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Photoactivated DNA Cleavage by Compounds Structurally Related to the Bithiazole Moiety of Bleomycin

James C. Quada, Jr, Didier Boturyn and Sidney M. Hecht*

Departments of Chemistry and Biology, University of Virginia, Charlottesville, VA 22904, USA

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Abstract—The syntheses of several novel halogenated bithiazoles structurally related to the bithiazole moiety of bleomycin A_5 are described. Also described is the ability of these compounds to mediate photoactivated DNA cleavage. Chlorinated bithiazole analogues were shown to be much more active than an analogous brominated derivative. DNA strand scission activity was strictly light dependent and was accompanied by dechlorination of the bithiazole nucleus, apparently in a stoichiometric fashion. Inhibition of DNA cleavage in the presence of DMSO, as well as photoaddition to 1-octene by both brominated and chlorinated bithiazole derivatives, suggest strongly that the initial step in photoactivated DNA cleavage involves homolysis of the thiazole carbon-halogen bond. The chlorinated bithiazoles were found to mediate sequence selective cleavage of a ^{32}P-end labeled DNA, although the selectivity observed was not the same as that of bleomycin itself. The implications of this observation are discussed.

The bleomycins (BLMs) are a class of antitumor agents[1] believed to mediate their cytotoxic effects at the level of DNA,[2] and possibly also RNA[3] degradation. Extensive mechanistic studies have focused on the molecular details of activation of the metal–O_2 complexes of bleomycin and subsequent DNA degradation,[2] as well as the structural elements in BLM that control sequence selective DNA interaction.[4] The bithiazole moiety of BLM has been shown to bind to DNA,[5] but is not the primary source of the characteristic sequence selective cleavage of DNA by BLM,[6] which involves the pyrimidine moieties of many 5′-GC-3′ and 5′-GT-3′ sequences.[7] To study the nature of bithiazole–DNA interaction, we sought to equip the bithiazole with a small reporter group, analogous to those used to study DNA binding by other ligands.[8]

Chlorpromazine is a member of the promazine class of antipsychotic drugs, which are characterized by phototoxic and photosensitizing side effects.[9] Chlorpromazine mediates photoactivated DNA cleavage which is unaffected by oxygen radical scavengers such as *t*-butanol, sodium benzoate, and sodium formate.[10] EPR experiments have shown that chlorpromazine photolysis (330 nm) does not involve active oxygen species, and that spin adducts characteristic of a phenyl free radical[10c] or of those resulting from UV photolysis of carbon tetrachloride[11] are formed. These results implicate homolysis of the carbon–chlorine bond of chlorpromazine as the initial event in photoactivated DNA cleavage.

We hypothesized that a chlorinated bithiazole irradiated in the presence of DNA could, like chlorpromazine, undergo photohomolysis to produce reactive species capable of DNA cleavage. Reported herein is the synthesis of halogenated bithiazole molecules (**1a–d**) structurally related to bleomycin A_5, and their ability to mediate remarkably efficient photoactivated DNA cleavage.[12] Also described are mechanistic experiments that support a cleavage mechanism involving initial photohomolysis of the carbon–halogen bond, and conditions that facilitate extensive DNA cleavage.

Results and Discussion

Synthesis of halogenated bithiazole derivatives

It has been shown that thiazoles can be deprotonated with lithium bases and substituted with a variety of electrophiles,[13] and experimental conditions were optimized for lithium diisopropylamide lithiation and in situ halogenation using hexachloroethane. *N*-Boc protected ethyl (2-aminoethyl)thiazole-4-carboxylate **2**[14] (Scheme 1) was treated with excess lithium diisopropylamide and hexachloroethane at −78 °C to produce chlorothiazole **3**

*Corresponding author. Tel.: +1-804-924-3906; fax: +1-804-924-7856; e-mail: sidhecht@virginia.edu

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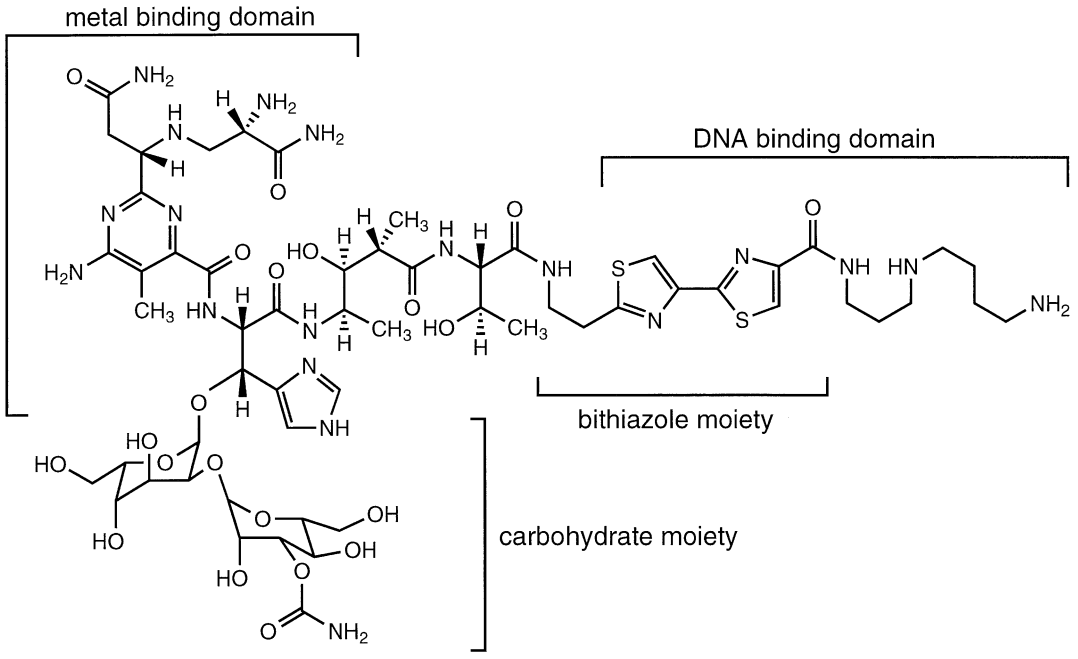
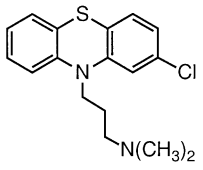
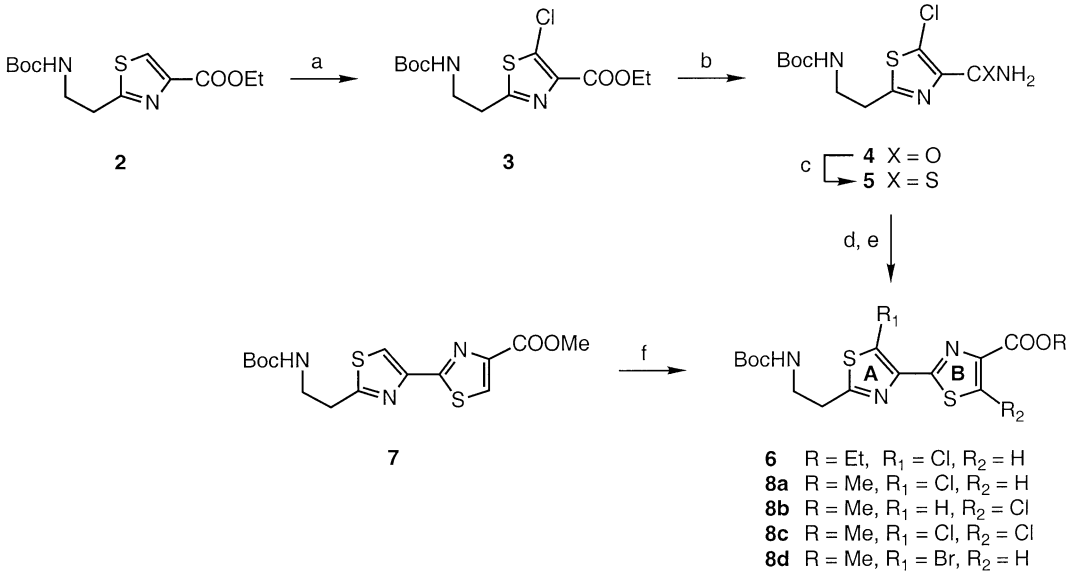




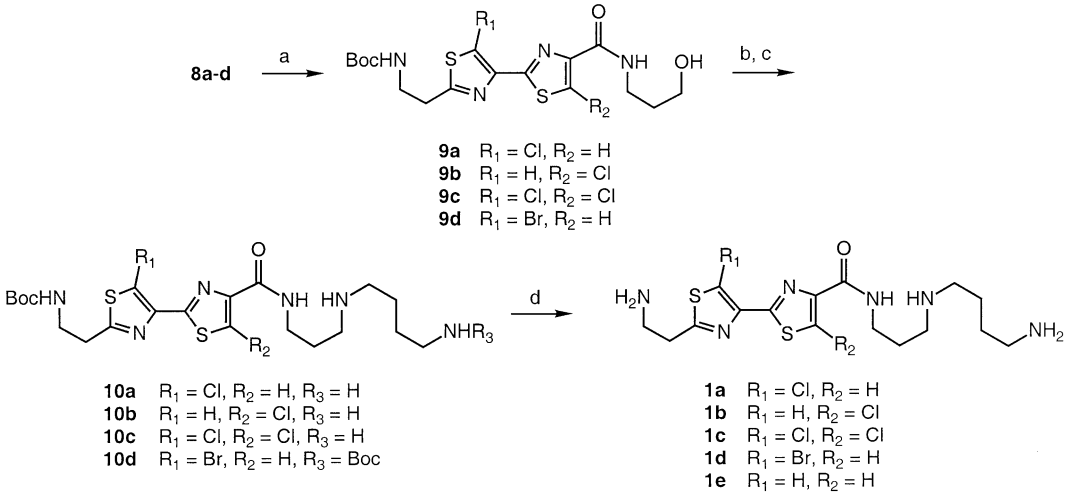
chlorpromazine

in 39% yield. Ester **3** was converted to amide **4** using ammonia, and then to thioamide **5** via the agency of Lawesson's reagent[15] (62% yield for two steps). Hantzsch cyclization of **5** with ethyl bromopyruvate was performed in the presence of 1,2-epoxybutane to trap HBr as it evolved during cyclization.[16] The crude hydroxylated intermediate was then dehydrated with trifluoroacetic anhydride in pyridine[17] at −20 °C to afford monochlorobithiazole ethyl ester **6** in 70% yield. Although not the most convenient method for preparing the requisite halogenated bithiazoles, this route established the position of chlorination unambiguously.

More direct access to the halogenated bithiazoles was sought starting from preformed bithiazole derivatives. Due to low yields and side reactions in the lithiation–halogenation method of preparing chlorothiazoles, other methods of halogenation were also explored. Following published reports[18,19] of radical halogenation of



Scheme 1. Halobithiazole synthesis: (a) iPr_2NLi, Cl_3CCCl_3, THF, 39%; (b) NH_3, EtOH, 92%; (c) Lawesson's reagent, THF, 67%; (d) ethyl bromopyruvate, 1,2-epoxybutane, to toluene; (e) $(CF_3CO)_2O$, 70%; (f) (**8a**) *N*-chlorosuccinimide, hν, 74%; (**8b**) iPr_2NLi, Cl_3CCCl_3, THF, 21%; (**8c**) iPr_2NLi, Cl_3CCCl_3, THF, 25%; (**8d**) *N*-bromosuccinimide, CH_3CN, hν, 42%.



Scheme 2. Synthesis of halobithiazoles **1a**–**1e;** (a) 3-aminopropanol; (b) *p*-toluenesulfonyl chloride, pyridine; (c) (Boc)1,4-diaminobutane; (d) CF_3COOH or HCl.

heterocyclic compounds, photohalogenation of *N*-Boc protected methyl 2-(2′-aminoethyl)-2,4′-bithiazole **7**[14] was attempted. A degassed acetonitrile solution of *N*-chlorosuccinimide and **7** was irradiated in Pyrex glassware using a 100 watt low pressure mercury lamp at 25 °C. This procedure provided chlorobithiazole methyl ester **8a** in 74% yield. Treatment of **8a** with excess 3-aminopropanol in methanol effected conversion into hydroxypropyl amide **9a** in 80% yield; the product was identical in all respects to **9a** produced by treatment of **6** with 3-aminopropanol. Hydroxypropyl amide **9a** was converted to the respective tosylate by treatment with 5 equiv of *p*-toluenesulfonyl chloride in dry pyridine at 0–4 °C for 22 h, then 50 equiv of 1,4-diaminobutane was added; this procedure afforded Boc-protected chlorobithiazole **10a** in 58% yield from **9a**. Boc deprotection was accomplished with trifluoroacetic acid in dichloromethane, providing chlorobithiazole **1a** as a light yellow solid in 49% yield.

While photohalogenation of the bithiazole moiety provided the N-terminal chlorobithiazole in good yield, no C-terminal chlorinated product was formed under any condition attempted. Although the lithiation-halogenation procedure used for the conversion **2**→**3** had proceeded in rather low yield, we nonetheless attempted this procedure on bithiazole **7**. It was found that lithiation and in situ halogenation of **7** with optimal amounts of base and halogenating agent could provide predominantly the C-terminal monochloroinated bithiazole **8b** or bischlorobithiazole **8c**, which could be separated chromatographically. Using 2.5 equiv of lithium diisopropylamide and 4 equiv of hexachlorethane, monochlorobithiazole **8b** was obtained in 21% yield, along with bischlorobithiazole **8c** (6%) and starting material (62% recovery).

Attempts to improve the yield of monochlorobithiazole with more lithium diisopropylamide and hexachloroethane resulted instead in increased yields of bischlorobithiazole **8c**. The best yield of bischlorobithiazole was achieved with 3.2 equiv each of lithium diisopropylamide and hexachlorethane, providing **8c** in 25% yield, as well as monochlorobithiazole (11%) and starting material (20% recovery).

Aminolysis of the mono- and bischlorobithiazole methyl esters (**8b** and **8c**, respectively) was accomplished using 50 equiv of 3-aminopropanol to provide hydroxypropylamides **9b** (68% yield) and **9c** (75% yield) (Scheme 2). That **9b** was chlorinated on ring B (cf. Scheme 1) was confirmed by comparison of the chemical shift of the thiazole proton in **9b** (7.78 ppm) with the thiazole proton in authentic **9a** (8.17 ppm); the latter was chlorinated on ring A. This chemical shift difference was consistent for each intermediate in both monochlorinated series. Tosylation of **9b** was accomplished using 5 equiv of *p*-toluenesulfonyl chloride in dry pyridine (0–4 °C) and provided the tosylate in 72% yield. Treatment with 50 equiv of 1,4-diaminobutane in dry pyridine afforded Boc-protected monochlorobithiazole **10b** in 63% yield. The tosylate derived from **9c** was not isolated, but was treated directly with 50 equiv of 1,4-diaminobutane, to give bischlorobithiazole **10c** in 60% yield. Deprotection of both **10b** and **10c** (HCl, CH_2Cl_2, 25 °C)[20] and purification by reversed-phase chromatography provided monochlorobithiazole **1b** in 67% yield and bischlorobithiazole **1c** in 78% yield.

The success of the photochlorination procedure in providing monochlorobithiazole derivatized on ring A led

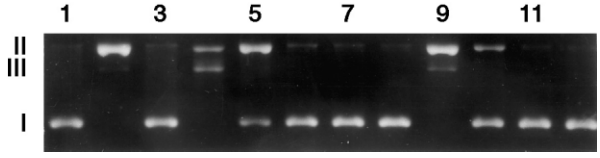


Figure 1. Relaxation of supercoiled pBR322 DNA by chlorobithiazole **1b**. The incubation mixtures including 200 ng of DNA were irradiated for 5 min with 1000 watt high pressure (lanes 3–7) or 100 watt low pressure (lanes 8–12) mercury lamps. Lanes 1 and 2 were not irradiated. Lane 1, DNA alone; lane 2, 10 μM Fe^{2+} + 55 mM H_2O_2; lane 3, DNA alone; lane 4, 100 nM **1b**; lane 5, 10 nM **1b**; lane 6, 1 nM **1b**; lane 7, 100 pM **1b**; lane 8, DNA alone; lane 9, 100 nM **1b**; lane 10, 10 nM **1b**; lane 11, 1 nM **1b**; lane 12, 100 pM **1b**.

to the attempted photobromination of **7** using *N*-bromosuccinimide. Equimolar amounts of *N*-bromosuccinimide and bithiazole **7** were irradiated in deoxygenated acetonitrile; monobromobithiazole **8d** was obtained in 42% yield. The thiazole proton of monobromobithiazole resonated at 8.26 ppm in the ^{1}H NMR spectrum, almost identical to the chemical shift of the thiazole proton of monochlorobithiazole **8a** (8.24 ppm). Ten equiv of aminopropanol in methanol was employed to convert monobromobithiazole ester **8d** to the respective hydroxypropylamide **9d** in 82% yield. The tosylate was prepared from **9d** in 58% yield using 5 equiv of *p*-toluenesulfonyl chloride in dry pyridine. In order to reduce the amount of diaminobutane for conversion of the tosylate to the requisite spermidine derivative **10d**, and thereby facilitate purification, tosylate **9d** was treated with 4.3 equiv of mono Boc protected 1,4-diaminobutane to afford *N,N′*-bis *tert*-Boc-protected bromobithiazole **10d** in 16% yield. Deprotection was accomplished with trifluoroacetic acid in dichloromethane to afford monobromobithiazole **1d** in 50% yield.

DNA cleavage activity

A preliminary report[12] illustrated a comparison of the DNA cleavage activity of photoactivated monochlorobithiazole **1b** and bischlorobithiazole **1c**, showing that the extent of chlorine substitution was directly proportional to the extent of DNA cleavage observed at a constant level of irradiation. Another DNA plasmid relaxation experiment is shown in Figure 1, in which a single chlorobithiazole was irradiated with lamps having different intensities. The figure illustrates DNA cleavage resulting from irradiation of a solution containing 100 nM to 100 pM concentrations of monochlorobithiazole **1b** with a 1000 watt high pressure mercury lamp or a 100 watt low pressure mercury lamp. Lanes 4 and 5 show greater extents of DNA plasmid relaxation (1000 watt lamp) than can be seen in lanes 9 and 10 (100 watt lamp). Other experiments (data not shown) have also demonstrated the requirement for light in chlorobithiazole-mediated DNA scission, and that increased irradiation time results in increased DNA cleavage.

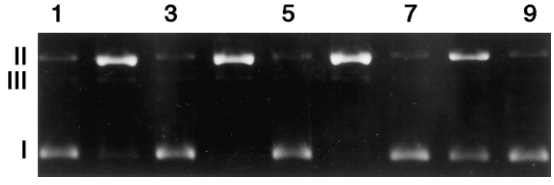


Figure 2. Relaxation of supercoiled pBR322 DNA by chlorobithiazoles **1b** and **8b**. The incubation mixtures including 200 ng of DNA were irradiated (lanes 3–9) with a 100 watt low pressure mercury lamp for 10 min. Lane 1, DNA alone; lane 2, 10 μM Fe^{2+} + 55 mM H_2O_2; lane 3, DNA alone; lane 4, 200 nM **1b**; lane 5, 200 nM **8b**; lane 6, 100 nM **1b**; lane 7, 100 nm **8b**; lane 8, 50 nM **1b**; lane 9, 50 nM **8b**.

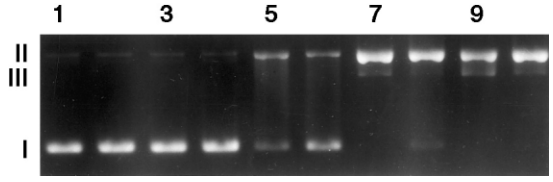


Figure 3. Relaxation of supercoiled pBR322 DNA by chlorobithiazoles **1a**–**c**. The incubation mixtures including 200 ng of DNA were irradiated with a 100 watt low pressure mercury lamp for 20 min. The reaction mixtures in lanes 2 and 4–10 were irradiated. Lanes 1 and 3 were not irradiated. Lanes 1 and 2, DNA alone; lanes 3 and 4, DNA + 10% DMSO; lane 5, 100 nM **1a**; lane 6, 100 nM **1a** + 10% DMSO; lane 7, 100 nM **1b**; lane 8, 100 nM **1b** + 10% DMSO; lane 9, 100 nM **1c**; lane 10, 100 nM **1c** + 10% DMSO.

It may be noted that greater than 50% conversion of Form I → Form II DNA was achieved using 10 nM **1b**. Since the concentration of DNA plasmid employed was ~3–4 nM, this represents an exceptionally efficient process for DNA nicking, and suggests that the bithiazole must be activated essentially stoichiometrically and also likely binds to its DNA target to facilitate cleavage. The putative association of the bithiazoles with DNA prior to cleavage is also supported by the observation that cleavage by **1b** was more efficient at 0 °C than at 25 °C (data not shown).

In order to define the structural nature of the apparent requirement for DNA binding, protected monochlorobithiazole **8b** was tested in a DNA plasmid relaxation assay in direct comparison with monochlorobithiazole **1b**. As shown in Figure 2, concentration dependent conversion of Form I to Form II DNA resulted when **1b** was employed (lanes 4, 6, and 8), but irradiation of protected analogue **8** (which should bind DNA weakly if at all since it lacks a spermidine substituent) in the presence of Form I DNA failed to produce DNA strand scission at identical concentrations (lanes 5, 7, and 9). These experiments begin to demonstrate the remarkable chemistry of these agents: very high efficiency transduction of light into DNA cleavage, which is proportional to irradiation time, extent of bithiazole halogenation and intensity of irradiation, suggesting that halogen may be intimately involved in the cleavage process. That DNA binding is required for cleavage to occur suggests that the active species may be formed in close proximity to the DNA target, assuming that both **8b** and **1b** are activated to the same extent as seems reasonable.

Mechanism of chlorobithiazole activation for DNA cleavage

Having defined the conditions required to obtain effective photoactivated DNA cleavage, experiments were

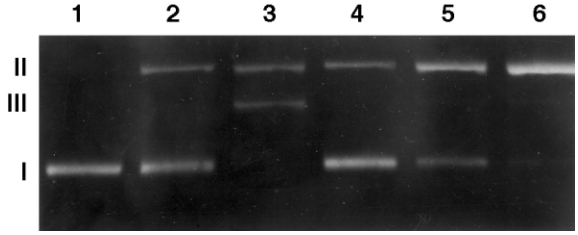


Figure 4. Relaxation of supercoiled pGEM3Zf(+) DNA by chlorobithiazole **1a** in the presence and absence of O_2. Lane 1 was not irradiated. Reaction mixtures including 200 ng of DNA in lanes 2–6 were irradiated under argon (lanes 4 and 6) or an ambient atmosphere. Lanes 1 and 2, DNA alone; lanes 3 and 4, 100 μM Fe(II)·BLM A_2; lanes 5 and 6, 50 nM **1a**.

designed to elucidate the mechanism of photoactivation. Dimethyl sulfoxide has been used with spin trapping reagents to probe the photoreactivity of chlorpromazine.[10a] The authors interpreted their results in terms of the reaction of promazine and chlorine radicals with DMSO. Therefore, an experiment was performed (Fig. 3) to determine the ability of DMSO to inhibit DNA cleavage by the chlorobithiazoles. Photoactivated DNA cleavage by all compounds except bischlorobithiazole **1c** was diminished in the presence of 10% (v/v) DMSO (lanes 6, 8, and 10).

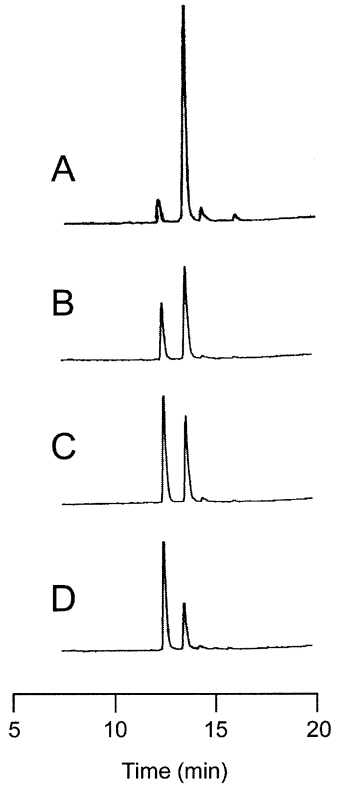
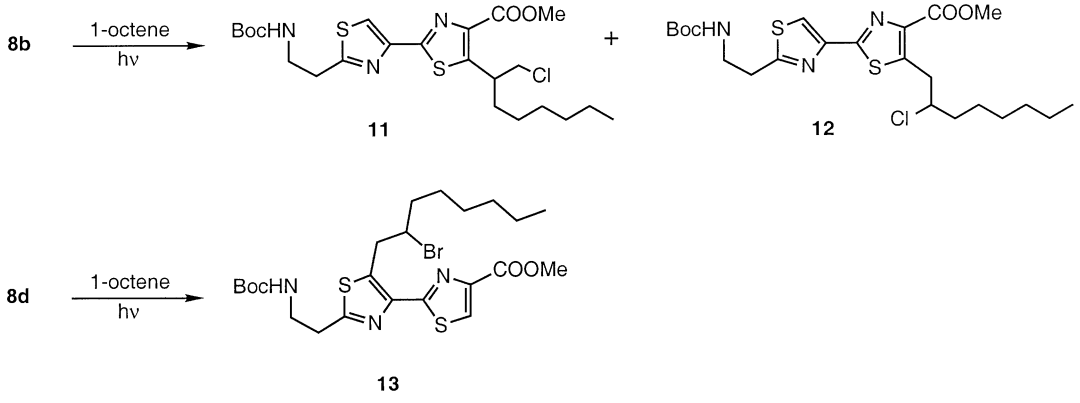


Figure 5. Dechlorination of chlorobithiazole **1b** during irradiation in the presence of calf thymus DNA. Aliquots of the irradiated solution were analyzed by C_{18} reversed-phase HPLC. A, nonirradiated control; B, 5 min irradiation; C, 10 min irradiation; D, 20 min irradiation. An authentic sample of **1e** was found to co-elute with the peak at 12.3 min.

Dimethyl sulfoxide has also been shown to trap active oxygen species,[21] so the requirement for oxygen in DNA cleavage by the chlorobithiazoles was studied. As shown in Figure 4, under conditions that were sufficiently anaerobic to substantially suppress the relaxation of supercoiled plasmid DNA by 100 μM Fe(II)•bleomycin A_2, DNA cleavage by **1a** was essentially unaffected by the presence or absence of dioxygen. In this particular experiment, a slight enhancement of cleavage was actually observed when oxygen was absent. An analogous effect has been documented in a study of chlorpromazine.[10a] The inhibition seen in the above experiment utilizing DMSO must, therefore, have arisen from the interaction of DMSO with some other species formed upon irradiation of the chlorobithiazoles.

In order to determine the fate of the C–Cl bond of the chlorobithiazole during irradiation with DNA, monochlorobithiazole **1b** (100 μM concentration) was irradiated in the presence of 400 μg of calf thymus DNA for 5, 10, or 20 min (Fig. 5). A control reaction was also performed in which the same amounts of DNA and **1b** were incubated together in the absence of light for 20 min. The DNA was precipitated and the supernatant was assayed by C_{18} reversed-phase HPLC. The control reaction resulted in trace A and the peak at 13.4 min corresponded to **1b** as judged by co-injection with authentic **1b**. With increasing irradiation time the peak corresponding to **1b** at 13.4 min diminished and a new peak (at 12.3 min) increased concurrently; few other peaks were found in the HPLC traces. Co-injection of the trace C sample (10-min irradiation) and a roughly equal amount of authentic nonchlorinated analogue **1e**[22] caused the peak at 12.3 min to approximately double in size. In addition, the UV spectra associated with the peaks at 12.3 and 13.4 min matched the UV spectra of nonchlorinated bithiazole **1e** and monochlorobithiazole **1b**, respectively. These results showed that in the presence of DNA, chlorobithiazole **1b** was dechlorinated with very few side products, under conditions utilized for DNA cleavage. Photohomolysis of the C–Cl bond in chlorobithiazole would produce chlorine atoms and carbon-centered thiazole radicals, which could be quenched by abstraction of deoxyribose hydrogen atoms from DNA in much the same manner as has been demonstrated for the enediyne antibiotics.[23]



Scheme 3. Photo-addition of **8b** and **8d** to 1-octene.

In fact, it has been shown that Cl• can mediate DNA cleavage.[24]

Having demonstrated C–Cl bond cleavage under the conditions of the photoactivated DNA cleavage reaction, analogue **1d** was prepared in which chlorine was replaced with bromine. The comparative photoactivated DNA cleavage of chlorobithiazole **1a** and bromobithiazole **1d** has been reported;[12] the bromobithiazole analogue induced no DNA cleavage at concentrations up to 200 nM, whereas chlorobithiazole analogue **1a** was able to completely convert supercoiled DNA into products under the same conditions. If photocleavage is initiated via homolysis of the C–X bond, diminished DNA cleavage by the bromobithiazole could mean that the bromobithiazole homolyzes to a lesser extent, or that the bromine atom, once formed, is less efficient as a DNA cleavage agent than the chlorine atom. These possibilities were investigated by studying the relative photochemical reactivies of the halobithiazoles. Photoinduced radical addition to olefins is known for organic halides.[25] Irradiation of monochlorobithiazole **8b** in deoxygenated 1-octene for 1 h (Scheme 3) produced two products. These were isolated and structures assigned as 2-bithiazolyl-1-chlorooctane **11** (31% yield) and 1-bithiazolyl-2-chlorooctane **12** (39% yield) on the basis of mass spectral and NMR data. As a control reaction monochlorobithiazole **8b** was heated at reflux in 1-octene for 1 h in the absence of light. There was no reaction and the starting material was recovered quantitatively, which provides support for a radical process. Monobromobithiazole **8d** was also shown to undergo photoinduced addition to the double bond of 1-octene, but only 1-bithiazolyl-2-bromooctane **13** was formed (34% yield). This is the same type of product that is formed by bromotrichloromethane[26] in olefin photoadditions. A mechanism that could account for the different product distribution in these photoadditions might involve initial attack on the olefin at C-1, to form the more stable stable 2° free radical intermediate. In the case of **8b**, either the thiazole radical or chlorine atom may initiate attack on the double bond, then the remaining two radicals could combine to form the observed products **11** and **12**. The formation of only one photoadduct from 1-octene and bromobithiazole **9** may indicate that only the thiazole radical was responsible for initial attack on the double bond, and the less reactive bromine atom quenched the intermediate 2° octenyl radical to complete the addition. Taken together with the observed difference between chlorobithiazole **1a** and bromobithiazole **1d** as DNA cleaving agents, the photoaddition data support the interpretation that both C–Cl and C–Br bonds are efficiently photohomolyzed by irradiation, but that the bromine atom so liberated is less reactive than the chlorine atom in the event that initiates DNA cleavage, presumably abstraction of a sugar hydrogen atom. While the enediyne antibiotics utilize an aryl radical to abstract H atoms from DNA, the present data argue that DNA cleavage by the chlorobithiazoles involves Cl•, a conclusion that is consistent with the observation of Armitage and Schuster.[24,27]

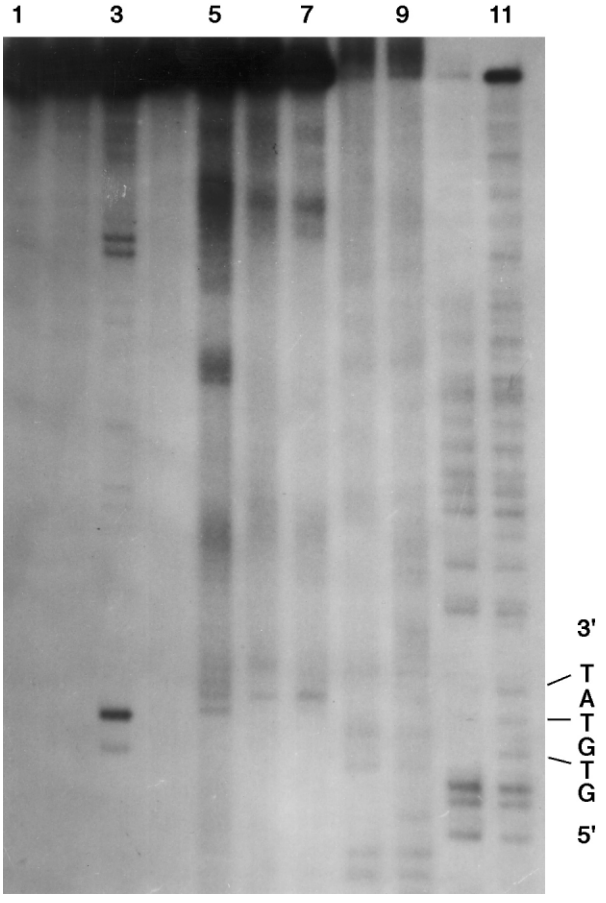


Figure 6. Cleavage of a 5′-^{32}P end labeled 158 base pair DNA duplex by Fe(II)•BLM A_2 or by nonchlorinated bithiazole **1e** and chlorobithiazoles **1a**–**c** during irradiation with a 100 watt low pressure mercury lamp apparatus for 15 min. The reaction mixtures in lanes 2 and 4–7 were irradiated. Lanes 1 and 2, DNA alone; lane 3, 2 μM Fe(II)•BLM A_2; lane 4, 1 μM nonchlorinated bithiazole **1e**; lane 5, 1 μM chlorobithiazole **1a**; lane 6, 1 μM chlorobithiazole **1b**; lane 7, 1 μM bischlorobithiazole **1c**; lanes 8–11, Maxam-Gilbert G, G + A, C, and C + T reactions, respectively.

Sequence selectivity of DNA cleavage

Also investigated has been the sequence selectivity of DNA cleavage by the chlorobithiazoles. The sequence selectivities of 5′-^{32}P end labeled DNA cleavage by chlorobithiazoles **1a**–**1c** are compared in Figure 6. DNA cleavage by 2 μM Fe(II)•bleomycin $A_2 + O_2$ is shown for comparison (lane 3), and lanes 5–7, respectively, demonstrate the patterns of DNA cleavage resulting from treatment with 1 μM **1a**–**1c**. At the 5′-GTGTAT-3′ site (nucleotides 82–87),[28] Fe(II)•BLM A_2 cleaves at T_{83} and T_{85}, monochlorobithiazole **1a** has a cleavage band at A_{86}, and monochlorobithiazole **1b** exhibits cleavage bands at T_{87}. At this position, the orientations of cleavage sites lend themselves to the interpretation that **1a**–**1c** bind in the minor groove of DNA in a single orientation, consistent with findings for chlorobleomycin derivatives prepared using these abbreviated bithiazoles.[29] That these bands are close to sites of Fe•BLM-mediated cleavage of this substrate (cf. lane 3) supports the idea[29] that the bithiazole moiety may in some cases reinforce the sequence selectivity of DNA binding defined by the metal binding domain of bleomycin, and thereby create strongly preferred sites for DNA cleavage.[6] However,

the gel in Figure 6 also reflects chlorobithiazole cleavage sites not close to any site of cleavage mediated by Fe(II)·BLM (cf. lane 3 and lanes 5–7), and vice versa, indicating that the sites preferred for DNA binding by the metal binding and bithiazole domains of bleomycin are not always the same.

The accumulated data support a mechanism for DNA cleavage initiated via carbon–halogen bond photohomolysis. Chlorobithiazole-derived radical species are among the most efficient DNA damaging agents known, and their utility in study of DNA–bleomycin interactions has the wherewithal to provide further insight into the nature of bleomycin–DNA interaction.

Experimental

All irradiations were performed in Pyrex glassware in a Rayonet preparative photochemical reactor, type RS, from Southern New England Ultraviolet Company that was equipped with four RUL 2537Å lamps, or with a high pressure mercury capillary lamp, Model BH61B, from TJ Sales. ^{1}H NMR spectra were recorded at 300 MHz; chemical ionization mass spectra were obtained using methane as reagent gas. Melting points are uncorrected. Blenoxane was obtained from Bristol-Myers Squibb Pharmaceuticals and was fractionated to provide bleomycin A_2 as described.[30] Supercoiled plasmid DNAs were obtained from Promega (Madison, WI). Reagent grade water for bioassays was produced from a Millipore Milli-Q system.

Ethyl 2-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5-chlorothiazole-4-carboxylate (3). A solution containing 1.6 g (5.4 mmol) of *N*-Boc-thiazole ethyl ester **2** and 5.71 g (24.1 mmol) of Cl_3CCCl_3 in 120 mL of dry THF was cooled to −78 °C with stirring under argon. Lithium diisopropylamide (33.8 mmol, 22.5 mL of a 1.5 M solution) was added dropwise over a period of 45 min. The reaction mixture was stirred for 30 min then diluted with 10 mL of water. The reaction mixture was partitioned between 100 mL of water and 250 mL of hexanes, and the organic layer was concentrated under diminished pressure. The residue was applied to a flash silica gel column,[31] and was washed with 1:2 ethyl acetate–hexanes to provide *N*-Boc-aminoethyl 5-chlorothiazole ester **3** as colorless needles from acetone–hexanes: yield 0.7 g (39%); mp 88 °C; ^{1}H NMR ($CDCl_3$) δ 1.38–1.47 (m, 12H), 3.15 (t, 2H), 3.52 (q, 2H), 4.43 (q, 2H), and 4.88 (br s, 1H); mass spectrum, *m*/*z* 335, 337 and 279. Anal. calcd for $C_{13}H_{19}ClN_2O_4S$: C, 46.63; H, 5.72; N, 8.37. Found: C, 46.69; H, 5.72; N, 8.37.

2-[2-(*tert*-Butoxycarbonyl)amino]ethyl 5-chlorothiazole-4-carboxamide (4). To a solution of 116 mg (0.35 mmol) of Boc-chlorothiazole ethyl ester **3** in 7 mL of ethanol in a heavy walled Pyrex tube cooled in dry ice was added ammonia gas for 5 min (until the volume increased by ~1 mL). The tube was then sealed and the solution was stirred at 25 °C for 3 days. The tube was again cooled in dry ice and opened, then warmed to 25 °C slowly with stirring to allow excess ammonia to evaporate. The solution was then concentrated under diminished pressure, and the residue was applied to a flash silica gel column, which was washed with 2:1 ethyl acetate–hexanes, affording Boc-protected 5-chlorothiazole amide **4** as a light yellow gum: yield 96 mg (92%); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.09 (t, 2H), 3.52 (t, 2H), 6.13 (br s, 1H) and 7.15 (br s, 1H); mass spectrum *m*/*z* 308 and 306 $(M+H)^+$, 252 and 250, and 208 and 206; HRMS *m*/*z* 307.0547 (M^+), $C_{11}H_{16}{}^{37}ClN_3O_3S$ requires 307.0570).

2-[2-(*t*-Butoxycarbonyl)amino]ethyl 5-chlorothiazole-4-thiocarboxamide (5)

A solution containing 96 mg (0.32 mmol) of Boc-protected 5-chlorothiazole amide and 78 mg (0.19 mmol) of Lawesson's reagent in 10 mL of dry THF under argon was heated at reflux with stirring for 75 min. The solution was then concentrated under diminished pressure and the residue was applied to a column of flash silica gel and washed with 1:1 ethyl acetate–hexanes, affording *N*-Boc 5-chlorothiazole thioamide **5** as a yellow solid: yield 67 mg (67%); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.22 (t, 2H) and 3.60 (q, 2H); mass spectrum *m*/*z* 324 and 322 $(M+H)^+$, 268 and 266, 232, and 224 and 222; HR-MS *m*/*z* 321.0394 (M^+) ($C_{11}H_{16}{}^{35}ClN_3O_2S_2$ requires 321.0372).

Ethyl 2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5′-chloro-2,4′-bithiazole-4-carboxylate (6). A solution containing 105 mg (0.33 mmol) of *N*-Boc 5-chlorothiazole thioamide **5**, 83 mg (0.42 mmol) of ethyl bromopyruvate, and 26 mg (0.36 mmol) of 1,2-epoxybutane in 10 mL of dry toluene under argon was heated at reflux for 30 min and then concentrated under diminished pressure. The residue was dissolved in 25 mL of ethyl acetate and the solution was washed with three 25-mL portions of water, three 25-mL portions of saturated brine, and then dried over anhydrous $MgSO_4$. Concentration under diminished pressure afforded a brown gum that was dissolved in 10 mL of dry CH_2Cl_2. Dry pyridine (129 mg, 1.6 mmol) was added to the mixture, which was then cooled to −20 °C. To this cooled solution was added dropwise 205 mg (0.98 mmol) of trifluoroacetic anhydride. The reaction mixture was stirred at −20 °C for 10 min and then concentrated under diminished pressure. The residue was applied to a flash silica gel column, that was washed with 2:1 hexanes–ethyl acetate to provide *N*-Boc protected 5′-chloro-2,4′-bithiazole ester **6** as colorless microcrystals from acetone–hexanes: yield 94 mg (70%); mp 92–93 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (m, 12H), 3.16 (t, 2H), 3.56 (q, 2H), 4.42 (q, 2H), 5.00 (br s, 1H) and 8.21 (s, 1H); mass spectrum *m*/*z* 420 and 418 $(M+H)^+$, 364 and 362, and 320 and 318. Anal. calcd for $C_{16}H_{20}ClN_3O_4S_2$: C, 45.98; H, 4.82; N, 10.05. Found: C, 46.07; H, 4.79; N, 10.04.

Methyl 2′-[2-(*t*-Butoxycarbonyl)amino]ethyl-5′-chloro-2,4′-bithiazole-4-carboxylate (8a). A solution containing 25 mg (69 μmol) of *N*-Boc protected bithiazole ester **7** and 12 mg (92 μmol) of *N*-chlorosuccinimide in 3 mL of acetonitrile in a test tube was stirred and purged with a stream of argon gas for 20 min, then irradiated at 25 °C

through a water filter for 40 min in a Rayonet apparatus. The solution was concentrated under diminished pressure and the residue was purified by preparative silica gel TLC (development was with 1:1 ethyl acetate–hexanes), to afford *N*-Boc protected 5′-chlorobithiazole ester **8a** as colorless microcrystals from ethyl acetate: yield 20 mg (74%); mp 132 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 3.16 (t, 2H), 3.57 (q, 2H), 3.97 (s, 3H), 4.96 (br s, 1H) and 8.24 (s, 1H); mass spectrum *m*/*z* 406 and 404 $(M+H)^+$, 350 and 348, and 306 and 304. Anal. calcd for $C_{15}H_{18}ClN_3O_4S_2$: C, 44.61; H, 4.49; N, 10.40. Found: C, 44.84; H, 4.50; N, 10.37.

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5′-chloro-2,4′-bithiazole-4-(3-hydroxypropyl)carboxamide (9a). A solution containing 600 mg (1.64 mmol) of *N*-Boc protected 5′-chlorobithiazole ester **8a** and 6.14 g (81.7 mmol) of 3-aminopropanol in 12 mL of methanol was stirred at 25 °C for 11 h, and was then concentrated under diminished pressure. The residue was applied to a column of flash silica gel, which was washed with ethyl acetate and acetone to afford 1.24 g of 5′-chlorobithiazole 3-hydroxypropylamide **9a** as a yellow solid that crystallized from acetone as colorless prisms: yield 586 mg (80%); mp 142 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.82 (m, 2H), 3.18 (q, 2H), 3.55–3.74 (m, 6H), 4.95 (br s, 1H), 7.73 (br s, 1H) and 8.17 (s, 1H); mass spectrum *m*/*z* 449 and 447 $(M+H)^+$, 393 and 391, and 349 and 347. Anal. calcd for $C_{17}H_{23}ClN_4O_4S_2$: C, 45.68; H, 5.19; N, 12.53. Found: C, 45.74; H, 5.17; N, 12.57.

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5′-chloro-2,4′-bithiazole-4-[3-(4-aminobutyl)aminopropyl]carboxamide (10a). A solution containing 455 mg (1.02 mmol) of 5′-chlorobithiazole 3-hydroxypropylamide **9a** and 975 mg (5.1 mmol) of *p*-toluenesulfonyl chloride in 40 mL of dry pyridine was stirred at 0–4 °C for 23 h. The reaction mixture was then treated with 4.4 g (51 mmol) of 1,4-diaminobutane and stirred at 25 °C for 16 h. The solution was concentrated under diminished pressure and the residue was applied to a flash silica gel column, which was washed successively with $CHCl_3$, 1:1 methanol–$CHCl_3$, 1:1:0.01 methanol–$CHCl_3$–NH_4OH, 99:1 MeOH–NH_4OH, and finally 9:1 MeOH–NH_4OH. The product, *N*-Boc 5′-chlorobithiazole **10a**, eluted from the column with the 9:1 methanol–NH_4OH wash; concentration afforded the product as a tan solid: yield 304 mg (58%); ^{1}H NMR ($CDCl_3$) δ 1.30–1.55 (m, 13H), 1.88 (m, 2H), 2.65–2.83 (m, 6H), 3.14 (t, 2H), 3.47–3.57 (m, 4H), 5.37 (br s, 1H), 7.97 (br s, 1H) and 8.14 (s, 1H); mass spectrum *m*/*z* 519 and 517 $(M+H)^+$, and 419 and 417; FAB HR-MS *m*/*z* 517.1851$(M+H)^+$ ($C_{21}H_{34}{}^{35}ClN_6O_3S_2$ requires 517.1824).

2′-Aminoethyl-5′-chloro-2,4′-bithiazole-4-[3-(4-aminobutyl)aminopropyl]carboxamide (1a). To a solution of 240 mg (0.46 mmol) of *N*-Boc 5′-chlorobithiazole **10a** in 20 mL of dry CH_2Cl_2 at 25 °C was added 6 mL of trifluoroacetic acid with stirring. The reaction mixture was stirred at 25 °C for 3 h and then concentrated under diminished pressure. The residue was coevaporated successively with portions of CH_2Cl_2, methanol, and then water under diminished pressure, and then the residue was applied to an Amberlite XAD-2 column (31×1.3 cm), which was washed successively with 5% aq NaCl, water and methanol. The water and methanol fractions containing the desired product were pooled and concentrated under diminished pressure. The residue was purified by preparative silica gel TLC, development with 4:1 methanol–NH_4OH to afford chlorobithiazole **1a** as a pale yellow solid: yield 96 mg (49%); ^{1}H NMR (D_2O) δ 1.51 (s, 4H), 1.80 (m, 2H), 2.58–2.78 (m, 6H), 2.98 (s, 4H), 3.30–3.42 (m, 2H) and 7.94 (s, 1H); mass spectrum *m*/*z* 419 and 417 $(M+H)^+$; HR-MS *m*/*z* 417.1291 $(M+H)^+$ ($C_{16}H_{26}{}^{35}ClN_6OS_2$ requires 417.1299).

Methyl 2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5-chloro-2,4′-bithiazole-4-carboxylate (8b). To 2.0 g (5.4 mmol) of bithiazole **7** and 5.14 g (21.7 mmol) of Cl_3CCCl_3 in 100 mL of dry THF was added 13.6 mmol (9.03 mL of a 1.5 M solution) of lithium diisopropylamide using the same procedure as described above for lithiation-halogenation of thiazole **2**, which gave after purification Boc-protected 5-chlorobithiazole (**8b**) as colorless needles from hexanes–acetone: yield 466 mg (21%), plus recovered starting material, 1.24 g (62%); mp 128.5–129 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.22 (t, 2H), 3.59 (q, 2H), 3.98 (s, 3H), 4.99 (br s, 1H) and 7.97 (s, 1H); mass spectrum *m*/*z* 406 and 404 $(M+H)^+$, 350 and 348, and 306 and 304. Anal. calcd for $C_{15}H_{18}ClN_3O_4S_2$: C, 44.61; H, 4.49; N, 10.40. Found: C, 44.72; H, 4.49; N, 10.41.

2′-[2-(*t*-Butoxycarbonyl)amino]ethyl-5-chloro-2,4′-bithiazole-4-(3-hydroxypropyl)carboxamide (9b). *N*-Boc protected 5-chlorobithiazole (**8b**) (455 mg, 1.1 mmol) and 4.24 g (56.5 mmol) of 3-aminopropanol in 25 mL of THF were allowed to react for 44 h and the reaction mixture was worked up as described for 5′-chlorobithiazole **9a** to provide *N*-Boc protected 5-chlorobithiazole hydroxypropylamide **9b** as colorless microcrystals from CH_2Cl_2–acetone: yield 345 mg (68%); mp 118–119 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 1.78 (m, 2H), 3.17 (t, 2H), 3.5–3.6 (m, 4H), 3.67 (t, 2H), 5.18 (br s, 1H), 7.73 (t, 1H) and 7.78 (s, 1H); mass spectrum *m*/*z* 449 and 447 $(M+H)^+$, and 349 and 347. Anal. calcd for $C_{17}H_{23}ClN_4O_4S_2$: C, 45.68; H, 5.19; N, 12.53. Found: C, 45.72; H, 5.15; N, 12.57.

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5-chloro-2,4′-bithiazole-4-[3-(*O*-*p*-toluenesulfonyl)propyl]carboxamide. A solution containing 173 mg (0.39 mmol) of *N*-Boc protected 5-chlorobithiazole hydroxypropylamide **9b** and 372 mg (1.95 mmol) of *p*-toluenesulfonyl chloride in 10 mL of dry pyridine under argon at 0–4 °C was stirred for 17 h, and was then diluted with 10 mL of dry toluene and concentrated under diminished pressure. The residue was applied to a flash silica gel column and washed with 2:1 ethyl acetate–hexanes to afford the tosylate as a colorless foam: yield 167 mg (72%); ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 1.97 (m, 2H), 2.39 (s, 3H), 3.18 (t, 2H), 3.45–3.6 (m, 4H), 4.13 (t, 2H), 5.01 (br s, 1H), 7.28 (d, J = 8.1 Hz, 2H), 7.69 (br s, 1H), 7.76 (d, J = 8.1 Hz, 2H) and 7.97 (s, 1H).

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5-chloro-2,4′-bithiazole-4-[3-(4-aminobutyl)aminopropyl]carboxamide (10b). A solution containing 100 mg (0.17 mmol) of 5-

chlorobithiazole tosylate and 736 mg (8.4 mmol) of 1,4-diaminobutane in 6 mL of dry pyridine under argon was stirred at 25 °C for 17 h, after which 6 mL of dry toluene was added and the mixture was concentrated under diminished pressure. The residue was applied to a flash silica gel column, which was washed successively with 2:1:0.01 $CHCl_3$–MeOH–NH_4OH, 1:1:0.01 $CHCl_3$–MeOH–NH_4OH, and finally 9:1 methanol–NH_4OH, affording *N*-Boc protected 5-chlorobithiazole **10b** as a colorless solid: yield 54 mg (63%); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.52 (m, 2H), 1.62 (m, 2H), 1.88 (m, 2H), 2.64–2.74 (m, 4H), 2.81 (t, 2H), 3.22 (t, 2H), 3.50–3.63 (m, 4H), 5.14 (br s, 1H), 7.91 (s, 1H) and 8.05 (br s, 1H); mass spectrum *m*/*z* 519 and 517 $(M+H)^+$, and 419 and 417; FAB HR-MS *m*/*z* 517.1846 $(M+H)^+$ ($C_{21}H_{34}{}^{35}ClN_6O_3S_2$ requires 517.1824).

2′-Aminoethyl-5-chloro-2,4′-bithiazole-4-[3-(4-aminobutyl)aminopropyl]carboxamide (1b). To a solution of 54 mg (0.1 mmol) of *N*-Boc 5-chlorobithiazole **10b** in 5 mL of dry CH_2Cl_2 under argon at 0–4 °C was added 2.1 mmol (2.1 mL of a 1.0 M solution in diethyl ether) of hydrogen chloride in a dropwise fashion. The reaction mixture was stirred at 25 °C for 50 min, and was then concentrated under diminished pressure. The residue was dissolved in dry CH_2Cl_2, and again concentrated. The residue was then applied to a column of C_2 bonded silica gel (Analytichem International) and washed with water, affording 42 mg of impure product and 9 mg of unreacted starting material. The impure product was purified further on an MPLC system (Rainin) using a C_8 reversed-phase column with water as the eluant; this afforded free chlorobithiazole **1b** as a cream-colored powder: yield 29 mg (67%); ^{1}H NMR (D_2O) δ 1.70 (s, 4H), 1.95 (br s, 2H), 2.90–3.10 (m, 6H), 3.32–3.46 (m, 6H) and 7.99 (s, 1H); mass spectrum *m*/*z* 419 and 417 $(M+H)^+$; FAB HR-MS *m*/*z* 419.1304 $(M+H)^+$ ($C_{16}H_{26}{}^{37}ClN_6OS_2$ requires 419.1269).

Methyl 2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5,5′-dichloro-2,4′-bithiazole-4-carboxylate (8c). To a solution containing 200 mg (0.54 mmol) *N*-Boc protected bithiazole ester **7** in 7 mL of dry THF under argon at −78 °C was added 186 mg (1.73 mmol) of lithium diisopropylamide dropwise as a 1.5 M solution in THF-cyclohexane over a period of 10 min. The reaction mixture was stirred for 10 min at −78 °C, then 410 mg (1.73 mmol) of Cl_3CCCl_3 was added as a solution in 1.5 mL of dry THF over a 5-min period. The reaction mixture was stirred at −78 °C for 10 min, quenched by the addition of 3 mL of water, and concentrated to 3 mL under diminished pressure. Twenty milliliters of ethyl acetate was added and the organic solution was washed with three 20-mL portions of water and two 20-mL portions of saturated brine, then dried over $MgSO_4$. The reaction mixture was concentrated under diminished pressure and the residue was applied to a flash silica gel column and washed with 1:1 ethyl acetate–hexanes to afford *N*-Boc protected 5,5′-dichlorobithiazole ester (**8c**) as colorless needles from acetone: yield 60 mg (25%); mp 136–137 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.15 (t, 2H), 3.56 (q, 2H), 3.97 (s, 3H) and 4.92 (br s, 1H); mass spectrum *m*/*z* 440 and 438 $(M+H)^+$ 384 and 382, and 340 and 338. Anal. calcd for $C_{15}H_{17}Cl_2N_3O_4S_2$: C, 41.10; H, 3.91 ; N, 9.59. Found: C, 41.71; H, 3.96; N, 9.49.

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5,5′-dichloro-2,4′-bithiazole-4-(3-hydroxypropyl)carboxamide (9c). *N*-Boc protected 5,5′-dichlorobithiazole ester **8c** (139 mg, 0.32 mmol) and 1.18 g (15.75 mmol) of 3-aminopropanol in 1 mL of methanol were allowed to react over a period of 24 h; purification was carried out as described for 5′-chlorobithiazole **9a** to afford *N*-Boc protected 5,5′-dichlorobithiazole **9c**, as colorless prisms from acetone–CH_2Cl_2: yield 114 mg (75%); mp 169 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.82 (m, 2H), 3.14 (t, 2H), 3.50–3.64 (m, 4H), 3.72 (br s, 2H), 5.02 (br s, 1H) and 7.75 (br s, 1H); mass spectrum *m*/*z* 483 and 481 $(M+H)^+$ and 381.

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5,5′-dichloro-2,4′-bithiazole-4-[3-(4-aminobutyl)aminopropyl]carboxamide (10c). To a solution of 114 mg (0.24 mmol) of *N*-Boc protected 5,5′-dichlorobithiazole hydroxypropylamide **9c** in 5 mL of dry pyridine at 0–4 °C was added 181 mg (0.95 mmol) of *p*-toluenesulfonyl chloride and the solution was stirred at 0–4 °C for 6.5 h. 1,4-Diaminobutane (1.04 g, 11.9 mmol) was then added and the resulting solution was stirred at 25 °C for 12 h. The reaction mixture was concentrated under diminished pressure and the residue was applied to a flash silica gel column and washed with 1:2:0.02 methanol–$CHCl_3$–NH_4OH to afford a product contaminated with diaminobutane. The product was again applied to a column of flash silica gel and washed with the same eluant, affording Boc-dichlorobithiazole **10c** as a yellow gum: yield 81 mg (60%); ^{1}H NMR ($CDCl_3$) δ 1.38–1.60 (m, 13H), 1.80 (m, 2H), 2.63 (q, 4H), 2.73 (t, 2H), 3.15 (t, 2H), 3.47–3.59 (m, 4H), 5.15 (br s, 1H) and 7.88 (br s, 1H); mass spectrum *m*/*z* 555, 553,and 551 $(M+H)^+$; FAB HR-MS *m*/*z* 551.1456 $(M+H)^+$ ($C_{21}H_{33}{}^{35}Cl_2N_6O_3S_2$ requires 551.1434).

2′-Aminoethyl-5,5′-dichloro-2,4′-bithiazole-4-[3-(4-aminobutyl)aminopropyl]carboxamide (1c). A solution containing 78 mg (0.14 mmol) of *N*-Boc protected dichlorobithiazole **10c** in 2 mL of dry CH_2Cl_2 under argon at 0–4 °C was treated with 2.76 mmol of hydrogen chloride (30 min reaction time) and purified as for Boc-5-chlorobithiazole **1b**, affording dichlorobithiazole **1c** as an off-white powder: yield 50 mg (79%); ^{1}NMR (D_2O) δ 1.70–1.82 (m, 4H), 2.03 (m, 2H), 2.98–3.17 (m, 6H) and 3.37–3.54 (m, 6H); mass spectrum *m*/*z* 453 and 451 $(M+H)^+$; FAB HR-MS *m*/*z* 451.0910 $(M+H)^+$ ($C_{16}H_{25}{}^{35}Cl_2N_6OS_2$ requires 451.0909).

Methyl 2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5′-bromo-2,4′-bithiazole-4-carboxylate (8d). A solution containing 1.00 g (2.71 mmol) of *N*-Boc protected bithiazole ester **7** and 496 mg (2.79 mmol) of *N*-bromosuccinimide in 100 mL of dry acetonitrile was stirred and purged with a stream of argon gas for 12 min, then irradiated at 0–4 °C with stirring through a water filter in a Rayonet apparatus for 30 min. The solution was concentrated under diminished pressure and the residue was applied to a flash silica gel column, which was washed with 1:1

ethyl acetate–hexanes to afford *N*-Boc protected 5′-bromobithiazole ester **8d** as colorless microcrystals from ethyl acetate: yield 510 mg (42%); mp 118–120 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.18 (t, 2H), 3.58 (q, 2H), 3.97 (s, 3H), 4.96 (br s, 1H) and 8.26 (s, 1H); mass spectrum *m*/*z* 450 and 448 $(M+H)^+$. Anal. calcd for $C_{15}H_{18}BrN_3O_4S_2$: C, 40.18; H, 4.05; N, 9.37. Found: C, 40.28; H, 4.02; N, 9.35.

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5′-bromo-2,4′-bithiazole-4-(3-hydroxypropyl)carboxamide (9d). A solution containing 386 mg (0.86 mmol) of *N*-Boc protected 5′-bromobithiazole and 646 mg (8.61 mmol) of 3-aminopropanol in 20 mL of methanol was stirred at 25 °C for 41 h and worked up as described above for 5′-chlorobithiazole **9a** to afford *N*-Boc protected 5′-bromobithiazole hydroxypropylamide **9d** as colorless microcrystals from acetone: yield 348 mg (82%); mp 142 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.83 (m, 2H), 3.20 (q, 2H), 3.55–3.74 (m, 6H), 4.96 (br s, 1H), 7.73 (br s, 1H) and 8.18 (s, 1H); mass spectrum *m*/*z* 493 and 491 $(M+H)^+$, and 393 and 391. Anal. calcd for $C_{17}H_{23}BrN_4O_4S_2$: C, 41.55; H, 4.72; N, 11.40. Found: C, 41.99; H, 4.72; N, 11.36.

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5′-bromo-2,4′-bithiazole-4-[3-(*O*-*p*-toluenesulfonyl)propyl]carboxamide. A solution containing 340 mg (0.69 mol) of *N*-Boc protected 5′-bromobithiazole hydroxypropylamide **9d** and 658 mg (3.45 mmol) of *p*-toluenesulfonyl chloride in 20 mL of dry pyridine was stirred at 0–4 °C for 8 h and was then concentrated under diminished pressure. The residue was applied to a flash silica gel column and washed with 2:1 ethyl acetate–hexanes to provide 5′-bromobithiazole tosylate as a colorless foam: yield 261 mg (58%); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.02 (m, 2H), 2.42 (s, 3H), 3.18 (t, 2H), 3.52–3.61 (m, 4H), 4.16 (t, 2H), 5.00 (br s, 1H), 7.32 (d, 2H, $J=8.1$ Hz), 7.66 (br s, 1H), 7.79 (d, 2H, $J=8.1$ Hz) and 8.13 (s, 1H); mass spectrum *m*/*z* 475 and 473 $(M\text{-}OTs)^+$.

2′-[2-(*tert*-Butoxycarbonyl)amino]ethyl-5′-bromo-2,4′-bithiazole-4-[3-(4-[*tert*-butoxycarbonyl]aminobutyl)aminopropyl] carboxamide (10d). A solution containing 260 mg (0.40 mmol) of bromobithiazole tosylate derivative and 325 mg (1.73 mmol) of mono-*N*-Boc-1,4-diaminobutane in 10 mL of dry pyridine was stirred at 25 °C for 26 h, after which 10 mL of water was added and the reaction mixture was concentrated under diminished pressure. The residue was applied to a flash silica gel column and washed with 8:1:0.01 $CHCl_3$–methanol–NH_4OH. The fractions containing the product were pooled and concentrated and the residue was purified by preparative silica gel TLC using 8:1:0.01 $CHCl_3$-methanol-NH_4OH for development. Bis *N*-Boc protected 5′-bromobithiazole **10d** was obtained as a colorless glass: yield 42 mg (16%); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H) 1.44 (s, 9H), 1.54 (m, 2H), 1.67 (m, 2H), 2.00 (m, 2H), 2.79 (m, 2H) and 2.87 (m, 2H), 3.09 (m, 2H), 3.17 (t, 2H), 3.53–3.63 (m, 4H), 4.94 (br s, 1H), 5.05 (br s, 1H), 7.82 (br t, 1H) and 8.17 (s, 1H); mass spectrum *m*/*z* 663 and 661 $(M+H)^+$, 563 and 561, and 463 and 461; FAB HR-MS *m*/*z* 661.1843 $(M+H)^+$ ($C_{26}H_{42}{}^{79}BrN_6O_5S_2$ requires 661.1843).

2′-Aminoethyl-5′-bromo-2,4′-bithiazole-4-[3-(4-aminobutyl)aminopropyl]carboxamide (1d). A solution of 42 mg (63.5 μmol) of bis *N*-Boc protected 5′-bromobithiazole **10d** in 15 mL of dry CH_2Cl_2 and 6 mL of trifluoroacetic acid was stirred at 25 °C for 1 h and was then concentrated under diminished pressure. The residue was coevaporated successively with portions of CH_2Cl_2 and water under diminished pressure, and the residue was then purified by preparative silica gel TLC using 4:1 methanol–NH_4OH for development, to provide mono bromobithiazole **1d** as a cream-colored powder: yield 15 mg (50%); ^{1}H NMR (D_2O) δ 1.61 (s, 4H), 1.75–1.98 (m, 2H), 2.68–3.08 (m, 8H), 3.26–3.46 (m, 4H) and 7.93 (s, 1H); mass spectrum *m*/*z* 463 and 461 $(M+H)^+$, and 383; FAB HR-MS *m*/*z* 463.0742 $(M+H)^+$ ($C_{16}H_{26}{}^{81}BrN_6OS_2$ requires 463.0772).

Agarose gel bioassay

DNA cleavage reactions were performed in 20 μL (total volume) of 0.5 mM Na cacodylate, pH 7.2, containing 200 ng of supercoiled pBR322 DNA, and the other components described in the figure legends. Reactions were performed with or without irradiation, using a Pyrex beaker as sample holder, a cooling bath, and a UV filter. All irradiations were performed with the reaction tubes packed in ice. Reaction mixtures were diluted with 5 μL of a loading buffer containing 0.27 M Tris–acetate, pH 7.8, 13 mM EDTA, 40% glycerol, 0.4% sodium dodecylsulfate, and 3.2% bromophenol blue, and the entire reaction mixture was then loaded onto a 1.2% (w/v) agarose gel containing 40 mM Tris–acetate, pH 7.8, 1 mM EDTA, and 1 μg/mL of ethidium bromide.[28] Horizontal gel electrophoresis was carried out in the same buffer and cleavage products were visualized by UV transillumination.

HPLC assay

A solution containing 400 μg of calf thymus DNA and 100 μM monochlorobithiazole **1b** in 200 μL of 4.2 mM Na cacodylate, pH 7.2, was irradiated at 0–4 °C for 5–20 min or incubated in the absence of light at 0–4 °C for 20 min. The DNA was removed by precipitation and the supernatant was concentrated to dryness under diminished pressure. C_{18} Reversed-phase HPLC analysis (100×4.6 mm column) utilized a flow rate of 1 mL/min and a linear gradient program of 0→40% CH_3CN in 0.1% aqueous trifluoroacetic acid over a period of 20 min with detection at 295 nm. A diode array detector recorded UV spectra at the apex of each peak.

Sequence gel bioassay

A 10-μL reaction mixture contained 5 mM Na cacodylate, pH 7.5, 5 μM (final nucleotide concentration) sonicated calf thymus DNA, and 5′-[^{32}P] labeled duplex

DNA 158 bp in length (derived from plasmid pBR322 by *Hin*dIII cleavage, dephosphorylation with alkaline phosphatase, ^{32}P labeling with T4 polynucleotide kinase, *Eco*RV cleavage, and native PAGE isolation).[32] Other conditions are described in the figure legends. Solutions of $Fe(NH_4)_2(SO_4)_2$ were prepared immediately before use. Reactions were performed as described above for the agarose gel bioassay. Then, 5 μL of loading solution (10 M urea, 1.5 mM EDTA, 0.05% (w/v) each of bromophenol blue and xylene cyanol) was added and one-half of each reaction mixture was applied to the gel. DNA sequencing reactions were performed according to the method of Maxam and Gilbert.[33] The denaturing 10% polyacrylamide gel (7.5 M urea) contained 89 mM Tris–borate, pH 8.0, and 2 mM EDTA.[28] Gels were run in the same buffer and analyzed by autoradiography.

Photolysis of chlorobithiazole in 1-octene

A solution of 1.0 mg (2.5 μmol) of chlorobithiazole methyl ester **8b** in 3 mL of 1-octene was purged with argon gas for 20 min. The solution was irradiated in a Rayonet apparatus for 1 h at 30 °C in a water bath with a Pyrex filter. The reaction mixture was concentrated under diminished pressure and the residue was purified by preparative silica gel TLC, development with 2:1 hexanes–ethyl acetate, to afford two products. The faster moving product was found to be 1-bithiazolyl-2-chlorooctane **12**, isolated as a colorless glass: yield 0.5 mg (39%); ^1H NMR ($CDCl_3$) δ 0.83 (t, 3H), 1.21 (s, 10H), 1.44 (s, 9H), 2.73 (dd, 1H, $J=15$, 6 Hz), 3.02 (dd, 1H, $J=15$, 10 Hz), 3.23 (t, 2H), 3.55–3.65 (m, 3H), 3.88 (s, 3H), 4.97 (br s, 1H) and 8.08 (s, 1H); mass spectrum m/z 581 and 516 $(M+H)^+$, and 462 and 460. The slower moving product was found to be the regioisomer, 2-bithiazolyl-1-chlorooctane **11**, also isolated as a colorless glass: yield 0.4 mg (31%); ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H), 1.25 (s, 10H), 1.45 (s, 9H), 2.48 (m, 1H), 2.78 (dd, 1H, $J=12$, 12 Hz), 3.12–3.25 (m, 3H), 3.57 (q, 2H), 3.92 (s, 3H), 4.97 (br s, 1H) and 8.08 (s, 1H); mass spectrum m/z 518 and 516 $(M+H)^+$, and 462 and 460.

Photolysis of bromobithiazole in 1-octene

A solution of 10 mg (23 μmol) of bromobithiazole methyl ester **8d** in 30 mL of 1-octene was treated and worked up as described above for the photolysis of chlorobithiazole **8d**, to afford 1-bithiazolyl-2-bromooctane **13** as a yellow oil: yield 4.5 mg (34%); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.29 (s, 10H), 1.45 (s, 9H), 3.12–3.20 (m, 2H), 3.55-3.6 (m, 3H), 3.95 (s, 3H), 5.09 (br s, 1H) and 8.15 (s, 1H); mass spectrum m/z 562 and 560 $(M+H)^+$, 506 and 504, and 462 and 460.

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References and Notes

1. (a) Umezawa, H. In *Bleomycin: Current Status and New Developments*; Carter, S. K., Crooke, S. T., Umezawa, H. Eds.; Academic: New York, 1978; pp 15–20. (b) Sikic, B. I.; Rozencweig, M., Carter, S. K., Eds. *Bleomycin Chemotherapy*; Academic: 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, 1993; pp 197–242. (d) Kane, S. A.; Hecht, S. M. *Prog. Nucleic Acid Res. Mol. Biol.* **1994**, *49*, 313. (e) Burger, R. M. *Chem. Rev.* **1998**, *98*, 1153.
3. (a) Magliozzo, R. S.; Peisach, J.; Ciriolo, M. R. *Mol. Pharmacol.* **1989**, *35*, 428. (b) 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. (c) Hüttenhofer, A.; Hudson, S.; Noller, H. F.; Mascharak, P. K. *J. Biol. Chem.* **1992**, *267*, 24471. (d) Holmes, C. E.; Carter, B. J.; Hecht, S. M. *Biochemistry* **1993**, *32*, 4293. (e) Dix, D. J.; Lin, P.-N.; McKenzie, A. R.; Walden, W. E.; Theil, E. C. *J. Mol. Biol.* **1993**, *231*, 230. (f) Hecht, S. M. *Bioconjugate Chem.* **1994**, *5*, 513. (g) Hecht, S. M. In *The Many Faces of RNA*; Eggleston, D. S., Prescott, C. D., Pearson, N. D., Eds.; Academic: London, 1998, pp 3–17 (h) Hecht, S. M. *J. Nat. Prod.* **2000**, *63*, 158.
4. (a) Takeshita, M.; Kappen, L. S.; Grollman, A. P.; Eisenberg, M.; Goldberg, I. H. *Biochemistry* **1981**, *20*, 7599. (b) Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310. (c) Shipley, J. B.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 25.
5. (a) Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641. (b) Lin, S. Y.; Grollman, A. P. *Biochemistry* **1981**, *20*, 7589. (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) Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278.
6. (a) 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. (b) Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S.-N. *J. Biol. Chem.* **1990**, *265*, 4193. (c) Guajardo, R. J.; Hudson, S. E.; Brown, S. J.; Mascharak, P. K. *J. Am. Chem. Soc.* **1993**, *115*, 7971.
7. (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.; Ting, A.; Huang, C.-H.; Mong, S.; Crooke, S. T. *Cancer Res.* **1982**, *42*, 2779.
8. (a) Youngquist, R. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 5528. (b) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 8266.
9. (a) Epstein, J. H.; Brunsting, L. A.; Peterson, M. C.; Schwarz, B. E. *J. Invest. Dermatol.* **1957**, *28*, 329. (b) Kirschbaum, B. A.; Beerman, H. *Am. J. Med. Sci.* **1964**, *248*, 445. (c) Satanove, M.; McIntosh, J. S. *J. Am. Med. Assoc.* **1967**, *200*, 209.
10. (a) Decuyper, J.; Piette, J.; Lopez, M.; Merville, M.-P.; van de Vorst, A. *Biochem. Pharmacol.* **1984**, *33*, 4025. (b) Chignell, C. F.; Motten, A. G.; Buettner, G. R. *Environ. Health Perspect.* **1985**, *64*, 103. (c) Motten, A. G.; Buettner, G. R.; Chignell, C. F. *Photochem. Photobiol.* **1985**, *42*, 9.

(d) Ciulla, T. A.; Epling, G. A.; Kochevar, I. E. *Photochem. Photobiol.* **1986**, *43*, 607.
11. Janzen, E. G.; Stronks, H. J.; Nutter, D. E.; Davis, E. R.; Blount, H. N.; Lee Poyer, J.; McCay, P. B. *Can. J. Chem.* **1980**, *58*, 1596.
12. Quada, J. C., Jr.; Levy, M. J.; Hecht, S. M. *J. Am. Chem. Soc.* **1993**, *115*, 12171.
13. (a) Knaus, G.; Meyers, A. I. *J. Org. Chem.* **1974**, *39*, 1192. (b) Dondoni, A.; Mastellari, A.R.; Medici, A.; Negrini, E.; Pedrini, P. *Synthesis* **1986**, 757 (c) Sawhney, I.; Wilson, J. R. H. *J. Chem. Soc., Perkin Trans. 1* **1990**, 329.
14. (a) Zee-Cheng, K. Y.; Cheng, C. C. *J. Heterocycl. Chem.* **1970**, *7*, 1439. (b) McGowan, D. A.; Jordis, U.; Minster, D. K.; Hecht, S. M. *J. Am. Chem. Soc.* **1977**, *99*, 8078. (c) Sakai, T. T.; Riordan, J. R.; Booth, T. E.; Glickson, J. D. *J. Med. Chem.* **1981**, *24*, 279.
15. Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061.
16. Schmidt, U.; Gleich, P.; Greisser, H.; Utz, R. *Synthesis* **1986**, 992.
17. Campagna, F.; Carotti, A.; Casini, G. *Tetrahedron Lett.* **1977**, *21*, 1813.
18. Anderson, A. G.; Tober, T. Y. *J. Org. Chem.* **1980**, *45*, 1695.
19. Phillips, R. S.; Cohen, L. A. *Tetrahedron Lett.* **1983**, *24*, 5555.
20. Stahl, G. L.; Walter, R.; Smith, C. W. *J. Org. Chem.* **1978**, *43*, 2285.
21. Repine, J. E.; Pfenninger, O. W.; Talmage, D. W.; Berger, E. M.; Pettijohn, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1001.
22. Bithiazole **1e** was prepared from **7**[14] in analogy with the syntheses of **1a**–**1d**. See: Kane, S. A.; Sasaki, H.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 9107.
23. (a) Kappen, L. S.; Napier, M. A.; Goldberg, I. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1970. (b) Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198. (c) Sugiura, Y.; Uesawa, Y.; Takahashi, Y.; Kuwahara, J.; Golik, J.; Doyle, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7672. (d) Sugiura, Y.; Shiraki, T.; Konishi, M.; Oki, T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3831.
24. Armitage, B.; Schuster, G. B. *Photochem. Photobiol.* **1997**, *66*, 164.
25. (a) Poutsma, M.L. In *Methods in Free-Radical Chemistry*; Huyser, E. S., Ed.; Academic: New York, 1969; Vol. 1, pp 163–186 (b) Thaler, W. A. In *Methods in Free-Radical Chemistry*; Huyser, E. S., Ed.; Academic: New York, 1969; Vol. 2, pp 214–218.
26. Kharasch, M. S.; Sage, M. *J. Org. Chem.* **1949**, *14*, 537.
27. It may be noted, however, that it is possible that the differences observed for bromobithiazole **1d** reflect its reaction via an ionic mechanism. See: Kropp, P. J. *Acc. Chem. Res.* **1984**, *17*, 131.
28. Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.
29. Zuber, G.; Quada, J. C., Jr.; Hecht, S. M. *J. Am. Chem. Soc.* **1998**, *120*, 9368.
30. Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641.
31. Still, W. C.; Khan, J.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
32. Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869.
33. Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.