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Asymmetric Alkene and Arene Halofunctionalization Reactions in Meroterpenoid Biosynthesis

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Abstract

Meroterpenoid natural products are important bioactive molecules with broad distribution throughout nature. In *Streptomyces* bacteria, naphthoquinone-based meroterpenoids comprise a simple yet structurally fascinating group of natural product antibiotics that are enzymatically constructed through a series of asymmetric alkene and arene halofunctionalization reactions. This account article highlights our discovery and characterization of a group of vanadium-dependent chloroperoxidase enzymes that catalyze halogen-assisted cyclization and rearrangement reactions and have inspired biomimetic syntheses of numerous meroterpenoid natural products.

Graphical Abstract

Keywords

biomimetic; haloperoxidase; α -hydroxyketone rearrangement; meroterpenoid; natural product biosynthesis

1 Introduction

The architectural beauty of nature's chemistry continues to motivate scientists to explore the logic of how exactly complex natural product molecules are constructed.¹ Such biosynthetic studies have unraveled many of the remarkable mysteries behind how natural compounds are made² and led to numerous discoveries and applications in chemistry and biology.³ The discovery of biosynthetic enzymes and pathways has inspired the development of new chemical reactions and reagents that have expanded the tools available for chemists and engineers to bring nature's chemistry to life in a flask.

A group of molecules that has intrigued me for over a decade are simple polyketide naphthoquinone–terpenoid hybrid compounds, or meroterpenoids, produced by a variety of *Streptomyces* bacteria.⁴ These gene-encoded small molecules, such as the antimicrobial

agents napyradiomycin B1 **1**,⁵ marinone **2**,⁶ and merochlorin A **3**,⁷ often harbor fascinating structural features suggestive of halofunctionalization reactions involving alkene and arene substrates (Figure 1). I've always marveled at the different strategies that nature invented to install halogens into natural products⁸ and wondered if these meroterpenoid molecules could teach us something new. Little did I know that after 10 years of working on the biosynthesis of these molecules that my laboratory would still be unraveling their secrets that have shown us that halogenating enzymes don't simply just perform straightforward substitution reactions but also initiate remarkable bond-forming construction reactions. In this account article, I highlight several intertwining biosynthetic stories that have helped identify vanadium-dependent haloperoxidases (VHPOs) as a new group of bacterial halogenating enzymes⁹ and guided the logic of natural product total synthesis through biomimetic approaches.

2 Early Biosynthetic Insights and the Characterization of Alkene Halofunctionalization in Napyradiomycin Biosynthesis

Amongst the first naphthoquinone-based meroterpenoid natural product groups that my laboratory interrogated were the napyradiomycins. These streptomycete compounds were first characterized in the mid 1980s,⁵ and new members continue to be reported from terrestrial soil and marine sediment strains because of their distinct chemical structures and biological properties in antimicrobial and anticancer assays.¹⁰ What caught our attention with this compound series were the cyclized terpene groups that were often halogenated with chlorine atoms. The placement of the halogen adjacent to the *gem*-dimethyl group of the precursor isoprene unit was suggestive of an oxidative halogenation reaction of the isoprene olefin resulting in an intramolecular ring cyclization with a nearby electron-rich oxygen or alkene. At that time in 2007, while there was no biosynthetic precedence in a bacterial system, an example enzymatic reaction had been established by the Butler group for the formation of the brominated snyderol terpenes in red algae by vanadium-dependent bromoperoxidases.¹¹

To explore the route of napyradiomycin biosynthesis, we set out to identify the corresponding biosynthetic gene cluster (BGC) in the marine isolate *Streptomyces* sp. CNQ525 that my Scripps colleagues Bill Fenical and Paul Jensen had discovered.^{10c} Earlier isotope labeling experiments by Shiomi et al. established the biosynthetic building blocks of the napyradiomycins being derived from the symmetrical polyketide 1,3,6,8-tetrahydroxynaphthalene (THN, **4**) and two isoprene chains assembled by the mevalonate pathway (Scheme 1).¹² We thus PCR-amplified THN synthase (THNS, a type III polyketide synthase) and prenyltransferase (PTase) gene fragments and identified a cosmid clone that fortuitously contained the majority of the 43-kb napyradiomycin BGC (*nap*).¹³ At that time in 2007, only two other THN meroterpenoid BGCs had been reported a year earlier by the Dai and Heide groups for the structurally related furaquinocin A **5**¹⁴ and furanonaphthoquinone I **6**¹⁵, respectively. While all three THN meroterpenoid BGCs similarly contained genes encoding THNS, PTase, and a full complement of enzymes for mevalonate isoprene diphosphate biosynthesis, the *nap* BGC uniquely encoded three VHPOs. The co-clustering of VHPO genes within a natural product BGC was a novel

observation at the time and would become the key to understanding the myriad of alkene and arene halofunctionalization reactions in napyradiomycin as well as many other meroterpenoid natural products.

We validated the *nap* locus by heterologous production of the napyradiomycins in the host *Streptomyces albus*, which allowed us to begin investigating the biosynthetic mechanism of halogenation.¹³ The first *nap*-encoded VHPO we characterized was NapH1 in 2011.¹⁶ We confirmed that NapH1 behaved like a eukaryotic VHPO in its requirement of vanadate and H₂O₂ for catalysis, and demonstrated its unique specificity and catalytic efficiency in the asymmetric halogen-assisted cyclization of SF2415B1 **7** to the napyradiomycin analogue SF2415B3 **8** (Scheme 1). This selective and efficient alkene halofunctionalization enzymatic reaction preferred chloride as the halogenating reagent, as bromide additionally yielded nonspecific byproducts and, moreover, deactivated the enzyme over time. Our discovery and characterization of the NapH1 vanadium-dependent chloroperoxidase (VCPO) as the first highly substrate-selective VHPO in natural product asymmetric biosynthesis prompted us to explore its substrate binding and catalytic mechanism. Through strategic mutagenesis experiments of the NapH1 protein, we showed that substrate binding and haloperoxidase activities could be decoupled, thereby establishing that NapH1 must selectively bind its substrate to ensure the strategic delivery of the oxidized chlorine reagent (proposed either as HOX or an *N*-halo lysine residue) to the substrate olefin (Scheme 2). However, many unsolved questions still remain about the exact delivery mechanism that we hoped would be solved with a high-resolution structure of the NapH1 protein. Unfortunately, although we solved the three-dimensional structures of the homodimeric NapH1 with and without vanadate several years ago (PDB codes 3W36 and 3W35), we have yet to visualize how this enzyme positions its substrate prior to the delivery of the halogen.

3 Discovery of the Merochlorin Natural Products and Enzymatic Aryl Halofunctionalization

The discovery of the napyradiomycin VCPO NapH1 motivated us to explore other biosynthetic systems for new examples of VHPOs in biosynthesis. Such an opportunity presented itself the following year in 2012. My Scripps colleague Bill Fenical and his team were in the process of isolating a series of very unusual meroterpenoid antibiotics called the merochlorins from the marine sediment strain *Streptomyces* sp. CNH-189.^{7,17} Over the last dozen years since my move to the Scripps Institution of Oceanography at UC San Diego, the close physical proximity of our laboratories has allowed our research groups to share their emerging results and pursue joint projects merging our small-molecule discovery and biosynthesis programs.¹⁸ So with the discovery of merochlorin A **3**, B **9**, C **10**, and D **11** as highly rearranged, chlorinated members of the naphthoquinone meroterpenoid family, we had the chance to discover and characterize new halogen-assisted cyclization reactions.

We quickly identified the merochlorin BGC (*mcl*) by genome scanning, which revealed that *Streptomyces* sp. CNH189 harbored a THN meroterpenoid gene locus with a single PTase and two VHPO-encoding genes.⁷ Through mutagenesis and heterologous expression, we confirmed that the *mcl* BGC controlled the biosynthesis of all merochlorin molecules,

thereby establishing that despite their different carbon skeletons they all shared the same biogenesis. The merochlorins can first be divided into two structure groups in which the unusually branched C₁₅ isoprene moiety is attached at either C-4 (**3** and **9**) or at C-3 (**10** and **11**) of the THN precursor (Figure 2). To account for the different terpene attachment sites in the two products, we proposed at the time, albeit incorrectly, that the lone PTase Mcl23 must have relaxed substrate specificity and deliver the terpene substrate to adjacent carbons of an oxidized THN molecule. While we later experimentally corrected this original theoretical proposal, we did show at the time of our original publication⁷ that the VHPO encoding gene *mcl40* participates in the macrocyclization of **11** to **10**. The asymmetric construction of the massive 15-membered cyclic ether ring in **10** represents, to the best of our knowledge, the largest natural olefin halofunctionalization reaction to result in a cyclized product, a remarkable feat in substrate control and stereoselectivity.

While we have not yet been able to functionally express the Mcl40 VCPO enzyme, as we did earlier with the napyradiomycin NapH1 VCPO,¹⁶ we did successfully characterize the second merochlorin biosynthesis VHPO encoded by *mcl24*, which resulted in an unanticipated observation.¹⁹ We originally hypothesized that Mcl24 would be a VCPO that would oxidatively chlorinate an alkene group of the precursor “pre-merochlorin” **12** intermediate to facilitate a series of cyclization reactions to give the multicyclic merochlorin A and B products.⁷ While somewhat true, what transpired turned out much more interesting.¹⁹

To interrogate the function of the Mcl24 VCPO, we fully recapitulated the biosynthesis of the merochlorins using recombinant enzymes in a one-pot enzymatic synthesis using just four enzymes (Scheme 3).^{19a} We showed that premerochlorin **12** is biosynthesized by the coupling of the substrates THN **4** and the unprecedented sesquiterpene isosesquilavandulyl diphosphate **13** by the PTase Mcl23. Needing both **12** and **13** for our biochemical studies, we teamed up with Phil Baran and his student Matthew Villaume from the nearby Scripps Research Institute. Having access to those novel synthetic standards allowed us not only to confirm their biosynthetic intermediacy, but also provided valuable material to interrogate the enzymatic reactivity of these labile molecules.

Remarkably, incubation of **12** with the VCPO Mcl24 directly gave **3** and **9** through a series of unprecedented biosynthetic transformations involving a site-specific naphthol chlorination followed by an oxidative dearomatization/terpene cyclization (Scheme 3).¹⁹ This sequence of reactions resulted in the construction of the stereochemically complex carbon framework of the merochlorin molecules in a single step. At the outset, we had not anticipated that the Mcl24 VCPO would be able to wholly catalyze this transformation as there was no literature precedent for secondary metabolic oxidative cyclization reactions involving an aromatic core. Yet despite identifying numerous oxidative enzyme-encoding genes in the merochlorin BGC, including an iron–sulfur cluster-containing protein, we clearly demonstrated that the Mcl24 VCPO solely performs both the oxidative cyclization and halogenation reactions.

We were curious if we could further develop a parallel chemical protocol to induce the oxidative cyclization of **12** through a chlorination event.^{19b} We interrogated a number of standard chlorination protocols that mostly failed until we added an amine to the reaction

mixture. Using *N*-chlorosuccinimide in the presence of either two equivalents of iPr_2NH or NaH gave 30 or 22% yield, respectively, of the merochlorin product mixture (Scheme 4). Interestingly, although **3** and **9** were synthetically generated, they were in the minority. Rather, the primary reaction products were four new merochlorin molecules – deschloro-merochlorin B **14**, isochloro-merochlorin B **15**, deschloro-merochlorin A **16**, and minor amounts of dichloro-merochlorin B **17**. All these synthetic merochlorin molecules were also produced enzymatically from Mcl24 and naturally by *Streptomyces* sp. CNH-189, albeit in those cases, they were minor products and were thus not originally characterized due to sample limitations. The inverted reaction profiles suggested that while the enzyme appears to preferentially chlorinate at C-2 in pre-merochlorin prior to cyclization, the synthetic treatment of pre-merochlorin primarily leads to oxidative cyclization followed by C-7 chlorination. In fact, we could exclusively produce the deschloro-merochlorin A **16** and B **14** derivatives by conducting the chemical chlorination reaction at low temperature ($-78\text{ }^{\circ}C$). Although we have yet to solve the molecular basis for the reactivity profile of the Mcl24 enzyme, we recently solved the high-resolution crystal structure of Mcl24 that we hope will shed light on its selective reactivity (V. Agarwal, S. Diethelm, B. S. Moore, unpublished).

The mechanistic rationale for the formation of merochlorin A, B and their derivatives is suggestive of the in situ generation of a chloramine intermediate as the active oxidant, both chemically with iPr_2NH and biochemically with a nearby lysine residue.^{19b} In the case of Mcl24, the product profiles suggest two halogenation events resulting in C-2 chlorination and an aromatic hypochlorite that upon loss of chloride would yield a benzylic carbocation intermediate **18** (Scheme 3). Finally, terpene cyclization of the carbocation via a common intermediate would give the merochlorin A and B scaffolds. Elegant total syntheses of **3** and **9** by the George²⁰ and Trauner²¹ laboratories, respectively, similarly took advantage of biomimetic approaches and the inherent reactivity of this molecular class.

4 Discovery and Development of Unifying THN-Based Meroterpenoid Biosynthesis and Synthesis Approaches

One of the more perplexing and unsolved questions with many of the THN-based meroterpenoids was the common placement of the isoprene unit on electron-poor C-3 carbon centers of the THN precursor. For instance in the merochlorin series, while **3** and **9** have their isoprenes attached to the electron-rich C-4 of THN, in **10** the same isoprene group is rather attached at C-3 (Figure 2).⁷ This same C-3 attachment is observed in most other meroterpenoid natural products, including the napyradiomycins,⁵ marinone,⁶ and naphterpin.²² We recently organized the THN meroterpenoids into two structural classes depending on the attachment site of the isoprene chain to the THN-derived naphthoquinone core with class I THN meroterpenoids having electron-rich C-2/C-4 isoprene attachments and class II's with electron-poor C-3 attachments.²³

A major breakthrough in solving the riddle of how electrophilic isoprene diphosphates are appended to the electron-deficient C-3 position of THN came with a key observation of an additional minor product of the VCPO Mcl24 enzymatic reaction. We observed that under basic reaction conditions a new Mcl24 product increased in amount, and that upon its

isolation and characterization, we identified its structure as a novel C-3 prenylated derivative **19** that exhibited dichlorination at C-2.²³ This unexpected finding suggested that the isoprene chain attached at C-4 in **12** migrates to the C-3 position (Scheme 2). This mechanism would necessitate the trapping of the previously proposed benzylic carbocation intermediate **18** with water followed by a second chlorination reaction at C-2 to give the geminal dichloride intermediate **19**. To provide evidence for this mechanism, we performed the reaction in ¹⁸OH₂ and showed that the new oxygen atom in the product was indeed water-derived. Finally, the C-4 prenylated intermediate is perfectly set up to undergo an α-hydroxyketone rearrangement to produce the C-3 substituted product.

The consequence that Mcl24 can additionally catalyze a halogenation-mediated α-hydroxyketone rearrangement, the first of its kind in nature, was that this remarkable enzyme is singularly responsible for generating the majority of the structural complexity and diversity in the entire merochlorin structural family (Scheme 3). Mcl24's ability to bombard the prenylated THN nucleus with successive halogen atoms ultimately is what is responsible for exposing pre-mero-chlorin to the observed series of carbocationic cascade reactions. This biochemical discovery opened our eyes to halogenating enzymes performing not just simple substitution reactions but also supporting diverse bond construction reactions, much like how a chemist would use halogens to direct chemical reactivity. This discovery also suggested that this α-hydroxyketone rearrangement might be universally responsible for the C-3 attachment of isoprenes in all class II THN meroterpenoids.

Around this time we had another pleasant surprise. Synthetic chemist Jonathan George from the University of Adelaide, whose lab had earlier completed the biomimetic synthesis of merochlorin A,²⁰ contacted me about a proposal to jointly explore napyradiomycin biosynthesis. His group had at the time recently established a new biomimetic synthesis of naphthomevalin **20** and predicted that their synthetic intermediates could be useful as chemical probes in our biosynthetic system.²³ Remarkably, they invoked an α-hydroxyketone rearrangement reaction as a key step in their synthesis and wondered if such a reaction could be catalyzed by an enzyme. What a coincidence of timing! Our groups enthusiastically shared ideas and materials to explore whether the geranyl group in napyradiomycin does indeed result from an α-hydroxyketone rearrangement from C-4 to C-3 and whether one of the remaining and uncharacterized napyradiomycin VHPOs catalyzes the reaction like we had just observed with Mcl24.

Thus armed with the knowledge of George's naphthomevalin biomimetic synthesis and access to their unprotected synthetic intermediates, we set out to interrogate whether naphthomevalin is indeed biosynthesized through an α-hydroxyketone rearrangement reaction.²³ The napyradiomycin BGC encodes two PTases, presumably for the selection and attachment of the two isoprene units. We thus first explored whether the C-4 geranylated substrate **21**, a deprotected synthetic intermediate of the naphthomevalin synthetic pathway, was a substrate for one of the uncharacterized *nap* PTases. We showed that **21** was a substrate for the NapT8 PTase, which catalyzed the stereoselective addition of dimethylallyl pyrophosphate to the C-2 position of **21** to produce **22** (Scheme 5). We next evaluated whether one of the orphan *nap* VHPO homologues could catalyze an analogous α-hydroxyketone rearrangement of **22** to give the naphthomevalin **20** natural product. Such a

reaction, however, would not require halogenation like in the merochlorin example as the naphthomevalin precursor **22** is already halogenated. We thus generated recombinant NapH3, a VCPO homologue with about 57% identity with the previously characterized NapH1, and showed that it efficiently supported the C-4 to C-3 α -hydroxyketone rearrangement to **20**. Moreover, this enzyme did not require H₂O₂ or Na₃VO₄ for activity, nor was it active in standard haloperoxidase assays.

These observations suggest that the merochlorin and napyradiomycin biosynthetic pathways share a central VCPO-assisted α -hydroxyketone rearrangement reaction but differ in how this reaction is catalyzed. In merochlorin, just a single VHPO (Mcl24) is needed to catalyze both the halogen-mediated oxidative dearomatization reaction and the α -hydroxyketone rearrangement reaction, while in napyradiomycin biosynthesis, this two-step, single enzyme process is more complex.²³ While we have yet to report the halogenating enzyme responsible for the construction of the chlorinated intermediate **21**, we have been able to show that the PTase NapT8 and the VHPO-homologue NapH3 are together required for its rearrangement. This observation was consistent with theoretical studies that showed that geminal disubstitution of C-2 is required for the α -hydroxyketone rearrangement to be thermodynamically favored. In contrast, the monochlorinated **21** was unreactive toward the 1,2-shift, as it possesses a conjugated π system, and hence the requirement of the PTase NapT8 to break the sp² character of C-2 with the addition of the dimethyl allyl group and thus set up the 1,2-suprafacial shift mechanism for the α -hydroxyketone rearrangement.

A number of biosynthetic questions still remain about napyradiomycin, however, that involve additional halofunctionalization reactions. First, what halogenating enzyme is responsible for generating **21**, and second, what enzyme catalyzes the asymmetric cyclization of the cyclohexyl ring in napyradiomycin B1 **1** from the linear geranyl diene precursor, napyradiomycin A1 **23**? Both of these reactions have hallmarks of VHPO biochemistry, and with one additional *nap* VHPO still uncharacterized, at least one of these reactions is bound to be catalyzed by NapH4. We are actively pursuing these remaining questions and hope to soon report the total enzymatic synthesis of napyradiomycin B1.

5 Insights into Naphterpin and Marinone Biosynthesis Involving Cryptic Aryl Halofunctionalization Reactions

The merochlorin and napyradiomycin α -hydroxyketone rearrangement reactions also suggested that a similar 1,2-suprafacial shift mechanism may take place in the naphterpin **24** and marinone **2** natural products. These molecules have long intrigued me for their asymmetry about the THN-derived naphthoquinone core.²⁴ Years ago when I first encountered these compounds, I initially questioned whether their structures had been misassigned, as I assumed based on straightforward biosynthetic logic that they were derived directly from flaviolin or another similarly oxidized THN molecule. If so, this would simply necessitate the swapping of the C-2 and C-3 substituents and thereby place the ether oxygen atom at C-3 (instead of at C-2) where it is in all other THN-derived meroterpenoids. NMR spectroscopy would have a difficult time differentiating between those two structure types. The structure and absolute configuration of naphterpin **24**, the maiden structure in this

natural product series, was rigorously established by X-ray crystallography and is correct as originally published.²² Thus another more exotic biosynthetic explanation is needed.

Early isotope labeling experiments established the symmetrical nature of the biosynthetic intermediate leading to the common naphthoquinone core in naphterpin²⁴ and marinone²⁵ and suggested the intermediacy of THN. Unfortunately, the BGCs for both molecules have not yet been reported, although three genes from the naphterpin BGC were described in 2005 by Kuzuyama and colleagues, including a THN synthase and a PTase.²⁶ The naphterpin PTase Orf2 (now renamed NphB²⁷) was examined and shown to have relaxed substrate specificity toward various naphthols, flavonoids, and other phenols, yet THN was never evaluated as a substrate.²⁶ In the case of marinone, the draft genome of the marinone-producing bacterium *Streptomyces* sp. CNQ-509 was recently reported²⁸ and shown to contain a BGC with a THN synthase, PTase and, significantly, genes encoding VHPOs homologous to Mcl24 and Naph3 that catalyze α -hydroxyketone rearrangements.

Thus, based on the established biochemistry of the merochlorin Mcl24 VCPO, we propose a similar reaction in the naphterpin and marinone biosynthetic pathways that only differ initially on the length of the terpene chain (Scheme 6). C-4 prenylation of THN with geranyl or farnesyl groups, like that in the construction of pre-mero-chlorin **12**,²³ would give intermediates **25** and **26** leading, respectively, to naphterpin and marinone. We propose that VCPO-catalyzed oxidative dearomatization and dichlorination at C-2 followed by α -hydroxyketone rearrangement would give the merochlorin analogues **27** and **28**. We anticipate at this point a series of rearrangement and redox reactions to effectively remove both chlorine atoms and replace them with an enone would set up an intramolecular hetero-Diels–Alder reaction to construct the tetracyclic nucleus of 7-demethylnaphterpin **29** and debromomarinone **30**.

Recently, the George lab completed a simple, biomimetic synthesis of ($\pm$)-7-demethylnaphterpin **29** following this biosynthetic proposal.²⁹ We are now actively working to-gether to interrogate the naphterpin and marinone biosynthetic systems to explore if VHPOs are indeed central to their biosyntheses and, if so, how many are involved in making the putative Diels–Alder substrate.

6 Closing Thoughts

Significant progress has been made over the past few years in establishing the biosynthetic logic of how the structurally diverse family of THN meroterpenoids is constructed at the genetic and biochemical levels. This work has taught us the central importance of VHPO enzymes in catalyzing a diversity of asymmetric alkene and arene halofunctionalization reactions involving cyclizations and rearrangements. Such reactions are presently difficult to control synthetically to achieve enantioselectivity and thus serve as guiding models for the synthetic and bioengineering communities. A major synthetic advance in asymmetric halofunctionalization of alkenes has taken place in recent years,³⁰ thus promising new strategies and opportunities in the controlled addition of hyperreactive oxidized halogen atoms.

Little is known, however, about how meroterpenoid VHPO enzymes control the precise stereochemical addition of halonium ions to their electrophilic, organic substrates. These enzymes were once considered nonselective for their indiscriminate production and delivery of hypohalous acid to electron-rich substrates,^{9,31} yet their discovery from *Streptomyces* meroterpenoid BGCs changed that perception for their asymmetric properties.¹⁶ We suspect that VHPOs will eventually fall into two groups based on their ability to control or not control the stereoselective addition of reactive halogen ions to their substrates (Scheme 2, routes A and B). We anticipate that the *Streptomyces* VHPO enzymes have evolved a specialized mechanism to trap “Cl⁺” species once generated by the vanadate/H₂O₂ oxidant, perhaps as a chloroamine attached to an active site lysine residue, to facilitate its controlled delivery to a bound substrate. How this biochemical tango takes place and whether it can be hijacked to allow for the creation of engineered VHPOs as broad-use, asymmetric halogenating biocatalysts remains to be seen. This field is only just starting to take shape.

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Biography

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Figure 1.
Structures of representative naphthoquinone-based meroterpenoids produced by *Streptomyces* bacteria

Figure 2.
Structures and biosynthetic grouping of merochlorins A–D

Scheme 1.

Biosynthesis of THN **4** and the NapH1-catalyzed alkene halofunctionalization of SF2415B1 **7** to napyradiomycin analogue SF2415B3 **8**

Scheme 2.

Proposed catalytic scheme for the oxidation of halide ions by VHPO enzymes (adapted from references 9 and 31a). Hydrogen peroxide in the first step reacts with the histidine-ligated vanadate center to form a vanadium-peroxo complex that reacts in the second step with a halide to give a vanadium-OX species in which $\text{X} = \text{Cl}, \text{Br}, \text{and I}$. VHPO enzymes are widely proposed to generate a reactive HOX species upon reacting with water to restore the vanadate active site (route A). The stereoselective *Streptomyces* VHPOs, like NapH1 and Mcl24, are suspected to generate an enzyme-bound halogen species (route B).

Scheme 3.

Biosynthesis of the merochlorins. The conversion of **19** to merochlorin D **11** is proposed and that from **11** to merochlorin C **10** has not yet been confirmed enzymatically.

Scheme 4.

Oxidative dearomatization through halofunctionalization of pre-merochlorin to merochlorins A, B, and analogues. NCS = *N*-chlorosuccinimide

Scheme 5.

Enzymatic biosynthesis of naphthomevalin **20** and other napyradiomycins through an α -hydroxyketone rearrangement catalyzed by NapH3. The conversions of **4** to **21** and **23** to **1** have not yet been biochemically established. DMAPP = dimethylallyl pyrophosphate

Scheme 6.
Proposed biosynthesis of naphterpin and marinone