Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Diversity-Oriented Routes to Thiopeptide Antibiotics: Total Synthesis and Biological Evaluation of Micrococcin P2.

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Supporting Information

Experimental Section

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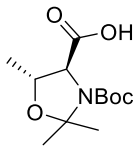
1. Experimental protocols

Unless otherwise noted, all reactions were carried out under Ar in flamed-dried glassware using anhydrous solvents. Anhydrous solvents were freshly distilled over the indicated drying agent (in parentheses) prior to use and were transferred under Ar: THF, Et_2O (Mg/anthracene), toluene (Na/K), CH_2Cl_2, MeOH (Mg). Triethylamine and DMF were dried over an adsorption solvent purification system based on molecular sieves. All other commercial compounds (Acros, Aldrich, Alfa Aesar, TCI) were used as received. Thin layer chromatography (TLC) was carried out with Macherey-Nagel precoated plates (POLYGRAM®SIL/UV254). Flash chromatography employed Merck silica gel 60 (40−63 μm) and technical grade solvents. NMR spectra were recorded on Bruker AV VIII 400 or 600 spectrometers in the solvents indicated. Solvent signals were used as references, and the chemical shifts were converted to the TMS scale ($CDCl_3$: δ_C = 77.0 ppm; residual $CHCl_3$ in $CDCl_3$: δ_H = 7.26 ppm; CD_3OD: δ_C = 49.0 ppm; residual CHD_2OD in CD_3OD: δ_H = 3.31 ppm; CD_2Cl_2: δ_C = 54.0 ppm; residual $CHDCl_2$ in CD_2Cl_2: δ_H = 5.32 ppm). FT-IR spectra were obtained on Thermo Scientific Nicolet 6700 and reported as wavenumbers (cm^{-1}). High-resolution mass spectra (HRMS) were recorded on an AB SCIEX Q-TOF 5600 mass spectrometer. Optical rotation ($[\alpha]_D^{20}$ and $[\alpha]_D^{25}$): Krüss P8000-T, 10 cm/1 mL cell. Melting points were determined on A. KRÜSS OPTRONIC M3000 and are uncorrected. MPLC purifications are performed by CombiFlash RF+ UV (Redisep® from Teledyne Isco).

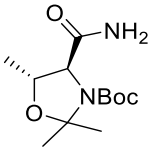
2. Synthesis and characterization of various intermediates

(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic acid (8).[1] To a



solution of N-Boc-(L)-threonine (100 mmol, 22.0 g) in THF (200 mL) was added 2,2-dimethoxypropane (1000 mmol, 123 mL, 10 equiv) and pyridinium *p*-toluenesulfonate (30 mmol, 7.50 g, 0.3 equiv) were added. The mixture was brought up to reflux and stirred for 18 hours. The mixture was cooled to room temperature, and concentrated *in vacuo*. The residue was partitioned between water (250 mL) and ethyl acetate (250 mL). The aqueous layer was further extracted with ethyl acetate (200 mL x 2). The combined organic layer was dried over Na_2SO_4, and concentrated *in vacuo* to afford the desired product as a white solid. [R_f – 0.30 (20% ethyl acetate : 80% hexane)]. Yield : 25.4 g (98%) **^{1}H NMR** (600 MHz, $CDCl_3$, ppm): δ 4.26 (d, *J* = 40.4 Hz, 1H), 4.05 – 3.86 (m, 1H), 1.68 – 1.52 (m, 6H), 1.51 – 1.37 (m, 12H) (note: An exchangeable proton is not present in the ^{1}H NMR); **^{13}C NMR** (151 MHz, $CDCl_3$, ppm): δ 176.0, 174.1, 152.8, 151.1, 95.5, 95.0, 81.7, 81.0, 74.0, 73.5, 66.1, 65.8, 28.4, 27.9, 26.7, 25.1, 24.1, 19.1. Due to restricted rotations, a greater number of ^{13}C peaks is observed. Spectral data are in accord with the literature data.

***tert*-Butyl (4*S*,5*R*)-4-carbamoyl-2,2,5-trimethyloxazolidine-3-carboxylate (9)**.[2] To a



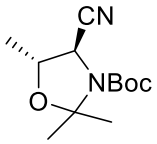
solution of (4*S*,5*R*)-3-(tert-butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic acid **(8)** (26.2 g, 101.4 mmol) and triethylamine (111.5 mmol, 15.5 mL, 1.1 equiv) in THF (200 mL) at 0 °C was carefully added ethyl chloroformate (111.5 mmol, 10.6 mL, 1.1 equiv) and the mixture was stirred for 1.5 hours. Then, aqueous ammonia (18M, 152 mmol, 8.40 mL, 1.5 equiv) was added and the mixture was allowed to warm to room temperature and stirred for 22 hours. The mixture was concentrated *in vacuo*, and the resulting aqueous suspension was extracted with ethyl acetate (150 mL x 3). The combined organic layer was washed with water (100 mL), brine (100 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford the desired product as a yellow solid. Yield : 22.8 g (87%), **^{1}H NMR** (600 MHz, $CDCl_3$, ppm): δ 5.47 (s, 1H), 4.40 (qd, *J* = 6.5, 2.3 Hz, 1H), 3.78 (d, *J* = 17.9 Hz, 1H), 1.62 (s, 3H), 1.59 (s, 3H), 1.46 (s, 9H), 1.41 (d, *J* = 6.1 Hz, 3H); **^{13}C NMR** (151 MHz, $CDCl_3$, ppm): δ 207.2, 174.0, 156.7, 95.1, 81.3, 80.7, 66.6, 61.3, 57.7, 34.6, 31.4, 31.1, 28.4, 25.7, 19.1, 18.3, 14.7. Due to restricted rotations, a greater

[1] Known compound: Nakamura, Y.; Shin, C. G.; Uemura, K.; Yoshimura, J. *Chem. Lett.* **1992**, 1005. See also: Ciufolini, M. A.; Shen, Y.-C. *Org. Lett.* **1999**, *1*, 1843.

[2] Known compound: Nicolaou, K. C.; Nevalainen, M.; Safina, B. S.; Zak, M.; Bulat, S. *Angew. Chem. Int. Ed.* **2002**, *41*, 1941.

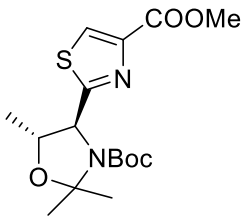
number of ^{13}C peaks is observed. Spectral data are in accord with the literature data.

***tert*-Butyl (4*R*,5*R*)-4-cyano-2,2,5-trimethyloxazolidine-3-carboxylate (10)**.[3] To a solution



of *tert*-butyl (4*S*,5*R*)-4-carbamoyl-2,2,5-trimethyloxazolidine-3-carboxylate **(9)** (88.2 mmol, 22.8 g) in DMF (88 mL) was carefully added cyanuric chloride (44.1 mmol, 8.13 g, 0.5 equiv) in portions. The mixture was stirred at room temperature for 30 minutes. Ice water (50 mL) was added and whereupon the solid was formed. The solids were filtered, and further washed with water (100 mL) and dried *in vacuo* to afford the desired product as a white solid. [R_f – 0.70 (25% ethyl acetate: 75% hexane)]. Yield : 19.5 g (92%) **^{1}H NMR** (600 MHz, $CDCl_3$, ppm): δ 4.47 – 4.40 (m, 1H), 4.02 (d, J = 7.4 Hz, 1H), 1.66 – 1.55 (m, 6H), 1.52 (s, 9H), 1.44 (d, J = 6.1 Hz, 3H); **^{13}C NMR** (151 MHz, $CDCl_3$, ppm): δ 150.7, 117.4, 96.0, 95.4, 82.4, 74.3, 74.0, 53.2, 28.4, 27.9, 26.6, 25.6, 24.6, 18.5. Spectral data are in accord with the literature data.

***tert*-Butyl (4*S*,5*R*)-4-(4-(methoxycarbonyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (12)**.[4] To a solution of *tert*-butyl (4*S*,5*R*)-4-cyano-2,2,5-trimethyloxazolidine-3-carboxylate **(10)** (81.2 mmol, 19.5 g) in the mixture of isopropyl alcohol : 0.1 M pH 6 sodium phosphate buffer (3 : 2 v/v 240 mL : 160 mL) was added L-cysteine methyl ester hydrochloride (122 mmol, 20.9 g, 1.5 equiv). The mixture was degassed with N_2 for a period of 10 minutes, and the mixture was stirred at 50 °C for 15 hours. The mixture was concentrated *in vacuo* to remove excess isopropyl alcohol, and the aqueous layer was extracted with ethyl acetate (150 mL x 2). The organic layer was washed with 1M HCl (60 mL), water (60 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford the thiazoline as a brown solid. The crude thiazoline **(11)** (77.6 mmol, 27.8 g) was dissolved in CH_2Cl_2 (260 mL) at 0 °C and was treated with $BrCCl_3$ (116.4 mmol, 11.5 mL, 1.5 equiv). DBU (116.4 mmol, 17.4 mL, 1.5 equiv) was carefully added dropwise. The mixture was then brought up to reflux and stirred for 4 hours. The mixture was quenched by the addition of 1M HCl (100 mL) and aqueous layer was extracted with CH_2Cl_2 (100 mL x 2). The combined organic layer was washed with water (100 mL), brine (100 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The isolated viscous crude material was kept in water (200 mL) for 30 minutes whereupon the crude was solidified. Then, the crude was washed through a suction filtration method by water (100 mL x 3),

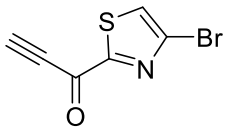


[3] Known compound: Merritt, E. A.; Bagley, M. C. *Synlett* **2007**, 954. See also footnote 4.

[4] Known compound: Christy, M. P.; Johnson, T.; McNerlin, C. D.; Woodard, J.; Nelson, A. T.; Lim, B.; Hamilton. T. L.; Freiberg, K. M.; Siegel, D. *Org. Lett*, **2020**, *22*, 2365.

followed by hexane (100 mL x 3). The desired product was isolated as a dark brown solid. [R_f – 0.60 (20% ethyl acetate : 80% hexane)]. Yield : 23.7 g (82% over 2 steps), **^{1}H NMR** (600 MHz, $CDCl_3$, ppm, **measured at 50 °C**): δ 8.13 (s, 1H), 4.78 (d, *J* = 7.6 Hz, 1H), 4.23 – 4.16 (m, 1H), 3.93 (s, 3H), 1.68 (d, *J* = 5.0 Hz, 6H), 1.41 (d, *J* = 6.1 Hz, 3H), 1.24 (brs, 9H) ; **^{13}C NMR** (151 MHz, $CDCl_3$, ppm, **measured at 50 °C**): δ 173.5, 161.8, 151.7, 146.8, 127.5, 95.4, 81.0, 77.9, 66.2, 52.4, 28.3, 27.0, 26.3, 18.2. ; **Melting point:** 121.4-122.6; **IR(neat):** 3099, 2980, 2934, 1699, 1490 (cm^{-1}); **HRMS:** Calcd for $C_{16}H_{25}N_2O_5S^+$ $[M+H]^+$ 357.1479, found 357.1480; $[\alpha]_D^{20}$: -48.8 (c=0.25, $CHCl_3$). Spectral data are in accord with the literature data.

1-(4-Bromothiazol-2-yl)prop-2-yn-1-one (15). A solution of 4-bromo-2-formyl thiazole



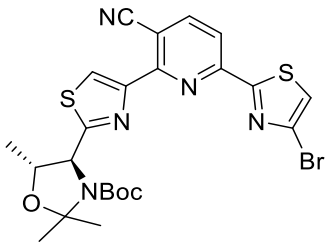
(104.0 mmol, 20.0 g) in THF (200 mL) was added dropwise to a commercial 0.5M solution of ethynylmagnesium bromide in THF (1.2 equiv, 125 mmol, 250 mL) at room temperature. The mixture was stirred for 30 min, and then quenched with aqueous saturated NH_4Cl solution (250 mL). The organic layer was separated and the aqueous phase was extracted with ethyl acetate (2 x 150 mL). The combined extracts were dried over Na_2SO_4 and concentrated *in vacuo.* MPLC (gradient 10 → 30% ethyl acetate : 90 → 70% hexane) of the residue afforded the desired alcohol as a white solid. (87 mmol, 19.0 g, Yield: 84%). ([R_f – 0.30 (30% ethyl acetate : 70% hexane)]. The resulting alcohol (87 mmol, 19.0 g) was dissolved in CH_2Cl_2 (400 mL). Manganese dioxide (11.3 equiv, 987 mmol, 85.0 g) was added at room temperature and the mixture was stirred for 12 hours. The mixture was filtered through Celite and the bed was further washed with ethyl acetate (300 mL) and CH_2Cl_2 (200 mL). The filtrate was concentrated *in vacuo* to afford the desired product as a brown solid. [R_f – 0.42 (30% ethyl acetate : 70% hexane)]. Crude is clean enough for a subsequent reaction. However, a clean sample is obtained by MPLC (30 → 50% ethyl acetate : 70 → 50% hexane) as a brown solid. [R_f – 0.42 (30% ethyl acetate : 70% hexane)]. Yield: 16.0 g (71% over 2 steps); **^{1}H NMR** (600 MHz, $CDCl_3$, ppm): δ 7.66 (s, 1H), 3.64 (s, 1H); **^{13}C NMR** (151 MHz, $CDCl_3$, ppm): δ 168.1, 165.8, 128.7, 125.9, 84.5, 78.9; **Melting point:** 148.2-149.3 °C; **IR(neat):** 3192, 3084, 2641, 2092, 1633 cm^{-1}; **HRMS**: Calcd for $C_6H_3BrNOS^+$ $[M+H]^+$ 215.9113, found 215.9107.

***tert*-Butyl (4*S*,5*R*)-4-(4-(2-cyanoacetyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (16)**. A commercial solution of *n*-BuLi (2.40 M in hexanes, 54.6 mL, 131 mmol, 2.1 equiv) was added over a period o f 10 minutes to a cold (–78 °C) solution of acetonitrile (135 mmol, 2.15 equiv 7.04 mL) in THF (120 mL), the mixture was stirred at -78ºC for 20 minutes, then a solution of *tert*-butyl (4*S*,5*R*)-4-(4-(methoxycarbonyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate **(12)** (62.6 mmol, 22.3 g) in THF (120 mL) was slowly added over a period of 10 minutes. The mixture was slowly brought up to room temperature over 1 hour, then it was carefully quenched with 1M HCl (250 mL). The mixture was diluted with ethyl acetate (300 mL), transferred to a separatory funnel and further acidified with 1M HCl to pH 3. Aqueous layer was further extracted with ethyl acetate (200 mL). The organic layer was collected, dried over Na_2SO_4 and concentrated *in vacuo*. Crude is clean enough for a subsequent reaction. However, authentic clean sample is obtained by MPLC (30 → 50% ethyl acetate : 70 → 50% hexane) as a brown solid. [R_f – 0.36 (30% ethyl acetate : 70% hexane)]. **^{1}H NMR** (600 MHz, $CDCl_3$, ppm, **measured at 50 °C**) δ 8.24 (s, 1H), 4.68 (brs, 1H), 4.21 – 4.10 (m, 3H), 1.67 (s, 3H), 1.65 (s, 3H), 1.59 – 0.97 (m, 12H); **^{13}C NMR** (151 MHz, $CDCl_3$,ppm, **measured at 50 °C**) δ 206.5, 182.2, 173.2, 151.6, 127.3, 113.8, 95.4, 81.2, 68.6, 65.9, 30.4, 28.3, 26.1, 18.1; **Melting point:** 98.1-100.6 °C; **IR(neat):** 3133, 2977, 2934, 2260, 1697, 1491 (cm^{-1}); **HRMS:** Calcd for $C_{17}H_{24}N_3O_4S^+$ $[M+H]^+$ 366.1482, found 366.1482; $[\alpha]_D^{20}$: -32.3 (c=0.25, $CHCl_3$).

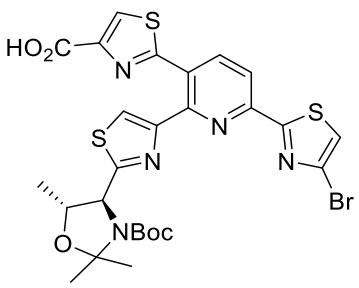
***tert*-Butyl (4*S*,5*R*)-4-(4-((*Z*)-1-amino-2-cyanovinyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (17)**. To a solution of *tert*-butyl (4*S*,5*R*)-4-(4-(2-cyanoacetyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate **(16)** (61.0 mmol, 22.3 g) in the mixture of toluene / acetic acid (5 :1 v/v 300 mL : 60 mL) was added ammonium acetate (610 mmol, 47.0 g, 10 equiv). The mixture was brought up to reflux and stirred for 1 hour. The mixture was cooled down, diluted with ethyl acetate (600 mL) and carefully washed with sat. aq. $NaHCO_3$ (350 mL). The organic layer was collected, dried over Na_2SO_4 and concentrated *in vacuo* to afford the desired product as a yellow oil (9:1 mixture of geometric isomers). Crude material is used for a subsequent reaction without further purification. However, an authentic clean sample is obtained by MPLC (30 → 50% ethyl acetate : 70 → 50% hexane) as a brown foam. [R_f – 0.36 (30% ethyl acetate : 70% hexane)]. **^{1}H NMR** (600 MHz, $CDCl_3$, **measured at 50 °C**) δ 7.54 (s, 1H), 5.46 (s, 2H), 4.68 (s, 1H),

4.53 (s, 1H), 4.23 – 4.16 (m, 1H), 1.69 (s, 3H), 1.67 (s, 3H), 1.40 (d, J = 6.1 Hz, 3H), 1.35 – 1.14 (m, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$, **measured at 50 °C**) δ 172.1, 153.5, 149.1, 147.1, 127.3, 122.0, 119.3, 117.3, 95.4, 81.1, 66.1, 61.9, 28.4, 26.0, 18.2; **IR(neat):** 3469, 3351, 3232, 3103, 2979, 2934, 2189, 1692, 1617, 1590 (cm^{-1}); **HRMS:** Calcd for $C_{17}H_{25}N_4O_3S^+$ $[M+H]^+$ 365.1642, found 365.1647; $[\alpha]_D^{20}$: -55.7 (c=0.25, $CHCl_3$).

***tert*-Butyl (4*S*,5*R*)-4-(4-(6-(4-bromothiazol-2-yl)-3-cyanopyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (18)**. To a solution of *tert*-butyl (4*S*,5*R*)-4-(4-((*E*)-1-amino-2-cyanovinyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (57.5 mmol, 21.0 g) in ethanol (300 mL) was added 1-(4-bromothiazol-2-yl)prop-2-yn-1-one (106.3 mmol, 22.9 g, 1.85 equiv) and the mixture was stirred at 60 °C for 1.5 hours. At this time, starting material is consumed and Michael adduct is clearly seen by TLC and LC/MS system. Then, acetic acid (30 % volume, 90 mL) was added and the mixture was brought up to reflux and stirred 16 hours to mediate the cyclization and aromatization. The excess ethanol and acetic acid was removed *in vacuo*. The crude was redissolved in CH_2Cl_2 (450 mL), and carefully quenched with sat. aq. $NaHCO_3$ (200 mL). The organic layer was collected and dried over Na_2SO_4, and concentrated *in vacuo*. MPLC (5 → 15% ethyl acetate : 95 → 85% hexane) of the residue afforded the desired product as a yellow solid. [R_f – 0.40 (20% ethyl acetate : 80% hexane)]. Yield : 16.5 g (47% over 3 steps); **^{1}H NMR** (600 MHz, $CDCl_3$,ppm, **50 °C**) δ 8.24 (s, 1H), 8.22 – 8.14 (m, 2H), 7.45 (s, 1H), 4.83 (d, J = 7.7 Hz, 1H), 4.39 (s, 1H), 1.72 (s, 6H), 1.54 – 1.49 (m, 3H), 1.47 – 1.21 (m, 9H); **^{13}C NMR** (151 MHz, $CDCl_3$, ppm, **50 °C**) δ 167.8, 153.3, 152.0, 151.7, 144.3, 144.0, 127.4, 121.8, 121.6, 118.0, 117.0, 107.6, 95.5, 80.9, 66.1, 28.5, 26.1, 18.2; **Melting point**: 185.3-187.7 °C; **IR(neat)**: 3124, 3062, 2979, 2933, 2221, 1681, 1582, 1558, 1490 (cm^{-1}); **HRMS:** Calcd for $C_{23}H_{25}BrN_5O_3S_2^+$ $[M+H]^+$ 562.0577, found 562.0558; $[\alpha]_D^{20}$: -45.2 (c=0.25, $CHCl_3$).

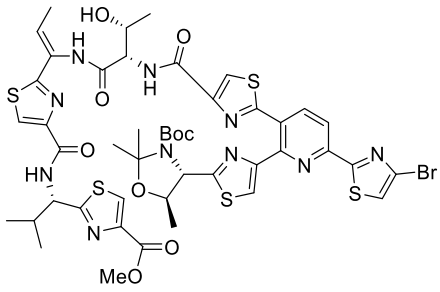


2-(6-(4-bromothiazol-2-yl)-2-(2-((4*S*,5*R*)-3-(*tert*-butoxycarbonyl)-2,2,5-trimethyloxazolidin-4-yl)thiazol-4-yl)pyridin-3-yl)thiazole-4-carboxylic acid (6). To a solution of tert-butyl (4*S*,5*R*)-4-(4-(6-(4-bromothiazol-2-yl)-3-cyanopyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate **(18)** (24.0 mmol, 13.5 g) in the mixture of ethanol / pH 7 sodium phosphate buffer / water (6 : 2 : 1 v/v 210 mL : 70 mL : 35 mL) was added L-cysteine (168 mmol, 20.3 g g, 7 equiv), sodium bicarbonate (96.0 mmol, 8.07 g, 4 equiv) and the mixture



was stirred at 100ºC overnight. Upon completion, excess solvent was removed *in vacuo* and mixture was diluted with ethyl acetate (500 mL). Organic layer was washed with 1M HCl (150 mL x 3) to get rid of excess cysteine, and dried over Na_2SO_4 to afford the crude desired thiazoline as a brown gum. The crude is pushed for a subsequent oxidation reaction. The crude thiazoline (23.5 mmol, 15.6 g) was dissolved in CH_2Cl_2 (260 mL) was treated with $BrCCl_3$ (94 mmol, 9.2 mL, 4.0 equiv). DBU (117.5 mmol, 17.4 mL, 5.0 equiv) was carefully added dropwise. The mixture was then brought up to reflux and stirred for 3 hours. The mixture was quenched by the addition of 1M HCl (200 mL) and aqueous layer was extracted with CH_2Cl_2 (200 mL). The combined organic layer was dried over Na_2SO_4, and concentrated *in vacuo* to afford the desired compound as a brown solid. This polar material was not purified and in crude form it was directly converted into the amide. **^{1}H NMR**[5] (400 MHz, $CDCl_3$, ppm) δ 8.35 – 8.05 (m, 3H), 7.89 (bs, 1H), 7.33 (s, 1H), 4.49 – 4.40 (m, 1H), 4.09 – 3.97 (m, 1H), 1.71 – 1.29 (m, 10H), 1.26 – 1.08 (m, 8H); **^{13}C NMR**[6] (100 MHz, $CDCl_3$, ppm) δ 181.3, 171.6, 168.6, 165.5, 163.7, 152.5, 151.4, 150.7, 150.3, 146.9, 140.3, 129.9, 128.7, 126.5, 121.0, 120.6, 118.4, 95.2, 80.8, 65.7, 43.0, 36.3, 30.5, 29.2, 28.3, 26.5, 25.7, 23.0, 17.8. Due to restricted rotations, a greater number of ^{13}C peaks is observed.

***tert*-Butyl (4*S*,5*R*)-4-(4-(6-(4-bromothiazol-2-yl)-3-(4-(((2*S*,3*R*)-3-hydroxy-1-(((*Z*)-1-(4-(((*S*)-1-(4-(methoxycarbonyl)thiazol-2-yl)-2-methylpropyl)carbamoyl)thiazol-2-yl)prop-1-en-1-yl)amino)-1-oxobutan-2-yl)carbamoyl)thiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (20)**. To a solution of tert-butyl (4*S*,5*R*)-4-(4-(6-(4-bromothiazol-2-yl)-3-cyanopyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate **(18)** (24.0 mmol, 13.5 g) in the mixture of ethanol / pH 7 sodium phosphate buffer / water (6 : 2 : 1 v/v 210 mL : 70 mL : 35 mL) was added L-cysteine (168 mmol, 20.3 g, 7 equiv), sodium bicarbonate (96.0 mmol, 8.07 g, 4 equiv) and the mixture was stirred at 100 ºC overnight. Upon completion, excess solvent was removed *in vacuo* and mixture was diluted with ethyl acetate (500 mL). Organic layer was washed with 1M HCl (150 mL x 3) to get rid of excess cysteine, and dried over Na_2SO_4 to afford the crude desired thiazoline as a brown gum. The crude is pushed for a subsequent oxidation reaction. The crude thiazoline (23.5 mmol, 15.6 g) was dissolved in CH_2Cl_2 (260 mL) and treated with $BrCCl_3$ (94 mmol, 9.2 mL, 4.0 equiv). DBU (117.5 mmol, 17.4 mL, 5.0 equiv) was carefully added



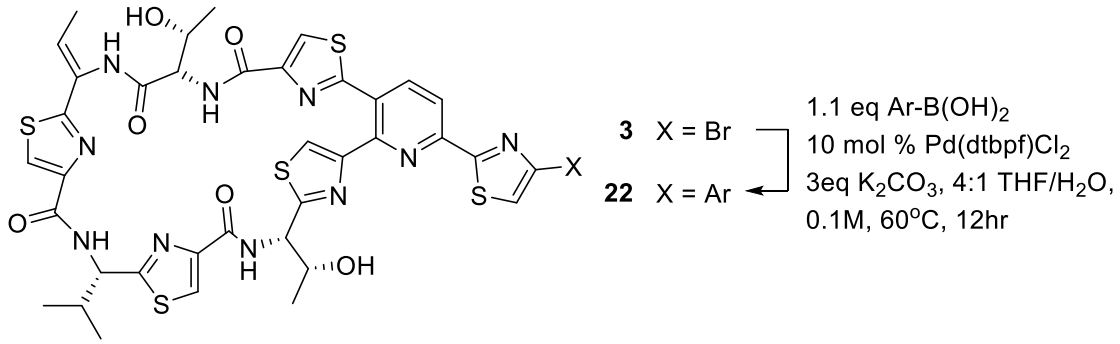
[5] Slow rotation about the N-BOC bond caused broadening of ^{1}H spectra and doubling of some ^{13}C signals

dropwise. The mixture was then brought up to reflux and stirred for 3 hours. The mixture was quenched by the addition of 1M HCl (200 mL) and aqueous layer was extracted with CH_2Cl_2 (200 mL). The combined organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The resulting crude thiazole was used for a subsequent amide coupling. The crude thiazole (23.5 mmol, 15.6 g) in acetonitrile (240 mL) was added **compound 5** (23.5 mmol, 11.3 g, 1.0 equiv) and N-methylimidazole (70.5 mmol, 5.61 mL, 3 equiv). Then, TCFH (30.6 mmol, 8.58 g, 1.3 equiv) was added and the mixture was stirred at room temperature for 30 minutes. Water (50 mL) was added to quench the reaction, and aqueous layer was extracted with CH_2Cl_2 (150 mL x 2). The organic layer was collected, dried over Na_2SO_4 and concentrated *in vacuo*. The crude was subjected to MPLC (1.5% → 2.3% MeOH : 98.5% → 97.7% CH_2Cl_2) of the residue afforded the desired product as a yellow solid. [R_f – 0.22 (4% MeOH : 96% CH_2Cl_2)]. Yield : 15.2 g (66% over 3 steps); **^{1}H NMR** (600 MHz, $CDCl_3$, ppm) δ 8.60 (s, 1H), 8.48 (d, *J* = 9.6 Hz, 1H), 8.32 – 8.08 (m, 4H), 8.07 (s, 1H), 7.96 (s, 1H), 7.94 – 7.90 (m, 1H), 7.40 (s, 1H), 6.45 (q, *J* = 7.0 Hz, 1H), 5.37 (dd, *J* = 9.6, 7.0 Hz, 1H), 4.85 (s, 1H), 4.63 (qd, *J* = 6.3, 2.6 Hz, 1H), 4.52 (s, 1H), 4.11 – 4.03 (m, 1H), 3.87 (s, 3H), 2.45 – 2.37 (m, 1H), 2.17 (brs, 1H), 1.87 (d, *J* = 7.1 Hz, 3H), 1.68 – 1.56 (m, 6H), 1.48 – 1.42 (m, 3H), 1.27 – 1.19 (m, 12H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.7 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$, ppm) δ 171.0, 168.9, 168.6, 166.2, 165.7, 161.6, 161.2, 160.5, 160.4, 152.5, 151.0, 150.2, 149.4, 148.9, 146.7, 140.0, 128.9, 128.6, 127.2, 126.9, 126.5, 125.8, 123.2, 120.6, 118.5, 95.2, 80.7, 67.4, 65.7, 58.1, 58.0, 52.4, 38.6, 34.1, 28.2, 26.5, 25.7,19.3, 18.7, 18.4, 17.8, 14.7; **Melting point:** 145.0-148.5 °C; **IR(neat):** 3381, 3116, 2973, 2932, 1656, 1579, 1531, 1477, 1429 (cm^{-1}); **HRMS:** Calcd for $C_{46}H_{52}BrN_{10}O_9S_5^+$ $[M+H]^+$ 1127.1700, found 1127.1725; $[\alpha]_D^{20}$: +22.4(c=0.25, $CHCl_3$).

(12*Z*,32*Z*,72*Z*,112*Z*,4*S*,8*S*,12*Z*,15*S*)-26-(4-bromothiazol-2-yl)-12-ethylidene-4,15-bis((*R*)-1-hydroxyethyl)-8-isopropyl-5,9,13,16-tetraaza-1(2,4),3,7,11(4,2)-tetrathiazola-2(3,2)-pyridinacycloheptadecaphane-6,10,14,17-tetraone (3). *tert*-butyl (4*S*,5*R*)-4-(4-(6-(4-bromothiazol-2-yl)-3-(4-(((2*S*,3*R*)-3-hydroxy-1-(((*Z*)-1-(4-(((*S*)-1-(4-(methoxycarbonyl)thiazol-2-yl)-2-methylpropyl)carbamoyl)thiazol-2-yl)prop-1-en-1-yl)amino)-1-oxobutan-2-yl)carbamoyl)thiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate **(20)** (21.2 g, 18.8 mmol) was dissolved in the mixture of THF (90 mL) / H_2O (90 mL) and LiOH · H_2O (56.3 mmol, 2.36 g, 3.0 equiv) was added and stirred at room temperature for 1.5 hours before it was

acidified with 1M HCl (150 mL). The compound was then extracted with ethyl acetate (3 x 150 mL), dried over Na_2SO_4, and concentrated *in vacuo* to afford the crude carboxylic acid. This was then dissolved in the mixture of CH_2Cl_2 (75 mL) / TFA (75 mL) and stirred at room temperature for 30 minutes before it was concentrated *in vacuo* to give the trifluoroacetate salt. This crude amine was then dissolved in DMF (135 mL), DPPA (28.2 mmol, 6.87 mL, 1.5 equiv) and sodium bicarbonate (225 mmol, 18.9 g, 12 equiv) were added and stirred overnight at room temperature before being diluted with 350 mL super solvent (4:1 $CHCl_3$: isopropyl alcohol). The organic layer was washed with water four times (150 mL x 4), dried over Na_2SO_4, and concentrated *in vacuo*. The crude was subjected to MPLC (2% → 5% MeOH : 98% → 95% CH_2Cl_2) to afford the desired product as a yellow solid. [R_f – 0.25 (5% MeOH : 95% CH_2Cl_2)]. Yield: 10.1 g (56% over 3 steps);**^{1}H NMR** (400 MHz, $CDCl_3$, ppm): δ 8.74 (s, 1H), 8.38 (d, *J* = 9.6 Hz, 1H), 8.20 – 8.17 (m, 2H), 8.15 – 8.06 (m, 3H), 8.00 – 7.86 (m, 3H), 7.39 (s, 1H), 6.37 (q, *J* = 7.0 Hz, 1H), 5.23 – 5.18 (m, 2H), 4.91 (d, *J* = 7.8 Hz, 1H), 4.71 – 4.55 (m, 2H), 4.39 – 4.28 (m, 1H), 2.93 (s, 1H), 2.46 (td, *J* = 13.4, 6.6 Hz, 1H), 1.78 (d, *J* = 7.0 Hz, 3H), 1.55 (d, *J* = 5.9 Hz, 3H), 1.17 (d, *J* = 6.4 Hz, 3H), 1.12 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H); (**^{13}C NMR** (151 MHz, $CDCl_3$, ppm) δ 170.6, 170.0, 168.8, 168.5, 166.5, 166.3, 161.4, 161.0, 160.5, 153.0, 150.6, 150.4, 150.2, 149.5, 149.0, 141.2, 129.0, 128.4, 127.9, 126.8, 125.1, 124.0, 123.9, 122.7, 120.9, 118.3, 69.0, 67.8, 58.5, 55.8, 54.8, 34.0, 20.1, 19.9, 19.2, 18.9, 14.6; **Melting point:** 237.7-239.9 °C; **IR(neat):** 3388, 3114, 2969, 2931, 1654, 1579, 1530, 1478, 1429 (cm^{-1}); **HRMS:** Calcd for $C_{38}H_{36}BrN_{10}O_6S_5^+$ $[M+H]^+$ 955.0601, found 955.0628; $[\alpha]_D^{20}$: +31.0 (c=0.25, $CHCl_3$).

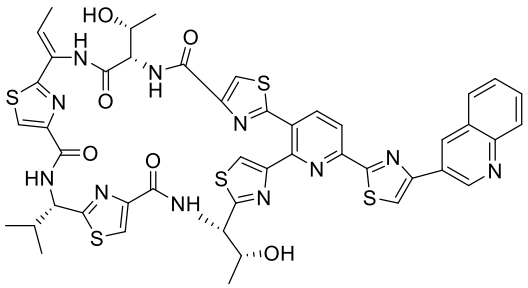
3. General Suzuki Coupling protocol and characterization of the products



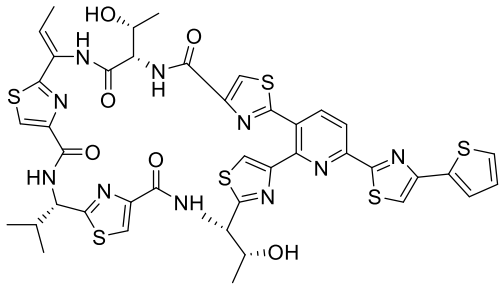
Compound **3** (0.031 mmol, 30 mg), a boronic acid (1.1 equiv) and K_2CO_3 (0.093 mmol, 12.8 mg, 3.0 equiv) were dissolved in the mixture of THF (0.25mL) / water (0.05 mL) and purged with N_2 for few minutes. Then, Pd(dtbpf)Cl_2 (0.0031 mmol, 2 mg, 10 mol %) was added and the mixture was brought up to 60 ºC and stirred overnight. The mixture was filtered through Celite, and the bed was further washed with ethyl acetate (5 mL). The organic layer was washed with 1M HCl (1 mL), dried over Na_2SO_4, and concentrated in *vacuo*. The crude was purified by MPLC (2% → 5% MeOH : 98% → 95% CH_2Cl_2) to afford the desired product.

Compound 22a: 70% yield, m.p. 233.9-237.8 ºC; $[\alpha]_D^{20}$: –3.3 (c=1, $CHCl_3$). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.46 – 8.43 (m, 1H), 8.40 – 8.34 (m, 2H), 8.26 (s, 1H), 8.17 (s, 1H), 8.15 – 8.12 (m, 3H), 8.11 (s, 1H), 8.08 (m, 3H), 7.98 (m, 2H), 7.79 (s, 1H), 6.40 (q, *J* = 6.9 Hz, 1H), 5.31 – 5.22 (m, 2H), 4.85 (d, *J* = 7.5 Hz, 1H), 4.65 – 4.61 (m, 1H), 4.49 – 4.45 (m, 1H), 3.95 (s, 3H), 3.81 – 3.49 (brs, 2H), 2.49 – 2.41 (m, 1H), 1.82 (d, *J* = 6.9 Hz, 3H), 1.54 (d, *J* = 6.3 Hz, 3H), 1.20 (d, *J* = 5.9 Hz, 3H), 1.13 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 170.0, 168.8, 168.0, 167.0, 166.7, 166.4, 161.4, 161.1, 160.6, 156.1, 151.7, 150.2, 149.4, 148.9, 140.9, 138.4, 130.4, 130.4, 129.9, 129.0, 128.6, 127.6, 126.4, 125.3, 124.2, 124.0, 122.5, 118.7, 118.5, 69.0, 67.8, 58.5, 55.8, 54.9, 52.4, 33.9, 29.8, 22.8, 20.0, 19.9, 19.2, 19.0, 14.6. **IR** (film): 3386, 3120, 2928, 1667, 1611, 1482, 1437, 1375, 1282. **HRMS**: Calcd for $C_{45}H_{43}N_{10}O_8S_5^+$ $[M+H]^+$ 1011.1863, found 1011.1867;.

Compound 22b: 68% yield, m.p. 246.0-247.7 °C; $[\alpha]_D^{20}$: +46.4 (c=0.25, DMSO). **^{1}H NMR** (600 MHz, DMSO-d_6)[6]: δ 9.66 (d, *J* = 2.4 Hz, 1H), 9.53 (s, 1H), 9.09 (s, 2H), 8.71 (s, 1H), 8.48 (s, 2H), 8.37 (s, 1H), 8.30 (s, 1H), 8.25 – 8.20 (m, 2H), 8.17 – 8.08 (m, 4H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.83 (ddd, *J* = 8.4, 6.7, 1.5 Hz, 1H), 7.70 (t, *J* = 7.5 Hz, 2H), 6.47 (q, *J* = 6.8 Hz, 1H), 5.20 – 5.05 (m, 2H), 4.69 (dd, *J* = 8.0, 3.6 Hz, 1H), 4.40 – 4.36 (m, 1H), 4.06 – 3.99 (m, 1H), 1.76 (d, *J* = 6.9 Hz, 3H), 1.38 (d, *J* = 6.4 Hz, 3H), 1.04 (d, *J* = 6.2 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO-d_6): δ 171.6, 170.0, 169.8, 168.5, 167.7, 166.3, 164.2, 160.5, 159.8, 153.0, 152.6, 151.0, 150.3, 149.6, 149.1, 148.5, 148.2, 146.5, 140.6, 133.0, 130.2, 129.6, 128.7, 128.4, 128.2, 127.7, 127.5, 127.0, 126.0, 125.3, 124.8, 124.4, 121.4, 120.0, 118.7, 67.5, 67.1, 59.8, 57.6, 56.1, 32.3, 20.6, 19.9, 19.6, 18.5, 13.8. **IR (film)**: 3385, 3120, 2972, 1665, 1485, 1376, 1205. **HRMS**: Calcd for $C_{46}H_{42}N_{11}O_6S_5^+$ $[M+H]^+$ 1004.1918, found 1004.1904.

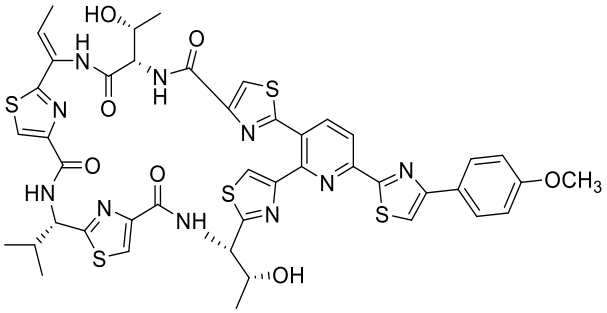


Compound 22c: 62% yield, m.p. 278.7-282.0 °C; $[\alpha]_D^{20}$: +15.3 (c=0.5, $CHCl_3$). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.46 (s, 1H), 8.37 (d, *J* = 8.9 Hz, 1H), 8.33 (d, *J* = 8.2, 1H), 8.23 (s, 1H), 8.18 (s, 1H), 8.16 – 8.11 (m, 1H), 8.09 (s, 1H), 7.99 – 7.81 (m, 3H), 7.54 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.52 (s, 1H), 7.33 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.11 (dd, *J* = 5.0, 3.6 Hz, 1H), 6.39 (q, *J* = 7.0 Hz, 1H), 5.29 – 5.22 (m, 2H), 4.84 (dd, *J* = 8.0, 2.1 Hz, 1H), 4.63 (m, 1H), 4.47 – 4.43 (m, 1H), 3.63 – 2.88 (brs, 2H), 2.45 (m, 1H), 1.81 (d, *J* = 7.0 Hz, 3H), 1.54 (d, *J* = 6.5 Hz, 3H), 1.19 (d, *J* = 6.3 Hz, 3H), 1.13 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 170.8, 170.0, 168.8, 167.6, 166.7, 166.3, 161.4, 161.0, 160.6, 153.1, 151.8, 151.6, 150.3, 149.8, 149.5, 149.0, 140.9, 138.2, 129.1, 128.3, 128.0, 127.4, 125.8, 125.0, 124.6, 124.0, 123.8, 122.5, 118.6, 115.3, 69.0, 67.8, 58.5, 55.8, 54.8, 33.9, 20.1, 19.9, 19.2, 18.9, 14.6. **IR (film)**: 3383, 2971, 1667, 1536, 1477. **HRMS**: Calcd for $C_{41}H_{39}N_{10}O_6S_6^+$ $[M+H]^+$ 959.1373, found 959.1374.

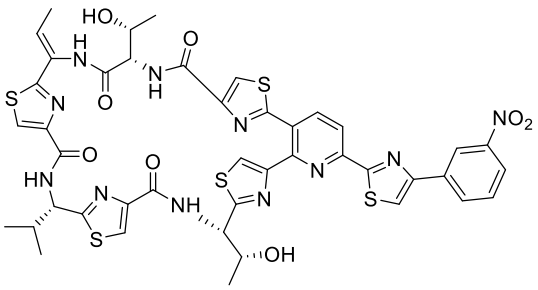


[6] Two exchangeable protons are invisible in this case

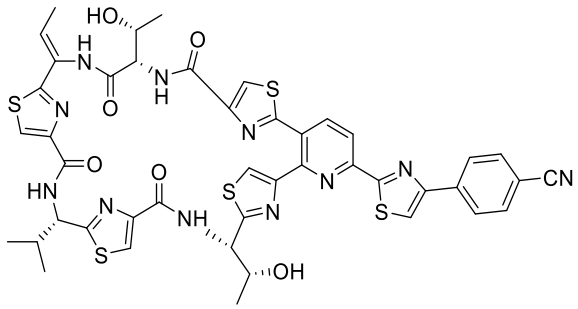
Compound 22d: 70% yield, m.p. 222.8-226.7 °C; $[\alpha]_D^{20}$: +1.6 (c=0.5, $CHCl_3$). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.40 (d, *J* = 8.1 Hz, 1H), 8.39 – 8.34 (m, 2H), 8.26 – 8.22 (m, 1H), 8.21 (s, 1H), 8.18 – 8.10 (m, 1H), 8.09 (s, 1H), 7.96 – 7.95 (m, 2H), 7.94 (m, 1H), 7.92 – 7.89 (m, 2H), 7.54 (s, 1H), 7.06 – 6.97 (m, 2H), 6.40 (q, *J* = 7.0 Hz, 1H), 5.31 – 5.23 (m, 2H), 4.81 (dd, *J* = 8.1, 2.0 Hz, 1H), 4.68 – 4.59 (m, 1H), 4.46 – 4.40 (m, 1H), 3.87 (s, 3H), 2.44 (m, 1H), 1.82 (d, *J* = 7.0 Hz, 3H), 1.53 (d, *J* = 6.5 Hz, 3H), 1.20 (d, *J* = 6.4 Hz, 3H), 1.13 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 170.6, 170.0, 168.8, 167.5, 166.9, 166.4, 161.4, 161.1, 160.5, 160.1, 157.1, 153.1, 151.9, 150.4, 149.6, 149.0, 141.0, 129.8, 129.0, 128.3, 127.9, 127.3, 125.0, 124.0, 123.8, 122.6, 118.6, 115.0, 114.4, 114.0, 69.1, 67.8, 58.5, 55.8, 55.5, 54.7, 34.0, 20.2, 19.9, 19.2, 18.9, 14.6. **IR (film)**: 3386, 2968, 1667, 1612, 1534, 1467, 1418, 1304, 1249; **HRMS**: Calcd for $C_{44}H_{43}N_{10}O_7S_5^+$ $[M+H]^+$ 983.1914, found 983.1916.



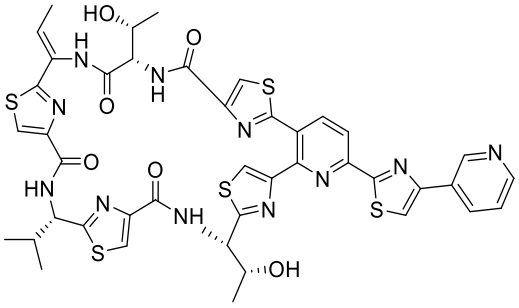
Compound 22e: 65% yield, m.p. 234.7-238.9 °C; $[\alpha]_D^{20}$: +7.43 (c=1, $CHCl_3$). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.86 (t, *J* = 2.0 Hz, 1H), 8.50 (s, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 8.35 (d, *J* = 9.8 Hz, 1H), 8.31 (dt, *J* = 7.8, 1.3 Hz, 1H), 8.25 – 8.19 (m, 2H), 8.15 (s, 1H), 8.10 (s, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.97 (s, 1H), 7.94 – 7.90 (m, 2H), 7.83 (s, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 6.41 (q, *J* = 6.9 Hz, 1H), 5.33 (d, *J* = 9.0 Hz, 1H), 5.25 (t, *J* = 9.2 Hz, 1H), 4.87 (d, *J* = 8.0, 1H), 4.66 – 4.62 (m, 1H), 4.52 (d, *J* = 6.3 Hz, 1H), 3.61 (br s, 2H), 2.45 (h, *J* = 6.8 Hz, 1H), 1.82 (d, *J* = 7.0 Hz, 3H), 1.56 (d, *J* = 6.5 Hz, 3H), 1.20 (d, *J* = 6.3 Hz, 3H), 1.13 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 171.4, 170.0, 168.8, 168.3, 166.4, 161.4, 161.2, 161.0, 160.5, 154.6, 152.7, 151.5, 150.3, 149.5, 149.1, 148.9, 140.8, 136.0, 132.1, 130.0, 130.0, 129.0, 128.6, 127.9, 125.2, 124.2, 123.8, 123.1, 122.4, 121.4, 118.8, 118.5, 69.0, 67.7, 58.4, 55.8, 55.0, 33.9, 20.0, 19.9, 19.3, 19.1, 14.6. **IR (film)**: 3394, 3119, 2970, 1663, 1534, 1483, 1419, 1349, 1246. **HRMS**: Calcd for $C_{43}H_{40}N_{11}O_8S_5^+$ $[M+H]^+$ 998.1659, found 998.1653.



Compound 22f: 67% yield, m.p. 246.3-245.7 °C; $[\alpha]_D^{20}$: -1.44 (c=0.46, $CHCl_3$). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.39 (d, 1H), 8.35 (s, 1H), 8.32 (d, 1H), 8.25 (s, 1H), 8.22 (s, 1H), 8.15 – 8.07 (m, 4H), 8.00 – 7.88 (m, 3H), 7.82 (s, 1H), 7.79 – 7.74 (m, 2H), 6.40 (qd, *J* = 6.8, 2.3 Hz, 1H), 5.28 – 5.19 (m, 2H), 4.83 (dd, *J* = 8.1, 2.1 Hz, 1H), 4.64 (td, *J* = 6.8, 2.2 Hz, 1H), 4.39 – 4.35 (m, 1H), 4.15 (d, *J* = 7.3 Hz, 1H), 3.08 (d, *J* = 3.1 Hz, 1H), 2.49 – 2.41 (m, 1H), 1.82 (d, *J* = 7.0 Hz, 3H), 1.54 (d, *J* = 6.5 Hz, 3H), 1.19 (d, *J* = 6.3 Hz, 3H), 1.13 (d, 3H), 0.96 (d, *J* = 6.5 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 170.3, 170.0, 168.8, 168.5, 166.7, 166.3, 161.4, 161.0, 160.5, 155.1, 153.3, 151.5, 150.5, 150.3, 149.6, 149.0, 141.2, 138.4, 132.9, 129.1, 128.3, 127.6, 127.0, 124.9, 123.9, 123.8, 122.7, 119.1, 119.0, 118.3, 111.8, 69.1, 67.8, 58.5, 55.8, 54.7, 33.9, 29.8, 20.5, 20.2, 19.9, 19.2, 18.8, 14.6. **IR (film)**: 3402, 2969, 2923, 2227, 1666, 1537, 1484, 1420, 1208. **HRMS**: Calcd for $C_{44}H_{40}N_{11}O_6S_5^+$ $[M+H]^+$ 978.1761, found 978.1776.



Compound 22g: 64% yield, m.p. 230.7-233.9 °C; $[\alpha]_D^{20}$: +49.3 (c=0.5, CH_3OH). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.17 (s, 1H), 8.56 (d, *J* = 3.5 Hz, 1H), 8.45 (s, 1H), 8.34 (t, *J* = 9.9 Hz, 1H), 8.25 – 8.11 (m, 4H), 8.09 – 7.94 (m, 3H), 7.91 – 7.82 (m, 2H), 7.67 (s, 1H), 7.27 (dd, *J* = 7.8, 4.9 Hz, 1H), 6.31 (q, *J* = 6.9 Hz, 1H), 5.29 – 5.07 (m, 2H), 4.79 (d, *J* = 8.3 Hz, 1H), 4.62 – 4.49 (m, 1H), 4.34 – 4.18 (m, 1H), 3.07 (s, 1H), 2.39 (dd, *J* = 14.8, 7.0 Hz, 1H), 1.75 (d, *J* = 7.0 Hz, 3H), 1.48 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.06 (d, *J* = 6.7 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$): δ 170.3, 170.0, 168.9, 166.8, 166.3, 161.3, 161.1, 160.5, 153.2, 151.5, 150.5, 150.2, 149.6, 149.0, 147.9, 143.2, 142.9, 141.2, 134.6, 130.6, 129.1, 128.3, 127.5, 125.0, 124.1, 123.9, 123.8, 122.7, 118.3, 118.1, 69.2, 67.8, 58.6, 55.8, 54.7, 34.0, 20.5, 20.3, 19.9, 19.2, 18.8, 14.6. **IR (film)**: 2972, 2901, 1664, 1536, 1478, 1408, 1249. **HRMS**: Calcd for $C_{42}H_{40}N_{11}O_6S_5^+$ $[M+H]^+$ 954.1761, found 954.1783.



Compound 22h: 61% yield, m.p. 238.8-242.5 °C; $[\alpha]_D^{20}$: +76.4 (c=0.25, DMSO). **^{1}H NMR** (400 MHz $CDCl_3$)[7]: δ 9.13 (s, 2H), 8.57 (s, 1H), 8.43 (d, *J* = 9.6 Hz, 1H), 8.34 – 8.30 (m, 2H), 8.26 (s, 1H), 8.22 (s, 1H), 8.16 – 8.09 (m, 3H), 7.99 (d, *J* = 7.0 Hz, 1H), 7.69 (s, 1H), 6.41 (q, *J* = 6.9 Hz, 1H), 5.31 – 5.21 (m, 2H), 4.89 (dd, *J* = 8.0, 1.6 Hz, 1H), 4.72 – 4.65 (m, 1H), 4.42 (dd, *J* = 15.5, 6.6 Hz, 1H), 4.10 (s, 3H), 2.48 (td, *J* = 13.3, 6.5 Hz, 1H), 2.12 – 1.96 (m, 1H), 1.89 – 1.77 (m, 3H), 1.57 (d, *J* = 6.5 Hz, 3H), 1.21 (d, *J* = 6.4 Hz, 3H), 1.15 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.3, 169.9, 168.7, 168.6, 166.6, 166.2, 165.3, 161.2, 160.9, 160.4, 157.1, 153.2, 151.3, 151.1, 150.3, 150.3, 149.5, 148.9, 141.0, 129.0, 128.1, 127.4, 124.8, 123.9, 123.7, 122.5, 118.2, 116.4, 68.9, 67.6, 63.7, 58.3, 55.7, 55.2, 54.7, 33.8, 19.8, 19.8, 19.2, 18.7, 14.5. **IR (film)**: 3368, 3119, 1667, 1603, 1539, 1482, 1404, 1330, 1219. **HRMS**: Calcd for $C_{42}H_{41}N_{12}O_7S_5^+$ $[M+H]^+$ 985.1819, found 985.1834.

[7] Two exchangeable protons are invisible in this case

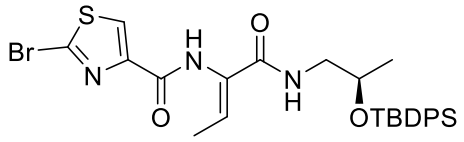
4. Total Synthesis of Micrococcins P2 and P1 by Suzuki Coupling

tert-Butyl ((2*S*,3*R*)-1-(((*R*)-2-((tert-butyldiphenylsilyl)oxy)propyl)amino)-3-hydroxy-1-oxobutan-2-yl)carbamate (27). N-Boc-L-threonine (9.20 mmol, 2.00 g) and (*R*)-2-((tert-butyldiphenylsilyl)oxy)propan-1-amine (11.0 mmol, 3.46 g, 1.2 equiv) was dissolved in CH_2Cl_2 (40 mL). EDCI (11.0 mmol, 2.10 g, 1.2 equiv), HOBt (11.0 mmol, 1.48 g, 1.2 equiv) and diisopropylethylamine (18.4 mmol, 3.20 mL, 2 equiv) was added and the mixture was stirred at room temperature for 2 hours. The mixture was quenched by the addition of water (10 mL), and further extracted with CH_2Cl_2 (40 mL). The crude mixture was purified by MPLC (20% → 40% ethyl acetate : 80% → 60% hexane) to afford the desired product as a white solid. [R_f – 0.50 (50% hexane : 50% ethyl acetate)]. Yield: 2.80 g (60%). **^{1}H NMR** (600 MHz, $CDCl_3$, ppm): δ 7.69 (m, 4H), 7.49 – 7.44 (m, 2H), 7.43 – 7.39 (m, 4H), 6.89 (brs, 1H), 5.47 (d, *J* = 7.9 Hz, 1H), 4.34 (qd, *J* = 6.4, 2.0 Hz, 1H), 4.05 – 3.97 (m, 2H), 3.34 – 3.30 (m, 1H), 3.24 (dt, *J* = 13.5, 4.5 Hz, 1H), 2.87 (brs, 1H), 1.48 (s, 9H), 1.18 (d, *J* = 6.4 Hz, 3H), 1.10 (s, 9H), 1.06 (d, *J* = 6.2 Hz, 3H); **^{13}C NMR** (151 MHz, $CDCl_3$, ppm): δ 172.0, 156.7, 135.9, 135.9, 134.1, 133.8, 130.0, 129.9, 127.9, 127.8, 80.4, 68.2, 66.8, 58.1, 46.4, 28.4, 27.1, 20.9, 19.3, 18.5. **IR (film):** 3337, 3070, 2968, 2931, 2893, 2859, 1814, 1658, 1488 (cm^{-1}); **HRMS:** Calcd for $C_{28}H_{43}N_2O_5Si^+$ $[M+H]^+$ 515.2936, found 515.2923. $[\alpha]_D^{20}$ = –3.7 (c = 1.0, $CHCl_3$).

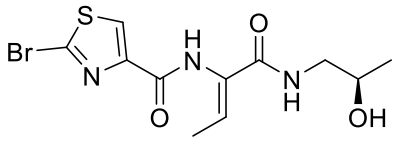
2-Bromo-*N*-((2*S*,3*R*)-1-(((*R*)-2-((tert-butyldiphenylsilyl)oxy)propyl)amino)-3-hydroxy-1-oxobutan-2-yl)thiazole-4-carboxamide (28). 2-bromothiazole-4-carboxylic acid (8 mmol, 1.66 g) was added to the solution containing (2*S*,3*R*)-2-amino-N-((*R*)-2-((tert-butyldiphenylsilyl)oxy)propyl)-3-hydroxybutanamide (which was synthesized by exposing **compound 27** in CH_2Cl_2 / TFA and used immediately) (8.8 mmol, 3.64 g, 1.1 equiv) and diisopropylethylamine (24 mmol, 4.20 mL, 3 equiv) in CH_2Cl_2 (20 mL). EDCI (8.8 mmol, 1.68 g, 1.1 equiv) and HOBt (8.8 mmol, 1.19 g, 1.1 equiv) were added, and the mixture was stirred at room temperature overnight. The mixture was quenched by the addition of water (10 mL), and extracted with CH_2Cl_2 (30 mL). The organic layer was then dried with Na_2SO_4 and concentrated *in vacuo*. The crude was subjected to MPLC (20% → 50% ethyl acetate: 80% → 50% hexane) to afford the desired product as a yellow foam. [R_f – 0.25 (45% ethyl acetate : 55% hexane)]. Yield: 3.00 g (63%) **^{1}H NMR** (600 MHz, $CDCl_3$, ppm) δ 8.08 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.69 – 7.65 (m, 4H), 7.48 – 7.44 (m, 2H), 7.42 – 7.37 (m, 4H), 6.92 – 6.90 (m, 1H), 4.44 (qd, *J* = 6.5, 1.9 Hz, 1H), 4.39 (dd, *J*

= 8.4, 1.9 Hz, 1H), 4.02 – 3.97 (m, 1H), 3.55 (brs, 1H), 3.29 (app.t, *J* = 5.3 Hz, 2H), 1.22 (d, *J* = 6.5 Hz, 3H), 1.05 (s, 9H), 1.03 (d, *J* = 6.5 Hz, 3H); **^{13}C NMR** (151 MHz, $CDCl_3$, ppm): δ 171.3, 160.8, 149.2, 136.4, 136.0, 135.9, 134.1, 133.9, 130.0, 129.9, 127.9, 127.8, 127.7, 68.3, 66.5, 56.8, 46.6, 27.1, 21.1, 19.3, 18.6; **IR** (Cast Film): 3375, 3071, 2931, 2857, 1734, 1652, 1533 (cm^{-1}); **HRMS**: Calcd for $C_{27}H_{35}BrN_3O_4SSi^+$ $[M+H]^+$ 604.1295, found 604.1281; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = -45.0 (c=0.1, $CHCl_3$).

(*R*,*Z*)-2-Bromo-*N*-(1-((2-((tert-butyldiphenylsilyl)oxy)propyl)amino)-1-oxobut-2-en-2-yl)thiazole-4-carboxamide (29). To a solution of 2-bromo-N-((2*S*,3*R*)-1-(((*R*)-2-((tert-butyldiphenylsilyl)oxy)propyl)amino)-3-hydroxy-1-oxobutan-2-yl)thiazole-4-carboxamide **(28)** (5.00 mmol, 1.50 g) in CH_2Cl_2 (40 mL) was added methanesulfonyl chloride (14.9 mmol, 1.15 mL, 3 equiv) and Et_3N (14.9 mmol, 2.10 mL, 3 equiv). The mixture was stirred at room temperature for 1 hour. Then, DBU (74.8 mmol, 11.2 mL, 15 equiv) was added and the mixture was further stirred at room temperature for 2 hours. The reaction was quenched by the addition of sat. aq. NH_4Cl (40 mL), and extracted with CH_2Cl_2 (100 mL). The organic layer was dried over Na_2SO_4. The crude mixture was purified by MPLC (30% → 50% ethyl acetate: 70 → 50% hexane) to afford the desired product as a white foam. [R_f – 0.38 (50% ethyl acetate : 50% hexane)] Yield: 2.20 g (75%) **^{1}H NMR** (600 MHz, $CDCl_3$, ppm): δ 8.32 (s, 1H), 8.12 (s, 1H), 7.66 – 7.64 (m, 4H), 7.48 – 7.41 (m, 2H), 7.40 – 7.37 (m, 4H), 6.53 (q, *J* = 7.1 Hz, 1H), 6.39 (app.t, *J* = 5.3 Hz, 1H), 4.05 – 4.01 (m, 1H), 3.41 (ddd, *J* = 13.6, 5.4, 3.6 Hz, 1H), 3.33 (dt, *J* = 13.6, 5.9 Hz, 1H), 1.79 (dd, *J* = 7.1, 0.7 Hz, 3H), 1.10 (d, *J* = 6.2 Hz, 3H), 0.99 (s, 9H); **^{13}C NMR** (151 MHz, $CDCl_3$, ppm): δ 164.3, 158.1, 149.3, 136.4, 135.9, 135.8, 133.9, 133.9, 130.2, 130.0, 129.9, 129.1, 128.1, 128.0, 127.8, 68.8, 46.8, 27.0, 21.2, 19.3, 14.1; **IR** (Cast Film): 3351, 3070, 2929, 2855, 1666, 1639, 1522 cm^{-1}; **HRMS**: Calcd for $C_{27}H_{33}BrN_3O_3SSi^+$ $[M+H]^+$ 586.1190, found 586.1198. $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +18.3 (c=0.25, $CHCl_3$).

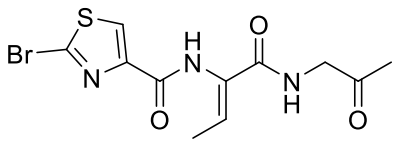


(*R*,*Z*)-2-Bromo-*N*-(1-((2-hydroxypropyl)amino)-1-oxobut-2-en-2-yl)thiazole-4-carboxamide (24). To a solution of (*R*,*Z*)-2-bromo-N-(1-((2-((tert-butyldiphenylsilyl)oxy)propyl)amino)-1-oxobut-2-en-2-yl)thiazole-4-carboxamide **(29)** (3.74 mmol, 2.20 g) in THF (30 mL) was added a 1 M solution of TBAF in THF (6.74 mmol, 6.74 mL, 1.8 equiv) and the mixture was stirred for 30 minutes before being diluted with ethyl acetate (80 mL) and sat. aq. NH_4Cl (40 mL). The organic layer was extracted, dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by MPLC (2% → 5%

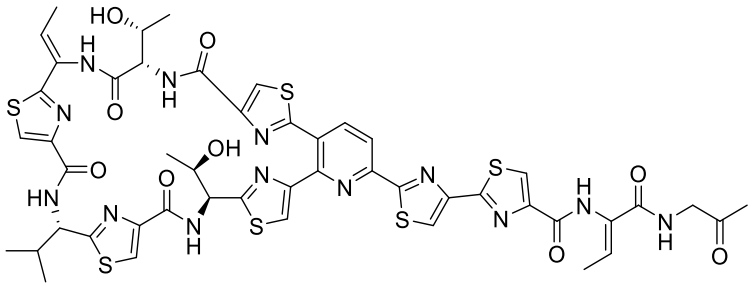


MeOH: 98% → 95% CH_2Cl_2) to afford the desired product as a colorless oil. [R_f – 0.23 (5% MeOH: 95% CH_2Cl_2)]. Yield: 800 mg (61%) **^{1}H NMR** (600 MHz, CD_3OD, ppm): δ 8.30 (d, *J* = 0.9 Hz, 1H), 6.71 (q, *J* = 7.1 Hz, 1H), 3.94 – 3.85 (m, 1H), 3.30 (dd, *J* = 13.5, 4.7 Hz, 1H), 3.20 (dd, *J* = 13.4, 7.1 Hz, 1H), 1.79 (d, *J* = 7.1 Hz, 3H), 1.16 (d, *J* = 6.3 Hz, 3H). Exchangeable protons (3H in total) are invisible in this case due to the deuterated methanol used; **^{13}C NMR** (151 MHz, CD_3OD, ppm): δ 167.2, 161.0, 150.4, 137.8, 132.2, 131.0, 130.2, 67.4, 48.1, 20.8, 13.7; **IR** (Cast Film): 3312, 2970, 2929, 1657, 1628, 1523 cm^{-1}; **HRMS**: Calcd for $C_{11}H_{15}BrN_3O_3S^+$ $[M+H]^+$ 348.0012, found 348.0008; $[\alpha]_D^{20}$ = -12.2 (c=0.5, CH_3OH).

(*Z*)-2-Bromo-N-(1-oxo-1-((2-oxopropyl)amino)but-2-en-2-yl)thiazole-4-carboxamide (25). To a solution of (*R*,*Z*)-2-bromo-N-(1-((2-hydroxypropyl)amino)-1-oxobut-2-en-2-yl)thiazole-4-carboxamide **(24)** (1.12 mmol, 386 mg) in CH_2Cl_2 (10 mL) was added Dess-Martin Periodinane (1.66 mmol, 706 mg, 1.5 equiv). The mixture was stirred at room temperature for 2 hours. The mixture was diluted with CH_2Cl_2 (20 mL) before being washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo.* The crude mixture was purified by MPLC (2% → 5% MeOH: 98% → 95% CH_2Cl_2) to afford the desired product as a white gum. [R_f – 0.33 (5% MeOH: 95% CH_2Cl_2)]. Yield: 356 mg (89%); **^{1}H NMR** (600 MHz, CD_3OD, ppm): δ 8.30 (s, 1H), 6.77 (q, *J* = 7.1 Hz, 1H), 4.07 (s, 2H), 2.18 (s, 3H), 1.81 (d, *J* = 7.1 Hz, 3H). Exchangeable protons (2H in total) are invisible in this case due to the deuterated methanol used; **^{13}C NMR** (151 MHz, CD_3OD, ppm): δ 206.5, 167.2, 161.0, 150.4, 137.8, 133.2, 130.2, 127.9, 50.6, 27.0, 13.8; **IR** (Cast Film): 3401, 3347, 3083, 2937, 2908, 1712, 1679, 1642, 1530 (cm^{-1}); **HRMS**: Calcd for $C_{11}H_{13}BrN_3O_3S^+$ $[M+H]^+$ 345.9856, found 345.9849.

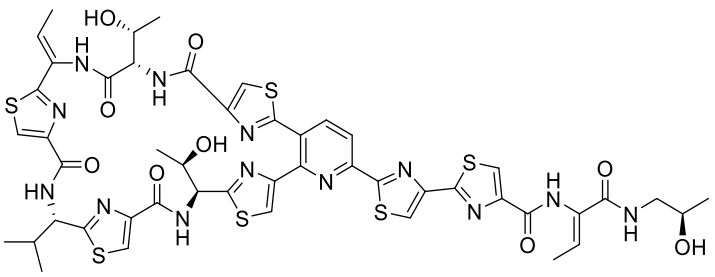


Micrococcin P2 (1) To a solution of (12*Z*,32*Z*,72*Z*,112*Z*,4*S*,8*S*,12*Z*,15*S*)-26-(4-bromothiazol-2-yl)-12-ethylidene-4,15-bis((*R*)-1-hydroxyethyl)-8-isopropyl-5,9,13,16-tetraaza-1(2,4),3,7,11(4,2)-tetrathiazola-2(3,2)-pyridinacycloheptadecaphane-6,10,14,17-tetraone **(3)** (1.30 mmol, 1.24 g) in dioxane (10 mL) was added B_2Pin_2 (1.69 mmol, 428 mg, 1.3 equiv), potassium acetate (1.95 mmol, 191 mg, 1.5 equiv), XPhos (0.195 mmol, 93.0 mg, 0.15 equiv) and purged with N_2 for few minutes. Then, $Pd_2(dba)_3$ (0.130 mmol, 119



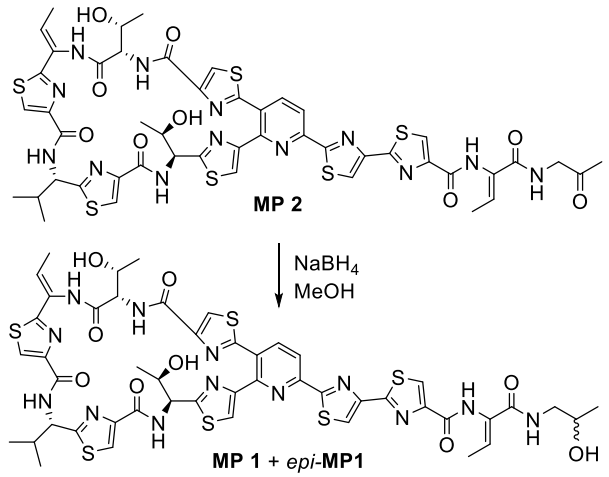
mg, 0.10 equiv) was added and the mixture was stirred at 90 ºC for 1.5 hours. The mixture was cooled to room temperature, diluted with CH_2Cl_2 (30 mL) and washed with H_2O (10 mL). The organic layer was collected and filtered through Celite. Then, the filtrate was dried over Na_2SO_4 and concentrated *in vacuo*. The resulting crude boronic acid is immediately treated with (1.30 mmol) compound **25** (1.43 mmol, 494 mg, 1.1 equiv), K_2CO_3 (3.90 mmol, 539 mg, 3 equiv) in THF (10 mL) / H_2O (2.5 mL) and purged with N_2 for few minutes. Then, Pd(dtbpf)Cl_2 (0.13 mmol, 84.7 mg, 0.1 equiv) was added and the mixture was brought up to 60 ºC and stirred for 12 hours. It was cooled to room temperature and was filtered through Celite, and the bed was further washed with CH_2Cl_2 (50 mL). The organic layer was washed with 1M HCl (1 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude was subjected to MPLC (2% → 5% MeOH : 98% → 95% CH_2Cl_2) to afford the desired product as a white solid. [R_f – 0.34 (5% MeOH : 95% CH_2Cl_2)]. Yield: 890 mg (60% over 2 steps) **^{1}H NMR** (600 MHz, CD_3OD, ppm): δ 8.40 – 8.31 (m, 4H), 8.27 (s, 1H), 8.22 (s, 1H), 8.13 (s, 1H), 7.96 (s, 1H), 6.82 (q, *J* = 7.1 Hz, 1H), 6.60 (q, *J* = 6.8 Hz, 1H), 5.24 (d, *J* = 2.9 Hz, 1H), 5.22 (d, *J* = 8.8 Hz, 1H), 4.78 (d, *J* = 3.1 Hz, 1H), 4.62 – 4.55 (m, 1H), 4.30 (qd, *J* = 6.3, 2.8 Hz, 1H), 4.10 (d, *J* = 13.0 Hz, 1H), 2.63 (dq, *J* = 13.4, 6.7 Hz, 1H), 2.19 (s, 3H), 2.20 – 2.14 (m, 1H), 1.92 (s, 1H) 1.86 (app. d, *J* = 7.0 Hz, 6H), 1.55 (d, *J* = 6.4 Hz, 3H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.13 (d, *J* = 6.7 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H). Exchangeable protons (3H in total) are invisible in this case due to the deuterated methanol used. **^{13}C NMR** (151 MHz, CD_3OD, ppm): δ 206.6, 172.4, 171.6, 171.0, 170.1, 168.0, 167.3, 166.2, 164.0, 163.2, 162.5, 162.5, 162.3, 155.2, 152.9, 151.8, 151.2, 151.2, 151.1, 150.4, 149.7, 141.3, 133.2, 131.1, 130.8, 130.6, 130.2, 126.5, 126.4, 125.8, 125.4, 122.3, 121.8, 120.0, 69.6, 69.2, 59.3, 57.4, 57.4, 50.6, 34.4, 27.1, 20.8, 20.7, 20.2, 19.3, 14.3, 13.9; **Melting point:** above 360 ℃, **IR (film):** 2923, 2851, 1590, 1464, 1352, 1134 cm^{-1} **HRMS:** Calcd for $C_{48}H_{48}N_{13}O_9S_6^+$ $[M+H]^+$ 1142.2017, found 1142.2045. $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = +120.0 (c = 0.1, CD_3OD).

Micrococcin P1 (2). To a solution of (12*Z*,32*Z*,72*Z*,112*Z*,4*S*,8*S*,12*Z*,15*S*)-26-(4-bromothiazol-2-yl)-12-ethylidene-4,15-bis((*R*)-1-hydroxyethyl)-8-isopropyl-5,9,13,16-tetraaza-1(2,4),3,7,11(4,2)-tetrathiazola-2(3,2)-pyridinacycloheptadecaphane-6,10,14,17-tetraone (1.30 mmol, 1.24 g) in dioxane (10 mL) was added B_2Pin_2 (1.69 mmol, 428 mg, 1.3 equiv), potassium acetate (1.95 mmol, 191 mg, 1.5 equiv), XPhos (0.195 mmol,



93.0 mg, 0.15 equiv) and purged with N_2 for few minutes. Then, $Pd_2(dba)_3$ (0.130 mmol, 119 mg, 0.10 equiv) was added and the mixture was stirred at 90 °C for 1.5 hours. The mixture was cooled to room temperature, diluted with CH_2Cl_2 (30 mL) and washed with H_2O (10 mL). The organic layer was collected and filtered through Celite. Then, the filtrate was dried over Na_2SO_4 and concentrated *in vacuo*. The resulting crude boronic acid is immediately treated with (1.30 mmol) compound **24** (1.43 mmol, 496 mg, 1.1 equiv), K_2CO_3 (3.90 mmol, 539 mg, 3 equiv) in THF (10 mL) / H_2O (2.5 mL) and purged with N_2 for few minutes. Then, Pd(dtbpf)Cl_2 (0.13 mmol, 84.7 mg, 0.1 equiv) was added and the mixture was brought up to 60 °C and stirred for 12 hours. It was cooled to room temperature and was filtered through Celite, and the bed was further washed with CH_2Cl_2 (50 mL). The organic layer was washed with 1M HCl (1 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude was subjected to MPLC (2% → 5% MeOH: 98% → 95% CH_2Cl_2) to afford the desired product as a white solid. [R_f – 0.30 (5% MeOH: 95% CH_2Cl_2)]. Yield: 892 mg (60% over 2 steps). This is synthesized to double confirm the feasibility of Suzuki coupling method to make MP1. **^{1}H NMR** (600 MHz, DMSO-d_6, ppm): δ 9.53 (br s, 1H), 9.52 (br s, 1H), 8.60 (s, 1H), 8.47 (d, J = 8.1 Hz, 2H), 8.45 (s, 1H), 8.38 (s, 1H) , 8.35 (d, J = 8.1 Hz, 1H), 8.30 (s, 1H), 8.24 (s, 1H), 8.21 (s, 1H), 8.12 (s, 1H), 7.92 (t, J = 5.6 Hz, 1H), 7.87 (d, J = 7.5 Hz, 1H), 6.53 – 6.46 (m, 2H), 5.16 – 5.12 (m,1 H), 5.11 – 5.08 (m, 1H), 4.70 (dd, J = 7,2 Hz, 3.0 Hz 1H), 4.41 – 4.34 (m, 2H), 4.03 – 3.96 (m, 2H), 3.74 – 3.69 (m, 2H), 3.12 – 2.95 (m, 2H), 1.75 (d, J = 6.9 Hz, 3H), 1.71 (d, J = 7.0 Hz, 3H), 1.39 (d, J = 5.9 Hz, 3H), 1.04 (d, J = 6.2 Hz, 3H), 1.03 (d, J = 6.2 Hz, 6H), 0.98 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H). Exchangeable protons were not detected in this case.

Sodium Borohydride Reduction of Micrococcin P2. To a solution of micrococcin P2 (20 mg, 0.018 mmol) in the mixture of CH_2Cl_2 / MeOH (1:1 v/v 100 µL:100 µL) was added $NaBH_4$ (4 eq, 0.070 mmol, 2.6 mg) at -78 °C. The mixture was stirred for 10 minutes and brought up to room temperature. The reaction was quenched by the addition of water, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was collected, dried over Na_2SO_4, and concentrated *in vacuo* to afford the desired product. Yield: 10 mg (50%).



Hydrolytic cleavage of MP2: 2'-((12Z,32Z,72Z,112Z,4S,8S,12Z,15S)-12-ethylidene-4,15-bis((R)-1-hydroxyethyl)-8-isopropyl-6,10,14,17-tetraoxo-5,9,13,16-tetraaza-1(2,4),3,7,11(4,2)-tetrathiazola-2(3,2)-pyridinacycloheptadecaphane-26-yl)-[2,4'-bithiazole]-4-carboxylic acid (30). To a solution of micrococcin P2 (23 mg, 0.020 mmol) in a mixture of THF / H_2O (1 : 1 v/v 100 µL : 100 µL) was added LiOH · H_2O (8 eq, 0.160 mmol, 6.7 mg), and the mixture was stirred at 50 ºC for 16 hours. Upon completion, the mixture was quenched by the addition of 1M HCl (300 µL). The mixture was transferred to a separatory funnel, and organic compounds were extracted with 1 mL x 2 super solvent (4:1 $CHCl_3$: isopropyl alcohol). There was thus obtained 14.8 mg (74%) of carboxylic acid **30** as a white solid, m.p. 258.9-261.6 ℃; $[\alpha]_D^{20}$: +42.0 (c=0.25, MeOH). No purification was necessary. Yield: 14.8 mg (74%). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) δ 9.55 (s, 1H), 8.59 – 8.51 (s, 2H), 8.50 – 8.35 (m, 3H), 8.35 – 8.27 (m, 2H), 8.28 – 8.17 (m, 2H), 8.13 (s, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 6.47 (q, *J* = 6.5 Hz, 1H), 5.20 – 4.96 (m, 2H), 4.73 – 4.63 (m, 1H), 4.42 – 4.33 (m, 1H), 4.04 – 3.96 (m, 1H), 1.76 (d, *J* = 6.3 Hz, 3H), 1.38 (d, *J* = 6.0 Hz, 3H), 1.00 (dd, *J* = 25.3, 6.1 Hz, 6H), 0.86 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR** (100 MHz, DMSO-*d*$_6$): δ 170.4, 170.3, 169.0, 168.6, 166.8, 164.5, 162.5, 162.3, 160.9, 160.2, 152.9, 151.5, 150.2, 150.0, 149.7, 149.5, 148.7, 148.7, 141.1, 130.0, 129.8, 129.1, 129.1, 126.5, 125.2, 124.8, 122.1, 122.0, 119.1, 68.0, 67.5, 58.1, 56.5, 55.9, 32.7, 21.1, 20.3, 20.1, 18.9, 14.2. **HRMS**: Calcd for $C_{42}H_{41}N_{12}O_7S_5^+$ $[M+H]^+$ 1004.1224, found 1004.1216.

^{1}H NMR Comparison of Synthetic and authentic MP1

Measured ^{1}H shifts (ppm, natural MP1[8])	Measured ^{1}H shifts (ppm, synthetic, this work)
9.53[a] (br s, 1H)	9.53 (br s, 1H)
9.48[a] (br s, 1H)	9.52 (br s, 1H)
8.59 (s, 1H)	8.60 (s, 1H)
8.46 (d, 1H)	8.47 (d, 1H)
8.46 (s, 1H)	8.45 (s, 1H)
8.38 (s, 1H)	8.38 (s, 1H)
8.37 (s, 1H)	8.37 (s, 1H)
8.35 (d, 1H)	8.35 (d, 1H)
8.30 (s, 1H)	8.30 (s, 1H)

[8] Data from: Lefranc, D. *Dissertation*, University of British Columbia, 2008. See also: Fenet, B.; Pierre, F.; Cundliffe, E.; Ciufolini, M. A. *Tetrahedron Lett.* **2002**, *43*, 2367.

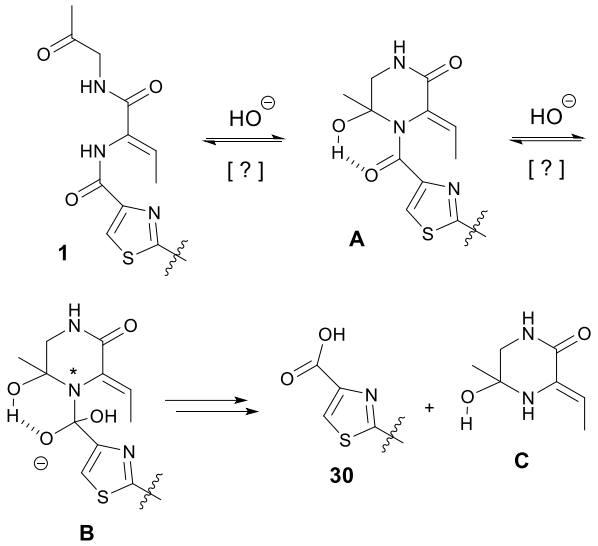
8.22[a] (s, 1H)	8.24 (s, 1H)
8.21 (s, 1H)	8.21 (s, 1H)
8.12 (s, 1H)	8.12 (s, 1H)
7.88[a] (br t, 1H)	7.92 (br t, 1H)
7.86[a] (br d, 1H)	7.87 (br d, 1H)
6.52 (q, 1H)	6.53 – 6.46 (m, 2H)
6.48 (q, 1H)	
5.42[b] (d, 1H)	not observed
5.15 (dd, 1H)	5.16-5.12 (m, 1H)
5.09 (dd, 1H)	5.11-5.08 (m, 1H)
4.80[b] (br d, 1H)	not observed
4.71 (dd, 1H)	4.70 (dd, 1H)
4.67[b] (d, 1H)	not observed
4.39 (m, 1H)	4.38 (m, 1H)
4.03 (m, 1H)	4.02 (m, 1H)
3.72 (m, 1H)	3.71 (m, 1H)
3.09 (m, 2H)	3.08 (m, 2H)
2.51 (m, 1H) – peak hidden in DMSO	peak hidden in DMSO
1.76 (d, 3H)	1.75 (d, 3H)
1.72 (d, 3H)	1.71 (d, 3H)
1.39 (d, 3H)	1.39 (d, 3H)
1.04 (d, 3H)	1.04 (d, 3H)
1.03 (d, 3H)	1.03 (d, 3H)
0.98 (d, 3H)	0.98 (d, 3H)
0.87 (d, 3H)	0.87 (d, 3H)

5. Mechanistic hypothesis for the difference in hydrolytic behavior between micrococcin P2 and micrococcin P1

The difference in question must be ascribed to the presence of the keto group in MP2. We speculate that base treatment of **1** promotes equilibration with a hemiamidal, which probably exists as intramolecularly hydrogen-bonded structure **A** (diagram below). The hydrogen bond in **A** is likely to activate the C=O group toward nucleophilic addition of hydroxide ion. The resulting tetrahedral intermediate **B** incorporates an N atom (starred) of significantly lower basicity than an ordinary 2-piperazinone.[9] Therefore, the starred N functions as a considerably

[9] The pKa of the protonated form of **C** may be estimated to be below 5 on the basis of the following: (a) the pKa of protonated nojirimycin (ca. 5.3: N. Ishida, K. Kumagai, T. Niida, T. Tsuruoka, H. Yumoto, *J. Antibiot. Ser. A*, 1967, **20**, 66) vs. that of 1-desoxynojirimycin (ca. 6.7: R. W. Wang, X. L. Qiu, M. Bols, F. Ortega-Caballero, F.-L. Qing, *J. Med. Chem.*, 2006, **49**, 2989), suggests that the OH group in a hemiaminal lowers the pKa of the protonated N atom by nearly 1.5 units; (b) the pKa of protonated 2-piperazinone is ca. 6.4; (I. Ledneczki, P. Tapolcsanyi, E. Gabor, J. Eles, I. Greiner, E. Schmidt, Z. Nemeth

better leaving group than an ordinary amino segment, accelerating the fragmentation of **B** into the ultimate **30** (which is rapidly converted into a salt) and **C**.[10] Work by Seto[1128] provides some support for this proposed mechanism.



Possible Mechanism for the Hydrolysis of 1

y, R. Soukupne Kedves, O. Balazs, V. Roman, G. Levay, S. Maho, *Bioorg. Med. Chem. Letters*, 2017, **27**, 4525; data in supplementary material). The pKa of **C** is thus likely to be lower than (6.4 – 1.5) = 4.9, and this without factoring in the pKa-lowering effect of the exocyclic double bond and of the Me group on the OH-bearing C atom

[10] The nature of this fragment remains speculative at this time. Of course, **33** could undergo further reactions under basic conditions, including dehydrative aromatization to an ultimate 3-ethyl-5-methyl-pyrazinone.

[11] M. Ghosh, J. L. Conroy, C. T. Seto, *Angew. Chem. Int. Ed.*, 1999, **38**, 514.

6. Biological studies

a. *In vitro* antibacterial activity test

For these studies, test organisms consisted of reference strains from the American Type Culture Collection (ATCC) and clinical isolates (*S. aureus, S. epidermidis, E. faecalis, E. faecium*). Details on the ATCC strains used in this study are described in Table 2.

In vitro susceptibility testing of organisms other than *C. difficile* were determined by microtiter broth serial dilution method in accordance with the Clinical and laboratory Standards Institute (CLSI) guidelines. The MIC for *C. difficile* was determined by the agar dilution method according to CLSI guidelines. The antimicrobial agents (vancomycin, linezolid and ciprofloxacin) were purchased from Sigma-Aldrich and used as the quality control agent. The experimental compounds were dissolved in DMSO (micrococcin P2, linezolid) or sterile water (ciprofloxacin, vancomycin). Each compound was tested in the concentration range prepared with a double series of dilution in the range 32–0.06 μg/mL

The medium employed for testing in the broth microdilution assay were cation-adjusted Mueller Hinton broth (BD Difco). A standardized inoculum of 5 X 10^5 CFU/ml in a volume of 0.1mL was added to each 96-well microtiter plate well and incubated with serially diluted antibiotic at 37°C for 18-24h. After 16–24 h we inspected the plates and the MIC was scored visually as the lowest concentration of antibiotic where no growth is apparent by visual inspection for turbidity.

C. difficile MIC test was performed with using brucella agar (BD Difco) supplemented with hemin(5 μg/mL), vitamin K1(1 μg/L), 5% sheep blood. Before MIC test, the liquid Brain Heart Infusion medium with yeast extract (BHIS; BD Difco) medium was placed in the anaerobic chamber to remove oxygen from the broth for a day. A day before the experiment, the bacteria were incubated in 4 ml of BHIS medium. Dilute the cultured bacteria using BHIS medium to make it about 4~5 X 10^7 CFU/ml. Diluted culture were dropped 10ul each on a brucella agar plate containing antibiotics, and the results were observed after incubating them in anaerobic chamber for more than 18 hours.

b. Inhibitory activity against *Mycobacterium tuberculosis*

M. tuberculosis stain H37Rv was obtained from ATCC (ATCC 27294). Clinical *M. tuberculosis* isolated strains was obtained from TB Specimen Biobank., Masan National Tuberculosis

Hospital. All the isolates were identified as *M. tuberculosis* using Acid-Fast Bacilli (AFB) stain and MTB immunochromatographic detection kit (SD BIOSENSOR).

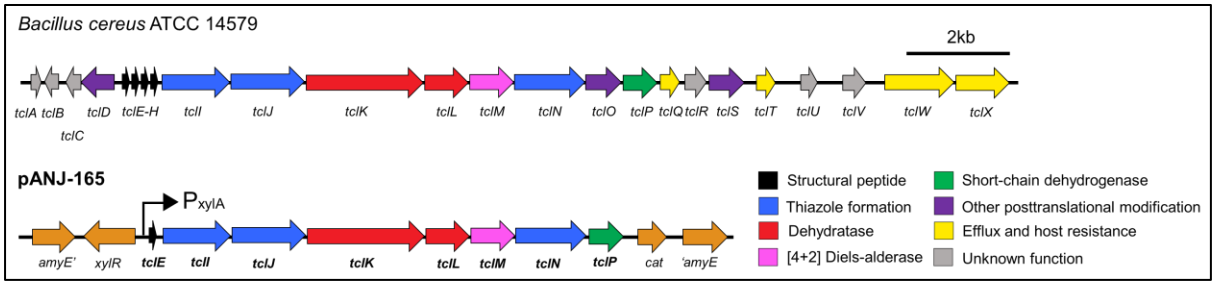
The drug susceptibility of *M. tuberculosis* strains was determined using the resazurin microtiter assay (REMA) under aerobic conditions. The resazurin solution was prepared in 0.02 % (wt/vol) solution in sterile distilled water using resazurin sodium salt powder (Sigma-Aldrich) and filter sterilized. Bacteria from exponential-phase cultures were harvested and adjusted to an OD600 of 0.0001. in wells of a 96-well microtiter plate. Two-fold serial dilutions of compounds were prepared from 10 uM to 10 nM Micrococcin P2. Plates were then incubated at 37°C. After 7 to 12 days of incubation, 40 μL of 0.025% resazurin was added to the wells. Fluorescence was measured using a SpectraMax® M3 Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA). Concentrations required to inhibit bacterial growth by 50% (IC_{50}) were determined by fitting the curves with a sigmoidal dose-response using GraphPad Prism software (version 6.05).

7. Molecular Biology Work

a. Table 1. List of primers used in the study

Primer	Sequence (5' to 3')	Role
1	GATTGTACTGAGAGTGCACCATATGTTTGCAAAACGATTCAAAACC	amyE-F
2	CCCGGGTACCGAGCTCGAATTCCGTATCATGCGACTCTACC	amyE-R
3	CGACCTGCAGGCATGCAAGCTTGCACCCATTAGTTCAACAAAC	cat-amyE-F
4	ATGACCATGATTACGCCAAGCTATCAATGGGGAAGAGAACCGC	cat-amyE-R
5	ACGGAATTCGAGCTCGGTACCGAACTCCTTTTTCATATGAGAAGG	xylR-CRE-F
6	TTCTAGAGGATACCCGGGTACCTTAGTAAACCACTTTGTTTGCGCTTCC	xylR-CRE-R
7	GATATACATATGAGTGAAATTAAAAAAGCATTAAATAC	BcTclE-F
8	GGAATTCCTCGAGTTAAGTTGTACAACAACTGCATG	BcTclE-R
9	GTTTACTAAGGTACCCGGGATCCGTTTGTTCCCTTTATAGAAAGG	BcTclI-F
10	TCAGTGGTGGTGGTGGTGGTGCTCGAGTTATGGGAAAGGATGAGGAGTTAC	BcTclJ-R
11	TCC TCA TCC TTT CCC ATA ACA TTT TTT ATG AAA GAT GG	BcTclK-F
12	TCAGTGGTGGTGGTGGTGGTGCTCGAGTCATCCCTTTCTACTCTTATACAATG	BcTclL-R
13	ATA AGA GTA GAA AGG GAT GAC TTA AAT GGA GCA GTA TC	BcTclM-F
14	TCAGTGGTGGTGGTGGTGGTGCTCGAGTCATACATAAAATTGTTCTCCTTTAGTT	BcTclN-R
15	TAAAGGAGAACAATTTTATGTATGACGGAAAACGGGTGAATC	TclP-F
16	TCAGTGGTGGTGGTGGTGGTGTCGAGATCTAGTGTAATGTATAACCACC	TclP-R
17	GGAGGAAGCGGAAGAATGAAG	Integration
18	GGTACCGAGCTCGAATTC	Integration
19	GGCTGCTTCCTAATGCAGG	Integration
20	CGGTAAGTCCCGTCTAGCCT	Integration

b. Vector map of pANJ-165

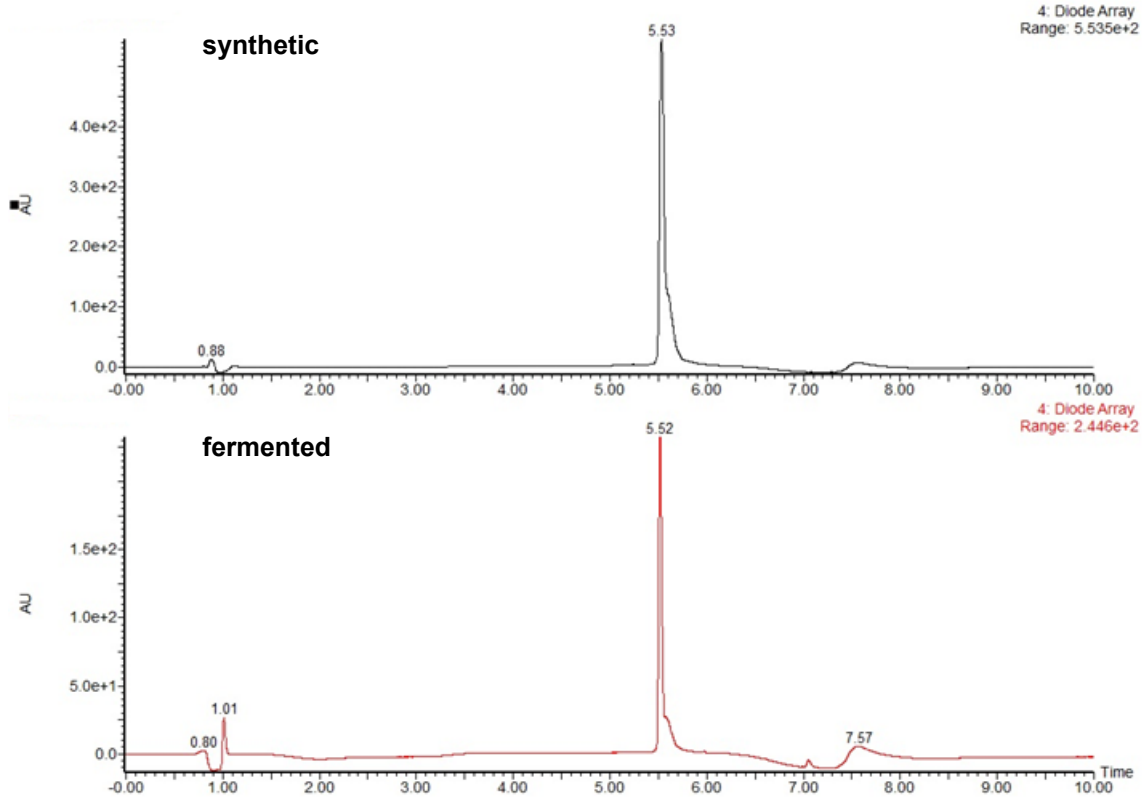


8. Fermentation, purification and analysis of MP2

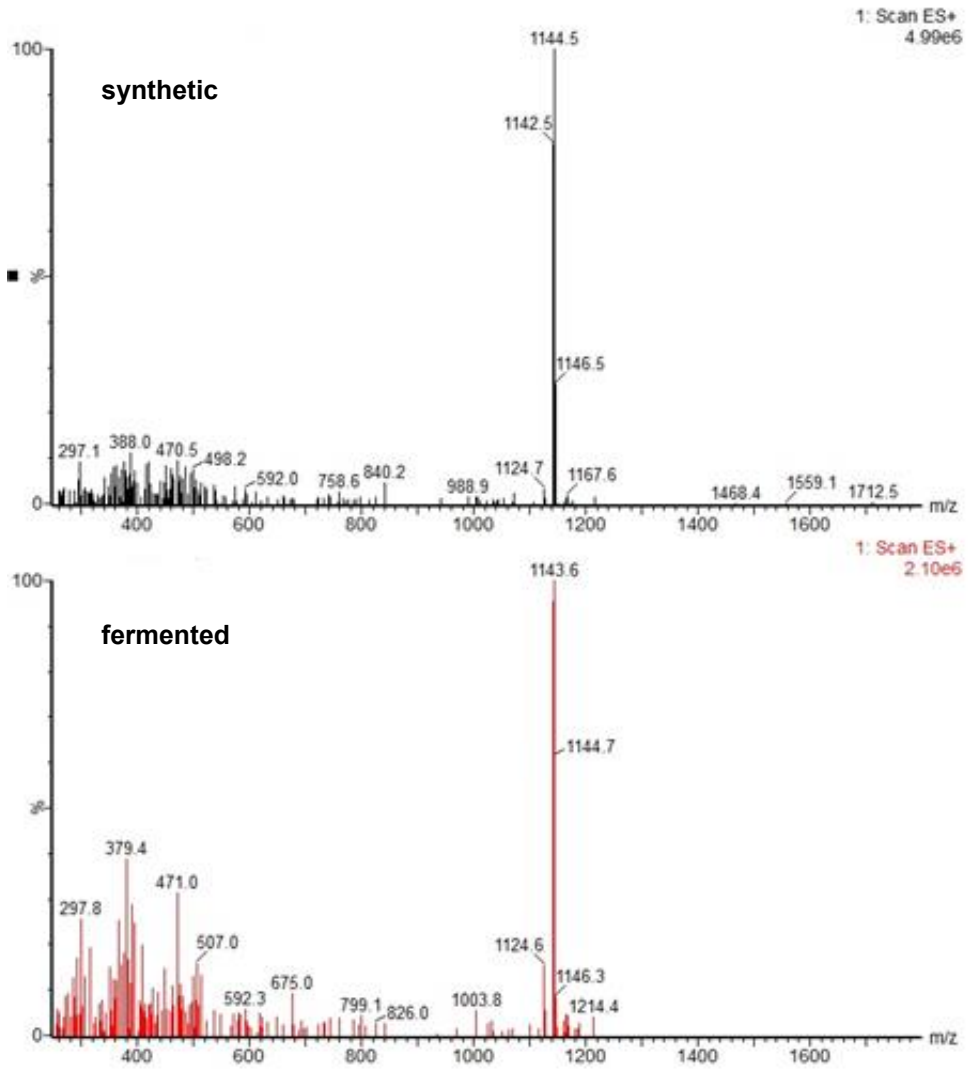
a. Fermentation procedure

MP2-producing starter cultures (3 mL) were grown in LB for 16 hours at 37 °C. Larger cultures (0.5 L LB in 2 L culture flasks) were inoculated with 500 µL of starter culture and grown for 24 hours at 37 °C with shaking at 200 rpm. (LB supplemented with 1% xylose, 5µg/mL chloramphenicol and 10 µg/mL tryptophan). Cells were harvested by centrifugation (4,000 x *g*, 30 min), resuspended in 50 mL methanol, vortexed vigorously and allowed to sit for at least 20 minutes. Sodium sulfate (15 g) was added to the methanolic extracts. The mixture was then filtered through Whatman filter paper (no. 1) and the methanol was removed by vacuum. Dried solid dissolved and extracted by ethyl acetate and removed on a rotary evaporator. The crude residue was then dissolved in 40 mL of 1:1 super solvent (CHCl3:IPA=3:1): water. The biphasic solution was transferred to a 5 mL separatory funnel, extraction and the organic layer removed. The aqueous layer was extracted twice and the combined organic solvents were dried with Na2SO4 and evaporated. Micrococcin P2 was finally purified by preparative RP-HPLC (30–100% acetonitrile in 0.1% trifluoroacetic acid, 60 min gradient) to obtain pure compounds. Biosynthesized MP2 was characterized by LC-MS and ^{1}H and ^{13}C NMR.

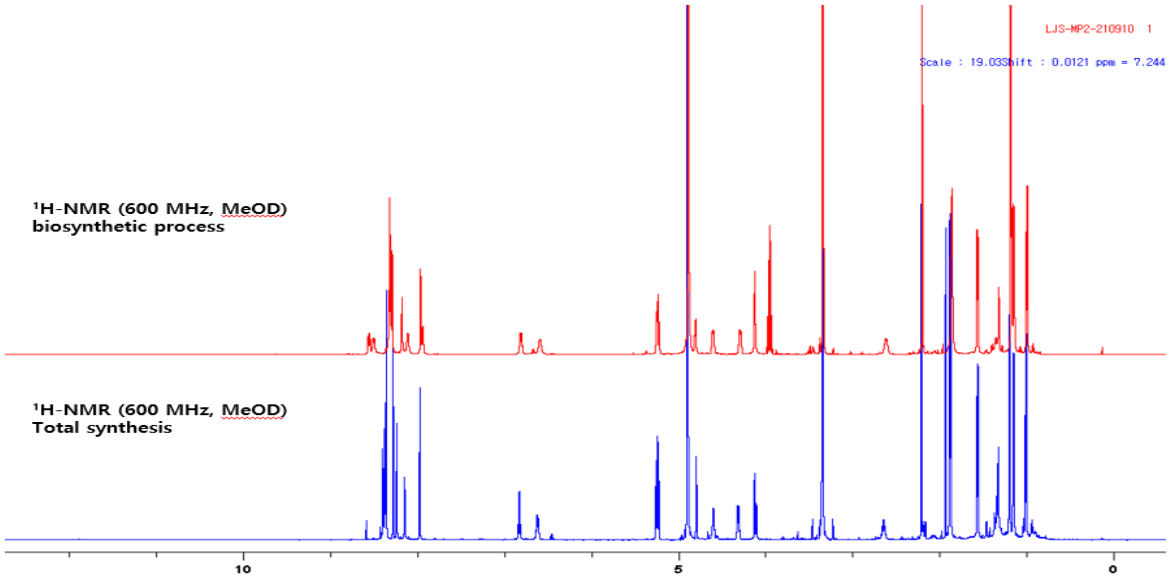
b. LC traces of synthetic and fermented MP2



c. Mass spectra of synthetic and fermented MP2

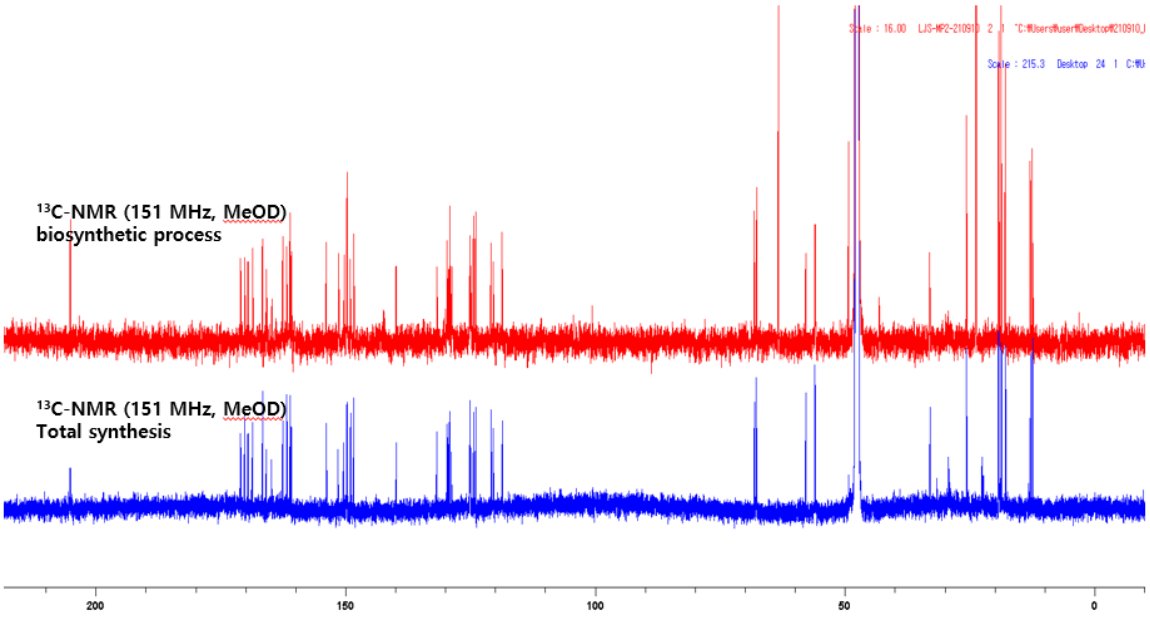


d. Comparison of NMR spectra of fermented and synthetic MP2 (see also *Spectra* section)



^{1}H NMR Spectra (CD_3OD, 600 MHz)

small differences are seen only for exchangeable (OH and NH) protons



^{13}C NMR Spectra (CD_3OD, 150 MHz)