Electronic Supplementary Information (ESI)

Towards a sustainable generation of pseudopterosin-type bioactives

Ringel *et al.*

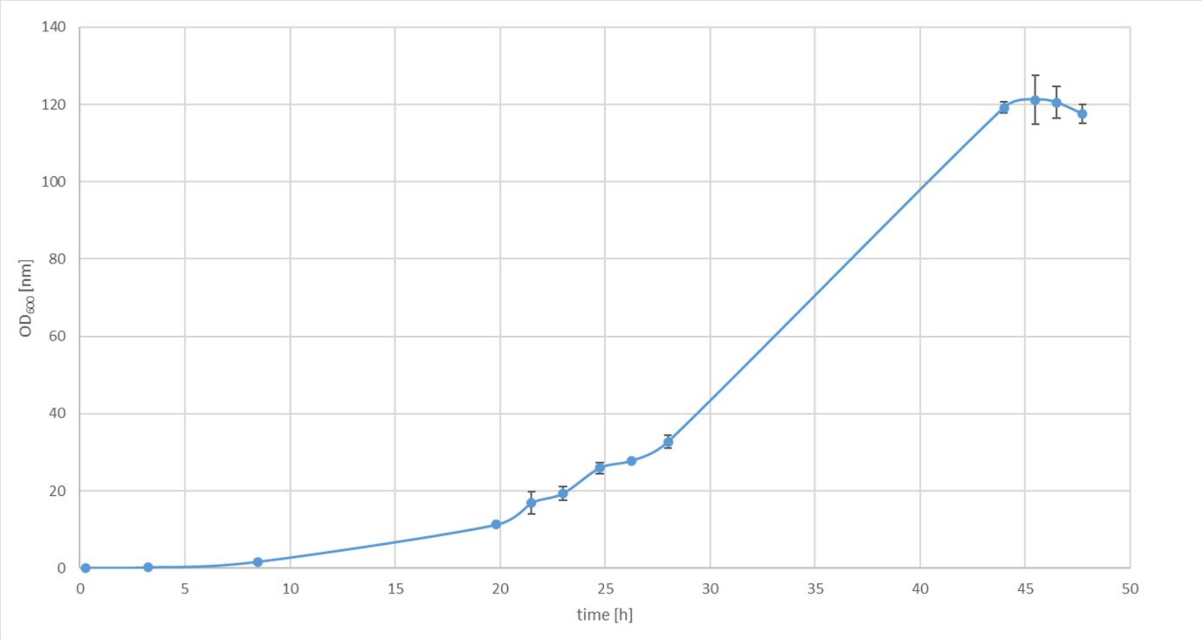


Figure S 1: Growth curve of *E. coli* host harbouring terpene biosynthesis precursor plasmid and plasmid coding for HpS; error bars show standard deviation calculated from triplicates (measurements of OD_{600}).

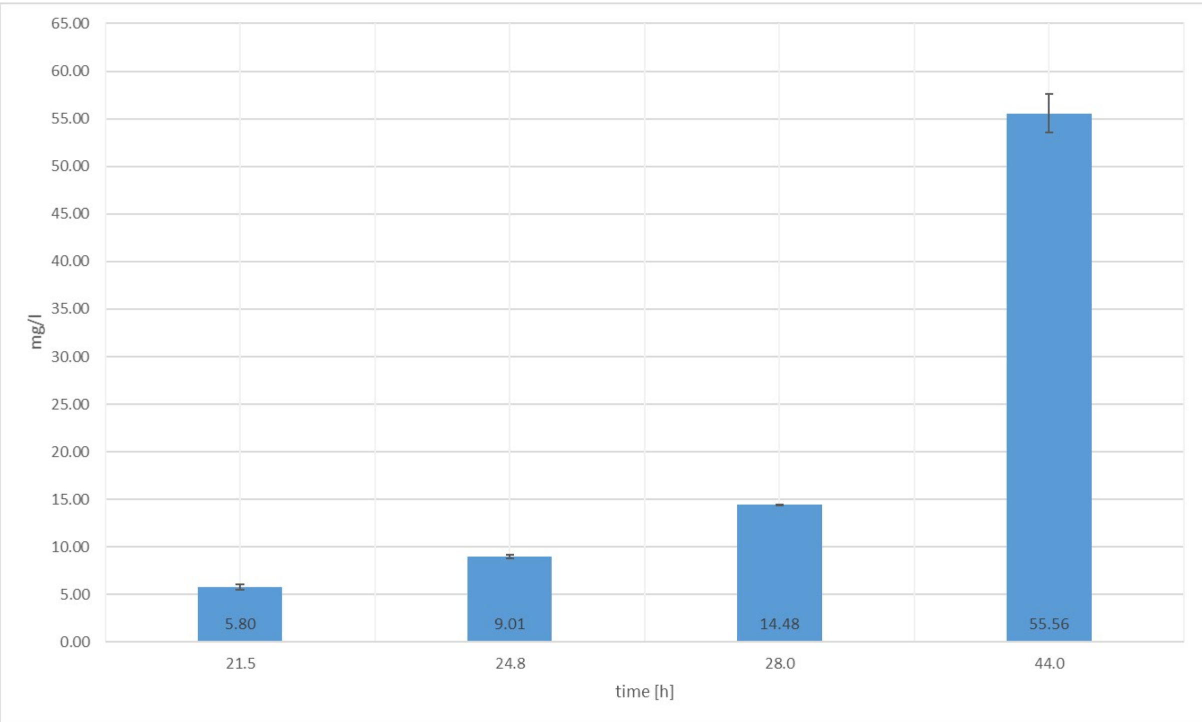


Figure S 2: Time dependent, total terpene yield from fermentation (HpS) shown in Figure S1; error bars show standard deviation calculated from duplicates (quantification from GC-FID measurements).

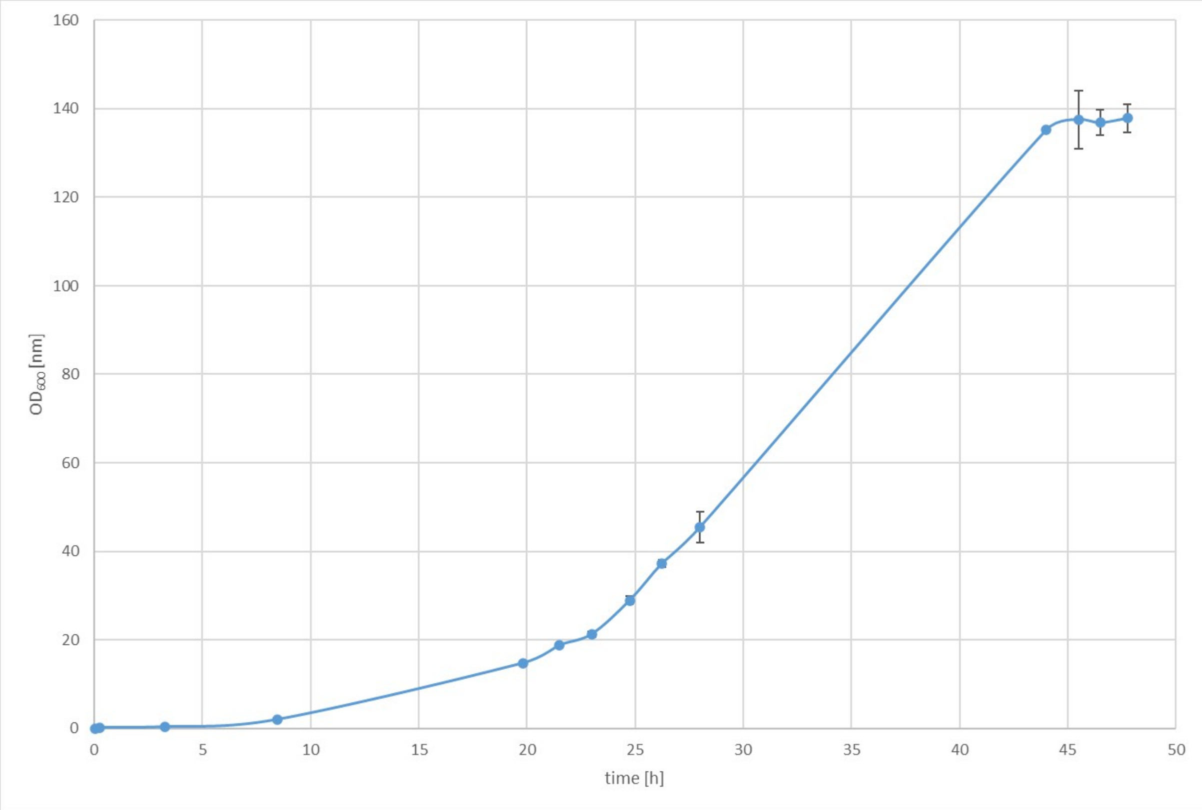


Figure S 3: Growth curve of *E. coli* host harbouring terpene biosynthesis precursor plasmid and plasmid coding for HpS M75L; error bars show standard deviation calculated from triplicates (measurements of OD_{600}).

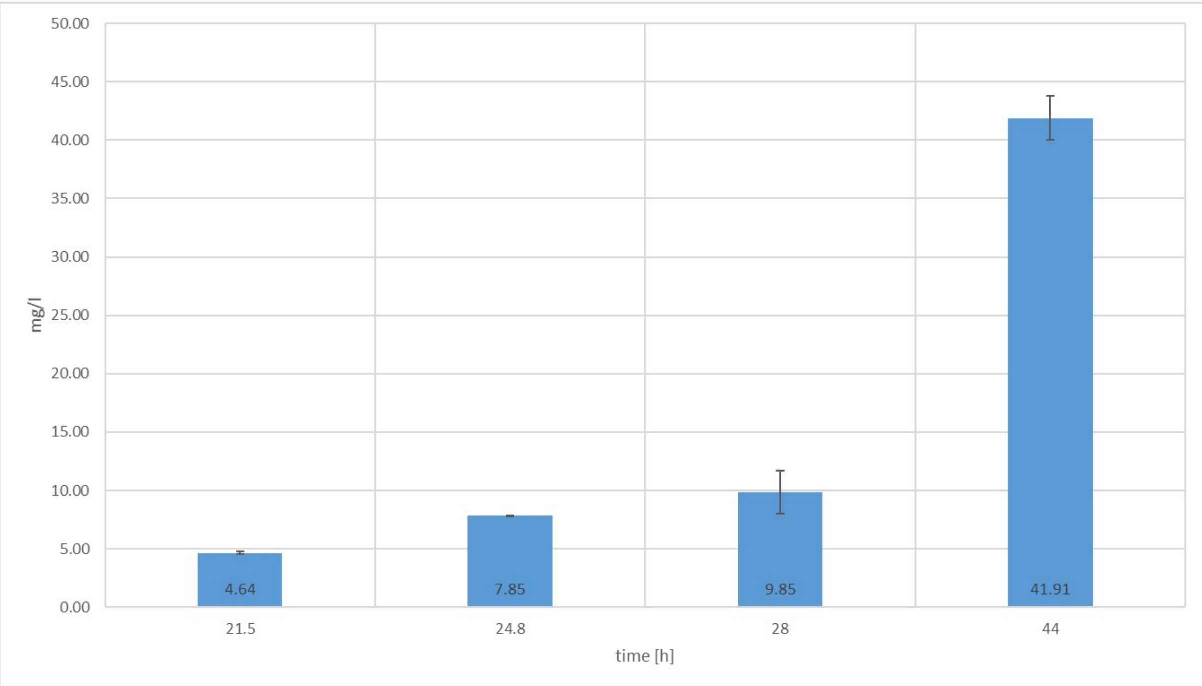


Figure S 4: Time dependent, total terpene yield from fermentation (HpS M75L) shown in Figure S3; error bars show standard deviation calculated from duplicates (quantification from GC-FID measurements).

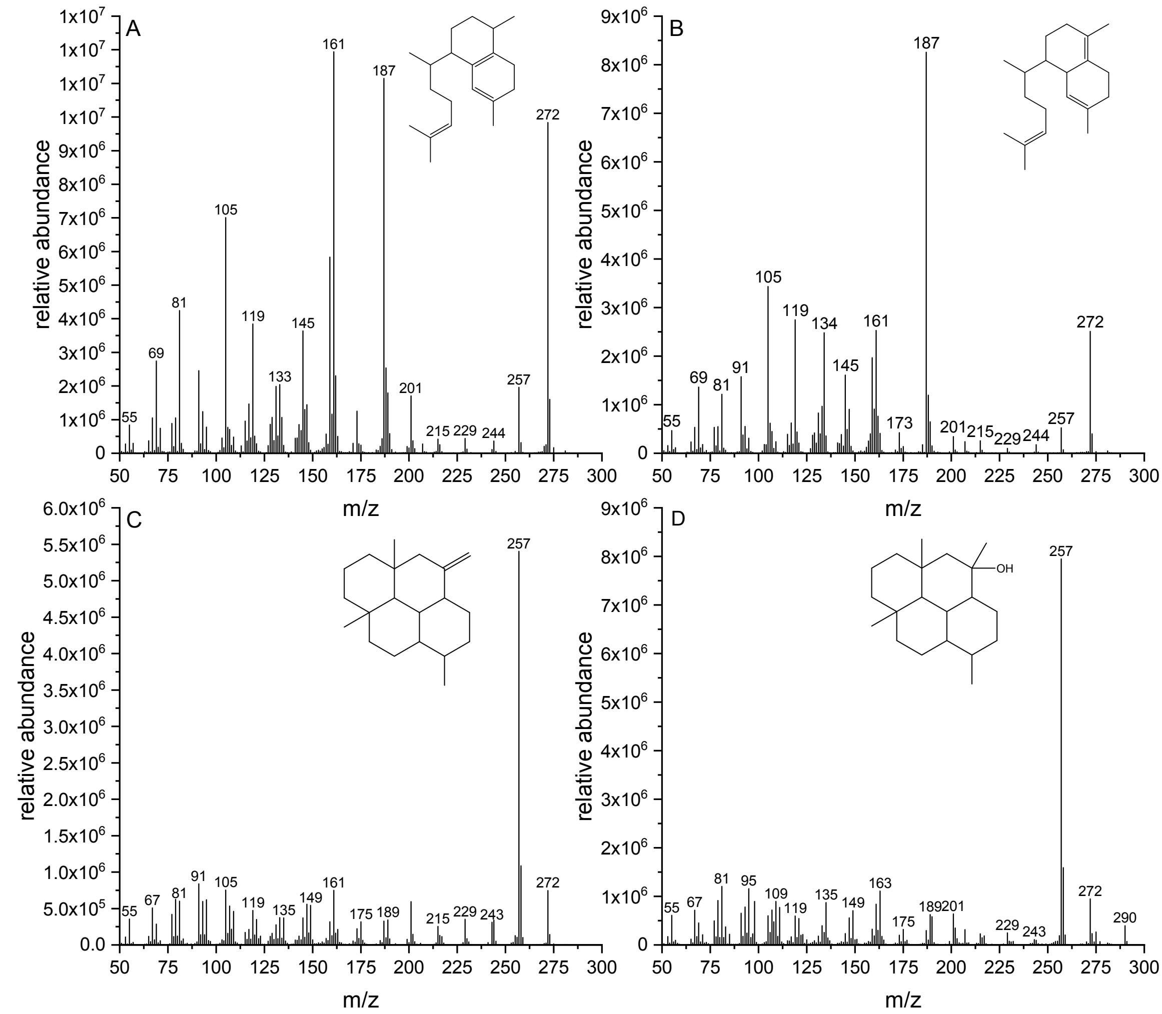


Figure S 5: GC-MS spectra of HpS’ main products (obtained in *E. coli*); (A) isoelisabethatriene A; (B) isoelisabethatriene B; (C) hydropyrene; (D) hydropyrenol

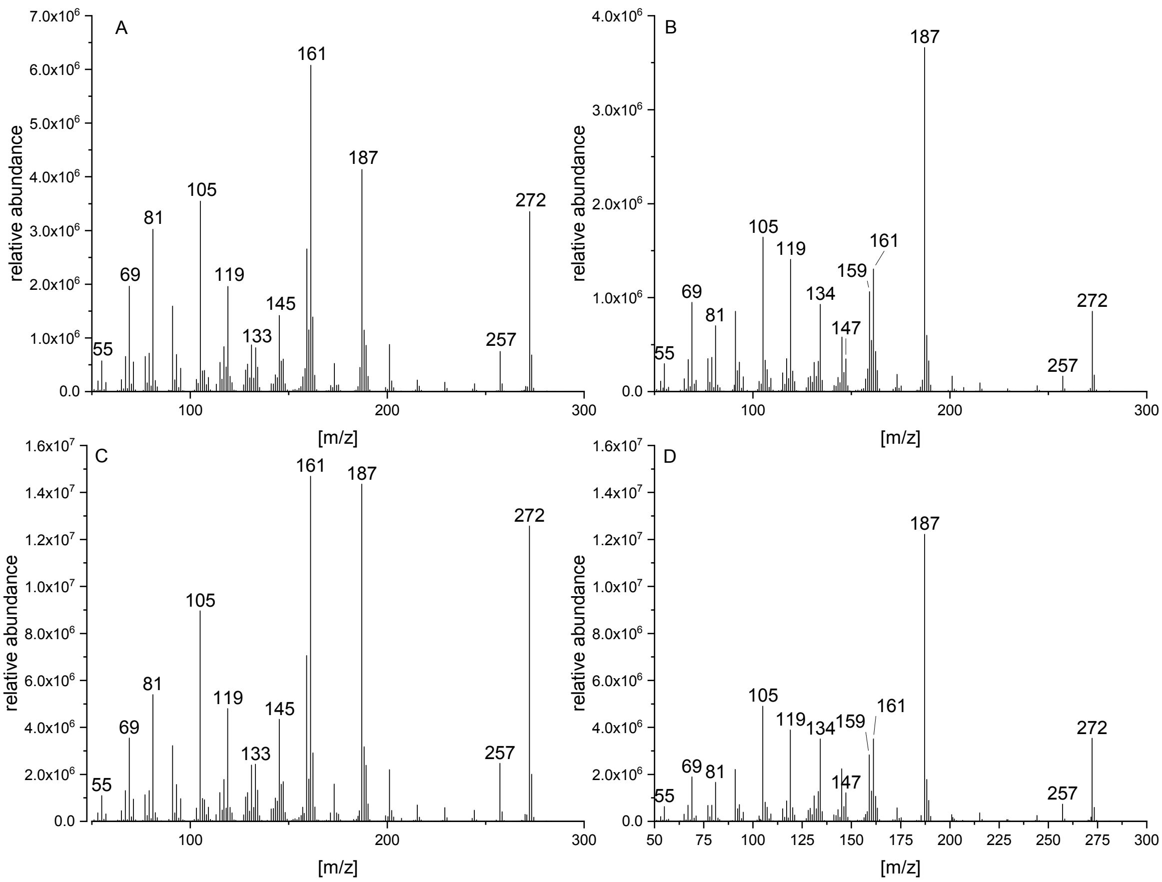
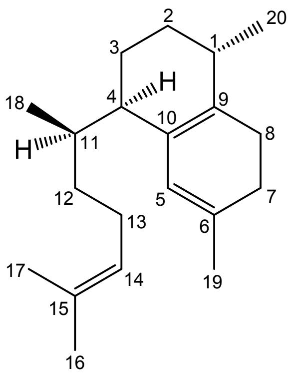


Figure S 6: Comparison of GC-MS spectra of isoelisabethatriene A (A and C) and B (B and D) from wild-type HpS and HpS M75L; (A) and (B): wt HpS; (C) and (D): HpS M75L. The isoforms produced in different enzyme variants show identical fragmentation patterns.

Table S 1: NMR data of isoelisabethatriene A (HpS wild-type); Standardised on solvent (benzene-d_6) peak: ^1H = 7.16ppm, ^{13}C = 128.06 ppm

C	Art	^1H [ppm]	Multiplet	Integral	^{13}C [ppm]
1	CH	2.18	m	1	34.07
2	CH_2	1.81 1.21	m m	1 1	31.87
3	CH_2	1.65 1.42	m m	1 1	21.17
4	CH	2.24	m	1	41.28
5	CH	5.78	s	1	122.77
6	C_q	-			132.33
7	CH_2	2.08 1.84	m m	1 1	29.22
8	CH_2	2.18 1.94	m m	1 1	27.50
9	C_q	-			133.38
10	C_q	-			130.31
11	CH	2.00	m	1	34.49
12	CH_2	1.44 1.37	m m	1 1	35.88
13	CH_2	2.10 2.07	m m	1 1	26.92
14	CH	5.27	m	1	125.57
15	C_q	-			130.94
16	CH_3	1.59	s	3	17.82
17	CH_3	1.71	s	3	25.90
18	CH_3	0.84	d	3	15.07
19	CH_3	1.76	s	3	23.36
20	CH_3	0.97	d	3	19.36



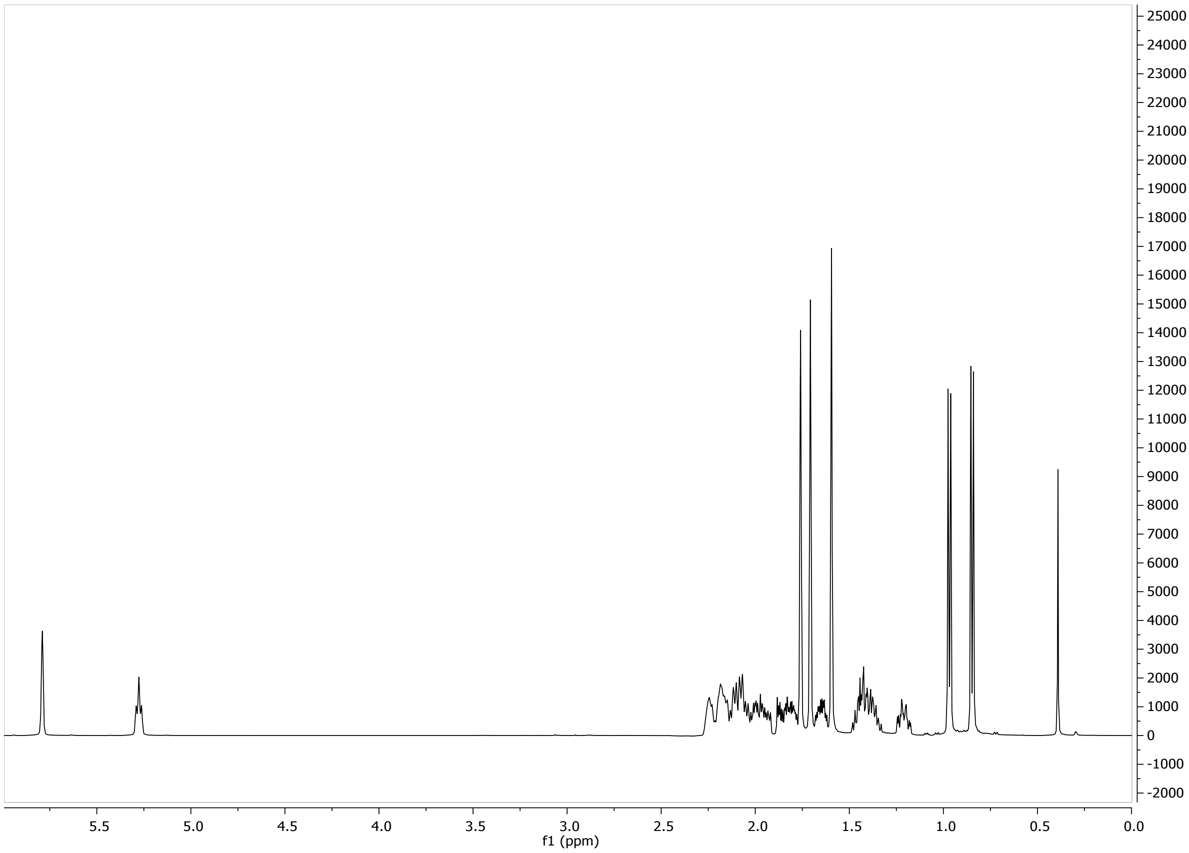


Figure S 7: ^{1}H NMR spectrum of isoelisabethatriene A produced by wild-type HpS.

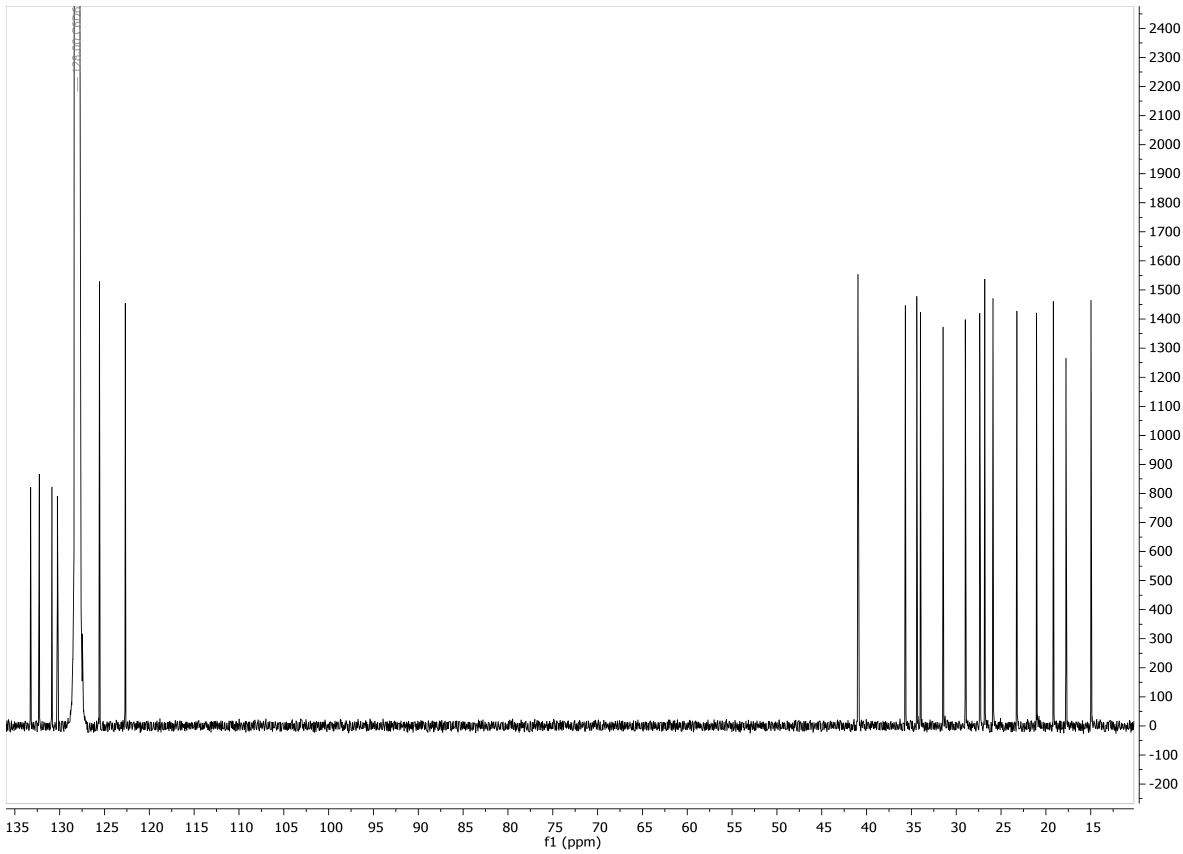


Figure S 8: ^{13}C NMR spectrum of isoelisabethatriene A produced by wild-type HpS.

Table S 2: Comparison of ^{13}C-NMR spectra of isoelisabethatriene A produced in HpS, HpS M75L and HpS M75F, respectively.

C	Literature[1]		Measurements conducted in C_6D_6		
	Art	WT δ ^{13}C (175 MHz)	WT δ ^{13}C (126MHz, Cryo)	M75L δ ^{13}C (75 MHz)	M75F δ ^{13}C (75 MHz)
1	CH	34.07	34.0	34.0	34.0
2	CH_2	31.87	31.5	31.5	31.5
3	CH_2	21.17	21.0	21.0	21.0
4	CH	41.28	41.0	41.0	41.0
5	CH	122.77	122.7	122.7	122.7
6	C_q	132.33	132.3	132.3	132.3
7	CH_2	29.22	29.0	29.0	29.0
8	CH_2	27.50	27.4	27.4	27.4
9	C_q	133.38	133.2	133.2	133.2
10	C_q	130.31	130.3	130.3	130.3
11	CH	34.49	34.4	34.4	34.4
12	CH_2	35.88	35.7	35.7	35.7
13	CH_2	26.92	26.8	26.8	26.8
14	CH	125.57	125.6	125.6	125.6
15	C_q	130.94	130.9	130.9	130.9
16	CH_3	17.82	17.8	17.8	17.8
17	CH_3	25.90	25.9	25.9	25.9
18	CH_3	15.07	15.0	15.0	15.0
19	CH_3	23.36	23.2	23.2	23.2
20	CH_3	19.36	19.2	19.2	19.2

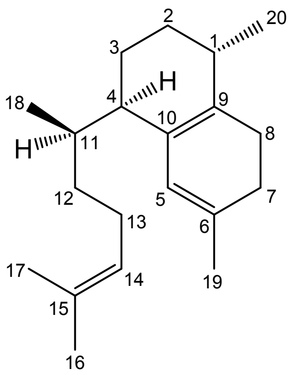
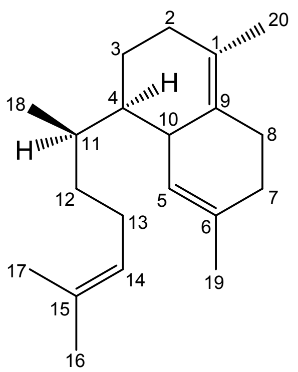


Table S 3: NMR data of isoelisabethatriene B (wild-type) Standardised on solvent (benzene-d_6) peak: ^1H = 7.16 ppm, ^{13}C = 128.06 ppm.

C	Art	^1H [ppm]	Multiplet	Integral	^{13}C [ppm]
1	C_q	-			130.20
2	CH_2	1.91 2.00	m m	1 1	32.73
3	CH_2	1.53 1.20	m m	1 1	21.63
4	CH	1.34	m	1	43.33
5	CH	5.61	m	1	124.68
6	C_q	-			133.77
7	CH_2	1.91 1.88	m m	1 1	32.09
8	CH_2	2.70	m	2	27.00
9	C_q	-			123.95
10	CH	2.67	m	1	39.61
11	CH	1.96	m	1	31.60
12	CH_2	1.37	m	2	36.01
13	CH_2	2.04	m	2	26.36
14	CH	5.22	m	1	125.29
15	C_q	-			130.69
16	CH_3	1.56	s	3	17.58
17	CH_3	1.69	s	3	25.53
18	CH_3	0.82	d	3	13.96
19	CH_3	1.66	s	3	23.57
20	CH_3	1.65	s	3	18.40



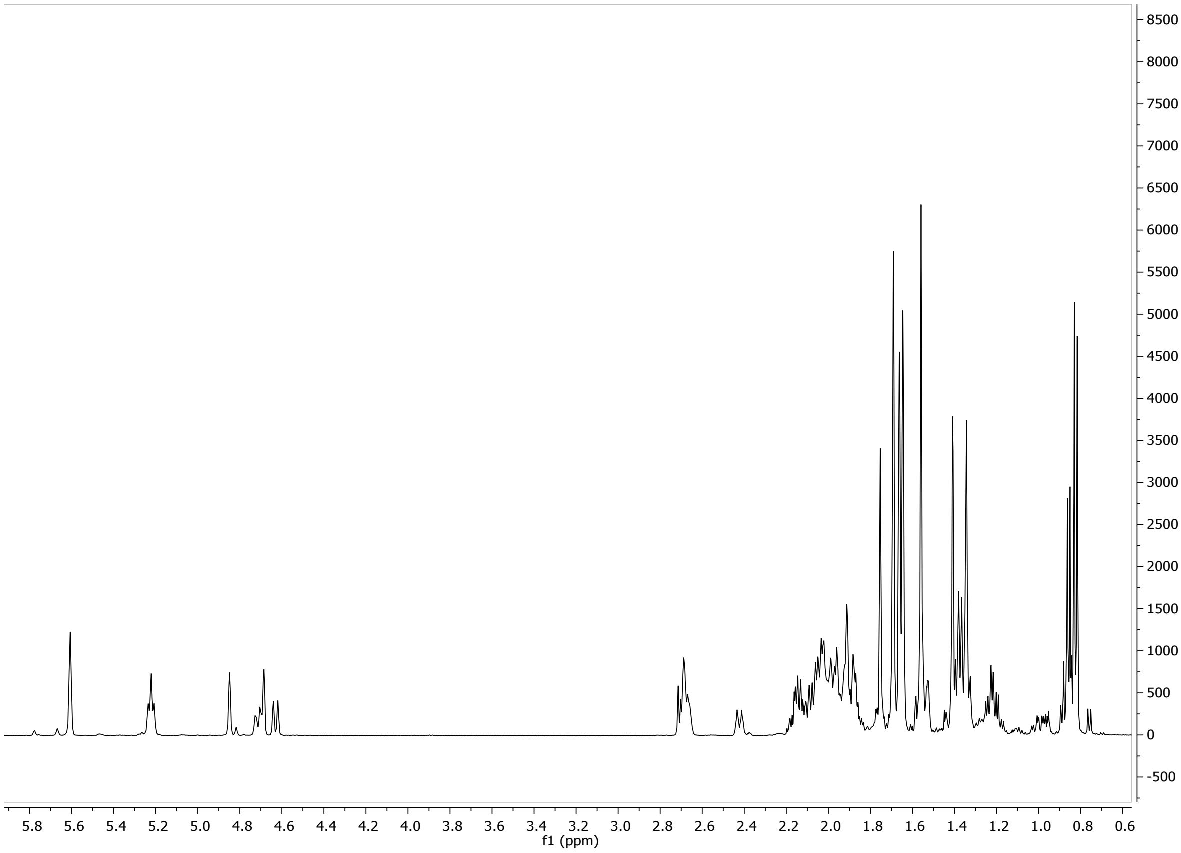


Figure S 9: ^{1}H NMR spectrum of isoelisabethatriene B produced by wild-type HpS.

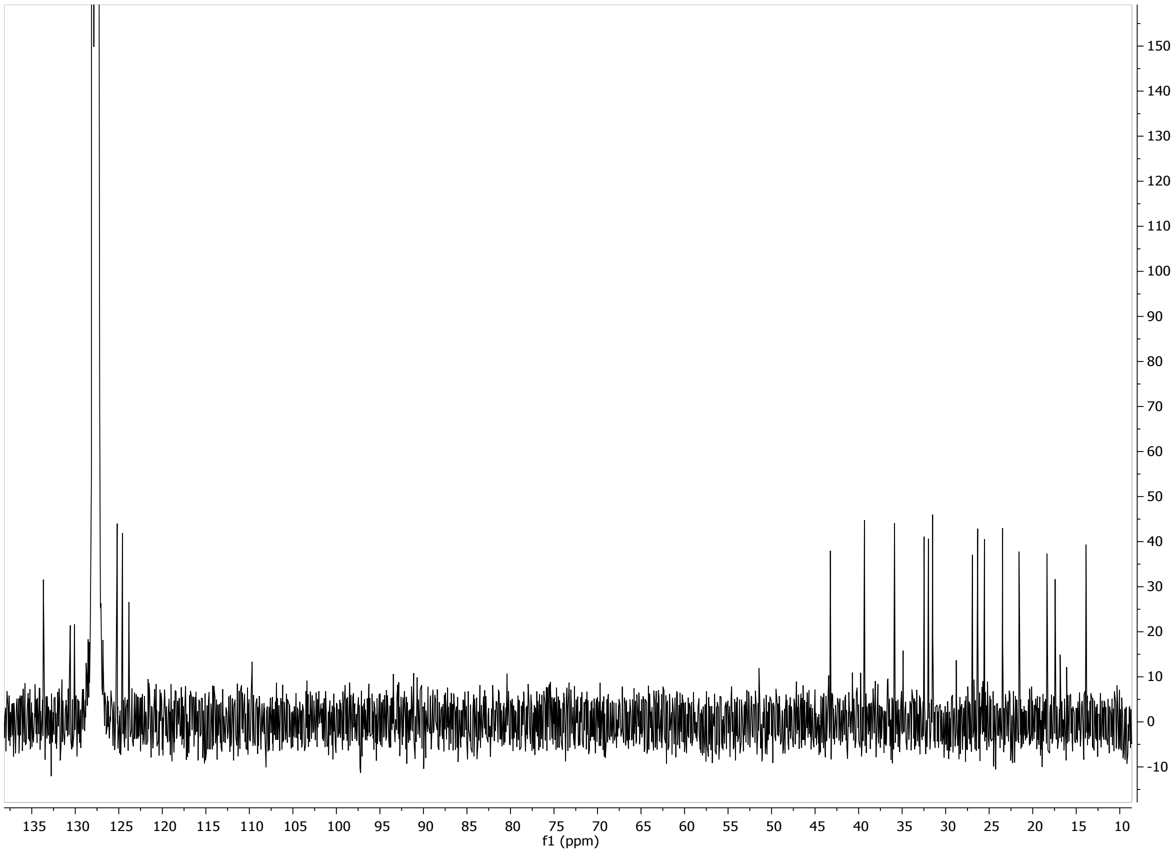


Figure S 10: ^{13}C NMR spectrum of isoelisabethatriene B produced by wild-type HpS.

Table S 4: Comparison of ^{13}C-NMR spectra of isoelisabethatriene B produced in HpS, HpS M75L and HpS M75F, respectively

Literature ($CDCl_3$)[2]			Measurements conducted in C_6D_6		
Nr. C	Art	δ ^{13}C	WT δ ^{13}C (75 MHz)	M75F δ ^{13}C (101 MHz)	M75L δ ^{13}C (75 MHz)
1	C_q	130.0	130.20	130.5	130.5
2	CH_2	32.4	32.73	32.9	32.9
3	CH_2	21.5	21.63	22.0	22.0
4	CH	43.2	43.33	43.6	43.6
5	CH	124.4	124.68	124.2	124.2
6	C_q	134.2	133.77	134.1	134.1
7	CH_2	31.9	32.09	32.4	32.4
8	CH_2	26.7	27.00	27.3	27.3
9	C_q	124.5	123.95	125.0	125.0
10	CH	39.2	39.61	39.7	39.7
11	CH	31.5	31.60	31.9	31.9
12	CH_2	35.8	36.01	36.3	36.3
13	CH_2	26.2	26.36	26.7	26.7
14	CH	124.9	125.29	125.6	125.6
15	C_q	131.2	130.69	131.0	131.0
16	CH_3	18.0	17.58	17.8	17.8
17	CH_3	25.7	25.53	25.9	25.9
18	CH_3	13.9	13.96	14.3	14.3
19	CH_3	23.5	23.57	23.9	23.9
20	CH_3	18.5	18.40	18.8	18.8

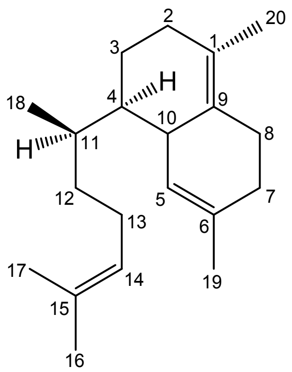


Figure S 11: Alignment of secondary structure elements of CotB2 and HpS. Red: α-helices; blue: β-sheet. The secondary structure of both diterpene synthases match in the positions of their respective α-helices (HHPred[3] and Ali2D using PSIPRED[4] and MEMSAT[5]).

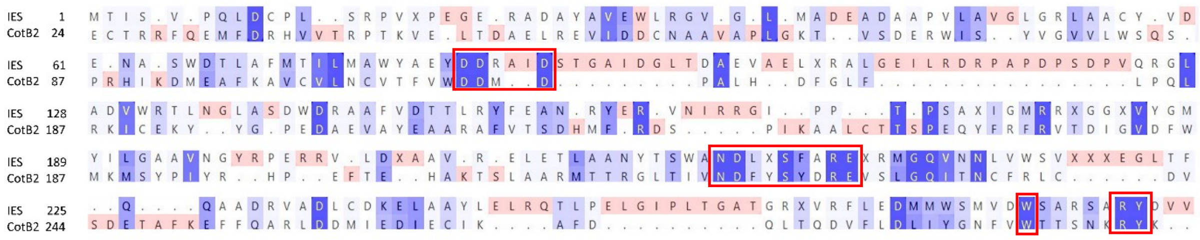


Figure S 12: Structure alignment CotB2 and HpS. Identical amino acids (AA) are highlighted in deep purple, similar AA are marked in light purple, AA with no corresponding match coloured in pink; highly conserved motifs are framed by a red box.

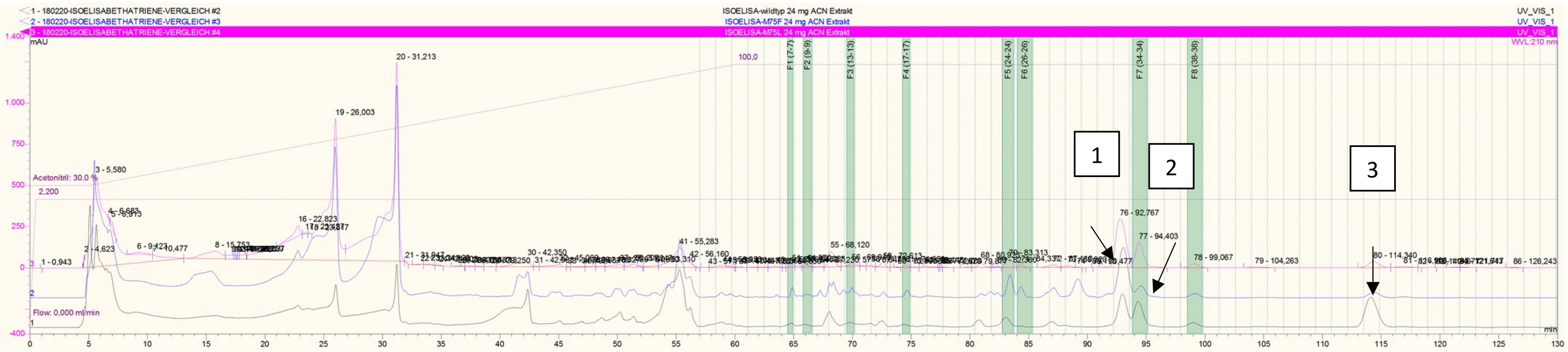


Figure S 13: Evaluation of the HpS' product spectrum (black: wild-type; pink: M75L and blue: M75F) with corresponding MS spectra. Whole spectrum of crude *E. coli* extract. (1) isoelisabethatriene B; (2) isoelisabethatriene A; (3) hydropyrene.

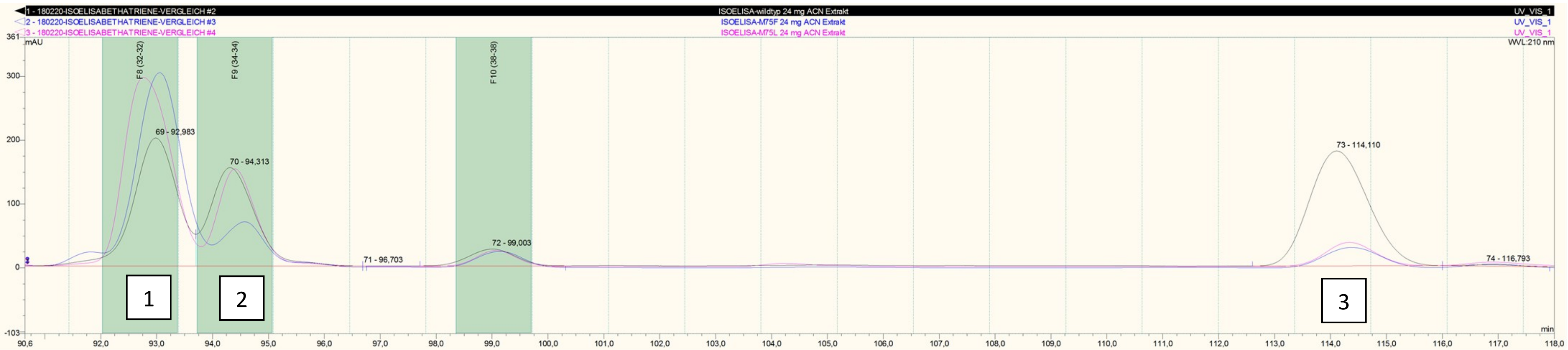


Figure S 14: Zoom into figure S 14 to enhance comparison of main products: (1) isoelisabethatriene B; (2) isoelisabethatriene A; (3) hydropyrene. Black: wild-type HpS; blue: HpS M75F; pink: HpS M75L.

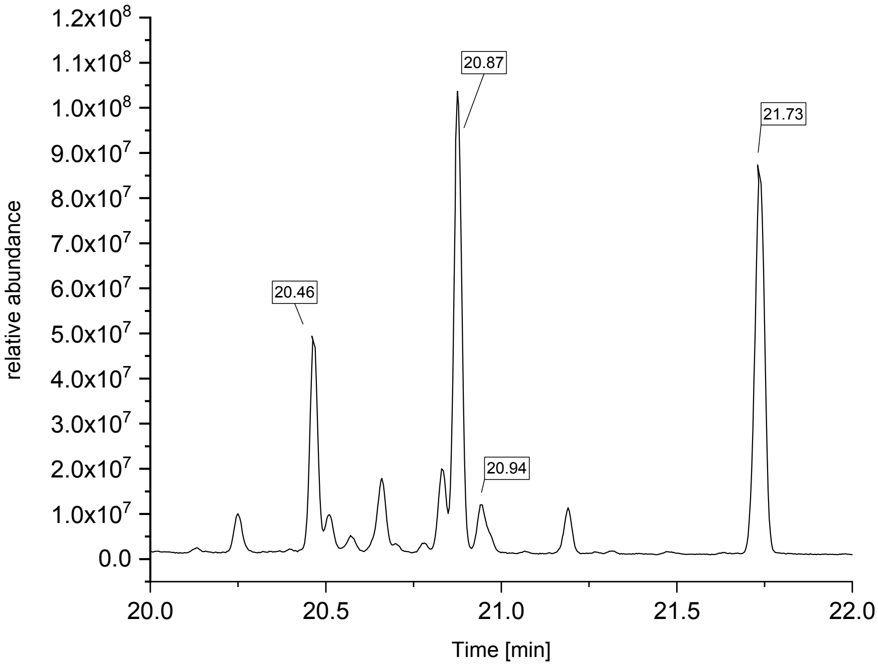


Figure S 15: GC chromatogram after 1 month storage of extract in hexane gained from HpS M75F. Analysis of compound with retention time 20.94 min (Figure S 16) showed spontaneous formation of erogorgiaene.

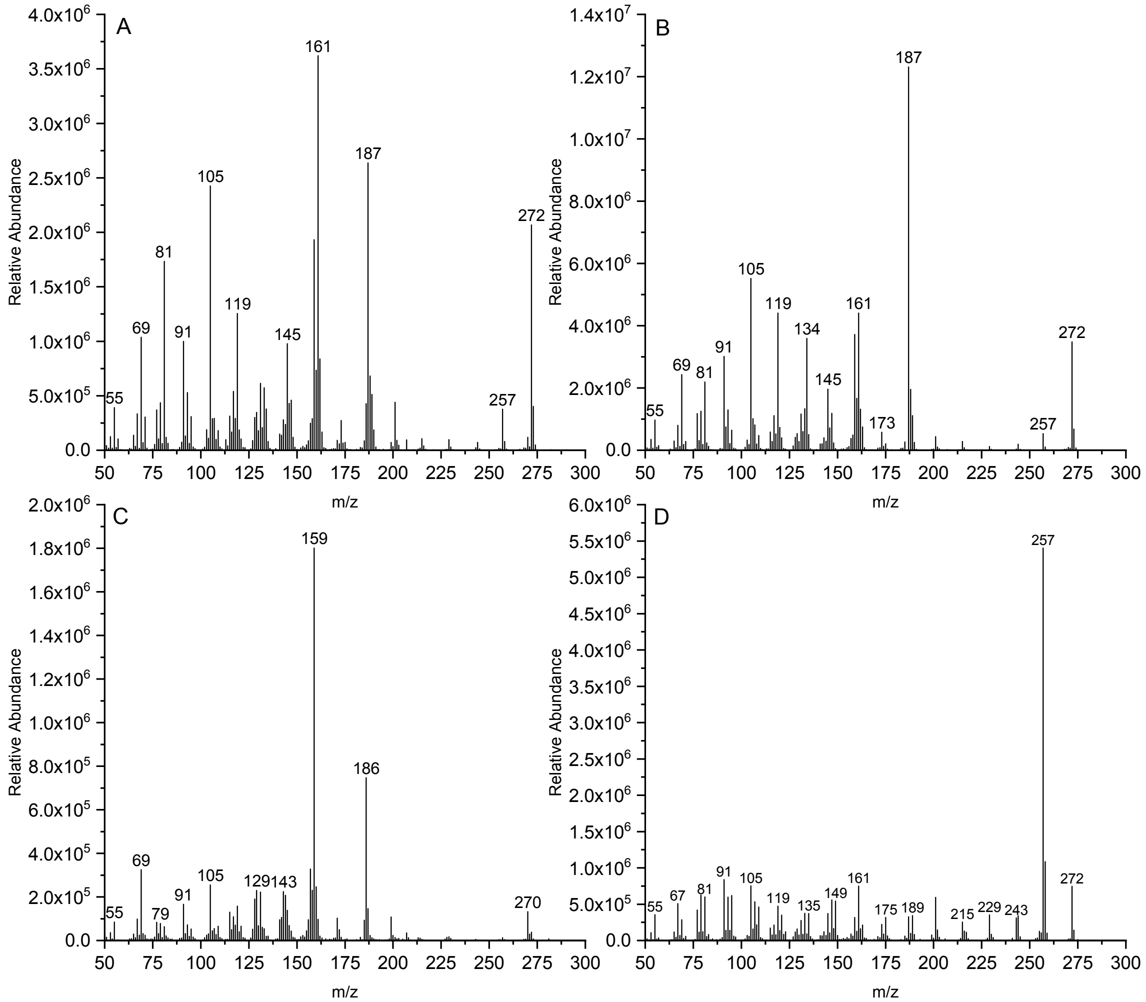


Figure S 16: GC-MS spectra analysis of compounds highlighted in figure S 16. (A) 20.46 min: isoelisabethatriene A, (B) 20.87 min: isoelisabethatriene B, (C) 20.94 min: erogorgiaene, (D) 21.73 min: hydropyrene

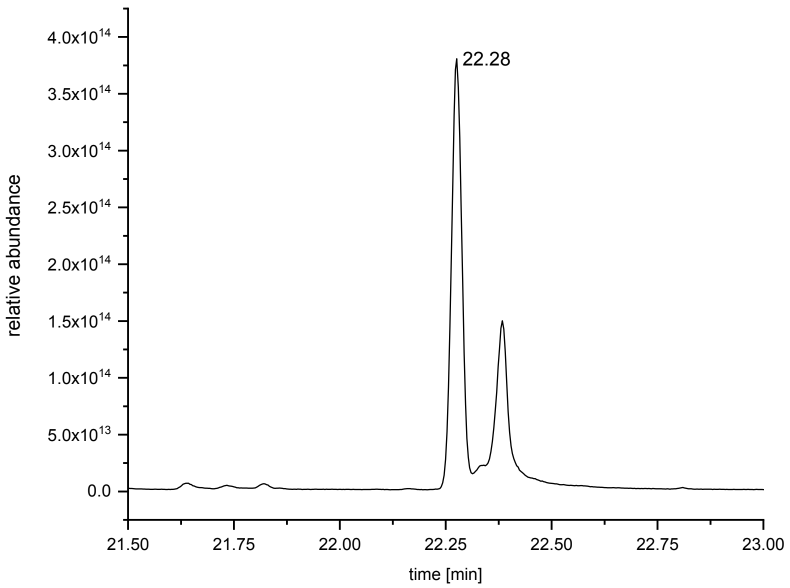


Figure S 17: GC chromatogram of mono hydroxylated diterpene (retention time 22.28 min) identified in HpS M75L extract.

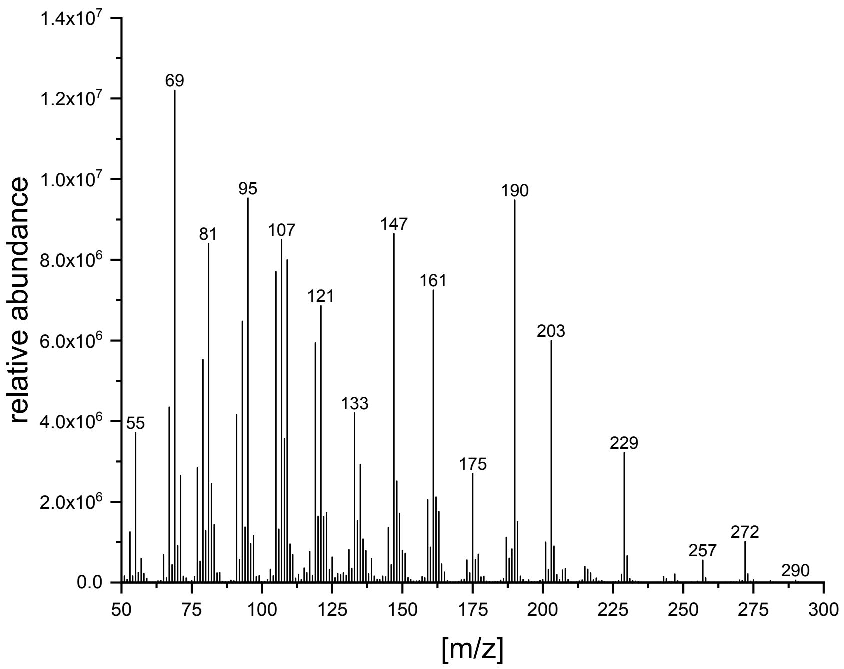


Figure S 18: GC-MS spectrum of hydroxylated diterpene (retention time 22.28 min) identified in HpS M75L extract with respective parent ion mass of 290.

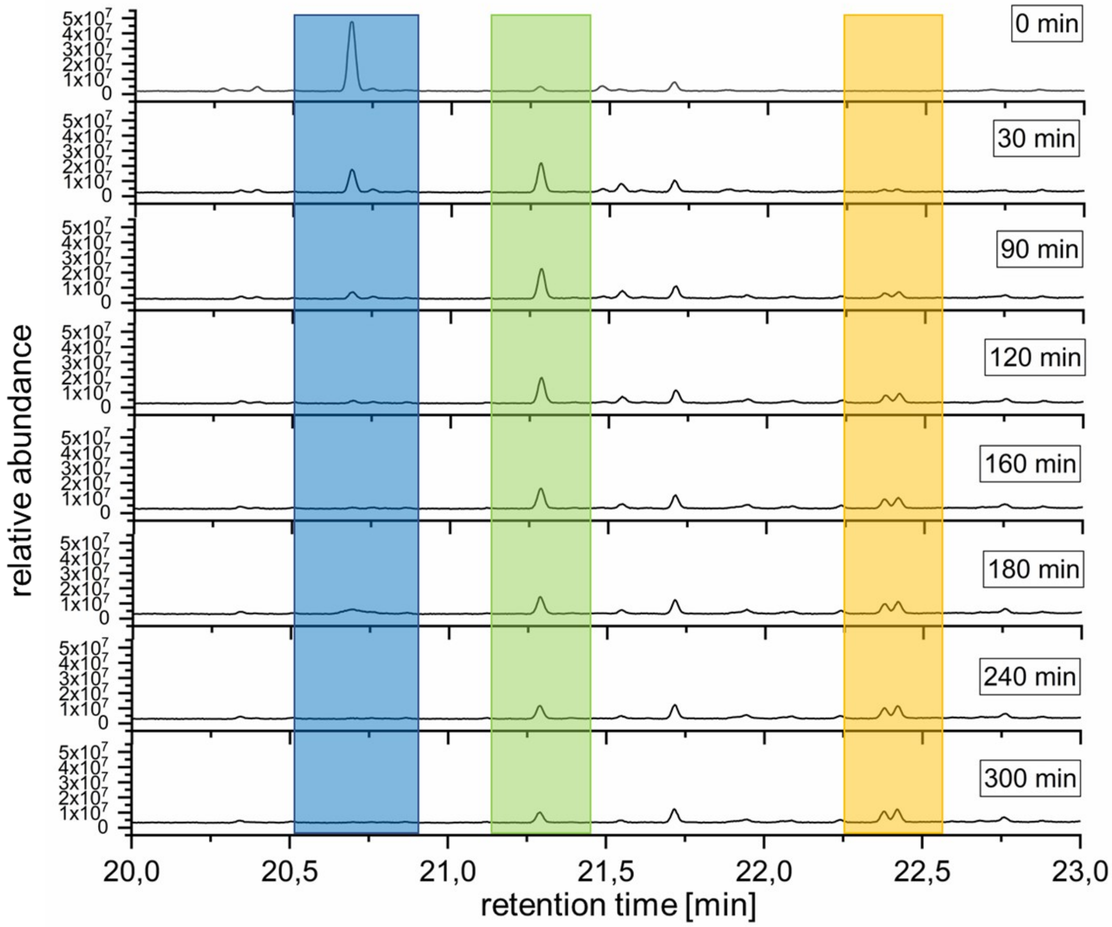


Figure S 19: Time resolved monitoring of CalB mediated conversion of isoelisabethatriene B. Blue: isoelisabethatriene B; green: isoelisabethatriene B monoepoxide; yellow: isoelisabethatriene B diepoxides.

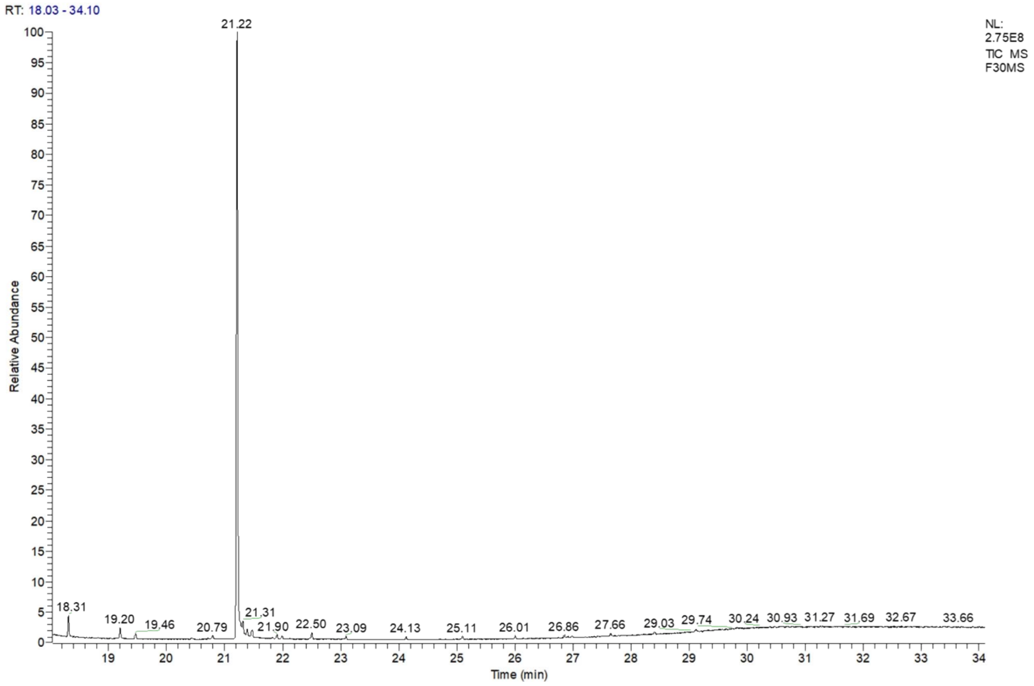


Figure S 20: GC-MS-chromatogram – isoelisabethatriene B monoepoxide; Total Ion Count (TIC) Recording started at 18 min.

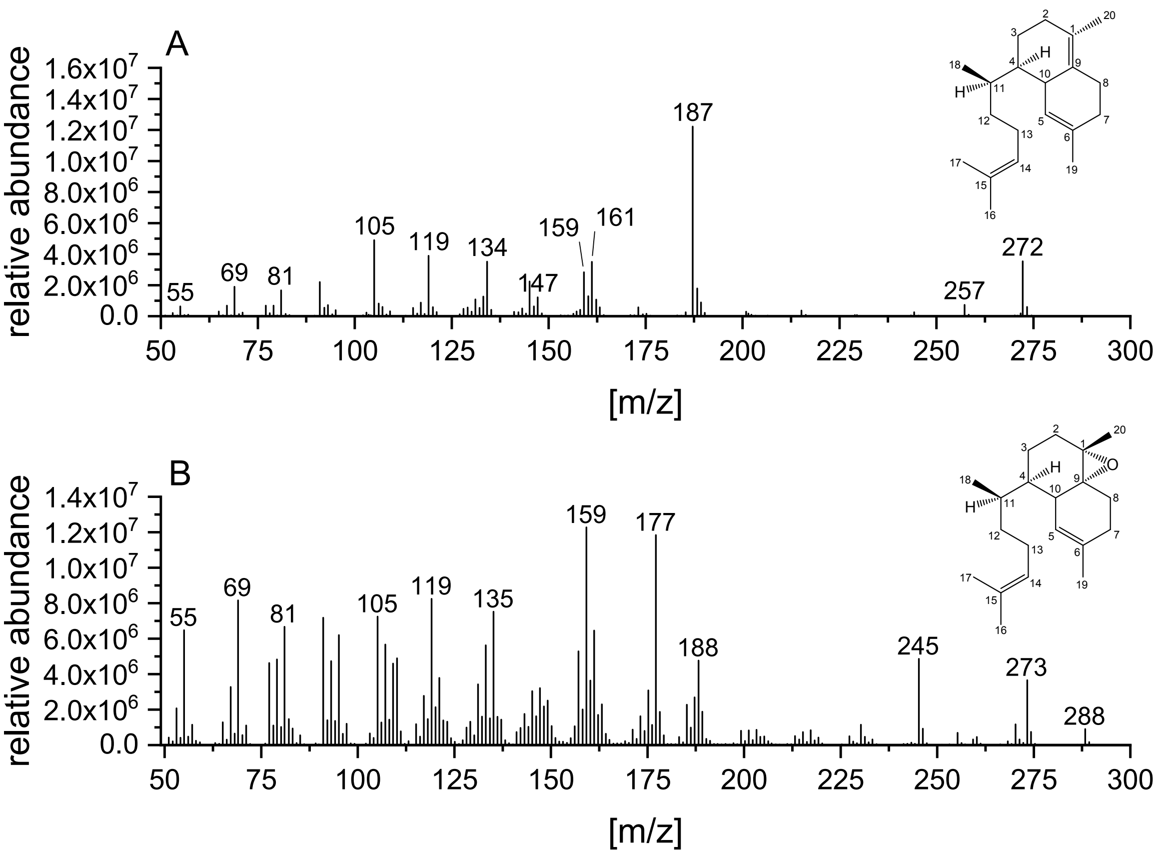


Figure S 21: Comparison of GC-MS spectra of isoelisabethatriene B (A) and the monoepoxide product 1*R*-epoxy-5,14- elisabethadiene (B), respectively.

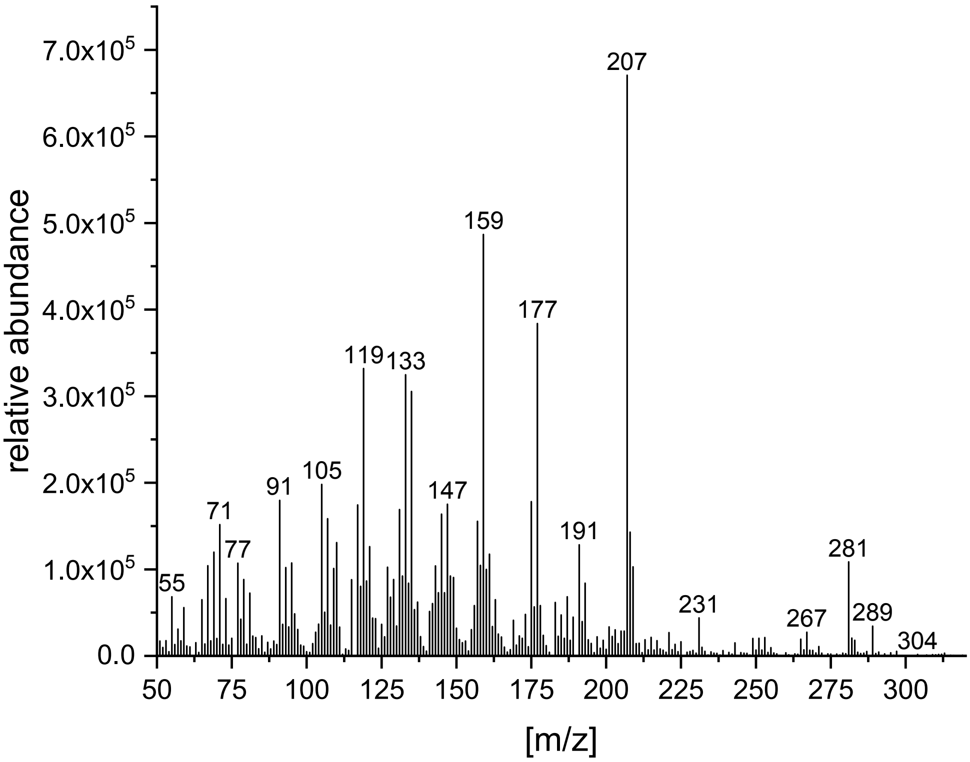
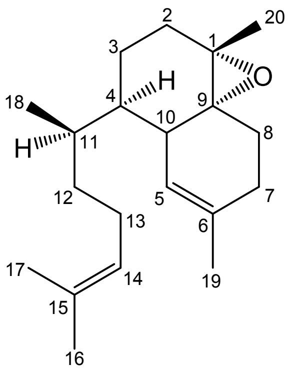


Figure S 22: GC MS spectrum of putative isoelisabethatriene B derived diepoxide with parent ion mass m/z 304; Retention time R_t = 22.42 min.

Table S 5: NMR data of 1*R*-epoxy-5,14- elisabethadiene; Standardised on solvent (benzene-d_6) peak: ^1H = 7.16 ppm, ^{13}C = 128.06 ppm.

Position	C [ppm]	H [ppm]	Multiplet	Integral	J-Coupling Constant [Hz]	COSY	HMBC	HSQC	NOESY
1	62.66	-					20		
2a	32.68	1.42	m	1		2b	20	yes	2b; 20
2b	32.68	1.87	m	1		3a; 2a	20	yes	2a
3a	18.02	1.60	m	1		3b; 4		yes	3b
3b	18.02	1.09	m	1		2b; 3a		yes	3a
4	44.83	1.03	m	1		10	18	yes	2a; 11; 12
5	125.89	5.42	s	1		10; 19	19	yes	8b;10; 19
6	132.90	-					19		
7a	29.87	1.74	m	1		8a	5	yes	
7b	29.87	1.94	m	1		8b		yes	
8a	29.68	1.97	m	1		7a		yes	5; 20
8b	29.68	1.66	m	1		7b		yes	10
9	64.21	-					5; 8; 20		
10	38.83	2.47	d	1	10.04	4; 5		yes	5; 18
11	33.33	1.67	m	1		12; 18	12. 18	yes	5; 18
12	35.85	1.29	m	2		11; 13	13; 18	yes	4; 14; 18
13	26.63	2.02	m	1		12; 14	14	yes	11; 14
14	125.44	5.20	t	1	7.14	13; 16;17	13; 16; 17	yes	12; 13; 16
15	131.04	-					16; 17		
16	25.98	1.69	s	3		14	15; 17	yes	14; 18
17	17.82	1.56	s	3		14	15; 16	yes	
18	14.67	0.81	d	3	6.83			yes	3b; 8b; 10; 11; 12
19	23.61	1.62	s	3		5	5; 6	yes	5
20	19.0	1.22	s	3			1; 2; 9	yes	2b;8b



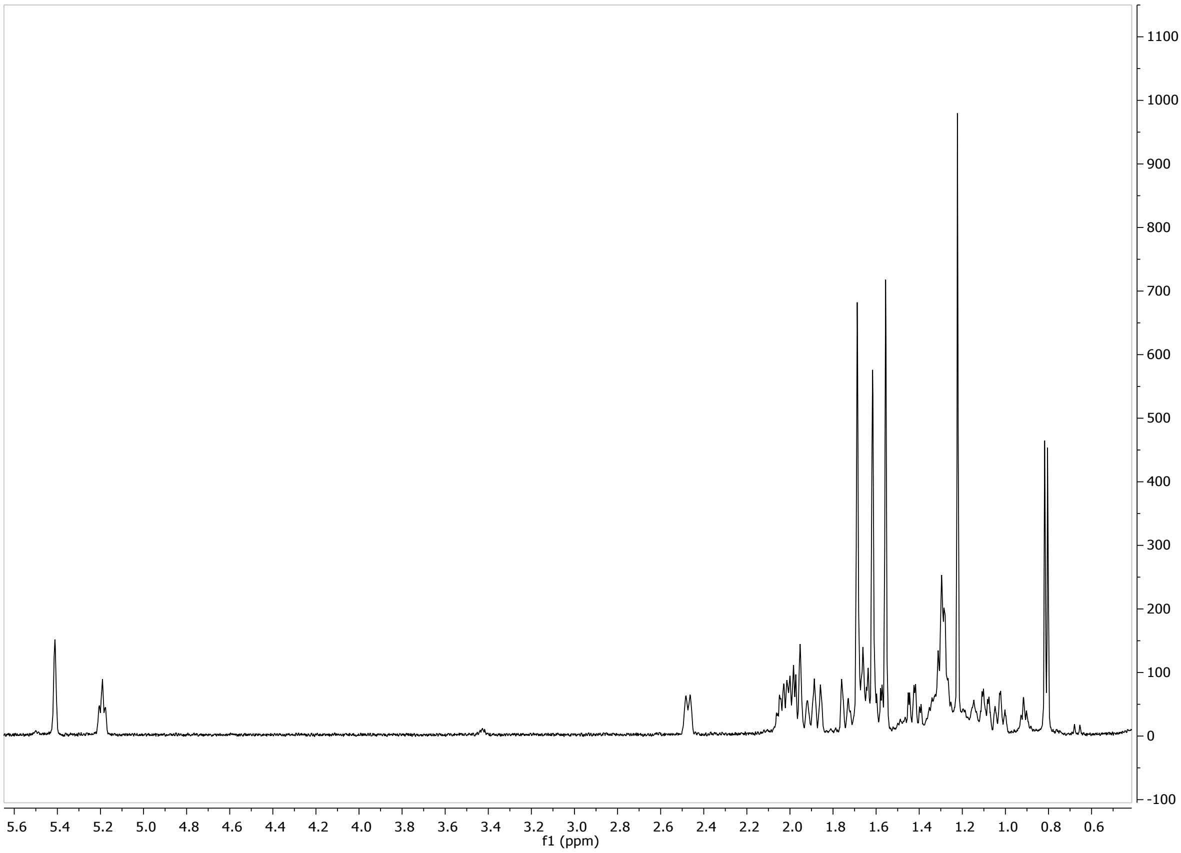


Figure S 23: ^1H NMR Spectrum of 1*R*-epoxy-5,14- elisabethadiene.

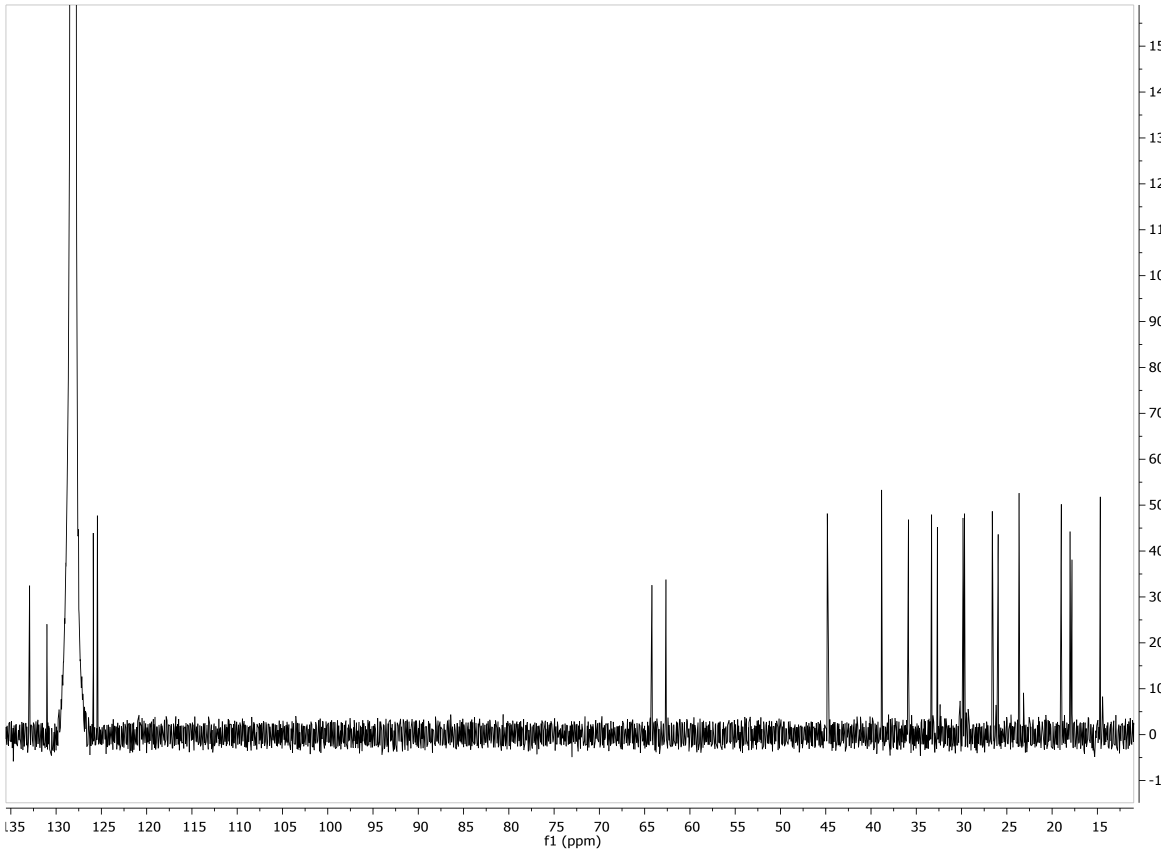


Figure S 24: ^{13}C NMR Spectrum of 1*R*-epoxy-5,14- elisabethadiene.

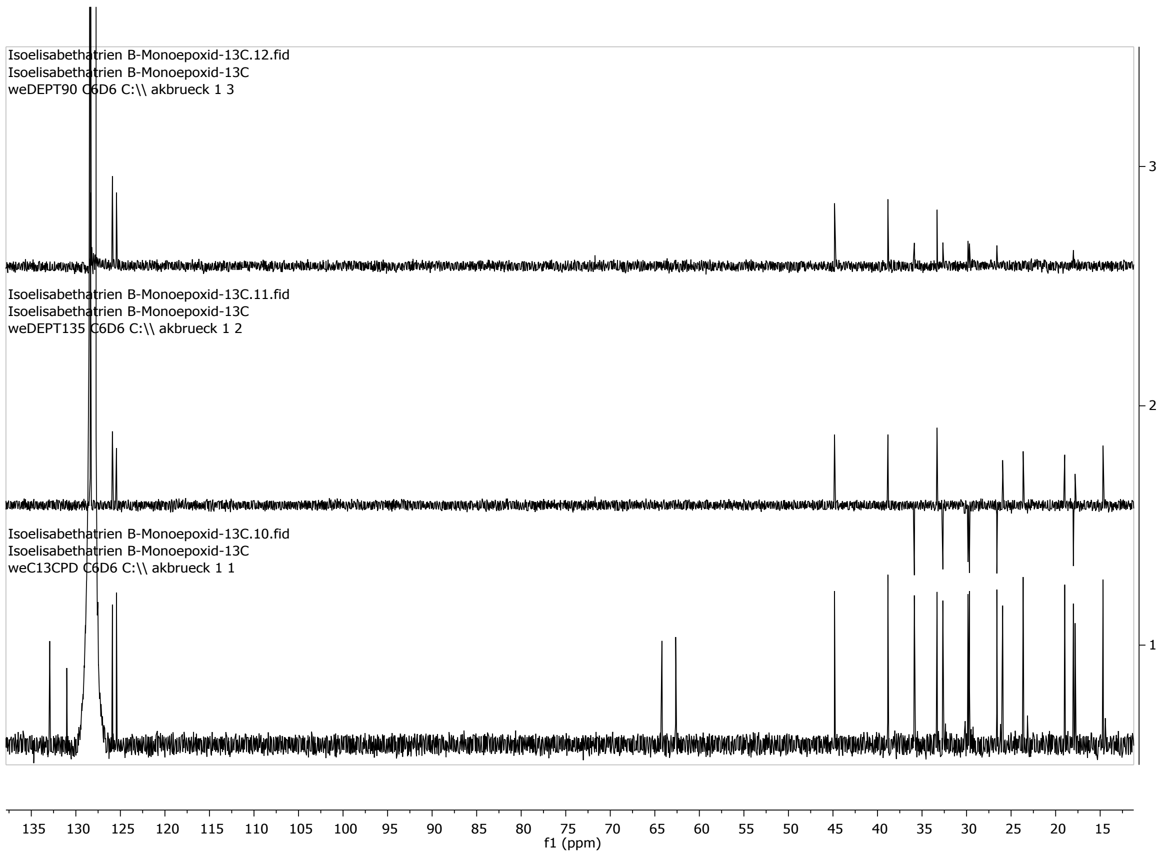


Figure S 25: ^{13}C DEPT spectra of 1*R*-epoxy-5,14- elisabethadiene.

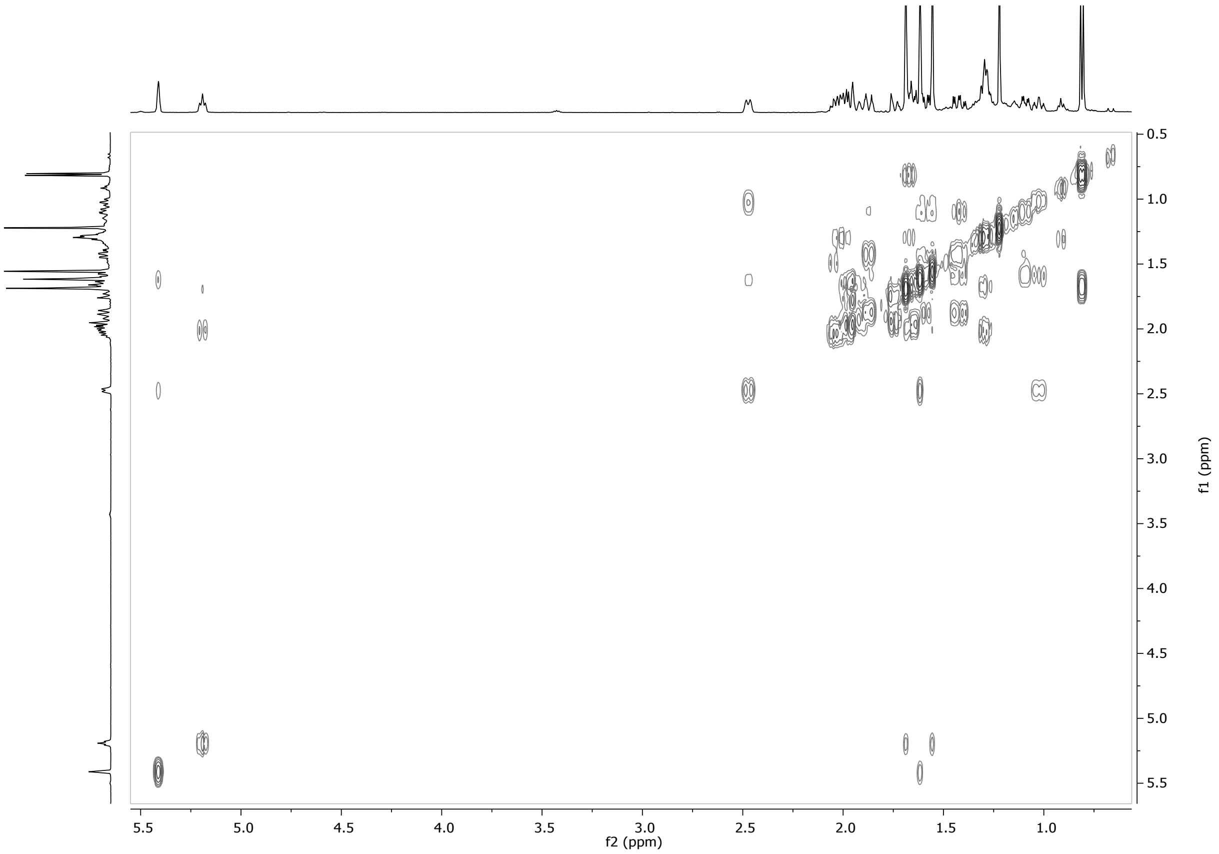


Figure S 26: ^{1}H-^{1}H COSY spectrum of 1*R*-epoxy-5,14- elisabethadiene.

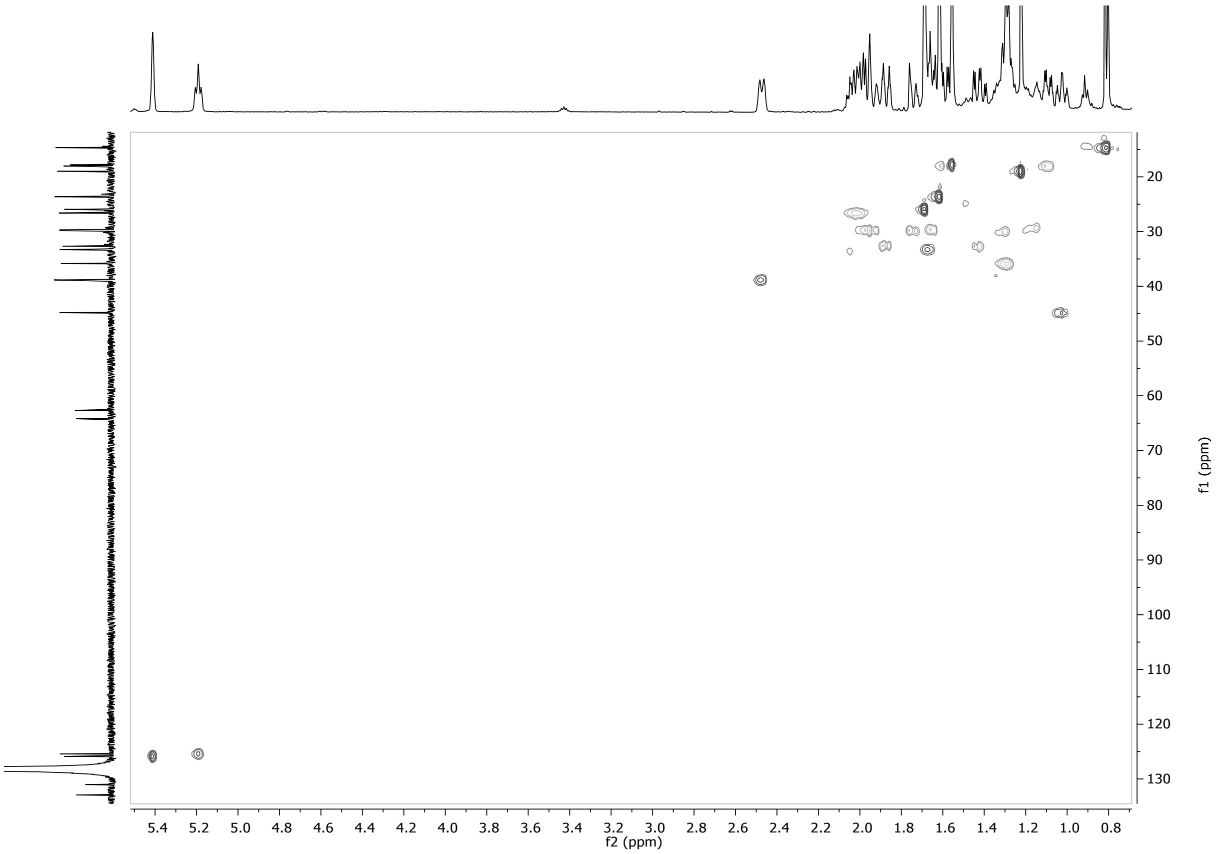


Figure S 27: ^{1}H-^{13}C HSQC spectrum of 1*R*-epoxy-5,14- elisabethadiene.

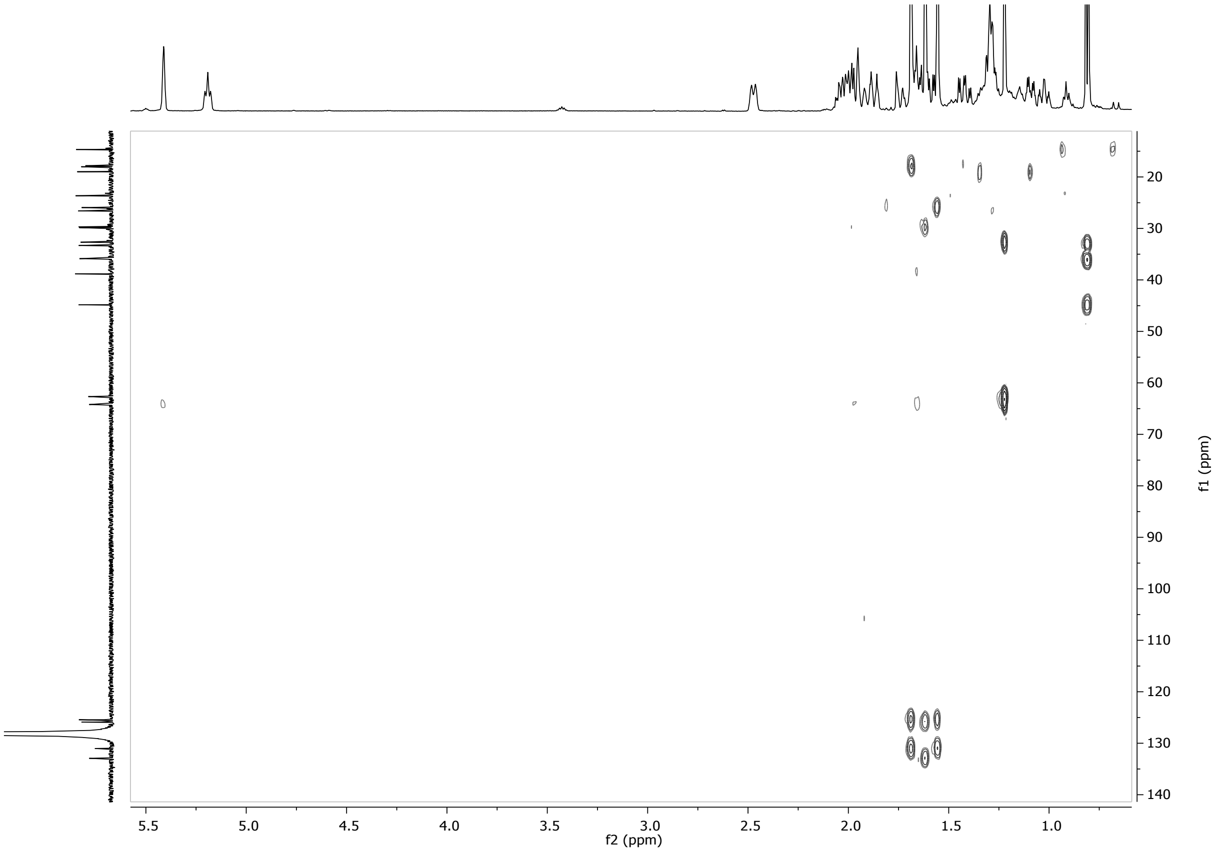


Figure S 28: ^1H-^{13}C HMBC spectrum 1*R*-epoxy-5,14- elisabethadiene.

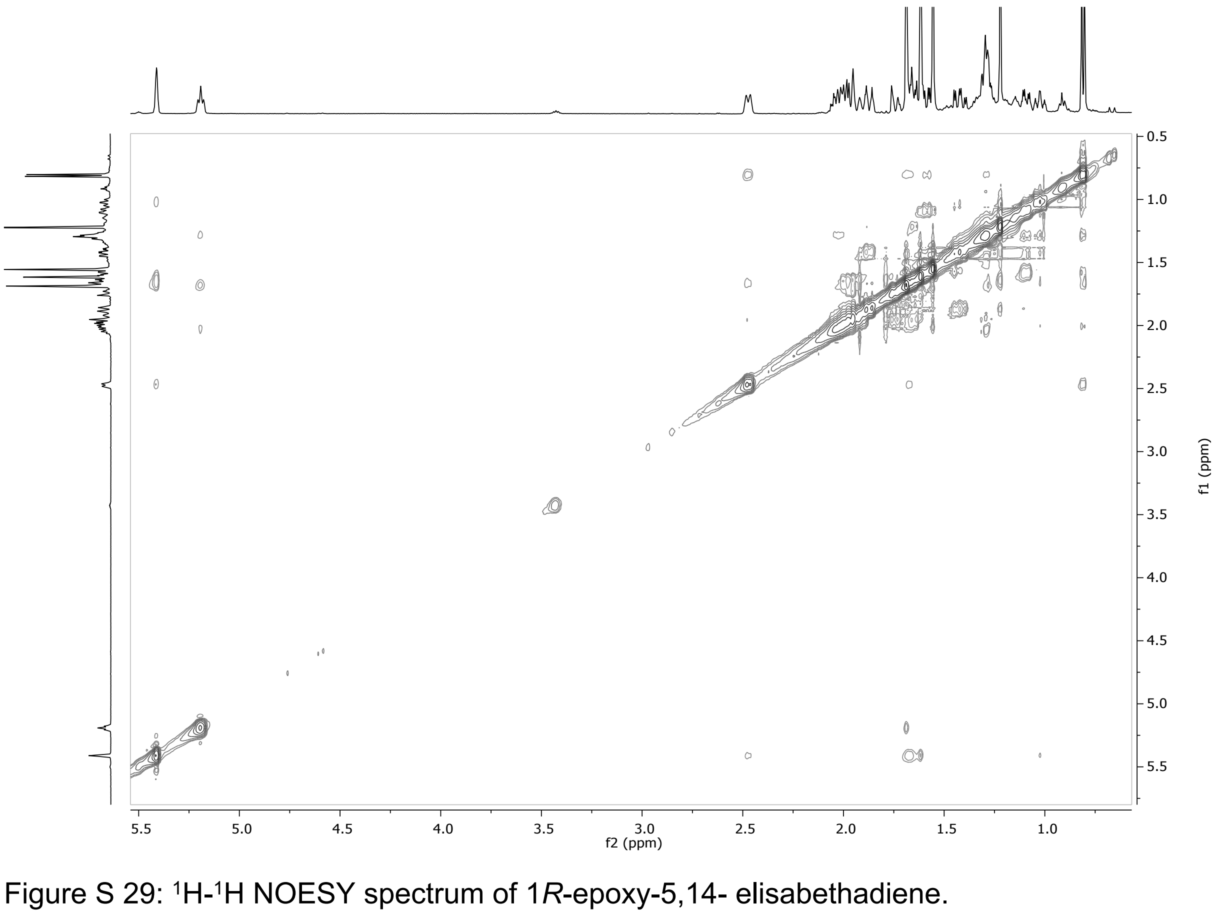


Figure S 29: ^1H-^1H NOESY spectrum of 1*R*-epoxy-5,14- elisabethadiene.

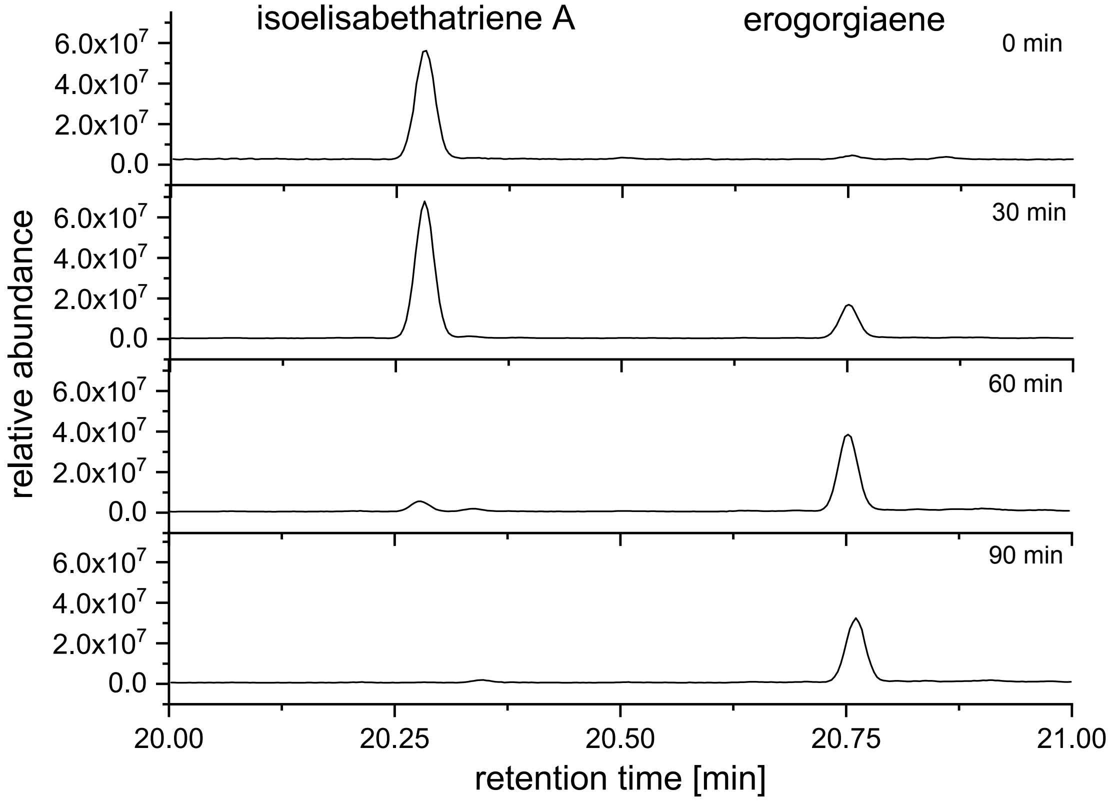


Figure S 30: Time resolved erogorgiaene formation upon treatment of isoelisabethatriene A with CalB (0 min to 90 min).

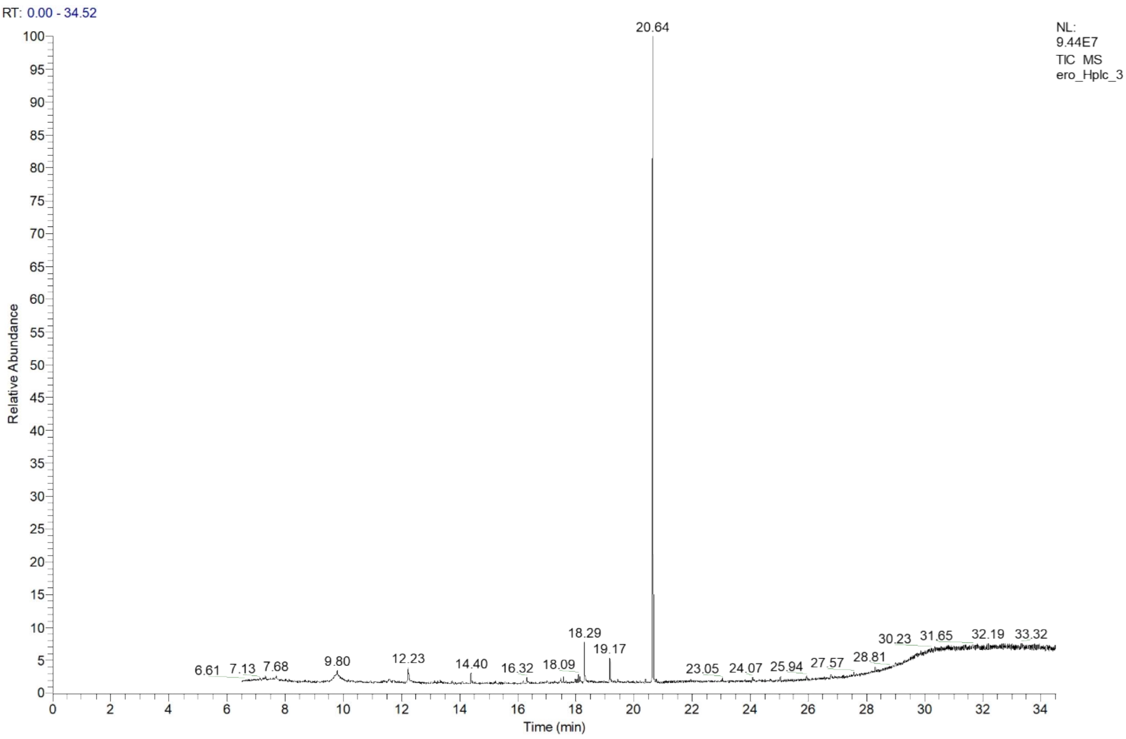


Figure S 31: GC-MS – chromatogram of erogorgiaene; Total ion Count recording started at 6 min.

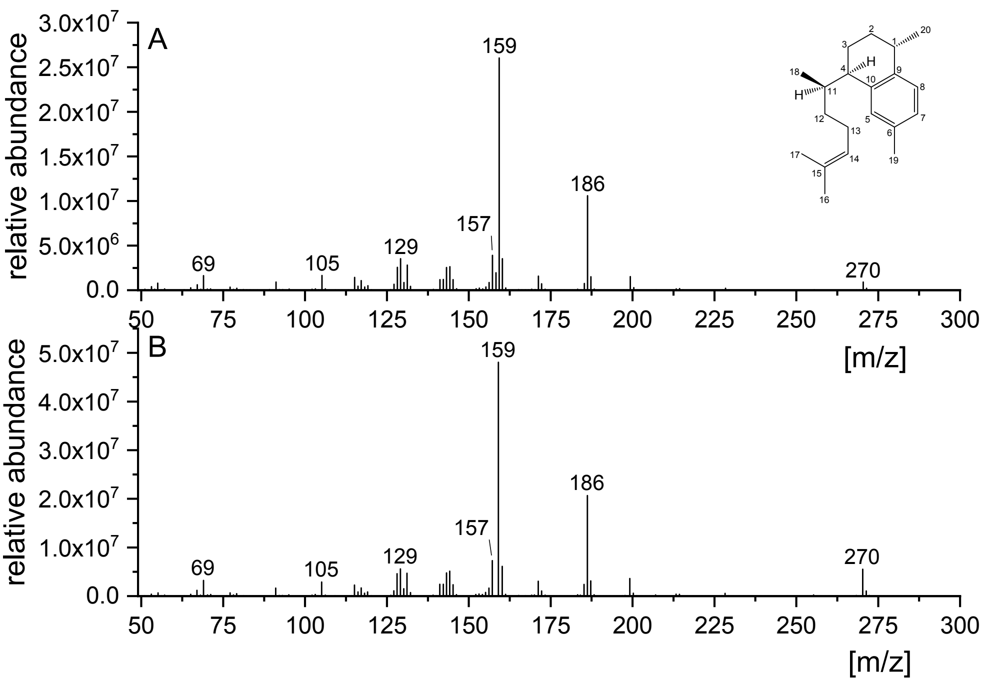
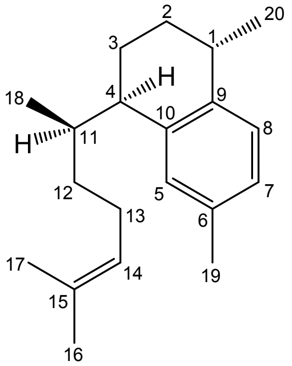


Figure S 32: Comparison of GC-MS spectra of erogorgiaene. (A) Derived from Lipase-mediated isoelisabethatriene A conversion. (B) Originating from a coral extract.

Table S 6: NMR data of erogorgiaene; Standardised on solvent ($CDCl_3$) peak: ^{1}H = 7.26 ppm, ^{13}C = 77.2 ppm.

Position	C [ppm]	H [ppm]	Multiplet	Integral	J-Coupling Constant [Hz]	COSY	HMBC	HSQC	NOESY
1	32.95	2.72	m	1	10.9	2b; 20	20	yes	2b; 3b; 8; 20
2a	31.95	1.34	m	1		2b	20	yes	2b
2b	31.95	1.94	m	1		1; 2a		yes	2a
3a	21.68	1.82	m	1		3b; 4		yes	3b
3b	21.68	1.55	m	1		3a		yes	3a
4	41.64	2.87	m	1	10.2	3a; 18	18	yes	2a; 3a; 5; 11; 12a
5	128.24	7.02	s	1		19	7; 19	yes	11
6	134.85	-					8; 19		
7	126.11	6.94	d	1	8.01	8;19	5; 19	yes	
8	126.55	7.13	d	1	8.01	7		yes	20
9	140.55	-					8; 20		
10	140.07	-					5; 8		
11	37.11	2.14	m	1		4; 12a; 18	18	yes	4; 5; 18
12a	35.38	1.44	m	1		11	18	yes	
12b	35.38	1.35	m	1		13		yes	
13a	26.46	2.08	m	1		12b; 14		yes	
13b	26.46	2.00		1				yes	
14	125.02	5.17	m	1	7.0	13; 16;17	16; 17	yes	12ab; 13ab; 17
15	131.45	-					16; 17		
16	25.98	1.72	s	3		14	15; 17	yes	
17	17.88	1.64	s	3		14	15; 16	yes	
18	14.67	0.64	d	3	6.83			yes	11; 2b; 3b
19	21.34	2.30	s	3		5; 7	5; 7	yes	
20	22.01	1.26	d	3	6.92	1		yes	1; 8



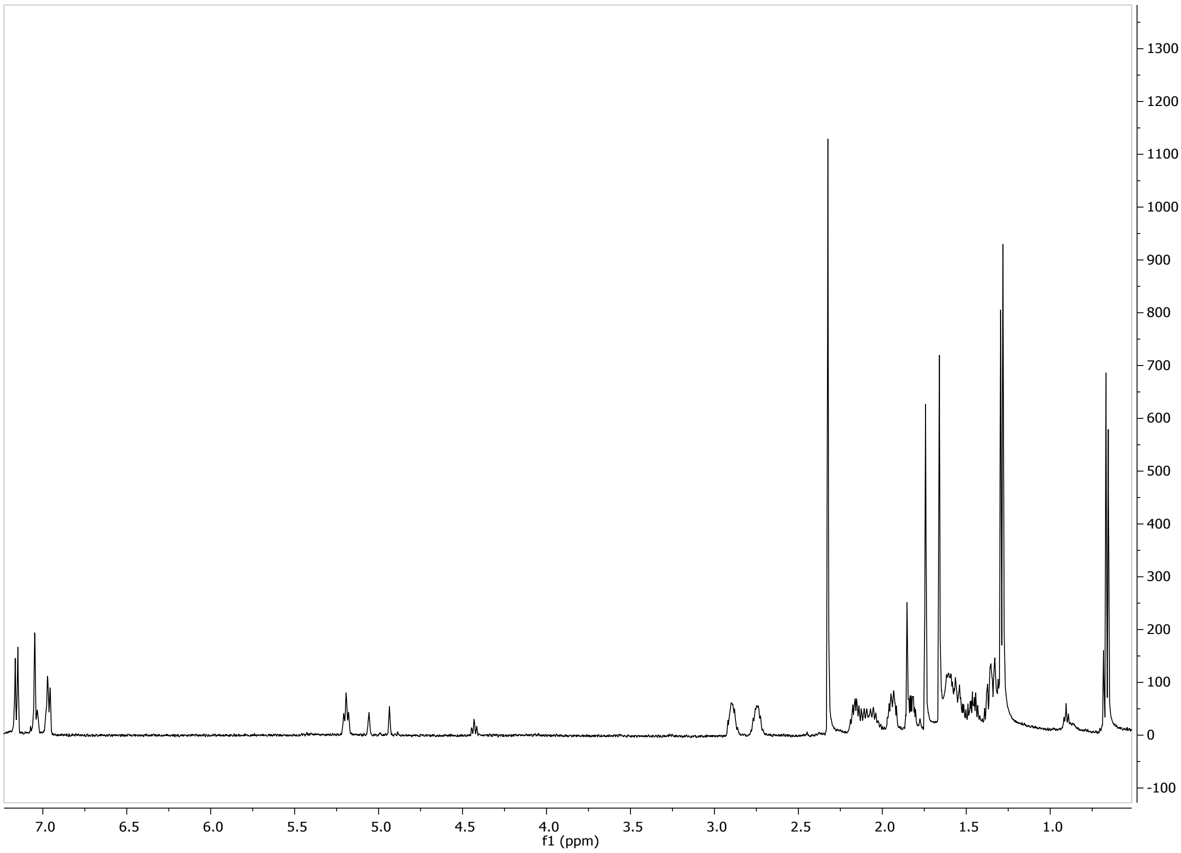


Figure S 33: ^{1}H NMR spectrum of erogorgiaene.

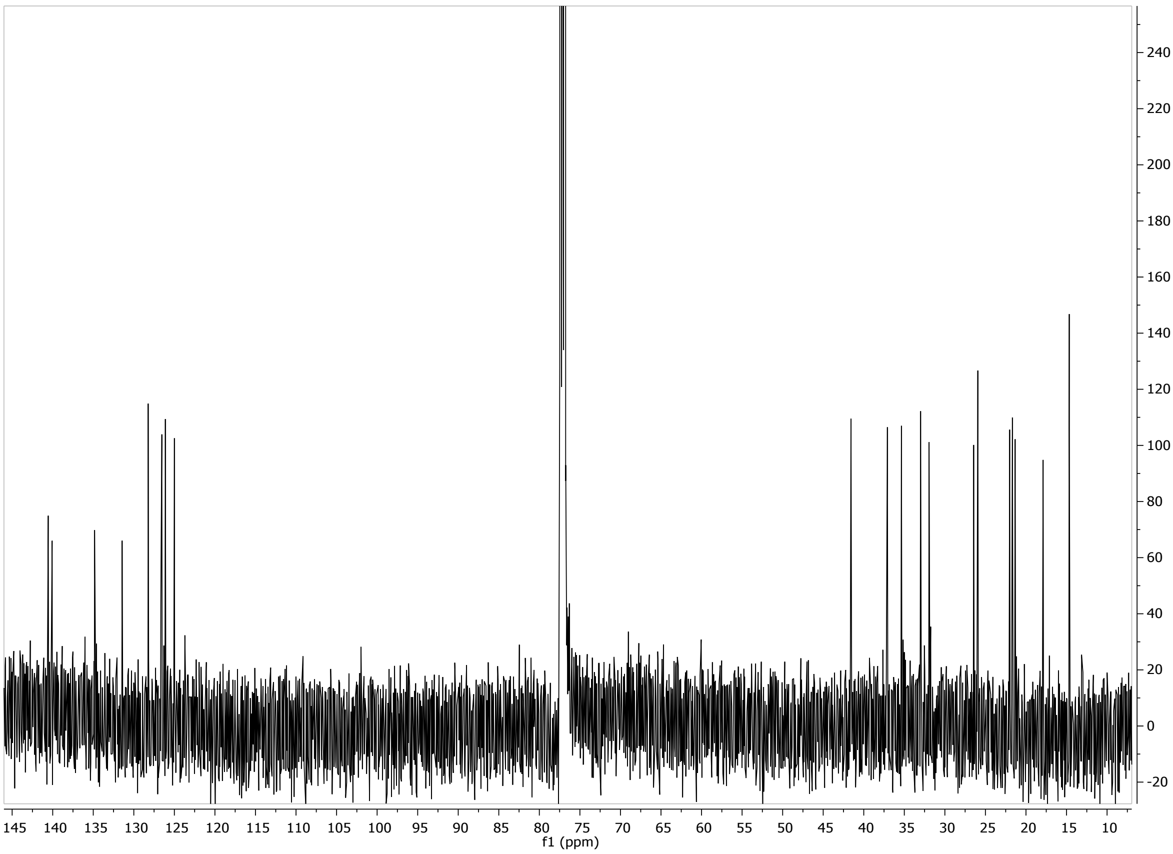


Figure S 34: ^{13}C NMR spectrum of erogorgiaene.

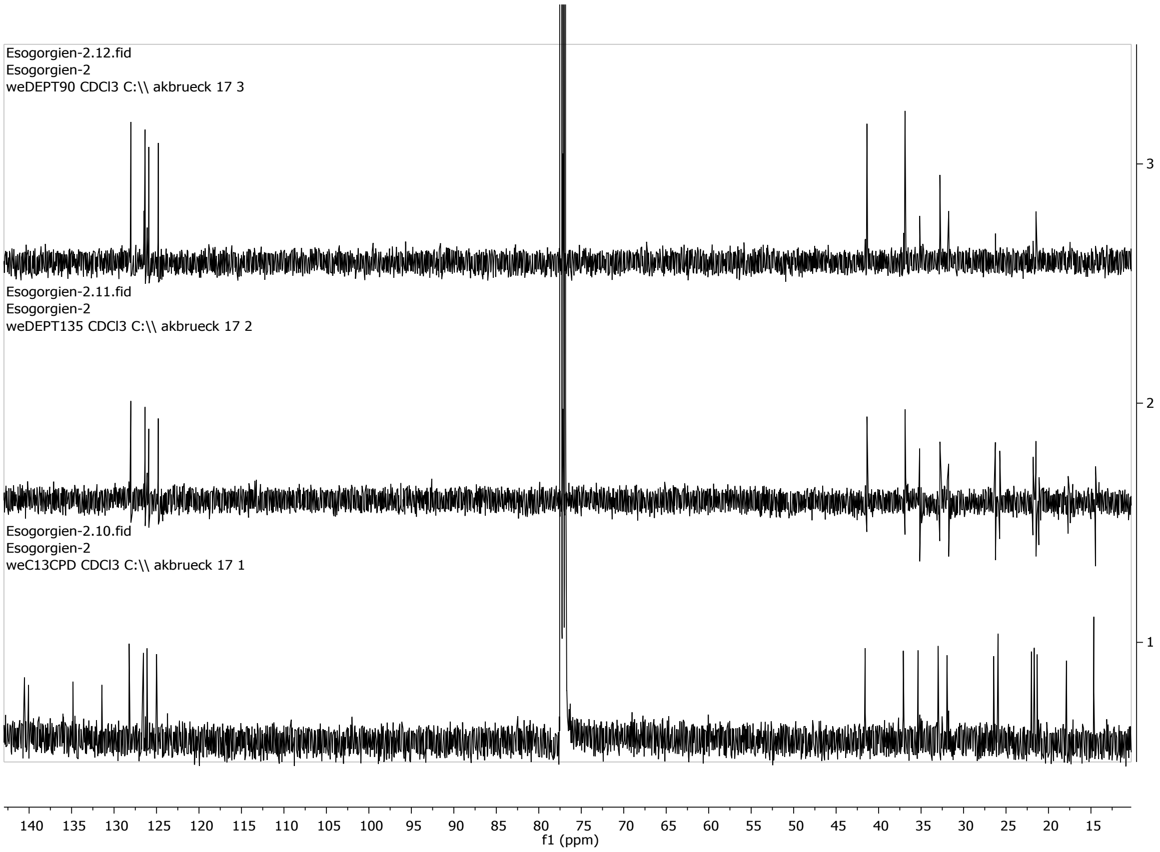


Figure S 35: ^{13}C DEPT spectra of erogorgiaene.

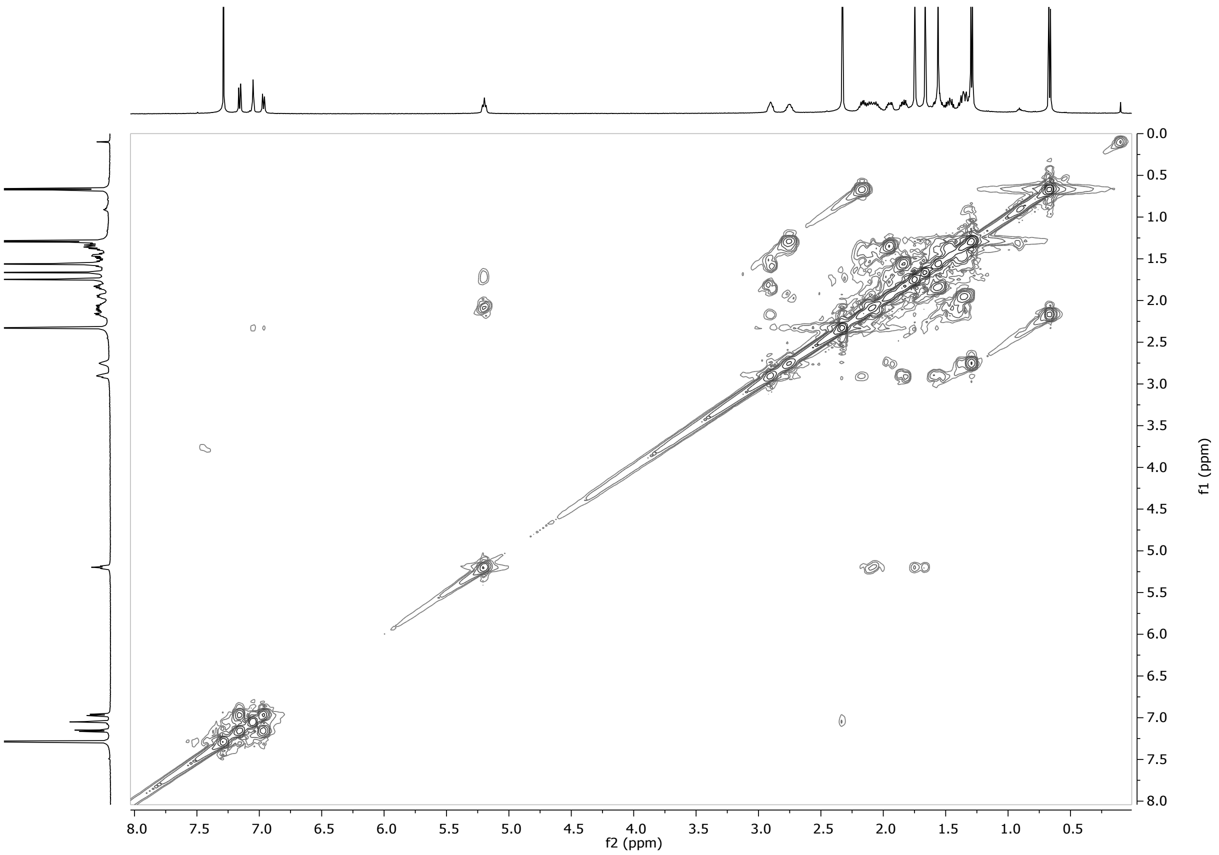


Figure S 36: ^{1}H-^{1}H COSY spectrum of erogorgiaene.

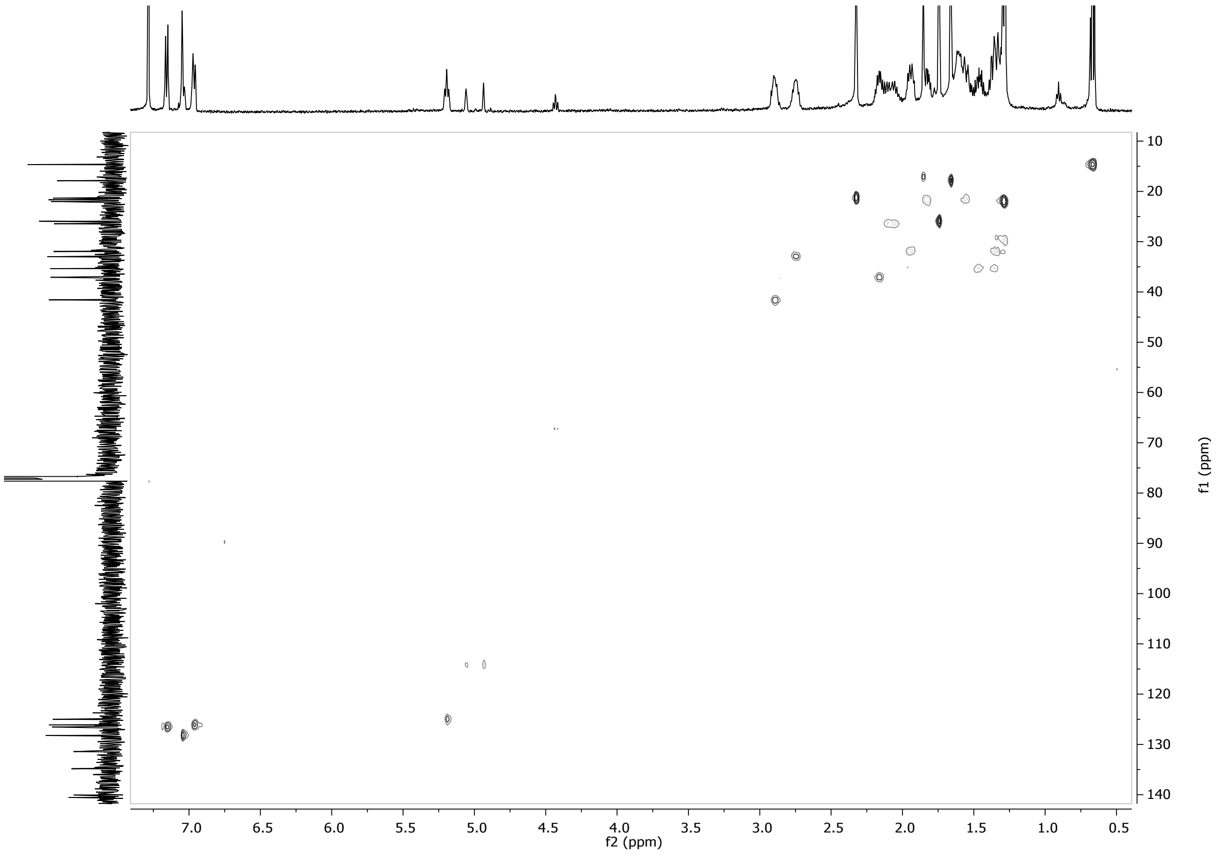


Figure S 37: ^{1}H-^{13}C HSQC spectrum of erogorgiaene.

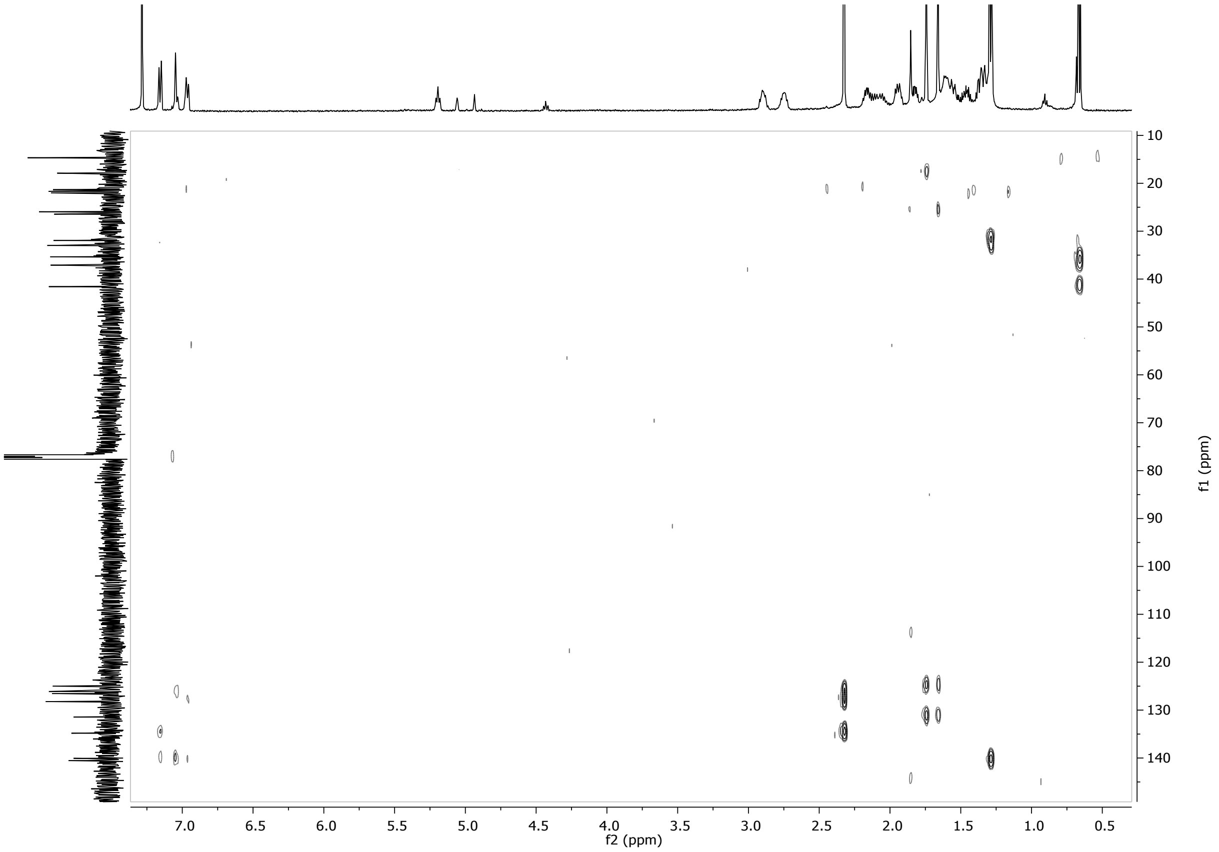


Figure S 38: ^{1}H-^{13}C HMBC spectrum of erogorgiaene.

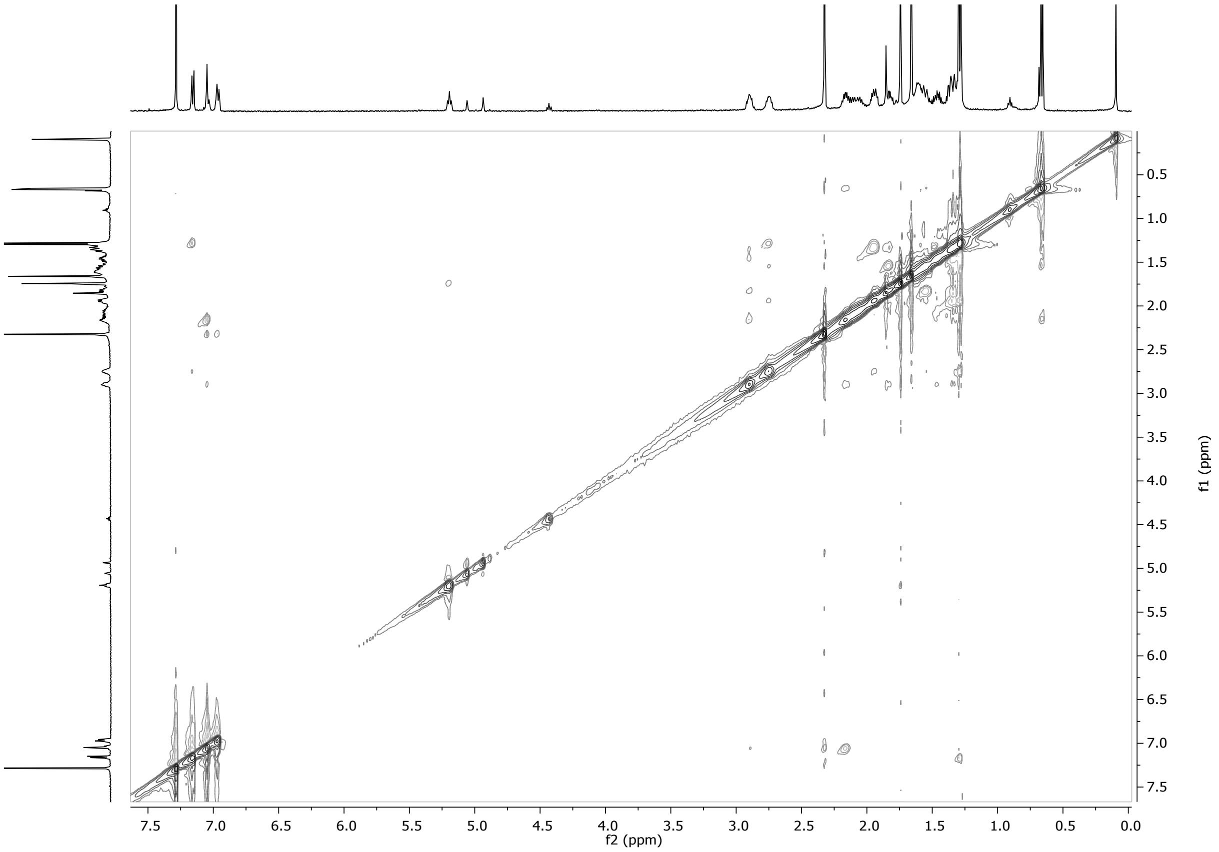


Figure S 39: ^1H-^1H NOESY spectrum of erogorgiaene.

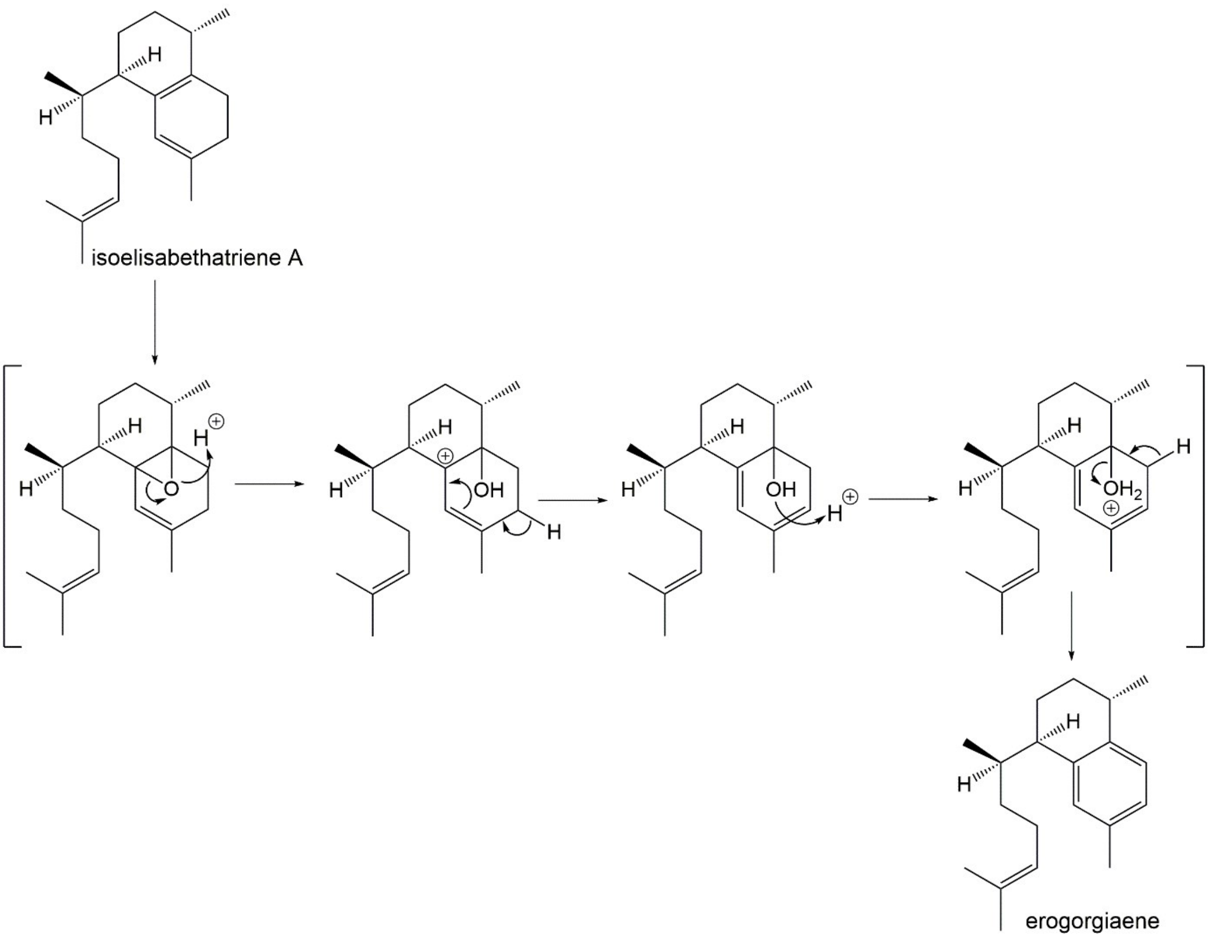


Figure S 40: Postulated mechanism for formation of (-)-erogorgiaene via epoxidation, dehydration and subsequent spontaneous aromatization.

Table S 7: Summary of approximate costs for biotechnological production of erogorgiaene

	#	Items	Description	Cost
SUMMARY	1	**CAPEX**	Total /10 years depreciation	1,449,732.11
	2	**OPEX**	Including: Maintenance, insurance and utility	1,464,014.73
	3	**RM**		23,055,842.10
			Total cost	**25,969,589**
		Revenues	**Kg of Product /yr.**	**2,829**
			Unit cost	**9,181.17**

Table S 8: Capital Investment for biotechnological production of erogorgiaene

Capital Investment										
#	Item	Unit Purchased	Quantity In process	Quantity Standby	Quantity Total	Cost	Installation factor	Installed Cost	Ref.
1	Fermenter, Vessel Volume = 200.00 m^3	2,266,000	2	0	2	4,532,000	1.6	7,251,200	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
2	Stirred Reactor Vessel Volume = 10 m3	53,000	1	0	1	53,000	1.3	68,900	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
3	Blending Tank, Vessel Volume = 150.00 m3	192,000	1	0	1	192,000	1.3	249,600	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
4	Decanter Centrifuge, Throughput = 200 m3/h	262,000	1	0	1	262,000	1.6	419,200	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
5	Distillation column,Volume = 100.00 m3	546,000	1	0	1	546,000	1.6	873,600	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
6	Inoculum preparation, Vessel Volume = 5.00 m3	-	1	0	1	-	1.3	-	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
7	Chromatography unit, 0.5D*2.5H	268,000	1	0	1	268,000	1.3	348,400	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
8	Centrifugal Extraction, Vol. 1000	462,000	1	0	1	462,000	1.6	739,200	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
9	Unlisted (10* of total PC)					631,500.0	1	631,500	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
	Total+ intrest 6%							**11,216,496**	

Table S 9: Total direct costs (TDC) for biotechnological production of erogorgiaene

#	Item	Unit cost	Quantity In process	Quantity Standby	Quantity Total		Factor	Cost	Ref.
1	Warehouse ,On-site storage of equipment and supplies.	448,660	1	-	1		0.04	448,659.84	Estimated as: 4.0%% of the ISBL
2	Site development, Includes fencing, curbing, parking lot, roads, well drainage, rail system, soil borings, and general paving. This factor allows for minimum site development assuming a clear site with no unusual problems such as right-of-way, difficult land clearing, or unusual environmental problems.	1,009,485	1	-	1		0.090	1,009,484.64	Estimated as: 9%% of the ISBL
3	Additional piping, Includes fencing, curbing, parking lot, roads, well drainage, rail system, soil borings, and general paving. This factor allows for minimum site development assuming a clear site with no unusual problems such as right-of-way, difficult land clearing, or unusual environmental problems.	504,742	1	-	1		0.045	504,742.32	Estimated as: 4.5%% of the ISBL
					Total			1,962,886.80	
	Total Direct Costs (TDC)							**13,179,382.80**	

Table S 10: Total capital investment (TCI) for biotechnological production of erogorgiaene

1	Indirect costs; Start-up and commissioning costs. Land, rights-of-way, permits, surveys, and fees. Piling, soil compaction/dewatering, unusual foundations. Sales, use, and other taxes. Freight, insurance in transit, and import duties on equipment, piping, steel, instrumentation, etc. Overtime pay during construction. Field insurance. Project team. Transportation equipment, bulk shipping containers, plant vehicles, etc.						0.100	1,317,938	Estimated as: 10% of total direct cost (TDC)
	Total							1,317,938.28	
							Total Capital Investment (TCI)	**14,497,321**	
							Depreciation 10 year	**1,449,732**	

Table S 11: Annual operation cost for biotechnological production of erogorgiaene

Annual Operation Cost									
#	**Item**	**Unit cost**	**Unit**	**Quantity**			**Factor**	**Cost**	**Ref.**
1	Maintenance						0.03	395,381.48	Estimated as3% of total direct fixed capital
2	Property insurance						0.007	101,481.25	Estimated as0.7% of total Capital Investment (TCI)
3	Std Power	0.1		4,760,231				476,023.11	the consumption amount based on SuperPro Designer (V10) . The cost of kW-h is based on German price of Std power
4	Steam	1.0	$/MT	34,233				34,232.91	
5	Chilled water	0.50	$/MT	911,016				455,508.20	
6	Consumable			1,388				1,387.77	
	Total								
							Total fixed operating costs	**1,464,015**	

Table S 12: Raw material cost for biotechnological production of erogorgiaene

RM Cost									
#	**Item**	**Unit cost**	**Unit**	**Quantity**			**Factor**	**Cost**	**Ref.**
1	Glycerol	0.3	Kg	313,971			1.00	78,492.86	biofuel waste stream
2	K2HPO4	1.5	Kg	31,114			1.00	46,671.43	Sigma with 30% save due to bulk procurement
3	KH2PO4	1.5	Kg	5,657			1.00	8,485.71	Sigma with 30% save due to bulk procurement
4	(NH4)2H2PO4	1.5	Kg	11,314			1.00	16,971.43	Sigma with 30% save due to bulk procurement
5	Citric acid	1.0	Kg	4,809			1.00	4,808.57	Sigma with 30% save due to bulk procurement
6	Yeast extract	3.0	Kg	14,143			1.00	42,428.57	Sigma with 30% save due to bulk procurement
7	propionic acid	1.0	L	16,398			1.00	16,397.52	Sigma with 30% save due to bulk procurement
8	urea-hydrogen peroxide	1.0	Kg	163,975			1.00	163,975.16	Sigma with 30% save due to bulk procurement
9	CalB (immobilized on beads)	30.0	Kg	163,975			1.00	4,919,254.66	Sigma with 30% save due to bulk procurement
10	Ethanol	13.0	L	67,347			95%	878,345.86	95% recovery and 5% loss
11	Hexane	33.7	L	101,020			90%	3,403,125.00	90% recovery and 10% loss
12	EtOAc	11.6	L	77,449			90%	899,957.14	90% recovery and 10% loss
13	Methanol	20.2	L	11,449			90%	231,078.57	90% recovery and 10% loss
14	Acetonitril	60.7	L	13,469			95%	817,928.57	95% recovery and 5% loss
					Total			11,527,921.05	
							Total RM Costs (TRM)	**23,055,842.10**	

Table S 13: Summary of approximate costs for chemical synthesis of erogorgiaene

SUMMARY	#	Items	Description	Cost
	1	**CAPEX**	Total /10 years depreciation	232,470.08
	2	**OPEX**	Including: Maintenance, insurance and utility	102,195.25
	3	**RM**		5,736,249.18
			Total cost	**6,070,915**
		Revenues	**Kg of Product /yr.**	**286**
			Unit cost	**21,194.54**

Table S 14: Capital investment for chemical synthesis of erogorgiaene

Capital Investment									
#	**Item**	**Unit Purchased**	**Quantity In process**	**Standby**	**Total**	**Cost**	**Installation factor**	**Installed Cost**	**Ref.**
1	Stirred Reactor Vessel Volume = 10 L	10,000	1	0	1	10,000	1.3	13,000	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
2	Stirred Reactor Vessel Volume = 20 L	12,000	1	0	1	12,000	1.3	15,600	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
3	Stirred Reactor Vessel Volume = 150 L	20,000	1	0	1	20,000	1.3	26,000	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
4	Stirred Reactor Vessel Volume = 500 L	300,000	1	0	1	300,000	1.3	390,000	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
5	Centrifugal Extraction, Vol. 100 l	20,000	1	0	1	20,000	1.3	26,000	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
6	Centrifugal Extraction, Vol. 1000	36,000	1	0	1	36,000	1.3	46,800	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
7	Chromatography unit, 0.5D*2.5H	268,000	1	0	1	268,000	1.3	348,400	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
8	Distillation column,Volume = 100.00 m3	546,000	1	0	1	546,000	1.3	709,800	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
9	Unlisted (10* of total PC)					121,200.0	1	121,200	SuperPro Designer (V10) Built-in cost model (Year analysis 2020)
							Total+ Interest 6%	**1,798,608**	

Table S 15: Total direct costs (TDC) for chemical synthesis of erogorgiaene

#	**Item**	**Unit cost**	**Quantity In process**	**Standby**	**Total**		**Factor**	**Cost**	**Ref.**
1	Warehouse ,On-site storage of equipment and supplies.	71,944	1	-	1		0.04	71,944.32	Estimated as: 4.0% of the ISBL
2	Site development, Includes fencing, curbing, parking lot, roads, well drainage, rail system, soil borings, and general paving. This factor allows for minimum site development assuming a clear site with no unusual problems such as right-of-way, difficult land clearing, or unusual environmental problems.	161,875	1	-	1		0.090	161,874.72	Estimated as: 9% of the ISBL
3	Additional piping, Includes fencing, curbing, parking lot, roads, well drainage, rail system, soil borings, and general paving. This factor allows for minimum site development assuming a clear site with no unusual problems such as right-of-way, difficult land clearing, or unusual environmental problems.	80,937	1	-	1		0.045	80,937.36	Estimated as: 4.5% of the ISBL
					Total			314,756.40	
							Total Direct Costs (TDC)	**2,113,364.40**	

Table S 16: Total capital investment (TCI) for chemical synthesis of erogorgiaene

1	Indirect costs; Start-up and commissioning costs. Land, rights-of-way, permits, surveys, and fees. Piling, soil compaction/dewatering, unusual foundations. Sales, use, and other taxes. Freight, insurance in transit, and import duties on equipment, piping, steel, instrumentation, etc. Overtime pay during construction. Field insurance. Project team. Transportation equipment, bulk shipping containers, plant vehicles, etc.						0.100	211,336	Estimated as: 10% of total direct cost (TDC)
	Total							211,336.44	
	Total Capital Investment (TCI)							**2,324,701**	
	Depreciation 10 year							**232,470**	

Table S 17: Annual operation cost for chemical synthesis of erogorgiaene

Annual Operation Cost									
#	**Item**	**Unit cost**	**Unit**	**Quantity**			**Factor**	**Cost**	**Ref.**
1	Maintenance						0.03	63,400.93	Estimated as3% of total direct fixed capital
2	Property insurance						0.007	16,272.91	Estimated as0.7% of total Capital Investment (TCI)
3	Uitility						0.01	21,133.64	the consumption amount based on SuperPro Designer (V10) . The cost of kW-h is based on German price of Std power
6	Consumable			1,388				1,387.77	
	Total								
	Total fixed operating costs							**102,195**	

Table S 18: Raw material cost for chemical synthesis of erogorgiaene

RM Cost									
#	Item	Unit cost	Unit	Quantity/yr.		Recovey factor	Bulk Factor	Cost	Ref.
1	triethylamine	114.13	L	2,940.35		1.00	0.70	234,907.66	
2	formic acid	50.85	L	1,345.00		1.00	0.70	47,875.18	
3	4-methylacetophenone	171.76	kg	454.48		1.00	0.70	54,642.59	
4	NaHCO3	5.65	kg	33,397.95		1.00	0.70	132,088.88	
5	MgSO4	5.65	kg	65,117.41		1.00	0.70	257,539.34	
6	THF	27.06	L	6,566.58		1.00	0.70	124,400.20	
7	sodium tert-butoxide	366.12	kg	35.22		1.00	0.70	9,026.51	
8	tert-butyl acrylate	186.45	L	596.96		1.00	0.70	77,912.44	
9	TMEDA	124.30	L	836.32		1.00	0.70	72,768.37	
10	MgBr2	1,785.40	kg	653.00		1.00	0.70	816,109.51	
11	KHSO4	101.70	kg	10,747.90		1.00	0.70	765,142.89	
12	toluene	22.04	L	22,430.40		0.10	0.70	34,597.77	
13	TBAF·3H2O	1,979.76	kg	1,300.96		1.00	0.70	1,802,916.16	
14	polyphosphoric acid	79.55	L	5,709.56		1.00	0.70	317,944.59	
15	HCl	339.00	L	1,058.10		1	0.70	251,087.78	
16	(R)-RuCl[(1S,2S)-pTsNCH (C6H5)CH(C6H5)NH2](η6-p-cymene)	187,580.00	kg	111.87		0.001	1.00	20,984.78	
17	EtOAc	18.76	L	455,331.59		0.100	0.70	597,877.70	
18	N,N-Diisopropylcarbamoyl chloride	7,774.40	kg	1,115.80		0.001	1.00	8,674.71	
19	CH2Cl2	115.26	L	178,968.01		0.100	0.70	1,443,949.69	
20	CuCl	11,920.00	kg	32.83		0.001	1.00	391.37	
21	DPEPhos	8,452.40	kg	65.67		0.001	1.00	555.03	
22	Bis(pinacolato)diboron	1,511.94	kg	1,092.44		0.001	1.00	1,651.70	
23	MeOH	32.58	L	3,845.29		0.100	0.70	8,770.01	
24	Et2O	60.77	L	33,726.59		0.100	0.70	143,466.90	
25	s-BuLi	299.45	L	4,005.29		0.001	1.00	1,199.38	
26	RuCl(p-cymene) [(S,S)-Ts-DPEN]	203,400.00	kg	79.11		0.001	1.00	16,090.40	
27	borane (S)-20	893.60	L	9,877.16		0.001	1.00	8,826.27	
28	pentane	65.92	L	79,017.27		0.10	0.70	364,598.87	
					Total			2,868,124.59	
							Total RM Costs (TRM)	5,736,249.18	

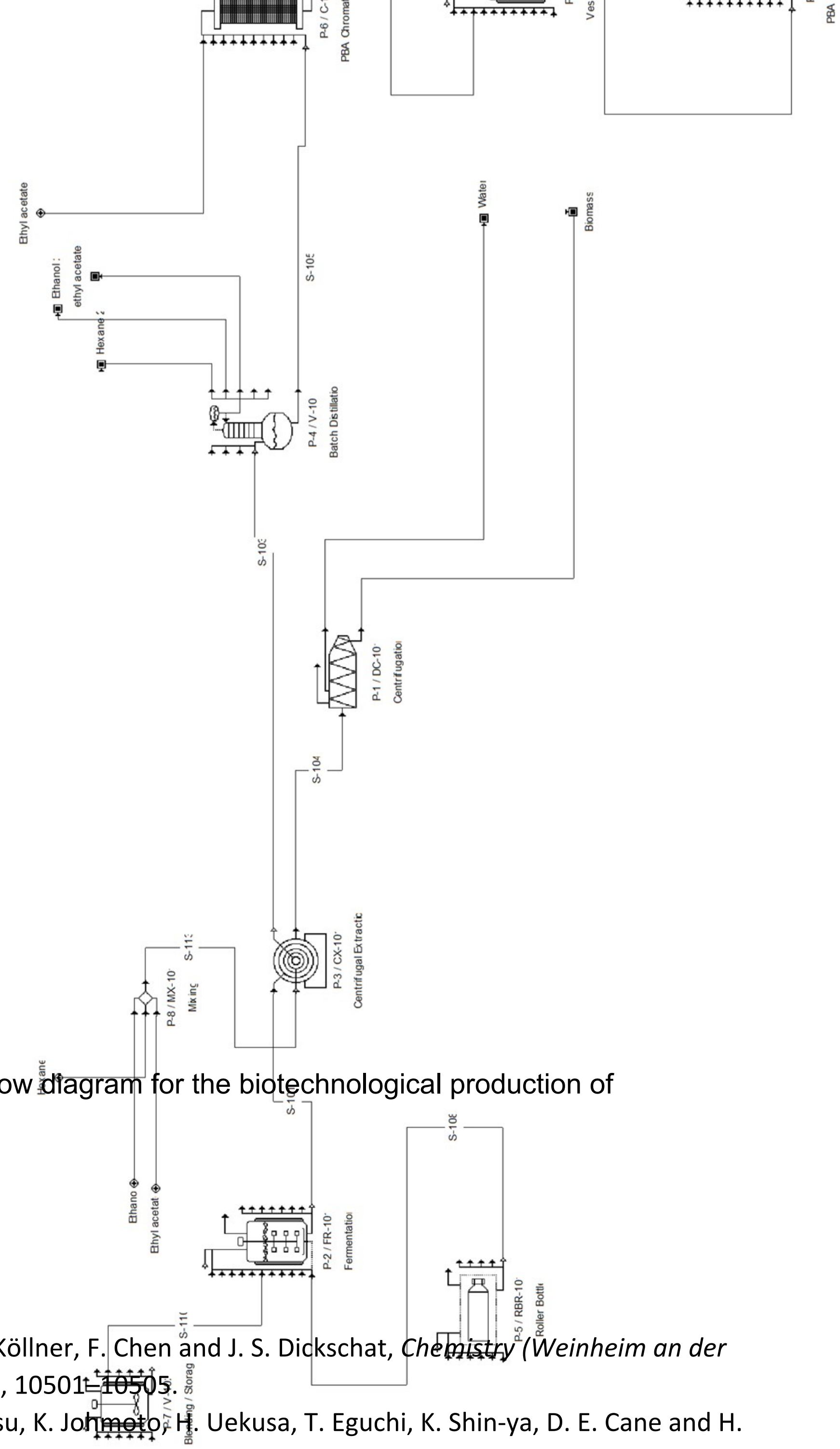


Figure S 41: Simplified process flow diagram for the biotechnological production of erogorgiaene

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