Supporting Information for:
The First Biosynthetic Studies of the Azinomycins – Acetate Incorporation into Azinomycin B
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**Experimental procedures:** A 100 ml seed culture of *S. sahachiroi* (NRRL 2485), maintained on GYM agar plates (glucose monohydrate, 4 g/L; yeast extract, 4 g/L; malt extract, 10 g/L; CaCO₃, 2 g/L; agar, 12 g/L; tap water to balance; adjusted to pH 6.8 with NaOH, 1 M before sterilisation) at 28 °C, was grown in PS5 medium (Pharmamedia, 5 g/L; starch, 5 g/L; tap water to balance; adjusted to pH 6.0) at 30 °C, 200 rpm for 24 hours then 25 ml was used to inoculate 500 ml of PS5 medium, which was grown at 30 °C, 200 rpm for 72 hours. Aqueous solutions of labeled precursors (purchased from Cambridge Isotope Labs) were added through a 0.2 µm filter at concentrations and times as outlined in the text. After centrifugation of the cultures, azinomycin B was isolated by extraction of the supernatant (pH 8.0) with an equal volume of chloroform at 4°C, concentration and then a series of precipitations. For each 100 ml of culture, the residue was precipitated from 600 µl chloroform/hexane (1:29), centrifuged at 2000 rpm and the supernatant discarded. This was repeated and then the residue dissolved in 600 µl chloroform/hexane (2:1), centrifuged and the supernatant retained. This residue was then dissolved in 600 µl chloroform/diethyl ether (1:4), centrifuged and the supernatant concentrated to give pure azinomycin B (~1.5 mg per 100 ml). ¹³C NMR spectra were obtained on a Bruker Advance 400 at 100.6 MHz in CDCl₃ or CD₂Cl₂, referenced to solvent.
1. $^{13}$C NMR spectrum in CDCl$_3$ of unlabeled azinomycin B.
2. $^{13}$C NMR spectrum in CD$_2$Cl$_2$ of unlabeled azinomycin B.
Resonances from an unidentified impurity are marked with X.
The expansion shows C12, C13, C18 and residual chloroform.
3. HMBC $^1$H-$^{13}$C correlation spectrum of the naphtoate fragment of azinomycin B. These data were used to reassign C4′a and C5′.
4. $^{13}$C NMR spectrum in $d_6$-DMSO of the naphthoate fragment of azinomycin B after feeding 1-$^{13}$C acetate.
5. $^{13}$C NMR spectrum in CDCl$_3$ of azinomycin B after feeding $^{1-^{13}}$C acetate.
Expansions of $^{13}$C NMR spectrum in CDCl$_3$ of azinomycin B after feeding $1^{13}$C acetate.
6. $^{13}$C NMR spectrum in CD$_2$Cl$_2$ of azinomycin B after feeding 2-$^{13}$C acetate.
7. $^{13}$C NMR spectrum in CDCl$_3$ of azinomycin B after feeding 1,2-$^{13}$C$_2$ acetate.
Expansions of $^{13}$C NMR spectrum in CDCl$_3$ of azinomycin B after feeding 1,2-$^{13}$C$_2$ acetate.
8. $^{13}$C NMR spectrum in CD$_2$Cl$_2$ of azinomycin B after feeding 1,2-$^{13}$C$_2$ acetate.

Resonances from an unidentified impurity are marked with X.
Expansions of $^{13}$C NMR spectrum in CD$_2$Cl$_2$ of azinomycin B after feeding 1,2-$^{13}$C$_2$ acetate.
9. $^{13}$C NMR spectrum in CDCl$_3$ of azinomycin B after feeding methyl-$^{13}$C-methionine.

10. ESMS spectrum of azinomycin B after feeding methyl-$^{13}$C-methionine.