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Activation of Leinamycin by Thiols: A Theoretical Study

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Reaction of thiols with the 1,2-dithiolan-3-one 1-oxide heterocycle found in leinamycin (**1**) results in the conversion of this antitumor antibiotic to a DNA-alkylating episulfonium ion (**5**). While the products formed in this reaction have been rationalized by a mechanism involving initial attack of thiol on the central sulfenyl sulfur (S2′) of the 1,2-dithiolan-3-one 1-oxide ring, the carbonyl carbon (C3′) and the sulfinyl sulfur (S1′) of this heterocycle are also expected to be electrophilic. Therefore, it is important to consider whether nucleophilic attack of thiol at these sites might contribute either to destruction of the antibiotic or conversion to its episulfonium ion form. To address this question, we have used computational methods to examine the attack of methyl thiolate on each of the three electrophilic centers in a simple analogue of the 1,2-dithiolan-3-one 1-oxide heterocycle found in leinamycin. Calculations were performed at the MP2/6-311+G(3df,p)//B3LYP/6-31G* level of theory with inclusion of solvent effects. The results indicate that the most reasonable mechanism for thiol-mediated activation of leinamycin involves initial attack of thiolate at the S2′-position of the antibiotic's 1,2-dithiolan-3-one 1-oxide heterocycle, followed by conversion to the 1,2-oxathiolan-5-one intermediate (**3**).

Introduction

Structurally novel natural products that possess potent biological activity are of significant interest because their mechanisms of action are often unique and unexpected.[1−3] For this reason, we have been inspired to study the DNA-damaging natural product leinamycin.[4−7] This natural product possesses a unique chemical structure, displays potent anticancer activity, and reacts with DNA by a completely novel sequence of reactions. DNA damage by leinamycin is triggered by attack of thiols on the 1,2-dithiolan-3-one 1-oxide heterocycle of the antibiotic (Scheme 1).[7−9] This reaction produces two crucial reactive intermediates: an 1,2-oxathiolan-5-one (**3**) and a hydrodisulfide (or persulfide) **4**.[10] The hydrodisulfide intermediate (**4**) mediates oxidative DNA damage via production of oxygen radicals,[11,12] while the 1,2-oxathiolan-5-one form of leinamycin (**3**) undergoes a unique rearrangement to produce a DNA-alkylating episulfonium ion (**5**).[13] The studies described here embrace the previous assumption[10,13,14] that the oxathiolanone **3** represents an "activated" form of leinamycin that serves as the immediate precursor to the episulfonium ion (**5**). This notion is supported by a wealth of precedents showing that sulfenyl carboxylates react readily with alkenes to yield episulfonium ions (Scheme 2).[15]

The products formed in the reaction of leinamycin with thiols have been rationalized by a mechanism involving initial attack of thiols on the central, sulfenyl sulfur (S2′) of the 1,2-dithiolan-3-one 1-oxide heterocycle as shown in Scheme 1.[10,13] However, the carbonyl carbon (C3′) and the sulfinyl sulfur (S1′) are also expected to be electrophilic. Thus, it is important to consider whether nucleophilic attack of thiol at these sites might contribute to either the activation or destruction of leinamycin. To

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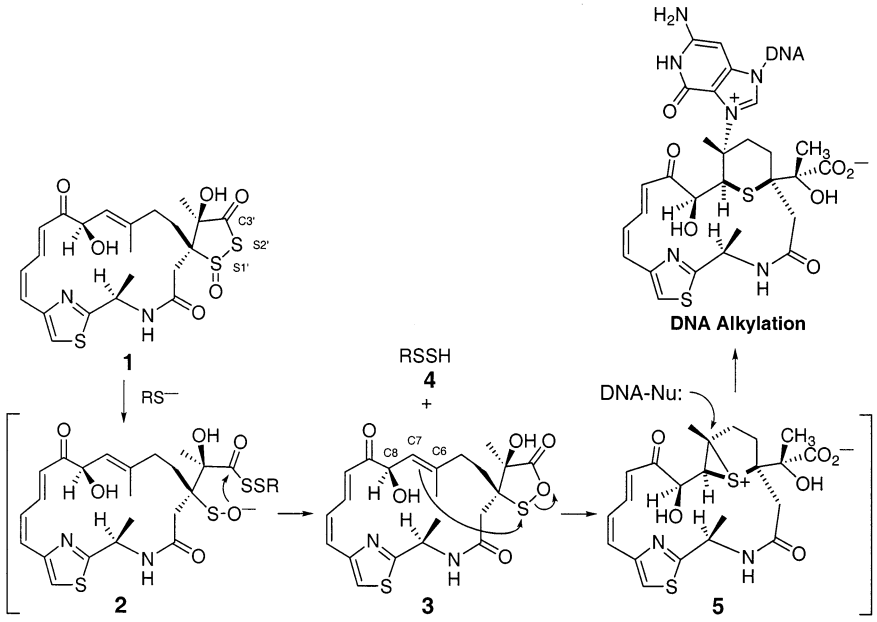
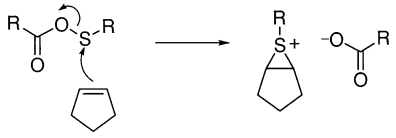
SCHEME 1



SCHEME 2



address this question, we have used computational methods to examine the attack of methyl thiolate on each of the three electrophilic centers in a simple analogue of the 1,2-dithiolan-3-one 1-oxide heterocycle found in leinamycin.

Computational Methods

All calculations were carried out utilizing Gaussian 94 or Gaussian 98.[16] All geometries were optimized and frequencies calculated at the B3LYP/6-31G* level of theory[17,18] in the solvent model using the self-consistent reaction field (SCRF) method based on the Onsager model.[19,20] Reoptimization of several representative structures at higher levels (B3LYP/6-31+G* or MP2/6-31G*) did not lead to significant changes in geometry. The representation of solvent used here is somewhat crude but nonetheless provides a reasonable estimate of the molecular geometry in solution. The solvent dielectric constant for dichloromethane ($\epsilon = 8.93$) was used because this solvent has been used in some of the experimental work on this system.[10]

Frequency calculations were performed at all stationary points at the B3LYP/6-31G* level using an Onsager model, and a correct number of imaginary frequencies were found. Zero-point vibrational and thermal corrections were calculated at the same level and were not scaled.[21] All transition states were analyzed either by calculating an intrinsic reaction coordinate (IRC) or by visual inspection of the transition vector with the program Vibrate.[22] Since entropy plays an important role in bimolecular reactions described here, all energies are reported as Gibbs free energy (ΔG) values at 298 K.

Solvation energies were calculated on the optimized geometries utilizing three SCRF models: integral equation formalism polarized continuum model (IEFPCM),[23] polarized continuum model (PCM),[24−26] and isodensity polarized continuum model (IPCM)[25] at the B3LYP/6-31G* level. In the models used, solvent is assumed to be a continuous medium with a dielectric constant ϵ that surrounds a cavity containing the solute and the shape of the cavity is adjusted to fit the shape of the solute molecule to afford more accurate solvation energies. All three models show qualitatively similar results, and PCM and IEFPCM afford nearly identical solvation energies (see Tables S1 and S2 and Scheme 4). Values shown in Scheme 4 are derived from IEFPCM calculations because this model is considered best for calculation of the solvation energies of charged molecules.[27]

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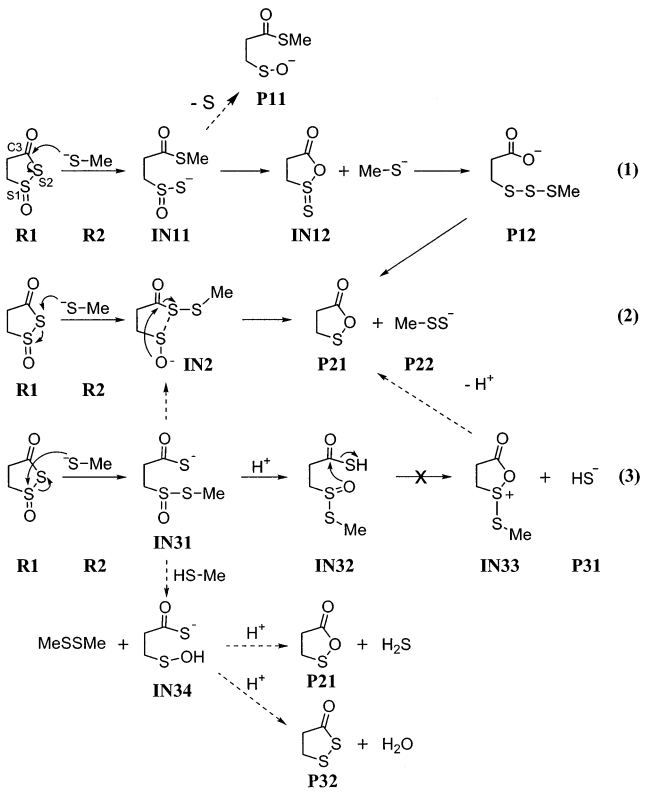
SCHEME 3



Single-point energies were calculated on the optimized geometries at the MP2 level with 6-311+G(2df,p) and 6-311+G(3df,p) basis sets. For molecules containing a sulfoxide moiety, calculations at the MP2 level with the triple-ζ basis set such as that used here generally give more accurate results than density functional theory with comparable or even larger basis sets.[28−30] Bond orders were estimated using Wiberg Bond Indices (WBI)[31] calculated with the NBO subroutine[32] of Gaussian 94 at the B3LYP/6-31G* level.

Results

Reaction of Thiolate at C3. Reactions of methyl thiolate with 1,2-dithiolan-3-one 1-oxide (**R1**, Scheme 3) were investigated at the MP2/6-311+G(3df,p)//B3LYP/6-31G* level with full optimization in the SCRF field based on the Onsager model ($\epsilon = 8.93$). Free energies (ΔG) were calculated at 298 K utilizing entropies from Hessians and corrected for solvation utilizing one of the SCRF models (PCM, IPCM, or IEFPCM). First, we investigated attack of thiolate on the carbonyl carbon (C3) of the 1,2-dithiolan-3-one 1-oxide heterocycle (**R1**; see Scheme 3). This reaction proceeds by an S_N2 mechanism that has been seen previously for nucleophilic substitution on acyl chlorides.[33] The transition state in this reaction (**TS11**, Scheme 4) is quite early judging by the long $S_{Nu}-C3$ distance (3.0 Å) and Wiberg Bond Index (WBI) of 0.17 for the $S_{Nu}-C3$ bond.[34] The resulting thiosulfinate (**IN11**) can, in principle, lose sulfur[35] to yield the sulfenate **P11**. However, unimolecular loss of sulfur from organic intermediates is typically not facile,[36] therefore, it seems more likely that **IN11** will decompose via unimolecular low barrier reactions involving either reversion to starting materials or rearrangement to **IN12** (Scheme 3).[37] The rearrangement of **IN11** to **IN12** is favored by 2.2 kcal/mol and proceeds through a very late transition state (**TS12**, Scheme 4) in which the $C3-S_{Nu}$ bond, at 4.71 Å, is completely broken and the C3−O bond, at 1.37 Å, is almost completely formed. We calculate the activation barrier for this reaction to be 7.8 kcal/mol. The overall conversion of **R1** to **IN12** represents a thiol-catalyzed isomerization reaction that is disfavored by 2.8 kcal/mol.

While loss of sulfur from an S═S bond such as that found in **IN12** has been observed previously,[38] we have not pursued such a possibility for **IN12** because such reactions are commonly believed to proceed via bimolecular (dimerization) mechanisms.[36,39] Under the physiological conditions in which we are interested (low drug concentrations and relatively high thiol concentrations), it is more likely that attack of thiolate on the terminal sulfur of the thiosulfoxide in **IN12** will lead directly to the trisulfide **P12**. This reaction encounters an energy barrier of 18.4 kcal/mol and is favored by 35.7 kcal/mol. In the transition state for the reaction of **IN12** with thiolate (**TS13**, Scheme 4), the S═S bond is left unchanged (1.95 Å, WBI 1.44, same as in **IN12**), but the S1−O bond is almost completely broken (2.14 Å, WBI 0.26) and the $S_{Nu}-S2$ bond is partially formed (3.38 Å, WBI 0.23). The relatively high barrier for this reaction (18.4 kcal/mol) can be rationalized by the ylide-type polarization of the S═S bond to create a partial negative charge on S2. Indeed, NBO analysis at the B3LYP/6-31G* level shows that the S═S bond is polarized with a natural charge of +0.51 on S1 and −0.24 on S2. Although we are unaware of any precedent for the attack of thiolate on a S═S bond, the analogous addition of a nitrogen nucleophile (morpholine) to the terminal sulfur of a S═S bond has been observed previously.[40]

Our calculations suggest that the resulting carboxytrisulfide **P12** cannot efficiently cyclize to the oxathiolanone **P21** and hydrodisulfide **P22** because this reaction is disfavored by 26.9 kcal/mol and must surmount an activation barrier of 36 kcal/mol (**TS14**, Scheme 4). Therefore, we suggest that conversion of the trisulfide **P12** to the oxathiolanone **P21** does not contribute significantly to the thiol-triggered activation of leinamycin,

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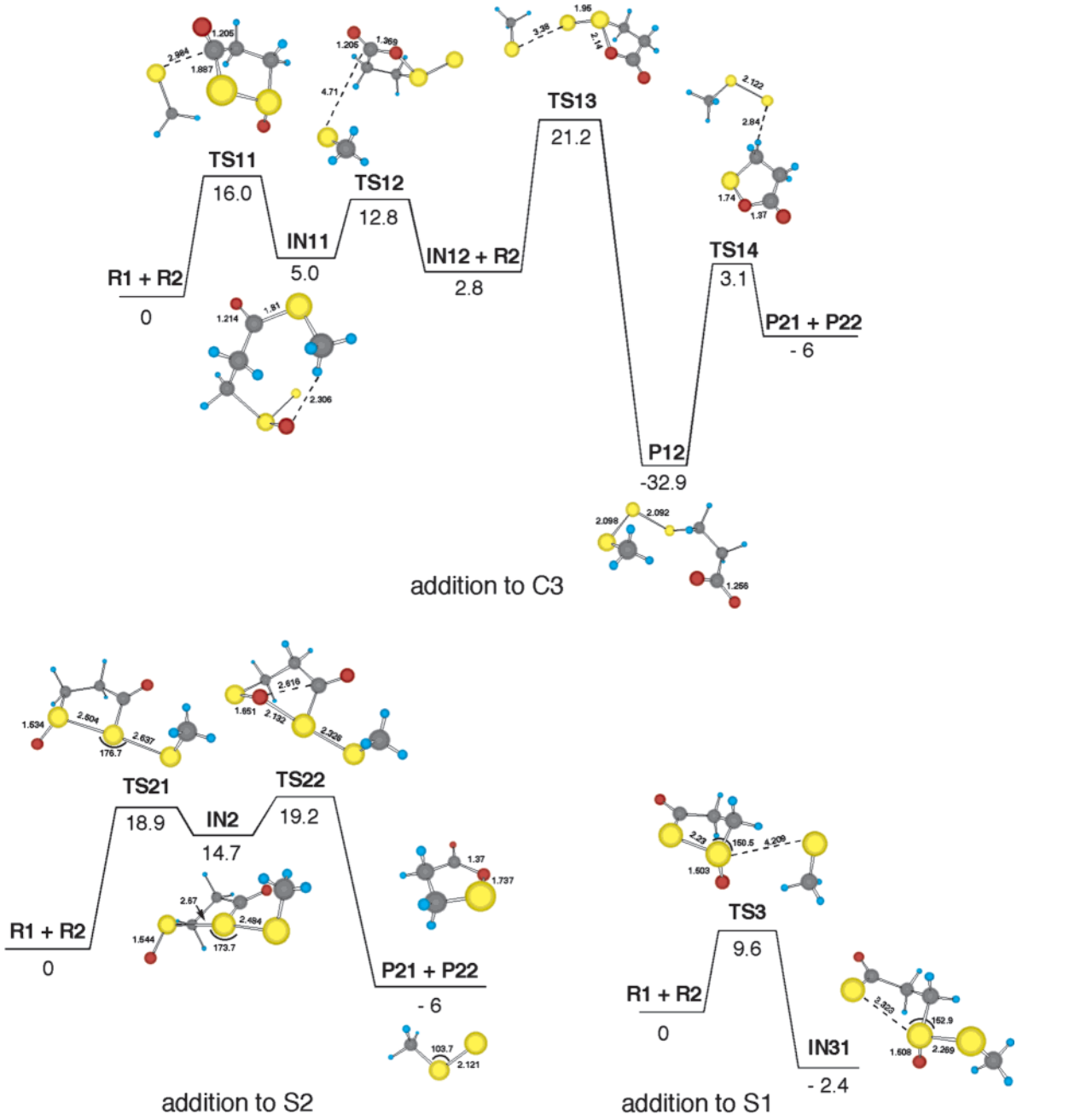
SCHEME 4



although we note that analogous reactions have been proposed in other systems.[41,42] In the Discussion section we consider the possibility that the trisulfide **P12** may be converted directly to activated leinamycin without the intermediacy of the oxathiolanone **P21**.

Reaction of Thiolate at S2. The mechanism of nucleophilic substitution on sulfur(II) has generally been described as S_N2,[43−45] although recent theoretical work by Bachrach[46,47] suggests that an addition−elimination mechanism also fits existing kinetic data and may, in fact, be operational. In our case, attack of thiolate on the sulfenyl sulfur (S2) of **R1** proceeds via a late transition state (**TS21**, Scheme 4) in which the S2−S_{Nu} distance is 2.6 Å and the WBI is 0.44 to yield the intermediate **IN2**. The angles between the incoming nucleophile and the leaving group (∠S_{Nu}−S2−S3) in **TS21** are close to 180° as required for S_N2 substitution; however, this reaction is probably most accurately described as an addition−elimination mechanism. This assessment is supported by the observation that S2 in **IN2** is clearly hypervalent with bonds to both S_{Nu} (2.484 Å, WBI 0.52) and S3 (2.67 Å,

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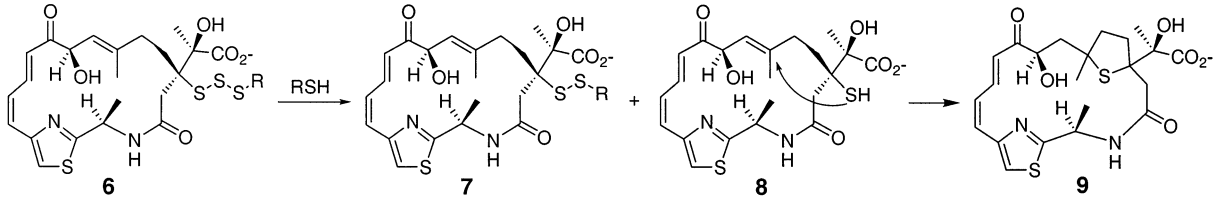
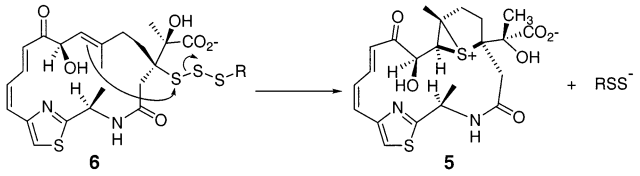
SCHEME 5



SCHEME 6



WBI 0.35). In this intermediate, the central S2 sulfur is trigonal-bipyramidal with its lone pairs in the equatorial positions and the nucleophile and leaving group in the axial positions as is typically seen in addition−elimination mechanisms.[46,47]

The hypervalent sulfenate-type intermediate **IN2** cyclizes directly to the oxathiolanone **P21**.[48] This reaction is favored by 20.7 kcal/mol and has an activation energy of only 4.5 kcal/mol. The overall reaction (**R1** + **R2** → **P21** + **P22**) is favored by 6 kcal/mol and encounters a modest activation barrier of 19.2 kcal/mol.[49]

Reaction of Thiolate at S1. Finally, we considered the consequences of thiol attack on the S1 sulfur of the 1,2-dithiolan-3-one 1-oxide (**R1**, Scheme 3). This reaction follows an S_N2 mechanism as seen previously for other systems involving nucleophilic attack at sulfinyl sulfur.[50,51] The transition state (**TS3**) is quite early, with a S1−S_{Nu} distance of 3.9 Å and a WBI of 0.03 for the S1−S_{Nu} bond. The reaction has a moderate barrier (9.6 kcal/mol) and is thermodynamically favored by 2.4 kcal/mol. Thus, attack of thiolate on S1 seems likely to occur but is readily reversible. Further reaction involving protonation of **IN31** to yield **IN32**, followed by conversion to **IN33** seems unlikely due to the large energy barrier (>40 kcal/mol) for the cyclization reaction.

There are several alternative reactions stemming from attack of thiolate at S1 that deserve consideration. First, isomerization of **IN31** to **IN2** is formally possible via a six-membered transition state. However, this reaction is not likely to compete with reversion to starting material because isomerization to **IN2** is disfavored by 17.1 kcal/mol and must surmount an energy barrier that is undoubtedly quite high because the preferred geometry for nucleophilic substitution at sulfur in which the angles between the incoming nucleophile and the leaving group are ∼180° cannot be attained in this cyclic system.[52,53] Alternatively, one can consider the possibility that a second equivalent of methyl thiolate attacks the sulfenyl sulfur of the thiosulfinate group in **IN32** to yield **IN34**. At physiological thiol concentrations (1 mM) this second-order reaction is not likely to compete favorably with unimolecular reversion to starting material.[54] To the extent that **IN34** is produced, it would be expected to yield the *S*-deoxygenated heterocycle **P32**.[55] Interestingly, this type of product has been observed in model reactions for the activation of leinamycin by thiols.[10] The alternate mode of cyclization, involving attack of the sulfenic acid on the thio acid group to yield **P21**, requires the protonated form of the thiocarboxylic acid (pK_a ∼ 3), which will be present in very low amount at neutral pH.[56] Thus, while one can imagine a formal route to the activated leinamycin intermediate **P21** stemming from initial attack of thiolate at S1 proceeding through **IN34**, this pathway does not explain the high yield and kinetically rapid nature of the thiol-mediated activation that is observed experimentally.[13,14]

Discussion

To gain a deeper understanding of the chemical mechanism by which reaction with thiol converts leinamycin into an alkylating agent, we have used computational methods to consider the energetics for attack of thiolate at each of the three electrophilic positions in leinamycin's 1,2-dithiolan-3-one 1-oxide heterocycle. Our findings are summarized and discussed below.

Attack at the sulfinyl sulfur (S1) is calculated to be a facile reaction that affords an energetically reasonable thiosulfinate species (**IN31**). However, conversion of this intermediate to new products seems unlikely to compete with its reversion to the starting material **R1**. The reversible attack of nucleophiles at the S1 position of the 1,2-dithiolan-3-one 1-oxide heterocycle predicted by our calculations is consistent with experimental data showing reversible intramolecular nucleophilic addition to this position in leinamycin under mild conditions.[57] The work

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(49) Due to overestimation of solvation energies for small ions such as MeS^- by dielectric continuum methods, the free energy of starting materials is probably too low. Additional errors will result from overestimation of entropies by Gaussian because all frequencies are treated as vibrations and changes of solvent entropy are not taken into account.

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(54) The rate constant for attack of BuS^- on a thiosulfinate is 2.9 × 10^6 M^{-1} s^{-1} (see: Kice, J. L.; Rogers, T. E. *J. Am. Chem. Soc.* **1974**, *96*, 8015−8019). At pH 7 only a small amount of RS^- will be present for biological thiols such as glutathione. Assuming a thiol concentration of 1 mM and a thiol pK_a of 9, k_{obs} for the "forward" reaction involving attack of thiolate on the thiosulfinate group of **IN31** will be about 30 s^{-1}. In contrast, application of the Eyring equation tells us that the competing "reverse" reaction to regenerate starting material **R1**, for which the free energy of activation is 12 kcal/mol, is expected to have a (much larger) rate constant of 8 × 10^3 s^{-1} at 25 °C.

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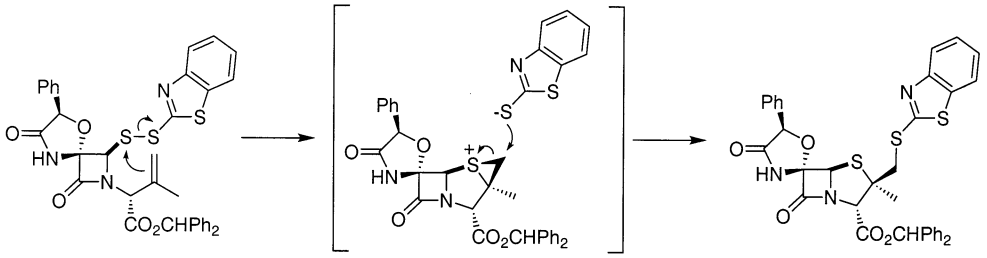
SCHEME 7



SCHEME 8

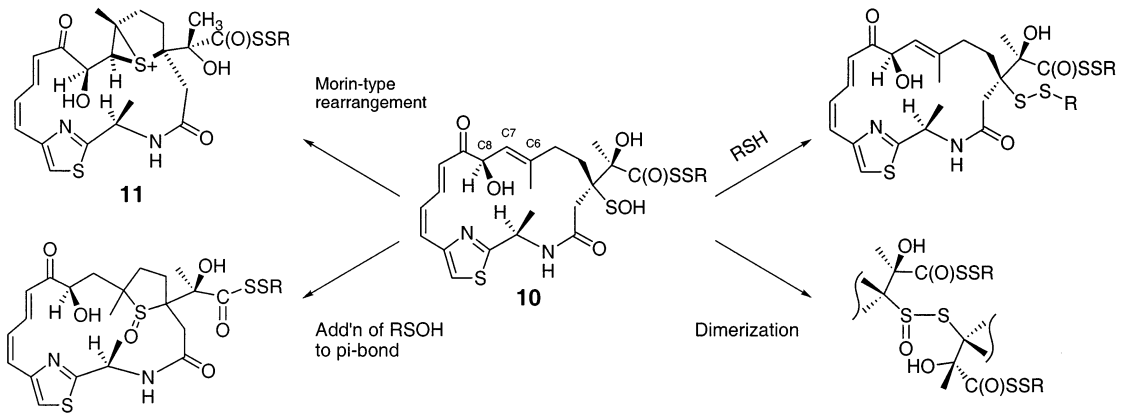


TABLE 1. Relative Energies, Enthropies, and Free Energies (kcal/mol) for the Stationary Points in Reactions 1−3[a]

	IEFPCM solvation		PCM solvation		IPCM solvation		
	Δ*H*, kcal/mol	Δ*G*, kcal/mol	Δ*H*, kcal/mol	Δ*G*, kcal/mol	Δ*H*, kcal/mol	Δ*G*, kcal/mol	Δ*S*, eu
R1 + R2	0	0	0	0	0	0	0
TS11	5.9	16.0	6.7	16.8	1.3	11.4	−34.1
P11	−5.0	5.0	−4.7	5.3	−12.9	−2.9	−33.5
TS12	6.1	12.8	6.5	13.2	5.6	12.3	−22.4
P12 + R2	3.0	2.8	3.2	3.0	3.4	3.2	0.5
TS13	13.1	21.2	13.5	21.6	11.1	19.2	−27.1
P13	−41.8	−32.9	−41.5	−32.6	−42.2	−33.3	−29.9
TS21	9.9	18.9	10.6	19.6	7.3	16.3	−30.2
IN2	6.0	14.7	3.2	11.9	1.7	10.4	−29
TS22	7.9	19.2	8.6	19.9	6.9	18.2	−37.9
P21 + P22	−3.5	−6.0	−3.1	−5.6	−4.4	−6.9	4.3
TS3	1.6	9.6	2.1	10.1	1.8	9.8	−25.7
IN31	−10.5	−2.4	−10.3	−2.2	−13.9	−5.8	−27.1

[a] All energies at 298 K (in kcal/mol) were calculated at the MP2/6-311+G(3df,p)//B3LYP/6-31G* level.

of Greer[58] suggests that the reaction of thiolate at the S1-position in leinamycin may be suppressed by interaction of the sulfinyl sulfur with the amide group of the antibiotic's macrocycle. Such an amide-S1 interaction is, of course, not possible in the simplified heterocycle **R1** used in our calculations. Regardless, it is important to note that our calculations suggest that reversible addition of thiolate to the S1-position is not likely to yield activated leinamycin.

Our computational results suggest that attack of thiolate on the carbonyl (C3) of leinamycin's sulfur heterocycle is a facile reaction that produces the thiosulfinate anion (**IN11**). This intermediate cyclizes to yield **IN12**, followed by conversion to the carboxytrisulfide (**P12**). The carboxytrisulfide **P12** is not expected to proceed to activated leinamycin via the oxathiolanone (**P21**) because this reaction is disfavored by 26.9 kcal/mol and must climb an energy barrier of 36 kcal/mol.

Thus, the data suggest that attack of thiolate on the C3′-position of leinamycin might produce the trisulfide **6** (Scheme 5) via an energetically reasonable pathway. However, it is important to note that there is no experimental evidence for the formation of this carboxytrisulfide (**6**) or its expected[46,59,60] breakdown products such as **7**, **8**, and **9** (Scheme 5). In experimental reactions of thiol with leinamycin, good mass balances have been recovered and the only products characterized to date are those stemming from attack of nucleophiles on the episulfo-

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nium ion **5** (isolated in ~70% yields).[13] The lack of experimental support for formation of the trisulfide **6** leads us to consider the possibility that our calculations overestimate the extent of thiol attack at the C3′-position of leinamycin. Along these lines, it is important to recognize that we employed a heterocycle lacking substituents adjacent to the carbonyl group. Literature precedents indicate that the α-substituents found in leinamycin could increase the activation barrier for attack of thiolate on the C3′-carbonyl, thereby diminishing the contribution of this reaction manifold.[61] Consistent with this notion, we find that hydrolysis of leinamycin is ~25 times slower than that for a leinamycin analogue lacking substitutents α to the C3′-carbonyl.[62]

Alternatively, it is possible that the trisulfide **6** forms and is subsequently converted directly to the episulfonium ion (**5**) by intramolecular reaction of the trisulfide moiety with the proximal C6−C7 alkene as shown in Scheme 6. It is unclear whether such a process can account for the kinetically rapid[14] thiol-triggered activation of leinamycin because, typically, reactions of this sort, involving electrophilic addition of S−S bonds to alkenes, require high temperatures or Lewis acid catalysis.[63−65] Interestingly, however, at least one literature example (shown in Scheme 7) indicates that an intramolecular reaction of this type can occur without catalysis and at relatively modest temperatures.[66] In this example, the sulfur leaving group (2-mercaptobenzothiazole anion) is comparable to the hydrodisulfide leaving group (RSS^-) in the leinamycin reaction as judged by the pK_a values of the conjugate acids.[67,68]

Finally, we considered attack of thiolate at the S2-position of the 1,2-dithiol-3-one 1-oxide heterocycle. Thiol-triggered activation of leinamycin has been suggested to proceed via initial attack of thiol at this site (Scheme 1).[10,13] Importantly, our calculations show that this reaction pathway is energetically reasonable. The overall conversion of the 1,2-dithiolan-3-one 1-oxide heterocycle (**R1**, Scheme 3) to the 1,2-oxathiolan-5-one (**P21**) is favored by 6 kcal/mol and must surmount a modest activation barrier of 19.2 kcal/mol.

Although the reaction of leinamycin with thiol has often been depicted[7,10,13] as proceeding through a leinamycin-sulfenic acid intermediate (RSOH) our calculations show that this may not be an entirely accurate portrayal. Rather, we find that the hypervalent sulfenate anion (RSO^-, **IN2**, Scheme 3) formed by initial attack of thiolate on leinamycin is likely to undergo immediate cyclization to the 1,2-oxathiolan-5-one **P21** without prior protonation to the sulfenic acid (RSOH). Cyclization of **IN2** to **P21** is calculated to have a very low activation barrier (4.5 kcal/mol) and, therefore, may compete effectively with protonation of the sulfenate (RSO^-) group in **IN21**.[69] Importantly, the predicted cyclization of **IN21** to **P21** bypasses the protonated leinamycin-sulfenic acid intermediate (**10**, Scheme 8), which is expected to be quite unstable. Sulfenic acids (RSOH) are notoriously unstable and, if formed, the leinamycin-sulfenic acid (**10**) might be expected to decompose into a complex mixture of products including those shown in Scheme 8.[54,55,70−73] Thus, our calculations provide a satisfying explanation for the fact that various sulfenic acid-derived decomposition products are not produced in the reaction of leinamycin with thiols.

Conclusions

Our calculations provide new, detailed insight into the thiolytic activation of the antitumor antibiotic leinamycin. We find that the previously proposed[7,10,13] mechanism (shown in Scheme 1) involving attack of thiolate on the central sulfur of leinamycin (S2′) represents the most reasonable route for thiol-triggered activation of the antibiotic. Reversible attack of thiolate at the S1′-position of the antibiotic may be possible, but is expected to be largely unproductive. The calculations revealed a previously unexpected reaction pathway in which attack of thiolate at the C3′-carbonyl of leinamycin can potentially produce the leinamycin-trisulfide **6**. The possibility that this trisulfide might be converted to the episulfonium ion (**5**) deserves further theoretical and experimental consideration but, based on literature precedents, this is not expected to be a primary pathway for thiol-triggered activation of the antibiotic.

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Supporting Information Available: Tables S1−S4 listing the geometries and total and relative energies for the compounds shown in Scheme 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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