Supporting Information

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SI Methods

Dehydrosqualene Synthase (CrtM) Expression, Purification, Inhibition and Site-Directed Mutagenesis. CrtM with a histidine tag was overexpressed in *Escherichia coli* BL21(DE3) cells. A 50-mL overnight culture was transferred into 1L LB medium supplemented with 100 μg/mL ampicillin. Induction was carried out with 1 mM IPTG for 4 h at 37 °C, when the cell culture reached an OD of 0.6 at 600 nm. The cell extract was loaded onto a Ni-nitrilotriacetate (Ni-NTA) column, and CrtM eluted by using a 100-mL linear gradient of 0–0.5 M imidazole in 50 mM Tris • HCl buffer, pH 7.4. CrtM inhibition assays were carried out by using 96-well plates with 200 μL reaction mixture in each well. The condensation of farnesyl diphosphate (FPP) was monitored by using a continuous spectrophotometric assay for phosphate releasing enzymes (1). The reaction buffer contained 25 mM Hepes, 0.5 mM $MgCl_2$, 250 μM FPP, pH 7.4. The compounds investigated (BPH-652, BPH-702) were preincubated with 2 μg CrtM for 30 min at 25 °C. The IC_{50} values were obtained by fitting the inhibition data to a normal dose-response curve in Origin 6.1 (OriginLab Corp.). K_i was calculated based on the IC_{50} value, and the reported kinetic constant of CrtM (2). IC_{50} values for BPH-651, BPH-673 in the presence of diphosphate were determined by using a radioactive assay, quantifying ^{3}H-labeled dehydrosqualene. In every 200-μL reaction, 2 μg CrtM and inhibitor were preincubated in reaction buffer plus 25 μM PPi for 30 min, then 25 μM FPP and 25 nM ^{3}H-FPP were added. After 30 min,, the reaction was quenched by EDTA and NaCl, ^{3}H-dehydrosqualene was extracted using hexane, and quantified using a scintillation counter. CrtM mutants were prepared by using the QuikChange site-directed mutagenesis kit (Stratagene) in conjunction with the *Staphylococcus aureus* CrtM gene template in the pET32Xa/LIC vector. The primers for performing site-directed mutagenesis were as follows: 5′-GTCATAAAATCATGAAGAAACATGCAAAAAGCTTTTCT-TACGC-3′ for S19A; 5′-CAAAAAGCTTTTCTTACGCTGCT-GACTTGTTACCAGAA-GATC-3′ for F26A; 5′-AAAGCGGTT-TGGGCAATTTTTGCTGTGTGTCGTAAAAT-3′ for Y41F; 5′-GCAATTTATGCTGTGTGTGCGAAAATTGATGAC-AGTAT-3′ for R45A; 5′-GCTGTGTGTCGTAAAATTGCTGACAGTAT-AGATGTT-TATGGCG-3′ for D48A; 5′-CGTAAAATTGATGA-CAGTATAGCTGTTTATGGCG-ATATTCAATT-3′ for D52A; 5′- CGGACGCTGAATTATTCGGAGCTTGTTATGGT-GTT-GCTGG -3′, for Y129A; 5′-AGGCTTGGTGAATCGTTGCTGT-TGATTAATAT-ATTAAG-3′ for Q165L; 5′-GCAATTGATTAA-TATATTAAGAGCTGTCGGTGAAG-ATTTTGAAA -3′ for D172A; 5′- ATTAAGAGATGTCGGTGAAGCTTTTGAAAAT-GAACGGAT-ATATT-3′ for D176A; 5′-GAATTAGCAGCACG-TATAGCTATTGAA-ATACTGGAC GAAG-3′ for 248A; and 5′-GCTAACTATACATTACATGAAGCGGTTTT-TGTGGAGAA-GAGGAA-3′ for R265A. Mutations were confirmed by sequencing and were expressed in the same way as for the wild-type protein.

Truncated Human Squalene Synthase (hSQS) Expression and Purification. The truncated hSQS (31–370) plasmid was the gift of Dolores González-Pacanowska (Instituto de Parasitología y Biomedicina López-Neyra, Spain). The pET28a-hSQS was transformed into BL21 (DE3) CodonPlus RP cells (Novagen). Transformed cells were inoculated in LB broth (Lennox) with 30 μg/mL kanamycin and 34 μg/mL chloramphenicol at 37 °C and incubated at 200 rpm overnight. Five milliliters of cells were then innoculated into 1L of LB broth with 30 μg/mL kanamycin and 34 μg/mL chloramphenicol at 37 °C and 120 rpm and were induced using 0.1 mM IPTG when the OD_{600} reached 0.6 ∼ 0.8. Induced cells were incubated at 24 °C overnight, then were harvested and frozen at −80 °C. For purification, frozen cells were thawed and resuspended with loading buffer [500 mM NaCl, 10 mM CHAPS, 2 mM $MgCl_2$, 10% glycerol, 10 mM β-mercaptoethanol, 10 mM imidazole, Complete Protease Inhibitor Cocktail tablet (Roche), benzonase nuclease, and 20 mM phosphate buffer, pH 7.4]. Resuspended cells were lysed by sonication, then centrifuged at $57{,}000 \times g$ for 30 min. After centrifugation, supernatants were loaded onto an Ni-NTA column (GE Healthcare). The Ni-NTA column was equilibrated with wash buffer A (500 mM NaCl, 10 mM CHAPS, 2 mM $MgCl_2$, 10% glycerol, 10 mM β-mercaptoethanol, 10 mM imidazole, and 20 mM phosphate buffer, pH 7.4). Impurity proteins were eluted by using 10% elution buffer A (500 mM NaCl, 10 mM CHAPS, 2 mM $MgCl_2$, 10% glycerol, 10 mM β-mercaptoethanol, 500 mM imidazole, and 20 mM phosphate buffer, pH 7.4), then the truncated $(His)_6$-hSQS eluted by using 100% elution buffer. The $(His)_6$-hSQS was then dialyzed against wash buffer B (150 mM NaCl, 10 mM β-mercapto-ethanol, 2 mM $MgCl_2$, 20 mM Tris, pH 7.4). After removal of the N-terminal poly-His tag by thrombin (Novagen), mature truncated protein was eluted using 100% elution buffer B (150 mM NaCl, 10 mM β-mercaptoethanol, 2 mM $MgCl_2$, 60 mM imidazole, 20 mM Tris, pH 7.4). Fractions containing pure truncated hSQS were identified by SDS-PAGE, after which the protein was dialyzed against storage buffer (0.1 mM EDTA, 1 mM DTT, and 20 mM Tris, pH 7.3) and stored at −80 °C.

X-Ray Crystallography. Wild-type and mutant CrtMs were eluted from Ni-NTA beads by incubation with Factor Xa (Novagen) to cleave it from the polyhistidine-containing N-terminal thioredoxin fusion tag. The cleaved product was equilibrated with buffer containing 150 mM NaCl, 5 mM DTT, 1 mM β-mercaptoethanol, 5% glycerol, and 20 mM Tris, pH 7.5, then concentrated to 15 mg/mL. One set of presqualene diphosphate (PSPP)/Y129A CrtM crystals were obtained by using 100 mM potassium sodium tartrate, 30% PEG 3,350, 1 mM PSPP, and 2 mM $MgCl_2$. A second set of PSPP/Y129A as well as S-thiolo-geranylgeranyl diphosphate (GGSPP)/F26A CrtM crystals were obtained by cocrystallization in the presence of 0.5 mM PSPP or GGSPP, with 11–21% PEG4K, 0.3–0.4 M $MgCl_2$, and 0.1 M Tris, pH 8.5. Cocrystallization of CrtM-DHS (dehydrosqualene) was achieved by incubating 1 mM PSPP + 5 mM $MnCl_2$ in room temperature for ∼3 h, then adding the crystallization solution (0.1 M potassium sodium tartrate, 30% PEG 3,350). Native (WT) CrtM crystals (space group $P3_221$) were grown by using the hanging-drop method by mixing equal amounts of reservoir with 0.12–0.58 M potassium sodium tartrate at room temperature. Inhibitor bound crystals (BPH-651, BPH-673, and BPH-702) were obtained by soaking with 0.05 mM ligand. All CrtM crystals (with the exception of GGSPP/F26A CrtM), belonged to the $P3_221$ space group and had similar lattice parameters. The BPH-652–bound hSQS cocrystal was obtained by using the hanging-drop method by mixing equal amount of protein and reservoir (containing 20% PEG 6,000, 0.2 M sodium tartrate dibasic, pH 7.3, and 0.5 mM BPH-652). Crystals appeared after three days. The data were indexed, integrated, and scaled by using the HKL2000 package (3) Structures were determined by using molecular replacement with the program Phaser (4). For CrtM, 2ZCP (minus ligands) was used as a starting model, whereas for hSQS, 1EZF (minus ligands) was used as template. Further model building and refinement employed Coot (5), Refmac (6, 7), CNS (8), and ProDRG server (9). Figures were obtained using PyMol (10).

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Residue#	Rank	1st half (%)	2nd half (%)	Score
Y129	1	0	6.66	0.997
Y41	2	12.2	10.5	0.979
Q165	3	0	0	0.976
D52	4	0	0	0.969
D176	5	0	7.23	0.965
R171	6	n/a	n/a	0.955
D48	7	0	0	0.948
R181	8	n/a	n/a	0.944
R45	9	30	0	0.941
D172	10	0	4.77	0.934
N168	11	n/a	n/a	0.930
D114	12	n/a	n/a	0.920
Y183	13	n/a	n/a	0.916
R265	14	78.7	227.45	0.843
Y248	15	18.8	27.4	0.798
S19	16	52.9	101.8	0.787

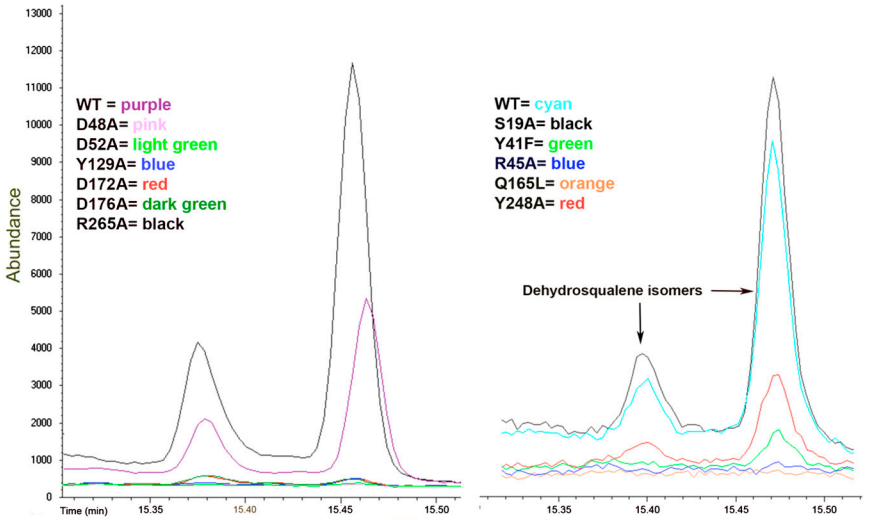


Fig. S1. Effects of site-directed mutagenesis on CrtM catalytic activity. The results in the table at the top shown CrtM residues rank-ordered by SCORECONS (11) conservation score together with the effects that mutations to Ala have on first- as well as second-half activity. Traces at bottom are GC/MS traces showing how mutations affect DHS formation. The two DHS isomer peaks shown below are the extracted ion chromatogram of $m/z = 408$ (C30H48+). The quantification of DHS is done by peak integration, using squalene (10 ug/mL, $m/z = 410$, C30H50+) as an internal standard.

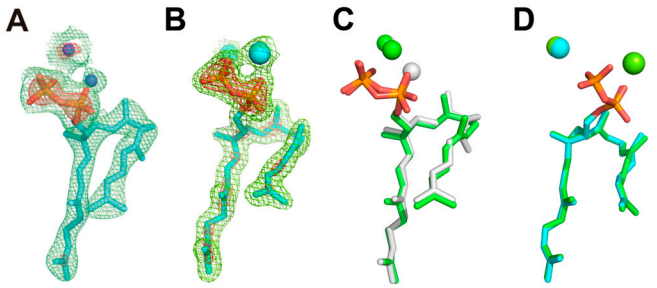
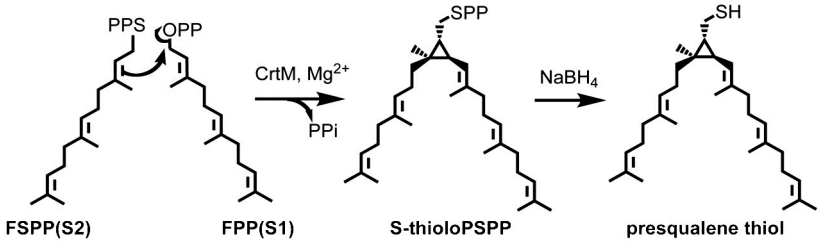
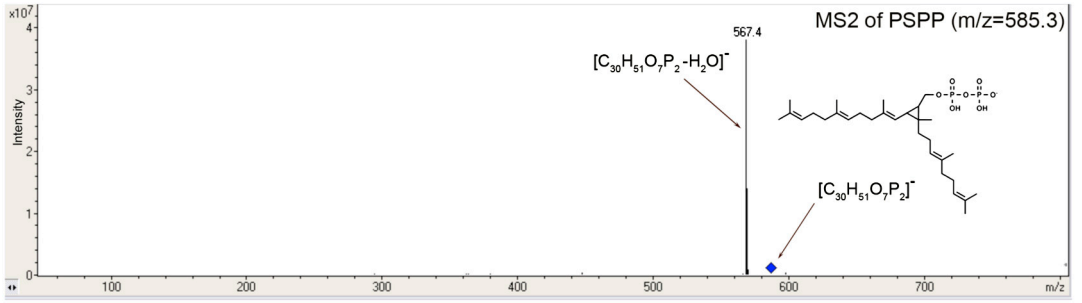


Fig. S2. Ligand electron densities of CrtM (Y129A)/PSPP (3LGZ), CrtM (WT)/PSPP (3NPR), and superposition of the three CrtM/PSPP structures. (*A*) The 2Fo-Fc map of CrtM (Y129A) /PSPP (PDB ID code 3LGZ). (*B*) The 2Fo-Fc map of CrtM (WT)/PSPP (PDB ID code 3NPR). (*C* and *D*) Superpositions: PDB ID code 3ADZ in green, 3LGZ in gray, 3NPR in cyan. The main difference between 3LGZ and 3ADZ is in the location of the second Mg^{2+} and likely arises due to the large difference in [Mg^{2+}] in the crystallization buffers (2 mM, 0.3–0.4 M), whereas 3NPR and 3ADZ are virtually identical.

Selected Isotopes : C H $O_{0-6}S_{0-2}$			Error Limit : 5 mmu		Unsaturation Limits : -0.5 to 30
Measured Mass	% Base	Formula	Calculated Mass	Error	Unsaturation
442.36363	4.10%	$C_{33}H_{46}$	442.35995	3.7	11.0
		$C_{26}H_{50}O_5$	442.36582	-2.2	2.0
		$C_{30}H_{50}S^a$	442.36332	0.3	6.0
		$C_{27}H_{54}S_2$	442.3667	-3.1	1.0

[a]The product is presqualene thiol (having six double bond equivalents) and is produced as follows:





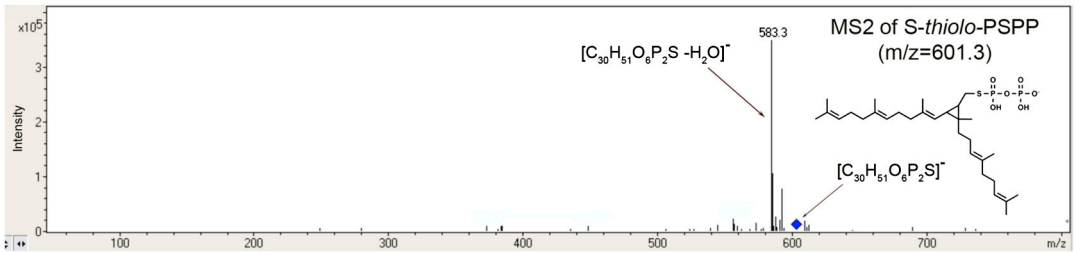


Fig. S3. High-resolution mass spectrometry results for S-thiolo-farnesyl diphosphate/FPP/CrtM reaction product (after purification and $NaBH_4$ reduction) and MS/MS spectra of S-thiolo-PSPP.

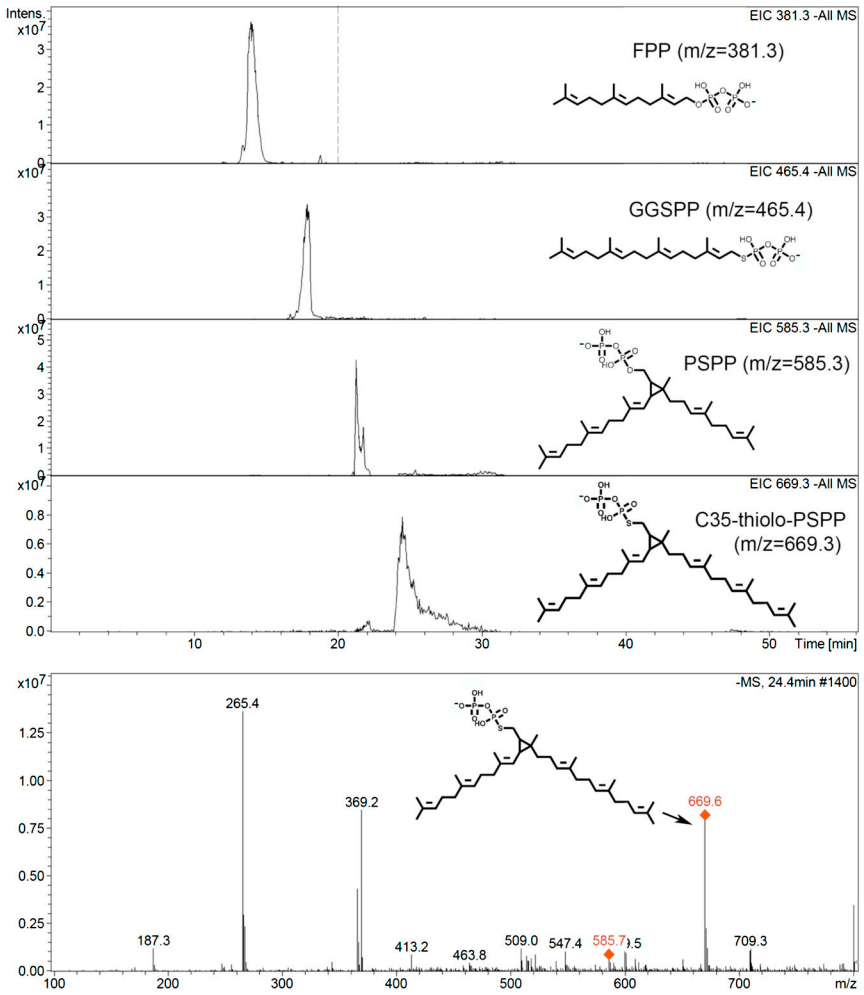


Fig. S4. Liquid chromatography/MS results for the FPP + GGSPP CrtM catalyzed reaction. The top four panels show the extracted ion chromatograms (EIC) for FPP, GGSPP, PSPP, and C35-*thiolo*-PSPP. The mass spectrum of the C35-*thiolo*-PSPP product (at 24.4 min is shown in the bottom panel.

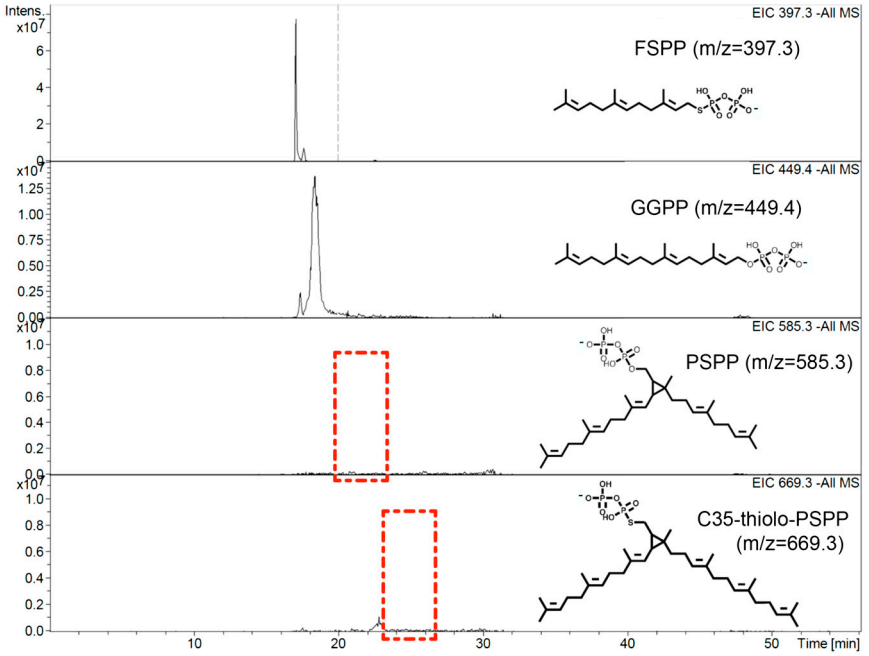


Fig. S5. Liquid chromatography/MS results for FSPP + GGPP (geranylgeranyl diphosphate) CrtM catalyzed reaction. Top two panels show the extracted ion chromatograms (EIC) of the substrates (FSPP, GGPP). PSPP and C35-*thiolo*-PSPP products were not detectable (third and fourth panels).

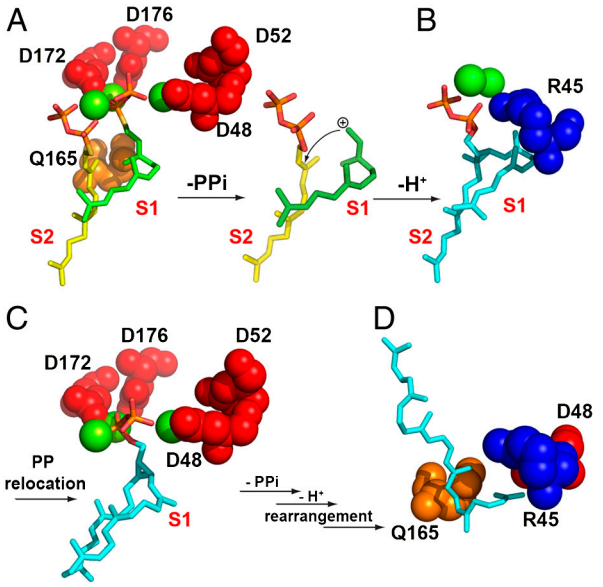


Fig. S6. Schematic illustration of spatial relationships between essential amino acids and FSPP, PSPP, and DHS product. (*A*) FSPP, (*B*) PSPP, (*C*) PSPP with PPi relocated to Mg^{2+}_3 cluster, (*D*) DHS product. *A*, *B*, and *D* from crystal structures; *C* from Glide docking.

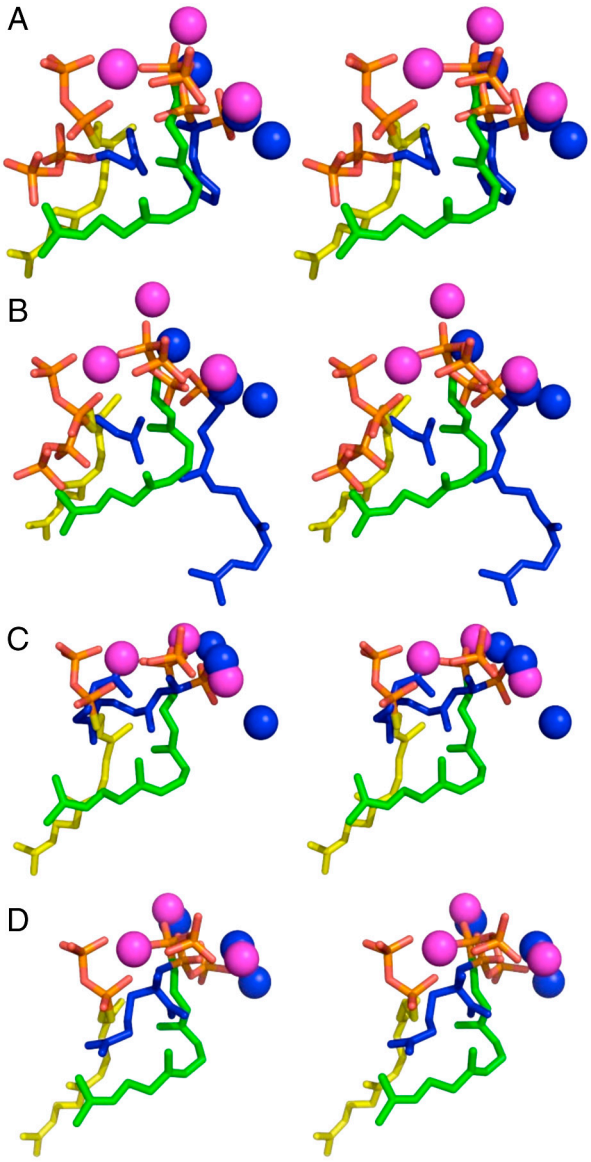


Fig. S7. Stereo views of the alignments of CrtM with various terpene synthases and cyclases, highlighting the location of the S1 (cationic, prenyl donor) and S2 (olefinic, prenyl acceptor) sites. (*A*) CrtM aligned to farnesyl diphosphate synthase (PDB ID code 2F8Z) containing a bisphosphonate inhibitor (zoledronate) and isopentenyl diphosphate (IPP). (*B*) Alignment of CrtM with human geranylgeranyl diphosphate synthase (PDB ID code 2Q80) containing geranylgeranyl diphosphate (GGPP) and IPP. (*C*) Alignment of CrtM (PDB ID code 2ZCP) with epiaristolochene synthase (PDB ID code 5EAU) from *Nicotiana tabacum* containing a substrate analog (trifluorofarnesyl diphosphate). (*D*) Alignment of CrtM with limonene synthase (PDB ID code 2ONG) from *Mentha spicata* containing dimethylhexyl trihydrogen diphosphate.

Table S1. Data collection and refinement statistics for CrtM with PSPP, GGSPP, and DHS

Crystals	CrtM(Y129A)/PSPP (3LGZ)	CrtM(Y129A)/PSPP (3ADZ)	CrtM(WT)/PSPP (3NPR)	CrtM (F26A)/GGSPP (3AE0)	CrtM(WT)/DHS (3NRI)
			Data collection		
Radiation source	APS 23ID-D	NSRRC BL13B1	APS 23ID-D	PF BL5A	APS 23ID-D
Wavelength, Å	1.0782	1.00000	1.0782	1.00000	1.0782
Space group	$P3_221$	$P3_221$	$P3_221$	$P3_121$	$P3_221$
a, Å	80.84	80.69	80.57	80.12	80.59
b, Å	80.84	80.69	80.57	80.12	80.59
c, Å	91.26	90.79	91.67	183.62	90.36
Resolution,* Å	30-2.40 (2.44-2.40)	30-1.89 (1.96-1.89)	30-2.00 (2.05-2.00)	30-2.37 (2.45-2.37)	30-2.85 (2.85-2.92)
No. of reflections	13,306 (685)	27,821 (2,720)	23,228 (917)	28,335 (2,697)	8,230 (224)
Completeness, %	96.3 (99.0)	99.9 (100)	97.06 (79.49)	99.1 (97.5)	98.9 (88.8)
Redundancy	3.6 (3.7)	7.3 (7.5)	4.1 (2.3)	6.2 (5.7)	10.6 (5.8)
R_{merge}, %	5.5 (34.2)	3.1 (32.3)	7.0 (45.5)	5.6 (58.1)	11.0 (44.4)
I/s(I)	31.8 (4.3)	49.4 (5.2)	37.4 (2.0)	32.5 (3.1)	23.8 (2.4)
			Refinement		
No. of reflections	12,637 (969)	26,389 (3,794)	21,778 (1,291)	26,857 (3,768)	7,776 (395)
R_{work}, %	20.3 (25.6)	16.8 (18.2)	19.76 (26.6)	21.9 (27.3)	20.7 (30.1)
R_{free}, %	27.8 (43.5)	22.2 (24.8)	26.85 (35.8)	28.1 (33.4)	30.7 (48.9)
			Geometry deviations		
Bond lengths, Å	0.018	0.007	0.006	0.007	0.004
Bond angles, °	1.66	1.4	1.2	1.5	0.5
			Mean B values (Å^2)/No.		
Protein atoms	42.33/2342	23.2/2385	39.21/2392	30.7/4772	48.9/2353
Compound atoms	54.45/39	28.2/39	31.9/39	67.7/116	64.0/30
Mg ions	56.51/2	32.8/2	42.5/2	56.3/6	—
Water molecules	46.28/68	33.3/263	45.0/203	30.8/70	—
			Ramachandran plot, %		
Most favored	95.5	95.2	96.8	93.0	94.9
Additionally allowed	4.5	4.8	2.8	7.0	4.0
Generously allowed	0	0	0.4	0	1.1

*Values in the parentheses are for the highest resolution shells.

Table S2. Reactivity of four isoprenoid diphosphates (FPP, FSPP, GGPP, GGSPP) bound to S1 or S2 in CrtM showing products formed

	FPP(S1)	FSPP(S1)	GGPP(S1)	GGSPP(S1)
FPP(S2)	PSPP			
FSPP(S2)	S-PSPP	NR		
GGPP(S2)	C35-PSPP	NR	NR [WT]; F26A shows activity in coupling assay	
GGSPP(S2)	C35-S-PSPP	NR	ND	ND

NR, no reaction; ND, not determined.

Table S3. Data collection and refinement statistics for CrtM and hSQS with inhibitors

Crystals	CrtM (WT)/ BPH-651 (3ACW)	CrtM/BPH-673 (3ACX)	hSQS/BPH-652 (3LEE)	CrtM/BPH-702 (3ACY)
		Data collection		
Radiation source	NSRRC BL13B1	NSRRCBL13B1	NSLS X29A	NSRRCBL13B1
Wavelength, Å	1.0000	0.9732	1.0809	0.9732
Space group	$P3_2$ 21	$P3_2$ 21	$P2_1$	$P3_2$ 21
a, Å	80.66	80.63	85.66	80.50
b, Å	80.66	80.63	153.42	80.50
c, Å	90.22	90.45	92.90	90.79
Resolution,* Å	30-1.63 (1.69-1.63)	30-1.31 (1.36-1.31)	30-3.20 (3.31-3.20)	30-1.84 (1.91-1.84)
No. of reflections	42,617 (4,208)	81,916 (8,101)	39,260 (3,898)	29,936 (2,955)
Completeness, %	99.5 (100)	99.9 (100)	100 (99.7)	99.7 (100)
Redundancy	3.4 (3.4)	9.5 (9.3)	7.3 (6.7)	4.6 (4.6)
R_{merge}, %	3.2 (48.7)	3.0 (22.2)	12 (44.9)	6.4 (48.6)
I/s(I)	35.4 (2.1)	66.3 (9.7)	16.6 (3.9)	29.1 (4.2)
		Refinement		
No. of reflections	40,455 (5,829)	77,731 (11,213)	37,261 (2,672)	28,389 (4,064)
R_{work}, %	17.4 (21.9)	16.0 (15.5)	20.6 (26.6)	16.7 (19.4)
R_{free} (%)	20.3 (28.2)	18.2 (19.5)	27.2 (37.0)	21.8 (28.8)
		Geometry deviations		
Bond lengths, Å	0.007	0.007	0.006	0.007
Bond angles, °	1.5	1.5	0.742	1.5
		Mean B-values (Å^2) / No.		
Protein atoms	22.5/2392	10.6/2392	68.89/15526	18.8/2392
Compound atoms	40.8/21	12.4/23	55.04/150	39.7/32
Mg ions	—	—	35.97/6	34.8/1
Water molecules	34.6/287	23.3/453	65.37/189	29.3/307
		Ramachandran plot (%)		
Most favored	95.9	95.6	91.1	95.6
Additionally allowed	3.7	4.1	8.6	4.4
Generously allowed	0.4	0.4	0.3	0

*Values in the parentheses are for the highest resolution shells.