Supporting Information

Generation of Complexity in Fungal Terpene Biosynthesis: Discovery of a Multifunctional Cytochrome P450 in the Fumagillin Pathway

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Supplementary Experimental Procedures

Chemicals and chemical analysis

All solvents and other chemicals used were of analytical grade. GC-FID was composed of a model 5890A gas chromatograph (Hewlett Packard) and a model 7673A automatic injector, sampler and controller (Hewlett Packard). Samples were separated through a DB-FFAP capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness; Agilent Technologies). Oven temperature was programmed from 50 to 200^{o}C at 10^{o}C/min, with a 4 minutes hold. Injection was performed at 280^{o}C in the split ratio 1:1; 1 μL of sample was injected. A flow of 2.0 mL/min of helium was used as carrier gas. Flame ionization detection (FID) was performed at 300^{o}C.

In silico analysis

The *A. fumigatus Af*CPR gene sequence (AFUA_6G10990) is retrieved by performing BLASTP search of the *A. fumigatus* Af293 genome on the NCBI database using a cytochrome P450 reductase (e.g. *A. terreus* CPR) as query sequence. Functional domains in the translated protein sequences were predicted using Conserved Domain Search (NCBI) or InterproScan (EBI). Details for analysis of fumagillin gene cluster are in Supporting Information of Lin et al.[1] Gene structure predictions (AFUA_8G00390, 470, 480, 490 and 510) were performed using the FGENESH program (Softberry) and manually checked by comparing with homologous gene/proteins in the GenBank database using BLASTX analysis. CLUSTALW or BIOEDIT software package was used for multiple sequence alignment. Protein structure was performed with Phyre2 server.[2]

Strains

Aspergillus fumigatus strains used in this study are listed in Table S1. Strains were maintained on glucose minimum media (GMM) or Czapek-Dox medium (Difco),[3] and stored as 30% glycerol stocks at -70°C.

Generation of deletion mutants

Deletion cassettes for AFUA_8G00470, AFUA_8G00480 and AFUA_8G00510 were generated using a fusion PCR technique previously described using *Aspergillus parasiticus pyrG* as the selectable marker.[4] Briefly, approximately 1.5kb of 5'UTR and 3'UTR were PCR

amplified from each targeted loci in the *A. fumigatus* genomic DNA using the corresponding primer pairs p1 & p2 and p3 & p4. *Aspergillus parasiticus pyrG* was PCR amplified from pSD38.1using the corresponding primer pairs p5 and p6 as previously described.[1] The three initial fragments were fused together using the corresponding primer pairs p7 & p8. All primers used in this study are listed in Table S2. Polyethylene glycol mediated transformation of an *A. fumigatus akuB*^{ku80}; *pyrG1* strain was performed as described previously (Szewczyk et al., 2006).[5] Gene replacements were confirmed by PCR (data not shown) and Southern blot analysis (Figures S1-S4).

Construction of plasmids for disrupting AFUA_8G00490

For plasmid construction of pKW6213, pRS423 was used as a delivery vector (2 μg) pre-digested with *Bam* HI (10 units) and *Eco* RI (10 units) at 37 °C for 30 min. The *ptrA* gene was amplified from pPTRII[6] by PCR. The four purified fragments (a delivery vector, Rec5', Rec3' and the selectable marker *ptrA* gene), each at 50 to 150 ng in a total volume of 45 μL, were mixed with 2 μg of a suitable pre-digested delivery vector (Table S2). The mixture was transformed into *Saccharomyces cerevisiae* BY4741 for constructing the plasmid possessing deletion cassette through *in vivo* homologous recombination. These four DNA fragments were joined *in situ* by the endogenous homologous recombination activity of *S. cerevisiae* through the 25-bp homologous sequences present at the ends of those DNA fragments. The desired transformants were selected for the presence of the selection marker. Transformants were selected on a histidine-deficient plate, because pRS423 carried a *HIS3* selection marker. The resulting plasmid pKW6213 was recovered from the yeast transformant and transferred to *Escherichia coli*. The plasmid was amplified in *E. coli* for subsequent characterization by restriction enzyme digestion and PCR amplification to confirm its identity.

Construction of plasmid for *Af*470 expression in *S. cerevisiae* (pKW20202)

To construct pKW20202 for expressing *Af*470 in yeast, *Af*470 ORF was amplified by PCR using from *A. fumigatus* A1159 cDNA (45 μl) with the pKW20202-F/pKW20202-R primer set (Table S2). The DNA fragment was mixed with the delivery vector pKW1250[7] (2 μg), which was digested with *Xho* I (10 units, restriction endonuclease is from Fermentas Inc./Thermo Fisher Scientific Inc.) at 37 °C for 8 h, for *in vivo* homologous recombination. The mixture was

transformed into *S. cerevisiae* BY4705. The two fragments were joined *in situ* by the endogenous homologous recombination activity of *S. cerevisiae* through the 25-bp homologous sequences present in both DNA fragments. The desired transformants were selected for the presence of the selection marker *URA3* on a uracil-deficient plate. The resulting plasmid pKW20202 carrying the *Af*470 gene was recovered from the yeast transformant and transferred to *E. col*i XL1-Blue. The plasmid was amplified in *E. coli* for subsequent characterization by restriction enzyme digestion and DNA sequencing (Macrogen Japan Corporation) to confirm its identity.

Preparation of microsomal fraction from *Af*470-producing *S. cerevisiae*

S. cerevisiae BY4705 was transformed with pKW20202. Selected cells were grown in 4 mL of SC medium without L-uracil at 30 °C for 48 h with 180 r.p.m. The culture was transferred into 100 mL of the fresh SC medium prepared using YNB with ammonium sulfate with DO supplement without L-leucine and the culture was incubated at 30 °C for 48 h. YPD medium (500 mL) was inoculated with the culture (100 mL) and incubated at 30 °C for 15 h. Then, expression of each gene was induced with 2% of D-galactose at 30 °C, and incubation was continued for another 72 h. The cells were harvested by centrifugation at 10,000 × *g* for 10 min. All subsequent procedures were performed at 4 °C or on ice. Harvested cells were resuspended in 200 ml of TEK buffer [0.1 M KCl, 50 mM Tris-HCl (pH 7.4) and 1 mM ethylenediaminetetraacetic acid (EDTA)]. Cells were incubated at 4 °C or on ice and collected by centrifugation at 2,500 × *g*. Cells resuspended in 50 ml of TES buffer [0.6 M sorbitol, 50 mM Tris-HCl (pH 7.4) and 1 mM EDTA] supplemented with 0.2 mM phenylmethylsulfonyl fluoride were disrupted by French Press, and the lysate was clarified by centrifugation at 8,000 × *g*. Then, the supernatant was fractionated by ultracentrifugation at 100,000 × *g* for 1 h. The pellet was resuspended in 2 ml of TEG buffer [20 % (v/v) glycerol, 50 mM Tris-HCl (pH 7.4) and 1 mM EDTA] to yield a microsomal fraction.

***In vitro* transformation of 4 with *Af*470**

A solution with a total volume of 500 μL comprised of 100 μL of microsomal fraction containing *Af*470, 100 μM of **4**, 0.1 M Tris-HCl (pH 7.4) and 0.1 M NaCl was incubated at 30 °C

for 14 h. Heat-inactivated Af470 was used in reaction as a negative control. After a 14-h incubation, the reaction mixture was quenched by addition of 500 μL of ethyl acetate. The organic layer was concentrated *in vacuo*. The dried material was dissolved in 100 μL of DMF and subjected to LC–MS analysis (Figure 4A).

Revised annotation of Fma-MT

Fma-MT was misannotated as two separated genes, AFUA_8G00390 (XM_742072.1) and AFUA_8G00400 (XM_742071.1), in NCBI database. Its CDS annotation in NC_007201.1 [complement(18493..18803,18862..19297) and complement(19384..19517,19571..19871)] was revised to the actual CDS annotation [complement (18493..18802,18861..>19322,19388..> 19515,19569..19871)]. The new GenBank ID is KJ187001.

Construction of plasmid for Fma-KR expression in *S. cerevisiae*

The Fma-KR expression plasmid, pHCfmaKR, was constructed using in vivo yeast recombination cloning. The complete Fma-KR cDNA was constructed by two overlapping fragments (Table S2). The larger 5′ end exon region (2160 bp) that was predicted to contain no intron was amplified from *A. fumigatus* gDNA using primer pair Af490-fg1-F with Af490-fg1-R (fragment designated as P_{ADH2}-Af490-fg1). The 3′ end region was predicted to contain introns and was thus amplified from cDNA. RNA was extracted from a four-day old culture in CYB medium using the RiboPure Yeast Kit (Ambion) following the manufacturer's instructions and residual gDNA in the total RNA was digested with DNase (2 U/μL) (Invitrogen) at 37 ^{o}C for four hours. The 3′ Fma-KR cDNA was synthesized using ImProm-II^{TM} Reverse Transcription System with oligo-dT reverse primer (Table S4). The cDNA was used as template for PCR. The 3′ and 5′ intron-free cDNA fragments were amplified using primer pair Af490-fg2-F/Af490-fg2-R (designated as Af490-fg2-T_{ADH2}). For in vivo yeast recombination, the two overlapping fragments, P_{ADH2}-Af490-fg1 and Af490-fg2-T_{ADH2}, were combined with a PmlI/NdeI-linearized YEplac195-derived 2μ expression plasmid (containing *URA3* marker) flanking with P_{ADH2} promoter and T_{ADH2} terminator (also encode a 6×His-tag and stop codon);[8] co-transfomation of *S. cerevisiae* BJ5464-NpgA using an *S. c* EasyComp™ Transformation kit (Invitrogen) yield the expression plasmid pHCfmaKR by in vivo recombination. The resulting pHCfmaKR plasmid

was recovered by Zymoprep™ Yeast Plasmid Miniprep (Zymo Research) for propagation in *E. coli* XL1 and verified by sequencing.

Construction of plasmid for *Af*CPR expression in *S. cerevisiae*

The *Af*CPR expression plasmid, pHCfmaCPR, was constructed using in vivo yeast recombination cloning with similar method as indicated above. The complete *Af*CPR cDNA was constructed by two overlapping fragments (Table S2). The *Af*CPR cDNA was synthesized using ImProm-II™ Reverse Transcription System with oligo-dT reverse primer (Table S2). The cDNA was used as template for PCR. The 3′ and 5′ intron-free cDNA fragments were amplified using primer pairs AfCPR-fg1-F/AfCPR-fg1-R and AfCPR-fg2-F/AfCPR-fg2-R (designated as P_{ADH2}-AfCPR-fg1 and AfCPR-fg2-T_{ADH2}, respectively). For in vivo yeast recombination, the two overlapping fragments, P_{ADH2}-AfCPR-fg1 and AfCPR-fg2-T_{ADH2}, were combined with an NdeI/HindIII-linearized YEplac112-derived 2 μ expression plasmid (containing *TRP1* marker) were combined; co-transformation of *S. cerevisiae* BJ5464-NpgA using an *S. c* EasyComp™ Transformation kit (Invitrogen) yield the expression plasmid pHCfmaCPR by in vivo recombination. The resulting pHCfmaCPR plasmid was recovered by Zymoprep™ Yeast Plasmid Miniprep (Zymo Research) for propagation in *E. coli* XL1 and verified by sequencing.

Construction of plasmid for Fma-P450 expression in *S. cerevisiae*

An intron-free Fma-P450 was constructed from cDNA which was synthesized with oligo-dT reverse primer using ImProm-II™ Reverse Transcription System. The cDNA was used as template for PCR and amplified by using primer pair Af510-F and Af510-R, which contained NdeI and PmeI restriction sites, respectively. To introduce 6×His-tag to the N-terminal of Fma-P450, the PCR product was digested with NdeI and PmeI and ligated into the a NdeI/PmeI-linearized YEplac118-derived 2μ expression plasmid (containing *Leu2* marker) flanking with P_{ADH2} promoter and T_{ADH2} terminator (also encode a 6×His-tag and stop codon) to yield plasmid pHCfmaP450.

Expression, and preparation of Fma-P450- and *Af*CPR-containing microsomes for in vitro assay

For expression of pHCfmaP450 and pHCfmaCPR, the cells were grown in YPD medium supplemented with 1% dextrose at 28^{o}C with shaking for 48 hours. The microsomes were prepared according to the protocol described previously.[5] Briefly, the cells were harvested by centrifugation (3,750 rpm at 4^{o}C for 10 mins) and the cell pellet was washed with 100 mL of TES buffer (50 mM Tris–HCl, pH, 7.5, 1 mM EDTA, 0.6 M sorbitol). The cells were centrifuged as above, resuspended in 100 mL of TES-M (TES supplemented with 10 mM 2-mercaptoethanol), and allowed to incubate at room temperature for 10 min. The yeast cells were centrifuged again at 3,750 rpm for 10 min, and the pellet was resuspended in 2.5 mL of extraction buffer (1% bovine serum albumin, fraction V, 2 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, all dissolved in TES). Zirconia/silica beads (0.5 mm in diameter, Biospec Products) were added until skimming the surface of the cell suspension. Cell walls were disrupted manually by hand shaking in a cold room for 10 min at 30-s intervals separated by 30-s intervals on ice. Cell extracts were transferred to a 50-mL centrifuge tube, the Zirconia/silica beads were washed three times with 5 mL of extraction buffer, and the washes were pooled with the original cell extracts. Finally, microsomes were obtained by differential centrifugation at 10,000*g* for 10 min at 4°C to remove cellular debris followed by centrifugation at 100,000*g* for 70 min at 4°C. The microsomal pellets were weighed prior to resuspension in 1.5 mL of TEG-M buffer (50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 20% glycerol, and 1.5 mM 2-mercaptoethanol) and stored frozen at -80 °C.

In vitro assays of Fma-P450 AfCPR, and Fma-KR

For in vitro synthesis of **6**, 10 mg/mL (wet weight) microsomal fractions containing *Af*510 and *Af*CPR, 10 μM Fma-KR, 1 mM substrates **8,** 2 mM NADPH and NADPH regeneration system (BD) solution A (5 μL) and B (1 μL) in 100 mM PBS, pH 7.4, were incubated in a total 100 μL reaction. The reaction was incubated at room temperature overnight and extracted with 100 μL ethyl hexanes-acetate (1:1) twice. The organic phase was dried and dissolved in 20 μL MeOH for analysis on LC-MS.

Determination of apparent rates of 21–23 reduction by Fma-KR

To compare the apparent rate of **21**-**23** reduction by Fma-KR, we set up a time course analysis of substrate-dependent NADPH consumption. In each reaction, 1 mM substrate, 2 mM NADPH and 5 μM Fma-KR were mixed together in 100 mM PBS, pH 7.4, in a total volume of 100 μL. The mixture was incubated at 28°C and NADPH oxidation was determined at 340 nm using a spectrophotometer (Beckman Coulter DU800). The apparent rate of **21**-**23** reduction at these conditions was measured to be 2.7×10^{-3}, 1.1×10^{-2} and 2.8×10^{-3} mM/min, respectively, as calculated using the NADPH extinction coefficient 6,220 $M^{-1}cm^{-1}$.

Purification and characterization of compounds

S. cerevisiae strain BJ5464-NpgA harboring pHCfmaTC, pHCfmaP450 and pHCfmaCPR plasmid was inoculated to 12 mL Yeast Synthetic Drop-Out medium without uracil, tryptophan and leucine. The cells were grown for 72 hours with constant shaking at 28°C. A 12 mL aliquot of the seed culture was inoculated to 12 L YPD medium supplemented with 1% dextrose. The culture was shaken at 28 °C for 96 hours.

The cells were harvested by centrifugation (3750 rpm, 10 minuntes, 4°C) and the cell pellet was extracted by actetone (1200 mL) at room temperature, filtered and concentrated *in vacuo*. The acetone extract was suspended in H_2O (200 mL) and partitioned with $CHCl_3$ (200 mL × 3). The $CHCl_3$ soluble fraction (1.60 g) was separated on a silica gel column (RediSep®, 40 g Flash Column) and eluted by 0-10% acetone with flow rate of 12 mL/min (CombiFlash® Rf 200) to give 5 fractions (Fr.C1−C5). Fr.C4 was pure **8** (36.5 mg). Fr.C2 was separated with PTLC (Silica gel 60 F_{254}, 2 mm, 20 × 20 cm, glass plates, Merck) and purified by semi-preparative silica gel HPLC column (Luna® 5 μm Silica (2) 100 Å, 250 × 10 mm) eluted by 3% acetone/hexanes with flow rate of 3.5 mL/min to give **17** (9.1 mg, t_R: 13.3 min).

The medium part (12 L) was subjected with 240 g Amberlite® XAD®-2 resins (Sigma-Aldrich) and shook at 150 rpm for 1h. The resins were filtered and extracted by acetone (1500 mL) and concentrated *in vacuo*. The acetone extract was suspended in H_2O (300 mL) and partitioned with $CHCl_3$ (300 mL × 3). The $CHCl_3$ soluble fraction (1.25 g) was separated on a silica gel column (RediSep®, 40 g Flash Column) and eluted by 0-15% acetone with flow rate of 12 mL/min (CombiFlash® Rf 200) to give 12 fractions (Fr.M1−M12). A semi-preparative silica gel HPLC column (Luna® 5 μm Silica (2) 100 Å, 250 × 10 mm) was used for further purification. Fr.M3

was eluted by 3% acetone/hexanes with flow rate of 4 mL/min to give **18** (4.0 mg, t_R: 60.9 min). Fr.M4 was eluted by 9% acetone/hexanes with flow rate of 4 mL/min to give **14** (8.0 mg, t_R: 14.4 min). Fr.M5 was eluted by 7% acetone/hexanes with flow rate of 4 mL/min to give **9** (2.2 mg, t_R: 35.1 min). Fr.M7 was eluted by 9% acetone/hexanes with flow rate of 4 mL/min to give **6** (0.4 mg, t_R: 25.2 min), **10** (8.0 mg, t_R: 30.1 min), **12** (0.4 mg, t_R: 24.1 min) and **16** (0.8 mg, t_R: 32.5 min). Fr.M8 was eluted by 9% acetone/hexanes with flow rate of 4 mL/min to give **15** (3.2 mg, t_R: 37.5 min) and **19** (1.1 mg, t_R: 42.0 min). Fr.M9 was eluted by 12% acetone/hexanes with flow rate of 4 mL/min to give **11** (12.8 mg, t_R: 39.2 min) and **13** (3.0 mg, t_R: 43.2 min).

AFUA_8G00470 gene deletion mutant was cultivated by MYG liquid medium (4 L) at 30°C for 150 hours. The mycelial cake was extracted by acetone and concentrated *in vacuo*. The acetone extract was suspended in H_2O and partitioned with EtOAc. The EtOAc was fractionated by HPLC column (Wakosil-II 5C18A, 250 × 20 mm) eluted by 20−100% MeOH-H_2O within 30 min with the flow rate of 8 mL/min and further purified with HPLC column (COSMOSIL 5C_{18}-MS-II, 250 × 20 mm) eluted by 70% MeCN-H_2O with the flow rate of 8 mL/min to give **4** (25.0 mg).

AFUA_8G00480 gene deletion mutant was cultivated by MYG liquid medium (5 L) at 30°C for 150 hours. The broth part was partitioned with EtOAc (5L). The EtOAc was fractionated by silica gel column and further purified with HPLC column (COSMOSIL 5C18-MS-II, 250 × 10 mm) eluted by 48% MeCN-H_2O with the flow rate of 4 mL/min to give **5** (1.6 mg).

Basic hydrolysis of 5

5 (0.6 mg, 1.4 μmol) was added to a 2 mL of 0.5N NaOH-Et_2O stirred at room temperature for 40 min. The resultant reaction mixture was diluted with Et_2O (3mL) and H_2O (2mL). Subsequently, the aqueous layer was further extracted with Et_2O (2 x 3 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude residue was purified by reversed-phase HPLC (Cosmosil 5C_{18} MS-II, φ 10 × 250 mm, CH_3CN/H_2O = 30/70 at a flow rate of 4 mL/min) to afford 0.1 mg of **6** as a colorless solid (29%).

Supplementary Tables

Table S1. *Aspergillus fumigatus* strains used in this study

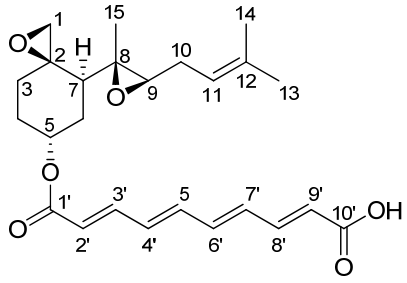
Name	Genotype	Source
ku80	*akuB*^{ku80}; *pyrG1*	gift from Robert Cramer
TSD51.1	*akuB*^{ku80}; *pyrG1, pyrG* $^{A.fumigatus}$	Lin et al., 2013
TSD55.1	*akuB*^{ku80}; *pyrG1*; ΔAfu8g00470::*pyrG* $^{A.parasiticus}$	This study
TSD56.1	*akuB*^{ku80}; *pyrG1*; ΔAfu8g00480::*pyrG* $^{A.parasiticus}$	This study
TSD57.1	*akuB*^{ku80}; *pyrG1*; ΔAfu8g00510::*pyrG* $^{A.parasiticus}$	This study
A1159	*akuA::loxP*	Purchased from FGSC
AfKW2	*akuA::loxP;* Afu8g00490::*ptrA*	This study

Table S2. Primers used in this study

Name	Sequence (5' → 3')
Afu440_p1	TCAGGCTGGGTTGAGACTGGAAACA
Afu440_p2	GTATAGTGCGATGCGGCAGACGCAG
Afu440_p3	CCGTCAATCGTAGGCGTACCCTGGA
Afu440_p4	TCTGGCGGTGGGCTCCTTTGAGGTA
Afu440_p5	CTGCGTCTGCCGCATCGCACTATACACCGGTCGCCTCAAACAATGCTCT
Afu440_p6	TCCAGGGTACGCCTACGATTGACGGGTCTGAGAGGAGGCACTGATGCG
Afu440_p7	TCTGTGACAGTGACCAAGGTGGCGG
Afu440_p8	GCCGATGCTGGTGATGGGCTTTC
Afu470_p1	CGGTGGACTTGGTGACCTTCAGACC
Afu470_p2	ACCTACGCAGCCAGCAACTCCTG
Afu470_p3	GGGAAACCCCTCCCCTCACCAACCA
Afu470_p4	CCAGGAGGACATTCCATTGCGGCCC
Afu470_p5	CAGGAGTTGCTGGCTGCGTAGGTACCGGTCGCCTCAAACAATGCTCT
Afu470_p6	TGGTTGGTGAGGGGAGGGGTTTCCCGTCTGAGAGGAGGCACTGATGCG
Afu470_p7	CCGTACATGCACACTTTCCTCCTCCCCTGC
Afu470_p8	CCCAAAACACATCAAGCGCCCGGACTGG
Afu480_p1	GGCGTCGAGGTCTTGACAGCGA
Afu480_p2	GCCACACAAGGAAACAGGACCGAC
Afu480_p3	AGGATTGCCTAACGAGCTGTCGGGG
Afu480_p4	CCTCTGTTGCCGCCACAGTCATCAG
Afu480_p5	GTCGGTCCTGTTTCCTTGTGTGGCACCGGTCGCCTCAAACAATGCTCT
Afu480_p6	CCCCGACAGCTCGTTAGGCAATCCTGTCTGAGAGGAGGCACTGATGCG
Afu480_p7	GCCGACTGGATAAGGGTCATTTGCCTG
Afu480_p8	CTTTGCGTAGCGATGCGAGAGCAGTCGG
Afu500_p1	CATTTGTGCCTCTCGCTTGGGC
Afu500_p2	CCTCCACCACCGAAGACCGATCTAG
Afu500_p3	CGGAGTCTCATCGCTCTTGCCGATG

Afu500_p4	CTGCTCCACCAGTCCAAGATAGTCC
Afu500_p5	CTAGATCGGTCTTCGGTGGTGGAGGACCGGTCGCCTCAAACAATGCTCT
Afu500_p6	CATCGGCAAGAGCGATGAGACTCCGGTCTGAGAGGAGGCACTGATGCG
Afu500_p7	GGAGGGCTATCAATGAGGCCGCTGC
Afu500_p8	GGAGACTTCTTATCCCGAGGGTGC
Afu510_p1	AGTCCATGCAACTCTTTGCCGCCATCTTGA
Afu510_p2	GGATGTGTGTCAAGTCTCTGTCTCGGATCG
Afu510_p3	TTCAACTCGGCGTTCGGAATTGTGTGAAGG
Afu510_p4	CAGCTTGGTATTACTCGGGTCCTC
Afu510_p5	CGATCCGAGACAGAGACTTGACACACATCCACCGGTCGCCTCAAACAATGCTCT
Afu510_p6	CCTTCACACAATTCCGAACGCCGAGTTGAAGTCTGAGAGGAGGCACTGATGCG
Afu510_p7	CCAACGTGCAGGAGATCGTGTTGGTGTAC
Afu510_p8	GAGTGACCGACAACCCACCCGATA
pKW6213-frg1-Fw	CTCACTATAGGGCGAATTGGGTACCTCAACCCGACCGCACTAGACC
pKW6213-frg1-Rv	CAAAGATGCAAGAGCGGCTCATCGTAGTTTGATTGAGCACGGAGATGAGC
pKW6213-frg2-Fw	GCTCATCTCCGTGCTCAATCAAACTACGATGAGCCGCTCTTGCATC
pKW6213-frg2-Rv	TTGTCGGGGGAACAGCTCAGAATCCGCAATTGATTACGGGATCCCATTG
pKW6213-frg3-Fw	CCAATGGGATCCCGTAATCAATTGCATTCTGAGCTGTTCCCCCGAC
pKW6213-frg3-Rv	CCCTCACTAAAGGGAACAAAAGCTGCGGAAGGAATGCTCAGGTCATC
pKW6213-PtrA-Fw-pos	GAACGTGCGCGTCTTCTATC
pKW6216-ptrA-Rv-pos	TGGCTGTGTCCCGTATG
Afu8g00490-Fw-2-neg	CCTCTGCCGATGATTCTCC
Afu8g00490-Rv-2-neg	GGTGGAGCAGTCGATGTAGG
Af470-pKW20202-F	TCACCATCACCATCATCATCATCATATGGATCAGTCGATGAAGCCCCTTC
Af470-pKW20202-R	TTCGCTTATTTAGAAGTGGCGCGCCTCAGTCATCTCTACCCAACCTTGCTTTAAAA
Af480-pKW20172-F1	AAGAAGGAGATATACATGACACTAAACATGGAAGACGCCCA
Af480-pKW20172-R1	TGGTGGTGGTGGTGCCCAGCGCTCACCGCAGTTGTACC
Af390400-pKW20174-F1	AAGAAGGAGATATACATGGCTGACATTGCCGAACAATTAAT
Af390400-pKW20174-R1	TGGTGGTGGTGGTGCCCTGGCAGCTCTGCCTCAATCAC
Af510-F	CATATGATGGCCTACGAGCTATCTACCCTTCAGCTTAG
Af510-R	GTTTAAACCTAGACTGTACTCGGTTTGAGAAGGCTTGCATG
AfCPR-fg1-F	ATCAACTATCAACTATTAACTATATCGTAATACCATATGGCGCAACTTGACACGCTCG
AfCPR-fg1-R	CGTTGGTAGGCCAGATAGCGATGTG
AfCPR-Fg2-F	GGCACACAATCCTTACATCGCTCC
AfCPR-Fg2-R	TGGTGGTGGTGACTCGCGACCTCATACACAAGCTTCGACCAGACATCCTCCTGGTAGC
Af490-fg1-F	CAATCAACTATCAACTATTAACTATATCGTAATACCATATGCTAGGATTGCCTAACGAGC
Af490-fg1-R	CTGGTTCGACCGTCCACAAGC
Af490-fg2-F	CCGACAAGGTTCTGCAGCTCC
Af490-fg2-R	TGTCATTTAAATTAGTGATGGTGATGGTGATGCACTATGATATGTGAAAAGAGCAAGCTC
anchored oligo-dT reverse primer	TTTTTTTTTTTTTTTTTTTTVN

Table S3. NMR data of compound **5** in $CDCl_3$. The molecular formula of **5** was established by mass data [ESI-MS: *m/z* 429 $(M+H)^+$; HRESIMS: *m/z* 429.2272 $(M+H)^+$, calcd. for $C_{25}H_{33}O_6^+$, 429.2272, Δ = 0.0 mmu].



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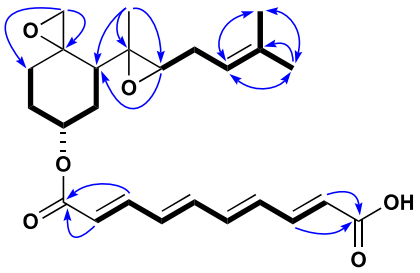
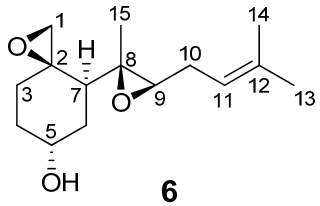


DQF-COSY
selected HMBC

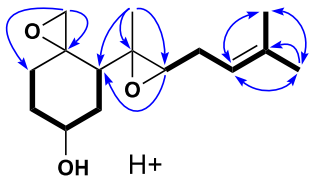
Position	δ_H [a]		mult. (*J* in Hz)	HMBC	δ_C [b]
1	2.56	1H	d (4.3)	2, 7	51.4
	2.91	1H	d (4.3)	2	
2					59.3
3	1.13	1H	m		30.3
	2.03	1H	dt (2.8, 13.0)		
4	1.94	2H	m		27.9
5	5.35	1H	m	3, 7	69.1
6	2.13	2H	m		29.9
7	1.83	1H	dd (3.7, 12.8)		43.4
8					60.7
9	2.75	1H	t (6.3)	3, 10, 11	64.3
10	2.14	1H	m	8, 9, 11, 12	27.5
	2.39	1H	m	8, 9, 11, 12	
11	5.18	1H	t (7.6)	13, 14	118.2
12					135.3
13	1.75	3H	s	11, 12, 13	25.8
14	1.65	3H	s	11, 12, 14	17.9
15	1.16	3H	s	3, 8, 9	13.7
1'					166.2
2'	5.96	1H	d (15.3)	1', 4'	123.8
3'	7.29	1H	dd (11.3, 15.3)	1', 5'	143.4
4'	6.47	1H	m		134.3
5'	6.64	1H	m		139.0
6'	6.62	1H	m		140.1
7'	6.49	1H	m		133.4
8'	7.37	1H	dd (11.3, 15.3)	6', 10'	145.5
9'	5.95	1H	d (15.3)	7', 10'	121.9
10'					170.7

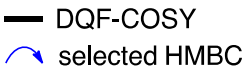
a) Recorded at 500 MHz. b) Recorded at 125 MHz.

Table S4. NMR data of compound **6** in $CDCl_3$. The molecular formula of **10** was established by mass data [ESI-MS: *m/z* 253 $(M+H)^+$; HRESIMS: *m/z* 253.1796 $(M+H)^+$, calcd. for $C_{15}H_{25}O_3^+$, 253.1798, Δ = 0.2 mmu].



6

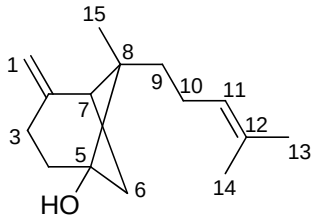




Position	δ_H[a)]		mult. (*J* in Hz)	HMBC	δ_C[b)]
1	2.86	1H	d (4.5)	2, 3	51.2
	2.53	1H	d (4.5)	2, 7	
2					59.8
3	2.24	1H	dt (4.5, 13.6))	6, 7	29.3
	1.08	1H	ddd (2.8, 3.9, 13.6)	1, 2, 3, 5, 6	
4	1.88	1H	ddd (2.8, 4.5, 13.0)		30.4
	1.79	1H	m		
5	4.31	1H	br.s	3, 7	65.8
6	1.95-2.00	2H	m		33.0
7	1.89	1H	dd (4.0, 12.5)		42.3
8					60.5
9	2.71	1H	dd (5.9, 7.0)	3, 8, 10, 11	64.3
10	2.39	1H	m	8, 9, 11, 12	27.7
	2.12	1H	m	8, 9, 11, 12	
11	5.18	1H	t (7.4)	9, 13, 14	118.5
12					135.0
13	1.75	3H	s	11, 12, 14	25.9
14	1.65	3H	s	11, 12, 13	18.1
15	1.15	3H	s	3, 8, 9	13.8

a) Recorded at 500 MHz. b) Recorded at 125 MHz.

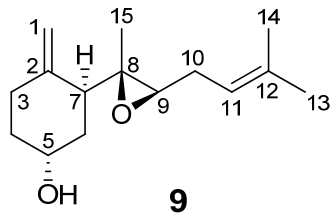
Table S5. NMR data of compound **8** in $CDCl_3$.
ESI-MS: *m/z* 203.0 $[M+H-H_2O]^+$



8

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	4.65 (br.s)	107.0 t	42.9, 25.2	2.61, 2.32, 0.78
1b	4.60 (br.s)		42.9, 25.2	2.47, 0.78
2		149.1 s		
3α	2.61 (m)	25.2 t	149.4, 42.9	4.65, 2.32, 1.77
3β	2.32 (br.dd, 18.1, 9.6)		149.4, 107.0, 76.0, 42.9, 30.4	2.61, 1.91, 0.78
4α	1.77 (dt, 11.7, 1.6)	30.4 t	149.1, 76.0, 49.1, 36.3, 25.2	2.61, 1.91
4β	1.91 (ddd, 11.7, 2.1, 5.0)		76.0, 49.1, 36.3, 25.2	2.32, 1.77, 0.78
5		76.0 s		
6α	1.83 (d,9.6)	36.3 t	149.4, 76.0, 49.3, 42.9, 30.4, 16.4	2.27
6β	2.27 (ddd, 9.6, 6.4, 2.7)		149.4, 76.0, 42.9, 30.4	1.83, 1.67
7	2.47 (d, 6.9)	42.9 d	149.4, 107.0, 76.0, 49.3, 33.2, 25.2, 30.4	2.27, 2.01, 1.67, 1.51
8		49.3 s		
9a	1.67 (m)	33.2 t	49.3, 23.2	2.27
9b	1.51 (m)		124.8, 49.3, 42.9, 23.2	2.01, 1.67, 0.78
10	2.01 (m)	23.2 t	131.6, 124.8, 49.1, 33.2	5.14, 1.67, 1.61, 1.51, 0.78
11	5.14 (br,t, 6.9)	124.8 d	33.2, 25.7, 23.2, 17.6	2.01, 1.68, 1.51
12		131.6 s		
13	1.68 (s)	25.7 q	131.6, 124.8, 17.6	5.14
14	1.61 (s)	17.6 q	131.6, 124.8, 25.7	2.01
15	0.78 (s)	16.4 q	76.0, 49.3, 42.9, 33.2	2.32, 2.01, 1.91, 1.51

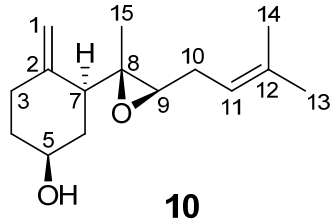
Table S6. NMR data of compound **9** in $CDCl_3$.
ESI-MS: *m/z* 218.9 $[M+H-H_2O]^+$, *m/z* 237.0 $[M+H]^+$



9

no.	δ_H (mult)	δ_C (mult)
1a	4.79 (br.s)	108.1 t
1b	4.56 (br.s)	
2		147.9 s
3α	2.40 (dd, 13.3, 4.5)	30.3 t
3β	2.12 (dt, 13.9, 4.5)	
4α	1.66 (m)	34.1 t
4β	1.74 (dtd, 13.6, 4.5, 2.0)	
5	4.20 (br.s)	66.0 d
6α	1.70 (m)	35.8 t
6β	1.89 (dtd, 13.3, 4.6, 2.0)	
7	2.18 (m)	46.0 d
8		61.3, s
9	2.77 (t, 6.5)	63.1 d
10a	2.35 (m)	27.9 t
10b	2.20 (m)	
11	5.18 (br.t, 7.2)	118.8 d
12		134.3 s
13	1.71 (br.s)	25.7 q
14	1.63 (br.s)	18.0 q
15	1.38 (br.s)	13.8 s

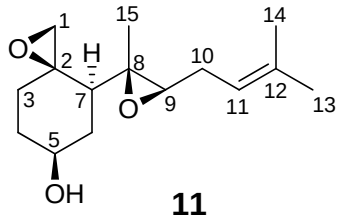
Table S7. NMR data of compound **10** in $CDCl_3$.
ESI-MS: *m/z* 218.9 $[M+H-H_2O]^+$, *m/z* 237.0 $[M+H]^+$



10

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	4.80 (br.s)	108.6 t	146.5, 108.6, 49.6, 33.0	4.58, 2.30
1b	4.58 (br.s)		146.5, 49.6, 33.0	4.80, 1.26
2		146.5 s		
3α	2.02 (m)	33.0 t	146.5, 108.6, 69.4, 36.1	3.74, 1.75, 1.44
3β	2.30 (br.dt, 14.1, 2.5)		146.5, 108.6, 69.4, 61.2, 49.6, 36.1	4.80, 2.02, 1.36
4α	1.44 (br.d, 11.4)	36.1 t	146.5, 69.4, 61.2, 49.6, 36.1	
4β	1.36 (dr.d, 11.4)		146.5, 69.4, 36.1	2.30, 1.95
5	3.74 (br.t, 9.4)	69.4 d		2.02, 1.75
6α	2.14 (br.t, 6.4)		146.5, 69.4, 49.6, 36.1	1.75
6β	1.95 (ddd, 9.6, 2.0, 4.0)	37.2 t	146.5, 69.4	1.36
7	1.75 (br.d, 12.2)	49.6 d	146.5, 108.6, 69.4, 61.2, 37.2, 13.3	3.74, 2.73, 2.14, 2.02
8		61.2 s		
9	2.73 (t, 6.4)	63.6 d	118.5, 61.2, 49.6, 27.7	1.75
10a	2.34 (br.dt, 14.2, 4.5)	27.7 t	134.5, 118.5, 63.6	2.20
10b	2.20 (br.dt, 14.2, 7.2		134.5, 118.5, 63.6	2.34
11	5.17 (br,t, 6.9)	118.5 d	63.6, 25.7, 18.0	2.20, 1.70
12		134.5 s		
13	1.70 (s)	25.7 q	134..5, 118.5, 18.0	5.17
14	1.62 (s)	18.0 q	134.5, 118.5, 25.7	
15	1.26 (d, 1.9)	13.3 q	61.2, 49.6	4.58

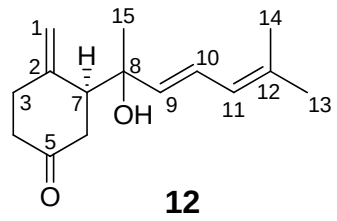
Table S8. NMR data of compound **11** in $CDCl_3$.
ESI-MS: *m/z* 234.9 $[M+H-H_2O]^+$, *m/z* 252.9 $[M+H]^+$



11

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	2.84 (d, 4.4)	50.5 t	58.4, 46.7, 32.8	2.53, 1.14
1b	2.53 (d, 4.4)		58.4, 46.7, 32.8	2.84
2		58.4 s		
3α	1.95 (br.d, 13.2)	32.8 t	69.6, 58.4, 32.6	3.71, 1.25, 1.63
3β	1.25 (br.dt, 14.3, 3.6)		69.6, 58.4, 46.7, 32.6	1.95, 1.82
4α	1.63 (m)	32.6 t	69.6, 32.8	1.95
4β	1.82 (br.dt, 13.8, 4.3)		69.6, 58.4	1.25
5	3.71 (br.tt, 10.5, 1.4)	69.6 d	32.6, 32.8	1.95, 1.39
6α	1.74 (m)	35.1 t	69.6, 58.4, 46.9, 32.6	2.17
6β	2.17 (br.ddd, 11.8, 6.2, 3.8)		69.6, 58.4, 46.9, 32.6	1.74, 1.14
7	1.39 (dd, 12.8, 3.6)	46.7 d	64.2, 60.3, 35.1, 13.5	2.64
8		60.3 s		
9	2.64 (dd, 7.2, 5.8)	64.2 d	118.2, 60.3, 46.7, 27.5	5.13, 1.39
10a	2.37 (br.dt, 14.5, 6.4)	27.5 t	135.0, 118.2, 64.2, 60.3	5.13
10b	2.08 (br.dt, 14.5, 7.5)		135.0, 118.2, 64.2, 60.3	5.13
11	5.13 (br.t, 7.5)	118.2 d	25.7, 18.0	2.64, 2.37, 2.08, 1.71
12		135.0 s		
13	1.71 (s)	25.7 q	135.0, 118.2, 18.0	5.13
14	1.62 (s)	18.0 q	135.0, 118.2, 25.7	
15	1.14 (s)	13.5 q	64.2, 60.3, 46.7	2.84, 2.17

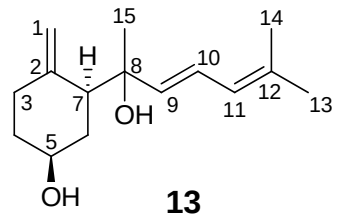
Table S9. NMR data of compound **12** in $CDCl_3$.
ESI-MS: *m/z* 216.9 $[M+H-H_2O]^+$



12

no.	δ_H (mult)	δ_C (mult)
1a	4.93 (br.s)	112.0 t
1b	4.87 (br.s)	
2		145.8 s
3α	2.30−2.60 (m)	30.9 t
3β	2.30−2.60 (m)	
4α	2.30−2.60 (m)	41.5 t
4β	2.30−2.60 (m)	
5		210.8 s
6α	2.30−2.60 (m)	45.1 t
6β	2.67 (dd, 15.1, 7.7)	
7	3.18 (br.d, 7.0)	50.7d
8		71.0 s
9	5.79 (d, 15.3)	141.1 d
10a	5.84 (br.d, 10.9)	125.8 s
11	6.44 (dd, 15.5, 10.6)	122.4 d
12		138.0 s
13	1.33 (br.s)	29.9 q
14	1.33 (br.s)	29.9 q
15	1.73 (s)	15.7 q

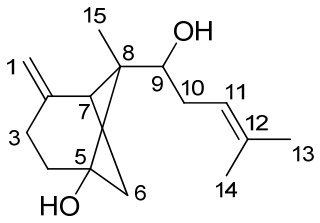
Table S10. NMR data of compound **13** in $CDCl_3$.
ESI-MS: *m/z* 218.9 $[M+H-H_2O]^+$



13

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	4.75 (br.s)	108.6 t	47.2, 30.2	4.52, 2.37, 2.15
1b	4.52 (br.s)		47.2, 30.2	4.74, 1.76
2		149.4 s		
3α	2.37 (ddd, 14.1, 9.8, 4.9)	30.2 t	149.4, 108.6, 66.6, 47.2, 35.4	4.52, 2.15, 1.72
3β	2.15 (m)		149.4, 108.6, 66.6, 47.2, 35.4	4.75, 2.37
4α	1.72 (m)	35.4 t	149.4, 66.6	4.10
4β	1.67 (m)		149.4, 66.6, 38.4	
5	4.10 (br.ddd, 9.0, 6.0, 3.2)	66.6 d		1.72
6α	1.72 (m)	38.4 t	149.4, 66.6, 47.2, 35.4	4.10, 1.95
6β	1.95 (br.ddd, 13.0, 9.3, 3.1)		149.2, 47.2, 35.4	1.72
7	3.09 (dd, 9.3, 4.5)	47.2 d	149.2, 139.8, 125.1, 10.6, 66.6, 38.4, 30.2, 16.2	5.85, 2.37, 1.76
8		70.9 s		
9	5.74 (d, 15.3)	139.8 d	125.1, 70.9, 29.9	6.48
10	5.85 (br.d, 10.8)	125.1 d	138.9, 122.9, 47.2, 16.2	4.52, 3.09, 1.95, 1.72
11	6.48 (dd, 15.3, 10.7)	122.9 d	139.8, 125.1, 70.9	5.74, 1.76, 1.33
12		138.9 s		
13	1.33 (s)	29.9 q	139.8, 70.9, 29.9	6.48
14	1.33 (s)	29.9 q	139.8, 70.9, 29.9	5.85
15	1.76 (s)	16.2 q	139.8, 125.1, 47.2	4.52, 3.09

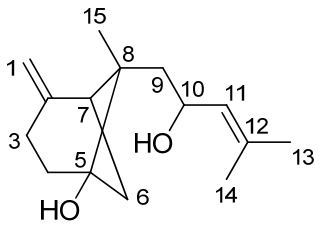
Table S11. NMR data of compound **14** in $CDCl_3$.
ESI-MS: *m/z* 218.9 $[M+H-H_2O]^+$



14

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	4.68 (br.s)	107.7 t	42.1, 25.2	2.61, 2.36, 0.84
1b	4.60 (br.s)		42.1, 25.2	
2		148.0 s		
3α	2.61 (ddd, 10.9, 7.3, 2.6)	25.2 t	148.0, 107.7, 31.5	4.68, 2.33, 1.89, 1.80
3β	2.33 (br.d, 10.9)		148.0, 76.6	2.61
4α	1.80 (br.t, 12.3)	31.5t	148.0, 76.6, 52.2, 36.4, 25.2	2.61
4β	1.94 (m)		36.4	0.84
5		76.6 s		
6α	1.89 (d, 9.8)	36.4 t	148.0, 76.6, 52.2, 42.1, 31.5, 11.4	2.61, 2.41
6β	2.41 (ddd, 9.8, 6.8, 2.1)		148.0, 76.6, 42.1, 31.5	
7	2.36 (d, 6.8)	42.1 d	148.0, 107.7, 76.6, 74.3, 52.2, 25.2	1.89, 0.84
8		52.2 s		
9	4.30 (d, 10.0, 2.5)	74.3 d	121.0, 11.4	5.23, 2.41, 2.20, 2.05, 0.84
10a	2.20 (m)	31.2 t	135.3, 121.0, 42.1	5.23, 4.30, 1.65, 0.84
10b	2.05 (br.dd, 13.7, 5.3)		135.3, 121.0	5.23, 4.30, 0.84
11	5.23 (br.t, 7.3)	121.0 d	26.0, 18.0	4.30, 2.20, 2.05, 1.74
12		135.3 s		
13	1.74 (br.s)	26.0 q	135.0, 121.0, 18.0	5.23
14	1.65 (br.s)	18.0 q	135.0, 121.0, 26.0	2.20
15	0.84 (s)	11.4 q	76.6, 74.3, 52.2, 42.1	2.36, 2.20, 2.05, 1.94

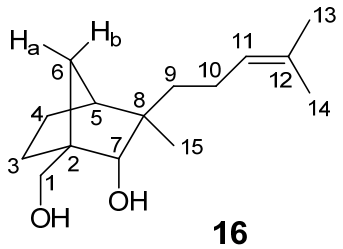
Table S12. NMR data of compound **15** in $CDCl_3$.
ESI-MS: *m/z* 218.9 $[M+H-H_2O]^+$



15

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	4.65 (br.s)	107.0 t	149.6, 44.1, 25.2	2.62, 2.31
1b	4.62 (br.s)		44.1, 25.2	2.63
2		149.6 s		
3α	2.62 (m)	25.2 t	149.6	2.31, 1.78
3β	2.31 (ddd, 17.9, 9.5, 0.9)		149.6, 107.0, 75.2, 44.1, 30.6	4.65, 2.62, 1.89
4α	1.78 (ddd, 12.9, 11.4, 1.5)	30.6 t	149.6, 75.2, 49.2, 36.1, 25.2	2.62, 1.89
4β	1.89 (m)		30.6	2.31, 1.78, 0.81
5		75.2 s		
6α	1.85 (d, 9.8)	36.1 t	149.6, 75.2, 49.2, 44.1, 30.6, 18.7	2.63, 2.39
6β	2.39 (ddd, 9.8, 6.9 2.2)		149.2, 75.2, 44.1, 30.6	4.67, 2.63, 1.96, 1.85
7	2.63 (d, 6.7)	44.1 d	149.6, 107.0, 75.2, 49.2, 42.4, 36.1, 30.6, 25.2	4.67, 4.62, 2.39
8		49.2 s		
9a	1.96 (dd, 14.8, 8.4)	42.4 t	129.0, 75.2, 66.9, 49.2, 44.1,18.7	5.29, 4.67, 2.39
9b	1.66 (br.dd, 14.8, 3.3)		75.2, 49.2, 44.1, 18.7	4.67, 0.81
10	4.67 (dt, 8.6, 3.2)	66.9 d	134.1, 49.2	2.63, 2.39, 1.96, 1.69, 1.66, 0.81
11	5.29 (br.dt, 8.7, 1.4)	129.0 d	42.4, 25.7, 18.1	4.67, 1.96, 1.70
12		134.1 s		
13	1.69 (d, 1.0)	18.1 q	134.1, 129.0, 25.7	4.67
14	1.70 (d, 1.0)	25.7 q	134.1, 129.0, 18.1	5.29
15	0.81 (s)	18.7 q	75.2, 49.2, 44.1, 42.4	4.67, 2.31, 1.96, 1.89, 1.66

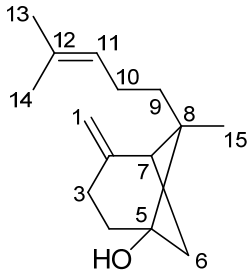
Table S13. NMR data of compound **16** in $CDCl_3$.
ESI-MS: *m/z* 239.0 $[M+H]^+$



16

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	3.82 (d, 10.6)	102.5 t	82.6, 53.7, 22.0	1.44, 1.17, 1.07
1b	3.74 (d, 10.6)		82.6, 53.7, 22.0	1.44, 1.07
2		53.7 s		
3α	1.87 (m)	22.0 t	82.6, 32.4	1.17, 0.86
3β	1.17 (m)		68.6	3.82, 3.74, 1.87
4α	1.72 (m)	25.7 t		1.45, 0.86
4β	1.45 (m)			1.72, 1.07
5	1.87 (m)	46.2 d	82.6, 32.4, 22.0	1.45, 1.44
6a	1.07 (dd, 10.0, 1.5)	36.4 t	82.6, 53.7, 46.2, 42.4, 25.7	3.82, 3.74, 1.45, 1.44
6b	1.44 (m)		42.4, 25.7, 22.0	3.82, 3.74, 3.62, 1.87, 1.29, 1.07
7	3.62 (br.s)	82.6 d	68.4, 42.6, 22.0	1.94, 1.44, 1.29, 1.17
8		42.4 s		
9	1.29 (m)	42.6 t	124.8, 82.6, 46.2, 22.8, 16.3	5.07, 3.62, 1.94, 1.87, 1.44
10	1.94 (m)	22.8 t	131.3, 124.8, 42.6	5.07, 3.62, 1.29, 0.86
11	5.07 (br.t, 7.3)	124.8 d	25.7, 22.8, 17.6	1.94, 1.69, 1.29
12		131.3 s		
13	1.67 (br.s)	17.6 q	131.3, 124.8, 17.6	5.07
14	1.59 (br.s)	25.7 q	131.3, 124.8, 25.7	
15	0.86	16.3 q	82.6, 46.2, 42.6	3.62, 1.94, 1.87, 1.72

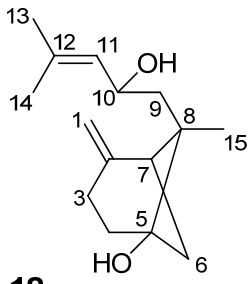
Table S14. NMR data of compound **17** in $CDCl_3$.
ESI-MS: *m/z* 203.0 $[M+H-H_2O]^+$



17

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	4.83 (br.s)	106.0 t	153.2, 29.0, 27.7, 13.2	2.18, 2.05, 1.80
1b	4.65 (br.s)		153.2, 29.0, 27.7, 13.2	2.18, 2.05
2		153.2 s		
3α	2.05 (m)		153.2, 102.7, 72.2, 40.0, 28.3, 27.7	2.18, 1.69
3β	2.18 (dd, 15.9, 9.0)	29.0 t	153.2, 102.7, 40.0, 28.3	4.65, 2.05, 1.91
4α	1.69 (d, 9.0)		153.2, 40.0, 13.2	2.05,1.91
4β	1.91 (br.dd, 21.1, 10.6)	27.7 t	72.5, 40.0, 29.0, 13.2	2.18, 1.69, 1.64, 1.13
5		72.5 s		
6α	0.61 (dd, 4.3, 3.7)		153.2, 72.5, 40.0, 27.7	2.05, 1.69, 0.97
6β	0.97 (ddd, 8.5, 4.6, 1.4)	13.2 t	153.2, 72.5, 40.0, 27.7	1.80, 1.13, 0.61
7	1.80 (dd, 8.7, 3.4)	28.3 d	153.2, 106.0, 72.5, 29.0	
8		40.0 s		
9	1.64 (m)	40.0 t	72.5, 40.0, 22.6	2.04, 1.91, 1.13
10	2.04 (m)	22.6 t	131.9, 124.3	5.10, 1.81, 1.64, 1.60, 1.13
11	5.10 (br,t, 7.1)	124.5 d	40.0, 25.7, 22.6, 17.7	2.04, 1.67, 1.13
12		131.9 s		
13	1.67 (br.s)	25.7 q	131.9, 124.5, 17.7	5.10
14	1.60 (br.s)	17.7 q	131.9, 124.5, 25.7	2.04
15	1.13 (s)	26.0 q	72.5, 40.0	2.04, 1.80, 1.64, 0.97

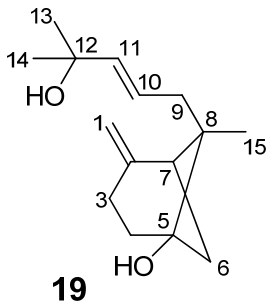
Table S15. NMR data of compound **18** in $CDCl_3$.
ESI-MS: *m/z* 218.9 $[M+H-H_2O]^+$



18

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	4.88 (br.s)	102.7 t	153.2, 29.4	4.68, 2.02
1b	4.68 (br.s)		153.2, 29.4	4.88, 2.20
2		153.3 s		
3α	2.11 (m)		153.3, 28.5	1.66
3β	2.20 (m)	29.4 t	29.4	4.68
4α	1.66 (m)	28.5 t	29.4, 10.8	2.17, 2.11
4β	2.17 (m)		153.3, 39.1, 29.4, 10.8	1.72, 1.66
5		72.2 s		
6α	0.54 (bt.t, 3.8)	10.8 t	153.3, 39.1	1.14
6β	1.14 (dd, 8.5, 4.0)		153.3, 39.1	0.54
7	2.02 (dd, 8.5, 2.8)	29.4 d	72.2, 29.4	4.88
8		39.1 s		
9a	1.84 (dd, 15.1, 1.7)	44.5 t	72.2, 29.4, 28.3	1.22
9b	1.72 (dd, 15.1, 11.1)		72.2, 67.0, 39.1, 29.4, 28.3	2.17, 1.22
10	4.72 (br.t, 9.9)	67.0 d	135.2, 44.5	1.84, 1.72, 1.62
11	5.17 (br,d, 8.7)	127.7 d	67.0, 25.7, 18.0	4.72, 1.72, 1.68
12		135.0 s		
13	1.68 (br.s)	25.7 q	135.0, 127.7	5.17
14	1.62 (br.s)	18.0 q	135.0, 127.7	4.72, 2.11
15	1.22 s	28.3 q	72.5, 44.5, 39.1, 29.4	1.84, 1.72

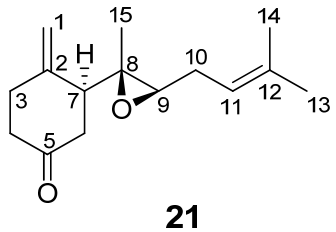
Table S16. NMR data of compound **19** in $CDCl_3$.
ESI-MS: *m/z* 218.9 $[M+H-H_2O]^+$



19

no.	δ_H (mult)	δ_C (mult)	HMBC	NOESY
1a	4.82 (br.s)	102.5 t	29.1, 29.0	1.76, 1.30, 1.28
1b	4.66 (br.s)		29.1, 29.0	2.19
2		153.7 s		
3α	2.05 (m)			2.19, 1.69, 0.57
3β	2.19 (m)	29.0 t	153.7, 102.5	2.05, 1.91
4α	1.69 (m)		153.7	2.05, 1.91
4β	1.91 (br.dd, 21.0, 10.2)	27.8 t	39.9, 12.5	5.65, 2.45, 2.24, 2.19, 1.69
5		72.0 s		
6α	0.57 (br.t, 4.0)		153.7, 27.8	1.00, 2.05
6β	1.00 (dd, 8.8, 4.5)	12.5 t	153.7, 39.9, 72.0	1.76, 1.18, 0.57
7	1.76 (m)	29.1 d	43.1, 29.0	1.18, 1.00
8		39.9 s		
9a	2.45 (dd, 15.1, 5.9)	43.1 t	141.6, 122.5, 72.0, 39.9, 26.5	5.65, 1.91, 1.18
9b	2.24 (m)		141.6, 122.5, 72.0, 39.9	5.65, 1.91, 1.18
10	5.65 (m)	122.5 t	70.6, 43.1	2.45, 2.24, 1.76, 1.18
11	5.67 (m)	141.6 d	70.6, 43.1	1.30, 1.28
12		70.6 s		
13	1.30 (br.s)	29.5 q	70.6, 29.6	5.67
14	1.28 (br.s)	29.6 q	70.7, 29.6	5.67
15	1.18 (s)	26.5 q	72.0, 43.1, 39.9	5.65, 2.45, 2.24, 1.76, 1.00

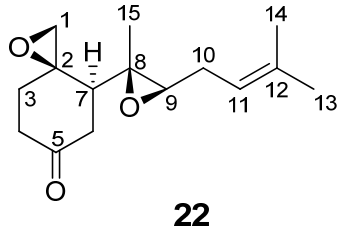
Table S17. NMR data of compound **21** in $CDCl_3$.
ESI-MS: *m/z* 216.9 $[M+H-H_2O]^+$, *m/z* 235.0 $[M+H]^+$



21

no.	δ_H (mult)	δ_C (mult)
1a	5.05 (br.s)	112.3 d
1b	4.91 (br.s)	
2		144.6 s
3α	2.29−2.51 (m)	31.8 t
3β	2.29−2.51 (m)	
4α	2.29−2.51 (m)	40.4 t
4β	2.67 (m)	
5		209.6 s
6α	2.29−2.51 (m)	41.7 t
6β	2.29−2.51 (m)	
7	2.29−2.51 (m)	48.8 d
8		61.4 s
9	2.86 (br.t, 6.4)	60.9 d
10a	2.29−2.51 (m)	27.8 t
10b	2.14 (br.dt, 14.7, 7.5)	
11	5.14 (br.t, 7.3)	118.4 d
12		134.7 s
13	1.71 (br.s)	25.7 q
14	1.63 (br.s)	18.0 q
15	1.25 (s)	14.3 q

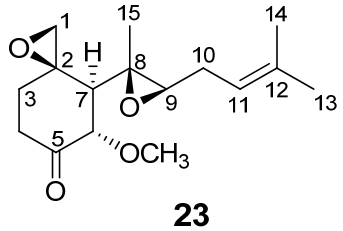
Table S18. NMR data of compound **22** in $CDCl_3$.
ESI-MS: *m/z* 232.9 $[M+H-H_2O]^+$, *m/z* 250.9 $[M+H]^+$



22

no.	δ_H (mult)	δ_C (mult)
1a	2.92 (d, 4.5)	51.5 t
1b	2.73 (d, 4.5)	
2		58.4 s
3α	1.99 (ddd, 13.6, 10.4, 5.9)	32.3 t
3β	2.35−2.73 (m)	
4α	2.35−2.73 (m)	38.6 t
4β	2.35−2.73 (m)	
5		208.3 s
6α	2.35−2.73 (m)	40.9 t
6β	2.35−2.73 (m)	
7	1.82 (dd, 10.7, 4.6)	47.6 d
8		60.1 s
9	2.35−2.73 (m)	62.2 d
10a	2.10 (br.dt, 14.8, 7.4)	27.5 t
10b	1.88 (ddd, 13.6, 6.5, 5.9)	
11	5.14 (br.t, 7.4)	118.1 d
12		135.1 s
13	1.73 (br.s)	25.8 q
14	1.63 (br.s)	18.0 q
15	1.24 (s)	13.8 q

Table S19. NMR data of compound **23** in $CDCl_3$.
ESI-MS: *m/z* 280.9 $[M+H]^+$



23

no.	δ_H (mult)	δ_C (mult)
1a	3.04 (d, 4.4)	53.7 d
1b	2.71 (d, 4.4)	
2		58.5 s
3α	2.04 (ddd, 13.6, 12.3, 5.4)	33.2 t
3β	1.69 (ddd, 13.7, 6.5, 4.0)	
4α	2.50 (ddd, 14.1, 5.4, 4.4)	36.9 t
4β	2.67 (ddd, 14.0, 6.6, 1.1)	
5		207.1 s
6	4.07 (dd, 10.6, 1.0)	83.2 d
7	1.86 (d, 10.5)	51.9 d
8		58.7 s
9	2.59 (br.t, 6.4)	60.5 d
10a	2.38 (br.dt, 14.5, 6.7)	27.4 t
10b	2.13 (br.dt, 15.0, 7.3)	
11	5.17 (br.t, 7.6)	118.3 d
12		135.1 s
13	1.73 (br.s)	25.7 s
14	1.63 (br.s)	18.0 s
15	1.27 (s)	13.9 s
-OMe	3.49 (s)	58.6 s

Supplementary Figures

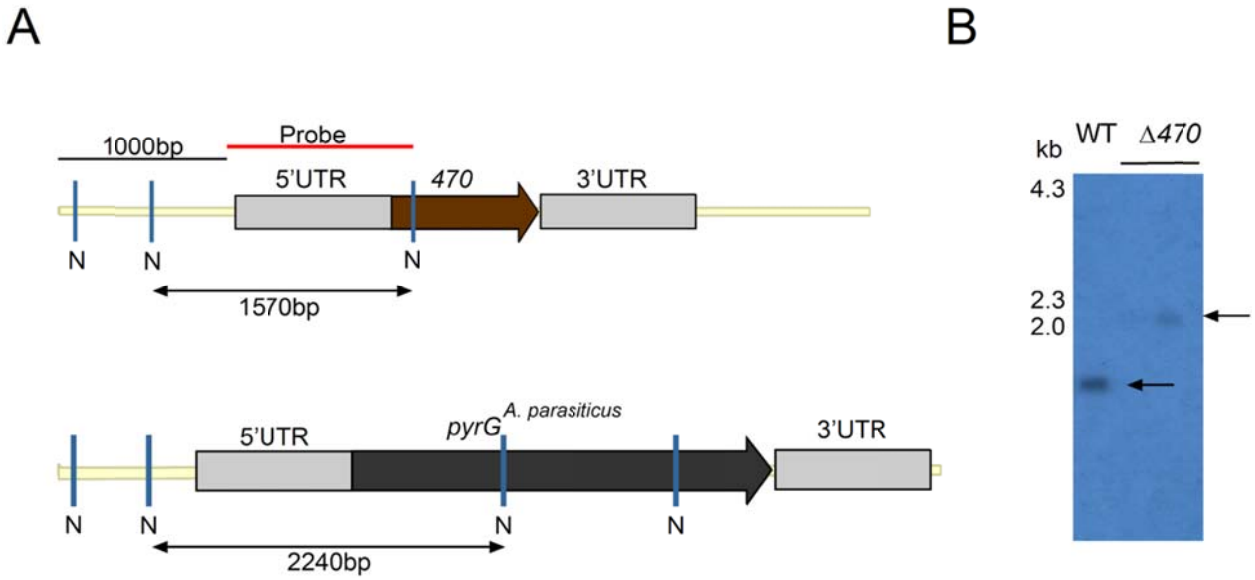


Figure S1. Targeted Afu8g00470 deletion. A) Diagram showing *Nco*I (N) sites in the *A. fumigatus* wild-type Afu8g00470 locus (indicated as 470) and modified locus after gene replacement of Afu8g00470 by the *A. parasiticus pyrG* gene. The fragment used as probe template for Southern blot analysis is indicated. Expected *Nco*I band sizes as also shown. B) Southern blot analysis. *Nco*I digested genomic DNA of control and transformant strain was hybridized with the indicated probe (A), containing 5'UTR of Afu8g00470.

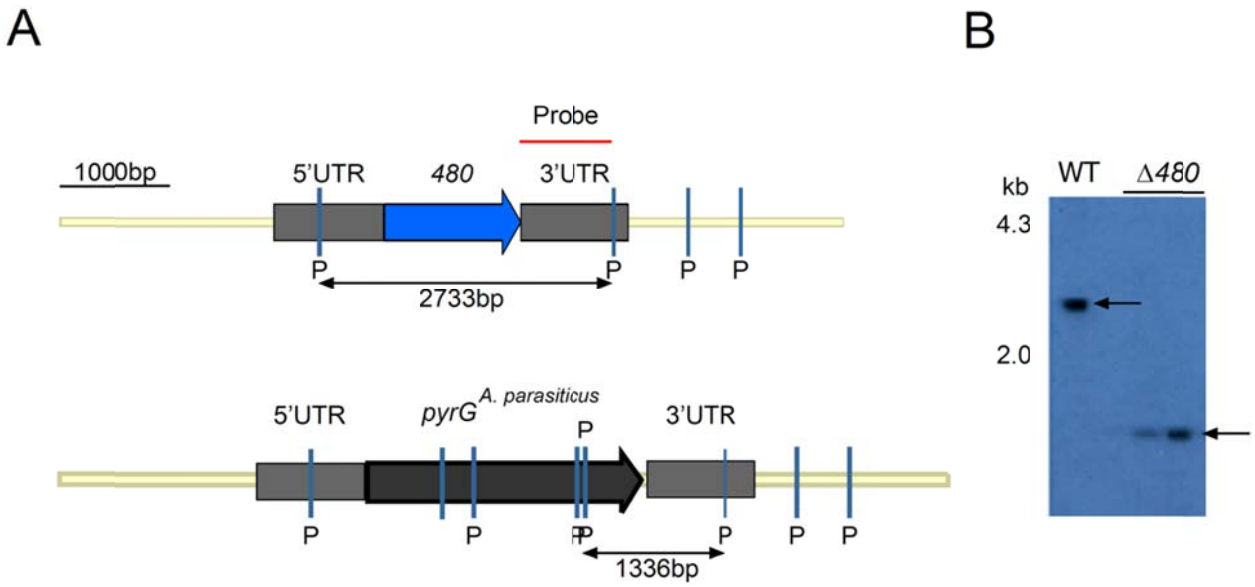


Figure S2. Targeted Afu8g00480 deletion. A) Diagram showing *Pst*I (P) sites in the *A. fumigatus* wild-type Afu8g00480 locus (indicated as 480) and modified locus after gene replacement of Afu8g00480 by the *A. parasiticus pyrG* gene. The fragment used as probe template for Southern blot analysis is indicated. Expected *Pst*I band sizes as also shown. B) Southern blot analysis. *Pst*I digested genomic DNA of control and transformant strain was hybridized with the indicated probe (A), containing 3'UTR of Afu8g00480.

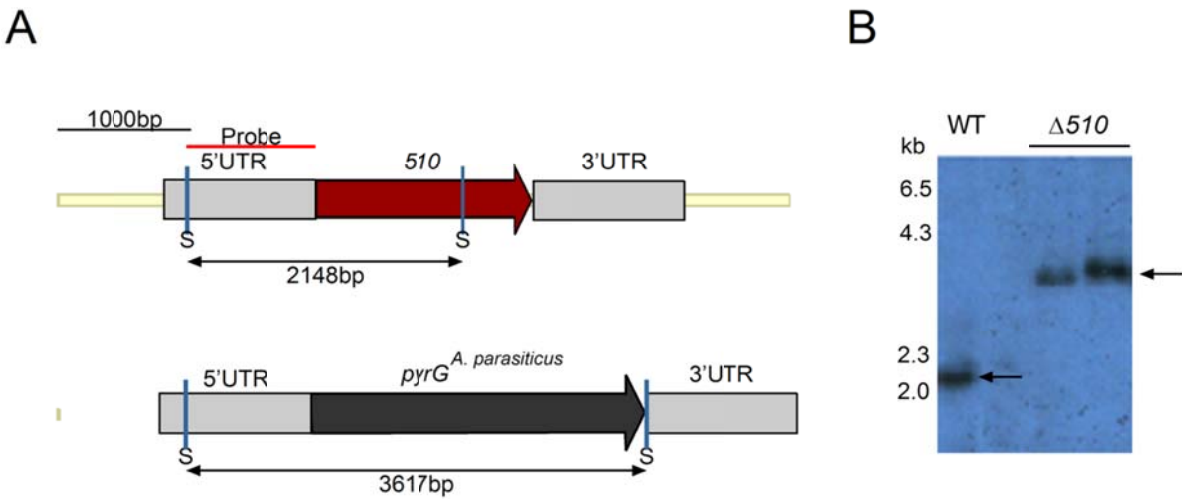


Figure S3. Targeted Afu8g00510 deletion. A) Diagram showing *Sal*I (S) sites in the *A. fumigatus* wild-type Afu8g00510 locus (indicated as 510) and modified locus after gene replacement of Afu8g00510 by the *A. parasiticus pyrG* gene. The fragment used as probe template for Southern blot analysis is indicated. Expected *Sal*I band sizes as also shown. B) Southern blot analysis. *Sal*I digested genomic DNA of control and transformant strain was hybridized with the indicated probe (A), containing 5'UTR of Afu8g00510.

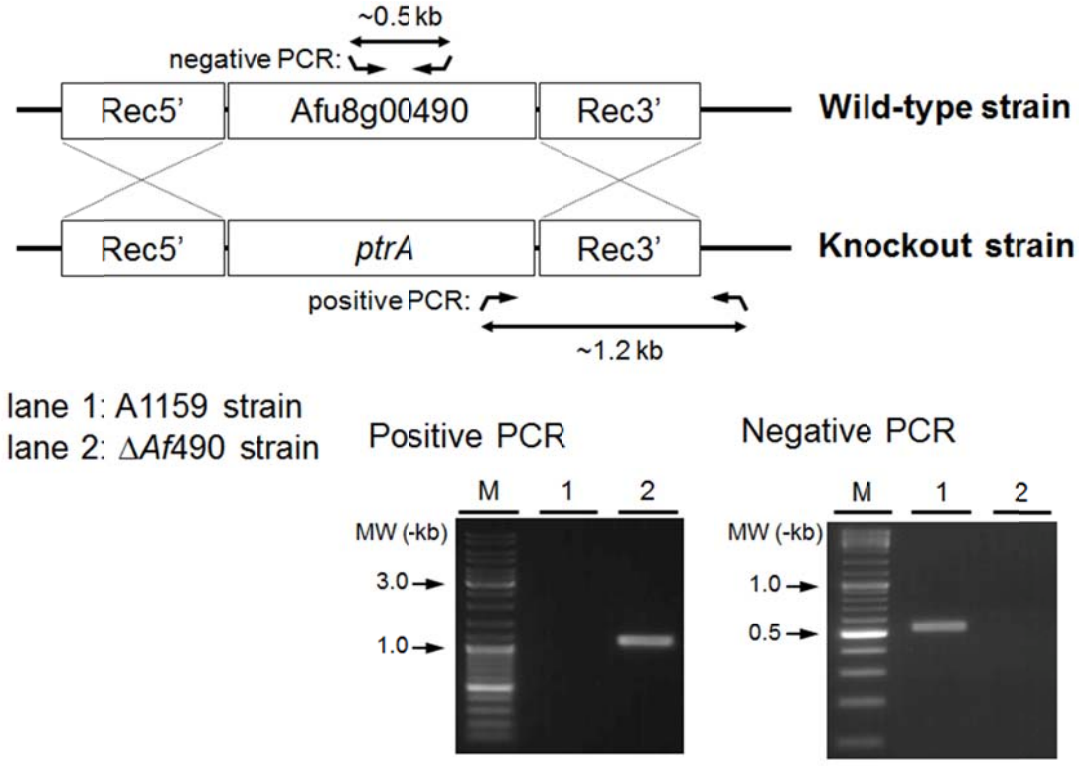


Figure S4. Targeted Afu8g00490 deletion.

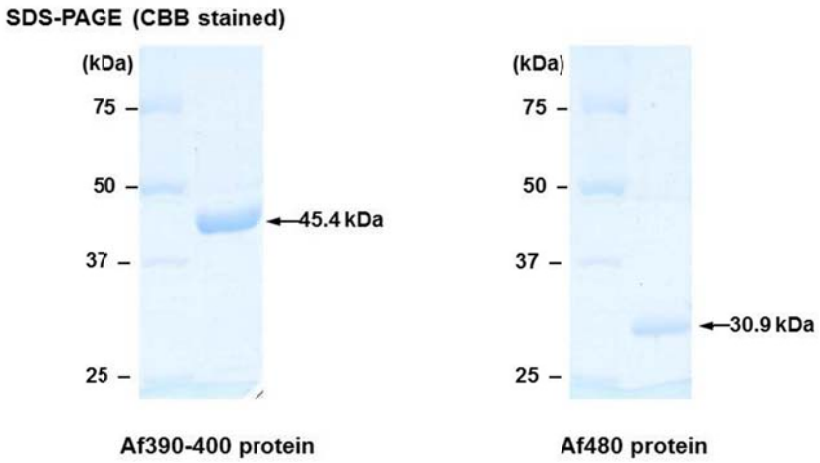


Figure S5. Recombinant Fma-MT (Af390-400) (45.4 kDa) and Fma-C6H (Af480) (30.9 kDa) purified from *E. coli*.

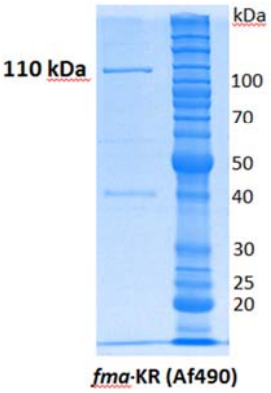
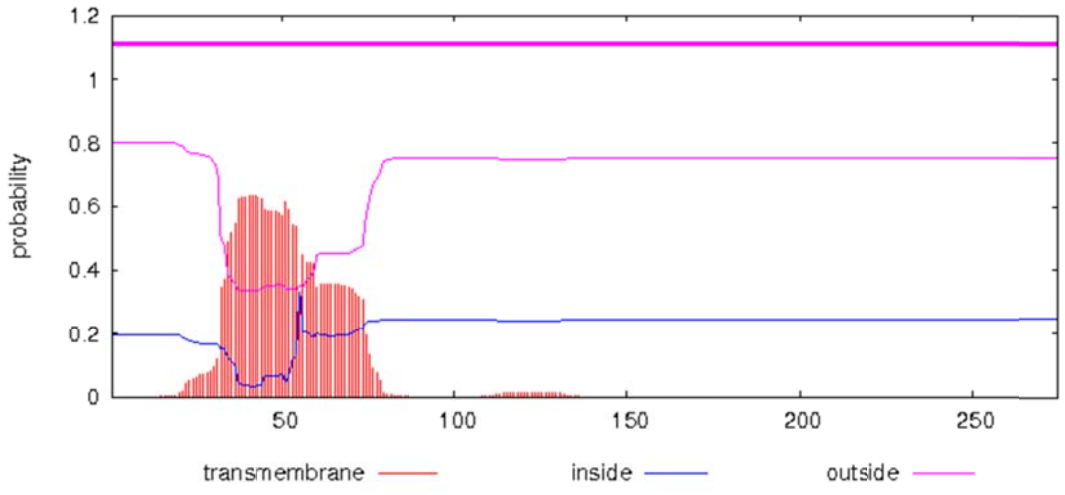
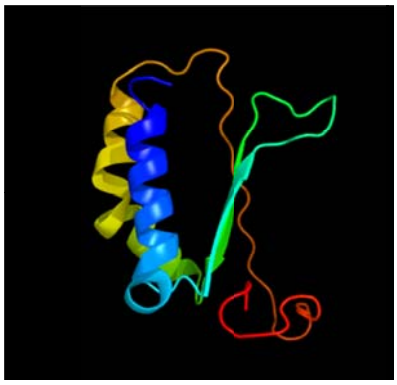


Figure S6. Recombinant Fma-KR (Af490) (110 kDa) from *S. cerevisiae*.

(A)



(B)



Homology model based on template c3a16C_: Chain C, aldoxime dehydratase (OxdRE) from *Rhodococcus sp*. N-771.

103 residues (37% of Af470 (*fma*-ABM) 275 residues protein sequence, aligned region shown above) have been modeled with 99.1% confidence by the single highest scoring template.

Figure S7. Protein structure prediction and analysis of Fma-ABM (Af470). (**A**) Transmembrane helices prediction of Fma-ABM with the TMHMM server (www.cbs.dtu.dk/services/TMHMM/). (**B**) Homology model of *fma*-ABM generated with Phyre2 protein fold recognition server (www.sbg.bio.ic.ac.uk/phyre2/) based on the top hit, *Rhodococcus sp*. N-771 aldoxime dehydratase (PDB_ID c3a16C_), as template.

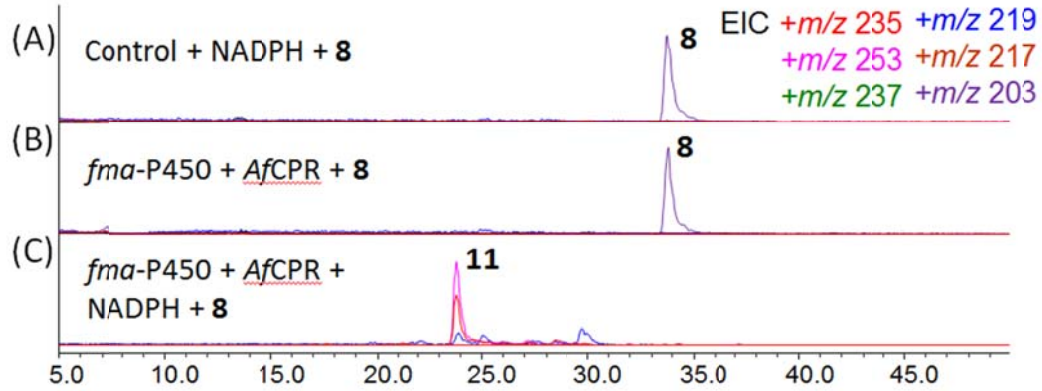


Figure S8. LC-MS analysis of in vitro assays of yeast microsomes containing *fma*-P450 and *Af*CPR with **8**. (**A**) Yeast microsome extracts and **8** with NADPH and (**B**) without NADPH (**C**) Yeast microsome extracts containing *fma*-P450 and *Af*CPR and **8** with NADPH.

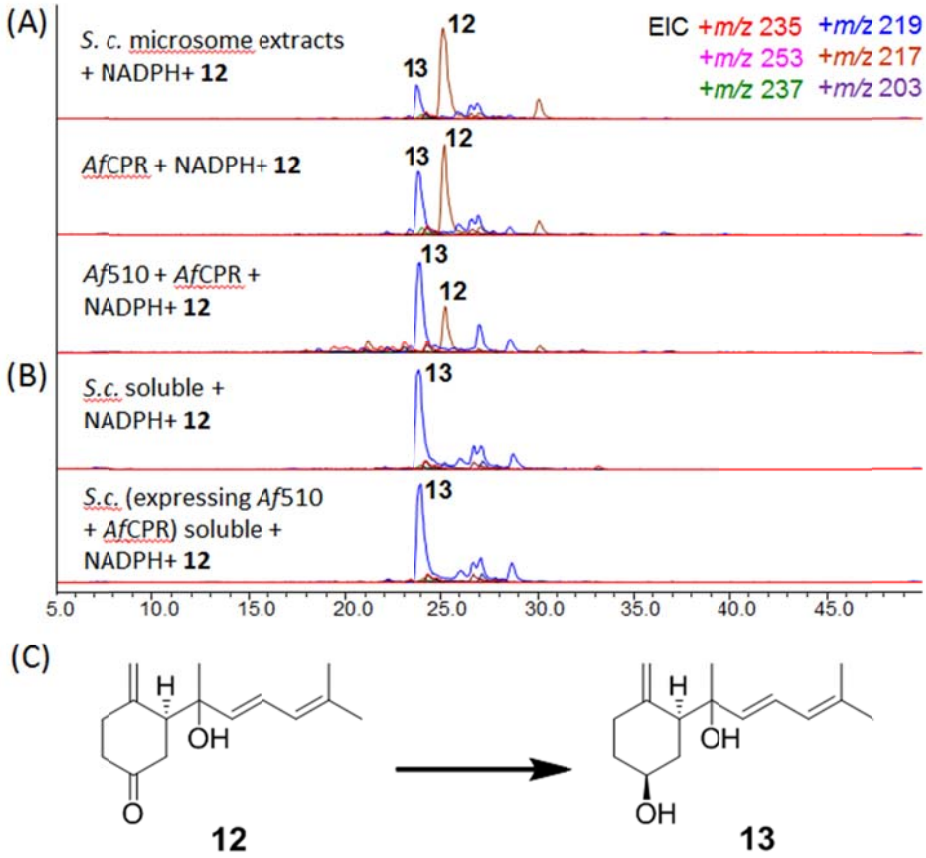


Figure S9. LC-MS analysis of in vitro assay of (**A**) yeast microsomes control with **12** and (**B**) soluble enzymes with **12**. (C) Conversion of **12** to **13**.

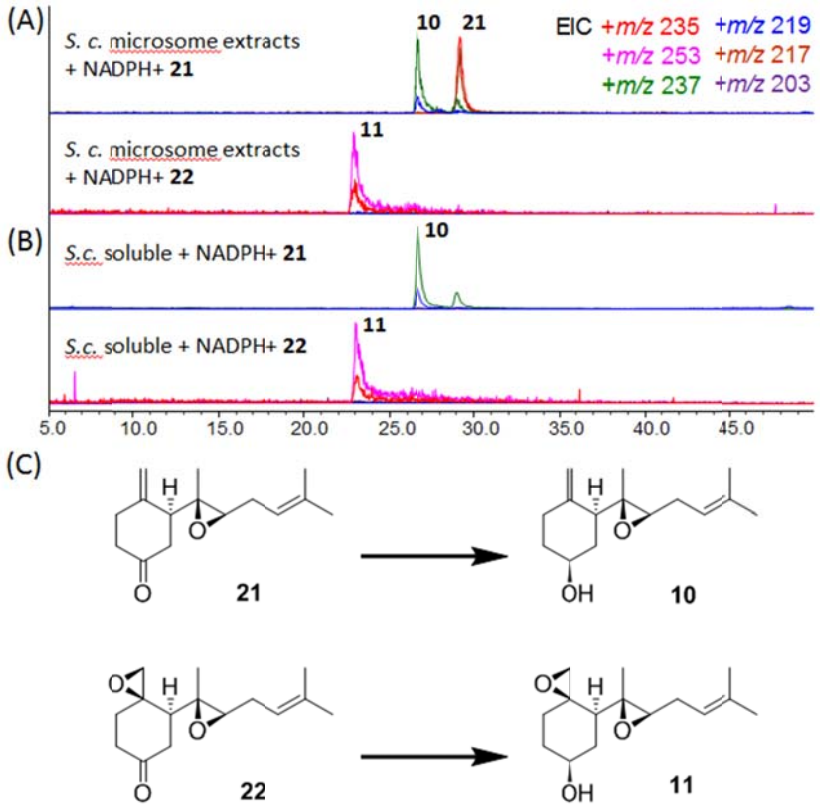


Figure S10. LC-MS analysis of in vitro assays of yeast microsomes (A) with **21** and **22**, respectively, and (**B**) soluble enzymes from yeast with **21** and **22**. (**C**) Conversion of **21** to **10**, and **22** to **11**.

.

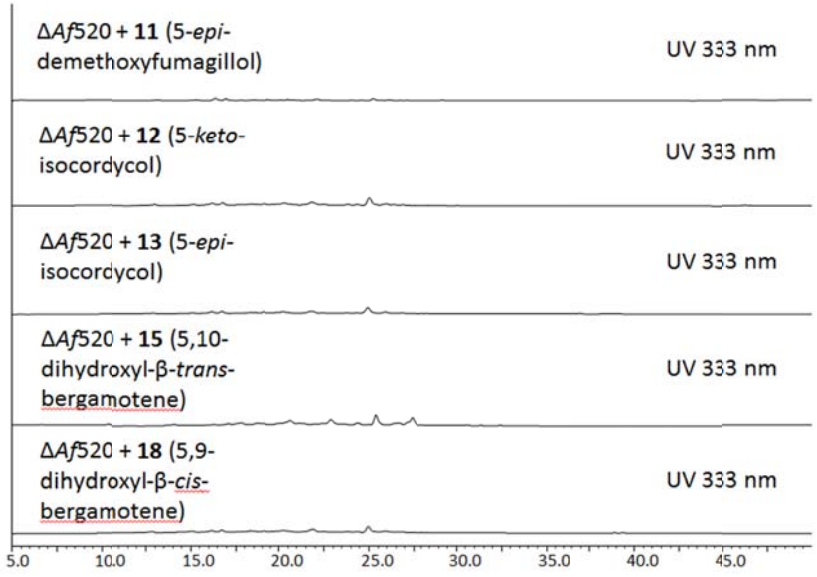


Figure S11. LC-MS analysis of metabolites produced as a result of chemical complementation of **11**, **12**, **13**, **15** and **18** to Δ*Af*520. Compounds are supplemented at ~40 μg/mL.

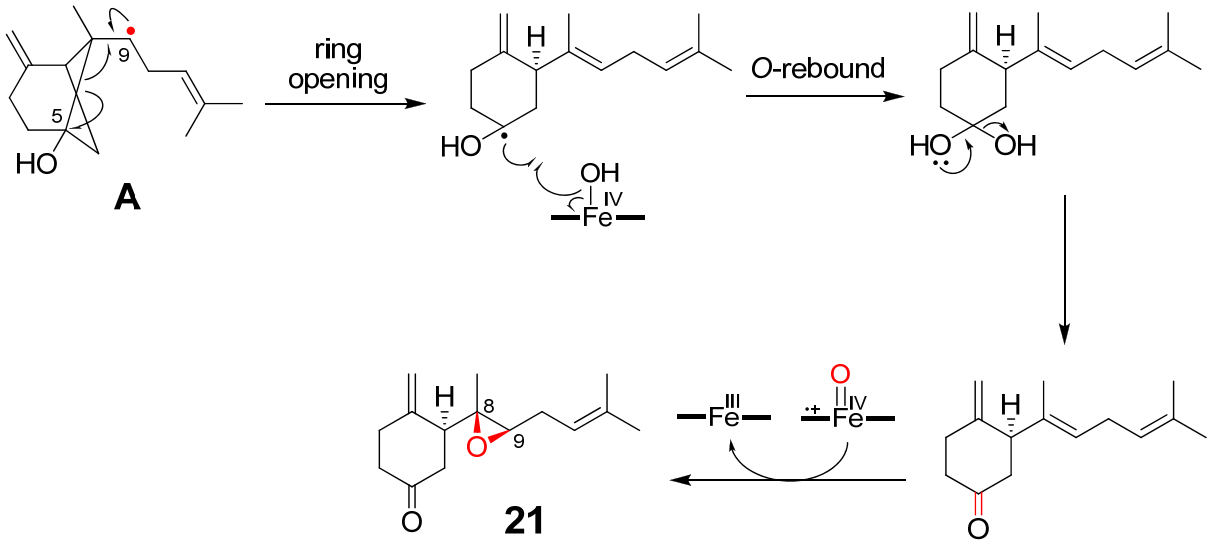


Figure S12. Alternative mechanism of Fma-P450 that converts radical intermediate **A** to **21**.

Figure S13. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of **5** in $CDCl_3$.

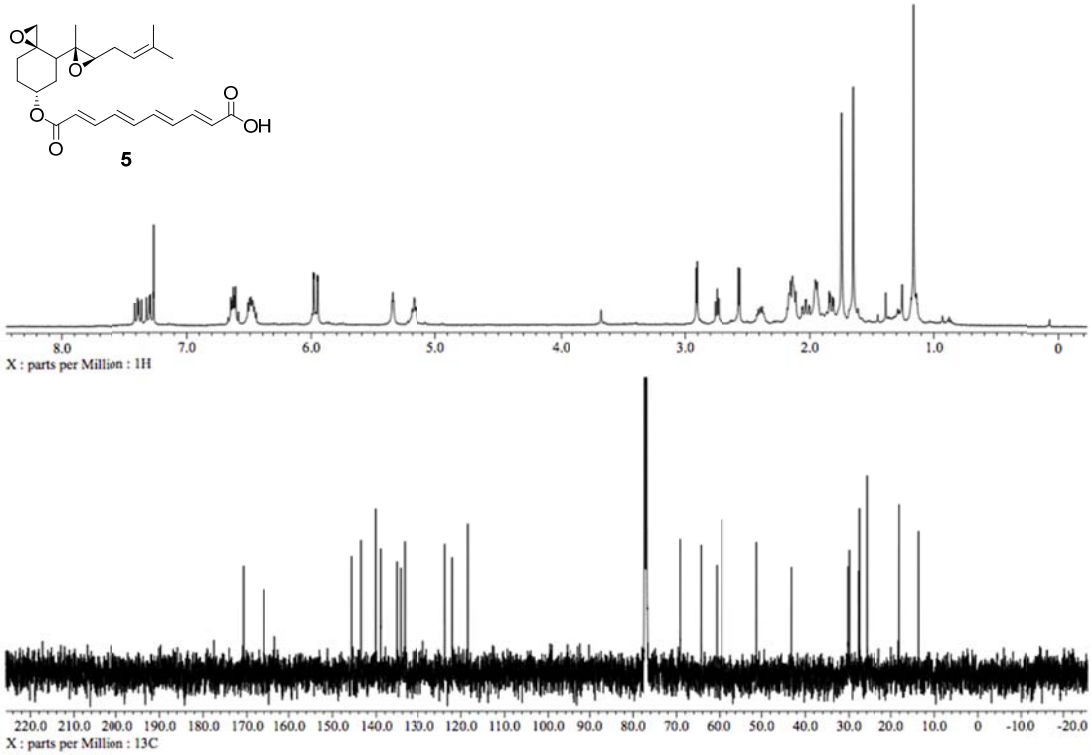


Figure S14. DQF–COSY spectrum of **5** in $CDCl_3$ (500 MHz).

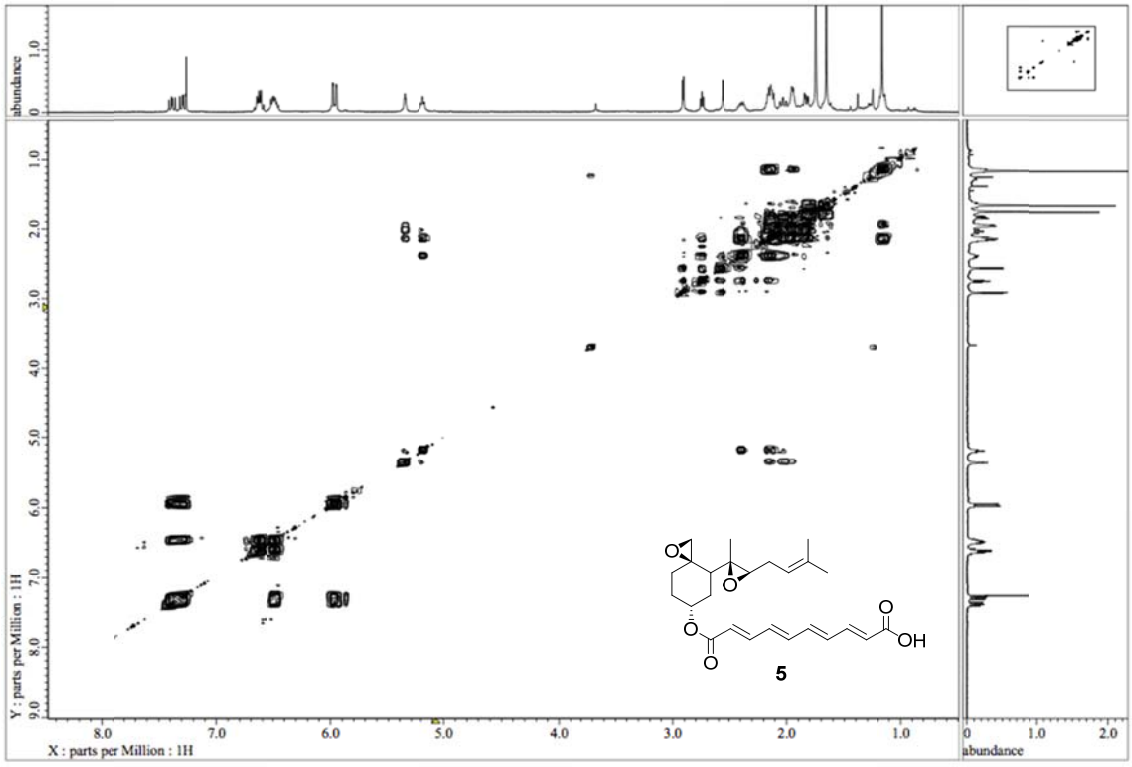


Figure S15. HMQC spectrum of **5** in $CDCl_3$ (500 MHz).

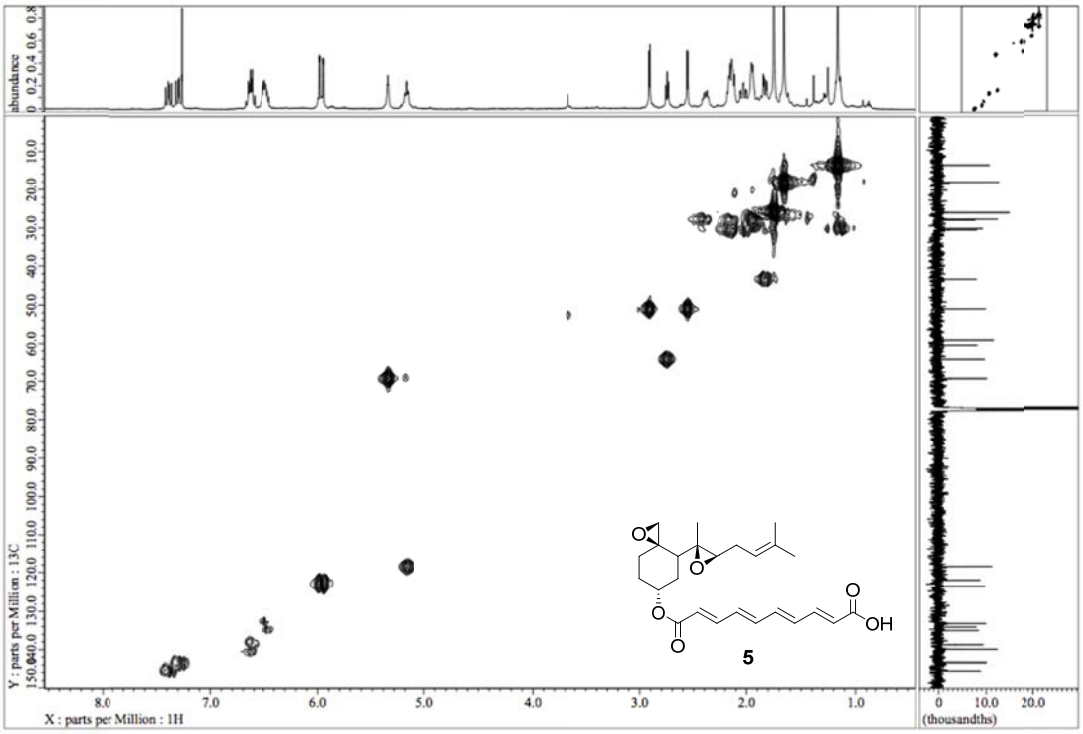


Figure S16. HMBC spectrum of **5** in $CDCl_3$ (500 MHz).

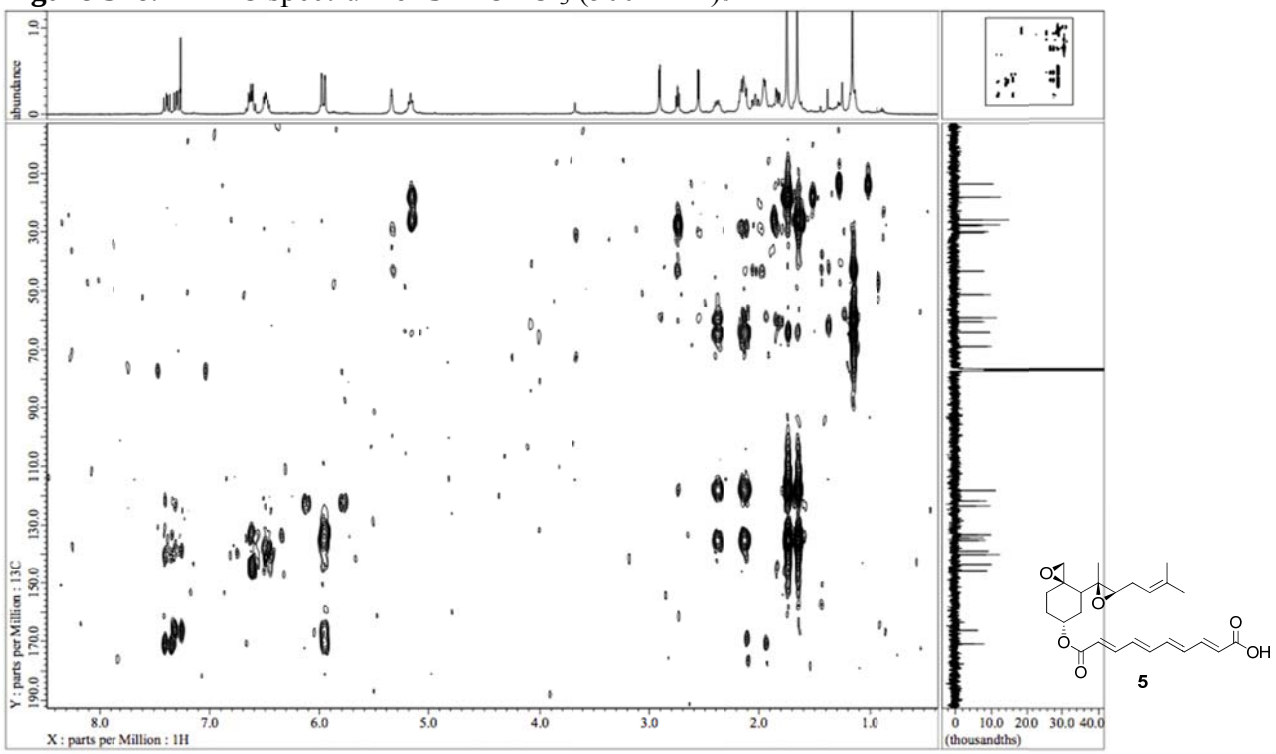


Figure S17. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of **6** in $CDCl_3$.

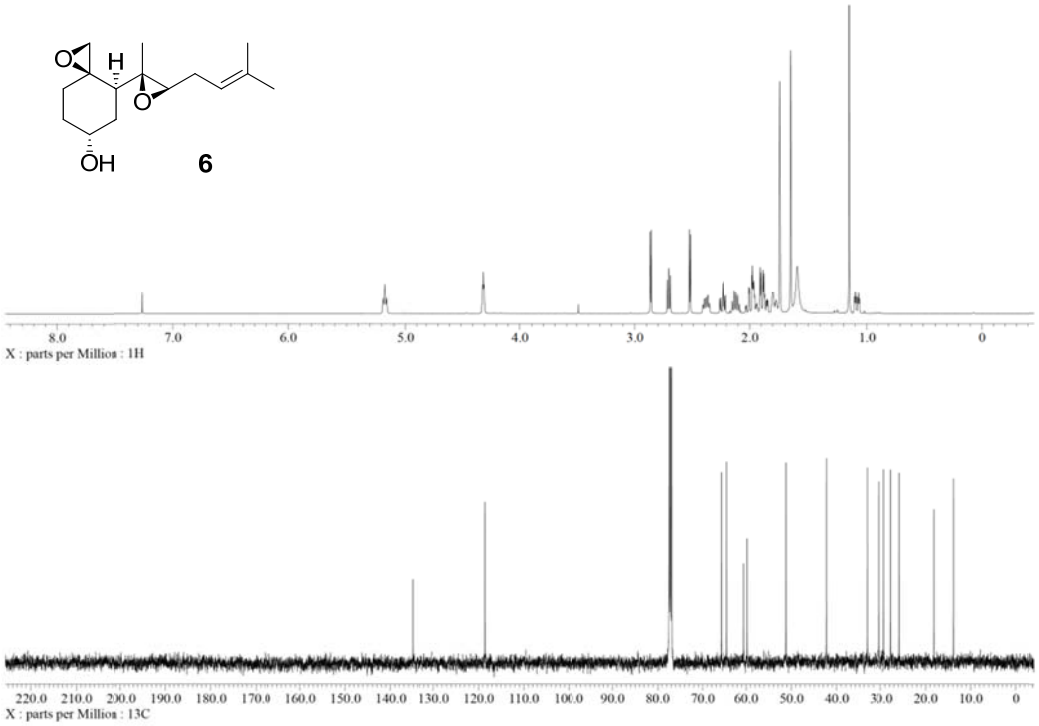


Figure S18. DQF–COSY spectrum of **6** in $CDCl_3$ (500 MHz).

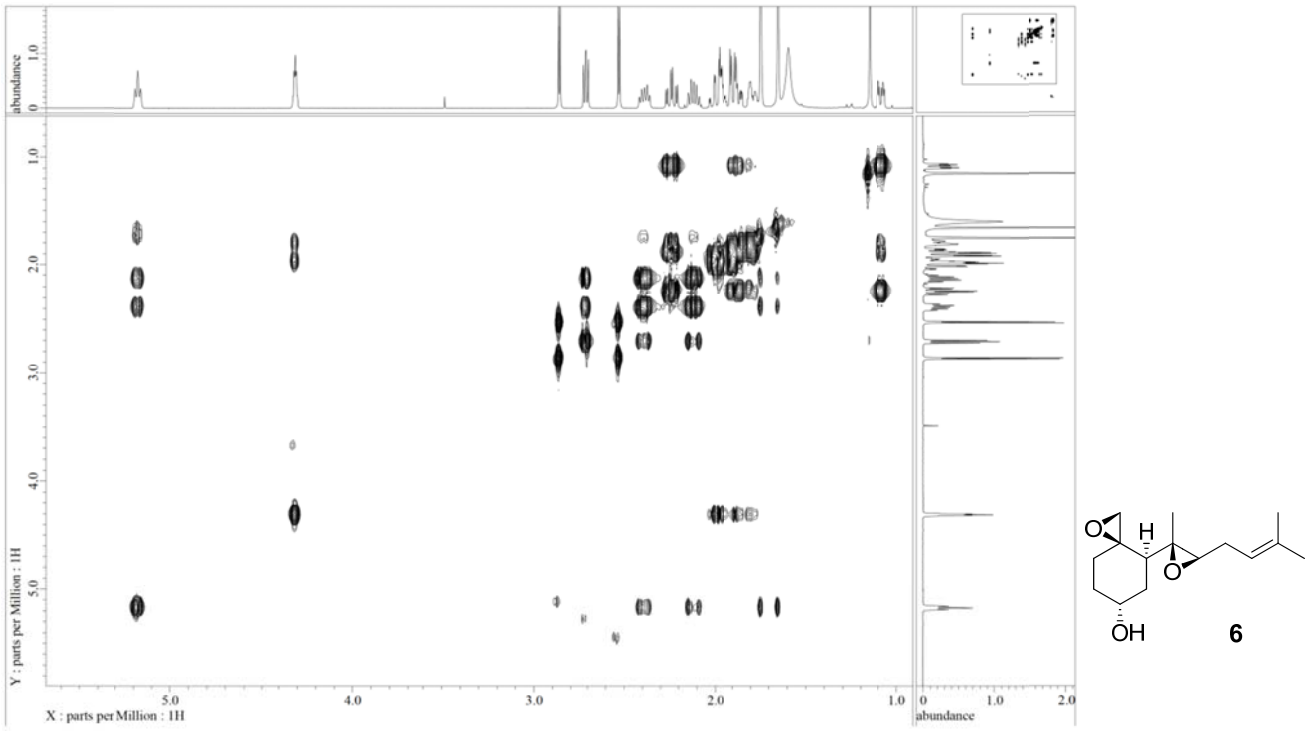


Figure S19. HMQC spectrum of **6** in CD_3OD (500 MHz).

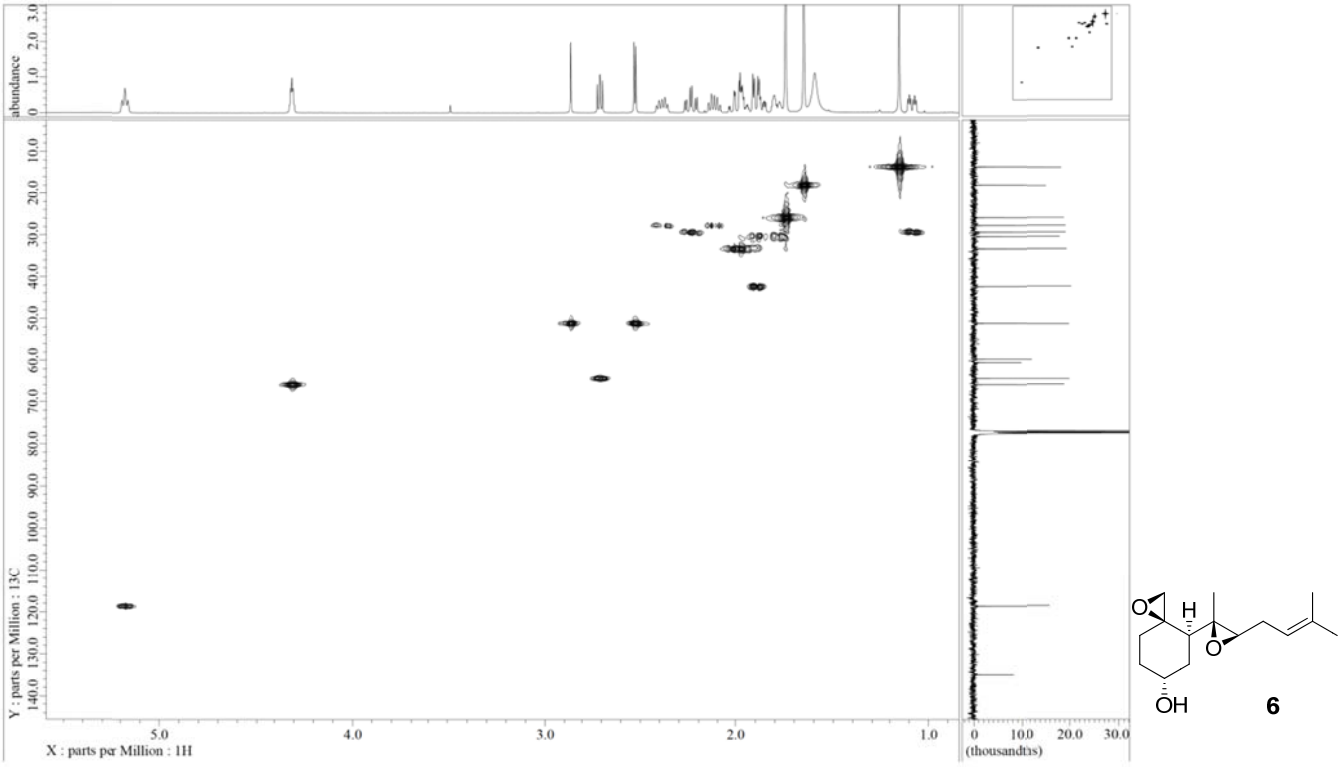


Figure S20. HMBC spectrum of **6** in $CDCl_3$ (500 MHz).

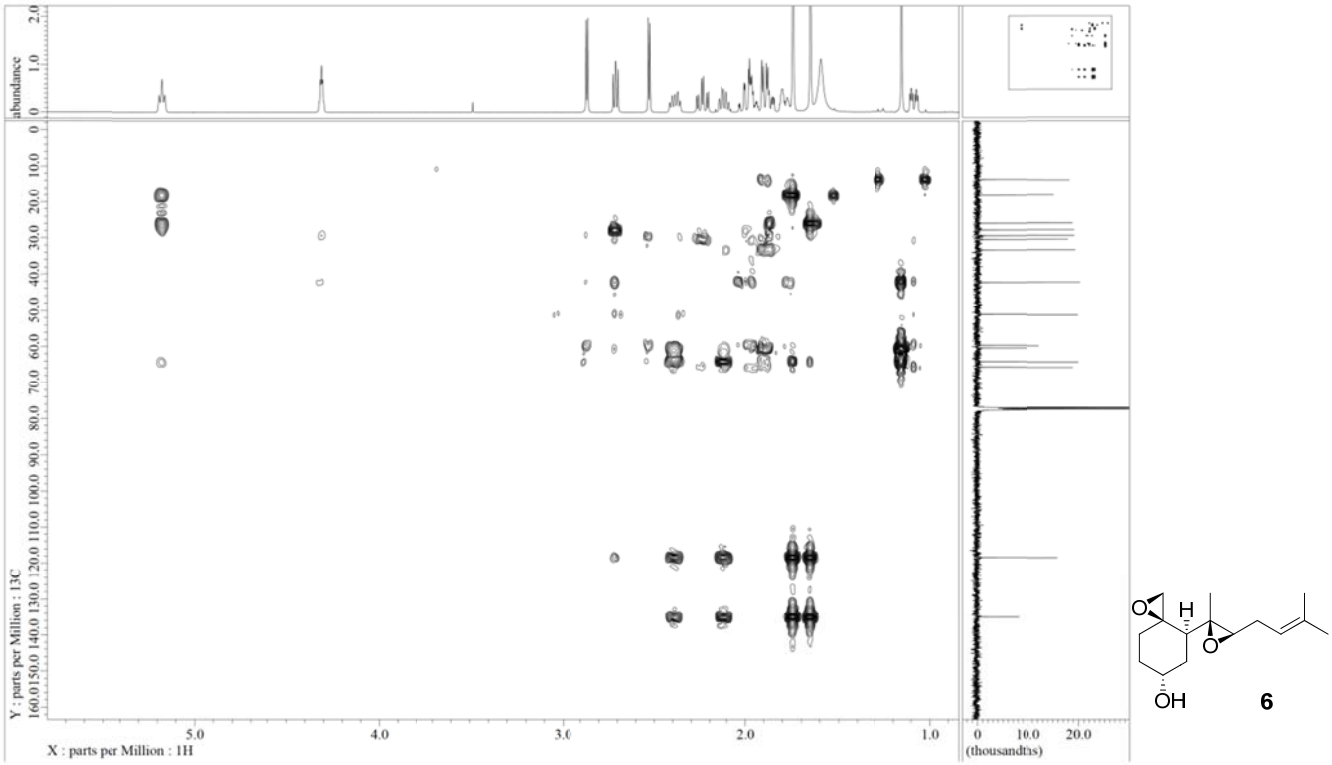


Figure S21. ^{1}H NMR spectrum of **8** ($CDCl_3$, 500 MHz).

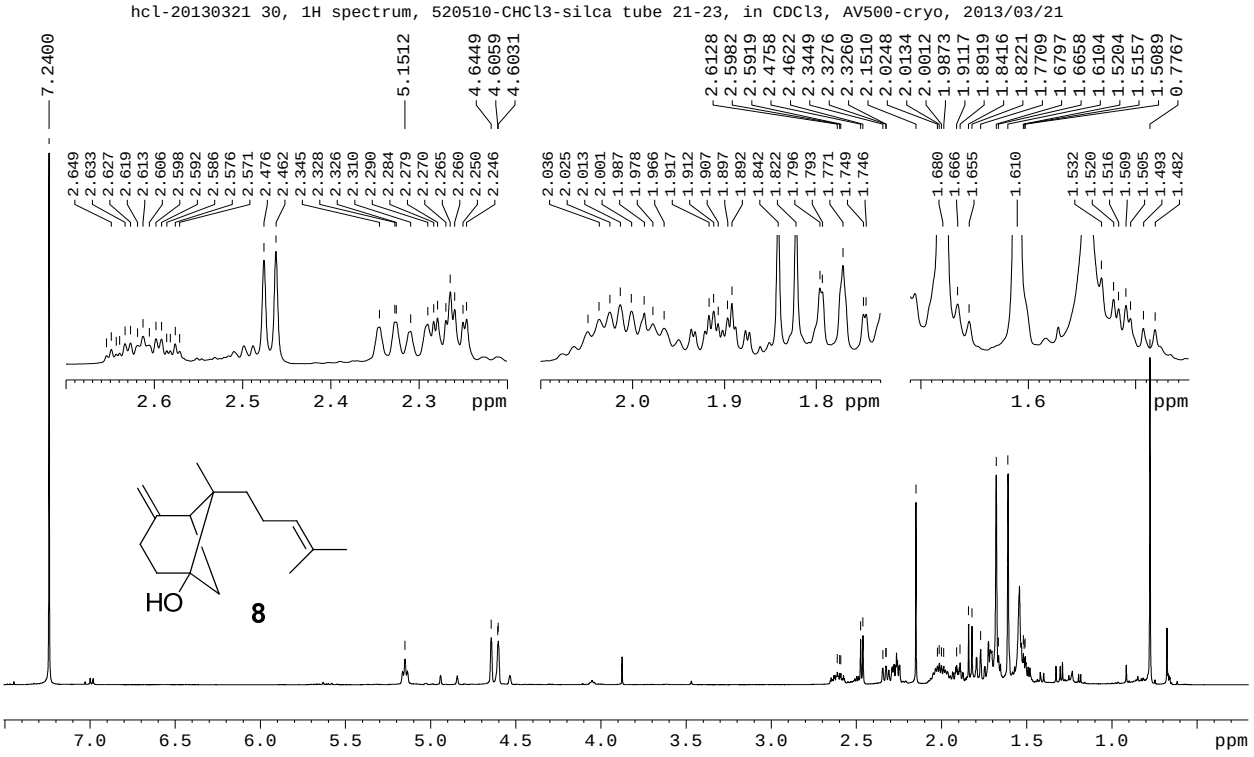


Figure S22. ^{13}C NMR spectrum of **8** ($CDCl_3$, 125 MHz).

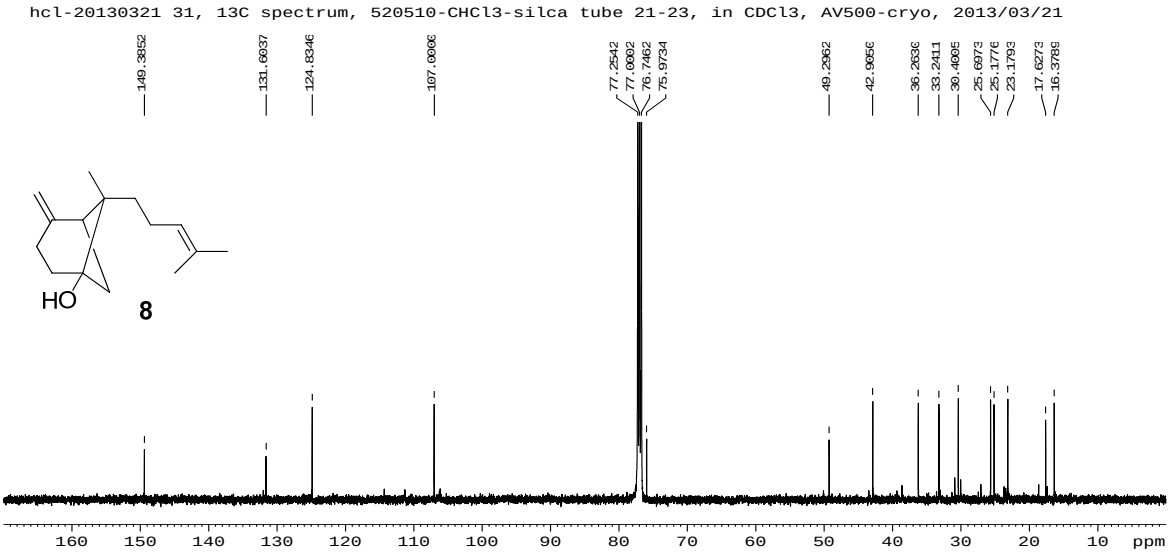


Figure S23. HSQC135 spectrum of **8** ($CDCl_3$, 500 MHz).

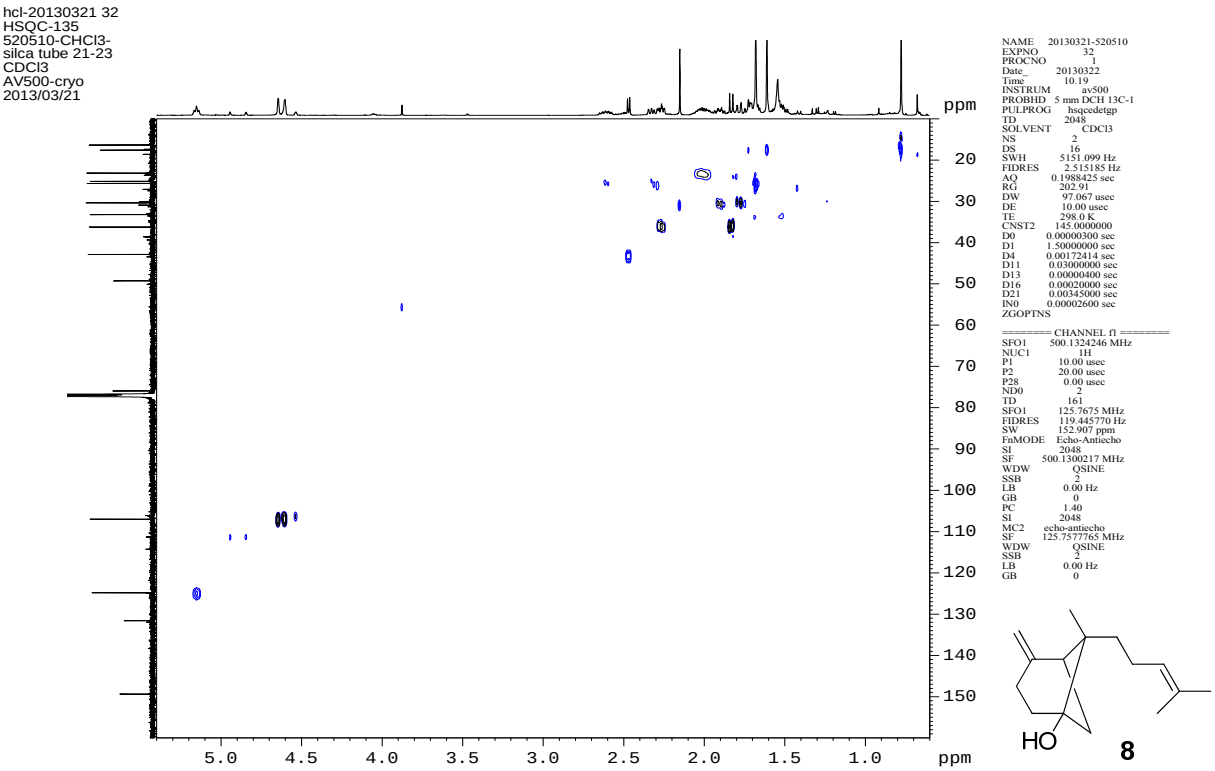


Figure S24. COSY spectrum of **8** ($CDCl_3$, 500 MHz).

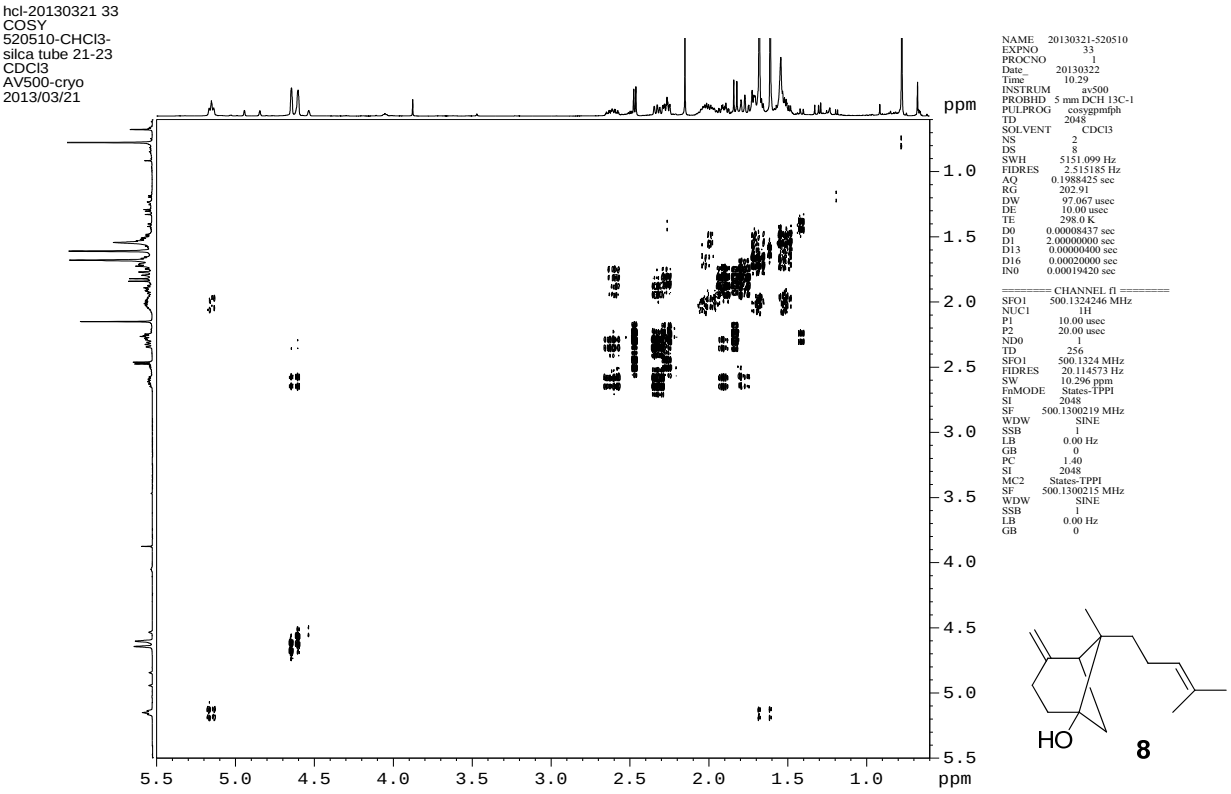


Figure S25. HMBC spectrum of **8** ($CDCl_3$, 500 MHz).

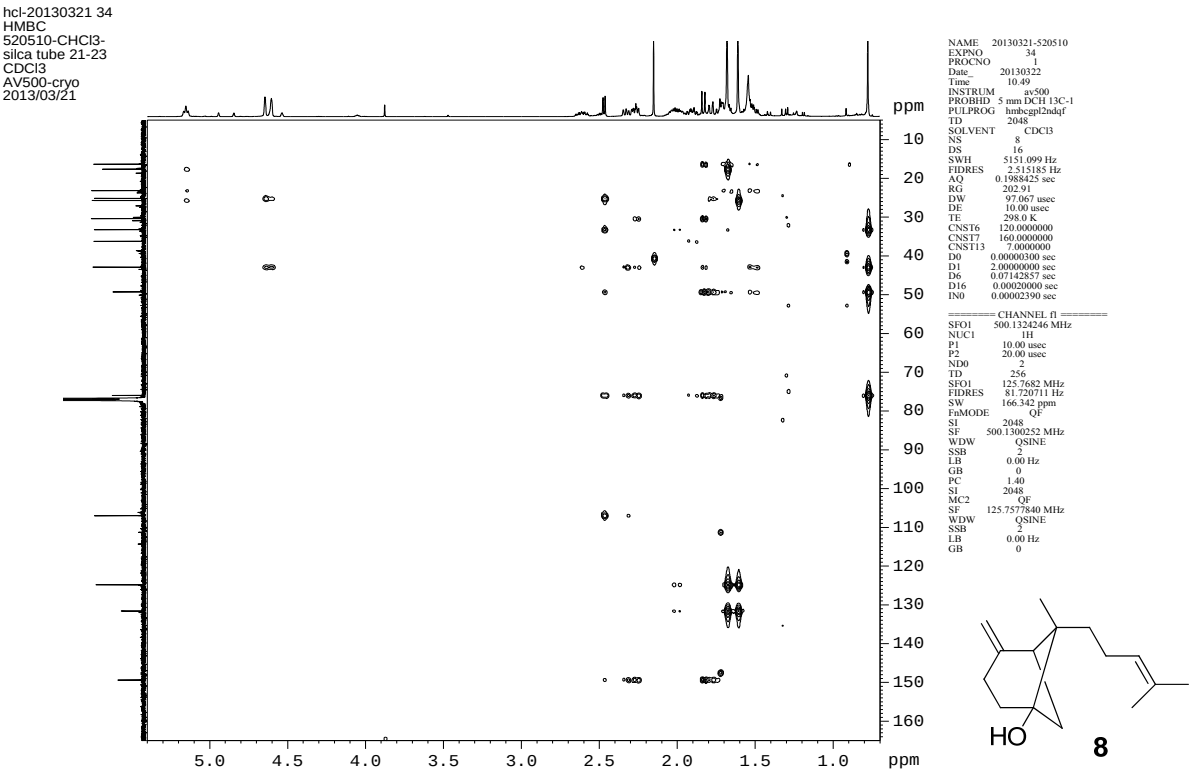


Figure S26. NOESY spectrum of **8** ($CDCl_3$, 500 MHz).

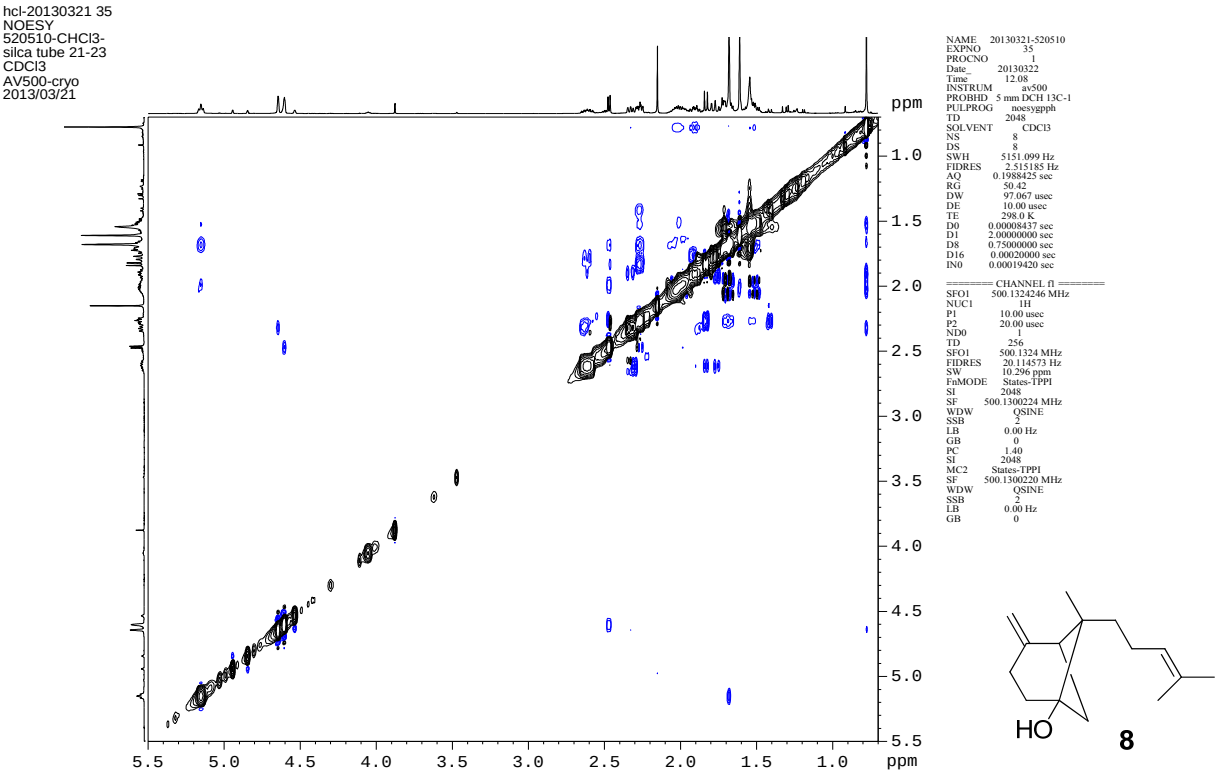


Figure S27. ^{1}H NMR spectrum of **9** ($CDCl_3$, 500 MHz).

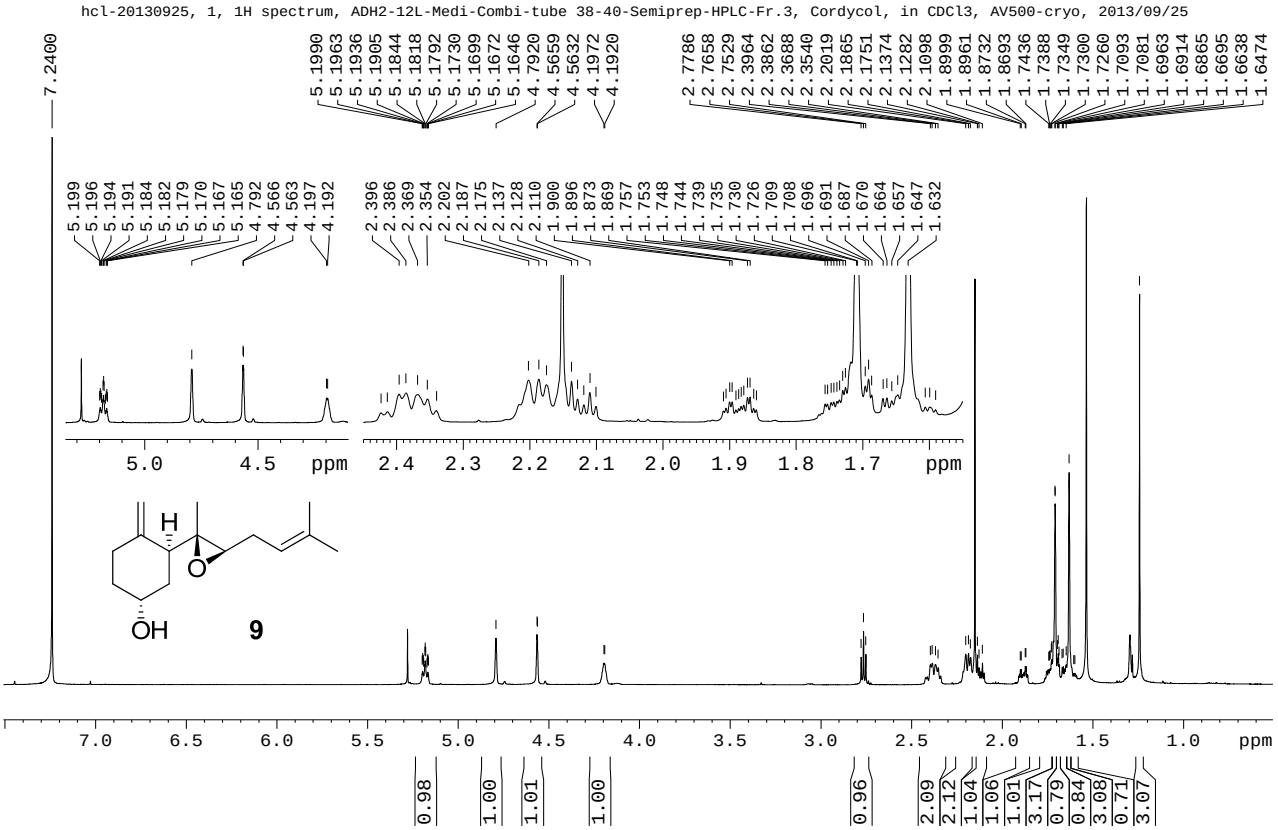


Figure S28. ^{13}C NMR spectrum of **9** ($CDCl_3$, 125 MHz).

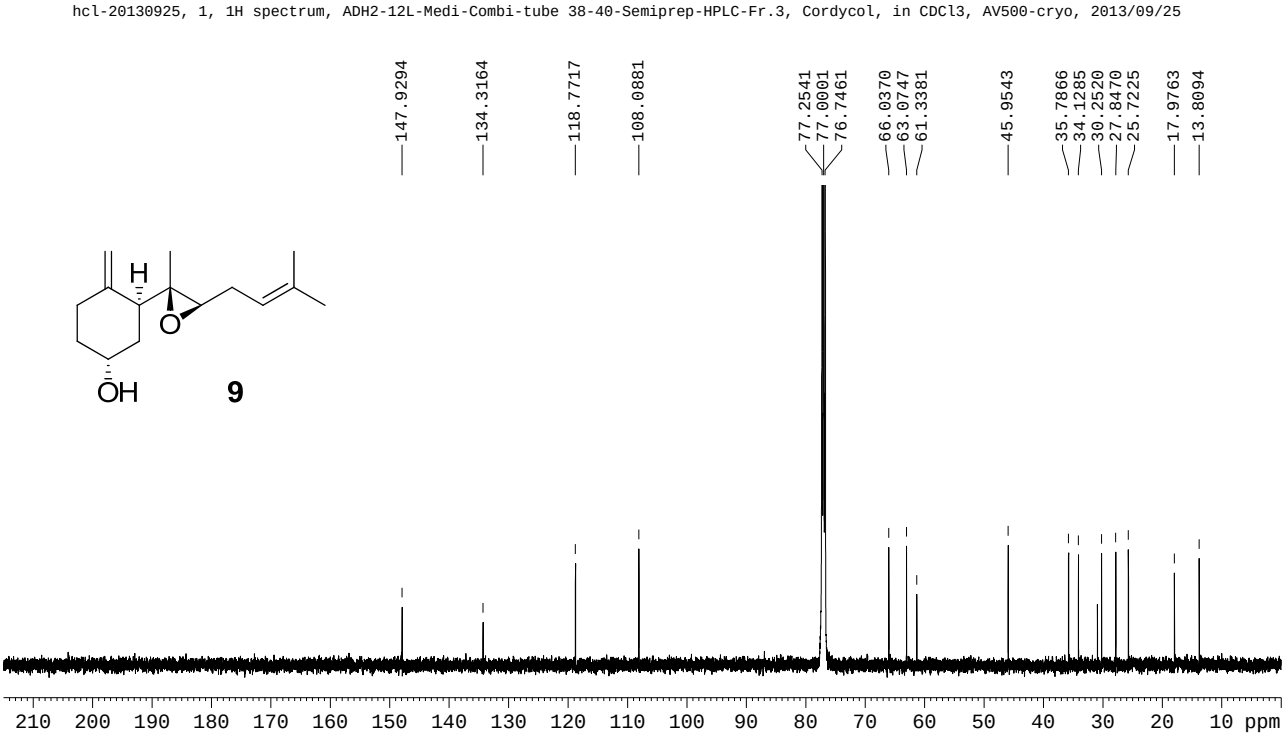


Figure S29. ^{1}H NMR spectrum of **10** ($CDCl_3$, 500 MHz).

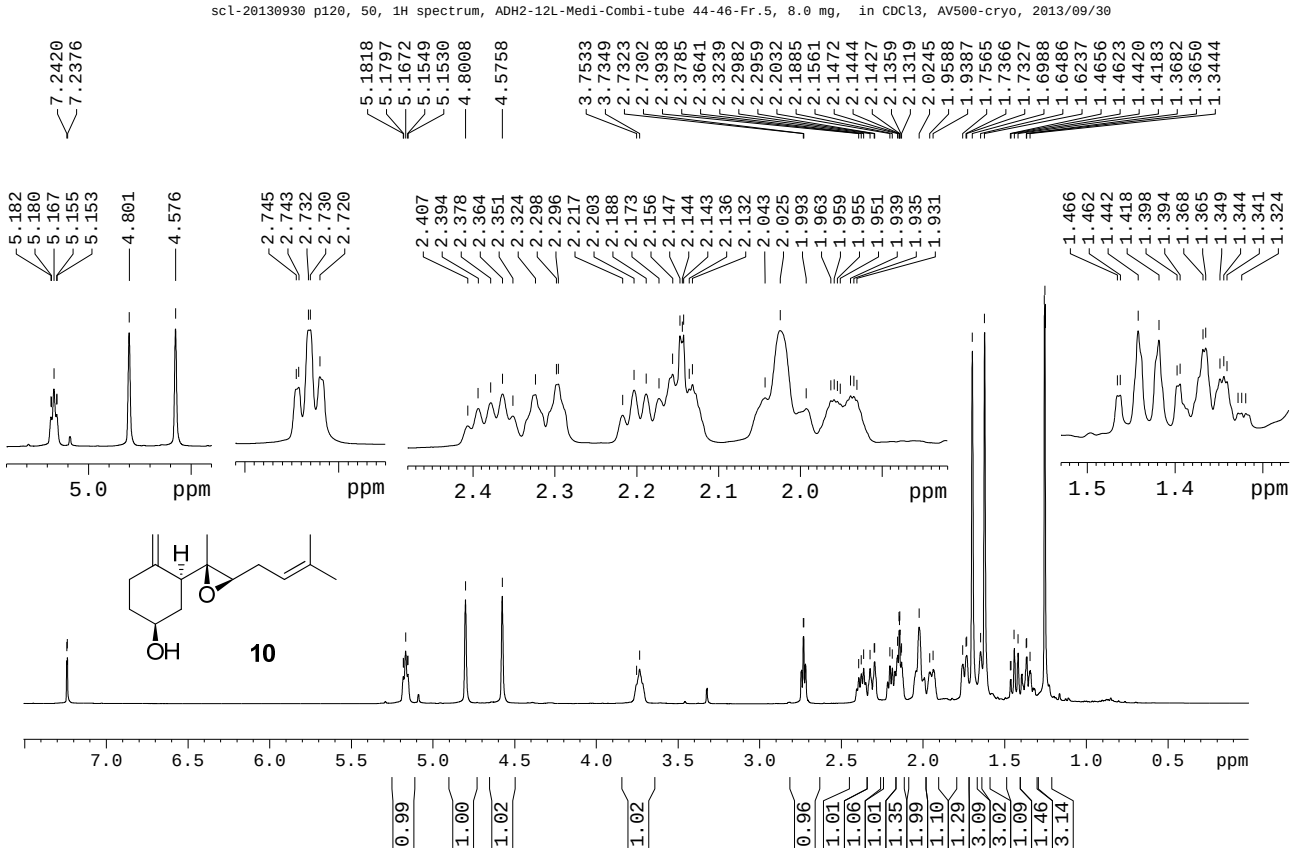


Figure S30. ^{13}C NMR spectrum of **10** ($CDCl_3$, 125 MHz).

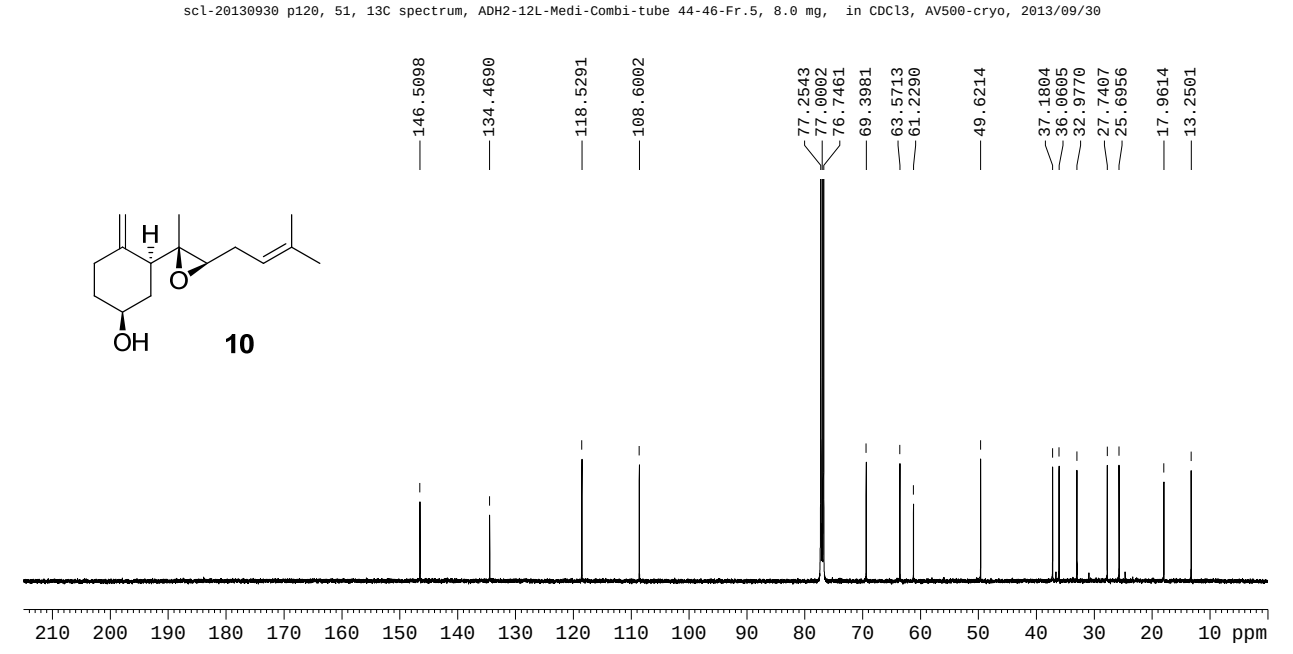


Figure S31. HSQC135 spectrum of **10** ($CDCl_3$, 500 MHz).

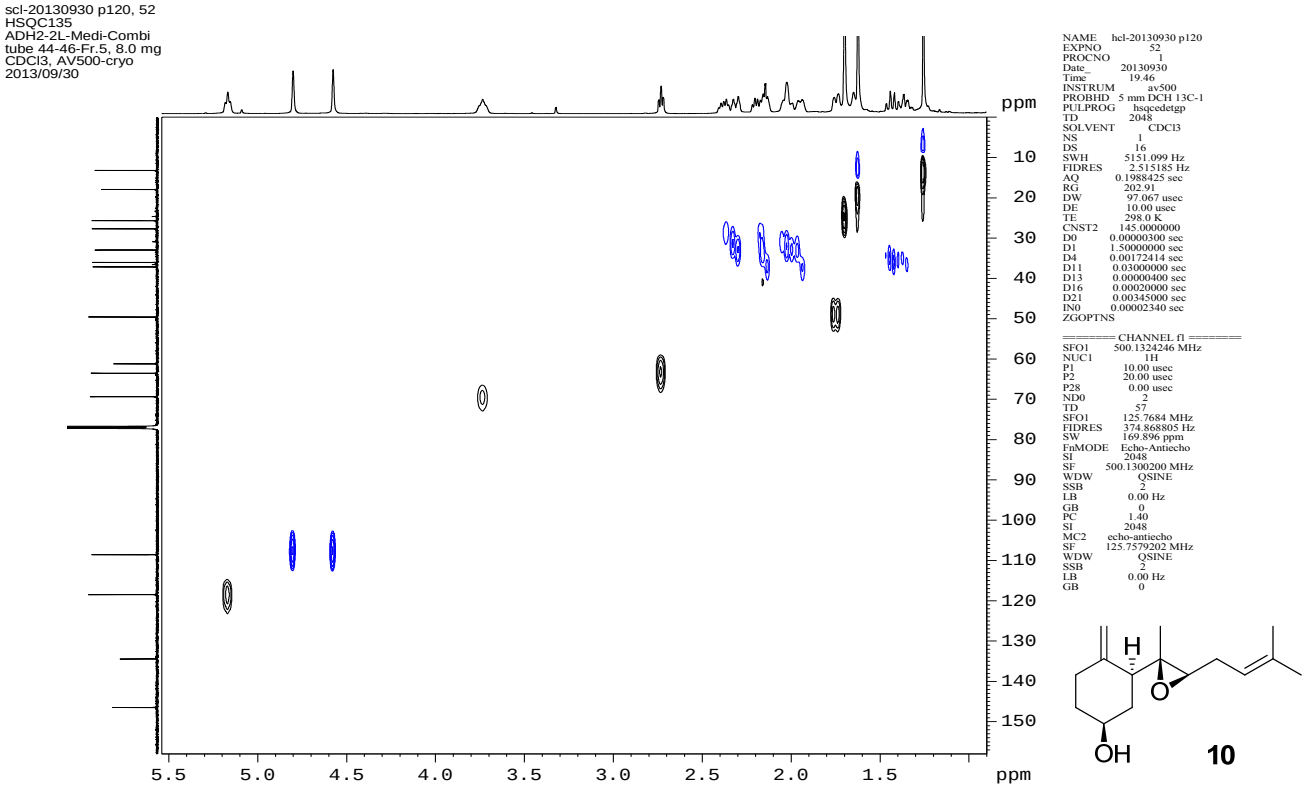


Figure S32. HMBC spectrum of **10** ($CDCl_3$, 500 MHz).

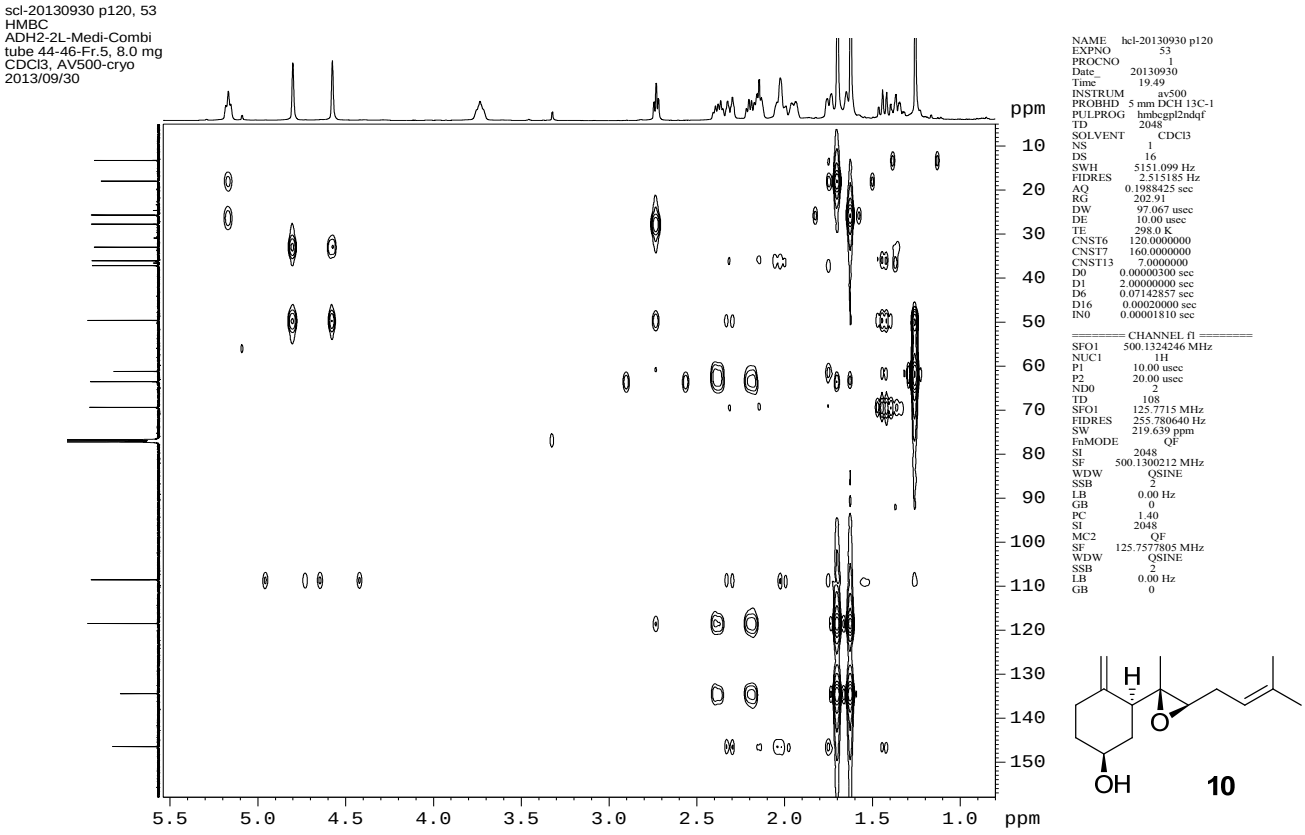


Figure S33. NOESY spectrum of **10** ($CDCl_3$, 500 MHz).

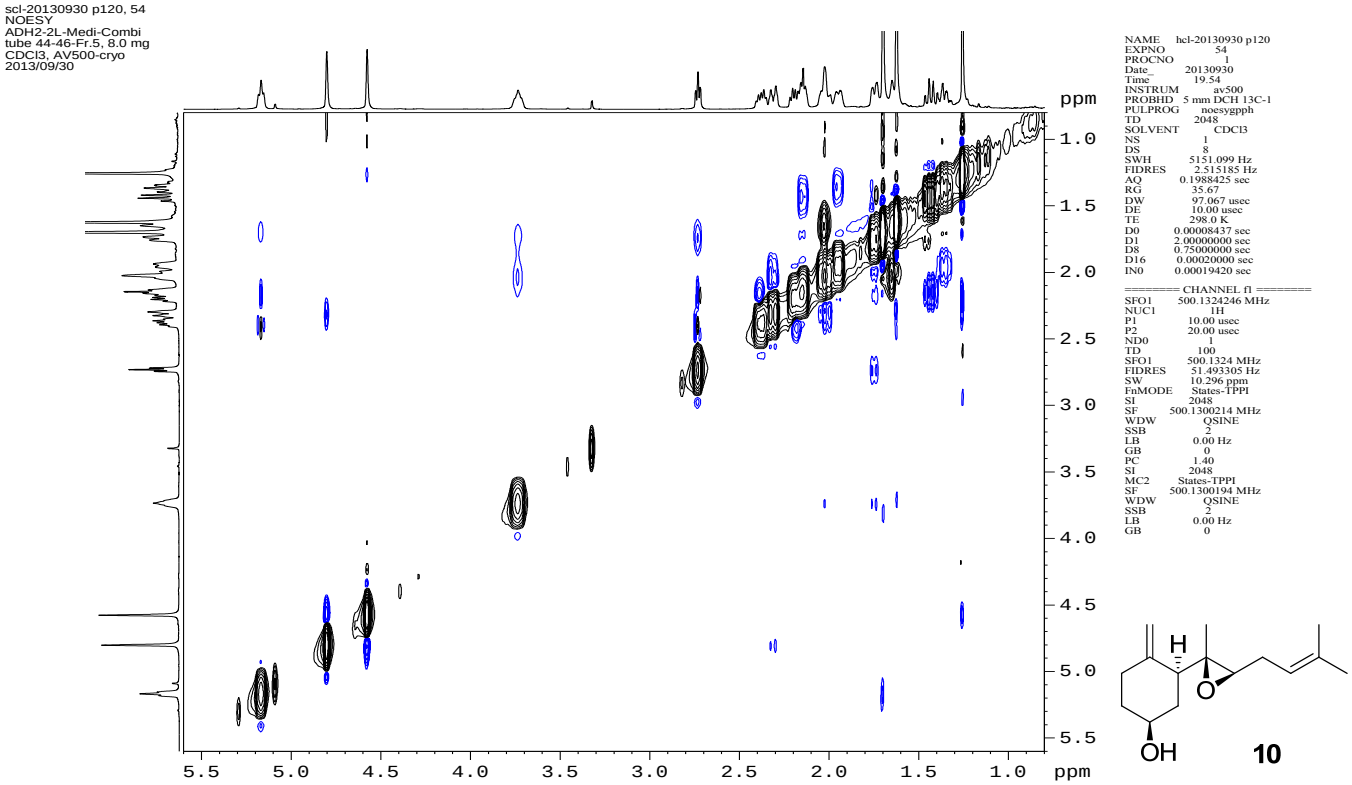


Figure S34. TOCSY spectrum of **10** ($CDCl_3$, 500 MHz).

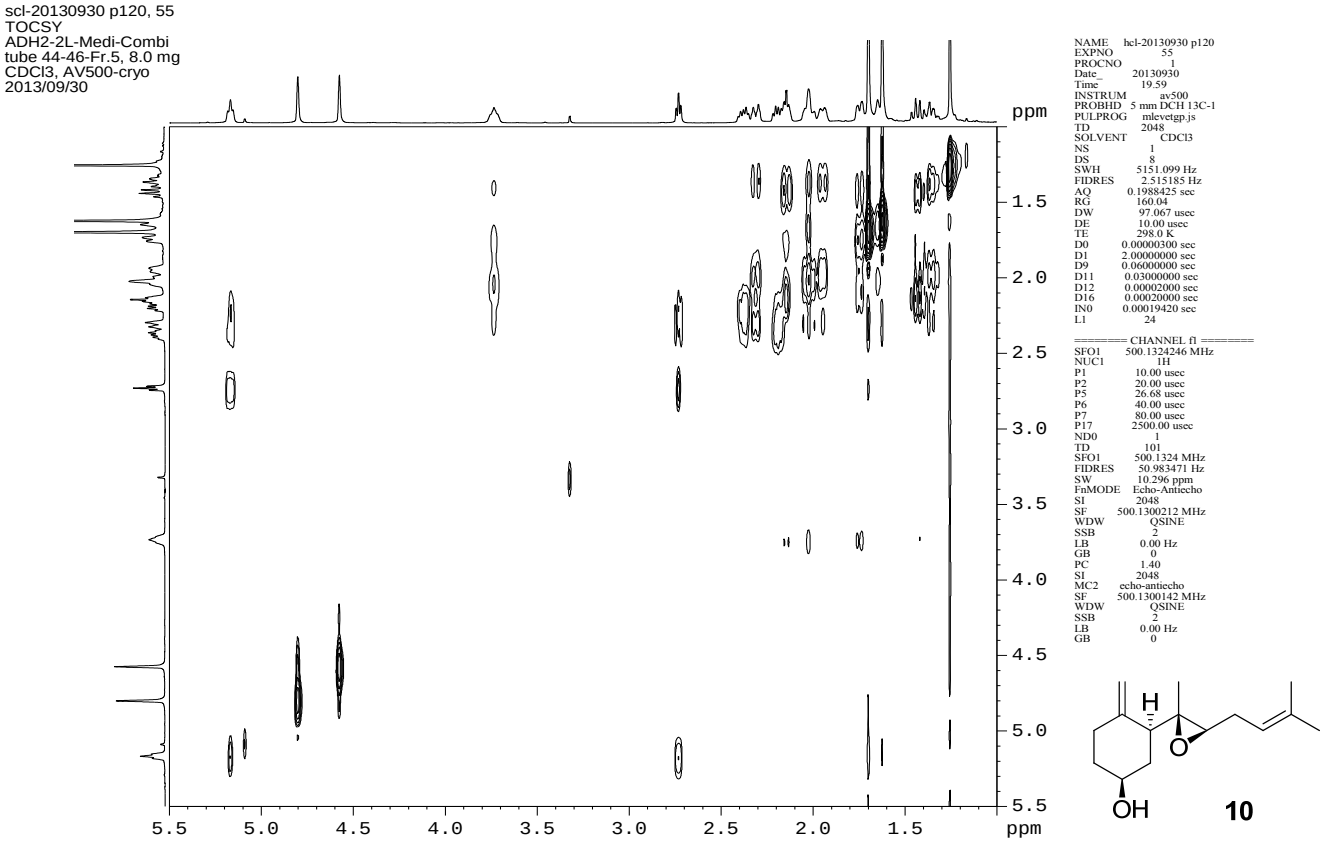


Figure S35. ^{1}H NMR spectrum of **11** ($CDCl_3$, 500 MHz).

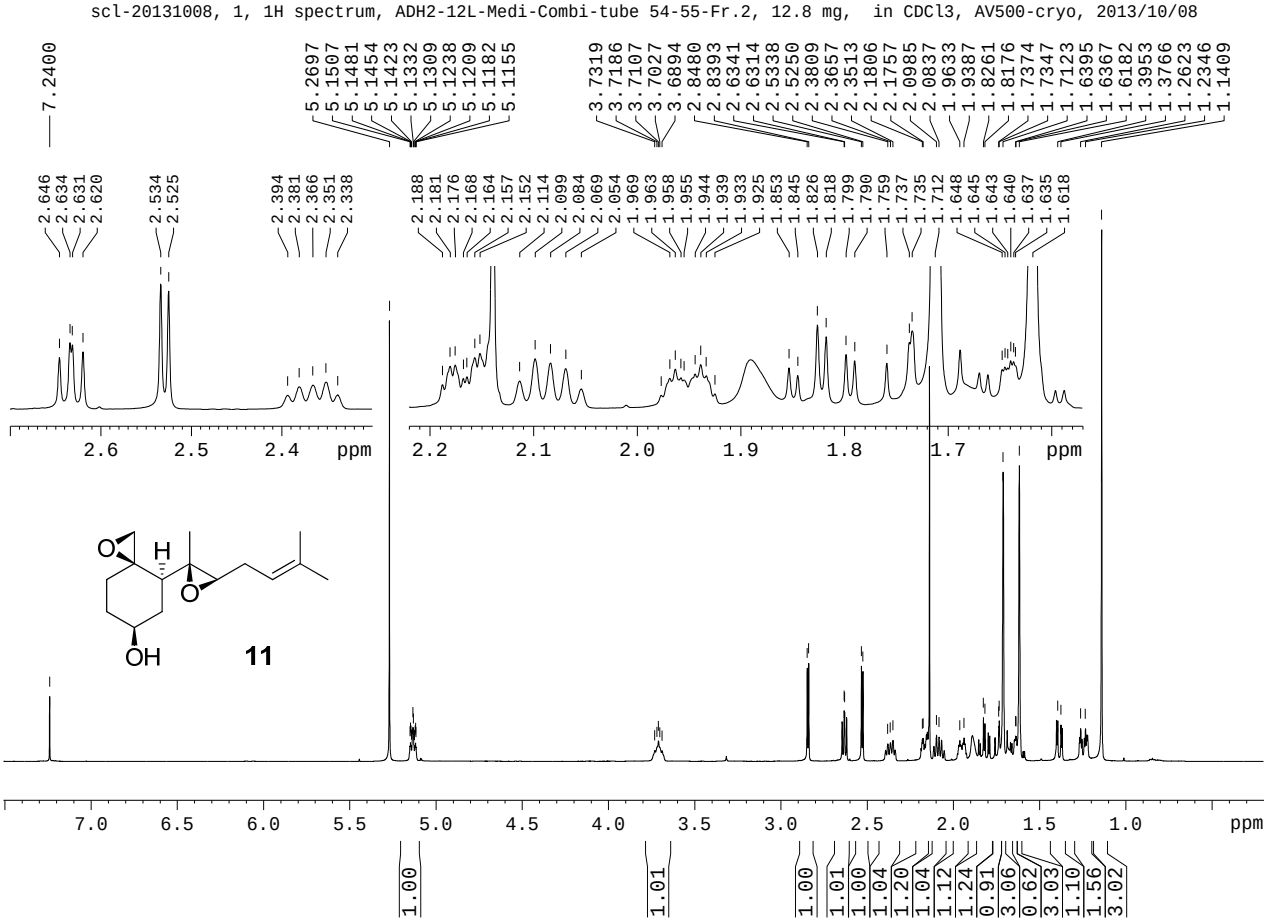


Figure S36. ^{13}C NMR spectrum of **11** ($CDCl_3$, 125 MHz).

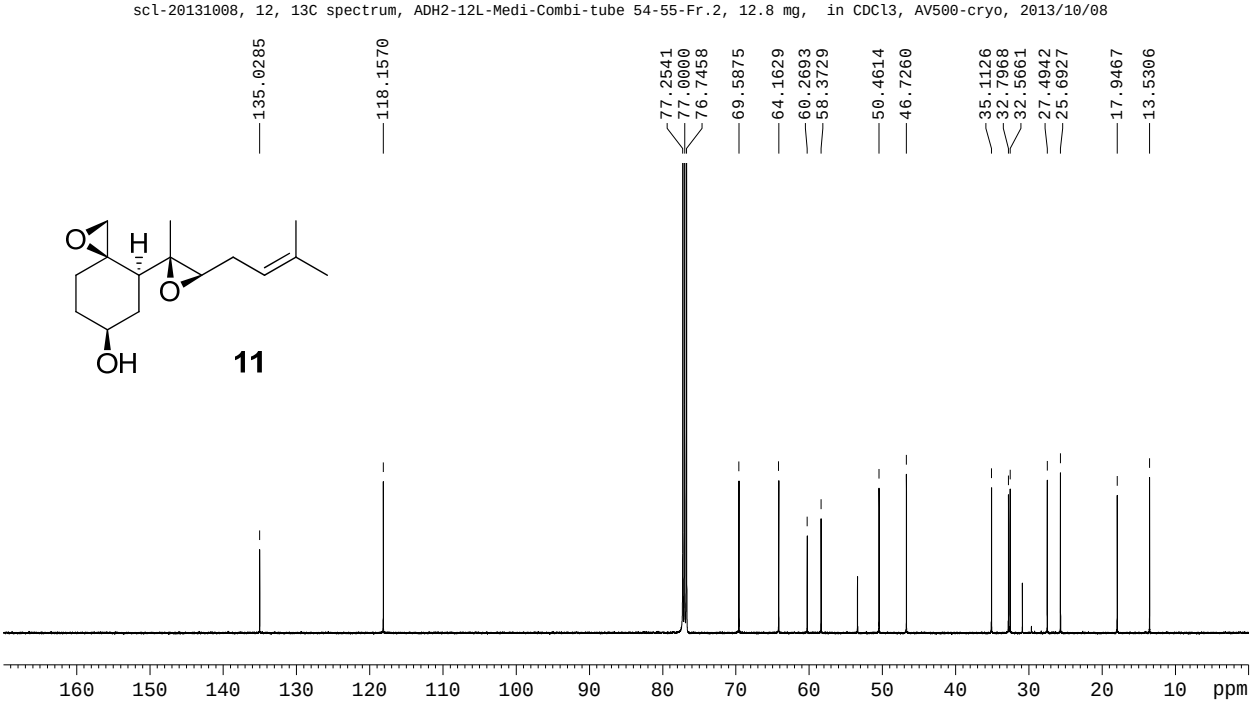


Figure S37. HSQC135 spectrum of **11** ($CDCl_3$, 500 MHz).

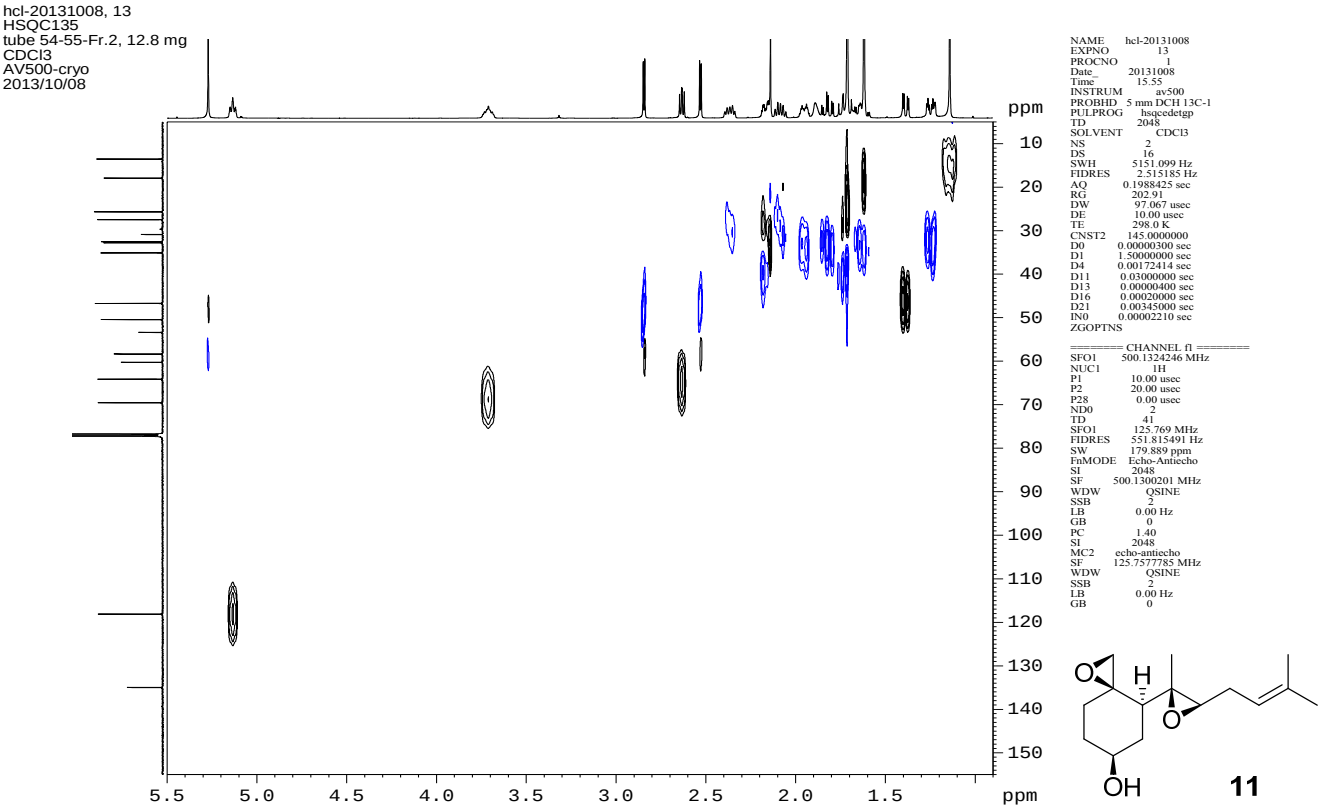


Figure S38. HMBC spectrum of **11** ($CDCl_3$, 500 MHz).

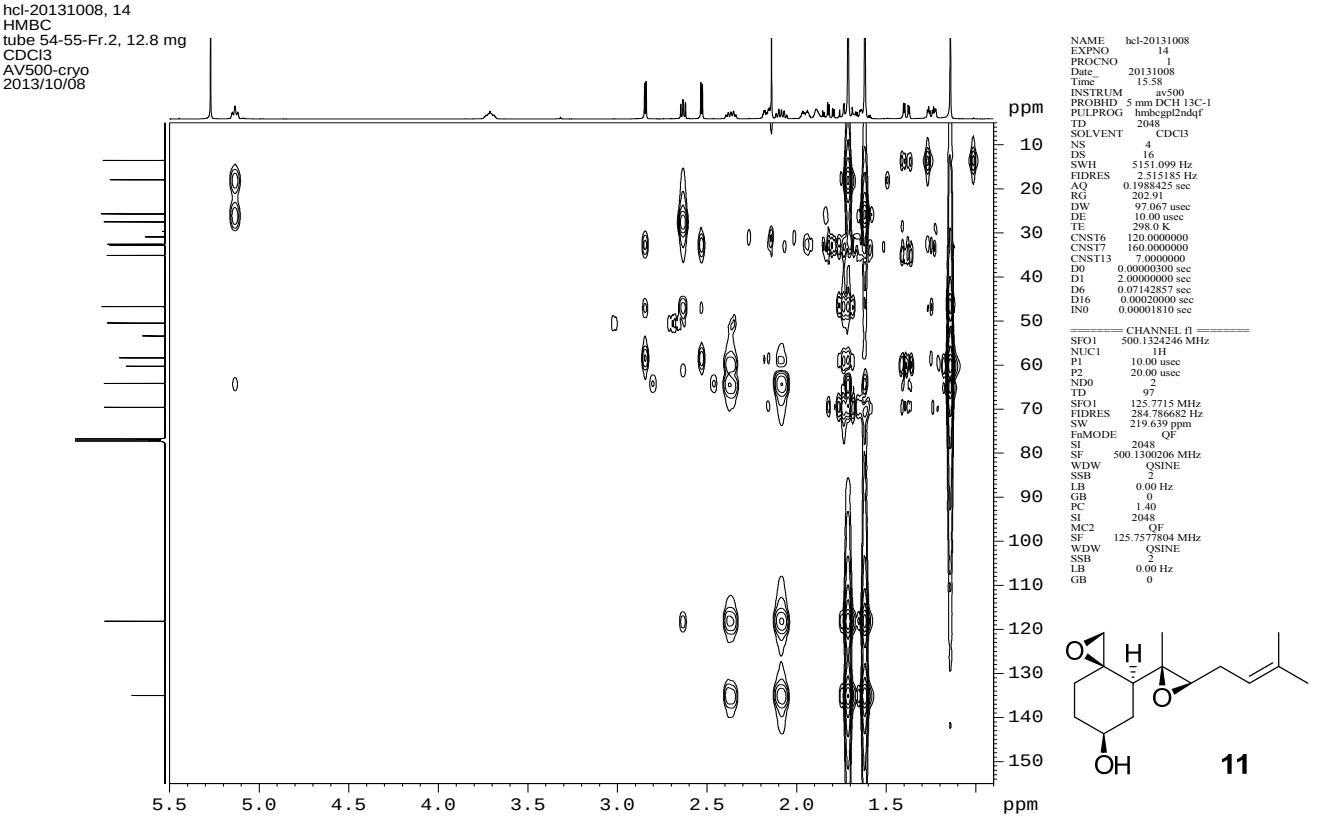


Figure S39. NOESY spectrum of **11** ($CDCl_3$, 500 MHz).

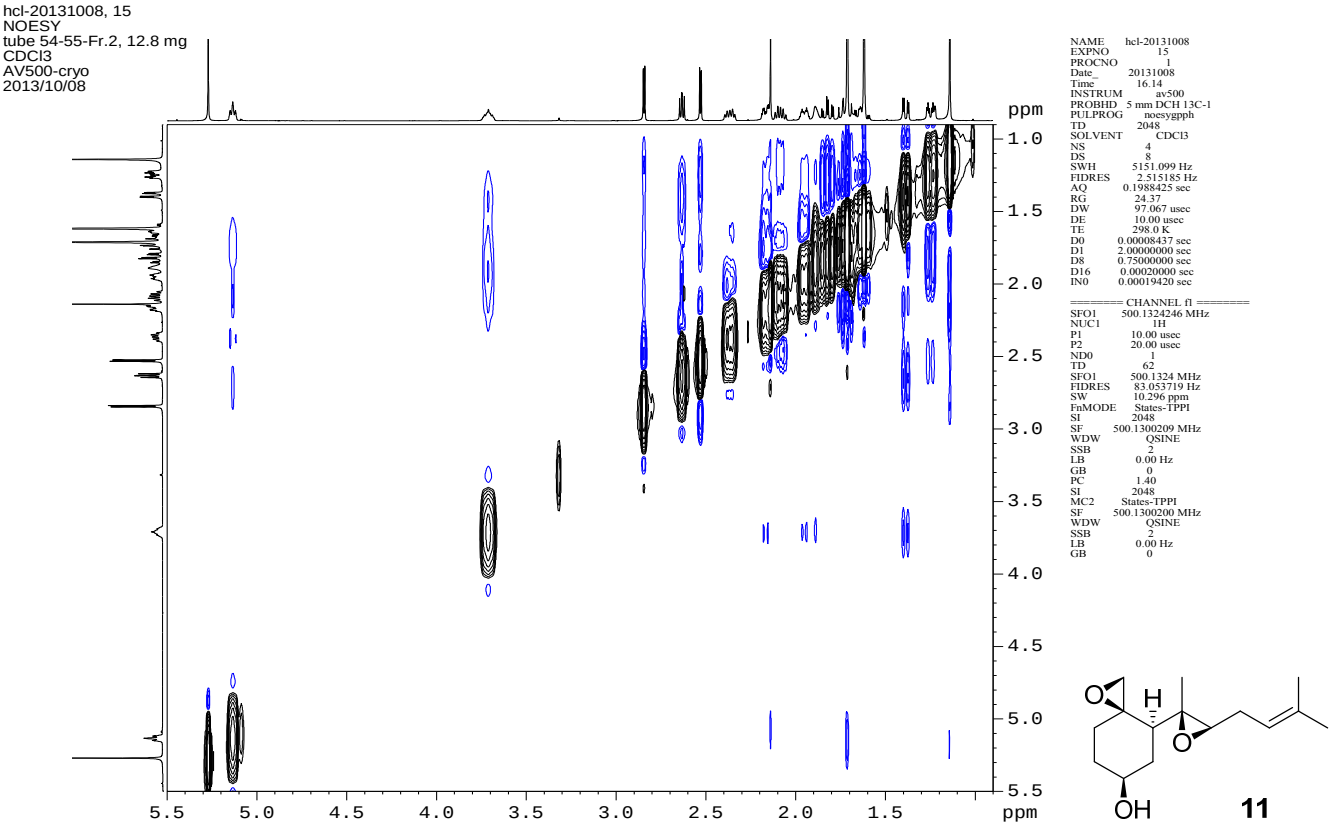


Figure S40. ^{1}H NMR spectrum of **12** ($CDCl_3$, 500 MHz).

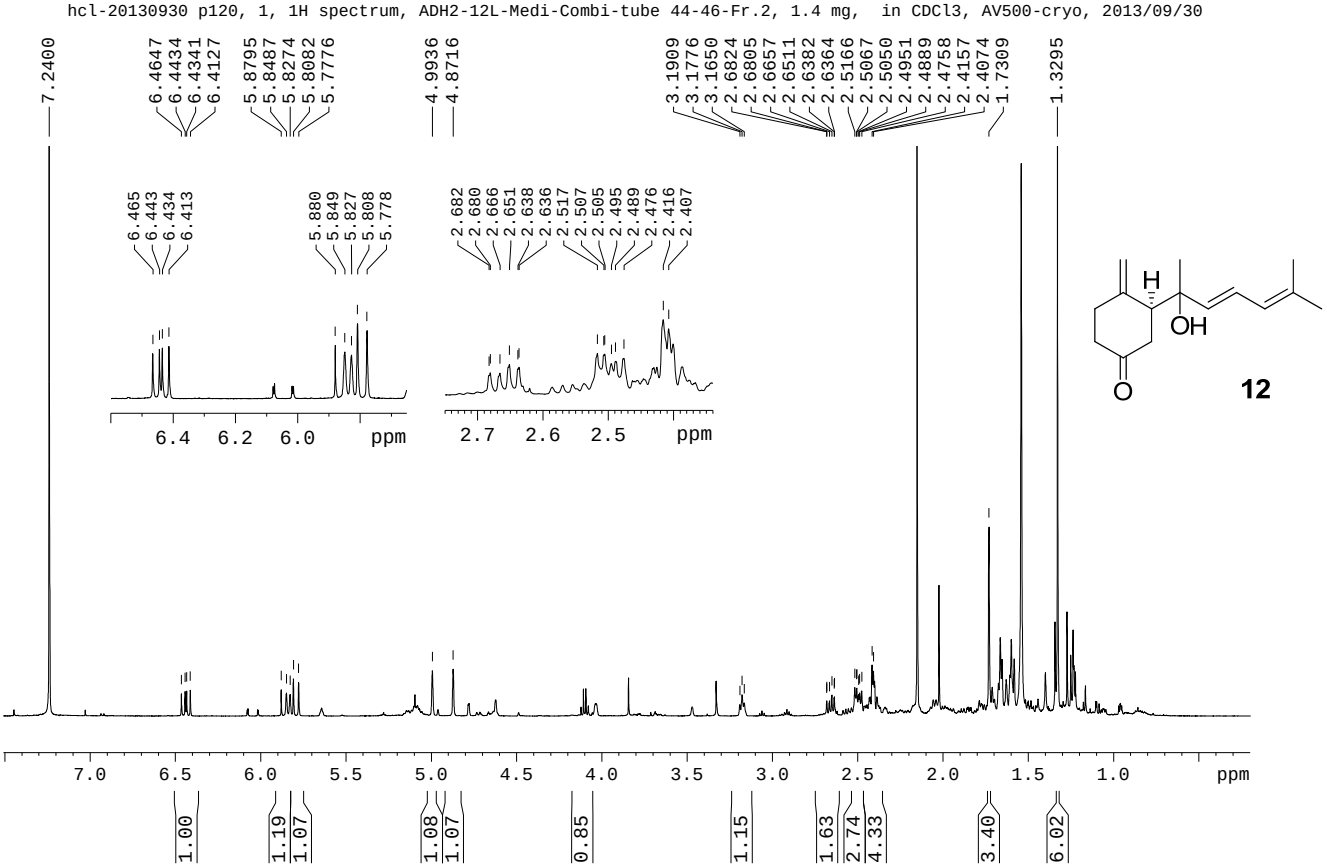


Figure S41. ^{13}C NMR spectrum of **12** ($CDCl_3$, 125 MHz).

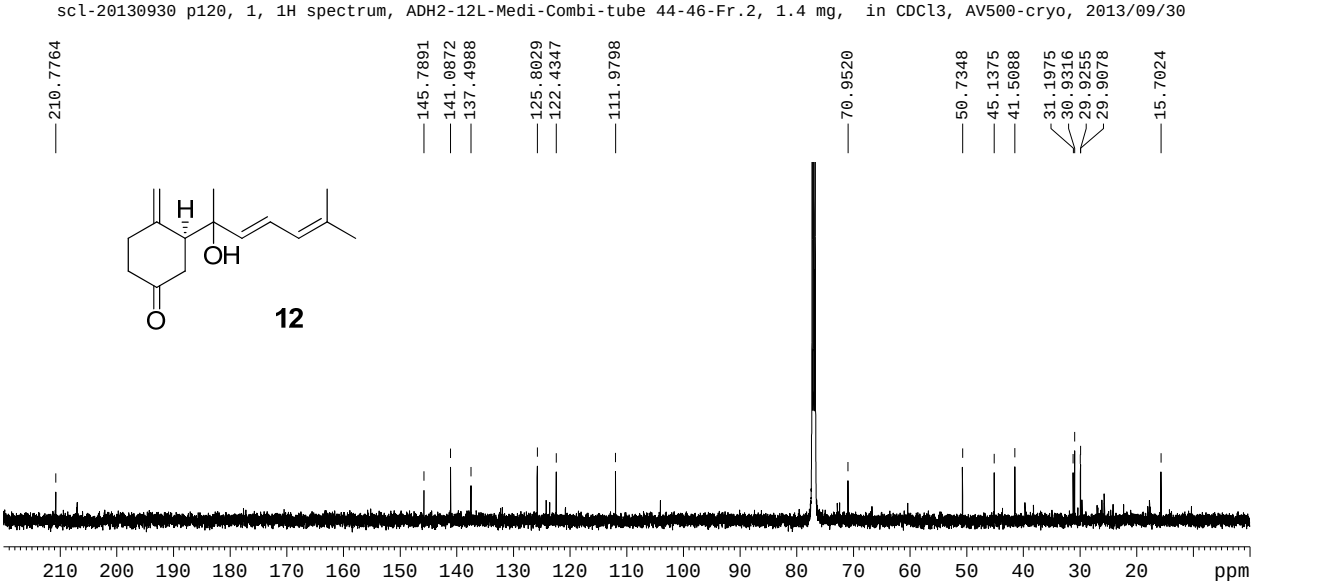


Figure S42. ^{1}H NMR spectrum of **13** ($CDCl_3$, 500 MHz).

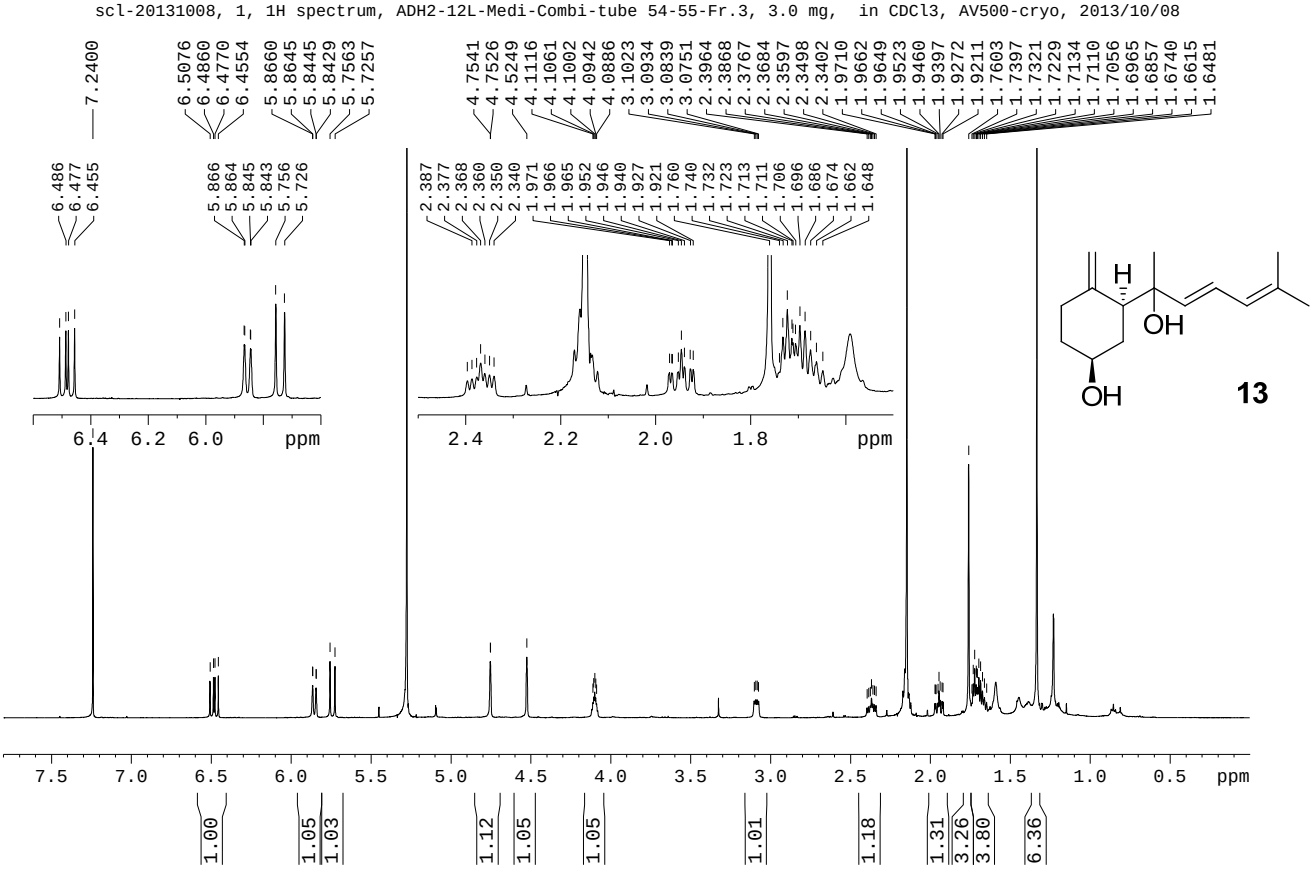


Figure S43. ^{13}C NMR spectrum of **13** ($CDCl_3$, 125 MHz).

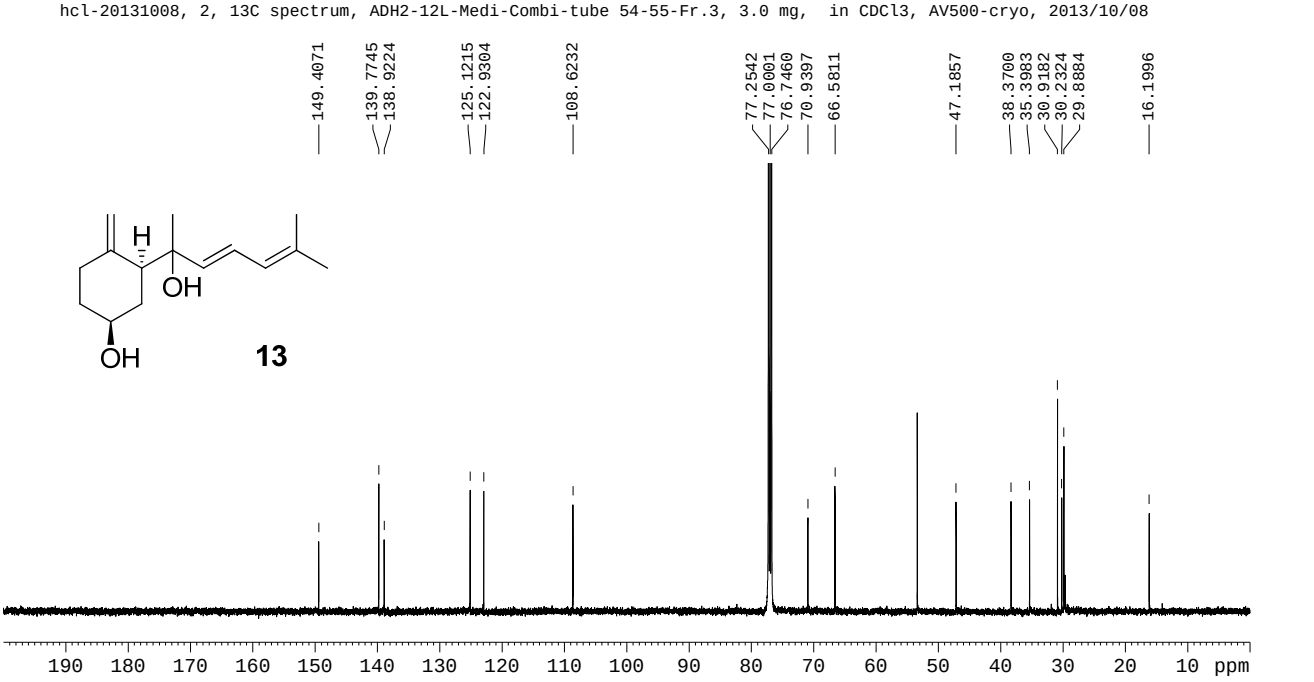


Figure S44. HSQC135 spectrum of **13** ($CDCl_3$, 500 MHz).

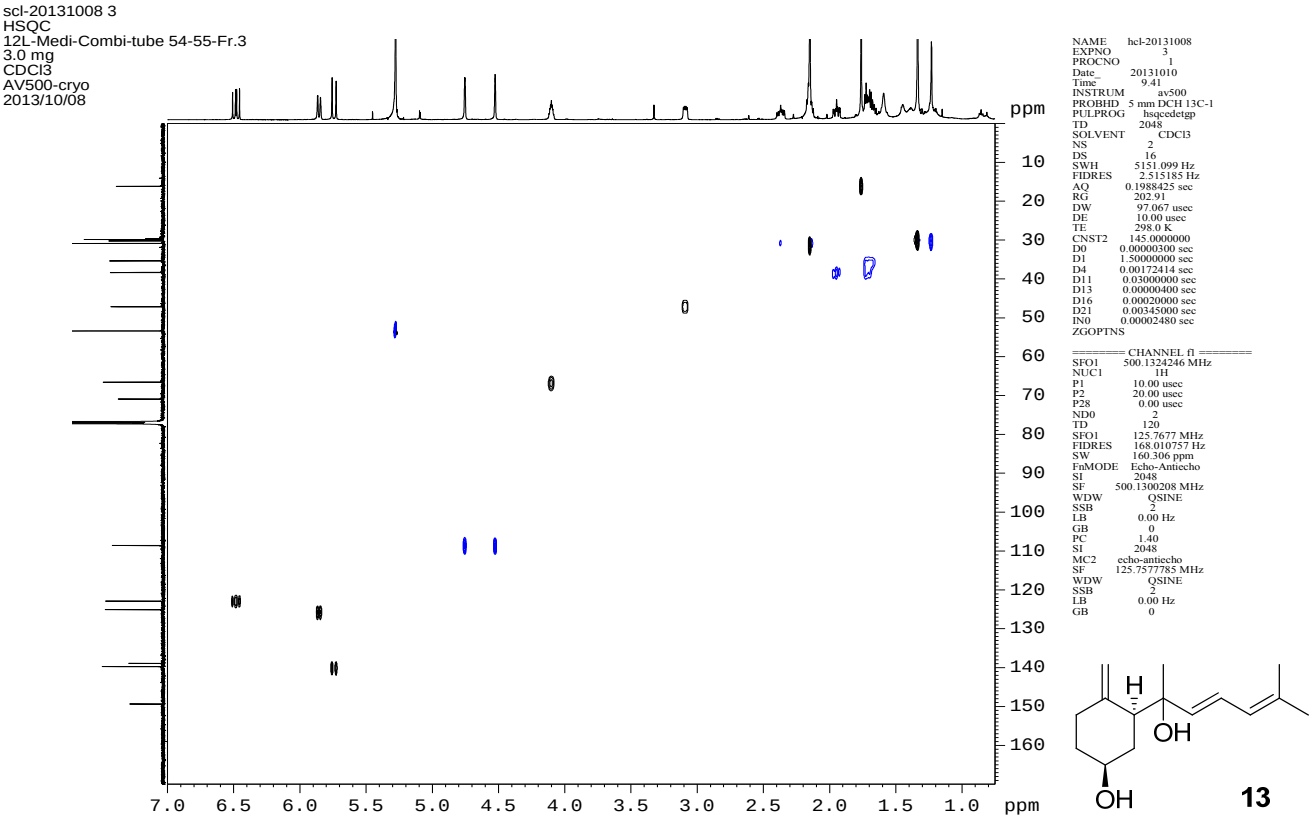


Figure S45. COSY spectrum of **13** ($CDCl_3$, 500 MHz).

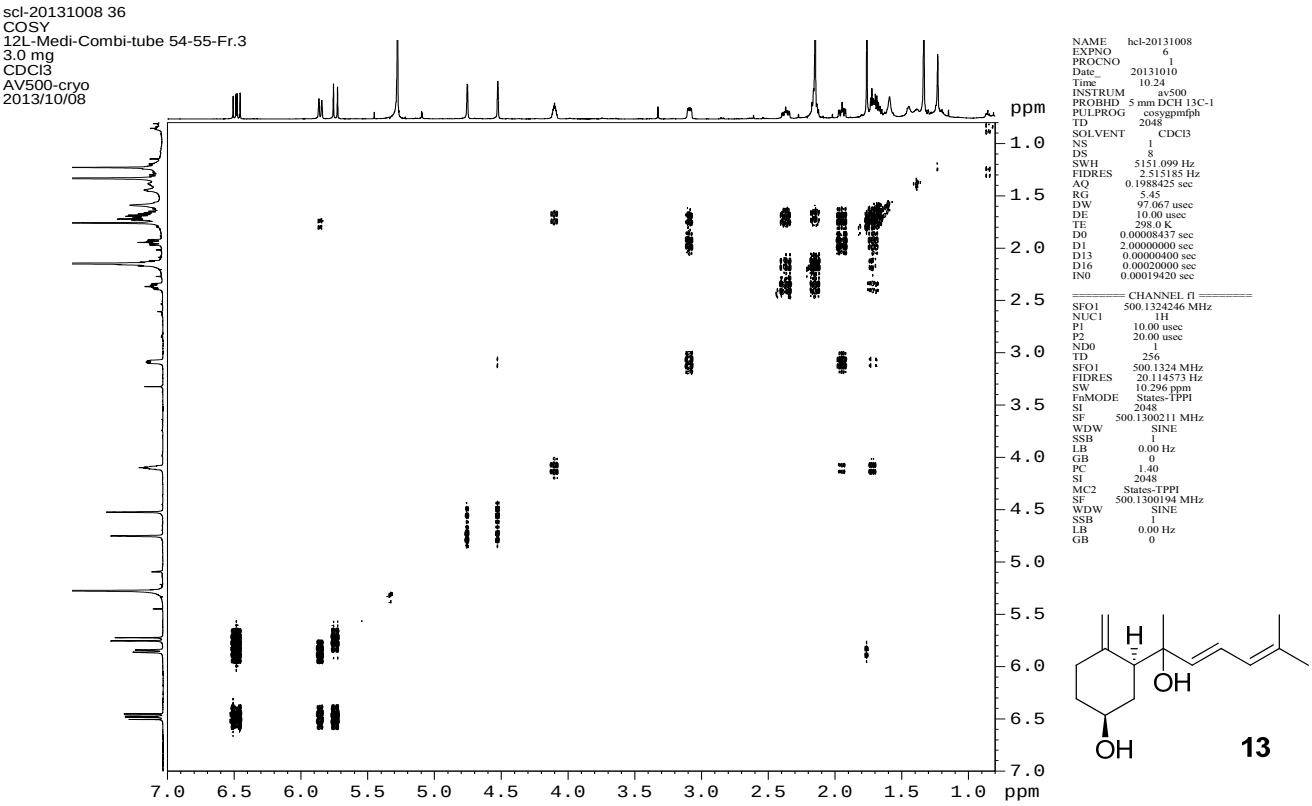


Figure S46. HMBC spectrum of **13** ($CDCl_3$, 500 MHz).

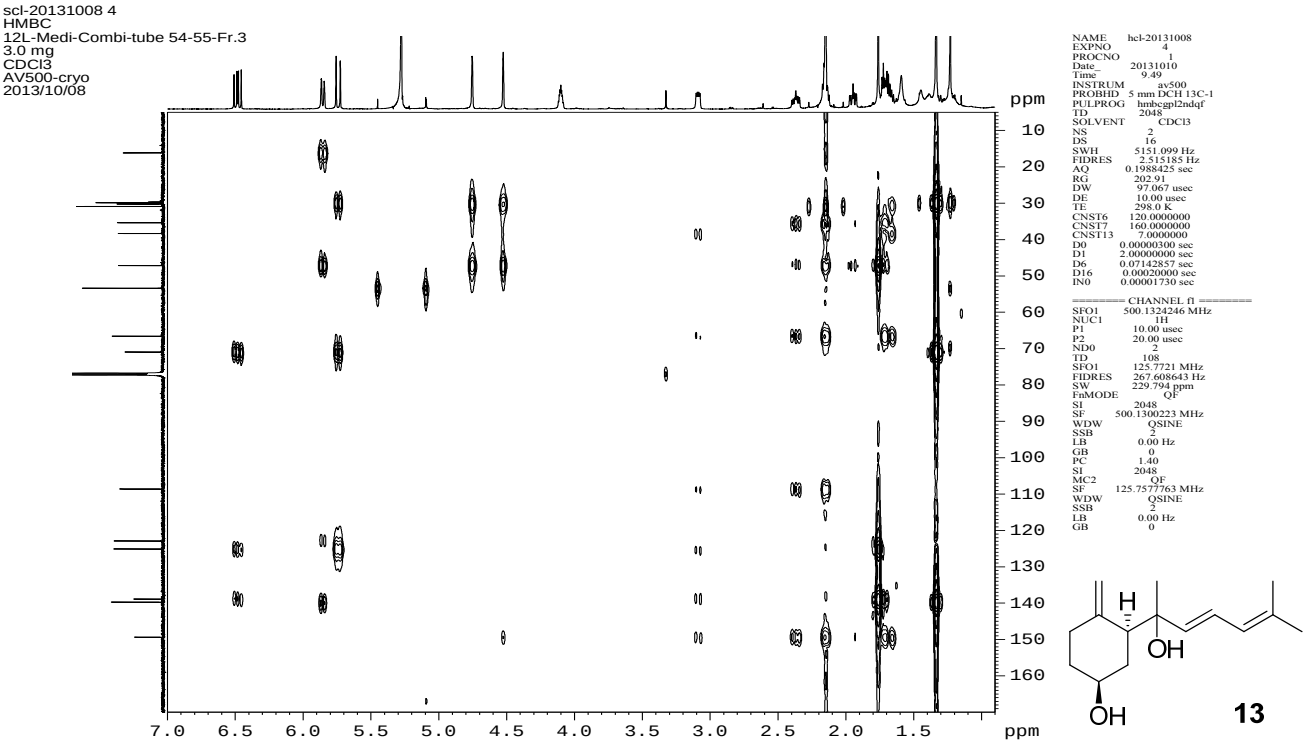


Figure S47. NOESY spectrum of **13** ($CDCl_3$, 500 MHz).

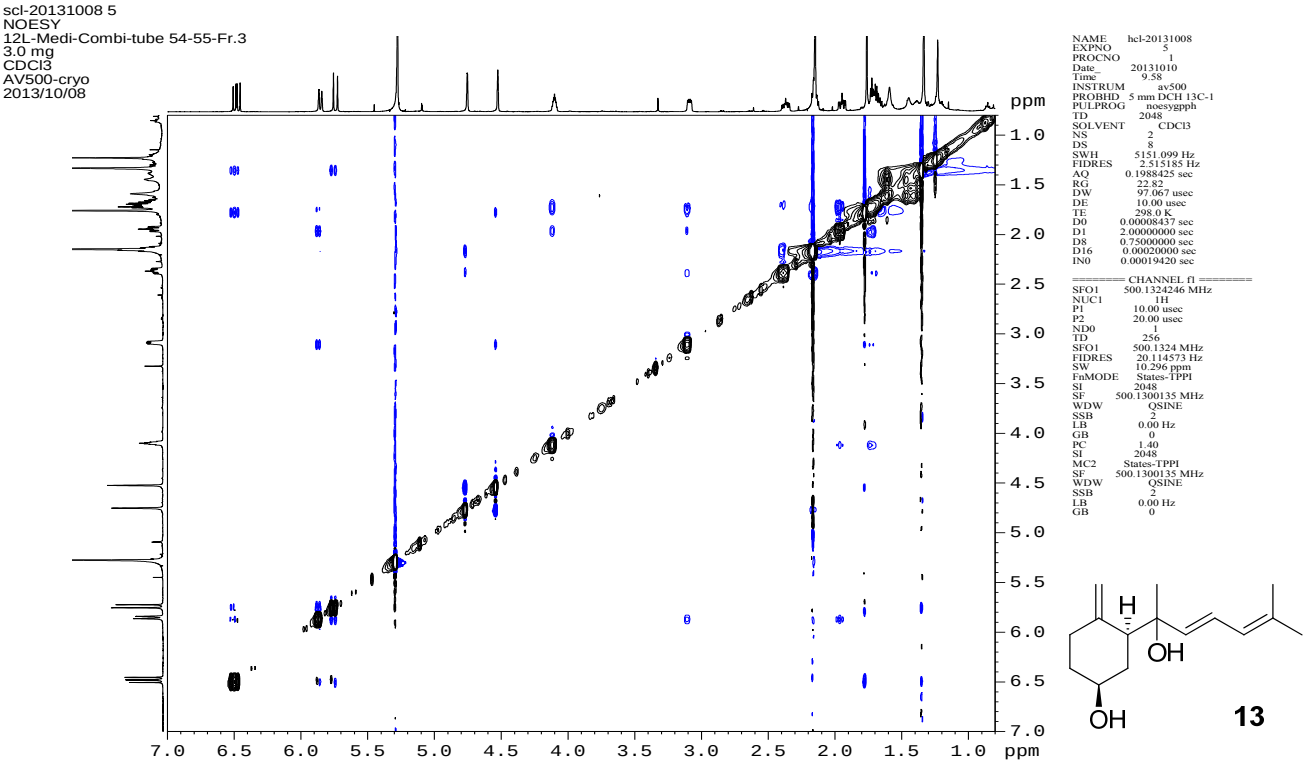


Figure S48. ^{1}H NMR spectrum of **14** ($CDCl_3$, 500 MHz).

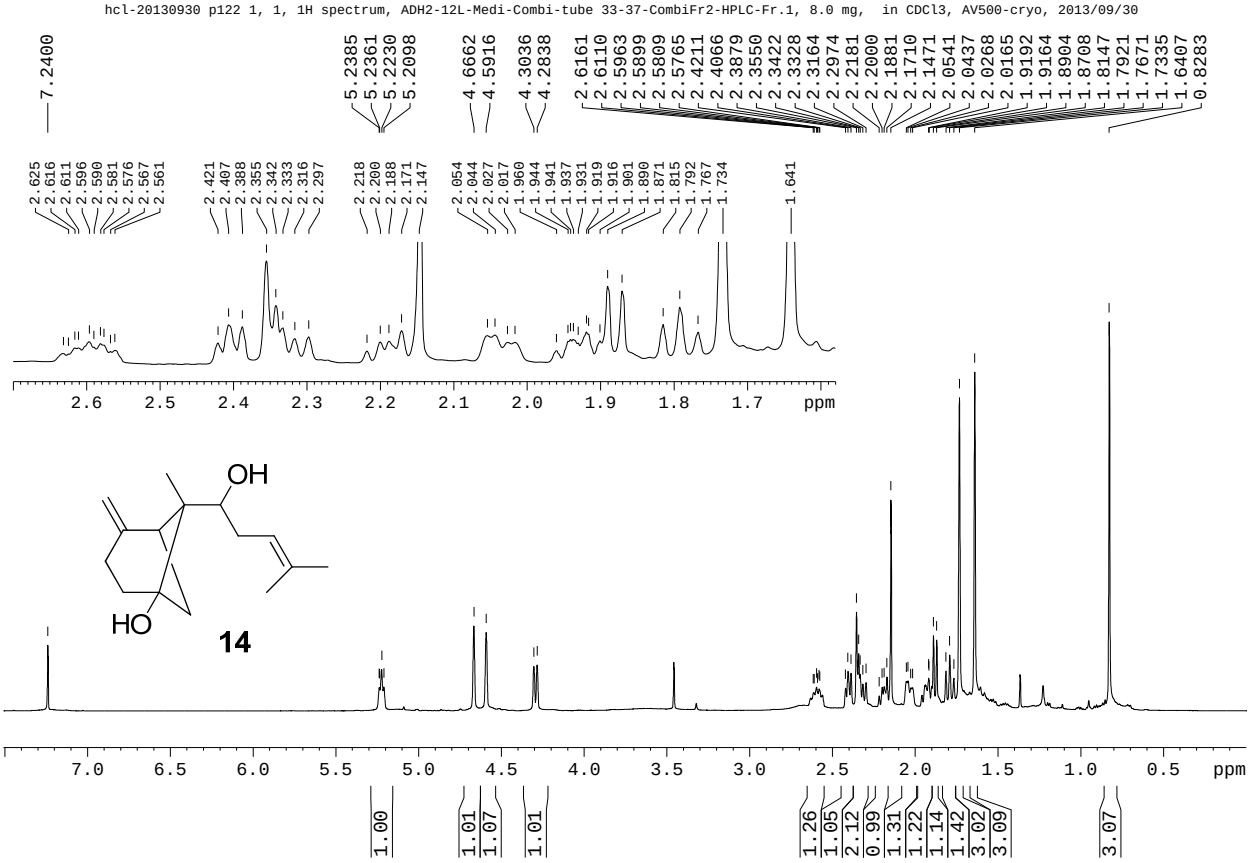


Figure S49. ^{13}C NMR spectrum of **14** ($CDCl_3$, 125 MHz).

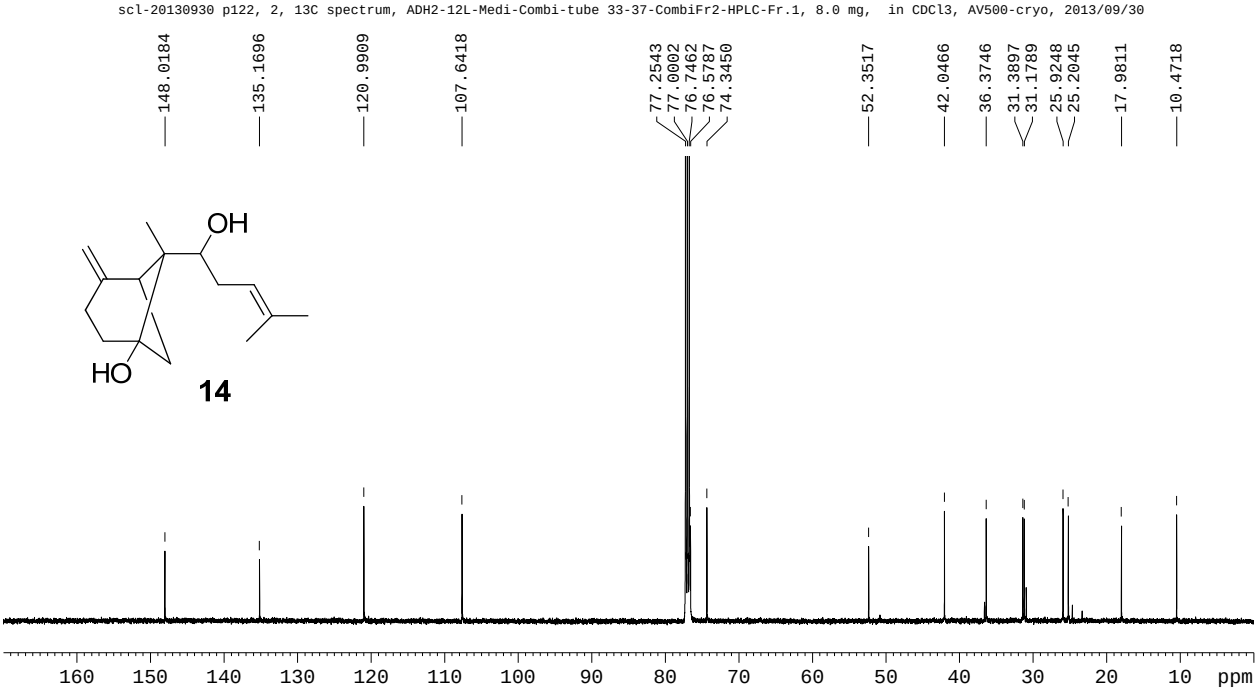


Figure S50. HSQC135 spectrum of **14** ($CDCl_3$, 500 MHz).

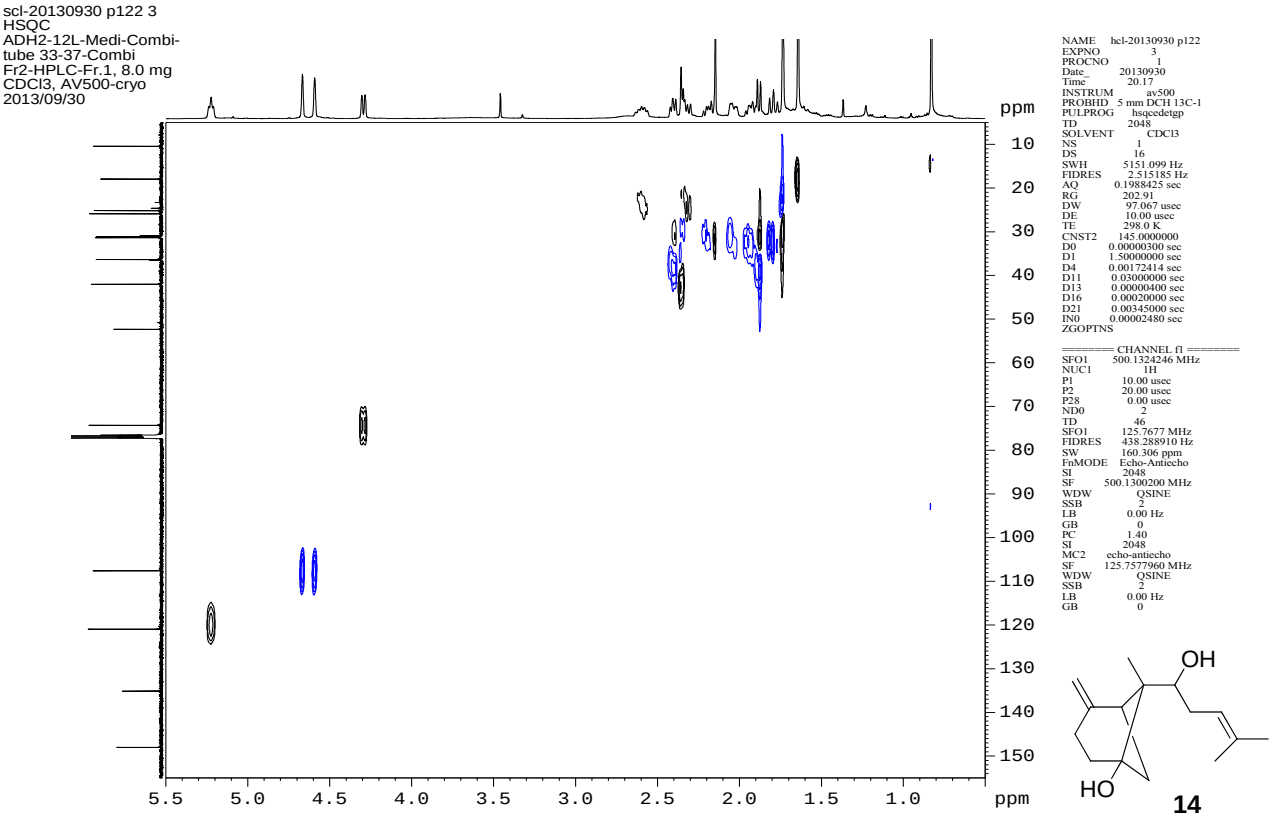


Figure S51. HMBC spectrum of **14** ($CDCl_3$, 500 MHz).

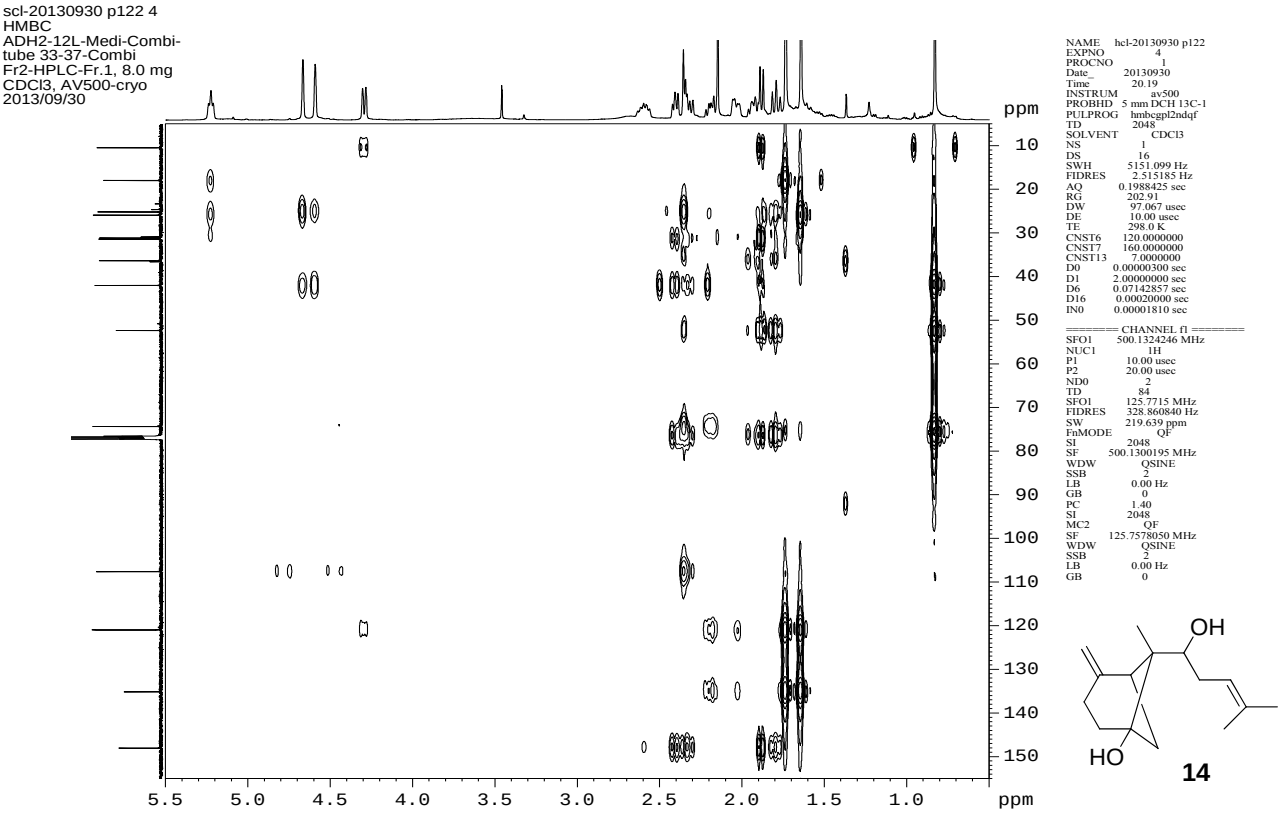


Figure S52. NOESY spectrum of **14** ($CDCl_3$, 500 MHz).

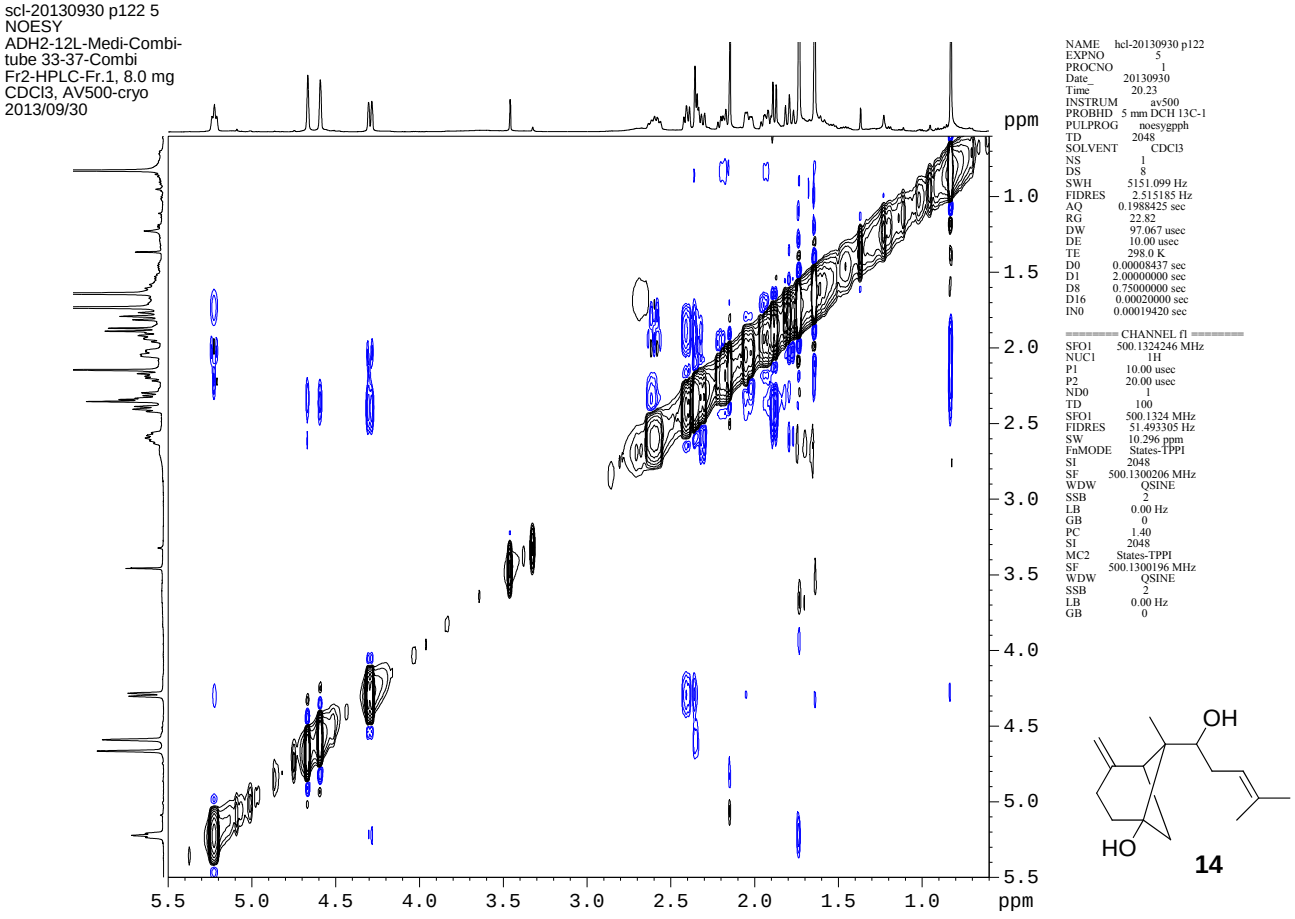


Figure S53. ^{1}H NMR spectrum of **15** ($CDCl_3$, 500 MHz).

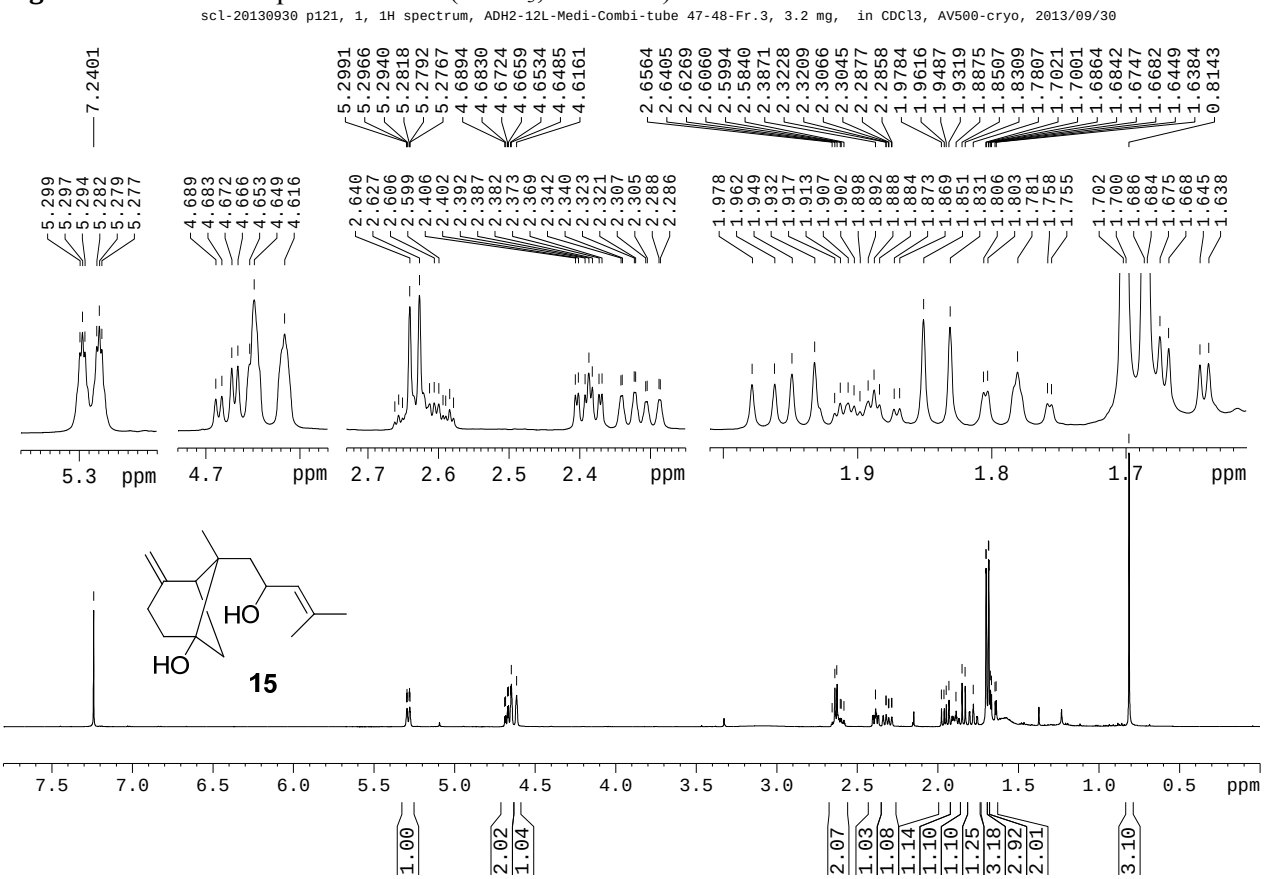


Figure S54. ^{13}C NMR spectrum of **15** ($CDCl_3$, 125 MHz).

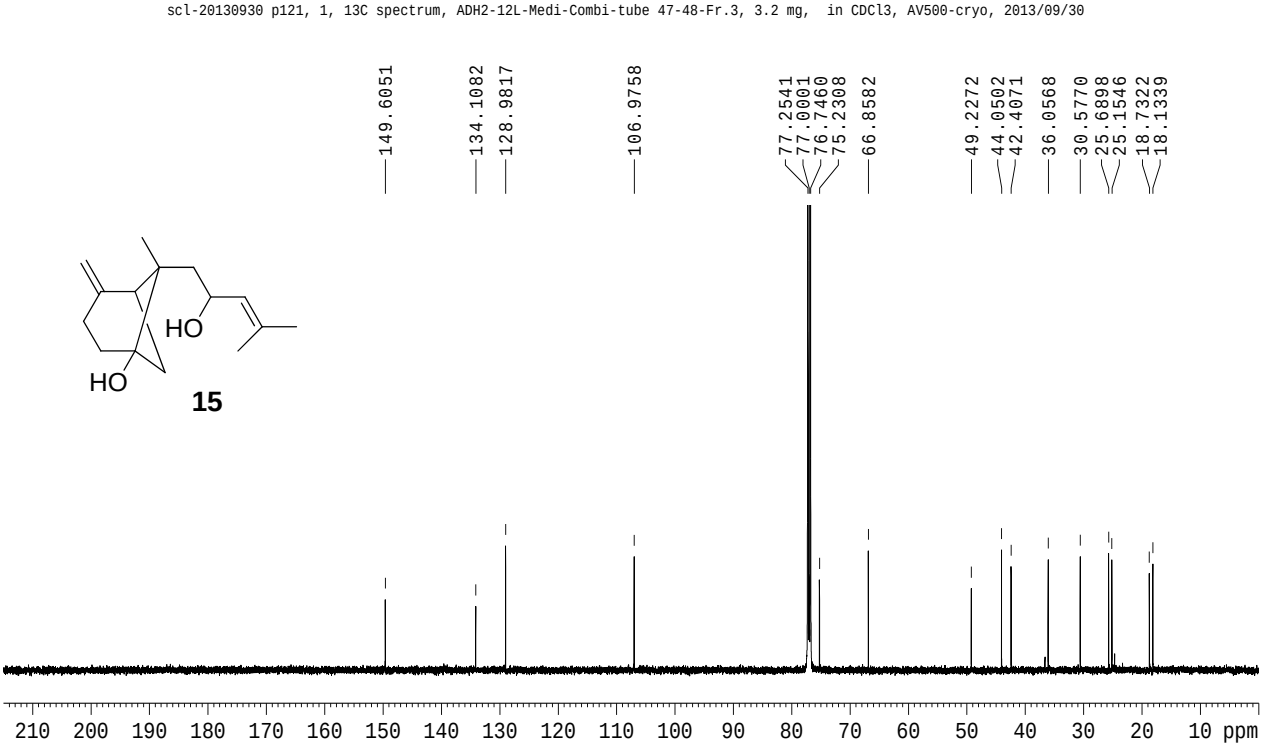


Figure S55. HSQC135 spectrum of **15** ($CDCl_3$, 500 MHz).

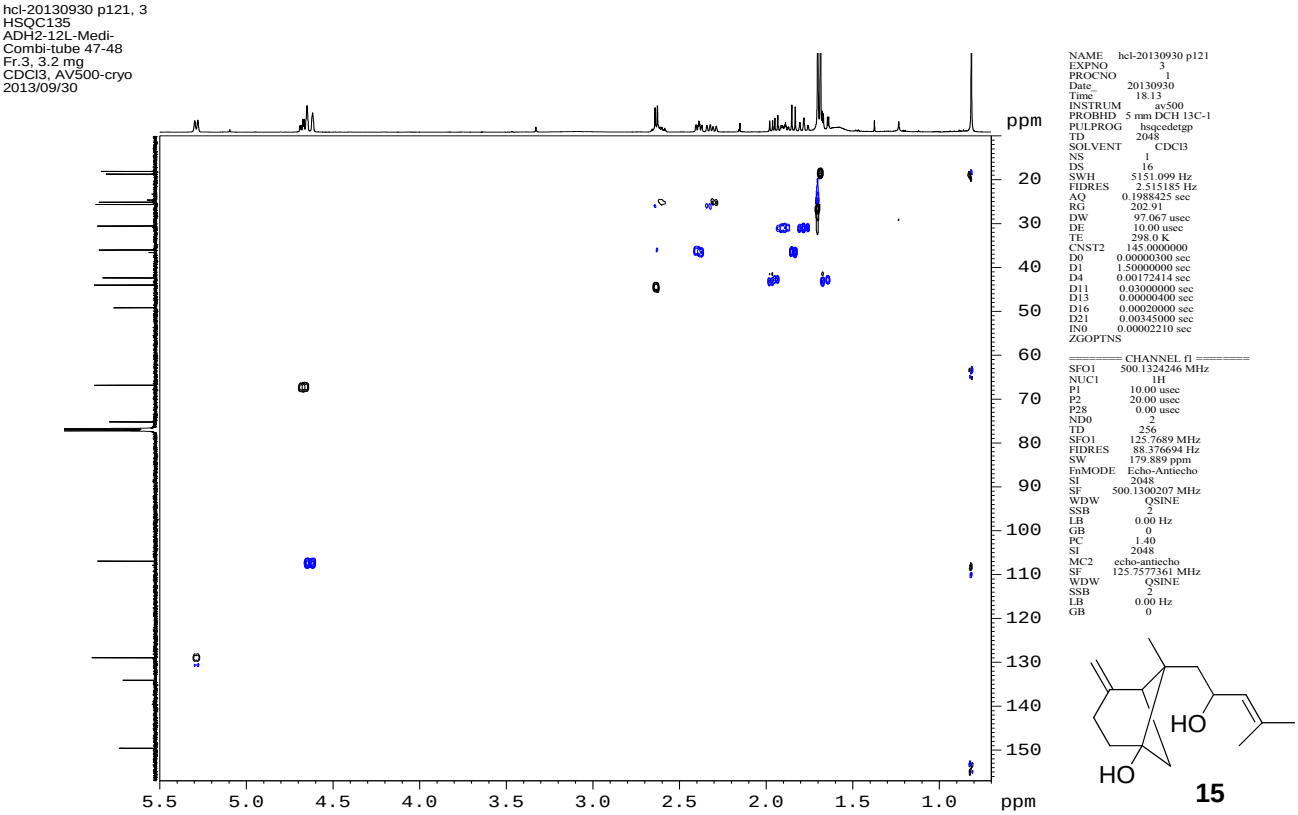


Figure S56. HMBC spectrum of **15** ($CDCl_3$, 500 MHz).

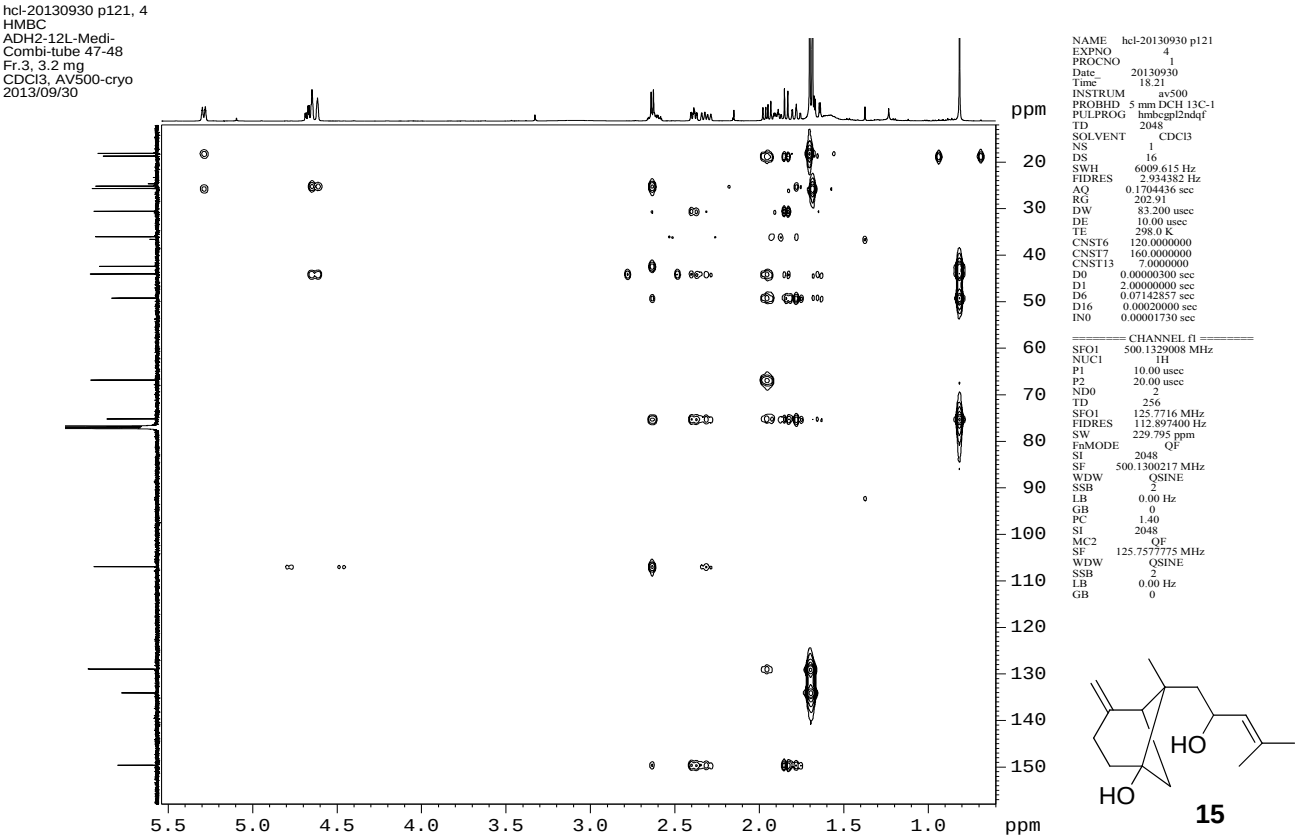


Figure S57. NOESY spectrum of **15** ($CDCl_3$, 500 MHz).

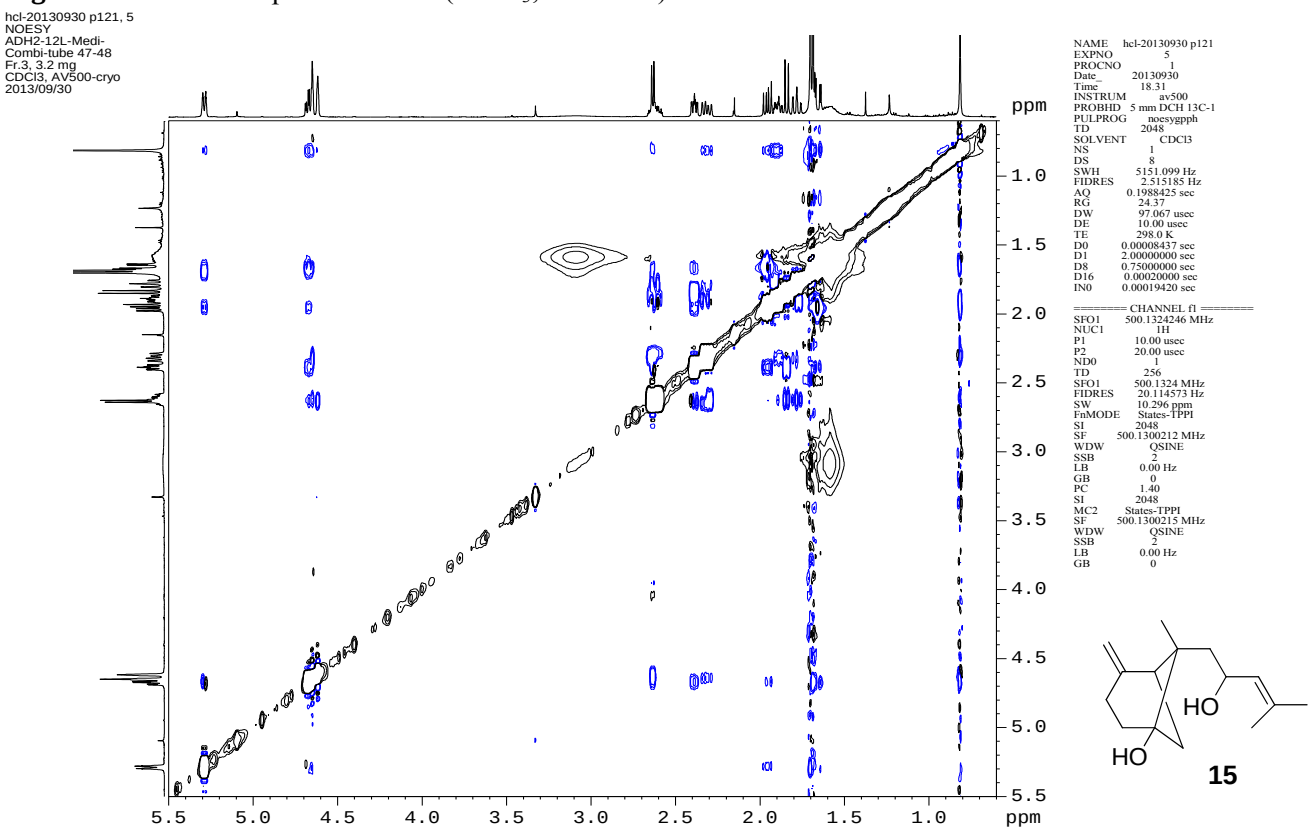


Figure S58. ^{1}H NMR spectrum of **16** ($CDCl_3$, 500 MHz).

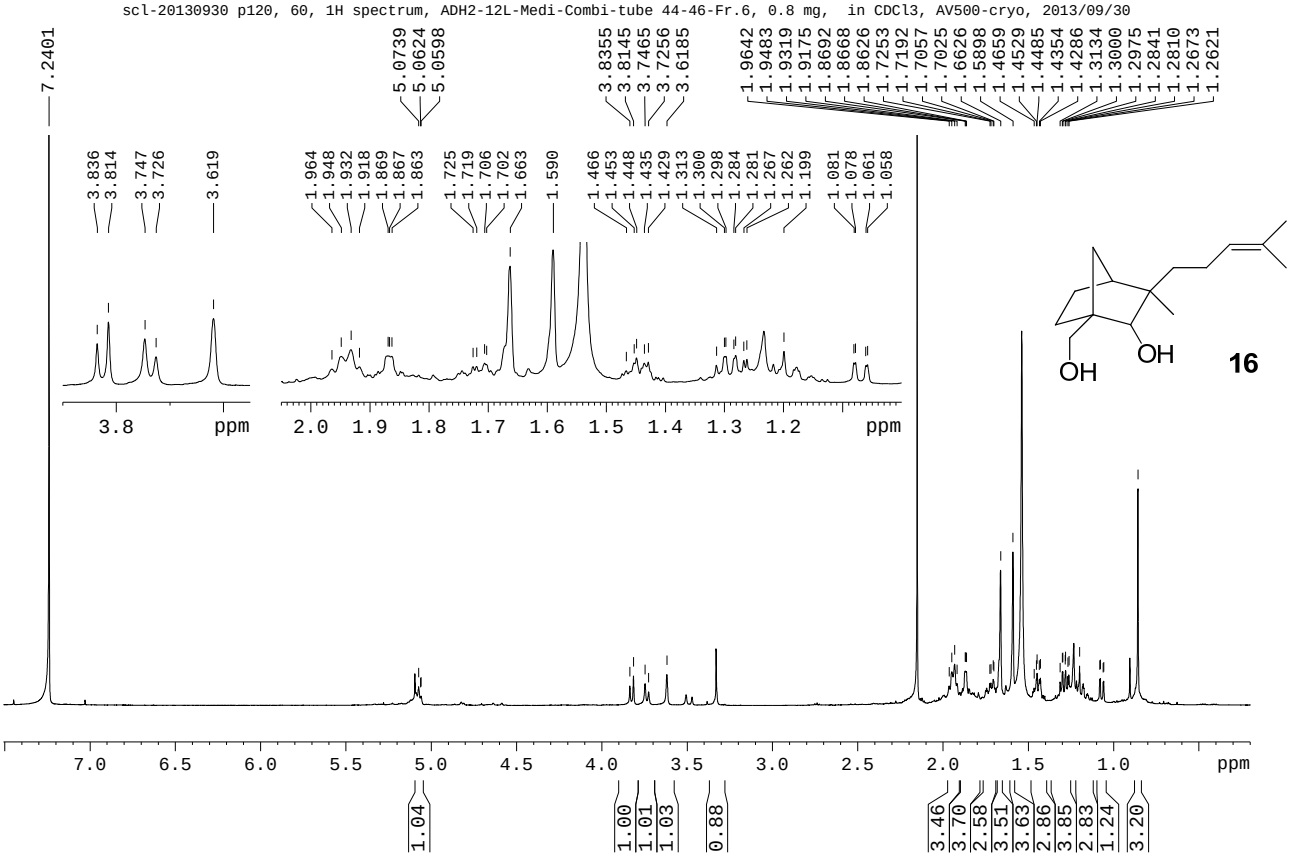


Figure S59. ^{13}C NMR spectrum of **16** ($CDCl_3$, 125 MHz).

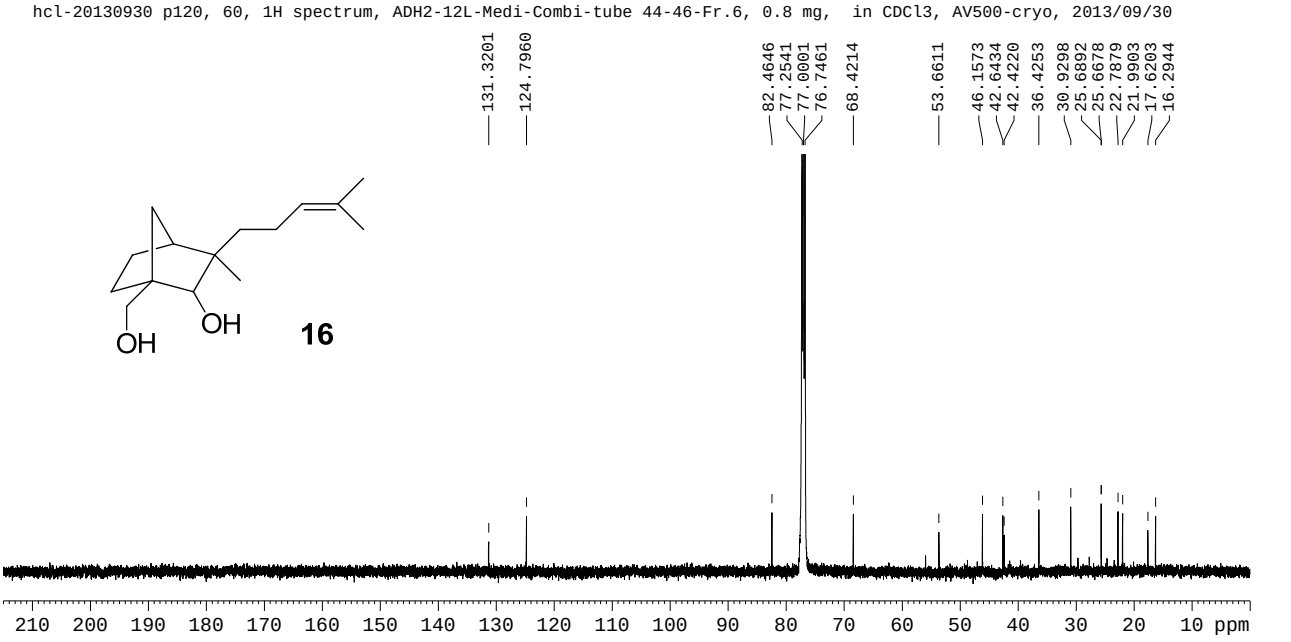


Figure S60. HSQC135 spectrum of **16** ($CDCl_3$, 500 MHz).

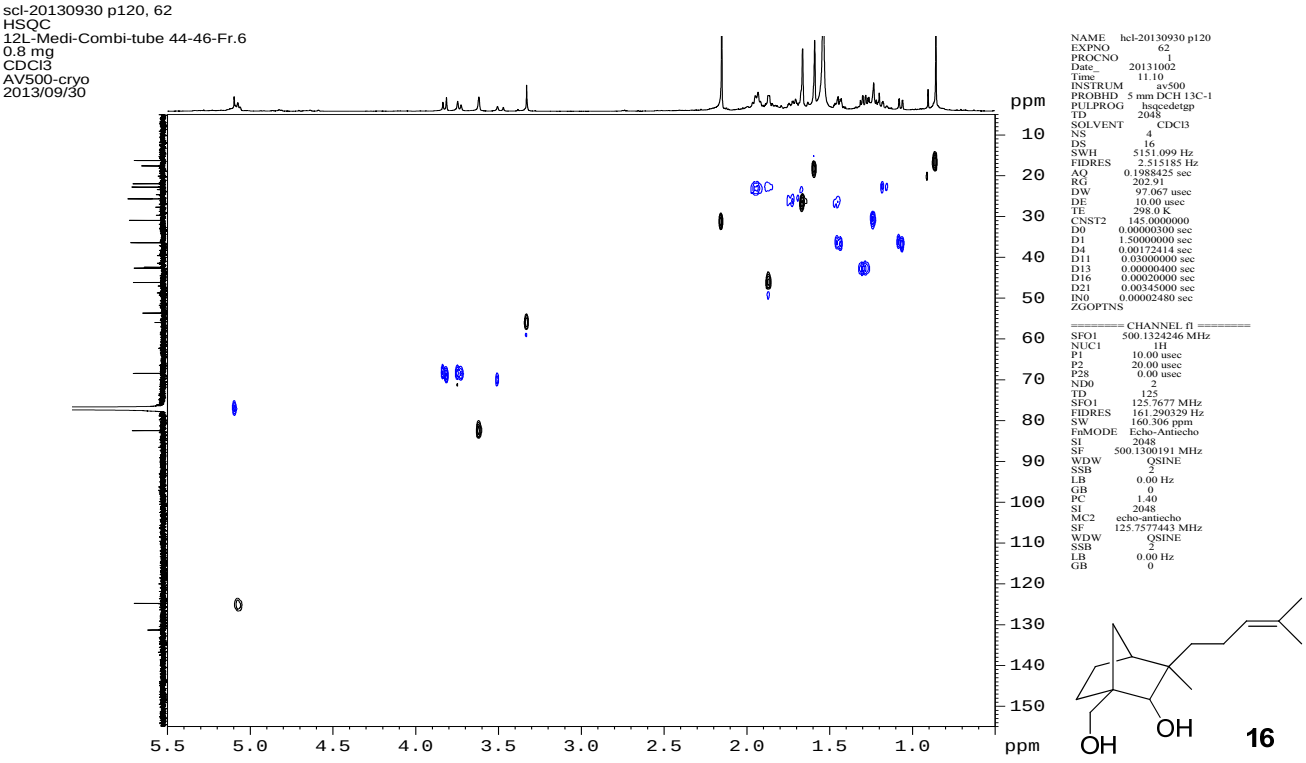


Figure S61. HMBC spectrum of **16** ($CDCl_3$, 500 MHz).

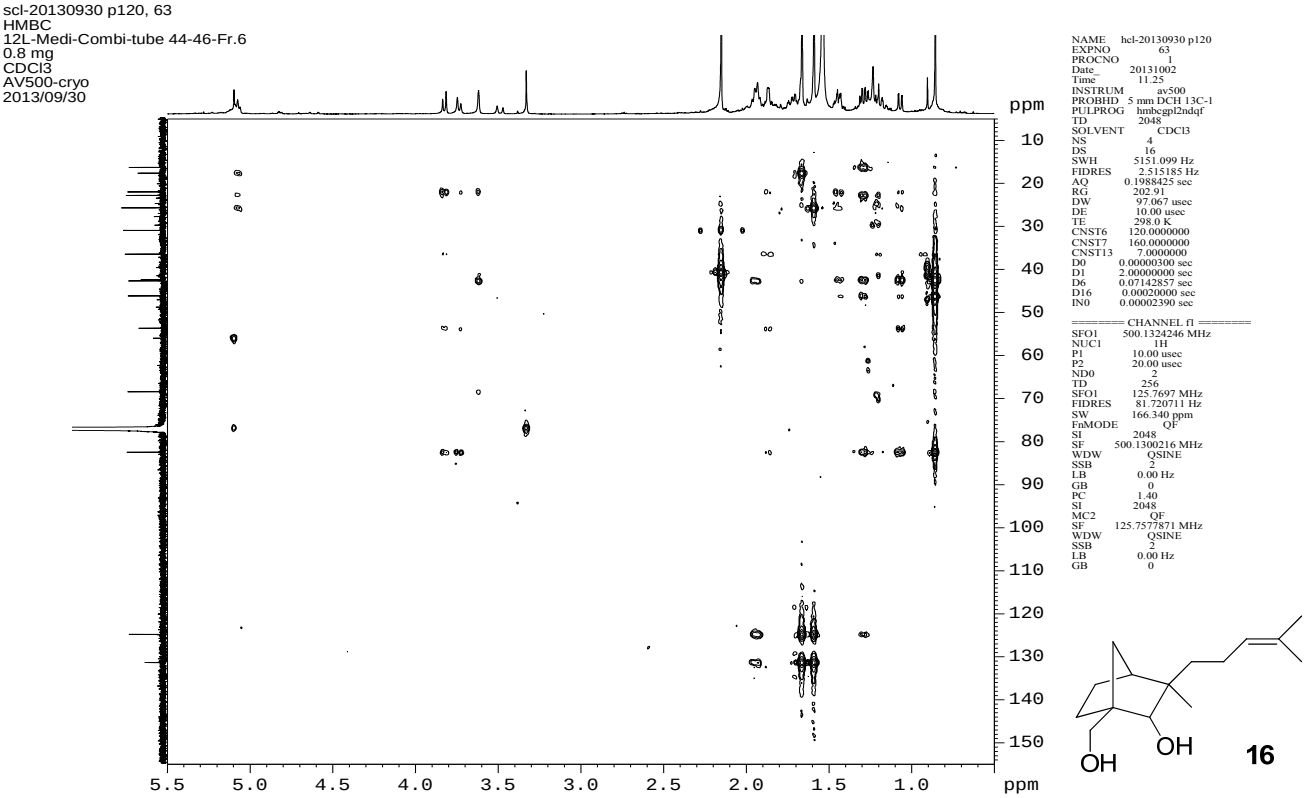


Figure S62. NOESY spectrum of **16** ($CDCl_3$, 500 MHz).

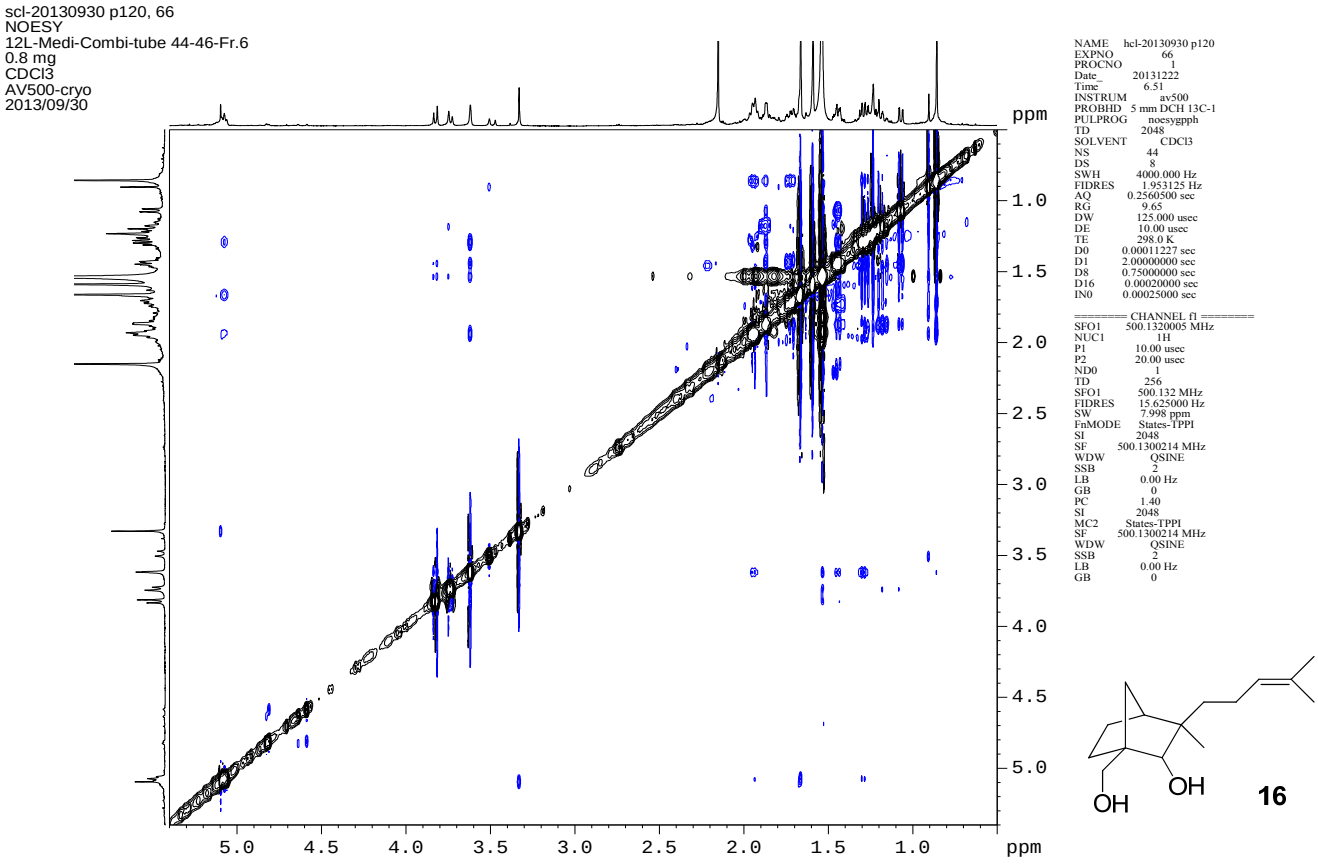


Figure S63. ^{1}H NMR spectrum of **17** ($CDCl_3$, 500 MHz).

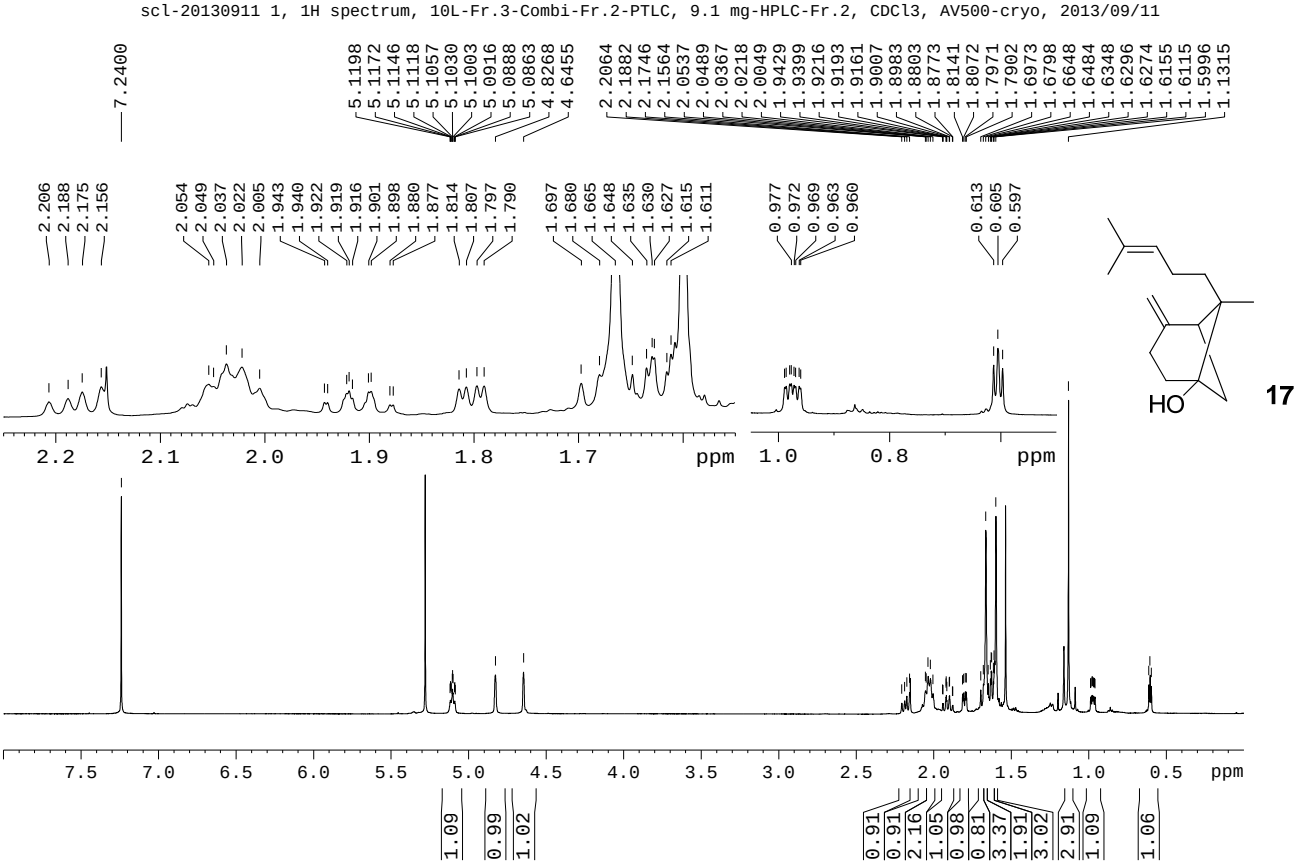


Figure S64. ^{13}C NMR spectrum of **17** ($CDCl_3$, 125 MHz).

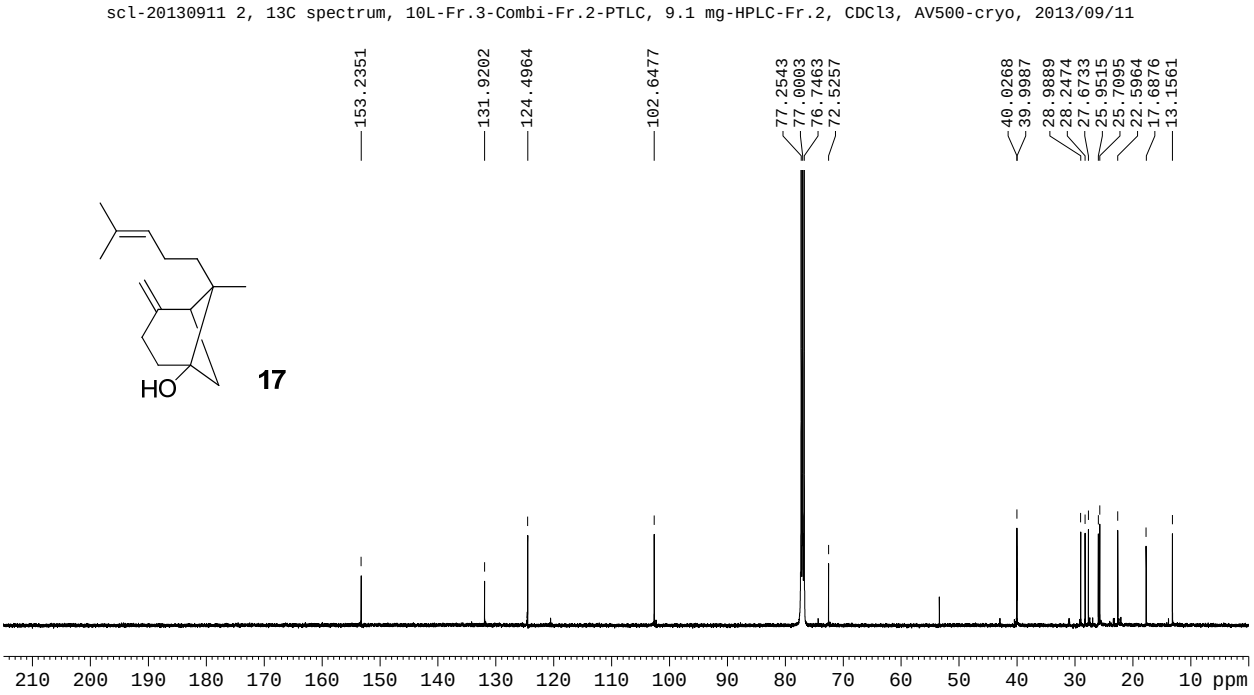


Figure S65. HSQC135 spectrum of **17** ($CDCl_3$, 500 MHz).

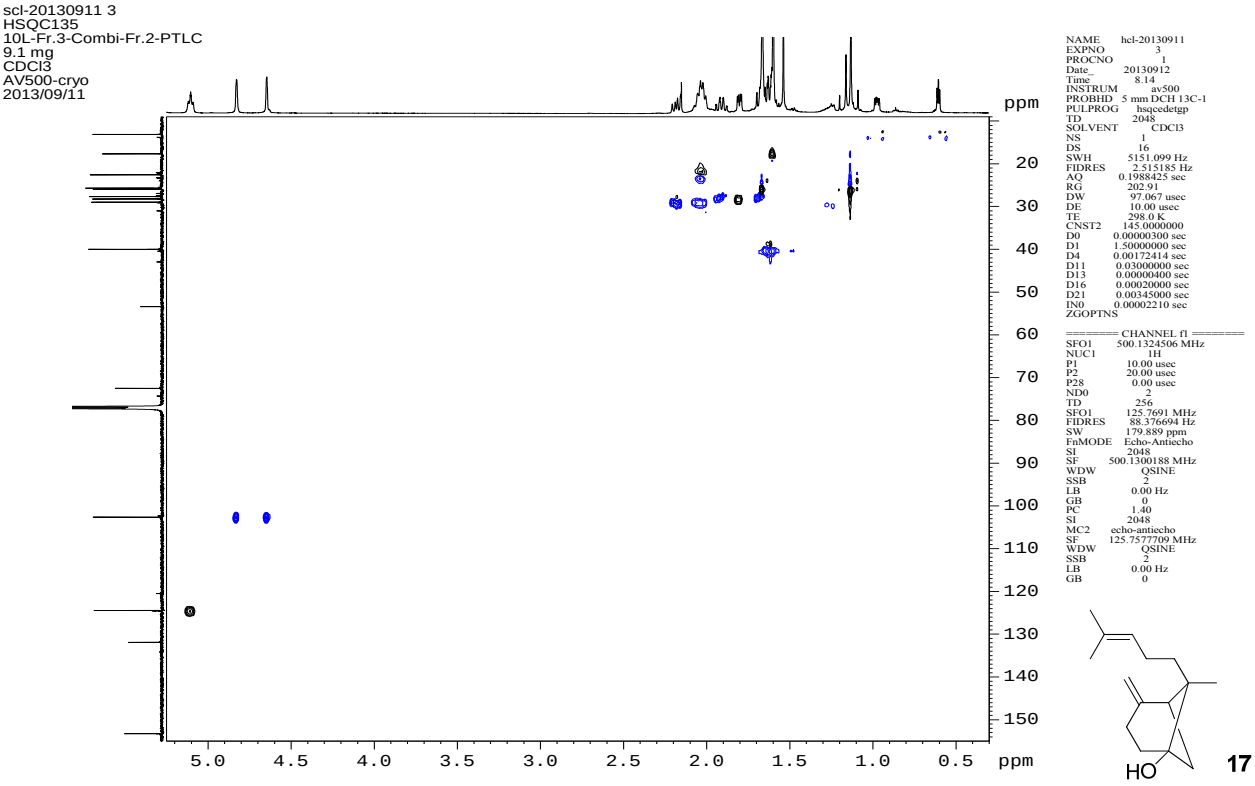


Figure S66. HMBC spectrum of **17** ($CDCl_3$, 500 MHz).

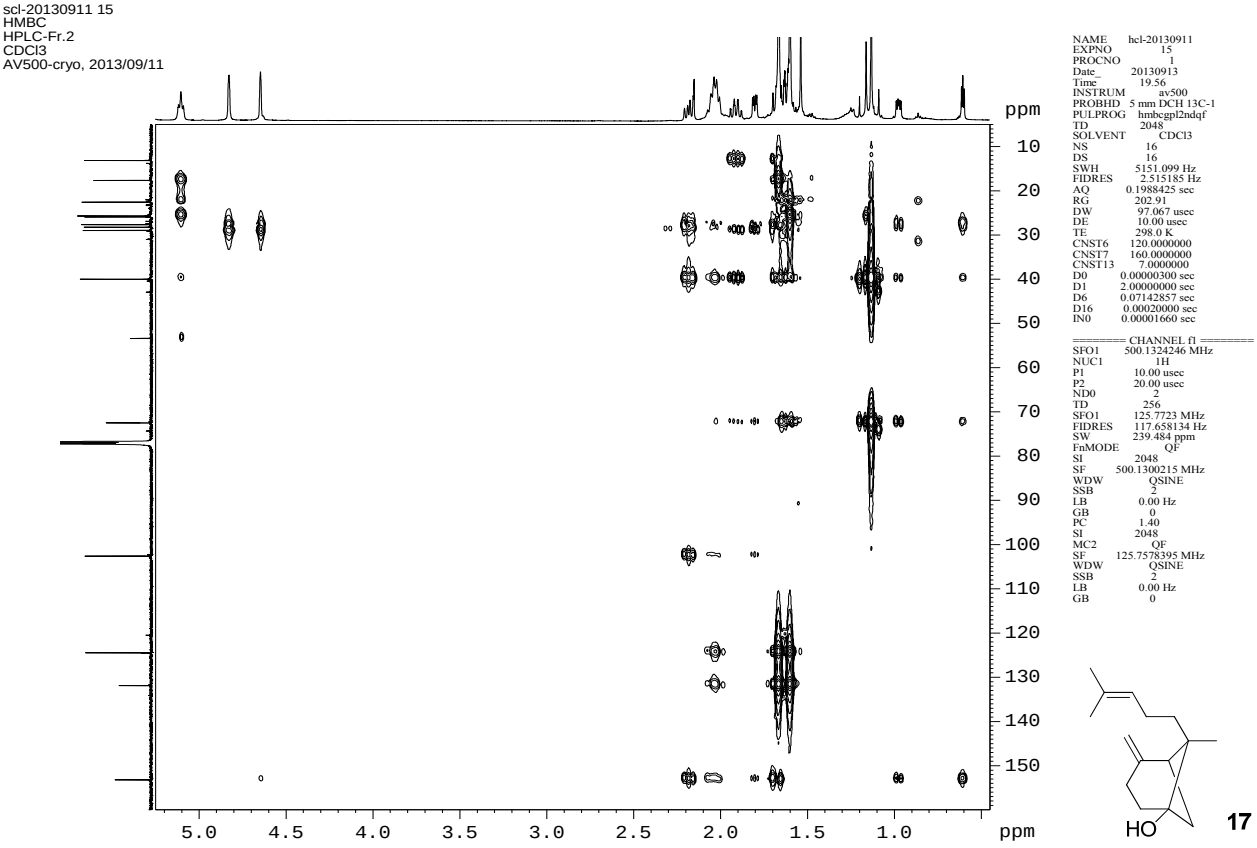


Figure S67. COSY spectrum of **17** ($CDCl_3$, 500 MHz).

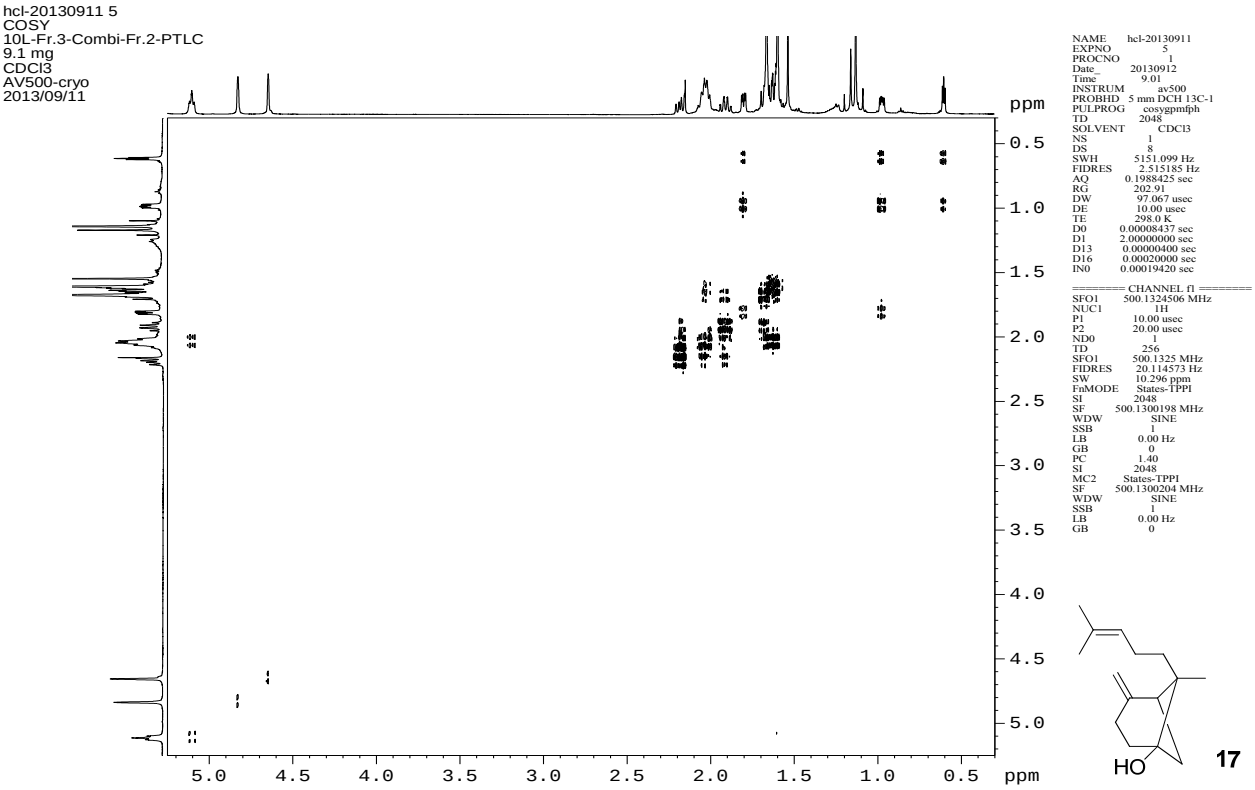


Figure S68. NOESY spectrum of **17** ($CDCl_3$, 500 MHz).

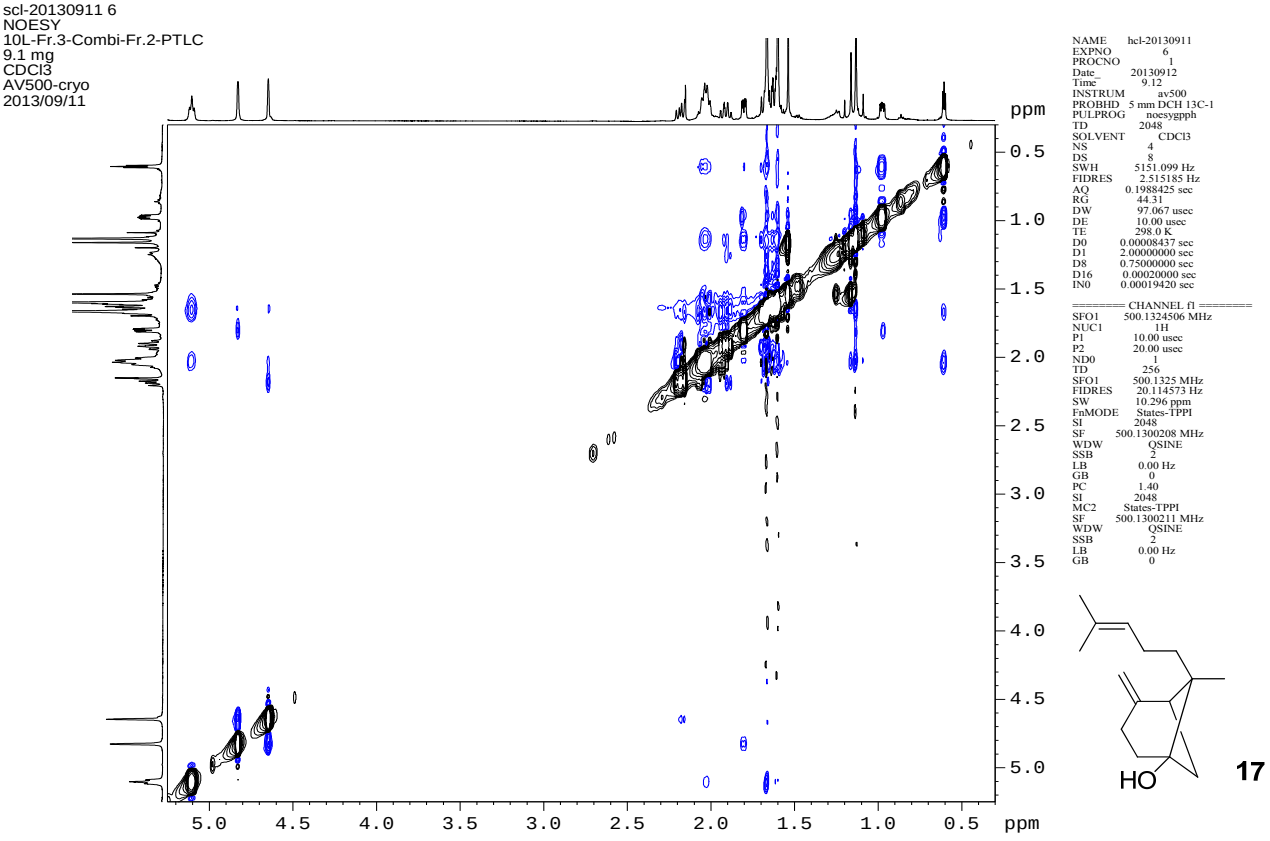


Figure S69. ^{1}H NMR spectrum of **18** ($CDCl_3$, 500 MHz).

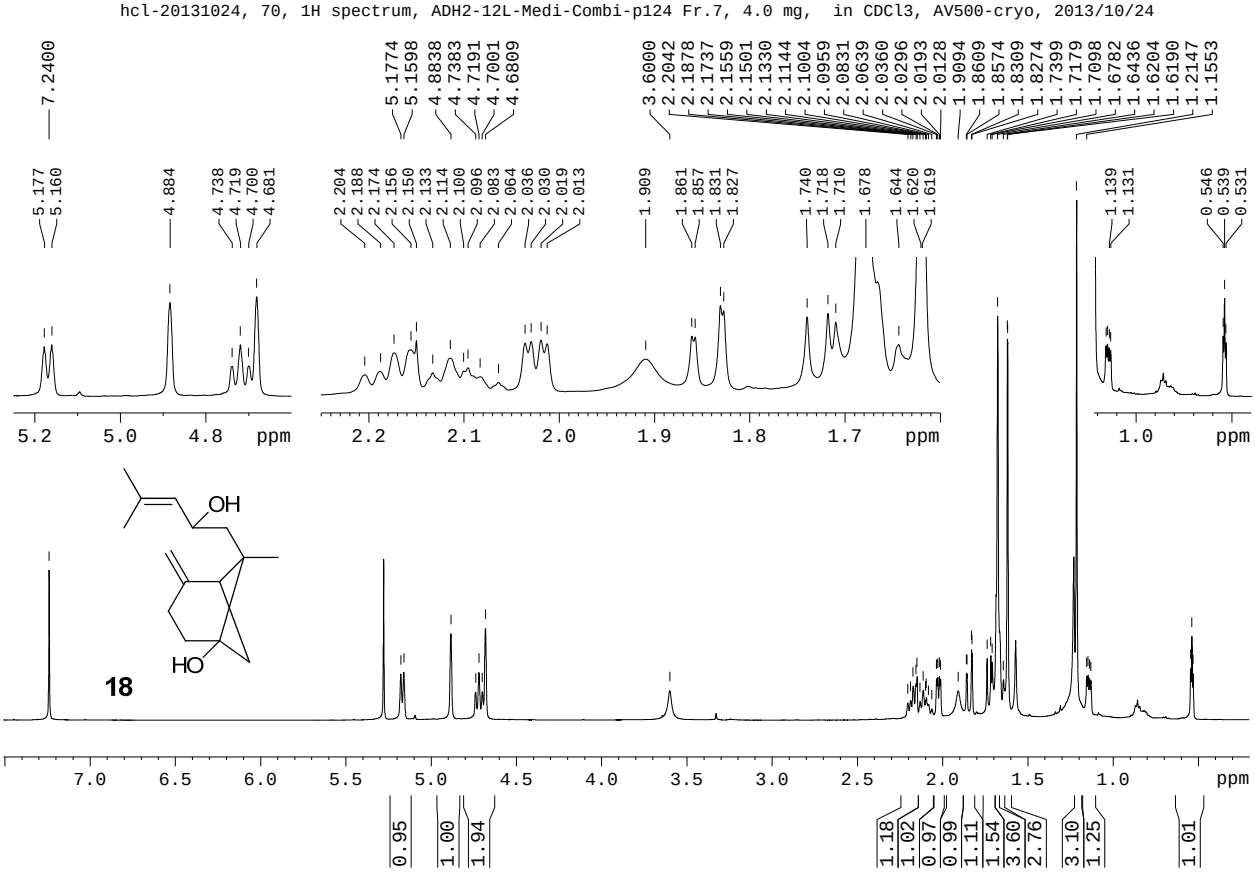


Figure S70. ^{13}C NMR spectrum of **18** ($CDCl_3$, 125 MHz).

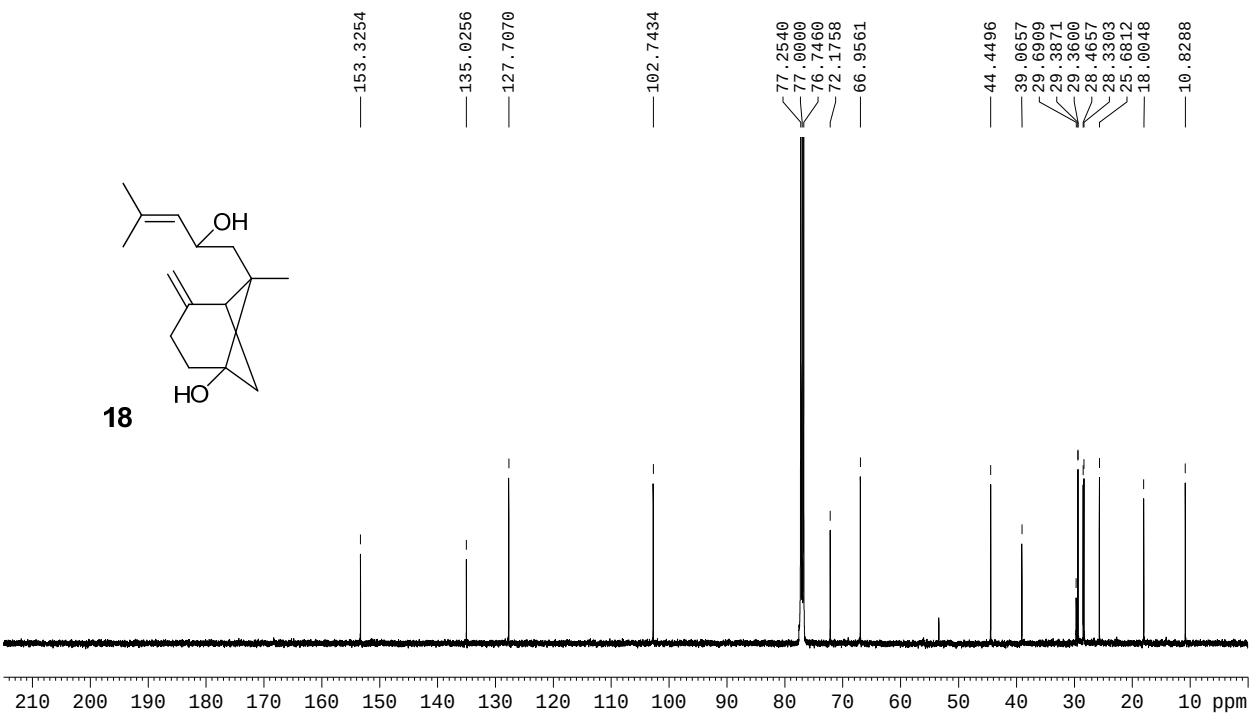
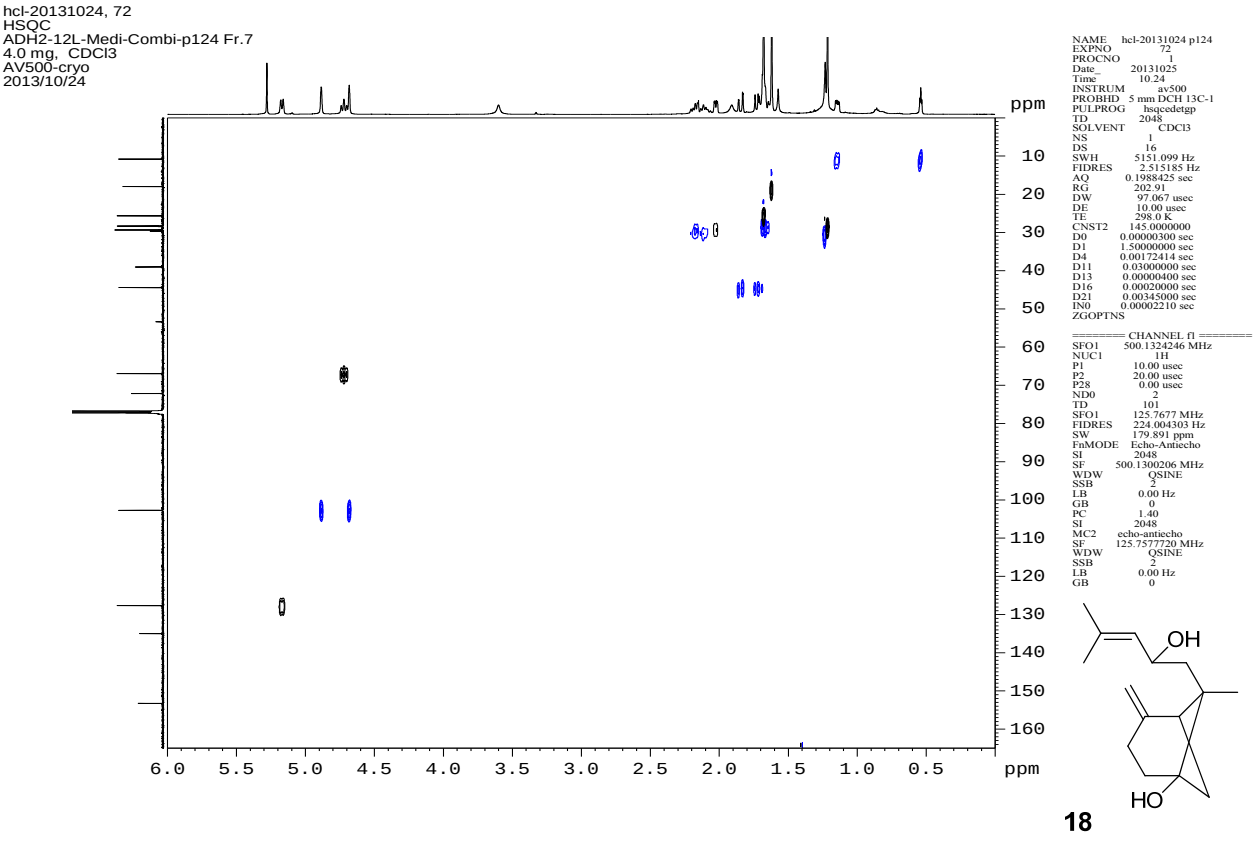
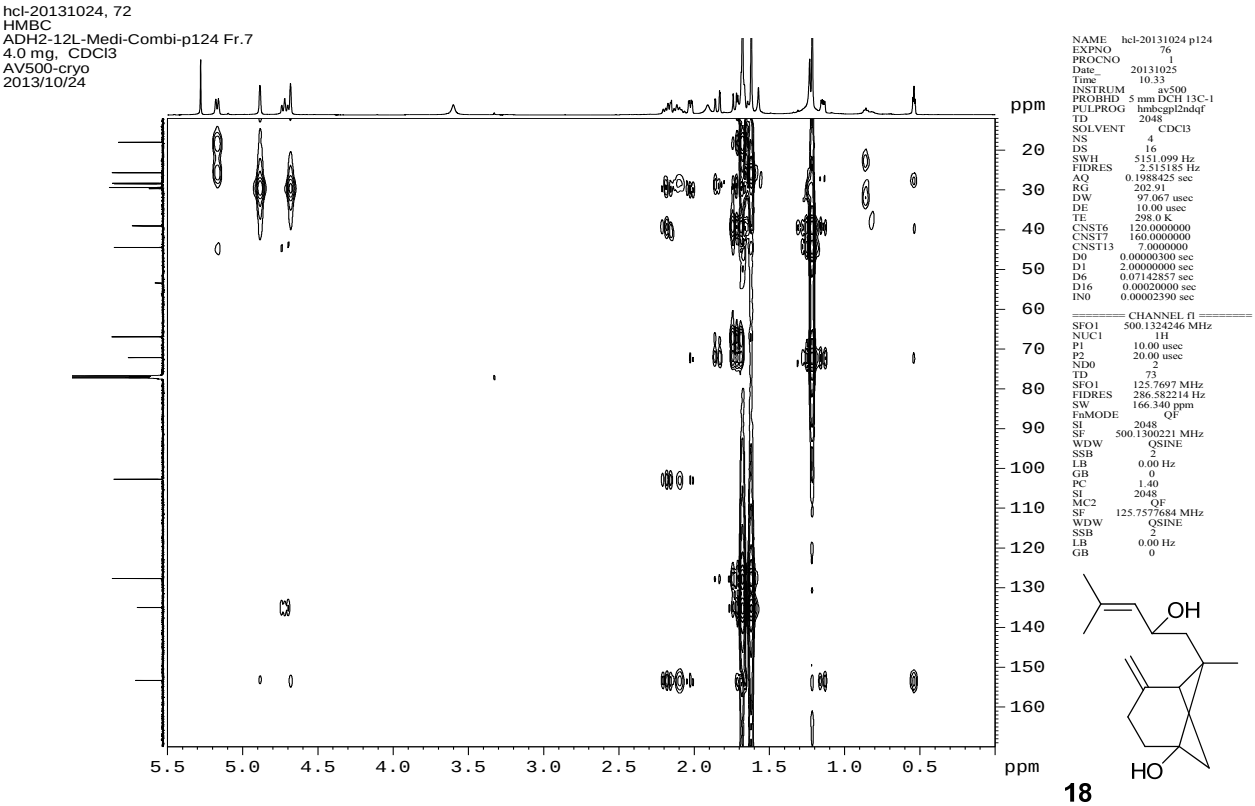


Figure S71. HSQC135 spectrum of **18** ($CDCl_3$, 500 MHz).



18

Figure S72. HMBC spectrum of **18** ($CDCl_3$, 500 MHz).



18

Figure S73. NOESY spectrum of **18** ($CDCl_3$, 500 MHz).

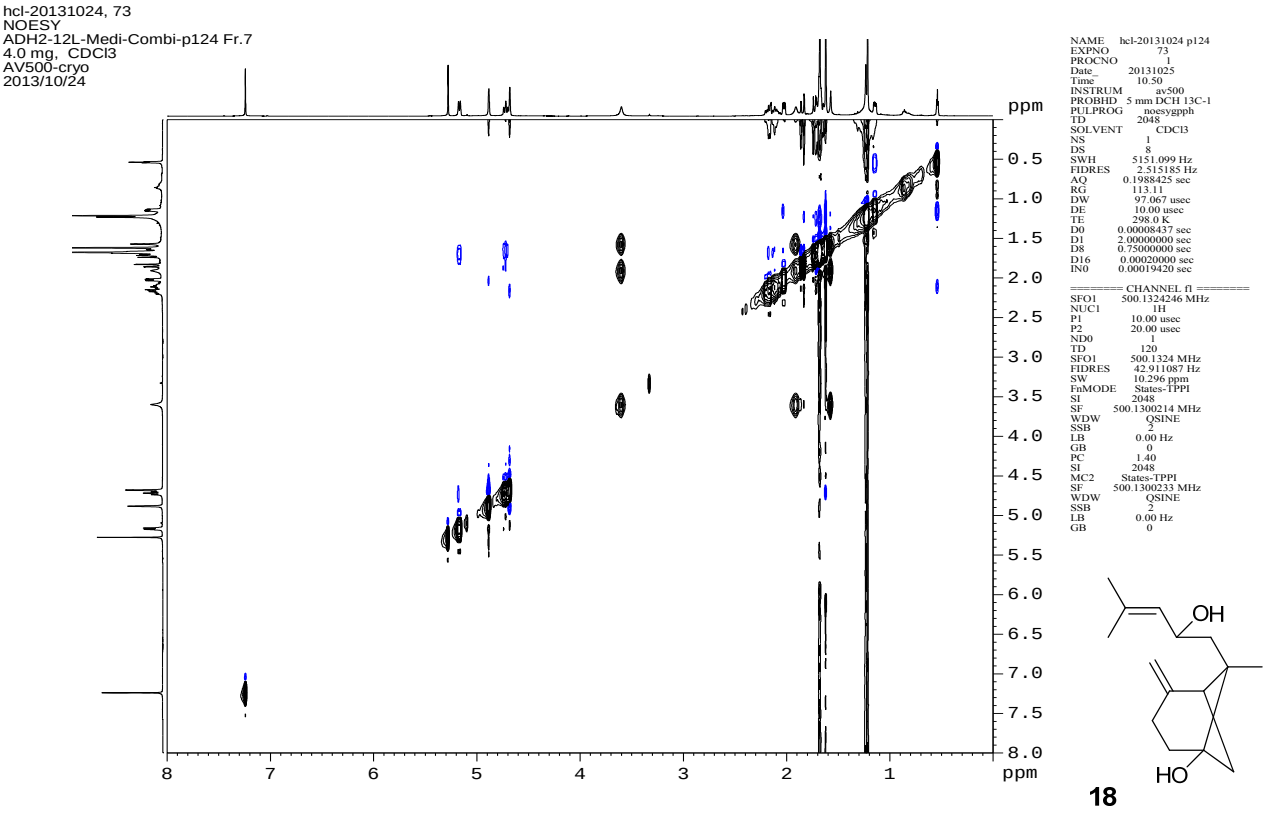


Figure S74.TOCSY spectrum of **18** ($CDCl_3$, 500 MHz).

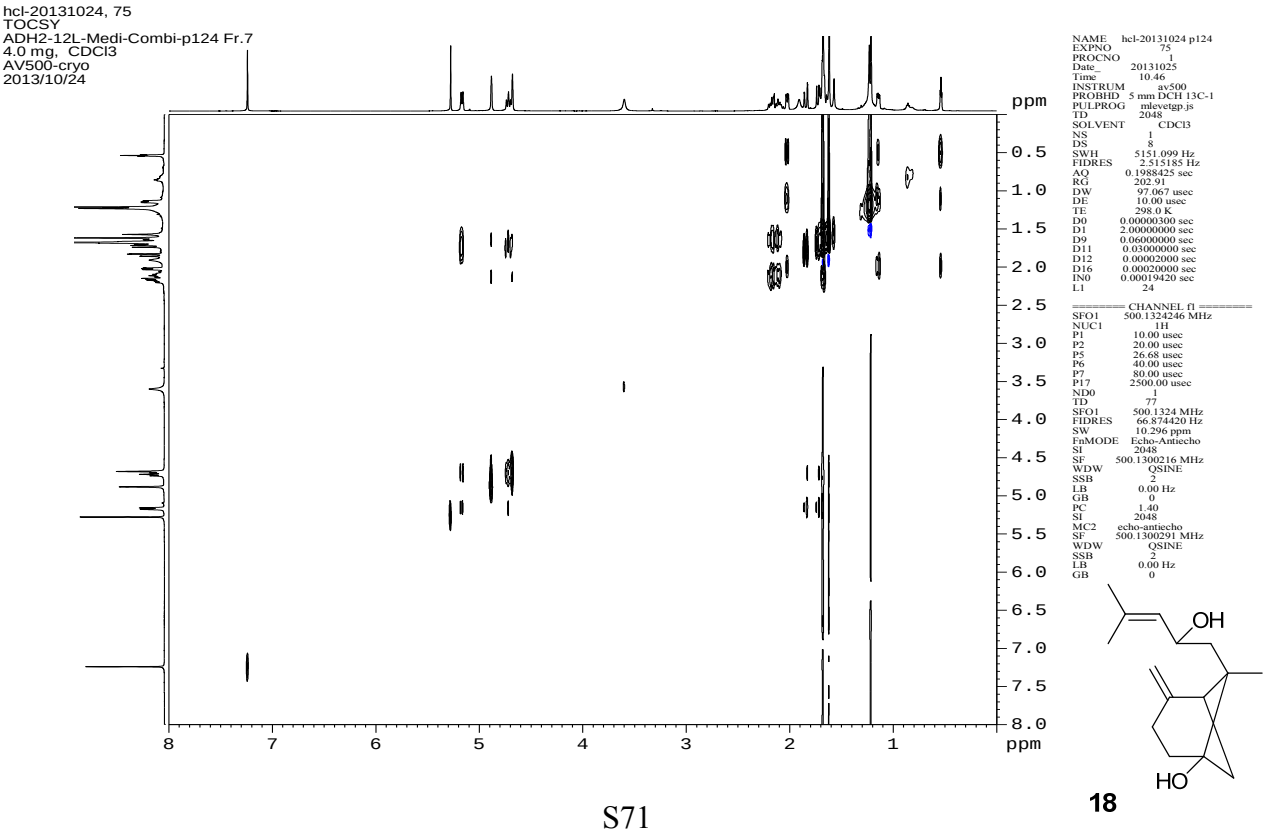


Figure S75. ^{1}H NMR spectrum of **19** ($CDCl_3$, 500 MHz).

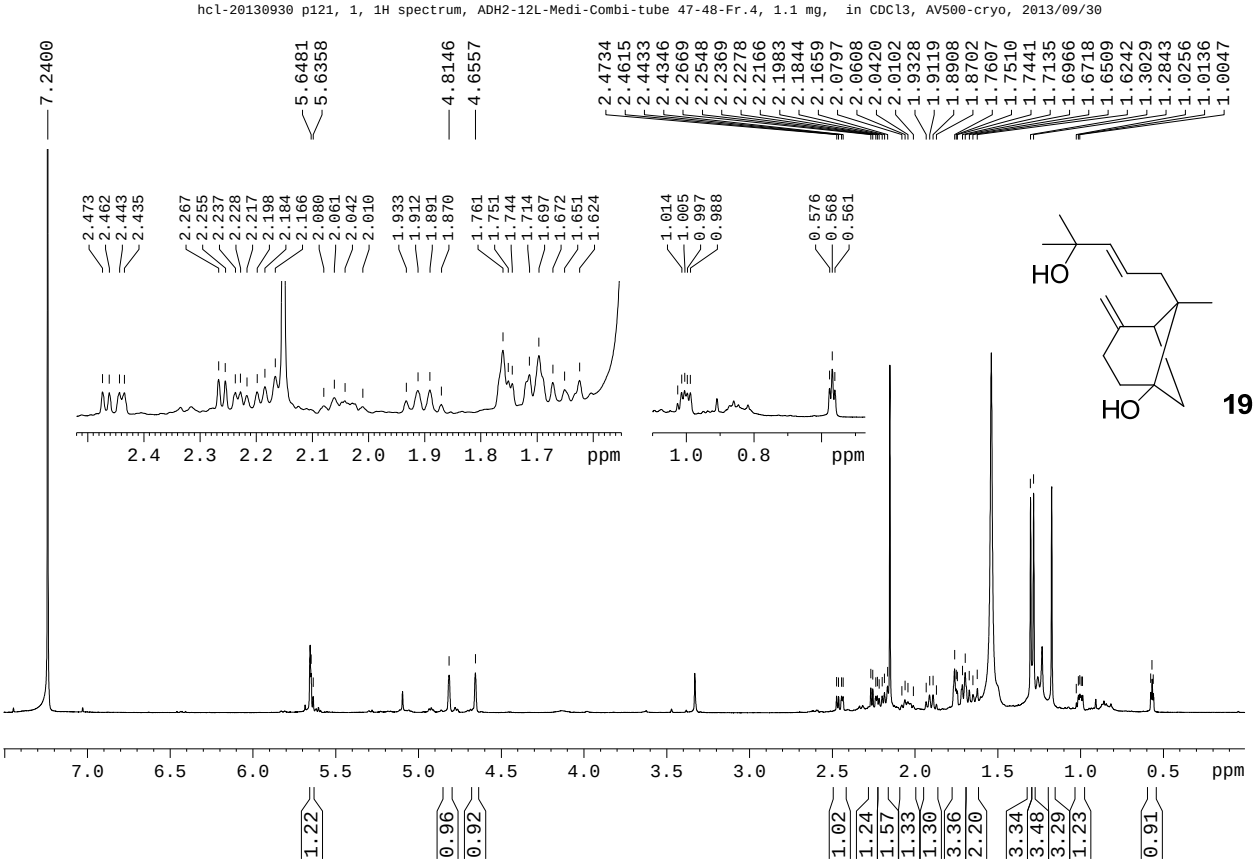


Figure S76. ^{13}C NMR spectrum of **19** ($CDCl_3$, 125 MHz).

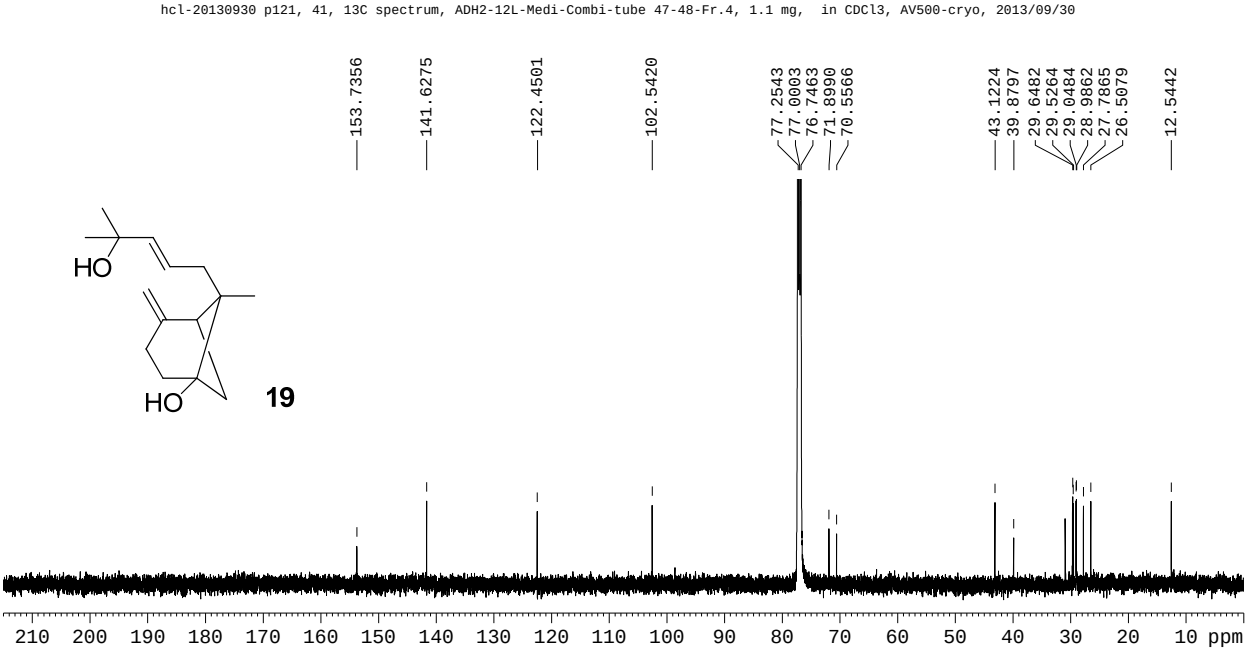


Figure S77. HSQC135 spectrum of **19** ($CDCl_3$, 500 MHz).

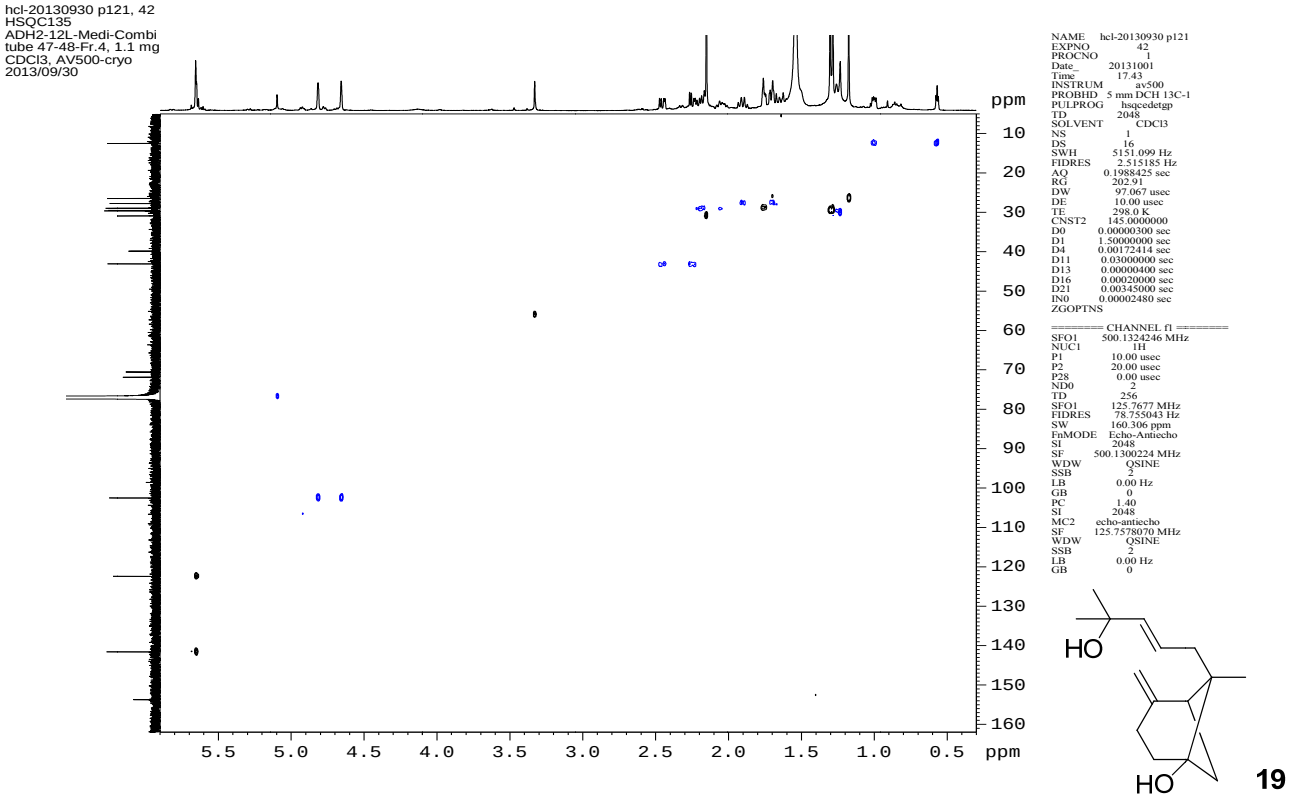


Figure S78. COSY spectrum of **19** ($CDCl_3$, 500 MHz).

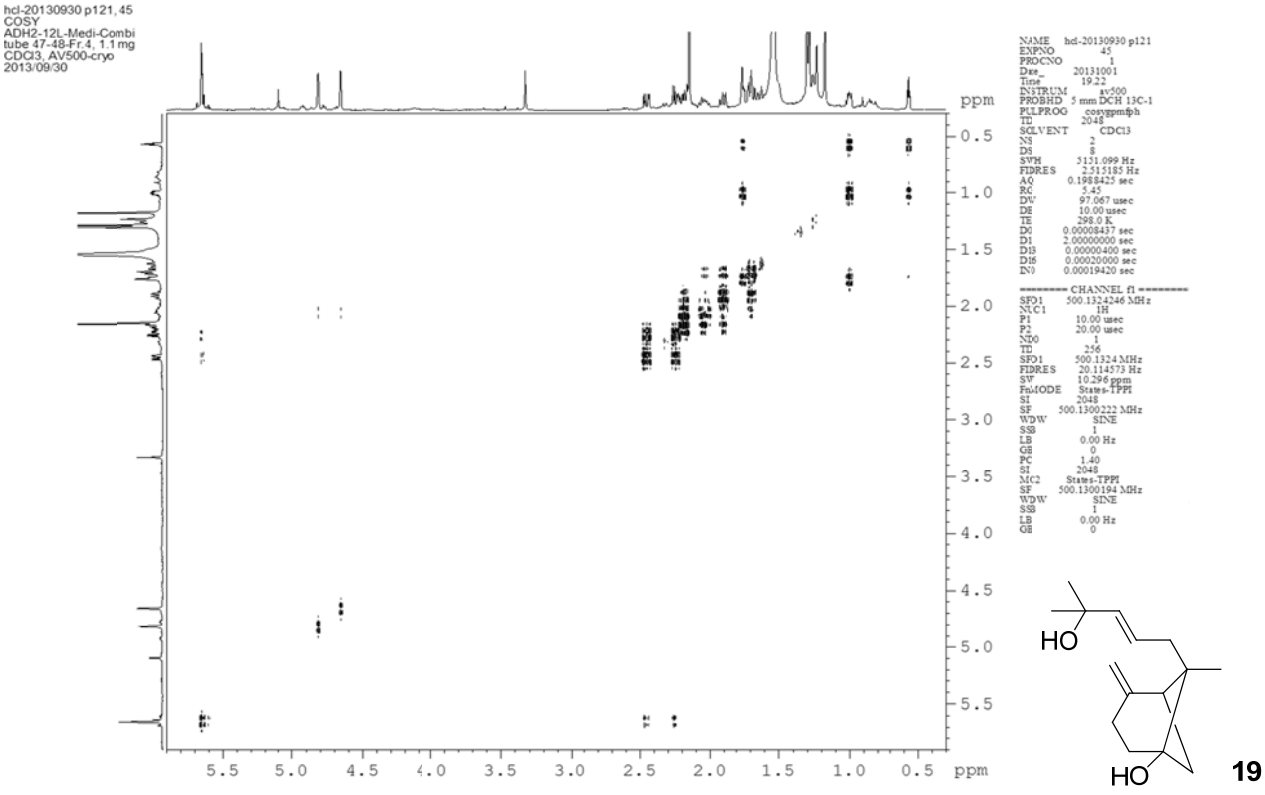


Figure S79. HMBC spectrum of **19** ($CDCl_3$, 500 MHz).

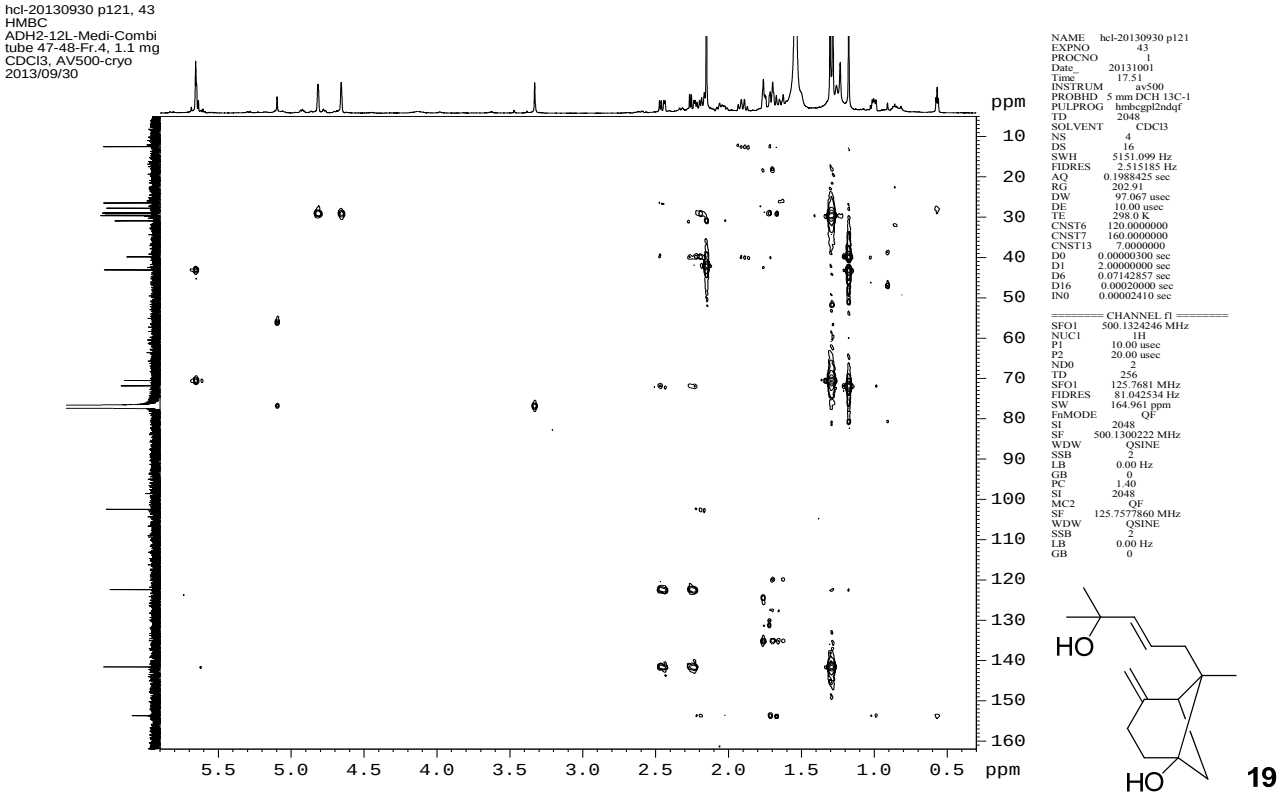


Figure S80. NOESY spectrum of **19** ($CDCl_3$, 500 MHz).

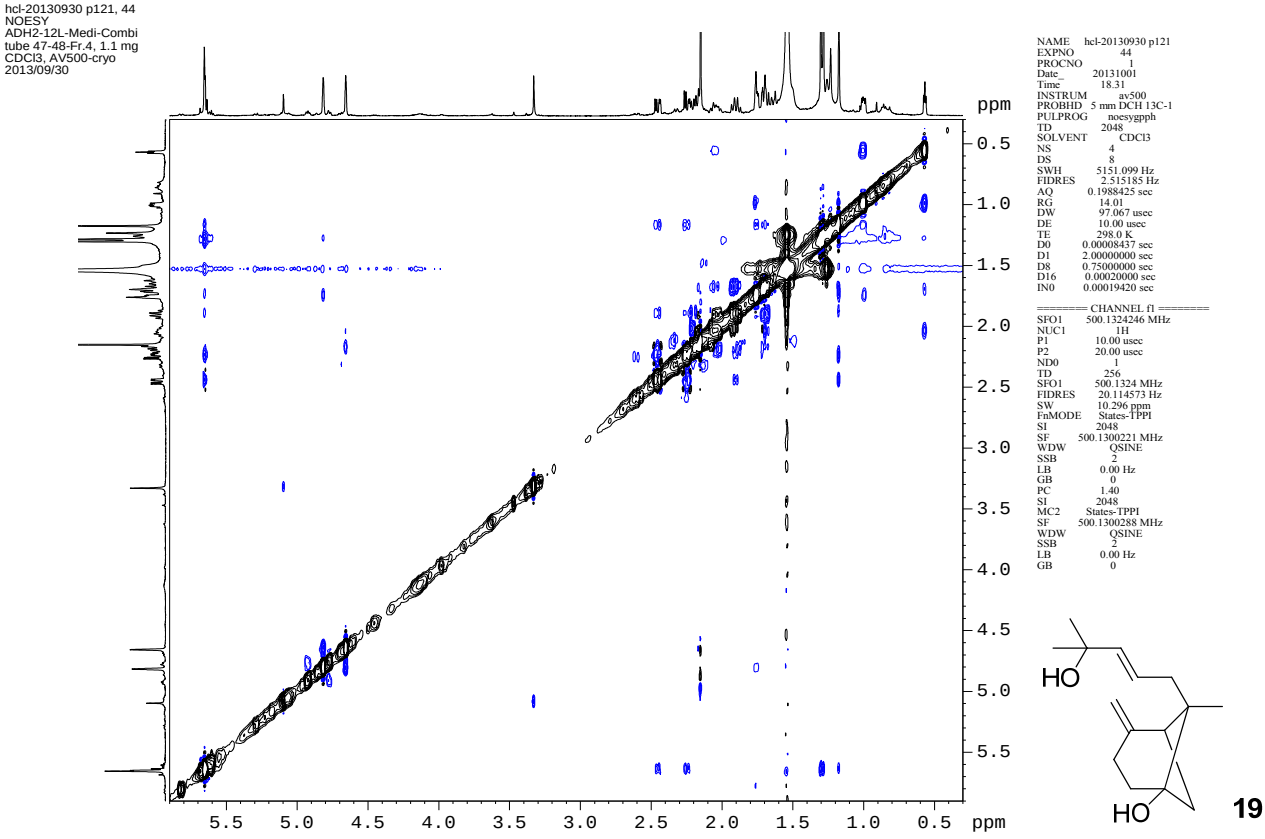


Figure S81. ^{1}H NMR spectrum of **21** ($CDCl_3$, 500 MHz).

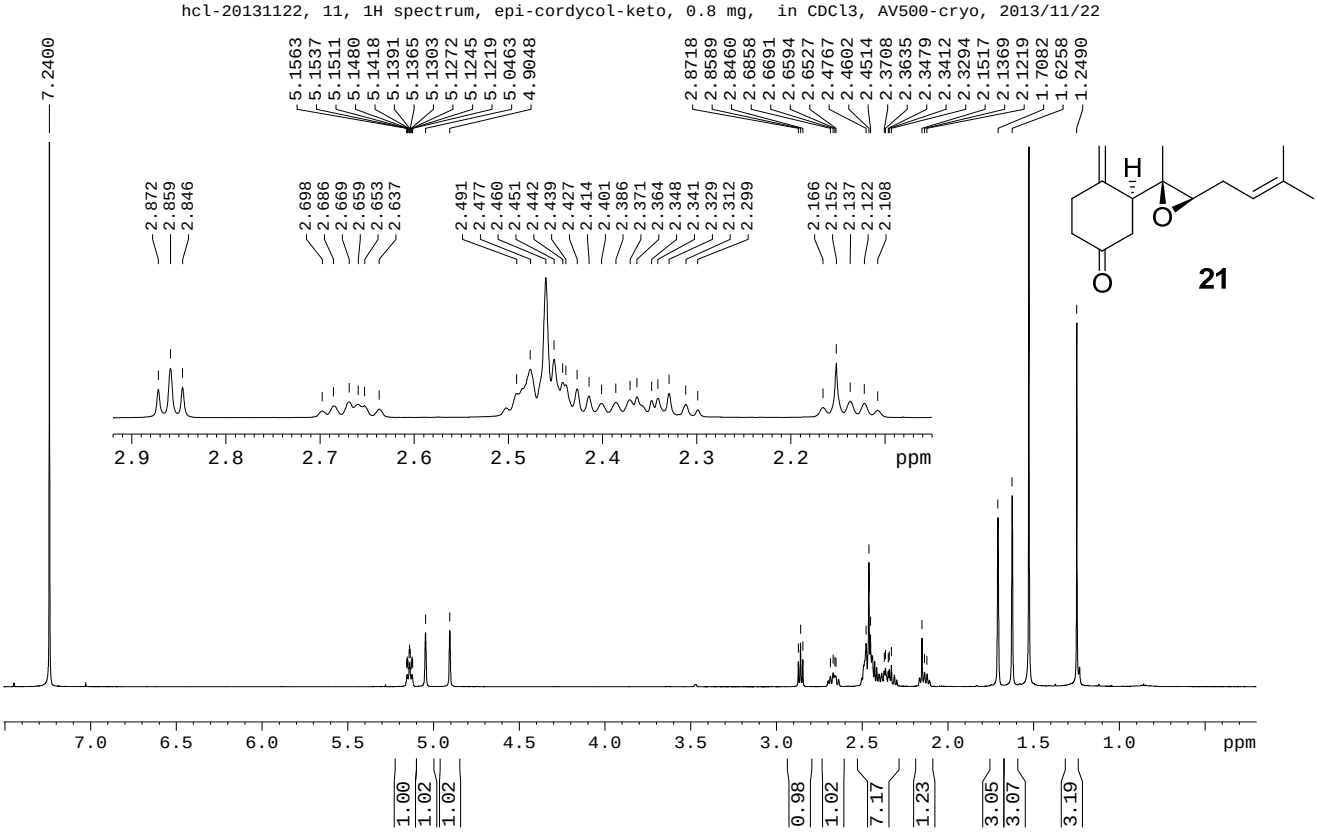


Figure S82. ^{13}C NMR spectrum of **21** ($CDCl_3$, 125 MHz).

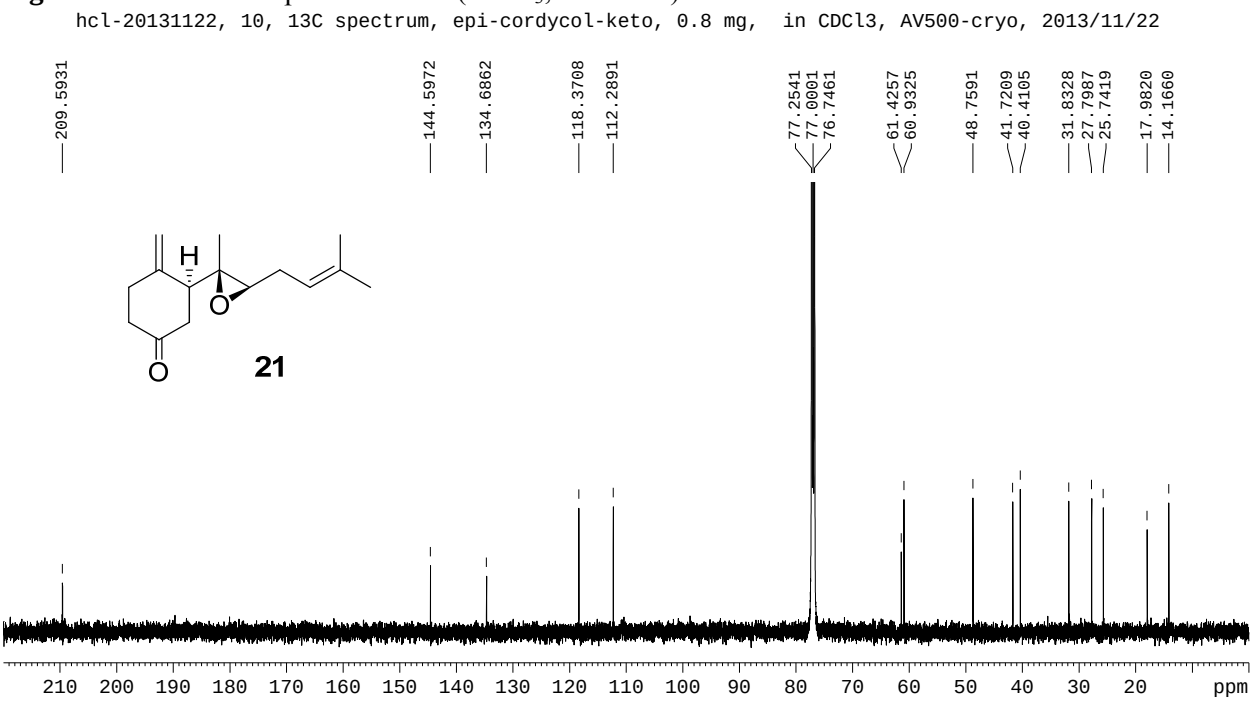


Figure S83. ^{1}H NMR spectrum of **22** ($CDCl_3$, 500 MHz).

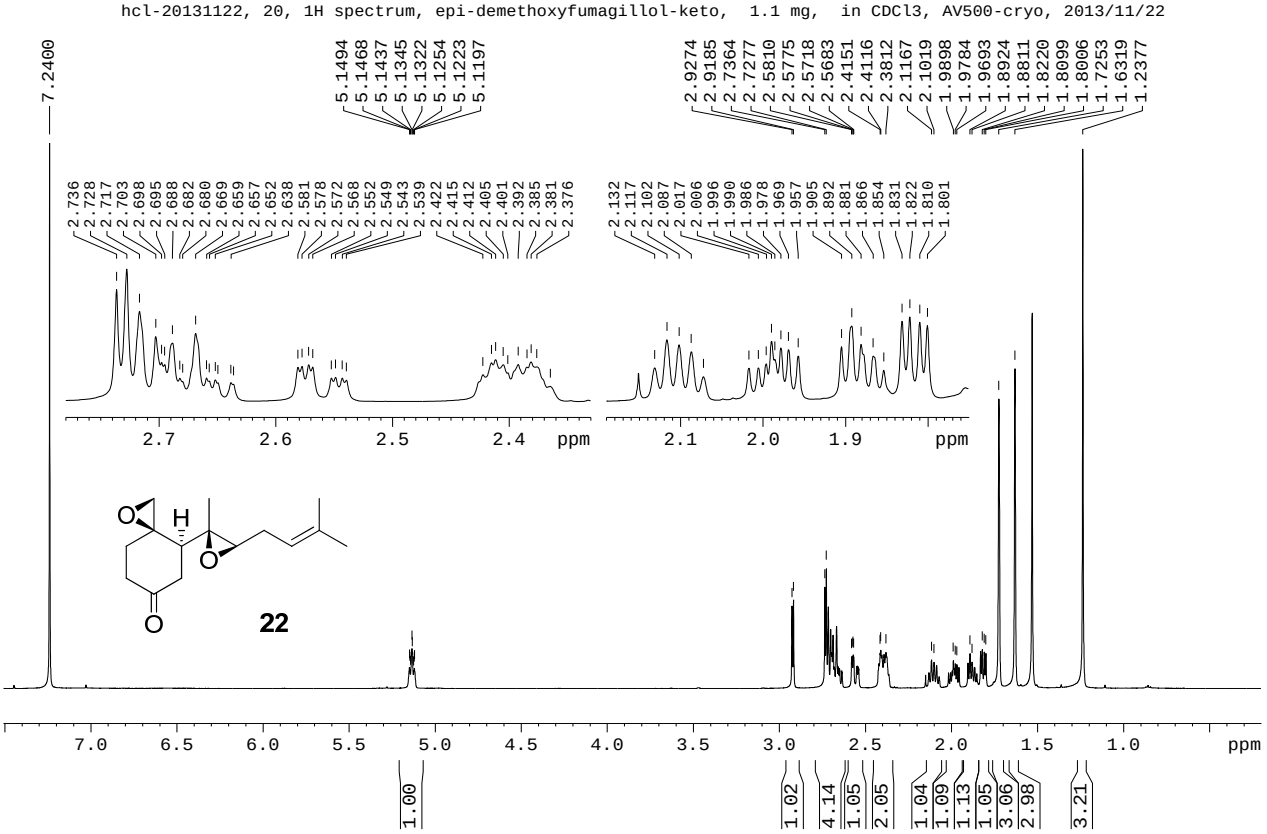


Figure S84. ^{13}C NMR spectrum of **22** ($CDCl_3$, 125 MHz).

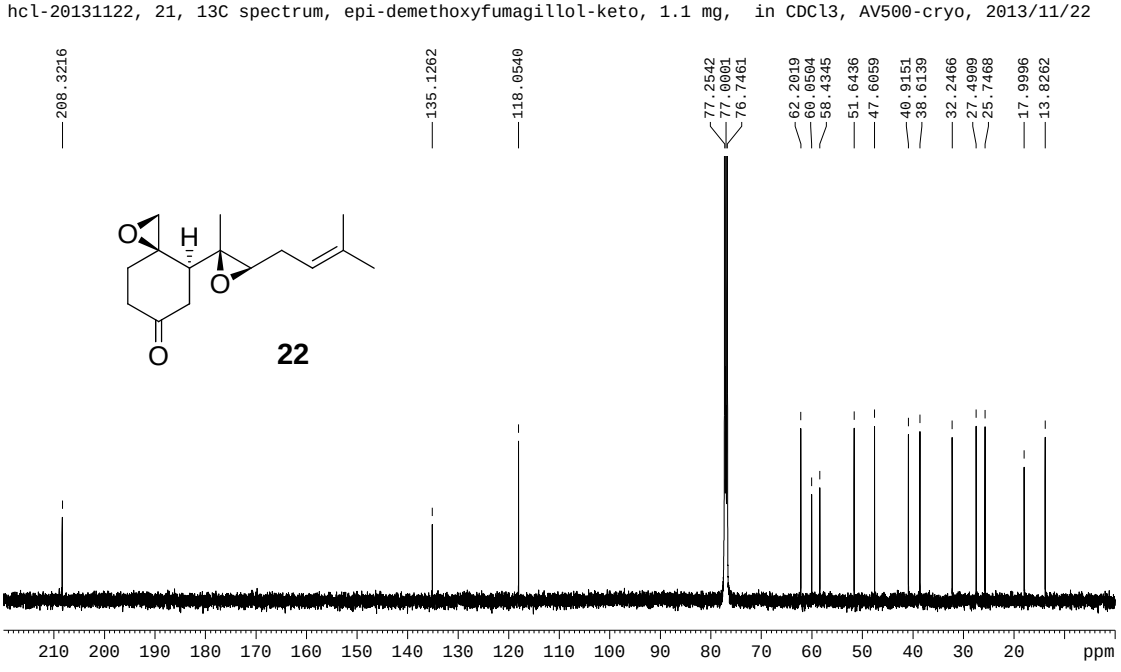


Figure S85. ^{1}H NMR spectrum of **23** ($CDCl_3$, 500 MHz).

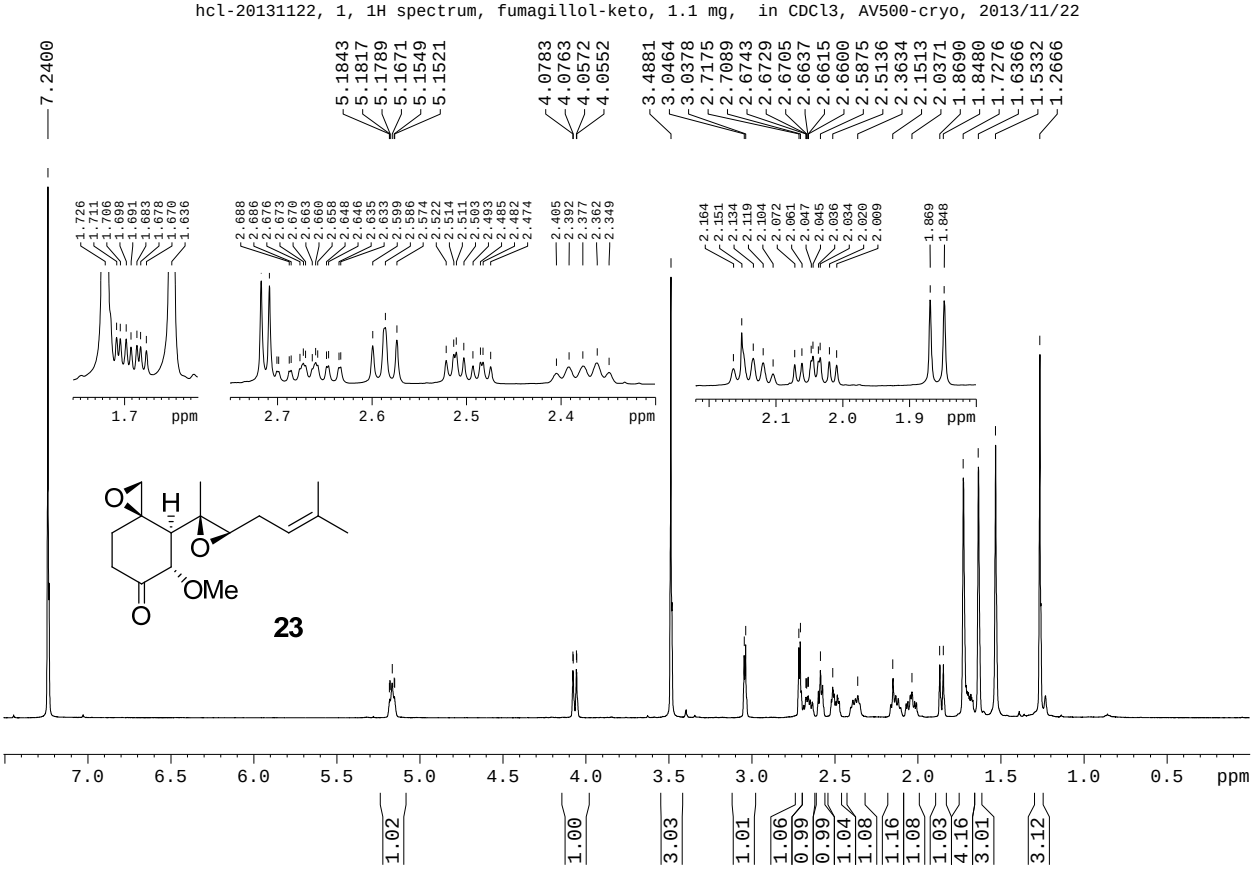
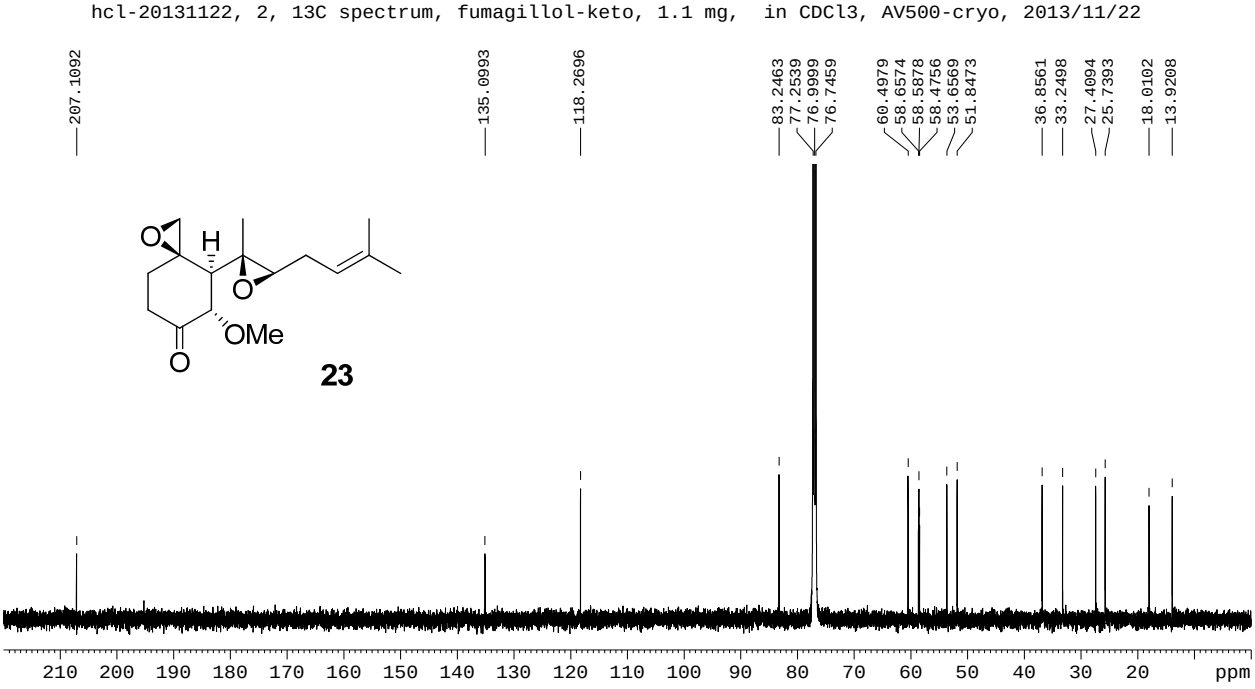


Figure S86. ^{13}C NMR spectrum of **23** ($CDCl_3$, 125 MHz).



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