Supporting Information for:

The T296V Mutant of Amorpha-4,11-diene Synthase is Defective in Allylic Diphosphate Isomerization but Retains the Ability to Cyclize the Intermediate (3*R*)-Nerolidyl Diphosphate to Amorpha-4,11-diene

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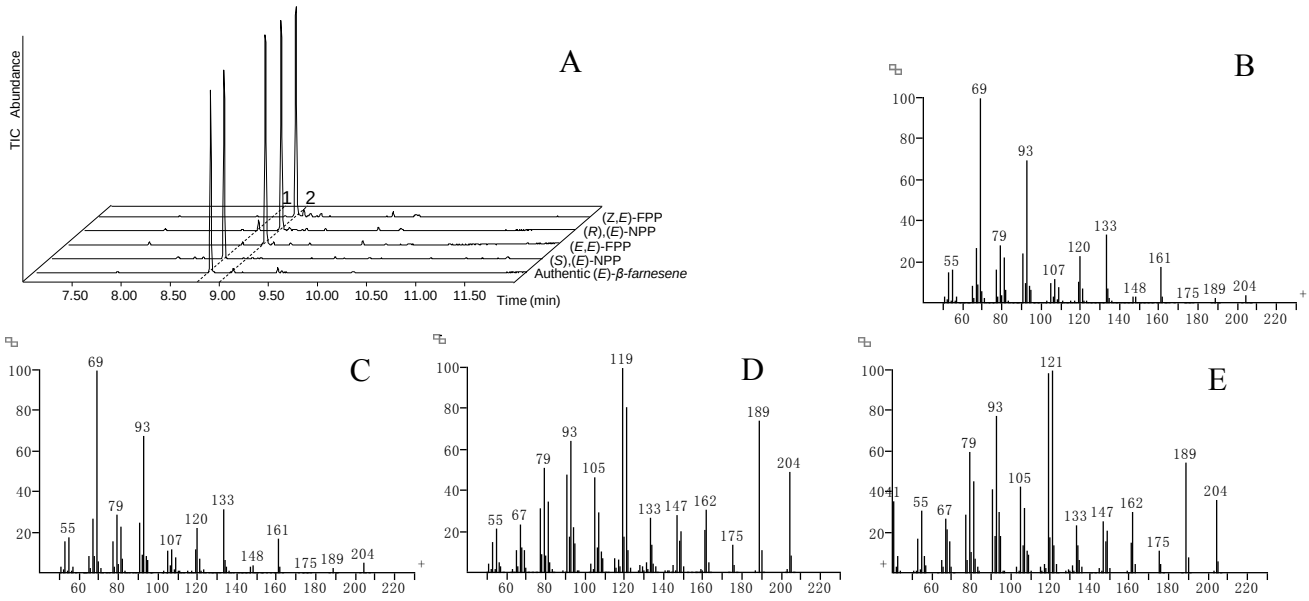


Figure S1. GC-MS analysis of authentic (*E*)- *β*-farnesene and products of ADS wild type using (*E*,*E*)-FPP, (*Z*,*E*)-FPP, (*R*),(*E*)-NPP and (*S*),(*E*)-NPP as substrates. A: Total ion chromatogram, B: MS spectrum of authentic (*E*)- *β*-farnesene peak 1; C: MS spectrum of peak 1 in A-(*S*),(*E*)-NPP; D: MS spectrum of peak 2 in A-(*E*,*E*)-FPP, (*Z*,*E*)-FPP and (*R*),(*E*)-NPP products; E: MS spectrum of amorpha-4,11-diene standard from MassFinder 4

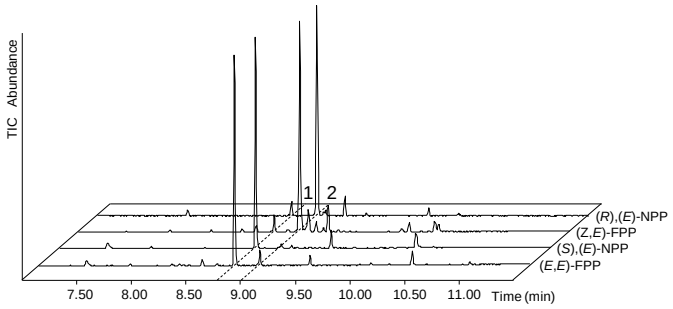


Figure S2. GC-MS TIC of ADS T296V mutant products using (*E*,*E*)-FPP, (*Z*,*E*)-FPP, (*R*),(*E*)-NPP and (*S*),(*E*)-NPP as substrates. Peak 1: (*E*)- *β*-farnesene; 2: amorpha-4,11-diene

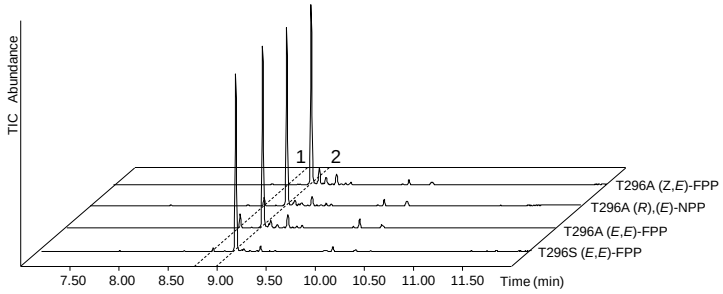


Figure S3. GCMS TIC of ADS T296S and T296A mutant products using (*E*,*E*)-FPP, or (*Z*,*E*)-FPP and (*R*),(*E*)-NPP as substrates. Peak 1: (*E*)-*β*-farnesene; 2: amorpha-4,11-diene

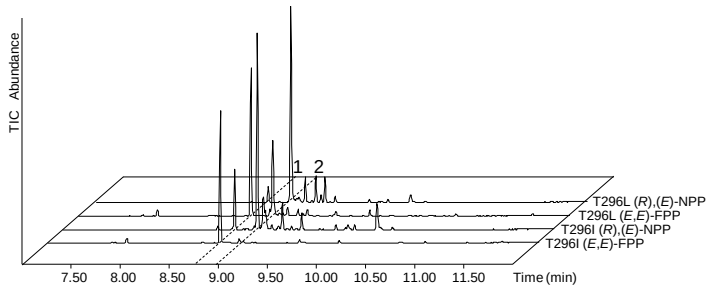


Figure S4. GCMS TIC of ADS T296I and T296L mutant products using (*E*,*E*)-FPP, and (*R*),(*E*)-NPP as substrates. Peak 1: (*E*)- *β*-farnesene; 2: amorpha-4,11-diene

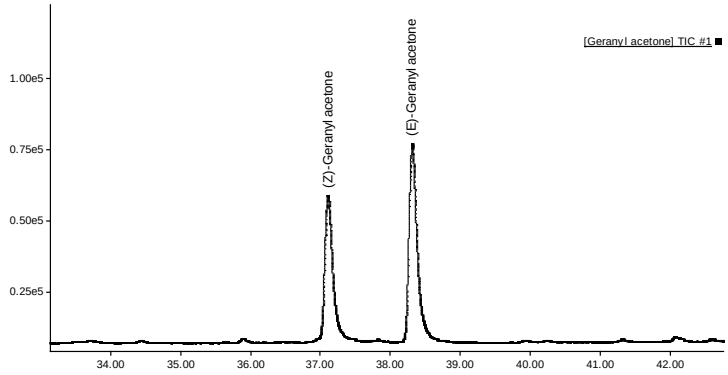


Figure S5. GC FID chromatograms of commercial geranyl acetone

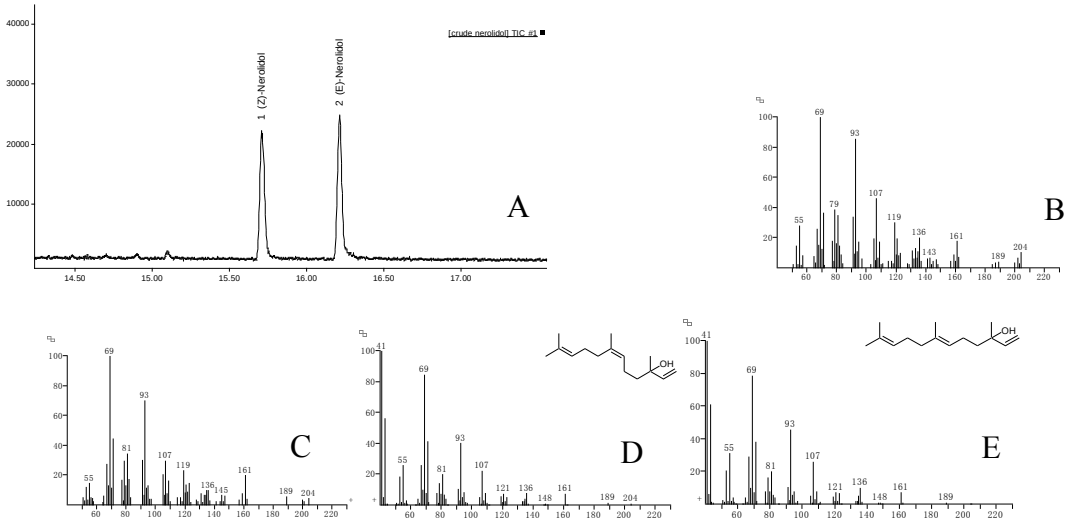
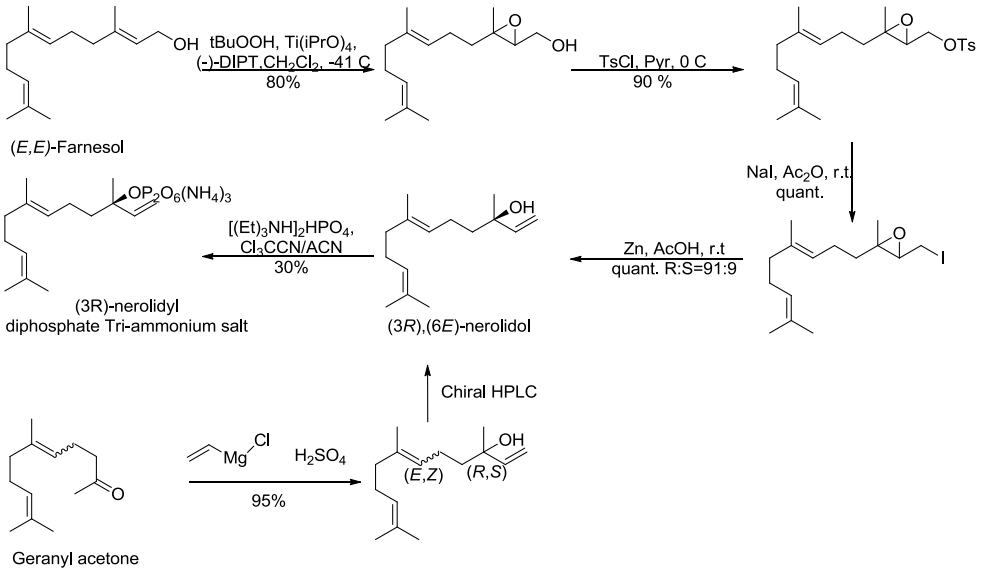


Figure S6. GC-MS total ion chromatogram of crude nerolidol and MS spectra of purified nerolidol and nerolidol isomers from the Massfinder 4 Library. A: TIC. B: MS spectrum of peak 1 in A. C: MS spectrum of peak 2 in A. D: MS spectrum of (*Z*)-nerolidol standard from MassFinder 4. E: MS spectrum of (*E*)-nerolidol standard from MassFinder 4.

Scheme S1. Synthesis of (*R*),(*E*)-nerolidyl diphosphate



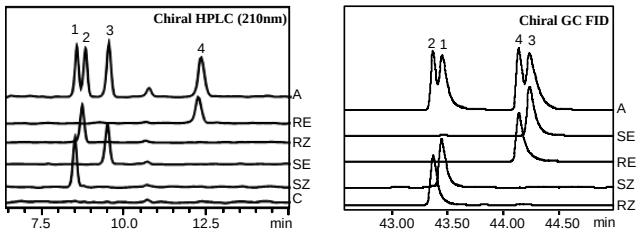


Figure S7. Chiral chromatogram of nerolidol purified using chiral HPLC 1: (3*S*, *Z*)-nerolidol($[\alpha]_D^{20}$=+16.5 in dichloromethane); 2: (3*R*, *Z*)-nerolidol; 3: (3*S*, *E*)-nerolidol; 4: (3*R*, *E*)-nerolidol ($[\alpha]_D^{20}$=-15.7 in dichloromethane); A: crude synthetic nerolidol; C: Petroleum ether.

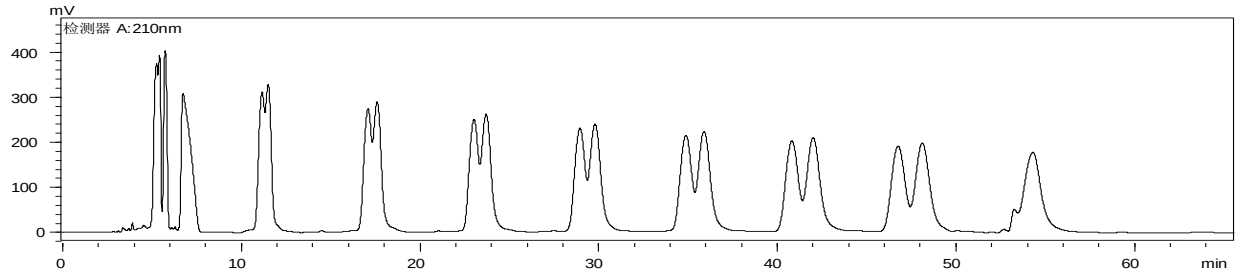


Figure S8. Chromatogram of one run in the preparation of optically pure (3*R*, *E*)-nerolidol by HPLC in the circulating mode.

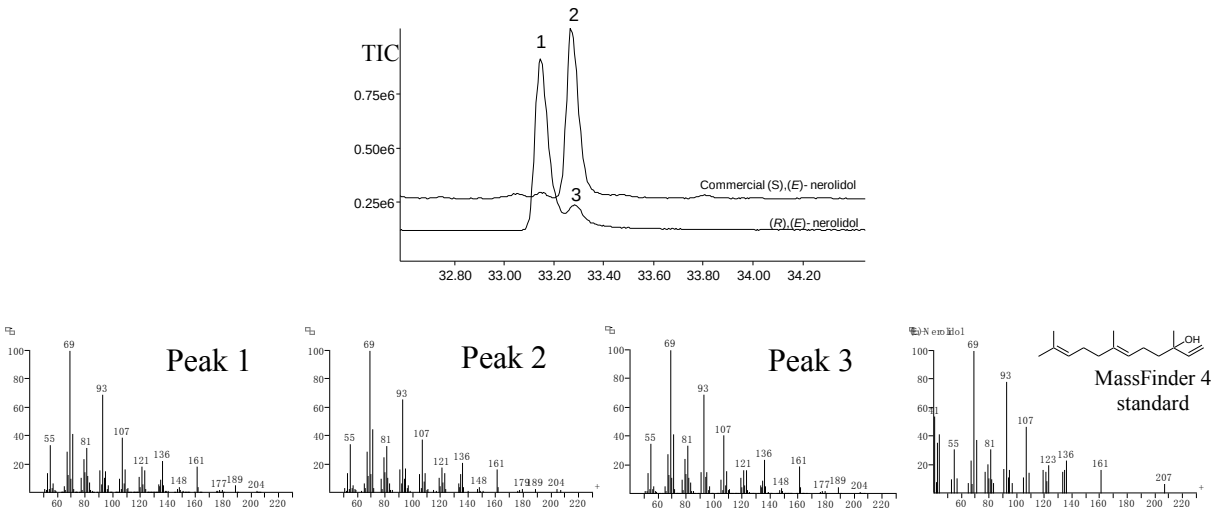


Figure S9. Chiral GC-MS analysis of commercial (3*S, E*)-nerolidol and of (3*R, E*)-nerolidol synthesized from geranyl acetone and 2,3-epoxyfarnesol by the Sharpless epoxidation method

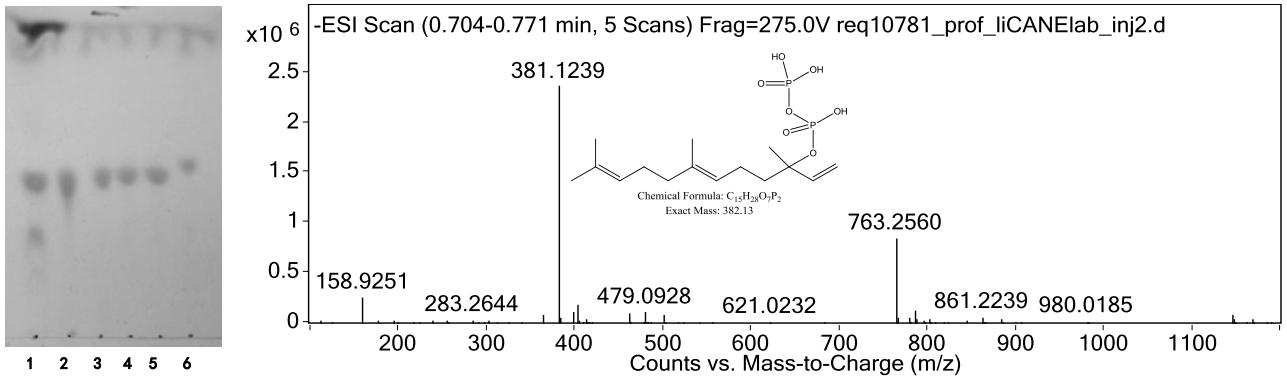


Figure S10. TLC and LC-MS analysis of synthesized nerolidyl diphosphate. Left: TLC 1: crude (3*R*, *E*)-nerolidyl diphosphate, 2: commercial (3*S*, *E*)-nerolidyl diphosphate; 3: (3*R*, *E*)-nerolidyl diphosphate; 4: (3*R*, *Z*)-nerolidyl diphosphate, 5: (3*S*, *Z*)-nerolidyl diphosphate; 6: commercial (2*E*, 6*E*)-farnesyl diphosphate. Right: LC-MS spectrum of (3*R*, *E*)-nerolidyl diphosphate

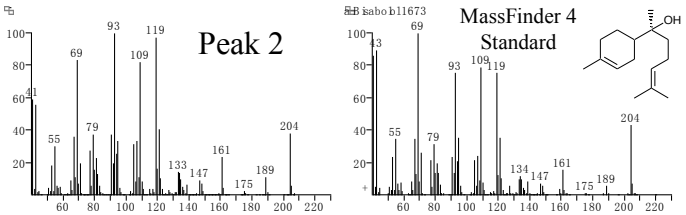


Figure S11. MS spectra of peak 2 in Figure 4 and α-bisabolol standard from Massfinder 4

Table S1. Mutagenic primers used for construction of ADS and AaBOS mutants

Name	Squence(5' --- 3')
ADS-T296V-F	TGTTGCTGTTATAGTTCTTATAGATGAC
ADS-T296V-R	GTCATCTATAAGAACTATAACAGCAACA
ADS-T296L-F	TGTTGCTGTTATACTTCTTATAGATGAC
ADS-T296L-R	GTCATCTATAAGAAGTATAACAGCAACA
ADS-T296A-F	CTGTTGCTGTTATAGCTCTTATAGATGAC
ADS-T296A-R	GTCATCTATAAGAGCTATAACAGCAACAG
ADS-T296I-F	TGTTGCTGTTATAATTCTTATAGATGACA
ADS-T296I-R	TGTCATCTATAAGAATTATAACAGCAACA
ADS-T296S-F	CTGTTGCTGTTATATCTCTTATAGATGAC
ADS-T296S-R	GTCATCTATAAGAGATATAACAGCAACAG
AaBOS-T296V-F	GTTATTGCGCTGGTTGTGCTGATTGATGACATC
AaBOS-T296V-R	GATGTCATCAATCAGCACAACCAGCGCAATAAC