

Incorporation of [$^2\text{H}_1$]-(*1R,2R*)- and [$^2\text{H}_1$]-(*1S,2R*)- glycerols into the antibiotic nucleocidin in *Streptomyces calvus*.

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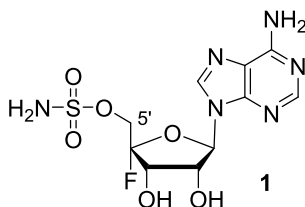
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Deuterium incorporations from [$^2\text{H}_1$]-(*1R,2R*) and [$^2\text{H}_1$]-(*1S,2R*) glycerols into the fluorine containing antibiotic nucleocidin, in *Streptomyces calvus* indicate that one deuterium atom is incorporated at the C-5' site of nucleocidin from each of these isotopomers of glycerol. Two deuteriums become incorporated at C-5' of nucleocidin after a feeding experiment with [$^2\text{H}_5$]-glycerol. These observations indicate that there is no obligate oxidation of the *pro-R* hydroxymethyl group of glycerol as it progresses through the pentose phosphate pathway and becomes incorporated into the fluorinated antibiotic.



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Nucleocidin **1**, is an antibiotic of the soil bacterium *Streptomyces calvus*.¹ It is one of the very rare natural products that contains a fluorine atom.² The biosynthesis of the fluorinated natural products has attracted interest as enzymatic methods for fluorination are rare and have biotechnological potential.³ Unlike the other fluorine containing natural products that have been described, nucleocidin **1** is the only metabolite that does not have an obvious origin from the enzyme that converts S-adenosyl-L-methionine to 5'-fluoro-deoxyadenosine.^{4,5} That fluorinase catalyses the first step in the biosynthesis of fluoroacetate and 4-fluorotheronine from *Streptomyces catleya*.⁶ It has now been well characterised and identified in a number of different bacteria.^{7,8} On the other hand, genome sequencing of the nucleocidin **1** producer indicates that there is not a related fluorinase within the *S. calvus* genome, implying a novel fluorination strategy.⁵

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Structurally too the presence of the tertiary fluorine at C-4' of the ribose ring in nucleocidin **1** suggests a distinct C-F bond forming reaction occurring during nucleocidin biosynthesis, whereas the previously characterised fluorinase delivers products carrying a fluoromethyl group (RCH₂F) or derived from a condensation of fluoroacetate (eg fluorocitrate). We recently reported isotopically

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[†] Electronic Supplementary Information (ESI) available: See <http://dx.doi.org/10.1039/b000000x/>

labelled glycerol incorporations into nucleocidin **1** after incubations with whole cell fermentations of *S. calvus*.⁹ In those experiments it was demonstrated that C-2 of glycerol became C-4' of nucleocidin, after a feeding experiment with [^{13}C]-glycerol. This was obvious from the large $^2J_{\text{CF}}$ coupling of 232 Hz in the ^{19}F -NMR spectrum of the isolated nucleocidin. Incorporation of perdeuterated [$^2\text{H}_5$]-glycerol and also (*R*)-[$^2\text{H}_2$]-glycerol into nucleocidin, resulted in incorporation of deuterium also into the C-5'-of nucleocidin, as determined by deuterium isotope induced chemical shifts in the resultant ^{19}F -NMR signal of nucleocidin. This demonstrated that the *pro-R* hydroxymethyl group of glycerol became the C-5' carbon of nucleocidin. There was no isotope incorporation after similar feeding experiments with (*S*)-[$^2\text{H}_2$]-glycerol. It follows that the *pro-S* hydroxymethyl group is lost in metabolism, consistent with glycerol processing through the pentose phosphate pathway and incorporation of the *pro-R* arm into the ribose moiety of adenosine. These deuterium incorporations could be determined from an upfield shift of 0.22 ppm in the resultant $^{19}\text{F}\{^1\text{H}\}$ -NMR spectrum of nucleocidin, induced by the presence of the isotope and its influence on the fluorine resonance. Based on the angular range (0.15-0.35 ppm)¹⁰ of magnitudes, from model compounds, of vicinal deuterium induced shift of the fluorine signal in ^{19}F -NMR, the 0.22 ppm shift suggested that a single deuterium atom had become incorporated into nucleocidin at C-5'. It became an objective then to explore the stereochemistry of the residual deuterium by evaluating incorporations from [$^2\text{H}_1$]-(*1R,2R*)-**2a** and [$^2\text{H}_1$]-(*1S,2R*)-**2b** glycerols. We have now prepared these isotopomers of glycerol following a previously reported synthesis protocol¹¹, and we have explored their incorporation into nucleocidin in feeding experiments in *S. calvus* fermentations.

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As before the isotopically labelled glycerols **2a-2c** were pulse fed to cultures of *S. calvus* to a final concentration of 5-7 mM. The antibiotic was isolated after 6 days, and extracted into butanol. The titres of nucleocidin **1** are consistent but low, however a very clear fluorine signal is evident in the ^{19}F -NMR spectrum at -119 ppm when it is produced. The resultant $^{19}\text{F}\{^1\text{H}\}$ -NMR spectra for the three glycerol feeding experiments and a control are illustrated in Figure 1. There is a clear and consistent heavy isotope induced fluorine signal of ~0.11 ppm upfield of the natural abundance signal, in the nucleocidins isolated after feeding experiments with glycerols **2a** and **2b**, and an upfield signal of twice the magnitude (~0.22 ppm) in the nucleocidin isolated after feeding glycerol **2c**.

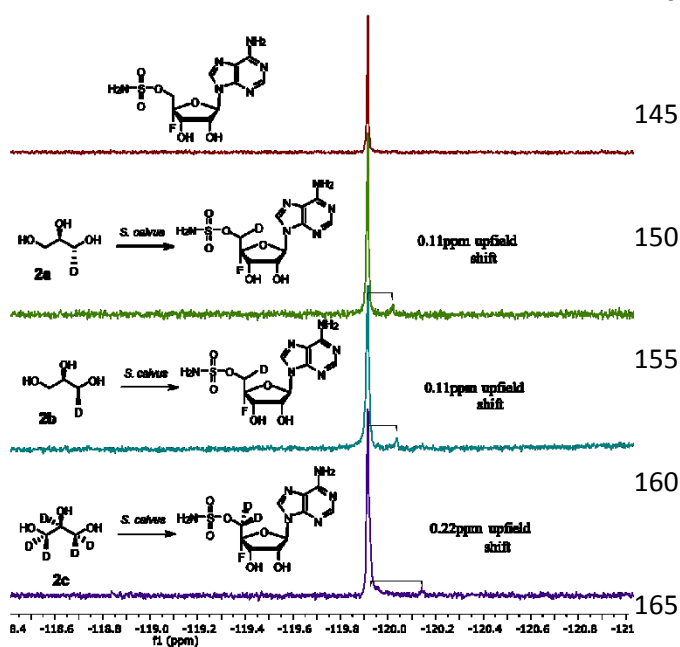
For glycerols **2a** and **2b** the deuterium atoms are diastereotopically located to distinguish the fate of the methylene hydrogens on the *pro-R* arm of glycerol. We find that in both cases isotope is retained at C-5' as evinced by the isotope induced shift (~0.11 ppm) in the ^{19}F -NMR signal of the resultant nucleocidin. The magnitude of this shift is below the expected threshold for a single deuterium, based on earlier calibration data,¹⁰ however the presence of a heavy atom induced shift

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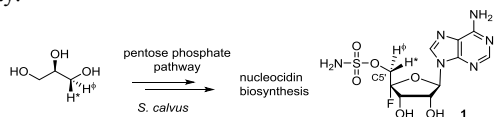
90 from each of these experiments is obvious and of similar magnitude in 130
 each case. A feeding experiment with $[^2\text{H}_5]$ -glycerol **2c** showed again
 a population of nucleocidin molecules with an 0.22 ppm upfield shift
 in the resultant ^{19}F -NMR signal, as observed previously.⁹ Since this is
 twice the magnitude of the induced shift relative to the feeding
 95 experiments with glycerols **2a** and **2b**, we are forced to conclude that
 two deuteriums were incorporated at C-5' of nucleocidin from $[^2\text{H}_5]$ -
 glycerol **2c** and also from (*R*)- $[^2\text{H}_2]$ -glycerol in our previous
 135 experiment,⁹ and that single deuteriums are retained from each of
 (*1R,2R*)- $[^2\text{H}_1]$ - **2a** and (*1S,2R*)- $[^2\text{H}_1]$ -**2b** glycerols.

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105 **Figure 1** $^{19}\text{F}\{^1\text{H}\}$ -NMR spectrum of nucleocidin **1** after *S. calvus* feeding
 experiments with glycerols, $[^2\text{H}_1]$ -(*1R,2R*)- **2a**; $[^2\text{H}_1]$ -(*1S,2R*)-**2b** and
 170 $[^2\text{H}_5]$ - **2c**. Both **2a** and **2b** result in a population (~8 %) of nucleocidin
 molecules with a shifted (0.11 ppm) signal suggesting retention of one
 deuterium at C-5' and **2c** results in a similar population of labelled
 110 molecules but with a larger isotope induced shift (0.22 ppm) suggesting
 retention of two deuteriums at C-5'.

The mechanism by which the fluorine becomes incorporated into
 nucleocidin **1** remains to be evaluated. However it follows from
 these observations that there is no evidence to suggest an obligate
 115 oxidation at C-5' of the ribose moiety of an adenosine precursor
 during nucleocidin **1** biosynthesis as previously implied,⁹ as there
 is a population of nucleocidin molecules carrying two deuterium
 atoms at C-5' after $[^2\text{H}_5]$ -glycerol **2c** incorporation. The retention
 of both hydrogens from the *pro*-(*R*) hydroxymethyl arm of glycerol
 120 into nucleocidin **1** is illustrated in Scheme 1, with the
 stereochemistry shown consistent with the pentose phosphate
 pathway.⁹



125 **Scheme 1.** Minimal representation of nucleocidin biosynthesis in *S. calvus*
 indicating the retention of both hydrogens from the *pro*-(*R*) hydroxymethyl arm
 of glycerol through the fluorination process..

Acknowledgement: We thank the Chinese Scholarship Council
 for Ph.D Studentship support (to XF).

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