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Supporting Information:

The first total synthesis of aeruginosamide

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Experimental

N-Boc-L-Pro-L-Serine methyl ester (**4**)

To a solution of *N*-Boc-L-proline (6.46 g, 30 mmol), L-serine methyl ester hydrochloride (4.68 g, 30 mmol) in dichloromethane (200 mL) at 0 °C, *N*-Hydroxybenzotriazole (HOBT) (6.05 g, 47 mmol), *N,N'*-Dicyclohexylcarbodiimide (DCC) (9.28 g, 45 mmol) and triethylamine (8.5 mL, 61 mmol) were added. The reaction mixture was stirred for 1 h at 0 °C and 12 hours at room temperature, during which time a white precipitate formed. This mixture was filtered; the filtrate was diluted with dichloromethane (300 mL), washed with saturated sodium hydrogen carbonate (100 mL), ammonium chloride (100 mL), brine (60 mL) and dried over sodium sulfate. The solvent was then evaporated and the residue was purified by flash on silica gel to give the dipeptide **4** (8.43 g, 89% yield). ¹H NMR (CDCl₃) (multiple rotamers): δ 1.44(9H, s); 1.85~2.35 (4H, m); 3.70~3.43 (4H, m); 3.82(3H, s); 4.71 (1H, m); 5.15(1H, m); ¹³C NMR (CDCl₃) (multiple rotamers): δ 24.6; 28.3; 29.1; 47.1; 55.2; 52.1; 60.3; 62.3; 80.7; 155.3; 170.7; 172.5; IR (cm⁻¹): 3418, 2977, 1747, 1681. EIMS: 316, 286, 260, 243, 215, 171, 156, 137; HRMS (EI): calcd for C₁₄H₂₄N₂O₆ 316.1634, found 316.1623 *N*-Boc-L-Pro-L-(OTBS)-Ser methyl ester (**5**)

To the solution of dipeptide **4** (8.10 g, 25.633 mmol) and TBS-Cl (4.44 g, 29.2 mmol) in dichloromethane (30 mL), DMAP (0.336 g, 2.254 mmol) and triethylamine (4.2 mL, 30 mmol) were added at 0 °C. The solution was stirred at room temperature for 24 hours, then diluted with dichloromethane (200 mL), washed with saturated sodium hydrogen carbonate (60 mL), ammonium chloride (60 mL), brine (60 mL), and dried over sodium sulfate. The organic solvent was evaporated and the residue was purified by flash chromatography on silica gel to give the title compound (10.2 g, 95% yield). [α]_D²⁰ - 2.78 (*c* 3.47, CHCl₃); ¹H NMR (CDCl₃) (multiple rotamers): δ -0.27 (6H, s); 0.82 (9H, s) 1.42 (9H, s); 2.28~1.80 (4H, m); 3.47(2H, m); 3.74 (3H, s); 4.12~3.99; 4.73(1H, m); 5.22(1H, m); 6.84&7.31(1H, br); ¹³C NMR (CDCl₃) (multiple rotamers): δ -6.1; -6.0; 17.7; 23.2; 25.2; 27.9; 30.6; 46.6; 59.8; 51.8; 53.8; 63.2; 79.9; 154.1; 170.0; 172.0; IR (cm⁻¹): 3418, 2878, 1747, 1680, 1519; EIMS: 430, 398, 386, 371, 330, 315, 317, 293, 273, 253, 114, 119, 170; HRMS (EI): calcd. for C₂₀H₃₈N₂O₆Si 430.2499, found 430.2499.

N-Boc-L-Pro(C=S)-L-Ser methyl ester (**6**)

To compound **5** (4.76 g, 11.423 mmol) in benzene (30 mL), a solution of Lawesson's reagent (C₁₄H₁₄O₂P₂S₄) in benzene (30 mL) was added. After the reaction mixture was under refluxed for 6 hours, the solvent was evaporated and the residue was purified by flash chromatography on silica gel to give the corresponding thioamide 3.21g. To the thioamide (3.21 g, 7.41 mmol) in THF (20 mL), TBAF (8 mL, 1 M in THF) was added. The solution was stirred for 12 hours and then diluted with ethyl acetate (100 mL), washed with ammonium chloride (2 x 20 mL), brine (20 mL) and dried over sodium sulfate. The solvent was evaporated and the residue was purified by flash chromatography on silica gel to give the title compound (2.32 g, 65% yield over two steps). [α]_D²⁰ -14.16 (*c* 1.25, CHCl₃). ¹H NMR (CDCl₃) (multiple rotamers): δ 1.42 (9H, s); 3.40~3.68 (4H, m); 2.30~1.86 (4H, m); 5.10~5.16 (1H, m); 4.67~4.77 (1H, m); 3.80 (3H, m); ¹³C NMR (CDCl₃) (multiple rotamers): δ 22.6; 28.1; 25.0; 47.4; 52.6; 59.6; 61.0; 67.1; 80.6; 154.9; 169.9; 205.2; IR (cm⁻¹): 3347, 2975, 1744, 1676, 1425, 1162. EIMS: 332, 259, 258, 215, 197, 231, 214, 170, 153, 142, 142, 136, 114. HRMS (EI): calcd. for C₁₄H₂₄N₂O₅S 332.1406, found 332.1404.

N-Boc-L-Pro-Thiazoline methyl ester (**7**)

To compound **6** (2.32 g, 7.0 mmol) in THF (10 mL) a solution of Burgess' reagent (C₈H₁₈N₂O₄S) (1.95 g, 8.13 mmol) in THF (30 mL) was added. The solution was heated under reflux for 4 hours, and then concentrated. The residue was purified by flash chromatography on silica gel to give thiazoline **7** (2.01 g, 85% yield). [α]_D²⁰ -3.99 (*c* 4.56, CHCl₃); ¹H NMR (CDCl₃) (multiple rotamers): δ 1.42 (9H, s); 1.83~2.30 (4H, m); 3.39~3.67 (4H, m); 3.80 (3H, m); 4.67 (1H, m); 5.12 (1H, m); ¹³C NMR (CDCl₃) (multiple rotamers): δ 23.8; 28.2; 32.6; 34.2; 46.6; 52.61&52.9; 67.6; 78.4; 80.1; 154.037; 170.9; 179.8&179.7; IR(cm⁻¹): 1710, 1640, 1456, 1206, 1141.

N-Boc-L-prolyl-thiazole methyl ester (**8**)

To compound **7** (1.2 g, 3.82 mmol) in dichloromethane (10 mL), a solution of activated manganese dioxide (2.1 g, 24 mmol) in dichloromethane (30 mL) was added. The reaction was monitored by tlc until the starting material was consumed. The reaction mixture

was then cooled to room temperature and the excess manganese dioxide was filtered. The solution was concentrated and the residue was purified by flash chromatography on silica gel to give thiazole **8** (1.17 g, 95% yield). $[\alpha]_{\text{D}}^{20}$ -91.78 (*c* 2.47, CHCl₃); ¹H NMR (CDCl₃) (multiple rotamers): δ 1.33&1.49 (9H, s); 1.96 (2H, m); 2.24 (2H, m); 3.63 (2H, m); 3.96 (3H, s); 8.11 (1H, s); ¹³C NMR (CDCl₃) (multiple rotamers): δ 23.0, 23.7; 28.1; 32.8&34.1; 46.9&46.6; 52.3; 59.5&59.0; 80.3; 126.9; 146.6; 154.0; 161.7; 177.1; IR (cm⁻¹): 2953, 1716, 1644, 1247, 1432, 860, 837; EIMS: 312, 281, 256, 170, 212, 153, 136, 195, 183, 157; HRMS (EI): calcd for C₁₄H₂₀N₂O₄S 312.1144, found 312.1149

N-Boc-L-Ile-L-Val-L-Pro-Thz methyl ester (**13**)

Thiazole **8** (32 mg, 0.1 mmol) was dissolved in 1:1 TFA/CH₂Cl₂ (0.5 mL) at 0 °C. The solution was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure and the residue was re-dissolved in CH₂Cl₂ and re-concentrated repeatedly to remove excess TFA. The dry sample was stored under high vacuum to give the TFA salt (**2**) which was combined with dipeptide acid **12** (35 mg, 0.1 mmol) in THF (10 mL) at 0 °C, and HOBt (27 mg, 0.2 mmol), EDCI (39 mg, 0.2 mmol) and DIPEA (0.175 ml, 0.1 mmol) were added. The reaction mixture was stirred at 0 °C for 1 hour and then at room temperature for 24 hours. Removal of the solvent *in vacuo* followed by chromatography on silica gel afforded the title compound (32 mg, 61% yield). $[\alpha]_{\text{D}}^{20}$ -60.85 (*c* 15.2, CHCl₃); ¹H NMR (CDCl₃): δ 0.86-1.02 (12H, m); 1.11&1.44 (2H, m); 1.43 (9H, s); 1.94 (1H, m); 2.05 (1H, m); 2.02-2.13 (2H, m); 2.42-2.46 (2H, m); 3.64-4.11 (2H, m); 3.93 (3H, s); 4.07 (1H, m); 4.61 (1H, dd, *J*₁ = 7.9 Hz, *J*₂ = 8.8 Hz); 5.03 (1H, d, *J* = 6.6 Hz); 5.47 (1H, dd, *J*₁ = 1.5 Hz, *J*₂ = 8.1 Hz); 6.70 (1H, d, *J* = 8.0 Hz); 8.03 (1H, s); ¹³C NMR (CDCl₃): δ 11.7; 15.8; 17.9; 19.8; 23.9; 24.4; 28.3; 30.7; 33.2; 37.2; 47.3; 52.3; 56.0; 59.0; 60.0; 79.4; 127.6; 146.5; 155.5; 161.9; 171.0; 171.6. IR (cm⁻¹): 3347, 3021, 2965, 1785, 1717, 1660, 1455, 754. EIMS: 524, 468, 451, 424, 381, 338, 296, 281, 240, 254, 212, 198, 170. HRMS(EI): calcd for C₂₅H₄₀N₄O₆S 524.2668, found 504.2681.

Aeruginosamide **1** (*cis* isomer)

N-Boc-L-Ile-L-Val-L-Pro-Thz methyl ester (**13**) (15 mg, 0.029 mmol) was dissolved in CH₂Cl₂ (1 mL) at 0 °C was added TFA (1 mL). The solution was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure and the residue was re-dissolved in CH₂Cl₂ and re-concentrated repeatedly to remove excess TFA. The residue was dissolved in DMF (5 mL) under nitrogen, followed by adding tetrabutylammonium iodide (23 mg, 0.058 mmol), 1-Bromo-3-methyl-2-butene (0.015 mL, 0.116 mmol), and Sodium bicarbonate (9.74 mg, 0.116 mmol) at 70 °C. The reaction was monitored by tlc. After consuming of the starting materials (30 minutes) and the reaction mixture was cooled back to ambient temperature. Removal of the solvent *in vacuo* followed by chromatography on silica gel afforded the *cis* isomer of aeruginosamide in nearly quantitative yield. $[\alpha]_{\text{D}}^{20}$ -68.1 (*c* 8.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 0.83 (3H, d, *J* = 6.6 Hz), 0.88 (3H, t, *J* = 7.4 Hz), 0.98 (3H, d, *J* = 6.7 Hz), 1.01 (3H, d, *J* = 6.7 Hz), 1.22 (1H, m), 1.63 (1H, m), 1.67 (6H, s), 1.70 (6H, s), 1.88 (1H, m), 2.06 (2H, m), 2.08 (1H, m), 2.22-2.31 (2H, m), 2.98 (1H, d, *J* = 6.8 Hz), 3.03 (2H, dd, *J*₁ = 7.9 Hz, *J*₂ = 14.3 Hz), 3.12 (2H, dd, *J*₁ = 4.7 Hz, *J*₂ = 14.3 Hz), 3.64-3.71 (1H, m), 3.93 (3H, s), 4.14 (1H, m), 4.62 (1H, dd, *J*₁ = 7.5 Hz, *J*₂ = 8.5 Hz), 5.13 (2H, t, *J* = 6.3 Hz), 5.47 (1H, d, *J* = 6.8 Hz), 6.76 (1H, d, *J* = 8.7 Hz), 8.01 (1H, m); ¹³C NMR (100 MHz, CDCl₃): δ 11.4, 15.8, 18.0, 18.1, 19.7, 24.1, 25.9, 26.6, 31.0, 32.1, 33.3, 47.3, 47.7, 52.3, 55.5, 59.0, 68.3, 122.2, 127.5, 134.6, 146.5, 161.9, 171.2, 172.0, 173.3.

Aeruginosamide **1** (*trans* isomer)

N-Boc-L-Ile-L-Val-L-Pro-Thz methyl ester (**13**) (15 mg, 0.029 mmol) was dissolved in CH₂Cl₂ (1 mL) at 0 °C was added TFA (1 mL). The solution was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure and the residue was re-dissolved in CH₂Cl₂ and re-concentrated repeatedly to remove excess TFA. The residue was dissolved in DMF (5 mL) under nitrogen, followed by adding tetrabutylammonium iodide (23 mg, 0.058 mmol), 1-Bromo-3-methyl-2-butene (0.015 mL, 0.116 mmol), and Sodium bicarbonate (9.74 mg, 0.116 mmol) at 70 °C. The reaction mixture was heated at 70 °C. for 18 hours. Removal of the solvent *in vacuo* followed by chromatography on silica gel afforded the *trans* isomer of aeruginosamide (15 mg, 92.4% yield). $[\alpha]_{\text{D}}^{20}$ -81.6 (*c* 8.7, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 0.83 (3H, d, *J* = 6.5 Hz), 0.84 (3H, t, *J* = 7.4 Hz), 0.93 (3H, d, *J* = 6.7 Hz), 1.0 (3H, d, *J* = 6.7 Hz), 1.18 & 1.59 (2H, m), 1.54 (6H, s), 1.66 (6H, s), 1.84 (1H, m), 2.04 (2H, m), 2.05 (1H, m), 2.22 (1H, m), 2.38 (1H, m), 2.94 (1H, d, *J* = 7.3 Hz), 3.00 (2H, dd, *J*₁ = 7.5 Hz, *J*₂ = 14.3 Hz), 3.10 (2H, dd, *J*₁ = 4.1 Hz, *J*₂ = 13.8 Hz), 3.74 & 3.85 (2H, m), 3.89 (3H, s), 4.64 (1H, dd, *J*₁ = 6.3 Hz, *J*₂ = 8.9 Hz), 5.11 (2H, t, *J* = 6.1 Hz), 5.45 (1H, dd, *J*₁ = 2.7 Hz, *J*₂ = 8.0 Hz), 6.83 (1H, d, *J* = 8.8 Hz), 8.02 (1H, s); ¹³C NMR (100 MHz, CDCl₃): δ 11.6, 16.0, 18.2, 18.2, 20.1, 24.7, 26.1, 27.0, 31.1, 31.9, 33.5, 47.8, 48.0, 52.6, 55.4, 59.1, 68.7, 122.4, 127.6, 134.6, 146.8, 162.0, 171.9, 172.7, 173.7