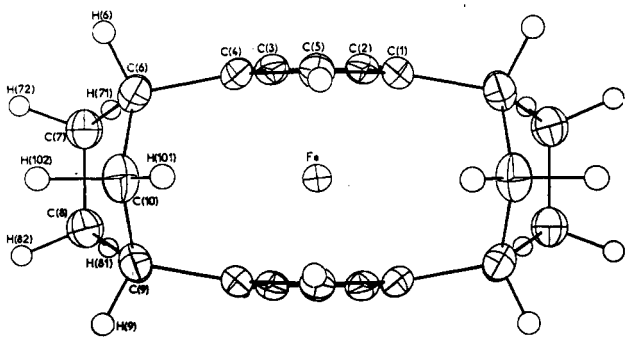

Figure 6. Ortep view of the X-ray crystal structure of the ferrocene cage **39** obtained by cleavage of ferrocene with aluminum chloride:(1,1′,3,3′-bis(cyclopentylene)ferrocene).[62]

ligands have similar electron-releasing properties, electrocatalytic exchange is possible provided it is driven by an irreversible step such as eq 20–21 (see also eq 22). The electrocatalytic chelation of monodentate

$$CpFe^{II}(CO)_2(\eta^1\text{-}SC(S)NMe_2) \xrightarrow[-CO]{Cp_2Fe^+(5\%)} CpFe^{II}(CO)(\eta^2\text{-}S_2CNMe_2) \quad (22)$$

dithiocarbamate is such an example driven by CO loss and chelation of dithiocarbamate.[60]

Stoichiometric oxidation leads to decomposition, except in the presence of PPh_3 which stabilizes $[CpFe^{III}(PPh_3)(\eta^2\text{-}S_2CNMe_2)]^+$ (compare eq 7). In order to improve the yields of ferricinium-catalyzed reactions, one must impede the radical-type reactions of ferricinium. Again steric bulk, as in the cage compound **39**, is desirable (Figure 6).

Redox catalysts are reversible redox systems conveying electrons in thermodynamically allowed processes which are kinetically inhibited by overpotential barriers.[56] For instance, nitrate ion cannot be reduced in basic aqueous medium on a mercury cathode. However, in the presence of catalytic amounts of **2**, NH_3 is formed. Under these conditions, **1** and **2** are stable. Although the thermodynamic potential of $NO_3^-/NO_3^{\bullet 2-}$, presumably ~2 V/SCE, is more negative than that of $1/1^+$, NO_3^- can be reduced to NH_3 by 1[61] since the overall process is driven by the irreversible reactions of $NO_3^{\bullet 2-}$

$$1 + NO_3^- \rightleftharpoons 2 + NO_3^{\bullet 2-} \xrightarrow[\text{reduction}]{\text{protonation,}} NH_3 \quad (23)$$

With the soluble redox catalyst **3**,[11] the system is homogeneous and stable ($k \sim 10^2$ mol^{-1} L s^{-1}).[61] The applied cathodic potential reduces **2** (or **3**) to **1** (or **4**) (~−1.8 V/vs. SCE).

These examples show that electron-rich (19-e$^-$) as well as electron-poor (17-e$^-$) sandwiches can function as electrocatalysts or redox catalysts.

Conclusion and Outlook

The syntheses of Jahn–Teller active 19-electron Fe(I) compounds, thermally stabilized by peralkylation of an aromatic ligand, provided extremely electron-rich molecules. These compounds activate dioxygen, allowing the examination of the reactivity of superoxide anion and the inhibition of this reactivity by the salt effect. In the near future, this unique possibility of generating cage ion pairs from Fe(I) compounds and various substrates should let us investigate the general influence of salts on the reactivity of such ion pairs. It is likely that the knowledge of these salt effects will provide selectivity in many reductions. The coupling of both ET catalysts **1** and $(Cp_2Fe^{II})^+$ in the same molecular unit such as **15** provides a bifunctional catalyst. The design of molecular materials with such multiple and stable oxidation states are important in the study of ET processes, electrode modification, and ET catalysis. Finally, stable Fe(I) compounds are excellent candidates as materials for piles and batteries on polymer supports.

It is a pleasure for me to thank the students, postdoctoral fellows, and scientists whose names appear in the references for their hard work, enthusiasm, and stimulating collaborations.

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The Chemistry of Activated Bleomycin

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The recognition that DNA serves as a target for small molecules in the initiation of cellular disorders, and in the therapy of certain diseases, has served to catalyze increased interest in the interaction of such species with DNA. These include natural[1] and synthetic[2] products

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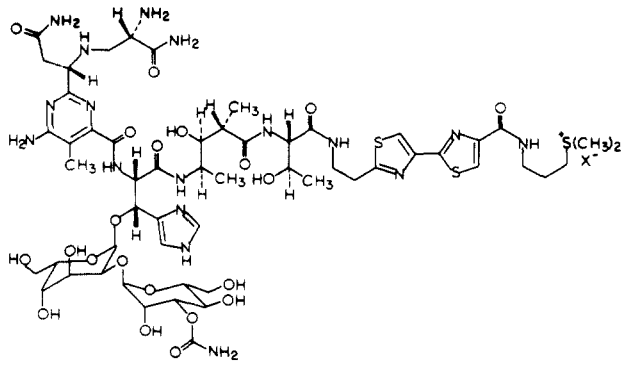
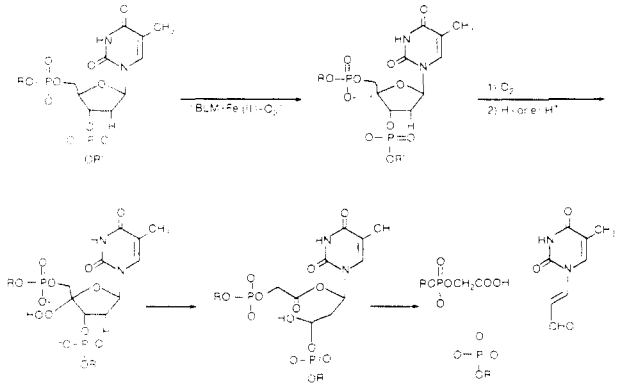


Figure 1. Bleomycin A_2.

Scheme I.
BLM-Mediated Degradation of DNA via a Putative C-4′ Hydroperoxide Intermediate



that bind to DNA directly, compounds that require metabolic activation prior to binding,[3] and small molecules that bind to DNA only in the presence of DNA binding proteins such as topoisomerase.[4] A subset of these DNA binding molecules mediate DNA strand scission subsequent to DNA binding, generally via metal ion or light-catalyzed processes.[5] For such DNA interactive agents, ongoing studies in a number of laboratories are focused on issues such as mode of DNA interaction, sequence recognition, and the chemistry of DNA strand scission or modification.

The bleomycins are a family of glycopeptide-derived antibiotics that have clinically useful antitumor activity.[5a,6] Bleomycin A_2 (BLM A_2; see Figure 1) is the major component of Blenoxane, the clinically used mixture of bleomycins; Blenoxane is used extensively for the treatment of neoplasms such as squamous cell carcinomas and malignant lymphomas. The antitumor activity of bleomycin is believed to derive from its ability to mediate DNA degradation, a transformation

Scheme II.
Cleavage of d(CGCT$_3$A$_3$GCG) by Fe(II)•BLM

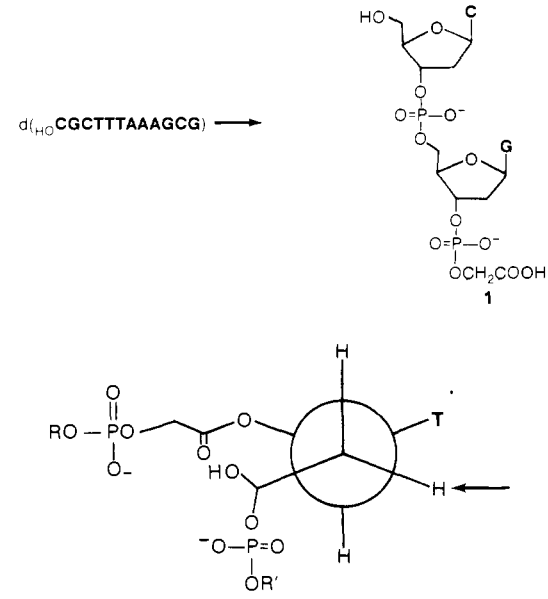


Figure 2. Formation of nucleoside 3′-(phosphoro-2″-*O*-glycolate) and 3-(thymin-1′-yl)propenal via a putative anti elimination.

that is metal ion and oxygen dependent.[7] Bleomycin-mediated DNA degradation is sequence selective, and results in preferential cleavage at certain...GT...and... GC...sequences;[8] interestingly, double-strand nicks are formed at a frequency in excess of what would be predicted from the random accumulation of single-strand breaks.[9] Described in this Account is the current understanding of several parameters of BLM activation and chemistry, including the stoichiometry of reaction with DNA and the nature of the products formed, the structural components in bleomycin that contribute to DNA sequence and strand selectivity, and the effect of DNA methylation on BLM-mediated strand scission.

Products of DNA Strand Scission

Scheme I outlines a postulated pathway for the degradation of a thymidine nucleotide in DNA by BLM;[10] it suggests the formation of a C-4′ hydroperoxide via a transient C-4′ radical. Scission of the C-3′–C-4′ ribose bond would then result from Criegee-type rearrangement[11] or some analogous process to give an intermediate (e.g., i) from which the observed products could form directly. This scheme predicts the formation of three types of products including a 5′-oligo-

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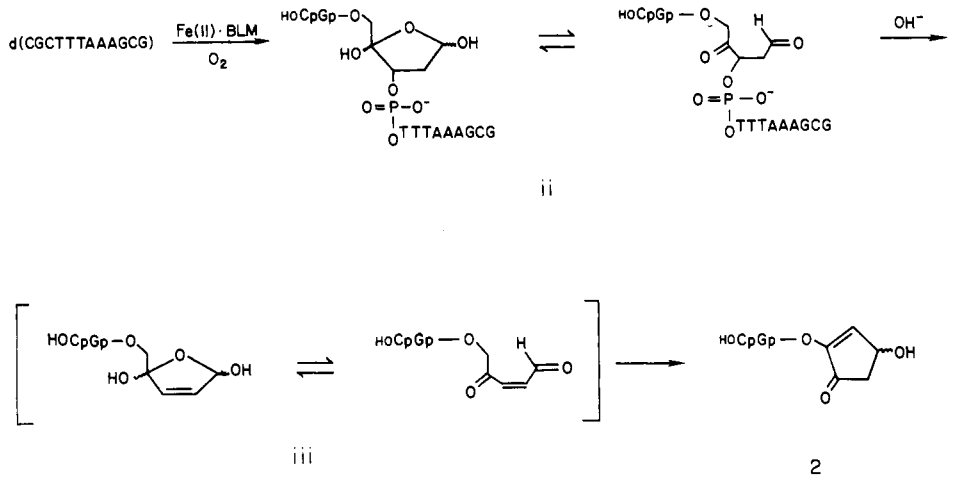
Scheme III.
BLM-Mediated DNA Degradation via a Putative C-4′ Hydroxyl Intermediate



nucleotide terminating at its 3′-end with a phosphoroglycolic acid moiety, a 3′-oligonucleotide containing a 5′-phosphate, and a 3-(thymin-9′-yl)propenal. When treated with Fe(II)·BLM, DNA has been shown to liberate all four possible base propenals with concomitant strand scission to give oligomers having 5′-phosphates.[5a,12] Identification of the third type of product predicted by Scheme I was more difficult technically, but has been accomplished following acid[13] or λ exonuclease[14] treatment of BLM-digested DNA. In an effort to develop a direct method of analysis, we synthesized a self-complementary dodecanucleotide having a putative BLM cleavage site close to its 5′-end (Scheme II). As anticipated, treatment of this oligonucleotide with Fe(II)·BLM resulted in cleavage at cytidine$_3$; the identity of putative dinucleotide **1** was verified by comparison with an authentic synthetic sample.[14,15]

Further support for Scheme I includes the observation by Wu et al.[16] that BLM-mediated DNA degradation involves abstraction of C-4′ H, and the report that products resulting from scission of the C-3′–C-4′ bond of ribose were formed only when sufficient O_2 was present.[17] That breaking of the C-1′–O-1′ bond occurs via an anti elimination (Figure 2) is supported by the findings that C-2′ αH is lost specifically[16] with the exclusive formation of the trans base propenals as primary reaction products.[14]

In addition to these products, Fe(II)·BLM-mediated DNA degradation also affords free bases and alkali labile lesions,[5a] the former of which do not result from decomposition of base propenals. While no DNA strand scission results from the BLM-mediated transformation that leads to the alkali labile lesions, subsequent base treatment has been noted to effect strand scission by further modification of the alkali labile lesions. Although the chemistry responsible for these transformations proved elusive for several years, the use of d(CGCTTTAAAGCG) as a substrate has now provided some insight. Successive treatments of this dodecanucleotide with Fe(II)·BLM and base resulted in the formation of diastereomeric dinucleotides **2** (Scheme III), identical with authentic synthetic samples.[18] Also outlined in Scheme III is a pathway that accommodates all experimental observations. Consistent with the suggestion of Wu et al.,[16] initial formation of a C-4′ hydroxyl derivative at cytidine$_3$ of the dodecanucleotide should result in facile elimination to cytosine and the formation of putative alkali labile lesion ii. Subsequent treatment with alkali could then afford strand scission with transient formation of iii, base-catalyzed rearrangement of which would provide diasteromers **2**. It is worth noting that while the transformation iii → **2** has considerable precedent[19] and was found to proceed with reasonable efficiency using an authentic synthetic sample of iii,[18] intermediate iii was never actually observed following digestion of d(CGCTTTAAAGCG) with Fe(II)·BLM and base. Therefore, it is also possible that the initial event resulting from base treatment of putative ii involves cyclopentanone formation.

As noted, the precursor of **2** (Scheme III) is presumably a derivative of d(CGCT$_3$A$_3$GCG) containing an OH group at C-4′ of cytidine$_3$. On the basis of studies with a DNA precursor radiolabeled at C-4′, and the observation that the formation of alkali labile product and nucleoside-3′-phosphoroglycolate were inversely related as a function of available O_2,[17] it was suggested that both pathways share a common C-4′ radical intermediate.[16] Recently, Burger et al.[20] have suggested a partitioning mechanism to account for formation of the putative C-4′ OH derivative, in which the C-4′ OH group would be derived from activated BLM. We have also investigated alternative mechanisms involving initial single-electron transfer from the putative C-4′

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Table I.
Quantitative Analysis of BLM-Mediated Product Formation from d($CGCT_3A_3GCG$)[a]

Fe(II)·BLM A_2, μM	cytosine, μM	cytosine propenal, μM	total prod,[b] μM	cytosine + cytosine propenal/total prod	total prod/ Fe(II)·BLM A_2
50	4.4	12	17	0.96	0.34
100	13	33	56	0.82	0.56
200	23	43	87	0.76	0.44
300	20	42	80	0.78	0.27
10[c]	7.7	24	37	0.86	3.7
30[c]	20	46	116	0.57	3.9

[a] Reaction mixtures contained d($CGCT_3A_3GCG$) (1mM final nucleotide concentration) and the indicated amount of Fe(II)·BLM A_2. [b] Total product is equal to the sum of all free bases and base propenals. [c] Carried out in the presence of 2 mM ascorbic acid.

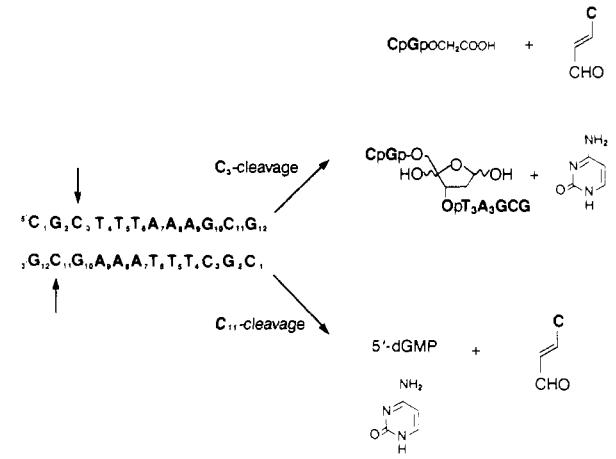
radical, or reductive cleavage of a C4′ peroxy intermediate. The results of these experiments were not conclusive.[21]

Bleomycin has been noted to carry out some chemistry closely analogous to that mediated by known monooxygenases (vide infra);[22] it seems possible that direct hydroxylation of the ribose C-4′ by activated bleomycin may be the source of the C-4′ OH group that leads to the alkali labile product. Consistent with this suggestion, it has recently been found that Fe(III)·BLM activated with cytochrome P-450 reductase, NADPH and O_2[23] under ambient conditions produced a much greater proportion of alkali labile product than did aerobically activated Fe(II)·BLM.[21] In this context it may be worth noting that a recent report has presented evidence consistent with the existence of two forms of activated Fe·BLM.[24]

Stoichiometry of Bleomycin Activation

In addition to the role that it played in product identification, the dodecanucleotide was also of utility in the quantification of products. Following an initial observation that d($CGCT_3A_3GCG$) was a much more efficient substrate for Fe(II)·BLM than was calf thymus DNA, we sought to define the course of dodecanucleotide degradation. On the assumption that degradation would occur largely at cytidine$_3$ and cytidine$_{11}$ (i.e., at a "double-strand recognition site"), a high performance liquid chromatography (HPLC) system was devised that would permit separation of the anticipated products (Scheme IV). These could be separated conveniently by reversed-phase HPLC and the use of carefully purified synthetic standards permitted direct quantitation of each product.[15a] As anticipated, degradation of the dodecanucleotide occurred predominantly (76–96%) at C_3 or C_{11} over a wide range of conditions.[15a] Admixture of 1 equiv of distamycin for each two dodecanucleotides precluded degradation within the AT-rich region;[25] >99% degradation occurred at C_3 or C_{11} with no diminution of overall product formation.[15a] This simplified the product analysis and permitted verification that there were no significant products resulting from Fe(II)·BLM degradation under ambient conditions beyond those included in Schemes I and III.

Scheme IV.
Products of d($CGCT_3A_3GCG$) Degradation by Fe(II)·BLM



Verification that the reaction products were limited to those in Schemes I and III further simplified the product analysis (even in the absence of distamycin), since reaction at any site produced a single equivalent of free base or base propenal (cf. Scheme IV). Accordingly, by simple quantitation of each base and base propenal, we determined the total number of "DNA events", as well as the percentage of those events that occurred at the "preferred cleavage site" (i.e., C_3 or C_{11}). The data obtained over a wide range of BLM concentrations is shown in Table I, which illustrates four interesting points. First, as noted, dodecanucleotide modification occurred predominantly at C_3 or C_{11}. Second, on the basis of oxygen consumption measurements, product and isotope labeling determinations and spectroscopic measurements, it has been suggested[15a,22,26] that activation of Fe(II)·BLM requires an additional electron; in the absence of any added reducing agent this electron would have to be obtained by disproportionation of two Fe(II)·BLM molecules (eq 1). The data in Table I were consistent with this

$$\text{Fe(II)·BLM} + \text{Fe(II)·BLM} \xrightarrow{O_2} \text{"active Fe·BLM"} + \text{Fe(III)·BLM} \quad (1)$$

suggestion. The efficiency of Fe·BLM utilization was never significantly greater than 0.5, and the formation of products diminished dramatically at 50 μM Fe(II)·BLM. Both of these observations are consistent with an obligatory bimolecular collision of two Fe(II)·BLM molecules to produce a single activated Fe·BLM. That the efficiency of dodecanucleotide degra-

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dation at 50 μM Fe(II)·BLM was actually limited by the availability of electrons can be appreciated from the significant potentiation of cleavage obtained by admixture of abscorbate (Table I). A third interesting point involved the efficiency of dodecanucleotide degradation by Fe(II)·BLM. Under the conditions employed in the absence of reducing agent, the maximum amount of product that could have been produced from the dodecanucleotide was ~84 μM[15a]; that this amount was produced by 200 μM added Fe(II)·BLM (presumably equivalent to 100 μM activated Fe·BLM) suggests a high efficiency of dodecanucleotide degradation. A fourth point was noted when Fe(II)·BLM (10–30 μM) was employed in the presence of 2 mM ascorbic acid. Under these conditions the number of DNA events was 3.7–3.8 times greater than the number of BLM molecules present.[27] Consistent with the observations of Povirk who quantified BLM-mediated base release from *E. coli* DNA,[28] this constituted direct evidence that bleomycin can act catalytically! The observation of catalytic activity also suggested strongly that the amount of product obtained in the absence of reducing agent (Table I) was not a consequence of BLM inactivation.

The accumulated data are consistent with eq 2. Because we have demonstrated that the Fe(III)·BLM resulting from this process is unaltered and can be re-

$$\text{Fe(II)·BLM} \xrightarrow[O_2]{e^-} \text{"active Fe·BLM"} \begin{cases} \xrightarrow{\text{DNA}} \text{Fe(III)·BLM} \\ \xrightarrow{\text{products}} \end{cases} \quad (2)$$

duced back to Fe(II)·BLM, each "DNA event" produced by Fe·BLM must require $2e^-$, as well as O_2.

Analysis of DNA Binding by Bleomycin

The interaction of BLM with DNA has been measured by a number of physicochemical techniques. These have included fluorescence quenching of the bithiazole moiety upon admixture of DNA,[29,30] which has permitted calculation of an affinity constant for BLM binding.[29] That the C-terminus of BLM is required for DNA binding can be judged from experiments using synthetic analogues of the C-terminal portion of BLM[31] and from analogues of BLM lacking the C-terminus.[32] The latter can be activated for oxygen transfer to small substrates in bimolecular reactions (vide infra) but fail to effect DNA degradation at reasonable concentrations. The nature of BLM binding to DNA has also been studied. Povirk et al. have reported that BLM caused DNA helix elongation but that the structurally related compound phleomycin did not.[33] Since phleomycin contains a nonplanar thiazolinylthiazole moiety, rather than a bithiazole, the results suggested that BLM is capable of DNA intercalation. A more recent analysis by Fisher et al. provided additional support for this mechanism of interaction.[31b] Nonetheless, it has also been suggested on the basis of a laser-light-scattering study that the DNA helix is shortened by BLM binding.[34] Also, the assertion of different DNA binding mechanisms for bleomycin and phleomycin is difficult to reconcile with their essentially identical patterns of DNA cleavage.[12,33b] In this context it may be worth noting that molecular modeling suggests the possibility that bleomycin may be able to bind to the minor groove of DNA; DNA helix unwinding has occasionally been noted for molecules believed not to be intercalators.[35]

Table II.
Position of d(CGCT$_3$A$_3$GCG) Cleavage by BLM Congeners[a]

BLM	total events at C_3, C_{11},[b] μM	specificity,[c] %	cleavage position, %	
			C_3	C_{11}
Fe(II)·BLM A_2	62	78	15	85
Fe(II)·BLM B_2			17	83
Fe(II)·deglyco BLM A_2	52	98	79	21
Fe(II)·decarbamoyl BLM A_2	60	90	72	28

[a] Reaction mixtures contained d(CGCT$_3$A$_3$GCG) (1μM final nucleotide concentration) and 300 μM BLM derivative. [b] Equal to the sum of cytosine + cytosine propenal. [c] Proportion of oligonucleotide modification occurring at C_3 or C_{11}.

In an effort to obtain additional information concerning BLM–DNA interaction, we analyzed several bleomycin congeners for their ability to cleave d(CGCTTTAAAGCG). Of particular interest was the extent of cleavage at C_3 vs. C_{11}, i.e., at the double strand recognition site; this could be determined readily following product analysis by hplc. As shown in Table II, when the dodecanucleotide was digested with Fe(II)·BLM A_2 reaction at this site was quite asymmetric: 85% of the chemical modification occurred at C_{11}, only 15% at C_3. Interestingly, alteration of experimental conditions (e.g., BLM or salt concentration, temperature, pH) had little effect on this ratio. Fe(II)·Bleomycin B_2 was studied next in the belief that alteration of the C-terminal portion of BLM might alter the "strand selectivity" at C_3 vs. C_{11}. However, the experimental finding was that the ratio of reaction at C_3 and C_{11} was virtually identical with that observed for BLM A_2, an observation consistent with their essentially identical cleavage of ^{32}P-end-labeled DNA restriction fragments.[12]

Repetition of the same experiments was carried out using analogues of BLM A_2 altered in that part of the molecule responsible for metal ion binding: the specific analogues studies were decarbamoyl BLM A_2 (i.e., lacking the mannose carbamoyl group) and deglyco BLM A_2 (lacking the disaccharide moiety), both of which have been shown to degrade DNA.[27,36] Remarkably, both gave results dramatically different than BLM A_2 (Table II). Moreover, while the efficiency of cleavage at the double strand recognition site was high

(27) Interestingly, when greater amounts of dodecanucleotide were added, as many as 10–11 turnovers of BLM were observed. Sugiura, H.; Kilkuskie, R. E.; Chang, L.-H.; Ma, L.-T.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1986**, *108*, 3852.
(28) Povirk, L. F. *Biochemistry* **1979**, *18*, 3989.
(29) Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641.
(30) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5616.
(31) (a) Kross, J.; Henner, W. D.; Haseltine, W. A.; Rodriguez, L.; Levin, M. D.; Hecht, S. M. *Biochemistry* **1982**, *21*, 3711; (b) Fisher, L. M.; Kuroda, R.; Sakai, T. T. *Biochemistry* **1985**, *24*, 3199.
(32) Kilkuskie, R. E.; Suguna, H.; Yellin, B.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 260 and references therein.
(33) (a) Povirk, L. F.; Hogan, M.; Dattagupta, N. *Biochemistry* **1979**, *18*, 96. (b) Povirk, L. F.; Hogan, M.; Dattagupta, N.; Buechner, M. *Biochemistry* **1981**, *20*, 665.
(34) Langley, K. H.; Patel, M. R.; Fournier, M. J. In *Biomedical Applications of Laser Light Scattering*; Satelle, D. B., Lee, W. I., Ware, B. R., Eds.; Elsevier Biomedical: Amsterdam, 1982; pp 37–49.
(35) Dougherty, G.; Pilbrow, J. R. *Int. J. Biochem.* **1984**, *16*, 1179.
(36) Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869.

in all cases, it also differed among the analogues; deglyco BLM A_2, e.g., produced >95% of all DNA events at C_3 or C_{11}.

One conclusion that seems inescapable on the basis of this data is that the choice of cleavage site on DNA must be influenced substantially by that portion of the molecule responsible for metal ion binding and oxygen activation. A corollary to this conclusion is that Fe-bound BLM, decarbamoyl BLM and deglyco BLM must possess geometries that differ substantially. This reinforces the conclusion made earlier for Fe(II)·CO·deglyco BLM and Fe·CO·BLM on the basis of ^1H NMR studies.[30,37] Since BLM and decarbamoyl BLM differ only in the carbamoyl moiety, the present indication of significant differences in geometry for the two would seem to revolve a longstanding issue in favor of participation of the carbamoyl group in the process of metal ligation.[30,37,38]

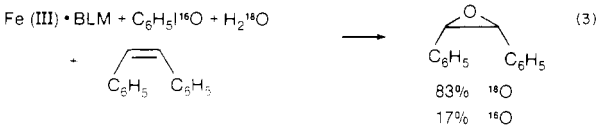
Also studied for the analogues listed in Table II was the nature of the chemistry mediated at C_3 vs. C_{11}, i.e., the formation of alkali labile product + free base or nucleoside 3′-phosphoroglycolate + base propenal. Significantly, for any single analogue, the chemical products produced at C_3 were in the same proportion as those produced at C_{11}. Although not conclusive, this fact, as well as the stoichiometry of DNA cleavage and the differences among analogues as regards strand selectivity at the double strand recognition site, argues for certain features in any model of DNA binding by BLM. These include two "antiparallel" binding orientations at the double-strand recognition site, one of which is responsible for chemical modification at C_3 and the other at C_{11}. Double-strand cleavage would necessarily involve two activated BLM molecules binding in complementary orientations at a double-strand recognition site (albeit not necessarily at the same time). Finally, because the chemistry at C-3 is mirrored exactly at C-11 (which is ~12 Å away), and since little chemistry is observed at adjacent positions on either strand, it seems likely that chemical modification is mediated by a ternary complex consisting of BLM, Fe and one or more oxygen atoms. While the oxygen(s) that react with DNA may have radical character, it seems unlikely that they are diffusible oxygen radicals as the latter are known to produce distinctive patterns during DNA degradation[39] which differ substantially from that observed for Fe·BLM.

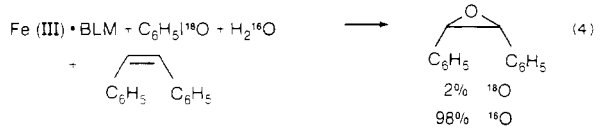
Chemical Transformations Mediated by Bleomycin

To date, most studies of BLM chemistry have involved the mechanism of DNA degradation. The overall picture that has emerged is that of a two-step process. First, bleomycin binds to its DNA substrate in a specific orientation in what is presumably the rate-limiting step for cleavage; oxidative strand scission then occurs in what is really an intramolecular process. The dilemma is that the chemistry observed is a function of what is allowable after binding, and possibly quite different than what would obtain in a process less constrained by orientation of the reacting species. Since some BLM congeners have already been shown to bind to DNA differently than the parent molecule (vide supra), an understanding of chemical transformations accessible to BLM might well facilitate the design of analogues capable of degrading DNA by novel processes.

Accordingly, we investigated the chemistry of activated BLM with small substrates; because of the analogies noted[40] between BLM and cytochrome P-450, we initiated our studies with known cytochrome P-450 substrates.[7d,22] As shown (Table III), when activated with the oxygen surrogate iodosobenzene, Fe(III)·BLM A_2 effected oxygen transfer to *cis*-stilbene. The major product obtained was *cis*-stilbene oxide, as was also true for the cytochrome P-450 analogue chloro-α,β,γ,δ-(tetraphenylporphinato)iron(III)(Fe(III)·TPP(Cl)). Further, neither Fe(III)·BLM + C_6H_5IO nor Fe(III)·TPP(Cl) + C_6H_5IO would mediate efficient oxygen transfer to *trans*-stilbene.[22,41] Because Fe(III)·BLM does not bind to olefins, much higher concentrations of BLM and substrate were employed to obtain significant oxidative transformation, relative to the conditions employed routinely for DNA strand scission. Other olefins were also studied with Fe(III)·BLM + C_6H_5IO; the products (Table III) were of the same type as those observed[41] for models of cytochrome P-450. Further, because C_6H_5IO was used in 30-fold molar excess relative to BLM in these experiments, product yields based on Fe(III)·BLM were as high as 1200–1800% for individual olefins. Thus BLM was used catalytically in this system as well.

Also investigated was the source of the oxygen atom incorporated into *cis*-stilbene oxide. When Fe(III)·BLM was activated with $H_2{}^{18}O$ and C_6H_5IO (eq 3), the iso-





lated *cis*-stilbene oxide was largely ^{18}O labeled. On the other hand, activation with $C_6H_5I^{18}O$ in $H_2{}^{16}O$ resulted in the formation of *cis*-stilbene oxide containing no more than 2% ^{18}O.[22] The observation of oxygen transfer with incorporation of label from solvent has also been reported for the C_6H_5IO-mediated hydroxylations of cyclohexene by cytochrome P-450 from rat liver,[42] and of camphor by *Pseudomonas putida* cytochrome P-450.[43] As is believed to be true for C_6H_5IO-

(37) Oppenheimer, N. J.; Chang, C.; Chang, L.-H.; Ehrenfeld, G.; Rodriguez, L. O.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 1606.

(38) (a) Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Iitaka, Y.; Umezawa, H. *Jpn. J. Antibiot.* **1978**, *31*, 1073. (b) Sugiura, Y.; Suzuki, T.; Otsuka, M.; Kobayashi, S.; Ohno, M.; Takita, T.; Umezawa, H. *J. Biol. Chem.* **1983**, *258*, 1328.

(39) (a) Schultz, P. G.; Taylor, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 6861. (b) Chu, B. C. F.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 963. (c) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 968.

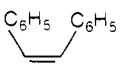
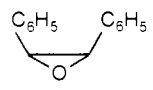
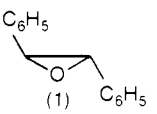
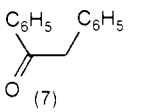
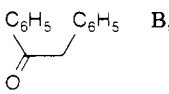
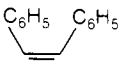
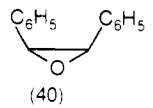
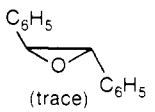
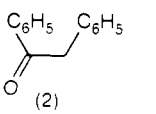
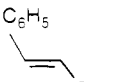
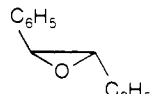
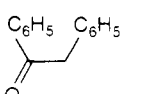
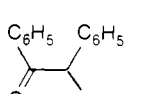
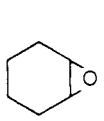
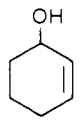
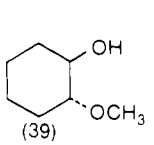
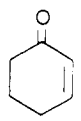
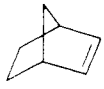
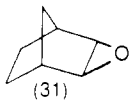
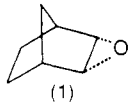
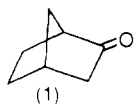
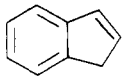
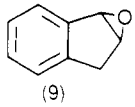
(40) These included the formation of a ternary complex with Fe(II) and CO,[30,37] and aerobic activation in analogy with cytochrome P-450. See ref 22 and 26 and Sausville et al. (Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 814).

(41) Groves, J. T.; Nemo, T.; Myers, R. S. *J. Am. Chem. Soc.* **1979**, *101*, 1032.

(42) Macdonald, T. L.; Burka, L. T.; Wright, S. T.; Guengerich, F. P. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 620.

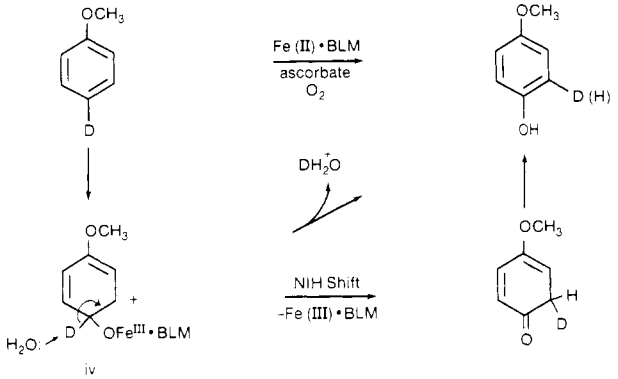
(43) Heimbrook, D. C.; Sligar, S. G. *Biochem. Biophys. Res. Commun.* **1981**, 99, 530.

Table III.
Olefin Oxidation by Fe(III) + BLM or TPP in the Presence of C_6H_5IO

oxidant	substrate	products, yields[a]				method of det yield[b]
Fe(III)·BLM A_2 + C_6H_5IO		 (25, 22)	 (1)	 (7)	 (5-7)	B, C, D
Fe(III)·TPP(Cl) + C_6H_5IO		 (40)	 (trace)	 (2)		B
Fe(III)·BLM A_2 + C_6H_5IO		 (3)	 (2)	 (2)		B, C, D
Fe(III)·BLM A_2 + C_6H_5IO		 (9)	 (12)	 (39)	 (trace)	A
Fe(III)·BLM A_2 + C_6H_5IO		 (31)	 (1)	 (1)		A
Fe(III)·BLM A_2 + C_6H_5IO		 (9)				C

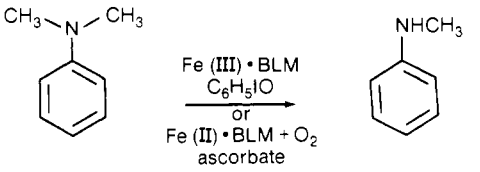
[a] Yields based on amount of added iodosobenzene; quantitative conversion was assumed. [b] (A) Gas chromatography–mass spectrometry, (B) HPLC analysis, (C) isolation, (D) 360-MHz ^1H NMR analysis.

Scheme V.
Aerobic Oxidation of *p*-Deuterioanisole by Fe(II)•BLM + Ascorbate



activated cytochrome P-450,[44] these observations are consistent with a C_6H_5IO-activated Fe·BLM that contains a single oxygen atom bound to Fe which is capable of undergoing solvent exchange. In a recent study, Heimbrook et al.[45] demonstrated that Fe(III)·BLM, activated with any of four oxidants, mediated both oxygen transfer and one-electron oxidation of *cis*-stilbene. Oxygen transfer from Fe·BLM resulted in the formation of *cis*-stilbene oxide, while electron transfer gave *trans*-stilbene or benzaldehyde in the absence and presence of O_2, respectively.

The aerobic activation of Fe·BLM for olefin oxidation has been accomplished using ascorbic acid as a source of electrons. Thus demethylation of *N*,*N*-dimethylaniline, which has been accomplished by the use of Fe(III)·BLM + C_6H_5IO (1100% yield, based on Fe(III)·BLM), was also demonstrated by the use of Fe(III)·BLM + O_2 + ascorbate (140% yield, based on bleomycin). Additionally, aerobically activated Fe·



BLM was shown to mediate the hydroxylation of aromatic substrates such as naphthalene and anisole,[22] a property noted earlier for microsomal monooxygenases.[44] The mechanism of these transformations was studied by the use of *p*-deuterioanisole (Scheme V). Oxidation of the deuteriated substrate (^2H content ~ 80%) with Fe(II)·BLM + O_2 + ascorbate gave *p*-methoxyphenol having a deuterium content that was somewhat variable (15–21%), but always much lower

(44) (a) Groves, J. T.; Krichnan, S.; Avaria, G. E.; Nemo, T. E. *Adv. Chem. Ser.* **1980**, *191*, 277. (b) Tabushi, I.; Koga, N. *Adv. Chem. Ser.* **1980**, *191*, 291. (c) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315 and references therein. (d) Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9.

(45) Heimbrook, D. C.; Mulholland, R. L.; Hecht, S. M. *J. Am. Chem. Soc.*, in press.

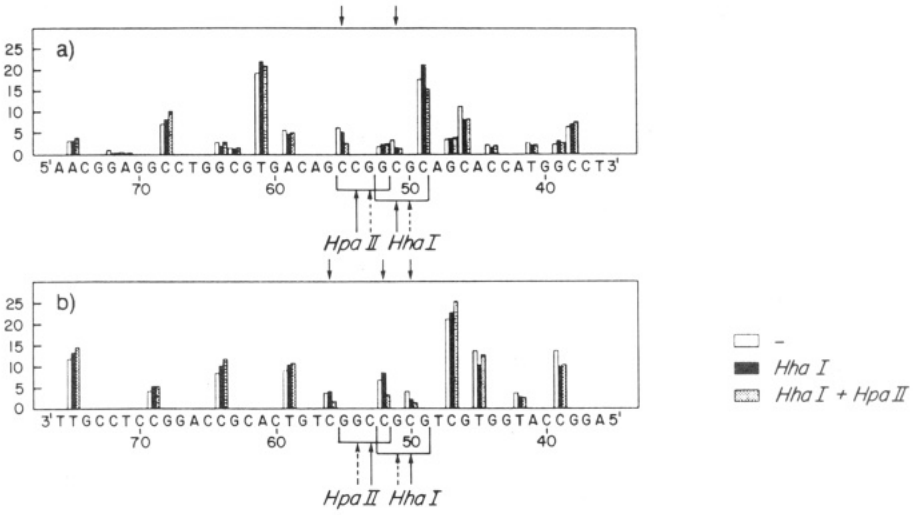


Figure 3. Analysis of the effects of DNA methylation on BLM A_2-mediated cleavage of a 113 base pair long DNA duplex.

than that of the starting material. These findings are consistent with the partitioning of an intermediate of type iv (Scheme V), which has also been implicated in other aromatic hydroxylations.

To date, studies of the oxidative transformation of small molecules by bleomycin have contributed to our understanding of the stoichiometry and chemical reactivity of the activated species involved. The facility of analysis of low-molecular-weight products of BLM-mediated oxidation reactions, and the ready availability of isotopically labeled substrates, suggests that further studies of this type may permit a more detailed analysis of the structure and chemistry of activated bleomycin.

Selectivity of Bleomycin Action

One aspect of the behavior of bleomycin that is of considerable interest is the source of its selectivity toward tumor cells. One possibility was noted by Tanaka[46] who summarized data showing that individual bleomycins distributed to individual organs to very different extents. Another possibility, that has been studied extensively, involves catabolism of bleomycin to an inactive form by bleomycin hydrolase. This enzyme activity varies widely from one tissue to another and may permit selective bleomycin action in those tissues where the enzyme activity is relatively deficient.[47]

In recent years, considerable effort has been expended in an effort to understand the way in which DNA methylation affects DNA function. It has been shown that 5-methylcytidine is the most prevalent methylated nucleotide and that methylation occurs via the action of restriction methylases.[48] DNA methylation has been shown to affect gene expression, e.g., by the inactivation of genes via promoter region methylation.[48,49] Importantly, increasing evidence suggests that specific genetic regions in tumor cells are undermethylated.[50] This finding provides a possible mechanism for selectivity toward tumor cells by DNA damaging agents, i.e., by selective recognition of DNA methylation patterns.

To determine whether recognition of DNA methylation might represent a possible basis for selectivity of BLM action, we investigated the effect of DNA methylation on the ability of BLM to degrade DNA.[51] DNA duplexes were obtained by restriction endonuclease treatment of intact DNA's. The linear duplexes were ^{32}P-end-labeled and then methylated with the restriction nuclease *Hha*I (recognition sequence: GCGC), or else with *Hha*I and *Hpa*II (recognition sequence: CCGG). The three types of DNA, differing only in extent of methylation, were then employed as substrates for Fe(III)·BLM cleavage and the patterns of cleavage analyzed by polyacrylamide gel electrophoresis. Individual gels were analyzed for cleavage at single positions by densitometry. Shown in Figure 3 is a histogram constructed from the densitometric analysis of a 113 base pair long DNA duplex derived from SV40 DNA. BLM-mediated cleavage of both strands is shown. A total of five BLM cleavage sites on both strands were diminished in intensity following methylation with *Hha*I or with both restriction methylases; all of these were in proximity to the site of methylation.

A detailed analysis of bleomycin-mediated cleavage of a number of unmethylated and methylated DNA's revealed some interesting points. These included the observations that diminution of BLM cleavage could be observed over as many as 14 base pairs in regions that were extensively methylated and that experimental conditions (e.g., high salt) known to stabilize non-B-form DNA structures contributed to lessened sensitivity to bleomycin. It is also of interest that the *Hpa*II site in Figure 3, whose methylation diminished cleavage by BLM, has been shown to regulate gene expression in vivo.[52]

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Metallobleomycins

Although Fe·BLM has been the most extensively studied of the metallobleomycins, bleomycin binds many metal ions, and a number of these are capable of mediating DNA strand scission. These include the cobalt,[53] manganese,[7d,54] vanadyl,[55] and copper[7b,c] complexes. Cu·BLM is of particular interest, because the rather high affinity of Cu(II) for bleomycin suggests that this metallobleomycin is actually the one present in situ after administration[56] and could be the therapeutically relevant form of the drug.

The ability of Cu·BLM's to mediate oxidative transformations has been questioned,[57] in spite of the successful enzymatic[23] and chemical[7c] activation of this species for oxidative transformation of DNA and small molecules. As demonstrated recently by Ehrenfeld et al.,[58] this discrepancy appears to be due to the failure of some workers to allow sufficient time for the reduction of preformed Cu(II)·BLM.

Concluding Remarks

The present Account describes the products of bleomycin-mediated DNA degradation and the chemistry that attends the strand scission event. Bleomycin cleaves DNA in a reaction that is both metal ion and oxygen dependent; the stoichiometry of Fe·BLM activation is suggested to involve $2e^-$ reduction of a ternary complex containing bleomycin, oxygen, and Fe(III).

Strand scission of DNA has been studied at high resolution by the use of a synthetic dodecanucleotide, $d(CGCT_3A_3GCG)$, which contains the preferred cleavage sequence...GC... Cleavage of this species occurs with high efficiency and considerable selectivity at GC. Further, by the use of segments of DNA methylated at specific sites by restriction methylases, it has been shown that Fe·BLM exhibits significantly diminished cleavage of methylated DNA, an observation that may represent an important source of selectivity for bleomycin as an antitumor agent.

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