

Supporting Information for

**Crystal Structure of (+)- δ -Cadinene Synthase from *Gossypium arboreum*
and Evolutionary Divergence of Metal Binding Motifs for Catalysis**

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Site-Directed mutagenesis of recombinant DCS cDNA

The Quickchange site-directed mutagenesis kit (Stratagene) was used to introduce the D307A, D308A, and D311A mutations (GAT to GCG) according to the manufacturer instructions. The mutagenic primers were as follows: for D307A, 5'-CAATGGCATCCATTGTAGCGGATACATATGACTCATATG-3' and 5'-CATATGAGTCATATGTATCCGCTACAATGGATGCCATTG-3'; for D308A, 5'-CAATGGCATCCATTGTAGATGCGACATATGACTCATATGCAAC-3' and 5'-GTTGCATATGAGTCATATGTTCGCATCTACAATGGATGCCATTG-3'; for D311A, 5'-CCATTGTAGATGATACATATGCCTCATATGCAACATATGAAGAGC-3' and 5'-GCTCTTCATATGTTGCATATGAGGCATATGTATCATCTACAATGG-3'; for D451A, 5'-CAATTATTTGTAGGTTTATGGCGGATGTTGCTGAACACAAGTTC-3' and 5'-GAACTTGTGTTTCAGCAACATCCGCCATAAACCTACAAATAATTG-3'; for D452A, 5'-GTAGGTTTATGGATGCGGTTGCTGAACACAAG-3' and 5'-CTTGTGTTTCAGCAACCGCATCCATAAACCTAC-3'; and for E455A, 5'-GTTTATGGATGATGTTGCTGCGCACAAGTTCAAGCATAGGAG-3' and 5'-CTCCTATGCTTGAACCTGTGCGCAGCAACATCATCCATAAAC-3'. Plasmids were purified from overnight LB/ampicillin cultures (5 mL) using the QIAGEN miniprep kit as described by the manufacturer. Mutations were confirmed by DNA sequence analysis using Walesbiogrid facilities (School of Bioscience, Cardiff University, UK).

Synthesis of 10F-FPP

Experimental details

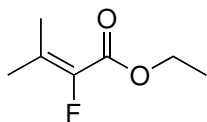
All chemicals were purchased from Sigma-Aldrich (UK) unless otherwise stated. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under nitrogen. Dichloromethane and triethylamine were distilled from calcium hydride under nitrogen. All other chemicals were of analar quality or better and used as received unless otherwise stated. Reactions were stirred at room temperature in air unless otherwise stated. All glassware was clean and dry before use.

Flash chromatography was performed by the method of Still.¹

¹H NMR spectra were measured on a Bruker Avance 500 NMR spectrometer or a Bruker Avance DPX400 NMR spectrometer and are reported as chemical shifts in parts per million downfield from tetramethylsilane, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (to the nearest 0.5 Hz) and assignment respectively. ¹³C NMR spectra were measured on a Bruker Avance 500 NMR spectrometer and are reported as chemical shift downfield from tetramethylsilane, coupling constant where appropriate and assignment. Assignments are made to the limitations of COSY, DEPT 90/135, gradient HSQC and gradient HMBC spectra. ¹⁹F and ³¹P NMR spectra were recorded on a Jeol Eclipse +300 NMR spectrometer and are reported in chemical shift downfield from CFCl₃ and 85% H₃PO₄ respectively followed by multiplicity and coupling constant (to the nearest 0.5 Hz) if appropriate. IR spectra were recorded on a Perkin ELMER 1600 series FTIR spectrometer and samples were prepared as thin films of neat liquid on sodium chloride discs for oils and as KBr disks for solids. EI⁺ mass spectra were measured on a Micromass LCT premiere XE mass spectrometer ES⁻ mass spectra were provided by the UK EPSRC mass spectrometry service, Swansea UK.

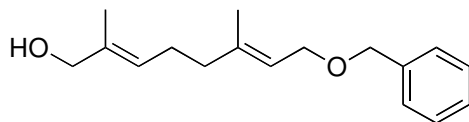
Reverse phase HPLC was performed on a system comprising of a Dionex P680 pump and a Dionex UVD170U detector unit.

Ethyl 2-fluoro-3-methylbut-2-enoate (1)



To a stirred solution of triethyl-2-fluoro-2-phosphonoacetate (4.20 cm³, 20.6 mmol) in anhydrous THF (40 cm³) was added a suspension of NaH (0.55 g, 22.7 mmol) in THF (5 cm³) was added at 0 °C under an argon atmosphere. After stirring of 30 min at RT, a mixture of acetone (1.82 cm³, 24.7 mmol) was added, and then the whole reaction mixture was stirred overnight. After quenching with cold saturated NH₄Cl solution (30 cm³), the result mixture was extracted with diethyl ether (3 x 30 cm³), and the organic phases were combined, washed with brine (30 cm³) and dried (MgSO₄), filtered and then concentrated under reduced pressure to give the title compound as a pale yellow oil, Purification of the crude product by flash column chromatography on silica gel with hexane and ethyl acetate (30 : 1) as eluent gave **1** as a colorless oil (1.34 g, 47%); TLC R_f 0.49 (Hexane : EtOAc = 9 : 1); ν_{\max} (thin film)/cm⁻¹ 2986.0, 2926.6, 1724.5, 1617.3, 1448.8, 1373.1, 1299.5, 1236.6, 1152.7, 1089.3, 1022.4, 934.0 and 864.6; δ_{H} (500 MHz, C²HCl₃) 1.35 (3 H, t, *J* 7.0, CH₂CH₃), 1.87 (3 H, d, *J*_{H-F} 4.0, CH₃C=CF), 1.61 (3 H, d, *J*_{H-F} 3.0, CH₃CH₂) and 4.27 (2 H, q, *J* 7.0 CH₂CH₃); δ_{C} (125 MHz, C²HCl₃) 14.17 (CH₂CH₃), 18.48 and 18.56 (2 x CH₃), 60.87 (CH₂CH₃), 129.49 (d, *J* 14.0, (CH₃)₂C=CF), 142.71 (d, *J* 244.0, (CH₃)₂C =CF) and 161.21 (d, *J* 34.0, C=O); d_{F} (283 MHz, C²HCl₃) -128.06; *m/z* (CI⁺) 164.1 (100%, [M + NH₄]⁺).

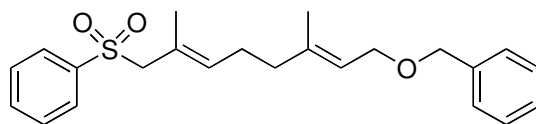
(2E,6E)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dien-1-ol (2)



To a stirred solution of benzyl geraniol (5.70 g, 23.4 mmol) in CH₂Cl₂ (40 cm³) was added selenium dioxide (0.26 g, 2.30 mmol), tert-butyl hydroperoxide (70% in H₂O, 55.6 cm³, 84.2 mmol) and acetic acid (0.13 cm³, 2.30 mmol) under an argon atmosphere. The whole reaction mixture was then stirred overnight at room temperature. Water (30 cm³)

was added and the resulting mixture was extracted with diethyl ether (3 x 30 cm³) and the organic phases were combined, washed with 10% aqueous potassium hydroxide (30 cm³), brine (30 cm³) and dried (MgSO₄), filtered and then concentrated under reduced pressure to give the title compound as a pale yellow oil. Purification of the crude product by flash column chromatography on silica gel with hexane and ethyl acetate (2 : 1) as eluent gave the title compound as a colorless oil (1.95 g, 32%); TLC R_f 0.26 (Hexane : EtOAc = 2 : 1); ν_{max} (thin film)/cm⁻¹ 3391.9, 2919.0, 2856.3, 1667.6, 1451.8, 1364.0, 1202.5, 1065.5, 1010.6, 736.0 and 697.8; δ_{H} (500 MHz, C²HCl₃) 1.67 (3 H, s, CH₃), 1.69 (3 H, s, CH₃), 2.11 (2 H, t, *J* 7.5, CHCH₂CH₂C), 2.19 (2 H, q, *J* 7.5, CHCH₂CH₂C), 4.00 (2 H, s, CH₂OH), 4.04 (2 H, d, *J* 7.0, CH₂OBn), 4.53 (2 H, s, OCH₂Ph), 5.40 (2 H, m, 2 x C=CHCH₂) and 7.29 (5 H, m, Ar-H); δ_{C} (125 MHz, C²HCl₃) 13.71 (CH₃), 16.46 (CH₃), 25.81 and 39.15 (CH₂CH₂), 66.57 (CHCH₂O), 68.95 (CH₂OH), 72.11 (OCH₂Ph), 121.17 and 125.63 (2 x C=CHCH₂), 127.57, 127.84 and 128.37 (Ar-CH) and 135.15, 138.52 and 139.94 (quaternary C); *m/z* (CI⁺) 278.3 (75%, [M + NH₄]⁺) and 135.0 (100).

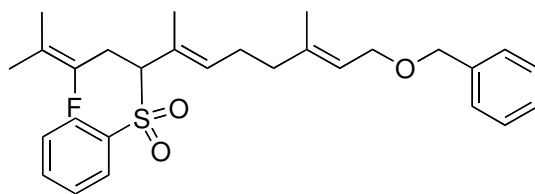
1-[(2*E*,6*E*)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dienylsulfonyl]benzene (3)



A stirred solution of alcohol **2** (1.60 g, 6.15 mmol) and triethylamine (1.71 cm³, 12.3 mmol) in anhydrous THF (40 cm³) was cooled to -45 °C and then MsCl (0.62 cm³, 8.01 mmol) was added. The resulting milky mixture was stirred at -45 °C for 45 min and then a solution of LiBr (2.14 g, 24.6 mmol) in THF (5 cm³) was added via a cannula at -45 °C. The suspension was allowed to warm to 0 °C and stirred for addition 1 h. Cold water (40 cm³) and hexane (40 cm³) were added. The two layers were separated, and the aqueous layer was extracted with hexane (2 x 30 cm³). The pooled organic layers were washed with saturated NaHCO₃ solution (30 cm³) and saturated NaCl solution (30 cm³) and then dried over anhydrous Na₂SO₄. Concentration of the solvent under reduced pressure gave the required allylic bromide as a light yellow oil which was used without further purification. The crude bromide and benzenesulfinic acid sodium salt (1.22 g,

7.38 mmol) were dissolved in anhydrous DMF (30 cm³). The mixture was stirred for 20 h and then hydrolyzed with water (30 cm³). The aqueous layer was extracted with diethyl ether (3 x 30 cm³), and the organic phases were combined, washed with water (30 cm³) and brine (30 cm³), dried (MgSO₄), filtered and then concentrated under reduced pressure to give the title compound as a pale yellow oil. Purification of the crude product by flash column chromatography on silica gel with hexane and ethyl acetate (2 : 1) as eluent gave the title compound as a colorless oil (1.91 g, 82%); TLC R_f 0.31 (Hexane : EtOAc = 2 : 1); HRMS (ES⁺, [M + NH₄]⁺) found 402.2097. C₂₃H₃₂O₃NS requires 402.2097; ν_{\max} (thin film)/cm⁻¹ 3061.7, 2918.5, 2855.5, 1666.7, 1585.9, 1448.9, 1386.4, 1310.5, 1255.3, 1134.0, 1086.9, 1026.2, 876.5, 740.8, 691.4 and 616.2; δ_{H} (500 MHz, C²HCl₃) 1.61 (3 H, s, CH₃), 1.78 (3 H, s, CH₃), 1.89 (2 H, t, *J* 7.5, CHCH₂CH₂C), 2.08 (2 H, q, *J* 7.5, CHCH₂CH₂C), 3.73 (2 H, s, CH₂SO₂), 4.01 (2 H, d, *J* 6.5, CHCH₂O), 4.52 (2 H, s, OCH₂Ph), 5.06 (1 H, t, *J* 7.0, C=CHCH₂CH₂), 5.32 (1 H, dt, *J* 6.5 and 1.0, C=CHCH₂O) and 7.29–7.86 (10 H, m, 2 x Ar-H); δ_{C} (125 MHz, C²HCl₃) 16.42 (CH₃), 16.71 (CH₃), 26.57 and 38.48 (CHCH₂CH₂C), 66.22 (CH₂SO₂), 66.54 (CHCH₂O), 72.20 (OCH₂Ph), 121.31 (CHCH₂O), 127.60, 127.81, 128.38, 128.52, 128.90 and 133.51 (Ar-CH), 135.64 (C=CHCH₂CH₂) and 123.61, 138.47, 138.49 and 139.35 (quaternary C); *m/z* (CI⁺) 402.3 (40%, [M + NH₄]⁺) and 135.1 (100).

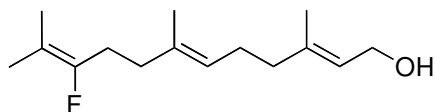
1-[(6*E*,10*E*)-12-(benzyloxy)-3-fluoro-2,6,10-trimethyldodeca-2,6,10-trien-5-ylsulfonyl]benzene (5)



To the mixture of sulfone **3** (1.57 g, 4.11 mmol) and bromide **4** (derived from compound **1** using standard literature methodology, 0.601 g, 3.60 mmol) in anhydrous THF (25 cm³) at -78 °C was added *n*-BuLi (2.2 M, 1.87 cm³, 4.11 mmol) dropwise over 15 min, and the mixture stirred for 2 h at -78 °C. The cooling bath was then removed and the

reaction mixture was allowed to warm slowly to 0 °C. Water (20 cm³) was added and the aqueous layer was extracted with diethyl ether (3 x 20 cm³), and the organic phases were combined, washed with water (20 cm³), brine (20 cm³) and dried (MgSO₄), filtered and then concentrated under reduced pressure to give the title compound as a pale yellow oil. Purification of the crude product by flash column chromatography on silica gel with hexane and ethyl acetate (4 : 1) as eluent gave the title compound as a light yellow oil (1.29 g, 76%); TLC R_f 0.29 (Hexane : EtOAc = 4 : 1); HRMS (ES⁺, [M + NH₄]⁺) found 488.2628. C₂₈H₃₉O₃NFS requires 488.2629; ν_{max} (thin film)/cm⁻¹ 2922.3, 2860.0, 1714.5, 1666.9, 1449.5, 1365.9, 1305.8, 1198.9, 1145.1, 1086.5, 737.7, 692.0 and 606.7; δ_H (500 MHz, C²HCl₃) 1.59 (9 H, m, 3 x CH₃), 1.69 (3 H, s, CH₃), 1.81 (2 H, t, J 7.5, CHCH₂CH₂C), 1.99 (2 H, m, CHCH₂CH₂C), 2.91 (2 H, m, CFCH₂), 3.79 (1 H, dd, J 11.0 and 4.0, CFCH₂CH), 4.00 (2 H, d, J 6.5, CHCH₂O), 4.52 (2 H, s, OCH₂Ph), 5.15 (1 H, t, J 7.5, C=CHCH₂CH₂), 5.31 (1 H, dt, J 6.5 and J 1.0, C=CHCH₂O) and 7.29–7.83 (10 H, m, 2 x Ar-H); δ_C (125 MHz, C²HCl₃) 13.79 (CH₃), 15.62 (d, J 9.0, CH₃), 16.40 (CH₃), 16.71 (d, J 12.5, CH₃), 25.38 (d, J_{C-F} 29.0, CFCH₂CH), 26.49 and 38.36 (CHCH₂CH₂C), 66.55 (CHCH₂O), 70.98 (CFCH₂CH), 72.23 (OCH₂Ph), 110.60 (d, J_{C-F} 17.5, C=CF), 121.21 (CHCH₂O), 127.58, 127.82, 128.38, 128.80, 128.81 and 133.51 (Ar-CH), 135.58 (C=CHCH₂CH₂), 126.36, 137.94, 138.50 and 139.46 (quaternary C) and 148.42 (d, J_{C-F} 240.0, C=CF); δ_F (283 MHz, C²HCl₃) –114.54 (dd, J_{F-H} 28.0); *m/z* (CI⁺) 488.3 (2%, [M + NH₄]⁺) and 94.1 (100).

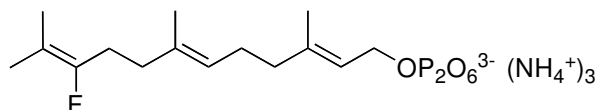
(2*E*,6*E*)-10-Fluoro-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (6)



Tert-butyl alcohol (2.0 cm³) and **5** (0.37 g, 0.79 mmol) were dissolved in anhydrous THF (10 cm³) were added via a cannula to finely chopped lithium metal (0.06 g, 8.57 mmol) with stirring. The reaction mixture was stirred under nitrogen atmosphere for 5 h, after which another portion of lithium (0.06 g, 8.57 mmol) was added to the reaction mixture together with tert-butyl alcohol (2.0 cm³) at 0 °C. The reaction mixture was then left to stir overnight. Cold water (10 cm³) was added and the result mixture was extracted with

diethyl ether (3 x 10 cm³), and the organic phases were combined, washed with brine (10 cm³) and dried (MgSO₄), filtered and then concentrated under reduced pressure to give the title compound as a pale yellow oil. Purification of the crude product by flash column chromatography on AgNO₃-impregnated silica gel (5% AgNO₃ on silica gel) with hexane and ethyl acetate (3 : 1) as eluent gave the title compound as a colorless oil (0.053 g, 28%); TLC R_f 0.29 (Hexane : EtOAc = 4 : 1); HRMS (EI⁺, [M - H₂O]⁺) found 222.1778. C₁₅H₂₃F requires 222.1784; ν_{max} (thin film)/cm⁻¹ 3333.7, 2932.4, 2862.2, 1714.5, 1667.9, 1445.9, 1383.6, 1301.9, 1258.2, 1152.4, 1102.7, 1005.5, 898.1, 798.4 and 737.6; δ_H (500 MHz, C²HCl₃) 1.44 (1 H, s, OH), 1.49 (3 H, d, J_{H-F} 2.5, CH₃), 1.55 (6 H, s, 2 x CH₃), 1.61 (3 H, s, CH₃), 1.96 (2 H, t, J_{H-H} 7.5, CHCH₂CH₂C), 2.02 (4 H, m, CHCH₂CH₂C and CFCH₂CH₂), 2.18 (2 H, dt, J_{H-F} 23.0, J_{H-H} 7.5, CFCH₂CH₂), 4.08 (2 H, d, J_{H-H} 7.0, CHCH₂OH), 5.06 (1 H, dt, J_{H-H} 7.0 and 1.0, C=CHCH₂CH₂) and 5.34 (1 H, tq, J_{H-H} 7.0 and 1.0, C=CHCH₂OH); δ_C (125 MHz, C²HCl₃) 15.45 (d, J_{C-F} 10.0, CH₃C=CF), 15.91 (CH₃), 16.26 (CH₃), 17.57 (d, J_{C-F} 6.5, CH₃C=CF), 26.32 (CHCH₂CH₂C), 27.51 (d, J_{C-F} 30.0, CFCH₂CH₂), 36.58 (CFCH₂CH₂), 39.44 (CHCH₂CH₂C), 59.38 (CHCH₂OH), 107.14 (d, J_{C-F} 17.5, C=CFCH₂CH₂), 123.40 (CHCH₂OH), 124.53 (C=CHCH₂CH₂), 134.41 and 139.67 (quaternary C) and 153.00 (d, J_{C-F} 240.0, C=CF); δ_F (283 MHz, C²HCl₃) -112.75 (t, J_{H-F} 23.0); m/z (EI⁺) 222.2 (5%, [M - H₂O]⁺) and 69.1 (100).

(2E,6E)-10-Fluoro-3,7,11-trimethyldodeca-2,6,10-trien-1-yl diphosphate tris ammonium salt (7)



This compound was prepared as previously described for 2F-FPP^{2,3} using the alcohol **6** (0.041 g, 0.171 mmol) to give the title compound as a white puffy solid (43 mg, 56%); HPLC *t*_R = 36.41 min; Purity 96.88 % by analytical RP HPLC detecting at 220 nm; HRMS (ES⁻, [M - H]) found 399.1124. C₁₅H₂₆O₇FP₂ requires 399.1138; ν_{max} (KBr disc)/cm⁻¹ 2818.0, 1716.4, 1670.0, 1456.4, 1201.1, 1120.7, 1090.0, 1025.5, 907.2, 804.7, 722.0, 596.3, 552.5 and 512.9; δ_H (500 MHz, ²H₂O at pH 8.5 buffered with N²H₄O²H) 1.41 (3 H, d, J_{H-F} 2.5,

$\text{CH}_3\text{C}=\text{CFCH}_2$), 1.43 (3 H, d, $J_{\text{H-F}}$ 3.0, $\text{CH}_3\text{C}=\text{CFCH}_2$), 1.48 (3 H, s, CH_3), 1.56 (3 H, s, CH_3),
 1.92 (2 H, t, $J_{\text{H-H}}$ 7.5, $\text{CHCH}_2\text{CH}_2\text{C}$), 1.98 (4 H, m, $\text{CHCH}_2\text{CH}_2\text{C}$ and CFCH_2CH_2), 2.20 (2 H,
 dt, $J_{\text{H-F}}$ 25.0, $J_{\text{H-H}}$ 7.0, CFCH_2CH_2), 4.31 (2 H, t, $J_{\text{H-H}}$ 6.5, CHCH_2O), 5.07 (1 H, t, $J_{\text{H-H}}$ 6.5,
 $\text{C}=\text{CHCH}_2\text{CH}_2$) and 5.31 (1 H, t, $J_{\text{H-H}}$ 7.0, $\text{C}=\text{CHCH}_2\text{O}$); d_{C} (125 MHz, $^2\text{H}_2\text{O}$ at pH 8.5
 buffered with $\text{N}^2\text{H}_4\text{O}^2$) 14.63 (d, $J_{\text{C-F}}$ 10.0, $\text{CH}_3\text{C}=\text{CFCH}_2$), 15.10 (CH_3), 15.64 (CH_3), 16.80
 (d, $J_{\text{C-F}}$ 6.5, $\text{CH}_3\text{C}=\text{CFCH}_2$), 25.74 ($\text{CHCH}_2\text{CH}_2\text{C}$), 26.52 (d, $J_{\text{C-F}}$ 29.0, CFCH_2CH_2), 35.75
 (CFCH_2CH_2), 38.79 ($\text{CHCH}_2\text{CH}_2\text{C}$), 62.51 (d, $J_{\text{C-P}}$ 5.0, CHCH_2O), 108.63 (d, $J_{\text{C-F}}$ 16.5,
 $\text{C}=\text{CFCH}_2\text{CH}_2$), 119.81 (d, $J_{\text{C-P}}$ 9.0, CHCH_2O), 125.04 ($\text{C}=\text{CHCH}_2\text{CH}_2$), 135.49 and 142.79
 (quaternary C) and 152.94 (d, $J_{\text{C-F}}$ 236.5, CFCH_2CH_2); d_{F} (283 MHz, $^2\text{H}_2\text{O}$ at pH 8.5
 buffered with $\text{N}^2\text{H}_4\text{O}^2\text{H}$) -113.98 (t, $J_{\text{H-F}}$ 25.0); d_{P} (122 MHz, $^2\text{H}_2\text{O}$ at pH 8.5 buffered with
 $\text{N}^2\text{H}_4\text{O}^2\text{H}$) -7.05 (d, $J_{\text{P-P}}$ 21.0) and -10.35 (d, $J_{\text{P-P}}$ 21.0); m/z (ES^-) 399.1 (100%, $[\text{M} - \text{H}]^-$).

GC-MS

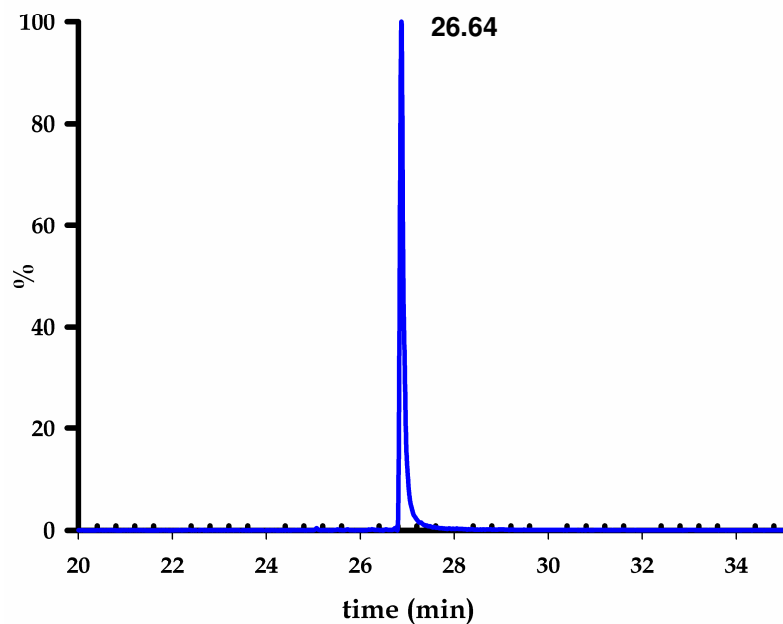


Figure S1. Total ion chromatogram of pentane extractable products from incubation of FPP with wild type DCS. (δ -cadinene retention time 26.6 min).

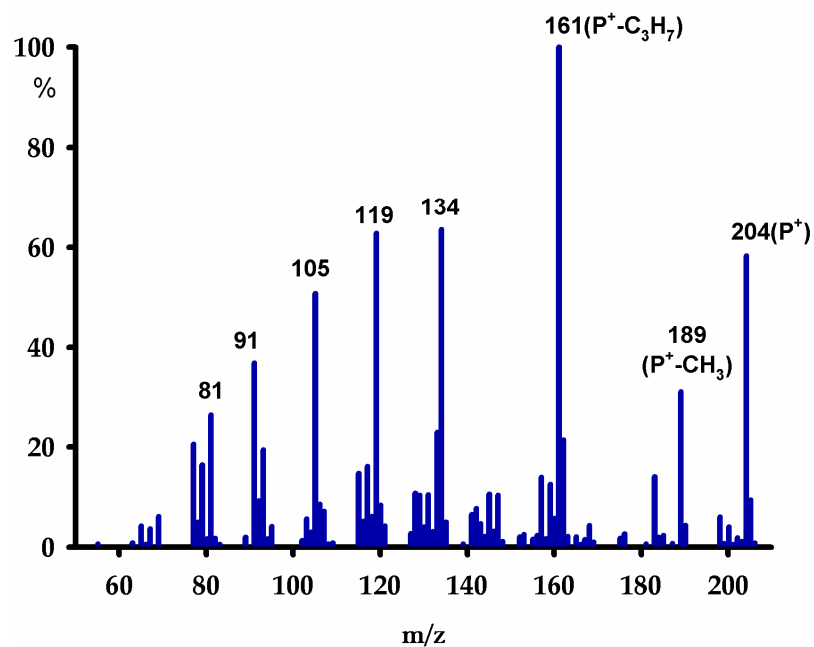


Figure S2. Mass spectrum of compound eluting at 26.6 min from incubation of FPP with WT DCS (δ -cadinene).

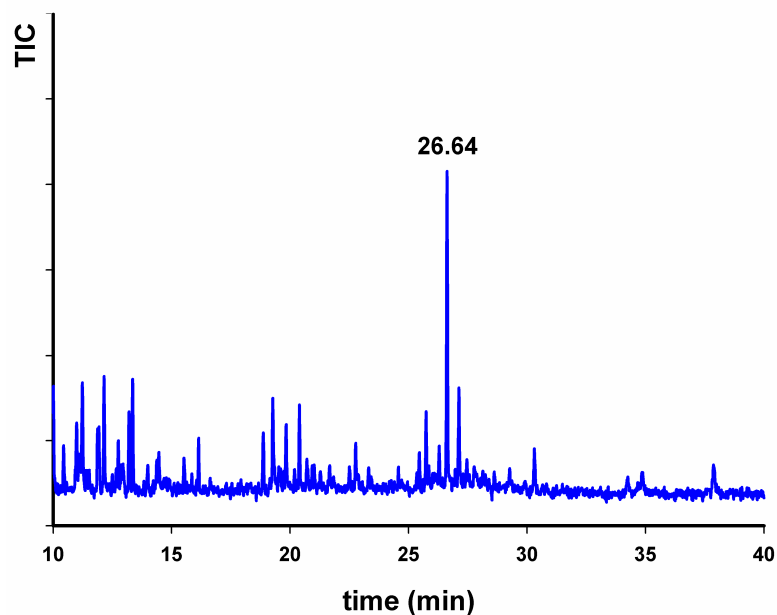


Figure S3. Total ion chromatogram of the pentane extractable product formed from incubation of FPP with D307A DCS. (δ -cadinene retention time 26.6 min).

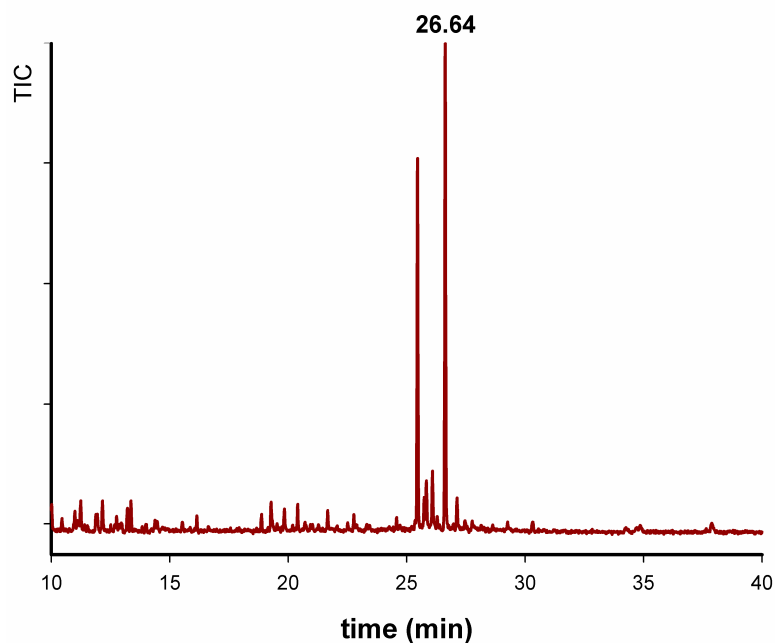


Figure S4. Total ion chromatogram of the pentane extractable product formed from incubation of FPP with D308A DCS (δ -cadinene retention time 26.6 min). Note that the peak at 25.4 min does not have m/z 204 and so is not a turnover product but a contaminant.

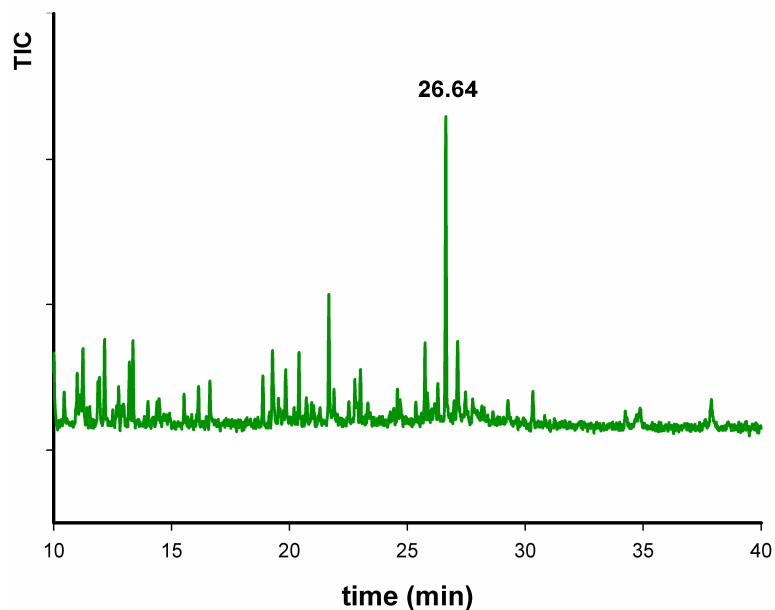


Figure S5. Total ion chromatogram of the pentane extractable products formed from incubation of FPP with D311A DCS (δ -cadinene retention time 26.6 min).

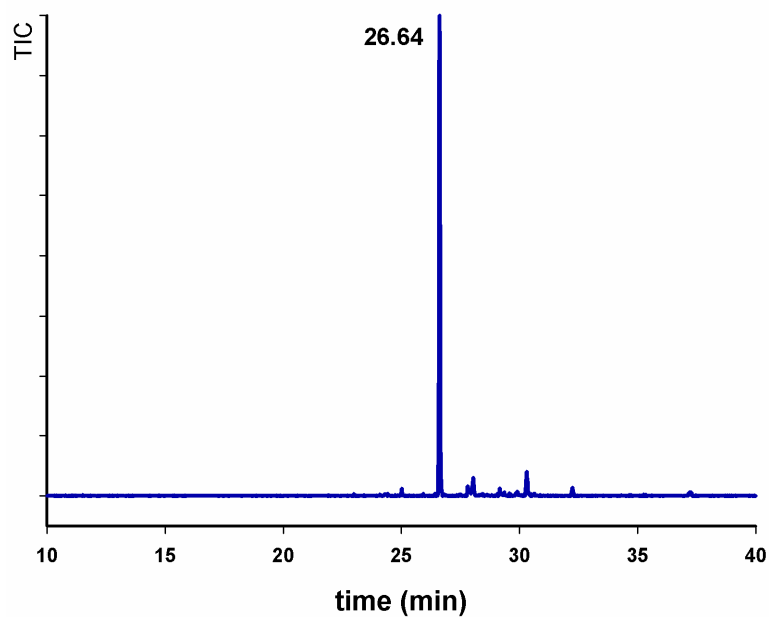


Figure S6. Total ion chromatogram of the pentane extractable products formed from incubation of FPP with D451A DCS (δ -cadinene retention time 26.6 min).

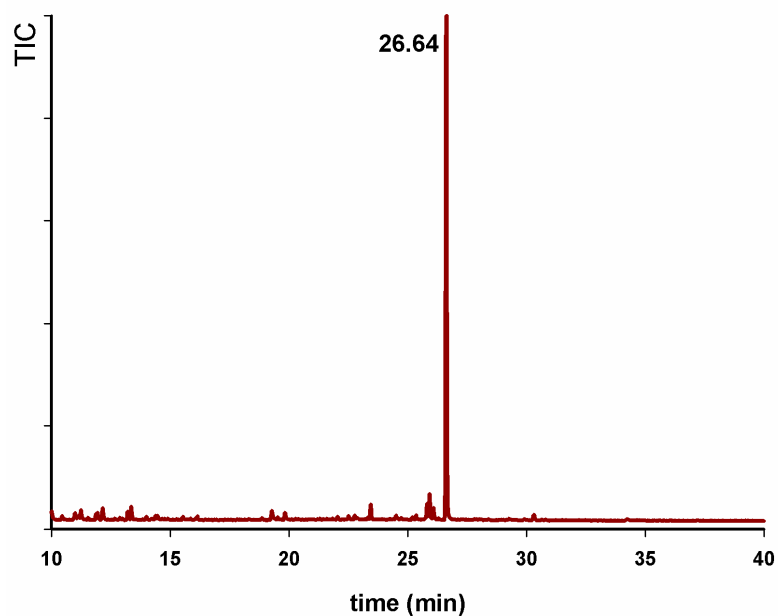


Figure S7. Total ion chromatogram of the pentane extractable products formed from incubation of FPP with D452A DCS (δ -cadinene retention time 26.6 min).

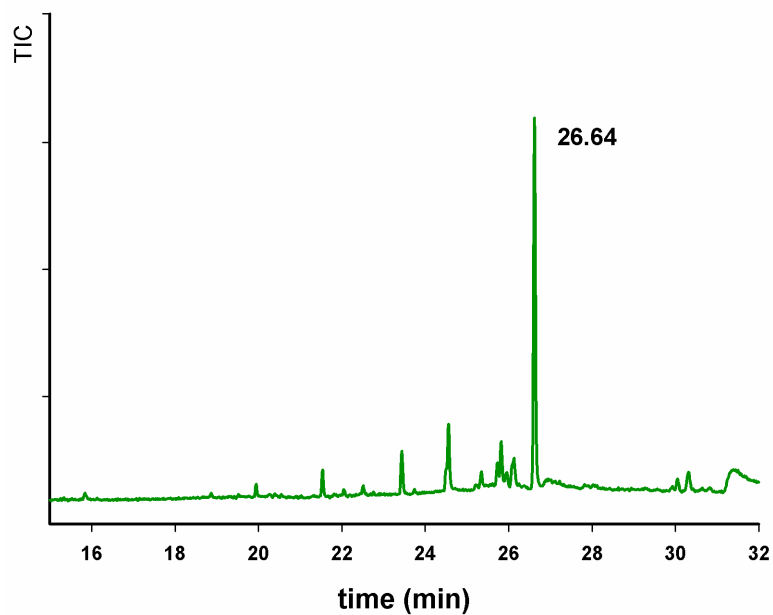


Figure S8. Total ion chromatogram of the pentane extractable products formed from incubation of FPP with E455A DCS (δ -cadinene retention time 26.6 min).

Plots of Kinetic Data

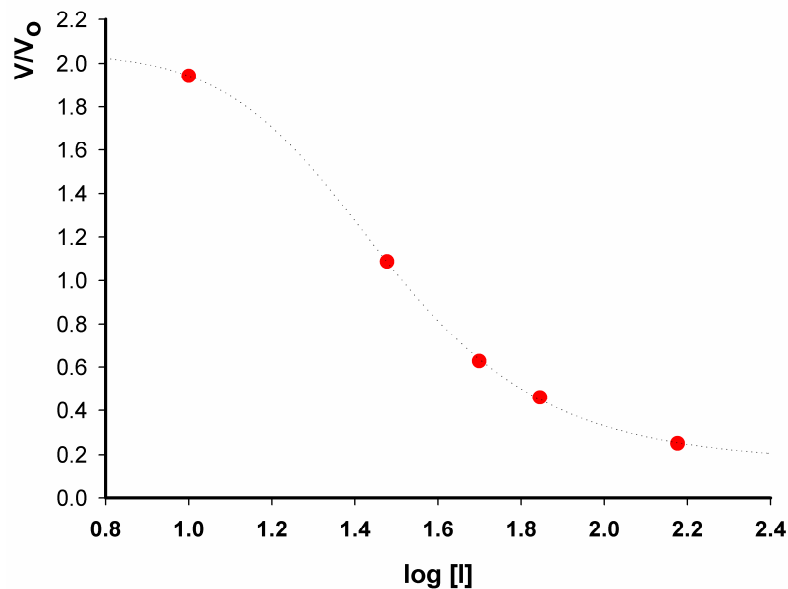


Figure S9. Dose-response curve for inhibition of DCS (3 μM) by 2F-FPP ([FPP] = 8 μM). $\text{IC}_{50} = 30 \mu\text{M}$.

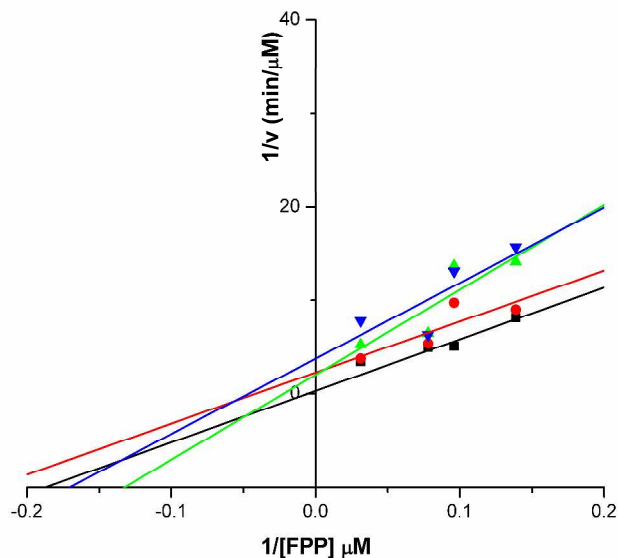


Figure S10. Typical double-reciprocal plot representations of the turnover of FPP by wild-type DCS at various concentrations of 2F-FPP. Black circles - no inhibitor; red circles, 40 μM 2F-FPP; green triangles - 80 μM 2F-FPP and blue triangles - 120 μM 2F-FPP. No consistent point of overlap between the double reciprocal plots could be observed despite repeated attempts at measuring these data.

References

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