

— SUPPORTING INFORMATION —

**Exploring the Influence of Domain Architecture on the  
Catalytic Function of Diterpene Synthases**

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Mustafa Köksal,<sup>†</sup> Alexander Genshaft,<sup>†</sup> David E. Cane,<sup>‡</sup> and David W. Christianson<sup>†,\*</sup>

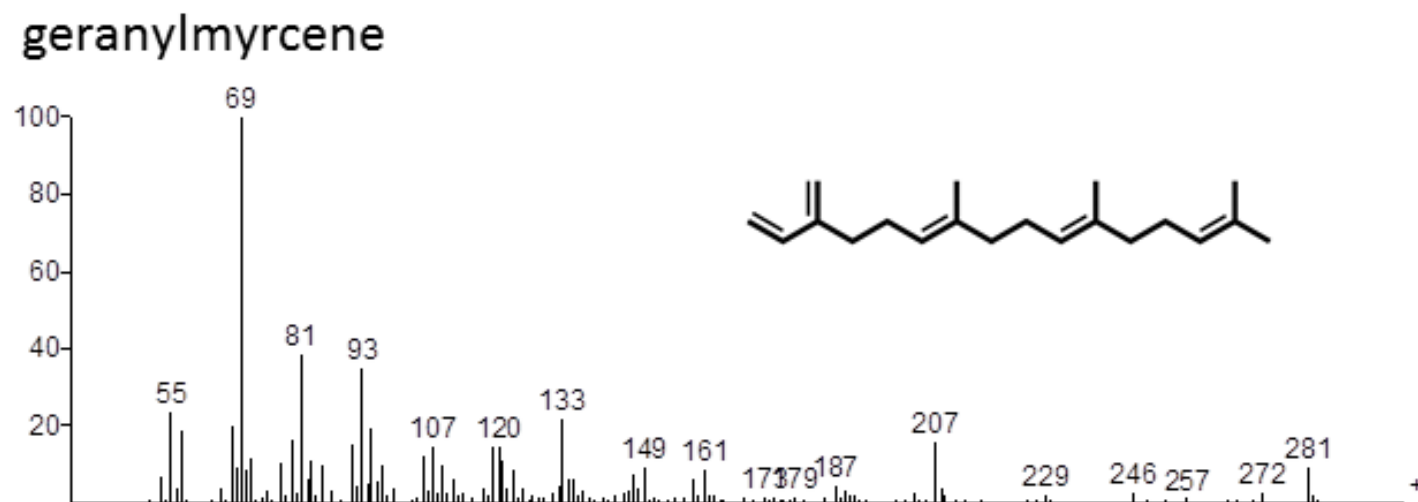
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United States

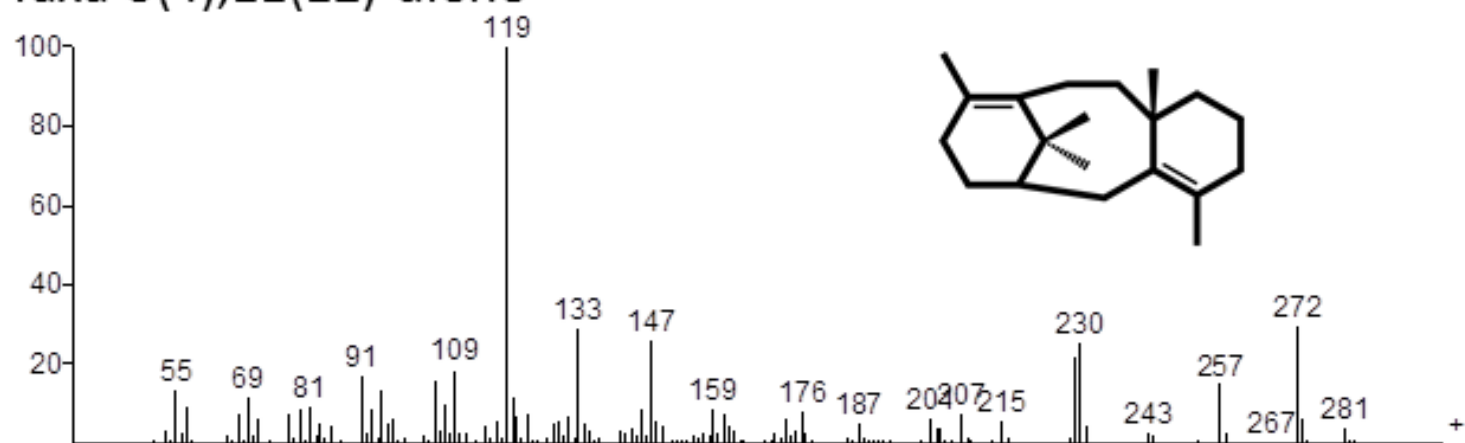
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<sup>¶</sup>Contributed equally to this work.

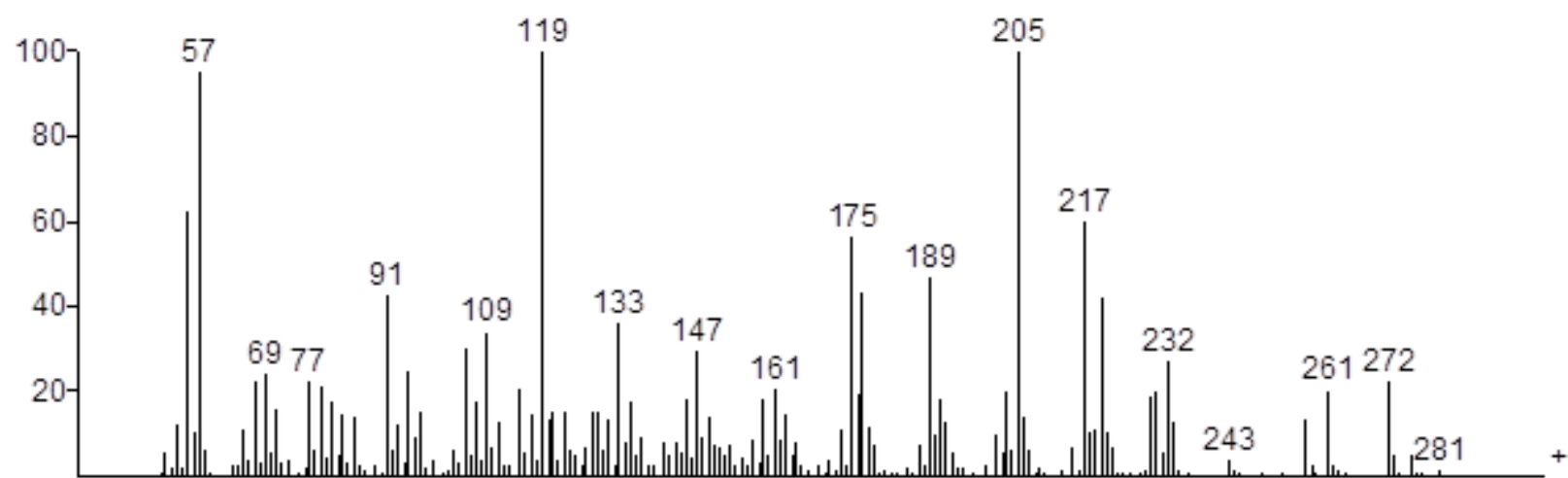
**Figure S2.** Mass spectra of diterpene products generated by TbTS mutants.



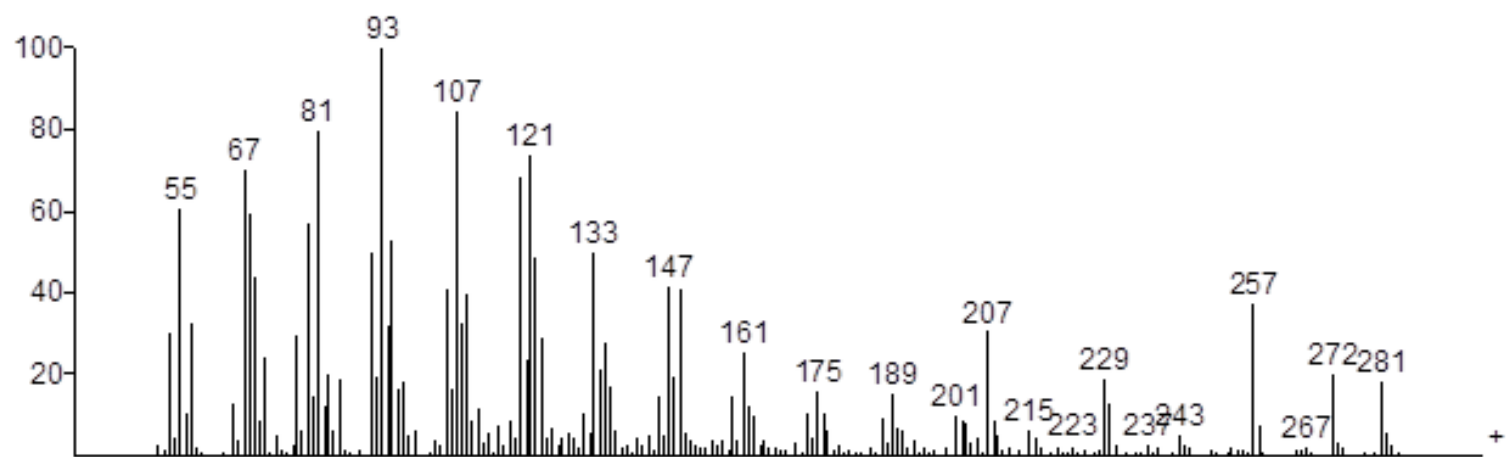
# Taxa-3(4),11(12)-diene



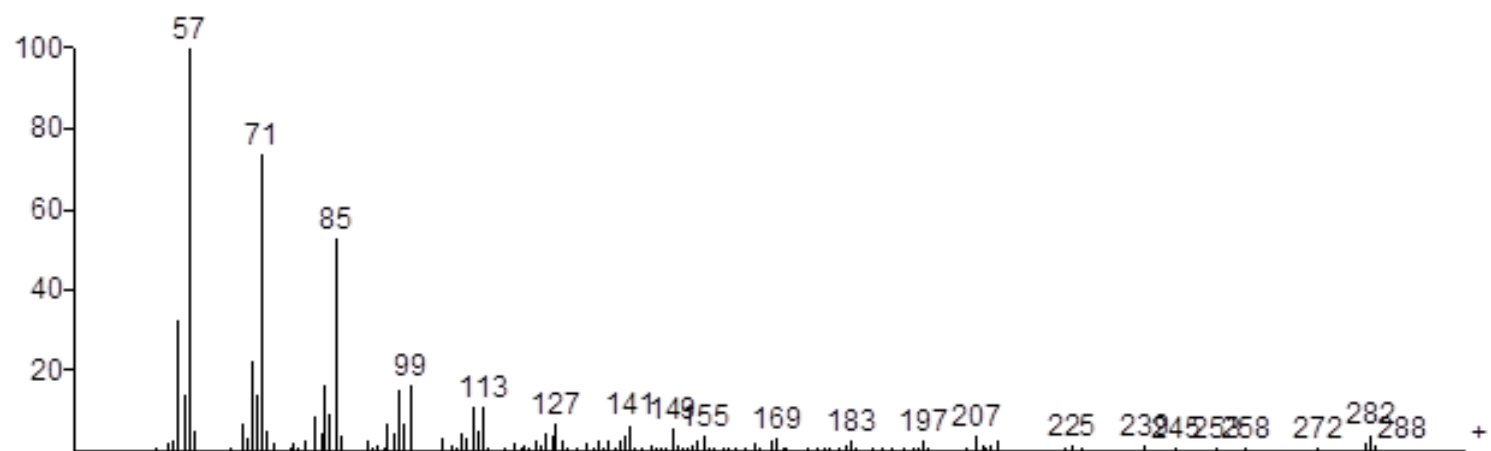
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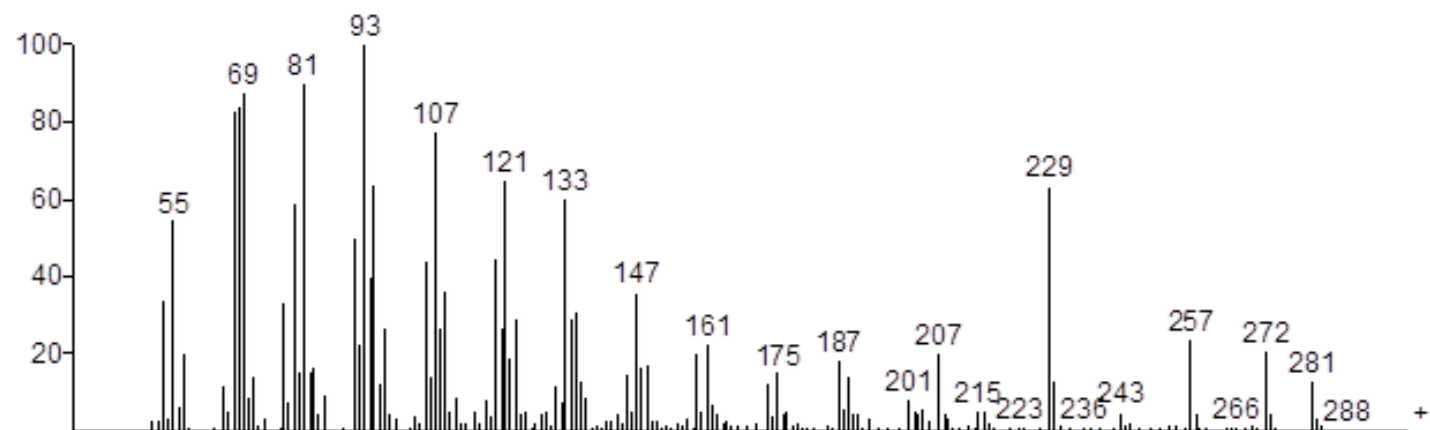
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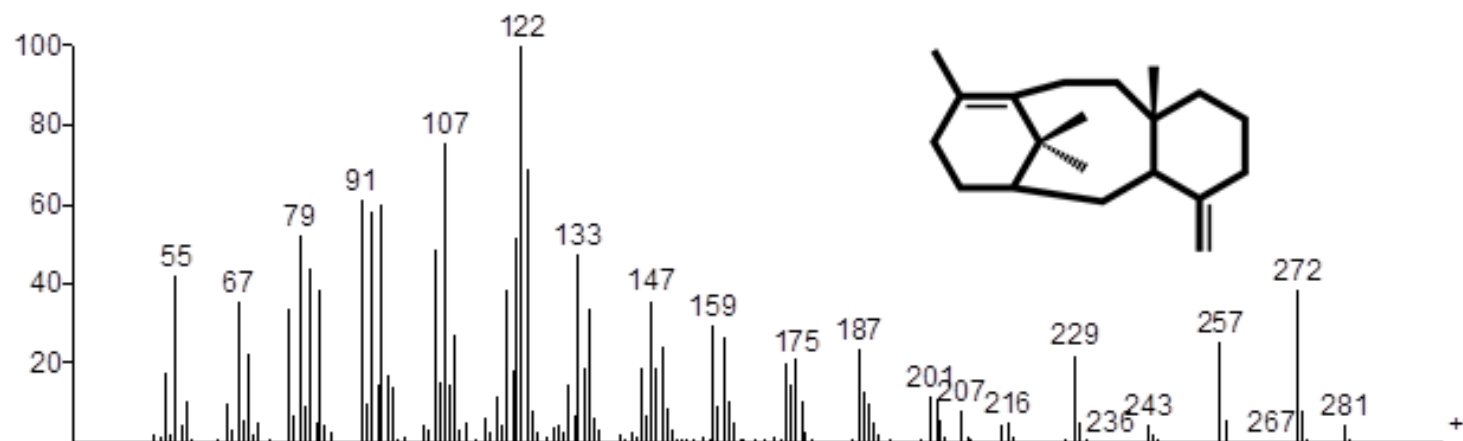
### Unknown diterpene 3



## Unknown diterpene 4

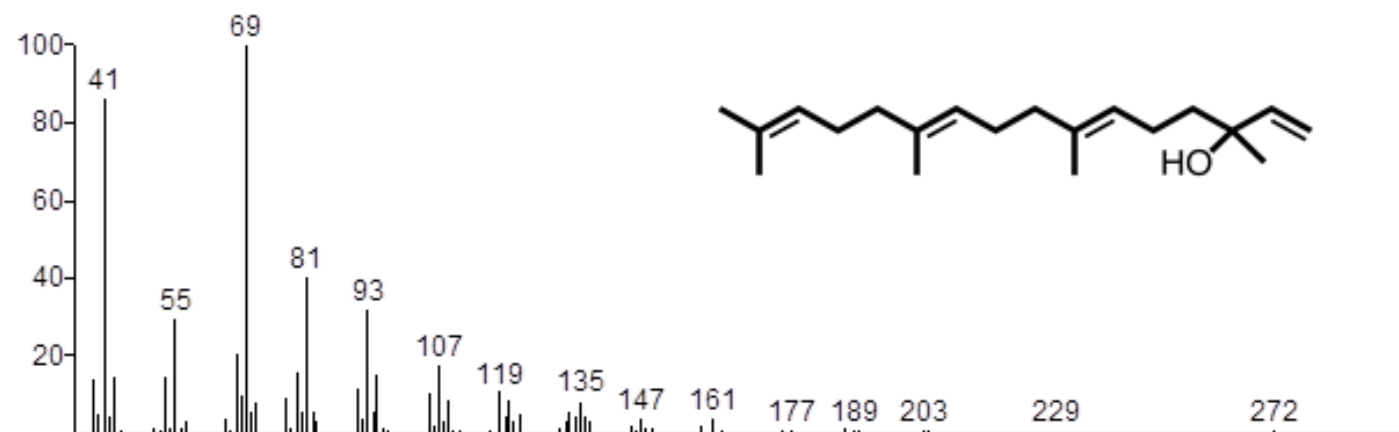


# taxa-4(20),11(12)-diene

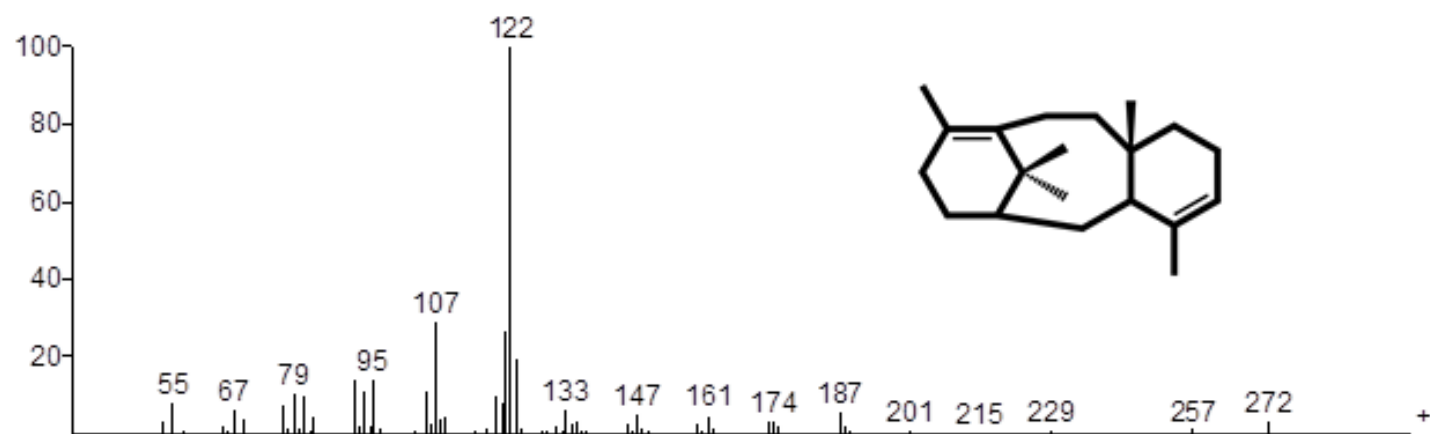




# geranylinalool



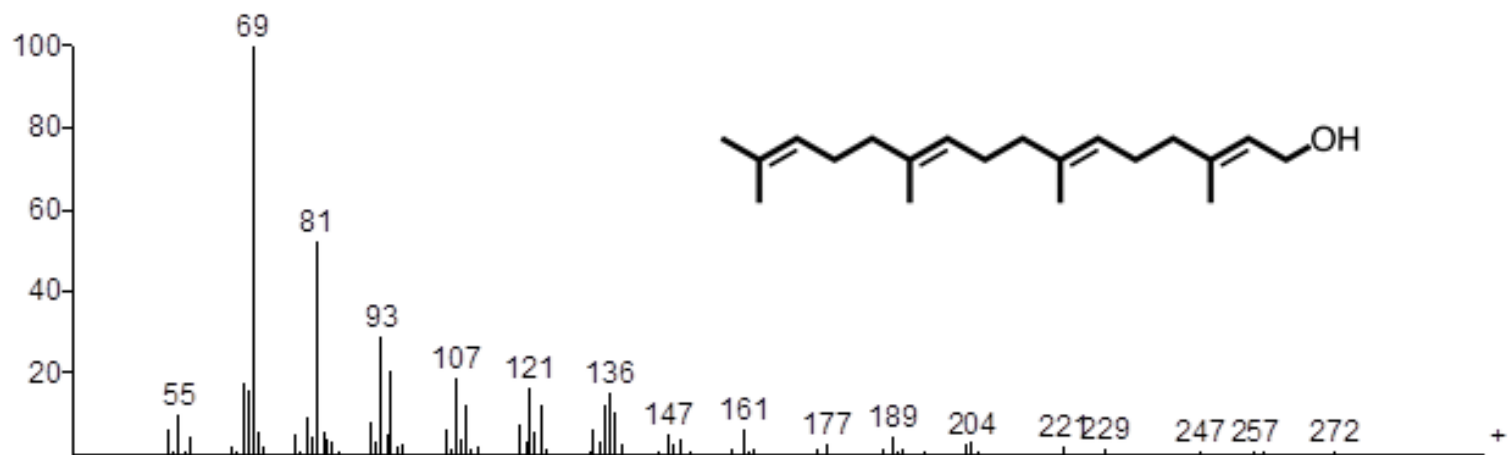
# taxadiene



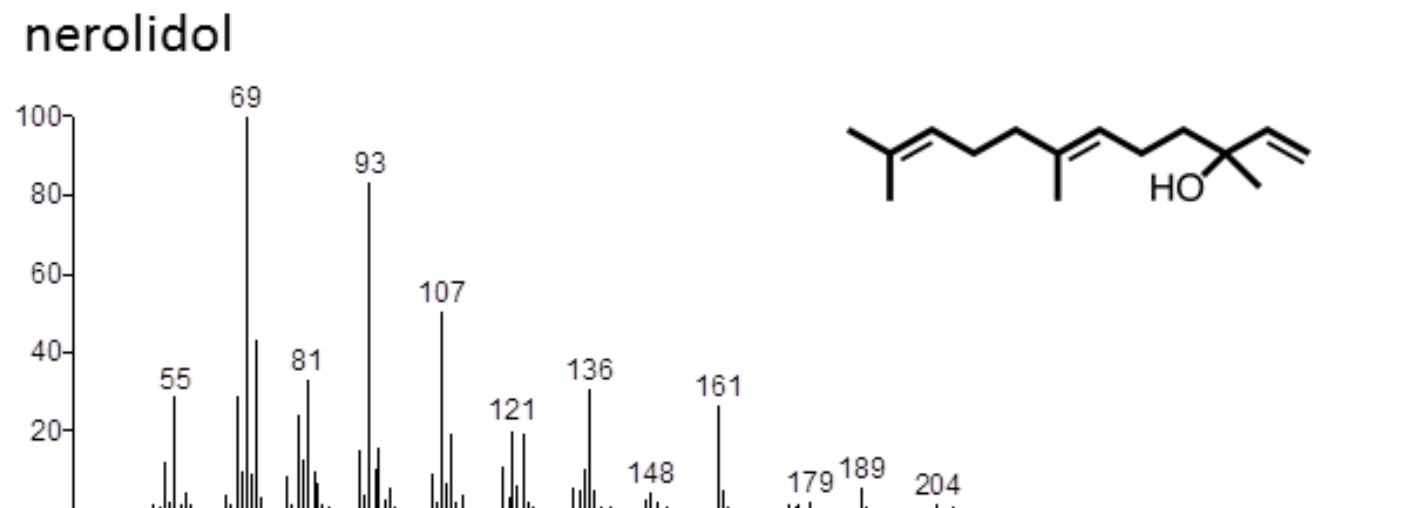
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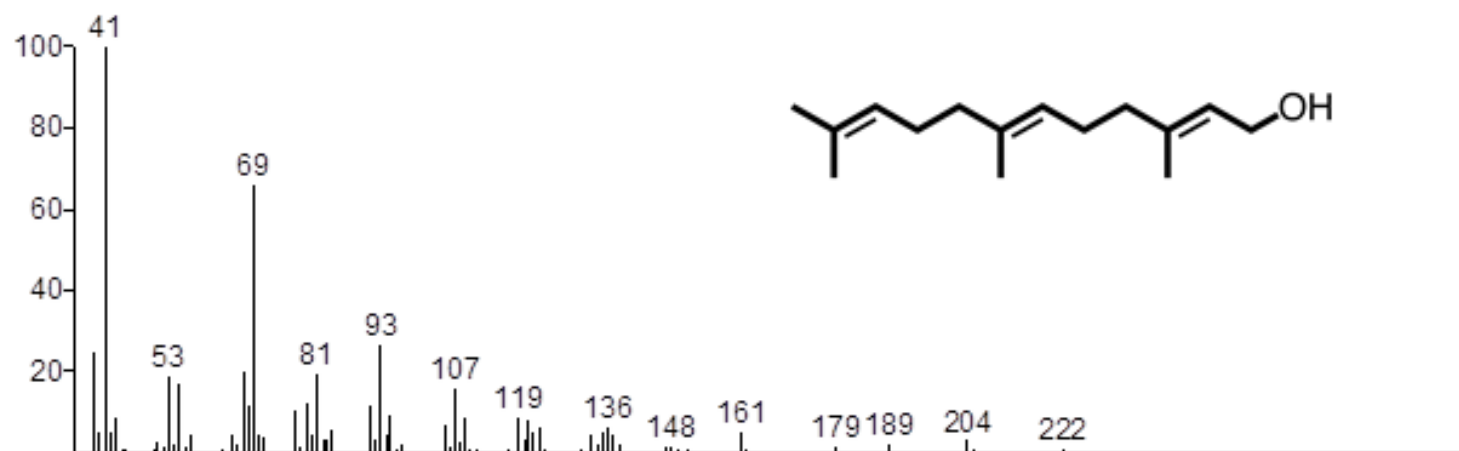
# geranylgeraniol



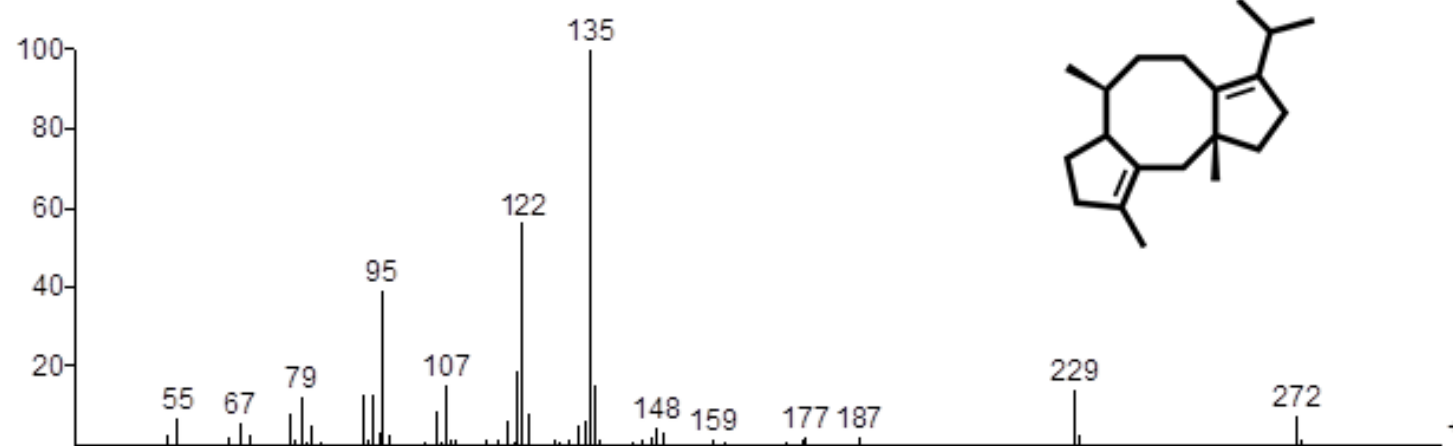
**Figure S3.** Mass spectra of sesquiterpene, diterpene, and sesterterpene products generated by PaFS and AcOS chimeras.



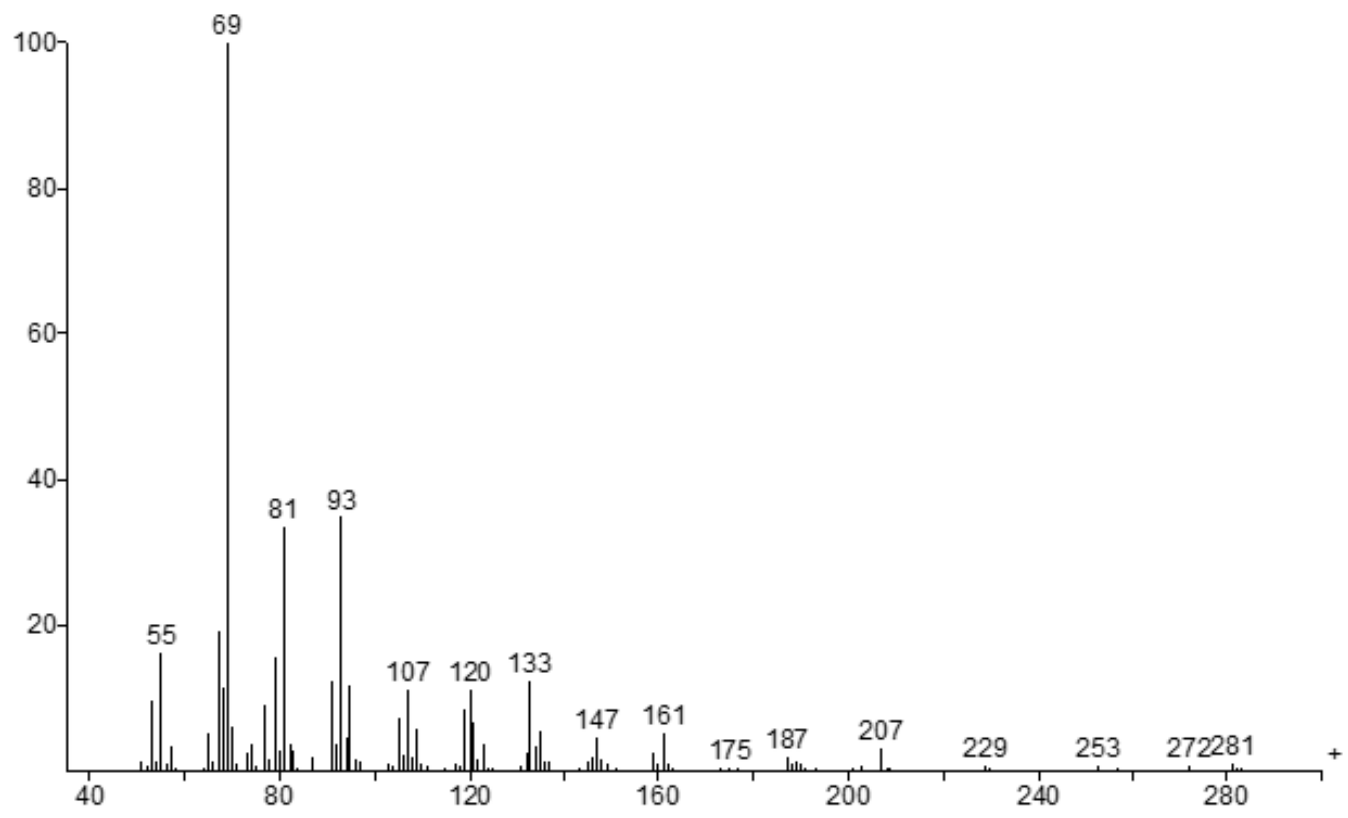
# farnesol



# fusicocadiene

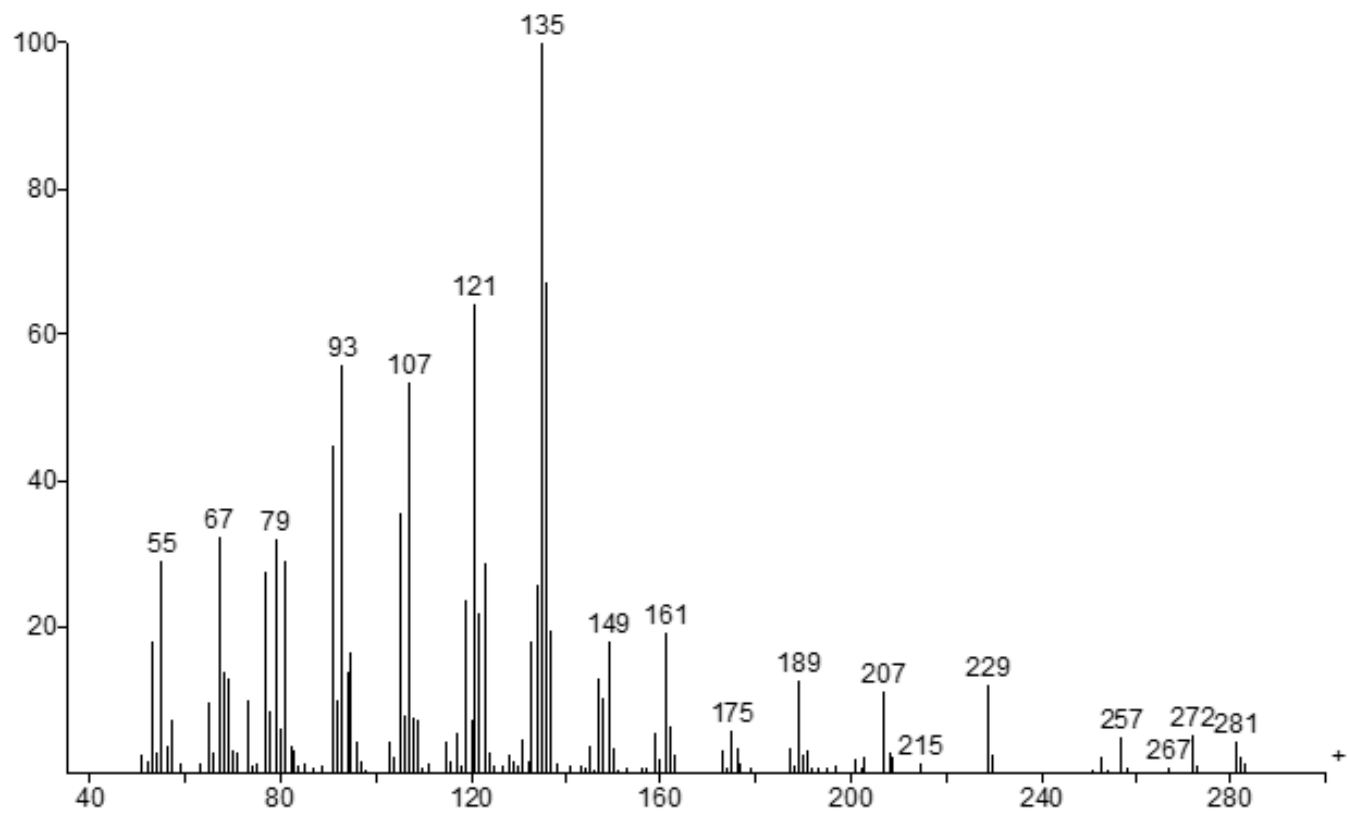


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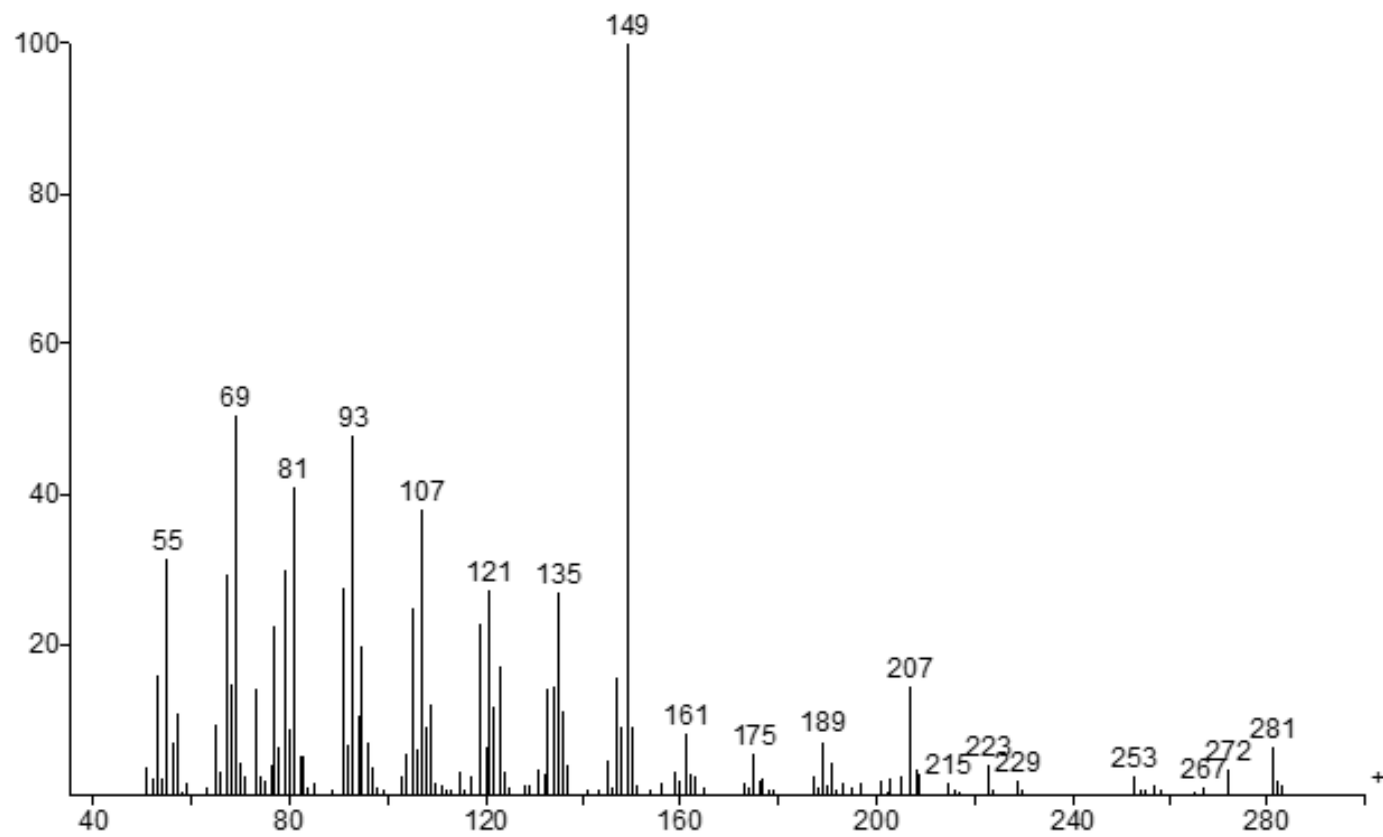




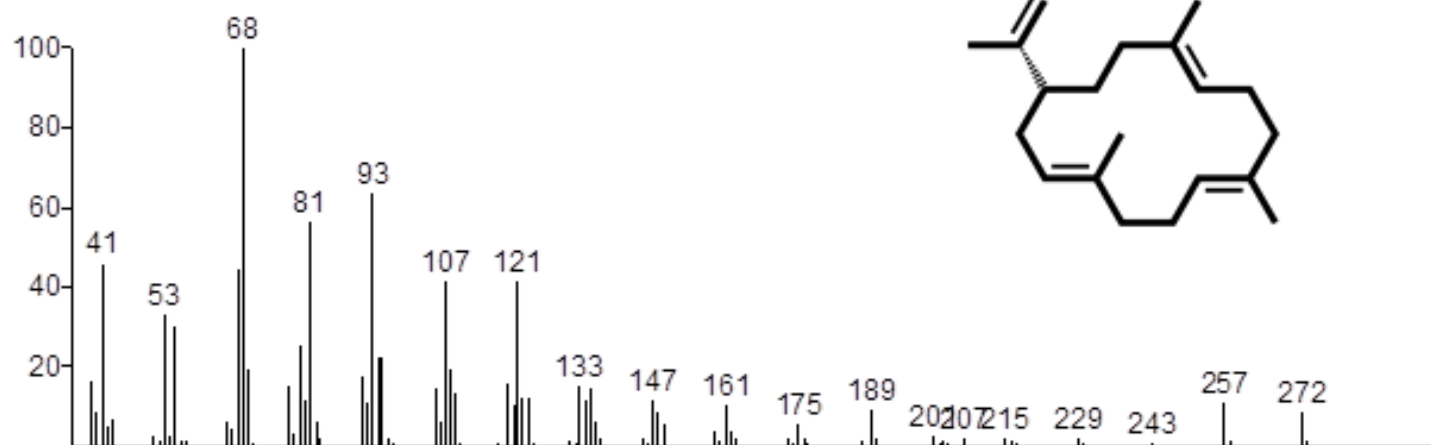
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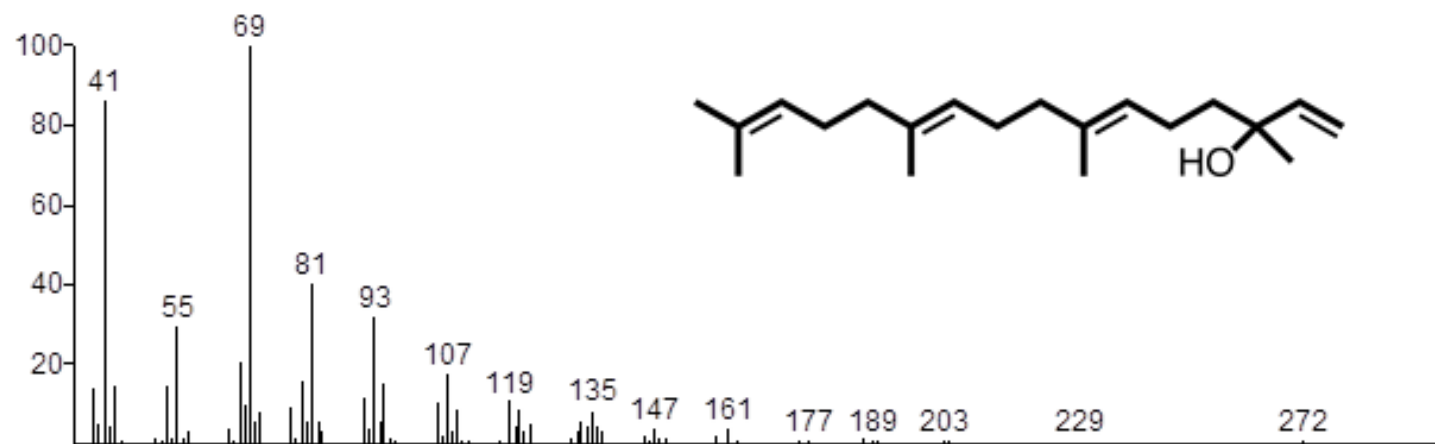
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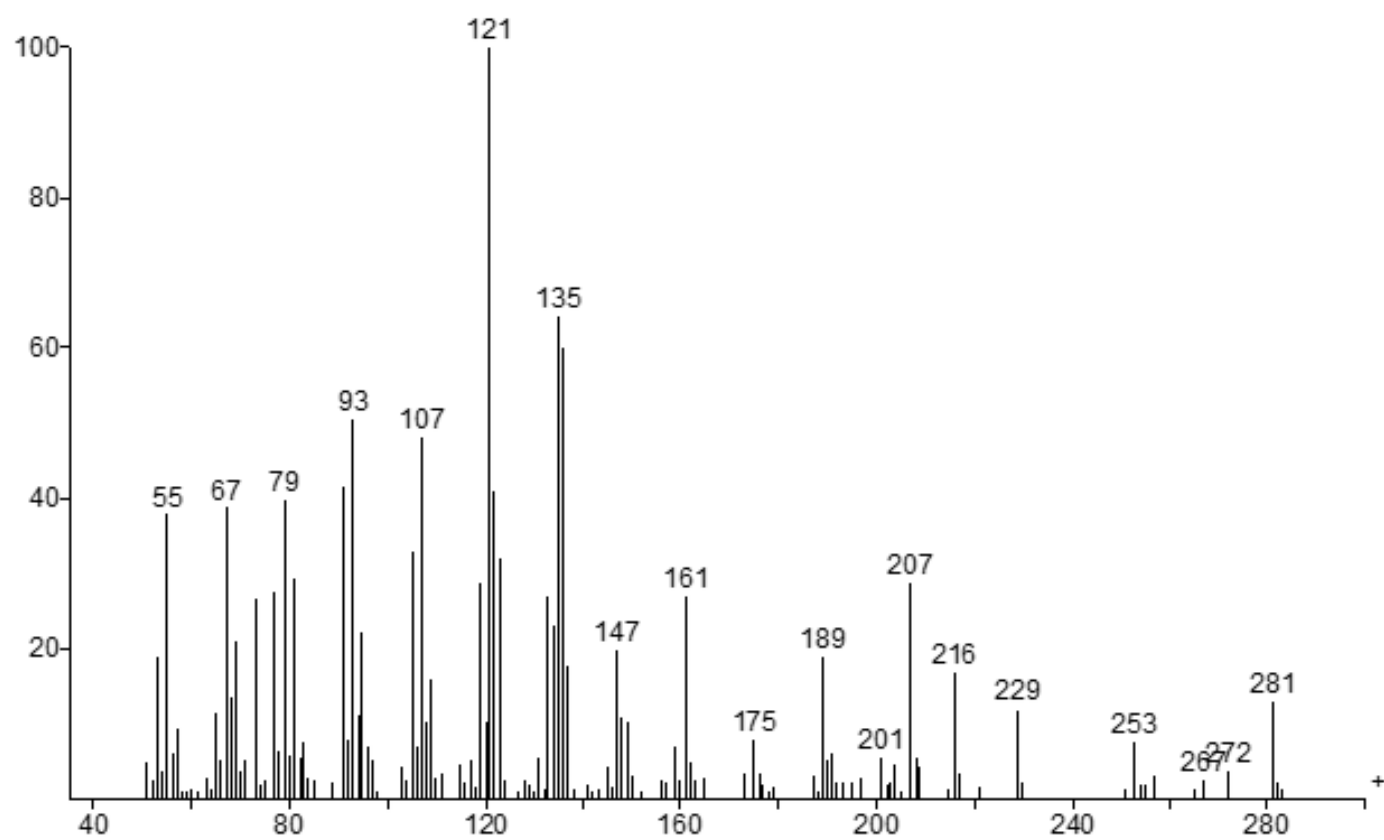
# cembrene A



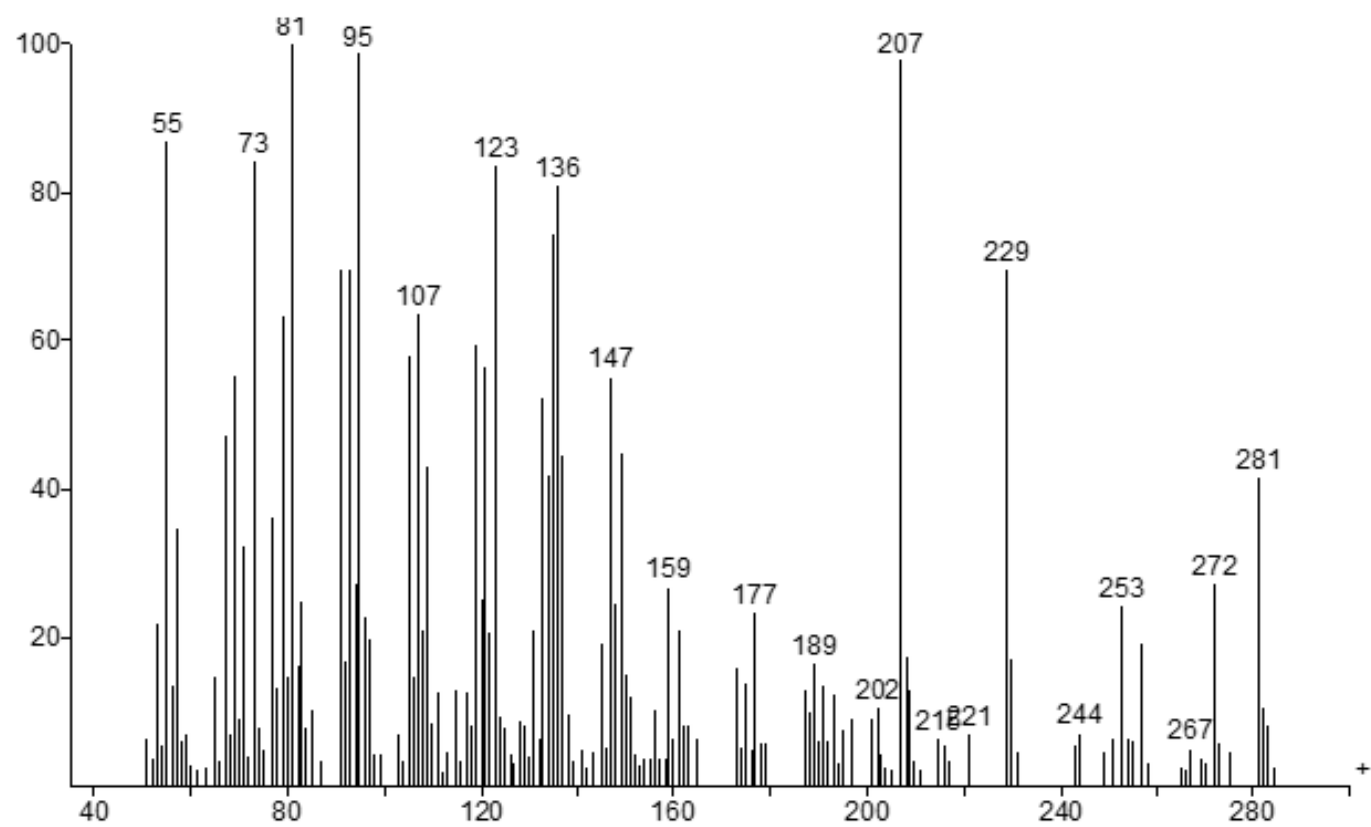
# geranylinalool



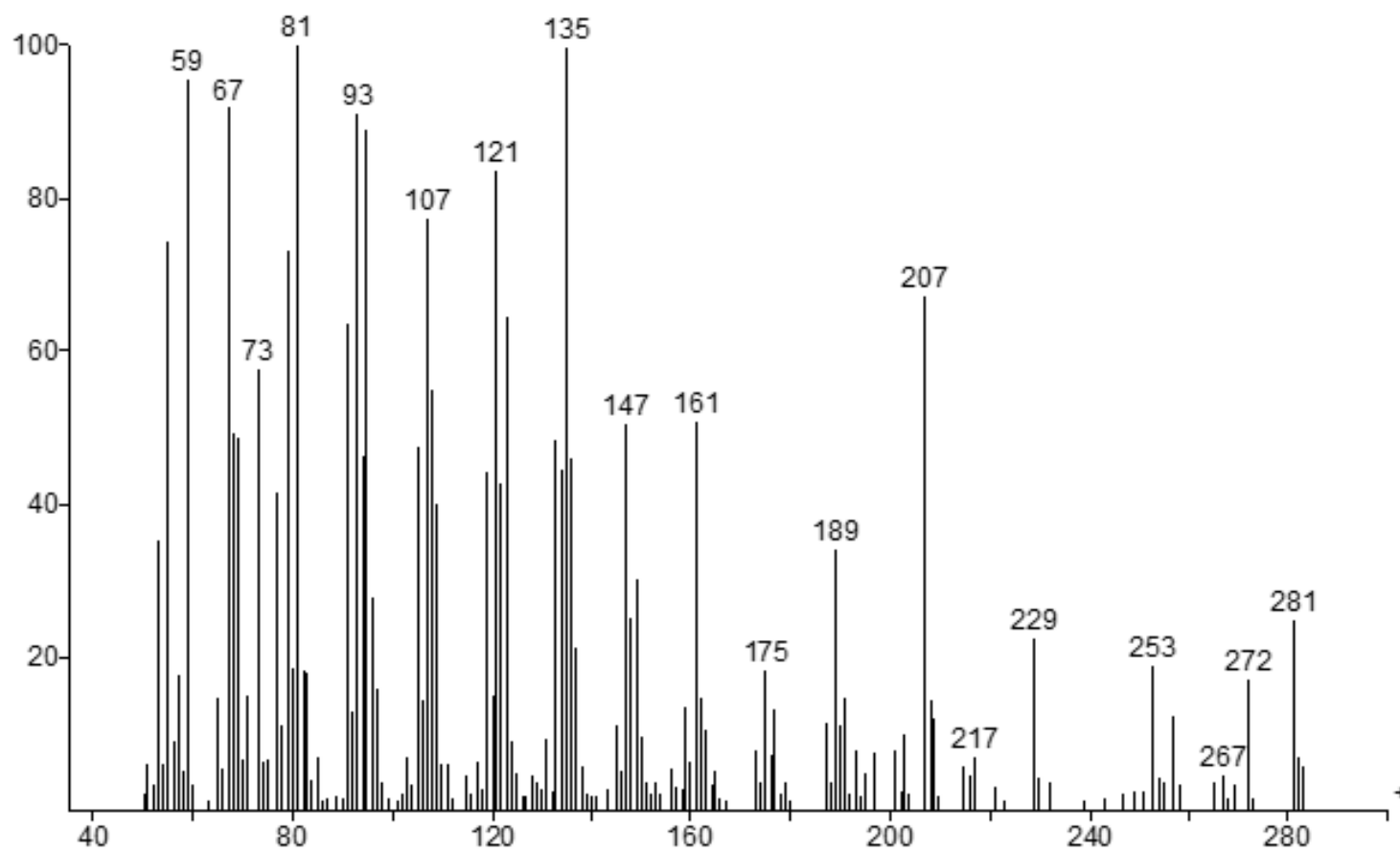
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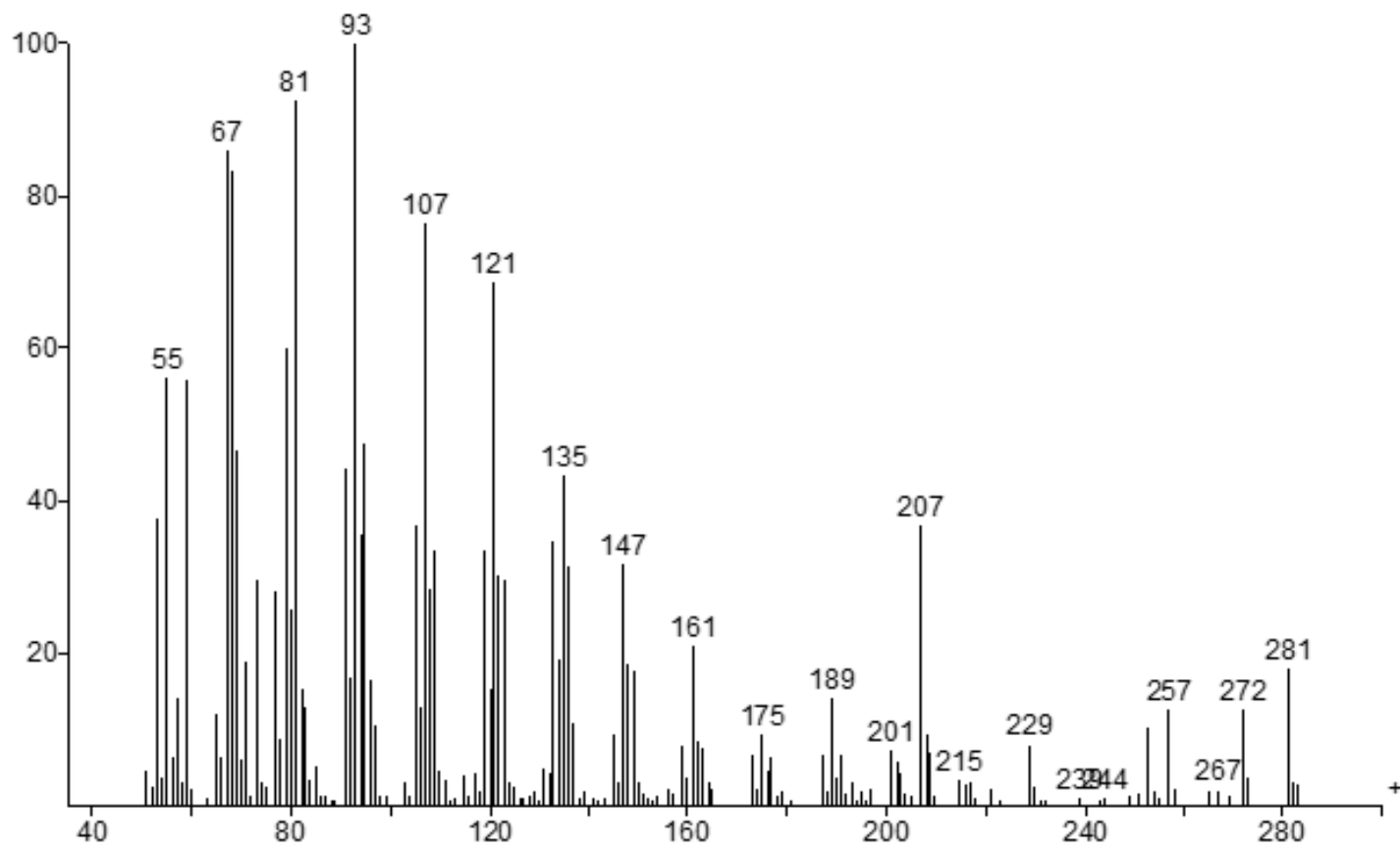
## Unknown diterpene 10



# Unknown diterpene 11

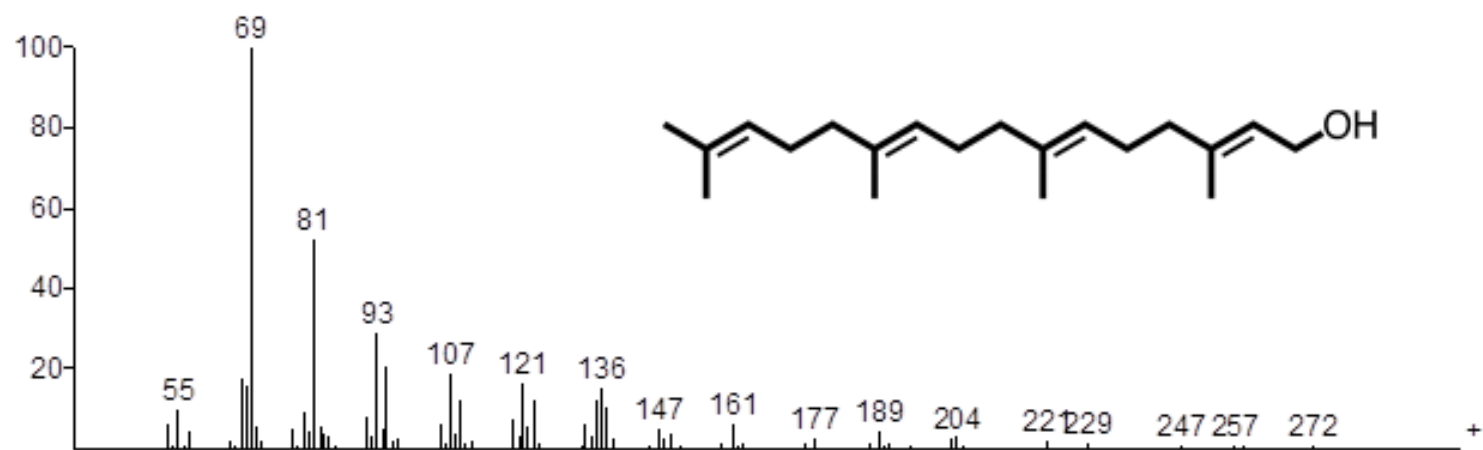


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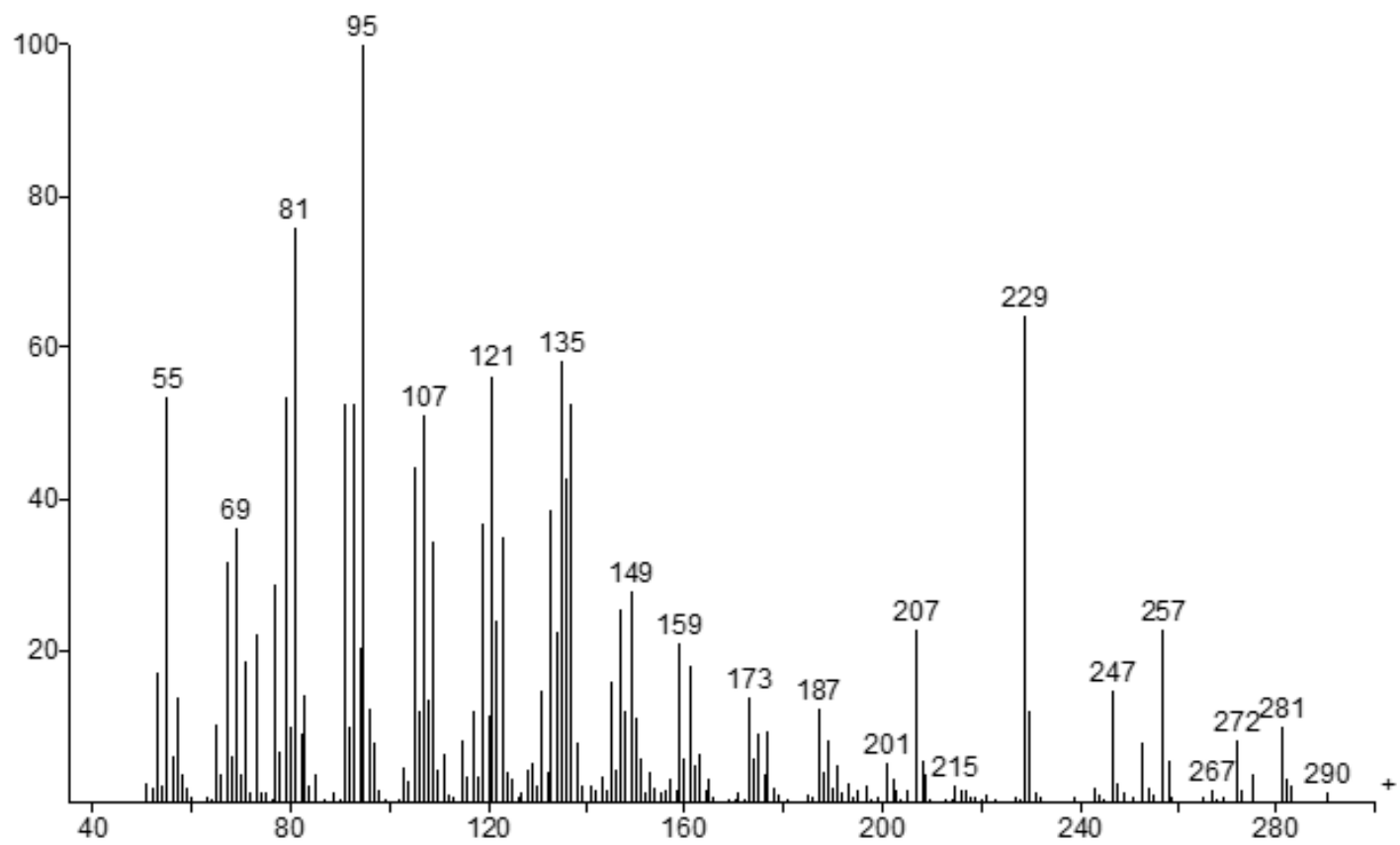




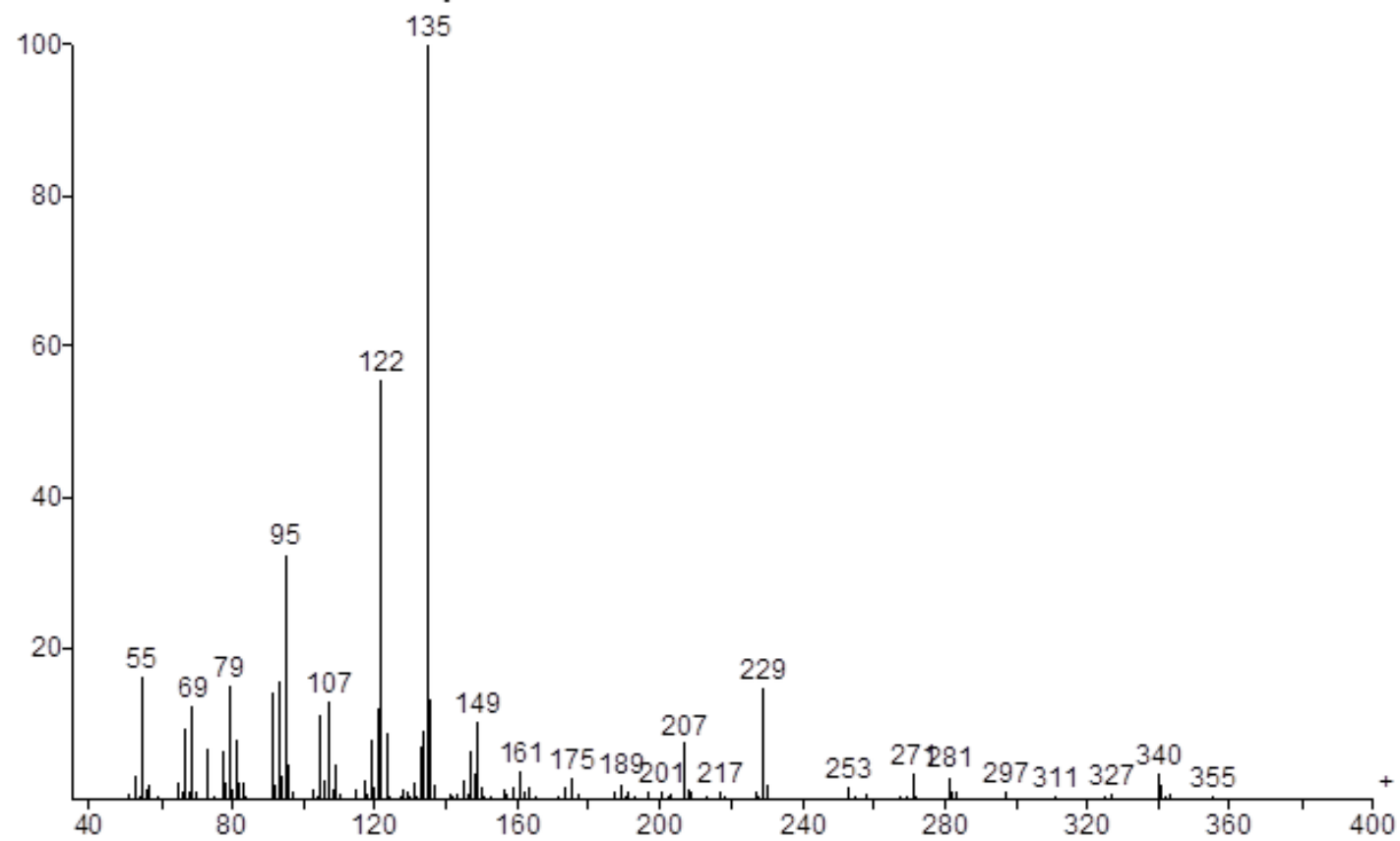
# geranylgeraniol



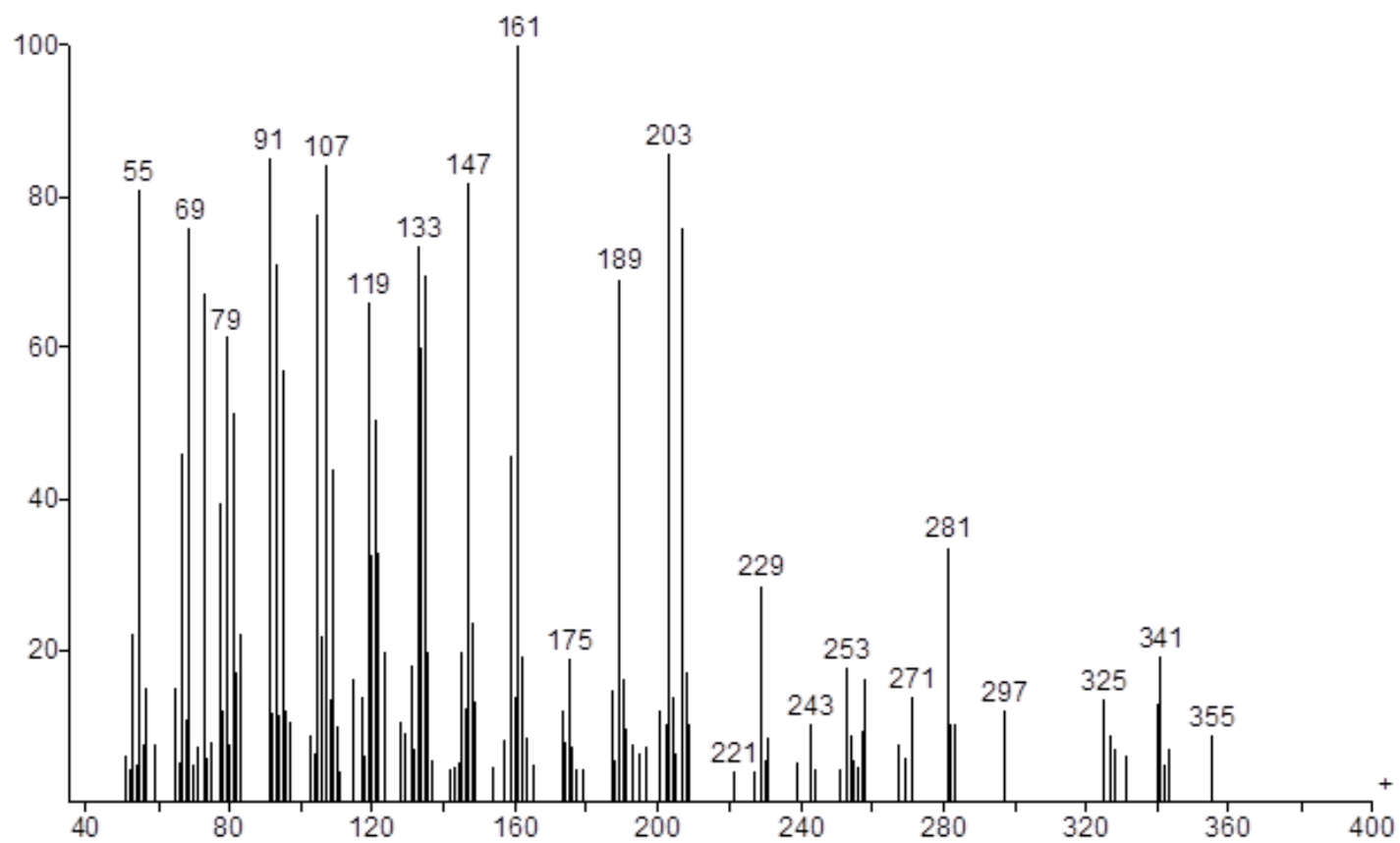
# Unknown diterpene alcohol



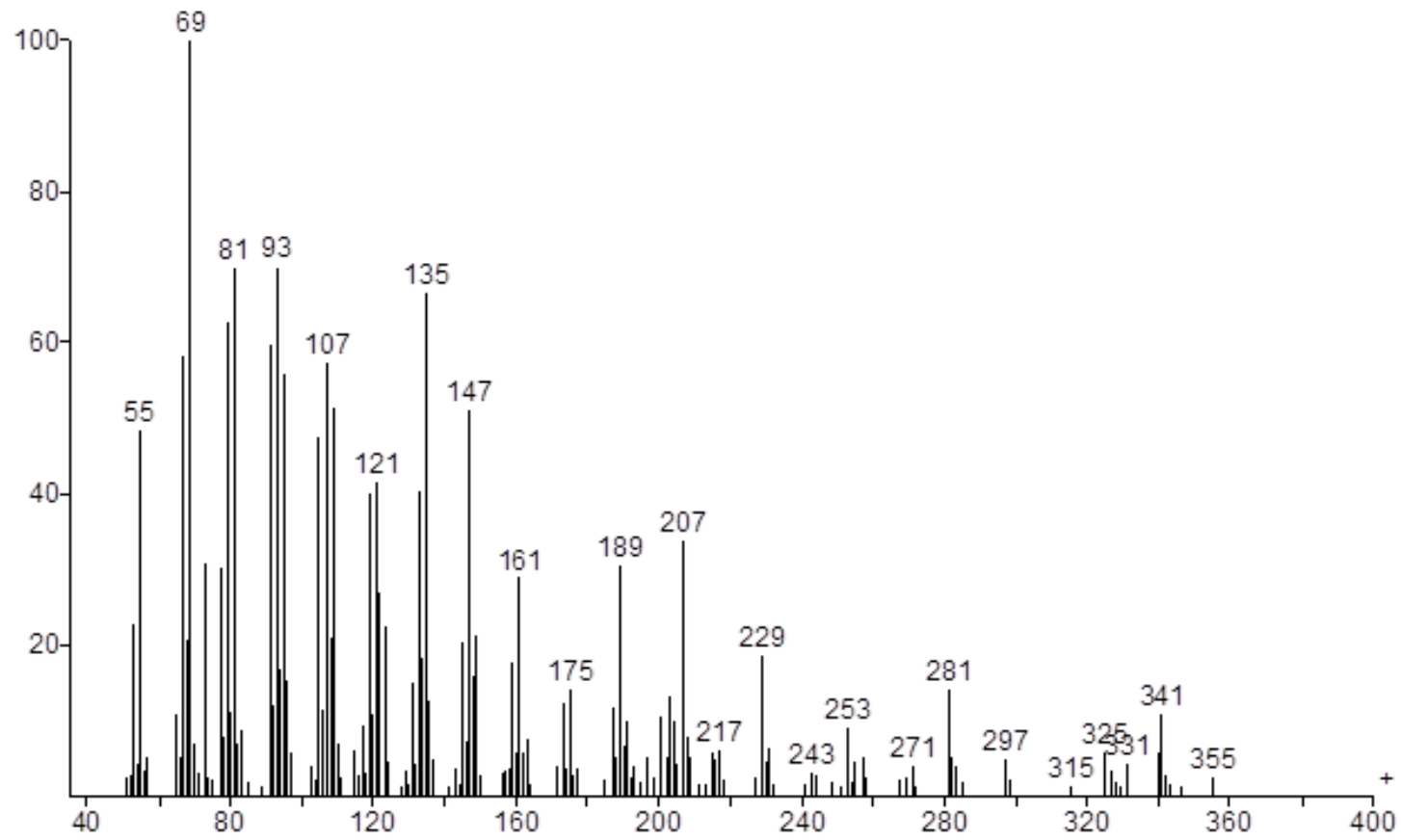
# Unknown sesterterpene 1



## Unknown sesterterpene 2



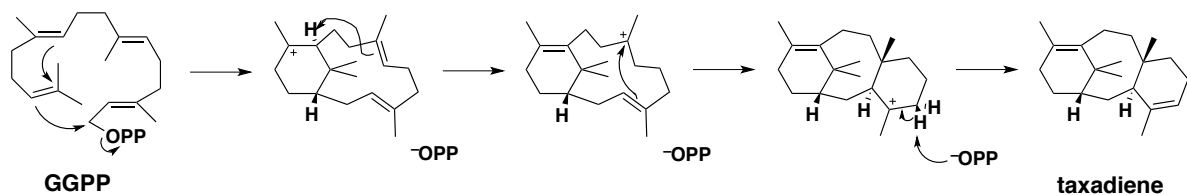
### Unknown sesterterpene 3



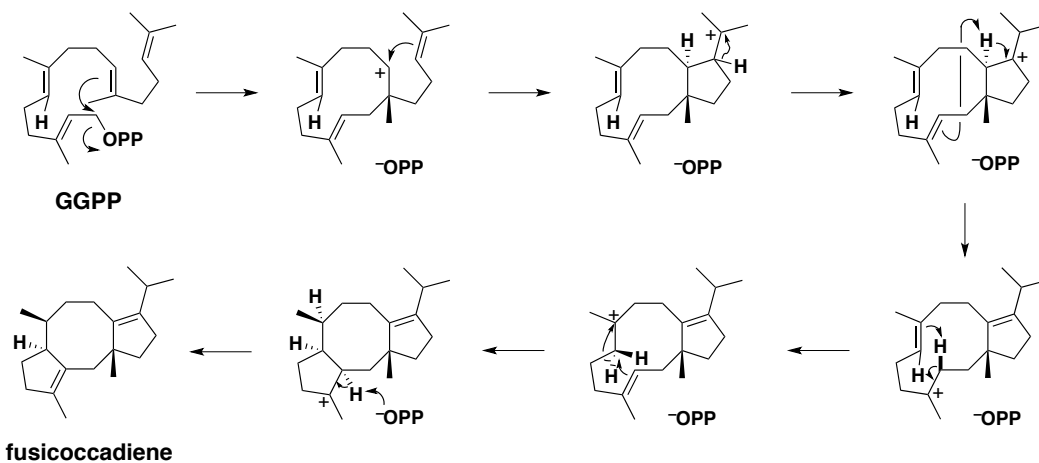
**Table S1.** Primers used for mutagenesis.

	<b>Primers</b>
TbTS $\alpha$ $\beta$	CGGCGGCATATGGATGATATCCCTCGACTCTCCGCCAATTATCATG GCGAAAGGCGTTCCAGCAGATCGATGGACCGTCTCCGAGCGCATTGAACATATC GATATGTTCAATGCGCTCGGAGACGGGTGATCGATCTGCTGGAACGCCTTTTCGC CCGCCGGGATCCTACTTGAATTGGATCAATATAAACTTTTCTTATATAGTCCTTAATCTCCTCATTG
TbTS $\alpha$ $\beta$ <sub>G</sub>	GTCGGAGACGGGGGATCGATCGATCTGC GCAGATCGATCGATCCCCCGTCTCCGAGC
TbTS $\alpha$ $\beta$ '	CGGCGGCATATGGATGATATCCCTCGACTCTCCGCC ATCGTCCATAGCTCTTTCGTCAGGAAATGCAAGAAATGAAGCTTCATATAGGCTTAGTAT ATACTAAGCCTATATGAAGCTTCATTTCTTGCAATTCCTGACGAAAGAGCTATGGACGAT CCGCCGGGATCCTACTTGAATTGGATCAATATAAAAC
TbTS $\alpha$	CGGCGGCATATGGATGATATCCCTCGACTCTCCGCCAATTATCATG GTCCAAATTTTGCCAATTTCTAAACATTTTGAATTAATCTCAAAGAATCTCCGTCTCCGAGCGCATTGAACAT ATGTTCAATGCGCTCGGAGACGGAGATTCTTTGAGTAATTCAAATGTTTAGAATTGGCAAATTTGGAC CCGCCGGGATCCTACTTGAATTGGATCAATATAAACTTTTCTTATATAGTCCTTAATCTCCTCATTG
PaFS $\alpha$ -EvSS $\alpha$	TACTTCCAATCCAATGCAGAGTTCAAATACTCGGAAGTCGTTG GGTTTTGATACCGTTTCGCGCTCAGATCCTCTGTAGAGTCCGATTCATCTGC GTACAGGACATGACTCCAAAGATGGCAGTCTTGACGCATCCATTCC GGAATGGATGCGTCAAGGACTGCCATCTTTGGAGTCATGTCCTGTAC GCAGATGAATCGGACTCTACAGAGGATCTGAGCGCGAACGGTATCAAAACC TTATCCAATTCCAATGTTATTAGCCGTTTTTCAGCAGCTCCATAATCA
PaFS $\alpha$ '-EvSS $\alpha$	GGCAAATGCCTTGATTGCTGTGGG CCCACAGCAATCAAGGCATTTGCC GATTGCTGCGGGCTTGCAGAAC GTTCTGCAAGCCCGCAGCAATC
AcOS $\alpha$ -PaFS $\alpha$	TACTTCCAATCCAATGCACATATGGCGTGCAAGTATAGCACCTGATTGATAGCAGCC GTCGTTTCCAGCAGGCTCGCCTGCACCAGCGCCGGAATGATC GATCATTCCGGCGCTGGTGCAGGCGAGCCTGCTGAACGAC CTGCCTACTTCCGCTTCCCAAGCTCTTCAGTTTCGTTCCGGCTTGCTC GAGCAAGCCGAACGAAGTGAAGAGCTTGGGAAGCGGAAGTAGGCAG TTATCCAATTCCAATGTTATTAGACTCGCAGAAGCTCCAGCAAAAGCCTCATGGCAGGGT
AcOS $\alpha$ '-PaFS $\alpha$	TACTTCCAATCCAATGCACATATGGCGTGCAAGTATAGCACCTGATTGATAGCAGCC GTCGTTTCCAGCAGGCTCACCTGCACCCACGCCGGAATGATC GATCATTCCGGCGTGGGTGCAGGTGAGCCTGCTGAACGAC CTGCCTACTTCCGCTTCCCAAGCTCTTCAGTTTCGTTCCGGCTTGCTC GAGCAAGCCGAACGAAGTGAAGAGCTTGGGAAGCGGAAGTAGGCAG TTATCCAATTCCAATGTTATTAGACTCGCAGAAGCTCCAGCAAAAGCCTCATGGCAGGGT

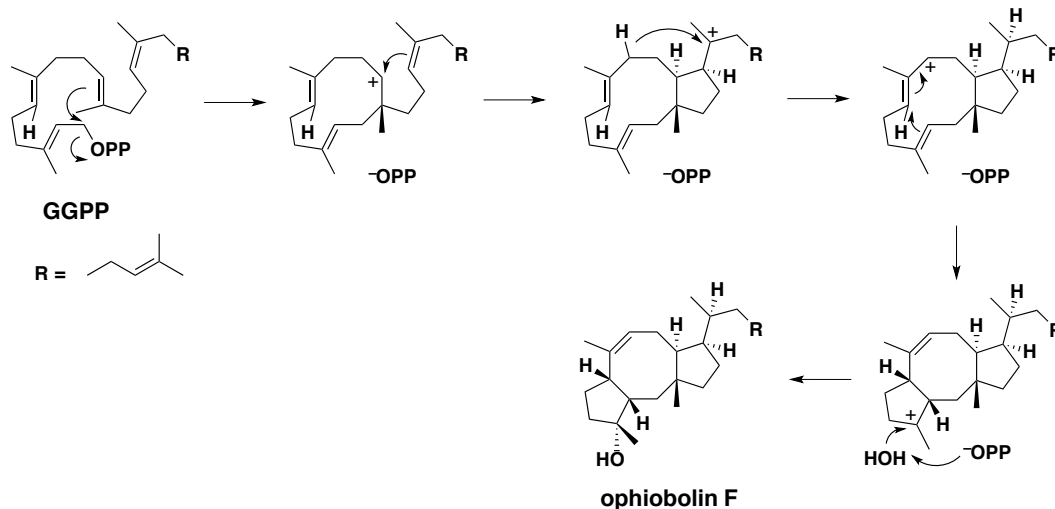
### Taxadiene Synthase Cyclization Mechanism



### Fusicocadiene Synthase Cyclization Mechanism



### Ophiobolin F Synthase Cyclization Mechanism



**Figure S1.** Cyclization mechanisms of taxadiene synthase, fusicocadiene synthase, and ophiobolin F synthase.