1. Materials

All CoA-esters (except F-Mal-CoA), β-NAD^+, NADH, α-ketoglutarate dehydrogenase (porcine heart) (αKGDH), α-ketoglutaric acid, thiamine pyrophosphate (TPP), and EDTA were purchased from Merck. BSA was from Serva. Restriction enzymes were bought from NEB biolabs. IPTG was from Carl Roth. Ni-NTA affinity resin was from Qiagen and 5 mL Strep-Tactin® columns were purchased from IBA technologies. Purity of CoA-esters was confirmed by HPLC-UV analysis before use.

Plasmid encoding DEBS M6+TE (pBL18) was kindly provided by the Khosla laboratory at Stanford University and the plasmid encoding the 4'-phosphopantetheinyl transferase Npt (UniProt code: A0A1Y2MXW0) was kindly provided by the Erb laboratory at the Max-Planck-Institute for terrestrial Microbiology, Marburg.

2. General molecular biology experiments

2.1 Cloning

Variations in the MAT domain were introduced by PCR based cloning. The template vector pAR70[1] was amplified with primer pairs, listed in Supplementary Table **5**. PCR products were treated with Dpn1 (NEB), purified by gel electrophoresis and DNA was extracted with the Wizard® SV Gel and PCR Clean-Up System (Promega). Purified DNA was transformed into *E. coli* Stellar™ Competent Cells, 5 mL LB cultures were grown and plasmids were isolated with the PureYield™ Plasmid Miniprep System (Promega) or the GeneJET Plasmid Miniprep Kit (Thermo Scientific). Sequences of all plasmids, listed in Supplementary Table 4, were confirmed with the "dye terminator" method.

Vectors encoding hybrid DEBS/FAS proteins (pMJD076 (**H2**) and pMJD077 (**H1**)) were produced by sequence and ligation independent cloning using the In-Fusion HD Cloning Kit (Takara Bio, USA). Briefly, pBL18 was amplified with primers: MJD101 and MJD102 or primers: MJD105 and MJD106. The corresponding inserts were generated by amplification of pAR264[1] with primers: MJD087 and MJD088 or primers: MJD091 and MJD092. PCR products were treated with Dpn1 (NEB), purified by gel electrophoresis and DNA was extracted with the Wizard® SV Gel and PCR Clean-Up System (Promega). Purified vector and insert DNA were combined in the In-Fusion reaction following the manufacturer's instructions. DNA was transformed into E. coli Stellar™ Competent Cells, 5 mL LB cultures were grown and plasmids were isolated with the PureYield™ Plasmid Miniprep System (Promega) or the GeneJET Plasmid Miniprep Kit (Thermo Scientific). Sequences of all plasmids, listed in Supplementary Table 4, were confirmed with the "dye terminator" method.

Vectors pAR432, pMJD091 and pMJD094 were also generated by sequence and ligation independent cloning using the In-Fusion HD Cloning Kit. For pAR432, the two fragments were obtained by amplification of pBL18 with primers: AR719 and AR722 or primers: AR721 and AR720. For pMJD091, the insert was generated by amplification of pET21a_Sppt with primers: MJD138 and MJD139 and the vector by amplification of pAR357 with primers: MJD136 and MJD137. For pMJD094, the insert was generated by amplification of pBL18 with primers: MJD145 and MJD146 and the linearized vector by digestion of pET-28a (Merck Millipore) with enzymes NdeI and EcoRI resembling the design of Kim *et al.* [2].

Vector pMSR001 **(H1.1)** was also generated by sequence and ligation independent cloning using the In-Fusion HD Cloning Kit, resembling the design of Koch *et al.*[3]. To generate pMSR001

(H1.1), pMJD077 (**H1**) was amplified with primers: PRMJD164 and PRMJD175 and the insert was generated by amplification of *Streptomyces venezuelae* (*Streptomyces sp.*, DSM 41110/ATCC 15439, purchased from DSMZ) genomic DNA with the primers: PRMJD177 and PRMJD178.

2.2 Heterologous expression and purification of murine KS-MAT and variants

All plasmids were transformed into chemically competent *E. coli* BL21 Gold (DE3) cells. Transformed cells were grown overnight at 37 °C in 20 mL LB (100 µg mL^{-1} ampicillin (amp) and 1 % (w/v) glucose) medium. Pre-cultures were used to inoculate 1 L TB medium (100 µg mL^{-1} amp). Cultures were grown at 37 °C until they reached an optical density (OD_{600}) of 0.5-0.6. After cooling at 4 °C for 20 min, cultures were induced with 0.25 mM IPTG, and grown for additional 16 h at 20 °C and 180 rpm. Cells were harvested by centrifugation (4,000 rcf for 20 min). The cell pellets were resuspended in lysis buffer (50 mM potassium phosphate, 200 mM potassium chloride, 10 % (v/v) glycerol, 1 mM EDTA, 30 mM imidazole (pH 7.0)) and lysed by French press. After centrifugation at 50,000 rcf for 30 min, the supernatant was mixed with 1 M $MgCl_2$ to a final concentration of 2 mM. The cytosol was transferred to Ni-NTA-columns and washed with 5 column volumes (CV) wash buffer (lysis buffer without EDTA). Bound protein was eluted with 2.5 CV elution buffer (50 mM potassium phosphate, 200 mM potassium chloride, 10 % (v/v) glycerol, 300 mM imidazole (pH 7.0). The eluent was transferred to Strep-Tactin-columns, and washed with 5 CV strep-wash buffer (250 mM potassium phosphate, 10 % (v/v) glycerol, 1 mM EDTA, (pH 7.0)). Proteins were eluted with 2.5 CV elution buffer (strep-wash buffer containing 2.5 mM D-desthiobiotin). After concentration to 10-20 mg mL^{-1}, the proteins were frozen in liquid nitrogen and stored at −80 °C. Samples were thawed at 37 °C for 30 min and further polished by size-exclusion chromatography (SEC) using a Superdex 200 GL10/300 column equilibrated with the strep-wash buffer. Fractions containing dimeric protein were pooled and concentrated to 10-20 mg mL^{-1} to be frozen in liquid nitrogen and stored in aliquots at −80 °C.

2.3 Heterologous expression and purification of FAS ACP and DEBS ACP6

FAS ACP for the activity assay was produced by co-expressing FAS ACP with Sfp from *Bacillus subtilis* bicistronically (pAR352) in *E. coli* BL21gold(DE3) cells [1]. Overnight cultures were grown in LB (100 µg/mL ampicillin and 1 % glucose) at 37 °C. 2 L TB medium (100 µg/mL amp) was inoculated and incubated at 37 °C until an optical cell density (OD_{600}) of 0.5-0.6 was reached. After cooling at 4 °C for 20 min, cultures were induced with 0.25 mM IPTG and cells were grown for 16 h at 20 °C. Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM sodium phosphate, 200 mM NaCl, 20 % (v/v) glycerol, 1 mM EDTA, 30 mM imidazole (pH 7.4) and lysed by French press. After centrifugation (50,000 rcf for 30 min), the supernatant (supplemented with 2 mM $MgCl_2$) was transferred to Ni-NTA-columns and washed with 5 CV wash buffer (lysis buffer without EDTA). The protein was eluted with 2.5 CV elution buffer (wash buffer containing 300 mM imidazole) and concentrated. Pooled fractions, were separated on a Superdex 200 HiLoad 16/60 or 26/60 SEC column equilibrated with buffer (50 mM sodium phosphate, 200 mM NaCl, 10 % (v/v) glycerol, 1 mM EDTA, 1 mM DTT). All fractions containing monomeric ACP were pooled and concentrated to 10-20 mg mL^{-1} (common yield was 20 mg purified protein per 1 L culture).

DEBS ACP6 was produced by co-expressing DEBS ACP6 (pMJD094) with Sfp from *Bacillus subtilis* (pAR357) or with 4'-phosphopantetheinyl transferase Npt from *Streptomyces platensis* (pMJD091) in *E. coli* BL21gold(DE3) cells. Expression and purification was performed

with the FAS ACP protocol, except that kanamycin (50 µg/mL kan) and spectinomycin (50 µg/mL spc) were used as antibiotics for cultivation. Phosphopantetheinylation of DEBS ACP6 was investigated by electrospray ionization (ESI) mass spectrometry (Supplementary Fig. 7), which showed that only co-expression with Npt yielded sufficiently activated protein. All assays were performed with this protein.

2.4 Heterologous expression and purification of DEBS M6+TE, its variants and DEBS KS^{C1661G}-AT

DEBS M6+TE and all its variant were produced by co-expression of DEBS M6+TE (pBL18, pMJD076, pMJD077) with Sfp from *Bacillus subtilis* (pAR357) in *E. coli* BL21gold(DE3) cells. Expression and purification was performed with the FAS ACP protocol, except that ampicillin (100 µg/mL amp) and spectinomycin (50 µg/mL spc) were used as antibiotics for cultivation. Further, the protein was purified in a different lysis buffer (50 mM sodium phosphate, 450 mM NaCl, 20 % (v/v) glycerol, 1 mM EDTA, 20 mM imidazole (pH 7.6)). The protein on the Ni-NTA column was washed with 5 CV washing buffer 1 (50 mM sodium phosphate, 450 mM NaCl, 20 % (v/v) glycerol, 20 mM imidazole (pH 7.6)) and 2 CV washing buffer 2 (50 mM sodium phosphate, 450 mM NaCl, 20 % (v/v) glycerol, 1 mM EDTA, 60 mM imidazole (pH 7.6)). The proteins were eluted with 2.5 CV elution buffer (50 mM sodium phosphate, 450 mM NaCl, 20 % (v/v) glycerol, 300 mM imidazole (pH 7.6)) and further polished and analyzed by size-exclusion chromatography (SEC) using a Superdex 200 Increase 10/300 GL, HiLoad 16/600 Superdex 200 or Superose 6 Increase 10/300 GL column equilibrated with the washing buffer 1. Fractions containing dimeric protein were pooled and concentrated to 1-10 mg mL^{-1} to be frozen in liquid nitrogen and stored in aliquots at −80 °C.

DEBS KS^{C1661G}-AT (pAR432) was expressed without Sfp in *E. coli* BL21gold(DE3) cells and purified following the protocol for the whole DEBS module 6.

2.5 Protein concentration

Protein concentrations were calculated from the absorbance at 280 nm, which was recorded on a NanoDrop 2000c (Thermo Scientific). Extinction coefficients were calculated from the primary sequence without *N*-formylmethionine with CLC Main workbench (Qiagen). Absorbance 1 g/L at 280 nm (10 mm): 1.053 for FAS KS-MAT; 0.475 for FAS ACP, 1.009 for DEBS M6+TE (**WT**), 0.474 for DEBS ACP6, 0.899 for DEBS KS6-AT6, 1.087 for **H1**, 1.069 for **H2** and 1.031 for **H1.1**.

2.6 Thermal shift assay

Thermal shift assays were performed as previously reported [4]. Briefly, 2 µL of protein solution (5-6 µM) were mixed with 21 µL of the respective buffer and 2 µL of SYPRO Orange protein gel stain (80 × diluted), then fluorescence was measured from 5 °C to 95 °C with a step gradient of 0.5 °C min^{-1}, with excitation wavelength set to 450-490 nm, and emission wavelength to 560-580 nm. Data was analyzed with the software CFX Maestro 1.0.

2.7 α-Ketoglutarate dehydrogenase coupled activity assay

The enzyme-coupled assay was performed as previously published [1]. Assays were run in 384-well Small Volume HiBase Microplates (Greiner Bio-one) with following settings for the microplate reader (ClarioStar, BMG labtech): 348-20 nm; emission: 476-20 nm; gain: 1500; focal height: 11.9 mm; flashes: 17; orbital averaging: off.

Briefly, four different solutions were prepared as 4-fold concentrated stocks in assay buffer (50 mM sodium phosphate, 10 % (v/v) glycerol, 1 mM EDTA (pH 7.6), filtered and degased). Solution 1 (Sol 1) contained the acyltransferase, supplemented with 0.1 mg mL^{-1} BSA. Solution 2 (Sol 2) contained 8 mM α-ketoglutaric acid, 1.6 mM NAD^+, 1.6 mM TPP and 60 mU/100 µL αKGDH. Solution 3 (Sol 3) contained the CoA-esters and Solution 4 (Sol 4) finally contained the respective ACP as standalone protein. The components (5 µL) were pipetted in order: Sol 1, Sol 2 and Sol 3, followed by manual mixing. The transfer reaction was initiated by injection of 5 µL Sol 4 with the dispenser. The final concentrations of all ingredients were 50 mM sodium phosphate, pH 7.6, 10 % (v/v) glycerol, 1 mM EDTA, 2 mM α-ketoglutaric acid, 0.4 mM NAD^+, 0.4 mM TPP, 15 mU/100 µL αKGDH, 0.03 mg mL^{-1} BSA, 1-5 nM FAS MAT or 250 nM DEBS AT6, 10-400 µM ACP, 0.1-25 µM X-CoA for FAS MAT and 2-130 µM for DEBS AT6 (where X refers to the respective acyl-moiety of the assay). Equidistant kinetic measurements were taken every 5 s for 5 min at 25 °C. Every data point was recorded in technical triplicates and the respective background noise of the assay set-up (assay buffer supplemented with 0.1 mg mL^{-1} BSA) was subtracted. The enzyme-mediated hydrolysis rate was not subtracted, as it is relatively low compared to transfer rates.

2.8 Analysis of AT-mediated transfer by global fitting

For every global fit, initial velocities were determined for eight different CoA-ester concentrations at four fixed ACP concentrations. Every data point reflects the result from one biological replicate and measurements were performed in at least two biological replicates ($n \geq 2$). Relative fluorescent units were converted into concentrations using a NADH calibration curve. Series of response curves were globally fit using all data without any parameter constraints. The global fit was performed with OriginPro 8.5 (OriginLab, USA) using the following equations for the ping-pong mechanism:

$$\boldsymbol{v} = \frac{\boldsymbol{k_{cat}}[\mathbf{AT_0}][\mathbf{XCoA}][\mathbf{ACP}]}{[\mathbf{XCoA}]\boldsymbol{K_{ACP}} + [\mathbf{ACP}]\boldsymbol{K_S} + [\mathbf{XCoA}][\mathbf{ACP}]} \qquad [1]$$

2.9 Specific ketoreductase activity

The specific ketoreductase activity of DEBS KR6 was measured fluorometrically by monitoring the consumption of NADPH at 25 °C according to previous reports [5]. Assays were performed in 384-well Small Volume HiBase Microplates (Greiner Bio-one) with following settings for the microplate reader (ClarioStar, BMG labtech): 348-20 nm; emission: 476-20 nm; gain: 1301; focal height: 12.4 mm; flashes: 17; orbital averaging: off. Two solutions were prepared as 4-fold concentrated stocks and *trans*-1-decalone was prepared as 2-fold concentrated stock, in the assay buffer (400 mM phosphate buffer, 20 % (v/v) glycerol, 2 mM DTT, 1 mM EDTA, 0.8 % DMSO (pH 7.2)) [6]. Solution 1, 2 and 3 contained the variants of DEBS M6+TE (1.2 µM), the NADPH (240 µM) and the *trans*-1-decalone (4 mM in assay buffer), respectively. 5 µL of enzyme and NADPH solution were mixed with 10 µL of *trans*-1-decalone solution (all incubated at 25 °C) to final concentrations of 0.3 µM enzyme, 60 µM NADPH and 2 mM *trans*-1-decalone. The consumption was monitored fluorometrically for 3 min and converted to concentrations using a NADPH calibration curve. Each of the biological triplicates was measured in technical replicates and the slope was corrected by the background noise of the assay set up (NADPH and *trans*-1-dealone in the respective concentrations without enzyme).

2.10 NADPH consumption assays following reduced triketide lactone (TKL) and reduced macrolactone production

The production rate of reduced TKLs and macrolactones were monitored fluorometrically by observing the consumption of NADPH. Assays were performed in 384-well Small Volume HiBase Microplates (Greiner Bio-one) with following settings for the microplate reader (ClarioStar, BMG labtech): 348-20 nm; emission: 476-20 nm; gain: 1301; focal height: 12.4 mm; flashes: 17; orbital averaging: off. Four solutions were prepared as 4-fold concentrated stocks and the X-CoA and NADPH solutions were combined to yield a 2-fold concentrated stock, in the assay buffer (400 mM phosphate buffer, 20 % (v/v) glycerol, 1 mM EDTA, 0.8 % DMSO (pH 7.2)). Solution 1, 2 and 3 contained the variants of DEBS M6+TE (16 µM), the diketide SNAC **2** (20 mM) or pentaketide **9** (4 mM) or hexaketide **21** (4 mM), MM-CoA or Mal-CoA or F-Mal-CoA or F-MM-CoA (400 µM) and the NADPH (120 µM), respectively. 5 µL of priming substrates and enzyme solutions were mixed with 10 µL of the X-CoA, NADPH stock (all incubated at 25 °C) to final concentrations of 4 µM enzyme, 5 mM **2** or 1 mM **9** or 1 mM **21**, 200 µM X-CoA and 60 µM NADPH. The fluorescence was monitored for 13-20 min and converted into concentrations by using a NADPH calibration curve. Each of the biological triplicates was measured in technical replicates and the slope was corrected by the background noise of the assay set up (NADPH, X-CoA and **2** without enzyme for TKL production and NADPH, **9** or **21** and enzyme without elongation substrate for macrolactones).

The NBOM protected hexaketide substrate was deprotected as decribed by Hansen *et al.*[7]. Briefly, 25 mM ascorbic acid, 4 mM sodium metabisulfite and 4 mM NBOM protected hexaketide were dissolved in water and irradiated for 20 min (254 nm and 365 nm switched on) to yield deprotected hexaketide **21**.

2.11 Chromatographic assay following non-reduced TKL production

Alternatively, we measured the production rates for non-reduced TKLs in the absence of NADPH by quantifying the consumption of CoA-ester substrates and liberation of CoA with HPLC-UV. The four stock solutions were prepared as described in the previous section in the assay buffer (400 mM phosphate buffer, 20 % (v/v) glycerol, 1 mM EDTA, 0.8 % DMSO (pH 7.2)). 80 µL of all solutions were mixed and incubated at 25 °C for 60 min. After 0, 5, 10, 15, 20, 30 and 60 min, 40 µL samples were taken and the reactions were stopped by the addition of 5 µL perchloric acid (70 %). Reaction mixtures were mixed thoroughly and subsequently neutralized with 5 µL NAOH (10 M). Hydoxybutyryl-CoA (final concentration: 50 µM) was added as an internal standard. Reaction mixtures were spun at 20000 rcf for 10 min. 30 µL of the supernatant was transferred into HPLC vials and measured by HPLC-UV.

HPLC-UV analysis of CoA esters was performed using a Dionex UltiMate 3000 RSLC with a UV detector. Chromatographic separation was performed on a Synchronis aQ-c18 column (4.6 × 250 mm, particle size 5 µm, ThermoFisher Scientific) with a mobile-phase system consisting of buffer A (0.2 M ammonium acetate (pH 6.0)) and buffer B (methanol). The column was equilibrated with 95 % buffer A and 5 % buffer B at a flow rate of 0.8 mL/min. X-CoA was purified using a linear gradient from 5 to 20 % B over 15 min and an increased gradient from 20 to 60 % B over 8 min (The column was regenerated 3 min at 60 % B and re-equilibrated again for the next sample). Absorbance of X-CoA was monitored at 260 nm and assigned by the elution time of purchased CoA ester references. Peak areas were correlated to the internal standard peak and converted into concentrations. Concentrations were plotted versus the time and fit with an exponential decay function in OriginPro 8.5 (OriginLab, USA). Initial velocities were obtained

from the deviation of the function and its slope at 0 min. Background measurements were performed in the absence of diketide SNAC **2**.

$$y = y_0 + A_1 e^{-x/t_1} \qquad [2]$$

2.12 HPLC-MS analysis of TKL and macrolactone products

All reaction mixtures were prepared as described in 2.10. Unreduced compounds were prepared with the same conditions but without NADPH. After overnight incubation, the reaction mixtures were extracted using EtOAc (2 times 400 µL for TKLs, 3 times 300 µL for macrolactones). Combined organic phases were evaporated in a SpeedVac *in vacuo*. Samples were dissolved in 50 µL methanol and spun at 20000 rcf for 20 min. 40 µL of the supernatant was transferred into HPLC vials and measured by HPLC-MS using the Ultimate 3000 LC (Dionex) system connected to a Acquity UPLC BEH C18 (2.1 × 50 mm, particle size 1.7 µm, Waters) for separation. After equilibration with 5 % acetonitrile in water, samples were purified using a linear gradient from 5-95 % within 16 min and subsequently injected into the AmaZonX (Bruker) or Impact II qTof (Bruker) for ESI.

2.13 HPLC-UV analysis and purification of macrolactone products

HPLC-UV analysis of macrolactones was performed using a Dionex UltiMate 3000 RS UHPLC with a RS Diode Array UV detector. Chromatographic separation was performed on a Synchronis aQ-c18 column (4.6 × 250 mm, particle size 5 µm, ThermoFisher Scientific) with a mobile-phase system consisting of buffer A (water) and buffer B (acetonitrile). The column was equilibrated with 95 % buffer A and 5 % buffer B at a flow rate of 0.8 mL/min. Macrolactones were purified using a linear gradient from 5 to 95 % B over 15 min and another 5 min at 95 % B. A linear gradient to 5 % B was used within 1 min and the column was re-equilibrated for five more minutes at 5 % B. Fractionation was performed with the UltiMate 3000 Autosampler in between 3 and 16 min in 20 s steps.

2.14 Enzymatic synthesis and analysis of compound **12**

In order to conduct NMR analysis of compound **12**, the reaction volume was scaled up to 10 mL and a purification strategy was established. Final concentrations of 300 µM pentaketide substrate **9** (1.05 mg, dissolved in DMSO), 400 µM Mal-CoA and 500 µM NADPH were dissolved in assay buffer (250 mM potassium phosphate, 10 % glycerol, pH 7) and 5 µM **H1** (9.16 mg, dissolved in 250 mM potassium phosphate, 10 % glycerol, pH 7) was added to a final volume of 10 mL. The reaction mixture (slightly cloudy emulsion) was incubated for 16 h at 25 °C and the progress of the reaction was monitored by HPLC (samples were prepared by quenching 20 µL mixture with 60 µL methanol). The reaction mixture was transferred to a 50 mL falcon tube and the aqueous phase was extracted with EtOAc (5 × 10 mL) by spinning for 1 min at 3000 rcf to separate the phases. The combined organic phases were transferred to a 250 mL round bottom glass flask, the solvent was evaporated *in vacuo* and the residual oil was dried by azeotropic evaporation with toluene (2 × 1 mL). After running a TLC (hexane/EtOAc 1:1 stained with $KMnO_4$-solution), the crude product was adsorbed to silica gel and purified by flash chromatography (silica column: 1.3 cm diameter and 15 cm height). A gradient was used for elution (hexane:EtOAc 20:1 → 10:1 → 7:1 → 5:1 → 3:1 → 2:1 → 1:1) and every fraction was carefully analyzed by TLC. All fractions containing the product were pooled, filtered and the solvent was evaporated *in vacuo* to yield compound **12** as white solid.

R_F-value (TLC; hexane/EtOAc: 1/1): 0.44
Retention time (HPLC; water/ACN): 13.74 min
^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.76 (dd, *J* = 15.7, 5.4 Hz, 1H), 6.44 (dd, *J* = 15.8, 1.2 Hz, 1H), 5.02 (ddd, *J* = 8.3, 6.0, 2.3 Hz, 1H), 4.05-4.02 (ddd, *J* = 11.2, 5.2, 1.2 Hz, 1H), 2.71-2.54 (m, 4H), 1.74-1.56 (m, 4H), 1.30-1.28 (m, 1H), 1.25 (d, J = 7.0 Hz, 3H), 1.13 (d, J = 6.9 Hz, 3H), 1.03 (d, J = 6.2 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H) ppm.
^{13}C NMR (500 MHz, Chloroform-*d*) δ 170.3, 147.5, 125.5, 74.5, 72.9, 45.3, 39.0, 38.1, 33.0, 32.5, 25.0, 17.7, 17.2, 10.3, 9.4 ppm.
MS (HR-ESI+) found 283.1900, for $[M+H]^+$ calculated 283.1910; found 305.1720, calculated for $[M+Na]^+$ 305.1729; found 265.1796, calculated for $[M-H_2O+H]^+$ 265.1804.

2.15 Enzymatic synthesis and analysis of compound **15/16**

In order to conduct NMR analysis of compound **15**/**16**, the reaction volume was scaled up to 50 mL and a purification strategy was established. Final concentrations of 600 µM pentaketide substrate **9** (10.46 mg, dissolved in DMSO), 4000 µM F-Mal-CoA and 1000 µM NADPH were dissolved in assay buffer (250 mM potassium phosphate, 10 % glycerol, pH 7) and 10 µM **H1** (90.57 mg, dissolved in 250 mM potassium phosphate, 10 % glycerol, pH 7) was added to a final volume of 50 mL. The reaction mixture (slightly cloudy emulsion) was incubated for 22.5 h at 25 °C and the progress of the reaction was monitored by HPLC (samples were prepared by quenching 20 µL mixture with 60 µL methanol). The reaction mixture was transferred to two 50 mL falcon tube and the aqueous phase was extracted with EtOAc (4-6 × 25 mL) by spinning for 1 min at 3000 rcf to separate the phases. The combined organic phases were transferred to a 250 mL round bottom glass flask, the solvent was evaporated *in vacuo*. The residual oil was dissolved in MeOH/CHCl3 (9/1) and purified with an uHPLC system (Thermo Fisher) on a Synchronis aQ-C18-LC column. Fractions containing the major fluorinated compound, based on ^{19}F-NMR analysis, were further purified with flash chromatography. The crude product was adsorbed to silica gel and purified (silica column: 1.3 cm diameter and 12 cm height) using a gradient for elution (hexane:EtOAc 20:1 → 15:1 → 10:1 → 7:1 → 5:1 → 4:1 → 3:1 → 1:1) and every fraction was carefully analyzed by TLC. All fractions containing product were pooled, filtered and the solvent was evaporated *in vacuo* to yield compound **16** as white solid.
R_F-value (TLC; hexane/EtOAc: 3/1): 0.22
Retention time (HPLC; water/ACN): 13.23 min
^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.70 (dd, *J* = 15.6, 8.0 Hz, 1H), 5.51 (dd, *J* = 15.7, 0.8 Hz, 1H), 4.84 (d, *J* = 48.4 Hz, 1H), 3.41 (ddd, *J* = 9.0, 5.5, 3.7 Hz, 1H), 2.54-2.47 (m, 1H), 2.41-2.34 (m, 1H), 2.07-2.00 (m, 1H), 1.92-1.87 (m, 1H), 1.66 (q, *J* = 12.8 Hz, 1H), 1.61-1.55 (m, 1H), 1.44-1.35 (m, 1H), 1.11 (d, *J* = 6.4 Hz, 3H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.98-0.95 (ovlp m, 6H)
^{19}F NMR (500 MHz, Chloroform-*d*) δ -206.21(d, *J* = 46,8 Hz)
^{13}C NMR from HSQC (500 MHz, Chloroform-*d*) δ 134.1, 131.5, 94.8/96.4, 76.5, 42.4, 42.2, 38.7, 36.9, 27.0, 13.4, 15.2, 10.4, 13.6
MS (HR-ESI+) found 273.1859, for $[M+H]^+$ calculated 273.1866; found 295.1677, calculated for $[M+Na]^+$ 295.1686; found 255.1754, calculated for $[M-H_2O+H]^+$ 255.1761; found 567.3462, calculated for $[M_2+Na]^+$ 567.3474

2.16 Enzymatic synthesis and analysis of compound **18**

In order to conduct NMR analysis of compound **18**, the reaction volume was scaled up to 50 mL and a purification strategy was established. Final concentrations of 600 µM pentaketide

substrate **9** (10.46 mg, dissolved in DMSO), 800 µM F-MM-CoA and 1000 µM NADPH were dissolved in assay buffer (250 mM potassium phosphate, 10 % glycerol, pH 7) and 7 µM **H1** (63.40 mg, dissolved in 250 mM potassium phosphate, 10 % glycerol, pH 7) was added to a final volume of 50 mL. The reaction mixture (slightly cloudy emulsion) was incubated for at least 4 h at 25 °C and the progress of the reaction was monitored by HPLC (samples were prepared by quenching 20 µL mixture with 60 µL methanol). The reaction mixture was transferred to two 50 mL falcon tube and the aqueous phase was extracted with EtOAc (5 × 25 mL) by spinning for 1 min at 3000 rcf to separate the phases. The combined organic phases were transferred to a 250 mL round bottom glass flask, the solvent was evaporated *in vacuo*. The residual oil was purified with flash chromatography. After running a TLC (hexane/EtOAc 2:1 stained with $KMnO_4$-solution), the crude product was adsorbed to silica gel and purified (silica column: 1.3 cm diameter and 12 cm height) using a gradient for elution (hexane:EtOAc 30:1 → 15:1 → 10:1 → 7:1 → 5:1 → 4:1 → 3:1 → 2:1 → 1:1) and every fraction was carefully analyzed by TLC. All fractions containing the product were pooled, filtered and the solvent was evaporated *in vacuo* to yield compound **18** as white solid. The educt could not be separated by this procedure as co-eluting with compound **18**.
Yield: 6 % (0.57 mg, 1.8 µmol). Note on the yield: Without any optimization, we yielded about 6 % of the fluorinated 10-deoxymethynolide (**18**). Recently, a similar yield was reported for the elongation of the pentaketide (**9**) with the native MM-CoA by wildtype DEBS M6, and the TE was identified as the bottleneck hindering macrolactonization [3]. These results indicate that the AT domain exchange is not responsible for the low yield.

The enzymatic reaction with **H1.1** was performed with slightly different conditions: In a 250 mL round-bottom flask, final concentrations of 300 µM pentaketide substrate **9** (8.67 mg, dissolved in 200 mM DMSO stock, added last), 600 µM F-MM-CoA (43.9 mg, dissolved in 60 mM H_2O stock) and 500 µM NADPH (34.58 mg) were dissolved in assay buffer (250 mM potassium phosphate, 10 % glycerol, pH 7.0). 9 µM hybrid **H1.1** (138 mg, dissolved in 250 mM potassium phosphate, 10 % glycerol, pH 7) was added and the slightly cloudy reaction mixture was slowly stirred for 16 h at 25 °C. The reaction mixture was transferred into 2 × 50 mL falcon tubes. The aqueous phase was extracted with EtOAc (5 × 20 mL) and phase separation was achieved by centrifugation (15,500 × g, 4 °C, 15 min). The organic phases were combined, transferred into a separation funnel and washed with H_2O (2 × 50 mL). The aqueous phase was discarded and the organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated *in vacuo*. Crude product was purified using a Synchronis™ Silica column (4.6 × 250 mm, particle size 5 µm, ThermoFisher Scientific; Part No.: 97005-254630) on a Dionex UltiMate 3000 RS UHPLC equipped with a RS Diode Array UV detector. The mobile phase system is consisting of buffer A (n-hexane) and buffer B (EtOAc). The column was equilibrated with 95 % buffer A and 5 % buffer B at a flow rate of 1.0 mL min^{-1}. Compound **18** was purified using a linear gradient from 5 to 100 % buffer B over 40 minutes and another 5 minutes at 100 % buffer B. A linear gradient to 5 % buffer B was used within 2 minutes and the column was re-equilibrated for another five more minutes at 5 % buffer B. Fractionation was performed using the UltiMate 3000 Autosampler in between 0 and 40 minutes in 60 seconds steps. Fractions containing compound **18** were pooled and the solvent was evaporated *in vacuo* to yield compound **18** in high purity.

Yield: 27 % (1.8 mg, 5.73 µmol)
R_F-value (TLC; n-hexane/EtOAc: 3/1): 0.29

Retention time (HPLC; n-hexane/acetone): 13 – 14 min
Retention time (HPLC; water/ACN): 14.65 min
^{1}H NMR (500 MHz, Chloroform-*d*) δ = 6.77 (dd, *J* = 15.7, 5.5 Hz, 1H), 6.47 (dd, *J* = 15.7, 1.3 Hz, 1H), 5.07 (ddd, 1H, *J* = 8.1, 5.5, 1.9 Hz), 3.67 (dd, *J* = 26.4, 1.1 Hz, 1H), 2.71 – 2.65 (m, 1H), 2.56 – 2.47 (m, 1H), 2.03 – 1.96 (m, 1H), 1.85 – 1.76 (m, 1H), 1.68 – 1.62 (m, 1H), 1.65 (d, *J* = 22 Hz, 3H), 1.44 – 1.38 (m, 1H), 1.31-1.24 (m, 1H), 1.20 (d, *J* = 7.0 Hz, 3H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm.
^{19}F NMR (471 MHz, Chloroform-*d*) δ = –172.46 (quintet, *J* = 22.5 Hz) ppm.
^{13}C NMR (126 MHz, $CDCl_3$) δ = 205.02, 171.22, 146.54, 126.42, 99.53, 78.46, 75.72, 45.25, 38.07, 33.26, 32.95, 25.43, 23.17, 18.07, 17.91, 10.46, 9.68 ppm.
MS (HR-ESI+) found 315.1961, for $[M+H]^+$ calculated 315.1972; found 337.1781, calculated for $[M+Na]^+$ 337.1791; found 297.1857, calculated for $[M-H_2O+H]^+$ 297.1866

Position	δ ^{1}H (mult., J Hz)	δ ^{13}C	COSY	HMBC	NOESY
1	-	171.1	-	-	-
2	-	99.5	-	-	-
3	3.67 (dd, *J* = 26.4, 1.1)	78.5	-	5, 14, 15	4, 14, 15
4	1.44 – 1.38 (m)	33.3	5, 15	16	3, 5',5'', 15
5'	2.03-1.96 (m)	33.0	5'', 6	3, 4, 6, 7, 15, 16	4, 5'', 6, 8, 16
5''	1.31-1.24 (m)	33.0	4, 5'	3	5', 6
6	2.56 – 2.47 (m)	45.3	5', 16	7	4, 5', 5'', 15, 16
7	-	205.0	-	-	-
8	6.47 (dd, J = 15.7, 1.3)	126.4	9, 10	7, 9, 10	5', 9, 10, 16, 17
9	6,77 (dd, J = 15.7, 5.5)	146.5	8, 10	7, 10, 17	8, 10, 11
10	2.71 – 2.65 (m)	38.1	8, 9, 11, 17	8, 9, 11, 17	8, 9, 11, 12', 13, 17
11	5.08 (ddd, *J* = 8.2, 5.5, 1.9)	75.7	10, 12', 12''	1, 17	9, 10, 12', 12'', 13
12'	1.85 – 1.76 (m)	25.4	11, 13, 12''	10, 11, 13	10, 11, 12'', 13, 17
12''	1.68 – 1.62 (m)	25.4	11, 13, 12'	13	11, 12', 13, 17
13	0.95 (t, *J* = 7.4)	10.5	12', 12''	11, 12	10, 11, 12', 12''
14	1.65 (d, J = 22)	23.2	-	1, 2, 3	3
15	1.02 (d, *J* = 6.7)	17.9	4	3, 4, 5	3, 4, 6, 5''
16	1.20 (d, *J* = 7.0)	18.1	6	5, 6, 7	5', 6, 8
17	1.17 (d, *J* = 6.9)	9.7	10	9, 10, 11	8, 10, 12', 12''

2.17 Biotransformation of compound **18**

The following biotransformation reaction was carried out in an analogous manner to the published procedures with minor modifications [8–10]. A 3 mL seed culture of SCM medium (20 g soytone, 15 g soluble starch, 10.5 g MOPS, 1.5 g yeast extract, 0.1 g $CaCl_2$, per 1 L water, pH 7.2) in 15 mL snap cap tube was inoculated with 3 µL of *Streptomyces venezuelae* strain DHS316 or YJ112 spore stock and shaken overnight at 28 °C (180 rpm). The OD600 was measured and this was used to inoculate a 10 mL biotransformation culture of SCM medium to an OD600 of ~0.1. The culture was incubated at 28 °C (180 rpm) for 1 h, prior to the addition of 2 µL of acetyl-narbonolide as a DMSO solution (20 mg/mL) followed by the macrolactone **18** as a DMSO solution (~75 µL). The cultures were incubated at 28 °C for 18 h (180 rpm) and centrifuged at 4,000 × g for 10 min to remove cell debris. The remaining aqueous solution was saturated with NaCl prior to adjusting the pH to 11 with 10 N NaOH. The solution was extracted with 3 × 10 mL of ethyl acetate, and the combined organic layers were dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure to yield crude product mixture. The reactions were analyzed by LCMS.

MS (HR-ESI+) 19 found 472.3082, for $[M+H]^+$ calculated 472.3075; **20** found 488.2997, calculated for $[M+H]^+$ 488.3024

3. Substrate synthesis

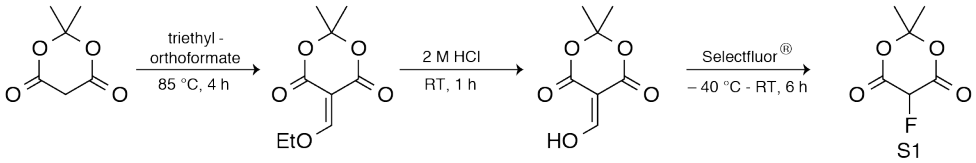
3.1 Chemicals

Reagents were purchased from Merck, Carl-Roth and abcr GmbH. All the reagents were used as purchased without any further purification. Reactions were carried out in oven-dried glassware under an inert Argon gas atmosphere. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and $KMnO_4$ stain & heat as developing agents. Room temperature when mentioned ranges from 22 to 25 °C. E. Merck silica gel (60, particle size 0.040-0.060 mm) was used for column chromatography. The NMR spectra were determined on Bruker DPX250, on Bruker AV400, on Bruker AV500 or on Bruker DRX600 using deuterated solvents from the company Deutero GmbH, Kastellaun. Chemicals shifts values are expressed in parts per million (ppm) relative to chloroform (δ 7.27), DMSO (δ 2.51) or water (δ 4.79). Multiplicities are explained using following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dq = doublet of quartet, ddd = doublet of doublet of doublet. High resolution Electrospray Ionization mass spectra were obtained on ThermoFisher Surveyor MSQ.

3.2 Fluoromalonyl-CoA

Fluoro-Meldrum's acid (S1)

Fluoro-Meldrum's acid (S1) was synthesized from Meldrum's acid in three steps using a previously described method [11].



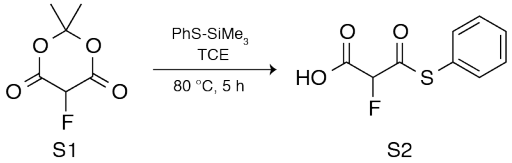
Yield (over three steps): 44 % (5.1 g), colorless solid.

^{1}H NMR (400 MHz, C_2D_6OS) δ = 6.69 (d, *J* = 41.6 Hz, 1H), 1.82 (s, 3H), 1.71 (s, 3H) ppm.

^{19}F NMR (300 MHz, C_2D_6OS) δ = –207.9 (d, *J* = 42 Hz, 1H) ppm.

Fluoromalonic acid thiophenyl halfester (S2)

The thiophenyl halfester (S2) was synthesized from fluoro-Meldrum's acid S1 and trimethylsilylthiophenol, which was produced utilizing the butyllithium method [11].



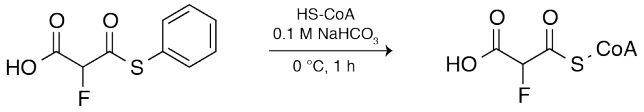
Yield: 53 % (0.7 g), yellowish powder.

^{1}H NMR (400 MHz, C_2D_6OS) δ = 7.64-7.50 (m, 3H), 7.49-7.46 (m, 2H), 5.95 (d, *J* = 47.2 Hz, 1H) ppm.

^{19}F NMR (300 MHz, C_2D_6OS) δ = –192.5 (d, *J* = 48 Hz) ppm.

Fluoromalonyl-CoA (1)

Fluoromalonyl-CoA (1) was synthesized via transacylation from the fluoromalonyl thiophenyl halfester S2 to free coenzyme A (CoASH), adapted from Dunn *et al.*[12].



S2 1

CoASH (30 mg; 38 µmol) and the fluoromalonyl thiophenyl halfester (44 mg; 205 µmol) were dissolved in 1 mL ice cold $NaHCO_3$ (0.1 M) at 0 °C. The pH was adjusted back to pH 8-9 with 1 mL NaOH (0.2 M) and the reaction was continued for 1 h at 0 °C. Then, the reaction mixture (2 mL) was poured into 15 mL acetone (-20 °C) in a 50 mL falcon and spun for 10 min at 3000 rcf. The supernatant was discarded and the pellet was washed with acetone (-20 °C) and dried by air stream. The pellet was dissolved in water and the yield was determined by the absorbance at 260 nm (ε = 16,400 M^{-1} cm^{-1}) [13].

Yield: 81 %, white crystals.

^{1}H NMR (400 MHz, D_2O) δ = 8.54 (s, 1H), 8.25 (s, 1H), 6.18-6.13 (m, 1H), 5.33 (d, *J* = 50.3 Hz, 1H), 4.83-4.79 (m, 2H), 4.58-4.53 (m, 1H), 4.25-4.17 (m, 1H), 3.99 (s, 1H), 3.83-3.76 (m, 1H), 3.55-3.47 (m, 1H), 3.47-3.39 (m, 2H), 3.37-3.30 (m, 2H), 3.10-3.03 (m, 2H), 2.47-2.37 (m, 2H), 0.85 (s, 3H), 0.70 (s, 3H) ppm.

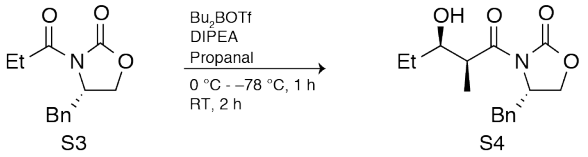
^{19}F NMR (300 MHz, D_2O) δ = –177.4 (d, *J* = 54 Hz), –182.0 (M) ppm.

HRMS (ESI+) found 872.1138, calculated for $[M+H]^+$ 872.1141.

3.3 Diketide SNAC (2)

(4*S*, 2'*S*, 3'*R*)-3-(2'-Methyl-3'-hydroxypentanoyl)-4-benzyl-2-oxazolidinone (S4)

Compound S4 was synthesized from Evans auxiliary S3 using the method of Sharma *et al.* [14]. The product was triturated with EtOAc, *n*-hexane and *n*-pentane after column chromatography.



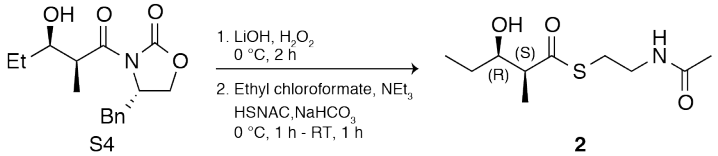
S3 S4

Yield: 69 % (4.3 g), white crystals.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37-7.21 (m, 5H), 4.75-4.69 (m, 1H), 4.27-4.19 (m, 2H), 3.90-3.86 (m, 1H), 3.81 (dq, *J* = 2.7, 7.0 Hz, 1H), 3.27 (dd, *J* = 3.4, 13.5 Hz, 1H), 2.81 (dd, *J* = 9.4, 13.4 Hz, 1H), 1.65-1.43 (m, 2H), 1.27 (d, *J* = 7.0 Hz, 3H), 0.99 (t, *J* = 7.5 Hz, 3H) ppm.

(2S, 3R) 3-hydroxy-2-methylpentanoyl-*S*-*N*-acetylcysteamine thioester (2)

Diketide SNAC **2** was synthesized in two steps from compound S4 following protocols of Sharma *et al.* and Peter *et al.* [14,15].



S4 **2**

3-hydroxy-2-methyl-pentanoic acid was obtained almost quantitatively following the instructions of Sharma *et al*. and was used without further purification. To the acid (220 mg, 1.66 mmol) in 5 ml THF cooled to 0 °C, 148 µL ethyl chloroformate (168 mg, 1.55 mmol) and 215 µL NEt_3 (157 mg, 1.55 mmol) were added at 0 °C. The reaction mixture was stirred excessively for 45 min at 0 °C. Then, 224 µL *N*-acetylcysteamine (251 mg, 2.00 mmol) and 54 mg $NaHCO_3$ dissolved in 2 mL H_2O were added and the reaction mixture was further stirred for 1 h at RT. The aqueous phase was extracted with EtOAc (3 × 20 mL) and the combined organic phases were washed with H_2O (20 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography (silica gel, DCM:Acetone 10:1 → 8:1 → 6:1 → 4:1) to yield 128 mg (33 %) product **2** as clear liquid.

Yield: 33 % (128 mg), clear liquid.
^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.76 (s, br, 1H), 3.88-3.83 (m, 1H), 3.53-3.41 (m, 2H), 3.10-2.99 (m, 2H), 2.78-2.72 (m, 1H), 1.98 (s, 3H), 1.57-1.43 (m, 2H), 1.23 (d, *J* = 7.0 Hz, 3H) 0.99 (t, *J* = 7.4 Hz, 3H) ppm.
MS (ESI+) found 234.12, for $[M+H]^+$ calculated 234.12; found 256.24, calculated for $[M+Na]^+$ 256.10.

3.4 Fluoromethylmalonyl-CoA (17)

Fluoromethyl-Meldrum's acid (S5)

2,2,5-Trimethyl-1,3-dioxane-4,6-dione (Methyl Meldrum's acid) was either synthesized from methylmalonic acid after the protocol of Bravo-Rodriguez *et al*. or purchased from Merck [16].
Yield: 73 % (1.96 g), white crystals.
^{1}H NMR (400 MHz, $CDCl_3$ δ = 3.58 (q, *J* = 7.0 Hz, 1H), 1.81 (s, 3H), 1.77 (s, 3H), 1.58 (d, *J* = 7.1 Hz, 3H) ppm.

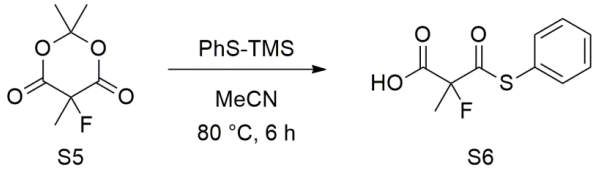
Fluoromethyl-Meldrum's acid (S5) was synthesized from compound methyl Meldrum's acid.

S5

A dry, nitrogen-flushed round bottom flask (500 mL) equipped with a septum was charged with Methyl Meldrum's acid (5 g, 31.6 mmol), the finely powdered Selectfluor (11.8 g, 33.2 mmol;) and 250 mL dry acetonitrile was added. The mixture was stirred for 4 h at RT and solids dissolved after 90 min. The solvent was evaporated and ca. 100 mL DCM and 50 mL water were added. The phases were separated and the aqueous phase was extracted with DCM (3 × 50 mL). Combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated *in vacuo* to yield crude Fluoromethyl-Meldrum's acid (5.1 g; 92 %, purity 90 %) as fine white crystals. The crude product still containing educt was adsorbed on silica and purified by column

chromatography (silica gel, hexane:ethylacetate 10:1 → 5:1 → 3:1 → 2:1 → 1:1 → 1:2 → 1:3) to yield 2.8 g (48 %) product S5 as white crystals.
Yield: 48 % (2.8 g), white crystals.
R_F-value (H:EE 1:1): 0.77 (slightly visible with $KMnO_4$-staining solution).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.93 (d, *J* = 24 Hz, 3H), 1.85 (s, 3H), 1.80 (s, 3H) ppm.
^{19}F NMR (300 MHz, $CDCl_3$) δ = –153.50 (q, *J* = 24 Hz) ppm.

Fluoromethylmalonic acid thiophenyl halfester (S6)

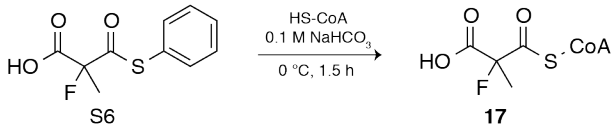


Fluromethyl-Meldrum's acid (S5) (4.5 g, 24.7 mmol, 1.0 eq.) was covered with 5.42 g ((Phenyl)thio)-trimethylsilane (5.63 mL, 29.7 mmol, 1.2 eq.) and 22.5 mL dry acetonitrile in a dry, argon-flushed round bottom flask. The reaction mixture was stirred at reflux for 6 h. The reaction mixture was cooled down with an ice bath and 80 mL DCM, 20 mL saturated $NaHCO_3$ (aq.) and 10 mL H_2O were carefully added. The mixture was transferred to a separation funnel and extracted under strong gas evaporation. Phases were separated and the aqueous phase was extracted with DCM (2 × 50 mL) until gas evaporation stopped. Combined organic phases were discarded and the aqueous phase was re-transferred to the round-bottom flask. The solution was cooled down with an ice bath and acidified with cold 0.1 M HCl to adjust pH 3.5-4. The aqueous phase was extracted with DCM (3 × 50 mL) to yield the defluorinated thioester. Organic phases were discarded and the aqueous phase was acidified with cold 1 M HCl to adjust pH 2.5-3. The aqueous phase was extracted with DCM (3 × 50 mL). Organic phases were combined, dried over $MgSO_4$, filtered and solvent was evaporated *in vacuo* to yield Fluoromethylmalonic acid thiophenyl halfester as colourless precipitate.

Yield: 21.5 % (1.2 g, 5.3 mmol)
^{1}H NMR (300 MHz, CDCl3) δ = 7.47 – 7.41 (m, 5H), 1.88 (d, *J* = 21.9 Hz, 3H) ppm.
^{19}F NMR (300 MHz, CDCl3) δ = –156.39 (q, J = 21.9 Hz) ppm.

Fluoromethylmalonyl-CoA (17)

Fluoromalonyl-CoA 17 was synthesized via transacylation from the fluoromalonyl thiophenyl halfester S6 to free coenzyme A (CoASH), adapted from Dunn *et al.* [12].



CoASH (200 mg; 255 µmol) and the fluoromethylmalonyl thiophenyl halfester S6 (200 mg; 876 µmol) were dissolved in 1 mL ice cold $NaHCO_3$ (0.1 M) at 0 °C. The pH was adjusted back to pH 8-9 with 1 mL NaOH (0.2 M) and the reaction was continued for 1.5 h at 0 °C. Then, the reaction mixture (ca. 2 mL) was poured into 20 mL acetone (-20 °C) in a 50 mL falcon and spun for 10 min at 3000 rcf. The supernatant was discarded and the pellet was washed with 1 mL

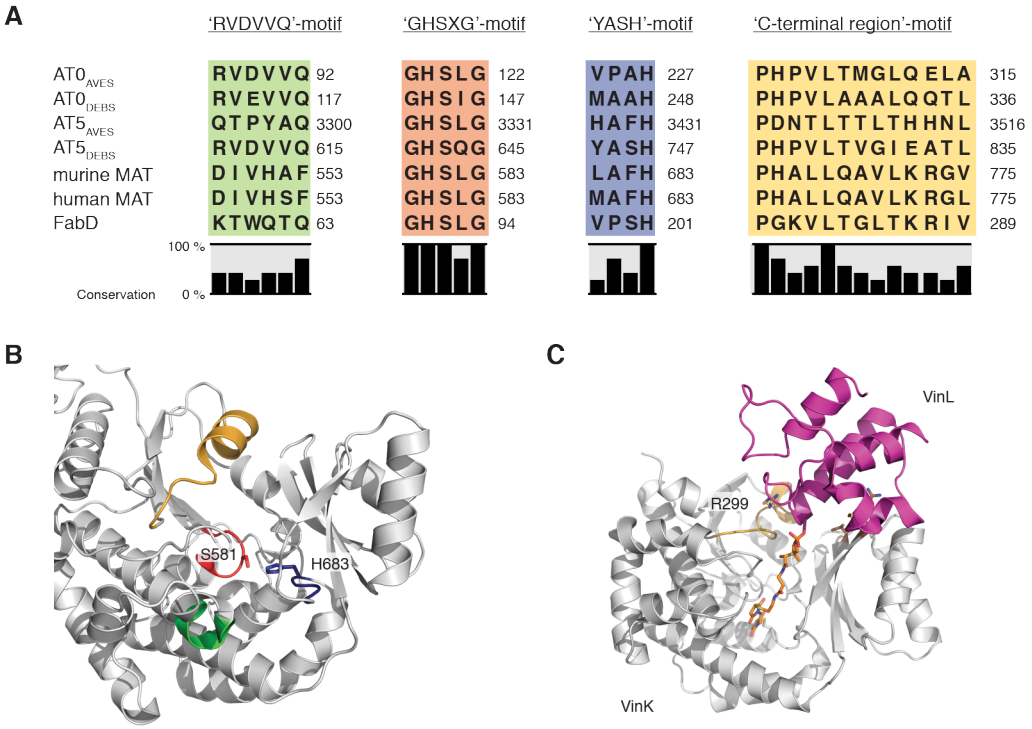
acetone (-20 °C) and dried *in vacuo*. The pellet was dissolved in water and the yield was determined by the absorbance at 260 nm (ε = 16,400 M^{-1} cm^{-1}) [13].

Yield: 93 %, white crystals.

^{1}H NMR (300 MHz, D_2O) δ = 8.57 (s, 1H), 8.28 (s, 1H), 6.19 (d, *J* = 6.9 Hz , 1H), 4.62-4.56 (m, 1H), 4.28-4.21 (m, 2H), 4.02 (s, 1H), 3.86-3.80 (m, 1H), 3.58-3.52 (m, 1H), 3.48-3.44 (m, 2H), 3.40-3.33 (m, 2H), 3.09-3.03 (m, 2H), 2.48-2.42 (m, 2H), 1.71 (d, *J* = 22 Hz, 3H), 0.88 (s, 3H), 0.73 (s, 3H) ppm.

^{19}F NMR (300 MHz, D_2O) δ = –146.7 (q, *J* = 22 Hz, 1H) ppm.

4. Supplementary Figures:

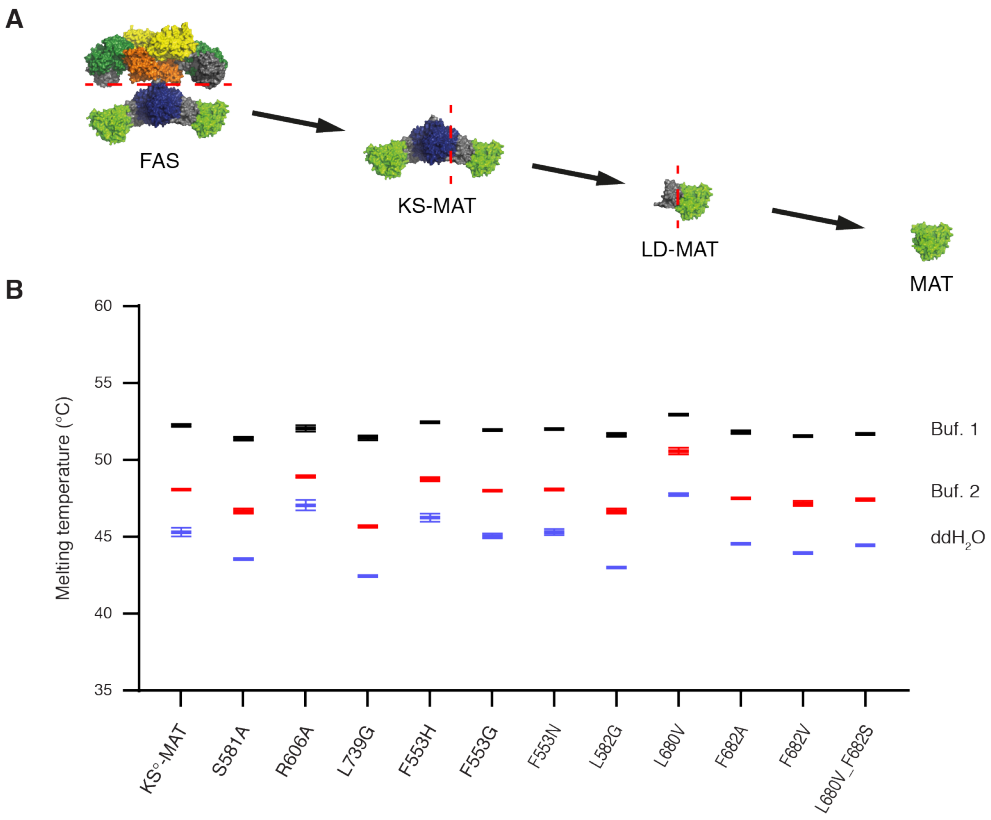


Supplementary Fig. 1: Substrate specificity of ATs.

(A) Sequence alignments of various ATs in regard to the four sequence motifs characterizing substrate specificity. Utilized primary sequences refer to loading-ATs: $AT0_{AVES}$ (Q79ZN1) and $AT0_{DEBS}$ (Q03131), specialized extender-ATs: $AT5_{AVES}$ (Q9S0R7), $AT5_{DEBS}$ (Q03133) and FabD (P0AAI9), and polyspecific ATs: murine MAT (P19096) and human MAT (P49327). The Uniprot code is given in brackets. (B) Cartoon depiction of the human MAT (PDB code: 3hhd) with sequence motifs colored as above. Active site serine (S581) is shown in sticks representation [17]. (C) Crystal structure of the VinK-VinL complex (PDB code: 5czd) in cartoon representation [18]. The ACP (VinL) was chemically cross-linked (sticks, orange) to the AT (VinK). The authors characterized the binding interface and identified crucial residues including R299 in the 'C-terminal' region (brightorange).

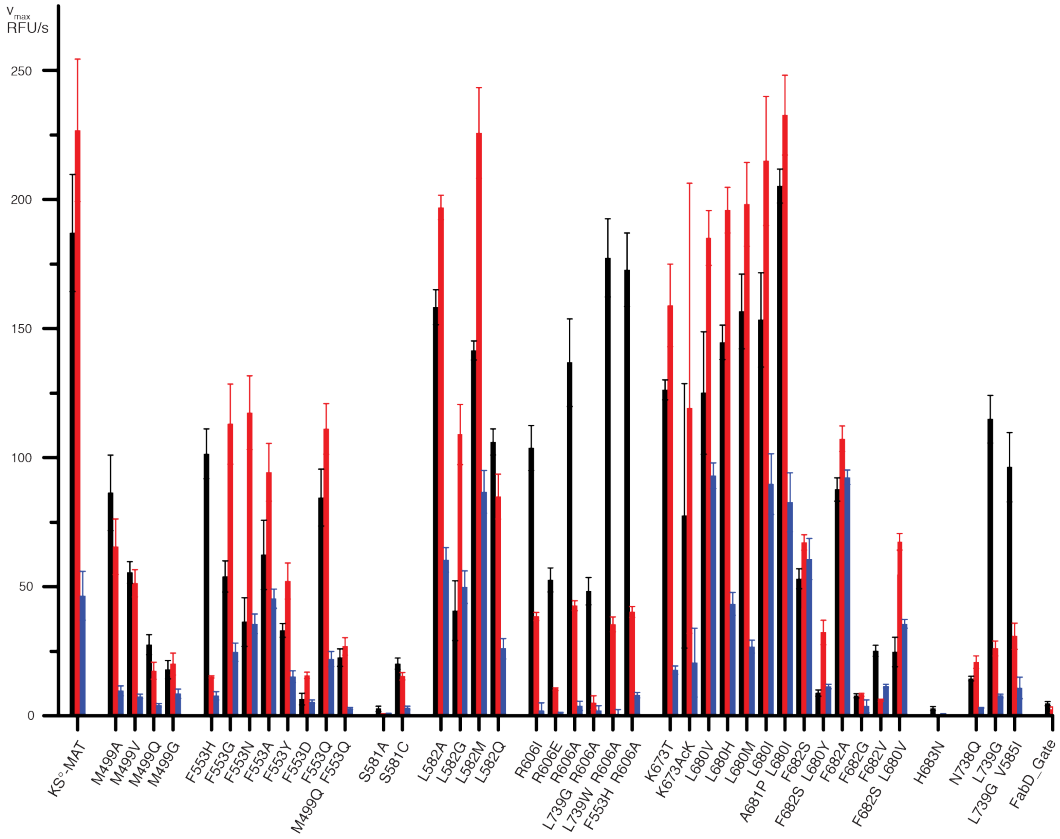
Supplementary Fig. 2: Evolution of acyltransferase.

Circular phylogram of select ATs is shown. Abbreviations refer to: RAPS – rapamycin polyketide synthase; LasA – Lasalocid modular polyketide synthase; PikA – Pikromycin polyketide synthase; DEBS – 6-deoxyerythronolide B synthase; AVES – avermectin polyketide synthase; DSZS – DisD protein; MCAT – mitochondrial malonyltransferase; FAS – type I fatty acid synthase. The UniProt code is given in brackets. The specificity is indicated by the background color. Phylogenetic tree was generated with CLC Main Workbench (version 6.9.1) using the UPGMA algorithm.



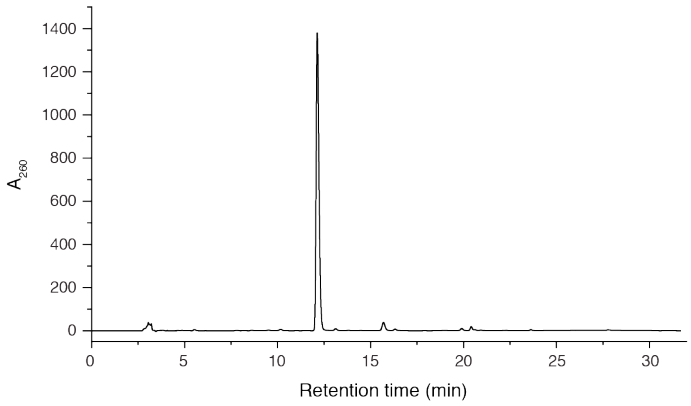
Supplementary Fig. 3: Robustness of murine MAT fold.

(A) The MAT domain can be released from the FAS fold and remains to be expressible in *E. coli* [4]. (B) Screen of melting temperatures of select MAT variants (KS^0-MAT) bearing point mutations. Mutations are well tolerated and the melting temperatures vary by about 2-3 °C. Buffer 1 refers to storage buffer (250 mM potassium phosphate, 10 % (v/v) glycerol, 1 mM EDTA, (pH 7.0)) and buffer 2 to αKGDH assay buffer (50 mM sodium phosphate, 10 % (v/v) glycerol, 1 mM EDTA (pH 7.6). Data were collected with enzyme from one preparation (n = 1) in technical replication (3 independent measurements) for each condition. Error bars show standard deviation.



Supplementary Fig. 4: Plasticity of the murine MAT domain.

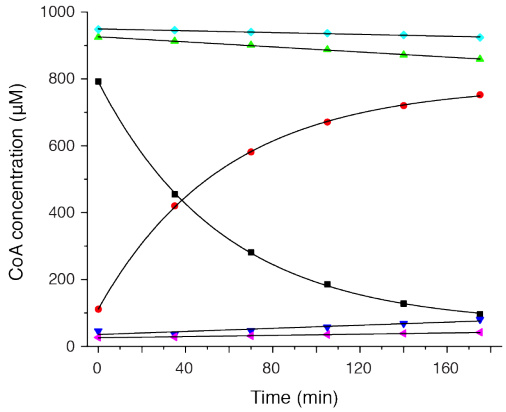
Initial velocity screen for the substrates acetyl- (black bar), malonyl- (red bar) and methylmalonyl-CoA (blue bar) were determined with the αKGDH assay. Standard conditions were applied with fixed concentrations of substrates and ACP of 20 µM and 60 µM, respectively. Data were collected with enzyme from one preparation (n = 1) in technical replication (3 independent measurements). Error bars show standard deviation.



Supplementary Fig. 5: Quality control of precipitated F-Mal-CoA.

F-Mal-CoA was purified with HPLC-UV using the gradient described in method section, showing almost quantitative conversion of CoA (15.5 min). F-Mal-CoA (12.1 min) has a comparable elution time to Mal-CoA (12.0 min).

A



B

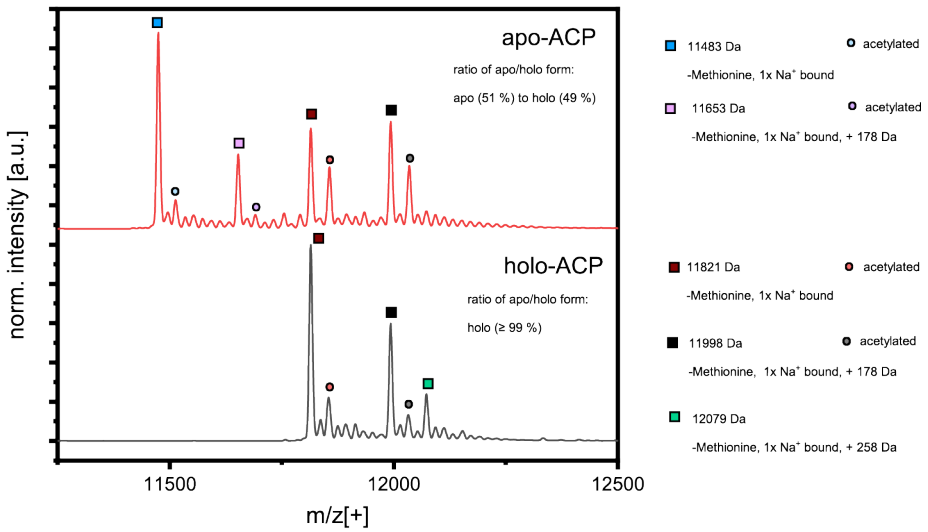
Hydrolyse	F-Mal-CoA	Temperature
with DTT (μM min^{-1})	12 ± 5	22 °C
with DTT (μM min^{-1})	0.11 ± 0.01	5 °C
w/o DTT (μM min^{-1})	0.31 ± 0.01	22 °C

C

Hydrolyse	Mal-CoA	MM-CoA	F-Mal-CoA
MAT-mediated (s^{-1})	1.13E-03	7.20E-04	1.81E-02
Total (μM min^{-1})	0.03	0.04	0.35

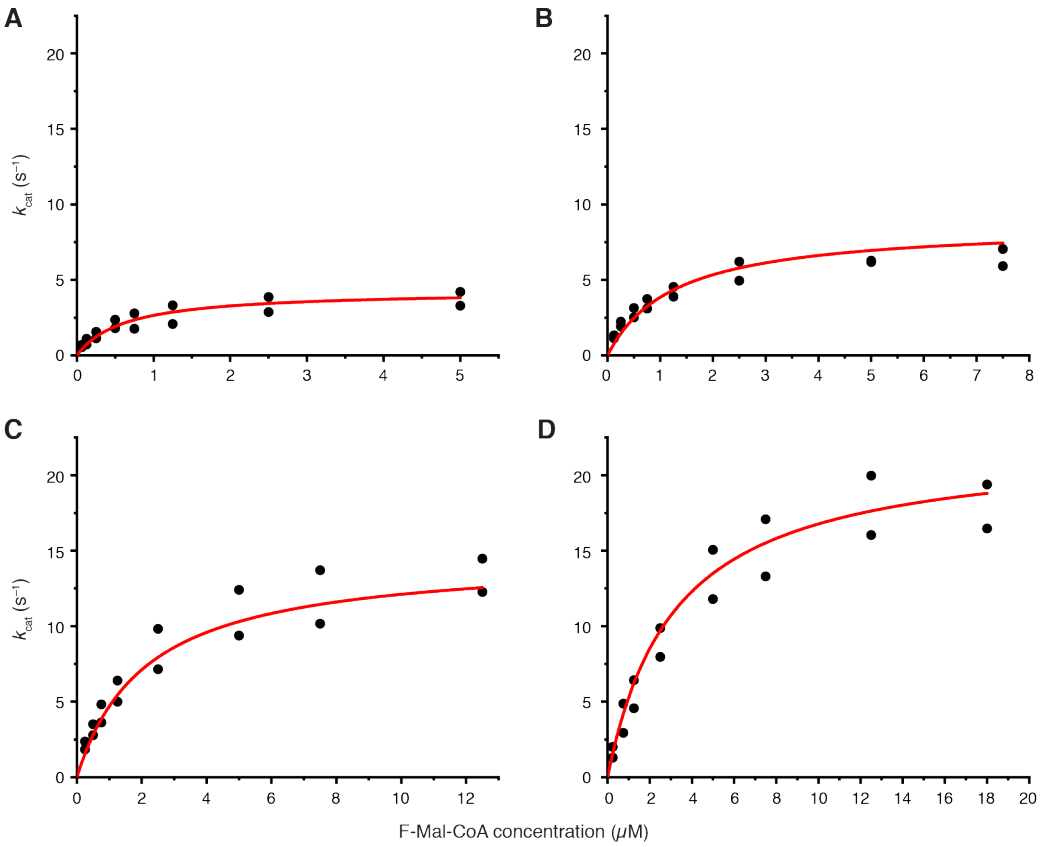
Supplementary Fig. 6: Further information on F-Mal-CoA.

(A) F-Mal-stability was monitored with HPLC-UV using the gradient described in the method section. Peak areas were converted to concentrations using a CoA calibration curve. F-Mal-CoA degradation (black) and its corresponding CoA liberation (red) in buffer with DTT at 22 °C was fitted with an exponential decay function in OriginPro 8.5. Degradation without DTT at 5 °C (cyan: F-Mal-degradation, purple: CoA-liberation) and at 22 °C (green: F-Mal-degradation, blue: CoA-liberation) was fitted by linear regression (buffer: (400 mM phosphate buffer, 20 % (v/v) glycerol, 2 mM or 0 mM DTT, 1 mM EDTA, 0.8 % DMSO (pH 7.2)). (B) Initial slope average of F-Mal-degradation and CoA-liberation of the corresponding condition (C) Hydrolysis rates of the extender substrates determined by the αKGDH assay. Final substrate concentrations in the assays were 20 µM X-CoA and 0.2 µM KS°-MAT. Fast F-Mal-CoA degradation by DTT was the reason why DTT was omitted in assays when using this substrate. (A-C) Data were collected with one batch of F-Mal-CoA.



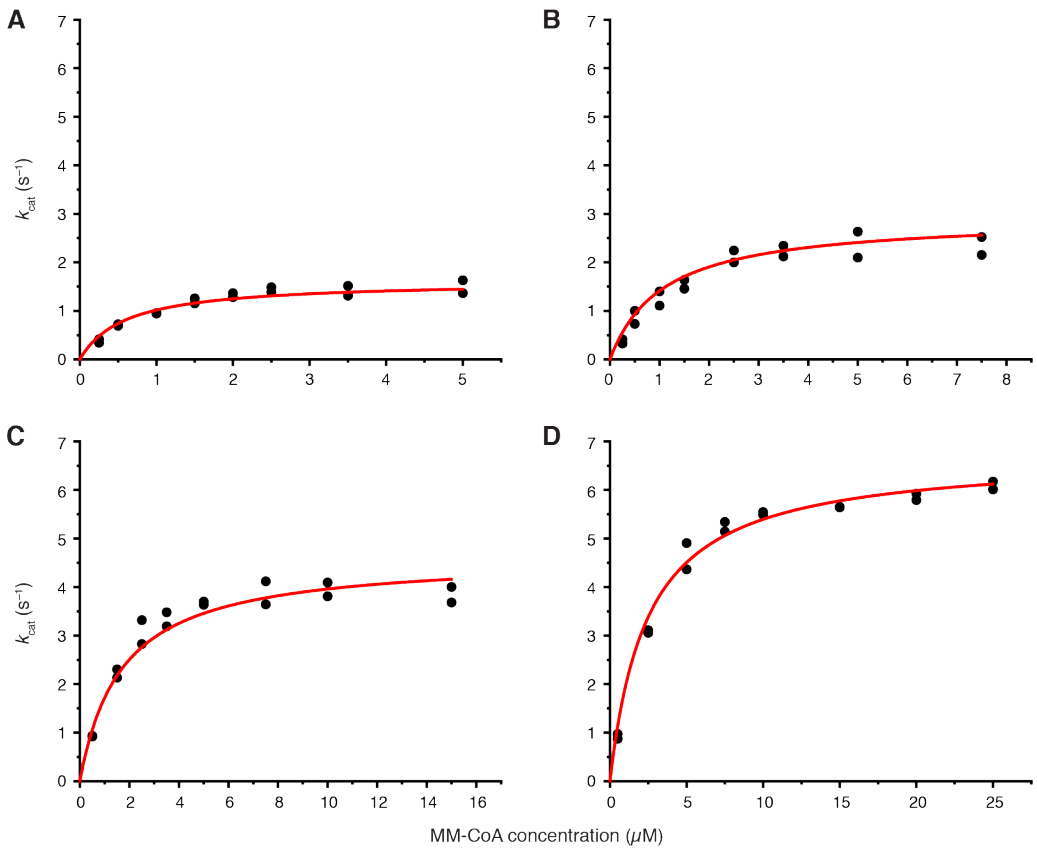
Supplementary Fig. 7: Quality control of DEBS ACP6.

Mass spectroscopic analysis of DEBS ACP6, co-expressed with Sfp (red) or Npt (black), was performed with ESI in positive mode (SYNAPT G-2, waters). Co-expression with Sfp led to a mixture of 49 % of phosphopantetheinlated (holo-form, brown square, theoretical mass: 11816.10 Da) and 51 % apo-form (blue square, theoretical mass = 11475.76 Da), while co-expression with Npt resulted in quantitative conversion to the holo-form. A mass shift of 178 Da (purple square for the apo-form and black square for the holo-form) and 258 Da (cyan square for the holo-form) could be explained by His-tag phosphogluconoylation in *E. coli*, whereas a mass shift of 42 Da corresponds to the acetylated form (circles with the corresponding color).



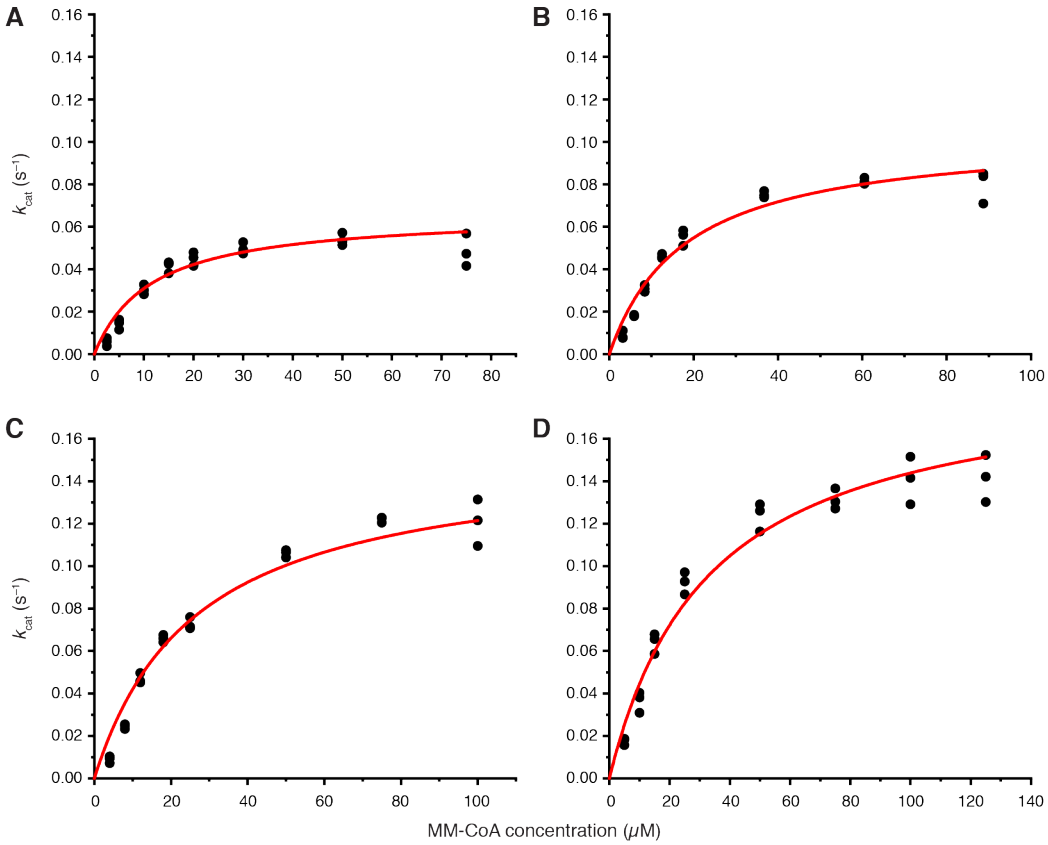
Supplementary Fig. 8: Global Michaelis-Menten fit of FAS MAT-mediated transacylation of fluoromalonyl moieties to FAS ACP.

Initial velocities were plotted against fluoromalonyl-CoA (F-Mal-CoA) concentrations at four fixed ACP concentrations: (A) 11 µM, (B) 24 µM, (C) 49 µM and 100 µM ACP. Data were fit globally with the Michaelis-Menten equation assuming a ping-pong bi-bi mechanism (n = 2).



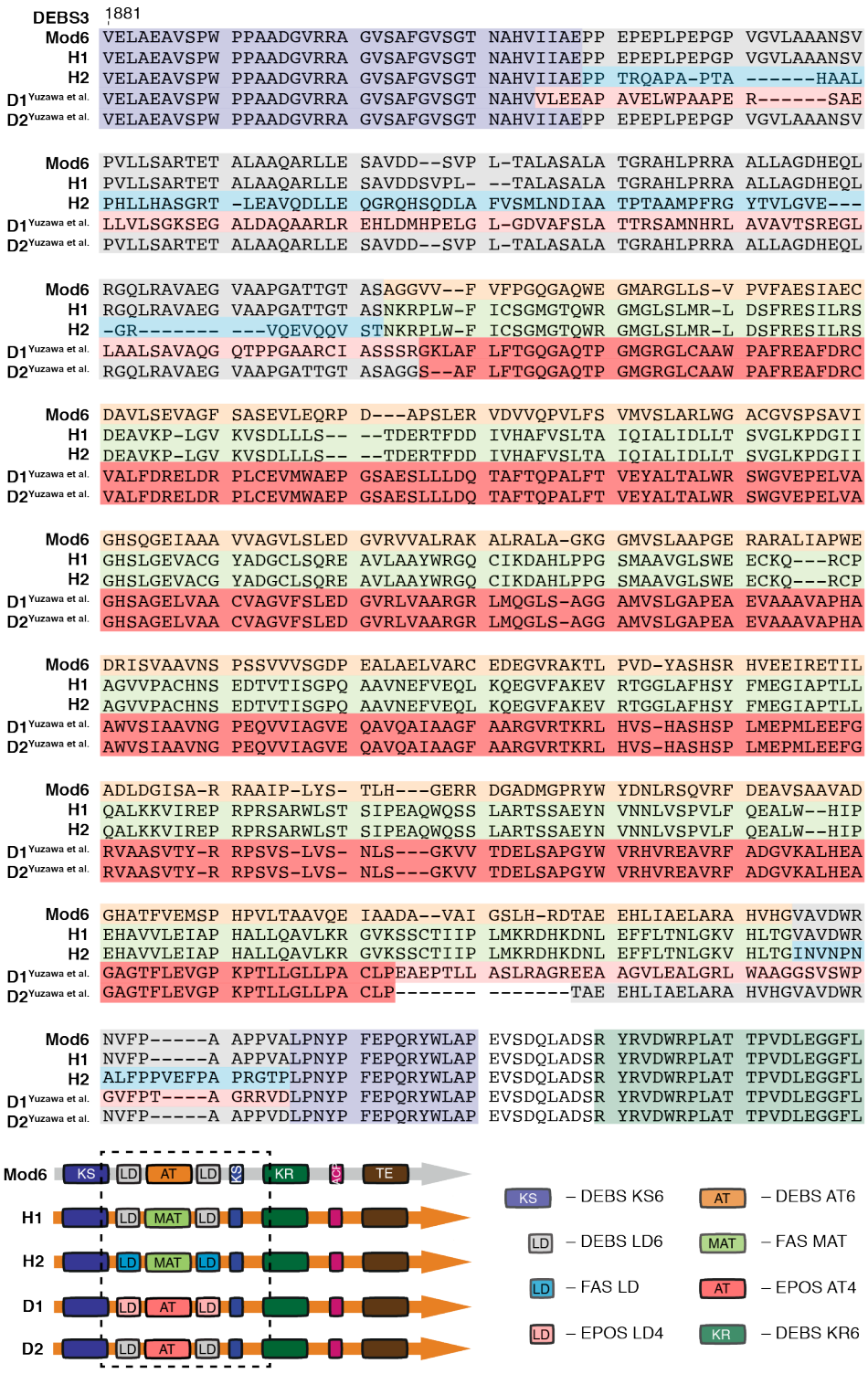
Supplementary Fig. 9: Global Michaelis-Menten fit of FAS MAT-mediated transacylation of methylmalonyl moieties to DEBS ACP6.

Initial velocities were plotted against methylmalonyl-CoA (MM-CoA) concentrations at four fixed ACP concentrations: (A) 53 µM, (B) 107 µM, (C) 200 µM and 373 µM ACP. Data were fit globally with the Michaelis-Menten equation assuming a ping-pong bi-bi mechanism (n = 2).



Supplementary Fig. 10: Global Michaelis-Menten fit of DEBS AT6-mediated transacylation of methylmalonyl moieties to DEBS ACP6.

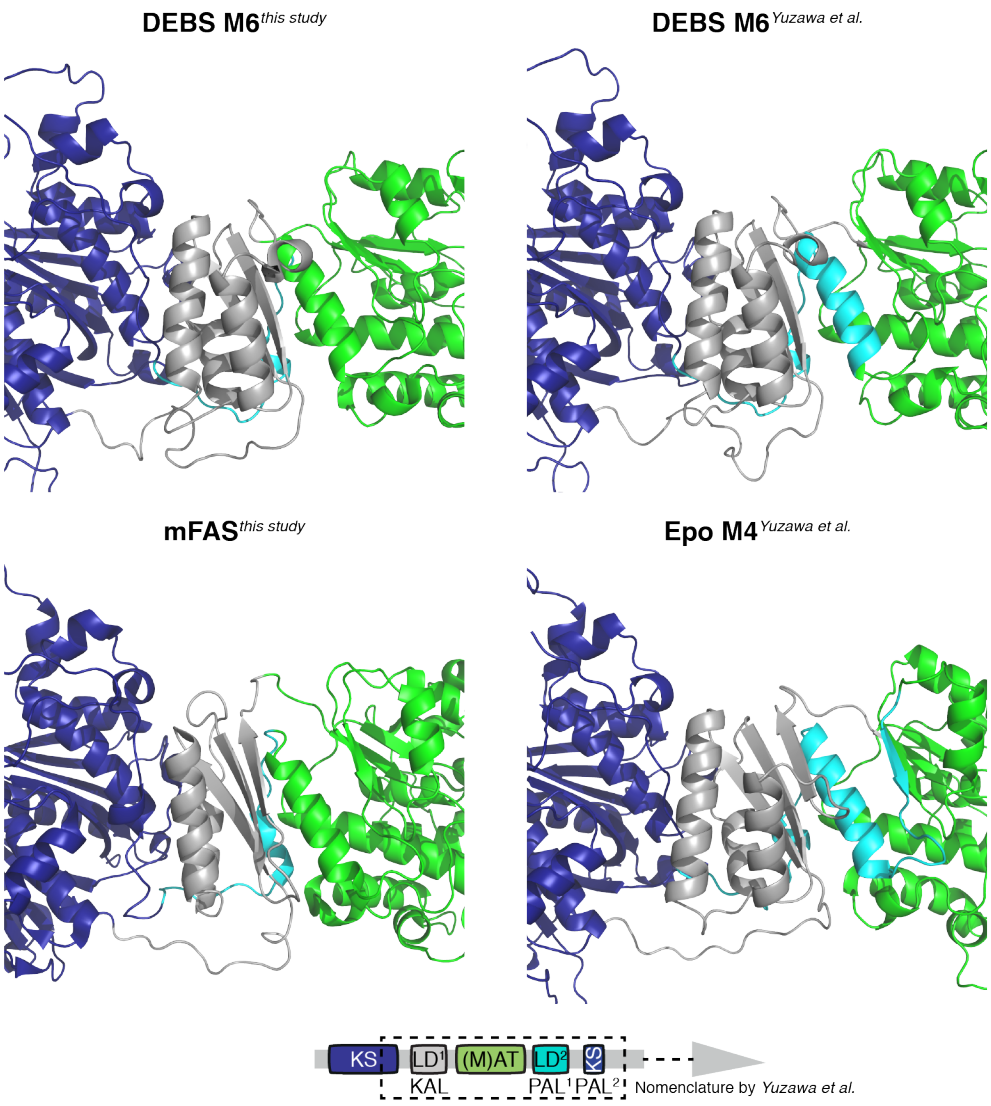
Initial velocities were plotted against methylmalonyl-CoA (MM-CoA) concentrations at four fixed ACP concentrations: (A) 64 µM, (B) 119 µM, (C) 238 µM and 406 µM ACP. Data were fit globally with the Michaelis-Menten equation assuming a ping-pong bi-bi mechanism (n = 3).



Supplementary Fig. 11: Design of DEBS/FAS hybrids.

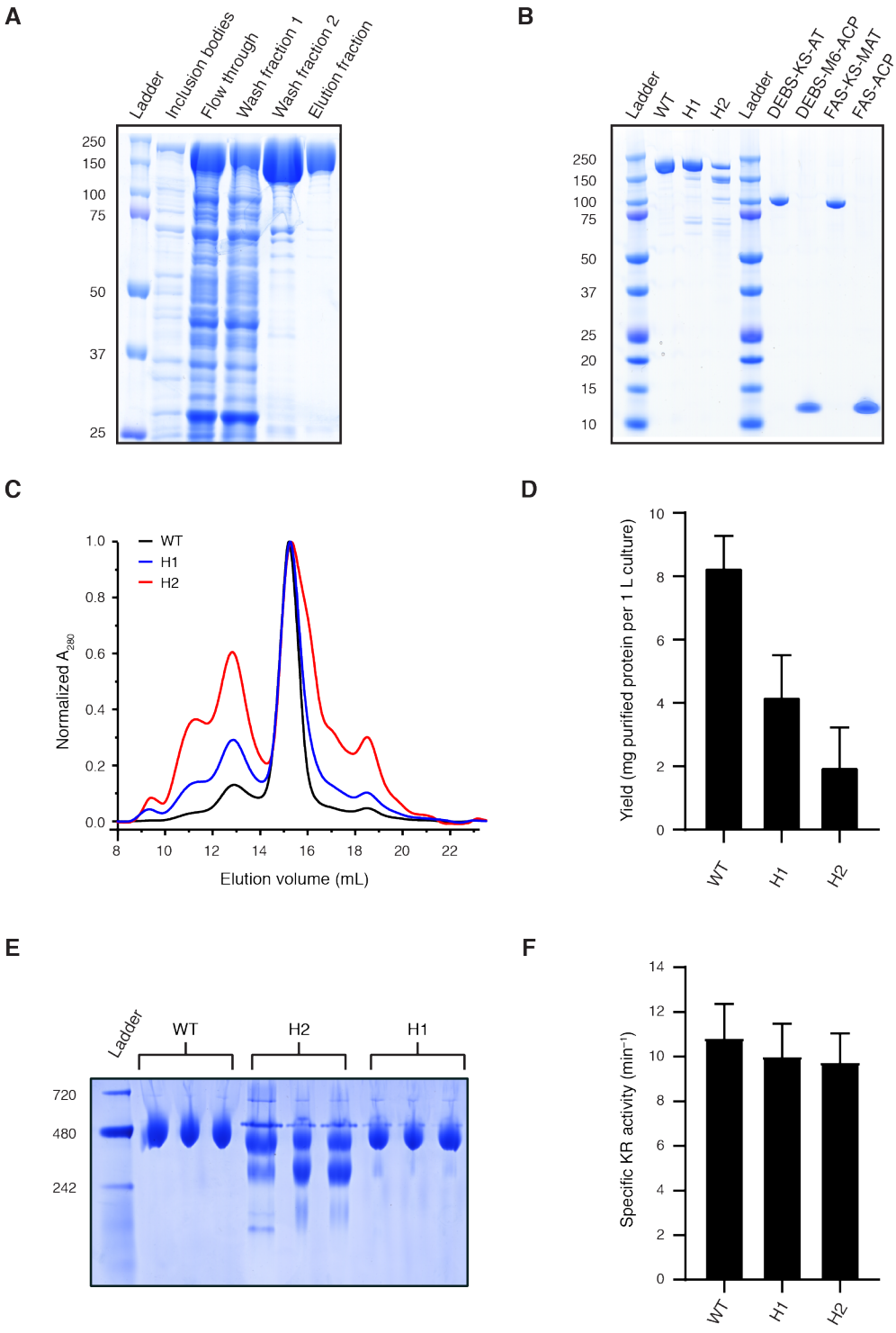
Sequence alignment of wildtype DEBS M6+TE (**Mod6**) with both hybrid DEBS/FAS constructs **H1** and **H2**. In construct **H1**, the AT6 domain of DEBS was exchanged by the MAT domain of

murine FAS. For construct **H2**, the AT6 domain of DEBS plus the adjacent linker domain was exchanged with LD-MAT from murine FAS. The sequence alignment is colored according to the color code of the attached domain architecture. Respective DNA sequences are shown in Supplementary Table 6. For comparison, we also integrated constructs **D1** and **D2** from Yuzawa *et al.* into the sequence alignment [6]. Our hybrid **H1** is similar to their **D2**, although we defined the terminal long α-helix as part of MAT and not as part of the post-AT linker.



Supplementary Fig. 12: Comparison of domain boundaries.

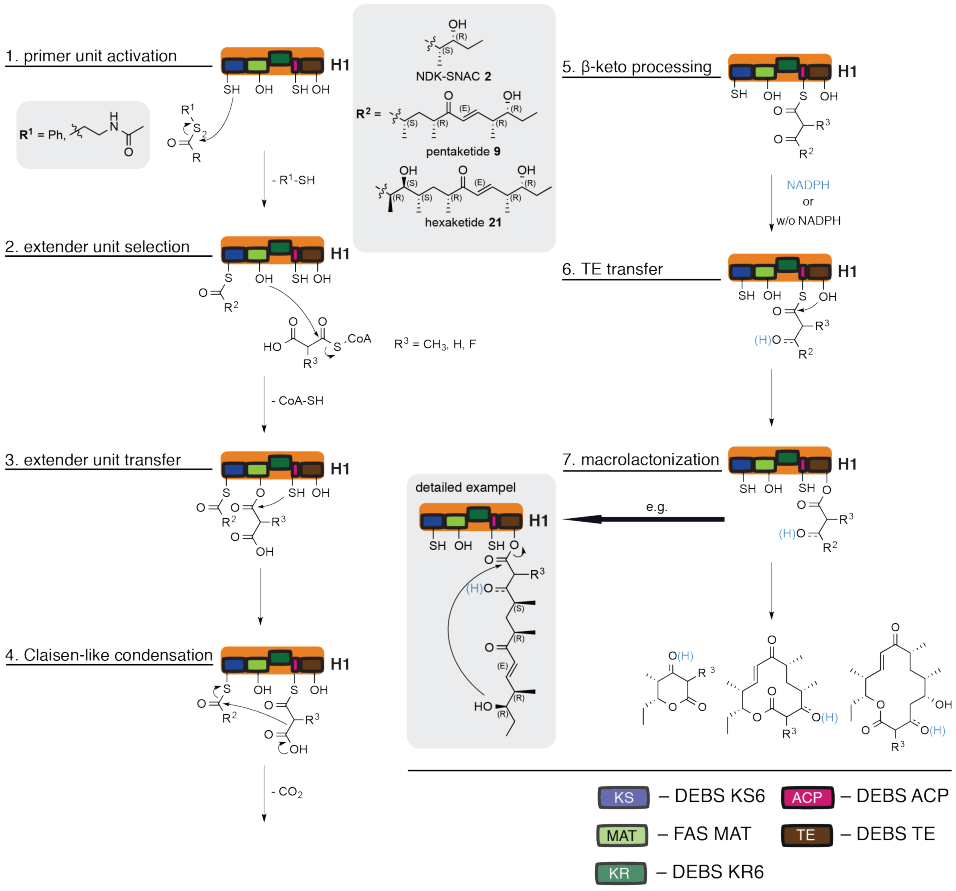
Cartoon depiction of the KS-AT architecture with different defined boundaries in respect of the linker domain. The KS-AT structures of DEBS M6 (based on DEBS M3, PDB code: 2qo3) and Epo M4 (based on curacin A PKS, PDB code: 4mz0) were modeled by Swissmodel[19–23] and compared to the X-ray structure of mFAS (5my0) [1]. Boundaries vary especially in the long terminal α-helix, which we defined as part of the (M)AT and *Yuzawa et al.* as part of the LD^2 (they used the nomenclature PAL^1) [6].



Supplementary Fig. 13: Purification and quality control of DEBS M6+TE and DEBS/FAS hybrids H1 and H2.

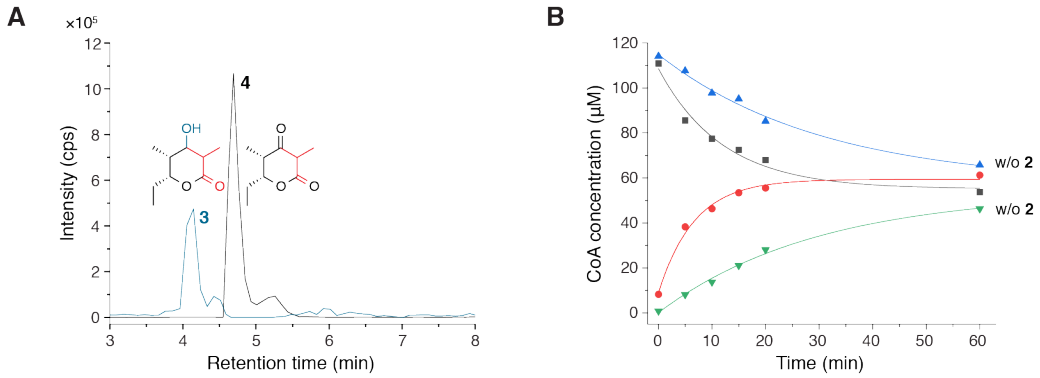
(A) Purification of DEBS M6+TE (WT) co-expressed with Sfp (SDS-PAGE (7.5 % Tris-glycine, 1 % SDS buffer). After centrifugation, supernatant was purified with Ni-chelating chromatography and the pellet was analyzed for inclusion bodies. DEBS M6+TE has little tendency to aggregate

and was received as a pure protein. (B) Purity of used enzymes after SEC (SDS-PAGE (NuPAGE 4-12 % Bis-Tris, Thermo Fisher)). (C) Oligomeric state of purified WT, **H1** and **H2** analyzed by SEC with absorbance normalized to the highest peak. While the chromatogram of H1 is similar to the WT, the main peak shoulder of H2 indicates the presence of monomers. (D) Comparison of yields after purification (IMAC, SEC) of three different expression cultures of DEBS-M6+TE and variants (n = 3). (E) Native gel electrophoresis (3-12 % Bis-Tris, Thermo Fisher) of DEBS-M6+TE and variants showing dimer for **WT**, **H1**, and **H2** as a mixture of dimer and monomer. Each enzyme is shown in biological triplicates (n = 3) (F) KR specific assay was performed with *trans*-1-decalone indicating 92 % KR-activity for **H1** and 90 % KR-activity for **H2** in biological and technical triplicates (n = 3) compared to the **WT**. Final substrate concentrations in the assay were 0.3 µM enzyme, 2 mM *trans*-1-decalone and 60 µM NADPH.



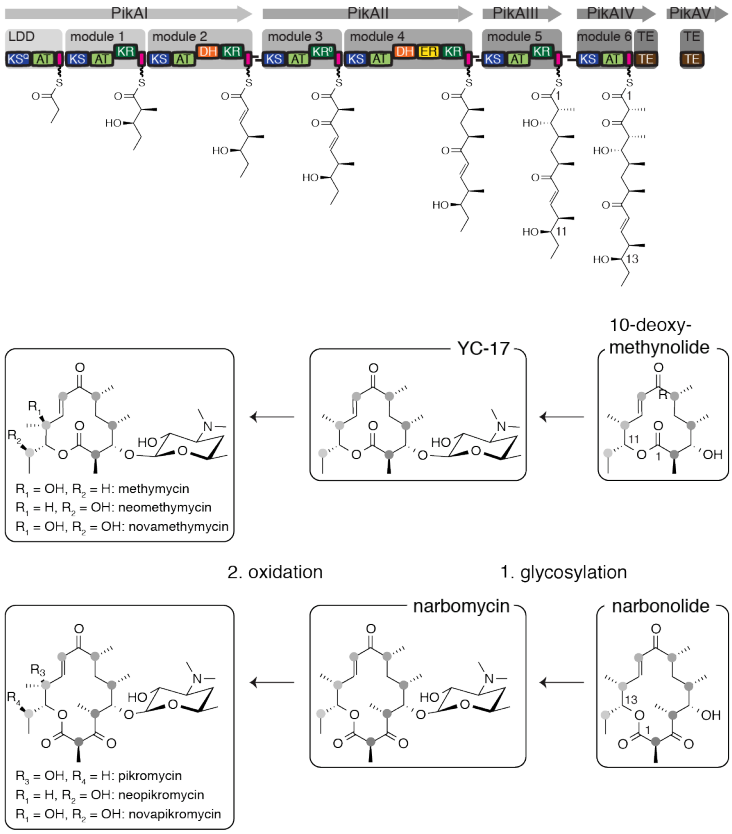
Supplementary Fig. 14: Proposed mechanism of polyketide formation.

(1) The priming units, *N*-acetylcysteamine-activated diketide, NDK-SNAC (**2**), and thiophenol-activated penta- (**9**) and hexaketide (**21**) bind directly to the active cysteine of the KS domain upon release of *N*-acetylcysteamine and thiophenol, respectively. (2) The extender units malonyl-CoA or derivatives thereof (MM-CoA, F-Mal-CoA and F-MM-CoA) are loaded by the substrate-promiscuous FAS-derived MAT domain upon release of free CoA, (3) and are subsequently transferred to the phosphopantetheine arm of the ACP domain. (4) The ACP-activated malonyl or malonyl-derivative binds to the KS domain for decarboxylative Claisen-like condensation with the priming unit. (5) The *β*-keto-group of the elongated acyl intermediate, bound to ACP, is then reduced by the KR to form a β-hydroxyl moiety if NADPH is present. (6) Ultimately, the processed acyl intermediate is transferred to the TE domain and (7) released from the hybrid protein by macrolactonization to yield the polyketide products. For clarity, the scheme was simplified to relevant residues and bond-forming steps. **H1** was chosen as an example for the enzyme mediated polyketide formation.



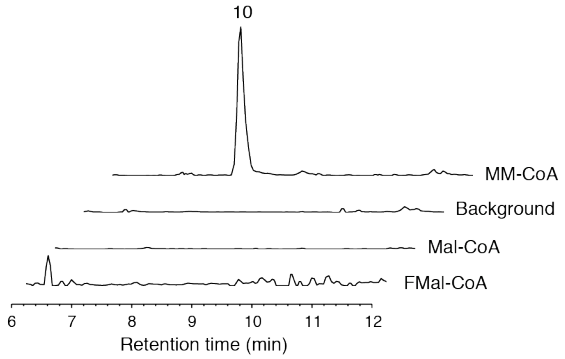
Supplementary Fig. 15: Functionality of DEBS M6+TE.

(A) Reductive WT-mediated chain extension of diketide-SNAC **2** with MM-CoA monitored by HPLC-MS (EIC: **3** $[M+Na]^+$ m/z = 194.98 and **4** $[M–H]^-$ m/z = 169.12). (B) Non-reductive enzyme-mediated chain extension of diketide-SNAC **2** with MM-CoA monitored by HPLC-UV exemplified by one preparation of **WT**. MMal-CoA consumption (blue and black) and CoA liberation (red and green) was tracked at A_{260} at defined time points. Enzyme-mediated hydrolysis was measured without **2** (blue and green). CoA concentrations of each time point were determined by using the internal standard HyBu-CoA and fitted with an exponential decay function in OriginPro 8.5. The sum of MMal-CoA and CoA concentrations of each sample was used as quality control and outliers were excluded.



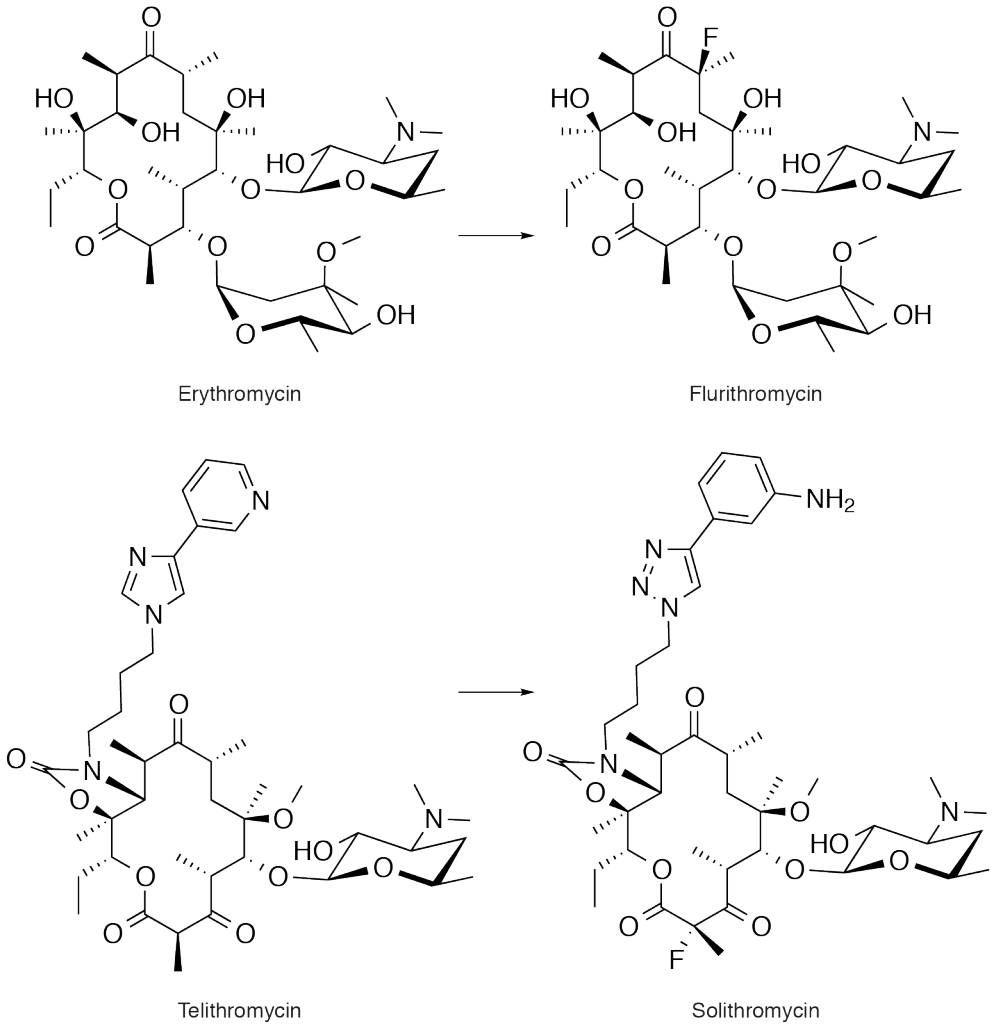
Supplementary Fig. 16: Biosynthesis of the pikromycin/methymycin precursor molecules.

The pikromycin synthase catalyze the assembly like biosynthesis of 10-deoxymethynolide and narbonolide, precursor molecules for YC-17 and narbomycin, which can be oxidized to various compounds. The enzymes are distributed on five peptide chains including a N-terminal loading module and two C-terminal thioesterases.



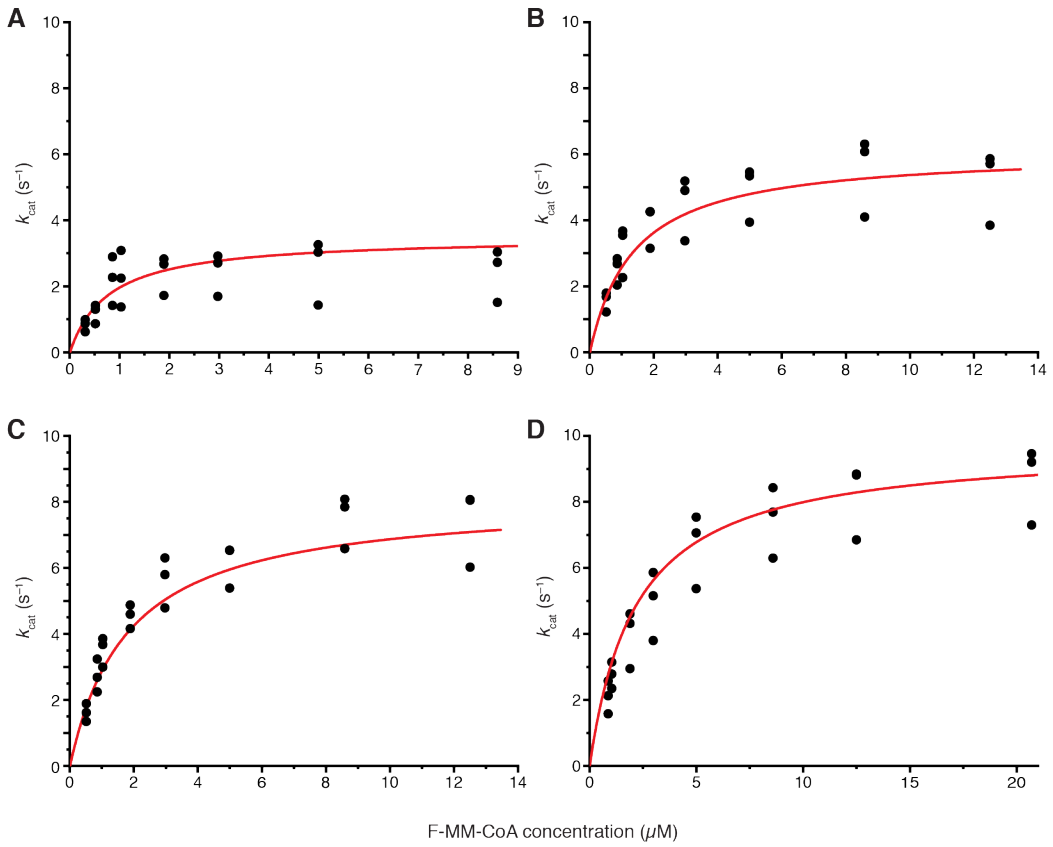
Supplementary Fig. 17: Extender substrate specificity of DEBS M6+TE (WT).

Reductive **WT**-mediated chain extension of pentaketide **9** with MM-CoA, Mal-CoA and F-Mal-CoA monitored by HPLC-MS. The background was performed without elongation substrate and only the EIC of the negative control is shown for the mass of compound **10** (elongation product with MM-CoA). EIC of compound **12** (elongation product of Mal-CoA) and **14** (elongation product of F-Mal-CoA) are shown although the masses were not found. Data are normalized with respect to the highest peak of **H1**. (EIC: **10** $[M+Na]^+$ m/z = 319.11; **12** $[M+Na]^+$ m/z = 305.09 and **14** $[M+Na]^+$ m/z = 323.08).



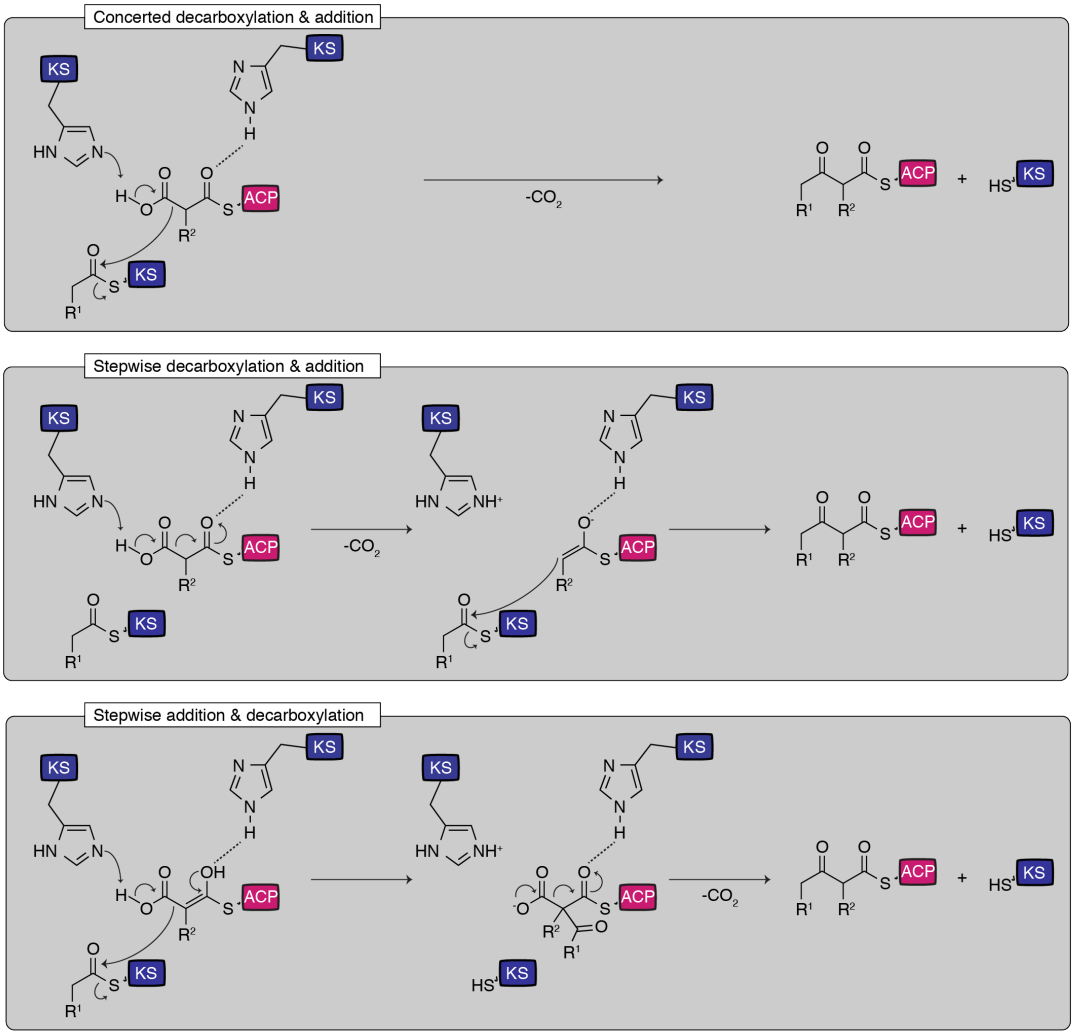
Supplementary Fig. 18: Chemical structures of erythromycin and derivatives.

The structures of the natural product erythromycin and the semi-synthetic second generation fluoro-derivative flurithromycin are shown. Telithromycin is also a semi-synthetic erythromycin derivative, which is a FDA-approved third generation antibiotic. Due to side effects of telithromycin the fourth generation antibiotic solithromycin was developed, which is under review for approval.

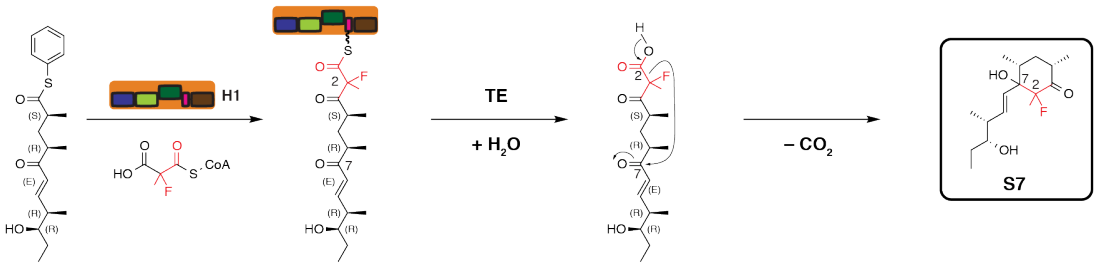


Supplementary Fig. 19: Global Michaelis-Menten fit of FAS MAT-mediated transacylation of fluoromethylmalonyl-CoA moieties to FAS ACP.

Initial velocities were plotted against fluoromethylmalonyl-CoA (FMM-CoA) concentrations at four fixed ACP concentrations: (A) 10 µM, (B) 25 µM, (C) 50 µM and 100 µM ACP. Data were fit globally with the Michaelis-Menten equation assuming a ping-pong bi-bi mechanism (n = 3).

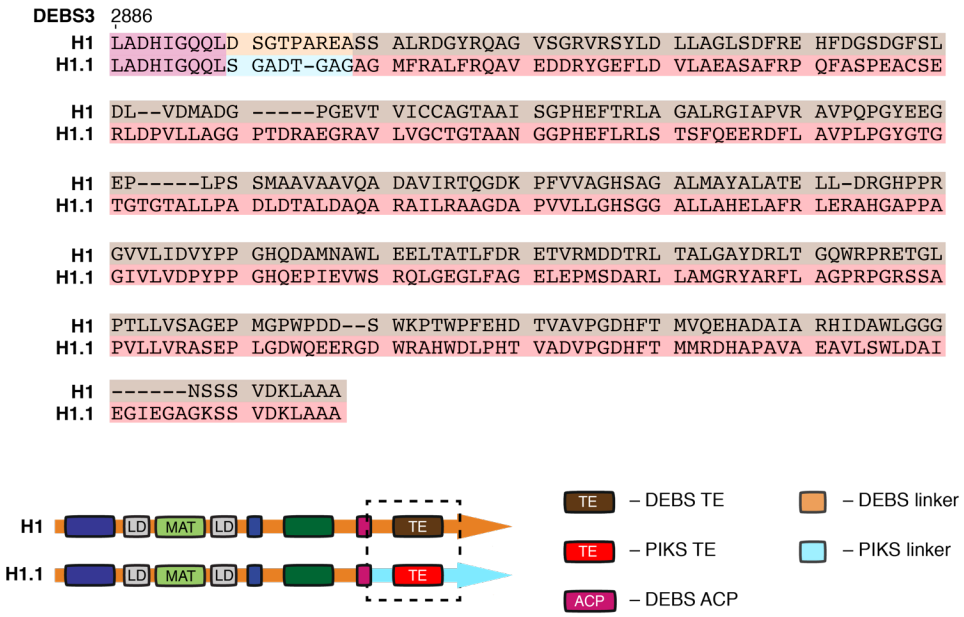


Supplementary Fig. 20: Possible mechanisms for the KS-catalyzed Claisen condensation.
After acylation of the KS with the starter substrate or intermediate and acylation of the ACP with the elongation substrate, three distinct mechanisms for the KS-catalyzed chain elongation are possible. In the first mechanism, the decarboxylation and C-C bond formation occur simultaneously. In contrast, two mechanisms are possible where either the decarboxylation or the C-C bond formation precede the other. Adapted from Blaquiere *et al*. [24].
We note that the biosynthesis of compound **18** is indicative of a Claisen condensation reaction that proceeds via decarboxylation to the enolate and nucleophilic C-C bond formation either occurring in concerted manner or in two steps by C-C bond formation succeeding decarboxylation. Demonstrating the direct condensation of disubstituted malonyl-CoA substrates by a conventional KS-domain expands the potential of PKS engineering strategies and enlarges the spectrum of accessible compounds.



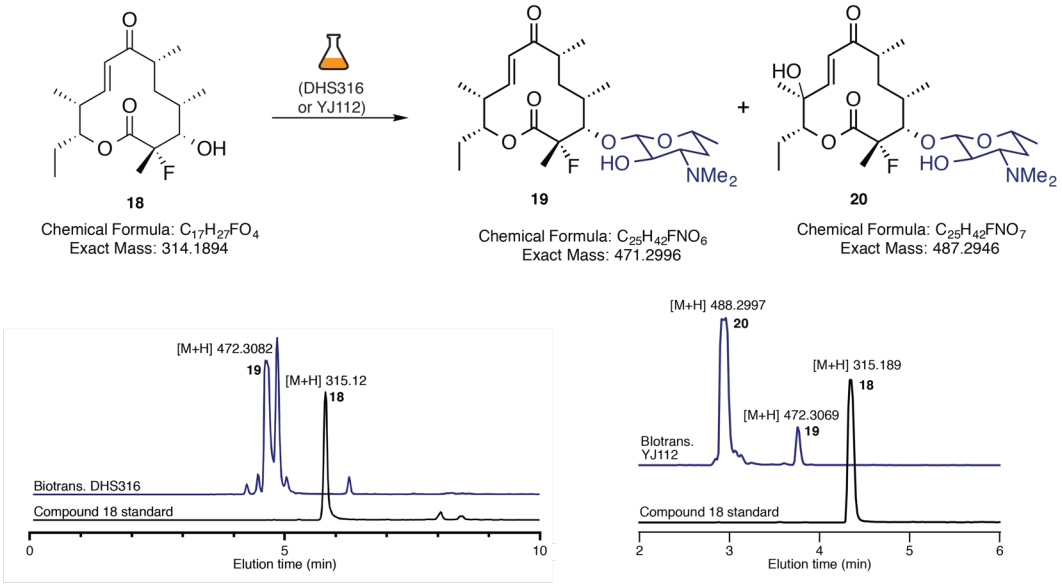
Supplementary Fig. 21: Postulated mechanism for the production of S7.

We note that elongating the pentaketide with F-MM-CoA in the absence of NADPH led to product **S7**, which is the respective derivative to the previously identified compound **16**. This data indicates that cyclohexanone ring formation of **S7** occurs after TE-catalyzed hydrolysis with subsequent spontaneous decarboxylation and ring closure, similar to the proposed formation of pacificanone, a side product of the rosamicin PKS [25].



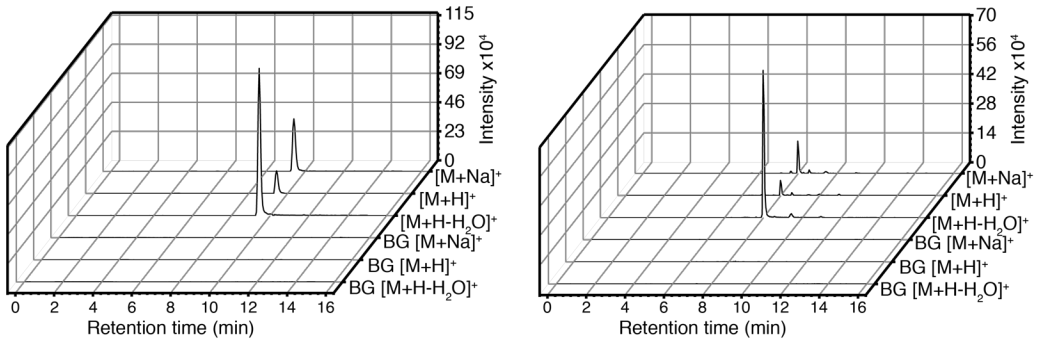
Supplementary Fig. 22: Design of TE exchange

Sequence alignment of DEBS/FAS hybrids **H1** and **H1.1**, the latter one includes the exchanged PIKS TE. The design of **H1.1** is similar to a previous study by Koch *et al.* and includes the PIKS ACP to TE linker [3]. The sequence alignment is colored according to the color code of the attached domain architecture. Respective DNA sequences are shown in Supplementary Table 6.



Supplementary Fig. 23: Biotransformation of compound 18.

Overlaid LC-MS traces of compound **18** and compounds after biotransformation with the *Streptomyces venezuelae* strains DHS316 (left) or YJ112 (right). Mass spectra were taken in positive mode with peaks for biotransformation and subsequent oxidation clearly displayed. According to Jung *et al*., the strain YJ112 produces next to methymycin also neomethymycin [10].



Supplementary Fig. 24: Conversion of hexaketide 21 to compounds 22-23

H1-mediated chain extension of hexaketide **21** with F-MM-CoA to macrolactone **22** (left, without NADPH) and **23** (right, with NADPH) was monitored by HPLC-HRMS. The background (BG) was performed in the absence of the elongation substrate F-MM-CoA. Found adducts are shown for the enzymatic reactions and background samples. (EIC: **22** $[M+Na]^+$ m/z = 393.2041; $[M+H]^+$ m/z = 371.2225; $[M+H\text{-}H_2O]^+$ m/z = 353.2117; **23** $[M+Na]^+$ m/z = 395.2201; $[M+H]^+$ m/z = 373.2384; $[M+H\text{-}H_2O]^+$ m/z = 355.2275).

5. Supplementary Tables:

Supplementary Table 1: Absolute kinetic parameters for MAT- and DEBS AT6-mediated transfer.

AT/MAT	Substrate	ACP	k_{cat} [s^{-1}]	K_S [μM]	K_{ACP} [μM]	k_{cat}/K_S [$M^{-1}s^{-1}$]
FAS	F-Mal-CoA	FAS	43.28 ± 3.04	6.3 ± 0.6	95 ± 11	6.9×10^6
FAS	MM-CoA	DEBS M6	14.08 ± 0.89	5.2 ± 0.4	408 ± 43	2.7×10^6
DEBS M6	MM-CoA	DEBS M6	0.29 ± 0.02	50.8 ± 5.9	217 ± 32	5.8×10^3
FAS	F-MM-CoA	FAS	12.1 ± 0.7	2.7 ± 0.3	24.6 ± 3.4	4.5×10^6

Supplementary Table 2: Table of compounds.

compound number	chemical formula	turnover rate (min^{-1})	calculated mass	observed mass	observed mass (HR)	retention time [min]
3	C9H16O3	WT = 0.29 ± 0.03 H1 = 0.19 ± 0.06 H2 = 0.07 ± 0.02	$[M+H]^+$ = 173,1178 $[M+Na]^+$ = 195,0997 $[M-H_2O+H]^+$ = 155,1072 $[M-H]^-$ = 171,1021	$[M+H]^+$ = 173,03 $[M+Na]^+$ = 194,98 $[M-H_2O+H]^+$ = 155,01	nd	4,1
4	C9H14O3	WT = 1.4 ± 0.4 H1 = 0.6 ± 0.1 H2 = 0.23 ± 0.02	$[M+H]^+$ = 171,1021 $[M+Na]^+$ = 193,0841 $[M-H_2O+H]^+$ = 153,0916 $[M-H]^-$ = 169,0865	$[M+H]^+$ = 171,00 $[M-H_2O+H]^+$ = 152,96 $[M-H]^-$ = 169.12	nd	4,8
5	C8H14O3	WT = 0.012 ± 0.003 H1 = 0.19 ± 0.02	$[M+H]^+$ = 159,1021 $[M+Na]^+$ = 181,0841 $[M-H_2O+H]^+$ = 141,0916 $[M-H]^-$ = 157,0865	$[M+Na]^+$ = 181,03	nd	3,3
6	C8H12O3	WT = 0.0 ± 0.1 H1 = 1.2 ± 0.3	$[M+H]^+$ = 157,0865 $[M+Na]^+$ = 179,0684 $[M-H_2O+H]^+$ = 139,0759 $[M-H]^-$ = 155,0708	$[M-H]^-$ = 155,16	nd	4,6
7	C8H13FO3	nd	$[M+H]^+$ = 177,0927 $[M+Na]^+$ = 199,0747 $[M-H_2O+H]^+$ = 159,0822 $[M-H]^-$ = 175,0771	-	-	-
8	C8H11FO3	nd	$[M+H]^+$ = 175,0771 $[M+Na]^+$ = 197,0590 $[M-H_2O+H]^+$ = 157,0665 $[M-H]^-$ = 173,0614	$[M-H]^-$ = 173,11	nd	4,8
10	C17H28O4	WT = 0.30 ± 0.05 H1 = 0.12 ± 0.01 H1 = 0.158 ± 0.001*	$[M+H]^+$ = 297,2066 $[M+Na]^+$ = 319,1886 $[M-H_2O+H]^+$ = 279,1960 $[M-H]^-$ = 295,1910	$[M+H]^+$ = 297,11 $[M+Na]^+$ = 319,11 $[M-H_2O+H]^+$ = 279,11	$[M+H]^+$ = 297,2056 $[M+Na]^+$ = 319,1876 $[M-H_2O+H]^+$ = 279,1950	8,1
11	C17H26O4	nd	$[M+H]^+$ = 295,1910 $[M+Na]^+$ = 317,1729 $[M-H_2O+H]^+$ = 277,1804 $[M-H]^-$ = 293,1753	$[M+H]^+$ = 295,09 $[M+Na]^+$ = 317,09 $[M-H_2O+H]^+$ = 277,08	$[M+H]^+$ = 295.1904 $[M+Na]^+$ = 317,1720 $[M-H_2O+H]^+$ = 277,1790	9,1
12	C16H26O4	WT = -0.009 ± 0.004 H1 = 0.22 ± 0.03	$[M+H]^+$ = 283,1910 $[M+Na]^+$ = 305,1729 $[M-H_2O+H]^+$ = 265,1804 $[M-H]^-$ = 281,1753	$[M+Na]^+$ = 305,09 $[M-H_2O+H]^+$ = 256,09	[M+H]+ = 283,1899 $[M+Na]^+$ = 305,1719 [M-H2O+H]+ = 265,1796	7,5
13	C16H24O4	nd	$[M+H]^+$ = 281,1753 $[M+Na]^+$ = 303,1573 $[M-H_2O+H]^+$ = 263,1647 $[M-H]^-$ = 279,1597	$[M+H]^+$ = 281,07 $[M+Na]^+$ = 303,08 $[M-H_2O+H]^+$ = 263,08	$[M+H]^+$ = 281,1741 $[M+Na]^+$ = 303,1559 $[M-H_2O+H]^+$ = 263,1637	7.2 / 9.5
14	C16H25FO4	WT = 0.003 ± 0.005 H1 = 0.056 ± 0.004 H1 = 0.04 ± 0.01*	$[M+H]^+$ = 301,1815 $[M+Na]^+$ = 323,1635 $[M-H_2O+H]^+$ = 283,1710 $[M-H]^-$ = 299,1659	$[M+H]^+$ = 301,10 $[M+Na]^+$ = 323,08 $[M-H_2O+H]^+$ = 283,08	$[M+H]^+$ = 301,1807 $[M+Na]^+$ = 323,1625 $[M-H_2O+H]^+$ = 283,1700	7.7 / 8.0
15	C16H23FO4	nd	$[M+H]^+$ = 299,1659 $[M+Na]^+$ = 321,1478 $[M-H_2O+H]^+$ = 281,1553 $[M-H]^-$ = 297,1502	$[M+H]^+$ = 299,08 $[M+Na]^+$ = 321,07 $[M-H_2O+H]^+$ = 281,06	$[M+H]^+$ = 299,1642 $[M+Na]^+$ = 321,1457 $[M-H_2O+H]^+$ = 281,1537	8.4 / 8.9
16	C15H25FO3	nd	$[M+H]^+$ = 273.1866 $[M+Na]^+$ = 295.1686 $[M-H_2O+H]^+$ =255.1761 $[M_2+Na]^+$ = 567.3474	nd	$[M+H]^+$ =273.1859 $[M+Na]^+$ = 295.1677 $[M-H_2O+H]^+$ =255.1754 $[M_2+Na]^+$ = 567.3462	7.0
18	C19H31FO5	H1 = 0.024 ± 0.016 H1.1 = 0.17 ± 0.06	$[M+H]^+$ = 315,1972 $[M+Na]^+$ = 337,1791 $[M-H_2O+H]^+$ = 297,1866 $[M-H]^-$ = 313,1815	nd	[M+H]+ = 315.1961 $[M+Na]^+$ = 337.1781 $[M-H_2O+H]^+$ = 297.1857	7.8
19	C25H42FNO6	nd	[M+H]+ = 472.3075 [M+Na]+ = 494.2894	nd	[M+H]+ = 472.3086 [M+Na]+ = 494.2877	4.6
20	C25H42FNO7	nd	[M+H]+ = 488.3024	nd	[M+H]+ = 488.2997	3.0
22	C20H31FO5	nd	[M+H]+ = 371.2234 [M+Na]+ = 393.2054 [M-H2O+H]+ = 353.2128	nd	[M+H]+ = 371.2225 [M+Na]+ = 393.2041 [M-H2O+H]+ = 353.2117	9.4
23	C20H33FO5	H1 = 0.16 ± 0.03	[M+H]+ = 373.2391 [M+Na]+ = 395.2210 [M-H2O+H]+ = 355.2285	nd	[M+H]+ = 373.2384 [M+Na]+ = 395.2201 [M-H2O+H]+ = 355.2275	8.0
S7	C16H27FO3	nd	[M+H]+ = 287,2023 [M+Na]+ = 309,1842 [M-H2O+H]+ = 269,1917 $[M_2+Na]$+ = 595,3787	nd	[M+H]+ = 287.2017 [M+Na]+ = 309.1836 [M-H2O+H]+ =269.1913 $[M_2+Na]$+ = 595.3779	7.0

Supplementary Table 3: Overview about substrates and constructs used in this study.

Substrate	MM-CoA	Mal-CoA	F-Mal-CoA	F-MM-CoA
NDK-SNAC	WT, H1, H2	WT, H1	WT, H1	-
Pentaketide-SPh	WT, H1	WT, H1	WT, H1	H1, H1.1
Hexaketide-SPh	-	-	-	H1

All constructs (**WT**, **H1**, **H2)** were used to generate the C2-methylated TKLs from NDK-SNAC and MM-CoA. Based on lower turnover rates achieved with **H2** and significantly compromised protein quality, we terminated efforts with this hybrid construct. Consequently, **WT** and **H1** were tested for C2-demethylated and C2-fluorinated TKL production from NDK-SNAC and Mal-CoA or F-Mal-CoA. Data are presented in Fig. 2b and 2c. **WT** and **H1** were also tested for the production of 12-membered macrolactones using the pentaketide starter substrate and MM-CoA, Mal-CoA or F-Mal-CoA as extender unit. Data are presented in Fig. 3. F-MM-CoA as extender substrate in combination with the pentaketide or hexaketide substrate was only tested with **H1**. To improve yields of compound **18**, produced with the pentaketide and F-MM-CoA, we used **H1.1** inspired by a recent report by Koch *et al.* [3], hybrid construct **H1.1** harbors the PIKS TE instead of the DEBS TE for improved macrolactonization. All reactions described above were tested in the presence and absence of the NADPH cofactor. Proteins colored in red did not produce any product with the used substrates.

Supplementary Table 4: Used plasmids

Construct	Name	Ref.
KS^{C161G}-MAT (WT)	pAR70_StrepI_m(KS(C161G)_MAT)_H8_pET22b	1
FAS ACP	pAR352_StrepII_mACP_H8_RBS_SFP_pET22b	1
DEBS ACP6	pMJD094_DEBS-M6-H8-ACP_W pET28a	This study
Sfp	pAR357_SFP_pCDF-1b	1
Npt	pMJD091_Sppt_pCDF	This study
DEBS $KS6^{C1661G}$-AT6	pAR432_DD2_KS6_C1661G_AT6_H6_pET	This study
DEBS M6+TE	pBL18_DEBS_M6_TE	Khosla Lab
FAS	pAR264_StrepI_Not1_mFASm_H8_pET22b	1
H2	pMJD076_DEBS_M6_TE_mfASm_LDAT	This study
H1	pMJD077_DEBS_M6_TE_mfASm_AT	This study
H1.1	pMSR001_ DEBS_M6_mFASm_AT_ PIKS_ACP-TE linker_ piks_TE	This study
R606A	pAR71_StrepI_m(KS(C161G)_ATmut(R(606A))_H8_pET22b	1
M499Q F553Q	pAR72_StrepI_m(KS(C161G)_MT(M499Q_F553Q)_H8_pET22b	This study
K673T	pAR91_StrepI_m(KS_K673T_MAT)_H8_pET22b	This study
R606E	pAR98_StrepI_m(KS(C161G)_ATmut(R(606E))_H8_pET22b	This study
MTeGate	pAR99_StrepI_m(KS(C161G)_MTeGate)_H8_pET22b	This study
F682S	pAR106_StrepI_m(KS(C161G)_MT(F682S))_H8_pET22b	This study
R606I	pAR109_StrepI_m(KS(C161G)_ATmut(R606I)_H8_pET22b	This study
N738Q	pAR122_StrepI_m(KS(C161G)_MT(N738Q))_H8_pET22b	This study
M499Q	pAR123_StrepI_m(KS(C161G)_MT(M499Q))_H8_pET22b	This study
F553Q	pAR124_StrepI_m(KS(C161G)_MT(F553Q))_H8_pET22b	This study
M499G	pAR134_StrepI_m(KS(C161G)_MT(M499G))_H8_pET22b	This study
L739G	pAR135_StrepI_m(KS(C161G)_MT(L739G))_H8_pET22b	This study
R606A L739W	pAR136_StrepI_m(KS(C161G)_ATmut(R606A_L739W))_H8_pET22b	This study
R606A L739G	pAR136B_StrepI_m(KS(C161G)_ATmut(R606A_L739G))_H8_pET22b	This study

V585I L739G	pAR137_StrepI_m(KS(C161G)_MT(V585I_L739G))_H8_pET22b	This study
M499A	pAR139_StrepI_m(KS(C161G)_MT(M499A))_H8_pET22b	This study
M499V	pAR140_StrepI_m(KS(C161G)_MT(M499V))_H8_pET22b	This study
F553G	pAR141_StrepI_m(KS(C161G)_MT(F553G))_H8_pET22b	This study
F553A	pAR142_StrepI_m(KS(C161G)_MT(F553A))_H8_pET22b	This study
F553D	pAR143_StrepI_m(KS(C161G)_MT(F553D))_H8_pET22b	This study
F553H	pAR144_StrepI_m(KS(C161G)_MT(F553H))_H8_pET22b	This study
F553N	pAR145_StrepI_m(KS(C161G)_MT(F553N))_H8_pET22b	This study
F553Y	pAR146_StrepI_m(KS(C161G)_MT(F553Y))_H8_pET22b	This study
F682S L680V	pAR147_StrepI_m(KS(C161G)_MT(L680Y_F682S))_H8_pET22b	This study
L680M	pAR148_StrepI_m(KS(C161G)_MT(L680M)_H8_pET22b	This study
L680V	pAR149_StrepI_m(KS(C161G)_MT(L680V)_H8_pET22b	This study
L680H	pAR150_StrepI_m(KS(C161G)_MT(L680H)_H8_pET22b	This study
S581C	pAR153_StrepI_m(KS(C161G)_MAT(S581C)_H8_pET22b	This study
S581A	pAR159_StrepI_m(KS_MAT(S581A))_H8_pET22b	1
H683N	pAR234_StrepI_m(KS(C161G)_MAT(H683N))_H8_pET22b	This study
L680I	pAR344_StrepI_m(KS(C161G)_MT(L680I)_H8_pET22b	This study
L680I A681P	pAR345_StrepI_m(KS(C161G)_MT(L680I)_(A681P)_H8_pET22b	This study
L582A	pAR347_StrepI_m(KS(C161G)_MT(L582A))_H8_pET22b	This study
L582G	pAR348_StrepI_m(KS(C161G)_MT(L582G))_H8_pET22b	This study
L582M	pAR349_StrepI_m(KS(C161G)_MT(L582M))_H8_pET22b	This study
L582Q	pAR351_StrepI_m(KS(C161G)_MT(L582Q))_H8_pET22b	This study
K673AcK	pDH01_StrepI_m(KS(C161G)_MAT(K673TAG))_H8_pET22b	This study
F682A	pDH02_StrepI_m(KS(C161G)_MAT(F682A))_H8_pET22b	This study
F682G	pDH03_StrepI_m(KS(C161G)_MAT(F682G))_H8_pET22b	This study
F682V	pDH04_StrepI_m(KS(C161G)_MAT(F682V))_H8_pET22b	This study

F553H R606A	pDH05_StrepI_m(KS(C161G)_ATmut(R606A)_(F553H))_H8_pET22b	This study
F682S L680V	pDH06_StrepI_m(KS(C161G)_MT(L680V)_(F682S)_H8_pET22b	This study
PMJD106	pMJD106_ DEBS_M6_mFASm_AT_DEBS_ACP-TE linker_piks_TE	This study

Supplementary Table 5: Primers

Nr.	Name	Sequence (5'3')	Template
AR26	AMP_infusion_for	GAG GAC CGA AGG AGC TAA CC	pBL18
AR27	AMP_infusion_rev	GGT TAG CTC CTT CGG TCC TC	pBL18
AR719	LE_H4_for	CTCGAGCACCACCACCAC	pBL18
AR720	DEBS_AT6_H4_rev	GTGGTGGTGCTCGAGGCTGT CGGCGAGCTG	pBL18
AR721	DEBS_AT6_C1661G_for	CACGGTGGACACGGCGGGC TCGTCGTCGTTGGTGG	pBL18
AR722	DEBS_AT6_C1661G_rev	CGCCGTGTCCACCG	pBL18
MJD087	mFASm_LDAT_fwd	acacggcaggccc	pAR264
MJD088	mFASm_LDAT_rev	aggagtccctcgggg	pAR264
MJD101	DEBS_LDAT_fwd	ccccgagggactcctCTGCCCAACT ACCCGTTCGAG	pBL18
MJD102	DEBS_LDAT_rev	caggggcctgccgtgtCGGGGGCTC GGCGAT	pBL18
MJD091	mFASm_AT_fwd	aacaagcgcccactctg	pAR264
MJD092	mFASm_AT_rev	tgtgaggtgcaccttgc	pAR264
MJD105	DEBS_AT_fwd	caaggtgcacctcacaGGCGTGGCC GTGGAC	pBL18
MJD106	DEBS_AT_rev	gagtgggcgcttgttGGAGGCGGTTC CGGTG	pBL18
MJD136	pCDFBB_fwd	TAATTAACCTAGGCTGCTGC CAC	pAR357
MJD137	pCDFBB_rev	GGTATATCTCCTTATTAAAG TTAAACAAAATTATTTC	pAR357
MJD138	Sppt_fwd	ATAAGGAGATATACCATGAT TGAGAAGTTACTCC	pET21a_Sppt
MJD139	Sppt_rev	AGCCTAGGTTAAttaCTCGAG TGCGGCCG	pET21a_Sppt
MJD145	ACP6_W_for	CCGCGCGGCAGCCATATGTG GGCGGCCCCG	pBL18
MJD146	ACP_W_rev	ACGGAGCTCGAATTCAGAGC TGCTGTCCTATGTGGTCG	pBL18
MJD164	DEBS_M6_fwd	GAGGGGGCGGGCAAGAGCT CCGTCGACAAGC	pMJD077
MJD175	DEBS_M6_rev	GGTGTCGGCCCCGGAGAGCT GCTGTCCTATGTGGTC	pMJD077
MJD177	PIKS_TE_fwd	TCCGGGGCCGACACCGGC	DSM41110
MJD178	PIKS_TE_rev	CTTGCCCGCCCCCTCGATGC C	DSM41110
AR168	mMAT(M499Q)_for	gttcatctgctcagggCAGggcacgcagtg gcgc	pAR70
AR169	mMAT(M499Q)_rev	gcgccactgcgtgccCTGccctgagcagat gaac	pAR70
AR170	mMAT(F553Q)_for	gatgacatcgtgcatgccCAGgtgagcctc actgcc	pAR70

AR171	mMAT(F553Q)_rev	ggcagtgaggctcacCTGggcatgcacgatgtcatc	pAR70
AR207	mMAT(K673T)_for	AGCTCGAGCGCCGCTGC	pAR70
AR208	mMAT(K673T)_rev	cgtacctcGGTggcaaacacaccttcttgcttttag	pAR70
AR223	mMat_R606E_for	ctgtgcttgcagcttactggGAAggccagtgcatcaaag	pAR70
AR224	mMat_R606E_rev	ctttgatgcactggccTTCccagtaagctgcaagcacag	pAR70
AR225	mMT_eGate_for	GGGCAAAGTGCTTACTGGCCTGACGaagcgaggcgtgaagtccag	pAR70
AR226	mMT_eGate_rev	AGTAAGCACTTTGCCCGGGCCGACctccagcaccacggcatgc	pAR70
AR238	mMAT_F682S_for	gaacaggaggcctggctTCCcactcctacttcatg	pAR70
AR239	mMAT_F682S_rev	catgaagtaggagtgGGAagccaggcctcctgttc	pAR70
AR240	mMAT_N738Q_for	ccgagtacaatgtcaacCAGctggtgagccctgtgc	pAR70
AR241	mMAT_N738Q_rev	gcacagggctcaccagCTGgttgacattgtactcgg	pAR70
AR244	mMAT_R606I_for	ctgtgcttgcagcttactggATTggccagtgcatcaaag	pAR70
AR245	mMAT_R606I_rev	ctttgatgcactggccAATccagtaagctgcaagcacag	pAR70
AR282	mMAT_M499G_for	ctcagggGGTggcacgcagtggcgcg	pAR70
AR283	mMAT_M499G_rev	gtgccACCccctgagcagatgaaccagag	pAR70
AR284	mMAT_L739G/W_for	tacaatgtcaacaacKGGgtgagccctgtgctcttcc	pAR70
AR285	mMAT_L739G/W_rev	gttgttgacattgtactcggcag	pAR70
AR286	mMAT_V585I_for	gagagATTgcctgtggctatgcagatgg	pAR70
AR287	mMAT_V585I_rev	cacaggcAATctctcccaaggagtgccc	pAR70
AR288	mMAT_M499A/V_for	ggttcatctgctcagggGYGggcacgcagtggcgcg	pAR70
AR289	mMAT_M499X_rev	ccctgagcagatgaaccagag	pAR70
AR290	mMAT_F553D/H/N/Y_for	gacatcgtgcatgccNACgtgagcctcactgccatcc	pAR70
AR291	mMAT_F553X_rev	ggcatgcacgatgtcatcaaagg	pAR70
AR292	mMAT_F553A/G_for	gacatcgtgcatgccGSCgtgagcctcactgccatcc	pAR70
AR293	mMAT_L680Y_S_for	gtacgaacaggaggcTATgctTCCcactcctacttcatg	pAR70
AR294	mMAT_L680X_rev	gcctcctgttcgtacctcc	pAR70
AR295	mMAT_L680M/V_for	gtacgaacaggaggcRTGgctttccactcctacttcatgg	pAR70
AR296	mMAT_L680H_for	gtacgaacaggaggcCACgctttccactcctacttcatgg	pAR70
AR300	mMAT_S581C_for	ggcatcattgggcactGcttgggagaggttgcctgtgg	pAR70
AR301	mMAT_S581X_rev	gtgcccaatgatgccgtcag	pAR70

AR310	mMAT_S581A_for	ggcatcattgggcacGccttgggagaggttg cctgtgg	pAR70
AR351	mMAT_H683X_rev	gaaagccaggcctcctgttc	pAR70
AR421	mMAT_H683N/D_for	ggaggcctggctttcRACtcctacttcatgg aaggaattgcc	pAR70
AR567	mMAT_L680I_for	gtacgaacaggaggcATTgctttccactcct acttcatgg	pAR70
AR568	mMAT_L680I_A681P_for	gtacgaacaggaggcATTCCGttccact cctacttcatggaagg	pAR70
AR569	mMAT_L582X_rev	ggagtgcccaatgatgccg	pAR70
AR570	mMAT_L582G_A_V_for	catcattgggcactccGBCggagaggttgc ctgtggc	pAR70
AR571	mMAT_L582M_T_for	catcattgggcactccAYGggagaggttgc ctgtggc	pAR70
AR572	mMAT_L582Q_for	catcattgggcactccCAGggagaggttgc ctgtggc	pAR70
DH01	mMAT_K673TAG_for	caagaaggtgtgtttgccTAGgaggtacga acaggaggcc	pAR70
DH02	mMAT_K673X_rev	ggcaaacacaccttcttgctttag	pAR70
DH03	mMAT_F682A_V_G_for	caggaggcctggctGBCcactcctacttcat ggaaggaattg	pAR70
DH04	mMAT_F682X_rev	agccaggcctcctgttcg	pAR70
DH05	mMAT_F553H_for	gacatcgtgcatgccCACgtgagcctcact gccatcc	pAR70
DH06	mMAT_L680V_F682S_for	gtacgaacaggaggcGTGgctAGCcact cctacttcatggaagg	pAR70

Supplementary Table 6: DNA Sequences

Construct	DNA Sequences
DEBS ACP6 (pMJD094)	ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATAT GTGGGCGGCCCCGGCGCGGGAGATGACGTCGCAGGAGTTGCTGGAGTTCACGCACTCGCAC GTCGCGGCGATCCTCGGGCATTCCAGCCCGGACGCGGTCGGGCAGGACCAGCCGTTCACCG AGCTCGGCTTCGACTCGCTGACCGCGGTCGGGCTGCGCAACCAGCTCCAGCAGGCCACCGG GCTCGCGCTGCCCGCGACCCTGGTGTTCGAGCACCCCACGGTCCGCAGGTTGGCCGACCACA TAGGACAGCAGCTCTGA
Npt (pMJD091)	ATGATTGAGAAGTTACTCCCGGCGCCAGTCAGAACGGCAGAGACTTTCGACGATGCGCCTTT ATCTGAAATGTTCCCCGAAGAGTGGGCGCAGGTTGCAAACGCTGTACCCAAACGCCAACGT GAGTTCGGTACTGTACGAGGGTGCGCTCGTCGTGCCCTGGCCGAGCTTGGCTTCGCTCCGGC ACCATTGCTGCCTGGACCTCATCGTGAGCCGCAGTGGCCAGATGGGGTTGTGGGCGCGATGA CGCACTGCGCGGGATATCGCGCTGTAGCGGTGGCACGCGCCGGCGAAGTTCGCACAATCGG CCTGGATGCCGAACCGAATCTCCCACTAAATGACCCGGGCGTTCTTGACCTGGTGACATTAC CGGAAGAACGGGACCAGATCCGGCGCCTCGCCGCCCTTCAACCGGAAGTCTGTTGGGATCG CTTGGTCTTTTCCGCAAAAGAAAGTGTCTACAAAGCCTGGTTTCCGCTGACGCGCCGTTGGTT GGATTTTGAAGAAGCACTGCTGACCTTTGATCCGACCAACGCGACCTTTACCGCGCAGCTGC TGGTGCCGGGCCCGGTGGTTGATGGTCGTGAACTGACCGAATTTTCGGGTCGTTGGCTGGTG GGTAGCGGTCTGGTCGTTACCGCGATTGTGGAAATGGTGTCAAAGCTTGCGGCCGCACTCGA Gtaa
DEBS KS6^{C1661G}-AT6 pAR432	ATGGCTAGCACTGACAGCGAGAAGGTGGCGGAGTACCTCCGTCGGGCGACGCTCGACCTGC GTGCCGCCCGGCAGCGCATCCGCGAGCTGGAATCCGACCCGATCGCGATCGTCGGCATGGC CTGCCGCTTCCCCGGCGGCGTGCACAACCCCGGTGAGCTGTGGGAGTTCATCGTCGGCGGCG GAGACGCCGTGACGGAGATGCCCACCGACCGCGGCTGGGACCTCGACGCGCTGTTCGACCC CGACCCGCAGCGCCACGGAACCAGCTACTCGCGACACGGCGCGTTCCTCGACGGGGCCGCC GACTTCGACGCGGCGTTCTTCGGGATCTCGCCGCGCGAGGCGCTGGCGATGGACCCGCAGCA GCGCCAGGTCCTGGAAACGACGTGGGAGCTGTTCGAGAACGCCGGCATCGACCCGCACTCG CTGCGGGGCAGCGACACCGGCGTCTTCCTCGGCGCCGCGTACCAGGGCTACGGCCAGGACG CGGTGGTGCCCGAGGACAGCGAGGGCTACCTGCTCACCGGCAACTCCTCCGCCGTGGTGTCC GGCCGGGTCGCCTACGTGCTGGGGCTGGAAGGCCCCGCGGTCACGGTGGACACGGCGGGCT CGTCGTCGTTGGTGGCCTTGCATTCGGCGTGTGGGTCGTTGCGTGACGGTGACTGCGGTCTTG CGGTGGCCGGTGGTGTGTCGGTGATGGCGGGCCCGGAGGTGTTCACCGAGTTCTCCCGCCAG GGCGGCTTGGCCGTGGACGGGCGCTGCAAGGCGTTCTCCGCGGAGGCCGACGGCTTCGGTTT CGCCGAGGGCGTCGCGGTGGTCCTGCTCCAGCGGTTGTCCGACGCCCGCAGGGCGGGTCGCC AGGTGCTCGGCGTGGTCGCGGGCTCGGCGATCAACCAGGACGGCGCGAGCAACGGTCTCGC GGCGCCGAGCGGCGTCGCCCAGCAGCGCGTGATCCGCAAGGCGTGGGCGCGTGCGGGGATC ACGGGCGCGGATGTGGCCGTGGTGGAGGCGCATGGGACCGGTACGCGGCTGGGCGATCCGG TGGAGGCGTCGGCGTTGCTGGCTACTTACGGCAAGTCGCGCGGGTCGTCGGGCCCGGTGCTG CTGGGTTCGGTGAAGTCGAACATCGGTCACGCGCAGGCGGCCGCGGGTGTCGCGGGCGTGA TCAAGGTGGTCCTGGGGTTGAACCGCGGCCTGGTGCCGCCGATGCTCTGCCGCGGCGAGCGG TCGCCGCTGATCGAATGGTCCTCGGGTGGTGTGGAACTTGCCGAGGCCGTGAGCCCGTGGCC TCCGGCCGCGGACGGGGTGCGCCGGGCCGGTGTGTCGGCGTTCGGGGTGAGCGGGACGAAC GCGCACGTGATCATCGCCGAGCCCCCGGAGCCCGAGCCGCTGCCGGAACCCGGACCGGTGG GCGTGCTGGCCGCTGCGAACTCGGTGCCCGTACTGCTGTCGGCCAGGACCGAGACCGCGTTG GCAGCGCAGGCGCGGCTCCTGGAGTCCGCAGTGGACGACTCGGTTCCGTTGACGGCATTGGC TTCCGCGCTGGCCACCGGACGCGCCCACCTGCCGCGTCGTGCGGCGTTGCTGGCAGGCGACC ACGAACAGCTCCGCGGGCAGTTGCGAGCGGTCGCCGAGGGCGTTGCGGCTCCCGGTGCCAC CACCGGAACCGCCTCCGCCGGCGGCGTGGTTTTCGTCTTCCCAGGTCAGGGTGCTCAGTGGG AGGGCATGGCCCGGGGCTTGCTCTCGGTCCCCGTCTTCGCCGAGTCGATCGCCGAGTGCGAT GCGGTGTTGTCGGAGGTGGCCGGGTTCTCGGCCTCCGAAGTGCTGGAGCAGCGTCCGGACGC GCCGTCGCTGGAGCGGGTCGACGTCGTACAGCCGGTGTTGTTCTCCGTGATGGTGTCGCTGG CGCGGCTGTGGGGCGCTTGCGGAGTCAGCCCCTCGGCCGTCATCGGCCATTCGCAGGGCGAG ATCGCCGCCGCGGTGGTGGCCGGGGTGTTGTCGCTGGAGGACGGCGTGCGCGTCGTGGCCCT GCGCGCGAAGGCGTTGCGTGCGCTGGCGGGCAAGGGCGGCATGGTCTCGTTGGCGGCTCCC GGTGAACGCGCCCGCGCGCTGATCGCACCGTGGGAGGACCGGATCTCCGTCGCGGCGGTCA ACTCCCCGTCCTCGGTCGTGGTCTCCGGCGATCCGGAGGCGCTGGCCGAACTCGTCGCACGT TGCGAGGACGAGGGCGTGCGCGCCAAGACGCTCCCGGTGGACTACGCCTCGCACTCCCGCC ACGTCGAGGAGATCCGCGAGACGATCCTCGCCGACCTCGACGGCATCTCCGCGCGGCGTGC CGCCATCCCGCTCTACTCCACGCTGCACGGCGAACGGCGCGACGGCGCCGACATGGGTCCGC GGTACTGGTACGACAACCTGCGCTCCCAGGTGCGCTTCGACGAGGCGGTCTCGGCCGCCGTC GCCGACGGTCACGCCACCTTCGTCGAGATGAGCCCGCACCCGGTGCTCACCGCGGCGGTGCA GGAGATCGCCGCGGACGCCGTGGCCATCGGGTCGCTGCACCGCGACACCGCGGAGGAGCAC CTGATCGCCGAGCTCGCCCGGGCGCACGTGCACGGCGTGGCCGTGGACTGGCGGAACGTCTT CCCGGCGGCACCTCCGGTGGCGCTGCCCAACTACCCGTTCGAGCCCCAGCGGTACTGGCTCG CGCCGGAGGTGTCCGACCAGCTCGCCGACAGCCTCGAGCACCACCACCACCACCACTGA
DEBS M6+TE (pBL18)	ATGGCTAGCACTGACAGCGAGAAGGTGGCGGAGTACCTCCGTCGGGCGACGCTCGACCTGC GTGCCGCCCGGCAGCGCATCCGCGAGCTGGAATCCGACCCGATCGCGATCGTCGGCATGGC CTGCCGCTTCCCCGGCGGCGTGCACAACCCCGGTGAGCTGTGGGAGTTCATCGTCGGCGGCG

	GAGACGCCGTGACGGAGATGCCCACCGACCGCGGCTGGGACCTCGACGCGCTGTTCGACCC CGACCCGCAGCGCCACGGAACCAGCTACTCGCGACACGGCGCGTTCCTCGACGGGGCCGCC GACTTCGACGCGGCGTTCTTCGGGATCTCGCCGCGCGAGGCGCTGGCGATGGACCCGCAGCA GCGCCAGGTCCTGGAAACGACGTGGGAGCTGTTCGAGAACGCCGGCATCGACCCGCACTCG CTGCGGGGCAGCGACACCGGCGTCTTCCTCGGCGCCGCGTACCAGGGCTACGGCCAGGACG CGGTGGTGCCCGAGGACAGCGAGGGCTACCTGCTCACCGGCAACTCCTCCGCCGTGGTGTCC GGCCGGGTCGCCTACGTGCTGGGGCTGGAAGGCCCCGCGGTCACGGTGGACACGGCGTGTT CGTCGTCGTTGGTGGCCTTGCATTCGGCGTGTGGGTCGTTGCGTGACGGTGACTGCGGTCTTG CGGTGGCCGGTGGTGTGTCGGTGATGGCGGGCCCGGAGGTGTTCACCGAGTTCTCCCGCCAG GGCGGCTTGGCCGTGGACGGGCGCTGCAAGGCGTTCTCCGCGGAGGCCGACGGCTTCGGTTT CGCCGAGGGCGTCGCGGTGGTCCTGCTCCAGCGGTTGTCCGACGCCCGCAGGGCGGGTCGCC AGGTGCTCGGCGTGGTCGCGGGCTCGGCGATCAACCAGGACGGCGCGAGCAACGGTCTCGC GGCGCCGAGCGGCGTCGCCCAGCAGCGCGTGATCCGCAAGGCGTGGGCGCGTGCGGGGATC ACGGGCGCGGATGTGGCCGTGGTGGAGGCGCATGGGACCGGTACGCGGCTGGGCGATCCGG TGGAGGCGTCGGCGTTGCTGGCTACTTACGGCAAGTCGCGCGGGTCGTCGGGCCCGGTGCTG CTGGGTTCGGTGAAGTCGAACATCGGTCACGCGCAGGCGGCCGCGGGTGTCGCGGGCGTGA TCAAGGTGGTCCTGGGGTTGAACCGCGGCCTGGTGCCGCCGATGCTCTGCCGCGGCGAGCGG TCGCCGCTGATCGAATGGTCCTCGGGTGGTGTGGAACTTGCCGAGGCCGTGAGCCCGTGGCC TCCGGCCGCGGACGGGGTGCGCCGGGCCGGTGTGTCGGCGTTCGGGGTGAGCGGGACGAAC GCGCACGTGATCATCGCCGAGCCCCCGGAGCCCGAGCCGCTGCCGGAACCCGGACCGGTGG GCGTGCTGGCCGCTGCGAACTCGGTGCCCGTACTGCTGTCGGCCAGGACCGAGACCGCGTTG GCAGCGCAGGCGCGGCTCCTGGAGTCCGCAGTGGACGACTCGGTTCCGTTGACGGCATTGGC TTCCGCGCTGGCCACCGGACGCGCCCACCTGCCGCGTCGTGCGGCGTTGCTGGCAGGCGACC ACGAACAGCTCCGCGGGCAGTTGCGAGCGGTCGCCGAGGGCGTTGCGGCTCCCGGTGCCAC CACCGGAACCGCCTCCGCCGGCGGCGTGGTTTTCGTCTTCCCAGGTCAGGGTGCTCAGTGGG AGGGCATGGCCCGGGGCTTGCTCTCGGTCCCCGTCTTCGCCGAGTCGATCGCCGAGTGCGAT GCGGTGTTGTCGGAGGTGGCCGGGTTCTCGGCCTCCGAAGTGCTGGAGCAGCGTCCGGACGC GCCGTCGCTGGAGCGGGTCGACGTCGTACAGCCGGTGTTGTTCTCCGTGATGGTGTCGCTGG CGCGGCTGTGGGGCGCTTGCGGAGTCAGCCCCTCGGCCGTCATCGGCCATTCGCAGGGCGAG ATCGCCGCCGCGGTGGTGGCCGGGGTGTTGTCGCTGGAGGACGGCGTGCGCGTCGTGGCCCT GCGCGCGAAGGCGTTGCGTGCGCTGGCGGGCAAGGGCGGCATGGTCTCGTTGGCGGCTCCC GGTGAACGCGCCCGCGCGCTGATCGCACCGTGGGAGGACCGGATCTCCGTCGCGGCGGTCA ACTCCCCGTCCTCGGTCGTGGTCTCCGGCGATCCGGAGGCGCTGGCCGAACTCGTCGCACGT TGCGAGGACGAGGGCGTGCGCGCCAAGACGCTCCCGGTGGACTACGCCTCGCACTCCCGCC ACGTCGAGGAGATCCGCGAGACGATCCTCGCCGACCTCGACGGCATCTCCGCGCGGCGTGC CGCCATCCCGCTCTACTCCACGCTGCACGGCGAACGGCGCGACGGCGCCGACATGGGTCCGC GGTACTGGTACGACAACCTGCGCTCCCAGGTGCGCTTCGACGAGGCGGTCTCGGCCGCCGTC GCCGACGGTCACGCCACCTTCGTCGAGATGAGCCCGCACCCGGTGCTCACCGCGGCGGTGCA GGAGATCGCCGCGGACGCCGTGGCCATCGGGTCGCTGCACCGCGACACCGCGGAGGAGCAC CTGATCGCCGAGCTCGCCCGGGCGCACGTGCACGGCGTGGCCGTGGACTGGCGGAACGTCTT CCCGGCGGCACCTCCGGTGGCGCTGCCCAACTACCCGTTCGAGCCCCAGCGGTACTGGCTCG CGCCGGAGGTGTCCGACCAGCTCGCCGACAGCCGCTACCGCGTCGACTGGCGACCGCTGGC CACCACGCCGGTGGACCTGGAAGGCGGCTTCCTGGTCCACGGGTCCGCACCGGAGTCGCTG ACCAGCGCAGTCGAGAAGGCCGGAGGCCGCGTCGTGCCGGTCGCCTCGGCCGACCGCGAAG CGCTCGCGGCGGCCCTGCGGGAGGTGCCGGGCGAGGTCGCCGGCGTGCTCTCGGTCCACAC CGGCGCCGCAACGCACCTCGCCCTGCACCAGTCGCTGGGTGAGGCCGGCGTGCGGGCCCCG CTCTGGCTGGTCACCAGCCGAGCGGTCGCGCTCGGGGAGTCCGAGCCGGTCGATCCCGAGC AGGCGATGGTGTGGGGTCTCGGGCGCGTCATGGGCCTGGAGACCCCGGAACGGTGGGGCGG TCTGGTGGACCTGCCCGCCGAACCCGCGCCGGGGGACGGCGAGGCGTTCGTCGCCTGCCTCG GCGCGGACGGCCACGAGGACCAGGTCGCGATCCGTGACCACGCCCGCTACGGCCGCCGCCT CGTCCGCGCCCCGCTGGGCACCCGCGAGTCGAGCTGGGAGCCGGCGGGCACGGCGCTGGTC ACCGGCGGCACCGGTGCGCTCGGCGGCCACGTCGCCCGCCACCTCGCCAGGTGCGGGGTGG AGGACCTGGTGCTGGTCAGCAGGCGCGGCGTCGACGCTCCCGGCGCGGCCGAGCTGGAAGC CGAACTGGTCGCCCTCGGCGCGAAGACGACCATCACCGCCTGCGACGTGGCCGACCGCGAG CAGCTCTCCAAGCTGCTGGAAGAACTGCGCGGGCAGGGACGTCCGGTGCGGACCGTCGTGC ACACCGCCGGGGTGCCCGAATCGAGGCCGCTGCACGAGATCGGCGAGCTGGAGTCGGTCTG CGCGGCGAAGGTGACCGGGGCCCGGCTGCTCGACGAGCTGTGCCCGGACGCCGAGACCTTC GTCCTGTTCTCGTCCGGAGCGGGGGTGTGGGGCAGTGCGAACCTCGGCGCCTACTCCGCGGC CAACGCCTACCTCGACGCGCTGGCCCACCGCCGCCGTGCGGAAGGCCGTGCGGCGACGTCC GTCGCGTGGGGCGCCTGGGCGGGCGAGGGCATGGCCACCGGCGACCTCGAGGGGCTCACCC GGCGCGGCCTGCGCCCGATGGCGCCCGAGCGCGCGATCCGCGCGCTGCACCAGGCGCTGGA CAACGGCGACACGTGCGTTTCGATCGCCGACGTCGACTGGGAGCGCTTCGCGGTCGGCTTCA CCGCCGCCCGGCCGCGTCCGCTGCTGGACGAGCTCGTCACGCCGGCGGTGGGGGCCGTCCCC GCGGTGCAGGCGGCCCCGGCGCGGGAGATGACGTCGCAGGAGTTGCTGGAGTTCACGCACT CGCACGTCGCGGCGATCCTCGGGCATTCCAGCCCGGACGCGGTCGGGCAGGACCAGCCGTT CACCGAGCTCGGCTTCGACTCGCTGACCGCGGTCGGGCTGCGCAACCAGCTCCAGCAGGCCA CCGGGCTCGCGCTGCCCGCGACCCTGGTGTTCGAGCACCCCACGGTCCGCAGGTTGGCCGAC CACATAGGACAGCAGCTCGACAGCGGGACTCCCGCCCGGGAAGCGAGCAGCGCTCTTCGCG ACGGCTACCGGCAGGCGGGCGTGTCGGGCAGGGTCCGGTCCTACCTCGACCTGCTGGCGGG GCTGTCGGACTTCCGCGAGCACTTCGACGGCTCCGACGGGTTCTCCCTCGATCTCGTGGACA

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H2 (pMJD076)	ATGGCTAGCACTGACAGCGAGAAGGTGGCGGAGTACCTCCGTCGGGCGACGCTCGACCTGC GTGCCGCCCGGCAGCGCATCCGCGAGCTGGAATCCGACCCGATCGCGATCGTCGGCATGGC CTGCCGCTTCCCCGGCGGCGTGCACAACCCCGGTGAGCTGTGGGAGTTCATCGTCGGCGGCG GAGACGCCGTGACGGAGATGCCCACCGACCGCGGCTGGGACCTCGACGCGCTGTTCGACCC CGACCCGCAGCGCCACGGAACCAGCTACTCGCGACACGGCGCGTTCCTCGACGGGGCCGCC GACTTCGACGCGGCGTTCTTCGGGATCTCGCCGCGCGAGGCGCTGGCGATGGACCCGCAGCA GCGCCAGGTCCTGGAAACGACGTGGGAGCTGTTCGAGAACGCCGGCATCGACCCGCACTCG CTGCGGGGCAGCGACACCGGCGTCTTCCTCGGCGCCGCGTACCAGGGCTACGGCCAGGACG CGGTGGTGCCCGAGGACAGCGAGGGCTACCTGCTCACCGGCAACTCCTCCGCCGTGGTGTCC GGCCGGGTCGCCTACGTGCTGGGGCTGGAAGGCCCCGCGGTCACGGTGGACACGGCGTGTT CGTCGTCGTTGGTGGCCTTGCATTCGGCGTGTGGGTCGTTGCGTGACGGTGACTGCGGTCTTG CGGTGGCCGGTGGTGTGTCGGTGATGGCGGGCCCGGAGGTGTTCACCGAGTTCTCCCGCCAG GGCGGCTTGGCCGTGGACGGGCGCTGCAAGGCGTTCTCCGCGGAGGCCGACGGCTTCGGTTT CGCCGAGGGCGTCGCGGTGGTCCTGCTCCAGCGGTTGTCCGACGCCCGCAGGGCGGGTCGCC AGGTGCTCGGCGTGGTCGCGGGCTCGGCGATCAACCAGGACGGCGCGAGCAACGGTCTCGC GGCGCCGAGCGGCGTCGCCCAGCAGCGCGTGATCCGCAAGGCGTGGGCGCGTGCGGGGATC ACGGGCGCGGATGTGGCCGTGGTGGAGGCGCATGGGACCGGTACGCGGCTGGGCGATCCGG TGGAGGCGTCGGCGTTGCTGGCTACTTACGGCAAGTCGCGCGGGTCGTCGGGCCCGGTGCTG CTGGGTTCGGTGAAGTCGAACATCGGTCACGCGCAGGCGGCCGCGGGTGTCGCGGGCGTGA TCAAGGTGGTCCTGGGGTTGAACCGCGGCCTGGTGCCGCCGATGCTCTGCCGCGGCGAGCGG TCGCCGCTGATCGAATGGTCCTCGGGTGGTGTGGAACTTGCCGAGGCCGTGAGCCCGTGGCC TCCGGCCGCGGACGGGGTGCGCCGGGCCGGTGTGTCGGCGTTCGGGGTGAGCGGGACGAAC GCGCACGTGATCATCGCCGAGCCCCCGacacggcaggcccctgcgcccactgcacacgctgcccttcccctttgctgcacgc cagtggacgcaccttagaggcagtgcaggacctgctggaacagggccgccagcacagccaggacctggcctttgtgagcatgctcaatgacattgcg gcaaccctacagcagccatgcccttcaggggttacactgtgctaggtgttgagggccgtgtccaagaagtgcagcaagtgtccaccaacaagcgccc actctggttcatctgctcagggatgggcacgcagtggcgcgggatggggctgagcctcatgcgcctggacagcttccgtgagtctatcctgcgctccgat gaggctgtgaagccgttgggagtgaaagtgtcagatctgctgttgagcacagatgagcgcacctttgatgacatcgtgcatgcctttgtgagcctcactgc catccagattgccctcatcgacctactgacttctgtgggactgaaacctgacggcatcattgggcactccttgggagaggttgcctgtggctatgcagatg gctgtctctcccagagagaggctgtgcttgcagcttactggcgaggccagtgcatcaaagatgcccacctcccgcctggatccatggcagctgttggtttg tcctgggaggaatgtaaacagcgctgccccgctggcgtggtgcctgcctgccacaactctgaggacaccgtgaccatctctggacctcaggctgcagtg aatgaatttgtggagcagctaaagcaagaaggtgtgtttgccaaggaggtacgaacaggaggcctggctttccactcctacttcatggaaggaattgccc ccacattgctgcaggctctcaagaaggtgatccgggaaccacggccgcgctcggctcgatggctcagcacctctatccctgaggcccagtggcagagc agcctggcccgcacatcttctgccgagtacaatgtcaacaacctggtgagccctgtgctcttccaggaagcactgtggcacatccctgagcatgccgtgg tgctggagattgcgccccacgcactgttgcaggctgtcctgaagcgaggcgtgaagtccagctgcaccatcattcccttgatgaagagggatcataaaga taacttggagttctttctcaccaaccttggcaaggtgcacctcacaggcatcaatgtcaaccctaacgccttgttcccacctgtggagttcccggctccccga gggactcctCTGCCCAACTACCCGTTCGAGCCCCAGCGGTACTGGCTCGCGCCGGAGGTGTCCGA CCAGCTCGCCGACAGCCGCTACCGCGTCGACTGGCGACCGCTGGCCACCACGCCGGTGGAC CTGGAAGGCGGCTTCCTGGTCCACGGGTCCGCACCGGAGTCGCTGACCAGCGCAGTCGAGA AGGCCGGAGGCCGCGTCGTGCCGGTCGCCTCGGCCGACCGCGAAGCGCTCGCGGCGGCCCT GCGGGAGGTGCCGGGCGAGGTCGCCGGCGTGCTCTCGGTCCACACCGGCGCCGCAACGCAC CTCGCCCTGCACCAGTCGCTGGGTGAGGCCGGCGTGCGGGCCCCGCTCTGGCTGGTCACCAG CCGAGCGGTCGCGCTCGGGGAGTCCGAGCCGGTCGATCCCGAGCAGGCGATGGTGTGGGGT CTCGGGCGCGTCATGGGCCTGGAGACCCCGGAACGGTGGGGCGGTCTGGTGGACCTGCCCG CCGAACCCGCGCCGGGGGACGGCGAGGCGTTCGTCGCCTGCCTCGGCGCGGACGGCCACGA GGACCAGGTCGCGATCCGTGACCACGCCCGCTACGGCCGCCGCCTCGTCCGCGCCCCGCTGG GCACCCGCGAGTCGAGCTGGGAGCCGGCGGGCACGGCGCTGGTCACCGGCGGCACCGGTGC GCTCGGCGGCCACGTCGCCCGCCACCTCGCCAGGTGCGGGGTGGAGGACCTGGTGCTGGTC AGCAGGCGCGGCGTCGACGCTCCCGGCGCGGCCGAGCTGGAAGCCGAACTGGTCGCCCTCG GCGCGAAGACGACCATCACCGCCTGCGACGTGGCCGACCGCGAGCAGCTCTCCAAGCTGCT GGAAGAACTGCGCGGGCAGGGACGTCCGGTGCGGACCGTCGTGCACACCGCCGGGGTGCCC GAATCGAGGCCGCTGCACGAGATCGGCGAGCTGGAGTCGGTCTGCGCGGCGAAGGTGACCG GGGCCCGGCTGCTCGACGAGCTGTGCCCGGACGCCGAGACCTTCGTCCTGTTCTCGTCCGGA GCGGGGGTGTGGGGCAGTGCGAACCTCGGCGCCTACTCCGCGGCCAACGCCTACCTCGACG CGCTGGCCCACCGCCGCCGTGCGGAAGGCCGTGCGGCGACGTCCGTCGCGTGGGGCGCCTG GGCGGGCGAGGGCATGGCCACCGGCGACCTCGAGGGGCTCACCCGGCGCGGCCTGCGCCCG ATGGCGCCCGAGCGCGCGATCCGCGCGCTGCACCAGGCGCTGGACAACGGCGACACGTGCG TTTCGATCGCCGACGTCGACTGGGAGCGCTTCGCGGTCGGCTTCACCGCCGCCCGGCCGCGT CCGCTGCTGGACGAGCTCGTCACGCCGGCGGTGGGGGCCGTCCCCGCGGTGCAGGCGGCCC

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H1 (pMJD077)	ATGGCTAGCACTGACAGCGAGAAGGTGGCGGAGTACCTCCGTCGGGCGACGCTCGACCTGC GTGCCGCCCGGCAGCGCATCCGCGAGCTGGAATCCGACCCGATCGCGATCGTCGGCATGGC CTGCCGCTTCCCCGGCGGCGTGCACAACCCCGGTGAGCTGTGGGAGTTCATCGTCGGCGGCG GAGACGCCGTGACGGAGATGCCCACCGACCGCGGCTGGGACCTCGACGCGCTGTTCGACCC CGACCCGCAGCGCCACGGAACCAGCTACTCGCGACACGGCGCGTTCCTCGACGGGGCCGCC GACTTCGACGCGGCGTTCTTCGGGATCTCGCCGCGCGAGGCGCTGGCGATGGACCCGCAGCA GCGCCAGGTCCTGGAAACGACGTGGGAGCTGTTCGAGAACGCCGGCATCGACCCGCACTCG CTGCGGGGCAGCGACACCGGCGTCTTCCTCGGCGCCGCGTACCAGGGCTACGGCCAGGACG CGGTGGTGCCCGAGGACAGCGAGGGCTACCTGCTCACCGGCAACTCCTCCGCCGTGGTGTCC GGCCGGGTCGCCTACGTGCTGGGGCTGGAAGGCCCCGCGGTCACGGTGGACACGGCGTGTT CGTCGTCGTTGGTGGCCTTGCATTCGGCGTGTGGGTCGTTGCGTGACGGTGACTGCGGTCTTG CGGTGGCCGGTGGTGTGTCGGTGATGGCGGGCCCGGAGGTGTTCACCGAGTTCTCCCGCCAG GGCGGCTTGGCCGTGGACGGGCGCTGCAAGGCGTTCTCCGCGGAGGCCGACGGCTTCGGTTT CGCCGAGGGCGTCGCGGTGGTCCTGCTCCAGCGGTTGTCCGACGCCCGCAGGGCGGGTCGCC AGGTGCTCGGCGTGGTCGCGGGCTCGGCGATCAACCAGGACGGCGCGAGCAACGGTCTCGC GGCGCCGAGCGGCGTCGCCCAGCAGCGCGTGATCCGCAAGGCGTGGGCGCGTGCGGGGATC ACGGGCGCGGATGTGGCCGTGGTGGAGGCGCATGGGACCGGTACGCGGCTGGGCGATCCGG TGGAGGCGTCGGCGTTGCTGGCTACTTACGGCAAGTCGCGCGGGTCGTCGGGCCCGGTGCTG CTGGGTTCGGTGAAGTCGAACATCGGTCACGCGCAGGCGGCCGCGGGTGTCGCGGGCGTGA TCAAGGTGGTCCTGGGGTTGAACCGCGGCCTGGTGCCGCCGATGCTCTGCCGCGGCGAGCGG TCGCCGCTGATCGAATGGTCCTCGGGTGGTGTGGAACTTGCCGAGGCCGTGAGCCCGTGGCC TCCGGCCGCGGACGGGGTGCGCCGGGCCGGTGTGTCGGCGTTCGGGGTGAGCGGGACGAAC GCGCACGTGATCATCGCCGAGCCCCCGGAGCCCGAGCCGCTGCCGGAACCCGGACCGGTGG GCGTGCTGGCCGCTGCGAACTCGGTGCCCGTACTGCTGTCGGCCAGGACCGAGACCGCGTTG GCAGCGCAGGCGCGGCTCCTGGAGTCCGCAGTGGACGACTCGGTTCCGTTGACGGCATTGGC TTCCGCGCTGGCCACCGGACGCGCCCACCTGCCGCGTCGTGCGGCGTTGCTGGCAGGCGACC ACGAACAGCTCCGCGGGCAGTTGCGAGCGGTCGCCGAGGGCGTTGCGGCTCCCGGTGCCAC CACCGGAACCGCCTCCaacaagcgcccactctggttcatctgctcagggatgggcacgcagtggcgcgggatggggctgagcctcatg cgcctggacagcttccgtgagtctatcctgcgctccgatgaggctgtgaagccgttgggagtgaaagtgtcagatctgctgttgagcacagatgagcgca cctttgatgacatcgtgcatgcctttgtgagcctcactgccatccagattgccctcatcgacctactgacttctgtgggactgaaacctgacggcatcattgg gcactccttgggagaggttgcctgtggctatgcagatggctgtctctcccagagagaggctgtgcttgcagcttactggcgaggccagtgcatcaaagat gcccacctcccgcctggatccatggcagctgttggtttgtcctgggaggaatgtaaacagcgctgccccgctggcgtggtgcctgccacaactctg aggacaccgtgaccatctctggacctcaggctgcagtgaatgaatttgtggagcagctaaagcaagaaggtgtgtttgccaaggaggtacgaacagga ggcctggctttccactcctacttcatggaaggaattgcccccacattgctgcaggctctcaagaaggtgatccgggaaccacggccgcgctcggctcgat ggctcagcacctctatccctgaggcccagtggcagagcagcctggcccgcacatcttctgccgagtacaatgtcaacaacctggtgagccctgtgctctt ccaggaagcactgtggcacatccctgagcatgccgtggtgctggagattgcgccccacgcactgttgcaggctgtcctgaagcgaggcgtgaagtcca gctgcaccatcattcccttgatgaagagggatcataaagataacttggagttctttctcaccaaccttggcaaggtgcacctcacaGGCGTGGCCG TGGACTGGCGGAACGTCTTCCCGGCGGCACCTCCGGTGGCGCTGCCCAACTACCCGTTCGAG CCCCAGCGGTACTGGCTCGCGCCGGAGGTGTCCGACCAGCTCGCCGACAGCCGCTACCGCGT CGACTGGCGACCGCTGGCCACCACGCCGGTGGACCTGGAAGGCGGCTTCCTGGTCCACGGG TCCGCACCGGAGTCGCTGACCAGCGCAGTCGAGAAGGCCGGAGGCCGCGTCGTGCCGGTCG CCTCGGCCGACCGCGAAGCGCTCGCGGCGGCCCTGCGGGAGGTGCCGGGCGAGGTCGCCGG CGTGCTCTCGGTCCACACCGGCGCCGCAACGCACCTCGCCCTGCACCAGTCGCTGGGTGAGG CCGGCGTGCGGGCCCCGCTCTGGCTGGTCACCAGCCGAGCGGTCGCGCTCGGGGAGTCCGA GCCGGTCGATCCCGAGCAGGCGATGGTGTGGGGTCTCGGGCGCGTCATGGGCCTGGAGACC CCGGAACGGTGGGGCGGTCTGGTGGACCTGCCCGCCGAACCCGCGCCGGGGGACGGCGAGG CGTTCGTCGCCTGCCTCGGCGCGGACGGCCACGAGGACCAGGTCGCGATCCGTGACCACGCC CGCTACGGCCGCCGCCTCGTCCGCGCCCCGCTGGGCACCCGCGAGTCGAGCTGGGAGCCGG CGGGCACGGCGCTGGTCACCGGCGGCACCGGTGCGCTCGGCGGCCACGTCGCCCGCCACCT CGCCAGGTGCGGGGTGGAGGACCTGGTGCTGGTCAGCAGGCGCGGCGTCGACGCTCCCGGC GCGGCCGAGCTGGAAGCCGAACTGGTCGCCCTCGGCGCGAAGACGACCATCACCGCCTGCG

	ACGTGGCCGACCGCGAGCAGCTCTCCAAGCTGCTGGAAGAACTGCGCGGGCAGGGACGTCC GGTGCGGACCGTCGTGCACACCGCCGGGGTGCCCGAATCGAGGCCGCTGCACGAGATCGGC GAGCTGGAGTCGGTCTGCGCGGCGAAGGTGACCGGGGCCCGGCTGCTCGACGAGCTGTGCC CGGACGCCGAGACCTTCGTCCTGTTCTCGTCCGGAGCGGGGGTGTGGGGCAGTGCGAACCTC GGCGCCTACTCCGCGGCCAACGCCTACCTCGACGCGCTGGCCCACCGCCGCCGTGCGGAAG GCCGTGCGGCGACGTCCGTCGCGTGGGGCGCCTGGGCGGGCGAGGGCATGGCCACCGGCGA CCTCGAGGGGCTCACCCGGCGCGGCCTGCGCCCGATGGCGCCCGAGCGCGCGATCCGCGCG CTGCACCAGGCGCTGGACAACGGCGACACGTGCGTTTCGATCGCCGACGTCGACTGGGAGC GCTTCGCGGTCGGCTTCACCGCCGCCCGGCCGCGTCCGCTGCTGGACGAGCTCGTCACGCCG GCGGTGGGGGCCGTCCCCGCGGTGCAGGCGGCCCCGGCGCGGGAGATGACGTCGCAGGAGT TGCTGGAGTTCACGCACTCGCACGTCGCGGCGATCCTCGGGCATTCCAGCCCGGACGCGGTC GGGCAGGACCAGCCGTTCACCGAGCTCGGCTTCGACTCGCTGACCGCGGTCGGGCTGCGCA ACCAGCTCCAGCAGGCCACCGGGCTCGCGCTGCCCGCGACCCTGGTGTTCGAGCACCCCACG GTCCGCAGGTTGGCCGACCACATAGGACAGCAGCTCGACAGCGGGACTCCCGCCCGGGAAG CGAGCAGCGCTCTTCGCGACGGCTACCGGCAGGCGGGCGTGTCGGGCAGGGTCCGGTCCTA CCTCGACCTGCTGGCGGGGCTGTCGGACTTCCGCGAGCACTTCGACGGCTCCGACGGGTTCT CCCTCGATCTCGTGGACATGGCCGACGGTCCCGGAGAGGTCACGGTGATCTGCTGCGCGGGA ACGGCGGCGATCTCCGGTCCGCACGAGTTCACCCGGCTCGCCGGGGCGCTGCGCGGAATCG CTCCGGTTCGGGCCGTGCCCCAGCCCGGCTACGAGGAGGGCGAACCTCTGCCGTCGTCGATG GCGGCGGTGGCGGCGGTGCAGGCCGATGCGGTCATCAGGACACAGGGGGACAAGCCGTTCG TGGTGGCCGGTCACTCCGCGGGGGCACTGATGGCCTACGCGCTGGCGACCGAACTGCTCGAT CGCGGGCACCCGCCACGCGGTGTCGTCCTGATCGACGTCTACCCGCCCGGTCACCAGGACGC GATGAACGCCTGGCTGGAGGAGCTGACCGCCACGCTGTTCGACCGCGAGACGGTGCGGATG GACGACACCAGGCTCACCGCCCTGGGCGCCTACGACCGCCTCACCGGTCAGTGGCGACCCC GGGAAACCGGGCTGCCGACGCTGCTGGTCAGCGCCGGCGAGCCGATGGGTCCGTGGCCCGA CGACAGCTGGAAGCCGACGTGGCCCTTCGAGCACGACACCGTCGCCGTCCCCGGCGACCAC TTCACGATGGTGCAGGAACACGCCGACGCGATCGCGCGGCACATCGACGCCTGGCTGGGCG GAGGGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCA CTGA
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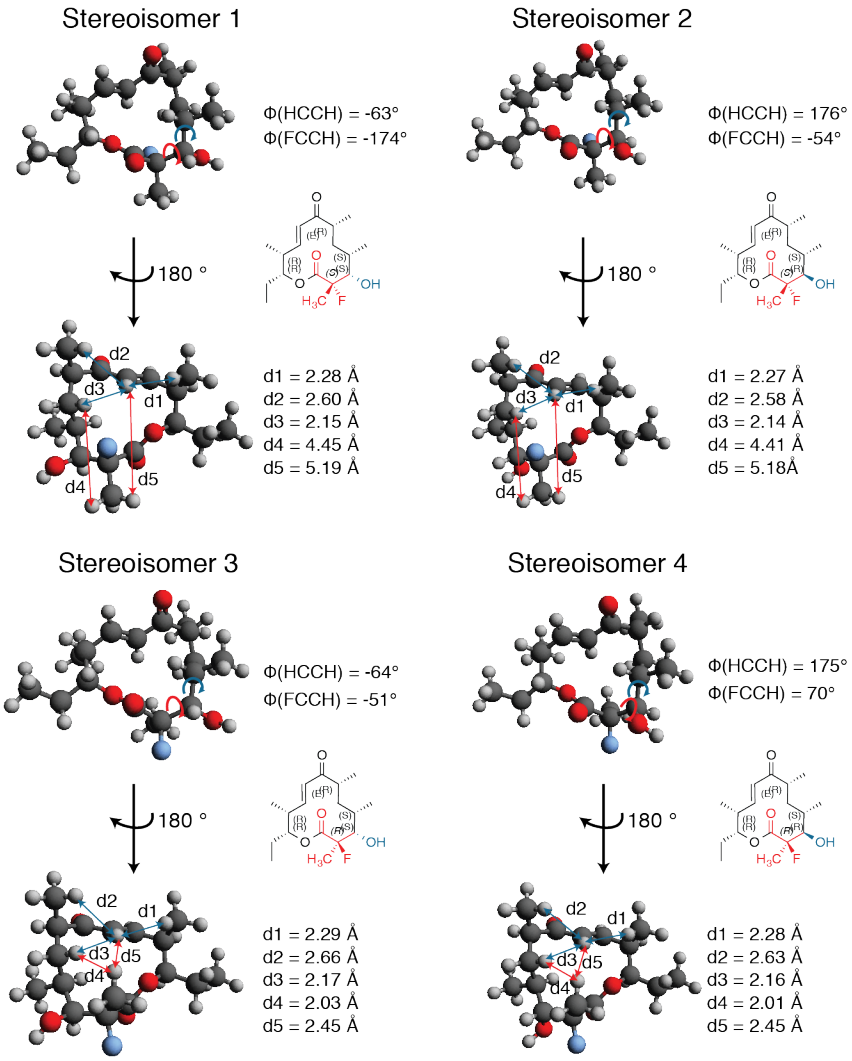
6.1 Supplementary note: Stereochemistry of 18

During the biosynthesis of compound **18**, two new stereo centers at position C2 and C3 are formed. ^{1}H-NMR and ^{19}F-NMR showed that only one stereoisomer was synthesized. To determine the stereochemistry of compound **18**, we predicted the 3D structures of all possible diastereomers using the software Avogadro with UFF force field (Steepest decent) algorithm and no constraints (see Supplementary Note Fig. 1). The template for the models was the structure of 10-deoxymethynolide, solved by crystallization of the pikromycin thioesterase (PDB code: 2hfk, E4H). We used the models to calculated characteristic distances (d1-d5) in the stereoisomers, more precisely, the distance of the hydrogen of the alkene group at C8 to the nearest neighbors (methyl groups at C6 and C9, methylene group at C5) and to the methyl group at C2, as well as the distance of the methyl group at C2 to the hydrogen of the methylene group at C5 (see Supplementary Note Fig. 1). Furthermore, we calculated the dihedral angel between the hydrogen of the CHOH group at C3 to the hydrogen of the $CHCH_3$ group at C4 (HCCH angle, blue arrow) and to the fluorine at C2 (HCCF angle, red arrow).

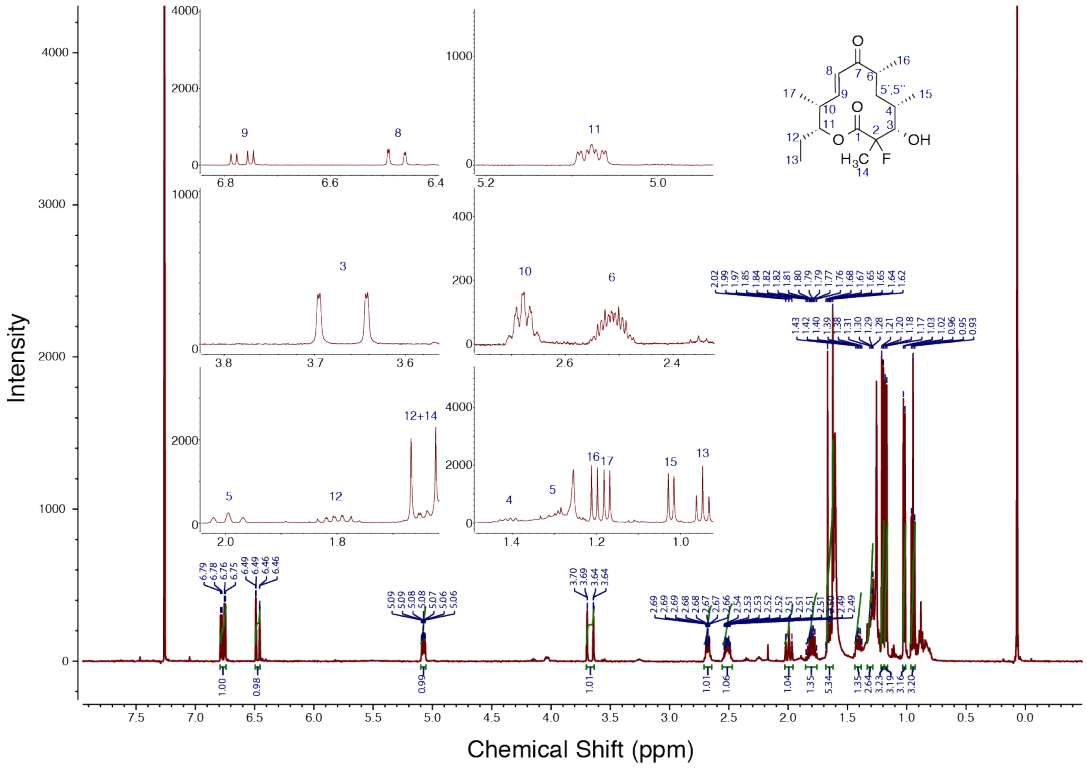
For the first analysis, we used the coupling constants of the ^{1}H-NMR to determine which stereoisomer was produced, more precisely the signal at 3.68 ppm, corresponding to the hydrogen at C3 (dd, $^{3}J_{FH} = 26.41$, $^{3}J_{HH} = 1.1$ Hz, 1H). With an improved Karplus equation, taking electronegativities of the substituents into account, we calculated the coupling constant of each stereoisomer model (Supplementary Note Table 1, equation 3) [26,27]. We received the values for the substituent's electronegativity from Altona and used the value of CH_2F for the CCH_3FC group and the value of CH_2X (0.65) for the CH_2C group [26]. Using that analysis we could clearly exclude stereoisomer 2 and 4, since the calculated coupling constants deviate strongly from the experimental ones ($^{3}J_{HH} = 1.1$ Hz).

Next, we used the $^{3}J_{FH}$ coupling constant of 26.41 Hz to further distinguish between stereoisomer 1 and 3. We considered a study, which developed a Karplus equation in respect to the CHHF torsion angle (equation 4) [28]. We again received the values for the substituent's electronegativity from Altona and used the value of $CHCH_3X$ for the $CHCH_3C$ group (0.6) (Supplementary Note Table 2, equation 4) [26]. The results confirmed the $^{3}J_{HH}$ coupling constant analysis, as the $^{3}J_{HF}$ coupling constant of stereoisomer 2 and 4 did not correlate with the experimental ($^{3}J_{FH} = 26.41$ Hz). Although the calculated $^{3}J_{FH}$ coupling constant for stereoisomer 1 fits much better to the experimental, we could not exclude stereoisomer 3, especially, because we used structural models. Therefore, we performed a NOESY experiments after another purification of the product in higher yields. We focused on the hydrogen of the alkene group at C8 (^{1}H NMR peak at 6.47 ppm) as the signal of the methyl group at C2 overlapped with the methylene group at C12. In the NOESY experiment, we found NOE signals to 1.18 ppm, 1.21 ppm and 2.00 ppm, which correspond to the methyl group at C10 (distance about 2.3 Å), to the methyl group of C6 (distance about 2.6 Å) and to the methylene group at C5 (distance about 2.2 Å, Supplementary Note Fig. 3-4). In contrast, we did not find a NOE signal of the methyl group at C2 (^{1}H NMR peak at 1.63 ppm) to the alkene group at C8 (distance 2.45 Å in stereoisomer 3) and no signal to the methylene group at C5 (distance 2.03 Å in stereoisomer 3). Therefore, we could exclude stereoisomer 3, as these signals should be observed for that case. The results show that hybrids **H1** and **H1.1** exclusively synthesize stereoisomer 1 with a 2S, 3S configuration.



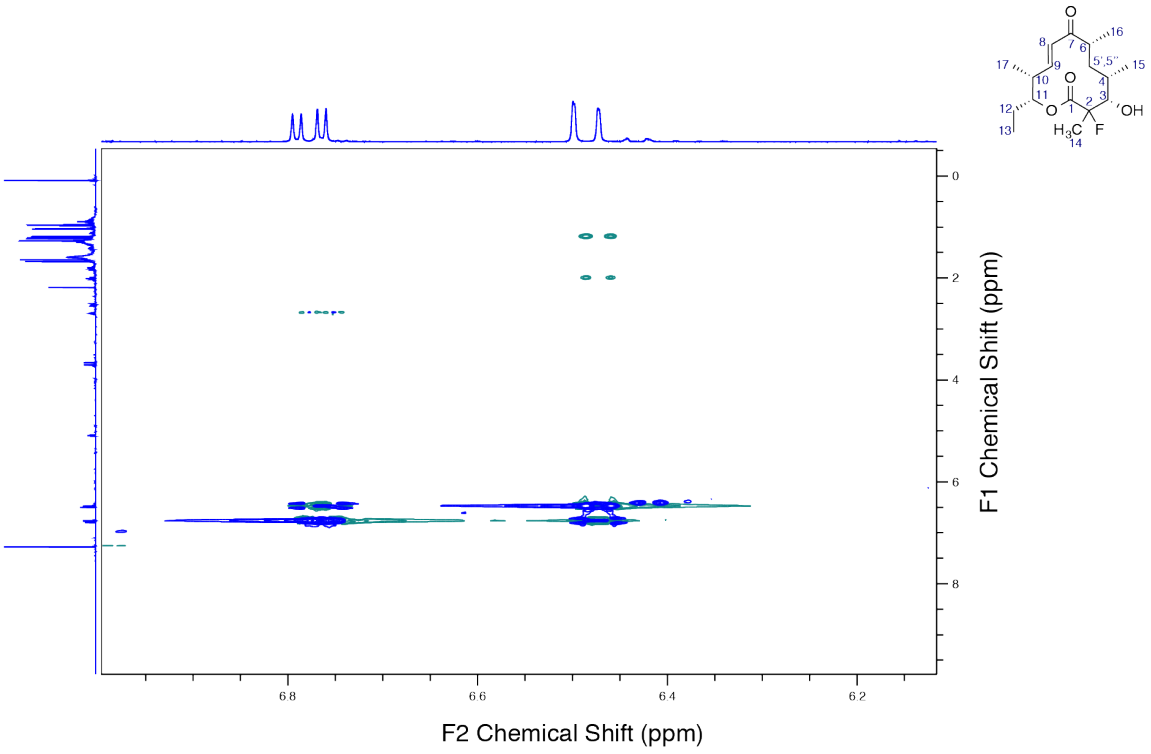
Supplementary Note Fig. 1: 2D and 3D structure of all possible stereoisomers, created by chemdraw and Avogadro.

Dihedral torsion angles between HCCH (C3-C4, blue arrow) and HCCF (C2-C3, red arrow) were calculated with Avogadro. Characteristic distances were also calculated by Avogadro and shown with blue arrows (d1-d3) for observed NOE signals and red arrows for non-existent NOE signals.

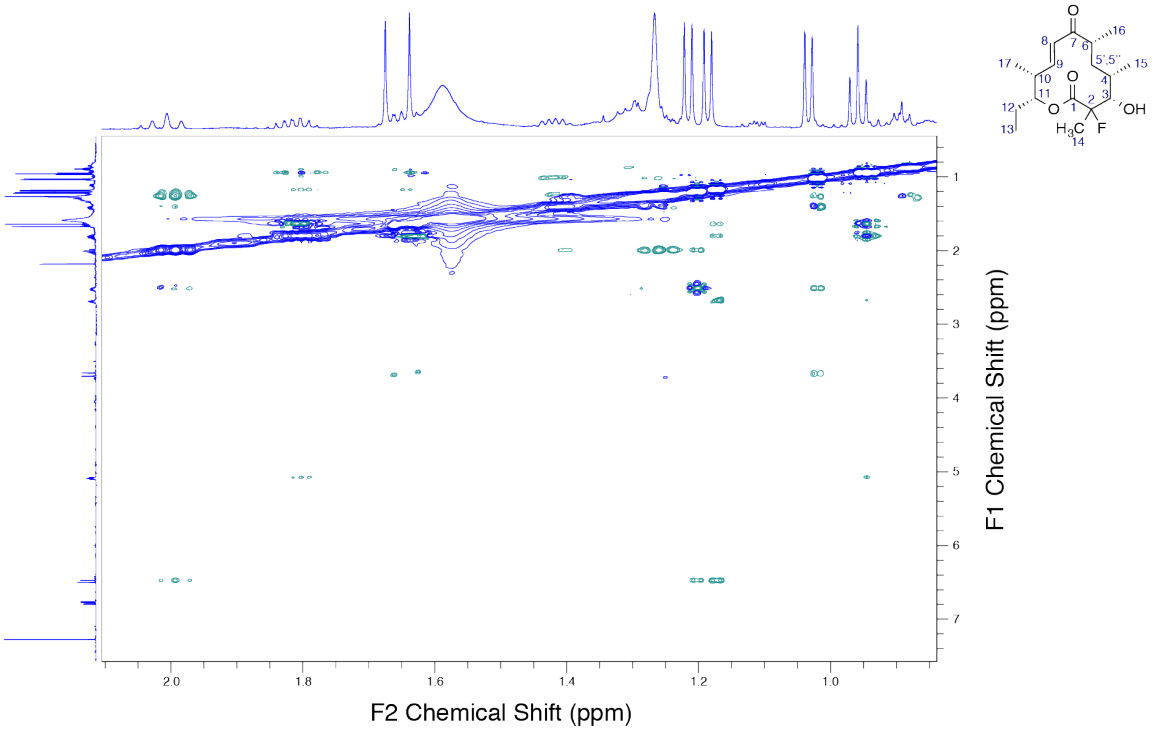


Supplementary Note Fig. 2: ^{1}H-NMR analysis of the product and assignment of the peaks.

The assignment was done with the help of the COSY and HSQC experiment (spectra in the appendix). NMR spectrum was measured on a 500 MHz instrument (Bruker) with the solvent $CDCl_3$ and processed with TopSpin (version 4.1.1) and structures were created with ChemDraw (version 14.0).



Supplementary Note Fig. 3: Selected view of the NOESY experiment with compound 18. NMR spectrum was measured on a 600 MHz instrument (Bruker) with the solvent $CDCl_3$ and processed with TopSpin (version 4.1.1).



Supplementary Note Fig. 4: Selected view of the NOESY experiment with compound 18.
NMR spectrum was measured on a 600 MHz instrument (Bruker) with the solvent $CDCl_3$ and processed with TopSpin (version 4.1.1).

Supplementary Note Table 1: HCCH dihedral angles (Φ) between C3 and C4 and experimental and calculated $^3J_{HH}$ coupling constants.

λ_i is the electronegativity and s_i the sign factor of the substituent [26,27].

	ϕ(HCCH)	$^3J_{HH}$	OH:λ_1;s_1	CCH_3FC:λ_2;s_2	CH_2C:λ_3;s_3	CH_3:λ_4;s_4
Exp.		1.1				
Isomer 1	-63	1.28	1.33; 1	0.65; -1	0.65; 1	0.8; -1
Isomer 2	176	12.47	1.33; -1	0.65; 1	0.65; 1	0.8; -1
Isomer 3	-64	1.09	1.33; 1	0.65; -1	0.65; 1	0.8; -1
Isomer 4	175	12.40	1.33; -1	0.65; 1	0.65; 1	0.8; -1

$$3J(H,H) = 14.64\,cos^2(\phi) - 0.78\cos(\phi) + 0.58 + \sum_i \lambda_i\,[0.34 - 2.31\,cos^2(s_i(\phi) + 18.40\,|\lambda i|)] \quad [3]$$

with λ_i = the electronegativity of the substituent, s_i = sign factor of the substituent [26,27].

Supplementary Note Table 2: HCCF dihedral angles (Φ) between C2 and C3 and experimental and calculated $^3J_{HF}$ coupling constants.

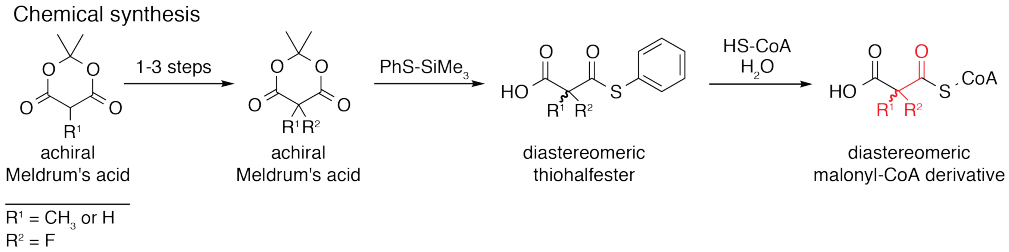
λ_i is the electronegativity, ξ_i the sign factor of the substituent and a_{FCC} and a_{HCC} the respective bond angles [26,28].

	ϕ(HCCF)	$^3J_{HF}$	OH:λ_1;s_1	$CHCH_3C$: λ_2;s_2	COOR:λ_3;s_3	CH_3:λ_4;s_4	a_{FCC};a_{HCC} (°)
Exp.		26.41					
Isomer 1	-174	26.37	1.33; -1	0.6; 1	0.42; 1	0.8; -1	111; 104.9
Isomer 2	-54	11.34	1.33; 1	0.6; -1	0.42; 1	0.8; -1	111; 107.5
Isomer 3	-51	22.41	1.33; -1	0.6; 1	0.42; -1	0.8; 1	105.9; 102.7
Isomer 4	70	7.99	1.33; 1	0.6; -1	0.42; -1	0.8; 1	105.9; 107.8

$$3J(H,F) = 40.61\, cos^2(\phi) - 4.22\cos(\phi) + 5.88 + \sum_i \lambda_i \left[-1.27 - 6.20\, cos^2(\xi_i(\phi) + 0.20\, \lambda_i)\right] - 3.72\left[\frac{(a_{FCC} + a_{HCC})}{2} - 110\right] cos^2(\phi) \quad [4]$$

with λ_i = the electronegativity of the substituent, ξ_i = sign factor of the substituent, a_{FCC} and a_{HCC} = bond angles [26,28].

6.2 Supplementary note: Stereochemistry of elongation substrates

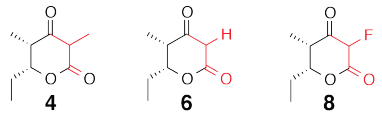


The malonyl-derivatives are synthesized via the achiral Meldrum's acid and the achiral, fluorinated Meldrum's acid derivatives. The two electrophilic carbonyl-carbon atoms in Meldrum's acid are equally likely for nucleophilic ring-opening with thiophenol, which gives a racemic mixture of thiohalfesters. We assume that the transesterification is insensitive to the stereocenter, and thus a diastereomeric mixture of CoA esters is obtained (diastereomers that are epimeric in the malonyl-moiety). Methylmalonyl-CoA was received from Sigma as a diastereomeric mixture.

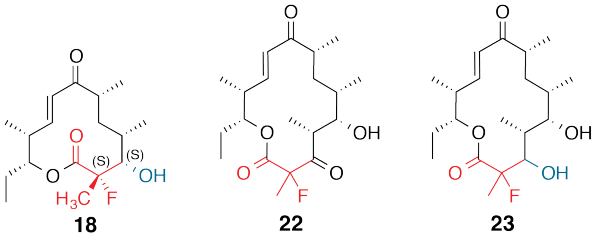
6.3 Supplementary note: Stereochemistry of TKLs and macrolactones

Enzymatic synthesis of macrolactones was performed with diastereomeric elongation units and enantiomerically pure di-, penta- and hexaketide substrates.

As reported previously, FAS from goose accepts (*R*)- and (*S*)-MM-CoA for fatty acid synthesis [29]. Therefore, we assume that the MAT of mouse FAS can transfer both stereoisomers, which is in contrast to ATs of DEBS that are specific for the *S*-configured extender unit [30]. The Claisen-like condensation reaction catalyzed by the KS proceeds with inversion of the C2 configuration [31].

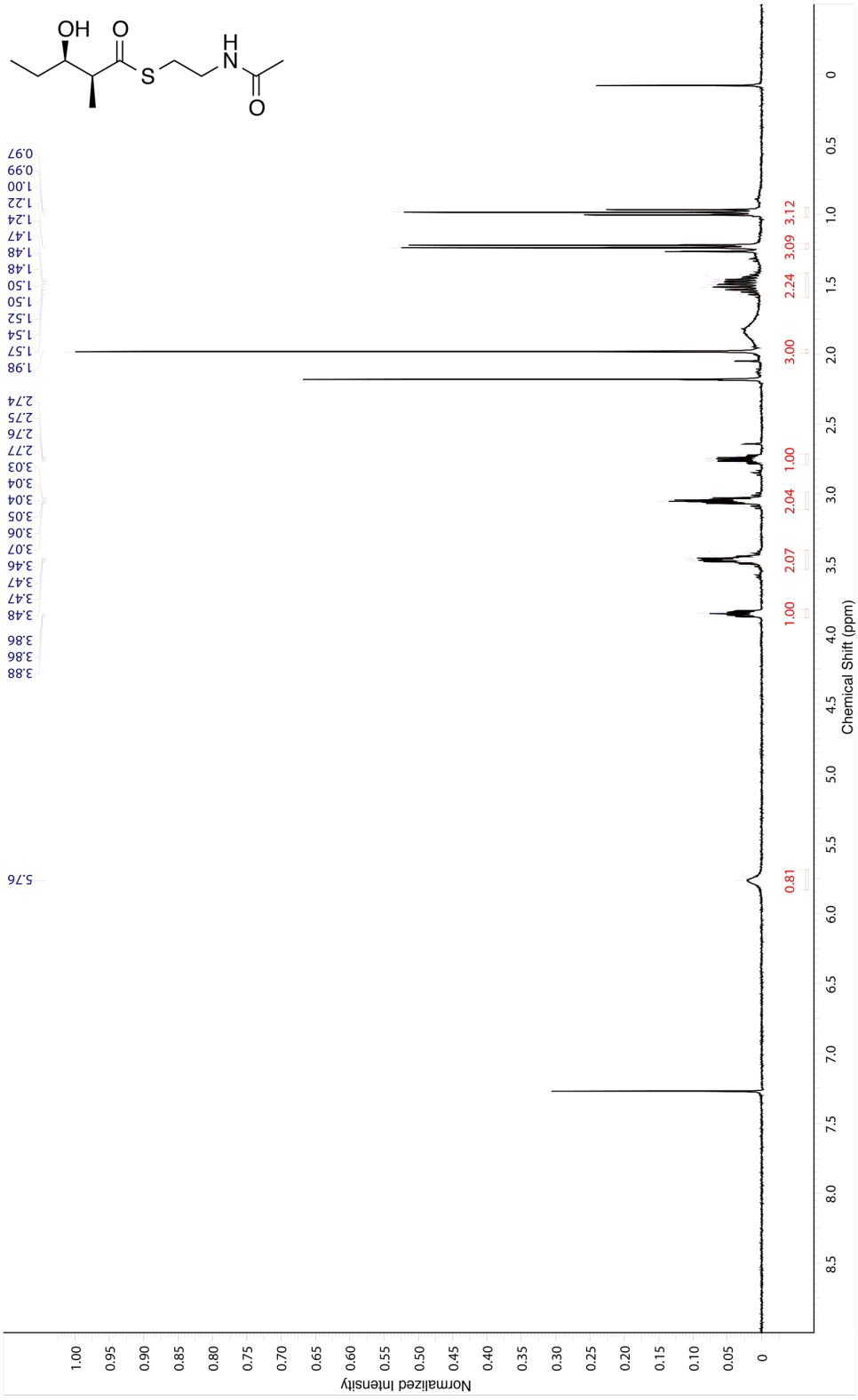


The unreduced TKLs **4** and **8** have been previously produced with DEBS module 6 by Chang and coworkers before (enzymatically and chemically) [32]. Compound **4** was mainly received in 2*R*-configuration, whereas compound **8** was received almost exclusively in 2*S*. The authors concluded that these configurations result from intrinsic stereoelectronic factors as a consequence of solvent dependent keto-enol tautomerism. We assume that compounds **11**, **15** and **16** are likely prone to keto-enol tautomerism; however, experimental evidence on the stereochemistry was not collected.

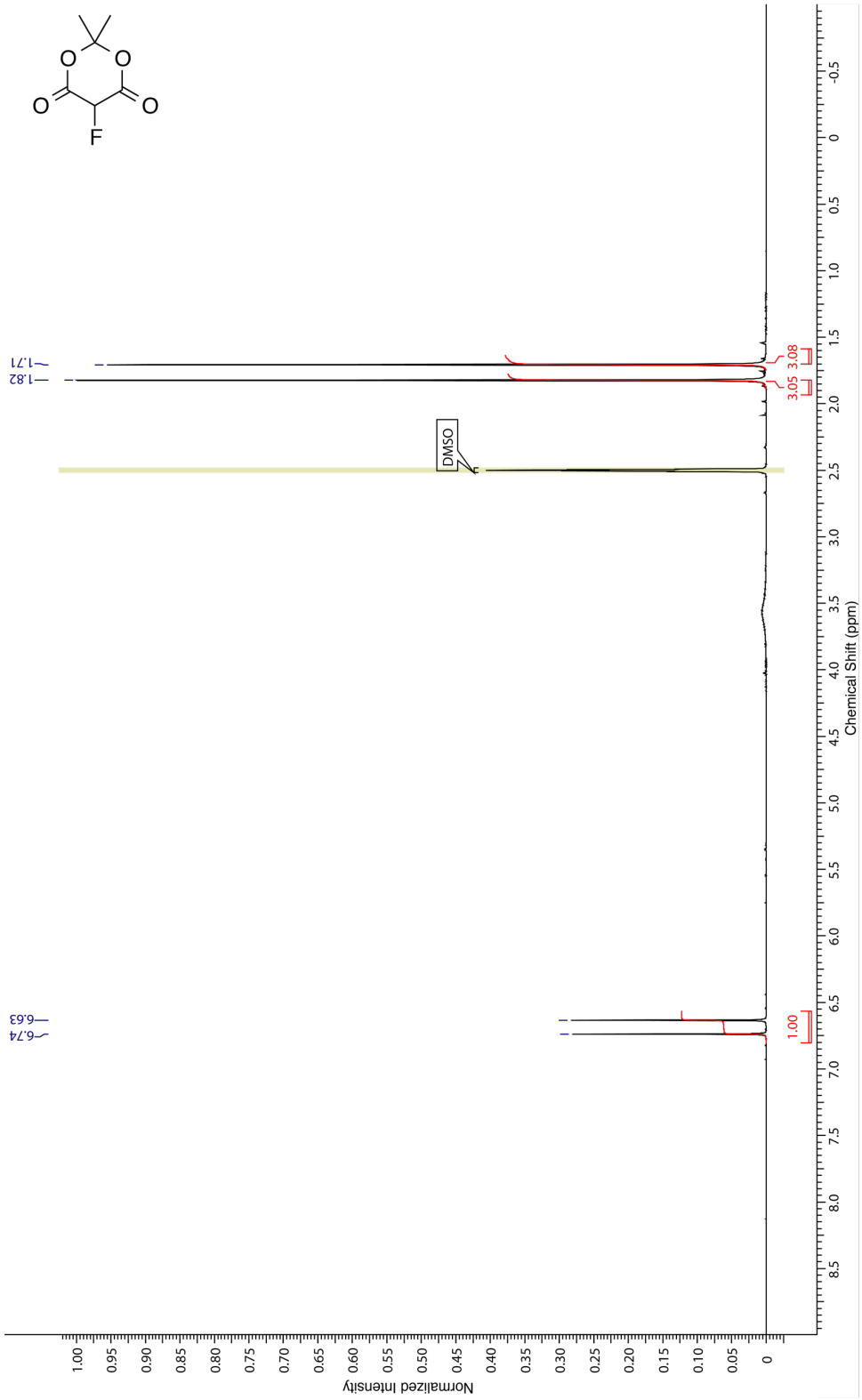


We found that **H1.1** produces 2-fluoro-10-deoxymethynolide (**18**) from the enantiomeric pentaketide and the diastereomeric F-MM-CoA in stereoselective manner, with compound **18** in 2*S*, 3*S*-configuration. In this reaction, we have not detected the 2*R*-epimer, nor any side product, which indicated that **H1.1** accepts just one F-MM moiety for elongation. We assume that stereoselectivity arises from the KS-mediated condensation: The *S*-configuration in position 2 of compound **18** can be explained by accommodating the F-MM moiety for substrate elongation with fluorine at the hydrogen position of the natively used methylmalonyl moiety. The stereochemistry in position 3 is as found in erythromycin. As described in the literature, the configuration at position 3 is most likely determined by the stereospecific KR-mediated reduction. Given the analogy to compound **18**, we expect compounds **22** and **23** to be obtained in 2*S*-configuration, but data to the stereochemistry of these compounds were not collected in this study.

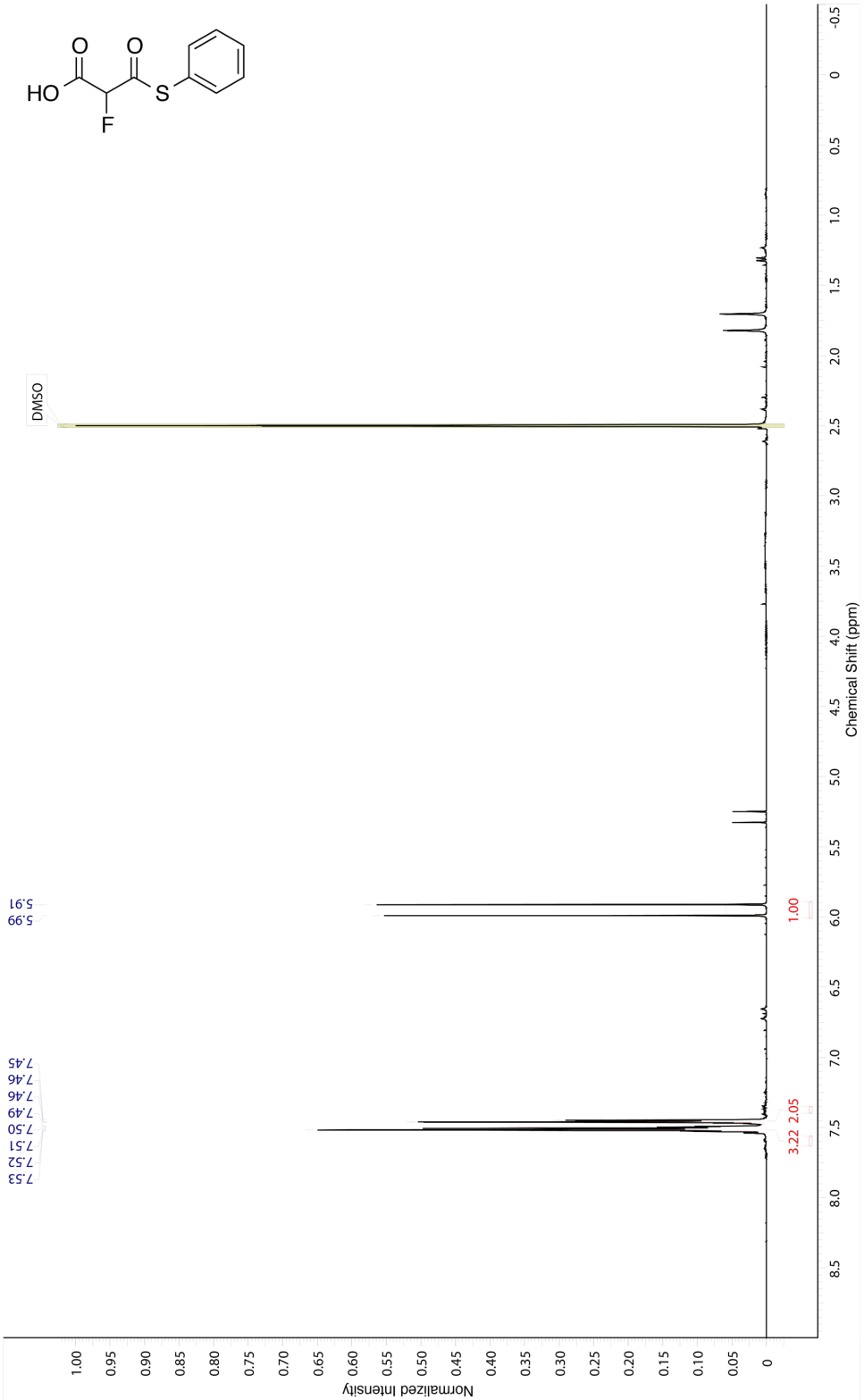
7. Spectra



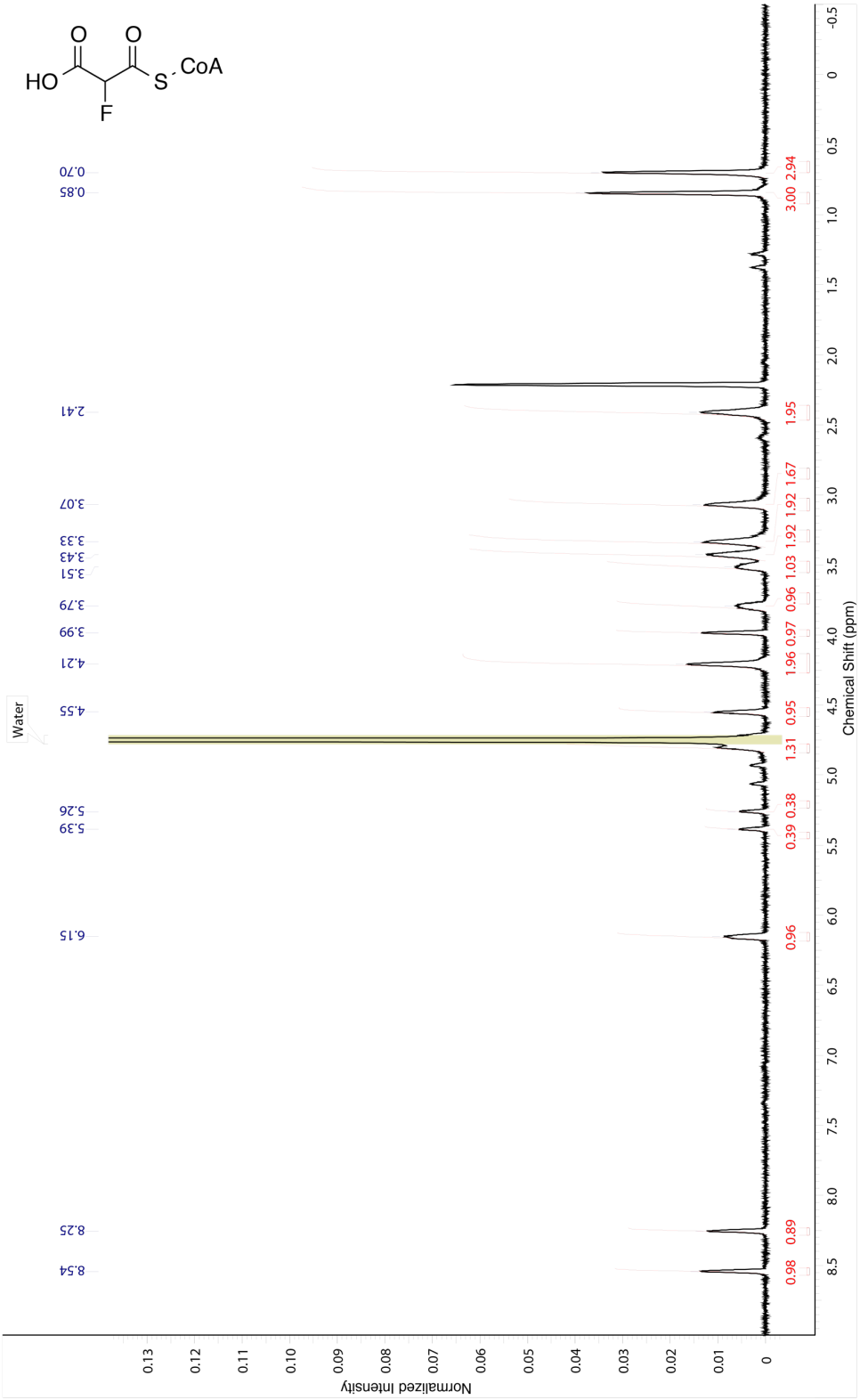
^{1}H-NMR experiment of natural diketide SNAC (**2**) was performed on a 400 Mhz instrument (Bruker) with the solvent $CDCl_3$.



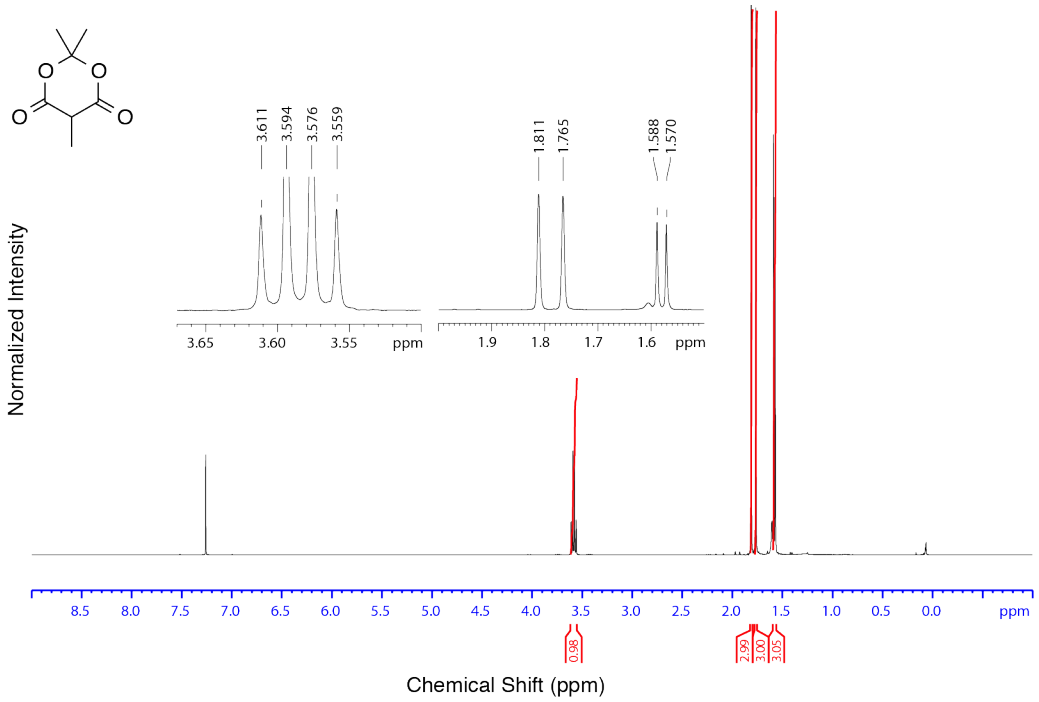
^{1}H-NMR experiment of fluoro-Meldrum's acid (**S1**) was performed on a 400 Mhz instrument (Bruker) with the solvent C_2D_6OS.



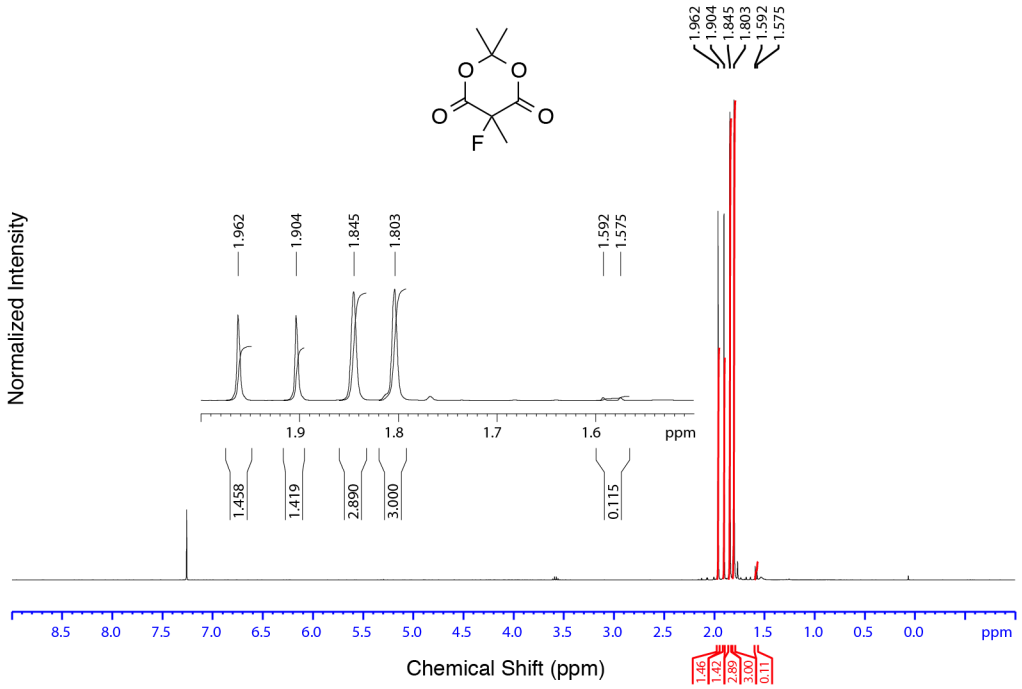
^{1}H-NMR experiment of fluoromalonic acid thiophenyl halfester (**S2**) was performed on a 400 Mhz instrument (Bruker) with the solvent C_2D_6OS.



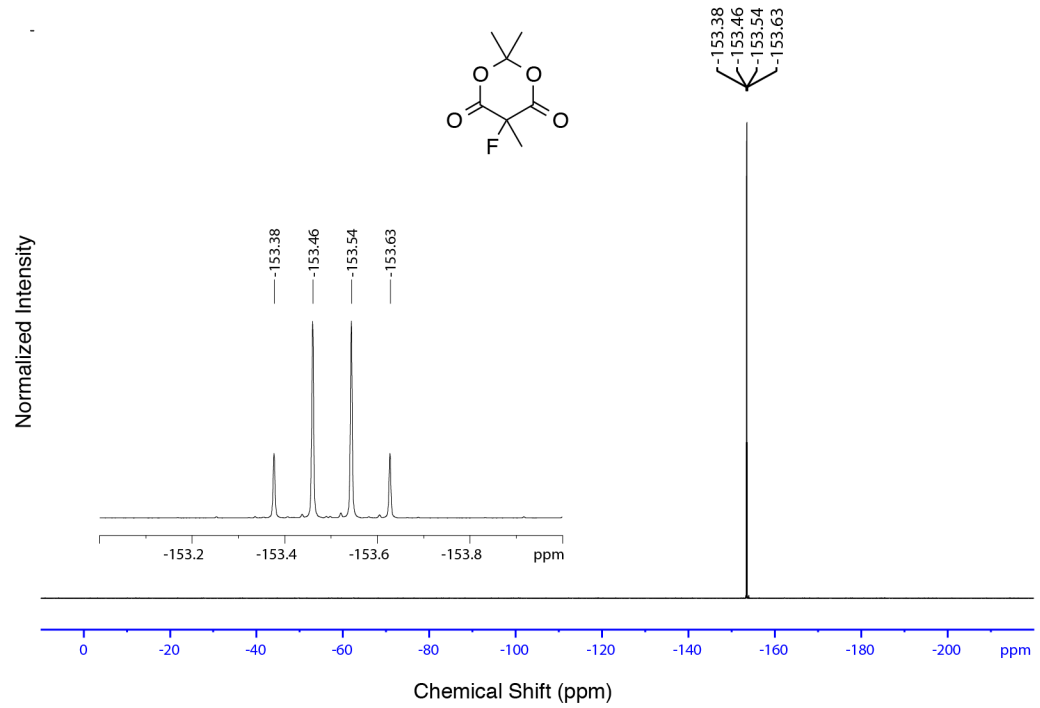
^{1}H-NMR experiment of fluoromalonyl-CoA (**1**) was performed on a 400 Mhz instrument (Bruker) with the solvent D_2O.



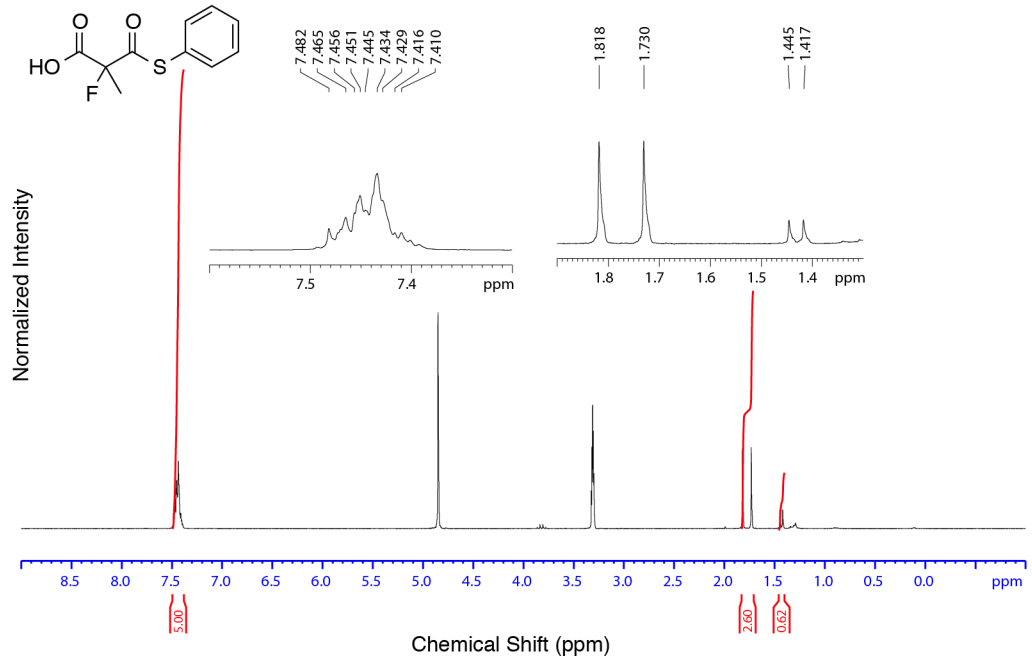
^{1}H-NMR experiment of methyl-Meldrum's acid was performed on a 400 Mhz instrument (Bruker) with the solvent $CDCl_3$.



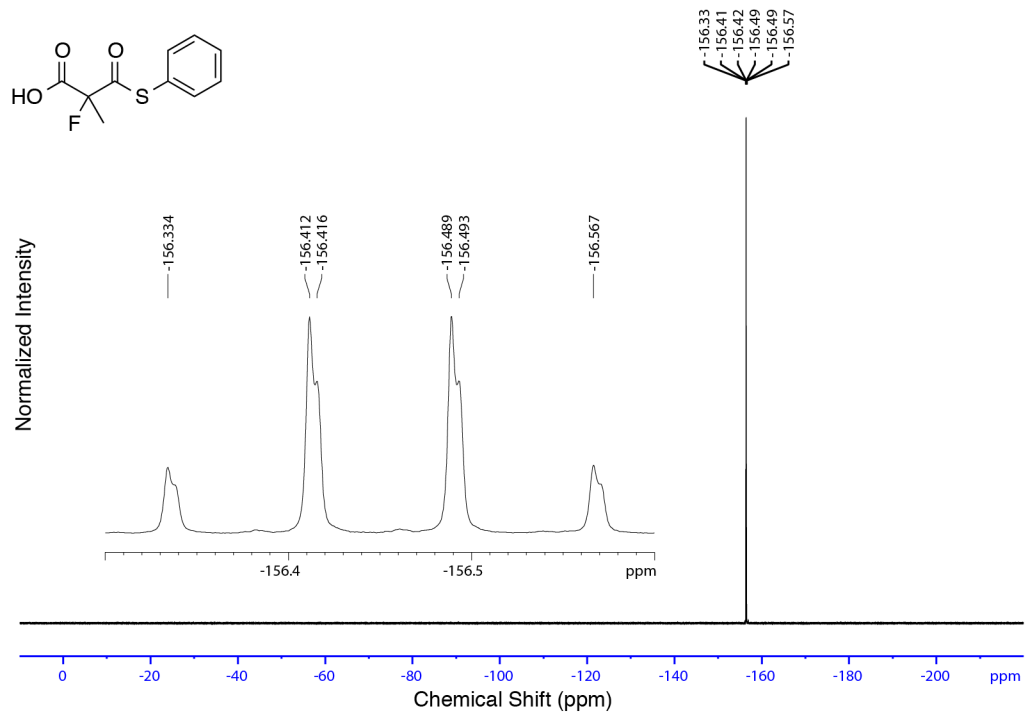
^{1}H-NMR experiment of fluoromethyl Meldrum's acid (**S5**) was performed on a 400 Mhz instrument (Bruker) with the solvent $CDCl_3$.



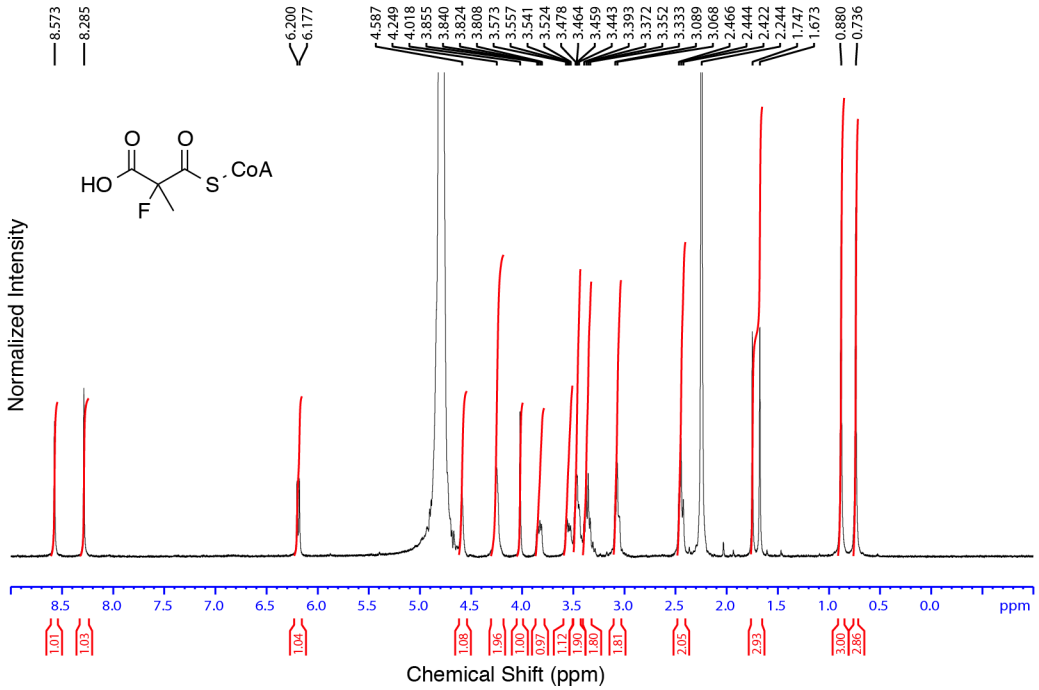
^{19}F-NMR experiment of fluoromethyl Meldrum's acid (**S5**) was performed on a 300 Mhz instrument (Bruker) with the solvent $CDCl_3$.



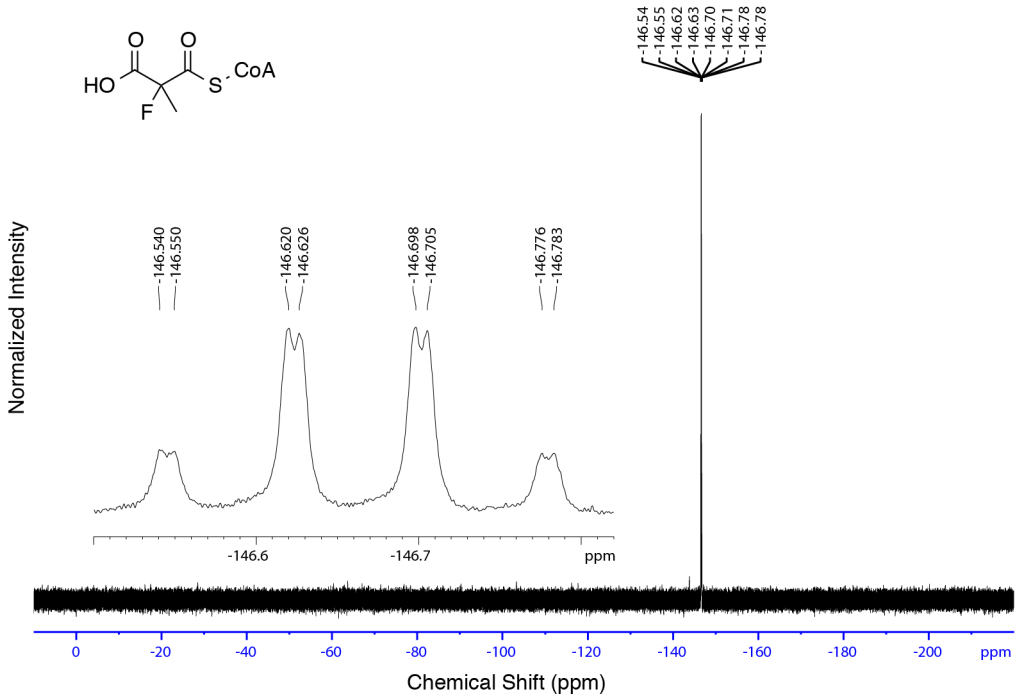
^{1}H-NMR experiment of fluoromethyl malonic acid thiophenyl halfester (**S6**) was performed on a 250 Mhz instrument (Bruker) with the solvent CD_3OD.



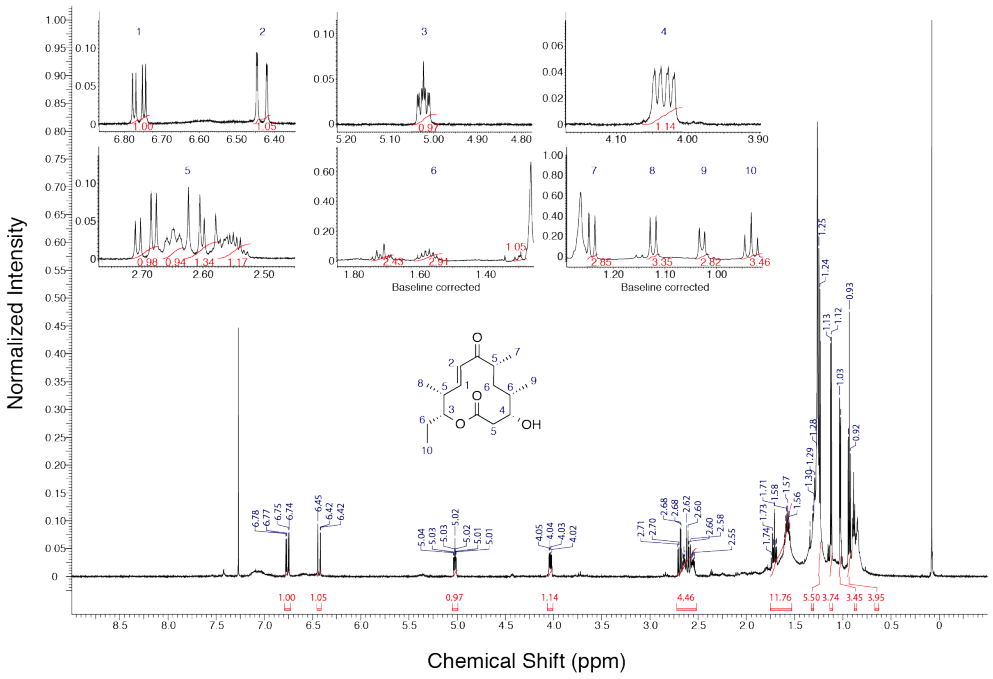
^{19}F-NMR experiment of fluoromethyl malonic acid thiophenyl halfester (**S6**) was performed on a 300 Mhz instrument (Bruker) with the solvent $CDCl_3$.



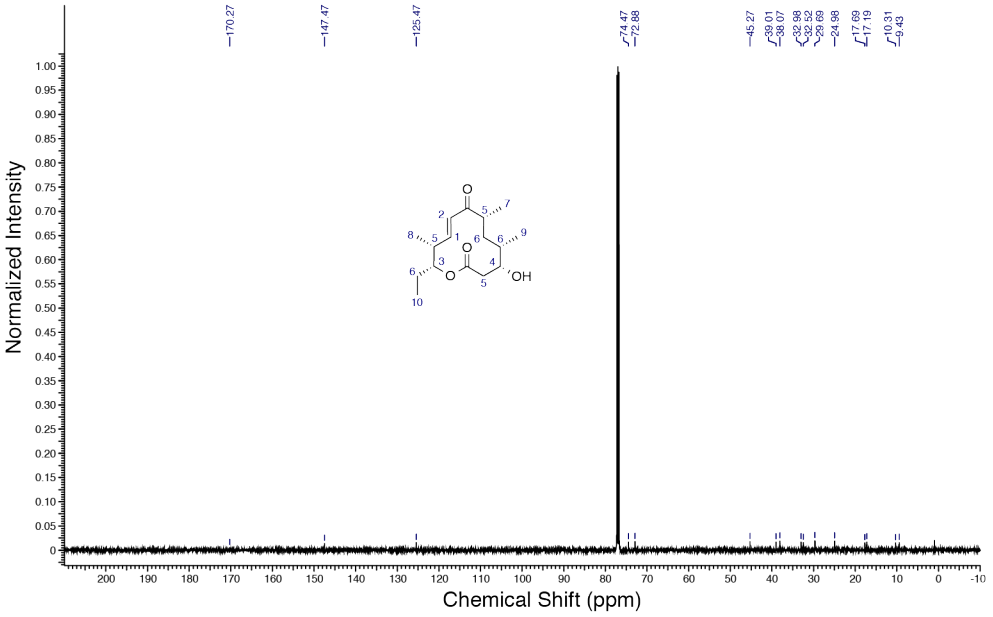
^{1}H-NMR experiment of fluoromethylmalonyl-CoA (**17**) was performed on a 300 Mhz instrument (Bruker) with the solvent D_2O.



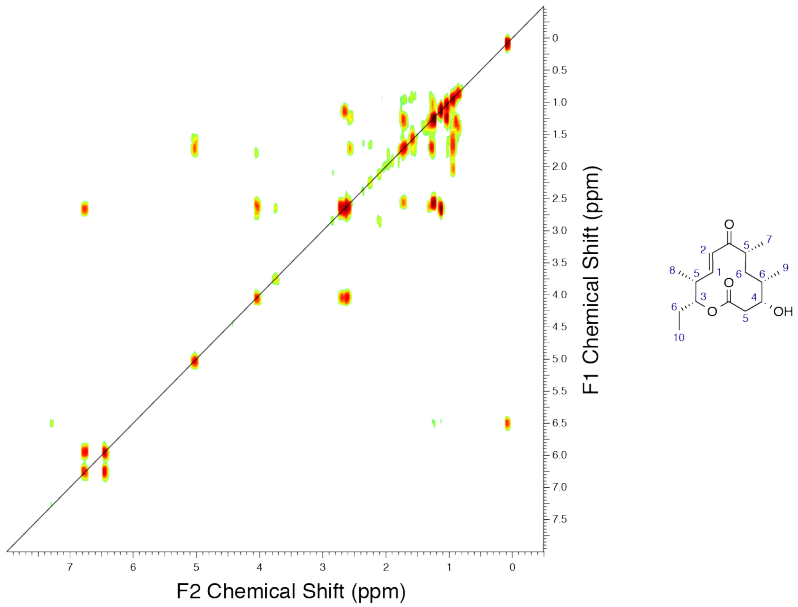
^{19}F-NMR experiment of fluoromethylmalonyl-CoA (**17**) was performed on a 300 Mhz instrument (Bruker) with the solvent D_2O.



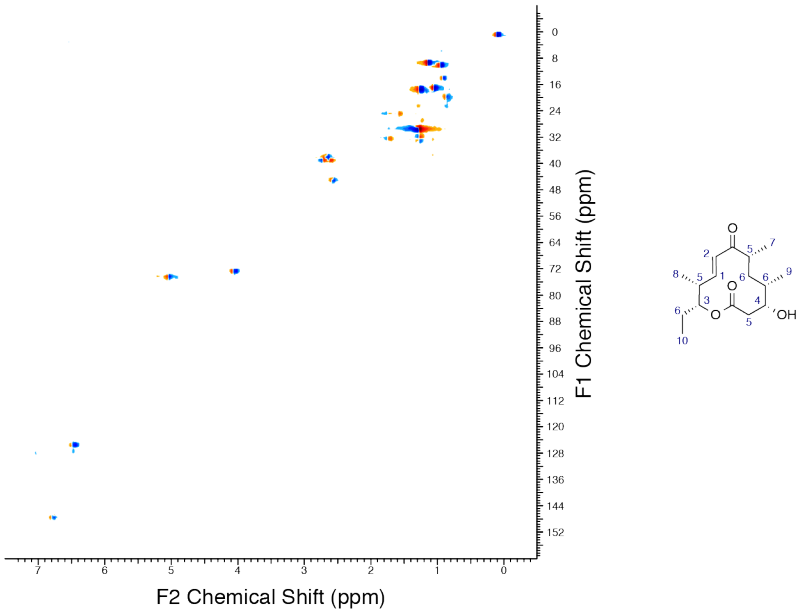
^{1}H-NMR experiment of compound **12** was performed on a 600 Mhz instrument (Bruker) with the solvent $CDCl_3$.



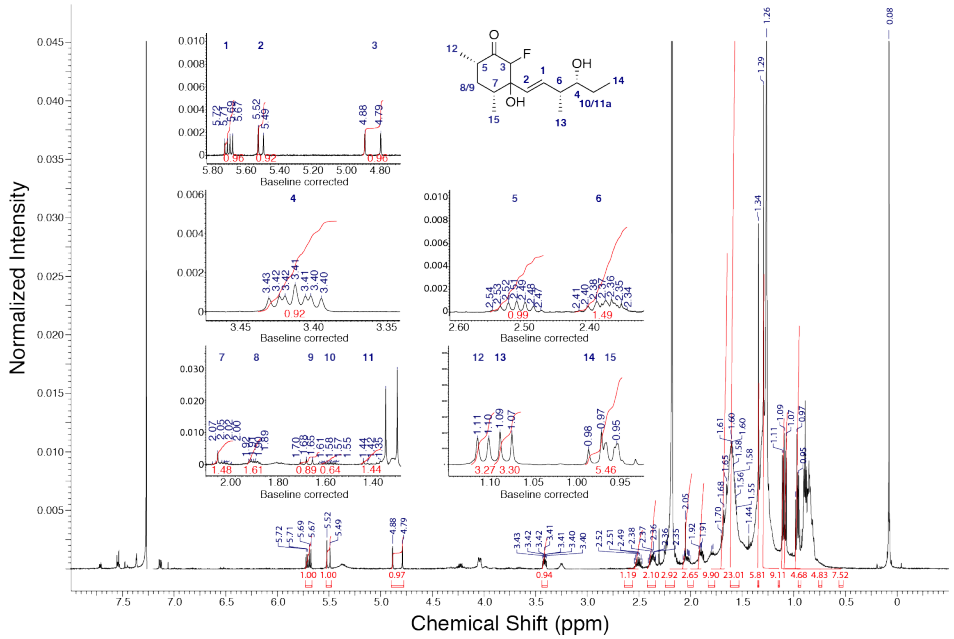
^{13}C-NMR experiment of compound **12** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



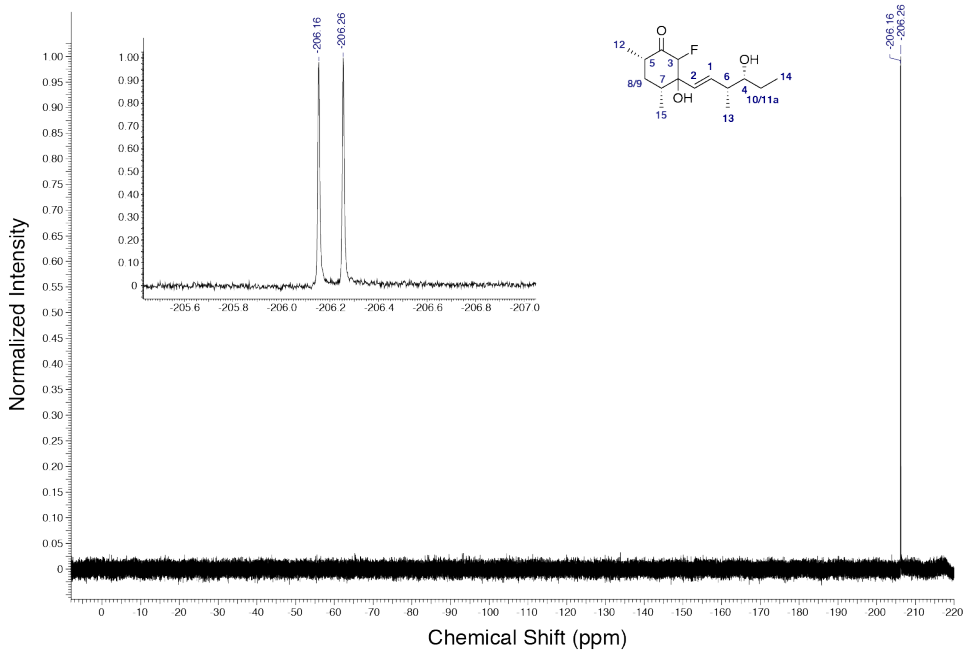
NOESY experiment of compound **12** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



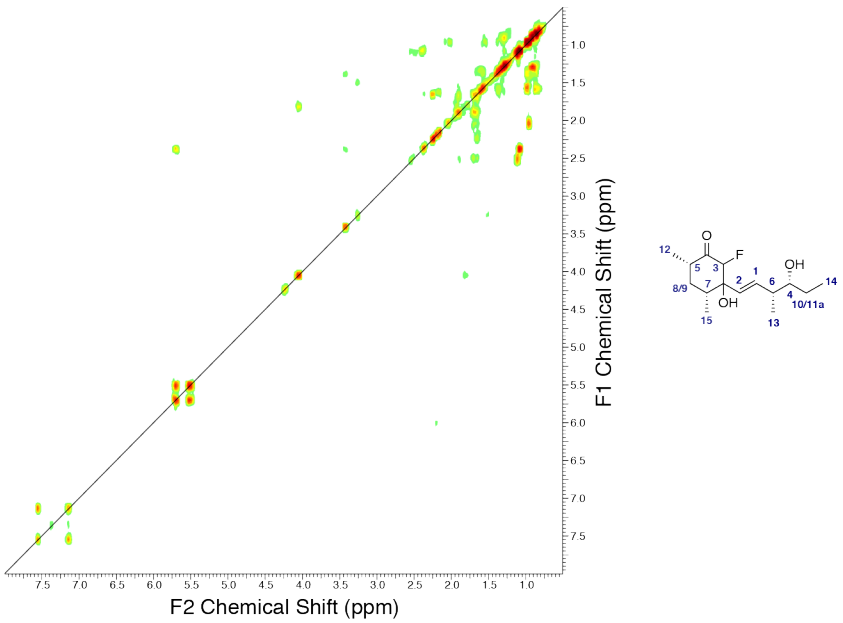
HSQC experiment of compound **12** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



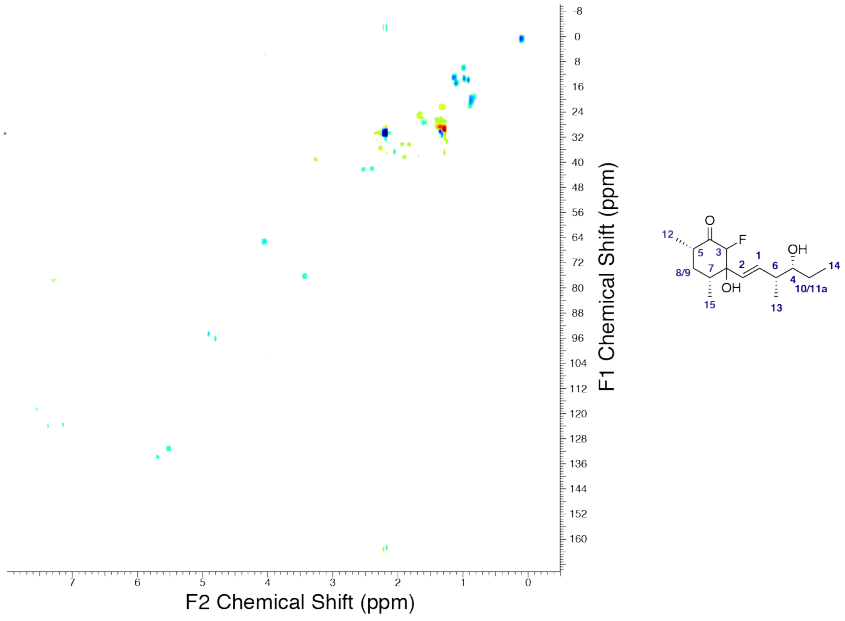
^{1}H-NMR experiment of compound **16** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



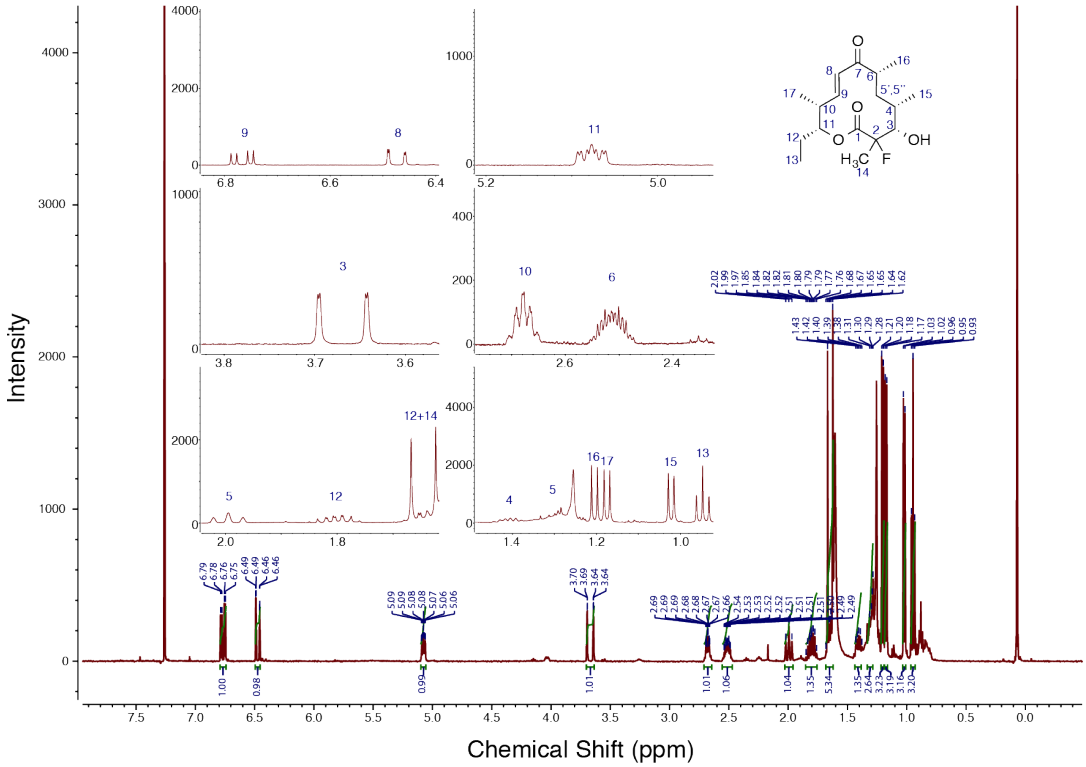
^{19}F-NMR experiment of compound **16** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



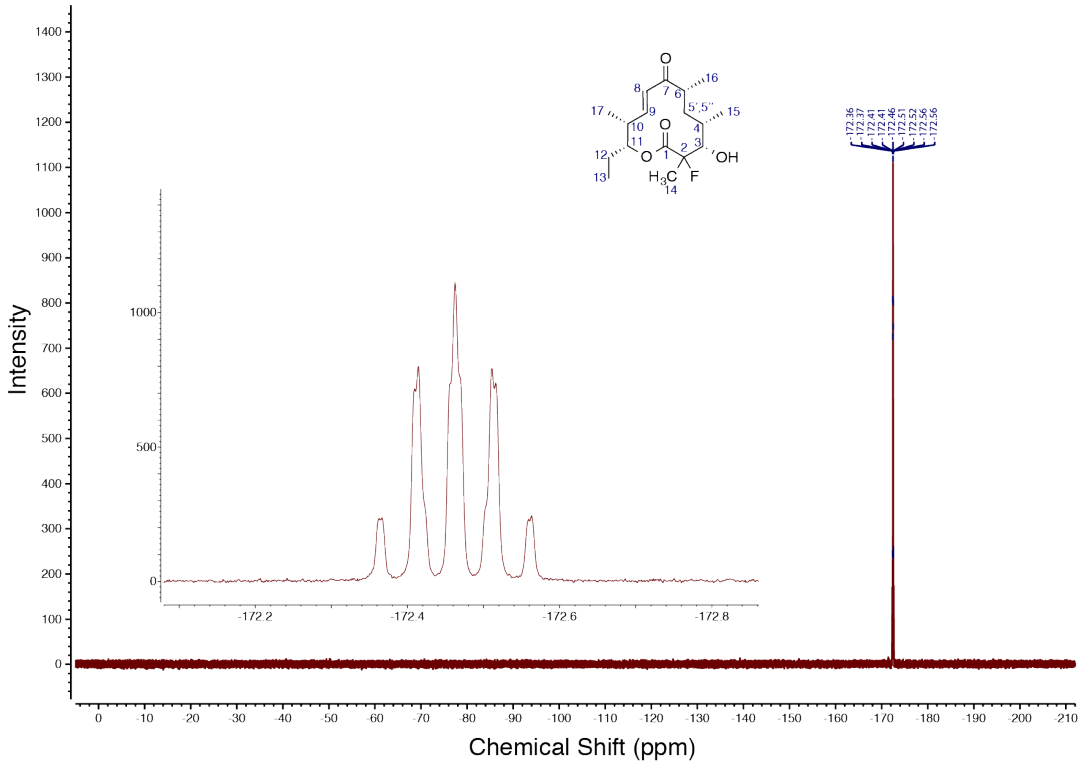
COESY experiment of compound **16** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



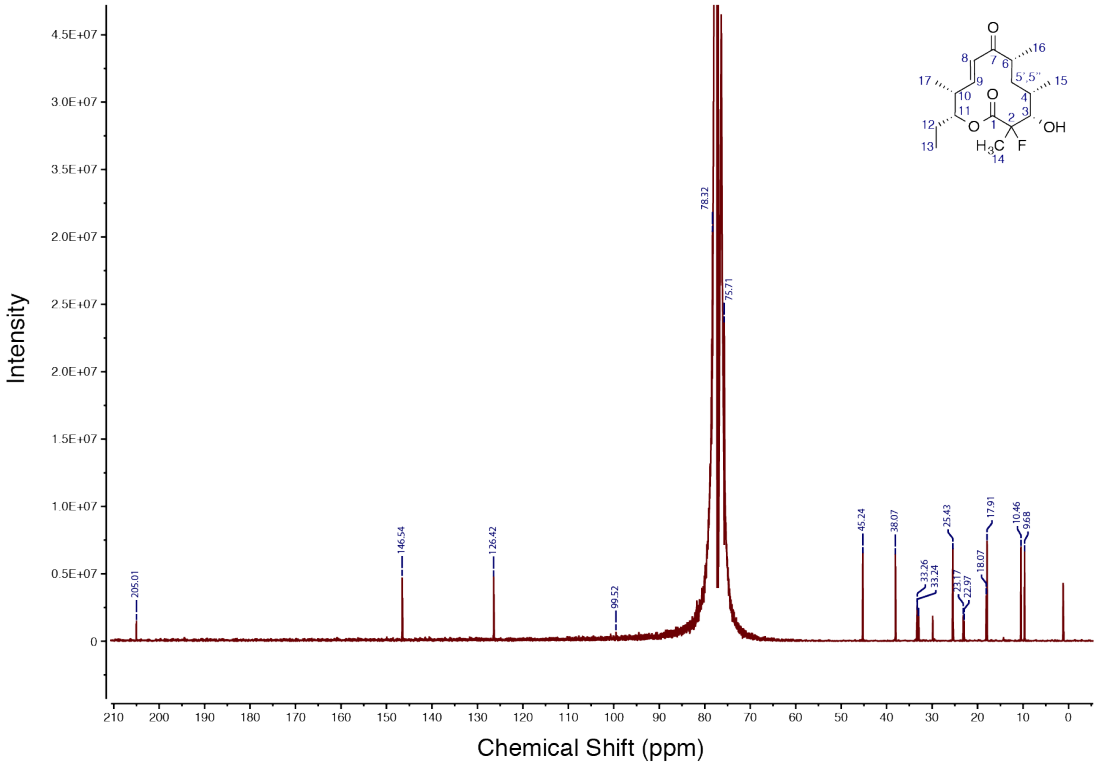
HSQC experiment of compound **16** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



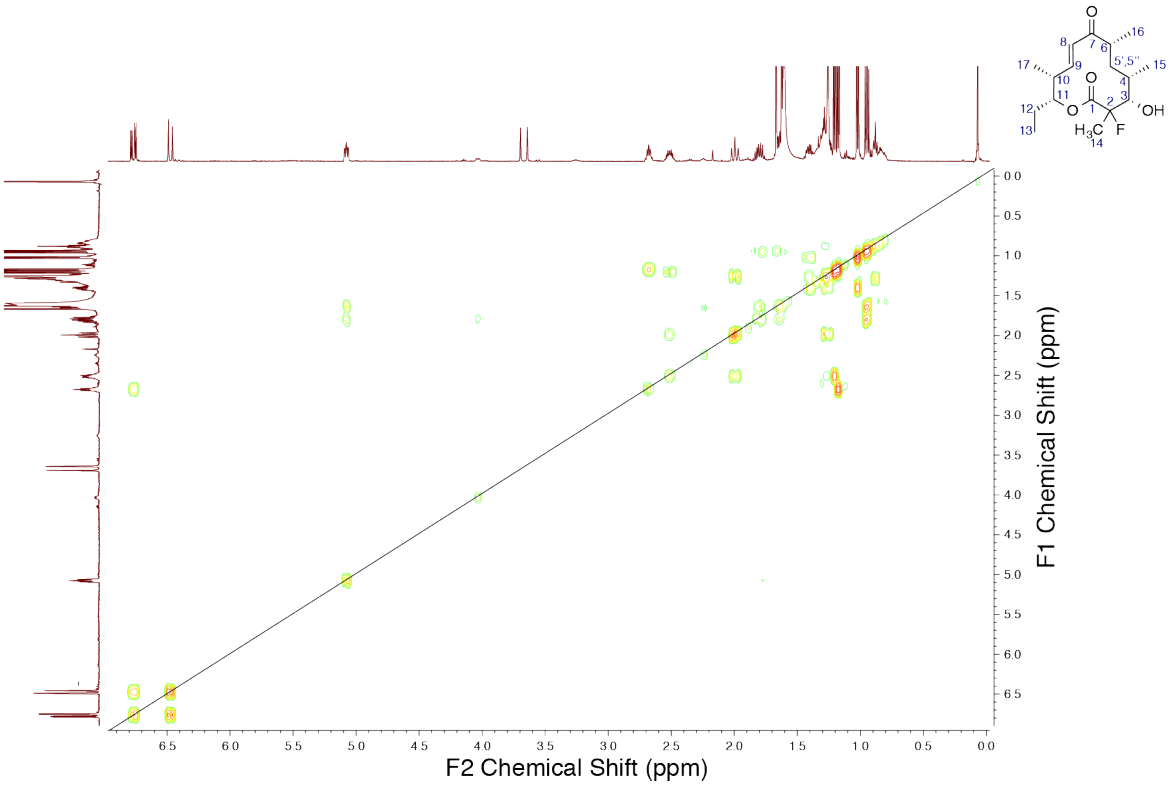
^{1}H-NMR experiment of compound **18** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



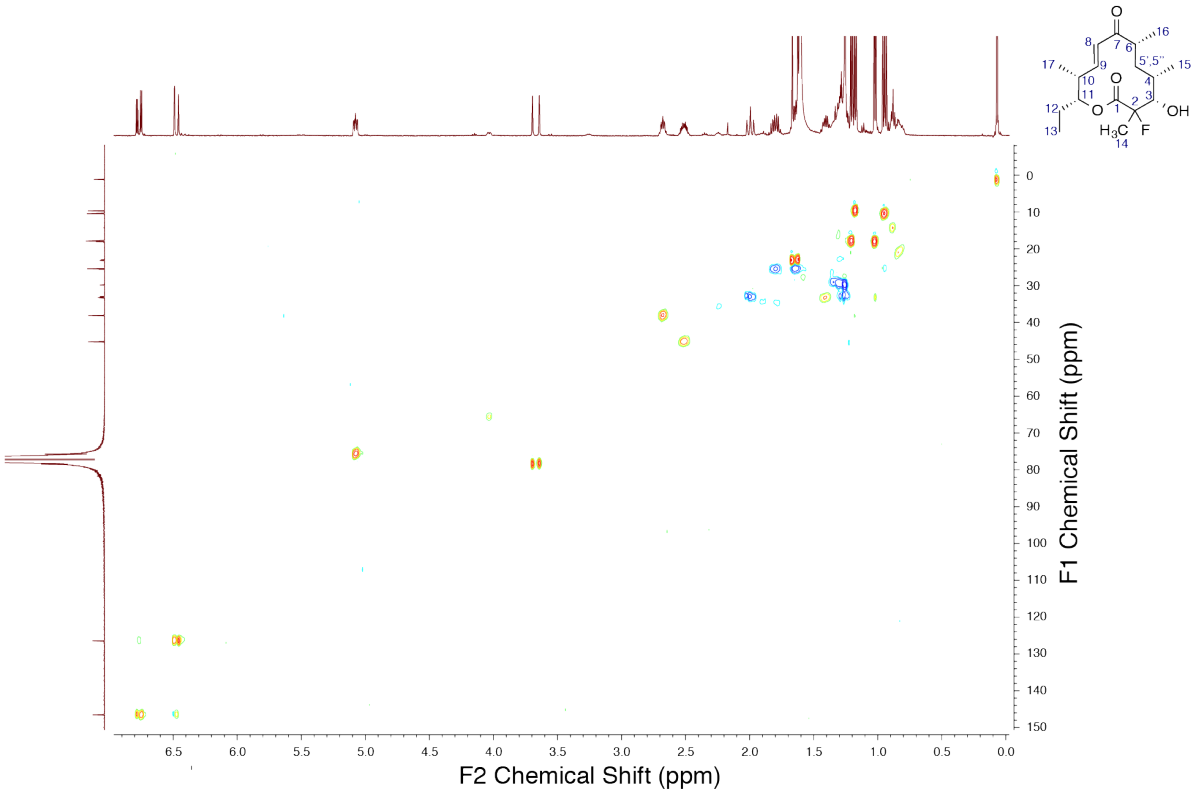
^{19}F-NMR experiment of compound **18** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



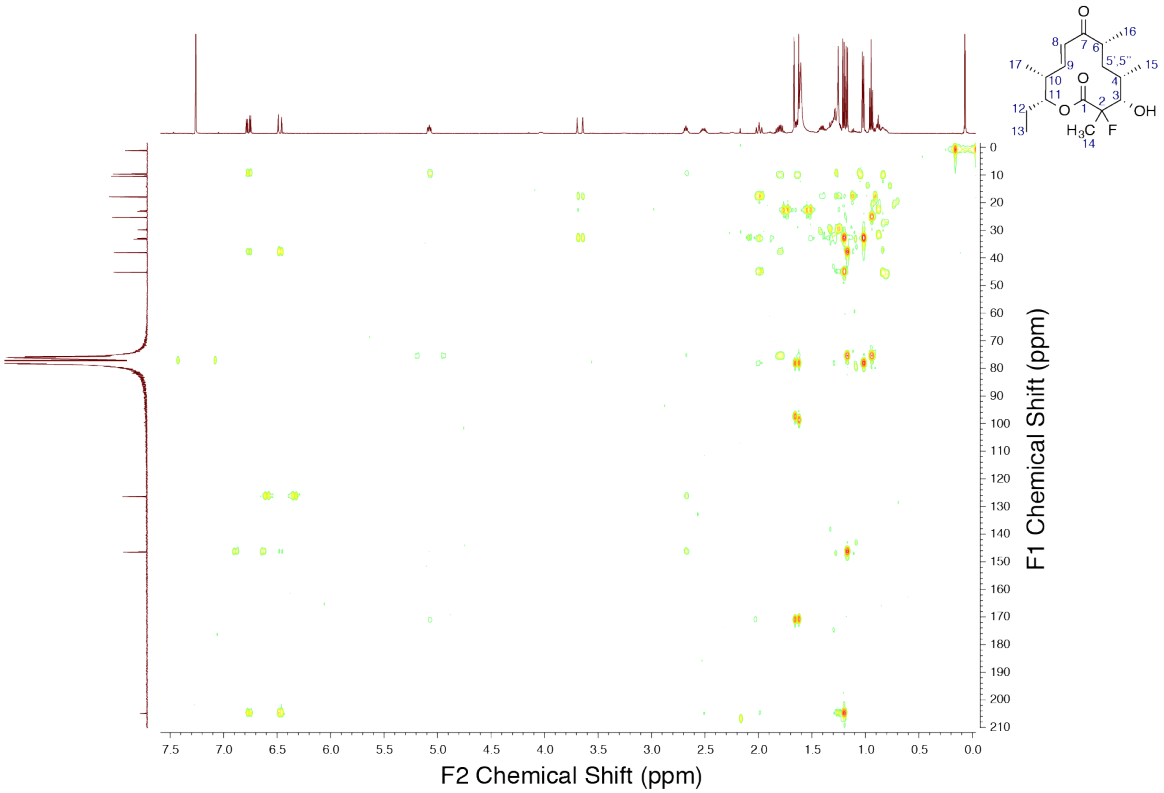
^{13}C-NMR experiment of compound **18** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



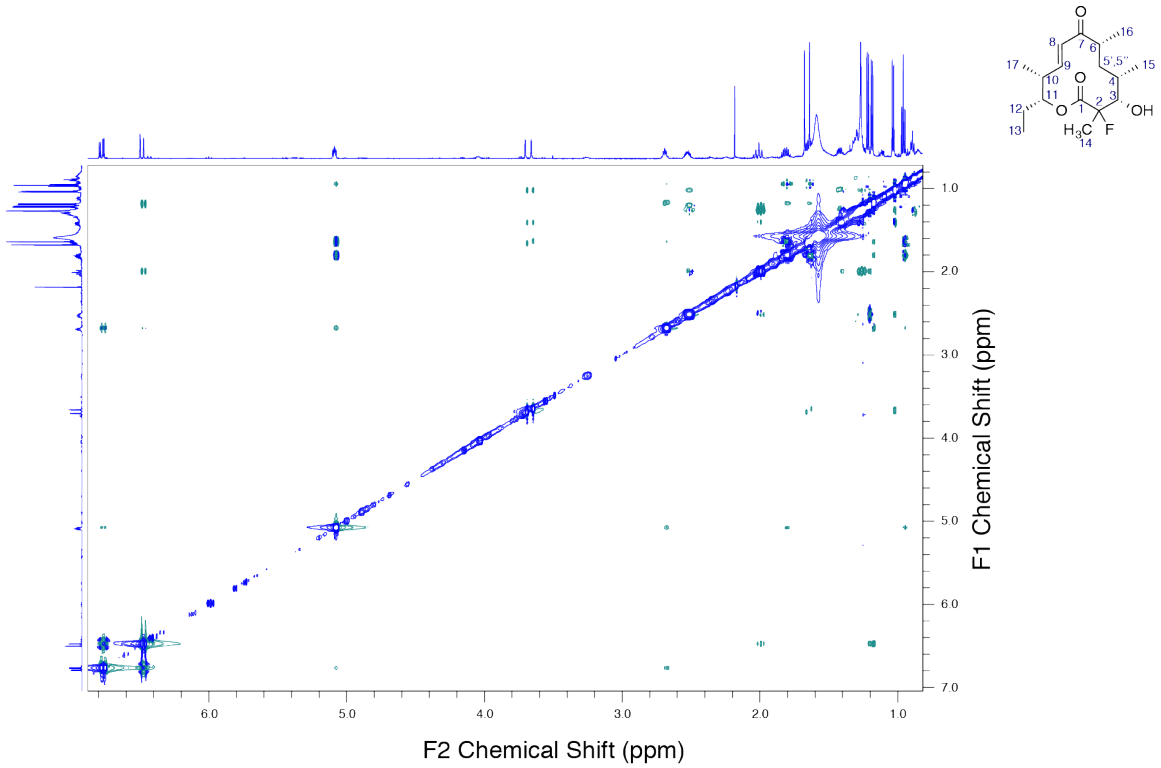
COSY experiment of compound **18** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



HSQC experiment of compound **18** was performed on a 500 Mhz instrument (Bruker) with the solvent $CDCl_3$.



HMBC experiment of compound **18** was performed on a 600 Mhz instrument (Bruker) with the solvent $CDCl_3$.



NOESY experiment of compound **18** was performed on a 600 Mhz instrument (Bruker) with the solvent $CDCl_3$.

8. References

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