Supplementary Information

Incorporation of [$^2H_1$]-(*1R,2R*)- and [$^2H_1$]-(*1S,2R*)- glycerols into the antibiotic nucleocidin in *Streptomyces calvus.*

Xuan Feng,[a] Nawaf Al Maharik,[a] Axel Bartholomé,[a] Jeffrey E. Janso,[b] Usa Reilly[b] and David O'Hagan.[a]*

[a] *University of St Andrews, School of Chemistry and Centre for Biomolecular Sciences, North Haugh, St Andrews, Fife, KY16 9ST, UK Fax: 01334 463800; Tel: 01334 467171; E-mail: do1@st-andrews.ac.uk.*
[b]*Pfizer, 220-3529, 445 Eastern Point Rd., Groton, CT 06340, 860-715-6375, USA.*

General experimental procedures

NMR spectra were recorded at 298 K on Bruker Avance II 400, Avance III HD 500 or Avance III HD 700 instruments. ^1H and ^{13}C NMR spectra were recorded in deuterated solvent as the lock and the residual solvent as the internal standard. ^{19}F NMR spectra were using $CFCl_3$ as an external reference. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are reported in Hertz (Hz).

Room temperature refers to 18-25 ^oC. All synthesis were carried out in flame-dried glassware. All evaporations and concentrations were performed under reduced pressure (*in vacuo*) by Büchi Rotavapor R-200. All reagents from Sigma Aldrich or Alfa Aesar were of synthetic grade and were used directly, without any further purification.

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

HPLC analysis was performed on a Shimazu LC-20-AT HPLC system. The samples were separated by Phenomenex Luna C-18 column. LC-MS analysis was performed on a Thermo UltiMate 3000 HPLC system coupled in Diode Array detector and LCQ Fleet mass spectrometer in ESI positive mode using the column indicated in the individual experiment.

All microbiological works were carried out in a Gallenkamp laminar flowhood, using standard sterile techniques. Glassware and consumables for biological operations were sterilised by autoclaving, flaming or wiping with 75% ethanol before using. Sterilised consumables were used as supplied. Media were sterilised by 121 ^oC， 15-min autoclaving. Cell cultures were

incubated in a temperature controlled incubator provided by New Brunswick Scientific. Centrifugation of 20 ml to 1 litre was processed by Beckman Avanti centrifuge. Hettich Mikro 200 bench-top centrifuge was applied to do the micro-centrifugation.

Growth of *Streptomyces calvus* on solid media

Streptomyces calvus was grown on solid ISP4 agar plates made by soluble starch (10 g), calcium carbonate (2 g), ammonium sulphate (2 g), sodium chloride (1 g), dipotassium phosphate (1 g), magnesium sulphate heptahydrate (1 g), ferrous sulphate (1 mg), manganese chloride (1 mg), zinc sulphate (1 mg), agar (20 g) and deionised water (to 1 L). The medium ISP4-agar was sterilised by autoclave before use. The plates were maintained at 30 ^{o}C in an incubator for 14 to 21 days. The spores were collected by means of sterilised cotton swabs and stored in a 25% glycerol solution at -80 ^{o}C.

The petri plates after harvesting the spores were stored at 4 ^{o}C for future use.

Seed culture of *Streptomyces calvus*

The seed culture was prepared in TSBY liquid medium composed of 3% tryptone soy broth, 10.3% sucrose and 0.5% yeast extract. The seed culture of *S. calvus* was obtained by inoculating (50 µl) spores into TSBY medium (50 ml), and the culture was allowed to grow at 28 ^{o}C for 2 days (250 ml conical flask shaking at 180 rpm).

Fermentation culture

S. calvus cell mass was obtained by inoculating a sterilised, defined medium (100 ml in 500 ml conical flask) with the seed culture described above (inoculate with 2 mL per 100 ml) and the culture was allowed to grow at 28 ^{o}C, 180 rpm for 8 days. The defined medium was composed of tap water (to 1 L), corn steep liquor (12.5 g), mannitol (10 g), sodium chloride (2 g), diammonium phosphate (2 g), monopotassium phosphate (1.5 g), magnesium sulphate heptahydrate (0.25 g), Hoagland's salt solution (1 mL), potassium fluoride solution (7.5 mL, 0.5 M).

Hoagland's salt solution contains deionised water (1 L), $MnCl_2.4H_2O$ (0.389 g), H_3PO_3 (0.611 g), $CuSO_4$ (0.056 g), $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.056 g), $NiSO_4.6H_2O$ (0.056 g), $ZnSO_4.7H_2O$ (0.056 g), $Al_2(SO_4)_3$ (0.056 g), $SnCl_2.2H_2O$ (0.028 g), $Co(NO_3)_2.6H_2O$ (0.056 g), TiO_2 (0.056 g), LiCl (0.028 g), KI (0.028 g) and KBr (0.028 g). Sterilised by autoclaving.

After 8 days of fermentation, the cells were discarded after centrifugation and the supernatant was extracted into *n*-butanol (20 ml per 100 ml). The *n*-butanol layer was concentrated under reduced pressure and the extract was re-dissolved in deuterated oxide (D_2O) and analyzed by ^{19}F-NMR.

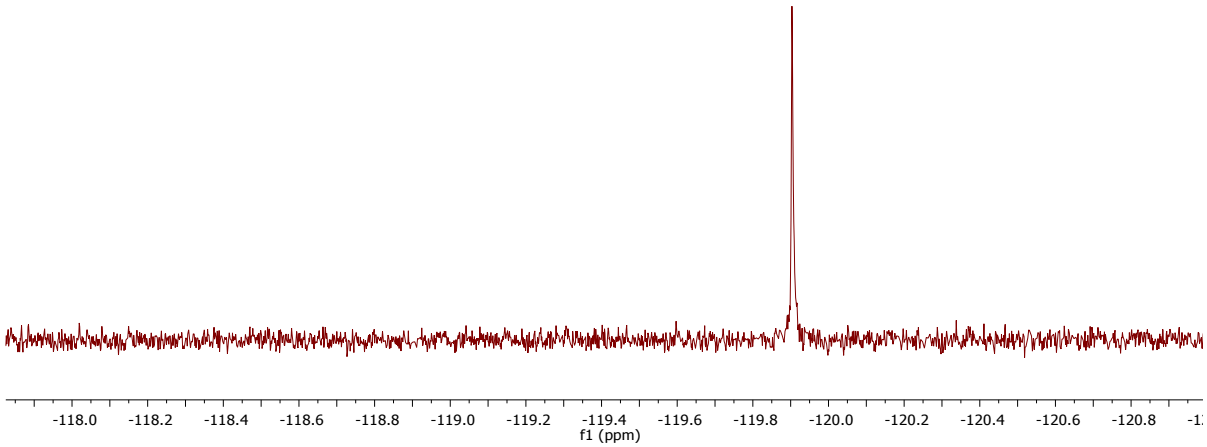


Figure S1. $^{19}F\{^{1}H\}$-NMR (376 MHz) of nucleocidin in D_2O from a *n*-butanol extract of a *S. calvus* culture.

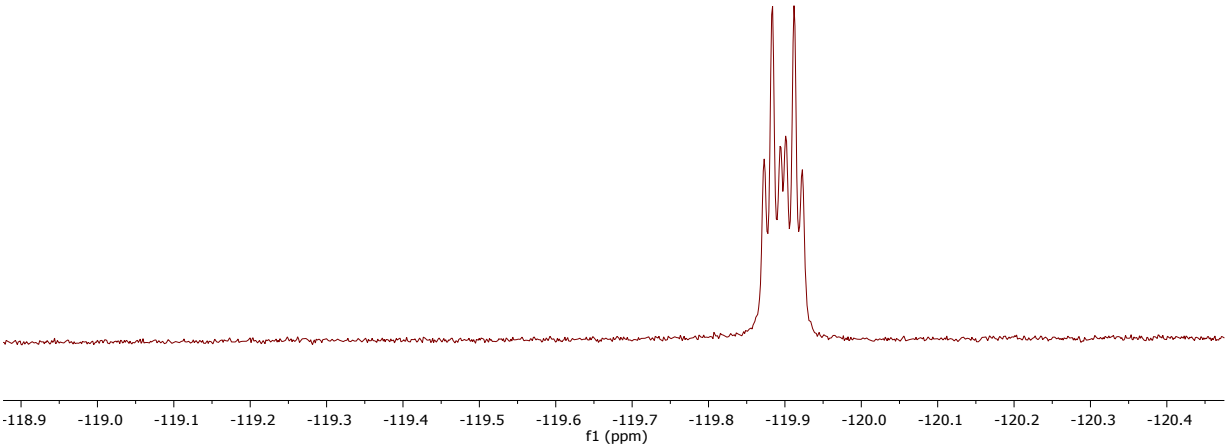
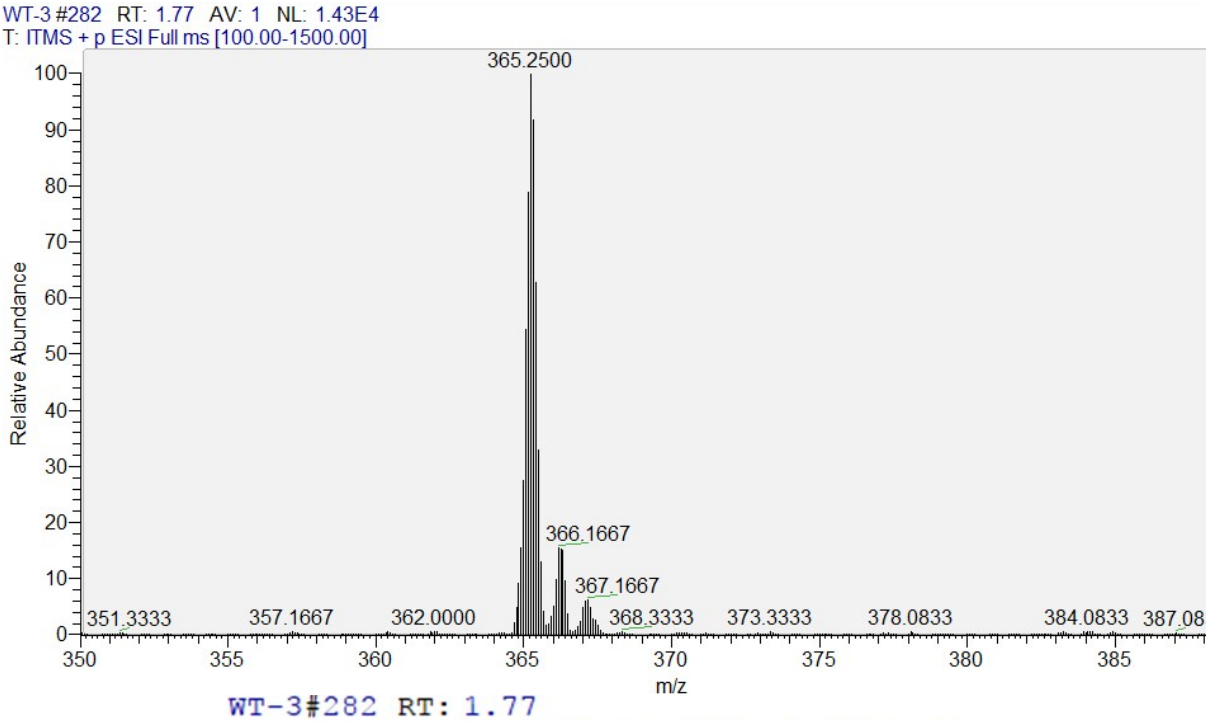


Figure S2. ^{19}F-NMR (658 MHz,) of nucleocidin in D_2O from a *n*-butanol extract of a *S. calvus* culture.

LC-MS for identification of nucleocidin from the *n*-butanol extract of the supernatant of a *Streptomyces calvus* fermentation



WT-3#282 RT: 1.77
T: ITMS + p ESI Full ms [100.00-1500.00]
m/z= 364.00-368.00

m/z	Intensity	Relative
365.0833	7803.8	54.45
365.1667	11297.8	78.84
365.2500	14330.8	100.00
365.3333	13150.6	91.76
365.4167	9001.6	62.81
366.0833	1414.7	9.87
366.1667	2211.7	15.43
366.2500	2195.6	15.32
366.3333	2161.9	15.09
366.4167	1385.3	9.67
367.0833	871.3	6.08
367.1667	888.0	6.20

Figure S3. LC-MS of nucleocidin in H_2O from the normal culture of *S. calvus*. $[M+H]^+$=365.2500 is the peak for nucleocidin.

Feeding pulse experiments

Cultures of *Streptomyces calvus* (100 mL in 250 mL conical flasks) were shaken at 30 ^{o}C at 180 rpm and the labelled glycerol was added from day 2, and then the same quantity every day for the next 5 days. The final concentration of labelled glycerol was 10 mM. The cultures were harvested after 8 days. The cells were discarded after centrifugation and the supernatant was extracted into *n*-butanol (20 mL). To ensure the reliability, a positive control of *Streptomyces calvus* was prepared with unlabeled glycerol under the same conditions. The organic *n*-butanol layer was concentrated under reduced pressure and the extract was re-solved in deuterium oxide (D_2O) and analysed by $^{19}F\{^{1}H\}$-NMR (376 MHz) and MHz ^{19}F-NMR (658MHz) to detect nucleocidin.

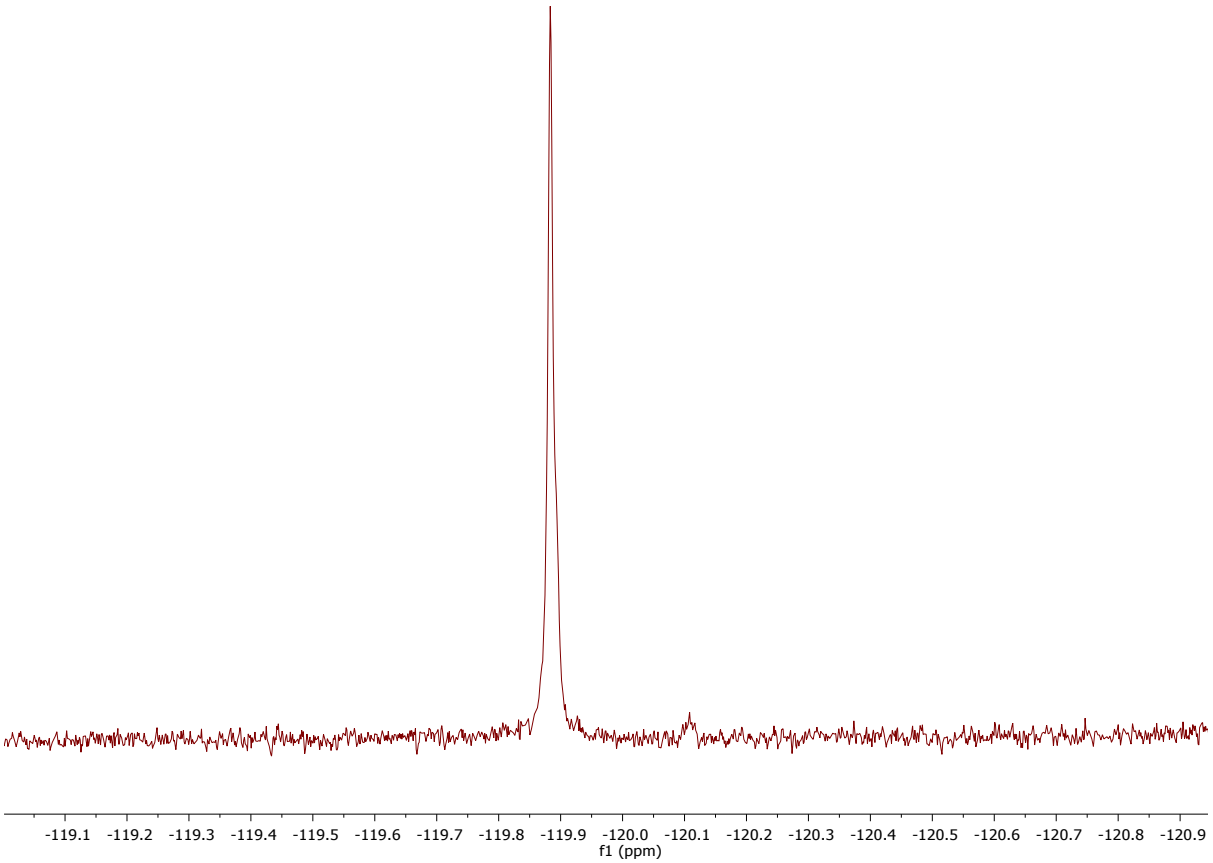


Figure S4. $^{19}F\{^{1}H\}$-NMR (376 MHz) of nucleocidin in D_2O from *S. calvus* after the $[^{2}H_5]$-glycerol **2c** feeding experiment.

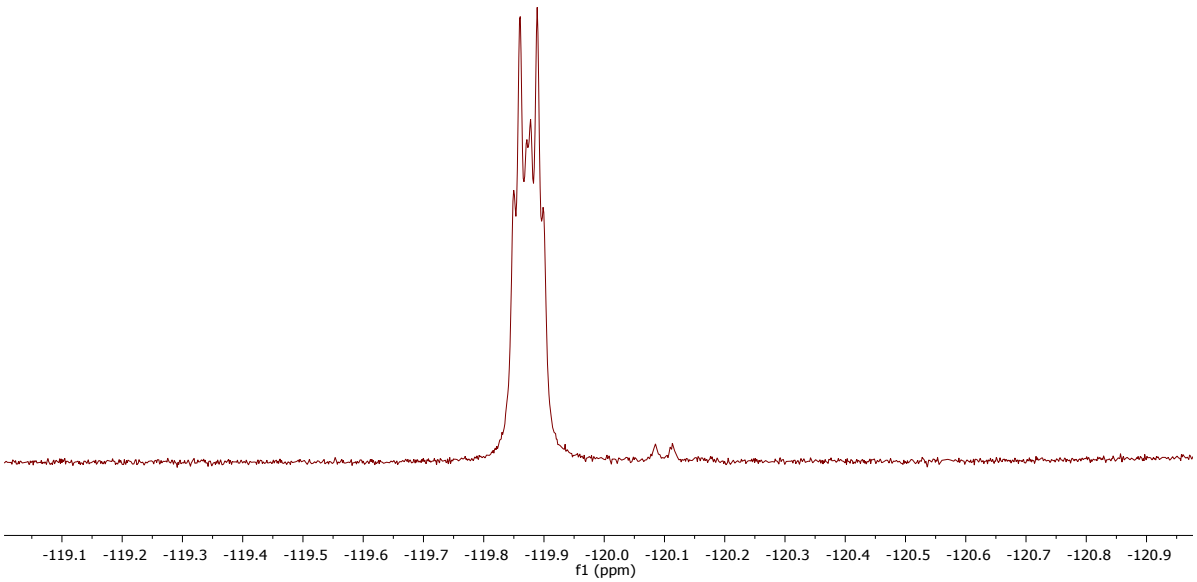


Figure S5. ^{19}F-NMR (658 MHz) of nucleocidin in D_2O from *S. calvus* after the $[^{2}H_5]$-glycerol **2c** feeding experiment.

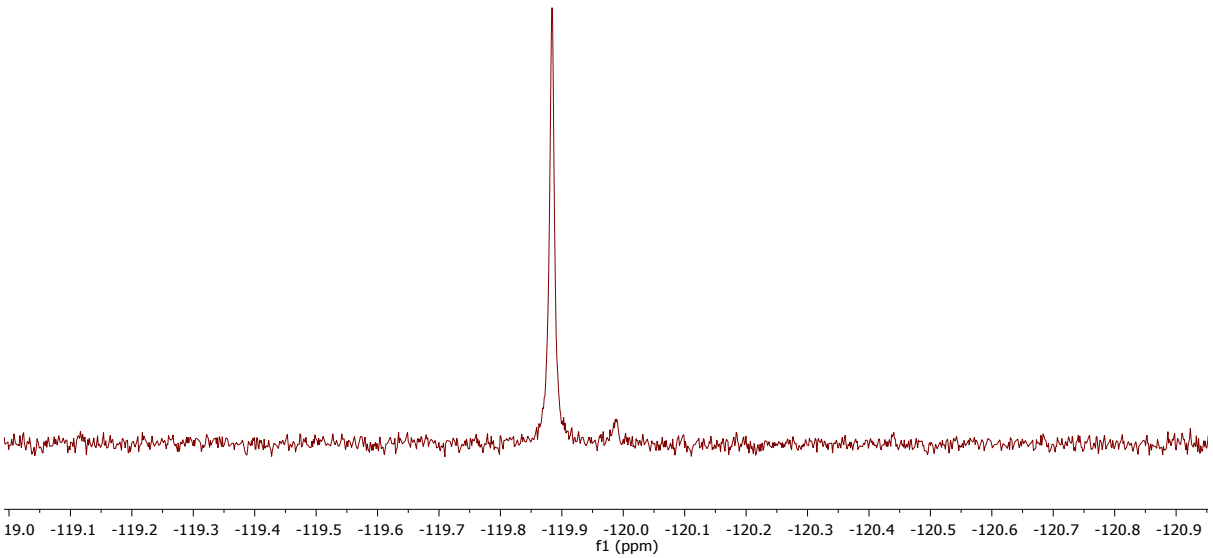


Figure S6. $^{19}F\{^{1}H\}$-NMR (376 MHz) of nucleocidin in D_2O from *S. calvus* after the (1*R*, 2*R*)-[$^{2}H_1$]-glycerol **2a** feeding experiment.

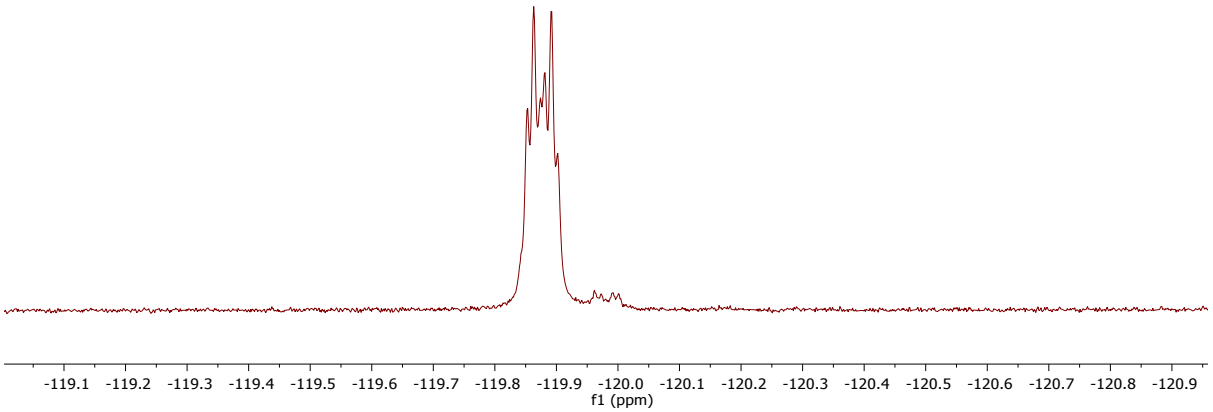


Figure S7. ^{19}F-NMR (658MHz) of nucleocidin in D_2O from *S. calvus* after the (1*R*, 2*R*)-[$^{2}H_1$]-glycerol **2a** feeding experiment.

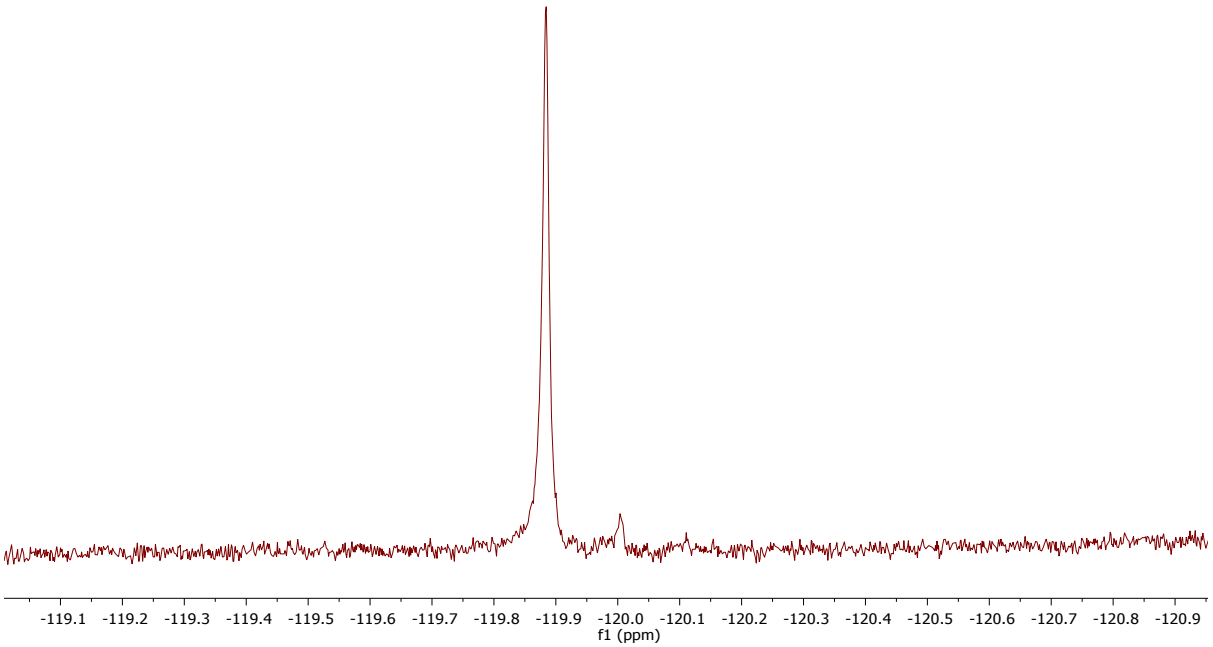


Figure S8. $^{19}F\{^{1}H\}$-NMR (376 MHz) of nucleocidin in D_2O from *S. calvus* after the (1*S*, 2*R*)-[$^{2}H_1$]-glycerol **2b** feeding experiment.

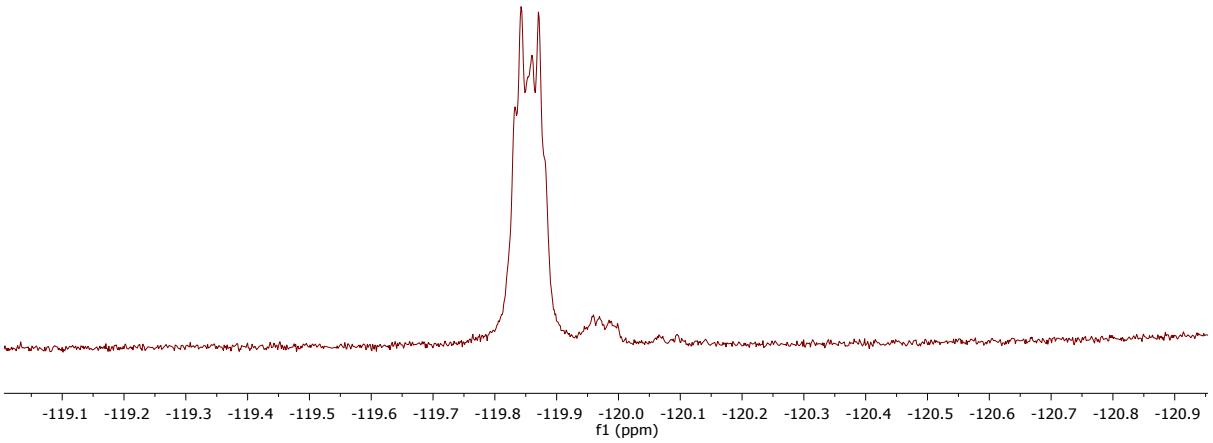
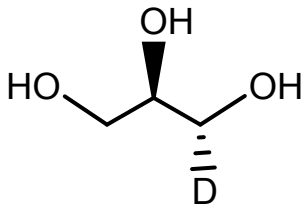


Figure S9. ^{19}F-NMR (658MHz) of nucleocidin in D_2O from *S. calvus* after the (1*S*, 2*R*)-[$^{2}H_1$]-glycerol **2b** feeding experiment.



(1*R*, 2*R*)-[$^{2}H_{1}$]-Glycerol 2a

The synthesis of **2a** following the method of Nieschalk and O'Hagan.[1]

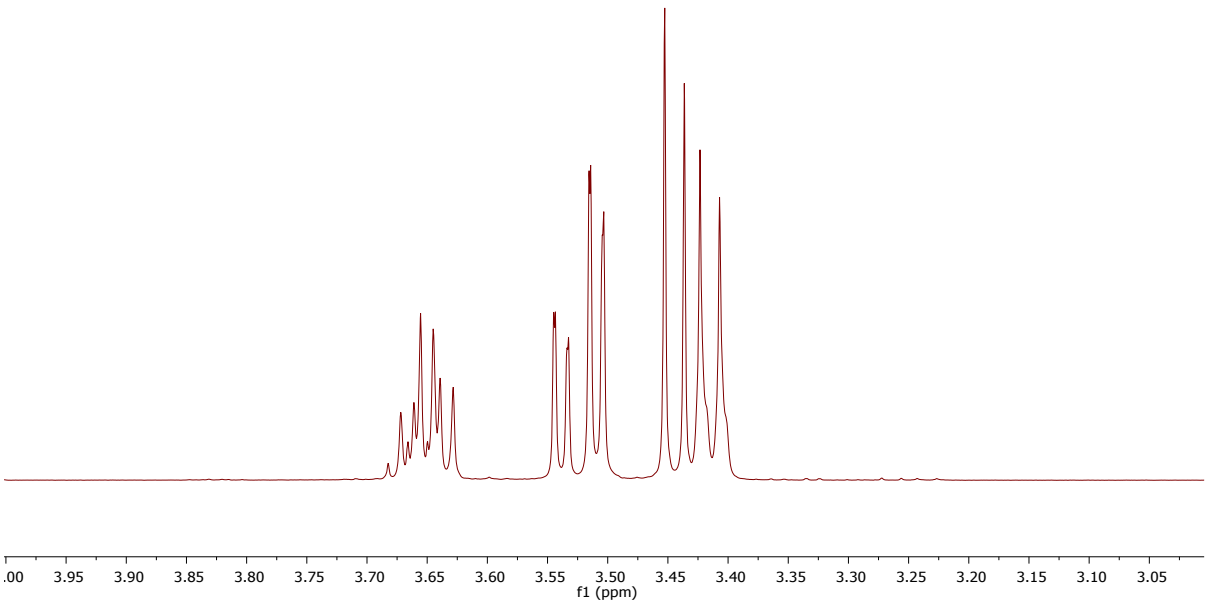
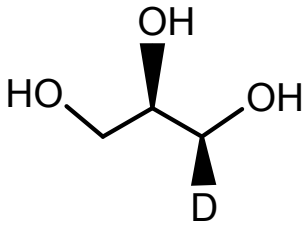


Figure S10. ^{1}H-NMR of **2a**



(1*S*, 2*R*)-[$^2H_1$]-glycerol 2b

The synthesis of **2b** following the method of Nieschalk and O'Hagan.[1]

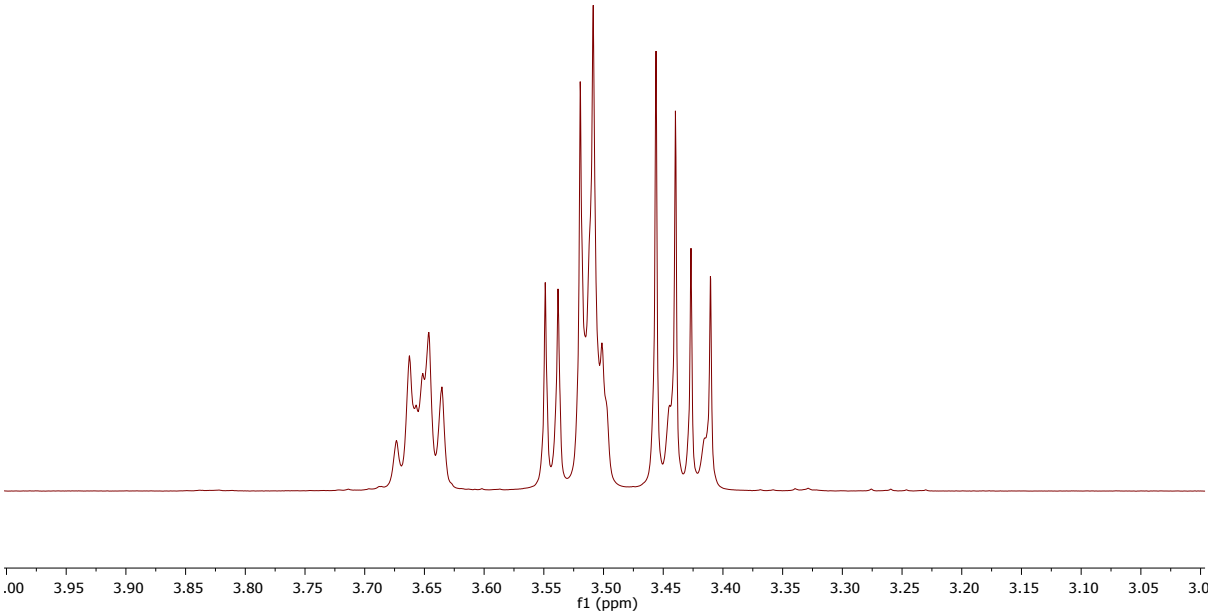


Figure S11. ^{1}H-NMR of **2b**

[$^2H_5$]-Glycerol 2c (≥ 98 atom % D) was obtained commercially from the Sigma-Aldrich Chemical Co. Ltd., product No-454524-1G.

Reference

1. J. Nieschalk, D. O'Hagan, *Tetrahedron: Asymmetry*, 1997, **14**, 2325-2330.