Supporting Information for:
Biosynthetic studies on the azinomycins: The pathway to the naphthoate fragment
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ESMS spectrum of 5a.
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\( ^2 \text{H} \) NMR spectrum of 4b.
ESMS spectrum of 4b.
Experimental procedures for feeding studies: 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 as their sodium salts were added through a 0.2 µm filter at concentrations 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).

¹H NMR spectra were obtained on a Bruker Advance 400 in CDCl₃, d₆-acetone or d₆-DMSO, referenced to solvent. ²H NMR spectra were obtained on a Bruker Advance 400 in CHCl₃, referenced to d₆-acetone. Electrospray ionisation mass spectrometry (ESI-MS) was performed on a MicroMass Platform LC. Samples were prepared in a solution generating positive ions (acetonitrile/H₂O 50:50 + 1% formic acid).

¹H NMR assignments for azinomycin B.

![Azinomycin B NMR](image)

δH (400 MHz; CDCl₃) 12.47 (1 H, br s, OH-4), 12.47 (1 H, s, H-5), 8.55 (1 H, dd, J 7.0, 3.6, H-8’), 8.20 (1 H, br s, H-16), 7.94 (1 H, d, J 2.9, H-2’), 7.48 (1H, d, J 2.5 Hz, H-4’), 7.32 (1 H, m, H-7’), 7.32 (1 H, m, H-6’), 5.50 (1 H, d, J 4.0, H-13), 5.12 (1 H, s, H-18), 4.64 (1 H, dd, J 4.8, 4.0, H-12), 3.96 (3 H, s, OCH₃-3’), 3.96 (1 H, OH-12), 3.36 (1 H, m, H-11), 2.98 (1 H, d, J 4.3, H-21b), 2.80 (1 H, d, J 4.3, H-21a), 2.70 (1 H, H-10b), 2.68 (3 H, s, CH₃-5’), 2.30 (1 H, H-10a), 2.30 (3 H, s, CH₃-1), 2.20 (3 H, s, CH₃-15), and 1.53 (3 H, s, CH₃-20).
ESMS of azinomycin B after feeding of $4a$, $5a$ and $6a$. 
$^2$H NMR of azinomycin B after feeding of 4a.
$^2$H NMR of azinomycin B after feeding of 5a.
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$^2$H NMR of the mixture of 4a and 6b used for competition feeding.
$^{2}$H NMR of azinomycin B after feeding of 4a and 6b.
$^2$H NMR of the mixture of 5a and 6b used for competition feeding.
$^2$H NMR of azinomycin B after feeding of 5a and 6b.
ESMS of azinomycin B after feeding of 4b.
$^2$H NMR of azinomycin B after feeding of 4b.
$^1$H NMR of azinomycin B after feeding of 4b.
$^1$H NMR spectrum of 6a.
$^2$H NMR spectrum of 6a.
ESMS spectrum of 6a.
$^1$H NMR spectrum of 5a.
$^2$H NMR spectrum of 5a.
ESMS spectrum of 5a.
H NMR spectrum of 4a.
$^2$H NMR spectrum of 4a.
ESMS spectrum of 4a.
$^{1}$H NMR spectrum of 6b.
$^2$H NMR spectrum of 6b.
EIMS spectrum of 6b.

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Landreau CL237 05/06/03
GCT03-0597 85 (1.416) Cm (75:85-20:50)
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103.0540

Elemental Composition Report

Single Mass Analysis (displaying only valid results)
Tolerance = 3.0 mDa / DBE: min = 0.0, max = 50.0

Monoisotopic Mass, Odd and Even Electron Ions
4029 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

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Operator's Comments

EI+
Probe at 100 deg C
$^1$H NMR spectrum of 4b.
$^2$H NMR spectrum of 4b.
ESMS spectrum of 4b.