Electronic Supplementary Information for:

First *in vitro* directed biosynthesis of new compounds by a minimal Type II polyketide synthase: evidence for the mechanism of chain length determination - Measuring or Counting?

T. P. Nicholson, C. Winfield, J. Westcott, J. Crosby, T. J. Simpson, and R. J. Cox*.
School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK; E-mail: r.j.cox@bris.ac.uk

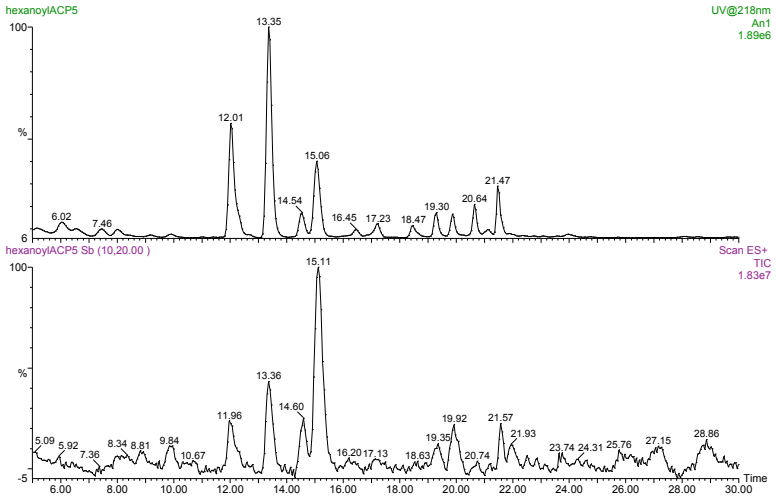
Appendix 1. **Experimental Procedures.**
Appendix 2. **LCMS Spectra for Hexanoyl ACP as starter unit.**
Appendix 3. **LCMS Spectra for □-keto Hexanoyl ACP as starter unit.**
Appendix 4. **LCMS Spectra for Butyryl ACP as starter unit.**
Appendix 5. **Putative Polyketides of *m/z* 290Da.**
Appendix 6. **Putative Polyketides of *m/z* 304Da.**

Appendix 1. Experimental procedures.

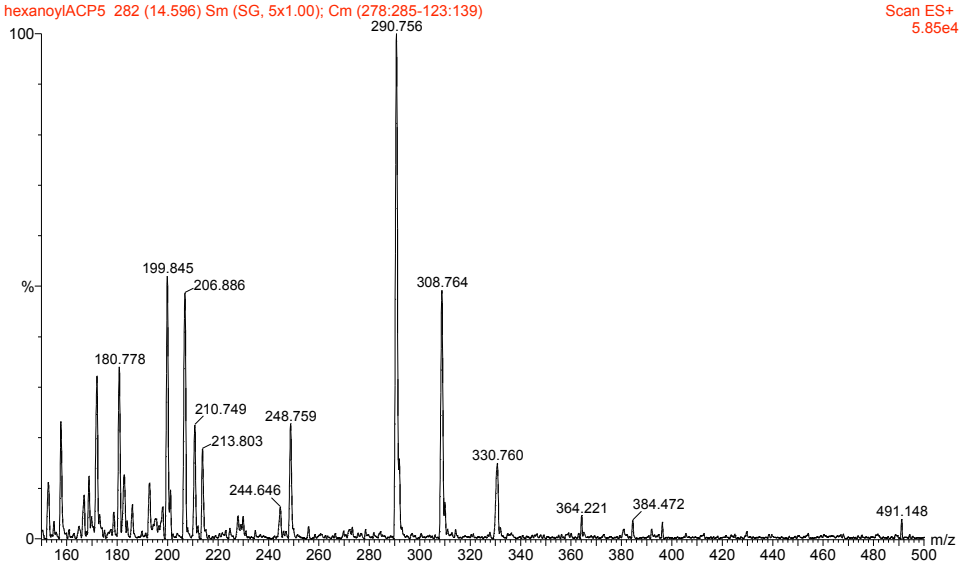
Assay conditions. Reactions were prepared in a total volume of 100□L of assay buffer (100 mM phosphate buffer pH 7.3; 10% glycerol; EDTA 2 mM; DTT 1 mM) containing actinorhodin $KS_{\square}/KS_{\square}$ (1□M) and actinorhodin (*holo*- or acyl-) ACP (50□M). The reaction was incubated for 10 min at 30˚C, then malonyl CoA (1mM) was added. After 2h the reaction mixture was diluted with assay buffer (100□L), acidified by the addition of NaH_2PO_4 (100mg) and extracted with EtOAc (3□300 □L, vortex + centrifuge). The organic layers were removed, combined and evaporated under a gentle stream of N_2. The residue was dissolved in HPLC grade CH_3CN/H_2O (1:9 *v/v*, 30□L) and examined by LCMS.

LCMS conditions. Buffer **A**, 0.05% trifluoroacetic acid (TFA) in H_2O. Buffer **B**, 0.05% TFA in HPLC grade CH_3CN. Buffers were filtered (0.4□m) and degassed prior to use. A Hewlett Packard (HP) 1050 HPLC running the following programme was used: Flow rate 300□L/min; 0.0□ 0.25min, 10%**B**; 0.25□ 30.0min, 10□ 55%**B**; 30.0□ 31.0min, 55□ 95%**B**; 31.0□ 34.0min, 95%**B**; 34.0□ 36.0min, 95□ 10%**B**; 36.0□ 40.0min, 10%**B**. The outlet of the column was run through a HP1050 uv detector (218nm) and then split, *ca* 1/2 passing into a Micromass Platform II ESMS instrument operating in ES^+ mode, detecting mass ions between 150 and 600 *m/z*. The column was a Waters Xterra RP_8 5□m, 2.1 □ 150mm.

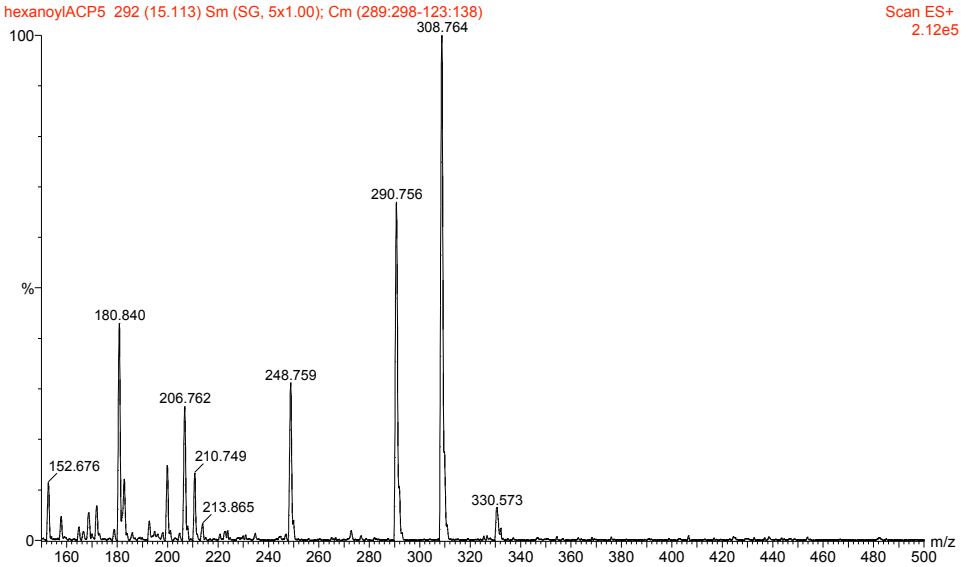
Appendix 2. LCMS Spectra for Hexanoyl ACP as starter unit.



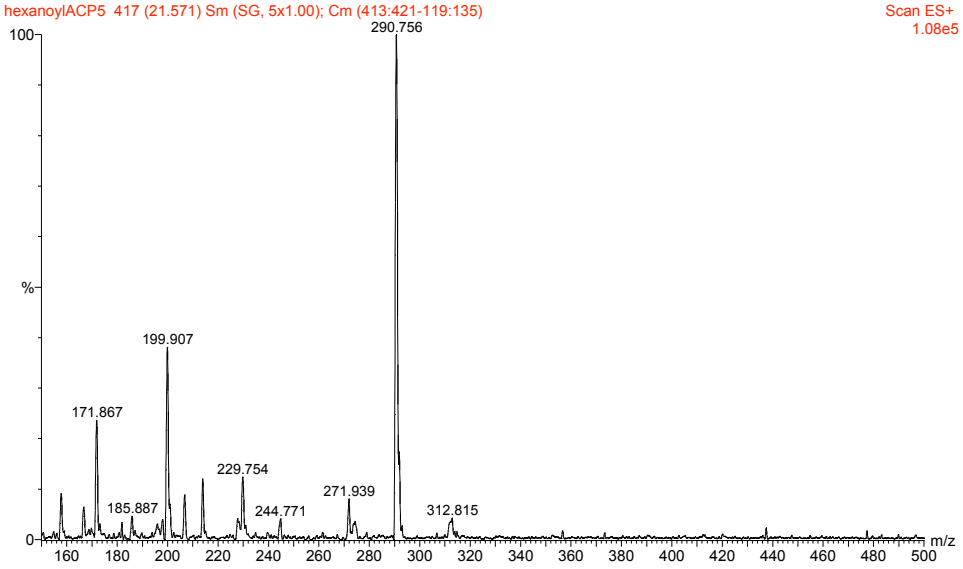
Peak 1 (14.59 min)



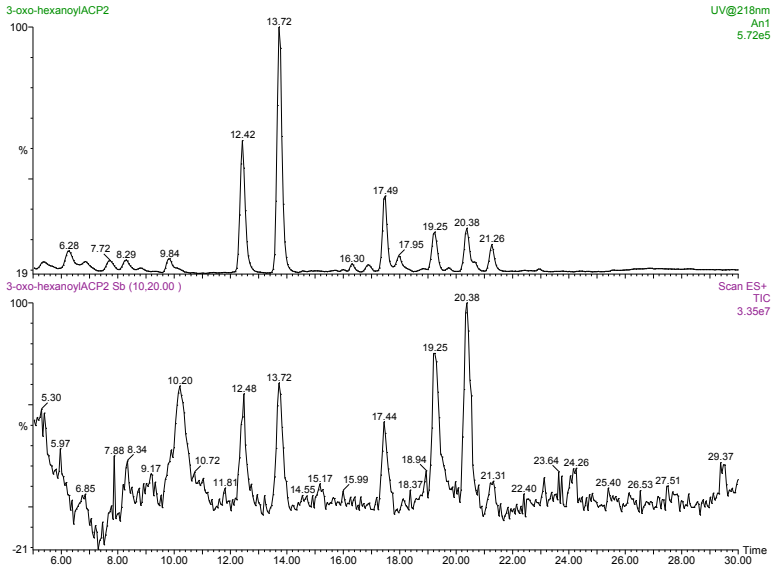
Peak 2 (15.11 min)



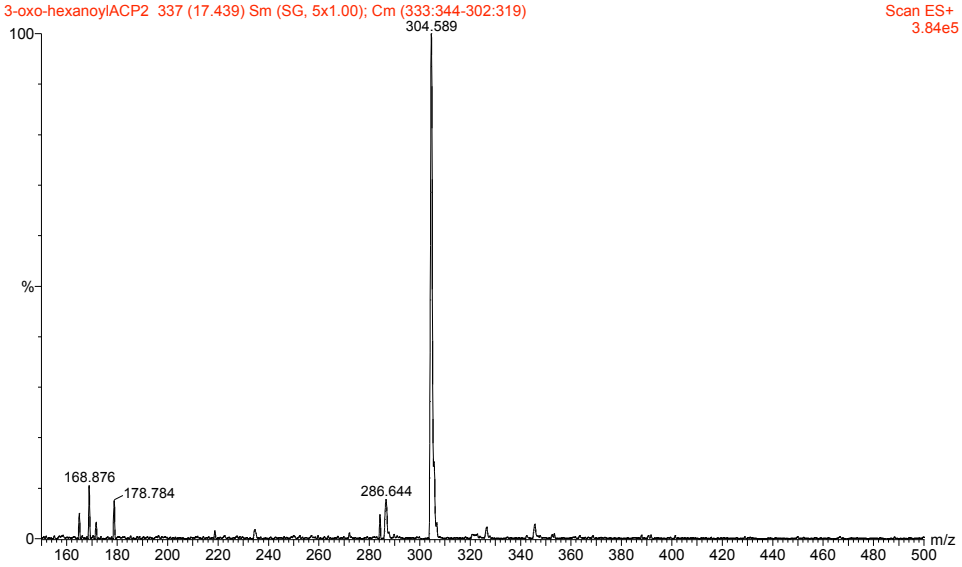
Peak 3 (21.57 min)



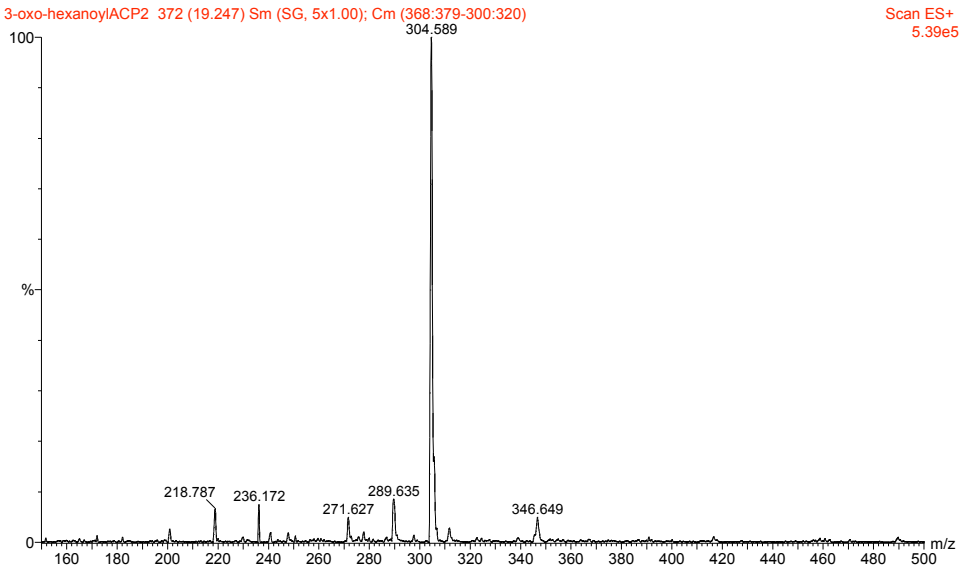
Appendix 3. LCMS Spectra for □-keto Hexanoyl ACP as starter unit.



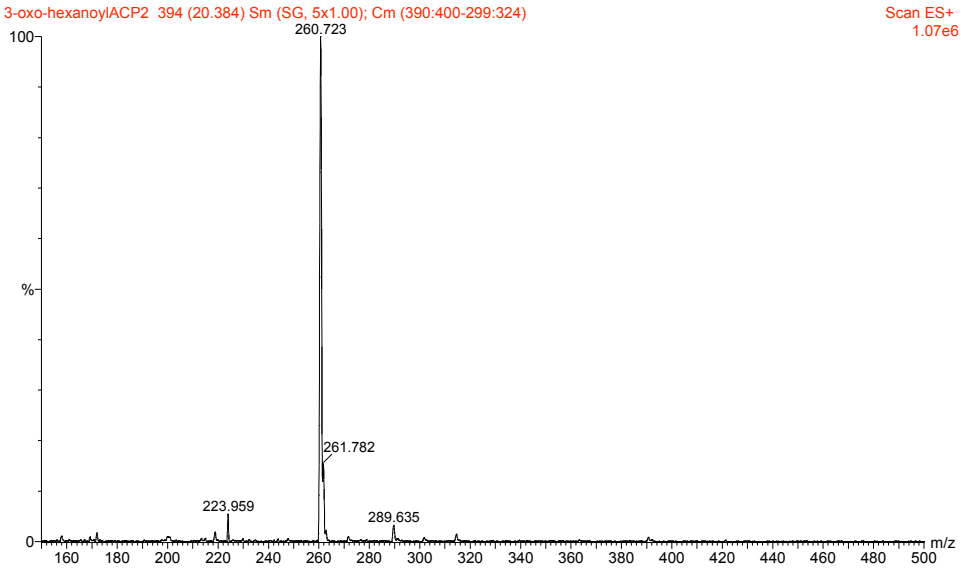
Peak 1 (17.44 min)



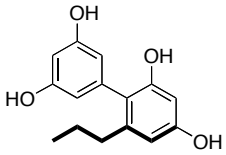
Peak 2 (19.25 min)



Peak 3 (20.38 min)

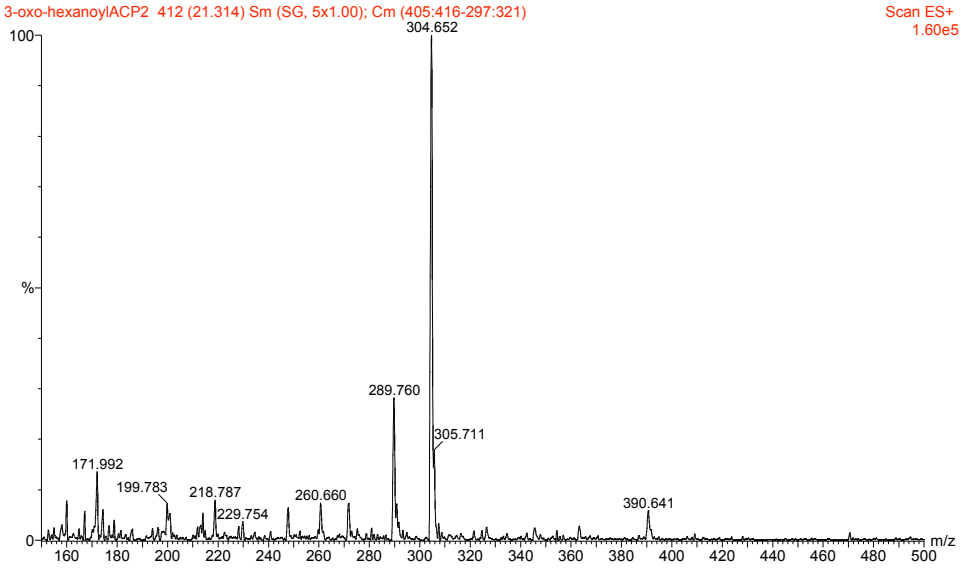


This compound, *m/z* 44 less than the other peaks, is a decarboxylated analogue, possibly:

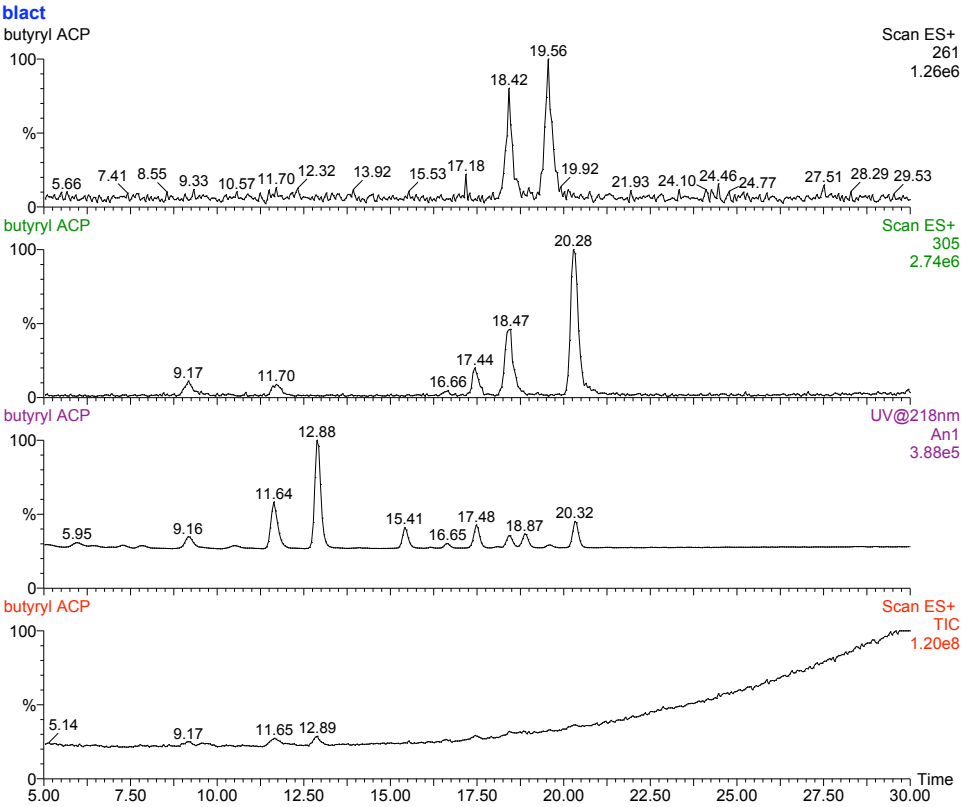


derived from **1c**, ***Appendix 6***

Peak 4 (21.31 min)

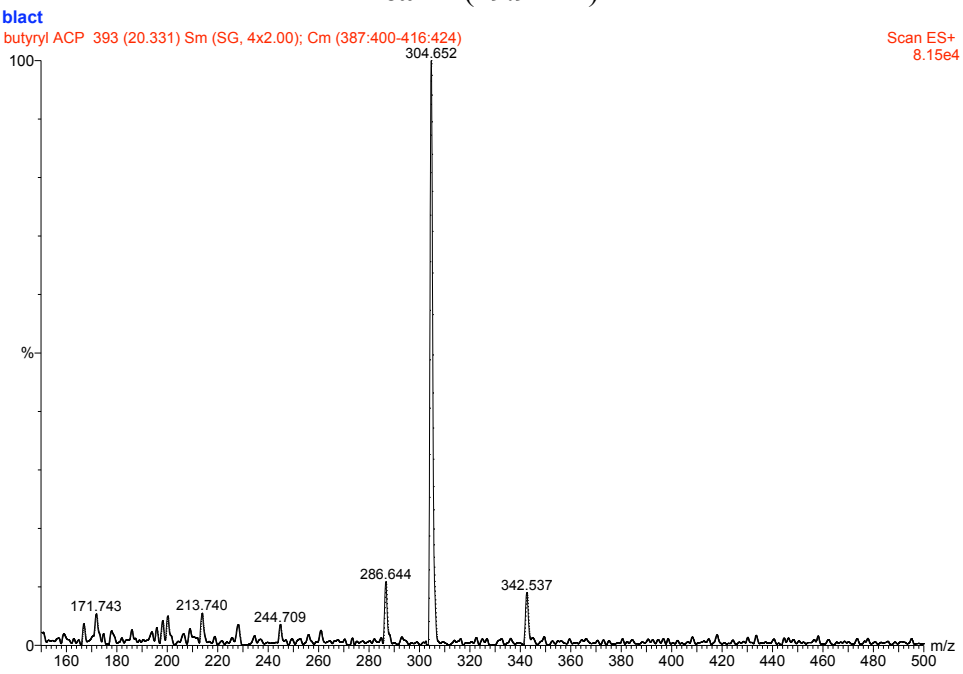


Appendix 4. LCMS Spectra for Butyryl ACP as starter unit.

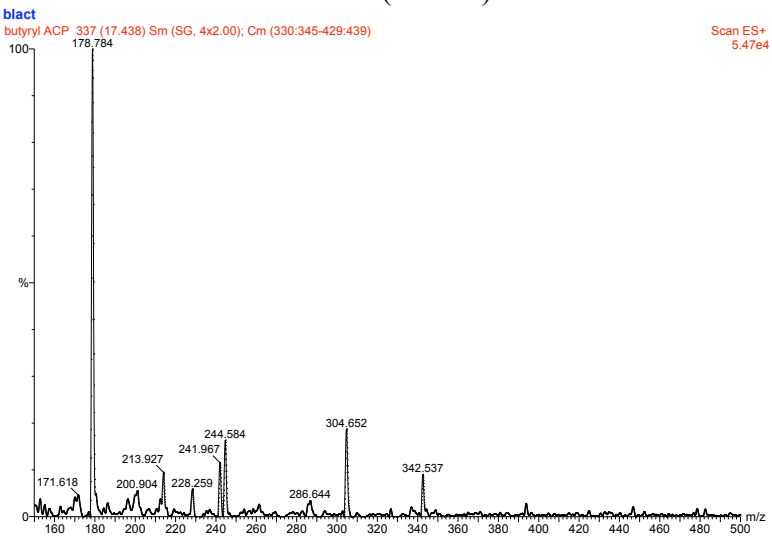


N.B. ES detection is weak, so single ion traces for *m/z* 305 and *m/z* 261 are also shown above.

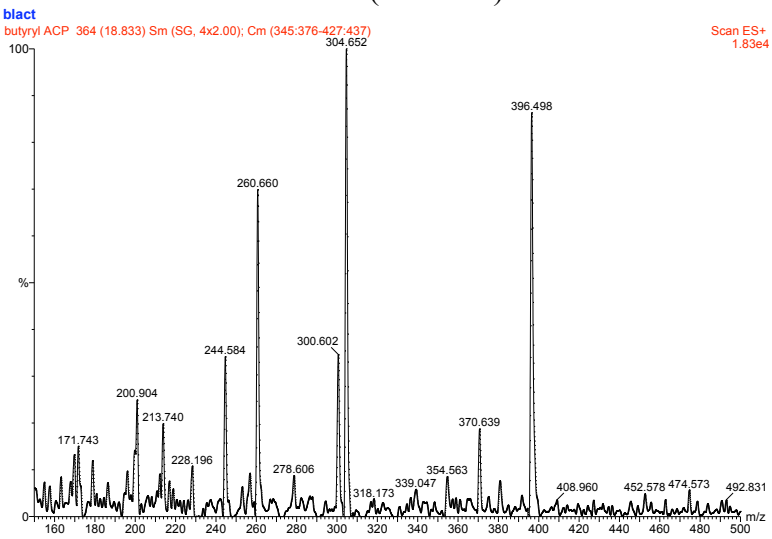
Peak 1 (19.9 min)



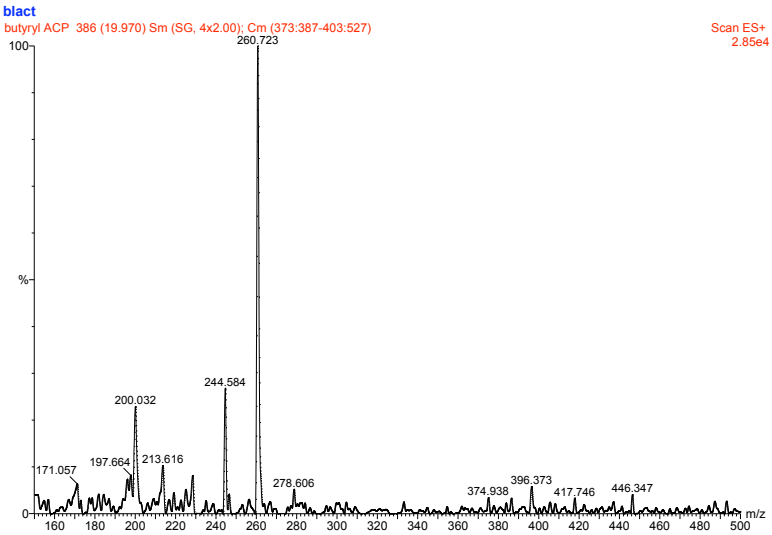
Peak 2 (17 min)



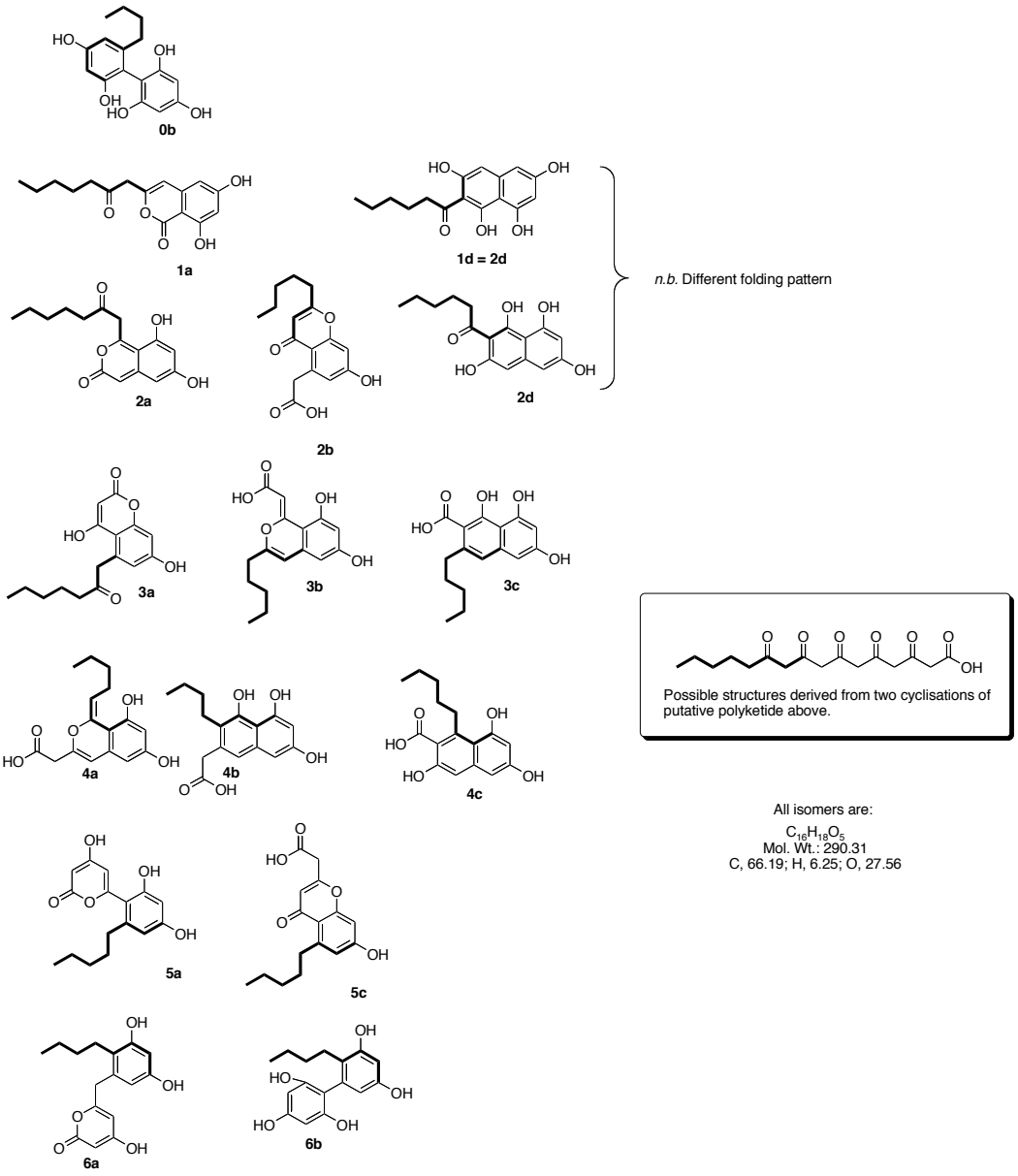
Peak 3 (18.4 min)



Peak 4 (19.5 min)



Appendix 5. Putative Polyketides of *m/z* 290Da.



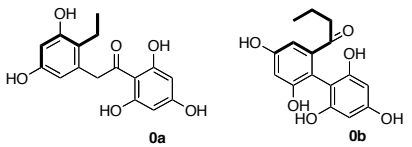
All isomers are:

$C_{16}H_{18}O_5$

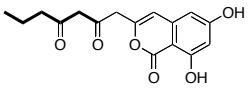
Mol. Wt.: 290.31

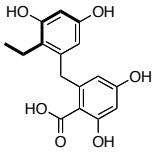
C, 66.19; H, 6.25; O, 27.56

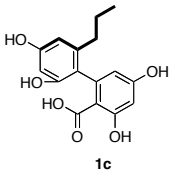
Appendix 6. Putative Polyketides of *m/z* 304Da.

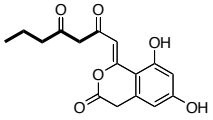

0a

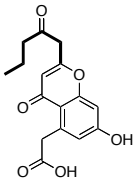
0b

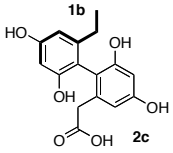

1a

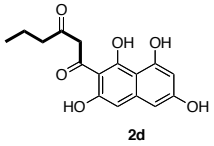

1b

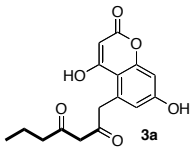

1c

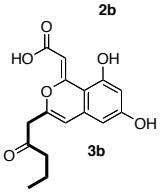

2a

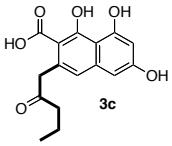

2b

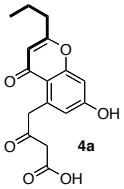

2c

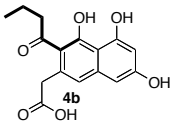

2d

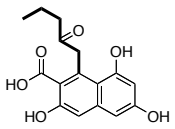

3a

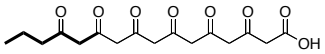

3b


3c


4a


4b

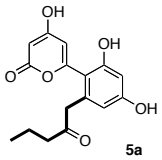

4c

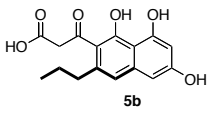


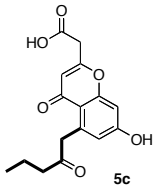
Possible structures derived from two cyclisations of putative polyketide above.

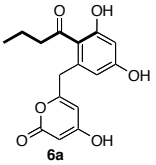
All isomers are:

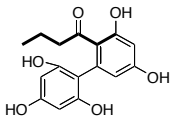
$C_{16}H_{16}O_6$
Mol. Wt.: 304.29
C, 63.15; H, 5.30; O, 31.55

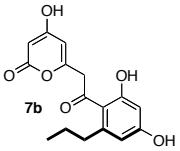

5a

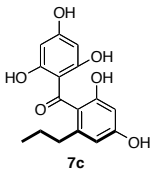

5b

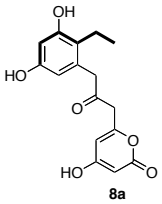

5c


6a


6b


7b


7c


8a