Biomimetic Alkaloid Synthesis

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A Concise, Biomimetic Total Synthesis of Stephacidin A and Notoamide B**

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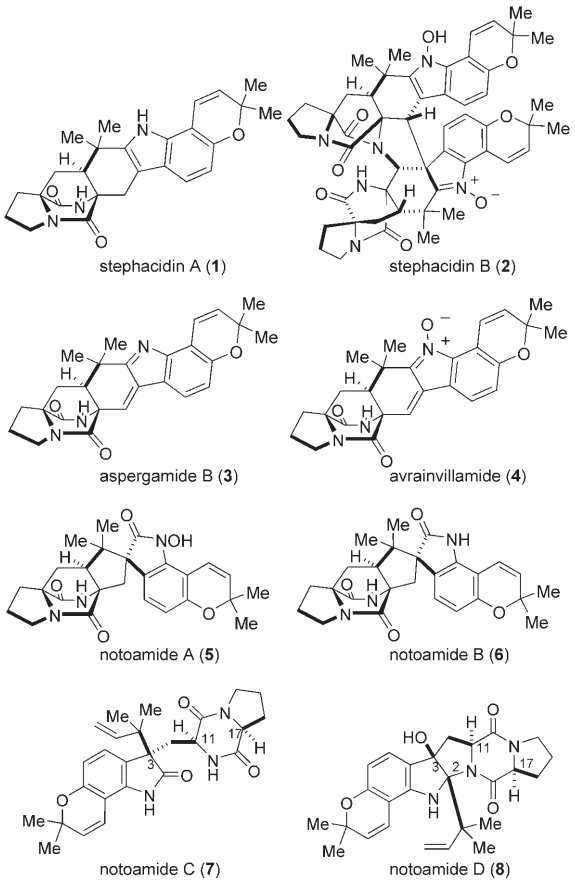
Dedicated to Professor Yoshito Kishi on the occasion of his 70th birthday

Marine organisms, particularly fungi, are abundant sources of biologically active natural products that possess complex and diverse ring systems. Prenylated indole alkaloids such as the paraherquamides[1] and brevianamides[2] are fungal metabolites whose synthesis and biogenetic origin have been extensively investigated.[3] Recently, several other structurally related prenylated indole alkaloids have been discovered from marine environments. Stephacidin A (**1**) and B (**2**) were isolated from the fungal strain *Aspergillus ochraceus* WC76466 and were shown to exhibit potent in vitro cytotoxicity against various human tumor cell lines (Scheme 1).[4] Stephacidin B (**2**) was found to be especially potent against testosterone-dependent prostate LNCaP cells (IC_{50} = 60 nM).[4] More importantly, the cytotoxic effects of these substances are not mediated by p53, mdr, bcl2, tubulin, or topoisomerase II, which is indicative of a novel mechanism of action.[4] The stephacidins possess remarkably similar structural features to those of the cytotoxic marine alkaloid, avrainvillamide (**4**), which had previously been isolated from *Aspergillus* sp.[5]

Very recently, Tsukamoto and co-workers[6] have reported the isolation of four new indole alkaloids, the notoamides A–D (**5**–**8**), from the marine-derived fungal strain *Aspergillus* sp. separated from the common mussel, *Mytilus edulis* obtained in the Sea of Japan. The notoamides A–C showed moderate cytotoxicity against a panel of tumor cell lines, while notoamide D was virtually inactive. Like stephacidins A and B, the notoamides possess a sensitive indolopyran ring system and a tryptophan/proline-derived bicyclo[2.2.2]diazaoctane embedded in their core. As a result of their inherent biological activity and structurally diverse ring systems, this family of prenylated indole alkaloids have become the subject of intense synthetic endeavors, including the first total synthesis of avrainvillamide (**4**) and stephacidin B (**2**) by Myers and Herzon,[7] and more recently stephacidin A (**1**) en route to avrainvillamide (**4**) and stephacidin B (**2**) by Baran and co-workers.[8] Our research group has extensively studied both the synthesis and biosynthesis of prenylated indole alkaloids of this family,[3] and in the preceding Communication reported the first biomimetic synthesis of



Scheme 1. Structures of the stephacidins, notoamides, and avrainvillamide.

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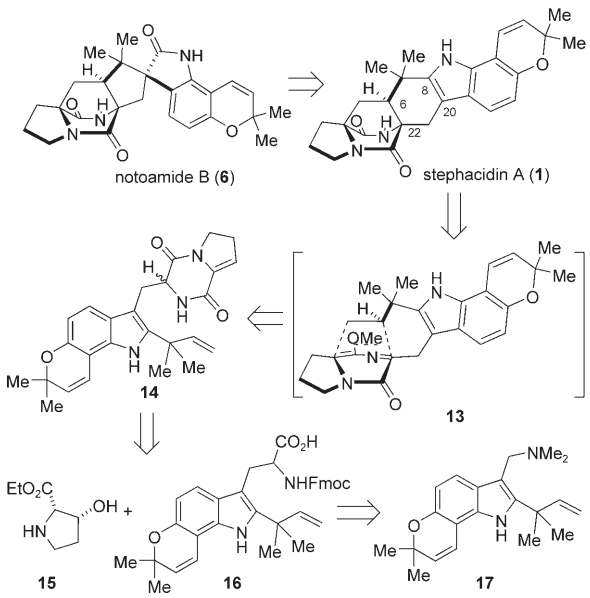
the notoamides C (**7**) and D (**8**).[9] Herein, we report the application of a general biomimetic strategy to the concise total synthesis of stephacidin A (**1**) and notoamide B (**6**).

Based on their structural similarities and their origin from the genus *Aspergillus*, it is quite interesting to contemplate some reasonable biogenetic relationships between the stephacidins and notoamides. A plausible biosynthetic pathway that interrelates these compounds is outlined in Scheme 2. Previous studies from our research group,[3] as well as those of the research groups of Birch[10] and Sammes,[11] suggest that the bicyclo[2.2.2]diazaoctane core of these alkaloids is likely to arise in nature from a biosynthetic intramolecular Diels–Alder (IMDA) reaction. Thus, one could imagine a [4+2] cycloaddition reaction of azadiene **10** (derived from **9**), which contains the reverse prenyl group, and which would directly furnish stephacidin A (**1**). Compound **1** would appear to be a clear and important biosynthetic precursor to avrainvillamide (**4**), stephacidin B (**2**), and notoamide A (**5**) and B (**6**). It has already been suggested that **1** undergoes an overall four-electron oxidation to the unsaturated nitrone **4**, which in turn spontaneously dimerizes to **2**.[12] The latter process has been experimentally corroborated by the research groups of Myers and Baran.[7,8] Notoamide B (**6**) may simply arise from the precursor **9** through two plausible pathways: 1) oxidation and tautomerization to the azadiene **10**, followed by an IMDA cycloaddition to give **1** and subsequent oxidative ring contraction to the spirooxindole; or **2**) oxidation and rearrangement to oxindoles such as notoamide C (**7**) and/or further oxidation to norgeamide A (**11a**) or B (**11b**), elimination/tautomerization to produce the azadiene **12**, which could then be trapped by the isoprenyl group to afford **6**.

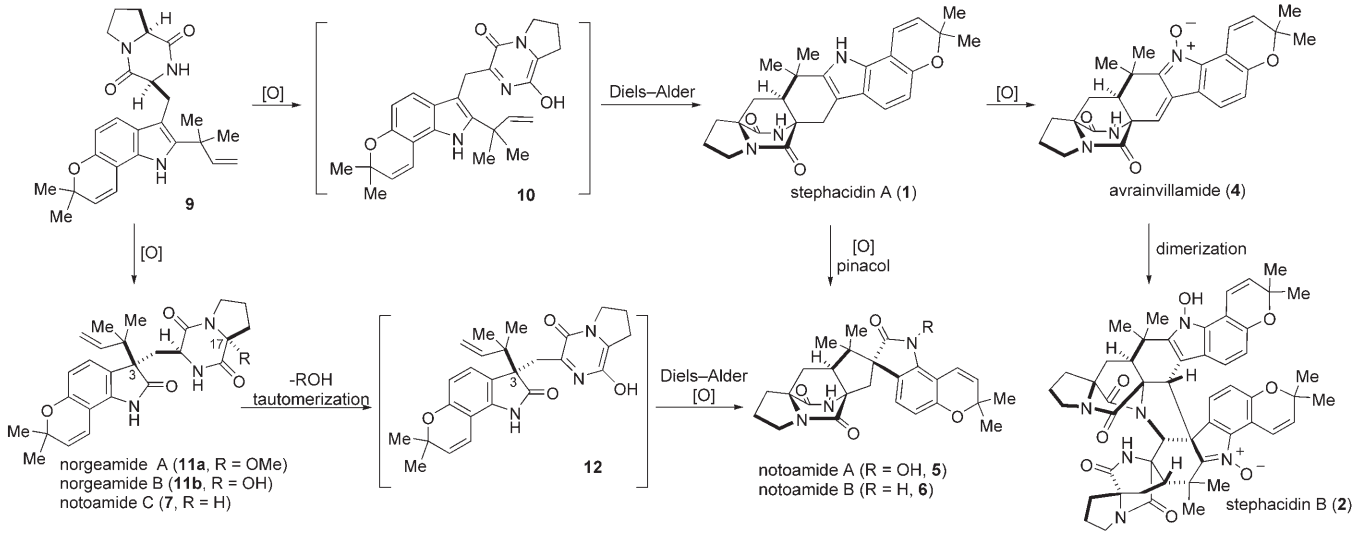
Intrigued by possible biosynthetic relationships between these families of alkaloids, along with their unique biological activities, we have explored their total synthesis with the objective of forming isotopically labeled substances for ongoing biosynthetic investigations. In the retrosynthetic analysis (Scheme 3), we envisaged that the spiroxindole of **6** could arise from a stereoselective oxidative ring contraction



Scheme 3. Retrosynthetic analysis. Fmoc = 9-fluorenylmethyloxycarbonyl.

of the C8–C20 fused indole of **1**. A biomimetic IMDA reaction of the azadiene **13** with the isoprene residue should proceed preferentially to produce the desired *syn* relative stereochemistry at the C6–C22 ring fusion required to access **1**. Our research group has previously reported related biomimetic Diels–Alder cyclizations,[3a,13] which culminated in the total synthesis of the fungal metabolite VM55599[13b] and brevianamide B. Based on previous experiments with similar substrates, it seemed unlikely that azadiene **13** would be stable, and therefore could be generated from enamide **14** through lactim ether formation, followed by a base-induced tautomerization.

Finally, enamide **14** should be available from coupling of the tryptophan derivative **16** with *cis*-3-hydroxy-L-proline



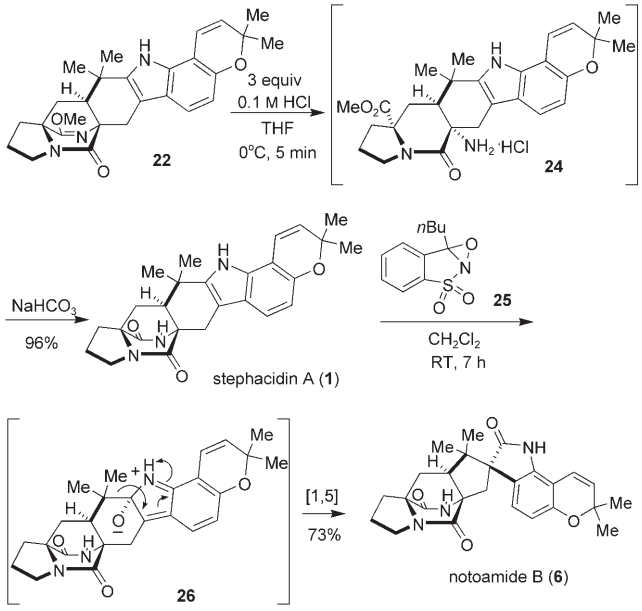
Scheme 2. Postulated biosynthetic relationships between the alkaloids.

ethyl ester (**15**), followed by removal of the Fmoc group, concomitant diketopiperazine formation, and dehydration. Compound **16** is readily available from the corresponding gramine **17**, and has been recently synthesized in our group on gram scale.[9,14]

Our synthesis commenced with the coupling of the L-proline derivative **15**[15] and acid **16** in the presence of BOPCl to afford amide **18** in 54% yield as an inseparable 1:1 mixture of diastereomers (Scheme 4). Various amounts of starting material (25%–35%) were recovered, and the reaction appeared more sluggish than an analogous coupling of **16** with the parent (*S*)-proline methyl ester hydrochloride as reported in the preceding Communication.[9] Peptide **18** was subjected to a solution of morpholine in THF at room temperature to effect removal of the Fmoc group and a concomitant cyclization of the resultant amine onto the ethyl ester provided diketopiperazine **19** as a separable mixture of diastereomers. Both diastereomers were then found to undergo a smooth Mitsunobu-type elimination (PBu_3, DEAD) to afford the enamide **14**, (Scheme 3) which was treated with Me_3OBF_4 and Cs_2CO_3 to provide the desired lactim ether **20** cleanly in good yield.

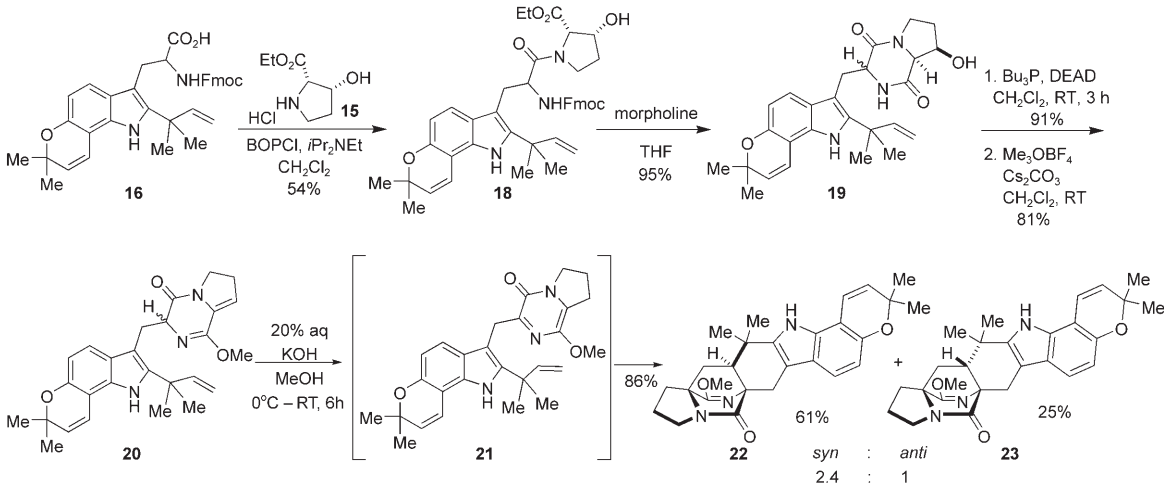
We next directed our attention toward the key cycloaddition reaction and the completion of the total synthesis of stephacidin A. We were delighted to find that treatment of **20** with 20% aqueous KOH in MeOH effected tautomerization to the intermediate azadiene **21**, which spontaneously suffered IMDA cycloaddition to produce the cycloadducts **22** and **23** as a 2.4:1 mixture of diastereomers favoring the desired *syn* stereoisomer. Interestingly, the intermediate azadiene **21** is a metastable substance that could be observed by both TLC and ^{1}H NMR analysis. During the course of the reaction, the lactim ether **20** ($R_f = 0.75$, EtOAc) disappeared within 1.5 h, as evident by TLC, and the azadiene **21** ($R_f = 0.25$, EtOAc) appeared. Then, the azadiene slowly disappeared, as evident by TLC, and the cycloadducts **22** and **23** ($R_f \approx 0.4$, EtOAc) appeared. The azadiene intermediate **21** was also observable by ^{1}H NMR spectroscopy through treatment of **20** with KOD in CD_3OD/D_2O in an NMR tube.

The tentative stereochemical assignment for cycloadduct **22** was confirmed upon its transformation to racemic stephacidin A (**1**). Thus, treatment of **22** with HCl in THF effected cleavage of the lactim ether to afford, through the corresponding secondary amide, stephacidin A (**1**) in 96% yield (Scheme 5). All ^{1}H and ^{13}C NMR spectroscopic data for



Scheme 5. Synthesis of stephacidin A and notoamide B.

synthetic stephacidin A (**1**) corresponded with those reported previously ([D_6]dimethylsulfoxide and $CDCl_3/CD_3OD$, 1:1).[4] Interestingly, this reaction appears to proceed through the ring-opened amino ester **24**, which spontaneously cyclizes to **1** during workup. Amino ester **24**, which could be observed by TLC ($R_f = 0.12$, 10% MeOH/CH_2Cl_2), slowly disappeared on concentration of the reaction mixture to give rise to **1** ($R_f = 0.68$, 10% MeOH/CH_2Cl_2). Our research group also encountered similar ring-opened amino ester intermediates in the total synthesis of VM55599.[13b]



Scheme 4. Construction of the ring system of **1** by using an IMDA cycloaddition. BOPCl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride, DEAD = diethyl azodicarboxylate.

With stephacidin A (**1**) in hand, we directed our attention to the stereoselective oxidation and pinacol rearrangement of **1** to notoamide B (**6**). Indeed, we were pleased to find that treatment of **1** with excess oxaziridine **25**[16] in CH_2Cl_2 cleanly provided **6** as a single product in 73% yield. The stereochemistry of the transformation can be rationalized by the epoxidation of the 2,3-disubstituted indole occurring from the less-hindered α face, followed by ring opening of the incipient epoxide to the 2-alkoxyindole intermediate **26**. A subsequent α-face ring contraction by a [1,5] sigmatropic shift successfully furnished **6** as a single diastereomer. Again, all ^1H and ^{13}C NMR spectroscopic data ([D_6]acetone) corresponded to those reported by Tsukamoto and co-workers.[6] Furthermore, the use of oxaziridine **25** for the one-step conversion of a 2,3-disubstituted indole into the corresponding spirooxindole is an unprecedented and potentially highly useful transformation. Standard methods for effecting such an oxidative ring contraction have been known for many years and typically involve treatment of the indole substrate with *tert*-butyl hypochlorite to give a 3-chloroindolenine that must be hydrated to a *syn*-chlorohydrin that subsequently undergoes a rearrangement to the spirooxindole.[17,18] Additional applications of this mild and direct reaction to form oxindoles are currently being explored.

In conclusion, we have completed a concise biomimetic total synthesis of stephacidin A (**1**) in 17 steps and 5.4% overall yield from the commercially available 6-hydroxyindole by using an azadiene IMDA reaction. In addition, we have also effected the biomimetic oxidation of stephacidin A (**1**) to the closely related fungal metabolite notoamide B (**6**). This study underscores the low activation barriers inherent in this specific class of azadiene IMDA reactions that have been strongly implicated in the construction of the bicyclo-[2.2.2]diazaoctane core ring system that is common to the paraherquamide/stephacidin/notoamide family of prenylated indole alkaloids. Further studies to experimentally corroborate the biogenetic relationship between the notoamides and stephacidins, as well as the validity of proposed biosynthetic intermediates, such as **1**, **7**, and **9**–**12** (Scheme 2) are currently under investigation and will be disclosed in due course.

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