Supporting Information for

Analogues of Bleomycin: Synthesis of Conformationally Rigid Methylvalerates

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(1*R*)-[1-(Methoxy-methylcarbamoyl)-but-3-enyl]-carbamic Acid *tert*-Butyl Ester (9).[14] To a stirred solution of 2.15 g (10.0 mmol) of *N*-(*tert*-butoxycarbonyl)-(*R*)-allylglycine in 30 mL of CH_2Cl_2 was added 1.01 g (10.0 mmol) of triethylamine. To this mixture was then added 5.20 g (10.0 mmol) of PyBOP, followed after 5 min by 1.12 g (11 mmol) of *N,O*-dimethylhydroxylamine hydrochloride. The pH of the reaction was monitored, and kept at a value ≥ 8.0. The progress of the reaction was monitored by thin layer chromatography, and usually was complete within 2 h. The mixture was then diluted with 250 mL of EtOAc and washed with three 30-mL portions of 1 N HCl, three 30-mL portions of saturated aqueous $NaHCO_3$ and three 30-mL portions of brine. The solution was dried over anhydrous $MgSO_4$, filtered, and the solution was concentrated under diminished pressure. The crude product was purified by flash chromatography (SiO_2, 40-70 μm, 30 × 3 cm, 1:2 EtOAc-hexanes) to afford a sticky white, low melting solid: yield 2.08 g (80.4%); silica gel TLC R_f 0.29 (1:2 EtOAc-hexanes); $[\alpha]^{25}_D$ -4.3 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.44 (m, 1H, J = 6.5 Hz), 2.50 (m, 1H, J = 6.9 Hz), 3.20 (s, 3H), 3.77 (s, 3H), 4.76 (m, 1H), 5.11 (m, 2H), 5.16 (d, 1H) and 5.73 (m, 1H); mass spectrum (chemical ionization), *m/z* 259 $(M + H)^+$, 203 and 159.

(1*R*)-(1-Formyl-but-3-enyl)-carbamic Acid *tert*-Butyl Ester (10).[15] To a stirred solution of 0.52 g (2.0 mmol) of compound **9** in 20 mL of anhydrous ether at -78 °C was added 95 mg (2.5 mmol) of lithium aluminum hydride. The reaction mixture was stirred and allowed to warm slowly to -20 °C, and the progress of the reaction was monitored by TLC. The reaction was usually complete within 2-3 h. Upon completion, the mixture was again cooled to –78 °C and treated slowly with a total of 10 mL of aqueous 0.35 M $KHSO_4$. The reaction mixture was then permitted to warm to room temperature. The reaction mixture was diluted with 50 mL of

ether, and the aqueous and organic phases were partitioned. The aqueous phase was extracted with three additional 50-mL portions of ether. The combined ether wash was rinsed with three 20-mL portions of 1 N HCl, three 20-mL portions of saturated aqueous $NaHCO_3$ and three 20-mL portions of brine. The solution was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to afford the desired product as a colorless oil; yield 350 mg (89%); $[\alpha]^{25}_D$ -49.6 (*c* 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.62 (m, 2H), 4.28 (m, 1H), 5.07 (m, 1H), 5.18 (m, 2H), 5.70 (d, 1H) and 9.59 (s, 1H); mass spectrum (chemical ionization), *m/z* 200 $(M + H)^+$,144 and 100.

(4*S*)-4-Isopropyl-3-pent-4-enoyl-oxazolidin-2-one (11).[11] To a stirred solution of 23.2 g (181 mmol) of (4*S*)-(–)-isopropyl-2-oxazolidinone in 450 mL of anhydrous THF (distilled from potassium spheres and benzophenone ketyl) at -78 °C under N_2 was added 72.4 mL of a 2.5 M solution of *n*BuLi in hexanes. The reaction mixture was allowed to warm to -20 °C and was then maintained at -20 °C for 20 min. The reaction mixture was again cooled to -78 °C, and a solution of 21.4 g (181 mmol) of 4-pentenoyl chloride in 20 mL of anhydrous THF was introduced carefully by dropwise addition over a period of 1 h. Once the addition was complete, the reaction mixture was stirred at -78 °C for 30 min and then allowed to gradually warm to room temperature over a period of 16 h. To the reaction mixture was then added 80 mL of a saturated NH_4Cl solution. The reaction mixture was extracted with three 400-mL portions of EtOAc. The combined organic extract was washed with two 100-mL portions of brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to yield a tan oil. The product was further purified by flash chromatography on a silica gel column (26 × 3 cm). Elution with 20% EtOAc-hexanes provided the desired product as an oil: yield 34.4 g (90%); silica gel TLC R_f 0.41 (1:2 EtOAc-hexanes); ^{1}H NMR ($CDCl_3$) δ 0.90 (dd, 6H, J = 12 Hz), 2.41

(m, 3H), 2.92–3.16 (m, 2H), 4.27 (m, 2H), 4.42 (m, 1H) and 5.05 (m, 2H) and 5.84 (m, 1H); mass spectrum (chemical ionization), *m/z* 212 $(M + H)^+$ and 130.

(1*R*, 2*S*, 3*S*, 4'*S*)-[1-Allyl-2-hydroxy-3-(4'-isopropyl-2'-oxo-oxazolidine-3'-carbonyl)-hex-5-enyl]-carbamic Acid *tert*-Butyl Ester (12). A solution of 1.00 g (4.75 mmol) of compound **11** in 5 mL of dry CH_2Cl_2 under N_2 at 0 °C was treated with 5.22 mL (5.22 mmol) of a 1 M solution of dibutylboron triflate in CH_2Cl_2, followed by 0.987 mL (5.70 mmol) of Hunig's base. After stirring for 45 min at 0 °C, the reaction mixture was cooled to -78 °C and permitted to equilibrate at this temperature for 15 min. A solution of 1.04 g (5.22 mmol) of compound **10** in 2 mL of CH_2Cl_2 was then added, and the reaction mixture was allowed to warm slowly from -78 °C to 25 °C overnight with stirring. The reaction was quenched by the addition of 10 mL of 50 mM phosphate buffer, pH 7.0, and was then extracted with three 10-mL portions of Et_2O. The organic washings were combined, rinsed with two 10-mL portions of brine and concentrated under diminished pressure. The resulting crude oil was dissolved in 20 mL of methanol at 0 °C. To this solution was added 7.5 mL of 30% aqueous H_2O_2. The reaction mixture was stirred at 0 °C for 4 h at which time 10 mL of H_2O was added, and the milky mixture was concentrated under diminished pressure to remove most of the methanol. The reaction mixture was diluted with 50 mL of EtOAc, partitioned, and the aqueous phase was extracted with four additional 25-mL portions of EtOAc. The combined organic extract was washed with two 25-mL portions of $NaHCO_3$, one 10-mL portion of brine, and then dried over anhydrous $MgSO_4$. Filtration and concentration of the filtrate under diminished pressure gave the crude product as an oil. Flash chromatography (SiO_2, 20 × 3 cm column, 1:2 EtOAc-hexanes) followed by recrystallization from EtOAc-hexanes afforded colorless crystals; yield 1.27 g (65 %); silica gel TLC R_f 0.25 (2:1 hexanes-ethyl acetate); mp 84-85 °C; $[\alpha]^{25}_D$ +63.9 ± 0.3; (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ

0.86 (dd, 6H, J = 12 Hz), 1.44 (s, 9H), 2.30 (m, 1H), 2.60 (m, 4H), 2.91 (bs, 1H), 3.75 (d, 2H), 4.21 (m, 3H), 4.47 (m, 1H), 4.59 (d, 1H), 5.08 (m, 4H) and 5.82 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.57, 18.04, 28.18, 28.32, 31.42, 35.56, 43.82, 51.71, 58.62, 63.01, 73.55, 79.60, 117.20, 118.25, 134.14, 135.34, 153.60, 155.63 and 175.92; mass spectrum (chemical ionization), *m/z* 411 $(M + H)^+$, 369, 311 and 130; mass spectrum (FAB), *m/z* 411.2511 $(M+H)^+$ ($C_{21}H_{34}N_2O_6$ requires 411.2495). Anal. Calcd for $C_{20}H_{36}O_6N_2$: C, 61.44; H, 8.35. Found: C, 61.51; H, 8.36.

(1*R*, 6*S*, 7*S*, 4'*S*)-[7-Hydroxy-6-(4'-isopropyl-2'-oxo-oxazolidine-3'-carbonyl)-cycloheptyl-3-enyl]-carbamic Acid *tert*-Butyl Ester (13). To a solution of 340 mg (0.83 mmol) of **12** in 100 mL of anhydrous CH_2Cl_2 under nitrogen was added 34 mg (0.042 mmol) of bis(tricyclohexylphosphine)benzylidine ruthenium(IV) dichloride. The reaction mixture was maintained at reflux for 48 h. The cooled reaction mixture was concentrated under diminished pressure and the residue was purified by flash chromatography (SiO_2, 40-75μm, 30 × 3 cm column, 1:2 EtOAc-hexanes) to afford **13** as an off-white foam: yield 263 mg (83 %); silica gel TLC R_f 0.20 (2:1 hexanes-EtOAc); $[\alpha]^{22}_D$ +85.2 ± 1.4 (c 0.44, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.89 (dd, 6H, J = 12 Hz), 1.42 (s, 9H), 1.97 (m, 2H), 2.30 (m, 1H), 2.80 (m, 2H), 3.55 (m, 1H), 3.67 (m, 2H), 4.26 (m, 3H), 4.41 (m, 1H), 5.28 (d, 1H, J = 9 Hz), 5.77 (m, 1H) and 5.93 (m, 1H); ^{13}C NMR ($CDCl_3$)14.90, 17.92, 23.71, 27.81, 28.42, 28.51, 46.24, 51.70, 58.13, 63.52, 72.35, 79.24, 129.31, 131.38, 153.09, 155.15, and 176. 43; mass spectrum (electrospray), *m/z* 405.3 $(M + Na)^+$, 383.1 $(M + H)^+$, 349.3 and 283.3; mass spectrum (FAB), *m/z* 383.2187 $(M+H)^+$ ($C_{19}H_{30}N_2O_6$ requires 383.2182).

(1*R*, 2*S*, 3*S*, 4'*S*)-[2-Hydroxy-3-(4'-isopropyl-2'-oxo-oxazolidine-3'-carbonyl)-cycloheptyl]-carbamic Acid *tert*-Butyl Ester (14). To a solution of 240 mg (0.62 mmol) of **13** in 25 mL of absolute ethanol was added 100 mg of 5% platinum-on-carbon. The reaction

mixture was evacuated and then purged with hydrogen three times, and then finally maintained under a H_2 atmosphere for 48 h. The mixture was filtered through a pad of Celite, and the Celite pad was washed with 100 mL of absolute ethanol. The filtrate was concentrated under diminished pressure and purified by flash chromatography (SiO_2, 40-75μm, 30 × 3 cm column, 1:2 EtOAc-hexanes) to afford **14** as a colorless foam: yield 212 mg (89.1%); silica gel TLC R_f 0.25 (1:2 EtOAc-hexanes); $[\alpha]^{23}_D$ +82.6 ± 1.1 (*c* 0.60, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.85 (dd, 6H, *J* = 10 Hz), 1.39 (s, 9H), 1.42-1.95 (m, 8H), 2.12 (m, 1H), 2.25 (m, 1H), 3.36 (s, 1H), 3.50 (m, 1H), 3.70 (m, 1H), 4.17 (m, 1H), 4.26 (m, 1H), 4.37 (m, 1H) and 5.21 (d, 1H); ^{13}C NMR ($CDCl_3$) 14.88, 17.93, 23.98, 23.08 24.01, 25.14, 28.43, 28.48, 28.82, 44.53 55.60, 58.10, 63.46, 71.89, 79.12, 153.23, 155.23 and 178.04; mass spectrum (electrospray), *m/z* 407.2 $(M + Na)^+$, 385.1 $(M + H)^+$, 329.3 and 285.2; mass spectrum (FAB), *m/z* 385.2346 $(M+H)^+$ ($C_{19}H_{32}N_2O_6$ requires 385.2339).

(1*S*, 2*S*, 3*R*)-3-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2-hydroxy-cycloheptanecarboxylic Acid (1). To a solution of 167 mg (0.43 mmol) of **14** in 4.5 mL of a 3:1 THF-H_2O mixture at 0 °C was added 55.0 mg (1.30 mmol) of lithium hydroxide monohydrate and 0.56 mL of a 30% aqueous hydrogen peroxide. The reaction mixture was stirred, and allowed to warm to room temperature overnight. The reaction mixture was then quenched by the sequential addition of 2.5 mL of saturated aqueous $NaHCO_3$ followed by 2.5 mL of aqueous 0.05 M Na_2SO_3. The mixture was extracted with three 10-mL portions of dichloromethane. The aqueous phase was acidified to a pH of 2-3 by the careful addition of 1N aqueous HCl. The acidified aqueous layer was then extracted with four 15-mL portions of EtOAc. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless oil. The recovered oil was dissolved

in 6 mL of dimethylsulfide and stirred for 5 min under nitrogen before 10 mL of redistilled trifluoroacetic acid was added. The reaction mixture was stirred under nitrogen overnight. Excess trifluoroacetic acid and dimethylsulfide were removed under a stream of nitrogen and the residue was co-evaporated with five 10-mL portions of toluene to remove any remaining traces of trifluoroacetic acid. The crude reaction product was dissolved in 7.5 mL of 3:1 dioxane-water. To this solution was added 203 mg (1.23 mmol) of $K_2CO_3 \cdot 1.5\ H_2O$. The pH of the reaction mixture was checked to insure basicity (pH ≈ 8.0). The reaction mixture was then stirred for 30 min, at which time 152 mg (0.45 mmol) of FmocOSu was added. The pH of the reaction mixture was checked once again, and the reaction mixture was then stirred overnight. The mixture was diluted with 5.0 mL of 2.0 M K_2CO_3 and then extracted with four 15-mL portions of ethyl acetate. The aqueous phase was carefully acidified to a pH of 1-2 by the addition of 1 N aqueous HCl, and was then extracted with four additional 25-mL portions of ethyl acetate. The combined ethyl acetate extract was washed with one portion of 1 N aqueous HCl, dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to provide the crude product as an oil. The recovered material was further purified by flash chromatography on a silica gel column (30 × 3 cm). Elution with 80% EtOAc - 20% hexanes - 0.1% AcOH afforded the purified product as a colorless solid: yield 66 mg (38.3 %); silica gel TLC R_f 0.26 (80% EtOAc-20% hexanes-0.1% AcOH); $[\alpha]^{25}_D$ +50.4 ± 1.9 (*c* 0.78, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.20–1.90 (m, 8H), 2.36 (m, 1H), 3.40 (m, 2H), 4.21 (m, 4H), 7.10 (d, 1H, J = 7 Hz), 7.32 (m, 4H), 7.66 (d, 2H, J = 7Hz) and 7.83 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 22.89, 23.23, 24.26, 27.95, 46.73, 47.22, 55.93, 65.32, 71.78, 120.07, 125.24, 127.04, 127.58, 140.70, 143.94, 155.15 and 175.65; mass spectrum (electrospray), *m/z* 418.4 $(M + Na)^+$ and 396.2 $(M + H)^+$; mass spectrum (FAB), *m/z* 396.1811 $(M+H)^+$ ($C_{23}H_{25}NO_5$ requires 396.1811).

(1*S*, 6*R*, 7*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-7-hydroxy-cyclohept-3-enecarboxylic Acid (3). To a solution of 167 mg (0.43 mmol) of **13** in 4.5 mL of a 3:1 THF–H_2O mixture at 0 °C was added 55.0 mg (1.30 mmol) of lithium hydroxide monohydrate and 0.56 mL of a 30% aqueous hydrogen peroxide solution. The reaction was stirred and allowed to warm to room temperature overnight. The reaction mixture was then quenched by the sequential addition of 2.5 mL of saturated aqueous $NaHCO_3$ followed by 2.5 mL of aqueous 0.05 M Na_2SO_3. The mixture was extracted with three 10-mL portions of dichloromethane. The aqueous phase was acidified to a pH of 2-3 by the careful addition of 1 N aqueous HCl. The acidified aqueous layer was then extracted with four 15-mL portions of EtOAc. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless oil. The recovered oil was dissolved in 6 mL of dimethylsulfide and stirred for 5 min under nitrogen before 10 mL of freshly distilled trifluoroacetic acid was added. The reaction mixture was stirred under nitrogen overnight. Excess trifluoroacetic acid and dimethylsulfide were removed under a stream of nitrogen and the residue was co-evaporated with five 10-mL portions of toluene to remove any remaining traces of trifluoroacetic acid. The crude reaction product was dissolved in 7.5 mL of 3:1 dioxane-water. To this solution was added 203 mg (1.23 mmol) of $K_2CO_3 \cdot 1.5\ H_2O$. The pH of the reaction mixture was checked to insure basicity (pH $\approx$ 8.0). The reaction mixture was then stirred for 30 min, at which time 152 mg (0.45 mmol) of FmocOSu was added. The pH of the reaction mixture was checked once again, and the reaction mixture was then stirred overnight. The mixture was diluted with 5.0 mL of 2.0 M K_2CO_3 and then extracted with four 15-mL portions of ethyl acetate. The aqueous phase was carefully acidified to a pH of 1-2 by the addition of 1 N aqueous HCl, and was then extracted with four additional 25-mL portions of ethyl acetate. The combined ethyl acetate

extract was washed with one portion of 1 N aqueous HCl, dried over anhydrous $MgSO_4$, filtered, and concentrated under diminished pressure to provide the the crude product as an oil. The recovered material was purified further by flash chromatography on a silica gel column (30 × 3 cm). Elution with 80% EtOAc-20% hexanes–0.1% AcOH afforded **3** as a colorless solid: yield 60 mg (35.6 % for 3 steps); silica gel TLC R_f 0.25 (80% EtOAc-20% hexanes-0.1% AcOH); $[\alpha]^{25}_D$ +72.1 ± 1.2 (c 0.46, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.76 (m, 1H), 2.03 (m, 1H), 2.29 (m, 1H), 2.58 (m, 1H), 3.19(m, 2H), 4.22 (m, 4H), δ 5.64 (m, 2H), 7.07 (d, 1H, J = 8 Hz), 7.34 (m, 4H), 7.66 (d, 2H, J = 7 Hz) and 7.84 (d, 2H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) 21.85, 27.09, 46.71, 47.56, 53.63, 65.34, 71.79, 120.07, 125.23, 127.04, 127.58, 129.13, 131.36, 140.70, 143.91, 155.15 and 174.91; mass spectrum (electrospray), *m/z* 418.4 $(M + Na)^+$ and 396.2 $(M + H)^+$; mass spectrum (FAB), *m/z* 394.1659 $(M+H)^+$ ($C_{23}H_{23}NO_5$ requires 394.1654).

References

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