Investigating the biosynthesis of Sch-642305 in the fungus *Phomopsis* sp. CMU-LMA

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Electronic Supplementary Information

1. General experimental procedures

All reagents and chemicals were purchased from Sigma Aldrich, Carl Roth or Thermo Fischer Scientific. All solvents used for HPLC were analytical grade. General molecular biology procedures were performed according to standard protocols or following the manufacturer´s instructions in case of commercially obtained equipment/kits. For polymerase chain reaction either OneTag polymerase (NEB; analytical purposes) or Q5 polymerase (NEB; cloning purposes) was used. All restriction enzymes deployed in this study were obtained from NEB and used according to the manufacturer´s protocol. All media used was prepared in deionized water and sterilized at 126 °C for at least 20 min. NMR analysis, HRMS analysis and analytical as well as preparative LC/MS was performed as published previously. All concentrations are *w/v* unless otherwise specified. (1)

1.1. Bioinformatics

Pairwise sequence identities of proteins were determined using Clustal Omega in default settings.(2) The putative *pvsch* BGC was identified in the publicly available genome of *P. verrucosum* (NCBI: BioProject PRJNA276626). ORFs within the cluster were manually annotated using BLAST(3) and Softberry FGENESH 2.6(4) with default settings and using *Penicillium chrysogenum* as reference organism. Domain analysis of *schPKS* was performed using the InterPro(5) tool.

1.2. Media and solutions

- Czapek Dox with sorbitol (CD+S; Czapek Dox broth 3.5%, D-sorbitol 18.22%, agar 1.5%)
- Czapek Dox with sorbitol, soft agar (CD+S soft agar; Czapek Dox broth 3.5 %; D-sorbitol 18.22%; agar 0.8%)
- CD/S agar (3.5% Czapek Dox broth; 1 M sorbitol; 0.05% adenine; 0.15% methionine; 0.1% $(NH_3)_2SO_4$)
- CZD/S agar (Czapek Dox broth 3.5%; D-sorbitol 18.22%; ammonium sulfate 0.10%; adenine 0,05%; L-methionine 0.15%; agar 1.5%)
- CZD/S1 agar (-Met) (Czapek Dox broth 3.5%; D-sorbitol 18.22%; ammonium sulfate (0.10%; L-methionine (optional) 0.15%; agar 1.5%)
- CZD/S softagar (Czapek Dox broth 3.5%; D-sorbitol 18.22%; ammonium sulfate 0.10%; adenine 0.05%; L-methionine 0.15%; agar 0.80%)
- CZD/S1 (-Met) Softagar (Czapek Dox broth 3.5%; D-sorbitol 18.22%; ammonium sulfate 0.10%; L-methionine (optional) 0.15%; agar 0.8%)
- CD1 agar (Czapek Dox broth 3.5%; NaCl 4.68%; ammonium sulfate 0.10%; adenine 0.05%; L-methionine 0.15%; agar 1.5%)
- CD2 agar (-Met) (Czapek Dox broth 3.5%; NaCl 4.68%, ammonium sulfate 0.10%; L-methionine 0.15%; agar 1.5%)
- DPY (dextrin from potato starch 2%; polypeptone 1%; yeast extract 0.5%; KH_2PO_4 0.5%; $MgSO_4$ 0.05%; agar 2.5%)
- LB agar (yeast extract 0.5%; tryptone 1%; NaCl 0.5%; agar 1.5%)
- Malt extract (ME; malt extract 1.28%; peptone ex soya 0.08%; glycerol 0.24%; detrin from potato starch 0.28%; agar 1.5%)
- M1D medium $Ca(NO_3)_2$ 1.2 mM; KNO_3 0.79 mM; KCl 0.87 mM; $MgSO_4$ 3 mM; NaH_2PO_4 0.14 mM; sucrose 87.6 mM; ammonium tartrate 27.1 mM; $FeCl_3$ 7.4 µM; $MnSO_4$ 30 µM; $ZnSO_4$ 8.7 µM; H_3BO_3 22 µM; KI 4.5 µM; pH 5.5)
- PDB medium (potato infusion 200 g L^{-1}; dextrose 20 g L^{-1}; pH 5.1).
- PDB agar (potato infusion 200 g L^{-1}; dextrose 20 g L^{-1}; pH 5.1; agar 1.5%)
- Supplement mixture minus uracil (SM-URA; yeast nitrogen base 0.17%; $(NH_4)_2SO_4$ 0.5%; D(+)-glucose monohydrate 2%; complete supplement mixture minus uracil 0.077%; agar 1.5%)
- YPAD (yeast extract 1%; tryptone 2%; D(+)-glucose monohydrate 2%; adenine 0.03%)

1.3. Strains and culture conditions

Phomopsis sp. CMU-LMA was provided by Dr. Jamal Ouazzani. Typically, the fungus was cultured on PDB agar at 28 °C. Formation of spores was observed after 10 d cultivation and spore suspensions containing 25% glycerol allowed long-term storage at -80 °C. For secondary metabolite production *Phomopsis* sp. CMU-LMA was inoculated into PDB medium (pH 5.2) and kept at 28 °C, 110 rpm for up to 8d (in particular used for *Phomopsis* sp. CMU-LMA wildtype and all KO strains except *Phomopsis* sp. CMU-LMA *ΔschR3).* Alternatively, *Phomopsis* sp. CMU-LMA was inoculated into M1D medium and kept at 28 °C and static conditions for 10 d (for *Phomopsis* sp. CMU-LMA *ΔschR3*). *Phomopsis* KO strains were always cultivated alongside a WT strain as positive control. *Aspergillus oryzae* NSAR1 was typically cultured on DPY agar (28 °C). Spores were harvested after 7d and mixed with glycerol to a final concentration of 25%, allowing long-term storage at – 80 °C. For secondary metabolite production spores were inoculated into 500 ml baffled flasks containing 100 ml DPY medium and kept at 28 °C and 110 rpm for 7d. *E. coli* TOP-10 cells were grown on solid or liquid LB medium supplemented with the appropriate selection marker. Typically, cultures were incubated at 37 °C and 225 rpm for 16 h. Glycerol stocks containing 50% glycerol (*v/v*) were prepared for long-term storage at -80 °C. *Saccaromyces cerevisisae* CEN.PK2 was cultured on YPAD agar at 30 °C for 2-3 d. Alternatively, a single colony was inoculated into 10 ml liquid YPAD medium and grown at 30 °C and 200 rpm for 16 h.

1.4. Chemical analysis of cultures

Mycelia of *Phomopsis* sp. CMU-LMA WT and KO strains was blended in the supernatant of producing cultures. Cell debris was removed by filtration and the filtrate was extracted two times with ethyl acetate (filtrate : ethyl acetate 1:5). Organic layers were combined and dried over anhydrous $MgSO_4$. Solvent was removed under reduced pressure and the crude extract was dissolved in acetonitrile for LC/MS analysis.

For *Aspergillus oryzae* NSAR1 mycelium and supernatant was separated by Büchner filtration. The supernatant was acidified to pH 2 (2M HCl), mixed with equal amounts of ethyl acetate and extracted twice. In parallel, the mycelium was stirred in acetone (100 ml per 100 ml culture) for 1h (RT) prior to evaporation of the solvent under reduced pressure. 100 ml ddH_2O was added to the remaining mycelium fraction, acidified to pH 2 (2M HCl) and extracted twice with equal amounts of ethyl acetate. Combined ethyl acetate extracts from supernatant and cells were dried over anhydrous magnesium sulfate and organic solvent was removed *in vacuo*. Crude extracts were dissolved in methanol to a final concentration of 10 mg ml^{-1}.

1.5. Isotopic feeding experiments

Sodium [1-^{13}C]-labelled acetate was obtained from Sigma Aldrich. Subsequently, growing cultures of *Phomopsis* sp. CMU-LMA were supplemented with the labelled isotope to a final concentration of 15 mM.

1.6. Yeast homologous recombination

Assembly of Knockout plasmids and expression plasmids was routinously achieved by yeast homologous recombination. The uracil auxotrophic *S. cerevisiae* CEN.PK2 strain was used. Typically, a single colony was inoculated into 10 ml YPAD medium and grown overnight at 30 °C and 160 rpm. This seed culture was mixed with 40 ml fresh YPAD medium and kept at 30 °C and 160 rpm for 4-5h. Next, the cells were collected by centrifugation and washed twice with ddH_2O prior to final resuspension in 1 ml ddH_2O. 100 µL were centrifuged (13,000 x g, 4 ˚C, 1 min) and resuspended in the transformation solution (240 µL PEG 3350 (50% w/v), 36 µL LiOAC (1M), 50 µL denatured salmon sperm DNA (2 mg ml^{-1}) and 34 µL DNA master mix). The DNA master mix contained linearized vector and DNA insert with a 5´and 3´overlap of 30 bp with the cut sites of the vector (fragments). Vectors were either linearized by *NotI* (pE-YA) or *AscI* (pTYGSarg/ade/met). The resulting mixture of DNA and yeast was incubated at 42 °C for 50 min. After centrifugation (13,000 x g, 4 ˚C, 1 min) the pellet was resuspended in 1 ml ddH_2O

and plated on SM-URA agar. After incubation at 30 °C for 2-3 days plasmid was isolated using the Zymoprep Yeast Plasmid Miniprep kit, transformed into *E. coli* TOP10 or *E. coli* ccdb cells, re-isolated from bacterial cells and submitted to DNA sequencing. DNA sequencing was performed by *Eurofins Genomics*.

1.7. Construction of *Phomopsis* sp. CMU-LMA knockout plasmids

KO-cassettes for gene disruption in *Phomopsis* sp. CMU-LMA were designed as displayed in supplementary figure S5. KO-cassettes were composed of three DNA fragments previously amplified by polymerase chain reaction: the hygromycin resistance cassette (here named HygR; comprising P_{gdpA} + *hph*, ~ 4 KBp) and two gene specific sequences homologous to the target gene (Target Left T_L and Target Right T_R; each ~ 1 KBp). HygR was designed to have overlaps with T_L and T_R at the 3´ and 5´ end respectively. T_L and T_R were designed to have additional overlaps with the destination vector pE-YA. Overlaps comprised 30 nt. The KO-cassette was cloned into the shuttle vector pE-YA by yeast homologous recombination. Primers used for the generation of KO plasmid are listed in supplementary table S8. Successful generation of KO plasmids was confirmed by DNA sequencing.

1.8. Construction of *A. oryzae* expression vectors

Total RNA was extracted from *Phomopsis* sp. CMU-LMA using oligo (dT) nucleotides and the RevertAid Premium Transcriptase kit (Thermo Fischer) was used according to the manufacturer´s instructions to transcribe RNA into cDNA. For *schPKS* the Maxima Reverse Transcriptase from NEB was used. Intron free cDNA was used for PCR amplification of biosynthetic genes. *schPKS* was assembled in the pE-YA vector and shuttled into the pTYGS expression vector by gateway cloning (Invitrogen Gateway LR Clonase II enzyme mix; manufacturer´s protocol with halved reaction scale).

For assembly in pE-YA *schPKS* (about 8 kb) was amplified in four fragments, each about 2 kb. Yeast homologous recombination yielded plasmid KELIII02A(No2_3) and DNA sequencing identified the presence of four predicted introns, suggesting that the cDNA used for amplification was contaminated with traces of gDNA. To remove the introns *schPKS* was reassembled in pE-YA. For this, *schPKS* was amplified from KELIII02A(No2_3) in seven fragments, omitting the predicted intron sequences and yielding plasmid KELIII07. Sequencing revealed that introns were indeed absent but that a point mutation was present (insertion of alanine at position 2490) leading to an undesired frame shift. The point mutation was manually repaired by yeast homologous recombination using *KpnI* digested KELIII07 and an amplified patch from KELIII02A(No2_3) having the correct sequence (supplementary figure S40). Repaired plasmid KELIII08 was confirmed by sequencing and shuttled into the pTYGSarg expression plasmid, yielding KELIII08a, using gateway cloning. Yeast homologous recombination was used to clone the tailoring biosynthetic genes into the respective multigene expression vectors pTYGSarg/ade/met under control of a constitutive promoter (either peno, padh, pgdpA; see supplementary figure S41). Expression vector KELIII08C was revealed to possess two point mutations in *schR3* causing a frame shift resulting in inactive *schR3*. This vector was only used in experiments lacking *schR3*. Details for construction of expression plasmids are outline in supplementary table S10 and oligonucleotides are noted in supplementary table S11.

1.9. Transformation of *Phomopsis* sp. CMU-LMA

For transformation of *Phomopsis* sp. CMU-LMA a protoplast mediated protocol was established. Mycelia from growing plates was inoculated into 50 ml PDB medium and incubated at 28 °C in static conditions for up to 5d. Cells were collected by filtration through sterile miracloth. Protoplast formation was accomplished by digestion of young mycelia with a mixture of lysing enzymes extracted from *Trichoderma harzianum* (10 mg ml^{-1}, Sigma Aldrich) and driselase from *Basidiomycetes* spp. (5 mg ml^{-1}, Sigma Aldrich) in osmotic buffer (0.8 M NaCl). The protoplasting solution was kept at 30 °C for 2-3 hours while being slowly inverted. Protoplasts were released from hyphal strands by careful pipetting followed by filtration through sterile miracloth. After centrifugation (3000 x g, 5 min, 4 ˚C) the pelleted protoplasts were resuspended in 100 µL Solution 1 (0.8M NaCl; 10 mM $CaCl_2$; 50 mM Tris-HCl pH 7.5)

and the concentration was assessed microscopically using a hemocytometer. Typically, 10 µg DNA were added to the protoplast solution and after incubation on ice for 2 minutes 1 ml of Solution II (60% w/v PEG3350; 0.8M NaCl; 10 mM Tris-HCl pH 7.5) was added and incubated at RT for another 20 minutes. Finally, 5 ml of molten CZD/S agar (at 50 °C) was added and the mixture was spread evenly on a 10 cm Petri dish. Plates were incubated at 28 °C overnight for cell wall regeneration and then overlayed with 10 ml CD agar containing Hygromycin B at 100 µg ml^{-1-}. Emerging single colonies were transferred to secondary plates (CD agar with Hygromycin B at 100 µg ml^{-1}). Colonies that grew healthily on secondary plates were transferred to medium without antibiotic and assessed for correct integration of the KO cassette.

1.10. Assessment of targeted gene disruption

Successful disruption of *sch* biosynthetic genes was confirmed using polymerase chain reaction. For this, gDNA was isolated from *Phomopsis* sp. CMU-LMA KO strains using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma Aldrich). Two PCR reactions were performed on each sample and WT gDNA as control. Whole PCR (wPCR) reveals successful disruption as it amplifies the entire target gene cassette (in case of *schPKS* a 1 kbp region within the internal region that is deleted upon successful gene disruption). In case of WT gDNA wPCR gives the correct insert size. In case of KO strains wPCR yields a larger PCR product as the whole disruption cassette is amplified, including the *hph* resistance gene. External PCR (extPCR) reveals whether recombination occurred at the desired site: primers are designed to amplify from 5´ end of the *hph* resistance gene to genomic DNA flanking the target gene. extPCR only gives a product in case of successful disruption in the correct locus. It does not give a PCR product using WT gDNA, as WT gDNA lacks the *hph* gene. For wPCR the gene specific primers T_L Fw and T_R Re were used (compare oligonucleotides used for construction of KO plasmids). For external PCR validation gene specific primers (supplementary table S9) in combination with either primer HygRP7.2 Fw or HygRP6.2 Re (both binding to hygromycin gene cassette) were used.

1.11. Biotransformation of SCH **1** by *Aspergillus oryzae* NSAR1

Aspergillus oryzae NSAR1 from a sporulating DPY agar plated was inoculated into 500 ml baffled flasks containing 100 ml DPY medium and kept at 28 °C, 110 rpm for 2d. SCH **1** (7 mg) was dissolved in 1 ml DMSO, sterilfiltrated and added to the growing *A. oryzae* culture. After another 3d cultures were extracted and analysed as described above. *Aspergillus oryzae* NSAR1 WT fed with pure DMSO was used as a control experiment.

1.12. Transformation of *Aspergillus oryzae* NSAR1

A PEG-mediated transformation protocol was used for the ectopic integration of DNA into *Aspergillus oryzae* NSAR1. For this, conidia from a sporulating plate was inoculated into 100 ml GN medium (250 ml baffled flask) and kept at 30 °C, 180 rpm overnight. Mycelia was collected by filtration through sterile Miracloth and incubated in 10 ml protoplasting solution (20 mg ml^{-1} trichoderma lysing enzyme; sterilized by filtration through a 0.45 µm syringe filter) at 30 °C and 70 rpm for 3h. Gentle pipetting with a wide-bore pipette released the protoplasts from the hyphal strains. Protoplasts were collected by centrifugation (10 min, 3000 x g, 4 ˚C) and resuspended in solution 1 (100 µL per transformation; 50 mM Tris-HCl pH 7.5; 800 mM NaCl; 10 mM $CaCl_2$). After addition of 10 µL purified plasmid DNA the mixture was incubated on ice for 2 min prior to addition of 1 ml solution 2 (60% w/v PEG3350, 50 mM Tris HCl pH 7.5, 800 mM NaCl, 10 mM $CaCl_2$). Additional incubation at room temperature for 20 min was followed by mixing with pre-warmed 5 ml CZD/S(1) softagar (-methionine) (50 °C) and overlayed onto pre-prepared CZD/S(1) plates (-methionine). Suitable agar was chosen according to auxotrophic resistance markers on the transformed plasmid DNA. All primary plates were kept at 28 °C for 3-5 days until visible formation of colonies. All colonies were subjected to two rounds of selection on arginine-(adenine or methionine) deficient media, depending on the nature of auxotrophic markers present on transformed plasmids. Prior to fermentation single colonies were grown on DPY agar for 1 week. For every colony, gDNA was isolated and ectopic integration of DNA was checked by PCR using gene specific primers (identical to those used for plasmid construction).

2. Supplementary Figures

Figure S1

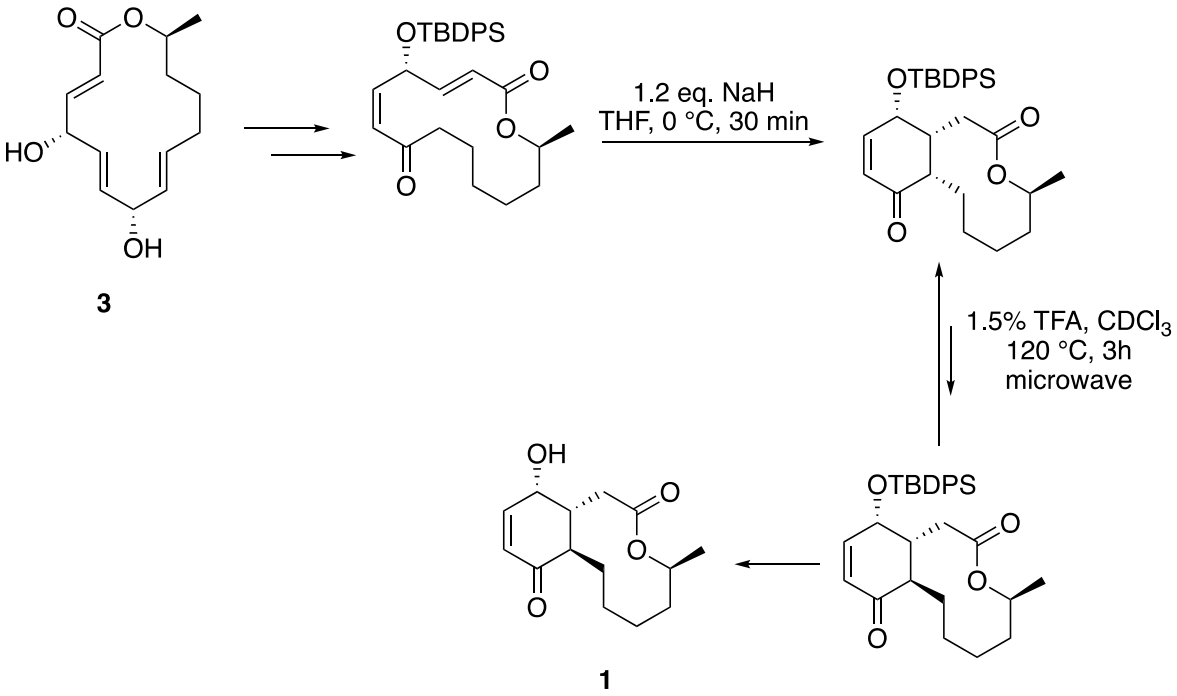


Figure S1 Biomimetic synthesis of SCH **1** by a key transannular Michael reaction reported by Snider and Zhou.(6)

Figure S2

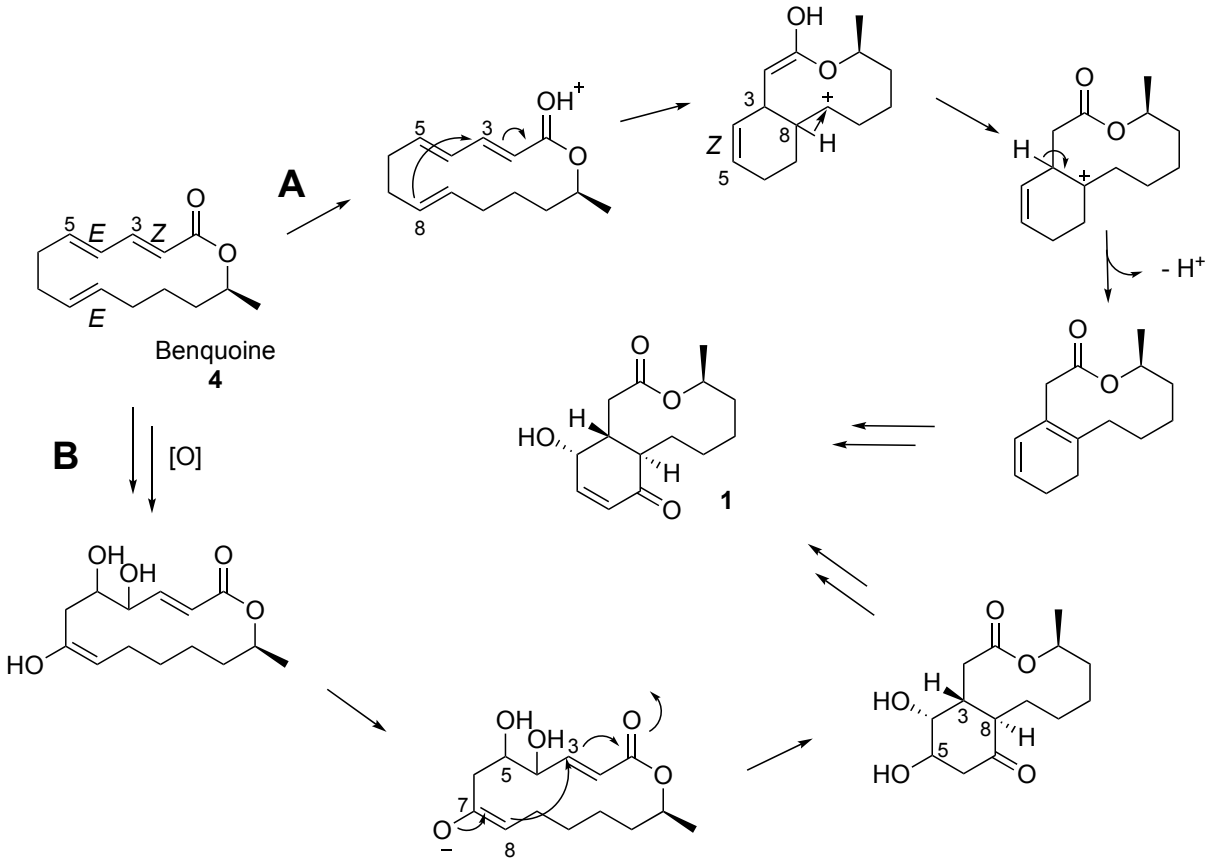


Figure S2 *Proposed mechanisms for SCH **1** biosynthesis based on Co-isolation of metabolites from* Phomopsis *sp. CMU-LMA*.(7)

Figure S3

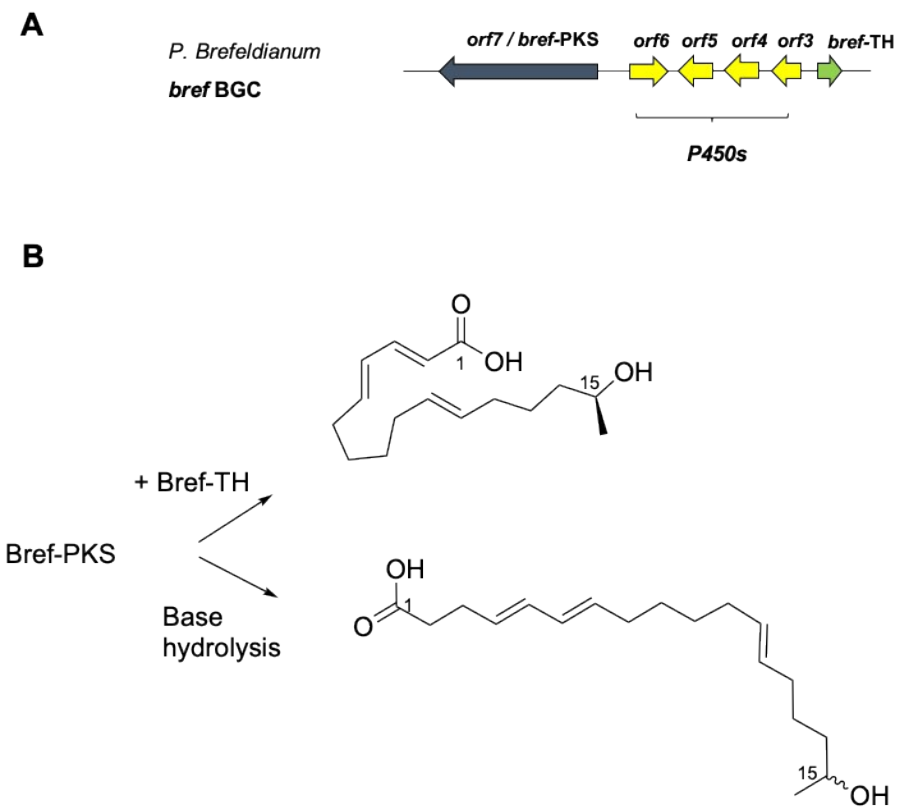


Figure S3 **A,** BGC for Brefeldin A from *P. brefeldianum*. **B,** chain-lenth control of the hrPKS product of brefeldin A biosynthesis.(8)

Figure S4

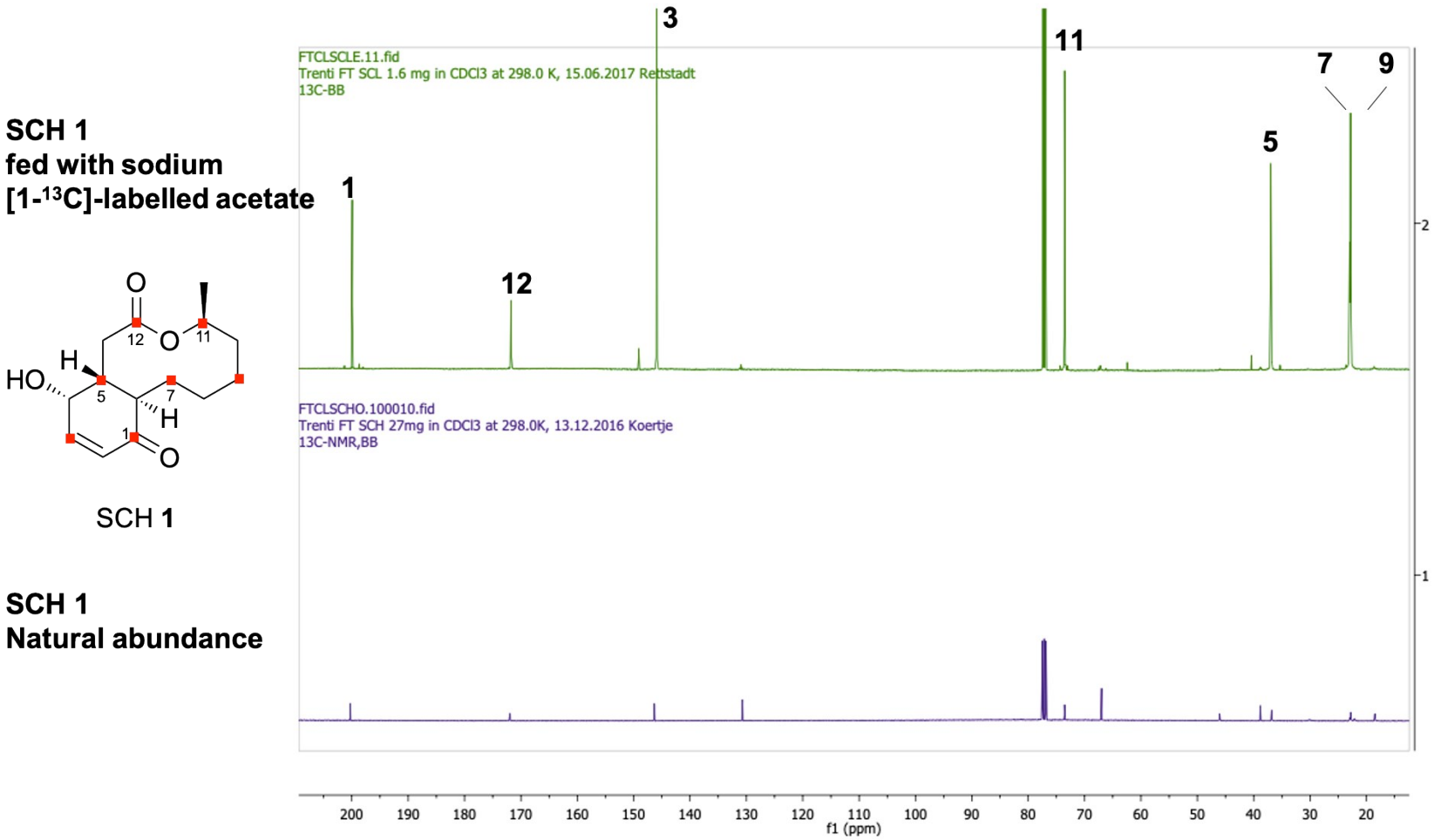


Figure S4 ^{13}C-NMR of labelled SCH **1** (top) in $CDCl_3$ (125 MHz) compared to ^{13}C-NMR of natural abundance SCH **1** (bottom).

Figure S5

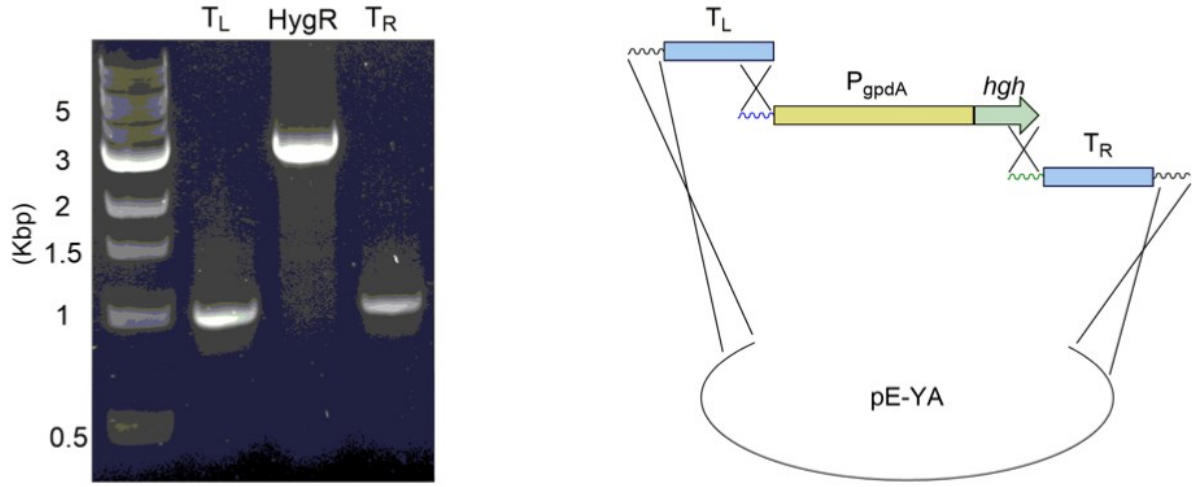


Figure S5 Construction of KO cassettes for targeted gene disruption in *Phomopsis* sp. CMU-LMA.

Figure S6

Comment [CS]: Ext. PCR data for PKS, schR1, schR2 missing. Do we need this?

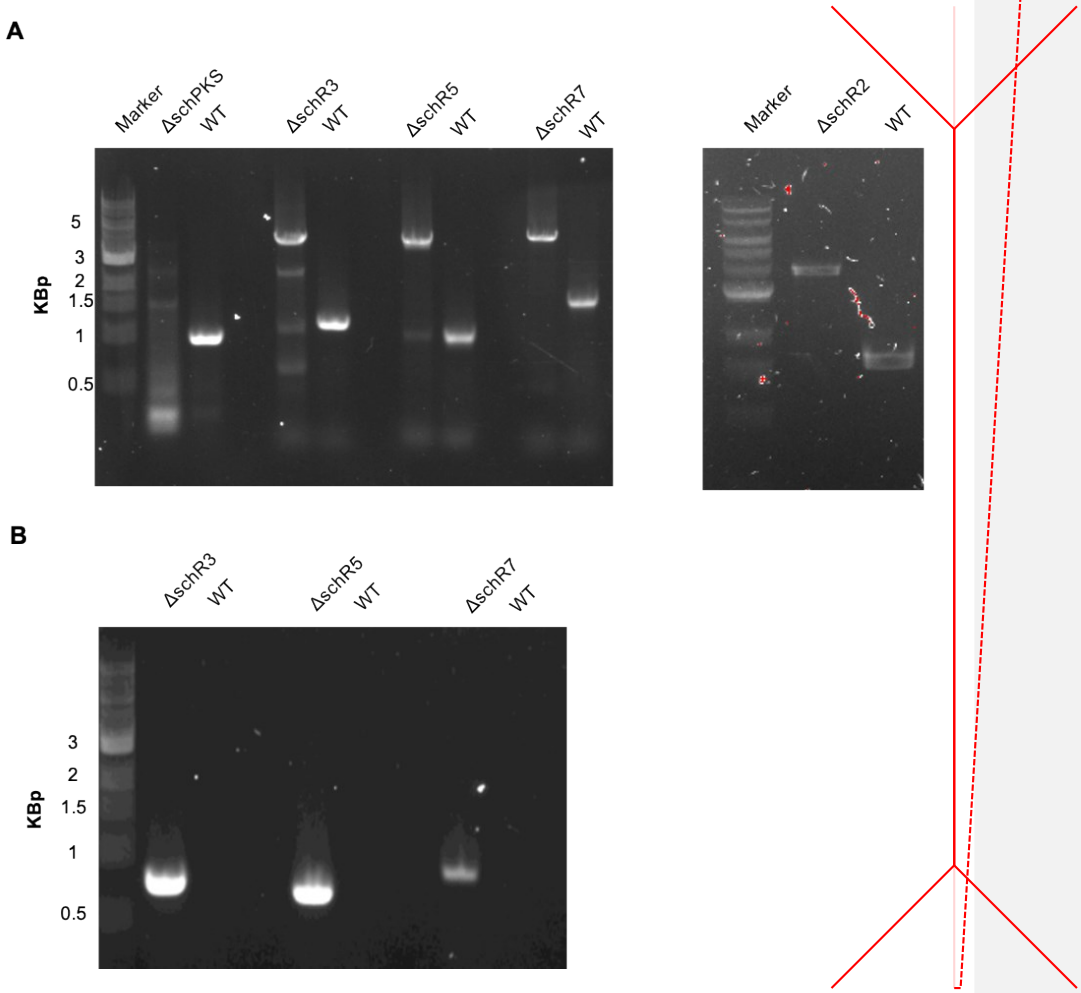


Figure S6 PCR validation of gene disruption in *Phomopsis* sp. CMU-LMA. **A,** whole PCR; **B,** ext PCR.

Figure S7

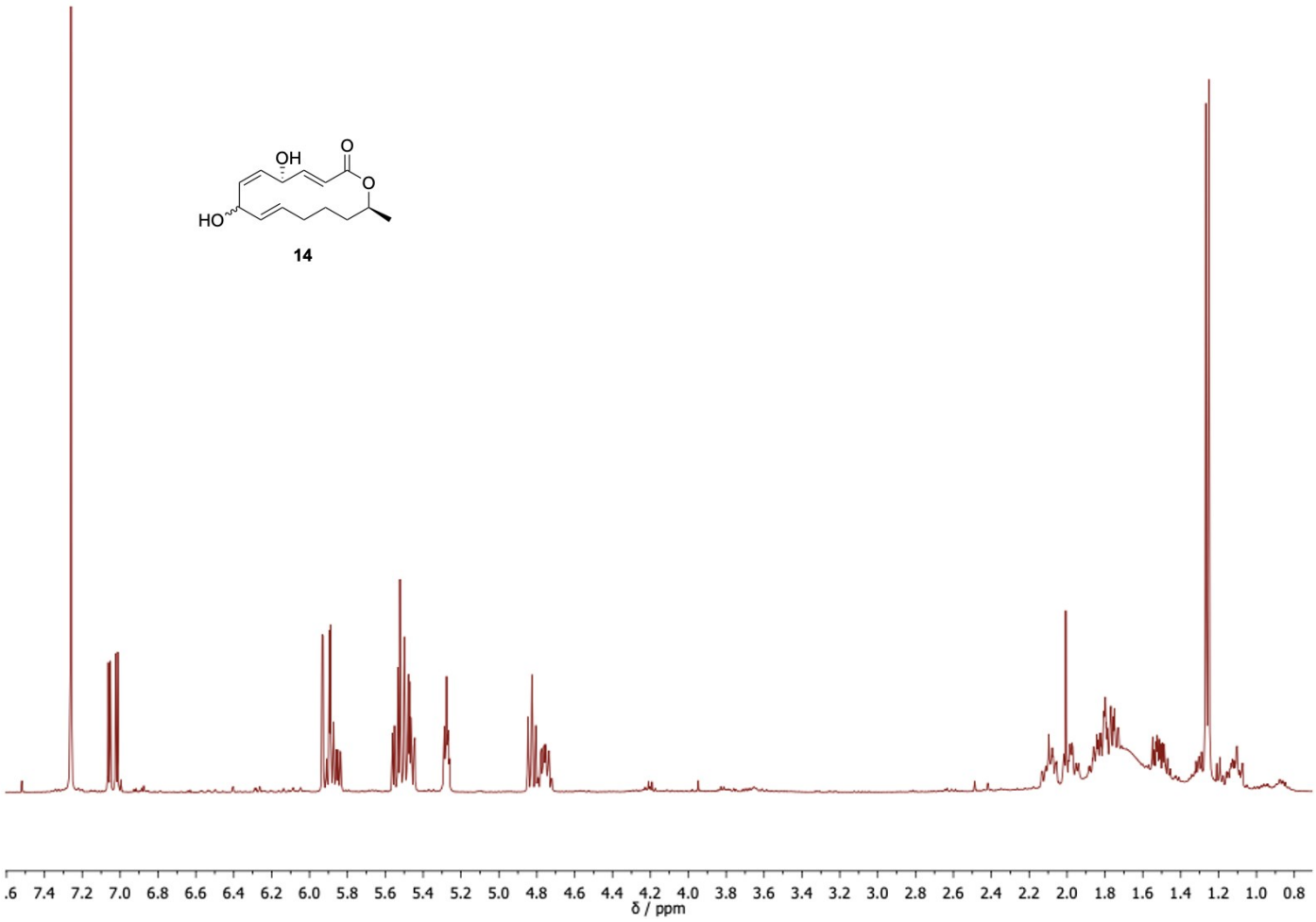


Figure S7 ^{1}H-NMR of compound **14** in $CDCl_3$ (500 MHz).

Figure S8

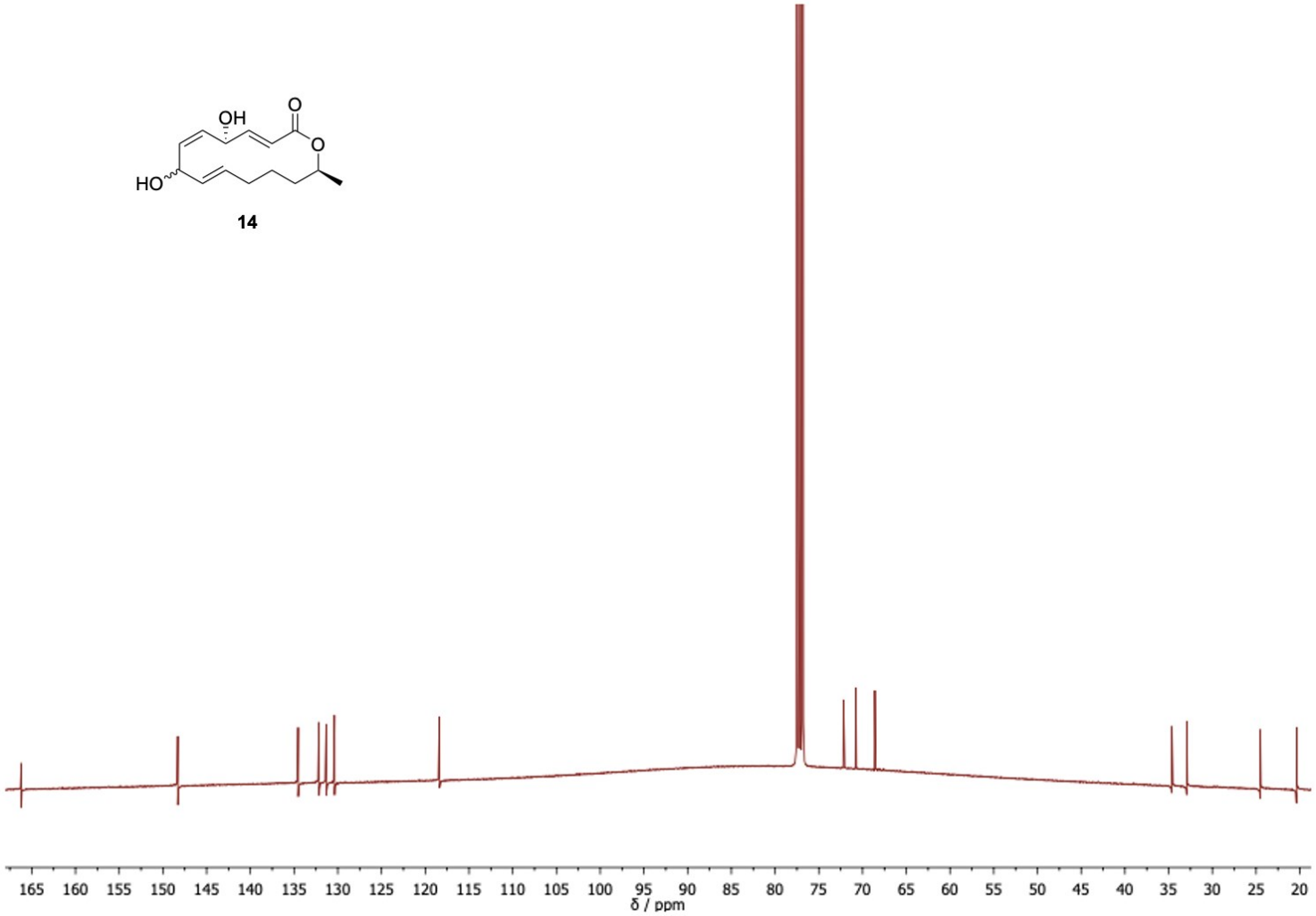


Figure S8 ^{13}C-NMR of compound **14** in $CDCl_3$ (500 MHz).

Figure S9

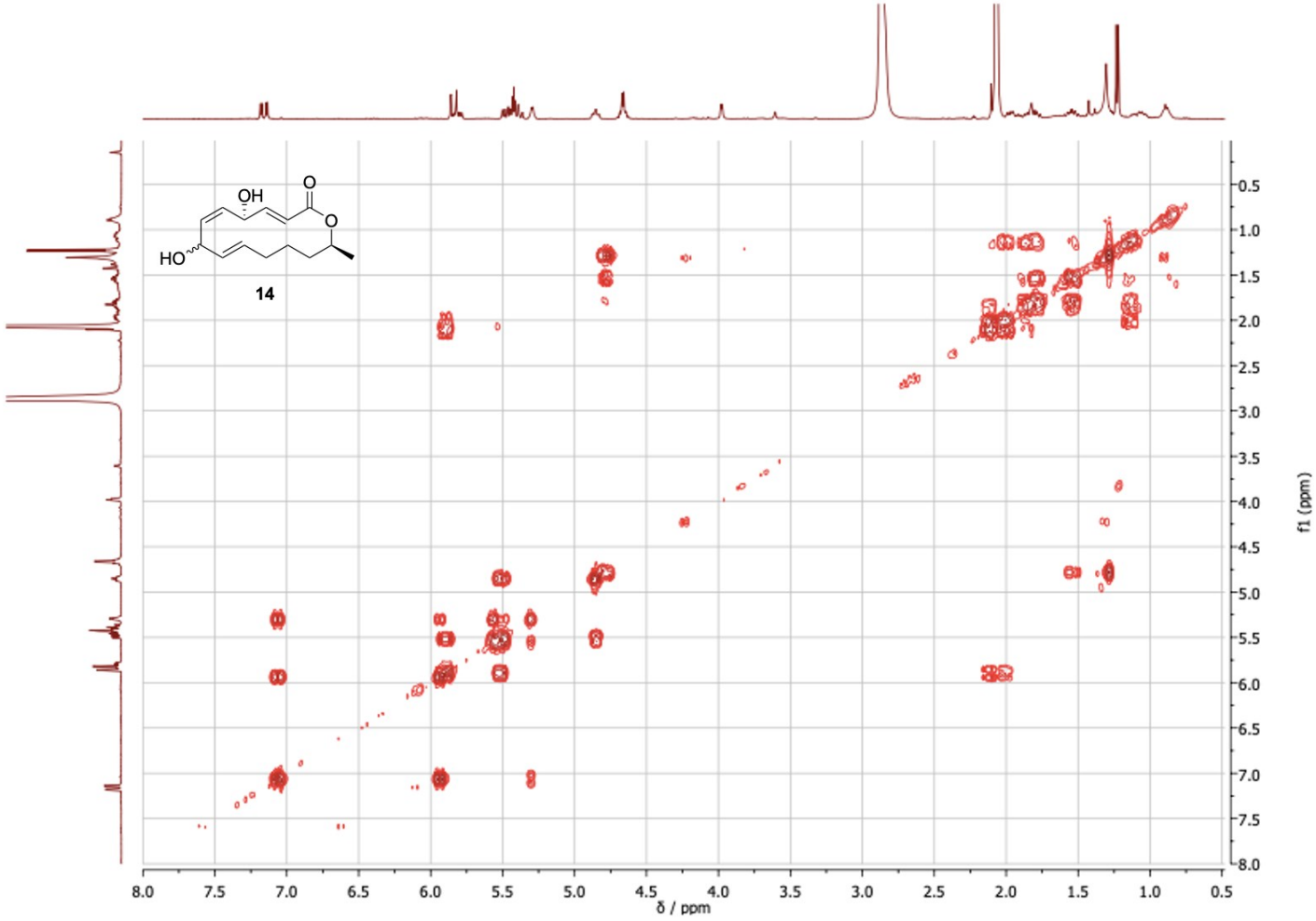


Figure S9 COSY-NMR of compound **14** in Acetone-d_6 (500 MHz).

Figure S10

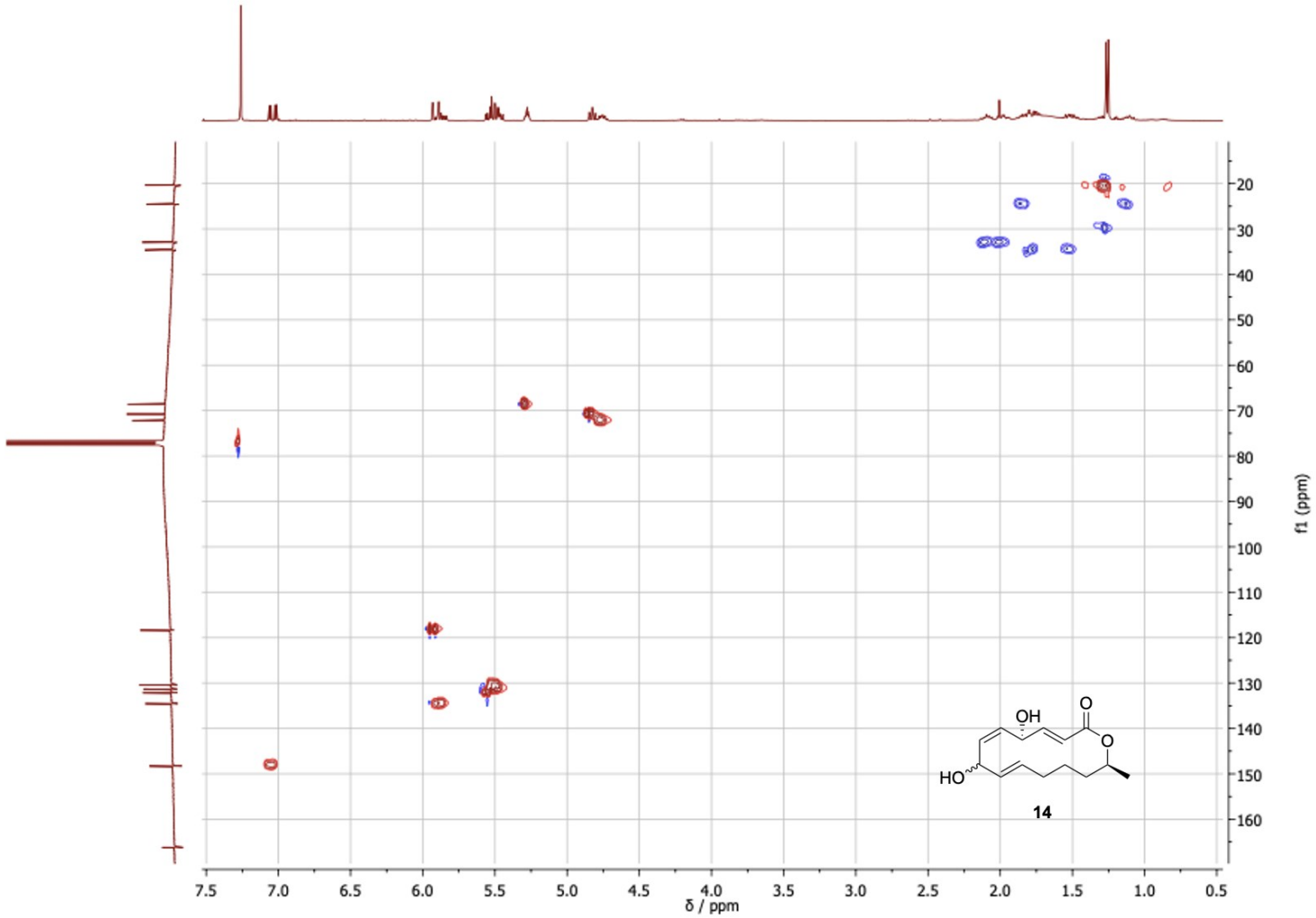


Figure S10 HSQC-NMR of compound **14** in Acetone-d_6 (500 MHz).

Figure S11

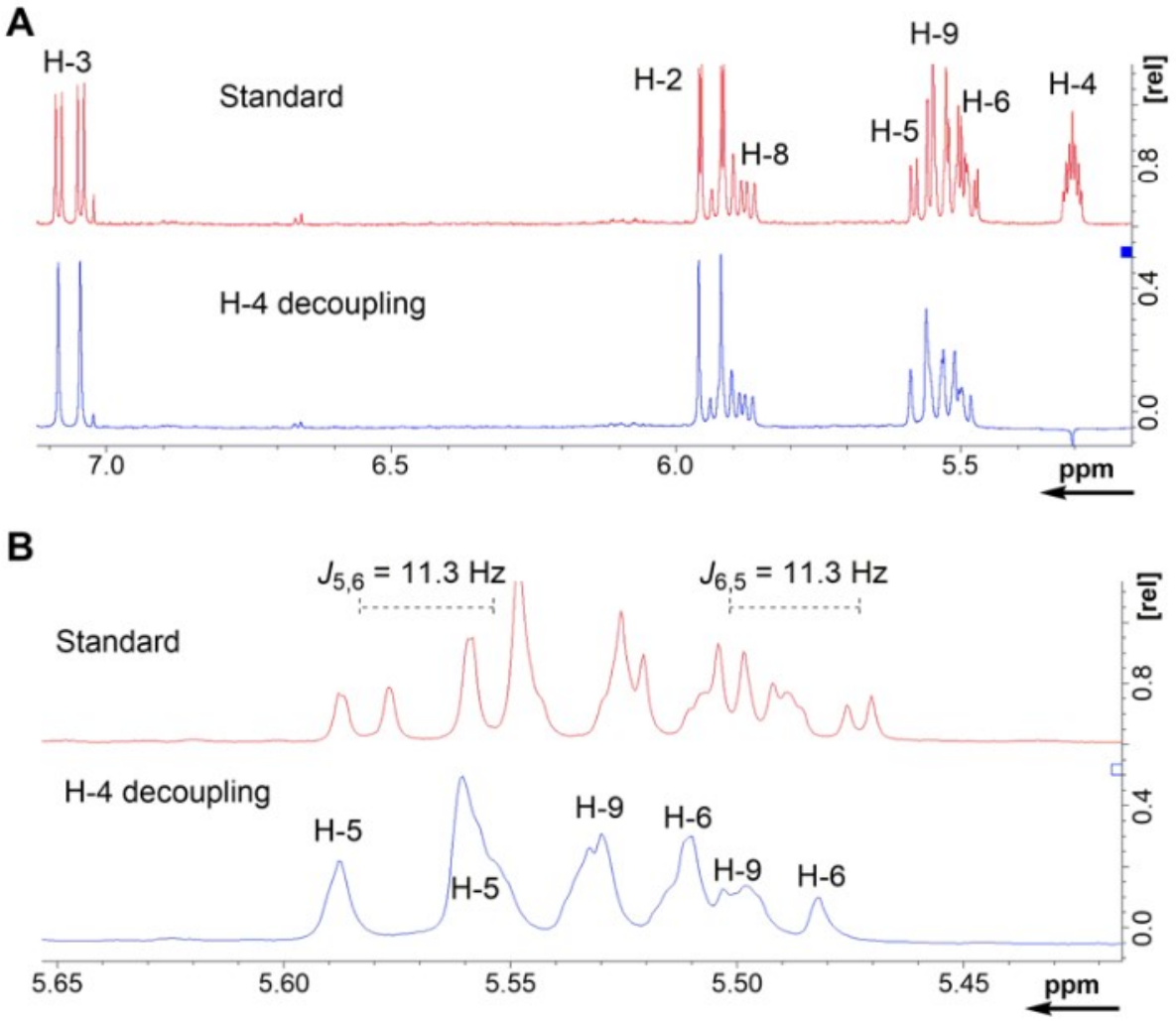


Figure S11 H-4 decoupling in ^{1}H-NMR for compound **14** and determination of key coupling constants. **A** and **B** are identical with **B** being the enlarged region between 5.40 and 5.65 ppm of **A.**

Figure S12

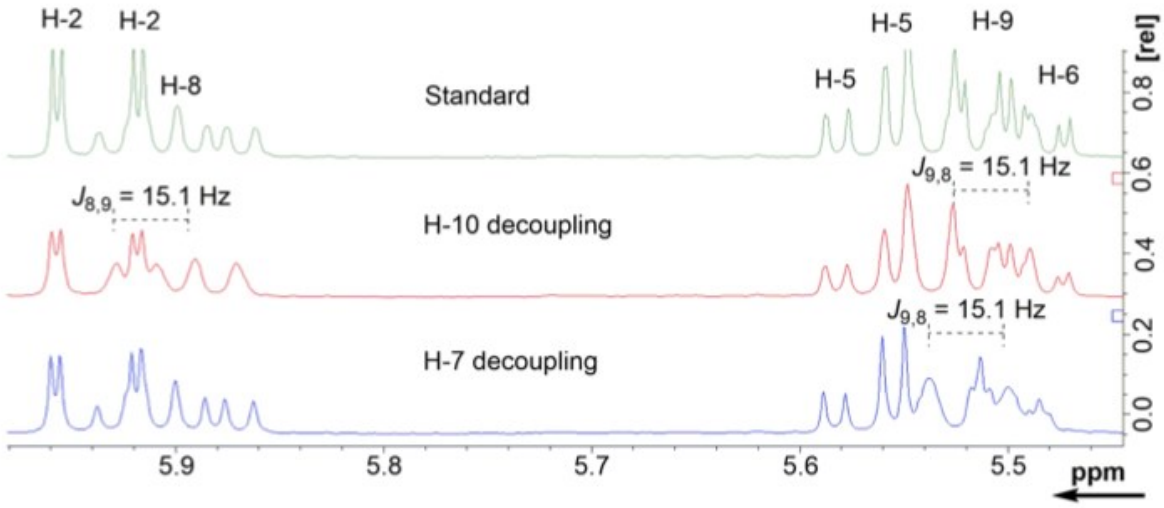


Figure S12 H-7/H-10 decoupling in ^{1}H-NMR for compound **14** and determination of key coupling constants.

Figure S13

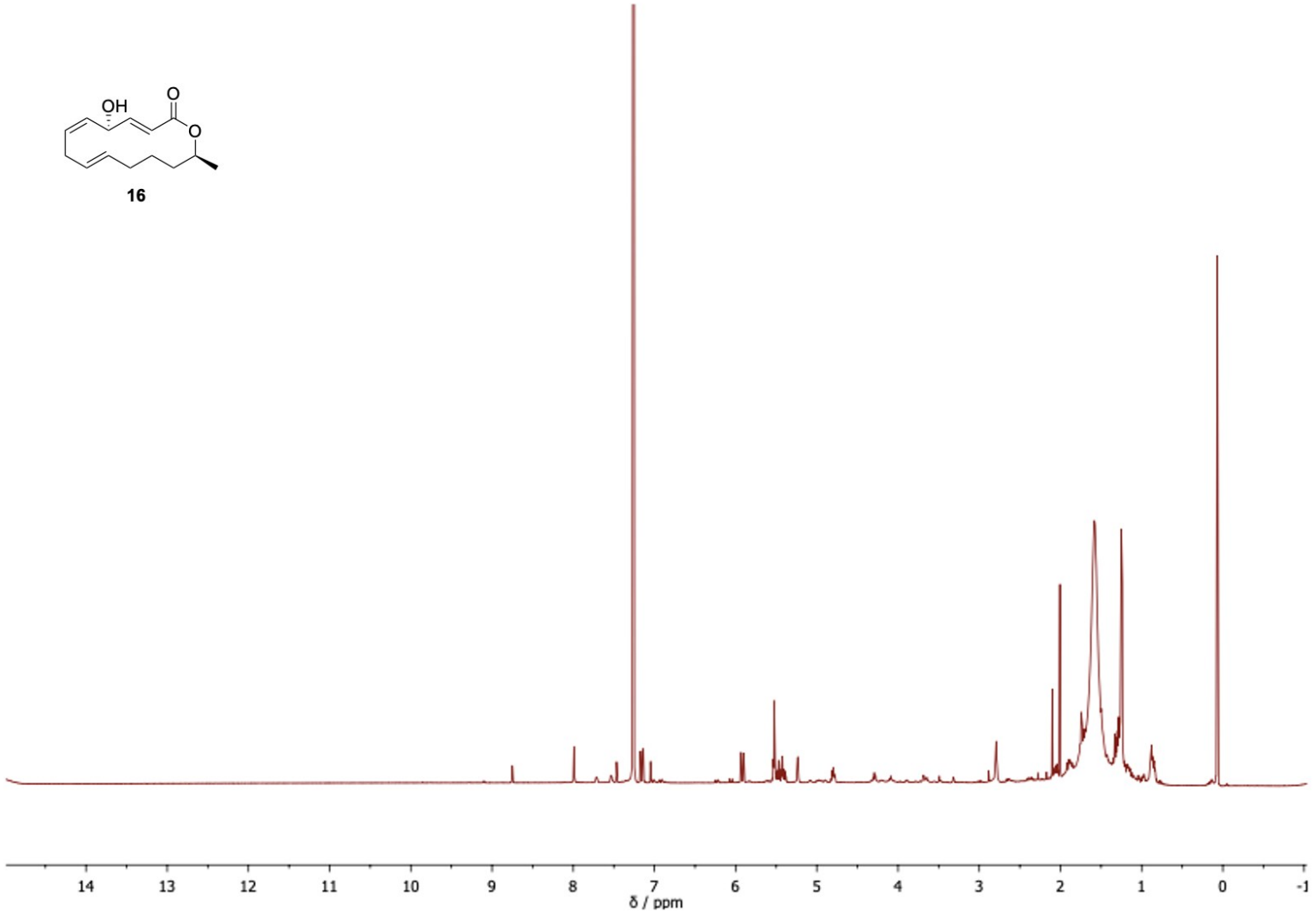


Figure S13 ^{1}H-NMR of compound **16** in $CDCl_3$ (500 MHz).

Figure S14

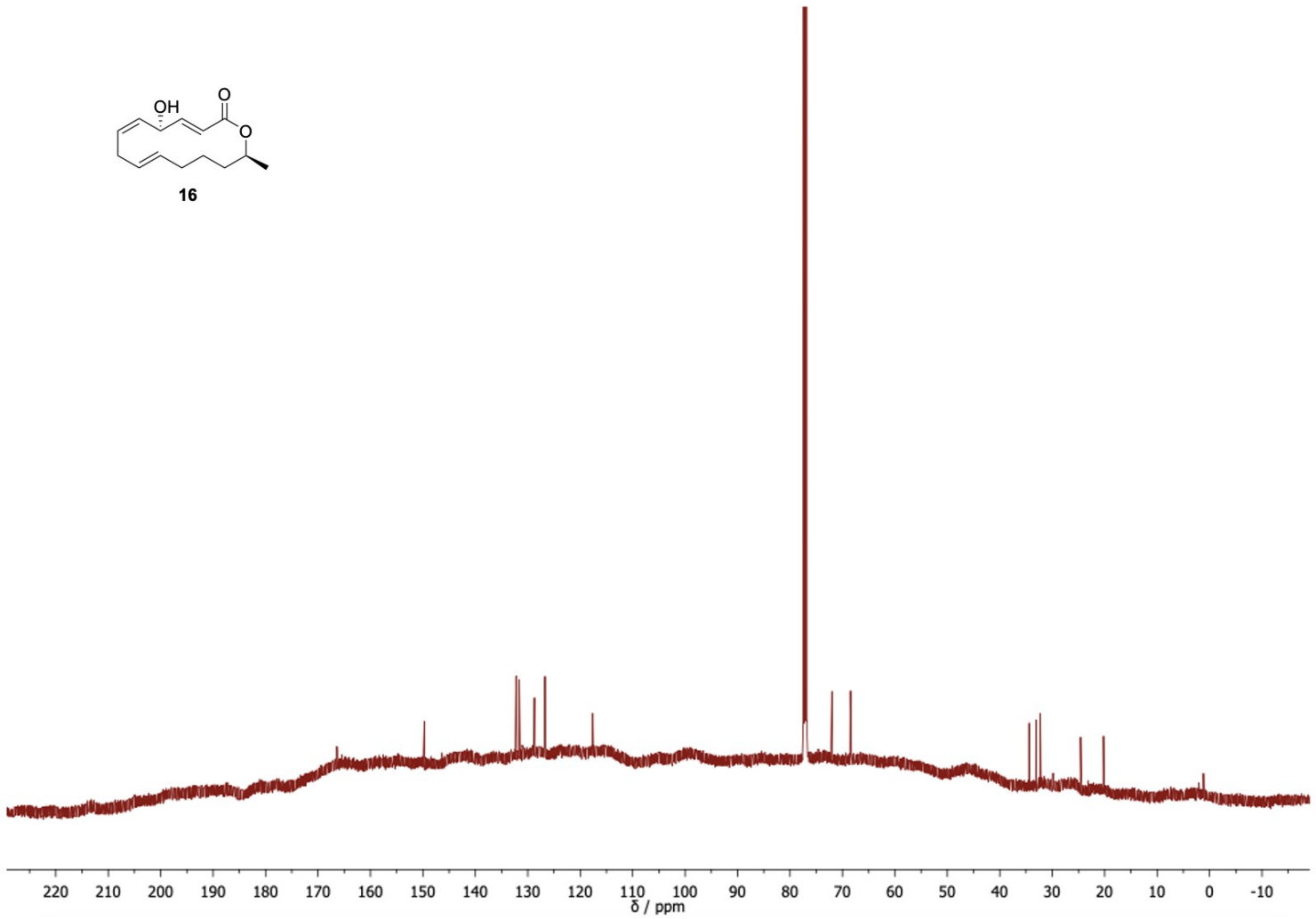


Figure S14 ^{13}C-NMR of compound **16** in $CDCl_3$ (500 MHz).

Figure S15

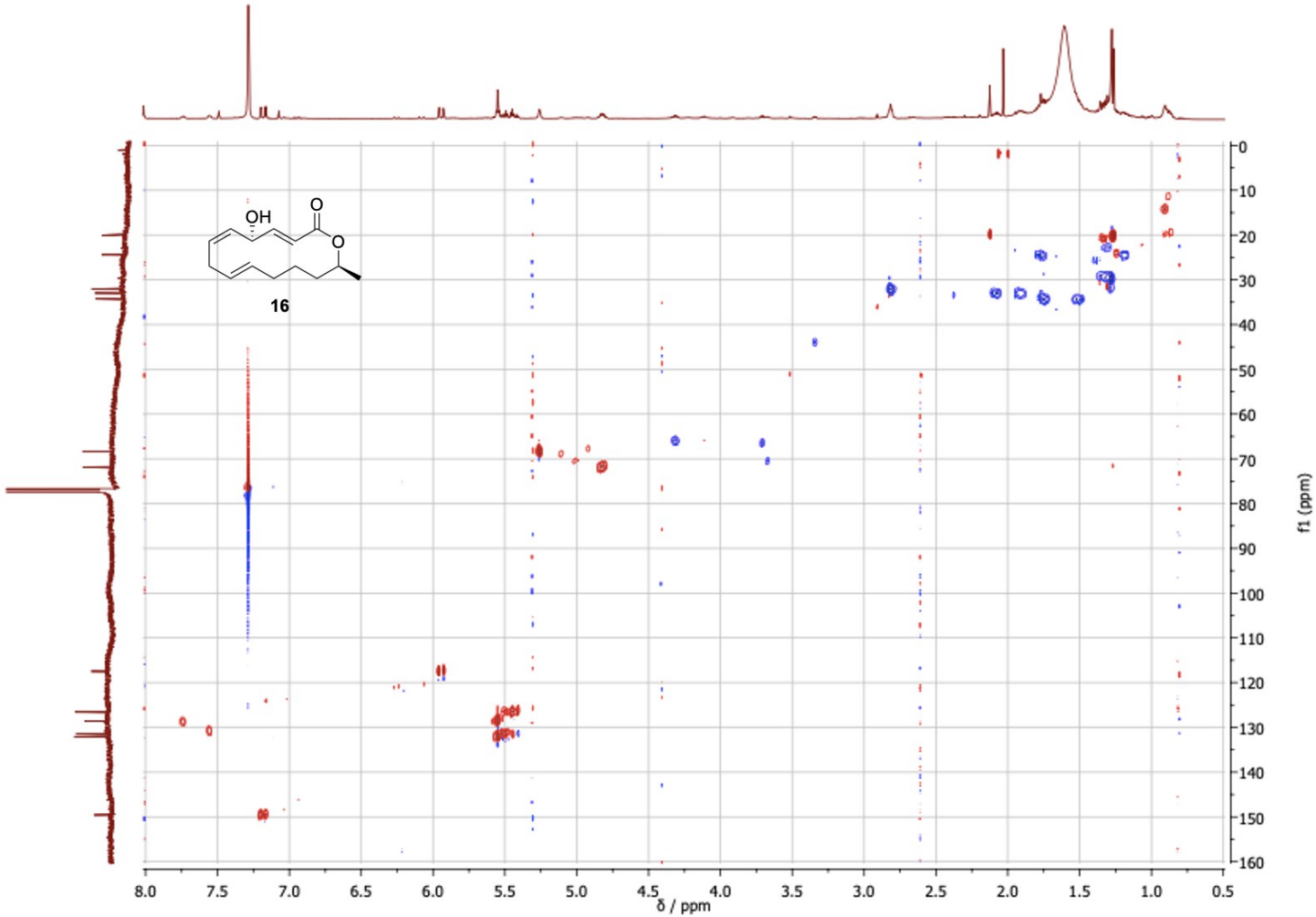


Figure S15 HSQC-NMR of compound **16** in $CDCl_3$ (500 MHz).

Figure S16

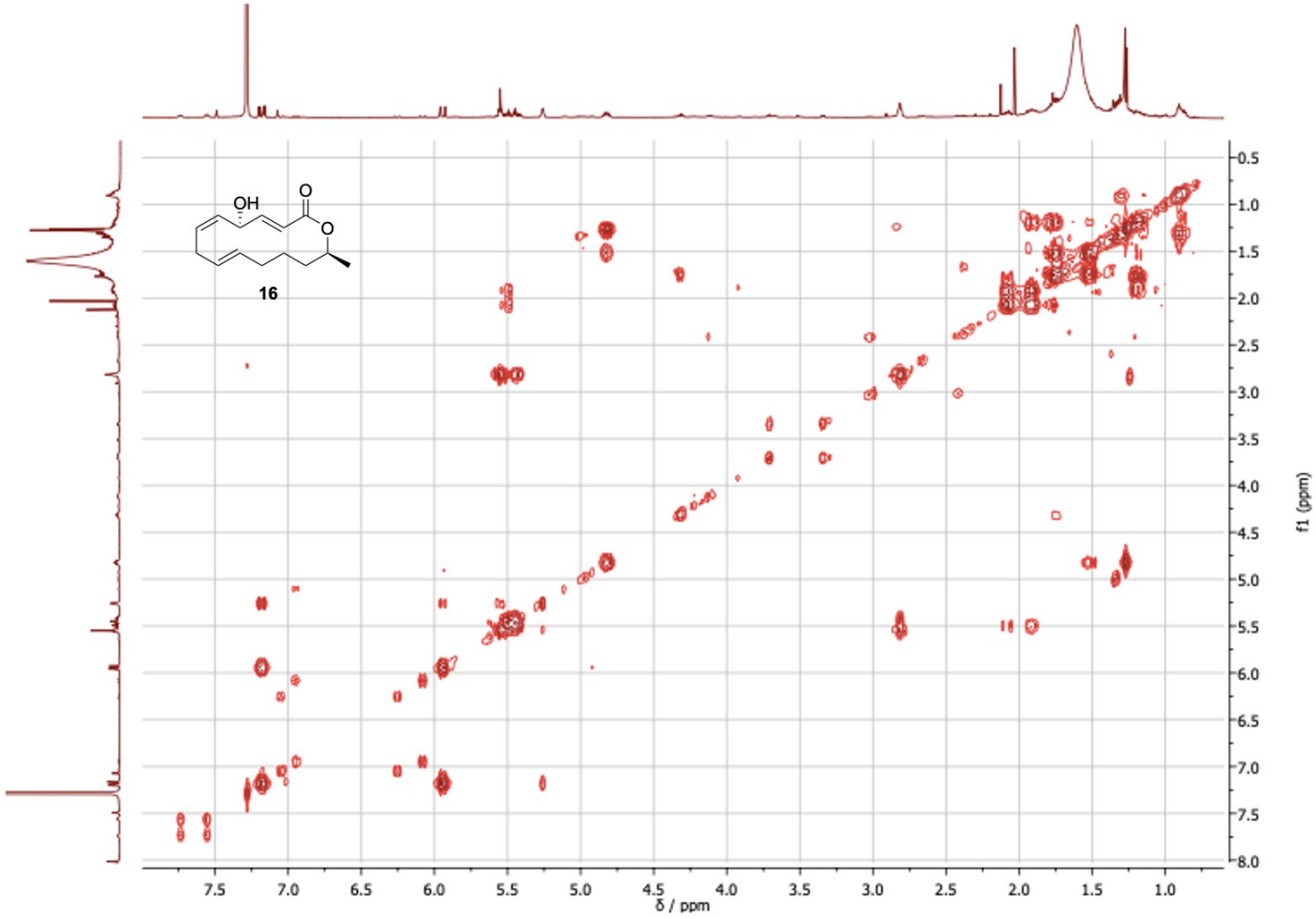


Figure S16 COSY-NMR of compound **16** in $CDCl_3$ (500 MHz).

Figure S17

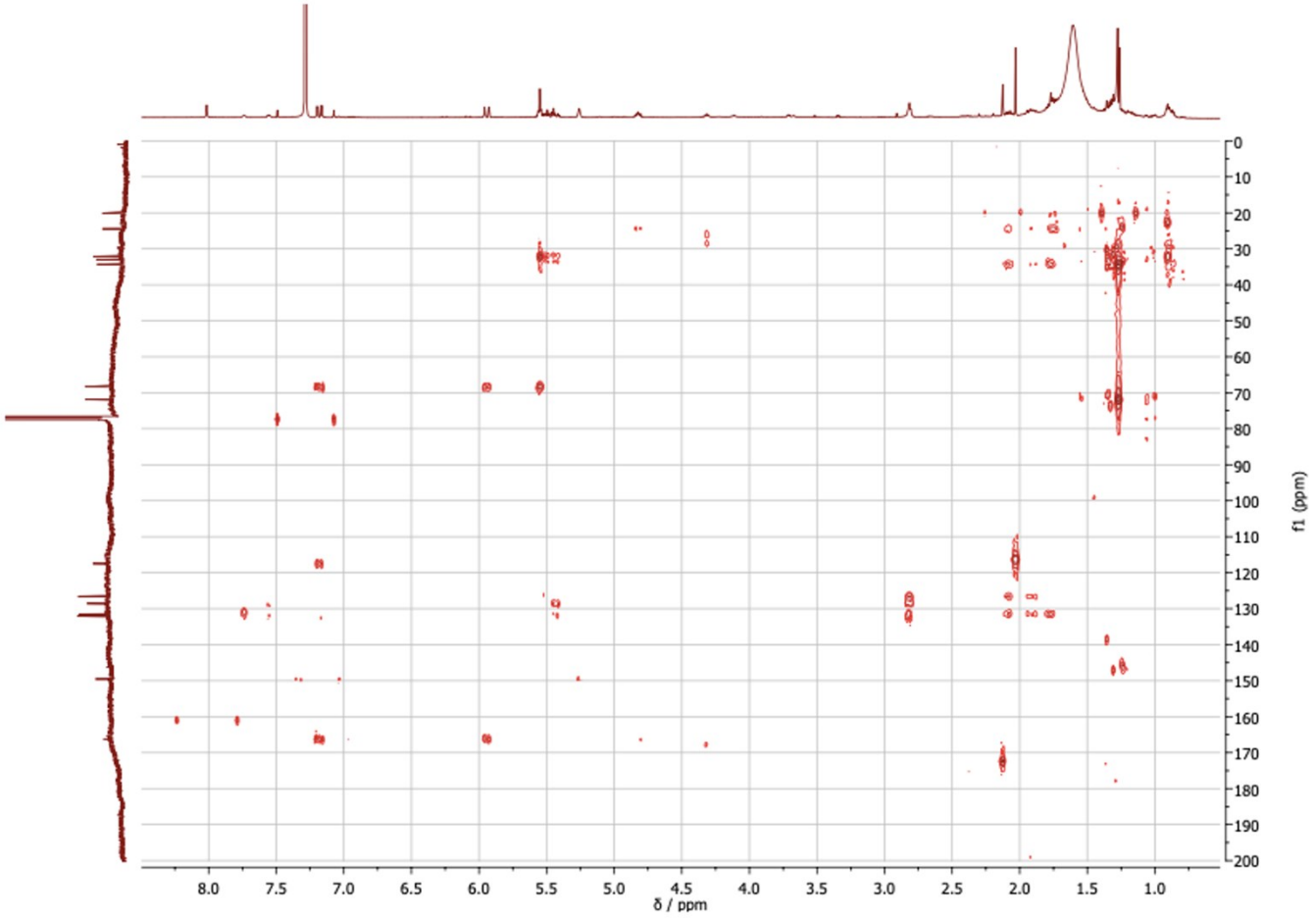


Figure S17 HMBC-NMR of compound **16** in $CDCl_3$ (500 MHz

Figure S18

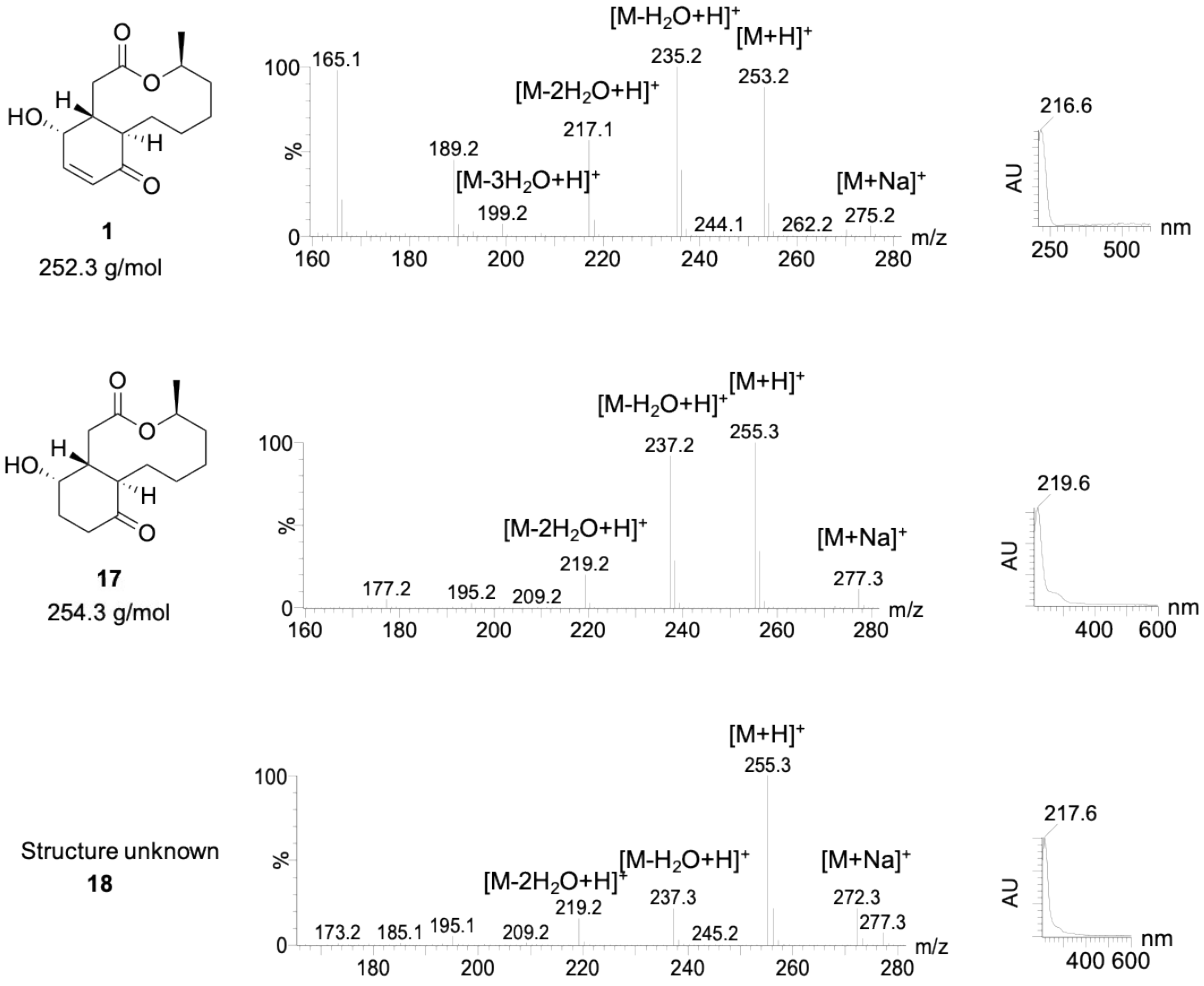


Figure S18 Mass spectra (ES^+) and UV chromatograms of **1**, **17**, **18**.

Figure S19

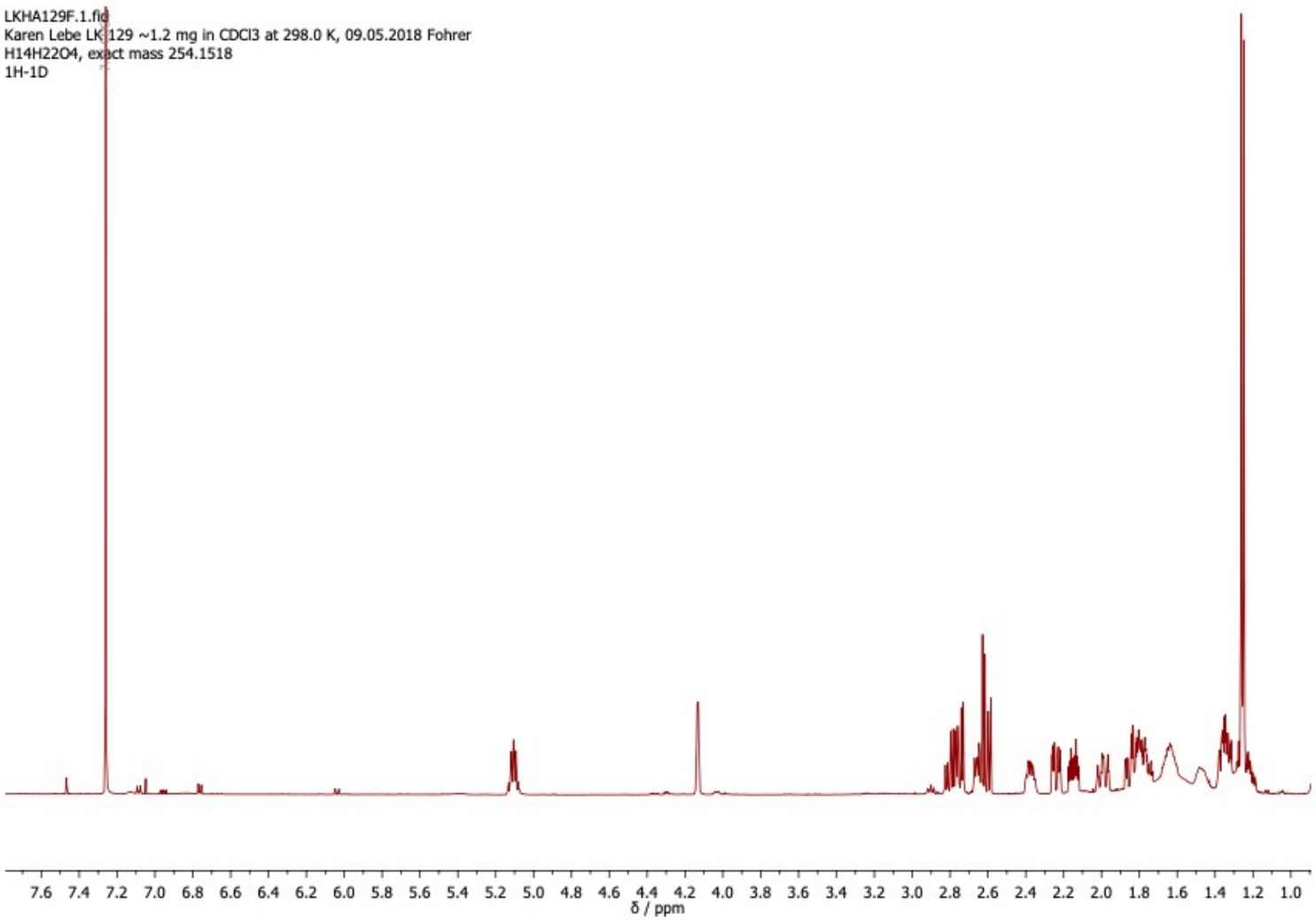


Figure S19 ^{1}H-NMR of compound **17** in $CDCl_3$ (500 MHz).

Figure S20

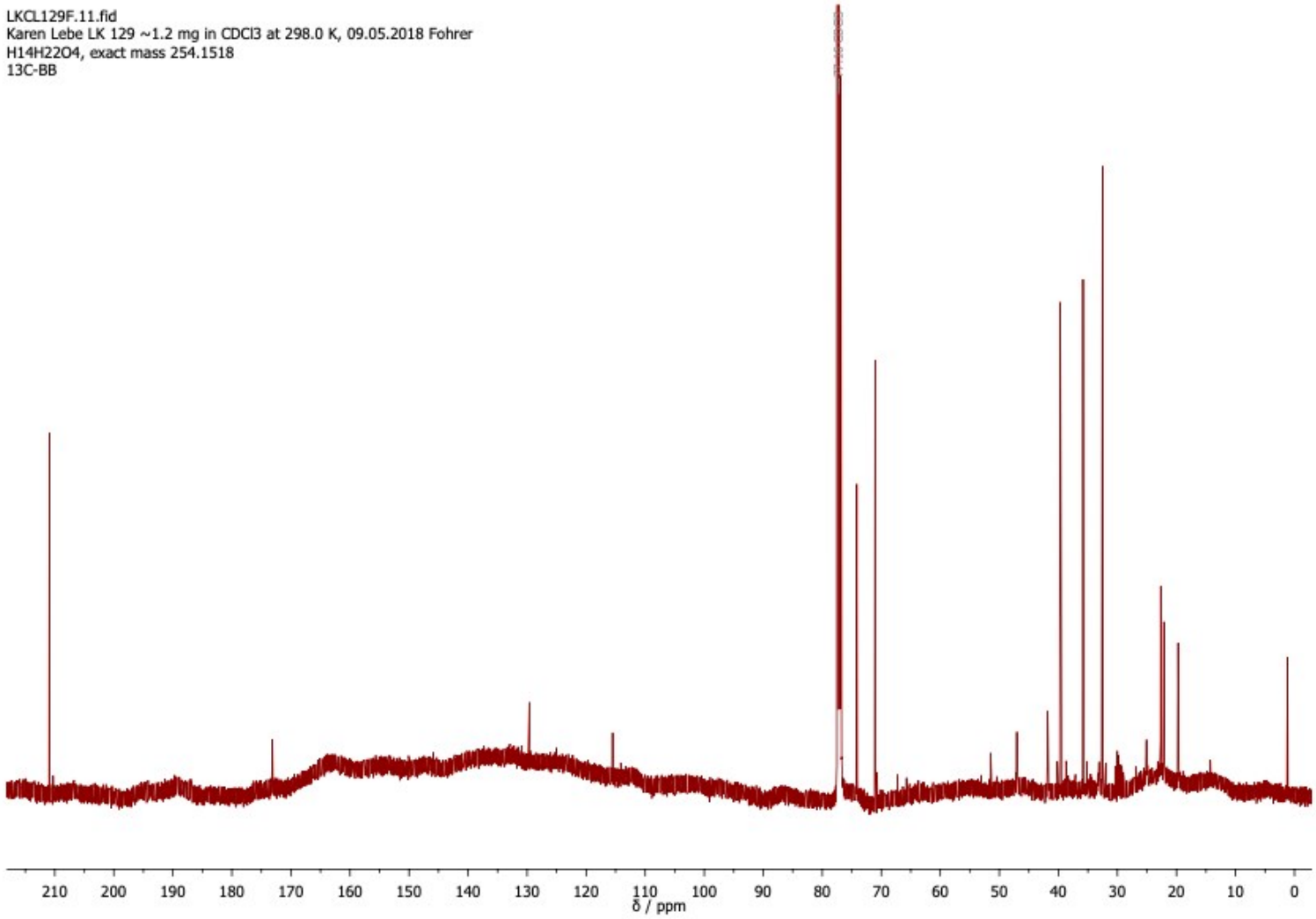


Figure S20 ^{13}C-NMR of compound **17** in $CDCl_3$ (500 MHz).

Figure S21

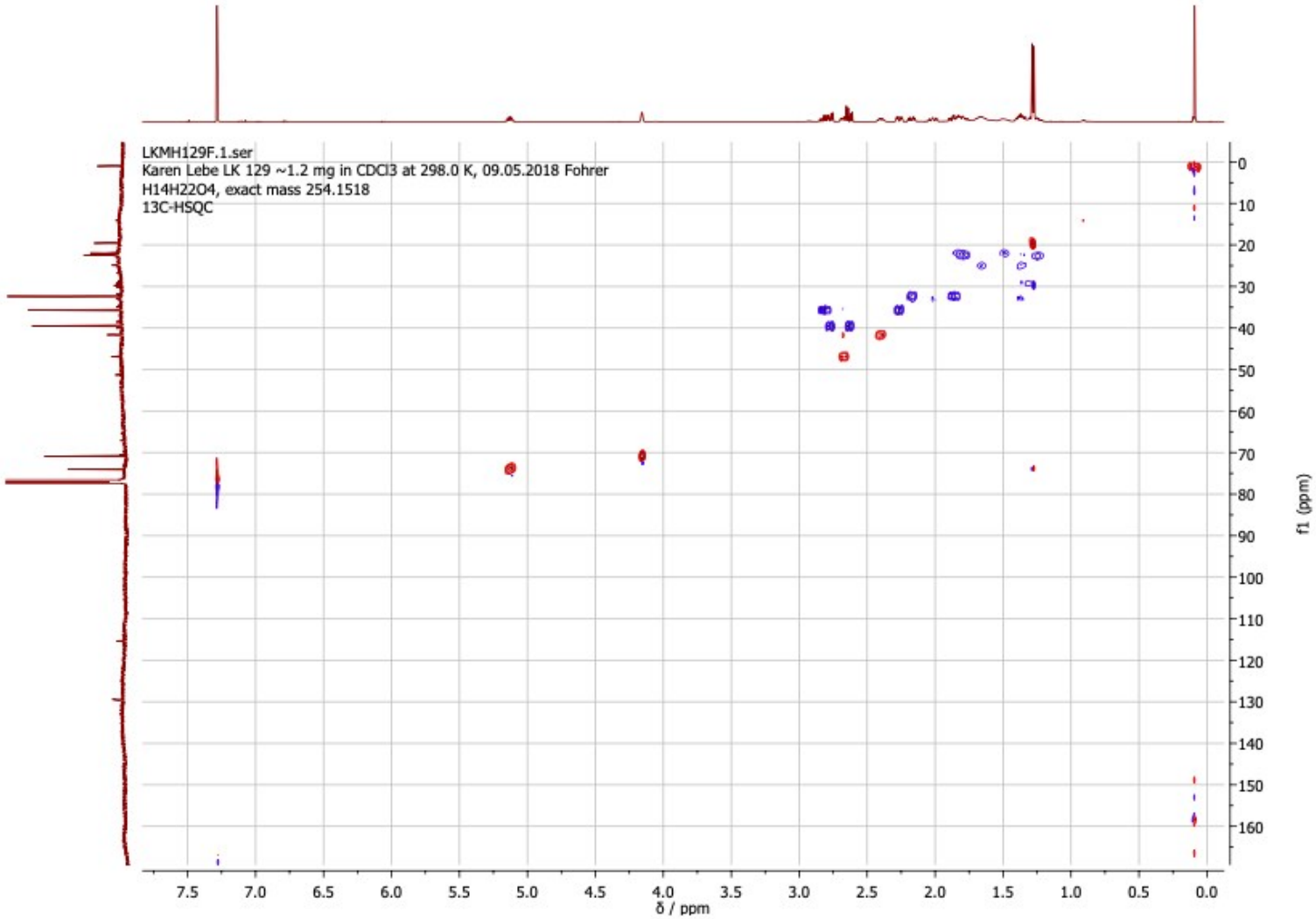


Figure S21 HSQC-NMR of compound **17** in $CDCl_3$ (500 MHz).

Figure S22

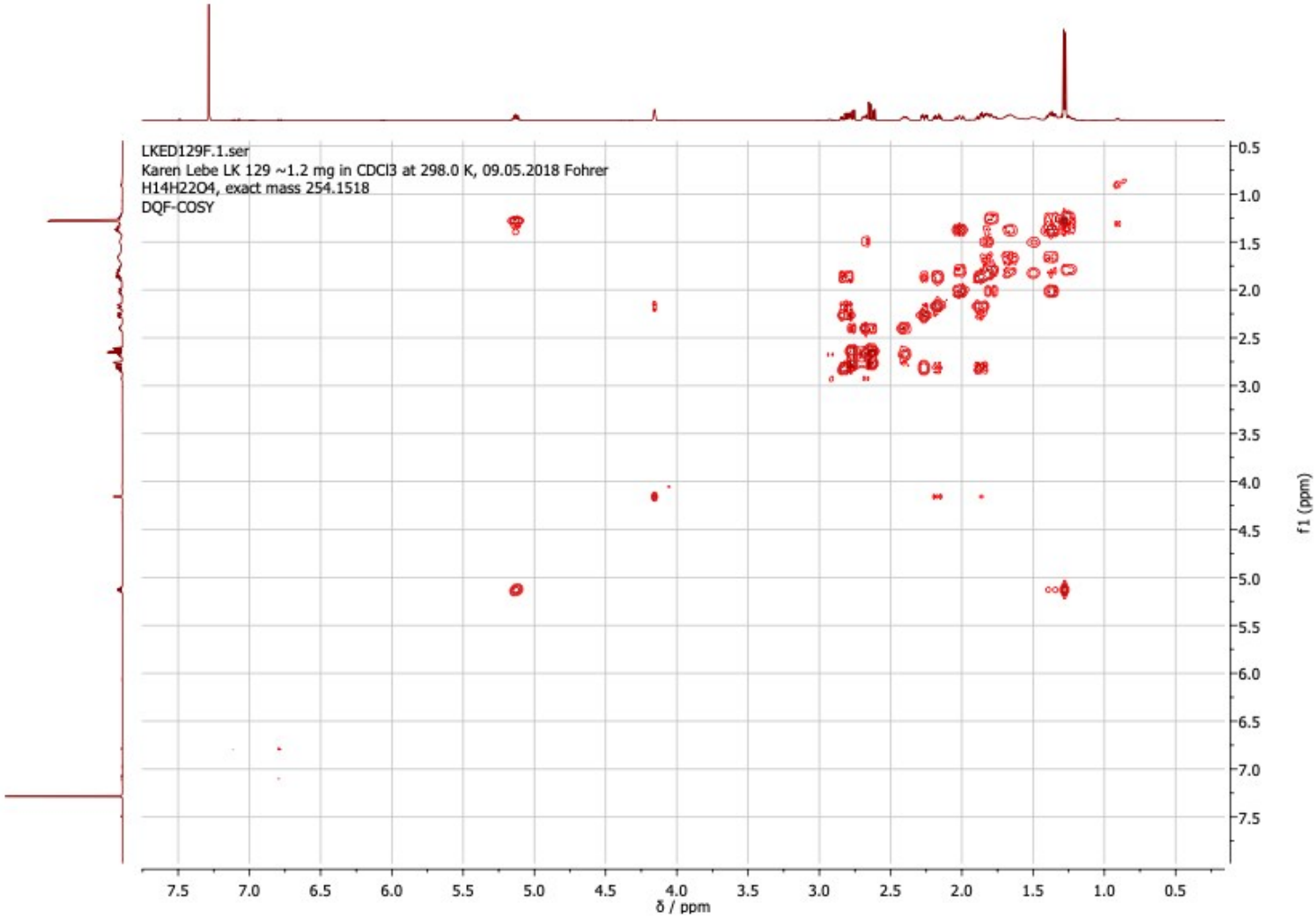


Figure S22 COSY-NMR of compound **17** in $CDCl_3$ (500 MHz).

Figure S23

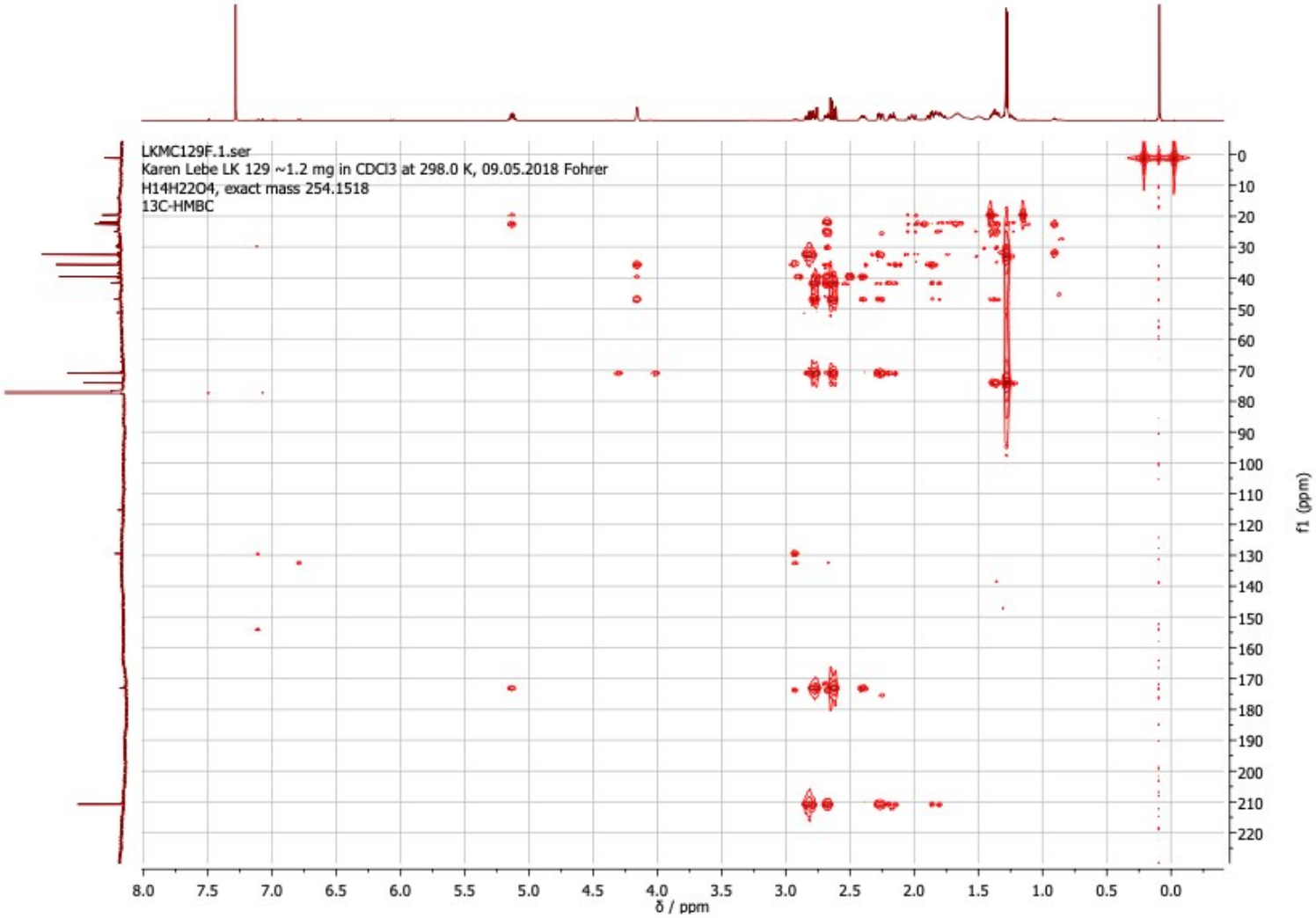


Figure S23 HMBC-NMR of compound **17** in $CDCl_3$ (500 MHz)

Figure S24

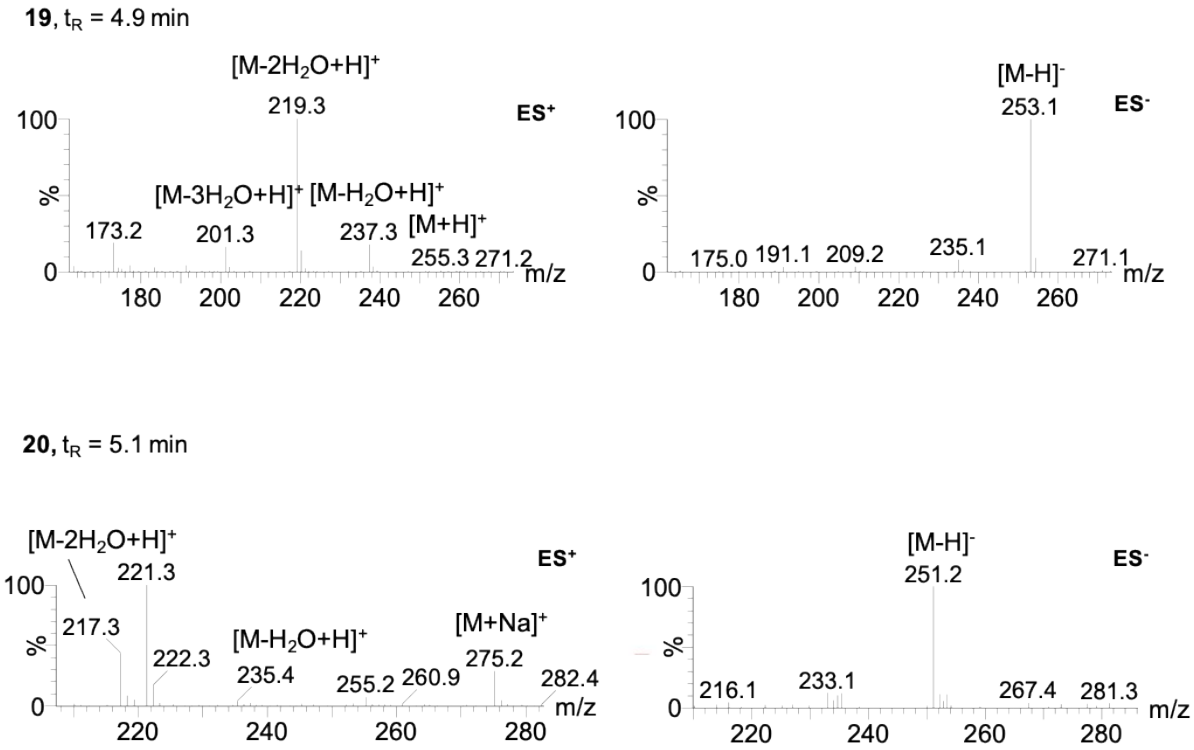


Figure S24 Mass spectra (ES^+ and ES^-) and UV chromatograms of **19** and **20**.

Figure S25

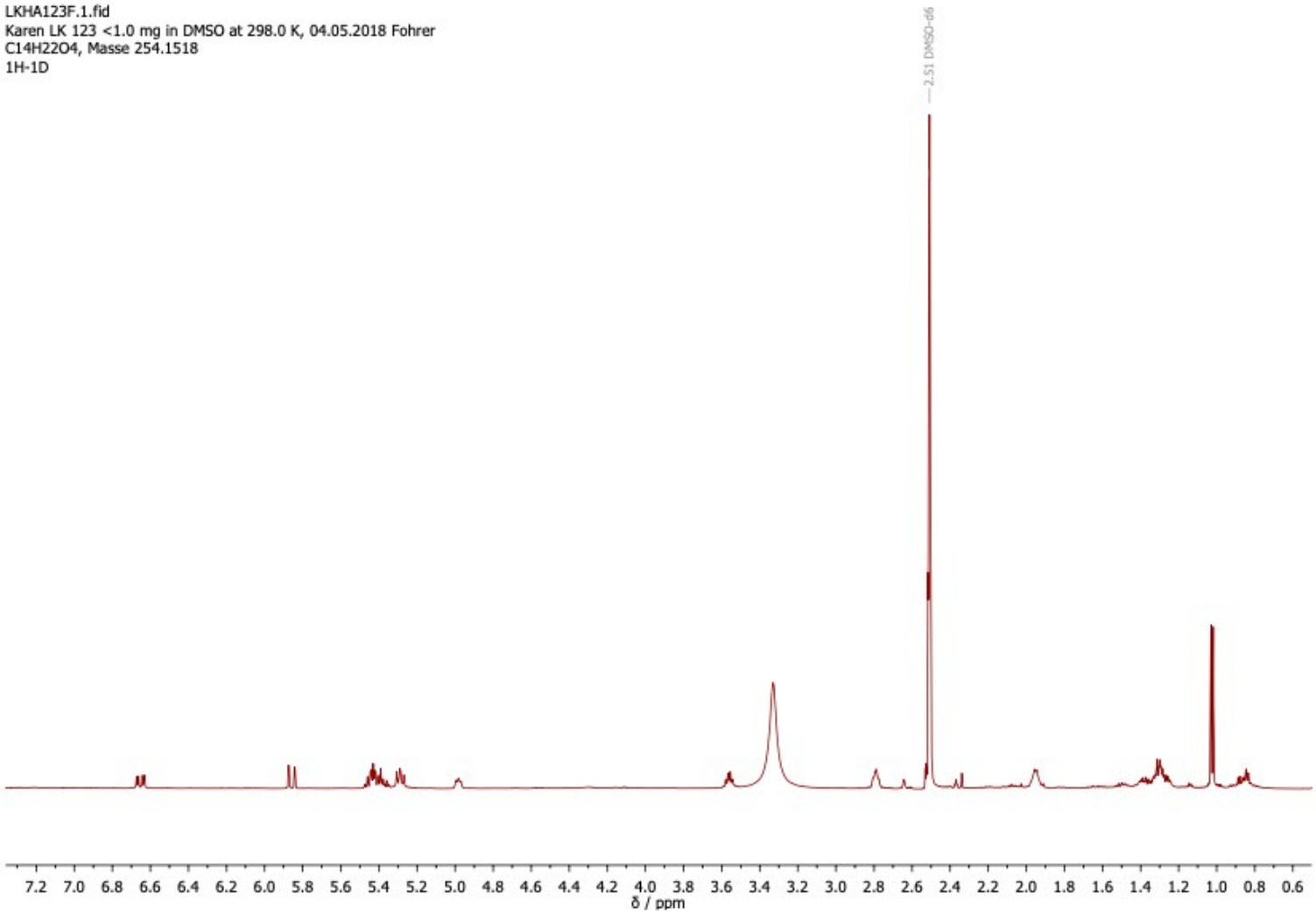


Figure S25 ^{1}H-NMR of compound **19** in $CDCl_3$ (400 MHz)

Figure S26

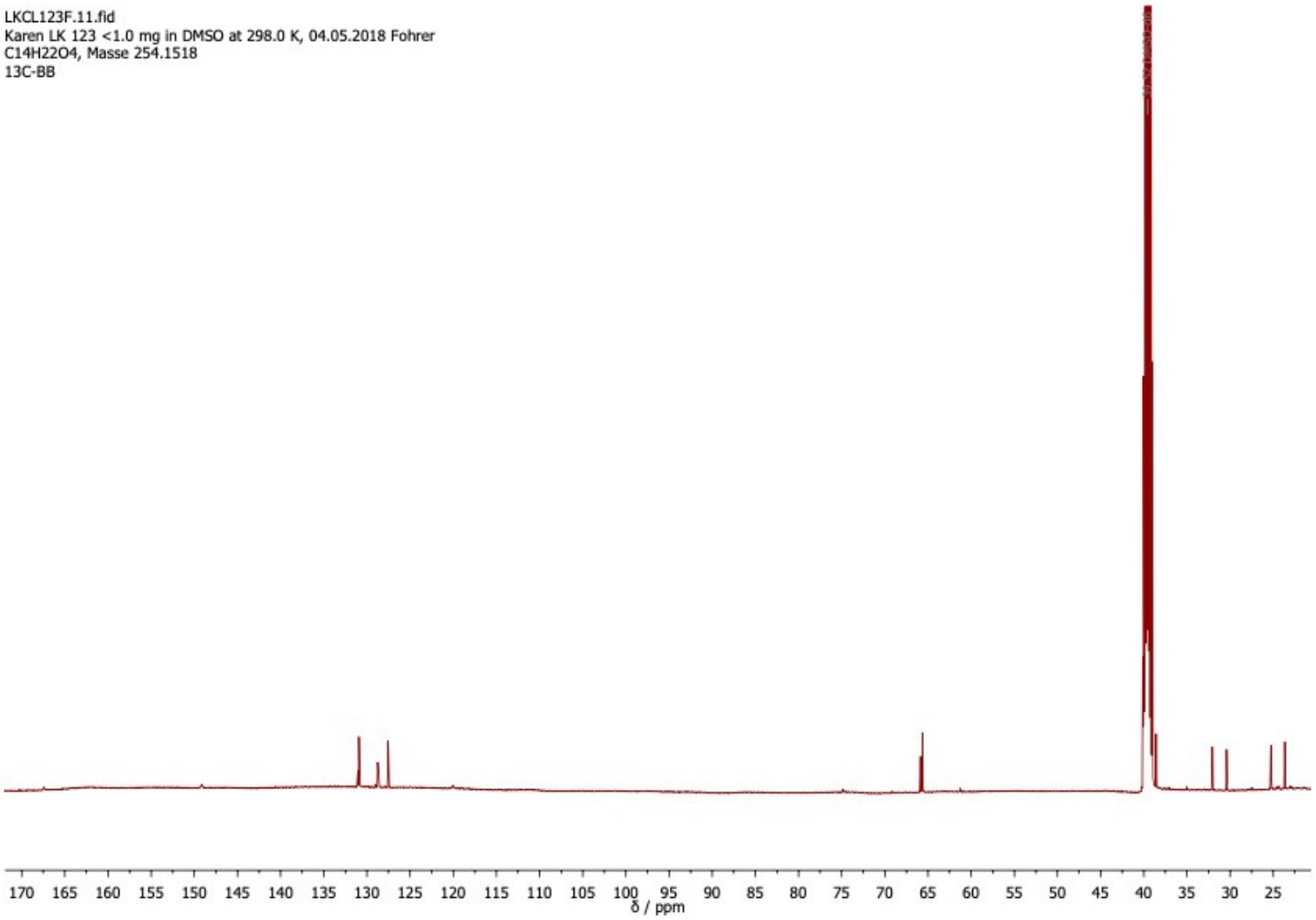


Figure S26 ^{13}C-NMR of compound **19** in $CDCl_3$ (100 MHz)

Figure S27

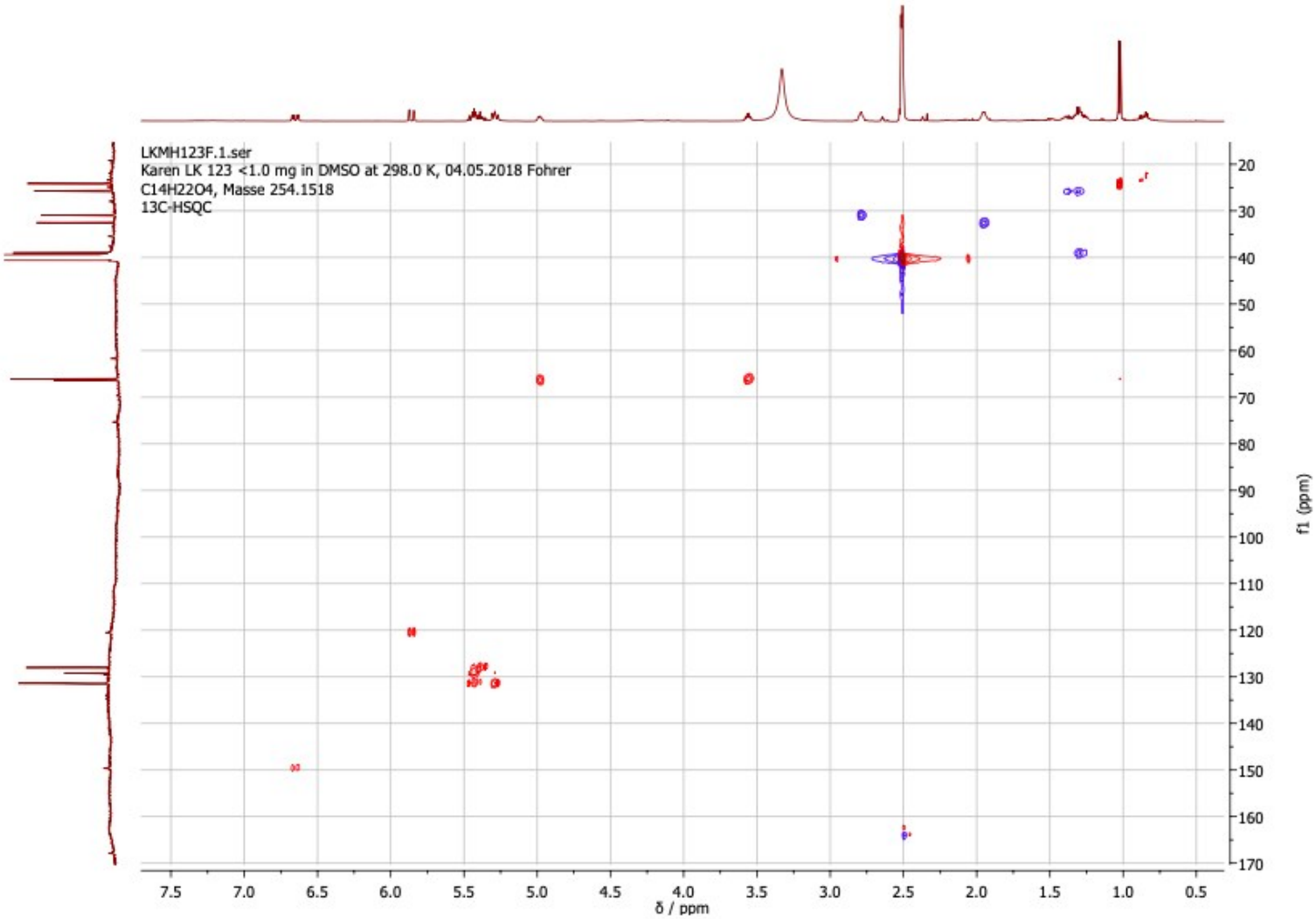


Figure S27 HSQC-NMR of compound **19** in $CDCl_3$ (400 MHz)

Figure S28

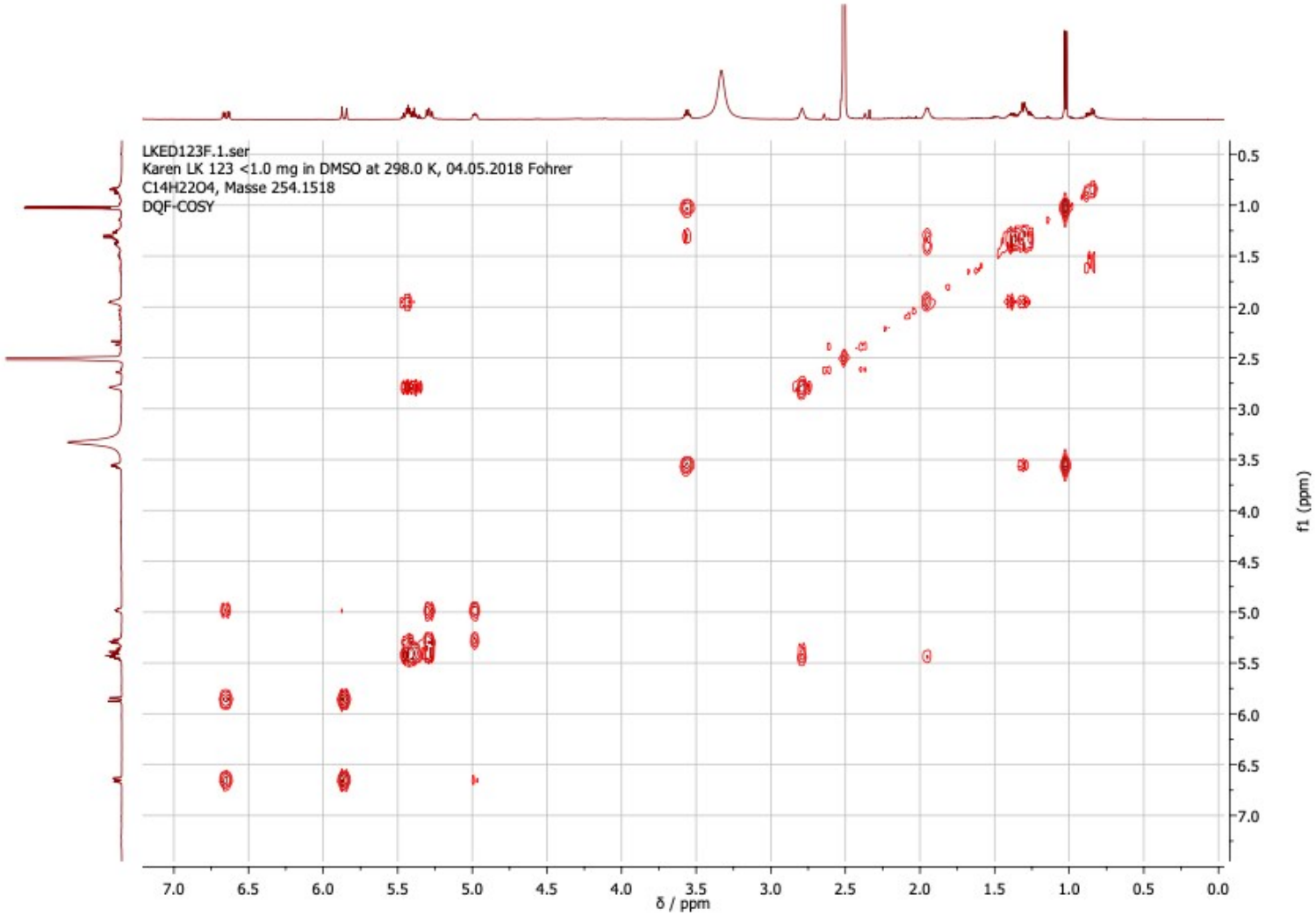


Figure S28 COSY-NMR of compound **19** in $CDCl_3$ (400 MHz).

Figure S29

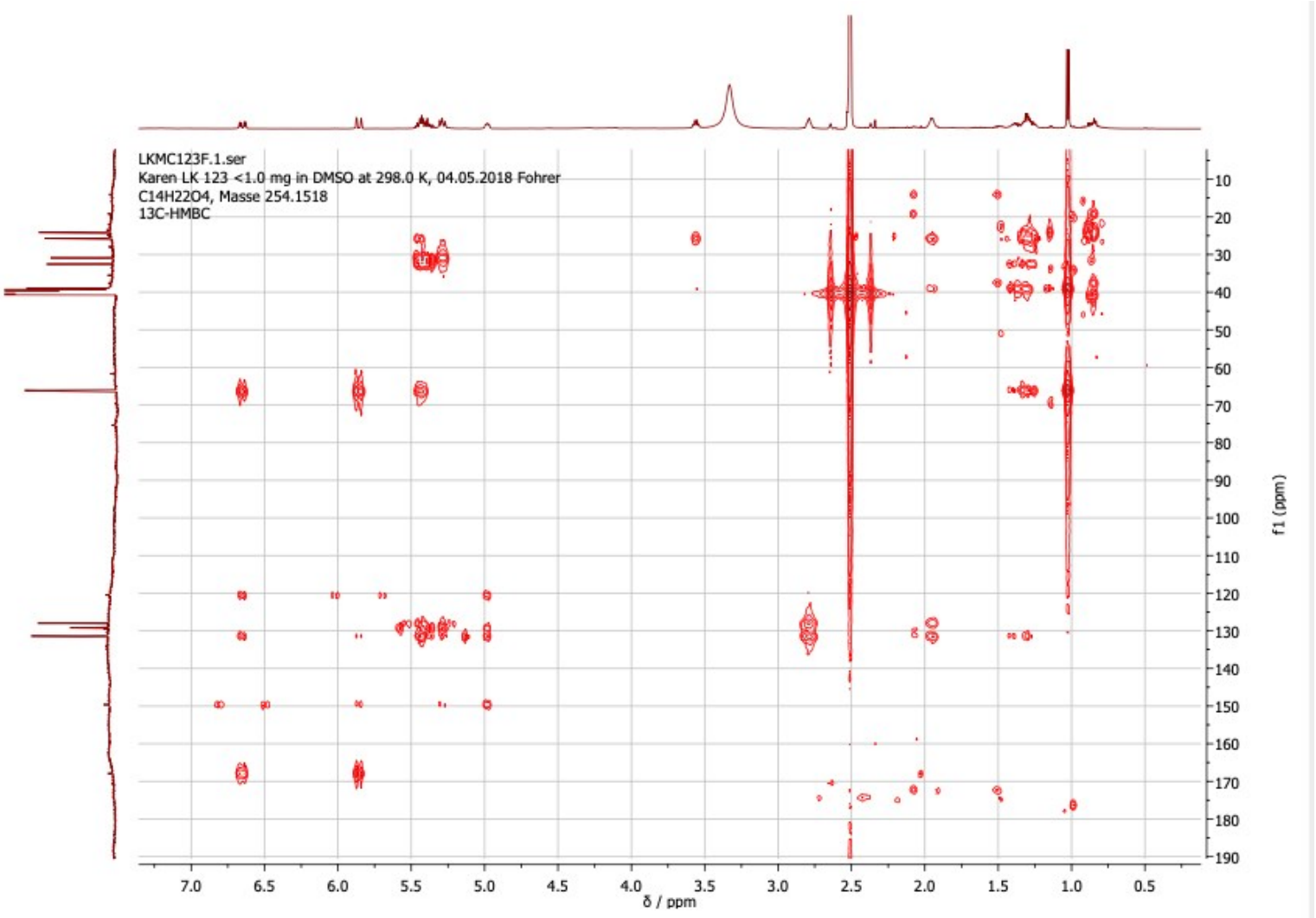
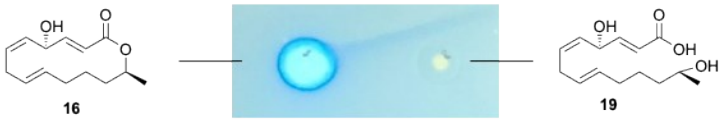
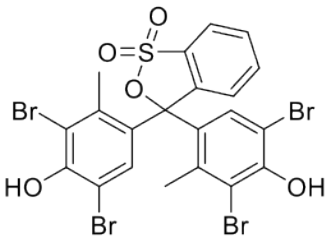


Figure S29 HMBC-NMR of compound **19** in $CDCl_3$ (400 MHz).

Figure S30



stain:



bromocresol green

Figure S30 Thin-layer-chromatography (TLC) plate with one drop of each **16** and **19**, stained with pH indicator Bromocresol green – a color change to yellow indicating an acid.

Figure S31

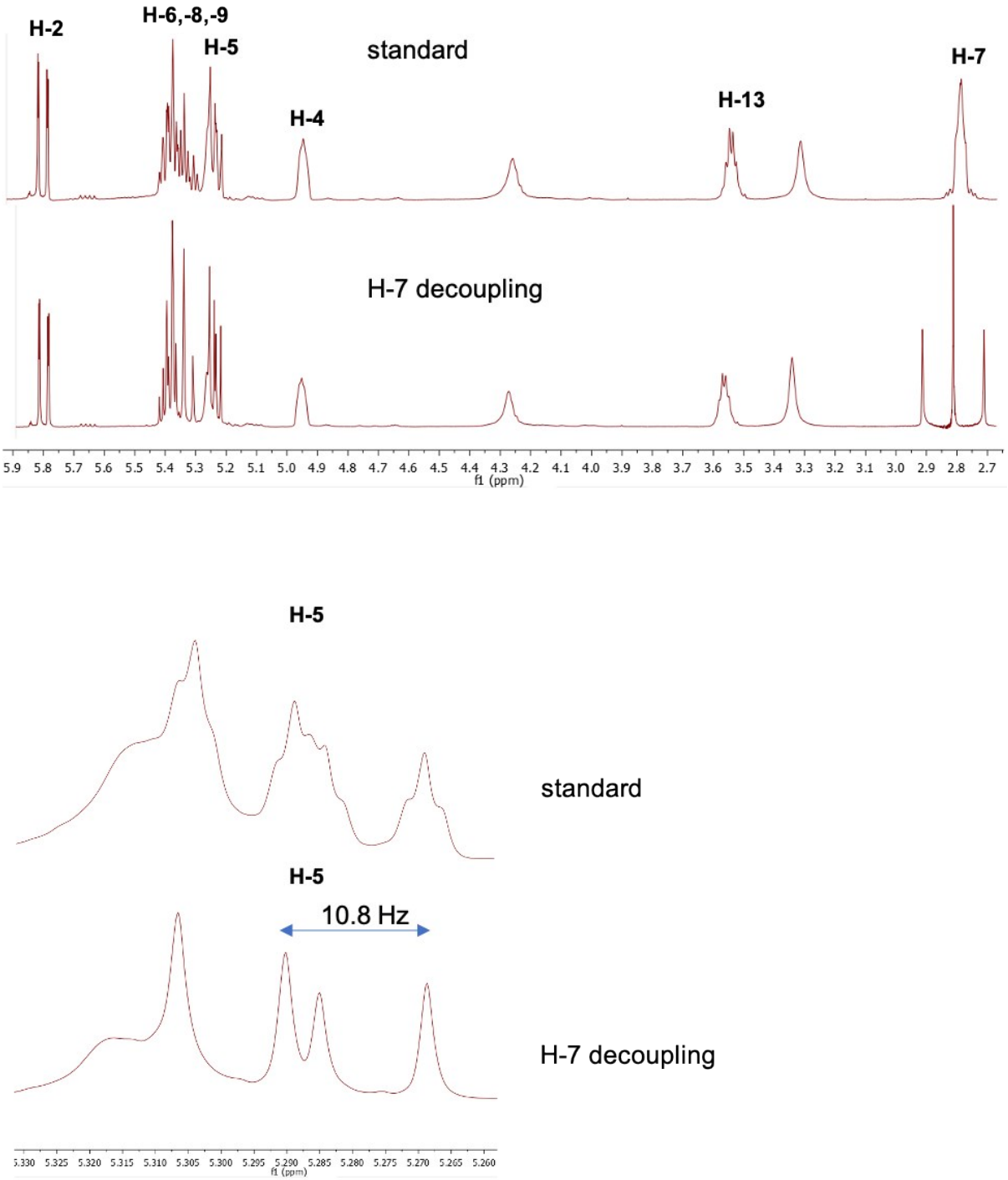


Figure S31 Decoupling of H-7 in compound **19** ^{1}H-NMR (500 MHz)

Figure S32

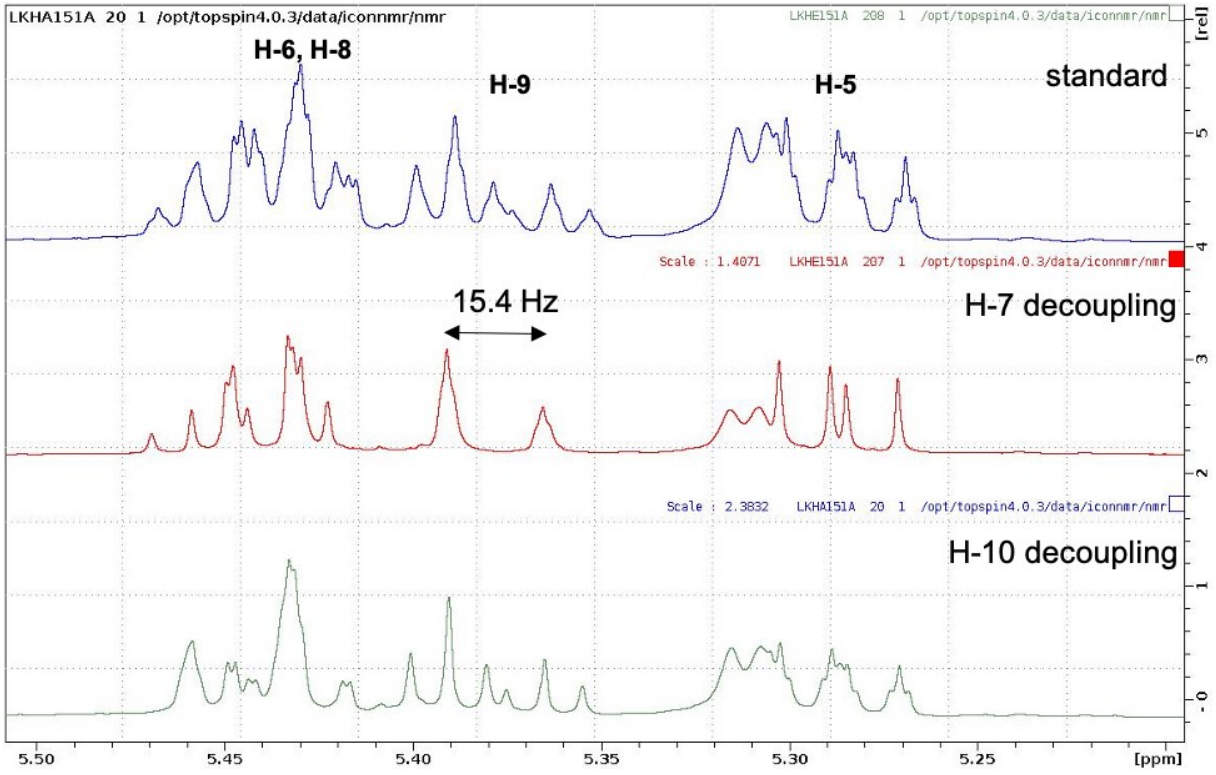


Figure S32 Decoupling of H-7 and H-10 in compound **19** ^{1}H-NMR (600 MHz)

Figure S33

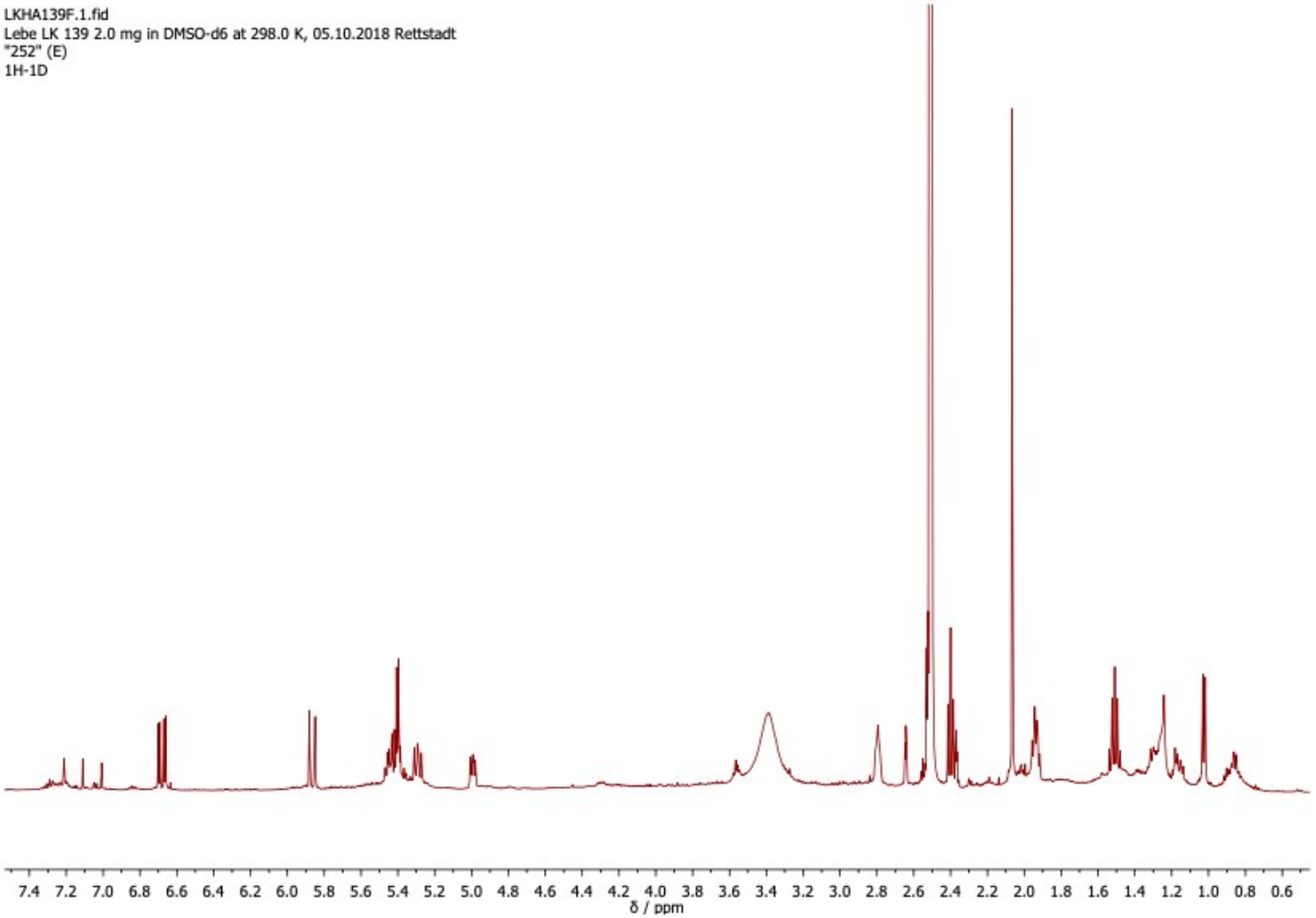


Figure S33 ^{1}H-NMR of compound **20** in DMSO-d_6 (500 MHz).

Figure S34

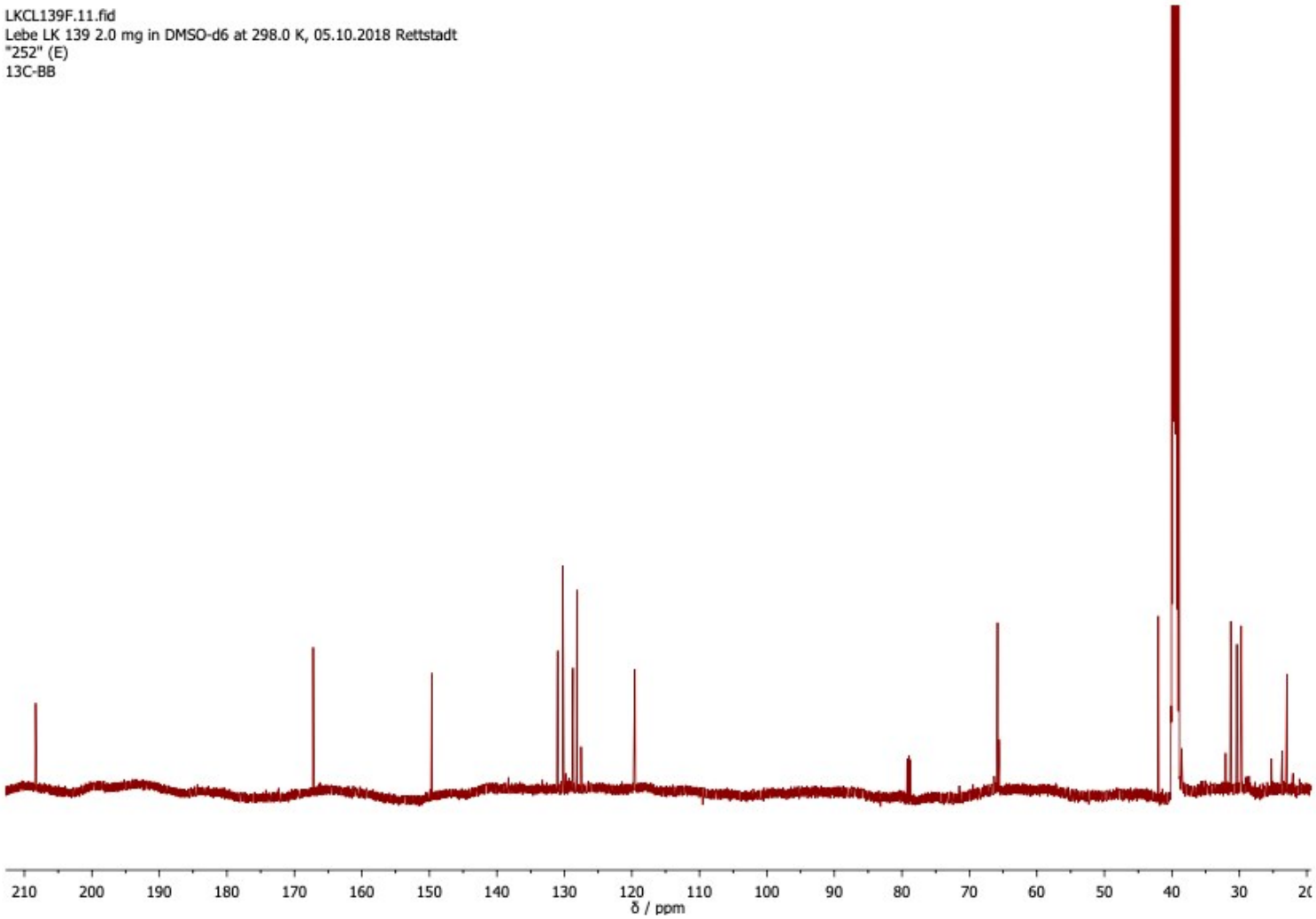


Figure S34 ^{13}C-NMR of compound **20** in DMSO-d_6 (500 MHz).

Figure S35

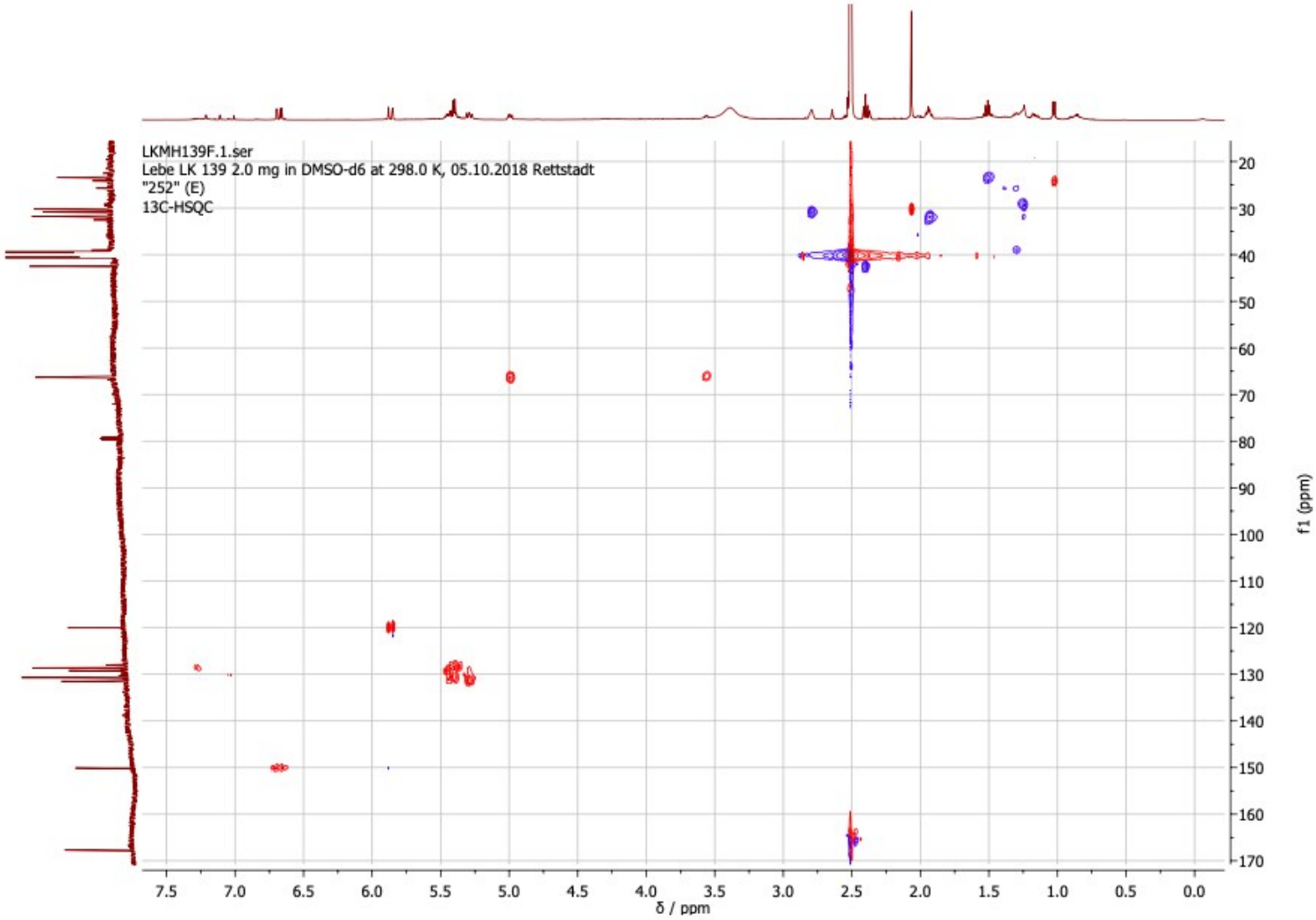


Figure S35 HSQC-NMR of compound **20** in DMSO-d_6 (500 MHz).

Figure S36

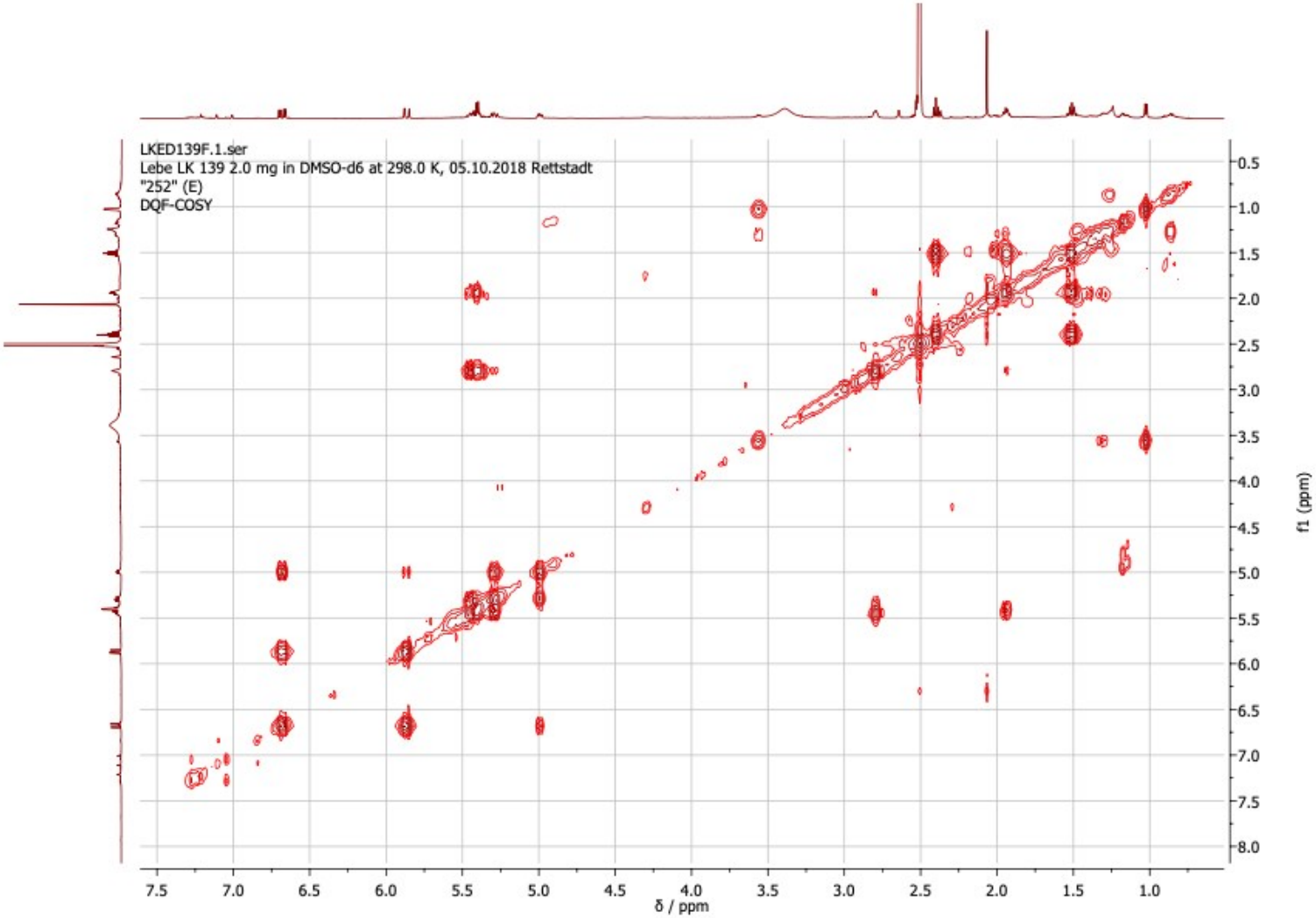


Figure S36 COSY-NMR of compound **20** in DMSO-d_6 (500 MHz).

Figure S37

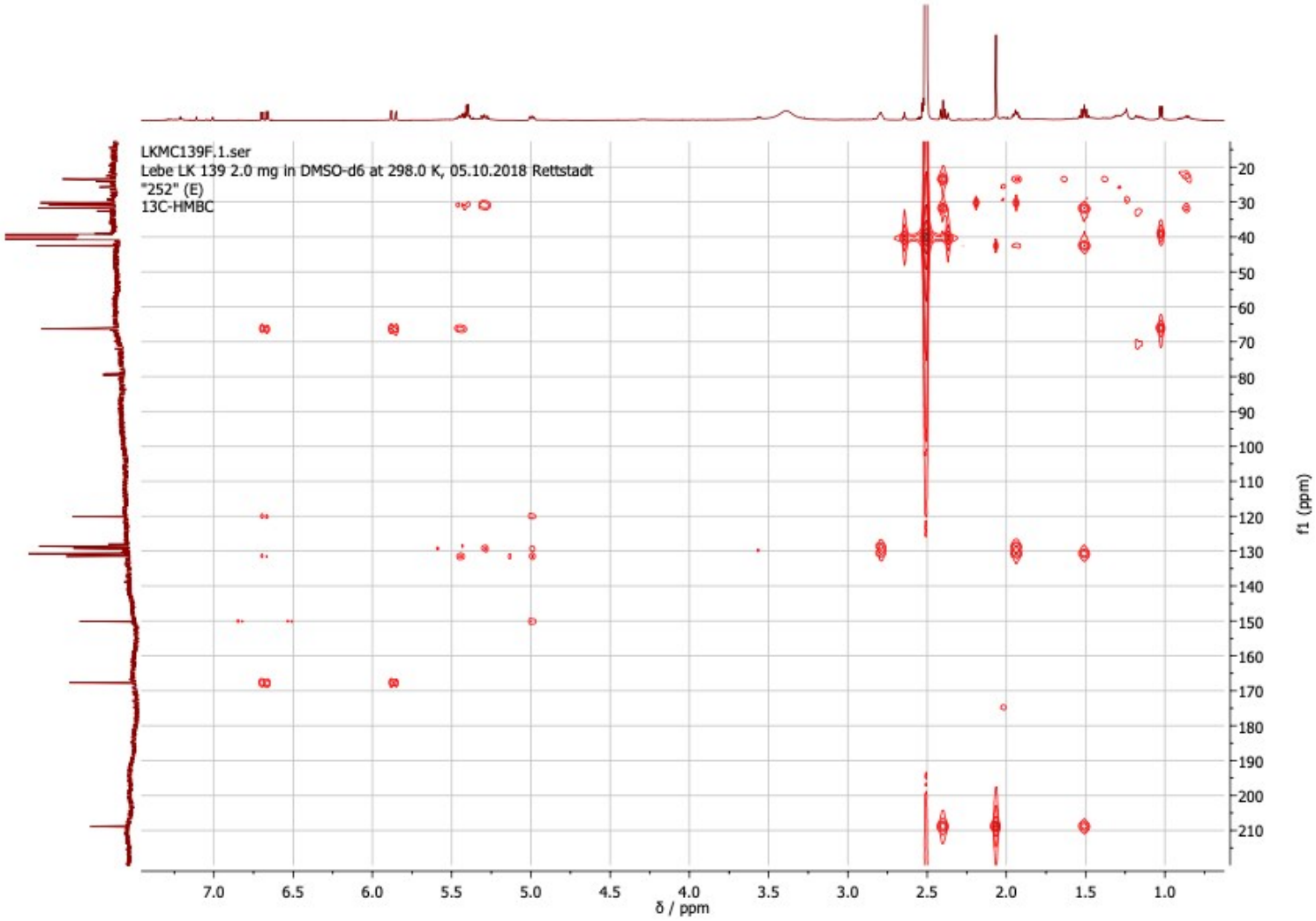


Figure S37 HMBC-NMR of compound **20** in DMSO-d_6 (500 MHz).

Figure S38

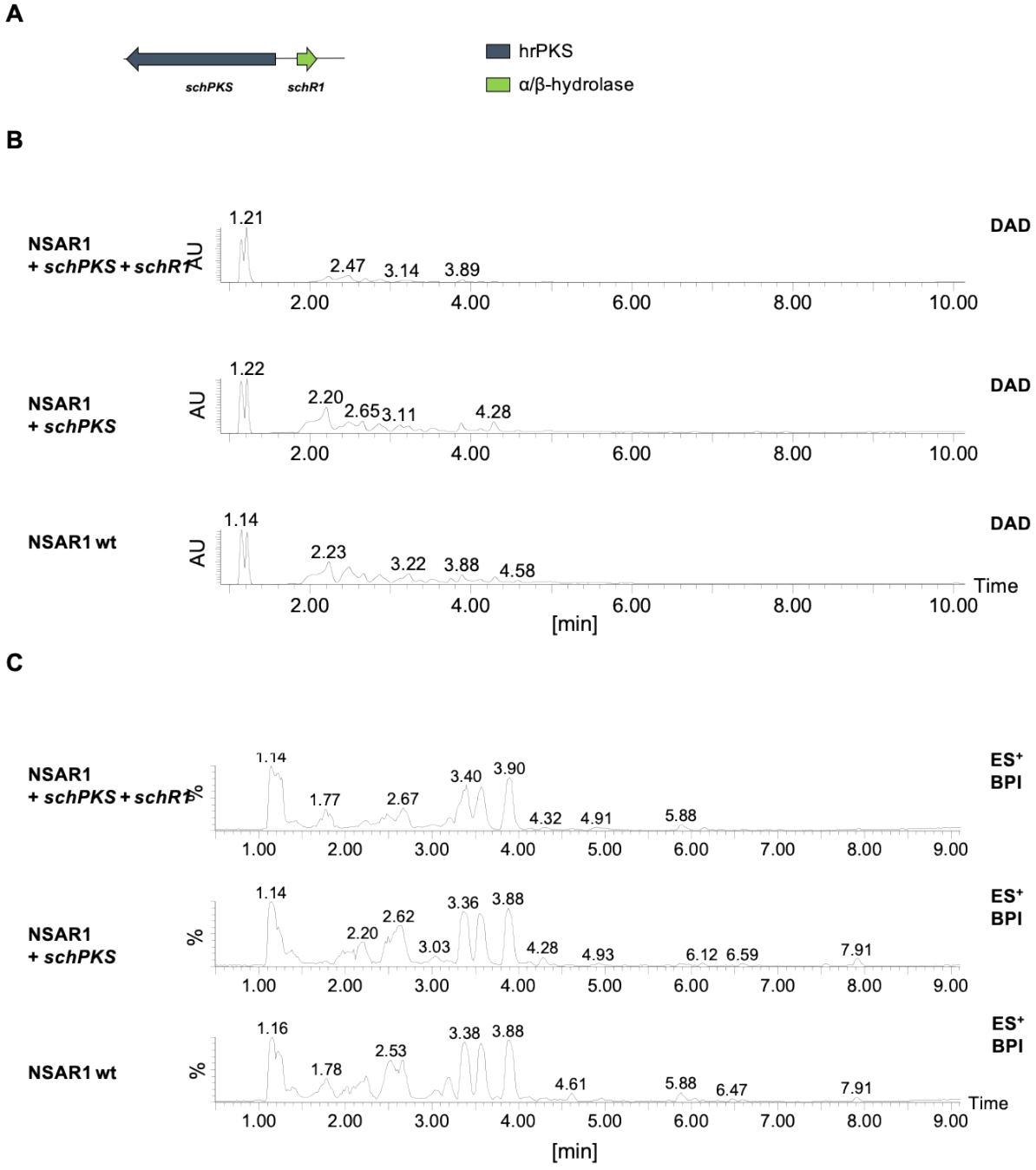


Figure S38 Chemical analysis of extracts from *A. oryzae* NSAR1 transformed with *schPKS* and *schPKS + schR1* compared to Aspergillus oryzae NSAR1 wt control. **A,** coexpresion of schPKS and schR1; **B,** DAD traces; **C,** ES^+ traces

.

Figure S39

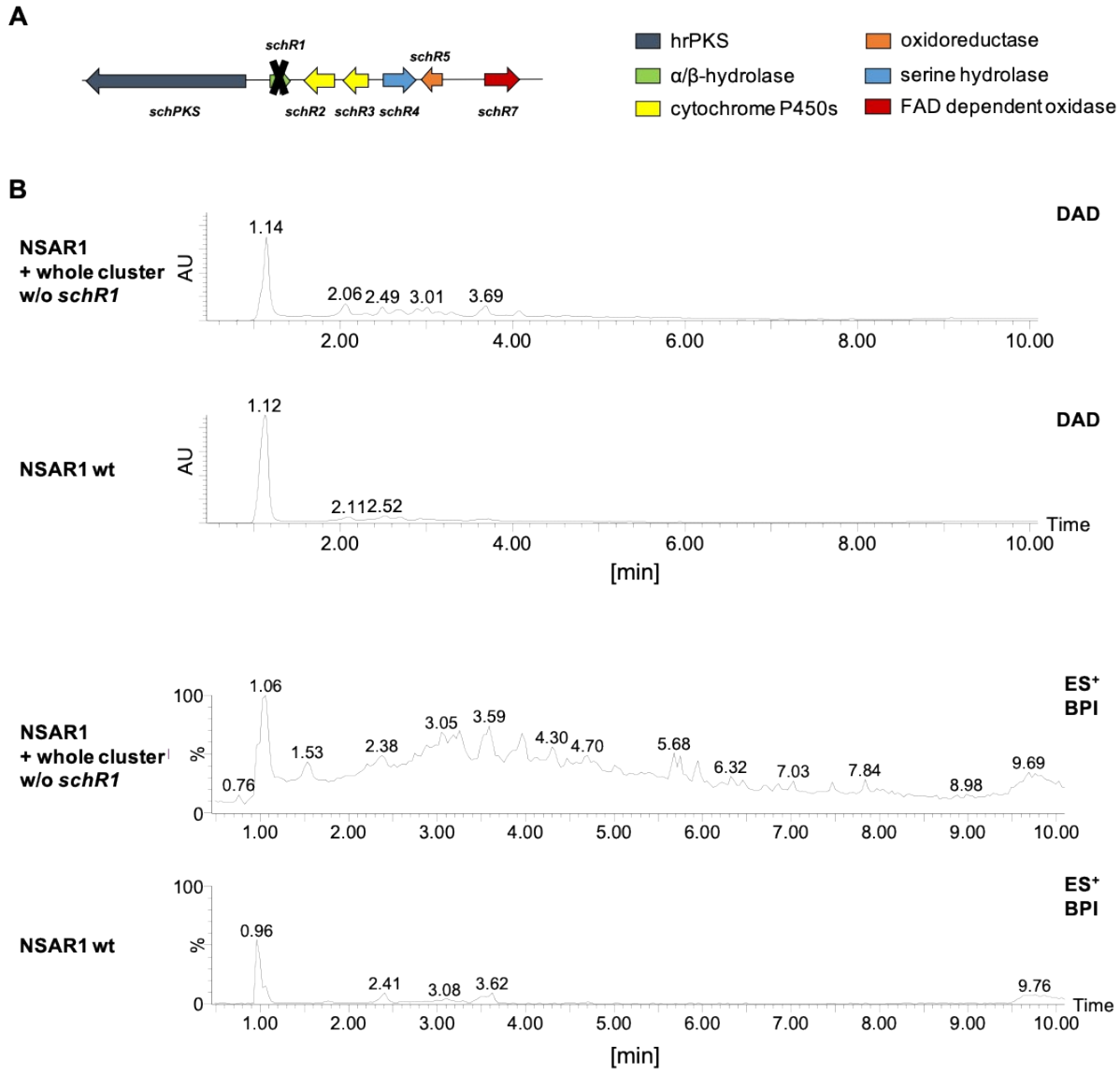


Figure S39 Chemical analysis of extracts from *A. oryzae* NSAR1 transformed with full *sch* BGC omitting only *schR1* and compared to Aspergillus oryzae NSAR1 wt control. **A,** transformed genes; **B,** DAD traces; **C,** ES^+ traces

Figure S40

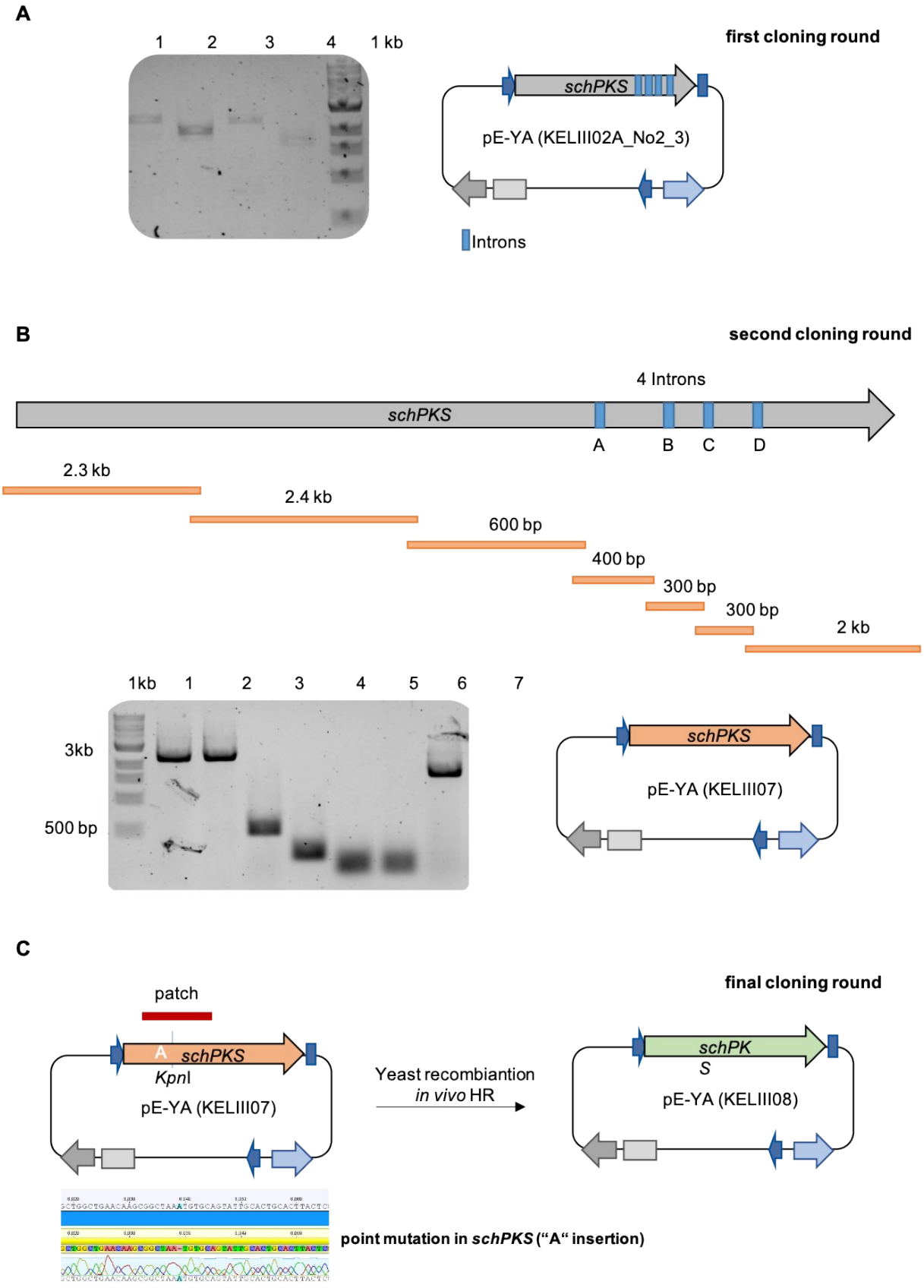


Figure S40 Cloning strategy *schPKS*.

Figure S41

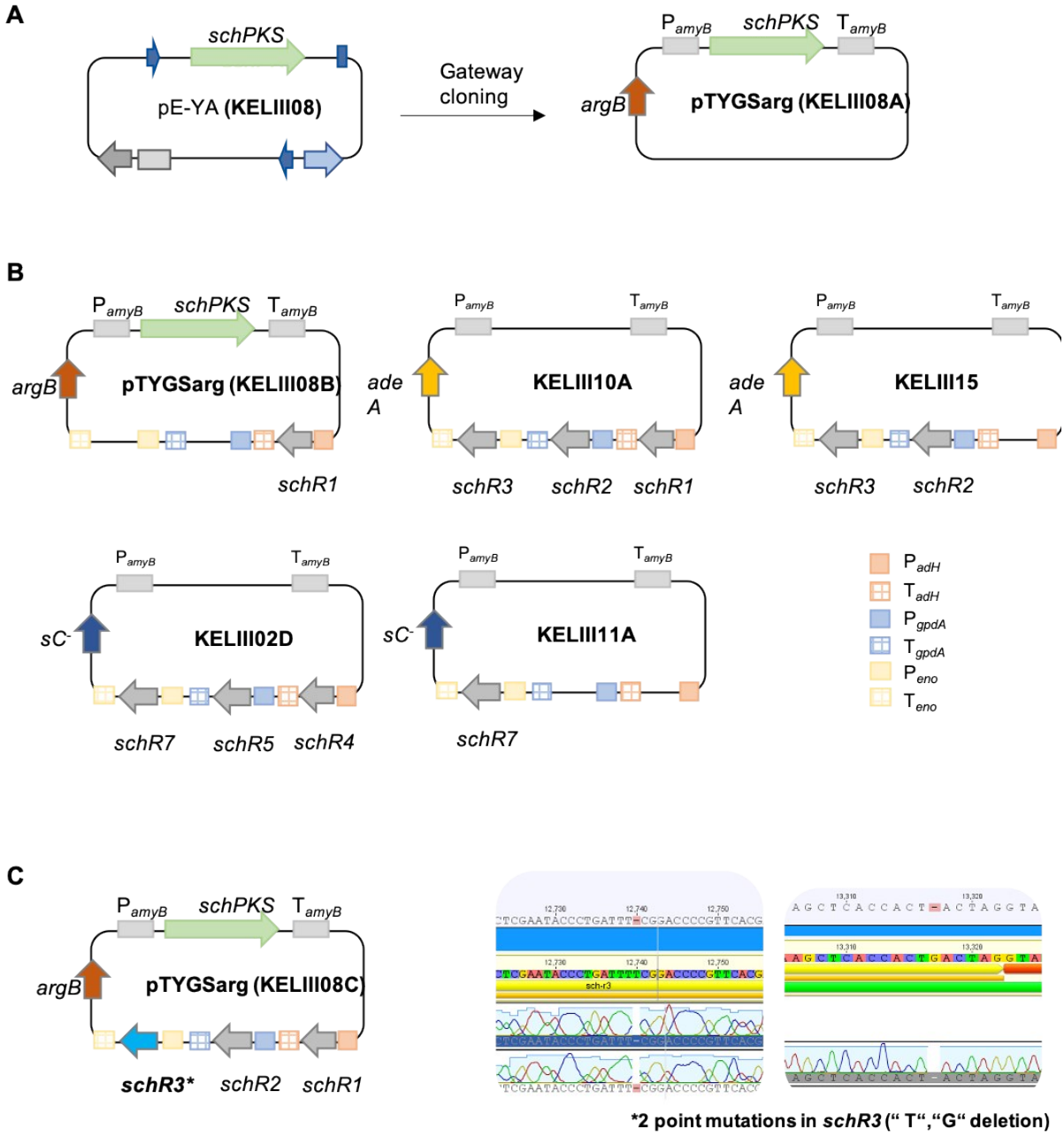


Figure S41 Overview of generated expression plasmids for transformation of *A. oryzae* NSAR1.

3. Supplementary Tables

Table S1

Alligned reads (all/paired ends)	54377401/23842462
Assembled bases	4201735013
PE-Size(s)	883±239; 884±239
Scaffolds (all/true)	2042/2042
Contigs (Scaffolded/Large (>500 Bp)/all)	5742/7578/28816
Bases in Scaffolds	64382824
Coverage	65.26 x
GC content (%)	52.92
Avg. Scaffold (Bp)	31529
N_{50} Scaffold	69395
Largest Scaffold	420401
Avg. Scaffold Contig	10656
Avg. Contig	8321
N_{50} Contig	19566
Largest Contig	119136

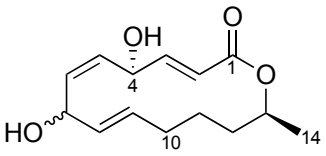
Table S1 Assembly details of *Phomopsis* sp. CMU-LMA WT.

Table S2

BGC type	Number of predicted BGC
PKS	60
Terpene	26
Fatty Acid	5
NRPS	17
PKS-Terpene	3
Linaridin	1
Lantipeptide	1
Siderophore	1
Type III PKS	1
PKS-NRPS	19
PKS-NRPS-Terpene	1
Other	21
Total	**156**

Table S2 AntiSMASH prediction of secondary metabolites BGC in *Phomopsis* sp. CMU-LMA.

Table S3



14

		$CDCl_3$		**Acetone-d_6**	
	Position	δ_C / ppm	δ_H / ppm	δ_C / ppm	δ_H / ppm
CO_2	1	166.1	-	166.7	-
CH=CH	2	118.2	5.91 (dd, 15.6, 1.8)	117.7	5.81 (dd, 15.6, 1.8)
CH=CH	3	148.2	7.03 (dd, 15.6, 4.5)	151.3	7.13 (dd, 15.6, 4.5)
CH-OH	4	68.5	5.27 (m)	68.5	5.26 (m)
CH=CH	5	132.0	5.54 (m)	132.9	5.45 (m)
CH=CH	6	131.2	5.46 (m)	132.8	5.36 (m)
CH-OH	7	70.6	4.82 (t)	70.5	4.82 (t, 16.0, 7.9, 3.4)
CH=CH	8	134.4	5.87 (m)	133.0	5.79 (m)
CH=CH	9	130.2	5.51 (m)	132.7	5.4 (m)
CH_2	10a	32.8	2.08 (m)	33.6	
	10b		1.97 (m)		1.9 (m)
CH_2	11a	24.4	1.82 (m)	25.5	1.79 (m)
	11b		1.12 (m)		1.04 (m)
CH_2	12a	34.5	1.75 (m)	35.2	1.7 (m)
	12b		1.5 (m)		1.5 (m)
CH-OH	13	72.05	4.76 (m)	72.3	4.6 (m)
CH3	14	20.2	1.26 (d, 6.2)	20.6	1.2 (d, 6.2)

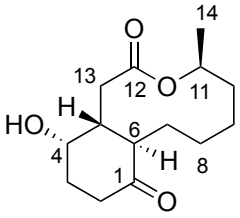
Table S3 NMR data compound **14** ($CDCl_3$, 500 MHz).

Table S4

	Position	δ_C / ppm	δ_H / ppm
CO_2	1	166.3	-
CH=CH	2	117.5	5.94 (dd, 15.7, 1.9)
CH=CH	3	149.6	7.18 (dd, 15.7, 3.9)
CH-OH	4	68.3	5.26 (m)
CH=CH	5	131.5	5.51 (m)
CH_2	6	128.6	5.54 (m)
CH=CH	7	32.1	2.82 (m)
CH=CH	8	132.1	5.55 (m)
CH=CH	9	126.6	5.43 (m)
CH_2	10a	32.9	2.08 (m)
	10b		1.91 (m)
CH_2	11a	24.4	1.78 (m)
	11b		1.19 (m)
CH_2	12a	34.3	1.74 (m)
	12b		1.51 (m)
CH-OH	13	71.8	4.82 (m)
CH_3	14	20.1	1.27 (d, 6.4)

Table S4 NMR data compound **16** ($CDCl_3$, 500 MHz).

Table S5

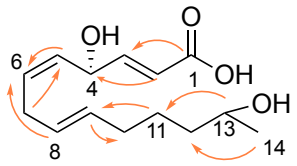


17
254.3 g/mol

Compound **17** (1.2 mg, $CDCl_3$, 500 MHz)				Adelin *et al.* (2011), $CDCl_3$		
Atom	δ_C / ppm	δ_H / ppm	*J* / Hz	δ_C / ppm	δ_H / ppm	*J* / Hz
1	210.5	-	-	211	-	-
2a	35.8	2.24	dddd (14.3, 4.8, 2.4, 0.7)	35.8	2.2	m
2b	35.8	2.6	ddt (14.3, 6.4, 0.7)	35.8	2.66	m
3a	32.5	1.85	ddd (14.1, 4.8, 2.2)	32.5	1.78	m
3b	32.5	2.15	dddd (14.0, 6.1, 3.5, 2.3)	32.5	1.94	m
4	71.0	4.13	q (2.7)	70.9	4.1	m
5	41.8	2.37	dtd (12.1, 6.6, 2.2)	41.7	2.36	q (7.3)
6	47.0	2.66	m	47.8	2.66	m
7	22.1	1.47	m	21.2	1.35	m
8	22.7	1.20, 1.76	m	22.2	1.35	m
9	25.2	1.40, 1.60	m	22.7	1.35	m
10	33.2	1.35	m	33	1.35	m
11	74.1	5.1	m	74.1	5.07	m
12	173.2	-	-	173.2	-	-
13	39.7	2.60, 2.76	m, d (5.4)	39.8	2.66	m
14	19.7	1.26	d (6.4)	19.7	1.23	d (6.4)

Table S5 Chemical shifts of 1.2 mg **17** in $CDCl_3$. Referenced to Adelin *et al*.(9)

Table S6

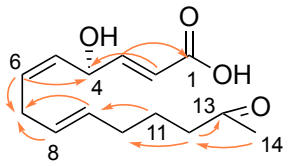


19
254.3 g/mol

Compound **19** (1.0 mg, DMSO, 500 MHz)					
Atom	δ_C / ppm	δ_H / ppm	*J* / Hz	^{1}H-^{1}H COSY	HMBC
1	167.4	-	-	-	2, 3
2	120.0	5.86	dd, 15.4, 1.6	3	1, 3, 4
3	149.1	6.67	dd, 15.4, 4.9	2, 4	1, 2, 4, 5
4	65.9	4.99	d, 6.2	3, 5	2, 3, 5, 6
5	131.0	5.29	m	6	3, 4, 6, 7
6	128.8	5.42	m	5, 7	4, 5, 7
7	30.4	2.79	m	6, 8	5, 6, 8, 9
8	130.9	5.39	m	7, 9, 10	6, 7, 9
9	127.5	5.42	m	10	10, 11
10	32.1	1.95	m	9, 11a, 11b, 12	9, 11
11a	25.3	1.36	m	10, 12	9, 10
11b	25.3	1.28	m	10, 12, 13	9, 10
12	38.6	1.28	m	10, 11	13, 14
13	65.6	3.56	m	12, 14	10, 11, 12, 14
14	23.7	1.02	d, 6.1	13	12, 13

Table S6 Chemical shifts of 1.2 mg **19** in $CDCl_3$. Referenced to Adelin *et al*.(9)

Table S7



20
252.3 g/mol

Atom	δ_C / ppm	δ_H / ppm	J / Hz	^{1}H-^{1}H COSY	HMBC
1	167.2	-	-	-	2, 3
2	119.6	5.86	dd, 15.6, 1.6	3	1, 3, 4
3	149.6	6.67	dd, 15.6, 4.9	2, 4	1, 2, 4, 5
4	65.8	4.99	m	3, 5	2, 3, 5, 6
5	131.0	5.28	ddt, 10.9, 8.2, 1.5	4, 6	3, 6, 7
6	128.7	5.44	m	5, 7	4, 5, 7
7	30.4	2.79	m	6	6, 8, 9
8	130.2	5.39	m	7, 9	7, 9
9	128.1	5.39	m	8, 10	7, 8, 10
10	30.3	1.93	m	9, 11	8, 9, 10, 11
11	23.0	1.50	p, 7.4	10, 12	9, 10, 12, 13
12	42.0	2.39	t, 7.3	11	10, 11, 13
13	208.3	-	-	-	11, 12, 14
14	29.7	2.06	S	-	12, 13

Table S7 Chemical shifts of 1.9 mg **20** in DMSO.

Table S8

schPKS T_L Fw	GCCAACTTTGTACAAAAAAGCAGGCTCCGCATGCCTTCGTCATACATACA
schPKS T_L Re	AAACGAAAGGCACAGAACAT
schPKS HygR Fw	CTCCTTTTCTATGTTCTGTGCCTTTCGTTTTCTAGTGGATCTTTCGACAC
schPKS T_R Fw	CCCAGCACTCGTCCGAGGGCAAAGGAATAGGGGCGACGTCGCCAAGGAAG
schPKS T_R Re	TGCCAACTTTGTACAAGAAAGCTGGGTCGGTCACGTAACAACCATCTTGG
schR2 T_L Fw	GCCAACTTTGTACAAAAAAGCAGGCTCCGCAACCGGACATCTCGACACTC
schR2 T_L Re	GGAACTCCGACTTCAGTCTG
schR2 HygR Fw	AGCTATGTCACAGACTGAAGTCGGAGTTCCTCTAGTGGATCTTTCGACAC
schR2 T_R Fw	CCCAGCACTCGTCCGAGGGCAAAGGAATAGGACCCAGATAGAATTCCTGC
schR2 T_R Re	TGCCAACTTTGTACAAGAAAGCTGGGTCGGCCAACTCCACATCCGACTTG
schR3 T_L Fw	GCCAACTTTGTACAAAAAAGCAGGCTCCGCCAATATTCATGCTCTTGGGC
schR3 T_L Re	AATCAGGGTATTCGAGAAAG
schR3 HygR Fw	TATCATATGACTTTCTCGAATACCCTGATTTCTAGTGGATCTTTCGACAC
schR3 T_R Fw	CCCAGCACTCGTCCGAGGGCAAAGGAATAGGACCCCGTTCACGACATCTA
schR3 T_R Re	TGCCAACTTTGTACAAGAAAGCTGGGTCGGCGGGGTCAAATTTACAATAC
schR4 T_L Fw	GCCAACTTTGTACAAAAAAGCAGGCTCCGCCAAATGATCTACCTCGGGAG
schR4 T_L Re	CAAGCTCATGGTGCAGTATC
schR4 HygR Fw	CAGCTTCCATGATACTGCACCATGAGCTTGCTATTCCTTTGCCCTCGGAC
schR4 T_R Fw	ACGTATTTCAGTGTCGAAAGATCCACTAGACTCTGTTCCAGTTTGCCATT
schR4 T_R Re	TGCCAACTTTGTACAAGAAAGCTGGGTCGGCAGGTCTCAGTATCACTTTTC
schR5 T_L Fw	GCCAACTTTGTACAAAAAAGCAGGCTCCGCGAGCAACAAACCATTCTGGT
schR5 T_L Re	GTCCAGTTGGGAATGCCACT
schR5 HygR Fw	CCTGAGGACAAGTGGCATTCCCAACTGGACTCTAGTGGATCTTTCGACAC
schR5 T_R Fw	CCCAGCACTCGTCCGAGGGCAAAGGAATAGCGTCCTTGCACATTCATGTC
schR5 T_R Re	TGCCAACTTTGTACAAGAAAGCTGGGTCGGGATTGCCTAACTTCGTCTTC
schR7 T_L Fw	GCCAACTTTGTACAAAAAAGCAGGCTCCGCGAACCCTTACTTTCAAAGCC
schR7 T_L Re	GTAGAGGTCCTCGTGCTCAG
schR7 HygR Fw	GCATCTCCAACTGAGCACGAGGACCTCTACTCTAGTGGATCTTTCGACAC
schR7 T_R Fw	CCCAGCACTCGTCCGAGGGCAAAGGAATAGGAGACATATACCGGGCCACT
schR7 T_R Re	TGCCAACTTTGTACAAGAAAGCTGGGTCGGCATTTATTCGGCAAAGAGTC

Table S8 Primers used for the construction of KO plasmids

Table S9

HygRP7.2 Fw	GCTTTCAGCTTCGATGTAGG
HygRP6.2 Re	CGTCAGGACATTGTTGGAG
schPKS ext	CTAGGCCTAGACTACTTGAC
schR2 ext	GAGAAAAGGCGATTTGAGTC
schR3 ext	GAATCACAAAGCCACCGTAATG
schR5 ext	GCATTAGCTCAACGGACC
schR7 ext	GCATTAGCTCAACGGACC

Table S9 Primers used for external PCR in KO validation

Table S10

Construct ID	**Vector backbone**	**Template**	**Oligonucleotides for construction in *S. cervisiae***
KELIII02A(No2_3)*	pE–YA	cDNA	*schPKS 4 fragments*: P966-P973
KELIII07*	pE–YA	KELIII02A(No2_3)	*schPKS 7 fragments*: P966+1103; P1104+1109, P1110+1079, P1080+1081, P1082+1083, P1084+1085, P1086+973
KELIII08	pE–YA	KELIII02A(No2_3)	*Patch*: P1104+1116; KELIII07 cut with KpnI
KELIII02B	pTYGSarg	cDNA	*schR1*: P975+976
KELIII02C*	pTYGSmet	cDNA	*schR1*: P975+977, *schR2*: P978+979, *schR3**: P980+981
KELIII02D	pTYGSmet	cDNA	*schR4*: P982+983, *schR5*: P984+985, *schR7*: P986+987
KELIII08A	pTYGSarg	-	LR with KELIII08 and KELIII02A
KELIII08B	pTYGSarg	-	LR with KELIII08 and KELIII02B
KELIII08C*	pTYGSarg	-	LR with KELIII08 and KELIII02C
KELIII10A	pTYGSade	KELIII08C, gDNA (*schR3*)	*schR1*: P975+977, *schR2*: P978+979, *schR3*: P980+981
KELIII10B	pTYGSade	KELIII08C	*schR1*: P975+977, *schR2*: P978+979, Patch_P*eno*: P87+88
KELIII10C	pTYGSade	KELIII08C	*schR1*: P975+977, Patch_P*gdpA*: P91+92, *schR3*: P980+981
KELIII11A	pTYGSmet	KELIII02D	Patch_P*adH*: P89+90, Patch_P*gdpA*: P91+92, *schR7*: P986+987
KELIII11B	pTYGSmet	KELIII02D	Patch_P*adH*: P89+90, *schR5*: P984+985, *schR7*: P986+987
KELIII11C	pTYGSmet	KELIII02D	*schR4*: P982+983, Patch_P*gdpA*: P91+92, *schR7*: P986+987
KELIII15	pTYGSade	KELIII10A	Patch_P*adH*: P89+90, *schR2*: P978+979, *schR3*: P980+981

*point mutations in gene

Table S10 Construction details for fungal transformation plasmids used for heterologous expression of SCH genes in *A. oryzae*

Table S11

87	CTTCTTAAATATCGTTGTAACTGTTCCTGA
88	CGAAGTATATTGGGAGACTATAGCTACTAG
89	CGAAGTATATTGGGAGACTATAGCTACTAG
90	GAGACGAAACAGACTTTTTCATCGCTAAAA
91	CTTTTCTTTTCTCTTTCTTTTCCCATCTTC
92	TGACCTCCTAAAACCCCAGTG
966	GCCAACTTTGTACAAAAAAGCAGGCTCCGCATGCCTTCGTCATACATACA
967	AGAAGTGGGTAGCACGTTCC
968	AGCCTCTCGTGACTGCATTG
969	TGCTGTAGTTGTGCTTGGAG
970	CCTCTCCAACGCCACGGTCT
971	CGTGAGCTGAAGATATTGGC
972	CACATTGGCGCCGAGATATT
973	TGCCAACTTTGTACAAGAAAGCTGGGTCGGTCACGTAACAACCATCTTGG
975	TTTCTTTCAACACAAGATCCCAAAGTCAAAATGGCGCCAACAGTCGAACC
976	GGTTGGCTGGTAGACGTCATATAATCATACCTAATTCCTTAATAAATGCT
977	TTCATTCTATGCGTTATGAACATGTTCCCTCTAATTCCTTAATAAATGCT
978	TAACAGCTACCCCGCTTGAGCAGACATCACATGGCATTACACGATATTTT
979	ACGACAATGTCCATATCATCAATCATGACCCTACTTGACGTGGAAGCGAA
980	GTCGACTGACCAATTCCGCAGCTCGTCAAAATGCTGAGCATTACAATATT
981	GGTTGGCTGGTAGACGTCATATAATCATACCTAGTCAGTGGTGAGCTTTT
982	TTTCTTTCAACACAAGATCCCAAAGTCAAAATGGTCCGAATTCTGTGTCT
983	TTCATTCTATGCGTTATGAACATGTTCCCTTCAGTCATAGTTTGCCATTG
984	AACAGCTACCCCGCTTGAGCAGACATCACCATGGAGCAACAAACCATTCT
985	ACGACAATGTCCATATCATCAATCATGACCTCATACAGCAAAAGATTGCC
986	GTCGACTGACCAATTCCGCAGCTCGTCAAAATGAACCCTTACTTTCAAAG
987	GGTTGGCTGGTAGACGTCATATAATCATACTTACAGCACTGCAGAGCTGA
1079	CAGGGACAATGCCCATGGTTACAGCAACGTCCTTGAAGTTCACACCCGCC
1080	AGCTGTACGCGGCGGGTGTGAACTTCAAGGACGTTGCTGTAACCATGGGC
1081	TCAAGAACACACTCGCAAGCGTCGATGCCTCCTCAAATGACATTGAGTCA
1082	ACCATATTCCTGACTCAATGTCATTTGAGGAGGCATCGACGCTTGCGAGT
1083	CCCTCCCGTGGCGGAATGAATCAAGACTCGATGACCCTTTTGGGTGTTTG
1084	TACGACCTGGCAAACACCCAAAAGGGTCATCGAGTCTTGATTCATTCCGC
1085	CTTCTCATCATTTCCGACGGTGGCGAATATCTCGGCGCCAATGTGCTGGC
1086	ATCCAGATTTGCCAGCACATTGGCGCCGAGATATTCGCCACCGTCGGAAA
1103	TGTAACCATCTGCTTTCTTATCAAAAGTGTTGTCCATATTATGCTCAGGG
1104	TGTACCTCAGCCCTGAGCATAATATGGACAACACTTTTGATAAGAAAGCA
1109	GTCGGGCTGGGTCACATCCAT
1110	GGGAGTCTCTCAAGGCCCTCC
1116	CGACCATGATGATACCACGGC

Table S11 Oligonucleotides used in the construction of fungal expression vectors

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