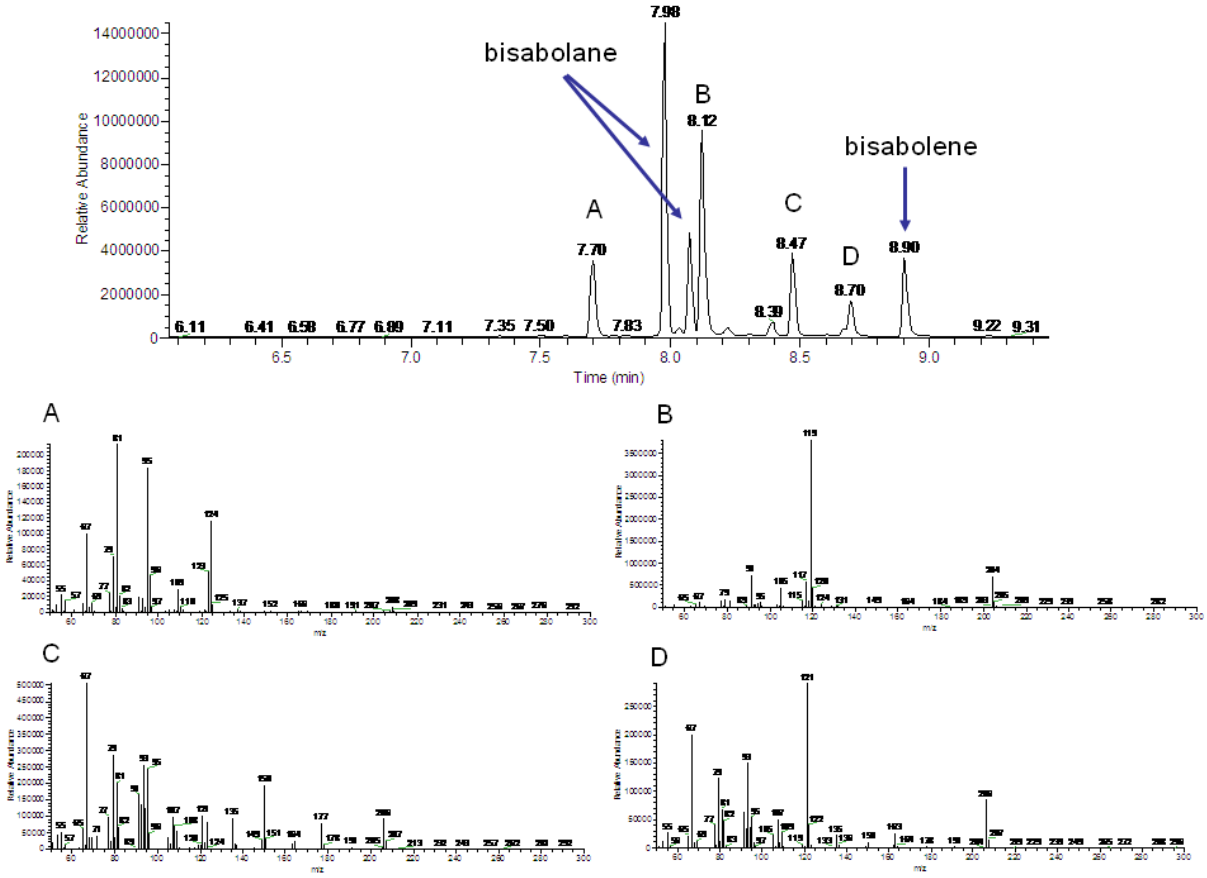
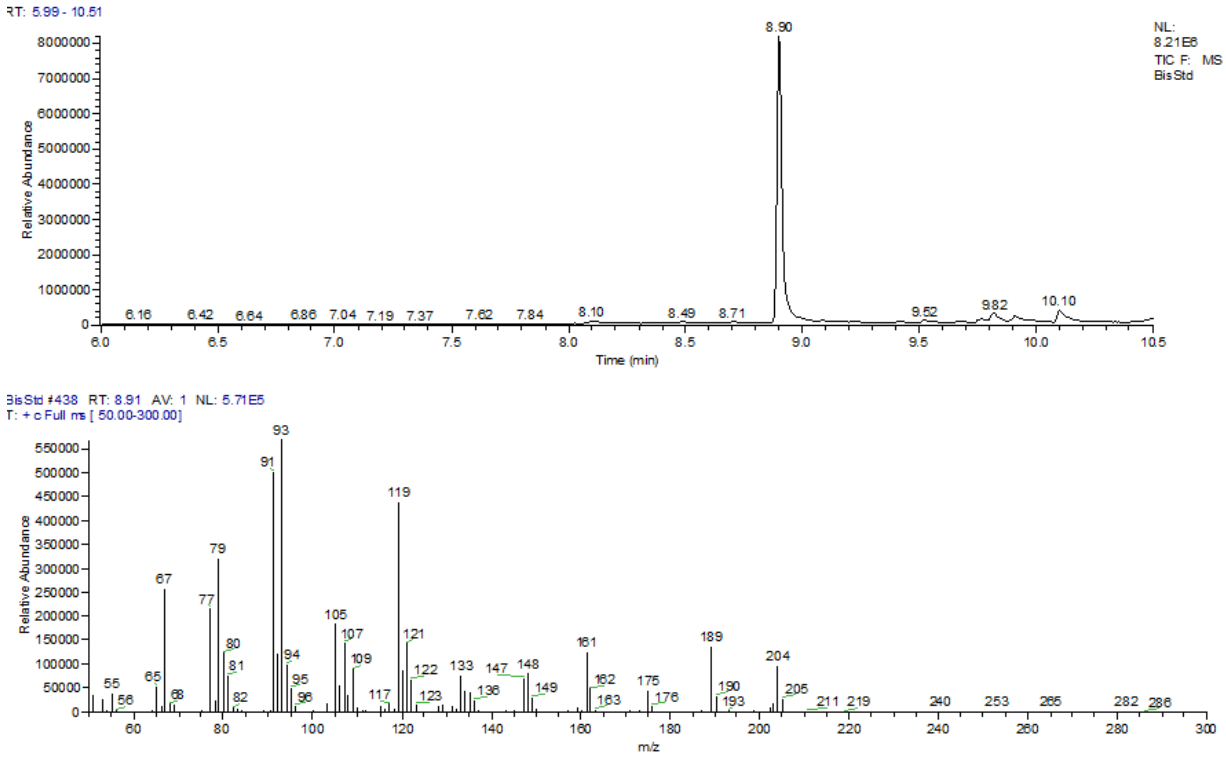
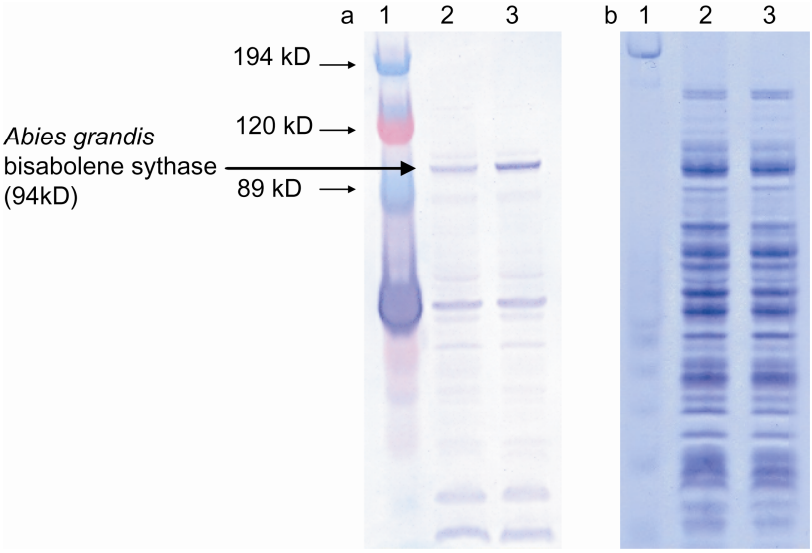


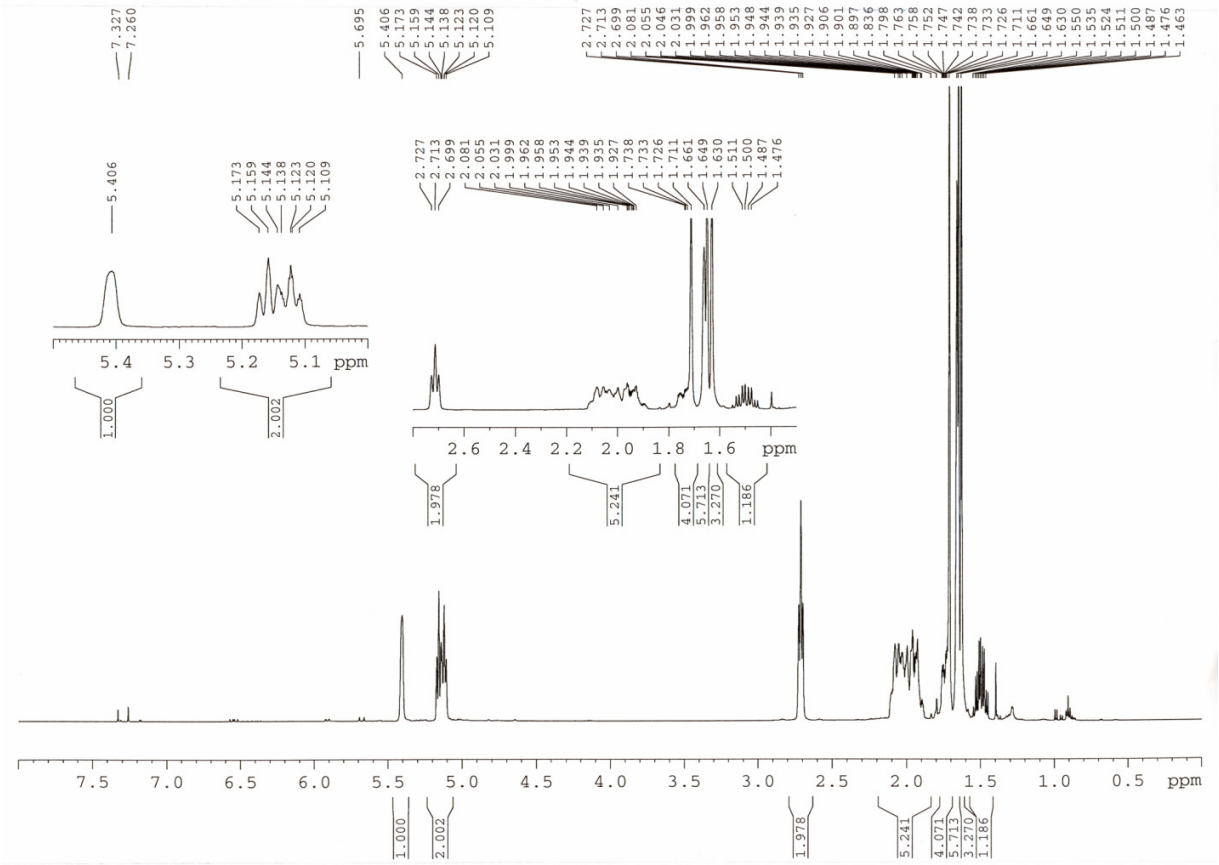
Supplementary Figure S1. Gas chromatography (GC) trace and mass spectrometry (MS) data of partially hydrogenated biosynthetic bisabolene. Partial hydrogenation of biosynthetic bisabolene was obtained using low hydrogen pressure and a short reaction time. Partially hydrogenated biosynthetic bisabolene generated fully hydrogenated bisabolenes (RT: 7.98 and 8.08), partially hydrogenated bisabolenes (A, C, and D at RTs: 7.70, 8.12, 8.47), aromatized bisabolanes (B at RT: 8.47), and unreacted bisabolene (8.90). Peak at RT: 7.70 is assigned as partially hydrogenated bisabolene with one double bond based on fragmentation pattern (methylcyclohexenyl at m/z 95). Peak at RT: 8.12 is aromatized bisabolene with fully hydrogenated side chain (mol wt: 204, and the fragment ion of methylphenyl at m/z 91). Peaks at RT: 8.47 and 8.70 are partially hydrogenated bisabolenes with two double bonds (mol wt: 206).



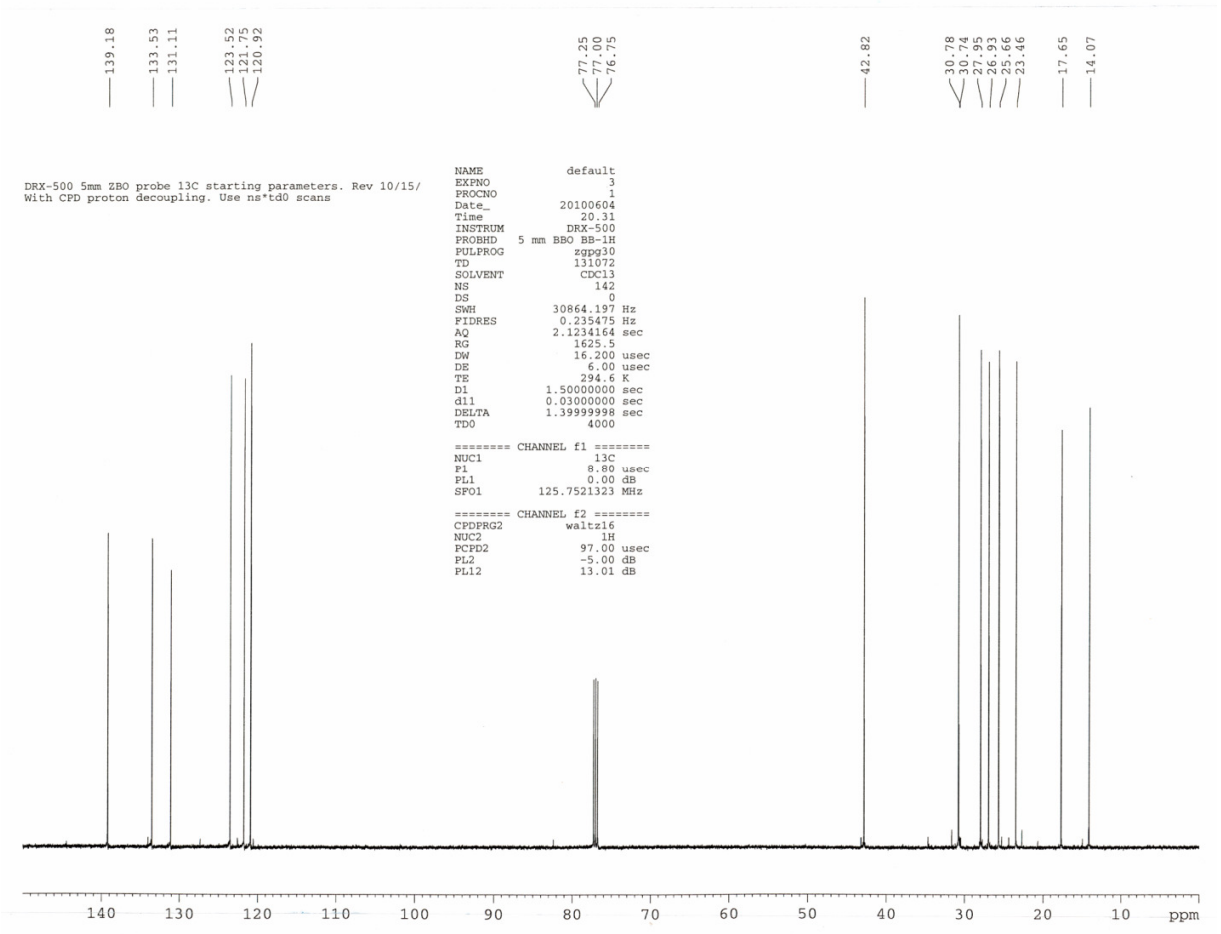
Supplementary Figure S2. Gas chromatography/ Mass spectrometry (GC/MS) of biosynthetic bisabolene. Top: GC of biosynthetic bisabolene (RT: 8.9) showing a single product, α-bisabolene. Bottom: MS of biosynthetic bisabolene.



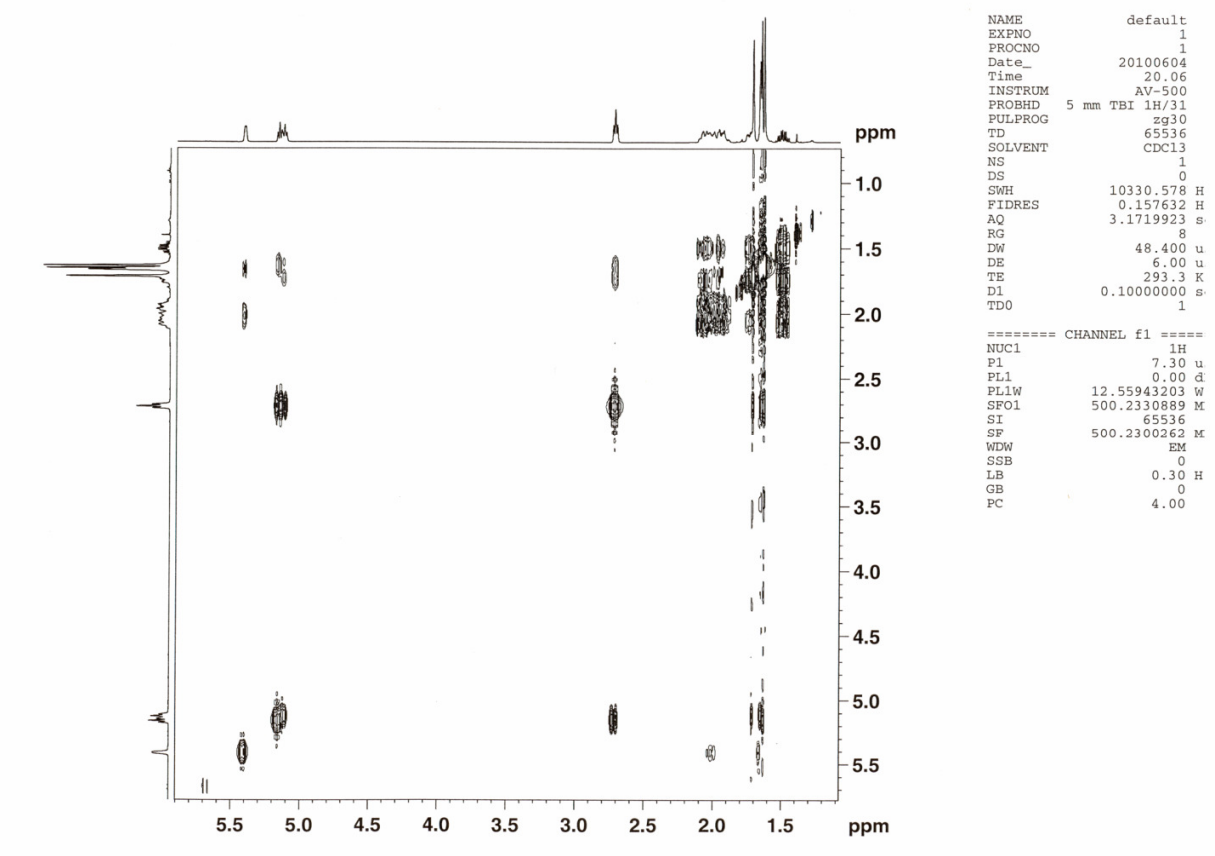
Supplementary Figure S3. *Abies grandis* bisabolene synthase soluble protein levels in *E. coli*. *E. coli* codon-optimized *A. grandis* bisabolene synthase (AgBIS) shows ~2-fold higher protein levels than original (plant) *A. grandis* bisabolene synthase (Ag1). **A**. Western Blot of 6His-tagged Ag1 and AgBIS. Lanes: 1: Kaleidograph ladder (Bio-Rad), 2: 6His-tagged Ag1, 3: 6His-tagged AgBIS. **B**. SDS-PAGE gel showing equal loading. Lanes: 1: Kaleidograph ladder (Bio-Rad), 2: His-tagged Ag1, 3: His-tagged AgBIS. Pre-cultures of *E. coli* DH1 harboring either 6His-tagged Ag1 (pJBEI-3867) or 6His-taged AgBIS (pJBEI-3868) were used to inoculated in a 1:100 dilution bisabolene production media (Teknova, EZ-Rich, 1% (v/v) glucose, amp^{100}, cm^{30}, 5 mL). The cultures were grown at 37°C for 5.5 h (180 rpm, OD_{600}=0.6-0.8) prior to induction with 500 µM IPTG. After growth for 3 h at 30°C, 1ml of cells were pelleted and analyzed for soluble bisabolene synthase protein.



Supplementary Figure S4. ^{1}H NMR of biosynthetic bisabolene. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (m, 1H), 1.63 (s, 3H), 1.65-1.66 (d, 6H), 1.71 (s, 3H), 1.73-1.74 (m, 1H), 1.93-2.08 (m, 5H), 2.71 (t, 2H), 5.41 (s, 1H), 5.11-5.17 (m, 2H)



Supplementary Figure S5. ^{13}C NMR of biosynthetic bisabolene. ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 17.7, 23.5, 25.7, 26.9, 28.0, 30.7, 30.8, 42.8, 120.9, 121.8, 123.5, 131.1, 133.5, 139.2

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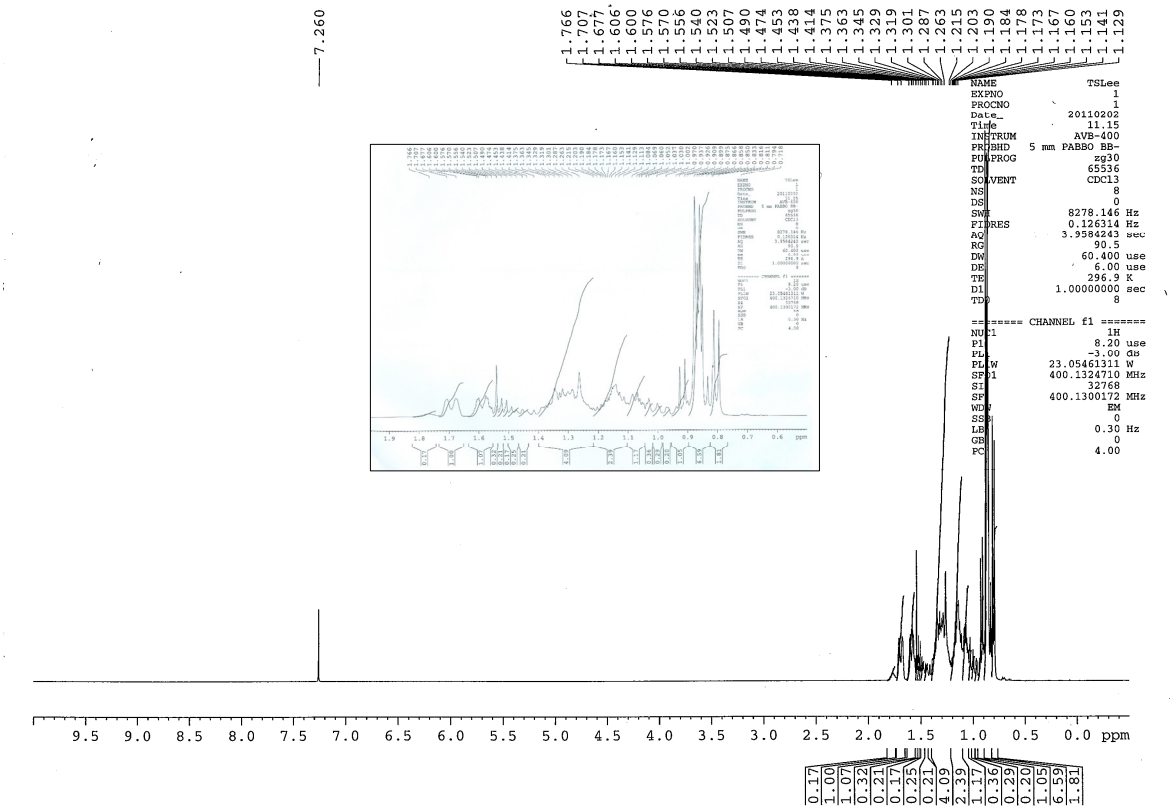
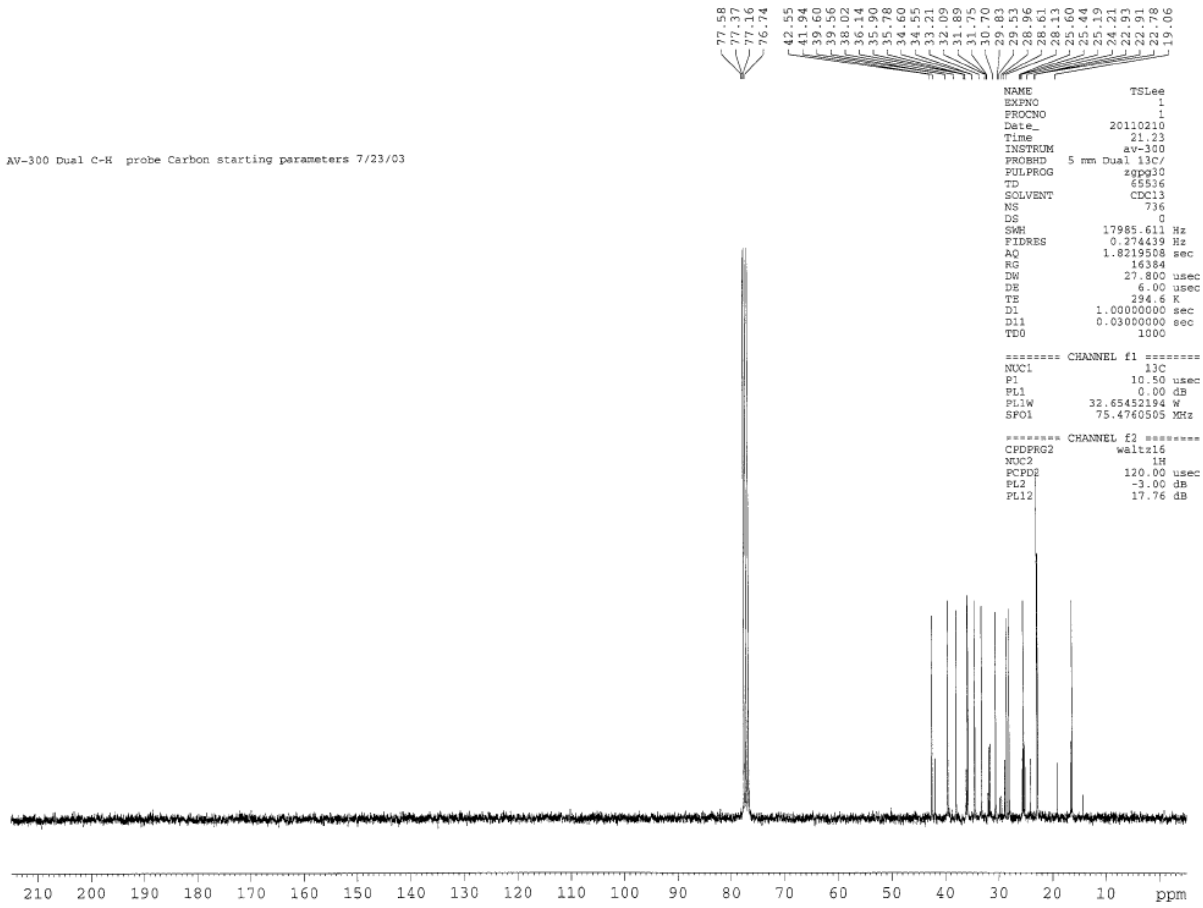
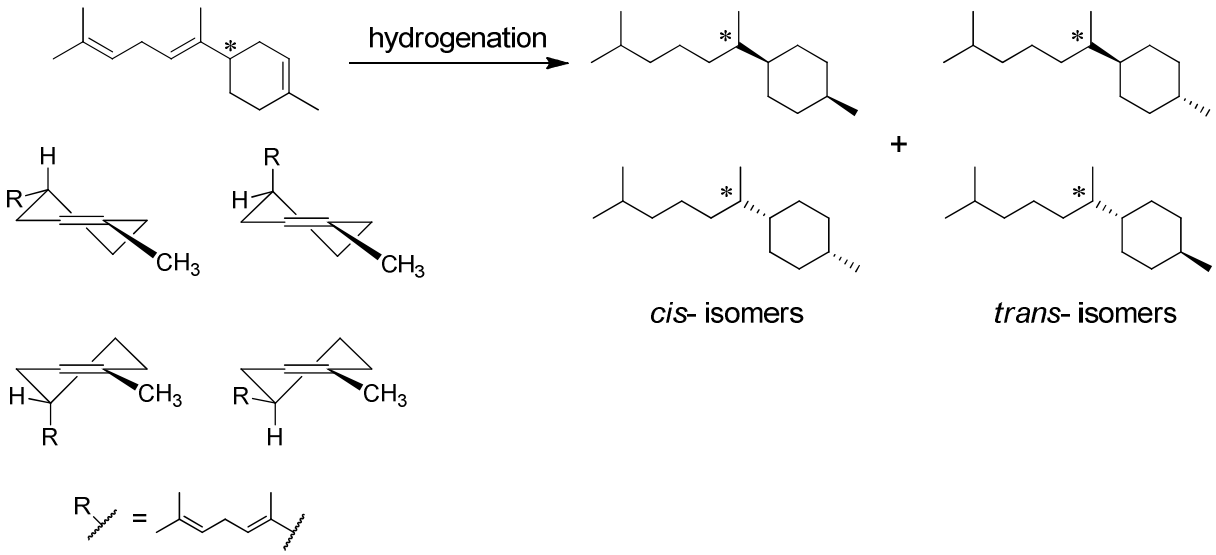
Supplementary Figure S6. COSY of biosynthetic bisabolene



Supplementary Figure S7. ^{1}H NMR of hydrogenated biosynthetic bisabolene (biosynthetic bisabolanes). The lack of vinylic protons (4.6-5.9 ppm) in the ^{1}H NMR spectra confirms the full hydrogenation of bisabolene into two geometric isomers of bisabolane. Individual peak assignment could not be performed due to the complexity of the spectrum. Inset zooms in at region from 0-1.9 ppm.



Supplementary Figure S8. ^{13}C NMR of hydrogenated biosynthetic bisabolene (biosynthetic bisabolanes). The lack of alkene carbons (115-140 ppm) in the spectra confirms the full hydrogenation of bisabolene into two geometic isomers of bisabolane. Two geometric isomers show two sets of peaks.



Supplementary Figure S9. Bisabolane geometric isomers. Based on the chemical structure of α-bisabolene and the reaction mechanism of Pd catalyzed hydrogenation, we speculate we obtained two bisabolane geometric isomers: cis- and trans- isomers on the 1 and 4 positions of the bisabolene cyclohexane ring.

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Supplementary Figure S10. *E. coli* codon optimized *Abies grandis* Ag1

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Supplementary Figure S11. *E. coli* codon optimized *Picea abies* TPS-BIS

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Supplementary Figure S12. *E. coli* codon optimized *Arabidopsis thaliana* TPS13

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Supplementary Figure S13. *E. coli* codon optimized *Arabidopsis thaliana* TPS12

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Supplementary Figure S14. *E. coli* codon optimized *S. cerevisiae* HMG synthase

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gcttagtcgccagcgccatgcgtggctgtaaggcgattaacgccggcggtggcgcgacgaccgtgttaaccaaggatggtatgacgcgcggtccggtc
gtccgcttcccaacgctgaagcgcagcggcgcgtgtaagatttggctggattctgaggagggccaaaacgcgatcaagaaagccttcaactctacgag
ccgtttcgcgcgtttacagcatatccagacctgcctggccggcgacctgctgttcatgcgcttccgcaccaccacgggcgatgcgatgggcatgaacatg
atcagcaagggcgtcgaatatagcctgaaacaaatggtggaagaatatggctgggaggacatggaggttgtctctgtgagcggcaactattgcaccgac
aagaagccggcagccattaactggattgagggtcgcggcaaaagcgtcgtggcagaagcgaccatcccaggcgacgtggtccgtaaggttctgaaga
gcgacgtcagcgccctggttgagttaaatatcgcgaaaaacctggtcggcagcgcgatggcgggcagcgtgggtggctttaacgcacatgcagcgaat
ctggttacggcggttttcttagccttaggtcaggacccagcccaaaatgtcgagagcagcaactgcattaccttaatgaaagaggttgacggtgacctgcg
catcagcgtttctatgccgtctatcgaggtcggcacgatcggcggcggcaccgttttagaaccgcaaggtgcgatgctggatctgctgggcgtgcgcgg
cccacatgcaacggccccaggcaccaatgcccgccaactggcccgtatcgtggcctgcgcggttctggcgggtgagctgagcctgtgcgccgcatta
gccgcgggccatttagttcaatctcacatgacccacaaccgcaagccggcagaaccaaccaagccaaataacctggacgcaaccgacattaaccgtct
gaaggatggcagcgtcacgtgcattaaaagctga

Supplementary Figure S15. *E. coli* codon optimized *S. cerevisiae* HMG reductase

atgtctctgccattcctgacgtctgcgccaggtaaggtgatcatcttcggcgagcactctgcggtgtacaataagccggccgtcgccgcctctgtgtctgc
gttacgcacctacctgctgatcagcgaatcttctgcaccggacacgatcgagctggactttccggacatcagcttcaaccacaagtggagcatcaacgac
ttcaacgcgatcacggaggaccaggtgaacagccaaaagctggccaaagcccagcaagcaaccgacggtctgtctcaggagctggtgtctctgctgg
acccgctgttagcgcagttaagcgagagcttccattaccacgccgcgttctgcttcctgtacatgttcgtttgcctgtgcccgcacgcaaagaacatcaagtt
cagcctgaagagcacgctgccgattggcgcaggcttaggctctagcgcatctatcagcgtgagcctggcgctggcgatggcctatctgggtggcctgat
tggcagcaacgacctggagaaactgagcgaaaacgacaagcacatcgtgaaccagtgggcctttatcggcgagaagtgcattcatggcaccccgagc
ggcattgacaacgcagttgccacgtatggcaacgccctgctgttcgagaaagacagccacaacggcacgatcaacacgaacaacttcaagttcctgga
cgacttcccggcgatcccgatgattctgacctacacccgtatcccacgcagcaccaaggatttagtcgcccgcgtgcgtgttttagtcaccgaaaagttcc
cggaggtgatgaagccgatcctggacgcgatgggcgagtgcgcgctgcagggtctggagatcatgaccaagctgagcaagtgcaagggcaccgac
gatgaggcggtggagaccaacaatgagctgtacgagcagctgctggagctgatccgtatcaatcacggcctgctggtctctatcggtgtgtctcacccgg
gcctggaactgatcaaaaacctgagcgacgacctgcgcattggctctacgaaattaacgggtgcaggtggcggtggctgctctttaacgctgctgcgcc
gtgacattacgcaggagcaaatcgacagcttcaagaagaagctgcaggacgacttcagctacgagacgttcgagacggacctgggcggcacgggctg
ttgcctgctgagcgccaaaaatctgaacaaggacctgaagatcaaaagcctggtgttccagctgttcgaaaacaagacgaccacgaagcagcagatcg
acgacctgttactgccgggtaacaccaatctgccgtggacgtcttaa

Supplementary Figure S16. *E. coli* codon optimized *S. cerevisiae* mevalonate kinase

atgagcgaattacgtgcattcagcgcgccaggtaaggcactgctggccggtggctacctggtgttagacaccaagtacgaggcgttcgtcgtcggcttat
ctgcccgtatgcatgcagttgcccacccgtatggtagcctgcagggctctgacaagttcgaagtgcgtgtgaagagcaagcagttcaaggacggcgagt
ggctgtaccacattagcccaaagagcggcttcatcccggttagcattggtggcagcaagaacccatttatcgagaaggtcattgccaacgtcttcagctac
ttcaagccgaatatggacgattactgcaaccgcaacctgttcgtcatcgacattttcagcgacgacgcgtaccacagccaagaggactctgttacggagc
atcgtggtaaccgccgcctgagcttccacagccatcgcattgaggaggtgccgaagacgggtctgggttctagcgccggtttagttaccgtcttaacgac
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gctctgcgacgtatggtagcaaactggcgcatctggtggacgaggaggactggaacatcaccattaagtctaatcacctgccgagcggcttaacgttatg
gatgggcgatatcaagaacggcagcgaaacggttaagctggtgcagaaagtgaaaaactggtacgacagccacatgccggaaagcctgaagatttac
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cgaaggagagcggcgcggatatcgaaccaccggtccagacgtctctgctggacgactgtcaaaccttaaagggcgtgttaacgtgcctgattccgggc
gcgggtggttacgacgccattgccgtcatcacgaaacaggacgtcgatctgcgcgcacaaacggccaacgacaaacgtttcagcaaagtccaatggct
ggatgttacgcaggccgactggggtgttcgcaaggagaaggacccggaaacgtatctggataagtga

Supplementary Figure S17. *E. coli* codon optimized *S. cerevisiae* phosphomevalonate kinase

Supplementary Table S1. Strains, plasmids and primers

	Description	Reference
Strains		
DH1	F⁻ *endA1 hsdR17 (rk−, mk+) sup*E44 *thi-1* λ⁻ *rec*A1 *gyr*A96 *rel*A1	Hanahan, D., *J Mol Biol* **166**, 557-80 (1983)
EPY300	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 PGAL1-tHMGR PGAL1-upc2-1 erg9::PMET3-ERG9 PGAL1-tHMGR PGAL1-ERG20	Ro, 2006
Plasmids		
JBEI-3360	pMB1, Amp^r, P_{Trc}, Ag1	This study
JBEI-3361	pMB1, Amp^r, P_{Trc}, AgBIS	This study
JBEI-3604	pMB1, Amp^r, P_{Trc}, TPS3	This study
JBEI-3177	pMB1, Amp^r, P_{Trc}, TPS-BIS	This study
JBEI-3167	pMB1, Amp^r, P_{Trc}, TPS13	This study
JBEI-3175	pMB1, Amp^r, P_{Trc}, TPS12	This study
JBEI-3867	pMB1, Amp^r, P_{Trc}, 6His-Ag1	This study
JBEI-3868	pMB1, Amp^r, P_{Trc}, 6His-AgBIS	This study
JBEI-2921	pRS425-Leu2d, P_{Gal1}-Ag1	This study
JBEI-2922	pRS425-Leu2d, P_{Gal1}-AgBIS	This study
JBEI-3869	pRS425-Leu2d, P_{Gal1}-TPS3	This study
JBEI-3870	pRS425-Leu2d, P_{Gal1}-TPS-BIS	This study
JBEI-3871	pRS425-Leu2d, P_{Gal1}-TPS13	This study
JBEI-3872	pRS425-Leu2d, P_{Gal1}-TPS12	This study
JBEI-2704	p15A, Cm^r, P_{LacUV5}, atoB-HMGS-HMGR-MK-PMK-PMD-idi-ispA	Redding-Johanson, 2011
JBEI-2997	p15A, Cm^r, P_{LacUV5}, atoB-HMGS(CO)-HMGR(CO)-MK(CO)-PMK(CO)-PMD-idi-ispA	This study
JBEI-2999	p15A, Cm^r, P_{LacUV5}, atoB-HMGS(CO)-HMGR(CO)-P_{TRC}-MK(CO)-PMK(CO)-PMD-idi-ispA	This study
Primers		
PPY82	ACGCCATGGCTGGCGTTTCTGCT	
PPY86	CGACCCCGGGTTACAGTGGCAGCGGTTC	
PPY207	CGACCCCGGGTTAGAGAGGAAGTGGTTCAA	
PPY208	ACGCCATGGCAGCTTCTACTCTTC	
PPY238	CCATGGCGGGTGTTTCTGC	
PPY239	CCCGGGTTACAGCGGCAGCGGTT	
PPY851	CCATGGAATCTCAGACCACC	
PPY852	CCCGGGTTACAGACGGATCGGGTC	
PPY853	CCATGGAATCTCAGACCAAATTC	
PPY854	CCCGGGTTACAGGTGGATCGGGTC	

PPY855	CCATGGCGACCTCTGTTTCTGTTGAATC
PPY856	CCCGGGTTACAGCGGCAGCGGTT
PPY1176	AGCCATGGCGCATCACCATCACCATCACGGTGTTTCTGC GGTTTCTA
PPY1177	ACGCCATGGCTCATCACCATCACCATCACGGCGTTTCTG CT
MO358	TCATGCGCTAGCATGGCTGGCGTTTCTGCTGTAT
MO359	CATGCACTCGAGTTACAGTGGCAGCGGTTCAATG
MO360	TCATGCGCTAGCATGGCGGGTGTTTCTGCGGTT
MO361	CATGCACTCGAGTTACAGCGGCAGCGGTTCGAT
MO465	CGTGCGCTAGC ATGGCAGCTTCTACTCTTCC
MO466	CTGCACTCGAGTTAGAGAGGAAGTGGTTCAATAAG
MO467	CGTGCGCTAGC ATGACCTCTGTTTCTGTTGAATC
MO468	CTGCACTCGAGTTACAGCGGCAGCGGTTCGA
MO469	CGTGCGCTAGCATGGAATCTCAGACCAAATTCG
MO470	CTGCACTCGAGTTACAGGTGGATCGGGTCGA
MO471	CGTGCGCTAGCATGGAATCTCAGACCACCTTC
MO472	CTGCACTCGAGTTACAGACGGATCGGGTCGAT

Supplementary Methods

- pAg1 (pJBEI-3360): The plant bisabolene synthase gene from *Abies grandis* was obtained from Prof. Croteau (pSBAg1) but was also commercially synthesized. The commercially synthesized plant Ag1 was used as a template and amplified with primers PPY82/PPY86.
- pAgBIS (pJBEI-3361): The *E. coli* codon optimized bisabolene synthase gene from *A. grandis* was commercially synthesized and amplified with primers PPY238/PPY239.
- pPmTPS3 (pJBEI-3604): The bisabolene synthase gene from *Pseudotsuga menziesii* obtained from Prof. Bohlmann (pPmeTPS3) and amplified with primers PPY207/PPY208.
- pPaTPS-BIS (pJBEI-3177): The *E. coli* codon optimized bisabolene synthase gene from *Picea abies* (AAS47689) was commercially synthesized and amplified with primers PPY855/PPY856. Primer PPY 855 introduces an alanine (GCG) after the start codon to generate the NcoI restriction site.
- pAtTPS13 (pJBEI-3167): The *E. coli* codon optimized bisabolene synthase gene from *Arabidopsis thaliana* TPS13 (NP_193066) was commercially synthesized and amplified with primers PPY853/PPY854.
- pAtTPS12 (pJBEI-3175): The *E. coli* codon optimized bisabolene synthase gene from *A. thaliana* TPS12 (NP_139064) was commercially synthesized and amplified with primers PPY851/PPY852.
- pHis_Ag1 (pJBEI-3867): Ag1 was amplified from pAg1 using primers PPY1177/PPY86. Primer PPY1177 introduces 6 His-Tag at the N-terminus of the protein.
- pHis_BIS (pJBEI-3868): AgBIS was amplified from pAgBIS using primers PPY1176/PPY239. Primers PPY1176 introduces a 6 His-tag at the N-terminus of the protein.
- pRSLeu2d-Ag1 (pJBEI-2921): The bisabolene synthase gene was amplified from pAg1 using primers MO358/MO359.
- pRSLeu2d-AgBIS (pJBEI-2922): The bisabolene synthase gene was amplified from pAgBIS using primers MO360/MO361.
- pRSLeu2d-PMTPS3 (pJBEI-3869): The bisabolene synthase gene was amplified from pPmTPS3 using primers MO465/MO466.
- pRSLeu2d-PaTPS-BIS (pJBEI-3870): The bisabolene synthase gene was amplified from pPaTPS-BIS using primers MO467/MO468.

- pRSLeu2d-AtTPS13 (pJBEI-3871): The bisabolene synthase gene was amplified from pAtTPS13 using primers MO469/MO470.
- pRSLeu2d-AtTPS12 (pJBEI-3872): The bisabolene synthase gene was amplified from pAtTPS12 using primers MO471/MO472.