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Synthesis and Bioconversions of Notoamide T: A Biosynthetic Precursor to Stephacidin A and Notoamide B

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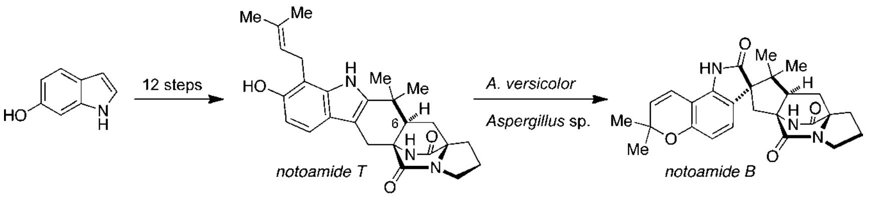
ABSTRACT



In an effort to further elucidate the biogenesis of the stephacidin and notoamide families of natural products, notoamide T has been identified as the likely precursor to stephacidin A. The total synthesis of notoamide T is described along with it is C-6-epimer, 6-*epi*-notoamide T. The chemical conversion of stephacidin A to notoamide T by reductive ring opening is described as well as the oxidative conversion of notoamide T to stephacidin A. Furthermore, [^{13}C]$_2$-notoamide T was synthesized and provided to *Aspergillus versicolor* and *Aspergillus* sp. MF297-2, in which significant incorporation was observed in the advanced metabolite, notoamide B.

The stephacidins and notoamides comprise a growing family of natural products that have been isolated in recent years from *Aspergillus ochraceus* WC76466,[1] *Aspergillus versicolor*,[2] and *Aspergillus* sp. MF297-2.[3] These natural products are members of a larger class of fungal-derived prenylated indole alkaloids that contain a bicyclo[2.2.2]-diazaoctane core structure.[4] This unique ring system has attracted the attention of a number of synthetic chemists[5] and has raised many interesting questions concerning the biogenesis of this core ring structure. Sammes[6] and Birch[7] originally suggested that the bicyclo[2.2.2]diazaoctane ring system is the product of an intramolecular Diels−Alder reaction (IMDA) of a 5-hydroxy-pyrazin-2(1*H*)-one, and more recent work from our laboratories has supported this hypothesis.[8]

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(1) (a) Qian-Cutrone, J.; Krampitz, K. D.; Shu, Y. Z.; Chang, L. P. U.S. Patent 6,291,461, 2001. (b) Qian-Cutrone, J.; Huang, S.; Shu, Y. Z.; Vyas, D.; Fairchild, C.; Mendendez, A.; Krampitz, K.; Dalterio, R.; Klohr, S. E.; Goa, Q. *J. Am. Chem. Soc.* **2002**, *124*, 14556.

(2) Greshock, T. J.; Grubbs, A. W.; Jiao, P.; Wicklow, D. T.; Gloer, J. B.; Williams, R. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 3573.

(3) (a) Kato, H.; Yoshida, T.; Tokue, T.; Nojiri, Y.; Hirota, H.; Ohta, T.; Williams, R. M.; Tsukamoto, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 2257. (b) Tsukamoto, S.; Kato, H.; Greshock, T. J.; Hirota, H.; Ohta, T.; Williams, R. M. *J. Am. Chem. Soc.* **2009**, *131*, 3834. (c) Tsukamoto, S.; Kato, H.; Samizo, M.; Nojiri, Y.; Onuki, H.; Hirota, H.; Ohta, T. *J. Nat. Prod.* **2008**, *71*, 2064. (d) Tsukamoto, S.; Kawabata, T.; Kato, H.; Greshock, T. J.; Hirota, H.; Ohta, T.; Williams, R. M. *Org. Lett.* **2009**, *11*, 1297. (e) Tsukamoto, S.; Umaoka, H.; Yoshikawa, K.; Ikeda, T.; Hirota, H. *J. Nat. Prod.* **2010**, *73*, 1438.

(4) Finefield, J. M.; Frisvad, J. C.; Sherman, D. H.; Williams, R. M. *J. Nat. Prod.* **2012**, *75*, 812.

(5) Miller, K. A.; Williams, R. M. *Chem. Soc. Rev.* **2009**, *38*, 3160.

(6) Porter, A. E. A.; Sammes, P. G. *J. Chem. Soc., Chem. Commun.* **1970**, 1103.

(7) Baldas, J.; Birch, A. J.; Russel, R. A. *J. Chem. Soc., Perkin Trans. 1* **1974**, 50.

(8) (a) Williams, R. M.; Stocking, E. M.; Sanz-Cervera, J. F. *Top. Curr. Chem.* **2000**, *209*, 97. (b) Williams, R. M. *Chem. Pharm. Bull.* **2002**, *50*, 711. (c) Williams, R. M.; Cox, R. J. *Acc. Chem. Res.* **2003**, *36*, 127.

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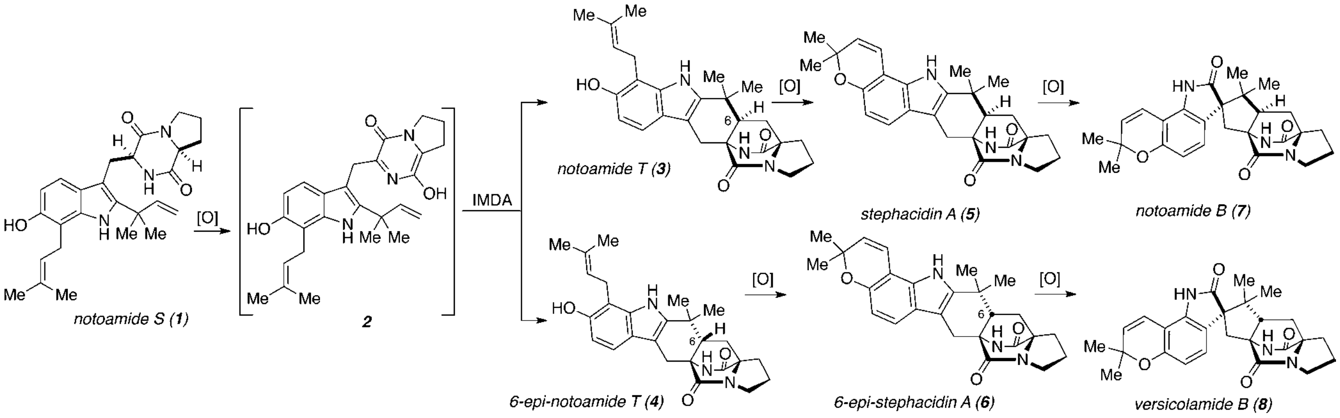
Scheme 1. Proposed Biosynthetic Pathway

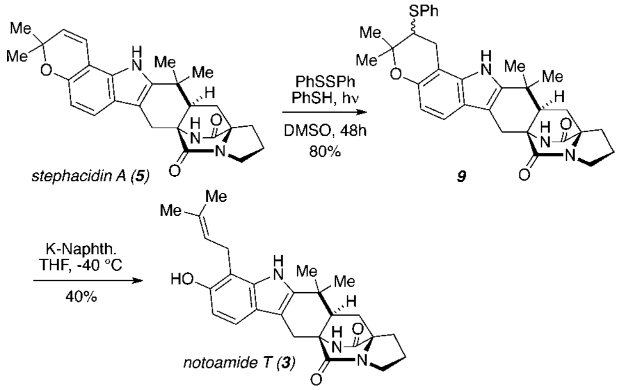


In our ongoing efforts toward determining the molecular details of the notoamide and stephacidin biosynthetic pathway,[9] we have utilized the genome-based characterization of the marine-derived Aspergillus sp. MF297-2 to identify a number of the early steps in the biogenesis of the stephacidins and notoamides.[10] The identification of NotC and NotF as the two indole prenyl transferase enzymes involved in this pathway led us to the discovery of notoamide S (**1**) as an important intermediate in the biosynthesis of the notoamide natural products.[9,10] Additionally, results from our precursor incorporation studies with double ^{13}C-labeled **1**[10] and double ^{13}C-labeled notoamide E[3b] have led us to propose that notoamide S is the direct precursor to the bicyclo[2.2.2]diazaoctane core structure found in stephacidin A (**5**) and versicolamide B (**8**) (Scheme 1). Thus, notoamide S is believed to undergo oxidation to give the achiral azadiene **2**, which would then undergo the IMDA reaction to give **3**, which we have named notoamide T, and 6-*epi*-notoamide T (**4**). Notoamide T can then undergo oxidation and cyclization to form the pyran ring and provide stephacidin A, which we have previously shown to be the precusor to notoamide B (**7**).[10] Versicolamide B would then be derived from **4** via an analogous pathway.

While the current evidence suggests that this is the likely biosynthetic pathway, to date, neither notoamide S nor notoamide T have been detected in the fungal extracts of *Aspergillus versicolor* or *Aspergillus* sp. MF297-2. In order to interrogate the potential intermediacy of notoamide T in the biosynthetic pathway, we sought to develop a synthesis that would provide us with authentic material, while being readily amenable to the synthesis of ample quantities of ^{13}C-labeled isotopomers of these putative metabolites for precursor incorporation studies.

Our initial efforts toward the synthesis of notoamide T focused on the degradation of stephacidin A (Scheme 2). In 1987 Whiting and co-workers published a two-step protocol for the ring opening of 2*H*-chromene systems similar to that found in stephacidin A,[11] and we felt this approach should be readily amenable to the synthesis of notoamide T. To this end, stephacidin A was treated with thiophenol and AIBN in refluxing benzene, but none of the desired **9** could be detected. This unexpected result was attributed to the poor solubility of stephacidin A in benzene. However, changing the solvent to DMSO, in which stephacidin A is soluble, did not improve the reaction. We then turned our attention to the light-mediated radical conditions described by Whiting, which ultimitely proved to be successful.

Scheme 2. Conversion of Stephacidin A into Notoamide T



When stephacidin A was irradiated in the presence of thiophenol and phenyl disulfide in DMSO for a period of 48 h, the desired sulfide was produced in 80% yield. After some experimentation, it was found that the reductive ring opening of **9** could be achieved by the addition of potassium naphthalide to a solution of **9** in THF at −40 °C to provide notoamide T in a modest 40% yield. The temperature of this reaction proved to be vital, and performing the reaction at higher temperatures resulted in the formation of varying amounts of the corresponding stryrene product resulting from isomerization of the double bond.

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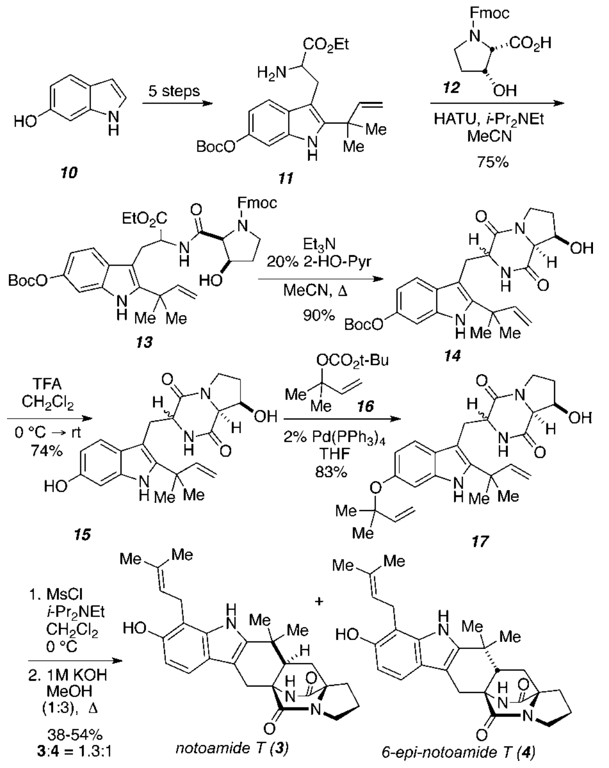
(9) McAfoos, T. J.; Li, S.; Tsukamoto, S.; Sherman, D. H.; Williams, R. M. *Heterocycles* **2010**, *82*, 461.

(10) Finefield, J. M.; Kato, H.; Greshock, T. J.; Sherman, D. H.; Tsukamoto, S.; Williams, R. M. *Org. Lett.* **2011**, *13*, 3802.

(11) Mohamed, S. E. N.; Thomas, P.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1987**, 432.

Scheme 3. Total Synthesis of Notoamide T and 6-*epi*-Notoamide T

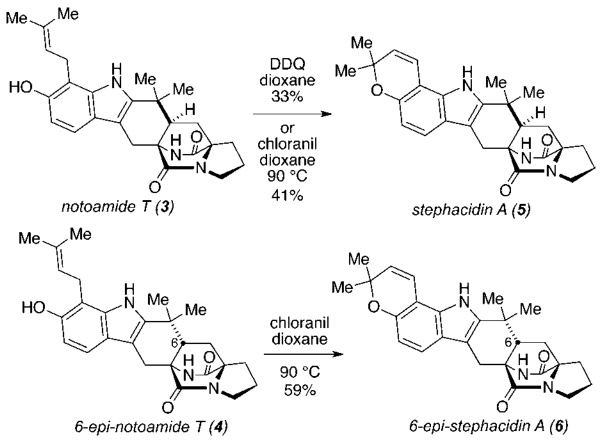


Although the conversion of stephacidin A into notoamide T provided us with access to this substance, we became interested in the possibility of developing a shorter route to notoamide T that utilized a late stage installation of the indole C-7 prenyl group via a one-pot IMDA/Claisen rearrangement sequence (Scheme 3). To this end, 6-hydroxyindole was converted to the tryptophan **11** in five steps following a slightly modified version of our previous approach.[12] Tryptophan **11** could then be coupled with *N*-Fmoc-3-hydroxyproline (**12**) to give dipeptide **13** in 75% yield. Dipeptide **13** was then deprotected and cyclized to dioxopiperazine **14** by treatment with triethylamine and catalytic 2-hydroxypyridine in refluxing acetonitrile. The Boc-group in **14** was then cleaved with TFA, providing the free phenol. The requisite prenyl group was then installed via a Pd-catalyzed allylic alkylation with carbonate **16**[13] to provide the reverse-prenylated phenol **17** in 84% yield. It is interesting to note that the Pd-catalyzed allylic alkylation of **15** was found to be readily reversible, and prolonged reaction times would eventually lead to the regeneration of **15**. The alcohol in **17** was then converted to its mesylate, and when the crude mesylate was treated with 1 M KOH in refluxing methanol the desired elimination/tautomerization/IMDA/Claisen rearrangement reaction sequence took place to provide a separable mixture (1.3:1) of notoamide T and its C-6 epimer in moderate yields. Thus, the syntheses of **3** and **4** were completed in 12 total steps from 6-hydroxyindole and 3-hydroxyproline.

With notoamide T in hand, we became interested in the conversion of this substance into stephacidin A. The cyclodehydrogenation of *ortho*-allylated phenols with DDQ or chloranil has been known for sometime,[14] and we anticipated this reaction to be readily applicable to the conversion of notoamide T into stephacidin A. When **3** was treated with DDQ in dioxane, the desired cyclized product was produced in 33% yield (Scheme 4). After some experimentation it was found that the reaction of **3** with chloranil in dioxane at 90 °C proceeded in slightly higher yields, and stephacidin A could be isolated in 41% yield. Under identical conditions, **4** could be converted into 6-*epi*-stephacidin A in 59% yield. The improved reactivity of **4** is attributed to the increased solubility of both **4** and **6**.

With the synthetic route to notoamide T established, attention was directed toward elucidating the role of this metabolite in the stephacidin biosynthetic pathway. As shown in Scheme 1, we propose that notoamide T serves as a biosynthetic precursor to stephacidin A, which is further converted to notoamide B. To determine the role of **3**, precursor incorporation studies were performed with *Aspergills versicolor* NRRL35600 and *Aspergillus* sp. MF297-2. Doubly ^{13}C-labeled D,L-notoamide T was prepared (see Supporting Information) and provided to both fungal cultures.

Scheme 4. Conversion of Notoamide T into Stephacidin A and Conversion of 6-*epi*-Notoamide T into 6-*epi*-Stephacidin A



Fungal extracts from the D,L-[^{13}C]$_2$-notoamide T precursor incorporation study with *A. versicolor* were analyzed via LC-MS and ^{13}C NMR spectroscopy. Further analysis of the electrospray mass spectrum showed significant intact incorporation of labeled notoamide T into advanced secondary metabolites (Scheme 5). Specifically, (+)-stephacidin A (4.7% incorporation) and (+)-notoamide

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(12) Finefield, J. M.; Williams, R. M. *J. Org. Chem.* **2010**, *75*, 2785.

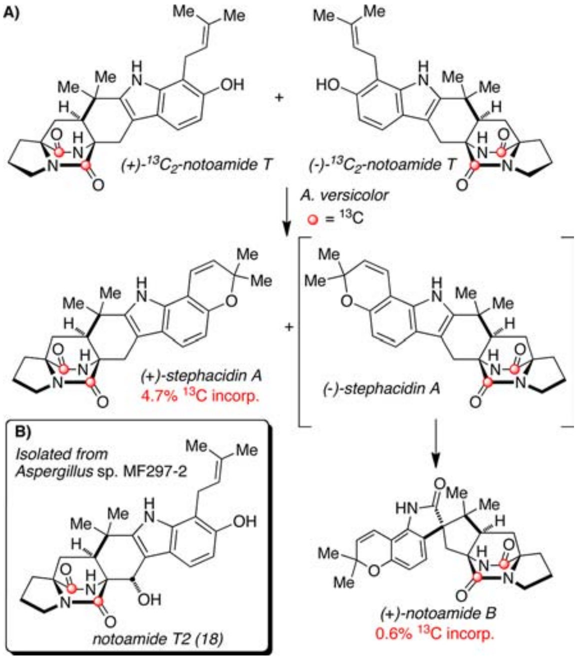
(13) Chantarasriwong, O.; Cho, W. C.; Batova, A.; Chavasiri, W.; Moore, C.; Rheingold, A. L.; Theodorakis, E. A. *Org. Biomol. Chem.* **2009**, *7*, 4886.

(14) Merlini, L. *Adv. Heterocycl. Chem.* **1975**, *18*, 159.

(15) Calculated according to the method outlined in: Lambert, J. B.; Shurvell, H. B.; Lightner, D. A.; Cooks, R. G. *Organic Structural Spectroscopy*; Prentice Hall: Upper Saddle River, NJ, 1998; pp 447–448.

B (0.6% incorporation) were isolated from the D,L-notoamide T precursor incorporation study.[15] Additional analysis of the labeled metabolites isolated *via* optical rotation revealed that the construction of the pyran moiety of stephacidin A must be mediated by a promiscuous oxidase that accepts either enantiomer of notoamide T as a substrate. In *A. versicolor*, complete consumption of D,L-[^{13}C]$_2$-notoamide T (**3**) was observed, as no unreacted material was recovered; however, one of the isolated bioconversion products, stephacidin A, displays the opposite rotation of the natural, endogenous metabolite from *A. versicolor* [recovered (+)-stephacidin A $[\alpha]_D^{25}$ +24 (*c* 0.0025, 1:1 MeOH/CH$_2$Cl$_2$); lit.[2] (−)-stephacidin A $[\alpha]_D^{25}$ −32 (*c* 0.05, 1:1 MeOH/CH$_2$Cl$_2$)]. As shown in Scheme 5, this result indicates that (±)-^{13}C$_2$-notoamide T was effectively converted to (±)-^{13}C$_2$-stephacidin A.

Scheme 5. (A) Bioconversion of Notoamide T into Stephacidin A and Notoamide B in *A. versicolor*; (B) Structure of Notoamide T2 Isolated from *Aspergillus* sp. MF297-2



To rationalize this outcome, it seems consistent that only the natural endogenous (to *A. versicolor*) enantiomer, (−)-stephacidin A, was converted to the natural, endogenous *A. versicolor* metabolite (+)-notoamide B. Since (+)-stephacidin A is not an endogenous natural metabolite produced in *A. versicolor* and displays the opposite absolute configuration of the natural notoamide B produced by this organism, it was not incorporated into the biosynthetic pathway and was, thus, isolated from the fungal extract.[12]

D,L-[^{13}C]$_2$-Notoamide T was also provided to *Aspergillus* sp. MF297-2 in a precursor incorporation study, in which analysis of the fungal extract revealed incorproation of D,L-[^{13}C]$_2$-notoamide T (**3**) into D,L-[^{13}C]$_2$-stephacidin A (**5**) (4.7% incorporation), D,L-[^{13}C]$_2$-notoamide B (7.1%), D,L-[^{13}C]$_2$-notoamide F (7.8%), and D,L-[^{13}C]$_2$-notoamide R (9.6%) (Scheme S2). From this precursor incorporation study, a new, hitherto undetected metabolite, D,L-[^{13}C]$_2$-notoamide T2 (Scheme 5), was also isolated. The structure of [^{13}C]$_2$-notoamide T2 (**18**) was elucidated on the basis of NMR (see Supporting Information) and mass spectral data. Upon further analysis of the isolated metabolites, it was noted that the specific rotations of all the metabolites were zero, which indicated that the metabolites were racemic mixtures. While this result was largely unexpected, it reveals that the orthologous gene products in *Aspergillus* sp. MF297-2 do not discriminate between the two enantiomers of notoamide T or of stephacidin A in the oxidative conversion to the advanced metabolites stephacidin A and notoamide B, respectively. The molecular basis for the distinct stereospecificities of the complementary gene products from the two respective fungi are not understood at this time and are the subject of ongoing interrogation.

In conclusion, we have demonstrated that notoamide T is readily accessed both from the degradation of stephacidin A and from a tandem IMDA/Claisen rearrangement sequence. Additionally, we have shown that notoamide T (**3**) and 6-*epi*-notoamide T (**4**) can be converted to stephacidin A and 6-*epi*-stephacidin A, respectively, providing corroborating support for their proposed biogeneses. Efforts are currently underway to explore the role of 6-*epi*-notoamide T as a biosynthetic precursor to versicolamide B. We are currently investigating the possible existence of 6-*epi*-stephacidin A as a natural metabolite in both *Aspergillus versicolor* and *Aspergillus* sp. MF297-2 and its role as a putative biosynthetic precursor.

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Supporting Information Available. Experimental details and copies ^{1}H and ^{13}C NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.