SUPPLEMENTARY MATERIAL

Synthesis of Norfijimycin A with Activity against
Mycobacterium tuberculosis

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General Methods and Materials

All reactions were carried out under an argon atmosphere and at room temperature (23 °C) unless the reaction was performed under aqueous conditions or unless otherwise specified. Reactions undertaken at –78 °C utilized a bath of dry ice and acetone. Reactions carried out at 0 °C employed a bath of water and ice. Anhydrous THF, CH₂Cl₂, DMF, and MeCN were obtained using a PureSolv® solvent purification system (water <10 ppm). Reactions were monitored by thin layer chromatography (TLC) on aluminium backed silica plates (Merck Silica Gel 60 F254). Visualisation of TLC plates was undertaken with an ultraviolet (UV) light at λ = 254 nm and staining with solutions of vanillin or phosphomolybdic acid (PMA), followed by exposure of the stained plates to heat. Silica flash column chromatography (Silica Gel 60 40 – 63 μm) was undertaken to purify crude reaction mixtures using solvents as specified. Separations were performed using a Biotage Isolera® purification system with a diode array detector and a fraction collector.

NMR spectra were obtained using a Bruker DRX 400 or DRX 500 at frequencies of 400 MHz or 500 MHz respectively in CDCl₃, CD₃OD, CD₃CN, or DMSO-d₆. Chemical shifts are reported in parts per million (ppm) and coupling constants in Hertz (Hz). The residual solvent peaks were used as internal standards (CDCl₃: δH = 7.26, δC = 77.16; CD₃OD: δH = 3.31, δC = 49.00; CD₃CN: δH = 1.94, δC = 118.26/1.32; DMSO-d₆: δH = 2.50, δC = 39.52 ppm).[1] ¹H NMR data is reported as follows: Chemical shift values (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) and relative integral. ¹³C NMR spectra were obtained using a Bruker DRX 300, DRX 400, or DRX 500 at 75.5 MHz, 100.6 MHz, or 125.8 MHz in CDCl₃, CD₃OD, CD₃CN, or DMSO-d₆. ¹³C NMR data is reported as chemical shift values (ppm). In the case of diastereomeric mixtures, the signals of the major diastereomer are reported unless otherwise noted.

Mass spectra were recorded on a Shimadzu 2020 (ESI) mass spectrometer operating in positive mode. High resolution mass spectra were recorded on a Bruker-Daltronics Apex Ultra 7.0T Fourier transform (FTICR) mass spectrometer.

Optical rotations were measured on a Perkin-Elmer 341 polarimeter at a wavelength of 589 nm.
IR spectra were recorded on a Bruker ALPHA FT-IR-spectrometer using a diamond ATR unit.

Melting points were determined with a SRS Optimelt melting point apparatus and are uncorrected.

Preparative RP-HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector. Programmable wavelength detector operating at 210 – 300 nm. Compounds were purified using a XBridge BEH C18 5 μm or a Sunfire C18 (19 × 150 mm or 30 × 150 mm) column operating at total flow rates of 32.0 mL·min⁻¹ or 50.0 mL·min⁻¹ respectively. A mobile phase of 0.1% trifluoroacetic acid in water (Solvent A) and 0.1% trifluoroacetic acid in acetonitrile (Solvent B) was used in all cases.

LC/MS was performed on a Shimadzu UPLC/MS instrument consisting of a LC-M20A pump and a SPD-M30A diode array detector coupled to a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. Separations on the UPLC/MS system were performed using a Waters Acquity UPLC BEH C18 1.7 µm column (2.1 × 50 mm at a total flow rate of 0.60 mL·min⁻¹). Separations were performed using a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B).

General Procedures

General Procedure 1: Solid phase peptide synthesis

Preloading 2-chlorotrityl chloride resin: 2-Chlorotrityl chloride resin was swollen in dry DCM for 30 min then washed with DCM (5 × 1 mL). A solution of Fmoc-AA-OH and iPr₂NEt (2.0 equiv. relative to resin functionalization) in DCM (final concentration 100 μM of amino acid) was added and the resin was shaken at rt for 16 h. The resin was washed with DMF (5 × 1 mL) and DCM (5 × 1 mL). The resin was capped by treating with a solution of DCM/CH₃OH/iPr₂NEt (17:2:1 v/v/v, 1 mL) for 1 h and washed with DMF (5 × 1 mL), DCM (5 × 1 mL), and DMF (5 × 1 mL). The resin was subsequently submitted to iterative peptide assembly (Fmoc-SPPS).

Estimation of amino acid loading: The resin was treated with 20% piperidine/DMF (2 × 1 mL, 3 min) and 50 μL of the combined deprotection solution was diluted to 10 mL
using 20% piperidine/DMF in a volumetric flask. The UV absorbance of the resulting piperidine-fulvene adduct was measured ($\lambda = 301$ nm, $\varepsilon = 7800$ M$^{-1}$ cm$^{-1}$) to estimate the amount of amino acid loaded onto the resin.

**General Procedure 2: Iterative peptide assembly (Fmoc-SPPS)**

**General amino acid coupling:** A solution of Fmoc-protected amino acid (4 equiv.), PyAOP (4 equiv.), HOAt (8 equiv.) and 4-methylmorpholine (NMM, 8 equiv.) in DMF (final concentration of resin-bound peptide 100 $\mu$M) was preactivated for 3 min before added to the resin. After 1 h (15 h in the case of $N$-methyl amino acids) the resin was washed with DMF ($5 \times 1$ mL), DCM ($5 \times 1$ mL) and DMF ($5 \times 1$ mL).

**Fmoc-deprotection:** The resin was treated with 20% piperidine/DMF ($2 \times 1$ mL, 3 min) and washed with DMF ($5 \times 1$ mL), DCM ($5 \times 1$ mL) and DMF ($5 \times 1$ mL).

**Capping:** Acetic anhydride/pyridine (1:9 v/v, 1 mL)) was added to the resin. After 3 min the resin was washed with DMF ($5 \times 1$ mL), DCM ($5 \times 1$ mL) and DMF ($5 \times 1$ mL).

**Cleavage:** TFA/TIS/H$_2$O (95:5:5 v/v/v, 3 mL) was added to the resin and shaken for 1 h. Then the resin was washed with TFA/TIS/H$_2$O (95:5:5 v/v/v, 4 $\times$ 2 mL). The combined solutions were concentrated with a stream of N$_2$, purified by preparative RP-HPLC and analyzed by LC/MS (ESI+).

**General Procedure 3: Alloc-deprotection:** A solution of Pd(PPh$_3$)$_4$ (10 mol–%) and PhSiH$_3$ (25 equiv.) in DCM (final concentration of resin-bound peptide 100 $\mu$M) was added to the resin. After 15 min the resin was washed with DCM ($10 \times 3$ mL), DMF ($10 \times 3$ mL), DCM ($10 \times 3$ mL), and DMF ($10 \times 3$ mL).

**General Procedure 4: Allyl-deprotection:** A solution of Pd(PPh$_3$)$_4$ (0.8 equiv.) and PhSiH$_3$ (40 equiv.) in DCM (final concentration of resin-bound peptide 100 $\mu$M) was added to the resin. After 1 h the resin was washed with DCM ($10 \times 3$ mL), DMF ($10 \times 3$ mL), DCM ($10 \times 3$ mL), and DMF ($10 \times 3$ mL).
Experimental and analytical data

(R)-Fmoc-N(Me)-Phg-OH (7)

A suspension of (R)-N-methylphenylglycine (495 mg, 3.00 mmol) and FmocOSu (1.11 g, 3.30 mmol) in THF/sat. NaHCO₃ (20 mL, 1:1, v/v) was stirred at rt for 15 h. The reaction mixture was diluted with H₂O (10 mL), then washed with Et₂O (25 mL). Subsequently, HCl (1M) was added dropwise to reach pH = 3. The reaction mixture was extracted with ethyl acetate (3 × 20 mL), the combined organic extracts were dried over anhydrous Na₂SO₄, and the solvent was removed in vacuo yielding 1.10 g (95%) of the Fmoc-protected amino acid 7 as a colorless solid.

[α]₂⁵ = −72.3 (c 1.0, CH₂Cl₂).

m.p. 124.5 – 126 °C.

FTIR (ATR): ν = 2929, 1743, 1667, 1479, 1447, 1399, 1351, 1305, 757, 739, 704 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ = 7.76 (d, J = 7.6 Hz, 2H), 7.60 (d, J = 6.6 Hz, 2H), 7.43 – 7.28 (m, 8H), 7.15 (br s, 1H), 6.15, 5.76 (rotamers, s, 1H), 4.58 – 4.44 (m, 2H), 4.30 (m, 1H), 2.78, 2.73, 2.68 (rotamers, s, 3H) ppm.

¹³C NMR (100.6 MHz, CDCl₃): δ = 175.4, 144.0, 143.9, 141.5, 129.2, 129 0, 128.8, 127.9, 127.2, 125.20, 125.16, 120.1, 68.2, 62.7, 47.4, 31.2 ppm.

MS (ESI+): m/z (%): 410.1 (100) [M + Na]⁺.

HRMS (ESI+): Calcd. for [C₂₄H₂₁NO₄ + Na]: m/z = 410.1363, found: 410.1365.
Allyl 3-O-Allyloxypicolinate

3-Hydroxypicolinic acid (3.06 g, 20.0 mmol) was added to a stirred mixture of NaH (60%, 2.00 g, 50.0 mmol) in DMF (100 mL) at rt and stirred for 20 min before allylbromide (3.62 mL, 42.0 mmol) was added and the resulting mixture was stirred for 15 h. After the addition of H₂O (2 mL), the solvent was removed in vacuo and the brown residue was taken up in sat. NaHCO₃ (100 mL) and extracted with CH₂Cl₂ (3 × 100 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and the crude product was purified by column chromatography on silica [hexanes/EtOAc = 90:10 → 0:100 (12 CV)] affording the title compound as a dark oil (2.52 g, 57%).

Rᶠ = 0.58 (hexanes/EtOAc = 1:1).

FTIR (ATR): ṽ = 1732, 1578, 1445, 1300, 1193, 1139, 1102, 989, 801, 737 cm⁻¹.

¹H NMR (500 MHz, CDCl₃): δ = 8.24 (s, 1H), 7.34 – 7.29 (m, 2H), 6.03 – 5.99 (m, 2H), 5.42 (app t, J = 15.2 Hz, 2H), 5.27 (app t, J = 12.6 Hz, 2H), 4.86 – 4.84 (m, 2H), 4.61 (app br s, 2H) ppm.

¹³C NMR (75.5 MHz, CDCl₃): δ = 164.7, 154.5, 141.4, 139.7, 132.1, 132.0, 126.9, 121.6, 119.1, 118.4, 69.7, 66.3 ppm.

MS (ESI+): m/z (%): 242.1 (100) [M + Na]⁺.

HRMS (ESI+): Calcd. for [C₁₂H₁₃NO₃ + Na]: m/z = 242.0788, found:242.0788

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CV = column volumes
3-Allyloxylicolic acid (12)

LiOH (320 mg, 13.4 mmol) was added to a solution of allyl 3-O-allyloxylicolate (2.18 g, 9.95 mmol) in THF/H2O (2:1, v/v) and the reaction mixture was stirred at rt for 15 h. After the addition of HCl (1M) to reach pH = 5, the solvent was removed in vacuo and the residue was purified by column chromatography on silica [CH2Cl2/MeOH = 95:5 → 85:15 (10 CV)] to yield the title compound as a colorless foam (1.71 g, 96%).

Rf = 0.34 (10% MeOH/CH2Cl2).

FTIR (ATR): δ = 3376, 1608, 1444, 1394, 1273, 1215, 1119, 982, 868, 803, 708, 667 cm⁻¹.

1H NMR (500 MHz, CD3OD, CDCl3): δ = 8.12 (dd, J = 4.4, 1.4 Hz, 1H), 7.49 – 7.16 (m, 2H), 6.02 (ddt, J = 17.3, 10.4, 5.0 Hz, 1H), 5.44 (dq, J = 17.3, 1.7 Hz, 1H), 5.28 (dq, J = 10.4, 1.4 Hz, 1H), 4.66 (dt, J = 5.0, 1.7 Hz, 2H) ppm.

13C NMR (125.8 MHz, CD3OD, CDCl3): δ = 168.4, 154.7, 141.7, 140.5, 132.7, 127.2, 122.8, 118.3, 70.1 ppm.

MS (ESI–): m/z (%): 178.6 (100) [M – H]–, [C9H9NO3 – H]–.

HRMS (ESI+): Calcd. for [C9H9NO3 + Na]: m/z = 202.0475, found: 202.0475.

Alloc-Thr-OH

L-Threonine (5, 2.38 g, 20.0 mmol) was suspended in a THF/sat. NaHCO3 (50 mL, 1:1 v/v) and cooled to 0 °C. Then allylchloroformate (2.13 mL, 20.0 mmol) was added dropwise and the mixture was stirred at this temperature for 10 min before the ice bath was removed and the reaction was allowed to stir at rt for 15 h. The reaction mixture was diluted with H2O (50 mL), then washed with Et2O (25 mL). Subsequently, HCl (5M) was added dropwise to reach pH = 1.5. The reaction mixture was extracted with ethyl acetate (3 × 100 mL), the combined organic extracts were dried over anhydrous Na2SO4, and the
solvent was removed in vacuo yielding 2.60g (64%) of the desired Alloc-protected amino acid as a colorless syrup which was used in the next step without further purification.

Alloc-Thr-OtBu (6)

A mixture of DIC (6.20 mL, 61.0 mmol), CuCl (226 mg, 2.28 mmol) and tert-BuOH (5.0 mL) was stirred at rt under the exclusion of light for 5 d. After dilution of the mixture with CH2Cl2 (25 mL), a solution of Alloc-Thr-OH (2.40 g, 11.8 mmol) in CH2Cl2 (15 mL) was added dropwise and the mixture was stirred at rt for 15 h. The mixture was filtered, washed with sat. NaHCO3 (2 ×25 mL), and the organic phase was dried over anhydrous Na2SO4. After reomval of the solvent in vacuo the residue was purified by flash chromatography on silica [hexanes/EtOAc = 85:15 → 25:75 (10 CV)] furnishing 6 as a colorless syrup (2.20 g, 72%).

Rf = 0.79 (hexanes/EtOAc = 1:1).

[α]D^25 = –26.9 (c 2.0, MeOH).

FTIR (ATR): ð = 2978, 1701, 1516, 1369, 1221, 1154, 1065, 993 cm⁻¹.

^1H NMR (500 MHz, CDCl3): δ = 5.93 (ddt, J = 17.4, 10.6, 5.7 Hz, 1H), 5.46 (d, J = 8.8 Hz, 1H), 5.39 – 5.28 (m, 1H), 5.22 (dq, J = 10.6, 1.4 Hz, 1H), 4.59 (dt, J = 5.7, 1.4 Hz, 2H), 4.26 (s, 1H), 4.19 (d, J = 8.8 Hz, 1H), 1.48 (s, 9H), 1.24 (d, J = 6.4 Hz, 3H) ppm.

^13C NMR (100.6 MHz, CDCl3): δ = 170.3, 156.7, 132.8, 117.9, 82.8, 68.5, 66.1, 59.6, 28.2, 20.1 ppm.

MS (ESI+): m/z (%): 282.1 (100) [M + Na]^+.

HRMS (ESI+): Calcd. for [C12H21NO5 + Na]: m/z = 282.1312, found: 282.1313.
O-((R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-2-phenylacetyl)-N-((allyloxy)carbonyl)-L-threonine (4)

To a stirred solution of Alloc-Thr-OtBu (6, 337 mg, 1.30 mmol) and (R)-Fmoc-α-phenylsarcosine (7, 620 mg, 1.60 mmol) in CH₂Cl₂ (6.50 mL) was added EDC·HCl (380 mg, 2.00 mmol) and DMAP (16.0 mg, 0.10 mmol) at 0 °C and the mixture was stirred at this temperature for 3 h. After removal of the solvent by a stream of N₂, the residue was redissolved in EtOAc (50 mL) and washed with HCl (50 mL, 1 M), sat. NaHCO₃ (50 mL), and H₂O (50 mL). The organic phase was dried over anhydrous Na₂SO₄ and the solvent was removed in vacuo. The residue was purified by chromatography on silica [hexanes → 30% EtOAc/hexanes (18 CV)] yielding tert-butylester 8 as a colorless oil and a mixture of diastereomers (600 mg 73%, dr 66:34).

Ester 8 (300 mg, 477 µmol, dr 66:34) was treated with a mixture of TFA/CH₂Cl₂ (10 mL, 1:1, v/v) and stirred at rt for 15 min. The solvent was removed in vacuo, the residue was redissolved in MeCN/H₂O (10 mL, 1:1) and purified by preparative reversed-phase HPLC (Sunfire C18, 30 × 150 mm) using an isokratic solvent combustion [MeCN/H₂O (0.1% TFA) = 55:45 total flow rate: 50 mL·min⁻¹] affording the desired diastereomer 4 (160 mg, 60%, dr > 99:1) and the minor component 9 (90.0 mg, 33%, dr 95:5) both as colorless oils. b

Preparative RP-HPLC: tᵣ = 9.3 min (9), 10.2 min (4).

RP-LC/MS: tᵣ = 2.70 min (9), 2.73 min (4), MeCN/H₂O (0.1% HCO₂H) = 0:0 (0.00–0.30 min) → 100:0 (3.00 min), total flow rate: 0.60 mL·min⁻¹.

[α]D²⁵ = +54.3 (c 0.4, MeOH).

FTIR (ATR): ν = 1649, 1450, 1149, 741, 691 cm⁻¹.

¹H NMR (500 MHz, Methanol-d₄): δ = 7.78 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.40 – 7.30 (m, 7H), 7.19 (br s, 1H), 7.03 (br s, 1H), 5.92, 5.60 (br s, 1H, rotamers), 5.90 – 5.84 (m, 1H), 5.52 (br s, 1H), 5.26 (d, J = 16.7 Hz, 1H), 5.14 (d, J = 10.8 Hz, 1H), 4.65 – 4.42 (m, 5H), 4.28 (m, 1H), 2.61 (s, 3H), 1.29 – 1.22 (m, 3H, rotamers) ppm.

b crude dr 66:34 (4:9).
\(^{13}\text{C NMR}\) (125.8 MHz, Methanol-\(d_4\)): \(\delta = 172.5, 170.7, 160.9, 158.7, 145.3, 142.7, 135.4, 134.1, 130.1, 129.9, 129.6, 128.8, 128.2, 126.0, 121.0, 117.8, 73.1, 69.0, 66.9, 63.8, 58.8, 48.5, 31.7, 17.1\) ppm.

**MS** (ESI\(^+\)): \(m/z\) (%): 595.3 (85) [M + Na]\(^+\).

**HRMS** (ESI\(^+\)): Calcd. for \([C_{32}H_{32}N_2O_8 + Na]\): \(m/z = 595.2051\), found: 595.2052.

**Norfijimyin A (3)**

![Norfijimyin A (3)](image)

Fmoc-Ala-OH (9.34 mg, 30 \(\mu\)mol) and DIPEA (10.5 \(\mu\)L, 60 \(\mu\)mol) were dissolved in anhydrous \(\text{CH}_2\text{Cl}_2\) (0.3 mL) and added to pre-swollen 2CTC-resin (12.5 mg). After 16 h the loading mixture was discharged from the fritted syringe and the resin was washed with \(\text{CH}_2\text{Cl}_2\) (5 \(\times\) 1 mL), DMF (5 \(\times\) 1 mL), and \(\text{CH}_2\text{Cl}_2\) (5 \(\times\) 1 mL). Then the resin was capped according to general procedure 1 and washed with \(\text{CH}_2\text{Cl}_2\) (5 \(\times\) 1 mL) and DMF (5 \(\times\) 1 mL). After deprotection of the first amino acid (Ala) according to general procedure 2 the resin loading was determined to be 0.3 mmol·g\(^{-1}\) (24 \(\mu\)mol). The linear peptide was elongated using coupling conditions according to general procedure 2 incorporating the commercially available amino acids Fmoc-\(\text{N}(\text{Me})\)-Leu-OH, Fmoc-Sar-OH, Fmoc-\(\text{D}\)-allo-hydroxyproline, and Fmoc-\(\text{D}\)-Leu-OH. After deprotection of \(\text{D}\)-leucine using the standard protocol, ester bond containing fragment 4 was incorporated.

Fragment 4 (32 mg, 56 \(\mu\)mol, 2.3 equiv.), PyAOP (29.2 mg, 56 \(\mu\)mol, 2.3 equiv.), HOAt (30.0 mg, 221 \(\mu\)mol, 9.2 equiv.), and NMM (12.2 \(\mu\)L, 106 \(\mu\)mol, 4.6 equiv.) were dissolved in anhydrous DMF (0.24 mL), and the resulting mixture was added to the resin. After 16 h resin-bound 11 was washed with DMF (5 \(\times\) 1 mL), \(\text{CH}_2\text{Cl}_2\) (5 \(\times\) 1 mL), DMF (5 \(\times\) 1 mL), and \(\text{CH}_2\text{Cl}_2\) (5 \(\times\) 1 mL).

Then, Alloc-protecting group was removed and the resin was washed according to general procedure 3 followed by coupling with 3-allyloxypicolinic acid (12) according to general
O-allyl protecting group was cleaved off according to general procedure 4 followed by cleavage of the peptide from the solid-support according to general procedure 2.

10% Palladium on activated charcoal (12.8 mg, 12 µmol, 50 mol–%) was added to the a solution of the N-terminal protected crude linear depsipeptide in Methanol (5 mL). The mixture was degassed by bubbling a stream of N₂ into the solution. Then, a hydrogen-filled balloon was attached and the reaction mixture was stirred at rt for 12 h before it was filtered using a PTFE-syringe filter (0.22 µm pore size). The filter was thoroughly washed with MeOH (20 mL) and the solvent was removed by a stream of N₂.

The crude linear depsipeptide 13 was purified by reversed-phase preparative HPLC (30 × 150 mm) using a focused gradient [MeCN/H₂O (0.1% TFA) = 0:0 (0.00–1.00 min) → 25:75 (4.00 min) → 45:55 (18.00 min), total flow rate: 50.0 mL·min⁻¹, tᵣ = 6.5 – 9.0 min (broad peak)] affording 13 (8.2 mg, 41%) as a colorless lyophilisate.

13 (6.0 mg, 6.0 µmol) was dissolved in a mixture of CH₂Cl₂/DMF (1.2 mL, 1:1, v/v, final concentration 5 mM). HATU (4.6 mg, 12 µmol), HOAt (4.9 mg, 36 µmol) and N,N-diisopropylethylamine (4.2 µL, 24 µmol) were added and the reaction mixture was stirred at rt for 24 h before the solvent was removed by a stream of N₂ and the crude cyclodepsipeptide was purified by preparative reversed-phase HPLC (19 × 150 mm) using a linear gradient [MeCN/H₂O (0.1% TFA) = 0:0 (0.00–1.00 min) → 60:40 (16.0 min), total flow rate: 32.0 mL·min⁻¹] affording two diastereomers (3.1 mg, 60% combined yield) as colorless lyophilisates (epi-3: 1.7 mg, 33%; 3: 1.4 mg, 27%)

Preparative RP-HPLC: tᵣ =14.0 min (epi-3), 16.0 min (3).

RP-LC/MS: tᵣ = 4.83 min (epi-3), 5.05 min (3), MeCN/H₂O (0.1% HCO₂H) = 0:0 (0.00–0.50 min) → 100:0 (8.00 min), total flow rate: 0.60 mL·min⁻¹.

Norfijimycin A (3, mixture of conformers):[2]

¹H NMR (400 MHz, CD₃CN): δ = 9.61 (s), 8.60 (d, J = 7.9 Hz), 8.48 (d, J = 7.2 Hz), 8.04 (d, J = 3.6 Hz), 8.00 – 7.93 (m), 7.37 – 7.04 (m), 5.88 (s), 5.68 (d, J = 11.3 Hz), 5.41 (br s), 5.29 (br s), 5.03 – 4.64 (m), 4.59 (br d, J = 8.0 Hz), 4.46 – 4.40 (m), 4.38 (s), 4.30 (br s),

Aliquots were taken at 2, 5, 18 and 24 h and analyzed by LC/MS: After 2 h reaction time a small amount of the linear depsipeptide was cyclized and only one diastereomer (tᵣ = 5.05 min) was formed, presumably Norfijimycin A (3). After 5 h reaction time the amount of cyclic peptide was increased whereas a small amount of a second diastereomer (tᵣ = 4.83 min), presumably epi-3, was also detected. The final 1:1 ratio of 3 and epi-3 was reached after 18 h. It is proposed that prolonged reaction time in the presence of coupling reagents led to increased epimerization.
4.25 (br d, $J = 6.2$ Hz), 4.19 (br s), 4.15 (br s), 4.11 – 4.05 (m), 4.03 (br d, $J = 3.8$ Hz), 4.01 – 3.99 (m), 3.98 (d, $J = 4.8$ Hz), 3.96 (br d, $J = 4.7$ Hz), 3.91 (dd, $J = 11.6$, 4.8 Hz), 3.88 (br s), 3.76 (dd, $J = 8.6$, 6.1 Hz), 3.67 – 3.62 (m), 3.54 (br d, $J = 10.3$ Hz), 3.41 (d, $J = 5.1$ Hz), 3.38 (d, $J = 10.2$ Hz), 3.36 (d, $J = 11.5$ Hz), 3.33 (d, $J = 6.0$ Hz), 3.16 (br s), 3.08 – 3.05 (m), 3.00 (br s), 2.92 (d, $J = 17.2$ Hz), 2.82 (d, $J = 14.4$ Hz), 2.76 (br s), 2.67 (br s), 2.21 (t, $J = 7.6$ Hz), 2.02 – 2.00 (m), 1.68 (p, $J = 2.6$ Hz), 1.50 – 1.47 (m), 1.42 – 1.31 (m), 1.25 (d, $J = 7.8$ Hz), 1.18 (br s), 1.12 (d, $J = 7.0$ Hz), 1.08 (br d, $J = 6.4$ Hz), 0.89 – 0.76 (m) ppm.

**MS** (ESI+): $m/z$ (%): 865.8 (24) [M + H]$^+$ (epi-3), 865.8 (48) [M + H]$^+$ (3).

**HRMS** (ESI+): Calcd. for [C$_{43}$H$_{60}$N$_8$O$_{11}$ + Na]: $m/z = 887.4274$, found: 887.4269 (3).

**Antimicrobial Screening: Resazurin Assay for Mtb**

The compounds were originally stored as 10 mM stock solutions in 100% DMSO. Two fold serial dilutions of the compounds were made in a 96 well plate using Middlebrook 7H9 medium supplemented with ADC (0.5% v/v glycerol and 0.05% v/v Tween-80). *M. tuberculosis* H37Rv was grown to mid-exponential phase to an OD$_{600}$ of 0.6–0.8 in 7H9 media at 37 °C. On the day of the assay, culture was diluted to an OD$_{600}$ of 0.002 and 100 µL of bacterial suspension was added to the 96 well plate containing 100 µL of the diluted compounds. The plate was incubated for 5 days at 37 °C in a humidified incubator and 30 µL of Resazurin (0.02% w/v) and 12.5 µL of Tween-80 was added to each well and incubated for further 24 h. On day 6, the fluorescence was read using a BMG Labtech Polarstar plate reader (excitation 530 nm and emission 590 nm). The results are presented as *M. tuberculosis* survival as a percentage of negative control.
Mass Spectra and Chromatograms

Generic Display Report

Analysis Info
Analysis Name: D:\Data\nick\2016-files\ESI_Positive\08-August\2016-08-29\2016-08-29-posesi-service_000037.d
Method: 1MW Positive ESI
Sample Name: AS4 100
Comment: MeCH 1M TOF delay 0.0008s, Q1 300 m/z

Acquisition Date: 26/08/2016 4:26:44 PM
Operator: Instrument: apex-Ultra

Bruker Compass DataAnalysis 4.0
printed: 26/08/2016 4:31:38 PM
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NL:
7.67E4
20160929_A84_160929133410
#12-54 RT: 0.32-1.51 AV: 43
T: FTMS + p ESI Full ms
[100.00-1400.00]

C₆H₆NO₅ Na⁺
C₆H₆N₂O₃ Na⁺
p (gss, s / p:40) Chrg 1
R: 200000 Res. Pwr.: @FWHM
C₄₀H₆₀N₂₀O₁₁ +Na:
C₄₃H₆₀N₂₀O₁₁ Na⁺
c (gss, s /p40)(Val) Chrg 1
R: 80000 Res. Pwr. @FWHM
NMR spectra
The image contains a 31P NMR spectrum with peaks labeled in ppm. The molecular structure of FmocMeN_{2}O_{2}COH is also shown. The spectrum shows various chemical shifts at different ppm values.
References
