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Enantioselective Synthesis of Stephacidin B

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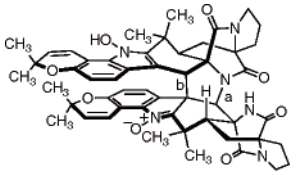
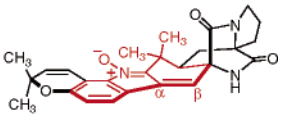
The complex alkaloid stephacidin B (**1**) was recently isolated from a fungal culture by a multistep process.[1,2] Ultimately, crystallization and X-ray analysis established the structure shown (**1**), save for absolute stereochemistry, which is unknown. It was recognized that **1** is potentially formed by dimerization of **2**. A mechanism for the putative dimerization reaction was advanced that involved protonation of **2** followed by formation of bonds b and a (see structure **1**), in that order, via cationic intermediates.[1b]



Stephacidin B (**1**)

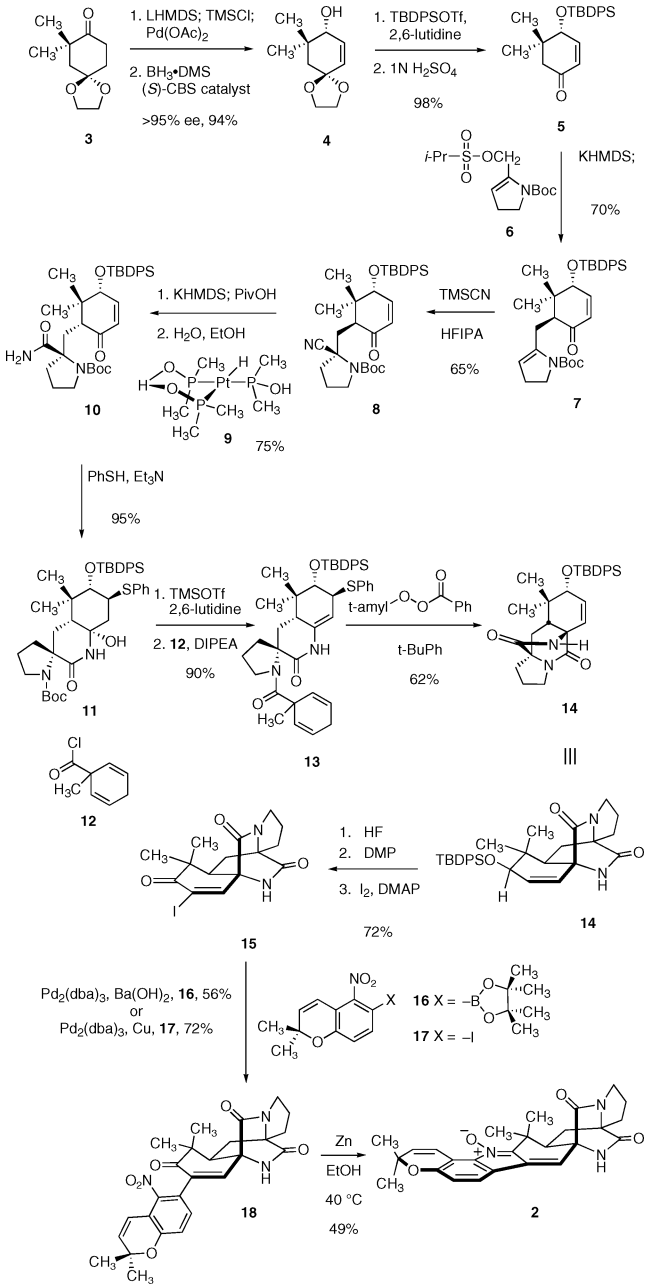


2 ("Avrainvillamide")

The structure **2** had previously appeared in the patent literature as the antiproliferative fungal isolate "avrainvillamide" (where it was depicted as *ent*-**2**; neither relative nor absolute stereochemical assignments were discussed)[3] and was later described by Sugie and co-workers as "CJ-17,665", an isolate from a different fungal strain (neither relative nor absolute stereochemistry was defined).[4] Both stephacidin B and avrainvillamide are reported to inhibit the growth of cultured human cancer cells (IC_{50} values ~50−100 nM), but side-by-side comparisons of these compounds have not been made, so far as we are aware. We have previously described methodology to synthesize the substructure depicted in red within structure **2** and found that the unsaturated nitrone (3-alkylidene-3*H*-indole 1-oxide) function within the model compound we synthesized readily underwent reversible addition of oxygen- and sulfur-based nucleophiles to the carbon labeled β, which suggested that the putative dimerization of **2** to form **1** might be initiated by bond formation to carbon β[5] (see also, ref 6), and not carbon α as originally proposed.[1b] Here, we describe an enantioselective synthesis of structure **2** (levorotatory, vide infra) and observe that (−)-**2** undergoes spontaneous dimerization to form (+)-stephacidin B (**1**) in the presence of triethylamine.[7]

Our synthetic route to **2** and stephacidin B (**1**) begins with the known, achiral cyclohexanone derivative **3**,[8] which was transformed via its trimethylsilyl enol ether into the corresponding α,β-unsaturated ketone in small-scale reactions by palladium-mediated oxidation (98% yield, 1.3-g scale, Scheme 1).[9] In larger scale preparations, **3** was oxidized directly with 2-iodoxybenzoic acid in the presence of 4-methoxypyridine *N*-oxide (70% yield, 10.4-g scale).[10] Enantioselective reduction of the α,β-unsaturated ketone produced by either method was achieved using the Corey−Bakshi−Shibata (CBS) catalytic protocol.[11] The stereochemistry of the single stereogenic center introduced in the CBS reduction step was subsequently relayed to all others within stephacidin B (**1**). Because neither the chirality of **1** nor **2** was known, we randomly selected the (*S*)-CBS catalyst to illustrate our enantioselective route to stephacidin B (**1**), forming the (*R*)-allylic alcohol **4** in >95% ee

Scheme 1



(96% yield).[12] Silyl ether formation and ketal hydrolysis then gave the α,β-unsaturated ketone **5** (98% yield, two steps).

In a key carbon−carbon bond-forming reaction, the ketone **5** was deprotonated with potassium hexamethyldisilazide (KHMDS) and the resulting enolate was trapped with the novel electrophile **6** [synthesized from *N*-(*tert*-butoxycarbonyl)-2,3-dihydropyrrole by a sequence involving α-lithiation,[13] formylation, reduction (boro-

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hydride), and *iso*-propylsulfonylation], producing the *trans*-coupling product **7** as a single diastereomer (70%, 4.4-g scale). Use of the methanesulfonate ester corresponding to **6** in the alkylation gave **7** in lower yield (50%), presumably due to competitive proton transfer from the methanesulfonate group. In a second critical transformation, the alkylation product **7** was found to undergo Strecker-like addition of hydrogen cyanide, but only in the solvent hexafluoroisopropanol (HFIPA, 0 °C, 4 days), forming the *N*-Boc amino nitrile **8** (65%) and 16% of the diastereomeric amino nitrile (not shown, yields of pure diastereomers, separated by flash-column chromatography). We know of no close precedence for Strecker-like additions to *N*-Boc enamine substrates such as **7**. To establish the stereorelationships required for synthesis of stephacidin B, the α-carbon of the ketone **8** was epimerized by deprotonation with KHMDS followed by quenching of the resultant enolate with pivalic acid (88%, 487-mg scale). The platinum catalyst **9** of Ghaffar and Parkins[14] then served to transform the nitrile group of the epimerized product into the corresponding primary amide (**10**, 85%). The latter transformation was conducted under essentially neutral conditions; its success within a complex substrate suggests that the method may be of value in extension to the hydrolysis of other Strecker-derived addition products (typically conducted at the extremes of pH).[15] Treatment of the primary amide **10** with thiophenol and triethylamine led to conjugate addition of thiophenol as well as cyclic hemiaminal formation, giving the tricyclic product **11** (95%). A strictly analogous transformation occurred when *p*-methoxythiophenol was used as nucleophile, giving a crystalline product, whose structure (including all relative stereochemical assignments) was solved by X-ray analysis (see Supporting Information). Dehydration of the cyclic hemiaminal **11** in the presence of trimethylsilyl triflate and 2,6-lutidine was accompanied by cleavage of the *N*-Boc protective group; acylation of the pyrrolidinyl amino group that was liberated with 1-methyl-2,5-cyclohexadiene-1-carbonyl chloride, an acyl radical precursor developed by Jackson and Walton,[16] then formed the amide **13** (90% yield, two steps). Heating of rigorously deoxygenated solutions of **13** and *tert*-amyl peroxybenzoate in *tert*-butyl benzene as solvent at 119 °C produced the bridged diketopiperazine core of stephacidin B in the form of the tetracyclic product **14** (62% yield, 144-mg scale). This key transformation (**13** → **14**) is believed to involve the formation of an aminoacyl radical intermediate, as would be expected based on precedent,[16] followed by attack of that aminoacyl radical upon the more substituted carbon of the enamide C−C double bond and expulsion of phenylthiyl radical, events that were less predictable. All efforts to prepare **14** using cyanide as the source of the final (bridging) carbon atom and intermediates such as **10**, **11**, or their derivatives as starting materials were unsuccessful.

With the development of an efficient synthetic sequence to the tetracyclic product **14**, completion of the synthesis of **2** and **1** was straightforward. First, **14** was transformed into the α-iodoenone **15** in a three-step sequence (72% yield, Scheme 1). Next, the α-iodoenone **15** was coupled in a Suzuki reaction with the arylboronic acid derivative **16**[17] (56% yield) or, more efficiently, by an Ullmann-like coupling[18] with the aryl iodide **17**[19] (10 mol % Pd_2dba_3, Cu powder, 72% yield). Finally, the nitroarene coupling product (**18**) was reduced in the presence of activated zinc powder,[5,20] forming the heptacyclic unsaturated nitrone **2** as a yellow solid in 49% yield (scale: 5−10 mg, 17 steps, 4.2% yield from **3**) after purification by flash-column chromatography. The chromatographically purified product **2** provided a clean ^{1}H NMR spectrum (Figure 1a) that was distinct from a published spectrum of stephacidin B in the same solvent (Figure 1c). Although there is no doubt that the synthetic material we have prepared corresponds

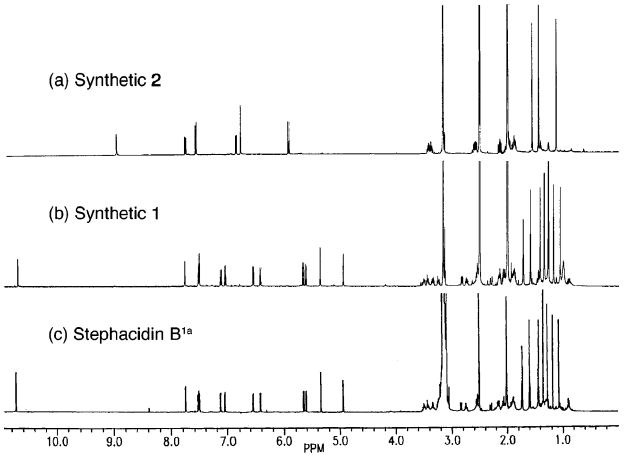
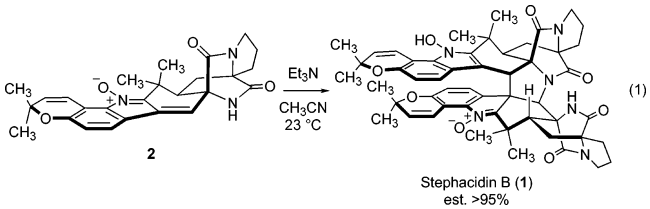


Figure 1. ^{1}H NMR spectra at 23 °C (500 MHz, 1:1 DMSO-d_6−CD_3CN) of (a) synthetic **2**, (b) synthetic **1**, (c) stephacidin B from a fungal source.[1a] The latter is a scanned spectrum from ref 1a. Note the peaks at δ 3.14, 2.49, and 1.99 in each spectrum correspond to residual water, DMSO-d_5, and HCD_2CN, respectively.

to structure **2** (*ent*-avrainvillamide, based on the structure depicted in ref 3, vide supra), spectroscopic data for avrainvillamide have not been published, and thus we cannot claim that our synthetic material and avrainvillamide are the same. While natural avrainvillamide is reportedly dextrorotatory (+10.6°, $CHCl_3$),[3] synthetic **2** was found to be levorotatory (−35.1°, $CHCl_3$, 25 °C). We draw no conclusions from the deviations in sign and magnitude of the two measurements, however, for the temperature of the rotational measurement of natural avrainvillamide was not reported and, more importantly, (−)-**2** and (+)-**1** are readily interconverted in solution (vide infra). Comparison spectra (^{1}H and ^{13}C NMR) of CJ-17,665, kindly provided by Dr. Yutaka Sugie (Pfizer Inc.), were similar in many respects to spectra we obtained from synthetic **2** (notably, all carbon resonances corresponded, within 0.6 ppm), but there was a lack of correspondence in the ^{1}H NMR spectra in the region δ 2.45−2.60, which is concerning. Because there are internal inconsistencies in this region in ^{1}H NMR spectra of CJ-17,665 provided to us, we cannot conclude at this time if synthetic **2** and CJ-17,665 are the same or different.

An unequivocal link between synthetic and natural materials was established when we observed that pure synthetic (−)-**2** was transformed into stephacidin B (**1**) in the presence of triethylamine at 23 °C (eq 1). Stirring a solution of (−)-**2** and a large excess of triethylamine (15 vol %, 22 mM in **2**) in acetonitrile at 23 °C led to gradual bleaching of the initially bright yellow solution with concomitant formation of a new, more polar material (TLC analysis). Concentration of the reaction mixture after 3.5 h and dissolution of the white solid residue obtained in a 1:1 mixture of DMSO-d_6−CD_3CN provided a nearly pure solution of stephacidin B (**1**, ^{1}H NMR analysis, est. >95%, Figure 1b).



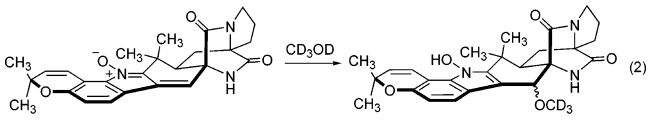
(1)

^{1}H NMR spectra of synthetic and natural stephacidin B (the latter from published data)[1a] corresponded exactly (cf., Figure 1b,c). Synthetic stephacidin B was found to be dextrorotatory ($[\alpha]^{24}_D$ = +91.0°, *c* 0.25, CH_3CN). Rotations of natural **1** have not been

reported; thus, the absolute configuration of stephacidin B remains unknown at this time.

Our preliminary studies leave little doubt that **1** and **2** are readily interconverted in solution. For example, concentration of an acetonitrile−water solution of pure synthetic stephacidin B (**1**) at 38 °C afforded a 2:1 mixture of **2** and **1**, as well as unidentified decomposition products. Also, whereas solutions of pure **1** in 50% DMSO-d_6−CD_3CN appeared to be stable for at least 48 h at 23 °C,[21] addition of powdered 3 Å molecular sieves led to partial retrodimerization, giving a 2:1 mixture of **1** and **2** within 1 h at 23 °C. We also observed partial transformation of **1** to form **2** upon exposure to silica gel (2D TLC analysis). From the data thus far, it is clear that (−)-**2** and (+)-**1** readily interconvert under mild conditions. This suggests that it is possible that the observed biological activity of stephacidin B may be attributable to **2** formed from **1** in vivo. In theory, the converse may be true, though this seems less likely, simply upon consideration of concentration effects.[22]

Finally, we have observed that solutions of **2** in pure methanol-d_4 rapidly (<10 min, 23 °C) form the diastereomeric products of 1,5-addition of methanol-d_4 (eq 2). The ratio of diastereomeric methanol-d_4 adducts was ~15:1 (stereochemistry not assigned). The ratio of these diastereomeric adducts combined to **2** remaining in solution suggests an equilibrium constant of 7.7 at 23 °C, although this value must be regarded as tentative for we have not yet conducted the experiments to establish that a true equilibrium exists (the solution decomposed upon concentration). The value 7.7 is somewhat larger than the equilibrium constant we had measured for the model unsaturated nitrone previously prepared ($K = 2$, 23 °C; the rate of methanol-d_4 addition was also faster: $t_{1/2} \ll 10$ min at 23 °C for **2** vs $t_{1/2} = 5$ h at 23 °C in the model system),[5] but these differences are not surprising given the structural dissimilarities of the two systems.



2 (2)

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Supporting Information Available: Detailed experimental procedures and tabulated spectroscopic data (^1H and ^{13}C NMR, FT-IR, and HRMS) for all new compounds, X-ray analysis of the *p*-methoxyphenylthio adduct corresponding to **11** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Qian-Cutrone, J.; Krampitz, K. D.; Shu, Y.; Chang, L.; Lowe, S. E. Stephacidin Antitumor Antibiotics. U.S. Patent 6,291,461, 2001. (b) Qian-Cutrone, J.; Huang, S.; Shu, Y.; Vyas, D.; Fairchild, C.; Menendez, A.; Krampitz, K.; Dalterio, R.; Klohr, S. E.; Gao, Q. *J. Am. Chem. Soc.* **2002**, *124*, 14556.
(2) For reviews of prenylated indole alkaloids from fungi, see: (a) Williams, R. M.; Cox, R. J. *Acc. Chem. Res.* **2003**, *36*, 127. (b) Stocking, E. M.; Williams, R. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 3078.
(3) Fenical, W.; Jensen, P. R.; Cheng, X. C. Avrainvillamide, a Cytotoxic Marine Natural Product, and Derivatives thereof. U.S. Patent 6,066,635, 2000.
(4) Sugie, Y.; Hirai, H.; Inagaki, T.; Ishiguro, M.; Kim, Y.; Kojima, Y.; Sakakibara, T.; Sakemi, S.; Sugiura, A.; Suzuki, Y.; Brennan, L.; Duignan, J.; Huang, L. H.; Sutcliffe, J.; Kojima, N. *J. Antibiot.* **2001**, *54*, 911.
(5) Myers, A. G.; Herzon, S. B. *J. Am. Chem. Soc.* **2003**, *125*, 12080.
(6) Independently proposed: Nussbaum, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 3068.
(7) For syntheses of alkaloids structurally related to **2**, see: (a) (−)-Brevianamide B: Williams, R. M.; Glinka, T.; Kwast, E.; Coffman, H.; Stille, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 808. (b) (+)-Paraherquamide B: Cushing, T. D.; Sanz-Cervera, J. F.; Williams, R. M. *J. Am. Chem. Soc.* **1996**, *118*, 557. (c) (±)-VM55599: Stocking, E. M.; Sanz-Cervera, J. F.; Williams, R. M. *J. Am. Chem. Soc.* **2000**, *122*, 1675. (d) (−)-VM55599: Sanz-Cervera, J. F.; Williams, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 2556. (e) Paraherquamide A: Williams, R. M.; Cao, J.; Tsujishima, H.; Cox, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 12172. (f) Stephacidin A: Baran, P. S.; Guerrero, C. A.; Ambhaikar, N. B.; Hafensteiner, B. D. *Angew. Chem., Int. Ed.* **2004**, *44*, 606.
(8) Nelson, L. A. K.; Shaw, A. C.; Abrams, S. R. *Tetrahedron* **1991**, *47*, 3259.
(9) Ito, Y.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011.
(10) Nicolaou, K. C.; Montagnon, T.; Baran, P. S. *Angew. Chem., Int. Ed.* **2002**, *41*, 993.
(11) (a) Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.* **1987**, *109*, 5551. (b) Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986.
(12) The absolute stereochemistry of **4** was determined by the modified Mosher method and is consistent with the established transition-state model for the oxazaborolidine-mediated reduction.[11b] (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512. (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
(13) Tae, J.; Paquette, L. A. *Can. J. Chem.* **2000**, *78*, 689.
(14) (a) Ghaffar, T.; Parkins, A. W. *Tetrahedron Lett.* **1995**, *36*, 8657. (b) Ghaffar, T.; Parkins, A. W. *J. Mol. Catal. A* **2000**, *160*, 249.
(15) Schaefew, F. C. In *The Chemistry of the Cyano Group;* Rappoport, Z., Ed.; The Chemistry of Functional Groups; Wiley and Sons: New York, 1970; p 239.
(16) (a) Jackson, L. V.; Walton, J. C. *Chem. Commun.* **2000**, 2327. (b) Bella, A. F.; Jackson, L. V.; Walton, J. C. *Org. Biomol. Chem.* **2004**, *2*, 421.
(17) Prepared from the aryl iodide **17** by the method of Sapountzis and Knochel: Sapountzis, I.; Knochel, P. *Angew. Chem., Int. Ed.* **2002**, *41*, 1610. See Supporting Information for details.
(18) Banwell, M. G.; Kelly, B. D.; Kokas, O. J.; Lupton, D. W. *Org. Lett.* **2003**, *5*, 2497.
(19) Iodide **17** was prepared in two steps following a route previously developed by others for the synthesis of similarly substituted chromene derivatives. (a) Elomri, A.; Michel, S.; Tillequin, F.; Koch, M. *Heterocycles* **1992**, *34*, 799. (b) Cox, R. J.; Williams, R. M. *Tetrahedron Lett.* **2002**, *43*, 2149. (c) See also, ref 7f. An alternative preparation of **17** has been reported: (d) Sun, H.; Qing, F.; Chen, X. *Synthesis* **1997**, 1249.
(20) Knochel, P.; Rao, C. J. *Tetrahedron* **1993**, *49*, 29.
(21) The merits of the DMSO-d_6−CD_3CN solvent system in stabilizing stephacidin B were discussed in ref 1b.
(22) Our results also leave open the possibility that stephacidin B is an artifact of the isolation of **2**; the converse may be true instead, though this would appear to be less likely.

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