Supplemental Information

Engineering the Biocatalytic Selectivity of Iridoid Production in *Saccharomyces cerevisiae*

John M. Billingsley[a], Anthony B. DeNicola[a], Joyann S. Barber[b], Man-Cheng Tang[a], Joe Horecka[c,d], Angela Chu[c,d], Neil K. Garg[b], Yi Tang[a,b,*]

[a] *Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, California 90095, United States*

[b] *Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, United States*

[c] *Stanford Genome Technology Center, Stanford University, Palo Alto, CA, USA*

[d] *Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA*

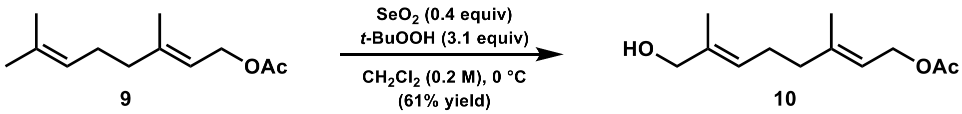
The Supplemental Information consists of 2 Supplementary Methods, 22 Supplementary Figures and 4 Supplementary Tables.

*Corresponding author. E-mail address: yitang@ucla.edu (Y. Tang)

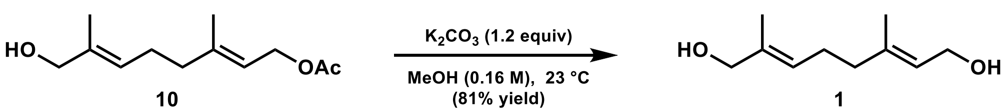
Supplementary Method 1. Construction of JHY651

In order to improve the capacity of BY4742 (Brachmann et al., 1998) for expression of heterologous natural product pathways, a number of genomic changes were implemented. Six previously identified quantitative trait loci (QTLs) were repaired to restore sporulation (MKT1(30G) RME1(INS-308A) TAO3(1493Q)) (Deutschbauer and Davis, 2005) and mitochondrial genome stability (SAL1+ CAT5(91M) MIP1(661T)) (Dimitrov et al., 2009) in BY4742. Additionally, a Ty1 element which inactivates the HAP1 regulatory gene in S288c derived strains (Gaisne et al., 1999) was repaired. The resulting strain, DHY214 was further modified by deletion of PRB1 and PEP4, which mediate the degradation of heterologous proteins (Jones, 1991), resulting in the parent strain used in this study, JHY651 (S1). Strain modifications were performed using the previously described 50:50 method (Horecka and Davis, 2014).

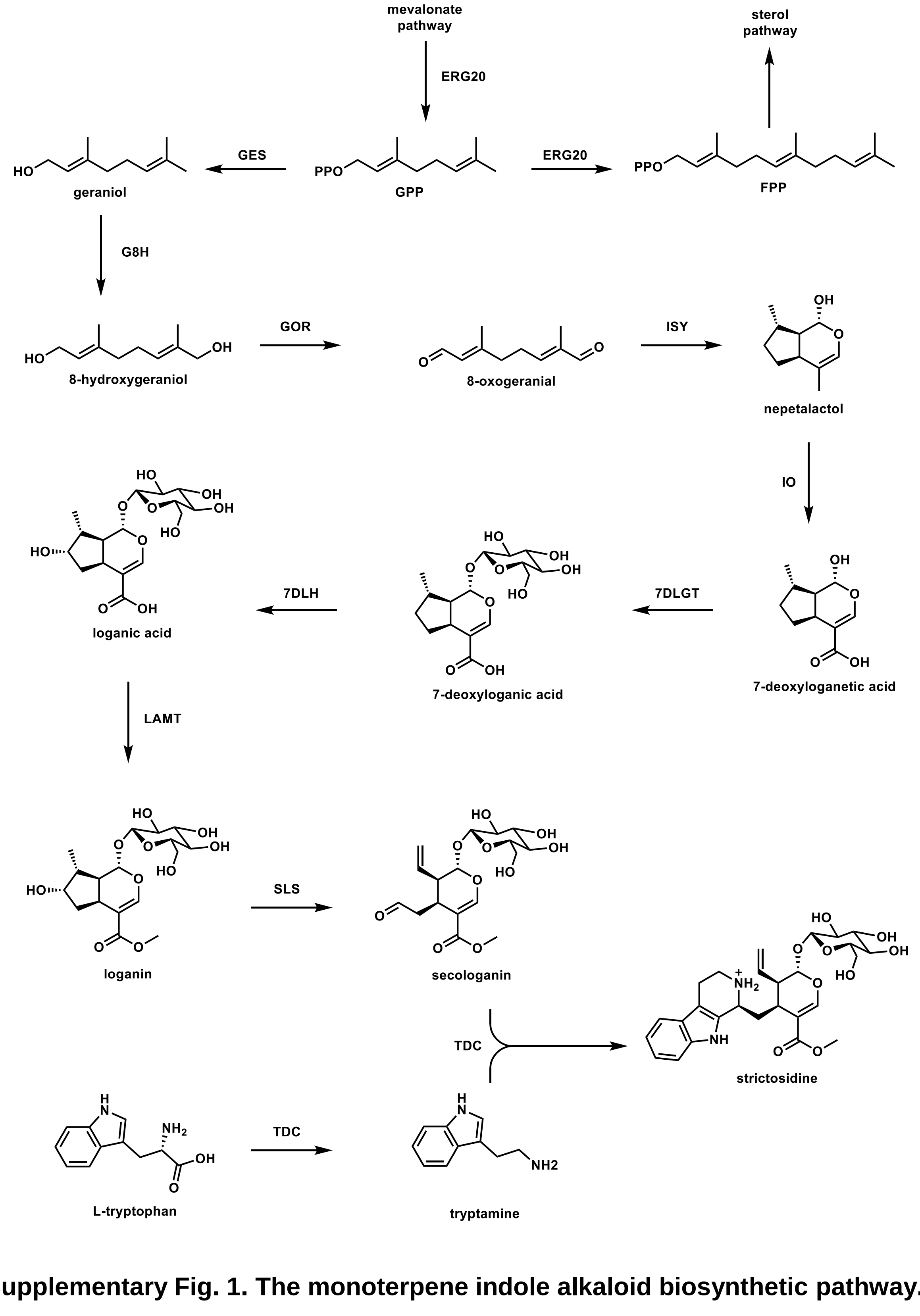
Supplementary Method 2. Synthesis of 8-hydroxygeraniol



Synthesis of **10**: To a solution of selenium dioxide (0.68 g, 6.12 mmol, 0.4 equiv) in CH_2Cl_2 (77 mL) was added *tert*-butyl hydroperoxide (5.5 M in decane, 8.6 mL, 47.5 mmol, 3.1 equiv). The solution was then cooled to 0 °C and geranyl acetate (**9**, 3.3 mL, 15.3 mmol, 1.0 equiv) was added. After stirring for 7 h at 0 °C, EtOAc (200 mL) was added and the reaction was transferred to a separatory funnel. The layers were separated and the organic layer was washed successively with deionized water (2 x 75 mL), saturated aqueous $NaHCO_3$ (1 x 75 mL), deionized water (1 x 50 mL), and brine (1 x 80 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting crude oil was purified by flash chromatography (9:1 → 1:1 hexanes:EtOAc) to afford allylic alcohol **10** (1.99 g, 61% yield) as a light yellow oil. Allylic alcohol **10**: R_f 0.44 (2:1 hexanes:EtOAc). Spectral data (Fig. S11) match those previously reported (Bogazkaya et al., 2014).



Synthesis of 8-hydroxygeraniol **1**: To a solution of allylic alcohol **10** (1.99 g, 9.39 mmol, 1.0 equiv) in MeOH (60 mL) was added potassium hydroxide (1.56 g, 11.3 mmol, 1.2 equiv) as a solid in one portion. After stirring for 2.5 h at 23 °C, deionized water (40 mL) was added and the reaction was transferred to a separatory funnel. The layers were separated and the aqueous layer was extracted with Et_2O (3 x 80 mL). The combined organic layers were washed successively with 0.5 M HCl (1 x 50 mL), saturated aqueous $NaHCO_3$ (1 x 100 mL), brine (1 x 50 mL), and deionized water (1 x 50 mL). The organic layers were then dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting crude oil was purified by flash chromatography (1:1 hexanes:EtOAc) to afford 8-hydroxygeraniol (**1**, 1.29 g, 81% yield) as a light yellow oil. **1**: R_f 0.14 (2:1 hexanes:EtOAc). Spectral data (Fig. S12) match those previously reported (Bogazkaya et al., 2014).



Supplementary Fig. 1. The monoterpene indole alkaloid biosynthetic pathway.

Supplementary Fig. 1 (continued). The monoterpene indole alkaloid biosynthetic pathway. 8-Hydroxygeraniol is converted to the dialdehyde by geraniol oxidoreductase (GOR). 8-oxogeranial undergoes reductive cyclization by iridoid synthase (ISY). The iterative P450 iridoid oxidase (IO) converts the C-4 methyl into a carboxy group. 7-deoxyloganitic acid glucosyltransferase (7-DLGT) stabilizes the hemiacetal. The P450 7-deoxyloganic acid hydroxylase (7-DLH) installs the hydroxyl, followed by O-methylation by loganic acid O-methyltransferase (LAMT). Secologanin synthase (SLS) then performs the oxidative cleavage of loganin. Meanwhile, tryptophan decarboxylase (TDC) produces the β-arylethylamine tryptamine. The Pictet-Spenglerase strictosidine synthase (STR) catalyzes the condensation of tryptamine with secologanin followed by ring closure to forge strictosidine.

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> codon optimized GOR gene sequence
ATGACTAAAACTAATTCTCCAGCCCCATCTGTCATTACTTGCAAGGCTGCTGTCGTTTGGAAATCCGGTGAACCACCAAAGGTCGAAGAGATCCAAGT
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> codon optimized ISY gene sequence
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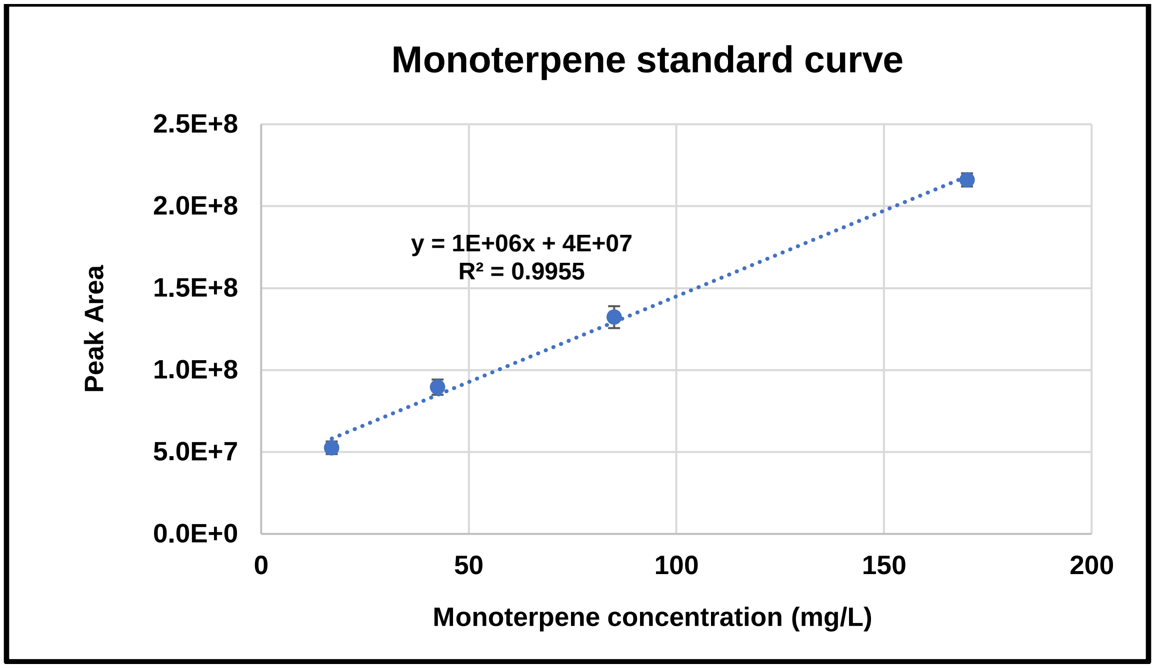
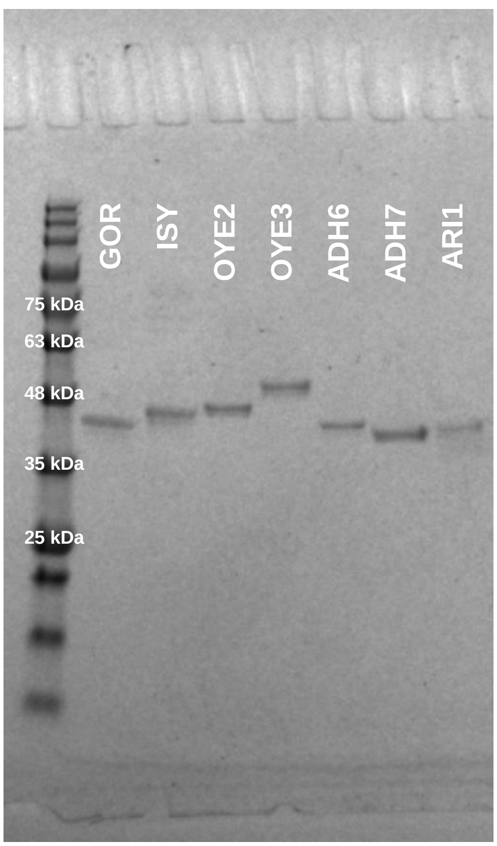
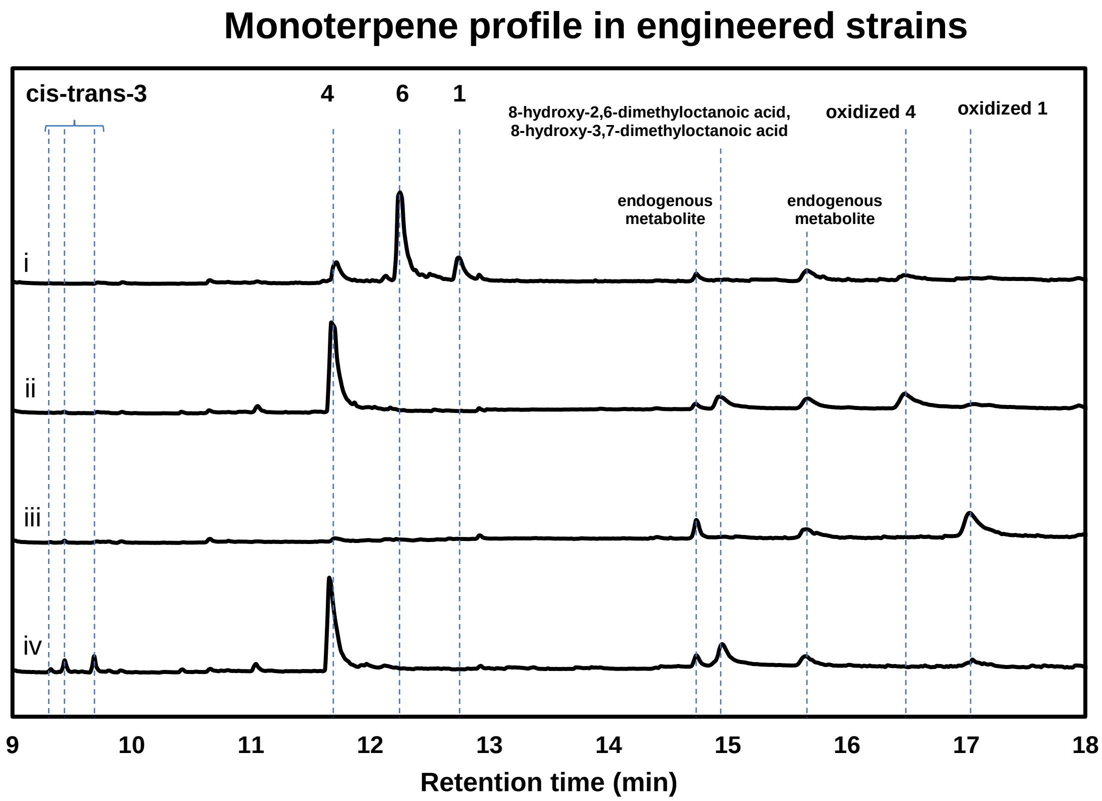
Supplementary Fig. 2. Gene sequences of GOR and ISY synthesized by Gen9.



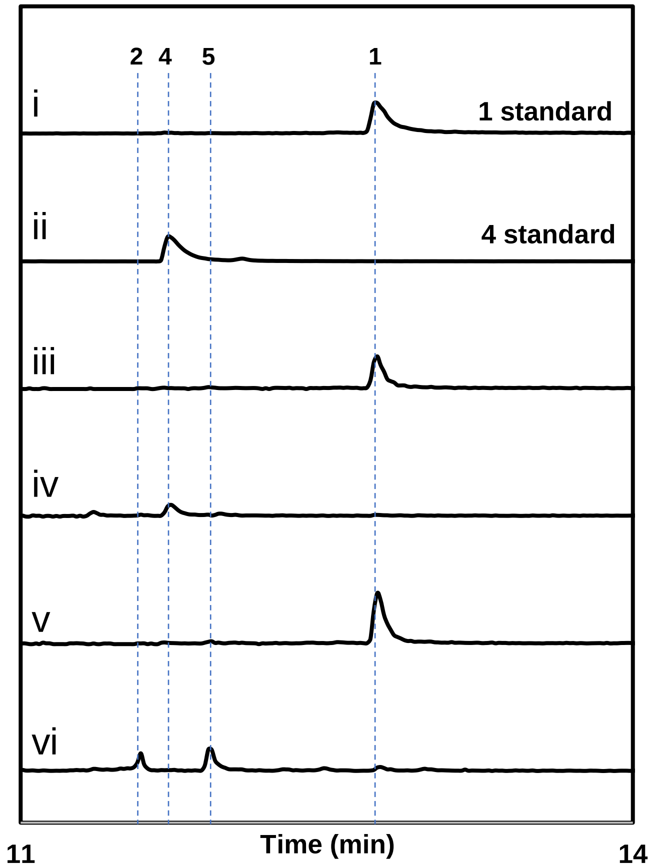
Supplementary Fig. 3. Monoterpene standard curve.



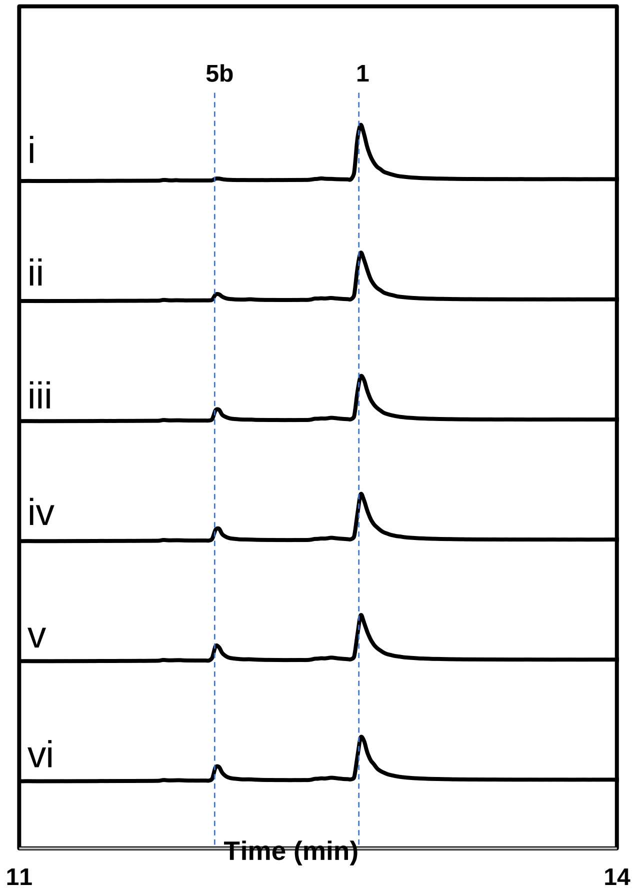
Supplementary Fig. 4. SDS-PAGE gel of purified enzymes used for *in vitro* assays.



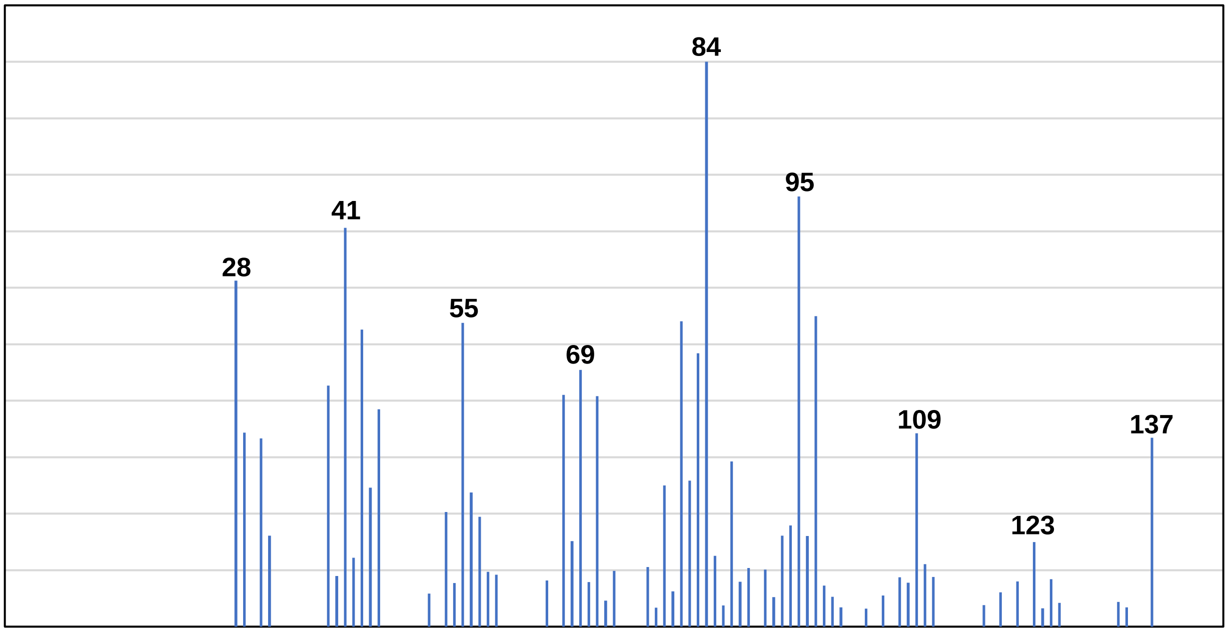
Supplementary Fig. 5. Monoterpene profile after bioconversion assay. (i) S1 with pJB031, (ii) S1 with pJB033, (iii) S4 with pJB033, (iv) S4 with pJB034.



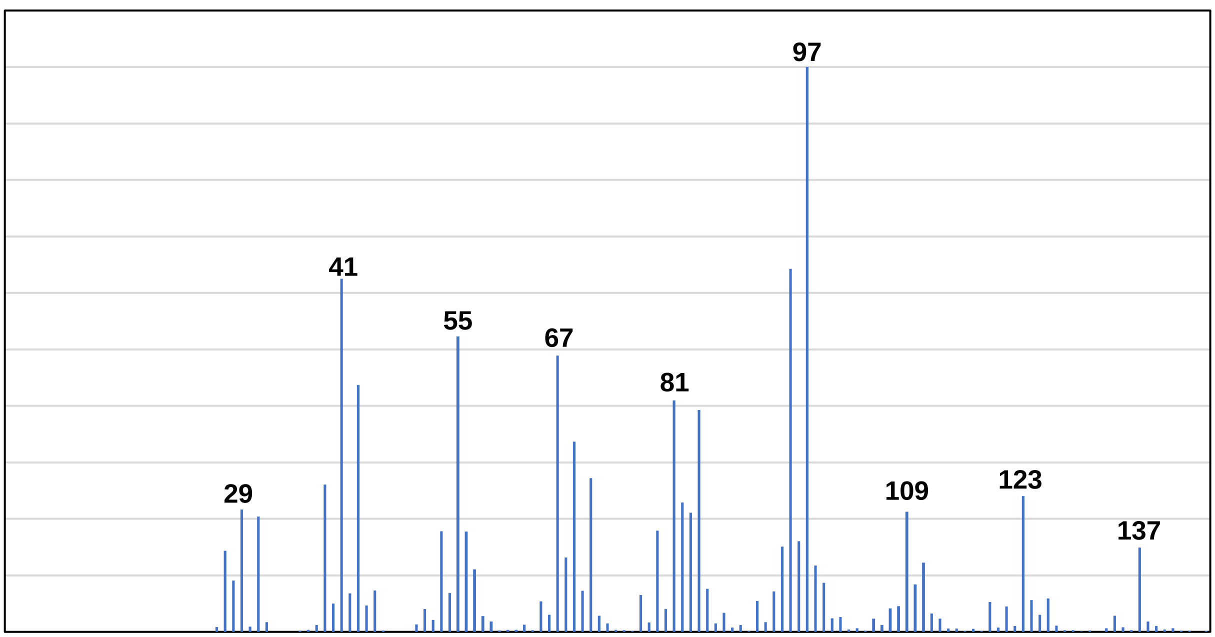
Supplementary Fig. 6. GOR and OYE cofactor dependence, (i) 8-hydroxygeraniol standard, (ii) 8-hydroxytetrahydrogeraniol standard, (iii) 8-hydroxygeraniol + GOR + OYE2, without NAD^+, (iv) 8-hydroxygeraniol + OYE2, without NADPH, (v) 8-hydroxygeraniol + OYE3, without NAD^+, (vi) 8-hydroxygeraniol + GOR + OYE2, without NADPH.



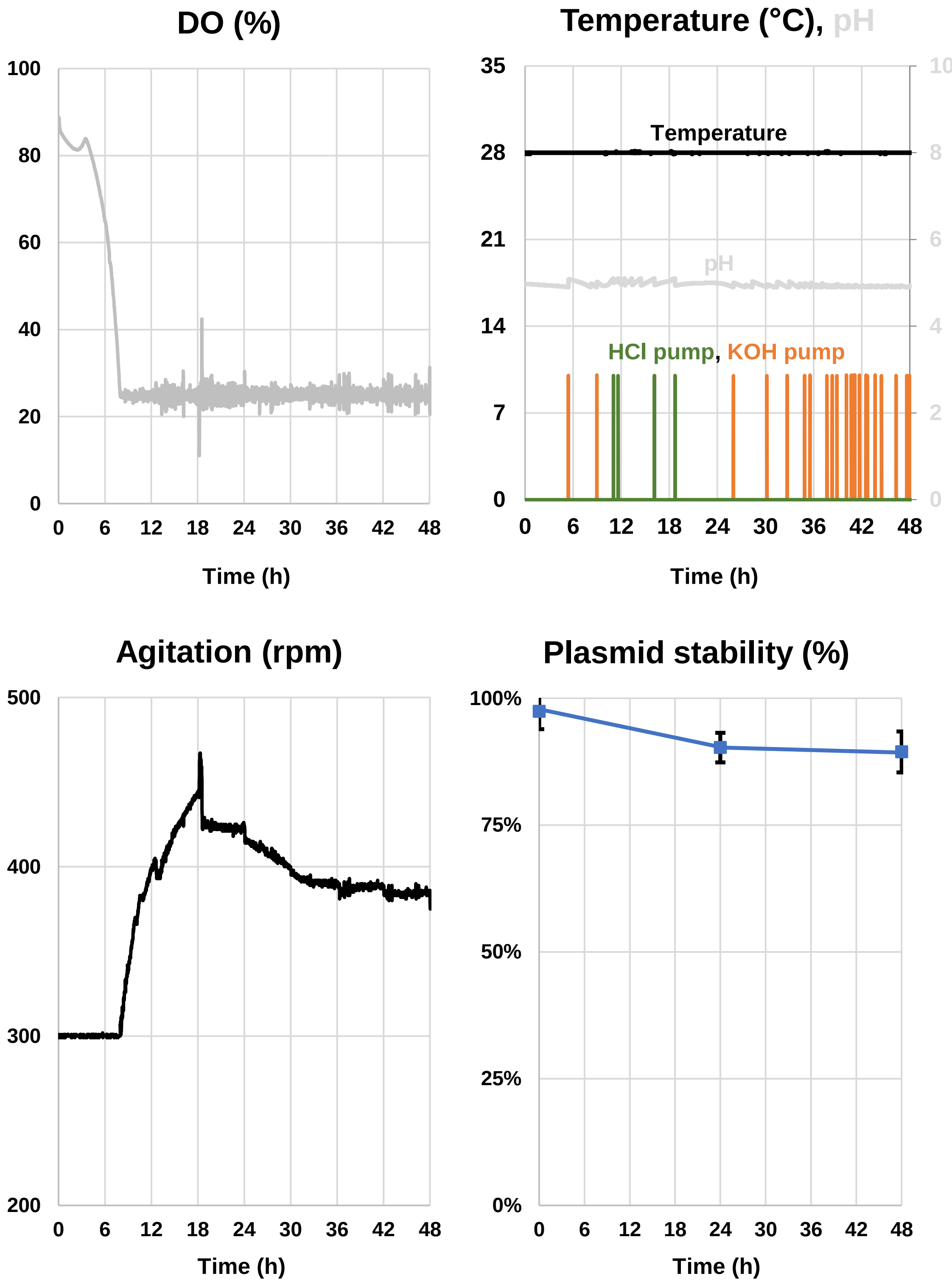
Supplementary Fig. 7. ARI1, ADH6, ADH7 cofactor dependence. (i) 8-hydroxygeraniol + ARI1 + NAD^+, (ii) 8-hydroxygeraniol + ADH6 + NAD^+, (iii) 8-hydroxygeraniol + ADH7 + NAD^+, (iv) 8-hydroxygeraniol + ARI1 + $NADP^+$, (v) 8-hydroxygeraniol + ADH6 + $NADP^+$, (vi) 8-hydroxygeraniol + ADH7 + $NADP^+$.



Supplementary Fig. 8. Mass spectra of compound 7 produced from 4 *in vitro* through the action of GOR and OYE.



Supplementary Fig. 9. Mass spectra of compound 8 produced from 4 *in vitro* through the action of ISY and ADH.



Supplementary Fig. 10. Additional fermentation data. Means and standard errors are reported for plasmid stability.

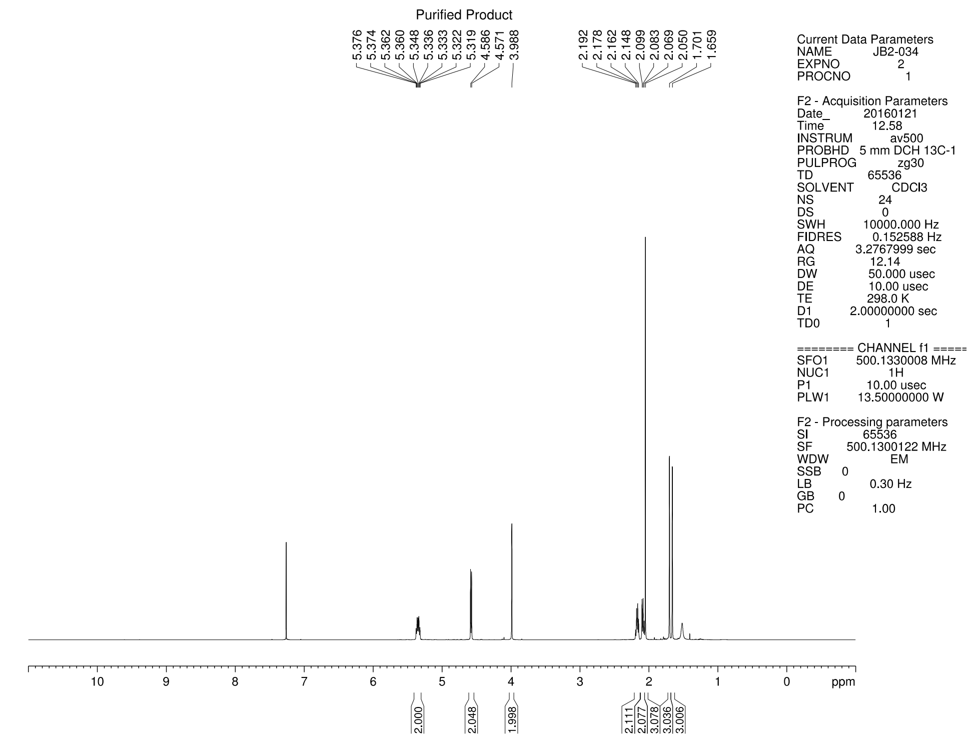
Supplementary Table 1. Primers used in this study.

Category	Primer	Sequence	Amplicon
GOR expression cassette assembly	P01	CAAAACGTAGGGGCAAACAAACG	ADH2p
	P02	GGTATTACGATATAGTTAATAGTTGATAGTTGATTG	
	P03	AACTATCAACTATTAACTATATCGTAATACCATGACTAAAACTAATTCTCCAGCCCCATC	GOR
	P04	TGTGCTAGTGTCTCCCGTCTTCTGTCTTAGAACTTAATAACAACTTTGACACAGTCTGGG	
	P05	GACAGAAGACGGGAGACACTAGCAC	PRM9t
	P06	GGCATTTTCAACATCGTATTTTCCGAAGC	
ISY expression cassette assembly	P07	CAATAGGAAAAAACCGAGCTTCCTTTC	PCK1p
	P08	GTTGTTATTTTATTATGGAATAATTAGTTGCGTG	
	P09	ACTCACGCAACTAATTATTCCATAATAAAATAACAACATGTCCTGGTGGTGGAAAAGGTC	ISY
	P10	AATCTTTGACTATTCAATCATTGCGCTTATGGAATGAATCTGTAGTCTCTCATCTTGTCG	
	P11	GCGCAATGATTGAATAGTCAAAGATT	CPS1t
	P12	ATTTGACACTTGATTTGACACTTCTTT	
pJB031 assembly	P13	GCGGCCGCAACGAAGCATCTGTGCTTCATTTTGTAG	NotI-pJB031
	P14	GCGGCCGCGTTTTTCCATAGGCTCCG	pJB031-NotI
pJB033 assembly	P15	TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCGCAAAACGTAGGGGCAAACAAACG	ADH2p-GOR-PRM9t
	P16	GTTCTACAAAATGAAGCACAGATGCTTCGTTGGCATTTTCAACATCGTATTTTCCGAAGC	
	P17	GATCCGATGATAAGCTGTCAAACATGAG	URA3
	P18	GGCGTTTTTCCATAGGCTCCG	
	P19	AACGAAGCATCTGTGCTTCATTTTGTAG	2μ
	P20	GAAGCATATTTGAGAAGATGCGGC	
pJB034 assembly	P21	TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCGCAAAACGTAGGGGCAAACAAACG	ADH2p-GOR-PRM9t
	P22	GGATGAAAGGAAGCTCGGTTTTTTCCTATTGGGCATTTTCAACATCGTATTTTCCGAAGC	
	P23	TTGTTCTACAAAATGAAGCACAGATGCTTCGTTATTTGACACTTGATTTGACACTTCTTT	PCK1p-ISY-CPS1t
	P24	GATCCGATGATAAGCTGTCAAACATGAG	URA3
	P25	GGCGTTTTTCCATAGGCTCCG	
	P26	AACGAAGCATCTGTGCTTCATTTTGTAG	2μ
	P27	GAAGCATATTTGAGAAGATGCGGC	
pJB097 assembly	P28	TTTCTAGCTCTAAAACTTTAGGACCACCCACAGCACGATCATTTATCTTTCACTGCGGAG	kanMX-LEU2
	P29	TGAATCGAGTCCCTCGATATTTCTCATACTAGTTCTAGAGCGCGCCAACAAATATATTGC	
	P30	ATGATCAAAGCGGTGACATCTCCGGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	LEU2-2μ
	P31	AAGAGTAAAAAAGGAGTAGAAACATTTTGAAGCTATGCAGACGAAAGGGCCTCGTG	
	P32	CTGCATAGCTTCAAAATGTTTCTACTCC	iCas9
	P33	TCTAGAACTAGTATGAGAAATATCGAGGGACTC	
pJB042 assembly	P34	CAGCCATATGACTAAAACTAATTCTCCAGCCCCATC	GOR
	P35	CGAGTGCGGCCGCTTAGAACTTAATAACAACTTTGACACAGTCTGGG	
pJB043 assembly	P36	AAGCTTGTCGTTATGGAATGAATCTGTAGTCTCTCATCTTGTCG	ISY

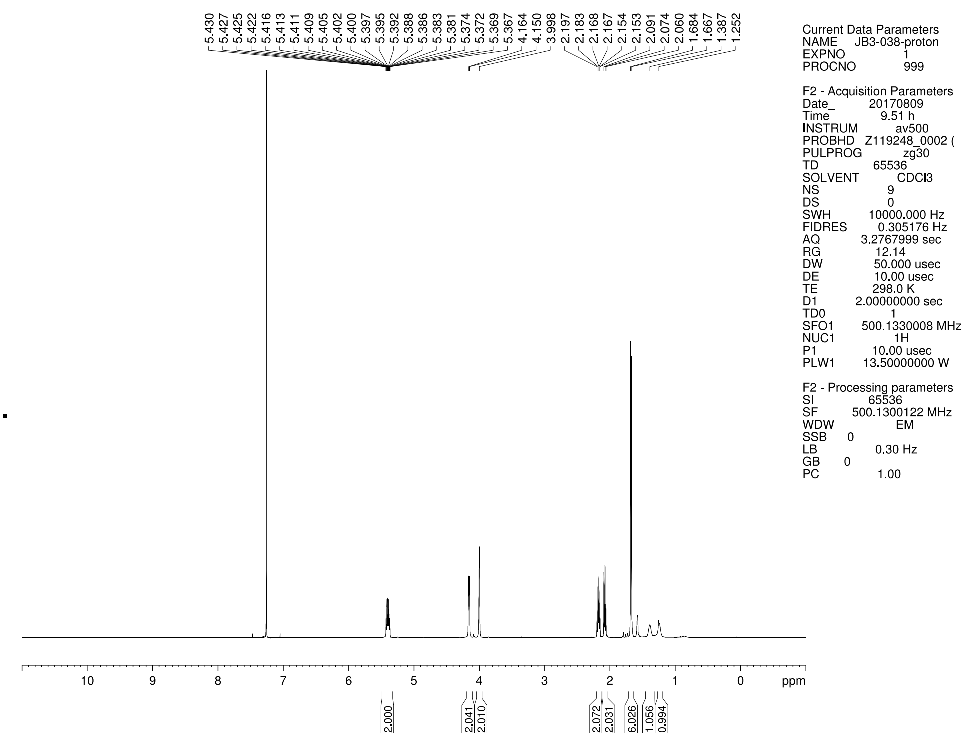
pJB043 assembly	P37	GGTGCTCGAGTTGGAATGAATCTGTAGTCTCTCATCTTG TCG	
pJB044 assembly	P38	TAATGCTAGCATGCCATTTGTTAAGGACTTTAAGCC	OYE2
	P39	TAATGAGCTCTTAATTTTTGTCCCAACCGAGTTTTAGAGC	
pJB045 assembly	P40	TAATGCTAGCATGCCATTTGTAAAAGGTTTTGAGCC	OYE3
	P41	TAATGAGCTCTCAGTTCTTGTTCCAACCTAAATCTACTGC	
pJB072 assembly	P42	TAATGCTAGCATGTCTTATCCTGAGAAATTTGAAGGTATC G	ADH6
	P43	TAATGAGCTCCTAGTCTGAAAATTCTTTGTCGTAGCC	
pJB071 assembly	P44	TAATGGTCTCGCTAGCATGCTTTACCCAGAAAAATTTCA GG	ADH7
	P45	TAATGAGCTCCTATTTATGGAATTTCTTATCATAATCGAC CAAAG	
pJB095 assembly	P46	TAATGCTAGCATGACTACTGATACCACTGTTTTCGT	ARI1
	P47	GCATACAATCAACTATCAACTATTAACTATATCGTAATAC CATGGGCAGCAGCCATCATC	
	P48	TGTGTGCTAGTGTCTCCCGTCTTCTGTCCTTAGGCTTCA TTTTGAACTTCTAACATTTGC	
S6 construction	P49	AGTAATTGTGCATTGTACAACTGTGCTAAACAGACTTAAA AAAGTAATAATTTAACCATTATTTTTTTCCTCAACATAACG AGAACACAC	ARI1p-LEU2
	P50	CTATAGATTTGCCTATTGGAGTGATCAAAAAAAACTTCAA TTAGCGTGATACGGATTTTCTTAACTTCTTCGGCGACAG	LEU2-ARI1t
S8 construction	P51	ATATTATTGAAATAAAAAAAAACATAGAACCACTGAAAAA TACAAAAAAAAATAACCATTATTTTTTTCCTCAACATAACG AGAACACAC	ADH7p-LEU2
	P52	TTCCGAGATTTGACATGCATTTTAAGAGATTCTGAAAAAT ATTACGTATATAGGATTTTCTTAACTTCTTCGGCGACAG	LEU2-ADH7t

Supplementary Table 2. gBlocks used for HICRISPR

gBlock	Sequence
Δoye2	CCTTTGGTCTCACCAAAACATAGCCTTGAAAATCTTGGTCCATTCTTTAATTTGTTCTTCGG ACCAGATCATTGTCGTAACCCCCAGATTGTGGAGAGGGAAAGGTACCTTCAGTGATATGT TCTTCGGACCAGATACCGTTTTAGAGAGAGACCTTTC
Δoye2	CCTTTGGTCTCACCAAAACAGACCTGGTACCATGATCATCACGGAAGGTACGTTTATTTCC CCTCAAGCATGACAACGCCCCTGGGATTTGGTCTGATGAGCAGGTCGCTGAGTGGAAGT TTATTTCCCCTCAAGCCGGGTTTTAGAGAGAGACCTTTC
Δgre2	CTTTGGTCTCACCAAAACTCCTTGCCGTGCTTTTGGAAAACATGGTCAAATGCGTCCAGCT TAGATATGGCCAGAGGTATAGACATAGCCAACTTCCATGGAGAATTTTGGGTTGTTACCAA AGGCCTCCGTTAAATTCGCGTCCAGCTTAGATATGTCGTTTTAGAGAGAGACCTTTC
Δadh6	CTTTGGTCTCACCAAAACAAGTTTGTTACCACATACAGTCAGCCTTATGAAGACGGCTATG TGTCGCAGACGTCTCACGGATCGTATATATGCAAACTACGTCAGAGTTCATGAACATTTTG TGGTGCCTATCCCAGAGGACGGCTATGTGTCGCAGGGGTTTTAGAGAGAGACCTTTC

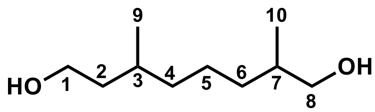


Supplementary Fig. 11 1H NMR of **10**.

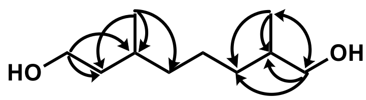


Supplementary Fig. 12 1H NMR of **1**.

Supplementary Table 3. NMR data for compound **4**.
^{1}H NMR spectrum (500 MHz), ^{13}C NMR spectrum (125 MHz), $CDCl_3$



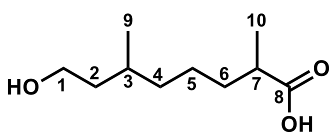
compound 4



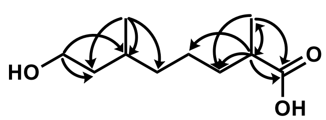
Key HMBC Correlations

Position	δ_H , mult (*J* in Hz)	δ_C	COSY
1	3.69, m	61.33	H2
2	1.37, m, overlap 1.59, m, overlap	40.09	
3	1.56, m, overlap	29.46	
4	1.14, m, overlap 1.29, m, overlap	37.37	
5	1.32, m, overlap	24.38	
6	1.08, m, overlap 1.37, m, overlap	33.38	
7	1.61, m, overlap	35.85	
8	3.43, m 3.50, m	68.53	H7
9	0.89, d (6.5)	19.76	H3
10	0.91, d (6.7)	16.70	H7

Supplementary Table 4. NMR data for the isolated saturated acid.
^{1}H NMR spectrum (500 MHz), ^{13}C NMR spectrum (125 MHz), $CDCl_3$

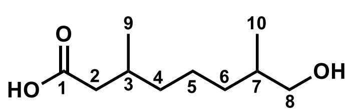


8-hydroxy-2,6-dimethyloctanoic acid

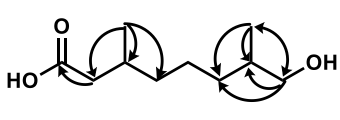


Key HMBC Correlations

Position	δ_H , mult (*J* in Hz)	δ_C	COSY
1	3.68, m	61.28	H2
2	1.35, m, overlap 1.58, m, overlap	39.91	
3	1.56, m, overlap	29.39	
4	1.15, m, overlap 1.32, m, overlap	36.93	
5	1.32, m, overlap	24.62	
6	1.39, m, overlap 1.65, m, overlap	33.83	
7	2.47, m	39.27	H6, H10
8		181.53	
9	0.89, d (6.4)	19.74	H3
10	1.18, d (7.0)	17.06	H7

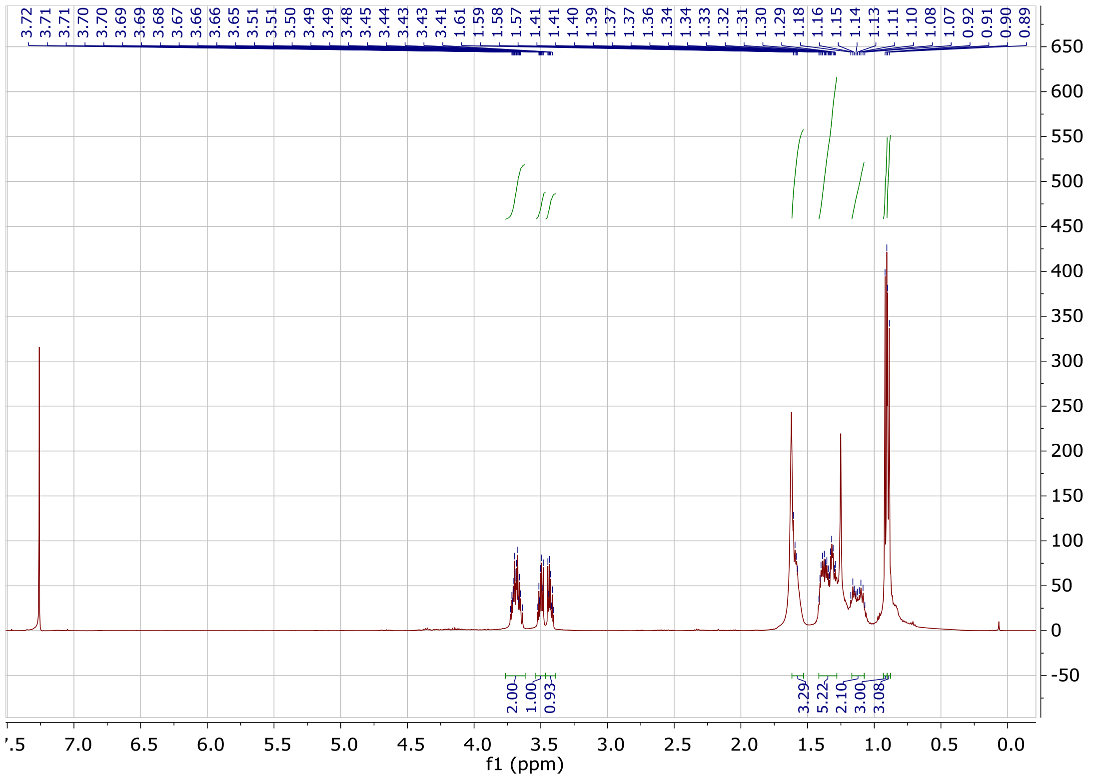


8-hydroxy-3,7-dimethyloctanoic acid

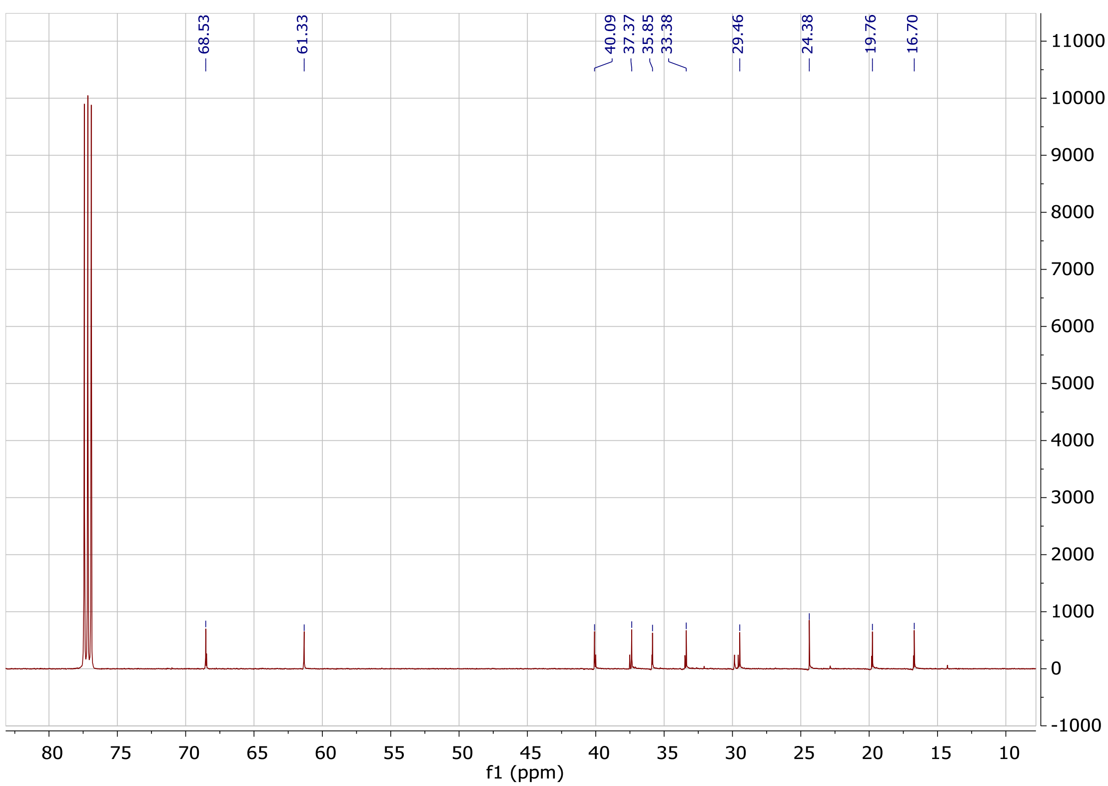


Key HMBC Correlations

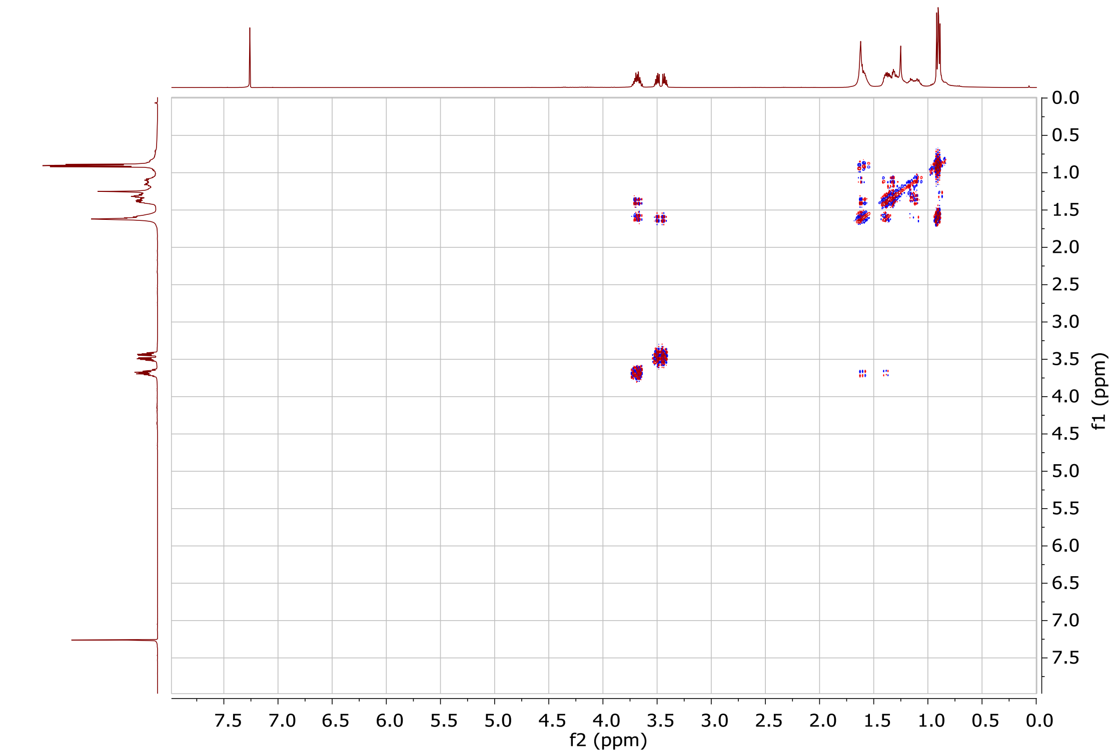
Position	δ_H , mult (*J* in Hz)	δ_C	COSY
1		177.89	
2	2.16, m 2.34, m	41.48	H3
3	1.97, m	30.19	H2, H9
4	1.15, m, overlap 1.32, m, overlap	37.01	
5	1.32, m, overlap	24.34	
6	1.09, m, overlap 1.39, m, overlap	33.18	
7	1.60, m, overlap	35.78	
8	3.44, m 3.50, m	68.46	H7
9	0.97, d (6.7)	19.87	H3
10	0.91, d (6.9)	16.67	H7



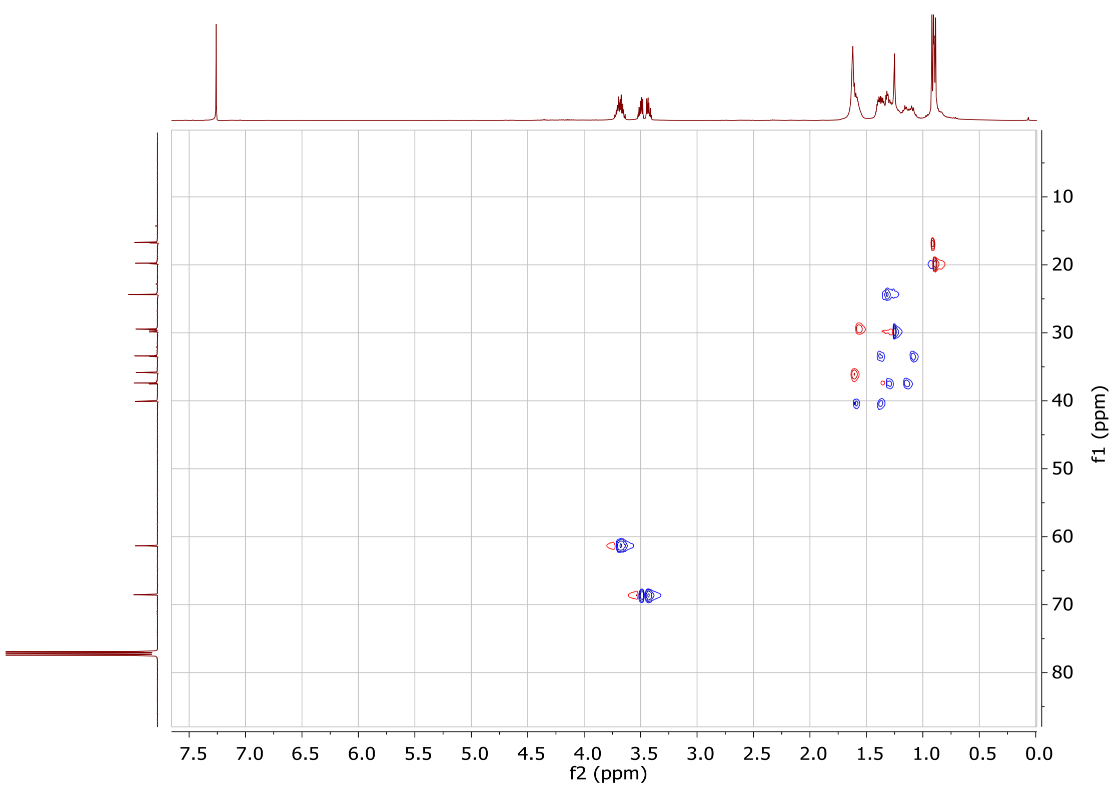
Supplementary Fig. 13. ^{1}H NMR Spectrum of Compound **4** in $CDCl_3$.



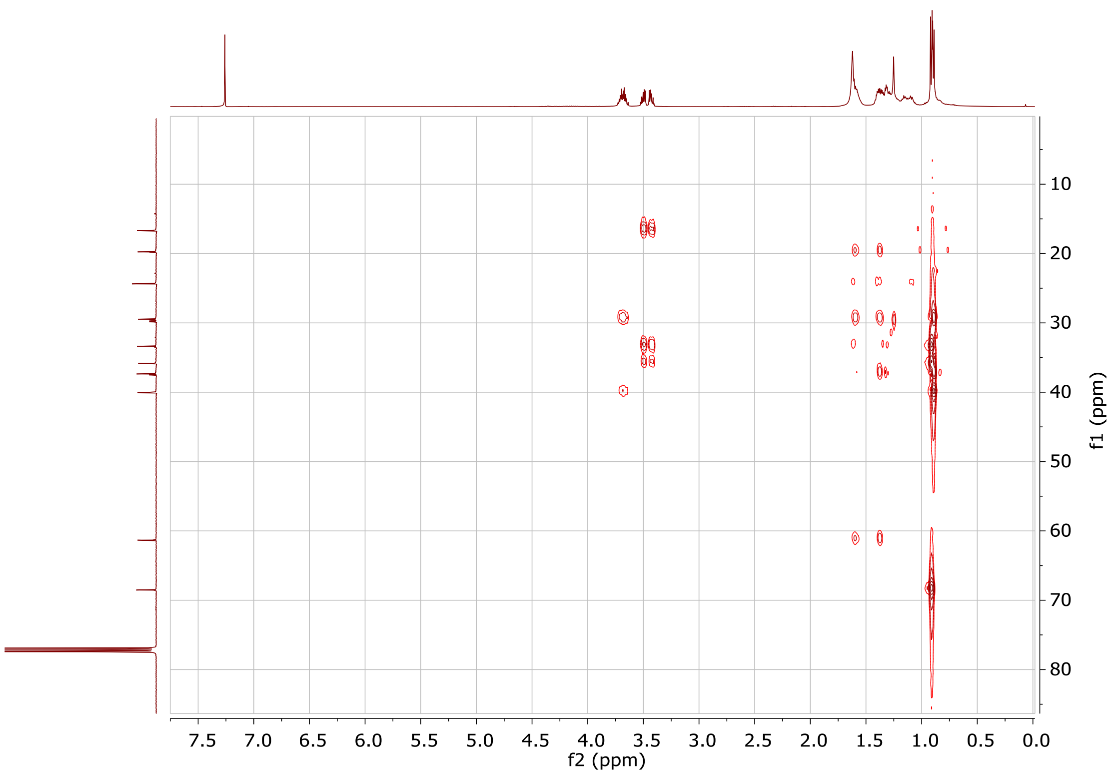
Supplementary Fig. 14. ^{13}C NMR Spectrum of Compound **4** in $CDCl_3$.



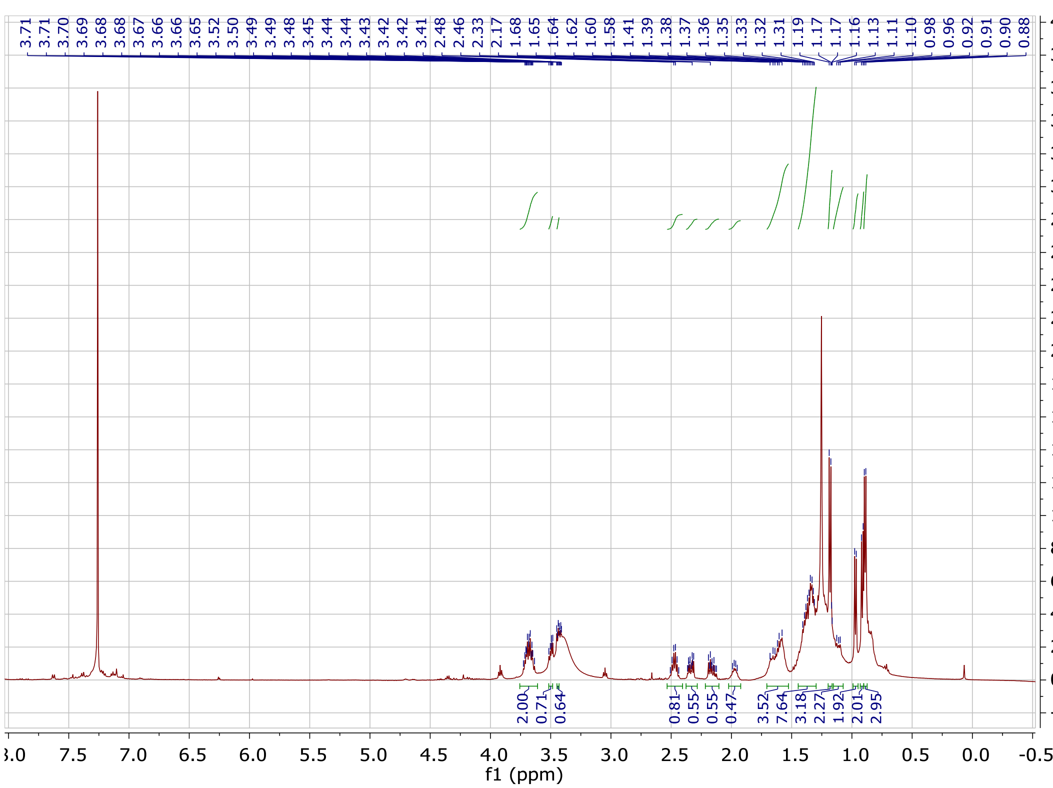
Supplementary Fig. 15. ^{1}H-^{1}H COSY Spectrum of Compound **4** in $CDCl_3$.



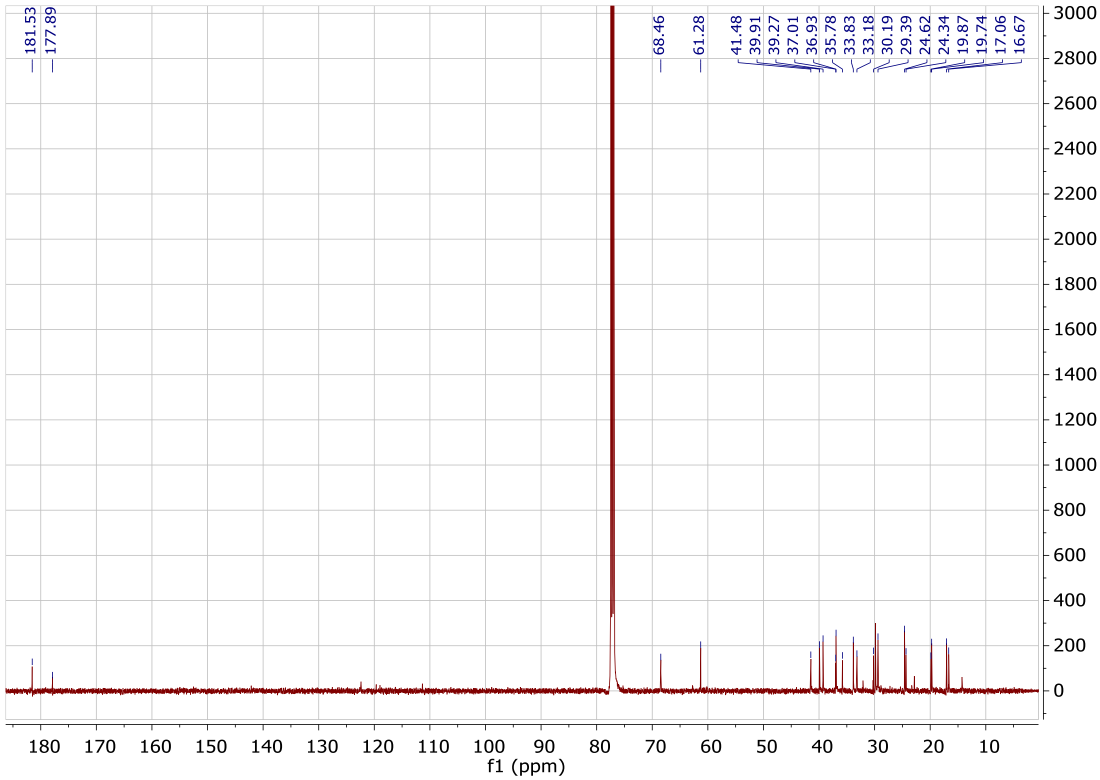
Supplementary Fig. 16. HSQC Spectrum of Compound **4** in $CDCl_3$.



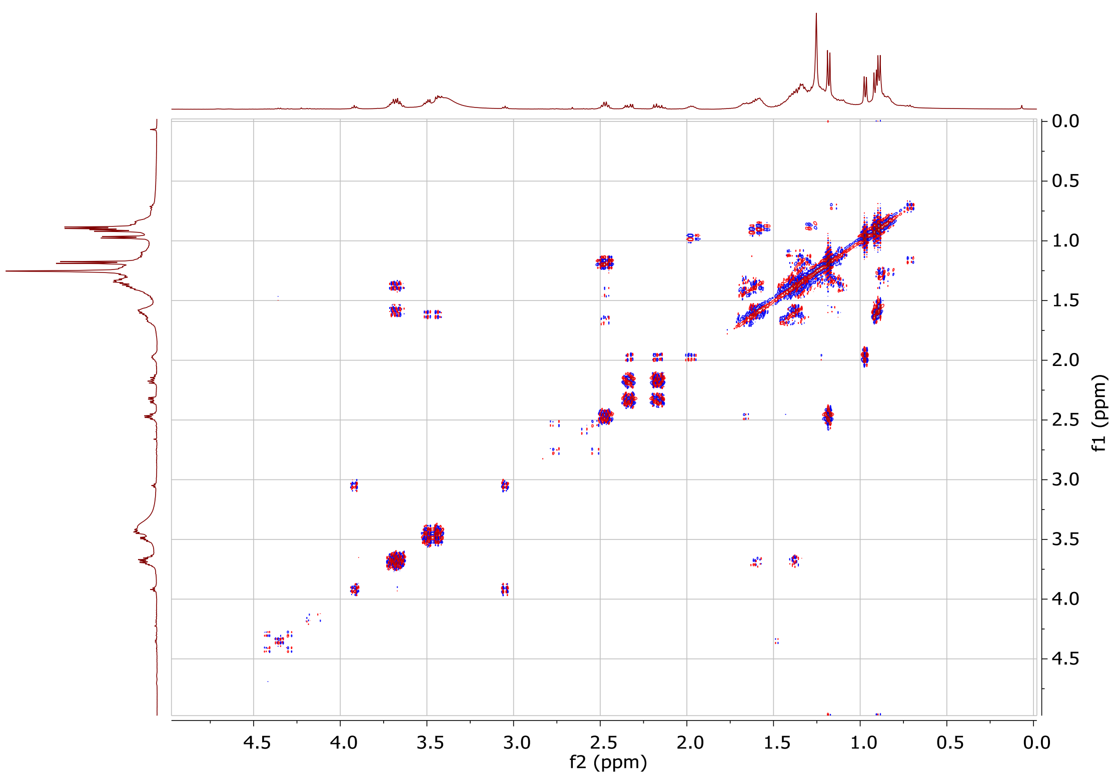
Supplementary Fig. 17. HMBC Spectrum of Compound **4** in $CDCl_3$.



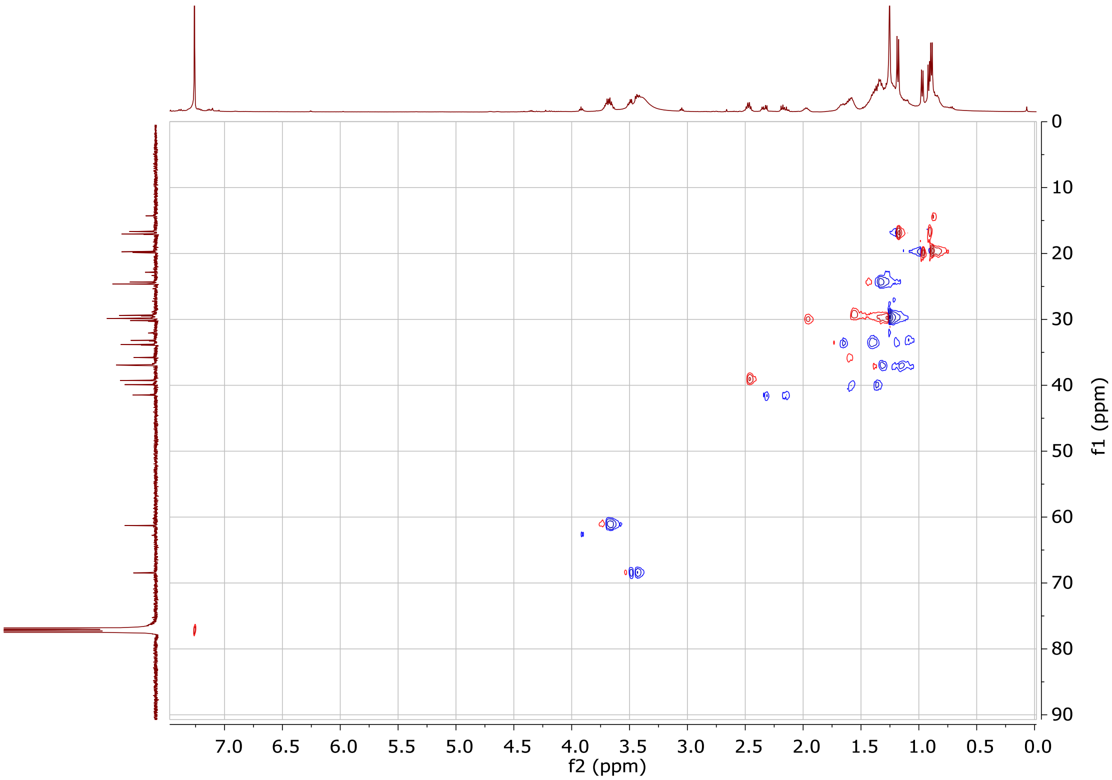
Supplementary Fig. 18. ^{1}H NMR Spectrum of the isolated saturated acid in $CDCl_3$.



Supplementary Fig. 21. ^{13}C NMR Spectrum of the isolated saturated acid in $CDCl_3$.



Supplementary Fig. 20. ^{1}H-^{1}H COSY Spectrum of the isolated saturated acid in $CDCl_3$.



Supplementary Fig. 21. HSQC Spectrum of the isolated saturated acid in $CDCl_3$.

Supplementary Fig. 22. HMBC Spectrum of the isolated saturated acid in $CDCl_3$.

Supplemental Information References

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