

SUPPLEMENTARY INFORMATION

An In Vitro Platform for the Rapid and Scalable Biocatalysis of Complex Polyketides

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Commercial materials

All restriction enzymes, T4 DNA ligase and Gibson Assembly reagents were purchased from New England Biolabs (NEB). KOD One PCR Master Mix was purchased from Toyobo. Luria–Bertani (LB) broth, tryptone, yeast extract, glycerol, sodium chloride, potassium phosphate, hydrochloric acid, glucose, sodium sulfate, glycine, ethyl acetate, hexanes, methanol, acetonitrile and formic acid were purchased from Fisher Scientific. Sodium citrate dihydrate was purchased from VWR. Methylmalonic acid and malonic acid were purchased from Oakwood Chemical. Polypeptone was purchased from Gibco. Isopropyl β -D-1-thiogalactopyranoside (IPTG) was purchased from Carbosynth. Milk filters were purchased from KenAG. Plasmid minipreps and gel extractions were performed using Promega Wizard SV kits. 3,5-dihydroxybenzoic acid, streptomycin sulfate, kanamycin sulfate, chloramphenicol, magnesium chloride, malonyl coenzyme A lithium salt, sodium propionate, myokinase, pyruvate kinase, phosphoenolpyruvate (PEP), adenosine triphosphate (ATP) and imidazole were purchased from Sigma-Aldrich. Coenzyme A, NADP⁺ and NADPH were purchased from Oriental Yeast Co., Ltd. HisPur Ni-NTA affinity resin, 4–20% Tris-glycine mini protein gels, tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) and chloroform-d were purchased from Thermo Fisher Scientific. SiliaFlash F60 was purchased from SiliCycle. Mono Q 5/50 and Superdex 200 Increase 10/300 columns were purchased from GE Healthcare. All plasmids and oligonucleotides used in this study are listed in **Tables S1** and **S2**.

Bacterial strains

E. coli DH5 α was used for plasmid construction. *E. coli* BL21(DE3) Star pLysS cells were used for expression of malonyl-CoA synthetase (MatB), glucose dehydrogenase (GDH), and (2S)-methylmalonyl-CoA epimerase. *E. coli* K207-3 cells were used for expression of PKS assembly-line polypeptides.

Construction of protein expression plasmids

The two-plasmid system previously reported by our laboratory for production of narbonolide (**1**) in *E. coli* K207-3 was used as the template for constructing the expression plasmids for each polypeptide in **NAR₁** (**Table S3**)¹. Thioesterase II (PikAV) and the **P3**, **P5** and **P6** docking domains used for construction of **NAR₂** were cloned from *Streptomyces venezuelae* ATCC 15439 genomic DNA, whereas the **E6**-derived region used for hybrid-platform construction was cloned from

Saccharopolyspora erythraea genomic DNA. Oligonucleotides used in this study were purchased from Sigma-Aldrich and are listed in **Tables S1** and **S2**. PCR amplification was performed using KOD One DNA polymerase according to the manufacturer's instructions. Amplified PCR products were assembled using Gibson Assembly or ligated using T4 DNA ligase. The resulting plasmids were transformed into *E. coli* DH5 α , purified and submitted for DNA sequencing by Plasmidsaurus.

Protein purification

Plasmids (**Tables S1** and **S2**) were transformed into *E. coli* K207-3 for expression of PKS polypeptides. Each transformant was pre-cultured for 6 h at 37 °C and 240 rpm in 5 mL of LB medium containing the appropriate antibiotics. A 1% seed culture was inoculated into 0.3–2 L of Terrific Broth (TB) medium [1.2% tryptone, 2.4% yeast extract, 0.56% (w/v) glycerol, 1.25% K₂HPO₄ and 0.23% KH₂PO₄] supplemented with the appropriate antibiotics and grown at 37 °C until the OD₆₀₀ reached 0.4–0.6. The phosphate salts, K₂HPO₄ and KH₂PO₄, were autoclaved separately from the other medium components and added after cooling. Cultures were cooled on ice for 10 min, induced with 100 μ M IPTG and further incubated at 16 °C overnight.

Cells were harvested by centrifugation at 4 °C and 4,000 $\times$ g for 20 min, washed with 20 mL of lysis buffer [50 mM phosphate buffer, 500 mM NaCl, 20 mM imidazole and 10% (v/v) glycerol, pH 7.5], and centrifuged again under the same conditions. The washed cell pellet was resuspended in lysis buffer supplemented with 1 mM TCEP and lysed by sonication on ice. Cell debris was removed by centrifugation at 4 °C and 30,000 $\times$ g for 50 min. A 2-mL slurry of HisPur Ni-NTA resin (Thermo Fisher Scientific) was added to the clarified lysate and incubated at 4 °C for 1 h. The mixture was loaded onto an Econo-Pac chromatography column (Bio-Rad), and the resin was washed with 30 mL of lysis buffer supplemented with 1 mM TCEP. Bound proteins were eluted with 10 mL of elution buffer [50 mM phosphate buffer, 500 mM NaCl, 250 mM imidazole, and 10% (v/v) glycerol, pH 7.5].

C-terminally His₆-tagged polypeptides, except PikAII, were further purified by size-exclusion chromatography using buffer containing 50 mM phosphate buffer, 150 mM NaCl, and 5% (v/v) glycerol, pH 7.5. Polypeptides containing loop His₆-tags were further purified by ion-exchange chromatography followed by size-exclusion chromatography. Ion-exchange chromatography was

performed using solvent A [50 mM phosphate buffer, pH 7.5, and 1 mM TCEP] and solvent B [50 mM phosphate buffer, pH 7.5, 1 M NaCl and 1 mM TCEP] with the following gradient: 10% B for 10 mL, 10–80% B over 25 mL and 100% B for 3 mL, at a flow rate of 0.5 mL min⁻¹. PikAV (TEII) was stored in buffer containing 100 mM phosphate buffer, pH 7.5, and 20% (v/v) glycerol. All purified proteins were concentrated using Amicon Ultra concentrators (Merck Millipore), aliquoted and stored at –80 °C. Protein purity was examined by SDS–PAGE (**Figs. 3b** and **S1**).

Polyketide purification for calibration curve construction

Purified standards of narbonolide (**1**), 10-dml (**2**), compounds **3–7**, and venemycin used for calibration-curve construction were prepared using *E. coli* K207 cells carrying plasmids encoding the corresponding PKSs. Cells were first pre-cultured for 6 h at 37 °C and 240 rpm in 5 mL of LB medium containing the appropriate antibiotics. A 1% seed culture was then inoculated into production medium [5 g L⁻¹ yeast extract, 10 g L⁻¹ polypeptone, 15 g L⁻¹ glycerol, 10 g L⁻¹ NaCl and 100 mM potassium phosphate buffer (pH 7.6)] containing the appropriate antibiotics in 2.8-L Pyrex Fernbach-style culture flasks fitted with milk filter disks. Cultures were grown at 37 °C and 240 rpm until the OD₆₀₀ reached 0.6. The cultures were then cooled to the production temperature, supplied with 60 mM sodium propionate and 0.1 mM IPTG, and incubated at 240 rpm for 6 d. Production was carried out at 19 °C for compounds **3**, **6**, **7**, and venemycin, and at 16 °C for compounds **1**, **2**, **4** and **5**. Purification of these compounds was performed as previously reported^{1–6}.

Time-course assays and design comparison for narbonolide-producing PKS platforms

Narbonolide (**1**)-producing reactions were performed in a defined in vitro system with a final volume of 100 µL containing 400 mM potassium phosphate buffer (pH 7.5), PKS polypeptides, and substrates at the indicated concentrations. All reactions were initiated by adding a substrate mixture containing methylmalonyl-CoA, malonyl-CoA and NADPH. For the TCEP-dependence assay, **NAR**₁ reactions were performed with 2 µM of each PKS polypeptide and final substrate concentrations of 1.2 mM methylmalonyl-CoA, 0.2 mM malonyl-CoA, and 1.2 mM NADPH, in the presence or absence of TCEP as indicated. For the **NAR**₁ synthase-concentration time-course

assay, each PKS polypeptide was tested at the indicated final concentrations (0.5, 1, 2, or 4 μ M synthase) using 6 mM methylmalonyl-CoA, 1 mM malonyl-CoA, and 6 mM NADPH.

For comparison of **NAR**₁–**NAR**₉ designs, each PKS polypeptide was used at 1 μ M, and reactions were quenched after 2 min. Further **NAR**₈ synthase-concentration assays were performed at 31 °C, and all **NAR**₈-related assays were conducted at this temperature. For TEII assays, reactions were performed in the presence or absence of 3.5 μ M TEII as indicated. **NAR**₈ reactions containing 4 μ M PKS were quenched after 1 min because of rapid consumption of substrates, whereas **NAR**₈ reactions containing 2 μ M PKS were quenched after 2 min. TEII assays with 2 μ M **NAR**₁ were also quenched after 2 min.

For time-course analysis, 100- μ L reaction mixtures were aliquoted into 10- μ L portions, and each aliquot was quenched at the indicated time point by rapid addition of acetonitrile. Precipitated material was removed by centrifugation at 15,000 $\times$ g for 10 min. When necessary, samples were further diluted with acetonitrile before quantification. The supernatant (10 μ L) was analyzed by LC/MS using a 6230 TOF LC/MS equipped with a ZORBAX Eclipse Plus C18 column (2.1 $\times$ 50 mm, 1.8 μ m). The following solvent system and gradient were used: solvent A, water with 0.1% (v/v) formic acid; solvent B, acetonitrile with 0.1% (v/v) formic acid; 2% B for 2 min, 2–100% B over 10 min and 100% B for 3 min, at a flow rate of 0.2 mL min⁻¹ in positive-ion mode. Quantification was performed using calibration curves generated from purified standards.

Time-course assays of engineered PKS platforms

Time-course assays for the engineered PKS (**DML**₁, **Δ P3**₁, **Δ P6**₁, and **ANH**₁) platforms were performed under the same general reaction and analytical conditions described above. Reactions contained 400 mM potassium phosphate buffer (pH 7.5), PKS polypeptides, and the substrate mixture required for each platform. Substrate compositions were adjusted according to the predicted extender-unit requirements of each engineered assembly line; malonyl-CoA was included only for platforms requiring a malonyl extender unit and omitted for platforms in which malonyl-CoA was not required. Reactions were initiated by addition of the appropriate substrate mixture and sampled at the indicated time points. Each aliquot was quenched with acetonitrile, centrifuged at 15,000 $\times$ g for 10 min, and diluted with acetonitrile as needed before LC/MS analysis. Product formation was quantified using calibration curves generated from purified standards.

In vitro reconstitution of venemycin

Reactions were performed in a final volume of 100 μ L containing 400 mM potassium phosphate buffer (pH 7.5), 5 mM TCEP, 15 mM MgCl_2 , 10 mM ATP, VemG, VemH, and substrates at the desired concentrations. Reactions were initiated by adding a substrate mixture to give final concentrations of 0.75 mM 3,5-dihydroxybenzoic acid (DHBA) and 2 mM malonyl-CoA. For time-course analysis, the reaction mixture was aliquoted into 10- μ L portions, and each aliquot was quenched at the indicated time point by rapid addition of methanol. Precipitated material was removed by centrifugation at $15,000 \times g$ for 10 min. The supernatant (10 μ L) was analyzed by HPLC using a Waters 1525 HPLC system equipped with a Microsorb-MV 300–5 C18 column (4.6 $\times$ 250 mm). The following solvent system and gradient were used: solvent A, water with 0.1% (v/v) formic acid; solvent B, acetonitrile with 0.1% (v/v) formic acid; 2% B for column equilibration, 2–100% B over 15 min, and 100% B for 3 min, at a flow rate of 1 mL min^{-1} .

In vitro assays for E6- and R5-derived domain-combination designs

For the comparison of **E6**- and **R5**-derived domain combinations, assays were conducted in a final volume of 20 μ L containing 400 mM potassium phosphate buffer (pH 7.5), PKS polypeptides, methylmalonyl-CoA, malonyl-CoA, and NADPH. A master mix containing the shared reaction components and PKS polypeptides was prepared and dispensed into individual reaction tubes. The design-specific exchanged polypeptides were then added to give a final concentration of 2 μ M for each PKS component. Reactions were initiated by adding a substrate mixture containing methylmalonyl-CoA, malonyl-CoA and NADPH to bring the final reaction volume to 20 μ L and to give final concentrations of 3 mM methylmalonyl-CoA, 0.5 mM malonyl-CoA, and 3 mM NADPH. Reactions were incubated for 4 min and quenched with acetonitrile. After centrifugation at $15,000 \times g$ for 10 min, the supernatants were diluted with acetonitrile as needed and analyzed by LC/MS using the method described above. Product formation was quantified using calibration curves generated from purified standards.

Component-titration assays for engineered PKS platforms

Component-titration assays were conducted in a final volume of 20 μ L containing 400 mM potassium phosphate buffer (pH 7.5), PKS polypeptides, and the indicated substrates. For each

titration series, a master mix containing the shared reaction components and non-titrated PKS polypeptides was prepared and dispensed into individual reaction tubes. The titrated polypeptides were then added at the indicated concentrations. For the $\Delta P3_3$ platform, non-titrated PKS components were kept at 1 μM , and $^C P2P4^N$ and $^C P4P5^N$ were varied as indicated. Reactions contained 3 mM methylmalonyl-CoA and 3 mM NADPH, without malonyl-CoA, and were incubated for 5 min. In ΔNH_3 , the core PKS components were kept at 1 μM , and the $^C P5R5^N$ and $^C R5P7$ were varied as indicated. ΔNH_3 reactions contained 3 mM methylmalonyl-CoA, 0.5 mM malonyl-CoA, and 3 mM NADPH, and were incubated for 4 min. Reactions were initiated by adding a substrate mixture to bring the final reaction volume to 20 μL , quenched with acetonitrile, and centrifuged at $15,000 \times g$ for 10 min. The supernatants were diluted with acetonitrile as needed and analyzed by LC/MS using the method described above. Product formation was quantified using calibration curves generated from purified standards.

HPLC analysis of MatB reactions and CoA quantification

All reactions were incubated at 23 °C and quenched by acidification to pH 2–3 with HCl. After pH adjustment, samples were centrifuged at $15,000 \times g$ for 10 min, and the supernatants were analyzed using a Waters 1525 HPLC system equipped with a Microsorb-MV 300–5 C18 column (4.6×250 mm). The following HPLC conditions were used: solvent A, water containing 0.1% (v/v) trifluoroacetic acid (TFA); solvent B, methanol containing 0.1% (v/v) TFA; 2% B for column equilibration, 2–67% B over 20 min and 100% B for 3 min, at a flow rate of 1 mL min⁻¹. Chromatographic signals were monitored at 259.6 nm.

Chiral chromatography

Products from in vitro reactions were analyzed using an Agilent 6230 TOF LC/MS system equipped with a Chiralcel OD-RH column (2.1×150 mm). The following solvent system was used: solvent A, water with 0.1% (v/v) formic acid; solvent B, acetonitrile with 0.1% (v/v) formic acid. Triketide lactone **7** was analyzed by isocratic elution at 4% B for 30 min at a flow rate of 0.8 mL min⁻¹. Products were detected using extracted ion chromatograms (EICs).

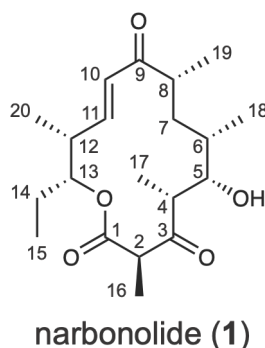
Scaled-up in vitro synthesis, purification, and characterization of narbonolide (1), 10-dml (2), and engineered polyketides (3–5, 8, and 9)

Scaled-up Extender unit to PolyKetide (EPK) reactions were performed using the optimized PKS platforms (**Fig. 6**). Narbonolide (**1**) was produced in a 10-mL reaction (**NAR₉**), whereas engineered polyketides (**DML₄**, **ΔP₃₃**, **ΔP₆₃**, and **ANH₃**) were produced in 20-mL reactions. Unless otherwise indicated, the standard EPK reaction contained 400 mM potassium phosphate buffer (pH 7.5), 4 μM MatB, 4 μM GDH, 2 μM methylmalonyl-CoA epimerase, 3 U (0.1 μM) myokinase, 10 U (0.3 μM) pyruvate kinase, 15 mM MgCl₂, 5 mM ATP, 60 mM methylmalonate, 10 mM malonate, 30 mM glucose, 40 mM phosphoenolpyruvate (PEP), 0.5 mM CoA, and 0.5 mM NADP⁺. For engineered polyketide production, modified EPK conditions were used in which GDH was increased to 10 μM and glycerol was included at 5% (v/v). PKS component concentrations and PikAV were used as indicated for each optimized platform (**Extended Data Table 1**). Reactions were initiated by addition of PKS polypeptides and PikAV where indicated, incubated at 23 °C for the indicated time, extracted with ethyl acetate and concentrated in vacuo. Semipreparative HPLC purification was performed using a Waters 1525 HPLC system equipped with a Waters Atlantis T3 OBD prep column (250 × 10 mm). Solvent A was water with 0.1% (v/v) formic acid, and solvent B was acetonitrile with 0.1% (v/v) formic acid. The flow rate was 4 mL min⁻¹.

Narbonolide (1)

Narbonolide (**1**) was produced in a 10-mL scaled-up EPK reaction using the optimized narbonolide-producing assembly line **NAR₉**. After incubation at 23 °C for 2 h, the reaction mixture was extracted three times with an equal volume of ethyl acetate. The combined organic extracts were dried and concentrated in vacuo. The crude extract was then purified by semipreparative HPLC under isocratic conditions using 50% solvent B, with UV absorbance monitored at 227 nm. Narbonolide (**1**) eluted as the major peak at 21 min, and this fraction was collected and concentrated. Removal of the solvent afforded 7.2 mg of narbonolide (**1**) as a white powder.

^1H NMR (400 MHz, CDCl_3) δ 6.97–6.83 (m, 1H), 6.10 (d, $J = 16.4$ Hz, 1H), 5.15 (m, 1H), 3.86 (t, $J = 5.1$ Hz, 1H), 3.72 (q, $J = 7.0$ Hz, 1H), 3.04 (s, 1H), 2.68 (d, $J = 12.7$ Hz, 2H), 1.76–1.56 (m, 6H), 1.51 (s, 1H), 1.36 (d, $J = 7.0$ Hz, 4H), 1.18–1.04 (m, 11H), 0.96–0.89 (m, 8H). The spectroscopic data were consistent with those previously reported for narbonolide. The identity and absolute configuration of narbonolide (**1**) were further confirmed by X-ray diffraction analysis (Fig. 6).

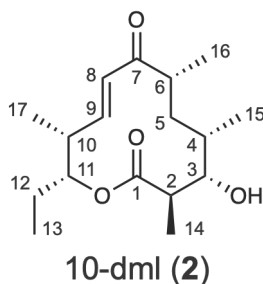


10-Deoxymethynolide (10-dml, **2**)

10-dml (**2**) was produced in a 20-mL scaled-up EPK reaction using the optimized engineered 10-dML-producing assembly line **DML**₄ under the modified EPK conditions described above. After incubation at 23 °C for 8 h, the reaction mixture was extracted three times with an equal volume of ethyl acetate. The combined organic extracts were dried and concentrated in vacuo. The crude extract was purified by semipreparative HPLC under isocratic conditions using 45% solvent B, with UV absorbance monitored at 227 nm. 10-dml (**2**) eluted as the major peak at 18 min, and this fraction was collected and concentrated. Removal of the solvent afforded 4.6 mg of 10-dml (**2**) as a colorless oil.

^1H NMR (500 MHz, CDCl_3) δ 6.75 (dd, $J = 15.7, 5.3$ Hz, 1H, H-9), 6.42 (d, $J = 15.7$ Hz, 1H, H-8), 5.00 (ddd, $J = 8.4, 5.5, 2.4$ Hz, 1H, H-11), 3.56 (d, $J = 10.4$ Hz, 1H, H-3), 2.58 (ddtd, $J = 37.8, 17.4, 7.1, 2.4$ Hz, 3H, H-10, H-2 and H-6), 1.65 (m, 3H, H-12ab and H-5a), 1.37–1.26 (m, 5H, H-4, H-5b and H-14), 1.22 (d, $J = 7.0$ Hz, 3H, H-16), 1.12 (d, $J = 6.8$ Hz, 3H, H-17), 1.01 (d, $J = 6.2$ Hz, 3H, H-15), 0.92 (t, $J = 7.5$ Hz, 3H, H-13).

^{13}C NMR (126 MHz, CDCl_3) δ 205.08 (C7), 174.78 (C1), 147.15 (C9), 125.65 (C8), 78.20 (C3), 73.70 (C11), 45.15 (C6), 43.34 (C2), 38.04 (C10), 33.20 (C5 and C4), 25.16 (C12), 17.72 (C16), 17.46 (C15), 16.43 (C14), 10.34 (C13), 9.59 (C17).

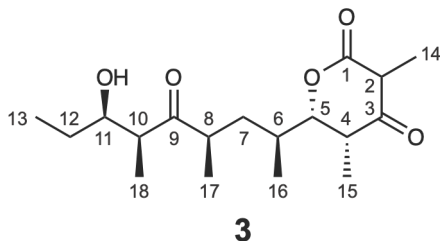


Compound **3**

Compound **3** was produced in a 20-mL scaled-up EPK reaction using the optimized ΔP3 platform, ΔP3_3 , under the modified EPK conditions described above. After incubation at 23 °C for 8 h, the reaction mixture was acidified to pH 2–3 with HCl before extraction. The acidified reaction mixture was then extracted three times with an equal volume of ethyl acetate. The combined organic extracts were dried and concentrated in vacuo. The crude extract was purified by semipreparative HPLC under isocratic conditions using 35% solvent B, with UV absorbance monitored at 248 nm. Compound **3** eluted as the major peak at 19 min, and this fraction was collected and concentrated. Removal of the solvent afforded 3.6 mg of compound **3** as a colorless oil.

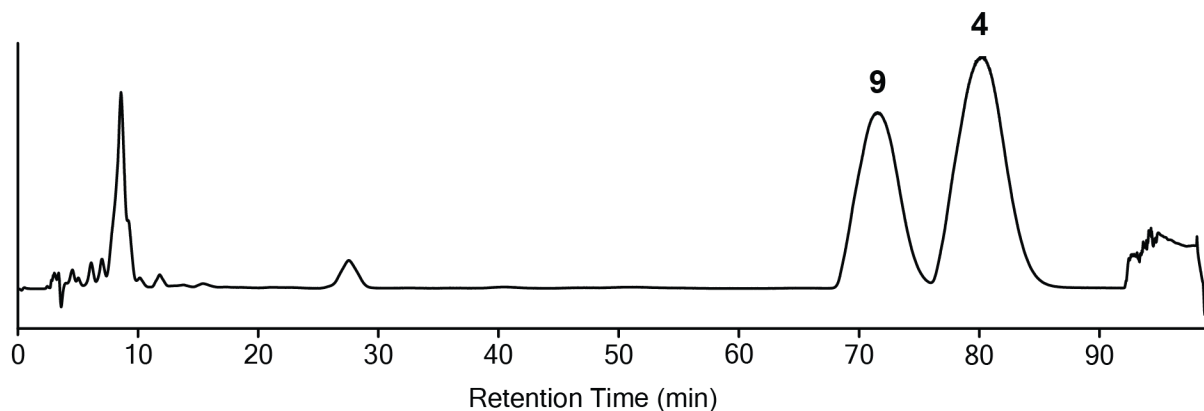
^1H NMR (500 MHz, CDCl_3) δ 4.34 (dd, J = 9.9, 2.6 Hz, 1H, H-5), 3.79 (ddd, J = 8.4, 5.6, 3.0 Hz, 1H, H-11), 3.61 (q, J = 6.6 Hz, 1H, H-2), 2.99 (q, J = 7.0 Hz, 1H, H-8), 2.82 (dt, J = 7.2, 3.6 Hz, 1H, H-10), 2.69 (qd, J = 7.5, 2.5 Hz, 1H, H-4), 2.30 – 2.21 (m, 1H, H-7a), 1.91 – 1.83 (m, 1H, H-6), 1.59 – 1.51 (m, 1H, H-12a), 1.42 (td, J = 7.9, 3.7 Hz, 1H, H-12a), 1.35 (d, J = 6.6 Hz, 3H, H-14), 1.20 – 1.15 (m, 4H, H-17, H-7b), 1.13 (m, 6H, H-15, H-18), 1.00 – 0.92 (m, 6H, H-13, H-16).

^{13}C NMR (126 MHz, CDCl_3) δ 205.43 (C3), 169.58 (C1), 81.74 (C5), 72.72 (C11), 50.24 (C2), 47.79 (C8), 43.88 (C10), 43.13 (C4), 37.10 (C7), 31.61 (C6), 26.94 (C12), 17.52 (C17), 15.93 (C13), 10.52 (C16), 9.88 (C15), 9.74 (C18), 8.07 (C14).



Compound 4 and its C-2 epimer, compound 9

Compound **4** and its C-2 epimer, compound **9**, were produced in a 20-mL scaled-up EPK reaction using the optimized ΔP6_3 platform under the modified EPK conditions described above. After incubation at 23 °C for 8 h, the reaction mixture was extracted three times with an equal volume of ethyl acetate. The combined organic extracts were dried and concentrated in vacuo. The crude extract was purified by semipreparative HPLC under isocratic conditions using 45% solvent B, with UV absorbance monitored at 227 nm. Two closely eluting major peaks were observed and collected separately. The earlier-eluting peak at 73 min afforded 1.3 mg of compound **9** as a colorless oil, whereas the later-eluting peak at 81 min afforded 2.7 mg of compound **4** as a white powder. Detailed 1D and 2D NMR analyses, including ^1H NMR, ^{13}C NMR, HSQC, COSY and HMBC, are provided and support assignment of compounds **4** and **9** as a pair of C-2 epimers that differ in the configuration of the methyl-bearing C-2 stereocenter. The identity and configuration of compound **4** were further supported by X-ray diffraction analysis.

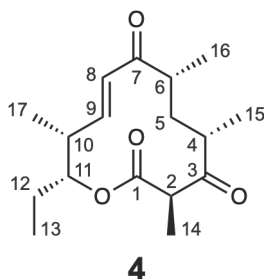


Compound 4

HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{17}H_{27}O_4^+$, 295.1904; found 295.1898 (-1.99 ppm).

1H NMR (500 MHz, $CDCl_3$) δ 6.84 (dd, $J = 15.8, 5.1$ Hz, 1H, H-9), 6.56 – 6.44 (m, 1H, H-8), 5.10 (ddd, $J = 8.4, 5.7, 2.5$ Hz, 1H, H-11), 3.57 (q, $J = 7.1$ Hz, 1H, H-2), 2.73 (t, $J = 6.8$ Hz, 1H, H-10), 2.61 (ddq, $J = 13.6, 6.9, 3.9$ Hz, 1H, H-6), 2.46 (dtt, $J = 12.6, 6.3, 4.0$ Hz, 1H, H-4), 1.95 (s, 1H, H-5a), 1.82 – 1.70 (m, 1H, H-12a), 1.62 (dd, $J = 7.7, 5.9$ Hz, 1H, H-12b), 1.33 (d, $J = 7.3$ Hz, 4H, H-5b and H-14), 1.23 (d, $J = 6.9$ Hz, 3H, H-16), 1.16 (d, $J = 6.8$ Hz, 3H, H-17), 1.03 (d, $J = 6.4$ Hz, 3H, H-15), 0.93 (t, $J = 7.4$ Hz, 3H, H-13).

^{13}C NMR (126 MHz, $CDCl_3$) δ 207.32 (C3), 204.03 (C7), 172.33 (C1), 147.78 (C9), 125.88 (C8), 75.47 (C11), 50.08 (C2), 45.18 (C6), 41.65 (C4), 38.29 (C5), 38.20 (C10), 25.11 (C12), 17.42 (C16), 14.19 (C15), 13.60 (C14), 10.32 (C13), 9.52 (C17).

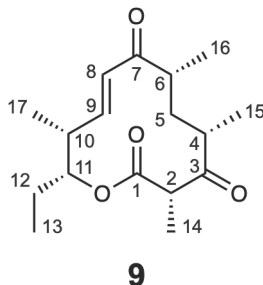


Compound 9

HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{17}H_{27}O_4^+$, 295.1904; found 295.1901 (-0.97 ppm).

1H NMR (500 MHz, $CDCl_3$) δ 6.67 (dd, $J = 15.9, 4.3$ Hz, 1H, H-9), 6.18 (dd, $J = 15.9, 1.8$ Hz, 1H, H-8), 5.14 (ddd, $J = 8.5, 5.5, 2.7$ Hz, 1H, H-11), 3.68 (q, $J = 6.9$ Hz, 1H, H-2), 2.61 (ddq, $J = 28.8, 7.0, 3.6$ Hz, 3H, H-4, H-6, and H-10), 2.22 – 2.12 (m, 1H, H-5a), 1.79 – 1.65 (m, 1H, H-12a), 1.58 (m, 1H, H-12b), 1.39 (ddd, $J = 14.9, 7.1, 3.2$ Hz, 1H, H-5b), 1.26 (d, $J = 6.8$ Hz, 3H, H-14), 1.14 (dd, $J = 12.5, 7.1$ Hz, 6H, H-16 and H-15), 1.04 (d, $J = 6.7$ Hz, 3H, H-17), 0.92 (t, $J = 7.5$ Hz, 3H, H-13).

^{13}C NMR (126 MHz, CDCl_3) δ 207.25 (C3), 204.78 (C7), 169.58 (C1), 147.05 (C9), 127.76 (C8), 76.20 (C11), 52.50 (C2), 43.67 (C6), 43.21 (C4), 38.56 (C10), 36.34 (C5), 24.80 (C12), 18.97 (C15), 18.31 (C16), 11.69 (C14), 10.27 (C13), 9.42 (C17).



Compound 5 and the related linear decarboxylated product (8)

Compound **5** was produced in a 20-mL scaled-up EPK reaction using the optimized ANH_3 platform under the modified EPK conditions described above. After incubation at 23 °C for 8 h, the reaction mixture was extracted three times with an equal volume of ethyl acetate. The combined organic extracts were dried and concentrated in vacuo. The crude extract was purified by semipreparative HPLC under isocratic conditions using 58% solvent B, with UV absorbance monitored at 227 nm. During purification of compound **5**, an additional product peak was observed and collected separately. The major peak corresponding to compound **5** eluted at 16 min and afforded 0.9 mg of compound **8** as a colorless oil. The additional peak eluting at 32 min afforded 5.1 mg of a related linear decarboxylated product as a colorless oil. Detailed 1D and 2D NMR analyses, including ^1H NMR, ^{13}C NMR, HSQC, COSY, and HMBC are provided and support assignment of this second product as a linear molecule derived from the fully elongated ANH_3 platform intermediate after decarboxylation rather than macrolactonization.

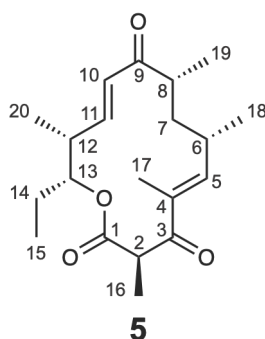
Compound 5

HRMS (ESI-TOF) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{31}\text{O}_4^+$, 335.2217; found 335.2218 (0.30 ppm).

^1H NMR (500 MHz, CDCl_3) δ 6.69 (dd, J = 16.7, 6.7 Hz, 1H, H-11), 6.39 (dd, J = 10.0, 1.5 Hz, 1H, H-5), 5.91 (dd, J = 16.7, 1.5 Hz, 1H, H-10), 5.04 (ddd, J = 8.7, 4.9, 3.4 Hz, 1H, H-13), 4.30 (q, J = 6.9 Hz, 1H, H-2), 2.85 (dtd, J = 12.4, 6.5, 2.6 Hz, 1H, H-6), 2.77 (dtt, J = 12.7, 6.3, 3.2 Hz,

1H, H-8), 2.70 (dddd, $J = 10.3, 8.1, 4.0, 2.4$ Hz, 1H, H-12), 1.90 (d, $J = 1.3$ Hz, 3H, H-17), 1.67 – 1.55 (m, 3H, H-14a, H-14b, and H-7a), 1.45 – 1.39 (m, 4H, H-7b and H-16), 1.13 (d, $J = 7.0$ Hz, 3H, H-20), 1.11 (d, $J = 6.4$ Hz, 3H, H-19), 1.07 (d, $J = 6.6$ Hz, 3H, H-18), 0.92 (t, $J = 7.4$ Hz, 3H, H-15).

^{13}C NMR (126 MHz, CDCl_3) δ 203.91 (C9), 195.85 (C3), 171.21 (C1), 148.72 (C11), 147.16 (C5), 138.06 (C4), 129.15 (10), 77.22 (C13, overlapped with CDCl_3), 45.66 (C2), 43.11 (C7), 39.17 (C8), 38.19 (C12), 32.87 (C6), 23.83 (C14), 21.24 (C18), 14.81 (C19), 13.85 (C16), 12.87 (C20), 12.42 (C17), 10.22 (C15).

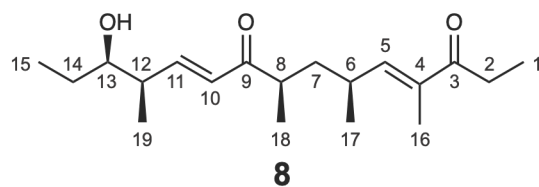


Compound 8

HRMS (ESI-TOF) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{33}\text{O}_3^+$, 309.2424; found 309.2430 (1.88 ppm).

^1H NMR (500 MHz, CDCl_3) δ 6.79 (dd, $J = 15.8, 7.9$ Hz, 1H, H-11), 6.27 (d, $J = 10.0$ Hz, 1H, H-5), 6.15 – 6.07 (m, 1H, H-10), 3.49 (dt, $J = 8.8, 4.4$ Hz, 1H, H-13), 2.66 (td, $J = 9.8, 6.0$ Hz, 4H, H-2b, H-2b, H-6, and H-8), 2.42 (q, $J = 6.7$ Hz, 1H, H-12), 1.85 (ddd, $J = 13.7, 9.6, 7.4$ Hz, 1H, H-7a), 1.78 (d, $J = 1.4$ Hz, 3H, H-16), 1.41 (ddt, $J = 32.8, 15.6, 6.4$ Hz, 3H, H-7a, H-14a, and H-14b), 1.08 (dd, $J = 7.1, 4.5$ Hz, 9H, H-1, H-18, and H-19), 1.04 (d, $J = 6.6$ Hz, 3H, H-17), 0.98 (t, $J = 7.4$ Hz, 3H, H-15).

^{13}C NMR (126 MHz, CDCl_3) δ 203.27 (C9), 203.04 (C3), 149.51 (C11), 147.25 (5), 135.58 (C4), 128.59 (C10), 75.93 (C13), 42.53 (C8), 42.35 (C12), 40.04 (C7), 32.37 (C6), 30.45 (C2), 27.29 (C14), 20.37 (C17), 17.62 (C18), 13.89 (C19), 11.62 (C16), 10.37 (C15), 8.67 (C1).



Crystallization of narbonolide (**1**) and compound **4**

For crystallization, purified narbonolide (**1**) was dissolved in hexanes/ethyl acetate (65:35), and compound **4** was dissolved in hexanes/ethyl acetate (75:25). Each solution was filtered through a cotton plug and allowed to evaporate slowly at room temperature overnight.

Table S1. Construction of the expression plasmids for PKSs that yield narbonolide (**1**), **3**, **4**, and **5**. Underlined sequences are homology sequences for Gibson assembly.

Plasmid	Method	Piece	Template	Primers
pJZ101 P1P2^N (NAR_I) vector: pET28b	2-piece Gibson	P1KS^Q+P1AT+P1ACP+ P1KS+P2AT+P2KR+P2 ACP+ S8^CDD+His₆	pCDF-1b- P1-P2- P3-P4	<u>TAAGAAGGAGATATACCATGTCTTC</u> <u>AGCCGGAATTACCAGGACC</u> <u>CAGTGGTGGTGGTGGTGGTGGCGCC</u> <u>GGAACCTCCGGTCGAT</u>
		pET28b	pET28b Vector	<u>TCGACCGGAAGTTCCGGCGCCACCA</u> <u>CCACCACCACCTGA</u> <u>CTGGTAATTCCGGCTGAAGACATGG</u> <u>TATATCTCCTTCTTAAAGTTAA</u>
pJZ102 ^CP2P3^N (NAR_I) vector: pET28b	2-piece Gibson	S8^NDD+P2KS+P3AT+P3 DH+ P3KR+P3ACP+S3^C DD+His ₆	pCDF-1b- P1-P2- P3-P4	<u>TAAGAAGGAGATATACCATGGCCAA</u> <u>TGAAGAAAAGCTCCGCGAG</u> <u>CAGTGGTGGTGGTGGTGGTGGGGAA</u> <u>GTTTCGAGATCGTTGTCGAG</u>
		pET28b	pET28b Vector	<u>ACAACGATCTCGAAGTTCCCCACCAC</u> <u>CACCACCACCTGA</u> <u>CGGAGCTTTTCTTCATTGGCCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>
pJZ103 ^CP3P4^N (NAR_I) vector: pET28b	2-piece Gibson	S3^NDD+P3KS+P4AT+ P4KR⁰+P4ACP+E3^CDD+ His ₆	pCDF-1b- P1-P2- P3-P4	<u>TAAGAAGGAGATATACCATGTTCGAA</u> <u>TGAAGAGAAGCTCCGGGAG</u> <u>CAGTGGTGGTGGTGGTGGTGATCGC</u> <u>CGTCGAGCTCCCGGCC</u>
		pET28b	pET28b Vector	<u>GCCGGGAGCTCGACGGCGATCACCA</u> <u>CCACCACCACCTGA</u> <u>CGGAGCTTCTTTCATTTCGACATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ104 ^CP4P5^N (NAR_i) vector: pET28b	2-piece Gibson	E3^NDD+P4KS+P5 AT+P5DH+P5KR +P5ER+P5ACP+E 5^CDD+His₆	pET28b-P4-P5-P6-P7	<u>TAAGAAGGAGATATACCATGACTGA</u> <u>CAGCGAGAAGGTGGCGG</u> <u>CAGTGGTGGTGGTGGTGGTGCAAAT</u> <u>CCTCTCCCCACCAAAAC</u>
		pET28b	pET28b Vector	<u>TTGGTGGGGGAGAGGATTTGCACCA</u> <u>CCACCACCACCACTGA</u> <u>GCCACCTTCTCGCTGTCAGTCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ105 ^CP5P6^N (NAR_i) (x₆) vector: pET28b	2-piece Gibson	E5^NDD+P5KS+P6 AT+P6KR+P6AC P+S5^CDD+His₆	pET28b-P4-P5-P6-P7	<u>TAAGAAGGAGATATACCATGAGCGG</u> <u>TGACAACGGCATGACCGAG</u> <u>CAGTGGTGGTGGTGGTGGTGGGACA</u> <u>GGTCGAGCTCGTTGTCG</u>
		pET28b	pET28b Vector	<u>ACAACGAGCTCGACCTGTCCACCA</u> <u>CCACCACCACCACTGA</u> <u>GTCATGCCGTGTGCACCGCTCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ106 ^CP6P7 (NAR_i) vector: pET28b (y₁)	2-piece Gibson	S5^NDD+P6KS+P7 AT+P7ACP+TE+ His₆	pET28b-P4-P5-P6-P7	<u>TAAGAAGGAGATATACCATGGCCAA</u> <u>TGAAGAAAAGCTCTTCGGCT</u> <u>CAGTGGTGGTGGTGGTGGTGCTTGCC</u> <u>CGCCCCCTCGATGCC</u>
		pET28b	pET28b Vector	<u>GCATCGAGGGGGCGGGCAAGCACCA</u> <u>CCACCACCACCACTGA</u> <u>AAGAGCTTTTCTTCATTGGCCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ107 PikAV (thioesterase II, TEII) vector: pACYCDue t-1	Restriction digestion + Ligation	PikAV	<i>Streptomyces venezuelae</i> ATCC 15439 genomic DNA	CCC GAATTC GACCGACAGACCTCTG AACGTGGA CCC AGCTT TCACCGTGGGTCTGCC ATCTCT
		pACYCDuet-1		

pJZ108 P1P2^N (NAR₂) vector: pET28b	2-piece Gibson	P1KS^Q+P1AT+P1ACP+ P1KS+P2AT+P2KR+P2 ACP+S8^CDD+His₆	pJZ101	<u>AAGAGGCCCCGGTGGTTGTCGAGG</u> <u>GTGCTTCGGTCGTCGAG</u> <u>AGCAGCGATTTCGATGCCGTTCTCCT</u> <u>CGTCACCGAGGAACTCGC</u>
		pET28b	pJZ101	<u>AGTTCCTCGGTGACGAGGAGAACG</u> <u>GCATCGAATCGCTGCTCG</u> <u>TCGACGACCGAAGCACCTCGACA</u> <u>ACCACCGGGCCTCTTCGA</u>

pJZ109 ^CP2P3^N (NAR₂) vector: pET28b	3-piece Gibson	P2KS	pJZ102	<u>GGAGCGCCAGGAGCACGACCCGATC</u> <u>GCGATCGTCGCGATGAGCTGCCGCT</u> A <u>TCGGCCCCAAGCACCTCACCAGCAG</u> <u>CATCCGGCGCCTGCTCCAGCA</u>
		P3AT+P3DH+P3KR+P3 ACP+P3^CDD	<i>Streptomyces</i> <i>venezuelae</i> ATCC 15439 genomic DNA	<u>AGCAGGCGCCGGATGCTGCTGGTGA</u> <u>GGTGCTTGGGGCCGATGAGGT</u> <u>CAGTGGTGGTGGTGGTGGTGGTCCGT</u> <u>GCTGGGGTCTTGGTCGAG</u>
		His ₆ +pET28b+S8 ^N DD	pJZ102	<u>ACCAGGACCCAGCACGGACCACCA</u> <u>CCACCACCACCTGAGAT</u> <u>ATCGCGACGATCGCGATCGGGTCGT</u> <u>GCTCCTGGCGCTCCAACCTCGT</u>

pJZ110 ^CP3P4^N (NAR₂) vector: pET28b	3-piece Gibson	P3^NDD+P3KS	<i>Streptomyces</i> <i>venezuelae</i> ATCC 15439 genomic DNA	<u>TAAGAAGGAGATATACCATGTCCAC</u> <u>GGTGAACGAAGAGAAGTACCTCGAC</u> T <u>GCCTCGGAGGCAGGGGTCTCCTCGG</u> <u>CCGCCGGGGCCTCTTCGA</u>
		P4AT+P4KR⁰+P4ACP	pJZ103	<u>AAGAGGCCCCGGCGGCCGAGGAGAC</u> <u>CCCTGCCTCCGAGGCGAC</u> <u>AGTTCCTCGAATGTCCCGGCACCGGC</u> <u>CTGCTCGCCCAGGAT</u>
		S3^CDD+His₆+pET28b	pJZ102	<u>TCCTGGGCGAGCAGGCCGGTGCCGG</u> <u>GACATTGAGGAACTCGACA</u> <u>TTCTCTTCGTTACCCGTGGACATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ111 ^CP4P5^N (NAR₂) vector: pET28b	3-piece Gibson	P4KS	pJZ104	<u>AGTCGGGAGAGCGGGAACCCGTCGC</u> <u>GATCGTCGGCATGGCGTG</u> <u>GGCTCGTCCGCCGGGGCGTCCTCGTC</u> <u>GACCGGGGCTTCTTCGAGCA</u>
		P5AT+P5DH+P5KR+P5ER+P5AC P+P5^CDD	pRB1 ⁵	<u>CGAAGAAGCCCCGGTCGACGAGGAC</u> <u>GCCCCGGCGGACGAGCCGT</u> <u>CAGTGGTGGTGGTGGTGGTGGAAAGT</u> <u>CGGAGTCGCCGACTCCTTGTC</u>
		His ₆ +pET28b+S3 ^N DD	pJZ103	<u>AGCTGGGCGACTCCGACTTCCACCAC</u> <u>CACCACCACCTG</u> <u>ATGCCGACGATCGCGACGGGTTCCC</u> <u>GCTCTCCCGACTCCGCCTCGT</u>

pJZ112 ^CP5P6^N (NAR₂) vector: pET28b	2-piece Gibson	PikAIII: P5^NDD+P5KS+P6 AT+P6KR+P6AC P+P6^CDD+His₆	pRB2 ⁵	<u>TAAGAAGGAGATATACCATGGCGAA</u> <u>CAACGAAGACAAGCTCCG</u> <u>CAGTGGTGGTGGTGGTGGTGGTGGT</u> <u>ACGGGGGCCGAGAGC</u>
		pET28b	pET28b	<u>GCTCTCGGCCCCGTAACACCCACCA</u> <u>CCACCACCACCTGA</u> <u>CGGAGCTTGTCTTCGTTGTTCCGCAT</u> <u>GGTATATCTCCTTCTAAAGTTAA</u>

pJZ113 ^CP6P7 (NAR₂) vector: pET28b	2-piece Gibson	PikAIV: P6^NDD+P6KS+P7 AT+P7ACP+TE+ His₆	pTM5 ⁴	<u>TAAGAAGGAGATATACCATGACGAG</u> <u>TTCCAACGAACAGTTGGTG</u> <u>CAGTGGTGGTGGTGGTGGTGGTGGC</u> <u>CGCCCCCTCGATGCC</u>
		pET28b	pET28b	<u>GCATCGAGGGGGCGGGCAAGCACCA</u> <u>CCACCACCACCTGA</u> <u>AACTGTTGTTGGAACCTCGTCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ114 PikAII (NAR ₃) vector: pET28b	3-piece Gibson	pET28b+ P3^NDD + P3KS+P4AT	pJZ110	<u>AGCTGGGCGACTCCGACTTCCACCAC</u> <u>CACCACCACCACTG</u> <u>CCGGTGACGAGACCGTGCCGTCGG</u> <u>CCTGCCACCACGGCGA</u>
		P4KR⁰+P4ACP	<i>Streptomyces venezuelae</i> ATCC 15439 genomic DNA	<u>CGCCGTGGTGGCAGGCCGACGGCAC</u> <u>GGTGCTCGTCACCGGT</u> <u>ATGCCGACGATCGCGACGGGCTCGT</u> <u>CGTCGACCCCGCCGTC</u>
		P4KS+P5AT+P5D H+P5KR+P5ER+P 5ACP+P5^CDD+His S ₆	pJZ111	<u>ACGGCGGGGTCGACGACGAGCCCGT</u> <u>CGCGATCGTCGGCAT</u> <u>CAGTGGTGGTGGTGGTGGGAAGT</u> <u>CGGAGTCGCCACGCTCCTTGTC</u>

pJZ115 PikAII (NAR ₅) vector: pET28b	3-piece Gibson	P4AT+P4KR⁰+P4 ACP	pJZ114	<u>AGGAGACCCCTCACCACCACCACCA</u> <u>CCATGCCGTCGAGCCGTCG</u> <u>GCTCGTCGTCGACCCCGCCGTCCAC</u>
		P4KS+P5AT+P5D H+P5KR+P5ER+P 5ACP+P5^CDD	pJZ114	<u>GGCGAGCAGCTTCCGGTGGACGGC</u> <u>TTGTTAGCAGCCGGATCTCAGAAGTC</u> <u>GGAGTCGCCACGCTCCTTG</u>
		pET28b+ P3^NDD + P3KS+His₆	pJZ114	<u>AGCTGGGCGACTCCGACTTCTGAGAT</u> <u>CCGGCTGCTAACAAGCCCG</u> <u>GGCTCGACGGCATGGTGGTGGTGGT</u> <u>GGTGAGGGGTCTCCTCGGCC</u>

pJZ116 P1P2^N (NAR ₆) vector: pET28b	3-piece Gibson	P1KS^Q+P1AT+P1ACP+ P1KS+His₆	pJZ108	<u>TAAGAAGGAGATATACCATGTCTTC</u> <u>AGCCGGAATTACCAGGACC</u> <u>ACATGGTGGTGATGGTGGTGAGCA</u> <u>CCCTCGACAACCACCGGGG</u>
		P2AT+P2KR+P2ACP+S 8^CDD	pJZ108	<u>CTCACCACCATCACCACCATGTTGG</u> <u>CGGGTCGGCGGTCGGCGGC</u> <u>TTGTTAGCAGCCGGATCTCAGCGCC</u> <u>GGAACTTCCGGTCGATGAG</u>
		pET28b	pJZ108	<u>TCGACCGGAAGTTCCGGCGCTGAG</u> <u>ATCCGGCTGCTAACAAGCCCG</u> <u>CTGGTAATTCCGGCTGAAGACATGG</u> <u>TATATCTCCTTCTTAAAGTTAA</u>

pJZ117 P1^N (NAR ₈) vector: pCDF-1b	2-piece Gibson	His ₆ + P1KS^Q + P1AT + P1A CP	pJZ108	<u>TGCATCACCACCACCATCACTCTTC</u> <u>AGCCGGAATTACCAGGACCGGT</u> <u>AGCCCGGCGAGGGCCGACGGCGCC</u> <u>GCCTCCCCGTGCACGACGAG</u>
		E3^CDD +pCDF-1b	pCDF-1b- P1-P2- P3-P4	<u>TCGTGCACGGGGAGGCGGCGCCGT</u> <u>CGGCCCTCGCCGGGCTGGAC</u> <u>GAGTGATGGTGGTGGTGCATG</u> <u>GTATATCTCCTTATTAAAGTTAA</u>

pJZ118 ^CP1P2^N (NAR ₈) vector: pET28b	2-piece Gibson	P1KS +His ₆ + P2AT + P2K R+ P2ACP + S8^CDD	pJZ116	<u>TCCGCGAGCTGGAATCCGACCCGGT</u> <u>GCGGATCGTCGGCATGGC</u> <u>TTGTTAGCAGCCGGATCTCAGCGCC</u> <u>GGAAGTTCCGGTCGATGAG</u>
		pET28b+ E3^NDD	pET28b- P4-P5-P6- P7	<u>TCGACCGGAAGTTCCGGCGCTGAG</u> <u>ATCCGGCTGCTAACAAAGCCCG</u> <u>ATGCCGACGATCGCCACCGGGTCG</u> <u>GATTCCAGCTCGCGGATGCG</u>

pJZ119 ^CP2P3^N (NAR ₈) vector: pET28b	2-piece Gibson	P3AT + P3DH + P3KR + P3 ACP+ P3^CDD	pJZ109	<u>AGCACCACCACCACCATACGGC</u> <u>TGGGACCTCCGAGGTGCGT</u> <u>TTGTTAGCAGCCGGATCTCAGTCCGT</u> <u>GCTGGGGTCTTGGTCGAG</u>
		pET28b+ S8^NDD + P2KS + His ₆	pJZ109	<u>ACCAGGACCCCAGCACGGACTGAGA</u> <u>TCCGGCTGCTAACAAAGCCCG</u> <u>GTATGGTGGTGGTGGTGGTGC</u> <u>T</u> CAG ACACCTCAGGCACCTCATCGG

pJZ120 ^CP3P4^N (NAR ₈) vector: pET28b	2-piece Gibson	P4AT + P4KR⁰ + P4ACP + S 3^CDD	pJZ110	<u>CTCACCACCACCACCATGCCGTC</u> <u>GAGCCGTGCGTCGGCG</u> <u>TTGTTAGCAGCCGGATCTCAGGGAA</u> <u>GTTCGAGATCGTTGTGCGAGGAG</u>
		pET28b+ P3^NDD + P3KS + His ₆	pJZ110	<u>ACAACGATCTCGAACTTCCCTGAGAT</u> <u>CCGGCTGCTAACAAAGCCCG</u> <u>GCATGGTGGTGGTGGTGGTGAGGGG</u> <u>TCTCCTCGGCCCGCCGGG</u>

pJZ121 ^CP4P5^N (NAR₈) vector: pET28b	2-piece Gibson	P5AT+P5DH+P5KR+P5ER+P5ACP+P5^CDD	pJZ111	<u>ACCACCACCACCACCACCATGAGCC</u> <u>GTCGGTCGGCGGTGTGGTG</u> <u>TTGTTAGCAGCCGGATCTCAGAAGTC</u> <u>GGAGTCGCCCAGCTCCTT</u>
		pET28b+S3 ^N DD+P4KS+His ₆	pJZ111	<u>AGCTGGGCGACTCCGACTTCTGAGAT</u> <u>CCGGCTGCTAACAAAGCCCG</u> <u>TCATGGTGGTGGTGGTGGTGGTTCGAC</u> <u>CGGGGCTTCTTCGAGCAC</u>

pJZ122 ^CP5P6^N (NAR₈) Vector: pACYCDuet-1	2-piece Gibson	P6AT+P6KR+P6ACP+P6^CDD	pRB2 ⁵	<u>CACCACCACCACCATCACGCCGGTG</u> <u>GCGGTGTGGTGCCG</u> <u>ACAGTCCAGCTTGGAGCGAACTG</u>
		pACYCDuet-1+P5 ^N DD+P5KS+His ₆	pRB2	<u>CTCAGTTCGGGTAGGCAGTTCG</u> <u>GTGATGGTGGTGGTGGTGGGAGTCC</u> <u>TCGACCGCCGGGGC</u>

pJZ123 ^CP6P7 (NAR₈) vector: pET28b	2-piece Gibson	pET28b+P6 ^N DD+P6KS+His ₆	pTM5	<u>CACCACCACCACCATCACGCCGGTG</u> <u>GCGGCGTGGTGCCGTGG</u> <u>TTGTTAGCAGCCGGATCTCACTTGCC</u> <u>CGCCCCCTCGATGCCCTC</u>
		P7AT+P7ACP+TE	pTM5	<u>GCATCGAGGGGGCGGGCAAGTGAGA</u> <u>TCCGGCTGCTAACAAAGCCCG</u> <u>GTGATGGTGGTGGTGGTGGGACTCCT</u> <u>CGACCGCCGGGGCCTC</u>

pJZ124 P1^N (NAR₉) vector: pET28b	2-piece Gibson	P1KS^Q+P1AT+P1ACP+E3^CDD+His₆	pJZ117	<u>TAAGAAGGAGATATACCATGTCTTC</u> <u>AGCCGGAATTACCAGGACC</u> <u>CAGTGGTGGTGGTGGTGGTGATCGC</u> <u>CGTCGAGCTCCCGGCC</u>
		pET28b	pET28b	<u>GCCGGGAGCTCGACGGCGATCACC</u> <u>ACCACCACCACCACTGA</u> <u>CTGGTAATTCCGGCTGAAGACATGG</u> <u>TATATCTCCTTCTTAAAGTTAA</u>

pJZ125 ^C P1P2^N (NAR₉) vector: pET28b	2-piece Gibson	P1KS+P2AT+P2KR+P2 ACP+ S8^CDD +His ₆	pJZ101	<u>TCCGCGAGCTGGAATCCGACCCGGT</u> <u>GGCGATCGTCGGCATGGC</u> <u>CAGTGGTGGTGGTGGTGGTGGCGCC</u> <u>GGAACTTCCGGTCGAT</u>
		pET28b+ E3^NDD	pJZ118	<u>TCGACCGGAAGTTCCGGCGCCACCA</u> <u>CCACCACCACCACTGA</u> <u>ATGCCGACGATCGCCACCGGGTCCG</u> <u>GATTCCAGCTCGCGGATGCG</u>

pJZ126 ^C P2P4^N (ΔP3₁) vector: pET28b	2-piece Gibson	S8^NDD+P2KS+P4AT+P4 KR ⁰ + P4ACP+E3^CDD +Hi S ₆	pCDF-1b- P1-P2-P4	<u>TAAGAAGGAGATATACCATGGCCAA</u> <u>TGAAGAAAAGCTCCGCGAG</u> <u>CAGTGGTGGTGGTGGTGGTGATCGC</u> <u>CGTCGAGCTCCGGGC</u>
		pET28b	pET28b	<u>GCCGGGAGCTCGACGGCGATCACCA</u> <u>CCACCACCACCACTGA</u> <u>CGGAGCTTTTCTTCATTGGCCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ127 ^C P5P7 (ΔP6₁) vector: pET28b	2-piece Gibson	P7AT+P7ACP+TE +His ₆	pJZ106	<u>AGGAGGCCCGGCGGTCGAGTCTAG</u> <u>TTCCCTGCGAGTTGAGCCGCCGG</u> <u>CAGTGGTGGTGGTGGTGGTGCTTGCC</u> <u>CGCCCCCTCGATGCC</u>
		pET28b+ E5^NDD+ P5KS	pJZ105	<u>GCATCGAGGGGGCGGGCAAGCACCA</u> <u>CCACCACCACCACTGA</u> <u>TCAACTGCAGGGGAAGTAGACTCGA</u> <u>CCGCCGGGGCCTCCTCAGG</u>

pJZ128 ^C P5R5^N (ANH₁) vector: pET28b	2-piece Gibson	E5^NDD+P5KS+R5 AT+ R5DH+R5KR + R5ACP+S5^CDD + His ₆	pET28b- P4-P5-R5-P7	<u>TAAGAAGGAGATATACCATGAGCGG</u> <u>TGACAACGGCATGACCGAG</u> <u>CAGTGGTGGTGGTGGTGGTGGGACA</u> <u>GGTCGAGCTCGTTGTCG</u>
		pET28b	pET28b Vector	<u>ACAACGAGCTCGACCTGTCCACCA</u> <u>CCACCACCACCACTGA</u> <u>GTCATGCCGTGTGTCACCGTCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ129 ^C R5P7 (ANH₁) (y ₂) Vector: pET28b	2-piece Gibson	S5^NDD+R5KS+P7 AT+ P7 ACP+TE+ His ₆	pET28b- P4-P5-R5-P7	<u>TAAGAAGGAGATATACCATGGCCAA</u> <u>TGAAGAAAAGCTCTTCGGCT</u> <u>CAGTGGTGGTGGTGGTGGTGCTTGCC</u> <u>CGCCCCCTCGATGCC</u>
		pET28b	pET28b Vector	<u>GCATCGAGGGGGCGGGCAAGCACCA</u> <u>CCACCACCACCACTGA</u> <u>AAGAGCTTTTCTTCATTGGCCATGGT</u> <u>ATATCTCCTTCTTAAGTTAA</u>

pJZ130 ^C P5E6^N (x ₁) vector: pET28b	2-piece Gibson	E5KS	<i>Saccharopolyspora</i> <i>erythraea</i> genomic DNA	<u>CCGGGCCGGTGAGCCAATTGCGATC</u> <u>GTCGGCATGGCGTGCCGGTTC</u> <u>GCCGGCTCGGGCTCACTAGAGTCGG</u> <u>CCTCGGGCGCCTCTTCGACGA</u>
		E6AT+E6KR+E6 ACP+ S5^CDD +His ₆ +pET28b+ E5^NDD	pJZ130	<u>AAGAGGCGCCCCGAGGCCGACTCTAG</u> <u>TGAGCCCCGAGCCGCCACCCGA</u> <u>GCACGCCATGCCGACGATCGCAATT</u> <u>GGCTCACC GGCCCGGTGCTCGA</u>

pJZ131 ^C P5E6^N (x ₂) vector: pET28b	2-piece Gibson	E5^NDD+P5KS+E6 AT+ E6KR+E6AC P+ S5^CDD +His ₆	pET28b- P4-P5-E6-P7⁶	<u>TAAGAAGGAGATATACCATGAGCGG</u> <u>TGACAACGGCATGACCGAG</u> <u>CAGTGGTGGTGGTGGTGGTGGGACA</u> <u>GGTCGAGCTCGTTGTCTG</u>
		pET28b	pET28b Vector	<u>ACAACGAGCTCGACCTGTCCACCA</u> <u>CCACCACCACCACTGA</u> <u>GTCATGCCGTTGTACCCGCTCATGGT</u> <u>ATATCTCCTTCTTAAGTTAA</u>

pJZ132 ^C P5E6^N (x ₃) vector: pET28b	2-piece Gibson	P5KS+P6AT	pJZ105	<u>TAAGAAGGAGATATACCATGAGCGG</u> <u>TGACAACGGCATGACCGAG</u> <u>TCCTCGTCCCGTGCGCGCCCGGGCGC</u> <u>GGAGCTCTGCAGCCAGAAGCGC</u>
		E6KR+E6ACP+S5^C DD +His ₆ +pET28 b+ E5^NDD	pJZ130	<u>GGCTGCAGAGCTCCGCGCCCGGGCGC</u> <u>CGCACGGGACGAGGACGAC</u> <u>GTCATGCCGTTGTACCCGCTCATGGT</u> <u>ATATCTCCTTCTTAAGTTAA</u>

pJZ133 ^c P5E6^N (x ₄) vector: pET28b	2-piece Gibson	E6KR	pJZ130	<u>GGCTGCAGAGCTCCGCGCCCCGGGCG</u> <u>CGCACGGGACGAGGACGAC</u> <u>GCGGCGTCGCCGGGAGCCGGGCCCT</u> <u>CCTCGCTCTGCCGGGCTTCC</u>
		P6ACP+S5^CDD+His₆+pET28b+E5^ND D+P5KS+P6AT	pJZ105	<u>CCCGGCAGAGCGAGGAGGGCCCGGC</u> <u>TCCCGGCGACGCCGCGT</u> <u>TCCTCGTCCCGTGCGCGCCCGGGCGC</u> <u>GGAGCTCTGCAGCCAGAAGCGC</u>

pJZ134 ^c P5E6^N (x ₅) vector: pET28b	2-piece Gibson	E6ACP+S5^CDD+His₆+pET28b	pJZ130	<u>TCGCCGCACCCGTCGGTGCCCCGGCG</u> <u>CTCGCCACGCGGCTCGCG</u> <u>GTCATGCCGTTGTACCCGTCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>
		E5^NDD+P5KS+P6AT+P6KR	pJZ105	<u>TAAGAAGGAGATATACCATGAGCGG</u> <u>TGACAACGGCATGACCGAG</u> <u>AGCCGCTGGGCGAGCGCCGGGGCAC</u> <u>CGACGGGTGCGGCGAGCGC</u>

pJZ135 ^c E6P7 (y ₂) vector: pET28b	2-piece Gibson	S5^NDD+E6KS+P7AT+P7ACP+TE+His₆	pET28b- P4-P5-E6-P7⁶	<u>TAAGAAGGAGATATACCATGGCCAA</u> <u>TGAAGAAAAGCTCTTCGGCT</u> <u>CAGTGGTGGTGGTGGTGGTGCTTGCC</u> <u>CGCCCCCTCGATGCC</u>
		pET28b	pET28b Vector	<u>GCATCGAGGGGGCGGGCAAGCACCA</u> <u>CCACCACCACCTGA</u> <u>AAGAGCTTTTCTTCATTGGCCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ136 ^c E6P7 (y ₃) vector: pET28b	2-piece Gibson	E6KS+E7AT	<i>Saccharopolyspora erythraea</i> genomic DNA	<u>GGAGTCAGGAGCCAATTGCCATCGT</u> <u>CGGCATGGCCTGCCGCTTCC</u> <u>GTGTGCGCGGGCGCCTCGCCGGACA</u> <u>CCTCCGGCGCGAGCCAGTAC</u>
		P7ACP+TE+His₆+pET28b+S5^NDD	pJZ106	<u>GGCTCGCGCCGGAGGTGTCCGGCGA</u> <u>GGCGCCCGCGCACACCGCTT</u> <u>CGGCAGGCCATGCCGACGATGGCAA</u> <u>TTGGCTCCTGACTCCGGCTCTC</u>

pJZ137 ^C P5R5^N (x ₁) vector: pET28b	2-piece Gibson	R4KS	pET28b- P4-R4-R5-P7	<u>CGAGCACCGGGCCGGTGAGCCAATT</u> <u>GCGATTGTGGGTATGGCGTGCCGT</u> <u>TCCTCTCCGCGGGACTAGACTGTGC</u> <u>GGGGGGTGCGCTCTCCAG</u>
		R5AT+R5DH+R5KR+R5ACP+S5^CDD+His₆+pET28b+E5^NDD	pJZ128	<u>AGAGCGCACCCCCCGCACAGTCTAG</u> <u>TCCCGCGGAAGAGGAGCAGCCT</u> <u>GCACGCCATACCCACAATCGCAATT</u> <u>GGCTCACCGGCCCGGTGCTC</u>

pJZ138 ^C P5R5^N (x ₃) vector: pET28b	2-piece Gibson	P5KS+P6AT	pJZ105	<u>TAAGAAGGAGATATACCATGAGCGG</u> <u>TGACAACGGCATGACCGAG</u> <u>GGATGACCATCCGCAGCCGCGGGCG</u> <u>CGGAGCTCTGCAGCCAGAA</u>
		R5DH+R5KR+R5ACP+S5^CDD+His₆+pET28b+E5^NDD	pJZ128	<u>GGCTGCAGAGCTCCGCGCCCCGCGGC</u> <u>TGCGGATGGTCATCCGTTGCT</u> <u>GTCATGCCGTTGTCACCGCTCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ139 ^C P5R5^N (x ₄) vector: pET28b	2-piece Gibson	R5DH+R5KR	pJZ138	<u>GGCTGCAGAGCTCCGCGCCCCGCGGC</u> <u>TGCGGATGGTCATCCGTTGCT</u> <u>GCGGCGTCGCCGGGAGCCGGGGCGG</u> <u>CCCTGCGAGCAATCGGCCGGT</u>
		P6ACP+S5^CDD+His₆+pET28b+E5^NDD+P5KS+P6AT	pJZ105	<u>CGATTGCTCGCAGGGCCGCCCGGCT</u> <u>CCCGGCGACGCCGCCGTG</u> <u>GGATGACCATCCGCAGCCGCGGGCG</u> <u>CGGAGCTCTGCAGCCAGAA</u>

pJZ140 ^C P5R5^N (x ₅) vector: pET28b	2-piece Gibson	R5ACP+S5^CDD+His₆+pET28b	pJZ138	<u>TCGCCGCACCCGTCGGTGCCAGGGC</u> <u>CGCCTCTACCGGTGACTCGT</u> <u>GTCATGCCGTTGTCACCGCTCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>
		E5^NDD+P5KS+P6AT+P6KR	pJZ105	<u>TAAGAAGGAGATATACCATGAGCGG</u> <u>TGACAACGGCATGACCGAG</u> <u>TCACCGGTAGAGGCGGCCCTGGCAC</u> <u>CGACGGGTGCGGCGAGCGCCT</u>

pJZ141 ^c P5P7 (ΔP6₂) vector: pET28b	2-piece Gibson	P7AT+P7ACP+TE +His₆	pJZ113	<u>AGGAGGCCCGGCGGTCGAGTCTAG</u> <u>TTCCCCCTGCAGTTGAGCCGCCGG</u> <u>CAGTGGTGGTGGTGGTGGTGCTTGCC</u> <u>CGCCCCCTCGATGCC</u>
		pET28b+ P5^NDD+ P5KS	pJZ112	<u>GCATCGAGGGGGCGGGCAAGCACCA</u> <u>CCACCACCACCACTGA</u> <u>TCAACTGCAGGGGAAGTAGACTCGA</u> <u>CCGCCGGGGCCTCCTCCAGG</u>

pJZ142 ^c P5P7 (ΔP6₃) vector: pET28b	2-piece Gibson	P7ACP+TE+His₆	pJZ113	<u>GGCTGCAGAGCTCCGCGCCCGGCGA</u> <u>GGCGCCCGCGCACACCGCTT</u> <u>CAGTGGTGGTGGTGGTGGTGCTTGCC</u> <u>CGCCCCCTCGATGCC</u>
		pET28b+ P5^NDD+ P5KS+P6AT	pJZ112	<u>GCATCGAGGGGGCGGGCAAGCACCA</u> <u>CCACCACCACCACTGA</u> <u>GTGTGCGCGGGCGCCTCGCCGGGCG</u> <u>CGGAGCTCTGCAGCCAGAAGCG</u>

pJZ143 ^c P5R5^N (ANH₃) vector: pET28b	2-piece Gibson	P5KS+P6AT	pJZ105	<u>TAAGAAGGAGATATACCATGAGCGG</u> <u>TGACAACGGCATGACCGAG</u> <u>GGATGACCATCCGCAGCCGCGGGCG</u> <u>CGGAGCTCTGCAGCCAGAA</u>
		R5DH+R5KR+R5 ACP+S5^CDD+His₆ +pET28b+P5^NDD	pJZ128	<u>GGCTGCAGAGCTCCGCGCCCGCGGC</u> <u>TGCGGATGGTCATCCGTTGCT</u> <u>GTCATGCCGTTGTACCCGCTCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

pJZ144 methylmalonyl-CoA epimerase vector: pET28b	2-piece Gibson	methylmalonyl-CoA epimerase	<i>Streptomyces coelicolor</i>	<u>TAAGAAGGAGATATACCATGCTGAC</u> <u>GCGAATCGACCACATCGGAA</u> <u>CAGTGGTGGTGGTGGTGGTGCTC</u> <u>AGGTGACTCAACGCCCGC</u>
		pET28b	pET28b	<u>CCGTTGAGTCACCTGAGCACCACCAC</u> <u>CACCACCACCACTGA</u> <u>ATGTGGTCGATTGCGGTCAGCATGGT</u> <u>ATATCTCCTTCTTAAAGTTAA</u>

Table S2. Primers for site-directed mutagenesis of ^CP6P7. Mutation sites are in red.

Plasmid	Template	Primers
pJZ145 Domains: P6KS^{C207A}+P7AT+P7ACP+TE (DML₁/DML₂) Vector: pET28b	pJZ106/ pJZ113	ACGGCG^{GCG}TCCTCTTCGCTCGTCGCCC TGCACCTCGCCC AGAGGA^{GCG}CGCCGTGTCCACGGTCAC GGCGGGTCCCT
pJZ146 Domains: P6KS^{C207A}+P7AT+P7ACP^{S980A}+TE (DML₃) Vector: pET28b	pJZ113	TTCGAC^{GCG}CTGACCGCCGTCGACTTCC GCAACCG GGTCAG^{GCG}GTCGAAGCCGATCTCCCGC AGCGGGC
pJZ147 Domains: P6KS^{C207A}+P7AT+TE (DML₄) Vector: pET28b	pJZ113	ACCGCTTCGCCGAGCCGTCGGATCACG AGCAGGCGGA CGGCTCGGCGGAAGCGGTGTGCGCGGG CGCCTCG

Table S3. Protein sequences for engineered pikromycin, erythromycin, and rapamycin PKS modules used in this study. **light teal**, **green**, **light orange**, **coral pink**, **dark orange**, **blue**, **lavender**, **yellow**, **yellow green**, **beige**, **dark green**, and **light brown** letters indicate modules **P1**, **P2**, **P3**, **P4**, **P5**, **P6**, **P7**, **E5**, **E6**, **E7**, **R4**, and **R5**, respectively (colored as in Figs. 1–6).

Spinosyn PKS S8 Docking Domain Sequence

Spinosyn PKS S3 Docking Domain Sequence

Erythromycin PKS E3 Docking Domain Sequence

Erythromycin PKS E5 Docking Domain Sequence

Spinosyn PKS S5 Docking Domain Sequence

HHHHHH (His₆-tag)

2 letters with double underline indicate aa's encoded by the introduced restriction sites

8 letters with double underline indicate aa's encoded by the *Hind*III-N₁₂-*Spe*I sequence

P1P2^N-[S8^CDD]-CterHis₆, (NAR₁)

MSSAGITRTGARTPVTGRGAAAWDTGEVRVRRGLPPAGPDHAEHSFSRAPTGDVRAELIRGEMST
VSKSESEEFVSNSNDAGSAHGTAEPVAVVGISCRVPGARDPREFWELLAAGGQAVTDVPADRWNA
GDFYDPDRSAPGRSNSRWGGFIEDVDRFDAAFFGISPREAAEMDPQQRLALELGWEALERAGIDPS
SLTGTRTGVFAGAIWDDYATLKHRQGGAAITPHTVTGLHRGIIANRLSYTLGLRGPSMVVDSGQSS
SLVAVHLACESLRRGESELALAGGVSLNLVPDSIIGASKFGGLSPDGRAYTFDARANGYVRGEGGG
FVVLKRLSRAVADGDPVLAVIRGSAVNNGGAAQGMTPDAQAQEAHLREHERAGTAPADVRYV
ELHGTGTPVGDPIEAAALGAALGTGRPAGQPLLVGSVKTNIGHLEGAAGIAGLIKAVLAVRGRAL
PASLNYETPNPAIPFEELNLRVNTEYLPWEPEHDGQRMVGVSSFGMGGTNAHVLEEAPGVVEG
ASVVESTVGGSAVGGGVVPWVVSASKSAAALDAQIERLAASFASRDRTDGDVADAGAVDAGAVDAGAV
ARVLAGGRAQFEHRAVVVGSGPDDLAAALAAPEGLVRGVASGVGRVAFVFPQGQTQWAGMGAE
LLDSSAVFAAAMAECEAALSPYVDWSLEAVVRQAPGAPTLERVDVVPVTFAVMVSLARVWQH
GVTPQAVVGHSQGEIAAAVYAGALSDDAARVVTLRSKSIAAHLAGKGGMLSLALSEDAVLERLA
GFDGLSVAAVNGPTATVVSGDPVQIEELARACEADGVRARVIPVDYASHSRQVEIIESELAEVLGL
SPQAPRPVFFSTLEGAWITEPVLDDGGYWYRNLRRHRVGFAFPAVETLATDEGFTHFVEVSAHPVLT
ALPGTGTGLATLRRDNGGQDRLVASLAEAWANGLAVDWSPLPSATGHHSDDLPTYAFQTERHWL
GEIEALAPAGEPAVQPAVLRTEAAEPAELDRDEQLRVILDKVRAQTAQVLGYATGGQIEVDRTFR
EAGCTSLTGVDLRNRINAAFGVRMAPSMIFDFPTPEALAEQLLLVVHGEAAANPAGAEPAPVAAA
GAVDEPVAIVGMACRLPGGVASPEDLWRLVAGGGDAISEFPQDRGWDVEGLYHPDPEHPGTSYV
RQGGFIENVAGFDAAFFGISPREALAMDPQQRLLLETSSWEAVEDAGIDPTSLRGRQVGVFTGAMT
HEYGPSLRDGGEGLDGYLLTGNTASVMSGRVSYTLGLEGPALTVDTACSSSLVALHLAVQALRKG
EVDMALAGGVAVMPTPGMFVEFSRQRLAGDGRSKAFAASADGTSWSEGVGVLLVERLSDARRN
GHQVLAVVRGSANQDQASNGLTAPNGPSQQRVIRRALADARLTSDVDVVEAHGTGTRLGDPIE
AQALIATYQGGRDDEQPLRLGSLKSNIGHTQAAAGVSGVIKVMQAMRHGLLPKTLHVDEPSDQID
WSAGAVELLTEAVDWPEKQDGGRLRAAVSSFGISGTNAHVLEEAPVVVKLAPAPTSEGASVVEP
SVGGSAGGGVTPWVVSASKSAAALDAQIERLAASFASRDRTDDADAGAVDAGAVAHVLADGRAQF
EHRAVALGAGADDLVQALADPDGLIRGTASGVGRVAFVFPQGQTQWAGMGAEELLDSSAVFAAA
MAECEAALSPYVDWSLEAVVRQAPGAPTLERVDVVPVTFAVMVSLARVWQHGGVTPQAVVG
SQGEIAAAVYAGALPLDDAARVVTLRSKSIAAHLAGKGGMLSLALNEDAVLERLSDFDGLSVA
AVNGPTATVVSGDPVQIEELAQACKADGFRARIIPVDYASHSRQVEIIESELAQVLGLSPQAPRPVFFS
TLEGTWITEPVLDDGTYWYRNLRRHRVGFAFPAIETLAVDEGFTHFVEVSAHPVLTMTLPETVTGLGT
LRREQGGQERLVTSLAEAWVNGLPVAWTSLLPATASRPGLPTYAFQAERYWLENTPAALATGDD
WRYRIDWKRLPAAEGSERTGLSGRWLAVTPEDHSAQAAAVLTALVDAGAKVEVLTAGADDRE
ALAARLTALTGDDGFTGVVSLDGLVPQVAWVQALGDAGIKAPLWSVTQGAVSVGRDLTPADPD
RAMLWGLGRVVALEHPERWAGLVDLPAQPDAAALHLVTALSGATGEDQIAIRTTGLHARRLAR
APLHGRRPTRDWQPHGTVLITGGTGALGSHAARWMAHHGAEHLLLVSRSGEQAPGATQLTAE
LTASGARVTIAACDVADPHAMRTLLDAIPAETPLTAVVHTAGALDDGIVDTLTAEQVRRRAHRAKAV
GASVDELTRDLDLDAFVLFSSVSSTLGIPQGQNYAPHNAYLDALAARRRATGRSAVSVAWGPWD
GGGMAAGDGVAERLRNHGVPGMDPELALAALESALGRDETAITVADIDWDRFYLAYSSGRPQPL

VEELPEVRRIIDARDSATSGQGGSSAQGANPLAERLAAAAPGERTEILLGLVRAQAAAVLRMRSPE
DVAADRAFKDIGFDSLAVELRNRLTRATGLQLPATLVFDHPTPLALVSLLRSEFLGDEEASNGIES
LLAELDRLDTTLAQGPSIPLEDQAKVAERLHALLAKWDGARDGTARATSPQSLTAATDDEIFDLID
RKFRRH

^CP2P3^N-[S8^NDD/S3^CDD]-CterHis₆, (NAR₁)

MANEEKLREYLKRVVVELEEAHERLHELERQEHDPIAIVAMSCRYPGDIRSPEDLWRMLSEGEGE
ITPFTDRGWDLGLYDADPDALGRAYVREGGFLHDAAEFDAEFFGVSPREALAMDPQQRMLLT
TSWEAFERAGIEPASLRGSSTGVFIGLSYQDYAARVPNAPRGVEGYLLTGSTPSVASGRIAYTFGLE
GPATTVDTACSSSLTALHLAVRALRSPECTMALAGGVAMMATPHMFVEFSRQALAPDGRSKAF
SADADGFGAAEGVGLLLVERLSDARRNGHPVLAVVRGTAVNQDGASNGLTAPNGPSQQRVIRQA
LADARLAPGDIDAVETHGTGTSLGDPPIEAQGLQATYGKERPAERPLAIGSVKSNIGHTQAAAGAAG
IKMVLAMRHGTLPKTLHADEPSPHVDWANSGLALVTEPIDWPAGTGPRRAAVSSFGISGTNAHV
VLEQAPDAASSGEVLGADEVPEVSETVAMAGTAGTSEVAEGSEASEAPAAPGSREASLPGHLPWV
LSAKDEQSLRGQAAALHAWLSEPAADLSADGPARLRDVGTYLATSRTAFAHRAAVTAADDRDGF
LDGLATLAQGGTSAHVHLDLTARDGTTAFLFTGQGSQRPAGRELYDRHPVFARALDEICAHLDG
HLELPLLDVMFAAEGSAAEALLDETTRYTQCALFALEVALFRLVESWGMRPAALLGHSVGEIAAAH
VAGVFSLADAARLVAAARGRLMQELPAGGAMLAVQAAEDEIRVWLETEERYAGRLDVAAVNGPE
AAVLSGDADAAREAEAYWSGLGRRTRALRVSHAFHSAHMDGMLDGFRAVLETVEFRRPSLTVVS
NVTGLAAGPDDLCDPEYWVRHVGRGTVRFLDGVVRVLRDLGVRTCLELGPDPGLTAMAADGLADT
PADSAAGSPVGPSPAGSPADSAAGALRPRPLLALLRRKRSETETVADALGRAHAHGTGPDWHAWF
AGSGAHRVDLPTYSFRRDRYWLDAADTAVDTAGLGLGTADHPLLGAUVSLPDRDGLLLTGRL
SLRTHPWLADHAVLGSVLLPGAAMVELAAHAAESAGLRDVRELTLEPLVLPHEGGVELRVTVG
APAGEPGGESAGDGARPVSLHSRLADAPAGTAWSCHATGLLATDRPELPVAPDRAAMWPPQGAE
EVPLDGLYERLDGNGLAFGLPFQGLNAVWRYEYGEVFADIALPATTNATAPATANGGGSAAAAPYG
IHPALLDASLHAIAVGGLVDEPELVVRVPFHWSGVTVHAAGAAAARVRLASAGTDAVSLSLTDGEG
RPLVSVERLTLPVTADQAAASRVGGLMHRVAWRPYALASSGEQDPHATSYGPTAVLGKDELKV
AAALESAGVEVGLYPDLAALSQDVAAGAPAPRTVLAPLPAGPADGGAEGVRGTVARTLELLQAW
LADEHLAGTRLLLVTGRGAVRDPEGSGADDGGEDLSHAAWGLVVRTAQTENPGRFGLLDLADDAS
SYRTLPSVLSDAGLRDEPQLALHDGTIRLARLASVRPETGTAAPALAPEGTVLLTGGTGGLGGLVA
RHVVGEGWGVRRLLLVSRRGTDAPGADELVHELEALGADVSAACDVADREALTAVLDAIPAEHP
LTAVVHTAGVLSDGTLPSMTTEDVEHVLRPKVDAAFLLDELSTPAYDLAAFVMESSAAAVFGGA
GQGAYAAANATLDALAWRRRAAGLPALSLGWGLWAETSGMTGELGQADLRMSRAGIGGISDA
EGIALLDAAALRDDRHPVLLPLRLDAAGLRDAAGNDPAGIPALFRDVVGARTVRARPSAASASTTAG
TAGTPGTADGAAETAAVTLADRAATVDGPARQRLLEFVVGEVAEVLGHARGHRIDAERGFLDL
GFDSLTAVELRNRLNSAGGLALPATLVFDHPSPAALASHLDAELPRGASASAGTFEELDRWAANLP
TLARDEATRAQITTRLQAILQSLADVSGGTGGGSVPDRLRSATDDELQLLDNDLELP

^CP3P4^N-[S3^NDD/E3^CDD]-CterHis₆, (NAR₁)

MSNEEKLREYLRRALVDLHQARERLHEAESGEREPIAIVGMACRLPGGVASPEDLWRLVAGGED
AISEFPQDRGWDEGLYDPNPEATGKSYAREAGFLYEAGEFDADFFGISPREALAMDPQQRLLLEA
SWEAFEHAGIPAATARGTSVGVFTGVMYHDYATRLTDVPEGIEGYLGTGNSGVSASGRVAYTLGL
EGPAVTVDTACSSSLVALHLAVQALRKGEVDMALAGGVTVMTSTPSTFVEFSRQRLAPDGRSKSF
SSTADGTSWSEGVGVLLVERLSDARRKGHRI LAVVRGTAVNQDGASSGLTAPNGPSQQRVIRRAL
ADARLTTSDDVDVEAHGTGTRLGDPPIEAQAVIATYGGQGRDGEQPLRLGSLKSNIGHTQAAAGVSG
VIKMOVAMRHGVLPKTLHVEKPTDQVDWSAGAVELLTEAMDWPKDGGGLRRAAVSSFGVSGT
NAHVVEEAPAAESSETPASEATPAVEPSVGAGLPWLVS AKTPAALDAQIGRLAAFASQGRTDAA
DPGAVARVLAGGRAEFEHRAVVLGTGQDDFAQALTAPEGLIRGTPSDVGRVAFVFPQGQTQWAG
MGAELLDVSKEFAAAMAECESALSRYVDWSLEAVVRQAPGAPTLE RVDVVPVTFAVMVSLAKV
WQHVGVTQPQAVVGHSGQEIAAAAYAGALTLDDAARVVTLSKSI AAHLAGKGMISLALSEAT
RQRIENLHGLSIAAVNGPTATVVSGDPTQIQELAQACEADGV RARIIPVDYASHSAHVETIESELAE
VLAGLSRPTPEVPFFSTLEGAWITEPVDGTQYWRNLRHVRVGFAPAVETLATDEGFTHFIEVSAHP
VLTMTLPETVTGLGTLRREQGGQERLVTSLAEAWTNGLTIDWAPVLPATGHHPPELPTYAFQRR
HYWLHDSPAVQGSVQDSWRYRIDWKRLAVADASERAGLSGRWL VVVPEDRSAEAPVLAALSGA
GADPVQLDVSPLGDRQLAATLGEALAAAGGAVDGVLSLLAWDES AHPGHPAPFTRGTGATLTL

VQALEDAGVAAPLWCVTHGAVSVGRADHVTSPAQAMVWGMGRVAALEHPERWGGGLIDLPSDAD
 RAALDRMTTVLAGGTGEDQVAVRASGLLARRLVRLASLPAHGTASPWWQADGTVLVTGAEEPAA
 AEAARRLARDGAGHLLHHTTPSGSEGAEGTSGAAEDSGLAGLVAELADLGATATVVTCDLTDAEA
 AARLLAGVSDAHPLSAVLHLPPTVDSEPLAATDADALARVVTAKATAALHLDRLLREAAAAGGRP
 PVLVLFSSVAAIWGGAGQGAYAAAGTAFDALAGQHRADGPTVTSVAWSPWEGSRVTEGATGERL
 RRLGLRPLAPATALTALDTALGHGDTAVTIADVWSSFAPGFTTARPGTLLADLPEARRALDEQQ
 STTAADDTVLSRELGALTGAEEQRRMQELVREHLAVVLNHPSPAEVDTGRAFRDLGFDLSLTAVEL
 RNRLKNATGLALPATLVFDYPTPRTLAEFLLAEILGEQAASPSALAGLDALEAALPEVPATEREEL
 VQRLERMLAALRPVAQAADASGTGANPSGDDLGEAGVDELLEALGRELDGDHHHHHH

^CP4P5^N-[E3^NDD/E5^CDD]-CterHis₆, (NAR₁)

MTDSEKVAEYLRRATLRLRAARQRIRELESPIAIVGMACRLPGGVASPEDLWRLVAGGEDAISGF
 PQDRGWDVEGLYDPPDASGRTYCRAGGFLDEAGEFDADFFGISPREALAMDPQQRLLLETSWEA
 VEDAGIDPTSLQGQQVGVFAGTNGPHYELLRNTAEDLEGYVGTGNAASIMSGRVSYTLGLEGPA
 VTVDTACSSSLVALHLAVQALRKGECLALAGGVTVMTPTTFVEFSRQRGLAEDGRSKAFAASA
 DGFPAEGVGMILLVERLSDARRNGHRVLAVVRGSAVNQDGASNGLTAPNGPSQQRVIRRALADA
 RLTTADVVDVEAHGTGTRLGDPIEAQALITYGQGRDTEQPLRLGSLKSNIGHTQAAAGVSGIHKM
 VQAMRHGVLPKTLHVDRPSDQIDWSAGTVELLTEAMDWPRKQEGGLRRAAVSSFGISGTNAHIV
 LEEAPVDEKLAPAPTSDAPADEPSVGGVVPWLVSAKTPAALDAQIGRLAAFASQGRDADADPGAV
 ARVLAGGRAQFEHRAVALGTGQDDLAAALAAPEGLVRGVASGVGRVAFVFPQGQTQWAGMGAE
 LLDVSKFAAAAMAECEAALAPYVDWSLEAVVRQAPGAPTLERVDVVQPVTFVAVMSLAKVWQH
 HGVTQAVVGHSGQGEIAAAAYVAGALSLLDDAARVVTILRSKSIGAHLAGQGGMLSLALSEAIVVERL
 AGFDGLSVAAVNGPTATVVSGDPTQIQELAQACEADGVRARIIPVDYASHSAHVETIESELADVLA
 GLSPQTPQVPFFSTLEGAWITEPALDGGYWYRNLHRVGFAPAVETLATDEGFTHFVEVSAHPVL
 TMAPETVTGLGTLRRDNGGQHRLTTSALAEAWANGLTVDWASLLPTTTTTHPDLPTYAFQTERYW
 PQPDLAAGDITSAGLGAAEHPLLGAVALADSDGCLLTGSLSLRTHPWLADHAVAGTVLLPGTA
 FVELAFRAGDQVGCIDLVEELTLDAPLVLPRRGAVRVQLSVGASDESGRRTFGLYAHPEADAPGEAE
 WTRHATGVLAARADRTAPVADPEAWPPPGAEPVDVDGLYERFAANGYGYGPLFQGVGRGVWRRG
 DEVFADVALPAEVAGAEGARFGLHPALLDAVQAAGAGRGVRRGHAAAVRLERDRLLYAVGATAL
 RVRLAPAGPDTVSVAADSSGQPVFAADSLTVLPVDPAPLAFAFSDPTLDALHLEWTAWDGAAQA
 LPGAVVLGGDADGLAAALRAGGTEVLSFPDLTDLVEAVDRGETPAPATVVLVACPAAGPDGPPEHVR
 EALHGSLALMQAWLADERFTDGRVLVLTDRDAVAARS GDGLRSTGQAAVWGLGRSAQTESPGRF
 VLLDLAGEARTAGDATAGDGLTTGDATVGGTSGDAALGSALATALGSGEPQLALRDGALLVPRL
 ARAAAPAAADGLAAADGLAALPLPAAPALWRLEPGTDGSLESITAAPGDAETLAPEPLGPGQVRI
 AIRATGLNFRDVLIALGMYPPDAPALMGTEGAGVVTATGPGVTHLAPGDRVMGLLSGAYAPVVVAD
 ARTVARMPEGWTFAGQASVPVVFLTAVYALRDLADV KPERLLVHSAAGGVGMAAVQLARHWG
 VEYHGTASHGKWDALRALGLDDAHIASSRTLDFESAFAAASGGAGMDVVLNSLAREFVDASLRL
 GPGGRFVEMGKTDVRDAERVAADHPGVGYRAFDLGEAGPERIGEMLAEVIALFEDGVLRHLPT
 TWDVRRARDAFRHVSQARHTGKVVLTMPSGLDPEGTVLLTGGTGALGGIVARHVVGWGVRR
 LLVSRRTDAPGAGELVHELEALGADVSAACDVADREALTAVLDSIPAHEHPLTAVVHTAGVLS
 GTLPSMTAEDVEHVLRPKVDAAFLDELDTSTPGYDLAAAFVMFSSAAAVFGGAGQGAYAAANATLD
 ALAWRRRTAGLPALSLGWGLWAETSGMTGGLSDTDRSRLARSGATPMDSELTLSLDDAAMRRD
 DPALVPIALDVAAALRAQQRDGMLAPLLSGLTRGSRVGGAPVNRRAAAGGAGEADTDLGGRLAA
 MTPDDRVAHLRDLVRTHVATVVLGHGTPSRVDLERAFRDTGFDSLTAVELRNRLNAATGLRLPATL
 VFDHPTPGELAGHLLDELATAAGASAVDIGDRLDELEKALEALSAEDGHDDVGQRLESLLRRWNS
 RRADAPSTSAISEDASDDELFSMLDQRFGGGEDLHHHHHH

^CP5P6^N-[E5^NDD/S5^CDD]-CterHis₆, (NAR₁; x₆)

MSGDNGMTEEKLRRLYLRKRTVTELDVYARLREVEHRAGEPIAIVGMACRLPGGVASPEDLWQLV
 AGDGDASEFPQDRGWDVEGLYDPPDASGRTYCRSGGLHDAGEFDADFFGISPREALAMDPQQR
 RLSTTAWEAIESAGIDPTALKGSGLVFVGWHTGYTSGQTTAVQSPELEGHLVSGAALGFLSG
 RIAYVLGTDGPALTVDTCSSSLVALHLAVQALRKGECDMALAGGVTVMPNADLFVQFSRQRGL
 AADGRSKAFATSADGFGPAEGAGVLLVERLSDARRNGHRILAVVRGSAVNQDGASNGLTAPHGPS
 QQRVIRRALADARLAPGDVDVVEAHGTGTRLGDPIEAQALITYGQEKSSQPLRLGALKSNIGHT
 QAAAGVAGVIKMOVQAMRHGLLPKTLHVDEPSDQIDWSAGTVELLTEAVDWPEKQDGGGLRRAAV

SSFGISGTNAHVLEEAPAVESSDSPAVEPPAGGGVVPWPVSAKTPAALDAQIGQLAAYADGRTDV
 DPAVAARALVDSRTAMEHRAVAVGDSREALRDALRMPEGLVRGTSSDVGRVAFVFPQGQTQWA
 GMGAELLDSSPEFAASMAECETALSRYVDWSLEAVVRQEPGAPTLDRVDVVQPVTFAVMVSLAK
 VWQHHGITPQAVVGHSQGEIAAAAYVAGALTDDAARVVTLRSKSIAAHLAGKGGMISLALDEAAV
 LKRLSDFDGLSVAAVNGPTATVVSGDPTQIEELARTCEADGVRARIIPVDYASHSRQVEIEKELAE
 VLAGLAPQAPHVPPFFSTLEGTWITEPVLDGTYWYRNLRRHVRGFAPAVETLAVDGFTHFIEVSAHPV
 LTMTLPETVTGLGTLRREQGGQERLVTSLAEAWANGLTIDWAPILPTATGHHPELPTYAFQTERF
 WLQSSAPTSAADDWRYRVIEWKPLTASGQADLSGRWIVAVGSEPEAEELGALKAAAGAEVDVLEAG
 ADDDREALAARLTALTGGDFTGVVSLDDLVQPQAVWQALGDAGIKAPLWSVTQGAVSVGRLD
 TPADPDRAMLWGLGRVVALEHPERWAGLVLDLPAQPDAAALAHLVTAALSGATGEDQIAIRTTGLH
 ARRLARAPLHGRRPTRDWQPHGTVLITGGTGALGSHAARWMAHHGAEHLLLVSRSGEQAPGAT
 QLTAELTASGARVTIAACDVADPHAMRTLDDAIPAETPLTAVVHTAGAPGGDPLDVTGPEDIARIL
 GAKTSGAEVLDDLLRGTPLDFAVLYSSNAGVWGSQGVYAAANAHLDALAARRRARGETATSV
 AWGLWAGDGMGRGADDAYWQRRGIRPMSPDRLDELAKALSHDETFFAVADVDERFAPFT
 VSRPSLLLDGVPEARQALAAPVGAPAGDAAVAPTQSSALAAITALPEPERRPALLTLVRTHAAA
 VLGHSSPDRVAPGRAFTELGFDSLTAVQLRNQLSTVVGNRLPATTVFDHPTPAALAAHLHEAYLAP
 AEAS^{TV}DSALAE^{LDRIE}QQLS^{MLT}GEARARDRIATRLRALHEKWN^{SAAE}VPTGADVLSTLDSATH
 DEIFE^{FID}NELDLS^{HHHHHH}

^CP6P7-[S5^NDD]-CterHis₆, (NAR₁; y₁)

MANEEKLFGYLKKVTADLHQTRQRLAAESRSQEPIAIVGMSCRFAGGIRSPEDLWDAVAAGKDL
 VSEVPEERGWIDISLYDPVPGRKGTITYVRNAAFLLDDAAGFDAAFFGISPREALAMDPQQRQLLEAS
 WEVFERAGIDPASVRGTDVGVYVGCYQDYAPDIRVAPEGTGGYVVTGNSSAVASGRIAYSGLGE
 GPAVTVDTACSSSLVALHLALKGLRNGDCSTALVGGVAVLATPGAFIEFSSQQAMAADGRTKGFA
 SAADGLAWGEGVAVLLLERLSDARRKGHVRLAVVRGSAINQDGASNGLTAPHGPSQQLRIRQAL
 ADARLTSSDVVDVEGHGTGTRLGDPIEAQALLATYQGGRAPGQPLRLGTLKSNIGHTQAASGVAG
 VIKMVQALRHGVLPKTLHVDEPTDQVDWSAGSVELLTEAVDWPERPGRLRRAGVSAFGVGGTNA
 HVVLEEAPAVEE^{SS}SPAVEPPAGGGVVPWPVSAKTSAAALDAQIGQLAAYAEDRTDVPDAVAARAL
 VDSRTAMEHRAVAVGDSREALRDALRMPEGLVRGTVTDPGRVAFVFPQGQTQWAGMGAELLD
 SPEFAAAMAECETALSPYVDWSLEAVVRQAPSAPTLDRVDVVQPVTFAVMVSLAKVWQHHGITPE
 AVIGHSQGEIAAAAYVAGALTDDAARVVTLRSKSIAAHLAGKGGMISLALSEEATRQRIENLHGLSI
 AAVNGPTATVVSGDPTQIQELAQACEADGIRARIIPVDYASHSAHVETIENELADVLAGLSPQTPQV
 PFFSTLEGTWITEPALDGGYWYRNLRRHVRGFAPAVETLATDEGFTHFIEVSAHPVLTMTLPDKVT
 GLATLRREDGGQHRLTTSALAEAWANGLALDWASLLPATGALSPAVPDLPTYAFQHRSYWISPAGP
 GEAPAHTASGREAVAETGLAWGPGAEDLDEEGRRSAVLAMVMRQAASVLRCDSPEEVPVDRPLR
 EIGFDSLTAVDFRNRVNRLTGLQLPPTVVFEHPTPVALAERISDELAERNWAVAEPDHEQAEEEK
 AAPAGARSGADTGAGAGMFRALFRQAVEDDRYGEFLDVLAEASAFRPQFASPEACSERLDPVLL
 AGGPTDRAEGRAVLVGCTGTAANGGPHEFLRLSTSFQEERDFLAVPLPGYGTGTGTGTALLPADL
 DTALDAQARAILRAAGDAPVVLLGHSGGALLAHELAFRLERAHGAPPAGIVLVDPPYPGHQEP
 IEVWSRQLGEGLFAGELEPMSDARLLAMGRYARFLAGPRPGRSSAPVLLVRASEPLGDWQEERGDWR
 AHWDLPHTVADVPGDHFTMMRDHAPAVAEAVLSWLDIAEGIEGAGK^{HHHHHH}

^P1P2^N-[S8^CDD]-CterHis₆, (NAR₂)

MSSAGITRTGARTPVTGRGAAAWDTGEVRVRRGLPPAGPDHAEHSFSRAPTGDVRAELIRGEMST
 VSKSESEEFVSVSNDAGSAHGTAEPVAVVGISCRVPGARDPREFWELLAAGGQAVTDVPADRWNA
 GDFYDPDRSAPGRSNSRWGGFIEDVDRFDAAFFGISPREAAEMDPQQLALELGWEALERAGIDPS
 SLTGTRTGVFAGAIWDDYATLKHRRQGGAITPHTVTGLHRGIIANRLSYTLGLRGPSMVVDSGQSS
 SLVAVHLACESLRRGESELALAGGVSLNLVPDSIIHASKFGGLSPDGRAYTFDARANGYVRGEGGG
 FVVLKRLSRAVADGDPVLAVIRGSAVNNGGAAQGMTPDAQAQEAVLREAHHERAGTAPADVRYV
 ELHGTGTPVGDPIEAAALGAALGTGRPAGQPLLVGSVKTNIGHLEGAAGIAGLIKAVLAVRGRAL
 PASLNYETPNPAIPFEELNLRVNTEYLPWEPEHDGQRMVVGVSFFGMGGTNAHVLEEAPGVVEG
 ASVVESTVGGSAVGGGVVPWVVSASAAALDAQIERLAAFASRDRTDGVDAAGAVDAGAVDAGAV
 ARVLAGGRAQFEHRAVVVGSGPDDLAAALAAPEGLVRGVASGVGRVAFVFPQGQTQWAGMGAE
 LLDSSAVFAAAMAECFAALSPYVDWSLEAVVRQAPGAPTLERVDVVQPVTFAVMVSLARVWQHH
 GVTPQAVVGHSQGEIAAAAYVAGALSDDAARVVTLRSKSIAAHLAGKGGMISLALSEDAVLRLA

GFDGLSVAAVNGPTATVVSGDPVQIEELARACEADGVRARVIPVDYASHSRQVEIIESELAEVLAGL
 SPQAPRVPPFFSTLEGAWITEPVLDDGGYWYRNLRRHVGFAFPAVETLATDEGFTHFVEVSAHPVLT
 ALPGTVTGLATLRRDNGGQDRLVASLAEAWANGLAVDWSPLLPSATGHHSDDLPTYAFQTERHWL
 GEIALAPAGEPAVQPAVLRTEAAEPAELDRDEQLRVILDKVRAQTAQVLGYATGGQIEVDRTFR
 EAGCTSLTGVDLRNRINAAGFVRMAPSMIFDFPTPEALAEQLLLTVHGEAAANPAGAEPAPVAAA
 GAVDEPVAIVGMACRLPGGVASPEDLWRLVAGGGDAISEFPQDRGWDVEGLYHPDPEHPGTSYV
 RQGGFIENVAGFDAAFFGISPREALAMDPQQRLLLETSWEAVEDAGIDPTSLRGRQVGVFTGAMT
 HEYGPSLRDGGEGLDGYLLTGNTASVMSGRVSYTLGLEGPALTVDACSSSLVALHLAVQALRKG
 EYDMALAGGVAVMPTPGMFVEFSRQRGLAGDGRSKAFAASADGTSWSEGVGVLLVERLSDARRN
 GHQVLAVVRGSAVNQDGASNGLTAPNGPSQQRVIRRALADARLTTSDDVDVVEAHGTGTRLGDP
 IEAQALITYGQGRDDEQPLRLGSLKSNIGHTQAAAGVSGVIKMOVQAMRHGLLPKTLHVDEPSDQID
 WSAGAVELLTEAVDWPEKQDGGRLRAAVSSFGISGTNAHVVEEAPVVVEGASVVEPSVGGSAVG
 GGVTPWVVSASAAAALDAQIERLAAFASRDRTDDADAGAVDAGAVAHVLADGRAQFEHRAVALG
 AGADDLVQALADPDGLIRGTASGVGRVAFVFPQGQTQWAGMGAELLDSSAVFAAAMAECEAALS
 PYVDWSLEAVVRQAPGAPTILERVDVVQPVTFAVMVSLARVWQHGGVTPQAVVGHSQGEIAAAVY
 AGALPLDDAARVVTLRSKSIAAHLAGKGGMLSLALNEDAVLERLSDFDGLSVAAVNGPTATVVSG
 DPVQIEELAQACKADGFRARIIPVDYASHSRQVEIIESELAQVLAGLSPQAPRVPPFFSTLEGTWIT
 EPVLDTGYWYRNLRRHVGFAPIETLAVDEGFTHFVEVSAHPVLTMTLPETVTGLGTLRREGQGG
 QERLVTSLAEAWVNGLPVAVTSLPATASRPGLTPTAFQAERYWLENTPAALATGDDWRYRIDWKR
 LPAAEGSERTGLSGRWLAVTPEDHSAQAAAVLTALVDAGAKVEVLTAGADDDREALAARLTALT
 TGDGFTGVVSLDDGLVPQVAVWVQALGDAGIKAPLWSVTQGAVSVGRLDTPADPDRAMLWGLGR
 VVALEHPERWAGLVDLPAQPDAAALAHVLTALSGATGEDQIAIRTTGLHARRLARAPLHGRRPTR
 DWQPHGTVLITGGTGALGSHAARWMAHHGAEHLVVSRSGEQAPGATQLTAELTASGARVTIAA
 CDVADPHAMRTLLDAIPAETPLTAVVHTAGALDDGIVDTLTAEQVRRAHRAKAVGASVLDDELTRD
 LDLDFAVFLSSVSSTLGIPQGNYAPHNAYLDALAARRRATGRSAVSVAWGPWDGGGMAAGDGV
 AERLRNHGVPGMDELALAALESALGRDETAITVADIDWDRFYLAYSSGRPQPLVEELPEVRRID
 ARDSATSGQGGSSAQGANPLAERLAAAAPGERTEILLGLVRAQAAAVLRMRSPEDVAADRAFKDI
 GFDSLAVGVELRNRLTRATGLQLPATLVFDHPTPLALVSLRSEFLGDEENGIESLLAELDRLDTTLA
 QGPSIPLEDQAKVAERLHALLAKWDGARDGTARATSPQSLTAATDDEIFDLIDRKFRHHHHHH

^CP2P3^N-[S8^NDD/P3^CDD]-CterHis₆, (NAR₂)

MANEEKLREYLKRVVVELEEHAHERLHELERQEHDPIAIVAMSCRYPGDIRSPEDLWRMLSEGGE
 ITFPPTDRGWDLGGLYDADPDALGRAYVREGGFLHDAAEFDAAEFFGVSPREALAMDPQQRMLLT
 TSWEAFERAGIEPASLRGSSTGVFIGLSYQDYAARVPNAPRGVEGYLLTGSTPSVASGRIAYTFGLE
 GPATTVDTACSSSLTALHLAVRALRSRECTMALAGGVAMMATPHMFVEFSRQALAPDGRSKAF
 SADADGFGAAEGVGLLLVERLSDARRNGHPVLAVVRGTAVNQDGASNGLTAPNGPSQQRVIRQA
 LADARLAPGDIDAVETHGTGTSLGDPPIEAQGLQATYGKERPAERPLAIGSVKSNIGHTQAAAGAAG
 IKMVLAMRHGTLPKTLHADEPSPHVDWANSGLALVTEPIDWPAGTGPRRAAVSSFGISGTNAHV
 VLEQAPDAAGEVLGADEVPEVSETVAMAGTAGTSEVAEGSEASEAPAAPGSREASLPGHLPWVLS
 AKDEQSLRGQAAALHAWLSEPAADLSADGPARLRDVGYYTLATSRTAFAHRAAVTAADDRDGLD
 GLATLAQGGTSAHVHLDATARDGTTAFLFTGQGSQRPAGAGRELYDRHPVFARALDEICAHLDGHL
 ELPLLDVMFAAEGSAAEALLDETRYTQCALFALEVALFRLVESWGMPPAALLGHSVGEIAAAHVA
 GVFSLADAARLVAARGRLMQELPAGGAMLAVQAAEDEIRVWLETEERYAGRLDVAAVNGPEAA
 VLSGDADAAREAEAYWSGLGRRTRALRVSHAFHSAHMDGMLDGFRAVLETVEFRRPSLTVVSNV
 TGLAAGPDDLCDPEYWVRHVRGTVRFLDGVVRVLRDLGVRTCLELGPDGVLTAMAADGLADTPA
 DSAAGSPVGPAGSPADSAAGALRPRPLLVALLRKRSETETVADALGRAHAHGTGPDWHAWFA
 GSGAHRVDLPTYSFRRDRYWLDAADTAVDTAGLGLGTADHPLLGAUVSLPDRDGLLLTGRLSL
 RTHPWLADHAVLGSVLLPGAAMVELAAHAAESAGLRDVRRELTLEPLVLPHEGGVLELRTVGP
 AGEPPGGSAGDGARPVSLHSRLADAPAGTAWSCHATGLLATDRPELVPAPDRAAMWPPQGAEEV
 PLDGLYERLDGNGLAFGPLFQGLNAVWRYEGEVFADIALPATTNATAPATANGGGSAAAAPYGIH
 PALLDASLHAIAVGGLVDEPELVRVPFHWSGVTVHAAGAAAARVRLASAGTDAVSLSLTDGEGRP
 LVSVLRLTLRPVTADQAAASRVGGLMHRVAVWRPYALASSGEQDPHATSYGPTAVLGKDELKVAA
 ALESAGVEVGLYPDLAALSQDVAAGAPAPRTVLAPLPAGPADGGAEGVRGTVARTLELLQAWLA
 DEHLAGTRLLLVTARGAVRDPEGSGADDGGEDLSHAAAAGLVRTAQTENPGRFGLLDLADDASSY
 RTLPSVLSDAGLRDEPQLALHDGTIRLARLASVRPETGTAAAPALAPEGTVLLTGGTGGLGLVAR
 HVVGEWGVRRLLLVSRRGTDAPGADELVHELEALGADVSAACDVADREALTAVLDAIPAHEPL

TAVVHTAGVLSDGTLPSTMTTEDVEHVLRPKVDAAFLDELDTSTPAYDLAAFVFMSSAAAVFGGAG
 QGAYAAANATLDALAWRRRAAGLPALSLGWGLWAETSGMTGELGQADLRMSRAGIGGISDAE
 GIALLDAAALRDDRHVPVLLPLRLDAAGLRDAAGNDPAGIPALFRDVGARTVRARPSAASASTTAGT
 AGTPGTADGAAETAAVTLADRAATVDGPQRQLLLEFVVGEVAEVLGHARGHRIDAERGFLDLG
 FDSLTAVELRNRLNSAGGLALPATLVFDHPSPAALASHLDAELPRGASDQDGAGNRNGNENGTTA
 SRSTAETDALLAQLTRLEGALVLTGLSDAPGSEEVLEHLRSLRSMVTGETGTGTASGAPDGAGSG
 AEDRPWAAGDGAGGGSEDGAGVPDFMNASAEELFGLLDQDPSTD **HHHHHH**

^CP3P4^N-[P3^NDD/S3^CDD]-CterHis₆, (NAR₂)

MSTVNEEKYLDYLRRATADLHEARGRLRELEAKAGEPVAIVGMACRLPGGVASPEDLWRLVAGG
 EDAISEFPQDRGWDVEGLYDPNPEATGKSYAREAGFLYEAGEFDADFFGISPREALAMDPQQRLL
 EASWEAFEHAGIPAATARGTSVGVFTGVMYHDYATRLTDVPEGIEGYLGTGNSGSVASGRVAYTL
 GLEGPVTVDTACSSSLVALHLAVQALRKGEVDMALAGGVTVMSTPSTFVEFSRQRGLAPDGRSK
 SFSSTADGTSWSEGVGVLLVERLSDARRKGHRILAVVRGTAVNQDGASSGLTAPNGPSQQRVIRRA
 LADARLTTSDDVDVVEAHGTGTRLGDPIEAQAVIATYGQGRDGEQPLRLGSLKSNIGHTQAAAAGVS
 GVIKMOVQAMRHGVLPKTLHVEKPTDQVDWSAGAVELLTEAMDWDPKGDGGLRRAAVSSFGVSG
 TNAHVVEEAPAAEETPASEATPAVEPSVGAGLVPWLVSAKTPAALDAQIGRLAAAFASQGRTDAA
 DPGAVARVLAGGRAEFEHRAVVLGTGQDDFAQALTAPEGLIRGTPSDVGRVAFVFPQGQTQWAG
 MGAELLDVSKEFAAAMAECESALSRYVDWSLEAVVRQAPGAPTTLERVDVVQPVTFVAVMVSLAKV
 WQHHGVTPQAVVGHSQGEIAAAAYVAGALTLDDAARVVTLRSKSIAAHLAGKGGMISLALSEEAT
 RQRIENLHGLSIAAVNGPTATVVSGDPTQIQELAQACEADGVRARIIPVDYASHSAHVETIESELAE
 VLAGLSPRTPEVPPFFSTLEGAWITEPVLDTGTWYRNLRRHRVGFAPAVETLATDEGFTHFIEVSAHP
 VLTMTLPETVTGLGTLRREQGGQERLVTSLAEAWTNGLTIDWAPVLPATGHHPELPTYAFQRR
 HYWLHDSPAVQGSVQDSWRYRIDWKRLAVADASERAGLSGRWL VVVPEDRSAEAAPVLAALSGA
 GADPVQLDVSPLGDRQRLAATLGEALAAAGGAVDGVLSLLAWDESAHPGHPAPFTRGTGATLTL
 VQALEDAGVAAPLWCVTHGAVSVGRADHVTSPAQAMVWGMGRVAALEHPERWGGGLIDLPSSDAD
 RAALDRMTTVLAGGTGEDQVAVRASGLLARRLVRASLPAHGTASPWWQADGTVLVTGAEEPAA
 AEAARRLARDGAGHLLHHTTPSGSEGAEGTSGAAEDSGLAGLVAELADLGATATVVTCDLTDAAE
 AARLLAGVSDAHPLSAVLHLPPTVDSEPLAATDADALARVVTAKATAALHLDRLLREAAAAGGRP
 PVLVLFSSVAIWWGGAGQGAYAAAGTAFLDALAGQHRADGPTVTSVAWSPWEGSRVTEGATGERL
 RRLGLRPLAPATALDALTALGHGDTAVTIADVDWSSFAPGFTTARPGTLLADLPEARRALDEQQ
 STTAADDTVLSRELGALTGAEEQQRMMQELVREHLAVVLNHPSPAEVDTGRAFRDLGFDLSLTAVEL
 RNRLKNATGLALPATLVFDYPTPRTLAEFLLAELGEQAGAGTFEELDRWAANLPTLARDEATRA
 QITTRLQAILQSLADVSGGTGGGSVPDRLRSATDDELQQLLDNDLELP **HHHHHH**

^CP4P5^N-[S3^NDD/P5^CDD]-CterHis₆, (NAR₂)

MSNEEKLREYLRRALVDLHQARERLHEAESGEREPVAIVGMACRLPGGVASPEDLWRLVAGGED
 AISGFPQDRGWDVEGLYDPDPDASGRTYCRAGGFLDEAGEFDADFFGISPREALAMDPQQRLLLE
 TSWEAVEDAGIDPTSLQGQVGVFAGTNGPHYELLRNTAEDLEGYVGTGNAASIMSGRVSYTLG
 LEGPAVTVDTACSSSLVALHLAVQALRKGEGLALAGGVTVMSTPTTFVEFSRQRGLAEDGRSKA
 FAASADGFGPAEGVGMILLVERLSDARRNGHRVLAVVRGSAVNQDGASNGLTAPNGPSQQRVIRR
 ALADARLTADVDVVEAHGTGTRLGDPIEAQALIAATYGQGRDTEQPLRLGSLKSNIGHTQAAAAGV
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^CP5P6^N-[P5^NDD/P6^CDD]-CterHis₆, (NAR₂)

MANNEDKL RDYLRVTAELQONTRRLREIEGRTHEPVAIVGMACRLPGGVASPEDLWQLVAGDG
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 TAWEAIESAGIDPTALKGSGLG VFGGWHTGYTSGQTTAVQSPELEGHLVSGAALGFLSGRIAYV
 LGTDGPALTVD TACSSSLVALHLAVQALRKGECDMALAGGVTVMPNADLFVQFSRQRGLAADGR
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 DGLSVA AVNGPTATVVS GDPTQIEELARTCEADGVRARIIPVDYASHSRQVEIIEKELAEVLAGLAP
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 DRVAPGRAFTELGFDSL TAVQLRNQLSTVVGNRLPATTVFDHPTPAALAAHLHEAYLAPAEPAPT D
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 MALGPRNT **HHHHHH**

^CP6P7-[P6^NDD]-CterHis₆, (NAR₂)

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 GLEGPVTVDTACSSSLVALHLALKGLRNGDCSTALVGGVAVLATPGAFIEFSSQQAMAADGR TK
 GFASAADGLAWGEGVAVLLLERLS DARRKGHRVLAVVRGSAINQDGASNGLTAPHGPSQQR LIR
 QALADARLTSSD VDVVEGHGTGTRLGDPIEAQALLATY GQGRAPGQPLRLGTLKSNIGHTQAASG
 VAGVIK MVQALRHGVLPKTLHVDEPTDQVDWSAGSVELLTEAVDWPERPGR LRRAGVSAFGVG
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PikAII-[P3^NDD/P5^CDD]-CterHis₆, (NAR₃)

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DRLEGVLASLAPAAGGRPELAARLRALAAALGDDGDDATDLDEASDDDLFSFIDKELGDSDF HHH
HHH

PikAII-[P3^NDD/P5^CDD]-LoopHis₆, (NAR₅)

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P1P2^N-[S8^CDD]-LoopHis₆, (NAR₆)

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 GAVDEPVAI VGMACRLPGGVASPEDLWRLVAGGGDAISEFPQDRGWDVEGLYHPDPEHPGTSYV
 RQGGFIENVAGFDAAFFGISPREALAMDPQQRLLLET SWEAVEDAGIDPTSLRGRQVGVFTGAMT
 HEYGPSLRDGGEGLDGYLLTGNTASVMSGRVSYTLGLEGPALTVD TACSSSLVALHLAVQALRKG
 EVDMALAGGVAVMPTPGMFVEFSRQRGLAGDGRSKAFAASADGTSWSEGVGVLLVERLS DARRN
 GHQVLAVVRGSAVNQDGASNGLTAPNGPSQQRVIRRALADARLTSDVDVVEAHGTGTRLGDPIE
 AQALIATYQGGRDDEQPLRLGSLKS NIGHTQAAAGVSGVIKMVQAMRHGLLPKTLHVDEPSDQID
 WSAGAVELLTEAVDWPEKQDGG LRRAAVSSFGISGTNAHVVLEEAPVVVEGAHHHHHHVGGSAV
 GGGVTPWVVS AKSAAALDAQIERLAAFASRDRTDDADAGAVDAGAVAHVLADGRAQFEHRAVAL
 GAGADDLVQALADPDGLIRGTASGVGRVAFVFPQGQTQWAGMGAE LLDSSAVFAAAMAECEAAL
 SPYVDWSLEAVVRQAPGAPTLERVDV VQPVTFAMVSLARVWQH HGVTPQAVVGHSQGEIAAAY
 VAGALPLDDAARVVTLRSKSIAAHLAGKGGMLSLALNEDAVLERLSDFDGLSVA AVNGPTATVVS
 GDPVQIEELAQA CKADGFRARIIPVDYASHSRQVEIIESELAQVLAGLSPQAPRPVFFSTLEG TWITE
 PVLDGTYWYRNLRHRVGFAPAIETLAVDEGFTHFVEVSAHPVLTMTLPETVTGLGTLRREQGGQ
 ERLVTS LAEAWVNGLPVAWTSLLPATASRPGLPTYAFQAERYWLENTPAALATGDDWRYRIDWK
 RLPAAEGSERTGLSGRWLAVTPEDHSAQAAAVLTALVDAGAKVEVLTAGADDDREALAARLTAL
 TTGDGFTGVVSLLDGLVPQVAWVQALGDAGIKAPLWSVTQGA VSVGRLDTPADPDRAMLWGLG
 RVVALEHPERWAGLVLDLPAQPDAAALAH LVTALSGATGEDQIAIRTTGLHARRLARAPLHGRRPT

RDWQPHGTVLITGGTGALGSHAARWMAHHGAEHLLLVSRSGEQAPGATQLTAELTASGARVTIA
ACDVADPHAMRTLLDAIPAETPLTAVVHTAGALDDGIVDTLTAEQVRRRAHRAKAVGASVLDLTR
DLDLDAFVLFSSVSSTLGIPGQGNYPHNAYLDALAARRRATGRSAVSVAWGPWDGGGMAAGDG
VAERLRNHGVPGMPELALAALESALGRDETAITVADIDWDRFYLAYSSGRPQPLVEELPEVRRRII
DARDSATSQGQSSAQGANPLAERLAAAAPGERTEILLGLVRAQAAAVLRMRSPEDVAADRAFKD
IGFDSLAVELRNRLTRATGLQLPATLVFDHPTPLALVSLLRSEFLGDEENGIESLLAELDRDLDTTL
AQGPSIPLEDQAKVAERLHALLAKWDGARDGTARATSPQSLTAATDDEIFDLIDRKFR

P1^N-[E3^CDD]-NterHis₆, (NAR₈)

MHHHHHHSSAGITRTGARTPVTGRGAAAWDTGEVRVRRGLPPAGPDHAEHSFSRAPTGDVRAEL
IRGEMSTVSKSESEEFVSVSNDAGSAHGTAEPVAVVGISCRVPGARDPREFWELLAAGGQAVTDVP
ADRWNAGDFYDPDRSAPGRSNSRWGGFIEDVDRFDAAFFGISPREAAEMDPQQRLELWEALE
RAGIDPSSLTGTRTGVFAGAIWDDYATLKHRRQGGAAITPHTVTGLHRGIIANRLSYTLGLRGPSMV
VDSGQSSSLVAVHLACESLRRGESELALAGGVSLNLVPDSIIGASKFGGLSPDGRAYTFDARANGYV
RGEGGGFVVLKRLSRAVADGDPVLAVIRGSVNNGGAAQGMTPDAQAEAVLREAHHERAGTAP
ADVRYVELHGTGTPVGDPPIEAAAALGAALGTGRPAGQPLLVGSVKTNIGHLEGAAGIAGLIKAVLA
VRGRALPASLNYETPNPAIPFEELNLRVNTEYLPWEPEHDGQRMVVGVSFSGMGGTNAHVVLEEA
PGVVEGASVVESTVGGSAVGGGVVPPVWSAKSAAALDAQIERLAAFASRDRTDGVDAVADAGA
VDAGAVARVLAGGRAQFEHRAVVVGSGPDDLAAALAAPEGLVRGVASGVGRVAFVFPQGQTQW
AGMGAELLDSSAVFAAAMAECEAALSPYVDWSLEAVVRQAPGAPTLEVRDVVQPVTFVAVMVS
RVWQHGHGVTTPQAVVGHSQGEIAAAAYVAGALSDDAARVVTLRSKSIAAHLAKGGMLSLALSED
AVLERLAGFDGLSVAAVNGPTATVVSGDPVQIEELARACEADGVRARVIPVDYASHSRQVEIESEL
AEVLAGLSPQAPRVPPFFSTLEGAWITEPVLDDGGYWRNLRHRVGFAPAVETLATDEGFTHFVEVS
AHPVLTMLPGTVTGLATLRRDNGGQDRLVASLAEAWANGLAVDWSPLPSATGHHSDLPTYAF
QTERHWLGEIEALPAGEPAVQPAVLRTAAEPAELDRDEQLRVILDKVRAQTAQVLGYATGGQI
EVDRTFREAGCTSLTGVDLRNRINAAFGVVRMAPSMIFDFPTPEALAEQLLLHVHGEAAPALAGLD
ALEAALPEVPATEREELVQRLERMLAALRPVAQAADASGTGANPSGDDLGEAGVDELLEALGREL
DGD

^CP1P2^N-[E3^NDD/S8^CDD]-LoopHis₆, (NAR₈)

MTDSEKVAEYLRRATLRLRAARQRIRELESDPVAIVGMACRLPGGVASPEDLWRLVAGGGDAISE
FPQDRGWDVEGLYHPDPEHPGTSYVRQGGFIENVAGFDAAFFGISPREALAMPQQRLLLETWE
AVEDAGIDPTSLRGRQVGVFTGAMTHEYGPSLRDGGEGLDGYLLTGNTASVMSGRVSYTLGLEG
PALTVDTACSSSLVALHLAVQALRKGEVDMALAGGVAVMPTPGMFVEFSRQRGLAGDGRSKAFA
ASADGTWSSEGVGVLLVERLSDARRNGHQVLAVVRGSVAVNQDGASNGLTAPNGPSQQRVIRRAL
ADARLTSDVDVVEAHGTGTRLGDPPIEAQALIAITYGQGRDDEQPLRLGSLKSNIGHTQAAAGVSG
VIKMQAMRHGLLPKTLHVDEPSDQIDWSAGAVELLTEAVDWPEKQDGGRLRAAVSSFGISGTN
AHVVLEEAPVVVEGAHHHHHHVGGSAVGGGVTPWVWSAKSAAALDAQIERLAAFASRDRTDDAD
AGAVDAGAVAHVLADGRAQFEHRAVALGAGADDLVQALADPDGLIRGTASGVGRVAFVFPQGQT
QWAGMGAELLDSSAVFAAAMAECEAALSPYVDWSLEAVVRQAPGAPTLEVRDVVQPVTFVAVMVS
LARVWQHGHGVTTPQAVVGHSQGEIAAAAYVAGALPLDDAARVVTLRSKSIAAHLAKGGMLSLALN
EDAVLERLSDFDGLSVAAVNGPTATVVSGDPVQIEELAQAACKADGFRARIIPVDYASHSRQVEIES
ELAQVLAGLSPQAPRVPPFFSTLEGTWITEPVLDDGTYWYRNLRHRVGFAPAIETLAVDEGFTHFVEV
SAHPVLTMTLPETVTGLGTLRREQGGQERLVTSLAEAWVNGLPVAWTSLLPATASRPGLPTYAFQ
AERYWLENTPAALATGDDWRYRIDWKRLPAAEGSERTGLSGRWLAVTPEDHSAQAAAVLTALVD
AGAKVEVLTAGADDDREALAARLTALTGDDGFTGVVSLDDGLVPQVAVWQALGDAGIKAPLWSV
TQGAVSVGRDLTPADPDRAMLWGLGRVVALEHPERWAGLVDLPAQPDAALAHVLTALSGATG
EDQIAIRTTGLHARRLARAPLHGRRPTRDWQPHGTVLITGGTGALGSHAARWMAHHGAEHLLLV
SRSGEQAPGATQLTAELTASGARVTIAACDVADPHAMRTLLDAIPAETPLTAVVHTAGALDDGIVD
TLTAEQVRRRAHRAKAVGASVLDLTRDLDLDAFVLFSSVSSTLGIPGQGNYPHNAYLDALAARR
RATGRSAVSVAWGPWDGGGMAAGDGVAERLRNHGVPGMPELALAALESALGRDETAITVADID
WDRFYLAYSSGRPQPLVEELPEVRRIIDARDSATSQGQSSAQGANPLAERLAAAAPGERTEILLG
LVRAQAAAVLRMRSPEDVAADRAFKDIGFDSLAVELRNRLTRATGLQLPATLVFDHPTPLALVS
LLRSEFLGDEENGIESLLAELDRDLDTTLAQGPSIPLEDQAKVAERLHALLAKWDGARDGTARATSP
QSLTAATDDEIFDLIDRKFR

^CP2P3^N-[S8^NDD/P3^CDD]-LoopHis₆, (NAR₈)

MANEEKLREYLKRVVVELEEHAHERLHELERQEHDPIAIVAMSCRYPGDIRSPEDLWRMLSEGGE
ITPFPTRDGRWDLGLYDADPDALGRAYVREGGFLHDAAEFDAAEFFGVSPREALAMDPQQRMLLT
TSWEAFERAGIEPASLRGSSTGVFIGLSYQDYAARVPNAPRGVEGYLLTGSTPSVASGRIAYTFGLE
GPATTVDTACSSSLTALHLAVRALRSPECTMALAGGVAMMATPHMFVEFSRQALAPDGRSKAF
SADADGFGAAEGVGLLLVERLSDARRNGHPVLAVVRGTAVNQDGASNGLTAPNGPSQQRVIRQA
LADARLAPGDIDAVETHGTGTSLGDPPIEAQGLQATYGKERPAERPLAIGSVKSNIGHTQAAAGAAG
IHKMVLAMRHGTLPKTLHADEPSPHVDWANSGLALVTEPIDWPAGTGPRRAAVSSFGISGTNAHV
VLEQAPDAAGEVLGADEVPEVSEHHHHHHTAGTSEVAEGSEASEAPAAPGSREASLPGHLPWVLS
AKDEQSLRGQAAALHAWLSEPAADLSADAGPARLRDVGITLATSRTAFAHRAAVTAADDRDGLD
GLATLAQGGTSAHVHLDRTARDGTTAFLFTGQGSQRPAGAGRELYDRHPVFARALDEICAHLDGHL
ELPLLDVMFAAEGSAAEALLDETRYTQCALFALEVALFRLVESWGMMPAALLGHSVGEIAAAHVA
GVFSLADAARLVAARGRLMQELPAGGAMLAVQAAEDEIRVWLETEERYAGRLDVAAVNGPEAA
VLSGDADAAREAEAYWSGLGRRTRALRVSHAFHSAHMDGMLDGFRAVLETVEFRRPSLTVVSNV
TGAAAGPDDLCDPEYWVRHVRGTVRFLDGVVRVLRDLGVRTCLELPGPDGVLTAAMAADGLADTPA
DSAAGSPVGPAGSPADSAAGALRPRPLLALLRRKRSETETVADALGRAHAHGTGPDWHAWFA
GSGAHRVDLPTYSFRRDRYWLDAPAADTAVDTAGLGLGTADHPLLGAUVSLPDRDGLLLTGRLSL
RTHPWLADHAVLGSVLLPGAAMVELAAHAAESAGLRDVRRELTLLEPLVLPEHGGVELRVTVGAP
AGEPGGESAGDGARPVSLHSRLADAPAGTAWSCHEVATGLLATDRPELVPAPDRAAMWPPQGAEEV
PLDGLYERLDGNGLAFGLPLFQGLNAVWRYEGEADIALPATTNATAPATANGGSSAAAPYGIH
PALLDASLHAIAGGLVDEPELVVRVFWHWSGVTVHAAGAAAARVRLASAGTDAVSLSLTDGEGRP
LVSVRELTLRPVTADQAAASRVGGLMHRVAWRPYALASSGEQDPHATSYGPTAVLGKDELKVAA
ALESAGVEVGLYPDLAALSQDVAAGAPAPRTVLAPLPAGPADGGAEGVRGTVARTLELLQAWLA
DEHLAGTRLLLVTRGAVRDPEGSGADDGGEDLSHAAAWGLVRTAQTENPGRFGLLDLADDASSY
RTLPSVLSADAGLRDEPQLALHDGTIRLARLASVRPETGTAAPALAPEGTVLLTGGTGGLGGLVAR
HVVGEWGVRRLLLVSRRGTDAPGADELVHELEALGADVSVAAACDVADREALTAVLDAIPAEHPL
TAVVHTAGVLSDGTLPSMTTEDVEHVLRPKVDAAFLLDLDELSTPAYDLAAFVMFSSAAAVFGGAG
QGAYAAANATLDALAWRRRAAGLPALSLGWGLWAETSGMTGELGQADLRMSRAGIGGISDAE
GIALDLAALRDDRHPVLLPLRLDAAGLRDAAGNDPAGIPALFRDVGARTVRARPSAASASTTAGT
AGTPGTADGAAETAAVTLADRAATVDGPARQRLLEFVVGGEVAEVLGHARGHRIDAERGFLDLG
FDSLTAVELNRNLSAGGLALPATLVFDHPSPAALASHLDAELPRGASDQDGAGNRNGNENGTTA
SRSTAETDALLAQLTRLEGALVLTGLSDAPGSEEVLEHLRSLRSMVTGETGTGTASGAPDGAGSG
AEDRPWAAGDGAGGGSEDGAGVPDFMNASAEELFGLLDQDPSTD

^CP3P4^N-[P3^NDD/S3^CDD]-LoopHis₆, (NAR₈)

MSTVNEEKYLDYLRRATADLHEARGRLRELEAKAGEPVAIVGMACRLPGGVASPEDLWRLVAGG
EDAISEFPQDRGWDVEGLYDPNPEATGKSYAREAGFLYEAGEFDADFFGISPREALAMDPQQRLLL
EASWEAFEHAGIPAATARGTSVGVFTGVMYHDYATRLTDVPEGIEGYLGTGNSGVSASGRVAYTL
GLEGPVAVTVDACSSSLVALHLAVQALRKGEVDMALAGGVVTVMSTPSTFVEFSRQRLAPDGRSK
SFSSTADGTSWSEGVGVLLVERLSDARRKGRILAVVRGTAVNQDGASSGLTAPNGPSQQRVIRRA
LADARLTTSDVDVVEAHGTGTRLGDPPIEAQAVIATYGQGRDGEQPLRLGSLKSNIGHTQAAAGVS
GVIKMVQAMRHGVLPKTLHVEKPTDQVDWSAGAVELLTEAMDWDPKGDGGLRRAAVSSFGVSG
TNAHVVEEAPAAEETPHHHHHHHAPEPSVGAGLVPWLVSAKTPAALDAQIGRLAAAFASQGRDA
ADPGAVARVLAGGRAEFEFHRAVVLGTGQDDFAQALTAPEGLIRGTSDVGRVAFVFPQGQTQWA
GMGAELLDVSKEFAAAMAECESALSRYVDWSLEAVVRQAPGAPTLEVRDVVQPVTFAVMVSLAK
VWQHGHGVTPQAVVGHSQGEIAAAAYVAGALTDDAARVVTLRKSIAAHLGKGGMISLALSEEA
TRQRIENLHGLSIAAVNGPTATVVS GDPTQIQELAQACEADGVRARIIPVDYASHSAHVETIESELA
EVLAGLSPRTPEVPFFSTLEGAWITEPVLDGTYWYRNLHRVGFAPAVETLATDEGFTHFIEVSAH
PVLMTLPETVTGLGTLRREQGGQERLVTSLAEAWTNGLTIDWAPVLPATGHHPELPTYAFQR
RHYWLHDSPAVQGSVQDSWRYRIDWKRLAVADASERAGLSGRWLVVVPEDRSAEAAPVLAALSG
AGADVPQLDVSPLGDRQLAATLGEALAAAGGAVDGVLSLLAWDESAHPGHPAPFTRGTGATLT
LVQALEDAGVAAPLWCVTHGAVSVGRADHVTSPAQAMVWGMGRVAALEHPPERWGGGLIDLPSDA
DRAALDRMTTVLAGGTGEDQVAVRASGLLARRLVRASLPAHGTASPWWQADGTVLVTGAEEPA
AAEAARRLARDGAGHLLLHTTPSGSEGAEGTSGAAEDSGLAGLVAELADLGATATVVTCDLTDAE
AAARLLAGVSDAHPLSAVLHLPPTVDSEPLAATDADALARVVTAKATAALHLDRLLREAAAAGGR

PPVLVLFSSVAAIWGGAGQGAYAAAGTAFLDALAGQHRADGPTVTSVAWSPWEGSRVTEGATGER
 LRRLGLRPLAPATALDTALGHGDTAVTIADVWSSFAPGFTTARPGTLLADLPEARRALDEQ
 QSTTAADDTVLSRELGALTGAEEQRRMQELVREHLAVVLNHPSPAEVDTGRAFRDLGFDLSLTAVE
 LRNRLKNATGLALPATLVFDYPTPRTLAEFLLAELGEQA**GAGTFEELDRWAANLPTLARDEATR**
AQITTRLQAILQSLADVSGGTGGGSVPDRLRSATDDELQLLDNDLELP

^CP4P5^N-[S3^NDD/P5^CDD]-LoopHis₆, (NAR₈)

MSNEEKLREYLRRALVDLHQARERLHEAESGEREPVAIVGMACRLPGGVASPEDLWRLVAGGED
 AISGFPQDRGWDVEGLYDPPDASGRTYCRAGGFLDEAGEFDADFFGISPREALAMDPQQRLLE
 TSWEAVEDAGIDPTSLQGQQVGVFAGTNGPHYEP LLRNTAEDLEGYVGTGNAASIMSGRVSYTLG
 LEGPAVTVDTACSSSLVALHLAVQALRKGECDMALAGGVTVMPNADLFVQFSRQRGLAADGR
 SKAFATSADGFGPAEGAGVLLVERLS DARRNGHRILAVVRGSAVNQDGASNGLTAPHGPSQQRVI
 RRALADARLAPGDVDVVEAHGTGTRLGDPIEAQAL IATYGGQESSEQPLRLGALKSNIGHTQAAA
 GVAGVIKMVQAMRHGLLPKTLHVDEPSDQIDWSAGTVELLTEAVDWPEKQDGGLRRAAVSSFGI
 SGTNAHVVEEAPAVEDS**HHHHHHH**AGGGVVPWPVSAKTPAALDAQIGQLAAYADGRD VDPAVA
 ARALVDSRTAMEHRAVAVGDSREALRDMPEGLVRGTSSDVGRVAFVFPQGQTQWAGMGAE
 LLDSSPEFAASMAECETALSRYVDWSLEAVVRQEPGAPTLDRVDVVPVTFAVMVSLAKVWQH
 GITPQAVVGHSQGEIAAAAYVAGALTLDDAARVVTLRSKSIAAHLAGKGMISLALDEAAVLKRLS

^CP5P6^N-[P5^NDD/P6^CDD]-LoopHis₆, (NAR₈)

MANNEDKL RDYLRVT AELQQNTRRLREIEGRTHEPVAIVGMACRLPGGVASPEDLWQLVAGDG
 DAISEFPQDRGWDVEGLYDPPDASGRTYCRSGGFLHDAGEFDADFFGISPREALAMDPQQRLSLT
 TAWEAIESAGIDPTALKGSGLG VFGGWHTGYTSGQTTAVQSPELEGHLVSGAALGFLSGRIAYV
 LGTDGPALTVDTACSSSLVALHLAVQALRKGECDMALAGGVTVMPNADLFVQFSRQRGLAADGR
 SKAFATSADGFGPAEGAGVLLVERLS DARRNGHRILAVVRGSAVNQDGASNGLTAPHGPSQQRVI
 RRALADARLAPGDVDVVEAHGTGTRLGDPIEAQAL IATYGGQESSEQPLRLGALKSNIGHTQAAA
 GVAGVIKMVQAMRHGLLPKTLHVDEPSDQIDWSAGTVELLTEAVDWPEKQDGGLRRAAVSSFGI
 SGTNAHVVEEAPAVEDS**HHHHHHH**AGGGVVPWPVSAKTPAALDAQIGQLAAYADGRD VDPAVA
 ARALVDSRTAMEHRAVAVGDSREALRDMPEGLVRGTSSDVGRVAFVFPQGQTQWAGMGAE
 LLDSSPEFAASMAECETALSRYVDWSLEAVVRQEPGAPTLDRVDVVPVTFAVMVSLAKVWQH
 GITPQAVVGHSQGEIAAAAYVAGALTLDDAARVVTLRSKSIAAHLAGKGMISLALDEAAVLKRLS

DFDGLSVAAVNGPTATVVSGDPTQIEELARTCEADGVRARIIPVDYASHSRQVEIEKELAEVLAGL
APQAPHVPPFFSTLEGTWITEPVLDDGTYWYRNLRHRVGFAPAVETLAVDGFTHFIEVSAHPVLTMT
LPETVTGLGLTLRREQGGQERLVTSLAEAWANGLTIDWAPILPTATGHHPELPTYAFQTERFWLQS
SAPTSAADDWRYRVWKPLTASGQADLSGRWIVAVGSEPEAEELGALKAAGAEVDVLEAGADDD
REALAARLTALTGGDGTGVVSLDDLVLPQVAWVQALGDAGIKAPLWSVTQGAVSVGRDTPAD
PDRAMLWGLGRVVALEHPERWAGLVLDLPAQPDAAALAHVLTALSGATGEDQIAIRTTGLHARRL
ARAPLHGRRPTRDWQPHGTVLITGGTGALGSHAARWMAHHGAEHLLLVSRSGEQAPGATQLTA
ELTASGARVTIAACDVADPHAMRTLLDAIPAETPLTAVVHTAGAPGGDPLDVTGPEDIARILGAKT
SGAEVLDDLLRGTPDLDAFVLYSSNAGVWVGSGSQGVYAAAHAHLDALAARRRRARGETATSVAWGL
WAGDGMGRGADDAYWQRRGIRPMSPDRALDELAKALSHDETFFVAVADVVDWERFAPAFTVSRPSL
LLDGVPEARQALAAPVGAPAPGDAAVAPTQGSSALAAITALPEPERRPALLTLVRTHAAAVLGHSS
PDRVAPGRAFTELGFDSLTAVALRNQLSTVVGNNRLPATTVFHDPTPAALAAHLHEAYLAPAEPAPT
DWEGRVRRALAEPLDRLRDAGVLDTVLRLTGIEPEPGSGSGSDGGAADPGAEPASIDDLDAEALI
RMALGPRNT

^CP6P7-[P6^NDD]-LoopHis₆, (NAR₈)

MTSSNEQLVDALRASLKENEELRKESRRRADRRQEPMAIVGMSCRFAGGIRSPEDLWDAVAAGK
DLVSEVPEERGWDIDSLYDPVPGRKGTTYVRNA AFLDDAAGFDAAFFGISPREALAMDPQQRQLL
EASWEVFERAGIDPASVRGTDVGVYVGGCYQDYAPDIRVAPEGTGGYVVTGNSSAVASGRIAYSL
GLEGPAVTVDACSSSLVALHLALKGLRNGDCSTALVGGVAVLATPGAFIEFSSQQAMAADGRTK
GFASAADGLAWGEGVAVLLERLSDARRKGHRVLAVVRGSAINQDGASNGLTAPHGPSQQRLIR
QALADARLTSSDVDVVEGHGTGTRLGDPIEAQALLATYQGQGRAPGQPLRLGTLKSNIGHTQAASG
VAGVIKMOVQALRHGVLPKTLHVDEPTQVDWSAGSVELLTEAVDWPWPGRRLRRAGVSAFGVG
GTNAHVVLEEAPAVEESH^{HHHHHH}AGGGVVPWPVSAKTSAALDAQIGQLAAYAEDRTDVPAAV
RALVDSRTAMEHRAVAVGDSREALRDALRMPEGLVRGTVTDPGRVAFVFPQGQTQWAGMGAE
LDSSPEFAAAMAECETALSPYVDWSLEAVVRQAPSAPTLDRVDVVQPVTFVAVMVSLAKVWQHGGI
TPEAVIGHSQGEIAAAVYVAGALTLDAAARVVTLSKSIAAHLAGKGGMISLALSEEATRQRIENLH
GLSIAAVNGPTATVVSGDPTQIQELAQACEADGIRARIIPVDYASHSAHVETIENELADVLGLSPQT
PQVPFFSTLEGTWITEPALDGGYWYRNLRHRVGFAPAVETLATDEGFTHFIEVSAHPVLTMTLPD
KVTGLATLRRREDGGQHRLTTS LAEAWANGLALDWASLLPATGALSPAVPDLPTYAFQHRSYWISP
AGPGEAPAHTASGREAVAETGLAWGPGAEDLDEEGRRSAVLAMVMRQAASVLRCDSPEEVPVDR
PLREIGFDSLTAVDFRNRVNRLTGLQLPPTVVFEHPTPVALAERISDELAERNWAVAEPDHEQAE
EEKAAAPAGARSGADTGAGAGMFRALFRQAVEDDRYGEFLDLVLAESAFAFRPQFASPEACSERLDP
VLLAGGPTDRAEGRAVLVGCTGTAANGGPHEFLRLSTSFQEERDFLAVPLPGYGTGTGTGTALLP
ADLDTALDAQARAILRAAGDAPVLLGHSGGALLAHELAFRLERAHGAPPAGIVLDPYPPGHQE
PIEVWSRQLGEGFLAGELEPMSDARLLAMGRYARFLAGPRPGRSSAPVLLVRASEPLGDWQEERG
DWRAHWDLPTVADVPGDHFTMMRDHAPAVAEAVLSWLD AIEGIEGAG

P1^N-[E3^CDD]-CterHis₆, (NAR₉)

MSSAGITRTGARTPVTGRGAAAWDTGEVRVRRGLPPAGPDHAEHSFSRAPTGDVRAELIRGEMST
VSKSESEEFVSVSNDAGSAHGTAEPVAVVGISCRVPGARDPREFWELLAAGGQAVTDVPADRWNA
GDFYDPDRSAPGRSNSRWGGFIEDVDRFDAAFFGISPREAAEMDPQQRALALELGWEALERAGIDPS
SLTGTRTGVFAGAIWDDYATLKHRRQGGAAITPHTVTGLHRGIIANRLSYTLGLRGPSMVVDSGQSS
SLVAVHLACESLRRGESELALAGGVSLNLVPDSIIIGASKFGGLSPDGRAYTFDARANGYVRGEGGG
FVVLKRLSRAVADGDPVLAVIRGS AVNNGGAAQGMTPDAQAQEAVLREAHHERAGTAPADVRYV
ELHGTGTPVGDPIEAAAALGAALGTGRPAGQPLLVGSVKTNIGHLEGAAGIAGLIKAVLAVRGRAL
PASLNYETPNPAIPFEELNLRVNTEYLPWEPEHDGQRMVVGVSFGMGGTNAHVVLEEAPGVVEG
ASVVESTVGGSAVGGGVVPWVVSAKSAAALDAQIERLAAFASRDRTDGDVAVDAGAVDAGAV
ARVLAGGRAQFEHRAVVVGSGPDDLAAALAAPEGLVRGVASGVGRVAFVFPQGQTQWAGMGAE
LLDSSAVFAAAMAEECEAALSPYVDWSLEAVVRQAPGAPTLERVDVVQPVTFVAVMVSLARVWQH
GVTPQAVAVGHSQGEIAAAVYVAGALSLLDAAARVVTLSKSIAAHLAGKGGMISLALSEDAVLERLA
GFDGLSVAAVNGPTATVVSGDPVQIEELARACEADGVRARIIPVDYASHSRQVEIESELAEVLGL
SPQAPRVPPFFSTLEGAWITEPVLDDGGYWYRNLRHRVGFAPAVETLATDEGFTHFIEVSAHPVLTMT
ALPGTVTGLATLRRDNGGQDRLVASLAEAWANGLAVDWSPLLP SATGHHS DLPTYAFQTERHWL
GEIEALAPAGEPAVQPAVLRTEAAEPAELDRDEQLRVILDKVRAQTAQVLGYATGGQIEVDRTFR

EAGCTSLTGVDLRNRINA AFGVRMAPSMIFDFPTPEALAEQ LLLVVHGEAA PSALAGLDALEAALP
EVPATEREELVQRLERMLAALRPVAQAADASGTGANPSGDDLGEAGVDELLEALGRELDGD **HHHH**
HHHH

^CP1P2^N-[E3^NDD/S8^CDD]-CterHis₆, (NAR₉)

MTDSEKVAEYLRRATL DLRAARQRIRELES DPVAIVGMACRLPGGVASPEDLWRLVAGGGDAISE
FPQDRGWDVEGLYHPDPEHPGTSYVRQGGFIENVAGFDAFFGISPREALAMDPQQRLLLET SWE
AVEDAGIDPTSLRGRQVG VFTGAMTHEYGPSLRDGGEGLDGYLLTGNTASVMSGRVSYTLGLEG
PALTVDTACSSSLVALHLAVQALRKGEVDMALAGGVAVMPTPGMFVEFSRQRGLAGDGRSKAFA
ASADGTSWSEGVGVLLVERLS DARRNGHQVLAVVRGSAVNQDGASNGLTAPNGPSQQRVIRRAL
ADARLTSDVDVVEAHGTGTRLGDPIEAQAL IATYGQGRDDEQPLRLGSLKSNIGHTQAAAAGVSG
VIKMOVQAMRHGLLPKTLHVDEPSDQIDWSAGAVELLTEAVDWPEKQDGGLRRAAVSSFGISGTN
AHVVLEEAPVVVEGASVVEPSVGGSAVGGGVTPWVVS AKSAAALDAQIERLA AFASRDRTDDADA
GAYDAGAVAHVLADGRAQFEHRAVALGAGADDLVQALADPDGLIRGTASGVGRVAFVFPQGQTQ
WAGMGAELLDS SAVFAAAMAEC EAALSPYVDWSLEAVVRQAPGAPT LERV DVVQPVTFAVMVSL
ARVWQH HGVTPQAVVGHSQGEIAAAYVAGALPLDDAARVVT LRSKSIAAHLAGKGGMLSLALNE
DAVLERLSDFDGLSVA AVNGPTATVVSGDPVQIEELA QACKADGFRARIIPVDYASHSRQVEIIESE
LAQVLAGLSPQAPRPFFSTLEGTWITEPVL DGTYWYRNL RHRVGFAPA IETLAVDEGFTHFVEVS
AHPVLTMTLPETVTGLGTLRREQGQERLV TSLAEAWVNGLPVAWTSLLPATASRPGLPTYAFQ
AERYWLENTPAALATGDDWRYRIDWKRLPAAEGSERTGLSGRWLAVTPEDHSAQAAAVLTALVD
AGAKVEVLTAGADDDREALAARLTALT TGDGFTGVVSLLDGLVPQVAVWVQALGDAGIKAPLWSV
TQGAVSVGRLDTPADPDRA MLWGLGRVVALEH PERWAGLVLDLPAQPDAAALAH LVTALSGATG
EDQIAIRTTGLHARRLARAPLHGRPRTRD WQPHGTVLITGGTGALGSHAARWMAHHGA EHL L LV
SRSGEQAPGATQLTAELTASGARVTIAACDVADPHAMRTLLDAIPAETPLTAVVHTAGALDDGIVD
TLTAEQVRRAHRAKAVGASVLD ELTRDLDLDAFVLFSSVSSTLGIPGQGNYPHNAYLDALAARR
RATGRSAVSVAWGPWDGGGMAAGDGVAERLRNHGVP GMDPELALAALESALGRDETAITVADID
WDRFYLAYSSGRPQPLVEELPEVRRIIDARDSATSGQGGSSAQGANPLAERLAAAAPGERTEILLG
LVRAQAAAVLRMRSPEDVAADRAFKDIGFDSL AGVELRNRLTRATGLQLPATLVFDHPTPLALVS
LLRSEFLGDEENGIESLLAELDRLDTTLAQGPSI PLEDQAKVAERLHALLAKWDGARDGTARATSP
QSLTAATDDEIFDLIDRKFR **HHHHHHH**

^CP6P7-[P6^NDD]-CterHis₆-C207A-P7ACPdeletion, (DML₄)

MTSSNEQLVDALRASLKENEELRKESRRR RADRRQEPMAIVGMSCRFAGGIRSPEDLWDAVAAGK
DLVSEVPEERGWDIDSLYDPVPGRKGT TYVRNAAFLDDAAGFDAFFGISPREALAMDPQQRQLL
EASWEVFERAGIDPASVRGTDVGVYVGC GYQDYAPDIRVAPEGTGGYVVTGNSSAVASGRIAYSL
GLEGPAVTVDACSSSLVALHLALKGLRNGDCSTALVGGVAVLATPGAFIEFSSQQAMAADGR TK
GFASAADGLAWGEGVAVLLERLS DARRKGHRVLAVVRGSAINQDGASNGLTAPHGPSQQR LIR
QALADARLTSSD VDVVEGHGTGTRLGDPIEAQALLATYGQGRAPGQPLRLGTLKSNIGHTQAASG
VAGVIKMOVQALRHGVLPKTLHVDEPTDQVDWSAGSVELLTEAVDWPERPGR LRRAGVSAFGVG
GTNAHVLEEAPAVEESPAVEPPAGGGVVPVPSAKTSAALDAQIGQLAAYAEDRTD VDPVAAR
ALVDSRTAMEHRAVAVGDSREALRDALRMPEGLVRGTVTDPGRVAFVFPQGQTQWAGMGAELL
DSSPEFAAAMAECETALSPYVDWSLEAVVRQAPSAPT LDRVDVVQPVTFAVMVSLAKVWQH HGIT
PEAVIGHSQGEIAAAYVAGALTLD DAARVVTLRSKSIAAHLAGKGGMISLALSEEATRQRIENLHG
LSIAAVNGPTATVVSGDPTQIQELAQACEADGIRARIIPVDYASHSAHVETIENELADVLAGLS PQTP
QVPFFSTLEGTWITEPALDGGYWYRNL RHRVGFAPAVETLATDEGFTHFIEVSAHPVLTMTLPDK
VTGLATLRREDGGQHRLT TSLAEAWANGLALDWASLLPATGALSPAVPDLPTYAFQHRSY WISPA
GPGEAPAHTASAEPSDHEQAEEEEKAAAPAGARSGADTGAGAGMFALFRQAVEDDRYGEFLDVL
AEASA FRPQFASPEACSERLDPVLLAGGPTDRAE GRAVLVGCTGTAANGGPHEFLRLSTS FQEERD
FLAVPLPGYGTGTGTGTALLPADLDTALDAQARAILRAAGDAPVLLGHSGGALLAHELA FRLER
AHGAPPAGIVLDPYPPGHQEPIEVWSRQLGEGLFAGELEPMSDARLLAMGRYARFLAGPRPGRS
SAPVLLVRASEPLGDWQEERG DWRAHWDLPHTVADVPGDHFTMMRDHAPAVAEAVLSWLD AIE
GIEGAG **HHHHHHH**

^CP2P4^N-[S8^NDD/E3^CDD]-CterHis₆, (ΔP3₁)

MANEEKLREYLKRVVVELEE AHERLHELERQEHD **PI**AIIVAMSCRYPGDIRSPEDLWRMLSEGGE G
ITPFPTDRGWDLDGLYDADPDALGRAYVREGGFLHDAAEFDAEFFGVSPREALAMDPQQRMLLT

TSWEAFERAGIEPASLRGSSTGVFIGLSYQDYAARVPNAPRGVEGYLLTGSTPSVASGRIAYTFGLE
 GPATTVDTACSSSLTALHLAVRALRSGETMALAGGVAMMATPHMFVEFSRQRALAPDGRSKAF
 SADADGFGAAEGVGLLLVERLSDARRNGHPVLAVVRGTAVNQDGASNGLTAPNGPSQQRVIRQA
 LADARLAPGDIDAVETHGTGTS LGDPIEAQGLQATYGKERPAERPLAIGSVKSNIGHTQAAAGAAG
 IIKMVLAMRHGTLPKTLHADEPSPHVDWANSGLALVTEPIDWPAGTGPRRAAVSSFGISGTNAHV
 VLEQAPDAASSETPASEATPAVEPSVGAGLVPWLVS AKTPAALDAQIGRLAAFASQGRDAAADPGA
 VARVLAGGRAEFEHRAVVLGTGQDDFAQALTAPEGLIRGTPSDVGRVAFVFPQGQTQWAGMGAE
 LLDVSKEFAAAMAECESALSRYVDWSLEAVVRQAPGAPT LERV DVVQPVTFAVMVSLAKVWQHH
 GVTPQAVVGHSQGEIAAAVYVAGALTDDAARVVT LRSKSIAAHLAGKGGMISLALSEEATRQRIEN
 LHGLSIAAVNGPTATVVSGDPTQIQELAQACEADGVRARIIPVDYASHSAHVETIESELAEVLAGLS
 PRTPEVPFFSTLEGAWITEPVLDGTYWYRNL RHRVGFAPAVETLATDEGFTHFIEVSAHPVLTMTL
 PETVTGLGTLRREQGGQERLVTSLAEAWTNGLTIDWAPVLP TATGHHPELPTYAFQRRHYWLHD
 SPAVQGSVQDSWRYRIDWKRLAVADASERAGLSGRWL VVVPEDRSAEAAPVLAALSGAGADPVQ
 LDVSP LGDRQRLAATLGEALAAAGGAVDGVLSLLAWDESAHPGHPAPFTRGTGATLT LTVQALD
 AGVAAPLWCVTHGAVSVGRADHVTSPAQAMVWGMGRVA ALEHPERWGGGLIDLPSDADRAALDR
 MTTVLAGGTGEDQVAVRASGLLARRLVRASLPAHG TASPWWQADGTVLVTGAEEPA AAEAAARR
 LARDGAGHLLLHTTPSGSEGAEGTSGAAEDSGLAGLVAELADL GATATVVTCDLTDAEAAAARLLA
 GVS DAHPLSAVHLPPPTVDSEPLAATDADALARVVTAKATAALHLDRLRLREAAAGGRPPVLVLF
 SSVAAIWGGAGQGAYAAAGTAFLDALAGQHRA DGPVTVTSAWSPWEGSRVTEGATGERLRLRLGLR
 PLAPATALTALDTALGHGDTAVTIADVWSSFAPGFTT ARPGTLLADLPEARRALDEQQSTTAADD
 TVLSRELGALTGAEEQRRMQELVREHLAVVLNHP SPEATVDTGRAFRDLGFDLSLTAVELRNRLKN
 ATGLALPATLVFDYPTPRTLAEFLLA EILGEQASPSALAGLDALEAALPEVPATEREELVQRLE
 MLAALRPVAQAADASGTGANPSGDDLGEAGVDELLEALGRELDGD HHHHHH

^CP5R5^N-[E5^NDD/S5^CDD]-CterHis₆, (ANH₁; x₂)

MSGDNGMTEEKLRRYLKRTVTELD SVTARLREVEH RAGEPIAIVGMACRLPGGVASPEDLWQLV
 AGDGD AISEFPQDRGWDVEGLYDPPDASGR TYCRSGGFLHDAGEFDADFFGISPREALAMDPQQ
 RLSLT TAWEAIESAGIDPTALKGSG LGVFVGGWHTGYTSGQTTAVQSPELEGLVSGAALGFLSG
 RIAYVLGTDGPALTVD TACSSSLVALHLAVQALRKGECDMALAGGVTVMPNADLFVQFSRQRL
 AADGRSKAFATSADGFGPAEGAGVLLVERLSDARRNGH RILAVVRGSAVNQDGASNGLTAPHGPS
 QQRVIRRALADARLAPGDVDVVEAHGTGTRLGDPIEAQAL IATYGQEKSSQPLRLGALKSNIGHT
 QAAAGVAGVIKMVQAMRHGLLPKTLHVDEPSDQIDWSAGTV ELLTEAVDWPEKQDGG LRRAAV
 SSFGISGTNAHV VLEEAPAVESSPAEEEEQPVETPVVASDVLPLVISAKTQPALTEHEDRLRAYLAASP
 GADTRAVASTLAVTRSVFEHRAVLLGDDAVTGTAVTDPR VVVFVFPQGWQWLGMGSALRDSSV
 FAERMAECAAAELSEFVDWDLFAVLDDPAVVD RVDVVQPASWAVMVSLAAVWQAAGVRPDAVIG
 HSQGEIAAACVAGAVSLRDAARIVTLRSQAIARGLAGRGAMASVALPAHEIELVDGAWIAAHNGP
 ASTVIAGTPEAVDHVLT AHEARGVRVRRTIVDYASHTPHVELIRDELLGITAGIGSQPPVVPWLSTV
 DGSWVDSPLDGEYWYRNLREPVG FHPAVSQLQAQGD AVFVEVSASPVLLQAMDDDVVTVATLRR
 DDGDATRMLTALAQA YVHGVTVDWPAILGTTTARVLDLPTYAFQH QRYWVKSVDRAAADGHPL
 LGAVVELPESDGVLLTGRVSLATHAWLADHAVWG SVLLPGTAFVELVVHAAGEVGCDVVD ELVI
 ETPLLLPQTGGVQLSVSVGEADESGHRVTVF SRADNADTWTRHVSATVRVSDTTVPPSDLTAWP
 PAQAKPVDVAGFYDQLTGMGYEYGPAFQGLQA AWRDGDTVFAEVALAEEQVREAAARYAVHPAL
 LDAALHACTLNASDAEVGVGLPFSWNGVRVHAGGSAMLRVAVTQAADGWSVRVADDIGRPVASV
 GSVLTRPVTDALGSAADDLLALTWAGIPTPQQTGLTVGRFEELVSDGDVPVPEVAVFTALPDND
 DDPLEQTRKLTGQVLQAVQE WLGGERFSDSTLVVRTGTGLAAAGVSGLMRSQAQSEHPGRFVLVE
 SDDDALAPDQLAAAVGLDEPRLRISDGRFEV PRLTRTHAAEPESEKVWDPDGTVLITGGSGVLGI
 AARHLVAERGVRHLLLLSR SAPDEALINQLGELGARVETAACDVSDRAALAQVLAGVSPEHPLTA
 VIHTAGALDDGVVESLTAQRLDAVLRPKADGAWN LHELTRDADLAAFV MYSSAAGVLGSAGQGN
 YAAANAFVDALAEQRRAEGLPALAVAWGLWEDASGLTANLTD TDRDRIRRGGLRAISAEYGMGL
 FDSASRHSEPVLVAAAMEPVRDAEVPALLRSLHRPIARRA ASTGDSSVQWLAALAPEERAKALLRV
 VCD SAATVLGHADIDSIPVTA AFKDLGVDSL TAVELRNSLAKATGLRLPATLVFDYPTPTALAARL
 DELFAPAEPASTVDSALAE LDRIEQQLSMLTG EARDRIATRLRALHEKWNSAAEVPTGADVLST
 LDSATHDEIFE FIDNELDLS HHHHHH

^CR5P7^N-[S5^NDD]-CterHis₆, (ANH₁; y₂)

MANEEKLFGYLKKVTTADLHQTRQRLAAESRSQEP^IAIVGMACRLPGGVSSPEDLWRLVESGTD
ISGFADRGWDVENLYDPDPEAAGKSYCVQGGFLDSAGGFDASFFGISPREALAMDPQQRLVLEAS
WEAFERAGIEPGSLRGSDTGVMGAYPGGYGVGADLGGFGATAGAVSVLSGRVSYFFGLEGPAV
TVDTACSSSLVALHQAGYALRQGECSLALVGGVTVMATPQSFVEFSRQRGLASDGRCKAFADAAD
GTGWAEGAGVLLVERLSAQAKGHQVLAVVRGSAVNQDGASNGLSAPNGPSQQRVIRAALSNA
LSTAEDVDVVEAHGTGTTLGDP^IEQAQALLATYGQDREQPLLLGSVKSNLGHTQAAAGVSGVIK
MALQHGLVPRTLHVDEPSR^HVDWTDGAVALVTENQPPWDMGRPRRAGVSSFGISGTNAHVILES
APPTQSSPAVEPPAGGGVVPWPVSAKTSAAALDAQIGQLAAYAEDRTDVP^AVAARALVDSRTAM
EHRAVAVGDSREALRDLRMPEGLVRGTVTDPGRVAFVFPQGQTQWAGMGAELLDSSPEFAAA
MAECETALSPYVDWSLEAVVRQAPSAPTLDRVDVVPVTFAVMVSLAKVWQHGHITPEAVIGHS
QGEIAAAYVAGALTLD^DDAARVVTLRKSIAAHLAGKGGMISLALSEEATRQRIENLHGLSIAAVNG
PTATVVSGDPTQIQELAQACEADGIRARIIPVDYASHSAHVETIENELADVLAGLSPQTPQVPFFSTL
EGTWITEPALDGGYWYRNLRRHVGFAVETLATDEGFTHFIEVSAHPVLTMTLPDKVTGLATLR
REDGGQHRLTTS^LAEAWANGLALDWASLLPATGALSPAVPDLPTAFQHRYSWISPAGPGEAPAH
TASGREAVAETGLAWGPGAEDLDEEGRRSAVLAMVMRQAASVLRCDSP^EEV^PDRPLREIGFDSL
TAVDFRN^RVNRLTGLQLPPTVFEHPTPVALAERISDELAERNWAVAEP^SDHEQAE^EE^EKAAAPAG
ARSGADTGAGAGMFRALFRQAVEDDRYGEFLDVLAESA^FRPQFASPEACSERLDPVLLAGGPTD
RAEGR^AVLVGCTGTAANGGPHEFLRLSTS^FQEERDFLAVPLPGYGTGTGTGTALLPADLDTALDA
QARAILRAAGDAPVLLGHSGGALLAHELAFRLERAHGAPPAGIVLVDPPYPPGHQEPIEVWSRQL
GEGLFAGELEPMSDARLLAMGRYARFLAGPRPGRSSAPVLLVRASEPLGDWQEERG^DWRAHWD
LPHTVADVPGDHFTMMRDHAPVAEAVLSWLD^AIEGIEGAGKHHHHHH

^CP5R5^N-[P5^NDD/S5^CDD]-CterHis₆, (ANH₃)

MANNEDKL^RDYLRVTAELQ^NTRRLREIEGRTHEPVAIVGMACRLPGGVASPEDLWQLVAGDG
DAISEFPQDRGWDVEGLYDPDPDASGRTYCRSGGFLHDAGEFDADFFGISPREALAMDPQQRLSLT
TAWEAIESAGIDPTALK^GSGLG^VFVGGWHTGYTSGQTAVQSPELEGHLVSGAALGFLSGRIAYV
LGTDGPALTVDTACSSSLVALHLAVQALRKGECDMALAGGVTVM^PNADLFVQFSRQRGLAADGR
SKAFATSADGFGPAEGAGVLLVERLS^DARRNGHRILAVVRGSAVNQDGASNGLTAPHGPSQQRVI
RRALADARLAPGDVDVVEAHGTGTRLGD^PIEQAQALIATYGQEK^SSEQPLRLGALKSNIGHTQAAA
GVAGVIK^MVQAMRHGLLPKTLHVDEPSDQIDWSAGTV^EELLTEAVDWPEKQDGG^LRRAAVSSFGI
SGTNAHV^VLEEAPAVEDSPA^VVEPPAGGGVVPWPVSAKTPAALDAQIGQLAAYADGRTDVDP^AVAA
RALVDSRTAMEHRAVAVGDSREALRDLRMPEGLVRGTSSDVGRVAFVFPQGQTQWAGMGAEL
LDSSPEFAASMAECETALSRYVDWSLEAVVRQEPGAPTLDRVDVVPVTFAVMVSLAKVWQHGH
ITPQAVVGHSQGEIAAAYVAGALTLD^DDAARVVTLRKSIAAHLAGKGGMISLALDEAAVLKRLSDF
DGLSVA^AVNGPTATVVSGDPTQIEELARTCEADGVRARIIPVDYASHSRQVEIEKELAEVLAGLAP
QAPHVPPFFSTLEGTWITEPVLDGTYWYRNLRRHVGFAVETLAVDGFTHFIEVSAHPVLTMTLP
ETVTGLGTLRREQGQERLVTSLAEAWANGLTIDWAPILPTATGHHPELPTAFQTERFWLQSSA
PAAADGHPLLGAVVELPESDGVLLTGRVSLATHAWLADHAVWGSVLLPGTAFVELVVHAAGEVG
CDVVDELVIETPLLLPQTGGVQLSVSVGEADESGHRVVTVFSRADNADTWTRHVSATVRVSDTTV
PPSDLTAWPPAQAKPVDVAGFYDQLTGMGYEYGP^AFQGLQA^AWRDGD^TVFAEVALAEEQVREA
ARYAVHPALLDAALHACTLNASDAEVGVLPFSWNGVRVHAGGSAMLRVAVTQAADGWSVRVA
DDIGRPVASVGS^LVTRPVTADALGSAADDLLALTWAGIPTPQQTGLTVGRFEELVSDGDVPVPEVA
VFTALPDNDDPLEQTRKLTGQVLQAVQE^WLGGERFSDSTLVVRTGTGLAAAGVSGLMRSAQSE
HPGRFVLVESDDDALAPDQLAAAVGLDEPRLRISDGRFEV^PRLTRTHAAEPESEK^VWD^PDPGTVLIT
GGSGVLAGIAARHLVAERGVRHLLLLSR^SAPDEALINQLGELGARVETAACDVSDRAALAQVLAG
VSPEHPLTAVIHTAGALDDGVVESLTAQRLDAVLRPKADGAWN^LHELTRDADLAA^FVMYSSAAG
VLGSAGQGN^YAAANAFVDALAEQRRAEGLPALAVAWGLWEDASGLTANLTD^TDRDRIRRGGLR
AISA^EYGMGLFDSASR^HSEPVLVAAAMEPVRDAEVPALLRSLHRPIARRA^ASTGDSSVQWLAALAP
EERAKALLRVCD^SAATVLGHADIDSIPVTA^FKDLGVDSLTA^VELRNSLAKATGLRLPATLVFDY
PTPTALAA^RLDELFAPEPASTVDSALAE^LDRIEQQLSMLTGEARARDRIATRLRALHEKWN^SAAE
VPTGADVLSTLDSATHDEIFE^FIDNELDLSHHHHHH

^CP2P4^N-[S8^NDD/S3^CDD]-CterHis₆, (ΔP3₃)

MANEEKLREYLKRVVVELEE~~AHERLHELERQEHD~~PIAIVAMSCRYPGDIRSPEDLWRMLSEGGE
ITPFPTRDGRWDLDGLYDADPDALGRAYVREGGFLHDAAEFD~~AEFFGVSPREALAMDPQQRMLLT~~
TSWEAFERAGIEPASLRGSSTGVFIGLSYQDYAARVPNAPRGVEGYLLTGSTPSVASGRIAYTFGLE
GPATTVDTACSSSLTALHLAVRALRSPECTMALAGGVAMMATPHMFVEFSRQRALAPDGRSKAF
SADADGFGAAEGVGLLLVERLS~~DARRNGHPVLAVVRGTAVNQDGASNGLTAPNGPSQQRVIRQA~~
LADARLAPGDIDAVETHGTGTSLGDP~~IEAQGLQATYGKERPAERPLAIGSVKSNIGHTQAAAGAAG~~
IHKMVLAMRHGTLPKTLHADEPSPHVDWANSGLALVTEPIDWPAGTGPRRAAVSSFGISGTNAHV
VLEQAPDAAS~~SE~~ETPASEATPAVEPSVGAGLVPWLVS~~AKTPAALDAQIGRLAAFASQGR~~TDAAADPGA
VARVL~~AGGRAE~~FEHRAVVLGTGQDDFAQALTAPEGLIRGTPSDVGRVAFVFPQGQTQWAGMGAE
LLDVSKEFAAAMAECESALSRYVDWSLEAVVRQAPGAPT~~LERVDVVQPVTF~~AVMVSLAKVWQH
GVTPQAVVGHSQGEIAAAYVAGALTLD~~DAARVVTLRSKSIAAHL~~AGKGGMISLALSEEATRQRIEN
LHGLSIAAVNGPTATVVSGDPTQIQELAQACEADGVRARIIPVDYASHSAHVETIESELA~~EVLAGLS~~
PRTPEVPPFFSTLEGAWITEPVLDGTYWYRNL~~RHRVGFAPAVETLATDEGFTHFIEVSAHPVLTMTL~~
PETVTGLGTLRREQGGQERLVTSLAEAWTNGLTIDWAPVLPTATGHHPELPTYAFQRRHYWLHD
SPAVQGSVQDSWRYRIDWKRLAVADASERAGLSGRWL~~VVPEDRSAEAPVLAALS~~GAGADPVQ
LDVSPLGDRQRLAATLGEALAAAGGAVDGVLSLLAWDESAHPGHPAPFTRGTGATLTLVQAL~~ED~~
AGVAAPLWCVTHGAVSVGRADHVTSPAQAMVWGMGRVA~~ALEH~~PERWGGGLIDLPSDADRAALDR
MTTVLAGGTGEDQVAVRASGLLARRLVRASLPAHGTASPWWQADGTVLVTGAEEPA~~AAEAARR~~
LARDGAGHLLLHTTPSGSEGAEGTSGAAEDSGLAGLVAELADLGATATVVTCDLTDAEAAARR
GVSDAHPLSAVLHLPPTVDSEPLAATDADALARVVTAKATAALHLDRL~~LLREAAAAGRP~~PVVLVLF
SSVAAIWGGAGQGAYAAAGTAFLDALAGQHRADGPTVTSVAWSPWEGSRVTEGATGERLRLGLR
PLAPATALTALDTALGHGDTAVTIADVWSSFAPGFTTARPGTLLADLPEARRALDEQQSTTAADD
TVLSRELGALTGAEEQRRMQELVREHLAVVLNHPSP~~EAVDTGRAFRDLGFD~~SLTAVELRNRLKN
ATGLALPATLVFDYPTPRTLA~~EFLLA~~EILGEQA~~AS~~AGTFEELDRWAANLPTLARDEATRAQITTRL
QAILQSLADVSGGTGGGSVPDRLRSATDDEL~~FQLLDNDLELP~~HHHHHHH

^CP3P4^N-[S8^NDD/S3^CDD]-CterHis₆, (ΔP3₂)

MANEEKLREYLKRVVVELEE~~AHERLHELERQEHD~~PIAIVGMACRLPGGVASPEDLWRLVAGGED
AISEFPQDRGWDVEGLYDPNPEATGKSYAREAGFLYEAGEFDADFFGISPREALAMDPQQRLLLEA
SWEAFEHAGIPAATARGTSVGVFTGVMYHDYATRLTDVPEGIEGYLGTGNSGSVASGRVAYTLGL
EGPAVTVDACSSSLVALHLAVQALRKGEVDMALAGGV~~VTMSTPSTFVEFSRQRGLAPDGRSKSF~~
SSTADGTSWSEGVGVLLVERLS~~DARRK~~GHRILAVVRGTAVNQDGASSGLTAPNGPSQQRVIRRAL
ADARLTSDVDVVEAHGTGTRLGDP~~IEAQAVIATYGQGRDGEQPLRLGSLKSNIGHTQAAAGVSG~~
VIKMOVQAMRHGVLPKTLHVEKPTDQVDWSAGAVELLTEAMDWPDKGDGGLRRAAVSSFGVSGT
NAHVVEEAPAAE~~SE~~ETPASEATPAVEPSVGAGLVPWLVS~~AKTPAALDAQIGRLAAFASQGR~~TDAA
DPGAVARVL~~AGGRAE~~FEHRAVVLGTGQDDFAQALTAPEGLIRGTPSDVGRVAFVFPQGQTQWAG
MGAELLDVSKEFAAAMAECESALSRYVDWSLEAVVRQAPGAPT~~LERVDVVQPVTF~~AVMVSLAKV
WQH~~HG~~VTQAVVGHSQGEIAAAYVAGALTLD~~DAARVVTLRSKSIAAHL~~AGKGGMISLALSEEAT
RQRIENLHGLSIAAVNGPTATVVSGDPTQIQELAQACEADGVRARIIPVDYASHSAHVETIESELA
VLAGLS~~PRTPEVPPFFSTLEGAWITEPVLDGTYWYRNL~~RHRVGFAPAVETLATDEGFTHFIEVSAHP
VLTMTLPETVTGLGTLRREQGGQERLVTSLAEAWTNGLTIDWAPVLPTATGHHPELPTYAFQRR
HYWLHDS~~PAVQGSVQDSWRYRIDWKRLAVADASERAGLSGRWL~~VVPEDRSAEAPVLAALS
GADPVQLDVSPLGDRQRLAATLGEALAAAGGAVDGVLSLLAWDESAHPGHPAPFTRGTGATLTL
VQAL~~EDAGVAAPLWCVTHGAVSVGRADHVTSPAQAMVWGMGRVA~~ALEHPERWGGGLIDLPSDAD
RAALDRMTTVLAGGTGEDQVAVRASGLLARRLVRASLPAHGTASPWWQADGTVLVTGAEEPA
AEAARRLARDGAGHLLLHTTPSGSEGAEGTSGAAEDSGLAGLVAELADLGATATVVTCDLTDAEA
AARLLAGVSDAHPLSAVLHLPPTVDSEPLAATDADALARVVTAKATAALHLDRL~~LLREAAAAGRP~~
PVLVLFSSVAAIWGGAGQGAYAAAGTAFLDALAGQHRADGPTVTSVAWSPWEGSRVTEGATGERL
RRLGLRPLAPATALTALDTALGHGDTAVTIADVWSSFAPGFTTARPGTLLADLPEARRALDEQQ
STTAADDTVLSRELGALTGAEEQRRMQELVREHLAVVLNHPSP~~EAVDTGRAFRDLGFD~~SLTAVEL
RNRLKNATGLALPATLVFDYPTPRTLA~~EFLLA~~EILGEQA~~AS~~PSALAGLDALEAALPEVPATEREEL
VQRLERMLAALRPVAQAADASGTGANPSGDDLGEAGVDELLEALGRELDGDHHHHHHH

^CP5P7-[E5^NDD]-CterHis₆, (ΔP6₁)

MSGDNGMTEEKLRRLRYLKRTVTELD^{SV}TARLREVEHRA^{GE}PIAIVGMACRLPGGVASPEDLWQLV
AGDGDAISEFPQDRGWDVEGLYDPPDASGRTYCRSGGFLHDAGEFDADFFGISPREALAMDPQQ
RLSLTTAWEAIESAGIDPTALKGSGLGVFVGGWHTGYTSGQTTAVQSPELEGHLVSGAALGFLSG
RIAYVLGTDGPALTVDTACSSSLVALHLAVQALRKGECDMALAGGVTVM^{PN}ADLFVQFSRQRGL
AADGRSKAFATSADGFGPAEGAGVLLVERLSDARRNGHRILAVVRGSAVNQDGASNGLTAPHGPS
QQRVIRRALADARLAPGDVDVVEAHGTGTRLGDPIEAQAL^{IATY}GQEKSS^{EQ}PLRLGALKSNIGHT
QAAAGVAGVIK^{MV}QAMRHG^{LL}PKTLHVDEPSDQIDWSAGTVELLTEAVDWPEKQDGG^{LR}RAAV
SSFGISGTNAHV^VLEEAPAVE^{SS}SPAVEPPAGGGVVPWPVSAK^{TS}AALDAQIGQLAAYAEDRTDVD
PAVAARALVDSRTAMEHRAVAVGDSREALRDALRMPEGLVRGTVTDPGRVAFVFPQGQTQWAG
MGAELLDSSPEFAAAMAECETALSPYVDWSLEAVVRQAPSAPT^{LD}RV^{DV}VQPVTFAVMVSLAKV
WQHHGITPEAVIGHSQGEIAAA^YVAGALTLDDAARVVT^{LR}SKSIAAHLAGKGGMISLALSEEATR
QRIENLHGLSIAAVNGPTATVVSGDPTQIQELAQACEADGIRARIIPVDYASHSAHVETIENELADVL
AGLSPQTPQVPFFSTLEG^{TW}ITEPALDGGYWYRNL^{RHR}VGFAPAVETLATDEGFTHFIEVSAHPVL
TMTLPDKVTGLATLRREDGGQHRLT^{TS}LAEAWANGLALDWASLLPATGALSPAVPDLPTYAFQH
RSYWISPAGPGEAPAHTASGREAVAETGLAWGPGAEDLDEEGRRSAVLAMVMRQAASVLRCDSP
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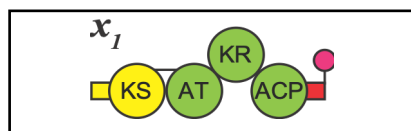
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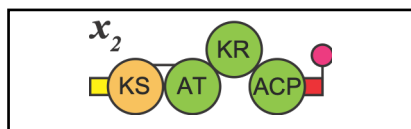
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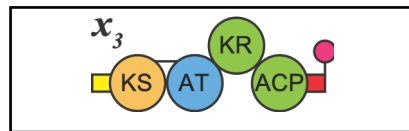


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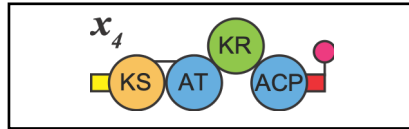


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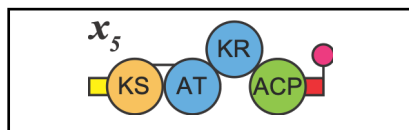
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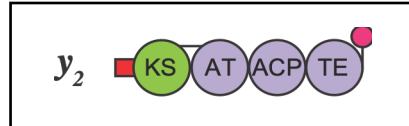


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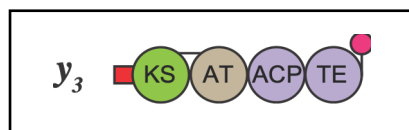


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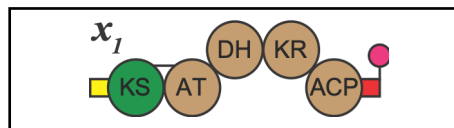


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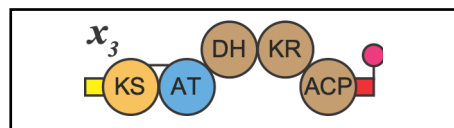


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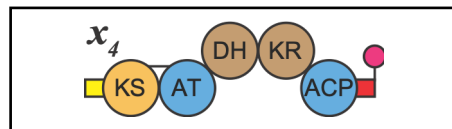


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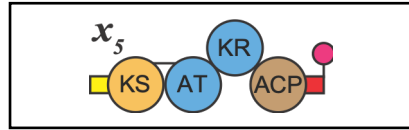


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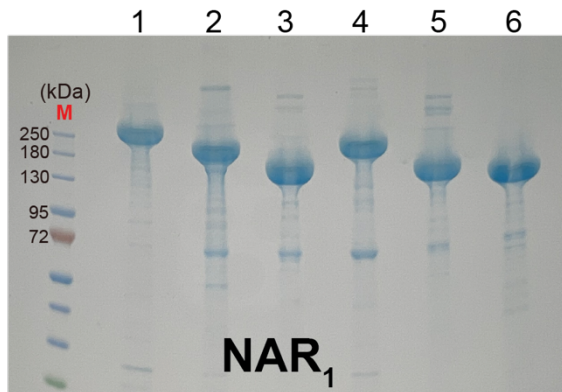
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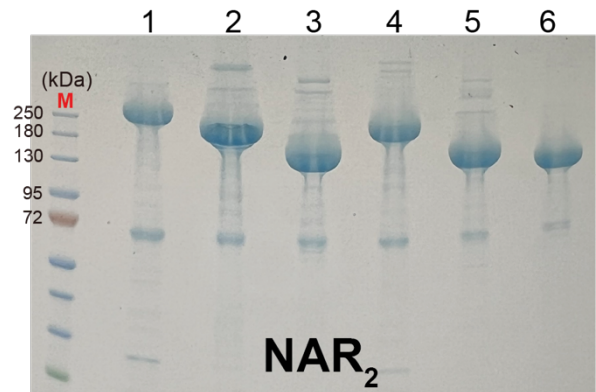
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Table S4. The approximate quantity of each protein that can be obtained from the purification of 1 liter of culture.

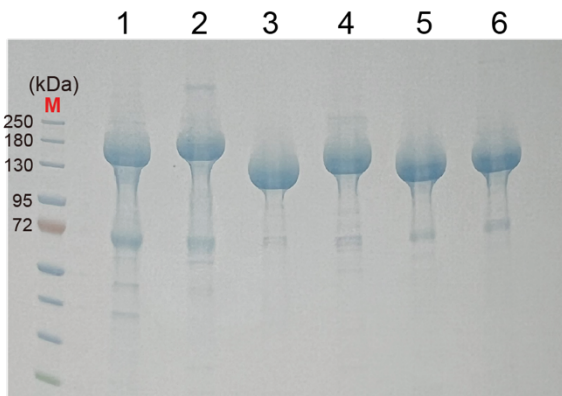
Protein	Approximate yield (mg/L of culture)	Protein	Approximate yield (mg/L of culture)	Protein	Approximate yield (mg/L of culture)
PIP2 ^N (NAR ₁)	4.2	^C P2P3 ^N (NAR ₁)	11.4	^C P3P4 ^N (NAR ₁)	13.5
^C P4P5 ^N (NAR ₁)	7.2	^C P5P6 ^N (NAR ₁ ; x ₆)	14.1	^C P6P7 (NAR ₁ ; y ₁)	20.3
PIP2 ^N (NAR ₂)	3.3	^C P2P3 ^N (NAR ₂)	12.6	^C P3P4 ^N (NAR ₂)	17.1
^C P4P5 ^N (NAR ₂)	8.8	^C P5P6 ^N (NAR ₂)	15.7	^C P6P7 (NAR ₂)	16.3
PikAII (NAR ₃)	1.2	PikAII (NAR ₅)	0.2	PIP2 ^N (NAR ₆)	0.6
P1 ^N (NAR ₈)	3	^C P1P2 ^N (NAR ₈)	4	^C P2P3 ^N (NAR ₈)	1.7
^C P3P4 ^N (NAR ₈)	2.9	^C P4P5 ^N (NAR ₈)	1.3	^C P5P6 ^N (NAR ₈)	2.1
^C P6P7 (NAR ₈)	5.0	P1 ^N (NAR ₉)	14.6	^C P1P2 ^N (NAR ₉)	16.2
^C P6P7_KS_C207A (DML ₁)	18.8	^C P6P7_KS_C207A (DML ₂)	17.8	^C P6P7_KS_C207A and ACP_S980A (DML ₃)	18.3
^C P6P7_KS_C207A and ACP deletion (DML ₄)	19.3	^C P2P4 ^N (ΔP3 ₁)	9.8	^C P3P4 ^N (ΔP3 ₂)	12.6
^C P2P4 ^N (ΔP3 ₃)	8.6	^C P5P7 (ΔP6 ₁)	17.5	^C P5P7 (ΔP6 ₂)	19.5
^C P5P7 (ΔP6 ₃)	18.7	^C P5R5 ^N (ANH ₁ ; x ₂)	8.8	^C R5P7 (ANH ₁ ; y ₂)	14.8
^C P5R5 ^N (x ₁)	13.0	^C P5R5 ^N (ANH ₂ ; x ₃)	10.1	^C P5R5 ^N (x ₄)	7.8
^C P5R5 ^N (x ₅)	14.7	^C P5R5 ^N (ANH ₃)	9.0	^C P5E6 ^N (x ₁)	11.4
^C P5E6 ^N (x ₂)	12.6	^C P5E6 ^N (x ₃)	13.7	^C P5E6 ^N (x ₄)	15.8
^C P5E6 ^N (x ₅)	14.5	^C E6P7 (y ₂)	15.2	^C E6P7 (y ₃)	14.3
TEII	60	malonyl-CoA synthetase (MatB)	66	glucose dehydrogenase (GDH)	57
methylmalonyl-CoA epimerase	50	VemG	0.3	VemH	13



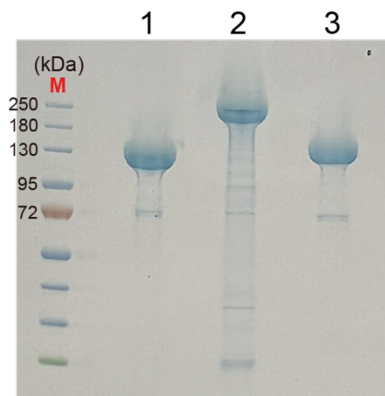
1. **P1P2^N** (278 kDa)
2. **^oP2P3^N** (208 kDa)
3. **^oP3P4^N** (168 kDa)
4. **^oP4P5^N** (231 kDa)
5. **^oP5P6^N** (164 kDa)
6. **^oP6P7** (142 kDa)



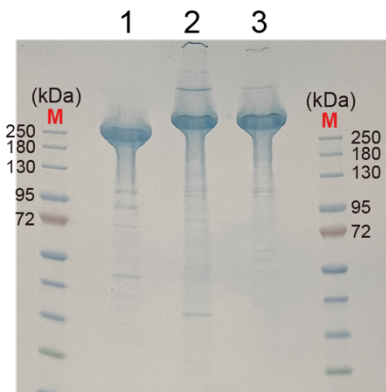
1. **P1P2^N** (277 kDa)
2. **^oP2P3^N** (212 kDa)
3. **^oP3P4^N** (168 kDa)
4. **^oP4P5^N** (231 kDa)
5. **^oP5P6^N** (164 kDa)
6. **^oP6P7** (143 kDa)



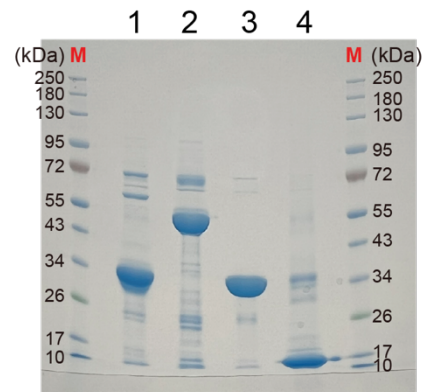
1. **^oP2P4^N (ΔP3₁)** (168 kDa)
2. **^oP5R5^N (ANH₁)** (187 kDa)
3. **^oR5P7 (ANH₁)** (141 kDa)
5. **^oP6P7_C207A (DML₁)** (142 kDa)
6. **^oP6P7_C207A_S980A (DML₃)** (142 kDa)



1. **^oP6P7_C207A_noACP (DML₄)** (130 kDa)
2. **VemG** (234 kDa)
3. **VemH** (141 kDa)



1. **P1P2^N_loopHis6 (NAR₆)** (276 kDa)
2. **PikAII (NAR₃)** (388 kDa)
3. **PikAII_loopHis6 (NAR₅)** (387 kDa)



1. **thioesterase II (PikAV)** (33 kDa)
2. **malonyl-CoA synthetase** (53 kDa)
3. **glucose dehydrogenase** (30 kDa)
4. **(2S)-methylmalonyl-CoA epimerase** (17 kDa)

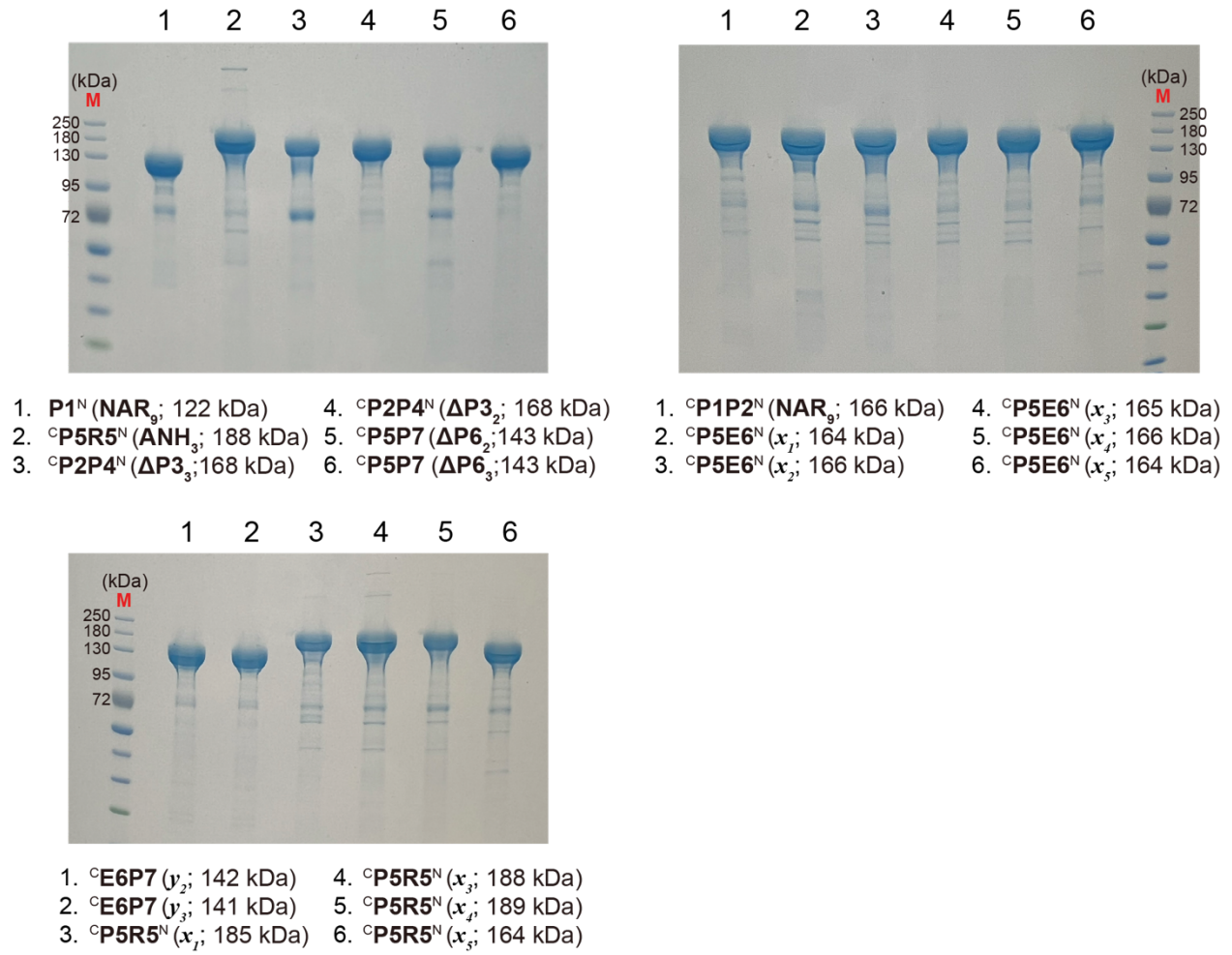


Figure S1. SDS-PAGE gels of the proteins used in this study.

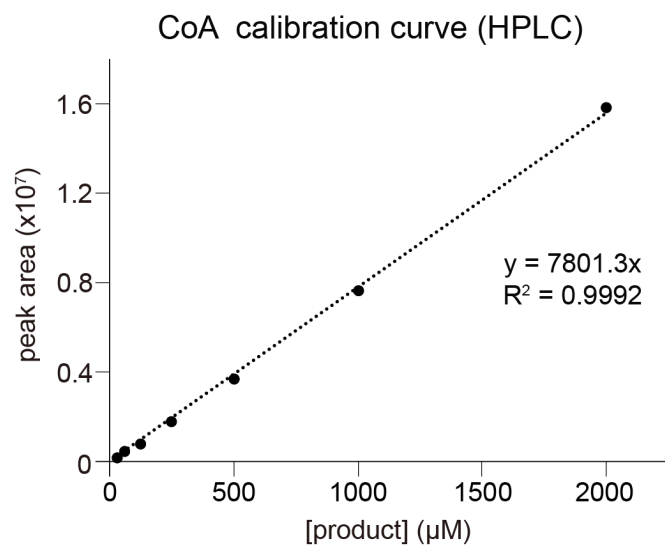


Figure S2. Calibration curve for CoA using $\gamma = 259.6$ nm.

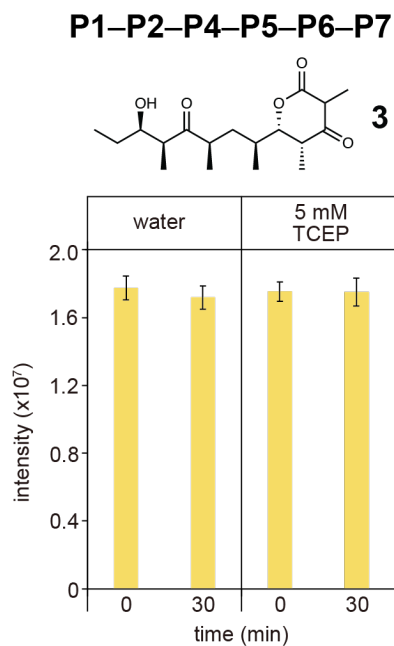


Figure S3. TCEP has no effect on compound **3**.

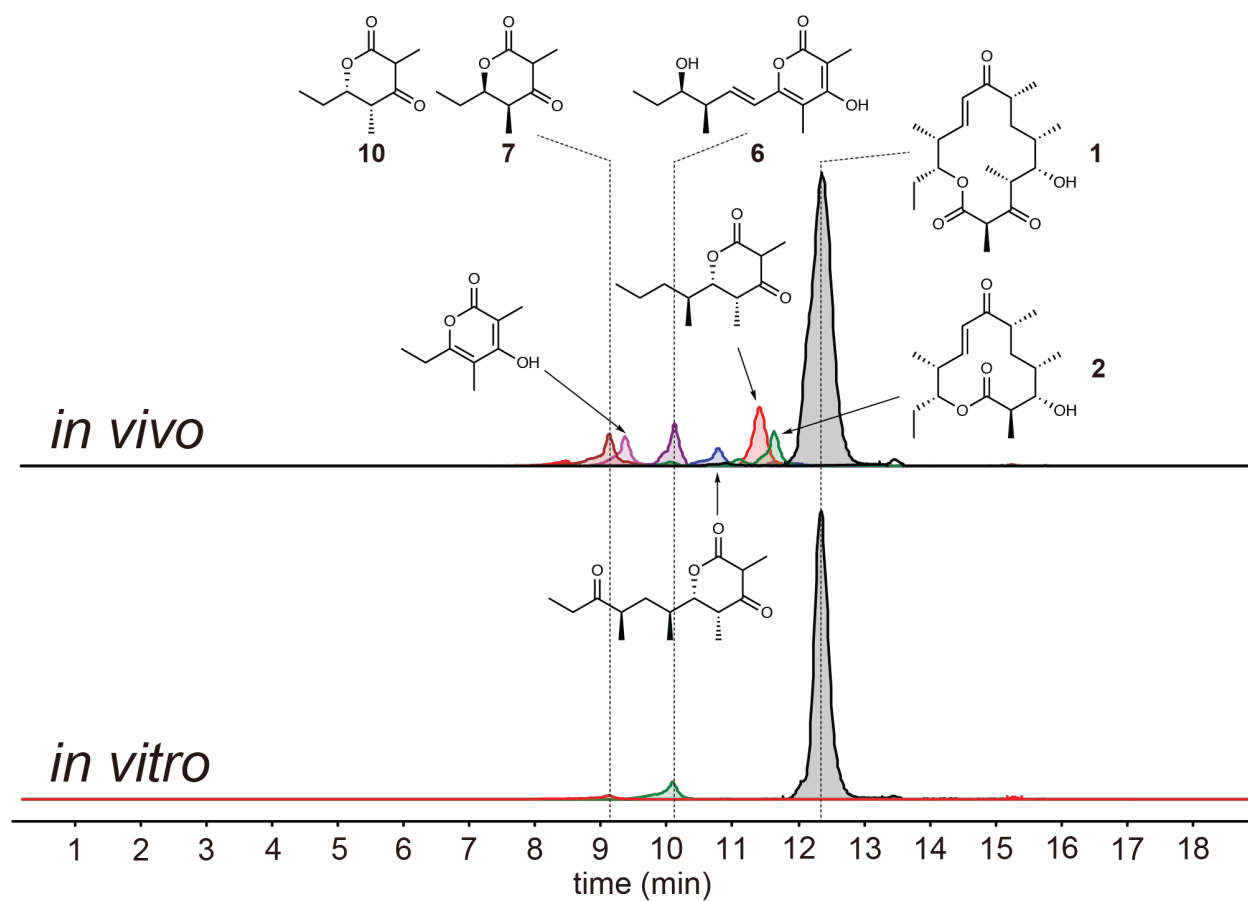


Figure S4. Products from the refactored pikromycin PKS *in vivo* vs. *in vitro*. The EICs of the products are overlaid. While 10-dml (2) is generated by *E. coli* K207-3 expressing the refactored pikromycin PKS, only trace is detected from *in vitro* reactions. The main byproducts from the *in vitro* reconstituted PKS are pentaketide 6 and triketide 7.

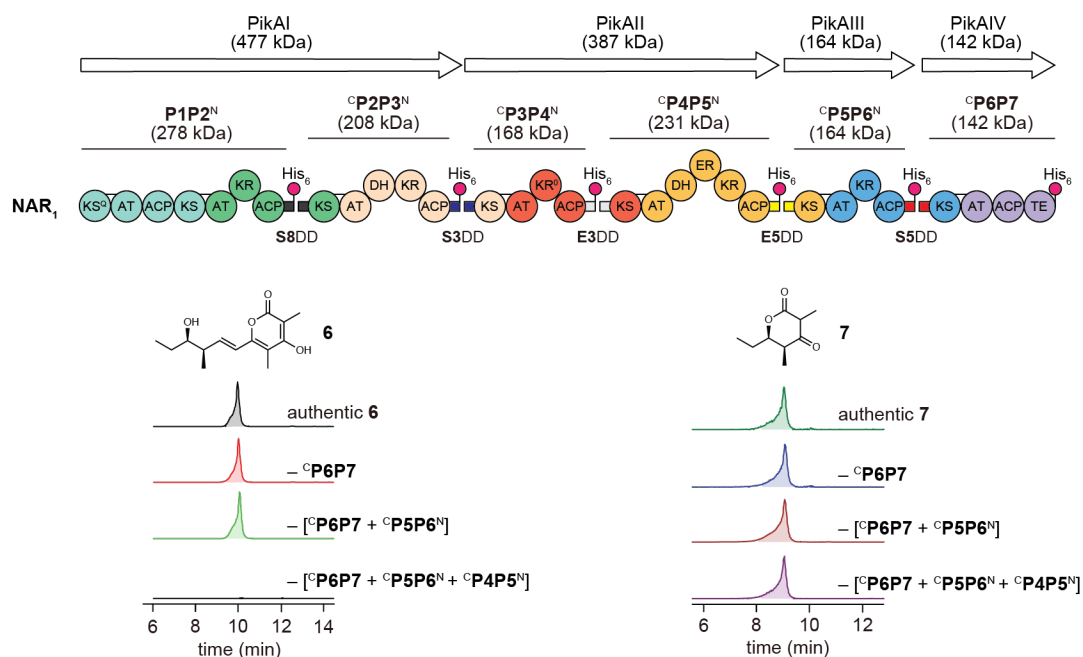


Figure S5. Production of byproducts **6** and **7** by **NAR₁**. a) Reactions in which the final polypeptide(s) of **NAR₁** were omitted are analyzed by LC/MS. Pentaketide **6** is generated when the final 2 polypeptides are omitted but not when the final 3 polypeptides are omitted, indicating that it is formed through ACP-independent extension between methylmalonyl-CoA and the **P4KS**-bound tetraketide intermediate.

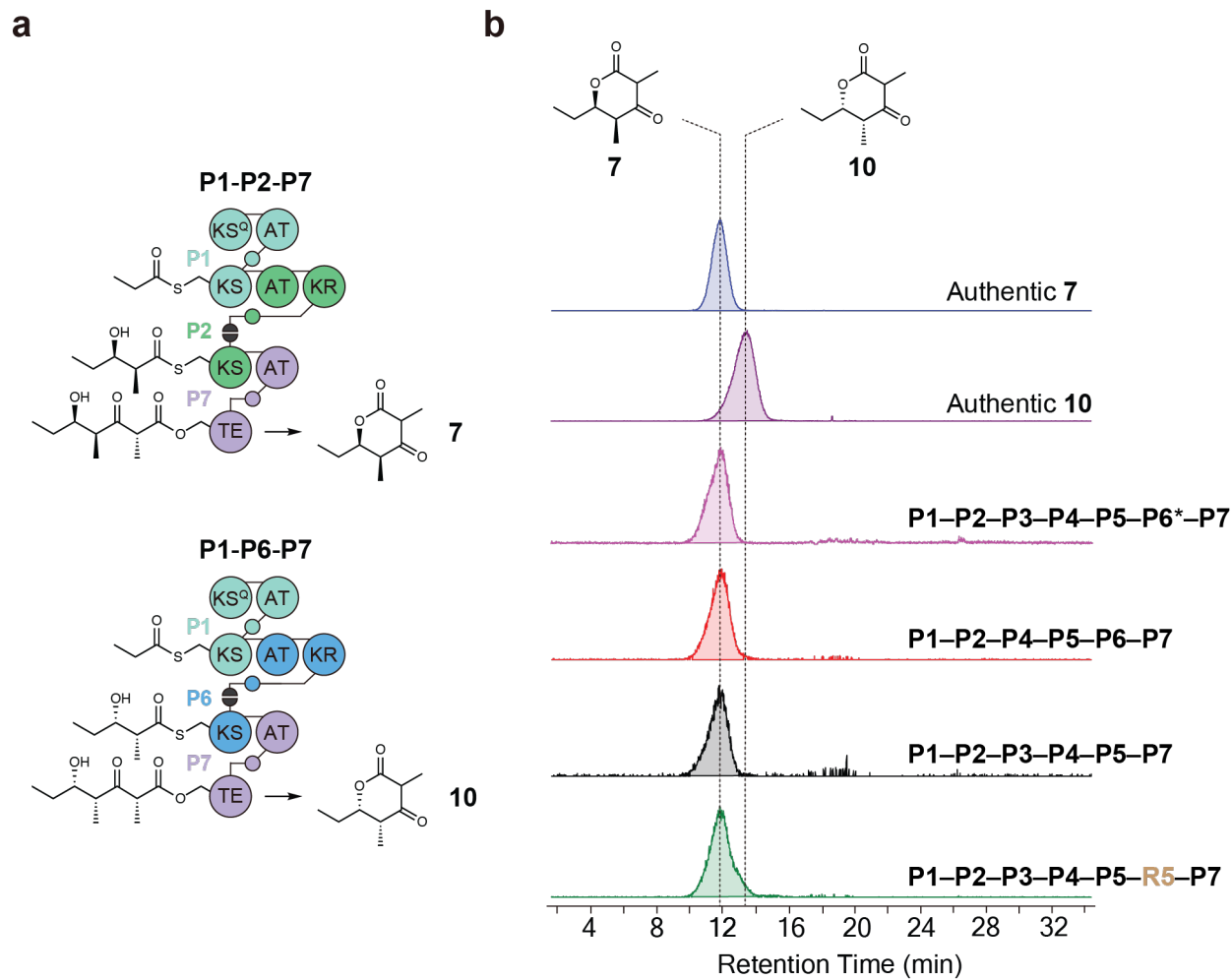
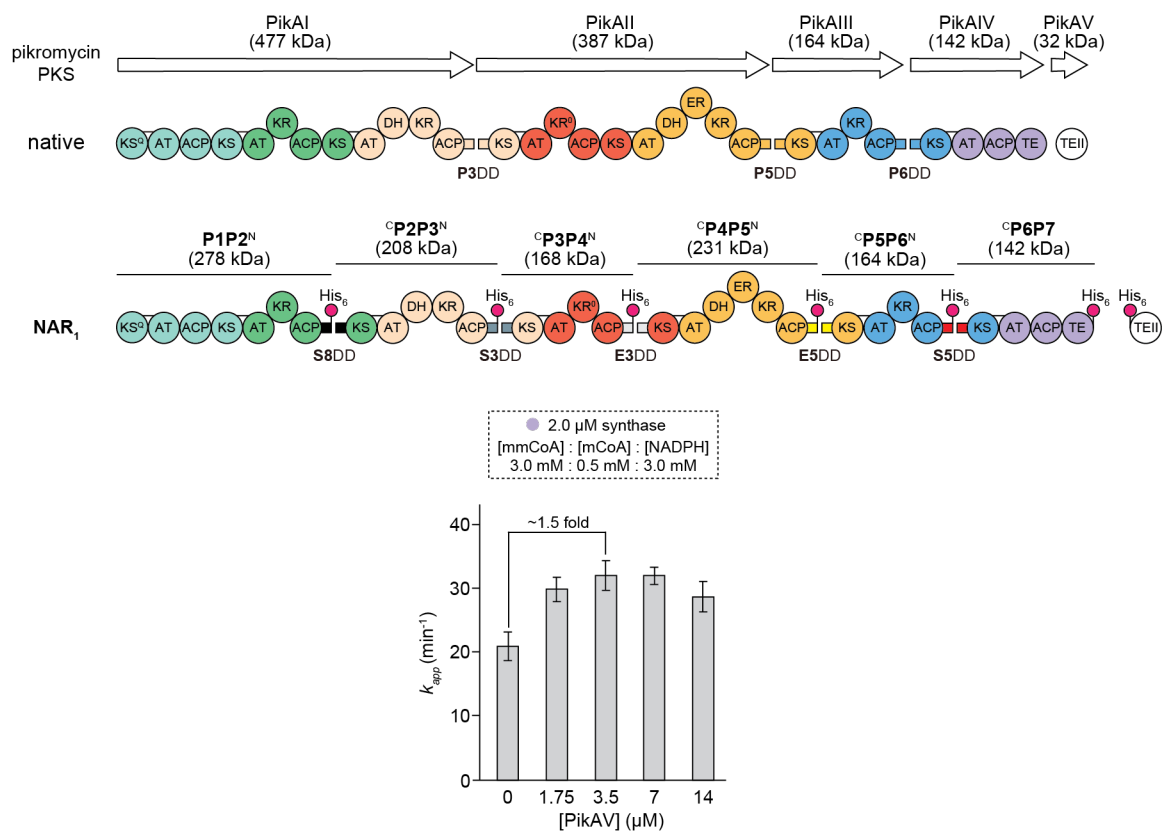


Figure S6. Chiral column analysis of triketide lactones generated by **P1-P2-P3-P4-P5-P6*-P7**, **P1-P2-P4-P5-P6-P7**, **P1-P2-P3-P4-P5-P7**, and **P1-P2-P3-P4-P5-R5-P7**.



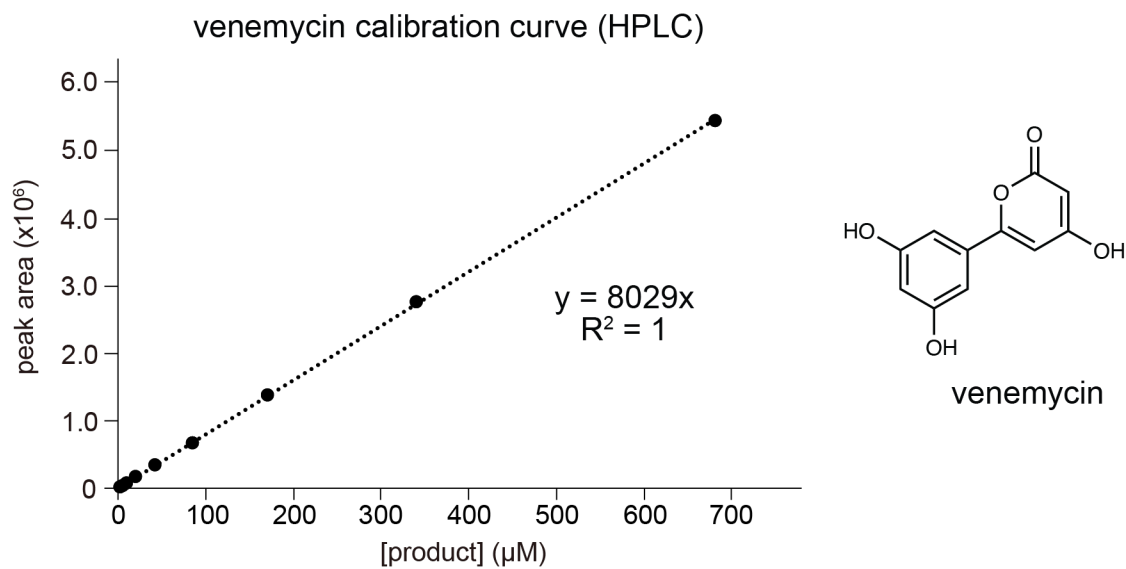
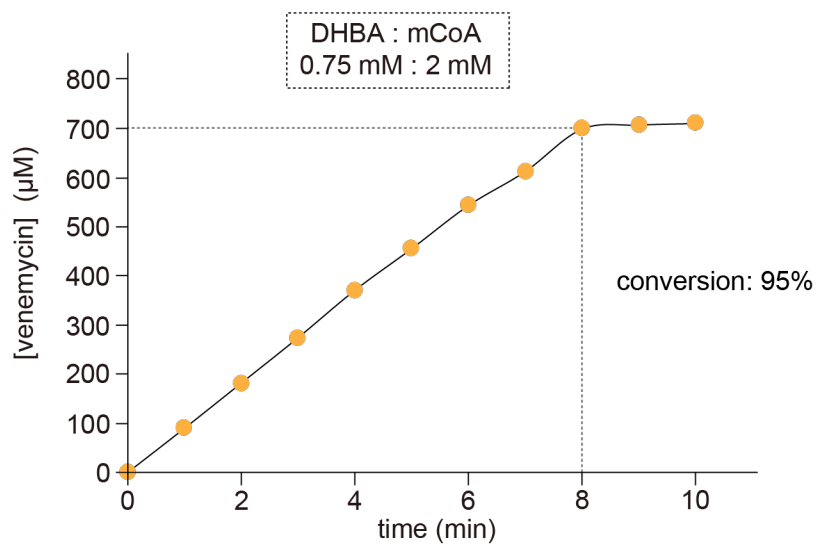
a**b**

Figure S8. a) Calibration curve for venemycin. b) The time course of venemycin biosynthesis *in vitro* at 2 μM concentrations of VemG and VemH.

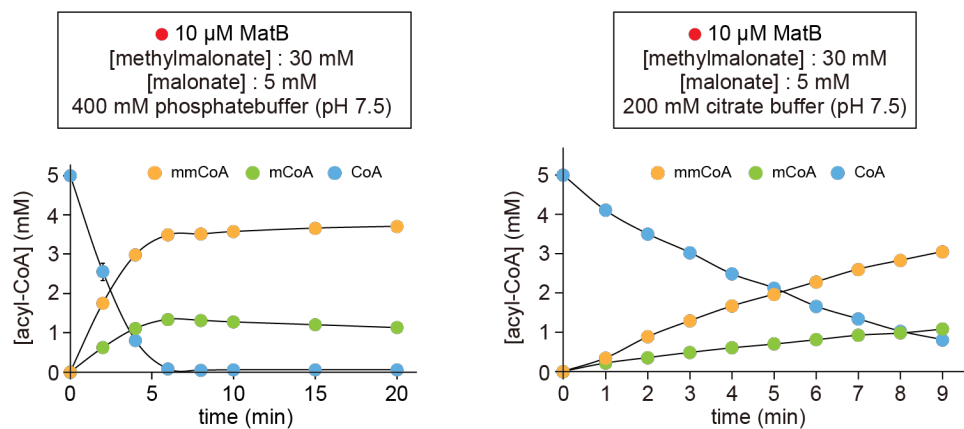


Figure S9. MatB-mediated acyl-CoAs formation in phosphate and citrate buffers. Time course of CoA conversion to methylmalonyl-CoA and malonyl-CoA using 5 mM coenzyme A, 10 μ M MatB, 5 mM malonate, and 30 mM methylmalonate in 400 mM phosphate buffer or 200 mM citrate buffer at pH 7.5.

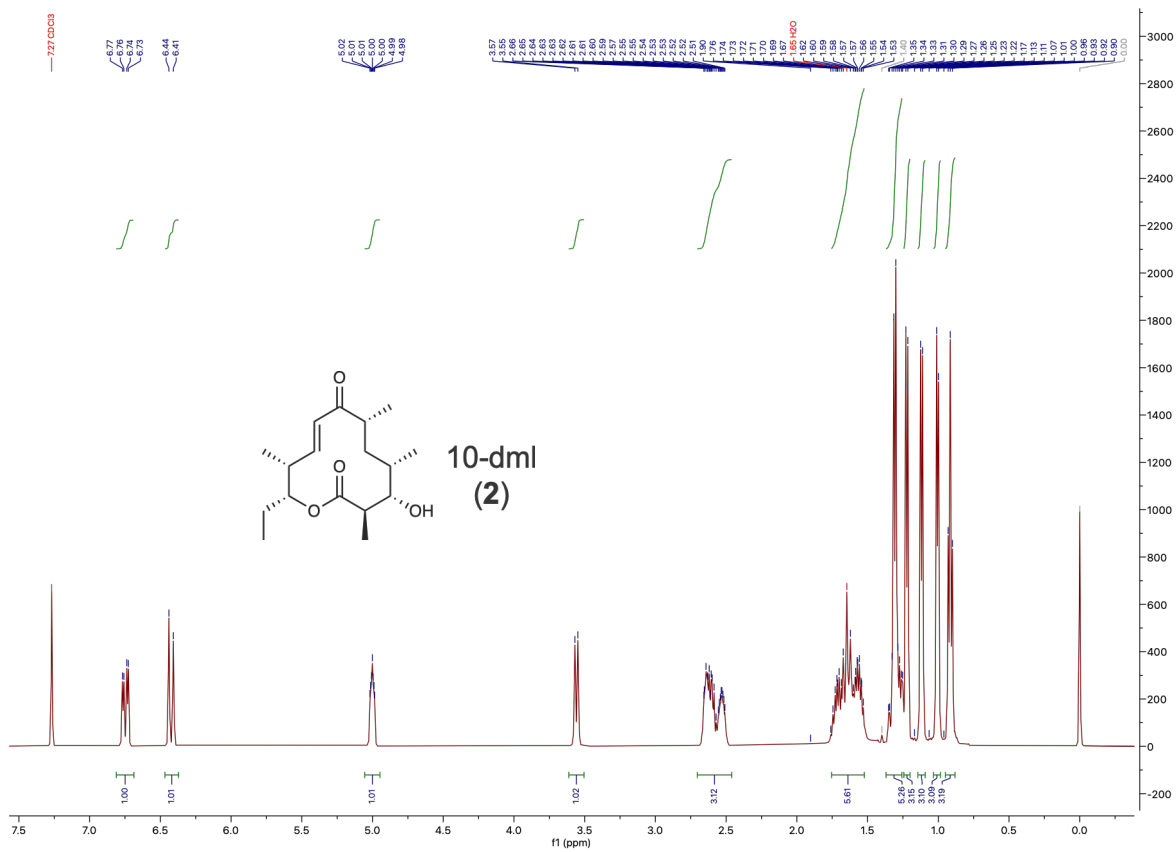


Figure S11. ¹H NMR spectrum of 10-dml (2) purified from EPK reaction.

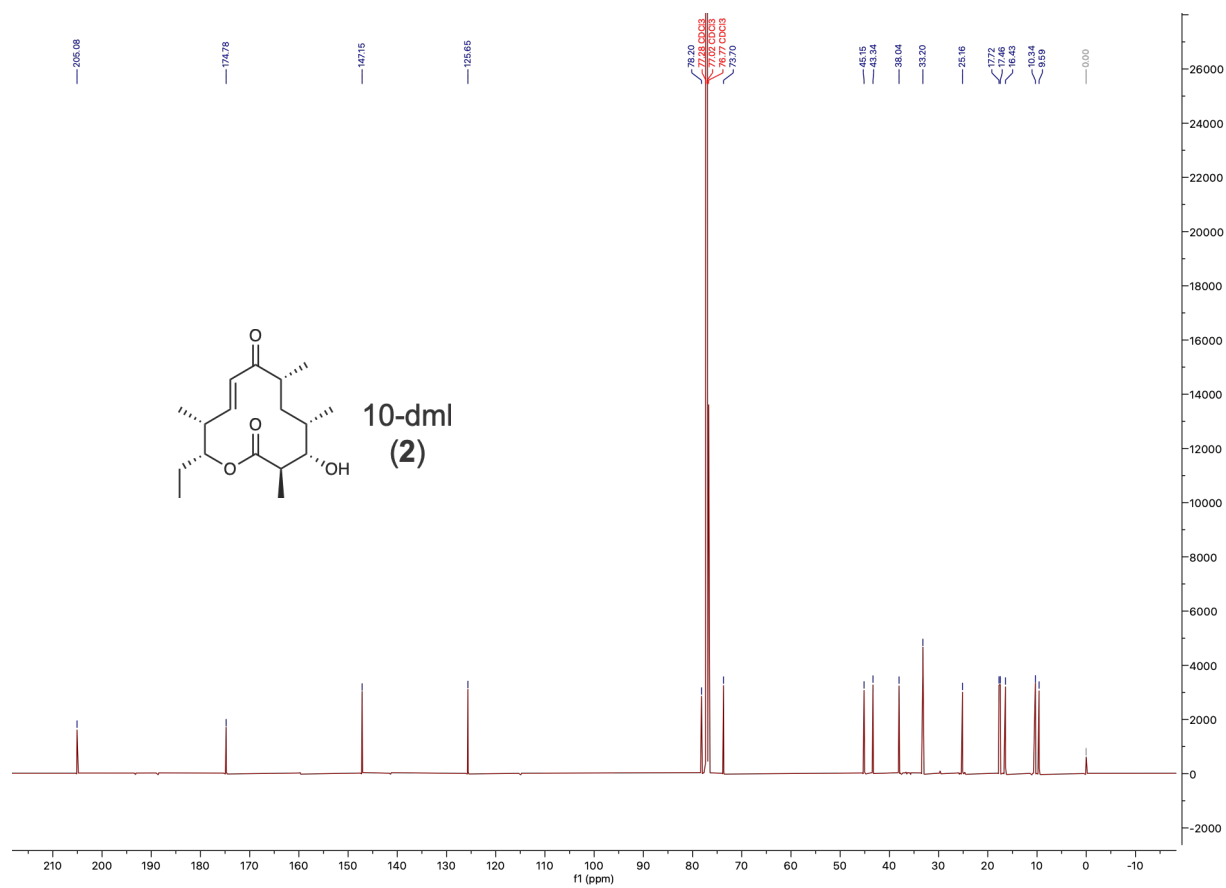
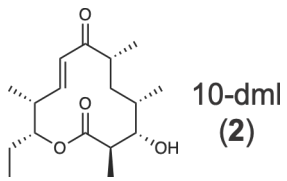


Figure S12. ^{13}C NMR spectrum of 10-dml (2) purified from EPK reaction.



S71

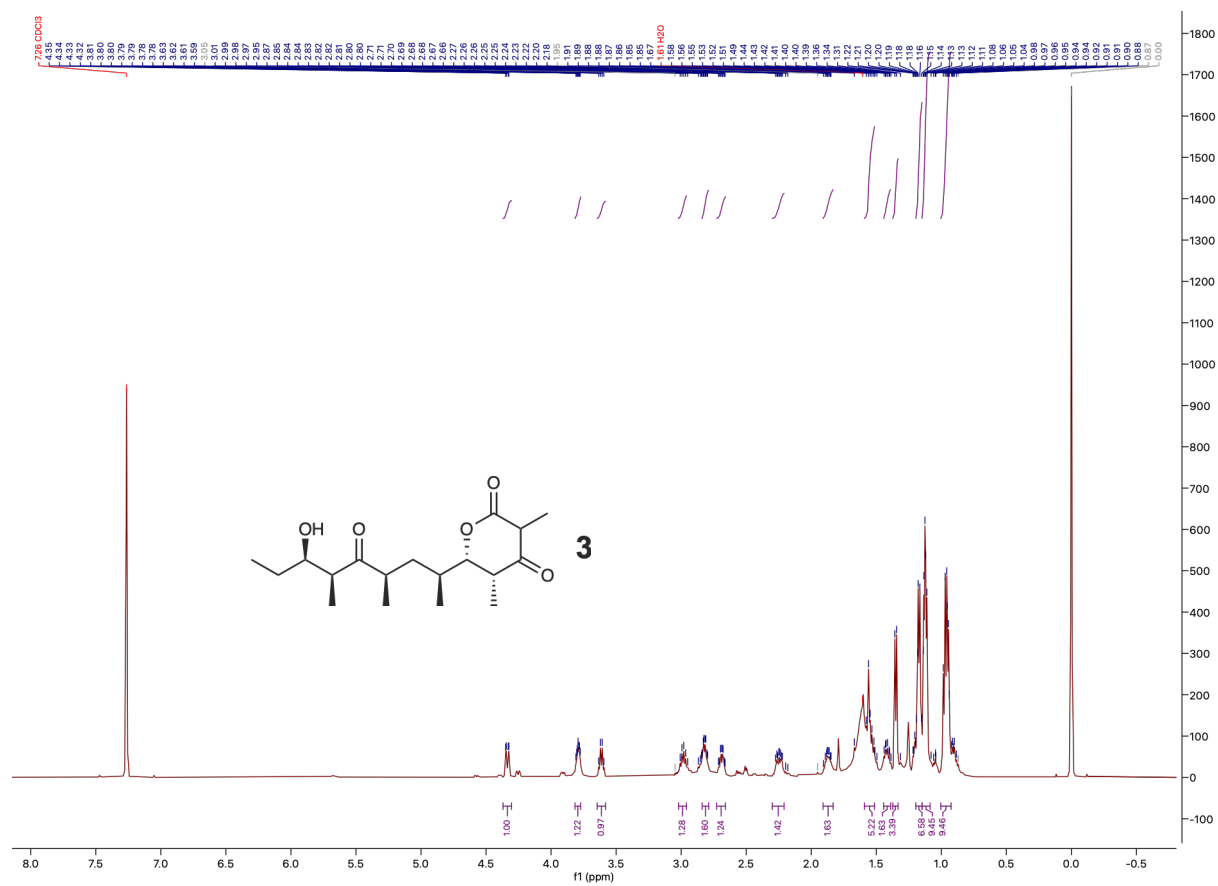


Figure S14. ^1H NMR spectrum of compound **3** purified from EPK reaction.

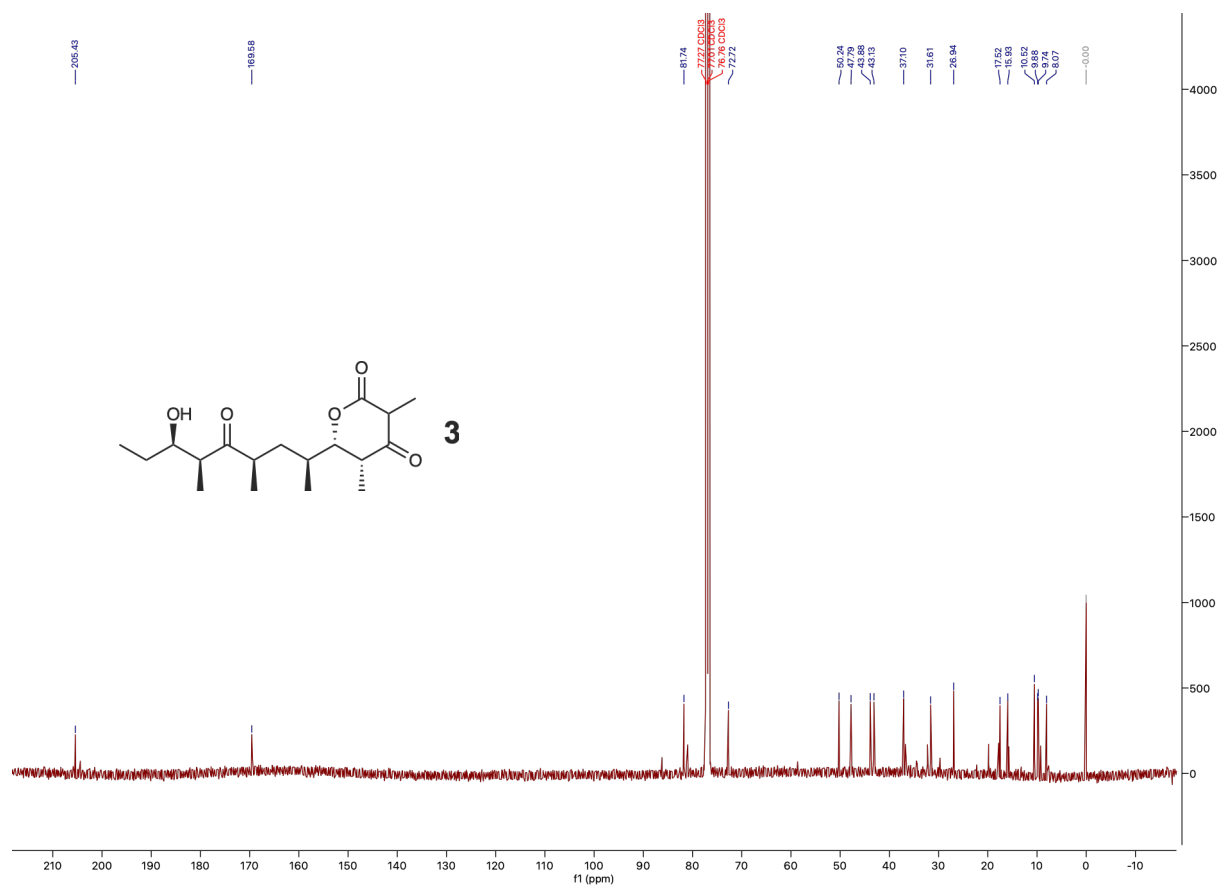


Figure S15. ¹³C NMR spectrum of compound **3** purified from EPK reaction.

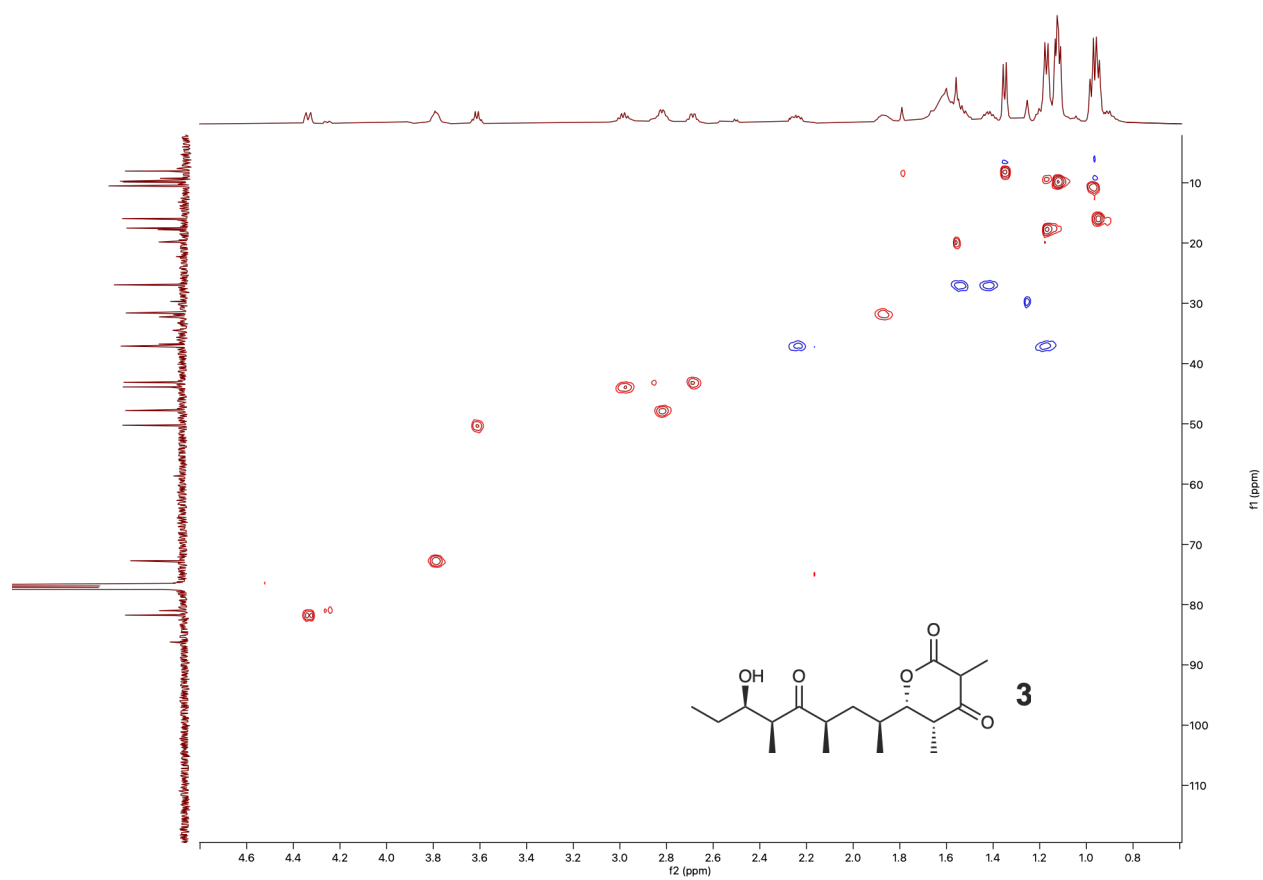


Figure S16. ^1H - ^{13}C HSQC spectrum of compound **3** purified from EPK reaction.

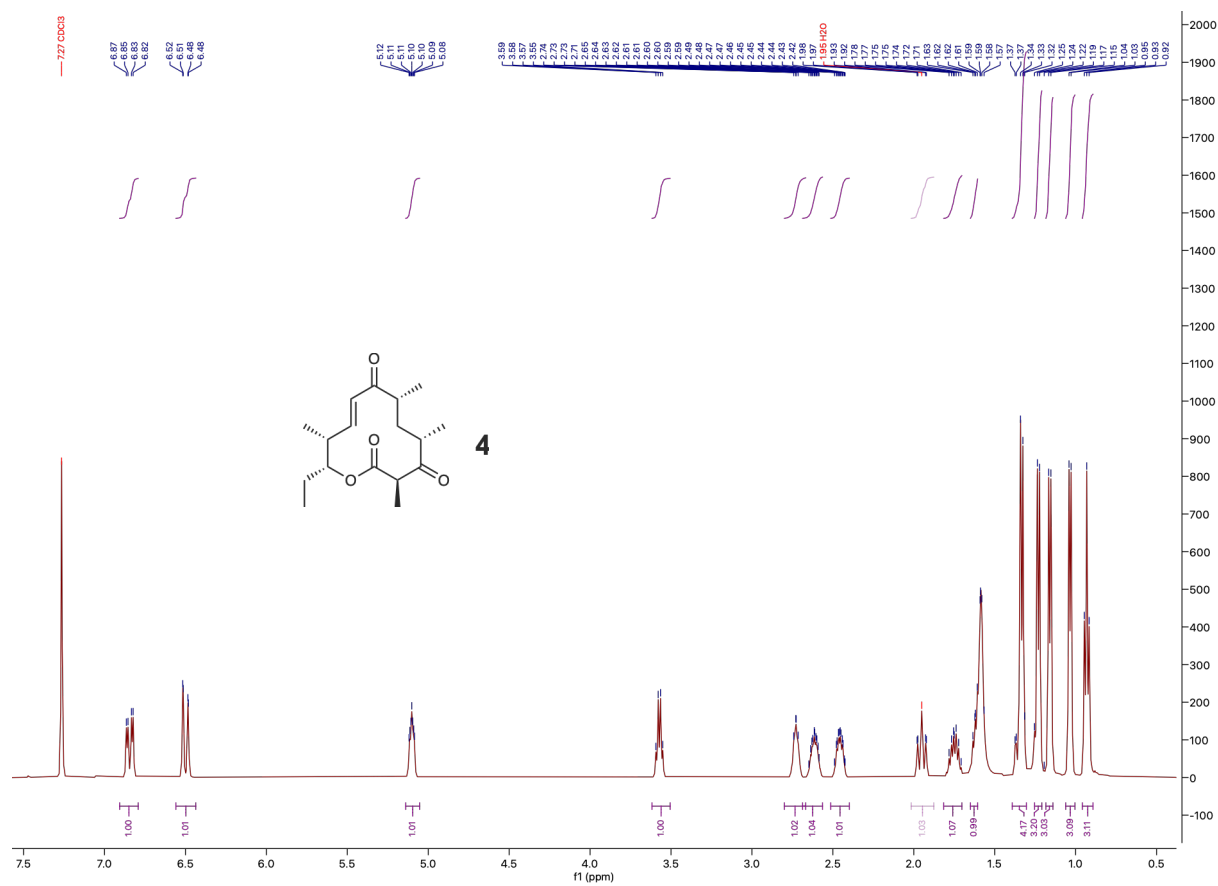


Figure S17. ¹H NMR spectrum of compound **4** purified from EPK reaction.

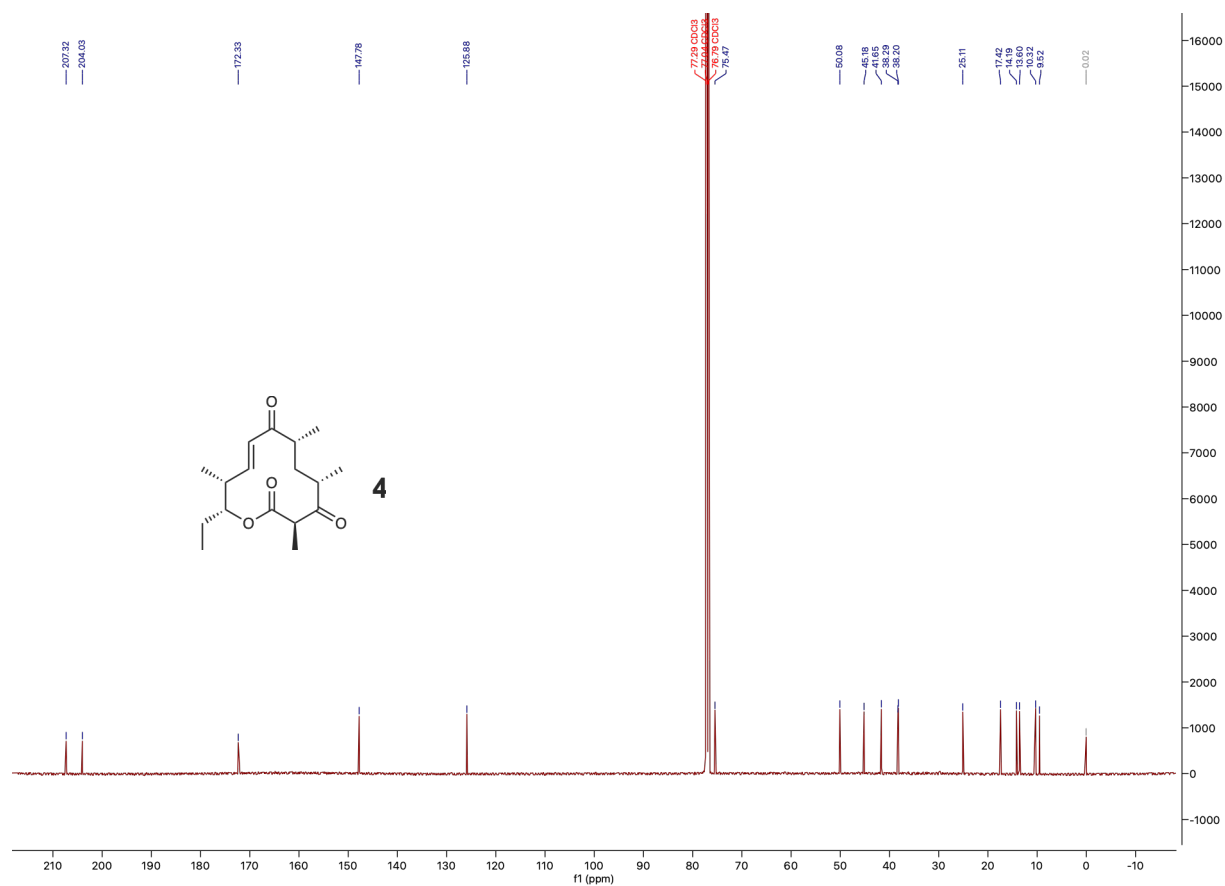


Figure S18. ^{13}C NMR spectrum of compound **4** purified from EPK reaction.

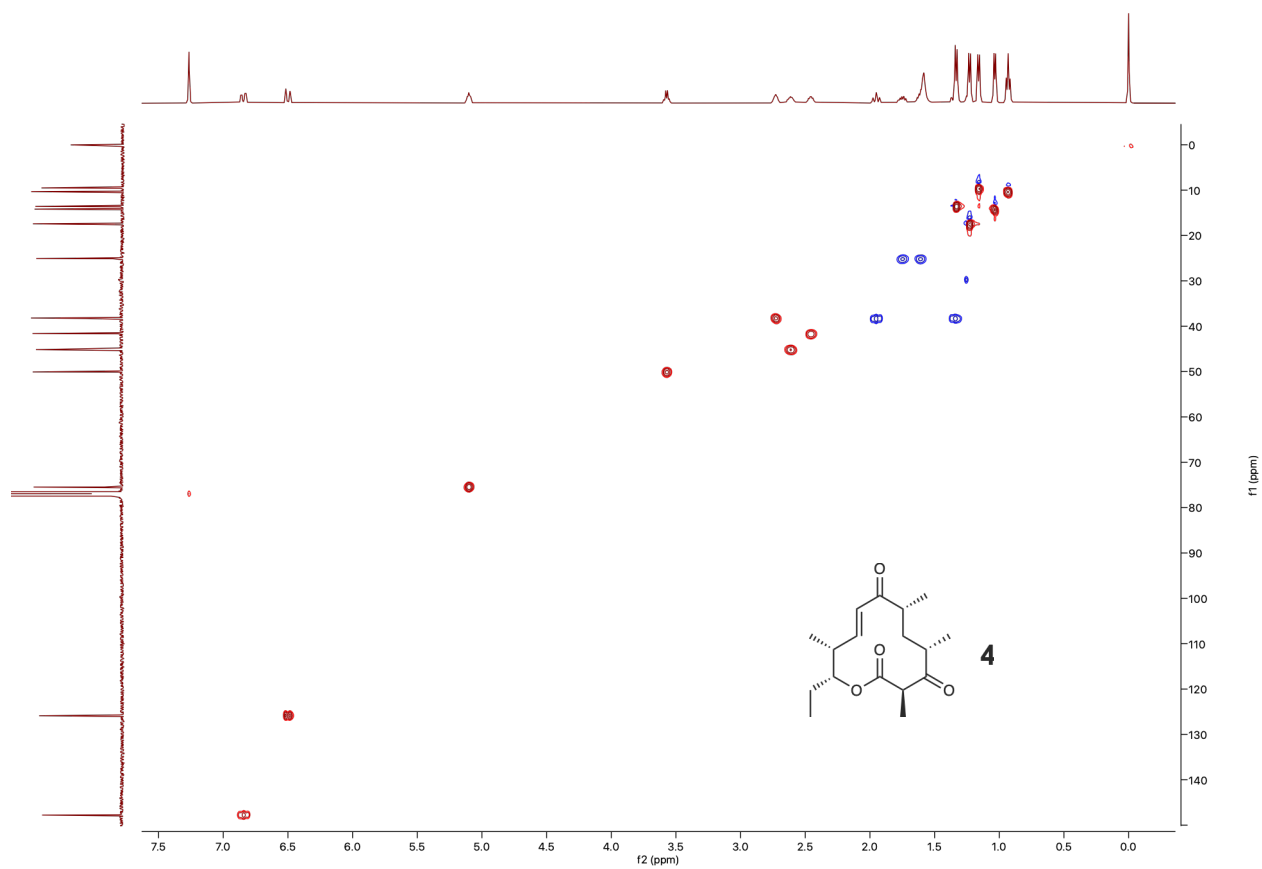


Figure S19. ^1H - ^{13}C HSQC spectrum of compound **4** purified from EPK reaction.

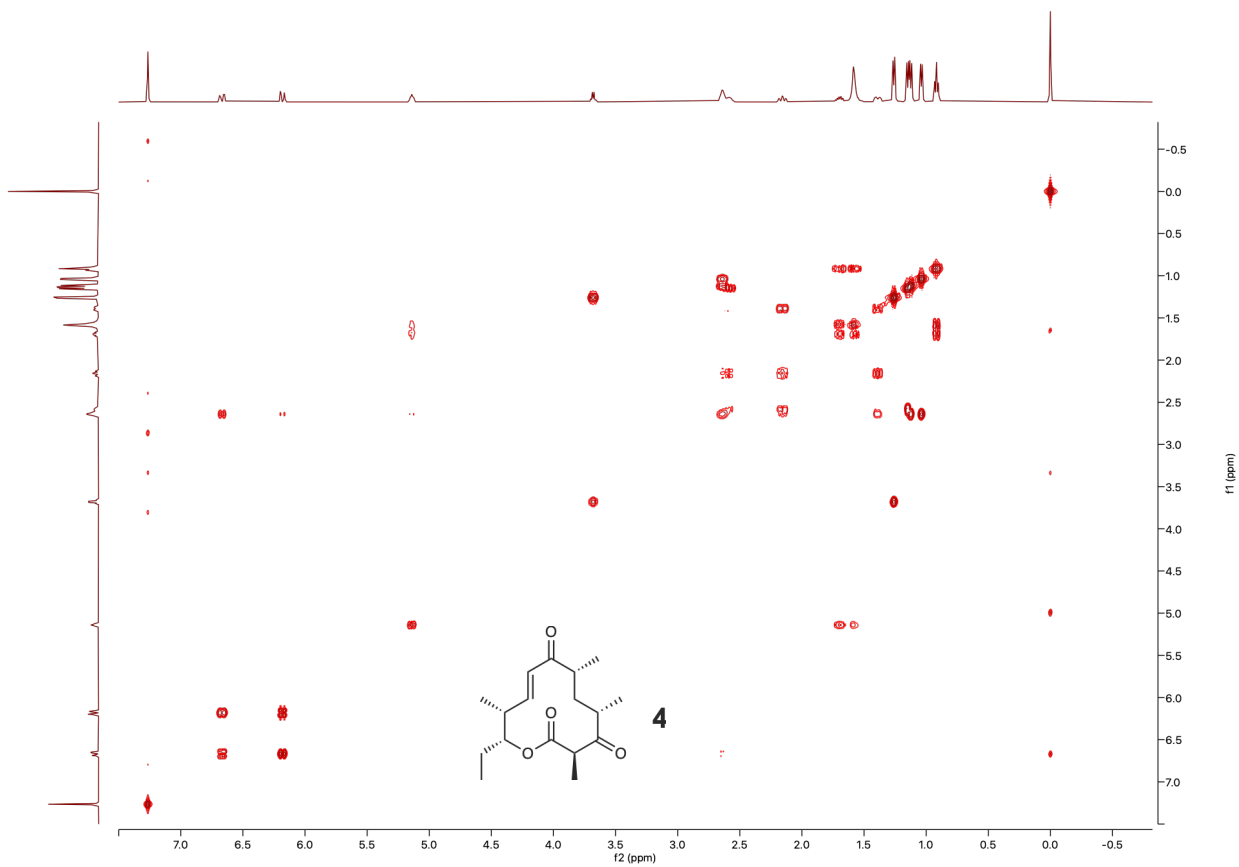


Figure S20. ^1H - ^1H COSY spectrum of compound **4** purified from the EPK reaction.

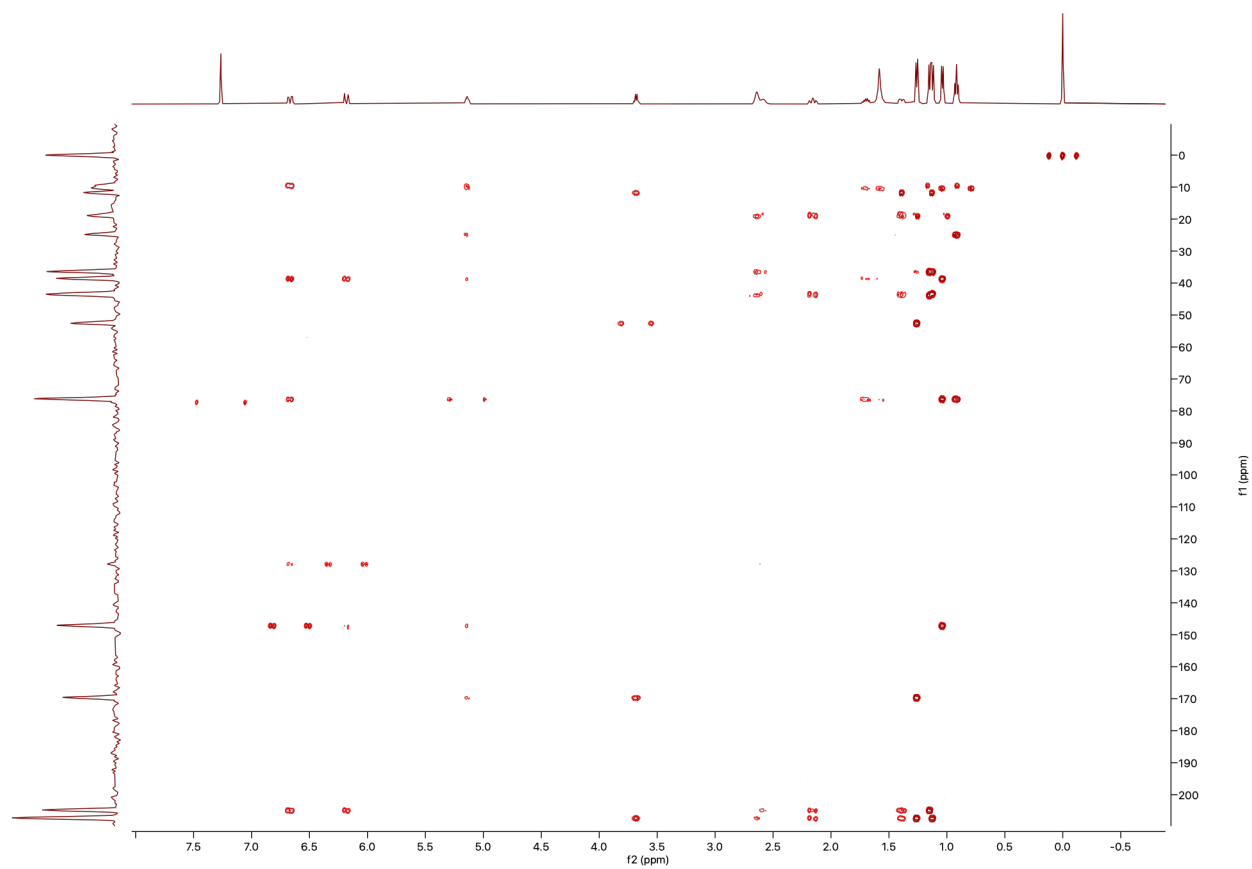


Figure S21. ^1H – ^{13}C HMBC spectrum of compound **4** purified from the EPK reaction.

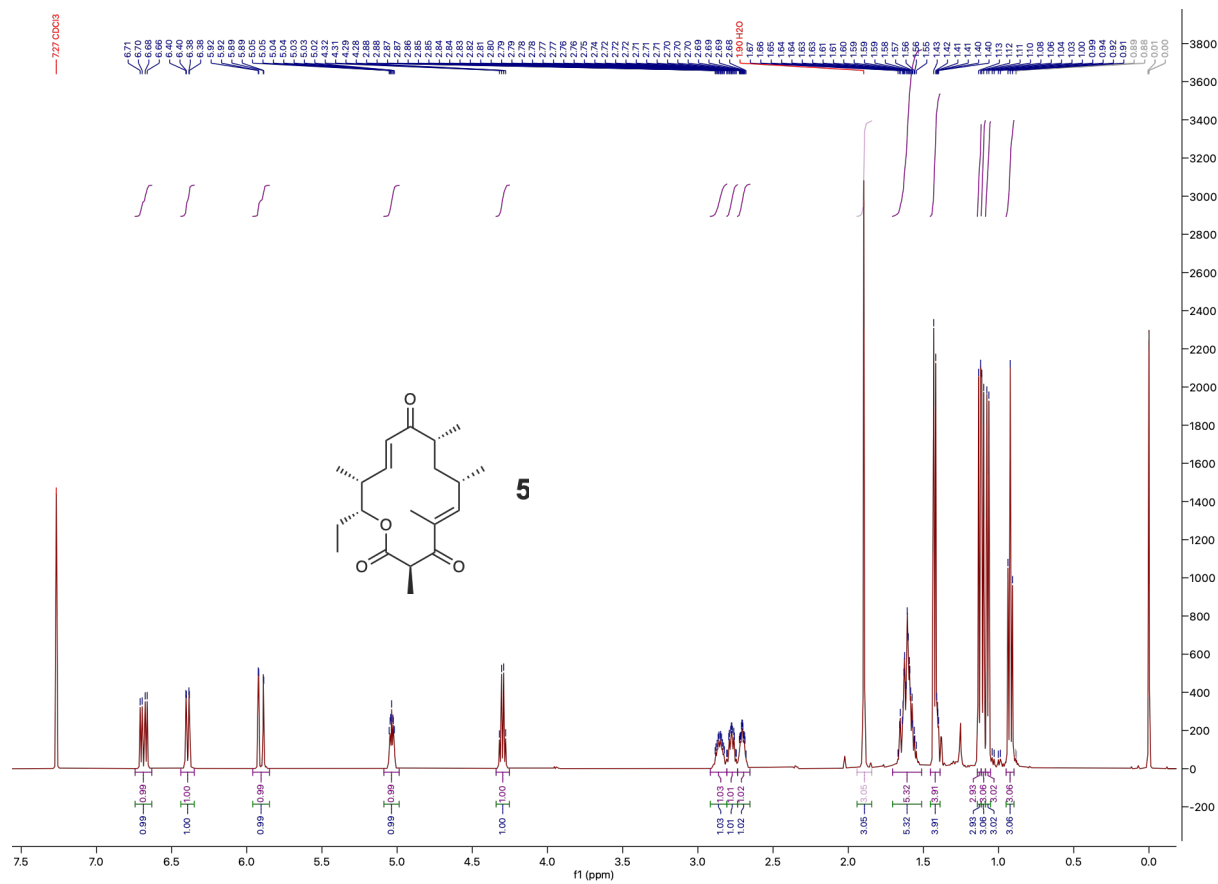


Figure S22. ¹H NMR spectrum of anhydronarbonolide (**5**) purified from EPK reaction.

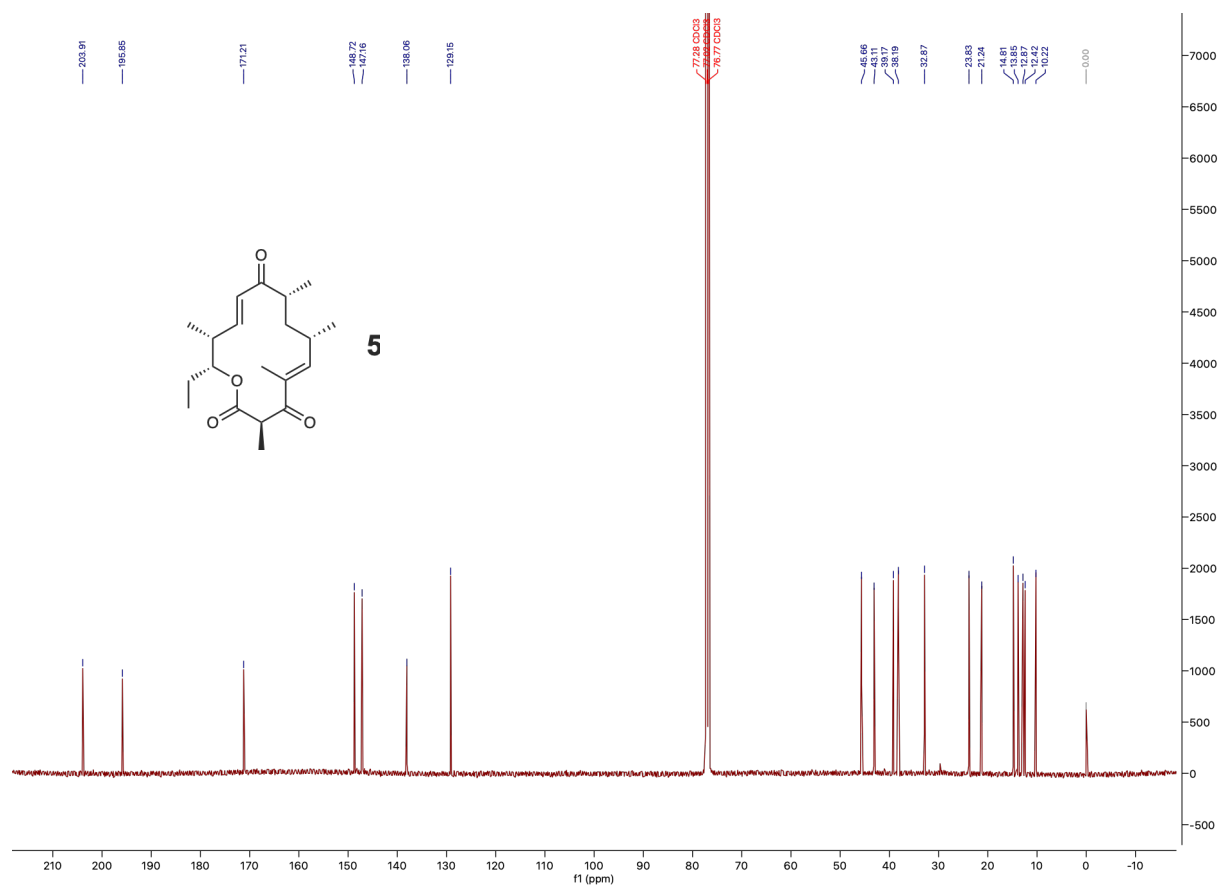


Figure S23. ^{13}C NMR spectrum of anhydronarbonolide (**5**) purified from EPK reaction.

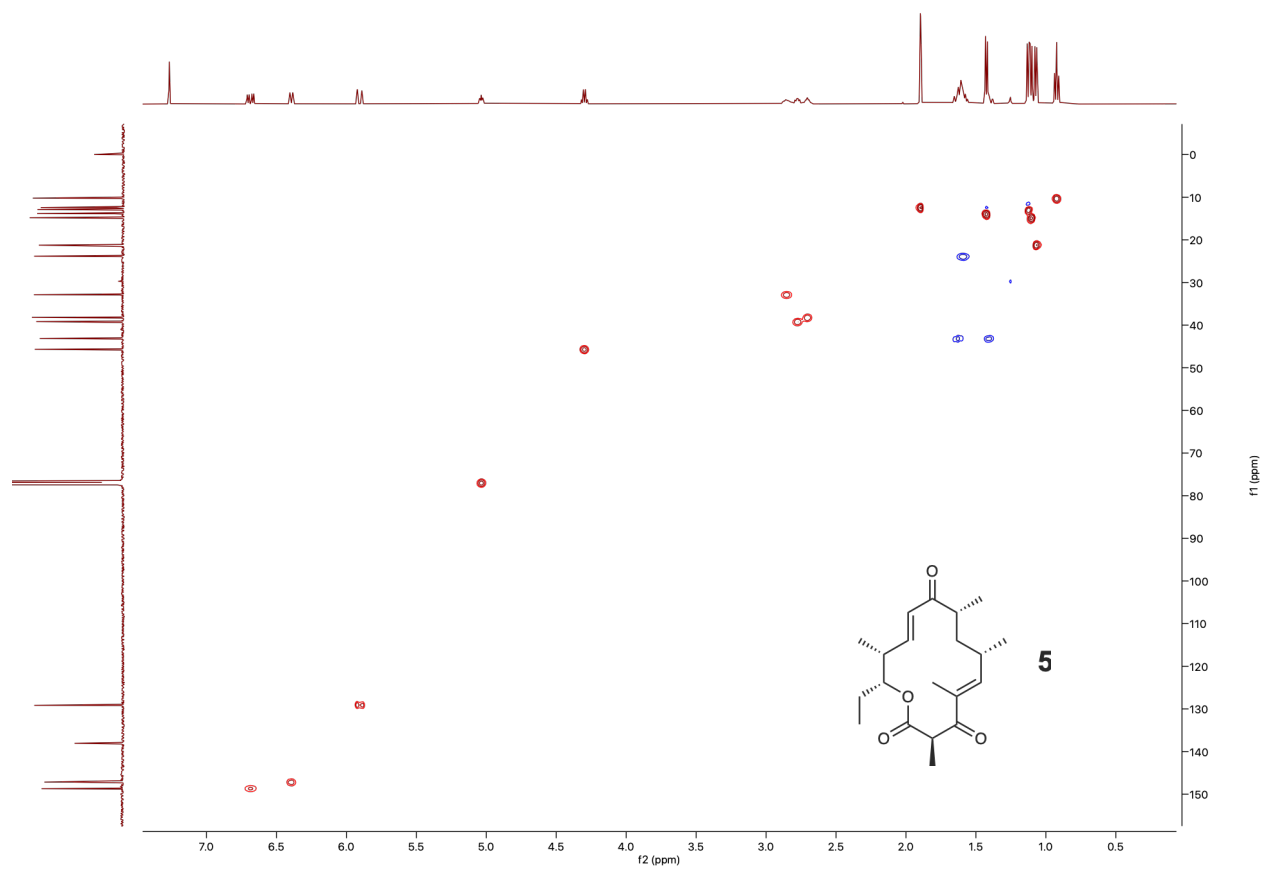
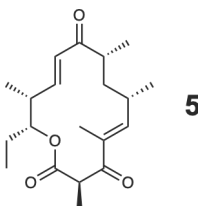


Figure S24. ^1H - ^{13}C HSQC spectrum of anhydronarbonolide (**5**) purified from EPK reaction.



5

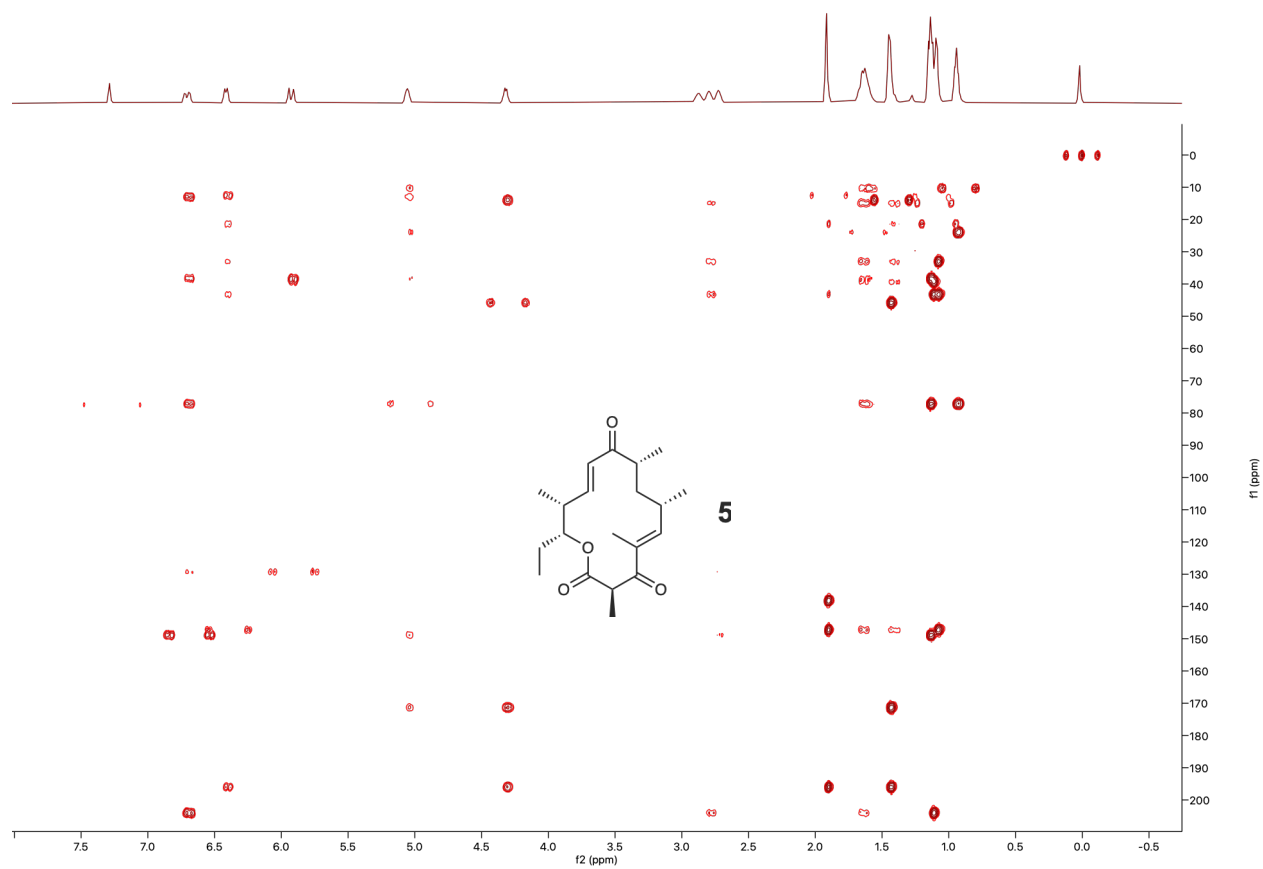


Figure S26. ^1H - ^{13}C HMBC spectrum of anhydronarbonolide (**5**) purified from the EPK reaction.

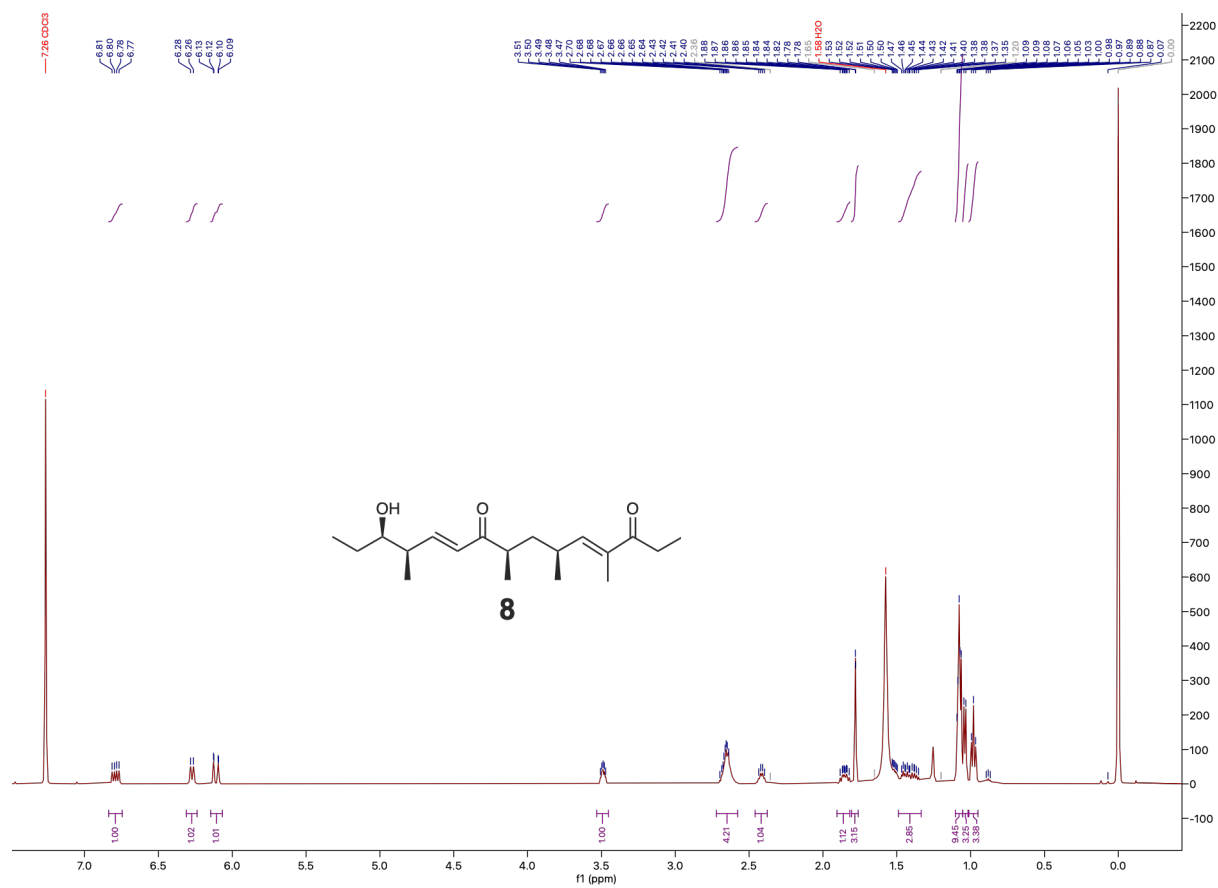


Figure S27. ¹H NMR spectrum of compound **8** purified from EPK reaction.

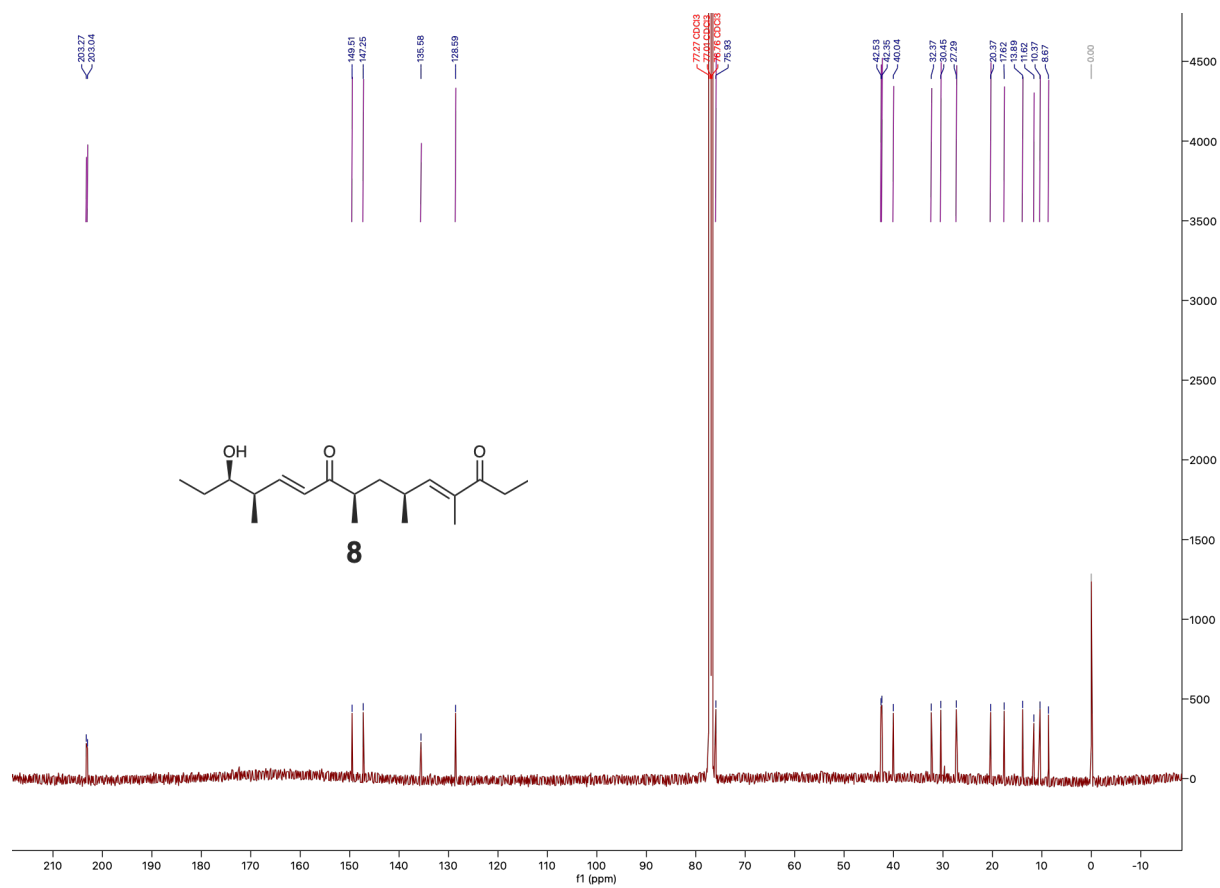


Figure S28. ¹³C NMR spectrum of compound **8** purified from EPK reaction.

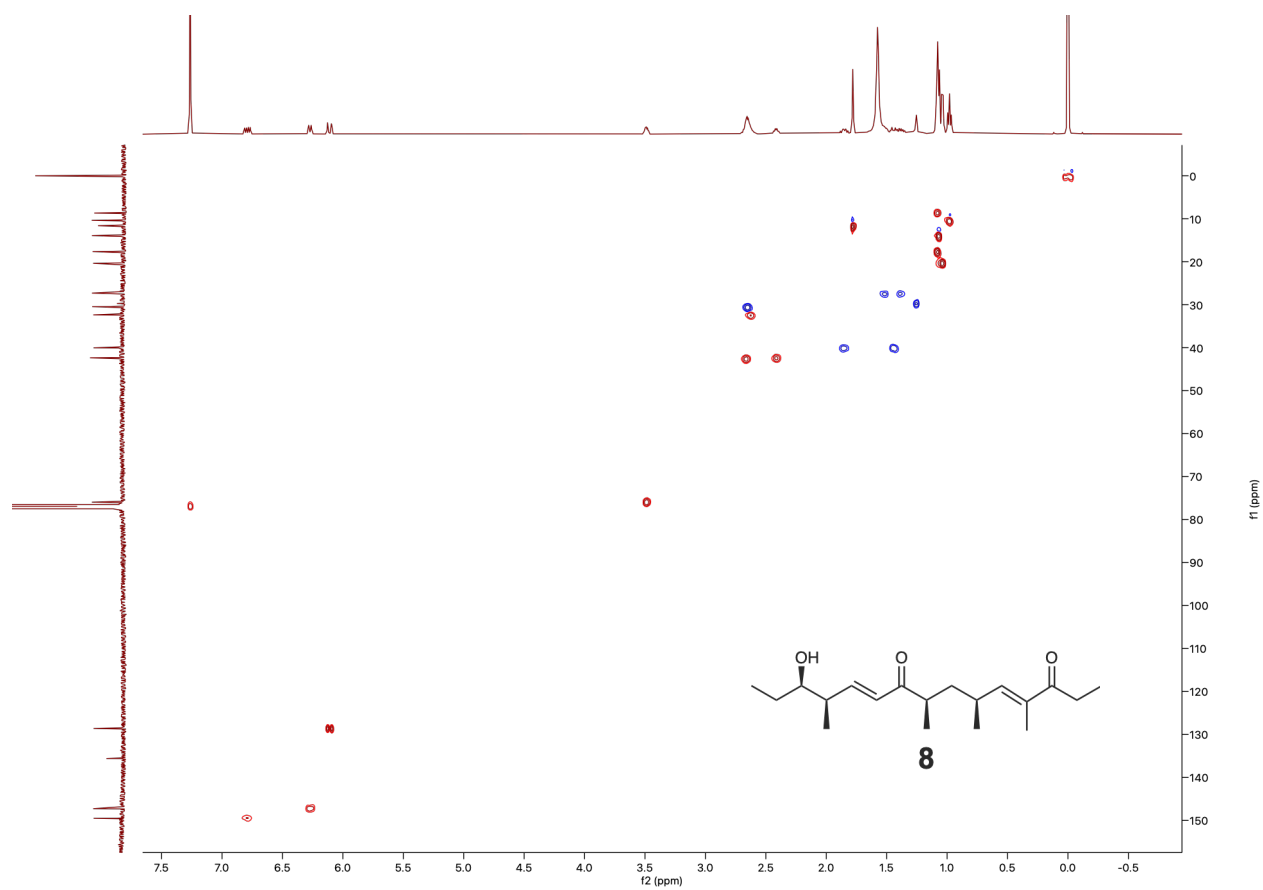


Figure S29. ^1H - ^{13}C HSQC spectrum of compound **8** purified from EPK reaction.

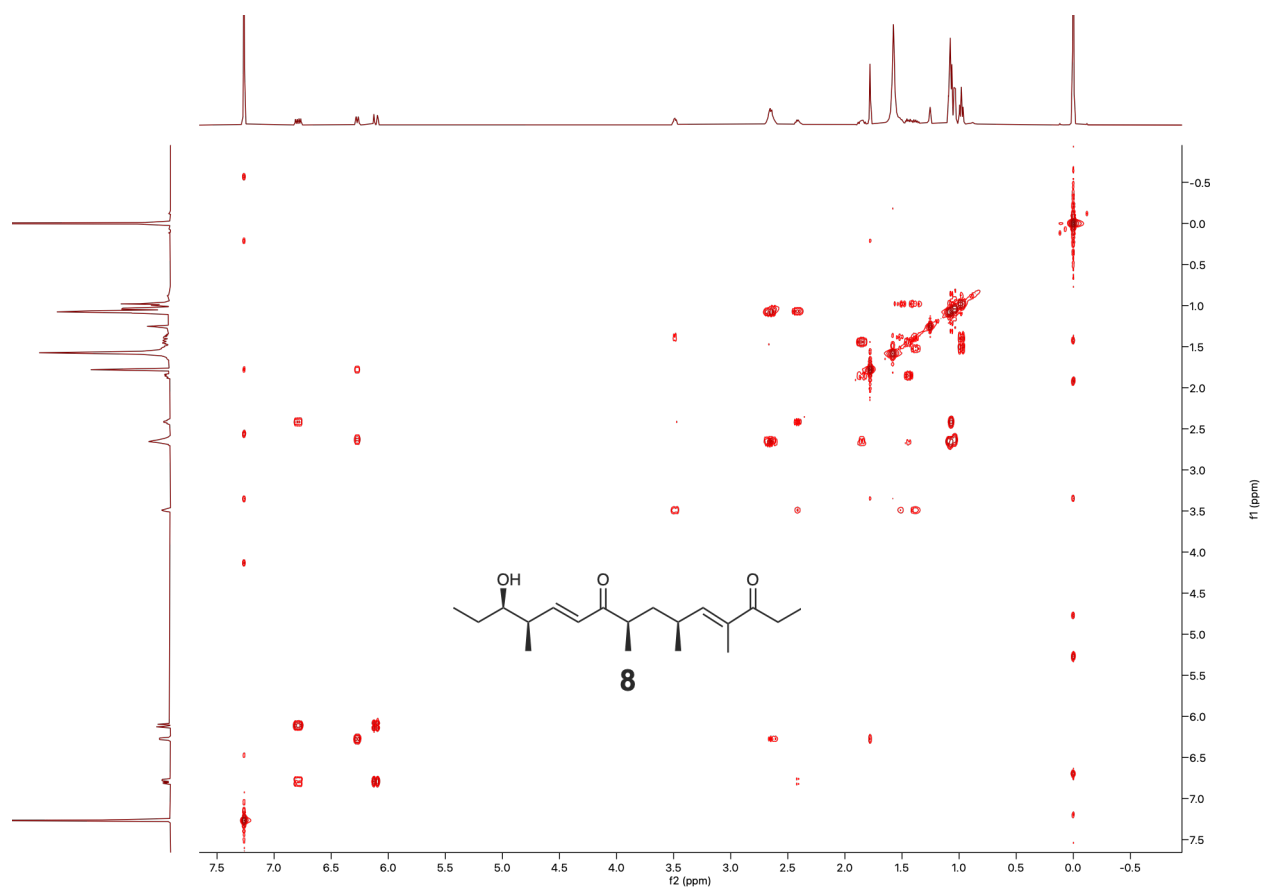


Figure S30. ^1H - ^1H COSY spectrum of compound **8** purified from the EPK reaction.

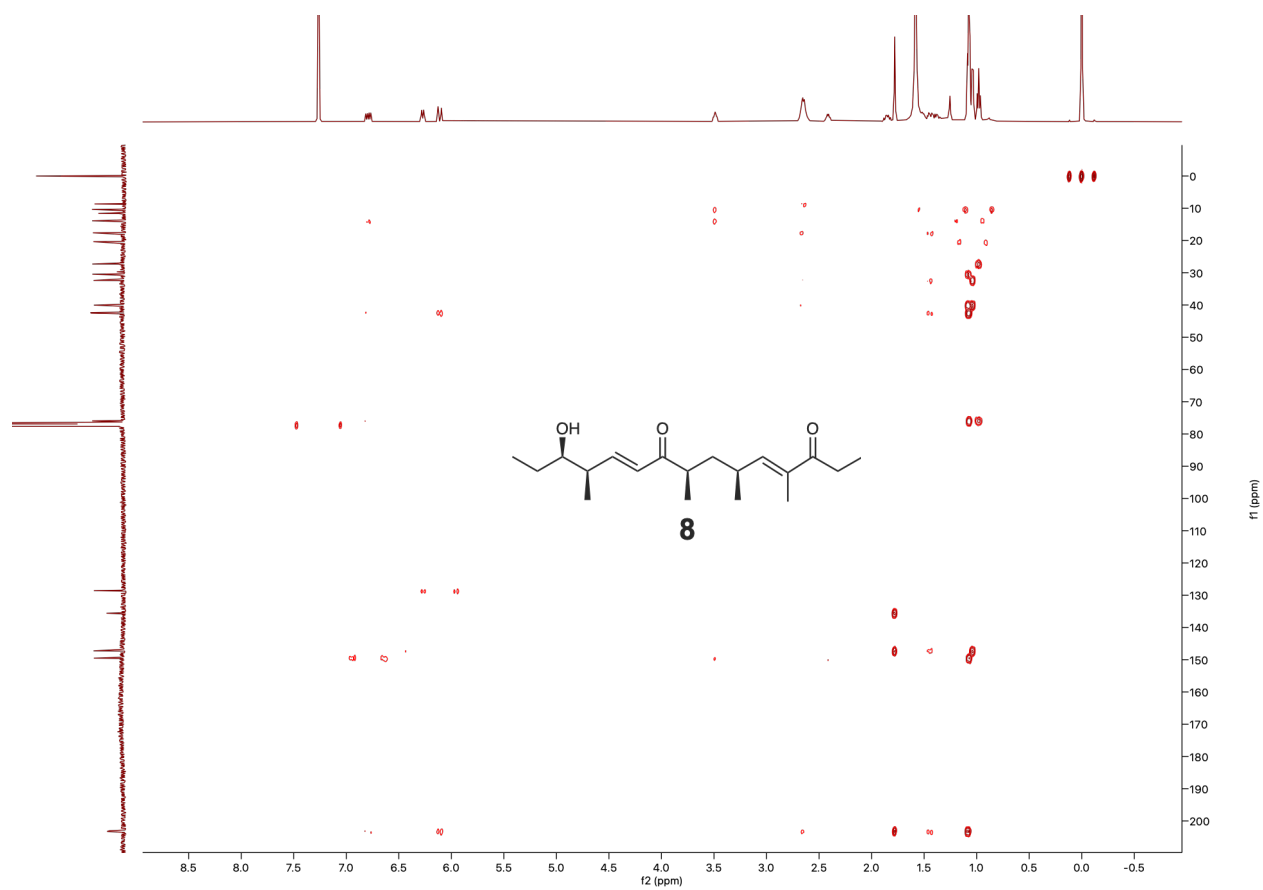


Figure S31. ^1H – ^{13}C HMBC spectrum of compound **8** purified from the EPK reaction.

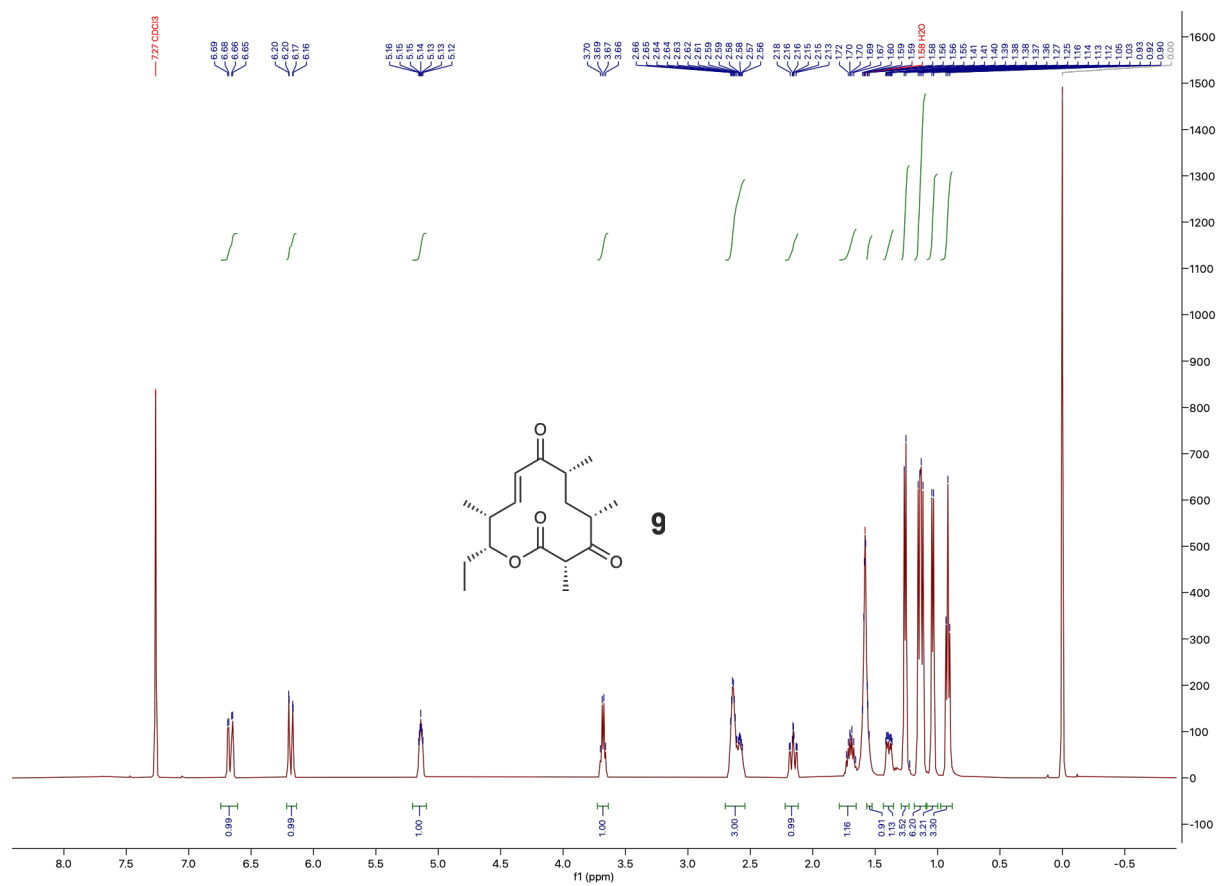


Figure S32. ^1H NMR spectrum of compound **9** purified from EPK reaction.

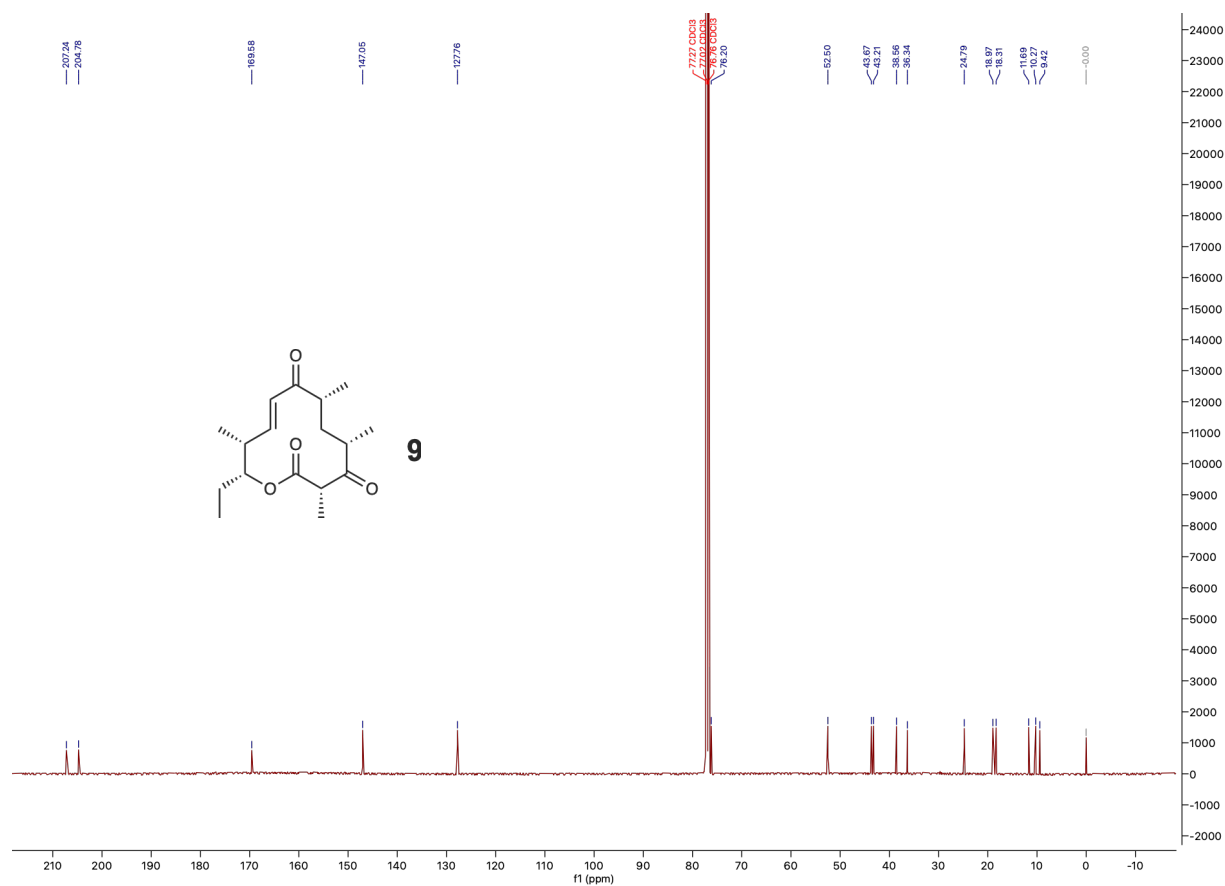


Figure S33. ^{13}C NMR spectrum of compound **9** purified from EPK reaction.

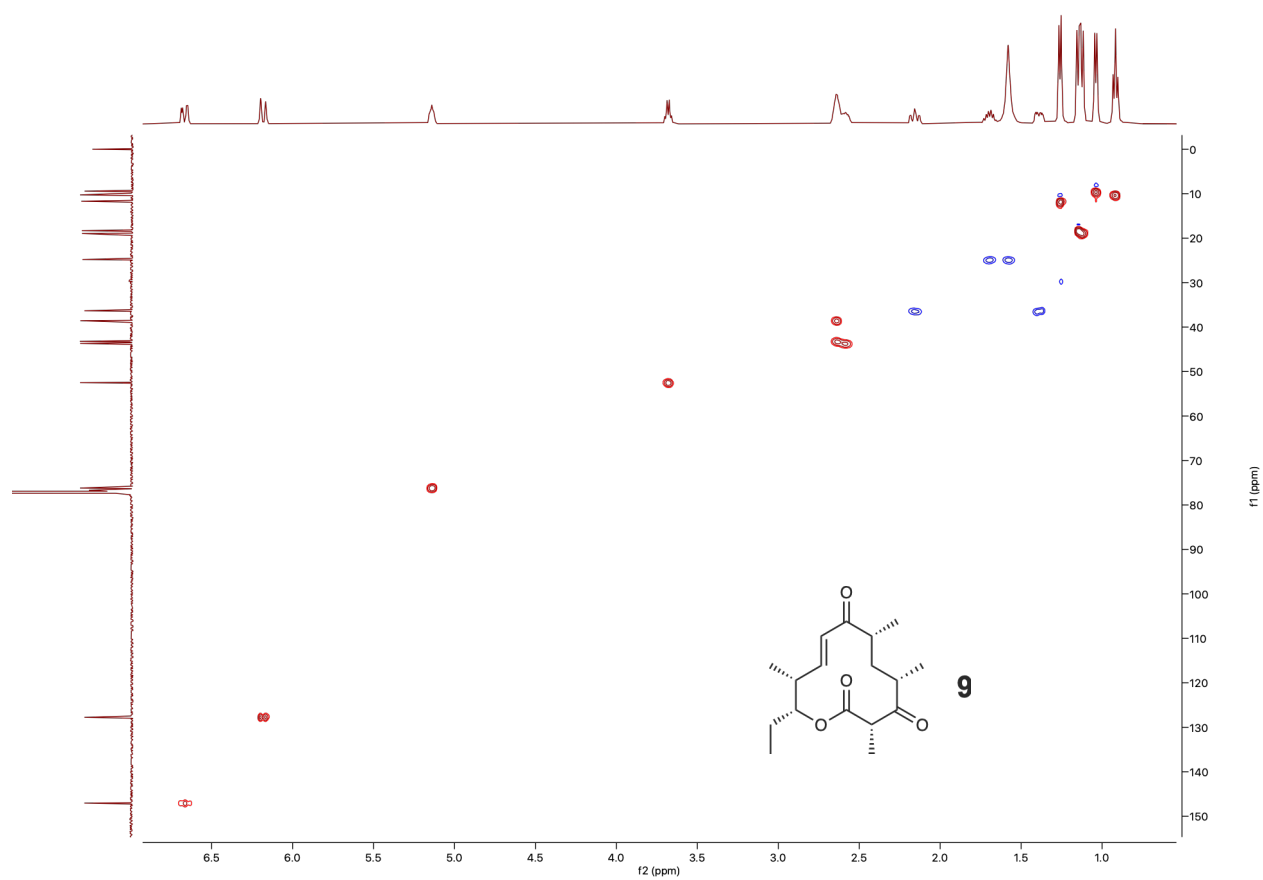


Figure S34. ^1H - ^{13}C HSQC spectrum of compound **9** purified from EPK reaction.

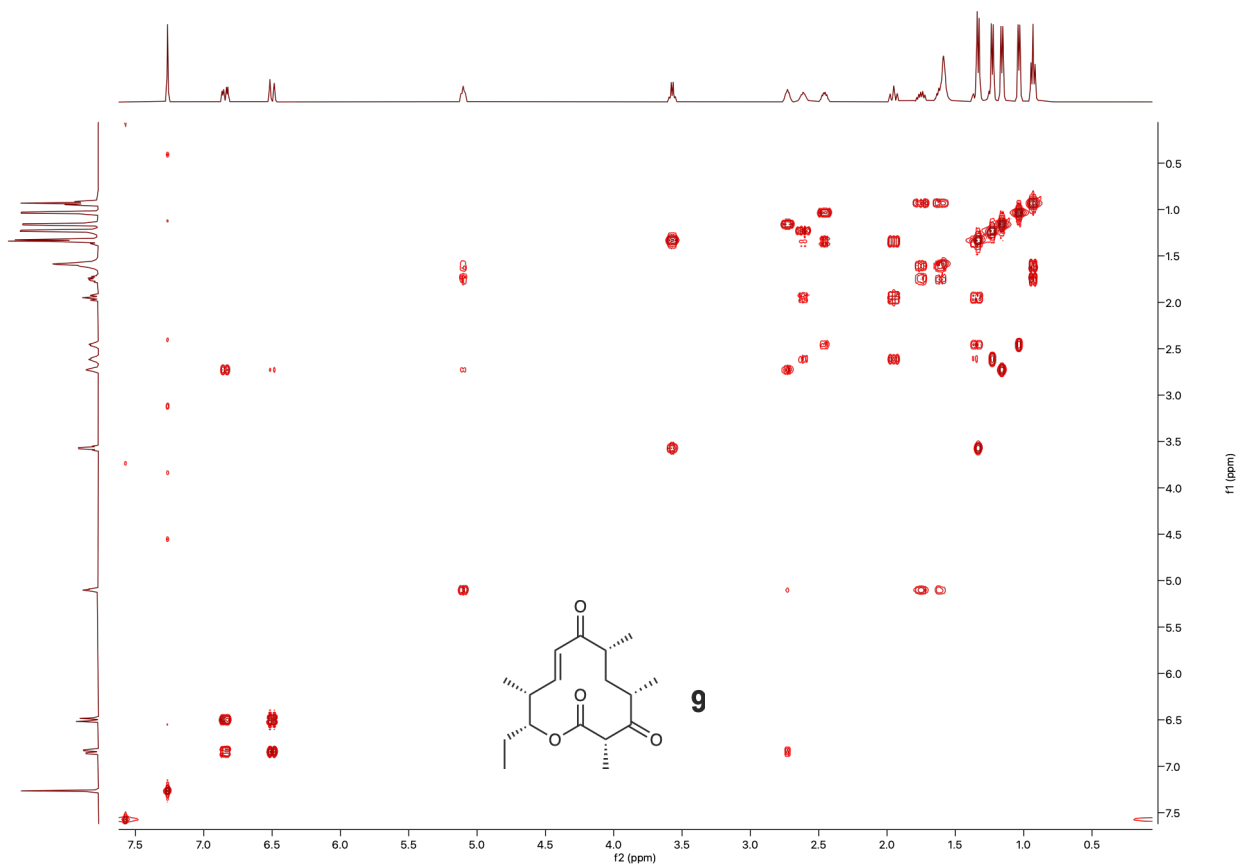


Figure S35. ^1H - ^1H COSY spectrum of compound **9** purified from the EPK reaction.

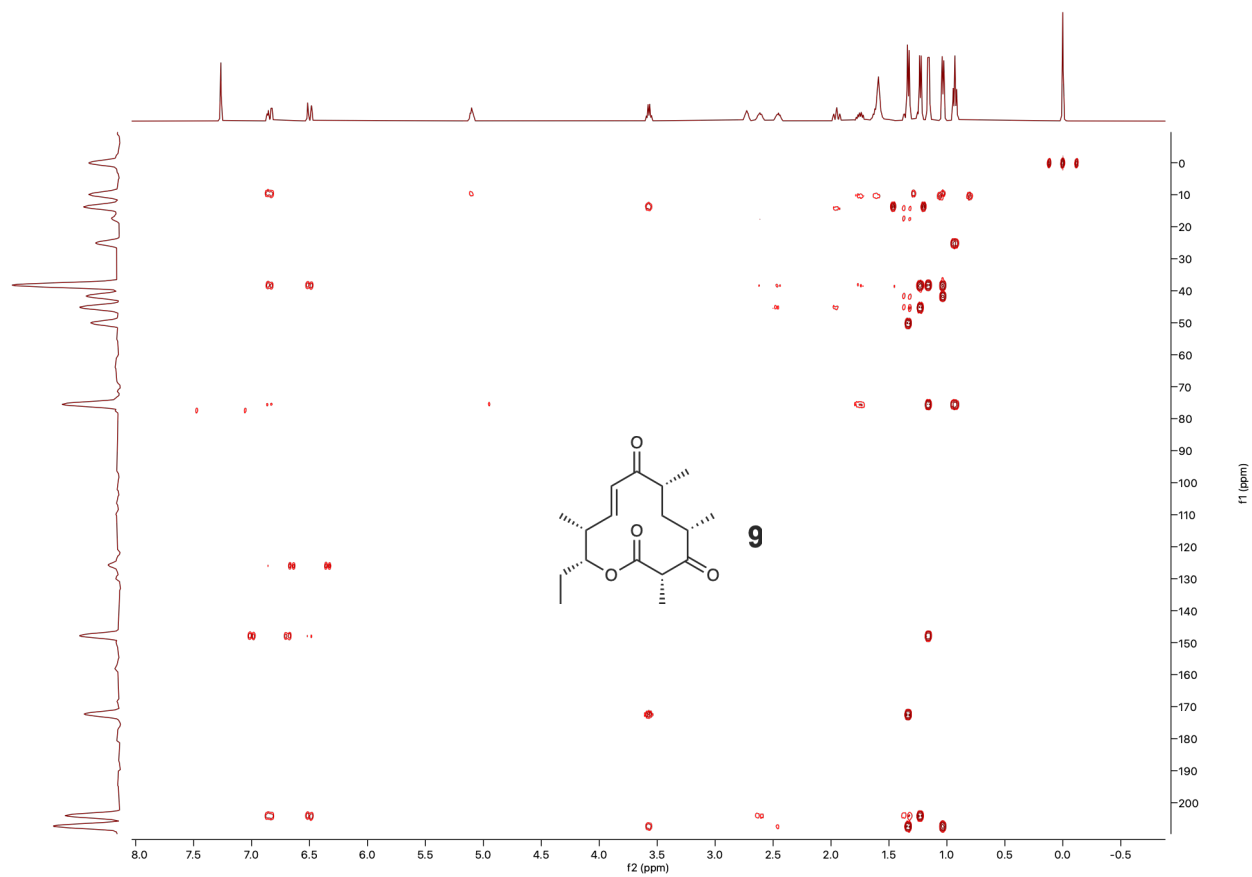


Figure S36. ^1H - ^{13}C HMBC spectrum of compound **9** purified from the EPK reaction.

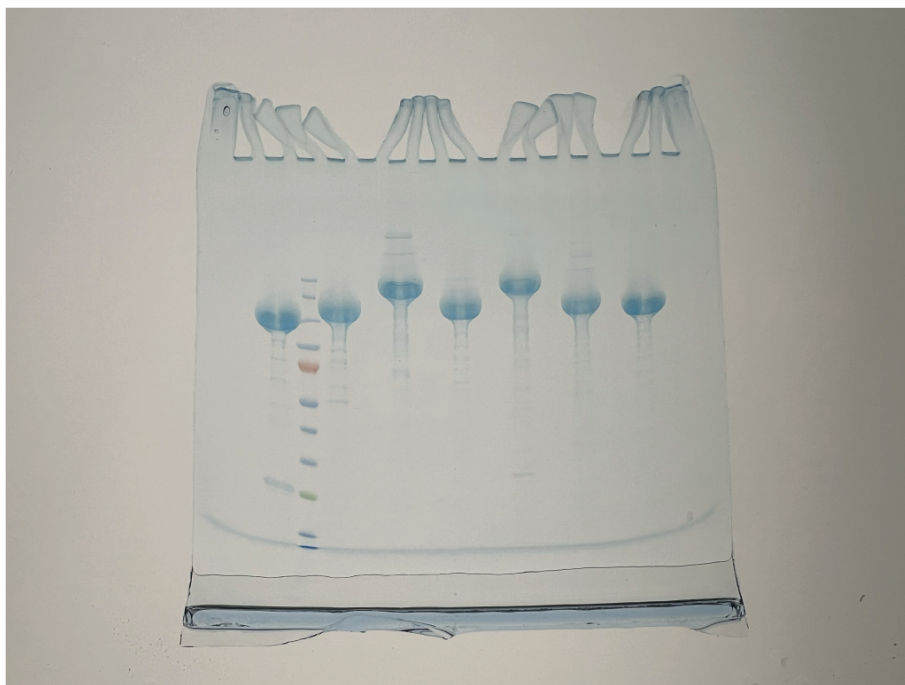
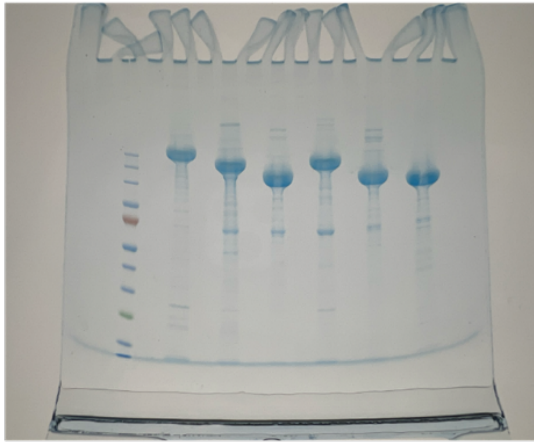


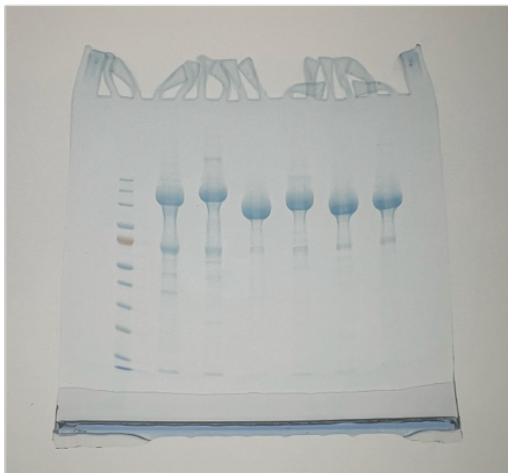
Figure S37. Uncropped, raw gel image that used to create Figure 3b.



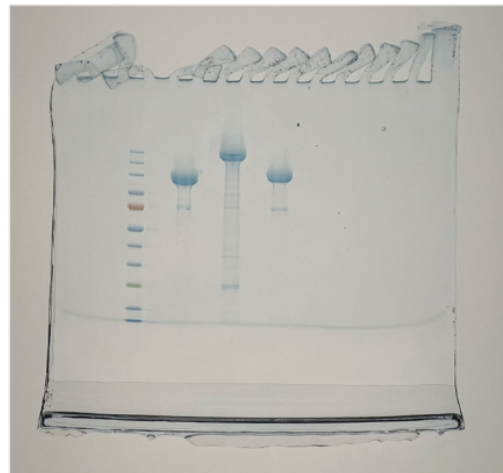
Panel 1



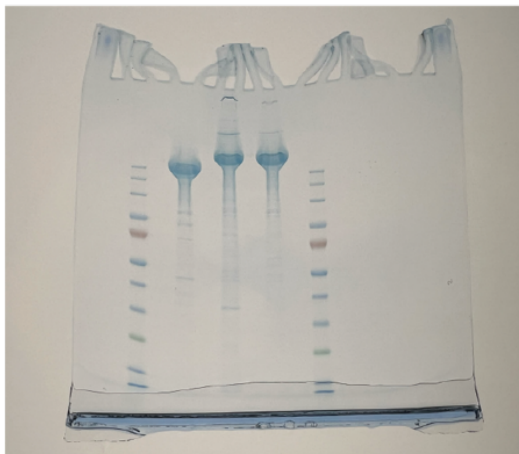
Panel 2



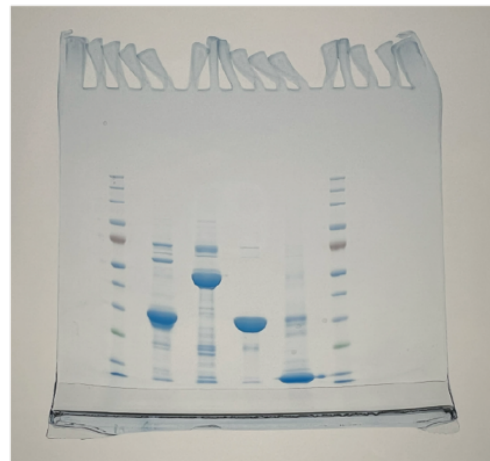
Panel 3



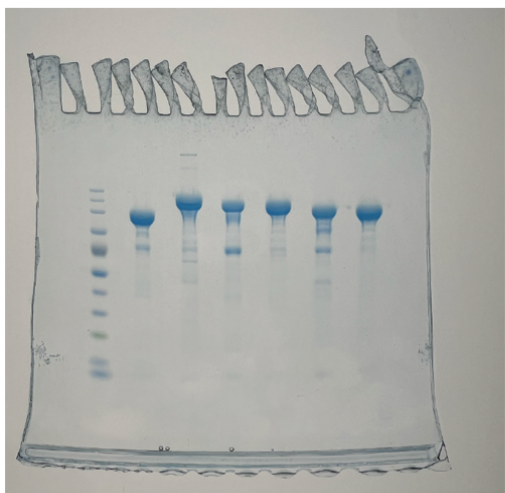
Panel 4



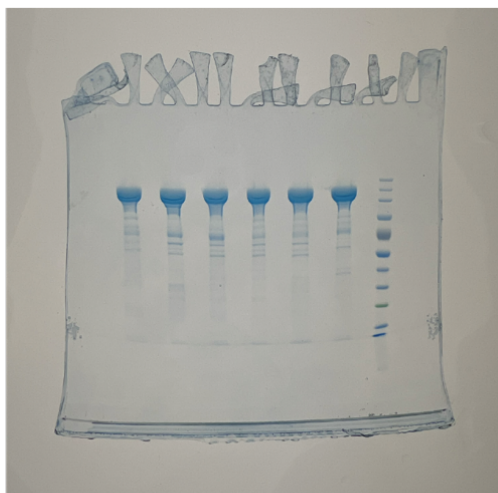
Panel 5



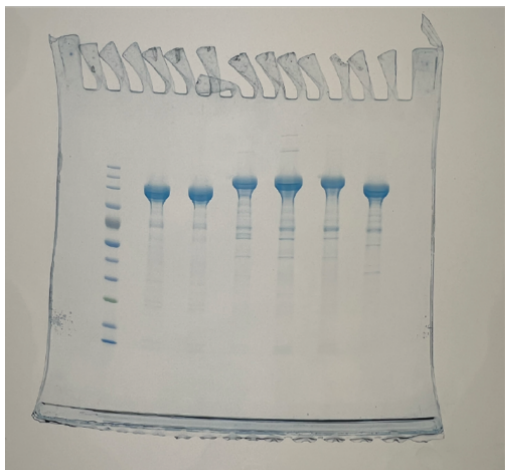
Panel 6



Panel 7



Panel 8



Panel 9

Figure S38. Uncropped, raw gel images used to create Figure S1.

References

1. Miyazawa, T., Bista, R., and Keatinge-Clay, A. T. (2026) Refactoring pikromycin synthase for the modular biosynthesis of macrolide antibiotics in *E. coli*, *Nature Synthesis*.
2. Ray, K. A., Lutgens, J. D., Bista, R., Zhang, J., Desai, R. R., Hirsch, M., Miyazawa, T., Cordova, A., and Keatinge-Clay, A. T. (2024) Assessing and harnessing updated polyketide synthase modules through combinatorial engineering, *Nat Commun* 15, 6485.
3. Bista, R., Zhang, J. & Keatinge-Clay, A. Exploring the limits of polyketide synthase engineering through swapping rapamycin modules into the pikromycin synthase. *Manuscript in preparation* (2025).
4. Miyazawa, T., Fitzgerald, B. J., and Keatinge-Clay, A. T. (2021) Preparative production of an enantiomeric pair by engineered polyketide synthases, *Chem Commun (Camb)* 57, 8762-8765.
5. Zhang, J., Bista, R., Miyazawa, T., and Keatinge-Clay, A. T. (2023) Boosting titers of engineered triketide and tetraketide synthases to record levels through T7 promoter tuning, *Metab Eng* 78, 93-98.
6. Miyazawa, T., Hirsch, M., Zhang, Z., and Keatinge-Clay, A. T. (2020) An in vitro platform for engineering and harnessing modular polyketide synthases, *Nat Commun* 11, 80.