Supporting Information for:

Mechanistic analysis of the *ent*-copalyl diphosphate synthases required for gibberellin plant hormone biosynthesis leads to novel product chemistry

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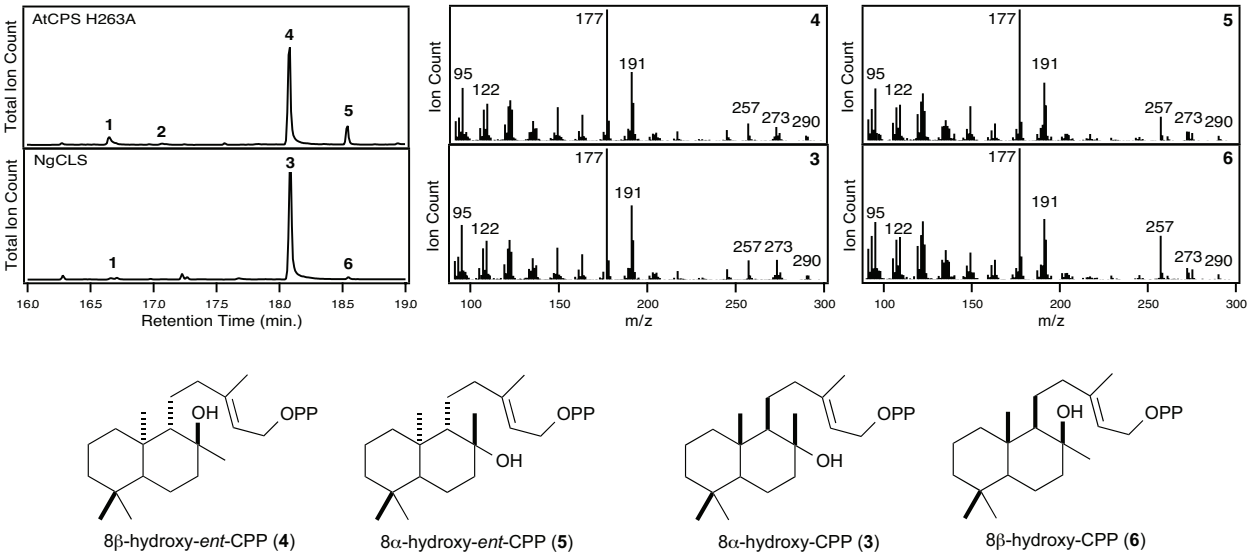
Figure S1: GC-MS based comparison of dephosphorylated AtCPS:H263A products with the pair of 8-hydroxy epimers of CPP previously reported from a tobacco species (*Nicotiana glutinosa*) cyclo-hydratase, NgCLS (ref. 3i). Also shown are the inferred direct enzymatic products, with numbering as defined in the text and **6** defined here as 8β-hydroxy-CPP.



Experimental NMR analysis

This was carried out much as previously described (ref. 3h). Briefly, to isolate sufficient amounts of *ent*-labd-13*E*-en-8α,15-diol for conformation by NMR spectral analysis, 2 x 1 L cultures were fermented, extracted twice with an equal volume of hexanes, with the phases separated in a separatory funnel, and the pool hexanes dried by rotary evaporation. The resulting extract was redissolved in 10 mL fresh hexanes and purified using a Reveleris automated flash chromatography system. The resulting fractions were analyzed by GC-MS, and that containing the targeted *ent*-labd-13*E*-en-8α,15-diol dried under N_2, yielding ~1.5 mg, which was redissolved in 0.5 mL $CDCl_3$. This sample was analyzed by NMR, using a Bruker Avance 700 spectrometer equipped with a 5-mm HCN cryogenic probe for ^1H and ^{13}C, one-dimensional ^1H acquired at 700 MHz, and one-dimensional ^{13}C acquired at 174 MHz using standard experiments from the Bruker TopSpin version 1.4 software. Chemical shifts were referenced using known chloroform (^{13}C 77.23, ^1H 7.24 ppm) signals offset from TMS (Table S1), and compared to those we previously found for the enantiomer (ref. 3h).

Table S1: ^1H and ^{13}C NMR data for *ent*-labd-13*E*-en-8α,15-diol

Position	δ_H	δ_C
1a **1b**	1.57 (1H, m) 0.89 (1H, dd, *J* = 11.8, 3.6 Hz)	40.3
2a **2b**	1.51 (1H, dt, *J* = 13.6, 3.6 Hz) 1.35 (1H, m)	19.0
3a **3b**	1.30 (1H, m) 1.07 (1H, dd, *J* = 13.6, 3.6 Hz)	42.6
4		33.8
5	0.84 (1H,dd, *J* = 12.2, 2.0 Hz)	56.7
6a **6b**	1.57 (1H, m) 1.19 (1H, m)	21.2
7a **7b**	1.78 (1H, br.d, *J* = 12.6Hz) 1.30 (1H, m)	45.2
8		74.7
9	0.97 (1H, t, *J* = 3.6 Hz)	61.9
10		
11a **11b**	1.45 (1H, m) 1.31 (1H, m)	24.2
12	2.01 (2H, m)	43.5
13		141.7
14	5.36 (1H, t, *J* = 7.0 Hz)	123.7
15	4.07 (2H, t, *J* = 7.8 Hz)	60.0
16	1.61 (3H, s)	17.1
17	1.05 (3H, s)	24.5
18	0.79 (3H, s)	34.0
19	0.70 (3H, s)	22.1
20	0.71 (3H, s)	16.1

Figure S2: ^{1}H 1D spectrum (black) with comparison to that previously recorded for labd-13*E*-en-8α,15-diol (blue; ref. 3h).

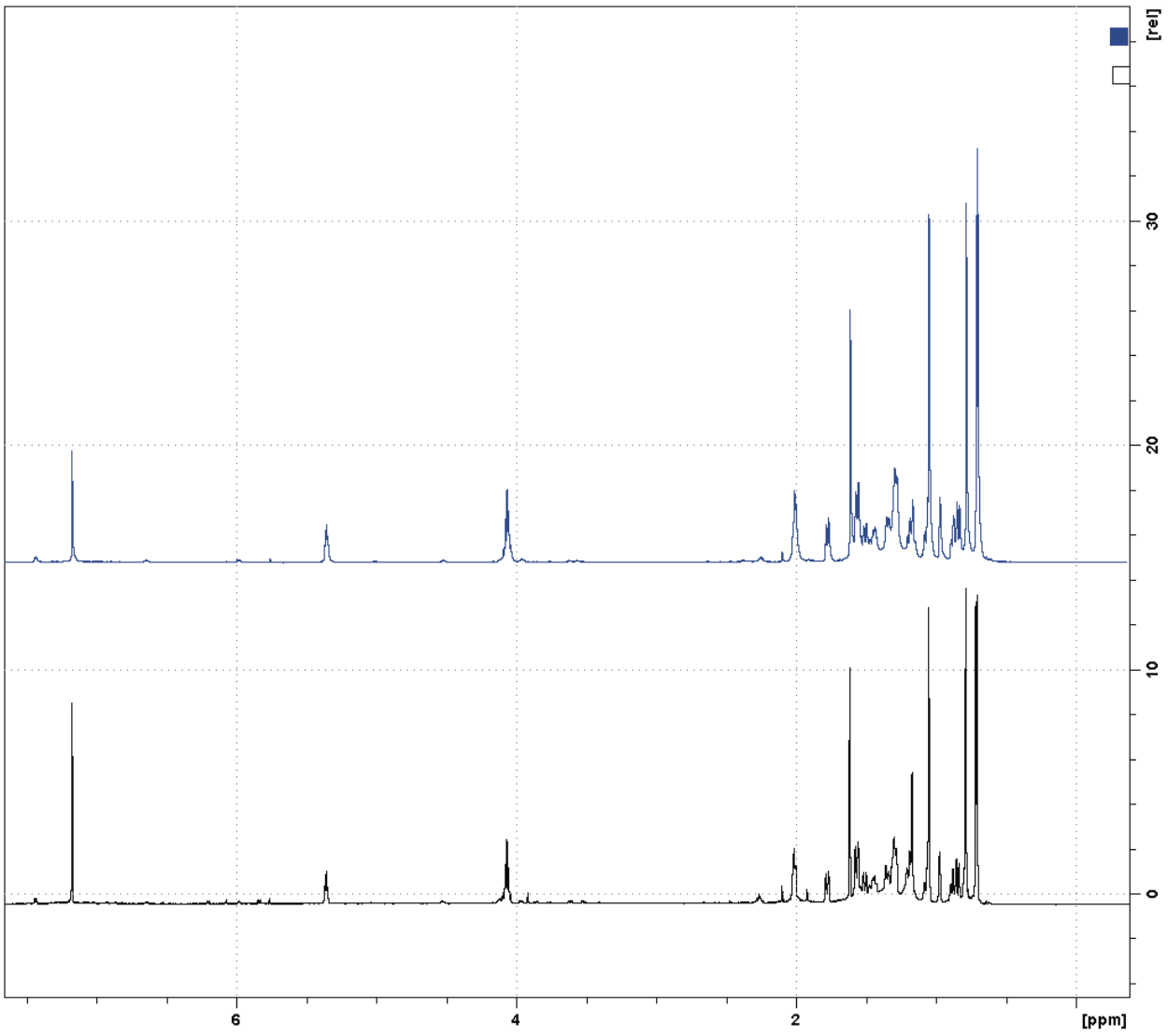
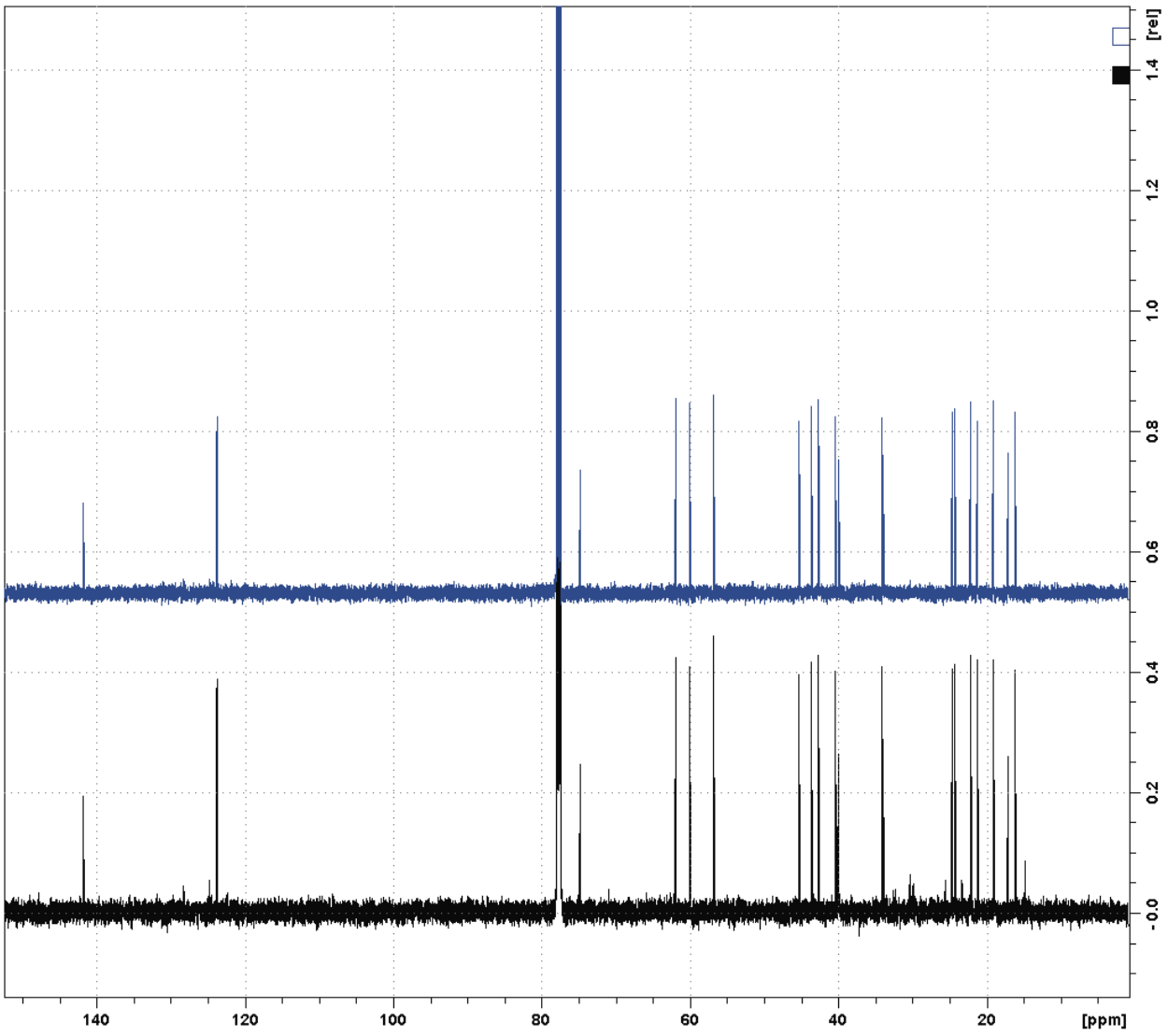


Figure S3: ^{13}C 1D spectrum (black) with comparison to that previously recorded for labd-13*E*-en-8α,15-diol (blue; ref. 3h).



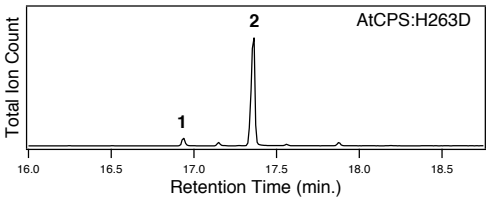


Figure S4: Effect of H263D mutation on AtCPS product outcome. Chromatograms from GC-MS analysis of the dephophorylated products (numbering as in text).

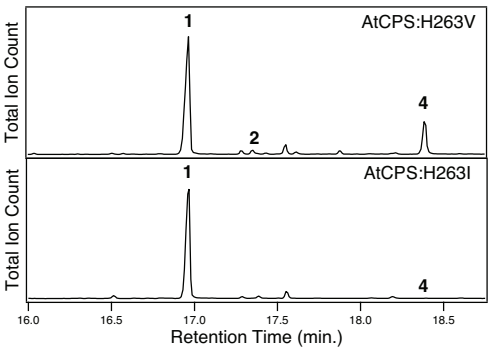


Figure S5: Effect of larger aliphatic residue substitutions for AtCPS:H263. Chromatograms from GC-MS analysis of the dephophorylated products (numbering as in text).

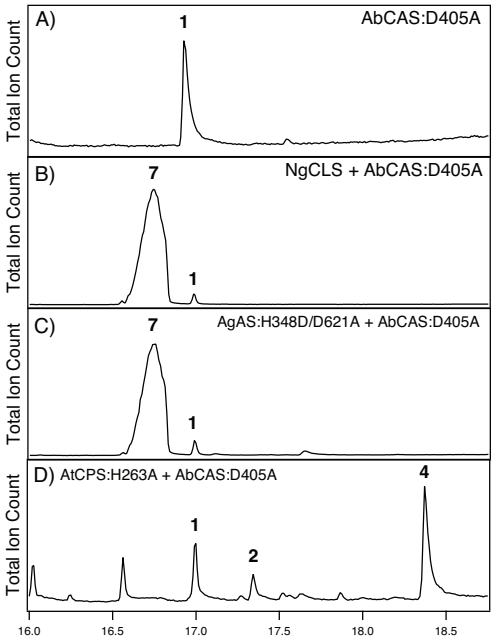


Figure S6: AtCPS:H263A produces *enantiomeric* 8-hydroxy-CPP. Chromatograms from GC-MS analysis of extracts from dephosphorylated products from reactions with the indicated enzyme(s). A) Mutation of "middle" aspartate from the DxDD motif knocks-out class II diterpene cyclase/hydratase activity of *Abies balsamea cis*-abienol synthase (AbCAS:D405A). B) Production of 8α-hydroxy-CPP (**3**) by NgCLS enables AbCAS:D405A to produce *cis*-abienol (**7**). C) Similarly, AgAS:H348D/D621A also produces **3**, leading to the production of **7** by AbCAS:D405A. D) AtCPS:H263A mutant products (e.g., **4**) are not further reacted upon by AbCAS:D405A, demonstrating a clear difference in stereochemistry.