SUPPORTING INFORMATION

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

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

*Contribution from the Department of Chemistry, The Scripps Research Institute, 10650 N. Torrey Pines Road, La Jolla, California 92037

**General Procedures.** All reactions were carried out under an inert nitrogen atmosphere unless otherwise stated. All reagents were purchased at the highest commercial quality and used as received unless otherwise stated. Dry methanol (MeOH), dichloromethane (CH₂Cl₂), benzene, toluene (PhMe), triethylamine (Et₃N), and tetrahydrofuran (THF) were obtained by passing these previously degassed solvents through activated alumina columns. Glacial acetic acid (AcOH) was purchased at ACS Plus commercial quality from Fisher Scientific and used as received. Acetone and 1,4-dioxane were distilled and stored in amber glass bottles. N, N-diisopropylethylamine was distilled over calcium hydride and stored over potassium hydroxide pellets in an amber glass bottle. Pd₃dba₅·CHCl₃ was prepared according to a published procedure. Lithium diisopropylamide (LDA; 0.5 M in THF) was generated as needed from distilled diisopropylamine and n-butyllithium (2.5 M in hexanes) by standard methods. Fe(acac)₃ was recrystallized from EtOH and dried under high vacuum for 12 hours prior to use. The Burgess reagent was prepared according to the published procedure. Sodium cyanoborohydride [NaBH₃CN], 95%, was purchased from Alfa Aesar and used without further purification. Sodium tungstate dihydrate (Na₂WO₄·2H₂O), ACS reagent grade, was purchased from Acros Organics and used without further purification. 35% aqueous hydrogen peroxide (H₂O₂) was purchased from Acros Organics, used as received, and stored at 0 °C. Selenium dioxide (SeO₂), 99%, was purchased from Aldrich and used as received. Yields refer to chromatographically and spectroscopically (¹H NMR) homogenous material, unless otherwise stated. Reactions were monitored by thin layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as the visualizing agent and an acidic anisaldehyde, acidic phshomolybdic acid, or basic aqueous potassium permanganate (KMnO₄) and heat as developing agents. E. Merck silica gel (60, particle size 0.043–0.063 mm) was used for flash column chromatography. Preparative thin layer chromatography (PTLC) separations were carried out on 0.25, 0.5, or 1.0 mm E. Merck silica gel plates (60F-254). Optical rotation measurements were recorded on a Perkin Elmer Model 341 polarimeter using a 10 cm (1 mL capacity) cell. NMR spectra were recorded on a Bruker DRX-500 instrument and calibrated using residual undeuterated solvent as internal reference. The following

abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. High resolution mass spectra (HRMS) were recorded on Agilent LC/MSD TOF mass spectrometer by electro spray ionization time of flight reflectron experiments.

**Model indoline 13. CAUTION: The following procedure should be conducted in a well-ventilated fume hood as hydrogen cyanide gas is evolved upon addition of the reducing agent to the reaction mixture.** Compound 12 (10.3 mg, 21.9 µmol) was dissolved in AcOH (220 µL, 0.1 M). NaBH₄CN (13.8 mg, 219 µmol) was added in one portion. The reaction was allowed to stir at 25 °C for 12 h after which it was diluted with EtOAc (10 mL) and poured into a separatory funnel. The solution was basified to pH 14 by the addition of 3 M aq. NaOH and gave a clear biphasic mixture. The layers were separated, and the aqueous portion was re-extracted with an addition 10 mL EtOAc. The combined organic portions were washed with sat. aq. NaCl, dried over anhydrous MgSO₄, and concentrated in vacuo. The residue was purified by PTLC (4:1 CH₂Cl₂:acetone) and furnished 5.5 mg (53%) of 13. We note that this reaction has not been optimized.

**Model α,β-unsaturated nitrone 15.** Compound 13 (3 mg, 6.4 µmol) was dissolved in methanol (200 µL). To this solution was added water (50 µL), H₂O₂ (6.2 µL), and Na₃WO₄•2H₂O (0.4 mg, 1.3 µL). Sonication was used to aid dissolution of the catalyst. The reaction was allowed to stir for 6 h under an oxygen atmosphere after which the solution was diluted with 5 mL of each water and EtOAc. The layers were separated in a separatory funnel and the aqueous portion was re-extracted with 10 mL additional EtOAc. The combined organic portions were washed with sat. aq. NaCl, dried over anhydrous MgSO₄, and concentrated in vacuo. The residue was purified by PTLC (4:1 CH₂Cl₂:acetone) and furnished 15 (ca. 1 mg, ca. 30% yield) as a bright yellow film. Rf = 0.22 (silica gel, EtOAc); IR (film) νmax 2928, 2260, 1732, 1682, 1513, 1455, 1394, 1326, 1249, 1158, 1138, 1034, 841, 778 cm⁻¹; ¹H NMR (500 MHz, CD₃CN) δ 7.73 (d, J = 8.01 Hz, 1 H), 7.59 – 7.53 (m, 2 H), 7.53 – 7.48 (m, 1 H), 7.18 (d, J = 8.71 Hz, 2 H), 6.92 (s, 1 H), 6.85 (d, J = 8.71 Hz, 2 H), 4.78 (d, J = 15.9 Hz, 1 H), 4.56 (d, J = 15.9 Hz, 1 H), 3.73 (s, 3 H), 3.53 – 3.47 (m, 1 H), 3.46 – 3.39 (m, 1 H), 2.81 – 2.73 (m, 1 H), 2.56 – 2.51 (m, 1 H), 2.21 – 1.95 (m, 5 H), 1.54 (s, 3 H), 1.12 (s, 3 H); ¹³C NMR (125 MHz, CDCl₃) δ 172.3, 167.0, 159.6, 130.4, 129.7, 129.3, 129.0, 128.8 (2 C), 125.0, 124.4, 120.7, 115.9, 115.8, 114.8, 114.3, 67.5, 67.0, 55.7, 52.1, 45.6, 45.0, 36.4, 31.7, 30.4, 24.9 (2 C); HRMS (ESI-TOF) calcd for C₂⁹H₃⁰N₅O₄ [M + H⁺]: 484.2231; found: 484.2227.
Avrainvillamide (3). **CAUTION**: The following procedure should be conducted in a well-ventilated fume hood as hydrogen cyanide gas is evolved upon addition of the reducing agent to the reaction mixture. To a solution of stephacidin A (1; 3.5 mg, 8.1 µL) in AcOH (650 µL, 0.0125 M) was added NaBH₄CN (20.4 mg, 325 µmol) in one portion. The reaction was allowed to stir for 16 h after which it was quenched by pouring it into 6 mL sat. aq. NaHCO₃. The mixture was diluted with 5 mL each of EtOAc and H₂O and poured into a separatory funnel. The layers were separated, and the aqueous portion was re-extracted with an additional 10 mL EtOAc. The combined organic portions were washed with sat. aq. NaCl, dried over anhydrous MgSO₄, and concentrated in vacuo. The conversion was >95% as judged by ¹H NMR (inconsequential mixture of diastereomers). To a solution of compound 16 (4 mg, 9.2 µmol) (prepared as described above) in 1,4-dioxane (461 µL) was added aq. H₂O₂ (11.8 µL) followed by SeO₂ (0.26 mg; 10.2 µL from a solution in water made by dissolving 10 mg SeO₂ in 400 µL H₂O). The reaction was allowed to stir for 40 h after which the solution was diluted with 3 mL of each water and EtOAc. The layers were separated in a separatory funnel and the aqueous portion was re-extracted with 3 mL additional EtOAc. The combined organic portions were washed with sat. aq. NaCl, dried over anhydrous MgSO₄, and concentrated in vacuo. The residue was purified by PTLC (SiO₂, 100% EtOAc) and furnished starting material 16 (2.0 mg, 50%) and avrainvillamide (3; 1.1 mg, 27% yield) as a bright yellow amorphous solid. Synthetic 3 was identical to natural 3 as judged by LCMS, TLC in several solvent mixtures, ¹H NMR, and optical rotation (synthetic 3 [α]_D = +11 (c 0.1, CHCl₃); natural 3 [α]_D = 10.6 (c 0.17, CHCl₃).

Stephacidin B (2). Procedure A:[⁶] To a solution of avrainvillamide (3, 0.5 mg, 1.1 µmol) in CH₃CN (85 µL) was added Et₃N (15 µL). The reaction was allowed to stir at room temperature for 45 min. The resulting solution was evaporated and dried under high vacuum for 30 min. The residue was purified by PTLC (SiO₂, 100% EtOAc). Procedure B: avrainvillamide (3) was absorbed onto a prep plate and allowed to stand for 1 hour before being eluted. Stephacidin B could be separated from avrainvillamide by eluting solvent twice during purification. This procedure gave a 15-20% yield of 2 along with recovered 3 (70 – 80%). Procedure C: avrainvillamide was dissolved in DMSO and then the solvent was removed in vacuo over 30 min to 1 h. ¹H NMR indicated a ratio of 2:1 (3:2) which could be separated by PTLC. Synthetic 2 was identical in all respects to a sample of natural 3 kindly provided by BMS (LCMS, TLC in several solvent mixtures, ¹H NMR, and optical rotation (synthetic (–)-2: [α]_D = –33 (c 0.1, CHCl₃); natural (–)-2: [α]_D = –21.1 (c 0.19, CDCl₃).
Synthetic (+)-3

Natural (+)-3

(−)-3 reported by Myers (ref 8)
CJ-17,665 as received from Pfizer
dissolved in DMSO-d6
CJ-17,665 as received from Pfizer dissolved in CD3CN/DMSO-d6 1:1

stephalidin B spectra reported by BMS dissolved in CD3CN/DMSO-d6 1:1
avainvillamide
in DMSO-d6

avainvillamide spectra provided by Prof. Fenical in DMSO-d6

$^{13}$C NMR spectrum of CCG33864 in DMSO-d6
13C NMR of natural avrainvillamide in CDCl₃