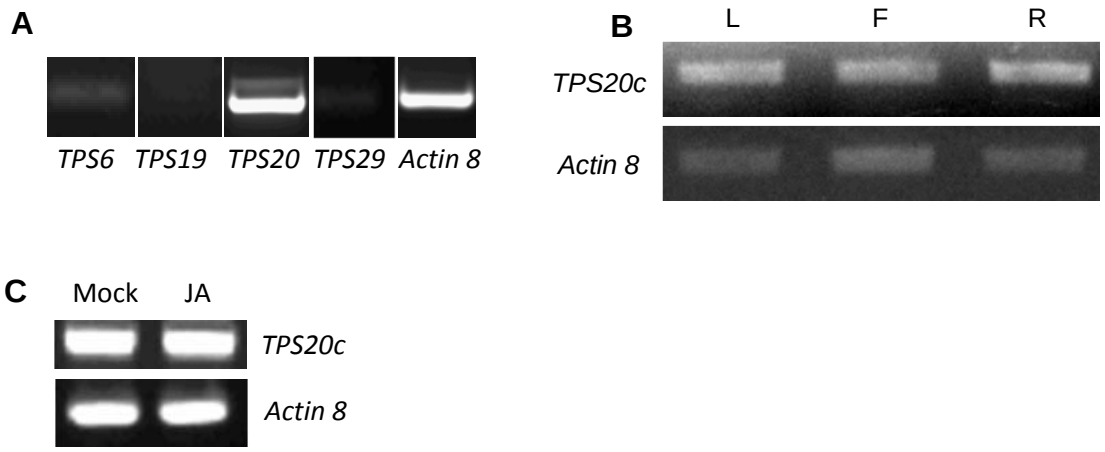


Figure S1. *TPS* gene expression in Cvi. (A) Expression of selected *TPS* genes in the Cvi root. (B) *TPS20c* gene expression in Cvi tissues. L: leaf; F: flower; R: root. (C) *TPS20c* gene expression in axenically grown roots of Cvi upon 24 h of treatment with 100 μM jasmonic acid.

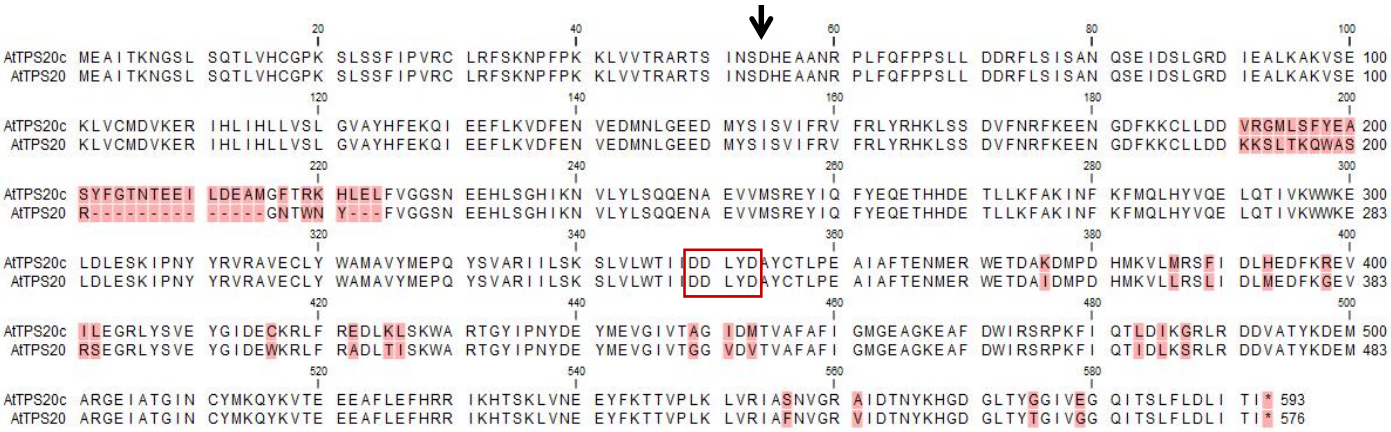


Figure S2. Amino acid sequence alignment of TPS20 (Col ecotype) and TPS20c (Cvi ecotype). Amino acid insertions and substitutions are highlighted in pink. The DDXXD motif is indicated with a red box. The arrow shows the position of the truncation of the transit peptide for protein expression in *E.coli* and transit peptide fusion to eGFP for transient expression in tobacco leaves.

Figure S3. Nucleotide sequence alignment of *TPS20* (ecotype Col) and *TPS20c* (ecotype Cvi). The *TPS20c* sequence has a single nucleotide deletion (675_676del) and a 52 bp insertion (571G to 622G) in comparison to the *TPS20* sequence. Nucleotide insertions, deletions, and substitutions are highlighted in pink.

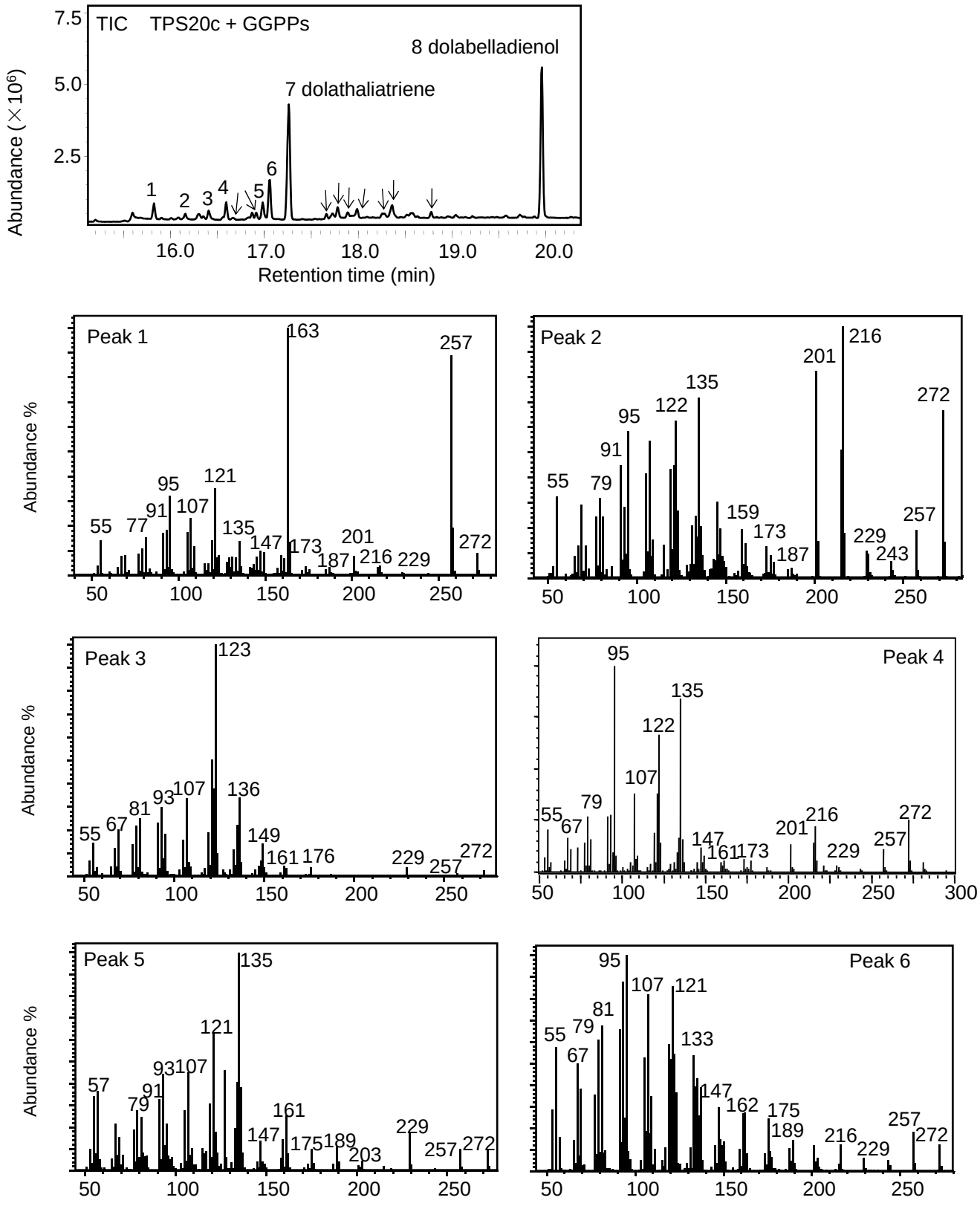


Figure S4. Mass spectra of minor diterpene products 1-6 of TPS20c.

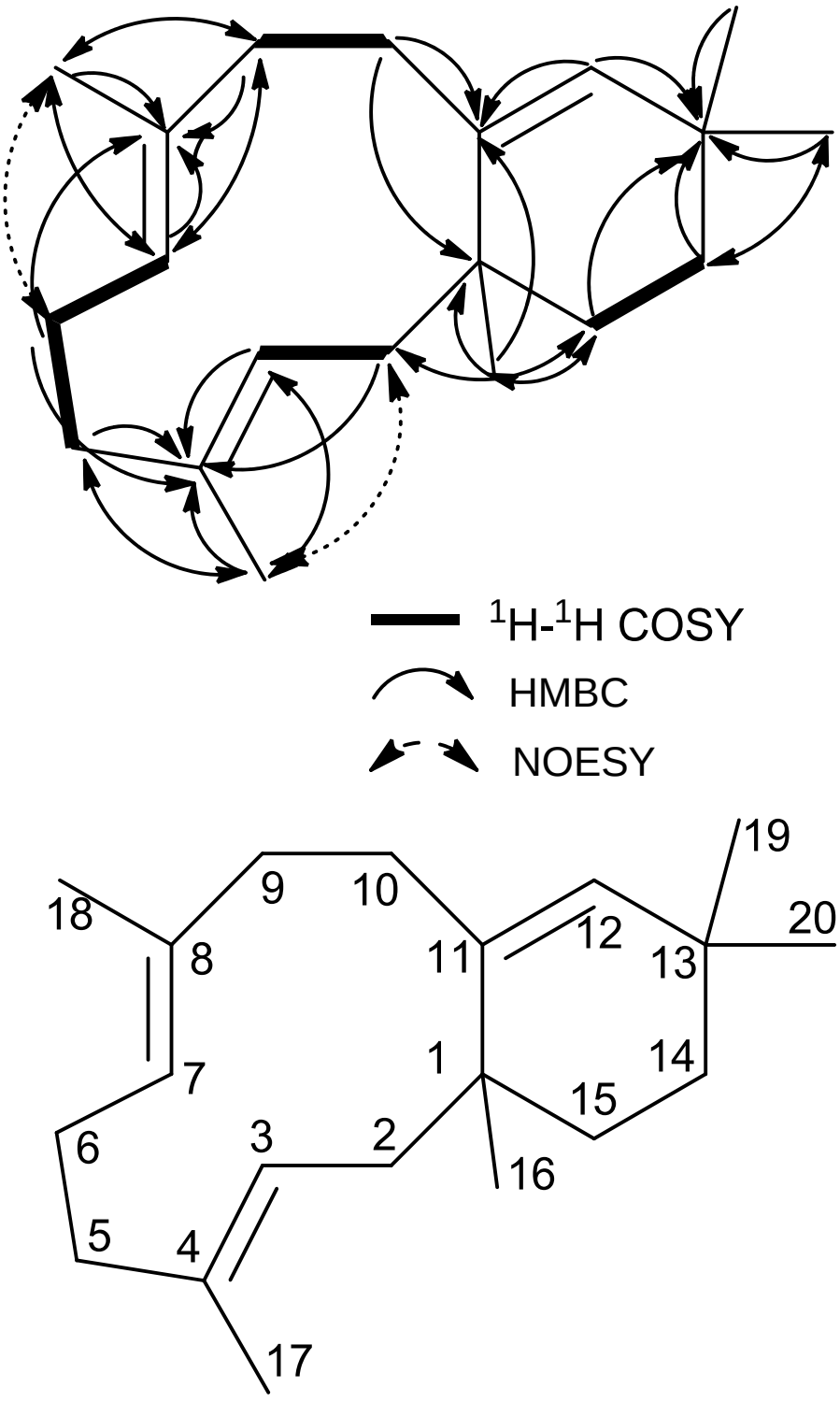


Figure S5. Important correlations of COSY, HMBC and NOESY observed for the major diterpene product of TPS20c.

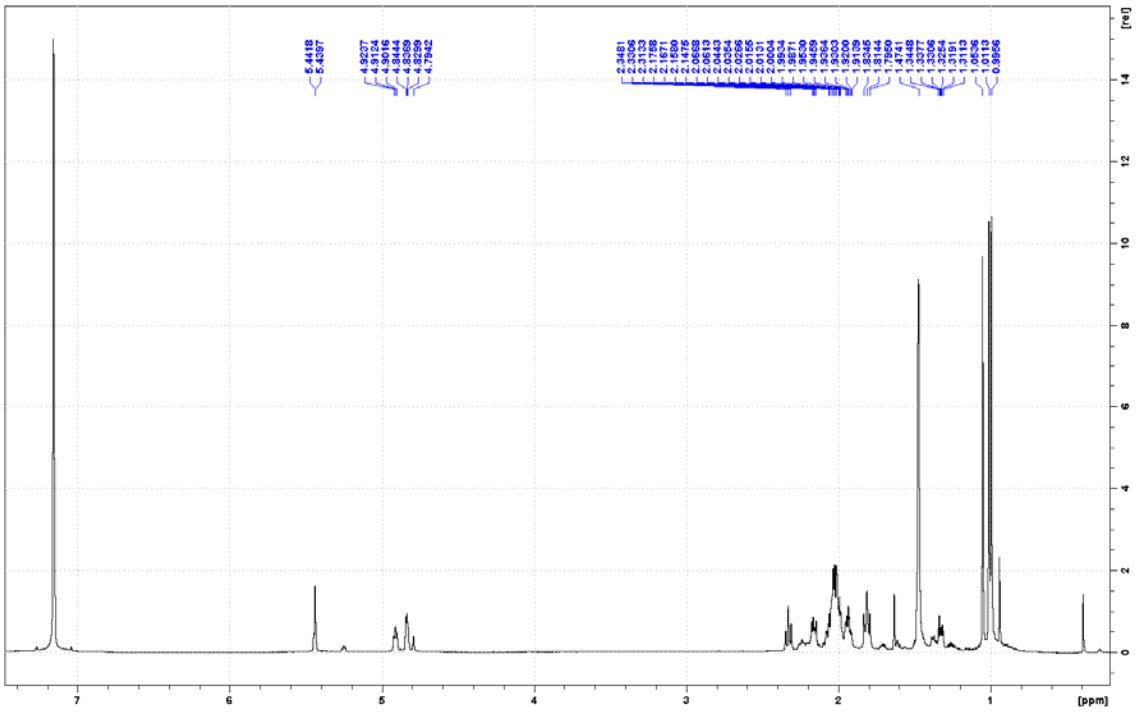


Figure S6. ^{1}H spectrum of the TPS20c major diterpene compound (solvent C_6D_6).

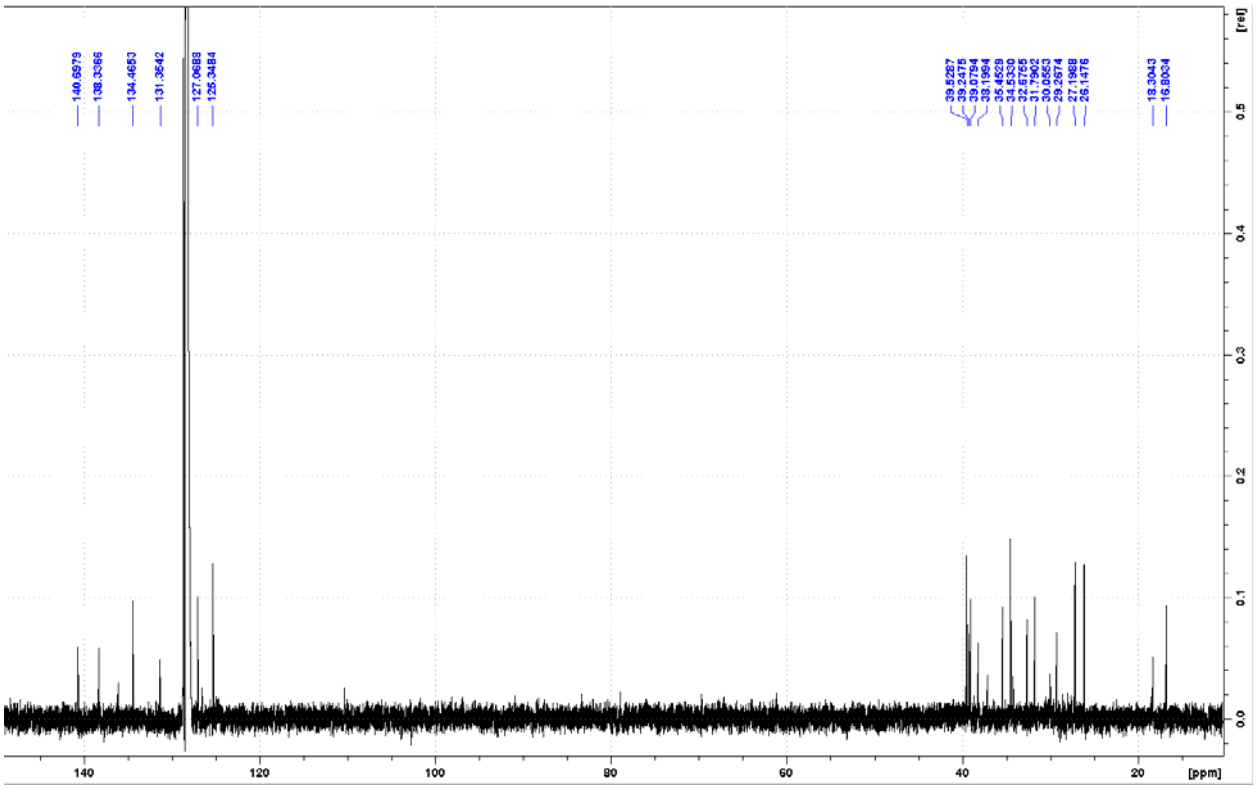


Figure S7. ^{13}C spectrum of the TPS20c major diterpene compound (solvent C_6D_6).

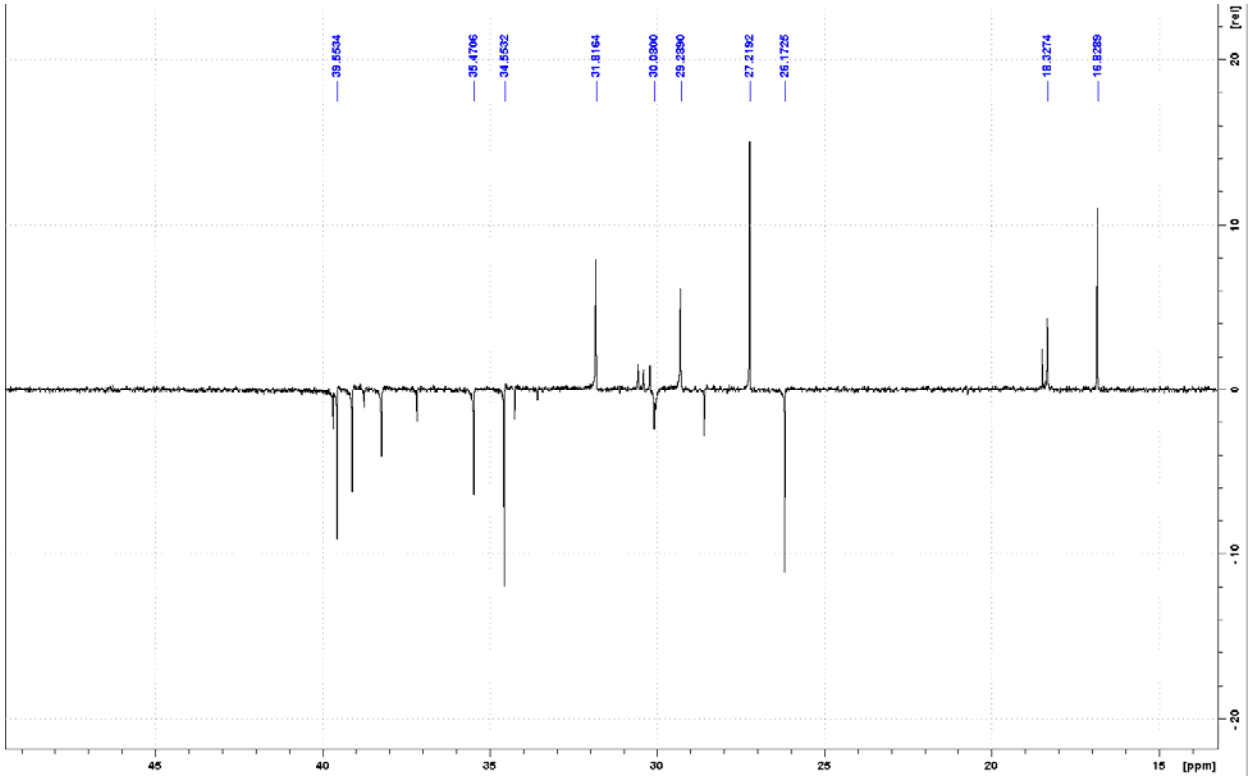


Figure S8A. ^{13}C DEPT135 of the TPS20c major diterpene compound (solvent C_6D_6).

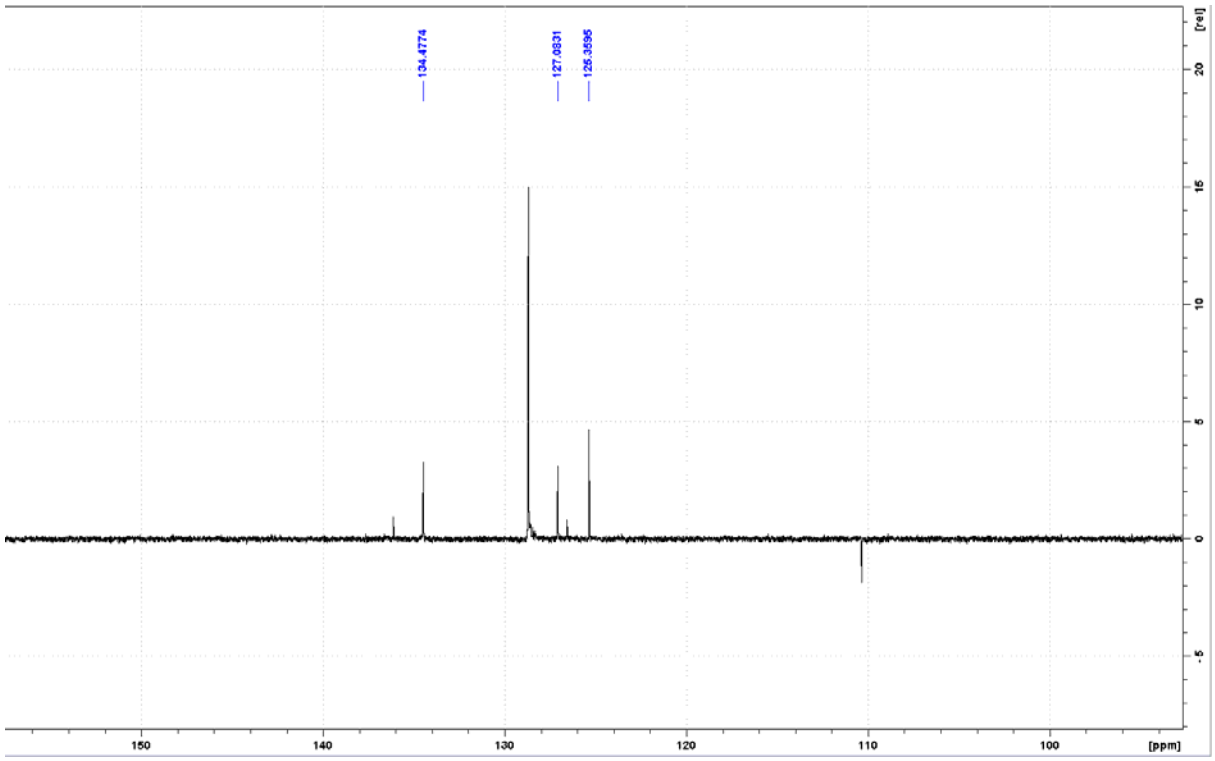


Figure S8B. ^{13}C DEPT135 of the TPS20c major diterpene compound (solvent C_6D_6).

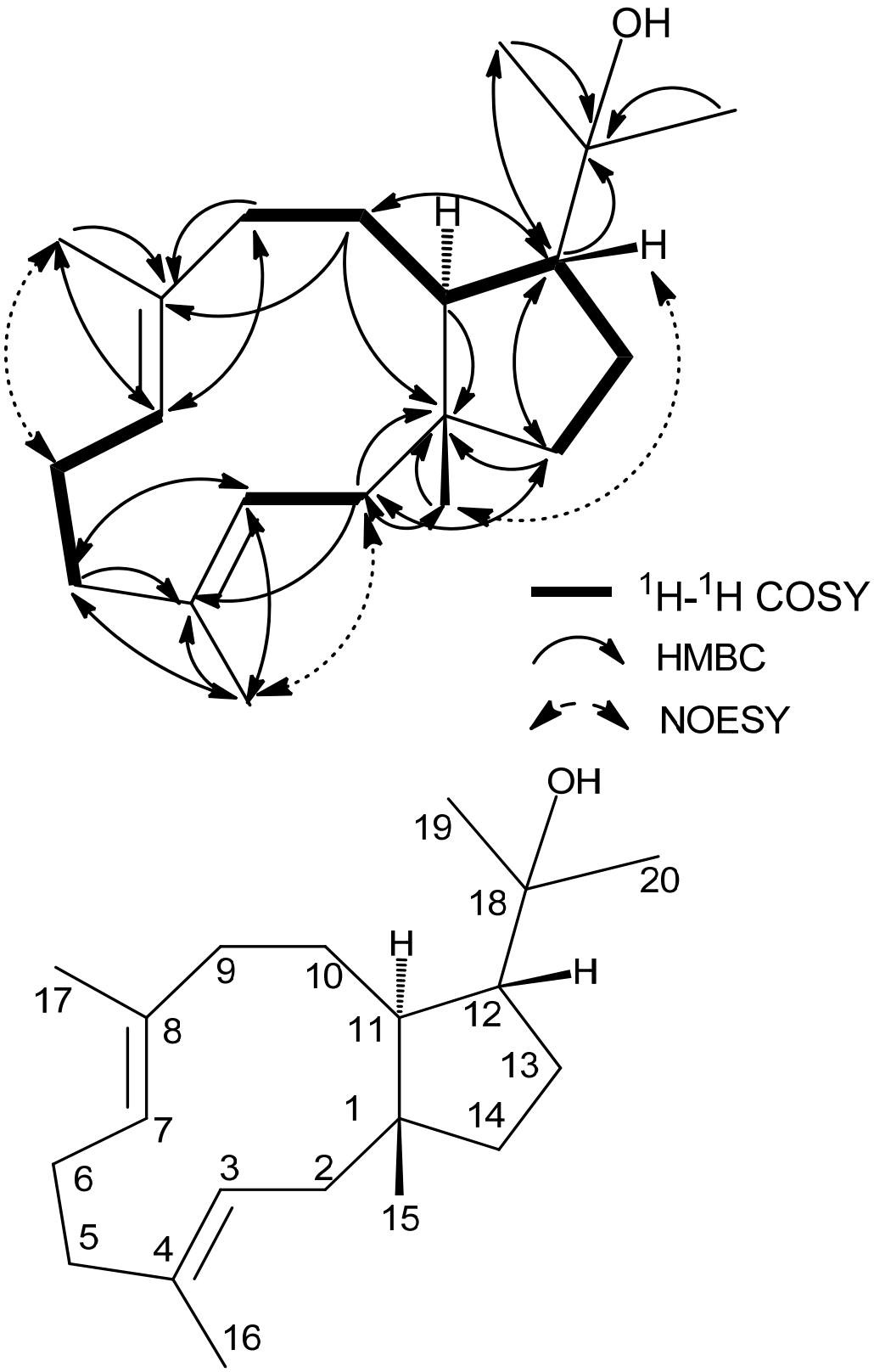


Figure S9. Important correlations of COSY, HMBC and NOESY observed for the major diterpene alcohol product of TPS20c.

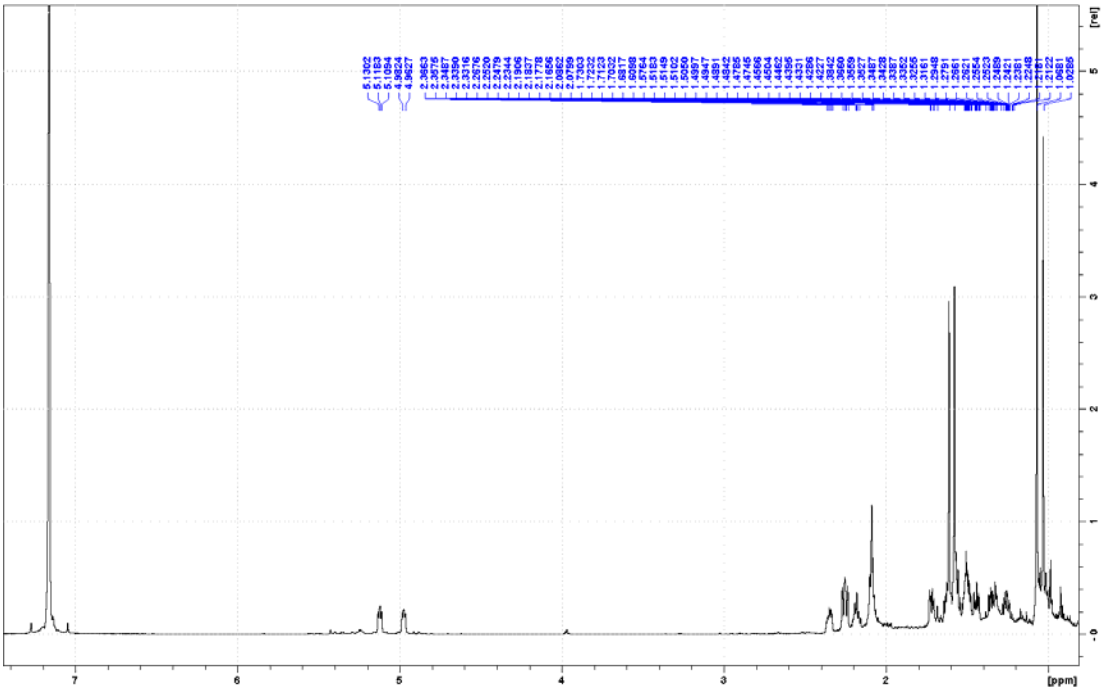


Figure S10. ^{1}H spectrum of the TPS20c diterpene alcohol compound (solvent C_6D_6).

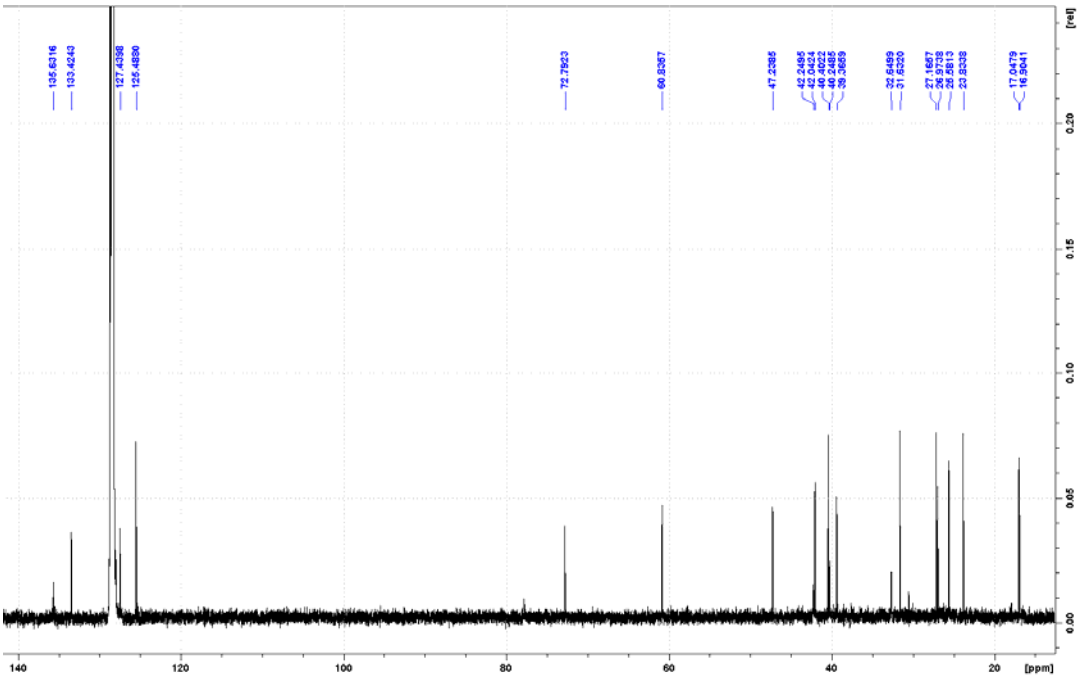


Figure S11. ^{13}C spectrum of the TPS20c diterpene alcohol compound (solvent C_6D_6).

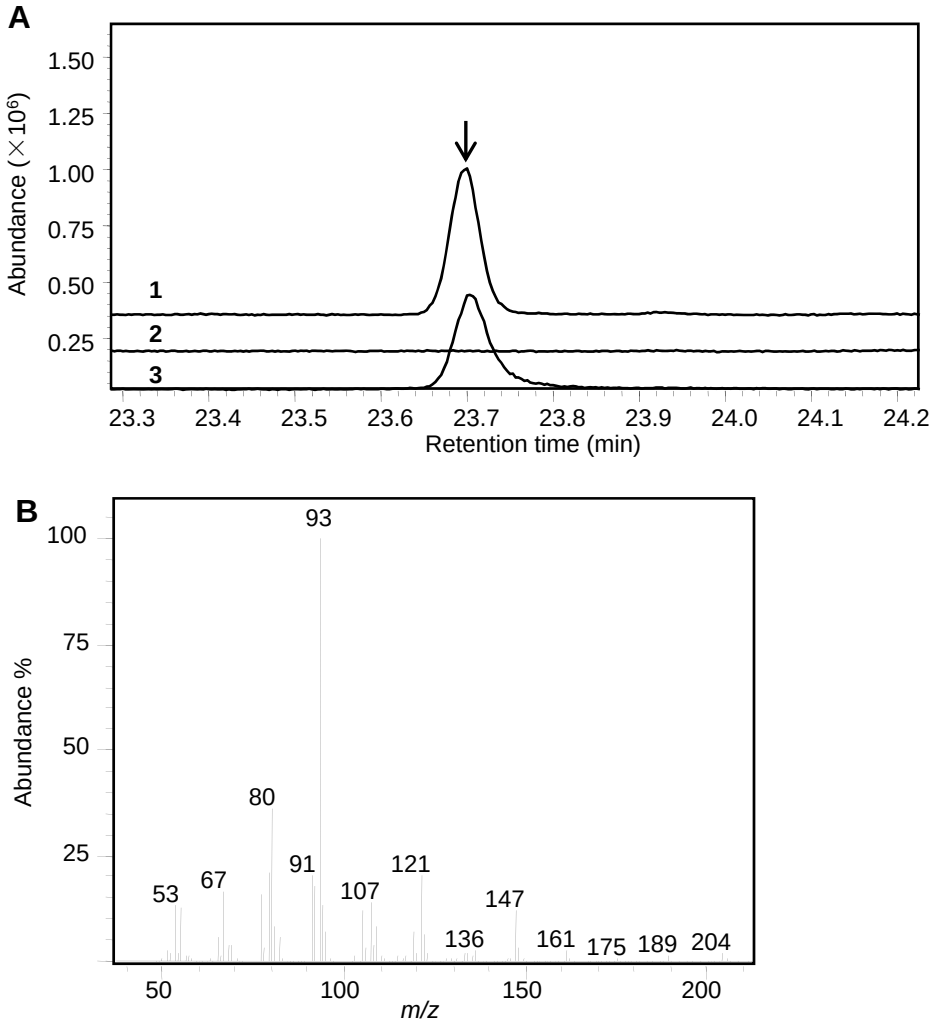


Figure S12. TPS20c reacts with (*E,E*)-FPP to produce α-humulene. (A) GC-MS chromatogram of an extract from *E. coli* coexpressing TPS20c and (*E,E*)-FPP synthase. 1, co-expression of TPS20c and (*E,E*)-FPP synthase. 2, co-expression of empty vector and (*E,E*)-FPP synthase. 3, standard of α-humulene. **(B)** Mass spectrum of α-humulene (arrow marked).

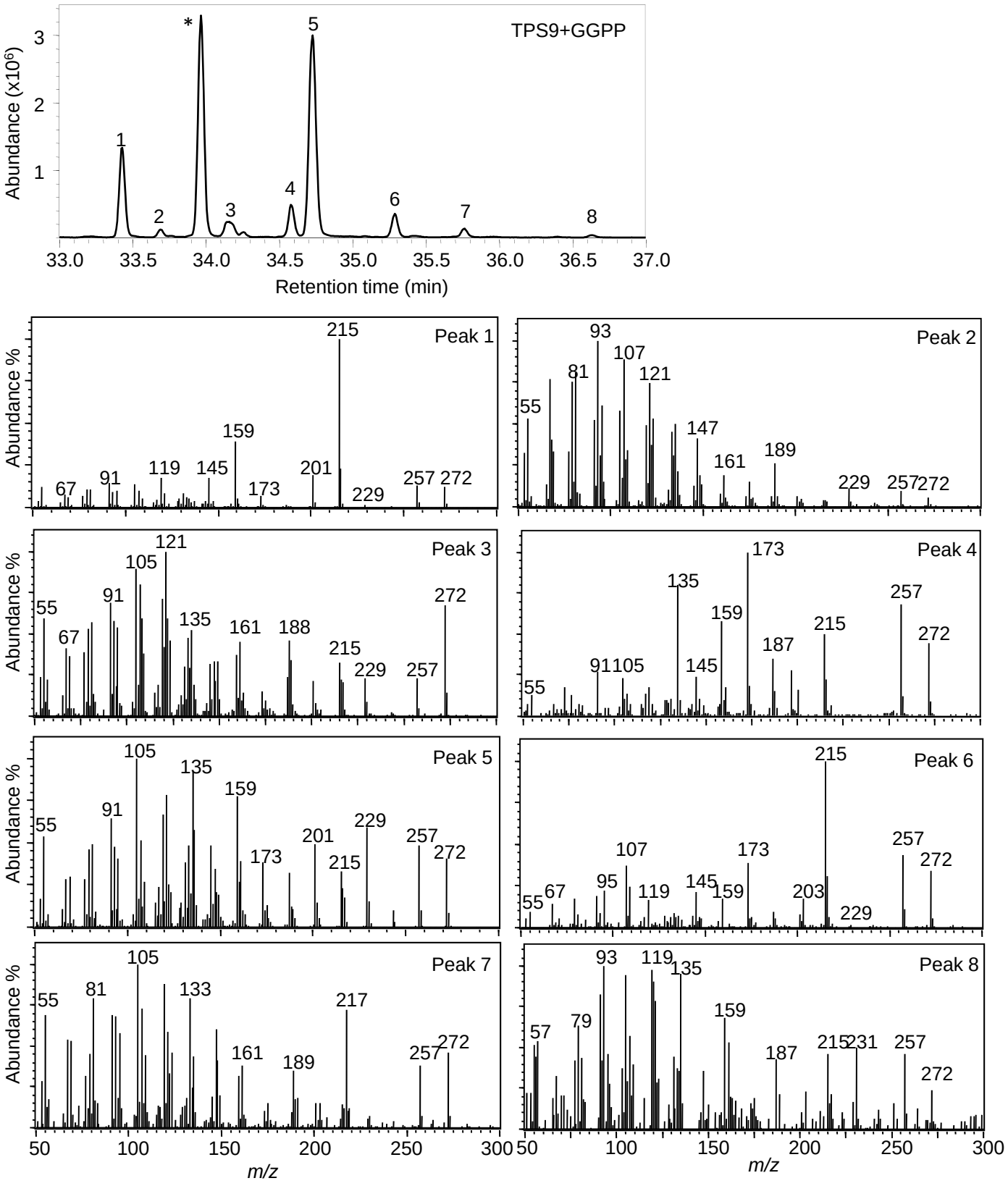


Figure S13. Mass spectra of minor diterpene products of recombinant TPS9.
The mass spectrum of the major diterpene product (labeled with asterisk) is shown in Figure 7C.

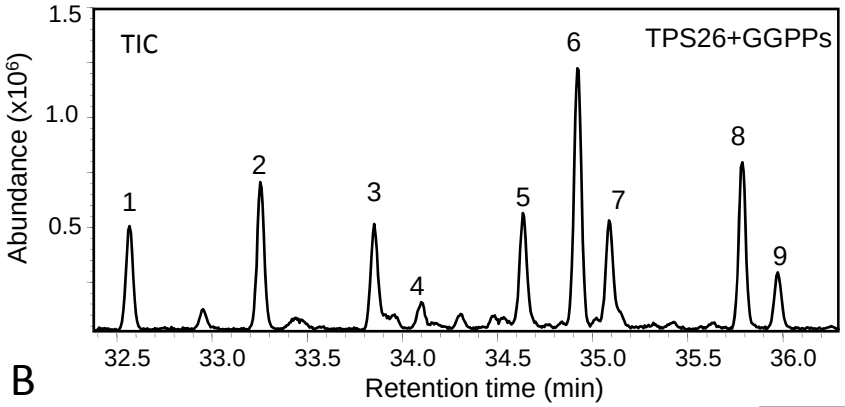
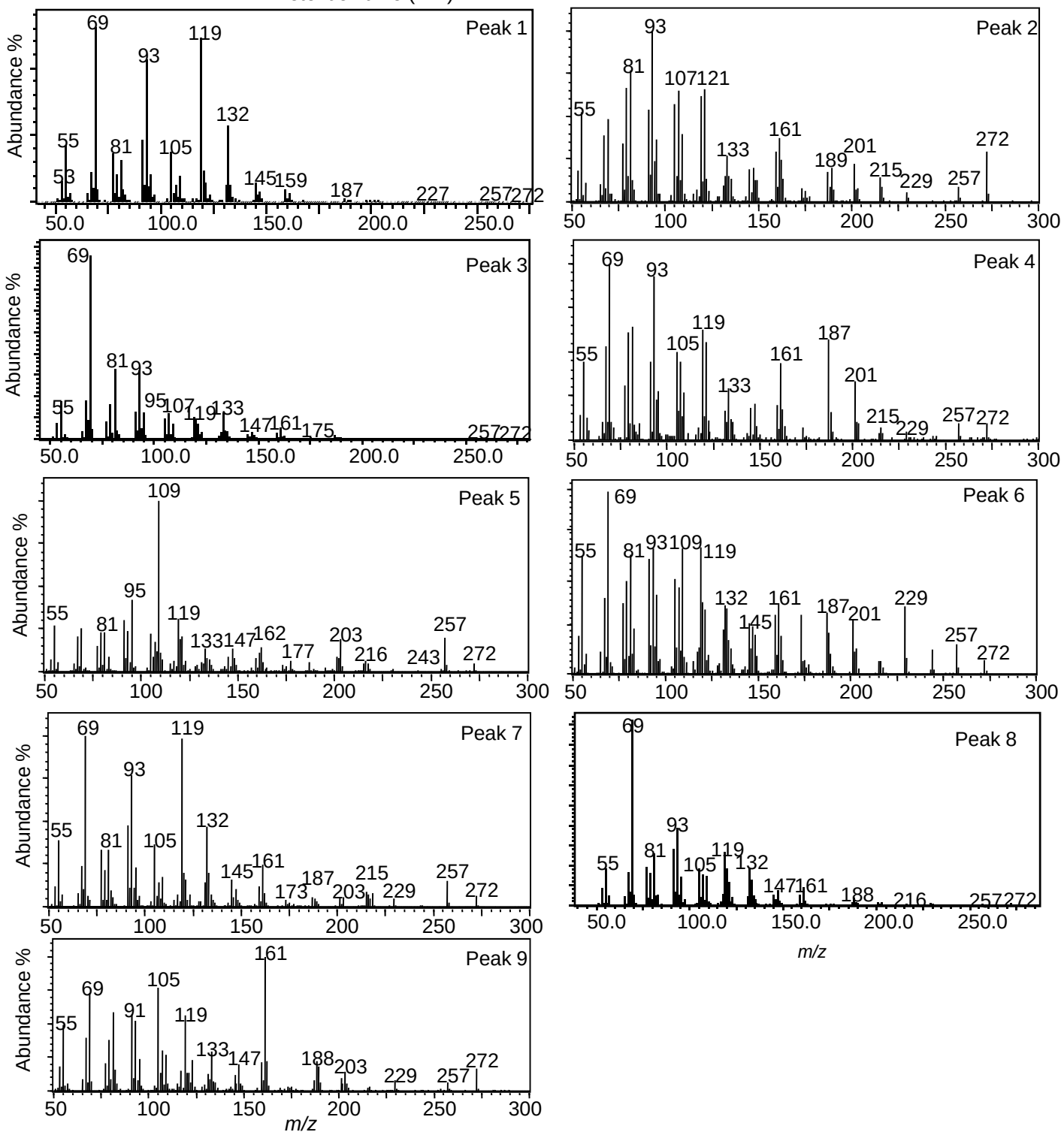


Figure S14. Diterpene synthase activity of Col TPS26. (A) GC-MS chromatogram of a culture extract from *E. coli* coexpressing TPS26 and GGPP synthase. (B) Mass spectra of TPS26 products.



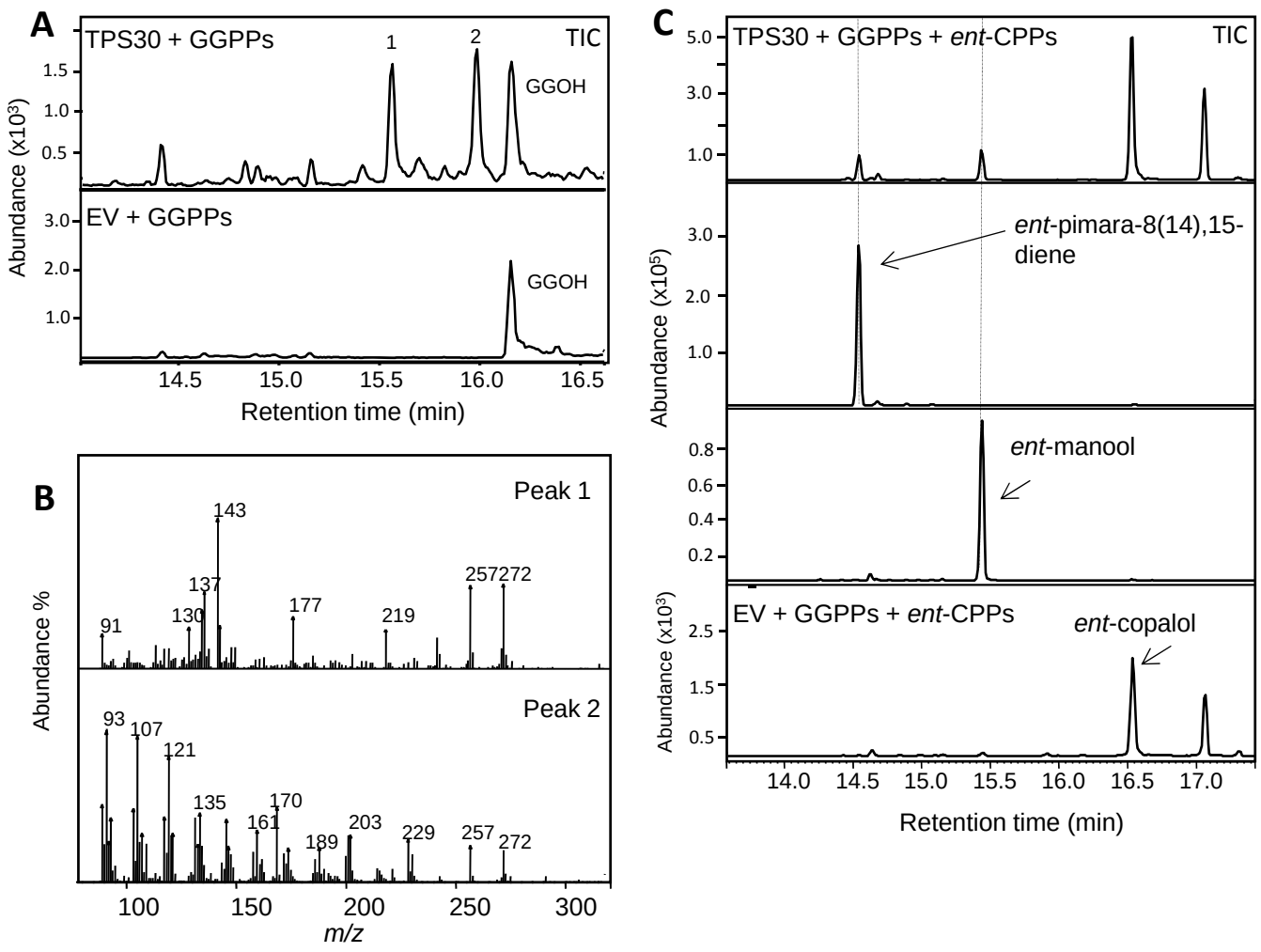


Figure S15. Diterpene synthase activity of Col TPS30. (A) Upper panel: GC-MS chromatogram of a culture extract from *E. coli* coexpressing truncated TPS30 and GGPP synthase. Lower panel: Extract of *E. coli* expressing only GGPP synthase. (B) Mass spectra of the TPS30 diterpene products 1 and 2 as shown in (A). (C) Upper panel: GC-MS chromatogram of a culture extract from *E. coli* coexpressing truncated TPS30, GGPP synthase and *ent*-CPP synthase. Diterpene products are marked with lines and arrows corresponding to authentic standards. Lower panel: Extract of *E. coli* expressing only GGPP synthase and *ent*-CPP synthase. EV, empty vector.

Table S1. Primers used in this study.

Primer name	Sequence 5'-3'
RT-PCR	
TPS6-F	GAGGTCGGGGTAGCTTCG
TPS6-R	CTCTCCAGCGAAGGTGTACC
TPS19-F	GCCACGTACCAACCTTTGATG
TPS19-R	GGGTATTGGGTGGACGTACAAG
TPS20-F	GTTGGAGGCAGCAATGAGGAAC
TPS20-R	CTAAATGGTGATTAGATCAAGG
TPS29-F	GTGTGAAACAAGTGGTAGAGAAG
TPS29-R	GGGTATAGGGTGGAGGTACAAG
Actin 8-F	ATGAAGATTAAGGTCGTGGCAC
Actin 8-R	GTTTTTATCCGAGTTTGAAGAGGC
Cloning of full length and truncated *TPS* cDNAs	
TPS6-F	AAAGTCGACATGGAGGCCATAACAAAATATG
TPS6-F-truncated	AACCATGGCAACGACGAATCCTACAGA
TPS6-R	AAACTCGAGTTAAAGAGTGATGAGATCGACA
TPS9-F-truncated	AACATATGGATGATAAAGAACGTACTCGC
TPS9-R	AACTCGAGCTACAACATTTTCAGAGTATGAT
TPS19-F	AACCATGGAAGCAACAAGAATGGGT
TPS19-F-truncated	AACCATGGCATCTACTAAGAGTAGTGATGATC
TPS19-R	AACTCGAGTTATGAAAGGGGTATTGGGTG
TPS20-F	AACCATGGAAGCAATAACTAAAAATGGG
TPS20-F-truncated	AACCATGGATCATGAAGCCGCAAATCGT
TPS20-F-truncated (His-tag)	AACATATGGATCATGAAGCCGCAAATCGT
TPS20-R	AAACTCGAGCTAAATGGTGATTAGATCAAGGA
TPS22-F-truncated	CACCACTCTTTCCCGGAGATCAA
TPS22-R	TCAAAGCGGAAGAGGATGGAAG
TPS25-F-truncated	CACCAACTTCTCTCTCTTTCCTTGC
TPS25-R	TCAAAGAGGTATTGGATGGAGG
TPS26-F-truncated	AACCATGGCACTTCGTTTAAAGGCCACTAATAC
TPS26-R	AAACTCGAG TCAAAGACCGATACGATCGA
TPS29-F	AACCATGGAAGCAATAAGAATAGGTTT
TPS29-F-truncated	AACCATGGCAAGTTCTTCTAAGAAGAGTA
TPS29-R	AACTCGAGTTATGAAAGGGGTATAGGGTG
TPS30-F	AACCATGGCAGTAGCAAGAACGGT
TPS30-F-truncated	AACCATGGCTACTACTAAGAGTTCGACT
TPS30-R	AACTCGAGTCAAACAGGAATGGGATGGAT

Table S2. ^{1}H-NMR and ^{13}C-NMR assignments for the major diterpene product of TPS20c (solvent: benzene-d_6).

position	δ_C (ppm)	δ_H (ppm)	Multiplicity, J in Hz
1	39.2	-	
2	39.1	2.01(2H)	m
3	127.1	4.84	t, 5.6
4	131.4	-	
5	39.5	1.93 (2H)	m
6	26.1	2.03	m
		2.07	m
7	125.3	4.91	t, 8.4
8	138.3	-	
9	38.2	2.33	m
		2.16	m
10	30.0	1.82	m
		2.02	m
11	140.7	-	
12	134.5	5.44	d, 1.24
13	32.7	-	
14	34.5	1.48 (2H)	m
15	35.4	1.33	m
		1.82	m
16	27.2	0.99(3H)	s
17	16.8	1.48(3H)	s
18	18.3	1.47(3H)	s
19	29.3	1.05(3H)	s
20	31.8	1.01(3H)	s

Table S3. ^{1}H-NMR and ^{13}C-NMR assignments for the major diterpene alcohol product of TPS20c (solvent: benzene-d_6).

position	δ_C (ppm)	δ_H (ppm)	Multiplicity, J in Hz
1	47.2	-	
2	40.2	1.72	dd, 13.5, 6.1
		2.25	dd, 13.5, 9.8
3	125.5	5.12	dd, 9.8, 6.1
4	133.4	-	
5	40.4	2.08 (2H)	m
6	25.6	2.09	m
		2.18	m
7	127.4	4.97	dd, 9.2, 5.6
8	135.6	-	
9	39.4	2.25	m
		2.35	m
10	32.6	1.49	m
		1.58	m
11	42.0	1.63	m
12	60.8	1.56	m
13	27.2	1.25	m
		1.50	m
14	42.2	1.35	m
		1.44	m
15	23.8	1.03(3H)	s
16	16.9	1.58(3H)	s
17	17.0	1.61(3H)	s
18	72.8	-	
19	27.0	1.07(3H)	s
20	31.6	1.07(3H)	s