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Enantioselective Total Synthesis of Avrainvillamide and the Stephacidins

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Abstract: In this article, full details regarding our total synthesis of avrainvillamide and the stephacidins are presented. After an introduction and summary of prior synthetic studies in this family of structurally complex anticancer natural products, the evolution of a final synthetic approach is described. Thus, a thorough description of three separate model studies is provided for construction of the characteristic bicyclo-[2.2.2]diazaoctane ring system common to these alkaloids. The first and second approaches sought to build the core using formal Diels−Alder and vinyl radical pathways, respectively. Although these strategies failed in their primary objective, they fostered the development of a new and mechanistically intriguing method for the synthesis of indolic enamides such as those found in numerous bioactive natural products. The scope and generality of this simple method for the direct dehydrogenation of tryptophan derivatives is described. Finally, details of a third and successful route to the core of these alkaloids are described which features oxidative C−C bond formation. Specifically, the first heterocoupling of two different types of carbonyl species (ester and amide) is accomplished in good yield, on a preparative scale, and with complete stereocontrol. The information gained in these model studies enabled an enantioselective total synthesis of stephacidin A. The absolute configuration of these alkaloids was firmly established in collaboration with Professor William Fenical. A full account of our successful efforts to convert stephacidin A into stephacidin B *via* avrainvillamide is presented. Finally, the first analogues of these natural products have been prepared and evaluated for anticancer activity.

Introduction

In 2002, scientists from Bristol Myers Squibb added a new entry to the list of structurally intriguing and bioactive indole alkaloids with the discovery of stephacidins A and B (**1** and **2** in Figure 1, respectively).[1] Isolated from a fungal species found in an Indian clay sample, they show obvious structural similarities to the brevianamides,[2] paraherquamides,[3] and asperparalines.[4] In 2000, the Fenical group at the Scripps Institution of Oceanography disclosed the structure of avrainvillamide (**3**) in a patent.[5] This compound was independently described in 2001 by a Pfizer group in Japan and named CJ-17,665.[6] The signature bicyclo[2.2.2]diazaoctane ring system common to these alkaloids has inspired numerous synthetic approaches.

Birch,[7] Sammes,[8] and Williams[9] have proposed that this ring system is derived biosynthetically from a Diels−Alder reaction. This hypothesis has influenced several creative synthetic strate-

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10.1021/ja061660s CCC: $33.50

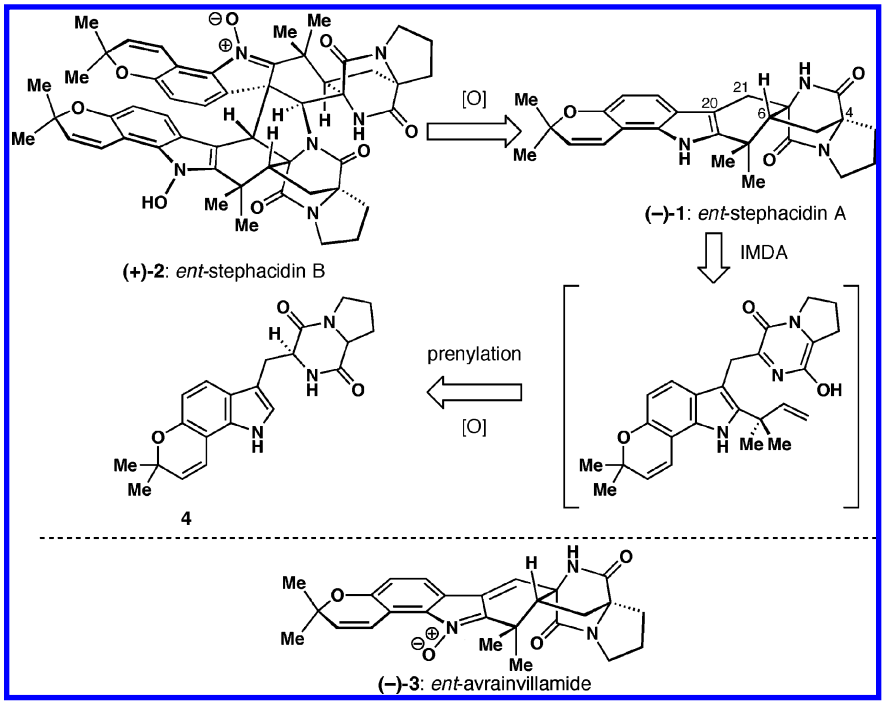

Figure 1. The stephacidins and their presumed biogenetic origins.

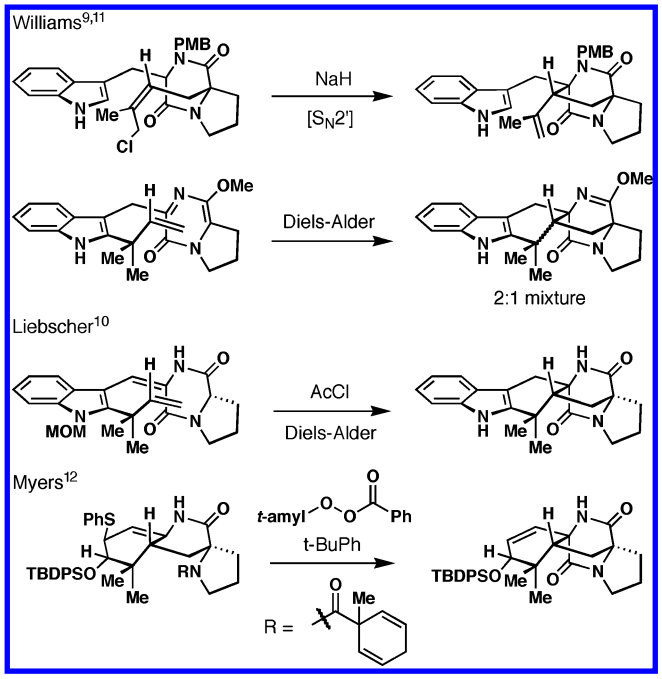

Figure 2. Approaches to construct the bicyclo[2.2.2]diazaoctane nucleus.

gies for its construction, as shown in Figure 2. Thus, the groups of Williams[9] and Liebscher[10] independently developed Diels−Alder strategies with varying levels of success; the Williams group also developed a novel stereoselective intramolecular S_N2' alkylation approach for the construction of this bicyclic core.[9,11] In Myers's recent total synthesis of stephacidin B, an elegant acyl radical approach was utilized.[12]

Our interest in these natural products initially stemmed from their considerable potential to inspire the development of new chemistry; they later became an ideal stage to showcase the power of oxidative enolate coupling in synthesis (*vide infra*). In this article we trace the evolution of our synthetic strategy, a journey which culminated in the total syntheses of stephacidins A and B and avrainvillamide.[13] Aside from successfully applying a strategy employing the first stereocontrolled oxidative enolate heterocoupling to forge the bicyclo[2.2.2]diazaoctane nucleus, several challenges were addressed including: (1) the design of a scalable route to the benzopyran-containing subunit; (2) precise selection and choreography of functional group masking devices; (3) chemo- and position-selective oxidative conversion of stephacidin A (**1**) into avrainvillamide (**3**); (4) the dimerization of avrainvillamide to stephacidin B (**2**); and (5) determination of the absolute configuration of this class of alkaloids. The difficulty of this final issue was compounded by the fact that the optical rotation of the stephacidins was not recorded.[1]

Results and Discussion

First Generation Retrosynthetic Analysis and Development of a New Method for Tryptophan Dehydrogenation. The obvious potential complications associated with a laboratory conversion of stephacidin A into stephacidin B were temporarily put aside, and the former was targeted for synthesis as the precursor to the latter. Our first generation retrosynthetic analysis, depicted in Figure 3, hinged on the use of a formal Diels−Alder reaction to construct both the bicyclic skeleton and prenylated indole subunits in one step.[14] Unlike the achiral intermedi-

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(11) For studies toward stephacidin A from the Williams group see: (a) Adams, L. A.; Gray, C. R.; Williams, R. M. *Tetrahedron Lett.* **2004**, *45*, 4489−4493. (b) Grubbs, A. W.; Artman, G. D., III; Williams, R. M. *Tetrahedron Lett.* **2005**, *46*, 9013−9016.
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(13) (a) Baran, P. S.; Guerrero, C. A.; Ambhaikar, N. A.; Hafensteiner, B. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 606−609. (b) Baran, P. S.; Guerrero, C. A.; Hafensteiner, B. D.; Ambhaikar, N. A. *Angew. Chem. Int. Ed.* **2005**, *44*, 3892−3895.
(14) For examples of Diels−Alder reactions of 3-vinyl indoles: (a) Noland, W. E.; Xia, G.-M.; Gee, K. R.; Konkel, M. J.; Wahlstrom, M. J.; Condoluci, J. J.; Rieger, D. L. *Tetrahedron* **1996**, *52*, 4555−4572. (b) Gonzalez, E.; Pindur, U.; Schollmeyer, D. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1767−1771. (c) Le Strat, F.; Maddaluno, J. *Org. Lett.* **2002**, *4*, 2791−2793. (d) Grieco, P. A.; Kaufman, M. D. *J. Org. Chem.* **1999**, *64*, 7586−7593. (e)

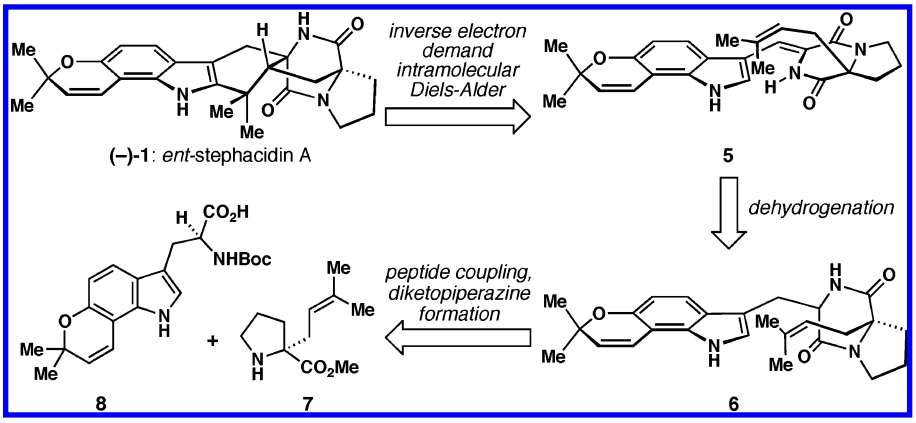
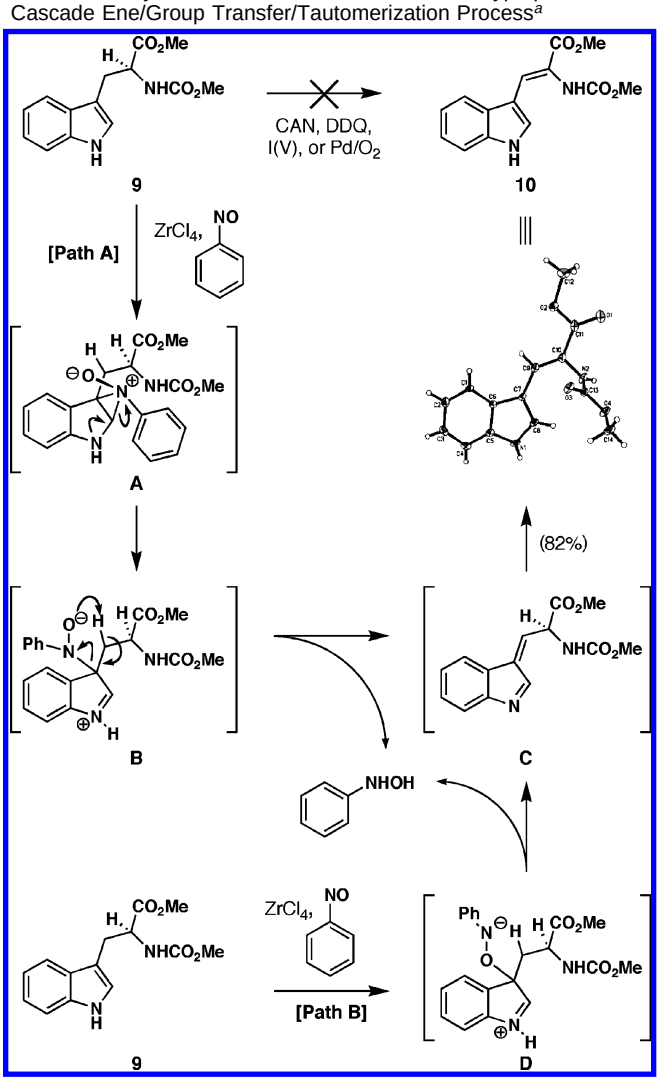


Figure 3. First generation retrosynthetic analysis for stephacidin A.

ates in the proposed biosynthesis, this cyclization event benefited from the presence of a quarternary center that could be used to govern the absolute stereochemical outcome of the reaction. This approach, although concise, was not without its potential pitfalls and drawbacks. Although molecular models showed that the diene/dienophile pretransition state assembly was nearly perfect, there was no reason to assume *a priori* that the dienophile would ever find itself in proximity to the diene. (This was based on the assumption that the enamide olefin could be isomerized to the *E* geometry (not shown) in the course of the cycloaddition. Alternatively, the directly accessible *Z* geometry (see **5**) would be acceptable if the proposed cycloaddition reaction took place in a stepwise rather than concerted manner.) Additionally, two quaternary stereocenters would be generated in the course of the cycloaddition. This consideration did not heavily influence our planning, however, because it had been proposed by Birch,[7] Sammes,[8] and Williams[9] that an equally complex event takes place in the biosynthesis of the brevianamides (Figure 2). The requisite substrate diene **5** could conceivably be accessed from saturated diketopiperazine **6** *via* a dehydrogenation event. Peptide coupling of the proline fragment **7** with indole **8** followed by diketopiperazine formation should furnish **6**.

The success of the "Diels–Alder" approach was contingent on the availability of a mild protocol for the dehydrogenation of tryptophans (e.g., **6** → **5**). Known methods allow access to these types of compounds; however, they typically involve multistep syntheses.[15] For instance, a published method for the synthesis of simple tryptophan derivative **10** (Scheme 1) utilizes a Wittig-type reaction with indole-3-carboxaldehyde. The enzymatic dehydrogenation of certain tryptophans has been reported by Hammadi using L-tryptophan-2′,3′-oxidase from *Chromobacterium violaceum* (ATCC 12472).[16] Using DDQ as the oxidant, certain indolic enamides have been shown to form

Scheme 1. Synthesis of Indolic Enamides from Tryptophans *via* a Cascade Ene/Group Transfer/Tautomerization Process[a]



[a] CAN = ceric ammonium nitrate, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

in good yields from tryptophans possessing strongly electron-withdrawing substituents such as a trifluoroacetyl group.[17] Such an approach, however, has not been employed in the dehydrogenation of diketopiperazines such as **6**. The model tryptophan

Pindur, U.; Otto, C.; Molinier, M.; Massa, W. *Helv. Chem. Acta* **1991**, *74*, 727–738. (f) Pindur, U.; Kim, M. H.; Rogge, M.; Massa, W.; Molinier, M. *J. Org. Chem.* **1992**, *57*, 910–915. (g) Fukazawa, T.; Shimoji, Y.; Hashimoto, T. *Tetrahedron: Asymmetry* **1996**, *7*, 1649–1658. (h) Beccalli, E. M.; Marchesini, A.; Pilati, T. *Tetrahedron* **1996**, *52*, 3029–3036. (i) Wolter, M.; Borm, C.; Merten, E.; Wartchow, R.; Winterfeldt, E. *Eur. J. Org. Chem.* **2001**, 4051–4060.

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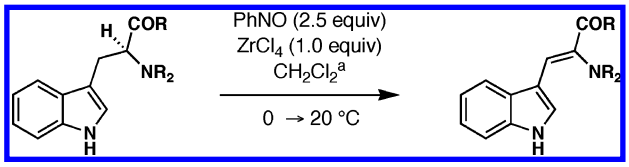
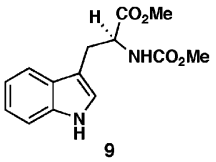
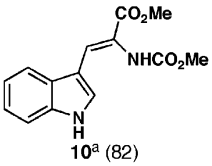
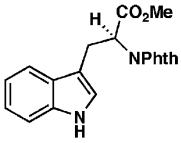
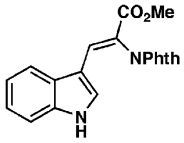
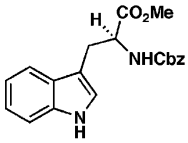
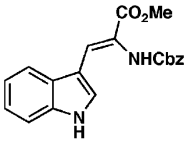
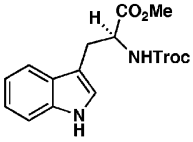
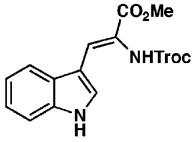
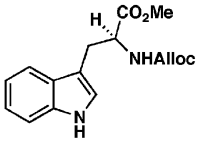
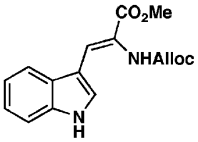
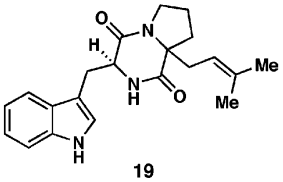
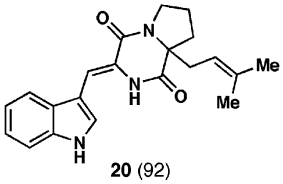
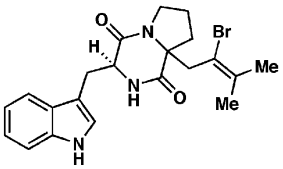
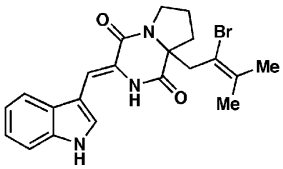
9 was screened with a variety of oxidants such as DDQ, CAN, IBX, and $Pd/C/O_2$ to elicit conversion to the enamide **10**. Although DDQ in THF[17] led to the disappearance of the starting material and the formation of polar byproducts, **10** could not be detected. Faced with these failures, a new method was invented which exploited the unique reactivity of these systems. In principle, the enophilic π bond of indole could be exploited such that it could form an adduct susceptible to elimination (Cope-like) followed by tautomerization to the desired α,β-unsaturated system. This strategy would fill the gap in current methodology and avoid the use of harsh oxidizing agents. Since indoles readily react with singlet oxygen[18] and triazolinediones[19] it was reasoned that nitroso compounds[20,21] would react with similar facility. Unfortunately, simply stirring nitrosobenzene and the tryptophan **9** in a wide variety of solvents at 20 °C or at elevated temperatures led to a full recovery of the starting material.

Inspired by Yamamoto's work on Lewis and Brønsted acid catalyzed reactions of enolates and enamines with nitroso compounds,[21] we decided to perform the above reaction in the presence of such acids. Indeed, when $ZrCl_4$ (1 equiv) was added to a solution of nitrosobenzene (2.5 equiv) and **9** in CH_2Cl_2, dehydrogenated product **10** was isolated in 82% yield as a single alkene isomer. The structure and *Z*-olefin geometry was confirmed by X-ray crystallography as shown in Scheme 1. Mechanistically, one of two pathways may be operative as depicted in Scheme 1 (paths A and B). These mechanisms differ by their initial step: electrophilic attack by the indole on the nitrogen or oxygen of nitrosobenzene. In path A, formation of aziridine *N*-oxide **A** followed by fragmentation and Cope-like elimination of phenylhydroxylamine from *N*-oxide **B** furnishes methylene indolenine **C** which undergoes immediate tautomerization to **10**. In path B, Lewis or Brønsted activation of nitrosobenzene at nitrogen elicits conversion to *O*-linked intermediate **D**, which might immediately fragment to **C** and phenylhydroxylamine. In light of Yamamoto's pioneering studies in this area, path B seems to be more likely as *O*-linked adducts are generally formed with nitroso compounds in the presence of acidic additives.

Several acids and solvents were screened using the phthalimide-protected tryptophan **11** (see Supporting Information for a tabular comparison of acids tested). The reaction appears to have reasonable generality using $ZrCl_4$ as shown in Table 1. Most importantly, this reaction is ideally suited to the task of dehydrogenating unprotected diketopiperazines (entries 6 and 7) and will be discussed in further detail below. It should be noted that other substituted nitroso compounds (nitrosotoluene and 4-nitrosodiphenylamine) did not promote the reaction. Significantly, this reaction may provide facile access to natural products[22] bearing unsaturated tryptophan or tryptamine subunits from their readily available saturated analogues.

Table 1. Direct Dehydrogenation of Tryptophan Derivatives



Entry	Reactant	Time (h)	Product (% yield)[b]
1	 **9**	2	 **10**[a] (82)
2	 **11**	1	 **12** (82)
3	 **13**	12	 **14**[a] (30,[b] 67[c])
4	 **15**	12	 **16**[a] (33,[b] 47[c])
5	 **17**	3	 **18** (45)
6	 **19**	2.5	 **20** (92)
7	 **21**	3	 **22** (79)

[a] Entries 1, 3, and 4 were run in toluene. [b] Isolated yield. [c] Yields based on recovered starting material.

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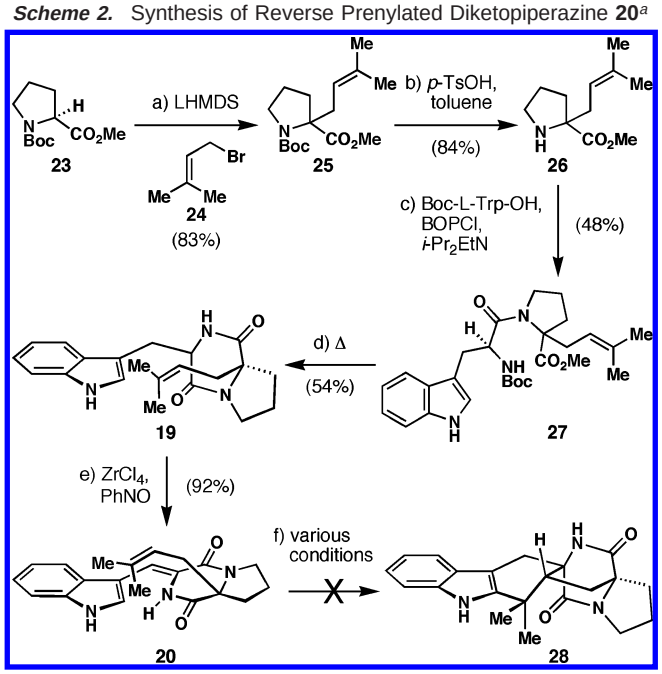
Scheme 2. Synthesis of Reverse Prenylated Diketopiperazine **20**[a]



[a] Reagents and conditions: (a) LHMDS (1.1 equiv), THF, −78 °C, 35 min; then **24** (1.1 equiv), −78 → 20 °C, 2 h, 83%; (b) *p*-TsOH (1.0 equiv), toluene, reflux, 2 h, 84%; (c) Boc-L-Trp-OH (1.0 equiv), BOPCl (1.1 equiv), *i*-Pr_2EtN (3.0 equiv), CH_2Cl_2, 20 °C, 12 h, 48%; (d) 190 °C, neat, 45 min, 54%; (e) PhNO (2.5 equiv), $ZrCl_4$ (1.0 equiv), CH_2Cl_2, 20 °C, 2.5 h, 92%. LHMDS = lithium bis(trimethylsilyl)amide, THF = tetrahydrofuran, *p*-TsOH = *p*-toulenesulfonic acid monohydrate, BOPCl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride.

With a simple and reliable method for dehydrogenation in hand, the model diene **20** (entry 6, Table 1) was constructed as shown in Scheme 2. Thus, *N*-Boc-L-proline methyl ester (**23**) was alkylated[23] with prenyl bromide (**24**) to afford racemic **25** in 83% yield. Subsequent *N*-Boc-deprotection with *p*-TsOH[24] afforded **26** (84%) which was coupled with *N*-Boc-L-tryptophan (1.1 equiv of BOPCl,[25] 3.0 equiv of *i*-Pr_2EtN, 12 h, 20 °C) to provide peptide **27** in 48% yield (unoptimized). Thermolysis of **27** at 190 °C for 45 min resulted in *N*-Boc-deprotection[26] and cyclization to diketopiperazine **19** in 54% yield (inconsequential mixture of two diastereomers). The key dehydrogenation proceeded uneventfully and efficiently (92% isolated yield) to afford Diels−Alder precursor **20**.

Several conditions were explored to promote the key cycloaddition such as refluxing in toluene, xylenes, or other high boiling solvents; the use of various Lewis acids ($AlCl_3$, $TiCl_4$, $ZrCl_4$) and protic acids; and also heating the substrate neat at high temperatures (300 °C). Because of the ambiguous electronic nature of **20**, rhodium(I)-catalyzed cycloisomerization was also attempted.[27] In addition, photochemical conditions were explored in the hopes of eliciting a formal Diels−Alder reaction *via* radical intermediates. Substrate **20** was surprisingly robust and emerged unscathed in these failed attempts. The bis-acetylated derivative of **20** was also prepared and found to be similarly unreactive. In retrospect, several factors may have contributed to the failure of this cycloaddition: (1) the diene-dienophile set may have been electronically mismatched; (2) the unreactive *s*-trans configuration of the diene (*s*-cis is depicted) might be highly favored to avoid steric clashing of the diketopiperazine N−H and indole C2−H; and (3) the loss of aromaticity and conjugation that would be required of the immediate Diels-Alder product prior to tautomerization.

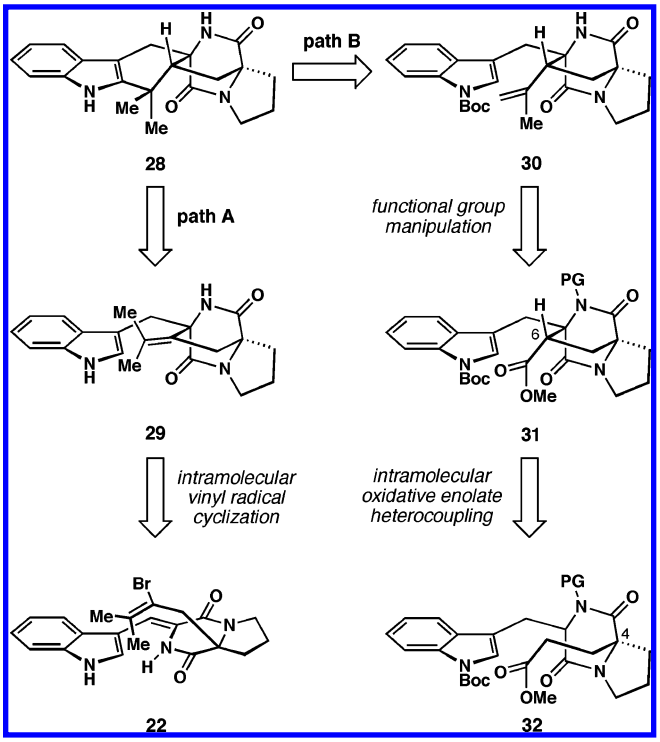


Figure 4. Second and third generation approaches toward the construction of the stephacidin A core.

Second Generation Retrosynthesis. The difficulties encountered in our first approach prompted a slight retreat to a radical-based strategy depicted in Figure 4 (path A). It was reasoned that the model compound **28** could be accessed *via* a vinyl radical cyclization[28] directly from **22** (entry 7, Table 1) or in a stepwise fashion *via* the isolated intermediate **29**. The synthesis of vinyl precursor **22** was carried out in a manner analogous to that used for the Diels−Alder precursor **20** as shown in Scheme 3.

Thus, *N*-Boc-L-proline methyl ester (**23**) was alkylated with the known allylic bromide **33**[29] giving **34** in 83% yield (Scheme 3). This racemic proline derivative was deprotected using *p*-TsOH[25] (85%) (giving **35**) and coupled with *N*-Boc-L-

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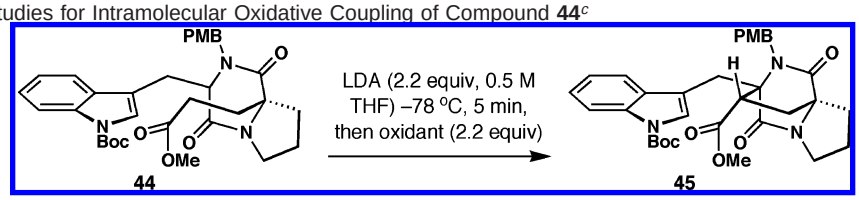
(29) Wijnberg, J. B. P. A.; Wiering, P. G.; Steinberg, H. *Synthesis* **1981**, *11*, 901−903.

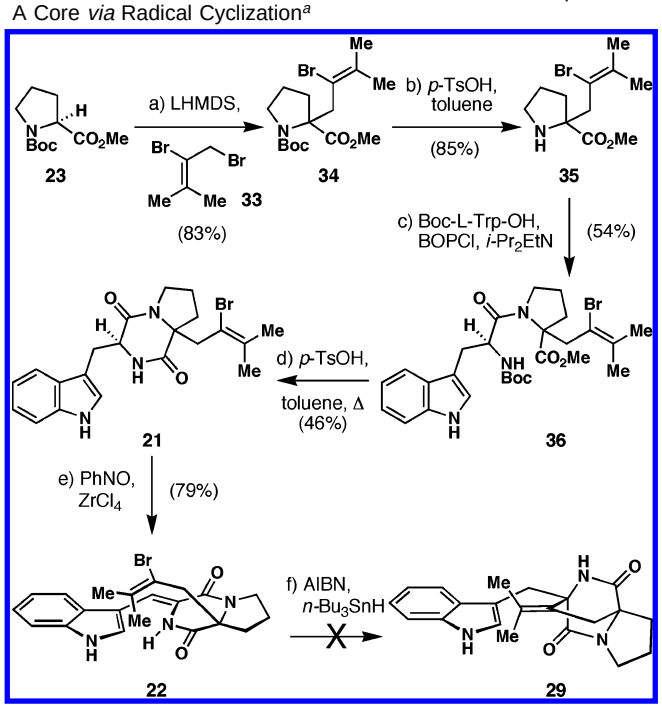
Table 2. Optimization Studies for Intramolecular Oxidative Coupling of Compound **44**[c]



Entry	Oxidant (concentration)	[O] Potential (eV)	Yield[a] (%)
1	I_2 (0.05 M THF)	−0.39	0
2	$FeCl_3$ (0.2 M DMF)	*b*	31
3	$Fe(Cp)_2PF_6$ (0.2 M DMF)	−0.28	26
4	$Fe(acac)_3$ (0.2 M THF)	−1.14	65
5	$CuCl_2$ (0.2 M DMF)	*b*	39
6	$Cu(OTF)_2$ (0.2 M DMF)	*b*	35
7	Cu(4-cyclohexylbutyrate)$_2$ (0.1 M 1:1 THF:DMF)	*b*	19
8	Cu(2-ethylhexanoate)$_2$ (0.1 M THF)	−1.65	29

[a] Isolated yield. [b] No meaningful data could be obtained. [c] Measured using cyclic voltammetry; potentials relative to ferrocene-ferrocenium standard.

Scheme 3. Model Studies toward the Construction of Stephacidin A Core *via* Radical Cyclization[a]



[a] Reagents and conditions: (a) LHMDS (1.1 equiv), THF, −78 °C, 35 min; then **33**[29] (1.1 equiv), −78 °C, 2 h, 83%; (b) *p*-TsOH (1.0 equiv), toluene, reflux, 2 h, 85%; (c) Boc-L-Trp-OH (1.0 equiv), BOPCl (1.1 equiv), *i*-Pr_2EtN (3.0 equiv), CH_2Cl_2, 20 °C, 12 h, 54%; (d) (i) *p*-TsOH (1.0 equiv), toluene, reflux, 2 h; (ii) toluene, reflux, 4 h, 46%; (e) PhNO (2.5 equiv), then $ZrCl_4$, CH_2Cl_2, 20 °C, 2 h, 79%. AIBN = azobis(isobutyronitrile).

tryptophan furnishing **36** (54%, unoptimized) which was then transformed to diketopiperazine **21** (46% yield) by *p*-TsOH-catalyzed Boc removal[25] and ring closure upon heating. Dehydrogenation proceeded smoothly as before using $ZrCl_4$/PhNO to afford vinyl bromide **22** in 79% yield. Unfortunately, we were unable to effect the conversion of **22** to even the monocyclized indole **29** under a variety of conditions. Ultimately, this strategy was not intensely pursued for two reasons. First, even if bicycle **29** could be generated, its efficient conversion to hexacycle **28** was uncertain since there was no reason to believe either face of the olefin in **29** would be more readily protonated or attacked by the indole. Second, an enticing strategy employing oxidative coupling was on the horizon and had the potential of revealing a relatively unexplored area of C−C bond formation and chemical reactivity.

Development of a Successful Strategy. In an ongoing project in our laboratory involving the total synthesis of the fischerindole,[30] hapalindole,[30] and welwitindolinone[31] alkaloids it was shown that enolates of carbonyl compounds undergo oxidative coupling with indoles and pyrroles[32] in the presence of metal oxidants such as Cu(II). This method directly merges sp^2 and sp^3 carbons, enabling the one-step preparation of a variety of α-linked heterocycles without using protecting groups or halogen atoms. Although the seminal work on oxidative dimerization of enolates was performend more than thirty years ago,[33] these represented the first synthetically useful heterocouplings of different types of enolates ("enamines" with ketones, esters, and amides). Oxidative enolate couplings had received very little attention in the chemical literature and had rarely been applied in the total synthesis of complex natural products.[34] Given our experience with these reactions, we wondered whether ester and amide enolates could be coupled together selectively in an intramolecular sense.

Although little is known regarding the pretransition state assembly during such an oxidative coupling event, we reasoned that the carbonyl oxygens (see **32** in Figure 4) of the two possible enolates would be oriented in a head-to-head fashion upon binding to a single, multivalent metal counterion. The two enolates should be forced together temporarily not only due to nonbonded interactions with the proline ring, but also by the Thorpe−Ingold effect imposed by the all-carbon quaternary center at C-4 (see **32**, Figure 4). In this arrangement, the sp^2-hybridized α-carbons of the enolized carbonyl species would be essentially stacked on top of each other and forced to react based on proximity. Models also indicated that the desired stereochemical outcome would arise independent of the ester

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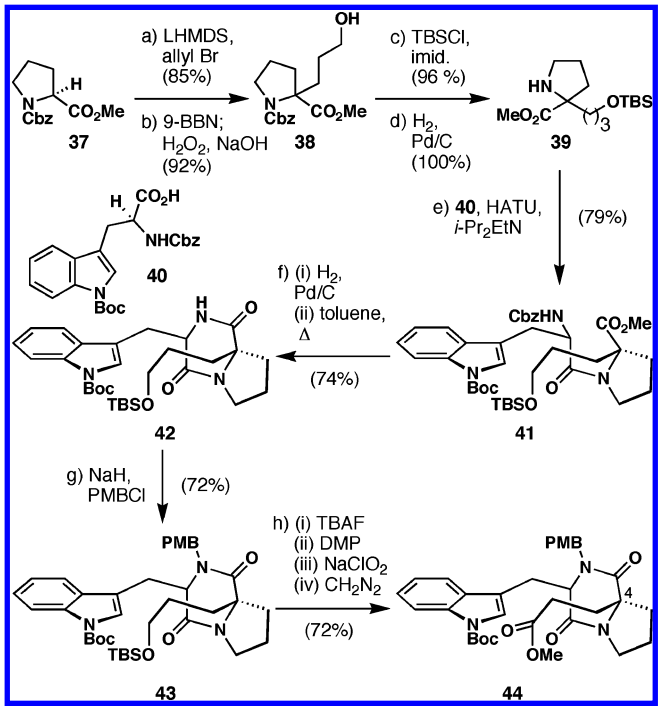
Scheme 4. Synthesis of Model Ester **44**[a]

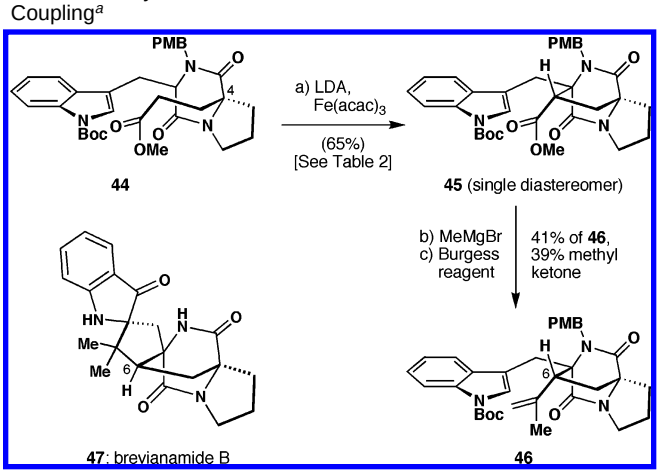


[a] Reagents and conditions: (a) LHMDS (1.0 equiv), THF, −78 °C, 35 min; then allyl bromide (1.1 equiv), −78 °C, 2 h, 85%; (b) 9-BBN (2.0 equiv), THF, 20 °C, 3 h; then 3 M aq. NaOH/35% aq. H_2O_2 (1:1), 1 h, 92%; (c) TBSCl (1.1 equiv), imidazole (1.2 equiv), CH_2Cl_2, 20 °C, 30 min, 96%; (d) H_2, 10% Pd/C (20% w/w), MeOH, 20 °C, 3 h, 100%; (e) compound **40** (1.0 equiv), HATU (1.1 equiv), *i*-Pr_2EtN (3.0 equiv), DMF, 20 °C, 12 h, 79%; (f) (i) H_2, 10% Pd/C (20% w/w), EtOAc/MeOH 1:1, 20 °C, 5 h; (ii) toluene, reflux, 4 h, 74%; (g) NaH (1.0 equiv), DMF, 0 °C, 30 min; then PMBCl (1.2 equiv), 0 °C, 2 h, 72%; (h) (i) TBAF (3.0 equiv), THF, 2 h; (ii) DMP (1.1 equiv), CH_2Cl_2, 20 °C, 1.5 h; (iii) $NaClO_2$ (2.8 equiv), $NaH_2PO_4 \cdot H_2O$ (3.0 equiv), 2-methyl-2-butene (20.0 equiv), THF/H_2O, 20 °C, 30 min; (iv) CH_2N_2, MeOH, 0 °C, 72%. 9-BBN = 9-borabicyclo[3.3.1]nonane; TBSCl = *tert*-butyldimethylsilyl chloride; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, DMF = *N*,*N*-dimethylformamide, PMBCl = *p*-methoxybenzyl chloride, TBAF = tetrabutylammonium fluoride, DMP = Dess−Martin periodinane.

enolate geometry (however, one would expect the *Z* enolate to be formed under kinetic enolization in the absence of HMPA, *vide infra*). Further encouragement came from observing that in Williams's studies,[9b] the stereochemical outcome of an S_N2' cyclization could be determined by proper cation selection and suitable tuning of the reaction conditions to prevent or promote tight ion pairing. Significantly, the quaternary stereocenter of the proline subunit would control the facial selectivity of the new bond being formed (relative to the diketopiperazine). From the standpoint of synthetic design, this implied that a racemic tryptophan could be utilized and only the quaternary proline stereocenter needed to be controlled for an eventual enantioselective synthesis. To evaluate these considerations, a synthesis of model compound **44** was undertaken (Scheme 4).

Thus, *N*-Cbz-L-proline methyl ester (**37**) was treated with LHMDS and allyl bromide to provide the racemic alkylated product in 85% yield (see Scheme 4). Hydroboration of the pendant olefin with 9-BBN[35] gave the alcohol **38** in 92% yield following oxidative workup. Subsequent TBS protection[36] of

Scheme 5. Synthesis of Olefin **46** *via* Intramolecular Oxidative Coupling[a]



[a] Reagents and conditions: (a) LDA (2.2 equiv), −78 °C, 5 min; then $Fe(acac)_3$ (2.2 equiv), THF, −78 → 20 °C, 65%; (b) (i) MeMgBr (5.0 equiv), toluene, 0 °C, 2 h; (ii) Burgess reagent (3.0 equiv), benzene, 50 °C, 20 min, 41% overall, 39% recovered methyl ketone. LDA = lithium diisopropylamide, $Fe(acac)_3$ = iron (III) acetylacetonate.

38 (96%) and deprotection of the Cbz group[37] (H_2, Pd/C) furnished amine **39** in quantitative yield. The coupling of amine **39** with the bis-protected tryptophan **40**[38] proceeded optimally using HATU[39] to furnish amide **41** in 79% yield. Alternative coupling agents such as EDC and BOPCl provided only modest yields of **41**. Cbz deprotection (H_2, Pd/C) liberated the free amine which cyclized upon heating in toluene to give diketopiperazine **42**. In preparation for the key oxidative coupling step, the free N−H of the diketopiperazine was protected with a PMB group (NaH, PMBCl)[40] to afford **43** in 72% isolated yield. The use of a PMB group would facilitate structure confirmation by comparison of our intermediates to those synthesized by Williams and co-workers (*vide infra*). The oxidative coupling precursor ester **44** was prepared from TBS-protected alcohol **43** by a standard deprotection/oxidation[41] sequence depicted in Scheme 4 which required a single purification and proceeded in 72% overall yield. To our delight, ester-amide **44** readily underwent intramolecular oxidative heterocoupling (LDA, −78 °C, 5 min then $Fe(acac)_3$, −78 → 20 °C) to furnish **45** as a single diastereomer in 65% yield (Scheme 5).

At the time, spectroscopic data indicated that the desired oxidation had taken place; however, the stereochemistry at C-6 (stephacidin A numbering, see Scheme 5) could not be decisively determined. Fortunately, in 1990 Williams[9b] and co-workers completed a synthesis of brevianamide B (**47**) in which *both* epimers (at C-6, stephacidin A numbering) of pentacycle **46** were synthesized. Correlating our data with those of the Williams group allowed for an unambiguous stereochemical determination. This comparison was made after compound **45** was converted to olefin **46** by tandem methyl Grignard addition[42] (5 equiv, MeMgBr, toluene, 20 °C) and dehydration with the

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(41) Ireland, R. E.; Meissner, R. S.; Rizzacasa, M. A. *J. Am. Chem. Soc.* **1993**, *115*, 7166−7172.

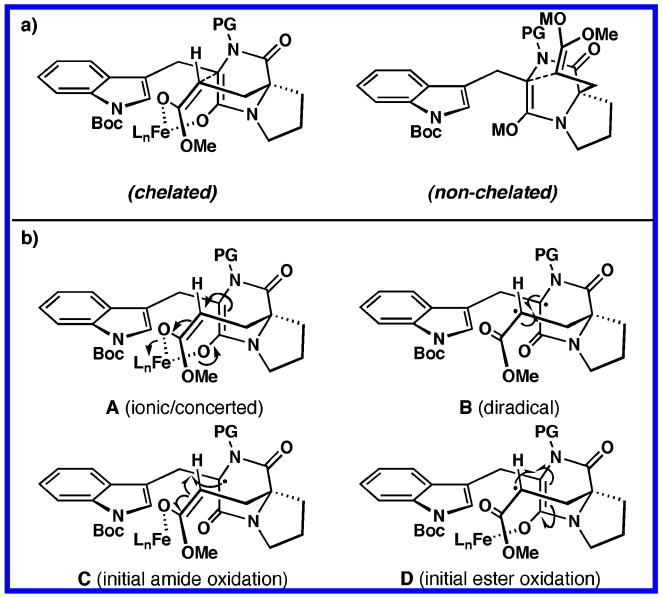


Figure 5. (a) Chelated and nonchelated pretransition state assemblies. (b) Possible bond forming scenarios.

Burgess reagent[43] (3 equiv, 50 °C) in 41% overall yield (unoptimized). Olefin **46** possessed the requisite stephacidin A C-6 stereochemistry, in contrast to that found in brevianamide B (**47**).

LDA (2.2 equiv) proved to be the base of choice to bring about bis-enolization at −78 °C. Several oxidants with varying oxidation potentials were investigated. The use of copper salts to oxidize the enolates showed encouraging results (Table 2) with significant product formation (isolated yields ranging from 19 to 39%, see Supporting Information for optimization studies). Perhaps due to its unique oxidation potential, $Fe(acac)_3$ furnished the highest yield. Notably, the use of more than 2.2 equiv of $Fe(acac)_3$ led to a decreased yield. The optimal enolization time was observed to be 5 min. When enolization times exceeded 5 min, undesired side products often formed, presumably arising from competing Dieckmann cyclization and α-oxidation.

To the best of our knowledge, this transformation represents the first example of an intramolecular oxidative heterocoupling of two different types of carbonyl enolates. The good yield of this reaction, which accomplishes the construction of adjacent quaternary and tertiary stereocenters with complete stereocontrol, might be due in part to exquisite selectivity of the iron oxidant used. It is possible that this oxidant succeeds by selectively or more rapidly oxidizing one of the enolate species (the amide or ester). The resulting radical and remaining enolate can then combine to give a ketyl-type radical anion that can be oxidized further to the final product. Lower yields may result from nonselective oxidation since either the amide or ester radical may not react as desired for steric or electronic reasons. The observed diastereoselectivity may be the result of a chelated transition state (see Figure 5) or simply the innate bias of the substrate. The former explanation is favored since the Williams group has been able to control the stereochemistry of alkylations in similar systems based on the presence or absence of metal

(42) (a) Bergmann, E.; Bondi, A. *J. Am. Chem. Soc.* **1936**, *58*, 1814. (b) Baran, J. S.; Laos, I.; Langford, D. D.; Miller, J. E.; Jett, C.; Taite, B.; Rohrbacher, E. *J. Med. Chem.* **1985**, *28*, 597−601.
(43) Burgess, E. M.; Penton, H. R., Jr.; Taylor, E. A. *J. Org. Chem.* **1973**, *38*, 26−31.

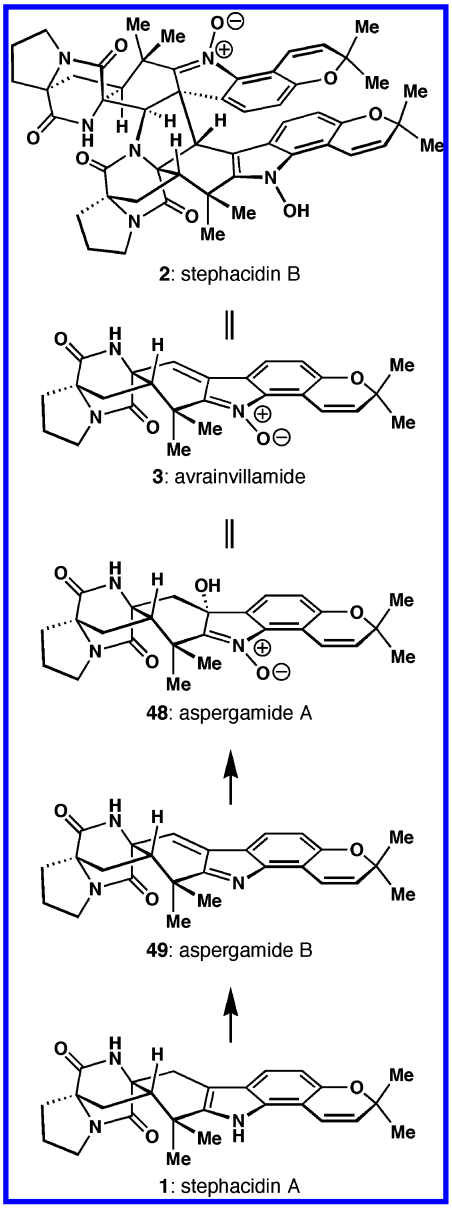


Figure 6. The stephacidins and related alkaloids listed in order of increasing oxidation state.

chelation (tight ion pairing).[9b] It should be noted that both C-6 isomers of **46** have similar overall steric energies based on MM3 computations. The success of this C−C bond forming event is impressive considering the wealth of possible side reactions that can occur such as oxidation of either enolate to an α-hydroxy species, dehydrogenation, dimerization, and inter- and intramolecular Dieckmann condensations. Since the mechanism and factors that govern oxidative enolate coupling are still poorly understood, further work in this field is underway.

At this point in our studies, we had established the viability of this strategy in forming the bicyclo[2.2.2]diazaoctane core in a stereoselective manner. Our next task was to apply our method to a system suitably functionalized to allow for eventual elaboration into stephacidin A. As shown in Figure 6 (natural series depicted), we planned to mimic the postulated biosynthesis of these alkaloids in the final conversion of stephacidin A to higher oxidized forms such as avrainvillamide. Although there was very little precedent for climbing such an oxidative "ladder," this was viewed as an opportunity for invention. Friedel−Crafts

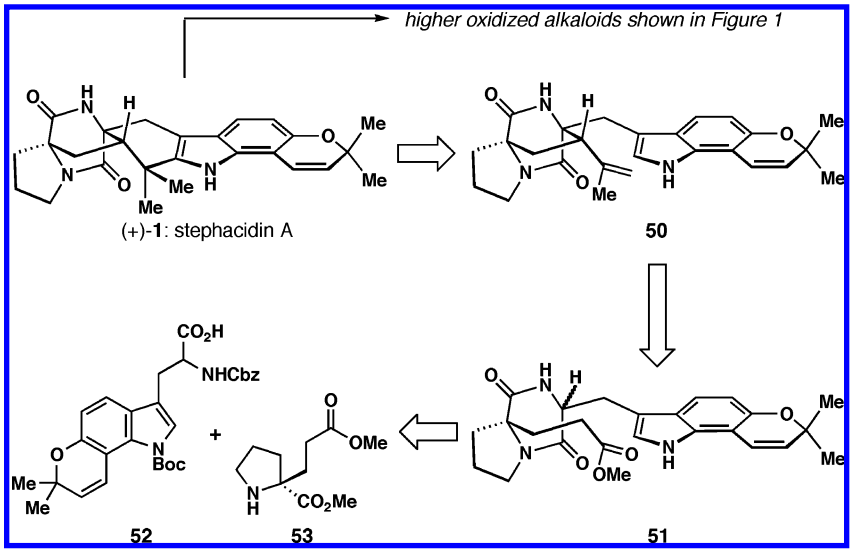
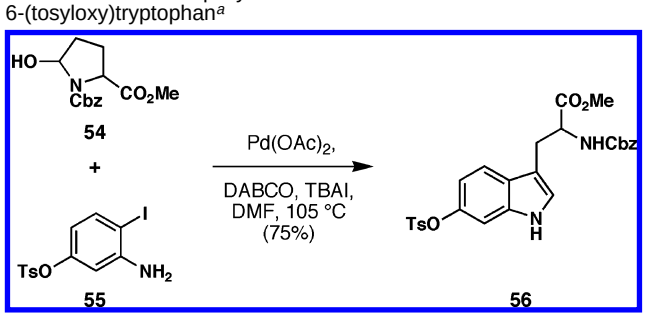


Figure 7. Retrosynthetic analysis of stephacidin A.

Scheme 6. One-Step Synthesis of a Protected 6-(tosyloxy)tryptophan[a]

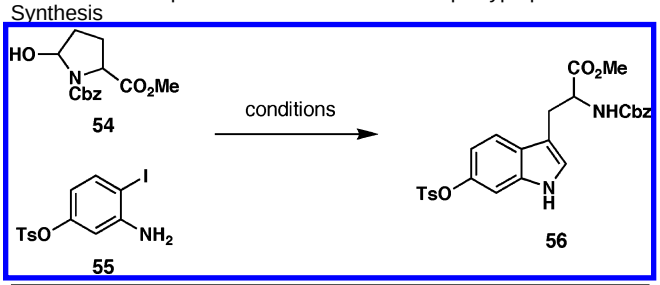


[a] Reagents and conditions: $Pd(OAc)_2$ (0.05 equiv), DABCO (3.0 equiv), TBAI (1.0 equiv), DMF, 105 °C, 4 h, 75%. DABCO = 1,4-diazabicyclo[2.2.2]octane, TBAI = tetra-*n*-butylammonium iodide.

cyclization of olefin **50** (see Figure 7), a tactic used successfully by Williams,[9b] should furnish stephacidin A. The requisite olefin **50** should be accessible from ester **51** by a sequence of oxidative C−C bond formation followed by functional group manipulations. Ester **51** could in turn be constructed from two simple building blocks: benzopyran tryptophan **52** and proline-derived ester **53**.

Development of a Scalable Route to Substituted Tryptophans. One of the first aims of this project was to develop an efficient and practical method for the synthesis of 6-hydroxytryptophan. Our motivation transcended the urgent need presented by the synthesis at hand, since dozens of indole alkaloids bear this oxidation pattern. With brevity and practicality in mind, we opted for a one-step pathway outlined in Scheme 6. Inspiration for this approach came from a publication by Reider and co-workers[44] at Merck for the palladium-catalyzed Heck-type synthesis of indoles from *o*-haloanilines and aldehydes. A glutamate-derived aldehyde (such as **54**) appeared to be an ideal surrogate for the tryptophan side chain,[45,46] while a suitably protected aniline such as **55** could serve as a precursor to the benzoypyran. In the event, reduced pyroglutamate **54** and

Table 3. Development of an Efficient One-step Tryptophan Synthesis



Entry	Conditions[a]	Product[b] (% yield)
1	$Pd(OAc)_2$ (5 mol %), DABCO (3 equiv), 105 °C , 3 h	35
2	$Pd(OAc)_2$ (5 mol %), DABCO (3 equiv), TBAI (1 equiv), 105 °C, 4 h	75
3	$Pd(OAc)_2$ (5 mol %), DABCO (3 equiv), TBAI (1 equiv), 105 °C, 2.5 h	18
4	$Pd(OAc)_2$ (5 mol %), DABCO (3 equiv), TBAI (1 equiv), $MgSO_4$ (5 equiv), 105 °C, 5 h	39
5	$Pd(PPh_3)_4$ (10 mol %), DABCO (3 equiv), TBAI (1 equiv), 105 °C, 10 h	8
6	$Pd(OAc)_2$ (5 mol %), Et_3N (3 equiv), TBAI (1 equiv), 105 °C, 1.5 h	34
7	Pd_2dba_3·$CHCl_3$ (10 mol %), DABCO (3 equiv), TBAI (1 equiv), 105 °C, 1.5 h	32

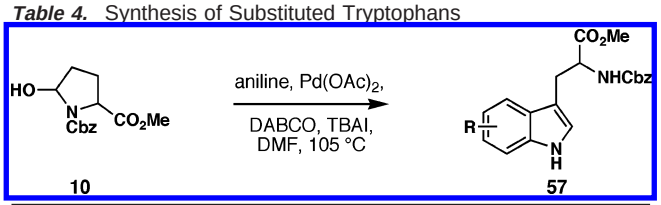
[a] All reactions were conducted in 0.3 M DMF. [b] Isolated yield.

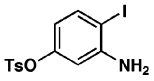
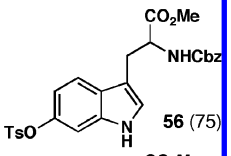
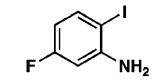
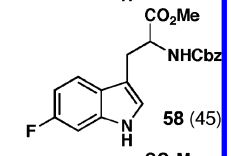
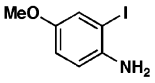
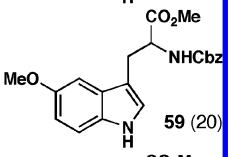
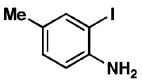
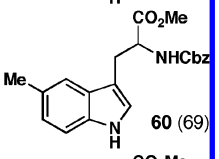
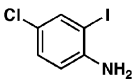
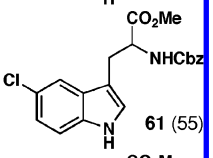
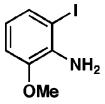
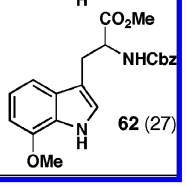
o-iodoaniline **55** could be efficiently coupled on a gram scale furnishing **56** in 75% yield under conditions arrived at through extensive optimization (see Table 3 for selected experiments). The generality of this method is exemplified in Table 4.

This reaction was initially conducted using the originally reported conditions,[44] furnishing **56** in modest (10−35%) yields. Investigation into the side products showed that a vast amount of material, both iodoaniline and intermediate enamine, was being stripped of iodine. With 6-hydroxytryptophan access being crucial to our forward progress, the problem of deiodination had to be overcome. In an attempt to elucidate the point of the reaction when deiodination was occurring, the iodoaniline and the preformed enamine were subjected to standard reaction conditions. It was interesting to find that the enamine gave deiodination products much more readily than did the iodoa-

(44) Chen, C.; Lieberman, D. R.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. *J. Org. Chem.* **1997**, *62*, 2676−2677.
(45) (a) Masumi, F.; Takeuchi, H.; Kondo, S.; Suzuki, K.; Yamada, S. *Chem. Pharm. Bull.* **1982**, *30*, 3831−3833. (b) Dieter, R. K.; Sharma, R. R. *J. Org. Chem.* **1996**, *61*, 4180−4184.
(46) While this manuscript was in preparation, a similar method for preparing A-ring substituted tryptophans was reported: Jia, Y.; Zhu, J. *Synlett* **2005**, 2469−2472.

Table 4. Synthesis of Substituted Tryptophans



Entry	Aniline	Time (h)	Product (% yield)[a]
1		4	 **56** (75)
2		4	 **58** (45)
3		3	 **59** (20)
4		1.5	 **60** (69)
5		1.5	 **61** (55)
6		3	 **62** (27)

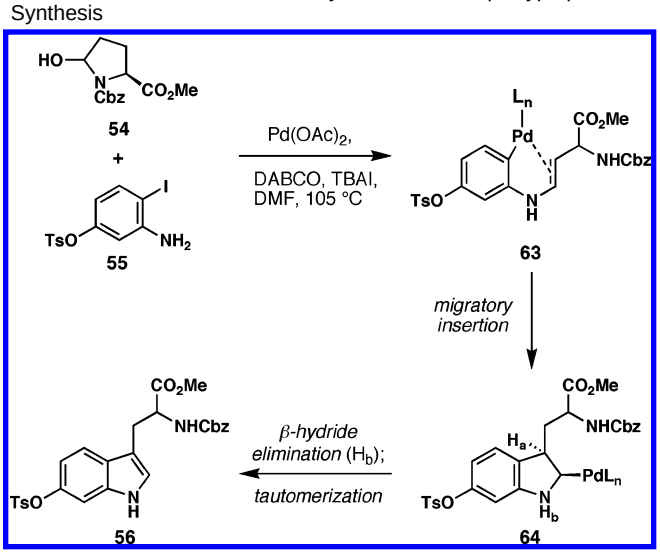
[a] Isolated yield

niline itself. The addition of tetraalkylammonium salts as described by Jeffery,[47] however, improved the yield, with tetra-*n*-butylammonium iodide (TBAI) showing the most promise by limiting the amount of deiodination. The addition of drying agents such as 4 Å MS or $MgSO_4$[44] did not improve the efficiency of the reaction. The screening of other additives, bases, and palladium sources indicated that the standard conditions with the addition of TBAI were ideally suited for the task at hand (see Table 3, entry 2).

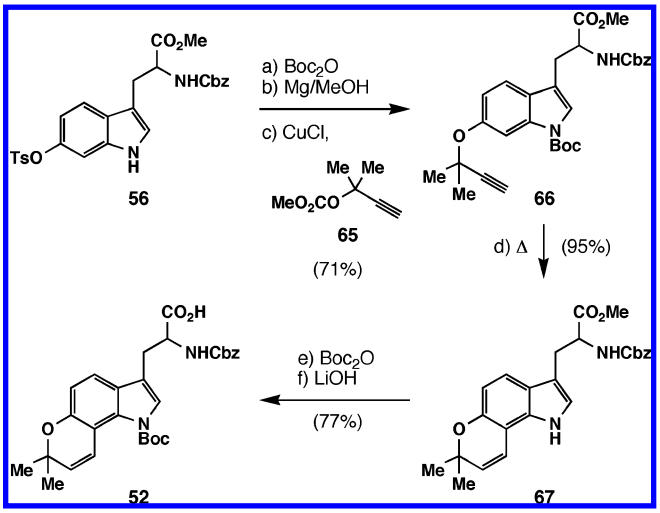
A likely mechanistic scenario for this process of indole synthesis is shown in Scheme 7. Base-catalyzed condensation of aniline **55** with the latent aldehyde of **54** followed by tautomerization to the *E*-enamine and oxidative addition to the iodine−aryl bond furnishes metalated intermediate **63**. Migratory insertion of the enamine olefin into the Pd−aryl bond generates *σ*-complex **64**, which can then undergo *β*-hydride elimination involving H_b. The benzylic proton H_a in **64** is not involved in this process since it is not *syn* to the metal. Tautomerization then leads to **56**.

(47) Jeffery, T. *Tetrahedron* **1996**, *52*, 10113−10130.

Scheme 7. Mechanistic Summary of the One-Step Tryptophan Synthesis



Scheme 8. Synthesis of Benzopyran **52** from 6-(tosyloxy)tryptophan **56**[a]

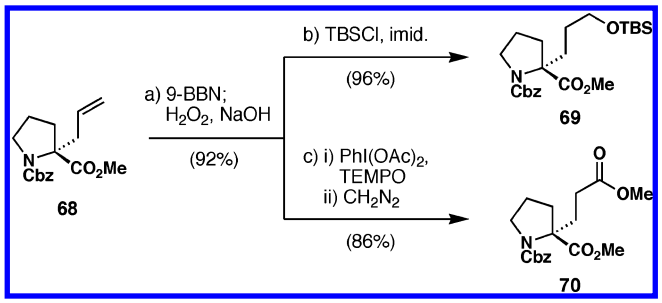


[a] Reagents and conditions: (a) Boc_2O (1.0 equiv), DMAP (0.01 equiv), CH_2Cl_2/MeCN (1:1), 20 °C, 10 min, 95%; (b) Mg^0 (10.0 equiv), MeOH, 0 → 25 °C, 2.5 h; (c) CuCl (0.001 equiv), **65** (3 equiv), DBU (3.0 equiv), CH_2Cl_2/MeCN (1:1), 0 °C, 24 h, 75% over two steps; (d) *o*-DCB, 190 °C, 10 min, 95%; (e) Boc_2O (1.0 equiv), DMAP (0.01 equiv), CH_2Cl_2/MeCN (1:1), 20 °C, 30 min, 77%; (f) LiOH (15.0 equiv), THF/H_2O (1:1), 0 °C, 3 h, 100%. Boc_2O = di-*tert*-butyl dicarbonate, DMAP = 4-(dimethylamino)pyridine, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, *o*-DCB = *ortho*-dichlorobenzene.

Total Synthesis of Stephacidin A. The next task, namely forging the benzopyran heterocycle onto tryptophan **56**, was accomplished as outlined in Scheme 8. Thus, Boc-protection (Boc_2O, DMAP)[48] and Mg-mediated deprotection of the tosyl group[49] permitted installation of the requisite benzopyran carbons by treating the crude phenol with the vinylidene carbene generated *in situ* from propargylic carbonate **65**, DBU, and catalytic CuCl[50] giving **66** in 71% overall yield from **56**. Allenyl Claisen rearrangement then took place smoothly and in 95% yield by heating in *o*-dichlorobenzene at 190 °C for 10 min

(48) Franzén, H. M.; Någren, K.; Grehn, L.; Långström, B.; Ragnarsson, U. *J. Chem. Soc., Perkin Trans. 1* **1988**, 497−502.
(49) Sridhar, M.; Kumar, B. A.; Narender, R. *Tetrahedron Lett.* **1998**, *39*, 2847−2850.
(50) Tisdale, E. J.; Vong, B. G.; Li, H.; Kim, S. H.; Chowdhury, C.; Theodorakis E. A. *Tetrahedron* **2003**, *59*, 6873−6887.

Scheme 9. Synthesis of Substituted Enantiomerically Pure Prolines **69** and **70**[a]



[a] Reagents and conditions: (a) 9-BBN (2.0 equiv), THF, 20 °C, 3 h; then 3 M aq. NaOH/35% aq. H_2O_2 (1:1), 1 h, 92%; (b) TBSCl (1.1 equiv), imid. (1.2 equiv), CH_2Cl_2, 20 °C, 30 min, 96%; (c) (i) $PhI(OAc)_2$ (2.2 equiv), TEMPO (0.2 equiv), MeCN/H_2O (3:1), 20 °C, 5 h; (ii) CH_2N_2, EtOAc, 20 °C, 86% overall. TEMPO = 2,2,6,6-tetramethyl-1-piperdinyloxy free radical.

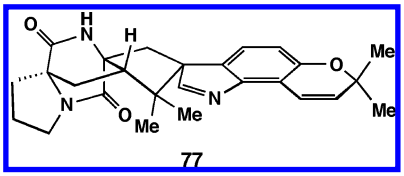
furnishing **67**. The Boc group was then re-appended, and the ester was saponified (LiOH) resulting in acid **52** in 77% yield from **67**.

While a viable and scalable route to the benzopyran-containing sector was developed, a route to the proline portion took shape as shown in Scheme 9. This sector of stephacidin A was prepared in enantiomerically pure form using Seebach's methodology for the asymmetric alkylation of amino acids.[51] The hydrochloride salt of (*R*)-allylproline (generated from L-proline) was esterified and protected as its benzyl carbamate delivering **68** (see Supporting Information for details). Hydroboration and oxidative workup gave the primary alcohol, a compound prepared previously in racemic form by Lectka.[52] At this point, the first and second generation syntheses diverged. Initially, it was feared that conversion of the primary alcohol to its carboxylic ester would be incompatible with the deprotected amine required for amide bond formation. This concern mandated that the alcohol be protected as its TBS ether (**69**) and be unmasked after peptide coupling and amide functionalization/protection. Our concerns, however, proved to be unfounded since the alcohol could be converted to diester analogue **70**, deprotected, and coupled without complication (*vide infra*).

Scheme 10 details the union of the tryptophan and proline fragments and their eventual conversion to *ent*-stephacidin A. While reductive removal of the Cbz group of **69** and peptide coupling with **52** proceeded uneventfully, amine diester **53** required swift peptide coupling with **52** immediately after liberation of the amine to avoid formation of the corresponding γ-lactam.[52] In coupling **71** to **52** in our first strategy, we elected to use BOPCl as the coupling agent allowing us to obtain **72** in 62% overall yield from **70**. However, as observed in more advanced model studies (*vide infra*), HATU was found to be the coupling reagent of choice and furnished amide **73** in 81% overall yield from **53**.

To form the diketopiperazine (DKP) ring system of **74**/**75**, chemoselective deprotection of the Cbz group of **72**/**73** was achieved using modified conditions of those reported by Ohfune[53] followed by dissolution and heating in a mixed solvent system of methanol (to hydrolyze the intermediate silyl carbamate) and DMF to elicit ring closure with loss of methanol giving **74**/**75**. Since Williams had reported difficulties in the removal of PMB protecting groups from DKPs,[9b] a MOM group was chosen and installed under standard conditions. Upon arrival at ester **75**, either directly *via* the route from proline **53** or through the more circuitous sequence from TBS-protected proline **71**, the stage was set for the key oxidative enolate coupling. In accordance with model studies, ester **75** reacted smoothly (LDA, followed by $Fe(acac)_3$) to furnish **76** as a single diastereomer in 61% isolated yield (along with 8% recovered **75**), even on a gram scale. After some optimization (*vide infra*), the MOM group could be reliably dismantled using *B*-bromocatecholborane[54] in 63% yield. The resulting free DKP was treated with an excess of MeMgBr (6 equiv) to furnish an intermediate tertiary alcohol that dehydrated upon treatment with the Burgess reagent[43] furnishing **50** in 88% overall yield.

A seemingly trivial Friedel−Crafts alkylation was planned to forge the seventh and final ring of **1**, based on extensive literature precedent.[55] Olefin **50** was unstable to all Brønsted acids, presumably due to the electron rich benzopyran ring (see Supporting Information for Friedel−Crafts alkylation optimization studies). Fortuitously, when **50** was simply heated in sulfolane at 200 °C for 1 h, we were able to isolate stephacidin A in variable yields (ca. 28−45%) along with recovered Boc-deprotected **50**. It is likely that this thermolytic sequence involves initial Boc-removal[26] followed by a formal ene reaction to generate a spirocyclic intermediate[56] such as **77** followed by a 1,2-shift[56] to afford **1**. It is also possible that trace quantities

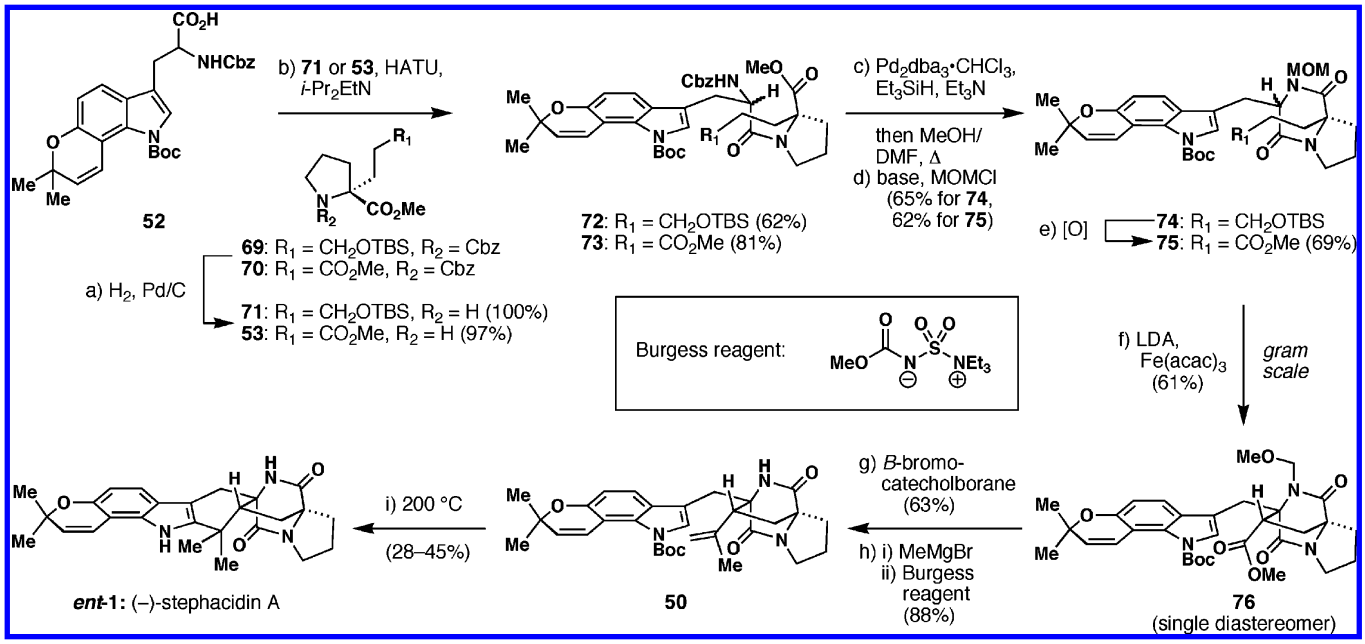


of acid are responsible for this transformation, although base-washed glassware gave a similar result. With the exception of optical rotation, which was not known at the time, synthetic **1** was found to be identical in all respects to a natural sample kindly provided by Professor Fenical (*vide infra*) and with data reported by the Bristol-Myers Squibb group.[1]

Determination of Absolute Configuration. As alluded to in the Introduction, optical rotation data were not reported for the stephacidins.[1] Although comparison of stephacidin A to other related natural products such as the paraherquamides, asperparalines, and others[9j] might have allowed an educated guess to be made with regards to its absolute configuration, we elected to use natural L-proline until this uncertainty was resolved. As of 2004 (or earlier), Bristol-Myers Squibb had exhausted their supply of stephacidin A.[57] Thus, the only means by which the absolute configuration of stephacidin A could have been determined would have been through reisolation or reduction of avrainvillamide (or one of the aspergamides) to the level of an indole followed by optical rotation measurement. This latter option was of course dependent on the assumption that these

(51) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390−5398.
(52) Cox, C.; Lectka, T. *J. Am. Chem. Soc.* **1998**, *120*, 10660−10668.
(53) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870−876.
(54) Boeckman, R. K., Jr.; Potenza, J. C. *Tetrahedron Lett.* **1985**, *26*, 1407−1410.
(55) See ref 9. Also see: (a) Mirand, C; Massiot, G.; Levy, J. *J. Org. Chem.* **1982**, *47*, 4169−4170. (b) Borschberg, H. J. *Helv. Chem. Acta* **1984**, *67*, 1878−1882. (c) Darbre, T.; Nussbaumer, C.; Borschberg, H. J. *Helv. Chem. Acta* **1984**, *67*, 1040−1052. (c) Stoermer, D.; Heathcock, C. H. *J. Org. Chem.* **1993**, *58*, 564−568.
(56) Jackson, A. H.; Smith, P. *Tetrahedron* **1968**, *24*, 2227−2239.
(57) Qian-Cutrone, J. Personal communication.

Scheme 10. Total Synthesis of *ent*-Stephacidin A from Proline **69** or **70** and Benzopyran Acid **52**[a]



[a] Reagents and conditions: (a) 10% Pd/C (20% w/w), H_2, toluene, 20 °C, 2 h, 100% from **69**; or 10% Pd/C (20% w/w), toluene, 20 °C, 2 h, 97% from **70**; (b) **71**, (1.5 equiv), BOPCl (1.1 equiv), *i*-Pr_2EtN (1.1 equiv), CH_2Cl_2, 0 → 20 °C, 10 h, 62% from **71**; or **53** (1.0 equiv), HATU (1.1 equiv), *i*-Pr_2EtN (3.0 equiv), DMF, 20 °C, 12 h, 81% from **53**; (c) Pd_2dba_3·$CHCl_3$ (0.2 equiv), Et_3SiH (40.0 equiv), Et_3N (2.0 equiv), CH_2Cl_2, 20 °C, 4 h; then MeOH, reflux, 30 min; then toluene, reflux, 2 h, 53% overall from **72**; or Pd_2dba_3·$CHCl_3$ (0.2 equiv), Et_3SiH (40.0 equiv), Et_3N (2.0 equiv), CH_2Cl_2, 20 °C, 2.5 h; then MeOH, DMF (2:1), reflux, 2.5 h, 85% from **73**; (d) NaH (1.2 equiv), DMF, 0 °C, 30 min; then MOMCl (1.1 equiv), 0 °C, 1 h, 65% from **74**; or NaHMDS (1.0 equiv), THF, −78 °C, 30 min; then MOMCl (1.3 equiv), −78 → 20 °C, THF, 1.5 h, 63% from **75**; (e) (i) TBAF (3.0 equiv), THF, 20 °C, 1 h; (ii) DMP (1.5 equiv), wet CH_2Cl_2, 20 °C, 2 h; (iii) $NaClO_2$ (2.8 equiv), NaH_2PO_4·H_2O (3.0 equiv), 2-methyl-2-butene (20.0 equiv), THF, 20 °C, 20 min; (iv) CH_2N_2, MeOH, 20 °C, 69% overall; (f) LDA (2.2 equiv), THF, −78 °C, 5 min; then $Fe(acac)_3$ (2.2 equiv), −78 °C, 5 min; then −78 → 20 °C, 20 min, 61% (plus 8% recovered **75**); (g) *B*-bromocatecholborane (1.5 equiv), CH_2Cl_2, 0 °C, 10 min, 63%; (h) (i) MeMgBr (6.0 equiv), toluene, 20 °C, 10 min; (ii) Burgess reagent (2.0 equiv), benzene, 50 °C, 30 min, 88% yield over two steps; (iii) 200 °C, sulfolane, 1 h, 28−45%. Pd_2dba_3·$CHCl_3$ = tris(dibenzylideneacetone) dipalladium(0) chloroform complex, MOMCl = chloromethyl methyl ether, NaHMDS = sodium bis(trimethylsilyl)amide.

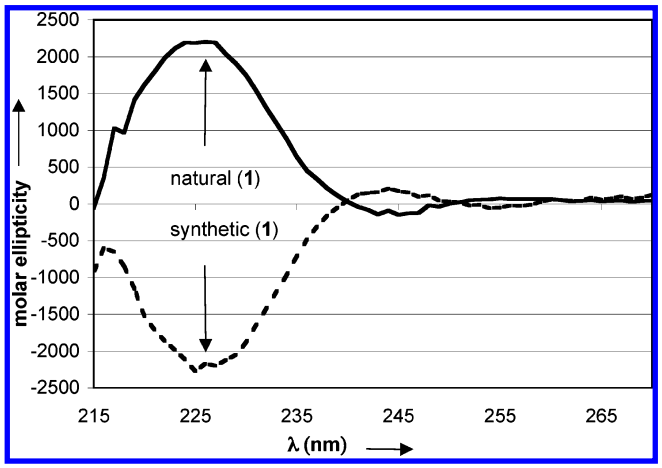


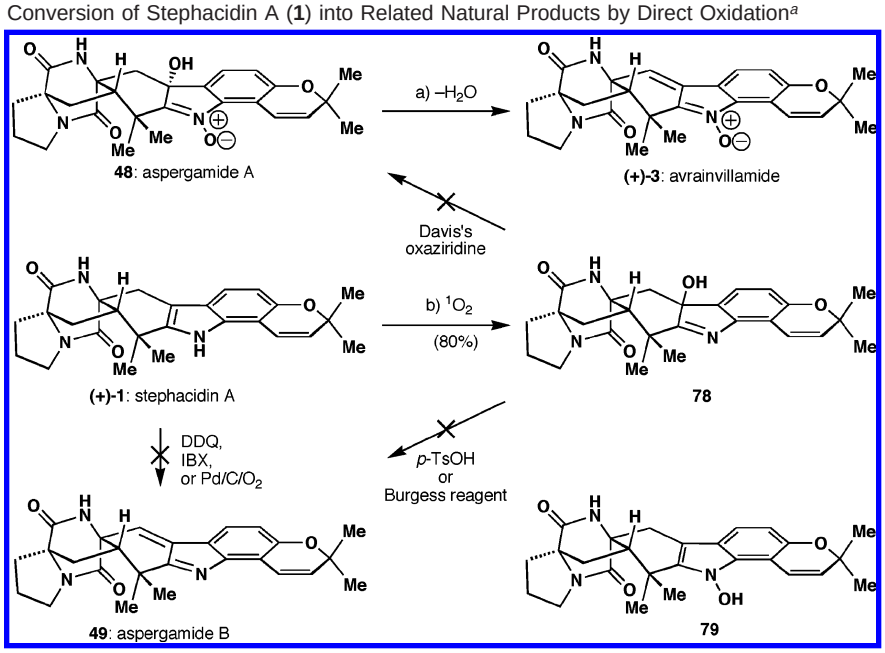
Figure 8. Comparison of synthetic and natural stephacidin A.

natural products were biogenetically related. Fortunately, Professor Fenical had previously isolated avrainvillamide[5] and still had active cultures of the organism, *Aspergillus sp.* CNC358, that produced the natural product. Fermentation of the organism and analysis of the broth by LCMS suggested the presence not only of the expected avrainvillamide but also of stephacidin A. Stephacidin B could not be detected. Purification of the extracts and ^{1}H NMR analysis confirmed these assignments. Comparison of the CD data (see Figure 8) of natural (+)-stephacidin A to those of our synthetic sample revealed that unnatural (−)-stephacidin A had been synthesized previously in our laboratories. We therefore rectified our synthesis by using D-proline from this point onward.

Total Synthesis of Avrainvillamide and Stephacidin B. As shown in Figure 6, stephacidin A is at the lowest oxidation state of this family of alkaloids. Initial analysis suggested that oxidation of **1** to either of the aspergamides[9j] (**48** or **49**, Figure 6) would be a logical pathway to avrainvillamide (**3**). Analysis of a sample of aspergamide A (**48**), kindly provided to us by Professor Zeeck, supported this reasoning since upon arrival it had dehydrated in quantitative yield to give avrainvillamide (based on ^{1}H NMR comparison; see Scheme 11). Aspergamide B (**48**) was initially targeted since, in principle, a chemoselective oxidation of the imine species would lead to **3**. This appeared to be an ideal opportunity to test the nitrosobenzene-mediated dehydrogenation of tryptophans (*vide supra*). Unfortunately, despite repeated attempts, this new oxidation method failed along with more conventional oxidants such as DDQ, *p*-chloranil, IBX, CAN, and Pd/C/O_2. In an effort to access aspergamide A, **1** was exposed to $^1O_2$ (generated *in situ* by bubbling dry O_2(g) in methanol while irradiating with a sunlamp in the presence of catalytic methylene blue) which cleanly delivered C-3 hydroxyindolenine **78** in 80% yield after reductive workup with Me_2S.[58] Since there are numerous methods for the oxidation of imines to nitrones,[59] the conversion of **78** into **48** was explored. After considerable experimentation, this approach was abandoned due to the lack of reactivity of the imine nitrogen and the propensity of the benzopyran olefin to

(58) See reference 18a.
(59) (a) Christensen, D.; Jørgensen, K. A. *J. Org. Chem.* **1989**, *54*, 126−131. (b) Boyd, D. R.; Coulter, P. B.; McGuckin, M. R.; Sharma, N. D.; Jennings, W. B.; Wilson, V. E. *J. Chem. Soc., Perkin Trans. 1* **1990**, 301−306. (c) Busqué, F.; de March, P.; Figueredo, M.; Font, J.; Gallagher, T.; Milán, S. *Tetrahedron: Asymmetry* **2002**, *13*, 437−445.

Scheme 11. Attempted Conversion of Stephacidin A (**1**) into Related Natural Products by Direct Oxidation[a]



[a] Reagents and conditions: (a) likely occurred gradually during storage/shipping, 100%; (b) sunlamp, cat. methylene blue, $^3O_2$, MeOH, −28 °C, 30 min; DMS (100 equiv), −28 → 20 °C, 10 min, 80%. DMS = dimethyl sulfide.

be oxidized. This set of failures prompted the conception of a new plan that would install the *N*-oxide functionality at an earlier stage. Specifically, we considered preparing **79**, the *N*-hydroxy analogue of **1**, since its tautomeric nitrone form bears a striking resemblance to avrainvillamide.

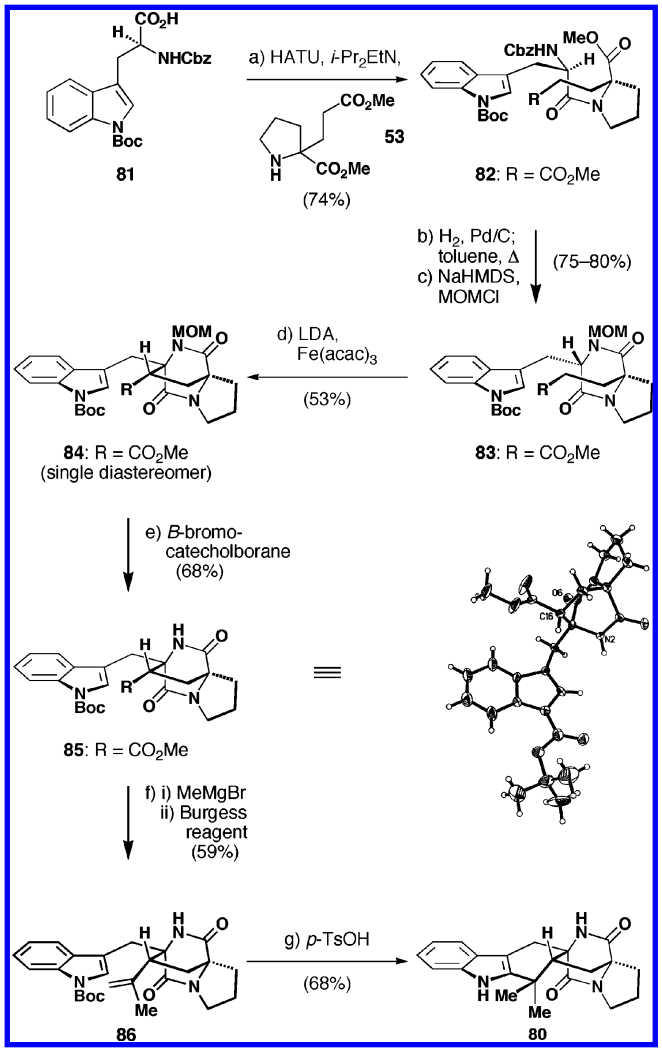
Keenly aware of Somei's pioneering 1-hydroxyindole hypothesis (*vide infra*) and accompanying methodology for the preparation of these elusive species,[60] we set forth on a model study. Using the reagents and general techniques of the stephacidin synthesis, the model compound **80** was prepared as shown in Scheme 12. Optimization of the deprotection of the MOM protecting group (see Supporting Information) was initially carried out on intermediate **84** and applied to the stephacidin A synthesis once satisfactory conditions were found (*vide supra*). X-ray analysis of crystalline **85** thus secured its relative configurational assignment and further bolstered the assignments of related systems (*vide supra*). Due to the absence of the benzopyran ring system, olefin **86** cleanly underwent Friedel−Crafts alkylation to stephacidin model **80** in 68% isolated yield when treated with *p*-TsOH in toluene at reflux. Hexacycle **80** possessed spectral properties that paralleled those of stephacidin A. Furthermore, its physical properties were also similar to those of **1**; both are white powders and have poor solubility in a number of common organic solvents. Thermal cyclization of **86** to **80** also effected cyclization, but in lower conversion (ca. 30%) than the acid-catalyzed process.

Somei's hypothesis concerning 1-hydroxyindoles states that these highly reactive species are widespread in Nature during the biosynthesis of alkaloids. Since there is no direct method for the conversion of an indole to its 1-hydroxy counterpart (in the laboratory), the indole is first reduced to the indoline[60,61] and then treated with catalytic $Na_2WO_4 \cdot 2H_2O$ and excess H_2O_2. Presumably, this reagent combination initially hydroxylates the indoline nitrogen after which further oxidation (presumably dehydrogenation) and tautomerization gives the desired 1-hydroxyindole. Model compounds lacking the benzopyran heterocycle with and without *N*-protecting groups on the DKP (**87** and **80**, respectively) were evaluated under Somei conditions (Scheme 13). Gribble reduction ($NaBH_3CN$) led cleanly to the indoline which was carried on in crude form following aqueous workup. Surprisingly, indolines **88** and **89** exhibited markedly different reactivities upon exposure to $Na_2WO_4 \cdot 2H_2O$ and H_2O_2. Whereas the unprotected indoline was oxidized readily to the 1-hydroxyindole (**91**, fully characterized), PMB-protected indoline **88** was oxidized directly to α,β-unsaturated nitrone **92**. As expected, hydroxyindole **91** was amendable to further oxidation to **93** under mild conditions (*p*-chloranil) in high yield (88%). The observed difference in reactivity between the indolines is difficult to explain but may be due to a subtle electronic effect.

Armed with useful data from the model series, the total synthesis of the natural products was pursued as shown in Scheme 14. Thus, reduction of the indole in stephacidn A (**1**) proceeded in quantitative yield to produce indoline **94** which could be carried on in crude form in the ensuing oxidation. When **94** was submitted to the Somei conditions ($Na_2WO_4 \cdot 2H_2O$), traces of **3** were sometimes observed but the overall result was usually complete decomposition. After a vast screening of different oxidation conditions it was discovered that SeO_2/H_2O_2[62] was capable of converting **94** into **3**, albeit in modest yield due to the instability of the product under the reaction conditions. Subsequently it was found that avrainvillamide (**3**) spontaneously dimerized (as did model **93**, *vide infra*) using PTLC or by simply dissolving in DMSO and drying *in vacuo*. Myers also observed a similar phenomenon and found that Et_3N enables complete conversion of avrainvillamide (**3**) to **2**.[12] Spectral data for synthetic **2** and **3** were found to be in agreement with those of natural samples (synthetic (−)-**2**: $[\alpha]_D = -33$ (*c* 0.1, CH_3CN); natural (−)-**2**: $[\alpha]_D = -21.1$ (*c* 0.19,

(60) Somei, M. *Adv. Heterocycl. Chem.* **2002**, *82*, 101−155.
(61) Gribble, G. W.; Lord, P. D.; Skotnicki, K.; Dietz, S. E.; Eaton, J. T.; Johnson, J. *J. Am. Chem. Soc.* **1974**, *96*, 7812−7814.
(62) Murahashi, S.-I.; Shiota, T. *Tetrahedron Lett.* **1987**, *28*, 2383−2386.

Scheme 12. Synthesis of a Stephacidin A Model System[a]



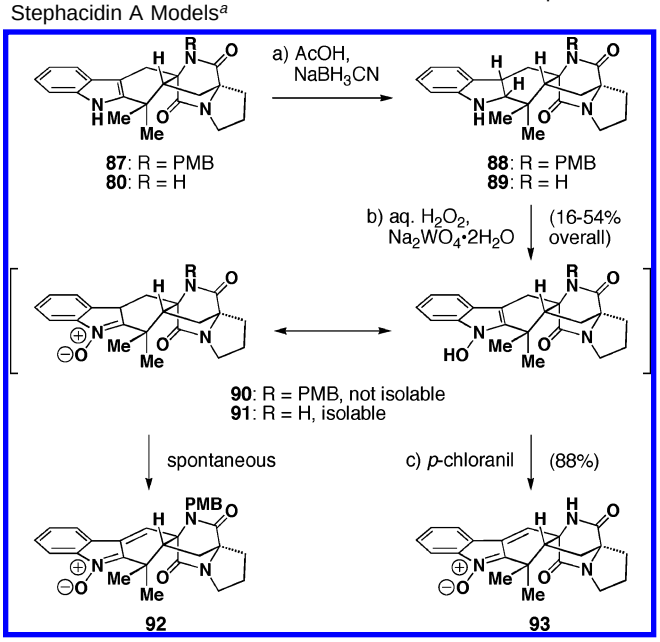
[a] Reagents and conditions; (a) proline **53** (1.0 equiv), HATU (1.1 equiv), *i*-Pr_2EtN (3.0 equiv), DMF, 20 °C, 12 h, 74%; (b) 10% Pd/C (20% w/w), H_2, toluene, 20 °C, 18 h; toluene, reflux, 2 h, 87%; (c) NaHMDS (1.05 equiv), MOMCl (1.2 equiv), THF, −78 °C → 20 °C, 2.5 h, 86−92%; (d) LDA (2.2 equiv), $Fe(acac)_3$ (2.2 equiv), −78 → 20 °C, 53%; (e) *B*-bromocatecholborane (2.0 equiv), CH_2Cl_2, 0 °C, 10 min, 68% after 1 recycle; (f) MeMgBr (10.0 equiv), toluene, 20 °C, 10 min; then Burgess reagent (6.0 equiv), benzene, 50 °C, 30 min, 59% overall; (g) *p*-TsOH (1 equiv), toluene, reflux, 10 h, 68%.

$CDCl_3$); synthetic (+)-**3**: $[\alpha]_D = +11$ (*c* 0.1, $CHCl_3$); natural (+)-**3**: $[\alpha]_D = +10.7$ (*c* 0.17, $CHCl_3$)).

With regard to the mechanism of dimerization of **3** or **93**, it seems most likely that the event takes place *via* a double Michael addition as shown in Scheme 15 in contrast to the cationic mechanism proposed by the isolation chemists.[1] Thus, attack of the secondary amide (25′) of one molecule of **3** onto electrophilic C-21 of the second molecule of **3** would generate a nucleophilic 1-hydroxyindole species. This 1-hydroxyindole, nucleophilic at C-20 (stephacidin A numbering), would be forced to react with the proximate Michael acceptor at C-21′ of the second molecule. The fact that the electrophilic carbons are sterically congested neopentyl sites may not be as significant in a conformationally locked system such as this one.

The model avrainvillamide **93** also dimerized readily as shown in Scheme 16. It is interesting to note that the dimerization of **93** occurred starting with an uneven mixture of enantiomers. These were generated as a result of carrying

Scheme 13. Initial Oxidation Studies Performed on Simplified Stephacidin A Models[a]



[a] Reagents and conditions: (a) $NaBH_3CN$ (10.0 equiv), AcOH, 20 °C, 12 h; (b) $Na_2WO_4 \cdot 2H_2O$ (0.2 equiv), aqueous 35% H_2O_2 (50 equiv), MeOH, H_2O, 20 °C, 6 h, 16% over two steps for **92**; $Na_2WO_4 \cdot 2H_2O$ (2.0 equiv), aqueous 35% H_2O_2 (50 equiv), MeOH, H_2O, 20 °C, 50 min, 54% over steps for **91**; (c) *p*-chloranil (2.0 equiv), THF, reflux, 30 min, 88%.

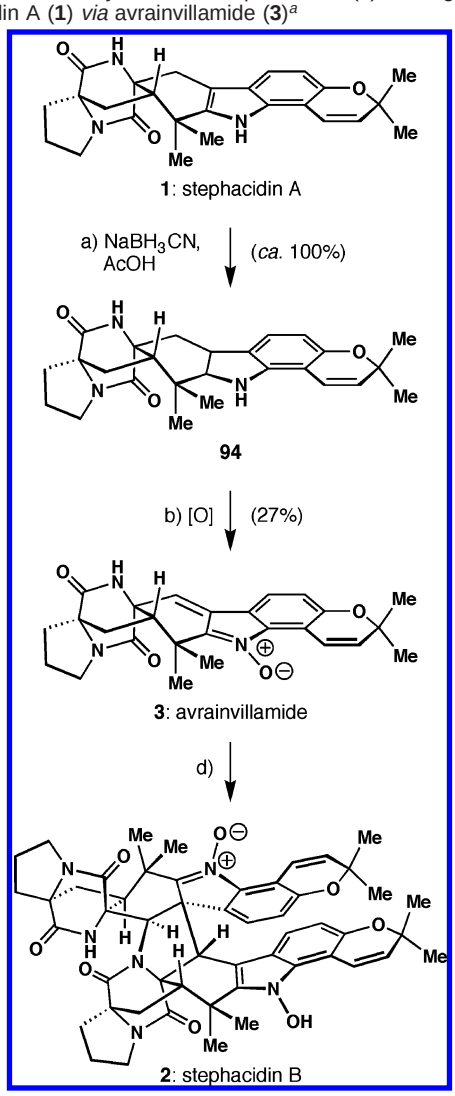
forward **83** as single diastereomers (resulting from the use of racemic **53**) for the oxidative coupling event to aid in purfication followed by combining the purified, oxidatively coupled products for further processing. Presumably, homodimerization occurs exclusively since stereochemical bias may retard the dimerization in the mismatched case.

Biological Evaluation of Avrainvillamide and Simplified Mimics. Biological assays of simplified analogues using the human colon HCT-116 cell line delivered exciting results. Although the benzopyran ring appears to be essential for the anti-cancer activity stephacidin A (compare entries 1 and 5), the higher oxidized analogs appear to restore activity. Interestingly, in the assay which was used, analogs **91**, **95**, and **93** appear to be more active than **1**. Of particular interest is that model compound (±)-**93** is approximately half as effective as (+)-avrainvillamide itself, a difference that may be ascribed to the fact that, in preparing **93** for our studies, racemic **53** was used (*vide supra*).

Avrainvillamide mimic **93** requires only nine steps for its preparation from *N*-Cbz-*N*′-Boc-tryptophan (**81**), is readily amenable to preparation on a multigram scale, and is qualitatively more shelf stable than avrainvillamide itself. As such it represents a potentially useful candidate for *in vivo* studies. Based on the data in Table 5, we propose that the sole source of bioactivity in these natural products (in anticancer screens) stems from the electrophilic α,β-unsaturated nitrone moiety of avrainvillamide. Indeed, Myers has shown this functionality to be readily susceptible to nucleophilic attack (even methanol adds in a conjugate fashion).[12] It is possible that *N*-hydroxyindole **91** is autoxidized to **93** slowly during testing and that the stephacidin B mimic (**95**) reverts to **93** to some extent, as seen in the natural series.

Extensive literature precedent would suggest that avrainvillamide functions by some sort of selective protein alkylation

Scheme 14. Total synthesis of stephacidin B (**1**) starting from stephacidin A (**1**) *via* avrainvillamide (**3**)[a]



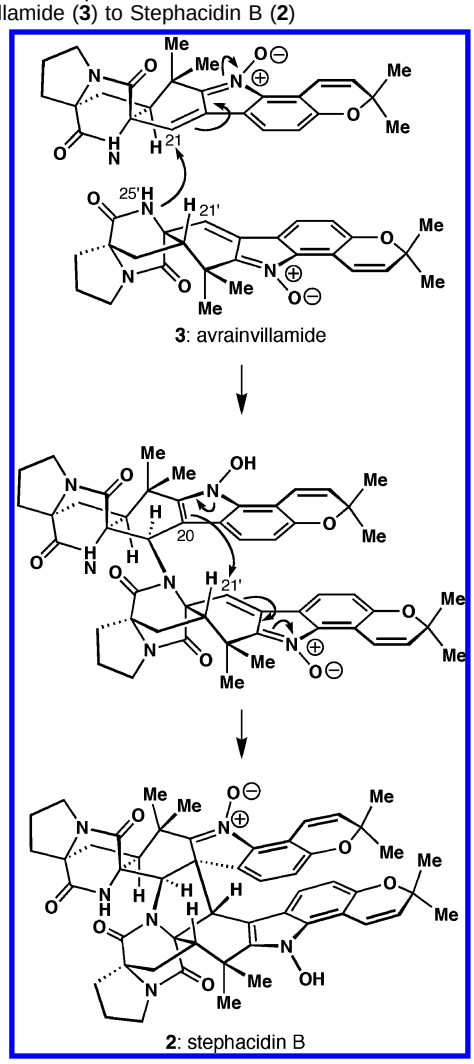
[a] Reagents and conditions: (a) $NaBH_3CN$ (40 equiv), AcOH, 20 °C, 24 h, >95%; (b) SeO_2 (0.25 equiv), 35% aqueous H_2O_2, (50 equiv), 1,4-dioxane, 20 °C, 40 h, 27% with 50% recovered **94**; (c) Procedure A: preparative TLC (SiO_2, EtOAc); Procedure B: Et_3N (excess), MeCN, 20 °C, 1 h; Procedure C: DMSO, then solvent removal *in vacuo*, approximately 2:1 mixture of **3** to **2**, purified by preparative TLC.

event, but further mechanistic studies are needed to confirm this suspicion.[63]

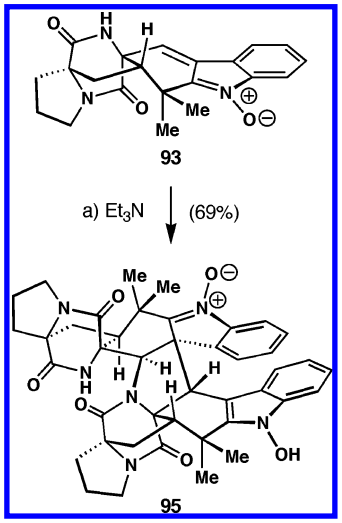
Conclusion

In this full account we have traced the evolution of our synthetic strategy to the bicyclic core of stephacidin alkaloids from a failed intramolecular Diels−Alder approach to a conceptually unrelated and successful strategy based on oxidative C−C bond formation. While the former approach did not proceed as designed, it did lead to the development of a new and mechanistically intriguing method for the direct dehydrogenation of tryptophans to enamides. The operational simplicity and stereoselectivity observed in this transformation is notable. The highlight of these model studies, however, is the interesting intramolecular oxidative coupling of **44** during the successful synthesis of **45**. This reaction reliably proceeds in good yield

Scheme 15. Proposed Mechanism for the Dimerization of Avrainvillamide (**3**) to Stephacidin B (**2**)



Scheme 16. Dimerization of Avrainvillamide Model **93** to Stephacidin B Analogue **95**[a]

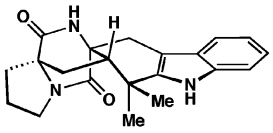
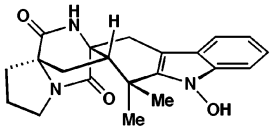
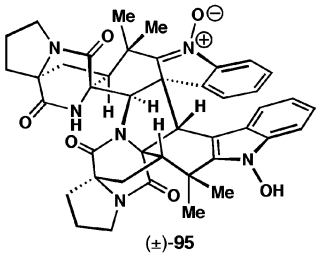
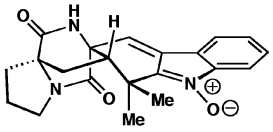


[a] Reagents and conditions: (a) Et_3N (100 equiv), MeCN, 20 °C, 1 h, 69%.

on a preparative scale with complete stereocontrol and represents the first union of different types of carbonyl groups in an enolate coupling. It is hypothesized that the high chemoselectivity in this reaction is controlled by the unique oxidation potential of

(63) Drahl, C.; Cravatt, B. F.; Sorensen, E. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 5788−5809.

Table 5. Preliminary Biological Activity for Simplified Analogues[a]

Entry	Substrate	Activity (μg/mL)
1	 (±)-**80**	no significant activity[b]
2	 (±)-**91**	9.36[b]
3	 (±)-**95**	3.95[b]
4	 (±)-**93**	5.47[b]
5	(+)-**1**: stephacidin A	10.4[b,c]
6	(–)-**2**: stephacidin B	0.41[1a]
7	(+)-**3**: avrainvillamide	2.0[3a]

[a] Activity = IC_{50} against human colon HCT-116 cell line. [b] Values are an average of three runs performed in the Fenical laboratory. [c] Researchers at Bristol-Myers Squibb reported a value of 0.91 μg/mL.[1a]

the Fe-based oxidant and that complete stereoselectivity is achieved by a metal chelated transition state.

Using this intramolecular oxidative enolate heterocoupling as a powerfully simplifying transform, we have succeeded in synthesizing avrainvillamide and the stephacidins. The issue of synthesizing a benzopyran-substituted tryptophan was addressed by extending the scope of a known indole synthesis. To this subunit was appended a modified and enantiomerically pure proline derivative that served as the source of chirality for the synthesis. With all the carbon atoms of stephacidin A in place, oxidative enolate coupling proceeded in good yield and excellent diastereoselectivity furnishing the hallmark bicyclo[2.2.2]-diazaoctane core. Further functional manipulations led to a penultimate olefinic intermediate whose reluctance to undergo Friedel–Crafts alkylation led to the discovery of a unique, reagent-free method for the annulation of 3-substituted indoles.

Before the more highly oxidized members of the stephacidin class of alkaloids were considered for synthesis, the absolute configuration of stephacidin A, the parent member, was elucidated by reisolation of the natural product. Once the identity of the natural series was confirmed, synthetic studies continued. Our inability to directly oxidize stephacidin A to either of the aspergamides forced a reevaluation of our strategy that prompted us to employ Somei's method for generating 1-hydroxyindoles. In some specific cases, indolines underwent unprecedented three-stage oxidation to α,β-unsaturated nitrones directly, an observation which could prove useful in other settings. Applying this method led to avrainvillamide, stephacidin B, and models thereof, making structure–activity studies possible for the first time in the course of these studies. The first analogues of these natural products have also been prepared and evaluated for biological activity. Further studies are underway to find more potent analogues and to probe the mechanism of action by which these complex alkaloids operate.

Acknowledgment. This work is dedicated to Professor William Fenical on the occasion of his 65th birthday. We are grateful to the Fenical lab (specifically John B. MacMillan, Paul Jensen, and Matt Woolery) for reculturing the avrainvillamide-producing organism (*Aspergillus sp.* CNC358) and providing us with the crude extracts and to Dr. J. Qian-Cutrone of Bristol-Myers Squibb for copies of spectra, discussions, and an authentic sample of **2**. We would like to thank Michael DeMartino and Professor M. G. Finn for providing the cyclic voltammetry data. We thank Dr. D. H. Huang and Dr. L. Pasternack for NMR spectroscopic assistance, and Dr. G. Siuzdak and Dr. Raj Chadha for mass spectrometric and X-ray crystallographic assistance, respectively. Biotage is acknowledged for a generous donation of microwave process vials used in this study. Financial support for this work was provided by The Scripps Research Institute, Amgen, Astra Zeneca, Bristol-Myers Squibb, DuPont, Eli Lilly, GlaxoSmithKline, Roche, the Searle Scholarship Fund, the Sloan Foundation, and the NIH/NIGMS (GM071498 and a predoctoral fellowship to C.A.G.).

Supporting Information Available: Full characterization, including copies of ^{1}H and ^{13}C NMR spectra, and experimental procedures for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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