Natural Product Synthesis

Total Synthesis of Avrainvillamide (CJ-17,665) and Stephacidin B**

Phil S. Baran, Carlos A. Guerrero, Benjamin D. Hafensteiner, and Narendra B. Ambhaikar*

The stephacidins and related alkaloids are a distinctive class of bioactive prenylated indole alkaloids[1] isolated from various terrestrial and marine fungal sources.[2–4] Recently, we reported an enantioselective route to stephacidin A (**1**, Figure 1).[5] Although our synthesis permitted the assignment of relative configuration, the lack of an authentic sample and an optical rotation measurement left the absolute configuration of **1** a mystery.[7] The highest oxidized member in this family of natural products, avrainvillamide (**3**, first isolated by Fenical et al.),[2c,6] also posed some unanswered questions. Specifically, the unique oxidation pattern (3-alkylidene-3*H*-indole-1-oxide) present in **3** is the first of its sort in a natural product[2] and required new methodology for its installation. Although it appeared logical to target **3** through a natural progression of oxidative transformations from the parent stephacidin A (**1**), the execution of this plan was uncertain. For instance, the benzopyran subunit of **1** is susceptible to oxidation under many of the conditions used for the oxidation of amines to nitrones.[8] Herein, we present the following results: 1) a streamlined enantioselective synthesis of both antipodes of **1**, 2) the absolute configuration of this family of alkaloids, 3) the verification of the structure of avrainvillamide (CJ-17,665; (+)-**3**) through reisolation and total synthesis, 4) an approach to the chemoselective conversion of (+)-**1** into (+)-**3**, and 5) the spontaneous dimerization of (+)-**3** to stephacidin B ((−)-**2**).

Our first synthesis[5] of (−)-**1** could be shortened and rendered more amenable to scale-up by making the modifications as illustrated in Scheme 1. The superfluous protection and oxidation of the ester side chain was avoided by using proline derivative **6**,[9] which was able to undergo peptide coupling with **5**[5] without intramolecular cyclization to form a γ-lactam. This undesired cyclization could be avoided when amine **6** was immediately subjected to peptide coupling to furnish **7**. The yield of the key enolate coupling (**8** to **9**) was improved (61% yield along with 8% recovered starting materials), and the reproducibility of the thermal annulation (**10** to **1**) was enhanced by using sulfolane as solvent at a higher temperature (240 °C). By this route, (+)-**1** or (−)-**1** could be prepared in seven steps (12% overall yield) from readily available **5** and (*R*)- or (*S*)-**6**, respectively. Although one could make an educated guess regarding the absolute configuration of these natural products by comparison to the paraherquamides, brevianamides, and other bicyclo-[2.2.2]diazaoctane alkaloids,[1] we elected to use natural (*S*)-proline until this uncertainty was resolved.

On the basis of the assumption that **3** is produced in nature by oxidation of **1**, we collaborated with Professor Fenical et al. (Scripps Institution of Oceanography) to obtain a sample of natural **3** as the original isolated compound was no longer available. Our hope was that **3** could be reduced to **1** to ascertain its absolute configuration. Careful analysis of the crude extracts indicated the presence of not only avrainvillamide (**3**) but also stephacidin A (**1**). Stephacidin B (**2**) was not detected by LC-MS. With a natural sample of (+)-**1** in hand the issue of absolute configuration could be addressed.[10] As shown in Figure 2, comparison of the CD spectra of synthetic (−)-**1** and natural (+)-**1** revealed that the enantiomer had been synthesized previously in these laboratories. The use of (*R*)-proline to secure the enantiomer of amine **6** eventually led to the preparation of (+)-**1** with the correct absolute configuration (synthetic (+)-**1**: $[\alpha]_D = +68.5$ ($c = 0.35$, 1:1 CH_2Cl_2/MeOH); natural (+)-**1**: $[\alpha]_D = +61.5$ ($c = 0.26$, 1:1 CH_2Cl_2/MeOH).

[*] Prof. Dr. P. S. Baran, C. A. Guerrero, B. D. Hafensteiner, Dr. N. B. Ambhaikar
Department of Chemistry
The Scripps Research Institute
10650 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-7375
E-mail: pbaran@scripps.edu

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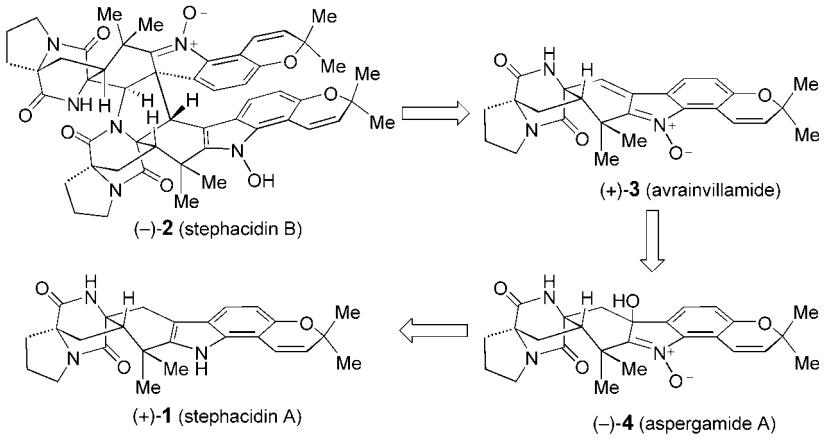
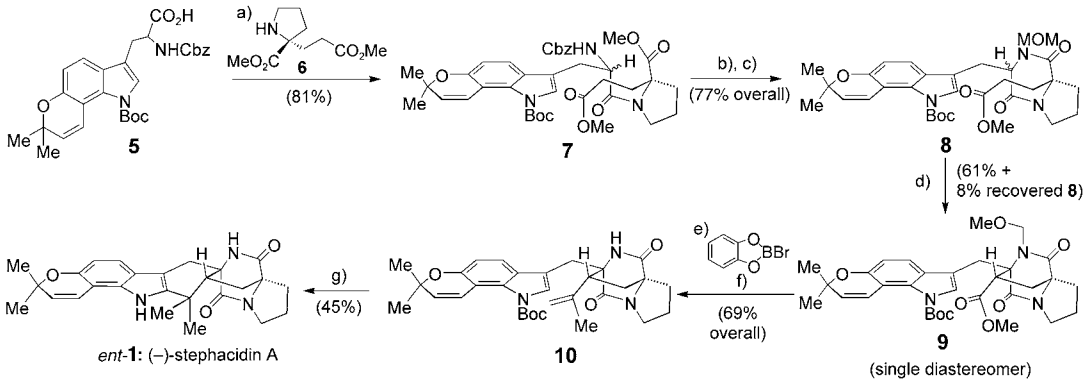


Figure 1. Structures of the stephacidins and related alkaloids along with their proposed[2,3] biogenetic relationships and absolute configuration.

With a shorter synthetic route and the absolute configuration of **1** determined, attention was turned to exploring its conversion into **3**. In the meantime, we had received a sample of aspergamide A (**4**) from Professor Axel Zeeck.[10] Surprisingly, analysis of the material indicated that it had transformed to (+)-**3** by dehydration (Scheme 2). We therefore targeted **4** as a logical precursor to **3**. In principle, the conversion of **1** into **3** could be carried out by chemoselective oxidation of the indole at C3 followed by conversion of the resulting C3-hydroxyindolenine into the corresponding nitrone. In the event, photooxidation with $^1O_2$[11] gave hydroxyindolenine **11** in good yield (Scheme 2). However, all attempts to carry out further oxidation to aspergamide A (**4**) met with failure. A variety of oxidants



Scheme 1. Second-generation enantioselective total synthesis of stephacidin A (**1**). Reagents and conditions: a) **6** (1.0 equiv), HATU (1.1 equiv), iPr$_2$EtN (3.0 equiv), DMF, 25 °C, 12 h, 81%; b) [Pd$_2$(dba)$_3$·CHCl$_3$] (0.2 equiv), Et$_3$SiH (40 equiv), Et$_3$N (2.0 equiv), CH$_2$Cl$_2$, 25 °C, 3.5 h; then DMF/MeOH (3:1), 4 h; 80% overall; c) NaHMDS (1.1 equiv), THF, −78 °C, 30 min then MOMCl (1.4 equiv), THF, −78→25 °C, 1.5 h, 95%; d) LDA (2.2 equiv), THF, −78 °C, 5 min then Fe(acac)$_3$ (2.2 equiv), THF, −78→25 °C, 1 h, 61% **9** with 8% recovered **8**; e) *B*-bromocatecholborane (1.5 equiv), CH$_2$Cl$_2$, 0 °C, 40 min, 78%; f) MeMgBr (5.0 equiv), toluene, 25 °C, 1 h, then Burgess reagent (2.0 equiv), benzene, 50 °C, 30 min, 88% overall; g) sulfolane, 240 °C, 1 h, 45%. Cbz = carbobenzyloxy; Boc = *tert*-butoxycarbonyl; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DMF = *N*,*N*-dimethylformamide; dba = *trans*, *trans*-dibenzylideneacetone; NaHMDS = sodium bis(trimethylsilyl) amide; MOM = methoxymethyl; LDA = lithium diisopropylamide; acac = acetylacetonate; Burgess reagent = MeO$_2$CN$^-$SO$_2$N$^+$Et$_3$.

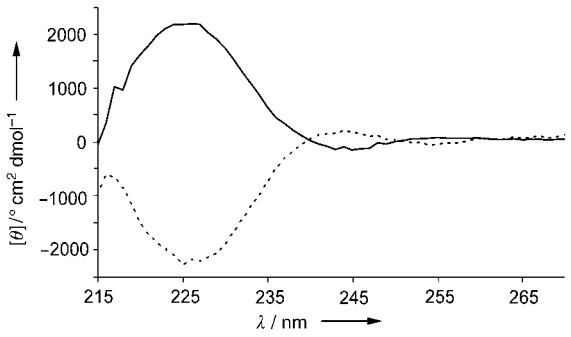
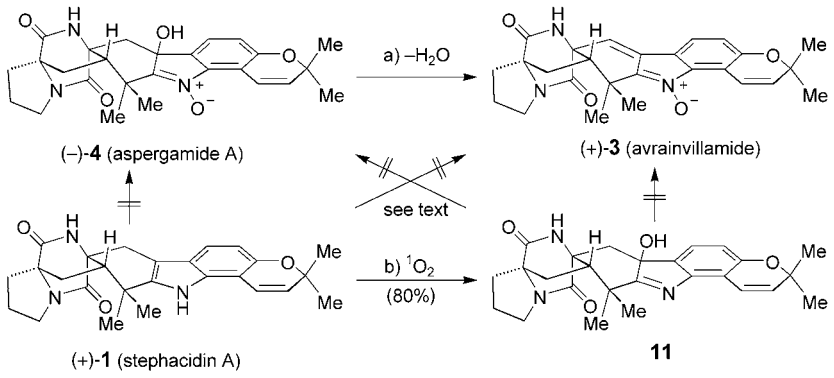


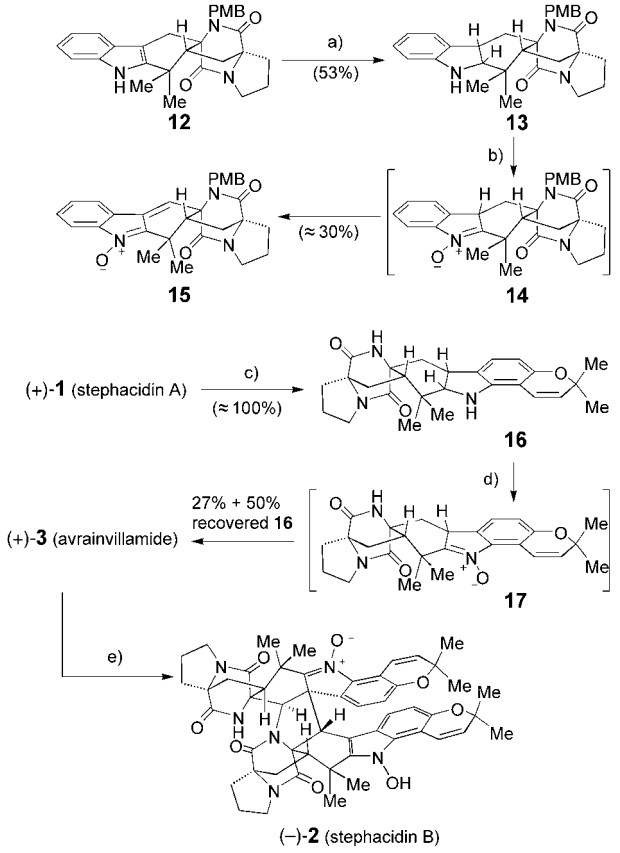
Figure 2. Circular dichroism (CD) spectra (CH$_2$Cl$_2$, 25 °C) of synthetic (−)-**1** (----) and natural (+)-**1** (——). [θ] = molar ellipticity.



Scheme 2. Attempted conversion of **1** into **3** or **4**. Reagents and conditions: a) likely occurred gradually during storage/shipping, 100%; b) sunlamp, cat. methylene blue, $^3O_2$, MeOH, −28 °C, 30 min; then DMS (100 equiv), −28→25 °C, 10 min, 80%. DMS = dimethyl sulfide.

that were screened to convert either **1** or **11** into either **3** or **4** were similarly unsuccessful. These shortcomings forced a reevaluation of our planned pathway to avrainvillamide.

In 1971, Somei put forth a provocative hypothesis for the role of 1-hydroxyindoles (tautomers of saturated indolic nitrones) in the biosynthesis and functionalization of indole alkaloids in nature.[12] These highly reactive species are susceptible to nucleophilic attack and dimerization, and undergo a variety of interesting rearrangements. These pioneering studies led us to hypothesize that perhaps such a species would be a viable precursor to **3**. As a proof of principle, model compound **12** was synthesized by a route that paralleled our synthesis of **1**.[13] As shown in Scheme 3, chemoselective reduction of the indole C2–C3 π bond with sodium cyanoborohydride in acetic acid (Gribble reduction)[14] gave indoline **13** (53% yield), poised for Somei oxidation.[12] Treatment of **13** with catalytic $Na_2WO_4{\cdot}2H_2O$ and excess H_2O_2 did not lead to appreciable amounts of the expected 1-hydroxyindole **14**. Instead, we were pleased to find that the major constituent in the crude reaction mixture was the bright yellow α,β-unsaturated nitrone **15** isolated in approximately 30% yield (unoptimized).



Scheme 3. Synthesis of simple avrainvillamide model **15** and the successful conversion of (+)-**1** into (+)-**3** and (−)-**2**. Reagents and conditions: a) $NaBH_3CN$ (10 equiv), AcOH, 25 °C, 12 h, 53%; b) $Na_2WO_4{\cdot}2H_2O$ (0.2 equiv), aq. 35% H_2O_2 (50 equiv), MeOH, H_2O, 25 °C, 6 h, ca. 30%; c) $NaBH_3CN$ (50 equiv), AcOH, 25 °C, 24 h, 93%; d) SeO_2 (0.25 equiv), 35% H_2O_2 (50 equiv), dioxane, 25 °C, 40 h, 27% **3** with 50% recovered **16**; e) Procedure A: Preparative TLC (SiO_2, EtOAc); Procedure B:[6] Et_3N (excess), CH_3CN, 25 °C, 1 h; Procedure C: DMSO, then solvent removal, approx. 2:1 mixture of **3** to **2**, purified by preparative TLC. PMB = *p*-methoxybenzyl; DMSO = dimethyl sulfoxide.

With a method in hand for the desired oxidative conversion, we turned our attention to stephacidin A (**1**) once again. Gribble reduction of synthetic (+)-**1** furnished indoline **16** (Scheme 3) in essentially quantitative yield as a separable but inconsequential mixture of diastereomers. This mixture was subjected to Somei oxidation, which unfortunately provided about 20% yield of (+)-**3** mixed with some inseparable impurities. Alternatively, indoline **16** could be treated with catalytic SeO_2[15] and excess H_2O_2 to provide pure (+)-**3** in 27% isolated yield along with 50% recovered **16** (spectroscopically identical to the samples obtained from Prof. Zeeck and Prof. Fenical and that reported by Myers;[6] synthetic (+)-**3**: $[\alpha]_D = +11$ ($c = 0.1$, $CHCl_3$); natural (+)-**3**: $[\alpha]_D = +10.6$ ($c = 0.17$, $CHCl_3$). We speculate that this cascade oxidation proceeds via the putative intermediate 1-hydroxyindole **17**, which is further oxidized directly to **3** or perhaps first to **4** (Figure 1) followed by loss of water to form (+)-**3**.

In accord with Herzon and Myers' observations in the unnatural series,[6] synthetic (+)-**3** underwent spontaneous dimerization to (−)-**2** under a variety of conditions, including exposure to silica gel (during preparative TLC), base (Et_3N),[6] or even simple evaporation from DMSO (synthetic (−)-**2** was spectroscopically identical to a sample obtained from BMS[2a] and to that reported by Myers;[6] optical rotation of synthetic (−)-**2**: $[\alpha]_D = -33$ ($c = 0.1$, $CDCl_3$); natural (−)-**2** (as received from BMS): $[\alpha]_D = -21.1$ ($c = 0.19$, $CDCl_3$); (+)-**2**:[6] $[\alpha]_D = +91$ ($c = 0.25$, CH_3CN)). The ease with which the dimerization took place actually hampered purification of **3**. Likewise, **2** underwent facile retrodimerization back to a mixture of **3** and **2** during chromatography. A final issue that needed to be addressed was the true identity of CJ-17,665[2d] as slight differences between synthetic **3** and the reported ^{1}H NMR spectra of CJ-17,665 were observed by both us and Herzon and Myers.[6] Comparison (LC-MS, TLC, NMR spectroscopy) with an authentic sample from Pfizer confirms that it is indeed **3**, and, perhaps not surprisingly, the sample contained approximately 20% of stephacidin B (**2**) as judged by ^{1}H NMR spectral analysis and LC-MS.[16] Interestingly, the sample from Pfizer was provided as a (yellow) solution in DMSO, whereas the sample from Professor Zeeck (see above) was a yellow–green powder and contained no stephacidin B (as judged by ^{1}H NMR spectroscopy), which implies that dimerization does not occur over time in the solid state.

The spontaneous (and reversible) dimerization of **3** to **2** is consistent with the known tendency of saturated indolic nitrones (a tautomeric form of a 1-hydroxyindole) to dimerize.[12] Taken together, these findings add further support for Somei's hypothesis regarding the potentially widespread significance of fleeting 1-hydroxyindoles in nature. The new selenium- and tungsten-based protocols reported herein to chemoselectively generate an unsaturated nitrone group from an easily accessible indoline should facilitate the synthesis of avrainvillamide and stephacidin mimics for biological explorations.[16]

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