

## SUPPORTING INFORMATION

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

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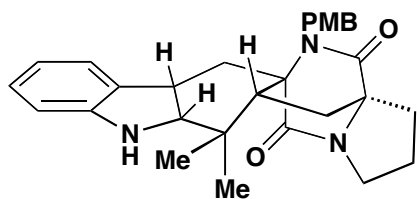
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**General Procedures.** All reactions were carried out under an inert nitrogen atmosphere unless otherwise stated. All reagents were purchased at the highest commercial quality and used as received unless otherwise stated. Dry methanol (MeOH), dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), benzene, toluene (PhMe), triethylamine (Et<sub>3</sub>N), 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. Pd<sub>2</sub>dba<sub>3</sub>•CHCl<sub>3</sub> was prepared according to a published procedure.<sup>1</sup> 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)<sub>3</sub> 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.<sup>2</sup> Sodium cyanoborohydride [NaBH<sub>3</sub>CN], 95%, was purchased from Alfa Aesar and used without further purification. Sodium tungstate dihydrate (Na<sub>2</sub>WO<sub>4</sub>•2H<sub>2</sub>O), ACS reagent grade, was purchased from Acros Organics and used without further purification. 35% aqueous hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was purchased from Acros Organics, used as received, and stored at 0 °C. Selenium dioxide (SeO<sub>2</sub>), 99%, was purchased from Aldrich and used as received. Yields refer to chromatographically and spectroscopically (<sup>1</sup>H 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 phosphomolybdic acid, or basic aqueous potassium permanganate (KMnO<sub>4</sub>) 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

<sup>1</sup> T. Ukai, H. Kawazura, Y. Ishii, J. J. Bonnet, J. A. Ibers, *J. Organomet. Chem.* **1974**, 65, 253 – 266.

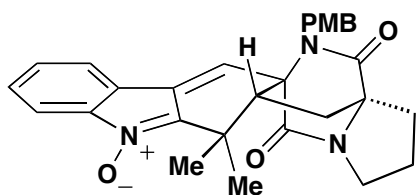
<sup>2</sup> E. M. Burgess, H. R. Penton, Jr., E. A. Taylor, *J. Org. Chem.* **1973**, 38, 26 – 31.

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 electrospray 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  $\mu\text{mol}$ ) was dissolved in AcOH (220  $\mu\text{L}$ , 0.1 M).  $\text{NaBH}_3\text{CN}$  (13.8 mg, 219  $\mu\text{mol}$ ) was added in one portion. The reaction was allowed to stir at 25  $^\circ\text{C}$  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  $\text{MgSO}_4$ , and concentrated *in vacuo*. The residue was purified by PTLC (4:1  $\text{CH}_2\text{Cl}_2$ :acetone) and furnished 5.5 mg (53%) of **13**. We note that this reaction has not been optimized.



**Model  $\alpha,\beta$ -unsaturated nitrone 15.** Compound **13** (3 mg, 6.4  $\mu\text{mol}$ ) was dissolved in methanol (200  $\mu\text{L}$ ). To this solution was added water (50  $\mu\text{L}$ ),  $\text{H}_2\text{O}_2$  (6.2  $\mu\text{L}$ ), and  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  (0.4 mg, 1.3  $\mu\text{L}$ ). 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  $\text{MgSO}_4$ , and concentrated *in vacuo*. The residue was purified by PTLC (4:1  $\text{CH}_2\text{Cl}_2$ :acetone) and furnished **15** (ca. 1 mg, ca. 30% yield) as a bright yellow film.  $R_f = 0.22$  (silica gel, EtOAc); IR (film)  $\nu_{\text{max}}$  2928, 2260, 1732, 1682, 1513, 1455, 1394, 1326, 1249, 1158, 1138, 1034, 841, 778  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ )  $\delta$  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);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  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  $\text{C}_{29}\text{H}_{30}\text{N}_3\text{O}_4$  [ $M + \text{H}^+$ ]: 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  $\mu\text{L}$ ) in AcOH (650  $\mu\text{L}$ , 0.0125 M) was added  $\text{NaBH}_3\text{CN}$  (20.4 mg, 325  $\mu\text{mol}$ ) 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.  $\text{NaHCO}_3$ . The mixture was diluted with 5 mL each of EtOAc and  $\text{H}_2\text{O}$  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  $\text{MgSO}_4$ , and concentrated *in vacuo*. The conversion was >95% as judged by  $^1\text{H}$  NMR (inconsequential mixture of diastereomers). To a solution of compound **16** (4 mg, 9.2  $\mu\text{mol}$ ) (prepared as described above) in 1,4-dioxane (461  $\mu\text{L}$ ) was added aq.  $\text{H}_2\text{O}_2$  (11.8  $\mu\text{L}$ ) followed by  $\text{SeO}_2$  (0.26 mg; 10.2  $\mu\text{L}$  from a solution in water made by dissolving 10 mg  $\text{SeO}_2$  in 400  $\mu\text{L}$   $\text{H}_2\text{O}$ ). 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  $\text{MgSO}_4$ , and concentrated *in vacuo*. The residue was purified by PTLC ( $\text{SiO}_2$ , 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,  $^1\text{H}$  NMR, and optical rotation (synthetic **3**  $[\alpha]_{\text{D}} = +11$  (*c* 0.1,  $\text{CHCl}_3$ ); natural **3**  $[\alpha]_{\text{D}} = 10.6$  (*c* 0.17,  $\text{CHCl}_3$ )).

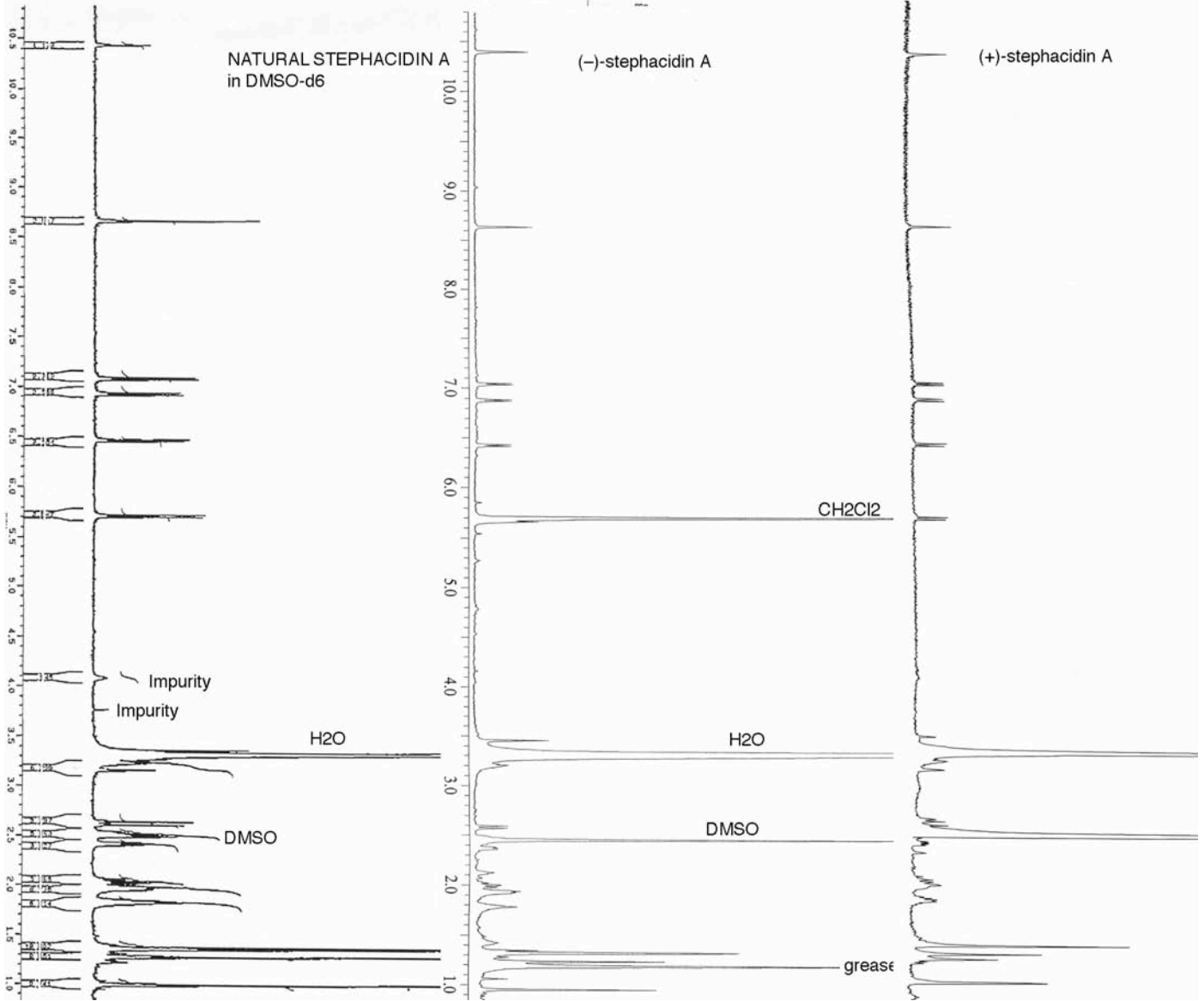
**Stephacidin B (2).** Procedure A:<sup>161</sup> To a solution of avrainvillamide (**3**, 0.5 mg, 1.1  $\mu\text{mol}$ ) in  $\text{CH}_3\text{CN}$  (85  $\mu\text{L}$ ) was added  $\text{Et}_3\text{N}$  (15  $\mu\text{L}$ ). The reaction was allowed to stir at room temperature for 45 min. The resulting solution was evaporated and dried under high vacuum for 30 min. The residue was purified by PTLC ( $\text{SiO}_2$ , 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.  $^1\text{H}$  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,  $^1\text{H}$  NMR, and optical rotation (synthetic (–)-**2**:  $[\alpha]_{\text{D}} = -33$  (*c* 0.1,  $\text{CHCl}_3$ ); natural (–)-**2**:  $[\alpha]_{\text{D}} = -21.1$  (*c* 0.19,  $\text{CDCl}_3$ )).

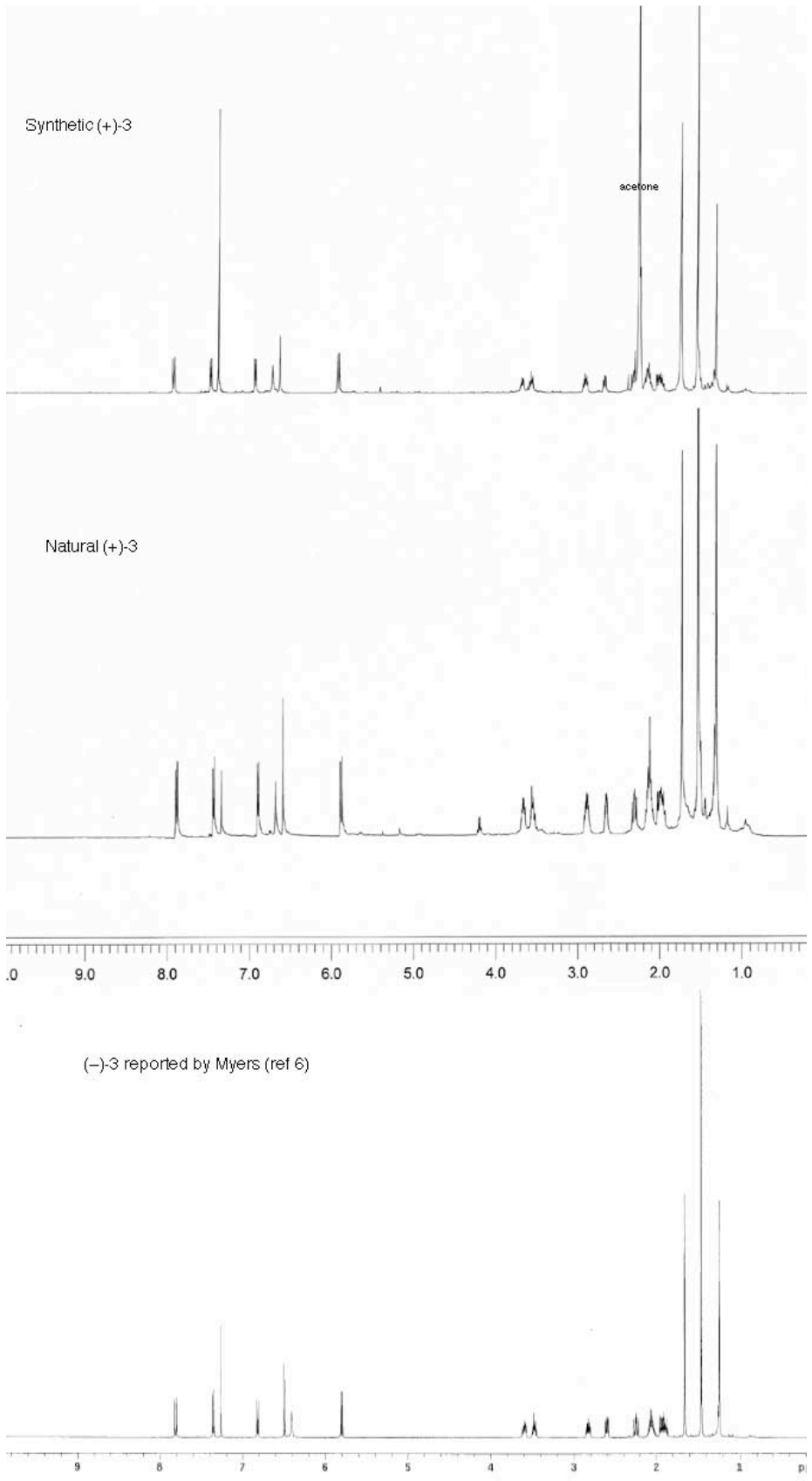
BRISTOL MYERS

NATURAL STEPHACIDIN A  
in DMSO-d6

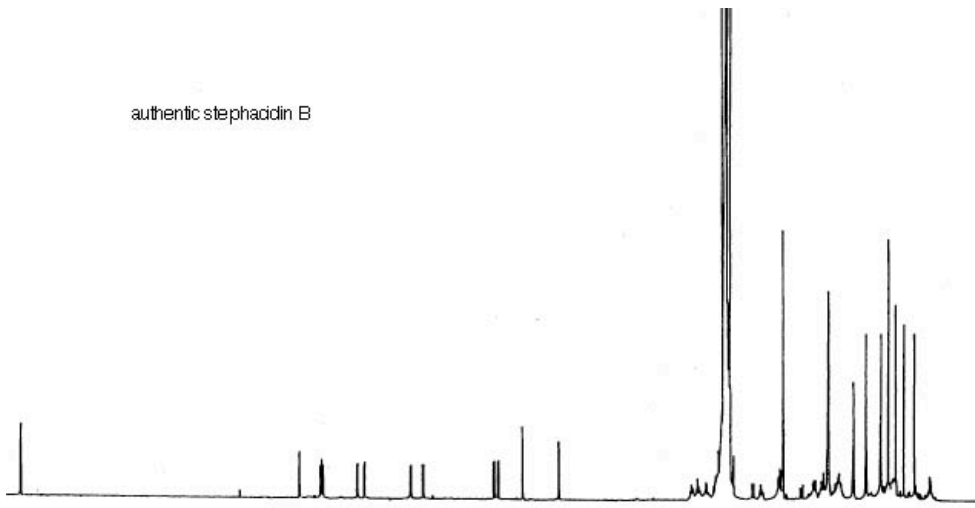
(-)-stephacidin A

(+)-stephacidin A

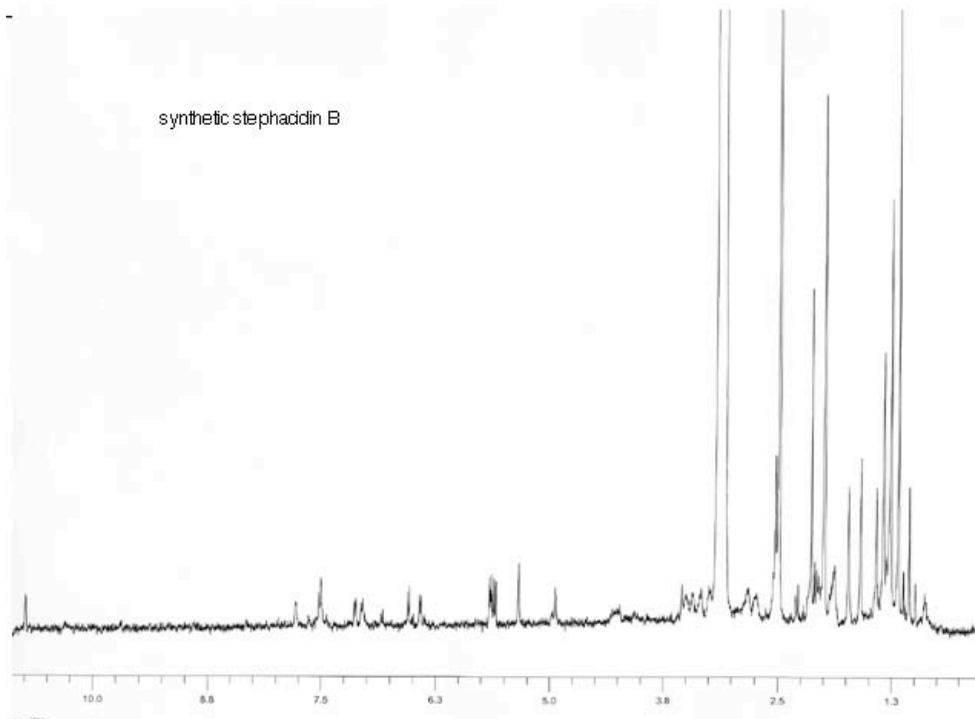


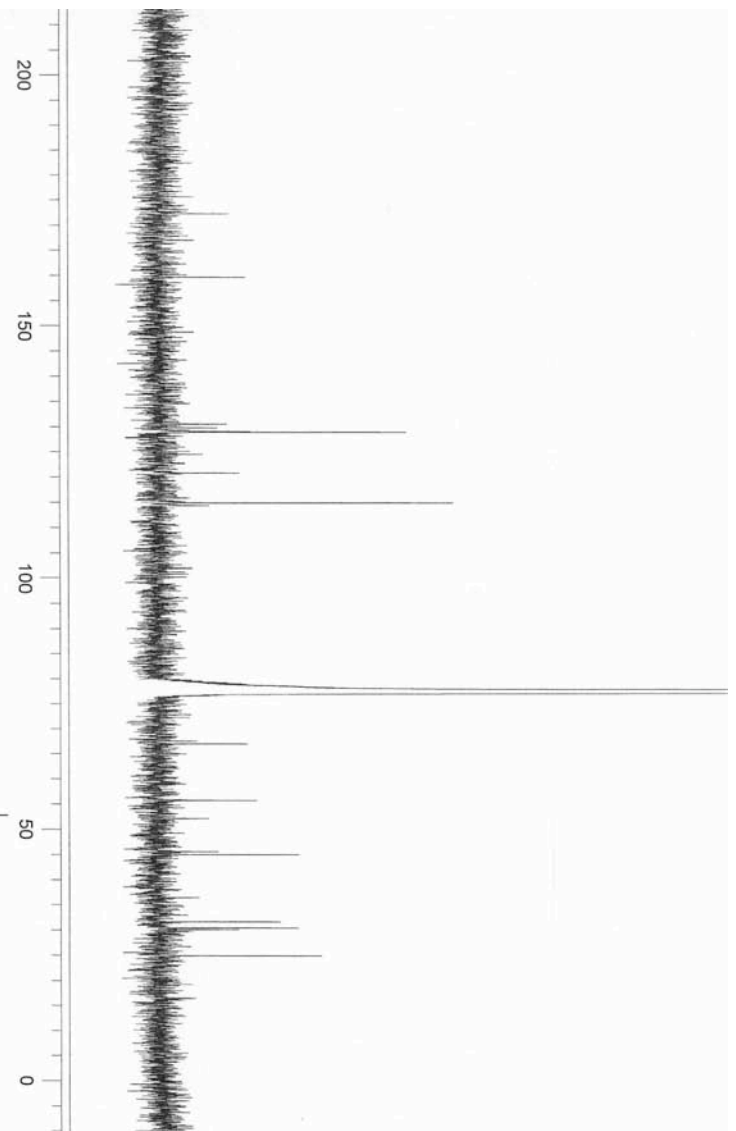
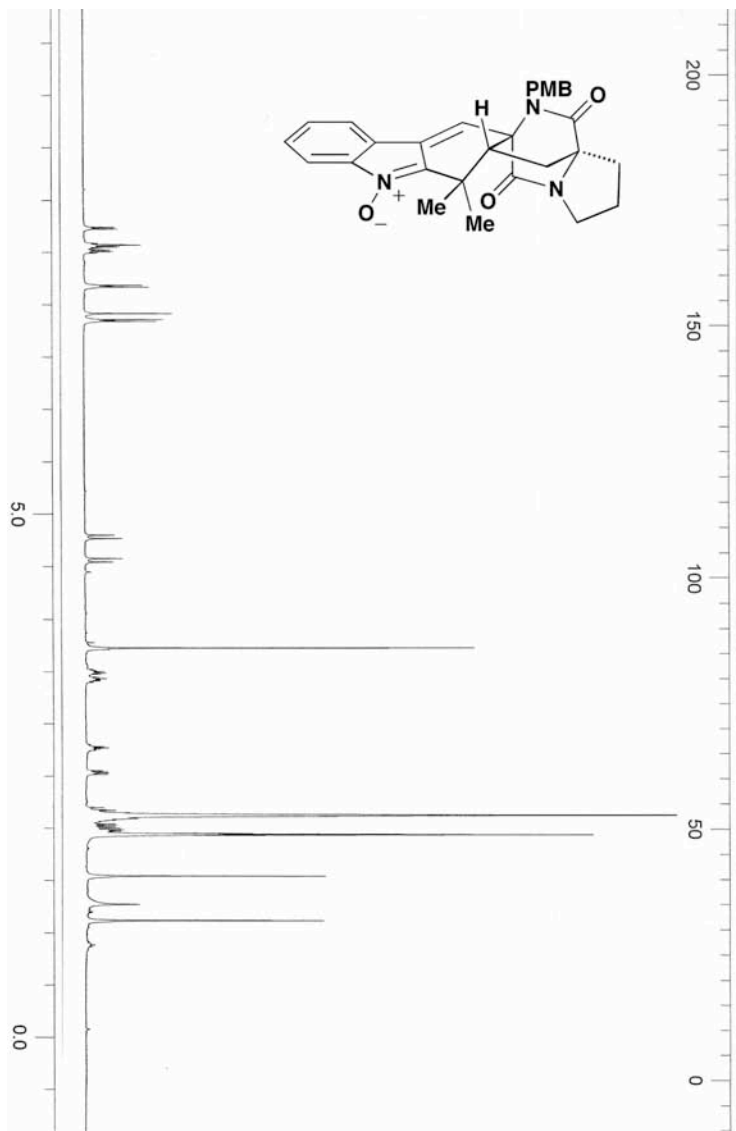
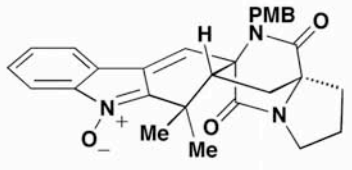


authentic stephadin B

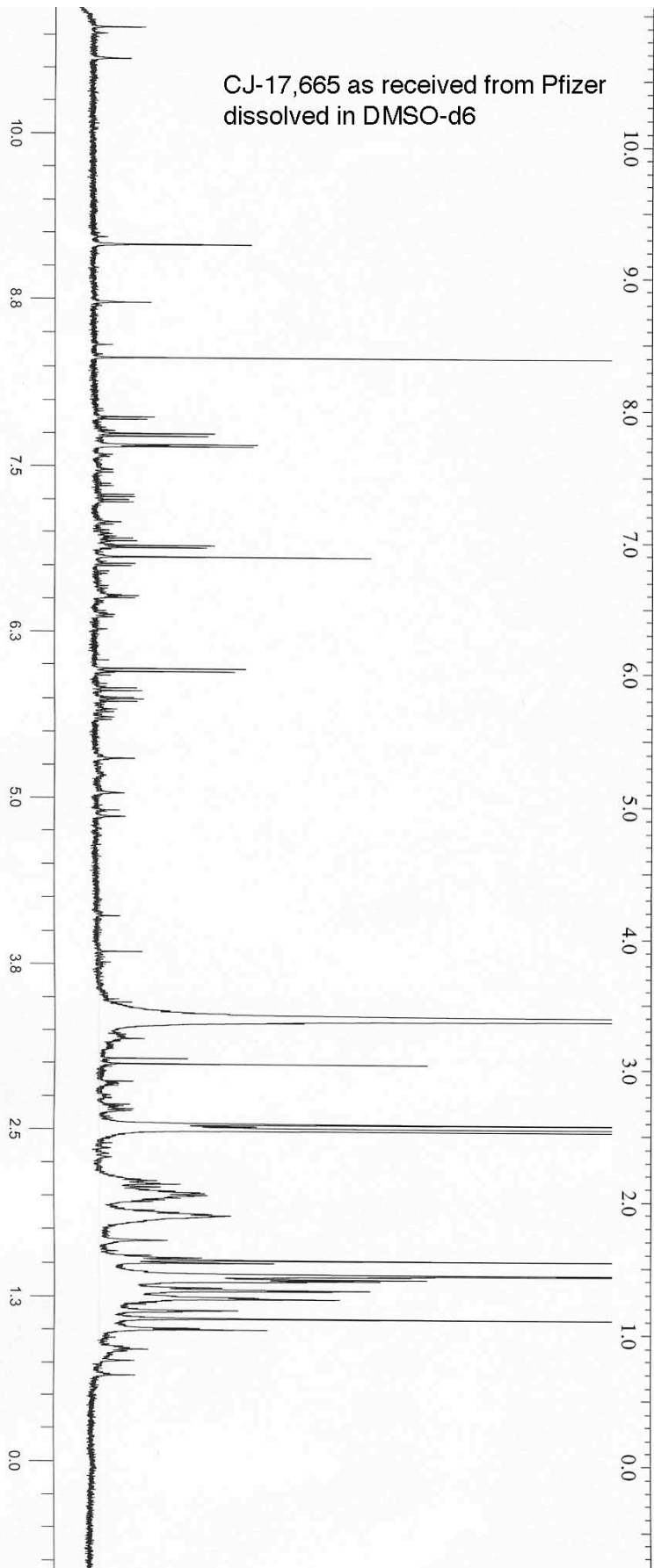


synthetic stephadin B

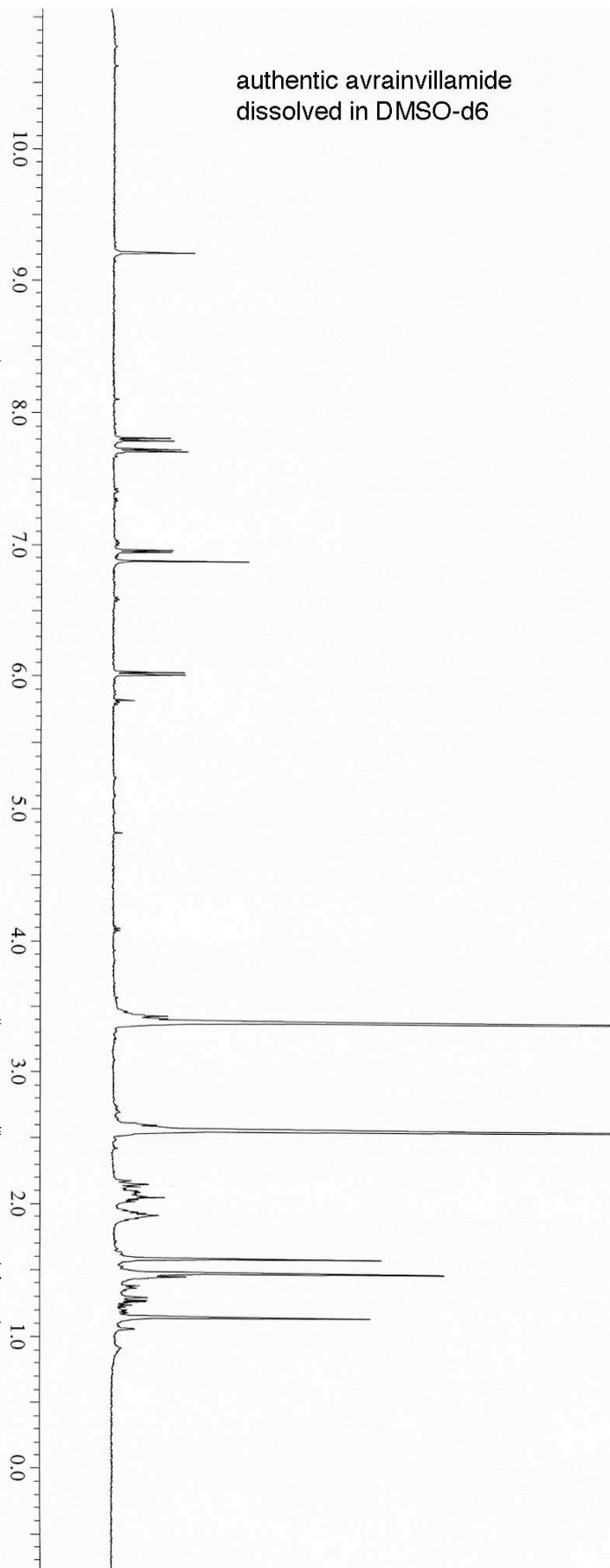


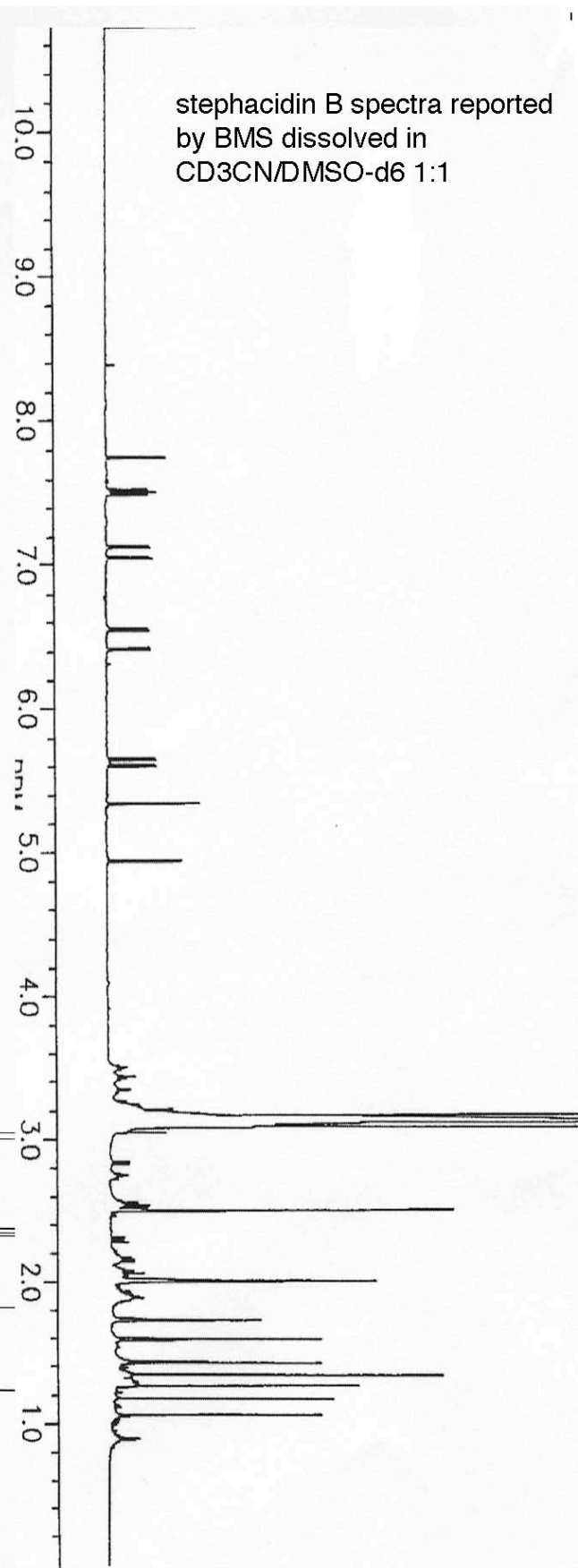
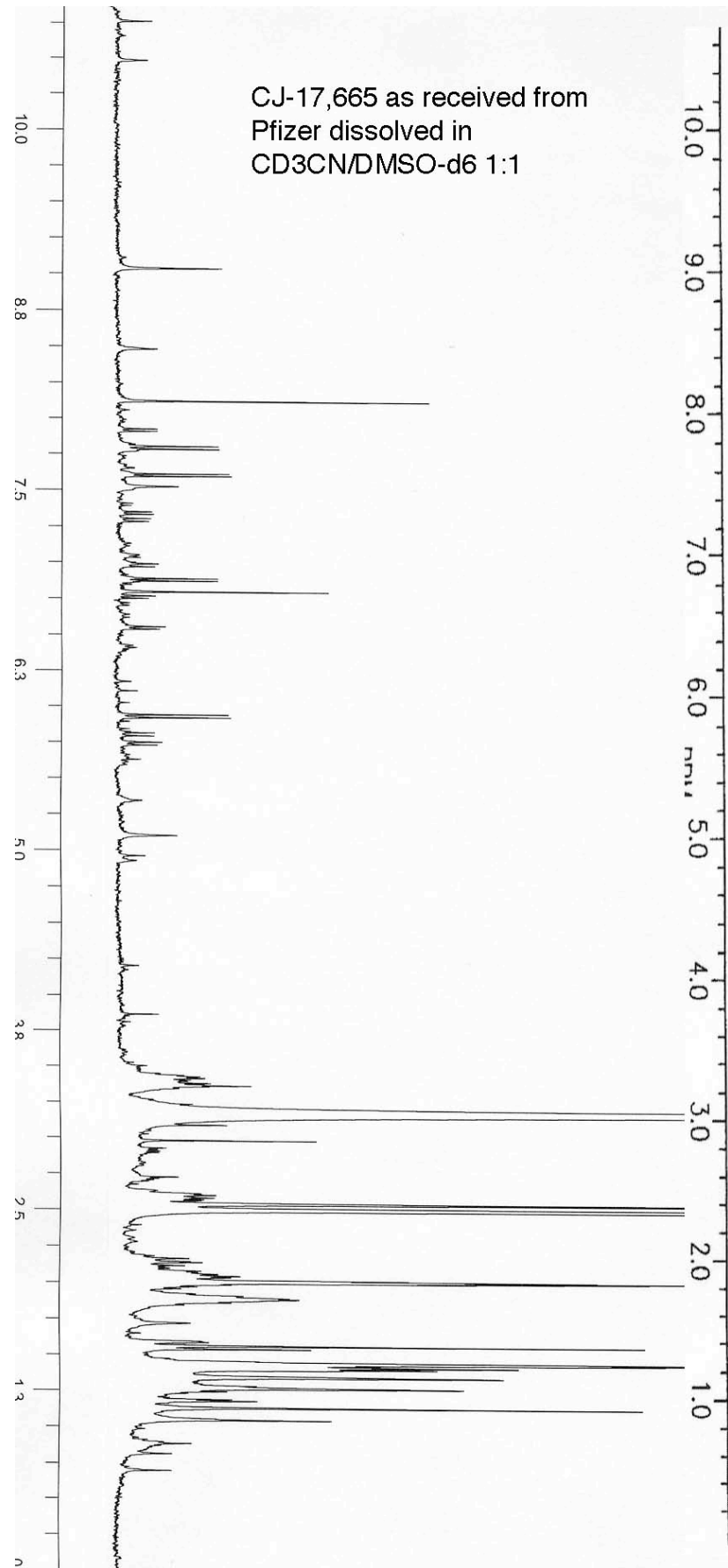


CJ-17,665 as received from Pfizer  
dissolved in DMSO-d6



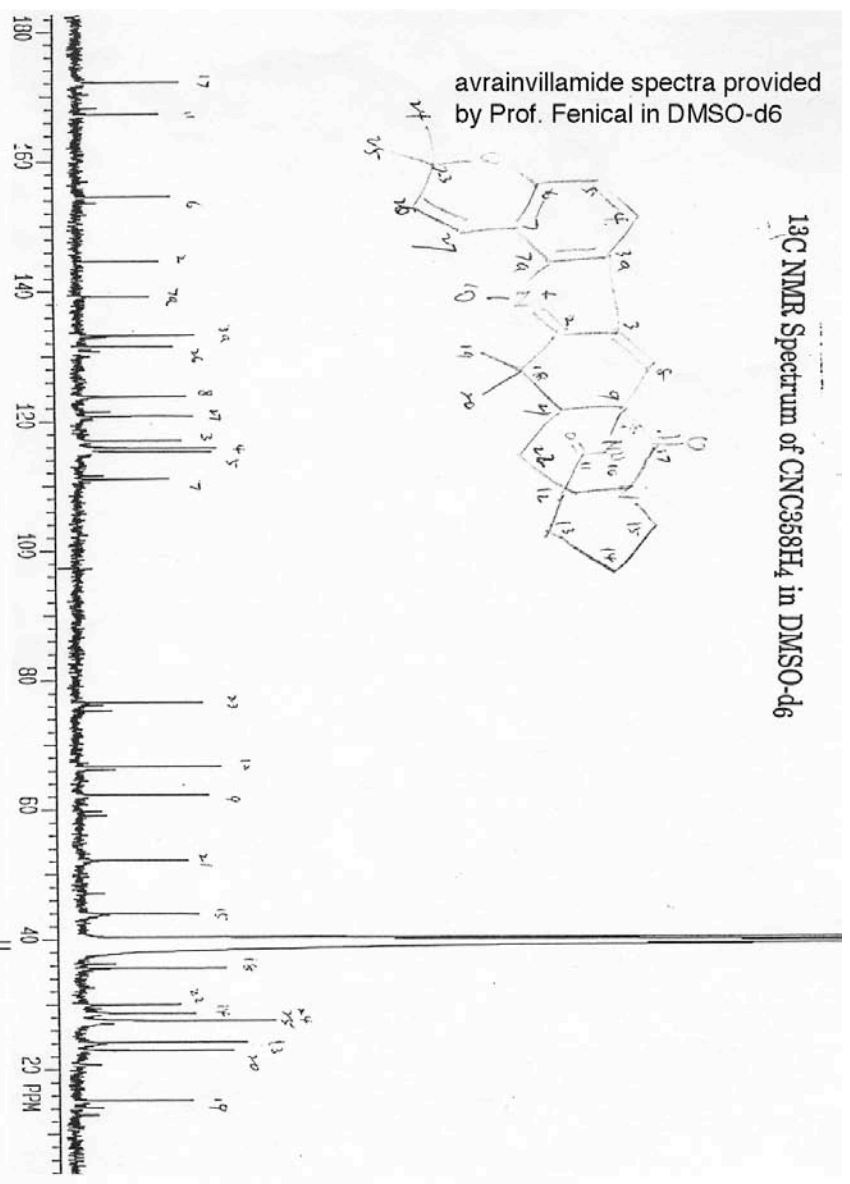
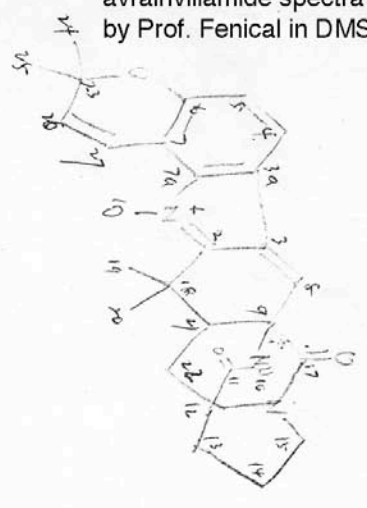
authentic avrainvillamide  
dissolved in DMSO-d6



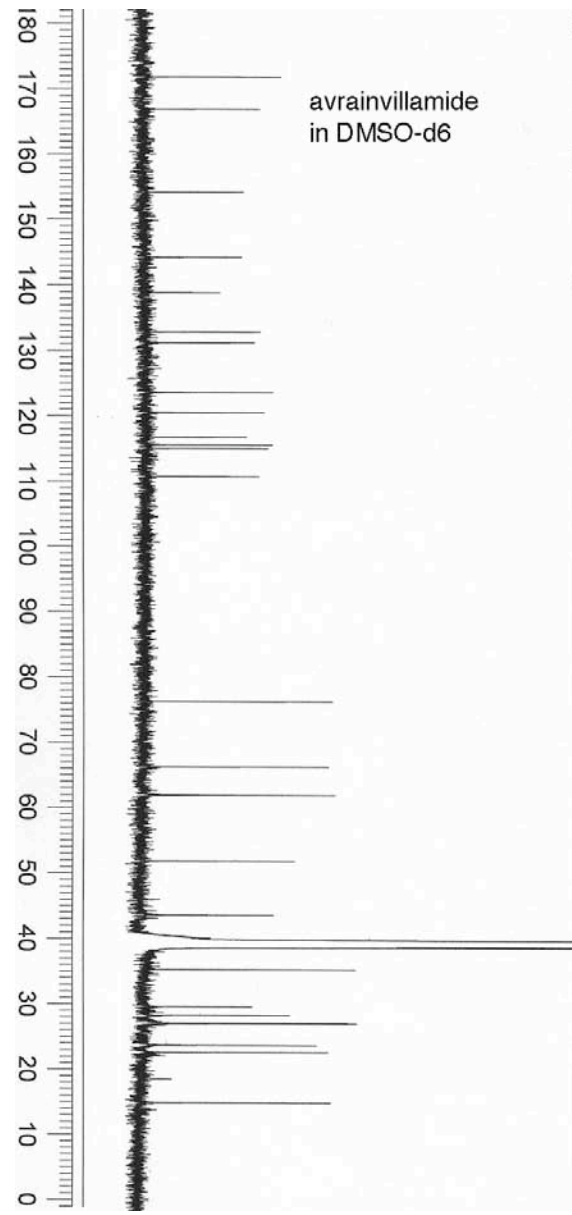


<sup>13</sup>C NMR Spectrum of CNC358H<sub>4</sub> in DMSO-d<sub>6</sub>

avrainvillamide spectra provided by Prof. Fenical in DMSO-d<sub>6</sub>



avrainvillamide in DMSO-d<sub>6</sub>



<sup>13</sup>C NMR of natural avrainvillamide in CDCl<sub>3</sub>

