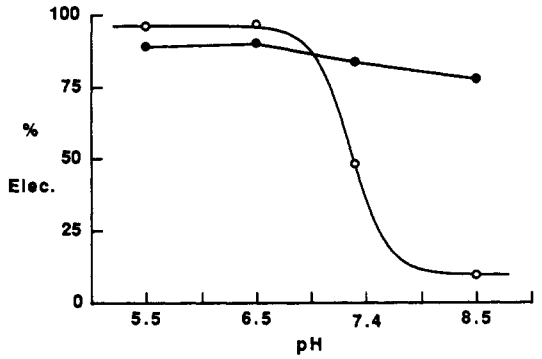
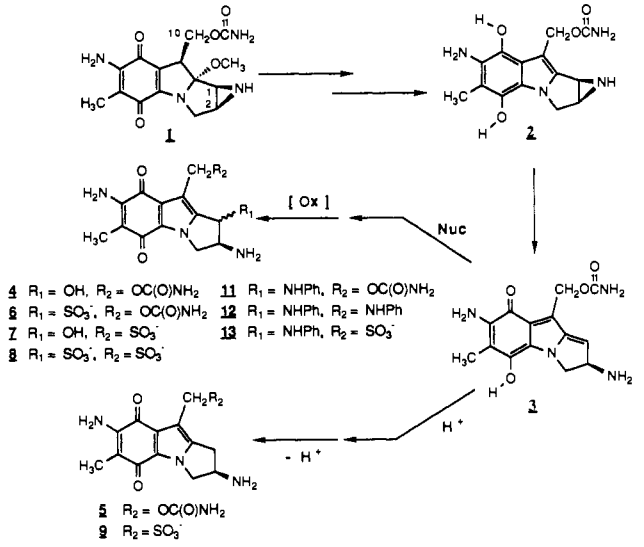


Figure 1. Percent C(1)-electrophilic mitosene products generated as a function of pH. The percent mitosene C(1)-electrophilic products generated with limiting amounts (0.2 equiv) of $Na_2S_2O_4$ (method A) was computed using the formula %(**5** + **9**)/%(**4** + **5** + **6** + **7** + **9**) (○); and with excess (1.2–2.0 equiv) $Na_2S_2O_4$ (method B), using the formula %(**5** + **9**)/%(**4** + **5** + **6** + **7** + **8** + **9**) (●). The reactions were run in duplicate, and the data was obtained using HPLC protocol 1.[10a]

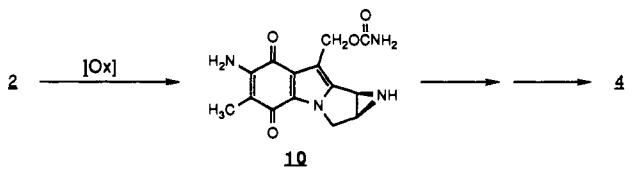
Scheme I. Previously Proposed Pathway for the Reduction of **1**[a]



[a] Reference 6a.

reaction mixture. The two procedures provided dramatically different pH–product profiles. When we limited the amounts of reductant, we observed the previously noted[6] change from predominantly electrophilic (**5**) to nucleophilic (**4**) compounds with increasing pH, whereas with excess $Na_2S_2O_4$ the C(1)-electrophilic adducts **5** and **9** were the major products (77.2–89.9%) at all pH values in the absence of external nucleophiles.[11]

To determine the origin of the product dependency as a function of pH with method A we monitored the reactions using a second HPLC protocol, which employed a more "basic" eluant system.[10b] At pH 8.5, we saw that *anti*- and *syn*-**10**[12] and **4** accounted for 24.9% and 62.4%, respectively, of the product profile after exclusion of unreacted mitomycin C (64.8%). Reducing the reaction time from 6 min to 20 s and then adding first oxygen and then aniline (10 equiv) led to the formation of **11** and **4** in a 2.9:1 ratio (combined product yield after exclusion of **1**: 87.2%) and decreased consumption of mitomycin C (16.9%). These results demonstrated that, in basic solutions where reduction of **1** is incomplete, the acid-promoted aziridine ring opening of **2** to **3** was slow enough to permit reoxidation of this species to **10**.[13] Compound **10** furnishes **4** rapidly in water (pH < 7.0).[12]



The propensity of reductively activated (i.e., 1.2–2.0 equiv of $Na_2S_2O_4$) mitomycin C to undergo C(1)-electrophilic substitution transformations in the presence of a competing nucleophile was assessed by incorporating excess aniline (4 equiv) in the original reaction mixture. Under these conditions, substantial amounts of C(1)-anilino nucleophilic products (**11**, **12**, **13**) were observed at pH 5.5 (69.9%), whereas the C(1)-electrophilic adduct **9**[8] predominated at pH 8.5 (70.8%). These results supported an earlier finding that mitomycin C–DNA cross-linking processes were promoted at lower pH values.[3]

These collective findings document that reductively activated mitomycin C in water in the absence of external nucleophiles undergoes principally electrophilic substitution processes. Previous observations[6] reporting the primary formation of *cis*- and *trans*-1-hydroxy-2,7-diaminomitosenes (**4**) at pH 7 and above are now principally, although not exclusively, attributed to the hydrolysis of the *oxidized* 7-aminoaziridinomitosene (**10**).[12] The decreased reactivity of **2** under mildly alkaline conditions raises the intriguing possibility that the effects of the medium or cellular constituents might influence drug activation and bonding by permitting **2** sufficient lifetime to translocate from the site of reduction[14] to the cellular nuclei before DNA bonding.

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(11) $Na_2S_2O_4$ reduction (1.5–2.0 equiv) of **4** under comparable conditions gave only **7**.[8]

(12) Compound **10** exists as a mixture of anti and syn isomers. These conformers differ in the orientation of the aziridine N–H proton in relation to the pyrrolo[1,2-*a*]indole ring system. Han, I.; Kohn, H. *J. Org. Chem.* **1991**, *56*, 4648. Kinetic studies performed in buffered methanol gave the following solvolysis $t_{1/2}$ values for **10**: "pH" 7.0, 3 min; "pH" 8.5, 228 min.

(13) Oxidation of **2** may have occurred by disproportionation with **1** or by reaction with adventitious amounts of oxygen.

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Site-Specific Isotopic Labeling of Proteins for NMR Studies

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NMR spectroscopy is a sensitive, site-specific probe of biomolecular structure. For relatively small proteins and peptides, the ^1H resonances can be assigned using the sequential method.[1] However, there are many cases, especially larger proteins, in which the spectra are too complex for complete, systematic resonance assignments. In some cases, assignments can be made by selective isotopic labeling (e.g., uniform incorporation of a ^{13}C-labeled amino acid) in conjunction with site-directed mutagenesis or ^{13}C,^{15}N double labeling of adjacent amino acids.[2,3] However,

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