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The macrocycle of leinamycin imparts hydrolytic stability to the thiol-sensing 1,2-dithiolan-3-one 1-oxide unit of the natural product

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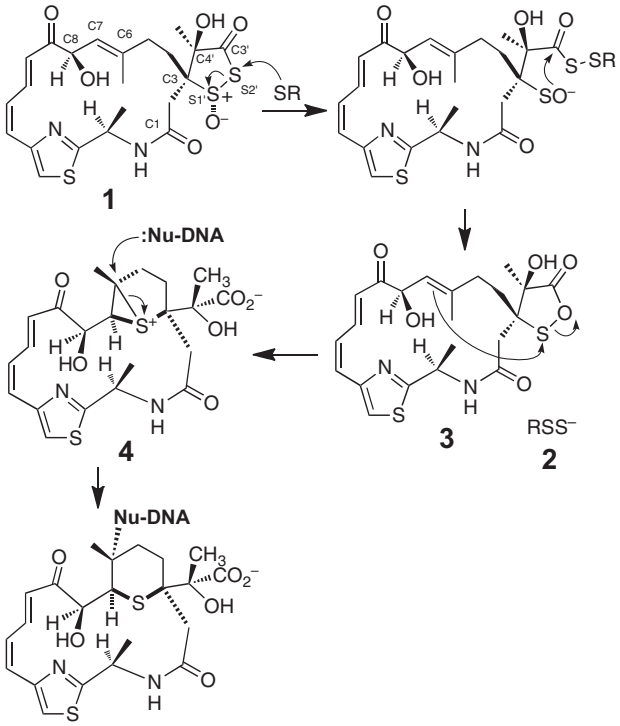
ABSTRACT

Reaction of cellular thiols with the 1,2-dithiolan-3-one 1-oxide moiety of leinamycin triggers the generation of DNA-damaging reactive intermediates. Studies with small, synthetic analogues of leinamycin reveal that the macrocyclic portion of the natural product imparts remarkable hydrolytic stability to the 1,2-dithiolan-3-one 1-oxide heterocycle without substantially compromising its thiol-sensing property.

The propensity to undergo intracellular bioactivation is an important property of many DNA-damaging natural products.[1–5] Characterization of these processes is relevant to both medicinal chemistry and toxicology as it has the potential to reveal novel and chemically interesting strategies for the intracellular generation of biologically active reactive intermediates.[6] The interior of cells is relatively rich in thiols[7] and reaction with intracellular thiols represents a common means for unmasking DNA-damaging intermediates from natural products. For example, calicheamicin, dynemicin, neocarzinostatin, acylfulvenes related to the illudins, myrocin C, 2-crotonyloxymethyl-2-cycloalkeneones, lissoclinotoxin A, varacin, thiarubrin C, and some analogues of mitomycin C can be activated by reactions with thiols.[8–24]

Leinamycin (**1**) is a thiol-activated, *Streptomyces*-derived secondary metabolite that displays potent activity against human cancer cell lines.[25–29] Leinamycin contains a unique 1,2-dithiolan-3-one 1-oxide heterocycle that serves as its 'thiol sensing' unit. Reaction of thiols with this moiety initiates rearrangement of leinamycin into a DNA-alkylating episulfonium ion **4** and converts the attacking thiol into a persulfide **2** that can mediate generation of reactive oxygen species (Scheme 1).[17,30–44] The 1,2-dithiolan-3-one 1-oxide heterocycle is hydrolytically labile; however, in the context of leinamycin this reaction is slow relative to the thiol-mediated activation of the natural product.[45] Indeed, it seems likely that *resistance to hydrolysis* coupled with *high thiol-reactivity* combine to allow efficient and selective bioactivation of leinamycin inside cells.



Scheme 1. Thiol-activated DNA alkylation by leinamycin.

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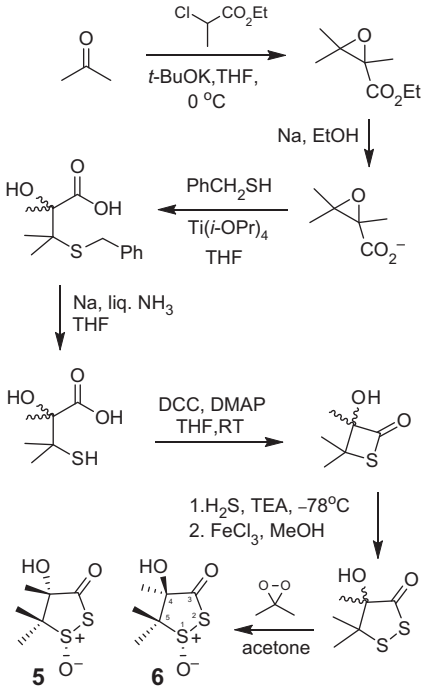

Scheme 2. Synthesis of 1,2-dithiolan-3-one 1-oxides related to leinamycin.

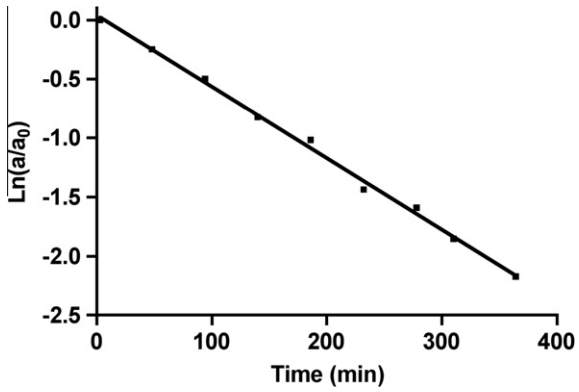

Figure 1. A representative plot showing the decomposition of **5** in aqueous buffered solution (**5** (70 μM); MOPS (250 mM, pH 7); MeCN (25% v/v); 24 °C). The disappearance of **5** was monitored by HPLC using a C18 reverse phase column (Varian microsorb-MV 100 Å pore size, 5 μm particle size, 250 mm length, 4.6 mm diam.) eluted with acetonitrile/water (35:65) at a flow rate of 1.0 mL/min.

To better understand the chemical events underlying the bioactivation of leinamycin, we examined the inherent reactivity of the thiol-sensing unit found in the natural product. Accordingly, we synthesized this fragment of leinamycin via a modification of the route described by Pattenden and Shuker (Scheme 2).[46] The published route employing NaSH for the epoxide ring-opening was ineffective in our hands, perhaps due to the notoriously impure nature of commercially available NaSH reagents.[47] Instead, we used benzyl mercaptan as a protected hydrogen sulfide surrogate. In addition, we employed dimethyldioxirane for the final oxidation step rather than *m*-CPBA.[48] The final oxidation reaction gave two major products and proton NMR analysis suggested that these were a 4:1 mixture of two diastereomers. These compounds were separable by thin layer chromatography and column chromatography on silica gel. The major isomer showed three methyl resonances at 1.74, 1.37, and 1.19 ppm, while resonances for the methyl groups in the minor isomer appeared at 1.58, 1.62, and 1.28 ppm. HMBC experiments were used to unambiguously assign the chemical shifts of the 4-methyl groups as the resonances at 1.37 ppm and 1.58 ppm for the major and minor isomer, respectively, via observation of three-bond coupling between methyl hydrogens and the carbonyl carbon. Literature precedents indicate that the proton NMR resonance of a methyl group in the *cis* orientation relative to a sulfoxide oxygen in a five-membered ring will be shifted downfield relative to a *trans* methyl group.[49–52] Thus, the NMR results immediately suggested that the major isomer has the methyl group in the 4-position in a *trans* relationship to the sulfoxide oxygen as shown in **5** (Scheme 2). Consistent with this assignment, an NOE experiment on the major isomer revealed a much stronger through space interaction of the 4-methyl substituent with the 5-methyl group that displays a resonance at 1.19 ppm (*trans* to the sulfoxide oxygen) than with the 5-methyl group at 1.74 ppm (*cis* to the sulfoxide oxygen). Analysis of ^{13}C-chemical shifts further supported the structural assignment. An HMQC experiment, along with the HMBC and NOE experiments described above, allowed us to assign the chemical shifts of each methyl group in the major isomer. The methyl group at C4 appears at 19.1 ppm. The methyl group at C5 with a *cis* relationship to the C4 methyl appears at 20.1 ppm and the methyl group at C5 with a *trans* relationship to the C4 methyl appears at 17.1. Literature precedents indicate that a methyl group with a *cis* relationship to the oxygen of an adjacent sulfoxide will appear upfield relative to a methyl group in the *trans* orientation.[49,53] Thus, our data showing that the shielded, upfield methyl at C5 is *trans* to the C4 methyl, suggested that, in the major isomer, the methyl group at C5 which has a *cis* relationship to the sulfoxide oxygen also has a *cis* relationship to the hydroxyl group at C4. This is consistent with structure **5** for the major isomer. Thus, we were able to assign the major isomer as **5**, in which the 4-hydroxyl group is *cis* to the sulfoxide oxygen, and the minor isomer as **6**, in which the 4-hydroxyl group is *trans* to the sulfoxide oxygen.[54] In the oxidation leading to **5** and **6**, hydrogen bonding may direct dimethyldioxirane to the same face as the hydroxyl substituent leading to favored production of **5** during the synthesis.[55] For the remainder of this Letter, **5** will be referred to as the *cis* isomer and **6** as the *trans* isomer.

With the *cis* and *trans* isomers of leinamycin's thiol-sensing unit in hand, we examined their stability at 24 °C in an aqueous buffered solution containing the compound (**5** or **6**, 70 μM), MOPS buffer (250 mM, pH 7), and acetonitrile (25% v/v). Disappearance of the compounds was monitored by HPLC over the course of three half-lives and the data fit to a first-order decay process to determine apparent rate constants and half-lives for the decomposition process (Fig. 1).[56]

The apparent rate constants for the decomposition of **5** and **6** under these conditions were measured at $k_{obs} = 13.2 \pm 0.01 \times 10^{-3}$ min^{-1} ($t_{1/2} = 53$ min) and $k_{obs} = 6.2 \pm 0.6 \times 10^{-3}$ min^{-1} ($t_{1/2} = 112$ min), respectively (Table 1). The decomposition rate of **6** increases with increasing buffer concentration ($k_{obs} = 1.33 \pm 0.02 \times 10^{-2}$ min^{-1} at 100 mM MOPS, $k_{obs} = 1.5 \pm 0.2 \times 10^{-2}$ min^{-1} at 300 mM MOPS, and $k_{obs} = 1.9 \pm 0.2 \times 10^{-2}$ min^{-1} at 500 mM MOPS). While the contribution of buffer is significant, at buffer concentrations used in our studies the buffer-independent reaction predominates. Near the physiological pH range, the rate at which **6** decomposes increases with increasing pH ($k_{obs} = 8.7 \times 10^{-3}$ min^{-1} at pH 6.5, $k_{obs} = 1.5 \times 10^{-2}$ min^{-1} at pH 7.0, and $k_{obs} = 4.7 \times 10^{-2}$ min^{-1} at pH 7.5; all at 300 mM MOPS). This mirrors the effect of pH observed previously on the stability of leinamycin in sodium phosphate buffer containing 10% methanol.[57] The pH effects are consistent with literature precedent indicates that hydroxide rather than water is the kinetically relevant species involved in the hydrolysis of thiosulfinates and thioesters.[58–60] We find that the decomposition of leinamycin under the same conditions used for the **5** and **6** above occurs

Table 1
Rate constants for **1**, **5**, and **6**

Compound	Conditions	k_{obs} (min^{-1})	$t_{1/2}$
5 No thiol	**5** (70 μM); MOPS (250 mM, pH 7); MeCN (25% v/v); 24 °C	$13.2 \pm 0.01 \times 10^{-3}$	53 min
6 No thiol	**6** (70 μM); MOPS (250 mM, pH 7); MeCN (25% v/v); 24 °C	$6.2 \pm 0.6 \times 10^{-3}$	112 min
1 No thiol	**1** (70 μM); MOPS (250 mM, pH 7); MeCN (25% v/v); 24 °C	$0.408 \pm 0.001 \times 10^{-3}$	27 h
5 + Thiol	**5** (70 μM); GSH (700 μM) MOPS (300 mM, pH 7); MeCN (25% v/v); 24 °C	$24.3 \pm 0.1 \times 10^{-2}$	2.8 min
6 + Thiol	**5** (70 μM); GSH (700 μM) MOPS (300 mM, pH 7); MeCN (25% v/v); 24 °C	$18.3 \pm 0.2 \times 10^{-2}$	3.8 min
1 + Thiol	**5** (70 μM); GSH (700 μM) MOPS (300 mM, pH 7); MeCN (25% v/v); 24 °C	$6.67 \pm 0.01 \times 10^{-2}$	10.4 min

with a rate constant of $k_{obs} = 0.408 \pm 0.001 \times 10^{-3}$ min^{-1} ($t_{1/2} = 27$ h). This value is generally consistent with a previous measurement of leinamycin's stability (100 μM **1** in HEPES, 250 mM, pH 7, 24 °C, containing no organic co-solvent) that gave a rate constant of 0.72×10^{-3} min^{-1} ($t_{1/2} = 16.1$ h).[45,61] Clearly, the 1,2-dithiolan-3-one 1-oxide heterocycle, when embedded in the context of the natural product, enjoys remarkably increased stability compared to the simple analogues **5** and **6**.

We next examined the reaction of **5**, **6**, and leinamycin (70 μM) with the biological thiol glutathione (GSH, 700 μM) in MOPS buffer (300 mM, pH 7) containing acetonitrile (25% v/v). The pseudo-first-order rate constants for the disappearance of **5** and **6** under these conditions were measured at $k_{obs} = 24.3 \pm 0.1 \times 10^{-2}$ min^{-1} ($t_{1/2} = 2.8$ min) and $k_{obs} = 18.3 \pm 0.2 \times 10^{-2}$ min^{-1} ($t_{1/2} = 3.8$ min), respectively. From these values, one can estimate second-order rate constants of 344 M^{-1} min^{-1} for **5** and 261 M^{-1} min^{-1} for **6**. The apparent rate constant for the reaction of leinamycin with GSH under these conditions was $k_{obs} = 6.67 \pm 0.01 \times 10^{-2}$ min^{-1} ($t_{1/2} = 10.4$ min, 95 M^{-1} min^{-1}). This is reasonably close to the value of 10.4 M^{-1} s^{-1} (624 M^{-1} min^{-1}) reported previously for the reaction of leinamycin with GSH (in HEPES buffer, 50 mM, pH 7, 24 °C, containing no organic co-solvent).[45]

The natural product leinamycin was isolated as the single stereoisomer shown in Scheme 1, with a *trans* relationship between the C4′-OH group and the S1′-sulfinyl oxygen.[62] Our work with **5** and **6** show that the naturally-occurring *trans* isomer is approximately two times more stable than the *cis* isomer in aqueous buffered solution. However, differences in the hydrolytic stability of the *cis*/*trans* isomers **5** and **6** are subtle compared to the dramatic effect that leinamycin's 18-membered macrocycle brings to the stability of the natural product in aqueous solution. Leinamycin is approximately 30 times more stable than **5** and 15 times more stable than **6** against decomposition in aqueous buffer. It is interesting to consider potential mechanisms by which the macrocycle stabilizes leinamycin against decomposition in aqueous solution. Based upon computational analysis of small model systems, Wu and Greer suggested that an $n_O \rightarrow \sigma^*_{S1'}$ interaction between the amide carbonyl in the macrocycle and the S1′-sulfinyl group of leinamycin stabilizes the 1,2-dithiolan-3-one 1-oxide ring system by ~6 kcal/mol.[63] Alternatively, or in addition, the macrocycle of the natural product may present a steric impediment to these approach of hydroxide to the 1,2-dithiolan-3-one 1-oxide heterocycle of leinamycin. The solution structure of leinamycin deserves further consideration in this regard. Regardless of mechanism, our results clearly establish that the macrocyclic portion of leinamycin stabilizes the natural product against decomposition in aqueous solution.

It is striking that the macrocycle increases the aqueous stability of leinamycin without compromising the ability of the natural product to react avidly with thiols. For example, leinamycin is only 3.6 times less reactive toward GSH than is compound **5**. This may reflect the fact that thiol-mediated activation of leinamycin proceeds via attack of the mercaptan at the sterically exposed S2′ position of the 1,2-dithiolan-3-one 1-oxide ring system,[41] while hydrolysis may proceed primarily via attack of hydroxide on S1′ or C3′—reactions that may be suppressed by the macrocyclic substituent.

Previous work established three mechanisms by which the macrocycle of leinamycin may facilitate efficient DNA alkylation. First, the *Z*,*E*-5-(thiazol-4-yl)-penta-2,4-dienone portion of the macrocycle presents a slightly twisted π-surface that confers non-covalent DNA-binding properties to the natural product.[32,64,65] Second, the hydroxyl group at C8 of the macrocycle engages the episulfonium ion **5** in a reversible thia-Payne reaction that may stabilize the episulfonium ion against hydrolytic destruction.[30] Third, the conformationally rigid macrocycle may accurately position the C6–C7 alkene for efficient reaction with the electrophilic sulfur of **3** in the generation of the alkylating agent **4** (Scheme 1).[66] The work presented here establishes an additional role for the macrocyclic portion of leinamycin in facilitating efficient thiol-triggered alkylation of cellular DNA. The macrocyclic portion of leinamycin imparts substantial aqueous stability to the 1,2-dithiolan-3-one 1-oxide without compromising its ability to act as a thiol-sensing unit. Thus, the 18-membered macrocycle of leinamycin enables efficient and selective bioactivation of the natural product in the thiol-rich environment found inside cells.

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54. Synthesis of 4-hydroxy-4,5,5-trimethyl-1,2-dithiolan-3-one-1-oxide (**5** and **6**). To a stirred solution of 4-hydroxy-4,5,5-trimethyl-1,2-dithiolan-3-one (50 mg, 0.28 mmol) in acetone (0.5 mL) in an ice bath was added freshly prepared dimethyldioxirane (dropwise using a pipette). The disappearance of the starting material was closely monitored by TLC (5:1 hexane/ethyl acetate) and the reaction was complete in about 5 min. Flash column chromatography on silica gel eluted with ethyl acetate/dichloromethane/hexane (1:3:4) gave the diastereomers **5** and **6** as colorless solids (50 mg total, 90%). Compound **5**: R_f = 0.36; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (3H, s, C5-CH3 trans to sulfoxide oxygen), 1.37 (3H, s, C4-CH_3), 1.74 (3H, s, C5-CH_3 *cis* to sulfoxide oxygen); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 201.8 (C=O), 84.9 (C4), 66.7 (C5), 20.1 (C5-CH_3 trans to sulfoxide oxygen), 19.1 (C4-CH_3), 17.1 (C5-CH_3 *cis* to sulfoxide oxygen); HRMS: (EI) calcd for $C_6H_{10}O_3S_2$ $[M+Li]^+$ 201.0225, found 201.0215. Compound **6**: R_f = 0.24 ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (3H, s), 1.58 (3H, s), 1.62 (3H, s), 2.9 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 201.9, 84.7, 65.9, 24.2, 18.8, 16.8; HRMS: (EI) calcd for $C_6H_{10}O_3S_2$ $[M+Na]^+$ 216.9963, found 216.9962.
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