

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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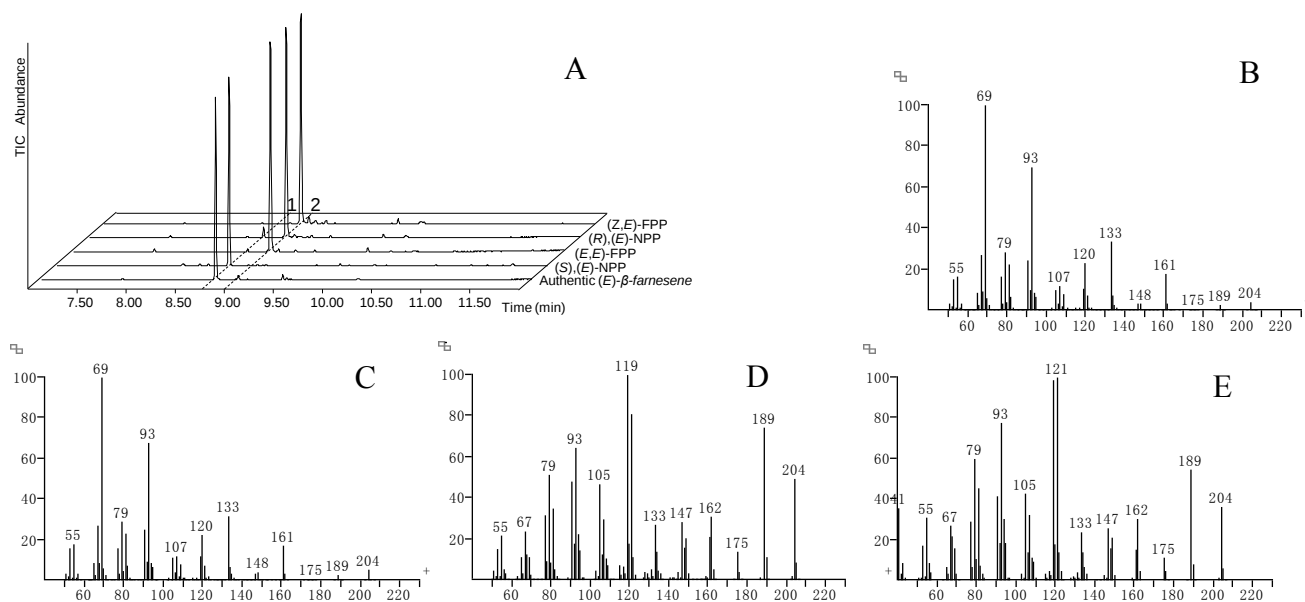


Figure S1. GC-MS analysis of authentic (*E*)- β -farnesene and products of ADS wild type using (*E,E*)-FPP, (*Z,E*)-FPP, (*R*),(*E*)-NPP and (*S*),(*E*)-NPP as substrates. A: Total ion chromatogram, B: MS spectrum of authentic (*E*)- β -farnesene peak 1; C: MS spectrum of peak 1 in A-(*S*),(*E*)-NPP;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

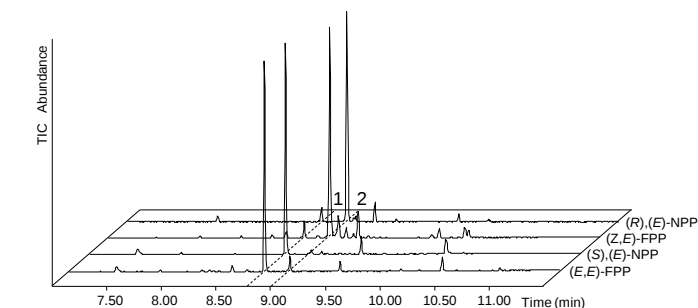


Figure S2. GC-MS TIC of ADS T296V mutant products using (*E,E*)-FPP, (*Z,E*)-FPP, (*R*),(*E*)-NPP and (*S*),(*E*)-NPP as substrates. Peak 1: (*E*)- β -farnesene; 2: amorpho-4,11-diene

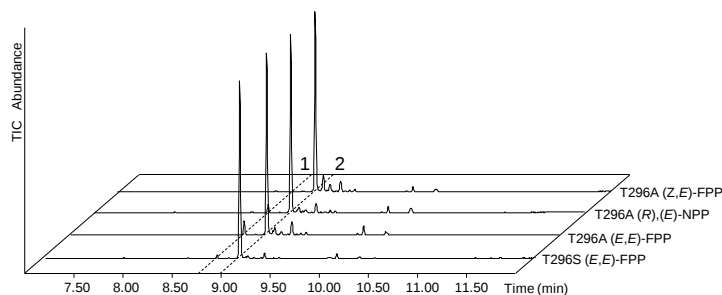


Figure S3. GCMS TIC of ADS T296S and T296A mutant products using (*E,E*)-FPP, or (*Z,E*)-FPP and (*R*),(*E*)-NPP as substrates. Peak 1: (*E*)- β -farnesene; 2: amorpho-4,11-diene

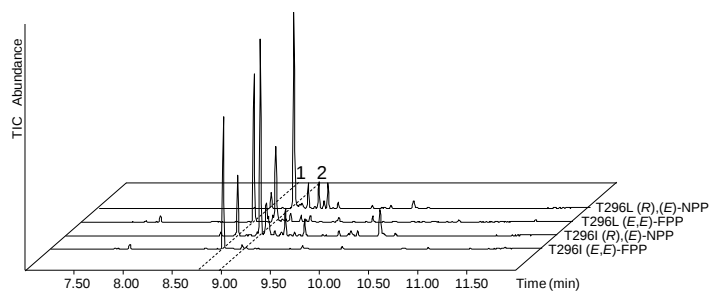


Figure S4. GCMS TIC of ADS T296I and T296L mutant products using *(E,E)*-FPP, and *(R),(E)*-NPP as substrates. Peak 1: *(E)*- β -farnesene; 2: amorpha-4,11-diene

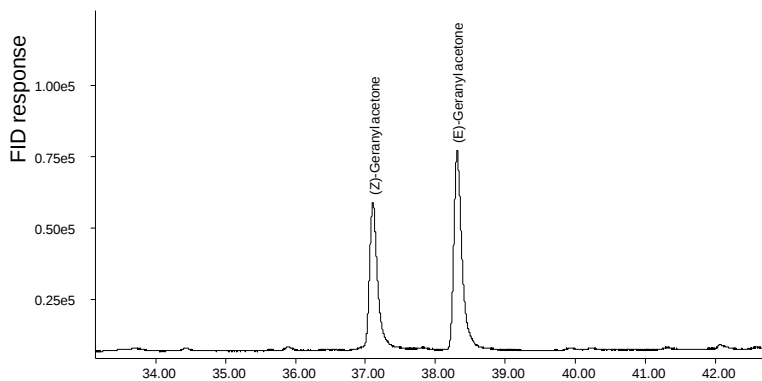


Figure S5. GC FID chromatograms of commercial geranyl acetone

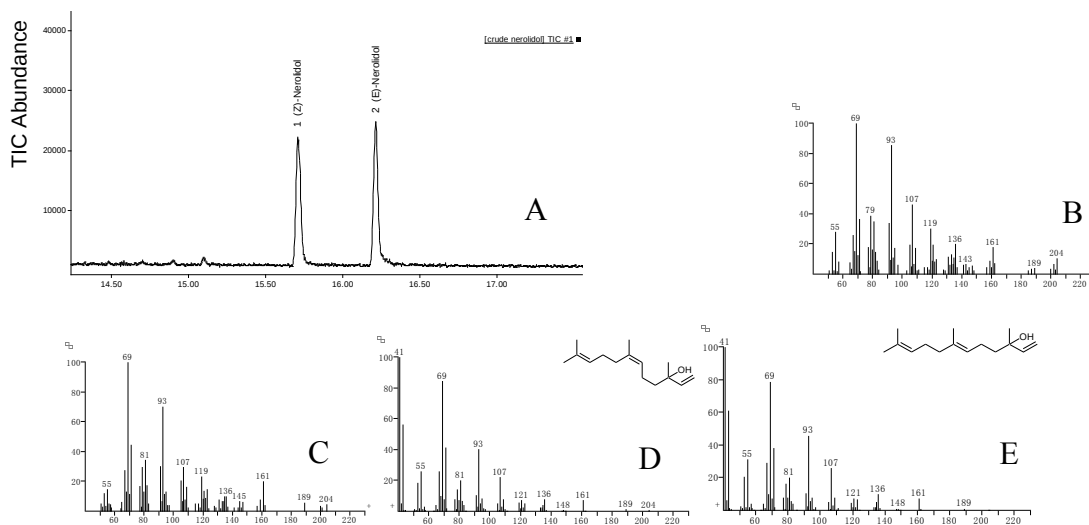


Figure S6. GC-MS total ion chromatogram of crude nerolidol and MS spectra of purified nerolidol and nerolidol isomers from the Massfinder 4 Library. A: TIC. B: MS spectrum of peak 1 in A. C: MS spectrum of peak 2 in A. D: MS spectrum of *(Z)*-nerolidol standard from MassFinder 4. E: MS spectrum of *(E)*-nerolidol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

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

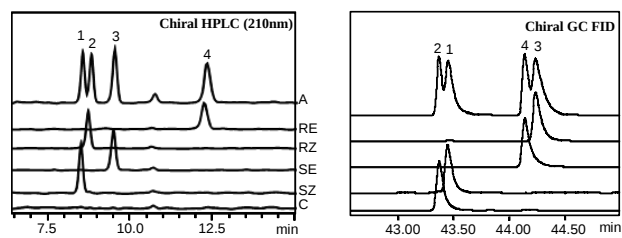
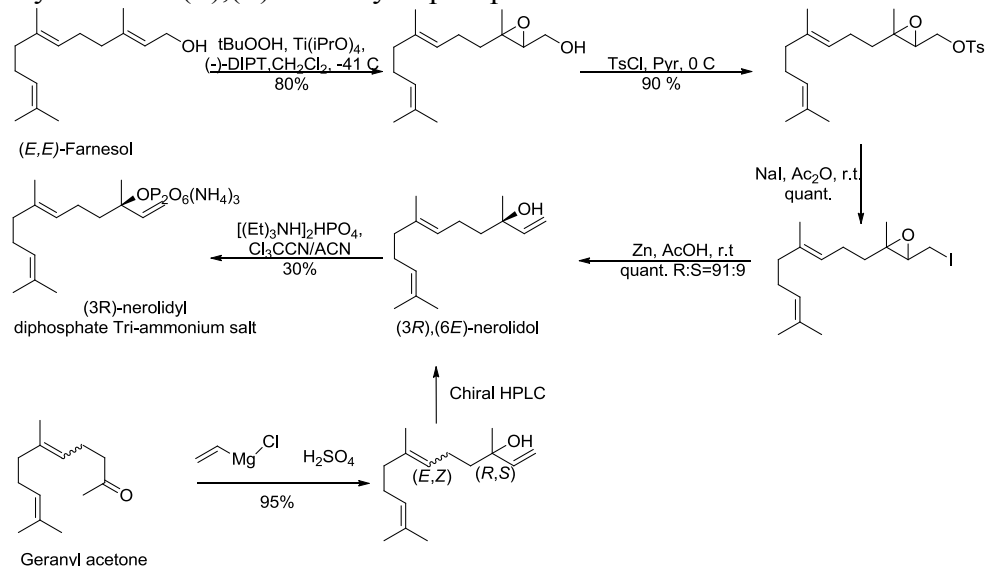


Figure S7. Chiral chromatogram of nerolidol purified using chiral HPLC 1: (*3S*, *Z*)-nerolidol ($[\alpha]_D^{20}=+16.5$ in dichloromethane); 2: (*3R*, *Z*)-nerolidol; 3: (*3S*, *E*)-nerolidol; 4: (*3R*, *E*)-nerolidol ($[\alpha]_D^{20}=-15.7$ in dichloromethane); A: crude synthetic nerolidol; C: Petroleum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

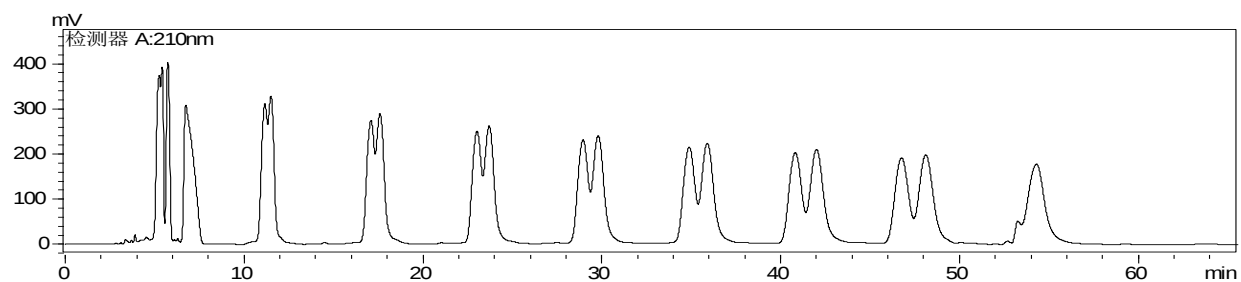


Figure S8. Chromatogram of one run in the preparation of optically pure (*3R*, *E*)-nerolidol by HPLC in the circulating mode.

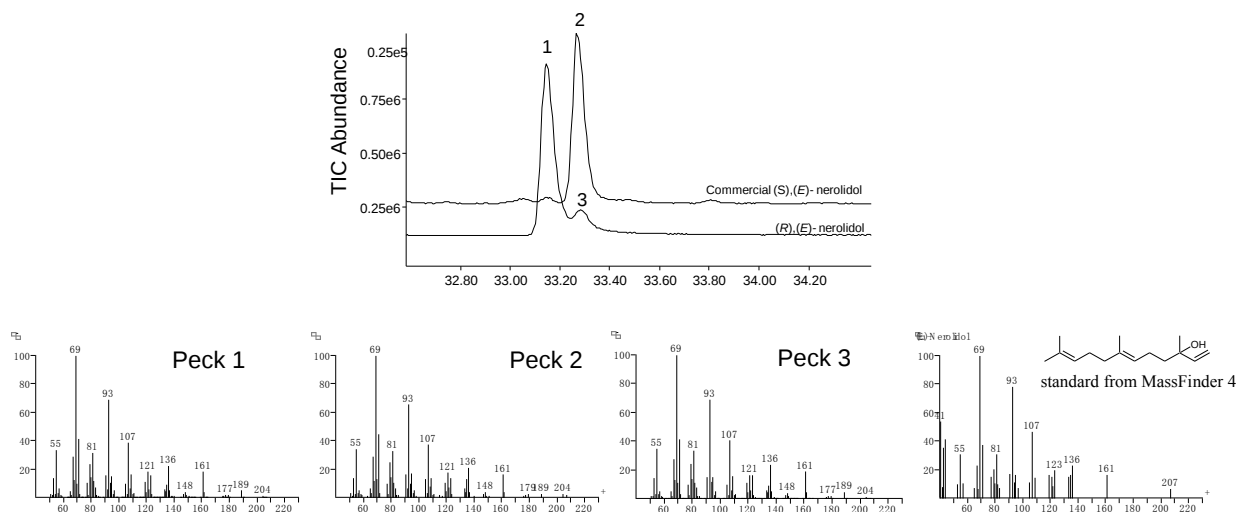


Figure S9. Chiral GC-MS analysis of commercial (3*S*, *E*)-nerolidol and of (3*R*, *E*)-nerolidol synthesized from geranyl acetone and 2,3-epoxyfarnesol by the Sharpless epoxidation method

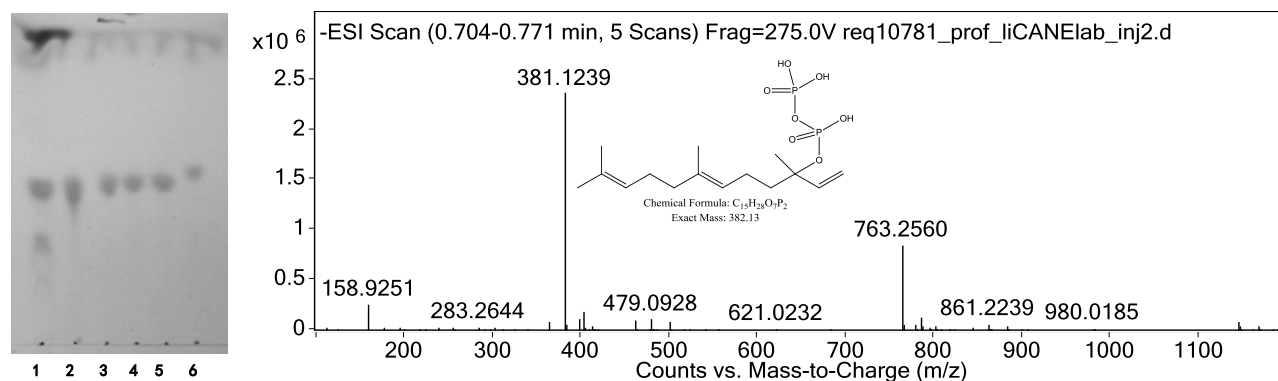


Figure S10. TLC and LC-MS analysis of synthesized nerolidyl diphosphate. Left: TLC 1: crude (3*R*, *E*)-nerolidyl diphosphate, 2: commercial (3*S*, *E*)-nerolidyl diphosphate; 3: (3*R*, *E*)-nerolidyl diphosphate; 4: (3*R*, *Z*)-nerolidyl diphosphate, 5: (3*S*, *Z*)-nerolidyl diphosphate; 6: commercial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

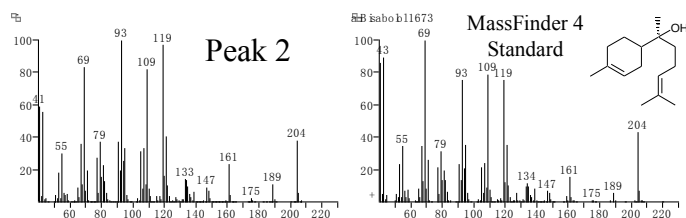


Figure S11. MS spectra of peak 2 in Figure 4 and α -bisabolol standard from Massfinder 4

Table S1. Primers used for construction of ADS and AaBOS mutants

Name	Sequence(5' --- 3')
ADS- <i>Bam</i> H I	GAAGGATCCCACCTTACAGAAGAAAAACC
ADS- <i>Xho</i> I	GCAGAGCTCTCATATACTCATAGGATAAACGAG
ADS-T296V-F	TGTTGCTGTTATAGTTCTTATAGATGAC
ADS-T296V-R	GTCATCTATAAGAAGTATAACAGCAACA
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

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1  ATGTCCCTGA CCGAAGAGAA GCCGATTCGT CCGATTGCAA ACTTTAGCCC
51  GTCTATTTGG GGTGACCAGT TCCTGATCTA CGATAACCAG GTGGAACAAG
101 GCGTGGAGCA GATTGTAAAA GACCTGAAGA AAGAAGTGCG TCAGCTGCTG
151 AAGGAGGCAC TGGACATTCC GATGAAACAC GCTAACCTGC TGAAGCTGGT
201 TGACGAGATT CAGCGTCTGG GTATTTTCCTA TCTGTTTCGAG CAAGAGATTG
251 ATCATGCCCT GCAACACATC TACGAAACCT ACGGCGATAA CTGGTCTGGC
301 GACCGCTCTT CTCTGTGGTT CCGTCTGATG CGTAAACAGG GCTACTTTGT
351 AACTTGCGAC GTGTTCAACA ACCACAAAGA CGAATCCGGT GTGTTCAAAC
401 AGAGCCTGAA GAACCACGTG GAGGGTCTGC TGGAACTGTA TGAAGCTACC
451 TCCATGCGCG TACCAGGTGA GATCATCCTG GAGGACGCGC TGGTATTAC
501 CCAATCCCAC CTGTCCATTA TCGCGAAAGA TACCCTGAGC ATCAACCCAG
551 CTCTGTCCAC CGAAATCCAG CGTGCACTGA AGAAACCGCT GTGGAAACGC
601 CTGCCACGTA TCGAAGCGGT GCAATACATC CCGTTCTACG AGCAGCAGGA
651 CTCCCATAAC AAGACCCTGA TCAAACTGGC GAAACTGGAG TTCAACCTGC
701 TGCAAAGCCT GCATCGTGAG GAACTGTCTC AGCTGTCTAA GTGGTGGAAA
751 GCGTTCGATG TGAAGAACAA CGCTCCGTAC AGCCGTGATC GCATCGTTGA
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851 CTCGCATCTT CCTGGCCAAA GTTATTGCGC TGGTTACGCT GATTGATGAC
901 ATCTACGATG CGTATGGCAC TTACGAAGAA CTGAAGATCT TCACCGAAGC
951 TATTGAACGT TGGTCTATTA CCTGTCTGGA TATGATCCCA GAATACATGA
1001 AACCGATCTA TAAGCTGTTC ATGGATACCT AACTGAAAT GGAGGAGATT
1051 CTGGCCAAAG AGGGCAAGAC CAACATCTTT AACTGCGGTA AAGAGTTCGT
1101 TAAAGATTTC GTACGTGTTT TGATGGTTGA GGCCAGTGG CTGAACGAAG
1151 GTCATATCCC GACCACCGAA GAGCTGGATT CCATCGCTGT TAACCTGGGT
1201 GGTGCCAACC TGCTGACCAC CACCTGCTAC CTGGGTATGT CCGACATCGT
1251 TACTAAGGAG GCTTTCGAGT GGGCTGTATC TGAACCACCA CTGCTGCGCT
1301 ACAAAGGTAT CCTGGGTCGT CGTCTGAACG ATCTGGCTGG TCATAAAGAA
1351 GAGCAGGAGC GTAAACATGT TTCCTCCTCC GTAGAATCTT ACATGAAAGA
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1451 AGGACCTGTG GAAAGACATC AACCCTGAGT ACCTGATTAC CAAGACCATC
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1551 ACTGTATGCA GAGAAGGACA ATTTCACTCG TATGGGCGAG GAATACAAGA
1601 ACCTGGTGAA ATCTCTGCTG GTTTACCCGA TGTCTATCTA A

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Figure S12. Codon optimized DNA sequence of *A. annua* α -bisabolol synthase