Supplementary Information

Incubation of 2-Methylisoborneol Synthase with the Intermediate Analogue 2-Methylneryl Diphosphate

Wayne K. W. Chou,[1] Colin A. Gould[1] and David E. Cane[1]

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

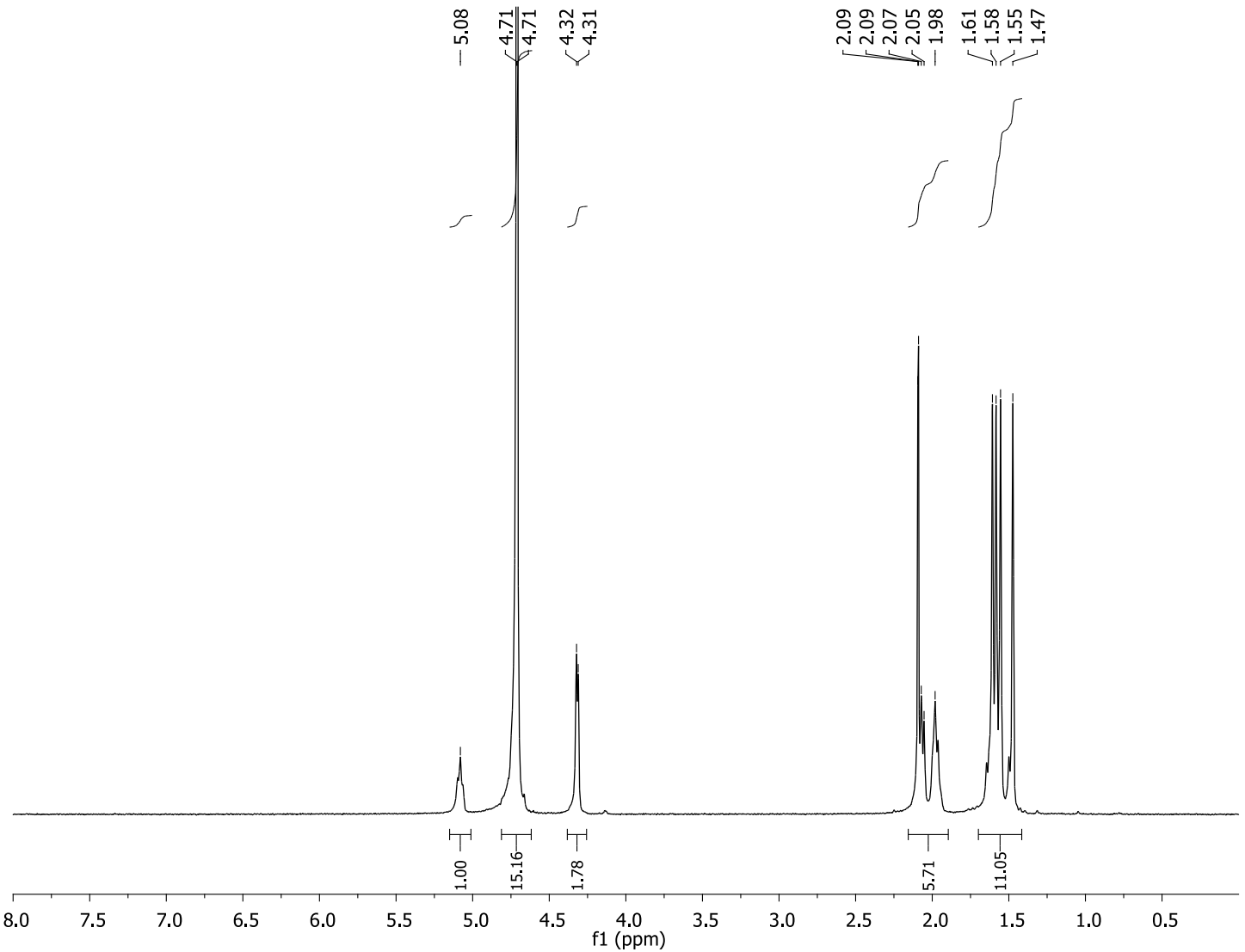

Figure S1. ^{1}H NMR spectrum (400 MHz, D_2O) of 2-MeNPP (**10**).

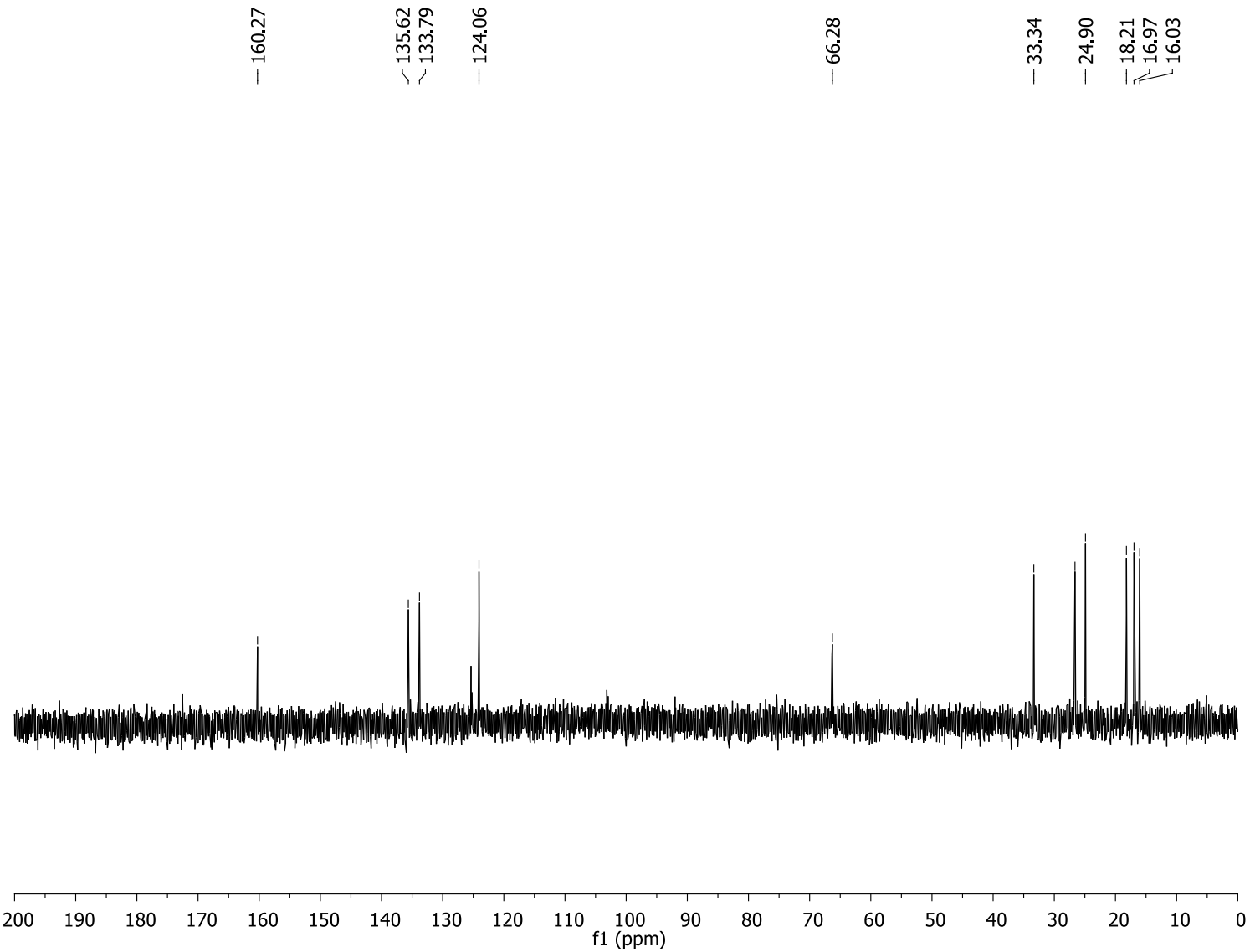

Figure S2. ^{13}C {^{1}H} NMR spectrum (75 MHz, D_2O) of 2-MeNPP (**10**).

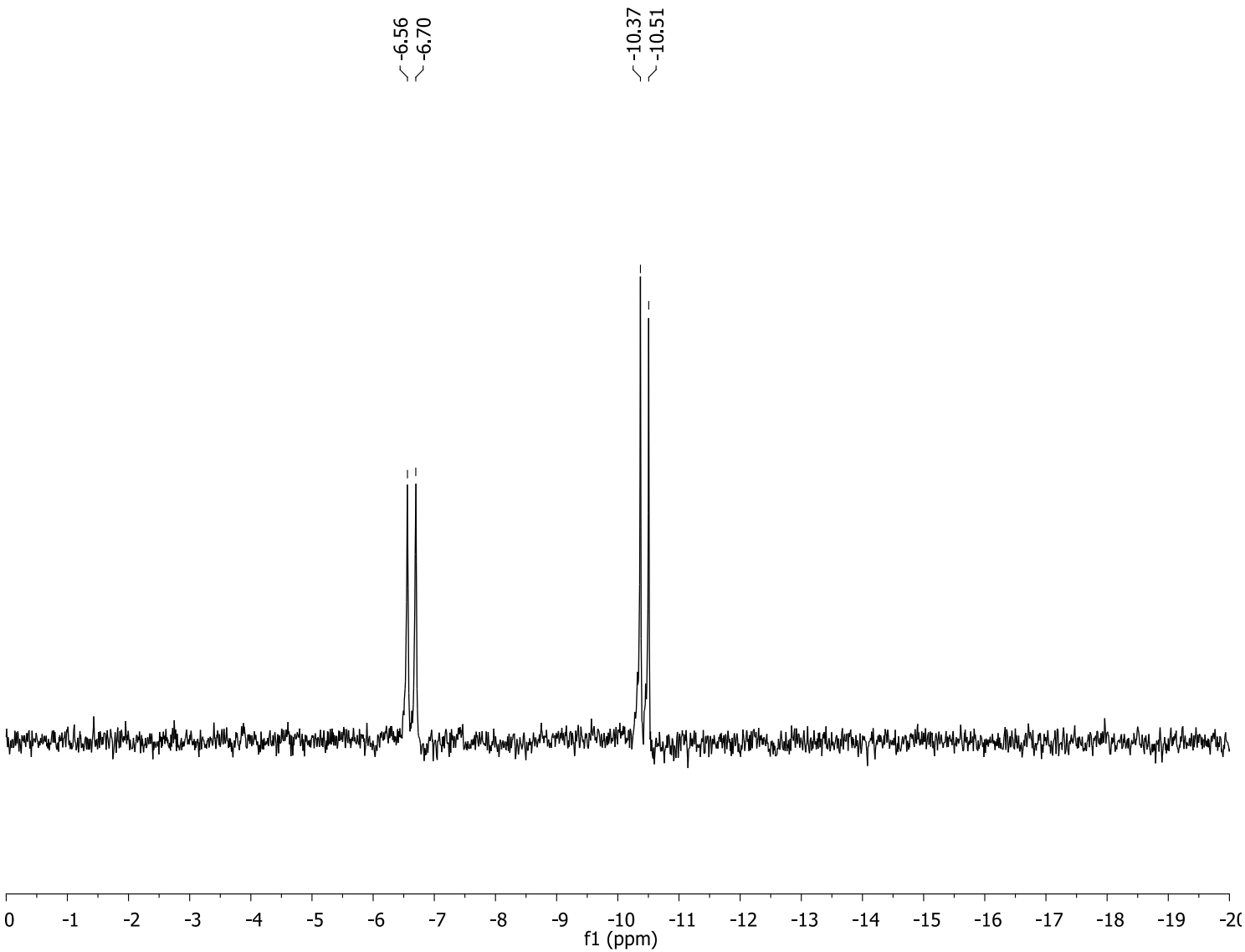

Figure S3. ^{31}P $\{^{1}H\}$ NMR spectrum (161 MHz, D_2O) of 2-MeNPP (**10**).

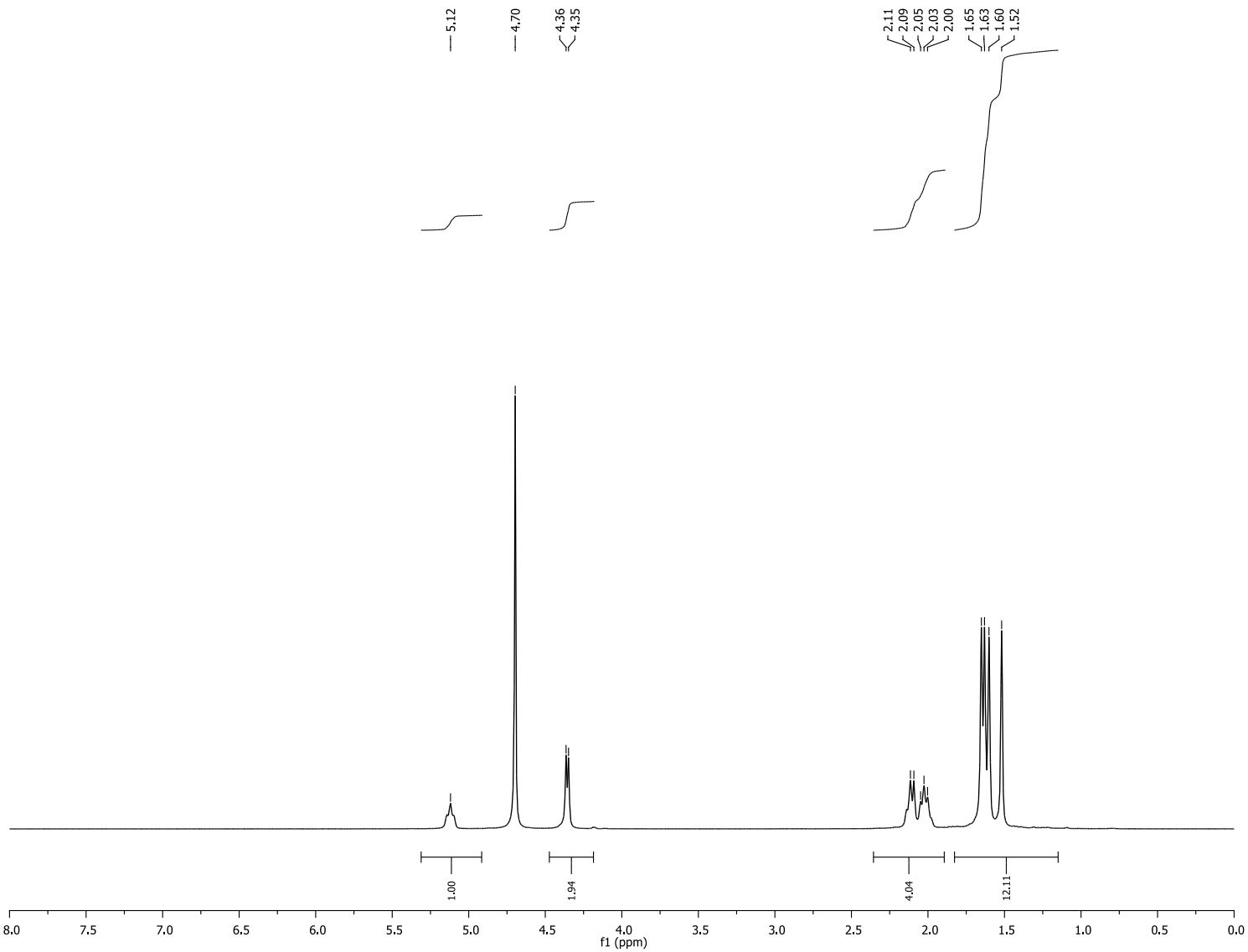

Figure S4. ^{1}H NMR spectrum (300 MHz, D_2O) of [1-^{3}H]-2-MeNPP (**10**).

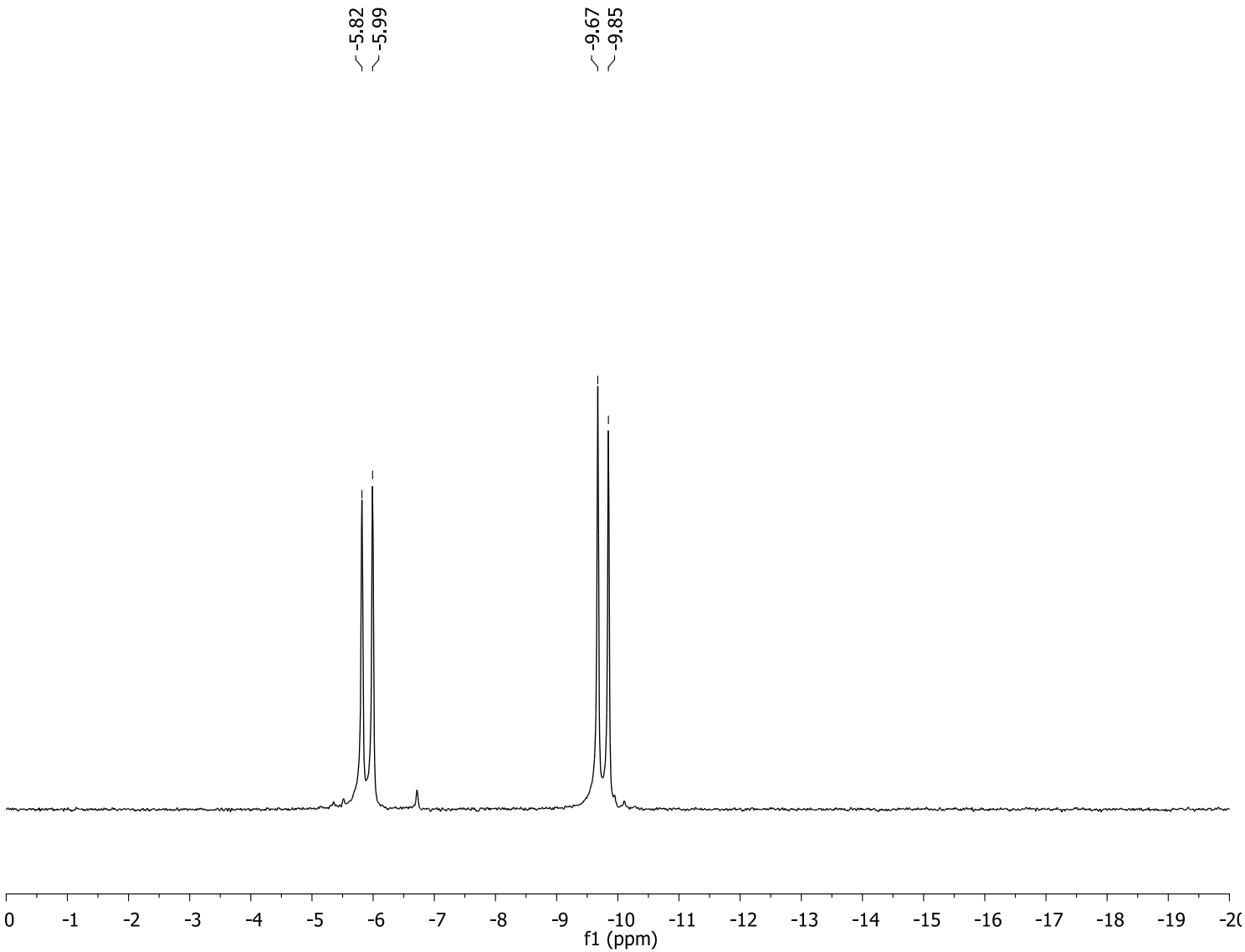

Figure S5. ^{31}P $\{^{1}H\}$ NMR spectrum (121 MHz, D_2O) of [1-^{3}H]-2-MeNPP (**10**).

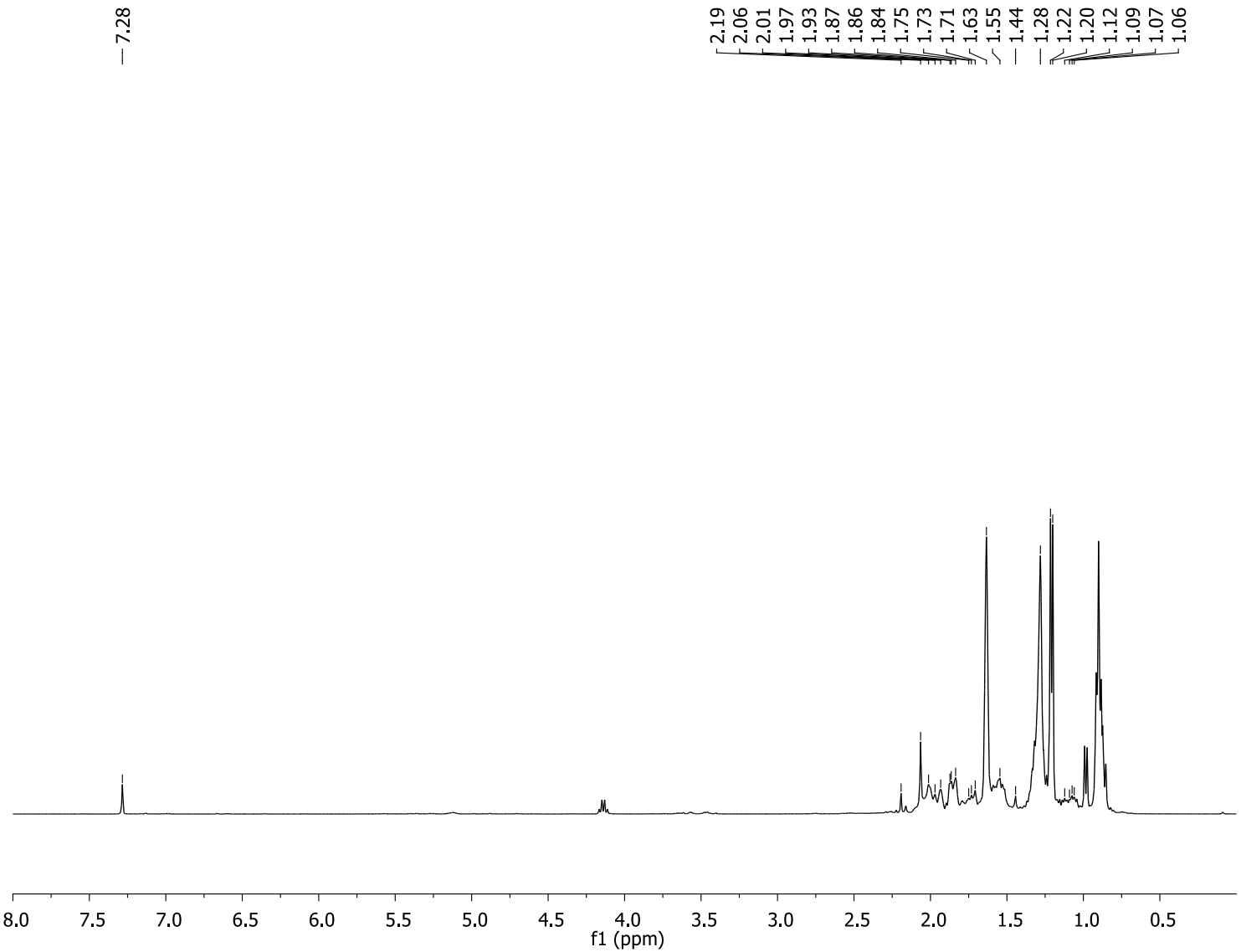

Figure S6. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 2-methyl-α-terpineol (**13**).

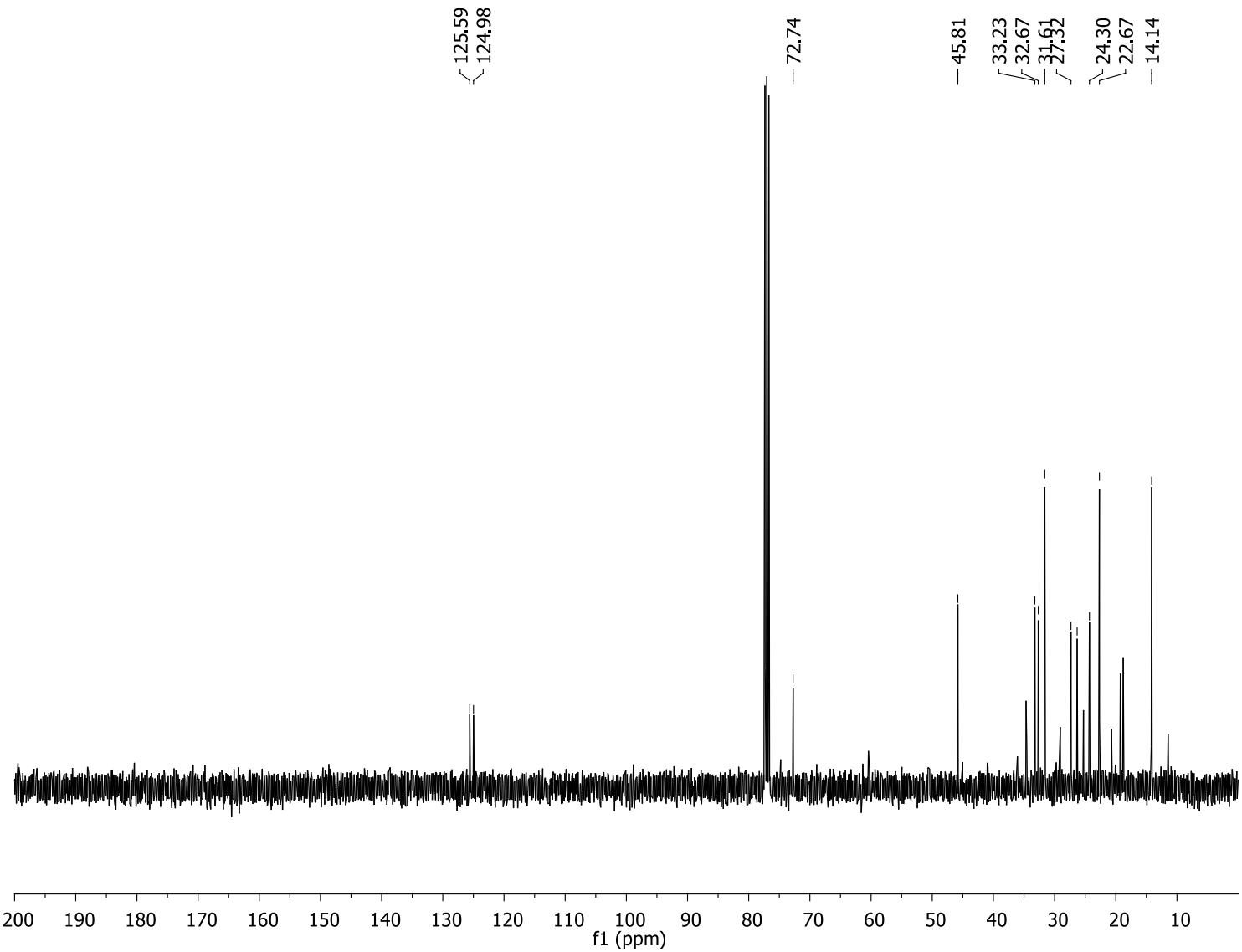

Figure S7. ^{13}C {^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of 2-methyl-α-terpineol (**13**).

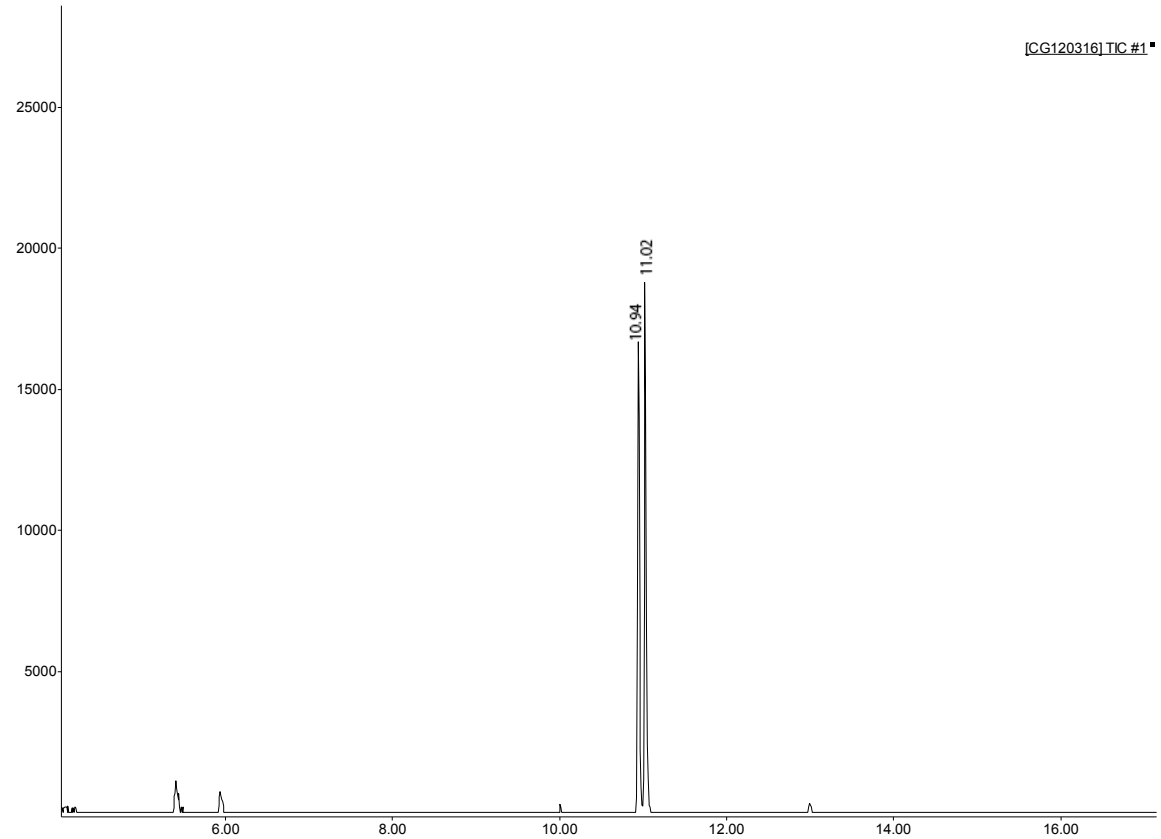

Figure S8. Chiral GC-MS TIC of synthetic racemic 2-methyl-α-terpineol (**13**), showing individual enantiomers at ret. time 10.94 and 11.02 min.

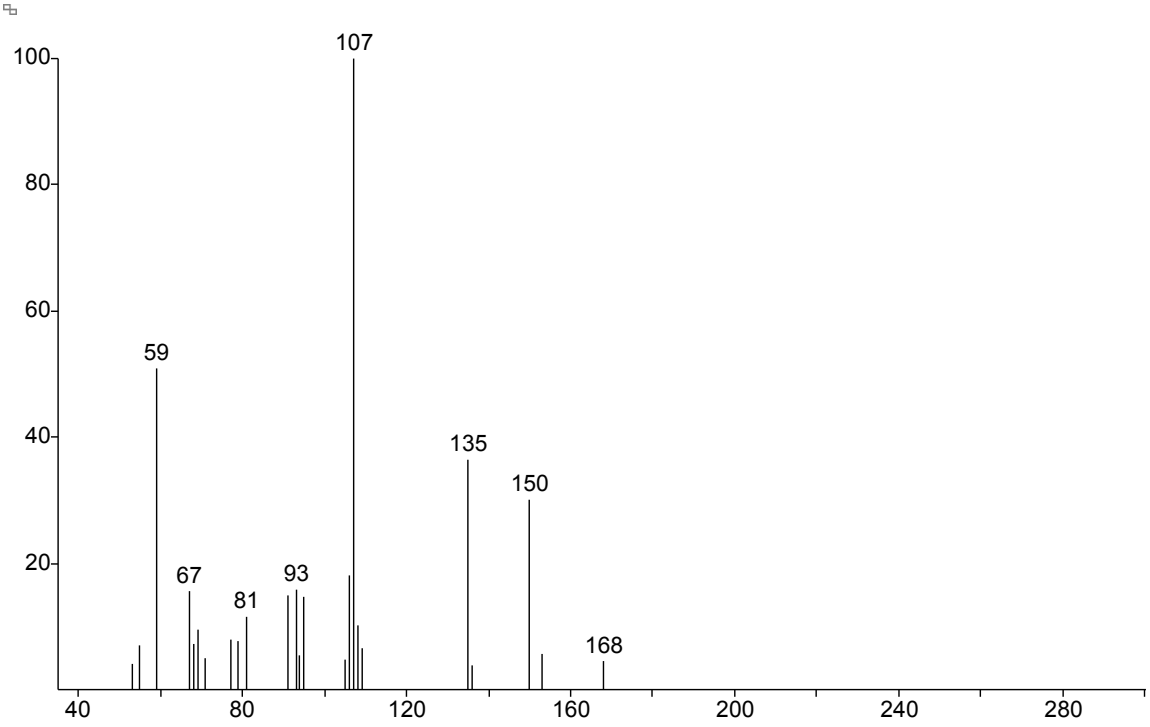

Figure S9. Mass spectrum of 2-methyl-α-terpineol (**13**) peak at 10.94 min.

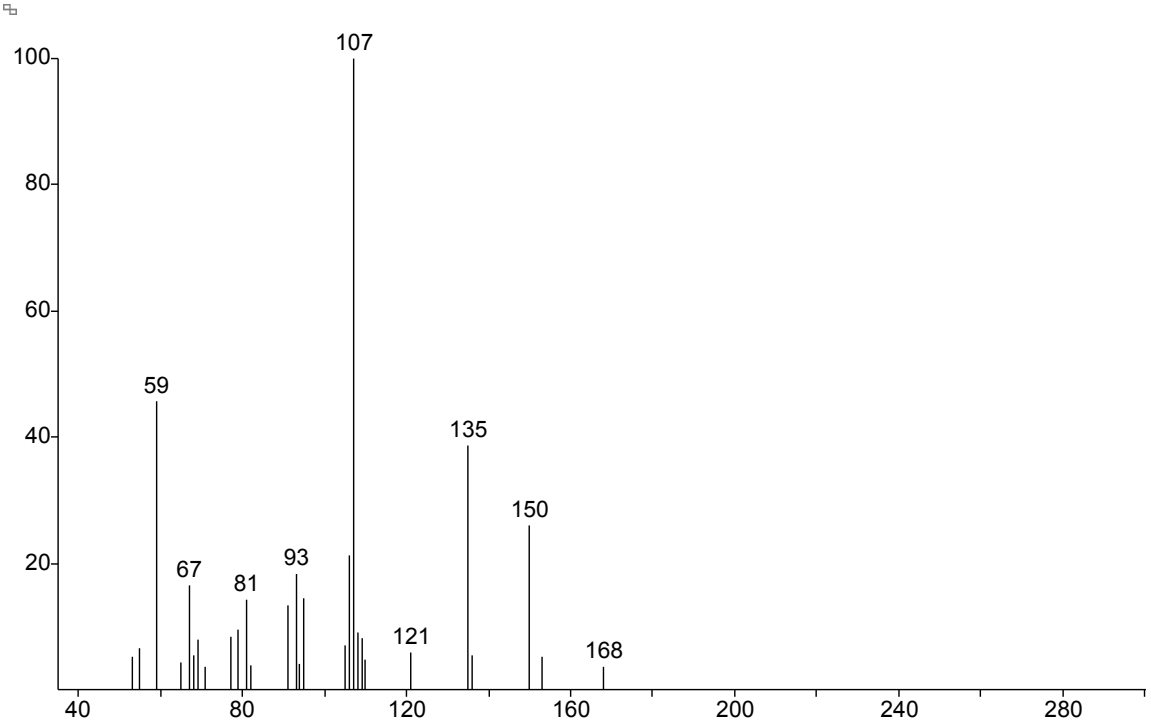

Figure S10. Mass spectrum of 2-methyl-α-terpineol (**13**) peak at 11.02 min.

Table S1. GC-MS analysis of percent distribution of products from the incubation of Sco7700 with 2-MeNPP (**10**), 2-MeGPP (**3**), and assay buffer alone.

Incubation	2-methyl-2-bornene (**5**)	1-methyl camphene (**6**)	2-methylene bornane (**4**)	2-methyl limonene (**15**)	2-methyl linalool (**14**)	2-methyl isoborneol (**1**)	2-methyl-α-terpineol (**13**)	2-methyl nerol (**11**)
rt (min)	5.07	5.16	5.46	6.41	6.93	6.94	7.70	7.81
RI (calc)	990	993	1027	1134	1196	1197	1299	1317
RI (ref)	989	995	1030	1129	1200	1178	1306	--
2-MeNPP control chiral	--	--	--	4 (2/2)[b]	10 (5/5)[b]	--	86 (44/42)[b]	--
2-MeNPP SCO7700 chiral	<1	<1	26	39 (22/17)[ab]	<1 (0/<1)[ab]	17	10 (10/0)[ab]	7
2-MeGPP Control chiral	--	--	--	--	100 (49/51)	--	--	--
2-MeGPP SCO7700 chiral	<1	<1	10	--	--	89	--	--

[a]percentages after subtraction Mg^{2+}-catalyzed background hydrolysis of 2-MeNPP.
[b]total percentage of product formed (percentage first enantiomer to elute/percentage second enantiomer to elute).

GC-MS spectra of co-injections.

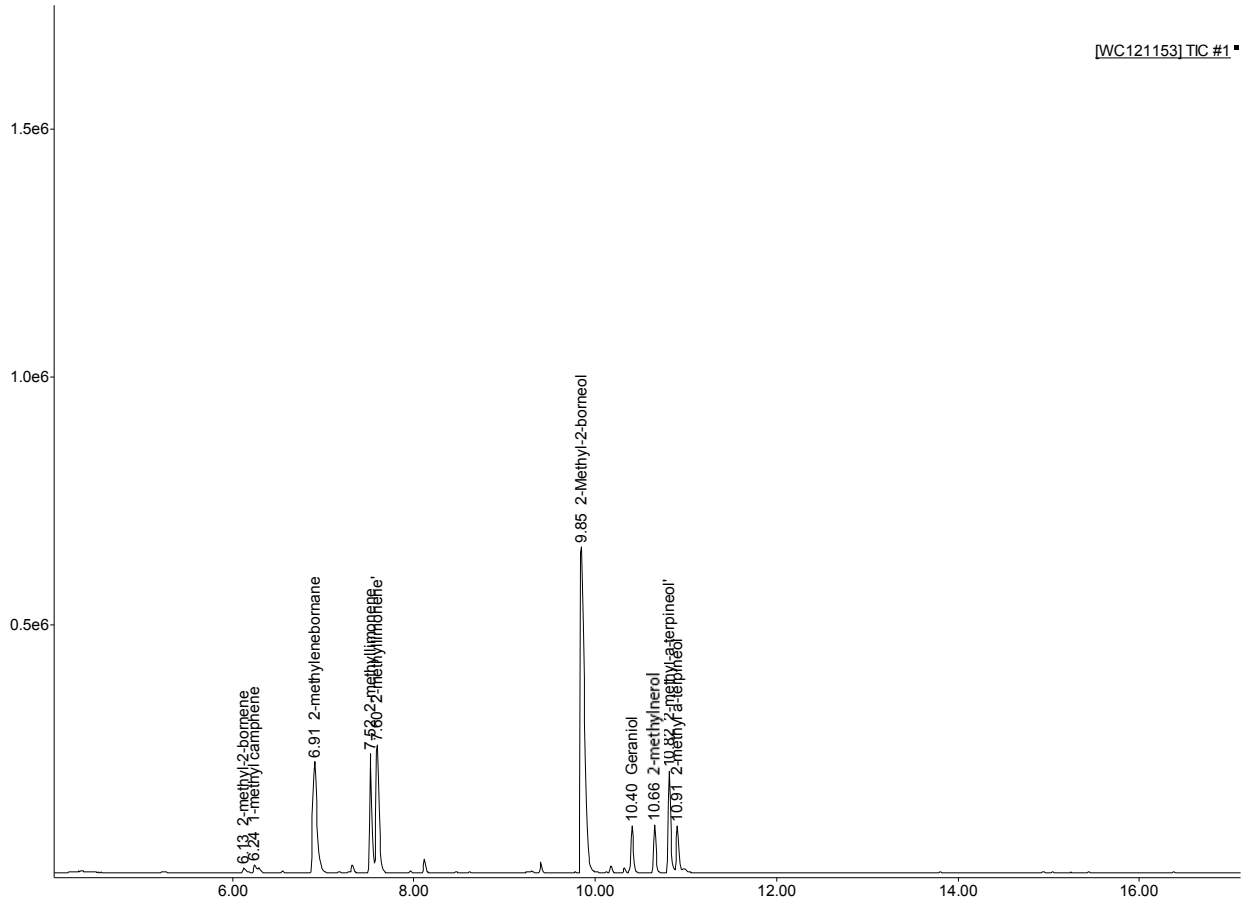

Figure S11. GC-MS TIC of the co-injected pentane extracts from the individual incubations of 2-MeNPP (**10**) and 2-MeGPP (**3**) with MIBS.

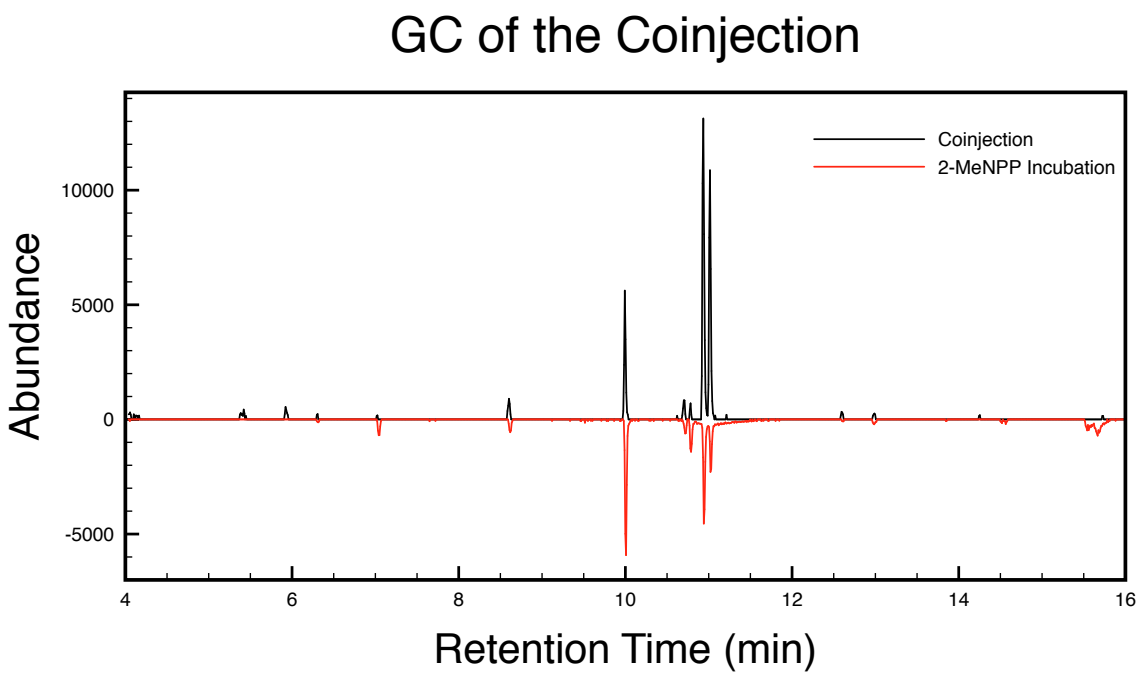

Figure S12. Chiral GC-MS TIC of the pentane extract from the incubation of 2-MeNPP with Sco7700 (bottom spectrum) compared to the co-injection of the extract with synthetic 2-methyl-α-terpineol (**13**) (top spectrum).

Representative GC-mass spectra.

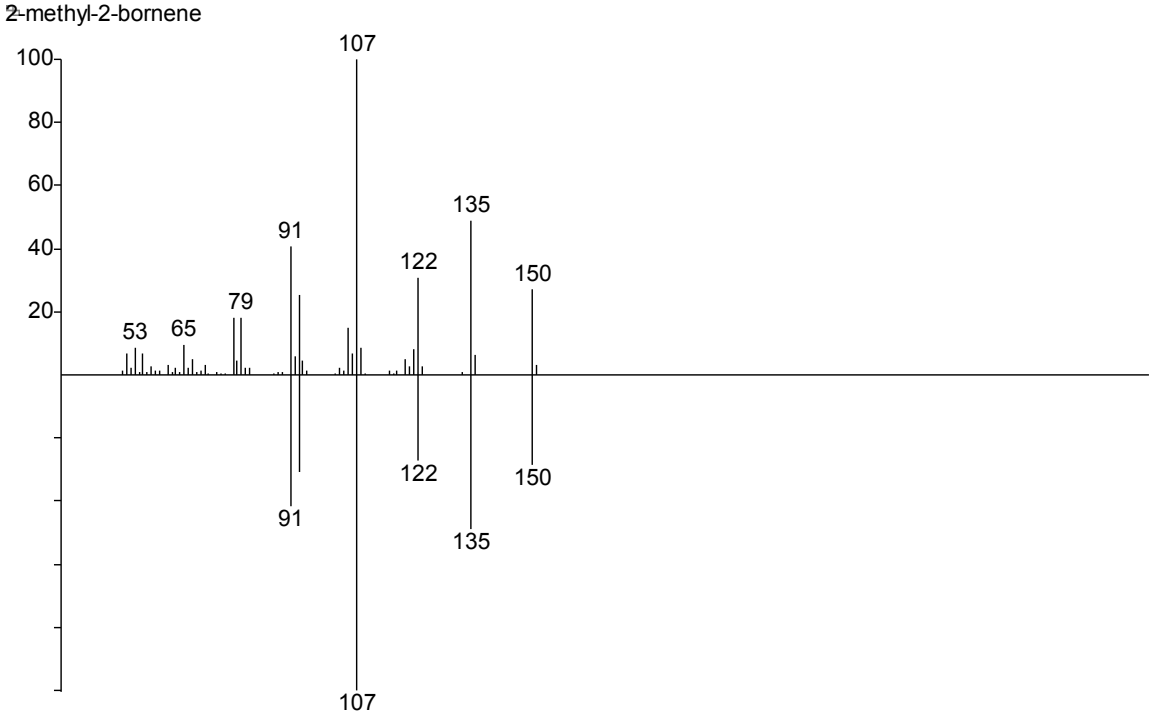

Figure S13. Mass spectrum of enzymatically generated 2-methyl-2-bornene (**5**) (lower spectrum) and 2-methyl-2-bornene standard (upper spectrum).

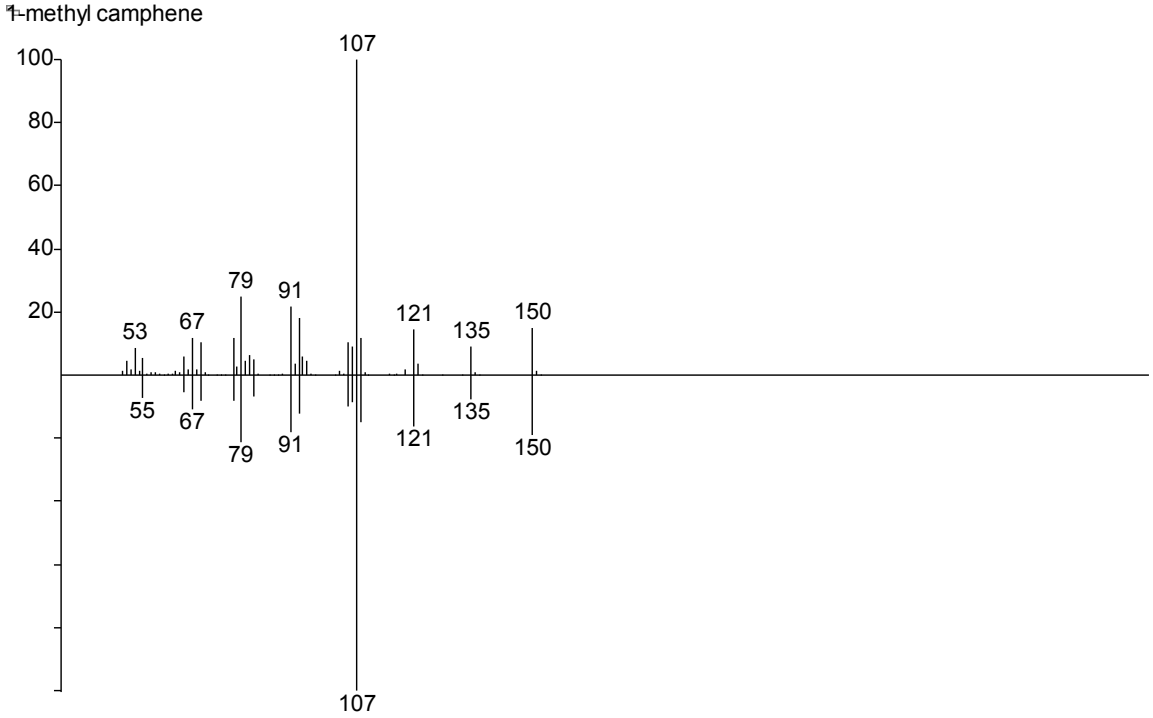

Figure S14. Mass spectrum of enzymatically-generated 1-methylcamphene (**6**) (lower spectrum) and 1-methylcamphene standard (upper spectrum).

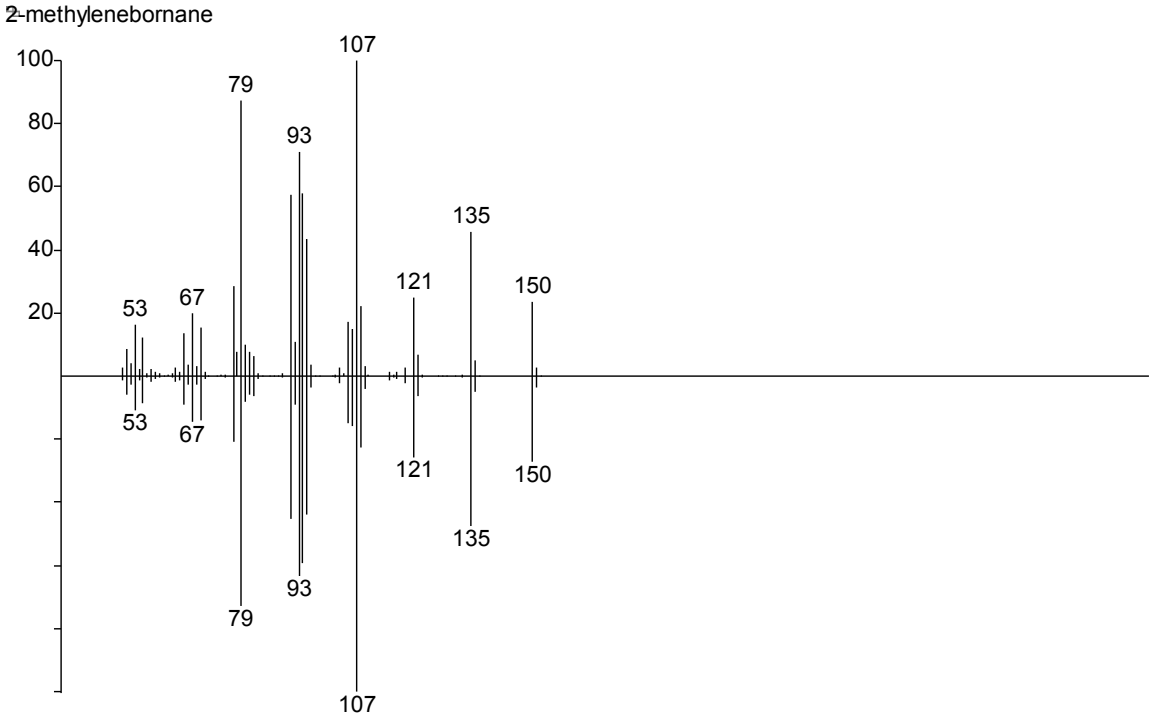

Figure S15. Mass spectrum of enzymatically-generated 2-methylenebornane (**4**) (lower spectrum) and 2-methylenebornane standard (upper spectrum).

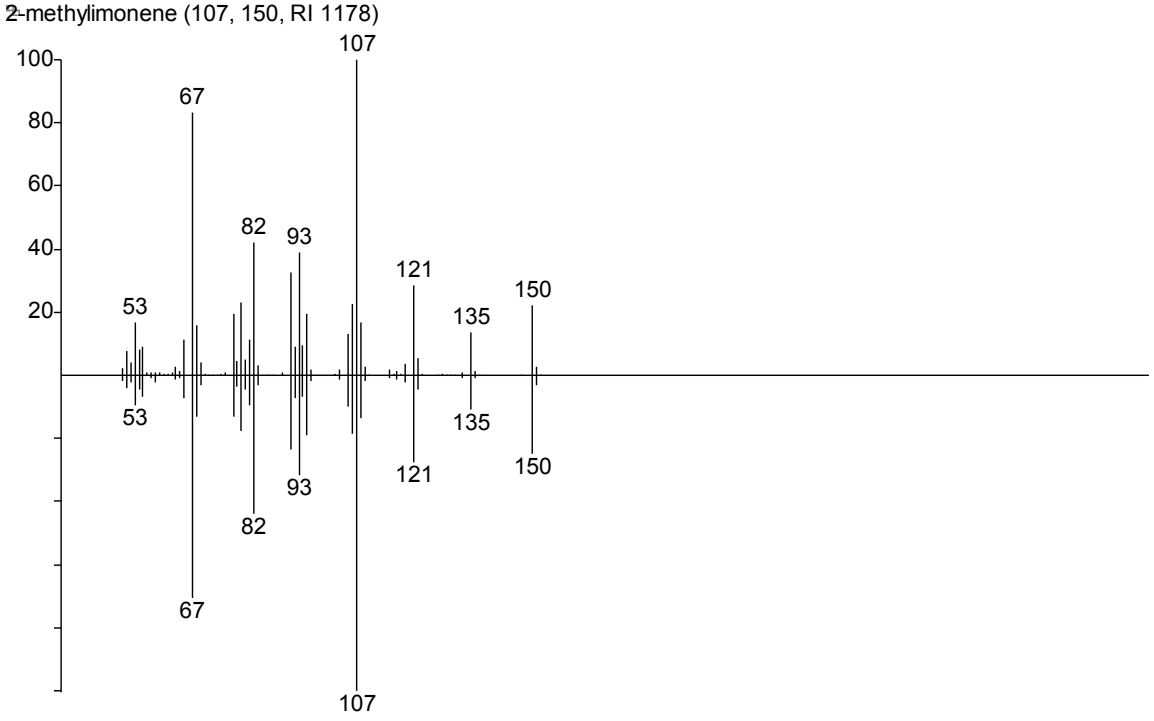

Figure S16. Mass spectrum of peak for enzymatically-generated 2-methyllimonene (**15**) (lower spectrum) and 2-methyllimonene standard (upper spectrum).

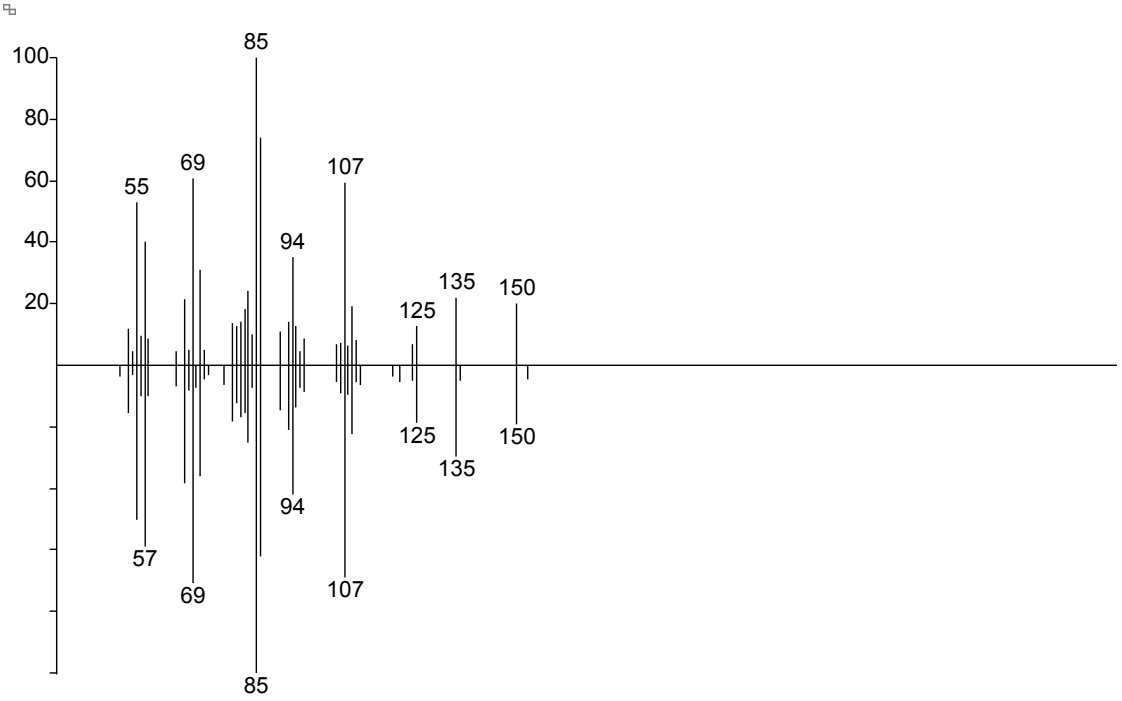

Figure S17. Mass spectrum for enzymatically-generated 2-methyllinalool (**14**) (lower spectrum) and 2-methyllinalool standard (upper spectrum).

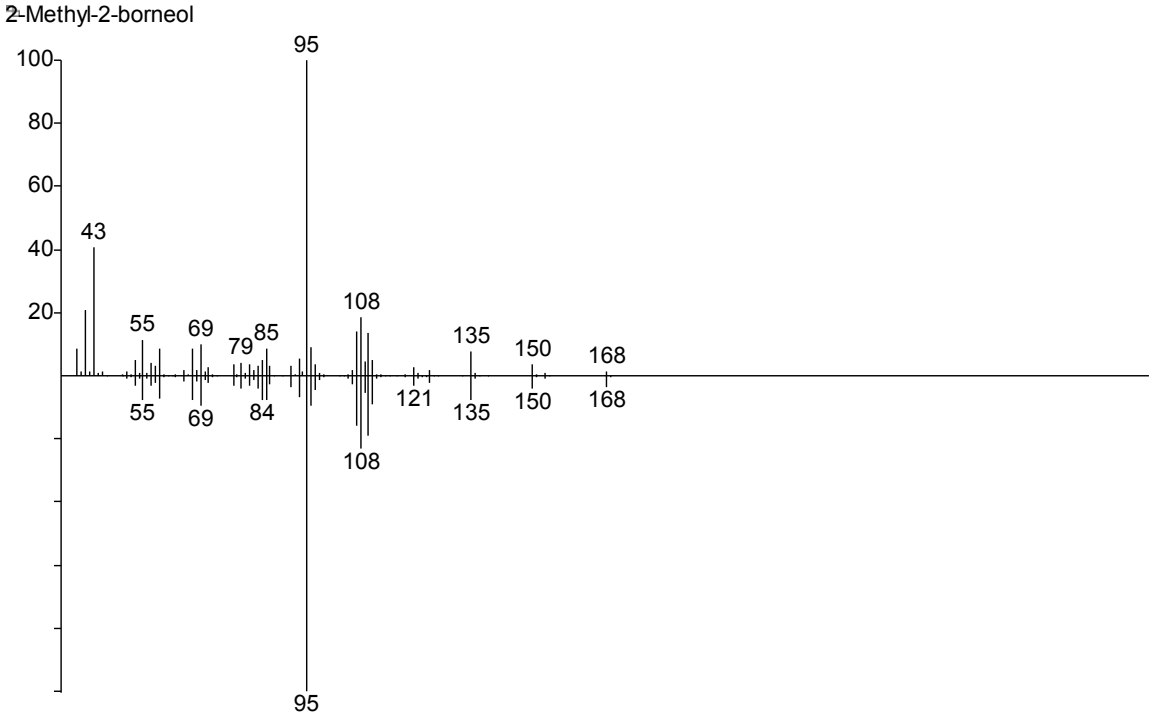

Figure S18. Mass spectrum of enzymatically-generated 2-methylisoborneol (**1**) (lower spectrum) and 2-methylisoborneol standard (upper spectrum).

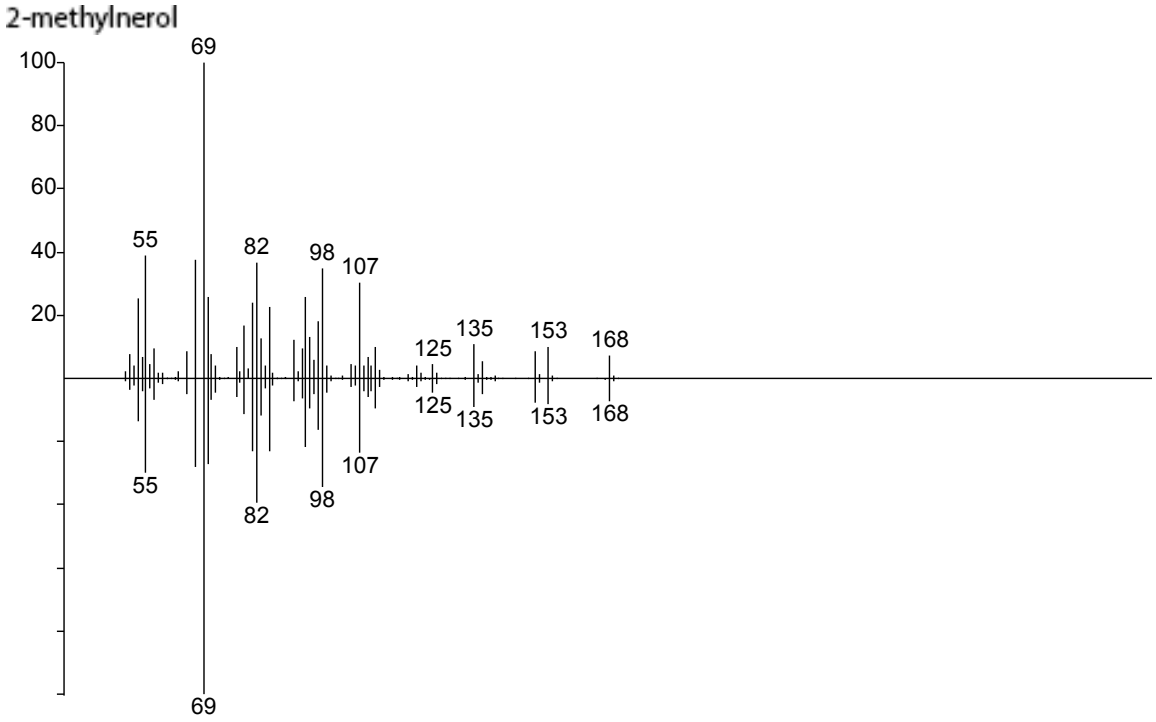

Figure S19. Mass spectrum of enzymatically-generated 2-methylnerol (**11**) (lower spectrum) and 2-methylnerol standard (upper spectrum).

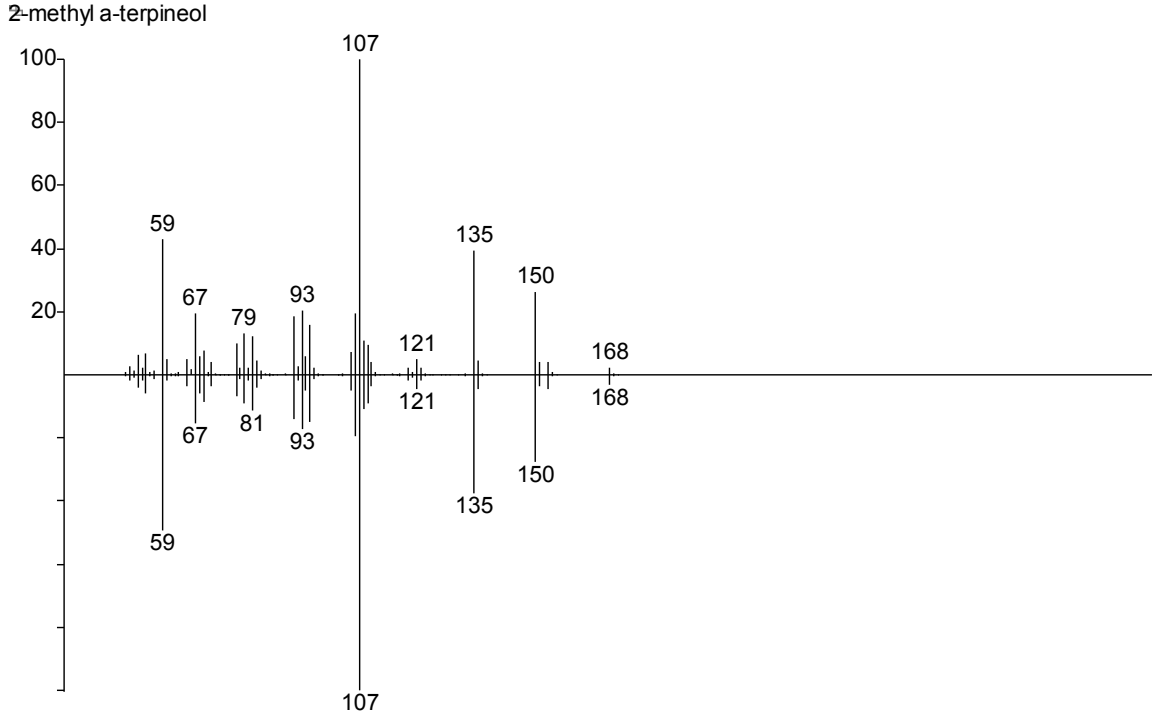

Figure S20. Mass spectrum of enzymatically-generated 2-methyl-α-terpineol (**13**) (lower spectrum) and 2-methyl-α-terpineol standard (upper spectrum).

Steady-state kinetics.

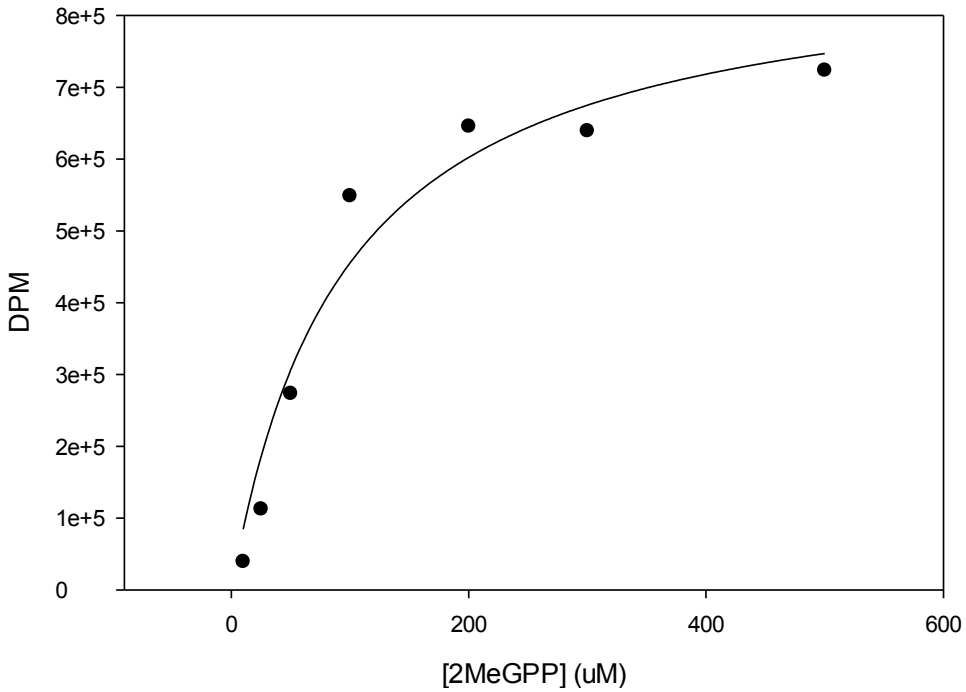

Figure S21. Michaelis-Menten plot of the reaction velocity for the MIBS-catalyzed formation of homo-monoterpenes as a function of the concentration of 2-MeGPP (**3**).

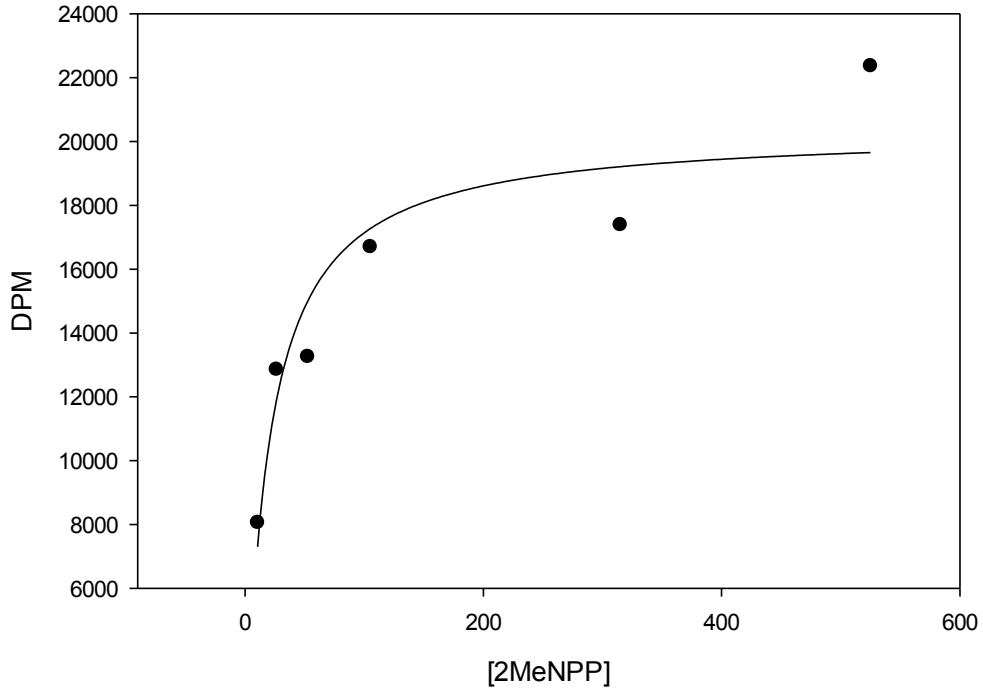

Figure S22. Michaelis-Menten plot of the reaction velocity for the MIBS-catalyzed formation of homo-monoterpenes as a function of the concentration of 2-MeNPP (**10**).