The dehydrogenation activity of Pd_n^+ exceeds that of Ni_n^+; e.g., Ni_4^+ removes only $2H_2$ whereas Pd_4^+ removes $3H_2$. It appears that Pt_n^+ is even more active, and already Pt_2^+ removes $3H_2$.

In order to ascertain whether the reactivity differences are intrinsic or due to different electronic or vibrational internal energies ("temperature")[4b,13] of the clusters, we have attempted to cool them by collisions with an inert room-temperature gas. The clusters were sputtered into a 1-cm-long pressure cell filled with argon ($>10^{-1}$ torr) and extracted with a weak electric field (2–5 V/cm) through a 1-mm orifice. It is estimated that the ions undergo between 10 and 100 collisions with argon atoms. In the absence of argon, virtually no ions are detected, confirming that collisions are indeed occurring and are necessary for the ions to reach the vicinity of the cell exit.

The results obtained with collision-cooled cluster ions are compared to those from the simple sputtering experiments in Table II. Directly sputtered ions are much more likely to undergo dissociative collisions leading to Ni_{n-1}^+ and thus clearly have higher internal energies. They are also more likely to cause C–C bond breaking in butane while the ions cooled by argon gas collisions are more likely to dehydrogenate it. The probability of an exoergic bimolecular reaction in the absence of an activation barrier increases as the total energy available to the reacting pair decreases.[14] The opposite is true for endoergic reaction coordinates. We find that dehydrogenation by the Ni_n^+ clusters follows the behavior expected of exoergic reactions with no activation barrier. Cracking, on the other hand, appears to be endoergic, becoming more probable as the energy of the system increases.

We conclude that the trends in dehydrogenation activity displayed in Table I reflect the intrinsic reactivities of the cluster ions, while the variation in their tendency to break C–C bonds may be at least in part due to differences in their internal energy.

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New Lumibleomycin-Containing Thiazolylisothiazole Ring

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In contrast to the recognition of AT-rich sites in the minor groove of B DNA by small molecules such as netropsin and distamycin,[1] GC recognition elements have not been well understood. Antitumor antibiotic bleomycin preferentially attacks guanine–pyrimidine (5′ → 3′) sequences, in particular GC sites.[2] The bithiazole moiety of bleomycin has been supposed to play a key role in its sequence-specific interaction with DNA.[3] However, there is much current controversy on the nature of the binding mode of the bithiazole group to DNA.[3-7] In this respect, a specific

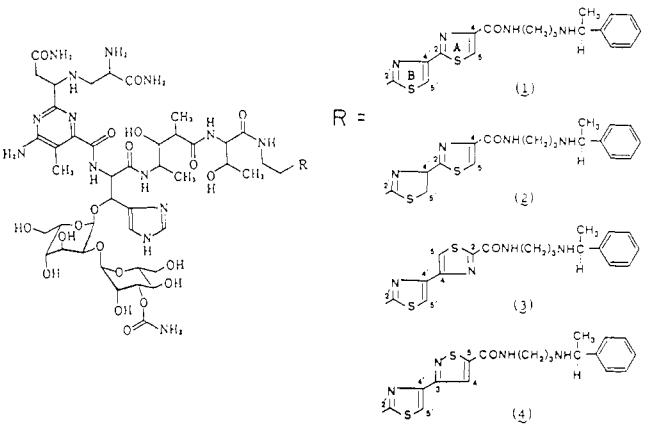

Figure 1. Chemical structures of original peplomycin (PEM, **1**), phleomycin (**2**), photoPEM (**3**), and lumiPEM (**4**).

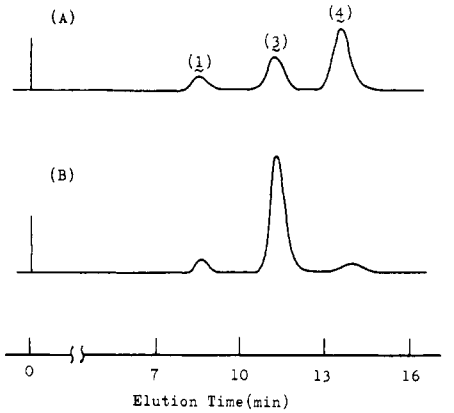

Figure 2. HPLC analyses of UV-irradiated 5:1 Cu(II)–PEM(A) and 1:1 Cu(II)–PEM(B) systems.

modification of the bithiazole group without alteration of the other part is extremely useful. Our previous work demonstrated that changing of the 2,4′-bithiazole group to 4,4′-bithiazole did not alter the sequence specificity in DNA cleavage.[8] We now wish to report a novel bleomycin, lumibleomycin, bearing a new ring system of thiazolylisothiazole as the DNA binding site. The chemical structures of bleomycin antibiotics used in this study are given in Figure 1.

In our previous paper,[8] light irradiation of a 1:1 peplomycin (PEM, **1**)–Cu(II) complex system resulted in an isomerization of the 2,4′-bithiazole ring to 4,4′-bithiazole to produce photoPEM (**3**) preferentially, together with a small amount of byproduct. As clearly shown in Figure 2, however, the present irradiation (302-nm light) of a 1:5 PEM–Cu(II) system dramatically enhanced the formation of this minor product (retention time, 13.4 min).[9] Thus the new phototransformed bleomycin, termed "lumiPEM", was isolated by preparative HPLC in 30% yield, and the structure was assigned as **4** on the basis of spectral data. Particularly useful for the structural assignment is the comparison of the spectral data with those of the model compound methyl

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(9) A solution containing PEM (5×10^{-5} M) and $CuSO_4$ (2.5×10^{-4} M) in 10 mM Tris-HCl buffer (pH 7.5, 5 mL) was irradiated with an ultraviolet 60-W transilluminator through a Pyrex tube for 15 min. After evaporation of the reaction solution, the residue was subjected to HPLC, using a Nova-PAK $_4C_{18}$ ODS column, and eluted with the solvent (1% aqueous CH_3COONH_4/CH_3CN, 85:15; flow rate, 2.5 mL/min).

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Table I. Representative ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Data for Heteroaromatic Rings of lumiPEM and Methyl 3-[4-(2-Methylthiazolyl)]isothiazole-5-carboxylate (Thiazolylisothiazole)[a]

	lumiPEM (**4**)		thiazolyliso-thiazole	
	^{13}C, δ^b	^{1}H, δ^b	^{13}C, δ^c	^{1}H, δ^c
isothiazole				
C-3	162.87		163.30	
C-4	123.92	8.27	126.38	8.25
C-5	159.53		157.08	
thiazole				
C-2′	166.88		166.48	
C-4′	151.52		149.95	
C-5′	119.69	8.05	117.26	7.76
pyrimidine				
C-2	165.09			
C-4	164.16			
C-5	113.99			
C-6	152.90			
imidazole				
C-2	137.80	7.90		
C-4	136.95	7.30		
C-5	119.25			

[a] ^{13}C chemical shifts were measured relative to the central peak of methanol-d_4 or chloroform-d_1 and converted to the tetramethylsilane scale (δ_{CD_3OD} 49.3 and δ_{CDCl_3} 76.9), and ^{1}H chemical shifts were given with respect to tetramethylsilane. [b] In CD_3OD. [c] In $CDCl_3$.

3-[4-(2-methylthiazolyl)]isothiazole-5-carboxylate,[10] which was obtained as minor product in light irradiation of methyl 2′-methyl-2,4′-bithiazole-4-carboxylate, a model compound of the bithiazole group of **1**. Table I lists the selected ^{1}H and ^{13}C NMR data of **4** in comparison with those of methyl 3-[4-(2-methylthiazolyl)]isothiazole-5-carboxylate. The chemical shifts and the multiplicities in the ^{1}H and ^{13}C signals of the heteroaromatic portion of **4** correspond well to those of the thiazolylisothiazole system of the model compound. Furhter, both lumiPEM (**4**) and PEM (**1**) showed the same numbers of proton-decoupled ^{13}C signals, and all the ^{1}H signals of **4** were correlated to the respective ^{1}H peaks of **1** except for their aromatic protons. The UV absorption and fluorescence emission spectra of **4** and the model thiazolylisothiazole also were similar: the former (in H_2O), λ_{max} 295 nm (log ϵ 3.85) and E_{ems}^{max} 420 nm (strong, excitation at 290 nm) and, the latter (in CH_3CN), λ_{max} 304 nm (log ϵ 3.66) and E_{ems}^{max} 416 nm (strong, excitation at 290 nm).[11] All the available data for lumiPEM(**4**) are fully consistent with the assigned structure.

Figure 3 shows the nucleotide sequence specificity in cleavage of a 301-base-pair pBR322 DNA fragment by the Fe(II)–O_2–reductant and the Co(III)–UV light systems of lumiPEM (**4**), in comparison with those of PEM (**1**), phleomycin (**2**), and photoPEM (**3**). LumiPEM (**4**) cleaved DNA preferentially at GC (5′ → 3′) and GT (5′ → 3′) sequences in both the metal complex systems. The sequence specificities observed for these four bleomycin derivatives (**1**–**4**) each bearing a different heterocyclic binding unit are considerably similar to each other in both the reaction systems. However, some differences are also apparent on closer examination. In the case of lumiPEM–iron complex system, the cleavages at two 5′-G*A*-3′ sites (base numbers 1992 and 1981), two 5′-T*T*-3′ sites (1988 and 1963), and one 5′-A*A*-3′ site (1976) were reduced in comparison with those of the original PEM system. Similar phenomena were also observed in the corresponding cobalt complex systems. The gel pattern also seems to suggest that some reorientation in the DNA site occurs with modification of the bithiazole. The results indicate that the bithiazole moiety of bleomycin participates in, but does not determine wholly, the site recognized along the strand. Perhaps

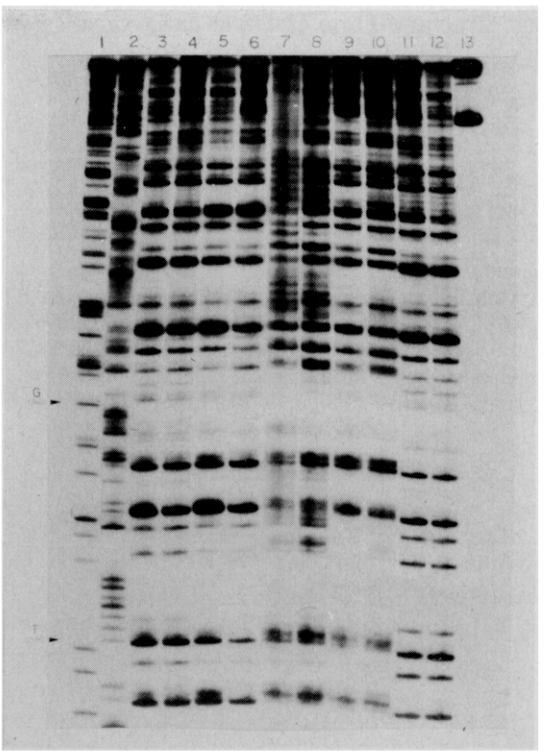

Figure 3. Autoradiogram of sequencing gel showing the cleavage of a 301-base-pair DNA fragment by iron and cobalt complexes of original PEM (**1**), phleomycin (**2**), photoPEM (3), and lumiPEM (**4**). The reaction samples contained 5′-end ^{32}P labeled DNA fragment, 1 μg of sonicated calf thymus DNA, 20 mM Tris-HCl buffer (pH 7.5), and the following additions: lane 3, 5 μM Fe(II)–**1** plus 1 mM 2-mercaptoethanol (2-ME); lane 4, 5 μM Fe(II)–**3** plus 1 mM 2-ME; lane 5, 5 μM Fe(II)–**2** plus 1 mM 2-ME; lane 6, 5 μM Fe(II)–**4** plus 1 mM 2-ME; lane 7, 10 μM Co(III)–**1**; lane 8, 10 μM Co(III)–**3**; lane 9, 10 μM Co(III)–**2**; lane 10, 10 μM Co(III)–**4**; lane 11, 5 μM Fe(II)–bleomycin A_2 (BLM) plus 1 mM 2-ME; lane 12, 5 μM Fe(II)–photoBLM plus 1 mM 2-ME; lane 13, none. The samples of the lanes 3–6 and 11,12 were incubated at 37 °C for 7 min, and then the samples of the lanes 7–10 were irradiated with UV light at 15 °C for 25 min. Lanes 1 and 2 exhibit the Maxam–Gilbert's chemical reactions for G and C + T, respectively. The number corresponds to the nucleotide number in the pBR322 entire sequence map.

rather than serving as a recognition element, the bithiazole then may be an anchor. In addition, photoPEM (**3**) and lumiPEM (**4**) have the ring-A sulfur atom in a different orientation from that of PEM (**1**). Nevertheless, they revealed a similar sequence-specific cleavage, suggesting that the ring-A sulfur does not participate in the sequence-selective binding. Another interesting feature is that these four derivatives have a common N–C–C–N unit across the two rings as indicated in the formulas. It is therefore not unreasonable to assume that these two ring nitrogens may participate in the sequence-specific binding of these bleomycin derivatives, even though a so-called "partial intercalation" of the heterocyclic binding units of **1**–**4** contributes significantly to the sequence-specific binding.[12]

In conclusion, the controlled phototransformation of the bithiazole group of bleomycin antibiotics leading to photobleomycin or lumibleomycin provides a new clue for the mechanistic investigation on the sequence recognition of bleomycins.

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