Mechanisms of DNA Damage by Leinamycin

Kent S. Gates*

Departments of Chemistry and Biochemistry, University of Missouri—Columbia, Columbia, Missouri 65211

Received April 17, 2000

Importance of DNA-Damaging Cytotoxins. DNA-damaging agents have historically played a central role in cancer therapy (*1*). Even as new approaches to cancer therapy become available, it seems likely that there will be a continued need for the study and development of novel DNA-damaging cytotoxins. These agents will see continued use due to their well-established role in treating various types of cancer and because many of the new approaches to cancer treatment such as inhibition of angiogenesis, immunotherapy, and modulation of the cell cycle are most effective when used in combination with traditional cytotoxins.

Leinamycin: A New Class of DNA-Damaging Agent. The large number of known DNA-damaging agents can be divided into a relatively small number of categories if they are classified on the basis of the functional groups and chemical reactions involved in their reactions with DNA (*2*). Well-known categories of DNA-damaging agents include enediynes, epoxides, imines, activated cyclopropanes, heterocyclic *N*-oxides, and quinones (*2*). From such a chemical perspective, the antitumor antibiotic leinamycin is of particular interest because this antibiotic represents a new structural type of DNA-damaging agent. Because structurally novel natural products often possess interesting and unexpected reactivity, leinamycin presents a unique opportunity to expand our understanding of the diverse chemical mechanisms by which anticancer agents, mutagens, and toxins can interact with DNA.

Leinamycin was isolated by researchers at Kyowa Hakko Kogyo Ltd. from a strain of *Streptomyces* found in soil samples collected near Miyagi, Japan (*3*). The structure of leinamycin was elucidated by NMR and IR spectroscopy and X-ray crystallography, and ultimately confirmed by total synthesis (*4*−*6*). The antibiotic contains a number of interesting structural features, including a 5-(thiazol-4-yl)penta-2,4-dieneone system embedded in its 18-membered macrolactam and a 1,2-dithiolan-3-one 1-oxide heterocycle that is unique to this natural product. Leinamycin displays potent antitumor and cytotoxic activities comparable to that of many clinically used agents (57% increased life span against murine leukemia P388 at 0.38 mg/kg, an IC_{50} of 0.014 μg/mL against HeLa S3 cells, and an LD_{50} of 2.8 mg/kg in mouse) (*7*) and remains in development as a potential anticancer agent (*8, 9*). Biological experiments suggest that DNA is the important biological target of leinamycin (*7*).

Interestingly, in vitro experiments revealed that leinamycin is a thiol-triggered DNA-damaging agent (*7*). Such thiol-dependent chemistry is biologically relevant because cells contain high concentrations of thiols such as glutathione (*10*). Upon first inspection of leinamycin's structure, chemical intuition suggests that the unique 1,2-dithiolan-3-one 1-oxide heterocycle is the most reactive portion of the antibiotic and, therefore, is likely to play a crucial role in thiol-triggered DNA cleavage. Firm evidence supporting this notion was provided by experiments showing that *S*-deoxyleinamycin possesses significantly diminished biological activity (IC_{50} = 2.1 μg/mL against HeLa cells) and does not cleave DNA in vitro at concentrations where leinamycin is effective (*7*). This finding and the results of other early experiments combined to suggest that nucleophilic attack of thiols on the sulfur heterocycle of leinamycin initiates a chain of chemical events that culminates in DNA damage.

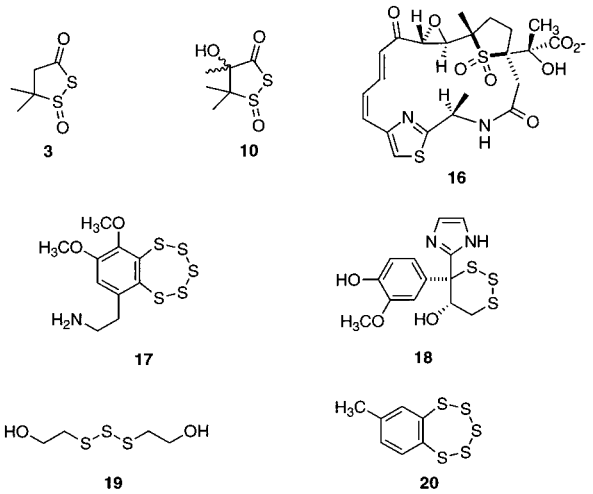


Figure 1. Structures of compounds discussed in the text.

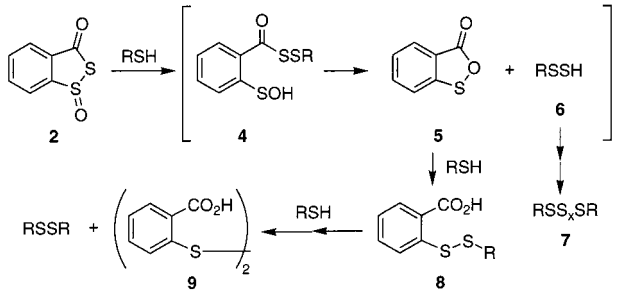
Reaction of Thiols with Leinamycin's 1,2-Dithiolan-3-one 1-Oxide Heterocycle. While early experiments suggested that attack of thiol on the 1,2-dithiolan-3-one 1-oxide heterocycle of leinamycin initiates DNA strand cleavage by the antibiotic, the detailed chemical events underlying DNA damage remained a mystery. At the time of leinamycin's discovery, nothing was known about the reactivity of the 1,2-dithiol-3-one 1-oxide heterocycle (*11*−*13*), and the first clues toward understanding thiol-triggered DNA damage by the antibiotic were provided by studies of the reaction between thiols and the simplified leinamycin model compounds **2** and **3** (*14, 15*) (Figure 1).

The major products stemming from the reaction of **2** with thiols are polysulfides (**7**), the 2-(alkyldithio)benzoic acid (**8**), and 2,2′-dithiosalicylic acid (**9**) (Scheme 1) (*15*). Importantly, compound **3**, whose structure closely resembles that of the essential sulfur heterocycle found in leinamycin, yields analogous products upon reaction with thiols. It was proposed (*15*) that the observed products

* To whom correspondence should be addressed. Phone: (573) 882-6763. Fax: (573) 882-2754. E-mail: GatesK@missouri.edu.

10.1021/tx000089m CCC: $19.00 © 2000 American Chemical Society
Published on Web 09/29/2000

Scheme 1. Reaction of Leinamycin Model Compound 2 with Thiol



arise from initial attack of thiol on the central (sulfenyl) sulfur of the heterocycle, followed by cyclization of the resulting sulfenic acid (**4**) to afford an unstable oxathiolanone (**5**) and a hydrodisulfide (persulfide, **6**) (Scheme 1). The final products of the reaction stem from attack of excess thiol on the oxathiolanone (**5**) to yield **8** and from decomposition of the hydrodisulfide (**6**) to polysulfides (**7**). When this chemistry is placed into the context of leinamycin, it was noted that the electrophilic oxathiolanone or the easily oxidized hydrodisulfide intermediate might be key intermediates in DNA damage by the natural product (*15*). Subsequent studies have revealed that, in fact, both of these reactive intermediates play key roles in thiol-triggered DNA damage by leinamycin.

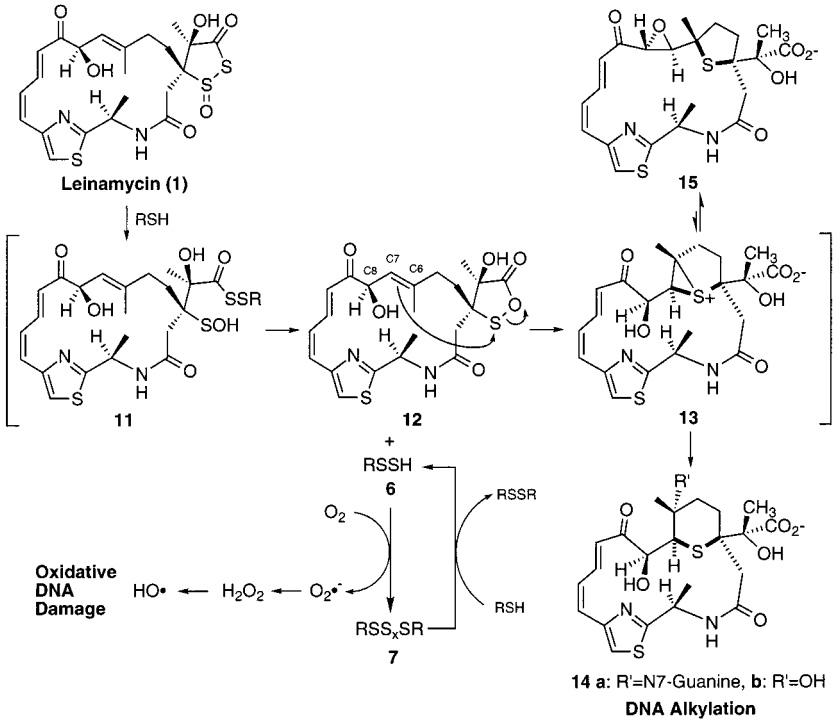
Thiol-Triggered Oxidative DNA Damage by Leinamycin. Investigation of the simple, synthetic leinamycin analogues **2**, **3**, and **10** showed that these compounds, like leinamycin, are thiol-triggered DNA-cleaving agents (*16*). The strand cleavage caused by these agents occurs by a general mechanism involving the conversion of molecular oxygen to DNA-cleaving oxygen radicals as shown in (unbalanced) eq 1.

$$O_2 \rightarrow O_2^{\bullet -} \rightarrow H_2O_2 + Mn^{+} \rightarrow HO^{\bullet} + M^{(n+1)+} \quad (1)$$

Leinamycin was subsequently shown to cause similar thiol-mediated oxidative DNA damage (*17*). Thiol-dependent production of DNA-cleaving oxygen radicals by leinamycin and its simple analogues (**2**, **3**, and **10**) is thought to involve O_2-mediated oxidation of the unstable hydrodisulfide intermediate (RSSH, **6**) produced in the initial reaction of the 1,2-dithiolan-3-one 1-oxide heterocycle with thiols (Scheme 2). Importantly, the resulting polysulfides (**7**) have been shown (*17*) to cause further thiol-dependent oxidative DNA damage via reactions with excess thiol that regenerate easily oxidized RS_xSH intermediates (Scheme 2). The mechanism and efficiency of thiol-dependent DNA cleavage by polysulfides (**7**) are comparable to those of cleavage by the intact heterocycles **2**, **3**, and **10** (*16*, *17*). Further support for the role of RSSH and polysulfides in oxidative DNA cleavage by leinamycin and its simple analogues **2**, **3**, and **10** has been provided by the observation that other agents expected to generate RSSH upon reaction with thiols damage DNA with a similar mechanism and efficiency (*18*).

The facile oxidation of hydrodisulfides (relative to thiols, for example) may result from the fact that, at physiological pH, the RSSH group ($pK_a \sim 6.2$) exists predominantly in the deprotonated form (RSS^-) (*19*). Analogous to the autoxidation of thiols (*20*), the anion (RSS^-) is expected to be the active substrate for trace-metal and oxygen-mediated oxidation. In addition, one-electron oxidation of RSS^- is thermodynamically favored over oxidation of the corresponding thiolate (RS^-) (*19*). It is significant that polysulfides might act as catalysts for the net transfer of electrons from thiols to molecular oxygen (Scheme 2). Such a process might induce oxidative stress through production of reactive oxygen species ($O_2^{\bullet -}$, H_2O_2, and $HO^{\bullet}$) and through depletion of cellular thiols. Finally, additional species, such as hydrogen sulfide, produced from the reaction (*21*) of thiols with the hydrodisulfide intermediate **6** may contribute to the production of oxygen radicals (*22*).

Scheme 2. Thiol-Triggered DNA Damage by Leinamycin



Thiol-Triggered DNA Alkylation by Leinamycin. In addition to the thiol-dependent oxidative DNA damage described above, leinamycin damages DNA by a second mechanism involving DNA alkylation (*23*). Thiol-triggered DNA alkylation by leinamycin and the accompanying deep-seated rearrangement of antibiotic can be rationalized by a chemical mechanism that is (in its initial stages) identical to the reaction of thiols with leinamycin model compounds **2** and **3** shown in Scheme 1 (*15*). Accordingly, initial attack of thiol on leinamycin is expected to produce the sulfenic acid (**11**) that cyclizes to the 1,2-oxathiolan-5-one **12** with concomitant release of a hydrodisulfide **6** (Scheme 2). Then, in a reaction not available to simple leinamycin analogues such as **2**, **3**, and **10**, the electrophilic oxathiolanone (**12**) undergoes intramolecular reaction with the C6−C7 alkene of the antibiotic's macrocycle to generate an episulfonium ion alkylating agent (**13**). In this regard, an important role of leinamycin's spiro-fused 18-member macrocycle must be to appropriately position the C6−C7 alkene for rapid reaction with the electrophilic sulfur of **12**. Generation of episulfonium ions by the reaction of analogous acyclic sulfur electrophiles (RSOCOR′) with alkenes has been reported in the chemical literature (*24*−*27*). The episulfonium ion of leinamycin (**13**) efficiently alkylates double-stranded DNA, forming a covalent attachment at the N7 position of deoxyguanosine residues (*23*). The reaction of leinamycin with 1.5 equiv of thiol in the presence of excess double-stranded DNA, followed by a thermal depurination workup, provides the leinamycin−guanine adduct in 75% yield. Alkylation occurs via the apparent backside attack of DNA on the episulfonium ion **13** and leads to the Markovnikov product (**14**). Consistent with an overall mechanism involving thiol-triggered release of hydrodisulfide (RSSH), it has been shown that the reaction of thiols with leinamycin produces polysulfides (*17*) and that significant yields of the leinamycin adduct resulting from the attack of RSSH on **13** are obtained when the reaction is carried out at high antibiotic concentrations (*23*). Leinamycin's reaction with thiols is rapid (200 μM leinamycin in pH 7 buffer is completely consumed by 1.2 equiv of thiol within 30 min) compared to hydrolysis ($t_{1/2} \sim 8$ h, pH 7, 37 °C) (*28*).

The episulfonium ion **13** exists in equilibrium with an epoxide form (**15**) resulting from intramolecular backside attack of the C8-hydroxyl on the episulfonium ion (Scheme 2) (*23*). Unlike intermediates **11**−**13**, the epoxide **15** can be directly observed by NMR and HPLC and has a significant lifetime in pH 7 buffer ($t_{1/2} \sim 3$ h) (*23*, *28*). In fact, the leinamycin epoxide (**15**) can be isolated in good yield from the reaction of leinamycin with thiol in organic solvents. This epoxide (**15**) modifies DNA, and as one would expect, this process does not require added thiol (*28*). Interestingly, hydrolysis of **15** yields the 3,7-sulfide **14**, indicating that hydrolysis occurs via the episulfonium ion **13** and not by direct attack of water on the epoxide residue of **15**. Similar neighboring group sulfide-assisted epoxide hydrolysis reactions have been reported in the literature (*29*). The sulfone analogue **16**, which lacks the sulfur lone pairs required for rearrangement to **13**, does not undergo hydrolysis under conditions where the sulfide **15** hydrolyzes readily (*28*). In addition, the sulfone **16** does not cause measurable DNA modification in a plasmid-based assay that readily detects thiol-triggered DNA alkylation by leinamycin or direct alkylation by **15**. Taken together, these data suggest that, although epoxides are well-known DNA alkylating agents (*2*), **15** alkylates DNA via the episulfonium ion **13** and not by direct reaction of DNA at the epoxide residue. Surprisingly, though the hydroxyl group at the C8 position of leinamycin is clearly required to establish the epoxide−episulfonium equilibrium shown in the upper right corner of Scheme 2, this group does not appear to be crucial for the activity of the antibiotic. Analogues bearing a protected hydroxyl group at C8 still undergo rearrangement to give the corresponding C8-protected analogue of **16b** and retain full biological activity (*8*, *9*).

Biological Relevance of DNA Damage by Leinamycin. The relevance of hydrodisulfides (**6**) and polysulfides (**7**) to the biological action of leinamycin is supported by the existence of a variety of cytotoxic polysulfide-containing natural products such as varacin (**17**) (*30*), the 1,2,3-trithiane **18** (*31*), and bis(2-hydroxyethyl)trisulfide (**19**) (*32*). Because attack of thiols on polysulfides is a facile process ($k \geq 1.8\ M^{-1}\ s^{-1}$) (*33*), it is reasonable to envision that these compounds react with endogenous cellular thiols as shown in Scheme 2. Various polysulfides display IC_{50} values in the range of 0.05−14 μg/mL against a range of cancer cell lines (*34*), and it is noteworthy that even the relatively unfunctionalized polysulfide, bis(2-hydroxyethyl)trisulfide (**19**), displays significant cytotoxicity (e.g., IC_{50} of 3 μg/mL against P388 mouse leukemia cells) (*32*).

The discovery that leinamycin-derived polysulfides serve as thiol-dependent DNA-cleaving agents inspired investigations into the biological chemistry of the natural product varacin (**17**). Varacin exhibits potent activity against a human colon cancer cell line (HCT-116, $IC_{90} =$ 0.05 μg/mL), and early experiments showing that varacin is selectively toxic to a cell line characterized as DNA repair-deficient led to the suggestion that this compound may derive its biological activity from the formation of single-strand breaks in DNA (*30*). Accordingly, recent studies have shown that 7-methylbenzopentathiepin (**20**), a synthetic varacin analogue stripped of all functionality except the critical polysulfur heterocycle found in the natural product, is a potent thiol-dependent DNA-cleaving agent under physiologically relevant conditions (*35*). Compound **20** reacts readily with thiols to yield a complex mixture of polysulfides and hydropolysulfides which are thought to be key intermediates in the observed oxidative DNA damage.

The alkylation of double-stranded DNA by leinamycin undoubtedly plays a key role in the toxicity of the antibiotic. In general, the cytotoxic effects of DNA alkylation are well-known (*36*). The only leinamycin−DNA adduct identified to date results from reaction of the compound at the N7 position of guanosine residues. Other antibiotics, such as pluramycin and hedamycin (*37*), that selectively modify the N7 position of guanosine residues, while generally not as active as leinamycin, display potent cytotoxic activities (e.g., hedamycin provides 13% increased life span against murine leukemia P388 at 0.63 mg/kg, and pluramycin shows anti-HeLa activity at 0.06−0.15 μg/mL and an LD_{50} of 12.5−25 mg/kg in mice). Leinamycin-derived polysulfur species may serve to potentiate the cytotoxicity arising from DNA alkylation by the antibiotic (*38*). It is possible that alkylation of biomolecules other than DNA contributes to the biological activity of leinamycin. Likewise, leinamycin-derived polysulfides may derive activity by causing general

oxidative stress or through reactions with thiol groups on enzymes and transcription factors.

Conclusions. As anticipated, the unique chemical structure of leinamycin confers unusual chemical reactivity on this natural product. Leinamycin damages DNA by (at least) two unprecedented chemical mechanisms involving (1) thiol-triggered release of hydrodisulfide and polysulfide species that cause oxidative DNA damage and (2) thiol-triggered generation of a DNA-alkylating episulfonium ion. Many intriguing aspects of leinamycin's chemistry and biology remain to be explored, including alternate chemical pathways for activation of the antibiotic, examination of noncovalent DNA binding, sequence specificity of DNA alkylation, identification of additional DNA adducts, and important chemical and biological properties of leinamycin−DNA adducts. With further study, it seems likely that leinamycin will reveal additional secrets that afford us a better understanding of the complex mechanisms by which small molecules can efficiently modify cellular DNA.

Acknowledgment. Our work on leinamycin is supported by the National Institutes of Health (Grants GM51565 and CA83925). I thank Dr. Yutaka Kanda and other researchers at Kyowa Hakko Kogyo Ltd. for providing us with samples of leinamycin, and I am grateful to my past and present co-workers for their enthusiasm and dedication.

References

(1) Foye, W. O., Ed. (1995) *Cancer chemotherapeutic agents*, American Chemical Society, Washington, DC.

(2) Gates, K. S. (1999) Covalent modification of DNA by natural products. In *Comprehensive Natural Products Chemistry* (Barton, D. H. R., Nakanishi, K., and Meth-Cohn, O., Eds.) Vol. 7, pp 491−552, Pergamon, New York.

(3) Hara, M., Takahashi, I., Yoshida, M., Kawamoto, I., Morimoto, M., and Nakano, H. (1989) DC-107, a novel antitumor antibiotic produced by a *Streptomyces* sp. *J. Antibiot.* **42**, 333−335.

(4) Hirayama, N., and Matsuzawa, E. S. (1993) Molecular structure of a novel antitumor antibiotic leinamycin. *Chem. Lett.* **11**, 1957−1958.

(5) Hara, M., Asano, K., Kawamoto, I., Takiguchi, T., Katsumata, S., Takahashi, K., and Nakano, H. (1989) Leinamycin: A new antitumor antibiotic from *Streptomyces*; Producing organism, fermentation and isolation. *J. Antibiot.* **42**, 1768−1774.

(6) Kanda, Y., and Fukuyama, T. (1993) Total synthesis of (+)-leinamycin. *J. Am. Chem. Soc.* **115**, 8451−8452.

(7) Hara, M., Saitoh, Y., and Nakano, H. (1990) DNA strand scission by the novel antitumor antibiotic leinamycin. *Biochemistry* **29**, 5676−5681.

(8) Kanda, Y., Ashizawa, T., Kakita, S., Takahashi, Y., Kono, M., Yoshida, M., Saitoh, Y., and Okabe, M. (1999) Synthesis and antitumor activity of novel thioester derivatives of leinamycin. *J. Med. Chem.* **42**, 1330−1332.

(9) Kanda, Y., Ashizawa, T., Saitoh, Y., Saito, H., Gomi, K., and Okabe, M. (1998) Synthesis and antitumor activity of leinamycin derivatives: modifications of C-8 hydroxy and C-9 keto groups. *Bioorg. Med. Chem. Lett.* **8**, 909−912.

(10) Bellomo, G., Vairetti, M., Stivala, L., Mirabelli, F., Richelmi, P., and Orrenius, S. (1992) Demonstration of nuclear compartmentalization of glutathione in hepatocytes. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4412−4416.

(11))Mitra, K., Pohl, M. E., MacGillivray, L. R., Barnes, C. L., and Gates, K. S. (1997) Synthesis and structure of functionalized derivatives of the cleft-shaped molecule dithiosalicylide. *J. Org. Chem.* **62**, 9361−9364.

(12) Mitra, K., and Gates, K. S. (1995) Novel syntheses of dithiosalicylide. *Tetrahedron Lett.* **36**, 1391−1394.

(13) Kim, W., Dannaldson, J., and Gates, K. S. (1996) Reactions of 3H-benzodithiol-3-one 1-oxide with amines and anilines. *Tetrahedron Lett.* **37**, 5337−5340.

(14) Behroozi, S. J., Barnes, C. L., and Gates, K. S. (1998) Crystal structure of 3H-1,2-benzodithiol-3-one 1-oxide. *J. Chem. Crystallogr.* **28**, 689−691.

(15) Behroozi, S. J., Kim, W., and Gates, K. S. (1995) The reaction of *n*-propanethiol with 3H-1,2-benzodithiol-3-one 1-oxide and 5,5-dimethyl-1,2-dithiolan-3-one 1-oxide: Studies related to the reaction of antitumor antibiotic leinamycin with DNA. *J. Org. Chem.* **60**, 3964−3966.

(16) Behroozi, S. J., Kim, W., Dannaldson, J., and Gates, K. S. (1996) DNA cleavage by 1,2-dithiolan-3-one 1-oxides: A class of thiol-activated DNA cleaving agents structurally related to the antitumor antibiotic leinamycin. *Biochemistry* **35**, 1768−1774.

(17) Mitra, K., Kim, W., Daniels, J. S., and Gates, K. S. (1997) Oxidative DNA cleavage by the antitumor antibiotic leinamycin and simple 1,2-dithiolan-3-one 1-oxides: Evidence for thiol-dependent conversion of molecular oxygen to DNA-cleaving oxygen radicals mediated by polysulfides. *J. Am. Chem. Soc.* **119**, 11691−11692.

(18) Breydo, L., and Gates, K. S. (2000) Thiol-dependent DNA cleavage by 3H-1,2-benzodithiol-3-one 1,1-dioxide. *Bioorg. Med. Chem. Lett.* **10**, 885−889.

(19) Everett, S. A., Folkes, L. K., Wardman, P., and Asmus, K. D. (1994) Free-radical repair by a novel perthiol: reversible hydrogen transfer and perthiyl radical formation. *Free Radical Res.* **20**, 387−400.

(20) Misra, H. P. (1974) Generation of superoxide free radical during the autooxidation of thiols. *J. Biol. Chem.* **249**, 2151−2155.

(21) Evans, M. B., and Saville, B. (1962) Nucleophilic displacements by thioanions on trisulfides. *Proc. Chem. Soc.*, 18−19.

(22) Zhang, J.-Z., and Millero, F. J. (1993) Products from the oxidation of H_2S in seawater. *Geochim. Cosmochim. Acta* **57**, 1705−1718.

(23) Asai, A., Hara, M., Kakita, S., Kanda, Y., Yoshida, M., Saito, H., and Saitoh, Y. (1996) Thiol-mediated DNA alkylation by the novel antitumor antibiotic leinamycin. *J. Am. Chem. Soc.* **118**, 6802−6803.

(24) Schank, K., Frisch, A., and Zwanenburg, B. (1983) α-Oxosulfones. 4. Correction of a pretended α-oxosulfone by an unambiguous synthesis of the revised structure. *J. Org. Chem.* **48**, 4580−4582.

(25) Morishita, T., Furukawa, N., and Oae, S. (1981) Reaction of thiolsulfinates with trihaloacetic anhydrides. II. Addition of sulfenyl trihaloacetates to olefins. *Tetrahedron* **37**, 2539−2546.

(26) Havlik, A. J., and Kharasch, N. (1956) Derivatives of sulfenic acids. XXIV. Stereochemical studies of certain *β*-chloroalkyl aryl sulfides. *J. Am. Chem. Soc.* **78**, 1207.

(27) Brydon, A., Cameron, G. G., and Hogg, D. R. (1972) Disulfenyl derivatives-bis(arenesulfenyl) terephthalates. *Int. J. Sulfur Chem. A* **2**, 289.

(28) Asai, A., Saito, H., and Saitoh, Y. (1997) Thiol-independent DNA cleavage by a leinamycin degradation product. *Bioorg. Med. Chem.* **5**, 723−729.

(29) Ikegami, S., Ohishi, J.-I., and Akaboshi, S. (1975) The effects of neighboring heteroatoms in ring opening of epoxides. *Chem. Pharm. Bull.* **23**, 2701−2710.

(30) Davidson, B. S., Molinski, T. F., Barrows, L. R., and Ireland, C. M. (1991) Varacin: a novel benzopentathiapin from *Lissoclinium vareau* that is cytotoxic toward a human colon tumor. *J. Am. Chem. Soc.* **113**, 4709−4710.

(31) Copp, B. R., Blunt, J. W., and Munro, M. H. G. (1989) A biologically active 1,2,3-trithiane derivative from the New Zealand ascidian *Aplidium* sp. D. *Tetrahedron Lett.* **30**, 3703−3706.

(32) Kohama, Y., Iida, K., Semba, T., Mimura, T., Inada, A., Tanaka, K., and Nakanishi, T. (1992) Studies on thermophile products. IV. Structural elucidation of cytotoxic substance, BS-1, derived from *Bacillus stearothermophilus*. *Chem. Pharm. Bull.* **40**, 2210−2211.

(33) Myers, A. G., Cohen, S. B., and Kwon, B. M. (1994) A study of the reaction of calicheamicin g1 with glutathione in the presence of double-stranded DNA. *J. Am. Chem. Soc.* **116**, 1255−1271.

(34) Tolstikov, G. A., Shults, E. E., and Tolstikov, A. G. (1997) Natural polysulfides. *Russ. Chem. Rev.* **66**, 813.

(35) Chatterji, T., and Gates, K. S. (1998) DNA Cleavage by 7-methylbenzopentathiepin: A simple analog of the antitumor agent varacin. *Bioorg. Med. Chem. Lett.* **8**, 535−538.

(36) Mitra, S., and Kaina, B. (1993) Regulation of repair of alkylation damage in mammalian genomes. *Prog. Nucleic Acid Res. Mol. Biol.* **44**, 109−142.

(37) Hansen, M. R., and Hurley, L. H. (1996) Pluramycins. Old drugs having modern friends in structural biology. *Acc. Chem. Res.* **29**, 249−258.

(38) Jevtovic-Todorovic, V., and Guenthner, T. M. (1992) Depletion of a discrete nuclear gluathione pool by oxidative stress, but not by buthionine sulfoximine. Correlation with enhanced alkylating agent cytotoxicity to human melanoma cells in vitro. *Biochem. Pharmacol.* **44**, 1383−1393.

TX000089M