Supporting Information

Geosmin Biosynthesis. Mechanism of the Fragmentation-Rearrangement in the Conversion of Germacradienol to Geosmin

*Jiaoyang Jiang and David E. Cane**

Department of Chemistry, Box H, Brown University, Providence, Rhode Island 02912-9108

E-mail: David_Cane@brown.edu

Materials and Methods. General materials and methods were as previously described.[1,2] Recombinant germacradienol/geosmin synthase (GS), encoded by the *sco6073* (SC9B1.20) gene of *S. coelicolor* A3(2) was expressed from *E. coli* BL21(DE3)pLysS/pRW31, resolubilized from inclusion bodies, purified to homogeneity, and assayed as previously described.[1,2]

Derivatization of Acetone – Optimization of pH. Acetone (10 μL, 27 mM) and 0.1545 g cysteamine hydrochloride (270 mM) were added to 5 mL reaction buffer (50 mM Tris-HCl, 1 mM EDTA, 20% glycerol, 5 mM $MgCl_2$, pH 8.2) in a 20-mL glass vial, and the pH of the solution was immediately adjusted to 8.0 with 0.1 N NaOH. The mixture was stirred at room temperature overnight. Parallel experiments were conducted at pH 5.0, 6.0, and 7.0. The reaction mixture was transferred to a test tube, 1 μL of α-humulene stock solution was added as internal standard, and the mixture was extracted with 3×3 mL CH_2Cl_2. The combined organic layers were dried by passage through a Pasteur pipette containing 1 g of $MgSO_4$ before concentration under reduced pressure at 0 °C to 200 μL. A 1-μL portion of the concentrated extract was analyzed by GC–MS (30 m × 0.25 mm DB5 capillary column, using a temperature program of 50–280 °C, 20 °C min^{-1}) (Figures S1 and S2).

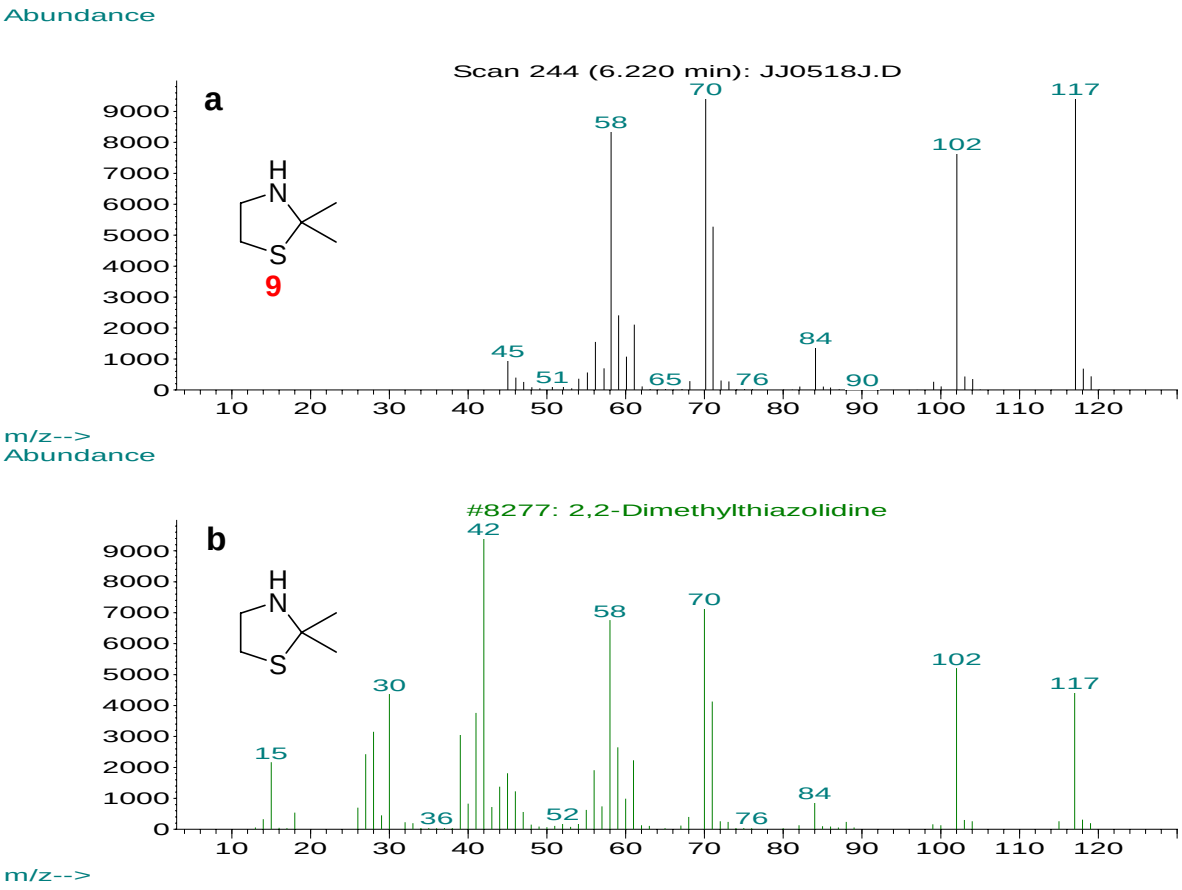


Figure S1. a) MS of 2,2-dimethylthiazolidine (**9**) derived from acetone. b) MS of standard 2,2-dimethylthiazolidine in the NIST/EPA/NIH Mass Spectral Library (2002 version).

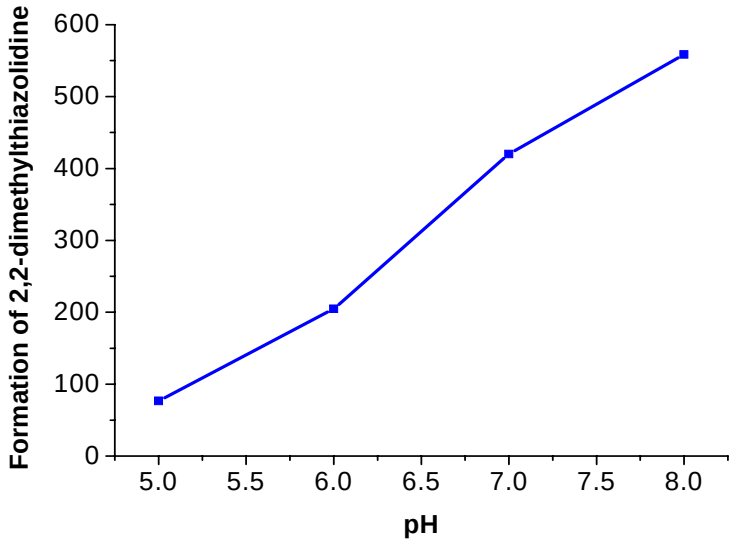


Figure S2. Relative yield of 2,2-dimethylthiazolidine (**9**) as a function of pH (internal standard α-humulene = 1.0).

Formation of Acetone in the Incubation of FPP with Germacradienol/Geosmin Synthase. FPP (66 μM) was incubated with germacradienol/geosmin synthase (10.2 μM) in 7.5 mL of buffer (50 mM Tris-HCl, 20% glycerol, 2 mM $MgCl_2$, pH 8.2) at 30 °C for 7 h 20 min, without a pentane overlay in order to avoid transfer of acetone out of the aqueous solution. At the end of the incubation period, 0.01 g cysteamine hydrochloride (11.7 mM) was added, and the pH was immediately adjusted to 8.0. The reaction mixture was stirred at room temperature overnight, and then extracted with 3×3 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ before being concentrated under reduced pressure at 0 °C to 200 μL and analyzed by GC-MS (Figure S3). A control incubation was carried out with 66 μM FPP and 10.2 μM boiled (98 °C, 30 min) protein.

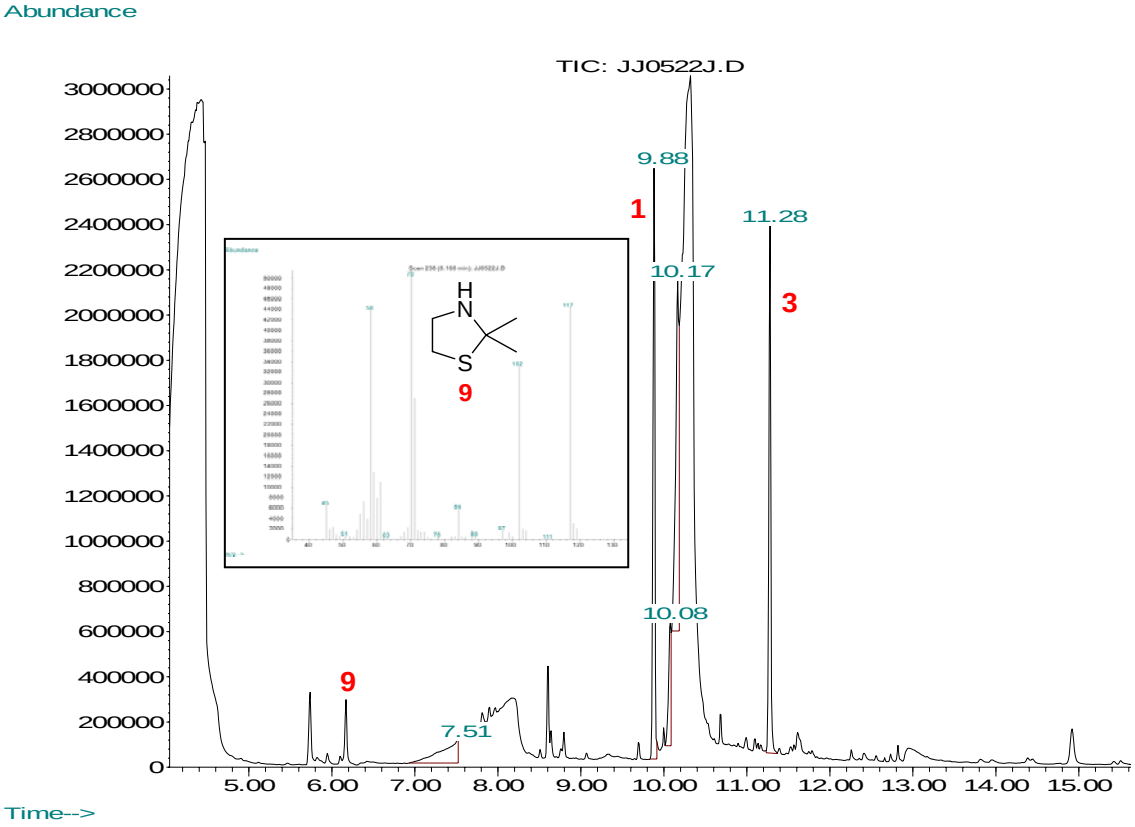
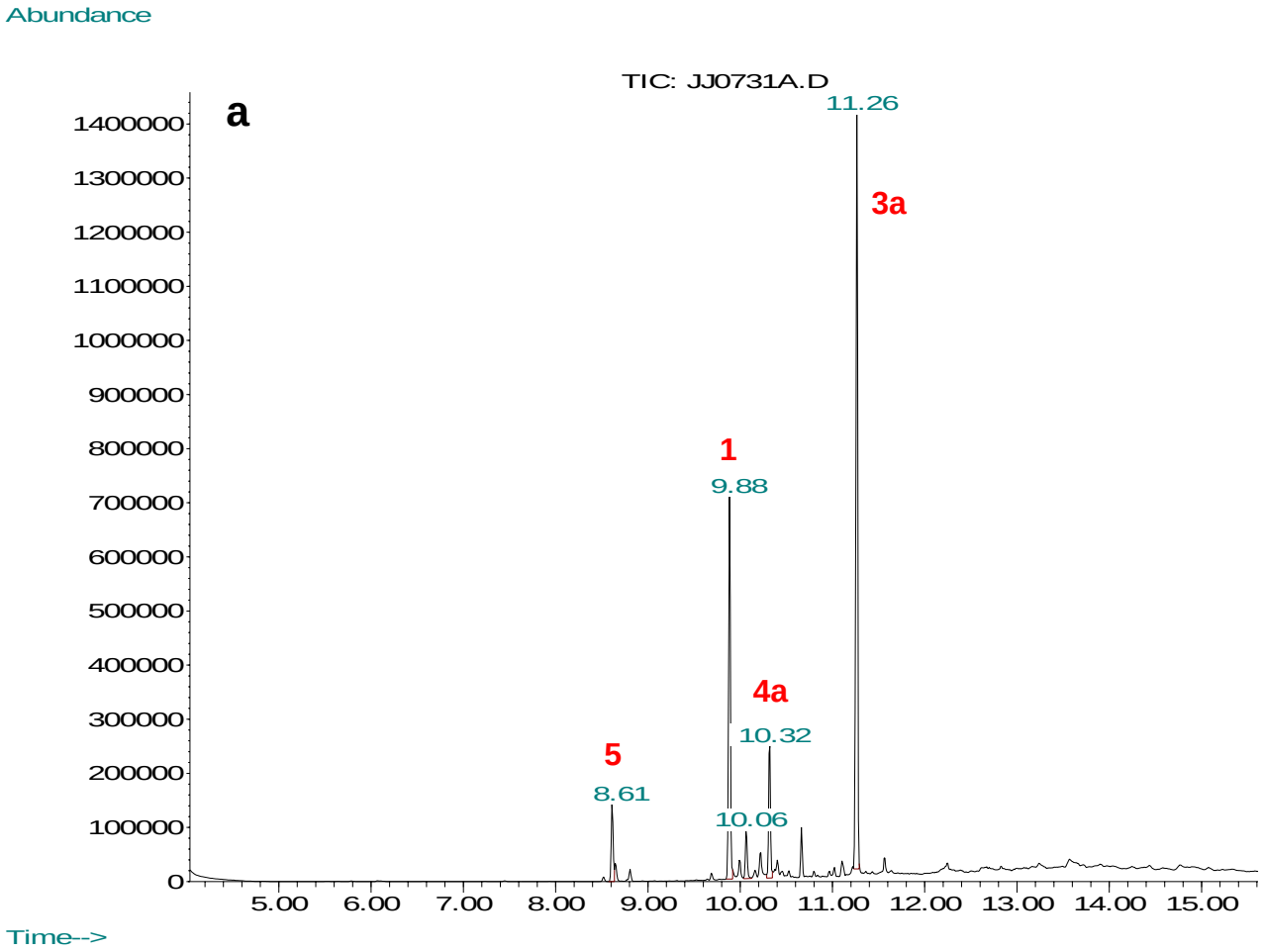


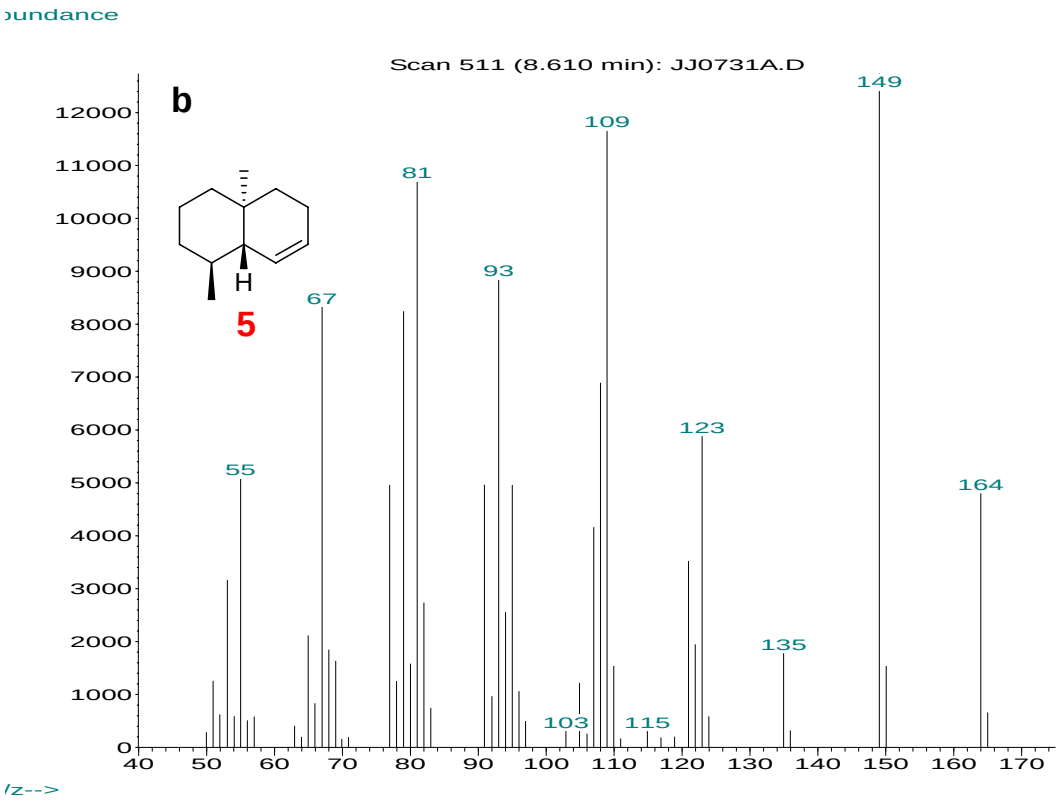
Figure S3. Trapping of acetone generated by the incubation of FPP with germacradienol/geosmin synthase. GC-MS of organic extract after treatment with cysteamine. Inset: EI-MS of 2,2-dimethylthiazolidine (**9**), ret. time 6.17 min.

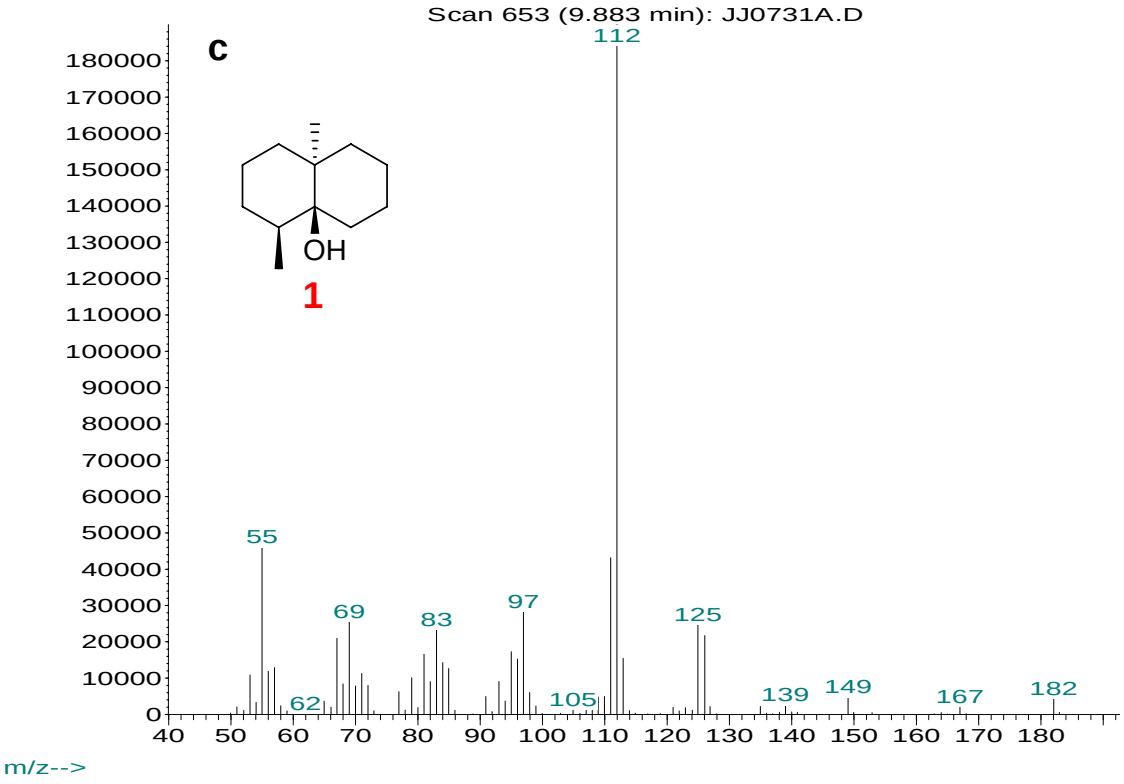
Incubation of [13,13,13-$^2H_3$]FPP with Germacradienol/Geosmin Synthase. [13,13,13-$^2H_3$]FPP (**2a**, 103 μM) was incubated with germacradienol/geosmin synthase (5.1 μM) in 5 mL of buffer (50 mM Tris-HCl, 20% glycerol, 3 mM $MgCl_2$, pH 8.2) at 30 °C for 6 h 15 min, with

a pentane overlay. The reaction mixture was extracted with 3×3 mL of pentane/CH_2Cl_2 (5:1) and analyzed by GC-MS (Figure S4).

To detect the formation of deuterated acetone **8a**, the acetone was converted into corresponding dimethylthiazolidine **9a**. FPP (**2a**, 62 μM) was incubated with germacradienol/geosmin synthase (7.8 μM) and in 8.3 mL of buffer was incubated at 30 °C for 7 h 35 min, without a pentane overlay. After the incubation, 100 mM cysteamine hydrochloride was added, and the pH was immediately adjusted to 8.0. The reaction mixture was stirred at room temperature overnight, extracted with 3×3 mL of CH_2Cl_2, and analyzed by GC-MS (Figure S5).




Scan 511 (8.610 min): JJ0731A.D
b
H
5
149
109
81
93
67
123
55
164
135
103
115
m/z-->


Abundance
Scan 653 (9.883 min): JJ0731A.D
c
OH
1
112
55
69
83
97
125
62
105
139
149
167
182
m/z-->

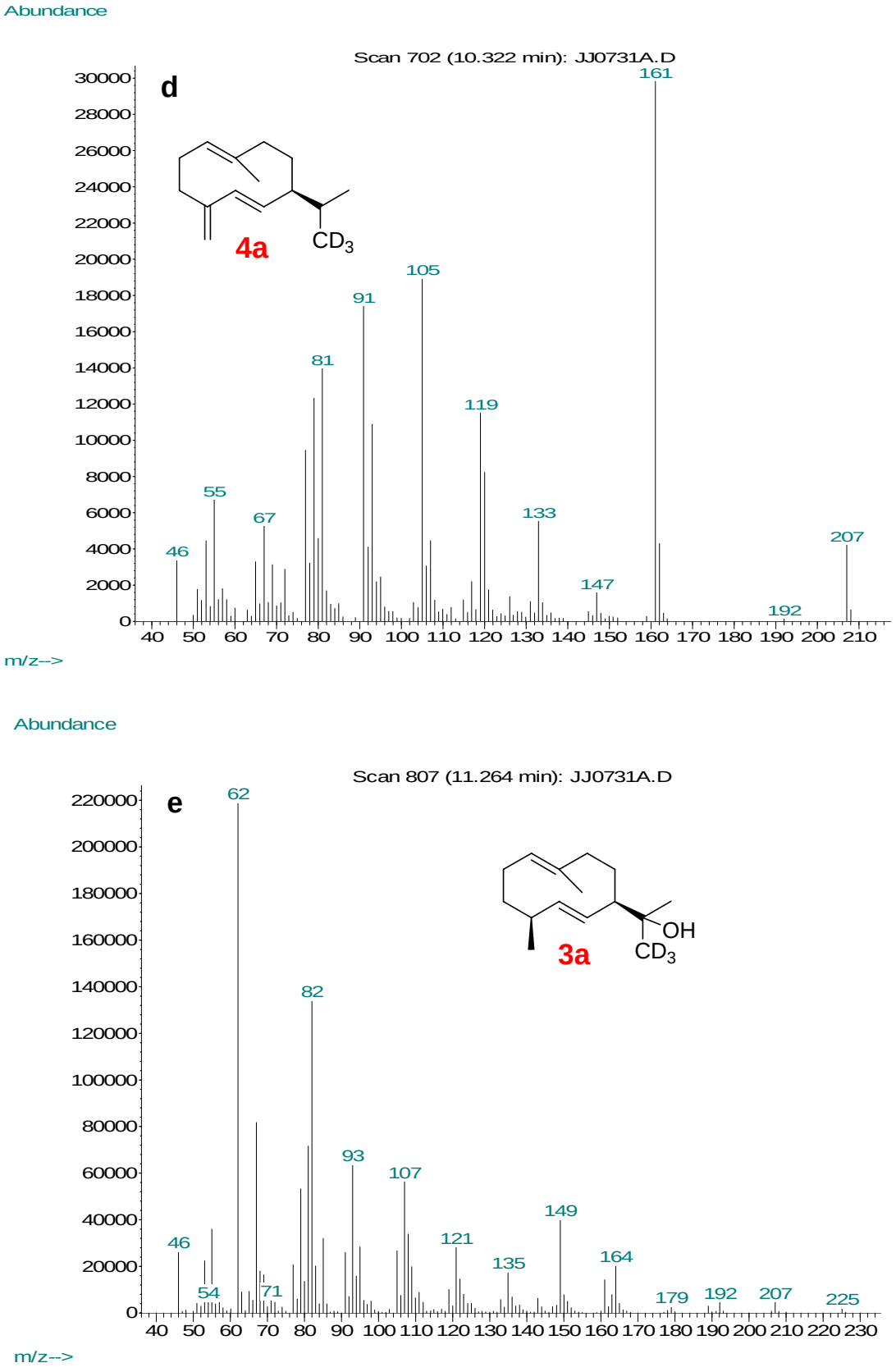


Figure S4. Incubation of 103 µM [13,13,13-$^{2}H_3$]FPP (**2a**) with 5.1 µM germacradienol/geosmin synthase with at 30 °C for 6 h 15 min. a) GC-MS. b) MS of octalin (**5**), ret. time 8.61 min. c) MS

of geosmin (**1**), ret. time 9.88 min. d) MS of germacrene D (**4a**), ret. time 10.32 min. e) MS of germacradienol (**3a**), ret. time 11.26 min.

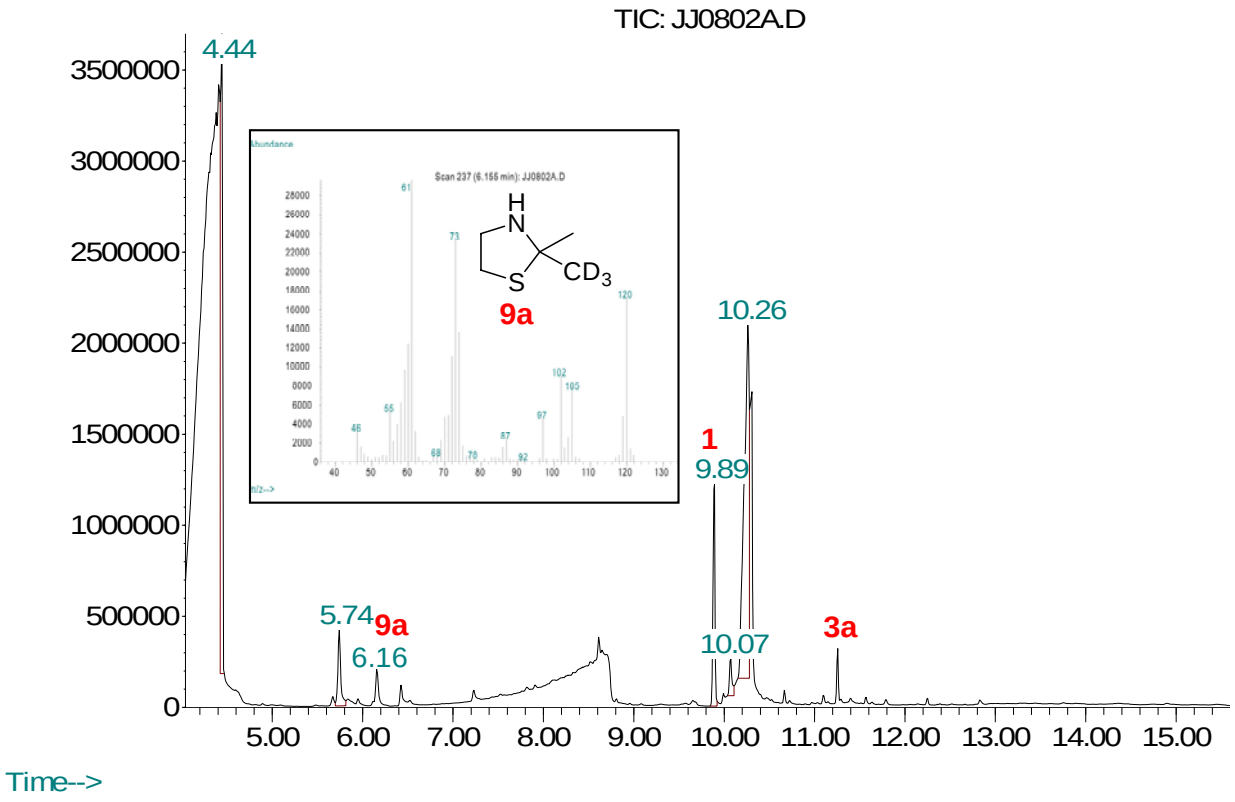
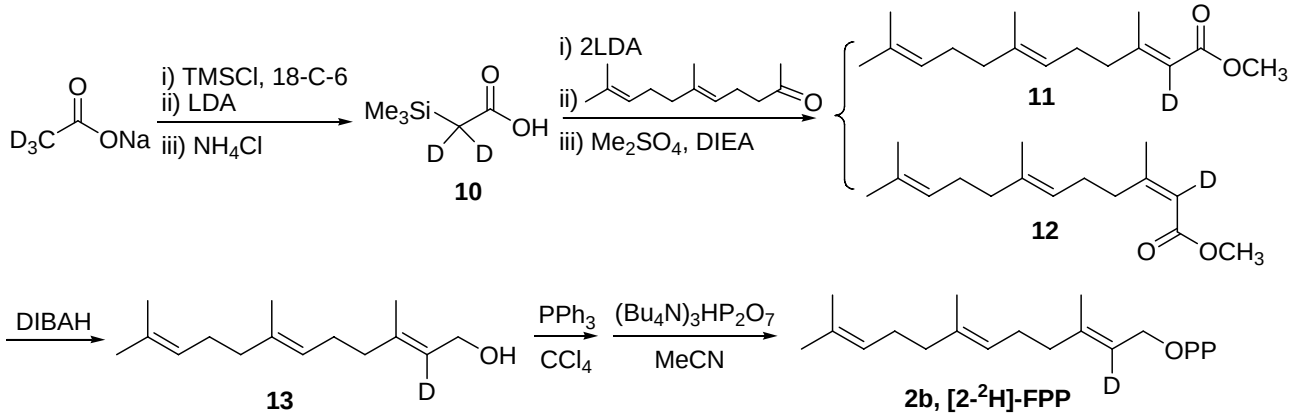


Figure S5. Trapping of acetone generated by the incubation of [13,13,13-$^2H_3$]FPP with germacradienol/geosmin synthase. GC-MS of organic extract after treatment with cysteamine. Inset: EI-MS of [$^2H_3$-Me]-2,2-dimethylthiazolidine (**9a**), ret. time 6.16 min.

Scheme S1. Synthesis of [2-^{2}H]FPP (**2b**)



Synthesis of [2-^{2}H]-FPP (2b)

*[2-$^2H_2$]Trimethylsilylacetic acid (**10**).* A mixture of 1.14 g (13.4 mmol) of vacuum-dried sodium [2-$^2H_3$]acetate (99 atom % ^{2}H, Aldrich), 1.46 g (13.4 mmol) of trimethylsilyl chloride, and 18-crown-6 ether (1.24 g, 4.7 mmol) in 80 mL of Et_2O was refluxed for 2.5 h under N_2. The reaction mixture was cooled and the solution transferred dropwise to another flask containing 14.7 mmol of lithium diisopropylamide (prepared by reaction of *n*-butyllithium with diisopropylamine) in Et_2O at –78 °C. The resulting white suspension was stirred for 30 min at –78 °C, then warmed to room temperature for an additional 30 min during which the precipitate dissolved. The yellow solution was refluxed for 2 h after which the reaction was quenched by addition of satd aq. NH_4Cl. The pH of the aq. phase was adjusted to 3, brine was added, and the solution was extracted with Et_2O (3 x 100 mL). Drying of the Et_2O extract ($MgSO_4$) and concentration under vacuum gave 856 mg (40%) of [2-$^2H_3$]trimethylsilylacetic acid which solidified at -20 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) are both consistent with the reported data.[3]

(E)- and (Z)-Methyl *[2-^{2}H]farnesoates (**11**) and (**12**).* [2-$^2H_2$]Trimethylsilylacetic acid (**10**, 1.38 g, 10.3 mmol) in 10 mL of THF was added dropwise to 1.9 eq. of lithium diisopropylamide in THF at –78 °C. The reaction mixture was stirred for 30 min at -78 °C and 1 h at 0 °C, then cooled to -78 °C. A solution of 2.0 g (10.3 mmol, 1.0 eq.) of geranylacetone in 8 mL of THF was added dropwise and the resulting solution was stirred for 1 h at -78 °C, 1 h at -10 °C, and 1 h at room temperature. The reaction was quenched by dropwise addition of 0.1 N HCl at 0 °C. After the mixture was stirred for an additional 10 min, the THF was evaporated *in vacuo*, and the oily product was taken up in hexane and poured into a mixture of 65 mL 0.5 N HCl and 190 mL of hexane. After extraction with hexane (3 x 200 mL), drying ($MgSO_4$), and concentration *in vacuo*, flash silica gel column chromatography (1-20% EtOAc:hexane, stepwise) gave 2.08 g of a mixture of *cis* and *trans* acids which was esterified without further purification. A mixture of the isomeric acids (1.80 g) was dissolved in 50 mL of MeCN at 0 °C, 938 mg (7.6 mmol) of diisopropylethylamine was added, and the solution was stirred for 20 min at 0 °C and 30 min at room temperature, then cooled to 0 °C. Dimethyl sulphate (1.92 g, 15.2 mmol) was added. After 30 min stirring at 0 °C, the mixture was warmed to room temperature and stirred for 3.5 h. After addition of 0.1 N aq. NH_4OH to destroy

excess Me_2SO_4, the MeCN was evaporated and the product mixture was taken up in Et_2O and partitioned into H_2O. After extraction with Et_2O, drying ($MgSO_4$), and concentration *in vacuo*, the crude product was purified by prep. TLC (10% Et_2O in hexane) to give 492 mg of a mixture of the methyl esters of *cis* and *trans* [2-^{2}H]farnesoic acid (**11** and **12**). Spectroscopic data for the unlabeled methyl esters, generated by identical procedures, were identical in all respects with authentic samples.

*[2-^{2}H]Farnesol (**13**)*. To a solution of 492 mg (1.96 mmol) of the (*E*)- and (*Z*)- methyl [2-^{2}H]farnesoate (**11** and **12**) in 37 mL of CH_2Cl_2 at –78 °C was added dropwise 4.51 mL (4.51 mmol) of diisobutylaluminum hydride (1 M, hexane). After stirring for 30 min at -78 °C, the reaction mixture was warmed to 0 °C with stirring for another 30 min, and the reaction was then quenched by addition of 9 mL of 0.5 M aq. sodium potassium tartrate followed by vigorous stirring of the mixture for 4 h at room temperature. After addition of H_2O, extraction with CH_2Cl_2, drying ($MgSO_4$) and concentration, the crude product was purified by column chromatography on $AgNO_3$-coated silica gel (20% EtOAc in hexane) to yield 88.6 mg *(E)*-[2-^{2}H]farnesol (**13**) (Figure S6).

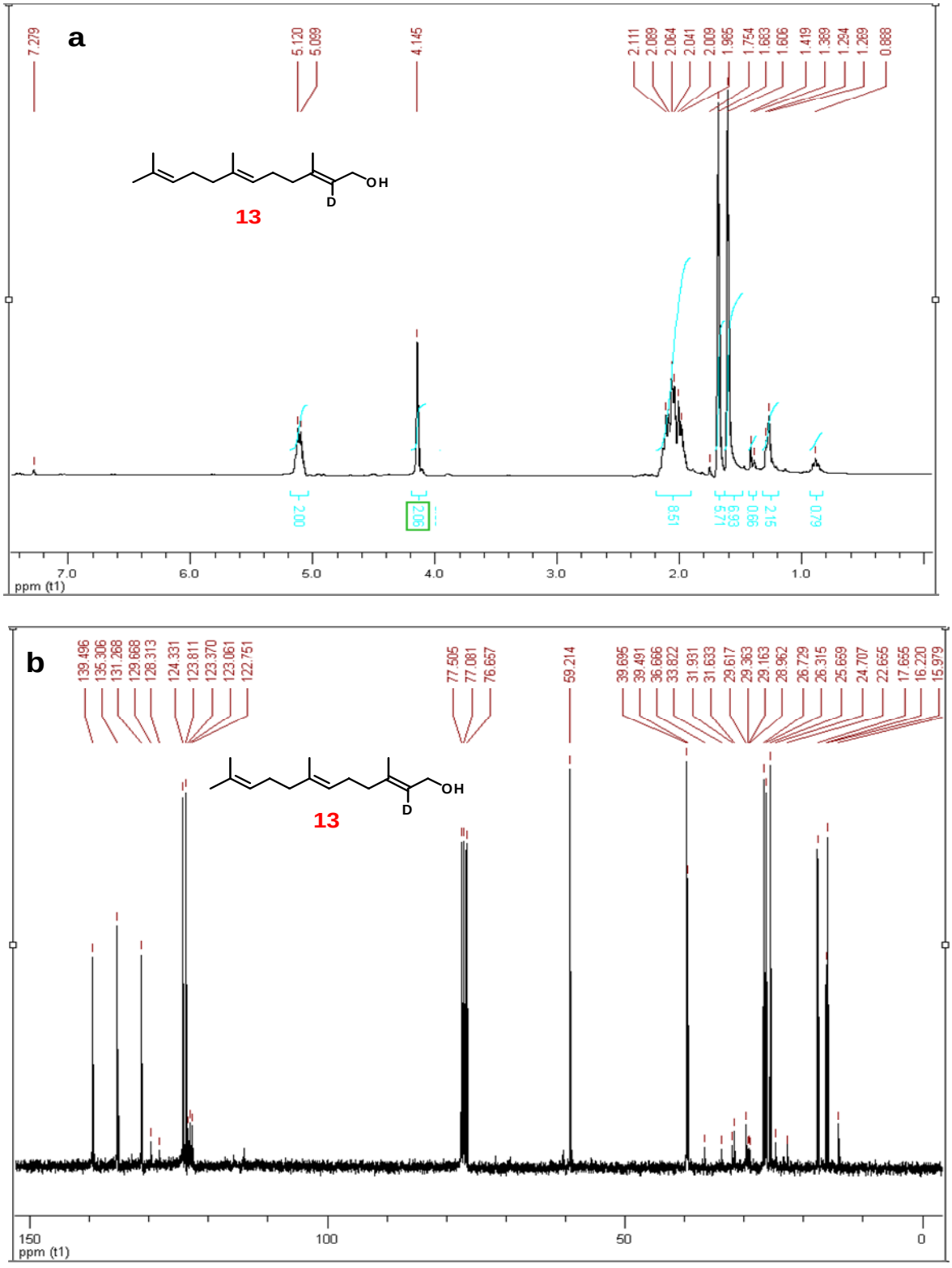


Figure S6. [2-^{2}H]farnesol (**13**) a) ^{1}H NMR (300 MHz, $CDCl_3$). b) ^{13}C NMR (75 MHz, $CDCl_3$).

*[2-^{2}H]Farnesyl diphosphate (**2b**)* [2-^{2}H]Farnesyl diphosphate (**2b**, 160 mg) was prepared from [2-^{2}H]farnesol (**13**) in ca 80% yield by the method previously described (Figure S7).[4]

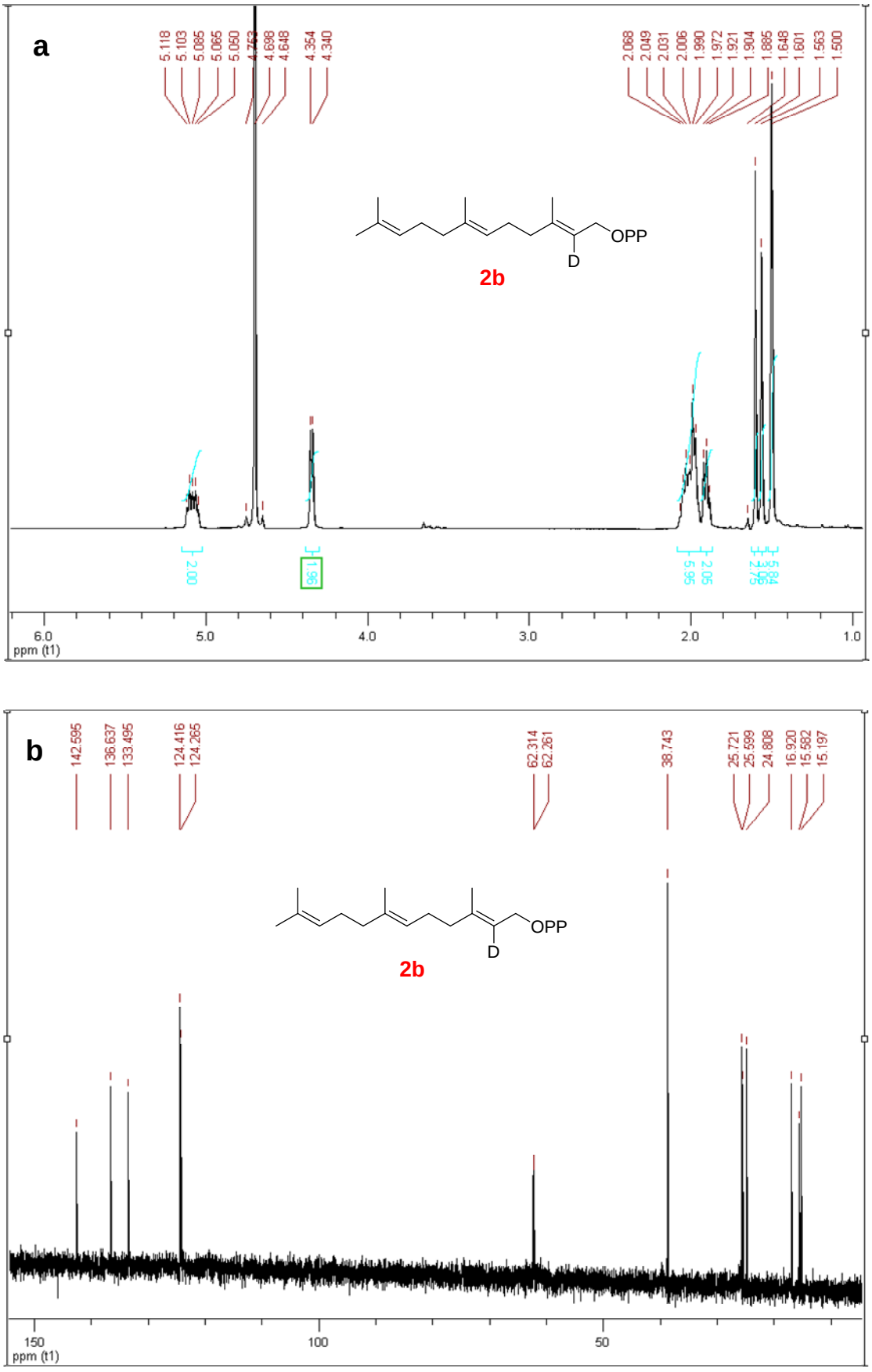
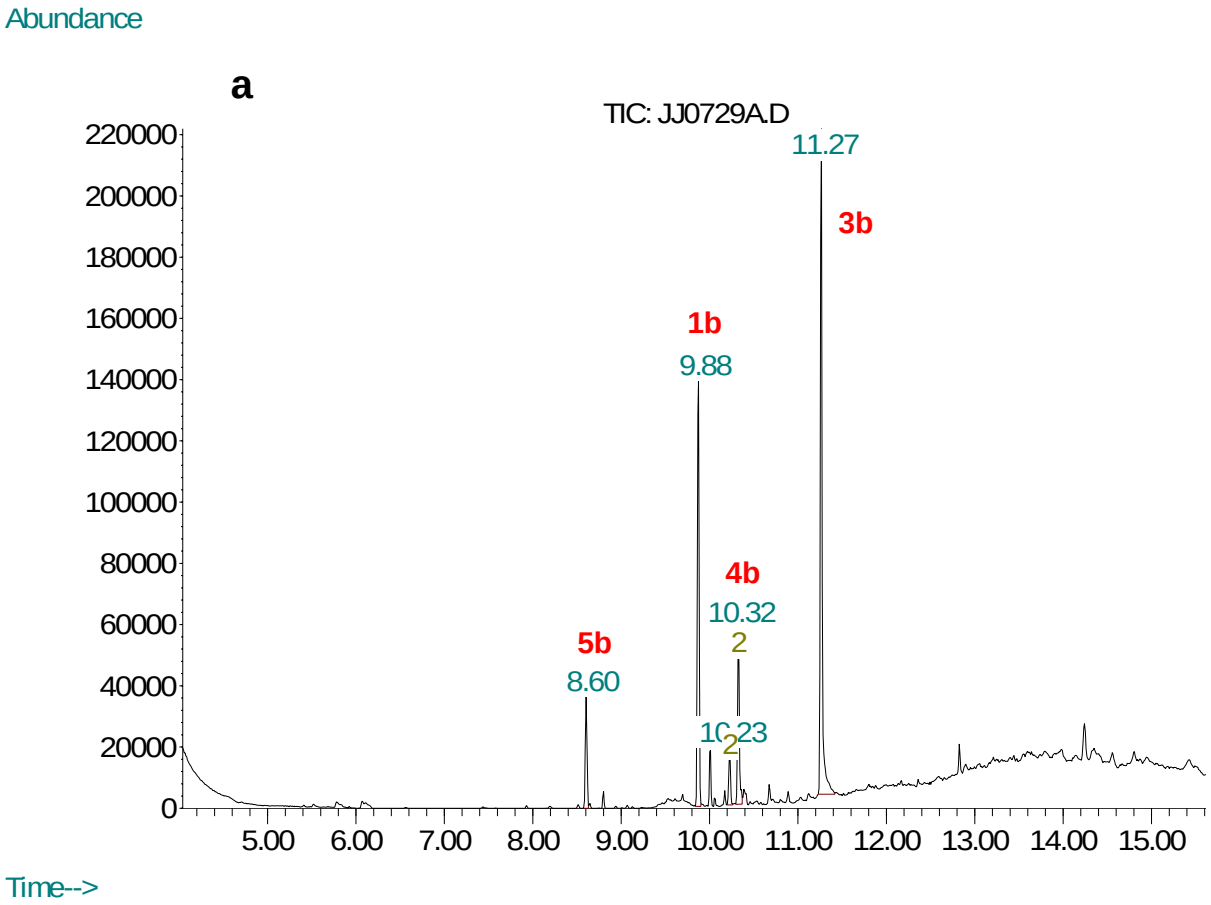
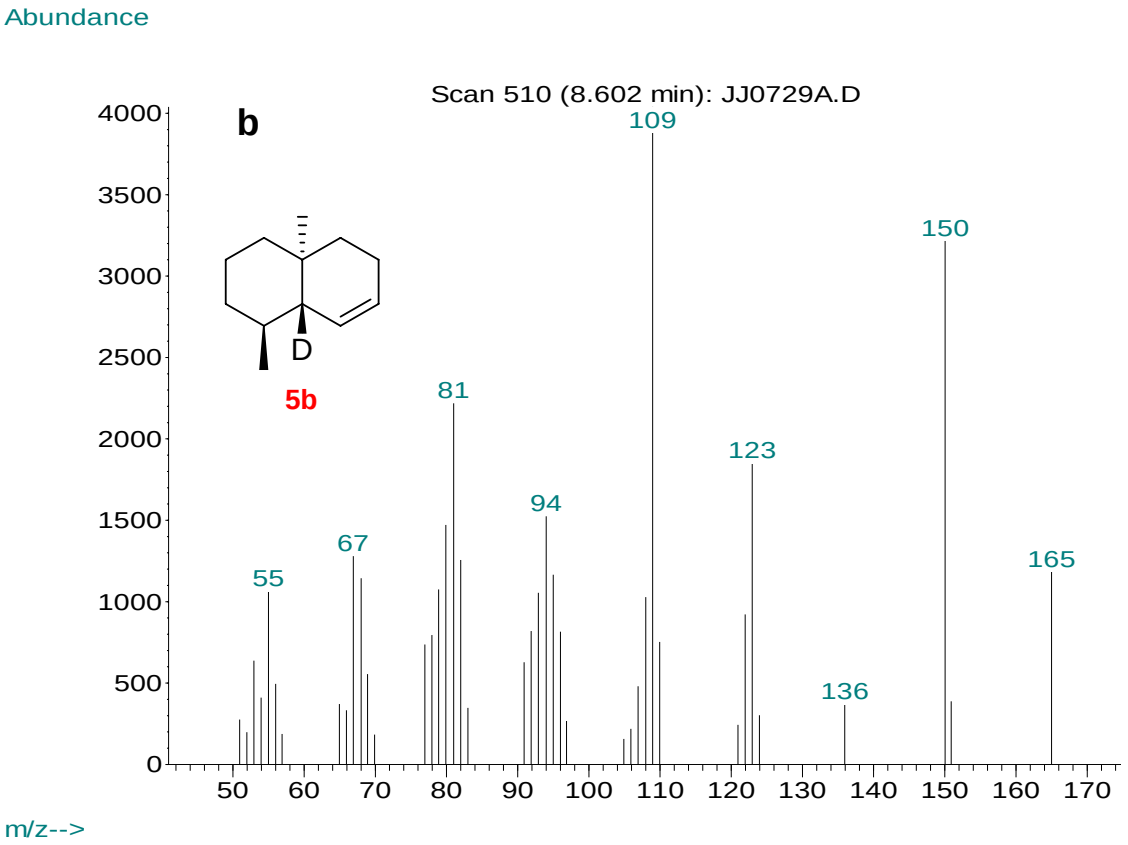
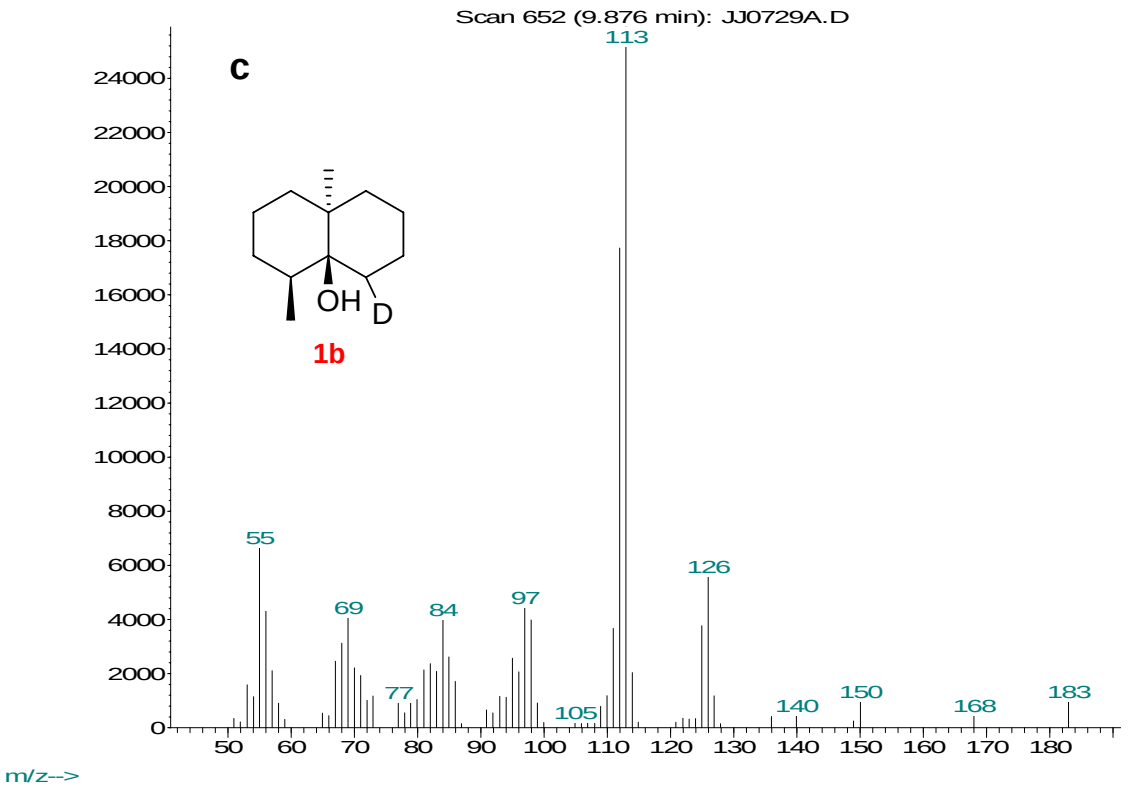


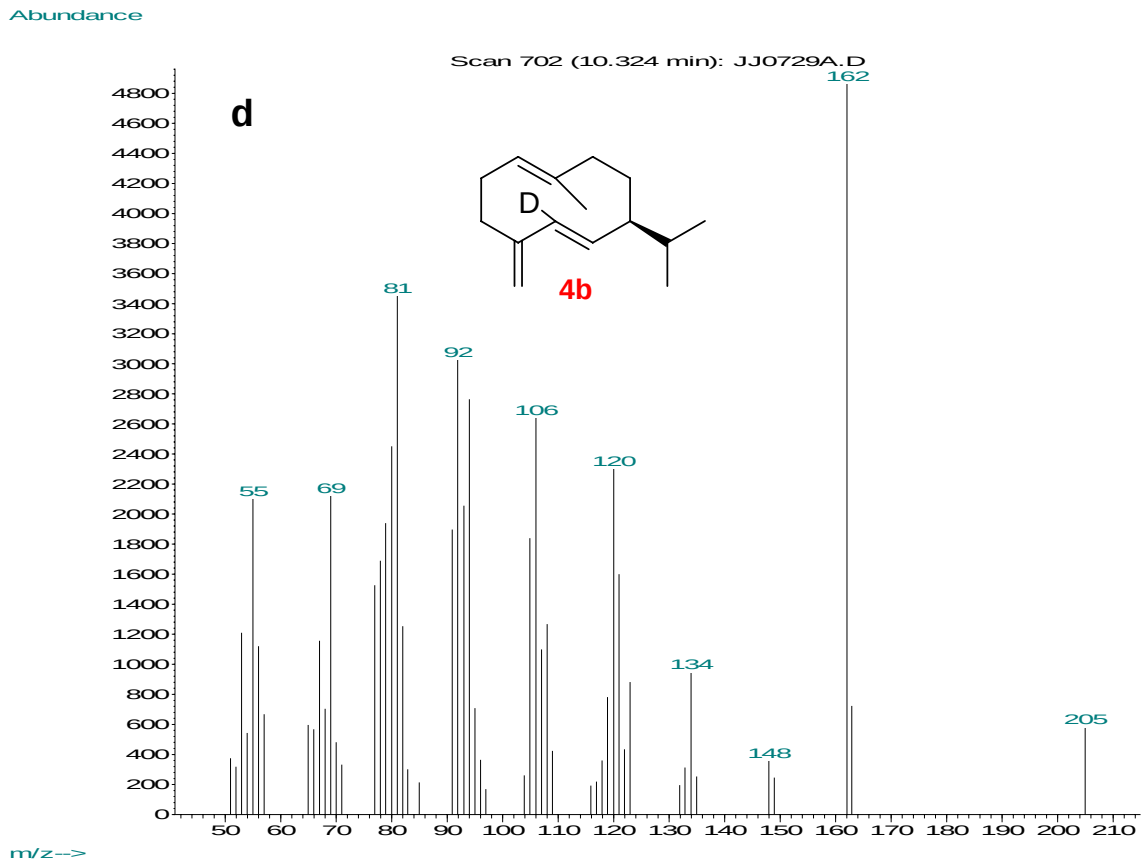
Figure S7. [2-^{2}H]FPP (**2b**). a) ^{1}H NMR (400 MHz, D_2O). b) ^{13}C NMR (100 MHz, D_2O).

Incubation of [2-^{2}H]FPP with Germacradienol/Geosmin Synthase. [2-^{2}H]FPP (**2b**, 99 μM) was incubated with germacradienol/geosmin synthase (7.0 μM) in 5 mL of buffer (50 mM Tris-HCl, 20% glycerol, 3 mM $MgCl_2$, pH 8.2) at 30 °C for 6 h 15 min with a pentane overlay. The reaction mixture was extracted with 3×3 mL of pentane/CH_2Cl_2 (5:1) and analyzed by GC-MS (Figure S8).




Abundance
Scan 510 (8.602 min): JJ0729A.D
b
D
5b
109
150
81
123
94
67
55
165
136
4000
3500
3000
2500
2000
1500
1000
500
0
50 60 70 80 90 100 110 120 130 140 150 160 170
m/z-->


Abundance
Scan 652 (9.876 min): JJ0729A.D
c
OH
D
1b
113
55
126
69
84
97
77
105
140
150
168
183
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
50 60 70 80 90 100 110 120 130 140 150 160 170 180
m/z-->


Abundance
Scan 702 (10.324 min): JJ0729A.D
d
D
4b
162
81
92
106
120
55
69
134
205
148
m/z-->

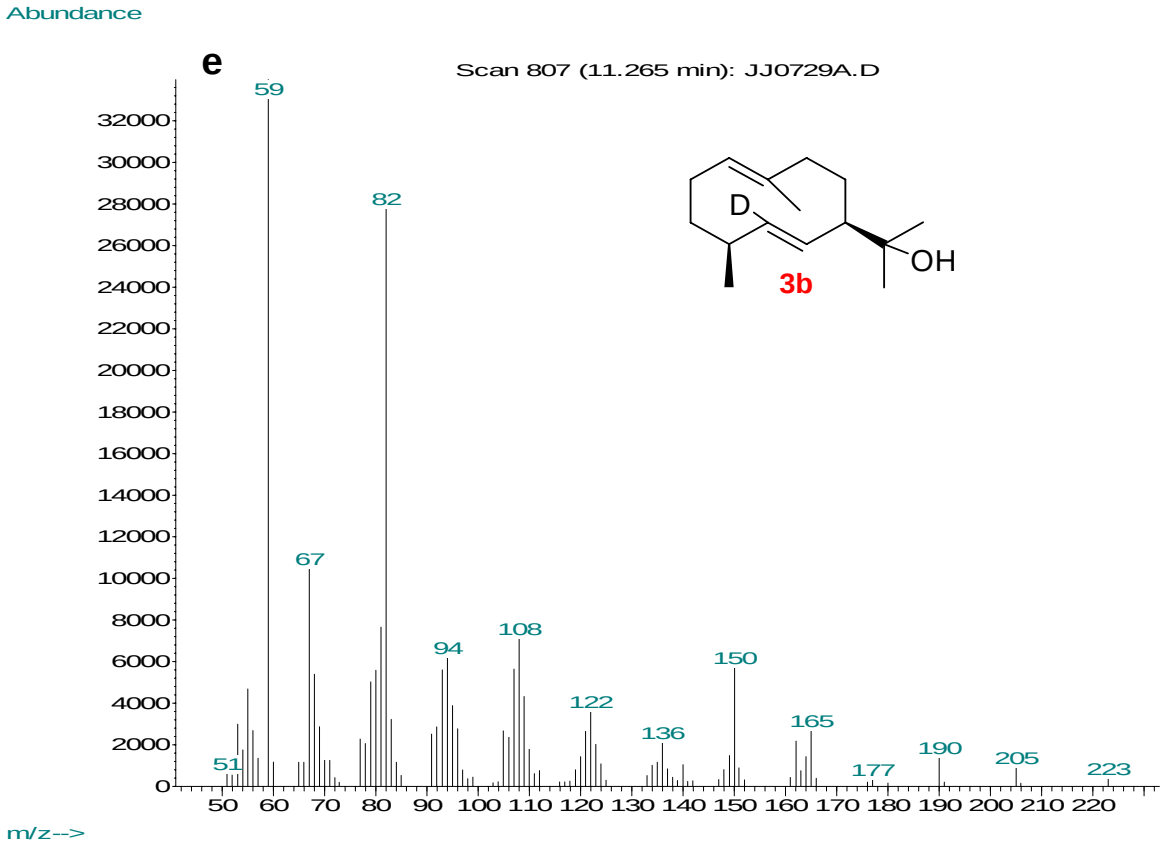

Abundance
e
Scan 807 (11.265 min): JJ0729A.D
D
OH
3b
59
82
67
94
108
150
122
136
165
190
205
177
51
223
m/z-->

Figure S8. Incubation of 99 μM [2-^{2}H]-FPP (**2b**) with 7.0 μM germacradienol/geosmin synthase at 30 °C for 6 h 15 min. a) (A) GC-MS. b) MS of octalin (**5b**), ret. time 8.60 min. c) MS of [6-^{2}H]geosmin (**1b**), ret. time 9.88 min. d) MS of germacrene D (**4b**), ret. time 10.32 min. e) MS of germacradienol (**3b**), ret. time 11.27 min.

References

(1) Cane, D. E.; Watt, R. M. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1547-1551.

(2) Jiang, J.; He, X.; Cane, D. E. *J. Am. Chem. Soc.* **2006**, *128*, 8128-8129.

(3) Arigoni, D.; Cane, D. E.; Shim, J. H.; Croteau, R.; Wagschal, K. *Phytochemistry* **1993**, *32*, 623-631.

(4) Croteau, R.; Felton, N. M.; Wheeler, C. J. *J. Biol. Chem.* **1985**, *260*, 5956-5962.