Published in final edited form as:

Nat Chem. 2018 January ; 10(1): 38–44. doi:10.1038/nchem.2862.

Bioinspired chemical synthesis of monomeric and dimeric stephacidin A congeners

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Abstract

Stephacidin A and its congeners are a collection of secondary metabolites that possess intriguing structural motifs. They stem from unusual biosynthetic sequences that lead to the incorporation of a prenyl or reverse-prenyl group into a bicyclo [2.2.2]diazaoctane framework, a chromene unit or the vestige thereof. To complement biosynthetic studies, which normally play a significant role in unveiling the biosynthetic pathways of natural products, here we demonstrate that chemical synthesis can provide important insights into biosynthesis. We identify a short total synthesis of congeners in the reverse-prenylated indole alkaloid family related to stephacidin A by taking advantage of a direct indole C6 halogenation of the related ketopremalbrancheamide. This novel strategic approach has now made possible the syntheses of several natural products, including malbrancheamides B and C, notoamides F, I and R, aspergamide B, and waikialoid A, which is a heterodimer of avrainvillamide and aspergamide B. Our approach to the preparation of these prenylated and reverse-prenylated indole alkaloids is bioinspired, and may also inform the as-yet undetermined biosynthesis of several congeners.

Secondary metabolites of terrestrial and marine origin (natural products) have served as the basis for many pharmaceuticals¹. Given the ease in collecting terrestrial flora and fauna relative to their marine counterparts², research on terrestrially derived natural products has historically outpaced that of marine-derived metabolites. However, over the past few decades^{3,4}, there has been an exponential increase in the number of natural products isolated from all sources, especially the marine environment, given the significant advances in collection, sequencing and analysis that have been made during this period.

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Author contributions

R.S., K.M. and D.P.S. designed the research with assistance from Y.H. and E.V.M.-M. All synthetic chemistry was performed by K.M., D.P.S., Y.H., E.V.M.-M., D.E.S., S.C.R. and N.K. Computational studies were conducted by D.E.S., K.G.M.K. and K.M. R.S. wrote the manuscript with contributions from all the authors; all the authors were actively engaged in the editing of the manuscript and gave their approval of the final version. K.M. and D.P.S. contributed equally to this work.

Additional information

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Competing financial interests

The authors declare no competing financial interests.

This increase in the number and diversity of secondary metabolites has also revealed diverse structural novelty, which probably stems from unusual biosynthetic sequences. In general, decoding the mechanisms for the biosynthesis of secondary metabolites is the purview of synthetic biologists, who have utilized synthetic chemistry to support their studies⁵. However, synthetic chemistry may also lead the way to unveiling biosynthetic connections that have been underappreciated or remained unsubstantiated. Thus, biosynthesis and chemical synthesis studies are of mutual benefit. Specifically, strategies for chemical synthesis may be inspired by biosynthetic understanding or, in turn, provide insight into the biosynthesis of secondary metabolites. Here we report an example of this fruitful intersection through the chemical syntheses of several indole-derived secondary metabolites related to stephacidin A (**1**) (Fig. 1).

Among the growing array of known marine indole-derived alkaloids is an intriguing subset of compounds isolated from *Penicillium aspergillus* that are enantiomeric with a series of terrestrial fungal-derived natural products⁶. These secondary metabolites are broadly referred to as ‘prenylated’ or ‘reverse-prenylated’ indole alkaloids because many contain a prenylated or reverse-prenylated indole or the vestige of this structural motif⁷. Reverse-prenylated indole alkaloids display a diverse range of biological activity. For example, stephacidin A (**1**) is cytotoxic towards various tumour cell lines (such as LNCaP cells; half-maximum inhibitory concentration (IC₅₀) = 1.0 μM) (refs 8,9), whereas sclerotiamide (**2**) activates caseinolytic protease P and could provide a starting point for the development of new antibiotics¹⁰. In a related vein, waikialoid A (**7**) is among the most potent natural product inhibitors of fungal biofilms and may present a novel approach to combating refractory infections¹¹. Perhaps the best known among the reverse-prenylated indole alkaloids from a bioactivity standpoint is paraherquamide A (**3**), a derivative of which, derquantel or Startect (which lacks the C2 carbonyl group), is used in veterinary medicine to rid sheep of worms¹². Numerous reverse-prenylated indole alkaloid congeners also display anthelmintic activity⁶.

The dizzying array of functional groups on the core framework of these compounds has spurred intense efforts to understand their biosynthesis. Over the past four decades, a clearer picture as to the biosynthesis of the reverse-prenylated indole alkaloids has begun to emerge⁶, led principally by the efforts of Birch¹³, Sammes¹⁴, Williams¹⁵, Sherman¹⁵, Tsukamoto¹⁵ and Kobayashi¹⁶. For example, the bicyclo[2.2.2]diazaoctane framework of these secondary metabolites is believed to arise through an intramolecular cycloaddition that involves a precursor such as **10** (Fig. 2a). It has also been demonstrated that stephacidin B (**8**) arises from avrainvillamide (**5**) through dimerization, as illustrated in Fig. 2b. This particularly fascinating biosynthetic homodimerization raises the question as to whether a similar process that involves the heterodimerization of aspergamide B (**4**) (the isolation of which was reported in 1995 by Fuchser and Zeeck¹⁷) and avrainvillamide (**5**) could account for the formation of waikialoid A (**7**) and its associated congeners.

We set out to answer this question using chemical synthesis, which required the preparation of various stephacidin A congeners to probe the biosynthesis of waikialoid A (**7**) and other related compounds. To accomplish such a goal, efficient syntheses of the dimerization partners (that is, **4** and **5**) needed to be achieved. We envisioned a unified approach from a

common precursor as the best strategy to prepare these natural product ‘addends’. Inspired by the emerging biosynthetic picture^{18–20}, stephacidin A (**1**), a lower-oxidation-level congener of the reverse-prenylated indole alkaloid family, could serve as a precursor to **2**, **4**, **5** and **6**. In turn, heterodimerization of **4** and **5**, homodimerization of **5** or formal heterodimerization of **5** and **6** would yield **7**, **8** or **9**, respectively.

Key to the success of a chemical synthesis approach that would deliver all these secondary metabolites is a high yielding and robust route to **1**. Given that several laboratories^{21–24}, including our own²⁵, have previously reported the total syntheses of **1**, we were optimistic that the quantities of **1** required to support our studies could be obtained through chemical synthesis. However, close examination of the existing syntheses of **1** reveals that no synthesis has demonstrated the ability to provide more than 2–10 mg in a single pass. As such, we concluded that the existing approaches were inadequate to provide the amounts of **1** (>200 mg) required for this study.

Generally, past approaches to **1** have relied on the early installation of the chromene unit or of a group (for example, a phenolic hydroxyl at C6 of the indole moiety) that could be employed to construct the chromene unit. As an alternative, we envisioned that late-stage chromene formation would simplify the task of preparing **1** down to the synthesis of ketopremalbrancheamide (**11**) (Fig. 3a)²⁶. A position-selective halogenation at C6 of **11** would then set the stage for not only the preparation of **1**, but also that of malbrancheamides C (ref. 27) and B (ref. 28) (**16** and **17**, respectively) (Fig. 3b). Gratifyingly, **11** can be accessed in a six-step sequence from D-proline and indole pyruvic acid following the succinct route developed by Frebault and Simpkins²⁹. We utilized and further optimized this sequence to provide **11** in a 17% overall yield, and have prepared >2 g in a single pass (Supplementary Section 2).

Our perusal of the literature revealed that, despite the substantial progress that has been made in site-selective indole C6 functionalization, a directing group is often required on the indole nitrogen to introduce, for example, a boron³⁰ or carbon substituent^{31–33} at C6. Biocatalysis has also been utilized to achieve C6 halogenation³⁴. Unfortunately, these methods are either restrictive (require a directing group) or are quite specialized (biocatalysis), which makes them non-ideal for our purposes. On the other hand, the existing (albeit underappreciated) precedent for the position-selective, metal-free and direct C6 bromination of 2,3-disubstituted indole derivatives (that is, without the need for N functionalization)^{35,36}, seemed perfectly suited for functionalization at C6 of ketopremalbrancheamide (**11**).

When **11** was treated with *N*-bromosuccinimide (NBS) (Fig. 3b), rapid conversion into a brominated compound was observed by liquid chromatography–mass spectrometry (LCMS) and NMR analysis. This intermediate was assigned as bromoindolenine **14**³⁶. Heating the reaction mixture to 70 °C provided the C6-brominated compound (**15**). This simple one-step sequence directly affords the C6 halogenated derivative without the need to functionalize the indole nitrogen first to introduce a directing group or to evolve an enzyme for this purpose. The mechanism by which this translocation of the halogen occurs is the subject of ongoing studies in our laboratory. Chemoselective reduction of the tertiary amide carbonyl group of

15 yields malbrancheamide C (**16**), which provided spectral data fully consistent with those of the naturally isolated material²⁷. This outcome also provided unambiguous evidence for the C6-selective indole bromination.

Curiously, we were unable to accomplish the analogous C6 chlorination of **11** via the chloroindolenine. However, we envisioned the chlorine group being readily installed via the pinacol boronic ester ultimately to afford malbrancheamide B (**17**). Although various methods for the conversion of aryl iodides and bromides into the corresponding pinacol boronic esters are known³⁷, we found that the photoirradiation methods of Larionov and co-workers³⁸ and of Li and co-workers³⁹ were the most effective for our purpose. The boronic ester was carried on to **17**, as illustrated in Fig. 3b. The spectral data for **16** and **17** prepared in our laboratory were fully consistent with those reported previously (Supplementary Tables 16 and 17 give the data for **17**).

Despite the utility of the C6-selective bromination strategy in delivering malbrancheamides B and C, the photoborylation step from bromide **16** provided an insuperably low yield. This motivated us to investigate the analogous iodination, which was anticipated to provide an aryl iodide better suited for borylation. Unlike in the bromination sequence, migration of the C3 iodine atom of an iodoindolenine (analogous to **14**) to C6 was slow to occur and led to a complex mixture of unidentifiable compounds. To accelerate this process and improve the selectivity, exogenous additives were investigated. Varying the position selectivity for translocation of the iodine atom to the benzenoid portion of the indole moiety was observed to depend on the additive. For example, the use of acetic acid^{35,36} provided only a complex mixture that included small amounts of the C5- and C6-halogenated isomers. On the other hand, the use of trifluoroacetic acid (TFA) resulted in a cleaner halogenation reaction. However, only a moderate selectivity between C5 and C6 iodination was observed. Gratifyingly, adding BF₃·OEt₂ (1.2 equiv.) gave, after optimization, the C6-iodinated material as the major product in a 1:9:1 ratio of iodinated C5:C6: starting material on a gram scale.

The inherent challenges associated with photochemical irradiation on a large scale in a batch reactor⁴⁰ inspired us to develop a flow apparatus akin to the efforts of Li and co-workers³⁹ that allowed us to achieve a higher throughput for the borylation reaction (Supplementary Section 2). For example, to prepare **1**, we desired a hydroxyl at C6 (**19**) (Fig. 4a). This was readily achieved from iodide **18** by photomediated borylation (conducted in flow) followed by oxidation of the resulting boronic ester to provide phenol **19** in a 55% yield over the two steps. Although it is possible to combine the borylation and oxidation steps by the direct addition of H₂O₂ to the reaction mixture after ultraviolet irradiation, the potential formation of explosive triacetone triperoxide caused us to resort to the two-pot process. Stephacidin A was easily obtained from **19** by installation of the chromene unit following the precedent of Cox and Williams⁴¹. Thus, the synthesis of **1** can now be accomplished in a 4.7% yield over 11 total steps from D-proline. Each reaction can also be conducted on a reasonable scale to lead to a total of 300 mg of **1** prepared to date.

Stephacidin A (**1**) has proved to be a versatile intermediate for the synthesis of several more-highly oxidized reverse-prenylated indole alkaloid congeners. First, following the precedent

of Baran *et al.*²⁰, avrainvillamide (**5**) was prepared through sequential reduction of stephacidin A (to 'dihydrostephacidin A') followed by oxidation to install the α,β -unsaturated nitron unit of **5**. This oxidation sequence is low yielding (as noted by Baran and co-workers^{20,22} and Williams and co-workers²³) and only provides, at best, **5** in a 27% yield over the two steps with a 50% recovery of dihydrostephacidin A. An alternative preparation of **5** would involve oxygenation of the α,β -unsaturated imine group of **4**. In this way, both **4** and **5** could be accessed in short order. As such, the preparation of **4** became a focus of our studies.

Previously, we had observed the formation of notoamide I (**21**) (Fig. 4b) on the treatment of **1** with manganese dioxide (MnO_2 , 120 equiv.) in wet ethyl acetate²⁵. Presumably, **21** results from the initial oxidation of **1** to give aspergamide B (**4**) followed by the conjugate addition of water to the α,β -unsaturated imine and oxidation of the resulting 'indolic' hydroxyl. The use of strictly anhydrous ethyl acetate as the solvent avoids the conjugate addition of water to afford **4** in 83% yield from **1**.

Although the mass spectral data for synthetic **4** is consistent with that obtained by Fuchser and Zeeck for the natural isolate reported as aspergamide B (**4**)¹⁷, the ^1H and ^{13}C NMR data obtained for our synthetic material differed substantially from that of the natural isolate. This prompted us to investigate the difference computationally. Utilizing density functional theory (DFT), the computed ^{13}C NMR spectrum⁴² for **4** is in better agreement with the spectrum for the material synthesized in our laboratory. Specifically, the corrected mean absolute deviations (CMADs) for the ^{13}C NMR resonances are 1.7 ppm for our synthetic **4** compared with 3.6 ppm with respect to the spectrum of the natural isolate ascribed as **4**. Interestingly, the ^{13}C NMR spectra of the natural isolate and avrainvillamide (**5**), isolated in 2000 by Fenical and co-workers^{43,44}, are nearly identical (mean absolute deviation of 0.1 ppm). Thus, based on the spectroscopic data, we believe that the natural isolate reported in 1995 by Fuchser and Zeeck¹⁷ to be avrainvillamide (**5**) (details given in Supplementary Sections 3 and 5).

Although an unimpeachable conclusion cannot be drawn about the reported isolation of **4**, our collected evidence from the chemical synthesis suggests that **4** may be a starting point for other reverse-prenylated indole alkaloid congeners. For example, notoamides F (**22**)^{45,46} and R (**23**)^{46,47} are probably produced from **4** through the conjugate addition of methanol or water, respectively. Indeed, we have demonstrated the feasibility and facility of these additions in the laboratory using TFA/MeOH and TFA/ H_2O solutions, respectively (Fig. 4b). The conditions that lead to these products (often employed as purification eluents) suggest that the natural products may be isolation artefacts that result from **4**⁴⁶. Subsequent oxidation or oxygenation of **23** may then lead to **21** or **2**, respectively. Support for this latter assertion comes from a recent report by Li and co-workers on the oxidative conversion of **23** to **2**⁴⁸.

With **4** and **5** in hand, we could now test the possible heterodimerization of these compounds to give waikialoid A (**7**) (Fig. 5). However, several challenges were anticipated. First, it was unclear whether homodimerization of either **4** or **5** would outcompete their heterodimerization to **7**. Second, should the heterodimerization of **4** and **5** proceed, either

compound could serve as the initial aza-Michael acceptor (Fig. 2b gives a depiction of an aza-Michael process), only one of which leads to **7**. Although there is a precedent for the homodimerization of **5** into stephacidin B (**8**)¹⁹ (Fig. 2b), homodimerization of **4** is without precedent. We found that treatment of **4** with triethylamine in acetonitrile yields the corresponding homodimer (**24**), which was unambiguously characterized by X-ray crystallographic analysis. Unlike **8**, which is known to revert to **5**, we have not found conditions that convert **24** back into **4**.

However, as the homodimerization of **5** is reversible and the homodimerization of **4** is kinetically fast, we expected that the heterodimerization of **4** and **5** would probably proceed if we maintained a reasonable concentration of **4** in large excess. This expectation assumes that the heterodimerization of **4** and **5** would be irreversible. In the event, mixing a 2:1 ratio of **4** and **5** in acetonitrile with triethylamine affords a 4:1 ratio of **24** to **7** (20% isolated yield of **7**), whereas mixing an 8:1 ratio of **4** and **5** affords an 11:1 ratio of **24** to **7** (29% isolated yield of **7** and 95% yield of **24**).

In sum, the shortest synthesis of stephacidin A (**1**) reported to date has been achieved, which has facilitated the first synthesis of the purported secondary metabolite aspergamide B (**4**). Although the report of the isolation of **4** is not fully certain, this probably fleeting secondary metabolite now serves as an important synthetic intermediate to many alkaloids in the reverse-prenylated indole alkaloid family. Specifically, access to **4** has led to syntheses of notoamides F (**22**), R (**23**) and I (**21**), as well as waikialoid A (**7**) and a formal synthesis of sclerotiamide (**2**). A key position-selective C6 halogenation of ketopremalbrancheamide (**11**) was critical to installing the chromene moiety present in stephacidin A and related congeners. This C6-selective halogenation has also yielded short syntheses of the natural products malbrancheamides C (**16**) and B (**17**).

Several questions as to the biosynthesis of several of the stephacidin A congeners described here have been prompted by this chemical synthesis study. Not least of which is how waikialoid A (**7**) and related dimeric congeners (for example, **9** (Fig. 1)) arise in nature. If the heterodimerization of **4** and **5** accounts for the formation of **7**, then it would be our expectation that the homodimer of aspergamide B (**24**) would also form rapidly. As **24** was not co-isolated with **7** from the producing organism, it may be the case that **7** arises through a different biosynthetic pathway or that **24** is converted in a facile manner into other congeners (for example, **7**) in the producing organism. This latter hypothesis is provocative as it may imply the oxidation of the imine group of **24** to the nitron found in stephacidin B (**8**), a transformation without biosynthetic precedent⁴⁹. This scenario also begs the question as to how avrainvillamide (**5**) arises in nature. Finally, the recent isolation from *Aspergillus tennesseensis* of versicoamides F–H (for example, **25** and **26**)⁵⁰, which are the formal kojic acid conjugates of **4** and **5**, strongly suggests that capture of **4** or **5** through a formal (3+2) cycloaddition (as may be the case for **7**) may be more widespread in the biosynthesis of related congeners. Should a heterodimerization of **4** and kojic acid account for the biosynthesis of **25**, one would have again expected the co-isolation of **24** from *A. tennesseensis*. Therefore, **24** may yet be isolated from a natural source.

Conclusion

Our chemical synthesis of a number of secondary metabolites in the reverse-prenylated indole alkaloid family has culminated in the synthesis of the heterodimer waikialoid A. These syntheses may inform the possible biosynthesis of several congeners structurally related to stephacidin A. For example, our studies provide insight into a possible site-selective halogenation that affords the malbrancheamides from the precursor ketopremalbrancheamide. Although bromination to afford malbrancheamide C may occur without the assistance of an enzyme, it may be that the analogous chlorination requires an enzyme. Efforts to better understand the biosynthesis of these molecules through a combination of chemical synthesis and biosynthetic engineering studies are underway.

Data availability.

The characterization data for new chemical compounds are given in Supplementary Information. The .cif file for **24** has been deposited at the Cambridge Crystallographic Data Centre (CCDC 1544164).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work is supported by a grant from the US National Institutes of Health (NIH) (NIGMS RO1 086374). We are grateful for a Japan Society for the Promotion of Science Postdoctoral Fellowship to K.M., a Science without Borders Postdoctoral Fellowship to D.P.S. (Brazil; CSF/CNPq 200205/2014-5), the US National Science Foundation (NSF) Graduate Research Fellowship Program, the American Chemical Society Division of Organic Chemistry and the Hellman Graduate Awards Program (University of California (UC) Berkeley) for awards to E.V.M.-M. and the Natural Sciences and Engineering Research Council of Canada for a Postdoctoral Fellowship to K.G.M.K. We are grateful to D. H. Sherman (University of Michigan) and R. M. Williams (Colorado State University), as well as D. Tantillo (UC Davis), for helpful discussions regarding the prenylated and reverse-prenylated indole alkaloids, and the NMR CMAD computational analysis, respectively. We thank R. Cichewicz (University of Oklahoma) for an authentic sample of waikialoid A as well as for an initial secondary metabolite screen on the producing *P. aspergillus* sp. A. Zeeck (Georg-August-Universität Göttingen) is graciously acknowledged for his tireless efforts to locate and obtain analytical data for the material reported by his laboratory in 1995 to be aspergamide B. X-ray crystallography instrumentation is supported by NIH Shared Instrumentation Grant S10-RR027172. The AV-600, AV-500, DRX-500, AVQ-400 and AVB-400 NMR spectrometers are partially supported by NIH Grants SRR023679A and 1S10RR016634-01 and NSF Grants CHE-9633007 and CHE-0130862. The 900 MHz NMR instrument is funded by NIH Grant GM68933. The Molecular Graphics and Computation Facility is funded by the NIH (S10OD023532). We thank K. Owens (UC Berkeley), K. Durkin (UC Berkeley) and Y. Olatunji-Ojo (UC Berkeley) for assistance with DFT computations and A. G. DiPasquale for X-ray crystallographic analysis.

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Figure 1 |. Selected reverse-prenylated indole alkaloid congeners.

Monomeric compounds **1-6** consist of a prenyl or reverse-prenyl group, a bicyclo[2.2.2] diazaoctane framework and a chromene unit. Homo- and heterodimeric compounds **7-9** arise from the non-symmetric association of monomeric units (bonds that give rise to the non-symmetric dimers are highlighted in red).

Figure 2 |. Hypotheses for the biosynthesis of stephacidin A congeners.

a, Prevailing biogenetic hypotheses for the biosynthesis of stephacidin A and 6-*epi*-stephacidin A. **b**, Proposed biosynthesis of stephacidin B from 2 equiv. avrainvillamide.

Figure 3 |. Synthetic strategy for stephacidin A and the application of C6 halogenation.

a, Retrosynthesis of (+)-stephacidin A from ketopremalbrancheamide (**11**) and the Simpkins route to **11**²⁹. **b**, Syntheses of malbrancheamide C (**16**) and malbrancheamide B (**17**) from **11**. DIBAL-H, diisobutylaluminium hydride.

Figure 4 |. Syntheses of stephacidin A and congeners.

a, Synthesis of (+)-stephacidin A from **11**. **b**, Syntheses of stephacidin congeners notoamide I, aspergamide B, notoamide F, notoamide R and sclerotiamide from **1**. B₂(pin)₂, bis(pinacolato)diboron; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; MS, molecular sieve; NIS, *N*-iodosuccinimide; TMEDA, tetramethylethylenediamine; TPAP, tetrapropylammonium perruthenate.

Figure 5 I. Conjugation possibilities for aspergamide B and avrainvillamide.

Homocoupling of aspergamide B (4) and avrainvillamide (5) afforded the aspergamide B dimer (24) and stephacidin B (8), respectively, whereas the heterodimerization of aspergamide B (4) and avrainvillamide (5) produced waikialoid A (7). We hypothesize that the newly isolated versicoamide G (25) and versicoamide H (26) would similarly arise from the dimerization of aspergamide B or avrainvillamide with formal kojic acid. The stereochemistry of the aspergamide B dimer (24) is supported by X-ray analysis.