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Thiol-Dependent DNA Cleavage by 3*H*-1,2-Benzodithiol-3-one 1,1-dioxide

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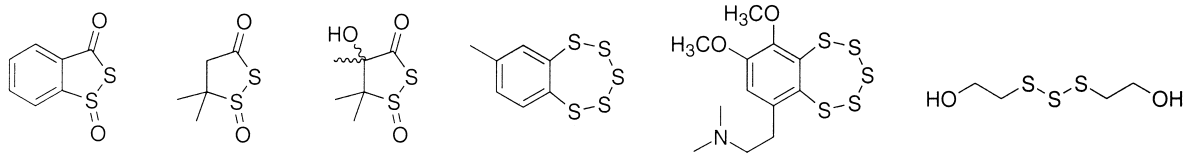
Abstract—Hydrodisulfides (RSSH) have previously been implicated as key intermediates in thiol-triggered oxidative DNA damage by the antitumor agent leinamycin. In an effort to better understand DNA damage by RSSH and to expand on the number and type of chemical systems that produce this reactive intermediate, the ability of 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (**11**) to serve as a thiol-dependent DNA cleaving agent has been investigated. The findings reported here indicate that reaction of **11** with thiols results in release of RSSH and subsequent oxidative DNA strand cleavage.

Reaction of thiols with the antibiotic leinamycin (**1**) triggers oxidative DNA cleavage and DNA alkylation via two chemically distinct pathways (Scheme 1).[1–4] Thiol-triggered oxidative DNA cleavage by leinamycin and by synthetic leinamycin analogues such as **5**, **6**, and **7** occurs by a general mechanism involving the conversion of molecular oxygen to DNA-cleaving oxygen radicals via superoxide, hydrogen peroxide, and a trace metal-catalyzed Fenton reaction.[2,4] Superoxide radical is presumably generated in this process by O_2-mediated degradation of unstable hydrodisulfide intermediates (RSSH, **3**; Scheme 1) that are produced during the reaction of the 1,2-dithiolan-3-one 1-oxide heterocycle with thiols.[4,5]

Hydropolysulfides (RS_xSH) are also suspected as key intermediates in thiol-triggered oxidative DNA cleavage by polysulfides such as 7-methylbenzopentathiepin[6] (**8**) and bis(2-hydroxyethyl)trisulfide (**10**).[4] DNA damage and/or general oxidative stress caused by the production of oxygen radicals in the decomposition of hydrodisulfide intermediates may play an important role in the biological activities of the antitumor agent leinamycin and various cytotoxic polysulfides such as varacin[7] (**9**) and lissoclinotoxin A,[8] and bis(2-hydroxyethyl)trisulfide (**10**).[9]

In an effort to better understand the DNA-cleaving properties of hydrodisulfides and to expand on the number and type of chemical systems capable of generating this reactive species, we have examined the ability of 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (**11**) to serve as a thiol-activated DNA-cleaving agent. We anticipated that attack of thiol on this sulfur heterocycle would result in the release of RSSH by the mechanism shown in Scheme 2. Although the reaction of thiols with **11** has not previously been reported, the mechanism proposed in Scheme 2 is directly analogous to that elucidated for the reaction of phosphites with this sulfur-transfer agent.[10]

We find that, in the presence of excess thiol, micromolar concentrations of **11** efficiently cause single-strand breaks in duplex DNA, as measured by the conversion



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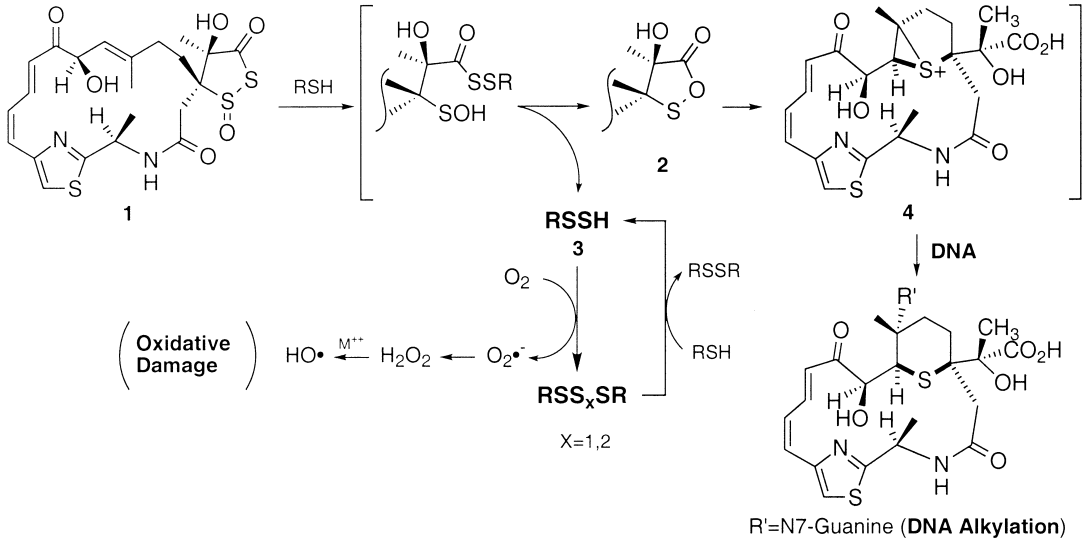
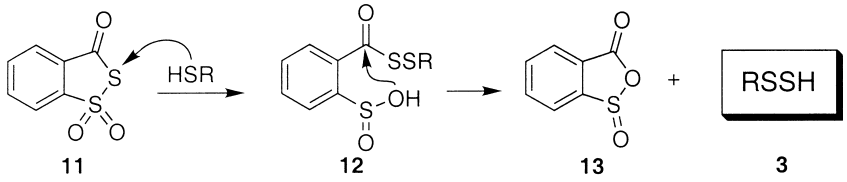


Scheme 1. Mechanisms of DNA damage by leinamycin.



Scheme 2. Proposed reaction of thiols with 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (**11**).

of supercoiled (Form I) plasmid DNA to the open circular form (Form II) (Fig. 1). In accord with the mechanism proposed in Scheme 2, efficient DNA cleavage by **11** is completely dependent upon added thiol (Fig. 2). As expected for the involvement of a hydrodisulfide intermediate (**3**), mechanistic experiments indicate that **11**, in conjunction with added thiols, mediates the conversion of molecular oxygen to DNA-cleaving oxygen radicals (Fig. 3). The involvement of reduced oxygen species ($O_2^{\bullet-} \rightarrow H_2O_2 \rightarrow HO^{\bullet}$) and a trace metal-catalyzed, Fenton-type conversion of hydrogen peroxide to DNA-cleaving radicals in thiol-mediated DNA cleavage by **11** is supported by several findings. Commonly used[11] oxygen radical scavengers such as methanol and mannitol significantly inhibit thiol-dependent DNA cleavage by **11**. Chelating agents such as diethylenetriaminepentaacetic acid (DETAPAC) inhibit strand scission in this system, presumably by sequestering adventitious trace metals in a non-redox-active form[11,12] that cannot participate in the Fenton reaction. Addition of the hydrogen peroxide-destroying enzyme catalase to the assay mixture almost completely inhibits DNA cleavage. Addition of superoxide dismutase (SOD) increases the efficiency of strand scission by **11**. The marked effect of SOD is consistent with the presence of superoxide radical in these reactions. SOD catalyzes the disproportionation of superoxide to hydrogen peroxide and O_2,[13] and the increase in cleavage efficiency can be rationalized by the fact that this enzyme facilitates the

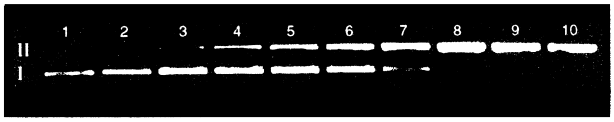


Figure 1. Thiol-dependent DNA cleavage by various concentrations of **11**. Supercoiled pBR322 DNA (38 μM bp) was incubated for 6 h at 24 °C with various concentrations of **11** and 5 equiv of 2-mercaptoethanol in sodium phosphate buffer (50 mM, pH 7.0), followed by agarose gel electrophoretic analysis as previously described.[2] Lane 1, DNA alone; lane 2, **11** (1 mM) (0.02 ± 0.01); lane 3, thiol (5 mM) (0.06 ± 0.01); lane 4, **11** (10 μM) + thiol (0.13 ± 0.02); lane 5, **11** (20 μM) + thiol (0.26 ± 0.05); lane 6, **11** (50 μM) + thiol (0.63 ± 0.11); lane 7, **1** (100 μM) + thiol (1.53 ± 0.64); lane 8, **1** (250 μM) + thiol (2.77 ± 0.69); lane 9, **11** (500 μM) + thiol (3.08 ± 0.61); lane 10, **11** (1 mM) + thiol (3.47 ± 0.78). The value in parentheses following each lane description represents the mean number of strand breaks per plasmid molecule (S) calculated using the equation $S = -\ln f_I$ where f_I is the fraction of plasmid present as form I. [15] The values are corrected for background nicks present in control DNA.

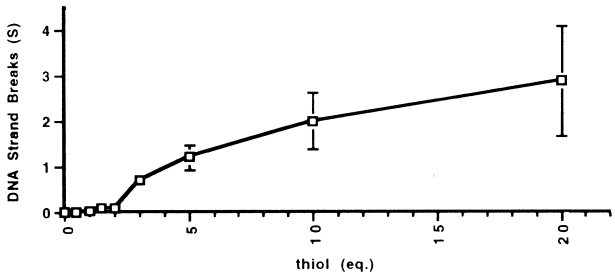


Figure 2. Effect of thiol concentration on DNA cleavage by **11**. Supercoiled pBR322 DNA (38 μM bp) was incubated for 6 h at 24 °C with **11** (100 μM) in sodium phosphate buffer (50 mM, pH 7.0) with various concentrations of 2-mercaptoethanol, followed by agarose gel electrophoretic analysis as previously described.[2]

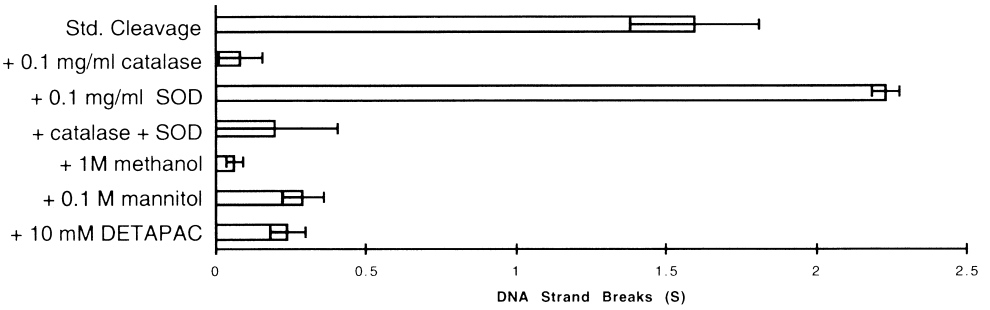


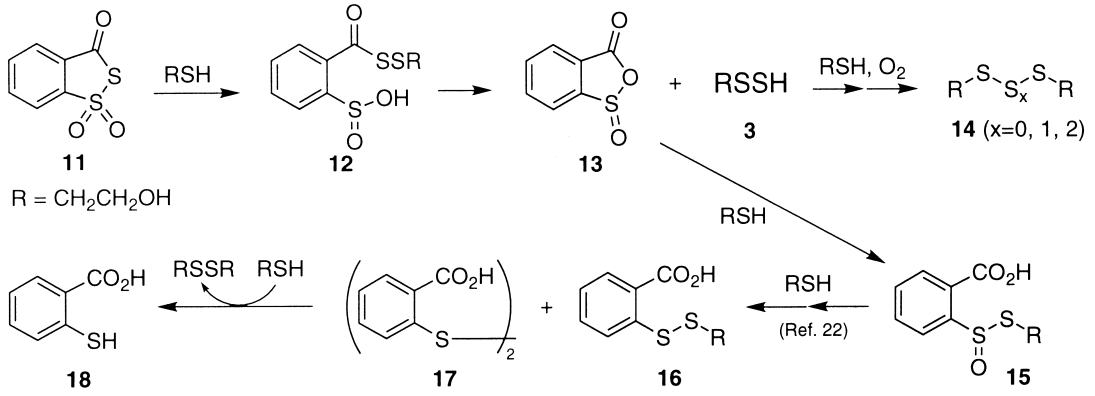
Figure 3. Effect of various additives on thiol-activated DNA cleavage by **11**. Supercoiled pBR322 DNA (38 μM bp) was incubated for 6 h at 24 °C with **11** (100 μM) and 2-mercaptoethanol (500 μM) in sodium phosphate buffer (50 mM, pH 7.0) in the presence of various additives, followed by agarose gel electrophoretic analysis as previously described.[2]

production of hydrogen peroxide.[14] The observation that >2 equiv of thiol are required for significant DNA cleavage (Fig. 2) suggests that secondary reactions of thiol with **3** may be important.

In order to characterize the chemical events underlying thiol-dependent oxidative DNA cleavage by **11**, we examined the products and intermediates resulting from reaction of this sulfur heterocycle with thiols. The reaction of **11** (40 mM) with excess thiol (10 equiv of 2-mercaptoethanol) in a 4:1 water:acetonitrile mixture is rapid (complete in seconds).[16] The resulting mixture of products was separated and characterized using standard methods, and the identity of the products was confirmed by comparison of NMR spectra with that of authentic samples prepared by independent routes. The aromatic carboxylic acids **16**, **17**, and **18** were isolated and characterized as their methyl ester derivatives following work up of the reaction mixture with diazomethane.[17–21]

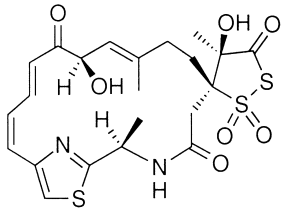
The products isolated from the reaction of **11** with thiols are consistent with those expected from the breakdown of the hydrodisulfide and 1,2-benzoxathiolan-3-one 1-oxide intermediates (**3** and **13**) shown in Scheme 2. Initial attack of thiol on the 1,2-benzoxathiolan-3-one 1-oxide intermediate **13** is expected to yield the thiosulfinate **15**. In accord with literature precedent,[22] this thiosulfinate reacts further with thiol to afford a mixture of disulfides **16** and **17**. The disulfides **16** and **17** are isolated in low yield because thiol-disulfide exchange[23] reactions with excess 2-mercaptoethanol in the mixture efficiently convert them to thiosalicylic acid **18**. Importantly, the production of polysulfides (**14**, X = 1,2) in this reaction is indicative of the formation of a hydrodisulfide intermediate (**3**). Hydrodisulfides readily decompose to yield mixtures of polysulfides.[24,25] Finally, in support of the proposed mechanism (Scheme 2), under conditions designed to favor its detection (0.5 equiv *n*-propanethiol, 0.5 equiv triethylamine, in dry CH_2Cl_2), we have directly observed the unstable 1,2-benzoxathiolan-3-one 1-oxide (**13**) as the major product stemming from the reaction of **11** with thiol.[26]

This work establishes the 3*H*-1,2-benzodithiol-3-one 1,1-dioxide heterocycle as a new type of thiol-activated DNA-cleaving agent. Examination of products and intermediates resulting from the reaction of 3*H*-1,2-benzodithiol-3-one 1,1-dioxide with thiol supports the notion that a DNA-cleaving hydrodisulfide species (RSSH) is released by attack of thiol on this ring system. The efficiency and mechanism of thiol-triggered DNA cleavage by **11** and **5** are very similar.[2] This observation is meaningful because RSSH is an intermediate generated by the attack of thiol on *both* of these heterocyclic systems and, therefore, thiol-triggered oxidative DNA damage by **11** and **5** is most reasonably attributed to this common reactive intermediate. Further studies designed to characterize the mechanism(s) by which hydrodisulfides generate oxygen radicals are currently underway in this laboratory.



Scheme 3. Products resulting from attack of thiol on 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (**11**).

Previous work has shown that **5** serves as an excellent model for the chemical reactivity of the 1,2-dithiolan-3-one 1-oxide heterocycle found in leinamycin (**1**).[1,3,4] Thus, our studies with **11** suggest that a 1,1-dioxide analogue of leinamycin (**19**) would retain the ability to produce RSSH upon reaction with thiols. On the other hand, such a leinamycin analogue would likely be devoid of DNA-alkylating properties because the electrophilic oxathiolanone intermediate (**2**, Scheme 1) required for the generation of the leinamycin-derived, DNA-alkylating episulfonium ion (**4**) is not produced by attack of thiol on the 1,1-dioxide system. Rather, attack of thiol on the 1,1-dioxide system produces a 1,2-oxathiolan-3-one 1-oxide (e.g., **13**) which, in the context of leinamycin, is not expected to lead to formation of a DNA-alkylating electrophile.[27] Consequently, the 1,1-dioxide analogue of leinamycin **19** could serve as a useful research tool to examine the biological effects stemming from hydrodisulfide production by leinamycin, in the *absence* of DNA alkylation by the natural product.



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Acknowledgements

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References and Notes

1. Behroozi, S. B.; Kim, W.; Gates, K. S. *J. Org. Chem.* **1995**, *60*, 3964.
2. Behroozi, S. J.; Kim, W.; Dannaldson, J.; Gates, K. S. *Biochemistry* **1996**, *35*, 1768.
3. Asai, A.; Hara, M.; Kakita, S.; Kanda, Y.; Yoshida, M.; Saito, H.; Saitoh, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6802.
4. Mitra, K.; Kim, W.; Daniels, J. S.; Gates, K. S. *J. Am. Chem. Soc.* **1997**, *119*, 11691.
5. Mitra, K.; Gates, K. S. *Recent Res. Devel. Org. Chem.* **1999**, *3*, 311.
6. Chatterji, T.; Gates, K. S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 535.
7. Davidson, B. S.; Molinski, T. F.; Barrows, L. R.; Ireland, C. M. *J. Am. Chem. Soc.* **1991**, *113*, 4709.
8. Searle, P. A.; Molinski, T. F. *J. Org. Chem.* **1994**, *59*, 6600.
9. Kohama, Y.; Iida, K.; Semba, T.; Mimura, T.; Inada, A.; Tanaka, K.; Nakanishi, T. *Chem. Pharm. Bull.* **1992**, *40*, 2210.
10. Iyer, R. P.; Phillips, L. R.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Org. Chem.* **1990**, *55*, 4693.
11. Halliwell, B.; Gutteridge, J. M. C. *Methods Enzymol.* **1990**, *186*, 1.
12. Graf, E.; Mahoney, J. R.; Bryant, R. G.; Eaton, J. W. *J. Biol. Chem.* **1984**, *259*, 3620.
13. Fridovich, I. *Acc. Chem. Res.* **1972**, *5*, 321.
14. In systems where addition of SOD inhibits DNA damage it is likely due to the fact that superoxide radical serves as a necessary reducing agent for trace metals involved in the Fenton reaction. In systems where other reducing agents (e.g., thiols) are present, addition of SOD can actually increase DNA-damage efficiency. See, for example: Eliot, H.; Gianni, L.; Myers, C. *Biochemistry* **1984**, *23*, 928. Parraga, A.; Orozco, M.; Portugal, J. *Eur. J. Biochem.* **1992**, *208*, 227. Weglarz, L.; Bartosz, G. *Int. J. Biochem.* **1991**, *23*, 663. Nagai, K.; Hecht, S. M. *J. Biol. Chem.* **1991**, *266*, 23994.
15. Hertzberg, R. P.; Dervan, P. B. *Biochemistry* **1984**, *23*, 3934.
16. Compound **11** undergoes hydrolysis slowly compared to thiolysis. In the DNA-cleavage reactions reported here, **11** was the final component added to thiol-containing reaction mixtures to ensure that thiol-triggered chemistry predominates.
17. In a typical reaction, 2-mercaptoethanol (1.4 mL, 20 mmol) in water (40 mL) was added to a stirred solution of **11**[10] (400 mg, 2 mmol) in acetonitrile (10 mL) to produce a cloudy mixture. Under these conditions, all **11** is consumed (TLC) within 1 min. After 10 min, 2 M HCl (2 mL, 4 mmol) was added, and resulting suspension was extracted with ether (5×50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to yield a white solid. To this solid was added diazomethane (50 mL of 0.3 M solution in ether), and the resulting yellow solution stirred for 5 min at 24 °C, concentrated under reduced pressure, and subjected to column chromatography (12:1 hexane:EtOAc→100% EtOAc) to yield 2-(methylthio)carboxymethylbenzene (**20**, the dimethyl derivative of **18**; 320 mg, 88%), dimethyl 2,2′-dithiodibenzoate (**21**, the methyl ester of **17**; 11 mg, 3%), 2-(2′-hydroxyethyldithio)carboxymethylbenzene (**22**, the methyl ester of **16**; 32 mg, 7%) and 2-(hydroxyethyl)di-, tri-, and tetrasulfides (**14** where $n = 0$, 1 and 2; as 90:9:1 mixture, 558 mg, 3.6 mmol). A trace amount (2 mg, 0.5%) of the dimethyl ester of *o*-carboxybenzenesulfinic acid, resulting either from direct hydrolysis of **11** or hydrolysis of intermediates **13** or **15**, was also detected. **2-(Methylthio)carboxymethylbenzene** (**20**): white solid (320 mg, 88% from **11**), mp 62–64 °C (lit: 63–65 °C);[18] ^{1}H NMR[19] (250 MHz, $CDCl_3$): δ 7.98 (d, $J = 7.5$ Hz, 1H), 7.45 (t, $J = 7.5$ Hz, 1H), 7.25 (d, $J = 7.5$ Hz, 1H), 7.13 (t, $J = 7.5$ Hz, 1H), 3.89 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 166.6, 143.1, 132.3, 131.1, 126.5, 124.1, 123.2, 51.8, 15.3; HRMS (EI) *m/z* calcd for $C_9H_{10}O_2S$ 182.0418, found 182.0401. The identity of **20** was further confirmed by comparison (NMR, TLC) to an authentic sample prepared via reaction of 2-mercaptobenzoic acid (Aldrich) with excess diazomethane (CAUTION: explosion hazard!). **Dimethyl 2,2′-dithiodibenzoate** (**21**): Initially isolated as a mixture containing∼10% dimethyl 2,2′-tri and tetrathiodibenzoate. An analytical sample of **21** was prepared by treating the mixture with triphenylphosphine to decompose the polysulfide impurities. This afforded **21** as white crystals (11 mg, 3% from **11**), mp 128–129 °C (lit: 131.5–133.5 °C);[20] ^{1}H NMR[21] (250 MHz, $CDCl_3$): δ 8.06 (dd, $J = 7.5$, 1 Hz, 2H), 7.76 (dd, $J = 7.5$, 1 Hz, 2H), 7.41 (td, $J = 7.5$, 1 Hz, 2H), 7.23 (td, $J = 7.5$, 1 Hz, 2H), 3.99 (s, 6H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 166.9, 140.3, 133.0, 131.4, 127.3, 125.8, 125.4, 52.4; HRMS (EI) *m/z* calcd for $C_{16}H_{14}O_4S_2$ 334.0333, found 334.0328. The identity of **21** was further confirmed by comparison (NMR, TLC) with an authentic sample prepared by esterification of 2,2′-dithiodibenzoic acid (Aldrich) with diazomethane. **2-(2′-Hydroxyethyldithio)carboxymethylbenzene** (**22**): clear oil (32 mg, 7% from **11**): ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.20 (d, $J = 7.5$ Hz, 1H), 8.02 (dd, $J = 7.5$ Hz, 1 Hz, 1H), 7.58 (td, $J = 7.5$ Hz, 1 Hz, 1H), 7.26 (td, $J = 7.5$ Hz, 1 Hz, 1H), 3.94 (s, 3H), 3.86 (m, 2H), 2.88 (t, $J = 6$ Hz, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 166.7, 141.3, 132.8, 131.4, 127.1, 125.6, 125.2, 60.2, 52.2, 40.5;

HRMS m/z calcd for $C_{10}H_{12}O_3S_2$ 244.0228, found 244.0232. **Bis(2-hydroxyethyl)di-,tri-** and **tetrasulfides** (**14**): As a clear oil. Analytical data for **14** (^{1}H NMR and HPLC retention time) matches that reported previously.[4,9] Obtained as a mixture where $n=0$, 1, and 2 (90:9:1, 558 mg, 3.6 mmol).
18. Lüdersdorf, R.; Martens, J.; Pakzad, B.; Praefcke, K. *Justus Liebig Ann. Chem.* **1992**, 1977
19. Ozaki, S.; Yang, H.-J.; Matsui, T.; Goto, Y.; Watanabe, Y. *Tetrahedron: Asymmetry* **1999**, *10*, 183.
20. Overman, L. E.; Matzinger, D.; O'Connor, E. M.; Overman, J. D. *J. Am. Chem. Soc.* **1974**, *96*, 6081.
21. Wentrup, C.; Bender, H.; Gross, G. *J. Org. Chem.* **1987**, *52*, 3838.
22. Kice, J. L.; Rogers, T. E. *J. Am. Chem. Soc.* **1974**, *96*, 8015.
23. Lees, W. J.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 642.
24. Derbesy, G.; Harpp, D. N. *Sulfur Lett.* **1992**, *14*, 199.
25. Kawamura, S.; Abe, Y.; Tsurugi, J. *J. Org. Chem.* **1969**, *34*, 3633.
26. To a stirred solution of **11** (100 mg, 0.5 mmol) in freshly distilled dichloromethane (15 mL) was added triethylamine (35 μL, 0.25 mmol) and 1-propanethiol (23 μL, 0.25 mmol). After 10 min, the reaction mixture was concentrated under reduced pressure and redissolved in $CDCl_3$. Analysis of the reaction mixture by ^{13}C NMR revealed that 1,2-benzoxathiolan-3-one 1-oxide (**13**) was the major product of the reaction. The ^{13}C NMR spectrum of **13** in $CDCl_3$ has previously been reported.[10]
27. Literature precedent suggests that a leinamycin-derived 1,2-oxathiolan-3-one 1-oxide (analogous to **13**) will not react with the C6-C7 alkene of the natural product. For example, other activated sulfinic acid derivatives, such as sulfinyl chlorides, react with alkenes but Lewis acid catalysis and relatively harsh conditions are required. Alternatively, thiol-triggered DNA alkylation by **19** could, in principle, occur via attack of thiol on a leinamycin-derived intermediate analogous to **15** to yield a carboxy-sulfenic acid which might cyclize to the activated form of the antibiotic (**2**). However, unless the cyclization reaction is very rapid, the sulfenic acid intermediate would decompose by reaction with excess thiol (to afford a mixed disulfide analogueueous to **16**),[22] and would not yield 'activated' leinamycin (**2**).