Supplementary information for

Ancient defensive terpene biosynthetic gene clusters in the soft corals

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Supplementary Results

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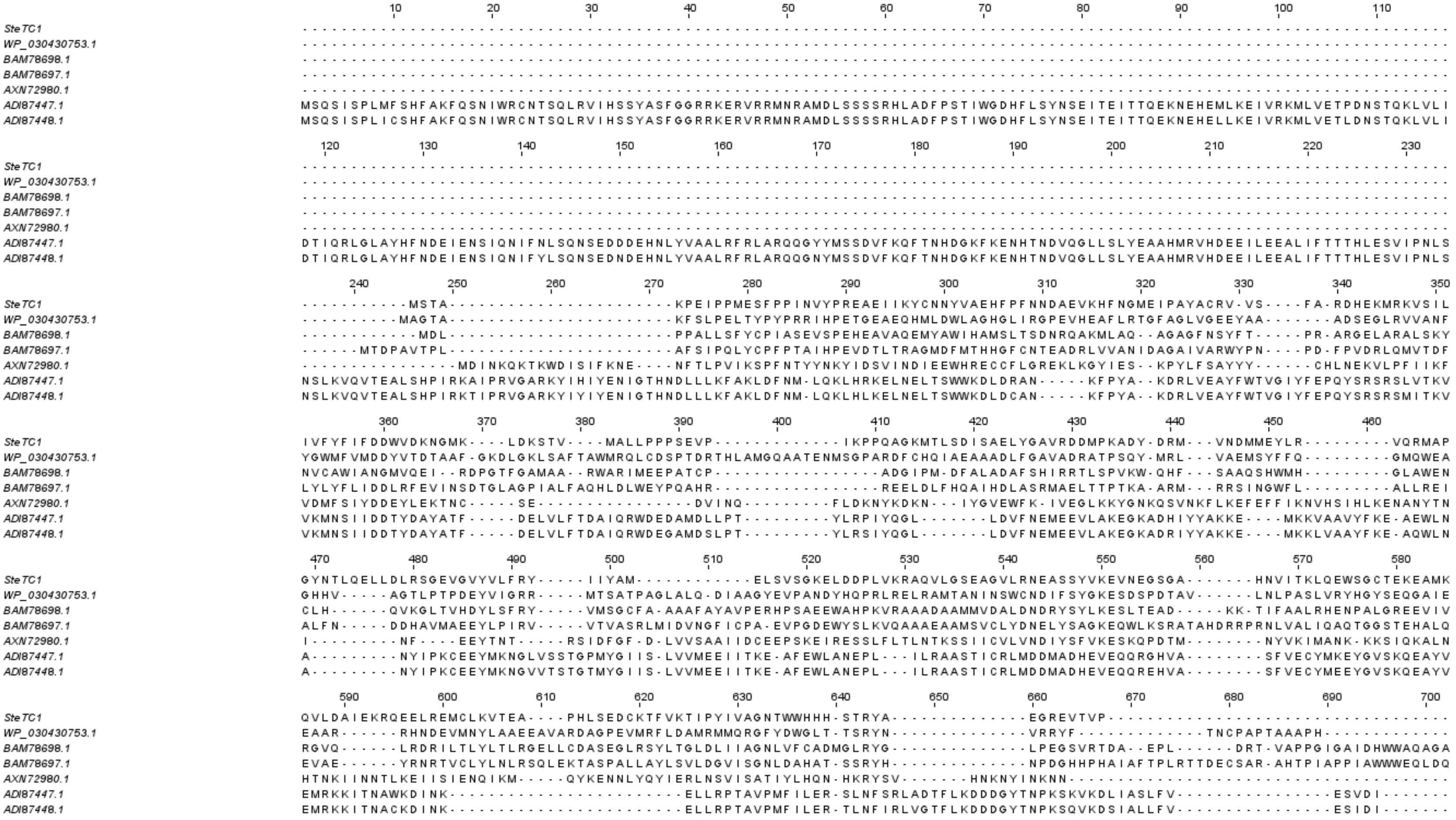
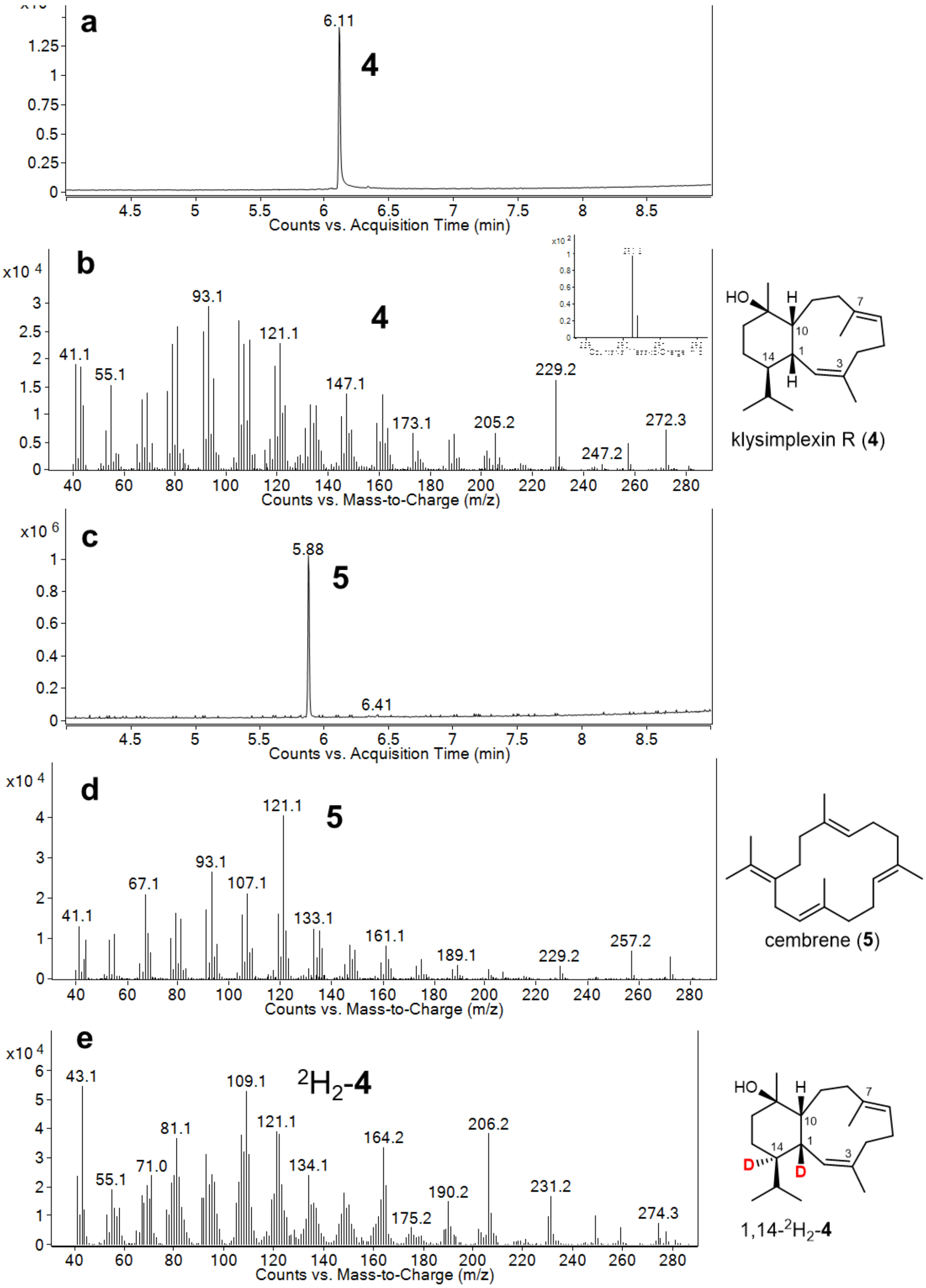
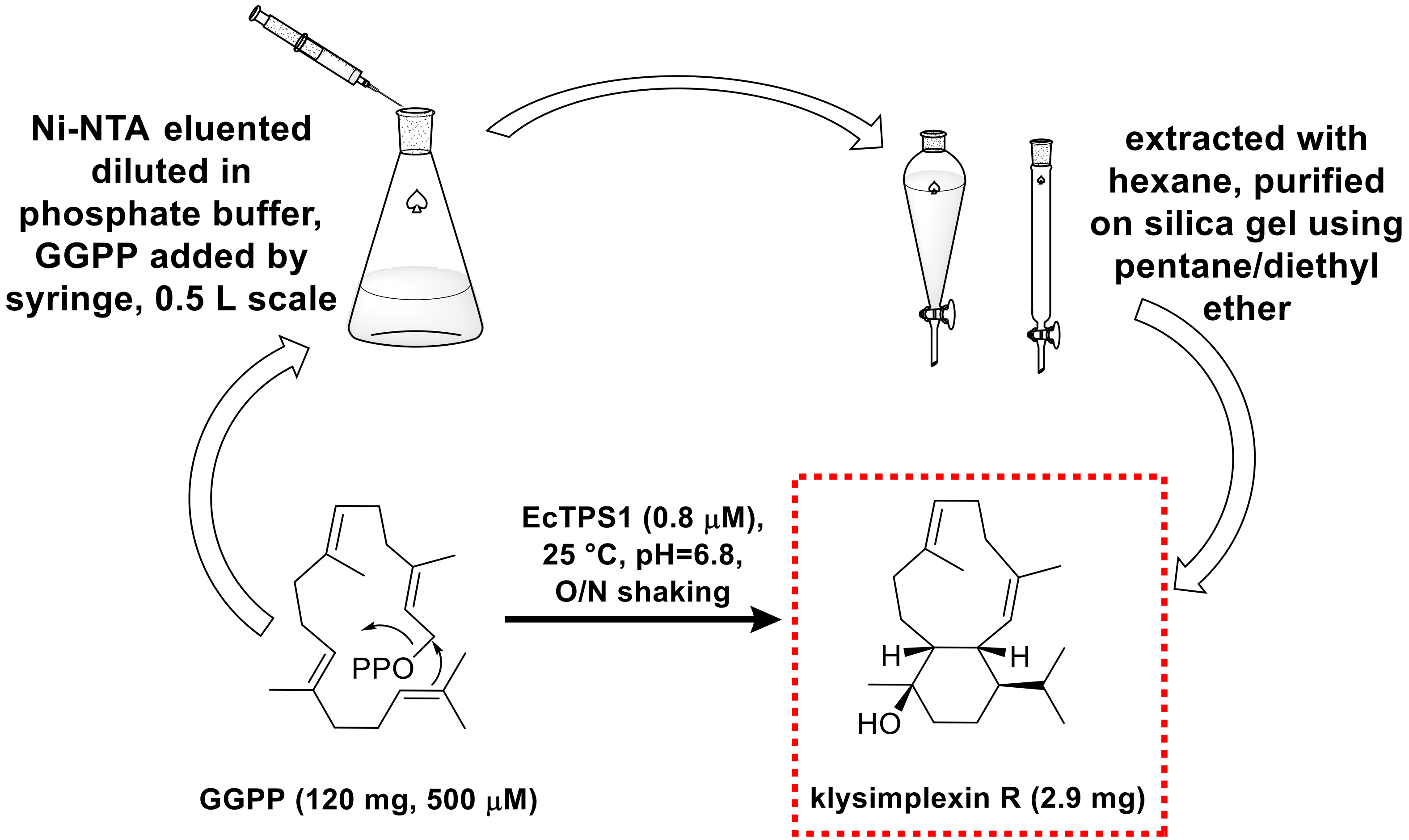


Supplementary Figure 1. Multiple sequence alignment of protein sequences from known cembrane producers used to build HMM profile.



Supplementary Figure 2. GCMS data for compounds **4**, **5** and $^2H_2$-**4**. **a**) GCMS TIC trace for **4**. **b**) EIMS spectrum for **4**. **c**) GCMS TIC trace for **5**. **d**) EIMS spectrum for **5**. **e**) EIMS spectrum for $^2H_2$-**4**.



Supplementary Figure 3. Schematic summary of scaled-up enzyme reaction used to produce milligram quantities of diterpenoids for structural characterization.

Supplementary Table 1. Protein sequences used to build multiple sequence alignment and hidden Markov models. Proteins were selected based on experimental evidence for the enzymatic production of cembranoids, which are structurally related to octocoral metabolites.

Name	Accession #	Species	Product	Ref.
DtcycA	BAM78698.1	*Streptomyces sp*. SANK 60404 (bacteria)	(-)-(*R*)-Nephthenol and cembrene isomer **9**[b]	12
DtcycB	BAM78697.1	*Streptomyces sp*. SANK 60404 (bacteria)	(-)-(*R*)-Nephthenol and (-)-(*R*)-Cembrene A[b]	12
CAS	WP_030430753.1	*Allokutzneria albata* (bacteria)	(+)-(*S*)-Cembrene A[b]	26
CBTS 2b	ADI87447.1	*Nicotiana sylvestris* (plant)	Cembratrienols[c]	27
CBTS 3	ADI87448.1	*Nicotiana sylvestris* (plant)	Cembratrienols[c]	27
SteTC1	[a]	*Stereum histurum* (fungus)	(-)-(*R*)-Nephthenol[b]	28
DpTPS11	AXN72980.1	*Dictyostelium purpureum* (amoeba)	(+)-(*S*)-Nephthenol[b]	29

[a] Accessed directly from Ref. *6* (found in supplemental information). [b] Supported by heterologous expression. [c] supported by gene knockout.

Supplementary Table 2. Results from i-TASSER modelling of EcTPS1 showing top 10 most similar structures from PDB, from both bacteria and plants.

Rank	PDB Hit	TM-score	RMSD	IDEN	Cov	Originating species
1	4okmA	0.779	1.85	0.141	0.810	*Streptomyces pristinaespiralis* ATCC 25486 (bacteria)
2	6tbdA	0.737	2.45	0.124	0.788	*Streptomyces* sp. CWA1 (bacteria)
3	3kb9A	0.692	2.59	0.116	0.759	*Streptomyces coelicolor* (bacteria)
4	3v1vA	0.691	2.67	0.114	0.754	*Streptomyces coelicolor* A3(2) (bacteria)
5	6vkzA	0.684	3.10	0.138	0.771	*Pseudo-nitzschia multiseries* (bacteria)
6	5a0iA	0.683	2.14	0.117	0.734	*Streptomyces* sp. (bacteria)
7	4zq8A	0.681	2.87	0.140	0.749	*Streptomyces lydicus* (bacteria)
8	1hm7B	0.673	2.15	0.099	0.722	*Streptomyces exfoliates* (bacteria)
9	6q4sA	0.672	2.56	0.097	0.737	*Streptomyces chartreusis* NRRL 3882 (bacteria)
10	5uv0A	0.671	3.35	0.100	0.773	*Citrus sinensis* (plant)

Supplementary Table 3. Accession numbers for *E. caribaeorum* genes and sequences reported in this study

Name	Accession #	Description
E. caribaeorum transcriptome	SRR15783032	SRA/raw reads of *E. caribaeorum* transcriptome
E. caribaeorum specimen 1 metagenome	SRR15817518	SRA/raw reads of *E. caribaeorum* metagenome
E. caribaeorum specimen 2 metagenome	SRR15817517	SRA/raw reads of *E. caribaeorum* metagenome
EcTPS1	OK081311	CDS
EcTPS6_contig	OK081312	Genomic contig
EcTPS2	OK081313	CDS
EcTPS3	OK081314	CDS
EcTPS4	OK081315	CDS
EcTPS4_1	OK081316	CDS
EcTPS5	OK081317	CDS
EcTPS7	OK081318	CDS
EcTPS8	OK081319	CDS
Dinoflagellate_TS	OK081320	CDS

Supplementary Table 4. Gene annotations for *elu* cluster contig by eggNOG-mapper (1.0.3-3-g3e22728).

Name	Length	Effective Length	TPM	#Reads	seed_eggNOG_ortholog	seed ortholog evalue	seed ortholog score	predicted gene name	KEGG_KOs	eggNOG annot
g1.t1	2824	2618.453	235.622667	83	45351.NEMVEDRAFT_v1g91817-PA	1.60E-130	427.6	RXFP1	K04306,K04307	Relaxin insulin-like family peptide receptor
g2.t1	288	91.309	19945.0362	245	6334.EFV56619	3.20E-36	112.8		K14803	PP2Cc
g3.t1	342	139.386	0	0						
g4.t1	531	325.456	2238.294772	98	7739.JGI235627	7.70E-65	211.3		K14803	protein phosphatase, Mg2 Mn2 dependent
g5.t1	648	442.456	33.600355	2	13735.ENSPSIP00000001475	4.60E-24	74.7			Reverse transcriptase (RNA-dependent DNA polymerase)
g6.t1	426	220.592	303.275544	9						
g7.t1	3645	3439.453	153.444995	71	9785.ENSLAFP00000004252	1.60E-160	531.2	TBC1D1	K18341	ecotropic viral integration site
g8.t1	6012	5806.453	48.647042	38						
g9.t1	783	577.453	0	0	7897.ENSLACP00000019709	1.90E-29	93.2			Endonuclease-reverse transcriptase HmRTE-e01
g10.t1	297	99.286	0	0	7897.ENSLACP00000007560	1.90E-21	68.3			Inherit from KOG: Cytochrome p450
g11.t1	546	340.456	0	0						
g12.t1	456	250.567	0	0	8090.ENSORLP00000024868	4.20E-41	131.2			Inherit from NOG: Retroelement
g13.t1	1836	1630.453	351.047751	77	45351.NEMVEDRAFT_v1g168885-PA	0	1078.2	IARS	K01870	isoleucyltRNA synthetase
g14.t1	249	61.247	2063.242038	17						
g15.t1	1533	1327.453	2777.450167	496	45351.NEMVEDRAFT_v1g168885-PA	1.20E-169	559.4	IARS	K01870	isoleucyltRNA synthetase
g16.t1	1554	1348.453	485.099259	88	313624.N9414_06939	5.60E-23	71.1			condensation domain protein
g17.t1	1221	1015.453	944.307921	129						should be terpene cyclase
g18.t1	291	93.841	633.695411	8						
g19.t1	1479	1273.453	828.875257	142	45351.NEMVEDRAFT_v1g234104-PA	1.20E-81	268.3	CYP1B1	K07408	cytochrome P450, family 1, subfamily
g20.t1	357	153.044	0	0						
g21.t1	345	142.162	0	0	13616.ENSMODP00000039345	3.00E-21	65.4			NA
g22.t1	861	655.453	11.340761	1						
g23.t1	519	313.456	0	0	400682.PAC_15700797	5.10E-59	189.8			Reverse transcriptase
g24.t1	435	229.591	129.505504	4	400682.PAC_15700797	3.40E-32	102.6			Reverse transcriptase
g25.t1	1797	1591.453	1237.758196	265	45351.NEMVEDRAFT_v1g213886-PA	3.40E-203	671	GK	K00864	Glycerol kinase
g26.t1	1773	1567.453	588.045474	124	7668.SPU_013953tr	1.00E-94	306.8		K18667	CUE
g27.t1	1023	817.453	0	0	7668.SPU_008342tr	3.40E-57	181.3			NA
g28.t1	555	349.456	0	0						
g29.t1	537	331.456	112.131495	5	45351.NEMVEDRAFT_v1g223755-PA	5.90E-36	116.6	ASCC2	K18667	activating signal cointegrator 1 complex subunit 2
g30.t1	375	170.145	910373.9189	20838	45351.NEMVEDRAFT_v1g190935-PA	1.40E-56	183.9	RPS25	K02975	Ribosomal protein S25
g31.t1	348	144.849	21705.45266	422.961	45351.NEMVEDRAFT_v1g186304-PA	1.90E-45	142.2			Calponin homology (CH) domain
g32.t1	1179	973.453	2184.210838	286.039	9103.ENSMGAP00000015929	8.60E-58	190.2	LRCH1		Leucine-rich repeats and calponin homology (CH) domain containing
g33.t1	1830	1624.453	21520.4616	4703	45351.NEMVEDRAFT_v1g88648-PA	2.90E-111	364.3	SLC16A10	K08187	solute carrier family 16, member 10 (aromatic amino acid transporter)
g34.t1	258	67.687	219.638225	2						
g35.t1	1146	940.453	10875.897	1376						

Supplementary Table 5. SRA data used in this study.

SRA accession number	Class	Scientific Names
DRR253190	*Dinophyceae*	*Durusdinium trenchii*
SRR8506632	*Dinophyceae*	*kawagutii*
SRR12021959	*Heteroscleromorpha*	*Cliona orientalis*
SRR6782832	*Hexacorallia*	*Agaricia lamarcki*
SRR6820379	*Hexacorallia*	*Alveopora japonica*
SRR5123105	*Hexacorallia*	*Amplexidiscus fenestrafer*
ERR2192493	*Hexacorallia*	*Coelastrea aspera*
SRR14295591	*Hexacorallia*	*Colpophyllia natans*
ERR2190350	*Hexacorallia*	*Dipsastraea favus*
ERR2190370	*Hexacorallia*	*Fungia fungites*
ERR2191368	*Hexacorallia*	*Galaxea fascicularis*
SRR4449115	*Hexacorallia*	*Goniopora columna*
SRR14295593	*Hexacorallia*	*Montastraea cavernosa*
SRR9330360	*Hexacorallia*	*Oculina patagonica*
SRR14295588	*Hexacorallia*	*Orbicella faveolata*
SRR10873896	*Hexacorallia*	*Pavona decussata*
SRR12904788	*Hexacorallia*	*Pocillopora damicornis*
SRR14295605	*Hexacorallia*	*Porites astreoides*
SRR14295600	*Hexacorallia*	*Siderastrea siderea*
SRR8297742	*Hexacorallia*	*Triactis producta*
SRR7585363	*Hexacorallia*	*Zoanthus sp.* QL-2018
SRR7174588	*Octocorallia*	*Briareum sp.* 1 RRV-2018
SRR7174589	*Octocorallia*	*Briareum sp.* 2 RRV-2018
SRR7174590	*Octocorallia*	*Briareum sp.* 3 RRV-2018
SRR7174591	*Octocorallia*	*Briareum sp.* 4 RRV-2018
SRR13925246	*Octocorallia*	*Callogorgia americana delta*
SRR8293935	*Octocorallia*	*Dendronephthya gigantea*
SRR8486075	*Octocorallia*	*Eleutherobia rubra*
SRR8486076	*Octocorallia*	*Eleutherobia rubra*
SRR8486077	*Octocorallia*	*Eleutherobia rubra*
SRR8486078	*Octocorallia*	*Eleutherobia rubra*
SRR8486079	*Octocorallia*	*Eleutherobia rubra*
SRR8486080	*Octocorallia*	*Eleutherobia rubra*
SRR8486081	*Octocorallia*	*Eleutherobia rubra*
SRR8486082	*Octocorallia*	*Eleutherobia rubra*
SRR8486083	*Octocorallia*	*Eleutherobia rubra*
SRR6039601	*Octocorallia*	*Eunicea calyculata*
SRR6039602	*Octocorallia*	*Eunicea calyculata*
SRR6039603	*Octocorallia*	*Eunicea calyculata*
SRR6039604	*Octocorallia*	*Eunicea calyculata*
SRR6039605	*Octocorallia*	*Eunicea calyculata*

SRR6039606	*Octocorallia*	*Eunicea calyculata*
SRR12876609	*Octocorallia*	*Eunicea flexuosa*
SRR12876610	*Octocorallia*	*Eunicea flexuosa*
SRR12876613	*Octocorallia*	*Eunicea flexuosa*
SRR12876614	*Octocorallia*	*Eunicea flexuosa*
SRR12876620	*Octocorallia*	*Eunicea flexuosa*
SRR12876621	*Octocorallia*	*Eunicea flexuosa*
SRR12876622	*Octocorallia*	*Eunicea flexuosa*
SRR12876624	*Octocorallia*	*Eunicea flexuosa*
SRR12876625	*Octocorallia*	*Eunicea flexuosa*
SRR12876628	*Octocorallia*	*Eunicea flexuosa*
SRR12876629	*Octocorallia*	*Eunicea flexuosa*
SRR12876631	*Octocorallia*	*Eunicea flexuosa*
SRR12876632	*Octocorallia*	*Eunicea flexuosa*
SRR12876633	*Octocorallia*	*Eunicea flexuosa*
SRR12876635	*Octocorallia*	*Eunicea flexuosa*
SRR12876636	*Octocorallia*	*Eunicea flexuosa*
SRR12876637	*Octocorallia*	*Eunicea flexuosa*
SRR12876638	*Octocorallia*	*Eunicea flexuosa*
SRR12876639	*Octocorallia*	*Eunicea flexuosa*
SRR12876640	*Octocorallia*	*Eunicea flexuosa*
SRR12876644	*Octocorallia*	*Eunicea flexuosa*
SRR12876647	*Octocorallia*	*Eunicea flexuosa*
SRR12876648	*Octocorallia*	*Eunicea flexuosa*
SRR12876649	*Octocorallia*	*Eunicea flexuosa*
SRR12876650	*Octocorallia*	*Eunicea flexuosa*
SRR12876653	*Octocorallia*	*Eunicea flexuosa*
SRR12876654	*Octocorallia*	*Eunicea flexuosa*
SRR12876657	*Octocorallia*	*Eunicea flexuosa*
SRR12876658	*Octocorallia*	*Eunicea flexuosa*
SRR12876659	*Octocorallia*	*Eunicea flexuosa*
SRR12876660	*Octocorallia*	*Eunicea flexuosa*
SRR12876661	*Octocorallia*	*Eunicea flexuosa*
SRR12876663	*Octocorallia*	*Eunicea flexuosa*
SRR12876664	*Octocorallia*	*Eunicea flexuosa*
SRR935078	*Octocorallia*	*Gorgonia ventalina*
SRR935079	*Octocorallia*	*Gorgonia ventalina*
SRR935080	*Octocorallia*	*Gorgonia ventalina*
SRR935081	*Octocorallia*	*Gorgonia ventalina*
SRR935082	*Octocorallia*	*Gorgonia ventalina*
SRR935083	*Octocorallia*	*Gorgonia ventalina*
SRR935084	*Octocorallia*	*Gorgonia ventalina*
SRR935085	*Octocorallia*	*Gorgonia ventalina*
SRR935086	*Octocorallia*	*Gorgonia ventalina*
SRR935087	*Octocorallia*	*Gorgonia ventalina*

SRR935088	*Octocorallia*	*Gorgonia ventalina*
SRR935089	*Octocorallia*	*Gorgonia ventalina*
ERR3040053	*Octocorallia*	*Heliopora coerulea*
ERR3040054	*Octocorallia*	*Heliopora coerulea*
SRR12587798	*Octocorallia*	*Heliopora coerulea*
SRR12587799	*Octocorallia*	*Heliopora coerulea*
SRR12587800	*Octocorallia*	*Heliopora coerulea*
SRR12587801	*Octocorallia*	*Heliopora coerulea*
SRR12587803	*Octocorallia*	*Heliopora coerulea*
SRR12587805	*Octocorallia*	*Heliopora coerulea*
SRR12587806	*Octocorallia*	*Heliopora coerulea*
SRR12587807	*Octocorallia*	*Heliopora coerulea*
SRR12587808	*Octocorallia*	*Heliopora coerulea*
SRR5949848	*Octocorallia*	*Heliopora coerulea*
SRR7521178	*Octocorallia*	*Paramuricea biscaya*
SRR7521179	*Octocorallia*	*Paramuricea biscaya*
SRR7521180	*Octocorallia*	*Paramuricea biscaya*
SRR7521181	*Octocorallia*	*Paramuricea biscaya*
ERR3664727	*Octocorallia*	*Paramuricea clavata*
ERR3664728	*Octocorallia*	*Paramuricea clavata*
ERR3664729	*Octocorallia*	*Paramuricea clavata*
ERR3664730	*Octocorallia*	*Paramuricea clavata*
SRR13925244	*Octocorallia*	*Paramuricea sp.* type B3
SRR12573942	*Octocorallia*	*Sarcophyton trocheliophorum*
SRR12573944	*Octocorallia*	*Sarcophyton trocheliophorum*
SRR12573945	*Octocorallia*	*Sarcophyton trocheliophorum*
SRR12573946	*Octocorallia*	*Sarcophyton trocheliophorum*
ERR3026434	*Octocorallia*	*Sinularia cruciata*
ERR3026435	*Octocorallia*	*Tubipora musica*
SRR9278440	*Octocorallia*	*Xenia sp.*
SRR9278441	*Octocorallia*	*Xenia sp.*
SRR9278446	*Octocorallia*	*Xenia sp.*
SRR9278447	*Octocorallia*	*Xenia sp.*
SRR8113906	*Octocorallia*	*Xenia sp.* KK-2018
SRR8113907	*Octocorallia*	*Xenia sp.* KK-2018
SRR8113908	*Octocorallia*	*Xenia sp.* KK-2018
SRR8113909	*Octocorallia*	*Xenia sp.* KK-2018

Supplementary Table 6. Results from i-TASSER modelling of EcAT1 showing top 10 most similar structures from PDB.

Rank	PDB Hit (Accession #)	TM-score	RMSD	IDEN	Cov	Description
1	7kvw	0.75	3.09	0.125	0.840	Nonribosomal didomain (holo-PCP-C) acceptor bound state
2	3fp0	0.75	4.11	0.095	0.888	Structural and functional characterization of TRI3 Trichothecene 15-*O*-acetyltransferase from *Fusarium sporotrichioides*
3	6n8e	0.73	2.93	0.089	0.808	Crystal structure of holo-ObiF1, a five domain nonribosomal peptide synthetase from *Burkholderia diffusa*
4	6ad3	0.73	3.48	0.095	0.830	Structural characterization of the condensation domain from monacolin K polyketide synthase MokA
5	5u89	0.72	3.30	0.097	0.822	Crystal structure of a cross-module fragment from the dimodular NRPS DhbF
6	6mfz	0.72	3.01	0.097	0.801	Crystal structure of dimodular LgrA in a condensation state
7	4zxh	0.72	3.45	0.101	0.822	Crystal structure of holo-AB3403 a four domain nonribosomal peptide synthetase from *Acinetobacter baumannii*
8	5t3e	0.72	3.03	0.118	0.805	Crystal structure of a nonribosomal peptide synthetase heterocyclization domain
9	5isw	0.72	3.57	0.099	0.824	Structure of the apo PCP-E didomain of the gramicidin S synthetase A
10	4jn3	0.71	2.94	0.107	0.793	Crystal structures of the first condensation domain of the CDA synthetase

Supplementary Table 7. Primers used in this study.

Primer Name	Primer Sequence	Target Name	Restriction Sites
308529	ATGTCGCATGTCTATTTTGCATC	EcTPS1	
308530	TTACATCAGGACACCATTGCC	EcTPS1	
309044	TAAGCACATATGTCGCATGTCTATTTTGCATC	EcTPS1	NdeI/XhoI
309045	TGCTTACTCGAGTTACATCAGGACACCATTGCC	EcTPS1	NdeI/XhoI
308527	ATGGCATTTAGTAGGCCCCTT	EcTPS2	
308528	TTATTTTATCAAGAACAACCCGTCC	EcTPS2	
308932	TAAGCACATATGGCATTTAGTAGGCCCCTT	EcTPS2	NdeI/XhoI
308933	TGCTTACTCGAGTTATTTTATCAAGAACAACCCGTCC	EcTPS2	NdeI/XhoI
308543	ATGGCATGCAGTAAGGAATTG	EcTPS3	
308532	TCATAGTTGTAGTAACTTGTCGAGG	EcTPS3	
308934	TAAGCACATATGGCATGCAGTAAGGAATTG	EcTPS3	NdeI/XhoI
308935	TGCTTACTCGAGTCATAGTTGTAGTAACTTGTCGAGG	EcTPS3	NdeI/XhoI
308537	ATGACGAACAAAAGCGAATTA	EcTPS5	
308538	TCAAGCGGGCAATAGATTTG	EcTPS5	
308937	TGCTTACTCGAGTCAAGCGGGCAATAGATTTG	EcTPS5	NdeI/NotI
308936	TAAGCAGCTAGCATGACGAACAAAAGCGAATTA	EcTPS5	NdeI/NotI
308536	ATGTTTCCTACAAGAGAAGTGC	EcTPS6	
308535	TTAATTCAATCCATAGATTAACACC	EcTPS6	
326330	TAAGCAGCTAGCATGTTTCCTACAAGAGAAGTGC	EcTPS6	NdeI/NotI
326331	TGCTTAGCGGCCGCTTAATTCAATCCATAGATTAACACC	EcTPS6	NdeI/NotI

Supplementary Note 1: Synthetic methods and compound characterization

General methods for NMR spectroscopy

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian iNOVA 500 (^{1}H 500 MHz) NMR spectrometer equipped with a 3 mm Nalorac MDBG probe or Varian iNOVA 600 (^{1}H 600 MHz) NMR spectrometer equipped with a 5 mm Varian inverse cold probe. ^{31}P NMR spectra were recorded on a Varian MERCURY 400 (^{1}H 400 MHz) NMR spectrometer equipped with a 5 mm Nalorac broadband probe, operated using VNMRJ 4.2. Data was processed and analyzed using MestreNova 9.1.0.

Synthesis of substrates for enzyme reactions

Synthetic methods are summarized in **Supplementary Note Scheme 1** and are based on reported methods, with changes as noted below[1,2]. Briefly, farnesyl chloride (**6**) was made from farnesol (**7**) by treating **7** with PCl_3 in place of PBr_3. Synthesis of **8**, **9** and **10** proceeded as previously reported except that farnesylacetone (**9**) was purified by vacuum distillation instead of chromatography. Reduction of ester **10** was performed using either diisobutylaluminum hydride (DIBAL) or lithium aluminum deuteride ($LiAlD_4$) to generate geranylgeraniol (GGOH) or deuterium labelled geranylgeraniol (D_2-GGOH). The pyrophosphate source tris(tetrabutylammonium) hydrogen pyrophosphate (TTBAHPP) was generated by passing a solution of sodium pyrophosphate through a column of DOWEX 50W X8 (H+ form) and titrating the resulting pyrophosphoric acid solution with tetrabutylammonium hydroxide to pH 7 as previously described. Preparation of GGPP from GGOH and TTBAHPP was performed as described allyl chloride **11** and provided a product with satisfactory ^{1}H, ^{13}C and ^{31}P-NMR spectra which was used for *in vitro* work without further purification after trituration from a mixture of acetonitrile and isopropanol. Preparation of D_2-GGPP from D_2-GGOH proceeded in a similar manner. Identity and purity of synthetic products were confirmed by analysis of ^{1}H and ^{13}C-NMR spectra and comparison to literature values. Reactions were monitored by thin layer chromatography (TLC), performed with silica plates (F254) and visualized with UV light a and/or iodine vapor.

Structure elucidation of enzyme reaction products

EcTPS1 enzyme fractions (100 mL total in elution buffer [50 mM sodium phosphates, 5 mM $MgCl_2$, 150 mM NaCl and 150 mM imidazole] obtained from a 30 L culture) were diluted with incubation buffer (400 mL containing 10% glycerol, 5 mM MgCl, 5 mM DTT and 20 mM sodium phosphate) to a final protein concentration of 0.8 µM, pH 6.8. Synthetic GGPP (120 mg) dissolved in water (50 mL) was slowly added dropwise at room temperature via syringe over 4 h to the enzyme solution with magnetic stirring. The reaction was then transferred to a shaking incubator at 25 °C and 180 RPM and left overnight. The reaction mixture was extracted with hexane (3x 200 mL). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure on a rotovap and the crude mixture fractionated by column chromatography on silica gel using a 4:1 pentane/diethyl ether mixture to yield klysimplexin R (**4**, 2.9 mg) as a colorless oil (**Supplementary Figure 3**).

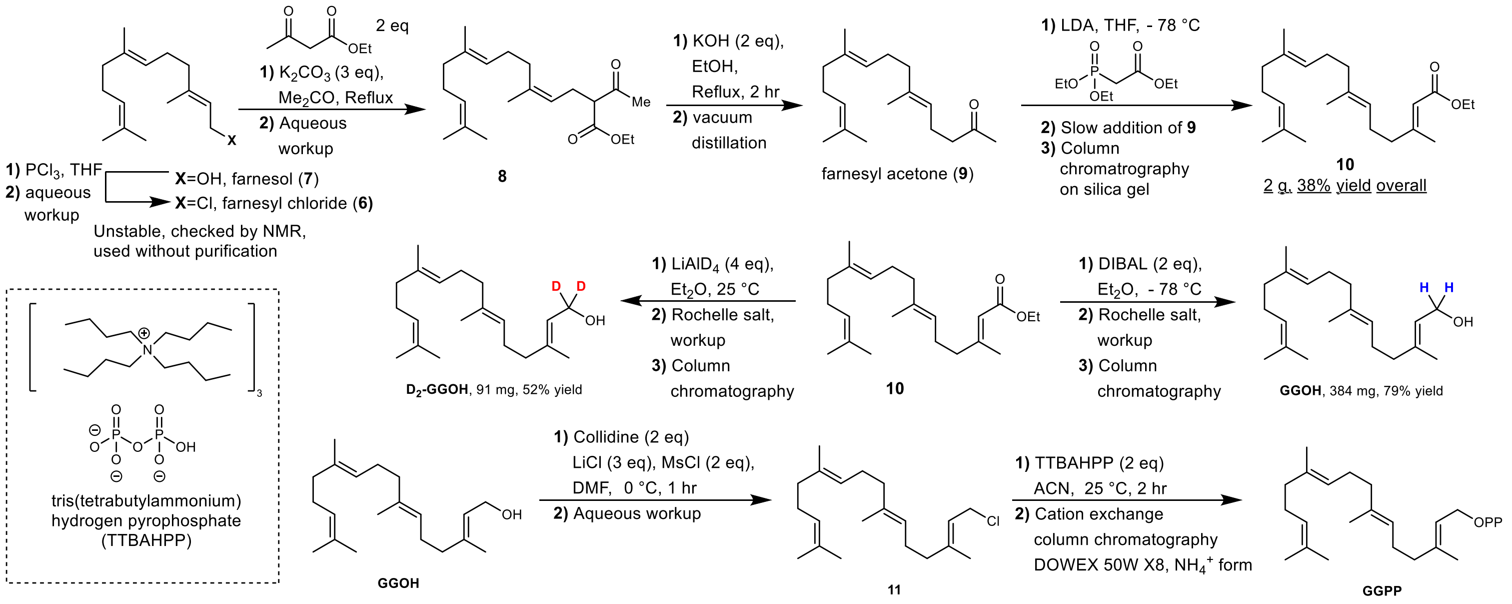
The molecular formula of **4** ($C_{20}H_{34}O$) was supported by preliminary analysis of the EIMS spectrum, with the fragment ion $[M-H_2O]^{+\cdot}$ at *m/z* 272.3, consistent with the diterpene backbone, and the faint parent ion $[M]^{+\cdot}$ at *m/z* 290.3 indicating the presence of a diterpene alcohol

(**Supplementary Figure 2**). This was further supported by HR-ESIMS (*m/z* 313.2535 $[M+Na]^+$, calcd. for $C_{20}H_{34}ONa$, 313.2502). The ^1H NMR spectrum (**Supplementary Note Figure 1**) of product **4** indicated the presence of two olefinic protons (δ_H 5.28 and 5.23). Two tertiary (δ_C 130.6 and 124.6) and two quaternary (δ_C 138.3 and 133.4) olefinic carbon signals were apparent in the ^{13}C NMR spectrum (**Supplementary Note Figure 2**), indicating that compound **4** is a bicyclic compound with two double bonds incorporated into rings. Two methyl groups exhibited resonances that are typical of allylic methyl groups (δ_H 1.71 and 1.52; each 3H, s; δ_C 25.0 and 17.0 respectively). Two additional methyl groups appeared to be aliphatic (δ_H, 0.97 and 0.77; each 3H, d, *J*=6.7; δ_C 22.1 and 18.5, respectively). A final methyl group was detected which indicated the presence of a tertiary alcohol (δ_H, 1.24; 3H, s; δ_C 26.1), which was corroborated by the presence of a single quaternary oxygenated carbon (δ_C 73.4). Sixteen of the remaining protons appeared in the region between δ_H 2.82 and 0.93 (**Supplementary Note Table 1**), and the final proton could not be detected and was presumed to be an exchangeable proton on an alcohol. The directly bonded carbon and hydrogen atoms were assigned based on the HSQC spectrum revealing three aliphatic methines, one allylic methine, three aliphatic methylenes and three allylic methylenes (**Supplementary Note Figure 3**). Using an extensive NMR spectroscopic analysis, including COSY and HMBC experiments (**Supplementary Note Table 1**), the structure of **4** was determined. The cross-peaks in the COSY spectrum between H_3-19/H_3-20 and H-18 as well as between H-18 and H-14 indicated the attachment of an isopropyl group to the ring system. Further cross-peaks in the COSY between H-1 and H-14 as well as H-10 eluded to the ring junction at C-1, while an elaborate array of HMBC correlations supported this branching point (cross-peaks between H-1 and C-2, C-3, C-9, C-10, C-11, C-13, C-14 and C-18). The remaining methylene carbons were assigned their respective positions by analysis of the COSY spectrum, with two separate spin-systems making up fragments of the carbon backbone. These fragments were connected by strategic use of the intense HMBC cross-peaks produced by the methyl resonances, with correlations between H_3-15 and C-2, C-3 and C-4 along with correlations between H_3-16 and C-6, C-7 and C-8 joining the two spin-systems and closing the macrocyclic ring. Finally, correlations between H_3-17 and C-10, C-11 and C-12 closed the remaining gap and revealed the cyclohexanol motif. The resulting structure and data were identical to those for the previously described octocoral metabolite klysimplexin R (**4**)[3].

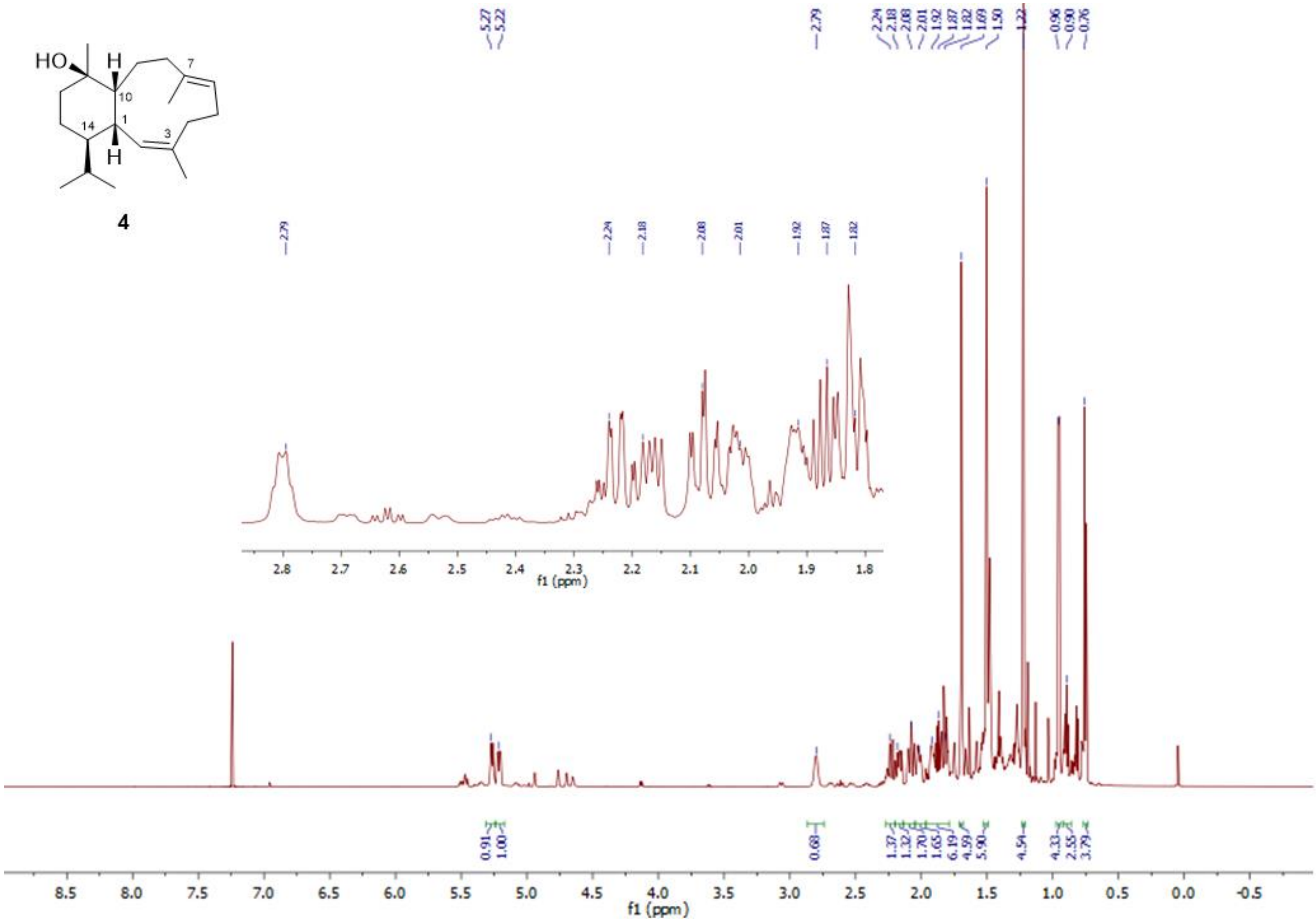
The product of EcTPS6 was determined in a similar manner without optimization. Scale-up protein purification and enzyme reactions followed extraction with hexane provided a yellow oil. While analytical scale enzyme reactions showed only a single peak by GCMS, at preparative scale a second, earlier eluting peak appeared on the GCMS chromatogram. Each peak showed an ion at *m/z* 272.3, which was assigned to the parent ion $[M]^{+\cdot}$ with formula $C_{20}H_{32}$ (**Supplementary Figure 2**). Purification of the crude enzyme product by silica gel column chromatography provided a clear oil eluting with pentane and showing a single spot by TLC, which upon further analysis by 1D ^1H-NMR and 2D HSQC spectroscopy showed only the presence of olefinic methine, allylic methyl and allylic methylene groups. In the ^1H-NMR spectrum collected in C_6D_6 at 600 MHz, two sets of resolved singlets between δ_H 1.75 and 1.45 were present, each attributed to five separate methyl resonances. Integration of each resonance indicated two pentamethylated products in 3:2 ratio. MS and NMR (collected in $CDCl_3$) data for the major isomer was consistent with a cembrene product reported from bacterial terpene cyclases (**Supplementary Note Figures 4 and 5, and Supplementary Note Table 2**)[4]. The other isomer was quite similar and is anticipated to be a double bond isomer, as has been previously observed in cembrene chemistry. This was supported by the presence of one methyl group shifted downfield (Me-15; δ_H 1.58/δ_C 15.7 in **5a** vs. δ_H 1.72/δ_C 23.8 in **5b**) olefin (H-14/H-15; δ_H 5.04/δ_C 124.6 in **5a** vs. δ_H 5.18/δ_C 125.2 in **5b**), as seen in (3*Z*)-cembrene A[5].

Mechanistic study of eunicellane skeleton formation

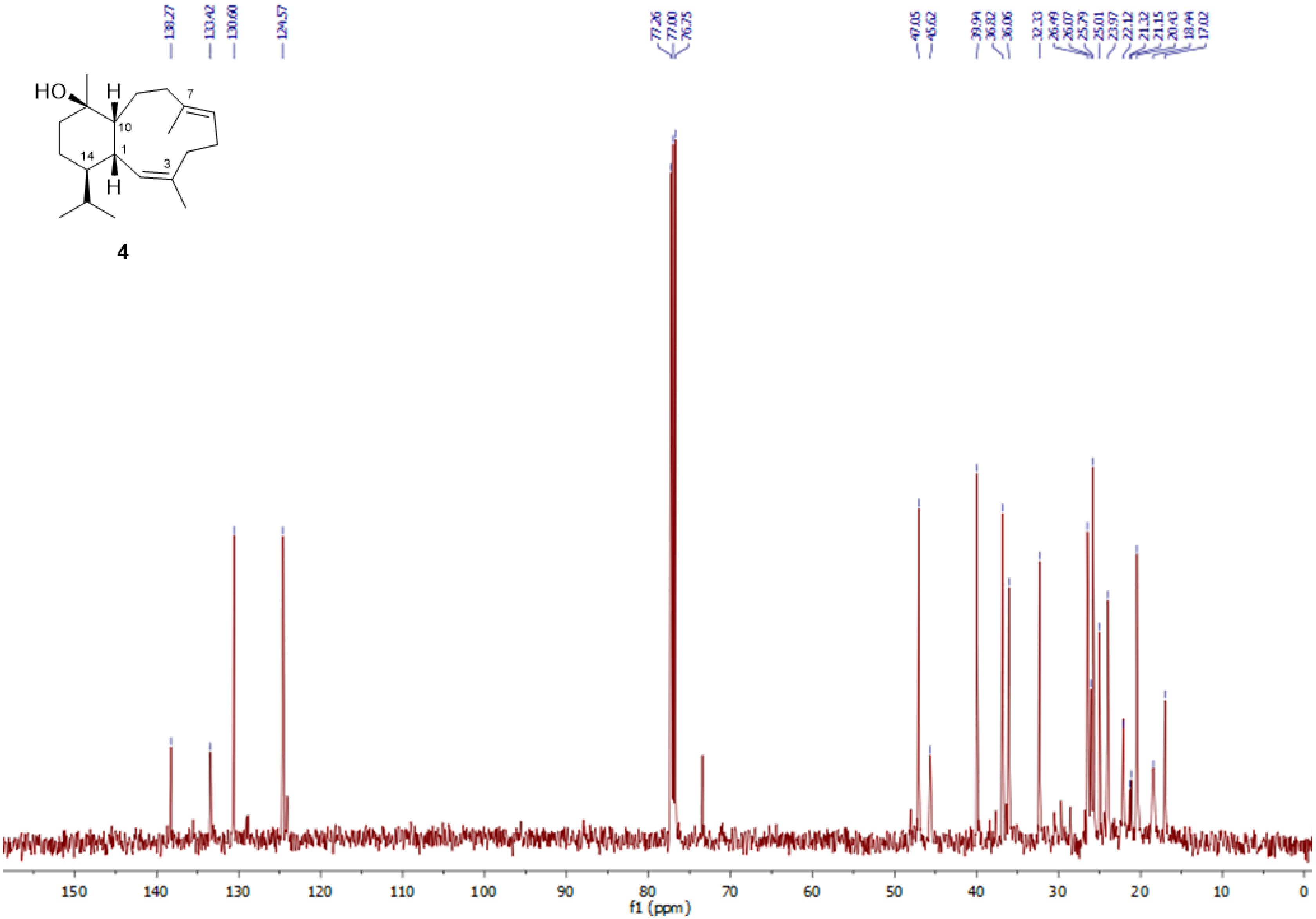
The $^2H_2$ -labelled isotopomer of 1,1-$^2H_2$-GGPP, (10 mg) was added to EcTPS1 in incubation buffer (20 mL). The mixtures were incubated for 16 h at 30 °C and extracted twice with pentane and the organic layer dried over sodium sulfate. The combined organic layers were analyzed by GCMS and NMR. The pattern of deuterium labelling was determined by analyzing the combined EI-MS and NMR data. The incorporation of two deuterium atoms overall was indicated by the observation of an EIMS fragment at 274.3 *m/z* ($[M\text{-}H_2O]^{+\bullet}$) (**Supplementary Note Figure 6**). The detection of an ion at 231.2 *m/z* ($[M\text{-}H_2O\text{-}C_3H_7]^{+\bullet}$) ruled out incorporation of deuterium at the isoproppyl side chain (ie. C-18). In the ^{1}H-NMR spectrum of the deuterated product (**Supplementary Figure 2**), the H-1 resonance (δ_H 2.82 in unlabeled product) was clearly absent and the H-2 resonance appeared as a broad singlet (δ_H 5.29, d, *J*=9.0 Hz in unlabeled product), indicating retention of deuterium at C-1 during the cyclization cascade. A DPFGSE 1D TOCSY experiment with selective irradiation on the H_3-19 resonance (δ_H 0.98) showed enhancement of the H-18 resonance (δ_H 1.88, septet, *J*=6.4 Hz in the labelled product), further confirming that deuterium was not present on the isopropyl side chain (**Supplementary Note Figure 7**). In this DPFGSE 1D TOCSY experiment, the H-14 resonance (δ_H 0.94) could not be observed. Taken together, these data indicate that deuterium is incorporated at C-1 as well as a second position on the cyclohexyl ring. Comparison of the ^{1}H-^{13}C HSQC spectra of the EcTPS1 products derived from both 1,1-$^2H_2$-GGPP and GGPP with natural isotopic abundance revealed the disappearance of the C-1 and C-14 correlations, consistent with deuteration at these positions (**Supplementary Note Figure 3**). All other resonances were present in the 1,1-$^2H_2$-GGPP-derived product. Finally, the position of the deuterium at C-14 was confirmed by careful comparison of the ^{1}H-^{13}C HMBC spectra for both 1,1-$^2H_2$-GGPP and GGPP derived products, revealing a chemical shift isotope effect at C-14 (**Supplementary Note Figure 8**). Accurate inverse measurements of the chemical shifts for the C-14, C-18 and C-19 resonances were determined from the 2D HMBC spectrum by extracting the 1D traces along F1 at 0.77 ppm (H_3-20 resonances). While the ^{13}C chemical shifts for C-18 and C-19 were nearly identical (δ_C 26.500 and 22.252±0.059, respectively) when measured by this method, the C-14 resonances for the 1,1-$^2H_2$-GGPP and GGPP derived products were significantly different, with measured values of δ_C 45.253 and 45.720, respectively. This equates to a chemical shift isotope effect of $\Delta\delta$ -467 ppb on the C-14 resonance of the 1,1-$^2H_2$-GGPP derived product, consistent with deuterium labelling at C-14. Thus, the incorporation of deuterium was determined to be at C-1 and C-14.



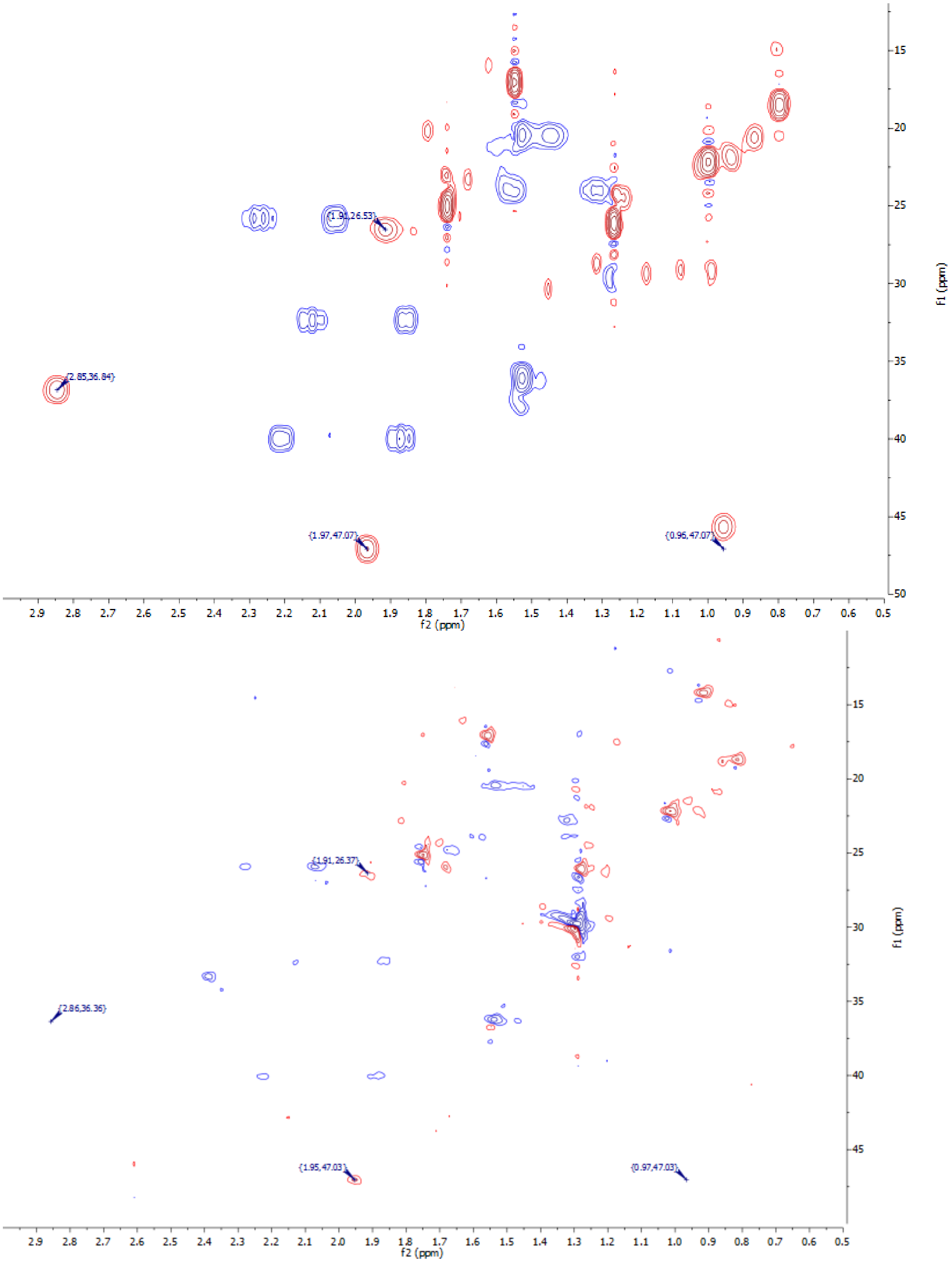
Supplementary Note Scheme 1. Synthesis of oligoprenyl pyrophosphate substrates starting from commercially available farnesol.



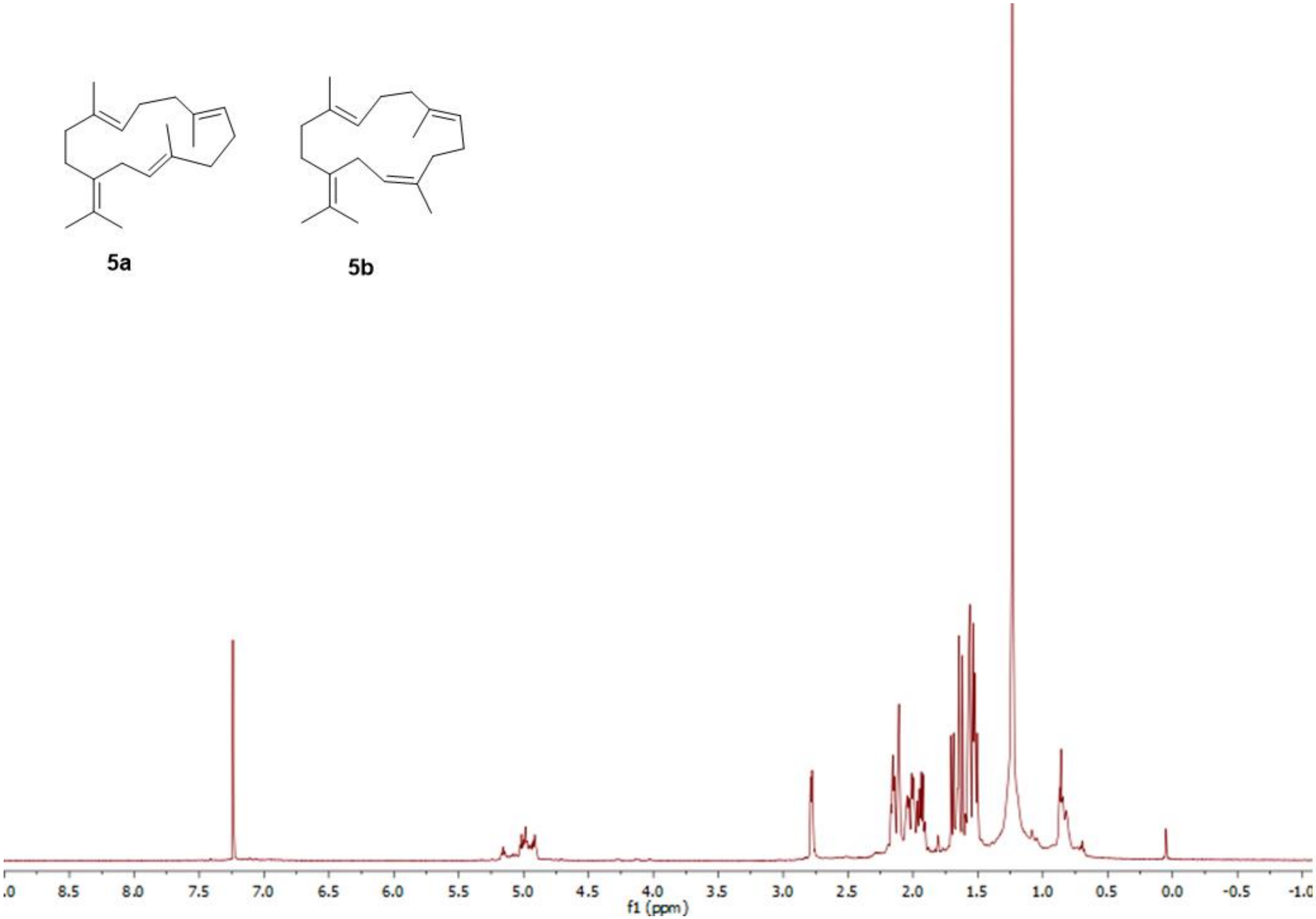
Supplementary Note Figure 1. ^{1}H NMR spectrum of **4** in $CDCl_3$ at 500 MHz.



Supplementary Note Figure 2. ^{13}C NMR spectrum of **4** in $CDCl_3$ at 125 MHz.



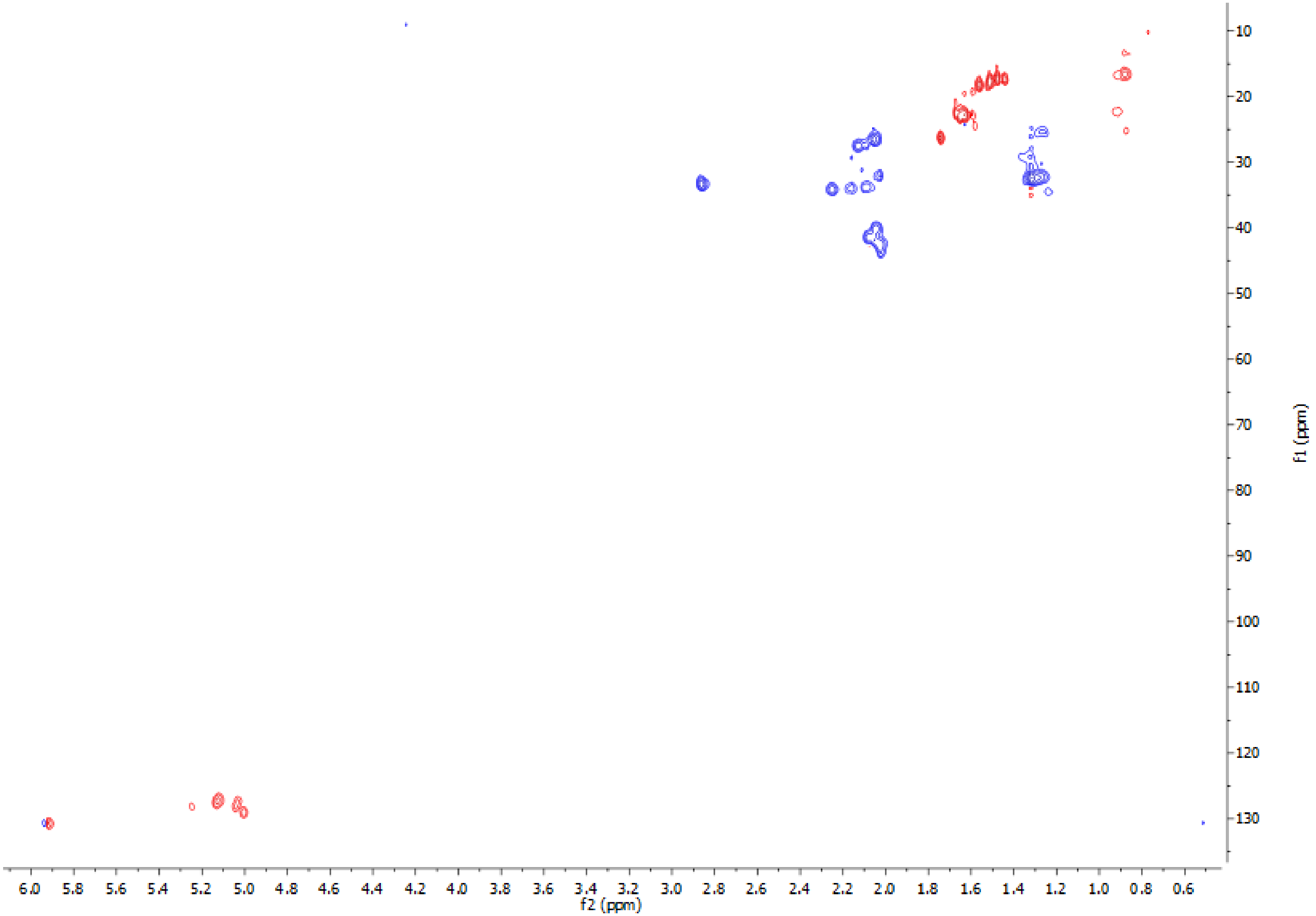
Supplementary Note Figure 3. ^{1}H-^{13}C HSQC NMR spectrum of **4** (top spectrum) and 1,14-$^{2}H_2$-**4** (bottom spectrum) in $CDCl_3$ at 500 MHz (^{1}H detection).



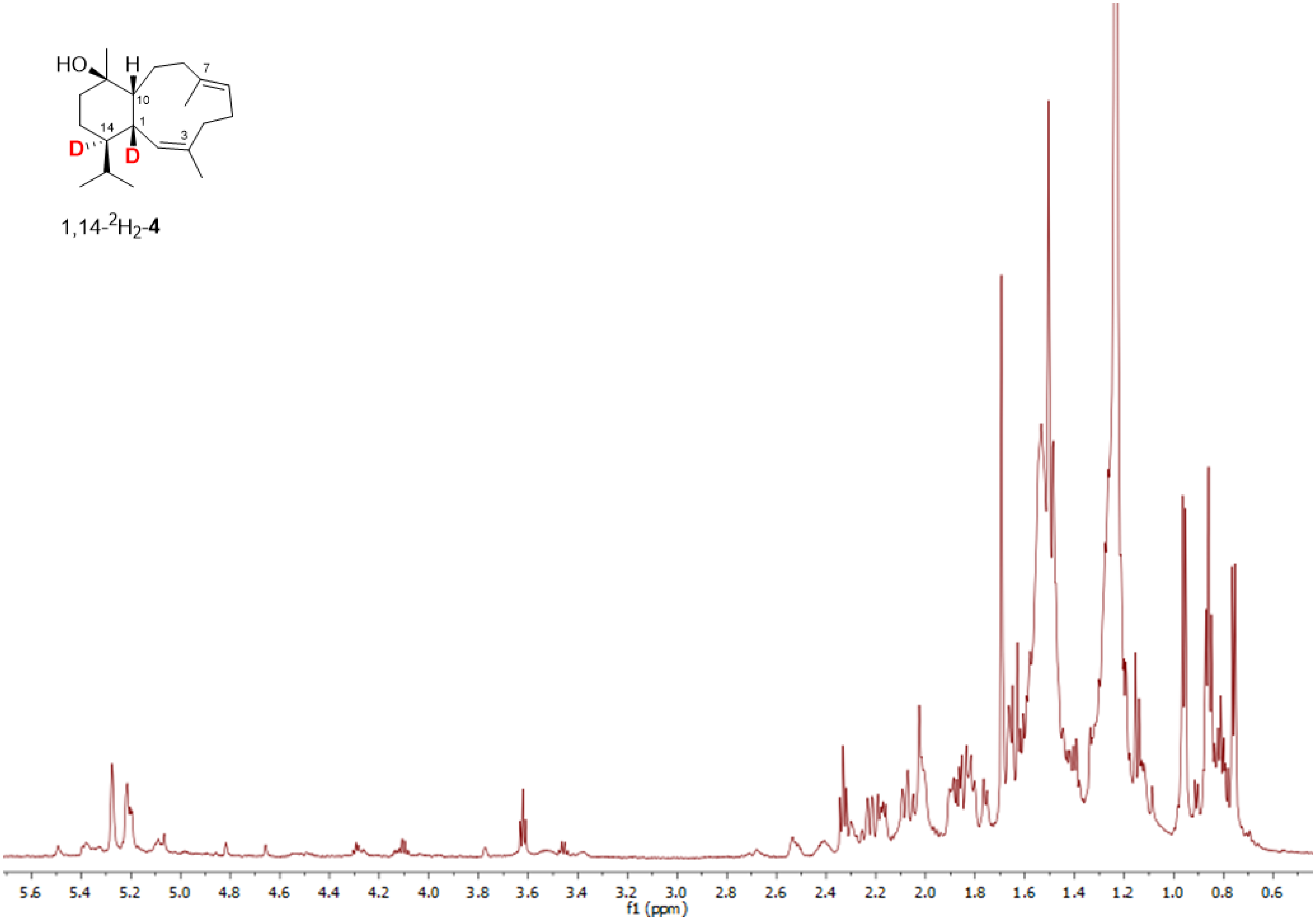
5a

5b

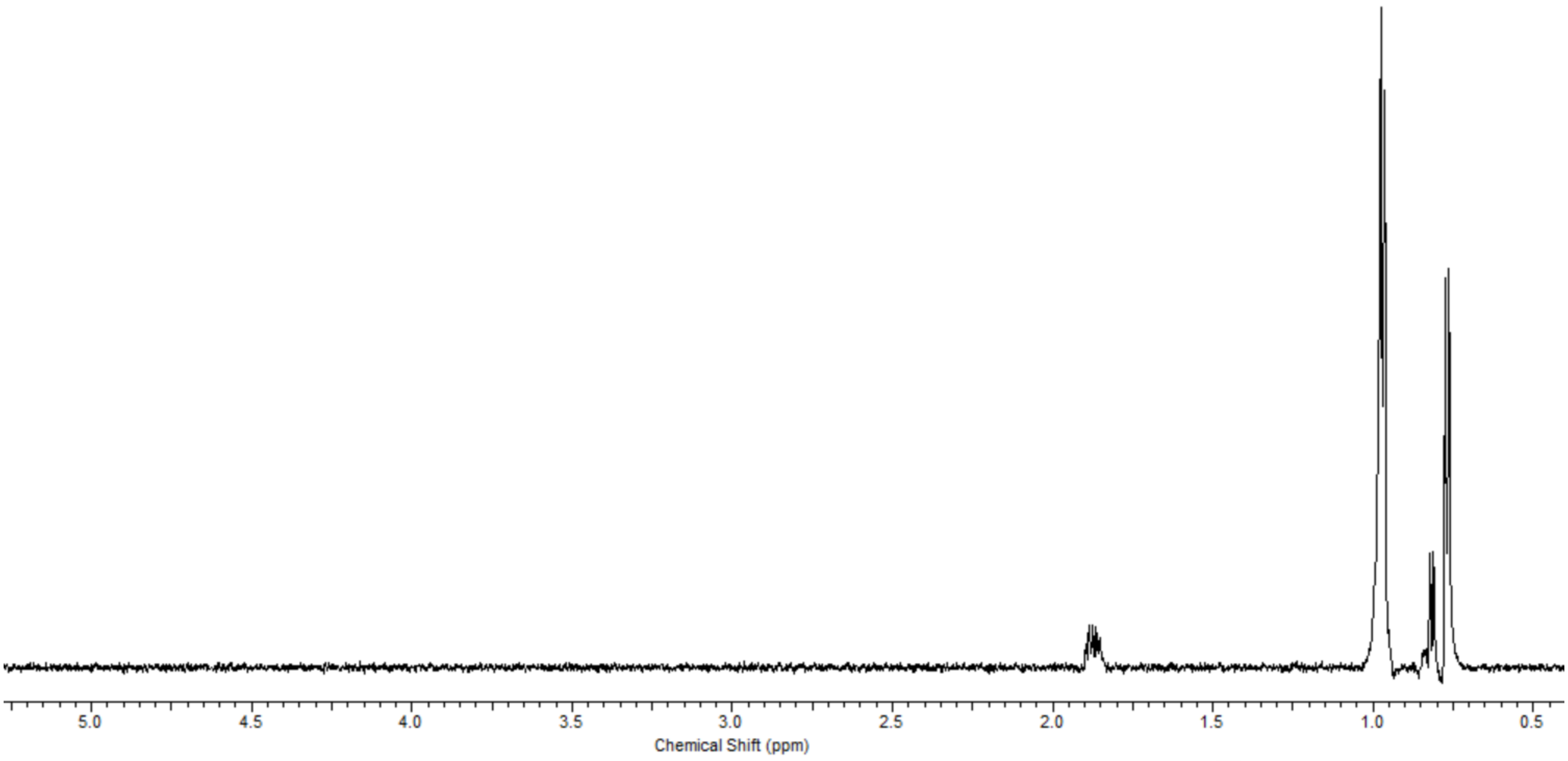
Supplementary Note Figure 4. ^{1}H NMR spectrum of **5a** and **5b** in $CDCl_3$ at 500 MHz.



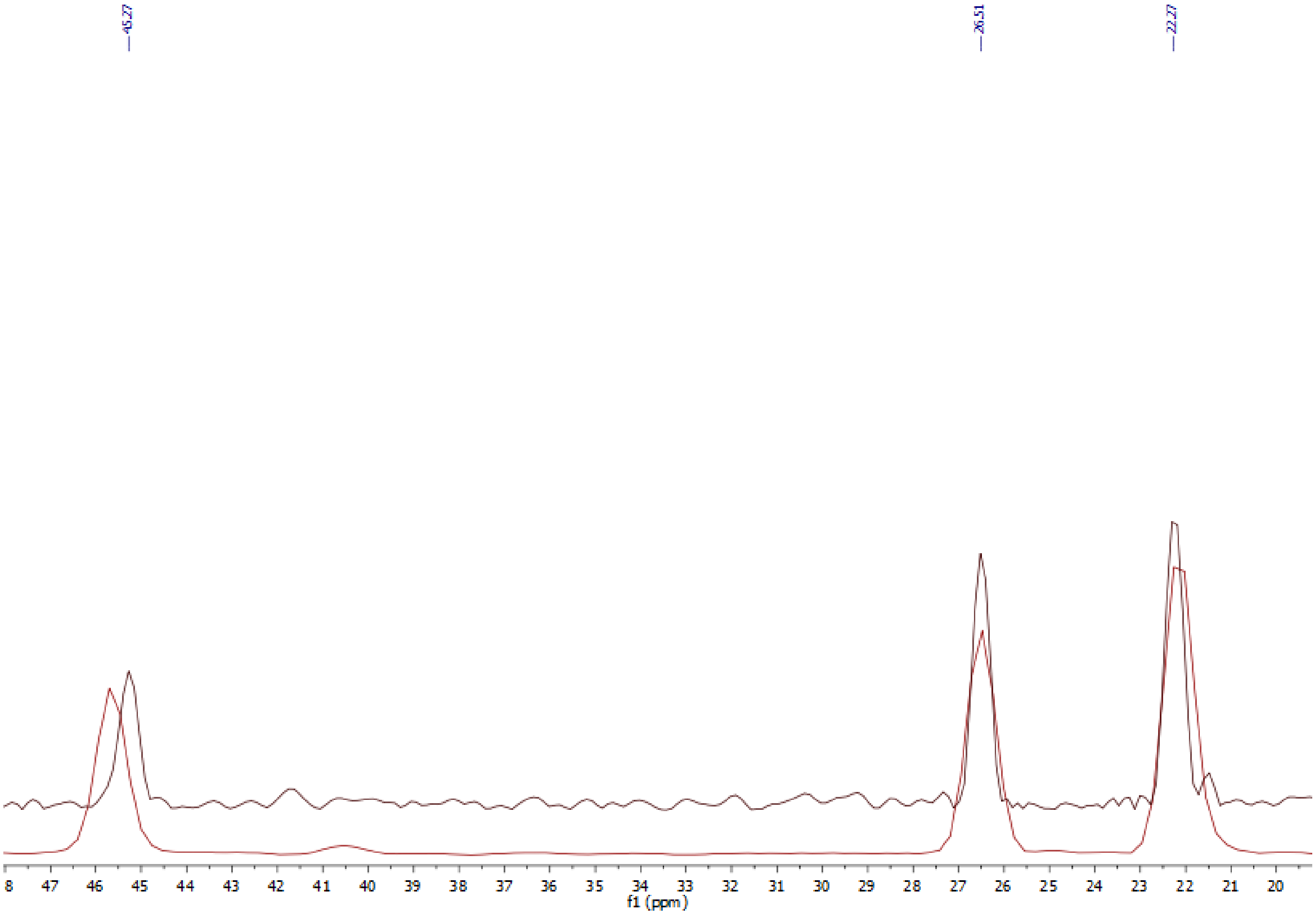
Supplementary Note Figure 5. ^{1}H-^{13}C HSQC NMR spectrum of **5** in $CDCl_3$ at 500 MHz (^{1}H detection).



Supplementary Note Figure 6. ^{1}H NMR spectrum of 1,14-$^{2}H_2$-**4** in $CDCl_3$ at 500 MHz.



Supplementary Note Figure 7. ^{1}H DPFGSE 1D TOCSY NMR spectrum of 1,14-$^2H_2$-**4** with selective excitation at 0.98 ppm in $CDCl_3$ at 500 MHz.

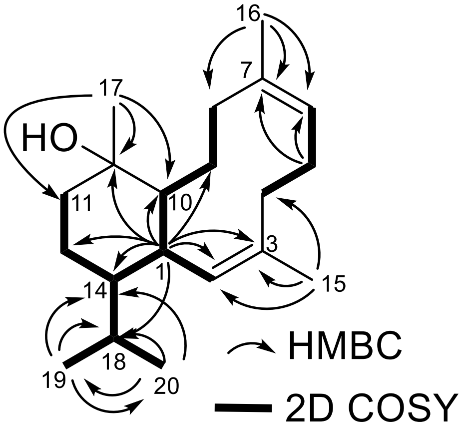


Supplementary Note Figure 8. 1D slices along F2 axis at 0.77 ppm from ^{1}H-^{13}C HMBC NMR spectrum of **4** (lower trace) and 1,14-$^{2}H_2$-**4** (upper trace) in $CDCl_3$ at 500 MHz (^{1}H detection).

Supplementary Note Table 1. ^{1}H and ^{13}C-NMR data for klysimplexin R (**4**) in $CDCl_3$ at 500 MHz.

Position	This work		Chen et. al.	
	δ_C[a]	δ_H[b]	δ_C	δ_H
1	36.8 (CH)	2.82 (br q, 6.5)	36.8	2.82 (m)
2	130.6 (CH)	5.28 (d, 8.8)	130.6	5.29 (d, 9.0)
3	133.4 (C)		133.4	
4	32.3 (CH_2)	2.10 (td, 12.2, 2.9)	32.3	2.10 (m)
		1.83 (dt, 11.7, 3.9)		1.83 (m)
5	25.8 (CH_2)	2.25 (qd, 12.0, 2.0)	25.8	2.25 (m)
		2.03 (m)		2.03 (m)
6	124.6 (CH)	5.23 (dd, 10.7, 4.9)	124.6	5.24 (dd, 11.0, 4.5)
7	138.3 (C)		138.3	
8	39.9 (CH_2)	2.19 (br dd, 11.7, 5.9)	39.9	2.19 (m)
		1.85 (br t, 13.5)		1.85 (m)
9	24.0 (CH_2)	1.53 (m)	24.0	1.57 (m)
		1.29 (m)		1.29 (m)
10	47.1 (CH)	1.94 (br q, 4.9)	47.0	1.94 (m)
11	73.4 (C)		73.5	
12	36.1 (CH_2)	1.50 (m)	36.1	1.48 (m)
13	20.4 (CH_2)	1.50 (m)	20.4	1.53 (m)
		1.42 (m)		1.42 (m)
14	45.6 (CH)	0.93 (td, 6.3, 2.4)	45.6	0.94 (m)
15	25.0 (CH_3)	1.71 (br s)	25.0	1.72 (s)
16	17.0 (CH_3)	1.52 (br s)	17.0	1.53 (s)
17	26.1 (CH_3)	1.24 (s)	26.1	1.25 (s)
18	26.5 (CH)	1.89 (m)	26.5	1.88 (m)
19	22.1 (CH_3)	0.97 (d, 6.7)	22.1	0.98 (d, 7.0)
20	18.4 (CH_3)	0.77 (d, 6.7)	18.5	0.77 (d, 7.0)

[a] Multiplicity determined by 2D HSQC. [b] Referenced to residual $CHCl_3$ at 7.26 ppm.



Selected 2D NMR correlations for **4**.

Supplementary Note Table 2. ^{1}H and ^{13}C-NMR data for **5a** and **5b** in $CDCl_3$ at 500 MHz.

Position	**5a** Kuzuyama et. al.		**5a** (This work)		**5b** (This work)	
	δ_C	δ_H	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
2	39.0	2.11 (t, 6.2)	38.9 (CH_2)	2.12[c]	31.0 (CH_2)[c, d]	1.97[c, d]
3	24.9	2.16 (m)	24.7 (CH_2)	2.15[c]	29.1 (CH_2)[c, d]	2.00[c, d]
4	126.3	4.92 (t, .6.8)	126.1 (CH)	4.94 (t, 7.0)	126.0 (CH)	4.96
6	40.0	2.00 (t, 5.8)	39.8 (CH_2)	2.00[c]	39.8 (CH_2)	2.00[c, d]
7	23.8	2.05 (m)	23.7 (CH_2)	2.06[c]	31.0 (CH_2)[c, d]	2.03[c, d]
8	124.1	4.99 (t, 7.6)	124.2 (CH)	5.01 (t, 5.0)	124.4 (CH)	4.98
10	37.4	1.93 (t, 7.6)	37.3 (CH_2)	1.93[c]	41.0 (CH_2)[c, d]	1.94[c, d]
11	31.4	2.16 (t, 7.6)	31.3 (CH_2)	2.18 (t, 7.6)	30.8 (CH_2)[c, d]	1.96[c, d]
13	30.5	2.80 (d, 7.6)	30.5 (CH_2)	2.80 (d, 7.0)	30.9 (CH_2)	2.12
14	124.8	5.02 (t, 7.6)	124.6 (CH)	5.04 (t, 7.0)	125.2 (CH)	5.18 (t, 7.6)
15	15.9	1.58 (s)	15.7 (CH_3)	1.59 (s)	23.8 (CH_3)	1.72 (s)
16	15.0	1.54 (s)	15.0 (CH_3)	1.56 (s)	15.0 (CH_3)	1.52 (s)
17	15.3	1.57 (s)	15.0(CH_3)	1.58 (s)	15.0 (CH_3)	1.54 (s)
19	20.5	1.63 (s)	20.2 (CH_3)	1.64 (s)	19.7 (CH_3)	1.71 (s)
20	20.7	1.65 (s)	20.4 (CH_3)	1.67 (s)	19.7 (CH_3)	1.68 (s)

[a] Measured by 2D ^{1}H-^{13}C HSQC. [b] Referenced to residual $CHCl_3$ at 7.26 ppm. [c] Multiplicity not reported due to signal overlap. [d] Position of assignments interchangeable.

Supplementary Note 2: Computational chemistry methods and data

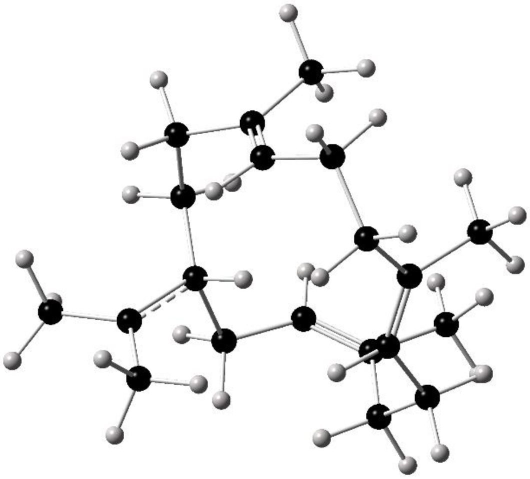
Computational methods

The deuterium label resulted in a model that was tested using quantum mechanical calculations. Initial conformer distributions were determined by MMFF methods implemented in Spartan 14 v1.1.2 software to provide starting structures. All further DFT calculations were performed using the Gaussian16 program.[6] Structures were optimized by DFT using the B3LYP functional and 6-31g(d) basis set in the gas phase. Stationary points were determined to be minima (no imaginary frequencies) or transition states (one imaginary frequency) by frequency analysis. The identity of transition states was further supported by IRC calculations. The Gibb's free energy of stationary points was computed as the sum of electronic and thermal free energies using single point frequency calculations with the mPW1PW91 functional and 6-311+g(d,p) basis set. These energies were calculated in gas phase as well as in water using the CPCM implicit solvent model (**Supplementary Information Table 10**).

Supplementary Note Table 1. Atomic coordinates and energies for DFT calculated intermediate $\mathbf{A^+}$**.**

Intermediate $\mathbf{A^+}$

C	-0.13515100	2.55735300	2.23455000
C	1.55613000	-2.01281100	0.42943200
C	2.19785700	0.41747400	1.30542200
C	1.77682900	-0.37495500	0.06210300
C	2.06258000	1.94723200	1.08416100
C	0.11073400	2.63387600	-0.28489200
C	2.21725000	-0.66547600	-2.41559500
C	4.17151900	-0.58864500	-0.80186800
C	-1.28072800	3.04659900	-0.67752700
C	0.36752000	-2.13963000	1.31085300
C	-0.88741300	-2.48679200	0.94225400
H	0.78815300	2.51706600	-1.13589200
H	0.79324500	-0.05961400	-0.29271500
C	-1.98435300	2.01245200	-1.59687300
C	-2.42668400	0.71605900	-0.94107100
C	-3.42902300	0.87358900	0.17780300
C	-1.99871000	-0.46873000	-1.41420400
C	-2.44365100	-1.85836800	-1.03264400
H	0.32350100	3.34078300	2.85254500
H	-0.10725100	1.63593700	2.83173800
H	-1.18380700	2.82348800	2.08996900
H	3.22415100	0.17034200	1.59959400
H	1.55701600	0.11776200	2.13941700
H	1.45188100	-2.58234300	-0.49487600
H	2.64482800	2.24340300	0.20135300
H	2.52717700	2.44727900	1.94350200
H	2.47378400	-2.32243700	0.93577000
H	2.64171700	-1.55835700	-2.89430500
H	2.61248700	0.18407800	-2.99788300
H	1.12913500	-0.66443300	-2.49481400
H	4.42543700	-1.10418200	0.13046000
H	4.53958100	0.44384700	-0.67670300
H	4.70936100	-1.03619800	-1.64054800
H	-1.21852800	3.98988500	-1.23776600
H	-1.90281400	3.25691600	0.19694800
H	0.55261800	-1.94162900	2.36424600
H	-1.33127000	1.79171000	-2.45118400
H	-2.87786100	2.49957100	-2.01366500
H	-4.25432900	1.52599400	-0.13505500
H	-3.86373800	-0.07323300	0.50492700
H	-2.97394000	1.34729600	1.05684800
H	-1.30195200	-0.44248800	-2.25689700
H	-2.82717400	-2.35039100	-1.93701000
H	-3.28126300	-1.83378700	-0.33107700
C	0.62045700	2.40371000	0.93639300
C	-1.32545500	-2.78457500	-0.47276600
H	-1.71476000	-3.81251600	-0.48487100
H	-0.47813800	-2.76911300	-1.16720700
C	-1.94133900	-2.65667800	2.00663600
H	-2.41369300	-3.64418400	1.93107400
H	-1.52968100	-2.54813000	3.01343700
H	-2.74333100	-1.91700300	1.88970900
C	2.70654000	-0.51757200	-1.02534600



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Calculation Method = RmPW1PW91
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Spin = Singlet
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Imaginary Freq = 0
Dipole Moment = 10.549386 Debye
Polarizability (?) = 336.62333 a.u.
Point Group = C1
Job cpu time: 0 days 19 hours 34 minutes 29.0 seconds.

Thermo Tab Data Section:
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Pressure = 1 atm
Frequencies scaled by = 1
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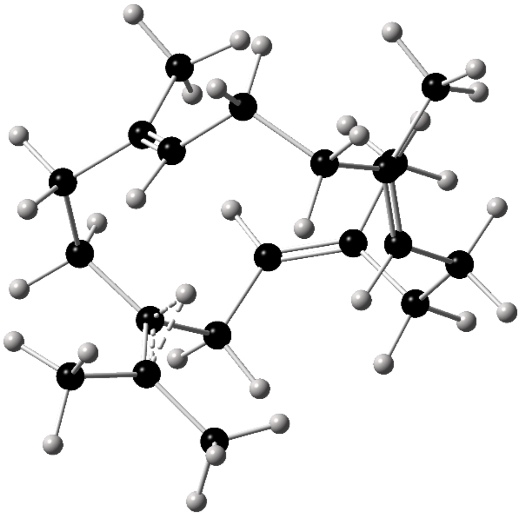
Zero-point Energy Correction = 0.482156 Hartree
Thermal Correction to Energy = 0.504822 Hartree
Thermal Correction to Enthalpy = 0.505766 Hartree
Thermal Correction to Free Energy = 0.43344 Hartree
EE + Zero-point Energy = -781.29052 Hartree
EE + Thermal Energy Correction = -781.26786 Hartree
EE + Thermal Enthalpy Correction = -781.26691 Hartree
EE + Thermal Free Energy Correction = -781.33924 Hartree
E (Thermal) = 316.781 kcal/mol
Heat Capacity (Cv) = 89.916 cal/mol-kelvin
Entropy (S) = 152.224 cal/mol-kelvin

Supplementary Note Table 2. Atomic coordinates and energies for DFT calculated transition state **TS$_{AB}$.**

Transition state TS$_{AB}$

C	0.67188200	2.91928000	1.38367600
C	0.51631100	-0.26791500	0.23205700
C	2.75876600	0.31825500	1.24015200
C	1.97654200	-0.70142400	0.40211300
C	2.73057200	1.69791000	0.51802600
C	0.57444000	1.56278100	-0.75440300
C	2.67349800	-1.15276600	-0.91955500
C	4.15071500	-1.51935000	-0.68960000
C	1.94986700	-2.35132900	-1.56016000
C	-0.73438500	2.19199600	-1.20588600
C	-0.37857800	-0.26208500	1.39500000
C	-1.41074100	-1.12572900	1.58693800
C	-2.19891900	-1.06336200	2.86932700
C	-1.80863700	-2.24775300	0.64549000
H	1.17068700	1.15554900	-1.57006200
H	0.04231700	-0.69313100	-0.64692500
C	-1.68118100	1.37314400	-2.11965600
C	-2.58733400	0.46098100	-1.32107200
C	-3.65113400	1.15159600	-0.49910900
C	-2.38793900	-0.86476300	-1.34290900
C	-2.92567700	-1.88180200	-0.38214000
H	1.21074500	2.84873900	2.33313800
H	0.73478700	3.96929200	1.05728400
H	-0.38644000	2.70077600	1.54391100
H	3.79325500	0.01044700	1.40356500
H	2.30222400	0.41687000	2.23173500
H	1.88903500	-1.61001800	1.02138500
H	3.25573600	1.62021500	-0.43823200
H	3.24089700	2.44440700	1.13481200
H	2.65121500	-0.32479100	-1.64271600
H	4.58086000	-1.93089200	-1.60789000
H	4.25368600	-2.28211300	0.09230200
H	4.76439500	-0.65859400	-0.40642100
H	0.90927200	-2.13938300	-1.82999000
H	1.95305100	-3.21651900	-0.88535000
H	2.45924500	-2.65149500	-2.48114600
H	-0.40993600	3.08104700	-1.76827100
H	-1.29664200	2.57684500	-0.35201000
H	-0.12780300	0.39420100	2.22557600
H	-2.04432400	-1.97310500	3.46393300
H	-3.27460000	-1.01693800	2.65424600
H	-1.93428800	-0.19995100	3.48575600
H	-0.94021400	-2.62014700	0.09172000
H	-2.16415000	-3.08785900	1.25420900
H	-1.09579400	0.80087300	-2.85009400
H	-2.27681200	2.09564100	-2.69226800
H	-4.35567300	0.45143600	-0.04638200
H	-3.23066900	1.76149000	0.31135100
H	-4.23026700	1.83377500	-1.13470800
H	-1.63941600	-1.25347500	-2.03788600
H	-3.20998300	-2.80593300	-0.89902800
H	-3.81452600	-1.52643200	0.14711900
C	1.29862200	2.06719800	0.32793400



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RMS Gradient Norm = 0.001495216 Hartree/Bohr
Imaginary Freq = 1
Dipole Moment = 9.1505554 Debye
Polarizability (?) = 313.756 a.u.
Point Group = C1
Job cpu time: 0 days 20 hours 0 minutes 48.5 seconds.

Thermo Tab Data Section:
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Temperature = 298.15 Kelvin
Pressure = 1 atm
Frequencies scaled by = 1
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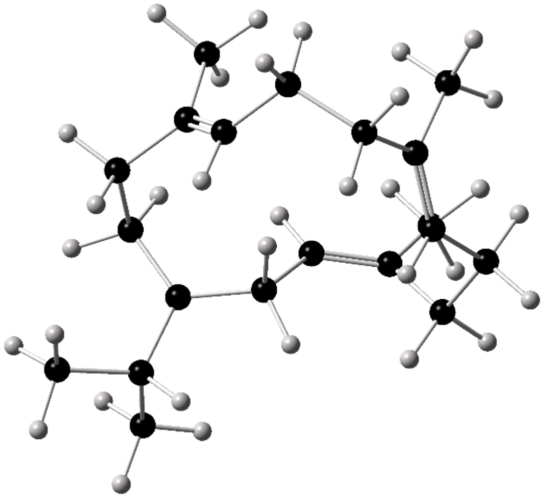
Zero-point Energy Correction = 0.480398 Hartree
Thermal Correction to Energy = 0.502321 Hartree
Thermal Correction to Enthalpy = 0.503265 Hartree
Thermal Correction to Free Energy = 0.432614 Hartree
EE + Zero-point Energy = -781.27868 Hartree
EE + Thermal Energy Correction = -781.25675 Hartree
EE + Thermal Enthalpy Correction = -781.25581 Hartree
EE + Thermal Free Energy Correction = -781.32646 Hartree
E (Thermal) = 315.211 kcal/mol
Heat Capacity (Cv) = 87.933 cal/mol-kelvin
Entropy (S) = 148.699 cal/mol-kelvin

Supplementary Note Table 3. Atomic coordinates and energies for DFT calculated intermediate **B$^+$.**

Intermediate B$^+$

C	-0.99541800	3.29345100	1.36351600
C	2.08543600	-1.02940400	-1.05843200
C	1.08851700	1.07399300	0.07417700
C	2.32841400	0.46055500	-0.63620100
C	0.57071100	2.42085500	-0.48073000
C	-1.85588400	2.17049600	-0.74127700
C	3.64799100	0.77657200	-0.08311700
C	3.85798900	1.93289700	0.80495800
C	4.83798200	-0.04109500	-0.41786800
C	-3.31339600	2.03105100	-0.41282700
C	1.74837100	-1.87819800	0.13914800
C	0.69439800	-2.69698100	0.31442600
H	-1.58805700	1.75808500	-1.71731200
H	1.26680500	-1.02288300	-1.78142000
C	-3.81847900	0.60846200	-0.77368000
C	-3.12936300	-0.54521100	-0.06227200
C	-3.36316900	-0.61609800	1.42727800
C	-2.43038800	-1.45090900	-0.77027800
C	-1.84556800	-2.76767500	-0.32160700
H	-0.62071700	4.32598800	1.33523800
H	-2.03290300	3.33333200	1.70175500
H	-0.41358300	2.77010800	2.13535000
H	1.25772900	1.13320400	1.15542300
H	0.28325900	0.34527900	-0.04964100
H	2.96242700	-1.41580000	-1.59251300
H	0.57859000	2.37460500	-1.57845800
H	1.22713100	3.25591400	-0.19683300
H	2.46340400	1.01769500	-1.60272500
H	4.79267900	2.45443800	0.56766600
H	4.01846100	1.50852200	1.81553600
H	3.01791500	2.62274300	0.85953300
H	4.91687400	-0.18469200	-1.50389700
H	4.70188400	-1.05376500	-0.00886400
H	5.76779000	0.38166700	-0.03495100
H	-3.90559000	2.74949700	-0.99782100
H	-3.51282000	2.25448400	0.64006400
H	2.46327600	-1.81002800	0.96321900
H	-3.72321900	0.47269400	-1.85804900
H	-4.89437000	0.56687300	-0.55142100
H	-4.43552000	-0.70588800	1.64680500
H	-2.85564900	-1.45762400	1.90450300
H	-3.02099900	0.30111900	1.92393700
H	-2.35824000	-1.28720600	-1.84817600
H	-2.43892500	-3.55860100	-0.80160600
H	-1.97446700	-2.91974500	0.75286700
C	-0.84979500	2.64355700	0.01050200
C	-0.35983600	-3.01085800	-0.73002200
H	-0.24588500	-4.08104000	-0.95467700
H	-0.15959700	-2.48997200	-1.67218500
C	0.57685400	-3.47331000	1.60659300
H	0.50383100	-4.55029700	1.40905300
H	1.43419100	-3.30523700	2.26486900
H	-0.32791200	-3.19635400	2.16101400



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RMS Gradient Norm = 0.001455913 Hartree/Bohr
Imaginary Freq = 0
Dipole Moment = 7.5293758 Debye
Polarizability (?) = 338.54433 a.u.
Point Group = C1
Job cpu time: 0 days 19 hours 2 minutes 1.8 seconds.

Thermo Tab Data Section:
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Temperature = 298.15 Kelvin
Pressure = 1 atm
Frequencies scaled by = 1
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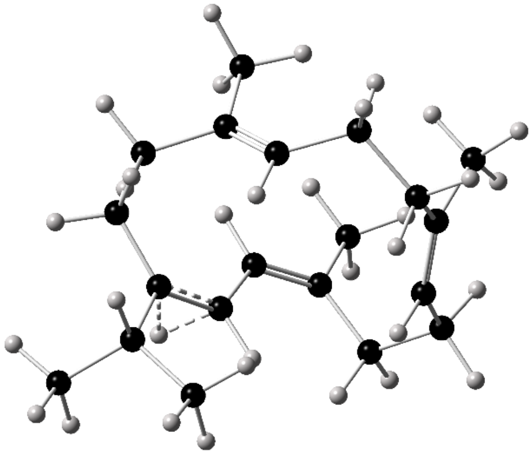
Zero-point Energy Correction = 0.482208 Hartree
Thermal Correction to Energy = 0.504707 Hartree
Thermal Correction to Enthalpy = 0.505651 Hartree
Thermal Correction to Free Energy = 0.433529 Hartree
EE + Zero-point Energy = -781.29141 Hartree
EE + Thermal Energy Correction = -781.26891 Hartree
EE + Thermal Enthalpy Correction = -781.26796 Hartree
EE + Thermal Free Energy Correction = -781.34008 Hartree
E (Thermal) = 316.708 kcal/mol
Heat Capacity (Cv) = 89.54 cal/mol-kelvin
Entropy (S) = 151.794 cal/mol-kelvin

Supplementary Note Table 4. Atomic coordinates and energies for DFT calculated transition state **TS$_{\mathrm{BC}}$.**

Transition state TS$_{\mathrm{BC}}$

C	0.08154500	1.46096200	2.43046100
C	1.11407400	-2.27716600	0.31537800
C	2.60405400	-0.42576800	1.34984600
C	2.01476100	-1.06454800	0.09550800
C	2.44836400	1.11960900	1.50400200
C	0.76983600	2.35563700	0.16546200
C	2.42735400	-0.69578600	-1.20225100
C	3.55679000	0.26817500	-1.46710000
C	1.87755800	-1.34448000	-2.45316200
C	-0.48900100	3.09324300	-0.20587300
C	-0.09492900	-2.07508500	1.19977400
C	-1.38644700	-2.12823000	0.83064200
H	1.58291100	2.48337100	-0.55436900
H	0.84100600	-2.73679000	-0.63219400
C	-1.29838700	2.45526600	-1.37107800
C	-2.18411900	1.28960200	-0.97466100
C	-3.39041900	1.66396600	-0.14519100
C	-1.91845600	0.04671200	-1.41076100
C	-2.70741400	-1.21738500	-1.19420500
H	0.41265200	2.02368300	3.31371400
H	-0.93295500	1.78042900	2.18775400
H	0.02962500	0.40767900	2.72799900
H	3.67227200	-0.67707000	1.38720900
H	2.14355100	-0.91711900	2.20892300
H	1.79615900	-2.99234000	0.80594700
H	3.14264300	1.63403900	0.83537300
H	2.78263300	1.35159700	2.52280200
H	1.38268900	-0.09020500	-0.53777700
H	3.19454800	1.18212400	-1.95040300
H	4.22213900	-0.22002100	-2.18930400
H	4.13859300	0.52918500	-0.58594300
H	0.83510000	-1.65127600	-2.37125100
H	2.47923200	-2.23703400	-2.66925800
H	1.97697000	-0.66647300	-3.30463700
H	-0.19057800	4.10200700	-0.52031300
H	-1.13676000	3.23055400	0.66427800
H	0.12219000	-1.93594800	2.25697300
H	-0.60586000	2.15097800	-2.16663000
H	-1.93209400	3.24322200	-1.80031000
H	-4.04136000	2.34404100	-0.71079500
H	-3.99461300	0.80580900	0.15483300
H	-3.10506500	2.20167000	0.76773000
H	-1.04559300	-0.07423200	-2.05921800
H	-3.08142800	-1.57323300	-2.16482900
H	-3.59450000	-1.03419800	-0.58283900
C	1.04850700	1.66604400	1.29075000
C	-1.88270800	-2.37866700	-0.57751400
H	-2.52393500	-3.27081400	-0.55919000
H	-1.05696400	-2.61927700	-1.25673800
C	-2.46462300	-1.97351000	1.87734400
H	-3.14788100	-2.83220200	1.85990300
H	-2.04781500	-1.89207900	2.88509800
H	-3.07555400	-1.08161800	1.69322200



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Imaginary Freq = 1
Dipole Moment = 7.6062157 Debye
Polarizability (?) = 327.273 a.u.
Point Group = C1
Job cpu time: 0 days 19 hours 29 minutes 15.6 seconds.

Thermo Tab Data Section:
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Temperature = 298.15 Kelvin
Pressure = 1 atm
Frequencies scaled by = 1
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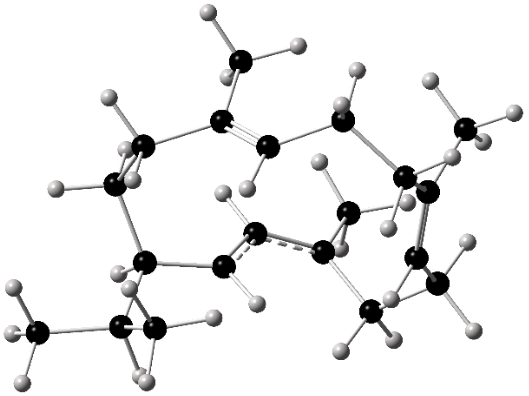
Zero-point Energy Correction = 0.480876 Hartree
Thermal Correction to Energy = 0.502866 Hartree
Thermal Correction to Enthalpy = 0.50381 Hartree
Thermal Correction to Free Energy = 0.432763 Hartree
EE + Zero-point Energy = -781.28408 Hartree
EE + Thermal Energy Correction = -781.26209 Hartree
EE + Thermal Enthalpy Correction = -781.26114 Hartree
EE + Thermal Free Energy Correction = -781.33219 Hartree
E (Thermal) = 315.553 kcal/mol
Heat Capacity (Cv) = 87.959 cal/mol-kelvin
Entropy (S) = 149.531 cal/mol-kelvin

Supplementary Note Table 5. Atomic coordinates and energies for DFT calculated intermediate **C^{+}.**

Intermediate C^{+}

C	0.85568500	-2.19715400	1.91844800
C	-1.18398700	0.20986900	-0.36399700
C	-2.04784700	-2.06364200	0.49976200
C	-2.24949800	-0.82726300	-0.43989300
C	-0.86603100	-3.00551700	0.20258600
C	1.42646400	-2.41915300	-0.53523400
C	-2.70678100	-1.08079000	-1.91147600
C	-1.65505500	-1.67926800	-2.86171200
C	-4.01128600	-1.89083300	-1.96236000
C	2.90628200	-2.17628700	-0.46126000
C	-0.89288400	0.95189800	0.77259200
C	-0.17393600	2.14904200	0.78122300
H	1.06901800	-2.67652200	-1.53363800
H	-3.10770300	-0.32377800	0.04500900
C	3.41185900	-0.92499600	-1.23550600
C	3.12040600	0.39488900	-0.55698300
C	3.87093200	0.64106300	0.72946500
C	2.27922000	1.27400200	-1.13108100
C	1.90679400	2.64223000	-0.64995400
H	0.76763600	-3.11509900	2.51492300
H	0.15916500	-1.47493900	2.36299300
H	1.86438100	-1.80500700	2.05945700
H	-2.97875400	-2.63744100	0.45843600
H	-1.97508700	-1.69833100	1.53103600
H	-0.70221900	0.49748400	-1.29563200
H	-0.93116200	-3.37843100	-0.82143200
H	-1.01734100	-3.88208600	0.85129000
H	-2.94455600	-0.07792500	-2.29793200
H	-1.98434100	-1.55036300	-3.89797200
H	-1.51859400	-2.75259300	-2.70110100
H	-0.67293800	-1.19995600	-2.77078100
H	-4.78566600	-1.45923900	-1.31721100
H	-3.85638700	-2.93197900	-1.65789500
H	-4.40398100	-1.90621200	-2.98399600
H	3.39459200	-3.04839100	-0.91848700
H	3.26032400	-2.14397800	0.57252000
H	-1.31972000	0.62587100	1.71946100
H	2.98686800	-0.93212400	-2.24616100
H	4.49905900	-1.02989300	-1.35143900
H	4.95244800	0.58153800	0.55147500
H	3.66660300	1.61526000	1.17826400
H	3.64129800	-0.12332600	1.48197000
H	1.83804400	0.99689700	-2.09039200
H	2.17282300	3.38664200	-1.41136000
H	2.44108000	2.92527300	0.26019500
C	0.52522000	-2.47336400	0.47101200
C	0.35749000	2.83083700	-0.43286700
H	0.18117400	3.90685400	-0.31249900
H	-0.16048700	2.51812800	-1.34214900
C	0.02294800	2.85510500	2.08246000
H	-0.68553100	3.69620800	2.14236900
H	-0.14448600	2.21004000	2.94784300
H	1.02175100	3.30217700	2.14412900



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Spin = Singlet
Solvation = scrf=(cpcm,solvent=water)
E(RmPW1PW91) = -781.78102 Hartree
RMS Gradient Norm = 0.001682506 Hartree/Bohr
Imaginary Freq = 0
Dipole Moment = 7.2346105 Debye
Polarizability (?) = 350.77367 a.u.
Point Group = C1
Job cpu time: 0 days 18 hours 58 minutes 24.2 seconds.

Thermo Tab Data Section:
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Temperature = 298.15 Kelvin
Pressure = 1 atm
Frequencies scaled by = 1
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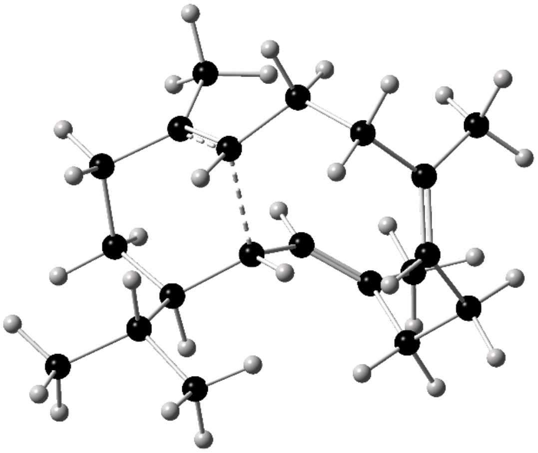
Zero-point Energy Correction = 0.483358 Hartree
Thermal Correction to Energy = 0.505889 Hartree
Thermal Correction to Enthalpy = 0.506833 Hartree
Thermal Correction to Free Energy = 0.434482 Hartree
EE + Zero-point Energy = -781.29766 Hartree
EE + Thermal Energy Correction = -781.27513 Hartree
EE + Thermal Enthalpy Correction = -781.27418 Hartree
EE + Thermal Free Energy Correction = -781.34653 Hartree
E (Thermal) = 317.45 kcal/mol
Heat Capacity (Cv) = 89.108 cal/mol-kelvin
Entropy (S) = 152.275 cal/mol-kelvin

Supplementary Note Table 6. Atomic coordinates and energies for DFT calculated transition state **TS$_{CD}$.**

Transition state TS$_{CD}$

C	0.96539200	-1.33959100	0.25949500
C	2.52376800	0.34089700	1.39605700
C	2.15253200	-0.54663900	0.24906700
C	2.21194300	1.84079700	1.03648600
C	0.28705100	2.40169400	-0.41007900
C	3.11150200	-0.60542100	-0.92905300
C	4.34003800	-1.49163500	-0.59352600
C	2.48697100	-1.01175900	-2.27152100
C	-1.06457500	2.88735100	-0.85698100
C	-0.07265700	-1.39830000	1.28757000
C	-1.26489600	-2.02390900	1.12723100
H	0.99300700	2.26058300	-1.23323800
H	0.72807500	-1.81039500	-0.68978700
C	-1.81727300	1.91334600	-1.80451600
C	-2.47900100	0.72637300	-1.13180800
C	-3.55663600	1.07858000	-0.13214500
C	-2.14445900	-0.52950400	-1.47501000
C	-2.73053600	-1.81832600	-0.96464000
H	3.59823900	0.26747900	1.59288400
H	1.99511400	0.06666400	2.31100700
H	1.94855900	-1.95601900	0.65365300
H	2.79391900	2.11704900	0.15153600
H	2.62651400	2.42104000	1.87056100
H	3.48319200	0.42340400	-1.02149900
H	5.07573800	-1.40661000	-1.39814100
H	4.04784100	-2.54594700	-0.51727400
H	4.83005500	-1.19794000	0.33937800
H	1.59643100	-0.41907700	-2.50504700
H	2.21990000	-2.07447200	-2.30054100
H	3.21634600	-0.84749100	-3.06986300
H	-0.90533500	3.82230900	-1.41241900
H	-1.69943100	3.15110800	-0.00661700
H	0.13687300	-0.92645600	2.24202500
H	-1.12994000	1.56745000	-2.58706500
H	-2.59744000	2.49514200	-2.31553900
H	-4.23097000	1.83780000	-0.54787900
H	-4.16891500	0.22395500	0.16293200
H	-3.13222000	1.50869300	0.78442700
H	-1.37210500	-0.65238100	-2.23810100
H	-3.05143500	-2.43504300	-1.81445700
H	-3.62532100	-1.64176700	-0.36220500
C	0.75106900	2.19795100	0.83503500
C	-1.72839500	-2.69039900	-0.14388300
H	-2.23213000	-3.62899600	0.11788000
H	-0.89013800	-2.96517300	-0.79275100
C	-2.24557300	-2.04220100	2.26809000
H	-2.45532800	-3.07771800	2.56678100
H	-1.88046800	-1.49812000	3.14296700
H	-3.20633100	-1.60657200	1.96854400
C	-0.04747500	2.38890800	2.10225800
H	0.04424600	1.52501500	2.77422300
H	0.32222500	3.25702100	2.66453300
H	-1.11131400	2.54463500	1.91432700



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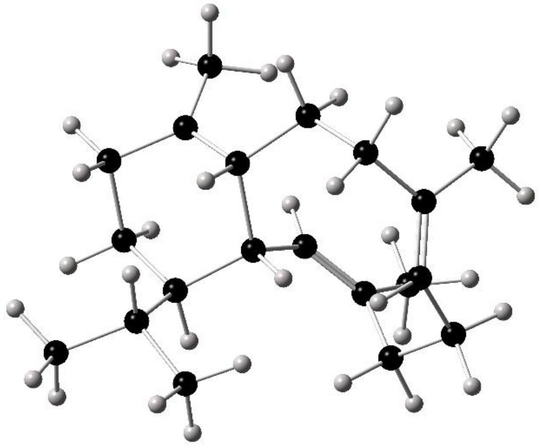
Calculation Method = RmPW1PW91
Basis Set = 6-311+G(d,p)
Charge = 1
Spin = Singlet
Solvation = scrf=(cpcm,solvent=water)
E(RmPW1PW91) = -781.77849 Hartree
RMS Gradient Norm = 0.001617416 Hartree/Bohr
Imaginary Freq = 1
Dipole Moment = 5.3776691 Debye
Polarizability (?) = 326.65867 a.u.
Point Group = C1
Job cpu time: 0 days 20 hours 1 minutes 47.0 seconds.

Thermo Tab Data Section:
Imaginary Freq = 1
Temperature = 298.15 Kelvin
Pressure = 1 atm
Frequencies scaled by = 1
Electronic Energy (EE) = -781.77849 Hartree
Zero-point Energy Correction = 0.484129 Hartree
Thermal Correction to Energy = 0.505264 Hartree
Thermal Correction to Enthalpy = 0.506209 Hartree
Thermal Correction to Free Energy = 0.437456 Hartree
EE + Zero-point Energy = -781.29436 Hartree
EE + Thermal Energy Correction = -781.27323 Hartree
EE + Thermal Enthalpy Correction = -781.27229 Hartree
EE + Thermal Free Energy Correction = -781.34104 Hartree
E (Thermal) = 317.058 kcal/mol
Heat Capacity (Cv) = 86.082 cal/mol-kelvin
Entropy (S) = 144.701 cal/mol-kelvin

Supplementary Note Table 7. Atomic coordinates and energies for DFT calculated intermediate **D$^+$.**

Intermediate D$^+$

C	0.98635500	3.21747200	0.98296900
C	0.44824900	-0.13940700	0.12116100
C	2.68584500	0.28132000	1.22127500
C	1.85644400	-0.72977000	0.42533400
C	2.86460600	1.61961900	0.38645900
C	0.63269300	1.32828200	-0.71368000
C	2.57997000	-1.33380400	-0.81858100
C	4.02259300	-1.76656400	-0.49894400
C	1.80832100	-2.53739500	-1.39009700
C	-0.64995600	2.03924000	-1.19468500
C	-0.46505400	-0.02885900	1.30662100
C	-1.45179400	-0.89467500	1.63132000
C	-2.25138000	-0.68452800	2.89335900
C	-1.83562400	-2.12679500	0.82910900
H	1.21640800	0.98028400	-1.57379400
H	-0.03673000	-0.73636900	-0.64625200
C	-1.60677300	1.24359900	-2.12420000
C	-2.57390000	0.38780300	-1.33142900
C	-3.64325700	1.14158600	-0.57499000
C	-2.40772500	-0.94202700	-1.28196200
C	-2.94710500	-1.88131800	-0.24532100
H	1.64931400	3.47702100	1.81098000
H	0.93101100	4.09267800	0.31030600
H	-0.03844400	3.06029200	1.33404800
H	3.68931600	-0.07812700	1.45851400
H	2.19761100	0.51350200	2.17359400
H	1.66623600	-1.57172100	1.10699300
H	3.36558100	1.35896600	-0.55049000
H	3.47129900	2.33207500	0.94993300
H	2.63569500	-0.57228400	-1.61232800
H	4.46023700	-2.28012000	-1.36061500
H	4.04854300	-2.46449100	0.34724400
H	4.68121500	-0.92405900	-0.26303700
H	0.79408600	-2.28577800	-1.71730300
H	1.73340100	-3.34116400	-0.64715200
H	2.33177400	-2.94304600	-2.26153300
H	-0.30857300	2.93063900	-1.73825800
H	-1.22151200	2.40747100	-0.33862400
H	-0.27507200	0.78161200	2.01003300
H	-2.09291300	-1.51273700	3.59624600
H	-3.32690300	-0.66905300	2.67330200
H	-1.99483600	0.24918600	3.40241100
H	-0.96185800	-2.55646000	0.32669800
H	-2.19504100	-2.89470000	1.52466800
H	-1.02706400	0.63135800	-2.82666300
H	-2.15045300	1.98179000	-2.72794500
H	-4.37436000	0.47817700	-0.10914000
H	-3.23333200	1.78242600	0.21674200
H	-4.19030300	1.80146500	-1.26095000
H	-1.65578000	-1.38319300	-1.94071300
H	-3.20878300	-2.85322800	-0.67952600
H	-3.84828400	-1.49437300	0.23944100
C	1.49224400	2.08988100	0.17181000



Calculation Type = FREQ
Calculation Method = RmPW1PW91
Basis Set = 6-311+G(d,p)
Charge = 1
Spin = Singlet
Solvation = scrf=(cpcm,solvent=water)
E(RmPW1PW91) = -781.78808 Hartree
RMS Gradient Norm = 0.001798224 Hartree/Bohr
Imaginary Freq = 0
Dipole Moment = 8.5242093 Debye
Polarizability (?) = 316.877 a.u.
Point Group = C1
Job cpu time: 0 days 20 hours 11 minutes 14.1 seconds.

Thermo Tab Data Section:
Imaginary Freq = 0
Temperature = 298.15 Kelvin
Pressure = 1 atm
Frequencies scaled by = 1
Electronic Energy (EE) = -781.78808 Hartree
Zero-point Energy Correction = 0.484982 Hartree
Thermal Correction to Energy = 0.506367 Hartree
Thermal Correction to Enthalpy = 0.507311 Hartree
Thermal Correction to Free Energy = 0.438158 Hartree
EE + Zero-point Energy = -781.30309 Hartree
EE + Thermal Energy Correction = -781.28171 Hartree
EE + Thermal Enthalpy Correction = -781.28076 Hartree
EE + Thermal Free Energy Correction = -781.34992 Hartree
E (Thermal) = 317.75 kcal/mol
Heat Capacity (Cv) = 87.143 cal/mol-kelvin
Entropy (S) = 145.546 cal/mol-kelvin

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