Kallio et. al. 2011 Supplemantal information

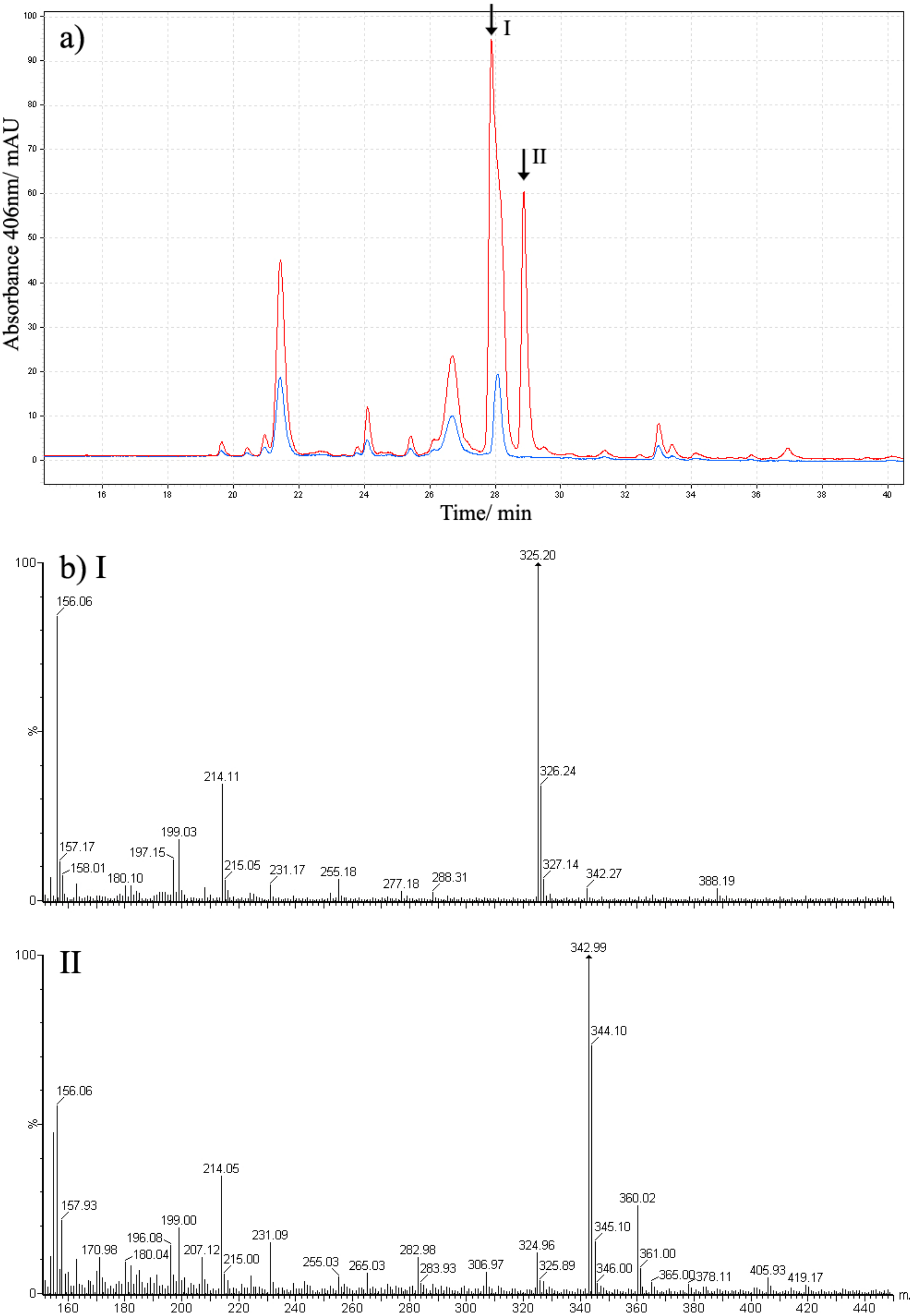

Figure S1: Analysis of *Streptomyces* TK24/pMC6BD expression profile a) HPLC profile of the crude extract before (red) and after (blue) incubation with PgaE/NADPH. The two metabolites accepted as substrates by PgaE are indicated by arrows. b) LC-MS (ES+) profiles of I and II are consistent with those expected for 2,3-dehydro-UWM6 (324.33g/mol) and UWM6 (342.34g/mol), respectively.

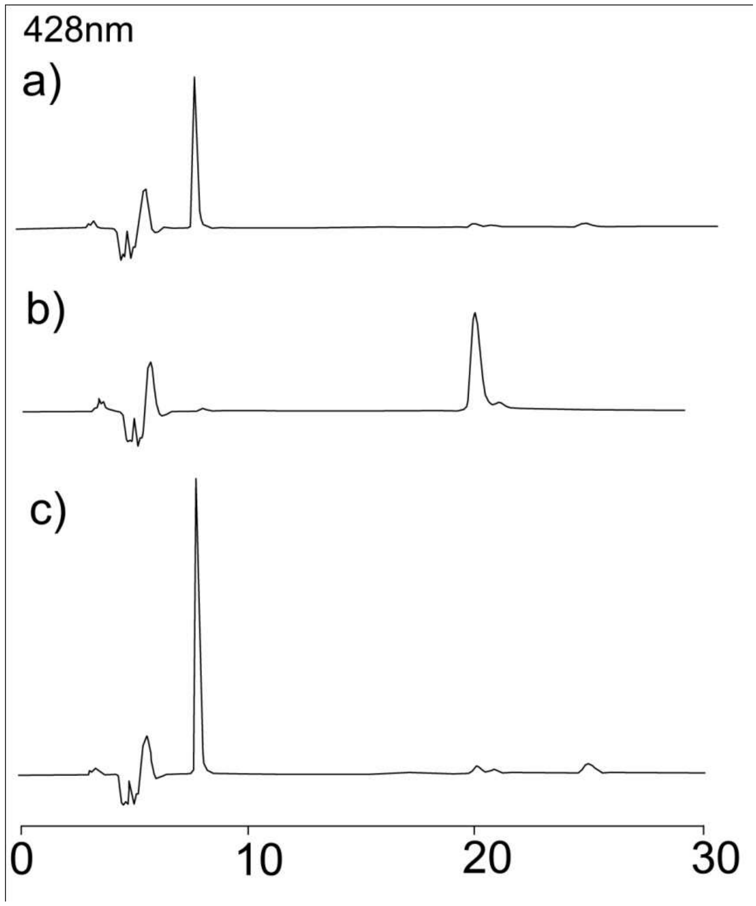

Figure S2. Inhibition of the second PgaE reaction by the initial substrate 2,3-dehydro-UWM6. a) coupled reaction PgaE/CabV results in complete conversion of 2,3-dehydro-UWM6 to gaudimycin C in 9 minutes b) When 2,3-dehydro-UWM6 is added during the reaction (at 4.5 min) gaudimycin C is not observed at the 9 minute time-point. Only a peak corresponding to the substrate is seen c) Prolonged incubation of reaction "b" results in the formation of gaudimycin C. The final peak size corresponds to the total amount of 2,3-dehydro-UMW6 supplied. The HPLC analysis is carried out in isogratic conditions (40% ACN).

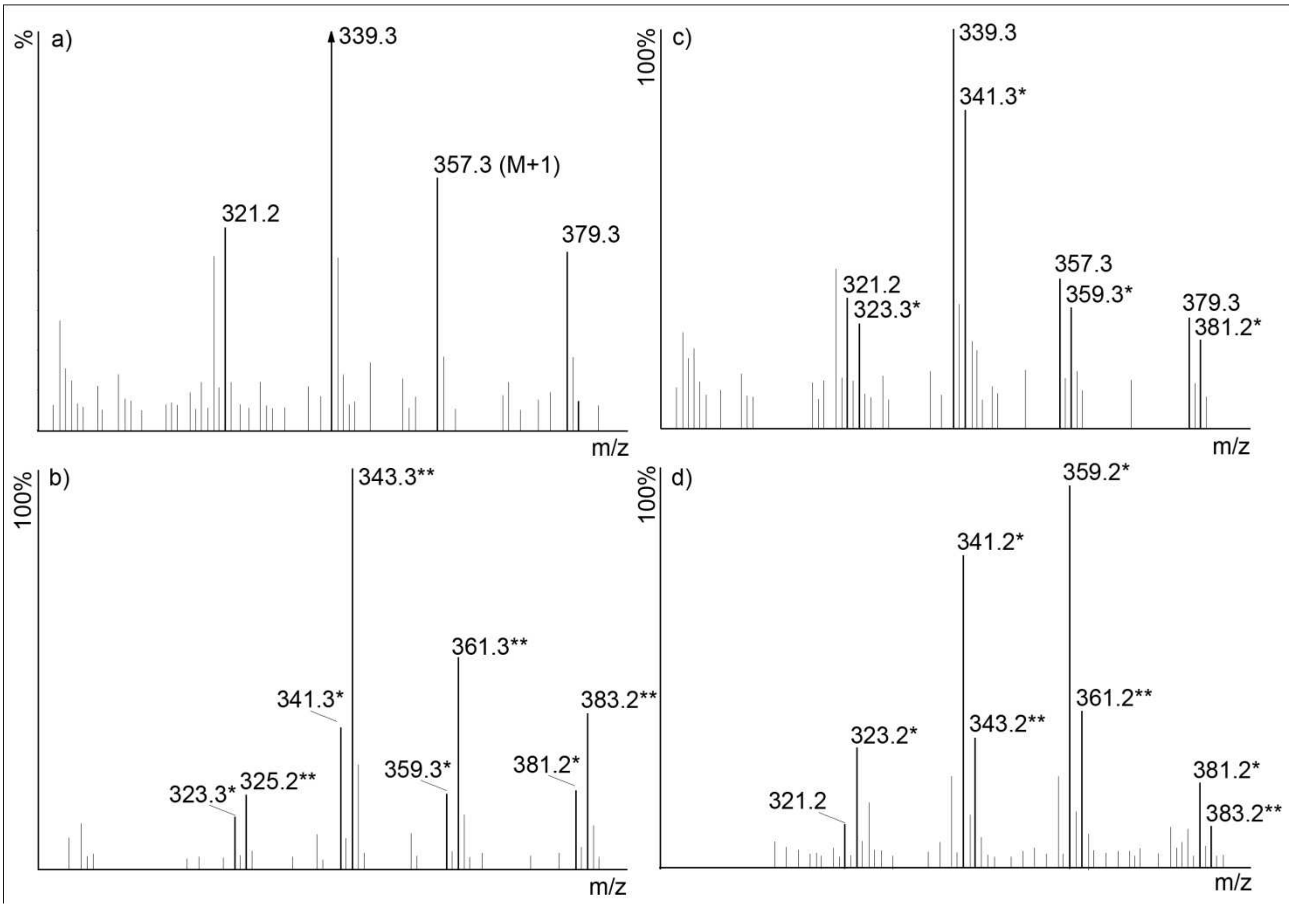

Figure S3. Typical LC-MS profiles showing heavy oxygen incorporation in gaudimycin C in PgaE/CabV/2-3-dehydro-UWM6 reactions carried out under different conditions. a) Control reaction under standard conditions b) Reaction carried out in $^{18}O_2$ c) Reaction carried out in 65% $H_2{}^{18}O$ d) Extracted gaudimycin C incubated for 2h in $H_2{}^{18}O$. The main peaks of interest have been highlighted and labelled. The fragments with 2Da mass increase denoted by "*" and 4Da mass increase by "**". The fragmentation peak masses have been rounded down to 1 decimal and calibrated to the molecular peak mass of "a".