Supporting Information

Genome mining of cryptic bisabolenes that were biosynthesized by intramembrane terpene synthases from *Antrodia cinnamomea*

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1.1 General molecular biology experiments

PCR was performed using Q5® High-Fidelity DNA Polymerase (New England Biolabs). PCR products were purified with a Zymoclean™ Gel DNA Recovery kit. DNA restriction enzymes were used as recommended by the manufacturer (New England Biolabs). DNA fragments were assembled using the NEBuilder® HiFi DNA Assembly Cloning kit (New England Biolabs). *E. coli* TOP10 (Invitrogen) and DH10B (Invitrogen) were used for cloning, following standard recombinant DNA techniques. RNA extraction was performed using a RiboPure Yeast Kit (Ambion). RT-PCR was performed using ImProm-II™ Reverse Transcription System (Invitrogen) to synthesize complementary DNA (cDNA) from total RNA.

1.2 Genome analysis

The genome sequence of *A. cinnamomea* was downloaded from NCBI database. Functional domains in the translated protein sequences were predicted using Conserved Domain Search. ClustalW was used for multiple sequence alignment.

1.3 Protein sequence of Tps1A

MSPRYSALLKVLCRMPGSLDGFVSIFRRFMQDPLGIRYHLHTLLLFTWADMKTILLPITA
FACSTAPLHSFSNLVQGMIWIWLHQLLCNVSNQARGKSEDALNKPWRPLPSGRLTEPQA
VILRWITVAVCLLLSATYGRDLLMTTVGLILTTLLYDELGMASHHIGKNLCNIGGYTTIE
VGATKLMGASRDLDYVSTVAVIISGVLIFTTIQAQDFPDIEGDAALGRVTFPIYAPEFSRIF
TFIVMPAWSIFLGWFWDIGVISRMVFAALGTYVGLRYYLWRTVDIDKRSYVFFNAWLT
LAHILPLSVRTGFLAF

1.4 Protein sequence of Tps2A

MASKRTFPVISIRPLLSLCFSALRNWIRTLILFTYTDYKTIVLPVSVFACVSAPVHSFVRFL
HAVLWIWLHLLQCNVSNQYRSVLEDAVNRPWRPLPSRLISVEHACILRWLLVPLCIGTS
LCYGWDVALASACLTLTTVCYDELGLAGHFLGKNLCNVPGYVSFEIGATKIMGSTTNL
DFIALESILCSAMVIFTTIQTQDFPDVAGDRALGRVTLPILCPEGSRHFTTCVLLFWSGFLS
YAWSIGLLSSAVLISLGIWVAYRYYRFRKVEEDKKSYLIYNIWLLFVHSLAAHARWNLM
AL

1.5 Protein sequence of Tps1H

MEGLQAIPSIFSLSAGVQSATALGIIVLVFYAIKGLLYPTSAARRFPPGPPQKPLVGNILEV
SPKGAWTRFTEYKEQYGDLVFFRGLGNNILVLNSMKVINDLLDKRGNVYSHRPGFTVV
GELMGLGQSMPLLPYGEEWRAHRKLAHVALSATAVKLYYTVQEDLAALLSKQLLETP

ADFFSHVRLIAGRIILSVTYGLAVDTAEDEYISHAEDTMLMIGRSTVPGAFICDFLPFLKH
LPSWVPFQREARKGKEMIERLVTKPFEHVKQQMEAGKALPSLTQHLLSSEEQVFNLEHR
VKWTAGAMYGAGGESTYATVLSFILAMALHPDKQRLAQAEIDSVIGVDRMPTIADRPN
LPYVGALIKETMRWHPVLPLSIARSTAQDDVYEGFFIPKSTTVMPNVWAIAFEPNDKYD
PHAFIPERFLDSTHPTLDPATYAFGFARRICPGKLLGENSLFILITTILAMFDVSPPEKGELT
PDFTLDLVSYPKPFECRITPRSDAKAKLIHLRAAHCAM

1.6 Protein sequence of Tps1D

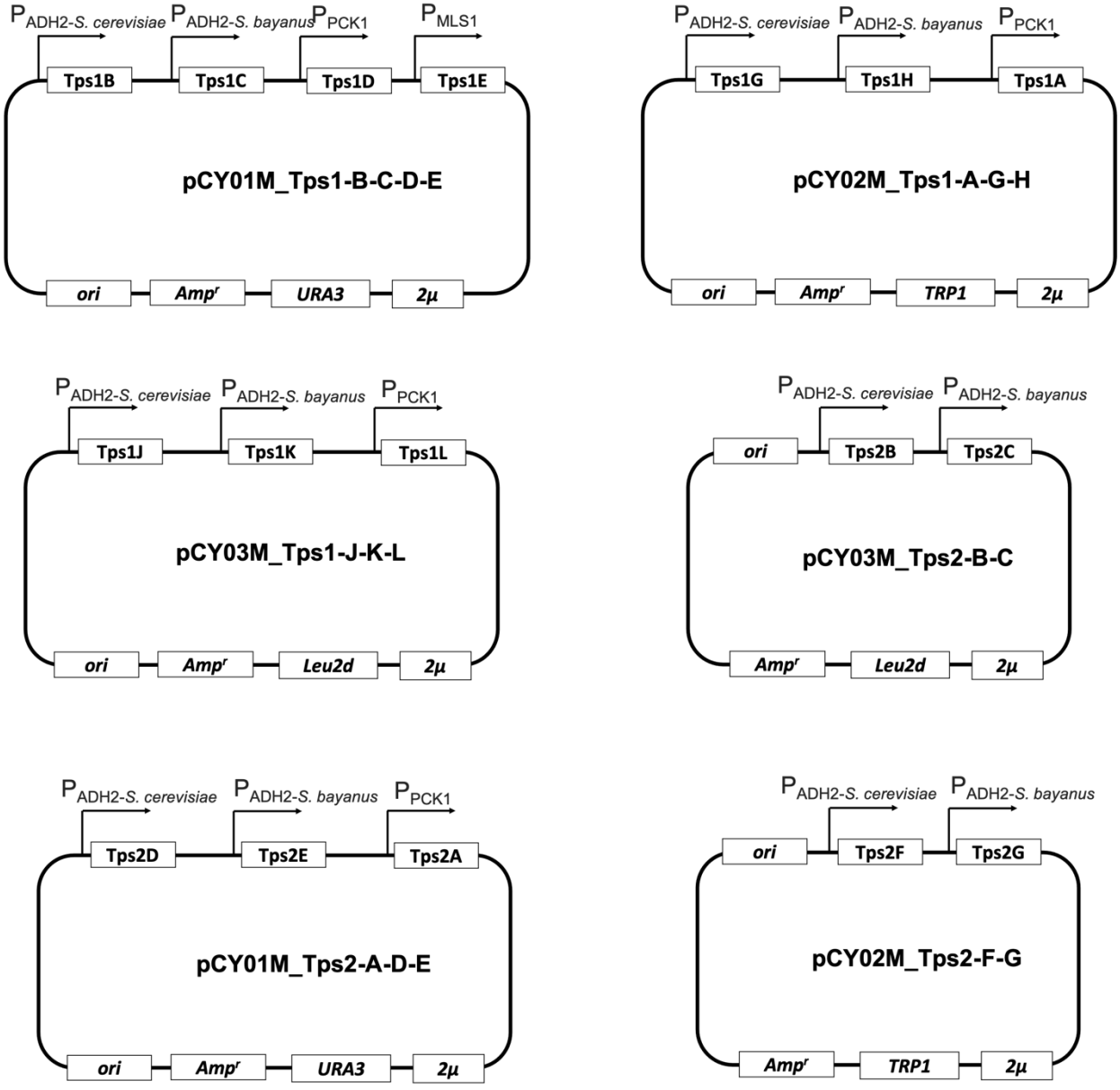
MSTNAELSQPGEAKTYAFQPAYKVPEETARLDELHAGITEYFGNRLSLAPLETISPVKILE
LGAGSGAWAIQAATEFPGAHVLAVDISPIPPRPLPSNLSFQQVDLTKPFALEKESFDVVH
ARLVMMHLPNGEDVLRRAIDLVKPGGWLLVEDPDDDNMVDGGKPLGPGMSAFVGAW
LKIVRSRGAEPSLGRELERILQSSSAFSEVNVMKVTIPISGKSQDPKENKLGLTWKTNML
RVARDLPGRFAEQGITEEVA

1.7 Construction of plasmids pCY01M-Tps1B-Tps1C-Tps1D-Tps1E, pCY02M-Tps1G-Tps1H-Tps1A and pCY03M-Tps1J-Tps1K-Tps1L for expression in *S. cerevisiae*

The cDNA of *tps1B*, *tps1C*, *tps1D* and *tps1E* were obtained by RT-PCR from the RNA extract of *A. cinnamomea* S27 and amplified using primer pairs, Tps1B-XhoI-$P_{ADH2\text{-}S.\ c}$-F/Tps1B-XhoI-T_{ADH2}-R, Tps1C-NotI-$P_{ADH2\text{-}S.b}$-F/Tps1C-NotI-T_{PGI1}-R, Tps1D-PmeI-P_{PCK1}-F/ Tps1D-PmeI-T_{ENO2}-R, and Tps1E-SmaI-P_{MLS1}-F/ Tps1E-SmaI-T_{TDH2}-R, respectively. pCY01M-Tps1B-Tps1C-Tps1D-Tps1E was constructed by assembling the intron-less *tps1B*, *tps1C*, *tps1D*, and *tps1E* DNA fragments to the XhoI/NotI/PmeI/SmaI linearized YEplac195-derived 2μ multiple expression plasmid (containing URA3 marker). The cDNA of *tps1G*, *tps1H* and *tps1A* were obtained by RT-PCR from the RNA extract of *A. cinnamomea* S27 and amplified using primer pairs, Tps1G-XhoI-$P_{ADH2\text{-}S.c.}$-F/Tps1G-XhoI-T_{ADH2}-R, Tps1H-NotI-$P_{ADH2\text{-}S.b}$-F/Tps1H-NotI-T_{PGI1}-R, Tps1A-PmeI-P_{PCK1}-F/ Tps1A-PmeI-T_{ENO2}-R, respectively. pCY02M-Tps1G-Tps1H-Tps1A was constructed by assembling the intron-less *tps1G*, *tps1H* and *tps1A* fragments to the XhoI/NotI/PmeI linearized YEplac112-derived 2μ multiple expression plasmid (containing TRP1 marker).

The cDNA of *tps1J*, *tps1K* and *tps1L* were obtained by RT-PCR from the RNA extract of *A. cinnamomea* S27 and amplified using primer pairs, Tps1J-XhoI-$P_{ADH2\text{-}S.c.}$-F/Tps1J-XhoI-T_{ADH2}-R, Tps1K-NotI-$P_{ADH2\text{-}S.b.}$-F/Tps1K-NotI-T_{PGI1}-R, Tps1L-PmeI-P_{PCK1}-F/Tps1L-PmeI-T_{ENO2}-R, respectively. pCY03M-Tps1J-Tps1K-Tps1L was constructed by assembling the intron-less *tps1J*, *tps1K* and *tps1L* DNA fragments to the XhoI/NotI/PmeI linearized YEplac195-derived 2μ

multiple expression plasmid (containing LEU2 marker). The above fragments were assembled using the NEBuilder HiFi DNA Assembly Cloning kit (New England Biolabs) for propagation in *E. coli* DH10β. Constructed plasmids were verified by sequencing. The primers used for amplification and cloning are listed in Table **S4**.



1.8 Construction of plasmid pXW55-Tps1A for expression in *S. cerevisiae*

The cDNA of *tps1A* was amplified using primer pair Tps1A-NdeI-PmlI-F/R. pXW55-Tps1A was constructed by assembling the intron-less *tps1A* DNA fragment to the NdeI/PmlI linearized YEplac195-derived 2μ expression plasmid (containing URA3 marker). The above fragment assembly experiments were performed using the NEBuilder® HiFi DNA Assembly Cloning kit (New England Biolabs) for propagation in *E. coli* DH10β. All plasmids were verified by sequencing. Primers used for the amplification and cloning are listed in Table **S4**.

1.9 Construction of plasmid pCY02M-Tps1A-Tps1H for expression in *S. cerevisiae*

The cDNA of *tps1A* and *tps1H* were amplified using primer pair Tps1A-PmeI-P_{PCK1}-F/Tps1A-PmeI-T_{ENO2}-R and Tps1H-NotI-P_{ADH2}-*S.b.*-F/Tps1H-NotI-T_{PGI1}-R, respectively. pCY02M-Tps1A-Tps1H was constructed by assembling the intron-less *tps1A* and *tps1H* DNA fragments to the NotI/PmlI linearized YEplac112-derived 2μ multiple expression plasmid (containing TRP1 marker). The above fragment assembly experiments were performed using the NEBuilder® HiFi DNA Assembly Cloning kit (New England Biolabs) for propagation in *E. coli* DH10β. All plasmids were verified by sequencing. Primers used for the amplification and cloning are listed in Table **S4**.

1.10 Construction of plasmid pXW02-Tps1D for expression in *S. cerevisiae*

The cDNA of *tps1D* was amplified using primer pair Tps1D-pXW02-NdeI-F/Tps1D-pXW02-PmlI-R. pXW02-Tps1D was constructed by assembling the intron-less *tps1D* DNA fragment to the NdeI/PmlI linearized YEplac195-derived 2μ expression plasmid (containing LEU2 marker. The above fragment assembly experiments were performed using the NEBuilder® HiFi DNA Assembly Cloning kit (New England Biolabs) for propagation in *E. coli* DH10β. All plasmids were verified by sequencing. Primers used for the amplification and cloning are listed in Table **S4**.

1.11 Construction of plasmid pXW55-Tps1A-D100A, pXW55-Tps1A-Y155A, pXW55-Tps1A-D215A, pXW55-Tps1A-D218A, pXW55-Tps1A-D222A for expression in *S. cerevisiae*

An alanine residue was replaced with the corresponding amino acid residue on the mutants Tps1A-D100A, Tps1A-Y155A, Tps1A-D215A, Tps1A-D218A and Tps1A-D222A. To construct the Tps1A-D100A mutant, the overlapping region was amplified using Tps1A-D100A-F/Tps1A-PmlI-R and Tps1A-NdeI-F/Tps1A-D100A-R. To construct the Tps1A-Y155A mutant, the

overlapping region was amplified using Tps1A-Y155A-F/Tps1A-PmlI-R and Tps1A-NdeI-F/Tps1A-Y155A-R. To construct the Tps1A-D215A mutant, the overlapping region was amplified using Tps1A-D215A-F/Tps1A-PmlI-R and Tps1A-NdeI-F/Tps1A-D215A-R. To construct the Tps1A-D218A mutant, the overlapping region was amplified using Tps1A-D218A-F/Tps1A-PmlI-R and Tps1A-NdeI-F/ Tps1A-D218A-R. To construct the Tps1A-D222A mutant, the overlapping region was amplified using Tps1A-D222A-F/Tps1A-PmlI-R and Tps1A-NdeI-F/Tps1A-D222A-R. The above DNA fragments were assembled with the PmlI/NdeI-linearized YEplac195-derived 2μ expression plasmid (containing URA3 marker). The above fragments assembly experiments were performed using NEBuilder® HiFi DNA Assembly Cloning kit (New England Biolabs) for propagation in *E. coli* DH10β. Constructed plasmids were verified by sequencing. The primers used for amplification and cloning are listed in Table **S4**.

1.12 Heterologous expression of point mutated Tps1A in *S. cerevisiae*

The mutant plasmids pXW55-Tps1A-D100A, Tps1A-Y155A, Tps1A-D215A, Tps1A-D218A, and Tps1A-D222A were transformed into the *S. cerevisiae* RC01 strain (= BJ5464-NpgA harboring *At*CPR) by using frozen-EZ yeast transformation II kit™ (Zymo Research), respectively. The transformants were inoculated into the corresponding 3 mL yeast synthetic drop-out medium and incubated with shaking 230 rpm at 28 °C for 3 days. A 3 μL aliquot of the seed culture was inoculated to 3 mL YPD medium (10 g/L yeast extract, 20 g/L peptone, 10 g/L glucose) and cultured 28 °C, 230 rpm for 3 days. The cultures were harvested and separated into cells and medium parts by centrifuge, respectively. The cells were extracted by acetone and concentrated *in vacuo*. The extracts were then dissolved in MeOH and analyzed by LC-MS as described in Chemical Analysis. For GCMS analysis, culture medium directly subjected to GCMS analysis using the SPME method.

1.13 Construction of plasmids pCY01M-Tps2D-Tps2E-Tps2A, pCY02M-Tps2F-Tps2G and pCY03M-Tps2B-Tps2C for expression in *S. cerevisiae*

The cDNA of the *tps2* gene cluster was obtained by RT-PCR from the RNA of *Antrodia cinnamomea* S27 and amplified using primer pairs, Tps2D-XhoI-$P_{ADH2\text{-}S.c}$-F/Tps2D-XhoI-T_{ADH2}-R, Tps2E-NotI-$P_{ADH2\text{-}S.b}$ -F/Tps2E-NotI-T_{PGI1}-R, and Tps2A-PmeI-P_{PCK1}-F/Tps2A-PmeI-T_{ENO2}-R respectively. The pCY01M-Tps2D-Tps2E-Tps2A was constructed by assembling the intronless *tps2D*, *tps2E*, and *tps2A* DNA fragments to the XhoI/NotI/PmeI linearized YEplac195-derived 2μ multiple expression plasmid (containing URA3 marker). The cDNA of *tps2F* and *tps2G* was

obtained by RT-PCR from the RNA of *A. cinnamomea* S27 and amplified using primer pairs, Tps2G-XhoI-$P_{ADH2\text{-}S.c}$ -F/Tps2G-XhoI-T_{ADH2}-R and Tps2F-NotI-$P_{ADH2\text{-}S.b}$-F/Tps2F-NotI-T_{PGI1}-R, respectively. pCY02M-Tps2G-Tps2F was constructed by assembling the intronless *tps2G* and *tps2F* fragments, to the XhoI/NotI linearized YEplac112-derived 2μ multiple expression plasmid (containing TRP1 marker). The cDNA of *tps2B* and *tps2C* were obtained by RT-PCR from the RNA of *A. cinnamomea* S27 and amplified using primer pairs, Tps2C-XhoI-$P_{ADH2\text{-}S.c}$-F/Tps2C-XhoI-T_{ADH2}-R and Tps2B- NotI-$P_{ADH2\text{-}S.\ b}$-F/ Tps2B-NotI-T_{PGI1}-R respectively. pCY03M-Tps2C-Tps2B was constructed by assembling the intronless *tps2B* and *tps2C* DNA fragments to the XhoI/NotI linearized YEplac195-derived 2μ multiple expression plasmid (containing LEU2 marker). The above fragments were assembled using the NEBuilder HiFi DNA Assembly Cloning kit (New England Biolabs) for propagation in *E. coli* DH10β. Constructed plasmids were verified by sequencing. The primers used for amplification and cloning are listed in Table **S5**.

1.14 Construction of plasmid pXW55-Tps2A for expression in *S. cerevisiae*

The cDNA of *tps2A* was obtained by RT-PCR from the RNA *Antrodia cinnamomea* S27 and amplified using primer pair Tps2A-NdeI-PmlI-F/R. pCY01-Tps2A was constructed by assembling the intronless *tps2A* DNA fragment to the NdeI/PmlI linearized YEplac195-derived 2μ expression plasmid (containing URA3 marker). The above fragment assembly experiments were performed using the NEBuilder® HiFi DNA Assembly Cloning kit (New England Biolabs) for propagation in *E. coli* DH10β. All plasmids were verified by sequencing. Primers used for the amplification and cloning are listed in Table **S5**.

1.15 Heterologous reconstitution of Tps2A gene cluster in *S. cerevisiae*

The *tps2* whole gene cluster or *tps2A* was co-transformed into the *S. cerevisiae* RC01 strain (= BJ5464-NpgA harboring *At*CPR)[1] by using frozen-EZ yeast transformation II kit™ (Zymo Research). The transformants were inoculated into the corresponding 2 mL yeast synthetic drop-out medium and incubated with shaking 250 rpm 28 °C for two days. A 20 μL aliquot of the seed culture was inoculated to 2 mL YPD medium (10 g/L yeast extract, 20 g/L peptone, 10 g/L glucose) and cultured at 28 °C, 250 rpm for 3 days. The cultures were harvested and separated into cells and medium parts by centrifuge, respectively. The cells were extracted by acetone and concentrated *in vacuo*. The extracts were then dissolved in MeOH and analyzed by LC-MS as described in Chemical Analysis. For GC-MS analysis, culture medium directly subjected to GCMS analysis using the SPME method.

1.16 Preparation of microsomal fractions of Tps1A, Tps2A and Tps1H from *S. cerevisiae*

A 2.5 mL aliquot of the *S. cerevisiae* RC01 strain[2] harboring pXW55-Tps1A, pXW55-Tps2A or pXW02-Tps1H was inoculated to 500 mL of YPD medium (10 g/L yeast extract, 20 g/L peptone, 10 g/L glucose) and was incubated for 3 days. The method of microsomal fraction was modified from the reported method.[3] The cells were harvested by centrifugation (3750 rpm at 4 °C for 15 minutes), and the pellet was washed with 25 mL TES buffer (50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 0.6 M sorbitol). The cell suspension was centrifuged (3750 rpm at 4 °C for 15 minutes), and resuspended in 25 mL of TES buffer with 10 mM β-mercaptoethanol followed by incubation at room temperature for 10 minutes. The cells were centrifuged again (3750 rpm at 4 °C for 15 minutes) and resuspended in 5 mL extraction buffer and transferred into a 50 mL falcon tube. The cells were disrupted by 0.5 mm dia. glass beads (BioSpec) manually handshaking at 4 °C for ten minutes at 30 seconds intervals separated by 30 seconds intervals on ice. The cell lysate was transferred into a new falcon tube, and the remaining lysate on beads was washed by 5 mL extraction buffer (50 mL TES buffer, 0.5 g bovine serum albumin, 2 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride) and then combined. The cell debris was removed by centrifugation (10,000 g at 4 °C for 20 minutes), and the microsomal fractions were obtained by centrifugation (at 100,000 g for 70 minutes at 4 °C). The microsomal fractions were resuspended in TEG-M buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 20% glycerol, 1.5 mM 2-mercaptoethanol) and stored at −80 °C.

1.17 *In vitro* assay of Tps1A with substrate FPP

The reaction of Tps1A was performed on a 50 μL scale. The reaction mixture contained 100 mg/mL microsomal fractions of Tps1A, 100 μM substrate farnesyl pyrophosphate, 5 mM $MgCl_2$ and 100 mM Tris-HCl buffer (pH 8.0). The reactions were incubated for 16 hours at 30 °C. For GCMS analysis, the reaction mixture was subjected to SPME analysis.

1.18 *In vitro* assay of yeast microsomal fractions with substrates 1, 2, and 3

The 50 μL reaction mixture contained 100 mg/mL yeast microsomal fractions, 100 μM of **1**, **2** or **3**, 2 mM NADPH, NADPH regeneration system (Corning Gentest^{TM}) (2.5 μL of solution A and 0.5 μL of solution B), 5 mM $MgCl_2$ and 100 mM Tris-HCl buffer (pH 8.0). The reactions were incubated for 16 hours at 30 °C and were then added by acetonitrile (100 μL).

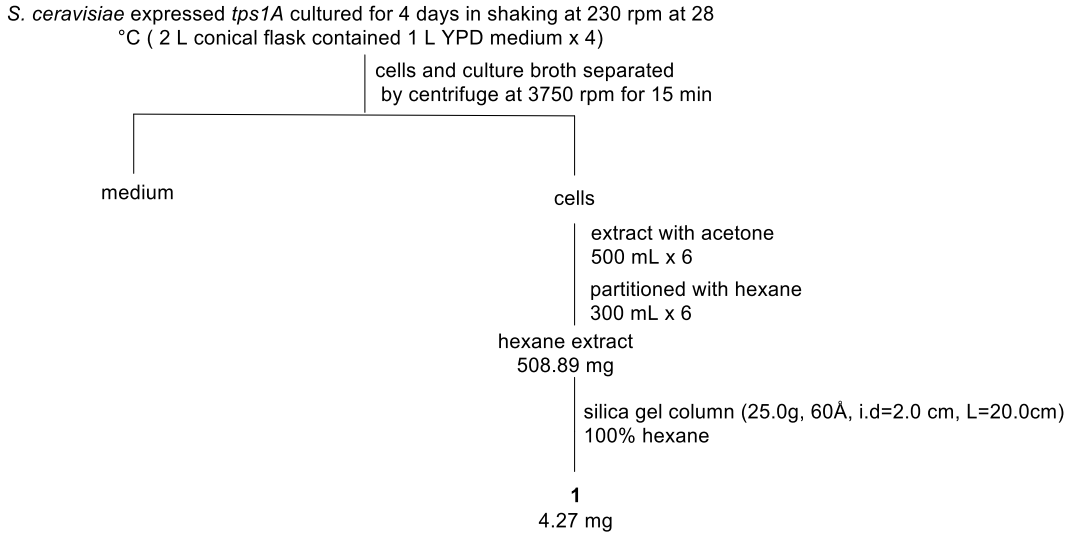
To analyze the generation of compound **4**, 5 μL of MeOH was incubated with compound **2** with or without 100 mg/mL yeast microsomal fractions. The extracts were evaporated, dissolved in acetonitrile, and analyzed by LC-MS. For GC-MS analysis, the reaction mixture was subjected to SPME analysis.

1.19 *In vitro* assay of Tps1H and Tps1D with compound 1

The 50 μL reaction mixture contained 100 mg/mL microsomal fractions of Tps1H, 10 μM Tps1D, 100 μM **1**, 100 μM *S*-adenosyl methionine, 2 mM NADPH, NADPH regeneration system (Corning Gentest^{TM}) (2.5 μL of solution A and 0.5 μL of solution B), 5 mM $MgCl_2$ and 100 mM Tris-HCl buffer (pH 8.0). The reactions were incubated for 16 hours at 30 °C and were then extracted by ethyl acetate (100 μL × 3). The extracts were evaporated, dissolved in methanol, and analyzed by LC-MS.

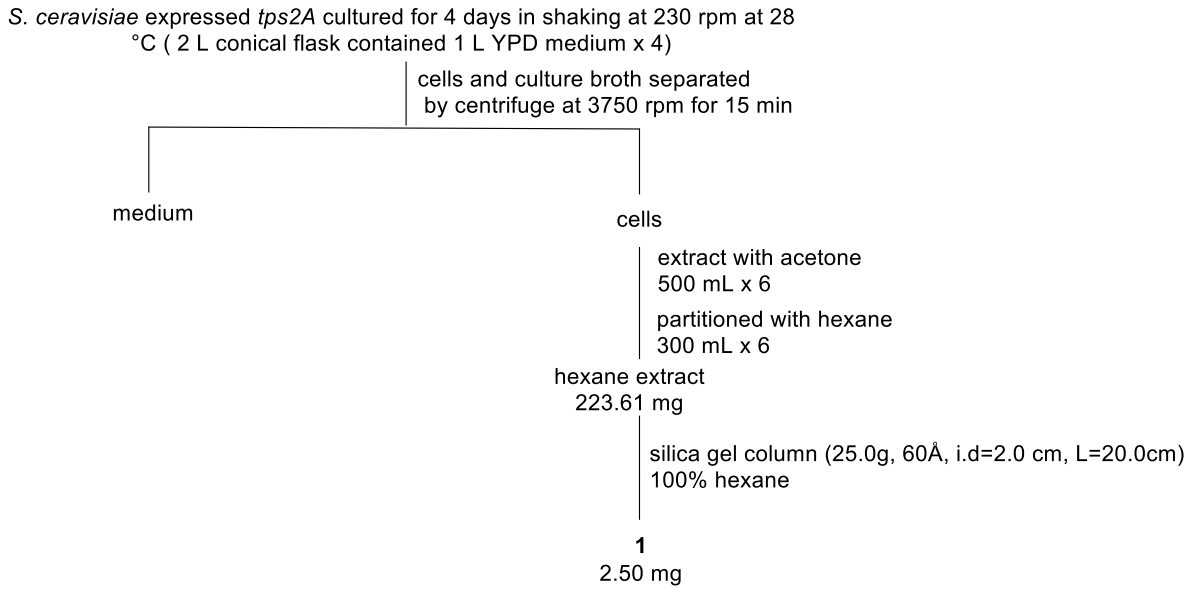
1.19.1 Isolation of (+)-(*S,Z)*-α-bisabolene (1)

The *S. cerevisiae* strain harboring pXW55-Tps1A was cultivated on YPD (4 L) for 4 days with constant shaking at 230 rpm at 28 °C. The cells were extracted with acetone six times. The acetone extracts was concentrated *in vacuo* then partitioned with hexanes and water for six times, and the hexanes part was concentrated *in vacuo* to give hexanes crude extracts (508.89 mg). The resulting hexanes-soluble products were separated by silica gel 60 column (230–400 mesh, 2 × 20 cm) eluted with 100% hexanes to give compound **1** (4.27 mg, colorless oil), (Scheme **S1**).



Scheme **S1**. Separation process of compound **1** from *tps1A* expressed yeast culture.

The *S. cerevisiae* strain harboring pXW55-Tps2A was cultivated on YPD (4 L) for 4 days with constant shaking at 230 rpm at 28 ^{O}C. The cells were extracted with acetone six times. The acetone extracts was concentrated *in vacuo* then partitioned with hexanes and water six times, and the hexanes part was concentrated *in vacuo* to give hexanes crude extract (223.61 mg). The resulting hexane-soluble products were separated by silica gel 60 column (230–400 mesh, 2 × 20 cm) eluted with 100% hexane to give compound **1** (2.50 mg, colorless oil), (Scheme **S2**).



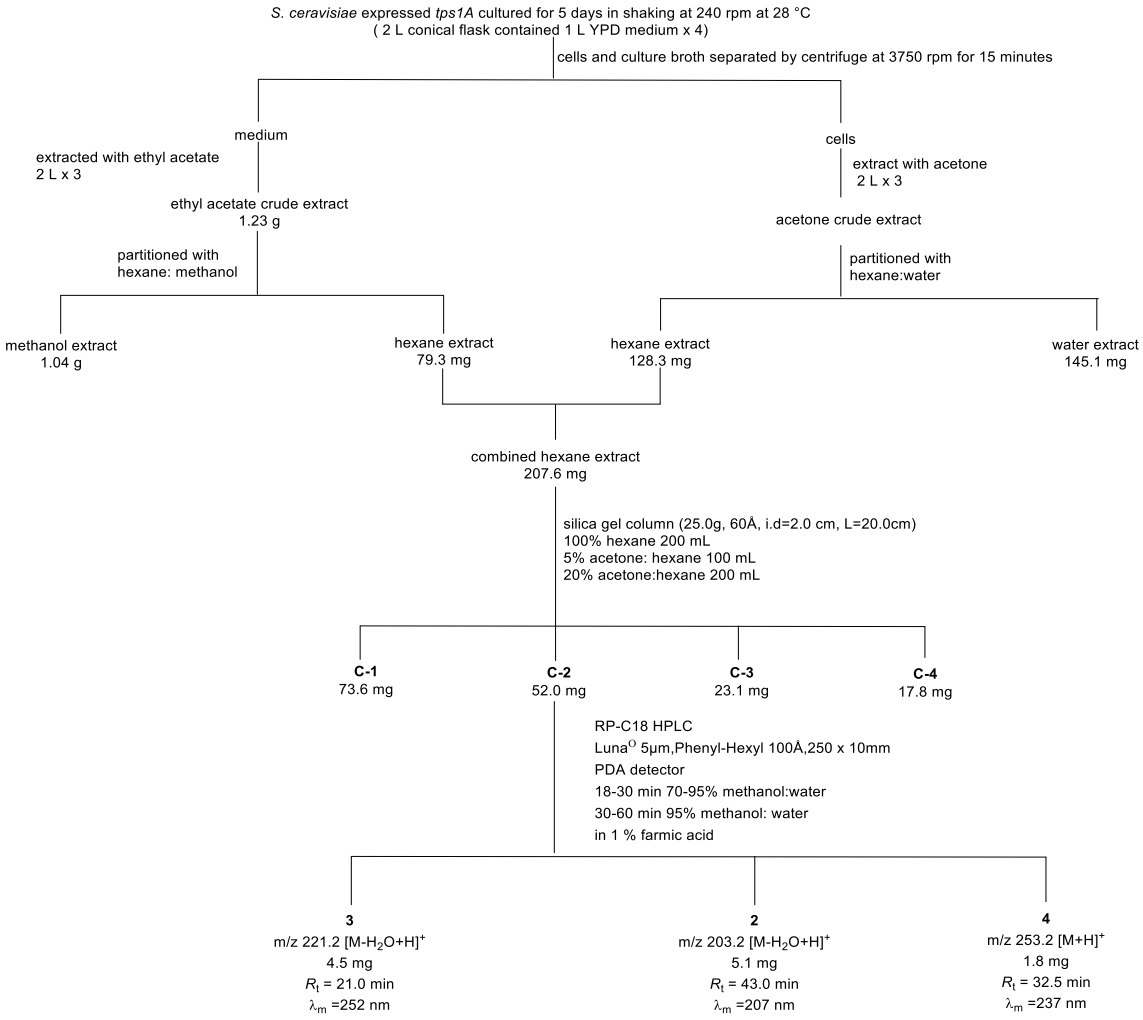
Scheme **S2**. Separation process of compound **1** from *tps2A* expressed yeast culture.

1.19.2 Isolation of compounds 2–4

The *S. cerevisiae* strain harboring pXW55-Tps1A was cultivated on YPD (4 L) for 5 days with constant shaking at 240 rpm at 28 ^{O}C. The cells part was extracted with acetone three times. The acetone extract was concentrated *in vacuo* and the residual water was partitioned with hexanes three times. The hexanes soluble part was concentrated *in vacuo* to give hexanes extracts (128.3 mg). The medium part was extracted with ethyl acetate three times. The ethyl acetate was concentrated *in vacuo* to give the crude extracts (1.23 g). The crude extracts were further partitioned with hexanes and methanol three times. The hexanes soluble part was concentrated in *vacuo* to give the hexanes extracts (79.3 mg). The above two hexanes soluble extracts from cells and medium extracts were combined and separated by silica gel 60 column (230–400 mesh, 2 ×

20 cm) eluted with 100% hexanes (200 mL), 5% acetone/hexanes (100 mL) and 20% acetone/ hexanes (200 mL) to give four fractions C1–C4. Fraction C2 was subjected to a semi-preparative RP-C18 HPLC column (Luna®, 5 μm Phenyl-Hexyl 100 Å , 250 × 10 mm) and eluted by 70% methanol/water (0–18 min), 70-95% methanol/water (18–30 min), and 95% methanol/water (30–60 min) with 1% formic acid at a flow rate of 1.0 mL/min to give compound **3** (4.5 mg, colorless amorphous solid, R_t = 21.0, PDA detector λ_m = 252 nm), compound **2** (5.1 mg, colorless oil, R_t = 43.0 minutes, PDA detector λ_m = 207 nm), and compound **4** (1.8 mg, colorless oil, R_t = 32.5 minutes, PDA detector λ_m = 237 nm) (Scheme **S3**).

To characterize the compounds **2** and **3** from Tps2A, the *S. cerevisiae* strain harboring pXW55-Tps2A was cultivated on YPD (2 L) for 5 days with constant shaking at 240 rpm at 28 ^{o}C. The cells were extracted with acetone three times. The acetone extract was concentrated *in vacuo* and the residual water was partitioned with hexane three times; The hexane part was concentrated *in vacuo* to give hexanes extracts (101.2 mg). The hexane extract was separated by silica gel 60 column (230–400 mesh, 2 × 20 cm) eluted with 100% hexane (200 mL), 5% acetone: hexane (100 mL), and 20% acetone:hexane (200 mL) to give three fractions E1-E3. Fraction E2 was subjected to a semi-preparative RP-C18 HPLC column (Luna®, 5 μm Phenyl-Hexyl 100 Å , 250 × 10 mm) and eluted by 70% methanol:water (0–18 min), 70-95% methanol:water (18-30 min), and 95% methanol:water (30-60 min) with 1% formic acid at a flow rate of 1.0 mL/min to give compound **3** (1.4 mg, colorless amorphous solid, R_t = 21.0, PDA detector λ_m = 252 nm) and compound **2** (1.8 mg, colorless oil, R_t = 43.0 minutes, PDA detector λ_m = 207 nm) (Scheme **S3**).

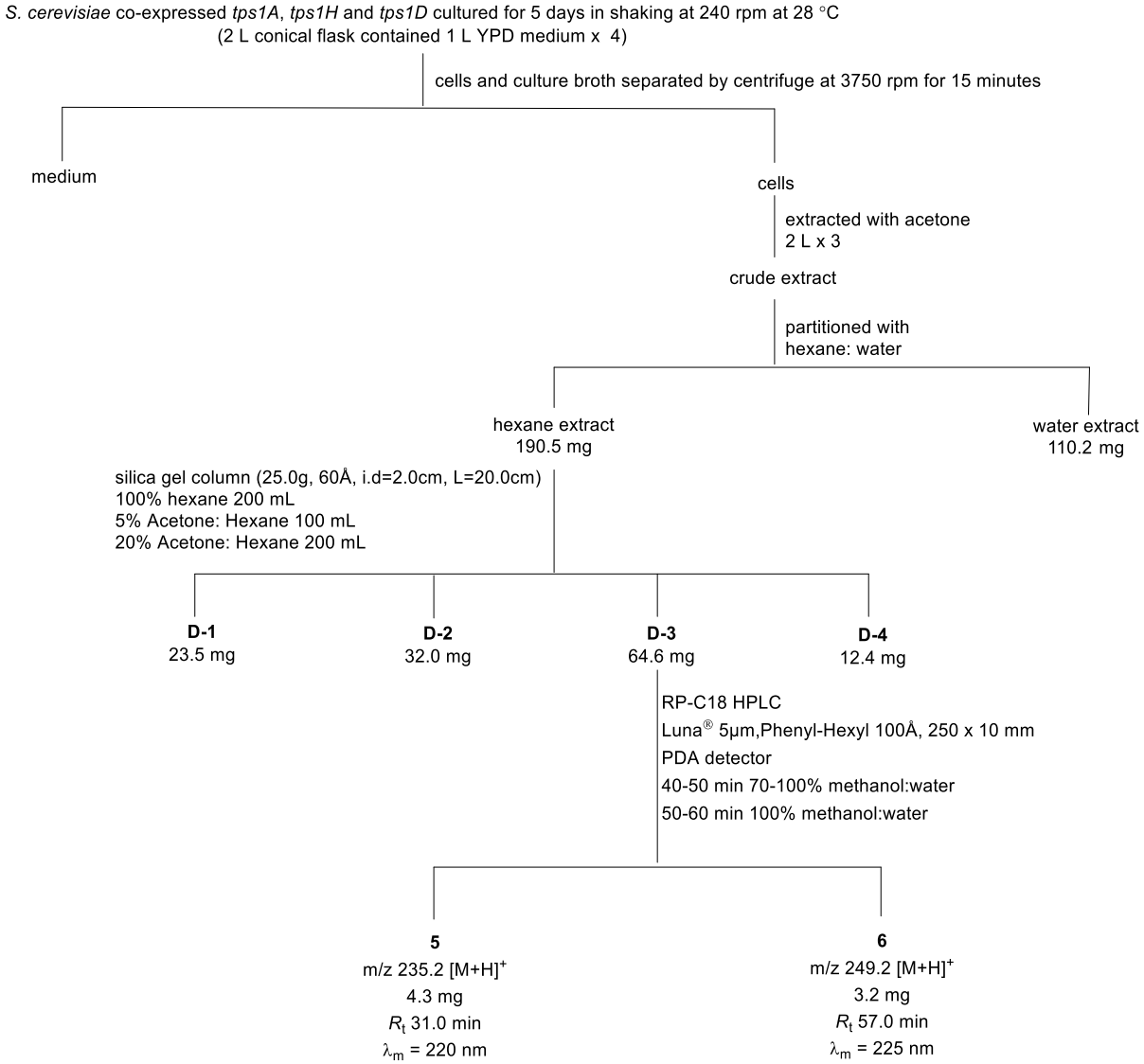


Scheme **S3**. Separation process of compounds **2**–**4** from *tps1A* expressed yeast culture.

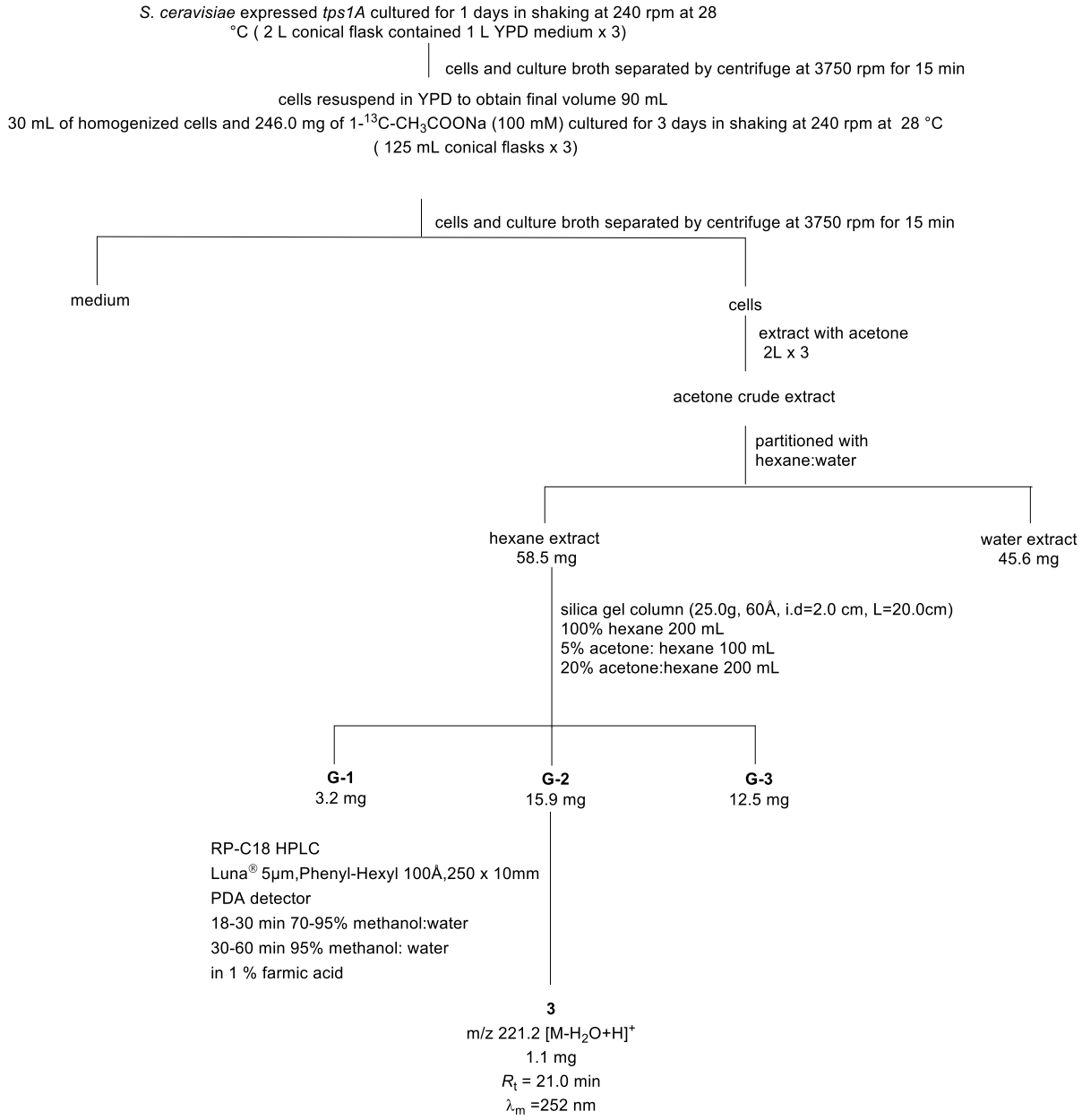
1.19.3 Isolation of compounds 5 and 6

The *S. cerevisiae* RC01 strain harboring pCY02M-Tps1A-Tps1H and pXW02-Tps1D was cultivated on YPD (4L) for 4 days with constant shaking at 250 rpm at 28 ºC. The cells were extracted with acetone three times. The acetone extract was concentrated *in vacuo*, and the resultant crude mixture was partitioned with hexanes and water three times. The hexanes soluble part was concentrated in *vacuo* to give hexanes extracts (190.5 mg) and then separated by a silica gel 60 column (230–400 mesh, 2 × 20 cm) eluted with 100% hexane (200 mL), 5% acetone/hexanes (100 mL), and 20% acetone/hexanes (200 mL) to give four fractions (fractions D1–D4). Fraction D3 was subjected to a semi-preparative RP-C18 HPLC column (Luna®, 5 µm

Phenyl-Hexyl 100 Å , 250 × 10 mm) and eluted by 70% methanol/water (0–40 min), 70–100% methanol/water (40–50 min) and 100% methanol (50–60 min) with flow rate of 1.0 mL/min to give compound **5** (4.3 mg, colorless oil, R_t = 31.0 minutes, PDA detector λ_m = 220 nm) and compound **6** (3.2 mg, yellow color oil, R_t = 57.0 minutes, PDA detector λ_m = 225 nm) (Scheme **S4**).



Scheme **S4**. Separation process of compounds **5** and **6** from *tps1A*, *tps1H*, and *tps1D* co-expressed yeast culture.



Scheme **S3**. Separation process of ^{13}C-labeled compound **^{13}C-3** from *tps1A* expressed yeast culture.

1.20 Method for homologs identification, sequence clustering, and phylogenetic analysis

Homologs of Tps1A were identified with BLASTp[4] against NCBI nr database at https://blast.ncbi.nlm.nih.gov/. A total of 1874 homologs with expected p values < E-10 were identified and protein sequences were downloaded from the website. The homologs of Tps1A were combined with Tps1A and Tps2B (total 1876 sequences) to build a database with which each of the sequences was aligned to all in this database with BLASTp on in-house server.

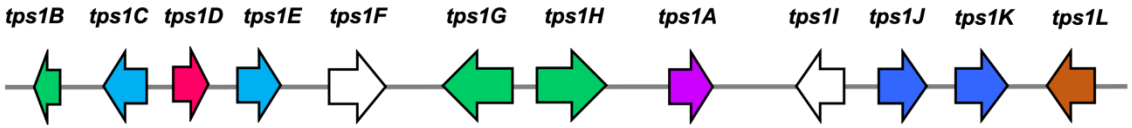
The expected p values calculated for each paired sequence alignment from BLASTp were transformed by taking the logarithm in base 10 and subsequently negating the sign. The transformed values are capped so that any E-value below 1e-200 is set to a maximum allowed edge weight of 200. A data matrix of 1876 by 1876 with the transformed p values was used for sequence clustering using Markov Cluster algorithm v14-137[5] with the inflation rate (I) set to 4.

One of the sequence clusters from MCL which contained 1077 homologs and included Tps1A and Tps1B was considered for phylogenetic analysis. To further reduce the number of homologs, the redundant or similar sequences with identity >0.7 were first grouped and the longest sequence was chosen as the representative for the group using CD-hit at http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi.[6] A total of 460 homologs of Tps1A (including 14 target proteins of interest) were used to build the phylogenetic tree with ETE3 v.3.1.2[7] using the standard fasttree workflow and the default parameters (Clustal Omega v.1.2.1)[8] with default parameters for multiple sequence alignment and FastTree v.2.1.8[9] for tree building). FastTree 2 is an approximately-maximum likelihood method and uses 1000 resamples to estimate the reliability of each split with the Shimodaira-Hasegawa test on the three alternate topologies (nearest-neighbor interchanges) around that split. Treeshrink v 1.3.7 (Mai et al. 2018) was used with alpha equal to 0.1 to remove the outliers (seven) which had unrealistically long branch lengths in the phylogenetic tree.

Final tree plot in the fan layout was generated with ggtree package in R v. 4.1.0 and the tree branches were colored according to the phylum which the protein homolog is belong to, based on the annotation from NCBI taxonomy database. The protein homologs missing the information of phylum in the database were removed from the tree so that only 459 sequences were shown. The phylogenetic tree was further trimmed down with Treemmer v. 0.3[10] to generate a simplified tree (50 sequences) based on the clades found in the tree with pre-determined maximal number of branches per clade.

Supplementary Tables

Table S1. Deduced functions of genes within the *tps1* gene cluster.



Antrodia cinnamomea S27, Scaffold 24

Gene name	Protein name	Size (gene/ protein)[a]	BLASTP homologs[b]	Identity/ similarity (%)[b]	Conserved domain[b]	Putative function[b]
tps1B	Tps1B	576/191	TBU28072.1	65/81	cl12078, cytochrome P450	cytochrome p450
tps1C	Tps1C	969/322	EEB90673.1	58/78	cl33968 , carboxylesterase	epoxide hydrolase
tps1D	Tps1D	870/289	XP_007400083.1	63/74	pfam13649, adenosylmethionine-dependent methyltransferase	methyltransferase
tps1E	Tps1E	1071/356	XP_024339819.1	59/74	pfam07859, α/β-hydrolase	α/β-hydrolase
tps1F	Tps1F		THH19420.1	59/72	hypothetical protein	hypothetical protein
tps1G	Tps1G	1584/527	BAK09392.1	71/83	cl12078, cytochrome P450	cytochrome p450
tps1H	Tps1H	1548/515	XP_007367894.1	67/81	cl12078, cytochrome P450	cytochrome p450
tps1A	Tps1A	948/315	KAF8273408.1	64/77	cd13965, UbiA family of prenyltransferases (PTases)	UbiA prenyltransferase
tps1I	Tps1I		TFK73510.1	35/45	hypothetical protein	hypothetical protein
tps1J	Tps1J	1173/370	XP_024339816.1	64/79	COG0673, predicted dehydrogenase	NAD-binding Rossmann fold oxidoreductase
tps1K	Tps1K	1113/390	TBU38724.1	52/70	COG0596, pimeloyl-ACP methyl ester carboxylesterase	α/β-hydrolase
tps1L	Tps1L	1029/342	XP_012183658.1	75/86	cl00470, aldo-keto reductase	aldo/keto reductase

[a] Open reading frames were predicted by FGENESH (Softberry).
[b] Based on analysis by NCBI Blastp and Conserved Domain Search.

Table S2. Deduced functions of genes within the *tps2* gene cluster.

Antrodia cinnamomea S27, Scaffold 2

Gene name	Protein name	Size (gene/ protein)[a]	BLASTP homologs[b]	Identity/ similarity (%)[b]	Conserved domain[b]	Putative function[b]
tps2B	Tps2B	1341/446	XP_012177243.1	70/80	pfam13532, 2OG-Fe(II) oxygenase	2OG-Fe(II) oxygenase superfamily
tps2C	Tps2C	1590/529	PIL27185.1	60/75	cl12078, cytochrome P450	cytochrome P450
tps2D	Tps2D	1530/509	EPS95741.1	65/80	cl12078, cytochrome P450	cytochrome P450/ hypothetical protein
tps2E	Tps2E	1638/545	KZT01991.1	62/81	cl12078, cytochrome P450	cytochrome P450
tps2A	Tps2A	906/302	TDL18470.1	65/76	cd13965, UbiA family of prenyltransferases (PTases)	UbiA prenyltransferase
tps2F	Tps2F	1008/335	EPS95742.1	54/71		hypothetical protein
tps2G	Tps2G	1632/543	KZT01991.1	64/80	cl12078, cytochrome P450	cytochrome P450

[a] Open reading frames were predicted by FGENESH (Softberry).
[b] Based on analysis by NCBI Blastp and Conserved Domain Search.

Table S3. Selected protein sequences of reported UbiA-type TPSs, the UbiA superfamily of intramembrane prenyltransferases and integral membrane TPSs for phylogenetic analysis.

Protein name	Function	Accession number	Source
AtyB	UbiA polyprenyltransferase	KZL66378.1	*Aspergillus sp.* PSU-RSPG185
MpaA	UbiA polyprenyltransferase	AJG44379.1	*Penicillium roqueforti*
AscA	UbiA prenyltransferase	BBF25313.1	*Acremonium egyptiacum*
Pyr6	Polyprenyl transferase	Q4WLD0.2	*Neosartorya fumigata*
ApUbiA	4-hydroxybenzoate polyprenyltransferase	WP_148679072.1	*Aeropyrum pernix*
AfUbiA	4-hydroxybenzoate polyprenyltransferase	WP_010879665.1	*Archaeoglobus fulgidus*
Coq2	4-hydroxybenzoate octaprenyltransferase	NP_014439.3	*Saccharomyces cerevisiae*
UbiA	4-hydroxybenzoate octaprenyltransferase	WP_000455227.1	*Enterobacteriaceae*
AusN	Polyprenyl transferase	Q5AR21.1	*Aspergillus nidulans*
Fma-TC	Fumagillin β-*trans*-bergamotene synthase	M4VQY9.1	*Aspergillus fumigatus*
Tps1A	UbiA prenyltransferase	KAF8273408.1	*Antrodia cinnamomea*
Tps2A	UbiA prenyltransferase	TDL18470.1	*Antrodia cinnamomea*
EriG	diterpene cyclase	ARE72244.1	*Hericium erinaceus*
StsC	UbiA family prenyltransferase	WP_010468024.1	*Streptomyces somaliensis*

Table S4. Primers used for *tps1* gene cluster study.

Antrodia cinnamomea S27, scaffold 24, *tps1* gene cluster	
Primer name	Sequence (5'→3')
pCY01M-Tps1B-Tps1C-Tps1D-Tps1E	
Tps1B-XhoI-$P_{ADH2\text{-}S.c}$-F	CTATTAACTATATCGTAATACACACTCGAGATGACAAGAGTACCATTCTTTCGTGTC
Tps1B-XhoI-T_{ADH2}-R	CGTAAAGACATAAGAGATCCGCCTCGAGTCAGATGATCCACCTTCTTCCGAATC
Tps1C-NotI-$P_{ADH2\text{-}S.b}$-F	CTCTATCCTCAAAATACAATACAAAGCGGCCGCATGAACCACTCAAACTACAAGGACC
Tps1C-NotI-T_{PGI1}-R	GGTATATATTTAAGAGCGATTTGTGCGGCCGCTTAGTTGAGAGGCAAAGTCAGGATCC
Tps1D-PmeI-P_{PCK1}-F	CTAATTATTCCATAATAAAATAACAACGTTTAAACATGTCTACGAACGCCGAACTGAG
Tps1D-PmeI-T_{ENO2}-R	CTAATAATTCTTAGTTAAAAGCACTGTTTAAACTTATACAATCCTCTTGCGGGACCATG
Tps1E-SmaI-P_{MLS1}-F	GTAAAAGCACATAAAAGAATTAAGAAACCCGGGATGGCGCAGTATAGCCACCTC
Tps1E-SmaI-T_{TDH2}-R	GTAACTTAAGGAGTTAAATCCCGGGTTATGACATCAGCAACAACCTGATCCC
pCY02M-Tps1G-Tps1H-Tps1A	
Tps1G-XhoI-$P_{ADH2\text{-}S.}$-F	CTATTAACTATATCGTAATACACACTCGAGATGGTTGCAGTCGATTCTCTGC
Tps1G-XhoI-T_{ADH2}-R	CGTAAAGACATAAGAGATCCGCCTCGAGTTAGAGAGCTTCAAGGATCACAGGG
Tps1H-NotI-$P_{ADH2\text{-}S.b}$-F	CTATCCTCAAAATACAATACAAAGCGGCCGCATGGAGGGACTTCAAGCTATACCG
Tps1H-NotI-T_{PGI1}-R	GGTATATATTTAAGAGCGATTTGTGCGGCCGCTCACATCGCGCAATGCGCAG
Tps1A-PmeI-P_{PCK1}-F	CTAATTATTCCATAATAAAATAACAACGTTTAAACATGTCTCCTCGCTATTCCGC
Tps1A-PmeI-T_{ENO2}-R	CTAATAATTCTTAGTTAAAAGCACTGTTTAAACTCAAAAGGCAAGGAAACCTGTTCTG
pCY03M-Tps1J-Tps1K-Tps1L	
Tps1J-XhoI-$P_{ADH2\text{-}S..c}$-F	CTATTAACTATATCGTAATACACACTCGAGATGGCACCCATCCGCTTAGG
Tps1J-XhoI-T_{ADH2}-R	GTAAAGACATAAGAGATCCGCCTCGAGTCACAAGACTACTTTCTTACCCTCCAATG
Tps1K-NotI-$P_{ADH2\text{-}S.b}$-F	CTCTATCCTCAAAATACAATACAAAGCGGCCGCATGGTGTTCACTGCGACCATG
Tps1K-NotI-T_{PGI1}-R	GTATATATTTAAGAGCGATTTGTGCGGCCGCCTACGAAAGACTTTCCAAGTAAGCCTG
Tps1L-PmeI-P_{PCK1}-F	CTAATTATTCCATAATAAAATAACAACGTTTAAACATGTCCGAACCAGTATTGGCTG
Tps1L-PmeI-T_{ENO2}-R	CTAATAATTCTTAGTTAAAAGCACTGTTTAAACTCAGAATCTGGGCGGCTGAGG
pXW02-Tps1D	
Tps1D-pXW02-NdeI-F	AATCAACTATCAACTATTAACTATATCGTAATACCATATGTCTACGAACGCCGAACTGAG

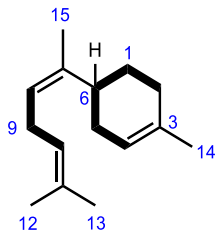
Tps1D-pXW02-PmlI-R	GGTGGTGGTGCACGTGTACAATCCTCTTGCGGGACCATG
pXW02-Tps1H	
Tps1H-pXW02-NdeI-F	ATCAACTATCAACTATTAACTATATCGTAATACCATATGGAGGGACTTCAAGCTATACCG
Tps1H-pXW02-PmlI-R	GTGGTGGTGGTGGTGCACGTGTCACATCGCGCAATGCGCAG
pXW55-Tps1A	
Tps1A-NdeI-F	ACCATATGTCTCCTCGCTATTCCGC
Tps1A-pXW55-PmlI-R	TGCACGTGTCAAAAGGCAAGGAAACCTGTTCTG
pXW55-Tps1A-D100A, Y155A, D215A, D218A, D222A	
Tps1A-D100A-F	CAAGCAAGAGGGAAGAGTGAGGCCGCCTTAAACAAACCATGGCGG
Tps1A-D100A-R	CCGCCATGGTTTGTTTAAGGCGGCCTCACTCTTCCCTCTTGCTTG
Tps1A-Y155A-F	CATCCTCACGACGTTGCTCGCAGACGAGCTTGGCATGG
Tps1A-Y155A-R	CCATGCCAAGCTCGTCTGCGAGCAACGTCGTGAGGATG
Tps1A-D215A-F	CAGGCACAAGCGTTCCCCGACATCGAAGGAGAC
Tps1A-D215A-R	CTTCGATGTCGGGGAACGCTTGTGCCTGAATGGTAGTG
Tps1A-D222A-R	CACGACCCAAGGCCGCCGCTCCTTCGATGTCGGGGAAG
Tps1A-D100A-F	CAAGCAAGAGGGAAGAGTGAGGCCGCCTTAAACAAACCATGGCGG
Tps1A-D100A-R	CCGCCATGGTTTGTTTAAGGCGGCCTCACTCTTCCCTCTTGCTTG
Tps1A-Y155A-F	CATCCTCACGACGTTGCTCGCAGACGAGCTTGGCATGG

Table S5. Primers used for *tps2* gene cluster study.

*Antrodia cinnamomea*_S27- scaffold 2-*tps2* gene cluster	
Primer name	Sequence (5'→3')
pCY01M-Tps2D-Tps2E-Tps2A	
Tps2D-XhoI-$P_{ADH2\text{-}S.c}$-F	TAACTATATCGTAATACACACTCGAGATGATGGCCACGACA GTCTATCTAC
Tps2D-XhoI-T_{ADH2}-R	GTAAAGACATAAGAGATCCGCCTCGAGTCAGGTGTCTTTG CGAAGAAGCTC
Tps2E-NotI-$P_{ADH2\text{-}S.b}$ -F	CCTCAAAATACAATACAAAGCGGCCGCATGCAGACCTACA CTATCATTGAAAGC
Tps2E-NotI-T_{PGI1}-R	GTATATATTTAAGAGCGATTTGTGCGGCCGCCTATGTGCCT GTTATCGGCCCG
Tps2A-PmeI-P_{PCK1}-F	CTAATTATTCCATAATAAAATAACAACGTTTAAACATGGCG TCGAAGCGTACTTTC
Tps2A-PmeI-T_{ENO2}-R	AATTCTTAGTTAAAAGCACTGTTTAAACTTAGAGAGCCAT GAGATTCCACCGTG
pCY02M-Tps2F-Tps2G	
Tps2G-XhoI-$P_{ADH2\text{-}S.c}$ -F	CTATTAACTATATCGTAATACACACTCGAGATGGAGACCAT GGCAATATTGAATGTG
Tps2G-XhoI-T_{ADH2}-R	GTAAAGACATAAGAGATCCGCCTCGAGTCAGATGCTCTTC ACCGGGCG
Tps2F-NotI-$P_{ADH2\text{-}S.\ b}$-F	CCTCAAAATACAATACAAAGCGGCCGCATGACAGTATCAT TGCAGAAGGCTG
Tps2F-NotI-T_{PGI1}-R	GTATATATTTAAGAGCGATTTGTGCGGCCGCTCAGACATGG GATGCCTCCGTG
pCY03M-Tps2B-Tps2C	
Tps2C-XhoI-$P_{ADH2\text{-}S.c}$ -F	CTATTAACTATATCGTAATACACACTCGAGATGGAACTGCT CAATGAGACCCTAC
Tps2C-XhoI-T_{ADH2}-R	GTAAAGACATAAGAGATCCGCCTCGAGCTACAACTGATGC AAATTCGAATCCTCAG
Tps2B- NotI-$P_{ADH2\text{-}S.\ b}$-F	CCTCAAAATACAATACAAAGCGGCCGCATGTCTTCAGCGA CAACGACCC
Tps2B-NotI-T_{PGI1}-R	TATATTTAAGAGCGATTTGTGCGGCCGCTCATGATACATTG GTATTTACATCGAGGGTTG
pXW55-Tps2A	
Tps2A-NdeI-F	CAACTATCAACTATTAACTATATCGTAATACCATATGGCGTC GAAGCGTACTTTC
Tps2A-pXW55-PmlI-R	GATGGTGATGGTGATGCACGTGGAGAGCCATGAGATTCCA CCGTG

Table S6. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) and 2D NMR spectroscopic data of (+)-(*S*,*Z*)-α-bisabolene (**1**) in $CDCl_3$.

A



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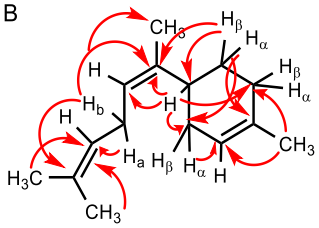

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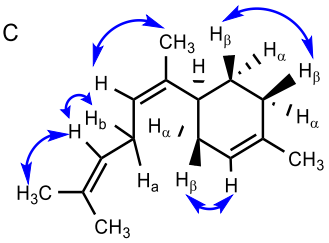
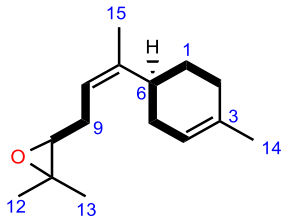


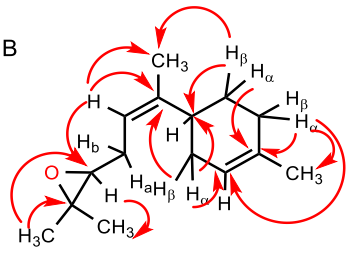
No	δ_H (mult, J in Hz)	δ_C (type)	HMBC	COSY	NOESY
1α	1.57 (m)	27.6 (CH_2)	139.3, 134.0, 35.4, 29.7	2.64, 2.01, 1.92	2.64
1β	1.59 (m)		139.3, 134.0, 35.4, 29.7		1.92
2α	2.01 (m)	30.8 (CH_2)	134.0, 121.8	1.59	
2β	1.92 (m)		134.0	1.57	
3		134.0 (CH)			
4	5.42 (m)	121.8 (CH)	35.4, 30.8	2.70, 1.66	2.00, 1.83, 1.66
5α	1.83 (m)	29.7 (CH_2)	121.8	2.64	1.66
5β	2.00 (m)				5.07
6	2.64 (m)	35.4 (CH)	139.3, 123.7, 29.8, 27.6, 19.4	2.00, 1.83, 1.57	
7		139.3 (C)			
8	5.09 (br t, 7.2)	123.7 (CH)	35.4, 26.5, 19.4,	2.70, 1.61	2.70, 1.61
9a	2.70 (m)	26.5 (CH_2)	139.3, 131.4, 123.7	5.08, 5.09	
9b	2.70 (m)		139.3, 131.4, 123.7		
10	5.08 br (t, 7.2)	123.7 (CH)	35.4, 17.8	2.70	2.70
11		131.4 (C)			
12	1.68 (s)	25.9 (CH_3)	131.4, 123.7, 17.8	5.08	
13	1.61 (br s)	17.8 (CH_3)	131.4, 123.7, 25.9	5.08	
14	1.66 (s)	23.8 (CH_3)	134.0, 121.8, 30.8	5.42	
15	1.61 (br s)	19.4 (CH_3)	139.3, 123.7, 35.4	5.09	

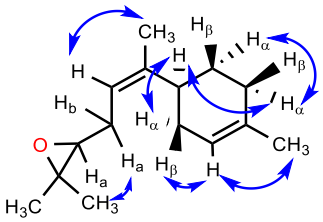
Table S7. Comparison of reported ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of (+)-(*S,Z)*-α-bisabolene (**1**).

	Reference[11, 12] (in $CDCl_3$) ^{1}H NMR: 500MHz,^{13}C NMR 125MHz		In this study (in acetone-d_6) ^{1}H NMR: 500MHz,^{13}C NMR 125MHz	
Carbon number	δ_H (mult, *J* in Hz)	$\boldsymbol{\delta}_C$	δ_H (mult, *J* in Hz)	$\boldsymbol{\delta}_C$
1α	1.60 (m)	27.7 (CH_2)	1.57 (m)	27.6 (CH_2)
1β	1.82 (m)		1.59 (m)	
2α	2.00 (m)	30.8 (CH_2)	2.01 (m)	30.8 (CH_2)
2β	2.00 (m)		1.92 (m)	
3		133.5 (C)		134.0 (CH)
4	5.46 (br s)	121.1 (CH)	5.42 (m)	121.8 (CH)
5α	2.00 (m)	29.6 (CH_2)	1.83 (m)	29.7 (CH_2)
5β	2.00 (m)		2.00 (m)	
6	2.68 (m)	35.5 (CH)	2.64 (m)	35.4 (CH)
7		138.9 (C)		139.3 (C)
8	5.11 (br t, 7.0)	123.8 (CH)	5.09 (br t, 7.2)	123.7 (CH)
9a	2.72 (t, 7.0)	26.4 (CH_2)	2.70 (m)	26.5 (CH_2)
9b	2.72 (t, 7.0)		2.70 (m)	
10	5.13 (br t, 7.0)	123.8 (CH)	5.08 br (t, 7.2)	123.7 (CH)
11		131.0 (C)		131.4 (C)
12	1.69 (s)	25.7 (CH_3)	1.68 (s)	25.9 (CH_3)
13	1.62 (s)	17.6 (CH_3)	1.61 (s)	17.8 (CH_3)
14	1.66 (s)	23.6 (CH_3)	1.66 (s)	23.8 (CH_3)
15	1.62 (s)	19.2 (CH_3)	1.61 (s)	19.4 (CH_3)

Table S8. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) and 2D NMR spectroscopic data of **2** in acetone-d_6.

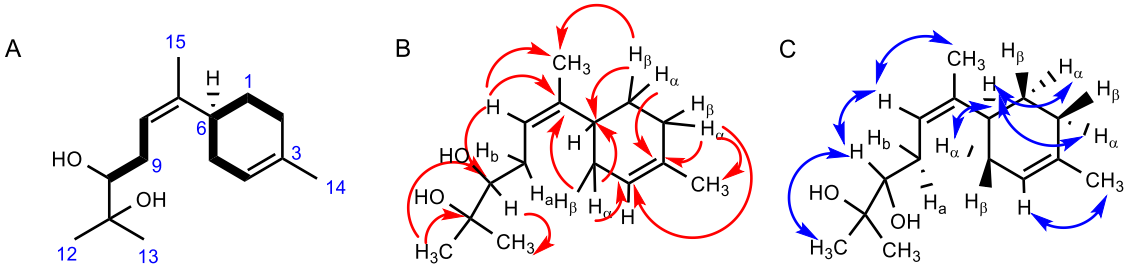
A 

B 

C 

No	d_H (mult, *J* in Hz)	d_C (type)	HMBC	COSY	NOESY
1	1.58 (m)	28.2 (CH_2)	134.1, 36.2, 30.2	2.65, 2.09, 1.94	1.94
2α	2.09 (m)	31.1 (CH_2)		1.94, 1.58	
2β	1.94 (m)		134.1, 121.6, 36.2, 23.7	2.09, 1.58	1.65, 1.63, 1.58
3		134.1 (C)			
4	5.40 (br s)	121.6 (CH)	134.1, 36.2, 31.1, 30.2, 22.8	1.82, 1.63	2.01, 1.82, 1.63
5α	2.01 (m)			2.65, 1.82, 1.63	5.40, 2.65
5β	1.82 (br.d, 17.0)	30.2 (CH_2)	134.1, 121.6, 36.2, 28.2	2.65, 2.01	5.40
6	2.65 (m)	36.2 (CH)	142.1, 120.7, 30.2, 28.2, 19.5	2.65, 1.82, 2.01	2.01
7		142.1 (C)			
8	5.20 (br.td, 7.4, 1.1)	120.7 (CH)	142.1, 64.0, 58.0, 36.2, 28.0, 19.5	2.24, 1.65	1.65
9	2.24 (m)	28.0 (CH_2)	142.1, 121.6, 64.0, 58.0	2.63, 1.65	2.63, 1.24
10	2.63 (t, 6.3)	64.0 (CH)	142.1, 121.6, 58.0, 28.0, 25.0	2.24	2.24, 1.24, 1.23
11		58.0 (C)			
12	1.24 (s)	18.9 (CH_3)	64.0, 58.0, 25.0		2.63, 2.24
13	1.23 (s)	25.0 (CH_3)	64.0, 58.0, 18.9		2.63
14	1.63 (br.s)	23.7 (CH_3)	134.1, 121.6, 31.9	5.40, 2.01	5.40, 1.94
15	1.65 (br.s)	19.5 (CH_3)	142.1, 120.7, 36.2	5.20, 2.24	5.20, 1.94, 1.83

Table S9. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) and 2D NMR spectroscopic data of **3** in acetone-d_6.



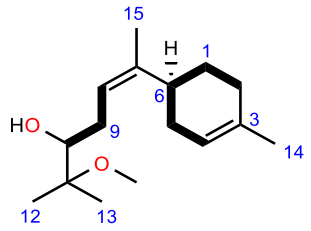
No	δ_H (mult, J in Hz)	δ_C (type)	HMBC	COSY	NOESY
1	1.56 (m)	28.3 (CH_2)	134.0. 36.1, 31.2, 30.3	2.66, 2.05, 1.94	2.66, 2.05, 1.94
2α	2.05 (m)	31.2 (CH_2)	134.0, 121.8	1.94, 1.56	1.56
2β	1.94 (m)		134.0, 121.8, 36.1	2.05, 1.56	1.62, 1.56
3		134.0 (C)			
4	5.39 (br.s)	121.8 (CH)	36.1, 31.2, 30.3, 23.7	2.02, 1.84, 1.62	1.84, 1.62
5α	2.02 (m)		134.0, 121.8	5.39, 2.66, 1.84	
5β	1.84 (br.d, 17.3)	30.3 (CH_2)	134.0, 121.8, 36.1	5.39, 2.66, 2.02	5.39, 2.02, 1.62
6	2.66 (m)	36.1 (CH)	140.3, 124.1, 31.2, 30.3, 28.3, 19.6	2.02, 1.84, 1.56	2.36, 2.02, 1.84, 1.56
7		140.3 (C)			
8	5.30 (br.t, 7.0)	124.1 (CH)	140.3, 36.1, 30.4, 19.6	2.36, 2.02, 1.62	2.36, 2.02, 1.62
9a	2.36 (br.dd, 14.8, 7.0)	30.4 (CH_2)	140.3, 124.1, 79.5, 72.7, 19.6	5.30, 2.02, 1.62	5.30, 2.66
9b	2.02 (m)		140.3, 124.1, 79.3, 72.7		
10	3.28 (dd, 10.1, 2.5)	79.5 (CH)	124.1, 72.7, 30.4, 25.8, 25.4	2.36, 2.02	2.36, 2.02, 1.13
11		72.7 (C)			
12	1.13 (s)	25.4 (CH_3)	79.5, 72.7, 25.8		3.28
13	1.13 (s)	25.8 (CH_3)	79.5, 72.7, 25.4		3.28
14	1.62 (br.s)	23.7 (CH_3)	134.0, 121.8, 31.2	5.39, 5.30, 2.36	5.39, 5.30, 1.94, 1.84
15	1.62 (br.s)	19.6 (CH_3)	140.3, 124.1, 36.1	5.39, 5.30, 2.36	5.39, 5.30, 1.94, 1.84

Table S10. Comparison of reported ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of (−)-(*S,Z)*-10,11-dihydroxy-bisabolene (**3**)[13].

	Reference[13] (in $CDCl_3$) ^{1}H NMR: 500MHz,^{13}C NMR 125MHz		In this study (in acetone-d_6) ^{1}H NMR: 500MHz,^{13}C NMR 125MHz	
Carbon number	δ_H (mult, *J* in Hz)	$\boldsymbol{\delta}_C$	δ_H (mult, *J* in Hz)	$\boldsymbol{\delta}_C$
1α	1.53 (m)	27.5 (CH_2)	1.54 (m)	28.3 (CH_2)
1β	1.58 (m)		1.57 (m)	
2α	2.05 (m)	30.5 (CH_2)	2.04 (m)	31.1 (CH_2)
2β	1.92 (m)		1.94 (m)	
3		133.8 (C)		134.0 (C)
4	5.38 (m)	120.8 (CH)	5.38 (br s)	121.8 (CH)
5α	2.02 (m)	29.9 (CH_2)	1.84 (m)	29.9 (CH_2)
5β	1.80 (m)		1.81 (m)	
6	2.60 (m)	35.4 (CH)	2.65 (sept, 5.5)	36.1 (CH)
7		143.5 (C)		140.3 (C)
8	5.18 (dd, 7.3, 7.0)	120.9	5.29 (t, 7.0)	124.1 (CH)
9a	2.20 (m)	29.8 (CH_2)	2.34 (m)	30.4 (CH_2)
9b	2.16 (m)		2.01 (m)	
10	3.36 (dd,8.9, 4.0)	77.9 (CH)	3.28 (dd,10.0, 2.4)	79.5 (CH)
11		72.6 (C)		72.7 (C)
12	1.16 (s)	23.7 (CH_3)	1.14 (s)	25.3 (CH_3)
13	1.22 (s)	26.6 (CH_3)	1.15 (s)	25.7 (CH_3)
14		(CH_3)	1.62 (s)	23.7 (CH_3)
15	1.66 (s)	19.5 (CH_3)	1.61 (s)	19.5 (CH_3)

Table S11. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) and 2D NMR spectroscopic data of **4** in acetone-d_6

A



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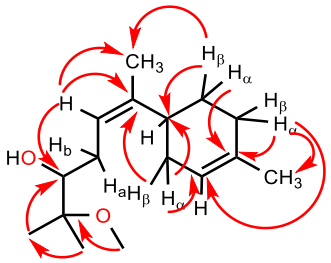

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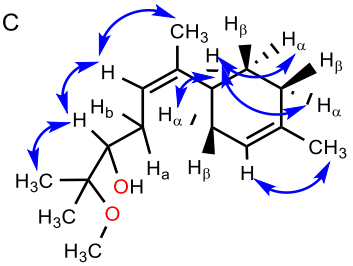
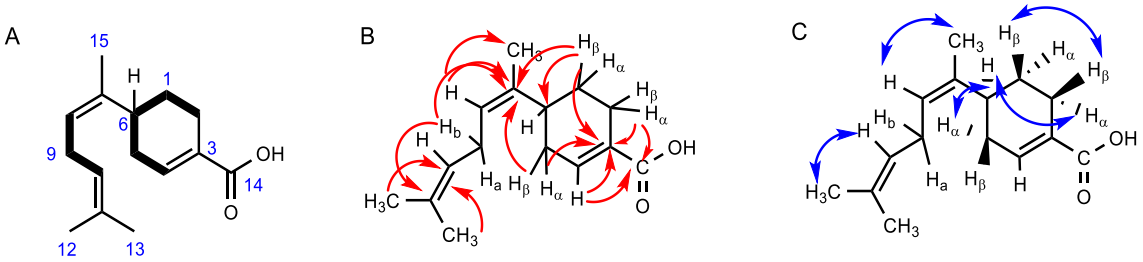


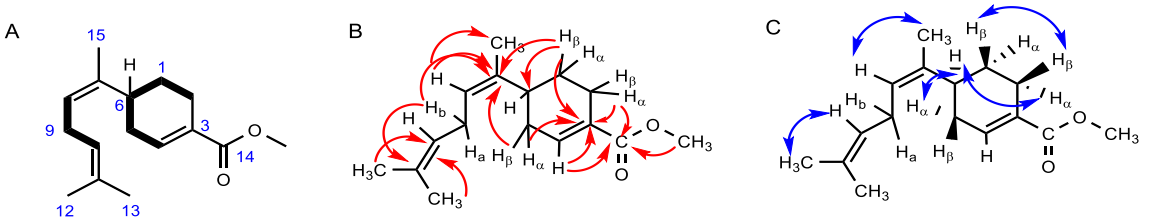
No	δ_H (mult, *J* in Hz)	δ_C (type)	HMBC	COSY	NOESY
1	1.57 (m)	28.3 (CH_2)	134.0, 30.1	2.65, 2.05, 1.94	2.65, 2.05, 1.94
2α	2.05 (m)	31.2 (CH_2)		1.94, 1.57	1.57
2β	1.94 (m)		134.0, 121.9	2.05, 1.57	1.62, 1.57
3		134.0 (C)			
4	5.39 (br.s)	121.9 (CH)	36.1, 31.2, 23.7	2.00, 1.84, 1.62	1.62
5α	2.00 (m)		134.0, 121.9, 28.3	5.39, 2.65, 1.84	2.65
5β	1.84 (br.d, 16.2)	30.0 (CH_2)	134.0, 121.9, 28.3	5.39, 2.65, 2.00	2.65, 1.62
6	2.65 (m)	36.1 (CH)	140.6, 124.2, 31.2, 30.0, 28.3, 19.6	2.00, 1.84, 1.57	2.33, 1.94, 1.84, 1.57
7		140.6 (C)			
8	5.30 (br.t, 7.4)	124.2 (CH)	77.7, 36.1, 30.1, 19.6	2.33, 1.97, 1.62	1.62
9a	2.33 (br.dd, 15.1, 7.4)	30.1 (CH_2)	140.6, 124.2, 77.8, 77.7, 19.6	5.30, 3.36, 1.97	2.65
9b	1.97 (m)		140.6, 124.2, 77.8, 77.7	5.30, 3.36, 2.33	3.36, 2.65, 1.12, 1.09
10	3.36 (dd, 9.9, 1.9)	77.7 (CH)	124.1, 77.8, 21.1, 20.1	2.33, 1.97, 1.12, 1.09	1.97, 1.12, 1.09
11		77.8 (C)			
12	1.09 (s)	20.1 (CH_3)	77.8, 77.7, 21.1		3.36, 3.17, 1.97
13	1.12 (s)	21.1 (CH_3)	77.8, 77.7, 20.1		3.36, 3.17, 1.97
14	1.62 (br.s)	23.7 (CH_3)	140.6, 134.0, 124.0, 121.9, 77.7, 36.1, 30.1	5.39, 5.30	5.39, 5.30, 1.94, 1.84
15	1.62 (br.s)	19.6 (CH_3)	140.6, 134.0, 124.0, 121.9, 77.7, 36.1, 30.1	5.39, 5.30	5.39, 5.30, 1.94, 1.84

Table S12. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) and 2D NMR spectroscopic data of **5** in methanol-d_4.



No	δ_H (mult, *J* in Hz)	δ_C (type)	HMBC	COSY	NOESY
1α	1.66 (m)	28.1 (CH_2)	31.0	2.72, 2.47, 2.19, 1.57	2.72, 2.19, 1.57
1β	1.57 (m)		35.9, 31.0, 26.0	2.72, 2.47, 2.19, 1.66	1.66
2α	2.19 (m)	26.0 (CH_2)	140.8, 131.5, 28.2, 35.9	2.47, 1.66, 1.57	1.66
2β	2.47 (m)		171.1, 140.8, 131.5, 35.9, 28.1	2.19, 1.66, 1.57	1.63
3		131.5 (C)			
4	7.02 (m)	140.8 (CH)	171.1, 132.0, 35.9, 31.0, 26.0	2.21, 2.07	2.20
5	2.20 (m)	31.0 (CH_2)	140.8, 131.5, 35.9, 28.1	7.02, 2.72	7.02
6	2.72 (m)	35.9 (CH)	139.0, 36.0, 31.0, 28.1, 19.2	2.20, 1.66, 1.57	1.66
7		139.0 (C)			
8	5.16 (br.td, 7.3, 1.3)	125.6 (CH)	124.5, 35.9, 27.3, 19.2	2.71, 1.63	2.71, 1.63
9	2.71 (m)	27.3 (CH_2)	139.0, 132.0, 124.5	5.16, 5.07	5.16, 1.63
10	5.07 (m)	124.5 (CH)	125.6, 27.3, 25.9, 17.8	2.71, 1.68, 1.62	2.71
11		132.0 (C)			
12	1.62 (br.s)	17.8 (CH_3)	132.0, 124.5, 25.9	5.07	
13	1.68 (br.s)	25.9 (CH_3)	132.0, 124.5, 17.8	5.07	5.07
14		171.1 (CH_3)			
15	1.63 (br.s)	19.2 (CH_3)	139.0, 125.6, 35.9	5.16	5.16, 2.47, 2.71

Table S13. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) and 2D NMR spectroscopic data of **6** in acetone-d_6.



No	δ_H (mult, J in Hz)	δ_C (type)	HMBC	COSY	NOESY
1α	1.67 (m)	27.6 (CH_2)	35.2, 30.5	2.74, 2.46, 1.57	2.46, 2.14, 1.57
1β	1.57 (m)		35.2, 30.5, 25.5	2.74, 2.46, 1.67	2.46, 1.67
2α	2.14 (m)	25.5 (CH_2)	167.7, 140.0, 130.7, 35.2, 27.6	6.96, 2.46	2.74, 2.46, 1.67
2β	2.46 (m)		167.7, 140.0, 130.7, 35.2, 27.6	2.14, 1.57	2.14, 1.67, 1.63, 1.57
3		130.7 (C)			
4	6.96 (m)	140.0 (CH)	167.6, 130.7, 35.2, 30.5, 25.5	2.20, 2.14	3.70, 2.74, 2.14
5	2.20 (m)	30.5 (CH_2)	140.0, 130.7, 35.2, 27.6	6.96, 2.14	2.74, 1.63
6	2.74 (m)	35.2 (CH)	140.0, 138.5, 131.5, 125.2, 124.3, 30.5, 27.6, 25.5, 19.1	2.20, 1.67, 1.63, 1.57	6.96, 2.20, 2.14, 1.67, 1.63
7		138.5 (C)			
8	5.15 (br.td, 7.3, 1.3)	125.2 (CH)	124.3, 35.2, 26.9, 19.1	2,72, 1.63	2,72, 1.63
9	2.72 (m)	26.9 (CH_2)	138.5, 131.5, 124.3	5.15, 5.08	5.15, 5.08
10	5.08 (m)	124.3 (CH)	125.2, 25.8, 17.7	2.72, 1.66	2.72, 1.61
11		131.5 (C)			
12	1.61 (br.s)	17.7 (CH_3)	131.5, 124.3		5.08
13	1.66 (br.s)	25.8 (CH_3)	131.5, 124.3, 17.7	5.08	
14		167.7 (CH_3)			
15	1.63 (br.s)	19.1 (CH_3)	138.5, 125.2, 35.2	5.15, 2.74	5.15, 2.74, 2.46, 2.20
15'	3.70 (s)	51.6 (CH_3)	167.7, 130.7		6.96

Supplementary Figures

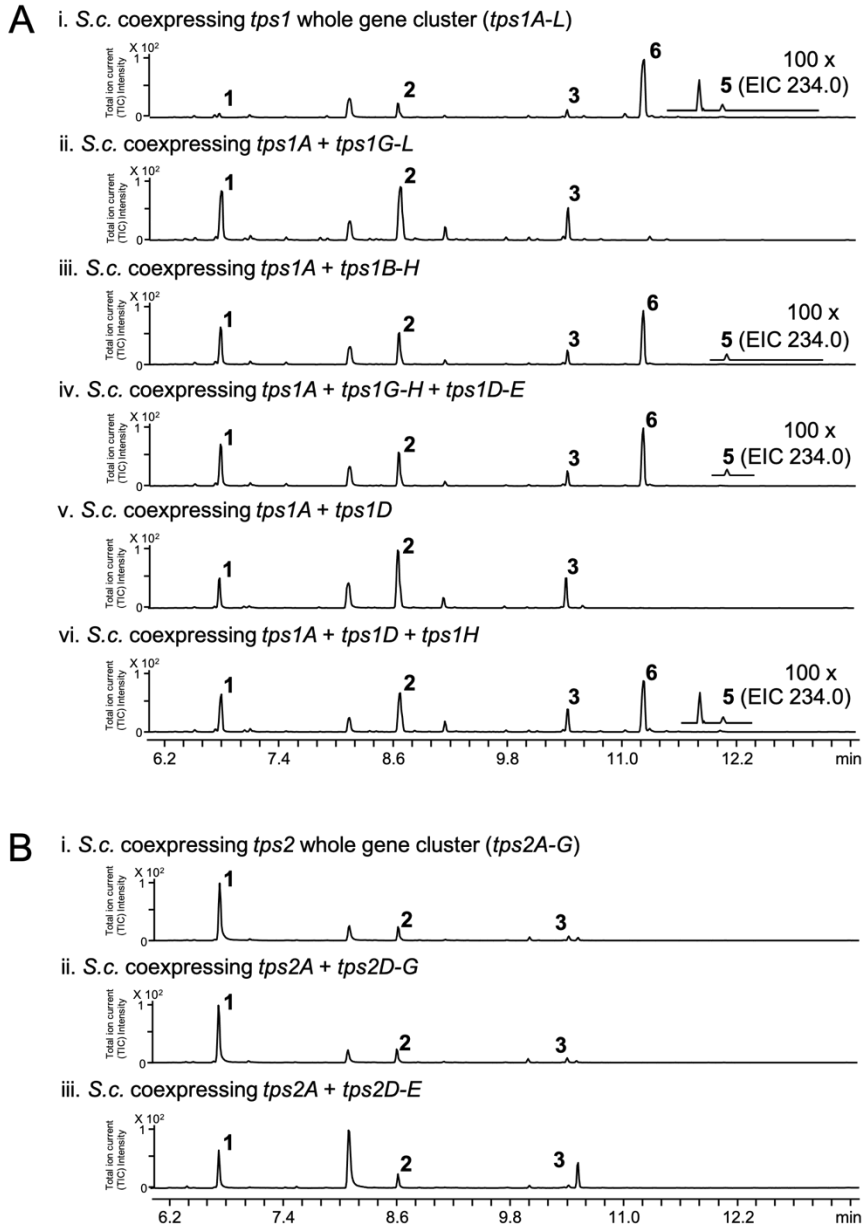


Figure S1. GC-MS analysis of yeast transformants (A) (i) *S. cerevisiae* co-expressing *tps1* whole gene cluster (*tps1A–tps1L*), (ii) *S. cerevisiae co*-expressing *tps1A* + *tps1G–tps1L*, (iii) *S. cerevisiae* co-expressing *tps1A* + *tps1B–tps1H*, (iv) *S. cerevisiae* co-expressing *tps1A* + *tps1G–tps1H* + *tps1D–tps1E*, (v) *S. cerevisiae* co-expressing *tps1A* + *tps1D*, and (vi) *S. cerevisiae* co-expressing *tps1A* + *tps1D* + *tps1H*; (B) (i) *S. cerevisiae* co-expressing *tps2* whole gene cluster (*tps2A–tps2G*), (ii) *S. cerevisiae co*-expressing *tps2A* + *tps2D–tps2G*, (iii) *S. cerevisiae* co-expressing *tps2A* + *tps2D–tps2E*.

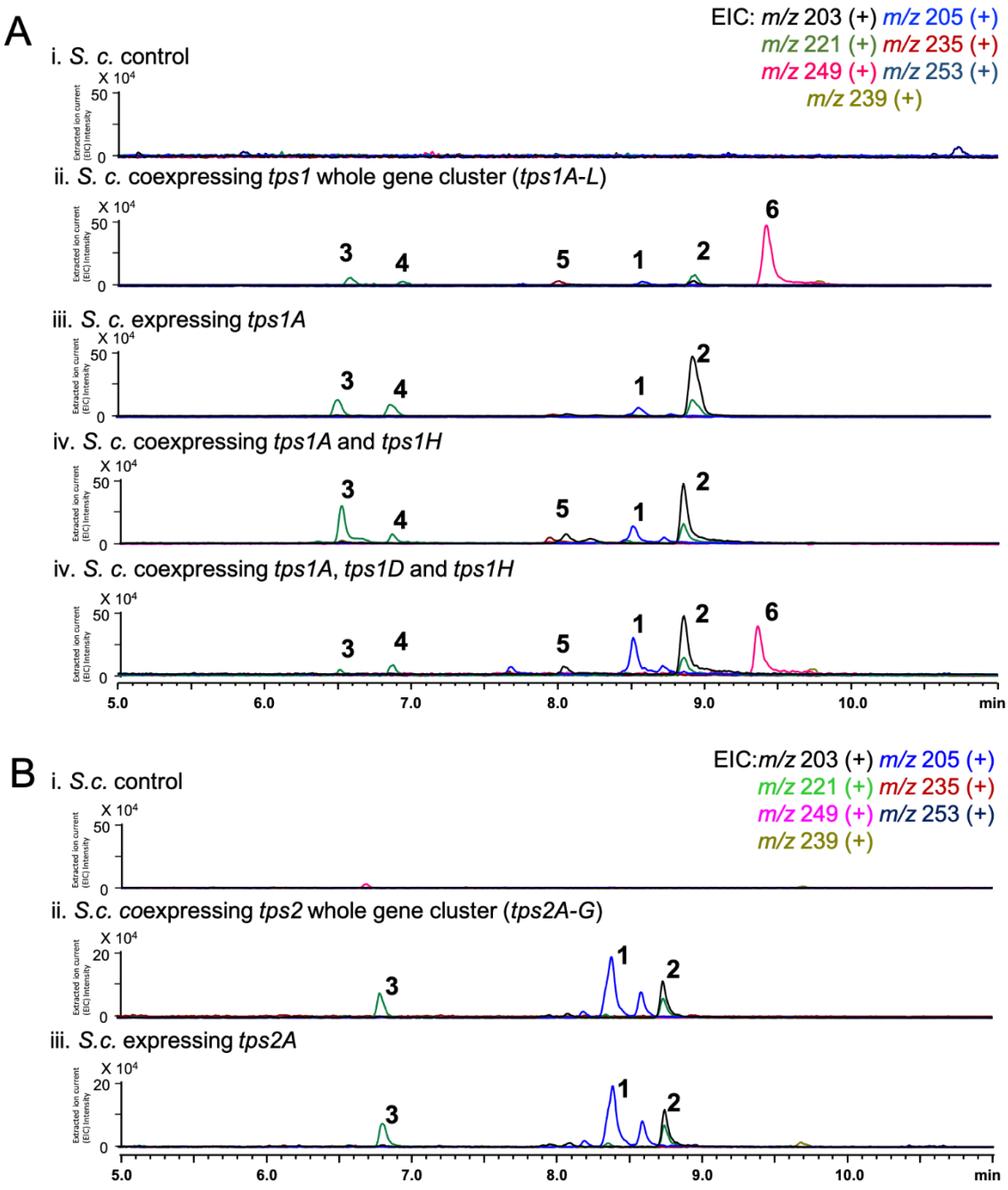


Figure S2. LC-MS analysis of yeast transformants, (A) (i) *S. cerevisiae* control (ii) *S. cerevisiae* co-expressing *tps1* whole gene cluster (*tps1A–tps1L*), (iii) *S. cerevisiae co*-expressing *tps1A,* (iv) *S. cerevisiae* co-expressing *tps1A* + *tps1H*, (v) *S. cerevisiae* co-expressing *tps1A* + *tps1D* + *tps1H*; (B) (i) *S. cerevisiae* control (ii) *S. cerevisiae* co-expressing *tps2* whole gene cluster (*tps2A–tps2G*), and (iii) *S. cerevisiae co*-expressing *tps2A*.

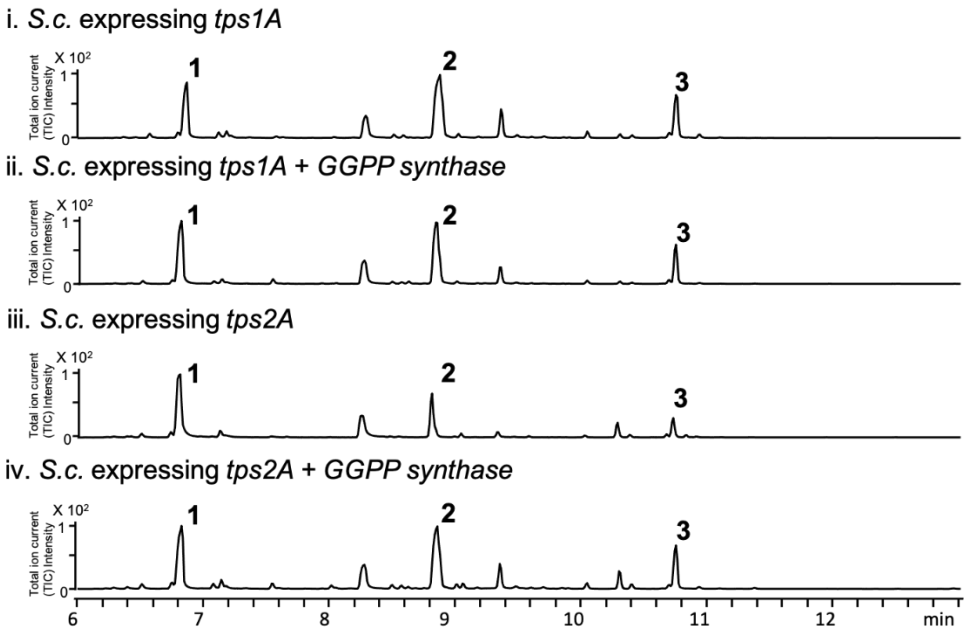


Figure S3. GC-MS analysis of yeast transformants, (i) *S. cerevisiae* expressing *tps1*A, (ii) *S. cerevisiae co*-expressing *tps1A* + *GGPP*S, (iii) *S. cerevisiae* expressing *tps2A*, (iv) *S. cerevisiae* co-expressing *tps2A* + *GGPP*S.

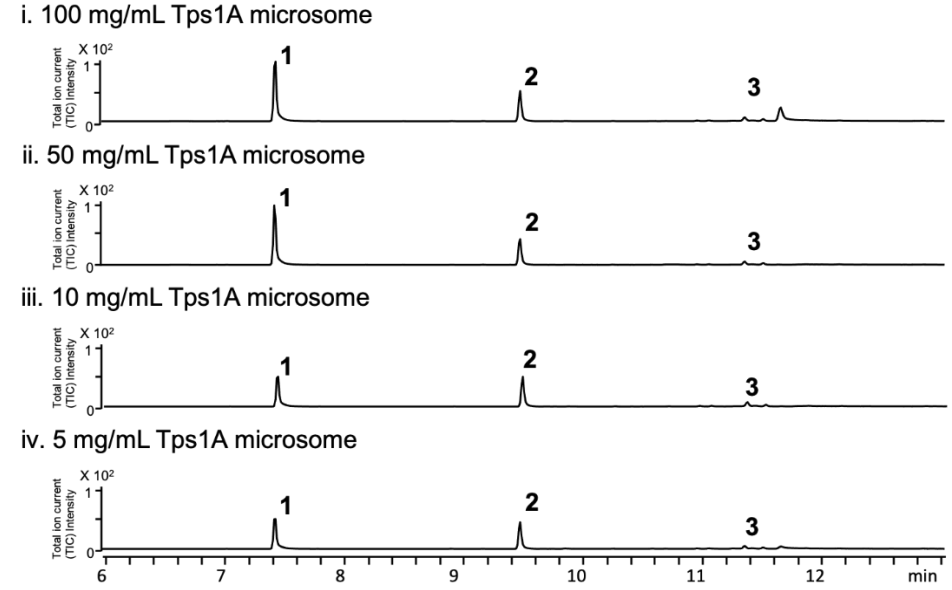


Figure S4. GC-MS analysis of *in vitro* assay with (i) 100 mg/mL, (ii) 50 mg/mL, (iii) 10 mg/mL and (iv) 5 mg/mL Tps1A microsomal fraction + FPP.

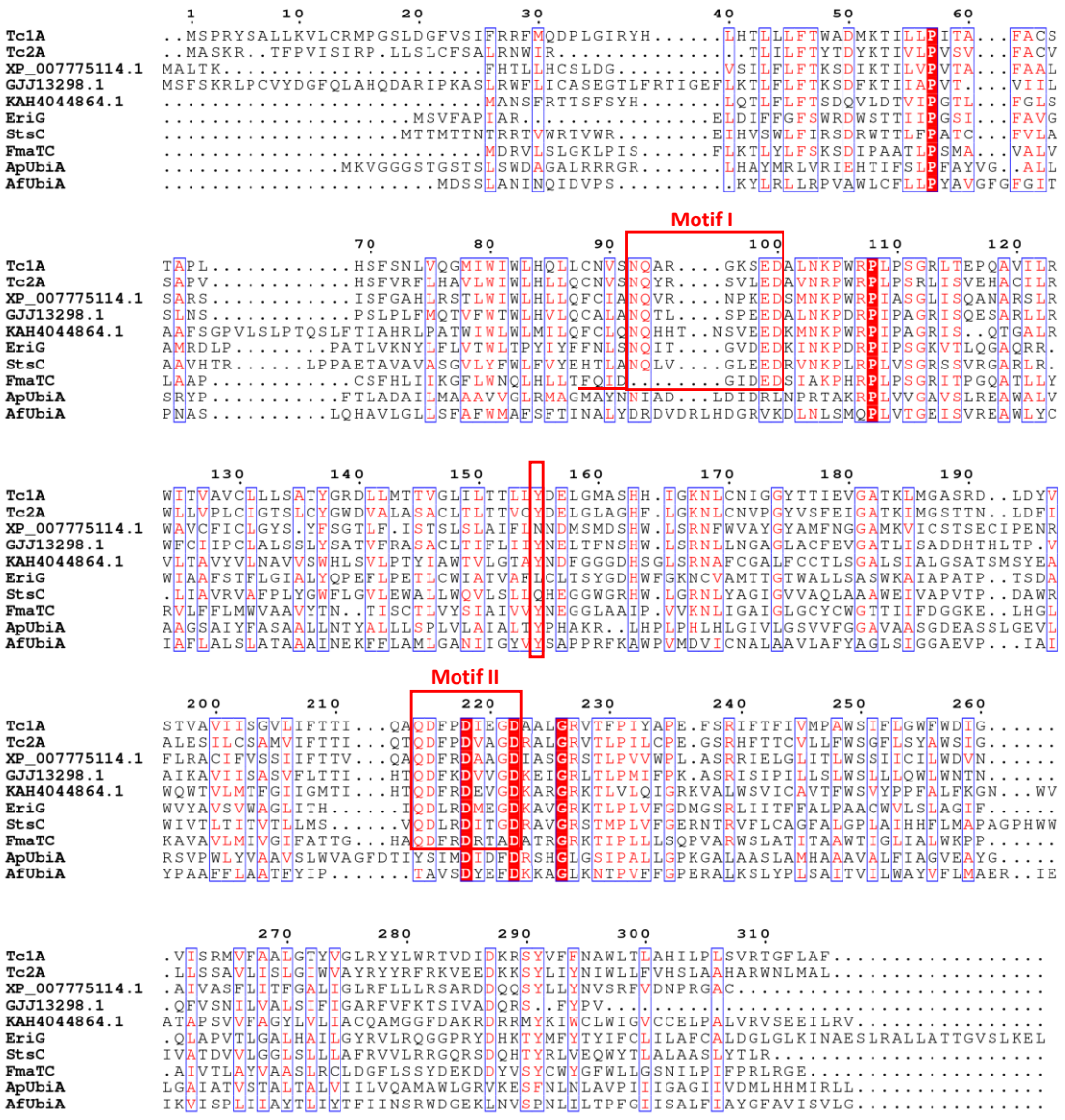
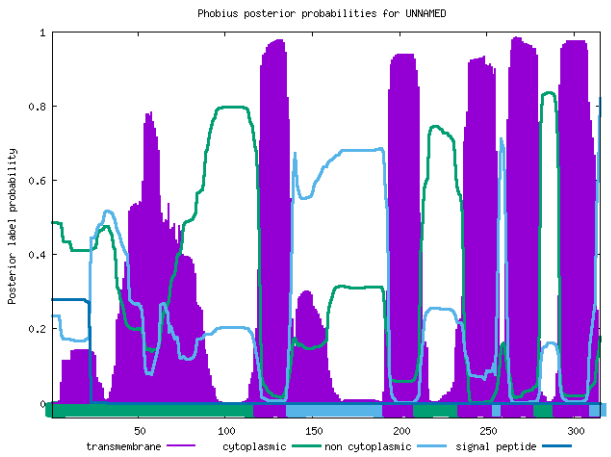


Figure S5. Amino acid sequences alignment of Tps1A, Tps2A, UbiA-type terpene synthases and UbiA prenyltransferases (ApUbiA and AfUbiA). Red squares show the putative active site motifs of Tps1A-like terpene synthases.

A

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FT   TRANSMEM    237    256
FT   TOPO_DOM    257    261       NON CYTOPLASMIC.
FT   TRANSMEM    262    280
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FT   TRANSMEM    292    312
FT   TOPO_DOM    313    315       NON CYTOPLASMIC.
//
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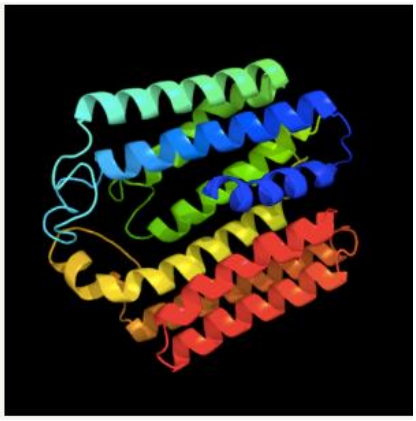
B



Homology model based on template c4od5C: 4-hydroxybenzoate octaprenyltransferase, a UbiA homolog from *Aeropyrum pernix* k1

256 residues (81% of (*tps1A*) 315 residues protein sequence, aligned region shown above) have been modelled with 99.9% confidence by the single highest scoring template.

C

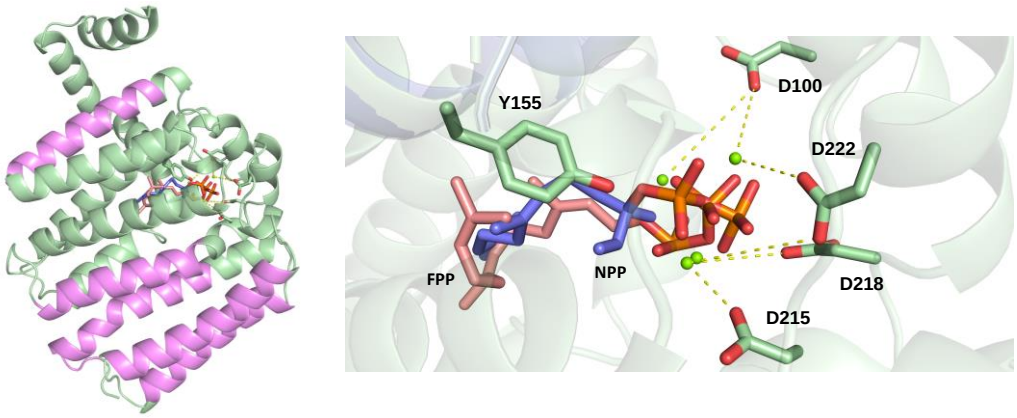


Figure S6. Protein structure prediction and analysis of Tps1A. (A) Transmembrane helices prediction of Tps1A with the Phobius server (https://phobius.sbc.su.se/). (B) Homology model of Tps1A generated with Phyre2 protein fold recognition server (www.sbg.bio.ic.ac.uk/ phyre2/) based on the top hit, *Aeropyrum pernix* k1 4-hydroxybenzoate octaprenyltransferase (PDB_ID c4od5C), as template. (C) Predicted Tps1A structure by AlphaFold[14] and simulated substrate binding by AutoDock Vina.[15] The docked substrates are farnesyl pyrophosphate (FPP) (color: salmon) and nerolidyl pyrophosphate (NPP) (color: slate). Magnesium metal ions are shown in green. The transmembrane region (color: violet) of the protein is based on the transmembrane regions predicted by Phobious.

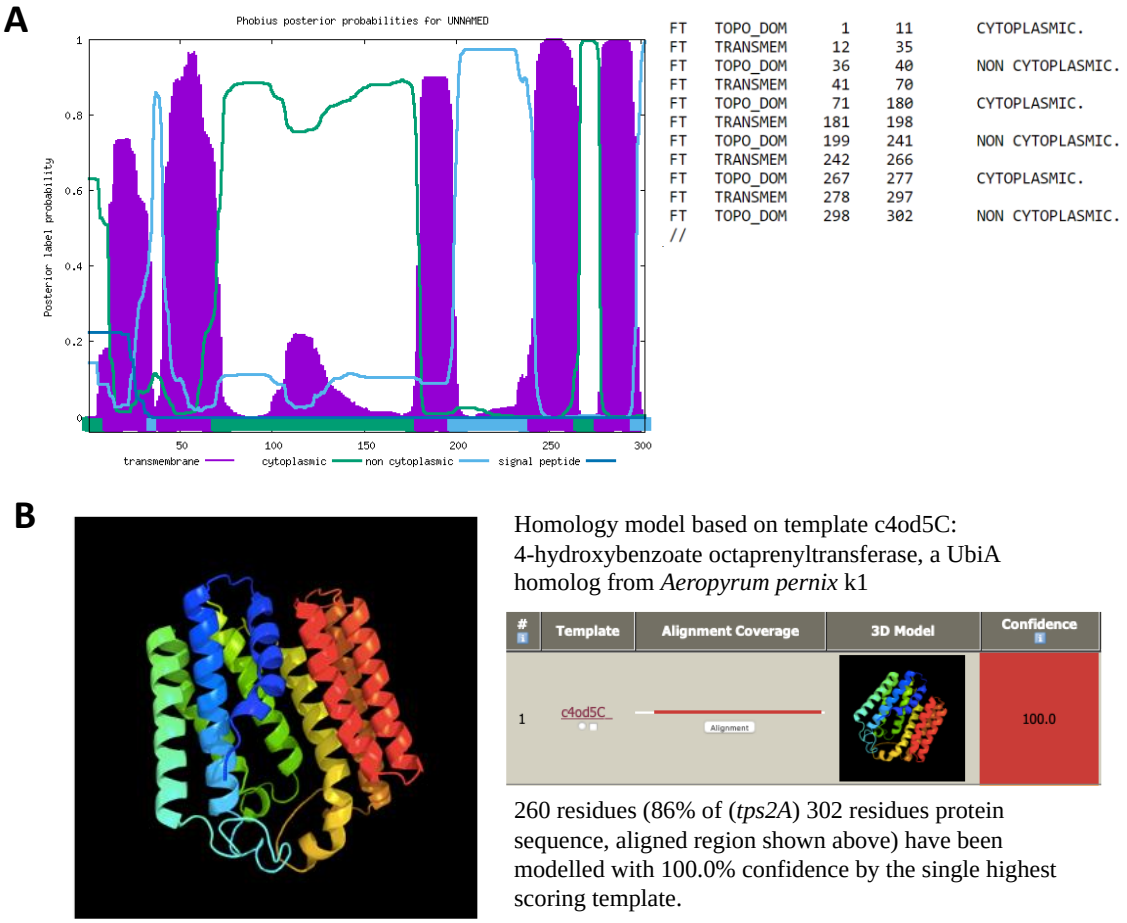


Figure S7. Protein structure prediction and analysis of Tps2A. (A) Transmembrane helices prediction of Tps2A with the TMHMM server (www.cbs.dtu.dk/services/TMHMM/), showing seven transmembrane helices. (B) Homology model of Tps2A generated with Phyre2 protein fold recognition server (www.sbg.bio.ic.ac.uk/ phyre2/) based on the top hit, *Aeropyrum pernix* k1 4-hydroxybenzoate octaprenyltransferase (PDB_ID c4od5C), as template.

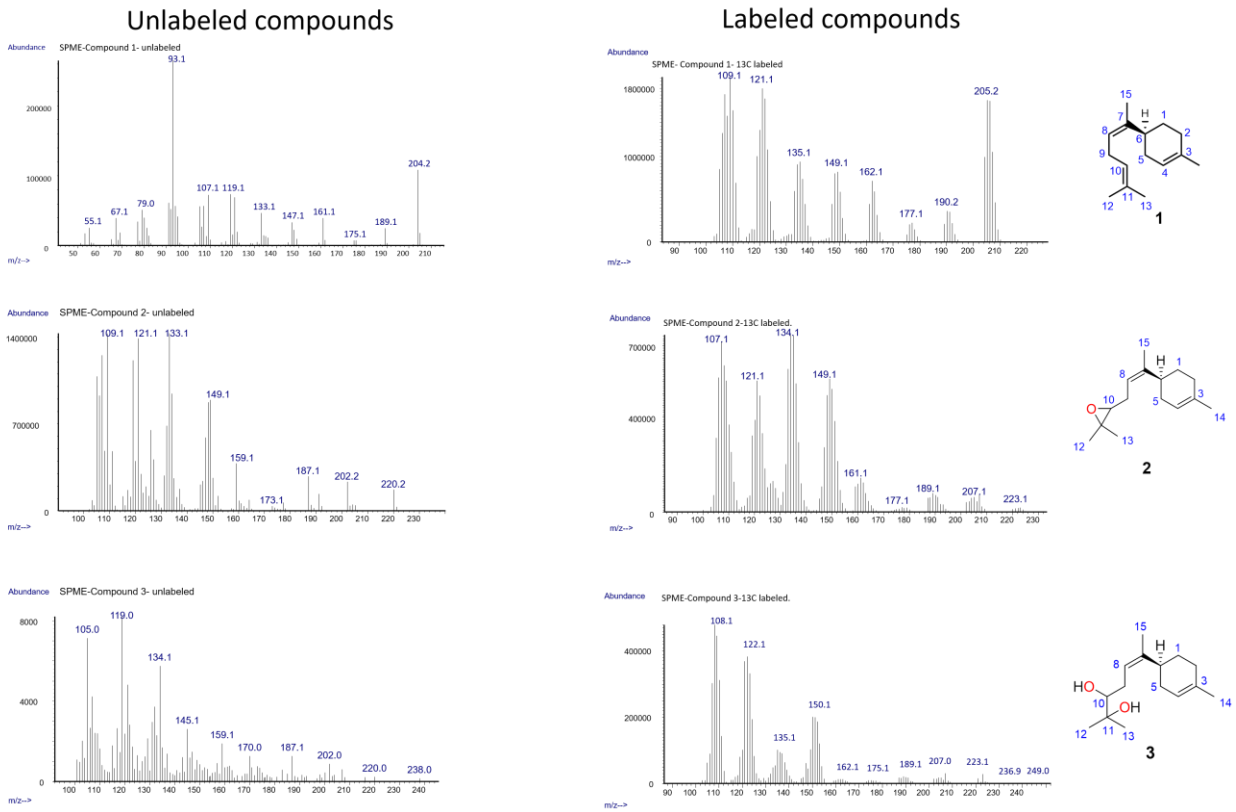


Figure S8. GC-MS spectra of unlabeled and ^{13}C-labeled compounds **1**, **2** and **3**.

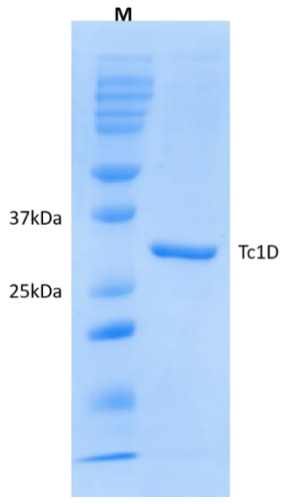


Figure S9. SDS-PAGE analysis of purified Tps1D (27.9 kDa).

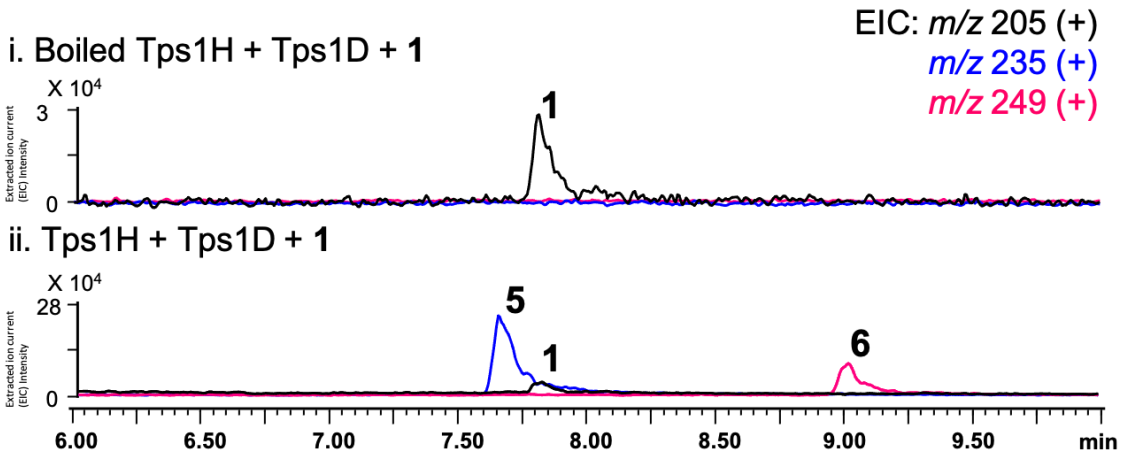


Figure S10. LCMS analysis of (i) boiled Tps1H and Tps1D with **1**; (ii) Tps1H and Tps1D with **1**.

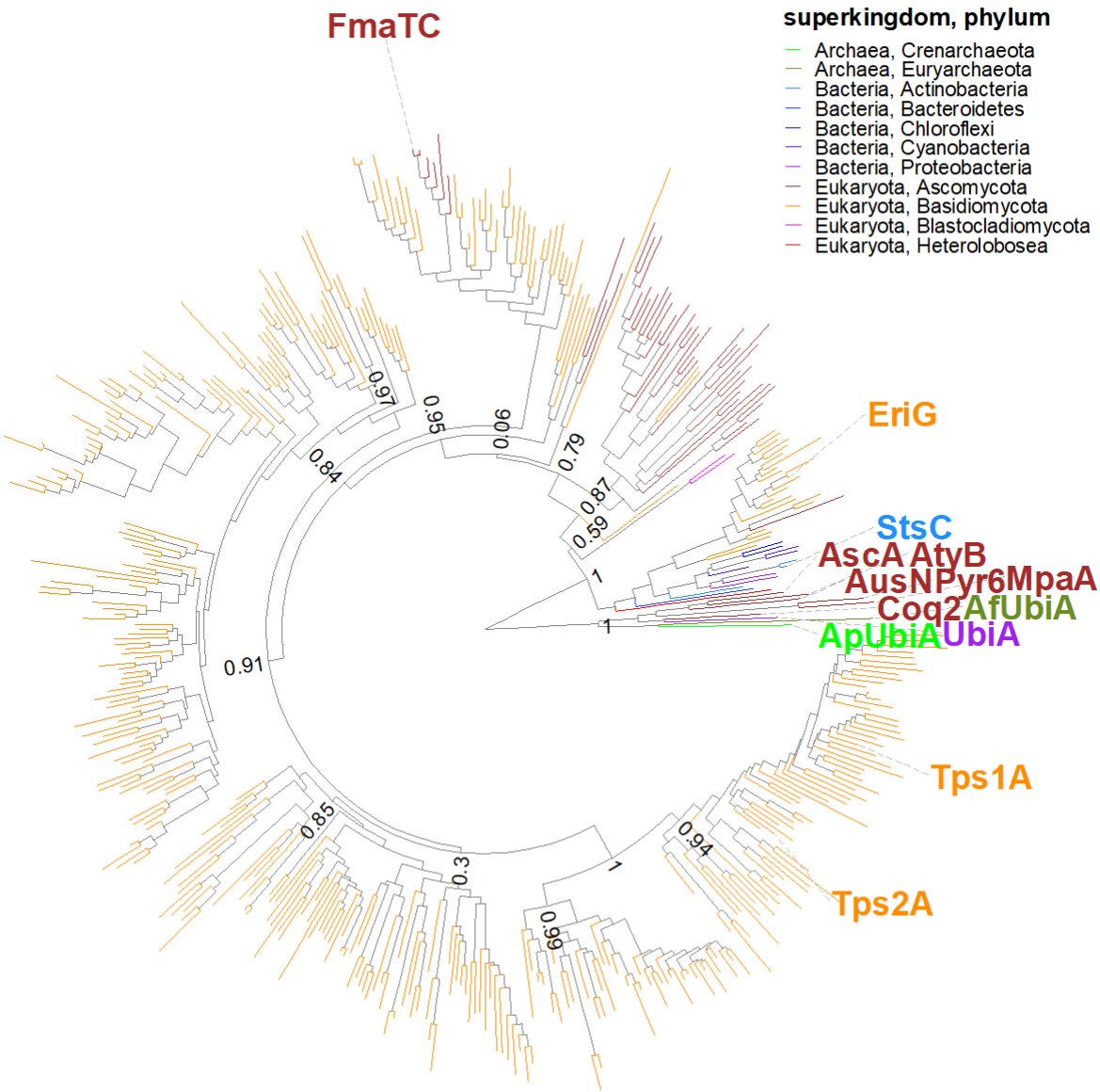


Figure S11. Phylogenetic analysis of Tps1A, Tps2A, other UbiA-type TPSs, integral membrane TPSs and UbiA prenyltransferases.

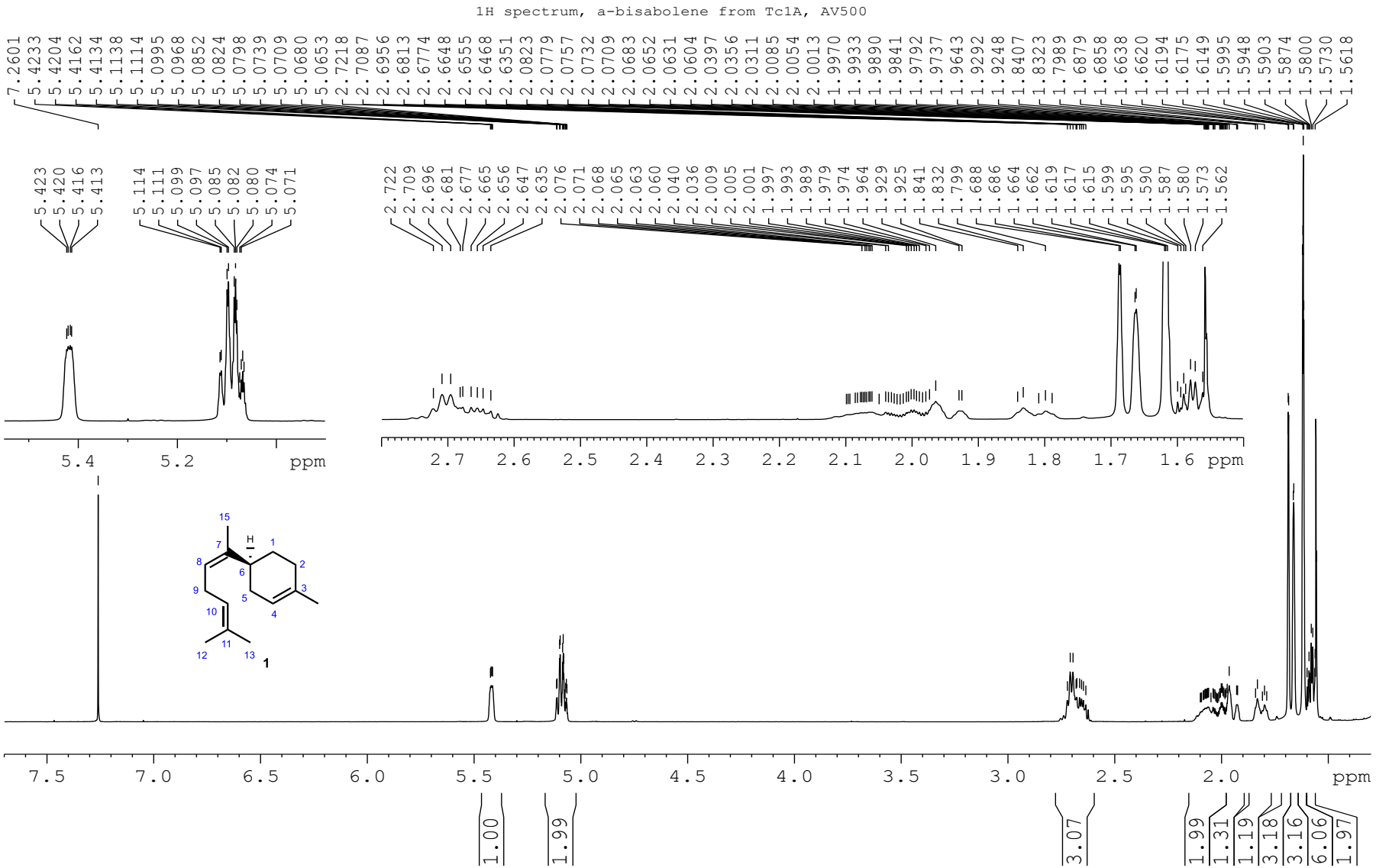


Figure S12. ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of **1** from Tps1A.

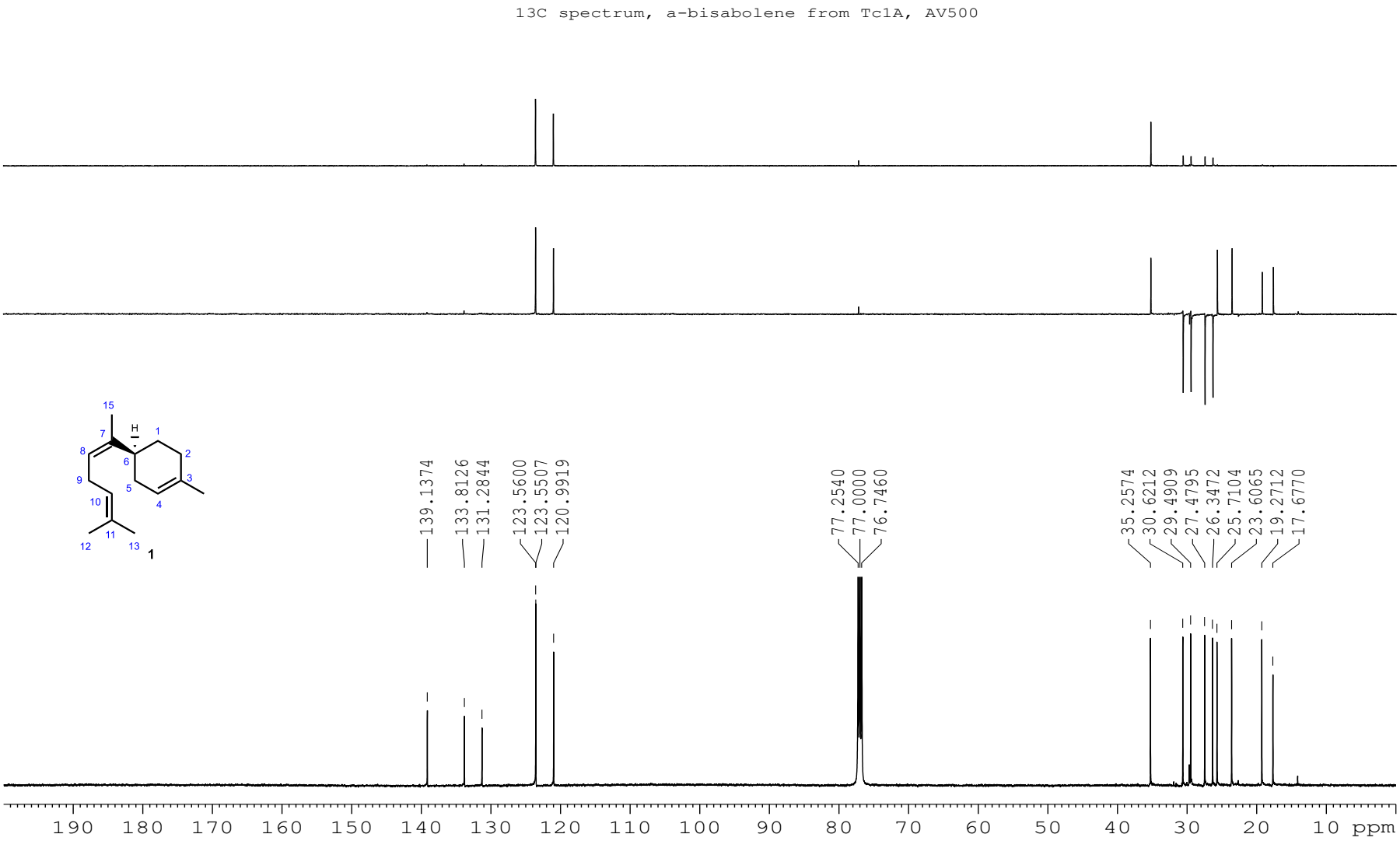


Figure S13. ^{13}C-NMR, DEPT135 and DEPT90 spectra ($CDCl_3$, 125 MHz) of **1** from Tps1A.

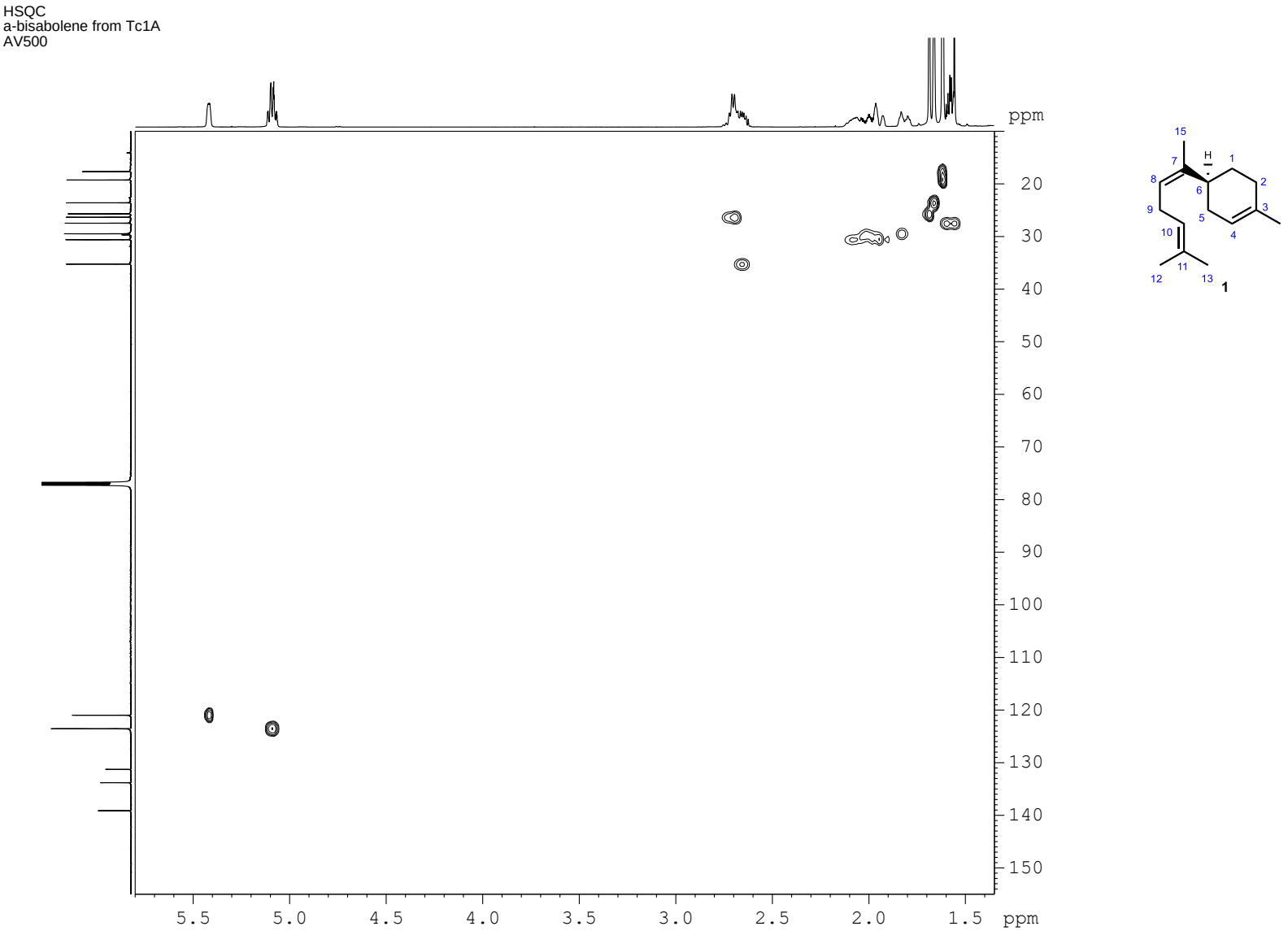


Figure S14. HSQC spectrum ($CDCl_3$, 500 MHz) of **1** from Tps1A.

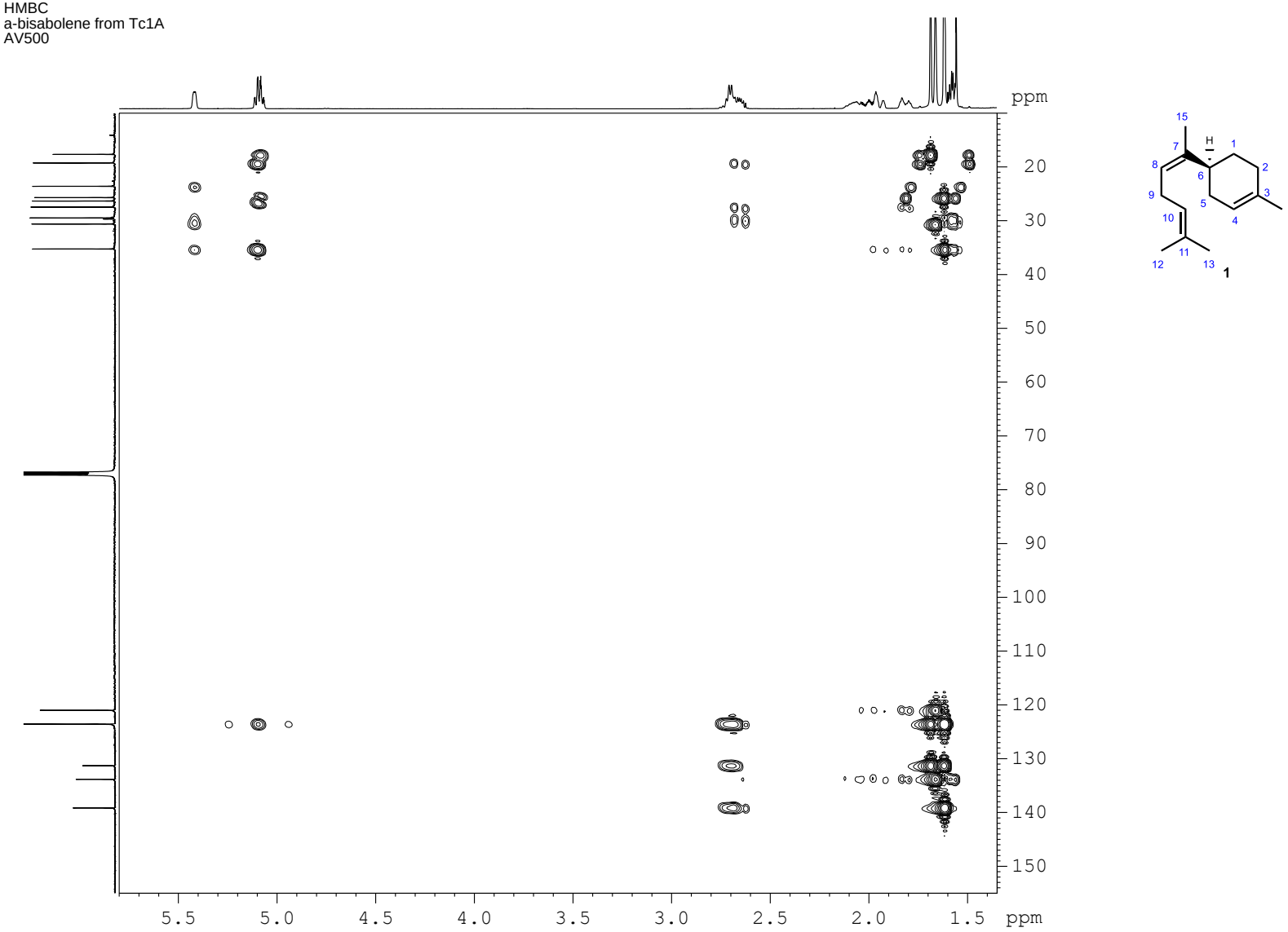


Figure S15. HMBC spectrum ($CDCl_3$, 500 MHz) of **1** from Tps1A.

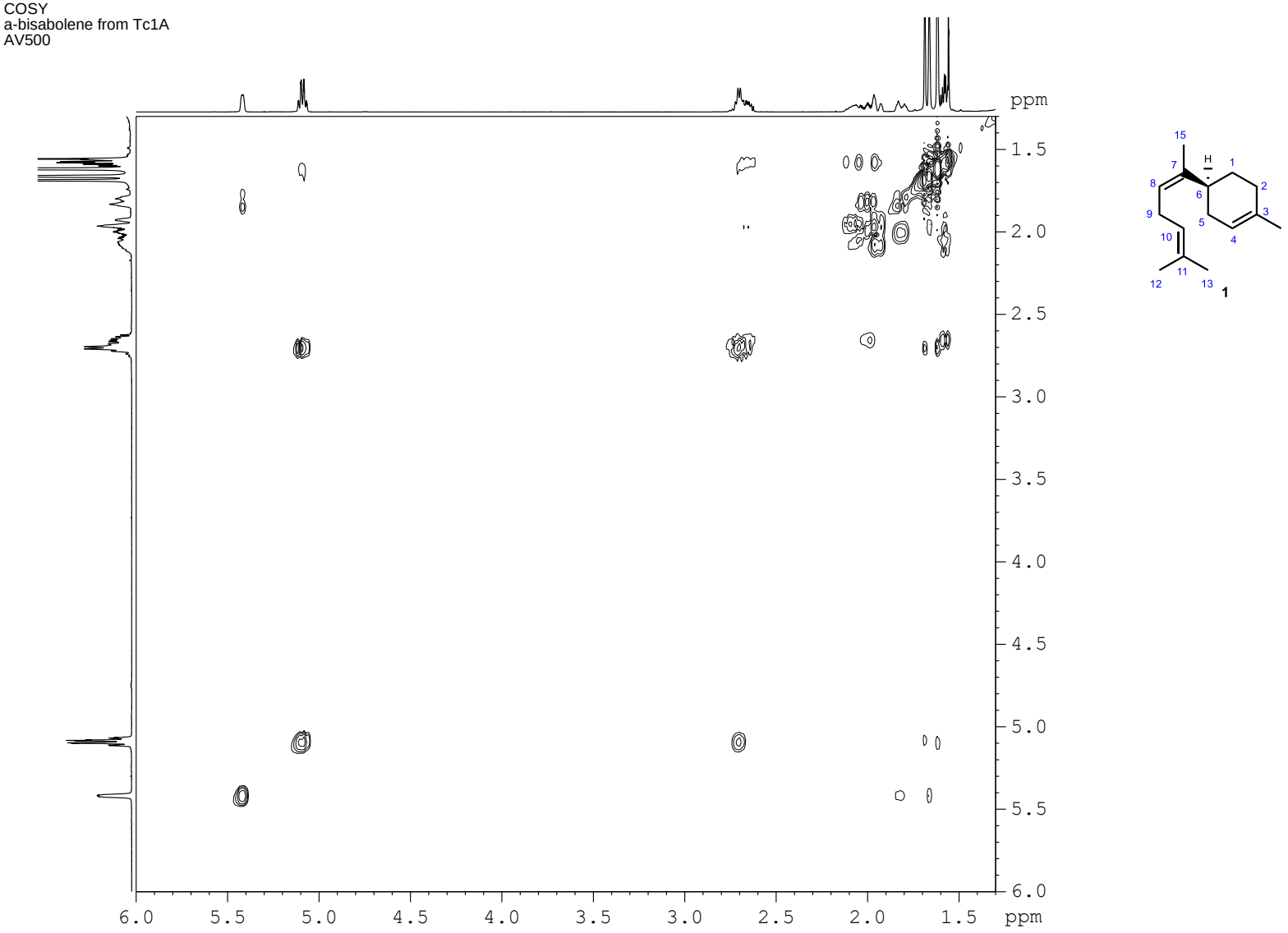


Figure S16. COSY spectrum ($CDCl_3$, 500 MHz) of **1** from Tps1A.

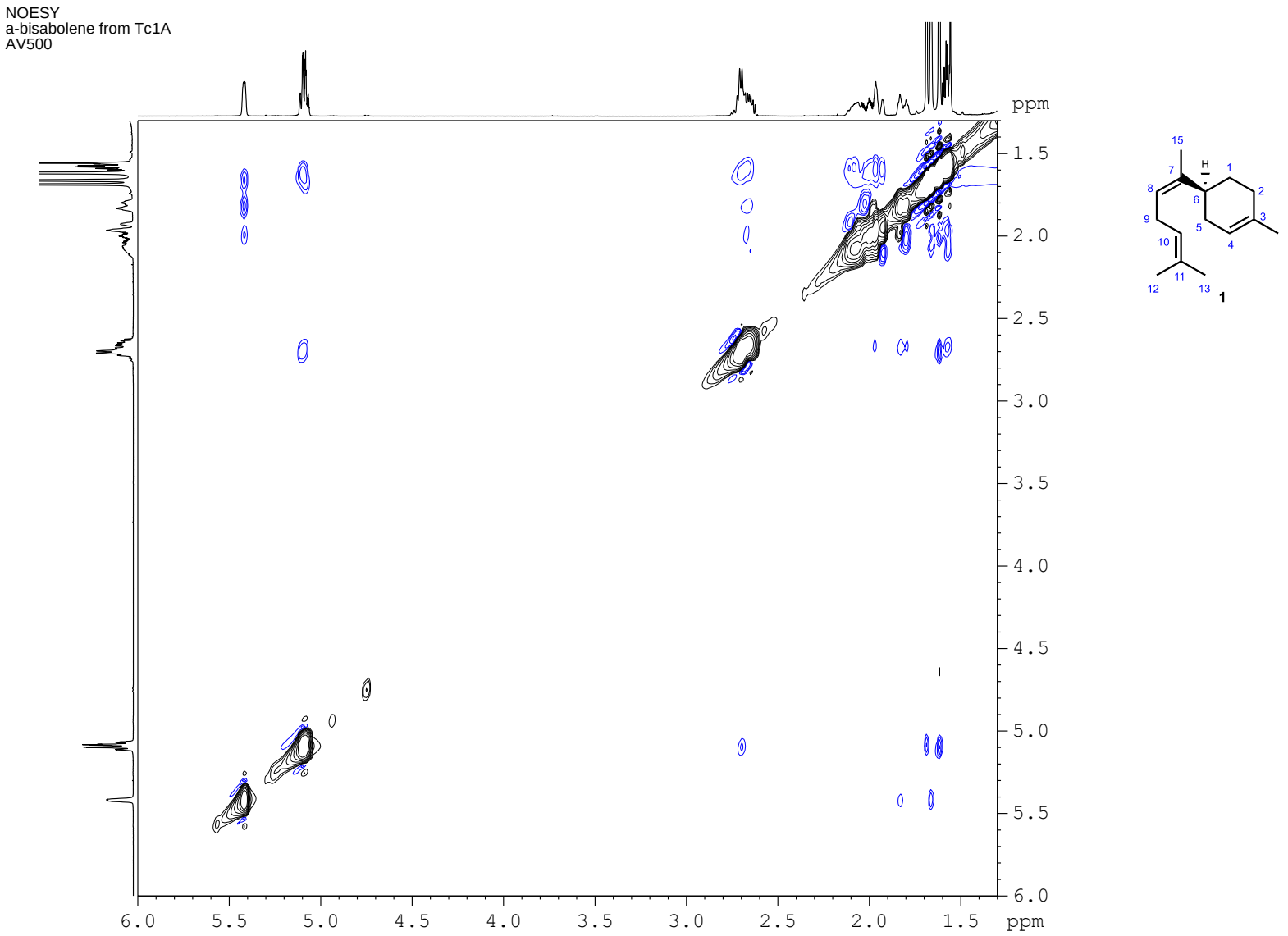


Figure S17. NOESY spectrum ($CDCl_3$, 500 MHz) of **1** from Tps1A.

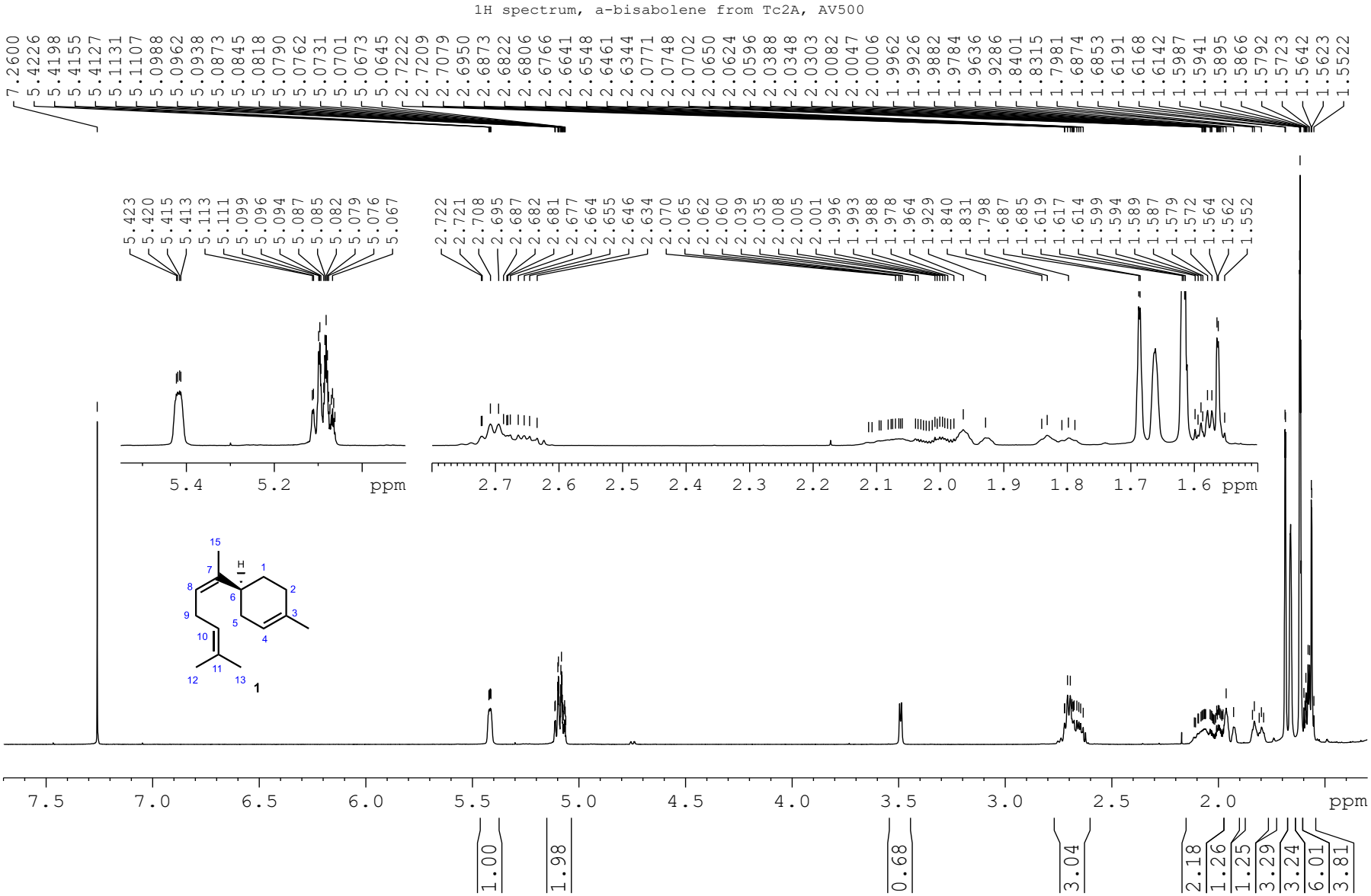


Figure S18. ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of **1** from Tps2A.

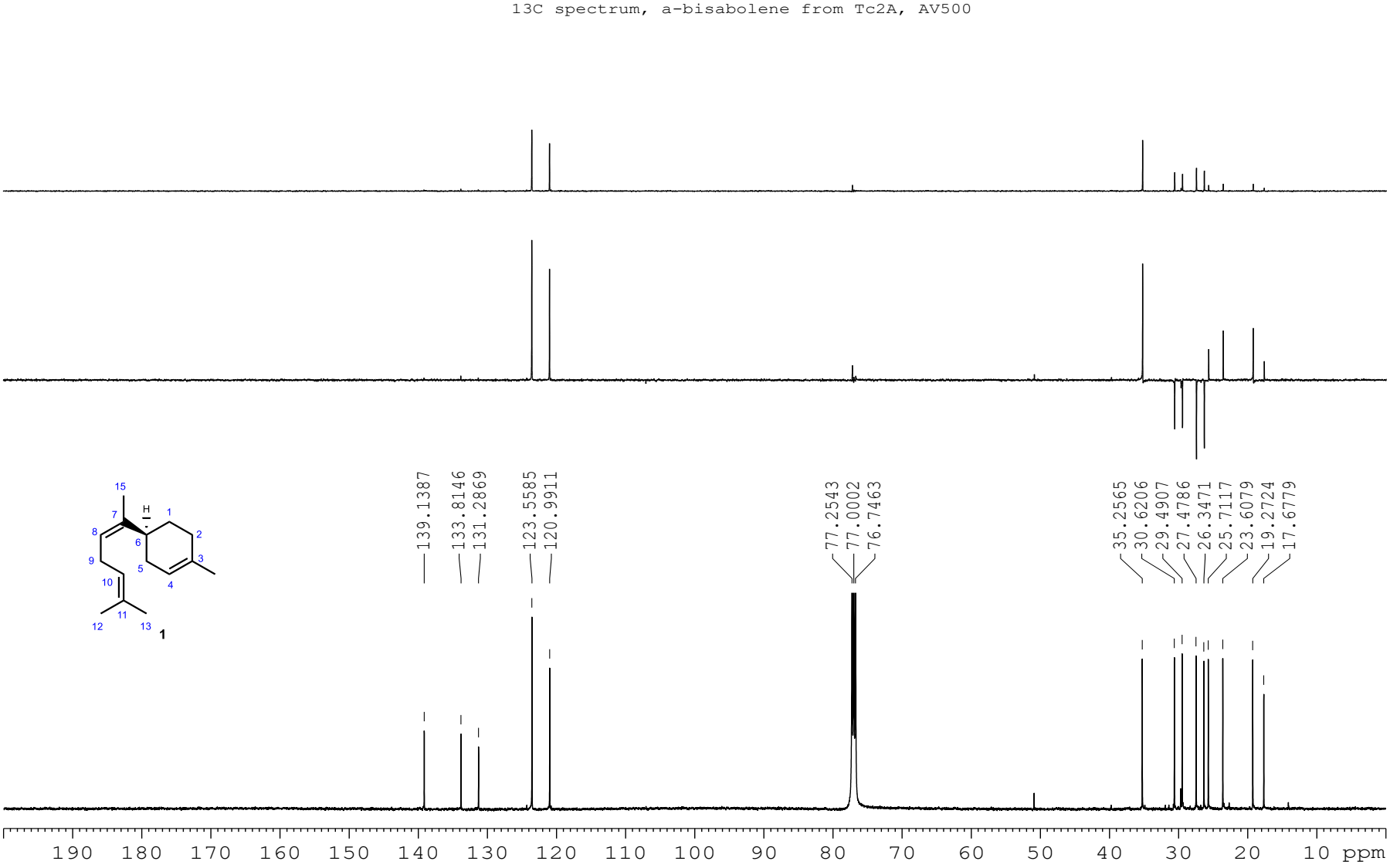


Figure S19. ^{13}C-NMR, DEPT135 and DEPT90 spectra ($CDCl_3$, 125 MHz) of **1** from Tps2A.

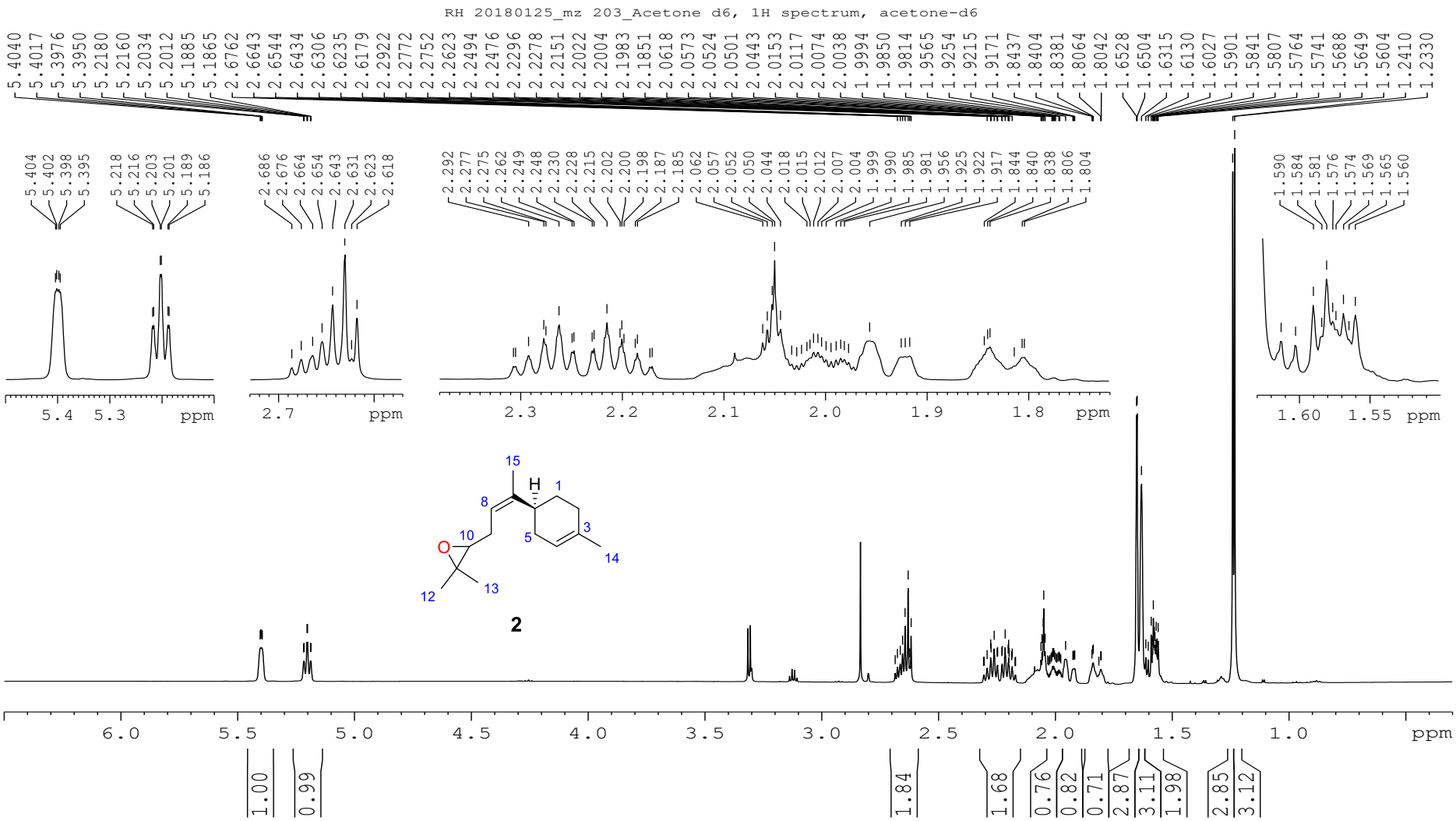


Figure S20. ^{1}H-NMR spectrum (acetone-d_6, 500 MHz) of **2** from *tps1A* expressed yeast culture.

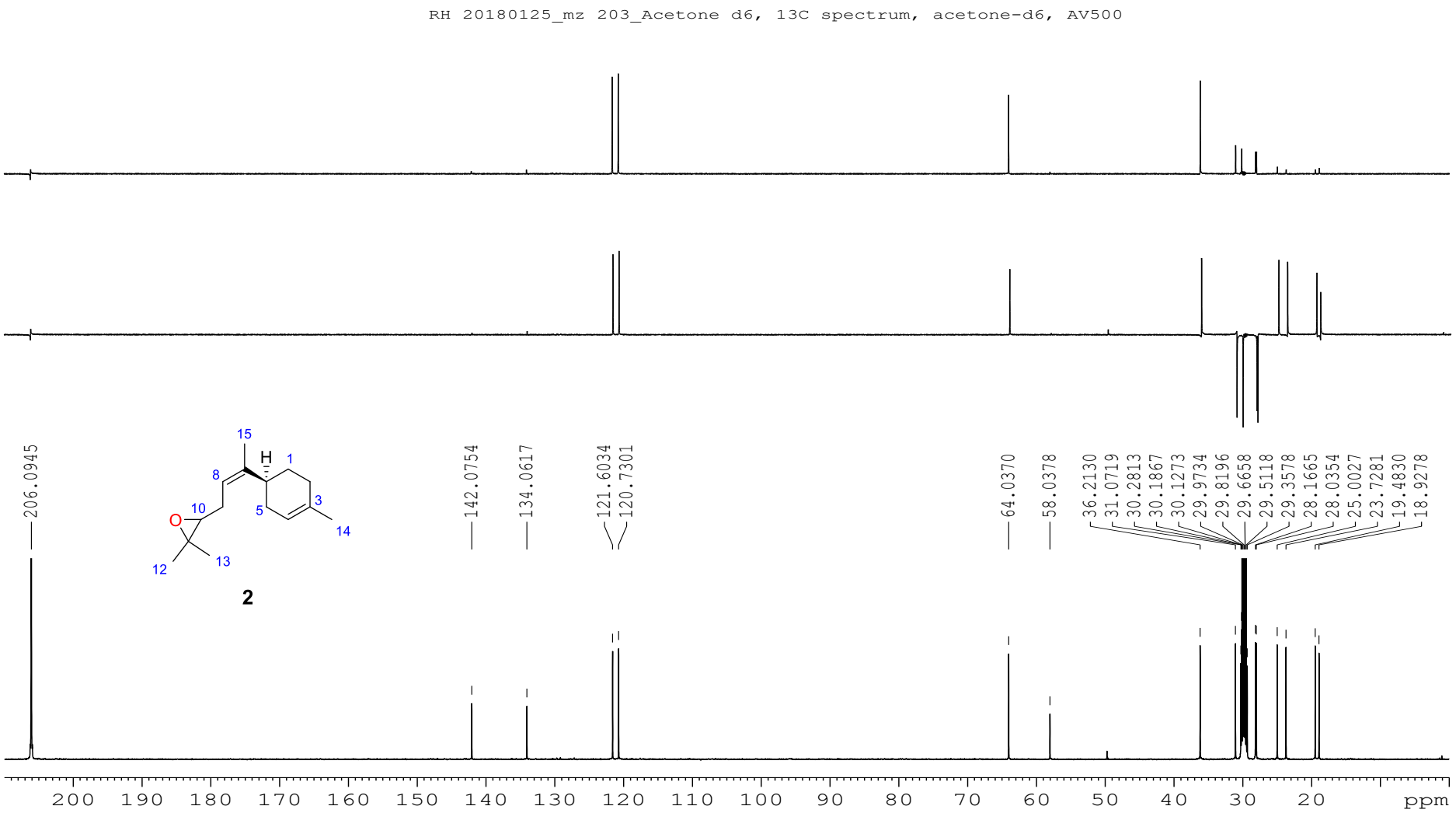
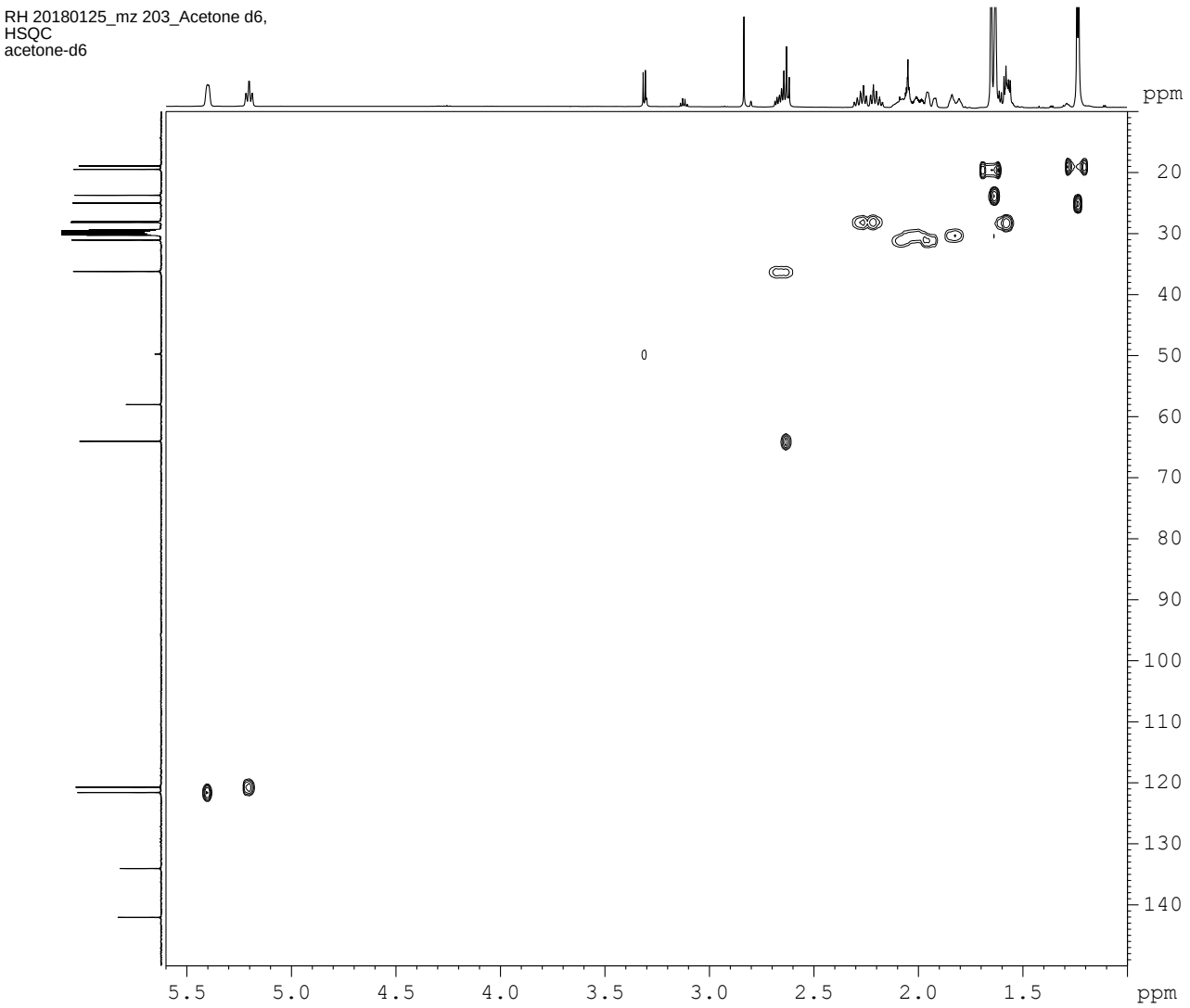
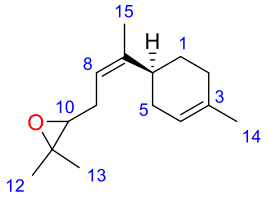


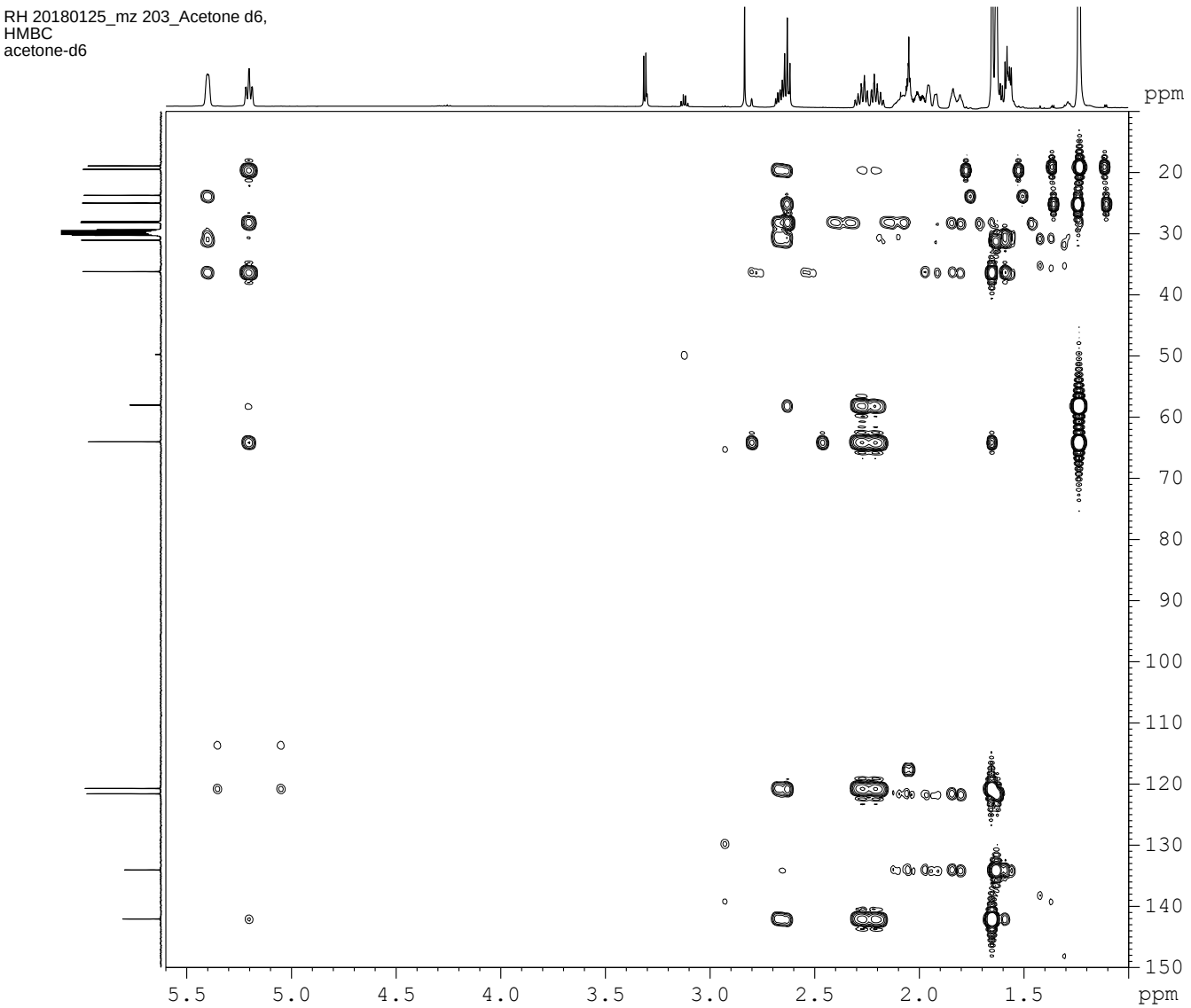
Figure S21. ^{13}C-NMR, DEPT135, and DEPT90 spectra spectrum (acetone-d_6, 125 MHz) of **2** from *tps1A* expressed yeast culture.

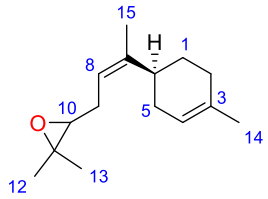




2

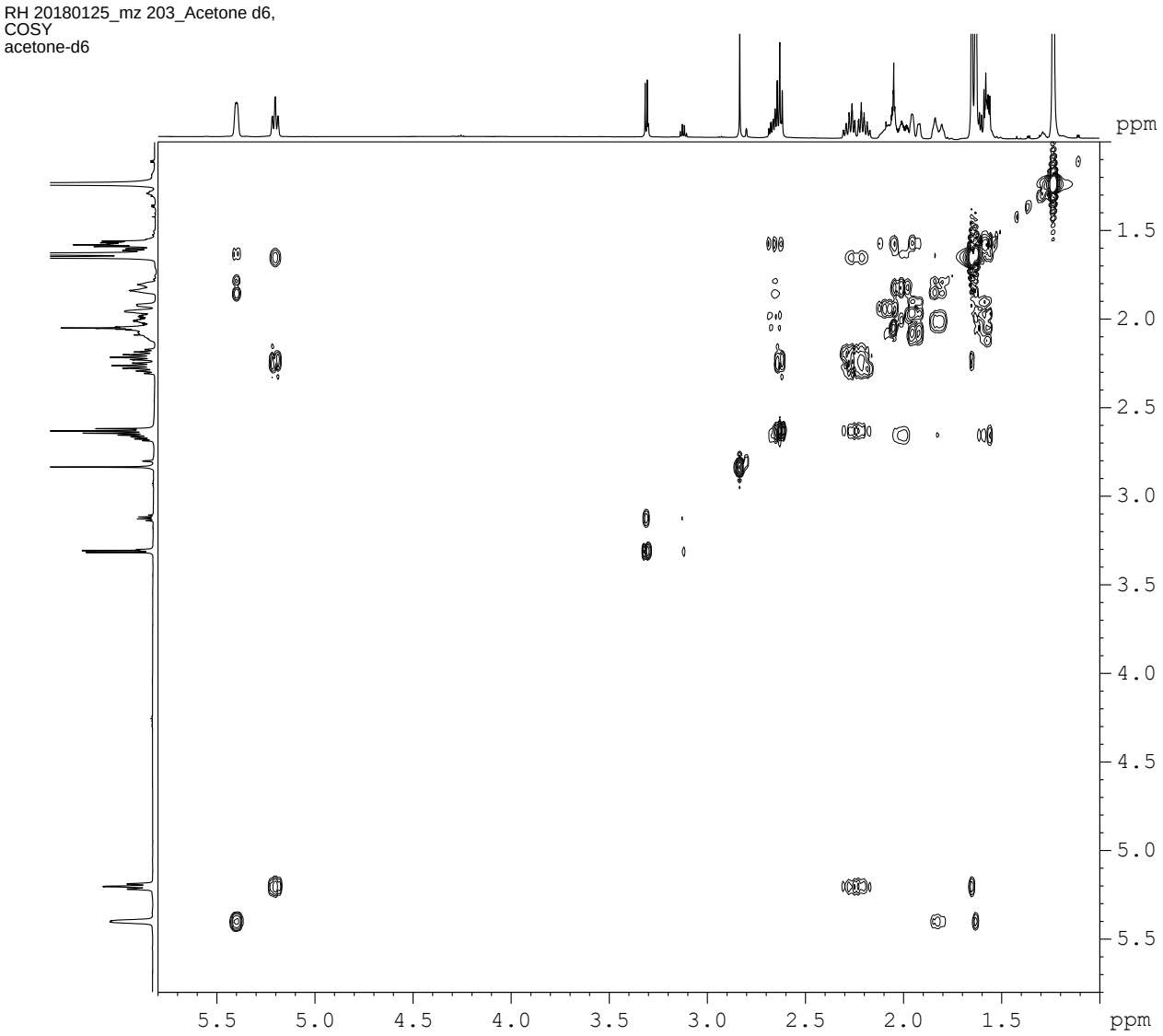
Figure S22. HSQC spectrum (acetone-d_6, 500 MHz) of **2** from *tps1A* expressed yeast culture.

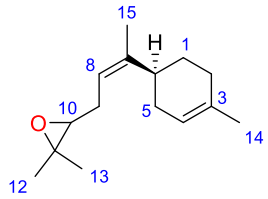




2

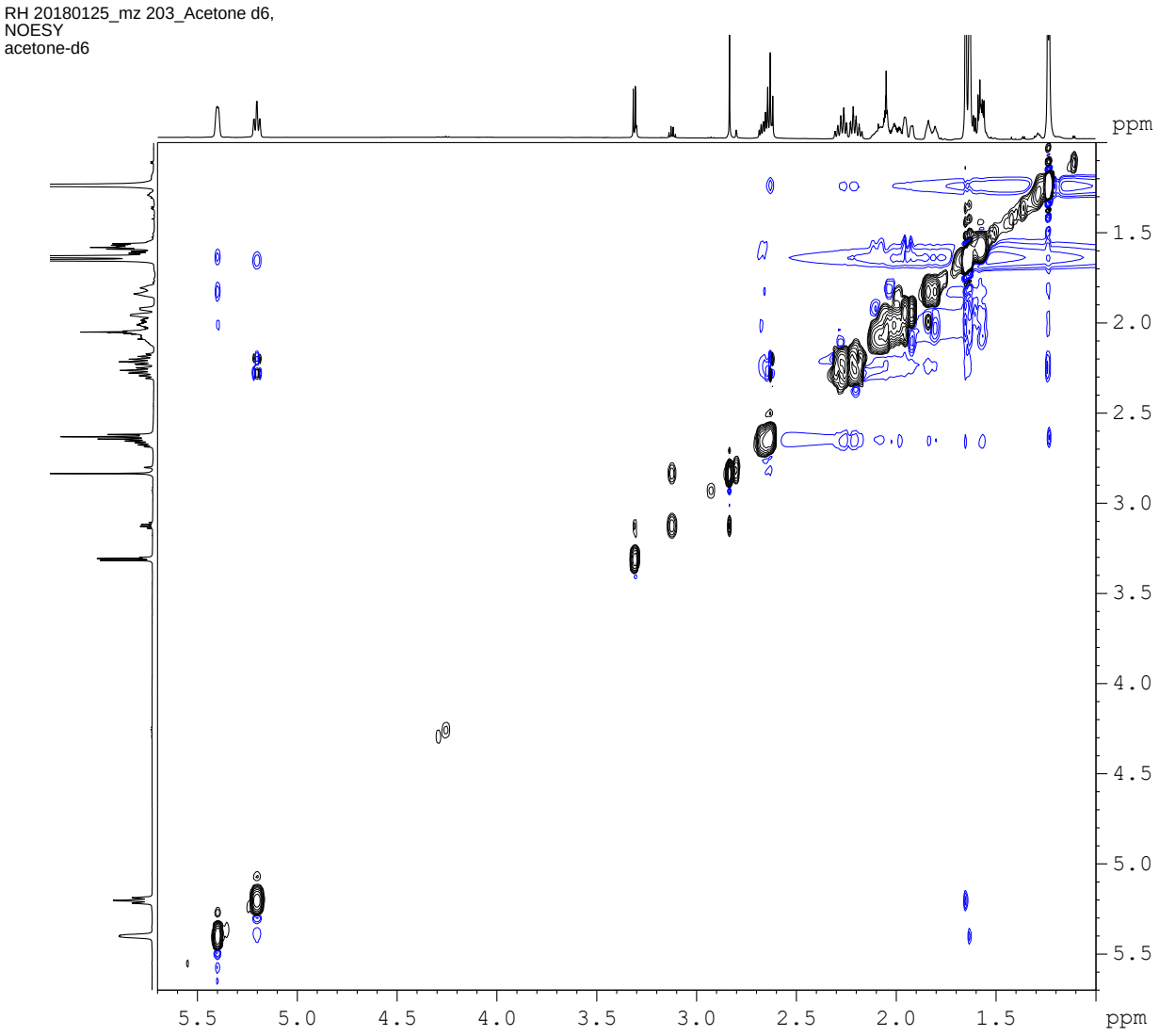
Figure S23. HMBC spectrum (acetone-d_6, 500 MHz) of **2** from *tps1A* expressed yeast culture.

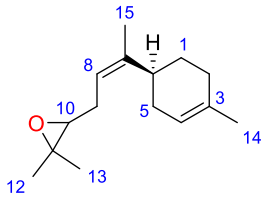




2

Figure S24. COSY spectrum (acetone-d_6, 500 MHz) of **2** from *tps1A* expressed yeast culture.





2

Figure S25. NOESY spectrum (acetone-d_6, 500 MHz) of **2** from *tps1A* expressed yeast culture.

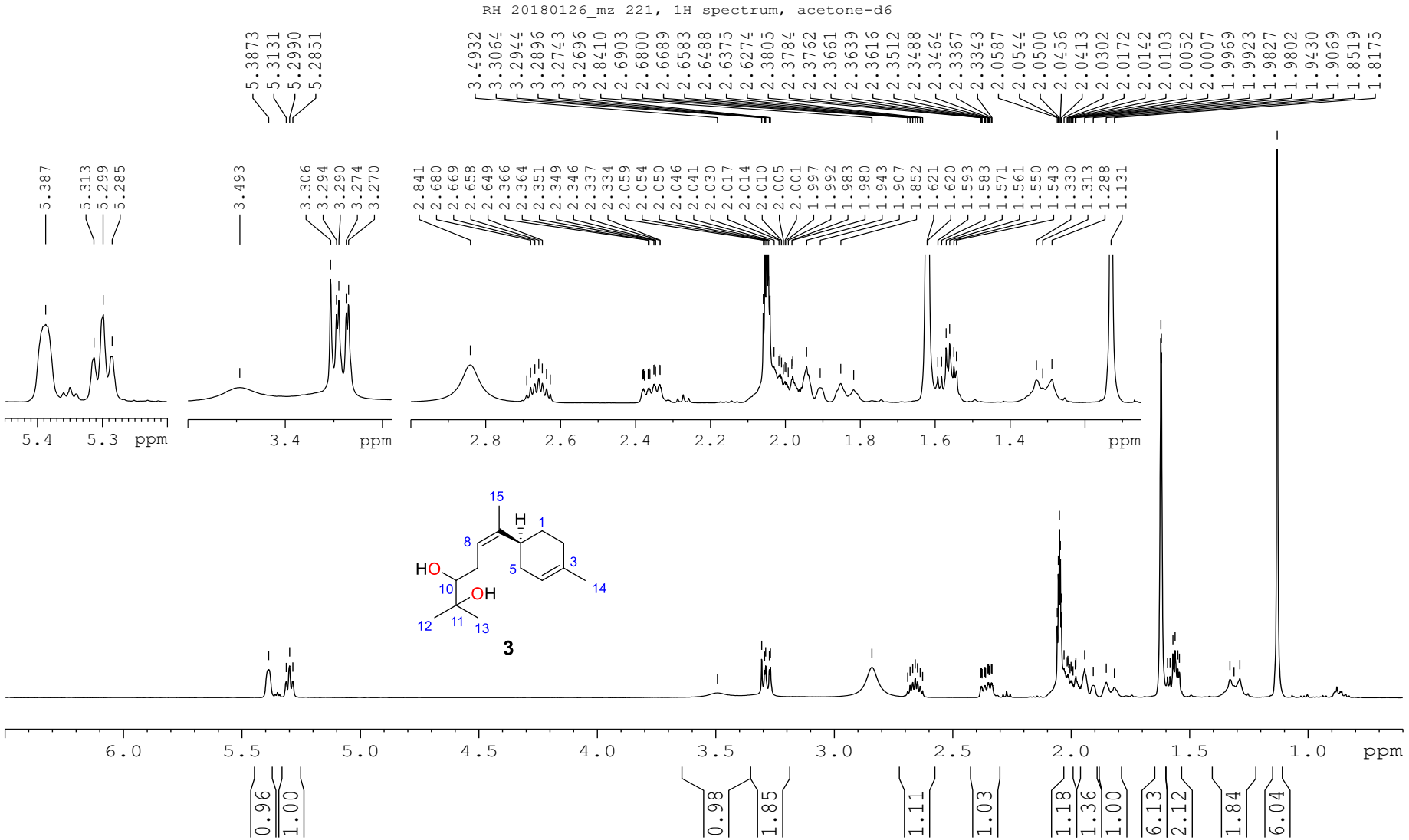


Figure S26. [1]H-NMR spectrum (acetone-d_6, 500 MHz) of **3** from *tps1A* expressed yeast culture.

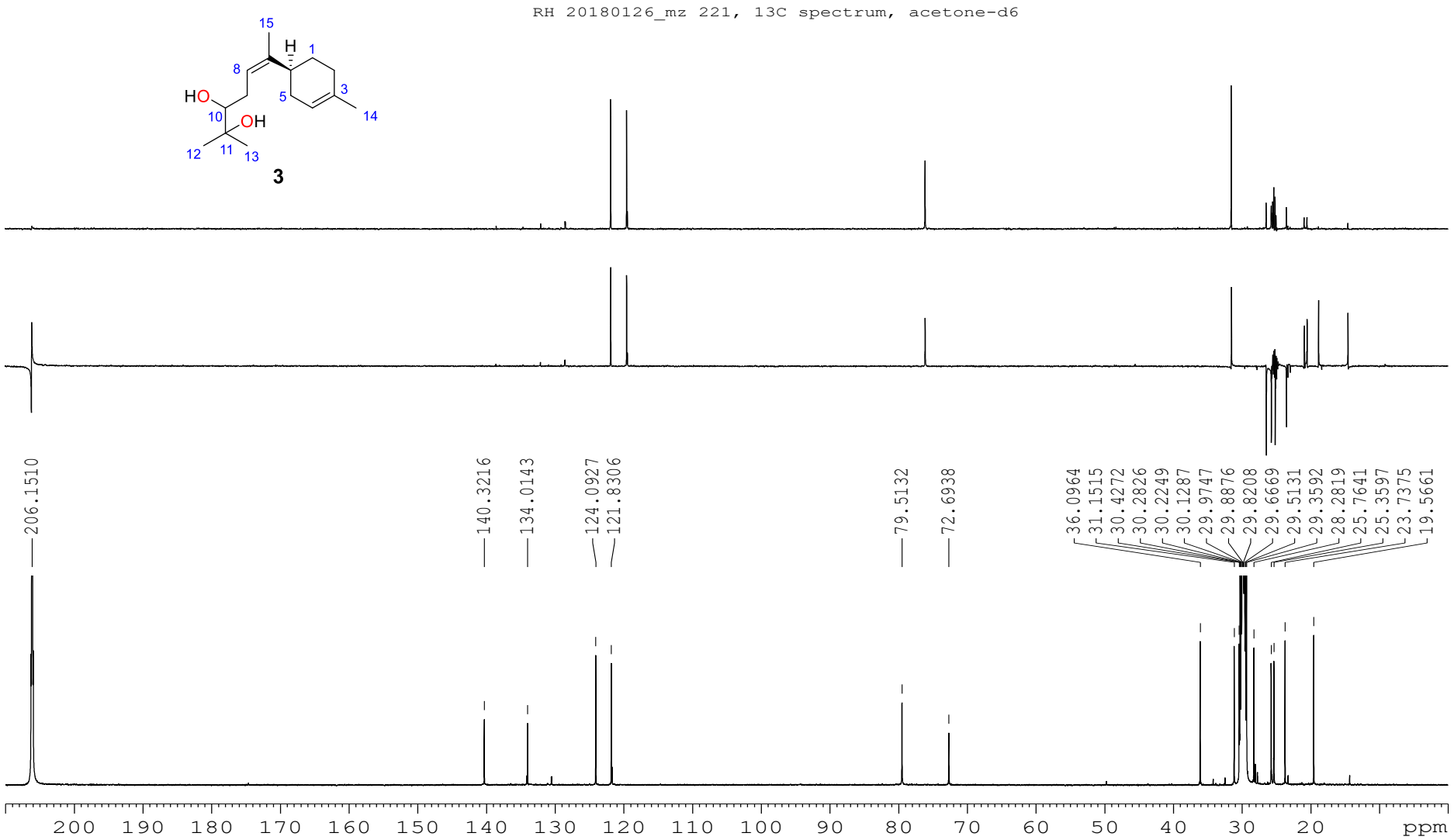
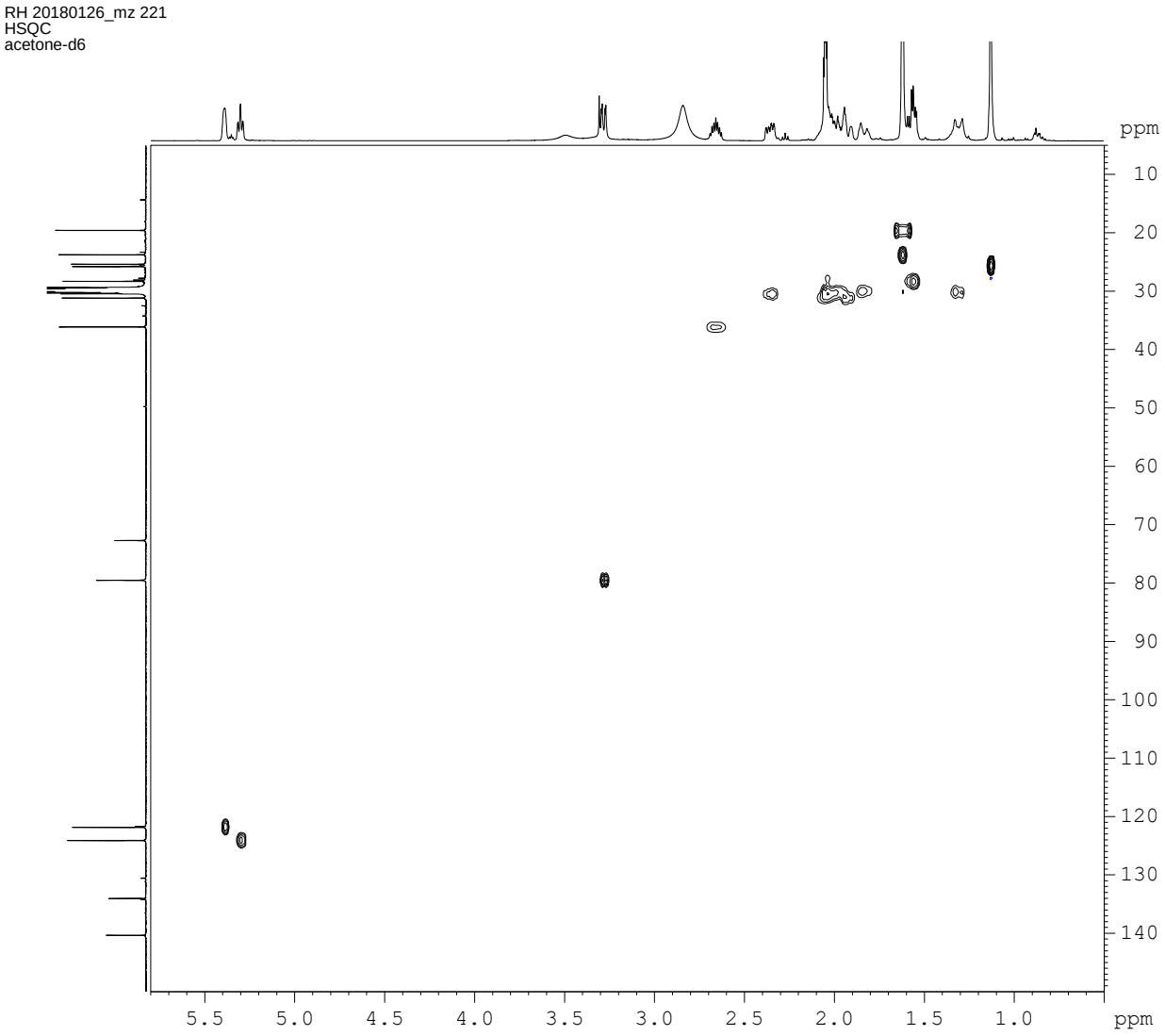
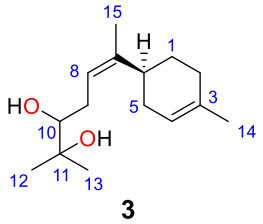


Figure S27. ^{13}C-NMR, DEPT135, and DEPT90 spectra (acetone-d_6, 125 MHz) of **3** from *tps1A* expressed yeast culture.





3

Figure S28. HSQC spectrum (acetone-d_6, 500 MHz) of **3** from *tps1A* expressed yeast culture.

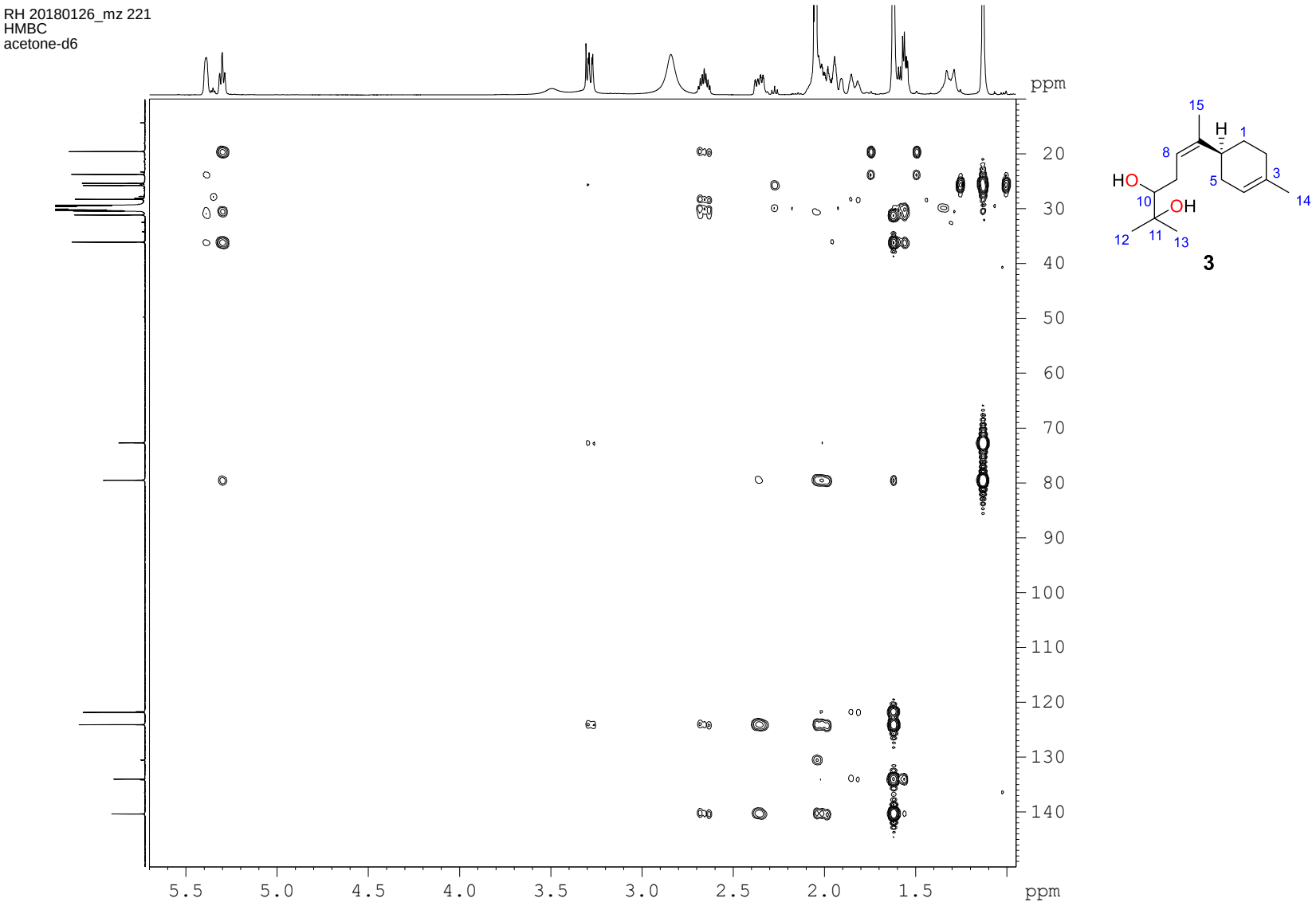
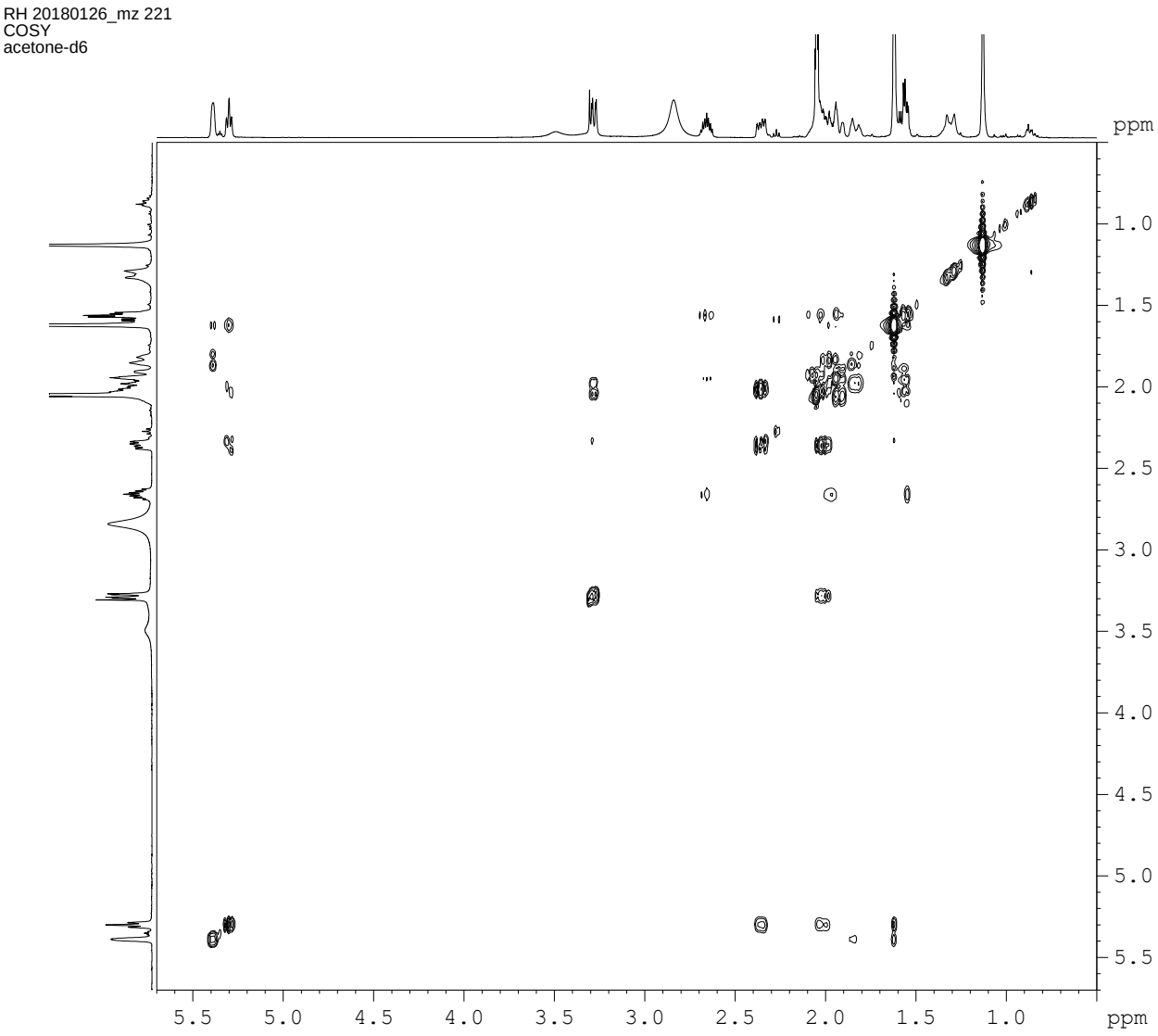
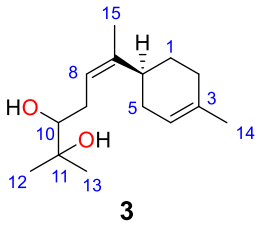


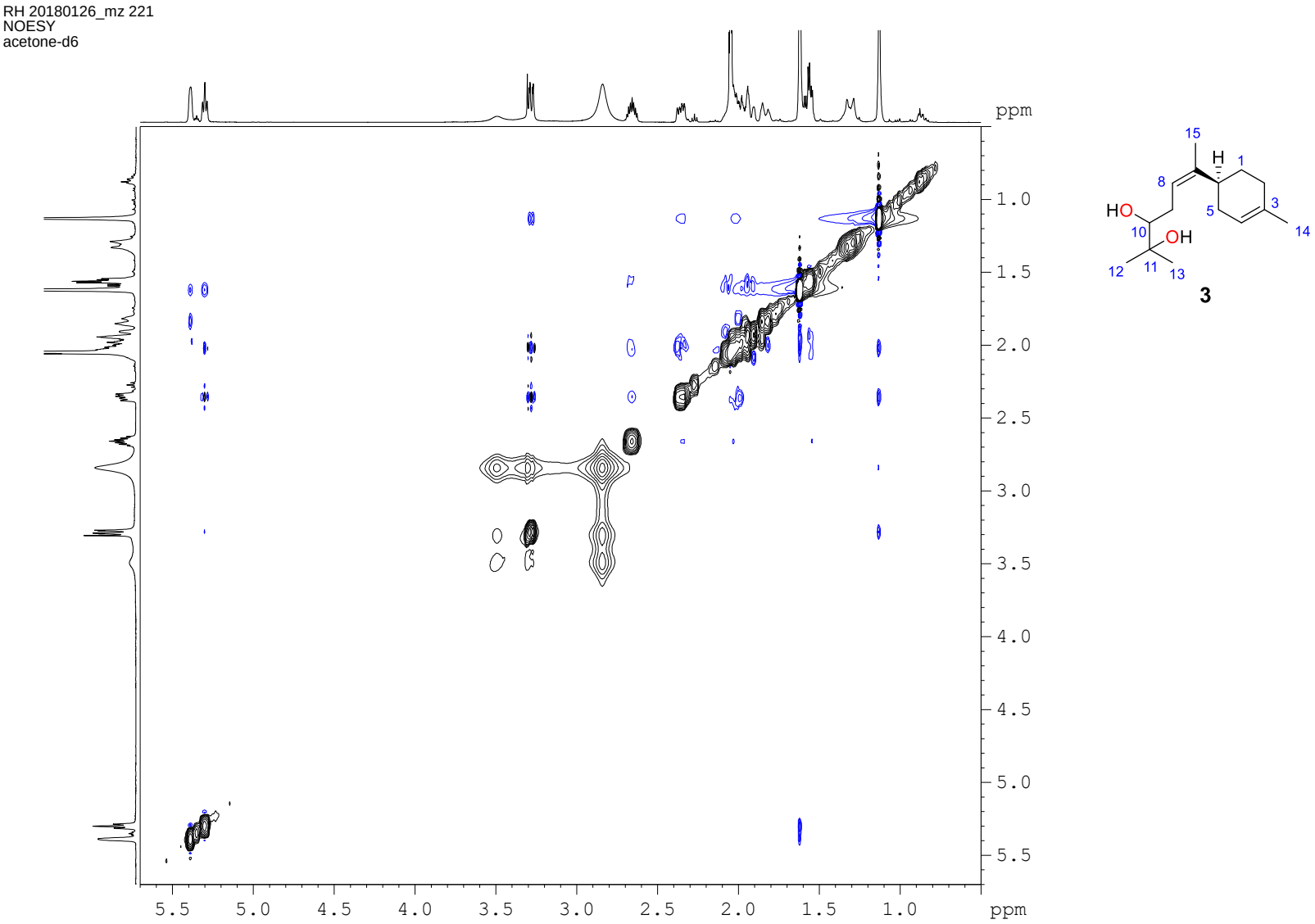
Figure S29. HMBC spectrum (acetone-d_6, 500 MHz) of **3** from *tps1A* expressed yeast culture.





3

Figure S30. COSY spectrum (acetone-d_6, 500 MHz) of **3** from *tps1A* expressed yeast culture.



3

Figure S31. NOESY spectrum (acetone-d_6, 500 MHz) of **3** from *tps1A* expressed yeast culture.

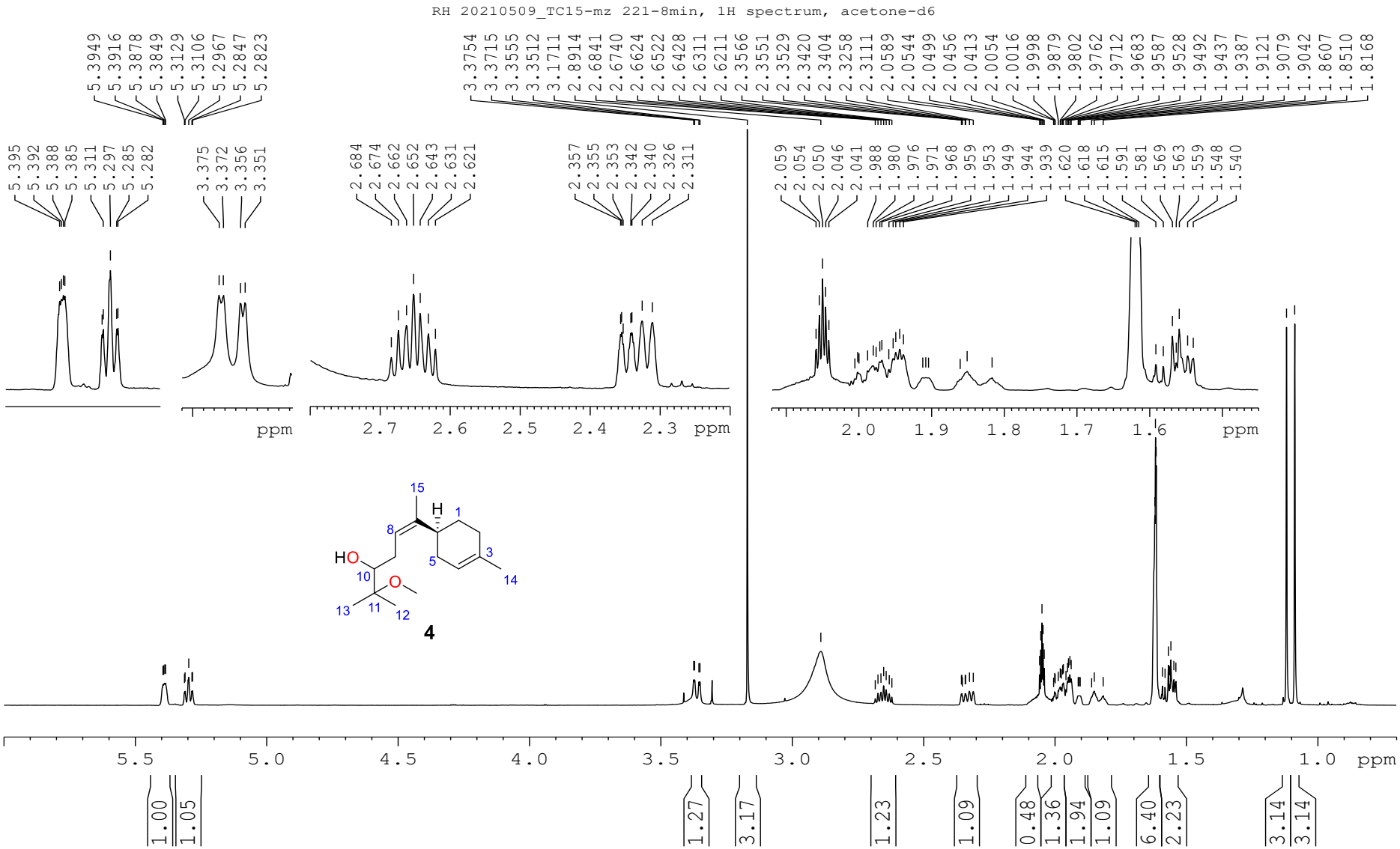


Figure S32. ^{1}H-NMR spectrum (acetone-d_6, 500 MHz) of **4** from *tps1A* expressed yeast culture.

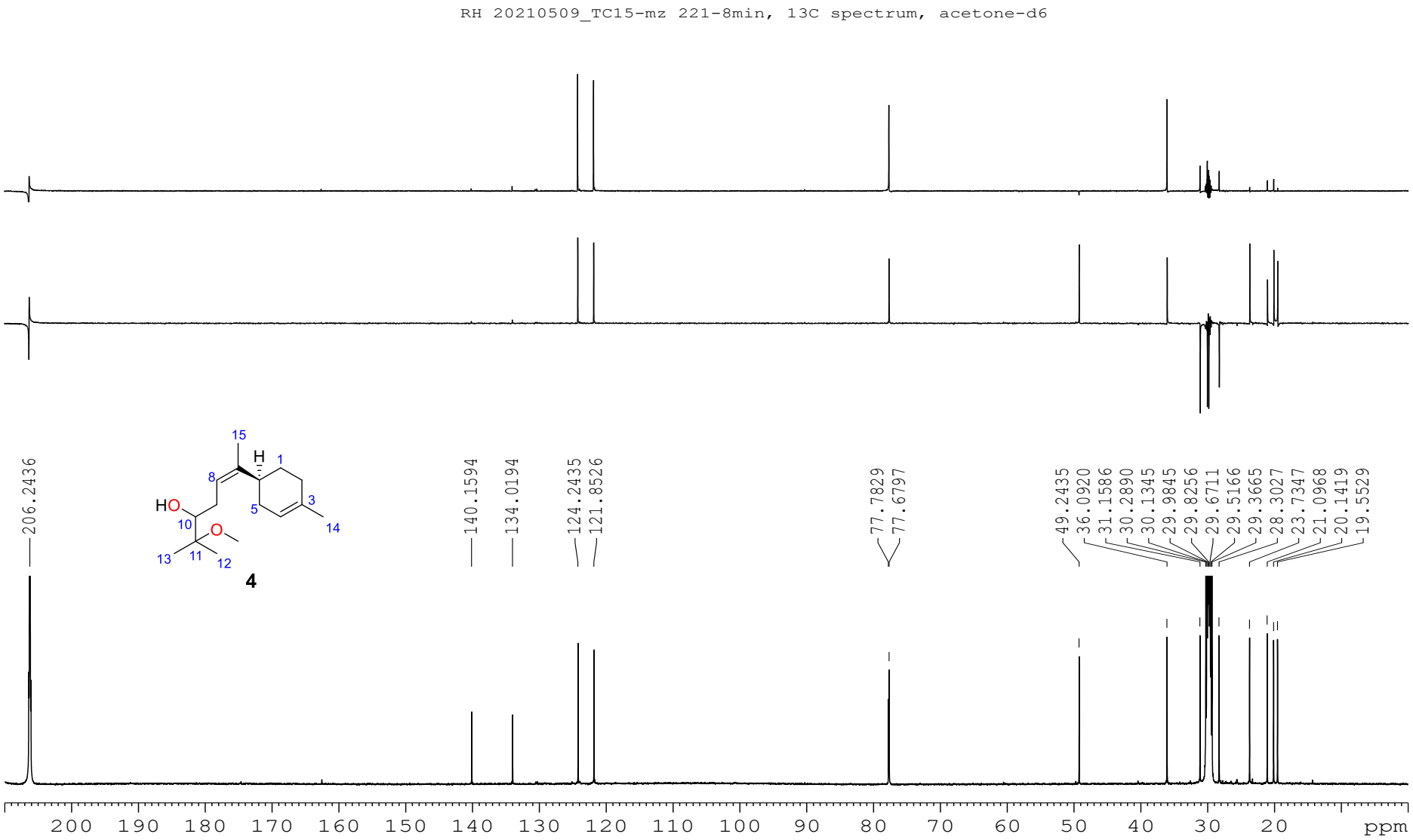


Figure S33. ^{13}C-NMR and DEPT135 spectra (acetone-d_6, 125 MHz) of **4** from *tps1A* expressed yeast culture.

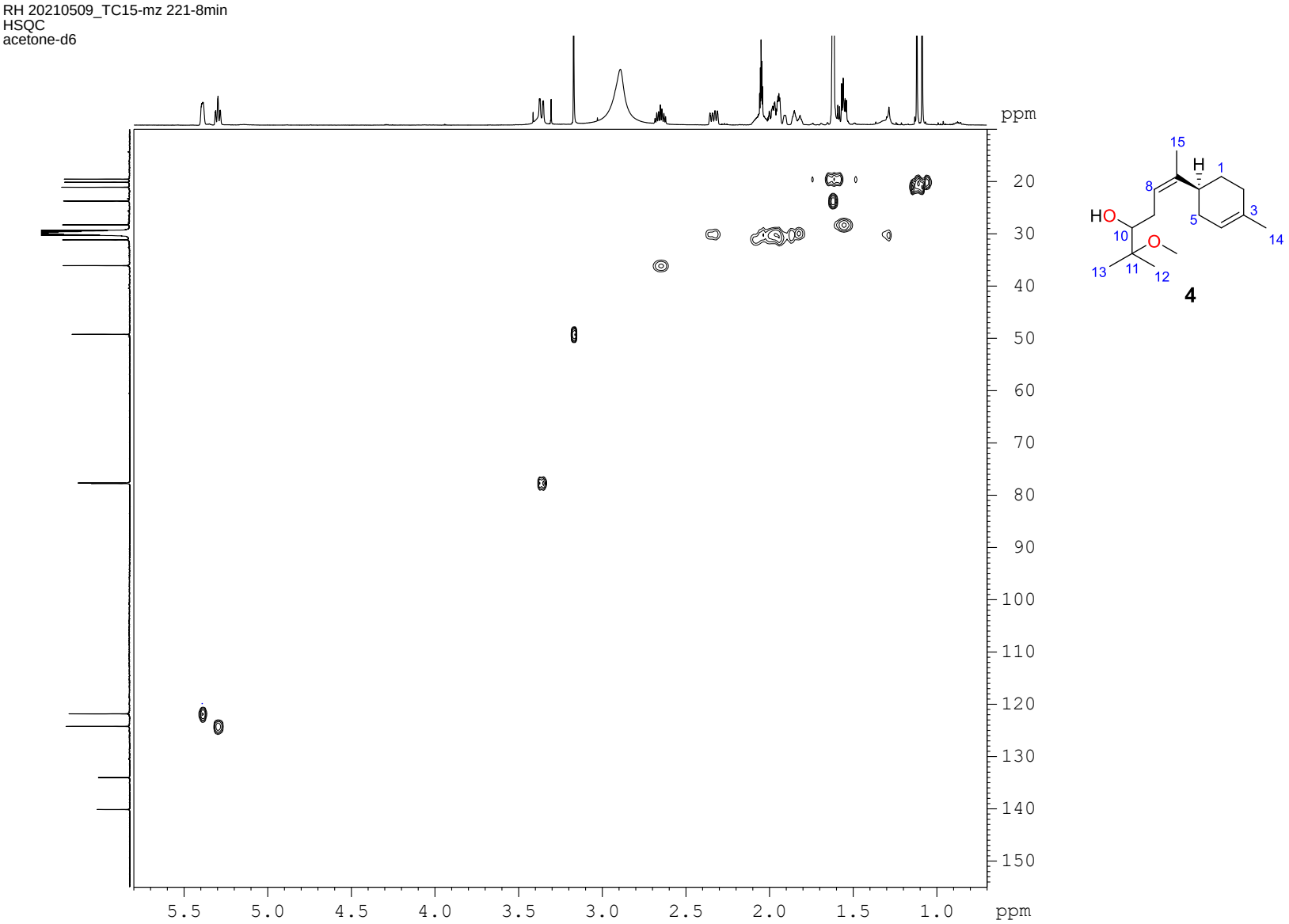
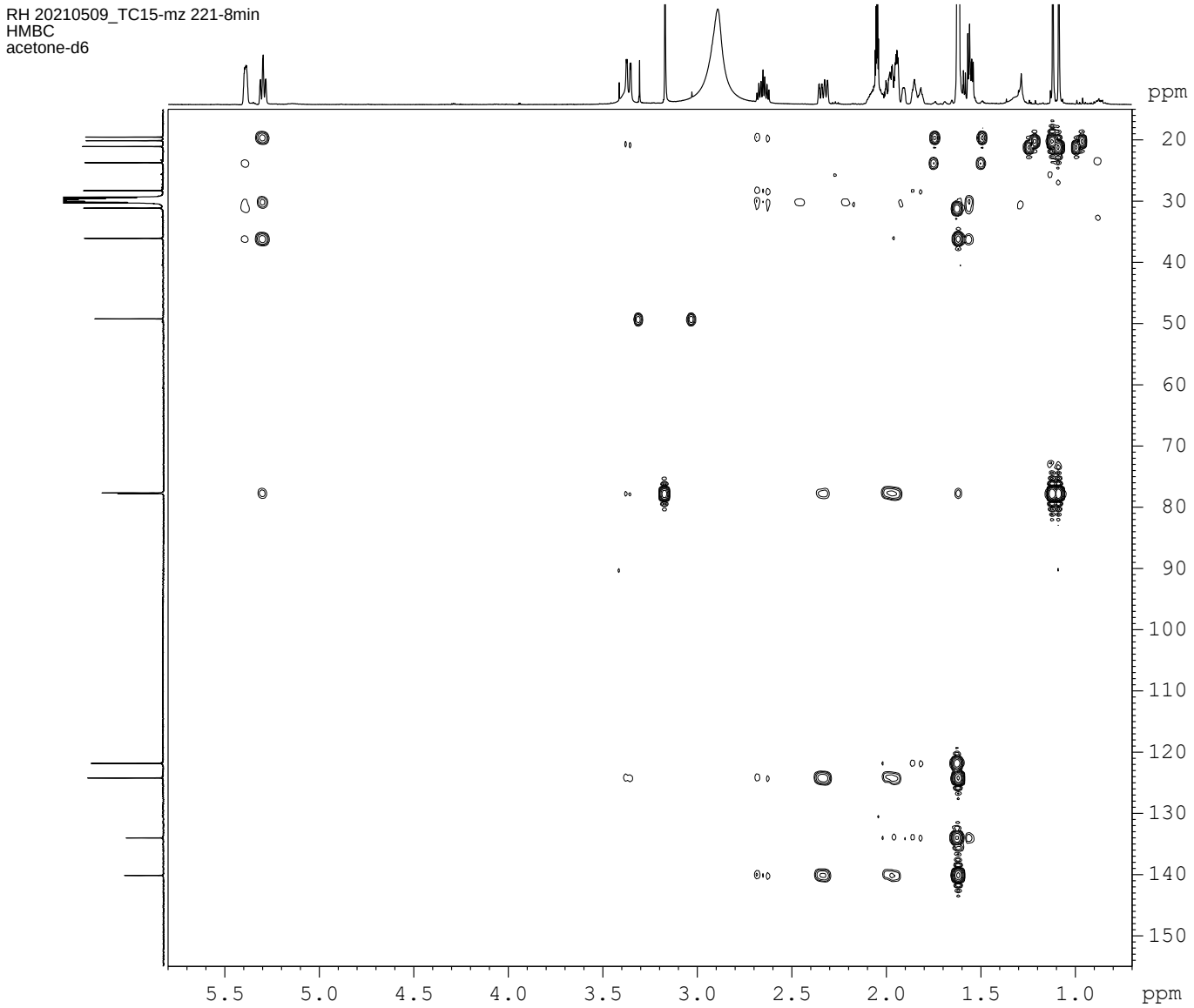
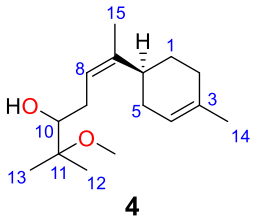


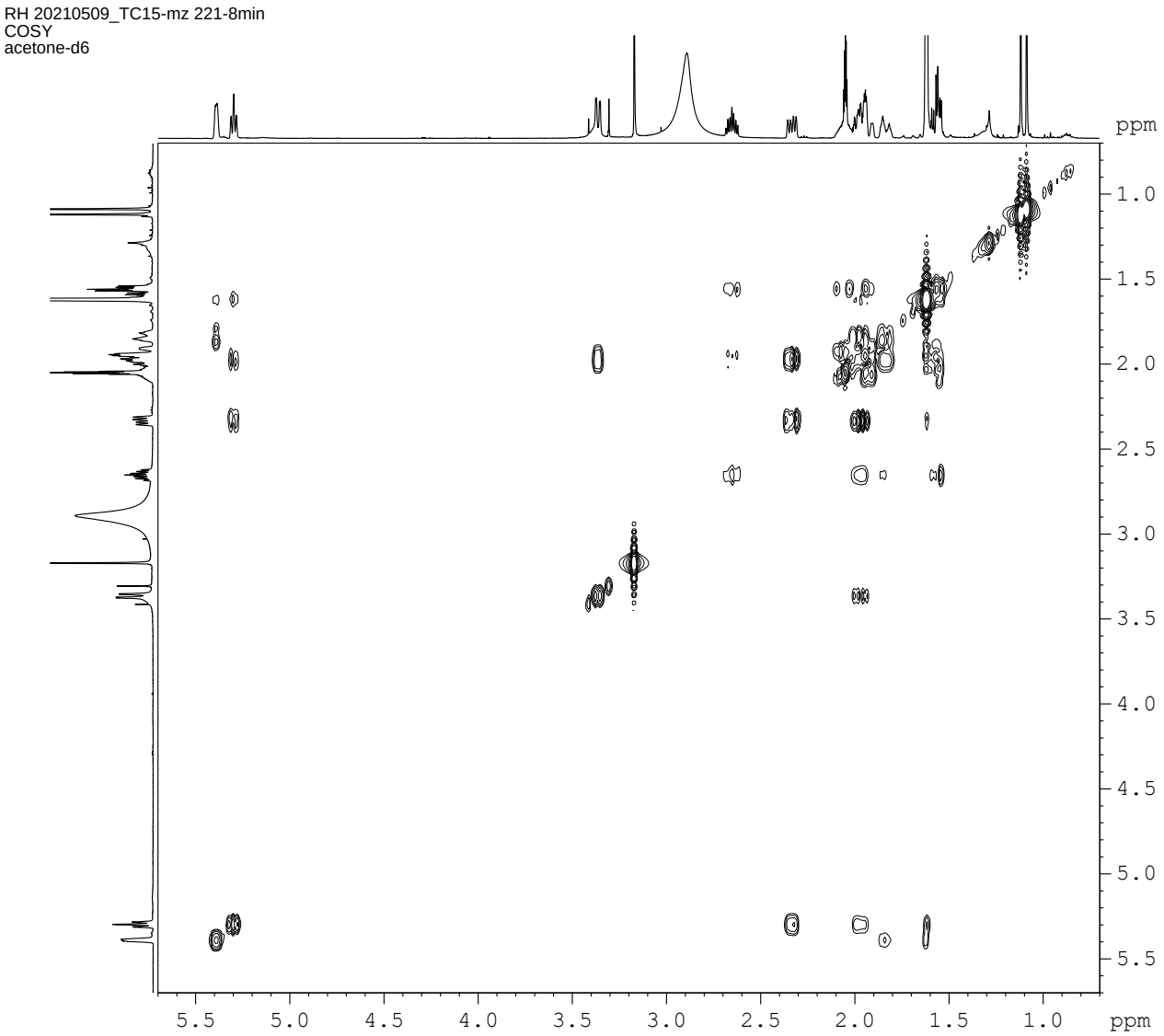
Figure S34. HSQC spectrum (acetone-d_6, 500 MHz) of **4** from *tps1A* expressed yeast culture.

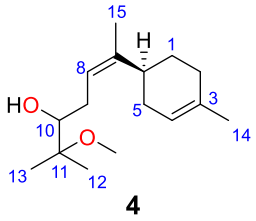




4

Figure S35. HMBC spectrum (acetone-d_6, 500 MHz) of **4** from *tps1A* expressed yeast culture.





4

Figure S36. COSY spectrum (acetone-d_6, 500 MHz) of **4** from *tps1A* expressed yeast culture.

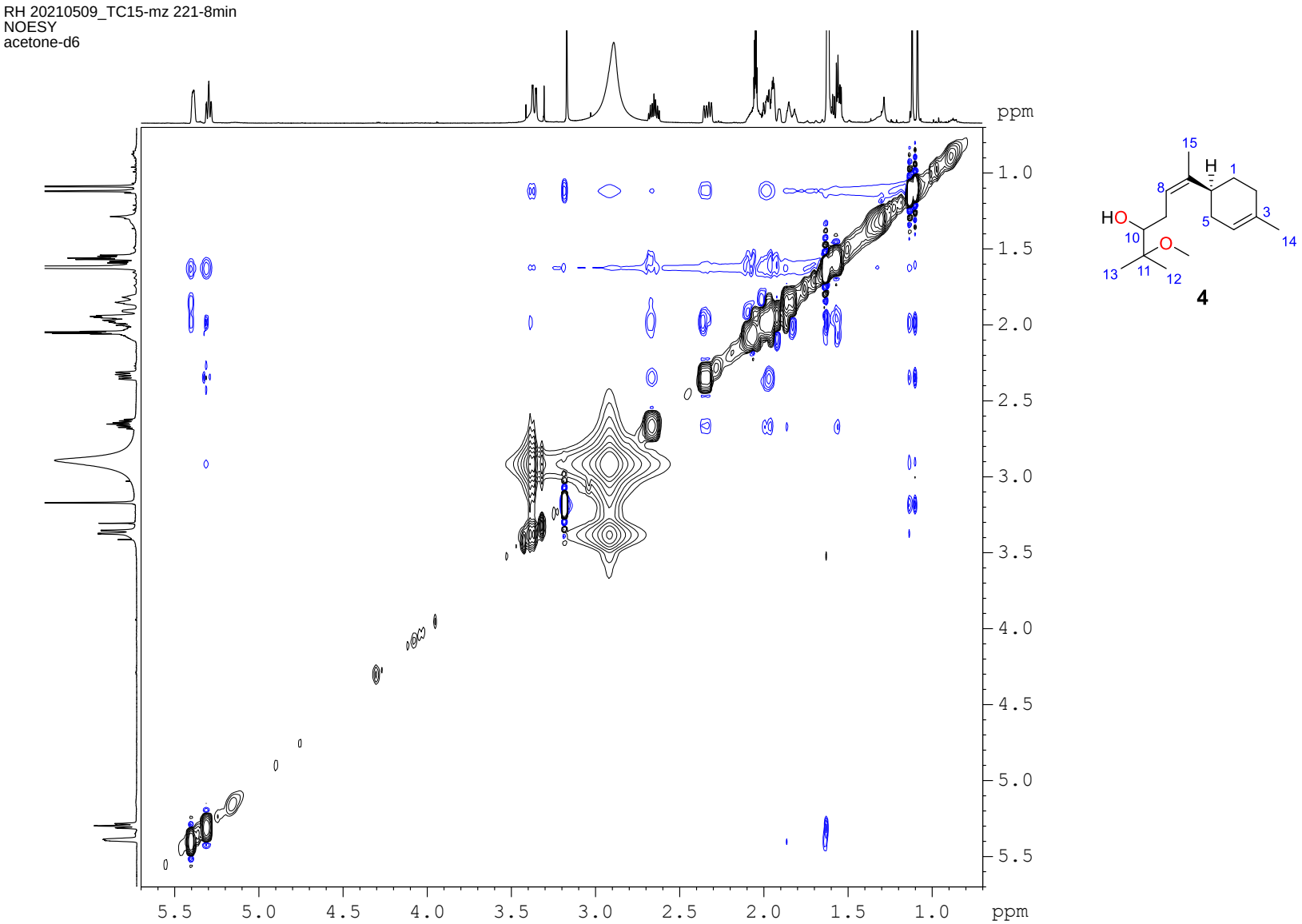


Figure S37. NOESY spectrum (acetone-d_6, 500 MHz) of **4** from *tps1A* expressed yeast culture.

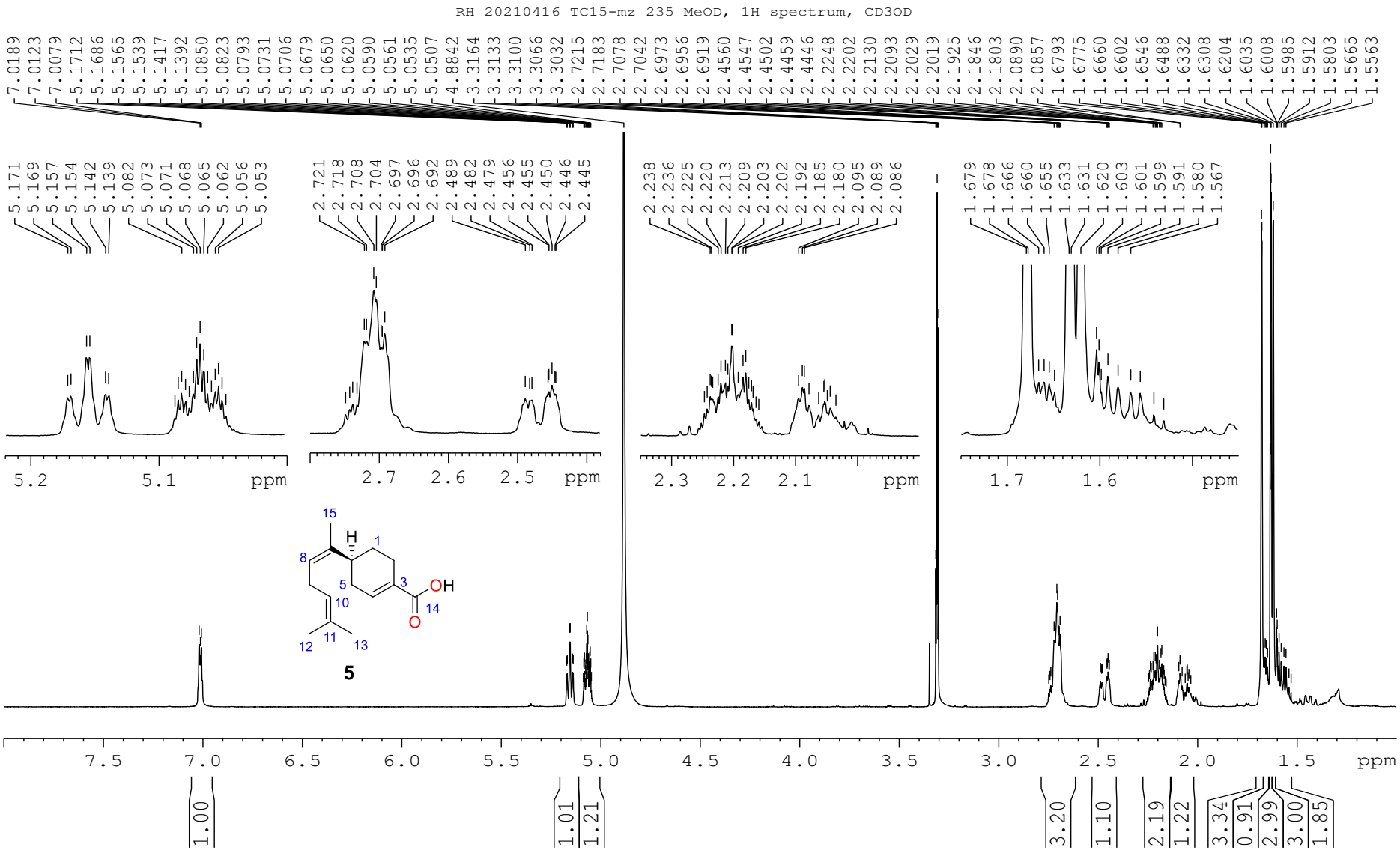


Figure S38. ^{1}H-NMR spectrum (MeOD, 500 MHz) of **5**.

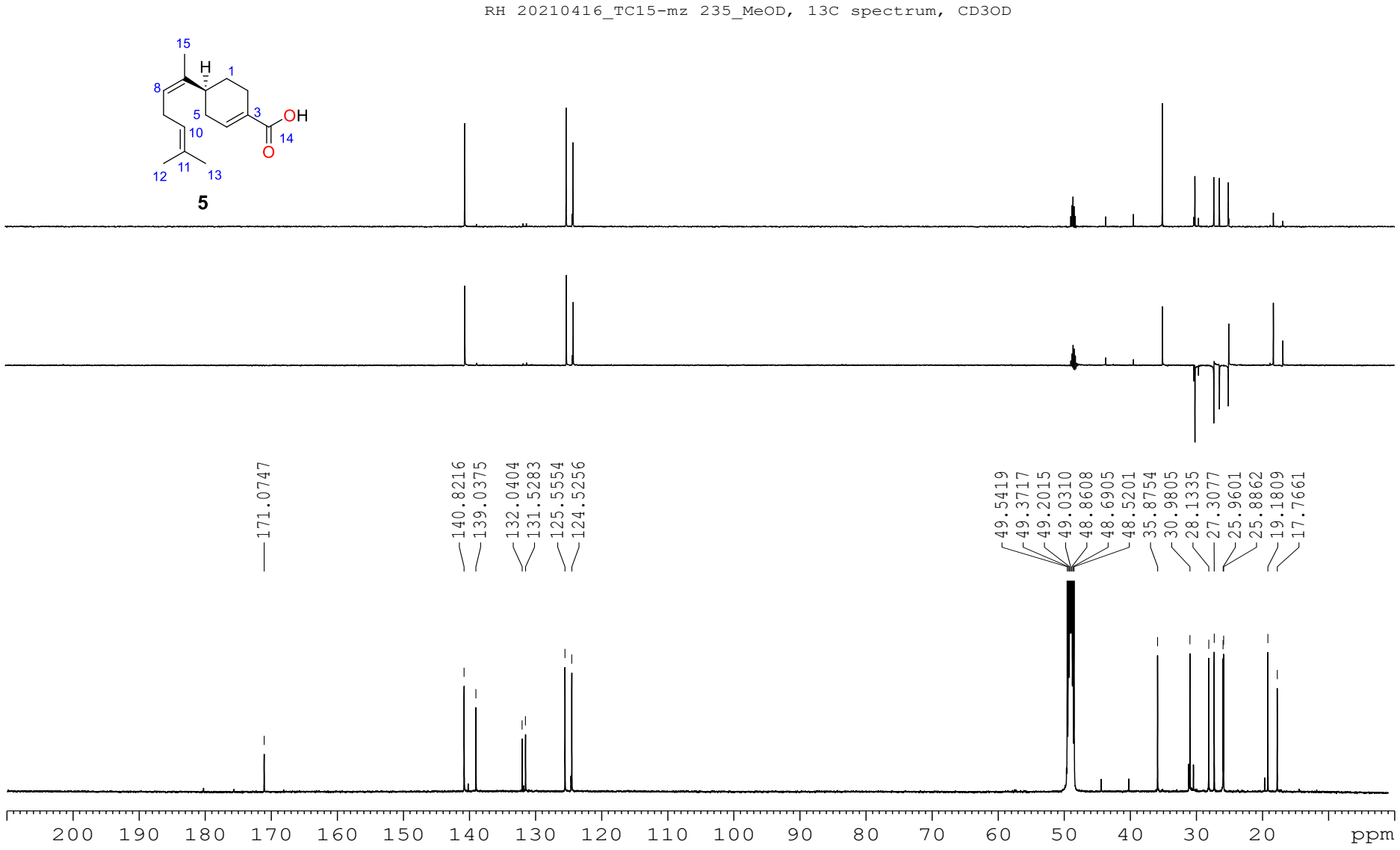
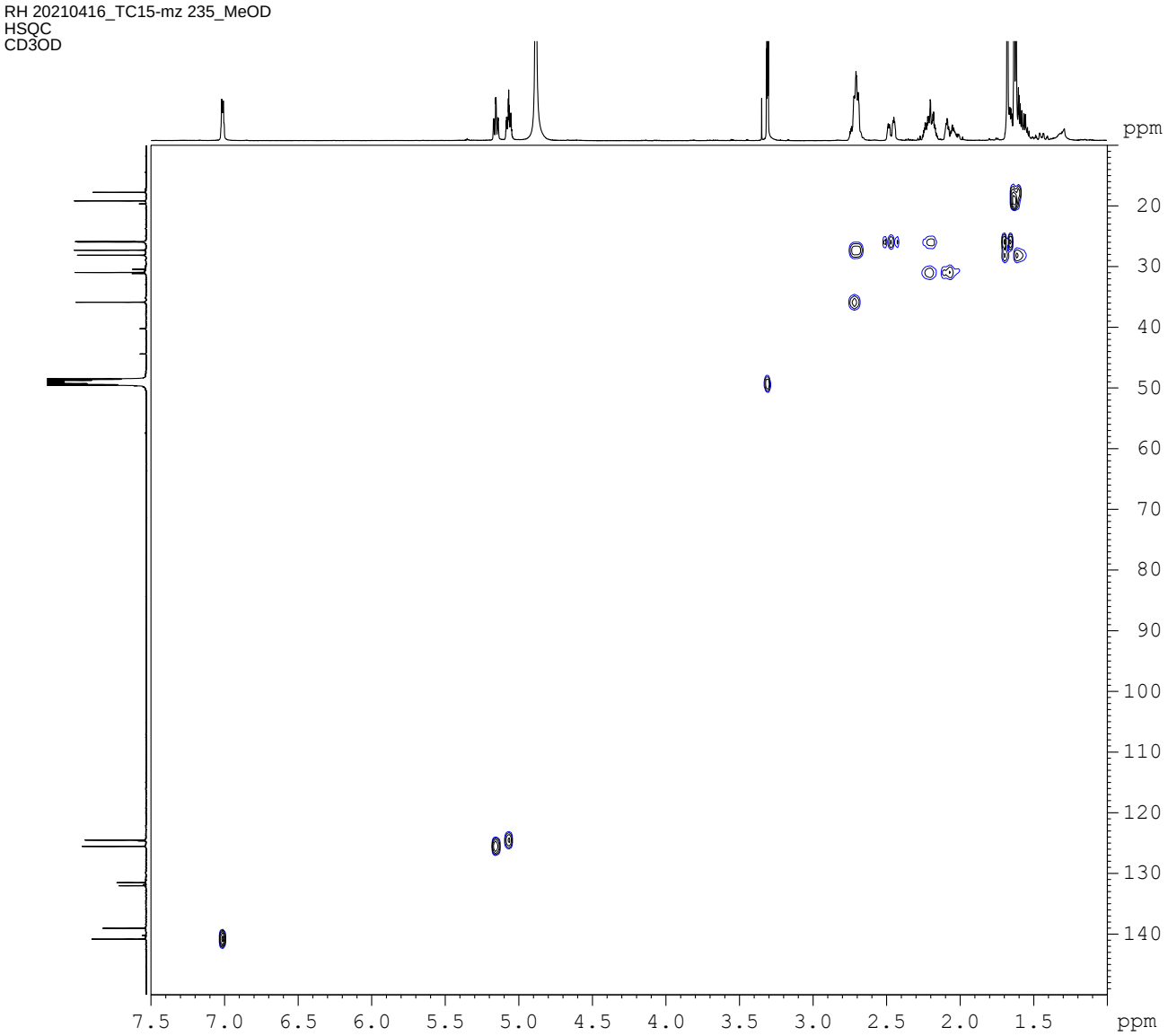
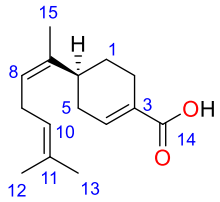


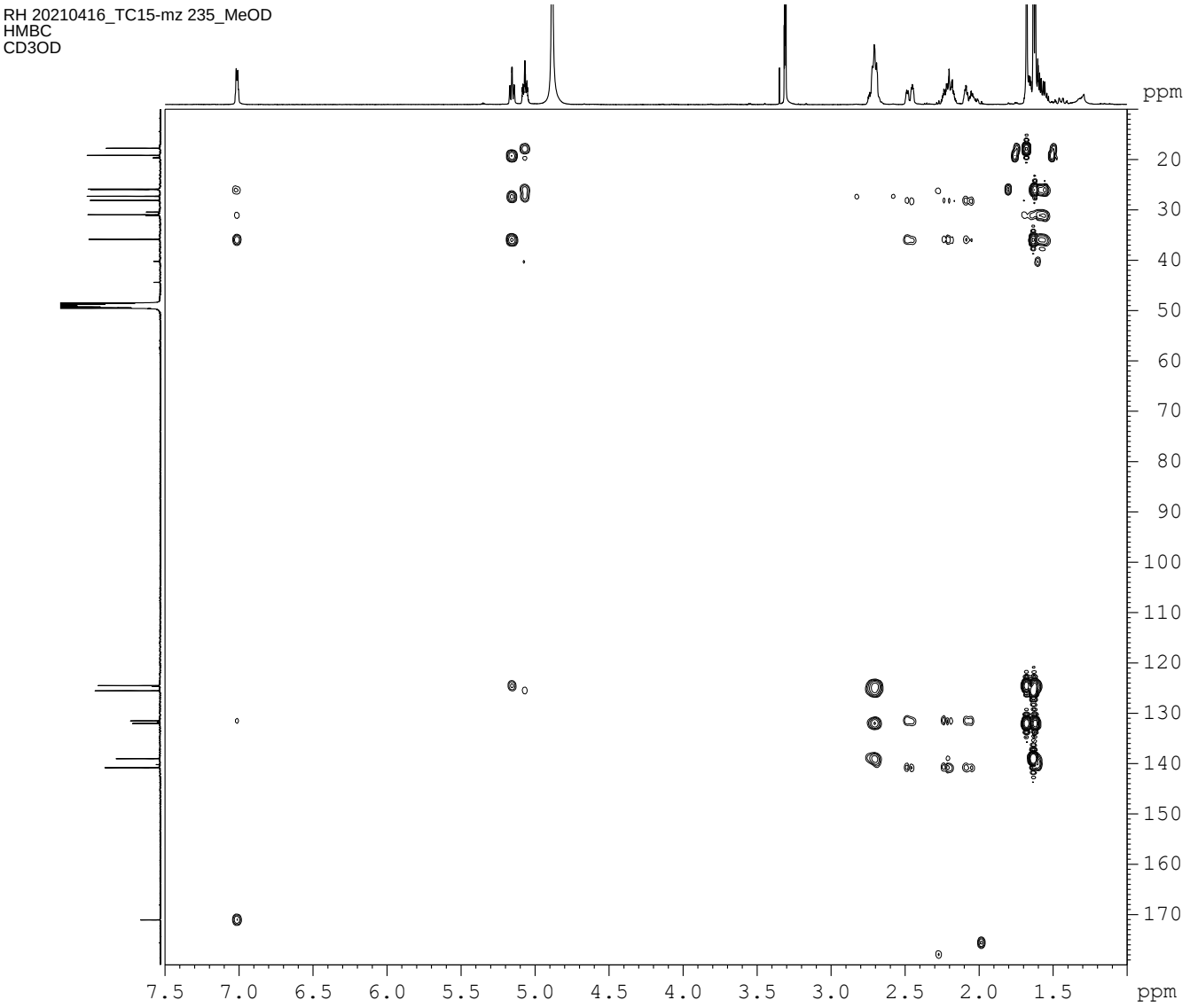
Figure S39. ^{13}C-NMR, DEPT135 and DEPT90 spectra (MeOD, 125 MHz) of **5**.

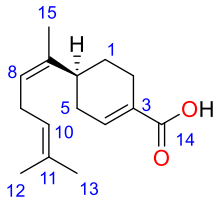




5

Figure S38. HSQC spectrum (MeOD, 500 MHz) of **5**.





5

Figure S39. HMBC spectrum (MeOD, 500 MHz) of **5**.

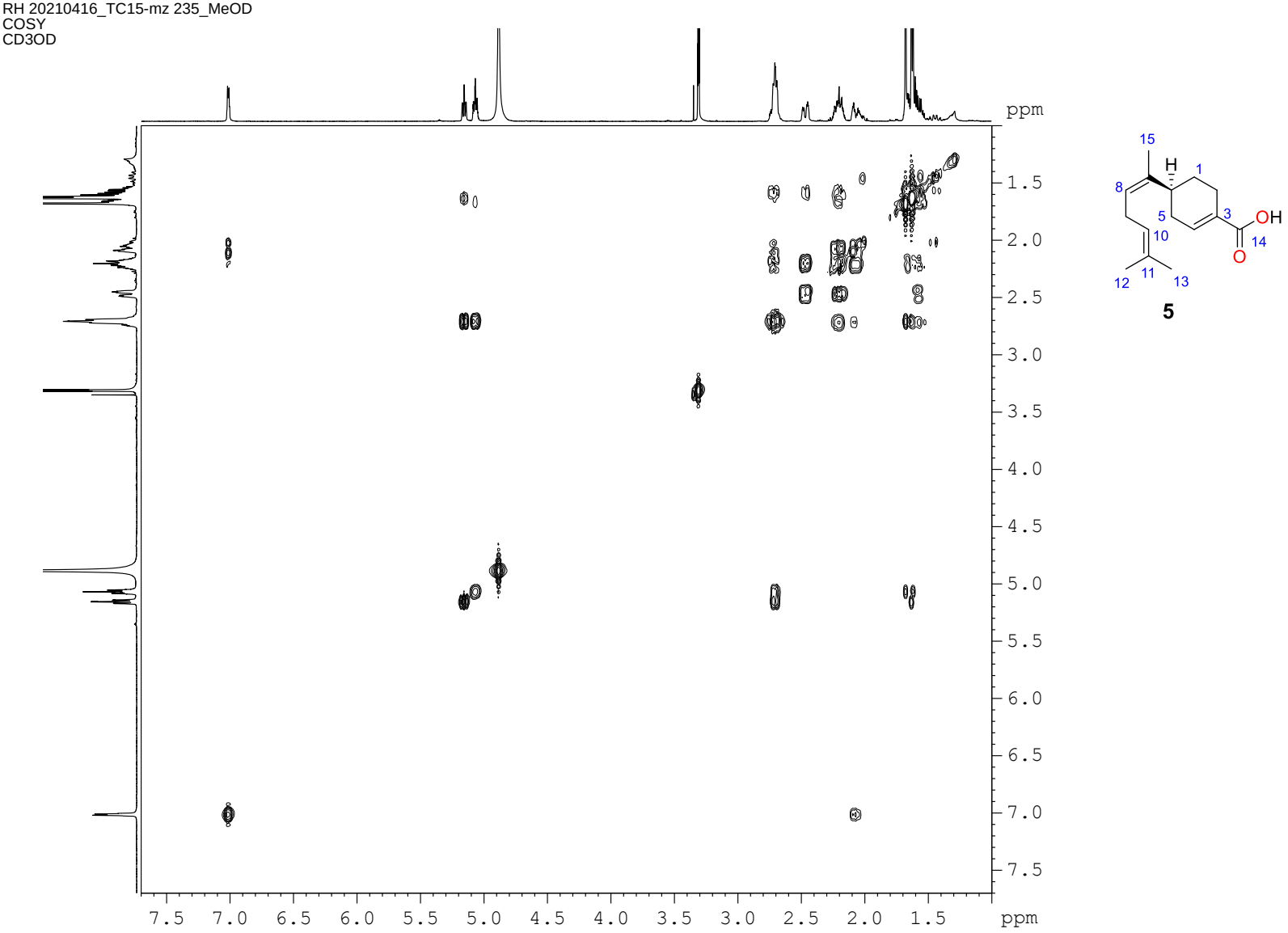


Figure S40. COSY spectrum (MeOD, 500 MHz) of **5**.

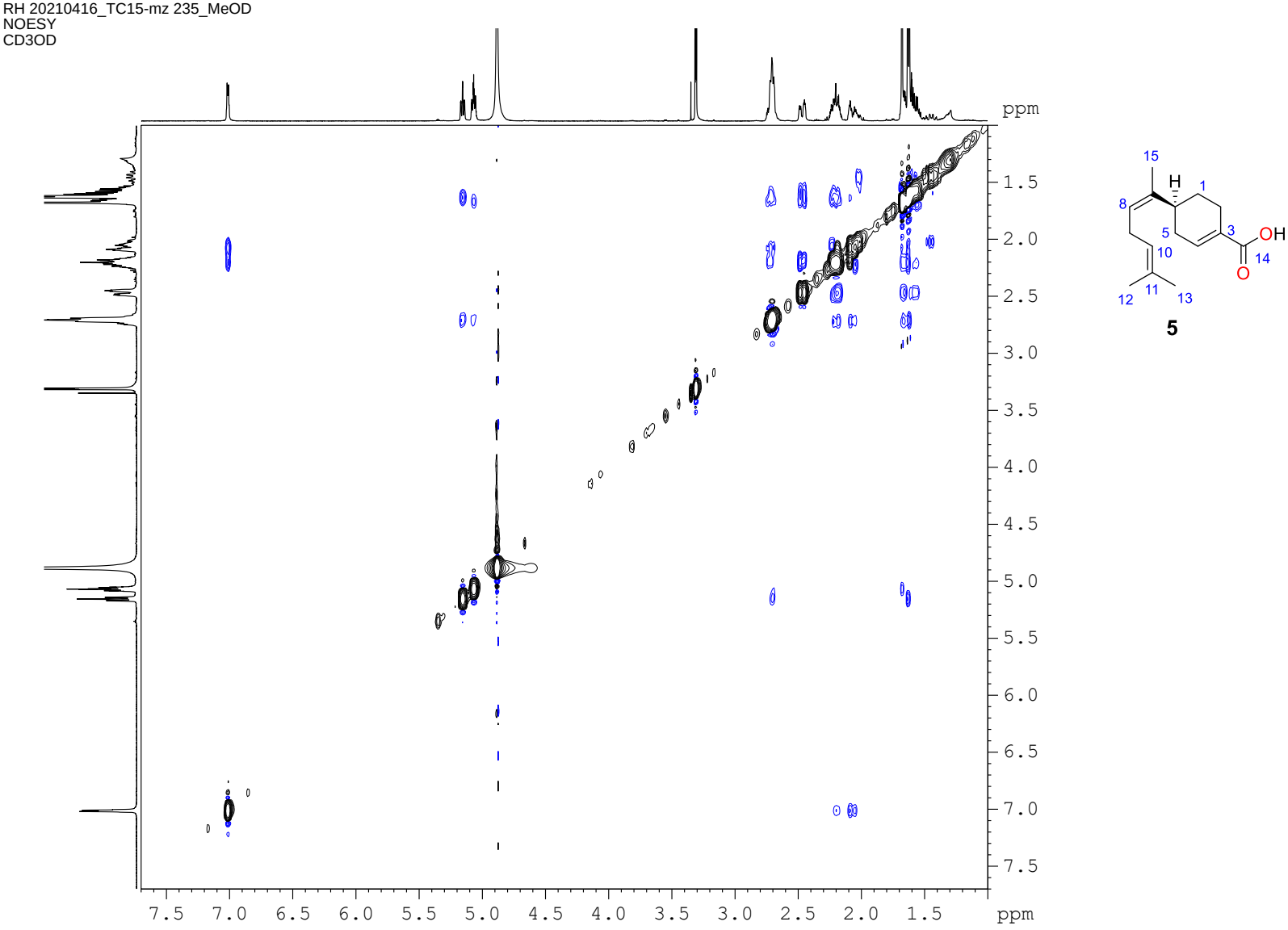


Figure S41. NOESY spectrum (MeOD, 500 MHz) of **5**.

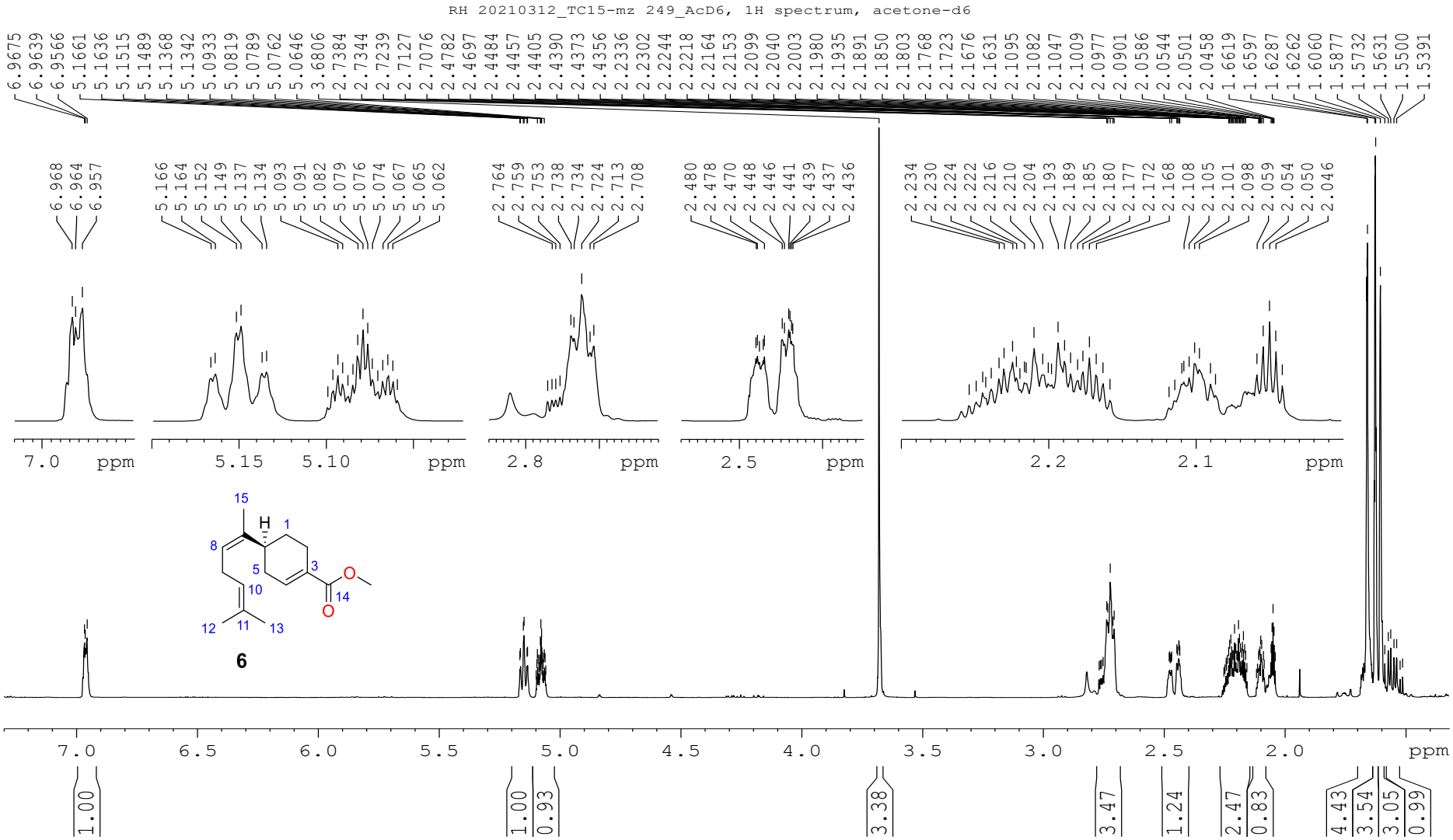


Figure S42. ^{1}H-NMR spectrum (acetone-d_6, 500 MHz) of **6**.

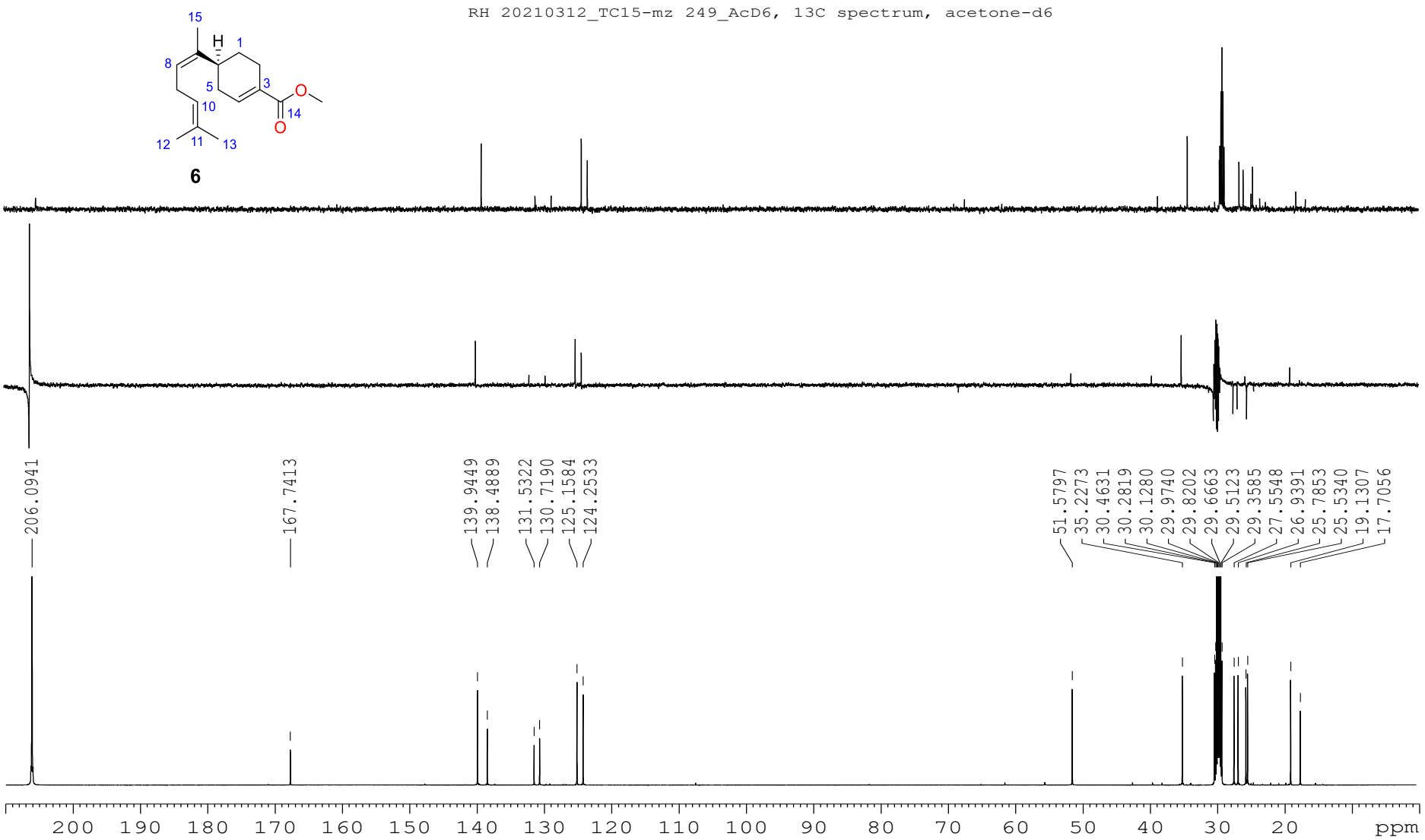


Figure S43. ^{13}C-NMR, DEPT135, and DEPT90 spectra spectrum (acetone-d_6, 125 MHz) of **6**.

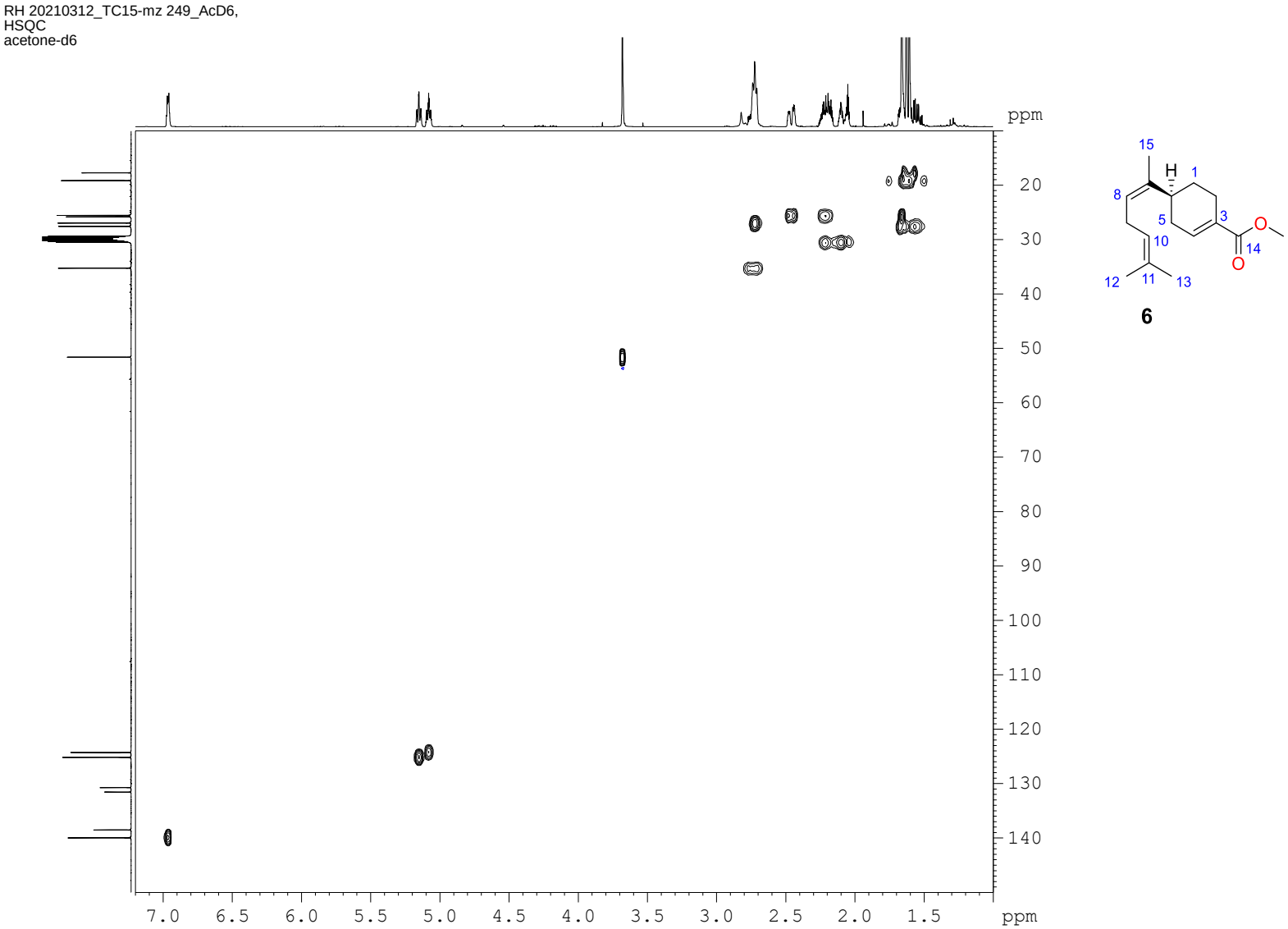
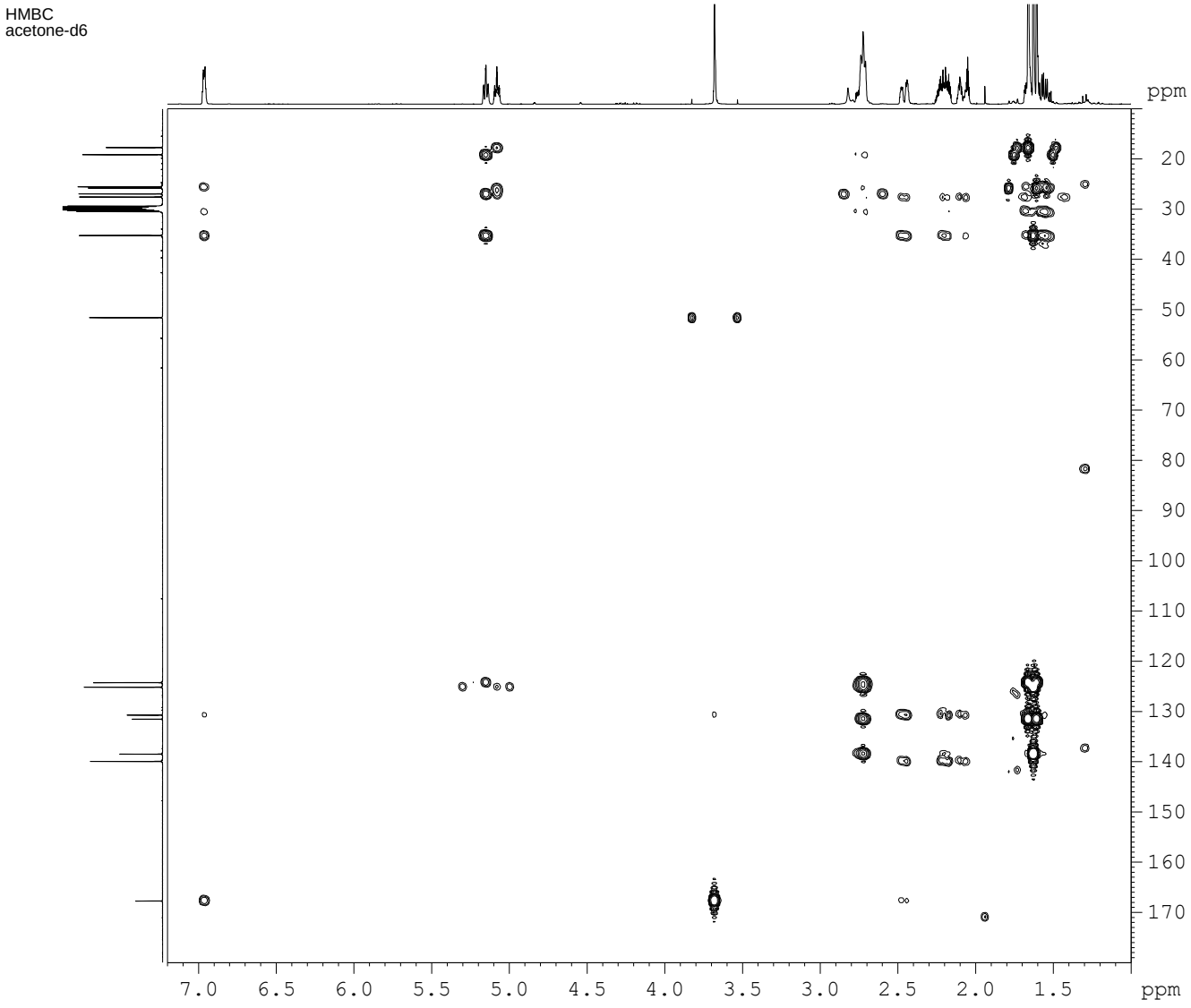
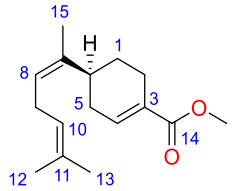


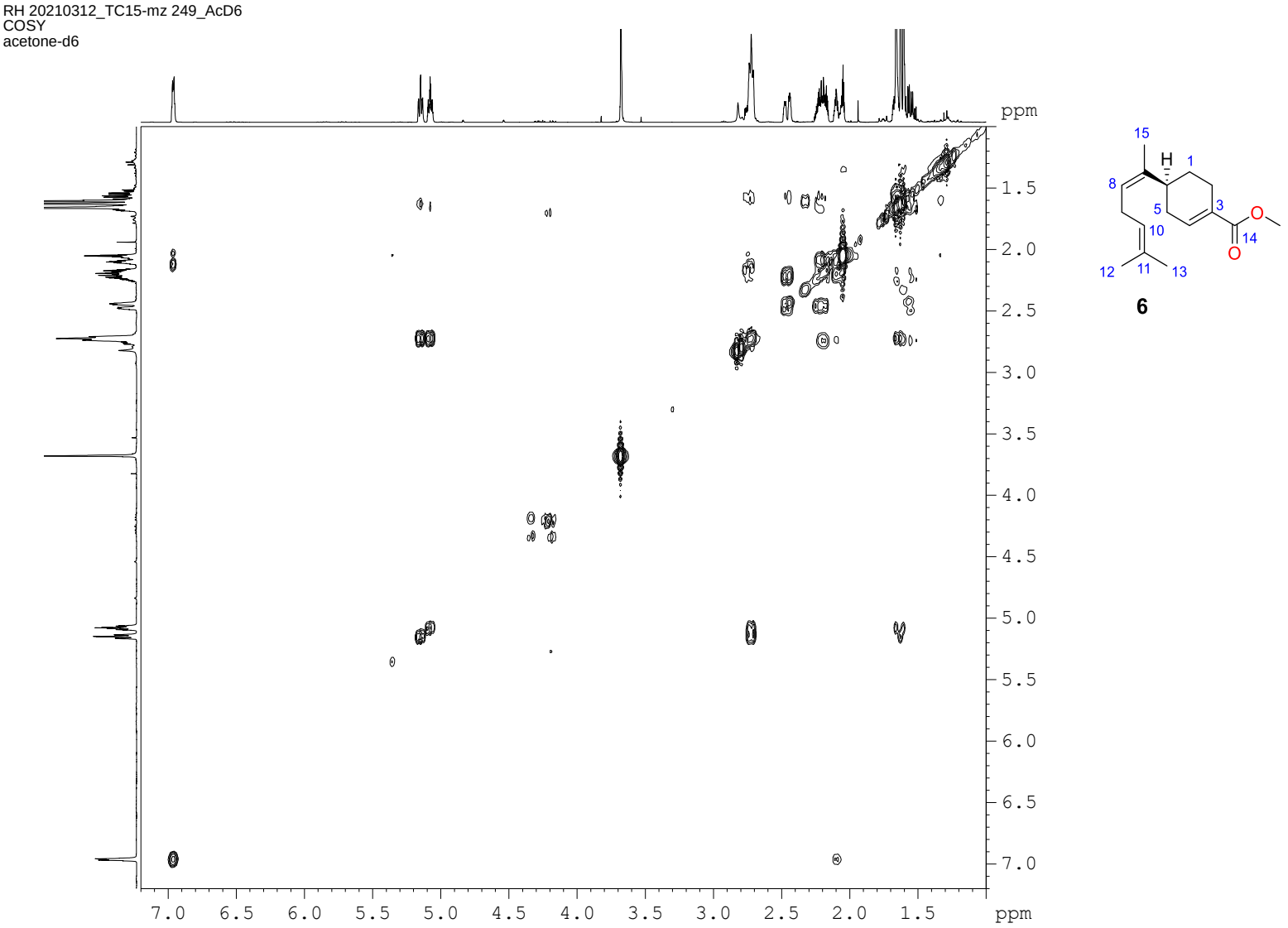
Figure S44. HSQC spectrum (acetone-d_6, 500 MHz) of **6**.





6

Figure S45. HMBC spectrum (acetone-d_6, 500 MHz) of **6**.



6

Figure S48. COSY spectrum (acetone-d_6, 500 MHz) of **6**.

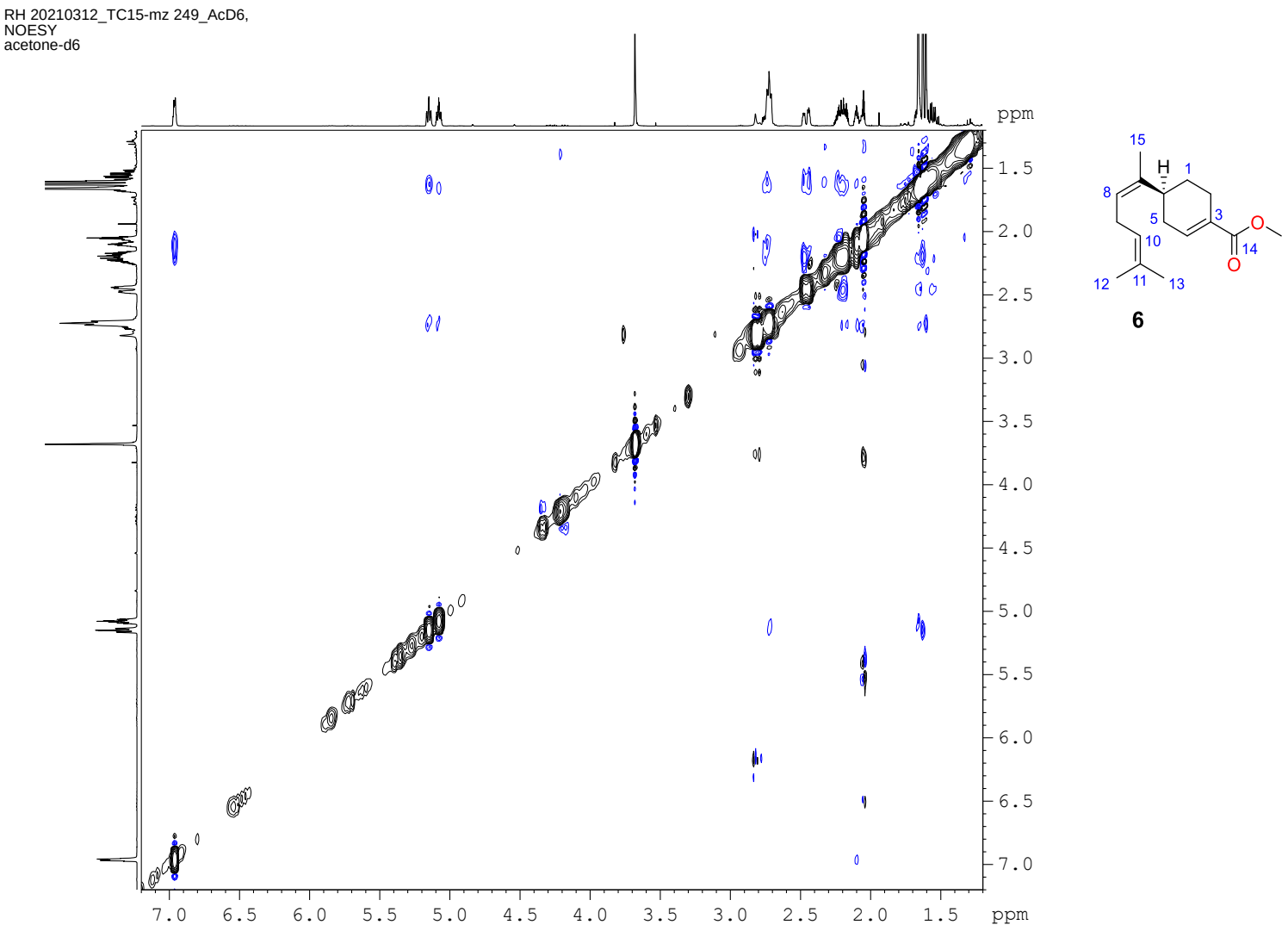


Figure S49. NOESY spectrum (acetone-d_6, 500 MHz) of **6**.

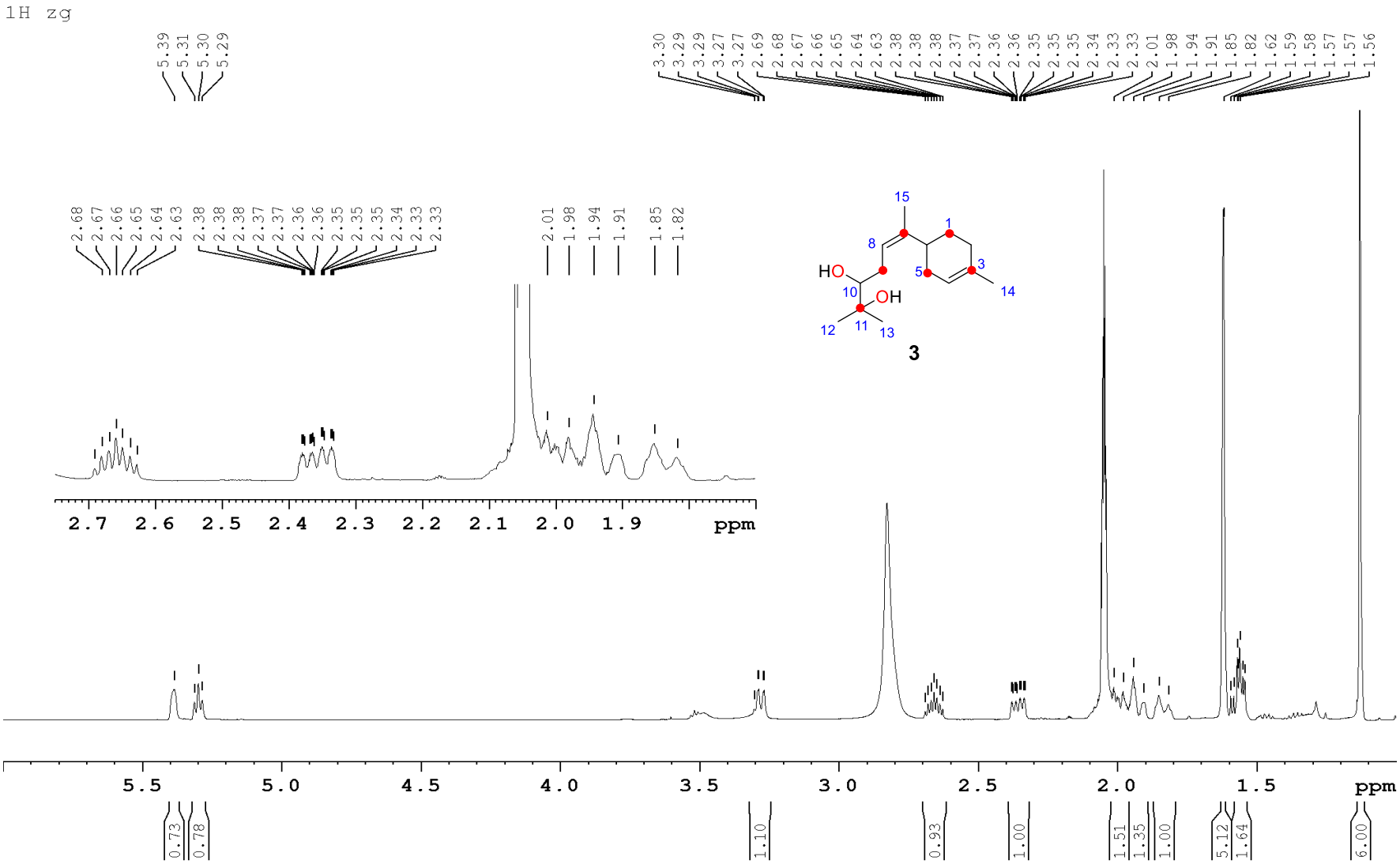


Figure S50. ^{1}H-NMR spectrum (acetone-d_6, 500 MHz) of ^{13}C-labeled **3**.

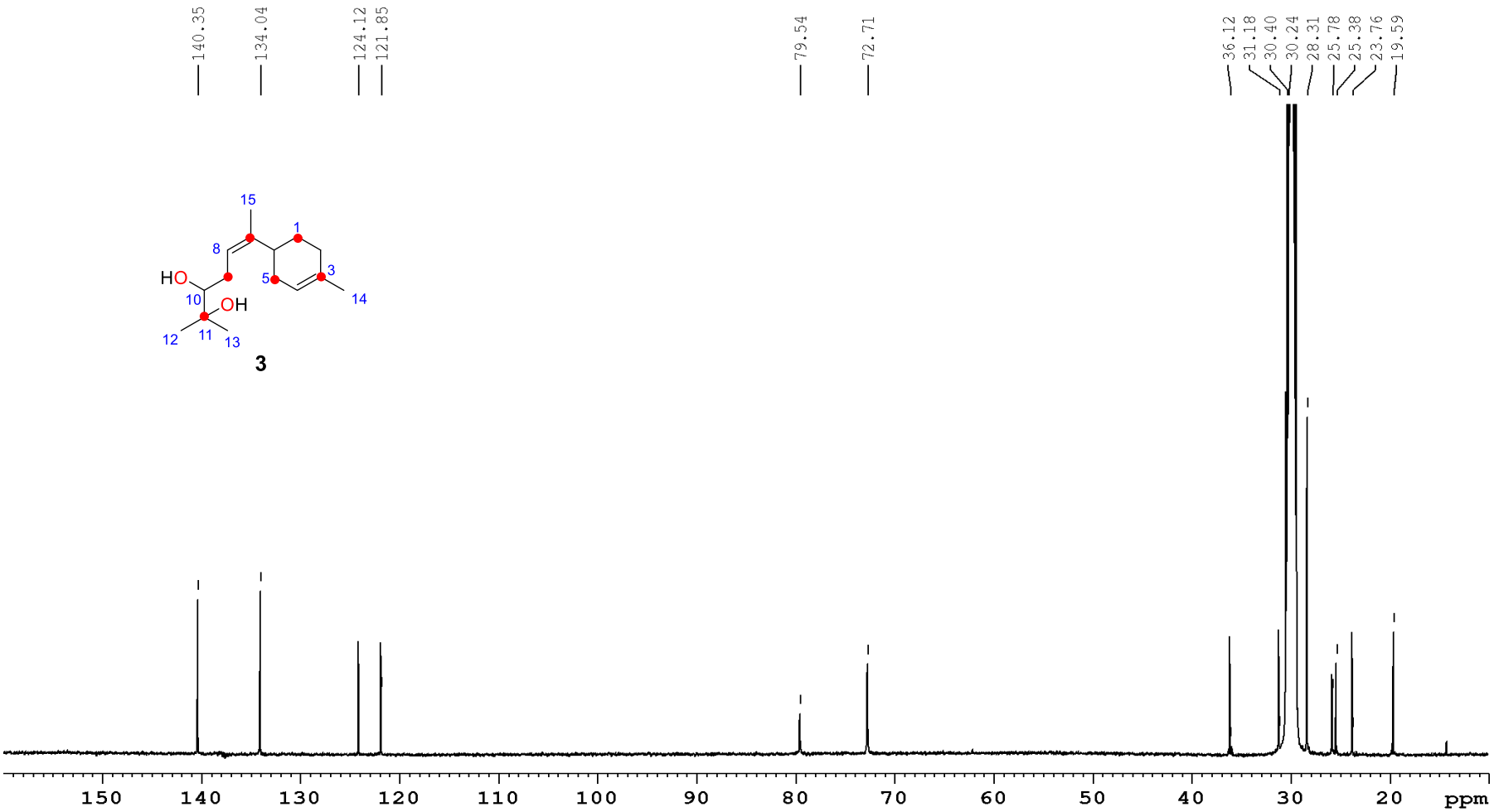
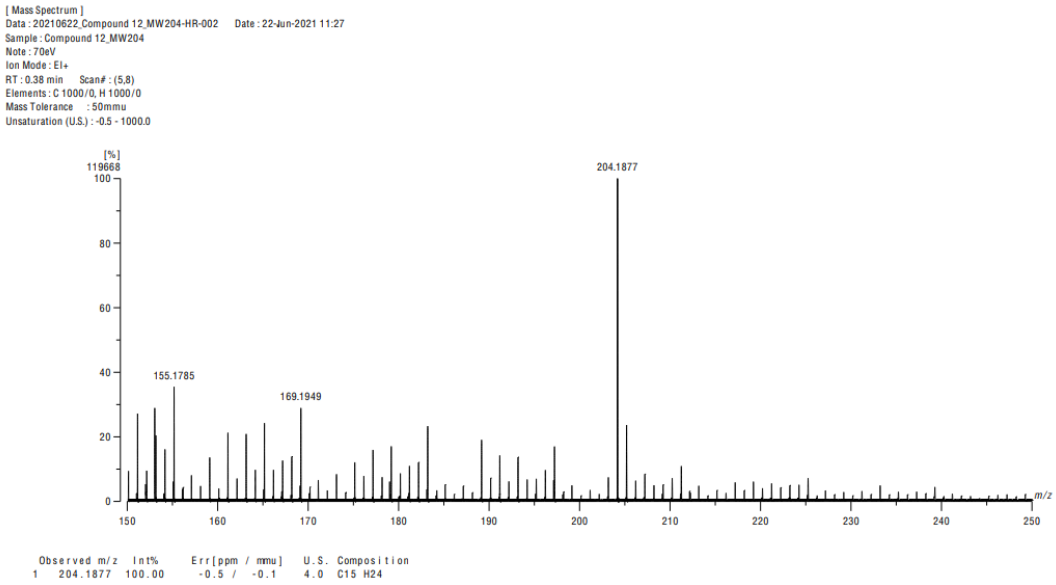


Figure S51. ^{13}C-NMR spectrum (acetone-d_6, 125 MHz) of ^{13}C-labeled **3**.

A (+)-(*S*,*Z*)-α-bisabolene (**1**), HRMS (EI) m/z: $[M]^+$ Calcd. for $C_{15}H_{24}$, m/z 204.1878; Found 204.1877



B

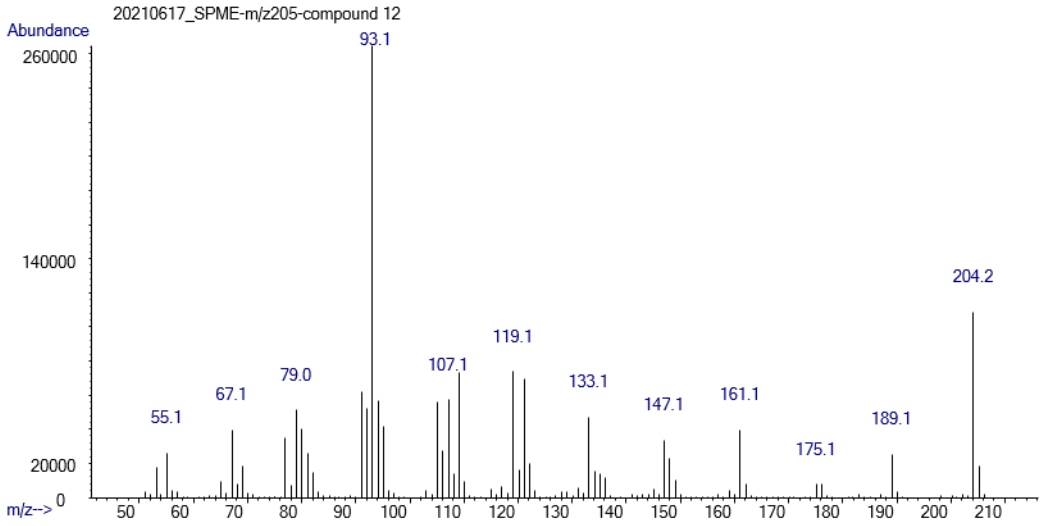
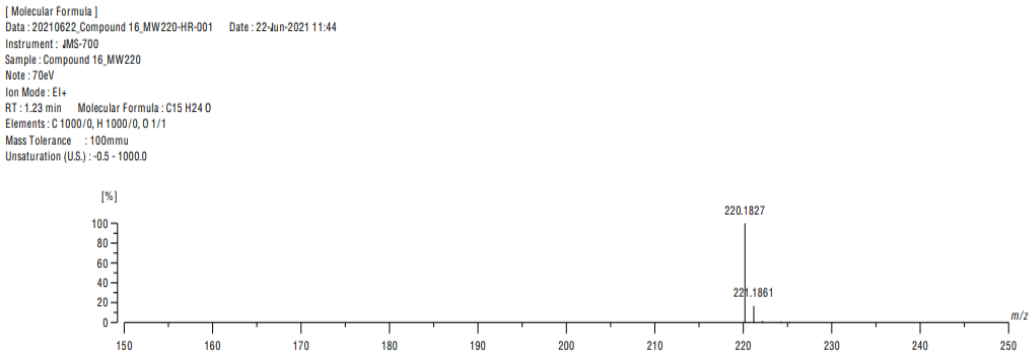


Figure S46. (A) HRMS and (B) EI-MS spectra of **1**.

A (–)-(*S*,*Z*)-10,11-epoxy-bisabolene (**2**), HRMS (EI) m/z: $[M]^+$ Calcd. for $C_{15}H_{24}O$, m/z 220.1827; Found 220.1827



B

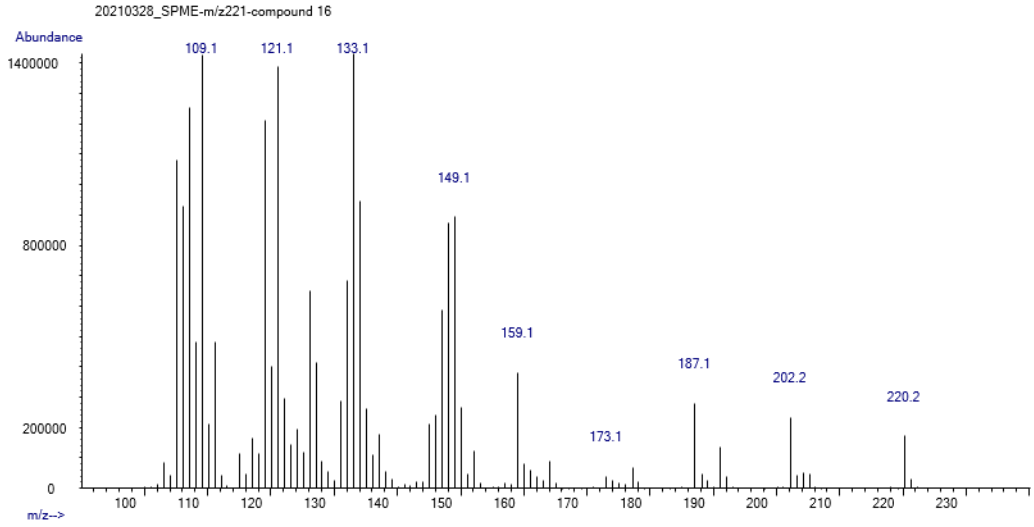
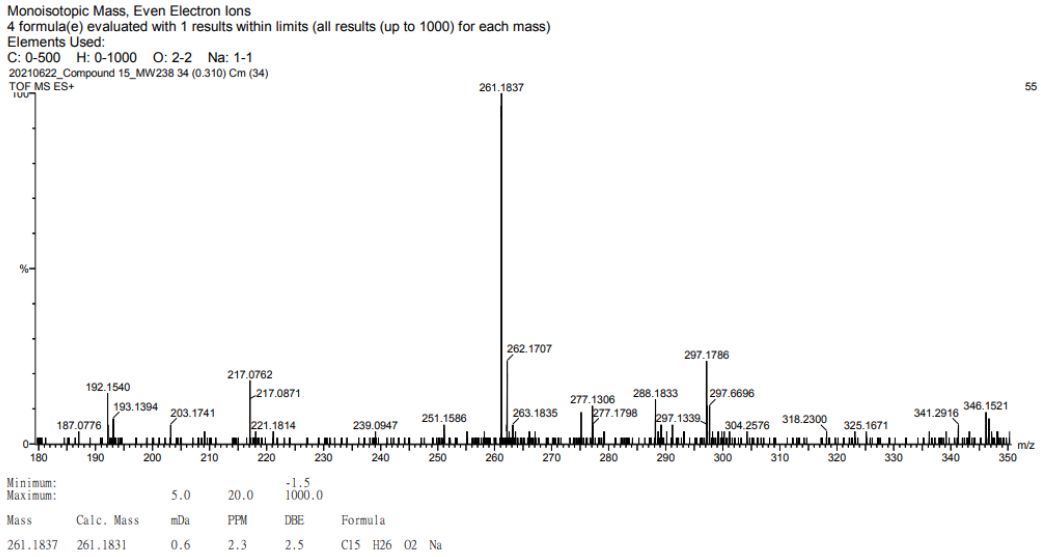


Figure S47. (A) HRMS and (B) EI-MS spectra of **2**.

A (–)-(*S*,*Z*)-10,11-dihydroxy-bisabolene (**3**), HRMS (ESI nanospray) m/z: $[M+Na]^+$ Calcd. for $C_{15}H_{26}O_2Na$, m/z 261.1830; Found 261.1837



B

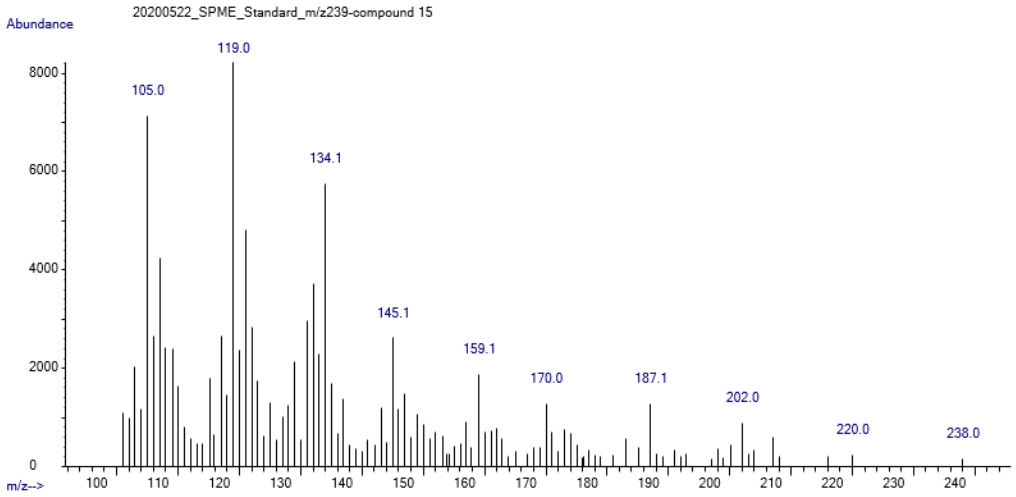
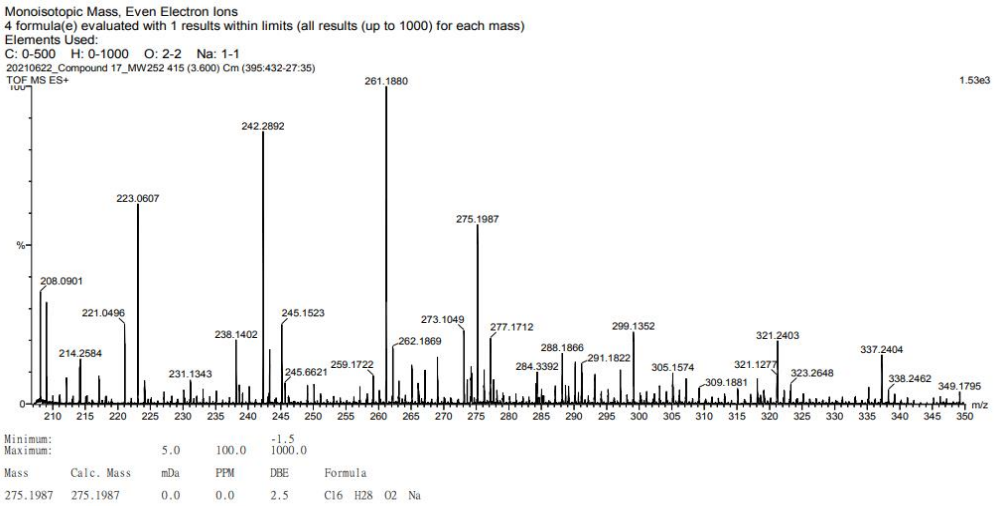


Figure S48. (A) HRMS and (B) EI-MS spectra of **3**.

A (–)-(*S*,*Z*)-10-hydroxy-11-methoxy-bisabolene (**4**), HRMS (ESI nanospray) m/z: $[M+Na]^+$ Calcd. for $C_{16}H_{28}O_2Na$, m/z 275.1987; Found 275.1987.



B

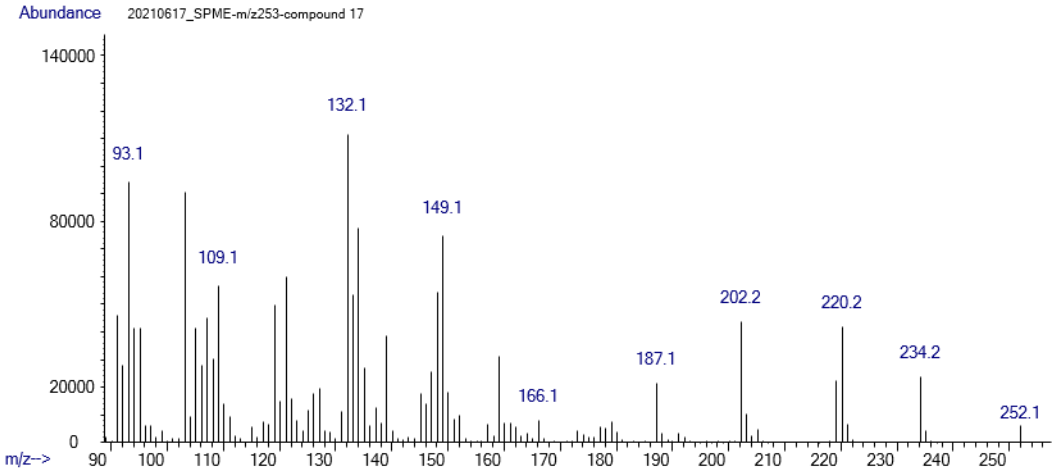
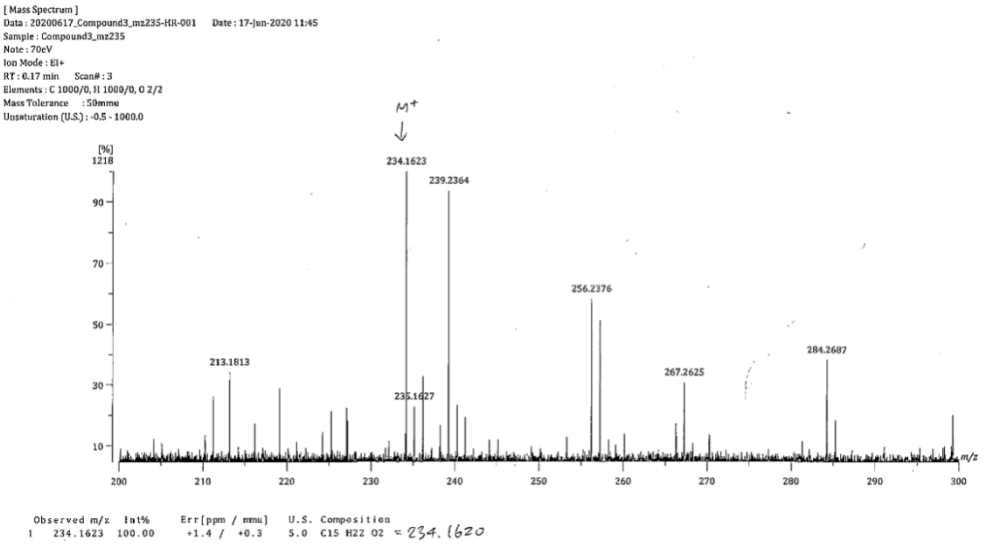


Figure S49. (A) HRMS and (B) EI-MS spectra of **4**.

A (–)-(*S,Z)*-14-bisabolenoic acid (**5**), HRMS (EI) m/z: $[M]^+$ Calcd. for $C_{15}H_{22}O_2$, m/z 234.1620; Found 234.1623



B

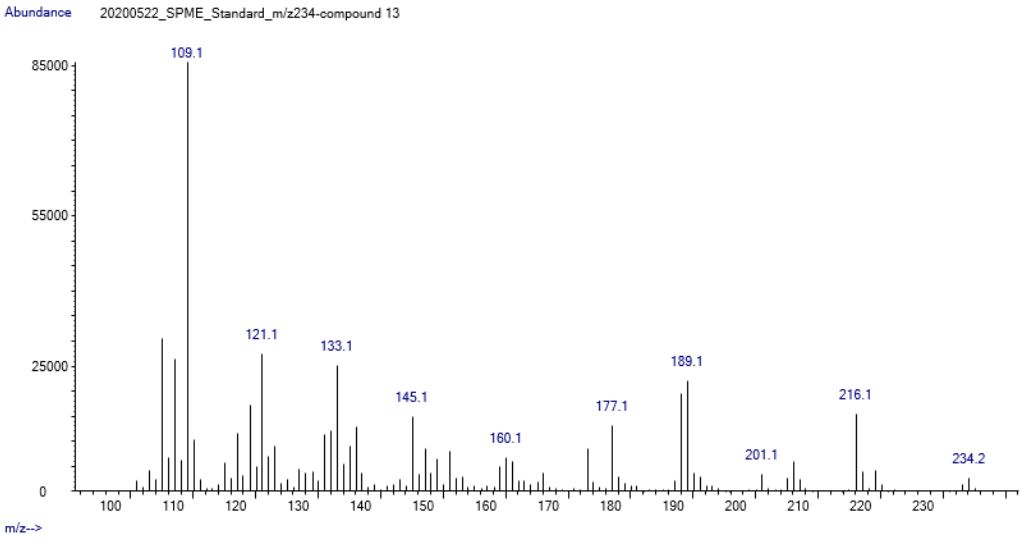
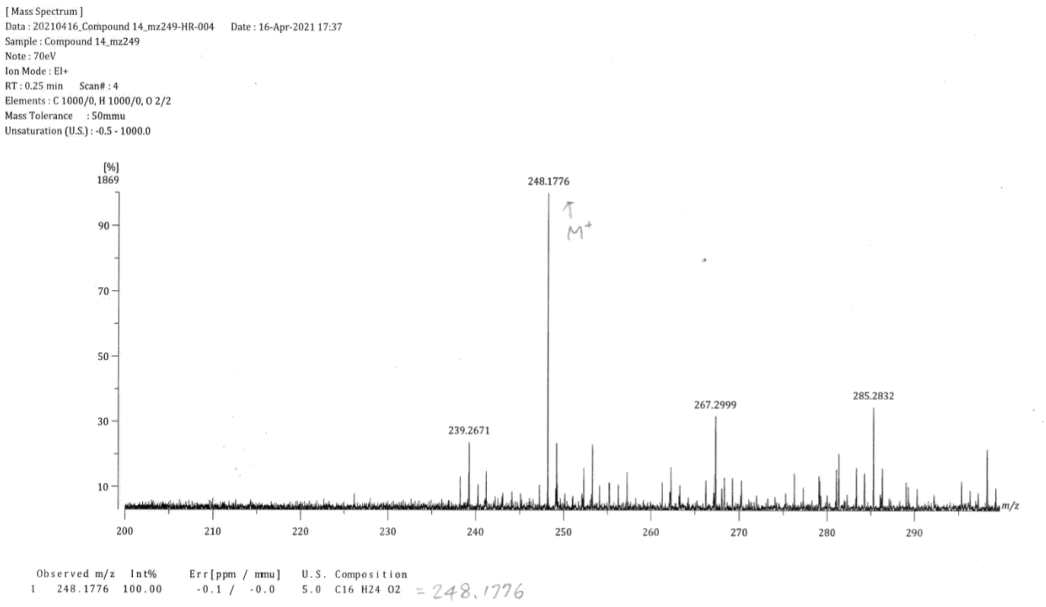


Figure S50. (A) HRMS and (B) EI-MS spectra of **5**.

A (–)-(*S*,*Z*)-14-bisabolenoic methyl ester (**6**) HRMS (EI) m/z: $[M]^+$ Calcd. for $C_{16}H_{24}O_2$, m/z 248.1776; Found 248.1776



B

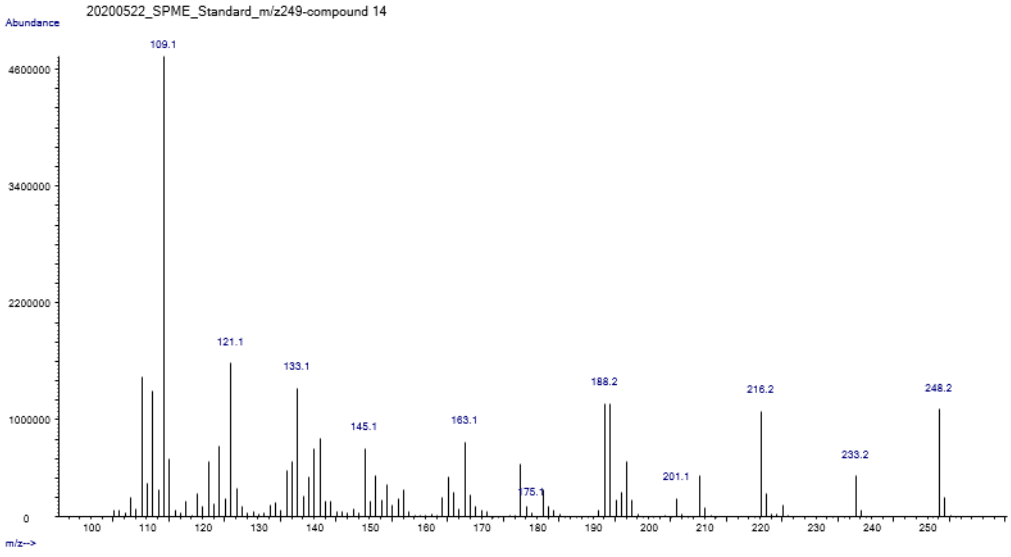
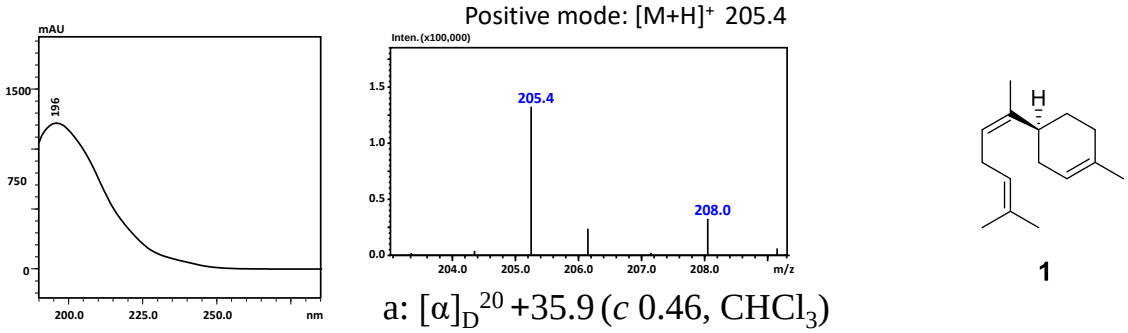


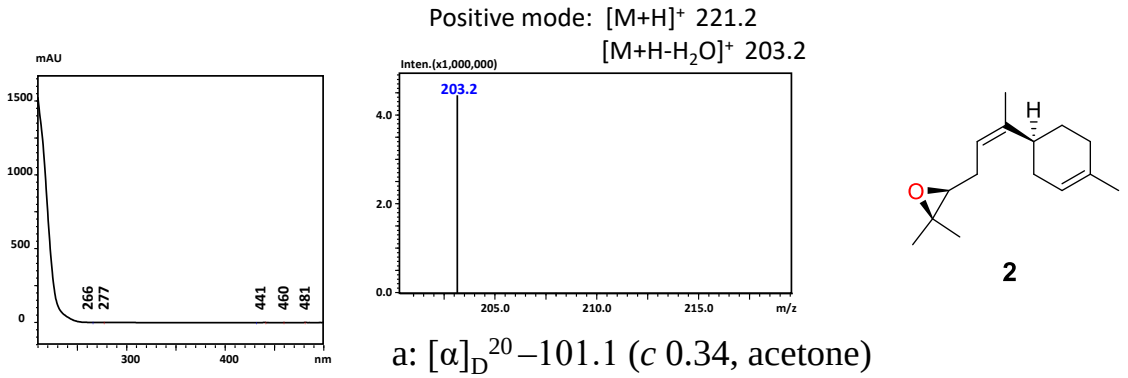
Figure S51. (A) HRMS and (B) EI-MS spectra of **6**.



1

a: $[\alpha]_D^{20}$ +35.9 (*c* 0.46, $CHCl_3$)

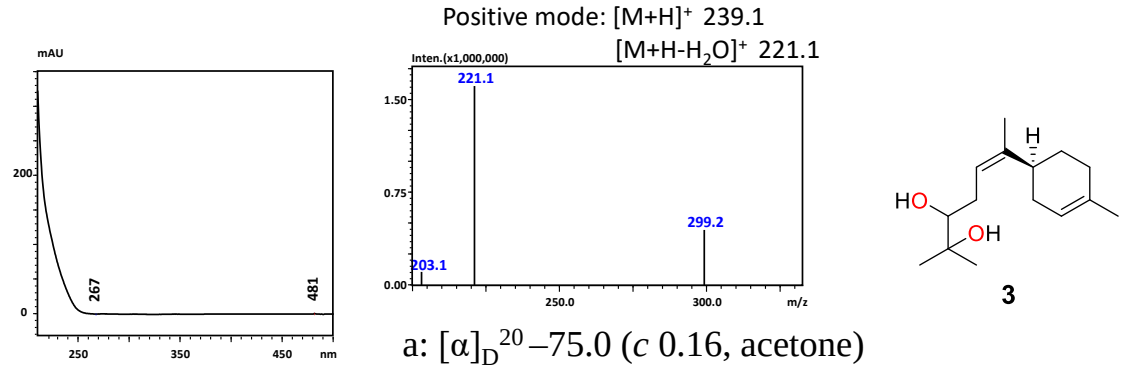
b: $[\alpha]_D^{20}$ +26.8 (*c* 0.25, $CHCl_3$)



2

a: $[\alpha]_D^{20}$ –101.1 (*c* 0.34, acetone)

b: $[\alpha]_D^{20}$ –92.3 (*c* 0.13, acetone)



3

a: $[\alpha]_D^{20}$ –75.0 (*c* 0.16, acetone)

b: $[\alpha]_D^{20}$ –58.1 (*c* 0.05, acetone)

Figure S52. UV and MS spectra and $[\alpha]_D^{20}$ of compounds **1**, **2** and **3** (a: Tps1 and b: Tps2).

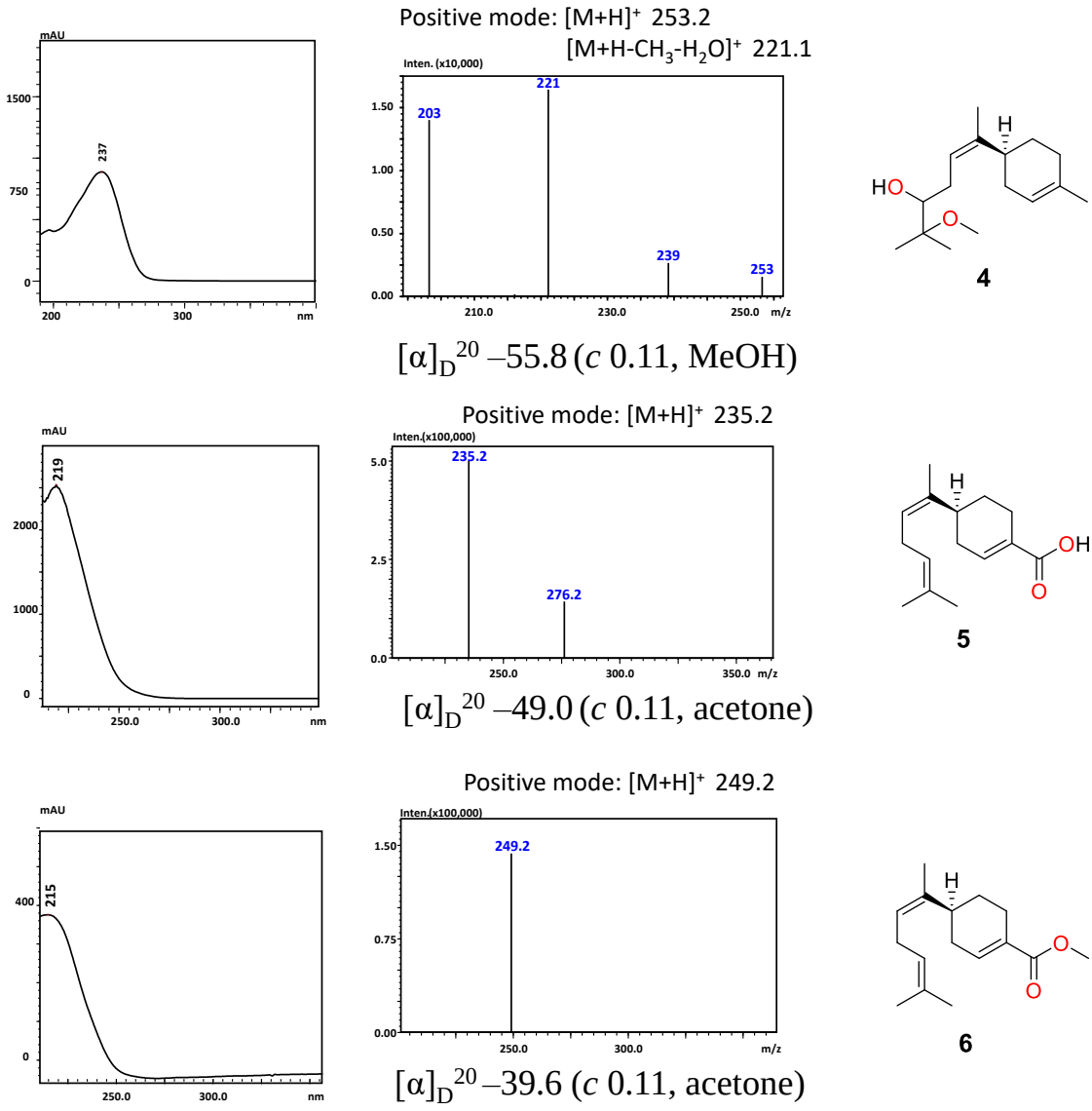


Figure S53. UV and MS spectra and $[\alpha]_D^{20}$ of compounds **4**, **5** and **6**.

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