Title: Synthesis of myrocin G, the putative active form of the myrocin antitumor antibiotics.

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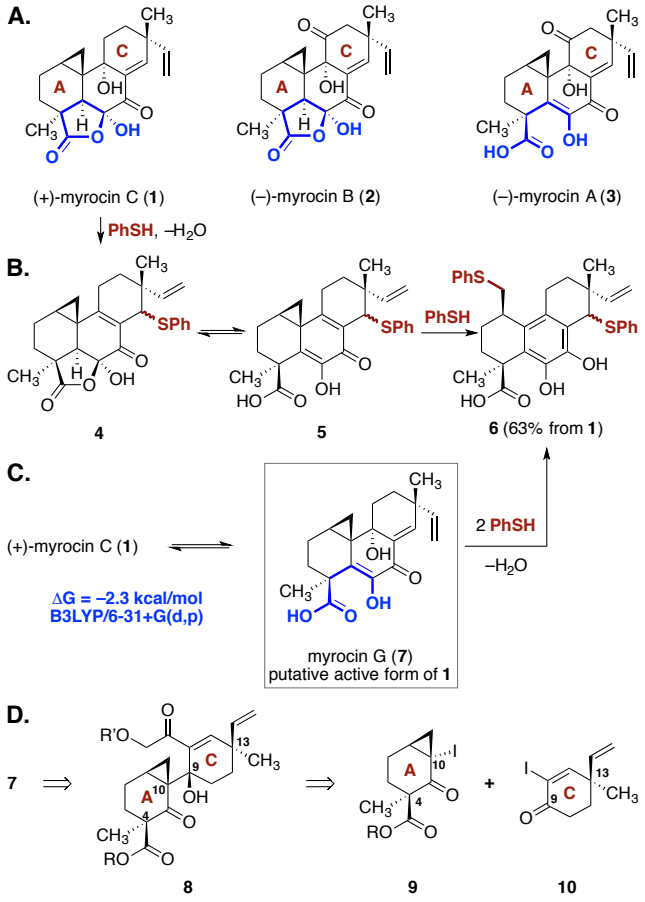
Abstract. The antiproliferative antimicrobial fungal metabolites known as the myrocins have been proposed to cross-link DNA by double nucleotide addition. However, the nature of the DNA-reactive species is ambiguous, as myrocins have been isolated as functionally-distinct 5-hydroxy-γ-lactone and diosphenol isomers. Based on computational studies and literature precedent, we hypothesized that the diosphenol **7** (assigned the trivial name myrocin G) is the biologically-active form of the representative isolate (+)-myrocin C (**1**). To probe this, we developed a 15-step enantioselective route to **7**. A complex fragment coupling reaction unites two synthetic precursors of similar complexity and forms the central ring of the target in a single step. In support of our hypothesis, **7** was efficiently transformed to the bis(sulfide) **6**, a product previously isolated from reactions of **1** with benzenethiol. This work provides the first direct access to the diosphenol **7**, sets the stage for elucidating the mode of interaction of the myrocins with DNA, and provides a foundation for the synthesis of other pimarane diterpenes.

Main text: Efforts to elucidate the mechanism of action of natural products are complicated when the metabolite can adopt two or more functionally-distinct forms. This issue is exemplified by the antiproliferative antimicrobial metabolites myrocins C (**1**)[1] and B (**2**),[2,3] fungal isolates that contain a sensitive 5-hydroxy-γ-lactone residue (Scheme 1A, blue in **1** and **2**). Literature indicates[4] this substructure undergoes facile ring-opening to the corresponding diosphenol under mildly acidic or basic conditions, raising uncertainty about its fidelity under biological conditions. Consistent with this, the diosphenol isomer of **2**, (–)-myrocin A (**3**), has been identified in fungal cultures.[5]

Following their landmark total synthesis of (±)-myrocin C (**1**),[6] Danishefsky and Chu-Moyer disclosed that treatment of synthetic (±)-**1** with excess thiophenol and triethylamine generated the bis(sulfide) **6** (63%, Scheme 1B).[7] The mechanism for formation of **6** was proposed to comprise S_N2' substitution of the tertiary hydroxyl group (**1**→**4**), isomerization to the diosphenol **5**, and addition to the resulting activated cyclopropane. This reactivity led the authors to speculate that the myrocins cross-link DNA by sequential nucleotide addition reactions.[8]

Scheme 1. A. Structures of myrocins A–C (**1**–**3**). B. Structure of the bis(sulfide) **6** and the originally proposed mechanism for its formation. C. The diosphenol **7** is 2.3 kcal/mol more stable than **1** and hypothesized to be the biologically-active form of **1**. D. Retrosynthetic analysis of **7**.



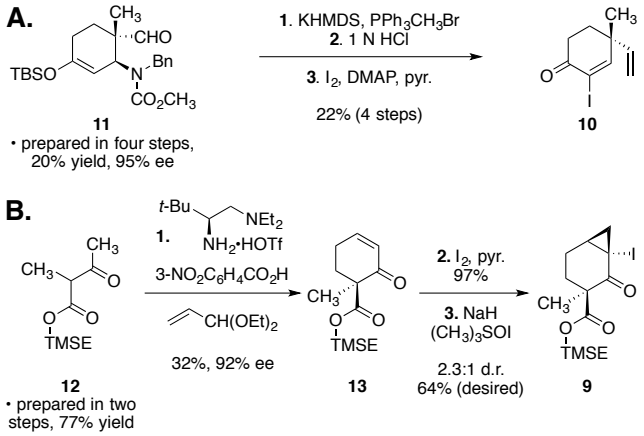
The isolation of **3** suggests the existence of an analogous diosphenol isomer of **1**, "myrocin G (**7**)". Consistent with earlier experimental studies,[4b] our own DFT calculations indicate that **7** is 2.3 kcal/mol more stable than the ring isomer **1** (Scheme 1C). This stability derives from a hydrogen-bonding interaction between the hydroxyl group and the adjacent carbonyl in **7**. These data suggested to us an alternative order of events for **1**→**6** wherein ring-opening of **1** to the diosphenol **7** precedes the initial alkylation.

Motivated by this analysis, we targeted **7** as the initial entry into this natural product family. In addition, we identified the diosphenol double bond as a strategic locus that could be converted retrosynthetically to the diketone **8** in a redox-neutral fashion (Scheme 1D). Further disconnection of the C9–C10 bond by a fragment coupling reaction reveals the α,β-cyclopropylketone **9** and the unsaturated

ketone **10** as two precursors of similar complexity. This synthetic strategy features the direct, redox-neutral installation of the C9 alcohol, high modularity, and independent introduction of the peripheral C4 and C13 quaternary centers.

The coupling fragments **9** and **10** were prepared in 3–4 steps from known compounds (Scheme 2). Beginning with the Diels–Alder adduct **11**,[14] Wittig olefination [potassium bis(trimethylsilyl)amide, methyl triphenylphosophium bromide], tandem enoxysilane hydrolysis and □-carbamate elimination (aqueous hydrochloric acid), and α-dehydroiodination (iodine, pyridine)[9] provided the C-ring fragment **10** (22% over four steps). The A-ring fragment **9** was synthesized from the □-ketoester **12**.[10] Stereoselective Robinson annulation[11] between **12** and acrolein diethylacetal provided the enone **13** (32%, 92% ee). α-Dehydroiodination[9] of **13** proceeded in 97% yield. Corey–Chaykovsky cyclopropanation[12] (trimethylsulfoxonium iodide, sodium hydride) provided a 2.3:1 mixture of diastereomeric α,β-cyclopropylketones. The major (desired) diastereomer **9** was isolated in 64% yield by recrystallization.

Scheme 2. Synthesis of the fragment coupling partners **9** and **10**. TMSE = 2-(trimethylsilyl)ethyl.



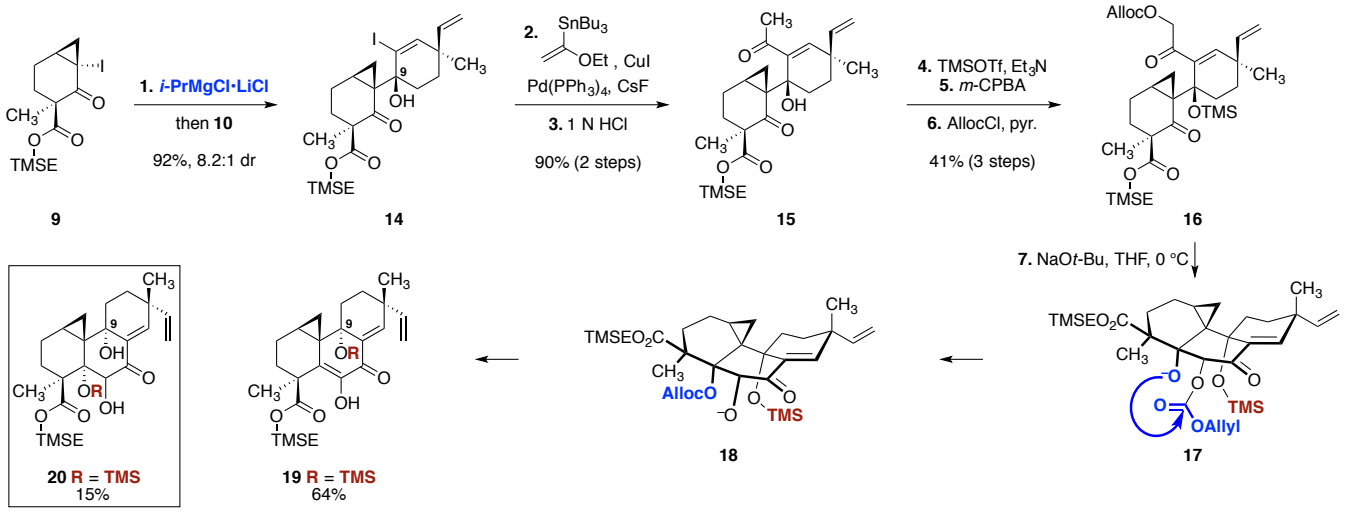
Treatment of the iodocyclopropane **9** with *iso*-propylmagnesium chloride–lithium chloride complex,[13,14] followed by addition of the iodoenone **10**, generated the fragment coupling product **14** (92%, 8.2:1 dr, Scheme 3). The stereoselectivity in the addition was anticipated based on the known

stereoelectronic preferences for nucleophilic addition to cycloalkanones[15] and consideration of non-bonded interactions in the transition state. It is noteworthy that retro-aldol reaction of **14** is precluded by the geometric constraints introduced by the cyclopropane ring.

The ring closure precursor **16** was prepared by a five-step sequence comprising Stille cross-coupling [tetrakis(triphenylphosphine)palladium-(0), copper(I) iodide, cesium fluoride] with tributyl(1-ethoxyvinyl) tin, hydrolysis of the vinyl ether product (aqueous hydrochloric acid), tandem alcohol silylation–enoxysilane formation (trimethylsilyl trifluoromethanesulfonate, triethylamine), Rubottom oxidation (3-chloroperoxybenzoic acid), and conversion of the primary alcohol to an allyl carbonate (allyl chloroformate, pyridine, 37% overall). The structure of the intermediate α-hydroxyketone was confirmed by X-ray analysis.[16]

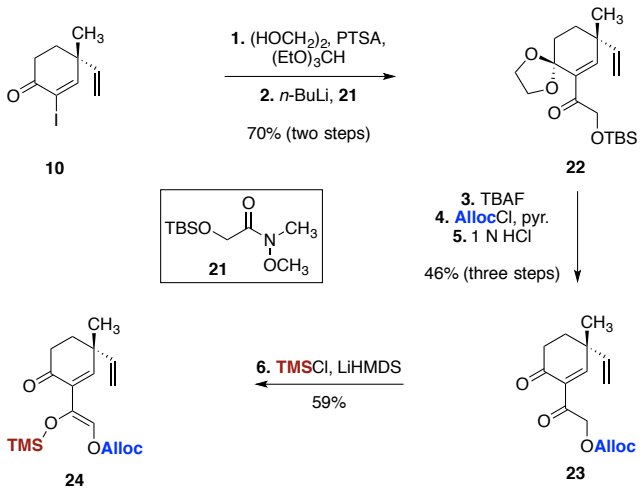
After much experimentation, we found that treatment of the allyl carbonate **16** with sodium *tert*-butoxide in tetrahydrofuran at 0 °C generated the diosphenol **19** (64%). Mechanistic studies suggest that **19** is formed via aldol addition (**16**→**17**), carbonate migration (**17**→**18**), and β-elimination. The silyl migration product **20** was isolated separately in 15% yield.

Scheme 3. Synthesis of 19.



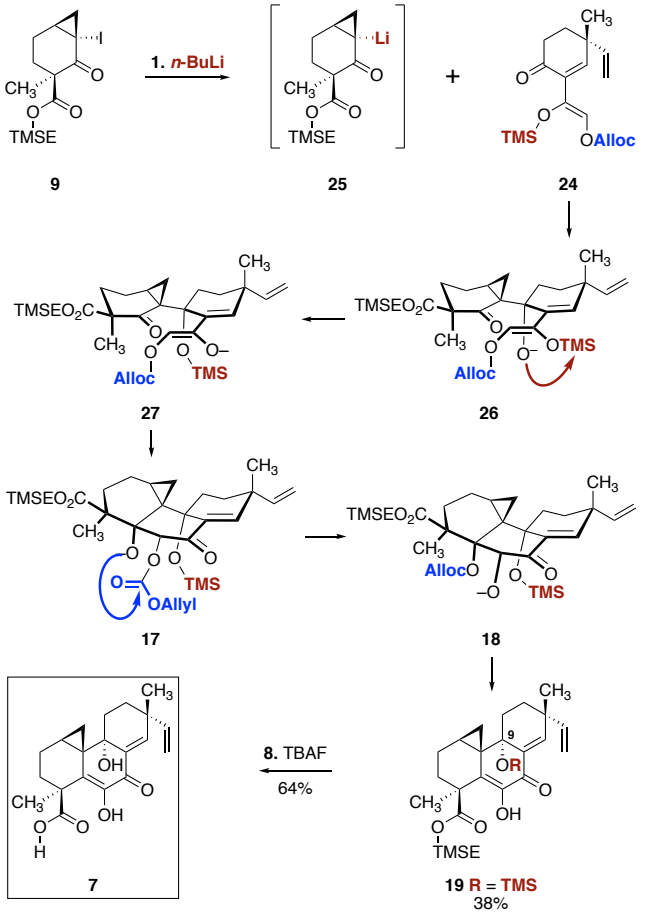
This cyclization cascade provides expedient access to a protected form of **7**. After some consideration, we recognized that the key fragment coupling–ring closure cascade could potentially be carried out in one flask by embedding a latent enolate nucleophile in the C-ring electrophile. Toward this end, we prepared the enoxysilane **24** by the sequence shown in Scheme 4. Beginning with the α-iodoenone **10**, ketalization (ethylene glycol, *p*-toluenesulfonic acid) followed by lithium–halogen exchange and addition of the Weinreb amide[17] **21** provided the α,β-unsaturated ketone **22** (70%). Removal of the silyl ether (tetra-*n*-butylammonium fluoride), installation of the allyl carbonate (allyl chloroformate, pyridine), and removal of the acetal (aqueous hydrochloric acid) generated the β-diketone **23**. Site-selective deprotonation of **12** (lithium hexamethyldisilazide) and trapping of the resulting enolate with chlorotrimethylsilane provided the target enoxysilane **24**.

Scheme 4. Synthesis of the enoxysilane **24**.

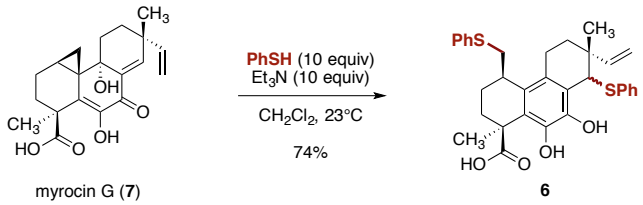


Attempts to effect the fragment coupling of the enoxysilane **24** with the organomagnesium reagent derived from **9** were unsuccessful. We found, however, that lithium–halogen exchange (*n*-butyllithium, –78 °C), followed by immediate addition of the enoxysilane **24** and warming to 0 °C provided the fully annulated product **19** in 38% yield (Scheme 5). The modest yield of this transformation is offset to some extent by the rapid increase in molecule complexity achieved. Deprotection of **18** (tetra-*n*-butylammonium fluoride) then provided the target **7** (64%). Subjecting synthetic **7** to the conditions disclosed by Danishefsky and Chu-Moyer[7] provided the bis(sulfide) **6** (74%, Scheme 6). This result provides support for the our hypothesis and indicates that the diosphenol **7** is a competent intermediate in the double nucleophilic addition of thiols. We reasoned that (+)-myrocin C (**1**) itself might be accessible by disrupting the diosphenol hydrogen bond, thereby making lactonization thermodynamically favorable,[18] although exploratory experiments toward this end were unsuccessful.

Scheme 5. One-step synthesis of **19** from **9** and **24**.



Scheme 6. Synthesis of the bis(sulfide) **6** from the diosphenol **7**.



In summary, we have developed a concise, enantioselective synthesis of "myrocin G" (**7**), the putative active form of the antiproliferative antimicrobial metabolite myrocin C (**1**). Key to the success of this approach was the development of a powerful annulation strategy that forges the central ring of the target in a single step. Future work will focus on elucidating the mode of interaction of **7** with DNA or its protein target(s) and applying this strategy to other pimarane diterpene natural products.

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References.

1. (a) Hsu, Y. H.; Hirota, A.; Shima, S.; Nakagawa, M.; Nozaki, H.; Tada, T.; Nakayama, M. *Agric. Biol. Chem.* **1987**, *51*, 3455. (b) Hsu, Y. H.; Hirota, A.; Shima, S.; Nakagawa, M.; Adachi, T.; Nozaki, H.; Nakayama, M. *J. Antibiot.* **1989**, *42*, 223. (c) Nakagawa, M.; Hsu, Y. H.; Hirota, A.; Shima, S.; Nakayama, M. *J. Antibiot.* **1989**, *42*, 218.

2. (a) Hsu, Y. H.; Nakagawa, M.; Hirota, A.; Shima, S.; Nakayama, M. *Agric. Biol. Chem.* **1988**, *52*, 1305. (b) Lehr, N.-A.; Meffert, A.; Antelo, L.; Sterner, O.; Anke, H.; Weber, R. W. S. *FEMS Microbiol. Ecol* **2006**, *55*, 105.

3. For a review of pimarane diterpenes, see: Wang, X.; Yu, H.; Zhang, Y.; Lu, X.; Wang, B.; Liu, X. *Chem. Biodiversity* **2018**, *15*, e1700276.

4. (a) Langschwager, W.; Hoffmann, H. M. R. *Liebigs Ann.* **1995**, 797. (b) Zander, N.; Langschwager, W.; Hoffmann, H. M. R. *Synth. Commun.* **1996**, *26*, 4577.

5. (a) Klemke, C.; Kehraus, S.; Wright, A. D.; König, G. M. *J. Nat. Prod.* **2004**, *67*, 1058. (b) Tsukada, M.; Fukai, M.; Miki, K.; Shiraishi, T.; Suzuki, T.; Nishio, K.; Sugita, T.; Ishino, M.; Kinoshita, K.; Takahashi, K.; Shiro, M.; Koyama, K. *J. Nat. Prod.* **2011**, *74*, 1645.

6. Chu-Moyer, M. Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 8333.

7. Chu-Moyer, M. Y.; Danishefsky, S. J. *Tetrahedron Lett.* **1993**, *34*, 3025.

8. For a review of DNa reactive natural products, see: Wolkenberg, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477.

9. Johnson, C. R.; Adams, J. P.; Braun, M. P.; Senanayake, C. B. W.; Wovkulich, P. M.; Uskokovic, M. R. *Tetrahedron Lett.* **1992**, *33*, 917.

10. (a) Ueda, Y.; Roberge, G.; Vinet, V. *Can. J. Chem.* **1984**, *62*, 2936. (b) Meng, Z.; Yu, H.; Li, L.; Tao, W.; Chen, H.; Wan, M.; Yang, P.; Edmonds, D. J.; Zhong, J.; Li, A. *Nat. Commun.* **2015**, *6*, 6096.

11. Xu, C.; Zhang, L.; Luo, S. *J. Org. Chem.* **2014**, *79*, 11517.

12. Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.

13. (a) Ren, H.; Krasovskiy, A.; Knochel, P. *Chem. Commun.* **2005**, 543. For a review, see: (b) Klatt, T.; Markiewicz, J. T.; Samann, C.; Knochel, P. *J. Org. Chem.* **2014**, *79*, 4253.

14. This addition drew inspiration from a related elegant fragment coupling employed by Baran and co-workers en route to (–)-maoecrystal V. See: Cernijenko, A.; Risgaard, R.; Baran, P. S. *J. Am. Chem. Soc.* **2016**, *138*, 9425.

15. Bürgi, H. B.; Dunitz, J. D.; Lehn, J. M.; Wipff, G. *Tetrahedron* **1974**, *30*, 1563.

16. See the Supporting Information.

17. Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.

18. Calculations [B3LYP/6-31+G(d,p)] suggest the diosphenol methyl ether of **7** is 1.7 kcal/mol higher in energy than the methyl ether of **1**.