Mannopeptimycins, Novel Antibacterial Glycopeptides from *Streptomyces hygroscopicus*, *LL*-AC98

Haiyin He,[†,*] R. Thomas Williamson,[†] Bo Shen,[†] Edmund I. Graziani,[†] Hui Y. Yang,[†] Subas M. Sakya,[‡] Pete J. Petersen,[‡] and Guy T. Carter[†]

Contribution from [†]the Department of Natural Products Chemistry, Chemical Sciences, and [‡]the Department of Infectious Disease Research, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965, U.S.A.

Supporting Information

Experimental

Purification. The AC98 complex (180 mg), was separated by reverse phase HPLC on a C18 column (YMC ODS-A, 20 x 250 mm, 5 μm particle size), using a linear gradient of 11–25% acetonitrile in water containing 0.02% trifluoroacetic acid (TFA) over 45 min. The flow rate of the mobile phase was maintained constant at 9 ml/min and the effluent was monitored by UV absorption at 226 nm. The individual components (TFA salts upon evaporation) were collected as follows: mannopeptimycins α (**1**) at 20 min (35 mg), β (**2**) at 28 min (29 mg), γ (**3**) at 32 min (25 mg), and δ (**4**) at 37 min (64 mg). The fraction eluted at 43 min was further separated by reverse phase HPLC using a different solvent (40–60% methanol in water with 0.02% TFA over 40 min) to afford TFA salts of mannopeptimycin ε (**5**) (5 mg). The pure components were all isolated as white amorphous powders. This procedure was repeated several times to generate enough quantities of pure **1–5** for structural elucidation and biological evaluation.

Mannopeptimycins α (1). $[\alpha]_D$ = -3.4 ° (c = 0.38, 1:1 H_2O/MeOH); ESIMS (positive) *m/z* 1295.5 $[(MH)^+]$, 648.3 $[(M+2H)^{2+}]$; HRFTICRMS (positive) *m/z* 648.26747 $[(M+2H)^{2+}$, $0.5*(C_{54}H_{78}N_{12}O_{25}+2H)$ requires 648.26733]; IR ν_{max} (KBr) cm^{-1} 3300, 2966, 1679, 1642, 1552, 1204, 1134. ^1H and ^{13}C NMR data see Table 1.

Mannopeptimycins β (2). $[\alpha]_D$ = -29.6 ° (c = 0.60, 1:1 H_2O/MeOH); HRFTICRMS (positive) *m/z* 971.42331 $[(MH)^+$, $(C_{42}H_{58}N_{12}O_{15}+H)$ requires 971.42174], 486.21481 $[(M+2H)^{2+}$, $0.5*(C_{42}H_{58}N_{12}O_{15}+2H)$ requires 486.21451]; IR ν_{max} (KBr) cm^{-1} 3277, 2969, 1679, 1633, 1553, 1204, 1136; ^1H NMR (300 MHz, 1:1, CD_3OD/D_2O, mult, *J* in Hz) δ Aiha-A 4.23 (m, H-2), 3.65 (m, H-3), 2.51 (br dd, 10, 7.4, H-4'), 3.51 (dd, 10, 7.4, H-5'), 3.15 (m, H-5'), Aiha-B 4.59 (d, 4.5, H-2), 4.24 (m, H-3), 4.23 (m, H-4'), 3.79, 3.37 (m, H_2-5'), Ser 4.49 (t, 5, H-2), 3.86 (2H, m, H2-3), Gly 4.05 (d, 11.2, H-2), 3.84 (d, 11.2, H-2), Mephe 4.38 (d, 10.6, H-2), 3.12 (m, H-3), 1.34 (3H, d, 7.0, 3-Me), 7.30 (5H, m, H-2', H-3', H-4', H-5', H-6'), 4.20 (m, H-2), 1.97 (dd, 12.4, 3.5, H-3), 2.42 (dd, 12.4, 11.4, H-3), 6.94 (2H, d, 8.2, H-2', H-6'), 6.73 (2H, d, 8.2, H-3', H-5'), N-Man 5.06 (d, 8.3, H-1), 4.20 (m, H-2),

4.02 (4.5, 3, H-3), 3.85 (m, H-4), 3.99 (ddd, 9, 4.2, 3, H-5), 4.15 (dd, 12.1, 9, H-6), 3.68 (m, H-6); ^{13}C NMR data see Table 3.

Mannopeptimycins γ (3). $[\alpha]_D$ = -7.1 ° (c = 0.35, 1:1 H_2O/MeOH); ESIMS (positive) *m/z* 1379.5 $[(MH)^+]$, 690.3 $[(M+2H)^{2+}]$; HRFTICRMS (positive) *m/z* 690.29631 $[(M+2H)^{2+}$, 0.5*($C_{59}H_{86}N_{12}O_{26}$+2H) requires 690.29609]; IR ν_{max} (KBr) cm^{-1} 3374, 3281, 2966, 1680, 1628, 1556, 1204, 1136; ^1H NMR (resolved signals only, 300 MHz, 1:1, CD_3OD/D_2O, mult, *J* in Hz) δ Man-B 5.33 (d, 1.2, H-1), 5.18 (dd, 3.3, 1.2, H-2), 3.92 (dd, 7.9, 3.3, H-3), other moieties 7.34 (2H, dd, 7.5, 7.5, H-3', H-5', Mephe), 7.27 (2H, d, 7.5, H-2', H-6', Mephe), 7.23 (dd, 7.5, 7.5, H-4', Mephe), 7.04 (4H, br s, H-2', H-3', H-5', H-6', Tyr), 5.43 (d, 1.2, H-1, Man-A), 5.05 (d, 8.3, H-1, N-Man), 4.56 (d, 5.1, H-2, Aiha-B), 4.49 (t, 5.3, H-2, Ser), 4.40 (d, 9.6, H-2, Mephe), 3.53 (dd, 11, 7.1, H-5', Aiha-A), 2.61 (br dd, 9, 7.1, H-4', Aiha-A), 2.52 (dd, 13.2, 10, H-3, Tyr), 2.32 (2H, d, 7.5, H_2-2, isovaleryl), 2.10 (m, H-3, isovaleryl), 1.33 (3H, d, 7.5, 3-Me, Mephe), 0.96 (6H, d, 6.5, CH_3-4, -5, isovaleryl); ^{13}C NMR data see Table 3.

Mannopeptimycins δ (4). $[\alpha]_D$ = -12.2 ° (c = 0.37, 1:1 H_2O/MeOH); ESIMS (positive) *m/z* 1379.5 $[(MH)^+]$, 690.3 $[(M+2H)^{2+}]$; HRFTICRMS (positive) *m/z* 690.29532 $[(M+2H)^{2+}$, 0.5*($C_{59}H_{86}N_{12}O_{26}$+2H) requires 690.29609]; IR ν_{max} (KBr) cm^{-1} 3374, 3277, 2962, 1681, 1634, 1554, 1204, 1135; ^1H NMR (resolved signals only, 300 MHz, 1:1, CD_3OD/D_2O, mult, *J* in Hz) δ Man-B 5.32 (br s, H-1), 4.12 (br s, H-2), 4.99 (dd, 9.8, 2.7, H-3), 3.88 (dd, 9.8, 9.8, H-4), other moieties 7.33 (2H, dd, 7.5, 7.5, H-3', H-5', Mephe), 7.27 (2H, d, 7.5, H-2', H-6', Mephe), 7.23 (dd, 7.5, 7.5, H-4', Mephe), 7.05 (4H, br s, H-2', H-3', H-5', H-6', Tyr), 5.44 (br s, H-1, Man-A), 5.05 (d, 8.4, H-1, N-Man), 4.56 (d, 4.5, H-2, Aiha-B), 4.50 (t, 4.9, H-2, Ser), 4.42 (d, 9.6, H-2, Mephe), 3.56 (dd, 10, 7, H-5', Aiha-A), 2.70 (br dd, 9.2, 7, H-4', Aiha-A), 2.55 (dd, 13.5, 10, H-3, Tyr), 2.33 (2H, d, 7.3, H_2-2, isovaleryl), 2.09 (m, H-3, isovaleryl), 1.32 (3H, d, 7.4, 3-Me, Mephe), 0.96 (6H, d, 6.6, CH_3-4, -5, isovaleryl); ^{13}C NMR data see Table 3.

Mannopeptimycins ε (5). $[\alpha]_D$ = -13.8 ° (c = 0.13, 1:1 H_2O/MeOH); ESIMS (positive) *m/z* 1379.5 $[(MH)^+]$, 690.2 $[(M+2H)^{2+}]$; HRFTICRMS (positive) *m/z* 690.29617 $[(M+2H)^{2+}$, 0.5*($C_{59}H_{86}N_{12}O_{26}$+2H) requires 690.29609]; IR ν_{max} (KBr) cm^{-1} 3373, 3280, 2962, 1679, 1636, 1553, 1204, 1136; ^1H NMR (resolved signals only, 300 MHz, 1:1, CD_3OD/D_2O, mult, *J* in Hz) δ Man-B 5.33 (d, 1.3, H-1), 4.15 (m, H-2), 3.87 (dd, 9.7, 2.4, H-3), 5.06 (dd, 9.7, 9.7, H-4), other moieties 7.32 (2H, dd, 7.5, 7.5, H-3', H-5', Mephe), 7.25 (2H, d, 7.5, H-2', H-6', Mephe), 7.22 (dd, 7.5, 7.5, H-4', Mephe), 7.04 (4H, br s, H-2', H-3', H-5', H-6', Tyr), 5.44 (d, 1.2, H-1, Man-A), 5.04 (d, 8.8, H-1, N-Man), 4.55 (d, 4.8, H-2, Aiha-B), 4.50 (t, 5.0, H-2, Ser), 4.43 (d, 9.5, H-2, Mphe), 3.56 (m, H-5', Aiha-A), 3.22 (dd, 10, 10, H-5', Aiha-A), 3.18 (m, H-3, Mephe), 2.83 (br dd, 9, 7, H-4', Aiha-A), 2.60 (dd, 12.8, 10, H-3, Tyr), 2.30 (2H, d, 7.3, H_2-2, isovaleryl), 2.20 (dd, 12.8, 4.5, H-3, Tyr), 2.06 (heptet, 7, H-3, isovaleryl), 1.33 (3H, d, 7.5, 3-Me, Mephe), 0.96 (6H, d, 6.5, CH_3-4, -5, isovaleryl); ^{13}C NMR data see Table 3.

Conversion of mannopeptimycins α (1) to β (2). A solution of **1** (2.0 mg) in 5 % aqueous HCl (1.4 ml) was stirred at 60 °C for 2 hrs. The aliquot was analyzed by LC/MS (YMC C18, 2 x 100 mm, 3 μm particle size, linear gradient 10–50% acetonitrile/water containing 0.025% formic acid over 15 min, 0.3 ml/min flow rate). The retention time (7.67 min) and the molecular ions [*m/z* (positive) 971.9, *m/z* (negative) 969.9] of the most abundant peak were identical to **2**.

Conversion of mannopeptimycins γ–ε (3–5) to α (1). A solution of **3** (2.0 mg) in 5% sodium carbonate (2.0 ml) was stirred at ambient temperature for 16 hrs. The aliquot was acidified and analyzed by LC/MS (YMC C18, 2 x 100 mm, 3 μm, linear gradient 10–50% acetonitrile/water containing 0.025% formic acid over 15 min, 0.3 ml/min flow rate). The retention time (3.73 min) and the molecular ions [*m/z* (positive) 1295.9, *m/z* (negative) 1293.9] were identical to **1**.

Aglycone (8). A solution of **2** (202 mg) and potassium periodate (270 mg, 5.3 eq) in 0.2 M sodium acetate/acetic acid buffer (35.0 ml, pH 5.5) was stirred at ambient temperature for 16 hrs. The reaction mixture was then loaded onto a chromatography column containing prewashed XAD-7 in water (bed volume ~200 ml). The column was washed with water (1.0 L) to remove the reagents and the product was eluted by 50 % acetonitrile/water (500 ml, containing 0.1% TFA). Evaporation of the eluent in vacuo afforded the dialdehyde **6** (121 mg) as a white powder. ESIMS *m/z* (positive) 939.5 (MH^+), 921.6 ($MH^+ - H_2O$), 865.5 [MH^+ – $CHO(CH_2OH)CH$], 849.5 (MH^+ – $CHO(CH_2OH)CHO$], 809.5 {MH^+ – $CHO[CHO(CH_2OH)CHO]CH$}; *m/z* (negative) 937.7 [$(M–H)^-$]. To a solution of **6** (91 mg) in MeOH (2.0 ml), sodium borohydride (18 mg, 5.0 eq) was added in 3 portions at ambient temperature. The reaction mixture was stirred for 2 hrs before being acidified by 5% TFA to pH 5. The resulting solution was then loaded onto the prewashed XAD-7 column. After the column was washed with water (1.0 L), the product **7** was eluted with a 50% acetonitrile/water containing 0.1% TFA (500 ml) giving a white powder upon evaporation (43 mg). ESIMS *m/z* (positive) 943.6 (MH^+), 809.5 {MH^+ – $CH_2OH[(CH_2OH)_2CHO]CH$}; *m/z* (negative) 941.6 [$(M–H)^-$]. A solution of **7** (34 mg) in 5% aqueous HCl (1.0 ml) was stirred at 80 °C for 4 hrs. The reactants were neutralized by 5% sodium carbonate, evaporated to dryness, and purified by reverse phase HPLC (YMC C18, 10 x 250 mm, 5 μm, linear gradient 10–50% acetonitrile/water containing 0.02% TFA) to afford the aglycone **8** as a white powder (17 mg). HR-FTICRMS *m/z* (positive) 809.3683 (MH^+, requires 809.3689); ^1H and ^{13}C NMR spectral data see Table 2.

Determination of the Absolute Stereochemistry.

(1). LC/MS analysis of Marfey's (FDAA) derivatives for mannopeptimycin α (**1**). Peptide Hydrolysis. Mannopeptimycin α (**1**) (1.0 mg) was dissolved in degassed 6 N HCl (0.5 ml) in a sealed vial and heated at 100 °C for 16 h. The solvent and the acid were removed in vacuo and the residue was dissolved in water (0.1 ml). The solution was then treated with 6.0% triethylamine (30 μl) and 1.0% 1-fluoro-2,4-dinitrophenyl-5-*L*-alanineamide (FDAA) in acetone (40 μl) at 40 °C for 1 hr. The reaction mixture was diluted with 1:1 acetonitrile/water (0.2 ml) and the resulting solution was analyzed by HPLC. YMC C18 column: 2.0 x 100 mm, 3 μm, linear gradient solvent: 20–100% $MeCN/H_2O$ with 0.025% formic acid over 15 min, flow rate: 0.3 ml/min. The mobile phase was monitored by both UV absorption and negative ESIMS. The retention times and the negative ions, $(M–H)^-$, of the FDAA derivatives of the amino acids are given in parentheses: Aiha (6.57 min, *m/z* 439), Aiha (6.59 min, *m/z* 439), *L*-Ser (8.10 min, *m/z* 356), Gly (8.75 min, *m/z* 326), *D*-Tyr (9.83 min, *m/z* 432; 12.74 min, *m/z* 684), Mephe (11.32 min, *m/z* 430).

(2). Preparation of (2*S*,3*S*)-β-methylphenylalanine (Mephe) and benzyl carbamates of Aiha methyl esters (**9** and **10**). A solution of **1** (202 mg) in 6 N HCl (20.0 ml) was stirred at 100 °C for 16 hrs. The solvent and the acid were evaporated in vacuo, and the residue was separated by reverse phase HPLC (YMC C18, 70 x 500 mm, 10 μm, linear gradient 3–80% acetonitrile/water containing 0.01% TFA in 45 min, 100 ml/min) to afford *D*-tyrosine (27.82 min, 24 mg) and (2*S*,3*S*)-β-methylphenylalanine (43.68 min, 27 mg). The Mephe prepared by above procedure was further

chromatographed by reverse phase HPLC (YMC C18, 30 x 250 mm, 5 μm, linear gradient 10–65% acetonitrile/water over 20 min) to afford neutral amino acid. $[\alpha]_D = -22^\circ$ (c 1.5, H_2O), ESIMS *m/z* 180.0 (MH^+), ^1H NMR (300 MHz, D_2O, mult, *J* in Hz) δ 7.44 (2H, dd, 7.5, 7.5), 7.37 (2H, d, 7.5), 7.36 (dd, 7.5, 7.5), 3.94 (d, 4.9), 3.53 (m), 1.39 (3H, d, 7.3).

The solvent front (10–15 min) was evaporated in vacuo to obtain a colorless solid (84 mg), which was dissolved in 5.0% aqueous Na_2CO_3 (2.0 ml). To this solution, benzyl chlorocarbamate (CBZCl, 100 mg) in ether (2.0 ml) was slowly added at 0 °C. The reaction mixture was stirred for 1 hr and partitioned between ether and water (5.0 ml each). The aqueous layer was acidified by 5.0% HCl to pH 6 and the carbamate products were extracted by *n*-butanol (2 x 5.0 ml). The combined organic extract (115 mg upon evaporation) was chromatographed by reverse phase HPLC (YMC C18, 70 x 500 mm, 10 μm, gradient 25–70% acetonitrile/water containing 0.01% TFA in 55 min, 100 ml/min) to afford a mixture of diastereoisomers of Aiha benzyl carbamates (22.7 min) as a white powder (46 mg). ESIMS *m/z* 323.3 (MH^+).

The powder was dissolved in dry DMSO (1.0 ml), and mixed with sodium carbonate (120 mg) and methyl iodide (5 drops, excess) at ambient temperature. The reactants were stirred for 16 hrs and filtered. The filtrate, after acidified by 5.0% TFA in MeOH, was repeatedly separated by reverse phase HPLC (YMC C18, 4.6 x 150 mm, 5 μm, gradient 10–50% acetonitrile/water containing 0.01% TFA in 22 min, 1 ml/min) to afford (**9**, 4.1 mg) and (**10**, 5.4 mg) at 7.22 and 7.40 min respectively. Benzyl carbamate of Aiha methyl ester (**9**). ESIMS *m/z* 337.3 (MH^+); ^1H NMR (300 MHz, CD_3OD, mult, *J* in Hz) δ 7.34 (5H, m, benzyl aromatic), 5.10 (2H, s, benzyl methylene), 4.32 (d, 5.9, H_α), 4.14 (ddd, 9.1, 6.8, 5.5, H-4'), 3.86 (dd, 5.9, 5.5, H_β), 3.75 (3H, s, OCH_3), 3.70 (2H, m, H_2-5'). Benzyl carbamate of Aiha methyl ester (**10**). ESIMS *m/z* 337.3 (MH^+); ^1H NMR (300 MHz, CD_3OD, mult, *J* in Hz) δ 7.34 (5H, m, benzyl aromatic), 5.13 (2H, s, benzyl methylene), 4.51 (d, 1.5, H_α), 4.07 (dd, 5.7, 1.5, H_β), 3.90 (ddd, 5.7, 5.7, 5.7, H-4'), 3.76 (3H, s, OCH_3), 3.68 (2H, d, 5.7, H_2-5').