In vitro reconstitution of the radical SAM enzyme MqnC involved in the biosynthesis of futalosine-derived menaquinone

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Supporting Information

Experimental Procedures

General Biological Materials. Molecular biology manipulations were carried out using standard techniques.[1,2] PCR amplifications were performed using an automatic thermocycler (Mastercycler, Eppendorf) and oligonucleotide primers were synthesized by Integrated DNA Technologies. *Phusion* DNA polymerase was obtained from New England BioLabs. The cloning vector pTHT is a derivative of pET-28, which was purchased from Novagen. Plasmid mini-prep and PCR purification kits were acquired from Qiagen or Fermentas. DNA fragments were purified from agarose gel with the Zymoclean Gel DNA Recovery Kit from Zymo Research. All restriction enzymes and T4 DNA ligase were purchased from New England Biolabs. DNA sequencing was performed using the Applied Biosystems Automated 3730 DNA Analyzer at the Cornell University Life Sciences Core Laboratories Center (Cornell BioResource Center). *Escherichia coli* strains DH5α or MachI (Invitrogen) were used as a recipient for transformations during plasmid construction as well as for plasmid propagation and storage. Growth media were obtained from Difco. Reagents were purchased from Sigma-Aldrich unless stated otherwise.

General Synthetic Methods and Materials. All reactions were carried out under argon with dry solvents (dried with molecular sieves). Yields refer to chromatographically and ^{1}H-NMR

homogeneous materials, unless otherwise stated. Reagents were purchased at a high commercial quality (typically 97% or higher) and used without further purification, unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV, ceric ammonium molybdate and/or iodine stains. Flash column chromatography was performed using silica gel 60 (Silicycle, 230-400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury or Inova spectrometer (300 MHz ^{1}H; 75 MHz ^{13}C) and were calibrated using residual undeuterated solvent as an internal reference. The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, p = pentet, br = broad. Melting points were recorded on an automated melting point apparatus (EZ-Melt, Stanford Research Systems) and are uncorrected. Optical rotation was obtained on a Jasco DIP-360 digital polarimeter at the D-line of sodium.

Synthesis of 3-(2-(triphenylphosphoranylidene)acetyl) benzoic acid 7. For use as a starting material during synthesis of DHFL, 3-(2-(triphenylphosphoranylidene)acetyl) benzoic acid (**7**) was prepared as outlined in Figure S1. A mixture of 5 mmol **18**, 5 mmol NBS and 7.5 mmol TsOH in 5 mL acetonitrile was refluxed for 2 h. Then solvent was removed at reduced pressure. The residue was dissolved in 50 mL of dichloromethane, washed with saturated solution of sodium bicarbonate and organic phase was dried with anhydrous sodium sulfate. Dichloromethane was removed at reduced pressure to give bromide **19** quantitatively. A mixture of 2 mmol **19** and 2.4 mmol triphenylphosphine in 50 mL THF was refluxed for 4 h, chilled to room temperature, and filtered to give crude solid **20** quantitatively. Pure white soft solid **20** was obtained after washing three times with 10 mL of THF and drying under vacuum. This

compound was used without further isolation and characterization.[3] A mixture of 1 equiv. **20** and 5 equiv. NaOH in 20 mL MeOH was stirred overnight at room temperature, evaporated to dryness under reduced pressure, and re-dissolved in 5 mL of water. After acidifying to pH 4-5, a suspension formed and compound **7** was extracted with dichloromethane. The organic phase was separated, dried with anhydrous sodium sulfate, and evaporated to give a sticky yellow solid (60-70% isolated yield). Additional purification was carried out by flash chromatography on silica gel (eluent dichloromethane/methanol), resulting in an overall yield of 50% (Figure S2). Yellow solid, m.p. = 138 °C with decomposition, R_f = 0.54 (DCM:MeOH, 9:1). ^{1}H NMR (300 MHz, $CHCl_3$): δ 8.60 (brs, 1H), 8.16 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.80 – 7.60 (m, 6H), 7.60 – 7.40 (m, 9H), 7.31 (t, J = 7.8 Hz, 1H), 7.02 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.4, 133.46 (d, J = 42 Hz), 133.2 (brs), 132.13 (d, J = 39 Hz), 131.8 (brs), 131.6, 129.38 (d, J = 48 Hz), 128.55 (d, J = 48 Hz), 128.2 (brs). ESI MS: calculated for $C_{27}H_{21}O_3P$ 424.12 m/z, observed 425.05 m/z ($[M-H]^+$).

Synthesis of DHFL 3 and [4-^{2}H]-DHFL. DHFL and [4-^{2}H]-DHFL were prepared using the same synthetic scheme starting with ribose or [4-^{2}H]-ribose, respectively (Scheme 2). Compound **8** was prepared by Swern oxidation of commercially available protected ribose (or [4-^{2}H]-ribose) as previously described.[3] It was used without additional purification. A mixture of Wittig reagent **7** (1 mmol) and aldehyde **8** (1 mmol) in 20 mL of dichloromethane was stirred for 60 h at room temperature and then the solvent was removed under reduced pressure. The resulting residue was dissolved in 10 mL ethyl acetate and filtered. It should be noted that **7** is difficult to dissolve in ethyl acetate thus unreacted **7** could be removed by filtering. The filtrate was extracted with saturated sodium carbonate (2 × 5 mL) and the aqueous phase containing **9** was separated. The

ethyl acetate layer contained the byproduct triphenylphosphosphine oxide. The basic aqueous phase was acidified to pH 5 and pure **9** was extracted with dichloromethane with 60% yield (white solid). It should be cautioned that the presence of **7** or triphenylphosphine oxide could slow down the subsequent hydrogenation step. Additional purification is desirable by flash column chromatography (eluent ethyl acetate/dichloromethane, or crystallization with ether/methanol mixtures). Compound **9** (0.35 g, 1 mmol) was stirred with 10 % palladium on charcoal (0.1 g) in ethyl acetate at room temperature under 1 atm of hydrogen gas for 4 h. The reaction mixture was filtered through a pad of celite and concentrated to afford a white solid product **10** quantitatively. Additional purification could be done on silica gel column (CH_2Cl_2/EtOAc), or by crystallization with mixtures of CH_2Cl_2/hexanes. It is important to mention that several different protected forms of DHFL were synthesized, however the deprotection step was very sensitive to degradation. The unique three-phase deprotection strategy employed and described herein was the most successful method. A mixture of 100 mg **10** in 1 mL of chloroform, 1 mL water, and 20 mg of DOWEX cation exchanged resin was stirred for 4 days at room temperature. The water layer was seperated, centrifuged, and lyophilized to give 7 mg pure DHFL (**3**) by HPLC and NMR. The same quantities of starting materials under microwave (MW) irradiation at 90 °C for 50 min provided 14 mg of pure DHFL. Use of 4 MW cycles with seperation of water layers and addition of new water each cycle provided 60 mg (70%) of pure DHFL. The chloroform layer did not contain starting compound **10**. DHFL is a white hygroscopic solid that is sensitive to acids, bases, and salts (e.g., degrades during lyophilization when dissolved in phosphate buffer). DHFL is stable as a water solution and as a solid at 4 °C for at least 5 months. DMSO and glycerol decomposed DHFL in aqueous solutions. Optical rotation: +30.5 (c = 0.5, MeOH, 19 °C). ^{1}H NMR (300 MHz, D_2O, suppressed water

signal): δ 8.35 (s, 0.7H), 8.33 (s, 0.3H), 8.07 (t, J = 7.6 Hz, 2H), 7.52 (t, J = 7.9 Hz, 1H), 5.26 (d, J = 3.9 Hz, 0.3H), 5.15 (d, J = 1.9 Hz, 0.7H), 4.06 (dd, J = 6.3 Hz, J = 4.8 Hz, 1H), 3.94 (dd, J = 4.8 Hz, J = 1.8 Hz, 1H), 3.92 – 3.80 (m, 1H), 3.20 – 3.00 (m, 2H), 2.10 – 1.70 (m, 2H). ^{13}C NMR (75 MHZ, D_2O): δ 202.8, 169.3, 136.3, 134.3, 132.7, 130.3, 130.1, 129.1, 125.6, 100.7, 95.8, 81.3, 81.0, 75.3, 73.7, 73.1, 70.5, 38.6, 34.5, 28.1. ESI MS: calculated for $C_{14}H_{16}O_7$ 296.09 m/z, observed 295.04 m/z ($[M-H]^-$), dimer 590.70 m/z ($[M-H]^-$), 303.12 m/z ($[M+Li]^+$).

Expression construct for His_6-MqnC. Chromosomal DNA from *Bacillus halodurans* C-125 (BAA-125D-5) was purchased from American Type Culture Collection (ATCC) and used as the template for PCR amplification of *mqnC* (BH_3143). The forward and reverse primers were designed with *Nde*I and *Xho*I restriction sites (indicated in underlined font), respectively, as *mqnC* FP: 5′-GGGTAGCATATGAGTATTGACGGAATCTTGGAGCGTG-3′ and *mqnC* RP: 5′-CCCTACTCGAGTTAATTTTGCATGACGAAATCTTTTTC-3′. The product containing the desired gene was excised from an agarose gel, purified and digested with the appropriate restriction enzymes, repurified, and then ligated into similarly digested and purified pTHT. The final construct encoding the gene product with six histidine residues and a TEV protease recognition sequence (underlined font) appended to the N-terminus: pTHT, MGSDKIHHHHHHSSGENLYFQGH. Competent *E. coli* DH5α cells were transformed with the sample. Cells were plated on LB agar (1.5%) supplemented with 50 μg/mL kanamycin (LabScientific) to select for positive clones. Subsequently, clones were screened by colony PCR and the desired gene products were confirmed by DNA sequencing.

Expression and purification of His_6-MqnC. Chemically competent *Escherichia coli* BL21(DE3)-T1R cells carrying pPH151, which contains the *Escherichia coli sufABCDSE* operon cloned into pACYCDuet-1,[4] were prepared using the calcium chloride method.[1] The cells were freshly transformed with pTHT-*mqnC* and plated on LB agar containing 40 μg/mL kanamycin and 15 μg/mL chloramphenicol. Single colony transformants were grown aerobically (200 rpm) at 37 °C for 12-15 h in 50 mL of LB medium supplemented with 50 μg/mL kanamycin and 25 μg/mL chloramphenicol (LB-kan-cam). Cells from the starter culture (10 mL) were added to 4 × 1.5 L of LB-kan-cam and expression cultures were grown aerobically (180 rpm) at 37 °C until the A_{600} was 0.4-0.5. Cultures were cooled at 4 °C for 30 min and then ferrous ammonium sulfate (160 mg), L-cysteine (160 mg), and 100 μM isopropyl-1-thio-β-D-galactopyranoside (IPTG, LabScientific) were added. Growth was continued with slow shaking (90 rpm) at 15 °C for 12-15 h. Cultures were cooled at 4 °C (1-2 hours) and then cells were harvested by centrifugation at 6,300×*g* in a Beckman Coulter Avanti J-26 XPI with a JLA-8.1000 rotor for 15 min at 4 °C. The medium was discarded and the cell paste (~ 1.0-1.5 g per liter of cell culture) was stored submerged in liquid nitrogen until use. Protein purification was carried out in an anaerobic chamber (Coy Laboratories) containing 5% hydrogen and 95% nitrogen. Cells were thawed and resuspended in 50 mL of Lysis Buffer (100 mM Tris, pH 7.5 at 25 °C) supplemented with 20 mg of lysozyme, 2000 units of benzonase, and 5 mM DL-dithiothreitol (DTT). After 1.5 hours of incubation on ice with continuous stirring, the cells were further lysed by sonication, while stirring on ice, for 6 × 60 s pulses with 3 min pauses using a QSonica Q55 (setting = 45) with a 1/4 inch microtip. Cell debris was removed by centrifugation at 31,000×*g* in a Beckman Coulter Avanti J-E with a JA-17 rotor for 45 min at 4 °C and the supernatant was clarified through a 0.45 μm syringe filter. The enzymes were purified by nickel affinity chromatography

using a stepwise gradient of increasing imidazole concentration with 2 × 5 mL HiTrap chelating HP column (GE Healthcare Life Sciences) arranged in tandem. The resin was charged with 2 column volumes (CV) of 0.1 M $NiSO_4$, washed with 5 CV of filtered water to remove excess nickel, and equilibrated with 5 CV of Lysis Buffer supplemented with 1 mM DTT. After loading the filtered protein sample, the resin was washed with 5 CV each of Wash Buffers (100 mM Tris, pH 7.5 at 25 °C, 300 mM NaCl, 1 mM DTT) containing 0 mM, 10 mM, 50 mM, and 250 mM imidazole. Brownish colored fractions of 0.5 mL were collected and analyzed for purity by 12% SDS-PAGE (Figure S3). His_6-MqnC eluted from the column in the 250 mM imidazole fractions. Nickel affinity column fractions containing the desired protein were pooled and the buffer was exchanged to Final Buffer (100 mM potassium phosphate, pH 7.5 at 25 °C, 1 mM DTT, 30% glycerol) via Econo-Pac 10DG size exclusion chromatography (Bio-Rad). Protein concentration was determined using the bicinchoninic acid (BCA) protein assay kit (Pierce) and A_{280} measurements. The theoretical extinction coefficient for A_{280} calculations was obtained from the ProtParam function on the ExPASy Proteomics Server: His_6-MqnC, ε_{280} = 56,380 M^{-1} cm^{-1}. Both methods gave the same estimates for protein concentration. Typical yields were ~ 30 mg of His_6-MqnC purified protein per liter of cell culture. Protein was stored submerged in liquid nitrogen.

Iron content determination for MqnC. Iron content was analyzed using the protocol herein, which was adapted from the previously described ferene assay.[5,6] The following reagents were prepared: A, 1.35 g SDS, 30 mL water, 450 µL saturated sodium acetate; B, 540 mg L-ascorbic acid, 18 mg sodium metabisulfite ($Na_2S_2O_5$; Fisher), 11.2 mL water, 800 µL saturated sodium acetate; C, 18 mg ferene (disodium salt of 5,5′-[3-(2-pyridyl)-1,2,4-triazine-5,6-diyl]bis-2-furansulfonic acid; MP Biomedicals) in 1 mL water. To 300 µL of assay solution containing 0 to

100 μM MqnC, 0 or 3.2 M guanidine hydrochloride, and 1 mM DTT was added 300 μL Reagent A and 300 μL Reagent B. After mixing by inversion, the samples were incubated for 15 min at 30 °C and then 15 μL Reagent C was added. The samples were incubated for 5 min at 25 °C, centrifuged at 16,110×*g* in an Eppendorf 5415 R with a F45-24-11 rotor for 5 min at 25 °C, and then the supernatant was diluted 10-fold with water. The absorbance at 593 nm was recorded using a Varian Cary Bio 300 UV-Visible Spectrophotometer. The iron content was finally determined by comparing the reading to a standard curve that was generated under identical conditions using $FeCl_3$ in 2% HNO_3 with a concentration range from 0 to 500 μM.

Sulfide content determination for MqnC. Sulfide content was analyzed using the protocol herein, which was adapted from the previously described methylene blue assay.[7] To 300 μL of assay solution containing 0 to 40 μM MqnC, 3.2 M guanidine hydrochloride, and 1 mM DTT was added 4 mM N,N-dimethyl-*p*-phenylenediamine (DMPD) monohydrochloride in 5 M HCl and 1 mM $FeCl_3$ in 1.2 M HCl (final volume: 400 μL). After mixing by inversion, the samples were centrifuged at 16,110×*g* in an Eppendorf 5415 R with a F45-24-11 rotor for 5 min at 25 °C. The supernatant was diluted 2-fold with buffer (50 mM potassium phosphate, pH 7.5 at 25 °C, 15% glycerol) and then the samples were incubated for 30-60 min at 25 °C. The absorbance at 670 nm was recorded using a Varian Cary Bio 300 UV-Visible Spectrophotometer. The sulfide content was finally determined by comparing the reading to a standard curve that was generated under identical conditions using sodium sulfide (Na_2S; Fisher) in 0.1 M NaOH with a concentration range from 0 to 100 μM.

EPR. Samples were prepared in an anaerobic chamber (Coy Laboratories) containing 5% hydrogen and 95% nitrogen. To ~ 280 μM MqnC in 100 mM potassium phosphate, pH 7.5 with 30% glycerol was added 0 or 40 mM sodium dithionite. After incubation for 1 h at 25 °C, ~ 150-200 μL of sample was transferred to EPR tubes (quartz, 4 mm OD, 3 mm ID). The samples were frozen using liquid nitrogen and then EPR data was collected using a Bruker EMX X-band spectrometer with an Oxford Instruments ER910 cryostat at a frequency of 9.45 GHz, power of 20 mW, and temperatures of 4 K, 10 K, and 50 K.

In vitro activity of MqnC. MqnC activity assays were carried out in an anaerobic chamber (Coy Laboratories) containing 5% hydrogen and 95% nitrogen. To 100 μM MqnC in 100 mM potassium phosphate, pH 7.5 with 30% glycerol was added sodium dithionite (8 mM), dehypoxanthine futalosine (DHFL, 1 mM), and *S*-adenosyl-L-methionine (SAM, 1.3 mM). Reactions were incubated for 2 to 6 h at 25 °C. Control reactions identical to those described above but lacking sodium dithionite, DHFL, SAM, or MqnC were also prepared. The samples (50 μL) were diluted 2-fold with assay buffer and then the protein was removed by centrifugal ultrafiltration through a 10 kDa MWCO membrane (Pall Life Sciences). A 50 μL aliquot of the filtrate was analyzed by reverse-phase HPLC. We attempted to further rebuild the Fe/S cluster *in vitro* by incubating the *in vivo*-Fe/S-reconstituted protein with Fe(II) and sulfide under anaerobic conditions.[8,9] No increase in enzyme activity was observed in these samples.

Analytical HPLC. Reaction mixtures were analyzed by reverse-phase HPLC on an Agilent 1200 HPLC system (ChemStation) equipped with a quaternary pump and thermostatted autosampler (10 °C). The system included a diode array UV-Vis detector (80 Hz full spectral sampling,

190 – 640 nm) and a fluorescence detector. The stationary phase was a Supelcosil LC-18-T column (15 cm × 4.6 mm, 3 μm particles), maintained at 22 °C. The LC eluent (1 mL/min flow rate) consisted of a gradient of methanol in 100 mM potassium phosphate buffer, pH 6.6 supplemented with 5 mM triethylamine. In the analytical method the percentages of phosphate buffer (*P*) and methanol (*M*) balanced with water at time *t* varied according to the following scheme: (*t*,*M*,*P*): (0,0,100), (4,0,90), (9,15,60), (14,65,10), (16,0,100), (21,0,100). Chromatograms were detected using the absorbance at 250 nm. UV-Vis spectra of substrate and product peaks were also collected and assessed.

LC-MS. Samples were analyzed by reverse-phase HPLC on an Agilent 1200 HPLC system (ChemStation) equipped with a diode array UV-Vis detector and thermostatted autosampler (10 °C). The stationary phase was a Supelcosil LC-18-T column (15 cm × 3 mm, 3 μm particles), maintained at 22 °C. The LC eluent (1 mL/min flow rate) consisted of a gradient of 75% methanol in 25% water with 5 mM ammonium acetate, pH 6.6. The percentages of ammonium acetate (*A*) and methanol (*M*) at time *t* varied according to the following scheme: (*t*,*M*,*A*): (0,0,100), (7,0,100), (15,20,80), (20,30,70), (26,100,0), (28,100,0); (29,0,100); (40,0,100). Chromatograms were detected using the absorbance at 254 nm. UV-Vis spectra (190 – 640 nm) were also collected and assessed. Mass data was collected using an in-line Bruker Daltonics micrOTOF-Q II ESI-Qq-TOF mass spectrometer (HyStar) in negative ion mode as indicated.

Purification of CDHFL from mqnD-Disruptant Strain. The SCO4326-disruptant strain,[10] in which the *mqnD* gene was replaced with the thiostrepton resistance gene by double-crossover homologous recombination, was kindly provided by Professor Tohru Diari from Hokkaido

University, Hokkaido, Japan. The strain was cultivated per the previously reported methodology.[10] Briefly, the SCO4326-disruptant stain was grown with agitation in M9 minimal medium (2 L) supplemented with thiostrepton (10 μg/mL, Tocris Bioscience) and menaquinone (MK-4, 200 μg/mL, Sigma) for 2 weeks at 30 °C. The culture medium was centrifuged at 6,300×*g* in a Beckman Coulter Avanti J-26 XPI with a JLA-8.1000 rotor for 15 min at 4 °C. The cells were discarded and the MK-4 was extracted (twice) from the supernatant with ethyl acetate. The aqueous layer was concentrated to dryness by lyophilization and then the dried material was dissolved in a small volume of distilled water. Samples were clarified through a 0.45 μm syringe filter and a 10 kDa MWCO membrane. Cyclic DHFL was purified by reverse-phase HPLC on an Agilent 1200 HPLC system equipped with a quaternary pump, and thermostatted autosampler (10 °C) and fraction collector (4 °C). The stationary phase was a Discovery C18 semi-prep reverse-phase column (25 cm × 10 mm, 5 μm particles), maintained at 22 °C. The LC eluent (2 mL/min flow rate) consisted of a gradient of methanol in 100 mM potassium phosphate buffer, pH 6.6 supplemented with 5 mM triethylamine. The percentages of phosphate buffer (*P*) and methanol (*M*) balanced with water at time *t* varied according to the following scheme: (*t*,*M*,*P*): (0,0,100), (2,0,90), (4,7,60), (16,8,60), (22,65,10), (25,0,100), (35,0,100). The methanol was removed by rotary evaporation and a second round of HPLC purification was performed to eliminate non-volatile buffer salts. The LC eluent (2 mL/min flow rate) consisted of a gradient of methanol in 5 mM ammonium formate, pH 6.6 (volatile salt). The percentages of methanol (*M*) and ammonium formate (*F*) balanced with water at time *t* varied according to the following scheme: (*t*,*M*,*F*): (0,0,100), (6,0,100), (9,15,80), (14,20,70), (20,75,0), (25,0,100), (30,0,100). Chromatograms were detected using the absorbance at 254 nm. UV-Vis spectra (190 – 640 nm) were also collected and assessed. The methanol was removed by rotary evaporation, the aqueous

layer was concentrated to dryness by lyophilization, and the resulting cyclic DHFL powder was stored at –80 °C.

Stoichiometry of MqnC Reactions. MqnC activity assays were carried out in an anaerobic chamber containing 5% hydrogen and 95% nitrogen as described above. Reactions were incubated at 25 °C and aliquots (50 μL) were quenched with 3.5 M guanidine hydrochloride (50 μL of 7 M) at 0, 5, 10, 15, 30, 60, 120, and 180 min. Control reactions lacking sodium dithionite, DHFL, SAM, or MqnC were also prepared. Protein was removed by centrifugal ultrafiltration through a 10 kDa MWCO membrane and a 50 μL aliquot of the filtrate was analyzed by reverse-phase HPLC. Since the small amount of CDHFL obtained from the *mqnD*-disruptant strain could not be weighted accurately, the commercially available model compound 3-acetylbenzoic acid (Sigma, **21**) was used to calculate the concentration of CDHFL (Figure S5). Calibration plots for **21** and 5′-dAd were used to convert HPLC peak areas to concentrations corresponding to CDHFL and 5′-dAd, respectively (Figure S6). The DHFL substrate was not necessary for cleavage of SAM but the production of 5′-dAd increased 4-fold upon addition of DHFL to the enzymatic assay.

References

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Supporting Information Figures

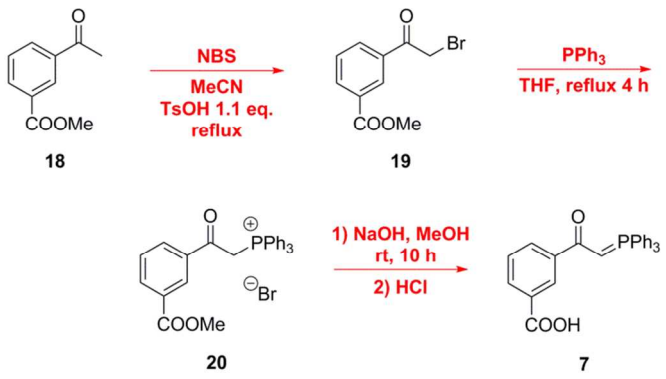

Figure S1. Synthesis of 3-(2-(triphenylphosphoranylidene)acetyl) benzoic acid (**7**).

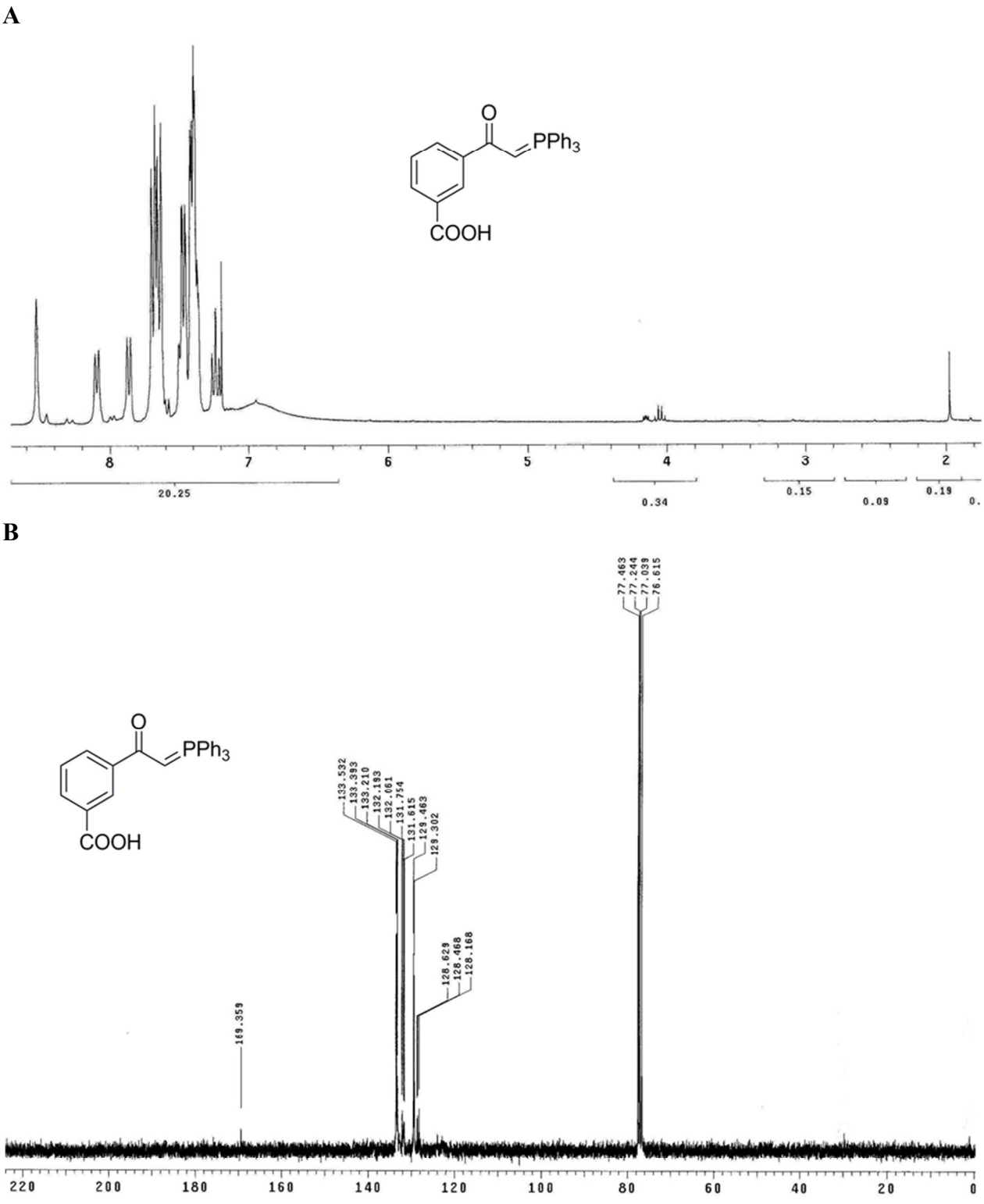

Figure S2. Synthetically prepared 3-(2-(triphenylphosphoranylidene)acetyl) benzoic acid (**7**). (A) ^{1}H NMR. (B) ^{13}C NMR.

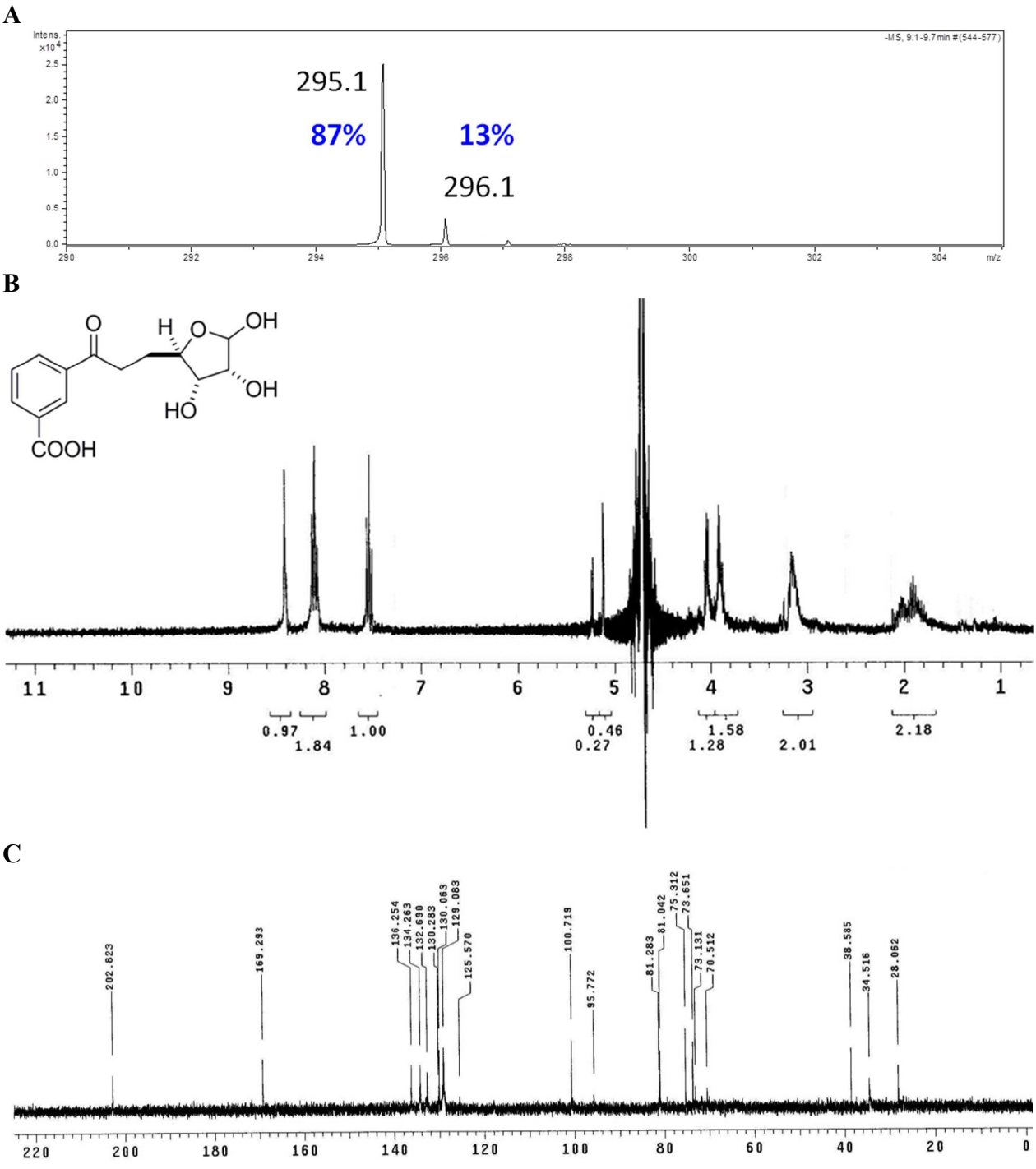

Figure S3. Synthetically prepared DHFL. (A) Mass spectrum. Calculated exact mass of DHFL: 295.1 m/z ($[M-H]^-$). (B) ^{1}H NMR. (C) ^{13}C NMR.

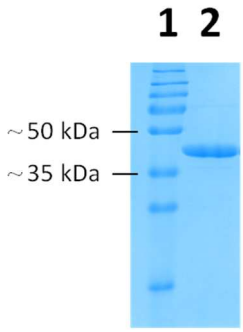

Figure S4. SDS-PAGE (12%) analysis for MqnC. Lane 1, broad-range protein standards (BioRad); lane 2, His_6-BhMqnC (44.7 kDa).

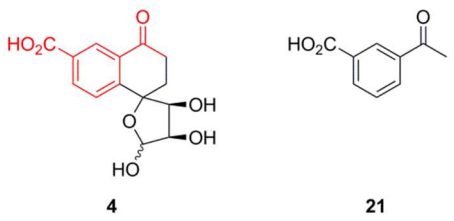

Figure S5. Structural comparison of CDHFL and 3-acetylbenzoic acid

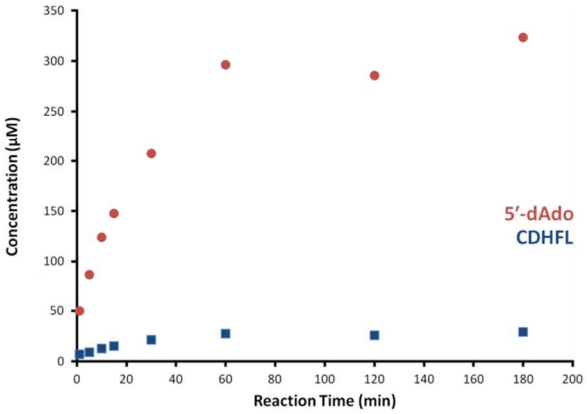

Figure S6. Time course for MqnC reaction illustrating the production of both 5′-dAd and CDHFL as a function of reaction time.