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) and B (2), fungal isolates that contain a sensitive 5-hydroxy-γ-lactone residue (Scheme 1A, blue in 1 and 2). Literature indicates 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.

Following their landmark total synthesis of (±)-myrocin C (1), Danishefsky and Chu-Moyer disclosed that treatment of synthetic (±)-1 with excess thiophenol and triethylamine generated the bis(sulfide) 6 (63%, Scheme 1B). 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.
The isolation of 3 suggests the existence of an analogous diosphenol isomer of 1, “myrocin G (7)”. Consistent with earlier experimental studies, 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, Wittig olefination [potassium bis(trimethylsilyl)amide, methyl triphenylphosphonium bromide], tandem enoxysilane hydrolysis and carbamate elimination (aqueous hydrochloric acid), and α-dehydroiodination (iodine, pyridine) provided the C-ring fragment 10 (22% over four steps). The A-ring fragment 9 was synthesized from the ketoester 12. Stereoselective Robinson annulation between 12 and acrolein diethylacetal provided the enone 13 (32%, 92% ee). α-Dehydroiodination of 13 proceeded in 97% yield. Corey–Chaykovsky cyclopropanation (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 iodo cyclopropane 9 with iso-propylmagnesium chloride–lithium chloride complex, 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\textsuperscript{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 \(\alpha\)-hydroxyketone was confirmed by X-ray analysis.\textsuperscript{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 \(\beta\)-elimination. The silyl migration product 20 was isolated separately in 15\% yield.
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 \( \alpha,\beta \)-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.
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-Moyer⁷ 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.
Acknowledgement.

Financial support from the National Institutes of Health (Ruth L. Kirschstein National Research Service Award F31CA213964-03 to C.E.) and Yale University is gratefully acknowledged.

References.


16. See the Supporting Information.


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.