



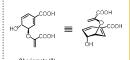
Synthetic and Biosynthetic Studies on Abyssomicin C

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Background

- Abyssomicin C (1) is a polyketide derived spirotetronate isolated from the marine microorganism Verrucosispora maris.
- It is a potent inhibitor of Gram-positive bacteria such as MRSA (MIC = 4 µg/mL) and VRSA (MIC = 13 µg/mL).
- It mimics chorismate and so inhibits the pABA/tetrahydrofolate biosynthetic pathway, making it an attractive target for novel antibiotics.¹
- In this project, we aim to elucidate the structures of key enzymes involved in the biosynthesis of 1 and investigate their use as biocatalysts in for the preparation of novel bioactive compounds.





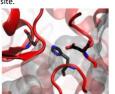
Proposed Biosynthesis² Module acetate (C-1) Feeding studies to determine building blocks to 1. DH =Dehydratase

AbyA5 Studies

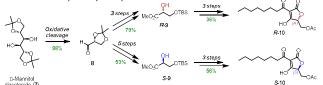
- Few enzymes have been reported for acetate elimination.^{3,4}
- DNA sequence of wildtype AbyA5 was transformed into E. coli for protein expression.
- AbyA5 structure was determined and it retains an alpha/beta hydrolase catalytic triad, with serine, histidine and aspartic acid residues housed in the active site





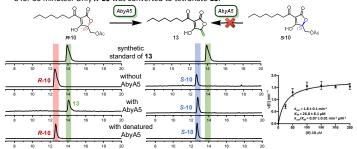


Tetronic acids R-10 and S-10 were synthesised from D-mannitol (7) in order to investigate the function and stereospecificity of AbyA5.5



A standard of ${f 13}$ was synthesised in 6 steps from commercially available lactone ${f 11}$ via formation of the exo-methylene on tetronate 12.

Tetronic acids *R*-10 and *S*-10 were incubated separately with AbyA5 in pH 7.5 Tris-HCl buffer at 25 °C for 30 minutes. Only R-10 was converted to tetronate 13



AbyU Studies

- Diels-Alder (DA) reactions are typically conducted in heat or Lewis acid-mediated reactions.
- AbyU was isolated and characterised as a homodimer containing 8-stranded antiparallel β-barrels.
- Protected tetronate 18 was synthesised in order to assess enzyme function of AbyU.
- AbyU catalyses the [4+2] cycloaddition of substrate 18 to spirotetronate 19

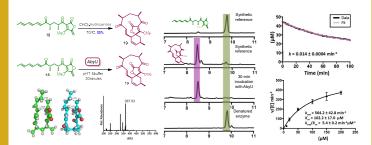






- Synthesis of tetronate 18 was conducted from commercially available alcohol 14 to give intermediate lactone 16 which was subsequently transformed to the linear tetronate 18.
- Synthetic standard of spirotetronate 19 was synthesised by heat mediated cyclisation of 18

- Incubation of 18 with AbyU in pH 7.5 Tris-HCl buffer at 25 °C for 30 minutes gave 19.



Conclusions and Future Work

- Enzymes AbyA5 and AbyU were successfully isolated and characterised using X-ray crystallography.
- Incubation of tetronic acids R-10 and S-10 with AbyA5 confirmed the function and stereospecificity of the enzyme, with R-10 giving 13 exclusively.
- AbyU efficiently catalyses the DA reaction of linear tetronate 18 to spirotetronate 19.
- Computational studies reveal contortion of linear substrate 18 within the AbyU active site to facilitate the [4+2] cycloaddition.
- Investigations into AbyU promiscuity and development into a biocatalyst are ongoing

References

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