

SUPPORTING INFORMATION

Product synthesis. Epimerisation of menthone with sodium hydroxide yielded a mixture of menthone and isomenthone (70/30), separable by column chromatography.¹ These compounds were stored at reduced temperature to retard the re-equilibration process. However even at cold temperatures, 5% of the alternative isomer is found to be present. Menthone and isomenthone were treated non-selectively with sodium borohydride to reduce the ketones to the respective secondary alcohols (SI Table 1).² These products were separable by column chromatography, including minor products derived from the contaminating isomer present in the starting material.

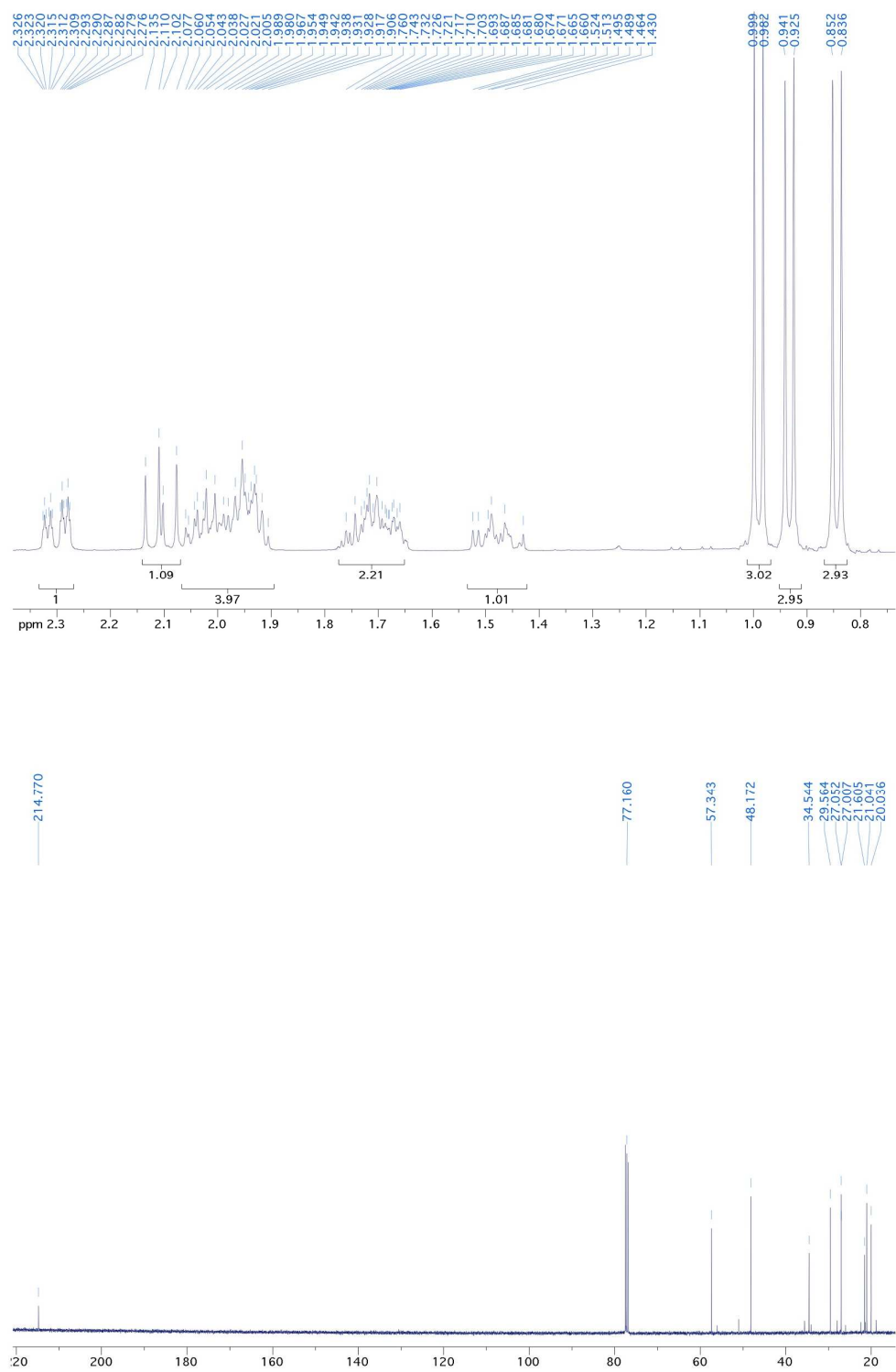
SI Table 1. Product distributions for sodium borohydride reduction of menthone and isomenthone.

Compound	Product Yield (%)			
	Menthol	Neomenthol	Isomenthol	Neoisomenthol
Menthone	60	35	-	4
Isomenthone	4	1.5	0.6	93

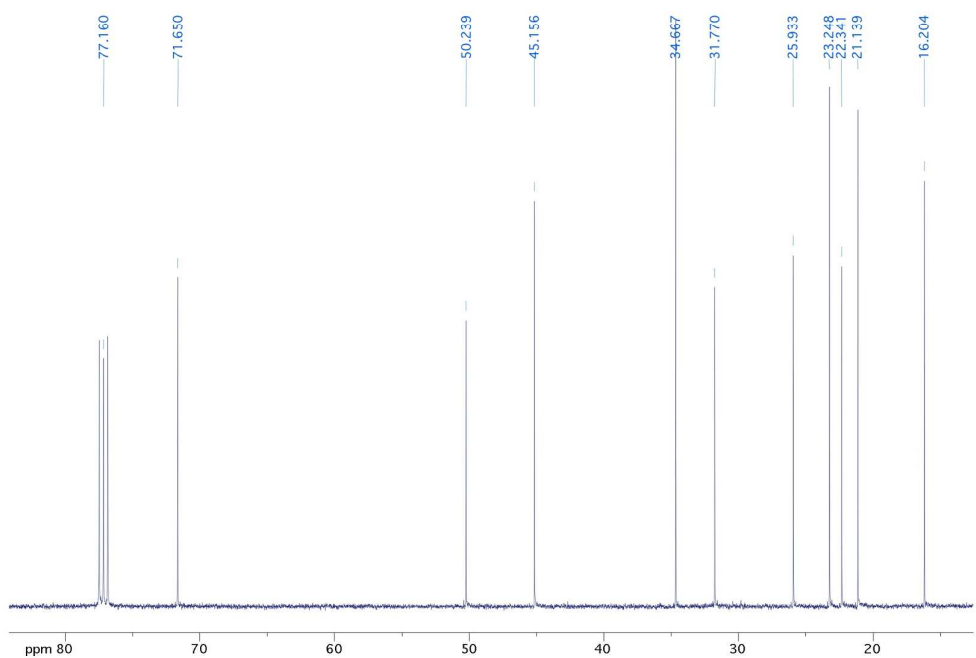
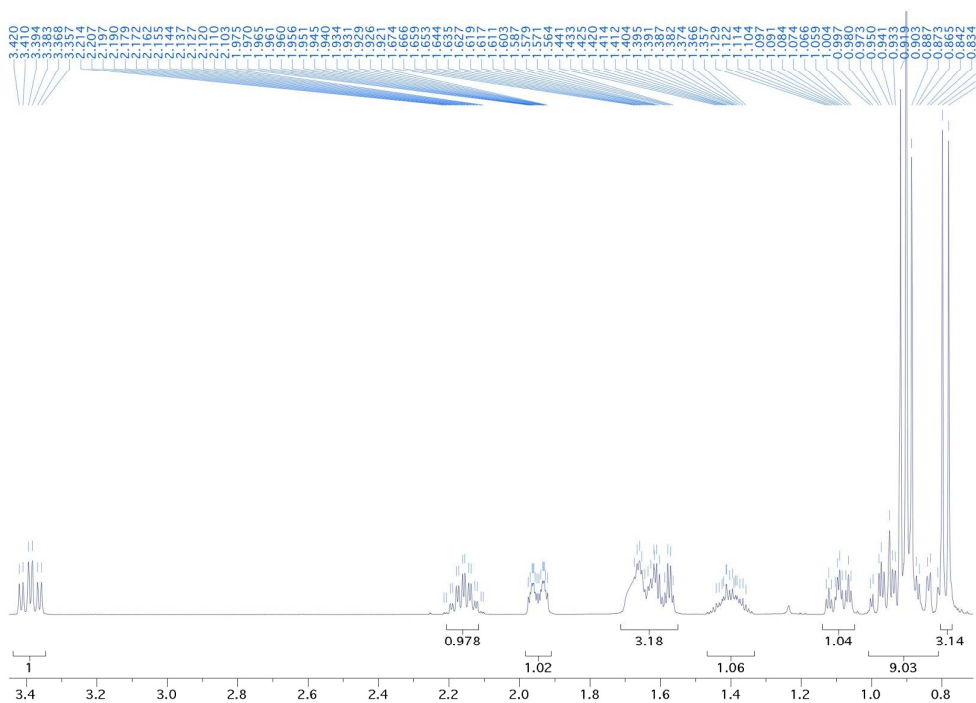
Menthone reduction resulted in a 2:1 ratio of menthol and neomenthol. In contrast, isomenthone reduction was highly selective yielding almost entirely the non-commercially available neoisomenthol (99%). A Mitsunobu reaction³ was carried out on neoisomenthol to produce the *p*-nitrobenzoate ester, with an inversion of configuration at the 3-position.⁴ Subsequent basic ester hydrolysis yielded the remaining compound isomenthol (SI Table 1).

SI Figure 1. ^1H and ^{13}C NMR spectra for synthesised compounds.

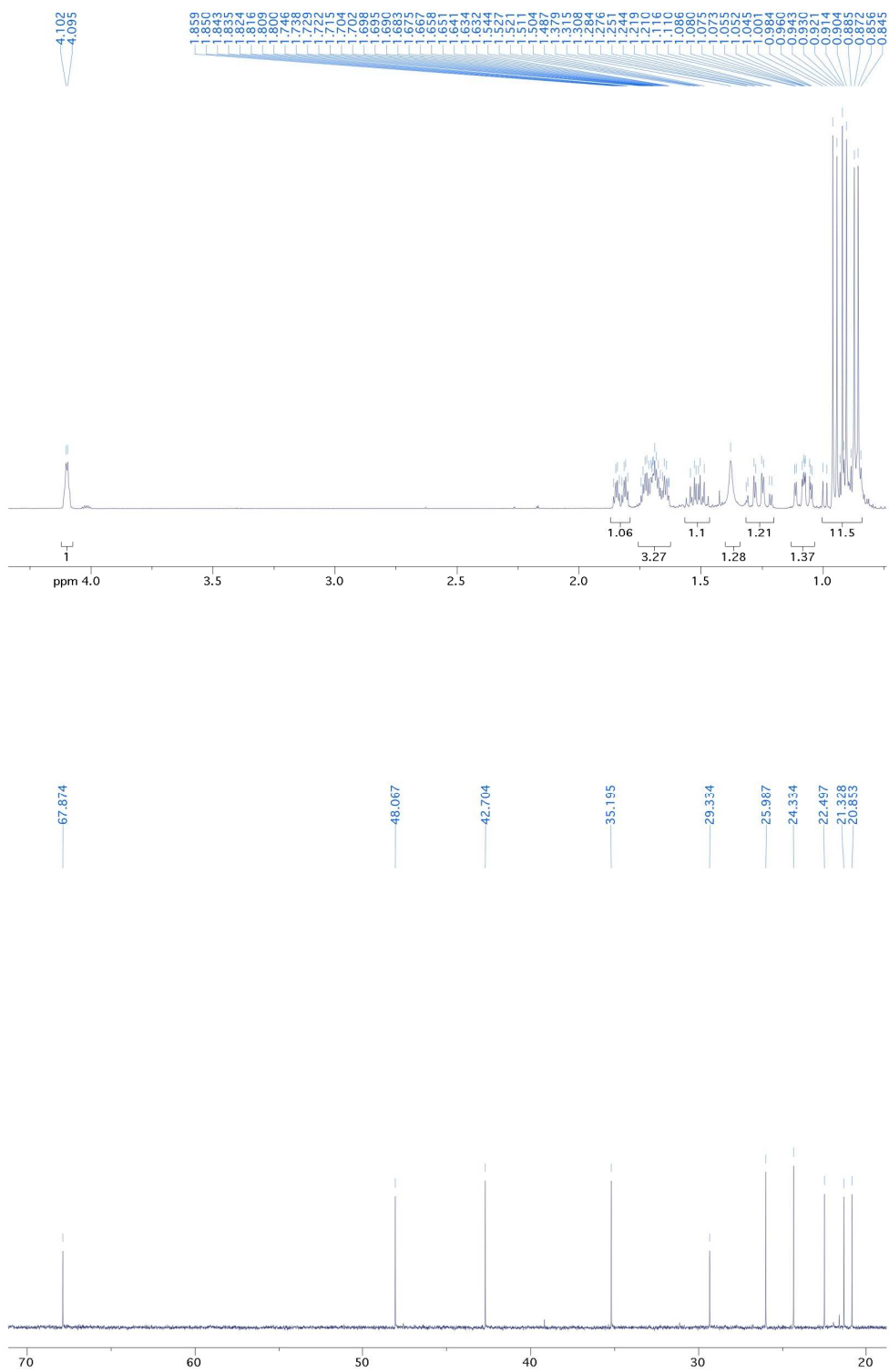
Menthone



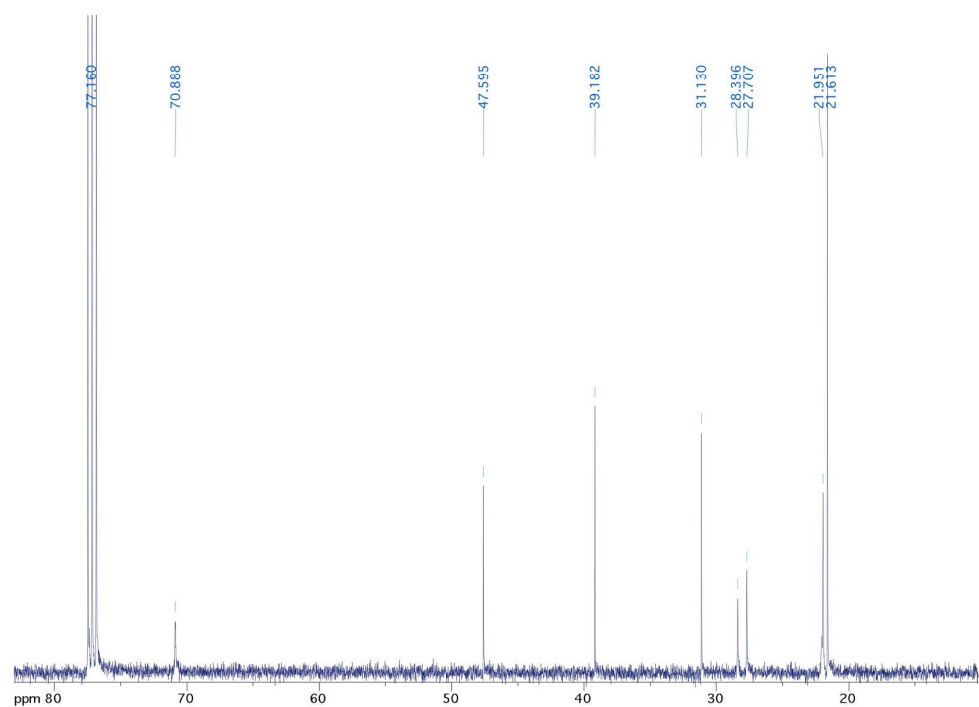
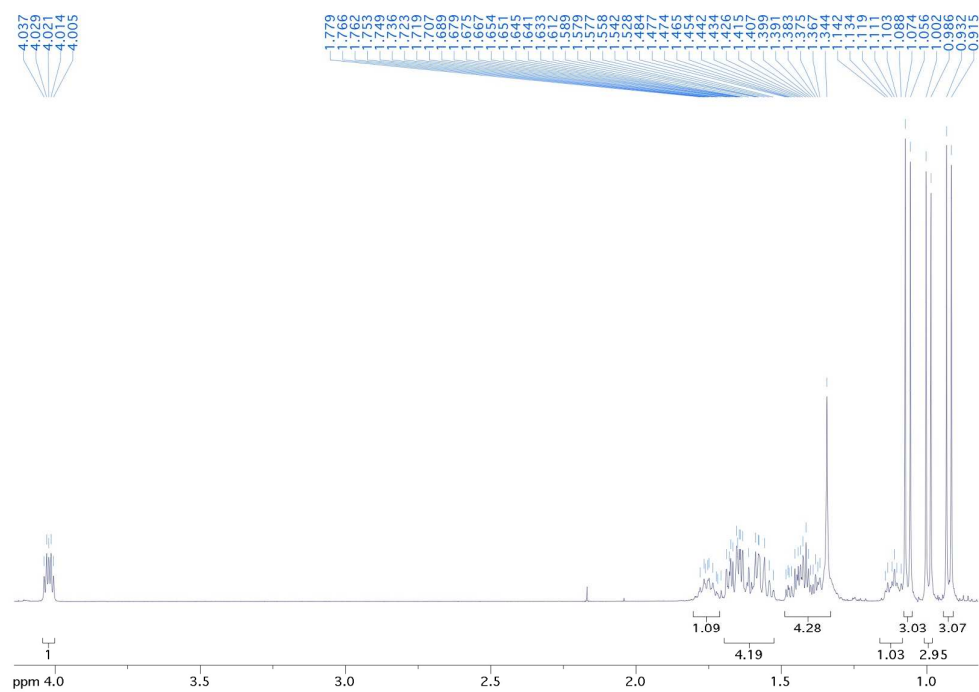
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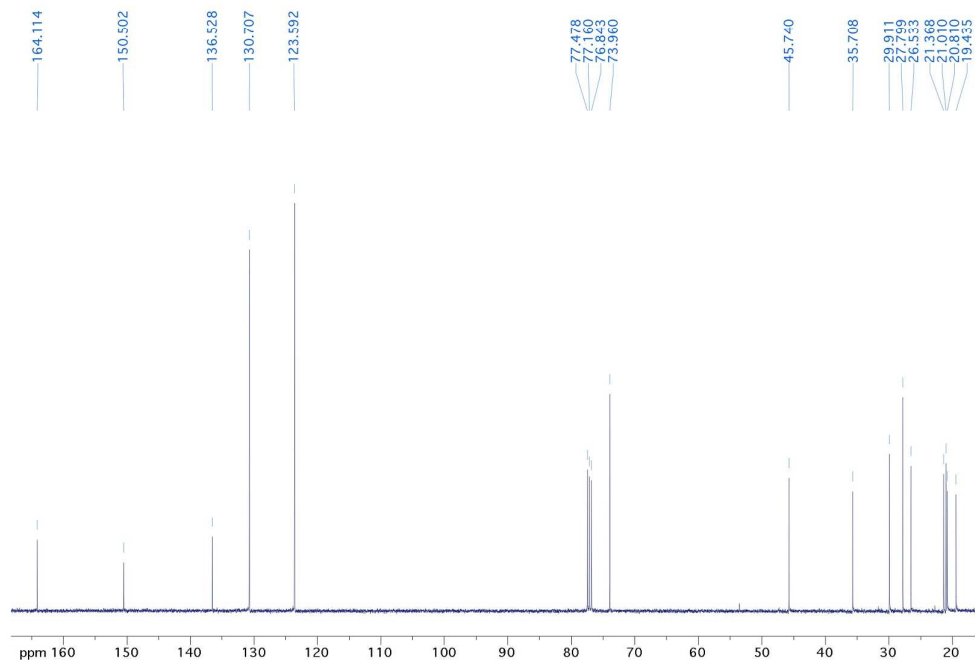
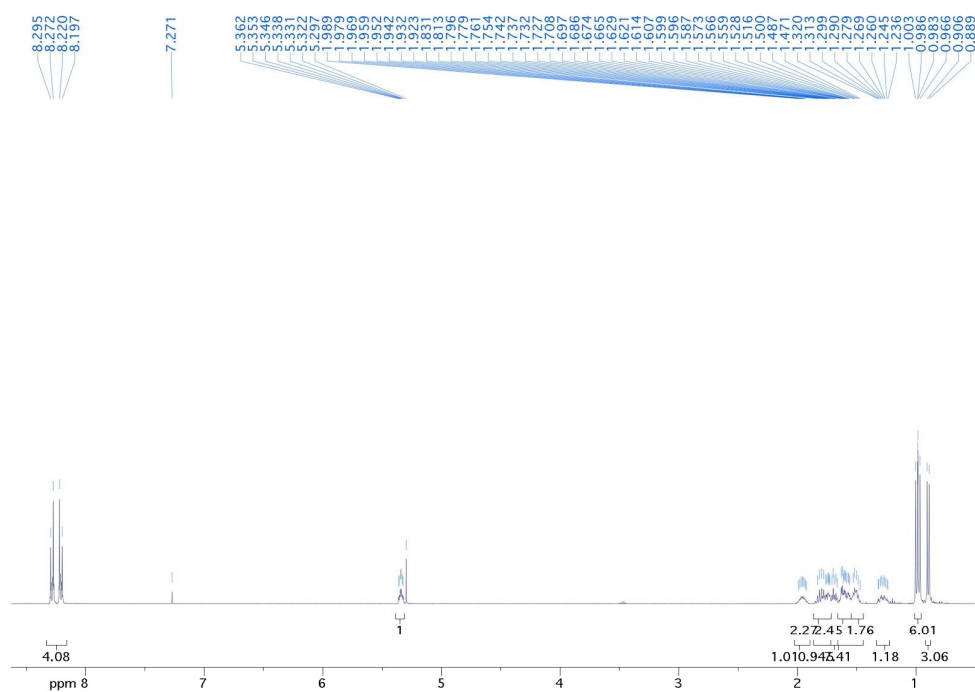
Neomenthol



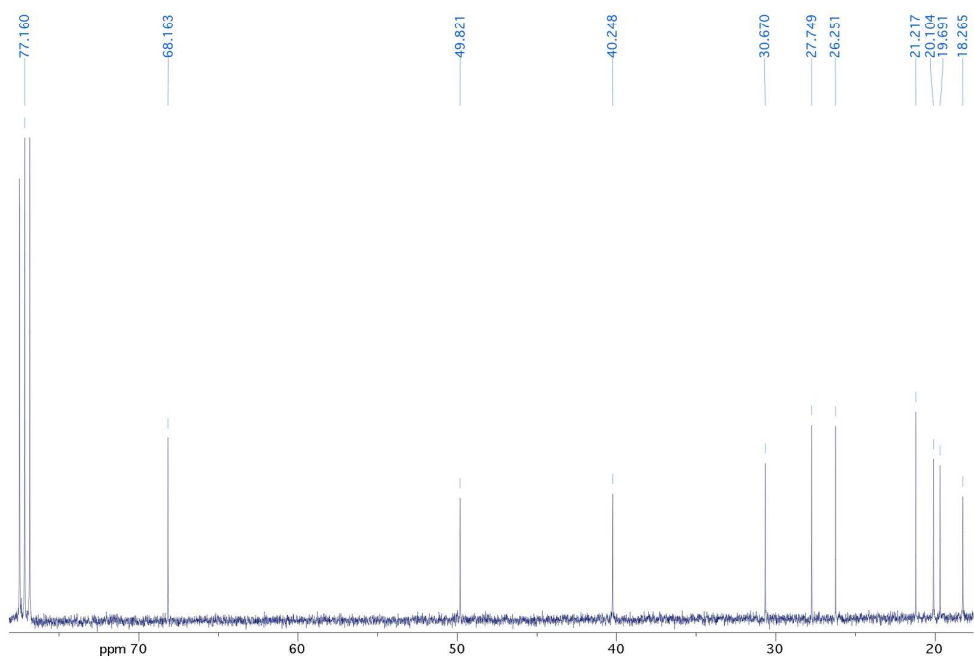
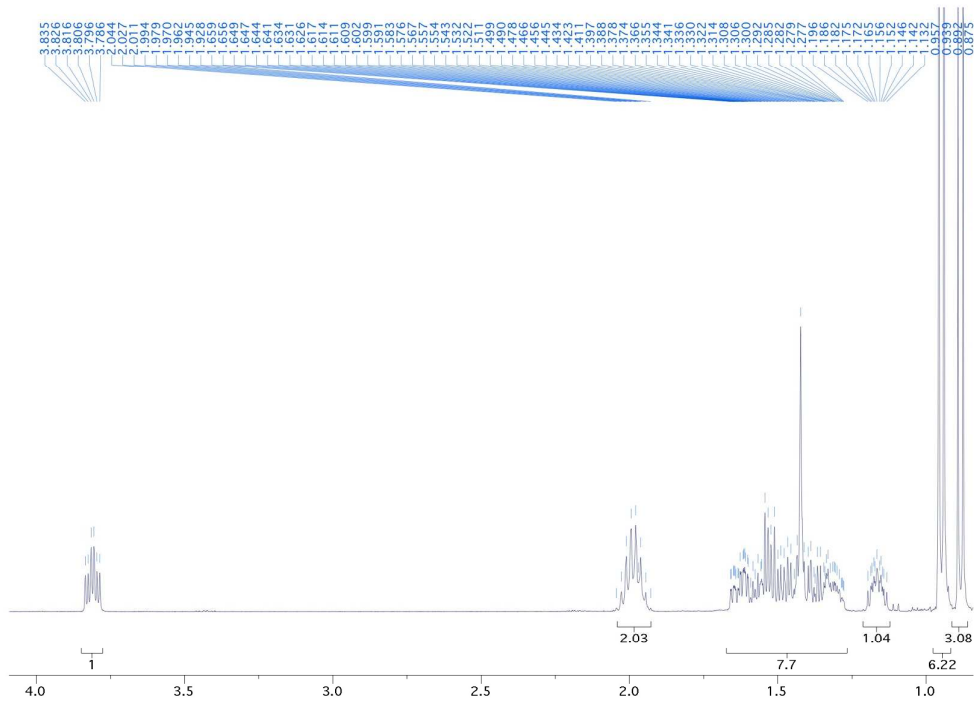
Neoisomenthol

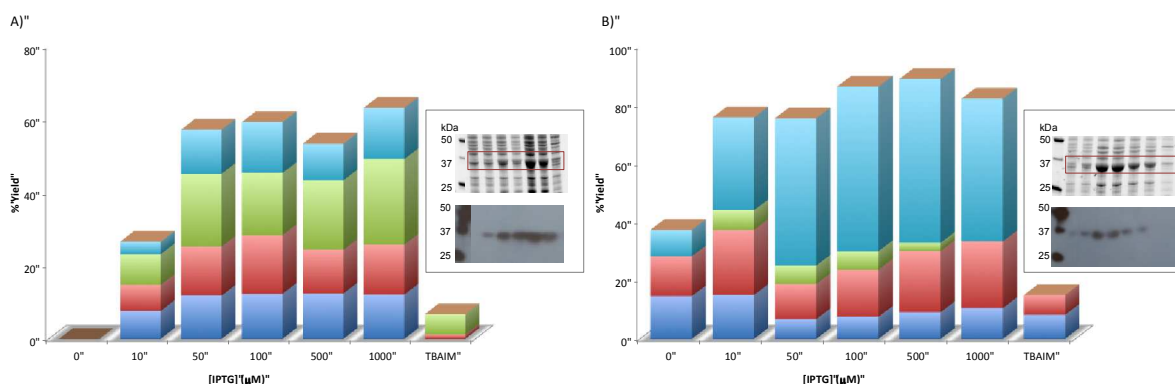


p-Nitrobenzoate-isomenthol

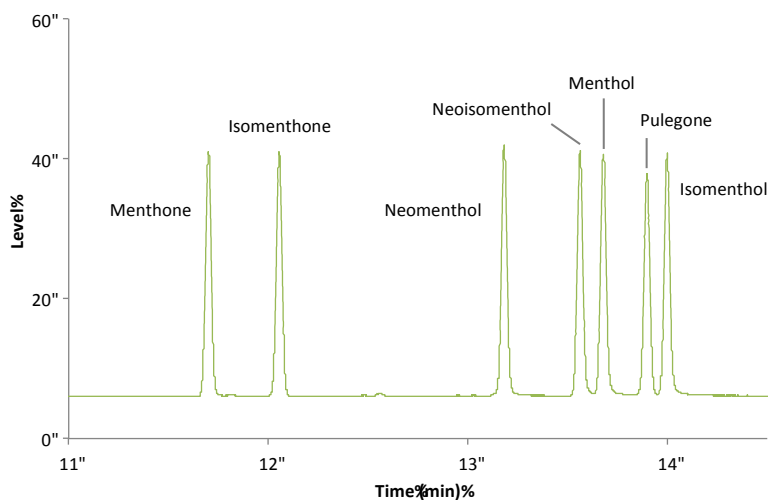


Isomenthol





SI Figure 2. Products formed during biotransformations of DMN cell extracts in strains A) 6 and B) 7 at different IPTG concentrations and TBAIM media. Inset: SDS-PAGE and Western blot analysis of the cell extracts from the biotransformations. Reactions (2 mL) were performed in buffer (50 mM Tris pH 7.0) containing monoterpenoid (1 mM), cell extracts (0.5 mL), NADP^+ (10 μM), glucose (15 mM) and GDH (10 U). The reactions were agitated at 30 °C for 24 h at 130 rpm. Product yields were determined by GC analysis using a DB-WAX column. Data point colours: menthone = dark blue; isomenthone = red; menthol = green; neoisomenthol = purple; neomenthol = light blue; isomenthol = orange.



SI Figure 3. GC trace showing the separation of seven monoterpenoids on a DB-WAX column. The internal standard *sec*-butylbenzene retention time is 8.77 minutes. Method: the injector temperature was at 220°C with a split ratio of 20:1 (1 μL injection). The carrier gas was helium with a flow rate of 1 mLmin^{-1} and a pressure of 5.1 psi. The program began at 40°C with a hold for 1 min followed by an increase of temperature to 220°C at a rate of 10°C/minute, with a hold at 210 °C for 1 min. The FID detector was maintained at a temperature of 250°C with a flow of hydrogen at 30 mL/min .

SI Table 2. Biotransformations of the purified enzymes.

Substrate	Product	Product Yield (μM)				Enzyme activity
		NtDBR	MMR	MNMR	Mixture ^a	
Pulegone	Menthone	437	-	-	0	NtDBR
	Isomenthone	360	-	-	201	NtDBR
	Menthol ^b	-	-	-	128	MMR
	Neoisomenthol ^c	-	-	-	71	MMR
	Neomenthol ^b	-	-	-	320	MNMR
	Isomenthol ^c	-	-	-	0	MNMR
Menthone	Menthol	-	786	0	122	MMR
	Neoisomenthol ^d	-	34	0	0	MMR
	Neomenthol	-	40	626	886	MNMR
	Isomenthol ^d	-	0	0	23	MNMR
Isomenthone	Menthol ^e	-	0	0	19	MMR
	Neoisomenthol	-	181	11	94	MMR
	Neomenthol ^e	-	34	16	46	MNMR
	Isomenthol	-	17	67	162	MNMR

Reactions (1 mL) were performed in buffer (50 mM Tris pH 7.0) containing monoterpenoid (1 mM), enzyme(s) (2 μM), NADP⁺ (10 μM), glucose (15 mM) and GDH (10 U). The reactions were agitated at 30 °C for 24 h at 130 rpm. Product yields were determined by GC analysis using a DB-WAX column. ^aEqual concentrations (2 μM) of NtDBR, MMR and MNMR; ^bProduct from a reaction with menthone; ^cProduct from a reaction with isomenthone; ^dProduct from a small amount (5%) of isomenthone in the substrate; ^eProduct from a small amount (5%) of menthone in the substrate.

SI Table 3. Biotransformations of cell extracts of DMN in twelve *E. coli* expression strains.

Substrate/Strain	Product Yield (μM)					
	Menthone	Isomenthone	Menthol ^a	Neoisomenthol ^b	Neomenthol ^a	Isomenthol ^b
Pulegone						
1	78 \pm 1	35 \pm 2	ND	ND	22 \pm 3	-
2	560 \pm 5	369 \pm 6	ND	ND	ND	-
3	439 \pm 2	342 \pm 2	ND	ND	ND	-
4	175 \pm 1	140 \pm 2	267 \pm 121	ND	394 \pm 6	-
5	ND	ND	ND	ND	ND	-
6	280 \pm 12	109 \pm 5	57 \pm 3	ND	322 \pm 6	-
7	211 \pm 7	121 \pm 21	237 \pm 67	ND	332 \pm 31	-
8	trace	76 \pm 47	159 \pm 40	trace	72 \pm 29	-
9	116 \pm 14	107 \pm 14	186 \pm 8	3.0 \pm 0.9	91 \pm 4	-
10	89 \pm 1	87 \pm 3	57 \pm 7	ND	36 \pm 2	-
11	332 \pm 15	231 \pm 11	ND	ND	38 \pm 4	-
12	23 \pm 1	ND	37 \pm 7	ND	ND	-
Menthone						
1	-	-	21 \pm 1	ND	212 \pm 8	ND
2	-	-	12 \pm 1	ND	11 \pm 1	ND
3	-	-	ND	ND	ND	ND
4	-	-	188 \pm 6	ND	698 \pm 24	ND
5	-	-	ND	ND	ND	ND
6	-	-	67 \pm 2	ND	822 \pm 10	ND
7	-	-	220 \pm 2	ND	778 \pm 10	ND
8	-	-	577 \pm 10	ND	340 \pm 4	ND
9	-	-	643 \pm 33	24 \pm 3	288 \pm 6	ND
10	-	-	537 \pm 32	ND	446 \pm 3	ND
11	-	-	ND	ND	185 \pm 4	ND
12	-	-	766 \pm 9	trace	26 \pm 12	ND
Isomenthone						
1	-	-	ND	ND	16 \pm 11	ND
2	-	-	ND	ND	ND	ND
3	-	-	ND	ND	ND	ND
4	-	-	142 \pm 4	276 \pm 7	110 \pm 2	229 \pm 14
5	-	-	ND	ND	ND	ND
6	-	-	25 \pm 1	ND	207 \pm 5	ND
7	-	-	86 \pm 1	87 \pm 1	118 \pm 8	51 \pm 1
8	-	-	ND	336 \pm 17	26 \pm 5	ND
9	-	-	ND	ND	40 \pm 3	ND
10	-	-	54 \pm 4	196 \pm 22	ND	ND
11	-	-	ND	ND	52 \pm 1	ND
12	-	-	75 \pm 14	278 \pm 96	trace	ND

Reactions (2 mL) were performed in buffer (50 mM Tris pH 7.0) containing monoterpenoid (1 mM), cell extracts (0.5 mL), NADP⁺ (10 μM), glucose (15 mM) and GDH (10 U). The reactions were agitated at 30 °C for 24 h at 130 rpm. Product yields were determined by GC analysis using a DB-WAX column. ^aProduct from a reaction with menthone; ^bProduct from a reaction with isomenthone. No product formation was observed with control extracts containing an empty pET21b vector. Strain identification can be found in the Methods section Table 4. ND = none detected; trace = <20 μM yield detected in some cultures.

SI Table 4. Biotransformations of cell extracts of DN produced under different growth conditions.

Substrate/Strain	Product Yield (μM)			
	Menthone	Isomenthone	Menthol ^a	Neoisomenthol ^b
<i>A) Medium</i>				
Minimal	$2.50 \pm 0.26(\text{ND})$	$3.77 \pm 0.50(\text{ND})$	$1.40 \pm 0.09(\text{ND})$	ND
LB	$0.67 \pm 0.35(\text{ND})$	$1.42 \pm 0.59(1.25)$	$0.92 \pm 0.25(1.51)$	ND
2YT	$0.60 \pm 0.04(\text{ND})$	$1.59 \pm 0.12(1.15)$	$1.33 \pm 0.28(1.60)$	ND
TB	$0.32 \pm 0.07(\text{ND})$	$1.28 \pm 0.38(0.31)$	$1.39 \pm 0.34(0.51)$	$0.11 \pm 0.17(\text{ND})$
SB	$0.04 \pm 0.09(\text{ND})$	$0.94 \pm 0.16(0.57)$	$1.31 \pm 0.29(0.93)$	$0.05 \pm 0.10(0.09)$
TBAIM	ND	1.04 ± 0.16	1.61 ± 0.39	0.23 ± 0.17
<i>B) IPTG concentration (μM)</i>				
0	ND	ND	ND	ND
10	0.46 ± 0.28	0.52 ± 0.38	0.42 ± 0.27	ND
50	0.48 ± 0.12	0.72 ± 0.08	0.46 ± 0.22	ND
100	0.27 ± 0.15	1.00 ± 0.19	0.91 ± 0.26	ND
500	0.94 ± 0.15	1.10 ± 0.06	0.32 ± 0.13	ND
1000	0.80 ± 0.09	0.80 ± 0.11	0.17 ± 0.01	ND
<i>C) Induction temperature ($^{\circ}\text{C}$)</i>				
15	ND	0.46 ± 0.03	0.91 ± 0.04	0.20 ± 0.01
20	ND	0.71 ± 0.08	1.32 ± 0.14	0.28 ± 0.03
25	0.33 ± 0.02	0.83 ± 0.05	0.82 ± 0.11	ND
30	1.17 ± 0.21	1.36 ± 0.20	0.60 ± 0.15	ND
37	0.84 ± 0.04	0.70 ± 0.23	ND	ND
<i>D) Cell growth at induction (OD 600 nm)</i>				
Initial	ND	0.97 ± 0.10	2.02 ± 0.40	0.54 ± 0.13
0.3	0.04 ± 0.08	1.16 ± 0.15	1.93 ± 0.54	0.37 ± 0.21
0.7	ND	0.78 ± 0.02	1.28 ± 0.18	0.25 ± 0.02
1.1	0.06 ± 0.07	0.79 ± 0.17	1.44 ± 0.40	0.25 ± 0.12
2.1	0.04 ± 0.08	0.75 ± 0.03	1.64 ± 0.24	0.36 ± 0.11
<i>E) Induction length (h)</i>				
3	ND	ND	1.11 ± 0.11	0.34 ± 0.06
5	ND	0.56 ± 0.04	1.27 ± 0.11	0.39 ± 0.07
16	0.13 ± 0.10	1.17 ± 0.09	1.80 ± 0.19	0.30 ± 0.07

Culture growth conditions and extract production protocols for each optimization trial are described in the Methods section. Reactions (1 mL) were performed in buffer (50 mM Tris pH 7.0) containing pulegone (1 mM), cell extracts (0.25 mL), NADP⁺ (10 μM), glucose (15 mM) and GDH (10 U). The reactions were agitated at 30 $^{\circ}\text{C}$ for 2 h at 130 rpm. Product yields were determined by GC analysis using a DB-WAX column. Menthol yields are expressed as the mg produced per g of protein in the cell extracts. Purity is the % menthol produced compared to the total product yields. Minimal = minimal medium; LB = lysogeny broth; 2YT = 2YT broth; TB = Terrific broth; SB = Super broth; TBAIM = Terrific broth auto induction medium; ND = none detected. Menthol yields in parentheses represents the activity detected in uninduced culture extracts.

SI Table 5. PCR primers used in the production of multi-gene expression constructs.

PCR Step	Forward Primers	Reverse Primers
1	GATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTG	ATT AGCTGT CTCTCA GTGGTGGTGGTGGTGGTCTC
2	GGAGGA CAGCTAA ATG GCGGATACGTTTACGCAGCGTTAC	GTTAGCAGCCGGATCT CAGTGGTGGTGGTGG
3	GGAGGA CAGCTAA ATG GGTAGCAGCC CATCATCATCATC	GTTAGCAGCCGGATCT CAGTGGTGGTGGTGG
4	GGAGGA CAGCTAA ATG CATATGGCGGATACGTTTAC GCAG	GTTAGCAGCCGGATCTCGAGGTACAGACTCAGGGCTTC ^a
5	GATCCGGCTGCTAACAAAGCCCGAAAGGAAGCT	CTCGAGGTACAGACTCAGGGCTTCTTCGCGAGAG
6	a) GGAGGA CAGCTAA ATG GGTAGCAGCC CATCATCATCATC b) CACCACCACCACCACTGA GGAGGA CAGCTAAATGGGTAGCA c) GAGTCTGTACCTCGAG CACCACCACCACCACCACTGA GGAG	a) GGATCTCA CTCGAGATACAGACAAAACGCTTCATCGCG b) AGCAGCCGGATCT CA CTCGAGATACAGACAAAACGCTTC

The step number refers to the PCR steps shown in SI Figure 1. ^aNo His₆-tag and stop codon on MMR. Non-complementary DNA overhangs are in italics. The Shine-Dalgarno sequence is shown in red. His₆-tags are shown in blue. Start (ATG) and stop (TGA) codons are shown in bold. PCR reaction 6 required 5 overlapping oligos to generate the large overhangs.

SI REFERENCES

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