Supporting Information

for

Solid-phase total synthesis and structural confirmation of antimicrobial longicatenamide A

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General remarks

$^1$H and $^{13}$C NMR spectra were recorded on a JEOL ECZ600 (600 MHz for $^1$H NMR and 150 MHz for $^{13}$C NMR) spectrometer. Chemical shifts are denoted in δ (ppm) relative to residual solvent peaks as internal standard (CDCl$_3$, $^1$H δ 7.25, $^{13}$C δ 77.2, DMSO-$d_6$, $^1$H δ 2.50, $^{13}$C δ 39.5). ESI-MS and LC–MS experiments were recorded on a Shimadzu LCMS-IT-TOF. Optical rotations were recorded on a JASCO P-2200 polarimeter. High performance liquid chromatography (HPLC) experiments were performed with a SHIMADZU HPLC system equipped with an LC-20AD intelligent pump. All reactions sensitive to air and/or moisture were conducted under nitrogen atmosphere using dry, freshly distilled solvents, unless otherwise noted. All reagents were used as supplied unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F$_{254}$ pre-coated plates. Silica gel column chromatography was performed using 40–50 μm silica gel 60N (Kanto Chemical Co., Inc.).

Synthesis and compound characterization

To a solution of compound 14 (2.66 g, 6.44 mmol) in THF (10 mL) was added NaHMDS (3.1 mL, 1.9 M in THF, 5.9 mmol) at 0 °C. After being stirred at 0 °C for 1 h, compound 13 (439 mg, 1.92 mmol) in THF (10 mL) was added dropwise to the solution. The reaction mixture was stirred at room temperature for 16 h, and then quenched with saturated aqueous NH$_4$Cl. The resulting solution was extracted with hexane/EtOAc 9:1 (three times). The combined organic layer was washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. The residue was roughly purified by silica gel column chromatography (hexane/EtOAc 97:3 to 90:10) to afford crude 15, which was used in the next reaction without further purification.

To a solution of the above compound 15 in CH$_2$Cl$_2$ (13 mL) were added TFA (6 mL) and H$_2$O (1 mL) at room temperature. After being stirred for 1 h, the mixture was concentrated and azeotroped with toluene (three times) to afford crude 16, which was used in the next reaction without further purification.

To a solution of the above compound 16 in THF/H$_2$O 2:1 (15 mL) were added NaHCO$_3$ (386 mg, 4.59 mmol) and FmocCl (594 mg, 2.30 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was treated with saturated aqueous NH$_4$Cl. The resulting mixture was extracted with EtOAc (three times). The combined organic layer was washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. The residue was roughly purified by silica gel column chromatography (hexane/EtOAc 75:25 to 55:45) to afford crude 17, which was used in the next reaction without further purification.

To a solution of the above compound 17 in EtOAc (15 mL) was added Pd/C (58.6 mg) and the mixture was stirred at room temperature under H$_2$ atmosphere for 5 h. The mixture was filtered through Celite and the filtrate
was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc 65:35) to afford of compound 18 (369 mg, 1.01 mmol, 53% for 4 steps) as a white powder: \([\alpha]^{20}_{D} = -7.3 (c 0.25, \text{MeOH}); \]

\[\text{H NMR (600 MHz, CDCl}_3\text{)} \delta 7.77 (d, 2H, \text{ } J = 7.6 \text{ Hz}), 7.60 (d, 2H, \text{ } J = 7.6 \text{ Hz}), 7.40 (t, 2H, \text{ } J = 7.6 \text{ Hz}), 7.32 (t, 2H, \text{ } J = 6.9 \text{ Hz}), 4.82 (d, 1H, \text{ } J = 6.9 \text{ Hz}), 4.43 (d, 2H, \text{ } J = 6.9 \text{ Hz}), 4.21 (t, 1H, \text{ } J = 6.9 \text{ Hz}), 3.68 (d, 2H, \text{ } J = 9.0 \text{ Hz}), 3.56 (m, 1H), 1.52 (m, 2H), 1.43 (m, 1H), 1.34 (m, 2H), 1.19 (m, 2H), 0.86 (d, 6H, \text{ } J = 6.9 \text{ Hz}); \]

\[1^C \text{NMR (150 MHz, CDCl}_3\text{)} \delta 156.9, 144.0, 141.4, 127.8, 127.1, 125.1, 120.1, 66.7, 65.7, 53.4, 47.4, 38.8, 31.6, 27.9, 23.9, 22.7; \]

\[\text{HRMS (ESI) } m/z: [M+H]^+ \text{ calcld for C}_{23}H_{30}NO_3^+ 368.2220, \text{ found 368.2207}. \]

To a solution of compound 18 (227 mg, 0.617 mmol) in CHCl\(_3\) (7 mL) was added Dess–Martin periodinane (392 mg, 0.924 mmol) at room temperature. After being stirred at room temperature for 1.5 h, the reaction mixture was added saturated aqueous solutions of Na\(_2\)S\(_2\)O\(_3\) and NaHCO\(_3\). The resulting mixture was extracted with EtOAc (three times). The combined organic layer was washed with brine, dried over MgSO\(_4\), filtered, and concentrated in vacuo to afford crude 19, which was used in the next reaction without further purification.

To a solution of the above compound 19 in t-BuOH (4.5 mL) and 2-methyl-2-butene (1.5mL) were added NaClO\(_2\) (80%, 416 mg, 4.60 mmol) and NaH\(_2\)PO\(_4\)·2H\(_2\)O (372.6 mg, 2.39 mmol) in H\(_2\)O (2.25 mL) at 0 °C. After being stirred at 0 °C for 2 h, to the reaction mixture was added saturated aqueous NH\(_4\)Cl solution. The resulting mixture was extracted with EtOAc (three times). The combined organic layer was washed with saturated aqueous NH\(_4\)Cl, dried over MgSO\(_4\), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc 80:20 to 60:40) to afford compound 10 (cas: 329270-51-1, 222 mg, 0.583 mmol, 95% for 2 steps) as a white solid.

To a solution of compound 21 (2.07 g, 5.90 mmol) in CHCl\(_3\) (50 mL) were added 22 (3.35 g, 23.6 mmol) and SnCl\(_2\) (565 mg, 2.98 mmol) at 0 °C. After being stirred at 0 °C for 15 h, to the reaction mixture were added 2% aqueous KHSO\(_4\) and brine. The resulting mixture was extracted with CHCl\(_3\) (five times). The combined organic layer was washed with brine, dried over MgSO\(_4\), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc 80:20 to 50:50) to afford compound 23 (1.45 g, 3.12 mmol, 53%) as a yellow oil: \([\alpha]^{19}_{D} +22 (c 1.0, \text{MeOH}); \)

\[\text{the compound gave complex NMR data due to the existence of multiple rotamers, therefore the structure was confirmed after conversion to 25, } \]

\[\text{H NMR (600 MHz, CDCl}_3\text{)} \delta \ldots\]

S3
MHz, CDCl₃) see Figure S3; ¹³C NMR (150 MHz, CDCl₃) see Figure S4; HRMS (ESI) m/z: [M+H]⁺ calecd for C₂₇H₃₃NO₆⁺ 466.2224, found 466.2221.

To a solution of compound 23 (2.37 g, 5.09 mmol) in THF (40 mL) was added K-Selectride (10.2 mL, 1 M in THF, 10.2 mmol) at −78 °C. After being stirred at −78 °C for 17 h, to the reaction mixture was added saturated aqueous NH₄Cl. The resulting mixture was extracted with EtOAc (three times). The combined organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc 80:20 to 60:40) to afford 24 (882 mg, 1.89 mmol, 37%, brsm 76%) as a yellow oil: [α]D₂₀ +8.7 (c 1.0, MeOH); the compound gave complex NMR data due to the existence of multiple rotamers, therefore the structure was confirmed after conversion to 25. ¹H NMR (600 MHz, CDCl₃) see Figure S5; ¹³C NMR (150 MHz, CDCl₃) see Figure S6; HRMS (ESI) m/z: [M+Na]⁺ calecd for C₂₇H₃₄NO₆Na⁺ 490.2200, found 490.2195.

To a solution of compound 24 (917 mg, 1.96 mmol) in MeOH (30 mL) was added p-TsOH·H₂O (100 mg) at room temperature. After being stirred at room temperature for 9 h, to the reaction mixture was added saturated aqueous NaHCO₃. The mixture was extracted with EtOAc (three times). The combined organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc 70:30 to 30:70) to afford 25 (465 mg, 1.09 mmol, 56%) as a yellowish oil: [α]D₂₀ +8.5 (c 0.16, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.77 (d, 2H, J = 6.9 Hz), 7.61 (d, 2H, J = 6.9 Hz), 7.41(t, 2H, J = 7.6 Hz), 7.33(t, 2H, J = 7.6 Hz), 5.49(d, 1H, J = 9.6 Hz), 4.43 (d, 2H, J = 6.9 Hz), 4.33 (d, 1H, J = 10.3 Hz), 4.22 (t, 1H, J = 6.9 Hz), 3.89 (dd, 1H, J = 3.4 Hz, 11.0 Hz), 3.76 (dd, 1H, J = 4.8 Hz, 11.0 Hz), 3.66 (m, 1H), 2.49 (dd, 1H, J = 10.3 Hz, 16.9 Hz), 2.42 (dd, 1H, J = 2.7 Hz, 17.2 Hz), 1.46 (s, 9H); ¹³C NMR (150 MHz, CDCl₃) δ 172.88, 156.84, 143.97, 141.43, 127.82, 127.17, 125.17, 120.11, 82.02, 69.93, 66.95, 65.00, 54.49, 47.36, 39.10, 28.17; HRMS (ESI) m/z: [M+Na]⁺ calecd for C₂₅H₂₉NO₆Na⁺ 450.1893, found 450.1892.
To a solution of compound 25 (663 mg, 1.55 mmol) in CH₂Cl₂ (15 mL) were added 2,6-lutidine (1.07 mL, 9.33 mmol) and TBSOTf (1.07 mL, 4.66 mmol) at 0 °C. After being stirred at 0 °C for 15 min, to the reaction mixture were added MeOH and saturated aqueous NaHCO₃. The resulting mixture was extracted with EtOAc (three times). The combined organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc 95:5 to 90:10) to afford 26 (899 mg, 1.37 mmol, 88%) as a colorless oil: [α]²⁰D +3.9 (c 1.0, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.77 (d, 2H, J = 6.9 Hz), 7.60 (d, 2H, J = 7.6 Hz), 7.40 (t, 2H, J = 6.9 Hz), 7.32 (t, 2H, J = 7.6 Hz), 5.03 (d, 1H, J = 8.3 Hz), 4.47 (t, 1H, J = 6.2 Hz), 4.37 (m, 2H), 4.26 (t, 1H, J = 6.9 Hz), 3.68 (m, 2H), 3.50 (t, 1H, J = 9.6 Hz), 2.45 (m, 2H), 1.44 (s, 9H), 0.91 (m, 18H), 0.09 (m, 12H); ¹³C NMR (150 MHz, CDCl₃) δ 170.55, 156.41, 144.36, 141.65, 128.00, 127.36, 125.47, 120.31, 84.07, 67.12, 61.56, 55.94, 47.60, 41.52, 28.42, 26.26, 18.50, 4.17, 4.61, –4.98, –5.07; HRMS (ESI) m/z: [M+H]+ calcld for C₃₆H₅₈NO₆Si₂ 656.3797, found 656.3796.

To a solution of 26 (158 mg, 0.241 mmol) in MeOH/CH₂Cl₂ (1:1, 6 mL) was added p-TsOH·H₂O (14.7 mg) at room temperature. After being stirred at room temperature for 80 min, the reaction mixture was added with Et₃N (100 µL). The mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography (hexane/EtOAc = 80:20) to afford 27 (88.2 mg, 0.163 mmol, 68%) as a colorless oil: [α]²⁰D +9.5 (c 1.00, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.77 (d, 2H, J = 7.6 Hz), 7.60 (d, 2H, J = 7.6 Hz), 7.41 (t, 2H, J = 7.6 Hz), 7.32 (t, 2H, J = 6.9 Hz), 5.24 (d, 1H, J = 8.3 Hz), 4.40 (m, 3H), 4.25 (t, 1H, J = 6.2 Hz), 3.80 (br, 1H), 3.70 (br, 1H), 3.63 (br, 1H), 2.63 (s, 1H), 2.48 (m, 2H), 1.45 (br, 9H), 0.92 (br, 9H), 0.14 (br, 6H); ¹³C NMR (150 MHz, CDCl₃) δ 170.54, 156.92, 143.98, 141.41, 127.81, 127.16, 125.19, 120.10, 81.24, 67.84, 67.00, 62.96, 56.24, 47.32, 40.79, 28.19, 25.88, 18.12, –4.39, –4.89; HRMS (ESI) m/z: [M+H]⁺ calcld for C₃₀H₄₄NO₆Si⁺ 542.2932, found 542.2933.

To a solution of compound 27 (454 mg, 0.838 mmol) in CH₂Cl₂ (10 mL) was added Dess–Martin
periodinane (535 mg, 1.26 mmol) at room temperature. After being stirred at room temperature for 1 h, to the reaction mixture were added saturated aqueous solutions of Na₂S₂O₃ and NaHCO₃. The resulting mixture was extracted with EtOAc (three times). The combined organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo to afford crude aldehyde, which was used in the next reaction without further purification.

To a solution of the above aldehyde in t-BuOH (6 mL) and 2-methyl-2-butene (2 mL) were added NaClO₂ (80%, 568 mg, 5.03 mmol) and NaH₂PO₄·2H₂O (471 mg, 3.02 mmol) in H₂O (3 mL) at 0 °C. After being stirred at 0 °C for 2 h, to the reaction mixture was added saturated aqueous NH₄Cl. The resulting mixture was extracted with EtOAc (three times). The combined organic layer was washed with saturated aqueous NH₄Cl, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (CHCl₃/MeOH 99:1) to afford 7 (460 mg, 0.828 mmol, 99%) as a colorless oil: [α]₂₀° +6.6 (c 0.50, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.77 (d, 2H, J = 7.6 Hz), 7.61 (t, 2H, J = 8.3 Hz), 7.40 (t, 2H, J = 6.9 Hz), 7.32 (t, 2H, J = 6.9 Hz), 5.50 (d, 1H, J = 8.9 Hz), 4.75 (t, 1H, J = 5.5 Hz), 4.54 (d, 1H, J = 8.3 Hz), 4.44 (m, 1H), 4.36 (m, 1H), 4.27 (t, 1H, J = 7.6 Hz), 2.55 (dd, 1H, J = 6.9 Hz, 16.5 Hz), 2.42 (dd, 1H, 6.2 Hz, 16.5 Hz), 1.45 (s, 9H), 0.89 (s, 9H), 0.12 (s, 6H); ¹³C NMR (150 MHz, CDCl₃) δ 169.77, 156.49, 143.99, 143.16, 141.39, 127.86, 127.23, 125.30, 120.12, 81.61, 69.28, 67.47, 47.20, 40.05, 28.19, 25.81, 18.02, −4.59, −5.00; HRMS (ESI) m/z: [M+H]⁺ calcd for C₃₀H₄₂NO₇Si 556.2725, found 556.2726.

Procedures for the solid-phase peptide synthesis (SPPS).

Step 1: To the solution of compound 6–11 (3 equiv) were added DIC (3 equiv, 0.50 M in NMP) and Oxyma (3 equiv, 0.50 M in DMF). After approximately 2–3 min of preactivation, the mixture was injected to the reaction vessel. The resulting mixture was stirred at 40 °C for 20 min, under microwave irradiation.

Step 2: The resin in the reaction vessel was washed with DMF (× 3) and CH₂Cl₂ (× 3).

Step 3: Fmoc group of the solid supported peptide was removed with 20% piperidine/DMF solution (8 min, room temperature).

Step 4: The resin in the reaction vessel was washed with DMF (× 3) and CH₂Cl₂ (× 3).

Amino acids were condensed onto the solid support by repeating steps 1–4.

2-Chlorotrityl resin (41.5 mg, 0.0647 mmol) in Biotage PP-reactor 2 mL with PTFE frit was swollen with CH₂Cl₂, and then excess solvent was removed by filtration. To the resin were added a solution of Fmoc-D-Trp-OH (6, 27.8 mg, 0.0528 mmol) and iPr₂NEt (26 µL, 0.15 mmol) in CH₂Cl₂ (0.23 mL), and stirred for 2 h. The reaction mixture was filtered, washed with DMF (× 3), CH₂Cl₂ (× 3), (CH₂Cl₂/MeOH/iPr₂NEt = 17:2:1) (× 3), DMF (× 3), CH₂Cl₂ (× 3) to afford compound 28.

The resin-bound D-Trp 28 was subjected to the SPPS protocol (steps 3 and 4), and then 5 cycles of the SPPS
protocol to afford resin-bound peptide 5.

To resin-bound peptide 5 was added TFA/CH₂Cl₂ 1:99 (0.5 mL). After being stirred for 30 min, the reaction mixture was filtered. This procedure was repeated twice. The filtrate was concentrated in vacuo and azeotroped with toluene (× 3) to afford crude peptide 30, which was used in the next reaction without further purification.

To a solution of peptide 30 in CH₂Cl₂/DMF 9:1 (40 mL) were added 2,4,6-collidine (53 μL, 0.499 mmol), HOAt (29.95 mg, 0.220 mmol), and PyBOP (106 mg, 0.204 mmol) at room temperature. After being stirred overnight, the reaction mixture was concentrated in vacuo, and the residue was diluted with EtOAc and saturated aqueous NH₄Cl. The mixture was extracted with EtOAc (three times). The combined organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. To the residue was added a mixture of TFA/iPr₃SiH/H₂O 95:2.5:2.5 (2 mL). After being stirred for 3 h, the reaction mixture was diluted with Et₂O (48 mL), centrifuged at 3500g for 5 min at 4 °C, and the Et₂O layer was removed by decantation. This procedure was repeated twice. The obtained crude 1 was purified by reversed-phase HPLC (PEGASIL ODS SP100 ø 20 × 250 mm, MeCN/H₂O/TFA 35:65:0.05, 19 mL/min, room temperature) to afford peptide 1 (13.3 mg, 36% for 15 steps) as a light orange powder: [α]₂₀° +8.3 (c 0.1, MeOH); ¹H NMR (600 MHz, DMSO-d₆) δ 10.8 (s, 1H), 8.55 (d, 1H, J = 5.5 Hz), 8.16 (d, 1H, J = 7.6 Hz), 7.99 (d, 1H, J = 6.2 Hz), 7.95 (d, 1H, J = 5.5 Hz), 7.70 (br, 1H), 7.68 (br, 1H), 7.50 (d, 1H, J = 7.6 Hz), 7.29 (d, 1H, J = 8.3 Hz), 7.16 (s, 1H), 7.04 (t, 1H, J = 7.6 Hz), 6.97 (t, 1H, J = 7.6 Hz), 4.39 (dt, 1H, J = 4.8 Hz, 9.6 Hz), 4.28 (m, 1H), 4.25 (m, 1H), 4.18 (t, 1H, J = 6.2 Hz), 4.02 (t, 1H, J = 7.6 Hz), 3.99 (br, 1H), 3.73 (dd, 1H, J = 4.1 Hz, 22.4 Hz), 3.66 (dd, 1H, J = 4.1 Hz, 17.2 Hz), 3.26 (dd, 1H, J = 4.1 Hz, 14.5 Hz), 2.90 (dd, 1H, J = 9.6 Hz, 14.1 Hz), 2.79 (br, 2H), 2.18 (m, 1H), 2.00 (m, 1H), 1.98 (m, 1H), 1.62 (m, 2H), 1.50 (m, 2H), 1.48 (br, 1H), 1.47 (br, 2H), 1.28 (m, 1H), 1.16 (m, 1H), 1.11 (m, 1H), 0.86 (t, 6H, J = 6.2 Hz), 0.83 (d, 6H, J = 6.2 Hz); ¹³C NMR (150 MHz, DMSO-d₆) δ 172.51, 171.27, 171.12, 170.37, 170.16, 168.32, 136.23, 126.94, 123.87, 121.02, 118.42, 118.09, 111.52, 110.01, 67.13, 58.34, 58.00, 54.04, 53.27, 52.31, 42.60, 38.64, 38.00, 31.36, 31.09, 29.37, 27.48, 26.82, 23.55, 23.17, 22.55, 22.48, 19.51; HRMS (ESI) m/z: [M+H]⁺ calcd for C₃₇H₅₅N₈O₉⁺ 743.4087, found 743.4087.
NMR spectra of new compounds

**Figure S1:** $^1$H NMR spectrum of 18 in CDCl$_3$ (600 MHz).

**Figure S2:** $^{13}$C NMR spectrum of 18 in CDCl$_3$ (150 MHz).
Figure S3: $^1$H NMR spectrum of 23 (mixture of the rotamer) in CDCl$_3$ (600 MHz).

Figure S4: $^{13}$C NMR spectrum of 23 (mixture of the rotamer) in CDCl$_3$ (150 MHz).
Figure S5: $^1$H NMR spectrum of 24 in CDCl$_3$ (600 MHz).

Figure S6: $^{13}$C NMR spectrum of 24 in CDCl$_3$ (150 MHz).
Figure S7: $^1$H NMR spectrum of 25 in CDCl$_3$ (600 MHz).

Figure S8: $^{13}$C NMR spectrum of 25 in CDCl$_3$ (150 MHz).
**Figure S9:** $^1$H NMR spectrum of 26 in CDCl$_3$ (600 MHz).

**Figure S10:** $^{13}$C NMR spectrum of 26 in CDCl$_3$ (150 MHz).
**Figure S11**: $^1$H NMR spectrum of 27 in CDCl$_3$ (600 MHz).

**Figure S12**: $^{13}$C NMR spectrum of 27 in CDCl$_3$ (150 MHz).
Figure S13: $^1$H NMR spectrum of 7 in CDCl$_3$ (600 MHz).

Figure S14: $^{13}$C NMR spectrum of 7 in CDCl$_3$ (150 MHz).
NMR spectra of 1

Figure S15: $^1$H NMR spectrum of 1 in DMSO-$d_6$ (600 MHz).

Figure S16: $^{13}$C NMR spectrum of 1 in DMSO-$d_6$ (150 MHz).
Figure S17: HSQC spectrum of 23 (mixture of the rotamer) in CDCl₃ (150 MHz).

Figure S18: HMBC spectrum of 23 (mixture of the rotamer) in CDCl₃ (150 MHz).