J_{PNP} = 4.0 Hz, J_{PNP} = 48.8 Hz; this peak became a triplet of doublet of doublets upon proton coupling, J_{POCH} = 20 Hz), 24.5 (1 P, doublet of doublets, J_{PNP} = 9.3 Hz, J_{PNP} = 48.8 Hz; this peak remained virtually unchanged upon proton coupling). ^{1}H NMR ($CDCl_3$ solution): $\delta(PCH_3)$ = 1.71 (3 H, doublet of triplets, J_{PCH} = 16.9 Hz, J_{PNPCH} = 3.3 Hz), δ(–N*H*$CH_2CH_2CH_2$O–) = 3.04 (unresolved multiplet), δ(–NHC*H*$_2$$CH_2CH_2$O–) = 3.30 (resolved multiplet), δ-(NHCH_2C*H*$_2$$CH_2$O–) = 1.85 (unresolved multiplet), δ(–NHCH_2CH_2C*H*$_2$O–) = 4.45 (unresolved multiplet). Infrared spectrum (cm^{-1}, KBr disk): 3310 (m, ν_{NH}), 2970 (m), 2940 (m), 2890 (w, ν_{CH}), 1190 (vs, ν_{PN}). Correct microanalytical data were also obtained.[10]

The assignment of an ansa structure to compound III comes from a careful inspection of the spectroscopic data for this compound, as well as a comparison of this data to that of known spiro derivatives.[1,2,7]

First, the PC*H*$_3$ resonance for compound III comes at δ 1.71. The region between δ 1.8–1.6 is where the methyl resonances for all geminally disubstituted compounds are found;[11] however, the resonance for a P(Cl)C*H*$_3$ group is found at δ 2.1.[9,11] From these facts alone it is clear that the nitrogen atom from the propanolamine residue is linked geminally to the methyl group. The proton NMR data for the propanolamine group is listed above, along with the assignments. The couplings for each peak are not informative. However, this is to be expected from a compound having an ansa type structure, where each and every proton is in a unique magnetic environment and thus would show an extremely complex set of resonances.

The ^{31}P NMR data can be interpreted in the following manner. The resonance at 31.2 ppm is assigned to the P-(CH_3)(NHR) group. This is the furthest downfield resonance, in the general area for an alkylated phosphorus,[11] although it is upfield shifted from a P(Cl)(CH_3) resonance.[9,11] This resonance is the most severely broadened upon proton coupling, which indicates the close proximity of both the methyl and the $NHCH_2$– protons. The resonance at 29.3 ppm is assigned to the P(Cl)O group, whereas the peak at 24.5 ppm is assigned to the $P(Cl)_2$ group. The argument is as follows: although the two resonances lie close together, they can be assigned simply on the basis of the proton-coupled spectrum. The resonance assigned to the $P(Cl)_2$ group is virtually unchanged, whereas the P(Cl)O resonance is split into a triplet, indicating the proximity of two protons. These coupling patterns can occur only if compound III has the ansa structure. A spiro compound would show a quartet for the P(Cl)(CH_3) resonance and a multiplet for the spiro phosphorus upon proton coupling; this is not the case.

The extension of this synthetic route to other ansa type phosphazene derivatives, together with their detailed structure determinations, is currently under investigation in our laboratory.

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Department of Chemistry
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

Paul J. Harris*
Kenneth B. Williams

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Activation of Oxygen and Mediation of DNA Degradation by Manganese-Bleomycin

Sir:

The bleomycins are a group of glycopeptide antibiotics employed clinically for the treatment of squamous cell carcinomas and Hodgkin's disease.[1] These agents appear to mediate their therapeutic effects at the level of DNA strand scission,[2] a transformation that requires a source of oxygen[3] and a metal cation.[3,4] While the metal ion(s) responsible for the action of bleomycin in situ are unknown, both the ferrous[4] and cuprous[5] complexes of bleomycin mediate DNA degradation in the presence of dioxygen. Recently, it has been shown that in the presence of oxygen surrogates such as iodosobenzene the corresponding ferric and cupric complexes will also effect the conversion of supercoiled covalently closed circular (form I) DNA to form II (linear duplex) DNA.[5b,6] In addition to the copper and iron complexes of bleomycin, a Co(III)–bleomycin complex has been reported to form an active complex capable of cleaving ϕX174 cccDNA in the presence of light.[7]

Studies performed in this laboratory[5,6,8] have probed the mechanistic similarities between bleomycin and cytochrome P-450.[9] Bleomycin and the porphyrin moiety of cytochrome P-450 both coordinate metal ions, are activated anaerobically by iodosobenzene or aerobically by dioxygen, and mediate the stereospecific epoxidation of olefinic compounds.[10,11] Further, both species form ferrous complexes that bind CO with attendant spectral changes, and it has recently been shown that at least two metallobleomycins can be activated by NADPH–cytochrome P-450 reductase.[8b] In an effort to extend further the analogy between cytochrome P-450 and bleomycin, additional metal ions (e.g., Mn[11,12]) known to form redox-active porphyrin complexes were tested for their ability to bind to bleomycin and effect oxygen-dependent transformation of olefinic substrates and DNA strand scission. Herein we report that Mn–bleomycin can mediate these oxidative transformations.

Figure 1 illustrates the HPLC elution profile of products formed from *cis*-stilbene[13] in the presence of Mn(III)–bleo-

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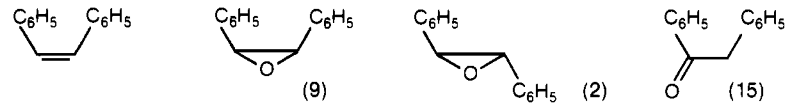
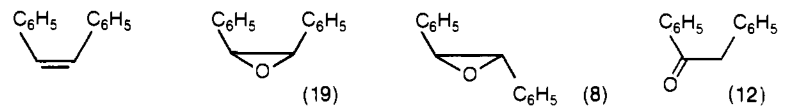
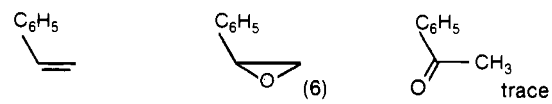
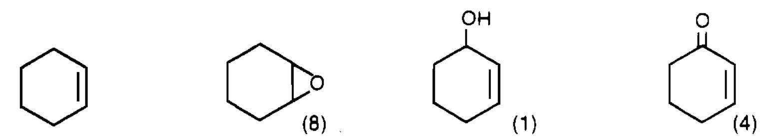
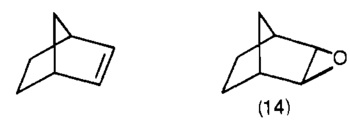
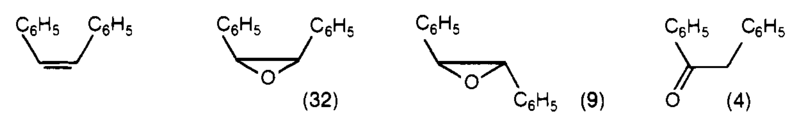
Table I. Olefin Oxidation by Mn(III)-Bleomycin and $Mn^{III}(TPP)Cl^{a,b}$

Oxidant	Substrate / Products (Yield)		
Mn (III) • BLM + C_6H_5IO		C_6H_5 CHO (<1)	a
Mn (III) TPP (Cl) + C_6H_5IO			a
Mn (III) • BLM + C_6H_5IO			b
Mn (III) • BLM + C_6H_5IO			c
Mn (III) • BLM + C_6H_5IO			c
Mn (III) • BLM + O_2 + ascorbate			a
Mn (III) • BLM + O_2 + ascorbate			a

[a] Yields were determined by (a) HPLC analysis, (b) isolation, and (c) gas chromatography–mass spectrometry. Small amounts of some of the products were observed in the presence of Mn(III) + C_6H_5IO. For iodosobenzene-mediated transformations, yields are based on the amounts of added C_6H_5IO. For entries 6 and 7, yields are based on the amount of bleomycin. [b] Although not detectable by HPLC analysis under the conditions employed here, the presence of O-methylhydrobenzoin (~3% yield) was also established by silica gel TLC (7% ethyl acetate in hexane).

mycin ($Mn^{III}BLM$) and iodosobenzene. In addition to some unreacted *cis*-stilbene not removed during workup, the products included *cis*- and *trans*-stilbene oxides, benzaldehyde, deoxybenzoin, and O-methylhydrobenzoin. The yields of products are summarized in Table I, which also illustrates the remarkably similar pattern of products obtained with (tetraphenylporphinato)manganese(III) chloride when the latter was employed for *cis*-stilbene oxidation using the same protocol described for $Mn^{III}BLM$.[13] Also shown in the table are the results of oxidation of styrene, cyclohexene, and norbornene with Mn(III)-bleomycin + C_6H_5IO, as well as the products formed from *cis*-stilbene and styrene following aerobic activation of Mn(III)-bleomycin in the presence of ascorbate.[14] As can be appreciated from the table, the results obtained for Mn(III)-bleomycin are quite similar to those obtained by other workers with (tetraphenylporphinato)manganese(III) derivatives[11a,14] and also for other metalloporphyrins[10] and metallobleomycins.[6,8a,c]

In common with the other active metallobleomycins,[4-7] when Mn(II)-bleomycin B_2 was incubated in the presence of SV40 form I DNA under aerobic conditions, DNA strand scission occurred, producing relaxed circular and linear duplex DNA (Figure 2) in a reaction that was both Mn(II) and bleomycin dependent.[15] No DNA strand scission was observed when O_2 was excluded from the system. On the basis of results obtained previously for Fe-bleomycin and Cu-bleomycin,[5b,6] it was also of interest to determine whether Mn(III)-bleomycin + C_6H_5IO would also effect DNA strand scission. As noted above for aerobically activated Mn-bleomycin, the activated species derived from Mn(III)-bleomycin B_2 + C_6H_5IO produced single- and double-strand nicks in SV40 form I DNA (data not shown).

Degradation of DNA by aerobically activated Fe-bleomycin (but not Cu-bleomycin[5b]) has been noted to result in concomitant formation of base propenal,[16] the formation of which can be quantitated following acid-catalyzed conversion to malondialdehyde and free base.[16a] DNA degradations me-

(13) In a typical experiment, 5 μmol of $Mn(OAc)_3$ and 5 μmol of bleomycin were dissolved in 4 mL of 5% aqueous CH_3OH under N_2 and admixed with *cis*-stilbene (100 mg, 0.55 mmol) in 2 mL of CH_3OH. Iodosobenzene (30 mg, 0.14 mmol) in 1.2 mL of CH_3OH was then added dropwise over a period of 15 min. The reaction was stirred at 25 °C for 1 h, and the crude reaction was concentrated to a small volume and applied to a preparative silica gel TLC plate (Merck, 0.25 mm). Development of the plate (7% ethyl acetate in hexane) permitted removal of excess substrate and iodobenzene; the mixture of products remaining was analyzed by HPLC on a 25-cm analytical Rainin Microsorb (5 μm) column (elution with 9:1 cyclohexane–chloroform at a flow rate of 2.0 mL/min).

(14) The procedure employed for aerobic activation was analogous to that employed previously for (tetraphenylporphinato)manganese(III) chloride. See Mansuy, D.; Fontecave, M.; Bartoli, J. F. *J. Chem. Soc., Chem. Commun.* **1983**, 253.

(15) The presence of forms II and III SV40 DNA in lane 5 are presumably due to adventitious metal binding by bleomycin B_2.

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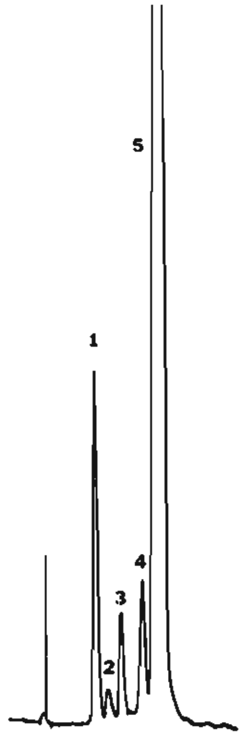

Figure 1. HPLC profiles of products formed from *cis*-stilbene following treatment with Mn(III)–bleomycin + C_6H_5IO. Peaks 1–5 correspond to unreacted *cis*-stilbene, *trans*-stilbene oxide, *cis*-stilbene oxide, benzaldehyde, and deoxybenzoin, respectively. The retention times of 1–5 were 2.4, 2.8, 3.5, 4.5, and 5.0 min, respectively. The response factors of individual products (UV detection 254 nm) differed substantially.

Figure 2. SV40 DNA strand scission by Mn(II)–bleomycin + O_2. Individual reaction mixtures contained, in addition to 50 mM sodium cacodylate buffer, pH 7.0, and 500 ng of SV40 form I DNA, the following: (lane 1) 25 μM bleomycin B_2 and 125 μM $Mn^{II}SO_4$; (lane 2) 10 μM bleomycin B_2 and 50 μM $Mn^{II}SO_4$; (lane 3) 1 μM bleomycin B_2 and 5 μM $Mn^{II}SO_4$; (lane 4) only DNA and buffer; (lane 5) 25 μM bleomycin B_2; (lane 6) 1 μM bleomycin B_2 and 5 μM $Fe^{II}(NH_4)_2(SO_4)_2$; (lane 7) 125 μM $Mn^{II}SO_4$. Reactions were initiated by the addition of bleomycin; the reaction mixtures were incubated at 25 °C for 30 min prior to analysis on a 1.4% agarose gel.

diated by Mn(II)–bleomycin + O_2 and by Mn(III)–bleomycin + C_6H_5IO produced no detectable malondialdehyde.[17]

The finding that Mn–bleomycin can mediate oxygen transfer to olefinic substrates and oxidative DNA strand scission in analogy with Fe–bleomycin and Cu–bleomycin is particularly important in that the corresponding metalloporphyrins have been studied in some detail[11,12,14] as analogues of cytochrome P-450. That three different metallobleomycins produced products from several olefinic substrates very similar to those observed with metalloporphyrins that are analogues of cytochrome P-450 strengthens the correlation between the two and suggests strongly that bleomycin can function as a monooxygenase.

As is evident from Figure 2 MnBLM was about 10-fold less active than FeBLM under the experimental conditions employed. This parallels the situation observed for the (TPP)-$Fe^{V}O$ complex, which has an oxidation potential approximately 0.3 V greater than that of the corresponding manganese–porphyrin complex.[18] On this basis, it would be expected that MnBLM might be a more selective reagent than FeBLM; the possibility that MnBLM might react only with a subset of those DNA sites modified by FeBLM seems worthy of investigation and might provide important insights into the design of more selective bleomycin congeners.

Acknowledgment. This work was supported by Research Grant CA-29235, awarded by the National Cancer Institute, Department of Health and Human Services.

(17) In addition to contributing to the characterization of Mn(II)–bleomycin, this experiment demonstrated that the activity of this metallobleomycin could not be due to contaminating Fe, as the latter is known to produce malondialdehyde (base propenal) concomitant with DNA cleavage. That the observed activity in DNA strand scission cannot be due to contaminating Cu may be appreciated from the fact that Cu(I)–bleomycin + O_2 does not cleave DNA in the absence of agents such as dithiothreitol.

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(19) To whom correspondence should be addressed at the Department of Chemistry.

Departments of Chemistry and Biology
University of Virginia
Charlottesville, Virginia 22901

Guy M. Ehrenfeld
Natesan Murugesan
Sidney M. Hecht*[19]

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Relation between the Electronic Structure and the Condensation of Metal Clusters in M_5X_4 Compounds

Sir:

Many transition-metal compounds have an octahedral cluster of transition-metal atoms as their basic unit. The clusters may be isolated, as in Mo_6S_8, or condensed at their corners, edges, or faces, depending on the metal to nonmetal ratio or/and the valence electron concentration (VEC) per metal atom.[1] In the family of M_5X_4 (M = Nb, Ta, V, Ti, Mo; X = S, Se, Te, As, Sb) compounds they condense at opposite corners to form infinite chains, with VEC in the range 2.4–3.6 for all known materials.[1] Why compounds only in this range of VEC crystallize in this structure is not clear, though Nohl et al.[2] have correlated the stability of these compounds with the occurrence of a band gap at the Fermi energy E_F by analogy with the Peierls instability argument. In this communication we discussed the matter further from the heat ΔH of condensation of clusters computed for two systems: (i) a model regular octahedral chain of metal atoms and (ii) a chain with the geometry of Nb_5Te_4, where the octahedra are somewhat squashed as in a bcc substructure.[1] We relate ΔH

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