Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

On the mechanism of ophiobolin F synthase and the absolute configuration of its product by isotopic labelling experiments

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General

Chemicals were purchased from Sigma Aldrich Chemie GmbH (Steinheim, Germany), Carl Roth (Karlsruhe, Germany) and used without purification. Solvents for column chromatography were purchased in p.a. grade and further purified by distillation. Thin-layer chromatography was performed with 0.2 mm precoated plastic sheets Polygram® Sil G/UV254 (Machery-Nagel (Düren, Germany)). Column chromatography was carried out using Merck (Darmstadt, Germany) silica gel 60 (70-200 mesh).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance I 500 MHz spectrometer and a Bruker Avance III HD 700 MHz Cryo spectrometer, and chemical shifts were referenced to the residual proton signal of C_6D_6 (δ = 7.16 ppm) for ^{1}H-NMR and the residual ^{13}C signal of C_6D_6 (δ = 128.06 ppm) for ^{13}C-NMR.[1]

GC-MS analyses were carried out with an Agilent (Santa Clara, USA) HP 7890B gas chromatograph fitted with a HP5-MS silica capillary column (30 m, 0.25 mm i. d., 0.50 μm film) connected to a HP 5977A inert mass detector. Used MS parameters were 1) transfer line: 250 ^{o}C, and 2) electron energy: 70 eV. The GC parameters were 1) inlet pressure: 77.1 kPa, He 23.3 mL min^{-1}, 2) temperature program: 5 min at 50 ^{o}C increasing at 5 or 10 ^{o}C min^{-1} to 320 ^{o}C, 3) injection volume: 1 μL, 4) splitless or split ratio 10:1, 60 s valve time and 5) carrier gas: He at 1 mL min^{-1}. Retention indices (*I*) were determined from a homologous series of n-alkanes (C8-C40). Optical rotary powers were recorded on a P8000 Polarimeter (Krüss).

In vivo functional analysis of candidate terpene synthase gene *acldOS*

Candidate sesterterpene synthase encoded gene *acldOS* (accession number: CEL04094.1) was identified by bioinformatics analysis in the genome of *Aspergillus calidoustus* CBS121601 using BLAST.

Gene cloning and plasmid construction:

Primers used for plasmid construction are listed in Table S1. PCRs were performed with Q5 polymerase (New England Biolabs, Ipswich, MA, USA) and ligations were performed with In-Fusion® HD cloning kit (Clontech, Saint-Germain-en-Laye, France) according to the manuals provided by the manufacturers. All plasmids were constructed in *Escherichia coli* Stellar (Clontech, SaintGermain-en-Laye, France) that was grown on LB agar medium with ampicillin.

The fungal expression vector pArgB-TAA, a vector for heterologous expression of genes in *Aspergillus oryzae* NSAR1, was derived from the pUC19 vector. First, the *argB* gene (accession number: P11803.1) from *Aspergillus nidulans* FGSC A4 was amplified from fungal genomic DNA using primers Arg-Fw and Arg-Rv and cloned into PCR linearised pUC19 by using primers pUC19-HindIII-Fw and pUC19-EcoRI-Rv to yield plasmid pArgB. Next, the promoter (609 bp) and terminator region (236 bp) of taka-amylase A (accession number: X12726.1) from *Aspergillus oryzae* RIB40 was cloned into pUC19 to yield pTAA-cst. The TAA-promoter was amplified by PCR using primers TAA-Prm-Fw and TAA-Prm-Rv. The TAA-terminator was amplified by PCR using primers TAA-Tmn-Fw and TAA-Tmn-Rv. pUC19 was linearised by PCR using primers pUC19-HindIII-Fw and pUC19-EcoRI-Rv. Finally, the cassette containing the TAA-promoter and terminator was amplified by PCR using primers TAA-Fw and TAA-Rv and cloned into pArgB linearised by PCR using primers pUC19-HindIII-Fw and pArgB-KpnI-Rv to yield pArgB-TAA.

Plasmid pArgB-AcldOS was constructed for the heterologous expression of the *acldOS* gene. The *acldOS* gene was amplified from genomic DNA of *A. calidoustus* CBS121601 by PCR using primers AcldOS-SmaI-Fw and AcldOS-SmaI-Rv, and cloned into pArgB-TAA linearised by single restriction enzyme digestion with SmaI to yield pArgB-AcldOS.

Table S1. Primers list

Primer	Sequence
AcldOS-SmaI-Fw	TCGAGCTCGGTACCCATGGAGTATAAGTACTCGACCATCGTCGACAGTTCCAAGTGGGACCCC
AcldOS-SmaI-Rv	ACTCTCCACCCTCCCTCAAACCTTCAGCAGCTCCAGCATCATCC
pUC19-HindIII-Fw	GGCGTAATCATGGTCATAGCTG
pUC19-EcoRI-Rv	ACTGGCCGTCGTTTTACAAC
Arg-Fw	AAAACGACGGCCAGTAACGAACGCTGTGTAAAGCGG
Arg-Rv	GACCATGATTACGCCTCTAGAGGATCCCCGGGTACCTGTCGTCGTCGTCAATGGG
TAA-Prm-Fw	GTTGTAAAACGACGGCCAGTTCATGGTGTTTTGATCATTTTAAATTTTTATATGGCG
TAA-Prm-Rv	CCCGGGTACCGAGCTCGAATTCGCCTTCTGTGGGGTTTATTGTTC
TAA-Tmn-Fw	GAATTCGAGCTCGGTACCCGGGAGGGTGGAGAGTATATGATGGTAC
TAA-Tmn-Rv	GCTATGACCATGATTACGCCGTAAGATACATGAGCTTCGGTGATATAATAC
TAA-Fw	TTGACGACGACGACATCATGGTGTTTTGATCATTTTAAATTTTTATATGGCG
TAA-Rv	GACCATGATTACGCCGTAAGATAC
pArgB-KpnI-Rv	TGTCGTCGTCGTCAATGGG
AcldOS-pYE-Fw	AGCATGACTGGTGGAATGGAGTATAAGTACTCGACCATCGTC
AcldOS-pYE-Rv	GGTGGTGCTCGAGTGTCAAACCTTCAGCAGCTCCAG
pYE-Fw	CACTCGAGCACCACCACCAC
pYE-Rv	TCCACCAGTCATGCTAGCCATATGG

Fungal transformation and product detection:

A. oryzae NSAR1 was kindly provided by Prof. K. Gomi (Graduate School of Agricultural Sciences, Tohoku University) and Prof. K. Kitamoto (Graduate School of Agricultural Sciences, The University of Tokyo).

Transformations of *A. oryzae* were performed with the protoplast–polyethylene glycol method, as reported previously.[2] Candidate transformants were stabilized in selective agar medium (M agar with methionine and adenine: 0.2 % NH_4Cl, 0.1 % $(NH_4)_2SO_4$, 0.05 % KCl, 0.05 % NaCl, 0.1 % KH_2PO_4, 0.05 % $MgSO_4$, 0.002%, $FeSO_4/7H_2O$, 2 % glucose, 0.15 % methionine, 0.01 % adenine, 1.5 % agar, pH=5.5) at 30 ^oC for 3 days, then inoculated in DPY medium (2 % dextrin, 1 % polypeptone, 0.5 % yeast extract, 0.05 % $MgSO_4$, 0.5 % KH_2PO_4) for further analysis.

The fungal expression plasmid pArgB-AcldOS was transformed into *A. oryzae* NSAR1 to give *A. oryzae* NSAR1-AcldOS. For a negative control experiment, the empty vector pArgB was also transformed into *A. oryzae* NSAR1 to generate *A. oryzae* NSAR1- NC. *A. oryzae* NSAR1-AcldOS and its negative control were cultivated in DPY medium and incubated at 30 ^oC with shaking at 220 rpm for 3 days. The mycelia were separated and dried and extracted with acetone under sonication. The extracts were subjected to GC/MS analysis (Figure S1).

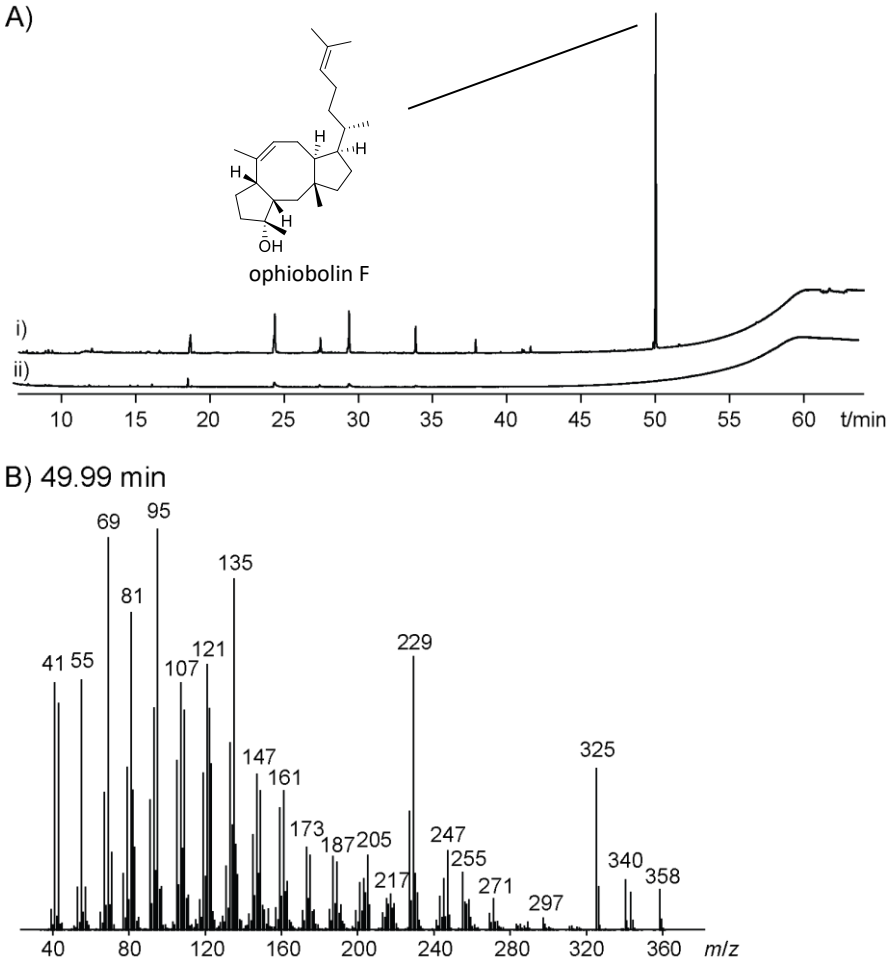


Figure S1. A) GC/MS chromatogram of crude extracts from i) *A. oryzae* NSAR1-AcldOS, and ii) *A. oryzae* NSAR1-NC. B) EI mass spectrum of ophiobolin F.

Product isolation and structure elucidation from metabolites accumulated in the *A. oryzae* NSAR1-AcldOS:

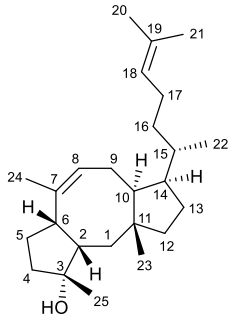
The *A. oryzae* transformant harboring *acldOS* was cultivated in 10 mL DPY medium and preincubated at 30 °C with shaking at 220 rpm for 3 days. The preculture was shaken thoroughly and then added into a 300 mL Erlenmeyer flask with 100 mL DPY medium and incubated at 30 °C with shaking at 220 rpm for 3 days. Finally, 100 mL DPY medium was added to an inducing culture of 3x1 L of CD-starch medium (0.3 % $NaNO_3$, 0.2 % KCl, 1.0 % polypepton, 0.05 % $MgSO_4/7H_20$, 0.1 % KH_2PO_4, 0.002 % $FeSO_4/7H_2O$, 2.0 % starch, pH=5.5) and incubated at 30 °C with shaking at 220 rpm for 5 days.

Mycelium was separated and extracted with acetone. The extract was suspended in ion-exchange water and re-extracted with cyclohexane. After removal of the solvent under reduced pressure, the extract was subjected to an open-column chromatography and eluted stepwise with cyclohexane/ethyl acetate (100/0 to 80/20). The fractions containing the target sesterterpene were collected and the solvents were evaporated to give 51 mg of clear oil. The pure compound was subjected to NMR spectroscopy.

Ophiobolin F:

Colourless oil. R_f [cyclohexane/ethyl acetate (80/20)] = 5.5. $[\alpha]_D^{20}$ = +15.7 (*c* 0.65, ethyl acetate), lit.: $[\alpha]_D^{25}$ = +25 (*c* 2.5, $CHCl_3$).[3] HRMS (EI): *m*/*z* = 358.3234 (calc. for $[C_{25}H_{42}O]^+$ 358.3230). GC (HP5-MS): *I* = 2720. MS (EI, 70 eV): *m*/*z* (%) = 358 (9), 343 (12), 340 (13), 325 (39), 297 (4), 289 (2), 271 (9), 255 (17), 247 (22), 229 (79), 227 (35), 217 (10), 205 (22), 189 (21), 187 (22), 175 (23), 173 (24), 161 (41), 159 (36), 153 (6), 149 (40), 147 (44), 135 (100), 133 (52), 131 (18), 121 (71), 119 (44), 109 (56), 107 (65), 105 (46), 95 (96), 93 (55), 91 (32), 81 (68), 79 (37), 77 (13), 69 (73), 67 (27), 55 (39), 43 (25), 41 (28). IR (diamond ATR): $\tilde{\nu}$ / cm^{-1} = 3611 (w), 3473 (w), 2952 (s), 2925 (s), 2876 (s), 2728 (w), 1733 (w), 1652 (w), 1455 (s), 1374 (s), 1285 (w), 1265 (w), 1223 (w), 1178 (m), 1152 (m), 1115 (m), 1083 (s), 1066 (m), 1035 (w), 1020 (w), 983 (w), 961 (w), 929 (s), 904 (w), 861 (s), 830 (s), 738 (w), 686 (w), 634 (w), 614 (w), 573 (w), 524.18 (m), 474 (w), 440 (w), 414 (w).

Table S2. NMR spectral data of ophiobolin F in C_6D_6 recorded at 298 K.



Ophiobolin F

C		^{1}H[a]	^{13}C[a]
1	CH_2	1.28 (m, 1H, H_α)*	36.85*
		1.41 (m, 1H, H_β)*	
2	CH	1.63 (m, 1H)	54.24
3	C		80.25
4	CH_2	1.68 (m, 1H, H_β)	41.95
		1.94 (m, 1H, H_α)	
5	CH_2	1.56 (m, 1H, H_β)*	26.14*
		1.94 (m, 1H, H_α)*	
6	CH	3.14 (t, $^{3}J_{H,H}$ = 8.29 Hz, 1H)	42.55
7	C		136.63
8	CH	5.62 (t, $^{3}J_{H,H}$ = 8.23 Hz, 1H)	129.18
9	CH_2	2.00 (m, 1H, H_α)*	23.87
		2.10 (m, 1H, H_β)*	
10	CH	1.67 (m, 1H)	55.89
11	C		43.95
12	CH_2	1.34 (m, 1H, H_β)	43.70
		1.42 (m, 1H, H_α)	
13	CH_2	1.44 (m, 1H, H_β)*	23.74*
		1.54 (m, 1H, H_α)*	
14	CH	2.30 (dtq, *J* = 13.67, 4.82, 4.78 Hz)	46.01
15	CH	1.65 (m, 1H)	33.42
16	CH_2	1.23 (m, 1H)	37.81
		1.36 (m, 1H)	
17	CH_2	2.04 (m, 1H)*	26.76
		2.09 (m, 1H)*	
18	CH	5.26 (ddt, *J* =7.11, 4.19, 1.32 Hz)	125.60
19	C		130.93
20	CH_3	1.70 (s, 3H)	25.96
21	CH_3	1.61 (s, 3H)	17.80
22	CH_3	0.83 (d, $^{3}J_{H,H}$ = 6.87 Hz)	17.22
23	CH_3	0.85 (s, 3H)	19.10
24	CH_3	2.00 (s, 3H)	21.99
25	CH_3	1.06 (s, 3H)	29.05

[a] Chemical shifts δ in ppm, multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, coupling constants *J* are given in Hertz. * Corrected assignments with respect to previously published data.[3]

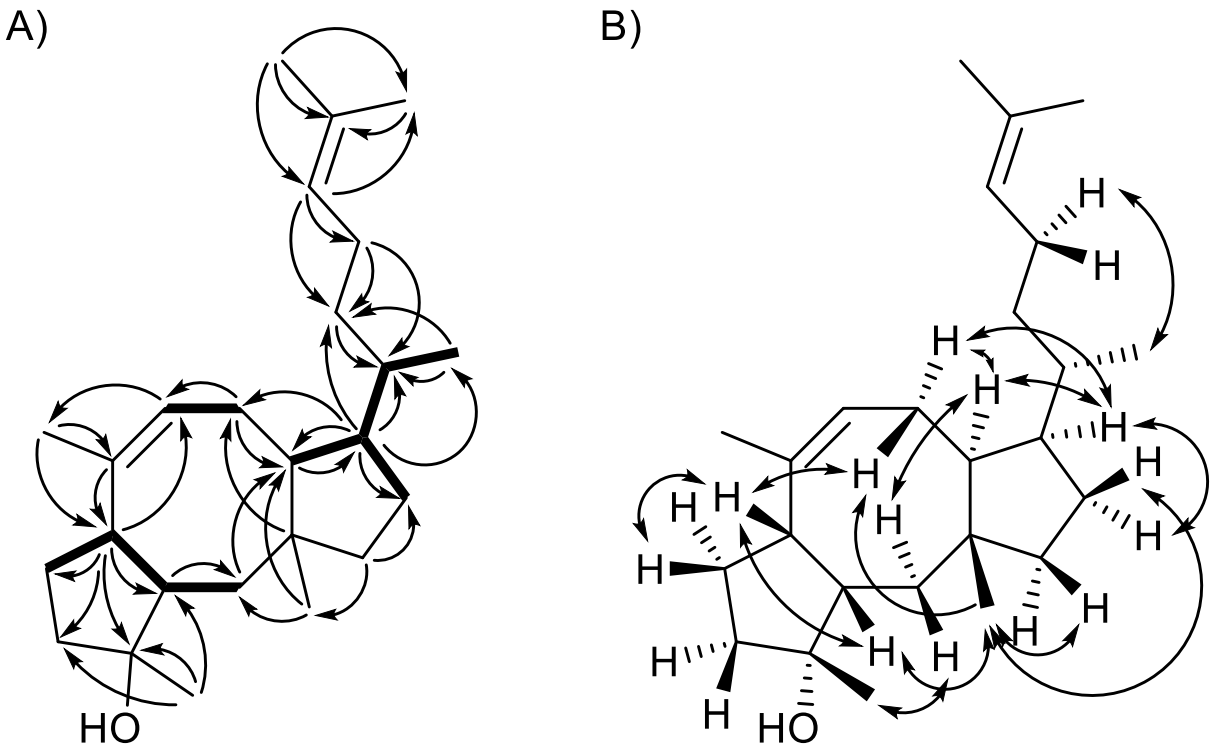


Figure S2. Correlations of A) ^{1}H,^{1}H-COSY and HMBC, B) ^{1}H,^{1}H-NOESY on ophiobolin F.

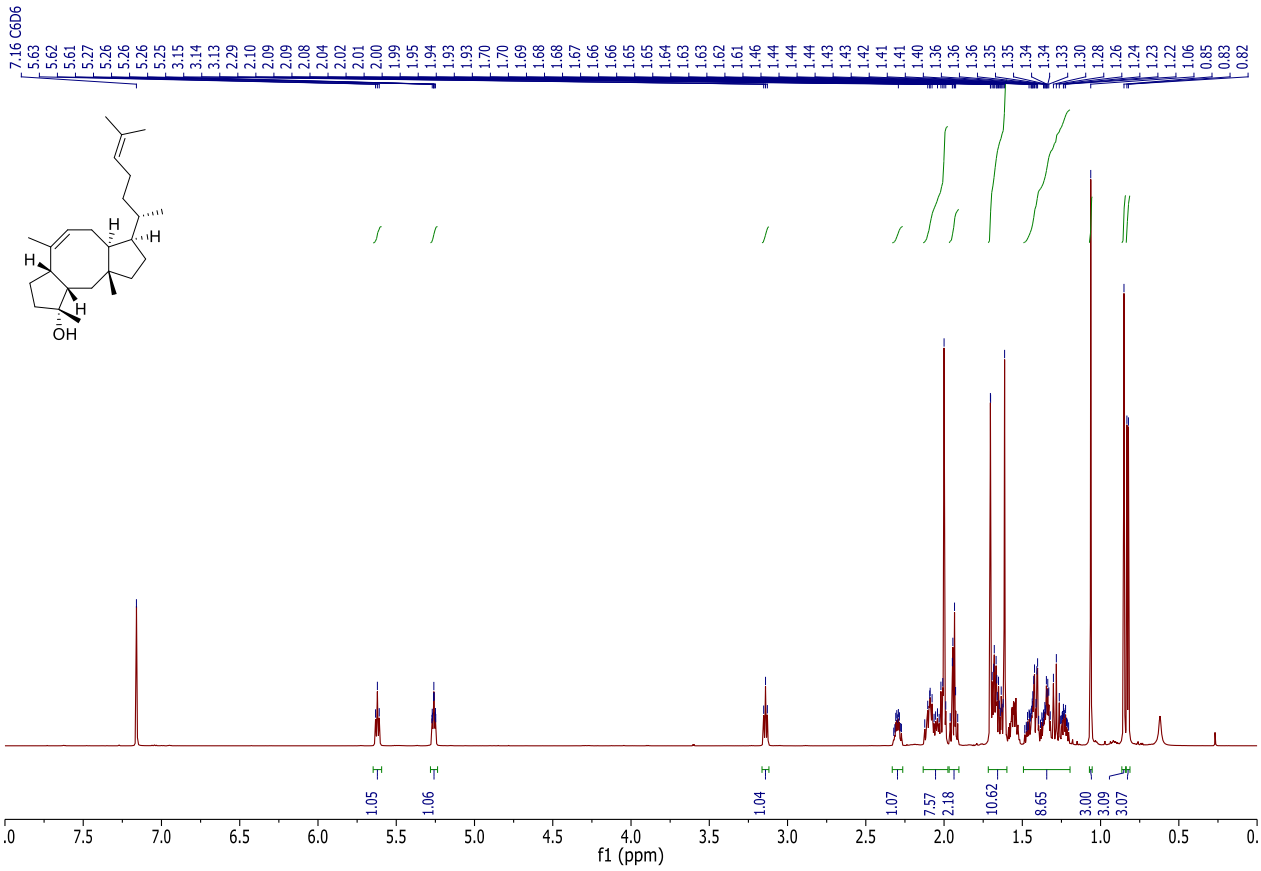


Figure S3. ^{1}H-NMR spectrum of ophiobolin F (700 MHz, C_6D_6).

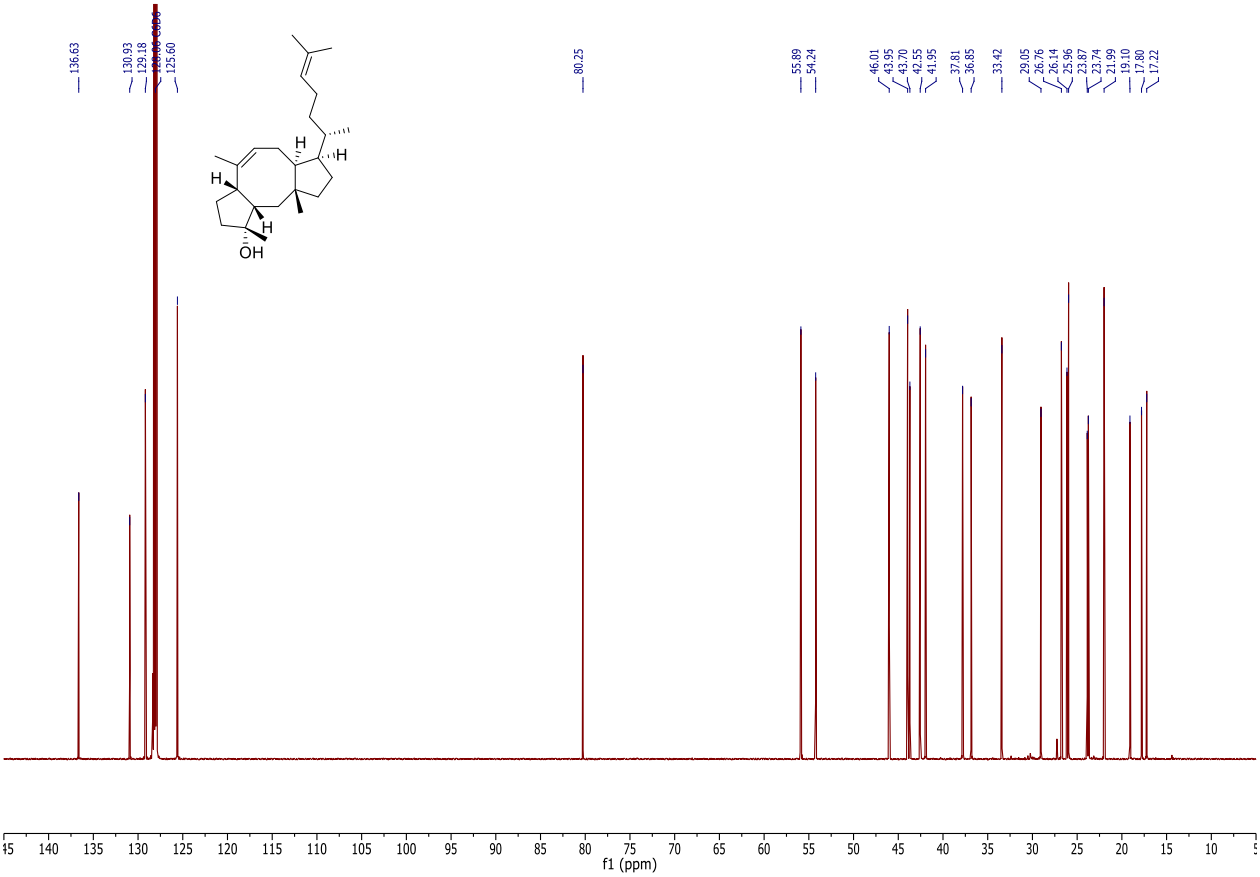


Figure S4. ^{13}C-NMR spectrum of ophiobolin F (700 MHz, C_6D_6).

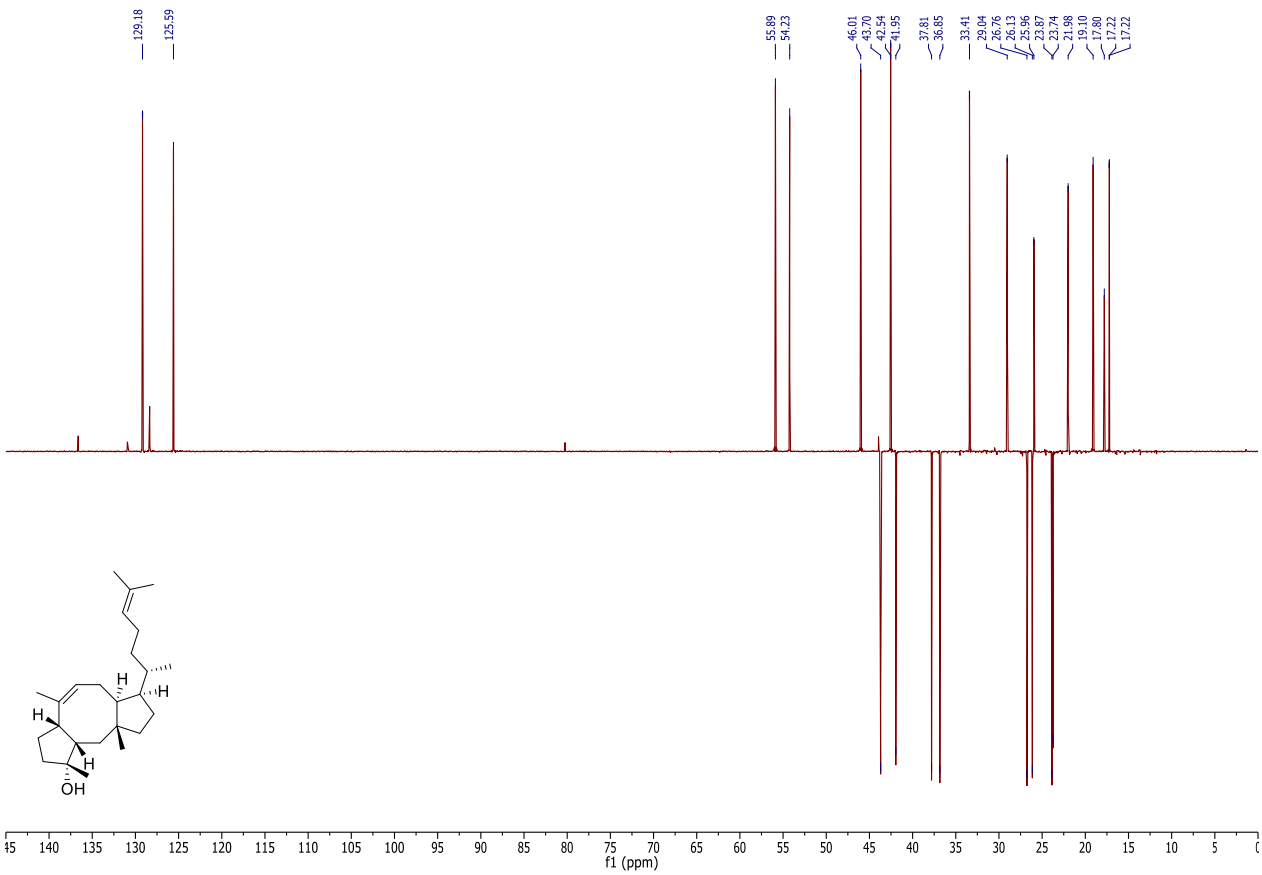


Figure S5. ^{13}C-DEPT-135 spectrum of ophiobolin F (700 MHz, C_6D_6).

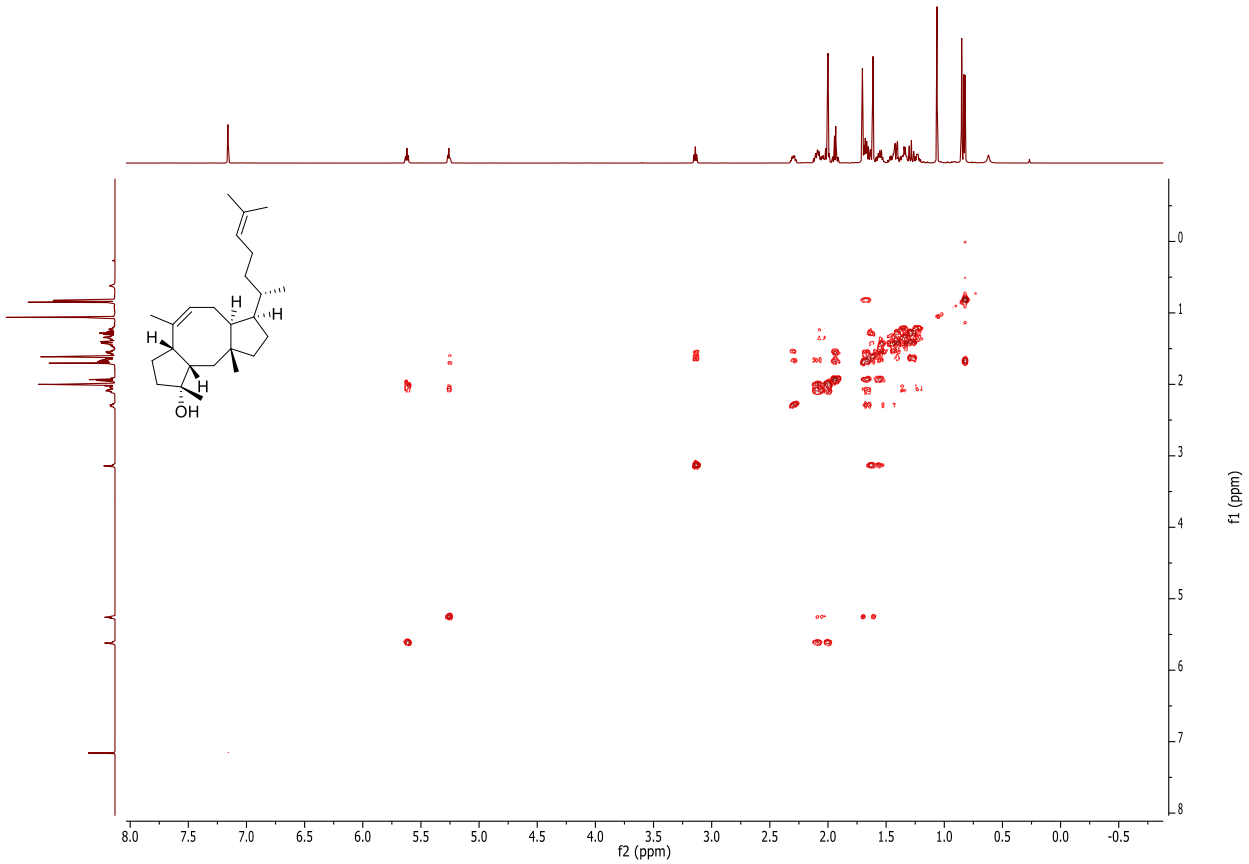


Figure S6. $^{1}H,^{1}H$-COSY spectrum of ophiobolin F (700 MHz, C_6D_6).

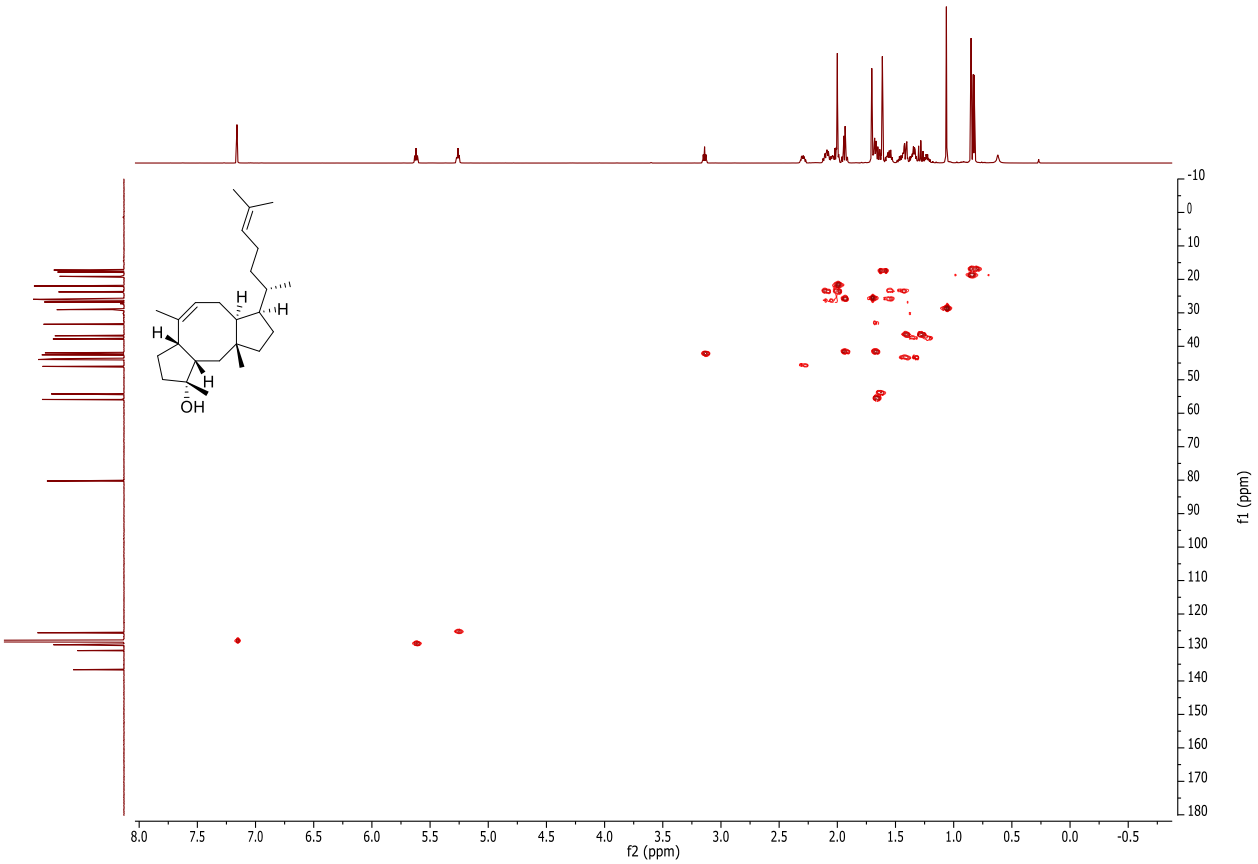


Figure S7. HMQC spectrum of ophiobolin F (700 MHz, C_6D_6).

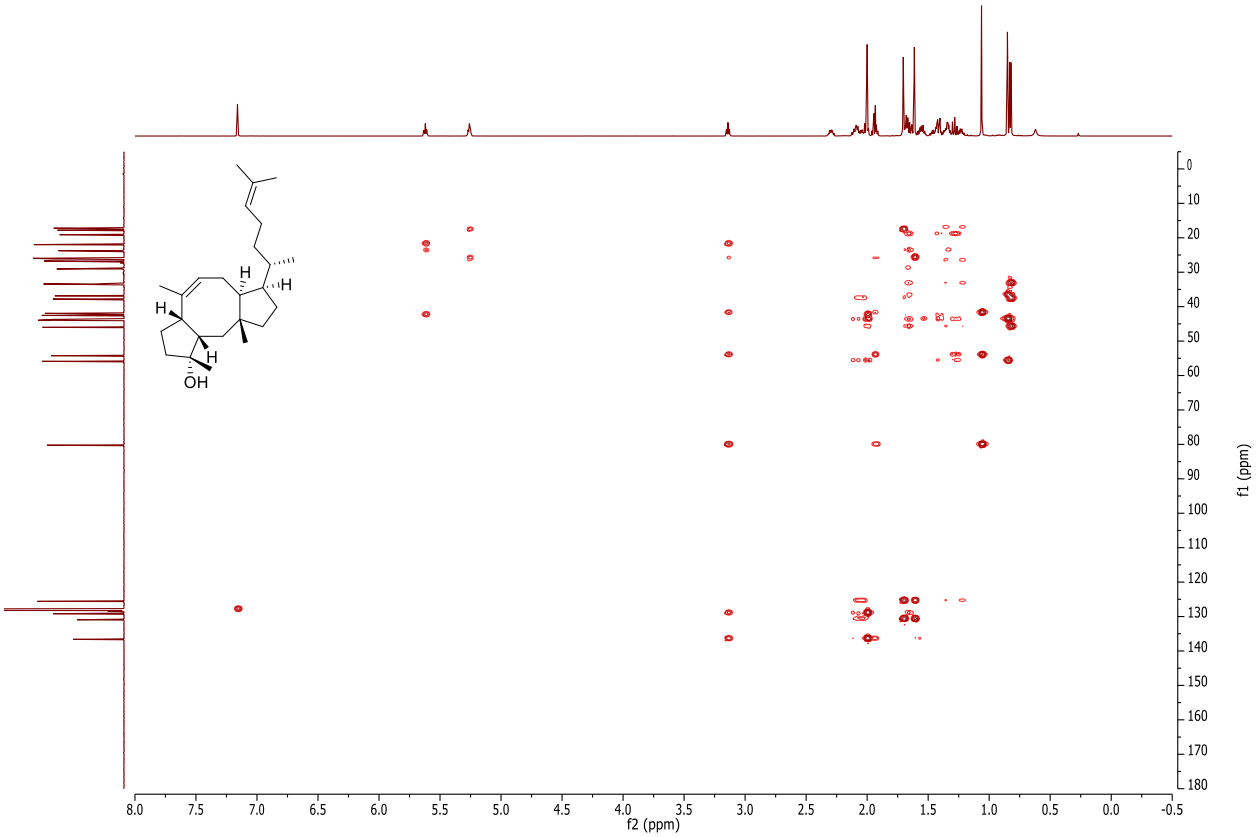


Figure S8. HMBC spectrum of ophiobolin F (700 MHz, C_6D_6).

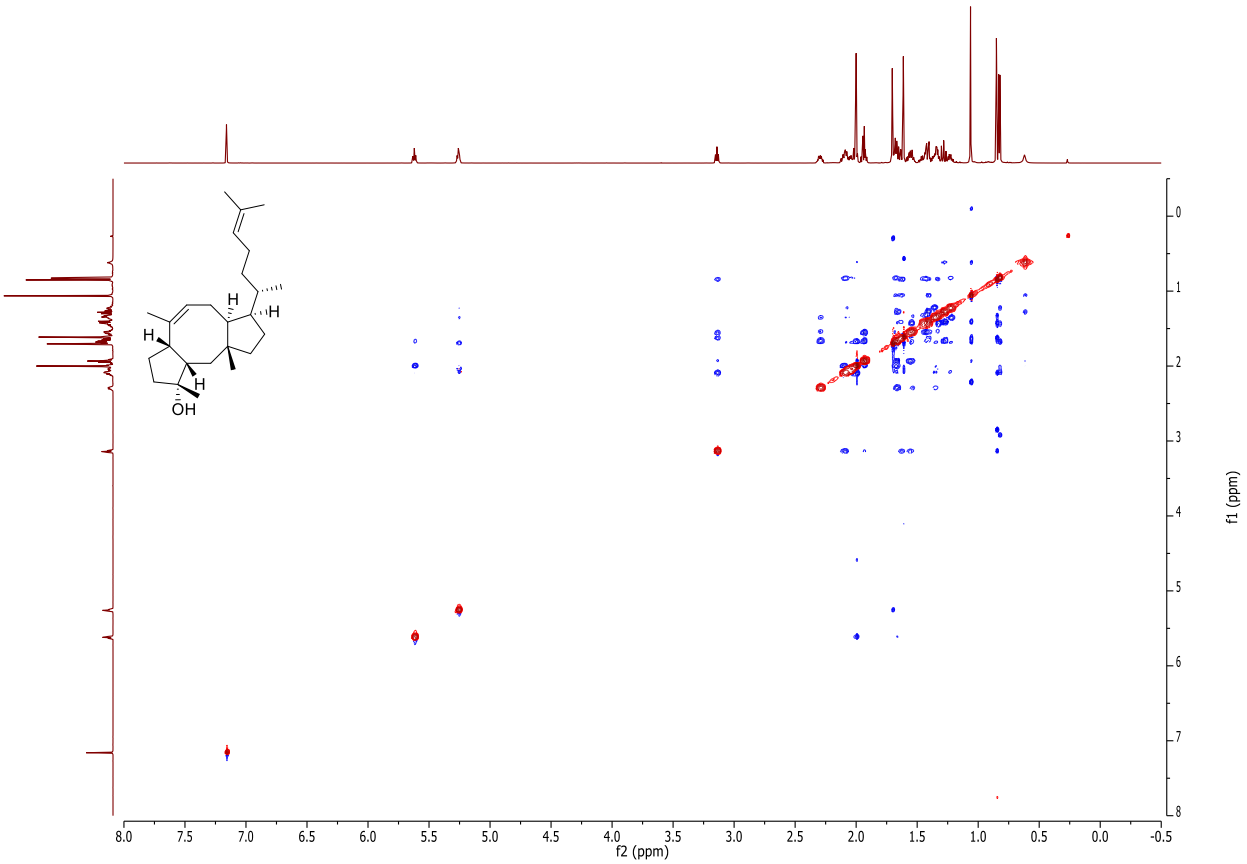


Figure S9. ^{1}H,^{1}H-NOESY spectrum of ophiobolin F (700 MHz, C_6D_6).

In vitro investigation of sesterterpene synthase AcldOS

RNA extraction and cDNA synthesis

A. oryzae NSAR1-AcldOS was cultivated in 10 mL DPY medium and preincubated at 30 °C with shaking at 220 rpm for 2 days. The whole culture was added to a 300 mL Erlenmeyer flask with 100 mL DPY and incubated at 30 °C with shaking at 220 rpm for 2 days. The mycelium was filtrated and washed with ion exchange water. The mycelium was frozen at –80 °C, and then powdered using a mortar. During this process the temperature was kept low by administration of liquid nitrogen. The powdered mycelium was collected for further extraction.

The extraction and purification of RNA was performed using TRIzol (Sigma Aldrich Chemie GmbH, Steinheim, Germany). Purified RNA was used as the template for cDNA reverse-transcription. cDNA synthesis was performed with SuperScript® III Reverse Transcriptase (Invitrogen, Carlsbad, California). All the operations were conducted under the manual provided by the manufacturers.

Plasmid construction and gene expression

Primers used for plasmid construction are listed in Table S1. The *E. coli* expression plasmid was constructed for protein expression and enzymatic investigation of AcldOS. The cDNA of the sesterterpene synthase AcldOS, which was amplified by PCR using the prepared cDNA as a template and primers AcldOS-pYE-Fw and AcldOS-pYE-Rv, was cloned into pYE-Express vector, which was linearised by PCR using primers pYE-Fw and pYE-Rv, to yield pYE-AcldOS. The pYE-AcldOS plasmid was constructed in *E. coli* Stellar that was grown on LB agar medium with kanamycin.

E. coli BL21(DE3) was transformed with plasmid pYE-AcldOS for protein expression. A preculture of the transformant was cultivated in 10 mL LB medium with kanamycin (50 µg mL^{-1}), and incubated at 37 °C overnight with shaking at 160 rpm. The preculture was used to inoculate larger culture volumes (1 mL L^{-1} culture) in 4x1 L LB medium containing kanamycin which were then grown for ~ 4 h until OD600 of 0.4 – 0.6 was reached. The cultures were chilled on ice and IPTG solution (400 mM, 1mL L^{-1}) was added to induce protein expression. Expression was carried out at 18 °C with shaking at 160 rpm overnight. The cultures were centrifuged (3.600 x g, 40 min) to separate the cells from the medium. The pellet was resuspended in binding buffer (10 mL L^{-1} culture, 20 mM Na_2HPO_4, 500 mM NaCl, 20 mM imidazole, 1 mM $MgCl_2$, pH = 7.4, 4 °C). Cell lysis was performed using ultra sonication (6 x 1 min) and the resulting suspension was centrifuged (14.610 x g, 2 x 7 min) to remove the cell debris. The supernatant was filtrated and transferred to a Ni^{2+}-NTA affinity column (Super Ni-NTA, Generon, Slough, UK). Undesired proteins were washed off the column with binding buffer (2 x 10 mL L^{-1} culture), the desired His_6-tagged protein was eluted with elution buffer (10 mL L^{-1} culture, 20 mM Na_2HPO_4, 500 mM NaCl, 500 mM imidazole, 1 mM $MgCl_2$, pH = 7.4, 4 °C).

The nucleotide sequences comparing of genomic DNA and the cDNA of the *acldOS* gene and the amino acid sequence of the sesterterpene synthase AcldOS are shown in Figure S10.

A) gDNA and cDNA of *acldOS*

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ATGGAGTATAAGTACTCGACCATCGTCGACAGTTCCAAGTGGGACCCCGAGGGCCTGATCGAGGGGATCCCTCTGCGGAAGCACGAAGCCGGGGACCTGGAAGAGGTCGGT
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GGCGTCGGCCGAGTATGCCCGCCAGAAGGGGTATGCGGAGGATCTGGACGAGGGCAAGTATTCGTTCACGCTGATTCATTGCATCAATACGCTCGAGGCGGAGGCTTCGCT

GGCGAGCGAGAAGATGGCCCTGCGCGCGTTTCTGATAAAGAGGCGGGTGGATTCGAGTCTGAGTAATGAGTCGAAGCGCGAGGTGTTGGATATCATGAAGAAGACCAAGAG
GGCGAGCGAGAAGATGGCCCTGCGCGCGTTTCTGATAAAGAGGCGGGTGGATTCGAGTCTGAGTAATGAGTCGAAGCGCGAGGTGTTGGATATCATGAAGAAGACCAAGAG

CCTGGAGTATACCCTGGGGGTGTTGCGGGCGTTGCAGGCGGAGCTCGAGAAGGAGGTGGATAGCCTCGAGGCCAAGTTTGGCGAGGAGAACTTTTCGCTCAGGATGATGCT
CCTGGAGTATACCCTGGGGGTGTTGCGGGCGTTGCAGGCGGAGCTCGAGAAGGAGGTGGATAGCCTCGAGGCCAAGTTTGGCGAGGAGAACTTTTCGCTCAGGATGATGCT

GGAGCTGCTGAAGGTTTGA
GGAGCTGCTGAAGGTTTGA
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B) AcldOS (CEL04094.1)

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MEYKYSTIVDSSKWDPEGLIEGIPLRKHEAGDLEEVGSFRVQEDWRRLVGPVENPFRGSLGPEISFITYTVPECLPERLEAISYGLDYGFLHDDEIDTKIEEAELDDVGAA
LAQGGSTGKIQEGTKSSGKRKMAAQLLREMMALDPERAMTLAKSWAQGVQHSARRVEEKDWKSLDEYIPFRCMDLGYMHWHGLVTFGCAITVPEEEEEERRTLLEPAVIAC
LMTNDLFSYEKEKNDNNPQNAVAVIMKIHKCSEEEARDICKQRIRLECRKYARIVKETLARTDISLDLKRYIEIMQYTVSGNWAWSTQCPRYHADAKFNELQMLRAEHGVA
KYPARYSLENRKNGANGVNGVNGINGVNGVNGVNGKRKRSGEETADDARTNGNGIKKPAHVLEYRDSLVLEDIVALSLDWNLPDLSDGVVVQPYKYLTSLPSKGFRDQAID
SLNTWLRVPTKTTKMIKDVIKMLHSASLMLDDIEDNSPLRRGKPSTHVIYGNAQTINSATYQYTEATGLAARLPNPTSLRIYLEEVQQLYIGQSYDLYWTHNALCPSIPEY
LKMVDQKTGGLFRMLTRLMVSESPARSSILDQTLYPLSHLIGRFFQIRDDYQNLASAEYARQKGYAEDLDEGKYSFTLIHCINTLEAEASLASEKMALRAFLIKRRVDSSL
SNESKREVLDIMKKTKSLEYTLGVLRALQAELEKEVDSLEAKFGEENFSLRMMLELLKV
```

Figure S10. A) Nucleotide sequences of up: gDNA and down: cDNA of *acldOS*. Introns are highlighted in yellow. B) Amino acid sequence of AcldOS. Highly conserved motifs are highlighted in yellow.[4-7]

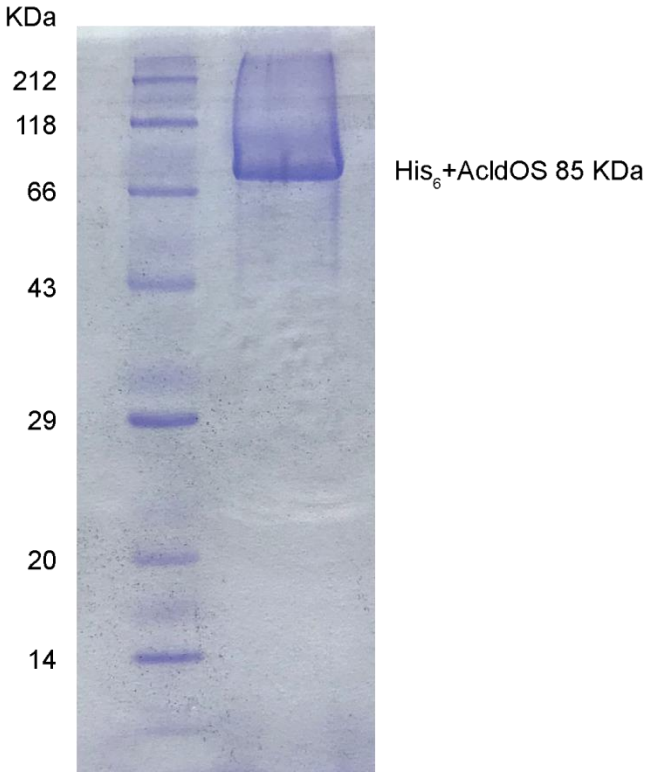


Figure S11. SDS-PAGE analysis of ophiobolin F synthase AcldOS with His_6-tag from *A*. *calidoustus* CBS121601.

Incubation experiments catalysed by AcldOS:

The sesterterpene synthase AcldOS is a bi-functional enzyme that contains a prenyltransferase domain and a terpene cyclase domain. Therefore, the combinations of DMAPP+IPP, GPP+IPP, FPP+IPP, GGPP+IPP, and GFPP were tested as substrates for it.

Incubation experiments were performed using freshly prepared enzyme solutions and all the combinations of substrates mentioned above (final concentration 0.25 mg mL^{-1}) dissolved in substrate buffer (25 mM NH_4HCO_3). The enzyme solution was diluted with an equal volume of incubation buffer (50 mM TRIS, 10 mM $MgCl_2$, 20% glycerol, pH 8.2, final concentration 0.2 mg mL^{-1}). The scale of the incubation was 4 mL and approximately 1 mg of each substrate was used. The mixtures were incubated at 30 ^{o}C with shaking at 160 rpm overnight. The reaction mixtures were extracted with benzene after incubation. The extracts were centrifuged (14.610 x g, 15 min) to remove water and subjected to the GC/MS analysis. The results are shown in Figure S12.

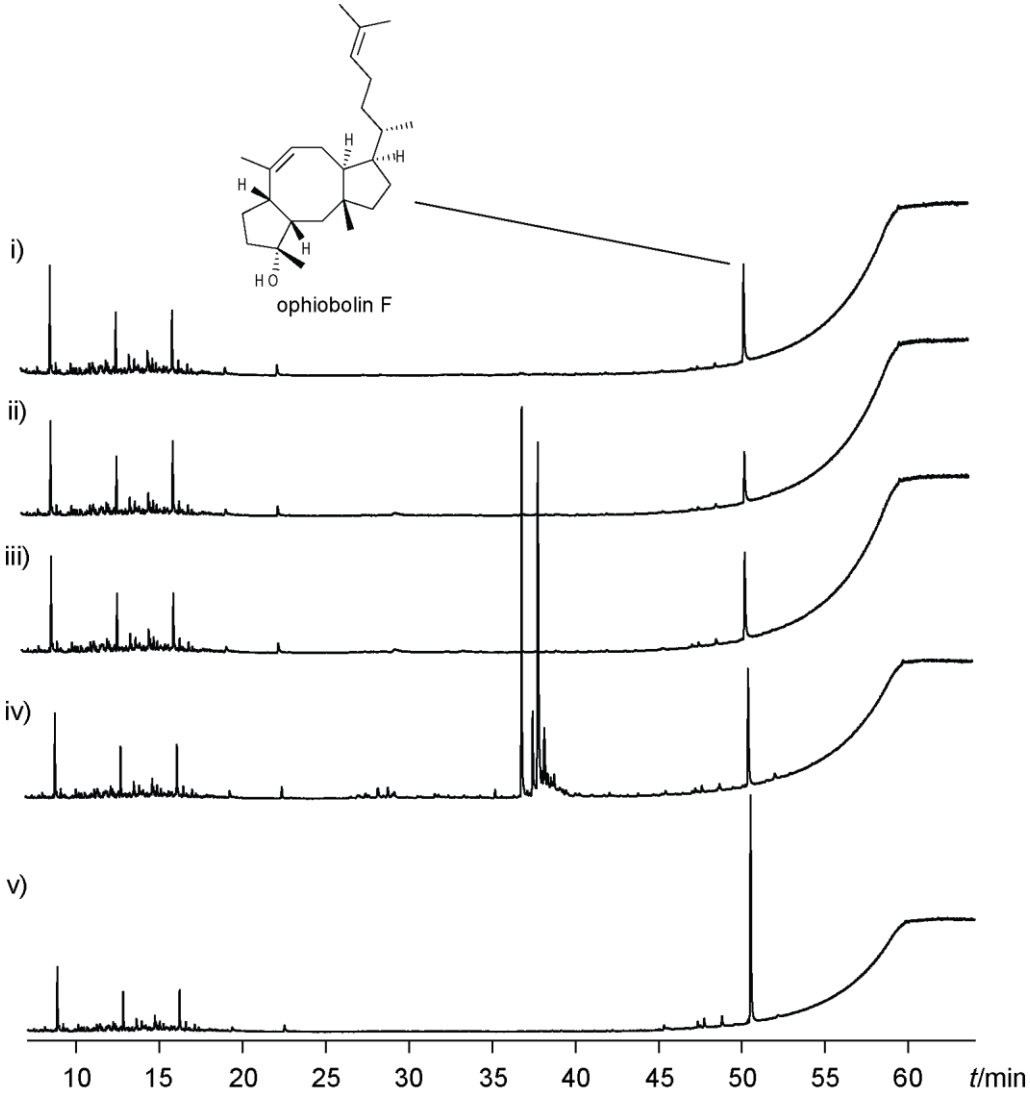


Figure S12. The GC/MS profile of reaction catalysed by AcldOS combined with: i) DMAPP+IPP, ii) GPP+IPP, iii) FPP+IPP, iv) GGPP+IPP, v) GFPP. The peaks between 35 min and 40 min in iv) are spontaneous hydrolysis products of GGPP.

Isotopic labelling experiments catalysed by AcldOS:

Isotopic labelling experiments were performed with combinations of ^{13}C and ^{2}H labelled terpene precursors incubated with AcldOS. Approximately 1 mg of each substrate was used in the reaction mixture. IDI was added as an additional enzyme, when needed. Incubation conditions were the same as described above for the unlabelled substrates. The reaction mixtures after incubation were extracted with C_6D_6 (0.6 mL). The extracts were centrifuged (14.610 x g, 15 min) to remove water and subjected to GC/MS and NMR analysis.

Table S3. Isotopic labelling experiments catalysed by AcldOS.

entry	substrates	additional enzyme	results shown in
1	DMAPP + (*E*)-(4-^{13}C,4-^{2}H)IPP[8]	-	Figure S13
2	DMAPP + (*Z*)-(4-^{13}C,4-^{2}H)IPP[8]	-	Figure S13
3	(*R*)-(1-^{13}C,1-^{2}H)IPP[9]	IDI[10]	Figure S14
4	(*S*)-(1-^{13}C,1-^{2}H)IPP[9]	IDI	Figure S14
5	GGPP + (1-^{13}C)IPP[11]	-	Figure S15
6	GGPP + (2-^{13}C)IPP[10]	-	Figure S15
7	GGPP + (3-^{13}C)IPP[11]	-	Figure S15
8	GGPP + (4-^{13}C)IPP[11]	-	Figure S15
9	(1-^{13}C)GGPP[a] + IPP	-	Figure S15
10	(2-^{13}C)GGPP[11] + IPP	-	Figure S15
11	(3-^{13}C)GGPP[a] + IPP	-	Figure S15
12	(4-^{13}C)GGPP[a] + IPP	-	Figure S15
13	(1-^{13}C)FPP[12] + IPP	-	Figure S15
14	(2-^{13}C)FPP[12] + IPP	-	Figure S15
15	(3-^{13}C)FPP[12] + IPP	-	Figure S16
16	(4-^{13}C)FPP[12] + IPP	-	Figure S16
17	(5-^{13}C)FPP[12] + IPP	-	Figure S16
18	(6-^{13}C)FPP[12] + IPP	-	Figure S16
19	(7-^{13}C)FPP[12] + IPP	-	Figure S16
20	(8-^{13}C)FPP[12] + IPP	-	Figure S16
21	(9-^{13}C)FPP[12] + IPP	-	Figure S16
22	(10-^{13}C)FPP[12] + IPP	-	Figure S16
23	(11-^{13}C)FPP[12] + IPP	-	Figure S16
24	(12-^{13}C)FPP[12] + IPP	-	Figure S16
25	(9-^{13}C)GPP[13] + IPP	-	Figure S17
26	(14-^{13}C)FPP[12] + IPP	-	Figure S17
27	(15-^{13}C)FPP[12] + IPP	-	Figure S17
28	(20-^{13}C)GGPP[11] + IPP	-	Figure S17
29	GGPP + (5-^{13}C)IPP	-	Figure S17
30	(7-^{13}C)FPP + (*E*)-(4-^{2}H)IPP[10]	-	Figure S18, S19
31	(7-^{13}C)FPP + (*Z*)-(4-^{2}H)IPP[10]	-	Figure S18, S19

[a] Synthesised in this study according to Schemes S1 and S2.

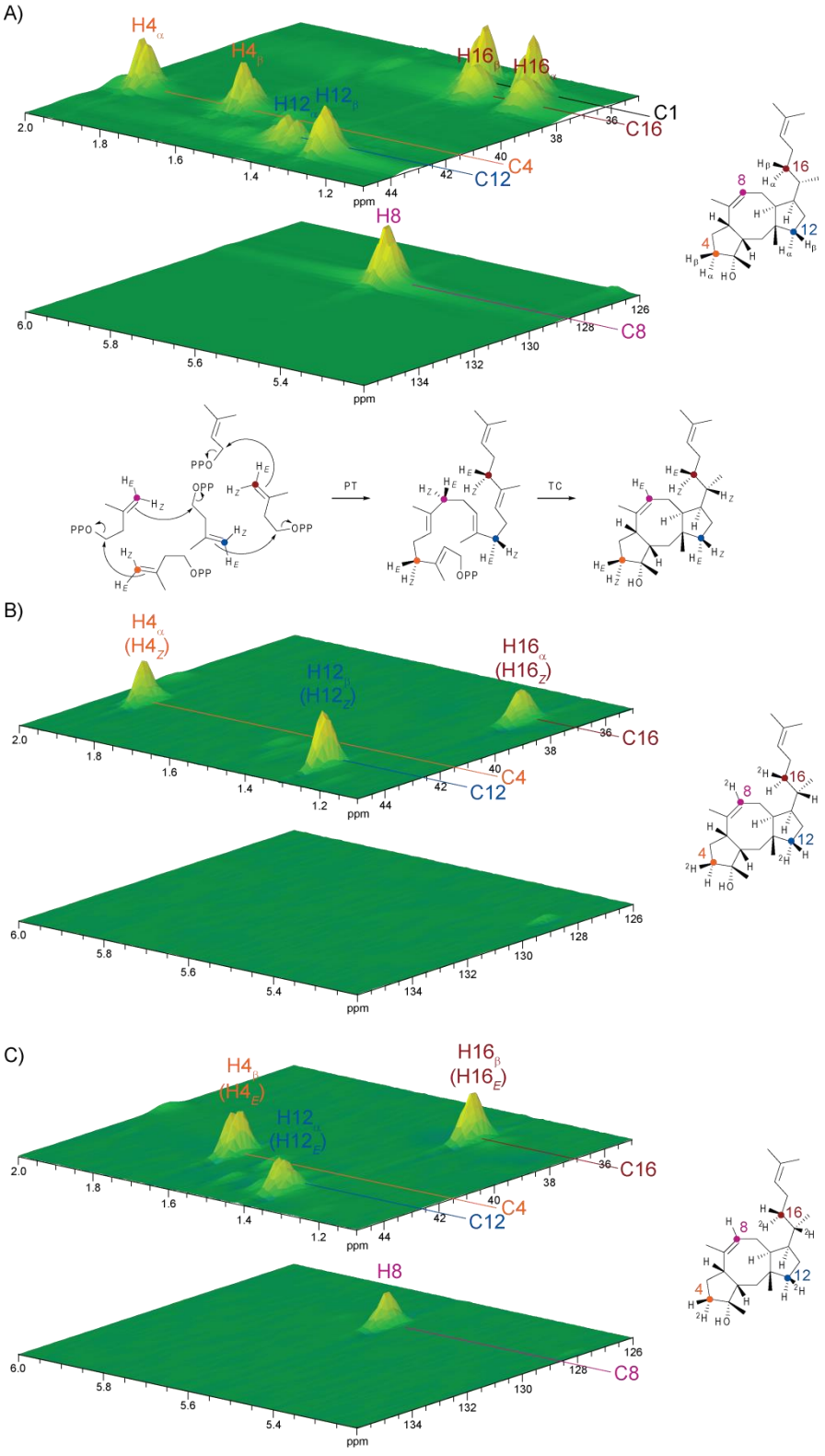


Figure S13. Partial HSQC spectra of A) unlabelled ophiobolin F, B) (4*S*,12*S*,16*S*)-(4,8,12,16-$^{13}C_4$, 4,8,12,16-$^{2}H_4$)ophiobolin F derived from DMAPP and (*E*)-(4-^{13}C,4-^{2}H)IPP incubated with AcldOS, and C) (4*R*,12*R*,16*R*)-(4,8,12,16-$^{13}C_4$,4,12,15,16-$^{2}H_4$)ophiobolin F derived from DMAPP and (*Z*)-(4-^{13}C,4-^{2}H)IPP incubated with AcldOS.

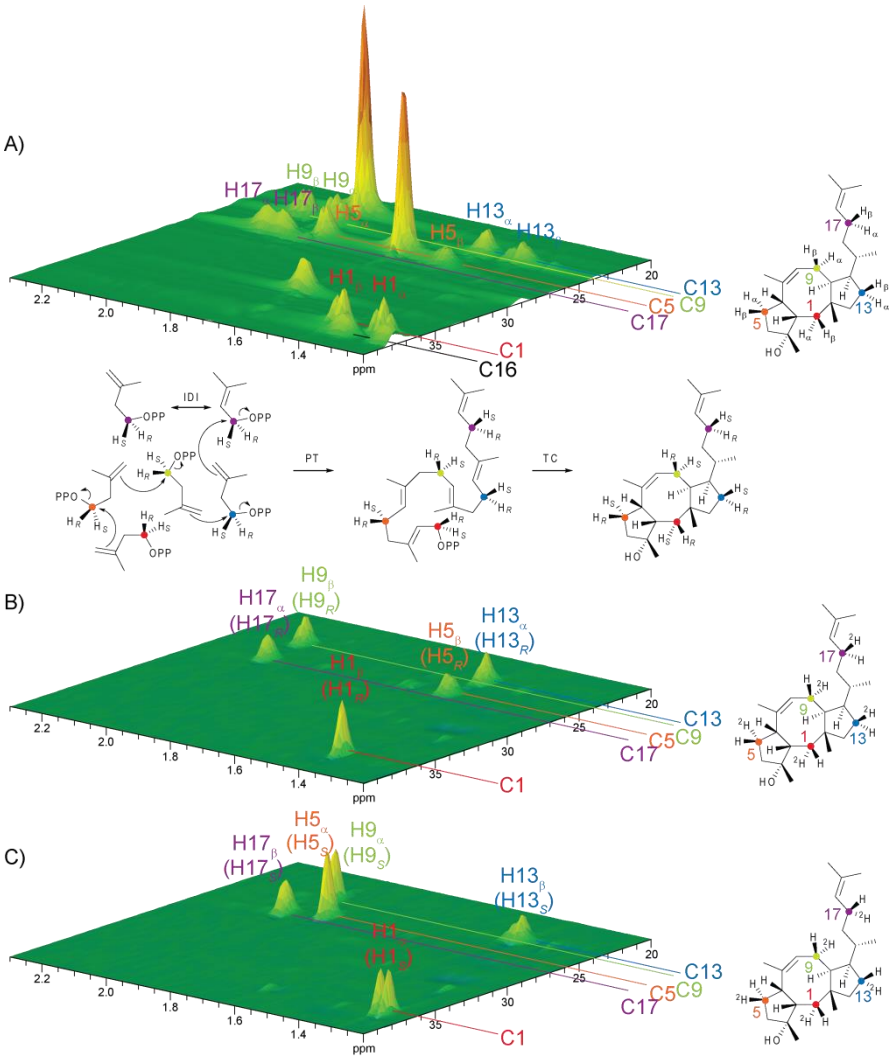


Figure S14. Partial HSQC spectra of A) unlabelled ophiobolin F, B) (1*R*,5*R*,9*R*,13*R*,17*R*)-(1,5,9,13,17-$^{13}C_5$,1,5,9,13,17-$^{2}H_5$)ophiobolin F derived from (*R*)-(1-^{13}C,1-^{2}H)IPP incubated with AcldOS and IDI, and C) (1*S*,5*S*,9*S*,13*S*,17*S*)-(1,5,9,13,17-$^{13}C_5$,1,5,9,13,17-$^{2}H_5$)ophiobolin F derived from (*S*)-(1-^{13}C,1-^{2}H)IPP incubated with AcldOS and IDI.

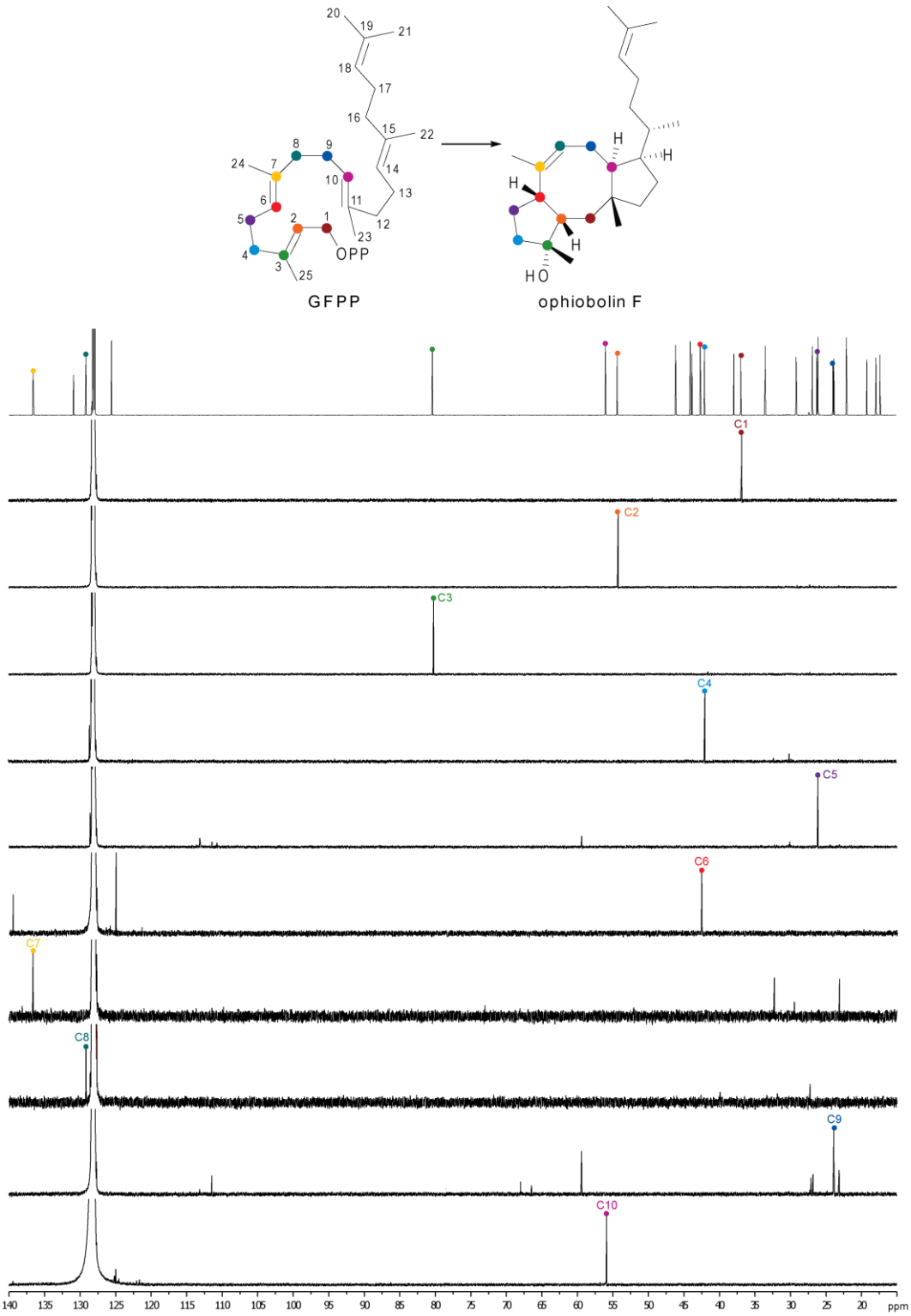


Figure S15. ^{13}C-NMR spectra of unlabelled ophiobolin F (top) and ^{13}C labelled ophiobolin F obtained from incubation experiments entries 5 - 14 in Table S3.

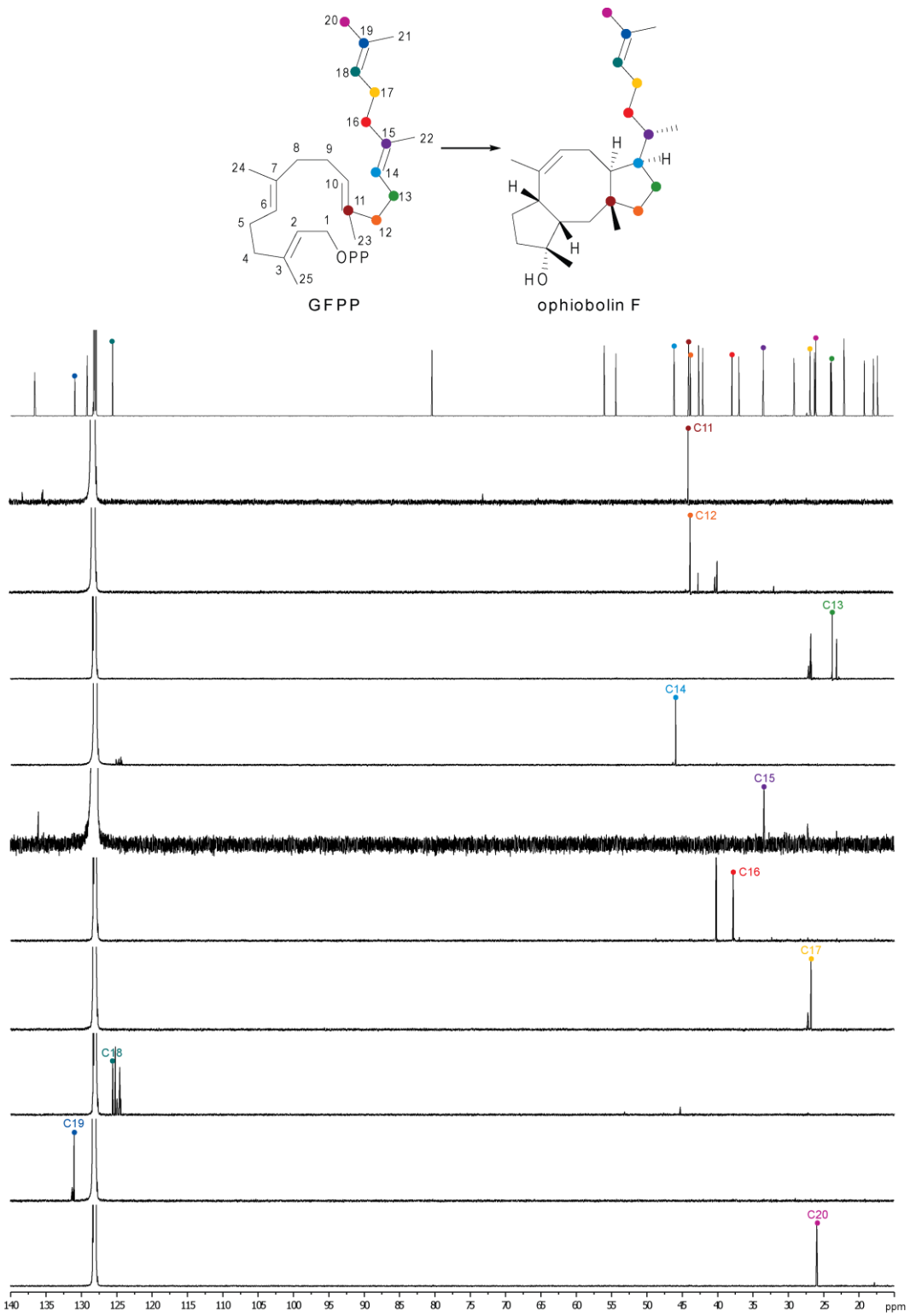


Figure S16. ^{13}C-NMR spectra of unlabelled ophiobolin F (top) and ^{13}C labelled ophiobolin F obtained from incubation experiments entries 15 - 24 in Table S3.

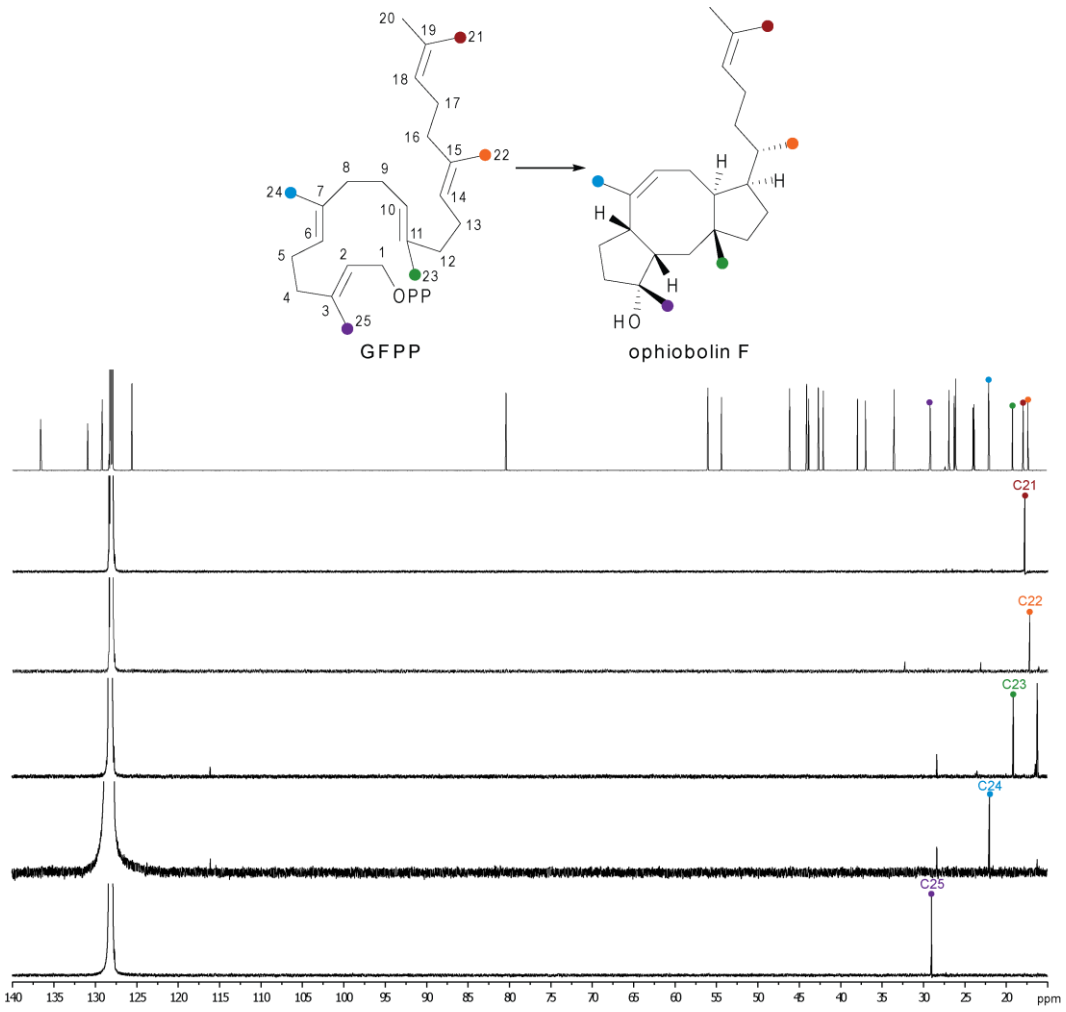
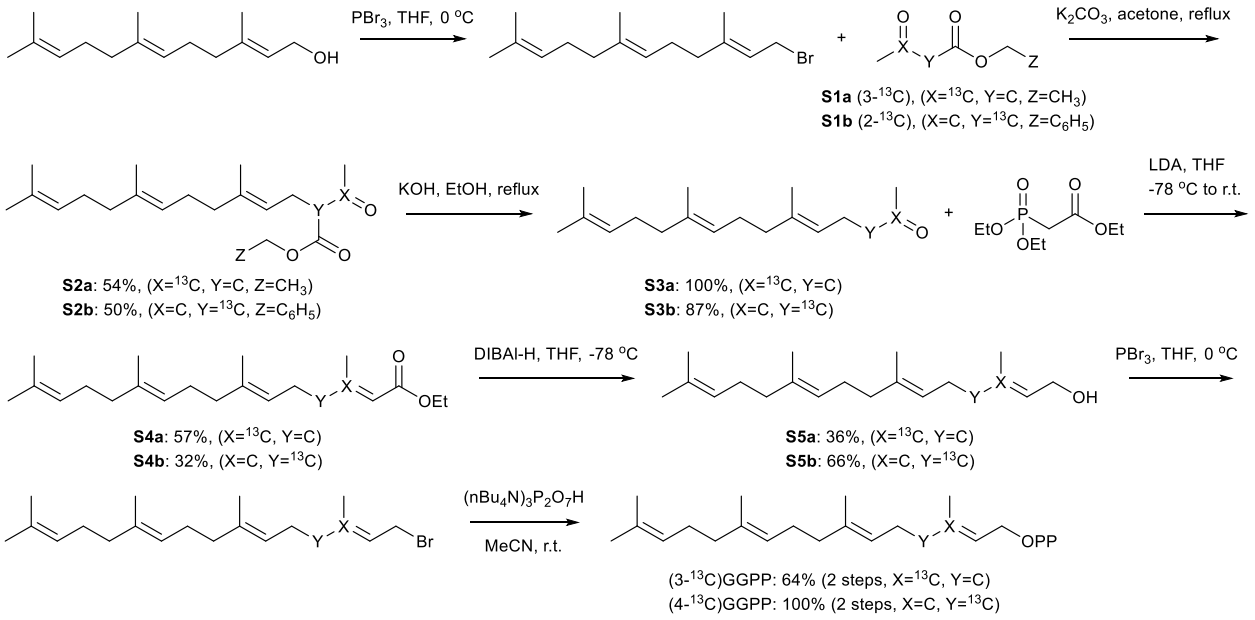


Figure S17. ^{13}C-NMR spectra of unlabelled ophiobolin F (top) and ^{13}C labelled ophiobolin F obtained from incubation experiments entries 25 - 29 in Table S3.

.

Total synthesis of substrates for isotopic labelling experiments:

Total synthesis of (2*E*,6*E*,10*E*)-(3-^{13}C)GGPP and (2*E*,6*E*,10*E*)-(4-^{13}C)GGPP



Scheme S1. Synthesis of (3-^{13}C)GGPP and (4-^{13}C)GGPP.

In the following general procedures the starting material is set to 1.0 eq. and the amounts of reagents are given in relative proportions. Solvent amounts and washing solutions are given in mL mmol^{-1} which is in all cases based on the starting material. The absolute amounts of transformed materials can be delineated from the yields.

Synthesis of ethyl (4*E*,8*E*)-(1-^{13}C)- and benzyl (4*E*,8*E*)-(2-^{13}C)-2-acetyl-5,9,13-trimethyltetradeca-4,8,12-trienoate (**S2a** and **S2b**)

Farnesol (1.0 eq.) was dissolved in dry tetrahydrofuran (THF, 10 mL mmol^{-1}) on ice and cooled to 0 °C. PBr_3 (0.4 eq.) was added dropwise. The reaction mixture was stirred on ice for 30 min. Then, the reaction mixture was poured onto ice. The aqueous phase was extracted two times using cold hexane. The combined hexane layers were dried with $MgSO_4$ and concentrated under reduced pressure to obtain (2*E*,6*E*)-1-bromo-3,7,11-trimethyldodeca-2,6,10-triene. K_2CO_3 (1.5 eq.) was suspended in acetone (10 mL mmol^{-1}) and obtained (2*E*,6*E*)-1-bromo-3,7,11-trimethyldodeca-2,6,10-triene was dissolved in this suspension. Ethyl (3-^{13}C)- or benzyl (2-^{13}C)-3-oxobutanoate (**S1a**, 3.75 mmol, or **S1b**, 2.07 mmol, 1.0 eq.) was added to the reaction mixture. The suspension was stirred overnight under reflux. After 14 h, the reaction suspension was filtrated after cooled to room temperature (r. t.), then concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting stepwise using cyclohexane/ethyl acetate from 100/0 to 80/20. The fractions containing the target compound were collected to give the product as colorless oil.

Ethyl (4*E*,8*E*)-(1-^{13}C)-2-acetyl-5,9,13-trimethyltetradeca-4,8,12-trienoate (**S2a**):

Yield: 675 mg (2.0 mmol, 54%). R_f [cyclohexane/ethyl acetate (9/1)] = 0.37. GC (HP5-MS): I = 2247. ^{1}H-NMR (500 MHz, C_6D_6): δ = 5.23 (m, 2H, 2xCH), 5.16 (m, H, CH), 3.90 (qd, $^3J_{H,H}$ = 7.12 Hz, $^4J_{H,H}$ = 1.54 Hz, 2H, CH_2), 3.30 (m, 1H,CH), 2.65 (m, 2H, CH_2), 2.21-2.07 (m, 6H, 3xCH_2), 2.00 (m, 2H, CH_2), 1.89 (d, $^2J_{C,H}$ = 6.04 Hz, 3H, CH_3), 1.69 (br s, 3H, CH_3), 1.58 (s, 6H, 2xCH_3), 1.55 (s, 3H, CH_3), 0.89 (t, $^3J_{H,H}$ = 7.10 Hz, 3H, CH_3) ppm. ^{13}C- NMR (125 MHz, C_6D_6): δ = 201.37 ($^{13}C_q$), 169.52 (d, $^2J_{C,C}$ = 1.61 Hz, C_q), 138.12 (C_q), 135.22 (C_q), 131.21 (C_q), 124.94 (CH), 124.53 (CH), 120.79 (d, $^3J_{C,C}$ = 2.69 Hz, CH), 61.01 (CH_2), 59.97 (d, $^1J_{C,C}$ = 36.01 Hz, CH), 40.22 (CH_2), 40.11

(CH_2), 28.69 (d, $^1J_{C,C}$ = 41.93 Hz, CH_3), 27.29 (d, $^2J_{C,C}$ = 1.27 Hz, CH_2), 27.25 (CH_2), 26.93 (CH_2), 25.89 (CH_3), 17.78 (CH_3), 16.15 (CH_3), 16.11 (CH_3), 14.05 (CH_3) ppm. MS (EI, 70 eV): *m/z* (%) = 335 (4), 290 (1), 289 (1), 274 (2), 248 (4), 211 (4), 204 (5), 198 (13), 193 (3), 189 (5), 178 (4), 177 (4), 174 (8), 167 (5), 161 (16), 155 (27), 152 (12), 149 (6), 144 (8), 136 (79), 124 (48), 121 (31), 109 (41), 95 (19), 93 (36), 86 (4), 81 (67), 77 (4), 69 (100), 55 (8), 44 (30), 41 (21).

Benzyl (4*E*,8*E*)-(2-^{13}C)-2-acetyl-5,9,13-trimethyltetradeca-4,8,12-trienoate (**S2b**):

Yield: 412 mg (1.0 mmol, 50%). R_f [cyclohexane/ethyl acetate (9/1)] = 0.4. ^{1}H-NMR (500 MHz, C_6D_6): δ = 5.23 (m, 2H, 2xCH), 5.12 (m, 1H, CH), 4.95 (s, 2H, CH_2), 3.31 (dt, $^1J_{C,H}$ = 131.39 Hz, $^3J_{H,H}$ = 7.5 Hz, 1H, CH), 2.65 (m, 2H, CH_2), 2.08 (m, 8H, 4xCH_2), 1.83 (d, $^3J_{C,H}$ = 1.01, 3H, CH_3), 1.68 (s, 3H, CH_3), 1.58 (s, 6H, 2xCH_3), 1.52 (s, 3H, CH_3) ppm. ^{13}C-NMR (125 MHz, C_6D_6): δ = 201.21 (d, $^1J_{C,C}$ = 36.99 Hz, C_q), 169.42 (d, $^1J_{C,C}$ = 56.34 Hz, C_q), 138.26 (d, $^3J_{C,C}$ = 3.51 Hz, C_q), 136.20 (C_q), 135.22 (C_q), 131.21 (C_q), 128.73 (CH), 128.62 (CH), 128.59 (CH), 128.46 (CH), 128.35 (CH), 124.94 (CH), 124.54 (CH), 120.62 (d, $^1J_{C,C}$ = 1.13 Hz, CH), 99.92 (C_q), 66.87 (CH_2), 59.92 (^{13}CH), 40.23 (CH_2), 40.08 (CH_2), 28.76 (d, $^2J_{C,C}$ = 13.22 Hz, CH_3), 27.28 (d, $^1J_{C,C}$ = 33.87 Hz, CH_2), 27.25 (CH_2), 26.91 (CH_2), 25.89 (CH_3), 18.70 (d, $^4J_{C,C}$ = 5.51 Hz, CH_3), 17.78 (CH_3), 16.13 (CH_3) ppm.

Synthesis of (5*E*,9*E*)-(2-^{13}C)- and (5*E*,9*E*)-(3-^{13}C)-6,10,14-trimethylpentadeca-5,9,13-trien-2-one (**S3a** and **S3b**)

Compound **S2a** or **S2b** (1.0 eq.) was dissolved in ethanol (10 mL mmol^{-1}). KOH aqueous solution (3 M, 2.0 eq.) was added dropwise to the reaction solution. The reaction mixture was stirred overnight under reflux. After 14 h, the reaction was quenched by adding HCl aqueous solution (1 M) until no gas was generated. The reaction mixture was extracted three times with ethyl acetate. The combined organic layers were washed with saturated aqueous NaCl and dried with $MgSO_4$, then concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with cyclohexane/ethyl acetate stepwise from 100/0 to 92/8. The fractions containing product were collected and the solvents were evaporated to give the target compound as colorless oil.

(5*E*,9*E*)-(2-^{13}C)-6,10,14-Trimethylpentadeca-5,9,13-trien-2-one (**S3a**):

Yield: 768 mg, (2.7 mmol, 100%): R_f [cyclohexane/ethyl acetate (9/1)] = 0.5. GC (HP5-MS): *I* = 1927. ^{1}H-NMR (500 MHz, C_6D_6): δ = 5.25 (m, 2H, 2xCH), 5.14 (tdd, $^3J_{H,H}$ = 7.19 Hz, $^4J_{H,H}$ = 2.51 Hz, $^4J_{H,H}$ = 1.21 Hz, 1H, CH), 2.24 (m, 2H, CH_2), 2.21-1.99 (m, 10H, 5xCH_2), 1.68 (br s, 3H, CH_3), 1.65 (d, $^2J_{C,H}$ = 5.80 Hz, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.56 (s, 3H, CH_3) ppm. ^{13}C-NMR (125 MHz, C_6D_6): δ = 205.85 ($^{13}C_q$), 135.89 (C_q), 135.12 (C_q), 131.19 (C_q), 124.95 (CH), 124.70 (CH), 123.62 (d, $^3J_{C,C}$ = 3.17 Hz, CH), 43.39 (d, $^1J_{C,C}$ = 39.12 Hz, CH_2), 40.24 (CH_2), 40.13 (CH_2), 29.37 (d, $^1J_{C,C}$ = 40.22 Hz, CH_3), 27.26 (CH_2), 27.02 (CH_2), 25.89 (CH_3), 22.77 (d, $^2J_{C,C}$ = 1.80 Hz, CH_2), 17.78 (CH_3), 16.14 (CH_3), 16.03 (CH_3) ppm. MS (EI, 70 eV): *m/z* (%) = 263 (4), 220 (3), 204 (4), 202 (4), 194 (5), 189 (2), 179 (10), 176 (9), 161 (7), 152 (4), 148 (3), 139 (16), 136 (56), 126 (25), 121 (20), 108 (32), 95 (23), 93 (25), 81 (45), 69 (100), 55 (6), 53 (5), 44 (49), 41 (21).

(5*E*,9*E*)-(3-^{13}C)-6,10,14-Trimethylpentadeca-5,9,13-trien-2-one (**S3b**):

Yield: 235 mg, (0.9 mmol, 87%). R_f [cyclohexane/ethyl acetate (9/1)] = 0.5. GC (HP5-MS): *I* = 1929. ^{1}H-NMR (500 MHz, C_6D_6): δ = 5.25 (m, 2H, 2xCH), 5.13 (m, 1H, CH), 2.24 (m, 2H, CH_2), 2.16 (m, 4H, 2xCH_2), 2.04 (m, 6H, 3xCH_2), 1.68 (br s, 3H, CH_3), 1.64 (d, $^3J_{C,H}$ = 1.33 Hz, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.56 (s, 3H, CH_3) ppm. ^{13}C-NMR (125 MHz, C_6D_6): δ = 205.84 (d, $^1J_{C,C}$ = 39.09 Hz, C_q), 135.89 (d, $^3J_{C,C}$ = 3.59 Hz, C_q), 135.12 (C_q), 131.19 (C_q), 124.95 (CH), 124.70 (CH), 123.63 (d, $^2J_{C,C}$ = 1.66 Hz, CH), 43.32 ($^{13}CH_2$), 40.24 (CH_2), 40.13 (CH_2), 29.37 (d, $^2J_{C,C}$ = 1.47 Hz, CH_3), 27.27 (CH_2), 27.03 (CH_2), 25.88 (CH_3), 22.77 (d, $^1J_{C,C}$ = 35.11, CH_2), 17.77 (CH_3), 16.14 (CH_3), 16.04 (CH_3) ppm. MS (EI, 70 eV): *m/z* (%) = 263 (9), 220 (7), 204 (9), 202 (7), 194 (10), 179 (19), 176 (16), 161 (13), 152 (8), 136 (82), 126 (45), 123 (28), 121 (35), 108 (54), 95 (30), 93 (44), 81 (75), 69 (100), 55 (11), 43 (85), 41 (41).

Synthesis of ethyl (2*E*,6*E*,10*E*)-(3-^{13}C)- and (4-^{13}C)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenoate (**S4a** and **S4b**)

Dry THF (10 mL mmol^{-1}) was added into a reaction vessel and cooled to –10 ^{o}C. Diisopropylamine (1.1 eq.) was dissolved in the cold THF and *n*-butyllithium (1.1 eq.) was added dropwise to the solution. The reaction mixture was stirred 1 h at –10 ^{o}C, then cooled to –78 ^{o}C. Triethyl phosphonoacetate (1.1 eq.) was added. The reaction mixture was stirred 2 h at –78 ^{o}C. The substrate (**S3a** or **S3b**, 1.0 eq.) in dry THF (10 mL mmol^{-1}) was added at –78 ^{o}C, and stirred overnight at r. t.. After 14 h, the reaction was quenched by adding H_2O (20 mL mmol^{-1}), then the aqueous layer was extracted four times with ethyl acetate. The combined organic layers were washed with saturated aqueous NaCl and dried with $MgSO_4$, then concentrated under reduced pressure. The residue was purified by silica gel chromatography using cyclohexane/ethyl acetate (stepwise from 100/0 to 92/8) to yield the products (mixture of (2*E*)- and (2*Z*)-stereoisomers) as colorless oil.

Ethyl (2*EZ*,6*E*,10*E*)-(3-^{13}C)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenoate (**S4a**):

Yield: 553 mg, (1.7 mmol, 57%). R_f (*E*+*Z*) [cyclohexane/ethyl acetate (9/1)] = 0.76 (*E*), 0.8 (*Z*). GC (HP5-MS): *I* (*E*) = 2320. ^{1}H-NMR (500 MHz, C_6D_6) for 2*E* stereoisomer: δ = 5.84 (dt, $^2J_{C,H}$ = 2.38 Hz, $^4J_{H,H}$ = 1.13 Hz, 1H, CH), 5.26 (m, 2H, 2xCH), 5.09 (m, 1H, CH), 4.06 (q, $^3J_{H,H}$ = 7.12 Hz, 2H, CH_2), 2.22 (dd, $^2J_{C,H}$ = 6.46 Hz, $^4J_{H,H}$ = 1.25 Hz, 3H, CH_3), 2.20-2.00 (m, 10H, 5xCH_2), 1.93 (m, 2H, CH_2), 1.69 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.02 (t, $^3J_{H,H}$ = 7.12 Hz, 3H, CH_3) ppm. ^{13}C-NMR (125 MHz, C_6D_6) for 2*E* stereoisomer: 166.49 (d, $^2J_{C,C}$ = 2.11 Hz, C_q), 159.36 ($^{13}C_q$), 136.02 (C_q), 135.13 (C_q), 131.19 (C_q), 124.95 (CH), 124.72 (CH), 123.58 (d, $^3J_{C,C}$ = 3.42 Hz, CH), 116.35 (d, $^1J_{C,C}$ = 72.20 Hz, CH), 59.39 (CH_2), 41.01 (d, $^1J_{C,C}$ = 39.99 Hz, CH_2), 40.24 (CH_2), 40.10 (CH_2), 27.27 (CH_2), 27.07 (CH_2), 26.26 (d, $^2J_{C,C}$ = 2.12 Hz, CH_2), 18.81 (d, $^1J_{C,C}$ = 40.12 Hz, CH_3), 17.77 (CH_3), 16.13 (CH_3), 16.06 (CH_3), 14.45 (CH_3) ppm. MS (EI, 70 eV) for 2*E* stereoisomer: *m*/*z* (%) = 333 (12), 318 (2), 290 (6), 288 (7), 264 (3), 260 (1), 251 (3), 248 (4), 244 (4), 222 (3), 220 (3), 218 (4), 216 (5), 210 (3), 204 (9), 190 (15), 176 (10), 191 (9), 154 (11), 149 (15), 142 (5), 136 (68), 129 (72), 122 (36), 114 (5), 109 (18), 101 (10), 95 (21), 93 (22), 81 (59), 69 (100), 55 (8), 53 (4), 41 (16).

Ethyl (2*EZ*,6*E*,10*E*)-(4-^{13}C)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenoate (**S4b**):

Yield (*E*+*Z*): 100 mg, (0.30 mmol, 32%). R_f (*E*+*Z*) [cyclohexane/ethyl acetate (9/1)] = 0.76 (*E*), 0.8 (*Z*). GC (HP5-MS): *I* (*E*) = 2325. MS (EI, 70 eV) for 2*E* stereoisomer: *m*/*z* (%) = 333 (5), 290 (3), 288 (3), 264 (1), 260 (1), 248 (2), 244 (2), 222 (1), 220 (1), 218 (2), 216 (3), 210 (2), 205 (5), 204 (5), 190 (9), 176 (6), 161 (6), 154 (5), 149 (10), 136 (46), 129 (48), 122 (27), 109 (14), 101 (7), 95 (17), 93 (18), 81 (53), 69 (100), 55 (9), 41 (20).

Synthesis of (2*E*,6*E*,10*E*)-(3-^{13}C)- and (2*E*,6*E*,10*E*)-(4-^{13}C)-geranylgeraniol (**S5a** and **S5b**)

Compound **S4a** or **S4b** (1.0 eq.) was dissolved in dry THF (10 mL mmol^{-1}). The solution was cooled to –78 ^{o}C. DIBAl-H (1.0 M in hexane, 2.2 eq.) was added dropwise to the solution at –78 ^{o}C. The reaction mixture was stirred 2 h at r. t.. The reaction mixture was cooled to 0 ^{o}C on ice, and quenched by slowly adding saturated sodium potassium tartrate solution while stirring. The mixture was extracted four times with ethyl acetate. The ethyl acetate layers were combined and washed with saturated aqueous NaCl, then dried with $MgSO_4$. The extract was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting stepwise using cyclohexane/ethyl acetate from 100/0 to 80/20 to give products **S5a** and **S5b** as colorless oils. Both (2*Z*)-stereoisomers were removed at this stage.

(2*E*,6*E*,10*E*)-(3-^{13}C)Geranylgeraniol (**S5a**):

Yield: 174 mg, (0.61 mmol, 36%). R_f (*E*+*Z*) [cyclohexane/ethyl acetate (4/1)] = 0.45 (*E*), 0.55 (*Z*). GC (HP5-MS): *I* = 2203. ^{1}H-NMR (500 MHz, C_6D_6): δ = 5.40 (dt, $^2J_{C,H}$ = 6.67 Hz, $^3J_{H,H}$ = 3.82 Hz, 1H, CH), 5.30 (tq, $^3J_{H,H}$ = 6.96 Hz, $^4J_{H,H}$ = 1.24 Hz, 1H, CH), 5.25 (m, 2H, 2xCH), 3.98 (s, 2H, CH_2), 2.24-2.06 (m, 10H, 5xCH_2), 2.01 (m, 2H, CH_2), 1.69 (br s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.59 (s, 3H,CH_3), 1.57 (s, 3H, CH_3), 1.48 (d, $^2J_{C,H}$ = 6.09 Hz, 3H, CH_3) ppm. ^{13}C-NMR (125 MHz, C_6D_6): δ = 138.16 ($^{13}C_q$), 135.33 (C_q), 135.09 (C_q), 131.18 (C_q), 124.97 (CH), 124.94 (d, $^1J_{C,C}$ = 72.99 Hz, CH), 124.80 (CH), 124.51 (d, $^4J_{C,C}$ = 3.51 Hz, CH), 59.39 (d, $^2J_{C,C}$ = 1.20 Hz, CH_2), 40.25 (CH_2), 40.19 (CH_2), 39.91 (d, $^1J_{C,C}$ = 42.03 Hz, CH_2), 27.28 (CH_2), 27.13 (CH_2), 26.79 (d, $^2J_{C,C}$ = 2.21 Hz, CH_2), 25.89 (CH_3), 17.78 (CH_3), 16.20 (d, $^1J_{C,C}$ = 41.77 Hz, CH_3), 16.14 (d, $^5J_{C,C}$ = 2.66 Hz, CH_3) ppm. MS (EI, 70 eV): *m*/*z* (%) = 291 (0.2), 273 (0.5), 260 (0.4), 258 (0.6), 248 (0.5), 230 (1), 222 (1), 204 (3), 149 (3), 177 (2), 162 (3), 149 (3), 136 (15), 123 (9), 121 (10), 107 (10), 95 (17), 93 (18), 81 (46), 69 (100), 55 (8), 53 (4), 41 (28).

(2*E*,6*E*,10*E*)-(4-^{13}C)Geranylgeraniol (**S5b**):

Yield: 60 mg (0.2 mmol, 66%). R_f (*E*+*Z*) [cyclohexane/ethyl acetate (4/1)] = 0.45 (*E*), 0.55 (*Z*). GC (HP5-MS): *I* = 2200. ^{1}H-NMR (500 MHz, C_6D_6): δ = 5.40 (m, 1H, CH), 5.29 (m, 1H, CH), 5.24 (m, 2H, 2xCH), 3.97 (d, $^3J_{H,H}$ = 4.02 Hz, 2H, CH_2), 2.19 (m, 2H, CH_2), 2.10 (m, 10H, 5xCH_2), 1.68 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.58 (s, 6H, 2xCH_3), 1.48 (d, $^3J_{C,H}$ = 2.97 Hz, 3H, CH_3) ppm. ^{13}C-NMR (125 MHz, C_6D_6): δ = 138.15 (d, $^1J_{C,C}$ = 42.01 Hz, C_q), 135.33 (d, $^3J_{C,C}$ = 3.64 Hz, C_q), 135.09 (C_q), 131.18 (C_q), 124.98 (CH), 124.97 (CH), 124.80 (CH), 124.51 (d, $^2J_{C,C}$ = 1.58 Hz, CH), 59.39 (d, $^3J_{C,C}$ = 4.76 Hz, CH_2), 40.23 (d, 4JC,C = 7.16 Hz, CH_2), 40.22 (CH_2), 39.92 ($^{13}CH_2$), 27.27 (CH_2), 27.12 (CH_2), 26.78 (d, $^1J_{C,C}$ = 33.7 Hz, CH_2), 25.89 (CH_3), 17.78 (CH_3), 16.22 (CH_3), 16.19 (CH_3), 16.14 (d, $^2J_{C,C}$ = 2.57 Hz, CH_2) ppm. MS (EI, 70 eV): *m*/*z* (%) = 291 (0.3), 273 (0.8), 258 (0.9), 248 (0.6), 230 (1), 222 (2), 204 (5), 189 (4), 277 (2), 162 (4), 149 (4), 136 (20), 123 (12), 121 (14), 107 (12), 95 (20), 93 (20), 81 (52), 69 (100), 55 (10), 41 (29).

Synthesis of trisammonium (2*E*,6*E*,10*E*)-(3-^{13}C)GGPP and trisammonium (2*E*,6*E*,10*E*)-(4-^{13}C)GGPP

The substrate **S5a** or **S5b** (1.0 eq.) was dissolved in dry THF (5 mL mmol^{-1}) and the solution was cooled on ice to 0 ^{o}C. PBr_3 (0.4 eq.) was added dropwise to the solution on ice. The reaction mixture was stirred 30 min at 0 ^{o}C. Then, the reaction mixture was poured onto ice. The reaction mixture was extracted two times with cold hexane. The hexane layers were combined and dried with $MgSO_4$, then concentrated under reduced pressure to obtain (2*E*,6*E*,10*E*)-(3-^{13}C) or (2*E*,6*E*,10*E*)-(4-^{13}C)geranylgeranyl bromide. (nBu$_4$N)$_3$P$_2$O$_7$H (1.2 eq.) was dissolved in dry acetonitrile (MeCN, 5 mL mmol^{-1}). (2*E*,6*E*,10*E*)-(3-^{13}C) or (2*E*,6*E*,10*E*)-(4-^{13}C)geranylgeranyl bromide was added to the solution. The reaction solution was stirred overnight at r. t.. After 14 h, the reaction was stopped by concentration under reduced pressure. The residue was loaded onto an ion exchange resin column (DOWEX® 50W-X8, 100-200 mesh, NH_4^+ form). The product was eluted with 1.5 column volumes of freshly prepared ion exchange buffer (0.025 M NH_4HCO_3 in 2% iPrOH/H_2O) and freeze-dried.

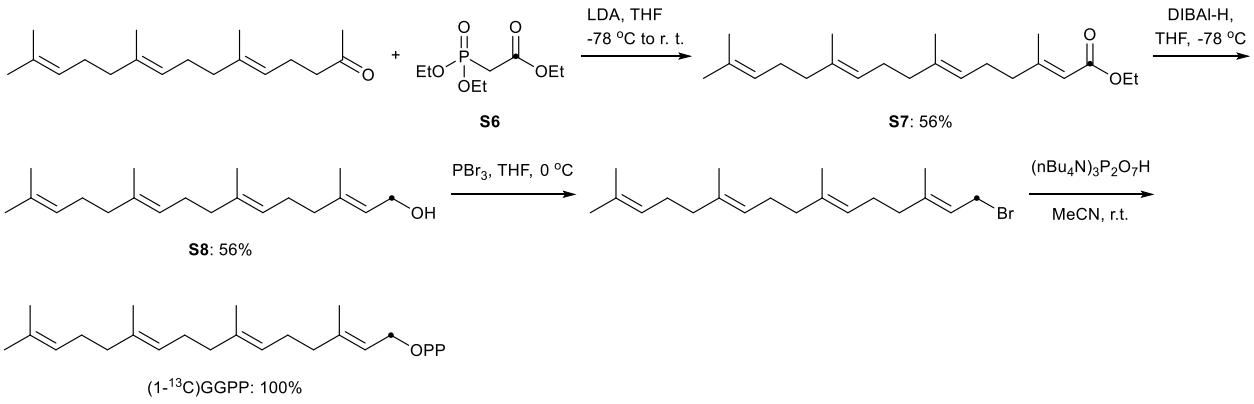
(2*E*,6*E*,10*E*)-(3-^{13}C)GGPP:

Yield: 227 mg, (0.5 mmol, 64%). ^{13}C-NMR (125 MHz, C_6D_6): δ = 139.86 ($^{13}C_q$) ppm. ^{31}P-NMR (202 MHz, D_2O): δ = –6.39 (d, $^2J_{P,P}$ = 20.36 Hz, 1P), –10.31 (d, $^2J_{P,P}$ = 21.76 Hz, 1P) ppm.

(2*E*,6*E*,10*E*)-(4-^{13}C)GGPP:

Yield: 92mg, (0.2 mmol, 100%). ^{31}P-NMR (202 MHz, D_2O): δ = –9.03 (1P), –10.67 (d, $^2J_{P,P}$ = 12.94 Hz, 1P) ppm.

Total synthesis of (2*E*,6*E*,10*E*)-(1-^{13}C)GGPP



Scheme S2. Synthesis of (1-^{13}C)GGPP.

Synthesis of ethyl (2*E*,6*E*,10*E*)-(1-^{13}C)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenoate (**S7**)

Dry THF (10 mL mmol^{-1}) was added into a reaction vessel and cooled to –10 °C. Diisopropylamine (1.1 eq.) was dissolved in cold THF and n-butyllithium (1.1 eq.) was added to the solution. The reaction mixture was stirred 1 h at –10 °C, then cooled to –78 °C. Ethyl 2-(diethoxyphosphoryl)-(1-^{13}C)-acetate (**S6,** 5 mmol, 1.0 eq.) was added. The reaction mixture was stirred 2 h at –78 °C. (5*E*,9*E*)-6,10,14-Trimethylpentadeca-5,9,13-trien-2-one (1.1 eq.) in dry THF (10 mL mmol^{-1}) was added at –78 °C, and the reaction mixture was stirred overnight at r. t.. After 14 h, the reaction was quenched by adding H_2O (20 mL mmol^{-1}), then extracted four times with ethyl acetate. The combined organic layers were washed with saturated aqueous NaCl and dried with $MgSO_4$, then concentrated under reduced pressure. The residue was purified by silica gel chromatography using cyclohexane/ethyl acetate (stepwise from 100/0 to 92/8) to yield products ((2*E*)- and (2*Z*)-stereoisomers mixture) as colorless oil.

Ethyl (2*EZ*,6*E*,10*E*)-(1-^{13}C)3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenoate:

Yield (*E*+*Z*): 940 mg, (2.8 mmol, 56%). R_f (*E*+*Z*) [cyclohexane/ethyl acetate (9/1)] = 0.76 (*E*), 0.8 (*Z*). GC (HP5-MS): *I* (*E*) = 2321. ^{1}H-NMR (500 MHz, C_6D_6) for 2*E* stereoisomer: δ = 5.84 (m, 1H, CH), 5.26 (m, 2H, 2xCH), 5.09 (m, 1H, CH), 4.05 (m, 2H, CH_2), 2.22 (m, 3H, CH_3), 2.20-2.00 (m, 10H, 5xCH_2), 1.93 (m, 2H, CH_2), 1.69 (s, 3H, CH_3), 1.61 (s,3H, CH_3), 1.58 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.02 (t, $^3J_{H,H}$ = 7.12 Hz, 3H, CH_3) ppm. ^{13}C-NMR (125 MHz, C_6D_6) for 2*E* stereoisomer: δ = 166.49 (^{13}Cq), 159.36 (d, $^2J_{C,C}$ = 2.07 Hz, C_q), 136.02 (C_q), 135.12 (C_q), 131.19 (C_q), 124.95 (CH), 124.72 (CH), 123.58 (CH), 116.37 (d, $^1J_{C,C}$ = 75.77 Hz, CH)59.39 (d, $^2J_{C,C}$ = 2.22 Hz, CH_2), 41.02 (d, $^3J_{C,C}$ = 7.02 Hz, CH_2), 40.23 (CH_2), 40.10 (CH_2), 27.27 (CH_2), 27.07 (CH_2), 26.26 (CH_2), 25.89 (CH_3), 18.82 (d, $^3J_{C,C}$ = 1.34 Hz, CH_3), 17.77 (CH_3), 16.13 (CH_3), 16.06 (CH_3), 14.45 (d, $^3J_{C,C}$ = 2.25 Hz, CH_3) ppm. MS (EI, 70 eV) for 2*E* stereoisomer: *m*/*z* (%) = 333 (3), 290 (2), 288 (2), 251 (1), 248 (1), 244 (2), 218 (2), 215 (2), 210 (2), 205 (4), 189 (10), 175 (5), 161 (7), 154 (7), 149 (10), 147 (10), 142 (3), 136 (41), 129 (54), 123 (25), 121 (41), 119 (6), 114 (4), 109 (14), 107 (18), 105 (7), 101 (8), 95 (21), 93 (24), 81 (59), 69 (100), 55 (8), 53 (5), 41 (17).

Synthesis of (2*E*,6*E*,10*E*)-(1-^{13}C)geranylgeraniol (**S8**)

Compound **S7** (1.0 eq.) was dissolved in dry THF (10 mL mmol^{-1}). The solution was cooled to –78 °C. DIBAl-H (1.0 M in hexane, 2.2 eq.) was added dropwise to the solution at –78 °C. The reaction mixture was stirred 2 h at r. t.. The reaction mixture was cooled to 0 °C on ice, and quenched by slowly adding saturated sodium potassium tartrate solution while stirring. The mixture was extracted four times with ethyl acetate. The ethyl acetate layers were combined and washed with saturated aqueous NaCl, then dried with $MgSO_4$. The extract was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting stepwise using

cyclohexane/ethyl acetate from 100/0 to 80/20 to give product **S8** as colorless oil. The (2*Z*)-stereoisomer was removed at this stage.

(2*E*,6*E*,10*E*)-(1-^{13}C)Geranylgeraniol (**S8**):

Yield: 553 mg, (1.9 mmol, 56%). R_f (*E*+*Z*) [cyclohexane/ethyl acetate (4/1)] = 0.45 (*E*), 0.55 (*Z*). GC (HP5-MS): *I* = 2206. ^{1}H-NMR (500 MHz, C_6D_6): δ = 5.40 (m, 1H, CH), 5.29 (m, 1H, CH), 5.25 (m, 2H, CH_2), 3.98 (dd, $^1J_{C,H}$ = 140.63 Hz, $^2J_{H,H}$ = 6.48 Hz, 2H, CH_2), 2.15 (m, 10H, 5xCH_2), 2.01 (m, 2H, CH_2), 1.69 (br s, 3H, CH_3), 1.61(s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.48 (s, 3H, CH_3) ppm. ^{13}C-NMR (125 MHz, C_6D_6): 138.16 (d, $^2J_{C,C}$ = 1.34 Hz, C_q), 135.33 (C_q), 135.09 (C_q), 131.18 (C_q), 124.98 (d, $^1J_{C,C}$ = 47.56 Hz, CH), 124.97 (CH), 124.80 (CH), 124.51 (CH), 59.39 ($^{13}CH_2$), 40.25 (CH_2), 40.19 (CH_2), 39.92 (d, $^3J_{C,C}$ = 4.78 Hz, CH_2), 27.27 (CH_2), 27.13 (CH_2), 26.80 (CH_2), 25.89 (CH_3), 17.78 (CH_3), 16.21 (d, $^3J_{C,C}$ = 4.23 Hz, CH_3), 16.15 (CH_3), 16.13 (CH_3) ppm. MS (EI, 70 eV): *m*/*z* (%) = 291 (0.4), 273 (0.7), 258 (1), 248 (0.9), 230 (2), 222 (2), 204 (6), 195 (1), 189 (5), 177 (4), 161 (5), 149 (6), 136 (24), 123 (16), 121 (19), 109 (15), 107 (17), 95 (27), 93 (26), 81 (70), 69 (100), 55 (12), 53 (6), 41 (38).

Synthesis of trisammonium (2*E*,6*E*,10*E*)-(1-^{13}C)GGPP:

Compound **S8** (1.0 eq.) was dissolved in dry THF (5 mL mmol^{-1}) and cooled on ice to 0 °C. PBr_3 (0.4 eq.) was added dropwise to the solution on ice. The reaction mixture was stirred 30 min at 0 °C. Then, the reaction mixture was poured onto ice. The reaction mixture was extracted two times with hexane. The hexane layers were combined and dried with $MgSO_4$, then concentrated under reduced pressure to obtain (2*E*,6*E*,10*E*)-(1-^{13}C)geranylgeranyl bromide. (*n*$Bu_4N)_3P_2O_7H$ (1.2 eq.) was dissolved in dry acetonitrile (MeCN, 5 mL mmol^{-1}). (2*E*,6*E*,10*E*)-(1-^{13}C)geranylgeranyl bromide was added to the solution. The reaction solution was stirred overnight at r. t.. After 14 h, the reaction was stopped by concentration under reduced pressure. The residue was loaded onto an ion exchange resin column (DOWEX® 50W-X8, 100-200 mesh, NH_4^+ form). The product was eluted with 1.5 column volumes of freshly prepared ion exchange buffer (0.025 M NH_4HCO_3 in 2% iPrOH/H_2O) and freeze-dried.

(2*E*,6*E*,10*E*)-(1-^{13}C)GGPP:

Yield: 180mg, (0.4 mmol, 100%). ^{13}C-NMR (125 MHz, D_2O): δ = 58.13 ($^{13}CH_2$) ppm. ^{31}P-NMR (202 MHz, D_2O): δ = –6.39 (d, $^2J_{P,P}$ = 20.35 Hz, 1P), –10.31 (d, $^2J_{P,P}$ = 19.69 Hz, 1P) ppm.

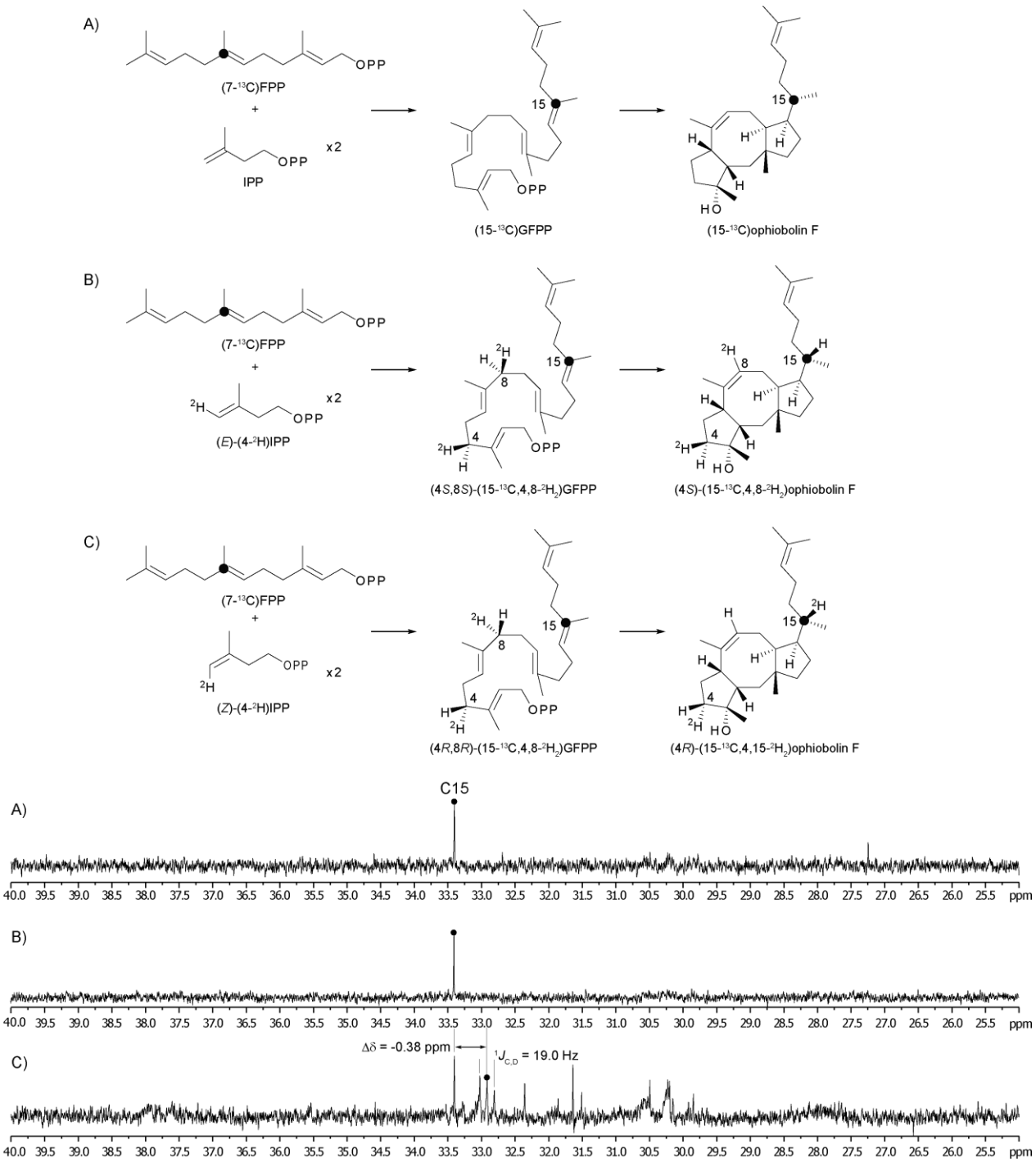
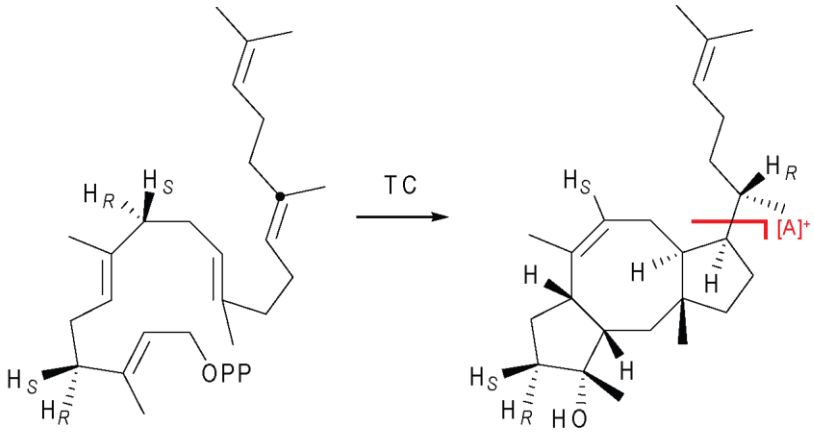
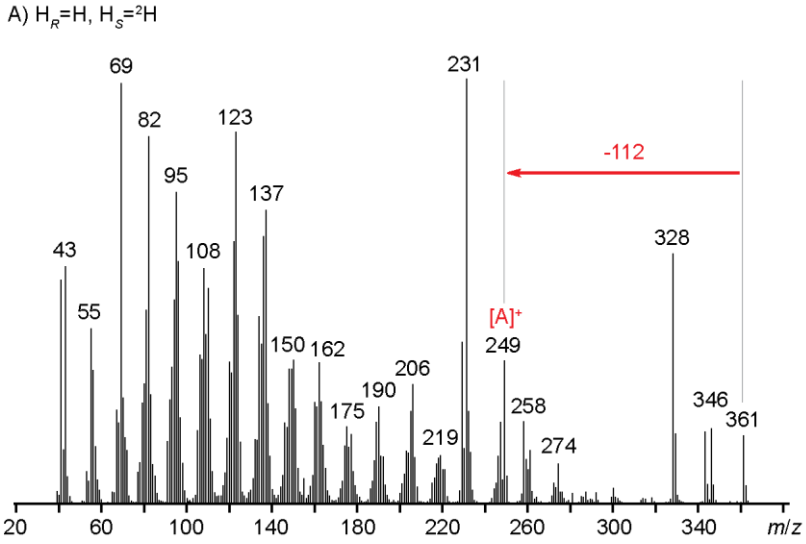


Figure S18. Partial ^{13}C-NMR of A) (15-^{13}C)ophiobolin F derived from (7-^{13}C)FPP+IPP incubated with AcldOS, B) (*S*)-(15-^{13}C,4,8-$^{2}H_2$)ophiobolin F derived from (7-^{13}C)FPP+(*E*)-(4-^{2}H)IPP incubated with AcldOS, and C) (*R*)-(15-^{13}C,4,15-$^{2}H_2$)ophiobolin F derived from (7-^{13}C)FPP+(*Z*)-(4-^{2}H)IPP incubated with AcldOS.





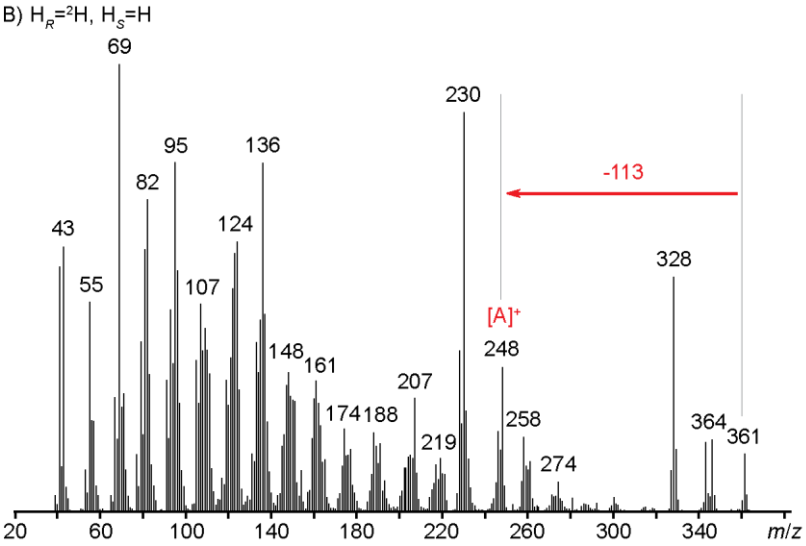


Figure S19. EI spectrum of ophiobolin F obtained from A) (7-^{13}C)FPP+(*E*)-(4-^{2}H)IPP, and B) (7-^{13}C)FPP+(*Z*)-(4-^{2}H)IPP incubated with AcldOS.

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