A Unifying Paradigm for Naphthoquinone-Based Meroterpenoid (Bio)synthesis


Over the past 30 years a large number of diverse and fascinating meroterpenoid natural products have been isolated from marine bacteria, including the merochlorins, the napyradiomycins (of which naphthomevalin is the simplest example), and the marinones (Figure 1). Professor Jonathan H. George, from the University of Adelaide (Australia), said: “These families of natural products are biosynthesized and derived from 1,3,6,8-tetrahydroxynaphthalene (THN), but the mechanisms of these biosynthetic pathways were unclear before our collaborative work with Professor Bradley S. Moore’s group at the University of California San Diego (USA).”

The George research group is broadly interested in the development of biomimetic cascade reactions for the rapid synthesis of complex meroterpenoid natural products. “In addition to synthetic efficiency, we firmly believe that this approach can give insight into biosynthetic pathways, as well as highlight possible structure revisions of biosynthetically dubious natural product assignments,” explained Professor George.

Professor Moore revealed: “This paper was a special treat. I’ve been fascinated with the chemical structures of the naphthoquinone-based meroterpenoid natural products for many years and have marveled on how microbes assemble these antimicrobial molecules by combining polyketide and terpenoid chemistry.” Little did he know then that half way around the world, Professor George’s lab was similarly intrigued with these molecules, yet from a synthetic point of view.

Professor George confirmed: “Our synthetic interest in THN-derived meroterpenoids began with merochlorin A, which possesses four contiguous stereocenters in a bicyclo[3.2.1]octanone core. The key step of our synthesis of merochlorin A was a cycloaddition triggered by oxidative dearomatization of the THN ring system.” The Moore group also completed their own biomimetic synthesis and biosynthetic studies of merochlorin A (and merochlorin B) in which they showed that a single multi-tasking vanadium-dependent haloperoxidase enzyme (VHPO) called McI24 controlled the chlorination, oxidative dearomatization and cycloaddition steps.

“Our two worlds collided in 2015 when Jonathan reached out to me after we had published the discovery and biosynthesis of the merochlorin antibiotics (*J. Am. Chem. Soc.* **2012**, *134*, 11988; *Angew. Chem. Int. Ed.* **2014**, *53*, 11019; *Angew. Chem. Int. Ed.* **2014**, *53*, 11023) and his lab had published an elegant biomimetic synthesis of merochlorin A (*Angew. Chem. Int. Ed.* **2013**, *52*, 12170),” explained Professor Moore, continuing: “In 2015 Jonathan’s lab shifted focus to another meroterpenoid compound, naphthomevalin, and in their biomimetic synthesis approach, they developed a key reaction involving the α-hydroxyketone rearrangement of a protected 4-prenylated tetrahydroxynaphthalene intermediate in which the isoprene chain shifted from C-4 to C-3.”

The Moore group’s original characterization of the McI24 VHPO enzyme revealed that it was singularly responsible for converting pre-merochlorin into merochlorins A and B. In those Cl⁺-induced cascade reactions, a site-selective naphthol chlorination is followed by an oxidative dearomatization/terpene cyclization sequence to construct the complex carbon

![Figure 1 Bacterial meroterpenoids derived from THN](image-url)
framework of the merochlorins in a single step. "My postdoc Stefan Diethelm, who was classically trained as a synthetic organic chemist turned biochemist, noticed that Mcl24 could do more and explain the origin of merochlorin D in which the terpene chain was attached to C-3 instead of C-4," remarked Professor Moore (Scheme 1). He continued: "When he assayed the Mcl24 enzyme at an elevated pH, a previously unrecognized minor product was produced in greatest abundance. Upon solving the structure of the dichlorinated 'merochlorin X', we were all surprised that the terpene chain had migrated to the C-3 position, just like that in the merochlorin D natural product. This result revealed a novel reaction in nature involving a halogen-mediated α-hydroxyketone rearrangement reaction and impressively showed that a single enzyme was responsible for constructing the diverse molecular diversity in the merochlorin series."

At the same time as the Moore group's discovery of the Mcl24-mediated dichlorination, oxidative dearomatization and α-hydroxyketone rearrangement of pre-merochlorin, the George lab completed their own biomimetic total synthesis of naphthomevalin using remarkably similar key steps (Scheme 2). "In our biomimetic synthetic design, we deliberately minimize the use of protecting groups. As well as cutting the step count of the overall route, this also allows us to explore the pre-disposed, innate reactivity of our proposed biosynthetic intermediates on route to the final natural product," explained Professor George. He continued: "However, in the case of our naphthomevalin synthesis we found that protection of the C-5 and C-7 phenols on the left-hand ring of the THN derivatives (e.g. compound 2) was necessary for selective oxidative dearomatization and chlorination steps. In our first-generation strategy (as yet unpublished) we used aryl methyl ethers to protect the C-5 and C-7 phenols – which worked perfectly until the final deprotection step!" After several false starts Professor George and co-workers were eventually forced to re-design the strategy to allow the use of more labile MOM-protecting groups instead. "The re-design and execution of the synthesis took almost a whole year of hard work from my very talented PhD student, Henry Pepper," he acknowledged.

The first key step of the successful synthesis was a one-pot oxidative dearomatization at C-4 of the THN derivative using Pb(OAc)₄ followed by dichlorination at C-2 using NCS to give 3. "This step is remarkably similar to the Mcl24-mediated reaction of pre-merochlorin shown previously in Scheme 1. We then removed one of the C-2 chlorine substituents using a highly selective LDA-mediated dechlorination, and we cleaved the acetate group using KOH to give 4," said Professor George. He continued: "Diastereoselective prenylation of 4 using prenyl bromide and NaH gave 5, which was deprotected to give 6 using mild acidic conditions to remove the MOM-protecting groups. A final α-hydroxyketone rearrangement then shifted the geranyl group from C-4 to C-3 under thermal conditions.
thus rationalizing the C-3 geranyl substitution pattern of naphthomevalin. We were not surprised by the success of the final 1,2-shift, as at that point we had conducted extensive studies of this reaction on model systems. We generally found that heating the α-hydroxyketone substrates overnight at reflux in toluene was required for 100% conversion, but the reaction is very clean with no by-products. However, the fact that the reaction did not occur appreciably at lower temperatures led us to speculate that it must be enzyme-catalyzed in nature.”

It was at this point that the Adelaide-based researchers contacted the Moore group, knowing they were interested (and had published previously) on meroclorin and napyradiomycin biosynthesis. “We both realized that our synthesis could give access to several possible biosynthetic intermediates via MOM deprotection at any point. These (racemic) substrates could be used to reveal the biosynthetic function of VHPOs (and other enzymes) discovered by the Moore group,” said Professor George.

This novel synthetic insight simplified beautifully the biogenesis of many of the naphthoquinone-based meroterpenoid natural products that had isoprene groups attached at both carbon centers.

“Remarkably, Stefan Diethelm in my lab had just identified the Mcl24 enzyme that catalyzed the tandem oxidative chlorination and α-hydroxyketone rearrangement reaction much like that accomplished by Henry Pepper in a fume hood in Australia,” said Professor Moore. “We had both independently discovered the same reaction, one by an enzyme and the other by a chemical reagent. Another postdoc in my lab, Zachary Miles, went on to show that this new enzymatic reaction was evident in both the napyradiomycin and meroclorin series and was also likely responsible for the construction of many more meroterpenoids in Streptomyces bacteria. It was this discovery that led to our *Nat. Chem.* article where we introduced a new, simplified paradigm for the assembly of naphthoquinone-based meroterpenoid natural products using enzymes and/or chemical reagents.”

Both labs found their collaboration enjoyable, as it brought many fresh ideas and complementary expertise to the table. Professor Moore remarked: “For us, our biosynthetic enzymology work on the meroclorins and napyradiomycins uncovered a number of novel biosynthetic transformations relating to asymmetric alkene and arene halofunctionalization reactions catalyzed by a rare class of vanadium-dependent chloroperoxidases in bacteria. These reactions have no precedence in the biochemical literature and at the time of their discovery were quite unexpected.” The George lab was able to provide synthetic material that allowed the Moore lab to rigorously interrogate some of their biosynthetic hypotheses.

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**Scheme 2** Biomimetic total synthesis of naphthomevalin
At the same time, they were able to use enzymes, such as the prenyltransferase NapT8 and the chloroperoxidases NapH3 and NapH1, to catalyze chiral resolving transformations on racemic synthetic material to ultimately give enantiopure naphthomevalin and napyradiomycin A1 (Scheme 3).

“One reviewer of our submitted manuscript asked for CD spectra of all enzymatic products,” said Professor George. “These CD spectra conclusively showed that when the NapT8 prenylation and the NapH3 α-hydroxyketone rearrangement were conducted on fully synthetic, racemic substrates 7 and 6, kinetic resolutions were observed. This shows that the prenylation and α-hydroxyketone rearrangement steps are both stereospecific and enzyme-catalyzed in napyradiomycin biosynthesis.”

Since completing the work that led to the Nat. Chem. publication, the two labs have continued their collaborative project. For three months at the end of 2017, graduate student Lauren Murray from the George lab in Adelaide visited the Moore lab at UC San Diego to pursue two related meroterpenoid target molecules called naphtherpin and marinone. Lauren is a synthetic chemist who recently completed the synthesis of naphtherpin via a route that she predicted was biomimetic. She then joined postdoc Shaun McKinnie from the Moore lab to interrogate the biosynthesis of marinone and they were able to quickly elucidate most of the pathway with the help of an assortment of synthetic materials. “That work is nearing completion and again shows the power and beauty of collaborative science between synthetic and biosynthetic laboratories,” said Professor Moore. “I think that our work also highlights the future of synthetic chemistry in which biosynthetic enzymes are added to the toolkit of synthetic chemists as they ponder the fastest and most efficient way to construct complex organic molecules.” He concluded: “I recently wrote a Synlett Account article entitled “Asymmetric Alkene and Arene Halo-functionalization Reactions in Meroterpenoid Biosynthesis” (Synlett 2018, 29, DOI: 10.1055/s-0036-1590919), which includes a short overview of this Nat. Chem. article in section 4. That article may provide the readers with some good additional backstory to this joint paper with the George lab.”
Literature Coverage

The George lab

Henry Pepper graduated with a BSc (First Class Honors) from the University of Adelaide (Australia) in 2011. He then earned his PhD in Chemistry in 2016 from the University of Adelaide, working under the supervision of Professor Jonathan George. His research interests focus on dearomatization strategies in the biomimetic synthesis of natural products. He was the recipient of the RACI Best PhD Thesis in Organic Chemistry Award in 2017 and was a Reaxys PhD Prize finalist in 2015.

David Huang received his BSc degree with First Class Honors and the University Medal from the University of Sydney (Australia) in 1998. He earned his PhD in Chemistry in 2002 from the University of California, Berkeley (USA), where he worked under the direction of Professor David Chandler as a Fulbright Scholar. After a stint as a scientific copyeditor for Springer-Verlag, he carried out postdoctoral research at the University of Lyon (France), then at the University of California, Davis (USA). He joined the Department of Chemistry at The University of Adelaide (Australia) in 2010, where he is now an Associate Professor. His research is broadly concerned with theory and computation of soft condensed matter, with a focus on applications in renewable energy and functional materials.

Jonathan George received an MChem degree from the University of Oxford (UK) in 2001, followed by a PhD in 2006 from University College London (UK), where he worked under the supervision of Professor Karl Hale. He then returned to Oxford to work as a postdoctoral researcher with Professor Sir Jack Baldwin and Dr. Robert Adlington. In May 2010, he joined the Department of Chemistry at the University of Adelaide (Australia), where he is now an Associate Professor. His research interests include the biomimetic synthesis of natural products, the development of cascade reactions, and biosynthesis.

The Moore Lab

Zachary Miles was born in Madison, WI (USA) and received his BS in biochemistry from the University of Wisconsin-Madison (USA) in 2009. He obtained a PhD under the guidance of Professor Vahe Bandarian at the University of Arizona (USA) in 2014, with his dissertation research entailing the enzymology in the biosynthesis of the modified nucleoside queuosine. He then undertook a position as a postdoctoral researcher in the laboratory of Professor Bradley Moore, wherein his research focused around the biosynthesis of marine natural products. Since 2017 he is applying his experience towards enzyme engineering for industrial purposes as a research scientist at BASF Enzymes in La Jolla, CA (USA).

Bradley Moore is Professor of Marine Chemical Biology at the Scripps Institution of Oceanography (USA) and Chair and Professor of Pharmaceutical Chemistry at the Skaggs School of Pharmacy and Pharmaceutical Sciences at UC San Diego (USA). Since 2015 he is working as a medicinal chemist at Idorsia Pharmaceuticals in Switzerland.

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Zachary Miles was born in Madison, WI (USA) and received his BS in biochemistry from the University of Wisconsin-Madison (USA) in 2009. He obtained a PhD under the guidance of Professor Vahe Bandarian at the University of Arizona (USA) in 2014, with his dissertation research entailing the enzymology in the biosynthesis of the modified nucleoside queuosine. He then undertook a position as a postdoctoral researcher in the laboratory of Professor Bradley Moore, wherein his research focused around the biosynthesis of marine natural products. Since 2017 he is applying his experience towards enzyme engineering for industrial purposes as a research scientist at BASF Enzymes in La Jolla, CA (USA).

Stefan Diethelm received his MSc in Biology from ETH Zürich (Switzerland) in 2009. He then joined the group of Professor Erick M. Carreira for PhD studies working on alkaloid natural product total synthesis. In 2014, he moved to the USA where he conducted postdoctoral research on natural product biosynthetic enzymology in the group of Professor Bradley S. Moore at the Scripps Institution of Oceanography in San Diego (USA). Since 2015 he is working as a medicinal chemist at Idorsia Pharmaceuticals in Switzerland.

Bradley Moore is Professor of Marine Chemical Biology at the Scripps Institution of Oceanography (USA) and Chair and Professor of Pharmaceutical Chemistry at the Skaggs School of Pharmacy and Pharmaceutical Sciences at UC San Diego (USA). He holds degrees in chemistry from the University of Hawaii (USA; BS 1988) and Washington (USA; PhD 1994), was a postdoctoral researcher at the University of Zurich (Switzerland; 1994–1995), and held prior faculty appointments at the Universities of Washington (1996–1999) and Arizona (USA; 1999–2005). Professor Moore has published over 180 papers on the chemistry, biochemistry, and genetics of natural product drug leads and toxins from (primarily) marine microbes.