

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 μ M jasmonic acid.

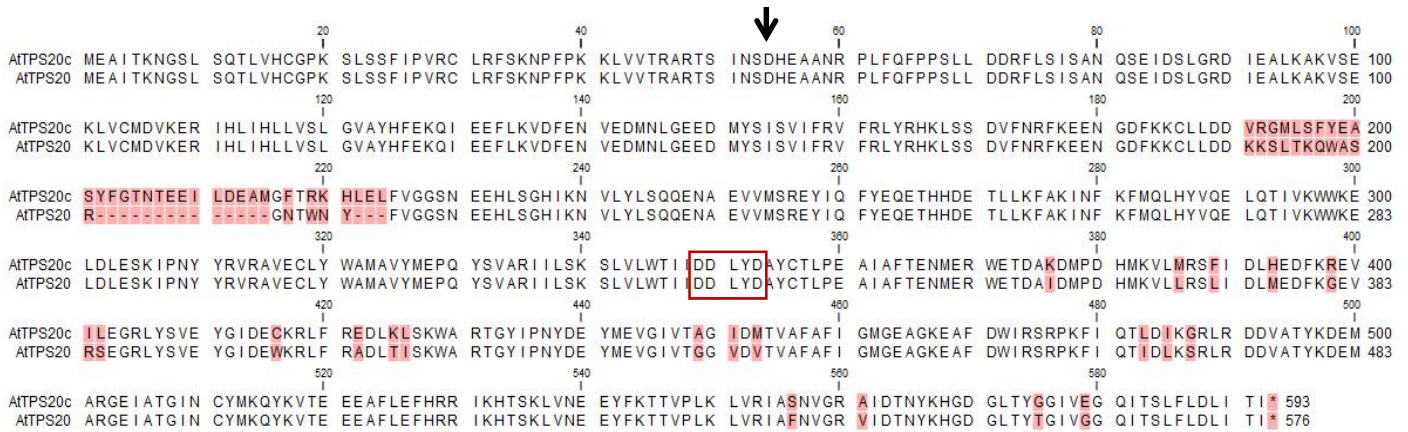


Figure S2. Amino acid sequence alignment of TPS20 (Col ecotype) and TPS20c (Cvi ecotype). Amino acid insertions and substitutions are highlighted in pink. The DDXXD motif is indicated with a red box. The arrow shows the position of the truncation of the transit peptide for protein expression in *E.coli*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

			20		40		60		80		100
AITPS20c	ATGGAAGCAA	TAACTAAAA	TGGGTCGCTC	TCTCAAACCTC	TTGTTTCATTG	TGGGCCAAAA	AGCTTGAGCT	CGTTCATCCC	AGTTTCGCTGT	CTTAGGTTTT	100
AITPS20	ATGGAAGCAA	TAACTAAAA	TGGGTCGCTC	TCTCAAACCTC	TTGTTTCATTG	TGGGCCAAAA	AGCTTGAGCT	CGTTCATCCC	AGTTTCGCTGT	CTTAGGTTTT	100
		120		140		160		180		200	
AITPS20c	CTAAGAATCC	GTTCCCCAAA	AAGTTGGTCC	TAAGTAGAGC	TAGGACGAGC	ATTAACAGTG	ATCATGAAGC	CGCAAATCGT	CCGTTATTTTC	AGTTTCCACC	200
AITPS20	CTAAGAATCC	GTTCCCCAAA	AAGTTGGTCC	TAAGTAGAGC	TAGGACGAGC	ATTAACAGTG	ATCATGAAGC	CGCAAATCGT	CCGTTATTTTC	AGTTTCCACC	200
		220		240		260		280		300	
AITPS20c	TTCCCTATTG	GATGATCGCT	TCCTTTCCAT	TTCCGCCAAC	CAGTCGGAAA	TTGATAGCCT	TGGGAGAGAT	ATCGAGGCAC	TTAAGGCTAA	AGTGAGTGAA	300
AITPS20	TTCCCTATTG	GATGATCGCT	TCCTTTCCAT	TTCCGCCAAC	CAGTCGGAAA	TTGATAGCCT	TGGGAGAGAT	ATCGAGGCAC	TTAAGGCTAA	AGTGAGTGAA	300
		320		340		360		380		400	
AITPS20c	AAGCTGGTTT	GTATGGACGT	AAAGGAGAGG	ATTCACCTGA	TCCATCTGCT	AGTCAGTCTT	GGAGTCGCTT	ACCATTTTCG	GAAACAGATC	GAAGAATTCC	400
AITPS20	AAGCTGGTTT	GTATGGACGT	AAAGGAGAGG	ATTCACCTGA	TCCATCTGCT	AGTCAGTCTT	GGAGTCGCTT	ACCATTTTCG	GAAACAGATC	GAAGAATTCC	400
		420		440		460		480		500	
AITPS20c	TGAAAGTTGA	TTTCGAGAAC	GTGGAGGATA	TGAACCTGGG	AGAAGAGSAT	ATGTATAGCA	TTTCTGTCAT	CTTCAGGGTT	TTTAGGCTGT	ACCGTCACAA	500
AITPS20	TGAAAGTTGA	TTTCGAGAAC	GTGGAGGATA	TGAACCTGGG	AGAAGAGSAT	ATGTATAGCA	TTTCTGTCAT	CTTCAGGGTT	TTTAGGCTGT	ACCGTCACAA	500
		520		540		560		580		600	
AITPS20c	GTTATCTTCC	GATGTGTTCA	ATAGGTTTAA	AGAAGAAAAT	GGGGATTTTA	AGAAATGCCT	TCTCGATGAT	GTAAGAGGTA	TGCTAAGCTT	CTACGAAAGT	600
AITPS20	GTTATCTTCC	GATGTGTTCA	ATAGGTTTAA	AGAAGAAAAT	GGGGATTTTA	AGAAATGCCT	TCTCGATGAT	-----	-----	-----	570
		620		640		660		680		700	
AITPS20c	TCATATTTTG	GGACGAATAC	AGAAGAAATC	CTTGACGAAG	CAATGGGCTT	CACGCGGAAA	CACCTGGAAC	TATTTGTTG	GAGGCAGCAA	TGAGGAACAT	699
AITPS20	-----	-----	AGAAGAAATC	CTTGACGAAG	CAATGGGCTT	CACGCGGAAA	CACCTGGAAC	TATTTGTTG	GAGGCAGCAA	TGAGGAACAT	648
		720		740		760		780		800	
AITPS20c	TTATCTGGGC	ACATAAAAAA	CGTGTGTGAT	CTTTCTCAGC	AAGAAAAATG	AGAGGTGGTG	ATGTCAAGAG	AATATATACA	GTTCTATGAA	CAAGAGACAC	799
AITPS20	TTATCTGGGC	ACATAAAAAA	CGTGTGTGAT	CTTTCTCAGC	AAGAAAAATG	AGAGGTGGTG	ATGTCAAGAG	AATATATACA	GTTCTATGAA	CAAGAGACAC	748
		820		840		860		880		900	
AITPS20c	ATCACGACGA	GACCTTGCTA	AAATTTGCCA	AGATCAATTT	CAAGTTCATG	CAGTTGCATT	ATGTTCAAGA	GCTTCAAAC	ATCGTGAAAT	GGTGGAAAGA	899
AITPS20	ATCACGACGA	GACCTTGCTA	AAATTTGCCA	AGATCAATTT	CAAGTTCATG	CAGTTGCATT	ATGTTCAAGA	GCTTCAAAC	ATCGTGAAAT	GGTGGAAAGA	848
		920		940		960		980		1,000	
AITPS20c	GCTTGACTTG	GAATCAAAGA	TCCCTAATTA	CTACAGAGTT	AGAGCCGTGG	AGTGCCTCTA	CTGGGCAATG	GCAGTGATA	TGGAGCCACA	ATATTCAGTT	999
AITPS20	GCTTGACTTG	GAATCAAAGA	TCCCTAATTA	CTACAGAGTT	AGAGCCGTGG	AGTGCCTCTA	CTGGGCAATG	GCAGTGATA	TGGAGCCACA	ATATTCAGTT	948
		1,020		1,040		1,060		1,080		1,100	
AITPS20c	GCTAGAATAA	TACTGAGCAA	GTCCTTGGTG	TTGTGGACCA	TTATAGATGA	CCTATATGAT	GCCTATTGTA	CTCTCCAGA	AGCTATTGCT	TTCAGTAAA	1099
AITPS20	GCTAGAATAA	TACTGAGCAA	GTCCTTGGTG	TTGTGGACCA	TTATAGATGA	CCTATATGAT	GCCTATTGTA	CTCTCCAGA	AGCTATTGCT	TTCAGTAAA	1048
		1,120		1,140		1,160		1,180		1,200	
AITPS20c	ATATGAAAAG	GTGGGAAACT	GATGCCAATG	ACATGCCAGA	TCATATGAAG	GTCCTCTATG	GGTCATTGAT	AGACCTTAC	GAAGATTTCA	AAGGAGAAGT	1199
AITPS20	ATATGAAAAG	GTGGGAAACT	GATGCCAATG	ACATGCCAGA	TCATATGAAG	GTCCTCTATG	GGTCATTGAT	AGACCTTATG	GAAGATTTCA	AAGGAGAAGT	1148
		1,220		1,240		1,260		1,280		1,300	
AITPS20c	GATTTTAGAA	GGGAGATTGT	ACAGTGTGGA	ATATGGAATA	GACGAGTGA	AAAGATTATT	CAGAGAGGAC	CTTAACTAT	CAAAGTGGGC	ACGCACGGGA	1299
AITPS20	GAGATCAGAA	GGGAGATTGT	ACAGTGTGGA	ATATGGAATA	GACGAGTGA	AAAGACTATT	CAGAGCGGAC	CTTACAATAT	CAAAGTGGGC	ACGCACGGGA	1248
		1,320		1,340		1,360		1,380		1,400	
AITPS20c	TACATACCAA	ACTATGACGA	ATACATGGAG	GTTGGAATAG	TGACTCGGGG	AATAGATATG	ACGGTTGCGT	TCGCCTTCAT	TGGTATGGGG	GAGGCAGGAA	1399
AITPS20	TACATACCAA	ACTATGACGA	ATACATGGAG	GTTGGAATAG	TGACTCGGGG	AGTAGATGTC	ACGGTTGCGT	TCGCCTTCAT	TGGTATGGGG	GAGGCAGGAA	1348
		1,420		1,440		1,460		1,480		1,500	
AITPS20c	AAGAAGCTTT	TGACTGGATA	AGATCAAGAC	CGAAATTCAT	TCAAACCTA	GATAAAAAAG	CAGCTCTCAG	AGATGATGTT	GCCACCTACA	AGGATGAAAT	1499
AITPS20	AAGAAGCTTT	TGACTGGATA	AGATCAAGAC	CGAAATTCAT	TCAAACCTA	GATCTAAAAA	CAGCTCTCAG	AGATGATGTT	GCCACCTACA	AGGATGAAAT	1448
		1,520		1,540		1,560		1,580		1,600	
AITPS20c	GGCTAGAGGA	GAGATTGCGA	CAGGAATCAA	CTGCTATATG	AAACAATATA	AAGTTACCGA	AGAAGAAGCG	TTTCTAGAGT	TTCATAGAAG	GATTAACAT	1599
AITPS20	GGCTAGAGGA	GAGATTGCGA	CAGGAATCAA	CTGCTATATG	AAACAATATA	AAGTTACCGA	AGAAGAAGCG	TTTCTAGAGT	TTCATAGAAG	GATTAACAT	1548
		1,620		1,640		1,660		1,680		1,700	
AITPS20c	ACTTCTAAGC	TAGTGAATGA	AGAGTATTTT	AAGACGACAG	TACCTTTGAA	ACTTGTGGGT	ATAGCTTCTA	ACGTTGGACG	GGCTATCGAT	ACCAACTION	1699
AITPS20	ACTTCTAAGC	TAGTGAATGA	AGAGTATTTT	AAGACGACAG	TACCTTTGAA	ACTTGTGGGT	ATAGCTTTTA	ACGTTGGACG	GGTTATCGAT	ACCAACTION	1648
		1,720		1,740		1,760		1,780		1,800	
AITPS20c	AGCATGGCGA	TGGATTAACC	TATGGAGGGA	TAGTAGAAGG	CCAGATCACC	TCTTTATTCC	TTGATCTAAT	CACCATTTAG			1779
AITPS20	AGCATGGCGA	TGGATTAACC	TATACAGGGA	TAGTAGAAGG	CCAAATCACC	TCTTTGTTCC	TTGATCTAAT	CACCATTTAG			1728

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.

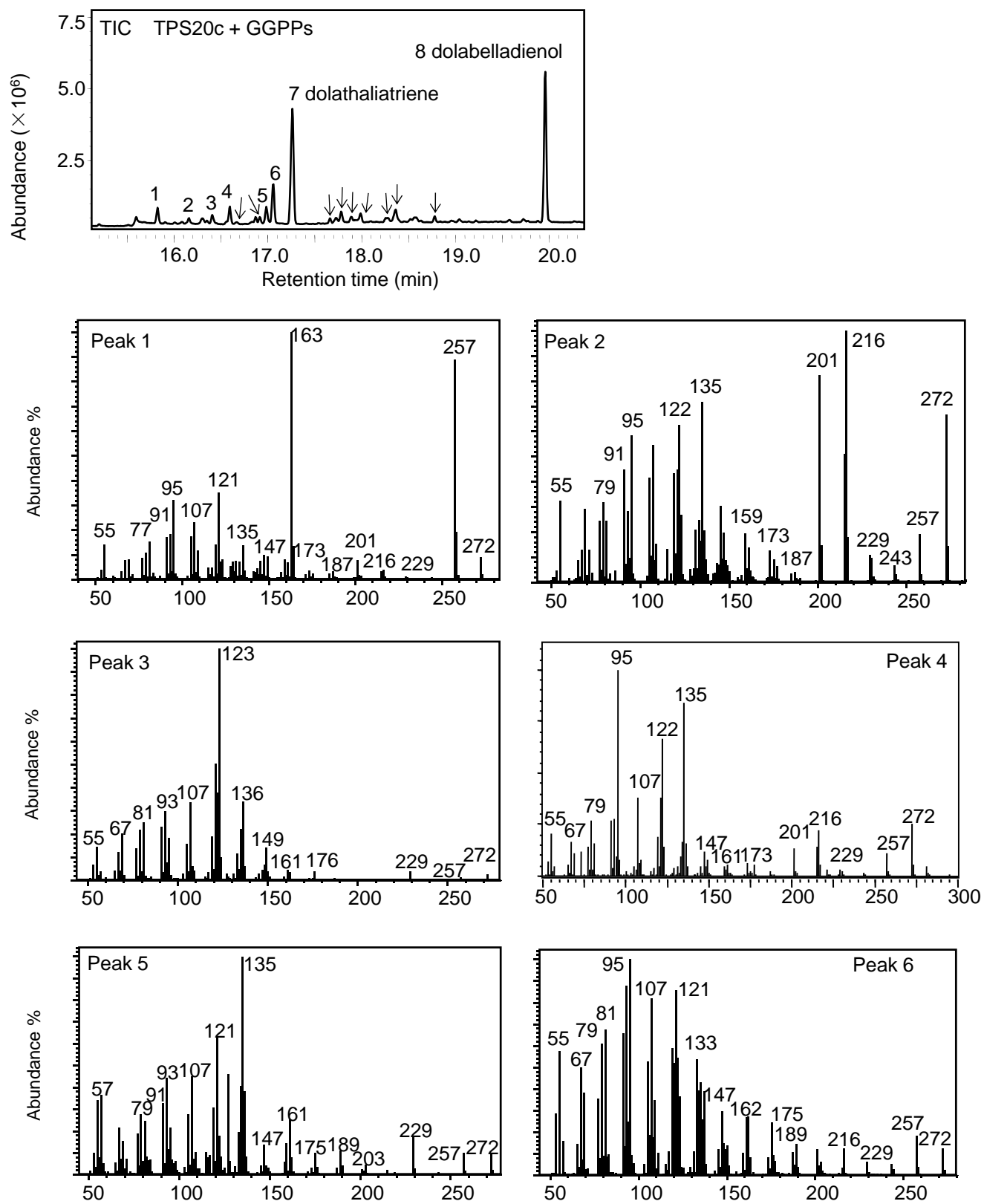


Figure S4. Mass spectra of minor diterpene products 1-6 of TPS20c.

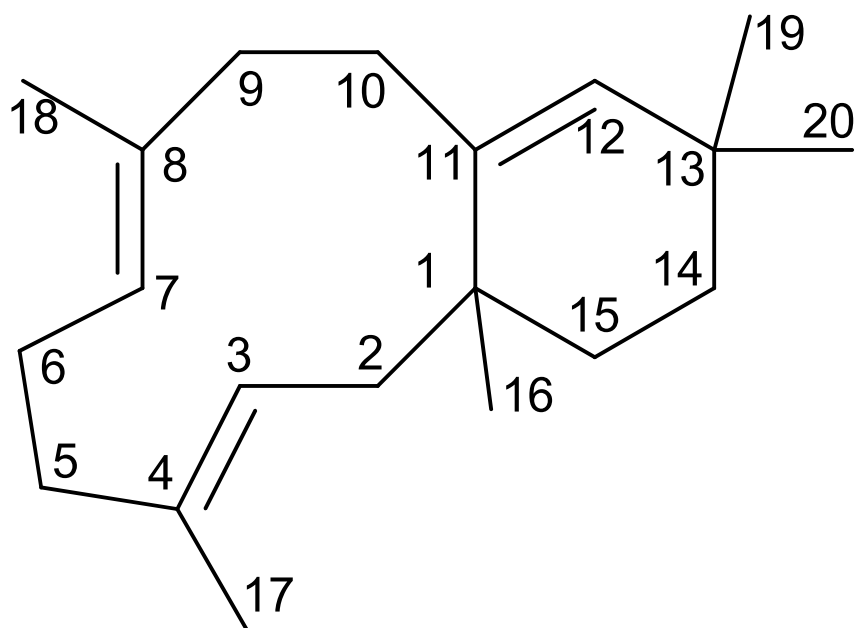
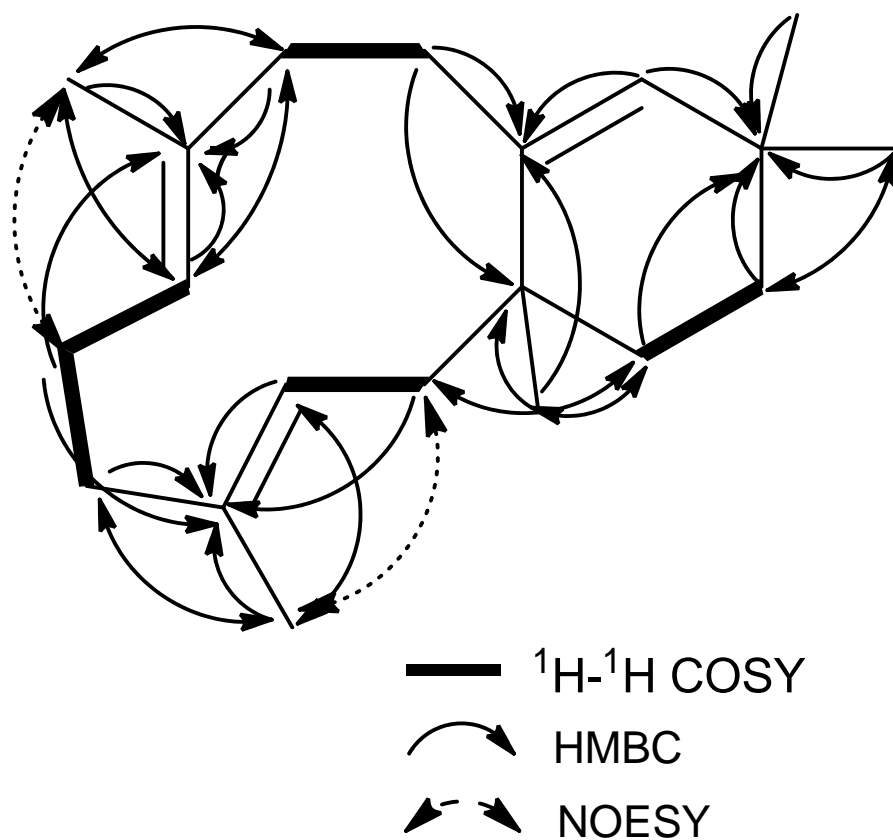


Figure S5. Important correlations of COSY, HMBC and NOESY observed for the major diterpene product of TPS20c.

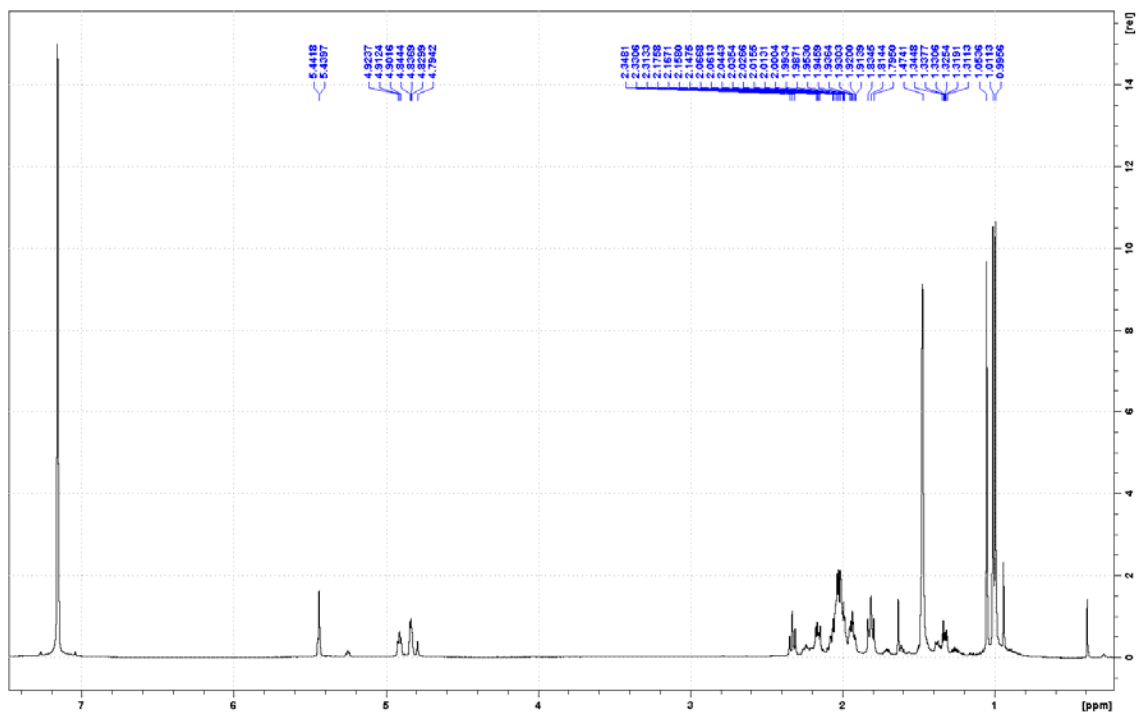


Figure S6. ^1H spectrum of the TPS20c major diterpene compound (solvent C_6D_6).

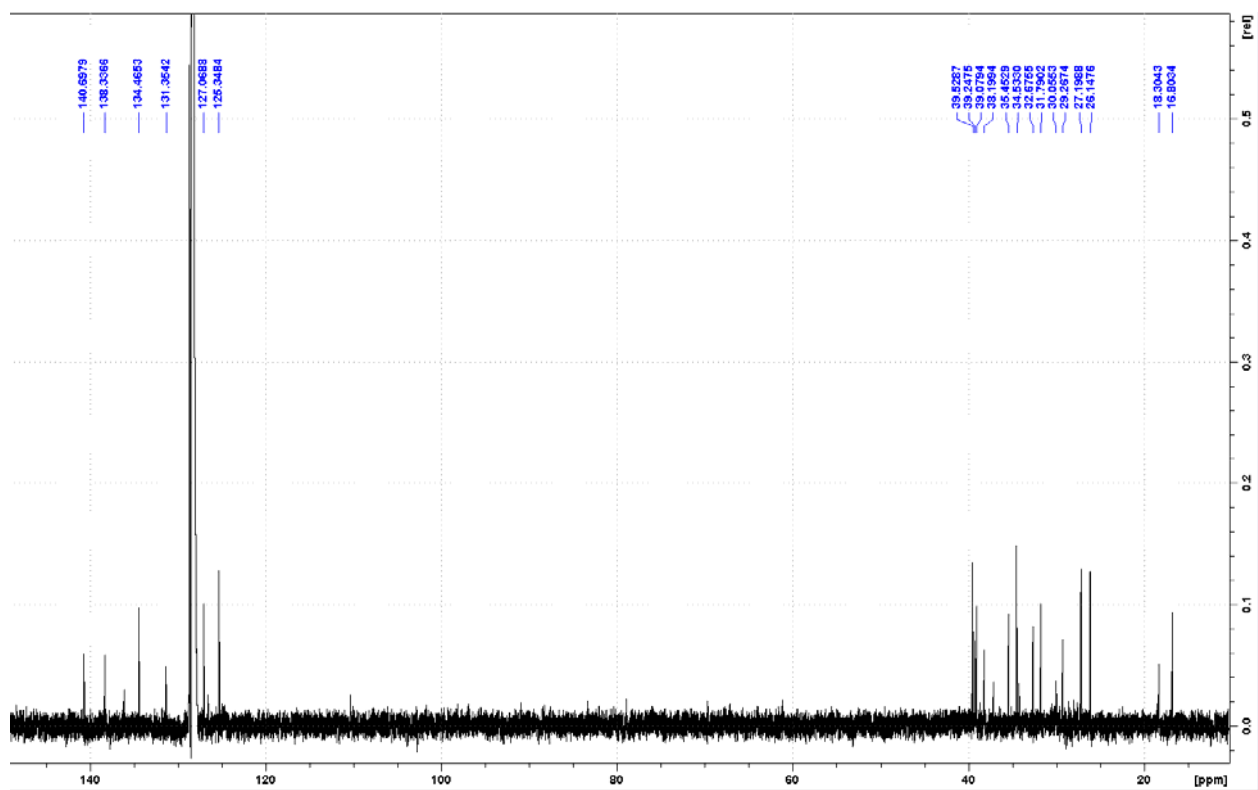


Figure S7. ^{13}C spectrum of the TPS20c major diterpene compound (solvent C_6D_6).

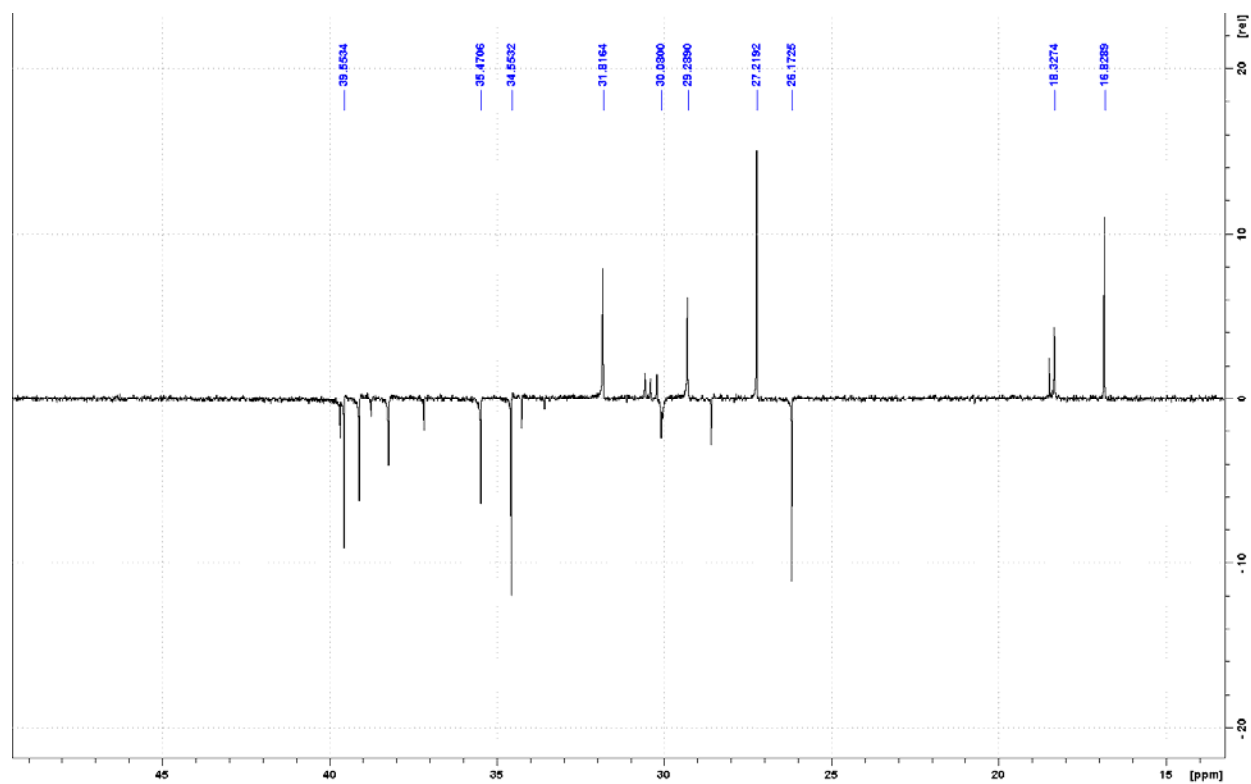


Figure S8A. ^{13}C DEPT135 of the TPS20c major diterpene compound (solvent C_6D_6).

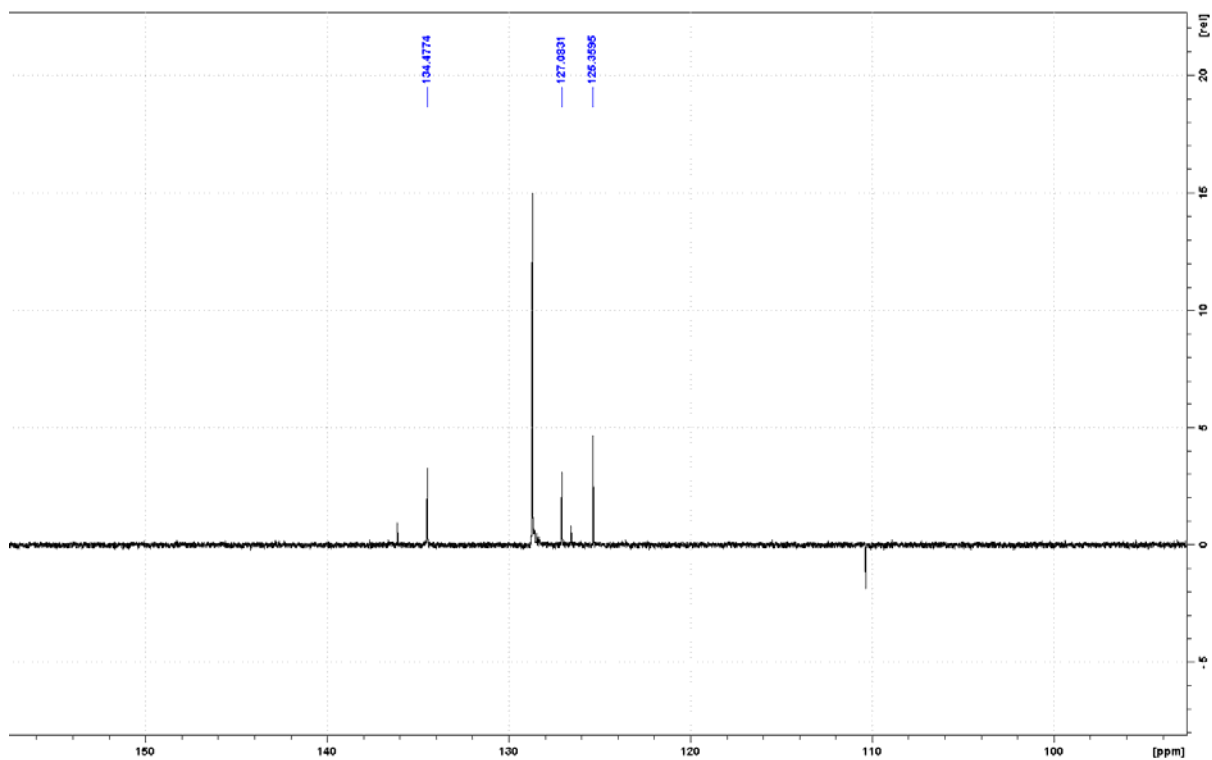


Figure S8B. ^{13}C DEPT135 of the TPS20c major diterpene compound (solvent C_6D_6).

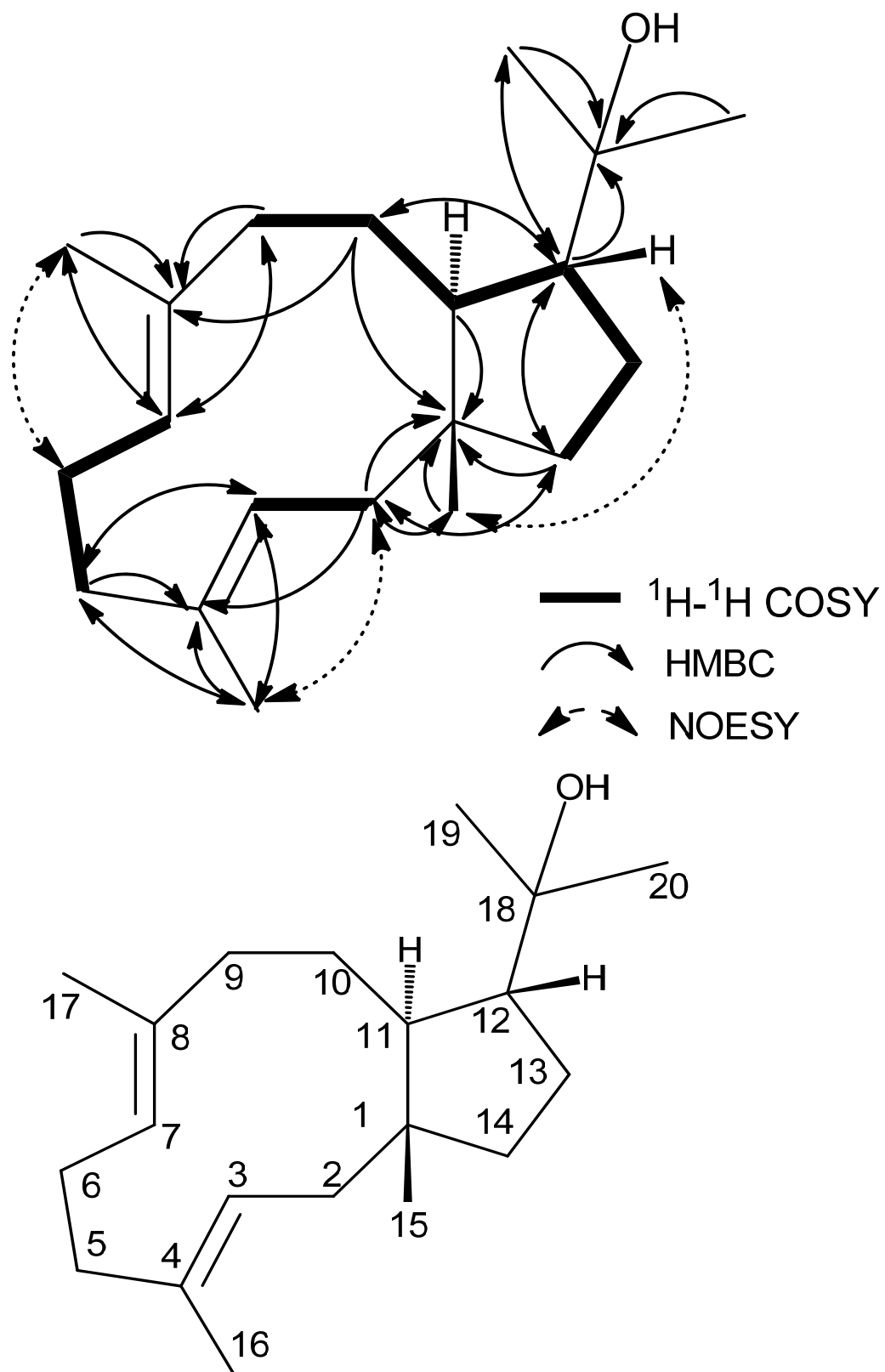


Figure S9. Important correlations of COSY, HMBC and NOESY observed for the major diterpene alcohol product of TPS20c.

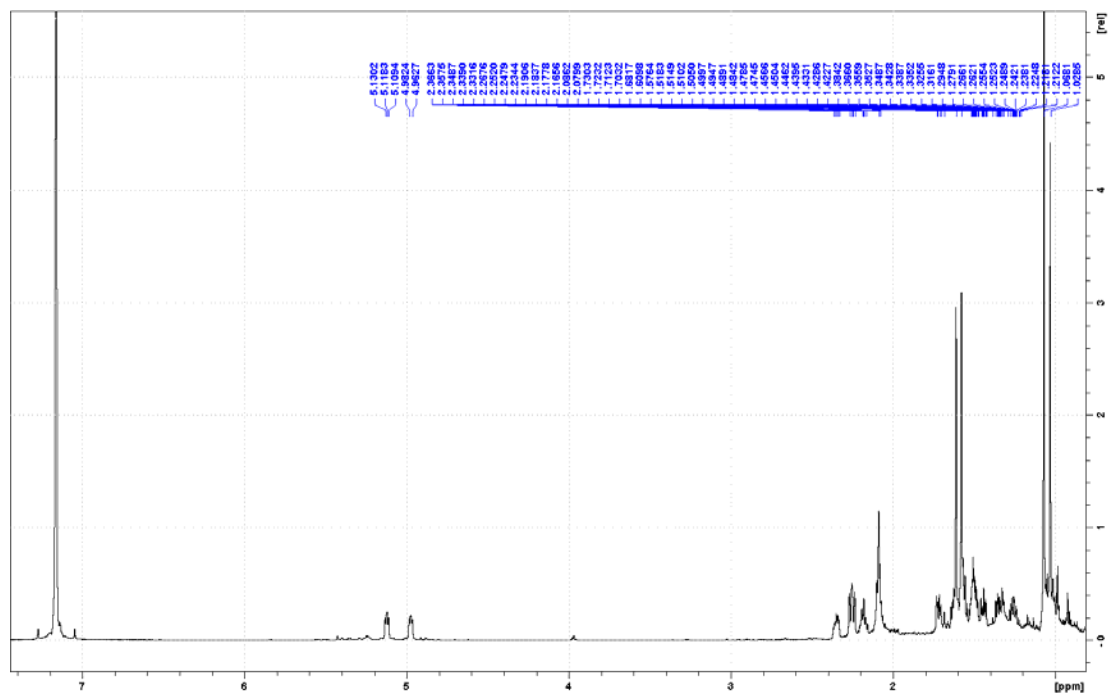


Figure S10. ^1H spectrum of the TPS20c diterpene alcohol compound (solvent C_6D_6).

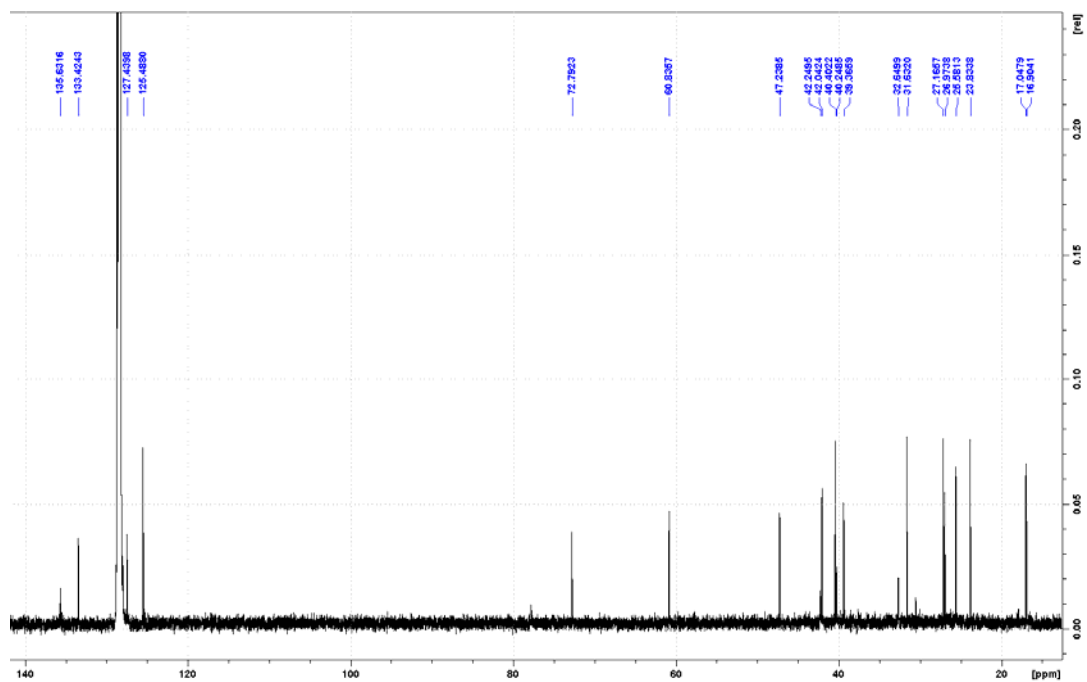


Figure S11. ¹³C spectrum of the TPS20c diterpene alcohol compound (solvent C₆D₆).

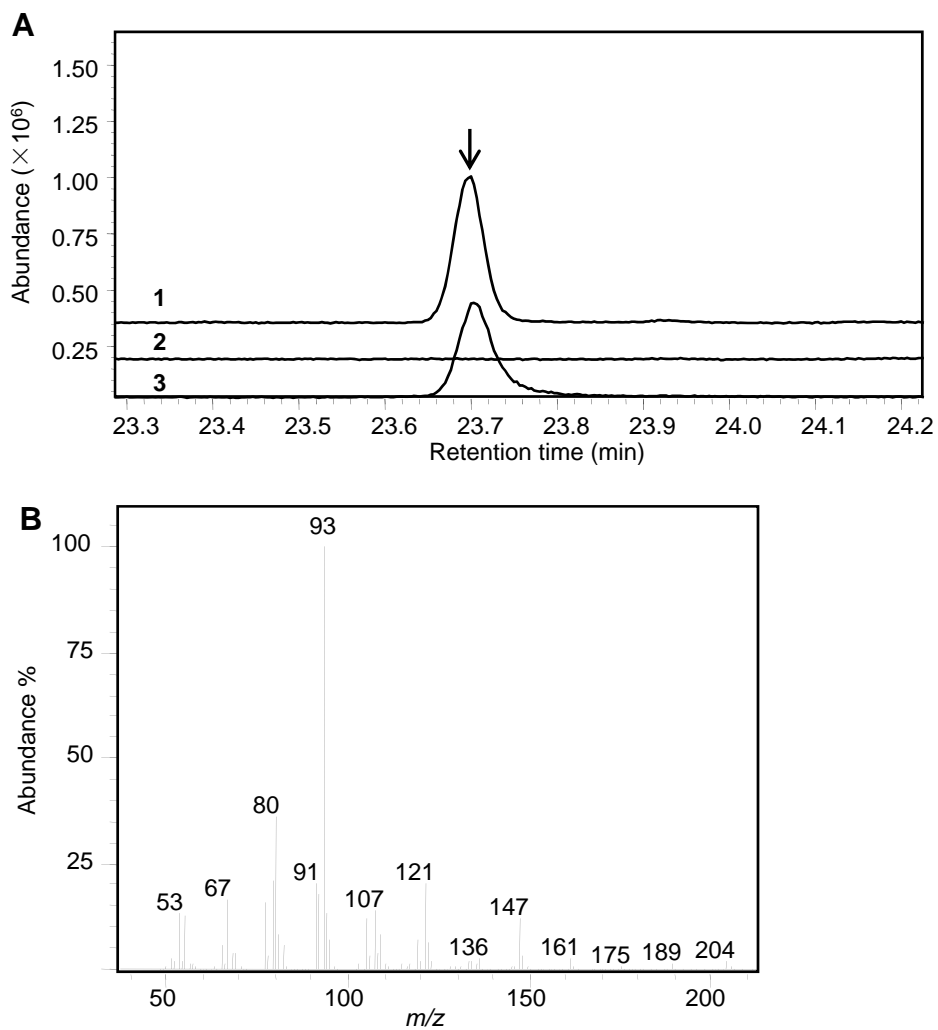


Figure S12. TPS20c reacts with (*E,E*)-FPP to produce α -humulene. (A) GC-MS chromatogram of an extract from *E. coli* coexpressing TPS20c and (*E,E*)-FPP synthase. 1, co-expression of TPS20c and (*E,E*)-FPP synthase. 2, co-expression of empty vector and (*E,E*)-FPP synthase. 3, standard of α -humulene.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

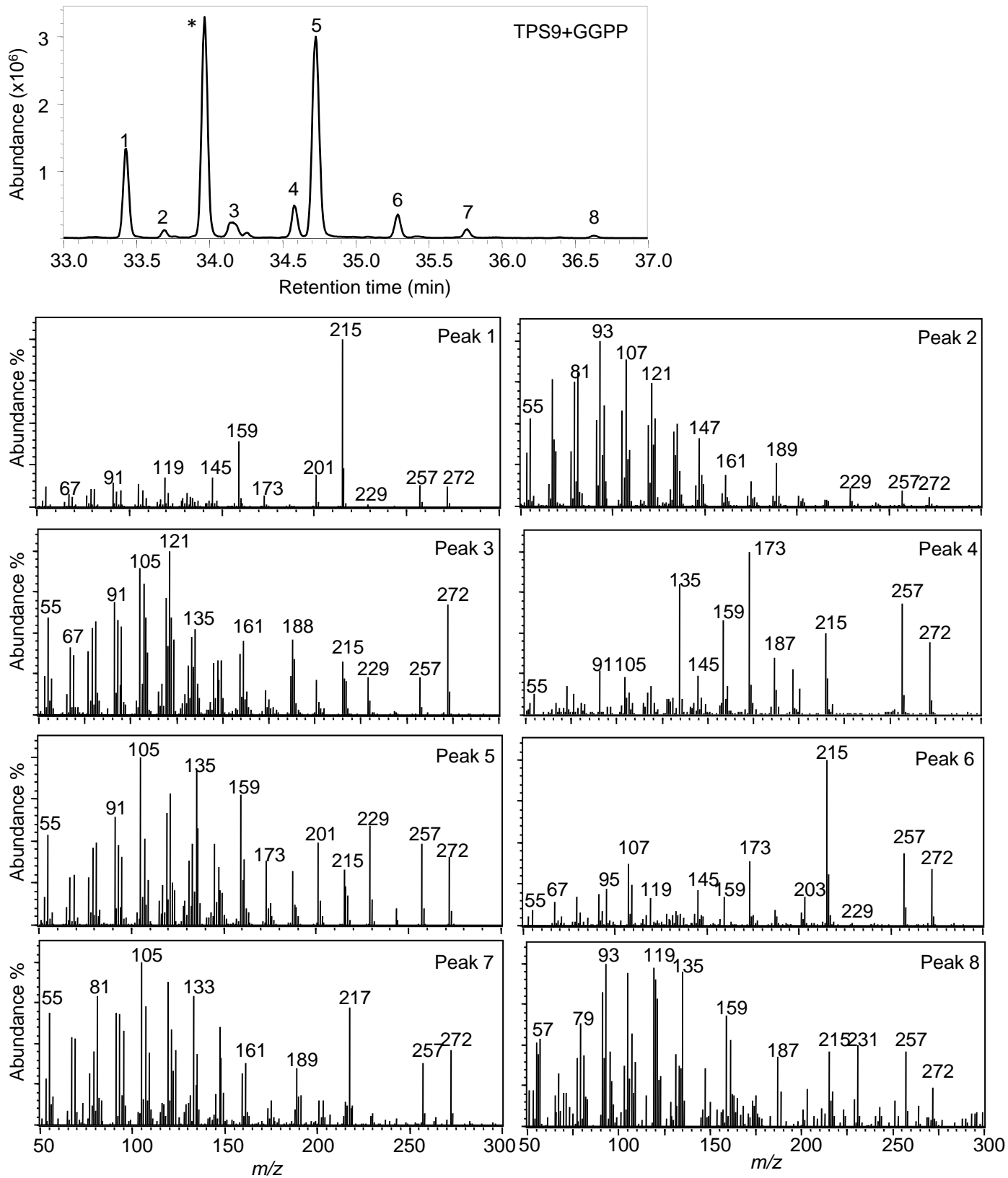
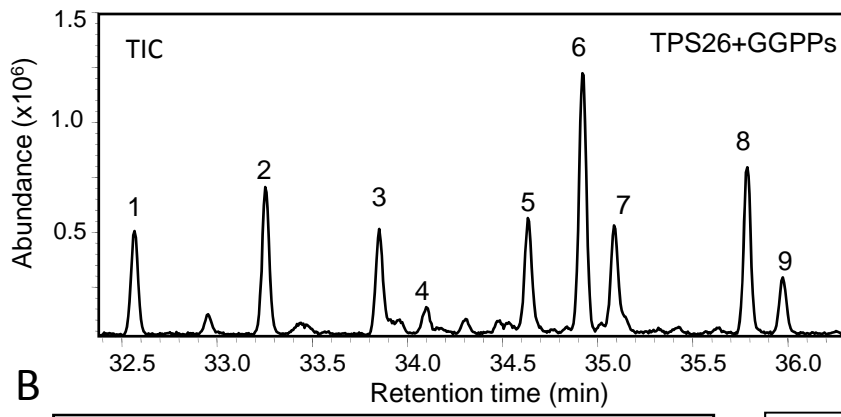
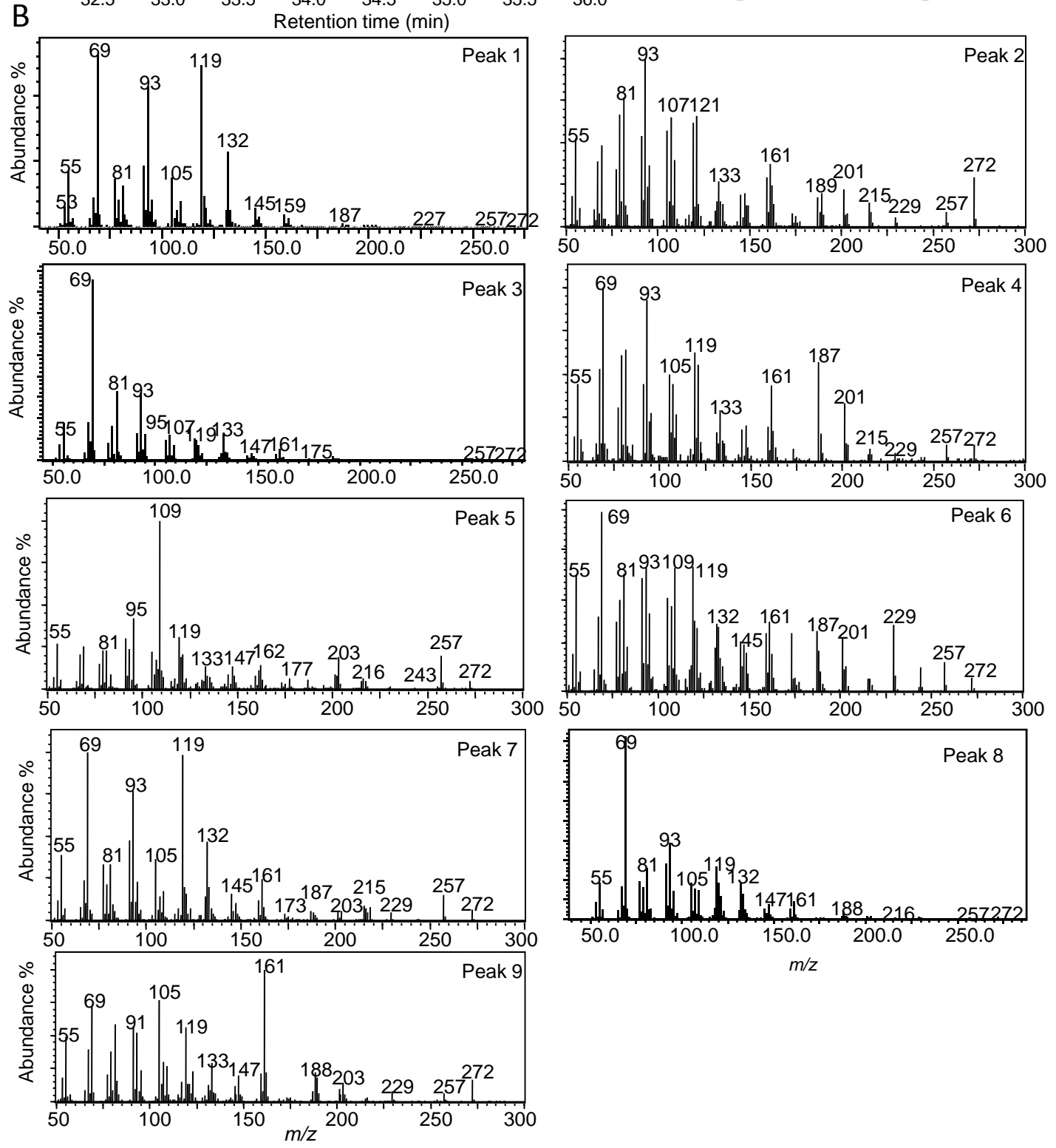


Figure S13. Mass spectra of minor diterpene products of recombinant TPS9.
 The mass spectrum of the major diterpene product (labeled with asterisk) is shown in Figure 7C.



A

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.



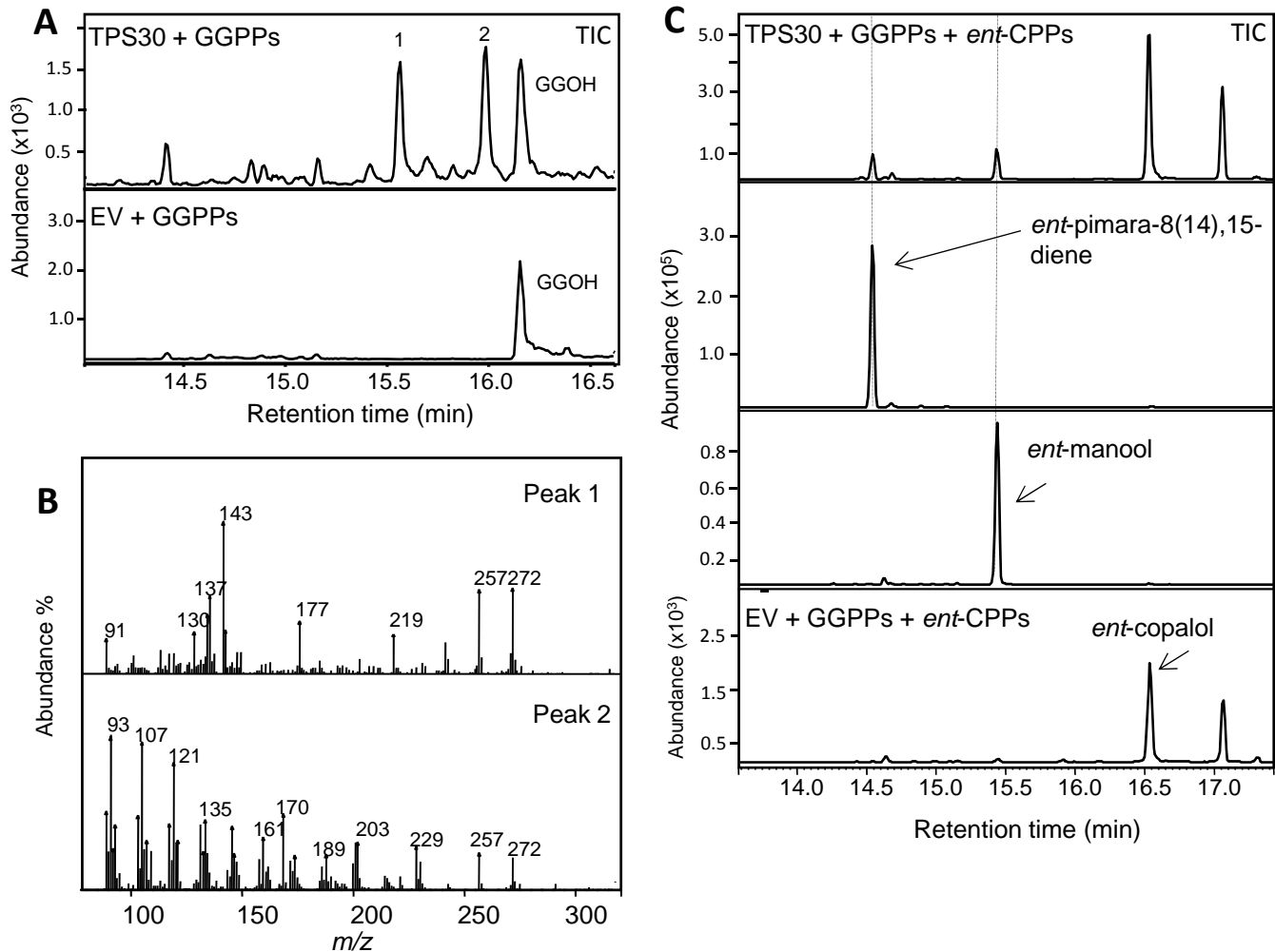


Figure S15. Diterpene synthase activity of Col TPS30. (A) Upper panel: GC-MS chromatogram of a culture extract from *E. coli* coexpressing truncated TPS30 and GGPP synthase. Lower panel: Extract of *E. coli* expressing only GGPP synthase. (B) Mass spectra of the TPS30 diterpene products 1 and 2 as shown 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	
<i>TPS6-F</i>	GAGGTCGGGGTAGCTTCG
<i>TPS6-R</i>	CTCTCCAGCGAAGGTGTACC
<i>TPS19-F</i>	GCCACGTACCAACCTTTGATG
<i>TPS19-R</i>	GGGTATTGGGTGGACGTACAAG
<i>TPS20-F</i>	GTTGGAGGCAGCAATGAGGAAC
<i>TPS20-R</i>	CTAAATGGTGATTAGATCAAGG
<i>TPS29-F</i>	GTGTGAAACAAGTGGTAGAGAAG
<i>TPS29-R</i>	GGGTATAGGGTGGAGGTACAAG
<i>Actin 8-F</i>	ATGAAGATTAAGGTCGTGGCAC
<i>Actin 8-R</i>	GTTTTTATCCGAGTTTGAAGAGGC
Cloning of full length and truncated <i>TPS</i> cDNAs	
<i>TPS6-F</i>	AAAGTCGACATGGAGGCCATAACAAAATATG
<i>TPS6-F-truncated</i>	AACCATGGCAACGACGAATCCTACAGA
<i>TPS6-R</i>	AAACTCGAGTTAAAGAGTGATGAGATCGACA
<i>TPS9-F-truncated</i>	AACATATGGATGATAAAGAACGTACTIONCGC
<i>TPS9-R</i>	AACTCGAGCTACAACATTTTCAGAGTATGAT
<i>TPS19-F</i>	AACCATGGAAGCAACAAGAATGGGT
<i>TPS19-F-truncated</i>	AACCATGGCATCTACTAAGAGTAGTGATGATC
<i>TPS19-R</i>	AACTCGAGTTATGAAAGGGGTATTGGGTG
<i>TPS20-F</i>	AACCATGGAAGCAATAACTAAAATGGG
<i>TPS20-F-truncated</i>	AACCATGGATCATGAAGCCGCAAATCGT
<i>TPS20-F-truncated (His-tag)</i>	AACATATGGATCATGAAGCCGCAAATCGT
<i>TPS20-R</i>	AAACTCGAGCTAAATGGTGATTAGATCAAGGA
<i>TPS22-F-truncated</i>	CACCACTCTTCCCGGAGATCAA
<i>TPS22-R</i>	TCAAAGCGGAAGAGGATGGAAG
<i>TPS25-F-truncated</i>	CACCAACTTCTCTCTTTTCTTGC
<i>TPS25-R</i>	TCAAAGAGGTATTGGATGGAGG
<i>TPS26-F-truncated</i>	AACCATGGCACTTCGTTTAAAGGCCACTAATAC
<i>TPS26-R</i>	AAACTCGAG TCAAAGACCGATACGATCGA
<i>TPS29-F</i>	AACCATGGAAGCAATAAGAATAGGTTT
<i>TPS29-F-truncated</i>	AACCATGGCAAGTTCTTCTAAGAAGAGTA
<i>TPS29-R</i>	AACTCGAGTTATGAAAGGGGTATAGGGTG
<i>TPS30-F</i>	AACCATGGCAGTAGCAAGAACGGT
<i>TPS30-F-truncated</i>	AACCATGGCTACTACTAAGAGTTCGACT
<i>TPS30-R</i>	AACTCGAGTCAAACAGGAATGGGATGGAT

Table S2. ^1H -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. ^1H -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