SUPPORTING INFORMATION

Synthesis of Cryptothilone 1, the First Cryptophycin-Epothilone Hybrid.

James D. White^{1*}, Helmars Smits¹ and Ernest Hamel²

¹*Department of Chemistry, Oregon State University, Corvallis, Oregon 97331*

²*Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, Maryland 21702*

(2E,4S,5R,6E)-4-*t*-Butyldimethylsilyloxy-5-methyl-7-phenylhepta-2,6-dienoic Acid (10). To a solution of **9** (1.9 mg, 4.6 mmol) in THF was added Et₃N (20 μL, 0.14 mmol) followed by slow addition of TMSOTf (23 μL, 0.14 mmol) and the mixture was stirred for 20 min. The mixture was extracted with Et₂O and the extract was washed with saturated aqueous NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Chromatography of the residue on silica (0.5-1% MeOH in CH₂Cl₂) gave 1.4 mg (87%) of **10** as a colorless oil: ¹H NMR (300 MHz, CDCl₃) δ 0.06 (s, 3H), 0.07 (s, 3H), 0.91 (s, 9H), 2.37-2.50 (m, 3H), 3.77 (td, J = 4, 6 Hz, 1H), 5.85 (d, J = 16 Hz, 1H), 6.16 (dd, J = 8, 16 Hz, 1H), 6.38 (d, J = 16 Hz, 1H), 7.07 (td, J = 8, 16 Hz, 1H), 7.19-7.38 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ -4.2, -4.0, 16.4, 18.5, 26.2, 38.0, 43.3, 75.3, 122.8, 126.4, 127.5, 128.9, 130.9, 132.2, 137.9, 149.5, 170.9

(2E,4S,5R,6E)-4-Triethylsilyloxy-5-methyl-7-phenylhepta-2,6-dienoic Acid (14). Ester **9** (13.5 mg, 23.5 μmol) was dissolved in *i*-PrOH-H₂O-AcOH (5:5:1, 4.4 mL) and the solution was refluxed for 36 h. The mixture was concentrated under reduced pressure and the resulting hydroxy acid was dried by evaporation with pyridine. The

residue was taken up in pyridine (1 mL), DMAP (10 mg, 82 μmol) and triethylsilyl chloride (12.6 μL , 75 μmol) were added at 0 $^{\circ}\text{C}$, and the mixture was stirred overnight at ambient temperature. MeOH (0.5 mL) was added and the mixture was evaporated under reduced pressure. The residue was taken up in CH_2Cl_2 -aqueous citric acid (pH 1) and the aqueous phase was extracted with CH_2Cl_2 (3X). The combined organic extracts were washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Chromatography of the residue on silica (0.5-1% MeOH in CH_2Cl_2) gave 8.2 mg (70%) of **14** as a colorless oil: $[\alpha]_{\text{D}}^{23} +91.0$ (c 1.19, CHCl_3); IR (neat) 2957, 2876, 1697, 1651, 1418, 1282, 1099 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 0.62 (q, J = 8 Hz, 6H), 0.97 (t, J = 8 Hz, 9H), 1.12 (d, J = 7 Hz, 3H), 2.35-2.51 (m, 3H), 3.81 (td, J = 4, 6 Hz, 1H), 5.85 (d, J = 16 Hz, 1H) 6.17 (dd, J = 8, 16 Hz, 1H), 6.38 (d, J = 16 Hz, 1H), 7.08 (dt, J = 8, 16 Hz, 1H), 7.20 (tt, J = 2, 7 Hz, 1H), 7.25-7.38 (m, 4H); ^{13}C NMR (75 MHz, CDCl_3) δ 5.5, 7.3, 16.4, 38.1, 43.4, 75.4, 122.8, 126.5, 127.5, 128.8, 130.9, 132.2, 137.9, 149.5, 171.0; HRMS (FAB) m/z 361.2194 (calcd for $\text{C}_{21}\text{H}_{33}\text{O}_3\text{Si}$: 361.2199)

Diester (18). To a solution of **14** (14.7 mg, 40.8 μmol) in THF (1.5 mL) under argon was added Et_3N (6.4 μL , 49.0 μmol) and 2,4,6-trichlorobenzoyl chloride (6.4 μL , 40.8 μmol), and the mixture was stirred for 2 h at ambient temperature. THF was removed under reduced pressure and the residue was dissolved in toluene (1.5 mL). A solution of DMAP (12.5 mg, 0.102 mmol) in toluene (0.5 mL) and a solution of **17** (24.5 mg, 41.6 μmol) in toluene (1.0 mL) were added and the mixture was stirred for 4.5 h at ambient temperature. Saturated aqueous NaHCO_3 was added and the mixture was stirred for 20 min. The aqueous layer was extracted with hexane and the combined organic phase was

dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Chromatography of the residue on silica (0-1.7% EtOAc in hexane) gave 29.0 mg (75%) of **18** as a colorless oil: $[\alpha]_D^{23} +6.47$ (c 0.68, CHCl₃); IR (neat) 2956, 2929, 2857, 1728, 1699, 1654, 1472, 1463, 1388, 1252, 1169, 1095 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.03 (s, 3H), 0.04 (s, 9H), 0.06 (s, 3H), 0.08 (s, 3H), 0.09 (s, 3H), 0.60 (q, J = 8 Hz, 6H), 0.86 (s, 9H), 0.91 (s, 9H), 0.96 (t, J = 8 Hz, 9H), 1.01 (d, J = 7 Hz, 3H), 1.06 (d, J = 7 Hz, 3H), 1.08 (s, 3H), 1.10 (d, J = 7 Hz, 3H), 1.70-1.79 (m, 1H), 2.25 (dd, J = 7, 16 Hz, 1H), 2.30-2.48 (m, 4H), 3.24 (quintet, J = 7 Hz, 1H), 3.78 (td, J = 4, 6 Hz, 1H), 3.88 (dd, J = 2, 7 Hz, 1H), 3.92 (dd, J = 8, 11 Hz, 1H), 4.09-4.21 (m, 2H), 4.31 (dd, J = 5, 11 Hz, 1H), 4.41 (dd, J = 3, 7 Hz, 1H), 5.83 (d, J = 16 Hz, 1H), 6.17 (dd, J = 8, 16 Hz, 1H), 6.37 (d, J = 16 Hz, 1H), 6.94 (dt, J = 8, 16 Hz, 1H), 7.21 (tt, J = 2, 7 Hz, 1H), 7.27-7.37 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ -4.3, -4.1, -3.3, -3.3, -1.2, 1.4, 5.5, 7.3, 14.5, 15.6, 16.5, 16.9, 17.6, 18.5, 18.8, 19.7, 23.0, 23.8, 26.4, 26.6, 30.1, 32.0, 37.8, 38.4, 40.9, 43.3, 46.5, 53.9, 63.1, 66.1, 74.2, 75.5, 76.6, 123.5, 126.4, 127.4, 128.9, 130.8, 132.3, 137.9, 146.6, 166.7, 172.5, 218.0; HRMS (FAB) m/z 947.6146 (calcd for C₅₁H₉₅O₈Si₄: 947.6104)

Hydroxy acid (19). To a solution of **18** (7.8 mg, 8.2 μ mol) in THF (2 mL) at 0 °C under argon was added TBAF (6 mg, 23 μ mol), and the mixture was stirred for 2 h at 0 °C. An aqueous solution of citric acid (pH 2) was added and the mixture was extracted with Et₂O, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Chromatography of the residue on silica (1-1.5% MeOH in CH₂Cl₂) gave 4.9 mg (82%) of **19** as a colorless oil: $[\alpha]_D^{23} +4.6$ (c 0.635, CHCl₃); IR (neat) 2956, 2929, 2856, 1716, 1653, 1472, 1257, 1092 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.05 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.10 (s, 3H), 0.87 (s, 9H), 0.91 (s, 9H), 1.03 (d, J = 7 Hz, 3H), 1.08 (d, J = 7 Hz, 3H), 1.14 (s, 3H), 1.15 (d, J = 7 Hz, 3H), 1.21 (s, 3H), 1.69-1.77 (m, 1H), 2.32 (dd, J

= 7, 16 Hz, 1H), 2.35-2.54 (m, 4H), 3.25 (quintet, $J = 7$ Hz, 1H), 3.67 (ddd, $J = 4, 6, 8$ Hz, 1H), 3.90 (dd, $J = 2, 7$ Hz, 1H), 3.94 (dd, $J = 8, 11$ Hz, 1H), 4.32 (dd, $J = 5, 11$ Hz, 1H), 4.40 (dd, $J = 3, 7$ Hz, 1H), 5.92 (dt, $J = 1, 16$ Hz, 1H), 6.12 (dd, $J = 9, 16$ Hz, 1H), 6.48 (d, $J = 16$ Hz, 1H), 7.01 (ddd, $J = 7, 8, 16$ Hz, 1H), 7.20-7.49 (m, 5H); ^{13}C NMR (100 MHz, CDCl_3) δ -4.2, -3.9, -3.4, -3.3, 15.8, 16.8, 17.1, 18.6, 18.9, 19.0, 24.0, 26.4, 26.6, 30.1, 37.6, 38.5, 40.3, 43.8, 46.3, 54.2, 66.1, 73.5, 74.3, 76.3, 124.1, 126.6, 127.9, 129.9, 131.1, 132.6, 137.3, 145.7, 166.7, 175.9, 218.3 HRMS m/z 733.4526 (calcd for $\text{C}_{40}\text{H}_{69}\text{O}_8\text{Si}_2$: 733.4531)

Macrolactone (20). To a solution of DMAP (1.7 mg, 13.9 μmol) and DMAP.HCl (1.4 mg, 8.8 μmol) in CHCl_3 (0.5 mL) was added diisopropyl carbodiimide (1.4 μL , 9.0 μmol), and the resulting mixture was brought to reflux. A solution of **19** (3.3 mg, 4.5 μmol) in CHCl_3 (0.5 mL) was added during 10 h using a syringe pump. After cooling the mixture to room temperature, MeOH (1 mL) and AcOH (0.1 mL) were added. The mixture was stirred for 30 min and concentrated under reduced pressure. Chromatography of the residue on silica (0.3-0.6% EtOAc in CHCl_3) gave 1.8 mg (56%) of **20** as a colorless oil: $[\alpha]_{\text{D}}^{23}$ -9.04 (c 0.365, CHCl_3); IR (neat) 2955, 2930, 2886, 2857, 1734, 1719, 1472, 1259, 1102 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 0.07 (s, 3H), 0.08 (s, 3H), 0.10 (s, 3H), 0.13 (s, 3H), 0.82 (s, 9H), 0.90 (s, 9H), 0.92 (s, 3H), 1.04 (d, $J = 7$ Hz, 3H), 1.05 (s, 3H), 1.10 (d, $J = 7$ Hz, 3H), 1.16 (d, $J = 7$ Hz, 3H), 1.82-1.95 (m, 1H), 2.48-2.72 (m, 5H), 3.48 (quintet, $J = 7$ Hz, 1H), 3.89 (d, $J = 7$ Hz, 1H), 3.99 (dd, $J = 4, 11$ Hz, 1H), 4.13 (t, $J = 11$ Hz), 4.64 (dd, $J = 2, 9$ Hz, 1H), 5.06 (ddd, $J = 3, 5, 11$ Hz, 1H), 5.88 (d, $J = 16$ Hz, 1H), 6.07 (dd, $J = 8, 16$ Hz, 1H), 6.44 (d, $J = 16$ Hz), 6.89 (ddd, $J = 7, 8, 16$ Hz, 1H), 7.20-7.38 (m, 5H); ^{13}C NMR (100 MHz, CDCl_3) δ -3.7, -3.5, -3.4, -2.3, 16.0, 16.6, 17.0, 18.6, 18.9, 21.2, 26.3, 26.6, 34.1, 37.6, 41.8, 42.8, 45.8, 55.6, 65.7, 73.2, 76.3,

Cryptothilone 1 (3). To a solution of **20** (7.0 mg, 9.8 μ mol) in THF (0.6 mL) was added TFA (0.5 mL), and the mixture was stirred for 16 h at ambient temperature. The solution was neutralized with saturated aqueous NaHCO₃ and the resulting mixture was extracted with CH₂Cl₂. The extract was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Chromatography of the residue on silica (0-0.5% MeOH in CH₂Cl₂) gave 3.4 mg (74%) of **3** as a colorless sticky solid: $[\alpha]_D^{23} +58.2$ (c 0.17, CHCl₃); IR (neat) 3502, 2969, 2927, 2361, 1732, 1717, 1700, 1457, 1261 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.94 (s, 3H), 0.99 (d, J = 7 Hz, 3H), 1.07 (d, J = 7 Hz, 3H), 1.09 (s, 3H), 1.14 (d, J = 7 Hz, 3H), 1.82-1.95 (m, 1H), 2.08 (d, J = 4 Hz, 1H), 2.24 (dd, J = 3, 15 Hz, 1H), 2.37 (dd, J = 10, 15 Hz, 1H), 2.50-2.72 (m, 3H), 3.18 (dq, J = 2, 7 Hz, 1H), 3.53 (d, J = 1 Hz, 1H), 3.70-3.75 (m, 1H), 4.04 (ddd, J = 3, 4, 10 Hz, 1H), 4.33 (dd, J = 3, 11 Hz, 1H), 4.47 (dd, J = 2, 11 Hz, 1H), 4.97 (dt, J = 5, 8 Hz, 1H), 5.86 (d, J = 16 Hz, 1H), 6.06 (dd, J = 9, 16 Hz, 1H), 6.36 (d, J = 16 Hz, 1H), 6.90 (ddd, J = 6, 9, 16 Hz, 1H), 7.21-7.38 (m, 5H); ¹³C NMR (75 MHz, CDCl₃) δ 10.7, 14.1, 17.1, 17.9, 20.7, 33.1, 37.2, 39.4, 41.0, 41.7, 54.1, 65.4, 70.4, 71.2, 76.4, 124.7, 126.3, 128.2, 129.3, 131.4, 131.7, 137.0, 144.5, 165.8, 170.3, 221.0; HRMS (FAB) *m/z* 487.2686 (calcd for C₂₈H₃₉O₇: 487.2696)