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Synthesis and characterization of a small analogue of the anticancer natural product leinamycin

Kripa Keerthi [a], Anuruddha Rajapakse [a], Daekyu Sun [c], Kent S. Gates [a,b,*]

[a] Department of Chemistry, University of Missouri, 125 Chemistry Building Columbia, MO 65211, United States
[b] Department of Biochemistry, University of Missouri, 125 Chemistry Building Columbia, MO 65211, United States
[c] Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, BIO5 Institute, Room 102, 1657 E. Helen Street, Tucson, AZ 85721, United States

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ABSTRACT

Leinamycin (**1**) is a *Streptomyces*-derived natural product that displays nanomolar IC_{50} values against human cancer cell lines. In the work described here, we report the synthesis and characterization of a small leinamycin analogue **19** that closely resembles the 'upper-right quadrant' of the natural product, consisting of an alicyclic 1,2-dithiolan-3-one 1-oxide heterocycle connected to an alkene by a two-carbon linker. The results indicate that this small analogue contains the core set of functional groups required to enable thiol-triggered generation of both redox active polysulfides and an episulfonium ion intermediate via the complex reaction cascade first seen in the natural product leinamycin. The small leinamycin analogue **19** caused thiol-triggered oxidative DNA strand cleavage in a manner similar to the natural product, but did not alkyate duplex DNA effectively. This highlights the central role of the 18-membered macrocycle of leinamycin in driving efficient DNA alkylation by the natural product.

1. Introduction

Natural products have played a central role in the discovery of anticancer, antifungal, and antibacterial drugs. Between 60% and 80% of the clinically used drugs in these categories are natural products or are derived from natural product leads.[1–4] In the fields of organic, medicinal, and natural product chemistry there is a rich history surrounding the preparation of small synthetic analogues of bioactive natural products.[5–10] In particular, studies of small, less functionalized—'stripped down'—analogues can help define the core functional groups that confer function upon the natural product and can facilitate the development of new derivatives with improved properties.[5–10] Here we describe the synthesis and characterization of a small analogue of the anticancer natural product leinamycin.

Leinamycin (**1**) is a *Streptomyces*-derived natural product that displays nanomolar IC_{50} values against human cancer cell lines.[11–16] Reaction of biological thiols with the 1,2-dithiolan-3-one 1-oxide heterocycle of leinamycin is thought to produce a sulfenic acid intermediate **2** that cyclizes[17] to eject a persulfide intermediate (**3**) that, in turn, generates reactive oxygen species (Scheme 1).[18–22] The 1,2-oxathiolan-5-one intermediate (**4**) formed in this process undergoes further rearrangement to an episulfonium ion (**5**) that efficiently alkylates guanine residues in duplex DNA (Scheme 1).[18,23–25] The resulting leinamycin–guanine adduct **6** undergoes rapid depurination to generate abasic sites in cellular DNA.[26–30]

Previous studies showed that the small leinamycin analogues **10–12** replicate the natural product's ability to generate reactive oxygen species upon reaction with thiols.[19,20,29] In the work described here, we report the synthesis and characterization of a small leinamycin analogue that closely resembles the upper right quadrant of the natural product (**1**, Scheme 1), consisting of an alicyclic 1,2-dithiolan-3-one 1-oxide heterocycle connected to an alkene moiety by a two-carbon linker. We felt this analogue was of interest because it nominally contains the minimum set of functional groups required to enable both the release of redox-active polysulfur species and generation of an episulfonium ion via the complex reaction cascade first seen in the natural product leinamycin.

2. Results and discussion

2.1. Synthesis of the leinamycin analogue 19

Our synthesis began with conjugate addition of benzyl mercaptan to commercially available geranic acid **14** in refluxing piperidine to give **15** in 95% yield (Scheme 2). Removal of the

* Corresponding author. Tel.: +1 573 882 6763; fax: +1 573 882 2754.
E-mail address: gatesk@missouri.edu (K.S. Gates).

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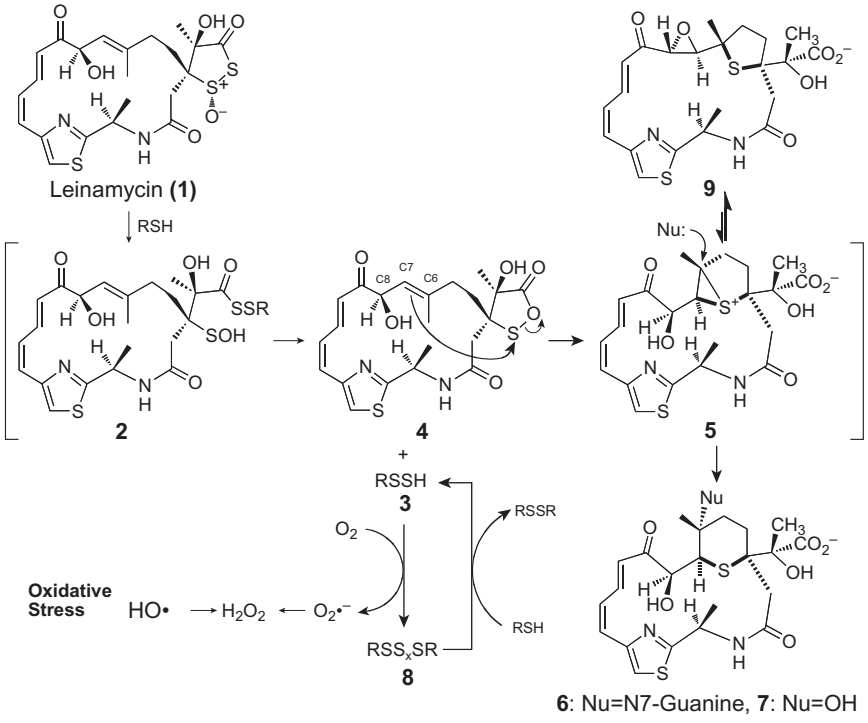
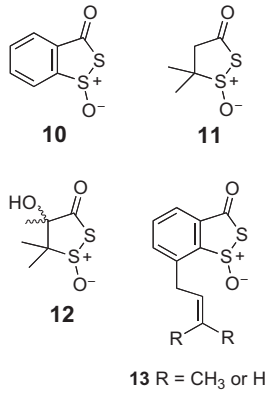
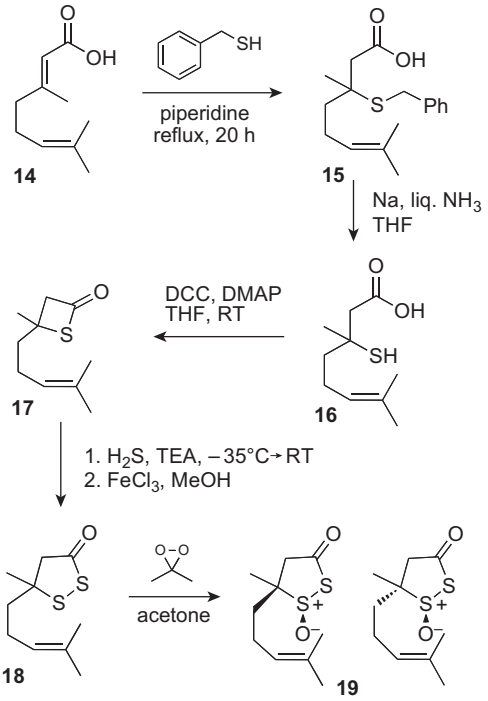


Scheme 1.



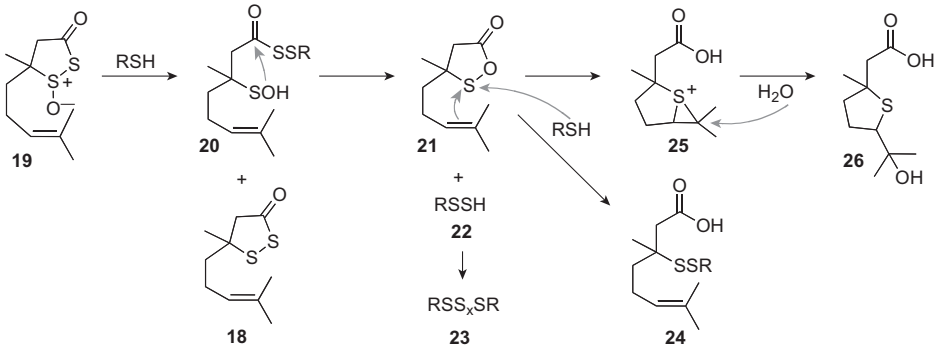
benzyl group from **15** using sodium in liquid ammonia gave the thiol derivative **16** in good yield (80%). Activation of the carboxylic acid in **16** with dicyclohexylcarbodiimide and DMAP in tetrahydrofuran converted this material to the thiolactone **17** in 75% yield. Treatment of **17** with H_2S and triethylamine, followed by oxidation with $FeCl_3$ in methanol gave the 1,2-dithiolan-3-one compound **18** in 60% yield for the two steps. Oxidation of this material with dimethyldioxirane gave a 1:1.7 diastereomeric mixture of the desired leinamycin analogue **19** in 80% yield (Scheme 2). Our approach represents a modified version of the route pioneered by Pattenden and Shuker for the synthesis of simple 1,2-dithionlan-3-one 1-oxides.[31] A similar route was used by Lee et al. for the preparation of analogues containing a pendent alkene.[32]



Scheme 2.

2.2. Reaction of 19 with mercaptans

We examined the reaction of **19** with several different thiols. Compound **19** was used as the mixture of diastereomers obtained in the synthesis above. A similar spectrum of products was



Scheme 3.

generated in reactions of **19** with 2-mercaptoethanol, ethanethiol, and benzyl mercaptan, although the relative yields of each product varied somewhat. The reaction with benzyl mercaptan is described here as a representative example. Compound **19** (20 mM) was mixed with benzyl mercaptan (100 mM) in a 1:1 mixture of sodium phosphate buffer (50 mM, pH 7)/acetonitrile. After 3 h, the mixture was treated with diazomethane to methylate carboxylic acid-containing products.

The reaction of **19** with benzyl mercaptan generated the deoxygenated product **18** in 15% isolated yield (Scheme 3). An analogous deoxygenation product was observed previously in the reaction of **10** with propanethiol.[18] Sulfoxides can be deoxygenated by reaction with thiols,[33,34] and **18** could arise via this route or by mechanisms unique to the 1,2-dithiolan-3-one 1-oxide heterocycle. The disulfide **24** was produced in 10% yield and the cyclized product **26** in 35% yield. In a separate reaction with 2-mercaptoethanol, GC/MS was used to detect the formation of polysulfides **23**. The products **18**, **23**, and **24** are analogous to those seen previously in the reactions of **10** with thiol.[18] In line with the conclusions from the early studies with **10**, we envision that the attack of a thiol group on the central, sulfenyl sulfur of the 1,2-dithiolan-3-one 1-oxide heterocycle in **19** gives rise to an 1,2-oxathiolan-5-one **21** and a persulfide **22** via the sulfenic acid intermediate **20** (Scheme 3).[17] Attack of a second equivalent of mercaptan on the 1,2-oxathiolan-5-one provides the disulfide **24**, while the persulfide **22** decomposes to yield a mixture of polysulfides **23**.[21] The alkene residue in **19** is clearly involved in the generation of the cyclized product **26**. Analogous to the reactions seen for leinamycin (**1**) and small synthetic leinamycin analogues **13**,[23,35] the formation of **26** is envisioned to proceed via an episulfonium ion intermediate **25** (Scheme 3) generated by reaction of the alkene with the electrophilic sulfur in the oxathiolanone ring.

2.3. Thiol-triggered DNA damage by 19

We employed a plasmid-based assay to examine the DNA-damaging properties of **19**. In this assay, single-strand cleavage converts supercoiled plasmid DNA (form I) to the open-circular form (form II).[36–38] The two forms of plasmid DNA are then separated using agarose gel electrophoresis, the gel stained with a DNA-binding dye such as ethidium bromide, and the relative amounts of cleaved and intact plasmid quantitatively determined by digital image analysis. The direct strand breaks (not requiring thermal or basic workup) monitored in this type of experiment typically arise via hydrogen atom abstraction from the 2′-deoxyribose residues in the backbone of DNA,[39–45] although the assay also can be used to detect abasic sites that arise via DNA alkylation processes.[46,47]

We found that **19** (used in this first experiment as the mixture of diastereomers) generated direct strand breaks when incubated with double-stranded plasmid DNA in the presence of 1.5 equiv of 2-mercaptoethanol at 24 °C in pH 7 sodium phosphate buffer (lane 4, Fig. 1). In the absence of thiol, **19** induced relatively little strand cleavage (lane 2, Fig. 1). Other control reactions showed that DNA in buffer alone, or DNA plus 2-mercaptoethanol was not significantly cleaved (lanes 1 and 3, respectively in Fig. 1).

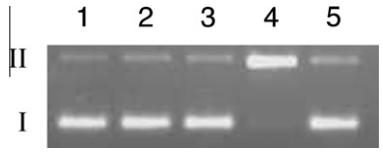


Figure 1. Thiol-dependent DNA strand cleavage by **19**. Assays were performed as described in the Section 4. Briefly, supercoiled double-stranded DNA (30 μM bp) was incubated with **19** and 2-mercaptoethanol in sodium phosphate buffer (50 mM, pH 7) containing acetonitrile (10% v/v) for 3 h at 37 °C. Form I and II plasmid DNA were resolved on an agarose gel and stained with ethidium bromide. The DNA was visualized by UV-transillumination and the amounts measured by digital image analysis. The number of single strand breaks per plasmid DNA molecule (S) was calculated using the equation $S = -\ln f_1$ where f_1 is the fraction of plasmid present as form I.[64] The numbers in the parentheses following the description of each lane indicate the number of strand breaks per plasmid molecule unless indicated otherwise. Lane 1: DNA in buffer alone ($S = 0.14 \pm 0.$), Lane 2: **19** alone (250 μM) (0.22 ± 0.09), Lane 3: 2-mercaptoethanol alone (375 μM) (0.26 ± 0.08), Lane 4: **19** (250 μM) + 2-mercaptoethanol (375 μM) (100% form II, S >4.6), Lane 5: **19** (250 μM) + 2-mercaptoethanol (375 μM) + catalase (100 μg/mL) (0.30 ± 0.02).

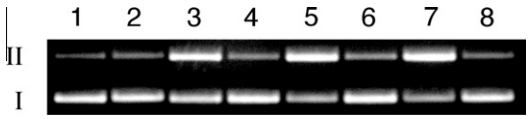


Figure 2. DNA strand cleavage by **10** or **19** in the presence of 2-mercaptoethanol. Assays were performed as described in the Section 4. Briefly, supercoiled double-stranded DNA (30 μM bp) was incubated with the indicated amounts of **10** or **19** and thiol in sodium phosphate buffer (50 mM, pH 7) containing acetonitrile (10% v/v) for 15 h at 37 °**C**. Form I and II plasmid DNA were resolved on an agarose gel and stained with ethidium bromide. The DNA was visualized by UV-transillumination and the amounts measured by digital image analysis. The number of single strand breaks per plasmid DNA molecule (S) was calculated using the equation $S = -\ln f_1$ where f_1 is the fraction of plasmid present as form I.[64] The numbers in the parentheses following the description of each lane indicate the number of strand breaks per plasmid molecule. Lane 1: DNA alone (0.21), Lane 2: 2-mercaptoethanol (375 μM) alone (0.2), Lane 3: **10** (250 μM) + 2-mercaptoethanol (375 μM) (0.89), Lane 4: **10** (250 μM) + 2-mercaptoethanol (375 μM) + catalase (100 μg/mL) (0.25), Lane 5: minor isomer of **19** (250 μM) + 2-mercaptoethanol (375 μM) (1.23), Lane 6: minor isomer of **19** (250 μM) + 2-mercaptoethanol (375 μM) + catalase (100 μg/mL) (0.27), Lane 7: major isomer of **19** (250 μM) + 2-mercaptoethanol (375 μM) (1.35), Lane 8: major isomer **19** (250 μM) + 2-mercaptoethanol (375 μM) + 100 μg/mL catalase (0.25).

Importantly, thiol-triggered strand cleavage by **19** was strongly inhibited by addition of the peroxide-destroying enzyme catalase (lane 5, Fig. 1). This provided evidence that thiol-dependent strand cleavage by **19** involves the generation of reactive oxygen species ($O_2^{\cdot -}$, H_2O_2, and $HO^{\cdot}$).[19,48] Yields of thiol-triggered strand cleavage by the minor diastereomer of **19** (lane 7, Fig. 2) and major diastereomer of **19** (lane 5, Fig. 2) were very similar and were comparable to that generated by the leinamycin model compound **10** (lane 3, Fig. 2) that lacks a pendent alkene.[19] Thiol-triggered strand cleavage by **10** and the two diastereomers of **19** was strongly inhibited by addition of the peroxide-destroying enzyme catalase. This confirms previous results showing that strand cleavage by **10** was inhibited by the presence of catalase in the reaction mixture and is consistent with the involvement of reactive oxygen species.[19] The thiol-dependent generation of reactive oxygen species in the case of **10** was previously attributed to thiol-driven redox cycling of the persulfide/polysulfide couple as shown in Scheme 1.[20] The extent to which catalase inhibited thiol-triggered strand cleavage was nearly identical for the two diastereomers of **19** and **10** (lanes 4, 6, and 8, Fig. 2). The presence of catalase brings strand cleavage yields almost down to the level of control lanes containing DNA incubated with buffer alone (lane 1, Fig. 2) or DNA incubated with just thiol (lane 2, Fig. 2). This result, coupled with the observation that polysulfides were generated in the reaction of thiol with **19**, suggested that thiol-dependent release of redox active polysulfur species from **19** generated DNA-cleaving reactive oxygen species as described previously for **1**, **10**, and **11** and shown in the unbalanced Equations 1–5, where *M* represents an adventitious transition metal ion such as iron or copper.[18]

$$RSSH \rightleftharpoons RSS^- + H^+ \quad (1)$$

$$RSS^- + M^{(n+1)+} \rightarrow RSS^{\cdot} + M^{n+} \quad (2)$$

$$M^{n+} + O_2 \rightarrow M^{(n+1)+} + O_2^- \quad (3)$$

$$O_2^- + HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \quad (4)$$

$$H_2O_2 + M^{n+} \rightarrow HO^{\cdot} + HO^- + M^{(n+1)+} \quad (5)$$

The observation that the peroxide-destroying enzyme catalase inhibits DNA strand cleavage by **19**, was consistent with the involvement of reactive oxygen species; however, alkylating agents also have the potential to generate strand cleavage in the plasmid assay.[47] Indeed, a separate control experiment showed that the DNA-alkylating[49] epoxide, glycidol (250 μM), generates measurable amounts of strand breaks (0.34 ± 0.2 above background) under our reaction conditions. However, strand cleavage by glycidol was not significantly altered by the presence of catalase (100 μg/mL; 0.42 ± 0.27 breaks above background). Thus, the observation that strand cleavage by **19** is almost completely quenched in the presence of catalase suggests that this compound damages DNA primarily via the generation of reactive oxygen species and not via DNA alkylation.

3. Conclusion

In this work, we prepared a simple analogue of leinamycin consisting of a 1,2-dithiolan-3-one 1-oxide bearing a pendent alkene. Our synthesis expands the scope of a route developed by Pattenden and Shuker for simple 1,2-dithiolan-3-one 1-oxides such as **11** and **12** related to leinamycin.[31] A similar approach was used by Lee et al. to prepare a leinamycin analogue similar to **19** bearing a pendent alkene; however, these researchers did not report the reactions of their product with thiols or DNA.[32]

The reaction of thiols with leinamycin generates two different types of DNA-damaging reactive intermediates: an episulfonium ion alkylating agent and redox-active polysulfides (Scheme 1).[20,23,24] Our findings suggest that the small leinamycin analogue **19** emulates both of these reaction manifolds. Specifically, the reaction of **19** with thiol leads to the generation of both polysulfides **23** and an episulfonium-derived product **26** (Scheme 3). In analogy with mechanisms proposed to explain the products arising from the reaction of thiols with leinamycin, we suggest that **26** arises via intramolecular reaction of the pendent alkene in **19** with the electrophilic sulfur atom in the putative 1,2-oxathiolan-5-one intermediate **21** (Scheme 3). Bimolecular versions of this process, involving reaction of an alkene with an *O*-acylsulfenic acid have been reported previously.[50–53] The yield of the episulfonium ion-derived product **26** (35%) generated in the reaction of **19** with 2-mercaptoethanol was somewhat lower than that for the analogous product generated from **13** (50%) under the same conditions.[35] Yields of the episulfonium ion-derived products from leinamycin are quite high (75% isolated yields).[23] Overall, the results may illustrate the role of leinamycin's 18-membered macrocycle in properly positioning the alkene of leinamycin for reaction with the electrophilic sulfur in **4**. In this regard, the solution structures of **1** and **4** deserve further consideration. DNA clearly is not required for episulfonium ion formation, as the hydrolysis product **7** forms efficiently in the absence of DNA;[23] nonetheless it may be interesting to consider how the noncovalent association of activated leinamycin with DNA affects episulfonium ion formation, position of the **5**/**9** equilibrium, or the rate at which **5** undergoes hydrolysis.

We found that the small, synthetic leinamycin analogue **19** generates strand cleavage in the presence of the thiol, 2-mercaptoethanol. Our evidence indicates that the strand cleavage process proceeds via reactive oxygen species ($O_2^{\cdot -}$, H_2O_2, and $HO^{\cdot}$). This is consistent with previous work showing that the reaction of thiols with **1**, **10**, **11**, and **12** leads to the release of redox active polysulfur species that generate DNA-cleaving reactive oxygen species via redox cycling (Scheme 1) and the release of H_2S.[8,18–21,54]

Our results further provided evidence that the incubation of **19** with duplex DNA in the presence of thiol did not result in efficient alkylation of the DNA substrate. Upon first consideration, the failure of **19** to carry out thiol-triggered DNA alkylation may seem surprising given the evidence for thiol-triggered generation of an episulfonium ion intermediate from this compound (Scheme 3). However, this result can easily be rationalized in light of existing data. First, small episulfonium ions are typically very inefficient DNA-alkylating agents because they primarily undergo hydrolysis in aqueous solution.[55,56] Second, the macrocycle of leinamycin plays critical roles in DNA alkylation by the natural product. The 18-membered macrocycle of leinamycin associates noncovalently with the double helix in a manner that drive efficient DNA alkylation by **5**/**9** (Scheme 1).[24,49,57] In addition, the DNA-alkylating episulfonium ion of leinamycin may be stabilized against hydrolysis via an equilibrium involving the C8 alcohol, in what can be classified as a reversible thia-Payne rearrangement (Scheme 1).[23] Finally, the macrocycle of leinamycin may stabilize the 1,2-dithiolan-3-one 1-oxide residue against hydrolytic degradation.[58,59] The simple leinamycin analogues synthesized and characterized here enjoy none of the benefits imparted by the 18-membered macrocycle found in the natural product and, therefore, are incapable of effective DNA alkyation.

The results presented here, along with our previous work,[35] provide evidence that the 1,2-dithiolan-3-one 1-oxide heterocycle with a pendent alkene represents the minimal functional unit required to enable thiol-triggered generation of both redox active polysulfides and an episulfonium ion intermediate via the sequence of rearrangement reactions first seen in the natural

product leinamycin. However, the relatively low yields of episulfonium-derived products from **19** and the failure of this simple analogue to carry out thiol-triggered DNA alkylation highlight the central roles played by the 18-membered macrocycle of leinamycin in driving efficient DNA alkylation by the natural product.

4. Experimental section

4.1. Materials and methods

Reagents were of highest purity available and were used without further purification unless otherwise noted. Materials were purchased from the following suppliers: solvents, Fisher; silica gel 60 (0.04–0.063 mm pore size) for column chromatography, Merck; TLC plates coated with general purpose silica containing UV_{254} fluorophore, Aldrich Chemical Company; catalase from Sigma–Aldrich Chemical Co.; agarose from Seakem, bromophenol blue, sucrose, and other chemicals were purchased from Aldrich Chemical Company. Water was distilled, deionized and glass redistilled. All reactions were carried out under an atmosphere of nitrogen, unless otherwise noted. Dimethyl dioxirane[60] (DMD) and diazomethane (CAUTION: explosion hazard)[61] were freshly prepared. The plasmid PGL2BASIC was prepared using standard methods.[62]

4.2. 3-Benzylsulfanyl-3,7-dimethyl-oct-6-enoic acid (15)

Benzyl mercaptan (9 g, 8.4 mL, 71 mmol) was added to geranic acid (**14**, 10 g, 10.3 mL, 59 mmol) in freshly distilled piperidine (15 mL). This mixture was heated at reflux under nitrogen for 12 h. The resulting mixture was cooled in an ice bath followed by addition of 10% HCl until the pH of the solution reached ~2–3 (~20 mL). The resulting suspension was extracted with diethyl ether (3 × 30 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a pale yellow oil. Flash column chromatography on silica gel eluted with 8:1 hexane:ethyl acetate, followed by 6:1 hexane:ethyl acetate gave **15** (13 g, 75%, R_f = 0.37, in 4:1 hexane:ethyl acetate, a streaky spot) as a thick colorless oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.36–7.23 (m, 5H), 5.08 (m, 1H), 3.77 (s, 2H), 2.68 (s, 2H), 2.20–2.05 (m, 2H), 1.75–1.71 (m, 2H), 1.69 (s, 3H), 1.64 (s, 3H), 1.48 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 175.7, 137.4, 132.0, 129.0, 128.5, 127.0, 123.5, 47.5, 45.1, 39.8, 32.8, 25.9, 25.6, 23.0, 17.6; HRMS (ESI) $C_{17}H_{24}O_2SNa^+$ calcd for 315.1389, found 315.1379.

4.3. 3-Mercapto-3,7-dimethyl-oct-6-enoic acid (16)

A solution of **15** (5 g, 17.1 mmol) in distilled THF (5 mL) was added to a dry flask equipped with a stir bar, calcium chloride drying tube, dry ice condenser, and an ammonia inlet. The flask was cooled in a −78 °C dry ice-acetone bath. Liquid ammonia (15 mL) was condensed into the flask and finely divided sodium metal (1 g, 43 mmol) was added. A deep blue color developed and the mixture was stirred at −78 °C for 1 h, followed by addition of solid ammonium chloride (5 g). The ammonia and THF was removed by blowing a stream of nitrogen gently on the solution. To the resulting white residue 10% HCl was added until the pH of the solution reached ~3 (10–15 mL). The mixture was then extracted with diethyl ether (3 × 20 mL), followed by washing the pooled ether extracts with brine (2 × 15 mL). The ether extract was further dried over anhydrous sodium sulfate and then concentrated under reduced pressure to afford **16** as a colorless oil (3.3 g, 95%, R_f = 0.34, in 3:1 hexane:ethyl acetate, a streaky spot faintly visible under UV and strongly visible using iodine stain): ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.00 (br s, 1H), 5.06 (m, 1H), 2.68 (s, 2H), 2.16–2.08 (m, 2H), 1.74–1.70 (m, 2H), 1.69 (s, 3H), 1.60 (s, 3H), 1.47 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 176.7, 132.2, 123.3, 48.2, 44.9, 43.8, 29.8, 25.6, 23.6, 17.6; MS-(ESI-) $C_{10}H_{18}O_2S$ (M−H) calcd for 202.10, found 201.11. If left in solution this compound cyclizes as described previously for 2-mercapto allylbenzene[63] and this material was used immediately in the next step without further purification.

4.4. 4-Methyl-4-(4-methyl-pent-3-enyl)-thietan-2-one (17)

To a stirred solution of **16** (3 g, 14.8 mmol) in dry, distilled THF (30 mL) under nitrogen, dicyclohexyl carbodiimide (3.7 g, 17.8 mmol) and a catalytic amount of 4-dimethylaminopyridine (181 mg, 1.5 mmol) were added. The reaction was stirred for 48 h and the solvent evaporated under reduced pressure. The resulting white, oily suspension was mixed with acetone and the dicyclohexylurea precipitate (white solid) removed by filtration. The filtrate was evaporated under reduced pressure to give a pale yellow oil. Flash column chromatography on silica gel eluted with 20:1 pentane:ether gave **17** as a colorless liquid (1.63 g, 60% yield, R_f = 0.54 in 10:1 pentane:ether): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.13 (m, 1H), 3.68 (d, 17 Hz, 1H), 3.60 (d, 17 Hz, 1H), 2.14–1.94 (m, 4H), 1.75 (s, 3H), 1.70 (s, 3H), 1.63 (s, 3H); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ 190.7, 132.8, 122.5, 66.1, 44.4, 43.7, 28.7, 25.6, 17.6; HRMS (EI) calcd for $C_{10}H_{16}OS$ (M+) 184.0922, found 184.0928.

4.5. 5-Methyl-5-(4-methyl-pent-3-enyl)-[1,2]dithiolan-3-one (18)

A vigorously-stirred solution of **17** (1.5 g, 8.1 mmol) in carbon tetrachloride (25 mL) at −35 °C was saturated with a stream of hydrogen sulfide gas for 30 min (CAUTION: hydrogen sulfide is highly toxic and the gas stream vented from the reaction should be scrubbed and the reaction performed in a well ventilated hood). Freshly distilled triethylamine (1.3 mL, 9.7 mmol) was then added dropwise. The resulting solution was maintained at −35 °C and bubbling with hydrogen sulfide gas was continued for 8 h. The resulting mixture was allowed to warm to room temperature and stirred for another 5 h. Hydrogen sulfide and solvent was removed by blowing a stream of nitrogen gas over the solution to give a pale yellow solid. This solid was dissolved in methanol (25 mL) and an aqueous solution of ferric chloride (0.05 M, 25 mL) was added over 30 min with vigorous stirring at room temperature (24 °C). The resulting orange solution was stirred for an additional hour and then extracted with diethyl ether (3 × 20 mL). The organic extract was washed with water (2 × 15 mL) and brine (2 × 15 mL) before drying over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure gave a pale yellow oil. Flash column chromatography on silica gel eluted with 20:1 hexane:ethyl acetate afforded **18** as a pale yellow oil (1.63 g, 60% yield, R_f = 0.67 in 9:1 hexane:ethyl acetate): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.10 (m, 1H), 2.83 (d, 16 Hz, 1H), 2.75 (d, 16 Hz, 1H), 2.18–2.11 (m, 2H), 1.95–1.89 (m, 1H), 1.83–1.75 (m, 1H), 1.70 (s, 3H), 1.63 (s, 3H), 1.59 (s, 3H); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ 206.3, 133.1, 122.6, 56.9, 56.3, 39.4, 25.6, 24.2, 24.1, 17.6 δ ppm; HRMS (EI) $C_{10}H_{16}OS_2$ (M+) calcd for 216.0643, found 216.0647.

4.6. 5-Methyl-5-(4-methyl-pent-3-enyl)-1-oxo-[1,2]dithiolan-3-one (19)

To a vigorously-stirred solution of dithiolanone **18** (276 mg) in HPLC grade acetone (3 mL) freshly prepared dimethyldioxirane (5 mL of an approximately 0.09 M solution in acetone) was added dropwise. The disappearance of the starting material was closely monitored by TLC, which revealed the appearance of two products.

When the starting material was consumed, the mixture was evaporated under reduced pressure to give a pale yellow oil. Flash column chromatography on silica gel eluted with 12:1 hexane:ethyl acetate afforded **19** as a 1:1.7 ratio of diastereomers. The isomers were separated by column chromatography on silica gel to give the minor isomer (100 mg, 30%, R_f = 0.35 in 4:1 hexane:ethyl acetate) and the major isomer (150 mg, 50%, R_f = 0.43 in 4:1 hexane:ethyl acetate), both as colorless oils. The minor isomer of **19**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.04 (m, 1H), 3.38 (d, 1H), 2.96 (d, 1H), 2.17–2.08 (m, 2H), 1.77–1.74 (m, 2H), 1.71 (s, 3H), 1.42 (s, 6H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 200.8, 133.7, 121.9, 68.4, 48.2, 34.7, 25.5, 23.1, 18.8, 17.7; the major isomer of **19**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.13 (m, 1H), 3.40 (d, 1H), 2.87 (d, 1H), 2.23–2.10 (m, 2H), 1.99–1.95 (m, 2H), 1.70 (s, 3H), 1.61 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 201.0, 133.7, 122.1, 67.6, 49.3, 34.4, 25.6, 22.8, 18.9, 17.7; HRMS (EI) calcd for HRMS (EI) $C_{10}H_{26}O_2S_2$ calcd for 232.0630, found 232.0589.

4.7. Reaction of 19 with benzyl mercaptan in aqueous buffered solution

In a typical reaction, compound **19** (1.27 mL of a 500 mM stock solution in acetonitrile) was added to a solution of sodium phosphate buffer (0.5 mL of 500 mM, pH 7 solution), water (2 mL), and benzyl mercaptan (1.23 mL of 620 mM stock solution in acetonitrile; final concentrations: **19**, 127 mM; sodium phosphate, 50 mM, pH 7; thiol, 152.5 mM; acetonitrile 50% v/v). The mixture was stirred for 3 h at room temperature, acidified with 5% HCl to a final pH of 2–3, and extracted with ethyl acetate (3 × 2 mL). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield a colorless oil. The product was treated with diazomethane (CAUTION: explosion hazard)[61] and the solvent removed by rotary evaporation to yield a pale yellow oil. Flash column chromatography on silica gel eluted with 4:1 hexane–ethyl acetate was used to isolate the major products of the reaction. The *S*-deoxy analogue **18** was obtained in 15%, yield (20 mg). Spectral data for this compound matched that for the material described above in the synthesis of **19**. In addition, several other products were obtained: 2-(5-(2-hydroxypropan-2-yl)-2-methyltetrahydrothiophen-2-yl) acetic acid methyl ester (**26**) as an inseparable (~1:1) mixture of diastereomers in 35% yield (22 mg): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.698 (s, 3H), 3.693 (s, 3H), 2.73–2.45 (m, 6H), 2.19–1.83 (m, 8H), 1.58 (s, 3H), 1.50 (s, 3H), 1.22 (6H), 1.19 (6H); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ 171.42, 171.38, 70.2, 69.9, 63.01, 62.98, 54.5, 54.0, 51.49, 51.45, 48.2, 47.2, 44.59, 44.53, 31.35, 31.32, 29.7, 29.4, 28.7, 26.0, 25.9; HRMS (EI) $C_{11}H_{20}O_3S$ (M+) calcd for 239.1133, found 239.1138. Using GC/MS equipped with an electron impact detector, a mixture of polysulfides RSS_xR (*m*/*z* 218, *x* = 4; *m*/*z* 186, *x* = 3; *m*/*z* 154, *x* = 2) was detected in the reaction of 2-mercaptoethanol with **19**. The properties of these products matched those characterized previously from the reaction of thiols with **1**, **10**, and **11**.[20] The compound 3-benzyldisulfanyl-3,7-dimethyl-oct-6-enoic acid methyl ester (methyl ester of **24**, R = CH_2Ph) was obtained in 10% yield (31 mg): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (m, 5H), 5.04 (m, 1H), 4.12 (m, 2), 3.78 (s, 3H), 2.89 (m, 2H), 2.06 (m, 2H), 1.69 (m, 2H), 1.67 (s, 3H), 1.54 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ 195.6, 137.3, 132.5, 129.0, 128.8, 127.3, 123.2, 63.3, 56.4, 56.3, 44.9, 33.6, 32.8, 25.7, 22.3, 17.6. An analogous reaction of **19** with ethanethiol gave the corresponding disulfide, 3-ethyldisulfanyl-3,7-dimethyl-oct-6-enoic acid methyl ester (methyl ester of **24**, R = CH_2CH_3) in 30% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.08 (m, 1H), 3.67 s, 3H), 2.74–2.65 (m, 4H), 2.12 (m, 1H), 1.73 (m, 2H), 1.67 (s, 3H), 1.62 (s, 3H), 1.43 (s, 3H), 1.29 (t, 7 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 171.0, 132.0, 123.5, 52.0, 51.4, 44.1, 38.6, 33.9, 25.6, 25.0, 23.1, 17.6, 14.3; HRMS (EI) $C_{13}H_{24}O_2S_2$ (M+) calcd for 276.1218, found 276.1217.

4.8. DNA-cleavage by the major and minor isomers of 19

In a typical assay, the major or minor isomer of **19** (2 μL of 2.5 mM stock solution in acetonitrile) was added to a solution containing 2-mercaptoethanol (2 μL of a 3.75 mM freshly prepared stock solution in water) and supercoiled plasmid DNA (2 μL of a 0.25 μg/μL solution in 1× TE buffer) and incubated for 3–15 h at 37 °C (final concentrations: **19**, 250 μM; 2-mercaptoethanol, 375 μM; sodium phosphate, 50 mM pH 7.0; acetonitrile, 10% v/v). In assays containing catalase, the enzyme was added (2 μL of 1 mg/mL freshly prepared stock in water) prior to **19**. Following incubation, glycerol loading buffer (5 μL) containing 0.25% bromophenol blue and 40% sucrose was added. The samples were then agitated on a vortex mixer for 1 s, spun in a bench-top centrifuge for 5 s, and loaded onto a 0.9% agarose gel. The gel was electrophoresed at 80 V for 3 h in 1× TAE buffer (40 mM Tris base, 20 mM acetate, 1 mM EDTA, pH 8.0) and then stained in an aqueous ethidium bromide solution (0.2 μg/mL) for 6–8 h. The DNA in the gel was then visualized by UV-transillumination and the amount of DNA in each band was quantitatively measured by digital image analysis.

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