Fluorometabolite biosynthesis: Isotopically labelled glycerol incorporations into the antibiotic nucleocidin in *Streptomyces calvus*.

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General experimental procedures

All reactions were carried out under an argon atmosphere in flame-dried glassware. Room temperature refers to 18-25 °C. All evaporations and concentrations were performed under reduced pressure (in vacuo). All reagents from Sigma Aldrich UK were of synthetic grade and were used without further purification. Anhydrous Et₂O was obtained from MBraun MB SPS-800 solvent purification system by passage through two drying columns and dispensed under an argon atmosphere.

NMR spectra were recorded at 298 K on Bruker Avance 300, Avance II 400, Avance 500 or Avance III HD 500 instruments. ¹H and ¹³C NMR spectra were recorded using deuterated solvent as the lock and the residual solvent as the internal standard. ¹⁹F NMR spectra were referenced CFCl₃ as an external standard. Chemical shifts are reported in parts per million (ppm) and coupling constants (J) are reported in Hertz (Hz). When necessary, resonances were assigned using two-dimensional experiments (COSY, HSQC, HMBC, TOCSY).

Optical rotations were measured with a Perkin Elmer 341 polarimeter in a 10.0 cm cell at the wavelength of the sodium D-line (λ = 589 nm). Specific rotations are reported in implied units of 10⁻¹ deg cm² g⁻¹ and concentrations (c) are reported in g/100mL.

High resolution electrospray ionisation mass spectra were obtained on a Micromass LCT or ThermoFisher Excalibur Orbitrap spectrometers operating in positive or negative mode, from solutions in CHCl₃, MeOH, ACN or water by the Mass Spectrometry Service at the University of St Andrews.

LC-MS analysis was performed on a Waters 2795 HPLC coupled in parallel to a Waters 2996 photodiode array detector and Micromass LCT TOF mass spectrometer in ESI positive mode using the column indicated in the individual experiment.
All microbiological works were carried out using standard sterile techniques under a Gallenkamp laminar flowhood. Glassware, equipment and consumables for biological works were sterilised by autoclaving, flaming or spraying with 70% ethanol as appropriate prior to use. Sterilised consumables were used as supplied. Media were sterilised by autoclaving. Cell cultures were incubated in a temperature controlled Gallenkamp orbit incubator or an Innova 2000 platform shaker. Centrifugation was carried out on a Beckman Avanti centrifuge.
Growth of *Streptomyces calvus* on solid media

*Streptomyces calvus* was grown on solid agar plates composed of soluble starch (10 g), dipotassium phosphate (1 g), magnesium sulphate VSP (1 g), sodium chloride (1 g), ammonium sulphate (2 g), calcium carbonate (2 g), ferrous sulphate (1 mg), manganese chloride (1 mg), zinc sulphate (1 mg), agar (20 g) and deionised water (1 L). The medium ISP4 was sterilised by autoclave before use. The plates were maintained at 30 °C where the bacteria mature after a period of 18 days. The spores were collected by means of sterilised cotton swabs and stored in a 50% glycerol solution at -80 °C.

After spore maturation the plates can be stored at 4 °C for future use.
**Fermentation culture**

A mass of the mycelium of *S. calvus* was obtained by inoculating a sterilised, defined medium (100 ml) with the spores obtained above (seed culture, inoculate with 1ml-5 mL per flask) and the culture was allowed to grow at 28 °C for 18 days (500 ml conical flask shaking at 180 rpm). The defined medium was composed of tap water (1 L), corn steep liquor (12.5 g), mannitol (10 g), sodium chloride (2 g), Hoagland’s salt solution (1 mL), potassium fluoride solution (7.5 mL, 0.5 M), magnesium sulphate (0.25 g), monopotassium phosphate (1.5 g) and diammonium phosphate (2 g).

Hoagland’s salt solution contains deionised water (1 L), H₃PO₃ (0.611 g), MnCl₂.4H₂O (0.389 g), CuSO₄ (0.056 g), ZnSO₄.7H₂O (0.056 g), Al₂(SO₄)₃ (0.056 g), NiSO₄.6H₂O (0.056 g), Co(NO₃)₂.6H₂O (0.056 g), (NH₄)₆Mo₇O₂₄.4H₂O (0.056 g), TiO₂ (0.056 g), LiCl (0.028 g), SnCl₂.2H₂O (0.028 g), KI (0.028 g) and KBr (0.028 g). Sterilised by autoclaving.

After 18 days of fermentation, the cells were discarded by centrifugation and the supernatant was extracted with *n*-butanol (20 mL). The organic layer was concentrated under reduced pressure. The extract was analysed by $^{19}$F-NMR (3600 scans).
Figure S1. $^{19}$F-$^1$H-NMR (470 MHz) of nucleocidin in D$_2$O from a butanol extract of a S. calvus culture.

Figure S2. $^{19}$F-NMR (470 MHz) of nucleocidin in D$_2$O from a butanol extract of a S. calvus culture.
LC-MS for identification of nucleocidin from the butanol extract of the supernatant of a *Streptomyces calvus* fermentation

**Figure S3.** Mass spectra of isolated nucleocidin from *S. calvus*. 
Feeding pulse experiments

Cultures of *S. calvus* (100 mL) were shaken at 30 °C and labelled glycerol was added after 4 days, and then the same quantity every two days for the next 12 days. The final concentration of labelled glycerol was between 8-10 mM. After 21 days of fermentation, the cells were discarded after centrifugation and the supernatant was extracted into *n*-butanol (20 mL). The organic layer was concentrated under reduced pressure and the extract was analysed by $^{19}$F-$^1$H-NMR (3600 scans) to detect nucleocidin.
Figure S4. $^{19}$F-$^1$H-NMR (470 MHz) of nucleocidin in D$_2$O from *S. calvus* after the [2-$^{13}$C]-glycerol 5a feeding experiment.

Figure S5. $^{19}$F-NMR (470 MHz) of nucleocidin in D$_2$O from *S. calvus* after the [2-$^{13}$C]-glycerol 5a feeding experiment.
Figure S6. $^{19}\text{F}[^1\text{H}]-\text{NMR}$ (470 MHz) of nucleocidin in D$_2$O from *S. calvus* after the [${}^2\text{H}_3$]-glycerol 5b feeding experiment.
Figure S7. $^{19}$F-{$^1$H}-NMR (470 MHz) of nucleocidin in D$_2$O from S. calvus after the (R)-[$^2$H$_2$]-glycerol 5e feeding experiment.

Figure S8. $^{19}$F-NMR (470 MHz) of nucleocidin in D$_2$O from S. calvus after the (R)-[$^2$H$_2$]-glycerol 5e feeding experiment.
Figure S9. $^{19}$F-$^1$H-$^1$NMR (470 MHz) of nucleocidin in D$_2$O from *S. calvus* after the (S)-[D$_2$]-glycerol 5d feeding experiment.

Figure S10. $^{19}$F-NMR (470 MHz) of nucleocidin in D$_2$O from *S. calvus* after the (S)-[D$_2$]-glycerol 5d feeding experiment.
**((S)-2,2-Dimethyl-4-hydroxy[2H]methyl-1,3-dioxolane)**

A solution of (S)-methyl 2,2-dimethyl-1,3-dioxolane-4-carboxylate (1.8 mL, 12.5 mmol, 1 eq.) in anhydrous diethyl ether (10 mL) was added dropwise to a suspension of LiAlD$_4$ (1.0 g, 23.8 mmol, 1.9 eq.) in anhydrous diethyl ether (8.5 mL). The mixture was heated under reflux for 1 h and then diluted with diethyl ether. After cooling the reaction mixture to 0 °C, water (1 mL) was slowly added, followed by aqueous NaOH (15% w/v, 1 mL) and water (3 mL) again. The reaction mixture was allowed to reach room temperature and was then stirred for 15 min. The reaction mixture was evaporated affording (S)-2,2-dimethyl-4-hydroxy[2H]methyl-1,3-dioxolane (1.58 g, 94%) as a yellow oil.

$R_f = 0.44$ (EtOAc/PE 1:1); $[\alpha]^{20}_D = -16.4^\circ$ (c 1.0, CHCl$_3$), Lit. $[\alpha]^{23}_D = -14.9^\circ$ (undiluted); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$H 4.21 (1H, t, $J = 6.6$ Hz, CH), 4.02 (1H, dd, $J = 8.2$ Hz, 6.6 Hz, CH$_2$), 3.77 (1H, dd, $J = 8.2$ Hz, 6.6 Hz, CH$_2$), 2.24 (1H, br s, OH), 1.42 (3H, s, CH$_3$), 1.35 (3H, s, CH$_3$); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$C 109.5 (C(CH$_3$)$_2$), 76.1 (CH), 65.8 (CH$_2$), 63.1 (CD$_2$, p, $J = 21.3$ Hz), 26.8 (CH$_3$), 25.4 (CH$_3$); IR $\nu_{\text{max}}$ (neat): 3454, 1395, 1380, 1049, 837 cm$^{-1}$; HRMS m/z (ES$^+$) (calculated C$_6$H$_{11}$D$_2$O$_3^+$ = 135.0985) found 135.0984 [M+H]$^+$. Data are in agreement with literature.$^{9b}$
(S)-1,1-Dideuteroglycerol

HCl (38%, 0.1 mL) was slowly added to a solution of (S)-2,2-dimethyl-4-hydroxy[2H]methyl-1,3-dioxolane (1.58 g, 11.8 mmol, 1 eq.) in water (1.9 mL) and the reaction mixture was stirred at room temperature for 3 h. The solution was then concentrated affording (S)-1,1-dideuteroglycerol (0.87 g, 78%) as a yellow oil.

\(^1\text{H NMR}\) (D\(_2\)O, 300 MHz) \(\delta_H\) 3.71 (1H, dd, \(J = 6.6\) Hz, 4.4 Hz, \(\text{CH}\)), 3.59 (1H, dd, \(J = 11.9\) Hz, 4.4 Hz, \(\text{CH}_2\)), 3.49 (1H, dd, \(J = 11.9\) Hz, 6.6 Hz, \(\text{CH}_2\)); \(^{13}\text{C NMR}\) (D\(_2\)O, 100 MHz) \(\delta_C\) 72.3 (CH), 62.8 (CH\(_2\)), 62.4 (CD\(_2\), p, \(J = 22.7\) Hz); \(^\text{HRMS}\) m/z (ES\(^+\)) (calculated \(\text{C}_3\text{H}_7\text{D}_2\text{O}_3^+\) = 95.0672) found 95.0672 [M+H]\(^+\). Data are in agreement with literature.\(^{9b}\)
(R)-2,2-Dimethyl-4-hydroxy[2H₂]methyl-1,3-dioxolane

A solution of (R)-methyl 2,2-dimethyl-1,3-dioxolane-4-carboxylate (0.9 mL, 6.3 mmol, 1 eq.) in anhydrous diethyl ether (5 mL) was added dropwise to a stirred suspension of LiAlD₄ (0.5 g, 12.6 mmol, 2 eq.) in anhydrous diethyl ether (4.5 mL). The mixture was heated under reflux for 1 h and then diluted with diethyl ether. After cooling to 0 °C, water (0.5 mL) was slowly added to the reaction, followed by a solution of aqueous NaOH (15% w/v, 1 mL) and water (1.5 mL). The reaction mixture was allowed to reach room temperature and was then stirred for 15 min. The reaction mixture was dried over MgSO₄ and filtered to remove salts. The residue was evaporated affording (R)-2,2-dimethyl-4-hydroxy[2H₂]methyl-1,3-dioxolane (0.5 g, 60%) as a colourless oil.

R_f = 0.43 (EtOAc/PE 1:1); [α]_D²⁰ : +15.9° (c 1.0, CHCl₃), Lit. [α]_D²³ : +15.3° (undiluted); ^1H NMR (CDCl₃, 300 MHz) δH 4.20 (1H, t, J = 6.6 Hz, CH), 4.01 (1H, dd, J = 8.2 Hz, 6.6 Hz, CH₂), 3.76 (1H, dd, J = 8.2 Hz, 6.6 Hz, CH₂), 2.29 (1H, br s, OH), 1.41 (3H, s, CH₃), 1.35 (3H, s, CH₃); ^13C NMR (CDCl₃, 100 MHz) δC 109.5 (C(CH₃)₂), 76.1 (CH), 65.8 (CH₂), 63.1 (CD₂, p, J = 21.8 Hz), 26.8 (CH₃), 25.4 (CH₃); IR νmax (neat): 3455, 1395, 1382, 1050, 837 cm⁻¹; HRMS m/z (ES⁺) (calculated C₆H₁₁D₂O₃⁺ = 135.0985) found 135.0984 [M+H]⁺. Data are in agreement with literature.¹
(R)-1,1-Dideuteroglycerol

HCl (38%, 0.1 mL) was added to a solution of (R)-2,2-dimethyl-4-hydroxy[2H2]methyl-1,3-dioxolane (0.50 g, 3.7 mmol, 1 eq.) in water (1.1 mL) and the reaction mixture was stirred at room temperature for 3 h. The solution was then concentrated affording (R)-1,1-dideuteroglycerol (0.25 g, 70%) as a colourless oil.

$^1$H NMR (D$_2$O, 300 MHz) $\delta$H 3.71 (1H, dd, $J = 6.5$ Hz, 4.4 Hz, CH), 3.59 (1H, dd, $J = 11.9$ Hz, 4.4 Hz, CH$_2$), 3.49 (1H, dd, $J = 11.9$ Hz, 6.5 Hz, CH$_2$); $^{13}$C NMR (D$_2$O, 100 MHz) $\delta$C 74.1 (CH), 64.0 (CH$_2$), 63.6 (CD$_2$, p, $J = 22.5$ Hz); HRMS m/z (ES$^+$) (calculated C$_3$H$_7$D$_2$O$_3^+$ = 95.0672) found 95.0672 [M+H]$^+$. Data are in agreement with literature.$^1$