Supporting Information for

Synthesis of 14-Azacamptothecin, A Water Soluble Topoisomerase I Poison

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Experimental Section

All solvents were analytical grade chemicals. ^{1}H NMR spectra were recorded in chloroform-*d* or DMSO-d_6 on a Varian 300 MHz spectrometer. Chemical shifts are reported in δ; coupling constants in Hz. Melting points are uncorrected. Mass determination was accomplished by electrospray ionization on a Finnigan 3200 Quadrupole mass spectrometer. High resolutin mass spectra were obtained at the Michigan State University Mass Spectrometry Facility. Anhydrous CH_2Cl_2 was distilled from CaH, and dry THF was distilled from potassium metal and benzophenone prior to use. All synthetic transformations were carried out under dry nitrogen. Flash chromatography was performed with E. Merck silica gel 60 (40-63 mm) and A. C. S. grade solvents. DMF was purchased from Acros Organics. All other chemicals were purchased from Aldrich Chemicals and were used without further purification.

2-Chloro-4-iodo-6-methoxypyrimidine-5-carboxaldehyde (7). To a solution containing 3.81 g (59.5 mmol) of *n*-BuLi in 300 mL of dry THF at -70 °C was added dropwise 10.5 mL (8.82 g, 62.5 mmol) of 2,2,6,6-tetramethylpiperidine. The reaction mixture was stirred at -70 °C for 1 h. To this was added at -70 °C a solution containing 6.30 g (23.3 mmol) of 2-chloro-4-iodo-6-methoxypyrimidine (**6**) in 50 mL of THF. The reaction mixture was stirred at -70 °C for 2 h. To the reaction mixture was added at -70 °C a solution containing 22.03 g (297.0 mmol) of ethyl formate in 50 mL of THF. The reaction mixture was stirred at -70 °C for 1 h. To this was added at -70 °C 303 mL of 45:129:129 37% HCl-EtOH-THF. The reaction mixture was allowed to warm to 25 °C for 1 h. To the reaction mixture was added 200 mL of a saturated solution of aqueous $NaHCO_3$. The reaction mixture was concentrated under diminished pressure nearly to dryness and then extracted three times with 300-mL portions of CH_2Cl_2. The combined organic extract was dried (Na_2SO_4) and concentrated under diminished pressure. The residue was

purified by flash chromatography on silica gel column (40 × 5.5 cm). Step gradient elution with 10→70% CH_2Cl_2 in hexanes gave formylated pyrimidine **7** as an off-white solid: yield 6.12 g (88%); silica gel TLC R_f 0.38 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 4.13 (s, 3H) and 10.09 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 56.3, 117.1, 132.6, 161.2, 168.5 and 188.7; mass spectrum (ESI) *m/z* 298.8 $(M+H)^+$; mass spectrum (FAB), *m/z* 298.9083 $(M+H)^+$ ($C_6H_5ClIN_2O_2$ requires 298.9084).

5-(But-2-enyloxymethyl)-2-chloro-4-iodo-6-methoxypyrimidine (8). To a solution of 6.12 g (20.5 mmol) of aldehyde **7** in 52 mL of dry dichloromethane at 0 °C was added 6.06 mL (5.12 g, 71.0 mmol) of crotyl alcohol, 6.55 mL (4.77 g, 41.0 mmol) of triethylsilane and 10.48 mL (15.51 g, 58.4 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 16 h and poured into 300 mL of a saturated aqueous solution of $NaHCO_3$. The resulting mixture was extracted three times with 300-ml portions of hexanes. The combined organic layer was dried (Na_2SO_4), filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (30 × 5.5 cm). Step gradient elution with 40→60% CH_2Cl_2 in hexanes gave **8** as an off-white solid: yield 5.23 g (72%); silica gel TLC R_f 0.28 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.72 (dd, 3H, J = 6.3, 1.0 Hz), 3.99 (dt, 2H, J = 5.4, 1.0 Hz), 4.01 (s, 3H), 4.46 (s, 2H), 5.61 (m, 1H) and 5.77 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 17.4, 55.1, 66.8, 71.3, 121.6, 126.7, 129.5, 134.7, 157.2 and 166.8; mass spectrum (ESI), *m/z* 354.8 $(M+H)^+$; mass spectrum (FAB), *m/z* 354.9712 $(M+H)^+$ ($C_{10}H_{13}ClIN_2O_2$ requires 354.9710).

2-Chloro-8-ethyl-4-methoxy-5*H*-pyrano[4,3-*d*]pyrimidine (9) and 2-Chloro-8-ethylidene-4-methoxy-7,8-dihydro-5*H*-pyrano[4,3-*d*]pyrimidine. To a solution of 5.24 g (14.8 mmol) of crotyl ether **8** in 150 mL of dry *N*,*N*-dimethylformamide at room temperature was added successively 4.76 g (14.8 mmol) of tetra-*n*-butylammonium bromide, 4.08 g (29.5 mmol) of anhydrous potassium carbonate and 332 mg (1.5 mmol) of palladium acetate. The

reaction mixture was stirred at 85 °C for 12 h. The reaction mixture was allowed to cool to room temperature, filtered through a pad of Celite, and rinsed with 250 mL of *ter*-butyl methyl ether. The filtrate was poured into 250 mL of ice water, extracted three times with 200-mL portions of *ter*-butyl methyl ether, and washed successively with 200 mL of water and 200 mL of brine. The combined organic phase was dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (25 × 5.5 cm). Step gradient elution with 50→100% CH_2Cl_2 in hexanes gave product **9** as an off-white solid: yield 1.05 g (31%) and the *exo*-ethylidene side product as an off-white solid: yield 0.89 g (26%). Compound **9**: silica gel TLC R_f 0.70 (1:5 hexanes-dichloromethane); ^{1}H NMR ($CDCl_3$) δ 1.11 (t, 3H, J = 7.2 Hz), 2.38 (dq, 2H, J = 7.5, 1.2 Hz), 3.98 (s, 3H), 5.07 (s, 2H) and 6.70 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 19.5, 54.7, 62.5, 103.5, 117.3, 150.7, 159.2, 160.3 and 165.1; mass spectrum (ESI), *m/z* 226.8 $(M+H)^+$; mass spectrum (FAB) *m/z* 227.0586 $(M+H)^+$ ($C_{10}H_{12}ClN_2O_2$ requires 227.0587). *Exo*-ethylidene side product: silica gel TLC R_f 0.50 (1:4 hexanes-dichloromethane); ^{1}H NMR ($CDCl_3$) δ 1.81 (d, 3H, J = 7.2 Hz), 4.00 (s, 3H), 4.52 (s, 2H), 4.63 (s, 2H) and 7.07 (q, 1H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 13.6, 54.9, 62.8, 65.4, 111.0, 128.4, 128.6, 158.8 and 167.1; mass spectrum (ESI), *m/z* 226.8 $(M+H)^+$ ($C_{10}H_{12}ClN_2O_2$ requires 227.0).

2-Chloro-8-ethyl-8-hydroxy-4-methoxy-5,8-dihydropyrano[4,3-*d*]pyrimidin-7-one (10). A solution containing 41.0 mg (46.5 μmol) of $(DHQD)_2$-PYR ligand, 4.58 g (13.9 mmol) of $K_3Fe(CN)_6$, 1.93 g (13.9 mmol) of K_2CO_3, 3.4 mg (9.3 μmol) of $K_2OsO_4 \cdot 2H_20$ and 444 mg (4.66 mmol) of $MeSO_2NH_2$ in 40 mL of 3:5 *t*-BuOH-H_2O was stirred at room temperature for 5 min. To the reaction mixture was added at 0 °C a solution of 1.05 g (4.63 mmol) of **9** in 10 mL of *t*-BuOH. The reaction mixture was stirred at 0 °C for 12 h and then at room temperature for 24

h. To the cold reaction mixture was added 5.9 g (23.4 mmol) of I_2 and 2.3 g (23.4 mmol) of $CaCO_3$. The reaction mixture was stirred at room temperature for 48 h. To the reaction mixture was added 4.44 g (28.1 mmol) of Na_2SO_3 at 0°C over a period of 5 min. The reaction mixture was stirred at room temperature for 1 h. To the reaction mixture was added 100 mL of 9:1 EtOAc-MeOH. The reaction mixture was then filtered through a pad of Celite, and rinsed four times with 100-mL portions of 9:1 EtOAc-MeOH. The combined organic phase was washed twice with 100-mL portions of brine, dried (Na_2SO_4), and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (29 × 4 cm). Step gradient elution with 1→2% methanol in dichloromethane gave **10** as an off-white solid: yield 696 mg (58%); silica gel TLC R_f 0.60 (1:20 methanol-dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.5 Hz), 1.88 (q, 2H, J = 7.5 Hz), 3.87 (s, 1H), 4.07 (s, 3H) and 5.30 (AB, 2H); ^{13}C NMR ($CDCl_3$) δ 7.4, 31.5, 55.4, 62.9, 73.8, 108.0, 160.3, 164.4, 165.7 and 171.3; mass spectrum (ESI), *m/z* 258.8 $(M+H)^+$; mass spectrum (FAB) *m/z* 259.0485 $(M+H)^+$ ($C_{10}H_{12}ClN_2O_4$ requires 259.0486).

The enantiomeric purity of **10** was determined as 80% ee by chiral HPLC on a Chirobiotic T column (250 x 4.6 mm); the mobile phase was 10% EtOH in hexane at a flow rate of 1.0 mL/min, UV detection was at 300 nm. The retention time of the desired isomer was 43.0 min; that of the optical antipode was 46.6 min. Crystallization from hexane–CH_2Cl_2 (70% yield) gave **10** having an optical purity >98% ee as determined by chiral HPLC.

8-Ethyl-8-hydroxy-4-methoxy-5,8-dihydropyrano[4,3-*d*]pyrimidin-7-one (11). A mixture of 140 mg (0.54 mmol) of lactone **10**, 150 μL (109 mg, 1.08 mmol) of triethylamine and 50 mg of 10% palladium-on-carbon in 30 mL of absolute ethanol was stirred at 25 °C under 40 psi of hydrogen for 10 h. The catalyst was filtered through a pad of Celite and washed with

ethanol. The combined organic fraction was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column. Elution with 5% methanol in dichloromethane gave **11** as a light yellow oil: yield 103 mg (85%); silica gel TLC R_f 0.50 (1:20 methanol-dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H, J = 7.2 Hz), 1.88 (q, 2H, J = 7.2 Hz), 3.85 (br, 1H), 4.07 (s, 3H), 5.31 (AB, 2H) and 8.82 (s, 1H); mass spectrum (ESI), *m/z* 224.8 $(M+H)^+$; mass spectrum (FAB), *m/z* 225.0874 $(M+H)^+$ ($C_{10}H_{13}N_2O_4$ requires 225.0875).

8-Ethyl-8-hydroxy-5,8-dihydro-3*H*-pyrano[4,3-*d*]pyrimidine-4,7-dione (12). A solution of 71 mg (0.32 mmol) of **11** in 2 mL of 6N HCl and 2 mL of MeOH was stirred at 75 °C for 3 h. The solution was neutralized with sat $NaHCO_3$ and then concentrated under diminished pressure. The residue was applied to a preparative silica gel TLC plate. Development with 1:10 methanol–dichloromethane (R_f 0.40) afforded **12** as a colorless solid: yield 34 mg (51%); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, J = 7.2 Hz), 1.88 (m, 2H), 4.00 (br, 1H), 5.25 (AB, 2H), 5.28 (s, 1H), and 8.31 (s, 1H); mass spectrum (ESI), *m/z* 210.8 $(M+H)^+$; mass spectrum (FAB), *m/z* 211.0718 $(M+H)^+$ ($C_9H_{11}N_2O_4$ requires 211.0719).

3-(2-Bromoquinolin-3-ylmethyl)-8-ethyl-8-hydroxy-5,8-dihydro-3*H*-pyrano[4,3-*d*]pyrimidine-4,7-dione (14). To a solution containing 34 mg (0.16 mmol) of **12** in 6 mL of dry dimethoxyethane at 25 °C was added dropwise a solution containing 22 mg (0.19 mmol) of potassium *tert*-butoxide in 0.5 mL of dimethoxymethane. The reaction mixture was stirred at room temperature for 30 min. To this was added 63 mg (0.21 mmol) of neat **13** in one portion. The reaction mixture was stirred at reflux for 12 h, then allowed to cool and concentrated under diminished pressure. The residue was applied to a preparative silica gel TLC plate and developed with 1:25 methanol–dichloromethane (R_f 0.50) to give product **14** as a colorless solid: yield 31 mg (45%); ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H, J = 7.2 Hz), 1.89 (m, 2H), 3.86 (br, 1H),

5.29 (AB, 2H), 5.39 (AB, 2H), 7.60 (dt, 1H, J = 6.9, 1.2 Hz), 7.75 (dt, 1H, J = 6.9, 1.5 Hz), 7.83 (d, 1H, J = 8.1 Hz), 8.01 (d, 1H, J = 8.1 Hz), 8.22 (s, 1H) and 8.64 (s, 1H); mass spectrum (ESI), *m/z* 429.8 $(M+H)^+$; mass spectrum (FAB), *m/z* 430.0401 $(M+H)^+$ ($C_{19}H_{17}BrN_3O_4$ requires 430.0402).

14-Azacamptothecin (5). To a solution of 13 mg (0.030 mmol) of **14** in 4 mL of benzene was added catalytic amount of AIBN and 9.3 μL (7.5 mg, 0.030 mmol) of tris (trimethylsilyl)silane. The reaction mixture was stirred at 79 °C for 3 h. Then a catalytic amount of AIBN and 9.3 μL (7.5 mg, 0.030 mmol) of tris (trimethylsilyl)silane was added to the solution and the reaction mixture was heated at reflux for 4 h. After concentration, the residue was applied to a preparative silica gel TLC plate developed with ethyl acetate (R_f 0.40) to give 14-aza CPT (**5**) as a colorless solid: yield 3 mg (28%); ^{1}H NMR ($CDCl_3$) δ 1.08 (t, 3H, J = 7.5 Hz), 2.03 (m, 2H), 4.05 (br, 1H), 5.31 (s, 2H), 5.47 (AB, 2H, J = 84.0, 16.5 Hz), 7.76 (dt, 1H, J = 7.2, 1.2 Hz), 7.91 (dt, 1H, J = 7.2, 1.2 Hz), 8.01 (d, 1H, J = 8.1Hz), 8.46 (d, 1H, J = 8.1 Hz) and 8.53 (s, 1H); mass spectrum (FAB), *m/z* 350.1140 $(M+H)^+$ ($C_{19}H_{16}N_3O_4$ requires 350.1141).