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Small Molecules That Mimic the Thiol-Triggered Alkylating Properties Seen in the Natural Product Leinamycin

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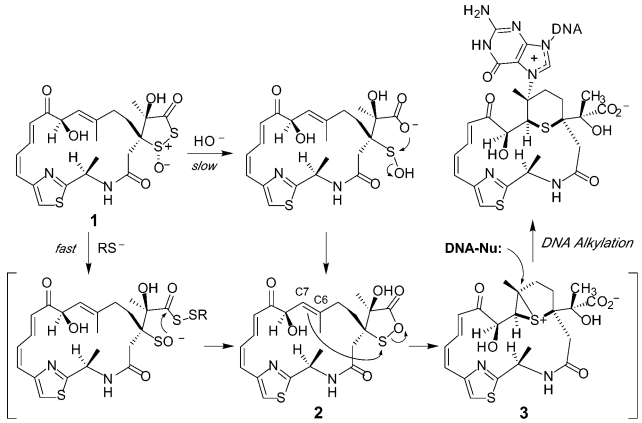
Identification and characterization of new chemical motifs that can efficiently modify biological macromolecules under physiological conditions is of general importance in medicinal chemistry, toxicology, and biotechnology. Biologically active natural products have long served as an inspirational source of chemical strategies for the intracellular generation of intermediates that react with proteins and nucleic acids.[1−4] In a recent example, studies of the antitumor natural product leinamycin have revealed a novel mechanism for the thiol-triggered generation of a DNA-alkylating episulfonium ion.[5−7] Reaction of thiols with the 1,2-dithiolan-3-one 1-oxide heterocycle found in leinamycin initiates a sequence of reactions that leads to the 1,2-oxathiolan-5-one intermediate **2** (Scheme 1).[8,9] This intermediate undergoes a profound rearrangement in which reaction of the C6−C7 double bond with the electrophilic sulfur of the 1,2-oxathiolan-5-one heterocycle yields an episulfonium ion (**3**) that efficiently alkylates the N7-position of guanosine residues in double-stranded DNA.[5] Leinamycin can also be transformed to the episulfonium ion **3** in the absence of thiol,[10] although, under typical cellular conditions (1−5 mM glutathione, pH ≈ 7.2), thiol-mediated activation of the antibiotic is approximately 1000 times faster than the thiol-independent process and also proceeds in higher yield.[10]

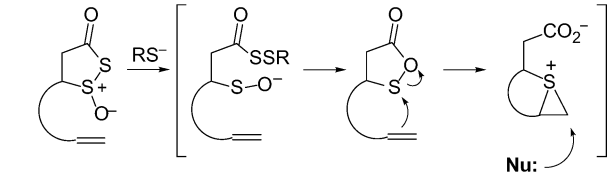
In leinamycin, nature has provided us with a novel assembly of functional groups that generates a potent alkylating agent upon exposure to the thiol-rich environment found inside cells. To establish whether the chemistry of leinamycin represents a general motif that can function in various molecular frameworks, we undertook the construction of greatly simplified leinamycin analogues containing only what we perceived to be the "core" functional groups required for thiol-accelerated production of an alkylating agent (Scheme 2).

We targeted compound **4** as the first "stripped-down" analogue of leinamycin (Scheme 3). Despite the obvious structural differences between the benzo-fused sulfur heterocycle found in **4** and the saturated heterocycle found in the natural product, previous work indicated that the 3*H*-1,2-benzodithiol-3-one 1-oxide system (**5**) would emulate key aspects of leinamycin's reactivity (specifically, thiol-mediated formation of an oxathiolanone intermediate).[8,11−13] Synthesis of the desired leinamycin analogue **4** was achieved as shown in Scheme 3. The diazonium salt of dibromoaniline (**6**) was reacted with trimethylsilylethanethiolate[14,15] in the presence of powdered copper[16] to provide the aromatic sulfide **7**. Metal−halogen exchange, followed by reaction of the resulting lithium anion with prenyl bromide, allowed installation of the alkene side chain in compound **8**. A second metal−halogen exchange reaction, followed by quenching of the lithium anion with carbon dioxide, provided the carboxylic acid derivative **9**. Conversion of the carboxylic acid to the acid chloride, followed by reaction with

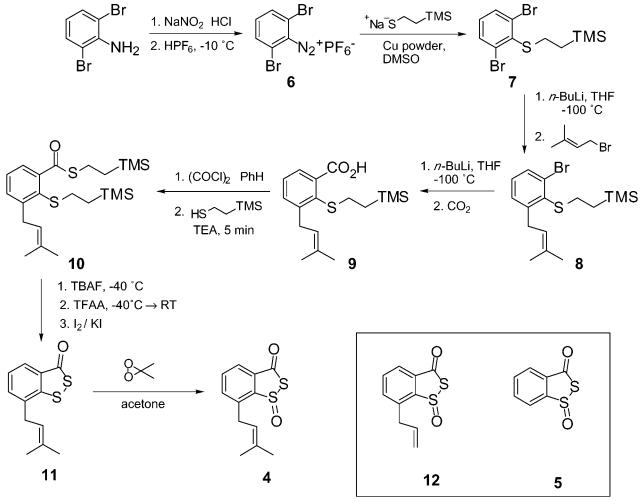
Scheme 1



Scheme 2



Scheme 3



trimethylsilylethanethiol, furnished the thioester **10**. Removal of both trimethylsilylethane protecting groups from **10** using tetrabutylammonium fluoride and trifluoroacetic anhydride,[17] followed by in situ oxidation with I_2/KI, resulted in formation of the 3*H*-1,2-benzodithiolan-3-one heterocycle **11** in modest yield. Finally, oxidation of **11** with dimethyldioxirane[18] in acetone gave the desired leinamycin analogue **4**. In addition, we prepared the allyl derivative **12** using the same general approach, with the exception that it was

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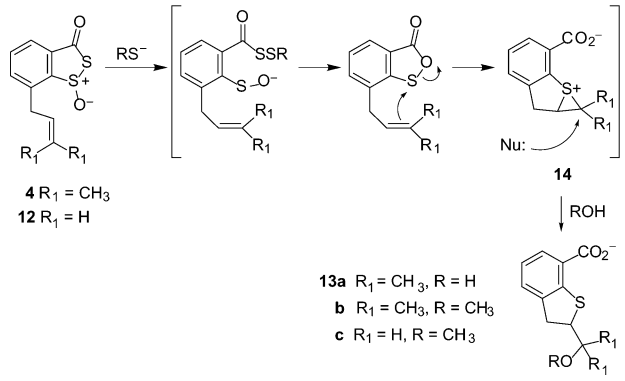
Scheme 4



necessary to employ the metalation−transmetalation procedure developed by Snieckus and co-workers[19] for installation of the allyl side chain into **7**.

Treatment of **4** with 1 equiv of thiol (either 2-mercaptoethanol or propanethiol) in a 1:1 mixture of acetonitrile and sodium phosphate buffer (100 mM, pH 7) led to rapid formation of the cyclized product **13a** which was isolated in approximately 50% yield as its methyl ester following treatment of the organic extract with diazomethane. This product is envisioned to arise from Markovnikov addition of water to the episulfonium ion intermediate **14** (Scheme 4).[20] It is noteworthy that the leinamycin-derived episulfonium ion **3** also yields Markovnikov products upon reaction with DNA or water.[5] Thiol-triggered conversion of **4** to an alkylating species also proceeds effectively in a 99:1 mixture of methanol/sodium phosphate buffer. In this case, the analogous product (**13b**) resulting from nucleophilic attack of methanol on the episulfonium ion **14** was obtained in 50% yield as the methyl ester after workup of the organic extract with diazomethane. Interestingly, we find that the allyl analogue **12** is also efficiently transformed to the methanol adduct **13c** under these reaction conditions. This indicates that formation of the intermediate episulfonium ion is not strongly dependent upon the presence of electron-donating alkyl substituents on the alkene.[21]

The leinamycin model compound **4** also emulates the thiol-*independent* activation chemistry reported recently for leinamycin.[10] Incubation of **4** in a 1:1 mixture of acetonitrile and sodium phosphate buffer (100 mM, pH 7) for 11 h in the absence of thiol, followed by diazomethane workup, affords **13a** in 35% yield. Similar to the natural product, thiol-independent conversion of **4** to the episulfonium ion is slower and somewhat less efficient than the thiol-triggered reaction. The reaction of **4** (100 μM) with thiol (10 equiv) is complete within 100 s under these conditions, whereas the half-life for the conversion of this compound to products in the absence of thiol is approximately 2 h.

Finally, it is important to report that **4** does *not* efficiently alkylate DNA. This is not surprising because existing data indicate that efficient DNA alkylation by leinamycin is driven by noncovalent association of the activated antibiotic (**3**) with the double helix.[5,22] Clearly, small analogues intended to recapitulate the DNA-alkylating properties of leinamycin will need to be equipped with additional functional groups that furnish noncovalent DNA binding.

In summary, we find that the relatively simple molecule **4** is able to effectively mimic key elements of leinamycin's chemical reactivity. This work helps define the "core" functional groups required for thiol-accelerated generation of an alkylating intermediate from leinamycin. The results suggest that substantially altered analogues of the natural product may retain DNA-alkylating properties. From a broader perspective, our findings provide evidence that the intriguing cascade of chemical reactions first seen in the context of leinamycin represents a *general* motif that can operate in a variety of molecular frameworks. This realization could facilitate the discovery of new natural and synthetic agents that effect thiol-triggered alkylation of macromolecular targets inside cells.

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Supporting Information Available: Experimental procedures and spectral data for all compounds, comments on the structure elucidation of **13**, and DNA-damage assays (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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