SUPPLEMENTARY MATERIAL

Syntheses of Both the Putative and Revised Structures of Aeruginosin EI461 Bearing a New Bicyclic α -Amino Acid

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Methyl (2*S*,3*aR*,6*R*,7*aR*)-1-Benzyl-6-hydroxyoctahydroindole-2-carboxylate (9). A solution of **7** (335 mg, 1.17 mmol) in MeOH (23 mL) was cooled to -78 °C and treated with NaBH₄ (90.5 mg, 2.39 mmol). The mixture was stirred for 6 h, concentrated and partitioned between H₂O (10 mL) and CH₂Cl₂ (4 x 25 mL). The dried organic extracts were concentrated and purified by chromatography. The first eluate (hexanes/EtOAc 1:1) gave the epimeric alcohol at C-6 (37 mg, 11%). Elution with hexane/EtOAc (2:3) gave alcohol **9** (241 mg, 72%): *R*_f = 0.25 (SiO₂, hexane/EtOAc, 1:1); [α]_D -36.5 (c 6, CHCl₃); IR (film) 3396, 1732, 1058 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, COSY, NOESY): 1.57 (m, 1H, H-4ax), 1.63 (m, 2H, H-5), 1.70 (m, 1H, H-4eq), 1.89 (ddd, *J* = 13, 8, 5 Hz, 1H, H-3), 1.99 (ddd, *J* = 13, 9.5, 5.5 Hz, 1H, H-3), 2.32 (dddd, *J* = 13, 7, 6, 5, 1 Hz, 1H, H-3a), 3.32 (q, *J* = 5.5 Hz, 1H, H-7a), 3.60 (dd, *J* = 10, 5 Hz, 1H, H-2), 3.70 and 4.09 (2d, *J* = 13 Hz, 1H each, NCH₂Ar), 3.74 (dddd, *J* = 5.5, 5.5, 4.5, 4.5 Hz, 1H, H-6eq), 7.26 (m, 5H, Ar); ¹³C NMR (50 MHz, CDCl₃, HSQC): 23.2 (C-4), 30.8 (C-5), 30.8 (C-7), 33.3 (C-3), 36.1 (C-3a), 51.5 (Me), 52.5 (CH₂Ar), 60.2 (C-7a), 61.4 (C-2), 68.0 (C-6), 127.1, 128.2, 128.7 and 138.3 (Ar), 174.8 (CO₂Me). Anal. Calcd for C₁₇H₂₃NO₃ (292.85).1/4 H₂O: C 69.67, H 7.85, N 4.78. Found: C 69.70, N 7.97, N 4.89.

Methyl (2*S*,3*aR*,6*R*,7*aR*)-1-*tert*-Butoxycarbonyl-6-hydroxyoctahydroindole-2-carboxylate (10). A suspension of **9** (310 mg, 1.05 mmol), Boc₂O (295 mg, 1.36 mmol) and Pd(OH)₂/C (75 mg) in EtOAc (10 mL) was stirred under hydrogen (atmospheric pressure) at room temperature for 48 h. The catalyst was removed by filtration through a short pad of Celite, which was washed with CHCl₃ (20 mL), EtOAc (20 mL), and MeOH (20 mL). The organic filtrates were concentrated and treated with a pH 3 buffer of AcOH/NaOAc (5 mL) at room temperature for 24 h. The aqueous solution was basified with Na₂CO₃ and extracted with CH₂Cl₂ (2×40 mL) and CHCl₃ (2×40 mL). The dried organic extracts were concentrated and purified by chromatography (SiO₂, hexane/EtOAc, 1:1) to give 285 mg (90%) of *N*-Boc derivative **10** as a yellow oil: *R*_f = 0.45 (SiO₂, EtOAc); [α]_D: -85.5 (c 1.45 in CH₃OH); ¹H NMR

(500 MHz, CDCl₃, COSY, NOESY) *trans-cis* rotamer 1.2:1 ratio, *trans* rotamer: 1.10 (q, *J* = 11.5 Hz, 1H, H-7ax), 1.29 (m, 1H, H-5ax), 1.32 (s, 9H, C(CH₃)₃), 1.68 (m, 4H, 2H-4, H-3 and H-5 eq), 2.05 (br dt, *J* = 12, 6.5 Hz, 1H, H-3a), 2.22 (ddd, *J* = 13.0, 9.0, 9.0 Hz, 1H, H-3), 2.38 (m, 1H, H-7eq), 3.49 (tt, *J* = 12, 4 Hz, 1H, H-6ax), 3.64 (s, 3H, OCH₃), 3.97 (ddd, *J* = 11, 6, 6 Hz, 1H, H-7a), 4.19 (dd, *J* = 9.5, 1 Hz, 1H, H-2); *cis* rotamer: 1.04 (q, *J* = 11.5 Hz, 1H, H-7ax), 1.40 (s, 9H, C(CH₃)₃), 1.68 (m, 5H, 2H-4, H-3, H-3a, and H-5 eq), 2.22 (ddd, *J* = 13, 9, 9 Hz, 1H, H-3), 2.34 (m, 1H, H-7eq), 3.50 (tt, *J* = 12, 4 Hz, 1H, H-6ax), 3.65 (s, 3H, OCH₃), 3.87 (ddd, *J* = 11, 6, 6 Hz, 1H, H-7a), 4.26 (d, *J* = 9 Hz, 1H, H-2); ¹³C RMN (50 MHz, CDCl₃, HMQC) *trans-cis* rotamer 1.2:1 ratio, *trans* rotamer: 22.7 (C-4), 28.2 (C(CH₃)₃), 29.5 (C-5), 32.0 (C-3), 33.6 (C-3a), 37.2 (C-7), 51.9 (OCH₃), 56.3 (C-7a), 58.4 (C-2), 68.1 (C-6), 79.9 (C(CH₃)₃), 152.9 (NCO₂), 173.5 (CO₂Me); *cis* rotamer: 22.9 (C-4), 28.4 (C(CH₃)₃), 29.9 (C-5), 30.9 (C-3), 34.3 (C-3a), 37.6 (C-7), 52.1 (OCH₃), 56.5 (C-7a), 57.9 (C-2), 68.4 (C-6), 153.7 (NCO₂), 173.1 (CO₂Me). Anal. calcd for C₁₅H₂₅NO₅ (299.36): C 59.83, H 8.77, N 4.39; found C 60.18, H 8.42, N 4.68.

Methyl (2*S*,3*aR*,6*R*,7*aR*) 1-*tert*-Butoxycarbonyl-6-acetyloxyoctahydroindole-2-carboxylate (11). Ac₂O (0.87 mL, 9 mmol) and DMAP (12.42 mg, 10 mol%) were added to a solution of **10** (270 mg, 0.90 mmol) in pyridine (2.70 mL). The solution was stirred at room temperature for 24 h, diluted with EtOAc (30 mL) and washed with saturated aqueous NaHCO₃ (3×20 mL). This aqueous phase was extracted with EtOAc (3×30 mL) and CHCl₃ (3×30 mL). The concentrated dried organic extracts gave **11** (300 mg, 97%): *R*_f 0.46 (SiO₂, EtOAc); ¹H NMR (200 MHz, CDCl₃, COSY, NOESY): *trans-cis* rotamer 1.1:1 ratio, *trans* rotamer: 1.20 (m, 1H, H-7ax), 1.39 (s, 9H, C(CH₃)₃), 1.70 (m, 4H, 2H-4, 2H-5), 2.05 (s, 3H, CH₃CO), 2.29 (m, 4H, H-7 eq, H-3a, 2H-3), 3.73 (s, 3H, OCH₃), 4.06 (m, 1H, H-7a), 4.20 (dd, *J* = 9.5, 1.5 Hz, 1H, H-2), 4.64 (m, 1H, H-6); *cis* rotamer: 1.45 (s, 9H, C(CH₃)₃), 2.02 (s, 3H, CH₃CO), 3.72 (s, 3H, OCH₃), 4.00 (m, 1H, H-7a), 4.26 (dd, *J* = 9.5, 1.5 Hz, 1H, H-2); ¹³C

NMR (50 MHz, CDCl₃, HSQC) *trans-cis* rotamer 1.1:1 ratio, *trans* rotamer: 21.2 (CH₃CO), 22.4 (C-4), 26.2 (C-5), 28.1 (C(CH₃)₃), 31.8 (C-3), 33.4 (C-7), 34.2 (C-3a), 51.8 (OCH₃), 56.0 (C-7a), 57.9 (C-2), 70.5 (C-6), 79.7 (C(CH₃)₃), 153.5 (NCO₂), 170.0 (CH₃CO₂), 172.8 (CO₂Me); *cis* rotamer: 21.1 (CH₃CO), 22.5 (C-4), 26.1 (C-5), 28.3 (C(CH₃)₃), 31.0 (C-3), 33.2 (C-7), 33.7 (C-3a), 52.0 (OCH₃), 55.8 (C-7a), 58.3 (C-2), 70.3 (C-6), 79.9 (C(CH₃)₃), 152.7 (NCO₂), 169.8 (CH₃CO₂), 173.2 (CO₂Me).

Boc-D-Leu-3,7-diepi-L-Choi(OAc)-OMe (12). TFA (3.40 mL, 44.27 mmol) was added to a cooled (0 °C) solution of **11** (308 mg, 0.90 mmol) in CH₂Cl₂ (7.5 mL), and the mixture was stirred for 2 h and concentrated. To a cooled (0 °C) solution of the resulting material (319.5 mg, 0.90 mmol) and Boc-D-Leu (366 mg, 1.58 mmol) in CH₂Cl₂ (18.5 mL), PyBOP (822.2 mg, 1.58 mmol) and NMM (0.37 mL, 3.33 mmol) were added. After the mixture had been stirred for 30 min at 0 °C, the temperature was allowed to rise to room temperature, and stirring was continued for 24 h. The solution was diluted with CH₂Cl₂ (60 mL) and washed successively with 5% aqueous NaHSO₄ (3×25 mL), saturated aqueous NaHCO₃ (2×25 mL), and brine (1×10 mL). The organic layer was dried and concentrated, and the residue was purified by chromatography (hexane/EtOAc 1:1) to give **12** (352 mg, 86%): *R*_f 0.63 (EtOAc); [α]_D: +7.1 (c 0.75, CHCl₃); ¹H NMR (500 MHz, CDCl₃, COSY, NOESY) *trans-cis* rotamer 2:1 ratio, *trans*-rotamer: 0.86 (d, *J* = 6.5 Hz, 3H, H-5 Leu), 0.89 (d, *J* = 6.5 Hz, 3H, H-5' Leu), 1.28 (m, 3H, H-7ax, H-5ax Choi, H-3 Leu), 1.37 (s, 9H, C(CH₃)₃), 1.53 (m, 1H, H-3' Leu), 1.61 (m, 1H, H-4 Leu), 1.74 (m, 4H, H-3 Choi, H-5eq Choi, 2 H-4 Choi), 1.96 (s, 3H, CH₃CO), 2.17 (m, 1H, H-7eq Choi), 2.22 (ddd, *J* = 13.5, 13, 9.5 Hz, 1H, H-3 Choi), 2.60 (m, 1H, H-3a Choi), 3.63 (s, 3H, OCH₃), 4.31 (d, *J* = 9 Hz, 1H, H-2 Choi), 4.42 (m, 2H, H-2 Leu, H-7a), 4.57 (m, 1H, H-6 Choi), 4.85 (d, *J* = 9.5 Hz, 1H, NH Leu); *cis* rotamer: 0.75 (d, *J* = 6.5 Hz, 3H, H-5 Leu), 0.83 (d, *J* = 6.5 Hz, 3H, H-5' Leu), 1.06 (q, *J* = 12 Hz, 1H, H-7ax), 1.28 (m, 1H, H-3 Leu), 1.35 (s, 9H, C(CH₃)₃), 1.53 (m, 1H, H-3'

Leu), 1.61 (m, 1H, H-4 Leu), 1.92 (s, 3H, CH₃CO), 2.43 (ddd, *J* = 13.5, 13, 9.5 Hz, 1H, H-3 Choi), 3.67 (s, 3H, OCH₃), 3.94 (ddd, *J* = 11, 9, 3.5 Hz, 1H, H-2 Leu), 4.18 (ddd, *J* = 12, 6, 6 Hz, 1H, H-7a Choi), 4.57 (m, 1H, H-6 Choi), 4.76 (d, *J* = 9.5 Hz, 1H, NH Leu), 4.97 (d, *J* = 9 Hz, 1H, H-2 Choi); ¹³C NMR (75 MHz, CDCl₃, HSQC) *trans-cis* rotamer 1.6:1 ratio, *trans* rotamer: 21.2 (C-5 Leu), 21.2 (CH₃CO Choi), 22.4 (C-4 Choi), 23.5 (C-5' Leu), 24.6 (C-4 Leu), 25.6 (C-5 Choi), 28.3 (C(CH₃)₃), 30.0 (C-3, Choi), 34.9 (C-3a Choi), 35.2 (C-7 Choi), 42.2 (C-3 Leu), 49.8 (OCH₃), 52.1 (C-2 Leu), 56.5 (C-7a Choi), 58.3 (C-2 Choi), 70.4 (C-6 Choi), 79.6 (C(CH₃)₃), 155.5 (NCO₂), 169.9 (CH₃CO₂)^{*}, 171.8 (C-1 Leu), 172.0 (CO₂Me)^{*}; *cis* rotamer: 21.2 (CH₃CO), 21.9 (C-5 Leu), 22.5 (C-4 Choi), 24.2 (C-4 Leu), 26.2 (C-5 Choi), 29.7 (C-3 Choi), 32.0 (C-7 Choi), 33.0 (C-3a Choi), 40.3 (C-3 Leu), 50.1 (OCH₃), 52.5 (C-2 Leu), 56.7 (C-7a, Choi), 58.8 (C-2 Choi), 70.5 (C-6 Choi), 155.8 (NCO₂), 170.1 (CH₃CO₂)^{*}, 172.1 (C-1 Leu), 172.8 (CO₂Me)^{*}. Anal. calcd for C₂₃H₃₈N₂O₇ (454.56): C 60.77, H 8.43, N 6.16. Found C 60.77, H 8.59, N 5.96.

AcO-L-Hpla(Bn)-D-Leu-3a,7a-diepi-L-Choi(OAc)-OMe (13). TFA (1.80 mL, 23.5 mmol) was added to a cooled (0 °C) solution of **12** (297 mg, 0.65 mmol) in CH₂Cl₂ (9 mL), and the mixture was stirred for 2 h and concentrated. A solution of the resulting material in CH₂Cl₂ (4.2 mL) with NMM (0.11 mL, 0.97 mmol) was added to a cooled (0 °C) solution of the L-Hpla derivative (*S*)-2-acetyloxy-3-(4-benzyloxyphenyl)propionic acid^{4c} (245.2 mg, 0.78 mmol) in CH₂Cl₂ (4.3 mL), which had been previously stirred at this temperature with PyBOP (406.1 mg, 0.78 mmol) and NMM (0.16 mL, 1.3 mmol) for 30 min. The mixture was stirred at room temperature for 24 h. CH₂Cl₂ (25 mL) was added, and the organic layer was successively washed with HCl (1 N, 3×20 mL), saturated NaHCO₃ solution (3×20 mL), and brine (1×15 mL). The concentrated dried extract was chromatographed (hexane/EtOAc 2:3)

^{*} The assignment of these signals can be inverted: major rotamer 169.9 and 172.0 (CH₃CO, CO₂Me); minor rotamer 170.1 and 172.8 (CH₃CO, CO₂Me).

to give **13** (345 mg, 82%): $R_f = 0.62$ (EtOAc); $[\alpha]_D^{25} -12.5$ (c 0.5 in CHCl_3); ^1H NMR (500 MHz, CDCl_3 , COSY, NOESY) *trans-cis* rotamer 2:1 ratio, *trans* rotamer: 0.88 (d, $J = 6.5$ Hz, 3H, H-5 Leu), 0.91 (d, $J = 6.0$ Hz, 3H, H-5' Leu), 1.38 (m, 3H, H-5ax Choi, H-3 Leu, H-7ax Choi), 1.48 (m, 1H, H-4 Leu), 1.60 (m, 1H, H-3' Leu), 1.79 (m, 3H, 2H-4 Choi, H-3 Choi), 1.84 (br s, 1H, H-5eq Choi), 1.94 (s, 3H, CH_3CO Choi)*, 2.03 (s, 3H, CH_3CO Hpla)*, 2.22 (ddd, $J = 12, 6, 5$ Hz, 1H, H-7eq Choi), 2.30 (ddd, $J = 13, 13, 9$ Hz, 1H, H-3 Choi), 2.64 (m, 1H, H-3a Choi), 2.97 (dd, $J = 14, 8$ Hz, 1H, H-3 Hpla), 3.15 (dd, $J = 14, 4.5$ Hz, 1H, H-3' Hpla), 3.68 (s, 3H, OCH_3), 4.39 (d, $J = 9.5$ Hz, 1H, H-2 Choi), 4.47 (ddd, $J = 12, 6.5, 6.5$ Hz, 1H, H-7a Choi), 4.67 (tt, $J = 12, 4.5$ Hz, 1H, H-6 Choi), 4.80 (ddd, $J = 11, 8.5, 3$ Hz, 1H, H-2 Leu), 5.02 (s, 2H, OCH_2Ar), 5.27 (dd, $J = 8.0, 4.5$ Hz, 1H, H-2 Hpla), 6.36 (d, $J = 9$ Hz, 1H, NH Leu), 6.89 (d, $J = 8.5$ Hz, 2H, H-6,8 Hpla), 7.11 (d, $J = 8.5$ Hz, 2H, H-5,9 Hpla), 7.31 (t, $J = 7$ Hz, 1H, *H**p*-Ar), 7.38 (m, 4H, 2*H**m*-Ar, 2*H**o*-Ar); *cis* rotamer: 0.76 (d, $J = 6.5$ Hz, 3H, H-5 Leu), 0.84 (d, $J = 6.5$ Hz, 3H, H-5' Leu), 1.11 (q, $J = 12.5$ Hz, 1H, H-7ax Choi), 1.38 (m, 3H, H-5ax Choi, H-4 Leu, H-3 Leu), 1.60 (m, 1H, H-3' Leu), 1.79 (m, 3H, 2H-4 Choi, H-3 Choi), 1.87 (br s, 1H, H-5 eq Choi), 2.05 (s, 3H; CH_3CO Hpla)*, 2.34 (m, 1H, H-3a Choi), 2.48 (ddd, $J = 13, 13, 9$ Hz, 1H, H-3 Choi), 2.64 (m, 1H, H-7eq Choi), 3.03 (dd, $J = 14, 8$ Hz, 1H, H-3 Hpla), 3.09 (dd, $J = 14, 4.5$ Hz, 1H; H-3' Hpla), 3.75 (s, 3H, OCH_3), 4.22 (ddd, $J = 12, 6.5, 6.5$ Hz, 1H, H-7a Choi), 4.28 (ddd, $J = 11, 8.5, 3$ Hz, 1H, H-2 Leu), 4.64 (tt, $J = 12, 4.5$ Hz, 1H, H-6 Choi), 5.04 (d, $J = 9.5$ Hz, 1H, H-2 Choi), 5.27 (dd, $J = 8.5, 4.5$ Hz, 1H, H-2 Hpla), 6.31 (d, $J = 8.5$ Hz, 1H, NH Leu), 6.88 (d, $J = 8.5$ Hz, 2H, H-6,8 Hpla), 7.05 (d, $J = 8.5$ Hz, 2H, H-5,9 Hpla); ^{13}C NMR (50 MHz, CDCl_3 , HSQC) *trans-cis* rotamer 1.2:1 ratio, *trans* rotamer: 20.7 (CH_3CO Hpla)*, 21.2 (CH_3CO_2 Choi)*, 22.3 (C-4 Choi), 23.3 (C-5 Leu), 23.4 (C-5' Leu), 24.6 (C-4 Leu), 25.6 (C-5 Choi), 29.9 (C-3 Choi), 34.7 (C-3a Choi), 35.1 (C-7 Choi), 37.1 (C-3 Hpla), 41.7 (C(3) Leu), 48.1 (C-2 Leu), 52.1 (OCH_3), 56.5 (C-7a Choi), 58.3 (C-2 Choi),

*The assignment of these signals can be inverted.

69.9 (OCH₂Ar), 70.3 (C-6 Choi), 74.5 (C-2 Hpla), 114.7 (C-6, C-8 Hpla), 127.4 (o-C), 127.8 (p-C), 128.2 (C-4 Hpla), 128.4 (m-C), 130.3 (C-5, C-9 Hpla), 136.9 (ipso-C), 157.6 (C-7 Hpla), 168.9 (C-1 Hpla), 169.2 (C-1 L), 169.9 and 170.3 (CH₃CO₂ Choi and Hpla), 171.6 (CO₂Me); *cis* rotamer: 20.8 (CH₃CO Hpla)*, 21.9 (C-5 Leu), 22.5 (C-4 Choi), 23.4 (C-5' Leu), 24.2 (C-4 Leu), 26.3 (C-5 Choi), 32.0 (C-3 Choi), 32.3 (C-7 Choi), 33.0 (C-3a Choi), 36.7 (C-3 Hpla), 39.8 (C-3 Leu), 48.7 (C-2 Leu), 52.6 (OCH₃), 56.7 (C-7a Choi), 58.8 (C-2 Choi), 70.5 (C-6 Choi), 74.3 (C-2 Hpla), 136.8 (ipso-C), 157.7 (C-7 Hpla), 169.3 and 169.4 (C-1 Hpla and C-1 Leu), 170.1 and 170.5 (CH₃CO₂ Choi and Hpla), 172.7 (CO₂Me). Anal.calcd for C₃₆H₄₆N₂O₉ (650.76): C 66.44, H 7.12, N 4.30. Found C 66.07, H 7.30 N 4.14.

L-Hpla-D-Phe-3a,7a-diepi-L-Choi-NH₂ [Aeruginosin EI461] (2). A cooled solution of LiOH (0.1 N, 22.5 mL, 2.25 mmol) was added dropwise to a cooled (0 °C) solution of peptide **13** (296 mg, 0.45 mmol) in THF (16 mL). After having been stirred at room temperature for 20 h, the solution was washed with Et₂O (2×30 mL) and acidified to pH 1 with 1 N HCl. The aqueous solution was extracted with EtOAc (4×60 mL) and CHCl₃/*i*-PrOH 4:1 (5×60 mL). The combined extracts were washed with brine, dried, and concentrated to give the corresponding acid (224 mg, 90%); ¹H NMR (CDCl₃, 200 MHz) *trans-cis* rotamer 1.4:1 ratio, *trans* rotamer: 0.91 (2d, *J* = 6 Hz, 6H, H-5, H-5' Leu), 1.10-2.0 (m, 9H, 2 H-5 Choi, H-7ax Choi, 2 H-4 Choi, H-3 Choi, 2 H-3 Leu, H-4 Leu), 2.25 (m, 2H, H-3 Choi, H-7 eq Choi), 2.53 (m, 1H, H-3a Choi), 2.75 (dd, *J* = 14, 8 Hz, 1H, H-3 Hpla), 3.07 (dd, *J* = 14, 4.5 Hz, 1H, H-3' Hpla), 3.64 (m, 1H, H-6 Choi), 4.03 (dd, *J* = 8.5, 4.5 Hz, 1H, H-2 Hpla), 4.22 (m, 1H, H-7a Choi), 4.39 (d, *J* = 9 Hz, 1H, H-2 Choi), 4.82 (m, 1H, H-2 Leu), 5.00 (s, 2H, OCH₂Ar), 6.88 (d, *J* = 8.5 Hz, 2H, H-6,8 Hpla), 7.14 (d, *J* = 8.5 Hz, 2H, H-5,9 Hpla), 7.37 (m, 5H, H-Ar); *cis* rotamer: 0.74 (d, *J* = 6 Hz, 3H, H-5 Leu), 0.81 (d, *J* = 6 Hz, 3H, H-5' Leu), 2.43 (m, 1H, H-3a Choi), 5.03 (s, 2H, OCH₂Ar).

To a solution of the above acid (224 mg, 0.405 mmol) in THF (5.3 mL) were added NMM (0.073 mL, 0.61 mmol), HOBT.H₂O (93 mg, 0.61 mmol) and EDC (117 mg, 0.61 mmol). After stirring at 0 °C for 1 h, the reaction mixture was treated with 0.12 mL of concentrated NH₄OH and allowed to slowly warm to room temperature. After 17 h, the reaction mixture was diluted with EtOAc (60 mL) and washed with 1 N HCl (3x30 mL), saturated aqueous NaHCO₃ (3x30 mL), and brine (1x10 mL). The EtOAc extract was dried and concentrated. Chromatography (EtOAc/MeOH 4%) of the residue gave HO-L-Hpla(Bn)-D-Leu-L-Choi-NH₂ (110 mg, 50%): *R*_f 0.70 (EtOAc/MeOH 1:1); ¹H NMR (500 MHz, DMSO-*d*₆, COSY, NOESY) *cis-trans* rotamer 1.6:1 ratio, *cis* rotamer: 0.71 (d, *J* = 7 Hz, 3H, H-5 Leu), 0.79 (d, *J* = 6.5 Hz, 3H, H-5' Leu), 0.80 (q, *J* = 12 Hz, 1H, H-7ax Choi), 1.22 (m, 1H, H-5ax Choi), 1.25 (m, 1H, H-3 Leu), 1.39 (m, 1H, H-4 Leu), 1.52 (m, 1H, H-3' Leu), 1.56 (m, 1H, H-5eq Choi), 1.61 (m, 2H, 2H-4 Choi), 1.70 (dd, *J* = 12.5, 6 Hz, 1H, H-3 Choi), 2.18 (m, 1H, H-3a Choi), 2.30 (td, *J* = 13, 9.5 Hz, 1H, H-3 Choi), 2.35 (m, 1H, H-7eq Choi), 2.64 (dd, *J* = 14, 7.5 Hz, 1H, H-3 Hpla), 2.84 (dd, *J* = 14, 4.5 Hz, 1H, H-3' Hpla), 3.27 (tq, *J* = 12, 4.5 Hz, 1H, H-6 Choi), 3.95 (ddd, *J* = 11.5, 6.5, 6.5 Hz, 1H, H-7a Choi), 4.05 (m, 1H, H-2 Hpla), 4.15 (ddd, *J* = 11.5, 9, 3 Hz, 1H, H-2 Leu), 4.55 (d, *J* = 4.5 Hz, 1H, 6-OH Hpla), 4.71 (d, *J* = 9 Hz, 1H, H-2 Choi), 4.71 (d, *J* = 4.5 Hz, 6-OH, Choi), 5.04 (s, 2H, OCH₂Ar), 5.34 (d, *J* = 6.5 Hz, 1H, 2-OH Hpla), 6.88 (d, *J* = 8.5 Hz, 2H, H-6,8 Hpla), 7.07 (d, *J* = 8.5 Hz, 2H, H-5,9 Hpla), 7.31 (t, *J* = 8Hz, 1H, H-*p*-Ar), 7.38 (dd, *J* = 7.5 Hz, 2H, H-*m*-Ar), 7.42 (d, *J* = 8.5 Hz, 2H, H-*o*-Ar), 7.59 (br s, 1H, NH₂ Choi), 7.60 (d, *J* = 8 Hz, 1H, NH Leu); *trans* rotamer: 0.86 (d, *J* = 6.5 Hz, 3H, H-5 Leu), 0.87 (d, *J* = 6.5 Hz, 3H, H-5' Leu), 1.17 (q, *J* = 12.0 Hz, 1H, H-7ax Choi), 1.22 (m, 2H, H-5ax Choi, H-3 Choi), 1.25 (m, 1H, H-3 Leu), 1.49 (m, 1H, H-4 Leu), 1.56 (m, 1H, H-5eq Choi), 1.61 (m, 2H, 2H-4 Choi), 1.64 (m, 1H, H-3 Leu), 2.01 (ddd, *J* = 12, 6, 5 Hz 1H, H-7eq Choi), 2.20 (m, 1H, H-3 Choi), 2.41 (br dt, *J* = 13.5, 6.5 Hz, 1H, H-3a Choi), 2.56 (dd, *J* = 14.0, 9.0 Hz, 1H, H-3 Hpla), 2.86 (dd, *J* = 14.0, 4.5 Hz, 1H, H-3' Hpla), 3.36 (m, 1H, H-6 choi), 4.05 (m, 1H, H-2 Hpla), 4.13 (d, *J* = 9 Hz, 1H, H-2 Choi), 4.24 (ddd, *J* = 11.5, 6.5, 6.5 Hz, 1H, H-7a Choi), 4.51

(ddd, $J = 11.0, 8.0, 4.0$ Hz, 1H, H-2 Leu), 4.71 (d, $J = 4.5$ Hz, 1H, 6-OH Hpla), 5.05 (s, 2H, OCH₂Ar), 5.40 (d, $J = 7.0$ Hz, 1H, 2-OH Hpla), 6.83 (br s, 1H, NH₂ Choi), 6.89 (d, $J = 8.5$ Hz, 2H, H-6,8 Hpla), 7.12 (d, $J = 8.5$ Hz, 2H, H-5,9 Hpla), 7.31 (t, $J = 8.0$ Hz, 1H, Hp-Ar), 7.38 (dd, $J = 7.5$ Hz, 2H; Hm-Ar), 7.42 (d, $J = 8.5$ Hz, 2H, Ho-Ar), 7.81 (d, $J = 8.0$ Hz, 1H, NH Leu); ¹³C NMR (100 MHz, DMSO-*d*₆, HSQC) *cis-trans* rotamer 1.6:1 ratio, *cis* rotamer: 21.2 (C-5 Leu), 22.7 (C-4 Choi), 23.7 (C-5' Leu), 23.7 (C-4 Leu), 29.9 (C-5 Choi), 32.7 (C-3a Choi), 33.3 (C-3 Choi), 36.2 (C-7 Choi), 40.0 (C-3 Leu), 40.3 (C-3 Hpla), 56.2 (C-2 Leu), 56.9 (C-7a Choi), 59.3 (C-2 Choi), 67.0 (C-6 Choi), 62.3 (OCH₂Ar), 72.1 (C-2 Hpla), 114.4 (C-6, C-8 Hpla), 127.7-128.6 (C-Ar, C-4 Hpla), 130.5 (C-5, C-9 Hpla), 137.5 (*ipso*-C), 157.0 (C-7 Hpla), 170.5 (C-1 Leu), 173.1 (C-1 Hpla), 173.9 (C-1 Choi); *trans* rotamer: 21.6 (C-5 Leu), 22.7 (C-4 Choi), 23.7 (C-5' Leu), 24.2 (C-4 Leu), 29.5 (C-5 Choi), 30.6 (C-3 Choi), 34.7 (C-3a Choi), 39.5 (C-7 Choi), 40.8 (C-3 Leu), 40.3 (C-3 Hpla), 56.2 (C-2 Leu), 56.9 (C-7a Choi), 59.3 (C-2 Choi), 62.3 (OCH₂Ar), 66.9 (C-6 Choi), 72.4 (C-2 Hpla), 114.5 (C-6, C-8 Hpla), 127.7-128.6 (C-Ar, C-4 Hpla), 130.4 (C-5, C-9 Hpla), 137.5 (*ipso*-C), 157.0 (C-7 Hpla), 170.2 (C-1 Leu), 173.7 (C-1 Hpla), 174.1 (C-1 Choi).

Pd/C (10%, 16 mg) was added to a solution of HO-L-Hpla(Bn)-D-Leu-L-Choi-NH₂ (53 mg, 0.096 mmol) in EtOAc/MeOH 1.5:1 (2 mL), and the mixture was stirred under hydrogen at atmospheric pressure for 22 h. Upon complete conversion, the catalyst was removed by filtration with Celite, which was washed with EtOAc/MeOH 1:1 and MeOH. The organic solution was concentrated to give 42 mg (95%) of aeruginosin EI461 (**2**) as a colorless oil; $R_f=0.57$ (EtOAc/MeOH 1:1); $[\alpha]_D^{25}$: -28.4 (c 0.7 in CH₃OH); ¹H NMR (600 MHz, DMSO-*d*₆, COSY, NOESY) *cis-trans* rotamer 3:2 ratio *cis* rotamer: 0.71 (d, $J = 6.5$ Hz, 3H, H-5 Leu), 0.78 (d, $J = 6.5$ Hz, 3H, H-5' Leu), 0.78 (q, $J = 12$ Hz, 1H, H-7ax), 1.21 (br dq, $J = 12.8, 4.2$ Hz, 1H, H-5ax Choi), 1.22 (m, 1H, H-3 Leu), 1.40 (m, 1H, H-4 Leu), 1.50 (m, 1H, H-3' Leu), 1.54 (m, 1H, H-5eq Choi), 1.63 (m, 2H, 2H-4 Choi), 1.69 (dd, $J = 12, 6$ Hz, 1H, H-3 Choi), 2.18 (m, 1H, H-3a Choi), 2.28 (td, $J = 12.5, 9$ Hz, 1H, H-3 Choi), 2.31 (br dd, $J = 12.0, 6.0$

Hz, 1H, H-7eq Choi), 2.54 (dd, $J = 14.0, 8.0$ Hz, 1H, H-3 Hpla), 2.79 (dd, $J = 14.0, 4.5$ Hz, 1H, H-3' Hpla), 3.25 (br t, $J = 11$ Hz, 1H, H-6 Choi), 3.94 (ddd, $J = 12.0, 6.5, 6.5$ Hz, 1H, H-7a Choi), 4.02 (m, 1H, H-2 Hpla), 4.14 (ddd, $J = 11.5, 8.5, 3.0$ Hz, 1H, H-2 Leu), 4.49 (m, 1H, 6-OH Choi), 4.71 (d, $J = 9.0$ Hz, 1H, H-2 Choi), 5.26 (d, $J = 6.5$ Hz, 1H; 2-OH Hpla), 6.61 (d, $J = 8.5$ Hz, 2H, H-6,8 Hpla), 6.95 (d, $J = 8.5$ Hz, 2H, H-5,9 Hpla), 7.10 (br s, 1H, NH₂ Choi), 7.58 (br s, 1H, NH₂ Choi), 7.59 (d, $J = 8.0$ Hz, 1H; NH Leu), 9.08 (s, 1H, 7-OH Hpla); *trans* rotamer: 0.86 (d, $J = 6.5$ Hz, 3H, H-5 Leu), 0.86 (d, $J = 6.5$ Hz, 3H, H-5' Leu), 1.17 (q, $J = 12$ Hz, 1H; H-7ax), 1.24 (br q, 1H, H-5ax Choi), 1.25 (m, 1H, H-3 Leu), 1.47 (m, 1H, H-3 Choi), 1.52 (m, 1H, H-4 Leu), 1.56 (m, 1H, H-3' Leu), 1.62 (m, 1H, H-5eq Choi), 1.63 (m, 2H, 2H-4 Choi), 2.40 (br dt, $J = 12, 6$ Hz, 1H, H-3a Choi), 2.17 (m, 1H, H-3 Choi), 2.01 (m, 1H, H-7eq Choi), 2.52 (dd, $J = 14, 8$ Hz, 1H, H-3 Hpla), 2.80 (dd, $J = 14.0, 4.5$ Hz, 1H, H-3' Hpla), 3.34 (br t, $J = 11$ Hz, 1H, H-6 Choi), 4.02 (m, 1H, H-2 Hpla), 4.12 (d, $J = 9.5$ Hz, 1H, H-2 Choi), 4.23 (ddd, $J = 12, 6.5, 5.5$ Hz, 1H, H-7a Choi), 4.49 (m, 1H, H-2 Leu), 4.72 (m, 1H, 6-OH Choi), 5.35 (d, $J = 6.5$ Hz, 1H, 2-OH Hpla), 6.62 (d, $J = 8.5$ Hz, 2H, H-6,8 Hpla), 6.82 (br s, 1H, NH₂ Choi), 6.88 (br s, 1H, NH₂ Choi), 6.98 (d, $J = 8.5$ Hz, 2H, H-5,9 Hpla), 7.79 (d, $J = 8$ Hz, 1H; NH Leu), 9.09 (s, 1H, 7-OH Hpla); ¹³C NMR (100 MHz, DMSO-*d*₆, HSQC) *cis-trans* rotamer 4:3 ratio, *cis* rotamer: 21.2 (C-5 Leu), 22.7 (C-4 Choi), 23.8 (C-5' Leu), 23.8 (C-4 Leu), 29.9 (C-5 Choi), 32.6 (C-3a Choi), 33.3 (C-3 Choi), 36.1 (C-7 Choi), 39.9 (C-3 Leu), 40.3 (C-3 Hpla), 40.8 (C-3 Leu), 48.1 (C-2 Leu), 56.5 (C-7a Choi), 59.3 (C-2 Choi), 67.0 (C-6 Choi), 72.3 (C-2 Hpla), 114.9 (C-6, C-8 Hpla), 128.4 (C-4 Hpla), 130.3 (C-5, C-9 Hpla), 155.7 (C-7 Hpla), 170.5 (C-1 Leu), 173.2 (C-1 Hpla), 173.9 (C-1 Choi); *trans* rotamer: 21.6 (C-5 Leu), 22.7 (C-4 Choi), 23.6 (C-5' Leu), 24.2 (C-4 Leu), 29.5 (C-5 Choi), 30.6 (C-3 Choi), 34.7 (C-3a Choi), 39.5 (C-7 Choi), 40.3 (C-3 Leu), 48.3 (C-2 Leu), 56.9 (C-7a Choi), 59.4 (C-2 Choi), 66.9 (C-6 Choi), 72.6 (C-2 Hpla), 114.9 (C-6, C-8 Hpla), 128.7 (C-4 Hpla), 130.4 (C-5, C-9 Hpla), 155.8 (C-7 Hpla), 170.2 (C-1 Leu), 173.7 (C-1 Hpla), 174.2 (C-1 Choi); HPLC:

Waters Nova-Pak C-18 analytical; A:H₂O, B: AcN/TFA 0.05%; A 30 min linear gradient elution, t_r =1.55 min (major rotamer) and t_r =1.15 (minor rotamer), ratio major-minor 3:1.

Marfey's analysis of aeruginosin EI461 and 2

I. A solution of 0.5 mg of aeruginosin EI461 was dissolved in 1 mL of 6 N HCl and stirred at 104 °C in a sealed tube for 20 h. After concentration to dryness, the residue was dissolved in 50 µL of water and 100 µL of 1% 1-fluoro-2,4-dinitrophenyl-5-L-alanine (L-FDAA) in acetone was added, followed by 20 µL of 1M NaHCO₃. The reaction mixture was stirred at 40 °C for 1 h, allowed to cool, and acidified with 10 µL of 2 N HCl. The solution was removed *in vacuo* and dissolved in water (100 µL).

II. A solution of **2** (0.5 mg) was treated in a similar way to natural aeruginosine EI461.

III. The *N*-[(-dinitrophenyl)-5-L-alanine amide]-amino acid (AA) derivatives, from hydrolysates of natural and synthetic (**2**) aeruginosine EI461, were analyzed on a Merck GmbH PurospherSTAR RP-18, 5 µm, 4.6 x 250 mm HPLC column, at a flow rate of 1 mL/min, using UV detection at 340 nm and a linear gradient elution from 9:1 50 mM triethylammonium phosphate (TEAP) buffer (pH 3)/acetonitrile to 1:1 TEAP/acetonitrile within 60 min. The hydrolysates of natural and synthetic (**2**) aeruginosine EI461 were tested separately and mixed to give identical chromatograms where the *diēp*-L-Choi derivative eluted from the column with retention time of 28.7 min and the D-Leu derivative with retention time of 54.1 min.

¹H NMR Data of Compound 1 and Aeruginosin EI461 (2) in DMSO-*d*₆

		Aeruginosin EI461 (2)		Compound 1	
Choi		<i>cis</i> (major)	<i>trans</i> (minor)	<i>trans</i> (major)	<i>cis</i> (minor)
	2	4.71 d (9)	4.12 d (9.5)	4.23 t (8.5)	4.31 t (8.5)
	3	α 2.28 td (12.5, 9)	2.17 m	2.02 m	
		β 1.69 dd (12, 6)	1.47 m	1.84 ddd (12, 10, 0.5)	
	3a	2.18 m	2.40 br dt (12, 6)	2.16 ddddd (12, 6, 6, 6, 0.5)	
	4	1.63 m	1.63 m	1.70 m	
	5	<i>eq</i> 1.54 m	1.62 m	1.62 m	
		<i>ax</i> 1.21 br dq (4.2, 12.8)	1.24 br q	1.23 ddddd (14, 10, 10, 4.5)	
	6	3.25 br t (11)	3.34 br t (11)	3.39 m	3.40 m
	7	<i>eq</i> 2.31 br dd (12, 6)	2.01 m	1.96 m	
		<i>ax</i> 0.78 q (12)	1.17 q (12)	1.57 m	
	7a	3.94 ddd (12, 6.5, 5.5)	4.23 ddd (12, 6.5, 5.5)	4.24 ddd (12, 6, 6)	4.45 ddd (12, 6, 6)
	6OH	4.49 m	4.72 m	4.75 d (4.5)	
	NH₂	7.58 br s	6.88 br s	7.31 br s	7.31 br s
		7.10 br s	6.82 br s	6.87 br s	6.85 br s
Leu	2	4.14 ddd (11.5, 8.5, 3)	4.49 m	4.56 m	4.59 m
	3	1.50 m	1.56 m	1.52 m	
		1.22 m	1.25 m	1.33 ddd (12, 8, 4)	
	4	1.40 m	1.52 m	1.48 m	
Hpla	5'	0.78 d (6.5)	0.86 d (6.5)	0.90 d (6.5)	0.94 d (6)
	5	0.71 d (6.5)	0.86 d (6.5)	0.89 d (6)	0.87 d (6.5)
	NH	7.59 d (8.0)	7.79 d (8.0)	7.73 d (8.5)	7.66 d (8)
	2	4.02 m	4.02 m	4.07 dd (8, 4)	4.14 m
	3	2.79 dd (14, 4.5)	2.80 dd (14, 4.5)	2.82 dd (14, 4.5)	2.94 dd (14.5, 3.5)
		2.54 .d (14, 8)	2.52 ddd (14, 8)	2.60 dd (13.5, 8)	2.49 dd (14, 9.5)
	5, 9	6.95 d (8.5)	6.98 d (8.5)	6.99 d (8.5)	7.03 d (8.5)
	6, 8	6.61 d (8.5)	6.62 d (8.5)	6.65 d (8.5)	6.68 d (8.5)
	2OH	5.26 d (6.5)	5.35 d (6.5)	5.38 d (6.5)	5.58 d (6.5)
	7OH	9.08 s	9.09 s	9.15 br s	

¹³C NMR Data of Compound 1 and Aeruginosin EI461 (2) in DMSO-*d*₆

Aeruginosin EI461 (2)			Compound 1		
		<i>cis</i> (major)	<i>trans</i> (minor)	<i>trans</i> (major)	<i>cis</i> (minor)
Choi	1	173.9	174.2	173.2	173.3
	2	59.3	59.4	59.3	59.3
	3	33.3	30.6	30.6	30.5
	3a	32.6	34.7	36.2	36.2
	4	22.7	22.7	22.8	22.7
	5	29.9	29.5	29.5	29.6
	6	67.0	66.9	67.1	67.1
	7	36.1	39.5	39.2	39.2
	7a	56.5	56.9	57.4	57.4
Leu	1	170.5	170.2	169.9	170.0
	2	48.1	48.3	47.7	47.8
	3	39.9	40.8	40.2	40.2
	4	23.8	24.2	24.1	24.2
	5'	23.8	23.6	21.8	21.9
	5	21.2	21.6	23.5	23.6
Hpla	1	173.2	173.7	173.1	173.3
	2	72.3	72.6	72.2	72.2
	3	40.3	40.3	41.6	
	4	128.4	128.7	128.3	128.4
	5, 9	130.3	130.4	130.3	130.3
	6, 8	114.9	114.9	114.8	114.9
	7	155.7	155.8	155.7	155.7