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Evidence for Facile Atropisomerism and Simple (Non-Nucleophilic) Biradical-Forming Cycloaromatization within Kedarcidin Chromophore Aglycon

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Kedarcidin is one of the most structurally complex and reactive of the known chromoprotein antiproliferative agents. When separated from its binding protein, kedarcidin chromophore rapidly decomposes. Leet et al. reported that solutions of the unbound chromophore quickly browned; decomposition was found to be greatly accelerated upon concentration.[1] For this reason, much of the early characterization of the natural product was conducted upon the stable cycloaromatization product that is formed upon treatment of the chromophore with sodium borohydride. Although elements of the originally proposed structure were later shown to be in error by the Hirama group,[2] the isolation and gross structural assignment of this extraordinarily reactive and complex molecule were nevertheless remarkable achievements.[1] The revised structures of the chromophore and its cycloaromatization product (borohydride-induced) are shown as **1** and **2**, respectively (Figure 1).[2]

In light of new data presented here, arising from a study of the series of synthetic intermediates **3**−**9** (as well as others, see Supporting Information),[3] we present two views of the dynamic behavior of kedarcidin chromophore that differ from what might reasonably be inferred from a reading of the current literature. The first concerns the issue of atropisomerism of the chloropyridine (ansa) bridge. The issue of atropisomerism is important in synthetic planning, and bears upon any detailed consideration of the binding of **1** to a molecular host, be this double-stranded DNA, the kedarcidin binding protein, or another molecule. On the basis of nOe studies, the borohydride-induced cycloaromatization product **2** was assigned the atropisomeric conformation shown,[1,2] with the chlorine atom projecting away, toward C12 rather than C10. Structural drawings depict **1** in the same atropisomeric conformation[1,2,4] although the existence of multiple-energy minima was specifically noted for **1**, but not further elaborated.[1] In conjunction with subsequent reports of an atrop-selective synthesis of an ansa-bridged kedarcidin model structure,[4] it would be reasonable to draw the conclusion that atropisomerism within **1** is not facile at ambient temperature. Here, we show that this is likely not the case.

The second issue we address concerns bioactivation of **1** by nucleophilic addition, a proposal given weight by the isolation of the cycloaromatized C12-(boro)hydride addition product **2**,[1] DNA-cleavage experiments incorporating "thiol activating agents,"[5] and the lack of any data to support a simple thermal cycloaromatization of **1**.[6] We present here the first experimental data showing that unimolecular, biradical-forming cycloaromatization of an ansa-bridged core structure (**8**) is rapid at physiologically relevant temperatures and that thiol activation of this substrate is slow relative to direct, non-nucleophilic cycloaromatization. These data clearly imply that proposals concerning the mechanism of action of **1** that invoke nucleophilic addition as a first step must be reevaluated, if not revised entirely.

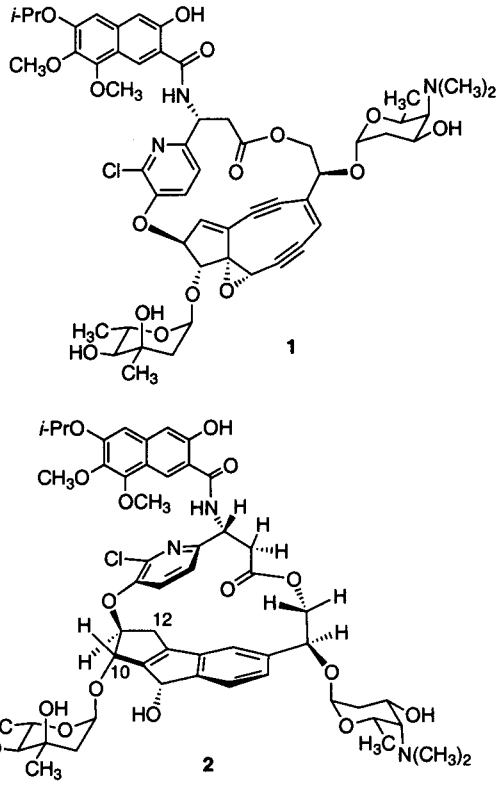

Figure 1. Kedarcidin chromophore (**1**) and the product of borohydride-induced cycloaromatization (**2**).

Evidence for the rapid interconversion of conformational isomers was first encountered in ^{1}H NMR analysis of the ansa-bridged macrolactone **3** (Figure 2), a key intermediate in the synthesis of the aglycon **8**.[3] Two different synthetic routes to the macrocycle **3** provided an identical mixture of two equilibrating conformational isomers ($K = 0.92$). Broadening and, eventually, coalescence of the two discrete sets of signals was observed in the ^{1}H NMR spectrum upon warming of the sample, establishing that the two isomers were indeed equilibrating. Two different NMR spin-transfer experiments (saturation transfer (ST) and inversion transfer (IT)) were conducted to evaluate the kinetics of conformer interconversion.[7] From these, the half-life for equilibration was determined to be ~0.2 s at 23 °C.[8] An Eyring analysis of the kinetics data from IT experiments, although obtained over a narrow temperature range

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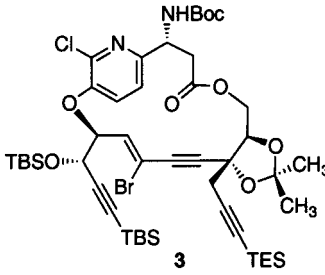


Figure 2. Thermodynamic and kinetic parameters for conformational isomerism of the ansa-bridged macrolactone **3**.

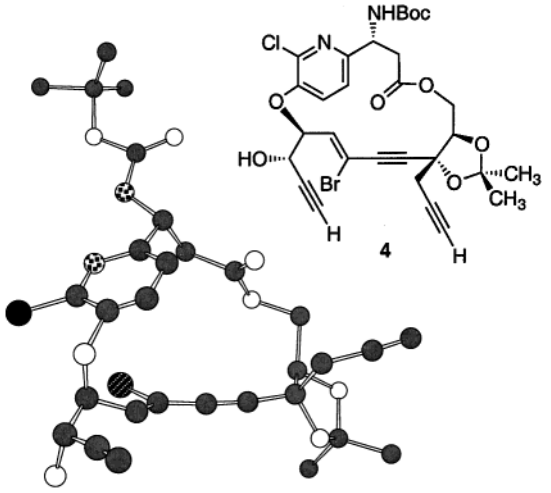


Figure 3. Solid-state structure of the ansa-bridged macrolactone **4**.

(10−35 °C), showed that there was a substantial entropic component to the isomerization barrier, as expected.

Greater insight into the delicate interplay of factors affecting the kinetics and thermodynamics of conformer interconversion was revealed by study of the crystalline macrocyclic intermediate **4**, produced by global desilylation of **3**. Single-crystal X-ray analysis of **4** provided the first and, to date, only crystallographic data for any kedarcidin ansa-bridged system (Figure 3). In the solid state, **4** was found to be a single conformer, with the mean plane of the macrocycle orthogonal to the chloropyridine ring, such that the chlorine atom projects toward C12, as proposed in **1** and **2**. Dissolution of solid **4** in benzene produced a nearly equal mixture (K = 0.70) of two rapidly equilibrating conformers ($t_{1/2}$ = 0.23 ± 0.01 s, 22 °C). The difference in energy between conformational isomers of **4** as well as the barrier to their interconversion were evidently both quite low. Apparently, intermolecular interactions (crystal packing forces) substantially bias the conformer ratio of **4**. It would therefore seem reasonable to consider that the distribution of atropisomeric forms of **1** might also vary with the environment (e.g., apoprotein- versus DNA-bound forms), provided that isomer interconversion can occur (vide infra).

The observation of rapid conformational isomerism within intermediates **3** and **4** was not surprising in light of their presumably more flexible macrocyclic rings as compared to those in **1**. However, analysis of more rigid macrobicyclic intermediates prepared en route to **8** revealed that rapid isomer interconversion occurred at ambient temperature in many of these compounds as well and that equilibrium ratios of atropisomers varied widely (see Supporting Information).[9] The point is perhaps most strikingly illustrated within the series of intermediates **5**−**7**. We observed that whereas the diene **5** and the epoxide **6** existed in a single atropisomeric conformation the regioisomeric epoxide **7**, the direct precursor to the aglycon **8**,

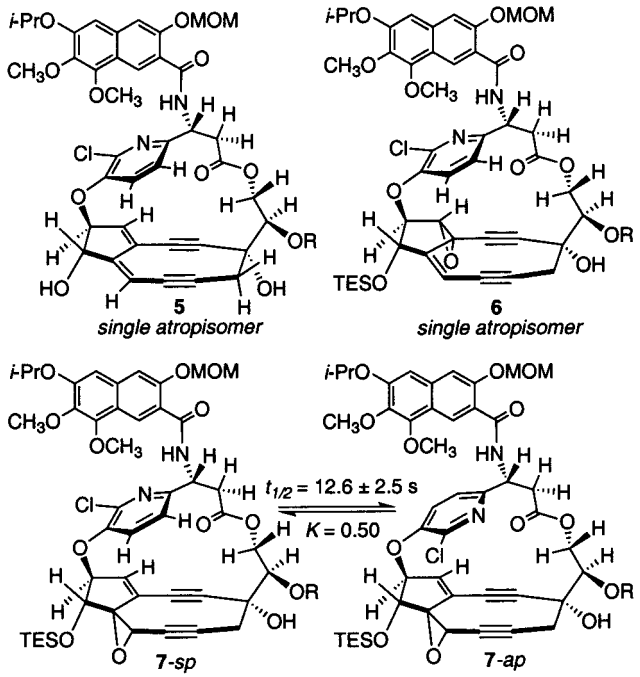
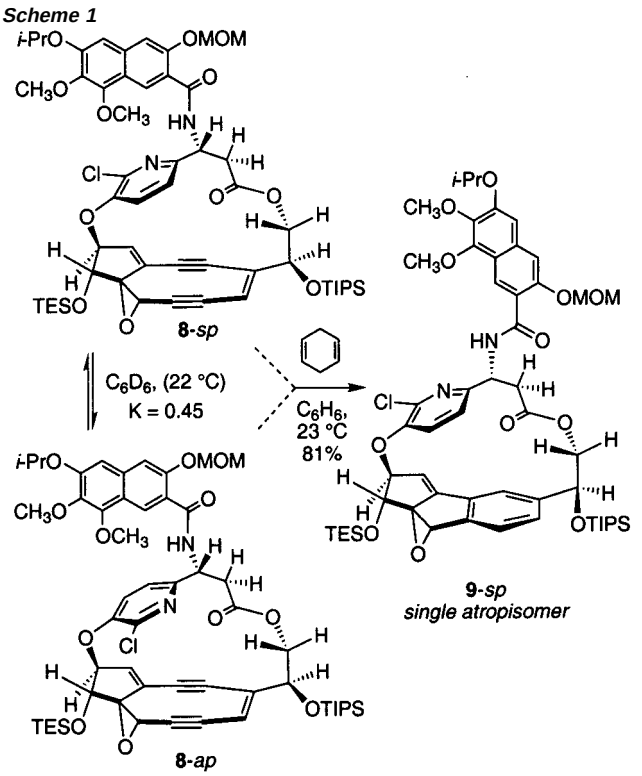


Figure 4. Kedarcidin advanced intermediates **5**−**7**, R = TIPS.

Scheme 1



existed as a mixture of atropisomers that equilibrated rapidly at ambient temperature (K = 0.50, $t_{1/2}$ = 12.6 ± 2.5 s). Thus, slight variations in the puckering of the unsaturated core in the two epoxides greatly influence the rotamer ratios. From the perspective of synthesis, however, a more important point is that the reaction of an atropisomerically "fixed" molecule (**5**, a single atropisomer) can form a dynamic mixture of atropisomeric products (**7**). Significantly, the fully functional ansa-bridged core system **8**, produced by elimination of water from **7**, also exists as an atropisomeric mixture (K = 0.45, Scheme 1). Tentative structural assignments of the major conformer as **8**-*sp* and the minor

conformer as **8**-*ap*[10] were based upon nOe data (obtained using the more stable precursor **7**). Indirect, but unequivocal, evidence that interconversion of atropisomeric forms of **8** (or, less likely, **9**) is facile at 23 °C comes from the observation that cycloaromatization of the mixture of atropisomers **8** in 1,4-cyclohexadiene−benzene (1:1) at 23 °C produces the epoxy indene **9** as a single atropisomer (81%). We estimate an upper limit of approximately 8×10^{-2} s^{-1} ($t_{1/2} > 10$ s) for the rate of atropisomerism of **8** at 23 °C.[11] The observation of facile interconversion of atropisomers **8** suggests that it is not only possible but also likely that **1** undergoes atropisomerism at ambient temperature as well. This view is also supported by tabulated nOe data in the original structure determination that are inconsistent with a single atropisomeric structure.[1]

As discussed above, the prevailing view in the literature is that nucleophilic activation of **1** precedes cycloaromatization.[6] Our studies of the direct cycloaromatization of **8** suggest that this need not be the case. We observed that the rate of cycloaromatization of **8** was strongly influenced by the solvent, in accord with the prior observations of Hirama et al. concerning the natural product C-1027 (also proposed to undergo cycloaromatization without nucleophilic activation) and a nonbridged kedarcidin model structure.[12a] For example, the rate of cycloaromatization of **8** in 5 M 1,4-cyclohexadiene at 22 °C was 2×10^{-4} s^{-1} ($t_{1/2} = 58$ min, 81% yield) whereas the rate in 0.1 M 1,4-cyclohexadiene at 22 °C was 5×10^{-5} s^{-1} ($t_{1/2} = 4$ h) (~65% yield). These results support the view that **8** (and possibly **1**) may be in equilibrium with its biradical form and that hydrogen-atom trapping is a kinetically significant process.[12]

To evaluate direct cycloaromatization versus nucleophilic activation pathways, **8** was incubated in the presence or absence of the thiol β-mercaptoethanol (BME) in DMSO−aqueous buffer (pH = 7.45, 37 °C), conditions designed to mimic earlier DNA cleavage experiments with **1**.[5] The water-soluble hydrogen-atom donor 1,4-cyclohexadiene 1,2 dimethanol ((CHDM), 2.5 M) was used in lieu of DNA.[13] Aliquots were removed at various time points, and the product distribution was analyzed by rp-HPLC. In the absence of BME, the aglycon **8** underwent cycloaromatization to form **9** in 50% yield. When **8** was treated with BME (1.4 mM)[5] the cycloaromatized product **9** was still formed as the major reaction product at a comparable rate, in 37% yield. This result shows that any nucleophilic activation pathway is, at best, competitive with direct cycloaromatization, under the conditions examined.[14]

Results described herein suggest that proposals involving nucleophilic activation of **1** should be regarded cautiously. In the event that **1** does undergo direct cycloaromatization in vivo, kedarcidin would join C-1027 as one of only two enediyne agents capable of biradical formation without chemical activation. In addition, in light of our experiments showing that the protected chromophore aglycon **8** exists as an atropisomeric mixture that equilibrates at ambient temperature, it is reasonable to conclude that such may be the case with **1** itself.

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Supporting Information Available: ^{1}H NMR data including kinetics and nOe determinations, where applicable, for **3**−**9** and three additional synthetic intermediates; experimental details for the cycloaromatization experiments with **8** (PDF) and a crystallographic information file (CIF) for **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Rate constants are reported as the rate of conversion of the major conformer to the minor conformer. In all cases, the two proton resonances of the chloropyridine ring (HC4′ and HC5′) showed a greater chemical shift difference in the minor conformer. In the case of compounds **3** and **7**, this was shown by nOe studies to correspond to the conformer in which the chlorine of the chloropyridine is pointing toward C(10) (Supporting Information).

(9) Careful NMR analysis of atropisomeric forms of **3** and **4** suggested that conformer movement in the region of the lactone group accompanied rotation of the chloropyridine bridge. However, in the more rigid compounds **7** and **8** NMR analysis suggests that isomerism is restricted to rotation of the chloropyridine bridge.

(10) Synperiplaner (*sp*). Antiperiplaner (*ap*). See Klyne, W.; Prelog, V. *Experientia* **1960**, *16*, 521.

(11) The failure to see saturation transfer suggests that the rate of exchange is at most 10% of the rate of relaxation of the resonance with the longest T_1. See ref 7b, page 54.

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(13) In DNA cleavage studies of neocarzinostatin, a high concentration of CHDM was necessary to mimic the efficiency of biradical trapping by DNA, as judged by the yield of cycloaromatized product. Myers, A. G.; Cohen, S. B.; Kwon, B.-M. *J. Am. Chem. Soc.* **1994**, *116*, 1670. In the absence of CHDM, **8** had limited thermal stability and suffered nonspecific decomposition ($t_{1/2} \approx 20$ min).

(14) We also observed little reaction of **8** in the presence of methylthioglycolate at 23 °C with or without added triethylamine, (THF-d_8, ^{1}H NMR analysis, gradual decomposition was observed). This result stands in contrast to our prior observations concerning neocarzinostatin chromophore, where thiol addition to C12 was rapid and clean even at −38 °C. (Myers, A. G.; Proteau, P. J. *J. Am. Chem. Soc.* **1997**, *119*, 2965.).

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