

Supporting Information for:

**Investigation of acid-base catalysis in the halimadienyl diphosphate synthase involved in
Mycobacterium tuberculosis virulence**

Cody Lemke,^{1,†} Kristin Roach,^{1,†} Teresa Ortega,² Dean J. Tantillo,² Justin B. Siegel^{2,3,4} and Reuben J. Peters^{1,*}

¹Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, IA 50011, United States

²Department of Chemistry, University of California-Davis, Davis, CA 95616, United States

³Department of Biochemistry and Molecular Medicine, University of California-Davis, Davis, CA 95616, United States

⁴Genome Center, University of California-Davis, Davis, CA 95616, United States

[†]These authors made equal contributions.

*Corresponding author: Email: rjpeters@iastate.edu

Table of contents

Figure S1. Depiction of *TerDockin* results for truncated **B** in MtHPS:Y328F/Y479F.

Figure S2. Coupled assay demonstrating stereochemistry of **4**.

Figure S3. Tukey Trimean plot for *TerDockin* total energy distribution with **B**. Intermediate **B** xyz coordinates.

Docking truncated intermediate **B** in MtHPS:Y328F/Y479F

The results for truncated intermediate **B** found D293 to be 5.1 Å from the proton removed to produce **3** (Figure 3a), potentially suggesting an intervening water molecule could serve as the deprotonating base. However, the energetically favorable poses lacked a reasonable angle for such activity (Figure 3b).

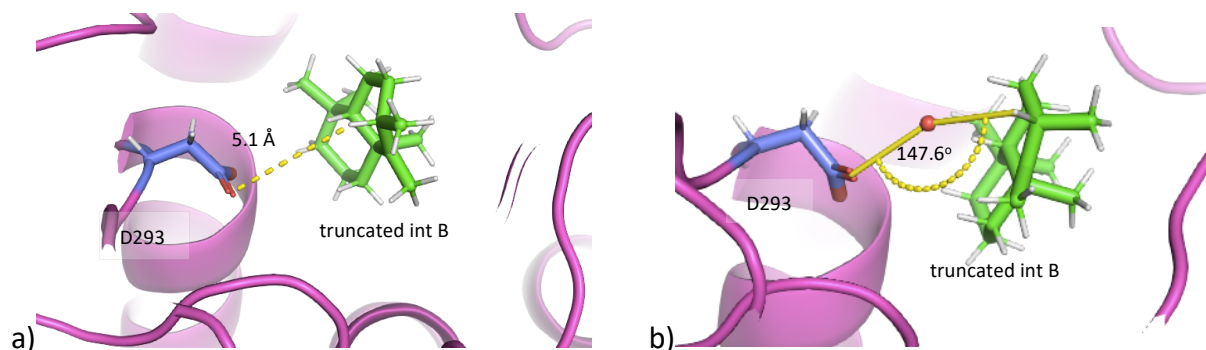


Figure S1. a) D293 carbonyl is 5.1 Å away from the proton removed to produce **3**. b) The resulting angle from acidic proton-oxygen-ASP 276 is chemically unreasonable for deprotonation.

Stereochemical analysis of **4**

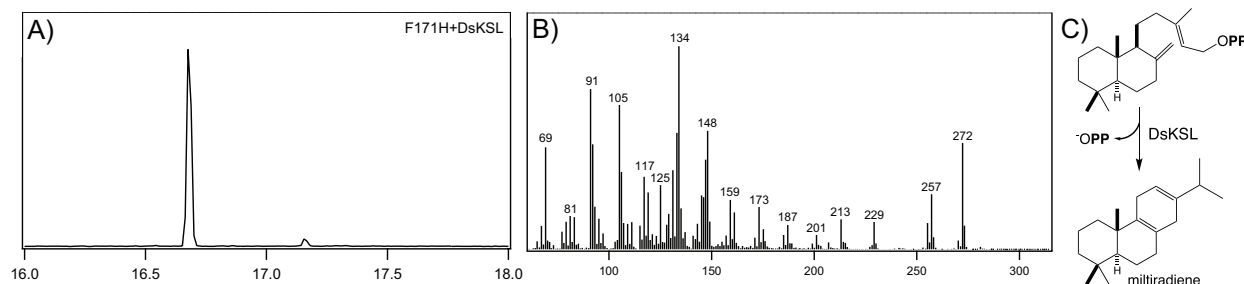


Figure S2. MtHPS:F171H produces normal CPP (**4**). A) Extracted ion ($m/z = 272$) chromatogram from GC-MS analysis of extract from *E. coli* engineered to produce **1** and expressing MtHPS:F171H and DsKSL, which is stereospecific for normal CPP. B) Mass spectra for resulting miltiradiene. C) Reaction catalyzed by DsKSL.

Tukey Trimean

The total energy distribution included outliers (Figure 1). As a result, the Turkey Trimean was calculated to estimate a better mean from -1250.9 ± 22.9 to -1263.4 ± 5.8 .

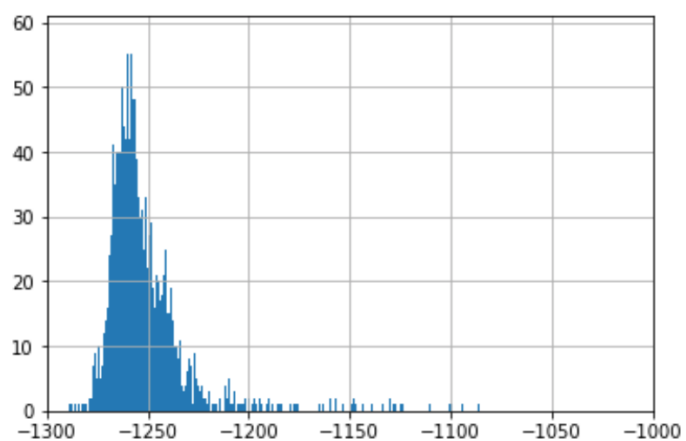


Figure S3. Total energy distribution of intermediate B.

Intermediate B coordinates

C	-0.04400	-0.08800	1.84700
C	0.40700	0.10800	-0.66400
C	2.32700	0.38600	1.06100
C	1.44300	-0.28000	2.12400
H	-0.32500	0.96600	1.95700
H	-0.61100	-0.62800	2.60700
H	1.66500	-1.35200	2.14900
H	1.71700	0.11900	3.10400
C	0.14200	-0.54700	-2.03800
C	-1.92000	-0.78800	0.17400
C	-2.08400	-1.42400	-1.22100
C	-1.35300	-0.67900	-2.33200
H	0.61800	0.02000	-2.84300
H	-1.47200	-1.21000	-3.28100
H	0.59300	-1.54800	-2.03500
H	-1.80100	0.30900	-2.48800
H	-1.71000	-2.45700	-1.17900
C	0.12300	1.67700	-0.76100
H	-0.26000	2.06700	0.17900
H	0.99400	2.27500	-1.05000
H	-0.61900	1.81400	-1.54800
C	-2.71500	0.52500	0.27100
H	-2.56400	1.01900	1.23600
H	-2.47300	1.24200	-0.51700
H	-3.78400	0.30900	0.18600

C	-2.51300	-1.77100	1.20100
H	-3.52200	-2.06100	0.89100
H	-1.91600	-2.68600	1.27900
H	-2.60200	-1.32900	2.19700
C	2.56000	1.90100	1.36100
H	3.13900	1.96500	2.28300
H	3.11700	2.40900	0.57000
H	1.61000	2.41600	1.51200
C	-0.39700	-0.62600	0.45900
C	1.83300	0.24700	-0.33200
H	-0.00600	-1.65600	0.42400
H	3.33000	-0.06300	1.06200
C	2.85800	0.27400	-1.39100
H	2.52000	0.71500	-2.32800
H	3.79900	0.71600	-1.06000
H	-3.15100	-1.49300	-1.45900
C	3.12500	-1.21300	-1.69200
H	2.35700	-1.63300	-2.34000
H	3.18900	-1.81500	-0.78600
C	4.47600	-1.30200	-2.42600
C	5.05100	-2.50900	-2.65100
C	5.17600	-0.01700	-2.90500
H	4.71700	-3.36800	-2.10800
C	6.18500	-2.64600	-3.68400
H	4.53000	0.51100	-3.57600
H	5.39900	0.60300	-2.06300
H	6.08300	-0.27300	-3.41100
H	7.05900	-2.14500	-3.32400
H	5.87800	-2.20700	-4.61000
F	6.46300	-3.95300	-3.87600