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Total Synthesis Establishes the Biosynthetic Pathway to the Naphterpin and Marinone Natural Products

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Abstract: *The naphterpins and marinones are naphthoquinone meroterpenoids with an unusual aromatic oxidation pattern that is biosynthesized from 1,3,6,8-tetrahydroxynaphthalene (THN). We propose that cryptic halogenation of THN derivatives by vanadium-dependent chloroperoxidase (VCPO) enzymes is key to this biosynthetic pathway, despite the absence of chlorine in these natural products. This speculation inspired a total synthesis to mimic the naphterpin/marinone biosynthetic pathway. In validation of this biogenetic hypothesis, two VCPOs were discovered that interconvert several of the proposed biosynthetic intermediates.*

The chemical reactions in a cell obey the same logic of organic chemistry as those that occur in a round-bottomed flask. As such, we can often use our knowledge of organic reactivity to predict a probable biosynthetic pathway to a natural product from its relatively limited pool of biochemical reagents. The total synthesis of a whole biosynthetic pathway offers an opportunity to interrogate a biogenetic hypothesis by providing both the substrates and the expected products for experiments with enzymes that are discovered through a genome mining approach. This collaborative approach gives insight into the chemistry of life, while also adding biosynthetic enzymes to the toolkit of synthetic chemists in the quest for the fastest and most efficient ways to construct complex organic molecules.

In the past 30 years, many naphthoquinone meroterpenoid antibiotics biosynthetically derived from 1,3,6,8-tetrahydroxynaphthalene (THN) have been isolated from marine and soil strains of *Streptomyces* bacteria, including the naphterpins,[1] marinones,[2] napyradiomycins,[3] and merochlorins.[4] 7-Demethylnaphterpin (**1**, also known as naphthgeranine A)[1b,c] is the simplest member of the naphterpin/marinone family (Figure 1). It possesses a naphthoquinone ring system oxygenated at C-2, C-6, and C-8 and is *cis*-fused to a geranyl side chain (attached by a C–C bond at C-3). Naphterpin[1a] (**2**) has an additional C-7 methyl substituent, and it is co-isolated with its oxidized analogues naphterpins B (**3**) and C (not shown).[1e]

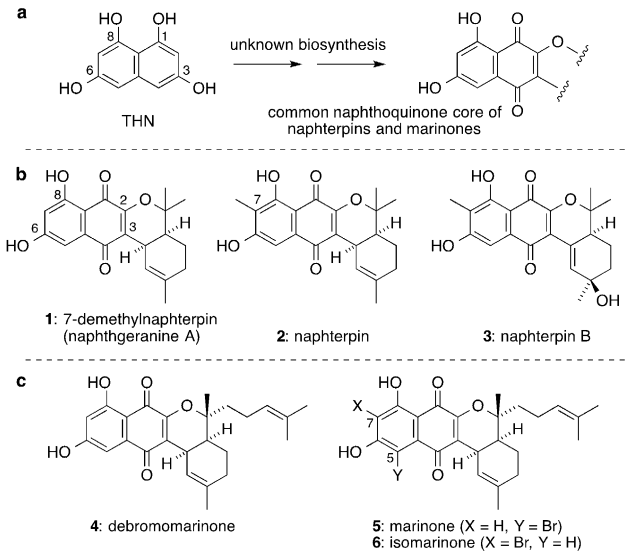

Figure 1. a) The mechanism of the biosynthesis of many naphthoquinone *Streptomyces* meroterpenoids from their biosynthetic precursor THN was previously unknown. This includes the naphterpins (b) and the marinones (c).

Marinones (**4**–**6**), isolated from *Streptomyces* sp. CNQ-509,[2] have an extra prenyl group compared to **1** that is derived from cyclization of a farnesyl side chain. Marinone (**5**) and isomarinone (**6**) have a bromine substituent at C-5 and C-7, respectively. Several structurally similar naphthoquinone meroterpenoids have been isolated from trees of the Bignoniaceae family, such as pyranokunthone A, which was synthesized by Trauner using an intramolecular hetero-Diels–Alder reaction.[5] Presumably, a similar hetero-Diels–Alder reaction is involved at a late stage of the biosynthesis of the naphterpins and marinones. However, in the case of these *Streptomyces* meroterpenoids, the mechanism of the biosynthesis of the highly oxidized naphthoquinone ring system is currently unknown. Although naphterpins and marinones have been shown to be biosynthesized from THN through ^{13}C labeling studies,[6] the oxidation pattern of THN does not obviously correlate with the oxidation pattern of these natural

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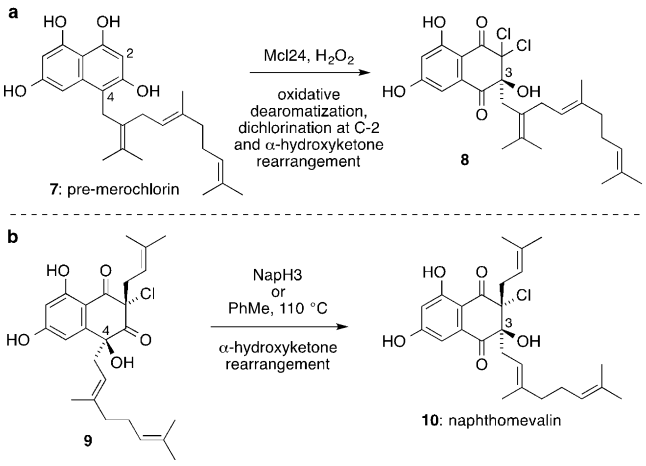
products. Furthermore, THN is nucleophilic at C-2 and C-4, but the naphterpins and marinones possess an isoprene substituent at the non-nucleophilic C-3 position.

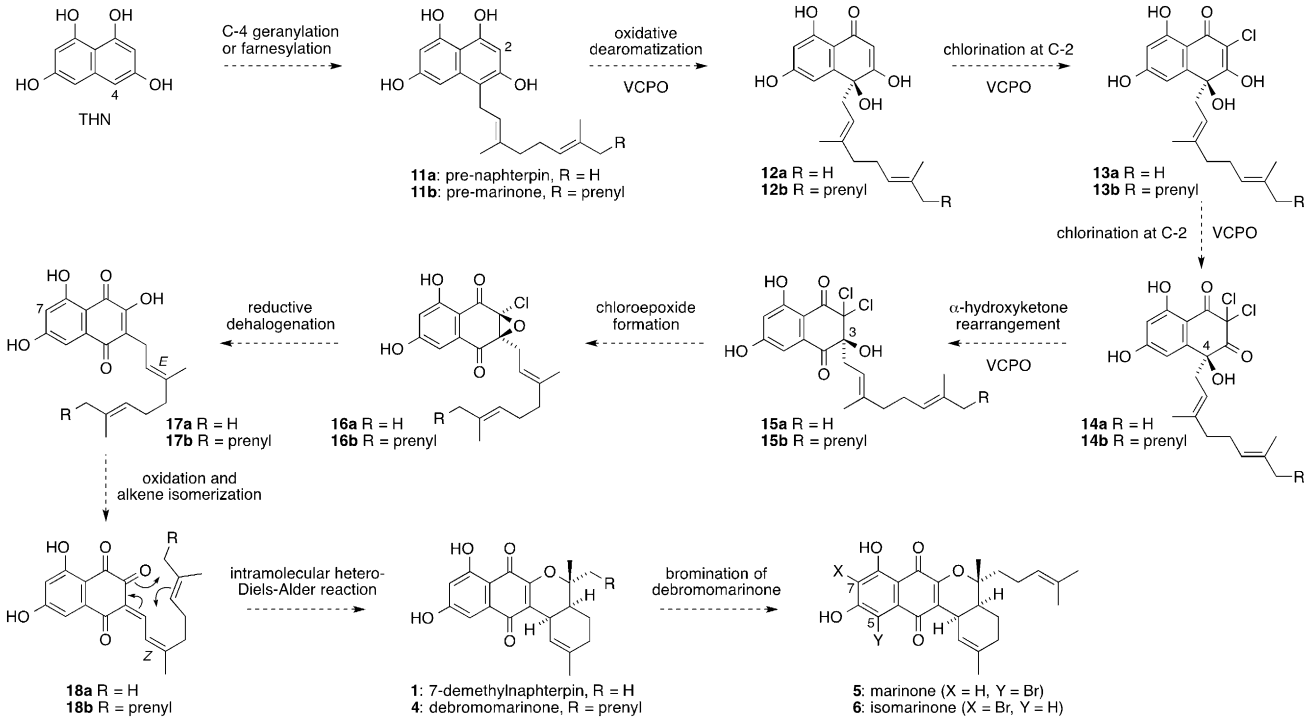
We recently reported two vanadium-dependent chloroperoxidase (VCPO)[7] enzymes, Mcl24 and NapH3,[8] that catalyze α-hydroxyketone rearrangements to shift terpene side chains[9] from C-4 to C-3 in merochlorin and napyradiomycin biosynthesis, respectively (Scheme 1). Mcl24 catalyzes



Scheme 1. VCPO enzymes known to catalyze α-hydroxyketone rearrangements a) Mcl24 in merochlorin biosynthesis. b) NapH3 in napyradiomycin biosynthesis.

oxidative dearomatization and dichlorination of the THN ring system of **7** followed by a 1,2-shift to give **8**, whereas NapH3 just catalyzes the 1,2-shift of **9** to give naphthomevalin (**10**), the simplest member of the napyradiomycin family. The discovery of these enzymes, combined with associated biomimetic synthetic work, led us to propose a biosynthesis of the napyradiomycins.[8] Herein, we extend this biosynthetic logic to include the naphterpins and marinones.

Our proposed biosynthesis of 7-demethylnaphterpin (**1**) and debromomarinone (**4**) from THN is outlined in Scheme 2. Firstly, THN is known to be biosynthesized by the condensation of five malonyl-coenzyme A units followed by aromatization of the resultant pentaketide under the control of a single type III polyketide synthase.[10] Next, we propose that THN undergoes geranylation or farnesylation at C-4 to give **11a** and **11b**. This reaction is putatively catalyzed in vivo by the NphB aromatic prenyltransferase in naphterpin biosynthesis[11] or by CnqP3 or CnqP4 in marinone biosynthesis.[12] We then propose that **11a**/**11b** are subjected to VCPO-catalyzed oxidative dearomatization to initially give **12a**/**12b**, followed by VCPO-catalyzed chlorination at C-2 to give monochlorides **13a**/**13b** and dichlorides **14a**/**14b**. A VCPO-catalyzed α-hydroxyketone rearrangement (which shifts the geranyl substituent from C-4 to C-3) would then give **15a**/**15b**. Computational studies have shown that this rearrangement is thermodynamically favorable, and the proposed multi-tasking VCPO chemistry is analogous to the known reactivity of the VCPO Mcl24 in merochlorin biosynthesis (Scheme 1a).[8] Exposure of **15a**/**15b** to mildly basic conditions should induce cyclization to give α-chloroepoxides **16a**/**16b**. A similar cyclization has been proposed to occur in the biosynthesis of A80915G from naphthomevalin.[13] A handful of marine α-chloroepoxide natural products have been previously reported,[14] but none have yet been proposed as intermediates in biosynthetic pathways. Next, we propose

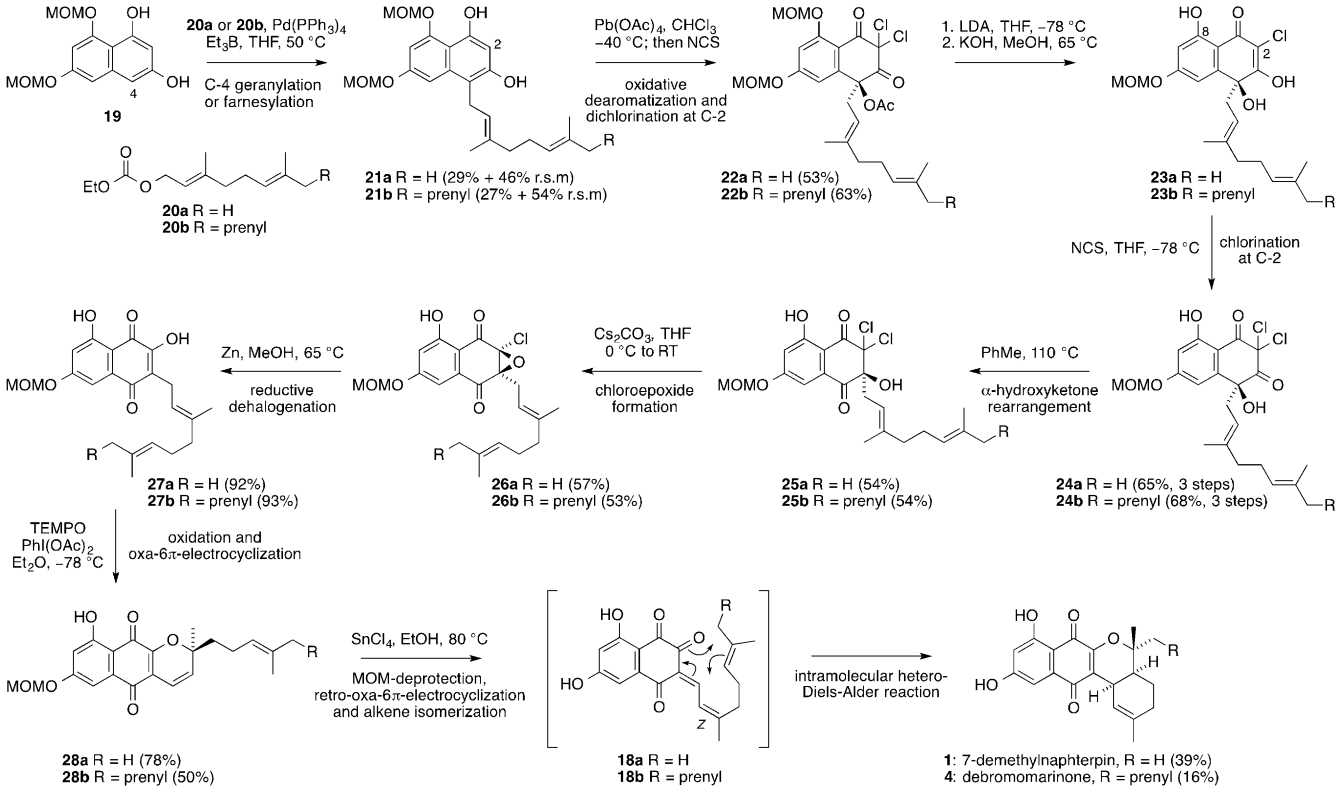


Scheme 2. Proposed biosynthesis of 7-demethylnaphterpin and debromomarinone from THN.

reductive dehalogenation of α-chloroepoxides **16a**/**16b** to give hydroxynaphthoquinones **17a**/**17b**. Related reductions of naphthoquinone epoxides to give naphthoquinones have been proposed in the biosynthesis and used in the biomimetic synthesis of zeylanone[15] and the juglocombins.[16] Importantly, the C-7 methylated analogue of **17a**, which we suggest is the direct biosynthetic precursor of naphterpin (**2**), has been isolated as a *Streptomyces* metabolite.[17]

Next, oxidation and facile *E*-to-*Z* double-bond isomerization of **17a**/**17b** would give the reactive enones **18a**/**18b**, which could be converted to 7-demethylnaphterpin (**1**) and debromomarinone (**4**) through intramolecular hetero-Diels–Alder reactions. This oxidative cyclization is similar to the biosynthesis of Δ^1-tetrahydrocannabinolic acid from cannabigerolic acid catalyzed by THCA synthase.[18] The proposed oxidation of **17a**/**17b** also has precedent in the biosynthesis of chlorizidine A in a marine strain of *Streptomyces*.[19] The naphterpins and marinones are isolated as enantiopure compounds, so the oxidative cyclization of achiral **17a**/**17b** must be under enzymatic stereocontrol. Finally, late-stage, vanadium-dependent bromoperoxidase-catalyzed bromination of **4** at C-5 or C-7 would give marinone (**5**) or isomarinone (**6**), respectively. Despite the absence of chlorine in the naphterpin and marinone natural products, we propose that this rather elaborate biosynthetic pathway involves cryptic chlorination[20] to selectively oxidize the THN ring and promote the α-hydroxyketone rearrangement. Genomic analyses of marinone-producing *Streptomyces* sp. CNQ-509 supports this biosynthetic hypothesis through the clustering of VCPO and aromatic prenyltransferase homologues with a THN synthase.[12] In the case of naphterpin biosynthesis, only three genes have yet been reported, none of which are VCPOs.[11a]

We completed a total synthesis of the whole biosynthetic pathway leading to 7-demethylnaphterpin (**1**) and debromomarinone (**4**) from a protected THN derivative using a sequence of key reactions that occur in the same order as the proposed biosynthesis. The synthesis was therefore designed to give access to as many proposed biosynthetic intermediates as possible, for use in later biosynthetic studies. Firstly, di-MOM-protected THN derivative **19** was geranylated[8] at C-4 with **20a** or farnesylated with **20b** to give **21a** and **21b**, respectively, which were then dearomatized with $Pb(OAc)_4$ and dichlorinated at C-2 with NCS in a one-pot process to give **22a**/**22b** (Scheme 3). LDA-mediated dechlorination and basic hydrolysis of **22a**/**22b** gave **23a**/**23b**, which were re-chlorinated using NCS to give **24a**/**24b** in good yield over 3 steps. Acetate hydrolysis of **22a**/**22b** to give **24a**/**24b** directly was impossible due to competing fragmentation through a haloform reaction. MOM removal at the C-8 phenol also occurred during the KOH-mediated acetate hydrolysis step. Heating **24a**/**24b** in PhMe at 110 °C induced a thermal α-hydroxyketone rearrangement to give **25a**/**25b**. The α-hydroxyketone rearrangement of **24a**/**24b** was not found to be catalyzed by protic or Lewis acids, and attempts to catalyze the reaction with base led to fragmentation. Treatment of **25a**/**25b** with Cs_2CO_3 in THF formed α-chloroepoxides **26a**/**26b**, which were reduced with Zn in MeOH to

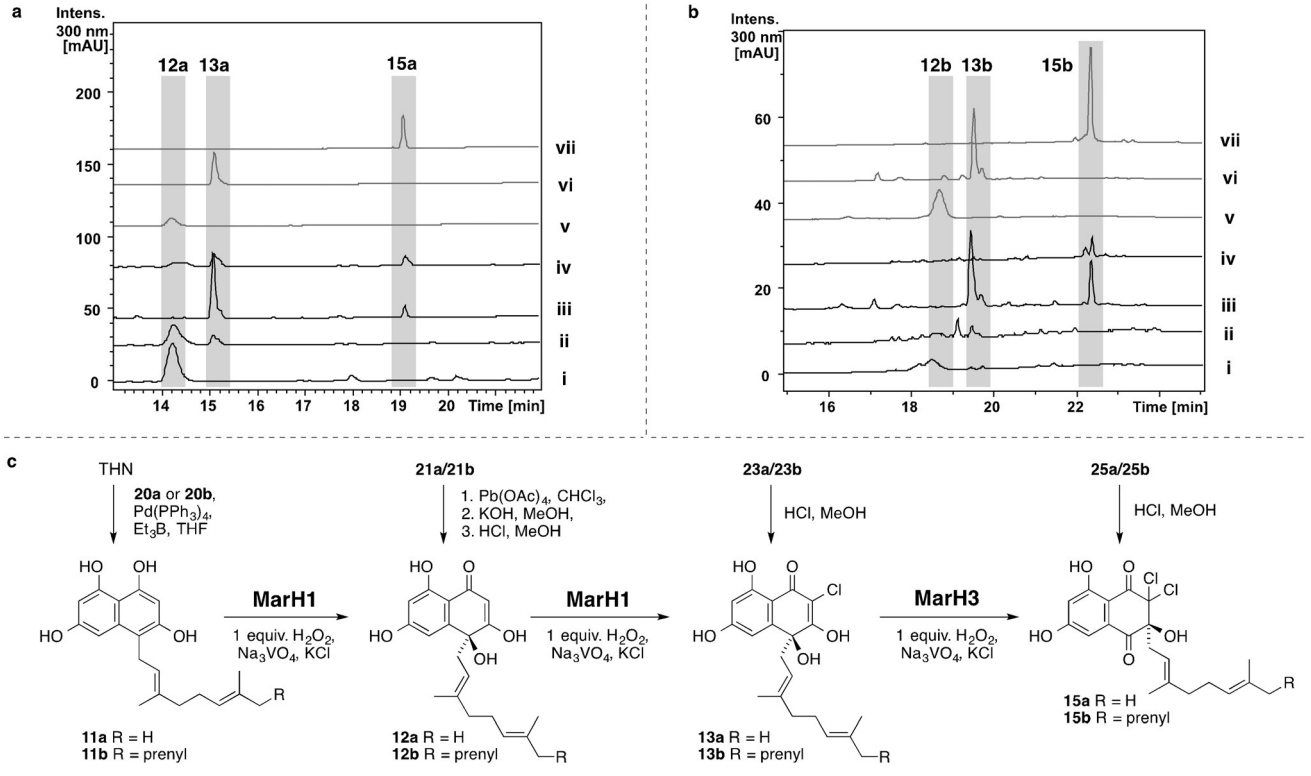


Scheme 3. Biomimetic total synthesis of the naphterpin/marinone biosynthetic pathway. MOM = methoxymethyl, THF = tetrahydrofuran, NCS = *N*-chlorosuccinimide, LDA = lithium diisopropylamide, TEMPO = (2,2,6,6-tetramethylpiperidin-1-yl)oxyl.

give **27a/27b** in high yield.[21] Oxidation of **27a/27b** with TEMPO/PhI(OAc)$_2$[22] in Et_2O then gave tricycles **28a/28b** through oxa-6π-electrocyclization. As has been observed previously, 6π-electrocyclization is usually kinetically favored over an intramolecular hetero-Diels–Alder reaction in such systems.[5] However, heating **28a/28b** with $SnCl_4$ in EtOH caused isomerization (and MOM removal) to give **1** in 39% yield (after recrystallization from EtOAc as an orange solid) or **4** in 16% yield through a cascade of retro-oxa-6π-electrocyclization, alkene isomerization, and a final intramolecular hetero-Diels–Alder reaction of the presumed intermediates **18a/18b**. The yield of **4** is lower than the yield of **1**, probably due to the instability of the exposed prenyl group of **4** in the presence of $SnCl_4$.

Our total syntheses of 7-demethylnaphterpin (**1**) and debromomarinone (**4**) are the first for members of the naphterpin/marinone family, but more efficient strategies for constructing these natural products can be envisaged. Indeed, a previous non-biomimetic synthesis of a protected form of **1** is shorter.[23] However, the value of our synthesis lies not in the final destination, but in the journey, since each step of the synthesis represents a potential biosynthetic intermediate (after facile MOM removal). The dearomatized biosynthetic intermediates **12a/b**, **13a/b** and **15a/b** were prepared through this route (Scheme 4c) for use as standards in biosynthetic studies. To interrogate the individual biosynthetic steps, three VCPO homologues: *marH1*, *marH2*, and *marH3*, were individually cloned from the putative *Streptomyces* sp. CNQ-509 marinone biosynthetic gene cluster and heterologously expressed in *Escherichia coli*. The recombinant proteins were purified using Ni^{2+} affinity chromatography and initially interrogated using the monochlorodimedone (MCD) assay (see Figures S4–S6 in the Supporting Information). Two VCPO homologues (MarH1, MarH3) showed halogenation activity and were further examined using synthetic substrates.

Thorough in vitro characterization of the reactions between pre-naphterpin (**11a**) and pre-marinone (**11b**)[24] and the recombinant MarH enzymes was performed using reversed-phase HPLC to monitor the formation of biosynthetic intermediates (Schemes 4a,b and Figures S7–S26).[25] In the presence of sodium vanadate and two stoichiometric equivalents of hydrogen peroxide, MarH1 catalyzed the oxidative dearomatization of **11a/11b** at C-4 and subsequent monochlorination at C-2, yielding **12a/12b** and **13a/13b**, respectively. Additional chlorination of **13a/13b** by MarH1 was not observed following incubation with excess hydrogen peroxide, so this substrate was then interrogated with MarH3. In vitro incubation of **13a/13b** with MarH3 subsequently catalyzed additional chlorination at C-2 and an α-hydroxyketone rearrangement, moving the terpene sidechain from C-4 to C-3 to produce **15a/15b**. Furthermore, a one-pot reaction of **11a/11b** with MarH1, MarH3, and sodium vanadate yielded the dichlorinated 1,2-shifted **15a/15b** as



Scheme 4. Reversed-phase HPLC chromatograms (300 nm) of MarH enzyme assays (i–iv) and comparison to synthetic standards (v–vii) with naphterpin (a) and marinone (b) substrates. i) **11a/11b**, no enzyme, 2 equiv. H_2O_2; ii) **11a/11b**, MarH1, 2 equiv. H_2O_2; iii) **13a/13b**, MarH3, 1 equiv. H_2O_2; iv) **11a/11b**, MarH1, and MarH3, 3 equiv. H_2O_2; v) **12a/12b**; vi) **13a/13b**; vii) **15a/15b**. c) Reaction conditions for the chemical syntheses (vertical arrows) and MarH enzymatic conversions (horizontal arrows) of biosynthetic intermediates **11a/11b**, **12a/12b**, **13a/13b**, and **15a/15b**. Additional control experiments can be found in Figures S7–S26.

the major product; progression along the biosynthetic pathway was directly correlated to the number of molar equivalents of hydrogen peroxide added (Figures S15, S24). MarH2 failed to show any catalytic halogenating or α-hydroxyketone rearrangement activities with meroterpenoid substrates, either individually or in combination with other MarH enzymes. We rationalize its inactivity despite high sequence similarity with other VCPO enzymes as being due to substitution of the key vanadate-coordinating histidine residue with an asparagine[26] (Figure S2).

In conclusion, we discovered that *Streptomyces* bacteria use VCPO enzymes to oxidize THN derivatives via cryptic chlorination in the biosynthesis of the naphterpin and marinone families of meroterpenoid natural products. The biosynthetic sequence is initiated by VCPO-mediated oxidative dearomatization and dichlorination, followed by an α-hydroxyketone rearrangement. Subsequent loss of chloride anion leaving groups allows the formation of the highly oxidized naphthoquinone core. We mimicked the entire biosynthetic pathway in biomimetic syntheses of 7-demethylnaphterpin and debromomarinone that use simple reaction conditions to exploit the predisposed reactivity of the intermediates in a logical manner. Several proposed biosynthetic intermediates were also synthesized and used as substrates and standards to help elucidate the function of two VCPO enzymes, MarH1 and MarH3, which initiate marinone biosynthesis. Further work to discover and characterize enzymes that control the final steps of naphterpin/marinone biosynthesis is underway.

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Conflict of interest

The authors declare no conflict of interest.

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