nature chemistry

ARTICLES

PUBLISHED ONLINE: 31 JULY 2017 | DOI: 10.1038/NCHEM.2829

A unifying paradigm for naphthoquinone-based meroterpenoid (bio)synthesis

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Bacterial meroterpenoids constitute an important class of natural products with diverse biological properties and therapeutic potential. The biosynthetic logic for their production is unknown and defies explanation via classical biochemical paradigms. A large subgroup of naphthoquinone-based meroterpenoids exhibits a substitution pattern of the polyketide-derived aromatic core that seemingly contradicts the established reactivity pattern of polyketide phenol nucleophiles and terpene diphosphate electrophiles. We report the discovery of a hitherto unprecedented enzyme-promoted α-hydroxyketone rearrangement catalysed by vanadium-dependent haloperoxidases to account for these discrepancies in the merochlorin and napyradiomycin class of meroterpenoid antibiotics, and we demonstrate that the α-hydroxyketone rearrangement is potentially a conserved biosynthetic reaction in this molecular class. The biosynthetic α-hydroxyketone rearrangement was applied in a concise total synthesis of naphthomevalin, a prominent member of the napyradiomycin meroterpenes, and sheds further light on the mechanism of this unifying enzymatic transformation.

Meroterpenoids are natural products of mixed biosynthetic origin that exhibit antimicrobial[1–6], anticancer[2,3,6,7] and antioxidant activities[8,9]. They are formed in nature by the regioselective addition of an electron-rich, polyketide-derived aromatic nucleophile to an electrophilic terpene diphosphate, a reaction catalysed by the ABBA prenyltransferase (PTase) class of enzymes[10,11]. Despite the impressive body of knowledge that pertains to terpenoid biosynthesis[12], surprisingly little is known about the biosynthesis of these hybrid compounds. Moreover, meroterpenoids are ubiquitous across all domains of life and are a prevalent source of natural product structural diversity in fungi[13]. Among bacteria, biosynthetic pathways to secondary metabolites that incorporate terpene fragments are often difficult to unravel with established terpene biosynthetic concepts[14–16].

Recently, a group of hybrid isoprenoid–polyketide natural products, which include naphthomevalin (**1**)[17], napyradiomycin A1 (**2**)[18,19], neomarinone (**3**)[20], naphterpin (**4**)[8,21] and merochlorins A, B and D (**5–7**)[1,22], have attracted the attention of the biosynthetic community because of their unique molecular architectures (Fig. 1). As demonstrated previously by ^{13}C-labelling studies[20,21,23], these naphthoquinone-based meroterpenoids derive from a tetrahydroxynaphthalene (THN) (**8**) precursor formed through the action of a single polyketide synthase using the substrate malonyl-coenzyme A (ref. 24). Single or multiple prenylation events, in addition to further embellishment by a multitude of predominantly uncharacterized modifying enzymes, adorn the THN precursor and form the complex final products. Curiously, the most-abundant subgroup of THN-derived meroterpenes (Fig. 1b, class II) exhibit a C3-prenylation pattern, which contradicts the expected C2/C4-nucleophilic reactivity of the parent THN molecule, as the C3 position is not nucleophilic and not poised for such an alkylation reaction. As such, they clearly distinguish themselves from the C2- or C4-prenylated meroterpenes, which include neomarinone (**3**) and merochlorins A (**5**) and B (**6**) (Fig. 1a, class I). To explain prenylation at the non-nucleophilic C3 carbon of the THN core, the involvement of 'promiscuous' PTases has been postulated[25–27]. To the best of our knowledge, no literature precedence exists for prenylation at such a non-nucleophilic aromatic carbon by a PTase, and therefore the lack of a satisfying explanation called for further investigation.

Herein we report the discovery of a halogenation-induced α-hydroxyketone rearrangement to account for the enigmatic substitution pattern of the prevalent class II meroterpenoid natural products (Fig. 1, bottom). This unifying transformation occurs in the biosynthesis of both the merochlorin and napyradiomycin classes of compounds through the action of the vanadium-dependent chloroperoxidase (VCPO) Mcl24 (merochlorin) and a homologous enzyme NapH3 (napyradiomycin) that does not harbour the same halogenation ability, yet catalyses a mirrored α-hydroxyketone rearrangement. In addition, our investigations identified a PTase, NapT8 (napyradiomycin), responsible for the upstream biosynthetic reaction in the napyradiomycin biosynthetic pathway, which includes prenylation of a halogenated meroterpenoid precursor and further underlines the novelty of biosynthetic transformations within bacterial meroterpenoid biosynthetic pathways.

In a joint effort that involves synthetic chemistry and biochemistry, we set out to exploit the unexpected discovery of this novel enzymatic rearrangement to develop a biosynthesis-inspired synthetic entry into the naphthomevalin class of meroterpenoids. As such, the presented synthesis is designed to follow a blueprint of enzymatic reactions, and is therefore in the strictest sense biomimetic. Moreover, our synthetic endeavour provides additional insight into the enzymatic transformation and lends credence to this rearrangement. We demonstrate that the amalgamation of biochemical investigations into an enzymatic reaction and its cognate synthetic application harbours great potential to address complex questions and advance the fields of both biosynthetic and synthetic chemistry[28,29].

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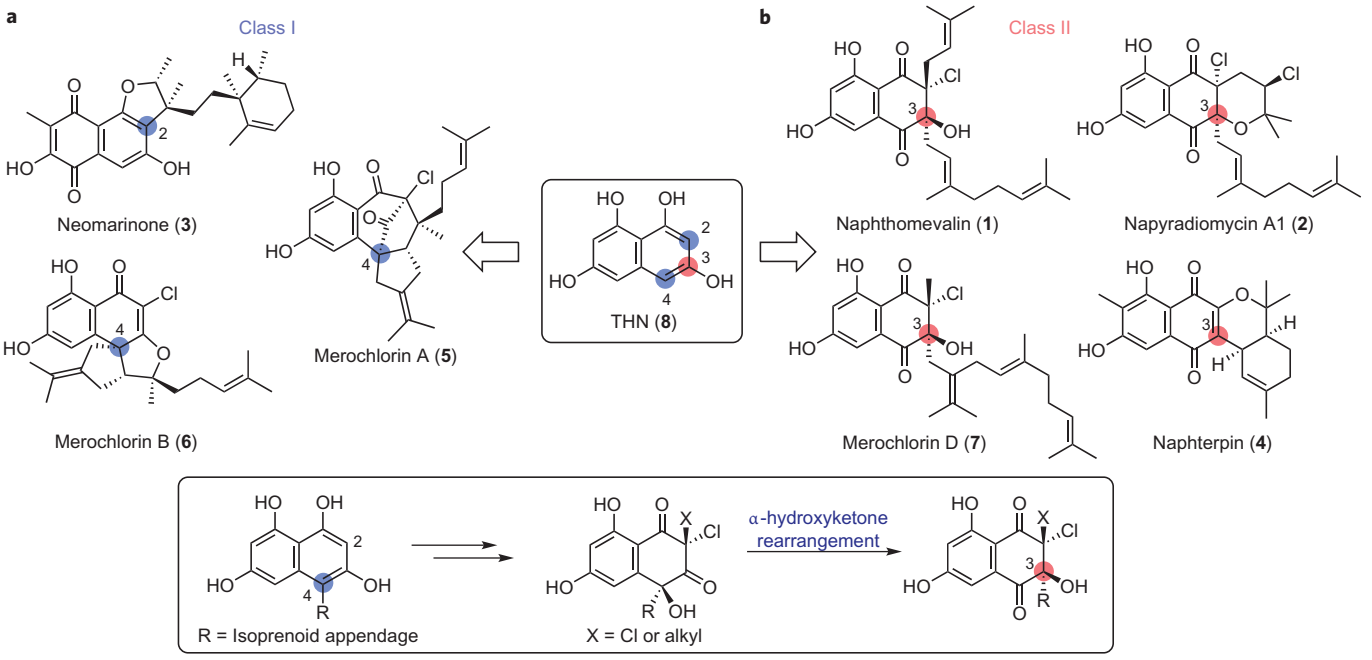

Figure 1 | Bacterial THN-derived meroterpenes. a,**b**, THN-derived meroterpenoids can be divided into two classes based on C2 or C4 prenylation (class I (**a**)) or C3 prenylation (class II (**b**)). The majority of naphthoquinone-based terpenoids are categorized as class II. Naphthomevalin (**1**) and napyradiomycin A1 (**2**) share features of both class I and II, with terpene units at both C2 and C3. All of the compounds could originate from a C4-prenylated molecule, with an α-hydroxyketone rearrangement of the prenyl appendage from C4 to C3 accounting for the creation of class II (bottom).

Results

Characterization of an additional Mcl24 product. The merochlorins are a group of recently isolated bacterial meroterpenoid antibiotics that include both the C4-prenylated members merochlorin A (**5**) and B (**6**), as well as the C3-substituted analogues merochlorin C (not shown) and D (**7**)[1]. Produced by a single biosynthetic gene cluster, this system offers a unique opportunity to study the chemistry that leads to the cryptic C3 prenylation observed in merochlorin D (**7**). We recently reported that the VCPO Mcl24 mediates a chlorination-induced oxidative dearomatization followed by intramolecular cyclization(s) to convert pre-merochlorin (**9**) into the stereochemically complex natural products **5** and **6** in a single enzymatic reaction (Fig. 2)[30,31]. However, the formation of the C3-prenylated merochlorin analogues remained elusive. Remarkably, the only PTase in the *mcl* gene cluster (Mcl23) showed selectivity for the prenylation of THN (**8**) at C4 to produce **9** exclusively[30]. Therefore, the possible promiscuity during the prenylation event could be excluded in the biosynthesis of the merochlorins.

During our investigations aimed towards a detailed characterization of Mcl24, we observed that a minor, previously uncharacterized, product of the reported reaction increased in abundance under basic reaction conditions (Fig. 2a and Supplementary Fig. 1). A detailed structural analysis indicated that the newly formed product **10** was a C3-prenylated THN derivative that exhibited dichlorination at C2 (Fig. 2b). In addition, a circular dichroism (CD) spectrum was obtained of **10**, and the pronounced features are indicative of an enantiopure chiral molecule, which demonstrates the enantiospecificity of Mcl24 (Supplementary Fig. 2). This finding suggests that **10** is formed through an α-hydroxyketone rearrangement, which accounts for the alkyl migration from C4 to C3 (Fig. 2c). We propose a mechanism wherein Mcl24 first chlorinates **9** at C2 and the C3 phenol to give intermediate **11**. Subsequent oxidative dearomatization to form a benzylic carbocation intermediate **12**, followed by intramolecular cyclization gives rise to **5** and **6**, as reported previously (path a)[31]. A similar benzylic carbocation intermediate was also proposed in previous total syntheses of both merochlorin A and B (refs 32,33). Alternatively, trapping of the intermediate benzylic carbocation **12** by water gives α-hydroxyketone **13**, which undergoes a second chlorination at C2 to give the geminal dichloride **14** (path b). The addition of water is supported by assays conducted in $^{18}OH_2$, wherein product **10** showed the requisite *m/z* shift of +2 of the primary ionization peak (Supplementary Fig. 3). The subsequent α-hydroxyketone rearrangement of **14** produces the C3-substituted THN derivative **10**, which shares the same carbon skeleton as merochlorin D (**7**). To the best of our knowledge, this is the first report of a halogenation-mediated α-hydroxyketone rearrangement in nature, and resembles a suggested α-hydroxyketone rearrangement in aurachin biosynthesis[34]. It is particularly remarkable that a single enzyme, Mcl24, encoded from a 41-open reading frame gene cluster, is responsible for generating a significant portion of the structural complexity and diversity of all of the merochlorin natural products.

Chemical chlorination of pre-merochlorin (9) and analogue 17. We previously established a chemical chlorination protocol using a combination of *N*-chlorosuccinimide (NCS) and *i*-Pr_2NH to mimic the Mcl24 chemistry[31]. Under these conditions, pre-merochlorin (**9**) was converted into deschloromerochlorin A (**15**) and deschloromerochlorin B (**16**) (Fig. 3). However, we did not observe the formation of a C3-prenylated product resulting from an α-hydroxyketone rearrangement under these conditions. We were curious to probe the influence that a change in the electronic properties of the substrate might have on the rearrangement chemistry. Such a study might be of interest, as some of the proposed intermediates en route to a rearranged α-hydroxyketone product possess polar character and would hence be influenced by such electronic changes in the substrate. Consequently, we subjected the less electron-rich analogue **17** to our chemical chlorination protocol. Interestingly, besides the expected merochlorin A analogue **18**, two additional products, **19** and **20**, were isolated.

We propose that the initial dichlorination of **17** gives intermediate **21**, which undergoes oxidative dearomatization to give benzylic carbocation **22**, in analogy to the chemistry performed by Mcl24 (Fig. 2c). Cyclization of **22** affords the merochlorin A analogue **18**. Alternatively, trapping of the carbocation intermediate **22** by

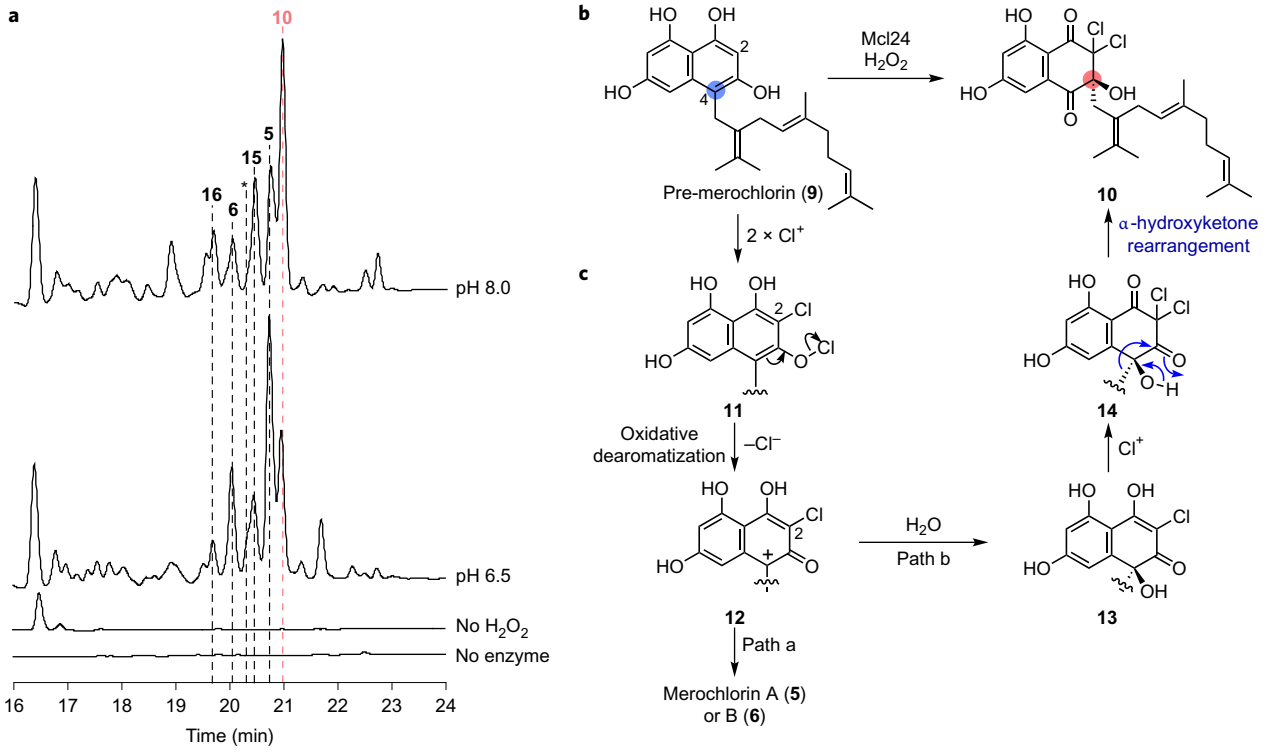

Figure 2 | Mcl24-mediated reaction of pre-merochlorin (9). a, Reversed-phase HPLC (254 nm) of the standard reaction of Mcl24 with **9** at pH 8.0, pH 6.5, excluding H_2O_2 and excluding enzyme. When assayed at a lower pH, the major product observed was merochlorin A (**5**). When assayed at a higher pH, the product distribution changed substantially, with **10** as the major product. Consistent with the reaction requirements of VHPO enzymes, the removal of hydrogen peroxide (or enzyme) resulted in no turnover. All of the products other than **10**, including isochloro-merochlorin B (asterisk) and the uncharacterized degradation product of **9** at ~16.5 min, were reported in a previous study[31]. **b**, Production of the C3-prenylated THN analogue **10** through an α-hydroxyketone rearrangement of **9**. **c**, The proposed mechanism involves a chlorination-induced enzymatic α-hydroxyketone rearrangement.

chloride at C4, followed by chlorination of the resultant chloroenol at C2, would deliver product **19**. Finally, the addition of water at C4 of carbocation **22** followed by C2 chlorination would give α-hydroxyketone **23**, which does not undergo an α-hydroxyketone rearrangement such as that observed in the enzymatic reaction. Instead, intramolecular hemiacetalization to form **24**, followed by fragmentation, gives rise to compound **20**. It seems probable that the presence of excess base influences the reaction outcome by promoting hemiacetalization at the expense of the desired α-hydroxyketone rearrangement. Furthermore, the different solvent properties of the enzymatic versus the chemical chlorination can influence the reaction outcome, and either prevent or promote the rearrangement chemistry by the impact on the stability of polar intermediates on the reaction path. Our failure to mimic the natural α-hydroxyketone rearrangement in these initial experiments raised questions about the thermodynamic requirements for the enzymatic 1,2-alkyl shift and motivated further synthetic studies.

Biomimetic total synthesis of (±)-naphthomevalin. The novel enzymatic α-hydroxyketone rearrangement discovered in the merochlorin system offered an opportunity to develop a general synthetic entry into the class II meroterpenes (Fig. 1b). A strategy that takes advantage of a biomimetic α-hydroxyketone rearrangement seemed particularly attractive, as it would exploit the predisposed C4-nucleophilic reactivity of THN to build up a precursor molecule efficiently, which would then undergo alkyl migration to arrive at the required C3-substitution pattern. Moreover, despite their promising antibacterial activity, only three total syntheses of naphthomevalin/napyradiomycin meroterpenoids have been reported to date[35–37].

As outlined in Fig. 4, our biomimetic synthesis of naphthomevalin (**1**) commenced with methyl (3,5-dimethoxyphenyl)acetate (**25**), which was converted into the methyl ketone **26** via Friedel–Crafts acylation[38] with Ac_2O followed by demethylation using $AlCl_3$. Protection of **26** as a bis-methoxymethyl (MOM) ether followed by base-induced aromatization via a Dieckmann-type cyclization then gave the protected THN derivative **27**. Selective C4 geranylation was achieved through a palladium-mediated allylation using ethyl geranyl carbonate (**28**) as the electrophile[30,39]. The resulting naphthol **29** was subsequently subjected to $Pb(OAc)_4$-mediated oxidative dearomatization[32,40] followed by *in situ* dichlorination of the intermediate enol at C2 using NCS to give dichlorodiketone **30**. Selective removal of one chlorine substituent from C2 of **30** was achieved using a lithium diisopropylamide (LDA)-mediated reduction to give a monochloride that is protected from further dechlorination by enolization[41]. Subsequent cleavage of the acetate protecting group delivered enol **31**, which was then prenylated at C2 to give α-hydroxyketone **32** as a single diastereomer because of the steric hindrance of one face of the enolate derived from **31** by the bulky geranyl substituent. Removal of the MOM protecting groups from **32** under mild acidic conditions then gave α-hydroxyketone **33**, and thus set the stage for the key biomimetic α-hydroxyketone rearrangement. Initial attempts to rearrange **33** using acidic or basic conditions failed to give C3-geranylated naphthomevalin (**1**). However, simply heating **33** in toluene at 110 °C for 16 hours efficiently produced (±)-naphthomevalin (**1**) in quantitative yield. All of the spectroscopic data for **1** matched the reported data of the natural product (Supplementary Information). The relative configuration of **1**, with a *trans* relationship between the C2-prenyl and C3-geranyl substituents, is supported by its facile conversion into the epoxide natural product A80915G under mild basic conditions[17] (Supplementary Information). Further experiments showed that the conversion of **33** into **1** occurred at a significantly

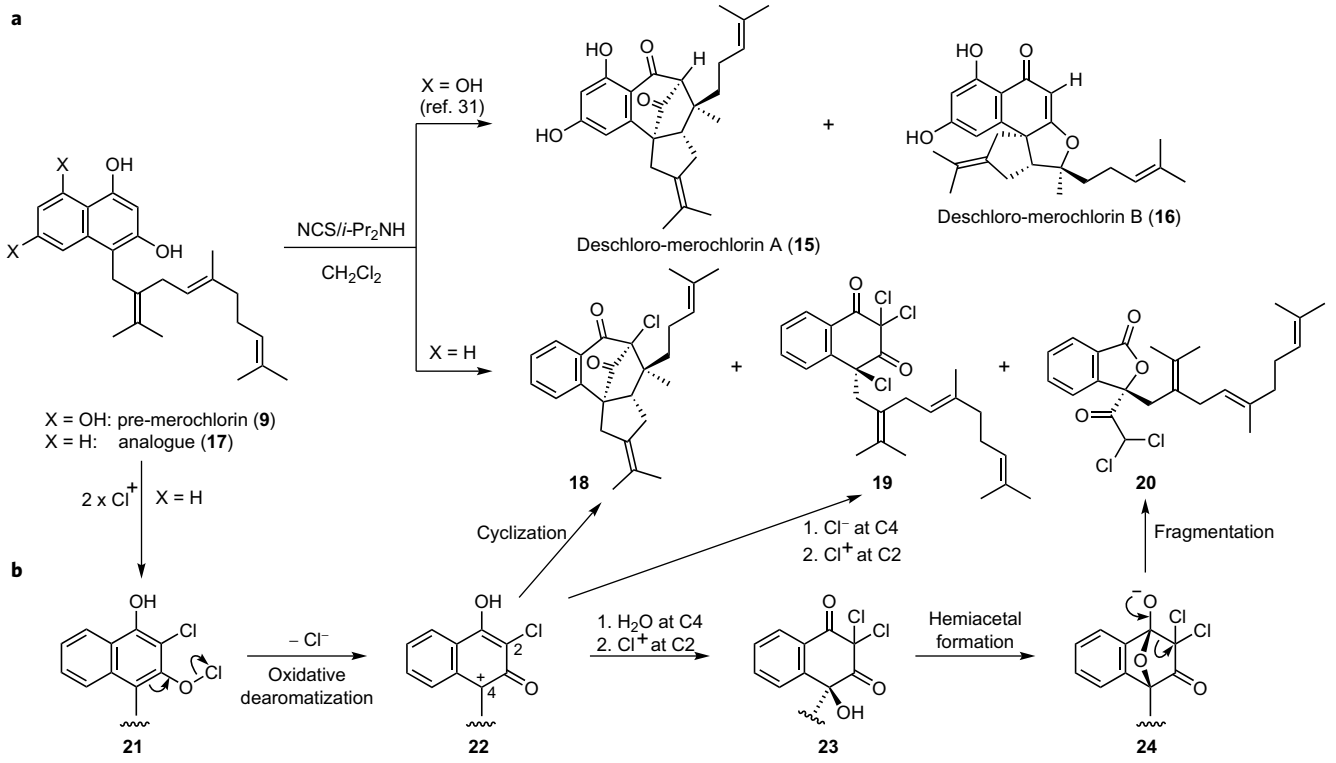

Figure 3 | Chemical chlorination of pre-merochlorin (9) or analogue 17. Pre-merochlorin (9) and its less electron-rich analogue 17 were subjected to our previously established NCS/*i*-Pr_2NH chemical chlorination conditions. **a**, An alternative outcome was observed for the NCS/*i*-Pr_2NH-mediated oxidative dearomatization of 9 and its analogue 17 (Supplementary Table 2 gives the product yields). Whereas 9 converted into the two deschloro-merochlorin analogues 15 and 16, chlorination of substrate 17 produced a mixture of products 18–20. Interestingly, no products that result from an α-hydroxyketone rearrangement were observed under these conditions. **b**, A possible mechanistic rationale for the formation of products 19 and 20 involves the initial formation of carbocation **22** through oxidation and chloride loss. Intermediate **22** can subsequently be trapped by chloride to provide product 19, or alternatively undergo water addition followed by a C–C bond cleavage to arrive at compound 20. NCS, N-chlorosuccinimide.

lower temperature (60 °C) when water was used as the solvent, and thus represented an unusual example of an 'on-water' catalysed α-hydroxyketone rearrangement[42].

Our synthesis of naphthomevalin (1) is biomimetic as it mirrors several steps of the proposed biosynthesis, namely C4 geranylation, oxidative dearomatization, C2 chlorination, C2 prenylation and α-hydroxyketone rearrangement. Thus, in conjunction with the synthesis of 1, we were able to prepare additional proposed biosynthetic intermediates free of protecting groups, which were instrumental in later enzymatic studies (*vide infra*).

With a synthesis of naphthomevalin and various analogues in hand, we envisioned further characterization of the thermal α-hydroxyketone rearrangement from a theoretical perspective. As shown in Fig. 5, three α-hydroxyketone derivatives (33, 34 and 35) were subjected to the originally established reaction conditions (110 °C in toluene) for the 1,2-alkyl shift. For each reaction, we also calculated kinetic and thermodynamic parameters using quantum chemical methods (Supplementary Information gives the full details). The calculations support a concerted 1,2-suprafacial-shift mechanism for the α-hydroxyketone rearrangement of both 33 and the dichlorinated derivative 35 (which closely resembles the putative intermediate **14** in the Mcl24 reaction of 9) with an internal proton transfer. The rearrangement of 33 and 35 is thermodynamically favoured, in part by the formation of a more-conjugated, planar structure in products 1 and 36. In contrast, monochlorinated derivative 34 was unreactive, as it possesses a near planar bicyclic structure, which indicates full conjugation of the π system. Clearly, geminal disubstitution at C2 is necessary for the migration to occur. An alternative two-step mechanism that involves a retro-[2,3]-Wittig rearrangement followed by an aromatic Claisen rearrangement has been proposed for a similar α-hydroxyketone rearrangement recently observed in aurachin biosynthesis[34,43].

Characterization of an α-hydroxyketone rearrangement in napyradiomycin biosynthesis. After unravelling the origin of C3 prenylation in merochlorin biosynthesis coupled with the successful application of the key α-hydroxyketone rearrangement in the total synthesis of naphthomevalin (1), it remained unclear if this rearrangement can, indeed, serve as a general rule for the C3-prenylation pattern in all naphthoquinone-derived meroterpenoids. We therefore set out to explore whether an analogous α-hydroxyketone rearrangement is involved in the biosynthesis of naphthomevalin (1) using the proposed biosynthetic intermediates generated in our biomimetic synthesis as substrates for enzymatic studies.

In 2007, we characterized the naphthomevalin-related napyradiomycin biosynthetic gene cluster[44] and identified genes that encoded two PTases and three vanadium-dependent haloperoxidases (VHPOs). The VCPO NapH1 was subsequently shown to catalyse the stereoselective chlorination–cyclization of the dimethylallyl group of a methylated analogue of naphthomevalin (1)[45]. Therefore, we set out to investigate the function of the putative ABBA aromatic PTases[10,11] encoded by *napT8* and *napT9*. We surmised that these genes encode enzymes responsible for the sequential addition of the dimethylallyl and geranyl moieties to THN at the electron-rich C2 and C4 positions (and not, as originally suggested, at the electron-deficient C3), which results in an intermediate primed for an α-hydroxyketone rearrangement. Indeed, NapT8 was cloned, heterologously expressed, purified and found to catalyse the addition of dimethylallyl pyrophosphate (DMAPP) to the C2 position of 34 to generate 33 (Fig. 6a). Furthermore,

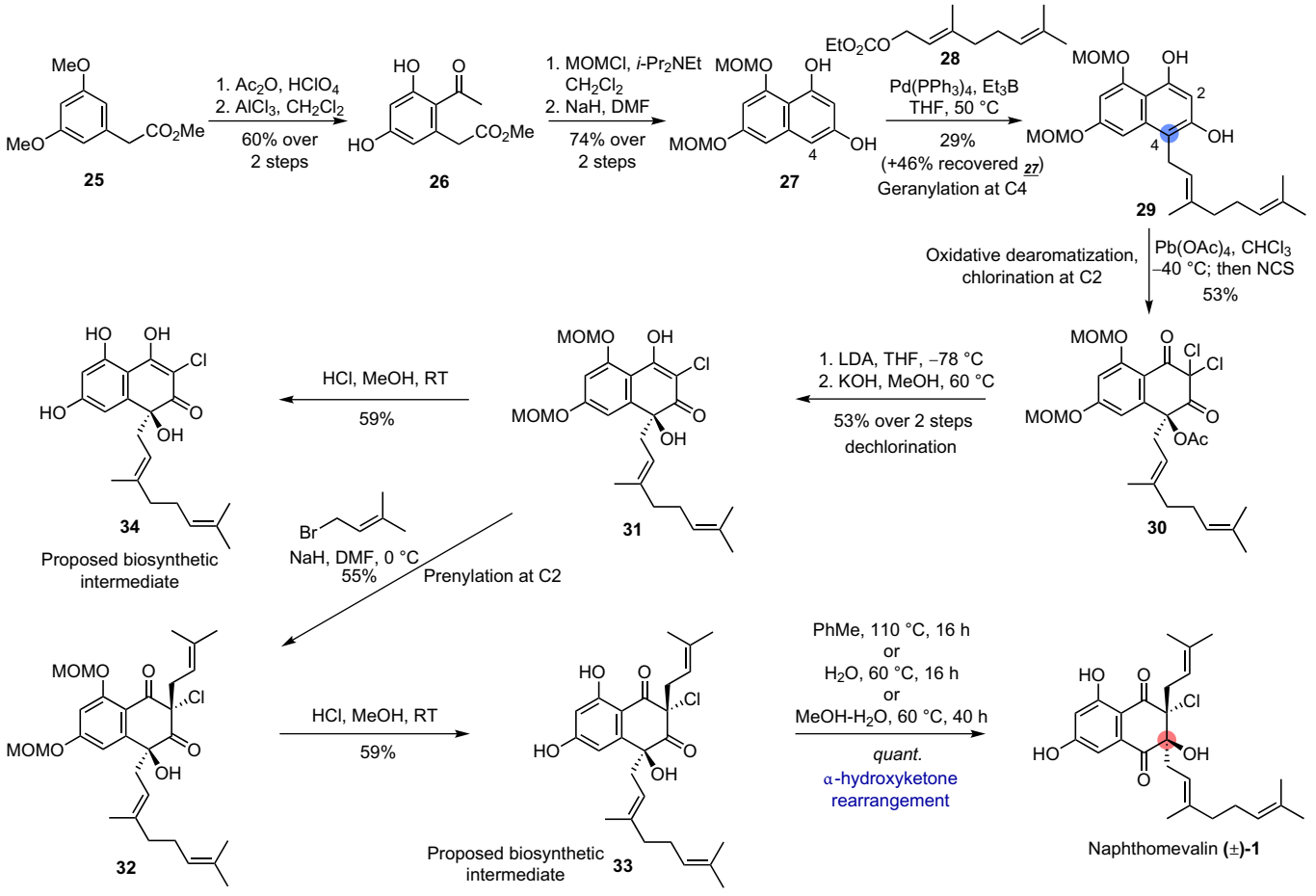

Figure 4 | Biomimetic total synthesis of (±)-naphthomevalin (1) via a thermal α-hydroxyketone rearrangement. This 11-step total synthesis of (±)-naphthomevalin was designed to incorporate several steps of its proposed biosynthesis, which include the C4 geranylation of a THN derivative followed later by an α-hydroxyketone rearrangement to shift the geranyl group to C3. In addition to the natural product, two putative biosynthetic intermediates were synthesized for use in enzymatic studies.

NapT8 was only active when the isoprenoid DMAPP was utilized and activity was stimulated by the presence of exogenous $MgCl_2$ (Supplementary Fig. 7). To the best of our knowledge, this represents the first example of a PTase that catalyses the prenylation of a halogenated carbon centre.

We next turned our attention to the identification of an enzyme that could potentially catalyse an analogous α-hydroxyketone rearrangement of **33** to give naphthomevalin (**1**). We immediately focused on the orphan napyradiomycin VHPO homologues encoded by *napH3* and *napH4* that share about a 57% pairwise amino acid sequence identity with the previously characterized NapH1 (ref. 46). Although NapH4 proved recalcitrant to all of the attempts at recombinant expression, we were able to clone, heterologously express and purify NapH3. Recombinant NapH3 was subjected to activity assays with putative synthetic pre-napyradiomycin substrates (Supplementary Figs 8 and 9), along with the enzymatic product of the NapT8 reaction (**33**). Of all of the substrates evaluated, NapH3 exhibited an efficient capacity to mediate the C4-to-C3 α-hydroxyketone rearrangement of the geranyl moiety only on incubation with both synthetic and NapT8-produced **33**, to form naphthomevalin (**1**) (Fig. 6a and Supplementary Fig. 8). Incubation of **33** with the VHPOs NapH1 and Mcl24, on the other hand, did not catalyse the formation of naphthomevalin (**1**) (Supplementary Fig. 10), although we could measure some slow, non-enzymatic conversion, as similarly observed in the synthetic conversion and NapT8 assays. To compare the non-enzymatic rate with that of the NapH3-catalysed reaction, the initial velocities under substrate-saturating conditions were measured (Supplementary Fig. 11). Under these conditions, NapH3 increased the rate nearly tenfold (0.374 ± 0.021 μM min^{-1} for the NapH3-catalysed reaction compared with 0.041 ± 0.002 μM min^{-1} for the non-enzymatic reaction) when assayed at a 10 μM enzyme concentration. The turnover number (k_{cat}) for NapH3 is 0.038 ± 0.002 min^{-1}, and remained constant when assayed with either synthetic or enantiopure (NapT8-produced) **33**. To ascertain that naphthomevalin (**1**) is, indeed, a biosynthetic intermediate, we further subjected racemic **1** to NapH1 to yield the natural product napyradiomycin A1 (**2**) (Fig. 6a). To gain insight into the stereochemistry of the enzymatic reactions of NapT8 and NapH3, CD spectra were measured for the enzymatically produced **33** (Supplementary Fig. 12) and **1** (Supplementary Fig. 13). The CD spectrum of **33** formed from racemic **34** and NapT8 demonstrated the presence of an enantiopure chiral molecule, that is, a kinetic resolution had taken place. The CD spectrum of **1** generated by NapH3 catalysis on racemic **33** also indicated an enantiopure chiral molecule. Furthermore, unreacted **33** re-isolated from the NapH3 reaction has the opposite CD spectrum to that of **33** generated from prenylation of **34** with NapT8. These observations prove conclusively that the prenylation and α-hydroxyketone steps are under strict enzymatic stereocontrol, dictated by NapT8 and NapH3, respectively.

In contrast to the standard assay conditions required for the halogenation activity of VHPOs[47], H_2O_2 or exogenous Na_3VO_4 were not required for NapH3 activity. Furthermore, this enzyme did not show chlorination or bromination activity when assayed

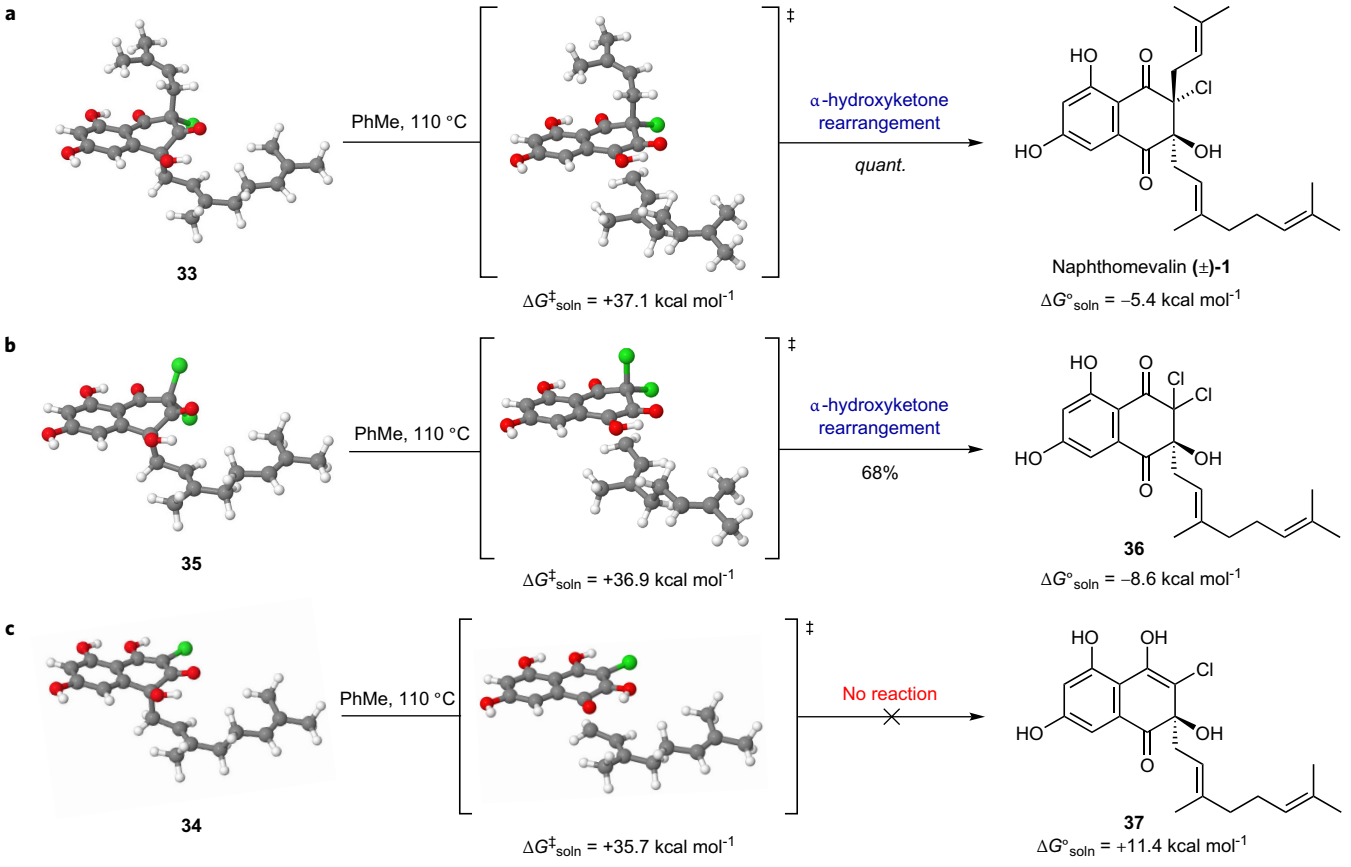

Figure 5 | Theoretical and experimental studies on the thermally induced α-hydroxyketone rearrangement. a–c, Attempted 1,2-shift of three α-hydroxyketone substrates (**33** (**a**), **35** (**b**) and **34** (**c**)), with $\Delta G^{\ddagger}_{soln}$ and ΔG°_{soln} denoting computed free energy changes from the reactant to the transition state and from the reactant to the product, respectively. Structures with and without the '‡' symbol are the computed transition state and reactant geometries, respectively. These studies show that geminal disubstitution at C2 is required for the α-hydroxyketone rearrangement to be thermodynamically favoured. soln, solution.

with monochlorodimedone (MCD), a commonly used substrate to assess the capacity of VHPOs to form hypohalous acid[48,49]. The observation that NapH3 catalyses the α-hydroxyketone rearrangement on an already chlorinated/dearomatized intermediate further supports the lack of halogenation ability. A multiple-sequence alignment of the VHPO homologues from both the merochlorin and napyradiomycin gene clusters revealed a conserved serine residue that is present as a phenylalanine residue (F378) only in NapH3 (Supplementary Fig. 14). This residue was found to be critical for the turnover in NapH1, wherein the mutation to histidine abolished the native activity[45]. To test if this single mutation would be sufficient to confer halogenation ability, the NapH3 F378S variant was expressed, purified and assayed using the standard MCD assay; however, no activity was observed (data not shown).

When taken together, the general biosynthetic transformation for both pathways first employs chlorination-mediated oxidative dearomatization, followed by subsequent α-hydroxyketone rearrangement through a presumed acid/base catalysis. There are very few examples of a rearrangement of a long-chain carbon moiety in natural product biosynthesis; it has only been observed as a reaction that results in a shunt product in hapalindole/ambiguine biosynthesis[29] and it is suggested in the biosynthesis of aurachin[34,43]. In merochlorin biosynthesis, both reactions are catalysed by a single enzyme, Mcl24. The increased production of **10** when the pH is increased from 6.5 to 8.0 implies a specific catalytic base residue with a p*K*a of ~7, such as a histidine residue. In napyradiomycin biosynthesis, NapH3 catalyses the 'second half' of the Mcl24 reaction and presumably participates in a solely acid/base catalysis to promote this novel α-hydroxyketone rearrangement. Yet the requirement for disubstitution of C2 remains in both parallel enzymatic transformations, which is in line with our previous synthetic and theoretical observations. Studies are ongoing to identify the origin of the initial chlorination event in napyradiomycin biosynthesis.

Discussion and conclusions

The merochlorin and napyradiomycin families of natural products have proved valuable model biosynthetic pathways to address the long-standing question of how a counterintuitive substitution pattern is generated in the largest subclass of bacterial meroterpenoids. We identified two enzymes with a highly conserved primary sequence, capable of mediating an α-hydroxyketone rearrangement that leads to a non-standard prenyl substitution of the parent THN core. Remarkably, these enzymes invoke similar, yet in one instance disjointed, chemistry. In the biosynthesis of the merochlorins, the VHPO Mcl24 acts as a multitasking halogenating enzyme that can catalyse both the oxidative dearomatization of a simple precursor molecule and the movement of a prenyl appendage. In contrast, its homologue in the napyradiomycin biosynthetic pathway, NapH3, requires a C2-chlorinated substrate that is predisposed for the rearrangement. Despite the close relationship between the two VHPO homologues, NapH3 appears to have lost its capability as a haloperoxidase and has evolved into an exclusive and selective catalyst for the requisite rearrangement chemistry. Furthermore, we have identified the PTase NapT8, which is capable of prenylating a halogenated carbon centre on the THN core, and thereby primes the substrate for the α-hydroxyketone rearrangement catalysed by NapH3.

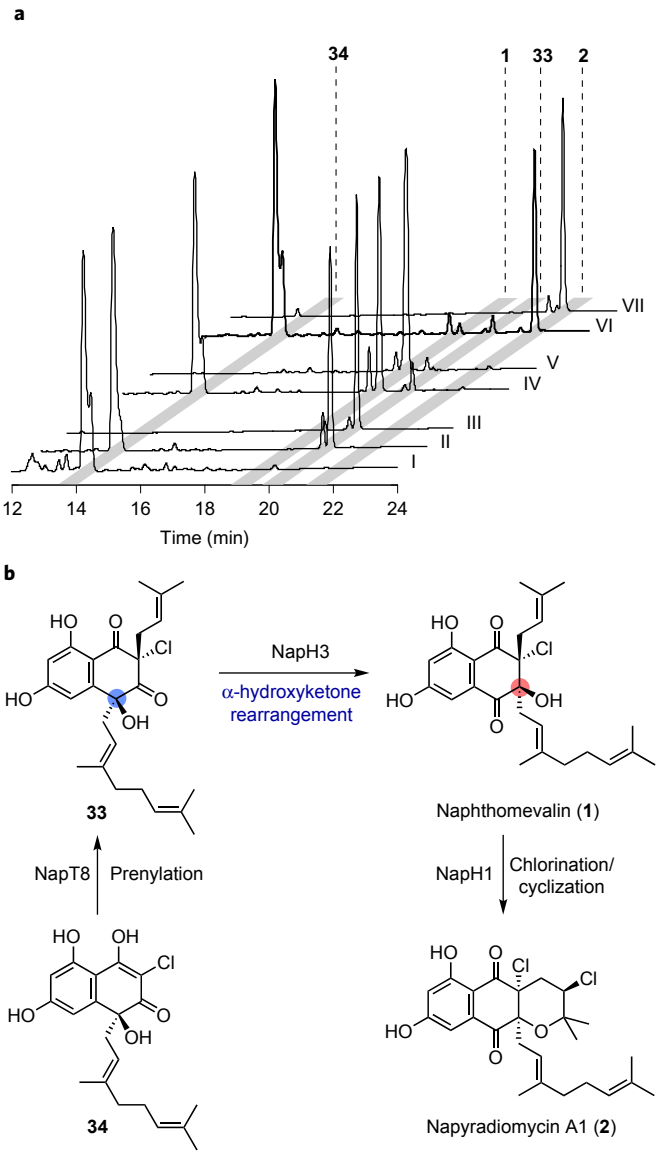

Figure 6 | NapT8-, NapH3- and NapH1-coupled assays with synthetic 34. **a**, Reversed-phase HPLC chromatogram (254 nm) of the standard reaction of racemic **34** with: I, no enzyme (control); II, NapT8; IV, NapT8 then NapH3; VI, NapT8, then NapH3, then NapH1; and in addition to standard compounds: III, **33**; V, naphthomevalin (**1**); VII, napyradiomycin A1 (**2**) (Supplementary Figs 4–6 give the complete ultraviolet/visible light and mass spectrometry characterizations and Supplementary Information gives the assay details). As evidenced through the *in vitro* assays on isolated reaction products, the sequential actions of NapT8, NapH3 and NapH1 are required for the formation of napyradiomycin A1 (**2**) from the synthetic substrate **34**. **b**, Schematic of the pathway from **34** to napyradiomycin A1 (**2**). The pathway also proceeds through an α-hydroxyketone rearrangement of a substrate that possesses disubstitution at C2, which parallels the reaction catalysed by Mcl24.

Through our synergistic approach of coordinating synthetic chemistry with biochemistry, we were able to discover and probe a novel biosynthetic reaction. Based on the presented results, we propose a new paradigm for the biosynthesis of an entire class of natural product compounds. We envision that all bacterial THN-derived C3-prenylated (class II) meroterpenoids proceed through an equivalent α-hydroxyketone rearrangement catalysed by a VHPO homologue, and thereby unify the class I and II meroterpenoid natural products.

Data availability. All data and any associated accession codes and references are available in the online version of this paper.

Received 12 October 2016; accepted 15 June 2017;
published online 31 July 2017

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Acknowledgements

We are grateful to our University of California San Diego colleagues B. Duggan for assistance with NMR measurements and X. Tang for helpful discussions. We are also grateful to M. Ghadiri and L. J. Leman at The Scripps Research Institute for their help in the collection of CD measurements. S.D. acknowledges the Swiss National Science Foundation for a postdoctoral fellowship. This research was supported by the US National Institutes of Health (R01-AI047818) and the Australian Research Council (DP160103393), and was undertaken with the assistance of resources from the National Computational Infrastructure, which is supported by the Australian Government.

Author contributions

Z.D.M., S.D., H.P.P., J.H.G. and B.S.M. designed the study and wrote the manuscript with input from all of the authors. Z.D.M., S.D. and H.P.P. performed the experiments. D.M.H. performed the computational studies.

Additional information

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Competing financial interests

The authors declare no competing financial interests.