Thirteen-Step Chemoenzymatic Synthesis of Gedunin

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ABSTRACT: The limonoids have attracted significant attention from the synthetic community owing to their striking structural complexity and medicinal potential. Recent efforts notwithstanding, synthetic access to many intact or ring-D seco limonoids still remains elusive. Here, we report the first de novo synthesis of gedunin, a ring-D *seco* limonoid with HSP90 inhibitory activity, that proceeds in thirteen steps. Two enabling features in our strategy are the application of modern catalytic transformations to set the key quaternary centers in the carbocyclic core and the application of site- and chemoselective enzymatic oxidation to establish the requisite oxidation pattern on the A ring. This work lays the foundation for efficient synthetic access to other limonoids and unnatural analogs to facilitate further pharmacological investigation of the family.

The limonoids are plant tetranortriterpenoid natural products with immense structural diversity that arises from skeletal oxidations and rearrangements (Figure 1A).[1] Many of the family members are known to demonstrate notable biological activities, including Tau aggregation inhibition by epoxyazadiradione,[2] TrkB agonism by deoxygedunin,[3] HSP90 inactivation[4] by gedunin (**1**) and more recently, inhibition of the E3 ligase RNF114 by nimbolide.[5] Recent efforts notwithstanding, the biosynthetic pathways towards complex limonoids are still not well-understood (Figure 1B). Initial conversion of squalene to the 30C protolimonoid melianol (**5**) had been elucidated,[6] but little is known about the downstream steps towards the more complex members of the family. This fundamental knowledge gap has hampered the development of tractable metabolic engineering strategies to synthetically produce high-value limonoids.

Conversely, several landmark total syntheses of complex limonoids have been reported,[7–16] which have also resulted in the development of elegant strategies and chemical methodologies. Nevertheless, analysis of prior chemical approaches to complex limonoids suggests that concise synthetic access has only been achieved for highly-rearranged limonoids and an efficient approach to intact or ring D-*seco* limonoids still remains an unmet challenge. For example, Corey's syntheses of azadiradione[7] (**3**) and protolimonoid[16] (**4**) required 27 and 20 steps respectively and no synthesis of **1** has been reported to date. To address this knowledge gap, we targeted the synthesis of gedunin as a prototypical ring D-*seco* limonoid. In addition to HSP90 inactivation, gedunin and its derivatives have also been reported to exhibit antimalarial and neuroprotective activities, to name a few.[17] As a synthetic target, **1** presents several unique challenges, namely the highly oxidized carbocyclic framework, the highly-congested B/C/D-ring tricycle, the presence of three quaternary centers at C8, C10 and C13 and the thermodynamically-unfavored boat-boat configuration of its C/D-bicycle. Thus, analog development has exclusively relied on semisynthetic derivatization. Here, we report a concise chemoenzymatic synthesis of gedunin that proceeds in thirteen steps with minimal functional group and protecting group manipulations. This work provides a strategic blueprint for the production of other complex limonoids that are previously inaccessible via synthetic means, as well as unnatural analogs with deep-seated modifications.

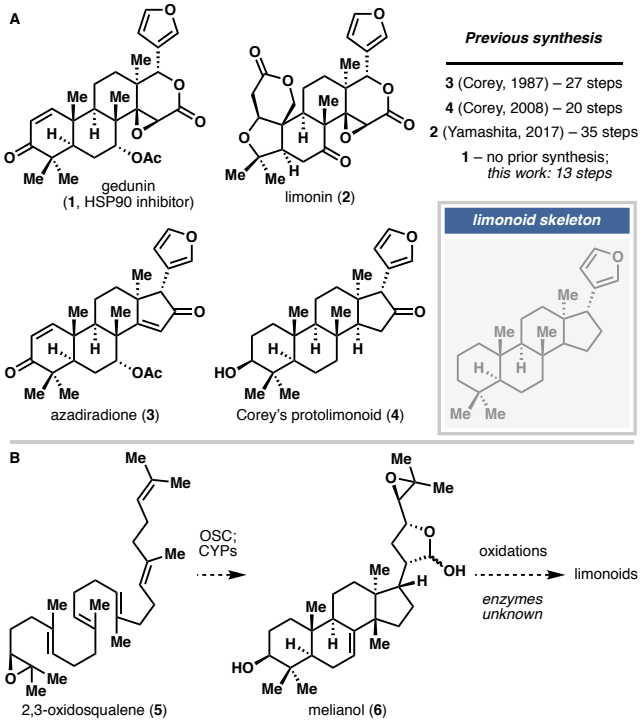


Figure 1. A. Representative limonoid triterpenes, highlighting prototypical intact limonoids (**3** and **4**) and ring D-*seco* limonoid (**1** and **2**). **B.** Current knowledge gap in limonoid biosynthesis.

Previous work from our lab has established the broad utility of biocatalytic hydroxylation in the preparation of complex meroterpenoids from sclareolide and sclareol.[18] However, a long-range search[19] for forward synthetic reactions to directly utilize intermediates from this work to access gedunin suggested that their use would lead to a completely linear approach with high step count. For this reason, an alternative approach that would satisfy two main criteria was sought. Firstly, the approach

would feature a union between two fragments of roughly equal complexity to maximize convergency and secondly, the route would be modular in nature to enable further adaptation in the assembly of other limonoids through a 'mix-and-match' approach. A strategy that would satisfy these criteria could be found in the convergent coupling of **7** and **8** in a 2-step annulation method (Figure 2). For maximum convergency, lactone **8** was also designed to contain all the requisite D-ring functionalities of **1**. With the plan of generating **7** from sclareolide in mind, only two quaternary center constructions had to be considered in our synthetic design. Here, early establishment of the quaternary center at C13 in lactone **8** was deemed strategic, as its presence would guide the diastereoselectivity of the C8–C14 bond construction event towards the desired configuration at the C/D-ring juncture. As a comparison, the use of Snider's radical cyclization on a linear polyene precursor led to sub-optimal diastereoselectivity at the C/D-ring juncture in Yamashita's synthesis of limonin (dr = 2.1:1 at C13).[15] The high-energy boat/boat C/D-ring configuration of **1** presented the next set of challenge, but we hypothesized that this issue could be addressed by carefully tuning the oxidation conditions in subsequent steps.

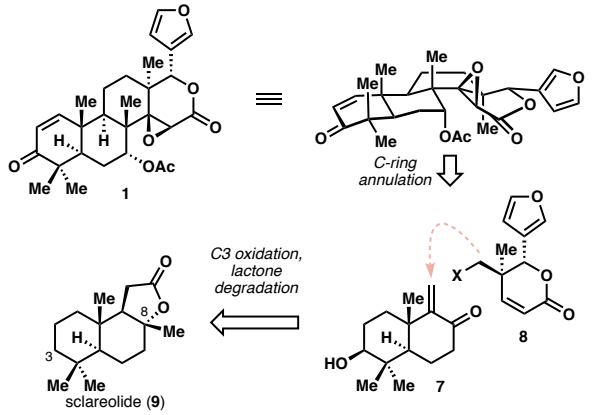
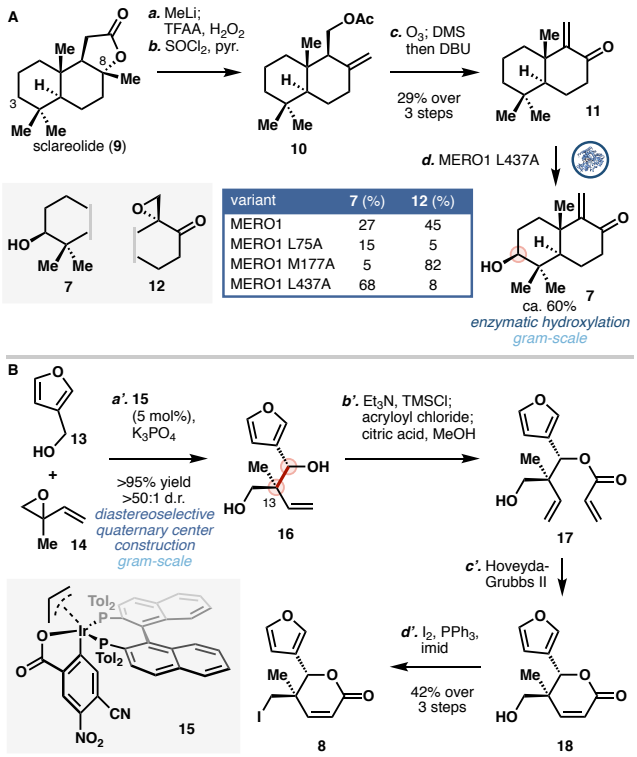


Figure 2. Retrosynthetic analysis of **1**.

Our effort commenced (Scheme 1) with the preparation of enone **7** from sclareolide (**9**). Despite prior precedents on C3 hydroxylation of **9** with $P450_{BM3}$ variants and its synthetic utility,[18,20] tactical considerations dissuaded us from introducing the C3 alcohol in the first step of the synthesis as this moiety would need to be protected before subsequent derivatizations. Instead, we elected to first manipulate the lactone motif of **9** to the corresponding B-ring enone before oxidizing at C3. This decision was not without its risks as P450s are known to catalyze the epoxidation of enones and dienones.[21,22] Though the conversion of **9** to **11** had been reported previously in the literature,[23] we developed a shorter sequence featuring: (i) methylketone generation at C12 via MeLi addition, (ii) conversion of the methylketone to a primary acetate via Baeyer–Villiger oxidation, (iii) tertiary alcohol elimination at C8 to the *exo*-methylene and (iv) ozonolytic cleavage of the resulting olefin with concomitant acetate elimination to the enone. Screening of enone **11** with several $P450_{BM3}$ variants in our library revealed that the efficiency and chemoselectivity of the enzymatic oxidation could be readily tuned through alanine scanning of the enzyme's active site. Thus, while the use of variant MERO1 M177A primarily led to enone epoxidation, variant MERO1 L437A was able to effect C–H hydroxylation at C3 with minimal formation of C9–C11 epoxide side product (See Table in Scheme 1). Importantly, introduction of the C3–OH at this stage bypassed the need for excessive protecting group manipulations in our synthesis of **1** (*vide infra*).

Scheme 1. A. Preparation of enone **7**. **B.** Preparation of iodide **8**.

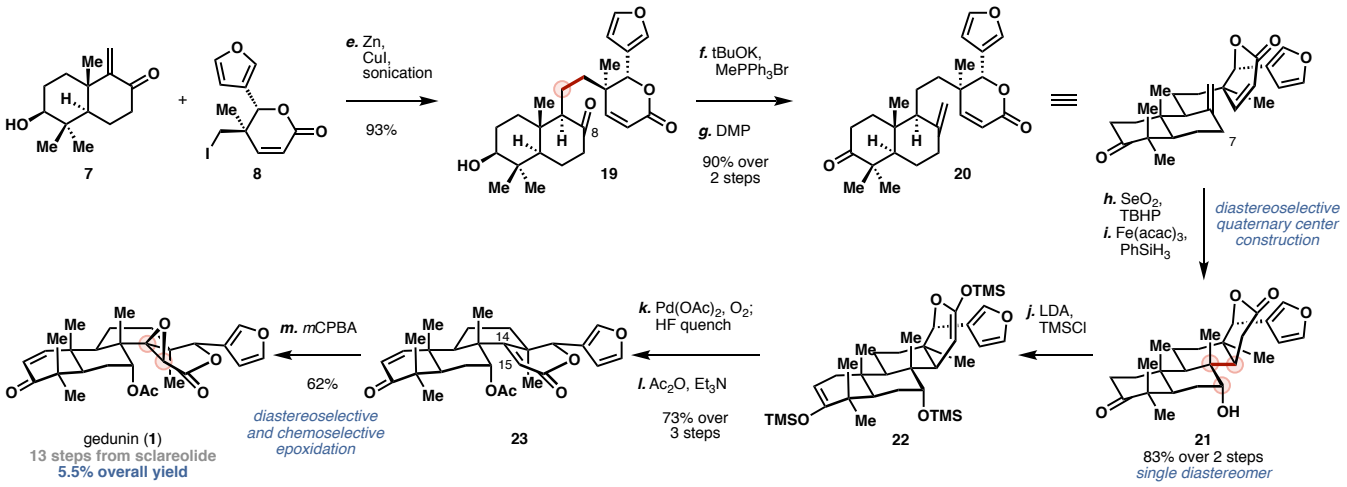


Construction of lactone **8** required the identification of a suitable method to set the C13 quaternary center in a rapid and stereoselective fashion. Lactone **8** bears a crotylation retron and prior work by Krische[24] has established the general utility and superior step-efficiency of transfer-hydrogen-based couplings in the asymmetric *tert*-(hydroxy)prenylation of alcohols. Subjection of furfuryl alcohol (**13**) and isoprene monoxide (**14**) to Krische's protocol successfully delivered the desired product in high diastereo- and enantioselectivity and excellent yield, thereby setting the key quaternary center at C13 in one step from commercial materials. The secondary alcohol on **16** was acrylated by way of transiently protecting the more reactive primary alcohol, and the resulting product was submitted to a ring closing metathesis with Hoveyda–Grubbs II catalyst to generate the desired unsaturated lactone. Conversion of the primary alcohol to the corresponding iodide delivered lactone **8** in just four steps from commercial materials and set the stage for the key union towards the ring D-*seco* limonoid framework.

Enone **7** and lactone **8** could be combined following Luche's protocol[25] to provide ketone **19** as a single diastereomer in 93% yield. Despite the presence of an electrophilic olefin on **8**, an approximately equimolar ratio of **7** and **8** could be employed in the reaction with no observable oligomerization of **8**, suggesting a remarkable selectivity of this transformation towards addition onto enones. Though a similar conjugate addition under photoredox conditions had been reported,[14] Luche's protocol was preferred due to economic reasons. Methylenation of the C8 ketone, SeO_2-mediated allylic oxidation at C7 and hydrogen-atom-transfer-based Giese coupling[26] completed the desired carbocyclic framework (compound **21**) while also setting both the key quaternary center at C8 and the highly-congested *anti*,*syn*-C/D-ring juncture with complete diastereoselectivity

and excellent yield. Initial efforts to effect a double desaturation of the A- and D-rings were met with significant challenges. With the C7-OH protected as the MOM ether, the use of PhSeCl, PhSeBr or Mukaiyama's reagent[27] led only to dehydrogenation of the A-ring with poor conversion. Success was finally realized by way of silyl enol ether formation–which also temporarily protected the secondary alcohol at C7–and Saegusa oxidation. A telescoped procedure was developed involving subsequent addition of HF at the end of the reaction to deprotect the TMS group at C7. Following acylation of the resulting free alcohol, selective epoxidation of the C14–C15 olefin was attempted. The use of DBU and TBHP on **23** led to non-selective epoxidation of both the A-ring enone and the D-ring enoate. However, treatment of **23** with *m*CPBA delivered the desired β-disposed epoxide on the D-ring with complete facial and chemoselectivity, completing our total synthesis of gedunin (**1**). Though the steric environment of the α- and the β-face of the substrate seemed similar, the epoxidation occurred with complete facial selectivity and this was attributed to the observation that the α-disposed epoxide would suffer from undesired lone pair-lone pair repulsion with the C7 OAc group.

Scheme 2. Conversion of **7** and **8** to deoxygedunin (**23**) and gedunin (**1**).



In this work, we established the first total synthesis of gedunin in 13 steps. Two key features that drive the efficiency of the route are the establishment of the two quaternary centers at C8 and C13 through the use of modern catalytic transformations (hydrogen-transfer-mediated alcohol functionalization and hydrogen-atom-transfer-based Giese addition) and the ability to biocatalytically introduce the C3 alcohol of **7** with minimal interference from enone epoxidation. These features combine to streamline protecting group manipulations and redox adjustments throughout the synthesis. All stereocenter-forming events also proceed with high levels of diastereoselectivity. This feature arises from the decision to set the C13 quaternary center early in the synthesis, which dictates the stereochemical outcome of subsequent C/D-ring assembly. Finally, the modularity of the route bodes well for future synthesis of other complex limonoids and unnatural analogs.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental details, analytical data, ^{1}H and ^{13}C NMR data (PDF).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript./ ‡These authors contributed equally.

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ABBREVIATIONS

*m*CPBA, *meta*-chloroperoxybenzoic acid; DMP, Dess-Martin periodinane; MOM, methoxymethyl; TBHP, *tert*-butyl hydroperoxide; TMSCl, trimethylsilyl chloride.

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