VOLUME 117, NUMBER 36
SEPTEMBER 13, 1995
© Copyright 1995 by the
American Chemical Society

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Guanosine-Specific DNA Damage by a Co(II)•Bithiazole Complex

Stefanie A. Kane, Hideaki Sasaki, and Sidney M. Hecht*

Contribution from the Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901

Received April 7, 1995[®]

Abstract: A bithiazole derivative structurally related to the bithiazole moiety of bleomycin (BLM) A_2 was prepared. This derivative contained a 2-(1,3-diaminopropyl) substituent, rather than the 2-(2-aminoethyl) substituent normally present in BLM, in order to facilitate metal coordination by the bithiazole moiety itself. In the presence of Co^{2+}, the modified bithiazole mediated the production of alkali labile lesions on double-stranded DNA. Following treatment with alkali, guanosine-specific DNA strand scission was observed. DNA degradation by this Co(II)•bithiazole complex was not light dependent but did require molecular oxygen. DNA cleavage studies employing scavengers of activated oxygen species suggested that the observed DNA damage did not result from diffusible, activated forms of oxygen. Moreover, ESR spectroscopy utilizing a spin trapping reagent demonstrated conclusively that the Co(II)•bithiazole complex did not produce diffusible oxygen radicals. Absorption spectroscopy with a thiazole analog of the modified bithiazole suggested that, in the presence of oxygen, a O_2•Co•thiazole complex was formed. These mechanistic studies suggested that the (oxygenated) Co(II)•bithiazole complex mediated the oxidative alteration or liberation of the guanine base, producing an alkali labile site. Further, the guanosine specificity appeared to derive from preferential *reactivity* at guanosine sites, as opposed to a guanosine binding selectivity of the bithiazole. The oxidative degradation of G residues in DNA appears to proceed by an inner sphere mechanism.

The bleomycins (BLMs, Figure 1) are a group of structurally related antitumor antibiotics that are used clinically for the treatment of certain forms of cancer.[1] The therapeutic effects of BLM appear to derive from the ability of the drug to mediate oxidative DNA strand scission[2] and possibly also from RNA degradation.[3] DNA degradation by Fe•BLM is sequence selective, resulting in the oxidative destruction of the pyrimidine nucleoside within $^{5'}$-GC-$^{3'}$ and $^{5'}$-GT-$^{3'}$ sequences.[4] The source of sequence selective DNA recognition by BLM is not entirely understood, although recent studies have provided some insight.[3d,5] The interaction of BLM with DNA is a two-step process that involves initial DNA binding followed by C-4′ H abstraction from the deoxyribose moiety that undergoes oxidative cleavage. The bithiazole moiety of BLM has been shown to participate in DNA binding.[6] Moreover, a BLM analog lacking the bithiazole has been shown to be incapable of mediating DNA

[®] Abstract published in *Advance ACS Abstracts*, August 15, 1995.
(1) Sikic, B. I., Rozencweig, M., Carter, S. K., Eds. *Bleomycin Chemotherapy;* Academic Press: Orlando, FL, 1985.
(2) (a) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383. (b) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107. (c) Natrajan, A.; Hecht, S. M. In *Molecular Aspects of Anticancer Drug-DNA Interactions*; Neidle, S., Waring, M., Eds.; MacMillan: London, 1980; pp 197−242. (d) Kane, S. A.; Hecht, S. M. *Prog. Nucleic Acid Res. Mol. Biol.* **1994**, *49*, 313.
(3) (a) Carter, B. J.; de Vroom, E.; Long, E. C.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9373. (b) Holmes, C. E.; Carter, B. J.; Hecht, S. M. *Biochemistry* **1993**, *32*, 4293. (c) Morgan, M. A.; Hecht, S. M. *Biochemistry* **1994**, *33*, 10286. (d) Hecht, S. M. *Bioconjugate Chem.* **1994**, *5*, 513.
(4) (a) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608. (b) Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983. (c) Mirabelli, C. K.; Huang, C.-H.; Crooke, S. T. *Biochemistry* **1983**, *22*, 300.
(5) (a) Wu, W.; Vanderwall, D. E.; Stubbe, J.; Kozarich, J. W.; Turner, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 10843. (b) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1994**, *116*, 10851.

0002-7863/95/1517-9107$09.00/0 © 1995 American Chemical Society

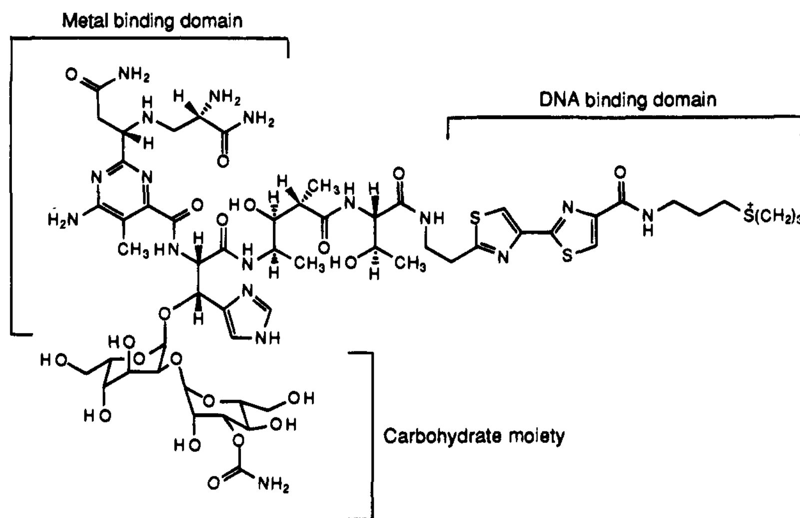


Figure 1. Bleomycin A_2.

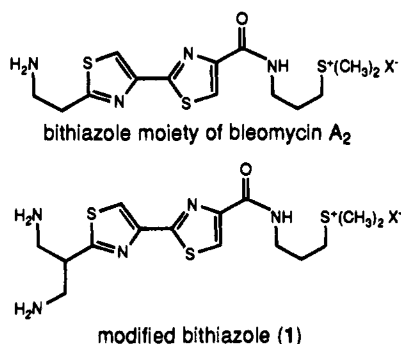


Figure 2. Modified bithiazole employed for Co ligation and DNA cleavage.

degradation, in spite of the fact that it bound Fe and mediated the oxidative transformation of *cis*-stilbene.[6d,7] Recently, two BLM analogs, each of which contains one of the two thiazole rings present in BLM, were shown to mediate the oxidative transformation of low molecular weight organic substrates.[8] However, these analogs cleaved DNA only at high concentrations and in a sequence neutral fashion. The lack of sequence selectivity appeared to derive from poor DNA binding as a result of disruption of the bithiazole ring system. However, oxygen transfer to small molecule substrates does not require initial binding[2a] and was, therefore, readily effected by the monothiazole BLMs.

These studies established that the bithiazole moiety is important for DNA binding by BLM, although the role of the bithiazole in DNA sequence recognition by BLM was still unclear. Several observations have suggested that the N-terminus of BLM not only contributes to DNA affinity[9] but actually may be dominant in determining the sequence selectivity of DNA strand scission.[7,10] Particularly compelling in this regard is a study by Mascharak and co-workers[7] in which a BLM analog (PMAH) lacking a bithiazole moiety was shown to afford the same sequence selectivity of DNA cleavage as BLM itself. Recent investigations employing bithiazole derivatives containing reactive functionalities[11] demonstrated that these species cleaved DNA essentially in a nonspecific fashion, suggesting that the bithiazole moiety itself displays little or no sequence binding selectivity. To further investigate the role of the bithiazole ring system of BLM in the recognition of $^{5'}$-Gpyr-$^{3'}$ sequences, we have prepared a bithiazole derivative (**1**) that is structurally related to that in BLM A_2 but contains an additional aminomethyl group attached to the 2-aminoethyl substituent. It was anticipated that the diamine functionality could serve as a metal ion chelator, thus promoting the formation of a complex capable of mediating DNA strand scission; hence, the sequence affinity of the bithiazole could be evaluated. Interestingly, the activation of bithiazole (**1**) for DNA cleavage could be effected only by Co(II). Toward this end, in an attempt to evaluate the sequence binding preference of the bithiazole, a novel approach for DNA cleavage was identified. Presently, we demonstrate that the Co(II)•bithiazole **1** complex mediates guanosine-specific DNA degradation by a reaction that is dependent on oxygen but which does not involve the participation of diffusible activated oxygen species. Moreover, the G specificity appears to derive from preferential reactivity at G sites, as opposed to a guanosine binding selectivity of the bithiazole.

Results

Synthesis of Bithiazole 1. The synthesis of bithiazole **1** is outlined in Scheme 1. 1,3-Diamino-2-propanol was protected with di-*tert*-butyl dicarbonate to afford Boc derivative **2** in 95% yield. The secondary alcohol was converted to its *p*-toluenesulfonate derivative **3**; the latter was obtained in 80% yield as colorless prisms. Displacement of the tosylate with CN^- was accomplished using NaCN in DMSO to give cyano derivative **4**, which was converted to thioamide **5** via the agency of hydrogen sulfide and triethylamine in ethanol. Thioamide **5**

(6) (a) Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641. (b) Povirk, L. F.; Hogan, M.; Dattagupta, N. *Biochemistry* **1979**, *18*, 96. (c) Kross, J.; Henner, W. D.; Haseltine, W. A.; Rodriguez, L.; Levin, M. D.; Hecht, S. M. *Biochemistry* **1982**, *21*, 3711. (d) Kilkuskie, R. E.; Suguna, H.; Yellin, B.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 260. (e) Fisher, L. M.; Kuroda, R.; Sakai, T. T. *Biochemistry* **1985**, *24*, 3199.

(7) See, however: Guajardo, R. J.; Hudson, S. E.; Brown, S. J.; Mascharak, P. K. *J. Am. Chem. Soc.* **1993**, *115*, 7971.

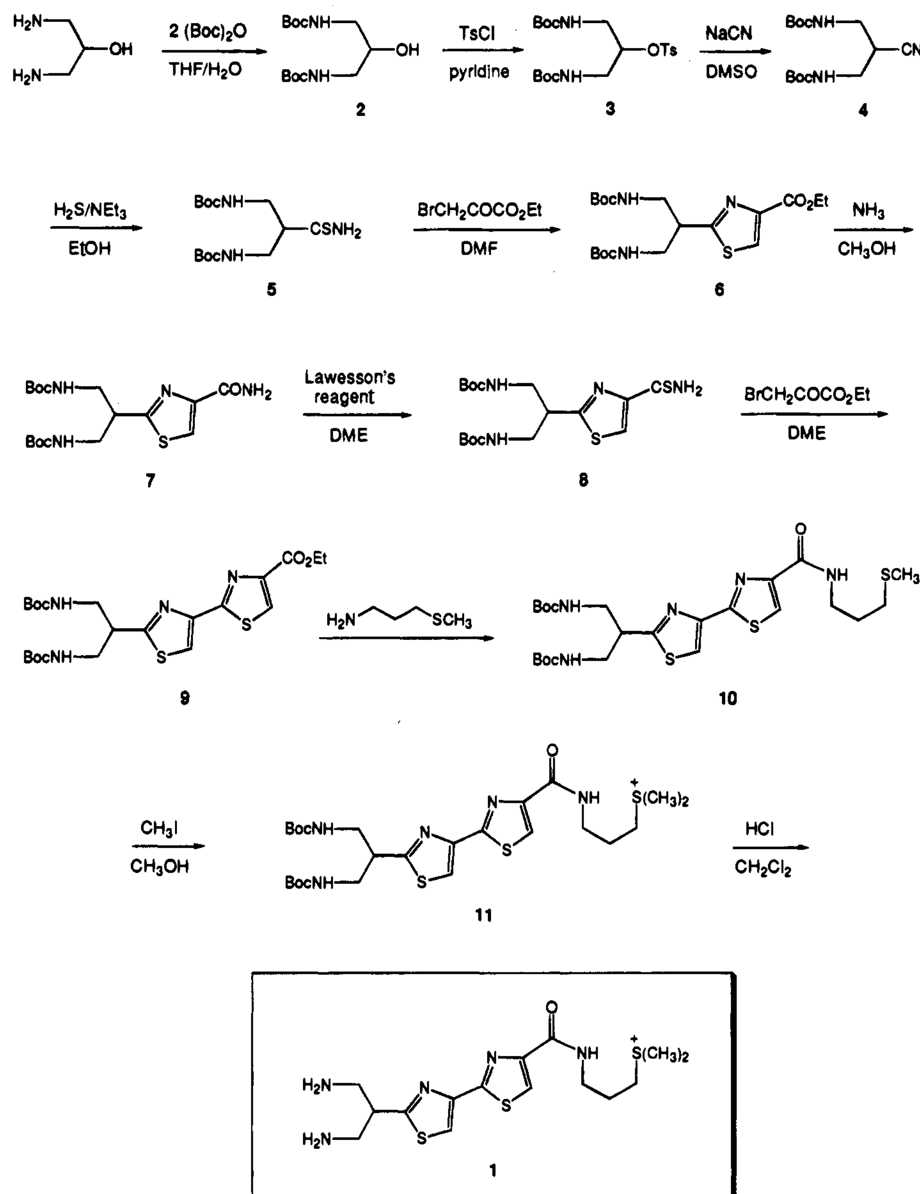
(8) Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278.

(9) Levy, M. J.; Hecht, S. M. *Biochemistry* **1988**, *27*, 2647.

(10) (a) Shipley, J. B.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 25. (b) Sugiyama, 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. (c) Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S.-N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193.

(11) (a) Kane, S. A.; Natrajan, A.; Hecht, S. M. *J. Biol. Chem.* **1994**, *269*, 10899. (b) Quada, J. C., Jr.; Levy, M. J.; Hecht, S. M. *J. Am. Chem. Soc.* **1993**, *115*, 12171. (c) Sasaki, H. *Tetrahedron Lett.* **1994**, *35*, 4401.

Scheme 1. Synthetic Route Used for the Preparation of Bithiazole 1



was condensed with ethyl bromopyruvate in DMF, which effected cyclization[12] to afford thiazole ester 6, which was isolated as colorless needles in 66% yield. The ester was converted to amide 7 using NH_3 in methanol at −78 °C; treatment of the resulting amide with Lawesson's reagent[13] in dimethoxyethane provided thioamide 8 in 96% yield. Condensation of thiazole thioamide 8 with ethyl bromopyruvate afforded bithiazole 9, as well as some deprotected material resulting from loss of the Boc groups and a small amount of putative Boc-protected bithiazole precursor that had undergone cyclization but not dehydration. The deblocked material in this mixture was reprotected using di-*tert*-butyl dicarbonate; following chromatographic separation of constituents of the reaction mixture, dehydrative aromatization of the putative thiazolylthiazoline was accomplished by heating a solution in toluene at reflux for 24 h. Boc-protected bithiazole ester 9 was thus obtained as pale orange microcrystals in 70% total yield from thioamide 8. Condensation of ester 9 with (3-(methylthio)-propyl)amine afforded bithiazole amide 10, which was methylated with methyl iodide in methanol to afford dimethylsulfonium salt 11. The Boc protecting groups were removed using anhydrous hydrogen chloride in dichloromethane to give 1. The final product was purified by chromatography on Dowex-50W (H^+ form); elution with HCl followed by lyophilization of the appropriate fractions afforded the hydrochloride salt of bithiazole 1 as a pale yellow powder. The purity of 1 was confirmed by analytical reversed phase HPLC analysis. The structure was verified by ^1H NMR spectroscopy and high-resolution mass spectrometric analysis.

DNA Degradation by Bithiazole 1. In order to determine whether the interaction of the bithiazole moiety of BLM with DNA confers sequence selectivity, the ability of the bithiazole itself to bind selectively to DNA was evaluated. It was anticipated that, if the diamine substituent of 1 were capable of binding an appropriate metal ion, DNA cleavage might be obtained in the presence of the metal; therefore, the sequence binding selectivity of the bithiazole could be evaluated. DNA cleavage by transition metal complexes is well documented.[2,14] The ability of bithiazole 1 to mediate DNA cleavage was assessed in the presence of various metal ions, as shown in

(12) (a) Zee-Cheng, K.-Y.; Cheng, C. C. *J. Heterocycl. Chem.* **1970**, *7*, 1439. (b) Babadjamian, A.; Gallo, R.; Metzger, J. *J. Heterocycl. Chem.* **1976**, *13*, 1205.

(13) Pederson, B. S.; Scheibye, S.; Clausen, K.; Lawesson, S.-O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 293.

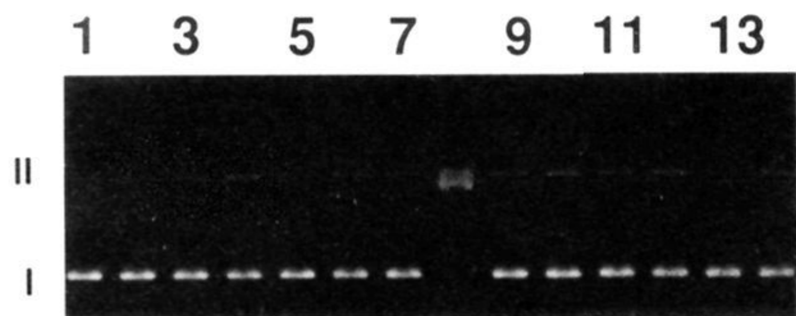


Figure 3. Relaxation of supercoiled pBR322 DNA by bithiazole **1** in the presence of metal ions: lane 1, DNA alone; lane 2, 10 μM **1**; lane 3, 10 μM Fe^{2+}; lane 4, 10 μM each Fe^{2+} and **1**; lane 5, 10 μM Ni^{2+}; lane 6, 10 μM each Ni^{2+} and **1**; lane 7, 10 μM Co^{2+}; lane 8, 10 μM each Co^{2+} and **1**; lane 9, 10 μM Mn^{2+}; lane 10, 10 μM each Mn^{2+} and **1**; lane 11, 10 μM Cu^{2+}; lane 12, 10 μM each Cu^{2+} and **1**; lane 13, 10 μM Zn^{2+}; lane 14, 10 μM each Zn^{2+} and **1**.

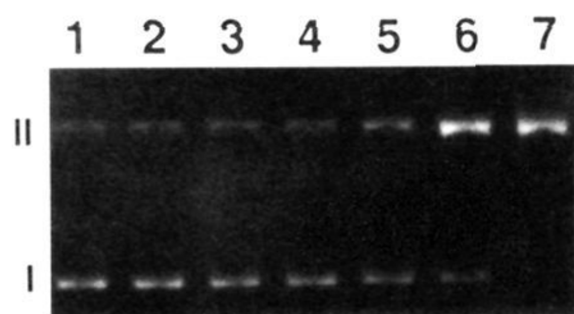


Figure 4. Relaxation of supercoiled pBR322 DNA by Co(II)•bithiazole **1**: lane 1, DNA alone; lane 2, 20 μM **1**; lane 3, 20 μM Co^{2+}; lane 4, 2.5 μM Co(II)•**1**; lane 5, 5 μM Co(II)•**1**; lane 6, 10 μM Co(II)•**1**; lane 7, 20 μM Co(II)•**1**.

Figure 3. In this assay, the metal and ligand concentrations were 10 μM. Interestingly, DNA cleavage by bithiazole **1** was observed only in the presence of Co(II) (lane 8). The other metals tested included Fe(II), Ni(II), Mn(II), Cu(II), and Zn-(II). Additional experiments demonstrated that neither prolonged incubation time nor increased concentration of metal complex enhanced the ability of the other metals to activate **1** for DNA cleavage. The concentration dependence of DNA cleavage by Co(II)•bithiazole is illustrated in Figure 4. That both the ligand and metal were required for DNA cleavage was demonstrated in lanes 2 and 3. Increasing concentrations of the Co(II)•bithiazole **1** complex effected increased DNA damage (lanes 4–7), with complete conversion of the supercoiled (Form I) DNA substrate to nicked (Form II) DNA in 15 min when employed at 20 μM concentration. Only single-strand breaks were observed. Neither increased concentrations of the Co(II) complex nor longer incubation times led to the production of linear Form III DNA.

The ability of the Co(II)•bithiazole **1** complex to mediate strand scission of a linear DNA duplex, as well as analysis of any possible sequence selectivity of cleavage, was investigated by the use of a 158-bp 5′-^{32}P end labeled DNA restriction fragment derived from plasmid pBR322. Degradation of the 5′-end labeled DNA fragment was carried out by incubation with 50 μM Co(II)•bithiazole **1** at 55 °C for 4 h; following precipitation with ethanol, the DNA was treated with 0.2 N NaOH at 90 °C for 10 min. The cleavage products were analyzed by 20% polyacrylamide gel electrophoresis (Figure 5). The DNA damage induced by the Co(II)•bithiazole **1** complex resulted in strand scission at all G residues (lane 11), following base treatment. Cleavage products were observed only after hot alkali treatment (compare lanes 8, 9, and 11). Control experiments demonstrated that these alkali-labile sites were not produced by the ligand or Co(II) separately, or in combination with NaOH (lanes 2–7). Also clear from this figure was the fact that the resulting DNA fragments comigrated exactly with those produced by the Maxam–Gilbert G-specific

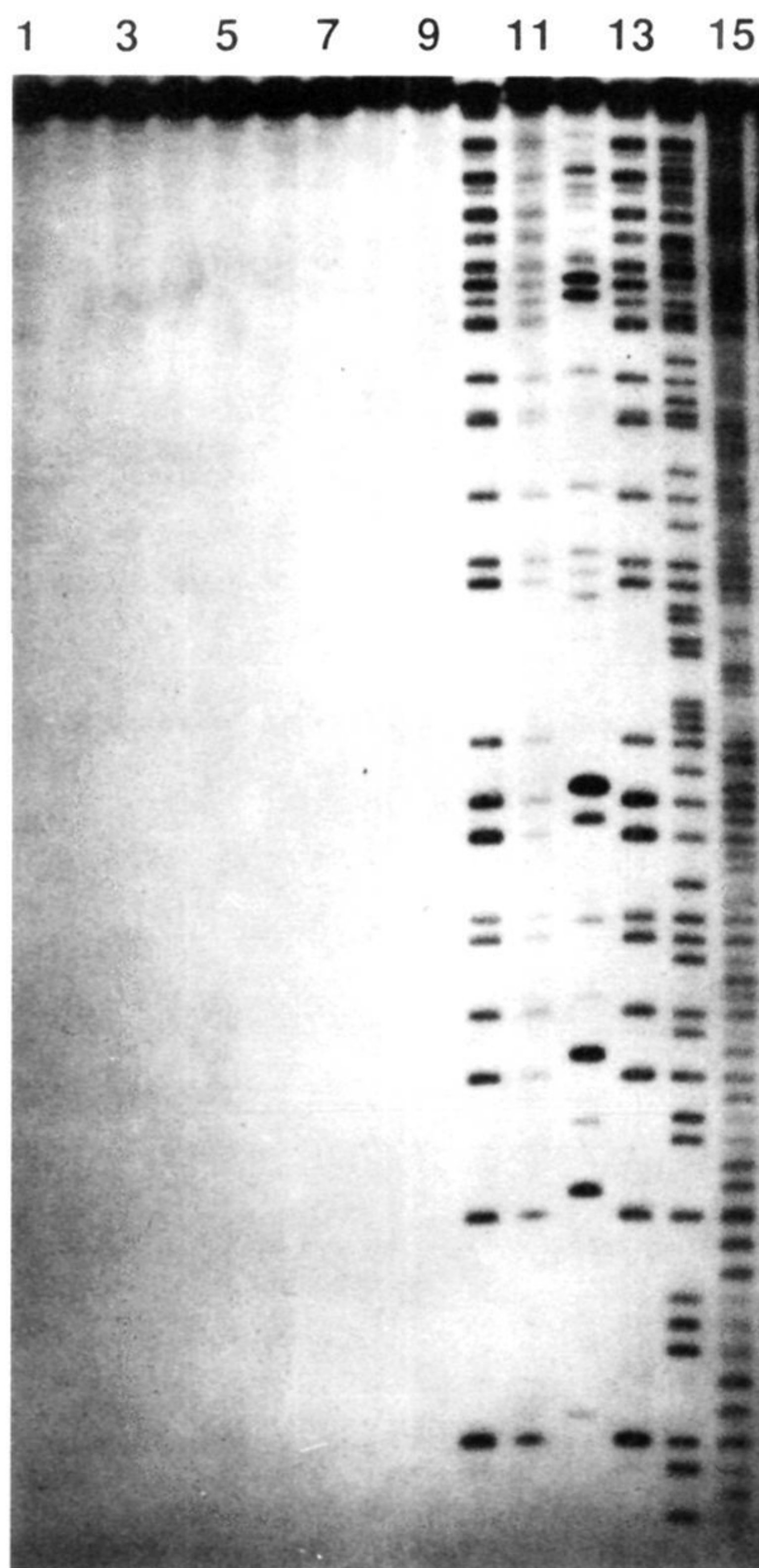


Figure 5. Autoradiogram of a 20% polyacrylamide gel demonstrating cleavage of a 5′-^{32}P end labeled DNA substrate by Co-(II)•bithiazole **1**. Reactions 2–9 contained 50 μM Co^{2+}, 50 μM **1**, or both: lane 1, DNA alone; lane 2, **1**; lane 3, **1**, followed by heating with alkali; lane 4, Co^{2+}; lane 5, Co^{2+}, followed by heating with alkali; lane 6, following incubation with **1**, Co^{2+} was added and the combined solution was heated with alkali; lane 7, following incubation with Co^{2+}, **1** was added and the combined solution was heated with alkali; lane 8, Co(II)•**1**; lane 9, Co(II)•**1**, followed by heating at 90 °C; lane 10, Maxam–Gilbert G lane; lane 11, Co(II)•**1**, followed by heating with alkali; lane 12, 5 μM Fe(II)•BLM A_2; lane 13, G lane; lane 14, G + A lane; lane 15, C lane; lane 16, C + T lane.

(14) For example: (a) Basile, L. A.; Barton, J. K. In *Metal Ions in Biological Systems*; Sigel, H., Sigel, A., Eds.; Marcel Dekker: New York, 1989; Vol. 25, pp 31–103. (b) Hertzberg, R. P.; Dervan, P. B. *Biochemistry* **1984**, *23*, 3934. (c) Barton, J. K. *Science* **1986**, *233*, 727. (d) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180. (e) Van Atta, R. B.; Bernadou, J.; Meunier, B.; Hecht, S. M. *Biochemistry* **1990**, *29*, 4783. (f) Chen, X.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1991**, *113*, 5884. (g) Pitié, M.; Pratviel, G.; Bernadou, J.; Meunier, B. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3967. (h) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303. (i) Cheng, C.-C.; Goll, J. G.; Neyhart, G. A.; Welch, T. W.; Singh, P.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 2970.

(15) (a) Maxam, A. M.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 560. (b) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499. (c) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700.

Figure 6. Autoradiogram of a 20% polyacrylamide gel demonstrating cleavage of a 3′-^{32}P end labeled DNA substrate by Co(II)·bithiazole **1**: lane 1, DNA alone; lane 2, 50 μM Co(II)·**1**; lane 3, 50 μM Co(II)·**1**, followed by heating at 90 °C; lane 4, 50 μM Co(II)·**1**, followed by alkali; lane 5: 50 μM Co(II)·**1**, followed by heating with alkali; lane 6, G lane; lane 7, 5 μM Fe(II)·BLM A_2.

reaction (compare lanes 10 and 11), the latter of which are known to be (oligo)nucleotide 3′-phosphates.[15]

Analysis of the damage induced on the opposite strand was accomplished using the same DNA duplex ^{32}P labeled on the opposing strand, but at the 3′-terminus. This also permitted assessment of the nature of the DNA damage mediated at the 5′-termini of the cleavage fragments (Figure 6). The same G-specific fragmentation pattern was observed for cleavage of the opposite strand. DNA cleavage fragments were produced only after hot alkali treatment (compare lanes 2−5); these fragments comigrated exactly with the analogous Maxam−Gilbert products (lane 6), the latter of which terminate in 5′-phosphates.[15] The fact that cleavage occurred only after base treatment suggested that the Co(II)·bithiazole **1** complex mediated the alteration or liberation of the guanine base, producing a modified base or apurinic site; these lesions are known to be

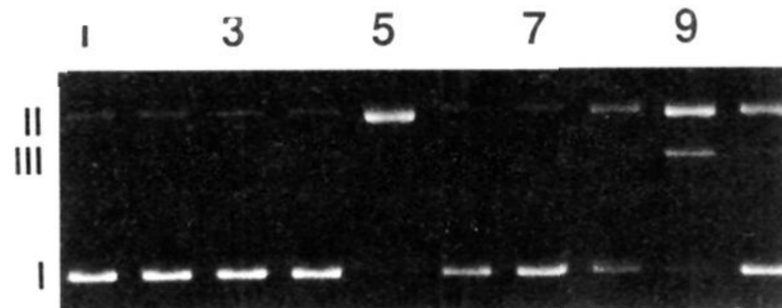


Figure 7. Relaxation of supercoiled SV40 DNA by Co(II)·bithiazole **1** under aerobic and anaerobic conditions. Reaction mixtures applied to lanes 1−5 were from experiments carried out under ambient oxygen conditions. Reaction mixtures applied to lanes 6−8 were from experiments carried out under an argon atmosphere: lane 1, DNA alone; lane 2, 5 μM **1**; lane 3, 5 μM Co^{2+}; lane 4, 5 μM Co(II)·**1** and 5 mM EDTA; lane 5, 5 μM Co(II)·**1**; lane 6, 5 μM **1**; lane 7, 5 μM Co^{2+}; lane 8, 5 μM Co(II)·**1**; lane 9, 0.5 μM Fe(II)·BLM A_2, aerobic; lane 10, 0.5 μM Fe(II)·BLM A_2, anaerobic.

alkali labile.[14ef,15,16] The production of cleavage fragments terminating in 5′- and 3′-phosphates after alkali treatment is also consistent with this thesis.

Mechanism of DNA Modification Mediated by Co(II)·Bithiazole 1. Having established the selectivity of DNA degradation mediated by Co(II)·bithiazole **1**, as well as the products of DNA strand scission, an investigation of the mechanism of DNA cleavage was carried out. Evidence concerning the ligands involved in Co(II) coordination was obtained by the use of the bithiazole moiety of BLM A_2, lacking a 2-aminomethyl substitutent (Figure 2). In the presence of Co(II) and over a wide range of concentrations, DNA damage was not observed using this bithiazole derivative (data not shown), providing strong evidence that the diamine substituent of **1** (cf. Figure 2) was essential for Co(II) chelation. Further, the bithiazole moiety itself did not appear to be involved in metal coordination. After incubation of the Co(II) and bithiazole ligand in the absence of DNA for up to 30 min, no change in the absorbance at 292 nm (λ_{max}) for the bithiazole ring system was observed, suggesting that ligation of the bithiazole to the Co(II) center was not occurring. Although the bithiazole moiety was not involved in metal coordination, a DNA cleavage experiment employing Co(II) and 1,3-diaminopropane, the diamine substituent of **1**, demonstrated that the bithiazole was required for DNA damage (data not shown); Co(II) in combination with 1,3-diaminopropane did not mediate DNA damage, presumably because the DNA affinity provided by the bithiazole was absent.

Although most cobalt-containing DNA cleaving agents require photoactivation,[17] experiments that rigorously excluded light demonstrated that Co(II)·bithiazole **1** does not require light to produce DNA damage (data not shown). However, oxygen was shown to be a necessary cofactor. The effect of oxygen on the relaxation of supercoiled DNA by Co(II)·bithiazole **1** was examined by comparison of the extent of cleavage obtained under normal atmospheric conditions and under an argon atmosphere (Figure 7). That the argon atmosphere was oxygen-depleted was verified by the fact that Fe(II)·BLM-mediated DNA cleavage was significantly diminished under these conditions (lanes 9 and 10). Comparison of lanes 5 and 8 demonstrated that DNA cleavage induced by the Co(II)·bithiazole **1** complex was suppressed under the argon atmosphere to approximately the same extent as the Fe(II)·BLM reaction. These results demonstrated that, like Fe(II)·BLM, Co(II)·bithiazole **1** required

(16) (a) Kawanishi, S.; Inoue, S.; Sano, S.; Aiba, H. *J. Biol. Chem.* **1986**, *261*, 6090. (b) Ito, K.; Inoue, S.; Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1993**, *268*, 13221. (c) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K. *J. Am. Chem. Soc.* **1995**, *117*, 6406.

(17) Chang, C.-H.; Meares, C. F. *Biochemistry* **1982**, *21*, 6332. (b) Chang, C.-H.; Meares, C. F. *Biochemistry* **1984**, *23*, 2268. (c) Barton, J. K.; Raphael, A. L. *J. Am. Chem. Soc.* **1984**, *106*, 2466. (d) Barton, J. K.; Raphael, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6460.

oxygen for activation leading to DNA cleavage. In a separate experiment, it was demonstrated that the formation of alkali-labile lesions on a ^{32}P-labeled DNA substrate was also an oxygen-dependent process (data not shown). The extent of DNA strand scission after alkali treatment when the reaction was carried out in argon-saturated solution was only 10% of that observed for a reaction carried out under ambient oxygen conditions, as determined by densitometric analysis of the polyacrylamide gel.

Having defined the requirement for oxygen for DNA damage mediated by the Co(II)•bithiazole **1** complex, the role of molecular oxygen was investigated. DNA cleavage mediated by transition metal complexes often involves the intermediacy of activated forms of oxygen.[14b,18] However, DNA nicking by Co(II)•bithiazole **1** was insensitive to the enzymes catalase and superoxide dismutase, suggesting that neither hydrogen peroxide nor superoxide were involved in mediating DNA damage (data not shown). The hydroxyl radical scavengers DMSO, *tert*-butyl alcohol, and mannitol,[19] as well as the singlet oxygen scavenger 2,5-dimethylfuran,[20] also failed to inhibit the relaxation of supercoiled DNA by Co(II)•bithiazole **1** (data not shown). Moreover, the absence of any cleavage enhancement when the reaction was carried out in 90% D_2O[18a,20,21] (data not shown), as well as the insensitivity of the cleavage reaction to light[18a,20,21] provided additional evidence that singlet oxygen was not responsible for the observed DNA damage mediated by Co(II)•bithiazole **1**.

It is well-known that Co(II)−amine and −polyamine complexes are capable of binding oxygen.[22] The earliest example was probably $[(NH_3)_5Co-O_2-Co(NH_3)_5]^{4+}$, first characterized in 1898.[23] Oxygenation of Co(II)−amine complexes can be detected by several methods, including absorption spectroscopy. The visible absorbance spectra of Co(II) complexes with ethylenediamine (en) and related ligands display an absorbance shoulder at ~360 nm, which is indicative of oxygen binding.[24] Attempts to apply this technique to the Co(II)•bithiazole **1** complex were unsuccessful, as the strong absorbance at 292 nm for the bithiazole ring system obscured the entire spectrum at the relatively high concentrations (300−500 μM) required for the control Co(II)•en system. At lower concentrations, no absorbance at 360 nm was observed for either complex. Since it was believed that neither thiazole ring was participating in Co(II) coordination (*vide supra*), it was anticipated that a *thiazole* analog of **1** would serve as a suitable model to study oxygen binding. Thiazole amide **7** (Scheme 1) was deblocked with 2:1 trifluoroacetic acid−dimethyl sulfide to afford the corresponding diaminopropyl thiazole amide, which was employed as a substrate for Co(II) coordination and oxygen binding. A 1:1 complex of Co(II) and deprotected **7** formed in oxygenated solution produced an absorption spectrum with λ_{max} at 239 (thiazole ring), 300 and 360 nm (supporting information, Figure 1). The peaks at 300 and 360 nm are indicative of an oxygenated Co complex containing a bridging peroxo group of the type $Co-O_2-Co$.[25] The intensity of the absorbance at 360 nm increased by 0.25 absorbance unit in the presence of oxygen, as compared with an analogous reaction carried out in argon-saturated solution. Increasing the ratio of deprotected **7**:Co(II) had no effect on the absorption spectrum.[26] This increase in absorbance at 360 nm in the presence of oxygen is quite similar to that observed for the 1:1 Co(II)•en complex (ΔA_{360} of 0.19, data not shown), suggesting that the Co(II) complex of deblocked **7** binds O_2 in a manner analogous to that of the ethylenediamine complex. By analogy, these results strongly suggest that Co(II)•bithiazole **1** also forms a cobalt−dioxygen complex.

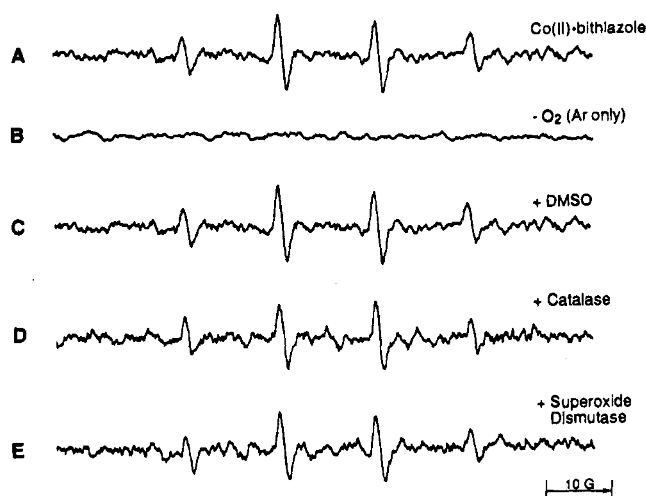


Figure 8. ESR spectra produced from Co(II)•bithiazole **1** in the presence of DMPO. Reactions contained 100 mM Na borate buffer (pH 8.0), 1 mM Co(II)•**1**, and 100 mM DMPO. Reactions C, D, and E contained 1 M DMSO, 3250 units/mL of catalase, and 125 units/mL of SOD, respectively.

ESR Studies. Although the results of the DNA relaxation assays suggested that diffusible activated oxygen species were not responsible for the observed DNA cleavage, it seemed conceivable that the ability of the bithiazole to bind to DNA facilitated a targeted delivery of reactive species directly to the DNA. In this event, only the freely diffusible species that did not encounter DNA would be trapped by scavenger molecules and inhibition of DNA cleavage would not be observed. In order to explore the possibility that the Co(II)•bithiazole **1** complex mediated the formation of activated oxygen species, ESR studies were carried out in the presence of a spin-trapping reagent, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO).[27]

Figure 8A demonstrates the four-line ESR signal produced from a 1:1 complex of Co(II)•bithiazole **1** in the presence of DMPO. This spectrum is analogous to the ESR spectrum produced from the DMPO/•OH adduct with hyperfine splitting constants of $a^N = a^H_\beta = 15$ G and a 1:2:2:1 intensity distribution.[27] The signal was comparable to that produced by Fe(II)•EDTA/H_2O_2, which is believed to generate •OH.[28] The signal continued to gain intensity for up to 5 min after mixing. Control experiments demonstrated that neither bithiazole **1** nor Co(II) separately, in the presence of DMPO, gave rise to an ESR signal. No additional signals, for example the DMPO/

(18) (a) Fleisher, M. B.; Waterman, K. C.; Turro, N. J.; Barton, J. K. *Inorg. Chem.* **1986**, *25*, 3549. (b) Mei, H.-Y.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1339.

(19) (a) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987. (b) Aruoma, O. I.; Halliwell, B.; Dizdaroglu, M. *J. Biol. Chem.* **1989**, *264*, 13024. (c) Aruoma, O. I.; Halliwell, B.; Gajewski, E.; Dizdaroglu, M. *J. Biol. Chem.* **1989**, *264*, 20509. (d) Sagripanti, J.-L.; Kraemer, K. H. *J. Biol. Chem.* **1989**, *264*, 1729.

(20) Wasserman, H. H.; Murray, R. W. *Singlet Oxygen*; Academic Press: New York, 1979; pp 597−641.

(21) Ingraham, L. L.; Meyer, D. L. *Biochemistry of Dioxygen*; Plenum Press: New York, 1985; pp 21−43.

(22) Jones, R. D.; Summerville, D. A.; Basolo, F. *Chem Rev.* **1979**, *79*, 139.

(23) Werner, A.; Mylius, A. *Z. Anorg. Chem.* **1898**, *16*, 745.

(24) Bijl, P.; de Vries, G. *J. Chem. Soc., Dalton Trans.* **1972**, 303.

(25) Lever, A. B. P.; Gray, H. B. *Acc. Chem. Res.* **1978**, *11*, 348.

(26) Varying the Co(II):bithiazole ratio from 1:1 had no effect on the extent of DNA cleavage observed in a plasmid DNA relaxation assay (data not shown), further supporting a stoichiometry of a 1:1 Co•bithiazole complex containing oxygen.

(27) Janzen, E. G. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1980; Vol. IV, pp 116−154.

(28) Croft, S.; Gilbert, B. C.; Lindsay Smith, J. R.; Whitwood, A. C. *Free Rad. Res. Commun.* **1992**, *17*, 21.

•OOH adduct, were observed.[29] Importantly, no signal was observed when the reaction was carried out using deoxygenated, argon-saturated solutions (Figure 8B).

The possible formation of •OH by the Co(II)•bithiazole **1** complex was investigated further. Recent reports that the superoxide adduct of DMPO decomposes to the DMPO/•OH adduct have raised some uncertainty as to whether authentic •OH was trapped in these systems.[30] The inclusion of DMSO in the reaction mixture obviates the problem.[30a,31] DMSO reacts with •OH, but not with superoxide, to yield $^{\bullet}CH_3$, the latter of which is trapped by DMPO to generate DMPO/$^{\bullet}CH_3$. Detection of the DMPO/$^{\bullet}CH_3$ adduct appears to be a more specific indicator of •OH spin trapping and is, therefore, the method of choice. Using DMSO in conjunction with DMPO, the Co(II)•bithiazole **1** complex generated the same four-line ESR signal (Figure 8C) as that produced in the absence of DMSO. Control experiments using authentic •OH generated from Fe(II)•EDTA/H_2O_2 confirmed that, in the presence of DMSO, only the distinctive six-line DMPO/$^{\bullet}CH_3$ adduct was observed (not shown). These results indicated that the ESR signal produced by the Co(II)•bithiazole **1** complex (Figure 8A) did not result from reaction of diffusible •OH with DMPO.

Further evidence regarding the formation of the DMPO/•OH adduct was obtained by performing the reactions in the presence of catalase and superoxide dismutase.[32] As illustrated in Figure 8D,E, catalase and superoxide dismutase failed to inhibit the formation of the DMPO/•OH adduct at enzyme concentrations shown to completely abolish the ESR signal generated by an equivalent amount of Co(II)/H_2O_2, a known superoxide-generating system. These results provide strong evidence that superoxide and H_2O_2 are not produced by the Co(II)•bithiazole **1** complex. The results of the ESR study suggest strongly that the observed DNA damage mediated by Co(II)•bithiazole **1** resulted from a mechanism other than the action of diffusible oxygen radicals.

Discussion

Sequence-selective DNA strand scission by Fe•BLM involves initial binding of the drug. Both the metal binding region and C-terminal substituent of BLM contribute to its DNA affinity.[2,6,8-10] While it seems clear that the bithiazole moiety of BLM is essential for DNA binding, the role of the bithiazole ring system in the recognition of $^{5'}$-GC-$^{3'}$ and $^{5'}$-GT-$^{3'}$ sequences by BLM has remained unclear. A model of BLM–DNA interaction has been suggested to involve specific recognition of guanosine residues through hydrogen bonding with the thiazole nitrogens.[33] However, recent findings employing chemically reactive bithiazole derivatives[11] suggest that the bithiazole moiety itself does not bind to DNA in a selective fashion. Since it seemed possible that the diffusible nature of the reactive species produced by these bithiazole derivatives could have obscured a weak binding selectivity of the bithiazole, we prepared an additional bithiazole derivative containing a diamine–metal chelation site. It was anticipated that, if this bithiazole derivative could bind an appropriate metal ion and form a nondiffusible reactive species, the selectivity of the bithiazole could be assessed more accurately. The synthetic route used for the preparation of bithiazole **1** is outlined in Scheme 1.

Initial studies with this bithiazole derivative focused on its ability to mediate DNA cleavage in the presence of a metal ion. As demonstrated in Figure 3, activation of bithiazole **1** for DNA cleavage could only be achieved in the presence of Co(II). Other metals examined included Fe(II), Ni(II), Mn(II), Cu(II), and Zn(II). Increasing concentrations of the Co(II) complex resulted in increased DNA damage (Figure 4). DNA degradation by Co(II)•bithiazole **1** was limited to single-strand breaks. No linear (Form III) DNA was observed, even after prolonged incubation times or at higher concentrations of the Co(II) complex (data not shown).

Having shown that bithiazole **1** could mediate DNA degradation in the presence of Co(II), the ability of the Co(II) complex to mediate strand scission of a linear 5′-^{32}P-labeled DNA duplex was next examined. As shown in Figure 5, 50 μM Co(II)•bithiazole **1** produced alkali-labile sites on the duplex DNA fragment; subsequent treatment of the modified DNA with alkali at 90 °C resulted in guanosine-specific DNA fragmentation. DNA strand scission was only observed after alkali treatment. Comparison of the resulting cleavage pattern with that produced by Fe(II)•BLM A_2 (compare lanes 11 and 12) demonstrated that the G specificity exhibited by the Co(II)•bithiazole **1** complex was not related to the $^{5'}$-Gpyr-$^{3'}$ selectivity of Fe(II)•BLM, i.e., all G residues in this DNA duplex were cleaved with equal facility by the Co(II)•bithiazole **1** complex. Moreover, the same G-specific cleavage pattern was also observed on the opposite strand (Figure 6). Also determined from the polyacrylamide gels in Figures 5 and 6 was the chemical nature of the termini of the cleavage fragments. After alkali treatment, the fragments resulting from strand scission comigrated with the products of the Maxam–Gilbert G-specific reaction, demonstrating that the products had 5′- and 3′-phosphate termini.[15]

As noted above, Co(II)•bithiazole **1** effected relaxation of supercoiled plasmid DNA, indicating the occurrence of strand scission under the reaction conditions. In comparison, significant strand scission of the ^{32}P-end labeled DNA's was observed only after treatment with alkali. We attribute this apparent difference to the much greater sensitivity of the plasmid relaxation assay; adventitious hydrolysis of a single alkali-labile lesion would afford the observed results. However, attempts to demonstrate the presence of multiple alkali-labile lesions within the treated plasmid DNA's were unsuccessful, due to the intrinsic sensitivity of the unmodified plasmid DNA's to hot alkali.

Mechanistic studies of DNA degradation mediated by Co(II)•bithiazole **1** established that the process was not light dependent but did require oxygen. The requirement for oxygen was established both from the DNA nicking assay (Figure 7) as well as for the formation of alkali-labile sites on a ^{32}P-labeled DNA fragment (data not shown). The requirement for oxygen suggested the possibility that the Co(II)•bithiazole **1** complex mediated the formation of activated oxygen species, which carried out the observed DNA degradation. However, the inclusion of catalase, SOD, •OH, or singlet oxygen scavengers had no effect on the ability of the Co(II)•bithiazole **1** complex to induce DNA nicks, suggesting that diffusible oxygen species were not responsible for the observed DNA cleavage.

To further explore the possible involvement of oxygen free radicals in Co(II)•bithiazole **1**-mediated DNA degradation, ESR

(29) The Co(II)/H_2O_2 system, which is believed to generate superoxide, was used to produce ESR spectra of the authentic DMPO/•OOH adduct. See: Kadiiska, M. B.; Maples, K. R.; Mason, R. P. *Arch. Biochem. Biophys.* **1989**, *275*, 98.

(30) (a) Britigan, B. E.; Rosen, G. M.; Chai, Y.; Cohen, M. S. *J. Biol. Chem.* **1986**, *261*, 4426. (b) Lloyd, R. V.; Mason, R. P. *J. Biol. Chem.* **1990**, *265*, 16733.

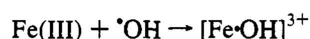
(31) Burkitt, M. J.; Mason, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8440.

(32) In order to determine the enzyme concentrations necessary to scavenge a comparable concentration of reactive species, the known superoxide-generating system Co(II)/H_2O_2 was used to calibrate the experiment[29] (data not shown).

(33) Kuwahara, J.; Sugiura, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 2459.

spectroscopy with a spin-trapping reagent (DMPO) was employed. As demonstrated in Figure 8, Co(II)•bithiazole **1** produced the four-line ESR signal corresponding to the DMPO/•OH adduct.[27] In the absence of oxygen, no signal was observed. The production of the ESR signal corresponding to the DMPO/•OH adduct was insensitive to 1 M DMSO, a fact which strongly indicated that the ESR signal did not result from the reaction of free •OH with DMPO.[30a,31] If •OH were present in the reaction mixture, reaction of •OH with DMSO would produce $^{\bullet}CH_3$ and the corresponding DMPO/$^{\bullet}CH_3$ adduct would be observed. Because DMSO had no effect on the ESR signal, *it is highly unlikely that •OH is produced by the Co(II)•bithiazole 1 complex*. Further, the fact that catalase and superoxide dismutase also failed to inhibit the production of the ESR signal demonstrated that neither H_2O_2 nor superoxide was produced by the Co(II)•bithiazole **1** complex as a species utilized subsequently for DNA degradation.

These mechanistic observations complemented the chemistry observed for DNA degradation, defining the absolute oxygen requirement for activation and eliminating the possibility that diffusible activated forms of oxygen were involved. Moreover, the production of an ESR signal characteristic of the DMPO/•OH adduct must have occurred by a mechanism other than addition of •OH to the spin trap. It has been shown that the simple Co(II)•en complex, in the presence of oxygen, also produces the DMPO/•OH adduct, although this was clearly shown to result from reaction between DMPO and water, promoted by the Co(II) complex.[34] When the reaction was carried out in the presence of $H_2{}^{17}O$, additional hyperfine splittings were observed corresponding to the DMPO/$^{\bullet 17}OH$ adduct. This type of spin trap hydration has also been observed following exposure of DMPO to ionizing radiation.[35] It seems possible that direct oxidation of DMPO by the oxygenated Co(II)•en complex occurred. The resulting unstable radical cation could then react with water to form the same adduct obtainable by direct attack of •OH on DMPO (Scheme 2A). Alternatively, coordination of DMPO by the Co(II)−amine complex could facilitate nucleophilic attack by water at the unsaturated carbon of the DMPO ring (Scheme 2B). Such a mechanism has been proposed for the reaction between Fe(III) and DMPO, which also generates the DMPO/•OH adduct.[36] For either mechanistic possibility, the nature of the species that is correspondingly reduced is unclear. An additional mechanism could involve direct attack of a metal-associated •OH or related species on DMPO. A similar metal-bound oxygen species has been proposed to result from reaction of Fe(III) and •OH:[37]

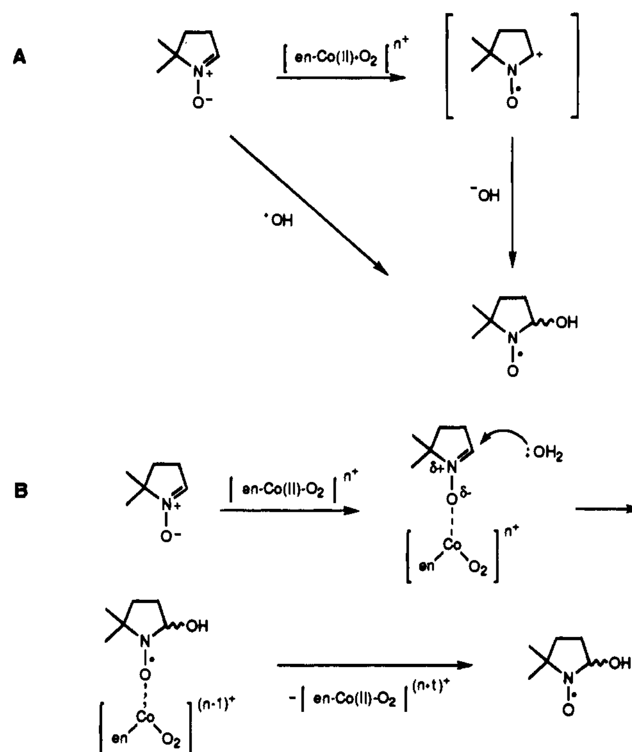


$$\mathrm{Fe(III)} + {}^{\bullet}\mathrm{OH} \rightarrow [\mathrm{Fe{\bullet}OH}]^{3+}$$

Whether one of these mechanisms can account for the formation of the DMPO/•OH adduct by Co(II)•bithiazole **1** remains unclear, although the possible involvement of diffusible oxygen radicals has been eliminated.

In the aggregate, the results of the ESR studies and the products of DNA degradation induced by the Co(II)•bithiazole **1** complex support a mechanism whereby the Co(II)•bithiazole **1** complex mediates the oxidative modification of the guanine moiety in double-stranded DNA. This oxidation is not the result of freely diffusible oxygen species but rather a result of an activated Co(II)•bithiazole **1** complex containing oxygen. The preferential reactivity of guanosine residues is consistent with an oxidative process. From electrochemical studies, guanine is known to be the most easily oxidized of the heterocyclic bases.[38] Moreover, the susceptibility of the four 2′-deoxynucleosides to oxidation by singlet oxygen was shown to decrease in the order dG ≫ dT > dC ∼ dA.[39] Additionally, the major site of base damage induced by diffusible •OH generated by ferric ion chelates in combination with the hypoxanthine/xanthine oxidase system[19b] or H_2O_2[19c] was dG.

Scheme 2. Possible Mechanisms for the Formation of the DMPO/•OH Adduct by Co(II)•en



The fact that DNA strand scission occurs only after hot alkali treatment supports a mechanism whereby the Co•bithiazole **1** complex modifies the guanine base, producing an alkali-labile site. Oxidatively modified guanine residues have been suggested to be the alkali-labile lesions induced by certain transition metal complexes.[14e,f,i] Additional known guanine-specific modifications that support this proposal include 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxodGuo)[40] and 2,2-diamino-4-[(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-5-(2*H*)-oxazolone,[41] base modifications resulting from oxidative DNA damage, and ionizing radiation-induced DNA damage. The modified nucleoside 8-oxodGuo has been incorporated into a single site in an oligonucleotide and shown to be base labile.[42] Recent experiments by Cadet and co-workers[41] revealed the novel oxazolone to be a predominant hydroxyl radical base oxidation product of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine; subsequent treatment of the oxazolone with alkali resulted in cleavage of the glycosidic bond and the release of free guanidine. Finally, the production of DNA fragments containing 5′-phosphate and 3′-phosphate termini is in complete agreement with modification of the heterocyclic base (*vide supra*).

(34) Hanna, P. M.; Kadiiska, M. B.; Mason, R. P. *Chem. Res. Toxicol.* **1992**, *5*, 109.
(35) Chandra, H.; Symons, M. C. R. *J. Chem. Soc., Chem. Commun.* **1986**, 1301.
(36) Makino, K.; Hagiwara, T.; Hagi, A.; Nishi, M.; Murakami, A. *Biochem. Biophys. Res. Commun.* **1990**, *172*, 1073.
(37) Youngman, R. J. *Trends Biochem. Sci.* **1984**, *9*, 280.
(38) Steenken, S. *Chem. Rev.* **1989**, *89*, 503.
(39) Cadet, J.; Teoule, R. *Photochem. Photobiol.* **1978**, *28*, 661.
(40) Halliwell, B.; Dizdaroglu, M. *Free Rad. Res. Commun.* **1992**, *16*, 75.
(41) Cadet, J.; Berger, M.; Buchko, G. W.; Joshi, P. C.; Raoul, S.; Ravanat, J.-L. *J. Am. Chem. Soc.* **1994**, *116*, 7403.
(42) Chung, M.-H.; Kiyosawa, H.; Ohtsuka, E.; Nishimura, S.; Kasai, H. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 1.

Additional evidence arguing against the participation of freely diffusible •OH was obtained from the DNA cleavage experiments. DNA damage induced by •OH, generated either by γ-irradiation or Fe(II)•EDTA/H_2O_2, produces a characteristic cleavage ladder, composed of two closely spaced bands at each cleavage site.[43] These doublet bands correspond to a mixture of fragments terminating in 3′-phosphates and 3′-phosphoroglycolates and are a result of *direct* strand scission.[14b,43] An alternative mechanism for DNA cleavage could involve H abstraction from the deoxyribose ring. This type of mechanism normally results directly in strand scission,[2b,14dh,44] as opposed, or in addition to the formation of alkali-labile sites; therefore, this type of mechanism can also be ruled out.

If the Co•(aminomethyl)bithiazole complex mediated a direct oxidation of the guanine base, the resulting guanine residues could be lost by solvolysis, generating apurinic lesions susceptible to strand scission upon alkali treatment. Alternatively, the formation of the alkali-labile 8-oxodGuo could proceed by one electron oxidation of guanine to generate the guanine radical cation, followed by hydration and a second one-electron oxidation.[45] Recent experiments by Thorp and co-workers[46] estimated a potential for the one-electron oxidation of G in duplex DNA to be between 0.9 and 1.0 V; these values are in good agreement with previous results obtained by direct electrochemical oxidation of adsorbed DNA.[47] Oxidation of cobalt μ-peroxo complexes by ferrous ion has been shown to occur readily, demonstrating a redox potential of ≥ 0.77 V for these types of complexes.[48] Although the exact nature of the Co•bithiazole **1**•oxygen complex is unknown, as is the redox potential for the complex, the results of these studies argue against a direct one-electron oxidation of guanine. Moreover, recent studies[16b,c] have demonstrated a preference for GG sites for the one-electron oxidation of guanine in double-stranded DNA. The Co•bithiazole **1** complex employed in the present study did not display a GG selectivity. Taken together, these studies suggest that oxidative degradation of G residues within DNA by the 1:1 Co•bithiazole complex most likely proceeds through an inner sphere mechanism, rather than a one-electron oxidation coupled to hydration. The O−O bond within dicobalt μ-peroxo complexes is expected to be weak;[49] homolytic cleavage, for example, would produce a cobalt−oxo moiety that could mediate direct oxo transfer to a guanine moiety.[14f]

Studies were carried out to identify oxidized guanine residues by HPLC with UV detection, but no oxidation products were identified prior to alkali treatment and no strand scission products were observed following alkali treatment. An alternative approach was carried out that involved incubation of a dodecanucleotide with the Co•bithiazole **1** complex followed by enzymatic digestion and dephosphorylation to produce mononucleosides,[50,51] which were analyzed by HPLC. Modified mononucleosides were not observed. Oxidation of the guanosine base often involves major decomposition to afford products that are difficult to analyze.[41] In this context, it should be noted that 8-oxodGuo and 2,2-diamino-4-[(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-5-(2*H*)-oxazolone are the only known stable oxidation products of guanine nucleosides. Moreover, the identification of these products required highly sensitive HPLC systems employing electrochemical detection for 8-oxodGuo[50,52] and differential refractometry for the oxazolone.[41]

This study has demonstrated a novel approach for DNA cleavage and has also provided important information regarding the binding selectivity of the bithiazole. Consistent with earlier findings for reactive bithiazole derivatives,[11] which suggested a lack of specificity in the binding of the bithiazole moiety to DNA, the present observation of the guanosine selectivity of DNA modification by the Co(II)•bithiazole **1** complex is probably best explained as arising from *preferential reactivity at guanosine sites, as opposed to a guanosine binding selectivity of Co•bithiazole 1*. This preference presumably reflects the redox potential of the reactive Co•bithiazole **1** complex. These results support a model of BLM−DNA interaction in which the bithiazole + C-terminus region contributes to DNA affinity but does not provide the structural basis for determining DNA sequence recognition.

Experimental Section

General Methods. Elemental analyses were carried out by Atlantic Microlab, Inc. Melting points were taken on a Thomas Hoover apparatus and are not corrected. ^{1}H NMR spectra were recorded on a General Electric QE300 spectrometer. Chemical shifts are referenced to $CHCl_3$ at 7.26 ppm, CH_3OH at 3.30 ppm, or HOD at 4.8 ppm. The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; q, quartet; qn, quintet; br, broad. Chemical ionization mass spectra were recorded on a Varian MAT-44 mass spectrometer using isobutane. 5, 5-Dimethyl-1-pyrroline *N*-oxide (DMPO), obtained from Aldrich Chemicals, was stored at −80 °C under argon and was used without further purification. Solvents were purified and dried according to standard procedures.

Blenoxane, obtained as a gift from Bristol Laboratories, was fractionated to obtain BLM A_2.[6a] Calf intestine alkaline phosphatase and *Eco*RV were purchased from Boehringer Mannheim Biochemicals. Plasmid pBR322 and SV40 supercoiled DNA's were obtained from GIBCO BRL. T4 Polynucleotide kinase, AMV reverse transcriptase, and *Hin*dIII were purchased from United States Biochemical. [γ-^{32}P]-ATP (7000 Ci/mmol) was obtained from ICN Biomedicals; [α-^{32}P]-dATP was from Dupont/NEN Products.

Agarose gel electrophoresis was carried out in 40 mM Tris−acetate buffer (pH 7.8), containing 5 mM EDTA; polyacrylamide gel electrophoresis was carried out in 90 mM Tris−borate buffer (pH 8.3) containing 5 mM EDTA.[53] Chemical DNA sequencing was carried out according to the method of Maxam and Gilbert.[15a,b,53] Enzyme reactions were performed according to the manufacturer's recommended protocol in the activity buffer provided. Distilled, deionized water from a Milli-Q system was used for all aqueous solutions and manipulations. Autoradiography was carried out with Kodak X-Omat film at −80 °C. Polyacrylamide gel loading solution: 10 M urea, 1.5 mM EDTA, 0.05% (w/v) xylene cyanol, 0.05% (w/v) bromphenol blue. Agarose gel loading buffer: 40 mM Tris-OAc (pH 7.8), 5 mM EDTA, 40% glycerol, 0.3% bromphenol blue.

1,3-Bis[*N*-(*tert*-butoxycarbonyl)amino]-2-propanol (2). A solution of di-*tert*-butyl dicarbonate (2.6 g, 12 mmol) in 30 mL of tetrahydrofuran (THF) was added dropwise to a stirred solution containing 0.45 g (5.0 mmol) of 1,3-diamino-2-propanol in 40 mL of 1:1 THF−water at room temperature. The combined solution was stirred at room

(43) Henner, W. D.; Rodriguez, L. O.; Hecht, S. M.; Haseltine, W. A. *J. Biol. Chem.* **1983**, *258*, 711.

(44) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191.

(45) Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692.

(46) Johnston, D. H.; Cheng, C.-C.; Campbell, K. J.; Thorp, H. H. *Inorg. Chem.* **1994**, *33*, 6388.

(47) (a) Brabec, V.; Dryhurst, G. *J. Electroanal. Chem.* **1978**, *89*, 161. (b) Brabec, V. *Biophys. Chem.* **1979**, *9*, 289.

(48) McLendon, G.; Martell, A. E. *Coord. Chem. Rev.* **1976**, *19*, 1.

(49) Reinauld, O. M.; Theopold, K. H. *J. Am. Chem. Soc.* **1994**, *116*, 6979.

(50) Mouret, J.-F.; Polverelli, M.; Sarrazini, F.; Cadet, J. *Chem.-Biol. Interact.* **1991**, *77*, 187.

(51) Comess, K. M.; Costello, C. E.; Lippard, S. J. *Biochemistry* **1990**, *29*, 2102.

(52) (a) Floyd, R. A.; Watson, J. J.; Wong, P. K.; Altmiller, D. H.; Rickard, R. C. *Free Rad. Res. Commun.* **1986**, *1*, 163. (b) Floyd, R. A.; West, M. S.; Eneff, K. L.; Hogsett, W. E.; Tingey, D. T. *Arch. Biochem. Biophys.* **1988**, *262*, 266. (c) Devasagayam, T. P. A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, *30*, 6283.

(53) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

temperature for 18 h. After removal of the solvent under diminished pressure, the residue was extracted with a mixture of ether (60 mL) and water (10 mL). The resulting organic layer was separated, washed with three 20-mL portions of brine, then dried ($MgSO_4$). The solution was concentrated under diminished pressure to yield a thick, colorless oil, which was purified by crystallization from ether. 1,3-Bis[(*tert*-butoxycarbonyl)amino]-2-propanol (**2**) was obtained as colorless prisms: yield 1.38 g (95%); mp 99–100 °C; silica gel TLC R_f 0.72 (9:1 $CHCl_3$–CH_3OH); ^1H NMR ($CDCl_3$) δ 1.45 (s, 18 H), 3.10–3.35 (m, 4 H), 3.67 (br s, 1 H), 3.73 (m, 1 H), and 5.14 (br s, 2 H); mass spectrum (chemical ionization) m/z 291 $(M + H)^+$. Anal. Calcd for $C_{13}H_{26}N_2O_5$: C, 53.78; H, 9.03; N, 9.65. Found: C, 53.62; H, 9.06; N, 9.78.

(1,3-Bis[*N*-(*tert*-butoxycarbonyl)amino]-2-propyl) *p*-Toluenesulfonate (3). *p*-Toluenesulfonyl chloride (5.0 g, 26 mmol) was added at once to a stirred solution of 5.8 g (20 mmol) of compound **2** in 60 mL of dry pyridine at 3 °C. The reaction mixture was allowed to warm to room temperature and was then stirred for 2 days. After removal of the solvent under diminished pressure, the residue was extracted with a mixture of ether (300 mL) and water (50 mL). The organic layer was separated and washed with three 50-mL portions of brine, then dried ($MgSO_4$). The solvent was concentrated under diminished pressure, and the crude residue was purified by silica gel chromatography. Elution with 3:2 hexane–ethyl acetate afforded tosylate **3**, which crystallized from ether as colorless prisms: yield 7.10 g (80%); mp 105–106 °C; silica gel TLC R_f 0.54 (3:2 hexane–ethyl acetate); ^1H NMR ($CDCl_3$) δ 1.40 (s, 18 H), 2.45 (s, 3 H), 3.10–3.50 (m, 4 H), 4.55 (m, 1 H), 5.00 (br s, 2 H), 7.35 (d, 2 H, J = 7.5 Hz), and 7.82 (d, 2 H, J = 7.5 Hz); mass spectrum (chemical ionization) m/z 445 $(M + H)^+$. Anal. Calcd for $C_{20}H_{32}N_2O_7S$: C, 54.04; H, 7.26; N, 6.30. Found: C, 53.99; H, 7.28; N, 6.42.

3-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[(N-(*tert*-butoxycarbonyl)-amino)methyl]propionitrile (4). Sodium cyanide (0.30 g, 6.1 mmol) was added in a single portion to a stirred solution of 2.2 g (5.0 mmol) of tosylate **3** in 10 mL of dry DMSO at room temperature. The reaction mixture was heated at 80 °C for 2 h with stirring. After cooling to room temperature, 400 mL of water was poured into the reaction mixture and the resulting solution was extracted with four 50-mL portions of ether. The ether extracts were combined, washed with three 20-mL portions of brine, then dried over $MgSO_4$. The solvent was concentrated under diminished pressure to afford a crude product that was purified by silica gel chromatography. Elution with 3:2 hexane–ethyl acetate afforded propionitrile **4**, which crystallized from ether as colorless prisms: yield 0.91 g (60%); mp 122-123 °C; silica gel TLC R_f 0.51 (3:2 hexane–ethyl acetate); ^1H NMR ($CDCl_3$) δ 1.43 (s, 18 H), 2.50–2.75 (m, 2 H), 3.20–3.50 (m, 2 H), 3.92 (m, 1 H), 4.93 (br s, 1 H) and 5.46 (br s, 1 H); mass spectrum (chemical ionization) m/z 300 $(M + H)^+$. Anal. Calcd for $C_{14}H_{25}N_3O_4$: C, 56.17; H, 8.42; N, 14.04. Found: C, 55.98; H, 8.31; N, 14.25.

3-[*N*-(*tert*-Butoxycarbonyl)amino]-2[(*N*-(*tert*-butoxycarbonyl)amino)methyl]thiopropionamide (5). A mixture of 5.2 g (17 mmol) of propionitrile **4** and 5.2 g (51 mmol) of triethylamine in 30 mL of ethanol was stirred and heated at 60 °C in a bottle under medium pressure in the presence of H_2S for 3 days. After cooling to room temperature, excess H_2S gas was removed under diminished pressure. The reaction mixture was cooled to 3 °C; the resulting precipitate was collected by filtration and was washed with ether and then with 2:1 ether–ethanol to afford 3.4 g of the desired product. The filtrate was concentrated under diminished pressure, then the residue was extracted with a mixture of ethyl acetate (60 mL) and water (20 mL). The organic layer was separated, washed with two 20-mL portions of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford a residue, which was purified by silica gel chromatography. Elution with 1:4 acetone–hexane afforded an additional quantity of the desired product. Thiopropionamide **5** was obtained as a colorless solid by precipitation of the isolated product from 3:1 ether–hexane: total yield 3.8 g (65%); mp 179–180 °C; silica gel TLC R_f 0.71 (9:1 $CHCl_3$–CH_3OH); ^1H NMR ($CDCl_3$) δ 1.42 and 1.45 (2 s, 18 H), 2.90–3.05 and 3.20–3.45 (m, 4 H), 3.79 (m, 1 H), 5.03 (br s, 1 H), 5.86 (br s, 1 H), 7.58 (br s, 1 H) and 9.12 (br s, 1 H); mass spectrum (chemical ionization) m/z 334 $(M + H)^+$. Anal. Calcd for $C_{14}H_{27}N_3O_4S$: C, 50.34; H, 8.16; N, 12.60. Found: C, 50.48; H, 8.13; N, 12.75.

Ethyl 2-[(1,3-Bis(*N*-(*tert*-butoxycarbonyl)amino))-2-propyl]thiazole-4-carboxylate (6). Ethyl bromopyruvate (0.19 g, 0.97 mmol) was added dropwise to a stirred solution of 0.27 g (0.81 mmol) of thiopropionamide **5** in 4 mL of dry DMF at room temperature. The reaction mixture was stirred at room temperature for 2 h. The solvent was concentrated under diminished pressure, then the residue was extracted with a mixture of ethyl acetate (30 mL) and water (10 mL). The organic layer was separated, washed successively with three 10-mL portions of saturated aqueous sodium bicarbonate and three 10-mL portions of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to yield a crude product, which was purified by silica gel chromatography. Elution with 4:1 ethyl acetate–hexane afforded thiazole carboxylate **6** as colorless needles: yield 0.23 g (56%); silica gel TLC R_f 0.73 (9:1 $CHCl_3$–CH_3OH); ^1H NMR ($CDCl_3$) δ 1.30–1.55 (m, 21 H), 3.15–3.40 (m, 4 H), 3.98 (m, 1 H), 4.42 (q, 2 H), 4.96 (br s, 1 H), 5.35 (br s, 1 H), and 8.08 (s, 1 H); mass spectrum (chemical ionization) m/z 430 $(M + H)^+$. Anal. Calcd for $C_{19}H_{31}N_3O_6S$: C, 53.13; H, 7.27; N, 9.78. Found: C, 53.25; H, 7.18; N, 9.92.

2-[(1,3-Bis(*N*-(*tert*-butoxycarbonyl)amino))-2-propyl]thiazole-4-carboxamide (7). Gaseous NH_3 was bubbled through a solution containing 0.13 g (0.30 mmol) of thiazole **6** in 9 mL of CH_3OH at −78 °C for 5 min. The solution was allowed to warm to room temperature and was stirred for an additional 20 h. The solution was concentrated under diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 4:1 ethyl acetate–hexane afforded thiazole carboxamide **7** as colorless needles: yield 0.11 g (93%); silica gel TLC R_f 0.28 (4:1 ethyl acetate–hexane); ^1H NMR ($CDCl_3$) δ 1.37 and 1.43 (2 s, 18 H), 3.10–3.40 (m, 4 H), 4.06 (m, 1 H), 5.22 (br s, 1 H), 5.44 (br s, 1 H), 6.28 (br s, 1 H), 7.31 (br s, 1 H), and 8.01 (s, 1 H); mass spectrum (chemical ionization) m/z 401 $(M + H)^+$. Anal. Calcd for $C_{17}H_{28}N_4O_5S$: C, 50.98; H, 7.05; N, 13.99. Found: C, 50.68; H, 7.06; N, 13.83.

2-[(1,3-Bis(*N*-(*tert*-butoxycarbonyl)amino))-2-propyl]thiazole-4-thiocarboxamide (8). Lawesson's reagent (0.04 g, 0.10 mmol) was added in one portion to a stirred solution containing 0.04 g (0.10 mmol) of thiazole carboxamide **7** in 2 mL of dimethoxyethane at room temperature. The combined solution was heated at reflux for 2 h with stirring. The solution was concentrated under diminished pressure, then the residue was extracted with a mixture of ethyl acetate (30 mL) and saturated aqueous sodium bicarbonate (10 mL). The organic layer was separated, washed with three 10-mL portions of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to give a crude product which was purified by silica gel chromatography. Elution with 1:1 ethyl acetate–hexane afforded thiazole thiocarboxamide **8** as pale yellow needles: yield 0.04 g (96%); silica gel TLC R_f 0.40 (3:2 ethyl acetate–hexane); ^1H NMR ($CDCl_3$) δ 1.42 and 1.44 (2 s, 18 H), 3.10–3.45 (m, 4 H), 4.10 (m, 1 H), 4.87 (br s, 1 H), 5.14 (br s, 1 H), 7.57 (br s, 1 H), 8.13 (s, 1 H), and 8.82 (br s, 1 H); mass spectrum (chemical ionization) m/z 417 $(M + H)^+$. Anal. Calcd for $C_{17}H_{28}N_4O_4S_2$: C, 49.02; H, 6.78; N, 13.45. Found: C, 49.46; H, 6.79; N, 13.30.

Ethyl 2′-[(1,3-Bis-(*N*-(*tert*-butoxycarbonyl)amino))-2-propyl]-2,4′-bithiazole-4-carboxylate (9). Ethyl bromopyruvate (0.54 g, 2.8 mmol) was added dropwise to a stirred solution of 0.83 g (2.0 mmol) of thiazole thiocarboxamide **8** in 40 mL of dimethoxyethane at room temperature. The combined solution was heated at 60 °C for 2 days, then heated at reflux for 3 h. At this point, deprotected product had precipitated from solution. This precipitate was collected by filtration, and the filtrate was concentrated under diminished pressure. The residue was extracted with a mixture of ethyl acetate (70 mL) and water (10 mL). The organic layer was separated, washed with three 10-mL portions of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford a crude product. The collected precipitate was dissolved in a mixture of THF (20 mL) and water (10 mL); then a solution of di-*tert*-butyl dicarbonate (0.50 g, 2.3 mmol) in 5 mL of THF was added, and the reaction mixture was stirred at room temperature for 3 h. After removal of the THF under diminished pressure, the residue was treated with 80 mL of ethyl acetate. The organic layer was separated, washed with two 20-mL portions of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure, and this residue was combined with the former

crude product; the mixture was purified by silica gel chromatography. The column was washed successively with 2:3 ethyl acetate−hexane, 1:1 ethyl acetate−hexane, and then 3:2 ethyl acetate−hexane. The 1:1 ethyl acetate−hexane eluate was concentrated to give 0.69 g (67%) of bithiazole **9**. The 3:2 ethyl acetate−hexane eluate was concentrated under diminished pressure to give 0.05 g (4.3%) of the "hydrated" bithiazole precursor, which was dissolved in toluene (10 mL) and heated at reflux for 1 day with stirring. The solvent was removed under reduced pressure, then a mixture of ethyl acetate (30 mL) and water (5 mL) was poured onto the residue. The organic layer was separated, washed with two 5-mL portions of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford a residue which was purified by silica gel chromatography. Elution with 1:1 ethyl acetate−hexane yielded an additional 0.04 g of bithiazole **9**. Crystallization of the crude product from ether afforded bithiazole **9** as pale orange microcrystals: total yield 0.73 g (70%); mp 152−153 °C; silica gel TLC R_f 0.71 (4:1 ethyl acetate−hexane); ^{1}H NMR ($CDCl_3$) δ 1.35−1.50 (m, 21 H), 3.20−3.40 (m, 4 H), 4.06 (m, 1 H), 4.45 (q, 2, J = 7.5 Hz), 5.01 (br s, 1 H), 5.43 (br s, 1 H), 8.06 (s, 1 H) and 8.22 (s, 1 H); mass spectrum (chemical ionization) m/z 512 (M^+). Anal. Calcd for $C_{22}H_{32}N_4O_6S_2$: C, 51.54; H, 6.29; N, 10.93. Found: C, 51.49; H, 6.27; N, 10.93.

2′-[(1,3-Bis(*N*-(*tert*-butoxycarbonyl)amino))-2-propyl]-2,4′-bithiazole-4-[*N*-(3-methylthio)propyl]carboxamide (10). A solution of bithiazole **9** (0.24 g, 0.47 mmol) in 0.5 mL of 3-(methylthio)-propylamine (Eastman Kodak Chemicals) was stirred at 25 °C for 12 h. The reaction mixture was partitioned between 200 mL of ethyl acetate and 20 mL of water. The organic layer was separated, washed with three 20-mL portions of brine, then dried over $MgSO_4$. The solution was concentrated under diminished pressure to afford a crude product which was purified by silica gel chromatography. Elution with 2:1 ethyl acetate−hexane afforded bithiazole **10**, which crystallized from ether as pale orange microcrystals: yield 0.19 g (70%); mp 121−122 °C; silica gel TLC R_f 0.75 (4:1 ethyl acetate−hexane); ^{1}H NMR ($CDCl_3$) δ 1.42 and 1.44 (2 s, 18 H), 1.97 (qn, 2 H, J = 6.6 Hz), 2.13 (s, 3 H), 2.61 (t, 2 H, J = 6.6 Hz), 3.20−3.40 (m, 4 H), 3.60 (q, 2 H, J = 6.6 Hz), 4.07 (m, 1), 5.14 (br s, 1 H), 5.43 (br s, 1 H), 7.50 (m, 1 H), 7.60 (s, 1 H) and 7.96 (s, 1 H); mass spectrum (chemical ionization) m/z 572 $(M + H)^+$. Anal. Calcd for $C_{24}H_{37}N_5O_5S_3$: C, 50.42; H, 6.52; N, 12.25. Found: C, 50.38; H, 6.46; N, 12.15.

2′-[(1,3-Bis-(*N*-(*tert*-butoxycarbonyl)amino))-2-propyl]-2,4′-bithiazole-4-carboxamide 11. Methyl iodide (0.88 g, 6.2 mmol) was added dropwise to a stirred solution of 0.18 g (0.31 mmol) of bithiazole **10** in 2 mL of methanol at room temperature. The reaction mixture was stirred at room temperature for 2 days and was then concentrated under diminished pressure to yield a dark yellow residue. This residue was purified by preparative silica gel TLC. Elution with 4:1:1 *n*-butanol−glacial acetic acid−water afforded bithiazole **11** as a colorless foam: yield 0.11 g (61%); silica gel TLC R_f 0.51 (4:1:1 *n*-butanol−glacial acetic acid−water); ^{1}H NMR (CD_3OD) δ 1.34 (s, 9 H), 1.44 (s, 9 H), 2.16 (qn, 2 H, J = 7.5 Hz), 2.97 (s, 6 H), 3.00−3.35 (m, 4 H), 3.43 (t, 2 H, J = 7.5 Hz), 3.59 (t, 2 H, J = 7.5 Hz), 4.00 (br s, 1 H), 8.17 (s, 1 H), and 8.19 (s, 1 H); mass spectrum (FABMS) m/z 586.222 ($C_{25}H_{40}N_5O_5S_3$ requires 586.219).

2′-(1,3-Diamino-2-propyl)-2,4′-bithiazole-4-carboxamide 1. To a stirred solution of 0.05 g (0.08 mmol) of bithiazole **11** in 3 mL of dry dichloromethane at 0 °C under argon was added 0.6 mL of 4 N anhydrous hydrogen chloride in dioxane (2.4 mmol). The reaction mixture was stirred at 0 °C for 1 h, then concentrated under diminished pressure to yield 0.05 g of crude **1**: silica gel TLC R_f 0.45 (1:1 10% NH_4HCO_3−CH_3OH). The crude product was dissolved in 1 mL of water and applied to a (6 × 80 mm) column of Dowex 50W (16−40 mesh, H^+-form). After washing with 100 mL of water, the column was eluted with a linear gradient of HCl (0−7 N; 10-mL fractions) at a flow rate of 25 mL/h. The column fractions were monitored for UV absorption at 292 nm; the appropriate fractions were combined and concentrated *in vacuo*. Lyophilization of an aqueous solution afforded 0.01 g of bithiazole **1** as a pale yellow solid. The purity of the sample was confirmed by analytical HPLC analysis using an Alltech Econosphere C-18 reversed phase column (100 × 4.6 mm); elution was with a 20-min gradient of 0.1% trifluoroacetic acid containing 10−50% acetonitrile at a flow rate of 1.4 mL/min. Detection at 292 and 260 nm demonstrated that only one peak eluted, with measured retention times at the two wavelengths of 3.40 and 3.38 min, respectively; ^{1}H NMR (D_2O) δ 2.15 (qn, 2 H, J = 7.0 Hz), 2.90 (s, 6 H), 3.37 (t, 2 H, J = 7.0 Hz), 3.49−3.71 (m, 6 H), 4.24 (qn, 1 H, J = 6.5 Hz), 8.17 (s, 1 H), 8.19 (s, 1 H); mass spectrum (FABMS) m/z 386.112 ($C_{15}H_{24}N_5OS_3$ requires 386.114).

Relaxation of Supercoiled DNA by Bithiazole 1 in the Presence of Metal Ions. Reaction mixtures (40 μL total volume) contained 0.2 μg of pBR322 Form I DNA, 5 mM Na borate (pH 8.0), and 10 μM each bithiazole **1** and metal ion. The metal salts employed were $Fe(NH_4)_2(SO_4)_2$, $Ni(OAc)_2$, $CoCl_2$, $Mn(OAc)_2$, $CuCl_2$, and $ZnCl_2$. Reaction mixtures were incubated at room temperature for 1 h, then quenched by the addition of EDTA to a final concentration of 5 mM, along with 8 μL of loading buffer. Samples were loaded onto a 1.2% agarose gel containing ethidium bromide (1 μg/mL). The gel was run at 50 V for 12 h and visualized by UV illumination.

Relaxation of Supercoiled DNA by Co(II)·Bithiazole 1. Reaction mixtures (40 μL total volume) contained 0.2 μg of pBR322 Form I DNA, 5 mM Na borate (pH 8.0), and 2.5, 5, 10, or 20 μM each bithiazole **1** and $CoCl_2$. Reaction mixtures were incubated at room temperature for 30 min, then quenched with EDTA to a final concentration of 5 mM, along with 8 μL of loading buffer. Samples were loaded onto a 1.2% agarose gel containing ethidium bromide (1 μg/mL). The gel was run at 50 V for 12 h and visualized by UV illumination.

Relaxation of Supercoiled DNA by Co(II)·Bithiazole 1 under Anaerobic Conditions. Reaction mixtures (80 μL total volume) contained 0.4 μg of SV40 Form I DNA, 5 mM Na borate (pH 8.0), 5 μM bithiazole **1**, and 5 μM $CoCl_2$, as indicated. Reaction mixtures were incubated at room temperature for 30 min, then quenched by the addition of EDTA to a final concentration of 5 mM. BLM reactions (80 μL total volume) contained 0.4 μg of SV40 Form I DNA, 5 mM Na cacodylate (pH 7.2), 0.5 μM BLM A_2, and 0.5 μM $Fe(NH_4)_2(SO_4)_2$. Reaction mixtures were incubated at room temperature for 30 min, then quenched by the addition of EDTA to a final concentration of 5 mM. Reactions performed anaerobically were carried out inside a glovebag which had been alternately evacuated by aspiration and filled with argon (three times). All solutions to be used in the experiment were lyophilized, then reconstituted inside the glovebag with degassed, argon-saturated water. All reactions were quenched by the addition of EDTA to a final concentration of 5 mM before exposure to air. Loading buffer (16 μL) was added to each reaction mixture prior to loading onto a 1.2% agarose gel containing ethidium bromide (1 μg/mL). The gel was run at 50 V for 12 h and visualized by UV illumination.

Preparation of a 5′-^{32}P End Labeled DNA Restriction Fragment. A 158-bp (bp = basepair) restriction fragment from pBR322 was isolated and 5′-^{32}P end labeled as described.[54] Briefly, plasmid pBR322 DNA[55] was linearized with restriction endonuclease *Hin*dIII, dephosphorylated with calf intestine alkaline phosphatase, then 5′-^{32}P end labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP. Digestion with *Eco*RV afforded the 158-bp fragment, which was purified on an 8% native polyacrylamide gel. The gel was visualized by autoradiography, the band of interest was excised from the gel, and the DNA was eluted into 0.6 M ammonium acetate containing 0.1 mM EDTA and 0.1% SDS and then recovered by ethanol precipitation.

Preparation of a 3′-^{32}P End Labeled DNA Restriction Fragment. pBR322 (30 μg) was digested simultaneously with 90 units each of restriction endonucleases *Hin*dIII and *Eco*RV (one unit is the amount of enzyme required to digest 1 μg of DNA substrate completely in 1 h at 37 °C) in a reaction mixture (200 μL total volume) containing 10 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 100 mM NaCl, and 1 mM β-mercaptoethanol. The reaction mixture was incubated at 37 °C for 6 h, and the DNA was recovered by ethanol precipitation.

The digested DNA was 3′-^{32}P end labeled[56] in a reaction mixture (50 μL total volume) containing 10 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 100 mM NaCl, 1 mM β-mercaptoethanol, and 0.25 mCi of [α-^{32}P]dATP. Seventy units of AMV reverse transcriptase (one unit

(54) Kane, S. A. Ph.D Thesis, University of Virginia, 1993.

(55) Sutcliffe, J. G. *Cold Spring Harbor Symp. Quant. Biol.* **1978**, *43*, 77.

(56) Lown, J. W.; Sondhi, S. M.; Ong, C.-W.; Skorobogaty, A.; Kishikawa, H.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 5111.

is the amount of enzyme required to incorporate 1 nmol of [^{3}H]TTP into acid precipitable material in 10 min at 37 °C) was added to initiate the reaction. The reaction mixture was incubated at 37 °C for 2 h.

Loading buffer (20 μL, 90 mM Tris−borate (pH 8.3), 50% glycerol, 0.1% bromophenol blue) was added to the reaction mixture, and the DNA fragment was purified on an 8% native polyacrylamide gel. The gel was visualized by autoradiography, the band of interest was excised from the gel, and the DNA was eluted into 0.6 M ammonium acetate containing 0.1 mM EDTA and 0.1% SDS and then recovered by ethanol precipitation.

DNA Strand Scission by Co(II)·Bithiazole 1. Reaction mixtures (10 μL total volume) contained 5 μM (nucleotide concentration) carrier pBR322 DNA, 5 × 10^4 cpm of 5′- or 3′-^{32}P end labeled 158-bp DNA, 5 mM Na borate (pH 8.0), 50 μM bithiazole **1**, and 50 μM $CoCl_2$, as indicated. Reaction mixtures were incubated at 55 °C for 4 h, then quenched by the addition of EDTA to a final concentration of 5 mM. Sonicated calf thymus DNA (4 μg) was added, and the DNA was recovered by ethanol precipitation. For reactions involving alkali treatment, NaOH was added to a final concentration of 0.2 N and the combined solution was heated at 90 °C for 10 min. After the solution was cooled, 4 μg of sonicated calf thymus DNA was added and the DNA was recovered by ethanol precipitation. After precipitation, the DNA was redissolved in 10 μL of water and 5 μL of loading solution. The resulting solution was heated at 90 °C for 10 min, chilled on ice, and then applied to a 20% denaturing polyacrylamide gel. The gel was run at 50 W for 2 h, then visualized by autoradiography. [The Fe(II)·BLM control reaction (10-μL total volume) contained 5 μM (nucleotide concentration) carrier pBR322 DNA, 5 × 10^4 cpm of 5′- or 3′-^{32}P end labeled 158-bp DNA, 5 mM Na cacodylate (pH 7.2), 5 μM BLM A_2, and 5 μM $Fe(NH_4)_2(SO_4)_2$. The reaction mixture was incubated at 0 °C for 1 h, then quenched by the addition of EDTA to a final concentration of 5 mM EDTA, along with 5 μL of loading solution. The resulting solution was heated at 90 °C for 10 min then chilled on ice before application to the gel.]

ESR Measurements. ESR spectra were recorded at room temperature using a Bruker ESP-300 spectrometer operating at 9.7 GHz with a 100 kHz modulation frequency. Instrumental settings were as follows: 20 mW microwave power, 0.35 G modulation amplitude, 0.327 s time constant, 2 × 10^4 gain, 100 G sweep width, 83.89 s sweep time, four scans collected. All samples contained 100 mM DMPO, which was thawed and used on the same day to avoid degradation at room temperature.

Reaction mixtures (150-μL total volume) contained 100 mM sodium borate buffer (pH 8.0), 1 mM bithiazole **1**, and 1 mM $CoCl_2$. The reaction was initiated by the addition of Co^{2+}, the sample was mixed, and 100 μL of the sample was transferred to a quartz ESR flat cell which was in turn placed in the spectrometer cavity. Sequential ESR data collection was then initiated. To obtain spectra under oxygen-depleted conditions, reaction mixtures were prepared with degassed, argon-saturated solutions, then transferred via a gastight syringe to a septum-sealed ESR cell that had been previously flushed with argon. When they were present, catalase and superoxide dismutase concentrations were 3250 and 125 units/mL, respectively. These reactions were carried out in Hepes buffer (pH 8.0) to ensure the stability of the enzymes. Control reactions indicated that the Co(II)·bithiazole **1** reaction worked equally as well in this buffer system.

Acknowledgment. We thank Dr. Laurie Kelsh, University of Virginia, for assistance with the ESR spectrometer. FAB mass spectral data were obtained at the Michigan State University Mass Spectrometry Facility, which is supported, in part, by a grant (DRR-00480) from the Biotechnology Research Technology Program, National Center for Research Resources, National Institutes of Health. This work was supported by Research Grant CA-53913, awarded by the National Cancer Institute, DHHS.

Supporting Information Available: Text describing protocol for the deprotection of thioamide **7** and absorption spectra for the oxygen adduct of the Co complex of deprotected **7** (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA951136D