Supporting Information for

Chemical Synthesis and Biological Activity of Analogues of the Lantibiotic Epilancin 15X

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General information.

Materials, reactions and purification: Standard Fmoc-amino acids and resins for solid-phase peptide synthesis (SPPS), amino acids for solution-phase synthesis, D-lactic acid and peptide coupling reagents 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEBPT), N,N-diisopropyl-carbodiimide (DIC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 1-hydroxy-7-azabenzotriazole (HOAt) and 1-hydroxybenzotriazole monohydrate (HOBt) were purchased from Chem-Impex International. 7-Azabenzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyAOP) was purchased from AAPPTec. Dimethylformamide (DMF), dichloromethane and tetrahydrofuran (THF) were purchased at reaction grade from Fisher Scientific and dried via a solvent dispensing system prior to use. Flow cytometry dyes 3,3′-diethyloxacarboxycyanine iodide (DiOC2(3)) and propidium iodide (PI) were purchased from Invitrogen. Other chemical reagents and solvents were purchased from Sigma Aldrich or Alfa Aesar and used without further purification. All reactions were run under an atmosphere of N2 unless otherwise stated. Reaction progress and chromatography fractions were monitored by thin layer chromatography (TLC) on silica-gel-coated glass plates with a F254 fluorescent indicator. Visualization was achieved by UV absorption by fluorescence quenching or permanganate stain (1.5 g KMnO4, 10 g K2CO3, 1.25 mL 10% NaOH in 200 mL of H2O). Flash chromatography was performed using Silicycle SiliaFlash P60, 230-400 mesh silica gel. Analytical reversed-phase high-performance liquid chromatography (RP-HPLC) was performed on an Agilent 1260 Infinity system with a Phenomenex Jupiter C12 analytical column with a flow rate of 1 mL/min and a solvent gradient of 2-100% solvent B over 45 min. Preparatory RP-HPLC was performed on a Waters 600 system with a Phenomenex Jupiter C12 preparative column with a flow rate of 10 mL/min and solvent gradients as described for each peptide. All HPLC solvents were filtered with a Millipore filtration system equipped with a 0.22 μm nylon membrane filter prior to use. HPLC solvent compositions: solvent A is 0.1% trifluoroacetic acid (TFA) in H2O; solvent B is 80:20 MeCN/H2O with 0.087% TFA.

Characterization: NMR spectra were recorded on a Varian Unity 400 or Unity Inova 500 spectrometer. Small molecules (MW < 1000 Da) were analyzed by electrospray ionization/time-of-flight (ESI-TOF) mass spectrometry on a Waters Quattro II quadrupole spectrometer. Peptides (MW > 800 Da) were analyzed by matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry on a Bruker Daltonics UltrafleXtreme TOF/TOF spectrometer using a matrix solution consisting of saturated α-cyano-4-hydroxycinnamic acid in 1:1:0.1 H2O/MeCN/TFA.
Compound 25: d-Serine (24, 2.10 g, 20.0 mmol) and sodium carbonate (3.18 g, 30.0 mmol) were dissolved in water (30 mL) and MeCN (15 mL) and chilled in an ice bath. A solution of allyl chloroformate (AlocCl, 2.1 mL, 20.0 mmol) in MeCN (15 mL) was added dropwise. The reaction was stirred for 8 h, gradually warming to room temperature. The reaction was concentrated under reduced pressure, then taken up in DMF (50 mL). Sodium bicarbonate (1.68 g, 20.0 mmol) was added, followed by allyl bromide (AllBr, 3.5 mL, 40.0 mmol). The reaction was stirred as a heterogeneous mixture under N\textsubscript{2} for 15 h. The reaction was concentrated under reduced pressure, then partitioned between water and EtOAc. The organic layer was separated, washed with saturated aqueous NaHCO\textsubscript{3}, 0.1 M KHSO\textsubscript{4} and brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated. Excess 4:1 hexane/EtOAc was added to precipitate phosphine oxide byproducts, which were removed via filtration through Celite. The filtrate was concentrated and purified by flash chromatography (SiO\textsubscript{2}, 2:1 hexane/EtOAc) to yield 25 (2.67 g, 11.7 mmol, 59\%) as a colorless oil. \textit{R}\textsubscript{f} 0.32 (1:1 hexane/EtOAc). Spectral data match those reported previously.\textsuperscript{1}

Compound 26: Compound 25 (2.00 g, 8.73 mmol) and carbon tetrabromide (3.47 g, 10.5 mmol) were dissolved in CH\textsubscript{2}Cl\textsubscript{2} (25 mL) and chilled in an ice bath. Triphenylphosphine (2.75 g, 10.5 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) and added dropwise to the chilled solution. The reaction was warmed to room temperature and stirred for 2.5 h, then washed with water and brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and concentrated under reduced pressure. Excess 4:1 hexane/EtOAc was added to precipitate phosphine oxide byproducts, which were removed via filtration through Celite. The filtrate was concentrated and purified by flash chromatography (SiO\textsubscript{2}, 5:1 then 4:1 hexane/EtOAc) to yield 26 (1.98 g, 6.78 mmol, 78\%) as a colorless oil. \textit{R}\textsubscript{f} 0.50 (3:1 hexane/EtOAc). Spectral data match those reported previously.\textsuperscript{1}

Compound 28: Synthesis was performed in two steps from l-cystine (27) as we have reported previously.\textsuperscript{2}
Compound 29: Tributylphosphine (410 μL, 1.64 mmol) was added to a solution of 28 (1.09 g, 1.37 mmol) in THF (15 mL) and stirred for 15 min. Water (1.5 mL) was added, and the reaction was stirred an additional 2.5 h, and then concentrated under reduced pressure. To the resulting oil was added 26 (0.80 g, 2.74 mmol) in N₂-sparged EtOAc (15 mL). Tetrabutylammonium bromide (3.55 g, 10.0 mmol) was dissolved in N₂-sparged 0.5 M aqueous NaHCO₃ (pH adjusted to 8.5, 15 mL), then added to the organic solution. The biphasic mixture was stirred for 18 h, then washed with water and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO₂, 4:1 hexane/EtOAc) to yield 29 (1.29 g, 2.11 mmol, 77% over two steps) as a colorless oil. Rₐ 0.28 (3:1 hexane/EtOAc). Spectral data match those reported previously.

Compound 2: To a solution of 29 (1.80 g, 2.95 mmol) in CH₂Cl₂ (15 mL) was added phenylsilane (400 µL, 3.24 mmol), followed by TFA (15 mL). The reaction was stirred for 2 h, then concentrated under reduced pressure to yield 2 (1.64 g, quant.) as a white solid after lyophilization from 1:1 benzene/MeCN. Rₐ 0.20 (25:1:0.1 CH₂Cl₂/MeOH/AcOH). Spectral data matched those reported previously.

Allyl-protected methyllanthionine building block 3

Compound 30: To a stirring suspension of N-Fmoc-L-serine (5, 8.19 g, 25.0 mmol) in EtOAc (125 mL) was added tert-butyl 2,2,2-trichloroacetimidate (8.95 mL, 50.0 mmol) in cyclohexane (50 mL) by an addition funnel over 15 min. The reaction was stirred for 18 h, then washed with saturated aqueous NaHCO₃, water, and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO₂, 3:1 hexane/EtOAc) to yield 30 (8.90 g, 23.2 mmol, 93%) as a white solid. Rₐ 0.58 (1:1 hexane/EtOAc). Spectral data match those previously reported.
Compound 6: Compound 30 (2.00 g, 5.22 mmol) and carbon tetrabromide (2.08 g, 6.26 mmol) were dissolved in CH₂Cl₂ (10 mL) and chilled in an ice bath. Triphenylphosphine (1.64 g, 6.26 mmol) was dissolved in CH₂Cl₂ (10 mL) and added dropwise to the chilled solution. The reaction mixture was warmed to room temperature and stirred for 2.5 h, then washed with water and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Excess 5:1 hexane/EtOAc was added to precipitate phosphine oxide byproducts, which were removed via filtration through Celite. The filtrate was concentrated and purified by flash chromatography (SiO₂, 15% EtOAc/hexane) to yield 6 (1.83 g, 4.10 mmol, 79%) as an amber oil. R<sub>f</sub> 0.56 (3:1 hexane/EtOAc). Spectral data match those reported previously.<sup>4</sup>

Compound 31: D-Threonine (7, 4.17 g, 35.0 mmol) and para-toluenesulfonic acid monohydrate (7.99 g, 42.0 mmol) were combined in toluene (90 mL). Allyl alcohol (AllOH, 24 mL, 350 mmol) was added, and the reaction was refluxed in an oil bath (110 °C) connected to a Dean-Stark apparatus for 15 h, then concentrated under reduced pressure and dried azeotropically with benzene. The residue was taken up in CH₂Cl₂ (175 mL) and chilled in an ice bath. Triethylamine (14.6 mL, 105 mmol) was added, and the reaction was allowed to stir for 10 min. 4-Nitrobenzenesulfonyl chloride (NsCl, 8.53 g, 38.5 mmol) was added portionwise as a solid, and the reaction was stirred for 4 h at 0 °C. The reaction mixture was washed with 1 M NaH₂PO₄, saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash chromatography (SiO₂, 7:3 then 3:2 hexane/EtOAc) to yield 31 (9.48 g, 27.5 mmol, 79% over two steps) as a yellow solid. R<sub>f</sub> 0.43 (1:1 hexane/EtOAc). Spectral data match those reported previously.<sup>5</sup>

Compound 32: A solution of 31 (2.70 g, 7.84 mmol) and triphenylphosphine (2.67 g, 10.2 mmol) in THF (30 mL) was chilled in an ice bath. Diisopropylazodicarboxylate (DIAD, 1.7 mL, 8.63 mmol) was added dropwise, and the reaction was allowed to stir for 10 min. The reaction mixture was washed with 1 M NaH₂PO₄, saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was taken up in EtOAc, washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash chromatography (SiO₂, 5:1 hexane/EtOAc) to yield 32 (2.13 g, 6.53 mmol, 83%) as a yellow solid. R<sub>f</sub> 0.54 (2:1 hexane/EtOAc). Spectral data match those reported previously.<sup>5</sup>

Compound 33: A solution of 32 (0.65 g, 2.00 mmol) and 4-methoxybenzyl mercaptan (MobSH, 1.12 mL, 8.00 mmol) in CH₂Cl₂ (20 mL) was chilled in an ice bath. Boron trifluoride diethyl etherate (0.74 mL, 6.00 mmol) was added dropwise to the stirring solution. The reaction was warmed to room temperature and stirred for 21 h at 4 °C, then washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO₂, 4:1 hexane/EtOAc) to yield 33 (0.82 g, 1.71 mmol, 86%) as a yellow solid. R<sub>f</sub> 0.47 (2:1 hexane/EtOAc). Spectral data match those reported previously.<sup>5</sup>
Compound 8: 4-Methoxybenzene thiol (PMP-SH, 1.84 mL, 15.0 mmol) and potassium carbonate (2.76 g, 20.0 mmol) were added to a stirring solution of 33 (2.40 g, 5.00 mmol) in 49:1 MeCN/dimethylsulfoxide (35 mL). The reaction was stirred as a heterogeneous mixture for 3 h, then concentrated under reduced pressure. The residue was taken up in EtOAc, washed with water and brine, dried over Na₂SO₄, filtered, and concentrated. The crude mixture was purified by flash chromatography (SiO₂, 3:2 then 2:3 hexane/EtOAc) to yield 8 (1.35 g, 4.57 mmol, 91%) as a colorless oil. Rf 0.30 (1:1 hexane/EtOAc). Spectral data match those reported previously.

Compound 9: Diisopropylethylamine (0.74 mL, 4.26 mmol) and allyloxy-carbonyloxy-succinimide (AlocOSu, 0.74 g, 3.73 mmol) were added to a solution of 8 (1.05 g, 3.55 mmol) in CH₂Cl₂ (20 mL) and the reaction was stirred for 12 h. The reaction was washed with water, 10% citric acid and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO₂, 4:1 hexane/EtOAc) to yield 9 (1.31 g, 3.45 mmol, 97%) as a colorless oil. Rf 0.58 (2:1 hexane/EtOAc). ¹H NMR (500 MHz, CDCl₃) δ 7.20 (dd, J = 9.5 Hz, 2.5 Hz, 2H), 6.84 (dd, J = 9.5 Hz, 2.5 Hz, 2H), 5.95-5.84 (m, 2H), 5.50 (d, J = 9.0 Hz, 1H), 5.37-5.20 (m, 4H), 4.66 (dd, J = 13.0 Hz, 6.0 Hz, 1H), 4.59-4.56 (m, 2H), 4.53 (dd, J = 9.5 Hz, 3.5 Hz, 1H), 3.79 (s, 3H), 3.67 (d, J = 6.0 Hz, 1H), 3.64 (d, J = 13.0 Hz, 1H), 3.31 (m, 1H), 1.30 (d, J = 7.0 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 170.6, 158.9, 156.4, 132.7, 131.5, 130.1, 129.7, 119.3, 118.0, 114.1, 66.4, 66.1, 58.6, 55.4, 42.2, 35.2, 19.7. HRMS (ESI) calc. for C₁₀H₁₆O₃S 380.1532, found 380.1532.

Compound 10: Compound 9 (0.68 g, 1.80 mmol) was dissolved in TFA (10 mL) and anisole (780 μL, 7.20 mmol). Mercury(II) acetate (1.15 g, 3.60 mmol) was added as a solid, and the purple solution was stirred for 4 h. Dithiothreitol (DTT, 0.56 g, 3.60 mmol) was then added, forming a grey precipitate. This heterogeneous mixture was stirred vigorously for 15 h, then diluted with CH₂Cl₂ and centrifuged (4600 ×g, 10 min) to remove the solids. The supernatant was concentrated under reduced pressure, taken up in CH₂Cl₂ and water, and neutralized by slow addition of saturated aqueous NaHCO₃ to pH 7. The organic layer was separated, dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash chromatography (SiO₂, 15% EtOAc/hexane) to yield 34, which was used directly for the next reaction without complete concentration or characterization due to its instability. Rf 0.52 (3:1 hexane/EtOAc). To the partially-concentrated 34 was added 6 (0.54 g, 1.20 mmol) and N₂-sparged EtOAc (6 mL). Tetrabutylammonium bromide (1.55 g, 4.80 mmol) was dissolved in N₂-sparged 0.5 M aqueous NaHCO₃ (pH adjusted to 8.5, 6 mL), then added to the organic solution. The biphasic reaction was stirred for 5 h, and the pH was adjusted to 8.5 as necessary with 1 M NaOH. Tributylphosphine (150 μL, 0.60 mmol) was added, and the reaction was stirred for an additional 17 h. The organic layer was isolated, washed with water and brine, dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash chromatography (SiO₂, 4:1 hexane/EtOAc) to yield 10 (0.48 g, 0.77 mmol, 64% over two steps) as a colorless foam. Rf 0.34 (3:1 hexane/EtOAc). ¹H NMR (500 MHz, CDCl₃) δ 7.77 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 5.95-5.88 (m, 2H), 5.62 (d, J = 7.5 Hz, 1H), 5.57 (d, J = 9.5 Hz, 1H), 5.37-5.20 (m, 4H), 4.70-4.53 (m, 5H), 4.48-4.37 (m, 3H), 4.24 (t, J
= 7.0 Hz, 1H), 3.43 (m, 1H), 3.02-2.89 (m, 2H), 1.48 (s, 9H), 1.34 (d, J = 7.0 Hz, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.4, 169.4, 156.4, 155.8, 144.0, 143.9, 141.4, 132.6, 131.4, 127.9, 127.2, 125.2, 120.1, 119.6, 118.1, 83.3, 67.3, 66.5, 66.2, 58.5, 54.3, 47.2, 44.0, 34.2, 29.8, 28.1, 19.8. HRMS (ESI) calc. for C$_{33}$H$_{40}$N$_2$O$_8$SNa 647.2403, found 647.2405.

Compound 3: To a solution of 10 (0.45 g, 0.72 mmol) in CH$_2$Cl$_2$ (3 mL) was added phenylsilane (95 µL, 0.76 mmol), followed by TFA (3 mL). The reaction was stirred for 2 h, concentrated under reduced pressure and repeatedly redissolved in CH$_2$Cl$_2$ and concentrated to remove residual TFA. The crude material was purified by flash chromatography (SiO$_2$, 1%-2% MeOH/CH$_2$Cl$_2$) to yield 3 (0.39 g, 0.69 mmol, 96%) as a white solid after lyophilization from 1:1 benzene/MeCN. $R_f$ 0.05 (2:1 EtOAc/hexane). $^1$H NMR (500 MHz, CD$_3$OD) δ 7.80 (d, J = 7.5 Hz, 2H), 7.69 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 5.99 - 5.88 (m, 2H), 5.38 - 5.29 (m, 2H), 5.24 - 5.16 (dd, J = 22.5 Hz, 10.5 Hz, 2H), 4.65 (m, 2H), 4.55 (d, J = 5.5 Hz, 2H), 4.47 (d, J = 4.0 Hz, 1H), 4.41 - 4.30 (m, 3H), 4.25 (t, J = 7.0 Hz, 1H), 3.45 (m, 1H), 3.08 (dd, J = 13.5 Hz, 4.5 Hz, 1H), 2.83 (dd, J = 13.5 Hz, 8.5 Hz, 1H), 1.31 (d, J = 7.0 Hz, 3H). $^{13}$C NMR (125 MHz, CD$_3$OD) δ 173.7, 171.7, 158.6, 158.4, 145.3, 145.2, 142.6, 142.5, 134.2, 133.1, 128.8, 128.2, 126.3, 120.9, 119.2, 117.8, 68.2, 67.2, 66.8, 60.2, 55.3, 48.4, 43.8, 34.2, 19.8. HRMS (ESI) calc. for C$_{29}$H$_{33}$N$_2$O$_8$S 569.1958, found 569.1959.

Nitrobenzyl-protected methylanthionine building block 4

Compound 35: Diisopropylethylamine (1.4 mL, 8.1 mmol) and 4-nitrobenzyl chloroformate (pNzCl, 1.46 g, 6.77 mmol) were added to a solution of 8 (2.00 g, 6.77 mmol) in CH$_2$Cl$_2$ (35 mL), and the reaction was stirred for 16 h. The reaction was washed with water, 10% citric acid and brine, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO$_2$, 4:1 hexane/EtOAc) to yield 35 (3.10 g, 6.53 mmol, 96%) as a colorless oil. $R_f$ 0.50 (2:1 hexane/EtOAc). $^1$H NMR (500 MHz, CDCl$_3$) δ 8.21 (d, J = 9.0 Hz, 2H), 7.51 (d, J = 9.0 Hz, 2H), 7.19 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2.0 Hz, 2H), 5.91-5.83 (m, 1H), 5.62 (d, J = 9.0 Hz, 1H), 5.36-5.32 (dd, J = 17.0 Hz, 1.0 Hz, 1H), 5.27 (dd, J = 10.5 Hz, 1.0 Hz, 1H), 5.23 (d, J = 13.5 Hz, 1H), 5.19 (d, J = 13.5 Hz, 1H), 4.63 (dd, J = 13.0 Hz, 6.0 Hz, 1H), 4.58 (dd, J = 13.0 Hz, 6.0 Hz, 1H), 4.53 (dd, J = 9.0 Hz, 3.5 Hz, 1H), 3.78 (s, 3H), 3.67 (d, J = 13.0 Hz, 1H), 3.63 (d, J = 13.0 Hz, 1H), 3.33 (m, 1H), 1.30 (d, J = 7.5 Hz, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.3, 158.9, 156.1, 147.7, 143.8,
Compound 11: Tetrakis(triphenylphosphine)palladium(0) (390 mg, 0.34 mmol) was added to a solution of 35 (3.20 g, 6.74 mmol) and N-methylaniline (1.5 mL, 13.5 mmol) in THF (60 mL). The reaction was stirred for 1.5 h, protected from light, then concentrated under reduced pressure. The resulting oil was taken up in DMF (25 mL). Sodium bicarbonate (1.13 g, 13.5 mmol) and 4-nitrobenzyl bromide (3.65 g, 16.9 mmol) were added as solids. The reaction was stirred for 30 h, with additional 4-nitrobenzyl bromide (pNbBr, 1.46 g, 6.74 mmol) added after 11 h. The reaction mixture was concentrated, taken up in EtOAc, washed with saturated aqueous NaHCO₃, water and brine, dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash chromatography (SiO₂, 30% EtOAc/hexane) to yield 11 (3.55 g, 6.23 mmol, 93% over two steps) as an amber foam. \( R_f 0.38 \) (2:1 hexane/EtOAc). \(^1\)H NMR (500 MHz, CDCl₃) \( \delta 8.19 \) (app. t, \( J = 8.5 \) Hz, 4H), 7.50 (d, \( J = 8.5 \) Hz, 2H), 7.45 (d, \( J = 9.0 \) Hz, 2H), 7.13 (d, \( J = 8.5 \) Hz, 2H), 6.78 (dt, \( J = 8.5 \) Hz, 3H), 5.60 (d, \( J = 9.0 \) Hz, 1H), 5.28-5.14 (m, 4H), 4.59 (dd, \( J = 9.0 \) Hz, 3.5 Hz, 1H), 3.75 (s, 3H), 3.64 (d, \( J = 13.0 \) Hz, 1H), 3.59 (d, \( J = 13.0 \) Hz, 1H), 3.33 (m, 1H), 1.33 (d, \( J = 7.0 \) Hz, 3H). \(^{13}\)C NMR (125 MHz, CDCl₃) \( \delta 170.3, 159.0, 156.0, 147.9, 147.8, 143.6, 142.2, 129.9, 129.3, 128.7, 128.2, 123.93, 123.89, 114.1, 66.0, 65.8, 58.8, 55.3, 41.7, 35.0, 19.4. \) HRMS (ESI) calc. for C_{27}H_{28}N_{2}O_{3}S 570.1546, found 570.1566.

Compound 36: Compound 11 (0.85 g, 1.50 mmol) was dissolved in TFA (6 mL) and anisole (650 μL, 6.00 mmol). Mercury(II) acetate (0.96 g, 3.00 mmol) was added, and the purple solution was stirred for 4 h. Dithiothreitol (0.46 g, 3.00 mmol) was added, immediately forming a grey precipitate. This heterogeneous mixture was stirred vigorously for 15 h, then diluted with CH₂Cl₂ and centrifuged (4600 xg, 10 min) to remove the solids. The supernatant was concentrated under reduced pressure, taken up in CH₂Cl₂ and water, and neutralized by slow addition of saturated aqueous NaHCO₃ to pH 7. The organic layer was separated, dried over Na₂SO₄, filtered, and concentrated. The crude material was purified by flash chromatography (SiO₂, 3:1 hexane/EtOAc) to yield 36, which was used directly for the next reaction. \( R_f 0.60 \) (1:1 hexane/EtOAc). \(^1\)H NMR (500 MHz, CDCl₃) \( \delta 8.23 \) (app. t, \( J = 8.5 \) Hz, 4H), 7.53 (d, \( J = 8.5 \) Hz, 4H), 5.66 (d, \( J = 9.0 \) Hz, 1H), 5.34-5.21 (m, 4H), 4.66 (dd, \( J = 9.0 \) Hz, 3.0 Hz, 1H), 3.65 (m, 1H), 1.68 (bs, 1H), 1.40 (d, \( J = 7.0 \) Hz, 3H). \(^{13}\)C NMR (125 MHz, CDCl₃) \( \delta 170.1, 156.2, 148.3, 143.5, 142.1, 128.8, 128.3, 124.1, 124.0, 66.2, 66.0, 59.8, 37.4, 22.1. \) HRMS (ESI) calc. for C_{19}H_{20}N_{3}O_{3}S 450.0971, found 450.0982.

Compound 12: Compounds 6 (0.45 g, 1.00 mmol) and 36 (assumed 1.50 mmol) were dissolved in N₂-sparged EtOAc (5 mL). Tetrabutylammonium bromide (1.29 g, 4.00 mmol) was dissolved in N₂-sparged 0.5 M aqueous NaHCO₃ (pH adjusted to 8.5, 5 mL), then added to the organic solution. The biphasic mixture was stirred under N₂ for 7 h, and the pH was adjusted to 8.5 as necessary with 1 M NaOH. Tributylphosphine (125 μL, 0.50 mmol) was added, and the reaction was stirred for an additional 17 h. The organic layer was isolated, washed with water and brine, dried over Na₂SO₄, filtered, and concentrated. The
crude material was purified by flash chromatography (SiO₂, 4:1 then 2:1 hexane/EtOAc) to yield **12** (0.69 g, 0.85 mmol, 85% over two steps) as a colorless foam. R_f 0.24 (2:1 hexane/EtOAc). 

**1H NMR** (500 MHz, CDCl₃) δ 8.17 (app. t, J = 8.5 Hz, 4 H), 7.75 (d, J = 7.5 Hz, 2H), 7.56 (m, 2H), 7.47 (app. d, J = 7.0 Hz, 4H), 7.39 (dt, J = 7.5 Hz, 3.0 Hz, 2H), 7.29 (t, J = 7.5 Hz, 2H), 5.78 (d, J = 9.0 Hz, 1H), 5.66 (d, J = 7.0 Hz, 1H), 5.34-5.15 (m, 4H), 4.57 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 4.44 (m, 1H), 4.34 (d, J = 7.0 Hz, 2H), 4.19 (t, J = 7.0 Hz, 1H), 3.48 (m, 1H), 3.01 (dd, J = 13.5 Hz, 4.5 Hz, 1H), 2.88 (dd, J = 13.5 Hz, 8.5 Hz, 1H), 1.47 (s, 9H), 1.35 (d, J = 7.0 Hz, 3H). 

**13C NMR** (125 MHz, CDCl₃) δ 170.3, 169.2, 156.1, 155.8, 148.0, 147.7, 143.8, 143.7, 143.5, 142.0, 141.4, 128.8, 128.1, 127.9, 127.2, 125.11, 125.08, 123.94, 123.87, 120.2, 83.5, 67.4, 66.1, 65.8, 65.8, 58.7, 54.5, 47.1, 43.5, 33.8, 28.1, 19.7. 

HRMS (ESI) calc. for C₄₁H₄₂N₄O₁₂SNa 837.2418, found 837.2416.

**Compound 4**: To a solution of **12** (0.65 g, 0.80 mmol) in CH₂Cl₂ (3 mL) and phenylsilane (105 µL, 0.84 mmol) was added TFA (3 mL). The reaction was stirred for 2 h, concentrated under reduced pressure and repeatedly redissolved in CH₂Cl₂ and concentrated to remove residual TFA. The crude material was purified by flash chromatography (SiO₂, 1%-2% MeOH/CH₂Cl₂) to yield **4** (0.58 g, 0.76 mmol, 95%) as a white solid. R_f 0.16 (EtOAc). 

**1H NMR** (500 MHz, CD₃OD) δ 8.12 (app. t, J = 8.5 Hz, 4 H), 7.75 (d, J = 7.5 Hz, 2H), 7.63 (d, J = 7.5 Hz, 2H), 7.45 (app. t, J = 7.0 Hz, 4H), 7.35 (t, J = 7.5 Hz, 2H), 7.27 (t, J = 7.5 Hz, 2H), 5.30-5.16 (m, 4H), 4.57 (d, J = 4.5 Hz, 1H), 4.39 (dd, J = 8.5 Hz, 4.5 Hz, 1H), 4.35-4.24 (m, 2H), 4.18 (t, J = 7.0 Hz, 1H), 3.49 (m, 1H), 3.09 (dd, J = 13.5 Hz, 4.5 Hz, 1H), 2.83 (dd, J = 13.5 Hz, 8.5 Hz, 1H), 1.33 (d, J = 7.0 Hz, 3H). 

**13C NMR** (125 MHz, CD₃OD) δ 173.6, 171.5, 158.4, 158.3, 149.0, 148.8, 145.7, 145.2, 145.1, 144.2, 142.5, 129.7, 129.0, 128.8, 128.2, 126.3, 124.6, 124.5, 120.9, 120.8, 120.6, 120.4, 120.2, 68.2, 66.8, 66.4, 60.4, 55.5, 48.3, 43.7, 34.1, 19.6. 

HRMS (ESI) calc. for C₃₇H₃₅N₄O₁₂S 759.1972, found 759.1964.

**Peptide fragment 13**

**Compound 38**: l-Threonine tert-butyl ester hydrochloride (37, 0.50 g, 2.36 mmol) was dissolved in CH₂Cl₂ (12 mL) and diisopropylethylamine (620 µL, 3.54 mmol). N-Boc-l-phenylalanine (0.63 g, 2.36 mmol), HOBt (0.36 g, 2.36 mmol) and EDC (0.45 g, 2.36 mmol) were added as solids. The reaction was stirred for 14 h, then washed with saturated aqueous NaHCO₃, 10% citric acid and water. Each aqueous wash was back-extracted with CH₂Cl₂. The organic fractions were combined, washed with brine, dried over Na₂SO₄, filtered, and concentrated, yielding **38** (0.98 g, 2.32 mmol, 98%)
as a white solid. $R_f 0.82$ (EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.30-7.19 (m, 5H), 6.67 (d, $J = 8.4$ Hz, 1H), 5.04 (d, $J = 7.6$ Hz, 1H), 4.43 (dd, $J = 8.4$ Hz, 3.2 Hz, 1H), 4.38 (m, 1H), 4.19 (m, 1H), 3.16-3.03 (m, 2H), 2.45 (bs, 1H), 1.46 (s, 9H), 1.39 (s, 9H), 1.15 (d, $J = 6.4$ Hz, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.9, 169.7, 155.7, 136.6, 129.5, 128.7, 127.0, 82.7, 80.4, 68.7, 58.1, 56.0, 38.1, 28.4, 28.1, 20.0. HRMS (ESI) calc. for C$_{23}$H$_{35}$N$_2$O$_6$ 423.2495, found 423.2499.

Compound 39: A two-step dehydration selective for the Z-olefin was performed based on the procedure of Pattabiraman et al.$^6$ Compound 38 (1.19 g, 2.82 mmol) was dissolved in CH$_2$Cl$_2$ (30 mL) and triethylamine (0.98 mL, 7.05 mmol) and chilled in an ice bath. Methanesulfonyl chloride (MsCl, 0.44 mL, 5.64 mmol) was added dropwise, and the reaction was stirred for 1 h, gradually warming to room temperature. The reaction was concentrated under reduced pressure, then taken up in 1,2-dichloroethane (DCE, 30 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 1.69 mL, 11.3 mmol). The reaction was heated to reflux in an oil bath (90°C) for 4 h, then concentrated. The residue was taken up in EtOAc, washed with 10% citric acid, then the combined organic layers were washed with 10% citric acid, saturated aqueous NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, filtered, and concentrated. The crude material was purified by flash chromatography (SiO$_2$, 7:1 then 4:1 hexane/EtOAc) to yield 39 (1.03 g, 2.55 mmol, 90% over two steps) as a white solid. $R_f 0.48$ (2:1 hexane/EtOAc). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.38 (s, 1H), 7.31-7.27 (m, 2H), 7.24-7.21 (m, 3H), 6.68 (q, $J = 7.0$ Hz, 1H), 5.00 (m, 1H), 4.49 (m, 1H), 3.19 (dd, $J = 13.5$ Hz, 6.0 Hz, 1H), 3.07 (m, 1H), 1.67 (d, $J = 7.0$ Hz, 3H), 1.46 (s, 9H), 1.40 (s, 9H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 169.5, 163.4, 156.5, 136.6, 132.6, 129.5, 128.8, 127.1, 126.9, 81.8, 80.5, 56.1, 38.2, 28.3, 28.1, 14.8. HRMS (ESI) calc. for C$_{22}$H$_{33}$N$_2$O$_5$ 405.2389, found 405.2392.

Compound 13: Compound 39 (1.27 g, 3.14 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL) and TFA (10 mL) and stirred for 1.5 h. The reaction was concentrated under reduced pressure, repeatedly taken up in CH$_2$Cl$_2$ and re-concentrated to remove residual acid. To the resulting residue was added sodium carbonate (0.67 g, 6.28 mmol), water (30 mL) and 1,4-dioxane (30 mL), and the system was chilled in an ice bath. N-(9-fluorenylmethoxycarbonyloxy)succinimide (FmocOSu, 1.06 g, 3.14 mmol) was then added portionwise as a solid. The reaction was stirred for 20 h, gradually warming to room temperature. Volatile components were removed under reduced pressure, then the system was diluted with H$_2$O and acidified to pH 2 with 2 M HCl. The aqueous suspension was extracted with EtOAc (3x), then the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated to ~20 mL. Hexane (~150 mL) was added to form a precipitate, which was isolated by filtration and dried to yield 13 (1.40 g, 2.98 mmol, 95% over two steps) as a white powder. $R_f 0.12$ (EtOAc). $^1$H NMR (400 MHz, CD$_3$OD) δ 7.75 (d, $J = 7.2$ Hz, 2H), 7.55 (m, 2H), 7.34 (t, $J = 7.2$ Hz, 2H), 7.29-7.15 (m, 7H), 6.82 (q, $J = 7.2$ Hz, 1H), 4.50 (dd, $J = 9.6$ Hz, 5.2 Hz, 1H), 4.30-4.16 (m, 2H), 4.11 (t, $J = 7.0$ Hz, 1H), 3.21 (dd, $J = 13.8$ Hz, 5.2 Hz, 1H), 2.89 (dd, $J = 13.8$ Hz, 9.6 Hz, 1H), 1.65 (d, $J = 7.2$ Hz, 3H). $^{13}$C NMR (125 MHz, CD$_3$OD) δ 173.1, 167.2, 158.2, 145.2, 142.5, 138.6, 136.8, 130.4, 129.4, 128.7, 128.4, 128.1, 127.7, 126.3, 120.9, 68.0, 57.8, 48.3, 39.1, 14.1. HRMS (ESI) calc. for C$_{28}$H$_{27}$N$_2$O$_5$ 471.1920, found 471.1923.
Peptide fragment 14

Compound 41: 1-Alanine methyl ester hydrochloride (40, 0.70 g, 5.00 mmol) was taken up in CH$_2$Cl$_2$ (25 mL) and disopropylethylamine (1.3 mL, 7.50 mmol) and stirred for 5 min. N-Boc-L-serine (1.03 g, 5.00 mmol), HOBt (0.84 g, 5.50 mmol) and EDC (0.96 g, 5.00 mmol) were added as solids. The reaction was stirred for 21 h, then washed with saturated aqueous NaHCO$_3$, 10% citric acid, water and brine. Each aqueous wash was back-extracted with CH$_2$Cl$_2$. The organic fractions were combined, dried over Na$_2$SO$_4$, filtered, and concentrated to yield 41 (1.24 g, 4.27 mmol, 85%) as a white solid. $R_f$ 0.45 (EtOAc). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.32 (d, $J$ = 7.0 Hz, 1H), 5.69 (d, $J$ = 6.5 Hz, 1H), 4.54 (pent, $J$ = 7.0 Hz, 1H), 4.22 (m, 1H), 3.95 (dd, $J$ = 11.0 Hz, 4.0 Hz, 1H), 3.71 (s, 3H), 3.65 (dd, $J$ = 11.0 Hz, 4.0 Hz, 1H), 1.41 (s, 9H), 1.38 (d, $J$ = 7.0 Hz, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.4, 171.1, 156.1, 80.4, 63.1, 55.3, 52.7, 48.3, 28.3, 17.8. HRMS (ESI) calc. for C$_{12}$H$_{22}$N$_2$O$_6$Na 313.1376, found 313.1377.

Compound 42: Compound 41 (1.08 g, 3.72 mmol) was dissolved in CH$_2$Cl$_2$ (40 mL) and triethylamine (1.3 mL, 9.30 mmol) and chilled in an ice bath. Methanesulfonyl chloride (580 µL, 7.44 mmol) was added dropwise, and the reaction was allowed to warm to room temperature and stirred for 1 h. DBU (2.8 mL, 18.6 mmol) was then added, and the reaction was stirred for an additional 2 h, then concentrated under reduced pressure. The residue was taken up in EtOAc, washed with 10% citric acid, saturated aqueous NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, filtered, and concentrated. The crude material was purified by flash chromatography (SiO$_2$, 7:3 hexane/EtOAc) to yield 42 (0.81 g, 2.97 mmol, 80% over two steps) as a pale yellow oil. $R_f$ 0.70 (1:1 hexane/EtOAc). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.25 (s, 1H), 6.69 (d, $J$ = 7.0 Hz, 1H), 6.03 (s, 1H), 5.12 (t, $J$ = 1.5 Hz, 1H), 4.61 (pent, $J$ = 7.0 Hz, 1H), 3.77 (s, 3H), 1.46-1.42 (m, 12H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.1, 163.7, 152.7, 134.5, 98.4, 80.6, 52.7, 48.6, 28.2, 18.2. HRMS (ESI) calc. for C$_{12}$H$_{20}$N$_2$O$_5$Na 295.1270, found 295.1275.

Compound 14: To a solution of 42 (0.54 g, 2.00 mmol) in 1,4-dioxane (5 mL) was added 1 M aqueous lithium hydroxide (5 mL). The reaction was stirred for 1 h, then volatile components were removed under reduced pressure. The system was diluted with water and acidified to pH 3 with 2 M HCl. The aqueous suspension was extracted with EtOAc (10x), then the combined organic layers were dried over Na$_2$SO$_4$, filtered, and concentrated to yield 14 (0.34 g, 1.32 mmol, 66%) as a colorless foam. $R_f$ 0.03 (EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.28 (s, 1H), 6.71 (d, $J$ = 7.2 Hz, 1H), 6.02 (s, 1H), 5.14 (s, 1H), 4.63 (pent, $J$ = 7.2 Hz, 1H), 1.51 (d, $J$ = 7.2 Hz, 3H), 1.45 (s, 9H). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 176.1, 164.1, 159.9, 134.7, 110.0, 81.0, 48.2, 28.4, 18.1. HRMS (ESI) calc. for C$_{11}$H$_{19}$N$_2$O$_5$ 259.1294, found 259.1288.
Synthetic procedures for epilancin 15X analogues.

Authentic epilancin 15X (1) was provided by Dr. Juan Velásquez (University of Illinois at Urbana-Champaign) after isolation from the producing organism Staphylococcus epidermidis 15X154 using a published procedure.\(^7\) HRMS (MALDI-TOF) calc. for C\(_{145}H_{234}N_{41}O_{33}S_3\) 3173.705, found 3173.860.

**Standard SPPS protocols:** Unless noted otherwise, standard cycles for SPPS were performed as follows, using a fritted glass reaction vessel equipped with a N\(_2\) inlet for resin/reagent agitation and a suction outlet for draining. Fmoc deprotection was achieved by agitating resin with 20\% piperidine in DMF for 20 min. After draining the reaction vessel, the resin was washed with DMF (3 x 30 s) and CH\(_2\)Cl\(_2\) (2 x 30 s). The appropriately side-chain protected Fmoc-amino acid (5 equiv.) in DMF (5-10 mL) was pre-activated with DIC and HOBT (5 equiv. each) for 5 min, then added to resin and agitated for 45-60 min. After draining the reaction vessel, the resin was washed as before. The completion of all couplings was assessed by a Kaiser test; double couplings were performed as needed but were generally not necessary. Test cleavages were performed after all cyclization steps by removing a small portion of dry resin from the reaction vessel and treating with 90:5:5 TFA/H\(_2\)O/triisopropylsilane for 1 h under N\(_2\). After removing the cleaved resin by filtration, the filtrate was concentrated under a stream of N\(_2\). The peptide was precipitated with cold Et\(_2\)O, isolated by centrifugation and dissolved in 1:1 H\(_2\)O/MeCN. An aliquot of this solution was spotted onto a MALDI-TOF MS target for analysis, while the remainder was lyophilized to dryness, taken up in 0.1\% TFA/H\(_2\)O and analyzed by analytical RP-HPLC.

**Intermediate 16:** The substitution of the Fmoc-Lys(Boc)-Wang resin (15, initial substitution 0.36 mmol/g) was first reduced such that 1 equiv. corresponded to 0.10 mmol/g. To ensure local as well as global reduction in resin substitution, the resin was first swelled in DMF for 20 min, followed by addition of Fmoc-Lys(Boc)-OH (1 equiv.) and Boc-Ala-OH (2 equiv.) that had been pre-activated with DIC and HOBT (3 equiv. each) for 5 min. The reaction was performed for 15 h. Any remaining free resin sites were capped with 1:2:7 Ac\(_2\)O/pyridine/DMF for 15 min. Adjusted resin substitution was calculated as follows: Fmoc-protected resin (10 mg) was agitated with 20\% piperidine/DMF (1.0 mL) for 15 min. A 20 \(\mu\)L aliquot of this solution was diluted 100:1 with DMF. The absorbance of this solution at 301 nm was recorded after blanking with pure DMF, and the resin substitution was calculated using the equation: substitution = 101(absorbance)/7.8(resin weight).\(^8\) After standard Fmoc deprotection and Fmoc-Gly-OH coupling/deprotection, fragment 13 (2 equiv.) was pre-activated with DIC and HOAt (2 equiv. each) and \(\text{Pr}_2\text{NET}\) (5 equiv.) in DMF for 10 min, reacted with the resin-bound peptide for 12 h, and deprotected by the standard protocol. Fmoc-His(Trt)-OH was coupled/deprotected by the standard protocol. MeLan building block 4 (1.5 equiv.) was pre-activated with DIC and HOAt (3 equiv. each) in DMF for 5 min, then reacted with the resin-bound peptide for 2 h and deprotected by the standard protocol. Fmoc-Gly-OH was coupled/deprotected by the standard protocol. MeLan building block 3 (1.5 equiv.) was pre-activated with DIC and HOAt (3 equiv.) in DMF for 5 min, then reacted with the resin-bound peptide for 2 h, but not Fmoc-deprotected, to yield resin-bound intermediate 16.
Monocyclic intermediate 17: The nitrobenzyl protecting groups of 16 were removed with two treatments of 6 M SnCl\textsubscript{2} and 5 mM HCl/dioxane in DMF (5 mL) for 1 h each. Following the second treatment, the reaction vessel was drained, and the resin was washed with 1:1 DMF/H\textsubscript{2}O (3 x 1 min), 1:1 THF/H\textsubscript{2}O (3 x 1 min), DMF (3 x 30 s) and CH\textsubscript{2}Cl\textsubscript{2} (2 x 30 s). The Fmoc group was removed by the standard protocol, followed by washing with DMF (5 x 30 s), CH\textsubscript{2}Cl\textsubscript{2} (3 x 30 s) and DMF (2 x 30 s) to remove all traces of piperidine. Cyclization was promoted by adding PyAOP and HOAt (5 equiv. each) in DMF to the resin and agitating for 5 min, then adding 2,4,6-collidine (10 equiv.) and agitating for 1.5 h. After draining, this treatment was repeated for 1.5 h to yield 17. Test cleavage analysis was performed to confirm completed cyclization. HRMS (MALDI-TOF) calc. for C\textsubscript{56}H\textsubscript{84}N\textsubscript{15}O\textsubscript{15}S\textsubscript{12} 1270.571, found 1270.572.

Bicyclic intermediate 18: Fmoc-Leu-OH was coupled to 17 by the standard protocol, but not Fmoc-deprotected. The allyl protecting groups were then removed by agitating resin with tetrakis(triphenylphosphine)palladium (0) (1 equiv.) and phenylsilane (10 equiv.) in 1:1 DMF/CH\textsubscript{2}Cl\textsubscript{2} (10 mL) for 2 h, protected from light. After draining the reaction vessel, the resin was washed with CH\textsubscript{2}Cl\textsubscript{2} (3 x 1 min), 0.5% diethyldithiocarbamate in DMF (3 x 1 min), DMF (3 x 30 s) and CH\textsubscript{2}Cl\textsubscript{2} (2 x 30 s). The Fmoc group was removed by the standard protocol, followed by washing with DMF (5 x 30 s), CH\textsubscript{2}Cl\textsubscript{2} (3 x 30 s) and DMF (2 x 30 s) to remove all traces of piperidine. Cyclization was promoted by adding PyAOP and HOAt (5 equiv. each) in DMF to the resin and agitating for 5 min, then adding 2,4,6-collidine (10 equiv.) and agitating for 2 h. After draining, this treatment was repeated for 2 h to yield 18. Test cleavage analysis was performed to confirm completed cyclization. HRMS (MALDI-TOF) calc. for C\textsubscript{55}H\textsubscript{85}N\textsubscript{16}O\textsubscript{13}S\textsubscript{2} 1241.592, found 1241.629.

Tricyclic intermediate 20: Fmoc-Phe-OH, Fmoc-Gly-OH and Fmoc-Arg(Pbf)-OH were coupled to 18 and deprotected by the standard protocol. Lan building block 2 (1.5 equiv.) was pre-activated with DIC and HOAt (3 equiv. each) in DMF for 5 min and coupled to the resin-bound peptide for 2 h, then deprotected by the standard protocol. Fmoc-Leu-OH and Fmoc-Lys(Boc)-OH were coupled but not Fmoc-deprotected. Removal of the allyl and Fmoc groups, peptide cyclization and test cleavage analysis were performed as described for 18 to yield resin-bound intermediate 20 HRMS (MALDI-TOF) calc. for C\textsubscript{96}H\textsubscript{152}N\textsubscript{29}O\textsubscript{21}S\textsubscript{3} 2143.087, found 2143.117.

Analogue 21: The next 10 residues were coupled to resin-bound 20 and deprotected by the standard protocol. The N-terminus was acylated by treatment with d-lactic acid (5 equiv.), DEPbt (5 equiv.) and iPr\textsubscript{2}NEt (10 equiv.) for 2 h. The peptide
was cleaved from resin and globally deprotected with 90:5:2.5:2.5 TFA/H₂O/triisopropylsilane/thioanisole under N₂ for 2 h. The cleaved resin was removed by filtration, and the filtrate was concentrated under a stream of N₂. The peptide was precipitated with cold Et₂O, isolated by centrifugation, dissolved in 1:1 H₂O/MeCN and lyophilized to dryness. Crude 21 was dissolved to 10 mg/mL in 5% MeCN/H₂O with 0.1% TFA and purified by preparatory RP-HPLC using a solvent gradient of 5% std. B for 1 min, then 5-25% over 4 min, then 25-50% over 25 min, then 50-100% over 1 min. Partially-pure 21 eluted in fractions collected over 25.3-29.2 min. These fractions were concentrated and re-purified under the same conditions, with pure product eluting over 27.1-28.0 min. Lyophilization yielded 21 (2.0 mg, 0.63 μmol, 1.6% from a 40 μmol scale synthesis, 93% per step over 59 steps) as a white powder (see Fig. S1 for HPLC and MS; see Fig. S4 for MS/MS). HRMS (MALDI-TOF) calc. for C₁₄₅H₂₄₀N₄₁O₃₃S₃ 3179.752, found 3179.794.

**Figure S1.** Characterization of analogue 21. (a) Analytical RP-HPLC chromatogram. (b) MALDI-TOF mass spectrum (insert: zoom-in on the expected product mass).

Anologue 22: The next 9 residues were coupled to resin-bound 20 and deprotected by the standard protocol. The N-terminal PyrAla moiety was incorporated by coupling fragment 14 (4 equiv.) with DIC and HOAt (4 equiv. each) in DMF for 3 h. To prevent reduction of the ketone formed upon Boc deprotection, anisole was used in place of triisopropylsilane in the cleavage cocktail. The peptide was cleaved from resin and globally deprotected with 90:5:2.5:2.5 TFA/H₂O/anisole/thioanisole under N₂ for 3 h. Crude 22 was isolated and purified as described for 21. Partially-pure 22 eluted in fractions collected over 27.2-30.0 min. These fractions were concentrated and re-purified under the same conditions, with pure product eluting over 27.5-28.5 min. Lyophilization yielded 22 (1.3 mg, 0.41 μmol, 1.6% from a 25 μmol scale synthesis, 93% per step over 57 steps) as a white powder (see Fig. S2 for HPLC and MS). HRMS (MALDI-TOF) calc. for C₁₄₅H₂₃₈N₄₁O₃₃S₃ 3177.736, found 3177.765.
Figure S2. Characterization of analogue 22. (a) Analytical RP-HPLC chromatogram. The peak centered at 15.0 min represents an instrument impurity. (b) MALDI-TOF mass spectrum (insert: zoom-in on the expected product mass).

Truncated analogue 23: Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH and Fmoc-Ile-OH were coupled to resin-bound 20 and deprotected by the standard protocol. The N-terminus was acetylated with 1:2:4 Ac₂O/ pyridine/DMF for 30 min. The peptide was cleaved from resin and globally deprotected with 90:5:2.5:2.5 TFA/H₂O/triisopropylsilane/thioanisole under N₂ for 2 h. Crude 23 was isolated and purified as for 21. Partially-pure 23 eluted in fractions collected over 14.4-17.0 min. These fractions were concentrated and re-purified under the same conditions, with pure product eluting over 16.1-17.0 min. Lyophilization yielded 23 (1.2 mg, 0.48 μmol, 1.9% from a 25 μmol scale synthesis, 92% per step over 45 steps) as a white powder (see Fig. S3 for HPLC and MS). HRMS (MALDI-TOF) calcd. for C₁₁₃H₁₈₂N₃₃O₂₅S₂ 2497.314, found 2497.405.

Figure S3. Characterization of analogue 23. (a) Analytical RP-HPLC chromatogram. (b) MALDI-TOF mass spectrum (insert: zoom-in on the expected product mass).
Figure S4. MALDI-MS/MS analysis of (a) authentic epilancin 15X (1), and (b) analogue 21. Similar fragmentation patterns are seen for both peptides. For a discussion of tandem MS for the analysis of lantipeptides, see Li et al.9
**Chiral gas chromatography-mass spectrometry analysis.**

The enantiomeric purity of Lan/MeLan amino acids produced by hydrolysis of 23 was confirmed by chiral GC/MS, using a procedure modified from previous reports.\(^2\),\(^10\),\(^11\) Lyophilized 23 (0.2 mg) was dissolved in 6 M HCl (3 mL) and heated at 100 °C in a sealed, high-pressure reaction vessel for 20 h. The reaction was cooled and concentrated with a stream of N\(_2\) over 4 h. Methanol (3 mL) was chilled in an ice bath, and acetyl chloride (1 mL) was added dropwise. This solution was added to the dry hydrolysate, and the mixture was sealed and heated at 100 °C for 1 h. The reaction was allowed to cool, then concentrated under reduced pressure. The dry residue was suspended in CH\(_2\)Cl\(_2\) (3 mL) and chilled in an ice bath. Pentafluoropropionic anhydride (1 mL) was added, and the mixture was sealed and heated at 100 °C for 20 min. The reaction was allowed to cool, then concentrated under reduced pressure. The residue was dissolved in methanol and re-concentrated, then dissolved again in methanol (200 μL) for analysis. Synthetic Lan/MeLan standards of differing stereochemical configurations (DD, DL and LL for Lan; DL and LL for MeLan), similarly derivatized as their pentafluoropropionamide methyl esters, were provided by Weixin Tang (University of Illinois at Urbana-Champaign) as solutions in methanol.\(^12\)

The derivatized hydrolysate and standards were analyzed by GC-MS using an Agilent 7890A gas chromatograph equipped with an Agilent 5975C Inert XL EI/CI MS detector and a Varian CP-Chirasil-L-Val fused silica column (25 m x 250 μm x 0.12 μm). Sample solutions in methanol were introduced to the instrument via splitless injection at an inlet temperature of 200 °C and flow rate of 2 mL/min helium gas. The temperature gradient used was held at 160 °C for 5 min, then ramped from 160 °C to 180 °C at 3 °C/min, then held at 180 °C for 6 min. The MS was operated in simultaneous scan/selected-ion monitoring (SIM) mode, monitoring at known and unique fragment masses of 365 Da for Lan and 379 Da for MeLan. All standards eluted as distinct peaks. For Lan, the DD-isomer eluted at 13.8 min, the DL-isomer at 14.1 min, and the LL-isomer at 14.3 min; for MeLan, the DL-isomer eluted at 11.1 min and the LL-isomer at 11.3 min. The derivatized hydrolysate of 25 confirmed the desired DL-configuration of both Lan and MeLan (Fig. S7). Small amounts of non-DL-configurations in the hydrolysate are believed to result from epimerization during hydrolysis, which has been reported previously.\(^11\),\(^13\)
Figure S7. GC-MS analysis of derivatized hydrolysate of analogue 23, confirming the desired DL-configuration of Lan/MeLan. (a) SIM at 365 Da of hydrolysate compared to synthetic Lan standards. (b) SIM at 379 Da of hydrolysate compared to synthetic MeLan standards. (c) Representative mass spectrum of derivatized Lan. (d) Representative mass spectrum of derivatized MeLan.

Liquid culture bioactivity assays.

Cultures of the indicator strain *Staphylococcus carnosus* TM300 (5 mL) were grown overnight at 37 °C in bovine heart infusion medium (BHI, 37 g/L), then diluted with fresh BHI to an optical density at 600 nm (OD$_{600}$) of 0.1. Lyophilized peptides were dissolved in sterile deionized water (SDW) to give stock solutions of 4 μM (for 1), 10 μM (for 21 and 22) or 100 μM (for 23). Two-fold serial dilutions were performed for each stock solution in SDW to give 11 concentrations at 4x final assay concentration. Corning-Costar 96 well flat-bottom assay plates were used to determine the activity of each peptide against *S. carnosus* TM300, and experiments were performed in triplicate. Experimental wells contained 150 μL of diluted culture and 50 μL of 4x peptide solution. Blank wells contained 150 μL BHI and 50 μL SDW. Positive control wells
contained 150 μL diluted culture and 50 μL SDW. OD_{600} was recorded at hourly intervals using a BioTek Synergy H4 plate reader, and plates were incubated at 37 °C between readings. After subtraction of blanks from experimental measurements, plots of OD_{600} vs. peptide concentration were fitted to a dose-response curve with the equation: y = A1 + (A2 - A1) / (1 + 10^{(\log x_0 - x)p}), where p = variable Hill slope. Half maximal inhibitory concentration (IC_{50}) and minimal inhibitory concentration (MIC) values were calculated from this fit for each peptide after 5 h incubation, and triplicate calculations were averaged.

| Peptide            | IC_{50} (nM) | MIC (nM) |
|--------------------|--------------|----------|
| Epilancin 15X (1)  | 95 ± 9.6     | 250      |
| Analogue 21        | 270 ± 23     | 625      |
| Analogue 22        | 354 ± 8      | 1250     |
| Truncated analogue 23 | Not determined | 12,500   |

**Flow cytometry analysis of pore-forming activities**

Cultures of S. carnosus TM300 were grown as for bioactivity assays and diluted with fresh BHI to an OD_{600} of 0.1. For membrane depolarization assays using the dye 3,3'-diethyloxacarbocyanine iodide (DiOC_{2}(3)),^{14} cells were combined with DiOC_{2}(3) (final concentration 2 μM), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, 1 mM) and glucose (1 mM) and incubated for 20 min at room temperature. Stock solutions of epilancin 15X analogues 21 or 23 were added to final concentrations of 0.1, 1.0 or 10 μM and incubated for an additional 20 min prior to analysis; water was added for the negative control. For membrane permeability assays using the dye propidium iodide (PI),^{15} cells were combined with PI (final concentration 25 μM), HEPES (1 mM), glucose (1 mM) and epilancin 15X analogues (0.1, 1.0, 10 μM), incubated for 15 min at room temperature and analyzed. Changes in cell-associated dye fluorescence were measured with a BD Biosciences LSR II flow cytometer, using excitation at 488 nm with an argon laser and measurement of emission through a band-pass filter at 530/30 nm for DiOC_{2}(3) or 695/40 nm for PI. A minimum of 25,000 events were detected for each sample, and each peptide concentration was repeated in triplicate. Data analysis to calculate the geometric mean fluorescence intensity (MFI) of each population was performed using FCS Express 3.00.0311 V Lite Stand-alone software.

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NMR spectra of novel small molecules (ordered by compound number)

Compound 3
Compound 4
Compound 9
Compound 11
Compound 12
Compound 13
Compound 14
Compound 35
Compound 36
Compound 38
Compound 39
Compound 41
