Electronic Supplementary information

A butenolide intermediate in methylenomycin furan biosynthesis is implied by incorporation of stereospecifically ^{13}C-labelled glycerols

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Experimetal Procedures

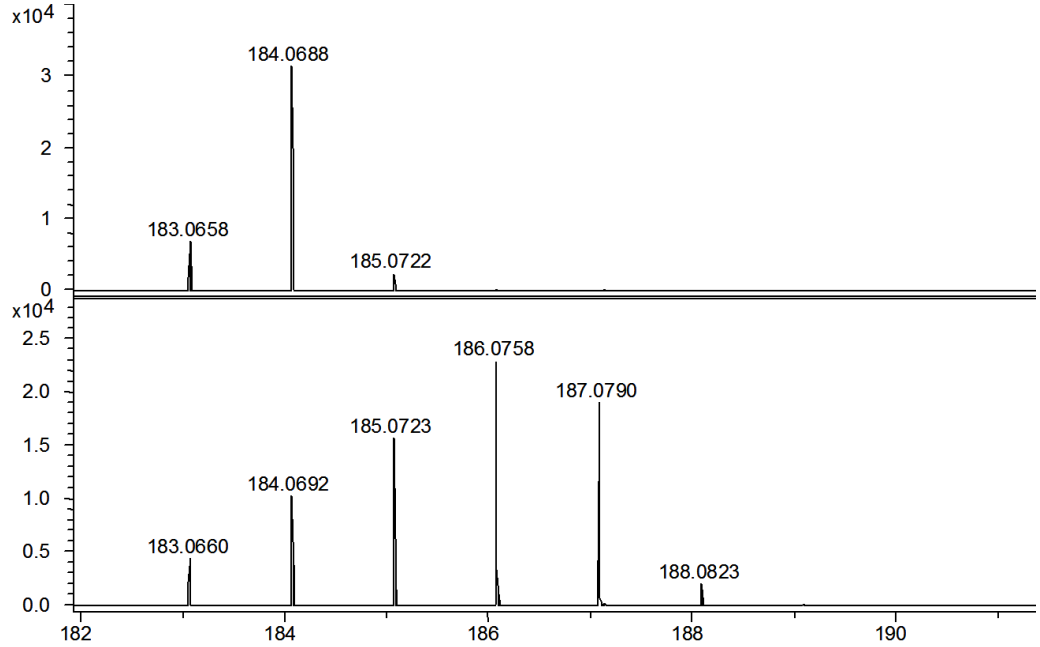
Compound **2** was synthesised according to the procedure of Sello and co-workers.[1] The O-deuterated derivative of **2** was prepared by suspending a small sample in a 1:1 mixture of CD_3OD/D_2O prior to high resolution ESI-MS/MS analysis.

For the incorporation experiements, fresh spores of *S. coelicolor* W74 were prepared from one MS agar[2] Petri plate by resuspension in sterile water and directly used to inoculate 50 mL SMM medium[2] containing 50 mg of [1-^{13}C]-L-glycerol or [3-^{13}C]-L-glycerol as the only carbon source. After 5 days of incubation at 30 ºC, both cultures were filtered and the acidified supernatants (pH 3) were extracted with EtOAc. After evaporation of the organic extract, **2** was purified from the residue according to a previously described procedure[3] and resuspended in 1:1 $MeOH/H_2O$ (1 mL) for high resolution ESI-MS/MS analysis.

High resolution ESI-MS analysis of MMF2 isolated from the feeding experiments

High resolution ESI-MS/MS analyses of synthetic and labelled **2** were carried out on a Bruker MaXis time-of-flight mass spectrometer using the following settings. End plate off-set: -500 V; Capillary: +4000 V; Nebulizer: 1.6 Bar; Dry gas: 8.0 L/min; Dry temperature: 180 °C; Ion transfer: Ion funnel RF: 200 Vpp; Multiple RF: 200 Vpp; Quadrupole: Ion Energy: 4.0 eV; Low Mass: 55 m/z; Collision Cell RF: 350 Vpp; Ion Cooler RF (Collision sweeping): 50 Vpp (50%) + 250 Vpp (50%); Transfer time: 120 µs; Pre-Pulse Storage: 1 µs; MRM setting: Isolation width: 1.00 Da; Collision Energy: 4.0 eV (Isolation) and 20.0 eV (Fragmentation).

Compound **2** isolated from the feeding experiment with [3-^{13}C]-L-glycerol was predominantly singly ^{13}C-labelled (*m/z* = 184.0688), with some unlabelled material (*m/z* = 183.0658) also present (top spectrum). Compound **2** isolated from the feeding experiment with [1-^{13}C]-L-glycerol contained a mixture of unlabelled (*m/z* = 183.0660), singly (*m/z* = 184.0692), doubly (*m/z* = 185.0723), triply (*m/z* = 186.0758) and quadruply (*m/z* = 187.0790) ^{13}C-labelled material (bottom spectrum).



References

1. J. B. Davis, J. D. Bailey and J. K. Sello, *Org. Lett.*, 2009, **11**, 2984-2987.
2. T. Kieser, M. J. Bibb, M. J. Buttner, K. F. Chater and D. A. Hopwood, *Practical Streptomyces Genetics*, John Innes Foundation, Norwich (2000).
3. C. Corre, L. Song, S. O'Rourke, K. F. Chater and G. L. Challis, *Proc. Natl Acad. Sci. USA*, 2008, **105**, 17510-17515.