Communication

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

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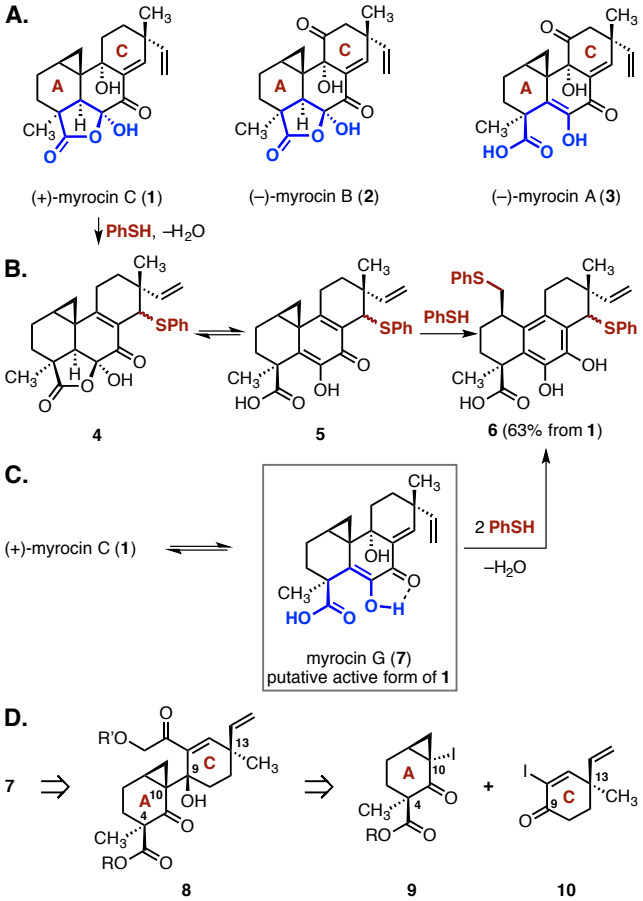
Supporting Information Placeholder

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 here the trivial name myrocin G) is the biologically-active form of the representative isolate (+)-myrocin C (**1**). To probe this, we developed a short enantioselective route to **7**. A powerful fragment coupling reaction that forms the central ring of the target in 38% yield and in a single step was developed. 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.

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 1.4 kcal/mol more stable than 1 and hypothesized to be the biologically-active form of 1. D. Retrosynthetic analysis of 7.

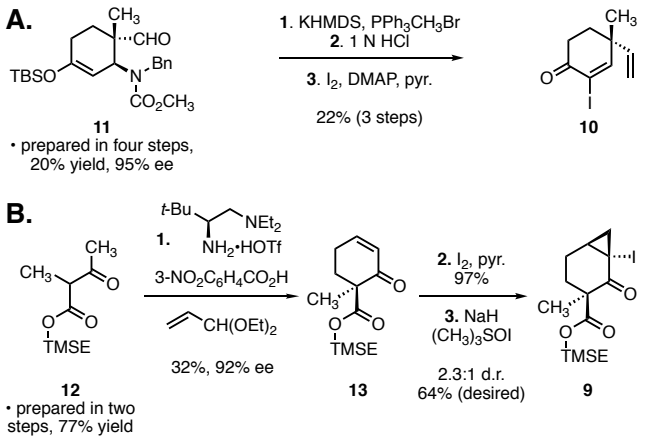


The isolation of **3** suggests the existence of an analogous diosphenol isomer of **1**, "myrocin G (**7**)" (Scheme 1C). Ring-opening of a lactone structurally related to **1** has been reported to occur under acidic or basic conditions.[4b] The diosphenol isomer may be stabilized by a strong intramolecular hydrogen bond between the hydroxyl group and the adjacent carbonyl. Collectively, 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[9] 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 β-carbamate elimination (aqueous hydrochloric acid), and α-dehydroiodination (iodine, pyridine)[10] provided the C-ring fragment **10** (22% over three steps, Scheme 2A). The A-ring fragment **9** was synthesized from the β-ketoester **12** (Scheme 2B).[11] Stereoselective Robinson annulation[12] between **12** and acrolein diethylacetal provided the enone **13** (32%, 92% ee). α-Dehydroiodination[10] of **13** proceeded in 97% yield. Corey–Chaykovsky cyclopropanation[13] (trimethylsulfoxonium iodide, sodium hydride) provided a 2.3:1 mixture

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

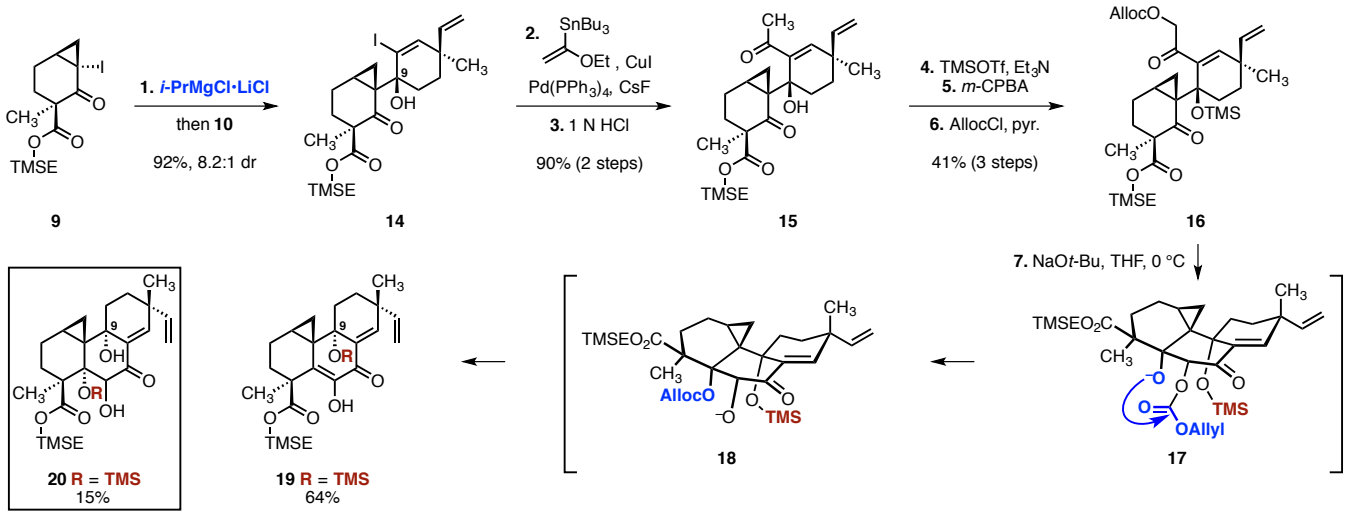


of diastereomeric α,β-cyclopropylketones. The major (desired) diastereomer **9** was isolated in 64% yield after recrystallization.

The fragment coupling product **14** was obtained by activation of the iodocyclopropane **9** with *iso*-propylmagnesium chloride–lithium chloride complex,[14] followed by addition of the iodoenone **10** (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. Notably, retro-aldol reaction of **14** is prevented 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,

Scheme 3. Synthesis of 19.



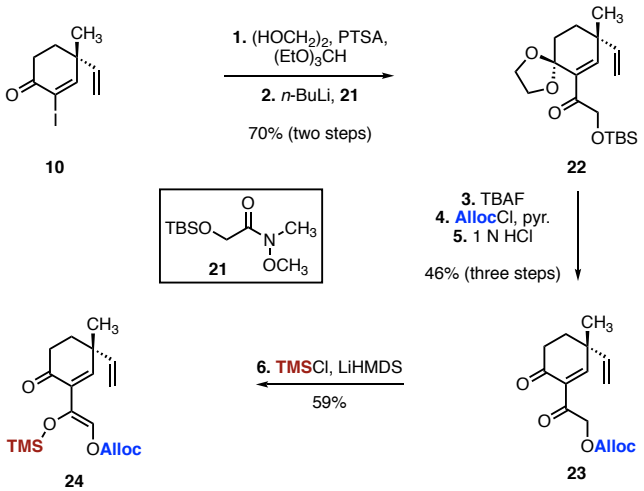
hydrolysis of the resulting vinyl ether (aqueous hydrochloric acid), tandem alcohol silylation–enoxysilane formation (trimethylsilyl trifluoromethanesulfonate, triethylamine), Rubottom oxidation (3-chloroperoxybenzoic acid), and conversion of the primary alcohol to an allyl carbonate (allyl chloroformate, 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.

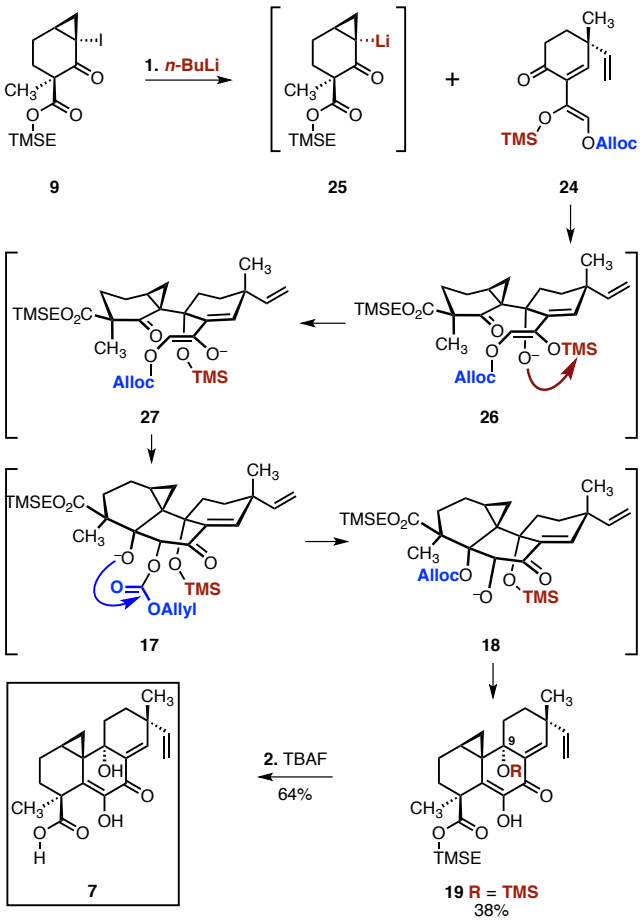
This cyclization cascade provides expedient access to a protected form of **7**. After some consideration, we recognized that the fragment coupling–ring closure cascade could potentially be carried out in one flask by embedding a latent enolate nucleophile in the C-ring electrophile. Toward this end, 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**.

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

Scheme 4. Synthesis of the enoxysilane 24.



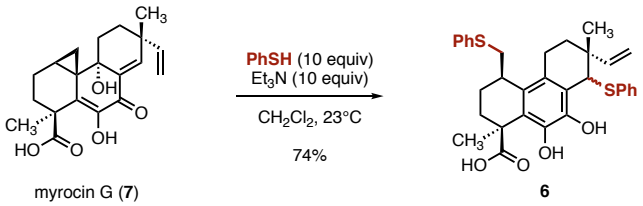
Scheme 5. One-step synthesis of 19 from 9 and 24.



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] generated 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 temporarily disrupting the diosphenol hydrogen bond in **19** or **7**, thereby making lactonization thermodynamically favorable. However, exploratory experiments toward this end have thus far been unsuccessful.

In summary, we have developed a concise, enantioselective synthesis of "myrocin G" (**7**), the putative active

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



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 from two synthetic precursors of similar complexity. With access to **7**, we are now in position to probe its antiproliferative activity and determine its biological target. The fragment coupling reaction we have developed should be amenable to the synthesis of other pimarane diterpenes.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website. The Supporting Information file contains detailed experimental procedures and characterization data for all new compounds.

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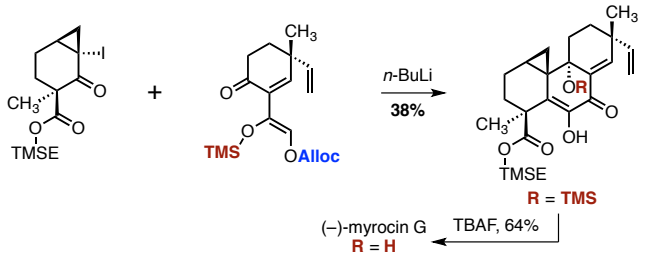
CH3
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OAlloc
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38%
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OR
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CH3
OH
O
TMSE
R = TMS
(–)-myrocin G
R = H
TBAF, 64%