Supporting information

Efficient Biosynthesis of Fungal Polyketides Containing the Dioxabicyclo-octane Ring System

Xu-Ming Mao[1,2‡], Zha-Jun Zhan[2,4‡], Matthew N. Grayson[3], Man-Cheng Tang[2], Wei Xu[2], Yong-Quan Li[1], Wen-Bing Yin[2], Hsiao-Ching Lin[2], Yit-Heng Chooi[2], K. N. Houk[3], Yi Tang[2,3*]

[1]Zhejiang University, College of Life Sciences, Hangzhou 310058, China, [2]Department of Chemical and Biomolecular Engineering, [3]Department of Chemistry and Biochemistry, University of California, Los Angeles CA90095, USA, [4]Zhejiang University of Technology, College of Pharmaceutical Science, Hangzhou 310014, China

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Supplementary Schemes

Experimental Procedures

Strains and culture media

Calcarisporium arbuscula strains in this study were listed in Table S3. *C. arbuscula* was grown in the potato dextrose broth (PDB, Fluka) at 25°C for aurovertin production. Spores were collected from the potato dextrose agar (PDA, PDB + 1.5% agar) medium at 25°C for 6 days. For gene knock-out in *C. arbuscula*, PDA plus 1.2 M sorbitol and 250 μg/ml hygromycin was used for protoplast regeneration and antibiotic resistance selection. *Saccharomyces cerevisiae* strain BJ5464-NpgA (MAT**α** *ura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*) was used for aurovertin biosynthesis pathway reconstitution or yeast expression plasmid construction by *in vivo* homologous recombination.[1] For yeast culture, the rich medium YPD was used for the routine growth of yeast strain BJ5464-NpgA and its derivatives at 30°C. SD-drop out media were used for selection of plasmids transformed into *S. cerevisiae*. For protein expression under *ADH2* promoter (*ADH2p*) and aurovertin biosynthetic pathway reconstitution *in S. cerevisiae*, the appropriate plasmids were introduced into yeast cells as described in Table S4. The yeast transformants were initially grown in the appropriate SD-drop out liquid media and then transferred to the liquid YPD media for further culture for 4 days. LB media were used for *Escherichia coli* culture. *E.coli* strain Topo10 was the host for routine plasmid sub-cloning according to the protocols described.[2]

Plasmid construction

Primers and plasmids were listed in Table S5 and S6, respectively. Yeast expression plasmids pXW02 (*LEU2* marker), pXW06 (*TRP1* marker) and pXW55 (*URA3* marker)[3] were used for construction of the heterologous expression plasmids for aurovertin biosynthetic enzymes by *in vivo* homologous recombination in yeast. All the enzymes were expressed under a strong promoter *ADH2p*. For polyketide synthase AurA expression, primers aurA F1 + aurA R1, aurA F2 + aurA R2, aurA F3 + aurA R3 were used to amplify three DNA fragments of *aurA* cDNA, and transformed into BJ5464-NpgA with *Nde*I/*Pml*I-digested pXW55 to create the plasmid pXM01 to express *aurA*. Primers aurB F + aurB R, aurC F + aurC R were used to amplify *aurB* and *aurC*, and co-transformed with *Nde*I/*Pme*I-digested pXW02, pXW06 backbones to give rise to plasmids pXM02 and pXM03, respectively. Primer aurD F + aurD R, aurG F + aurG R were used to amplify *aurD*, *aurG* cDNA and also co-transformed with *Nde*I/*Pml*I-digested pXW55 backbone to create plasmids pXM04 and pXM05, respectively. *aurD* expression cassette including *ADH2p* and *ADH2t* was amplified with primers 55 F + 55 R from the plasmid pXM04 and ligated to *Eco*RI-digested pXM03 to create the plasmid pXM06. All the cDNA was amplified from the wild type reverse transcript prepared as described below. Yeast competent cell preparation and transformation were demonstrated with *S.c.* EasyComp Transformation kit (Invitrogen) according to the manufacture's protocols. Yeast plasmids were prepared by Zymoprep™ Yeast Plasmid Miniprep kit (Zymo Research) and transformed into *E. coli* strain Topo10 for propagation.

To construct the plasmids for gene knock-out in *C. arbuscula* based on the split-marker strategy, the plasmid pAN7-1[4] was used as the template to amplify *hph* upstream fragment with primers hph-up F and hph-up R, and *hph* downstream fragment with primers hph-dn F and hph-dn R. Both fragments were digested with *Not*I/*Sac*II and *Sac*I/*Not*I, respectively, and ligated to T-vector pTA2 to generate plasmids pXM11 and pXM12. The upstream DNA fragments of *aurB*, *aurC*, *aurD* and *aurG* genes were amplified with primer pairs aurB-up F + aurB-up R, aurC-up F + aurC-up R, aurD-up F + aurD-up R and aurG-up F + aurG-up R. All the above PCR products were digested with *Bam*HI/*Not*I and ligated to the *Bam*HI/*Not*I site of pXM11 for plasmids pXM15, pXM17, pXM19 and pXM21, respectively. Meanwhile, the downstream DNA fragments of *aurB*, *aurC*, *aurD* and *aurG* genes were amplified with primer pairs aurB-dn F + aurB-dn R, aurC-dn F + aurC-dn R, aurD-dn F + aurD-dn R,and aurG-dn F + aurG-dn R, digested with *Not*I/*Hind*III and ligated to pXM12 to create plasmids pXM16, pXM18, pXM20 and pXM22, respectively.

Construction of *C. arbuscula* mutants

The entire *aurA* gene was deleted via homologous recombination based on the deletion cassette from the overlap PCR.[5] Primers aurA-up F1, aurA-up R1 and aurA-dn F1, aurA-dn R1 and hph F, hph R were used to amplify *aurA* upstream, downstream homologous regions from fungal genomic DNA and hygromycin resistance gene from plasmid pUCH2-8.[6] After purification of DNA fragments from agarose gel, primers aurA-up F1 and aurA-dn R1 were used to amplify the whole deletion cassette. The PCR product was gel purified and solubilized in STC buffer (1.2 M sorbitol, 10 mM $CaC1_2$, 10 mM Tris-HCl, pH 7.5). All other mutants were constructed in *C. arbuscula* based on the hygromycin split-marker strategy.[7] The upstream and downstream split-marker DNA for each gene were amplified with universal primer pair T7p + T3p with the high fidelity DNA polymerase. The PCR products were precipitated with ethanol and dissolved in STC buffer. Split-marker DNA was introduced into *C. arbuscula* by protoplast transformation. *C. arbuscula* spores were collected on PDA (Fluka) for 6 days at 25°C, and induced to young germ tubes in PDB (Fluka) at 25°C for 7 hours with 180 rpm agitation. Mycelia were collected, washed twice with the osmotic medium (1.2 M $MgCl_2$, 10 mM sodium phosphate, pH 5.8) and resuspended in the enzyme cocktail solution (3 mg/ml Lysing Enzymes, 3 mg/ml Yatalase in osmotic medium) at 30°C for 4 hours. After twice wash with STC buffer, protoplasts were gently mixed with DNA and incubated for 1 hour on ice. 300 μl of PEG 4000 solution (60% PEG 4000, 50 mM $CaC1_2$, 50 mM Tris-HCl, pH 7.5) was added to 100 μl of protoplast mixture, incubated at room temperature for 30 min and plated on the regeneration selection medium (PDA, 1.2 M sorbitol, 250 μg/ml hygromycin B). After incubation at room temperature for about 2 weeks, the transformants were inoculated on PDB medium with stationary incubation for about 1 week to confirm the genotype by PCR after preparation of the genomic DNA.

***C. arbuscula* genomic DNA preparation**

Mycelia collected from PDB medium were lyophilized on FreeZone Freeze Dry Systems (Labconco) over night and broken up with 1.5 ml microcentrifuge pestles. 700 μl of LETS buffer (10 mM Tris-HCl, pH 8.0, 20 mM EDTA, pH8.0, 0.5% SDS, 0.1 M LiCl) was added to solubilize the lysate, followed by two rounds of extraction with phenol : chloroform : isoamyl alcohol (25 : 24 : 1). Genomic DNA was precipitated from the supernatant with 2 volumes of ethanol. After wash with 70% ethanol and air dry, genomic DNA was solubilized in sterile water.

***C. arbuscula* RNA preparation and reverse transcription-PCR (RT-PCR)**

Mycelia of *C. arbuscula* were inoculated in PDB medium for stationary culture at 25°C for 7 days, and collected for lyophilization. The mycelia was grounded after freezing with liquid nitrogen, and solubilized in Trizol (Invitrogen). 1/5 volume of chloroform was added, vortex and centrifuged at 15000 rpm for 15 min. The supernatant was extracted once again with chloroform. RNA was precipitated from the supernatant with ethanol and resuspended in RNase-free water. Genomic DNA was further removed by digestion with RNase-free DNase I (Ambion). RNA was purified by acid phenol (Ambion) extraction and ethanol precipitation. RNA integrity was confirmed by electrophoresis on the TBE (Tris-boric acid-EDTA) agarose gel, and the concentration was determined by Nanodrop (Thermo Scientific). Reverse transcript was prepared from 500 ng of total RNA by SuperScript® II Reverse Transcriptase (Invitrogen) with random primers as described by the manufacture. PCR was performed with Phusion® High-Fidelity DNA Polymerase (New England Biolabs) in the presence of 25 ng of reverse transcribed RNA. Primers were listed in Table S5.

Yeast reconstitution of aurovertin biosynthesis pathway

Saccharomyces cerevisiae strain BJ5464-NpgA was transformed or co-transformed with appropriate plasmids as described in Table S4. For polyketide synthase product, yeast cells containing plasmid pXM01 were initially cultured in the SD uracil-drop out medium overnight and transferred to 50 ml of liquid YPD medium, with/without 20 mM sodium acetate or 20 mM sodium propionate, for additional 4-day culture. For other yeast strains with co-expression of various enzymes, yeast cells were also initially cultured in the appropriate SD-drop out medium overnight and transferred to 50 ml of liquid YPD medium with 20 mM sodium propionate for further 4 days. Samples about equal to 0.5 ml of culture were loaded for LC/MS analysis.

Intermediate feeding assays

For feeding of *C. arbuscula* strains with compounds (solubilized in DMSO), spores of *ΔaurA* or *ΔaurC* were inoculated in 10 ml of PDB together with 10 μg/ml compound **2**, **9** or **10** and further cultured for 7 days at 25 °C. The mycelia and medium were extracted for LC/MS analysis. For feeding assays of yeast, BJ5464-NpgA with/without plasmid pXM05 (expressing *aurG*) were inoculated in the appropriate SD-drop out medium overnight, and further cultured in 50 ml of YPD for 3 days. Then the cultures were concentrated to 3 ml and 10 μg/ml compound **4** (final concentration) was added for further culture for 2 days, followed by organic extraction and LC/MS analysis.

LC/MS analysis

C. arbuscula and *S. cerevisiae* cells were extracted with methanol : acetate ethyl (10 : 90). After brief centrifugation, the supernatant organic phase was dried by speed vacuum and solubilized in methanol for LC/MS loading. All LC-MS analyses were performed on a Shimadzu 2020 EVLC-MS (Phenomenex® Luna, 5μ, 2.0 × 100 mm, C18 column) using positive and negative mode electrospray ionization with a linear gradient of 5–95% MeCN-H_2O in 15 minutes followed by 95% MeCN for 5 minutes with a flow rate of 0.3 mL/min.

Compounds purification

Chemicals and chemical analysis

All solvents and other chemicals used were of analytical grade. All LC-MS analyses were performed on a Shimadzu 2020 EVLC-MS (Phenomenex® Luna, 5μ, 2.0 × 100 mm, C18 column) using positive and negative mode electrospray ionization with a linear gradient of 5–95%

MeCN-H_2O in 15 minutes followed by 95% MeCN for 5 minutes with a flow rate of 0.3 mL/min. ^1H, ^{13}C and 2D NMR spectra were obtained on Bruker AV500 spectrometer with a 5 mm dual cryoprobe or a Bruker DRX500 spectrometer with a 5 mm broadband probe at the UCLA Molecular Instrumentation Center.

Purification of aurovertin E (4) from *C. arbuscula ΔaurG* mutant.

The *Δaur*G mutant was cultivated on PDB (2 L) at 25°C for 10 d. The culture was filtered through cheesecloth. The mycelium was extracted with acetone for three times. The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (500 mL) was extracted with $CHCl_3$ (3 × 400 mL) and concentrated *in vacuo*. The crude products were purified by a Silica gel column (RediSep®, 40 g Flash Column, 8:2→6:4 hexane/acetone gradient) to afford aurovertin E (**4**) (33 mg). All spectral data for **4** (Table S8, Figure S22-S23) was consistent with that in the literature.[8]

Purification of aurovertins 1-6 and 9 from *C. arbuscula*.

C. arbuscula wild type strain was cultivated on PDB (6 L) at 25 °C for 10 d. The culture was filtered through cheesecloth. The mycelium was extracted with acetone for three times. The combined extracts were evaporated to dryness under reduced pressure to afford the residue (15.6 g).The residue was dissolved in H_2O (1 L) to form a suspension, which was then extracted with $CHCl_3$ (4 × 500 mL). The combined organics were dried over MgSO4 and concentrated *in vacuo*. The crude products (6.0 g) were separated by a RP-18 column (Redi*Sep* Rf Gold C18 Column, 20–40μ, 86 g, MeCN-H_2O 2:8→5:5) to afford four fractions (Fr.1–4). Fr.1 was purified by flash chromatography (MeCN-H_2O 1:9→3:7) to provide aurovertins D (**3**) (33 mg) and E (**4**) (13 mg). Fr.2 was further purified on a semi-preparative RP-18 HPLC column (Luna®, ODS-3, 5 *μ*, 250 × 10 mm) eluted by MeCN-H_2O (45:55→55:45) with flowrate of 3.5 mL/min to give aurovertin J (**5**) (1.1 mg, *t*R:15.9 min) and a subfraction (*t*R: 15.6–16.2 min) containing two compounds with the molecular weight 402. The two compounds can be separated by semi-preparative HPLC column (Luna®, silica gel, 5 *μ*m, 250 × 10 mm) eluted by hexane-acetone (75:25→65:35) with flow rate of 5.0 mL/min (peak1:*t*R:17.6 min; peak2:*t*R:22.1 min, Figure S10a). After separation, normal phase HPLC and LC-MS analysis all showed each peaks contained two same compounds with MW 402 (Figure S10). These showed that the two compounds with MW 402 from wild type can be interchanged quickly. Finally, the two compounds (total 1.3 mg) were checked by mixture NMR, and one of dominant compounds was determined as **9**. Aurovertin B (**2**) (110 mg) was obtained from Fr. 3 by recrystallization with MeCN-H_{2O} (9:1). Fr.4 was applied to flash chromatography (MeCN-H_{2O} 3:7→5:5) to give aurovertins A (**1**) (5.0 mg) and M (**6**) (11.0 mg). All spectral data (Table S7-S9, Figure S16-S27) for **1-6** were consistent with those in the literatures.[8-12] The structure of **9** was determined by 1D and 2D NMR (Table S10, Figure S36-S41).

Purification of compound 10 from yeast cells.

The yeast strain BJ5464-NpgA + AurABC were cultured in YPD (20 L) supplemented with 20 mM sodium propionate at 30°C for 5 d. The culture was filtered through cheesecloth. The yeast cells were extracted with acetone for three times. The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (1200 mL) was extracted with $CHCl_3$ (3 × 600 mL) and concentrated *in vacuo*. The crude products were purified by a RP-18 column (Redi*Sep* Rf Gold C18 Column, 20–40μ, 86 g, MeCN-H_2O 2:8→5:5) to afford four fractions (Fr.1−5).The Fr.3 was separated by a Sephadex LH-20 column (35 × 1.2 cm, MeOH) followed by purification on a semi-preparative RP-18 HPLC column (Luna®, ODS-3, 5 *μ*m, 250 × 10 mm) eluted by MeCN-H_2O

(15:85→40:60) with flow rate of 3.5 mL/min to yield **10** (1.3 mg, *t*R:15.9 min).The structure of **10** was determined by 1D and 2D NMR (Table S10, Figure S42-S47).

Purification of compound 7 from yeast

The yeast cells expressing AurA were cultured in YPD (10 L) with 20 mM sodium propionate at 30°C for 5 d. The culture was filtered through cheesecloth. The yeast cells were extracted with acetone for three times. The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (500 mL) was extracted with $CHCl_3$ (3 × 400 mL) and concentrated *in vacuo*. The crude products were purified by a Silica gel column (RediSep®, 40 g Flash Column, 9:1→7:3 hexane:acetone gradient) to afford compound **7** (31 mg). The structure of **7** was determined by NMR (Table S11, Figure S28-S29).

Purification of compound 8 from yeast.

The yeast cells expressing AurA and AurB were cultured in YPD (10 L) with 20 mM sodium propionate at 30°C for 5 d. The culture was filtered through cheesecloth. The yeast cells were extracted with acetone for three times. The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (500 mL) was extracted with $CHCl_3$ (3 × 400 mL) and concentrated *in vacuo*. The crude products were purified by a Silica gel column (RediSep®, 40 g Flash Column, 9:1→8:2 hexane:acetone gradient) to afford compound **8** (28 mg). The structure of **8** was determined by 1D and 2D NMR (Table S11, Figure S30-S35).

Computational details

Quantum mechanical calculations were performed using Gaussian 09 (Revision D.01).[13] All geometries were optimized using M06-2X,[14] within the IEFPCM model (water),[15] and the 6-31G(d) basis set. Single point energies were calculated using M06-2X,[14] within the IEFPCM model (water),[15] and the 6-311++G(d,p) basis set. The resulting energies were used to correct the gas phase energies obtained from the M06-2X optimizations.[16-18] Previous computational work on epoxide-opening cyclization reactions with similar methods provided results in accord with experiment.[19] Computed structures are illustrated with CYLView.[20]

Supplementary Tables

Table S1. Deduced gene functions within the *aur* gene cluster.

Gene name	Deduced protein function	Identity to *M. anisopliae* homolog
aurA	Polyketide synthase with KS-AT-DH-MT-KR-ACP modules	75%
aurB	SAM-dependent methyl-transferase	76%
aurC	flavin-dependent monooxygenase	77%
aurD	α/β hydrolase	72%
aurE	SnoaL-like protein	45%
aurF	Putative DNA-binding protein	45%
aurG	*O*-acyl-transferase	No homolog

Table S2. Accession numbers of all *aur* homologs from *C. arbuscula*, *M. anisopliae* and *A. terreus*.

Gene from *C. arbuscula*	*C. arbuscula*	Homologs in *M. anisopliae*	Homologs in *A. terreus* NIH2624
aurA	KT581574	EFY96172	XP_001218239
aurB	KT581575	EFY96171	XP_001218240
aurC	KT581576	EFY96168	XP_001218242
aurD	KT581577	EFY96169	XP_001218241
aurE	KT581578	No accession number[a]	XP_001218243
aurF	KT581579	EFY96170	No homolog nearby
aurG	KT581580	No homolog nearby	No homolog nearby

[a]No *C. arbuscula* AurE homolog in *M. anisopliae* was predicted near the gene cluster in the database. But the homolog was identified after BLAST was run against *Aspergillus nidulans* in Softberry (linux1.softberry.com/) with the query of *M. anisopliae* strain E6 contig00020 (accession number JNNZ01000020) containing the putative *aur* gene cluster.

Table S3. *Calcarisporium arbuscula* strains used in this study.

Strain	Genotype	Reference
Wild type	*Calcarisporium arbuscula* wild type, NRRL 3705, ATCC® 46034™	ATCC
ΔaurA	*ΔaurA::hph*, the entire *aurA* was replaced with *hph*	This study
ΔaurB	*ΔaurB::hph*, the entire *aurB* was replaced with *hph*	This study
ΔaurC	*ΔaurC::hph*, the entire *aurC* was replaced with *hph*	This study
ΔaurD	*ΔaurD::hph*, the entire *aurD* was replaced with *hph*	This study
ΔaurG	*ΔaurG::hph*, the entire *aurG* was replaced with *hph*	This study
ΔaurF	*ΔaurF::hph*, the entire *aurF* was replaced with *hph*	This study

Table S4. *S. cerevisiae* strains for aurovertin biosynthetic enzyme expression

Genotype	Description
Yeast + *aurA*	*S. cerevisiae* BJ5464-NpgA + pXM01
Yeast + *aurA* + *aurB*	*S. cerevisiae* BJ5464-NpgA + pXM01 + pXM02
Yeast + *aurA* + *aurC*	*S. cerevisiae* BJ5464-NpgA + pXM01 + pXM03
Yeast + *aurA* + *aurB* + *aurC*	*S. cerevisiae* BJ5464-NpgA + pXM01 + pXM02 + pXM03
Yeast + *aurA* + *aurB* + *aurC* + *aurD*	*S. cerevisiae* BJ5464-NpgA + pXM01 + pXM02 + pXM06
Yeast + *aurG*	*S. cerevisiae* BJ5464-NpgA + pXM05

Table S5. Primers used in this study.

Name	Sequence
aurA-up F1	GGGGAGATTAAAGGTGAGGC
aurA-up R1	GATGAGCGCATTGTTAGATTTCATACACGGTGCCTGGAGAGAAATGTCGATGAGAAC
aurA-dn F1	CATGATGTCAGGCCATTTTCATATGGCAATGCGCAGGTTGTTAGGTGGTCGGTCAAC
aurA-dn R1	GAAGGAGTGGGTTGATGAAG
hph F	GCACCGTGTATGAAATCTAAC
hph R	ACCTGCGCATTGCCATATG
hph-up F	ACCTGGCGGCCGCTACAACGACCATCAAAGTC
hph-up R	ACTGACCGCGGTACCGTCTGCTGCTCCATACAA
hph-dn F	CAGGGAGCTCGGTACCTCGGAGGGCGAAGAATC
hph-dn R	GAATGCGGCCGCAGGATTACCTCTAAACAAGTG
aurB-up F	CGGTACGACCTTGTCTAATC
aurB-up R	GAATAGCGGCCGCTTGAGAGTGAGGTTGTTG
aurB-dn F	GAATAGCGGCCGCTGGACAAAGACATCATAAC
aurB-dn R	TATTGAAGCTTGAATGAACTACAGTAAGAG
aurC-up R	GAATAGCGGCCGCTCCAGTTGGTCCTTCTGAG
aurC-dn F	GATGTGCGGCCGCAGCCTTGCCTATCAACTATG
aurD-up F	TATTAGGATCCACAGATTACGAGAATGAAG
aurD-up R	GAATAGCGGCCGCTTTGGGAGAGGGAAGAAA
aurD-dn F	TATTGAAGCTTAGTGGTGAGGAGATGAGG
aurD-dn R	GAATAGCGGCCGCTCTTCCAAGACGATGTTG
aurG-up F	TATTAGGATCCGTGCCATCAGACATTAC
aurG-up R	GAATAGCGGCCGCAAACGACGAGGACCAGC
aurG-dn F	CTCTAGCGGCCGCTGTGGTTTCTCTGGACATC
aurG-dn R	TATCTAAGCTTTGGTTCACGCACTCATTC
aurA F1	ATGGCTAGCGATTATAAGGATGATGATGATAAGACTAGTCCACCTAACAACATGACACC
aurA R1	TCGGAATAGCGTTTCAATACC
aurA F2	GGAAAATACCGAAGGAGAGTG
aurA R2	CCATGTCTGCTAAACACAATG
aurA F3	TAACAGACAAGACCTCGTATC
aurA R3	ATTTAAATTAGTGATGGTGATGGTGATGCACGTGCCTCTTCACGTTTGGAATGAG
aurB F	ATCAACTATCAACTATTAACTATATCGTAATACCATATGATCATGACCAAAGAATC
aurB R	GATAATGAAAACTATAAATCGTGAAGGCATGTTTAAACCTCACCTATCAATGTCTGG
aurC F	ATCAACTATCAACTATTAACTATATCGTAATACCATATGGGAGCGTATTCATTC
aurC R	TTGATAATGGAAACTATAAATCGTGAAGGCATGTTTAAACTCAGAAGGACCAACTG
aurD F	ATGGCTAGCGATTATAAGGATGATGATGATAAGACTAGTATGGCCTGGTACGACGAG
aurD R	ATTTAAATTAGTGATGGTGATGGTGATGCACGTGTTTTGTCTTTTCTTTAGCAC
aurG F	ATGGCTAGCGATTATAAGGATGATGATGATAAGACTAGTCTCTGGCTGGTGCTGG
aurG R	ATTTAAATTAGTGATGGTGATGGTGATGCACGTGTTGGACATGGCGACTCTG
55 F	TATTAGAATTCGTAGGGGCAAACAAACGG
55 R	CGAAAGGGGGATGTGCTG
T7p	ATACGACTCACTATAGGGC

T3p	TAACCCTCACTAAAGG

Table S6. Plasmids used in this study.

Plasmid name	Description	Reference
pXW02	*E. coli*-yeast shuttle expression vector, *ADH2p*, 2μ, *LEU2* marker	[3]
pXW06	*E. coli*-yeast shuttle expression vector, *ADH2p*, 2μ, *TRP1* marker	[3]
pXW55	*E. coli*-yeast shuttle expression vector, *ADH2p*, 2μ, *URA3* marker	[3]
pXM01	*aurA* cDNA cloned in *Nde*I/*Pml*I-digested pXW55	This study
pXM02	*aurB* cDNA cloned in *Nde*I/*Pme*I-digested pXW02	This study
pXM03	*aurC* cDNA cloned in *Nde*I/*Pme*I-digested pXW06	This study
pXM04	*aurD* cDNA cloned in *Nde*I/*Pml*I-digested pXW55	This study
pXM05	*aurG* cDNA cloned in *Nde*I/*Pml*I-digested pXW55	This study
pXM06	*aurD* expression cassette ligated to pXM03 at *Eco*RI site	This study
pTA2	*E. coli* T-vector	Toyobo
pAN7-1	*E. coli* plasmid vector with hygromycin resistance gene *hph*	[4]
pXM11	*hph* upstream 1.9 kb fragment cloned in *Not*I/*Sac*II site of pTA2	This study
pXM12	*hph* downstream 1.8 kb fragment cloned in *Sac*I/*Not*I site of pTA2	This study
pXM15	*aurB* upstream 1.8 kb cloned in *Bam*HI/*Not*I site of pXM11	This study
pXM16	*aurB* downstream 4.2 kb cloned in *Not*I/*Hin*dIII site of pXM12	This study
pXM17	*aurC* upstream 3.6 kb cloned in *Bam*HI/*Not*I site of pXM11	This study
pXM18	*aurC* downstream 1.5 kb cloned in *Not*I/*Hin*dIII site of pXM12	This study
pXM19	*aurD* upstream cloned in *Bam*HI/*Not*I site of pXM11	This study
pXM20	*aurD* upstream cloned in *Bam*HI/*Not*I site of pXM12	This study
pXM21	*aurG* upstream 1.2 kb cloned in *Bam*HI/*Not*I site of pXM11	This study
pXM22	*aurG* downstream 2.5 kb cloned in *Not*I/*Hin*dIII site of pXM12	This study

Table S7. NMR spectroscopic data of compounds **2**–**3** in $CDCl_3$

	2		3	
Position	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1	1.07 (3H, t, 7.5)	11.8	1.37 (3H, t, 6.5)	22.9
2	1.67 (2H, m)	20.1	4.23 (1H, m)	65.3
3	3.90 (1H, dd, 8.5, 4.0)	85.5	3.76 (1H, d, 7.5)	86.8
4	-	82.7	-	83.1
5	4.79 (1H, s)	80.5	4.78 (1H, s)	81.1
6	-	83.4	-	83.5
7	3.28 (1H, t, 7.5)	76.4	3.31 (1H, t, 8.5)	76.3
8	4.13 (1H, t, 7.5)	78.0	4.14 (1H, t, 8.0)	78.0
9	5.91 (1H, dd, 15.0, 6.5)	134.1	5.90 (1H, dd, 15.0, 7.5)	133.9
10	6.43 (1H, m)	131.7	6.44 (1H, m)	131.7
11	6.48 (1H, m)	137.0	6.47 (1H, m)	136.9
12	6.38 (1H, m)	132.1	6.38 (1H, m)	132.2
13	7.15 (1H, dd, 15.0, 11.5)	135.7	7.16 (1H, dd, 16.0, 11.0)	135.6
14	6.34 (1H, d, 15.0)	119.6	6.33 (1H, d, 15.0)	119.7
15	-	154.3	-	154.3
16	-	108.1	-	108.2
17	-	169.9	-	169.9
18	5.48 (1H, s)	88.8	5.48 (1H, s)	88.8
19	-	163.7	-	163.8
20	1.18 (3H, s)	16.4	1.37 (3H, s)	17.7
21	1.25 (3H, s)	15.1	1.24 (3H, s)	15.0
22	1.96 (3H, s)	8.9	1.95 (3H, s)	8.9
OMe	3.80 (3H, s)	56.4	3.82 (3H, s)	56.2
Ac	2.15 (3H, s)	20.8	2.15 (3H, s)	20.8
Ac	-	170.6	-	170.6

Table S8. NMR spectroscopic data of compounds **4** and **1** in $CDCl_3$

	4		**1**	
Position	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1	1.06 (3H, t, 7.5)	11.9	1.08 (3H, t, 6.5)	11.8
2	1.65 (2H, m)	20.3	1.67 (1H, m)	20.0
3	3.96 (1H, dd, 8.5, 4.5)	84.8	3.92 (1H, dd, 8.5, 4.5)	85.5
4	-	83.7	-	82.6
5	3.45 (1H, s)	80.4	4.89 (1H, s)	80.6
6	-	84.1	-	83.0
7	3.15 (1H,brs)	76.4	4.79 (1H, d, 8.5)	76.3
8	4.12 (1H, t, 7.5)	77.9	4.32 (1H, t, 7.5)	78.0
9	5.90 (1H, dd, 14.0, 6.0)	135.0	5.73 (1H, dd, 15.0, 7.0)	132.9
10	6.39 (1H, m)	131.5	6.41 (1H, m)	132.4
11	6.45 (1H, m)	137.4	6.45 (1H, m)	136.6
12	6.35 (1H, m)	131.9	6.33 (1H, m)	132.5
13	7.14 (1H, dd, 15.0, 10.5)	135.8	7.15 (1H, dd, 15.0, 11.0)	135.4
14	6.32 (1H, d, 15.0)	119.4	6.31 (1H, d, 15.0)	119.9
15	-	154.4	-	154.2
16	-	108.2	-	108.2
17	-	170.8	-	169.6
18	5.50 (1H, s)	88.7	5.48 (1H, s)	89.0
19	-	164.0	-	163.6
20	1.27 (3H, s)	16.5	1.12 (3H, s)	16.3
21	1.36 (3H, s)	14.6	1.18 (3H, s)	15.1
22	1.96 (3H, s)	8.9	1.95 (3H, s)	8.9
OMe	3.82 (3H, s)	56.3	3.82 (3H, s)	56.2
5-Ac			2.12 (3H, s)	20.8
5-Ac			-	170.5
7-Ac			2.04 (3H, s)	20.8
7-Ac			-	169.8

Table S9. NMR spectroscopic data of compounds **5**, **6** in $CDCl_3$

	5		**6**	
Position	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
1	1.06 (3H, t, 7.5)	11.8	1.08 (3H, t, 7.5)	11.8
2	1.70 (2H, m)	20.2	1.66 (2H, m)	20.2
3	3.90 (1H, dd, 8.5, 4.5)	85.5	3.90 (1H, dd, 8.0, 5.0)	85.6
4	-	82.7	-	82.8
5	4.79 (1H, s)	80.5	4.81 (1H, s)	80.3
6	-	83.4	-	83.4
7	3.24 (1H, t, 7.5)	76.4	3.29 (1H, t, 8.0)	76.4
8	4.11 (1H, t, 7.5)	77.9	4.12 (1H, t, 8.0)	78.0
9	5.92 (1H, dd, 15.0, 6.5)	134.5	5.92 (1H, dd, 15.0, 8.0)	134.1
10	6.43 (1H, m)	131.5	6.44 (1H, m)	131.7
11	6.48 (1H, m)	137.7	6.49 (1H, m)	137.0
12	6.38 (1H, m)	131.5	6.39 (1H, m)	132.1
13	7.13 (1H, dd, 15.0, 11.5)	136.0	7.16 (1H, dd, 16.0, 10.0)	135.7
14	6.02 (1H, d, 15.0)	122.0	6.33 (1H, d, 15.0)	119.6
15	-	158.6	-	154.3
16	-	101.0	-	108.1
17	-	169.8	-	170.6
18	5.48 (1H, s)	88.7	5.50 (1H, s)	88.9
19	-	164.0	-	163.6
20	1.16 (3H, s)	16.5	1.18 (3H, s)	16.4
21	1.23 (3H, s)	15.1	1.27 (3H, s)	15.0
22	-	-	1.97 (3H, s)	8.9
OMe	3.79 (3H, s)	55.9	3.82 (3H, s)	56.2
Ac	2.13 (3H, s)	20.8		
Ac	-	171.0		
Pr			1.19 (3H, t, 7.5)	9.3
Pr			2.44 (2H, q, 7.5)	27.6
Pr			-	173.3

Table S10. NMR spectroscopic data of compounds **7** and **8**

	8[a]		**7**[b]	
Position	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
1	1.00 (3H, t, 8.0)	14.0	0.97 (3H, t, 7.5)	13.9
2	2.13 (2H, m)	21.7	2.10 (2H, m)	21.2
3	5.44 (1H, t, 7.5)	135.1	5.45 (1H, t, 7.5)	134.7
4	-	132.3	-	132.2
5	6.00 (1H, s)	138.0	6.03 (1H, s)	138.1
6	-	132.9	-	132.7
7	6.38 (1H, d, 15.5)	141.2	6.41 (1H, d, 15.0)	140.5
8	6.28 (1H, m)	127.2	6.48 (1H, m)	127.7
9	6.45 (1H, dd, 14.5, 10.5)	137.0	6.62 (1H, dd, 15.5, 11.5)	137.4
10	6.34 (1H, m)	130.8	6.50 (1H, m)	132.0
11	6.54 (1H, dd, 14.5, 12.0)	138.6	6.67 (1H, dd, 15.5, 12.0)	134.9
12	6.31 (1H, m)	131.3	6.44 (1H, m)	131.4
13	7.21 (1H, dd, 15.0, 11.5)	136.1	6.98 (1H, dd, 15.5, 10.5)	136.4
14	6.32 (1H, d, 15.0)	118.5	6.38 (1H, d, 15.0)	119.8
15	-	154.7	-	154.7
16	-	107.6	-	108.2
17	-	170.6	-	169.8
18	5.48 (1H, s)	88.6	5.36 (1H, s)	89.8
19	-	163.8	-	162.1
20	1.80 (3H, s)	16.8	1.78 (3H, s)	16.6
21	1.94 (3H, s)	13.9	1.89 (3H, s)	13.7
22	1.95 (3H, s)	8.9	1.90 (3H, s)	
OMe	3.82 (3H, s)	56.1		8.7

[a] measured in $CDCl_3$; [b] measured in DMSO-d_6;

Table S11. NMR spectroscopic data of compounds **9**, **10** in $CDCl_3$

	9		**10**	
Position	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1	1.05 (3H, t, 7.5)	11.6	1.10 (3H, t, 7.5)	11.3
2	1.56 (2H, m)	21.5	1.55 (2H, m)	21.3
3	3.68 (1H, dd, 8.0, 5.0)	85.0	3.54 (1H, m)	84.8
4	-	81.8	-	81.1
5	3.79 (1H, brs)	85.2	3.75 (1H, m)	86.6
6	-	83.5	-	83.7
7	5.97 (1H, d, 15.0)	142.0	5.85 (1H, d, 14.0)	135.9
8	6.46 (1H, m)	127.6	6.44 (1H, m)	129.5
9	6.32 (1H, m)	135.0	6.40 (1H, m)	134.9
10	6.31 (1H, m)	132.1	6.32 (1H, m)	131.9
11	6.48 (1H, m)	138.1	6.52 (1H, dd, 15.5, 11.5)	137.9
12	6.32 (1H, m)	133.0	6.37 (1H, m)	132.7
13	7.18 (1H, dd, 16.0, 11.0)	136.0	7.21 (1H, dd, 16.0, 11.0)	135.9
14	6.34 (1H, d, 15.0)	119.4	6.34 (1H, d, 15.0)	119.2
15	-	154.6	-	154.4
16	-	108.2	-	108.0
17	-	170.8	-	170.6
18	5.46 (1H, s)	88.8	5.49 (1H, s)	88.8
19	-	163.9	-	163.7
20	1.22 (3H, s)	18.4	1.21 (3H, s)	19.2
21	1.28 (3H, s)	21.5	1.46 (3H, s)	27.7
22	1.95 (3H, s)	9.1	1.96 (3H, s)	8.9
OMe	3.80 (3H, s)	56.4	3.82 (3H, s)	56.2

Supplementary Figures

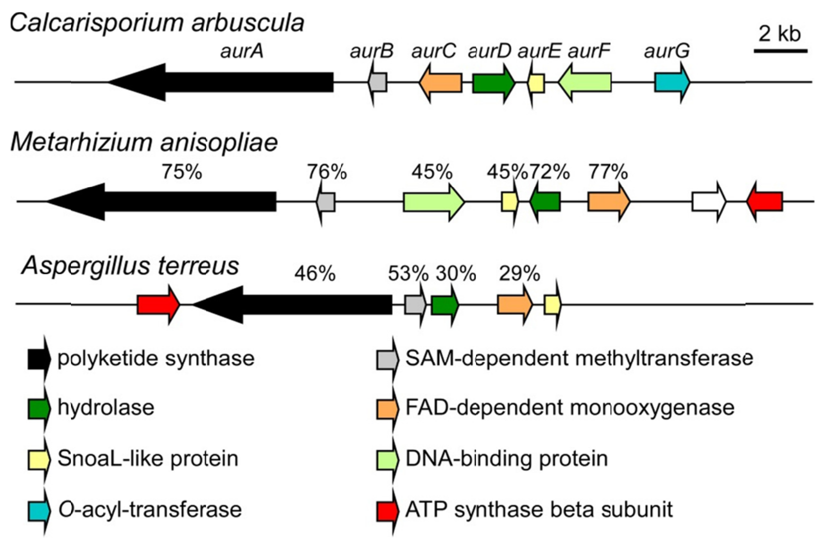


Figure S1. The homologous gene clusters among fungi producing aurovertins (*C. arbuscula* and *M. anisopliae*) and citreoviridin (*A. terreus*). The identity of each homolog to the *C. arbuscula* counterpart is shown.

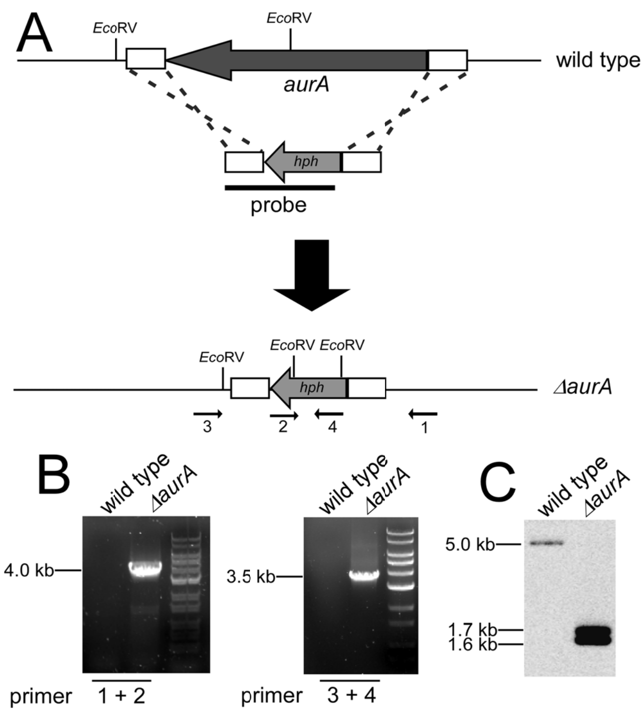


Figure S2. Deletion of the entire *aurA* gene in *C. arbuscula*. The genotype was confirmed by PCR and Southern blot.

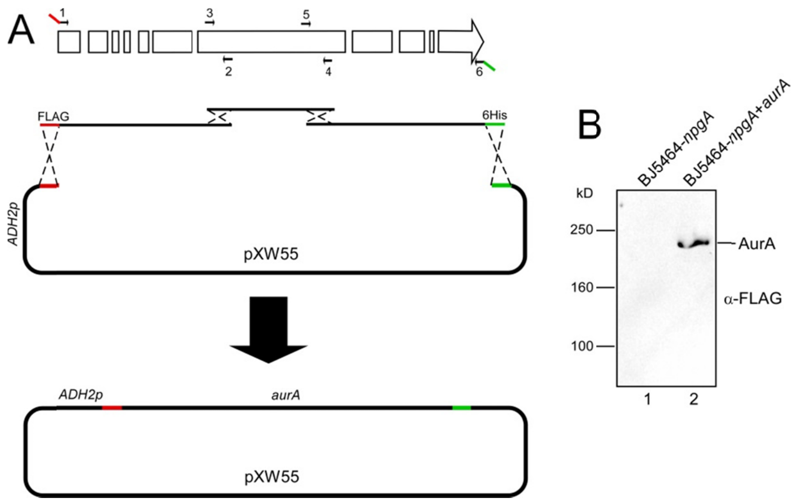


Figure S3. Heterologous expression of AurA in *S. cerevisiae* confirmed by Western blot.

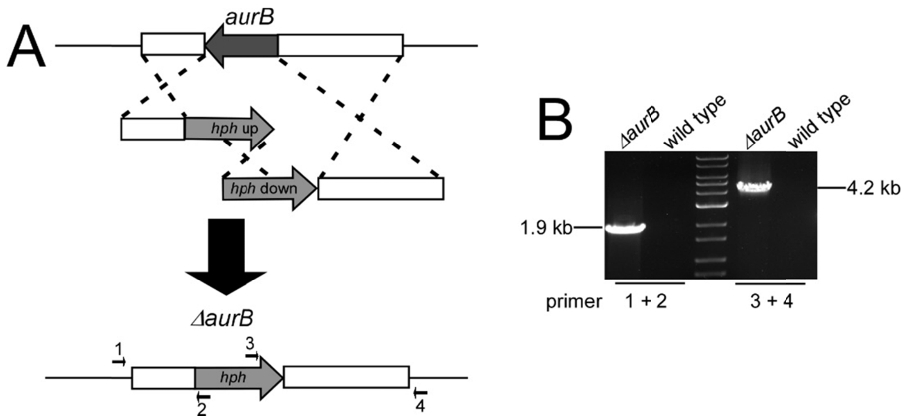


Figure S4. Deletion of *aurB* in *C. arbuscula*. The genotype was confirmed by PCR with primers as indicated.

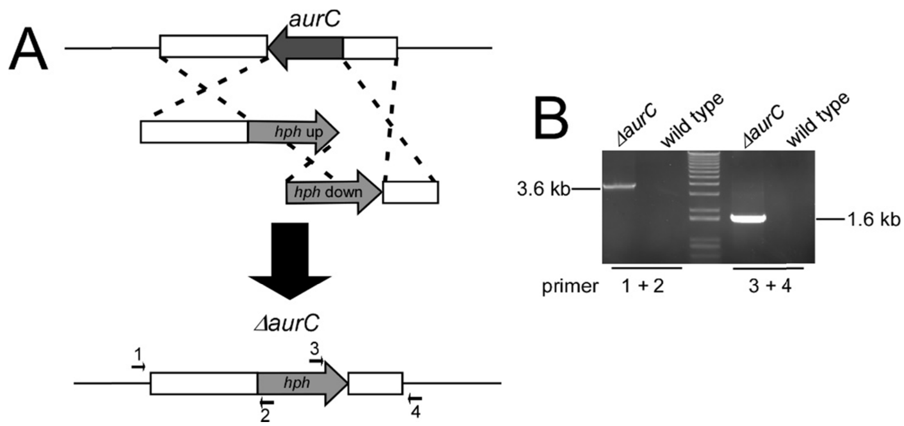


Figure S5. Deletion of *aurC* in *C. arbuscula*. The genotype was confirmed by PCR with primers as indicated.

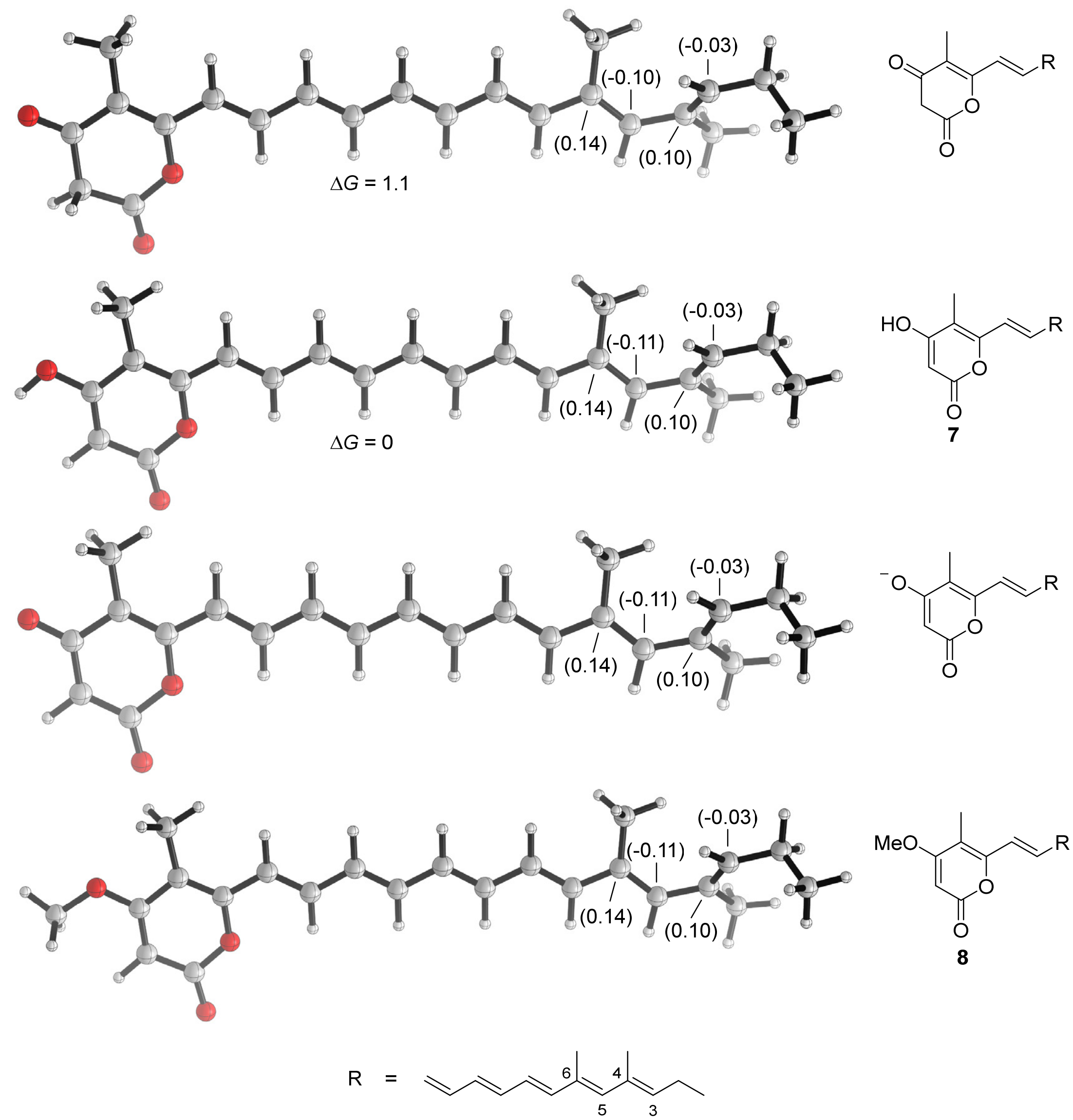


Figure S6. Lowest energy conformations of pyrone-polyene **7** and its alkoxide and β-ketoester forms prior to O-methylation, and that of **8**. M06-2X/6-311++G(d,p)–IEFPCM(water)//M06-2X/6-31G(d)–IEFPCM(water). All energies in kcal mol^{-1}. Mulliken atomic charges (with hydrogens summed into heavy atoms) in parentheses, calculated at the M06-2X/6-31G(d)–IEFPCM(water) level of theory.

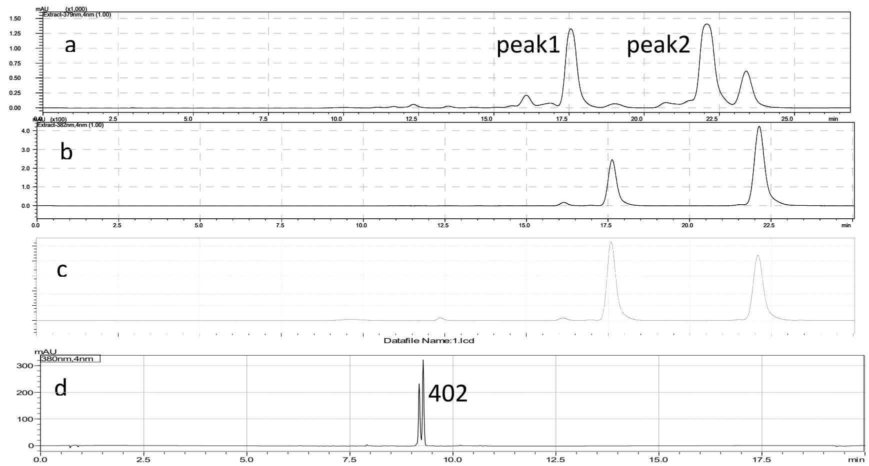


Figure S7. Compound **9** exists as an equilibrium of two forms**.** a: Semi-purified compound **9** from *C. arbuscula*; b: peak 1 checked by normal phase HPLC after purification; c: peak 2 checked by normal phase HPLC after purification; d: LC-MS spectrum *m/z* $[M+H]^+$ =403 ion after purification.

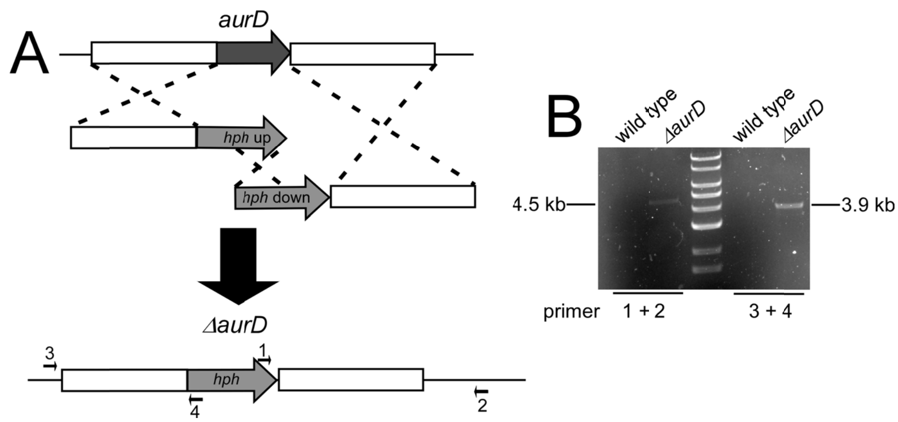


Figure S8. Deletion of *aurD* in *C. arbuscula*. The genotype was confirmed by PCR with primers as indicated.

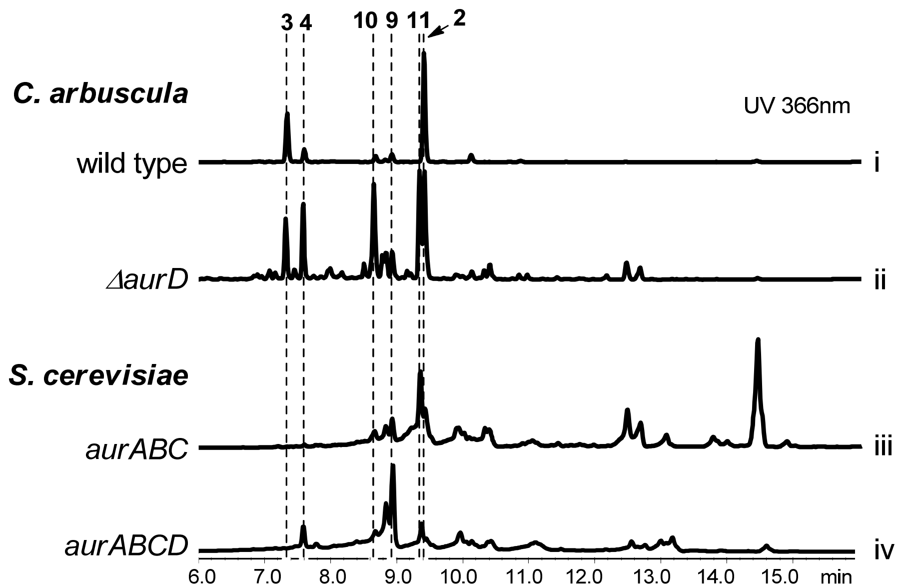


Figure S9. HPLC profile of *ΔaurD C. arbuscula* strain.

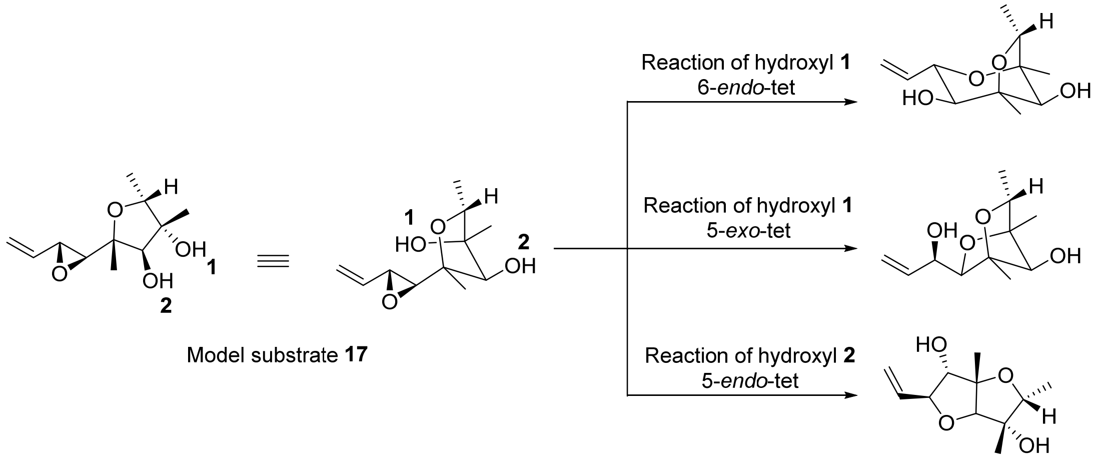


Figure S10. Possible epoxide-opening cyclization reactions with a model substrate.

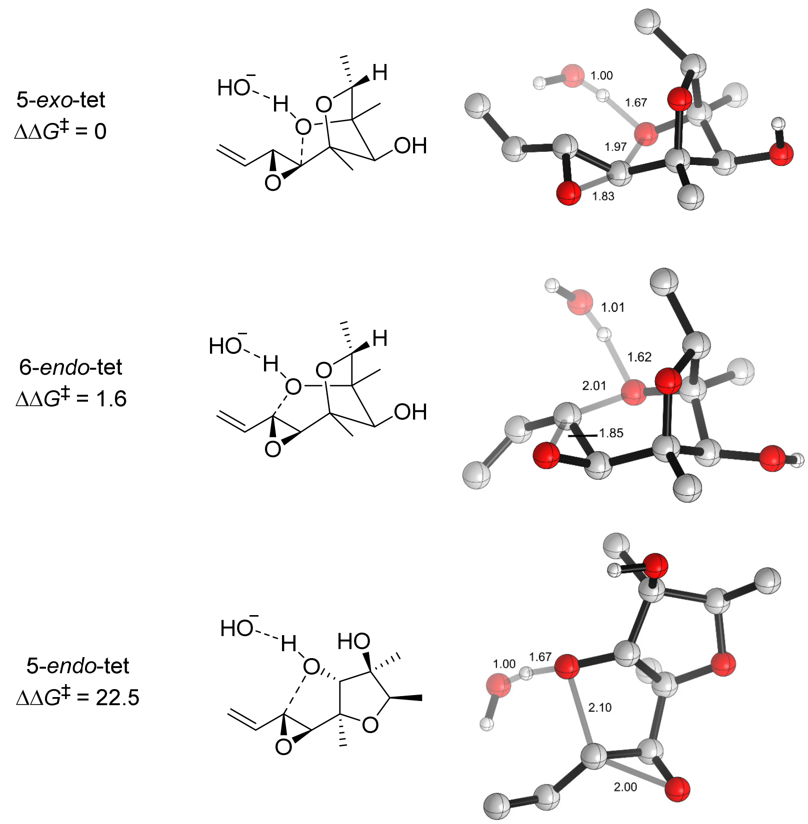
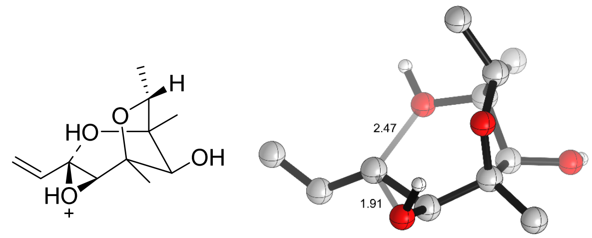
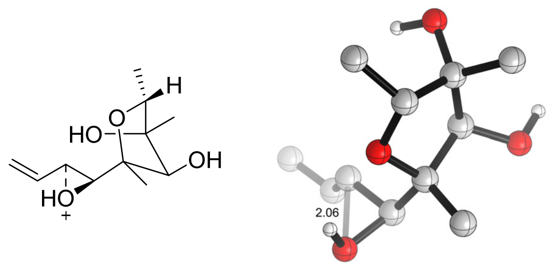


Figure S11. Base-catalyzed epoxide-opening cyclization reactions. M06-2X/6-311++G(d,p)–IEFPCM(water)//M06-2X/6-31G(d)–IEFPCM(water). Non-critical hydrogen atoms omitted for clarity. All energies in kcal mol^{-1}.

6-*endo*-tet
$\Delta\Delta G^{\ddagger}$ = 0



C-O bond cleavage
$\Delta\Delta G^{\ddagger}$ = 5.3



5-*exo*-tet
$\Delta\Delta G^{\ddagger}$ = 7.9

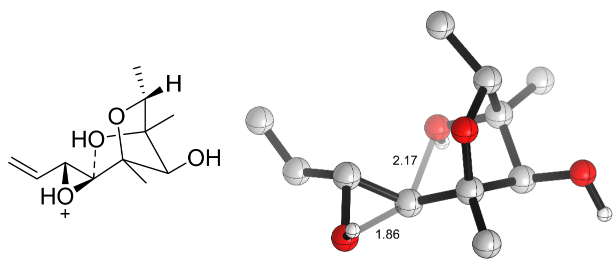


Figure S12. Acid-catalyzed epoxide-opening reactions. M06-2X/6-311++G(d,p)–IEFPCM(water)//M06-2X/6-31G(d)–IEFPCM(water). Non-critical hydrogen atoms omitted for clarity. All energies in kcal mol^{-1}.

6-*endo*-tet
$\Delta\Delta G^{\ddagger} = 0$

5-*exo*-tet
$\Delta\Delta G^{\ddagger} = 3.1$

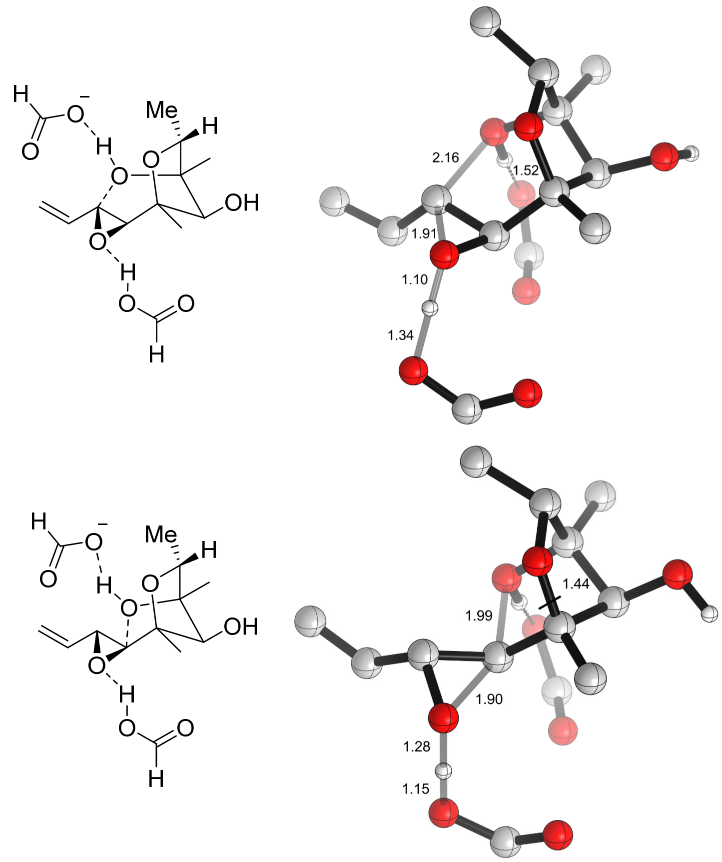


Figure S13. Simultaneous general acid/base catalysis in epoxide-opening cyclization reactions (formic acid and formate ion as acid and base respectively). M06-2X/6-311++G(d,p)–IEFPCM(water)//M06-2X/6-31G(d)–IEFPCM(water). Non-critical hydrogen atoms omitted for clarity. All energies in kcal mol^{-1}.

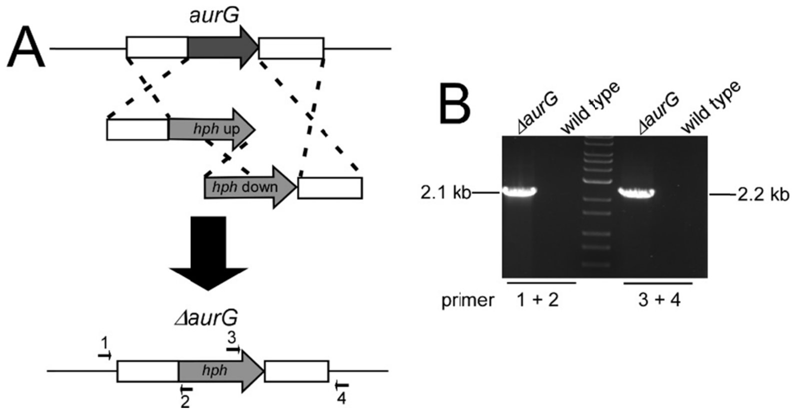


Figure S14. Deletion of *aurG* in *C. arbuscula*. The genotype was confirmed by PCR with primers as indicated.

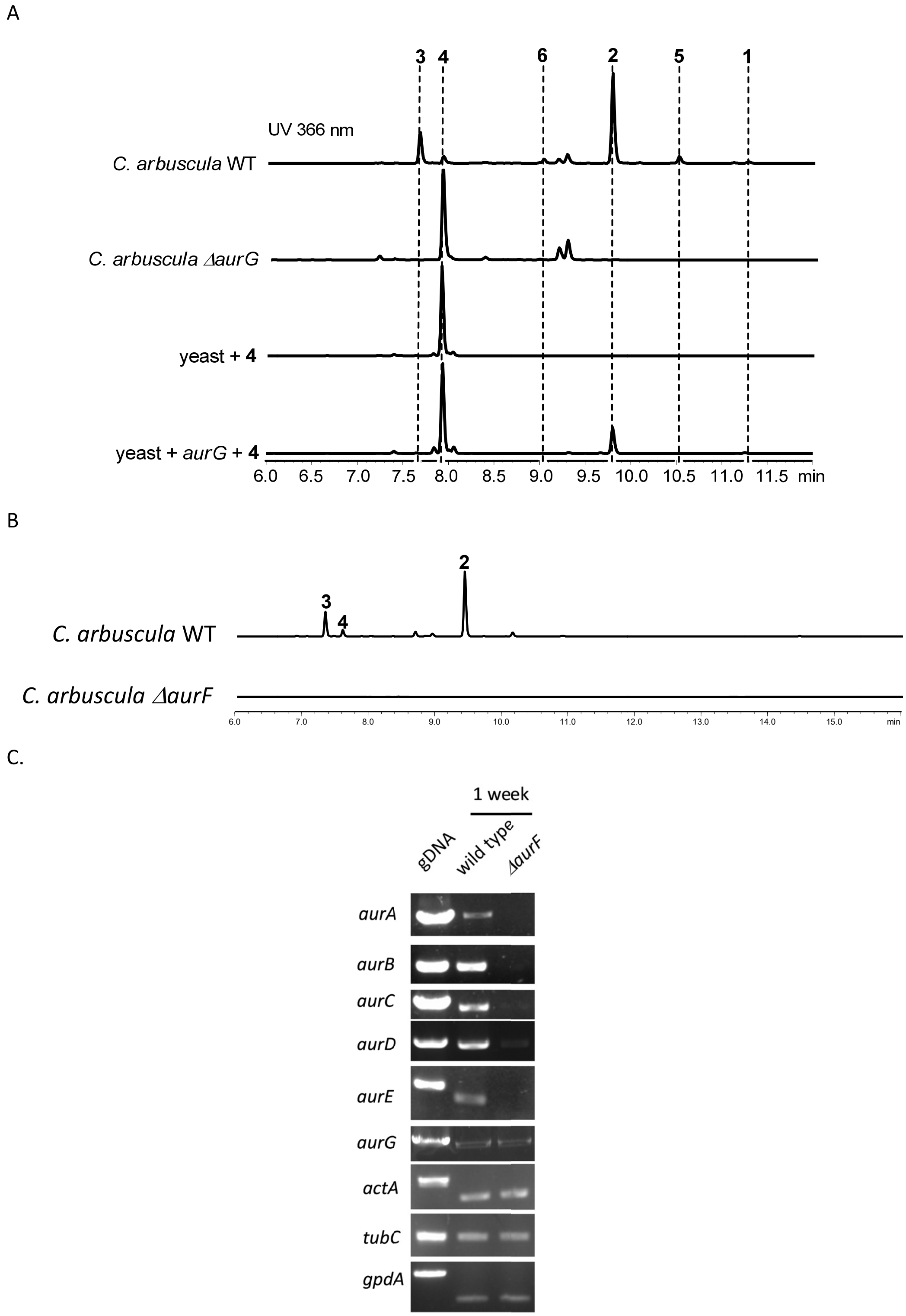


Figure S15. Functional verifications of AurG and AurF. (A) AurG is an *O*-acyltransferase involving in transformation of **4** to **2**. (B) AurF is the likely transcriptional factor that regulates the expression of the entire cluster. Knockout of *aurF* eliminates production of aurovertin production. (C) Transcription analysis of *aur* genes and housekeeping genes in wild type and *ΔaurF* mutant.

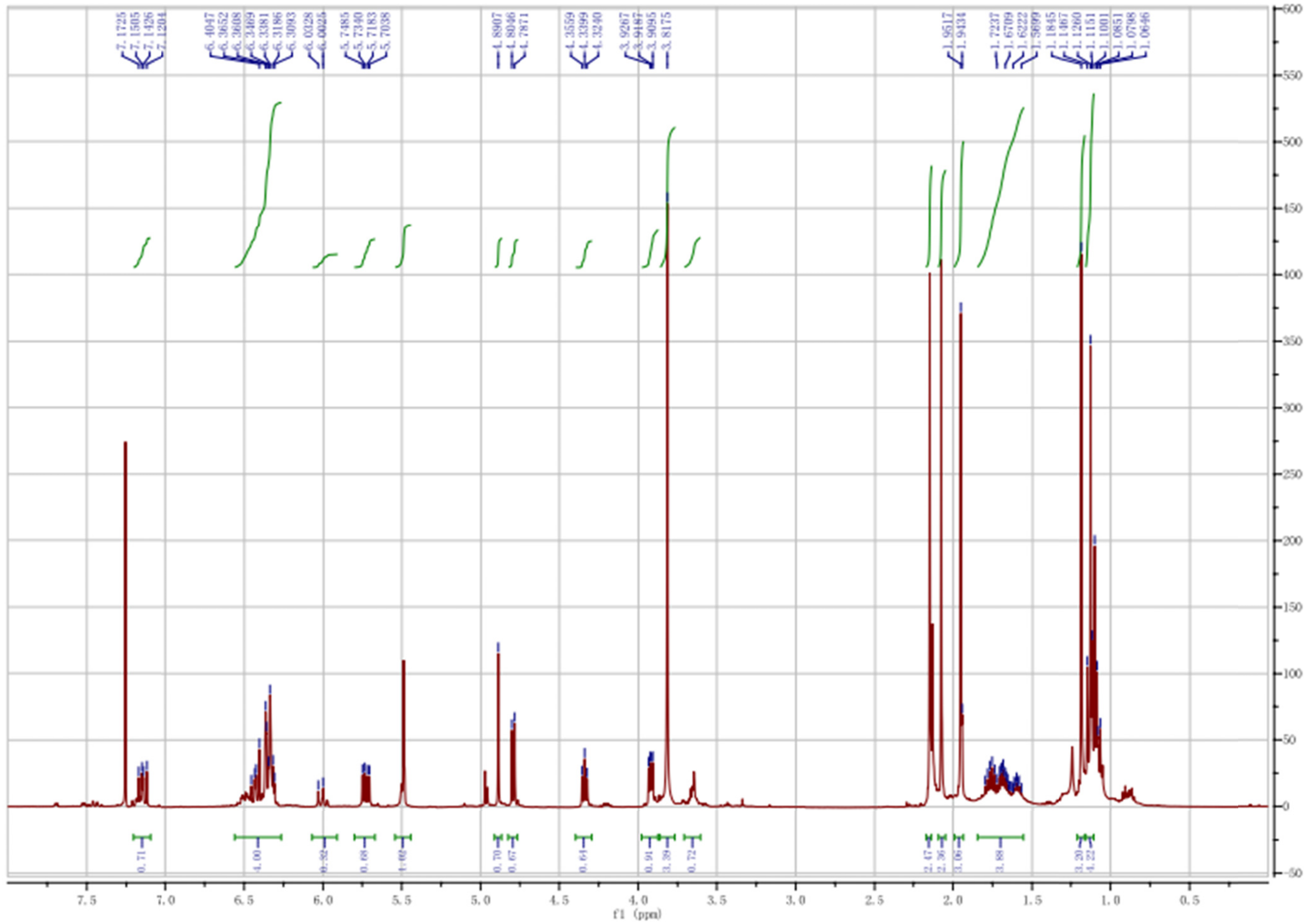


Figure S16. ^{1}H NMR of aurovertin A (**1**) in $CDCl_3$ (500 MHz)

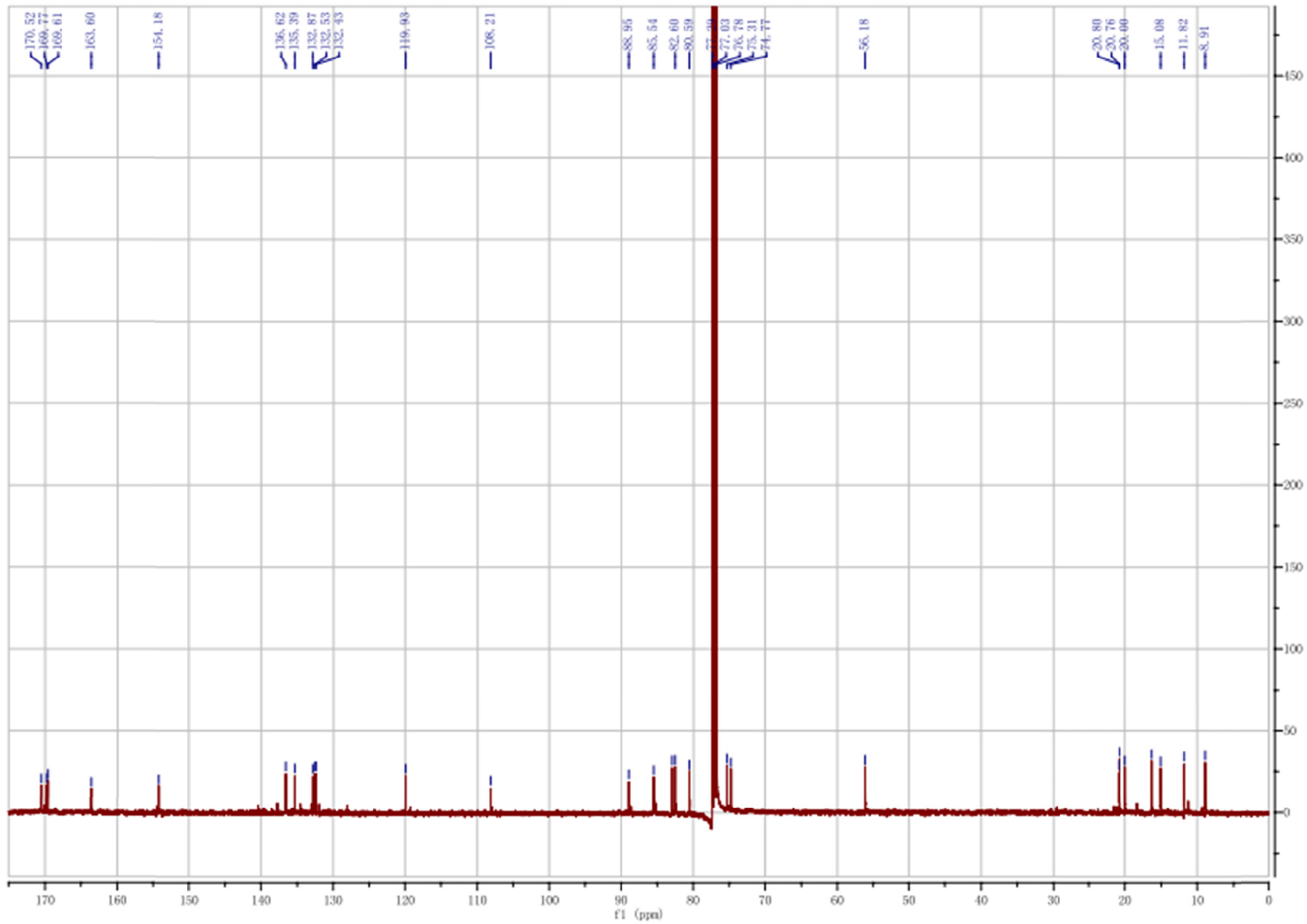


Figure S17. ^{13}C NMR of aurovertin A (**1**) in $CDCl_3$ (125 MHz)

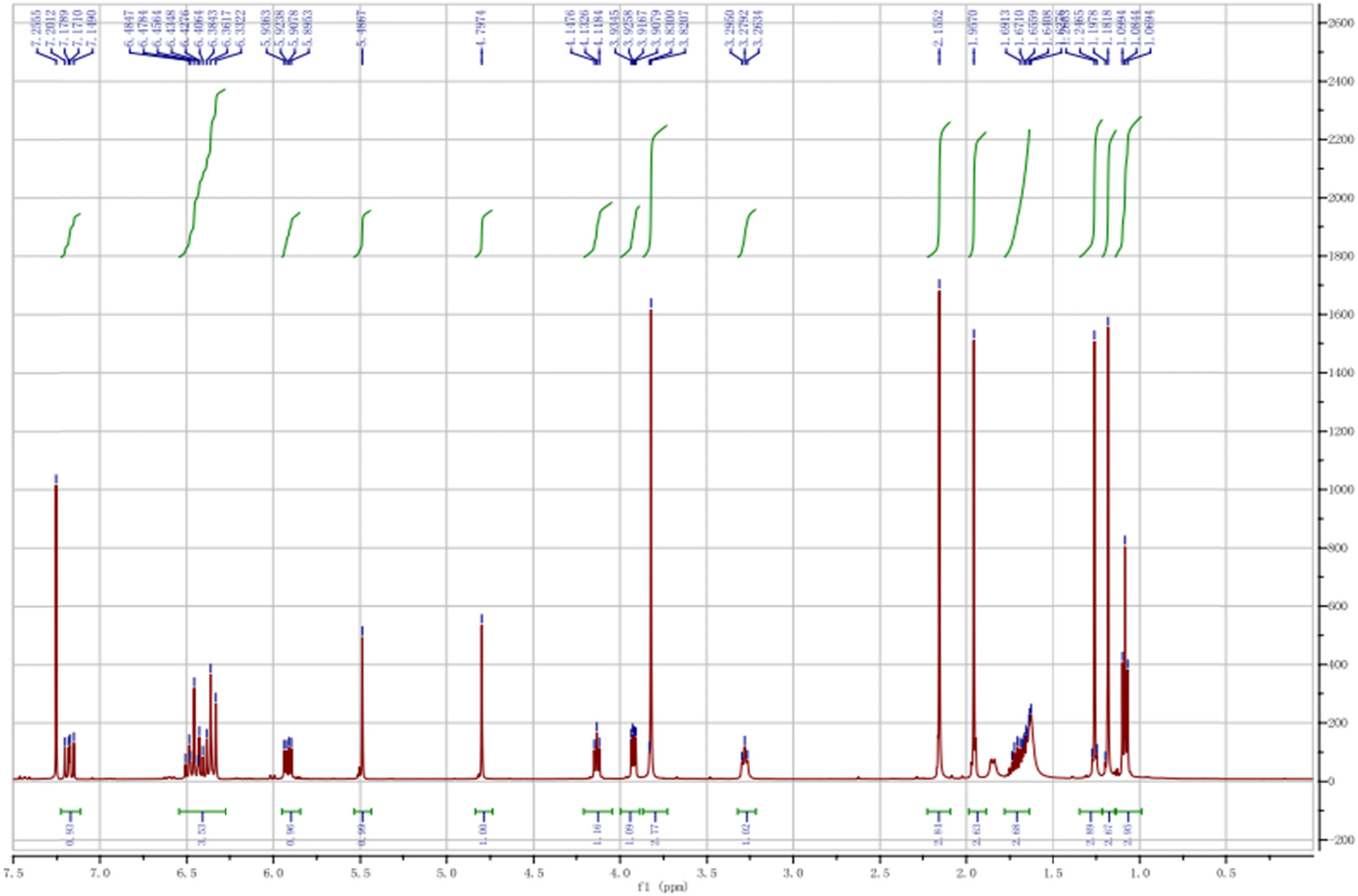


Figure S18. ^{1}H NMR of aurovertin B (**2**) in $CDCl_3$ (500 MHz)

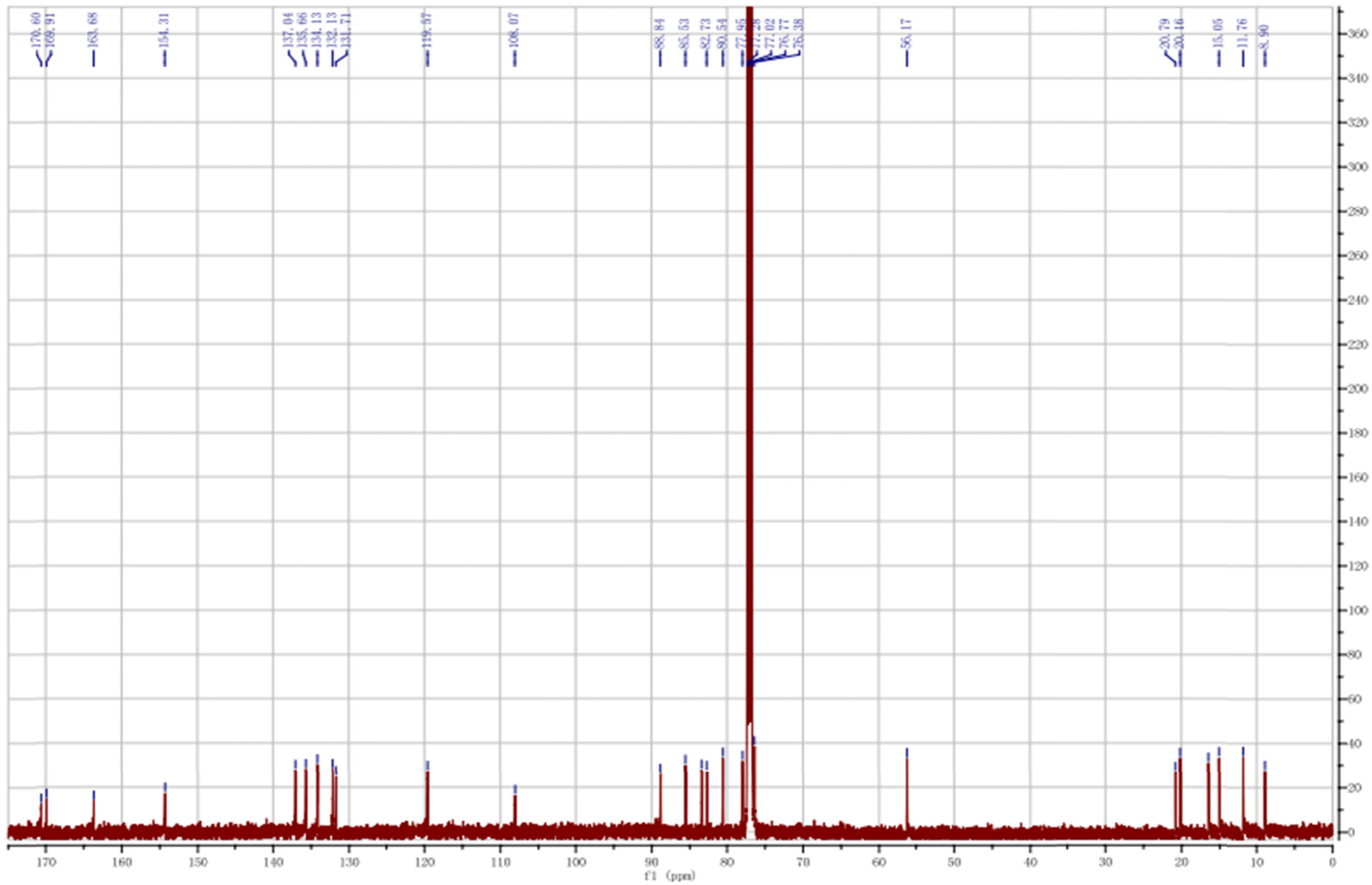


Figure S19. ^{13}C NMR of aurovertin B (**2**) in $CDCl_3$ (125 MHz)

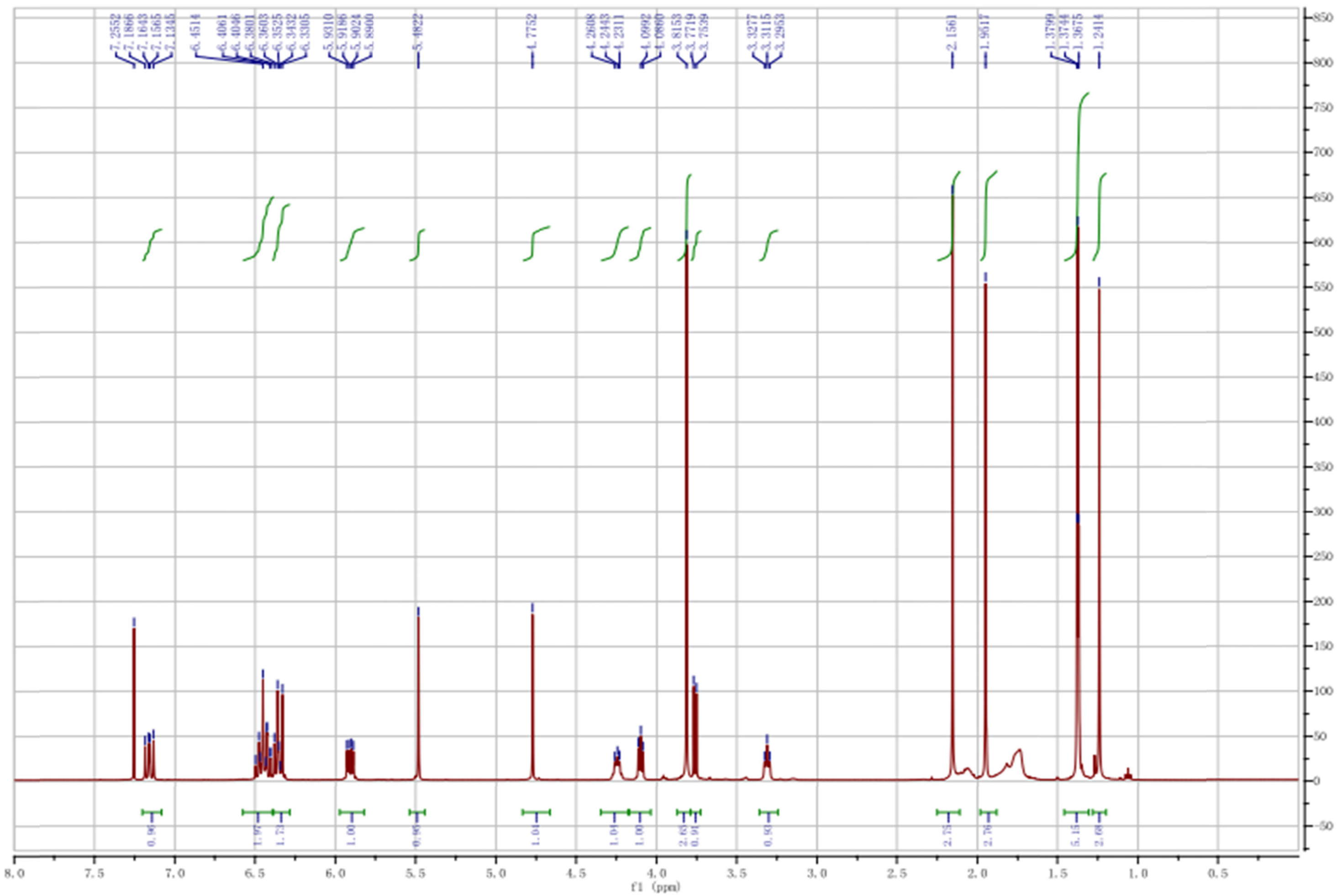


Figure S20. ^{1}H NMR of aurovertin D (**3**) in $CDCl_3$ (500 MHz)

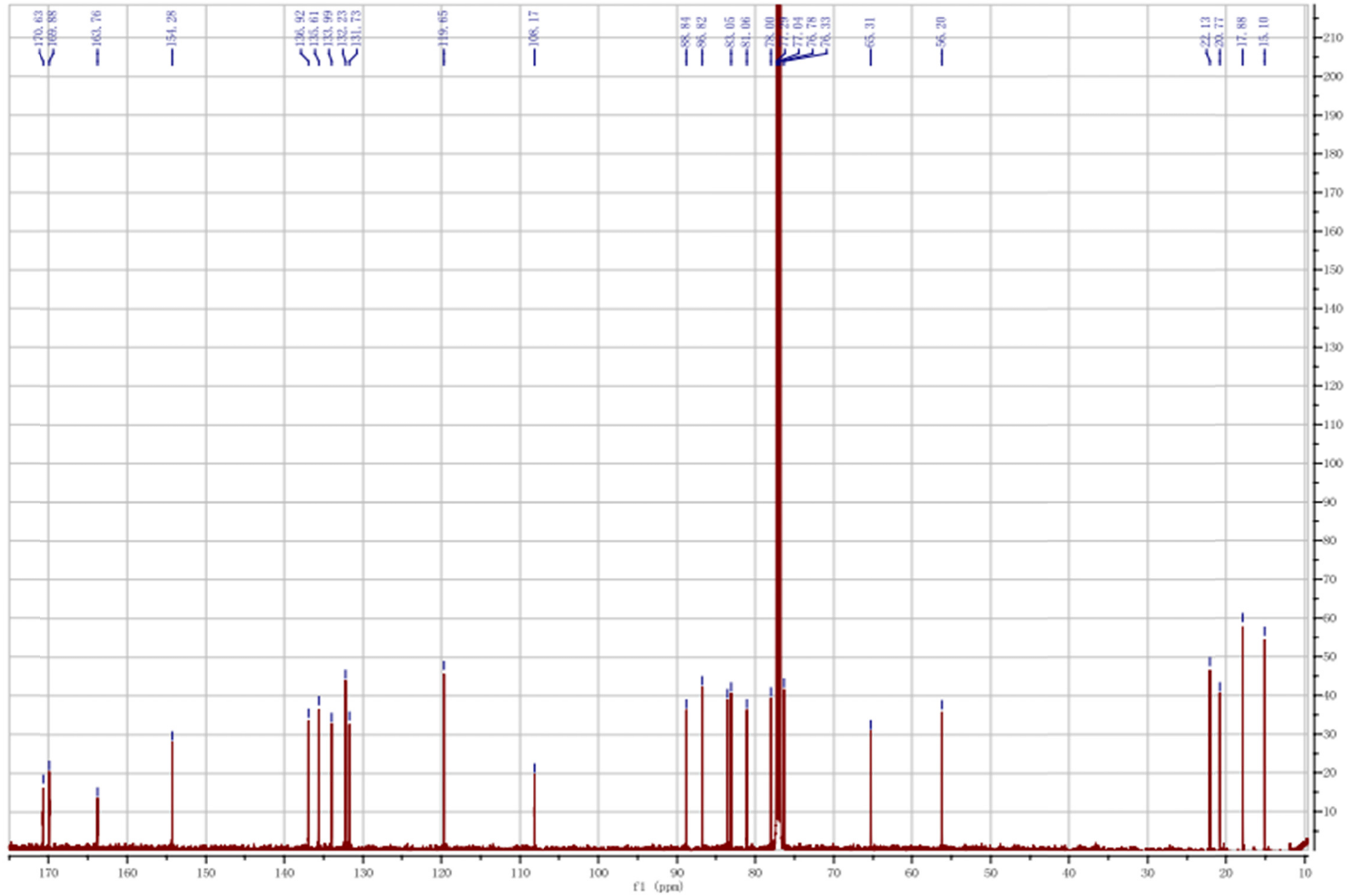


Figure S21. ^{13}C NMR of aurovertin D (**3**) in $CDCl_3$ (125 MHz)

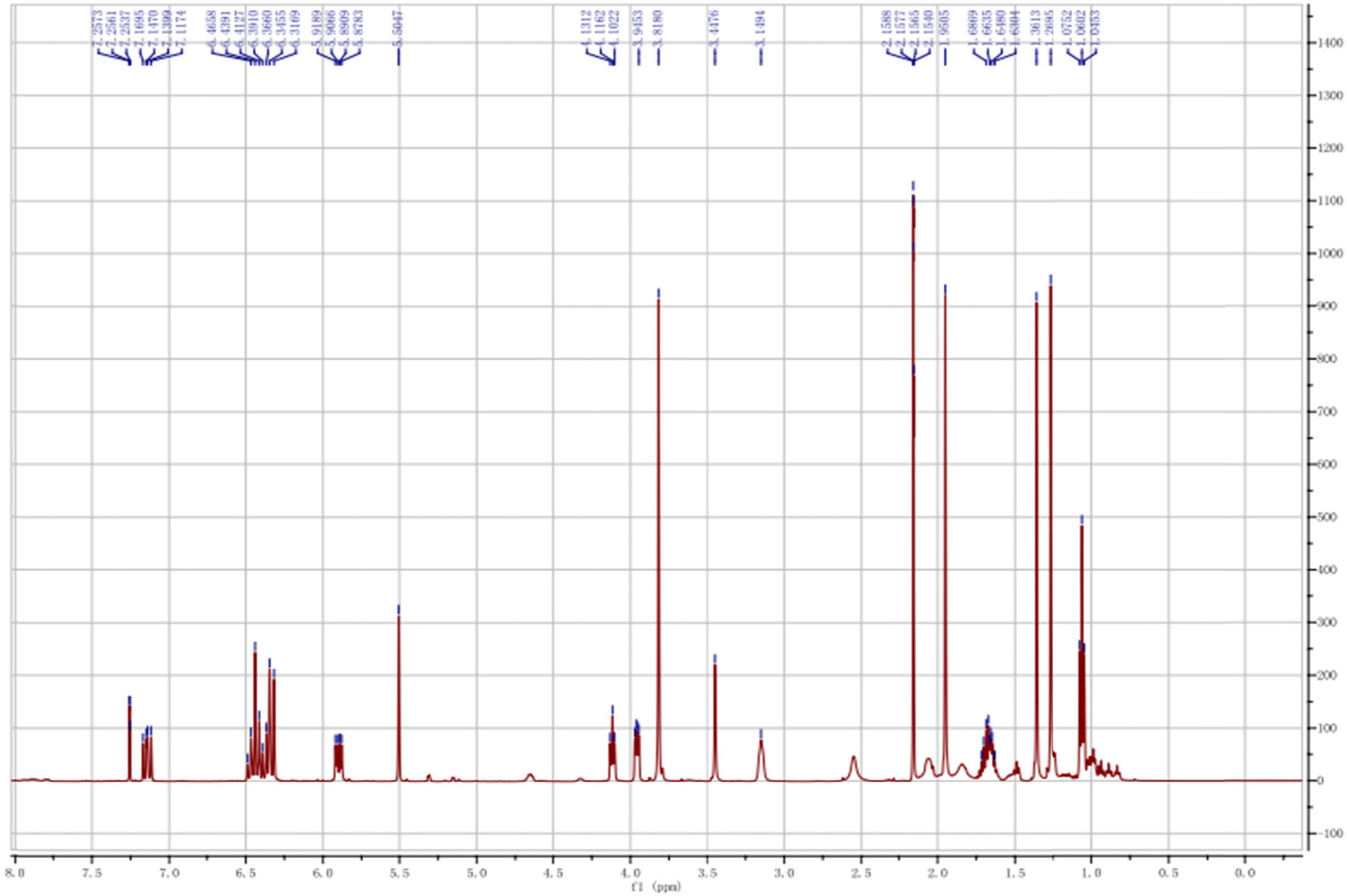


Figure S22. ^{1}H NMR of aurovertin E (**4**) in $CDCl_3$ (500 MHz)

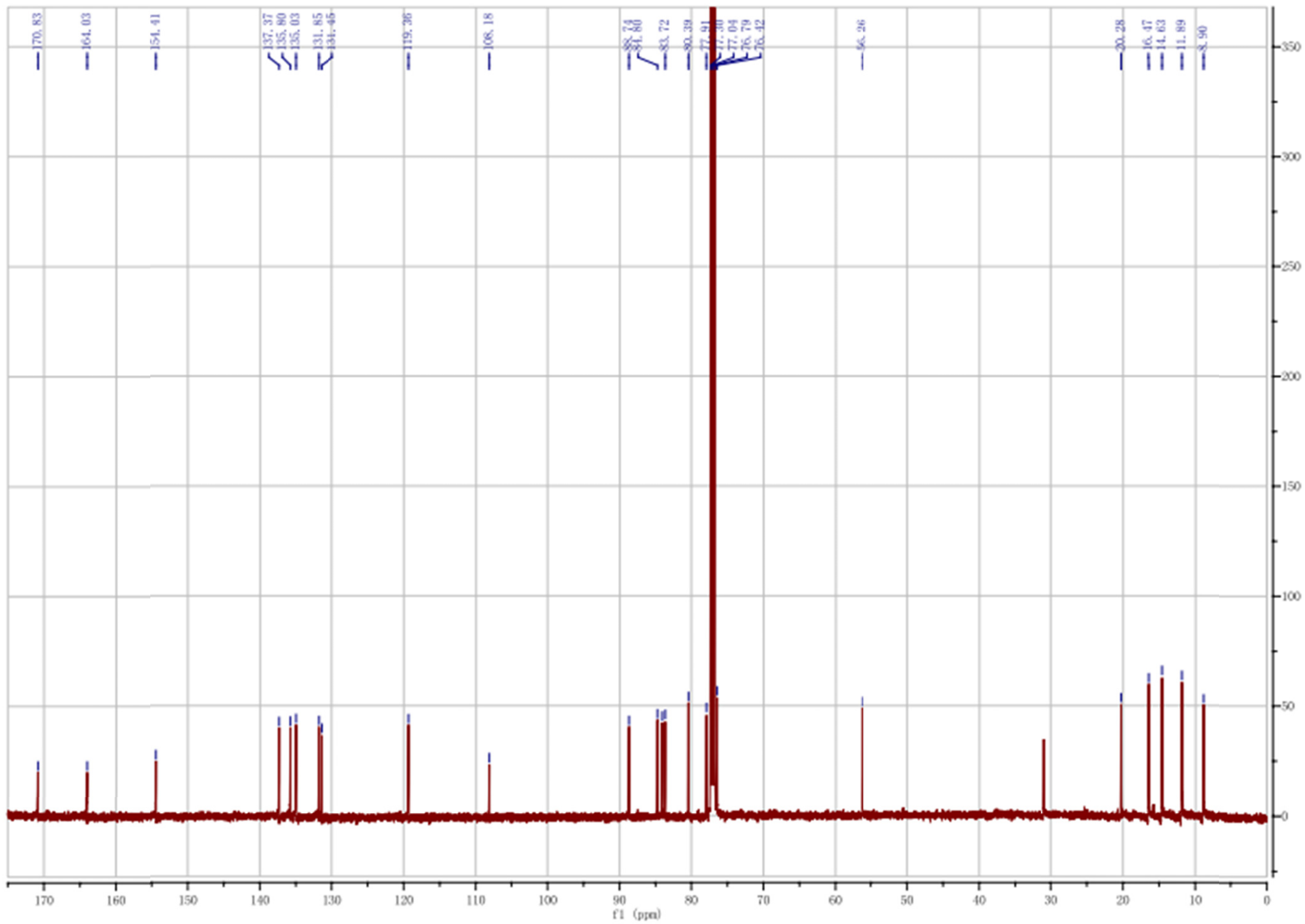


Figure S23. ^{1}H NMR of aurovertin E (**4**) in $CDCl_3$ (125 MHz)

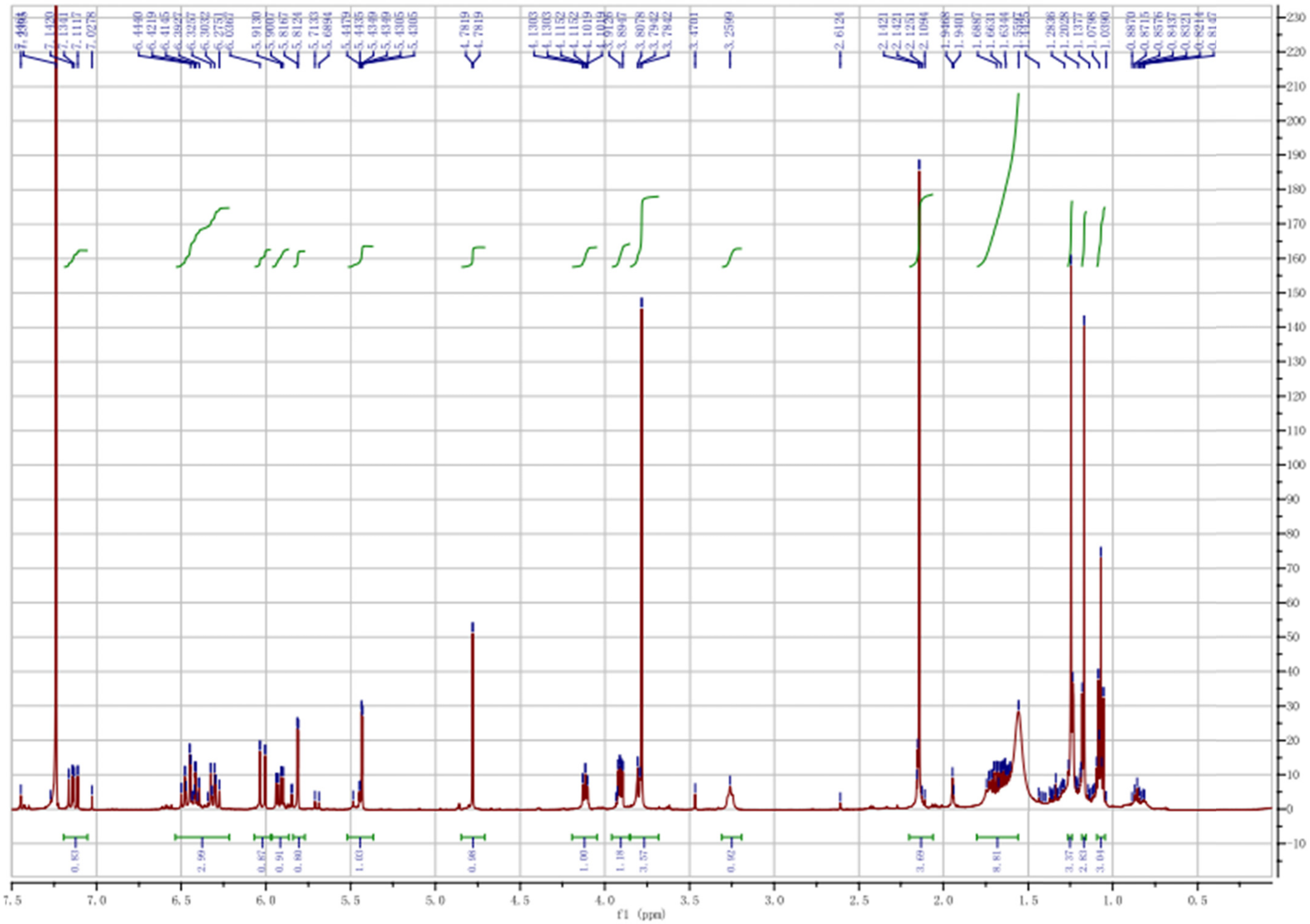


Figure S24. ^{1}H NMR of aurovertin J (**5**) in $CDCl_3$ (500 MHz)

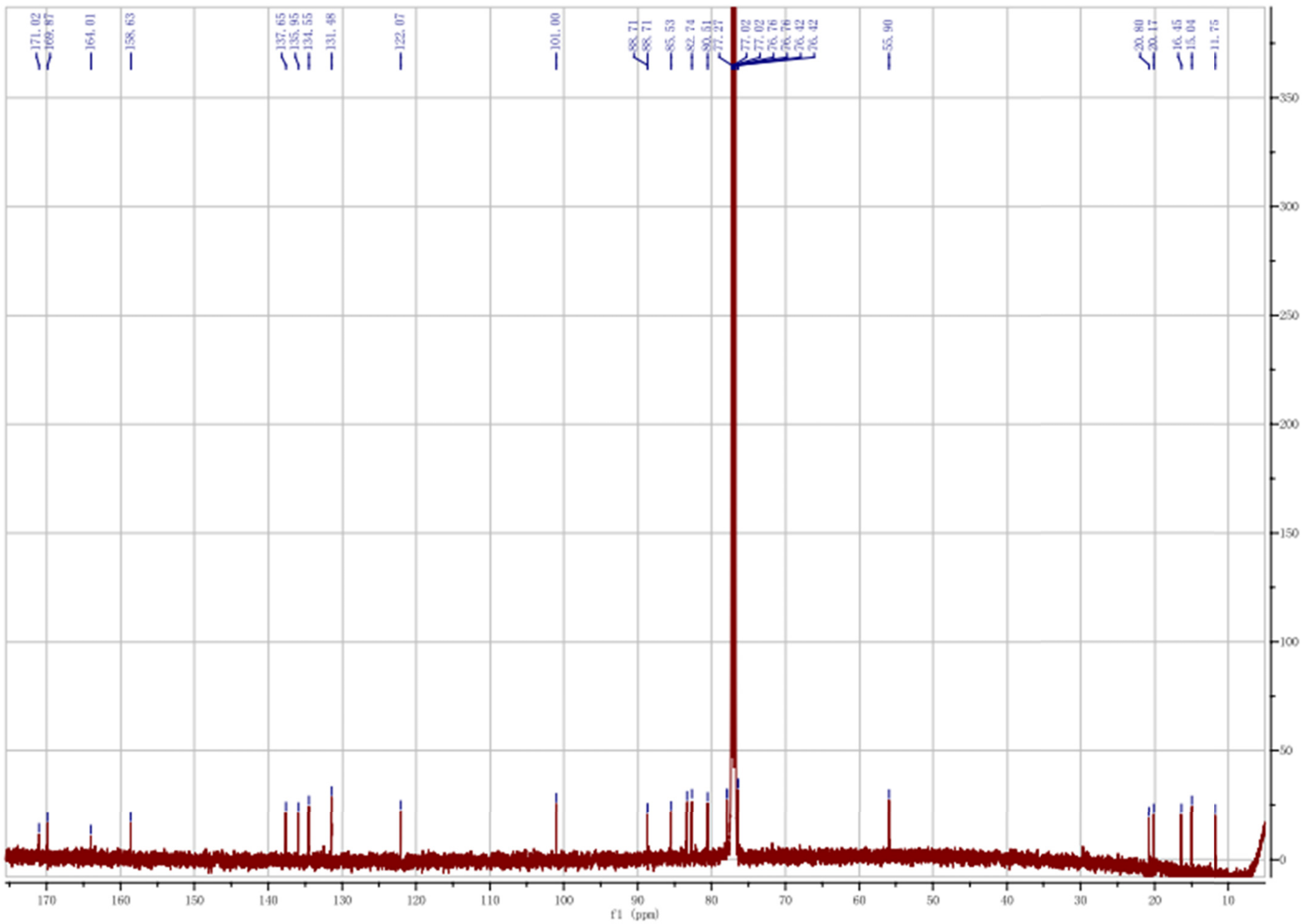


Figure S25. ^{13}C NMR of aurovertin J (**5**) in $CDCl_3$ (125 MHz)

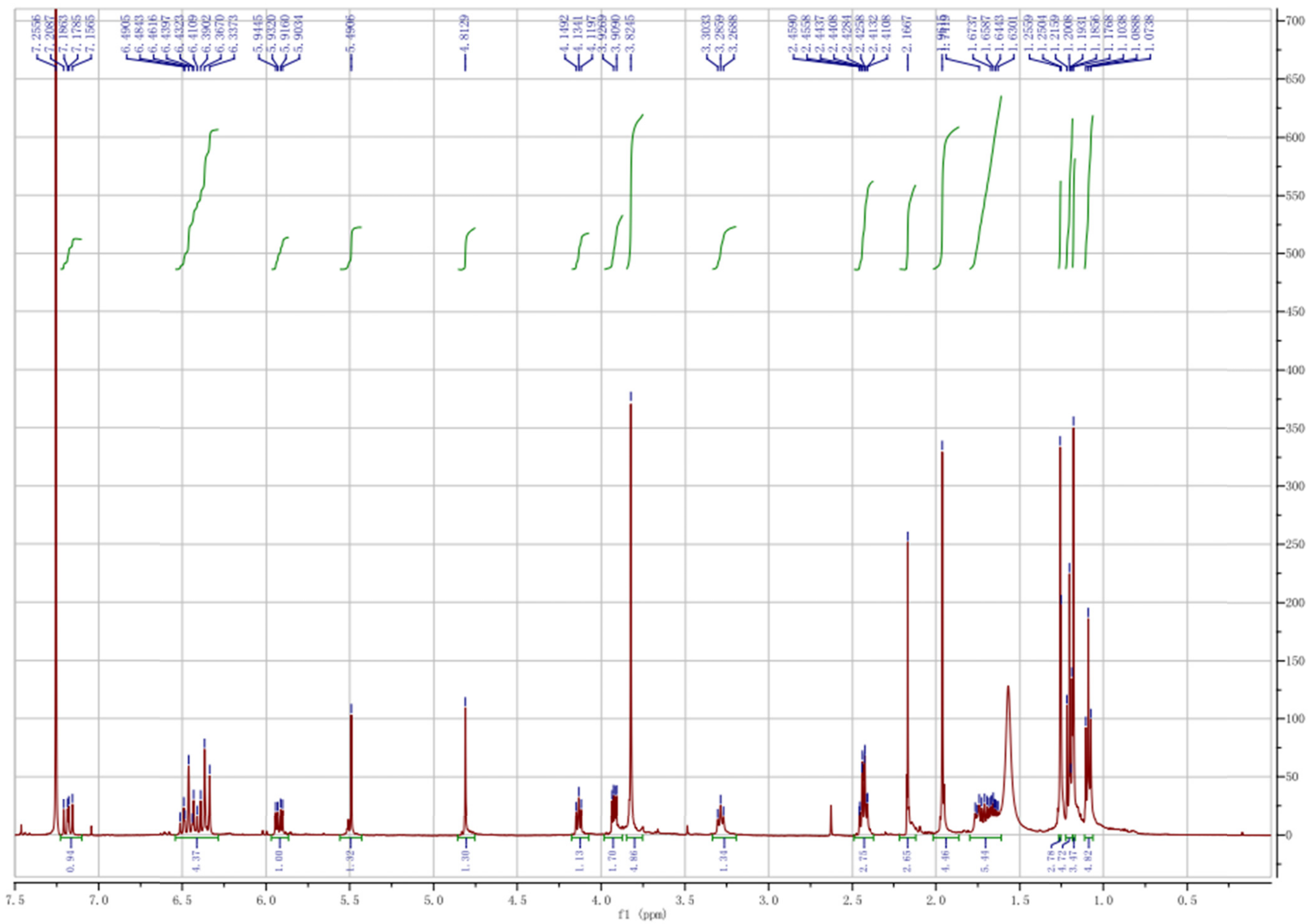


Figure S26. ^{1}H NMR of aurovertin M (**6**) in $CDCl_3$ (500 MHz)

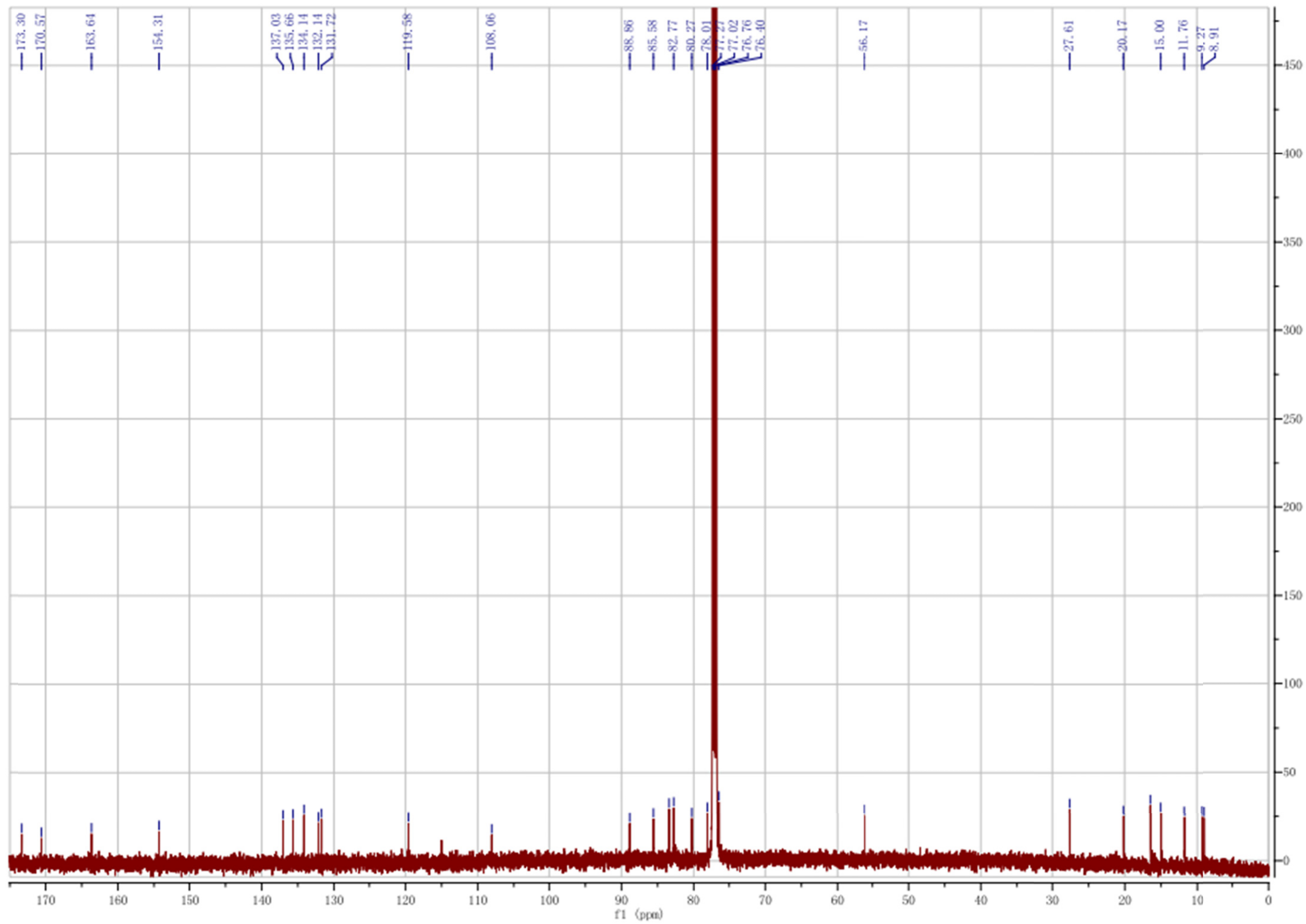


Figure S27. ^{13}C NMR of aurovertin M(**6**) in $CDCl_3$ (125 MHz)

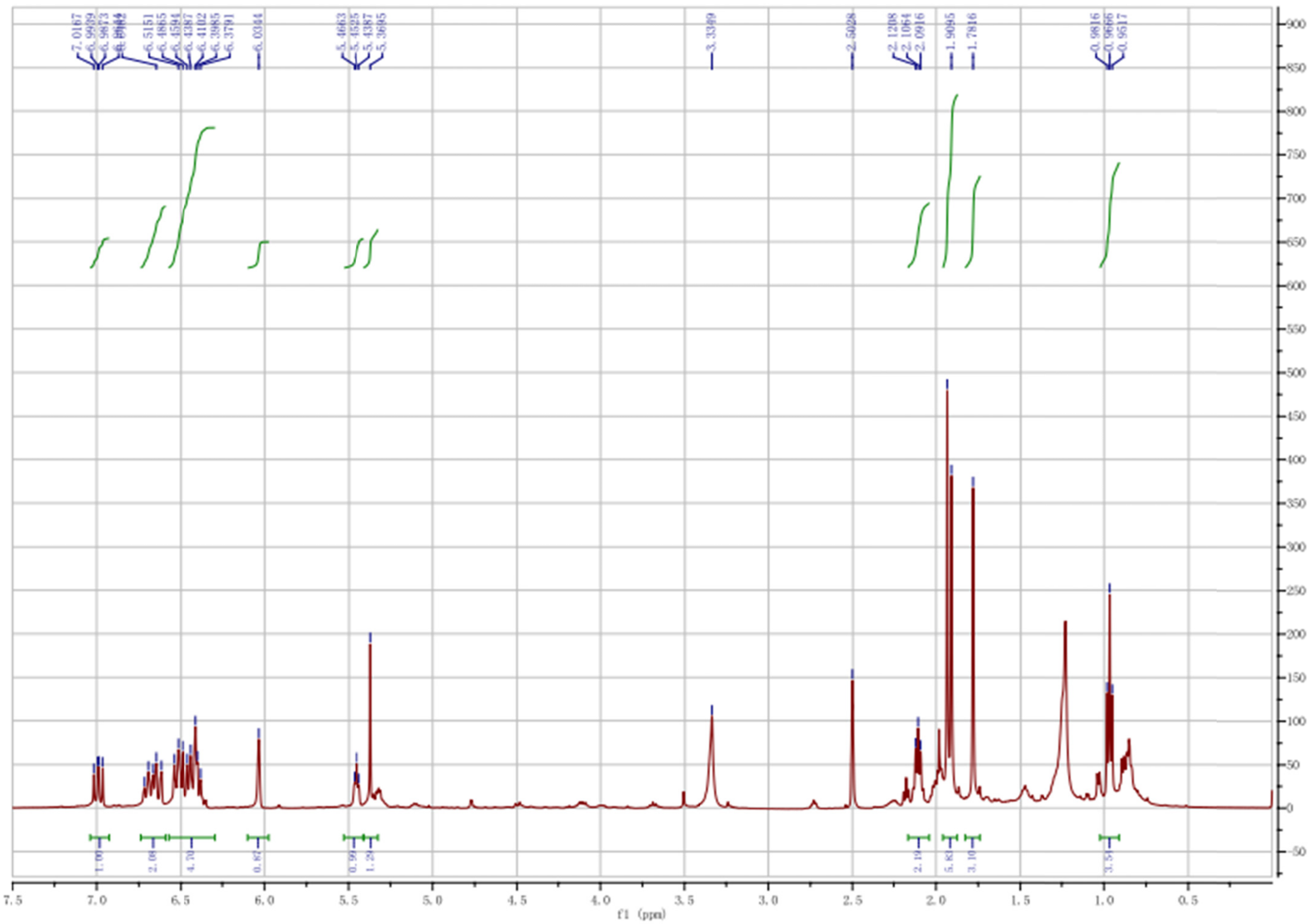


Figure S28. ^{1}H NMR of compound **7** in DMSO-d6 (500 MHz)

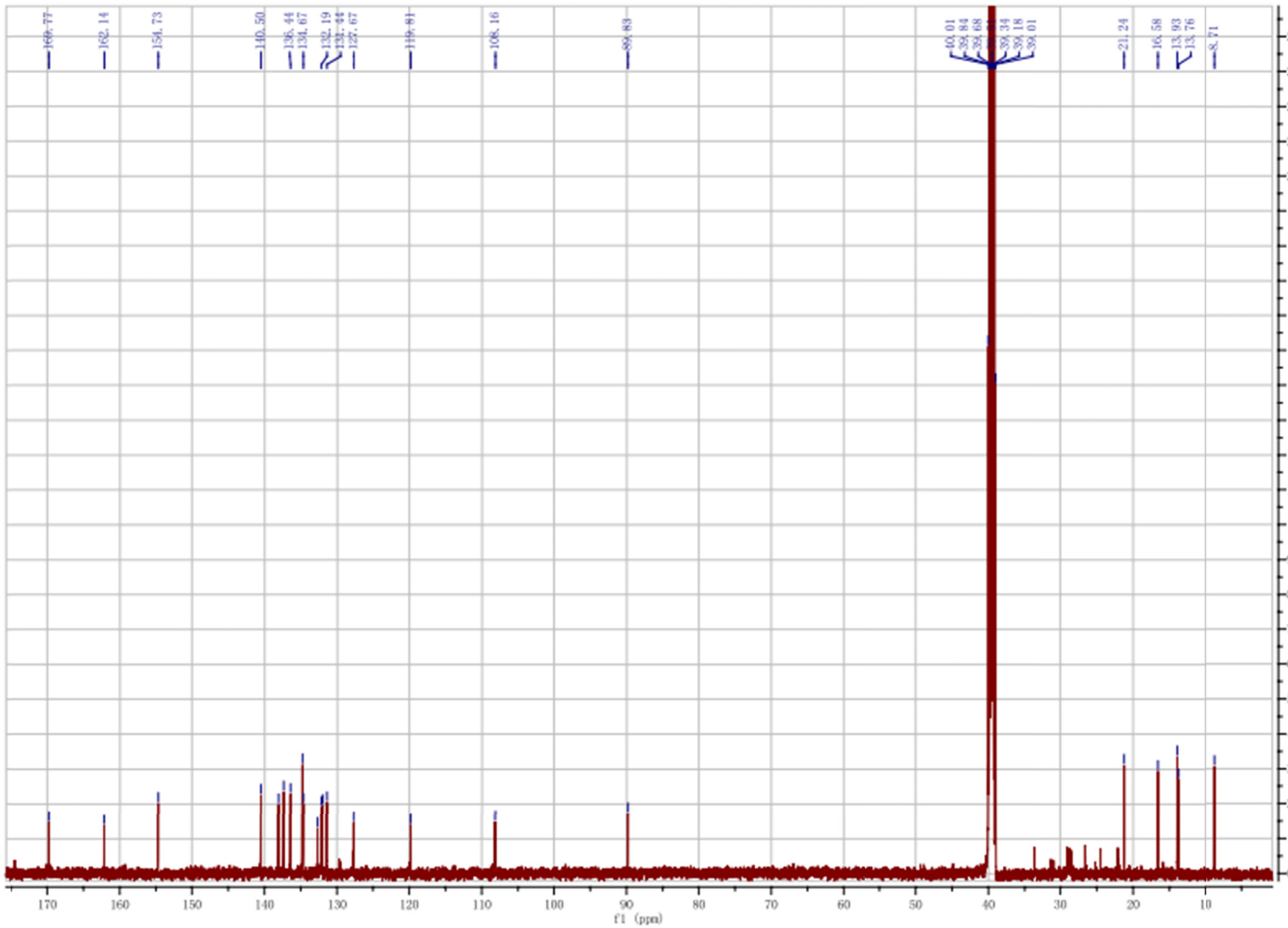


Figure S29. ^{13}C NMR of compound **7** in DMSO-d6 (125 MHz)

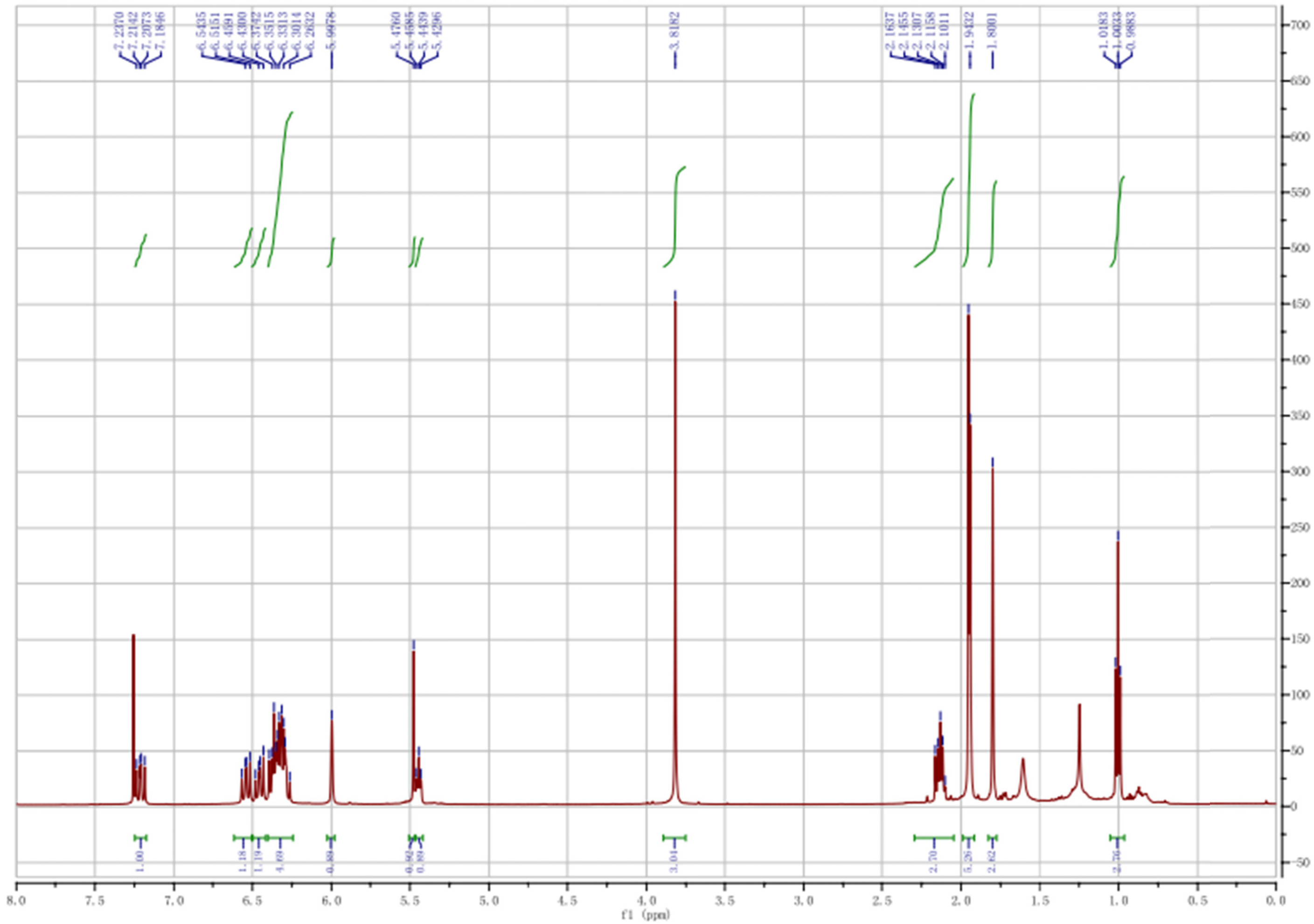


Figure S30. ^{1}H NMR of compound **8** in $CDCl_3$ (500 MHz)

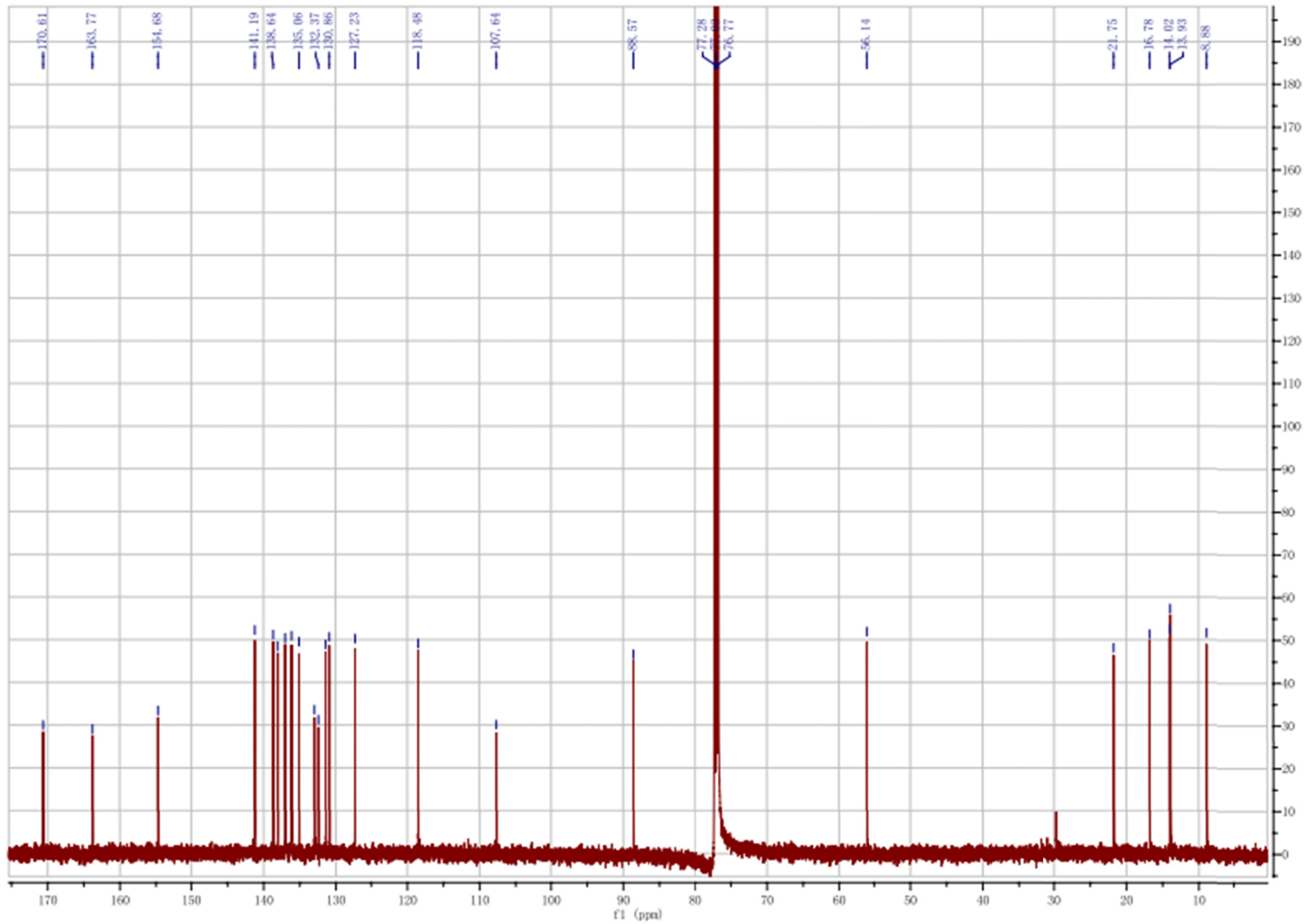


Figure S31. ^{13}C NMR of compound **8** in $CDCl_3$ (125 MHz)

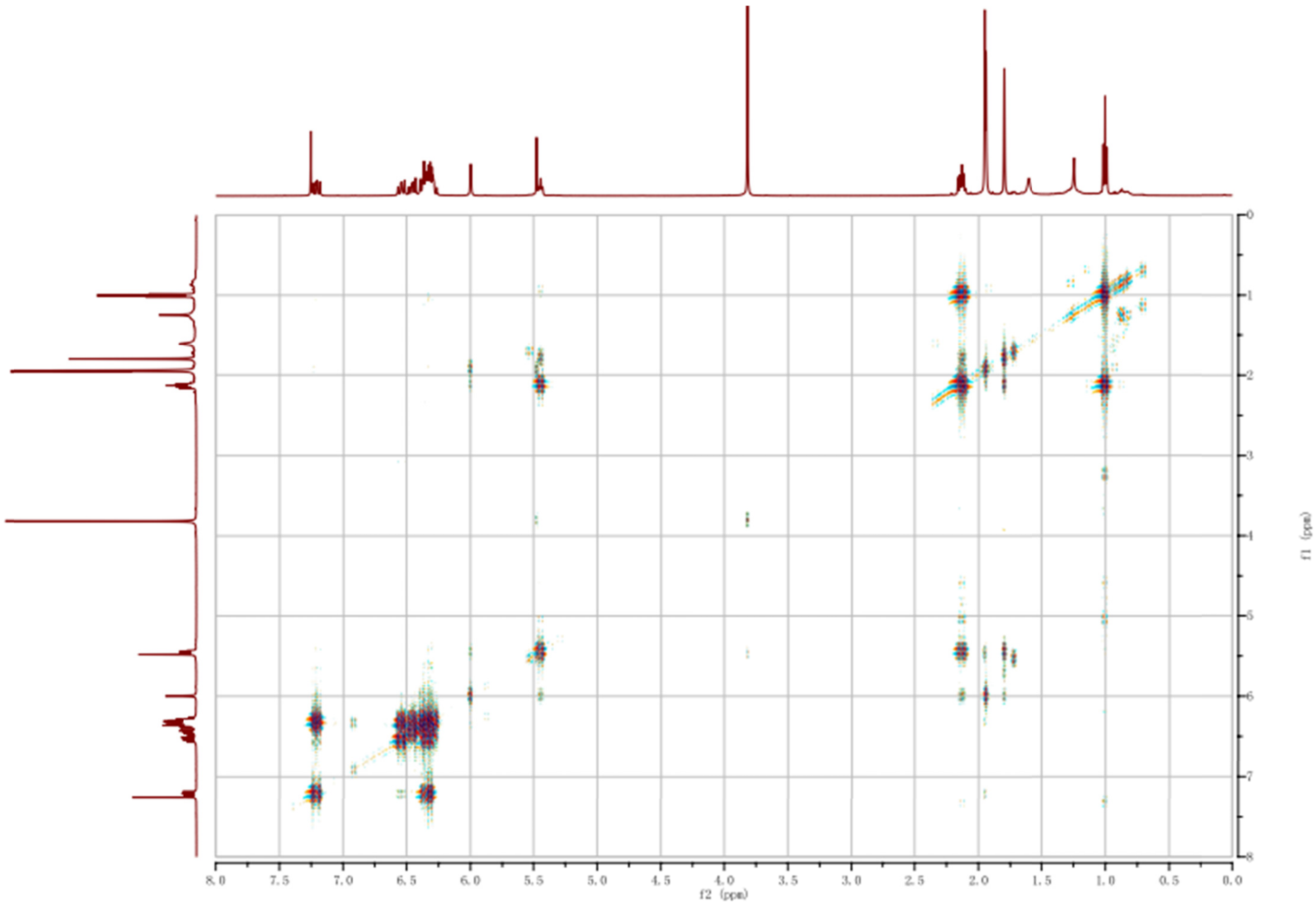


Figure S32. ^{1}H ^{1}H-COSY of compound **8** in $CDCl_3$ (500 MHz)

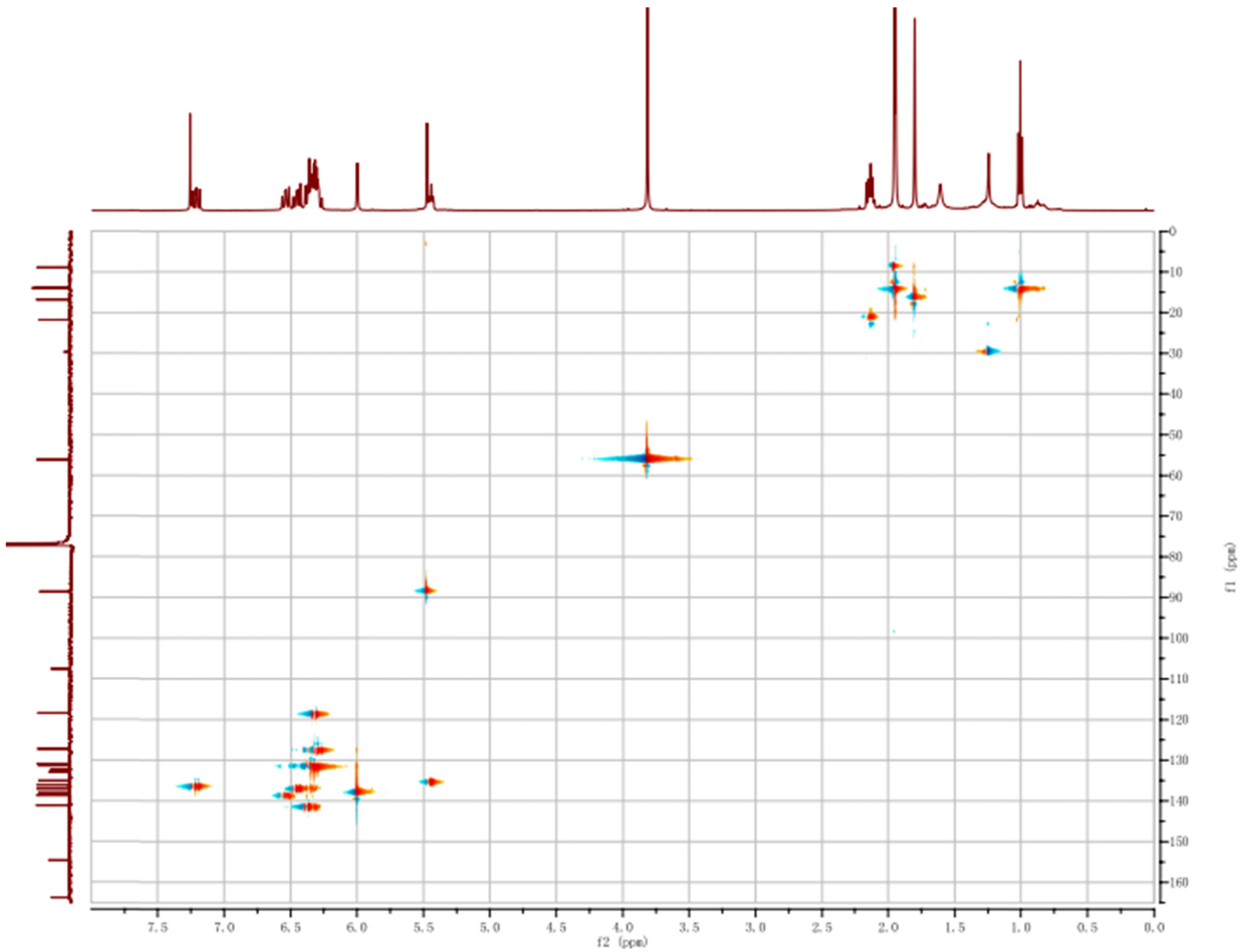


Figure S33. HSQC of compound **8** in $CDCl_3$ (500 MHz)

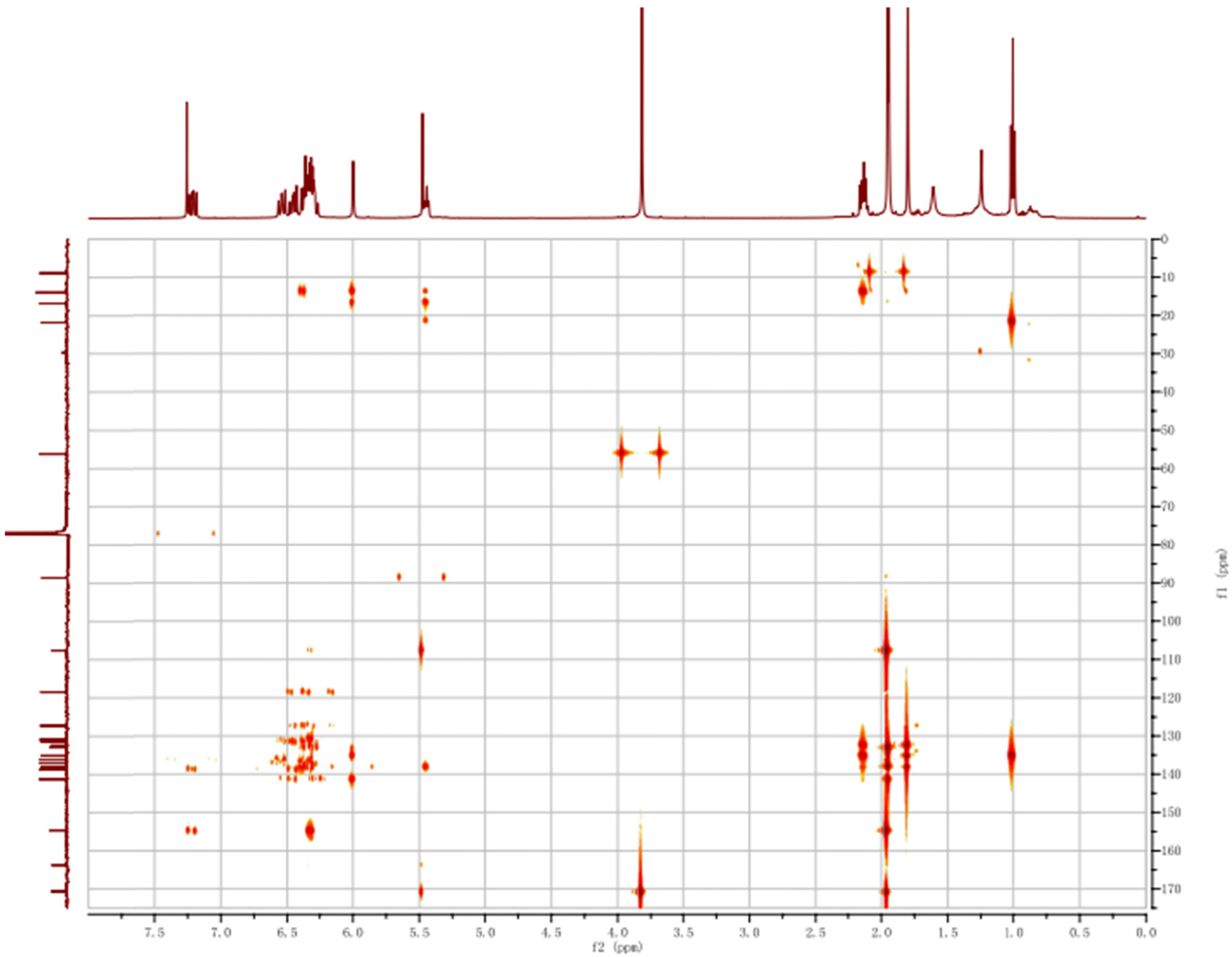


Figure S34. HMBC of compound **8** in $CDCl_3$ (500 MHz)

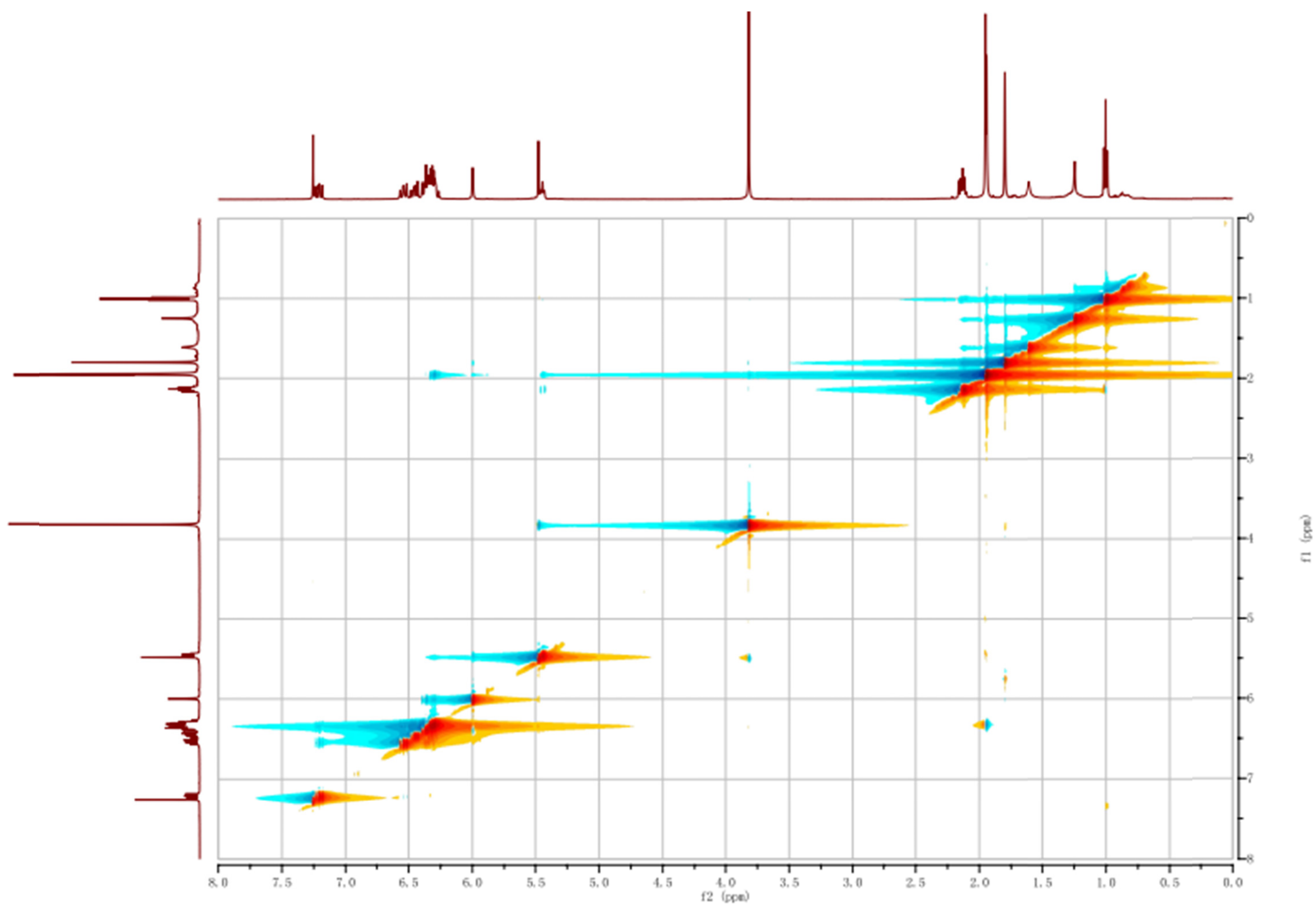


Figure S35. NOESY of compound **8** in $CDCl_3$ (500 MHz)

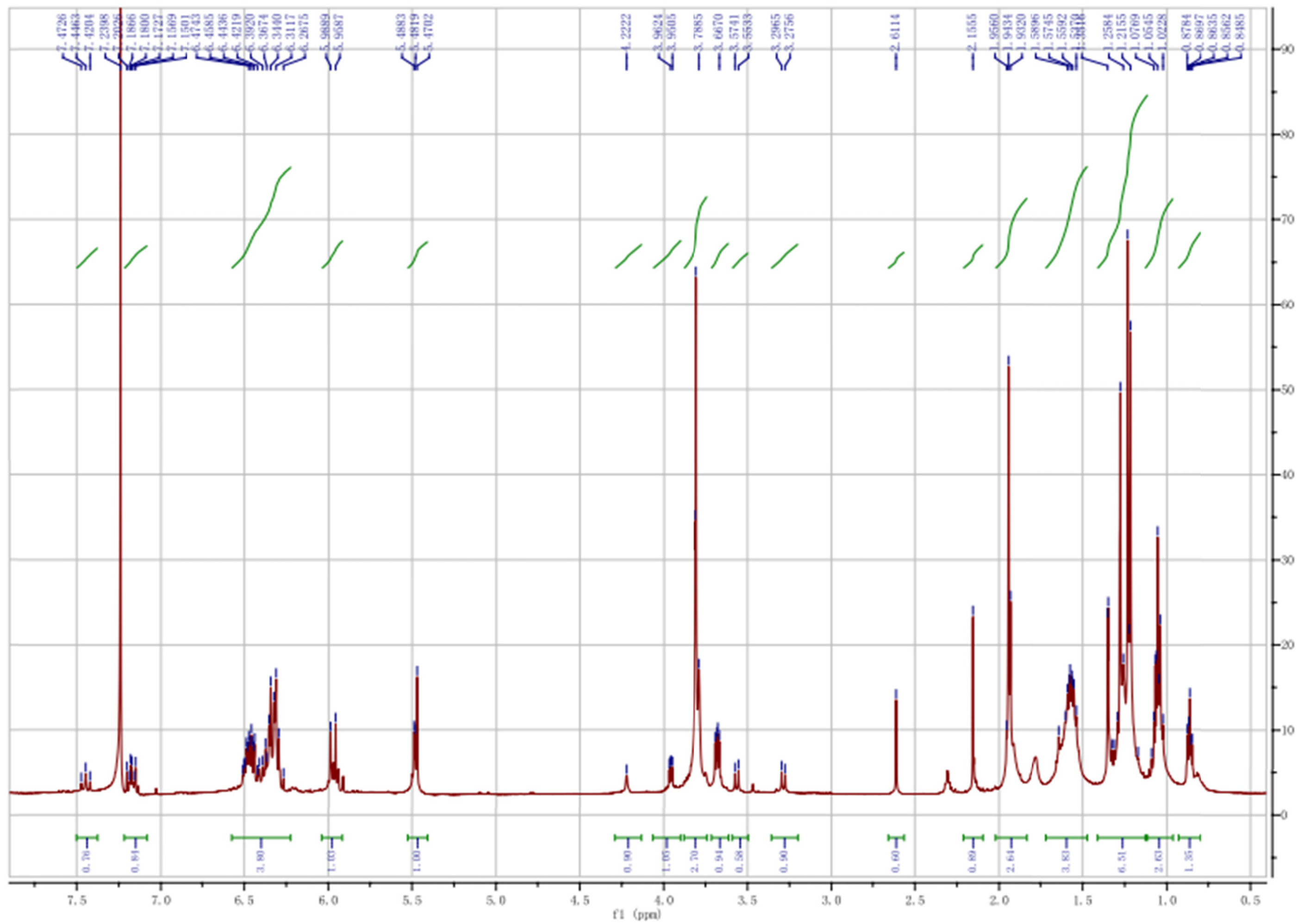


Figure S36. ^{1}H NMR of compound **9** in $CDCl_3$ (500 MHz)

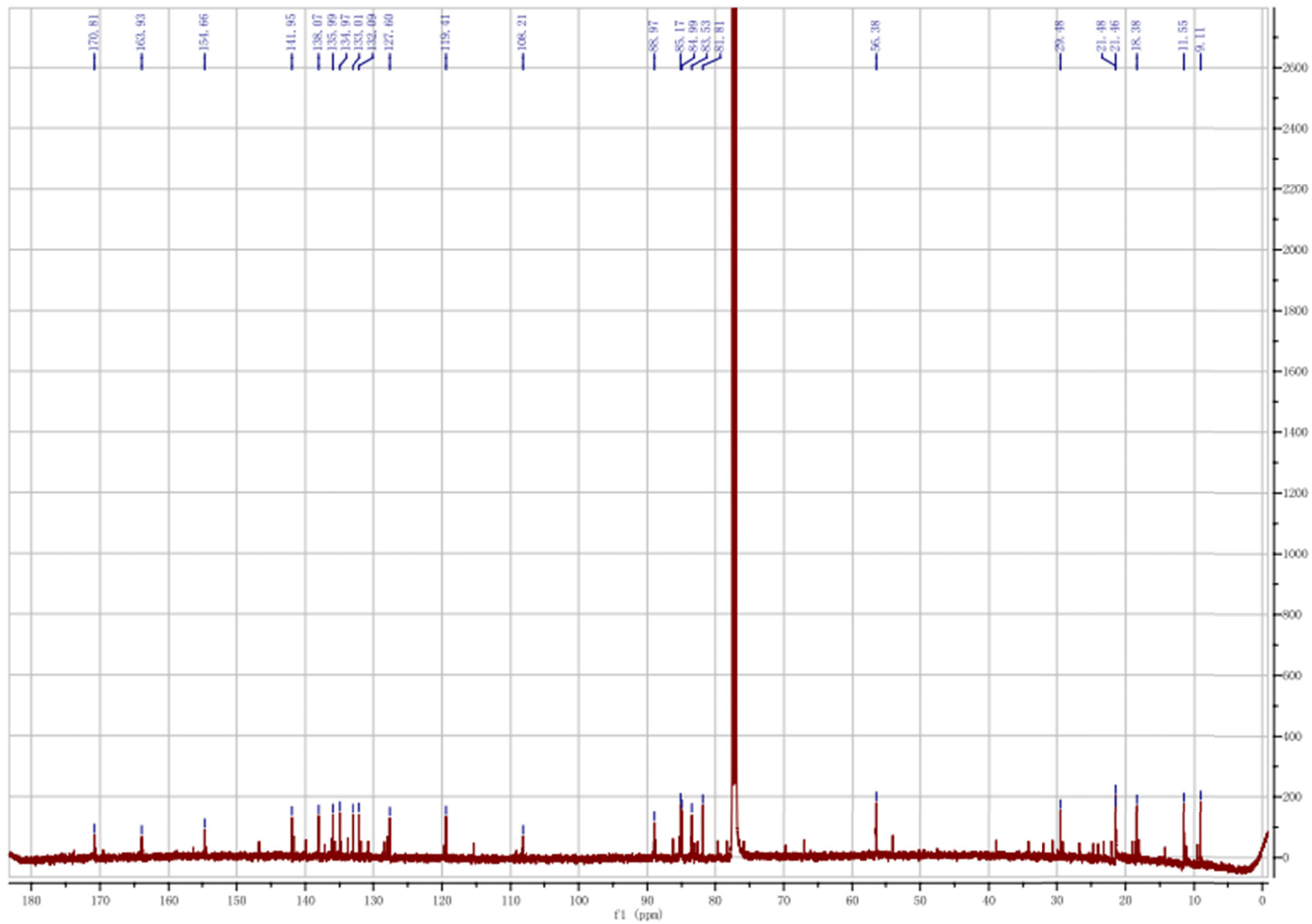


Figure S37. ^{13}C NMR of compound **9** in $CDCl_3$ (125 MHz)

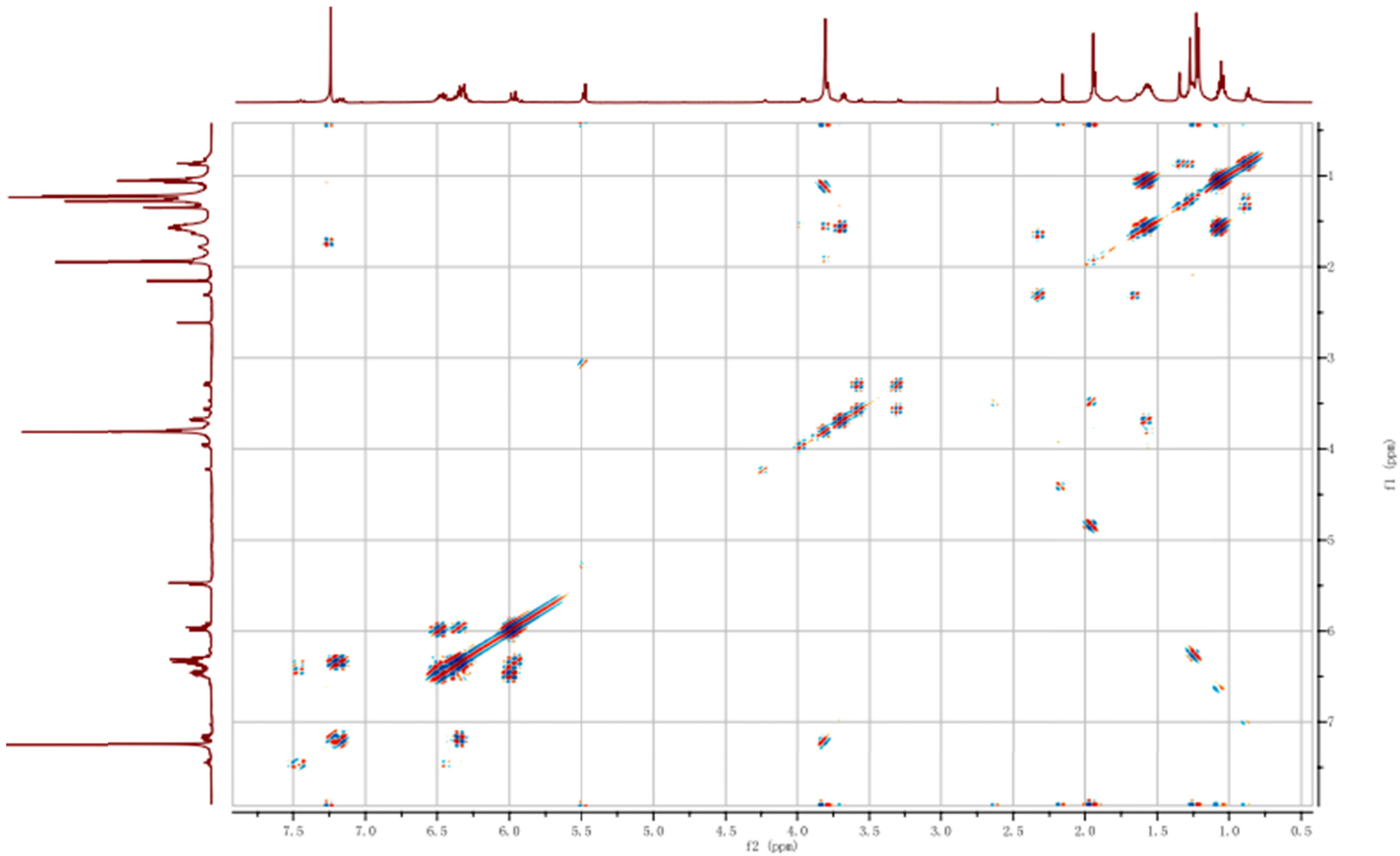


Figure S38. ^{1}H ^{1}H-COSY of compound **9** in $CDCl_3$ (500 MHz)

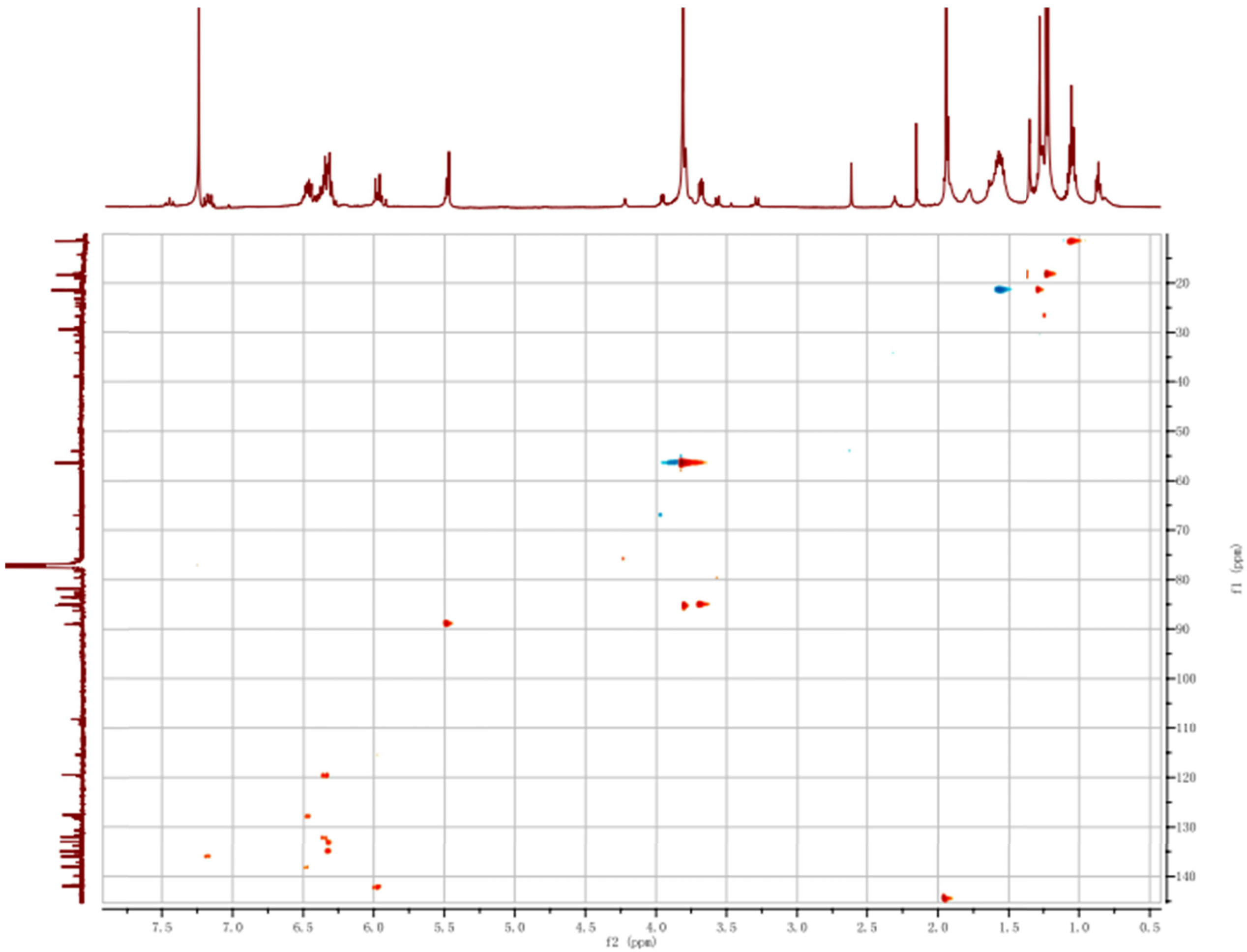


Figure S39. HSQC of compound **9** in $CDCl_3$ (500 MHz)

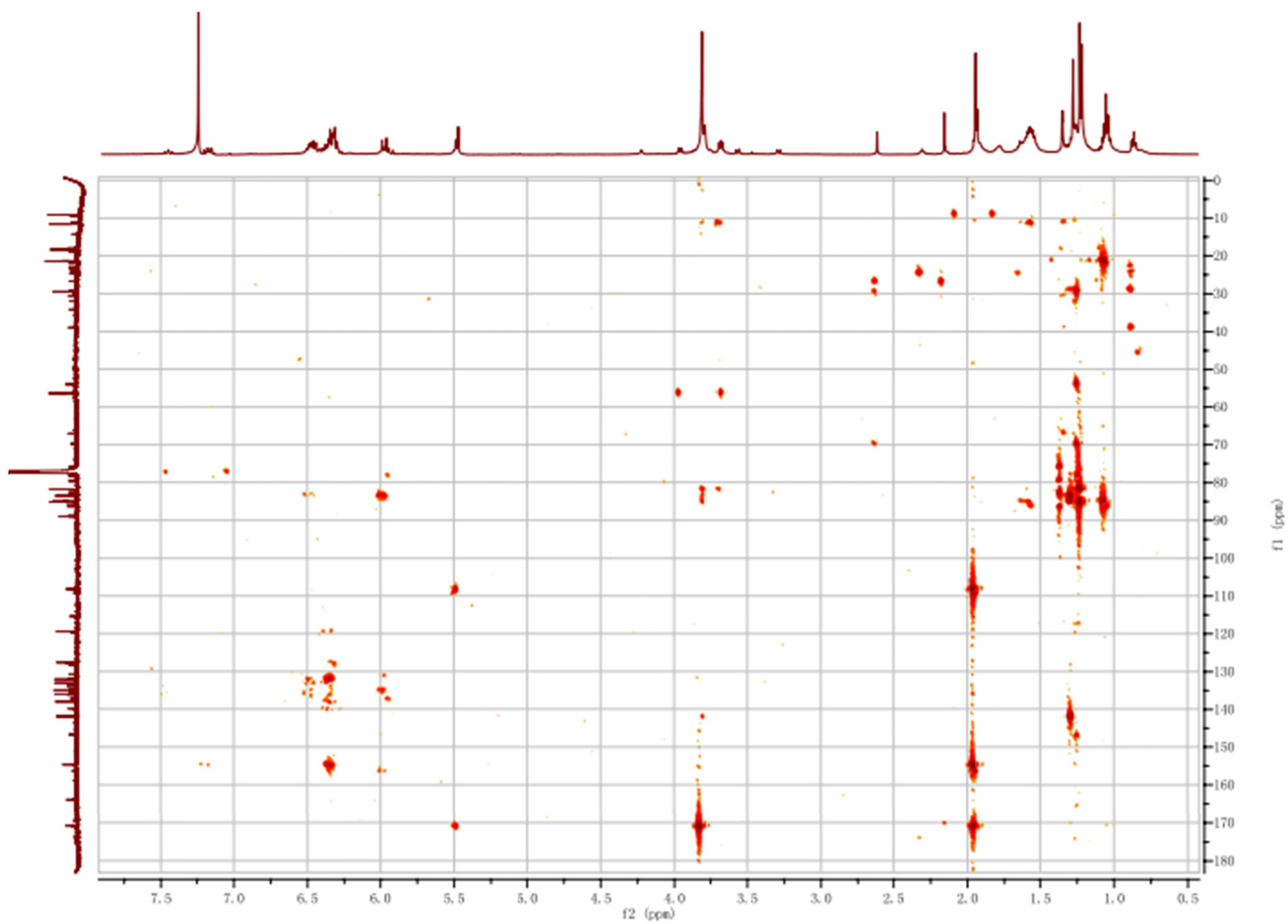


Figure S40. HMBC of compound **9** in $CDCl_3$ (500 MHz)

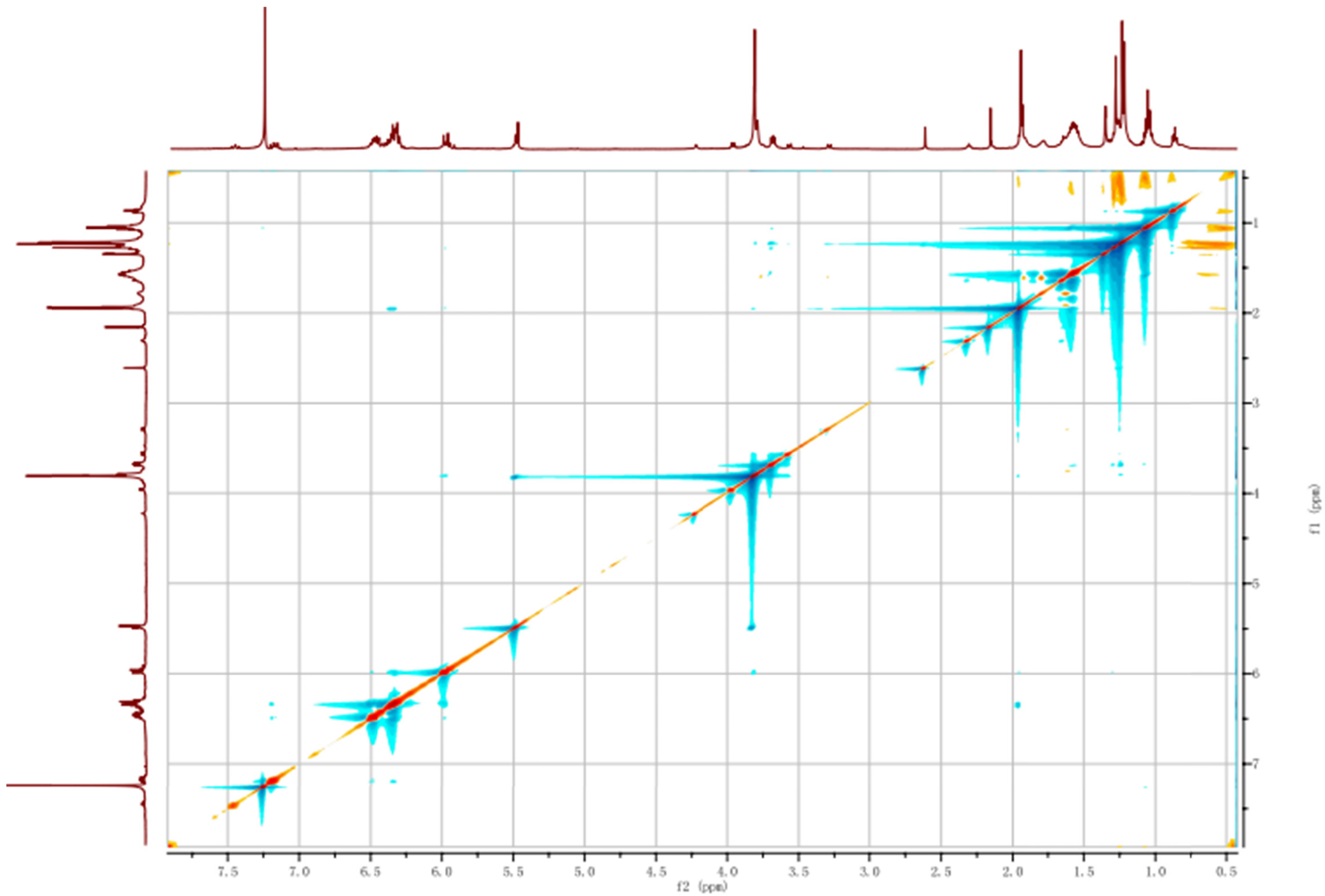


Figure S41. NOESY of compound **9** in $CDCl_3$ (500 MHz)

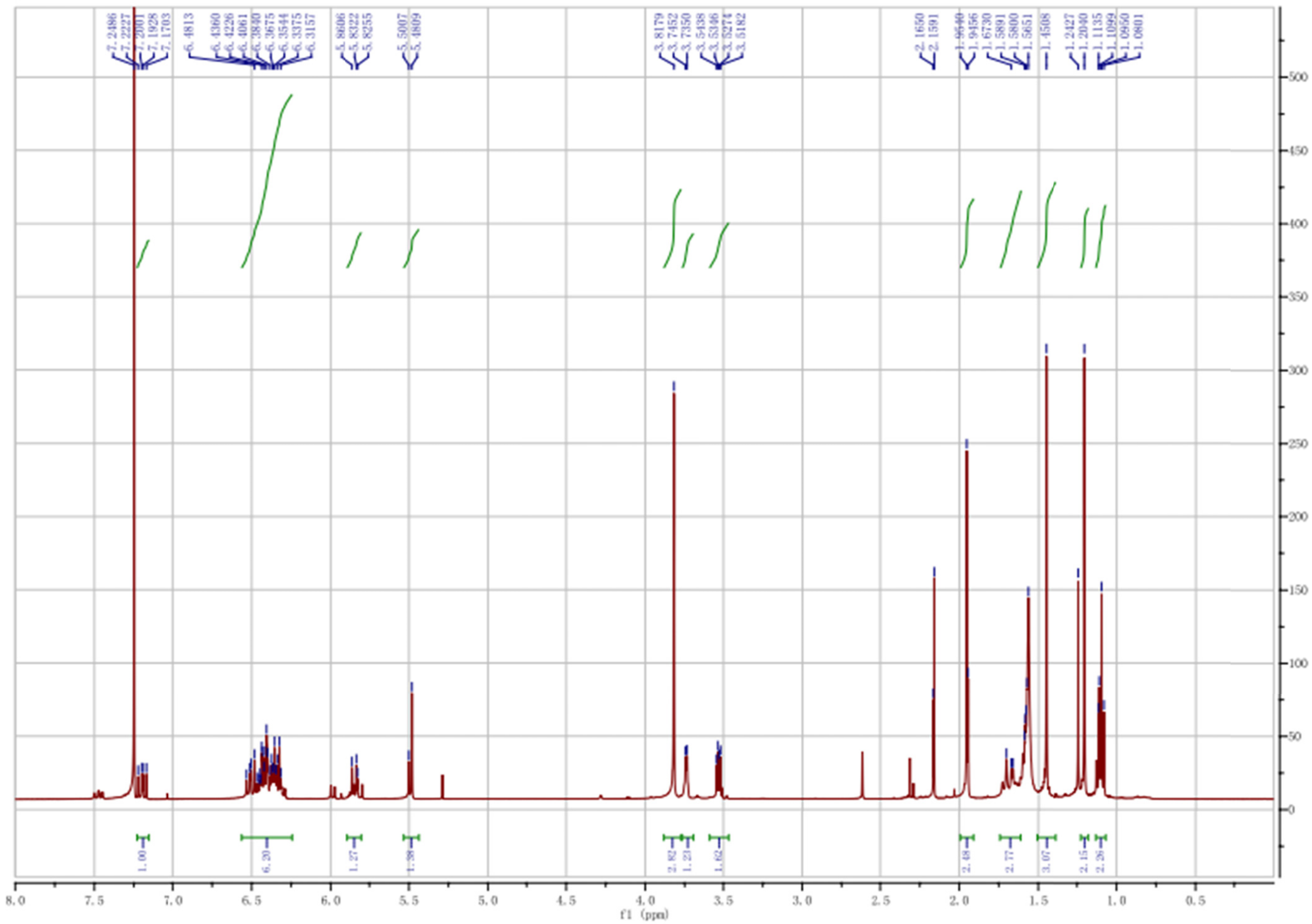


Figure S42. ^{1}H NMR of compound **10** in $CDCl_3$ (500 MHz)

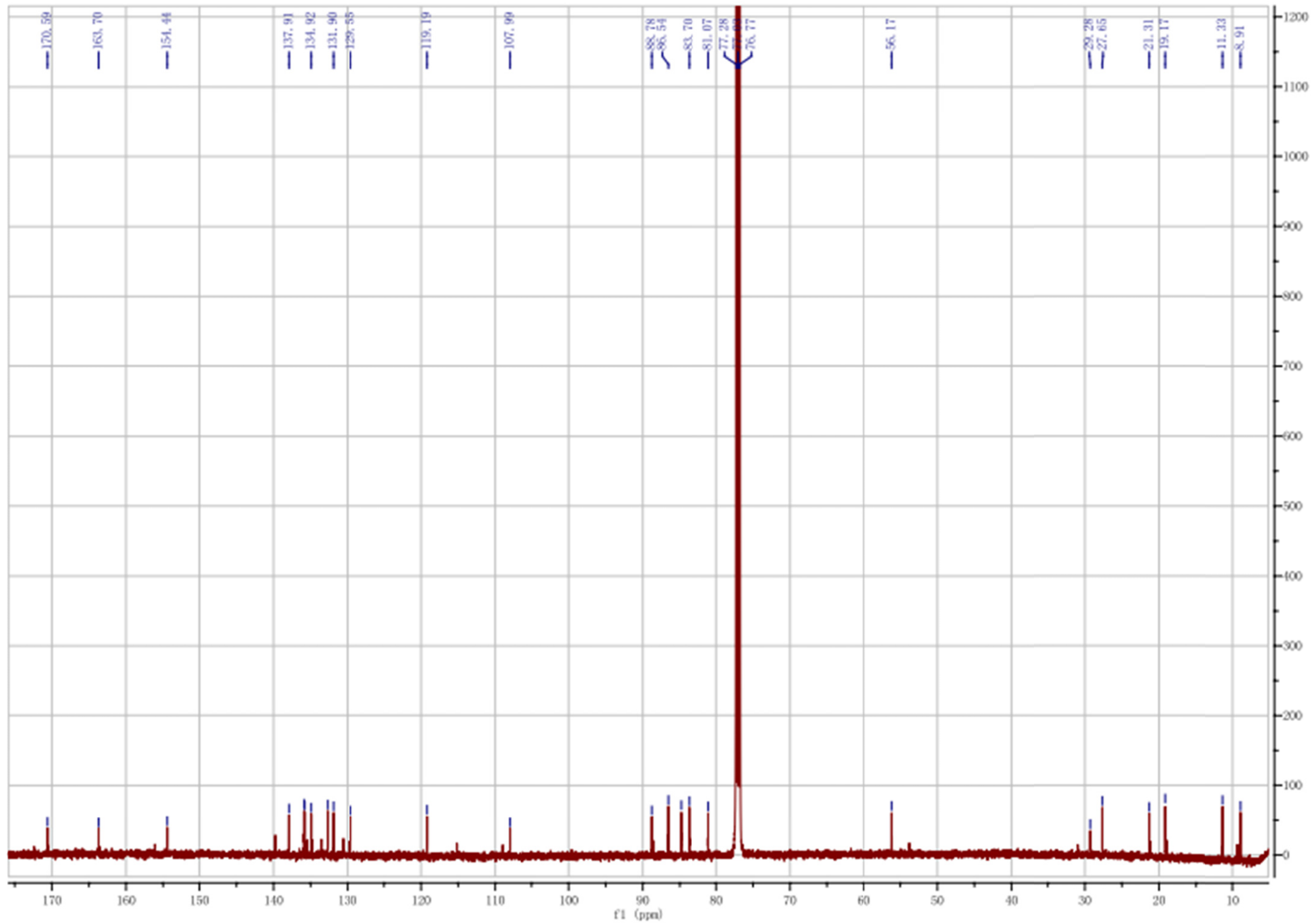


Figure S43. ^{13}C NMR of compound **10** in $CDCl_3$ (125 MHz)

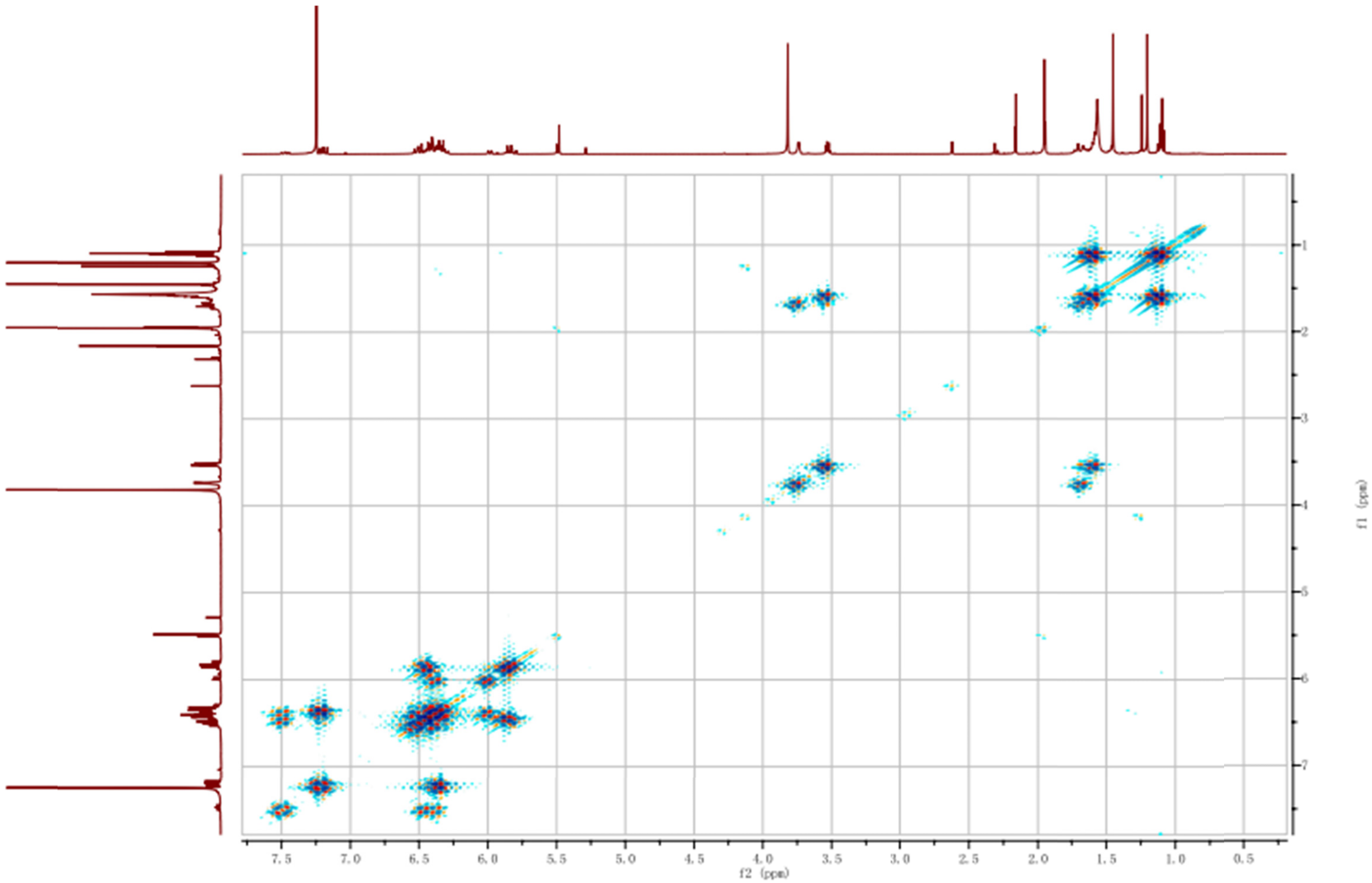


Figure S44. ^{1}H ^{1}H-COSY of compound **10** in $CDCl_3$ (500 MHz)

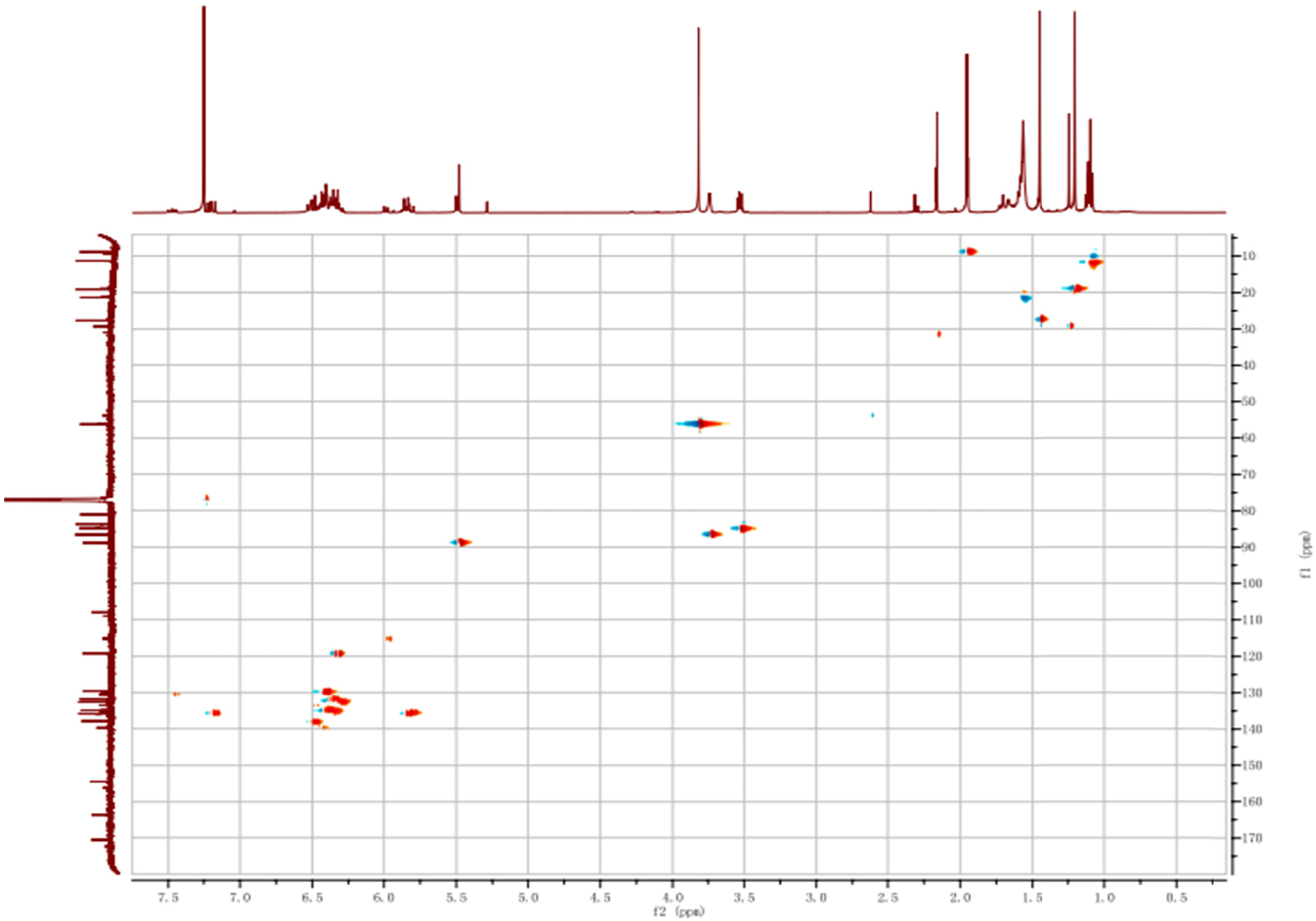


Figure S45. HSQC of compound **10** in $CDCl_3$ (500 MHz)

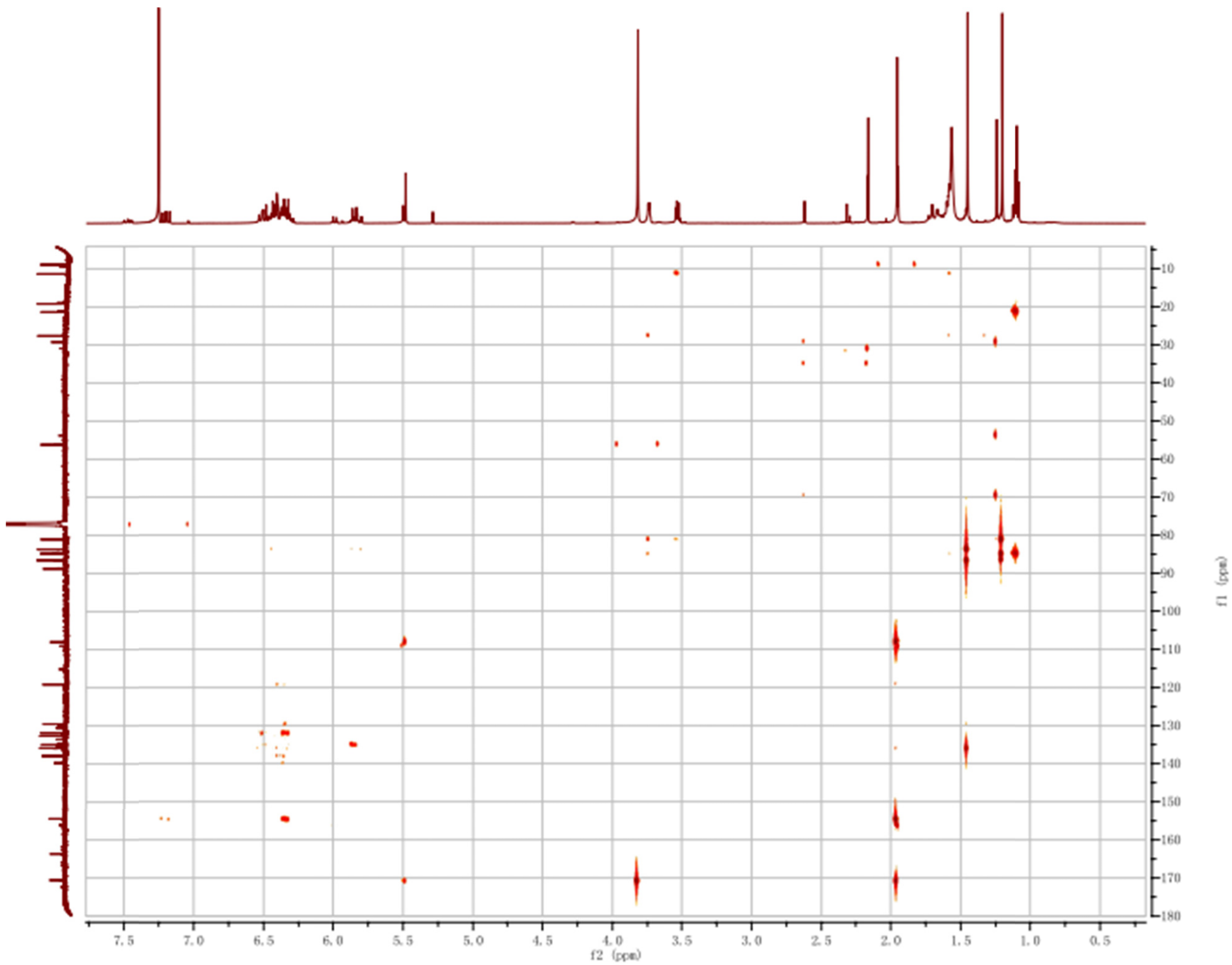


Figure S46. HMBC of compound **10** in $CDCl_3$ (500 MHz)

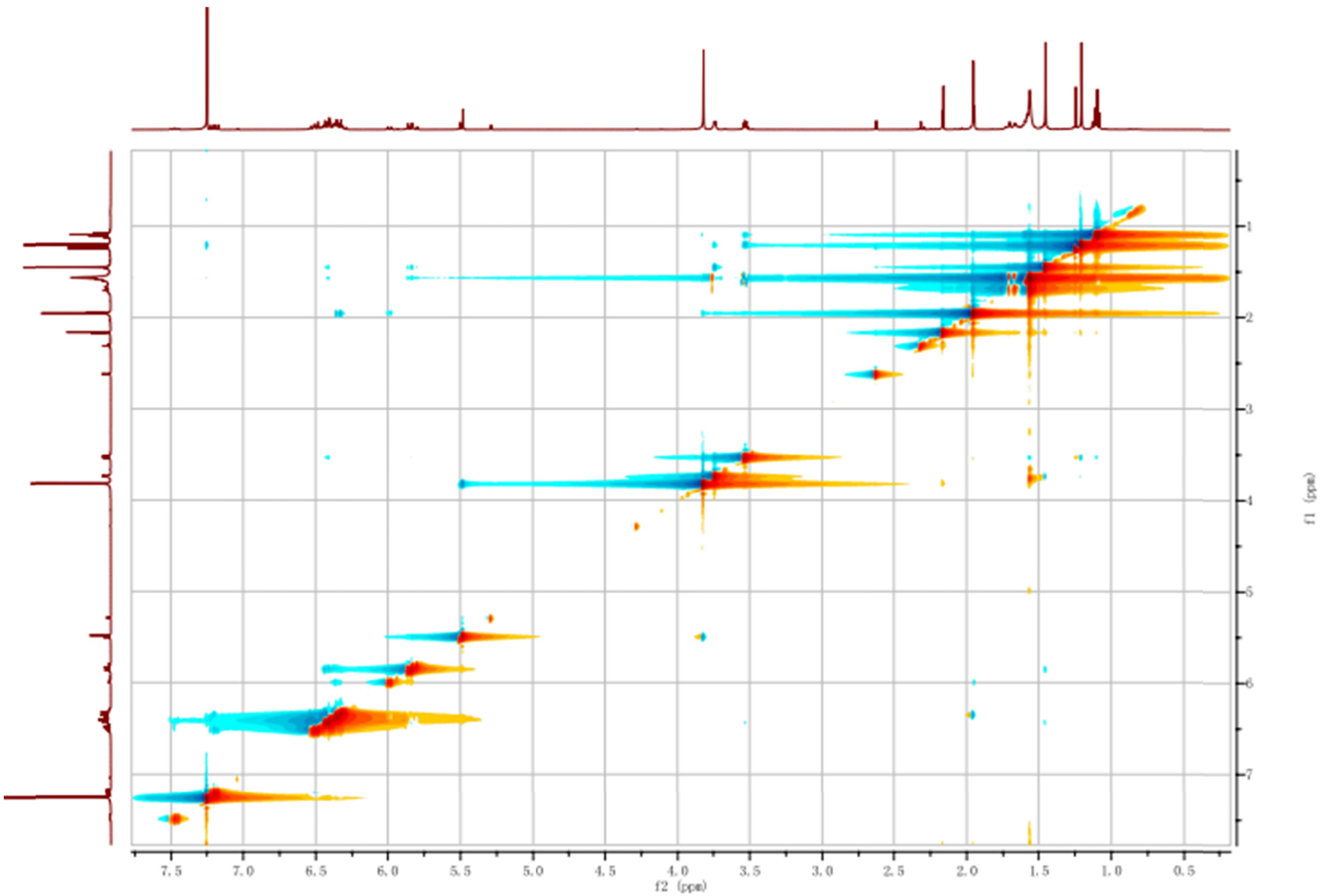
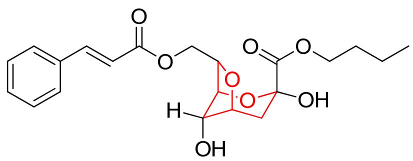
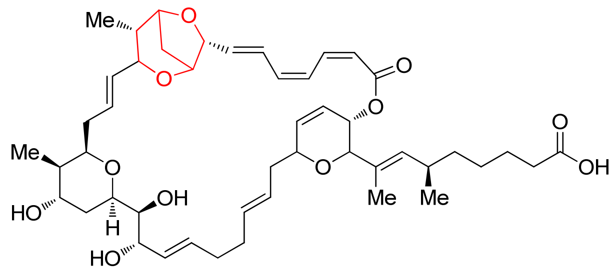


Figure S47. NOESY of compound **10** in $CDCl_3$ (500 MHz)



decurrenside A



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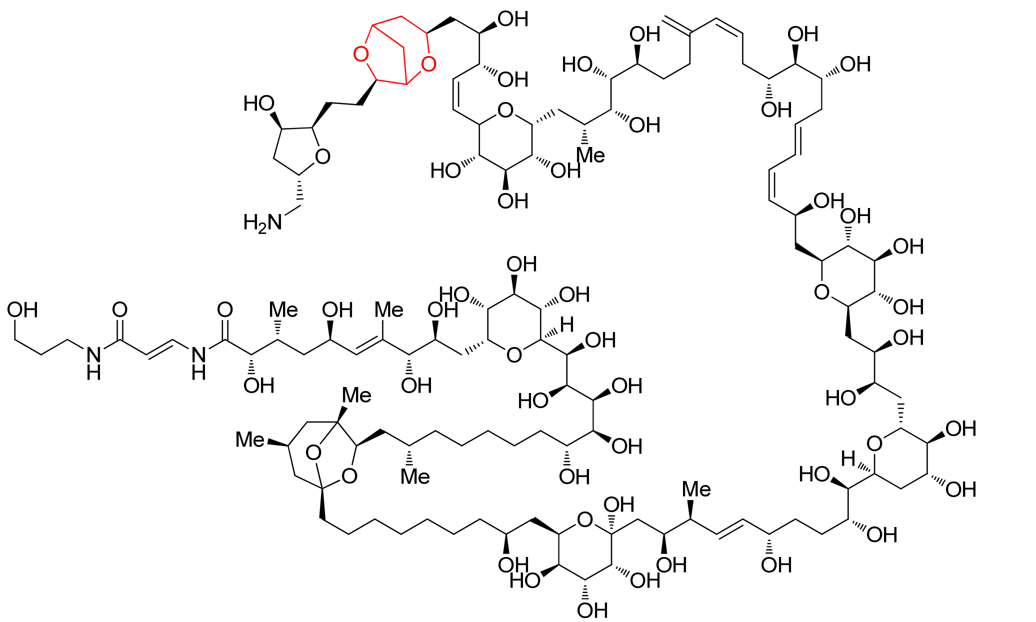
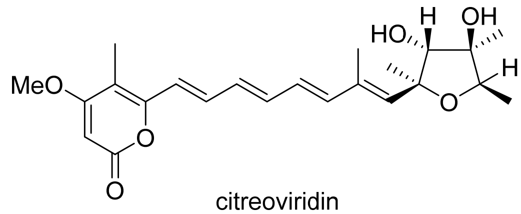
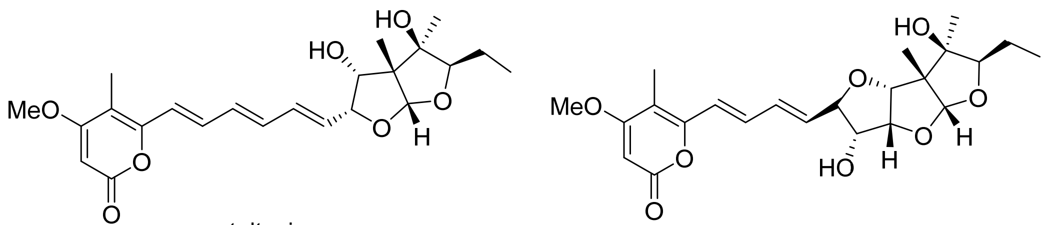


palytoxin

Scheme S1. Structures of decurrenside A, sorangicin A and palytoxin, which all contain a 2,6-dioxabicyclo[3.2.1]-octane (DBO) ring. The DBO moiety is shown in red.





Scheme S2. Structures of citreoviridin, asteltoxin and asteltoxin B.

Supplementary References

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Full list of authors in the Gaussian09 reference

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Supplementary Nucleotide and Protein Sequence Data

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8401 acgtgaagag gtag

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                 TVLLFKAEKPRH"
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     61 tttgtccagg tcctcaccaa ttccttctgg tggcgatgtt caaccaaggg catcctcgtc
    121 cctttttttc tcgacaacag tactaccaac cacatggaag ttggagctgg aaccggttac
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    301 aaccgcatct cccccgttcg ccccaaccgc gtactcgcca acatcatgga gccaattcct
    361 ctcaagggcc agaaattcga ctccatcgcc atcatgtacg tcttgcactg tattgctgcc
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    541 ttgatgtggt tgtataacta catcggcatg tttgataact gggacgatgg gaaagaagat
    601 tttttgaagc ctttgaggga gcattttgaa gttgttgaga gtgaggttgt tgggactgtg
    661 ctgctcttca aggcagagaa gcccagacat tga
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                    RYVMGHCGDLLVGNLARIVAGGGVLDFIPLTARSGKDWPPCPWQHSWGISESIDFCKK
                    FAVASLIVLIVVLARALDSPAGLSSGIRSSSWSF"
BASE COUNT     394 a   383 c   438 g   414 t
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       61 gcccattgcc ttgatcgcgc tggcattgac tatgttattc ttgagaaaca caaggacatt
      121 tttgcagagc ctggaatttc cattggactc atgcccaacg gctcccgcat cttggagcag
      181 ctaggaatat actcggacgt tcacgccctc tttgagggta ttaaaaagat ataccaatac
      241 atgcccgatg ggtactgtat cgagactgac agtccagtca acatagttga taggcaaggc
      301 tctgcttccc aaaaaaaaaa gaagaagaaa aaaaagagaa aaacctggag ctgactagcg
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BASE COUNT     391 a   406 c   386 g   434 t
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BASE COUNT     151 a   138 c   119 g   172 t
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BASE COUNT      442 a    558 c    568 g    372 t
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     1681 cactcttcac actccacagc gggaacagat gctaaaccac gtacagaaag aataccacgg
     1741 tcaccgctgc catgatcaat gaaatcaagc tggccattca acggtcggag acggggctga
     1801 tgagcctgct gagcttggcc aaccccattg cggagagcct atttctcgac caaggatacg
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     1921 caggggaggg ggttgcgtga
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```

Supplementary Computation Data

Cartesian coordinates, energies, free energies (1 atm, 298 K) and number of imaginary frequencies of all stationary points and values of imaginary frequencies of all transition structures.

Acid-catalyzed 5-*exo*-tet TS

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-730.918173
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-730.663185
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-731.148140
Number of Imaginary Frequencies =	1 (-437.56)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	-0.509872	0.439603	1.320640
C	-0.438501	1.164373	0.105885
C	-1.601577	0.581550	-0.755361
C	-1.353772	-0.892398	-0.428661
C	-1.178241	-0.825025	1.093262
C	-0.578125	2.655061	0.366153
O	-2.849272	0.966752	-0.260967
C	-2.383899	-1.878645	-0.924167
O	-0.043169	-1.148208	-0.964988
C	-0.388543	-1.956845	1.715591
H	-2.173892	-0.757240	1.545950
H	-1.486720	0.797255	-1.823762
H	-1.590255	2.836159	0.732115
H	0.124214	2.984080	1.134975
H	-0.405474	3.231992	-0.545599
H	-3.162461	1.743099	-0.747779
H	-3.366123	-1.605126	-0.533040
H	-2.434137	-1.863950	-2.017439
H	-2.135054	-2.891328	-0.596434
H	-0.113748	-1.365447	-1.911233
H	0.575650	-2.086528	1.218097
H	-0.220366	-1.747774	2.774730
C	2.042402	0.326589	0.080917
C	0.841274	0.799729	-0.607538
H	0.956468	1.063013	-1.655836
H	-0.949067	-2.892952	1.637794

H	1.863089	-0.021870	1.097519
C	3.064222	-0.446653	-0.664195
C	3.669045	-1.498377	-0.120518
H	3.278358	-0.123001	-1.680301
H	3.450964	-1.816189	0.895874
O	2.290684	1.774437	0.038047
H	2.115296	2.156181	0.918322
H	4.400582	-2.077213	-0.674008

Base-catalyzed 5-*exo*-tet TS

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-806.388506
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-806.137205
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-806.674425
Number of Imaginary Frequencies =	1 (-598.97)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	0.962457	-0.623191	1.286603
C	0.962336	-1.170253	-0.045265
C	1.893884	-0.183268	-0.770587
C	1.133942	1.080553	-0.340257
C	1.062912	0.819224	1.187897
C	1.473143	-2.593591	0.013826
O	3.232465	-0.281668	-0.322390
C	1.792548	2.401369	-0.700899
O	-0.108594	0.911782	-0.921665
C	-0.092733	1.497527	1.893752
H	2.007680	1.113213	1.672499
H	1.897684	-0.298941	-1.858126
H	2.507971	-2.607279	0.367059
H	0.837058	-3.181702	0.673913
H	1.434294	-3.041518	-0.983369
H	3.192364	-0.432682	0.636177
H	2.822274	2.449372	-0.331836
H	1.801738	2.518756	-1.789179
H	1.228064	3.239121	-0.274936
H	-1.318123	2.051855	-0.722076
H	-1.017829	1.360043	1.329424
H	-0.211709	1.094642	2.904287
C	-1.632875	-1.204225	0.133225
C	-0.423850	-1.018237	-0.662090

H	-0.534996	-1.151221	-1.730081
H	0.100379	2.573082	1.976970
H	-1.530693	-0.926471	1.194539
C	-2.926065	-0.688983	-0.421343
C	-3.870476	-0.099503	0.312713
H	-3.085863	-0.863696	-1.486736
H	-3.726380	0.063825	1.379519
O	-1.335110	-2.529064	-0.173851
O	-2.129349	2.622767	-0.622936
H	-2.801827	1.956351	-0.418639
H	-4.813633	0.234570	-0.111328

Base-catalyzed 5-*endo*-tet TS

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-806.344667
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-806.094020
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-806.637885
Number of Imaginary Frequencies =	1 (-572.66)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	-1.478213	-1.379119	0.676877
C	-0.245750	-0.665945	0.743716
C	-0.344707	0.247085	-0.478092
C	-1.791081	0.764043	-0.440837
C	-2.483472	-0.390873	0.365147
C	-0.109706	0.053788	2.085587
O	0.748450	1.059326	-0.612306
C	-1.953948	2.142403	0.185242
O	-2.354124	0.809145	-1.756510
C	-3.623790	-1.082786	-0.352235
H	-2.857710	0.029705	1.310990
H	-0.401520	-0.484026	-1.318815
H	-0.997727	0.631624	2.351005
H	0.050829	-0.688383	2.876093
H	0.752652	0.727411	2.066189
H	1.615789	1.895930	0.549169
H	-1.541946	2.196510	1.194919
H	-1.423060	2.878988	-0.427251
H	-3.013756	2.416387	0.221350
H	-1.741398	1.340148	-2.288796
H	-3.276225	-1.503617	-1.297958

H	-4.020915	-1.886383	0.274636
C	1.881827	-0.705525	-0.504983
C	1.085446	-1.434854	0.513011
H	1.627621	-1.466297	1.481551
H	-4.428291	-0.373176	-0.564624
H	1.522985	-0.785899	-1.518587
C	3.311381	-0.460188	-0.314663
C	4.068384	0.099917	-1.263626
H	3.730071	-0.690652	0.663578
H	3.653311	0.344071	-2.238525
O	1.238052	-2.577589	-0.216433
H	5.111904	0.345192	-1.092747
O	2.314631	2.312094	1.123659
H	3.119521	1.879227	0.805929

5-*endo*-tet product

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-730.528795
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-730.282782
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-730.761069
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	1.123653	1.318627	0.764290
C	-0.120250	0.654364	0.629879
C	0.157316	-0.235486	-0.564323
C	1.479109	-0.906009	-0.266430
C	2.116766	0.261201	0.620239
C	-0.547657	-0.017859	1.930358
O	-1.074671	-0.865621	-0.824399
C	1.410134	-2.250003	0.444514
O	2.156473	-1.065971	-1.505674
C	3.370544	0.871259	0.038351
H	2.323935	-0.138661	1.621700
H	0.389222	0.451390	-1.393539
H	0.288053	-0.512919	2.427846
H	-0.933573	0.744965	2.613540
H	-1.339152	-0.750907	1.752724
H	0.974301	-2.989799	-0.232136
H	2.419239	-2.580379	0.717744
H	0.809208	-2.217396	1.354039

H	2.979495	-1.551660	-1.341572
H	3.177907	1.249372	-0.968047
H	3.709996	1.692415	0.675039
C	-2.007189	0.238616	-0.755283
C	-1.305149	1.417808	0.033139
H	-1.977582	1.850019	0.784694
H	4.171272	0.127474	-0.014724
H	-2.171136	0.625292	-1.771855
C	-3.312032	-0.209097	-0.170799
C	-3.566923	-1.441242	0.258518
H	-4.079844	0.562414	-0.130703
H	-2.808105	-2.216387	0.213033
O	-0.881880	2.403022	-0.884622
H	-4.538595	-1.707345	0.661473
H	-0.031263	2.740332	-0.554987

5-*exo*-tet product

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-730.552673
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-730.307905
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-730.784526
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	-0.763764	0.593517	1.300589
C	-0.438392	1.152018	0.016838
C	-1.594201	0.670130	-0.866788
C	-1.361675	-0.794798	-0.459250
C	-1.449292	-0.656434	1.068015
C	-0.210836	2.634807	0.143449
O	-2.813511	1.227217	-0.451951
C	-2.196152	-1.874583	-1.094032
O	0.022229	-0.970869	-0.807575
C	-0.815911	-1.781939	1.859517
H	-2.499841	-0.530689	1.360308
H	-1.400843	0.829814	-1.935259
H	-1.140923	3.116808	0.455538
H	0.570454	2.835199	0.879069
H	0.103225	3.052939	-0.817284
H	-3.503266	0.934606	-1.065663
H	-3.253810	-1.717737	-0.861337

H	-2.065526	-1.861316	-2.179479
H	-1.903867	-2.860613	-0.720502
H	-0.738074	-1.500272	2.913221
H	0.182689	-2.012817	1.479113
C	1.908594	0.091045	0.316230
C	0.686182	0.288170	-0.577541
H	1.038777	0.698911	-1.534755
H	-1.432800	-2.683741	1.794221
H	1.549590	-0.143026	1.328701
C	2.761948	-1.045499	-0.184648
C	3.979138	-0.888552	-0.696222
H	2.313956	-2.034029	-0.106809
H	4.428842	0.095884	-0.789365
O	2.586269	1.334579	0.303438
H	3.322416	1.274605	0.930986
H	4.562801	-1.736256	-1.040135

Acid-catalyzed 6-*endo*-tet TS

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-730.930163
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-730.674640
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-731.161283
Number of Imaginary Frequencies =	1 (-250.22)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	-0.476090	0.540809	1.276692
C	-0.365397	1.238854	0.033434
C	-1.210634	0.448173	-0.989543
C	-1.226768	-0.964263	-0.377314
C	-1.303792	-0.628019	1.120324
C	-0.804793	2.685535	0.206718
O	-2.498438	1.012806	-1.030087
C	-2.351292	-1.853689	-0.872496
O	0.045642	-1.506519	-0.729096
C	-0.835409	-1.705594	2.073078
H	-2.339697	-0.344372	1.347712
H	-0.742330	0.431459	-1.982058
H	-0.758097	3.214990	-0.748689
H	-1.831373	2.707762	0.571010
H	-0.158594	3.195497	0.926118
H	-2.967790	0.639848	-1.791274

H	-3.325218	-1.416364	-0.641660
H	-2.266370	-1.997235	-1.953568
H	-2.299377	-2.836776	-0.392653
H	0.102035	-2.425166	-0.419308
H	0.186952	-2.023247	1.846175
H	-0.857288	-1.333853	3.099887
C	1.901125	-0.009634	-0.070601
C	1.114579	1.197646	-0.335843
H	1.352128	1.713238	-1.265841
H	-1.494263	-2.576832	2.013200
H	1.621225	-0.569106	0.814067
C	3.066805	-0.378582	-0.806030
C	3.767275	-1.457437	-0.412624
H	3.333116	0.187504	-1.692372
H	3.488972	-2.021189	0.473541
O	1.967372	1.734902	0.707109
H	1.512409	1.567883	1.560597
H	4.625477	-1.807970	-0.976447

Base-catalyzed 6-*endo*-tet TS

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-806.385620
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-806.133997
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-806.672276
Number of Imaginary Frequencies =	1 (-625.71)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	1.050110	-0.535351	1.325338
C	0.896719	-1.320305	0.125916
C	1.362989	-0.395905	-1.004397
C	0.852601	0.978554	-0.494028
C	1.297270	0.837973	0.982844
C	1.673179	-2.612539	0.288327
O	2.778883	-0.470135	-1.107386
C	1.527809	2.164547	-1.183583
O	-0.521572	1.058937	-0.668181
C	0.605637	1.765300	1.959904
H	2.384335	1.005642	1.034839
H	0.888668	-0.646140	-1.963348
H	2.722237	-2.411000	0.512763
H	1.229402	-3.190613	1.104560

H	1.617906	-3.209265	-0.628402
H	3.047069	0.138481	-1.811132
H	2.616117	2.170044	-1.058627
H	1.291066	2.154402	-2.253290
H	1.133413	3.097458	-0.766073
H	-1.376116	2.353134	-0.199273
H	-0.478665	1.726489	1.838800
H	0.862067	1.504874	2.991129
C	-1.452957	-0.487885	0.220668
C	-0.593561	-1.600759	-0.113621
H	-0.729781	-2.000475	-1.135917
H	0.924012	2.797793	1.779549
H	-1.243426	-0.028535	1.173634
C	-2.764881	-0.263596	-0.423661
C	-3.585932	-1.229644	-0.828395
H	-3.015686	0.787294	-0.556674
H	-3.333468	-2.270618	-0.649657
O	-1.354260	-2.221669	0.855847
O	-2.021475	3.074891	0.071790
H	-2.380587	2.757174	0.911269
H	-4.525273	-1.003974	-1.323257

6-*endo*-tet product

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-730.561084
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-730.314996
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-730.792331
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	-1.093413	0.667446	1.116339
C	-0.731882	1.154176	-0.198660
C	-1.054207	-0.012200	-1.137000
C	-0.565361	-1.175181	-0.255090
C	-1.193331	-0.770809	1.087883
C	-1.481578	2.440789	-0.476125
O	-2.418581	-0.106311	-1.482299
C	-0.951970	-2.555147	-0.738438
O	0.861668	-1.135607	-0.231066
C	-0.607551	-1.405801	2.333225
H	-2.261829	-1.033341	1.047181

H	-0.492900	0.032615	-2.074708
H	-2.560222	2.273933	-0.444807
H	-1.219386	3.195521	0.268306
H	-1.215283	2.820899	-1.466902
H	-2.953903	0.044964	-0.687501
H	-0.466241	-2.756274	-1.697327
H	-0.629667	-3.315131	-0.020607
H	-2.033664	-2.621740	-0.870827
H	0.479871	-1.325561	2.372181
H	-1.030181	-0.933491	3.223747
C	1.481682	0.045558	0.250233
C	0.787775	1.352916	-0.195535
H	1.121563	1.647726	-1.196172
H	-0.871930	-2.467100	2.356165
H	1.435803	0.066499	1.349816
C	2.925585	0.048290	-0.157911
C	3.515473	-0.901265	-0.876246
H	3.477770	0.924768	0.177223
H	2.965128	-1.774834	-1.210030
O	1.175516	2.390242	0.681166
H	0.741896	2.197274	1.530795
H	4.563833	-0.830841	-1.147667

β-keto ester conformer 1

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1079.128235
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1078.760482
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1079.431647
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

C	8.236914	-0.256017	0.140567
C	8.148151	1.214095	0.499263
C	6.833605	1.886823	0.227872
O	5.732348	1.089708	0.140855
C	5.805742	-0.285013	0.056885
C	6.972966	-0.980200	0.026167
C	4.495216	-0.890926	-0.025072
C	3.340373	-0.187193	-0.041768
C	2.037456	-0.797141	-0.111558
C	0.895670	-0.070968	-0.139034

C	-0.426852	-0.640474	-0.203548
C	-1.551119	0.110152	-0.240409
C	-2.888242	-0.433709	-0.299427
C	-3.990901	0.345073	-0.338710
C	-5.372324	-0.124837	-0.383602
C	-6.353516	0.807560	-0.357202
C	-7.812330	0.609390	-0.346652
C	-8.389753	-0.326853	0.426404
C	-9.860565	-0.607129	0.563646
C	-10.455717	0.088855	1.794773
C	-8.582515	1.581277	-1.209461
C	-5.608825	-1.609374	-0.481857
C	7.018175	-2.475428	-0.138959
O	6.680265	3.072583	0.131311
O	9.330569	-0.783660	0.017184
H	8.062066	-2.790522	-0.170622
H	6.529459	-2.990240	0.694086
H	6.534150	-2.797981	-1.065632
H	8.945421	1.778180	0.012277
H	8.320279	1.290813	1.582283
H	4.468587	-1.975121	-0.070853
H	3.378370	0.899501	-0.000329
H	1.985832	-1.884943	-0.142675
H	0.964437	1.017204	-0.110156
H	-0.506464	-1.727295	-0.222500
H	-1.459114	1.196972	-0.223822
H	-2.978324	-1.518747	-0.302914
H	-3.855381	1.427133	-0.318449
H	-6.036284	1.852496	-0.373602
H	-6.638733	-1.829294	-0.765737
H	-4.938666	-2.049577	-1.227429
H	-5.405341	-2.111221	0.471502
H	-8.374310	1.411142	-2.271824
H	-9.661046	1.518789	-1.055819
H	-8.269796	2.607866	-0.986149
H	-7.741250	-0.915269	1.075460
H	-10.005068	-1.689575	0.660230
H	-10.406513	-0.299904	-0.333085
H	-11.511909	-0.166800	1.920696
H	-9.922423	-0.206345	2.703997

H	-10.373088	1.175928	1.696342

β-keto ester conformer 2

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1079.127541
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1078.758822
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1079.431114
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

C	-8.252711	-0.027328	0.143009
C	-8.114682	1.431544	-0.244090
C	-6.750584	2.036772	-0.078776
O	-5.688529	1.183432	-0.074173
C	-5.826924	-0.183985	0.040746
C	-7.022995	-0.815314	0.175872
C	-4.548435	-0.859035	0.033397
C	-3.358746	-0.221509	-0.056569
C	-2.091034	-0.904162	-0.065853
C	-0.909798	-0.247284	-0.144187
C	0.377992	-0.893352	-0.148384
C	1.545273	-0.212269	-0.213516
C	2.848431	-0.833835	-0.210771
C	3.995892	-0.120565	-0.254779
C	5.350352	-0.664725	-0.230493
C	6.366121	0.234104	-0.204537
C	7.821421	0.077615	-0.102207
C	8.569128	1.175841	-0.336045
C	10.065309	1.284472	-0.267889
C	10.527834	1.845649	1.084609
C	8.450101	-1.233362	0.309964
C	5.474056	-2.167564	-0.244979
C	-7.132094	-2.303305	0.372584
O	-6.529678	3.213243	-0.002325
O	-9.360449	-0.495483	0.352388
H	-8.180643	-2.556900	0.534525
H	-6.777845	-2.854955	-0.504103
H	-6.558221	-2.642522	1.239903
H	-8.844087	2.043101	0.289582
H	-8.360083	1.503807	-1.313329
H	-4.576929	-1.941850	0.103467

H	-3.339578	0.864475	-0.123862
H	-2.099370	-1.991815	-0.002743
H	-0.917207	0.841720	-0.205304
H	0.394860	-1.981670	-0.091546
H	1.516180	0.876885	-0.268052
H	2.873687	-1.920990	-0.159698
H	3.917355	0.966687	-0.291580
H	6.060416	1.279512	-0.264367
H	6.486623	-2.509094	-0.440924
H	5.140040	-2.600328	0.705666
H	4.829695	-2.581421	-1.027718
H	8.531616	-1.935570	-0.526983
H	9.454664	-1.077062	0.707613
H	7.857934	-1.716502	1.091817
H	8.045904	2.103592	-0.571008
H	10.407037	1.952435	-1.066718
H	10.540706	0.315757	-0.447676
H	11.614457	1.969252	1.107402
H	10.068958	2.820401	1.277204
H	10.239626	1.173095	1.898658

Enol conformer 1

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1079.127453
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1078.757563
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1079.435479
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

C	8.172823	-0.124276	0.119986
C	8.111470	1.235803	0.219011
C	6.842640	1.896854	0.236744
O	5.735037	1.073477	0.145215
C	5.794767	-0.281115	0.047557
C	6.989100	-0.939731	0.032115
C	4.479913	-0.891379	-0.033754
C	3.329510	-0.184726	-0.030712
C	2.021731	-0.788704	-0.113098
C	0.881308	-0.062107	-0.119994
C	-0.443104	-0.628655	-0.201304
C	-1.567774	0.120998	-0.216479

C	-2.905116	-0.421761	-0.296104
C	-4.009004	0.355521	-0.312453
C	-5.389948	-0.114483	-0.379002
C	-6.373378	0.814082	-0.324262
C	-7.831903	0.613061	-0.329011
C	-8.413411	-0.351866	0.404648
C	-9.885291	-0.637918	0.518203
C	-10.495134	0.021261	1.762336
C	-8.599342	1.614169	-1.160533
C	-5.623410	-1.594971	-0.531158
C	7.158889	-2.428985	-0.070837
O	6.630605	3.089247	0.321474
O	9.324752	-0.810904	0.096170
H	7.720355	-2.806456	0.789174
H	6.206313	-2.954549	-0.113017
H	7.731535	-2.686700	-0.966989
H	4.440330	-1.972832	-0.102247
H	3.371489	0.900596	0.035663
H	1.967665	-1.875506	-0.173194
H	0.950458	1.024888	-0.061381
H	-0.522422	-1.714638	-0.253605
H	-1.477185	1.206938	-0.165798
H	-2.993681	-1.506325	-0.336764
H	-3.875701	1.436577	-0.254651
H	-6.058275	1.859572	-0.302385
H	-6.651515	-1.806200	-0.827958
H	-4.948535	-2.007253	-1.288379
H	-5.423735	-2.130380	0.404624
H	-8.391328	1.477972	-2.227864
H	-9.678125	1.549406	-1.009232
H	-8.284242	2.632248	-0.904239
H	-7.769302	-0.962856	1.036963
H	-10.029683	-1.722815	0.581187
H	-10.421474	-0.304786	-0.375311
H	-11.552848	-0.237451	1.867865
H	-9.972905	-0.301096	2.668774
H	-10.411076	1.110733	1.697250
H	8.999354	1.853086	0.284123
H	10.079380	-0.203165	0.163112

Enol conformer 2

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1079.126743
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1078.755480
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1079.434937
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

C	-8.183789	0.087937	0.133140
C	-8.066141	1.443557	0.024991
C	-6.772549	2.045961	-0.080348
O	-5.700285	1.171874	-0.062527
C	-5.816368	-0.178280	0.044611
C	-7.036097	-0.781055	0.143913
C	-4.530219	-0.851185	0.039282
C	-3.347900	-0.205174	-0.050428
C	-2.072853	-0.880014	-0.051294
C	-0.893836	-0.222043	-0.131147
C	0.395893	-0.867892	-0.128824
C	1.564637	-0.191190	-0.197867
C	2.866454	-0.817810	-0.190657
C	4.017637	-0.111826	-0.241442
C	5.369679	-0.663515	-0.215329
C	6.391314	0.228511	-0.200032
C	7.846340	0.063779	-0.101796
C	8.600389	1.154297	-0.350081
C	10.097606	1.254204	-0.289898
C	10.570360	1.832479	1.051704
C	8.468029	-1.246895	0.322093
C	5.484340	-2.167166	-0.217596
C	-7.266995	-2.260866	0.263778
O	-6.512457	3.227377	-0.182564
O	-9.363227	-0.542547	0.237438
H	-7.893735	-2.614923	-0.560392
H	-6.338595	-2.829198	0.253174
H	-7.797431	-2.489787	1.193122
H	-4.539843	-1.932821	0.115024
H	-3.336823	0.880483	-0.124559
H	-2.076622	-1.967613	0.018911
H	-0.901953	0.866535	-0.199617
H	0.411174	-1.956052	-0.064367
H	1.539555	0.897664	-0.260258

H	2.886426	-1.904803	-0.131380
H	3.945571	0.975599	-0.286654
H	6.092204	1.275382	-0.267829
H	6.494645	-2.516548	-0.411395
H	5.148275	-2.590228	0.736701
H	4.836754	-2.583462	-0.996393
H	8.543586	-1.958038	-0.507870
H	9.474382	-1.092798	0.716145
H	7.874518	-1.718667	1.109859
H	8.082238	2.082930	-0.592827
H	10.439858	1.907948	-1.100267
H	10.566104	0.280006	-0.457648
H	11.657821	1.949841	1.067462
H	10.118362	2.812780	1.232082
H	10.282129	1.173945	1.877113
H	-8.927650	2.100183	0.015268
H	-10.088795	0.103134	0.227177

Pyrone-polyene 11, conformer 1

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1118.410291
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1118.013022
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1118.722816
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

C	-7.742345	0.050712	0.103381
C	-7.629795	1.408781	-0.001290
C	-6.334221	2.014049	-0.101913
O	-5.256553	1.151013	-0.082866
C	-5.366526	-0.201382	0.021133
C	-6.582134	-0.809559	0.114812
C	-4.075405	-0.865806	0.018952
C	-2.896700	-0.213232	-0.067430
C	-1.618257	-0.882404	-0.064236
C	-0.441195	-0.220914	-0.141168
C	0.850184	-0.863883	-0.134238
C	2.018206	-0.185883	-0.200904
C	3.320557	-0.811615	-0.188954
C	4.471768	-0.105692	-0.238369
C	5.823764	-0.657487	-0.207868

C	6.845691	0.234167	-0.193747
C	8.300569	0.069137	-0.093015
C	9.055576	1.157973	-0.345546
C	10.552829	1.257095	-0.284585
C	11.025133	1.841403	1.054532
C	8.921055	-1.239875	0.337817
C	5.938074	-2.161164	-0.205305
C	-6.800551	-2.291655	0.229881
O	-6.084471	3.198092	-0.201165
O	-8.901248	-0.607628	0.204039
H	-7.422422	-2.648701	-0.596532
H	-5.866950	-2.851371	0.219732
H	-7.331576	-2.528006	1.156879
H	-4.077925	-1.947475	0.094610
H	-2.890707	0.872413	-0.141752
H	-1.618089	-1.970014	0.006811
H	-0.451984	0.867567	-0.210714
H	0.867143	-1.951964	-0.068185
H	1.992298	0.902844	-0.265106
H	3.340765	-1.898497	-0.127388
H	4.399903	0.981637	-0.286238
H	6.547123	1.280932	-0.265548
H	6.949015	-2.511449	-0.394068
H	5.598083	-2.581341	0.748869
H	5.293436	-2.579572	-0.985438
H	8.998267	-1.954659	-0.488868
H	9.926610	-1.084303	0.733350
H	8.325740	-1.708104	1.126334
H	8.538279	2.085785	-0.593222
H	10.896140	1.906710	-1.097831
H	11.020720	0.281774	-0.447425
H	12.112679	1.958010	1.070456
H	10.573798	2.822907	1.229967
H	10.735880	1.187071	1.882917
H	-8.477678	2.078450	-0.013950
C	-10.103657	0.154964	0.208083
H	-10.206129	0.713997	-0.726654
H	-10.912689	-0.566941	0.301117
H	-10.117268	0.844089	1.057649

Pyrone-polyene 11, conformer 2

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1118.411018
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1118.014574
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1118.723365
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

C	7.729913	-0.117170	0.095788
C	7.665018	1.244333	0.196989
C	6.390377	1.899609	0.220917
O	5.283120	1.080020	0.132774
C	5.345134	-0.275830	0.033402
C	6.539254	-0.931367	0.012408
C	4.029332	-0.885930	-0.043411
C	2.879061	-0.179485	-0.037163
C	1.570903	-0.783941	-0.115806
C	0.430071	-0.058218	-0.121250
C	-0.894273	-0.625758	-0.199714
C	-2.019569	0.122835	-0.215039
C	-3.356629	-0.421186	-0.292474
C	-4.461226	0.354999	-0.310207
C	-5.841856	-0.116337	-0.375043
C	-6.826117	0.811473	-0.322860
C	-8.284500	0.609151	-0.327007
C	-8.865250	-0.353881	0.409730
C	-10.336880	-0.640945	0.524151
C	-10.947428	0.021787	1.766057
C	-9.052680	1.606928	-1.161837
C	-6.074091	-1.597465	-0.522806
C	6.705645	-2.420818	-0.093446
O	6.181345	3.092438	0.308913
O	8.865481	-0.821465	0.063304
H	7.264154	-2.802095	0.766721
H	5.751614	-2.943342	-0.139624
H	7.280344	-2.677586	-0.988390
H	3.988900	-1.967381	-0.111340
H	2.920762	0.905855	0.028591
H	1.517212	-1.870871	-0.174699
H	0.498525	1.028904	-0.064062
H	-0.972832	-1.711916	-0.250047
H	-1.929893	1.208953	-0.166516

H	-3.444270	-1.505934	-0.330506
H	-4.328879	1.436322	-0.255200
H	-6.511970	1.857313	-0.304004
H	-7.102179	-1.810506	-0.818367
H	-5.399268	-2.011352	-1.279200
H	-5.873376	-2.129946	0.414425
H	-8.843949	1.467850	-2.228657
H	-10.131470	1.541399	-1.010897
H	-8.738870	2.626119	-0.908365
H	-8.220632	-0.962258	1.044046
H	-10.480236	-1.725769	0.590740
H	-10.873376	-0.311311	-0.370467
H	-12.004852	-0.237739	1.872532
H	-10.424791	-0.296891	2.673562
H	-10.864598	1.111129	1.697238
H	8.535781	1.881000	0.261292
C	10.095329	-0.108154	0.136826
H	10.162964	0.443408	1.079160
H	10.879178	-0.861711	0.089179
H	10.189222	0.582639	-0.706303

5-*exo*-tet TS formic acid system, conformer 1

M06-2X/6-31G(d)−IEFPCM(water) Energy = -1109.429332

M06-2X/6-31G(d)−IEFPCM(water) Free Energy = -1109.144622

M06-2X/6-311++G(d,p)−IEFPCM(water) Energy = -1109.807916

Number of Imaginary Frequencies = 1 (-712.70)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	-0.530516	-1.659941	0.232935
C	0.064498	-0.915282	-0.834163
C	1.566995	-1.222168	-0.651000
C	1.623302	-0.915068	0.851964
C	0.393276	-1.695313	1.348547
C	-0.500564	-1.376779	-2.163640
O	1.892630	-2.548656	-0.983930
C	2.921497	-1.242002	1.558130
O	1.291654	0.465543	0.926086
C	-0.251763	-1.153504	2.608149
H	0.675690	-2.745470	1.508453

H	2.209185	-0.555887	-1.233859
H	-1.590308	-1.346912	-2.136398
H	-0.145993	-0.724841	-2.966571
H	-0.166132	-2.397540	-2.362736
H	1.197502	-3.125210	-0.626004
H	2.824772	-1.089064	2.637439
H	3.195188	-2.283631	1.370074
H	3.715975	-0.591006	1.182913
H	2.119923	1.020980	0.596800
H	-1.188338	-1.683037	2.802768
H	0.413013	-1.307779	3.463991
C	-1.257979	1.147343	0.085421
C	-0.099793	0.572512	-0.589487
H	0.537318	1.243162	-1.162632
H	-0.453853	-0.084516	2.513150
H	-1.817171	0.455990	0.719366
C	-1.134874	2.506281	0.682570
C	-1.689267	2.824021	1.849011
H	-0.561702	3.236300	0.111602
H	-2.268925	2.093047	2.408473
O	-1.737939	1.135665	-1.263831
H	-2.613593	0.395418	-1.315608
H	-1.590303	3.814454	2.282003
C	-4.292175	-0.536403	-0.354922
O	-3.584490	-0.420744	-1.422253
O	-4.117501	0.027196	0.722724
H	-5.145207	-1.234672	-0.474420
C	3.354724	2.020265	-1.049529
H	4.287551	2.523972	-1.387673
O	2.473797	1.807402	-1.896062
O	3.334633	1.731387	0.187149

6-*endo*-tet TS formic acid system

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1109.432981
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1109.144631
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1109.813660
Number of Imaginary Frequencies =	1 (-544.61)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	1.245022	-1.748880	0.936213
C	0.363334	-1.487797	-0.168835
C	1.190175	-0.647833	-1.159572
C	2.115347	0.124953	-0.195046
C	2.459594	-0.997172	0.789823
C	-0.166701	-2.798061	-0.718899
O	1.921456	-1.520072	-2.002625
C	3.324977	0.764394	-0.858068
O	1.358198	1.116477	0.488864
C	2.959987	-0.550786	2.147783
H	3.209500	-1.642135	0.309951
H	0.555928	0.036661	-1.736592
H	0.654049	-3.414595	-1.087399
H	-0.697117	-3.328408	0.076175
H	-0.873036	-2.604982	-1.531811
H	2.309928	-0.987873	-2.711977
H	2.993520	1.528670	-1.568365
H	3.950346	1.249066	-0.102186
H	3.930055	0.020434	-1.383156
H	1.066562	1.826833	-0.201353
H	3.061820	-1.412607	2.812702
H	3.942407	-0.078710	2.048590
C	-0.487136	0.365082	1.331648
C	-0.790827	-0.613202	0.300160
H	-1.364526	-0.221808	-0.541505
H	2.275389	0.170897	2.599863
H	0.137772	0.014410	2.142142
C	-1.238840	1.600733	1.486830
C	-1.085772	2.344736	2.585944
H	-1.873137	1.895627	0.656560
H	-0.425968	2.030208	3.390915
O	-1.630981	-1.168208	1.306584
H	-2.648145	-0.834857	1.038748
H	-1.603427	3.290104	2.712567
C	-4.110080	-0.651788	-0.536871
O	-3.828312	-0.321595	0.668420
O	-3.422212	-1.318609	-1.311429
H	-5.095346	-0.271115	-0.881963
C	-0.346649	2.739130	-1.819771
H	-0.601100	3.542040	-2.550880

O	-1.155109	1.806562	-1.689134
O	0.758198	2.909052	-1.222115

5-*exo*-tet TS formic acid system, conformer 2

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1109.428867
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1109.142060
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1109.807172
Number of Imaginary Frequencies =	1 (-727.23)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	-1.623099	0.999455	-1.486653
C	-0.571236	0.040447	-1.361774
C	-1.283408	-1.205996	-0.787373
C	-2.048865	-0.458567	0.304019
C	-2.637999	0.704619	-0.507290
C	0.093442	-0.177180	-2.703149
O	-2.181924	-1.796623	-1.693048
C	-3.045510	-1.256928	1.115043
O	-1.009188	0.093370	1.108228
C	-2.970066	1.944955	0.298012
H	-3.528845	0.345974	-1.039345
H	-0.572669	-1.926917	-0.363077
H	-0.629453	-0.623044	-3.392345
H	0.435592	0.779361	-3.099670
H	0.965594	-0.825571	-2.584752
H	-1.683844	-2.380221	-2.283716
H	-3.561869	-0.609797	1.830691
H	-3.785171	-1.712930	0.451953
H	-2.527685	-2.044301	1.669547
H	-0.610002	-0.669165	1.729375
H	-3.283144	2.751293	-0.370800
H	-3.791553	1.740150	0.992034
C	0.757548	1.875578	-0.057803
C	0.381584	0.477449	-0.264883
H	1.056202	-0.274730	0.141129
H	-2.098303	2.271275	0.870529
H	0.000675	2.593041	-0.393568
C	1.315882	2.244067	1.279958
C	0.918108	3.314726	1.961625
H	2.077078	1.574119	1.679143

H	0.162896	3.988829	1.562633
O	1.699804	1.586583	-1.077348
H	2.698930	0.956698	-0.582860
H	1.326471	3.557815	2.937818
C	3.669975	-0.822236	-0.539658
O	3.513655	0.364997	-0.034590
O	3.167184	-1.254180	-1.564600
H	4.352558	-1.446835	0.064261
C	0.861168	-2.340336	2.176412
H	1.210418	-3.154745	2.849092
O	1.479615	-2.153713	1.119689
O	-0.153249	-1.706625	2.609959

Acid-catalyzed C-O bond cleavage TS

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-730.918124
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-730.663466
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-731.151952
Number of Imaginary Frequencies =	1 (-86.20)

M06-2X/6-31G(d)−IEFPCM(water) Geometry

O	-0.216005	-0.082715	1.248663
C	0.030569	0.934691	0.283923
C	-0.648523	0.400626	-0.989343
C	-1.824752	-0.483666	-0.459414
C	-1.565668	-0.542712	1.072261
C	-0.500872	2.289633	0.737678
O	-0.999278	1.470333	-1.824912
C	-3.190234	0.101787	-0.770152
O	-1.808621	-1.760678	-1.075583
C	-1.706442	-1.904749	1.712040
H	-2.237407	0.169184	1.570099
H	0.045707	-0.279388	-1.511908
H	-0.008579	2.584517	1.668278
H	-0.310728	3.045462	-0.026562
H	-1.578558	2.247534	0.912780
H	-1.280731	1.111230	-2.679889
H	-3.370125	0.089728	-1.848090
H	-3.267311	1.131973	-0.415971
H	-3.962911	-0.501398	-0.283757
H	-0.957890	-2.189540	-0.895089

H	-1.528652	-1.831302	2.787478
H	-2.715847	-2.290599	1.548468
C	2.236140	-0.300349	-0.089909
C	1.547449	0.984890	0.168801
H	1.897942	1.811273	-0.450350
H	-0.989981	-2.614413	1.290244
H	1.788760	-1.177913	0.377878
C	3.465399	-0.417631	-0.755428
C	4.027720	-1.652030	-0.836160
H	3.912488	0.447985	-1.232808
H	3.571716	-2.509388	-0.348761
O	2.178650	1.063432	1.453253
H	4.939022	-1.824511	-1.399943
H	1.590393	0.593645	2.080747

Alkoxide conformer 1

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1078.658639
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1078.302257
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1078.977105
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

C	8.308580	-0.228763	0.135989
C	8.201073	1.177409	0.248479
C	6.976435	1.862442	0.260111
O	5.820345	1.078734	0.147298
C	5.859508	-0.279427	0.041893
C	7.027069	-0.969615	0.031096
C	4.523677	-0.856215	-0.050255
C	3.386681	-0.130843	-0.051050
C	2.067993	-0.716716	-0.136946
C	0.930096	0.012093	-0.146340
C	-0.395580	-0.555590	-0.225376
C	-1.525653	0.184929	-0.242188
C	-2.859003	-0.370989	-0.314263
C	-3.973184	0.390882	-0.331522
C	-5.348555	-0.098592	-0.385632
C	-6.346055	0.814786	-0.334784
C	-7.801596	0.590892	-0.327504
C	-8.363901	-0.371438	0.424233

C	-9.830282	-0.679538	0.552024
C	-10.442312	-0.012338	1.790787
C	-8.589317	1.566936	-1.169774
C	-5.561775	-1.583887	-0.520645
C	7.146280	-2.460415	-0.081759
O	6.762754	3.070853	0.355078
O	9.387093	-0.865637	0.121805
H	7.700051	-2.853732	0.777003
H	6.186823	-2.974404	-0.140140
H	7.733606	-2.721921	-0.968120
H	4.457254	-1.937284	-0.121123
H	3.447699	0.953367	0.017337
H	2.004448	-1.803890	-0.195645
H	0.999884	1.099193	-0.089628
H	-0.470626	-1.642591	-0.272592
H	-1.444598	1.271894	-0.197056
H	-2.935199	-1.457027	-0.346638
H	-3.854618	1.474160	-0.282835
H	-6.047389	1.865331	-0.325782
H	-6.589515	-1.813441	-0.804695
H	-4.888199	-1.993992	-1.280270
H	-5.344540	-2.106634	0.418391
H	-8.381408	1.420424	-2.235781
H	-9.666458	1.485006	-1.014808
H	-8.291869	2.593868	-0.927929
H	-7.706336	-0.962810	1.061452
H	-9.956698	-1.765619	0.631806
H	-10.378377	-0.368471	-0.342193
H	-11.494684	-0.287234	1.908432
H	-9.907849	-0.312175	2.697834
H	-10.377207	1.077319	1.709005
H	9.103197	1.773058	0.331085

Alkoxide conformer 2

M06-2X/6-31G(d)−IEFPCM(water) Energy =	-1078.657858
M06-2X/6-31G(d)−IEFPCM(water) Free Energy =	-1078.300274
M06-2X/6-311++G(d,p)−IEFPCM(water) Energy =	-1078.976507
Number of Imaginary Frequencies =	0

M06-2X/6-31G(d)−IEFPCM(water) Geometry

C	-8.329517	0.013324	0.109760
C	-8.148979	1.413650	0.022416
C	-6.890812	2.030946	-0.050875
O	-5.776386	1.181303	-0.036947
C	-5.886197	-0.174451	0.044648
C	-7.087946	-0.799225	0.117951
C	-4.582452	-0.827306	0.041215
C	-3.407107	-0.169664	-0.032353
C	-2.123025	-0.833398	-0.031459
C	-0.944502	-0.175440	-0.101331
C	0.346366	-0.822692	-0.097789
C	1.518834	-0.153408	-0.163730
C	2.817810	-0.788380	-0.157574
C	3.974835	-0.093185	-0.213389
C	5.323264	-0.655719	-0.191780
C	6.353375	0.226164	-0.191162
C	7.808213	0.048523	-0.105715
C	8.570781	1.126993	-0.378195
C	10.069564	1.213311	-0.337278
C	10.564306	1.822799	0.982184
C	8.420874	-1.262081	0.332002
C	5.425437	-2.160305	-0.184687
C	-7.282408	-2.283860	0.206287
O	-6.615659	3.228225	-0.126483
O	-9.440124	-0.562088	0.179637
H	-7.859394	-2.637990	-0.654683
H	-6.349544	-2.845818	0.249550
H	-7.876218	-2.527650	1.092784
H	-4.574276	-1.910932	0.102432
H	-3.407681	0.916625	-0.094976
H	-2.121527	-1.922228	0.031088
H	-0.951497	0.913618	-0.163339
H	0.357830	-1.911519	-0.036384
H	1.500906	0.935752	-0.224251
H	2.829518	-1.875651	-0.096679
H	3.912204	0.994772	-0.261255
H	6.063647	1.275346	-0.264651
H	6.432142	-2.519533	-0.379453
H	5.088711	-2.574449	0.773298
H	4.771609	-2.576150	-0.958532

H	8.488010	-1.984129	-0.489298
H	9.429833	-1.111397	0.720882
H	7.826115	-1.719413	1.127296
H	8.059432	2.057163	-0.629672
H	10.408379	1.841643	-1.169077
H	10.526943	0.230606	-0.484712
H	11.652827	1.931381	0.981337
H	10.122677	2.811359	1.142198
H	10.281165	1.188969	1.828384
H	-9.018769	2.060517	0.013184