Total Synthesis and DNA-Cleaving Properties of Thiarubrine C

Yamin Wang and Masato Koreeda*,†

Department of Chemistry, The University of Michigan, Ann Arbor, Michigan 48109-1055

Tonika Chatterji and Kent S. Gates*,‡

Departments of Chemistry and Biochemistry, University of Missouri−Columbia, Columbia, Missouri 65211

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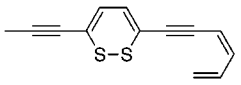
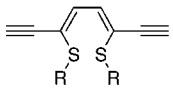
The thiarubrines are structurally intriguing antibiotics that have been isolated from a variety of plant sources and have been found to possess interesting antibacterial, antifungal, anticancer, and antiviral properties.[1,2] The mode of action of 1,2-dithiins such as the thiarubrines has been considered;[1,3,4] however, the cellular target(s) involved in the antibiotic action of these compounds remains uncertain.



thiarubrine C (**1**)



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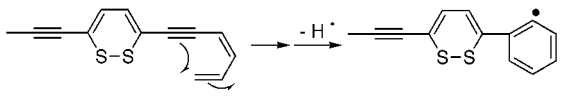
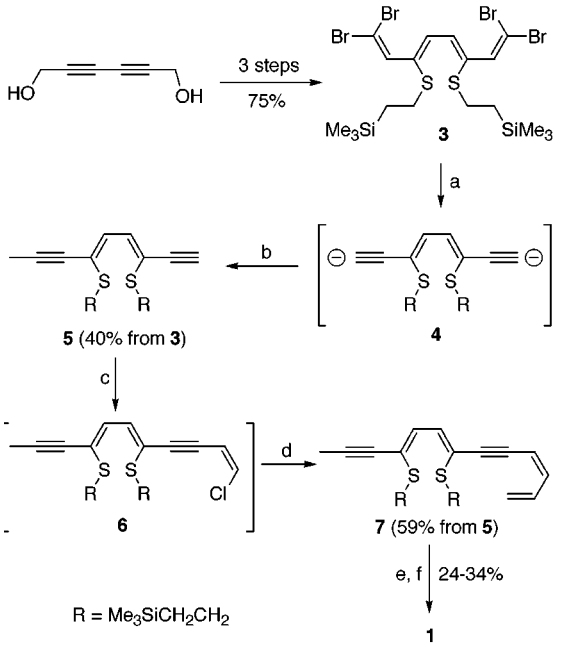
It has been reported that thiarubrine C[5] causes DNA strand scission,[6] although to our knowledge relevant experimental conditions and data supporting this statement have not been published. It was further suggested that this process might involve an enediyne-like[7] cyclization of the diene-yne side-chain of thiarubrine C that generates, with or without loss of a hydrogen radical, DNA-cleaving radical(s) (see Scheme 1).[6] Intrigued by the unusual, seemingly high-energy process proposed to explain DNA damage by thiarubrine C, we examined DNA cleavage by this class of natural products. We report here on the first total synthesis and DNA-cleaving properties of thiarubrine C.

The synthesis of thiarubrine C (**1**) was achieved following the strategy employed for the synthesis of thiarubrine A.[8] Thus, the element of dissymmetry was introduced by monoalkylation of a symmetrical intermediate such as **2** via its dianion. The precursor to this dianion, tetrabromide **3**, was obtained in three steps in 75% overall yield from 2,4-hexadiyne-1,6-diol (Scheme 2).[8] Treatment of the dianion **4**, generated in situ from **3** with 4.0 equiv of *n*-BuLi, with 1.1 equiv of MeI, provided monomethyl derivative **5** in 40% yield from **3**, together with dimethylated and nonmethylated, protonated products in 18 and 15% yield, respectively. This apparent lack of control for the selective monomethylation

Scheme 1



Scheme 2



Conditions and reagents: a) *n*-BuLi (4.0 equiv)/THF, -78 °C to rt, 1 h; b) MeI (1.1 equiv)/THF, -78 °C to rt, 12 h; c) *cis*-1,2-dichloroethene (1.5 equiv), $Pd(PPh_3)_4$ (5 mol%), CuI (10 mol%), *n*-$BuNH_2$ (2.0 equiv)/benzene, rt, 1 h ; d) H_2C=CHMgBr (2.0 equiv)/THF, $Pd(PPh_3)_4$ (5 mol%)/benzene, rt, 12 h; e) TBAF (12.6 equiv), $(F_3CCO)_2O$ (6.3 equiv)/THF, -40 °C to rt, 4 h; f) I_2 (10 equiv)/10% aq KI, rt, 15 min.

of the dilithio acetylide generated from tetrabromide **3** must be the reflection of ineffective orbital overlap between the two carbanionic orbitals with the rest of the diyne−diene π-system. However, in view of its directness and overall yield, this approach was judged preferable over an alternate selective sequence which would have added a few extra steps. The sequential Pd(0)-mediated alkenylation of **5** furnished the (*Z*)-diene-yne **7** in 59% overall yield. Deprotection to regenerate the two thiol groups proved quite problematic. Treatment of **7** with excess TBAF resulted in only the mono-deprotection, and the subsequent I_2-catalyzed oxidation provided the two dimeric disulfides. This problem of sluggish reactivity toward fluoride ion-mediated deprotection was circumvented by activation through acylation of the sulfur atoms. Thus, treatment of **7** with TBAF and trifluoroacetic anhydride followed by oxidation of the resulting bis-thioenolate with iodine provided thiarubrine C (**1**) in 24−34% overall yield from **7**. Apparently, the bis-$SCOCF_3$ intermediate was readily hydrolyzable to the bis-thioenolate during the aqueous sodium bicarbonate treatment prior to the oxidation.

The DNA-cleaving properties of thiarubrine C thus obtained were examined using a plasmid-based assay in which strand scission causes the conversion of supercoiled DNA (form I) to the open circular form (form II). It was found that thiarubrine C (concentrations up to 1 mM) does not efficiently cause DNA strand scission in our assays, thus suggesting that the previously proposed cyclization mechanism[6] is not significant under these conditions (Figure 1,

† Masato Koreeda: phone (734) 764−7371; fax (734) 764−7371; e-mail: koreeda@umich.edu.

‡ Kent S. Gates: phone (573) 882−6763; fax (573) 882−2754; e-mail: GatesK@missouri.edu.

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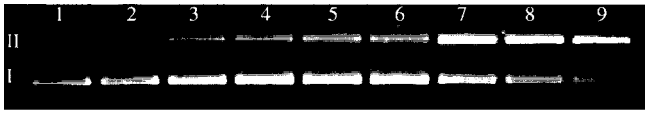
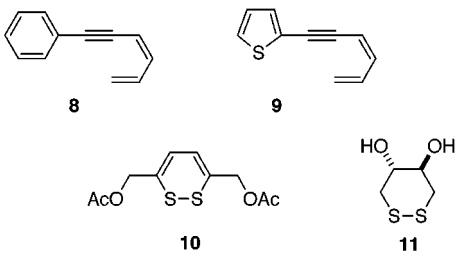


Figure 1. Thiol-dependent DNA Cleavage by various concentrations of thiarubrine C (**1**). Supercoiled pBR322 DNA (38 μM bp) was incubated for 14 h at 37 °C with various concentrations of thiarubrine C and 20 equiv of 2-mercaptoethanol in sodium phosphate buffer (50 mM, pH 7) containing 10% acetonitrile (by volume). Agarose gel electrophoresis was performed as described in the Supporting Information. The reactions were prepared under red (darkroom) light and were incubated in the dark. The number in parentheses following the description of each lane below indicates the *S*-value (mean number of strand breaks per plasmid molecule) for each lane and was calculated using the equation $S = -\ln f_I$, where f_I is the fraction of plasmid in a given lane that is present as form I. Values reported here represent the average of multiple experiments, and the standard error in these measurements is less than 2%. Lane 1, DNA alone (0.2); lane 2, 100 μM thiarubrine C (0.2); lane 3, 2 mM 2-mercaptoethanol (0.2); lane 4, 1 μM thiarubrine C + thiol (0.2); lane 5, 5 μM thiarubrine C + thiol (0.3); lane 6, 10 μM thiarubrine C + thiol (0.3); lane 7, 25 μM thiarubrine C + thiol (0.4); lane 8, 50 μM thiarubrine C + thiol (0.6); lane 9, 100 μM thiarubrine C + thiol (0.8).

lane 2).[9] The notion that the diene-yne substituent of thiarubrine C is not capable of causing significant DNA damage was further corroborated by our observation that other non-1,2-dithiin derivatives with the same diene-yne side chain, **8**[10] and **9**[11] (100 μM concentrations), do not induce significant DNA cleavage in our assays.



8 **9** **10** **11**

Interestingly, it was found that, in the presence of biologically relevant[12] concentrations of thiol, thiarubrine C does cause DNA strand scission (Figure 1, lanes 4−9). Some insight regarding the chemical mechanism of thiol-dependent DNA cleavage by thiarubrine C was provided by performing the reaction in the presence of various additives. The hydrogen peroxide-destroying enzyme catalase, the chelator of adventitious trace metals desferal, and the known radical scavengers ethanol and mannitol all significantly inhibit thiol-mediated DNA cleavage by thiarubrine C. The enzyme superoxide dismutase, which catalyzes the disproportionation of superoxide into hydrogen peroxide and molecular oxygen, has little effect on the cleavage reaction. When considered together, our experiments suggest that DNA cleavage in this system results from a pathway involving reduction of molecular oxygen to superoxide radical, followed by spontaneous disproportionation of superoxide to hydrogen peroxide which ultimately undergoes a trace metal-catalyzed Fenton reaction to yield DNA-cleaving species such as hydroxyl radical.[13−15]

The nature of the products formed in the reaction of thiarubrine C with thiols remains under investigation; however, one can imagine that the chemistry underlying thiol-dependent formation of oxygen radicals by thiarubrine C is conceptually similar to the thiol-driven production of DNA-cleaving oxygen radicals reported previously for the epidithiapiperazine-2,5-dione-containing antibiotics gliotoxin and sporidesmin.[16,17] In the case of the epidithiapiperazine-2,5-diones, reaction of thiols with the disulfide linkage[18] of the antibiotic is thought to yield a thiol species that is able to efficiently react with adventitious trace metals and molecular oxygen to produce reactive oxygen species.[19−21] The notion that the 1,2-dithiin moiety of thiarubrine C may be the key site of thiol attack is supported by the observation that the simple 1,2-dithiin analogue **10**[22] is comparable to the natural product in its ability to serve as a thiol-dependent DNA-cleaving agent. It is noteworthy that the alicyclic six-membered disulfide *trans*-1,2-dithiane-4,5-diol (**11**, oxidized DTT) is a relatively poor DNA-cleaving agent under the conditions employed here.

In conclusion, our work provides evidence that the thiarubrines can produce DNA-cleaving reactive oxygen species under physiologically relevant conditions. This is the first report of thiol-dependent DNA cleavage by these antibiotics. In general, the production of reactive oxygen species has important biological consequences;[13] however, it must be noted that the efficiency of DNA cleavage by thiarubrine C in our assays is not comparable to some other agents known to derive activity through DNA damage,[23] and our results should not necessarily be taken as evidence that DNA represents a biologically relevant target for these antibiotics. Finally, our findings do not support the previous postulation[6] that the diene-yne substituent found in the thiarubrines can efficiently cleave DNA involving a cyclization mechanism.

Acknowledgment. We thank the National Institutes of Health (GM51565, K.S.G.) for partial support of this work.

Supporting Information Available: Experimental details for the synthesis of thiarubrine C (**1**), from the known tetrabromide **3**, and yne-diene analogues **8** and **9**; characterization data for compounds **5**, **7**, **1**, **8**, and **9**; experimental procedures for DNA cleavage by thiarubrine C and **10** (19 pages).

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(10) Prepared from phenylacetylene in two steps [1. *cis*-1,2-dichloroethene (1.25 equiv), $Pd(PPh_3)_4$ (5 mol %), CuI (10 mol %), *n*-$BuNH_2$ (2.5 equiv)/benzene, rt, 1.5 h; 2. $H_2C{=}CHMgBr$ (2.0 equiv)/THF, $Pd(PPh_3)_4$ (5 mol %)/benzene, rt, 12 h] in 85% overall yield.

(11) Prepared from 2-iodothiophene in four steps [1. (trimethylsilyl)-acetylene (1.2 equiv), $Pd(PPh_3)_2Cl_2$ (4 mol %), CuI (8 mol %), *n*-$BuNH_2$ (4.0 equiv)/benzene, rt, 12 h; 2. KOH (1.0 equiv)/H_2O/MeOH, rt, 2 h; 3. *cis*-1,2-dichloroethene (2.0 equiv), $Pd(PPh_3)_4$ (5 mol %), CuI (10 mol %), *n*-$BuNH_2$ (2.0 equiv)/benzene, rt, 1.5 h; 4. $H_2C{=}CHMgBr$ (2.0 equiv)/THF, $Pd(PPh_3)_4$ (5 mol %)/benzene, rt, 12 h] in 35% overall yield.

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(20) The observed metal dependence in our reactions (indicated by inhibition of DNA cleavage by the metal chelator desferal) may reflect the requirement for transition metal ions such as copper or iron in an initial thiol oxidiation reaction as well as in the subsequent Fenton reaction. See ref 13.

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