Supporting Information

Biosynthesis of Heptacyclic Duclauxins Requires Extensive Redox Modifications of the Phenalenone Aromatic Polyketide

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Experimental Procedures:

1. Strains and Culture Conditions.

The *Talaromyces stipitatus* strain (syn. *Penicillium stipitatum*) was obtained from Agriculture Research Service Culture Collection (NRRL 1006) and was used as the parental strain in our study. Both the wild-type and its mutant strains were grown on MEPA medium (3% malt extract broth, BD; 0.3% soy flour, agar 15 g/L) for both production of secondary metabolites and mRNA extraction at 28 °C. For gene knock-out in *T. stipitatus*, potato dextrose agar (PDA, BD) with 1.2 M sorbitol and 400 μg/mL hygromycin was used for protoplast regeneration and antibiotic resistance selection. *Escherichia coli* DH10B (Invitrogen) and Trans1-T1 (TransGen) was used for routine plasmid cloning. *Saccharomyces cerevisiae* strain BJ5464-NpgA (*MATα ura3-52 his3-Δ200 leu2-Δ1 trp1pep4::HIS3 prb1Δ1.6R can1 GAL*) was used for *in vivo* yeast DNA recombination cloning and the yeast expression host[1]. YPD (20 g/L peptone, 10 g/L yeast extract, 20 g/L dextrose) was used for the routine growth of yeast strain BJ5464-NpgA and its derivatives at 30 °C. SD dropout semisynthetic medium was used for selection of plasmids transformed into *S. cerevisiae*.

2. *T. stipitatus* RNA Isolation, Purification and cDNA Preparation.

Mycelia of *T. stipitatus* were inoculated in MEPA medium, incubated at 28 °C for 5 days, and collected for lyophilization. The mycelia were ground after freezing with liquid nitrogen, and solubilized in 1 mL Trizol (Invitrogen), and vortexed for 3 min. 200 μL of chloroform was added, and the mixture was vortexed and centrifuged at 13, 000 rpm for 15 min. The supernatant (600 μL) was extracted once again with an equal volume of chloroform. RNA was precipitated from the supernatant with an equal volume of isopropanol and resuspended in RNase-free water. The genomic DNA was further digested with RNase-free DNase I Kit (Takara). Genomic DNA was further removed by digestion with RNase-free DNase I (Takara). RNA was purified by RNAclean purification kit (Tiangen). RNA integrity was confirmed by electrophoresis on TAE buffer (Tris-acetate-EDTA) agarose gel, and the concentration was determined by Nanodrop (Thermo Scientific). The first-strand cDNA was synthesized from 500 ng of total RNA by EasyScript® reverse transcriptase (Transgen) with random primers and oligo-dT_{18} primer (Takara) according to the manufacturer's instructions. PCR was performed with Q5 high-fidelity DNA polymerase (New England Biolabs) in the presence of 30 ng of reverse transcribed RNA. Primers are listed in Table S1 (Supplementary information). The gene expression level was analyzed by PCR using the specific primers (Supplementary Table S1) and cDNA as template.

3. Plasmid Construction.

Primers and plasmids were listed in Supplementary Table S1 and S3, respectively. For construction of the plasmids for gene knock-out in *T. stipitatus* which based on the split-marker strategy, [1-2] the plasmid pAN7-14 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. [1] For constructions of the upstream DNA fragments (Up) and downstream DNA fragments (Dn) of specific genes, including *duxD*-Up, *duxD*-Dn, *duxG*-Up, *duxH*-Up, *duxJ*-Up, *duxL*-Up, duxL-Dn, *duxM*-Up and *duxM*-Dn, *in vivo* yeast recombination cloning was performed by transforming the *S. cerevisiae* BJ5464-NpgA with DNA fragments with >35 bp overlaps and includes a 2 μm plasmid backbone (derived from pRS426) using an *S.C.* EasyComp Transformation kit (Zymo Research). The plasmid in the correct transformant screened by colony PCR was rescued using Yeast Plasmid Miniprep kit (Solarbio). For construction of knockout cassettes of *duxA*-Up and *duxA*-Dn, the homologous regions were amplified and digested by *Sac*I/*Pst*I and *Hind*III/*Pst*I, respectively. The Hph-Up fragment and Hph-Dn were digested with *Pst*I/*Hind*III and *Pst*I/*EcoR*I, simultaneously. Then, the above fragments, *i.e. duxA*-Up/Hph-Up and *duxA*-Dn/Hph-Dn were colligated into pET30a which was digested by *Sac*I/*Hind*III and *Sac*I/ *EcoR*I, respectively. The same strategy was used for construction of the knockout cassettes of *duxG*-Dn, *duxH*-Dn, *duxI*-Up, *duxI*-Dn, and *duxJ*-Dn. The correct plasmids were used as template to amplify the knockout cassettes.

Yeast expression plasmids pXW02 (LEU2 marker), pXW06 (TRP1 marker), pXW55 (URA3 marker), pGSS1 and pGSS4 were used for construction of the heterologous expression plasmids by *in vivo* homologous recombination in yeast. [1] All the enzymes were expressed under a *ADH2p* promoter and a *ADH2t* terminator. For DuxM expression, primers De-25 + De-26 was used to amplify the DNA fragments of *duxM* cDNA, and ligated into *Nde*I/*Pme*I-digested pXW02 to create the plasmid pGSS41. The *ADH2p-duxM-ADH2t* cassette was amplified by the primer pair Not-F/Not-R and then ligating into the pGSS4 digested with *Not*I to give pGSS49. For DuxJ expression, primer pair De-20a/De-21a was used to amplify the DNA fragments of *duxJ* cDNA, and transformed into *Nde*I/*Pml*I-digested pXW02 to create the plasmid pGSS40 using yeast homologous recombination. For DuxL expression, primer pair *dux*L-XW55-F/*dux*L-XW55-R was used to amplify the DNA fragments of *duxJ* cDNA, and transformed into *Spe*I/*Pme*I-digested pXW55 to create the plasmid pGSS92. For DuxB' expression, primer pair *duxB*'-2-XW02-F+*duxB*'-2-XW02-R was used to amplify the DNA fragments of *duxB*' cDNA, and transformed into *Nde*I/*Pme*I-digested pXW02 to create the plasmid pGSS117 using yeast homologous recombination.

For heterologous expression in *A. nidulans* A1145 *Δ*ST*Δ*EM, plasmids pYTU, pYTP, pYTR were used as backbones to insert genes which contain auxotrophic markers for uracil (pyrG), pyridoxine (pyroA), and riboflavin (riboB), respectively. Genes to be expressed were amplified through Polymerase Chain Reaction (PCR) using the gDNA of *T. stipitatus* as a template. The pieces were mixed with the corresponding backbone digested with *Pac*I and *Swa*I and assembled using yeas homologous recombination with BJ5464-NpgA. Primer pair of *duxD*-pYTU-F+*duxD*-pYTU-R was used to amplify *duxD* gDNA, and inserted into *Pac*I/*Swa*I-digested pYTU using yeast homologous recombination to create the plasmid pGSS119. Primer pairs of A31-Dux-P+A32-Dux-U, A23-Dux-P+A24-Dux-P, A25-Dux-P+A26-Dux-P, A27-Dux-P+A28-Dux-P, and A29-Dux-P+A30-Dux-P were used to amplify *duxD*, promoter *GlaA*, *duxM*, promoter *AmyB*, *duxJ* with gDNA as templates, respectively, and then inserted into *Pac*I/*Swa*I-digested pYTR using yeast homologous recombination to create the plasmid pGSS101. Primer pairs including A1-Dux-P+A2-Dux-P, A3-Dux-P+A4-Dux-P, A5-Dux-P+A6-Dux-P, A7-Dux-P/A8-Dux-P were used to amplify *duxE*, promoter *GlaA*, and *duxI* with gDNA as template, respectively, and then inserted into *Pac*I/*Swa*I-digested pYTR using yeast homologous recombination to create the plasmid pGSS100. Primer pair A35-Dux-U/A36-Dux-U was used to amplify *duxG* with gDNA as template and inserted into *Pac*I/*Swa*I-digested pYTU using yeast homologous recombination to create the plasmid pGSS102.

4. Construction of *T. stipitatus* Mutants.

All the mutants were constructed in *T. stipitatus* based on the hygromycin split-marker strategy. [1-2] The upstream split-marker DNA for KO of *duxA*, *duxD*, *duxG*, *duxH*, *duxI*, *duxJ*, *duxL*, and *duxM* genes were amplified with primer pairs including AKC *Uko* for+S2, DKC *Uko* for+S2, GKC *Uko* for+S2, HKC *Uko* for+S2, IKC *Uko* for+S2, JKC *Uko* for+S2, LKC *Uko* for+S2, and MKC *Uko* for+S2 from plasmids AKC *Uko*, DKC *Uko*, GKC *Uko*, HKC *Uko*, IKC *Uko*, JKC *Uko*, LKC *Uko*, and MKC *Uko*, respectively. The downstream split-marker DNA for KO of *duxA*, *duxD*, *duxG*, *duxH*, *duxI*, *duxJ*, *duxL*, and *duxM* genes were amplified with primer pairs including AKC *Dko* rev+S3, DKC *Uko* for+S3, GKC *Dko* rev+S3, HKC *Dko* rev+S3, IKC *Dko* rev+S3, JKC *Dko* rev+S3, LKC *Dko* rev+S3, and MKC *Dko* rev+S3 from plasmids AKC *Dko*, DKC *Dko*, GKC *Dko*, HKC *Dko*, IKC *Dko*, JKC *Dko*, LKC *Dko*, and MKC *Dko*, respectively. The PCR products of knockout cassettes were recovered by gel recycling kit (Axygen) and dissolved in 50 μL STC buffer. Split-marker DNA was introduced into *T. stipitatus* by protoplast transformation. Fresh spores of *T. stipitatus* were collected on PDA for 14 days at 28 °C, and then induced to young germ tubes in PDB (BD) with concentration of 10^8 spores mL-1 for 36 hours at 28 °C with 180 rpm agitation. Mycelia were harvested, 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, 2 mg/mL yatalase in osmotic medium) and incubated at 30 °C for overnight. The solution was transferred to a 50 mL tube and an equal volume of trapping buffer (0.6 M sorbitol, 0.1 M Tris-HCI, pH 7.0)

was added before centrifuging at 4 °C (3,750 rpm) for 10 min. Protoplasts in the supernatant were transferred to a new tube and an equal volume of STC buffer (1.2 M sorbitol, 10 mM $CaCl_2$, 10 mM Tris HCl, pH 7.5) was added before centrifuging at 4 °C (3,750 rpm) for 10 min. After washing twice with STC buffer, protoplasts were gently mixed with DNA and incubated for 50 minutes on ice. One milliliter of PEG solution (60% PEG 4000, 50 mM $CaC1_2$, 50 mM Tris-HCI, pH 7.5) was added to 100 µL of protoplast mixture, and the mixture was incubated for 20 min at room temperature and spread on the regeneration selection medium (PDA, 1.2 M sorbitol, 400 µg/mL hygromycin B). After incubation at 28 °C for 4-5 days, the transformants were inoculated on fresh PDA selection medium with stationary incubation for about 4 days to confirm the genotype by diagnostic PCRs after miniprep genomic DNA. Primers used for the generation of gene deletion cassettes and diagnostic PCRs are listed in Table S1.

5. Yeast Reconstitution of Phenalenone Biosynthesis Pathway.

S. cerevisiae strain BJ5464-NpgA was transformed with appropriate plasmid(s) as described in supplementary Table S3. Yeast cells containing transformed plasmid(s) were initially cultured in the drop out medium overnight and transferred to 50 mL of liquid YPD medium for additional 3-day culture. Extracted samples from approximately 0.5 mL of culture were loaded for LC/MS analysis.

6. Heterologous Production in *A. nidulans* A1145 ΔSTΔEM.

Protoplasts were generated by scraping spores from a solid CD medium (10 g/L glucose, 50 mL/L 20x nitrate salts, 1 mL/1L trace elements, 20% agar) plate. The spores were transferred to 25 mL of liquid CD medium and incubated for 12-13 hours at 37 °C at 250 rpm. After incubation, the germlings were harvested and washed with 10 mL of Osmotic medium (1.2 M $MgSO_4$, 10 mM $NaPO_4$) twice. The germlings were then transferred into 10 mL of Osmotic medium containing 30 mg of lysing enzyme from *Trichoderma* and 20 mg of Yatalase. The culture was incubated for 12 hours at 28 °C at 80 rpm. The cells were poured into a 30 mL Corex tube and overlaid with 10 mL of Trapping buffer (0.6 M Sorbitol, 0.1 M Tris-HCl). The tube was centrifuged at 5,000 rpm. The protoplasts were then removed from the interface of the two buffers and transferred to sterile tubes. 2 volumes of STC buffer (1.2 M sorbitol, 10 mM $CaCl_2$, 10 mM Tris-HCl) was added to the protoplasts. DNA and 60% PEG4000 solution were added to the protoplast solution and incubated at room temperature for 20 min. The cells were then plated onto solid CD-sorbitol medium (10 g/L glucose, 50 mL/L 20x nitrate salts, 1 mL/1 L trace elements, 20% agar, 1.2 M sorbitol). Until transformants being seen, the spores were re-streaked onto solid CD-ST production medium (20 g/L starch, 20 g/L peptone, 50 mL/L nitrate salts, 1 mL/1 L trace elements). Deuterium labeled precursors used for feeding were purchased from Cambridge Isotope Laboratories.

7. ***In Sillico* Genomic Analysis.**

The PhnA protein sequence, as described in previous work, [1] was used for local BLASTP query of *Talaromyces stipitatus* genome (NZ_ABAS00000000.1). [3] AntiSMASH platform was used for genome mining and bioinformatic analysis of secondary metabolites biosynthetic clusters.[4] Gene predictions of the coding sequences were performed *via* FGENESH program (www.softberry.com) or 2ndFind platform (http://biosyn.nih.go.jp/2ndFind/) and manually checked based on homologous gene/proteins in the NCBI database. The function of the protein was further analyzed in Phyre2 database (http://www.sbg.bio.ic.ac.uk/phyre2/html).

8. Chemical Complementation Studies.

For chemical complementation of *ΔduxI* strain of *T. stipitatus* with compounds (solubilized in DMSO), spores of *ΔduxI* were inoculated in modified MEPA plate together with 10 µg/mL compounds **7, 14, 15, 19, 8, 13** and **12,** and further cultured for 3 days at 28 °C. The mycelia and medium were extracted for LC/MS analysis.

9. LC/MS Analysis.

Cultures of *Aspergillus nidulans*, *S. cerevisiae* or *E. coli* cells were extracted with acetone:ethyl acetate (20 : 80). After brief centrifugation, the supernatant organic phase was dried and solubilized in DMSO for LC/MS injection. LC-MS analysis 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% acetonitrile (MeCN)-H_2O in 15 minutes followed by 95% MeCN for 5 minutes with a flow rate of 0.3 mL/min. Cultures of wild type strain, or deletant mutants of *T. stipitatus* cells were extracted with ethyl acetate. After 12,000 rpm, 10 min centrifugation, the supernatant organic phase was dried and solubilized in acetonitrile for LC-MS analyses. LC-MS analyses were performed on a Waters ACQUITY H-Class UPLC-MS with a PDA detector and a QDA mass detector (ACQUITY UPLC®BEH, 1.7 μm, 2.1× 50 mm, C18 column) using positive and negative mode electrospray ionization with a linear gradient of 5-99% acetonitrile-H_2O (v/v, 0.02% formic acid) for 8 minutes followed by 99% acetonitrile-H_2O (v/v, 0.02% formic acid) for 4 minutes with a flow rate of 0.4 mL/min.

10. Protein Expression and Purification of DuxM from *E. coli* strain BL21 (DE3).

The expression plasmid pGSS17 was transformed into *E. coli* strain BL21 (DE3) for expression of DuxM. Cells in LB medium (1 L) supplemented with ampicillin (100 mg/L) inoculated with BL21(DE3)/pGSS17 were grown to an OD_{600} of 0.6. Protein expression was then induced with 0.12 mM of isopropylthio-*β*-D-galactoside (IPTG, Sigma-Aldrich), and continued cultivation with shaking (250 rpm) for additional 16 h at 16 °C. All of the enzyme purification steps were performed at 4 °C using Nickel-NTA affinity chromatography following standard protocols. Purified proteins were concentrated and exchanged into buffer C (50 mM Tris-HCl, pH 7.9, 50 mM NaCl, and 5% glycerol) with Centriprep filters (Amicon). The protein was stored in buffer C at -80 °C. Protein concentration was determined by Bradford assay using bovine serum albumin as a standard.

11. Protein Expression and Purification of DuxJ from *S. cerevisiae* strain BJ5464-NpgA.

The expression plasmid pGSS31 was transformed into *S. cerevisiae* strain BJ5464-NpgA for expression of DuxJ. Cells in YPD medium inoculated with BJ5464-NpgA/pGSS31 were grown for 3 days for protein expression. *S. cerevisiae* cells were harvested by centrifugation (5,000 rpm, 15 min, and 4 °C), re-suspended in 300 mL yeast lysis buffer and lysed with sonication on ice. Cellular debris was removed by centrifugation (17,000g, 1 h, and 4 °C). FLAG-tagged proteins were purified by using Anti-FLAG®M1 Agarose Affinity Gel (Sigma-Aldrich), following the manufacture's protocols. Purified proteins were concentrated, buffer exchanged into 50 mM potassium phosphate buffer (pH 7.0) with 10% glycerol, concentrated, sub-aliquoted and flash frozen. Protein concentrations were determined using the Bradford assay.

12. Expression and Preparation of DuxL-containing Microsomes for *in vitro* Assay.

For expression of DuxL, the cells were grown in YPD medium supplemented with 1% dextrose at 28 °C with shaking for 48 hours. The microsomes were prepared according to the protocol described previously.[1] Briefly, the cells were harvested by centrifugation (3,750 rpm at 4 °C for 10 min) and the cell pellet was washed with 100 mL of TES buffer (50 mM Tris–HCl, pH, 7.5, 1 mM EDTA, 0.6 M sorbitol). The cells were centrifuged as above, resuspended in 100 mL of TES-M (TES supplemented with 10 mM 2-mercaptoethanol), and allowed to incubate at room temperature for 10 min. The yeast cells were centrifuged again at 3,750 rpm for 10 min, and the pellet was resuspended in 2.5 mL of extraction buffer (1% bovine serum albumin, fraction V, 2 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, all dissolved in TES). Zirconia/silica beads (0.5 mm in diameter, Biospec Products) were added until skimming the surface of the cell suspension. Cell walls were disrupted manually by hand shaking in a cold room for 10 min at 30-s intervals separated by 30-s intervals on ice. Cell

extracts were transferred to a 50-mL centrifuge tube, the Zirconia/silica beads were washed three times with 5 mL of extraction buffer, and the washes were pooled with the original cell extracts. Finally, microsomes were obtained by differential centrifugation at 10,000g for 10 min at 4°C to remove cellular debris followed by centrifugation at 100,000g for 70 min at 4°C. The microsomal pellets were resuspended in 1.5 mL of TEG-M buffer (50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 20% glycerol, and 1.5 mM 2-mercaptoethanol) and stored frozen at -80 °C.

13. *In vitro* Activity Assay for DuxM, DuxJ and DuxL.

DuxM activity was assayed by monitoring the conversion of substrates into products as analyzed by LC/MS. A typical 100 μL assay solution contained 100 mM potassium phosphate buffer (pH 8.0), 30 μM DuxM and 1mM substrate. The reactions were performed at 28 °C and quenched with methanol.

For *in vitro* activity assay of DuxJ, a typical 100μL assay solution contained 100 mM phosphate buffer (pH 8.0), 4 mM NADPH and 1 mM **14**. The reaction was performed at 28 °C and quenched with equal volume of methanol. Protein precipitate from the reactions was removed by centrifugation. The supernatant was then analyzed on LC-MS. LC-MS analyses were performed on a Shimadzu 2020 EV LC-MS (Kinetex™ 1.7 μm C18 100 Å, LC Column 100×2.1 mm) 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 3 minutes with a flow rate of 0.3 mL/min.

For *in vitro* synthesis of **12**, 12.5 mg/mL (wet weight) microsomal fractions containing DuxL and Pe-CPR, 0.5 mM **11**, 4 mM NADPH and NADPH regeneration system (BD) solution A and B in 100 mM PBS, pH 7.4, were incubated in a total 100 μL reaction. The reaction was incubated at room temperature overnight and extracted with 100 μL ethyl hexanes-acetate (1:1) twice. The organic phase was dried and dissolved in 20 μL MeOH for analysis on LC-MS.

14. Chemical Analysis and Compound Isolation.

From yeast**,** the ethyl acetate extract from a 4 L YPD liquid medium was evaporated to dryness to yield the crude extract. From *T. stipitatus*, the acetone extract from a 2 L PDA solid agar extract of mutant was evaporated to dryness and partitioned between ethyl acetate/H_2O three times. To purify the desired compound, crude extracts were separated by silica chromatography, Sephadex-LH20, reverse phase-C18 and additional HPLC steps as required. The purity of each compound was checked by LC-MS, and the structure was confirmed by NMR. ^{1}H, ^{13}C and 2D NMR spectra were obtained using DMSO-d_6 or CD_3OD as solvent on Bruker AV500 spectrometer with a 5 mm dual cryoprobe at the UCLA Molecular Instrumentation Center.

15. Purification of 14.

The crude extract was subjected to HPLC chromatography on a Phenomenex Luna column (250×10 mm, 5 μm, 40 ºC, flow 4 mL/min) eluted with isocratic wash of 30% MeCN in water for 20 minutes to yield a sub-fraction. The sub-fraction was further subjected to HPLC chromatography on Phenomenex Luna column (250×10 mm, 5 μm, 40 ºC, flow 4 mL/min) with isocratic wash of 50% MeOH in water for 20 minute. The purified fraction was cooled immediately on ice. The solvent was quickly removed under reduced pressure, and remaining water was frozen and lyophilized to yield 0.7 mg of **14**.

16. Computational Methods.

DFT calculations were performed using Gaussian 09.[5] Geometry optimizations and frequency calculations were performed using unrestricted B3LYP (UB3LYP)[6-8] with the 6-31G(d) basis set. Entropies were calculated for 1 atm and 298.15 K. Single point energy calculations were performed using the dispersion-corrected functional (U)B3LYP-D3(BJ)[9-10] with the 6-311++G(d,p) basis set, within the CPCM polarizable conductor model (diethyl

ether, ε = 4)[11-12] to have an estimation of the dielectric permittivity in the enzyme active site. The use of a dielectric constant ε=4 has been proved to be a good and general model to account for electronic polarization and small backbone fluctuations in enzyme active sites.[13-14] All stationary points were verified as minima or first-order saddle points by a vibrational frequency analysis.

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TableS1. Primers used in this study

Primer	Sequence of primer (5'-3')
De-25	ATATCATATGGACCAAAAACACAAA
De-26	ATATGTTTAAACCTATATGTCGATATCCACA
De-20a	TGATAATGAAAACTATAAATCGTGAAGGCATGTTTAAACCTACTCGCCTGTCTCCAAG
De-21a	ATCAACTATCAACTATTAACTATATCGTAATACCATATGCAGGCAGTTTCGATA
Orf12-XW55-F	ATATATACTAGTATGGATTCATCAAATACCGGCC
Orf12-XW55-R	ATATATCACGTGTCAGTTCTGACCACTCAAAATG
Orf-2-2-XW02-R	ATCAACTATCAACTATTAACTATATCGTAATACCATATGTCTCAAATACGAGTCTT
Orf-2-2-XW02-F	ATCAACTATCAACTATTAACTATATCGTAATACCATATGTCTCAAATACGAGTCTT
Orf4-pYTU-F	GAGCCTGAGCTTCATCCCCAGCATCATTACACCTCAGCATTATGAATTCCGAGGTTTGTG
Orf4-pYTU-R	CTTCAACACAGTGGAGGACATACCCGTAATTTTCTGGGCATGTACCACATGGGTCATCGA
A31-Dux-P	CATTACCCCGCCACATAGACACATCTAAACATTAATTAAATGAATTCCGAGGTTTGTGAG
A32-Dux-U	TCATAGGTCGCCAGGTACGACCAGTTCGGAAGATCAGGATCGACCAGTGGACCTTTTAG
A23-Dux-P	ATAATAAGCTCTCCCAACTAAAAGGTCCACTGGTCGATCCTGATCTTCCGAACTGGTCG
A24-Dux-P	TGTAAGGCTTTGATGTACTCTTTGTGTTTTTGGTCCATTGCTGAGGTGTAATGATGCTG
A25-Dux-P	AGCCTGAGCTTCATCCCCAGCATCATTACACCTCAGCAATGGACCAAAAACACAAAGAG
A26-Dux-P	TATTGTTATATCATTTATAGCTCGTTCGGCACCTTTAATCATATATATTACATTTTCGA
A27-Dux-P	GATGCAAGAAACAACACCGTCGAAAATGTAATATATATGATTAAAGGTGCCGAACGAGC
A28-Dux-P	AAATTCCGTAACCCAGGGCTCCAATGACCAGAAATCATAAATGCCTTCTGTGGGGTTTA
A29-Dux-P	CTCCCTTCTCTGAACAATAAACCCCACAGAAGGCATTTATGATTTCTGGTCATTGGAGC
A30-Dux-P	TAAAGGGTATCATCGAAAGGGAGTCATCCAATTTAAATAGACAAGCTACGTGTCTGCTG
A1-Dux-P	TCTGAACAATAAACCCCACAGAAGGCATTTTTAATTAAATGGAGTCAAAACTGCCACAA
A2-Dux-P	TCATAGGTCGCCAGGTACGACCAGTTCGGAAGATCAGGGACTCGCGAACTGGACATGAT
A3-Dux-P	CCCTACTTAGATGGCCGATCATGTCCAGTTCGCGAGTCCCTGATCTTCCGAACTGGTCG
A4-Dux-P	GTCGGAGCCTCCGCGAAGGCCACAGCTGTGCCCGCCATTGCTGAGGTGTAATGATGCTG
A5-Dux-P	AGCCTGAGCTTCATCCCCAGCATCATTACACCTCAGCAATGGCGGGCACAGCTGTGGCC
A6-Dux-P	TAGCATCCTGAAATTTTACTGGCTGACGACACGCGTTTGTAAGATAGGTACAGTCGAAT
A7-Dux-P	ATTCGACTGTACCTATCTTACAAACGCGTGTCGTCAGCCAGTAAAATTTCAGGATGCTA
A8-Dux-P	GATGAGACCCAACAACCATGATACCAGGGGATTTAAATGGGTGGTTTACATAGATACAA
A35-Dux-U	CTTCATCCCCAGCATCATTACACCTCAGCATTAATTAAATGATTGCAAACAACCGCATT
A36-Dux-U	GAGGACATACCCGTAATTTTCTGGGCATTTGCGGCCGCGTAACATGTTAATTTGGATGG
For *duxA* knockout	
AKC Up for	AGGAGCTCGAACCCGCTCCTTGTACT (*Sac*I)
AKC Up rev	ATCTGCAG CAAGCGAGCATCCAGTTC (*Pst*I)
Hph-Up F	ATCTGCAG GGAGGTCAACACATCAATG 3(*Pst*I)
Hph-Up R	AGAAGCTTGTCTGCTGCTCCATACAAGCCAAC (*Hind*III)
AKC *Uko* for	GAACCCGCTCCTTGTACT
S2	GTCTGCTGCTCCATACAAGCCAAC
AKC Dn for	AGAAGCTTCGGAGGGCGAAGAATCTCGT (*Hind*III)
AKC Dn rev	ATCTGCAG AAATTGACGCTTAGACAACTT *(Pst*I)
Hph-Dn F	ATCTGCAG ATTGGGCAGATAGAAGAC (*Pst*I)
Hph-Dn R	ACGAATTC ACTCGGGATGTTGTCAC (*EcoR*I)
S3	TCGGAGGGCGAAGAATCTCGT
AKC *Dko* rev	ACTCGGGATGTTGTCAC
Check LsA for	GCCTCGTTGTTGCTTGTA
Check LsA rev	ACTGGCGTCGGTCTCATT
Check RsA for	CGACAGCCGAGAACAAAG
Check RsA rev	TCAAAACCGGAGAGAATG
Check *hph* for	TCGGAGGGCGAAGAATCTCG
Check *hph* rev	GTTGGCTTGTATGGAGCAGCAGAC
For *duxD* knockout	
DKC Up for	ATATATCCCGGGATATCTTTGTGCAACAGAAT
DKC Up rev	CTATACGACTTTGATGGTCGTTGTAGCGGCCGCTATTACGGTTCATCTGATAT
Hph-Up for	TATATCAGATGAACCGTAATAGCGGCCGCTACAACGACCATCAAAGTCGTATAG
Hph-Up rev	GGAACAAAAGCTGGAGCTCCACCGCGGTACCGTCTGCTGCTCCATACAAGCCAAC
DKC Uko for	ATATCTTTGTGCAACAGAAT
S2	GTCTGCTGCTCCATACAAGCCAAC
AKC Dn for	TTCCCAATACGTATTGGGAATTCCTGCAGCCCGGGCTCTTCCTGACCACGTGGAA
AKC Dn rev	CAGGTACACTTGTTTAGAGGTAATCCTGCGGCCGCATGAAGAGAAAATACCGGAT
Hph-Dn for	ATCCGGTATTTTCTCTTCATGCGGCCGCAGGATTACCTCTAAACAAGTGTACCTG
Hph-Dn rev	TCACTAAAGGGAACAAAAGCTGGAGCTCGGTACCTCGGAGGGCGAAGAATCTCGT
S3	TCGGAGGGCGAAGAATCTCGT
AKC Dko rev	CTCTTCCTGACCACGTGGAA
Check LsD for	CGGAATCTCATCGCCCTT
Check LsD rev	CCACGAGTAGTCCCCGAG
Check RsD for	AAATTGAACCACCGAACA
Check RsD rev	CCCCAGCATCCACTCTAA
For *duxG* knockout	
GKC Up for	GCCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTTCAGACGGAGACGATCCTC
GKC Up rev	GGTCGTTGTAGCGGCCGCGGCATTTGCCATGTAGCTGCCTACATTGCCTCCAGC
Hph-Up for	GCTGGAGGCAATGTAGGCAGCTACATGGCAAATGCCGCGGCCGCTACAACGACC
Hph-Up rev	TAAAGGGAACAAAAGCTGGAGCTCCACCGCGGTACCGTCTGCTGCTCCATACAA
GKC Uko for	TCAGACGGAGACGATCCTC

S2	GTCTGCTGCTCCATACAAGCCAAC
GKC Dn for	ATCTGCAG GCTGGCAATTTTGAGGAC (*Pst*I)
GKC Dn rev	AGAAGCTT ACGACCCCATGAGTATTT (*Hind*III)
Hph-Dn for	ACGAATTC TCGGAGGGCGAAGAATCTCGT (*EcoR*I)
Hph-Dn rev	ATCTGCAG AAATTGACGCTTAGACAACTT (*Pst*I)
S3	TCGGAGGGCGAAGAATCTCGT
AKC Gko rev	ACGACCCCATGAGTATTT
Check LsG for	CGGTTTCCCATTTTTCAC
Check LsG rev	GGGGAATAGCCACTGAGA
For *duxH* knockout	
HKC Up for	CGGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTTCAGACGGAGACGATCCTC
HKC Up rev	GGTCGTTGTAGCGGCCGCGGCATTTGCCATGTAGCTGCCTACATTGCCTCCAGC
Hph-Up for	GCTGGAGGCAATGTAGGCAGCTACATGGCAAATGCCGCGGCCGCTACAACGACC
Hph-Up rev	TAAAGGGAACAAAAGCTGGAGCTCCACCGCGGTACCGTCTGCTGCTCCATACAA
HKC Uko for	TCAGACGGAGACGATCCTC
S2	GTCTGCTGCTCCATACAAGCCAAC
HKC Dn for	ATCTGCAG GCTGGCAATTTTGAGGAC (*Pst*I)
HKC Dn rev	AGAAGCTT ACGACCCCATGAGTATTT (*Hind*III)
Hph-Dn for	ACGAATTC TCGGAGGGCGAAGAATCTCGT (*EcoR*I)
Hph-Dn rev	ATCTGCAG AAATTGACGCTTAGACAACTT (*Pst*I)
S3	TCGGAGGGCGAAGAATCTCGT
HKC Gko rev	ACGACCCCATGAGTATTT
Check LsH for	ATGCCCATTTCGCTGATC
Check LsH rev	ACGTTGCGAAGACCTGAC
Check RsH for	TCGTCACCGTTGGAAATC
Check RsH rev	AAGGGGAAACAGGAAACT
For *duxI* knockout	
IKC Up for	ACGAATTC CGGTTTGTCATGGTTTAG (*EcoR*I)
IKC Up rev	ATCTGCAG GGTCCGTATTATCTAGGC (*Pst*I)
Hph-Up for	ATCTGCAG GGAGGTCAACACATCAATG (*Pst*I)
Hph-Up rev	AGAAGCTTGTCTGCTGCTCCATACAAGCCAAC (*Hind*III)
IKC Uko for	CGGTTTGTCATGGTTTAG
S2	GTCTGCTGCTCCATACAAGCCAAC
IKC Dn for	ACGAATTC TCGGAGGGCGAAGAATCTCGT (*EcoR*I)
IKC Dn rev	ATCTGCAG AAATTGACGCTTAGACAACTT (*Pst*I)
Hph-Dn for	ATCTGCAG AAAAGGAGCGAGTTCATG (*Pst*I)
Hph-Dn rev	AGAAGCTTATGCGAAGTTACGGCTGT (*Hind*III)
S3	TCGGAGGGCGAAGAATCTCGT
IKC Gko rev	ATGCGAAGTTACGGCTGT
Check LsI for	CGCTGGCAATTTTGAGGA
Check LsI rev	GTCCGCAGTCAACCTCTA
Check RsI for	GAGGGCTGCGTTGTCTTT
Check RsI rev	TGTTCGAGCCGCAATTAA
For *duxJ* knockout	
JKC Up for	GGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTACTCAGATTATTAGTAGAT
JKC Up rev	TGGTAGCTATACGACTTTGATGGTCGTTGTAGCGGCCGCACGATGAAGACCTTGGACG
Hph-Up for	CGTCCAAGGTCTTCATCGTGCGGCCGCTACAACGACCATCAAAGTCGTATAGCTACCA
Hph-Up rev	TAAAGGGAACAAAAGCTGGAGCTCCACCGCGGTACCGTCTGCTGCTCCATACAAGCCAAC
JKC Uko for	ACTCAGATTATTAGTAGAT
S2	GTCTGCTGCTCCATACAAGCCAAC
JKC Dn for	ATCTGCAG AGCAAGGACGAAGTACCC (*Pst*I)
JKC Dn rev	AGAAGCTTAGCACACCGTCACGATAG (*Hind*III)
Hph-Dn for	ACGAATTC TCGGAGGGCGAAGAATCTCGT (*EcoR*I)
Hph-Dn rev	ATCTGCAG AAATTGACGCTTAGACAACTT (*Pst*I)
S3	TCGGAGGGCGAAGAATCTCGT
JKC Gko rev	AGCACACCGTCACGATAG
Check LsJ for	TTACCTTTGCGCTGTTGA
Check LsJ rev	ACCGCCACCGAGAGATAG
Check RsJ for	GGAATGGGCCTGTCACAC
Check RsJ rev	CGTCGGTATGAGATTTCC
For *duxL* knockout	
LKC Up for	TTCCCAATACGTATTGGGAATTCCTGCAGCCCGGGTGACTATCGATCAGCTAGCC
LKC Up rev	GCTATACGACTTTGATGGTCGTTGTAGCGGCCGCTGGCATTGGTTGATCGATTCA
Hph-Up for	TGAATCGATCAACCAATGCCAGCGGCCGCTACAACGACCATCAAAGTCGTATAGC
Hph-Up rev	GGAACAAAAGCTGGAGCTCCACCGCGGTACCGTCTGCTGCTCCATACAAGCCAAC
LKC Uko for	TGACTATCGATCAGCTAGCC
S2	GTCTGCTGCTCCATACAAGCCAAC
LKC Dn for	ATTCCCAATACGTATTGGGAATTCCTGCAGCCCGGGGTGTATCTTACATCTGTTT
LKC Dn rev	CACAGGTACACTTGTTTAGAGGTAATCCTGCGGCCGCTGGCTTTCAGAATCAGAT
Hph-Dn for	ATCTGATTCTGAAAGCCAGCGGCCGCAGGATTACCTCTAAACAAGTGTACCTGTG
Hph-Dn rev	TCACTAAAGGGAACAAAAGCTGGAGCTCGGTACCTCGGAGGGCGAAGAATCTCGT
S3	TCGGAGGGCGAAGAATCTCGT
LKC Gko rev	GTGTATCTTACATCTGTTT
Check LsL for	TCAAAACGAACGCCTTAG
Check LsL rev	TCTCCACGCAGGCATATT
Check RsL for	GAGCGATGCGGACAAATG

Check RsL rev	TGGCCGTTGTTCTTCTTAAT
For *duxM* knockout	
MKC Up for	GGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCCATCTCTTATGATCCCA
MKC Up rev	TGTAGCGGCCGCTCTAGAACTAGTGGATCCCCCGGGCTTGAAAAAGTCAATGATC
Hph-Up for	GATCATTGACTTTTTCAAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCTACA
Hph-Up rev	GGAACAAAAGCTGGAGCTCCACCGCGGTACCGTCTGCTGCTCCATACAAGCCAAC
MKC Uko for	GCCATCTCTTATGATCCCA
S2	GTCTGCTGCTCCATACAAGCCAAC
MKC Dn for	CAGGTACACTTGTTTAGAGGTAATCCTGCGGCCGCGATGAAGATACCATCATGAA
MKC Dn rev	TTCCCAATACGTATTGGGAATTCCTGCAGCCCGGGATCGGGGCAAGACATGGAGC
Hph-Dn for	TCACTAAAGGGAACAAAAGCTGGAGCTCGGTACCTCGGAGGGCGAAGAATCTCGT
Hph-Dn rev	TTCATGATGGTATCTTCATCGCGGCCGCAGGATTACCTCTAAACAAGTGTACCTG
S3	TCGGAGGGCGAAGAATCTCGT
MKC Gko rev	ATCGGGGCAAGACATGGAGC
Check LsM for	TAGGAACCCGCCCGTGTC
Check LsM rev	TGCCCCAATCTCCGCCAT
Check RsM for	GAGGGGCCGGTATTTCAT
Check RsM rev	ATCCAAGCGTCATCAATG
For DuxI expression in *S. cerevisiae*	
duxI-S1 for	AACTATTAACTATATCGTAATACCATCATATGGCGGGCACAGCTGTGGCCTTCGCGGAG
duxI -S1 rev	CGACATTGTTTGAAGGCTGATTAAAGCAGCCTATCTCACAGCCACTGC
duxI -S2 for	GCAGTGGCTGTGAGATAGGCTGCTTTAATCAGCCTTCAAACAATGTCG
duxI -S2 rev	ATGGAAACTATAAATCGTGAAGGCATGTTTAAACTCATGCTAATGCCCGGTGAAGGAAAC
Primers used for RT-PCR	
duxA for	ATGTTTATCAAGACCCAATG
duxA Rev	CTTCTGTACTATCTCCTCCACT
duxB for	GTGCGATTGGACTGAAAG
duxB Rev	GAGCCATAGAAAGCGGTA
duxC for	TCTAACATGCGAAACAAAC
duxC Rev	TGGAATGAGGCTGAGGTA
duxD for	CATACCCGACAAAGACGC
duxD Rev	TCAATCACTTCGCCACCC
duxE for	TACCATACGCCAAGGGAC
duxE Rev	CCATTGCGGAATACAGGA
duxF for	CGCGAGTCTACTGTCTAA
duxF Rev	AAACAGCTTCTCCATTG
duxG for	ATAACAGCCAGAGCAACTT
duxG Rev	CTTCGTAATGGTCCAATCT
duxH for	ACTGTTATACGGGATTCTTA
duxH Rev	GTCTCCTCTTCAACCAAA
duxI for	GGCAGTGGCTGTGAGATA
duxI Rev	CCCTTAGTGAGGCAGAAAT
duxJ for	ACTTGACAATACCGACTACCT
duxJ Rev	ACCTTTGAACCGCTCTTA
duxK for	AGATACTGCCAAACCACTG
duxK Rev	GTCCACCAATCTCCACAA
duxL for	TCGCTCACAATGAAGTCG
duxL Rev	GATAAGATACAATCCACCAAGT
duxM for	GGCCACGTTCGACCAGAT
duxM Rev	TCCCATTGGCCTTGCTTT
orf14 for	CAAGAACCACTGGGCACG
orf14 Rev	CGACGGTTGATCGTTTGG
orf15 for	TGCTACAGGTTGTTTCGTG
orf15Rev	ATGTTTGTTGCGGTATCTT
duxN for	GCTGTGTCCCAGTGACTT
duxN Rev	CACTGGCAGCGTGAATAG
duxA' for	GTGGACTGCGTGTTTCTG
duxA' Rev	TCTGCAAAGAGGCGCCCA
duxB' for	GTCCTCGCAATCAACCTC
duxB' Rev	CAGCCTAGTATGACGTAT
duxC' for	ACAAGCCCAAATGATGAC
duxC' Rev	AGAAGTGCCATTTCGGAT
duxD' for	CGACAAAGACGCCTGCTC
duxD' Rev	TAACAGGTGCGTCGTAAT
duxG' for	AGACGGGCAAACACACTG
duxG' Rev	TGGATTGGGAGGCTTAGA
duxI' for	GGCATCTGAAACTCCTAAA
duxI' Rev	CTCGCTTGGCTCATCCTT
duxL' for	GCCGCAGCTACGAAGGAT
duxL' Rev	ATCTGCCACTTCCATCTC
duxM' for	GCAATACCTTTACAGAAT
duxM' Rev	TGTCAACTCCATCGTCTC
duxN' for	GCTTGGCAGTCACTTTCC
duxN' Rev	TTTTTCTTCAGCCCACAA
duxO for	GTCTGTGCTTTCATCATA
duxO Rev	GCCCAGCTTGTGATTGCC

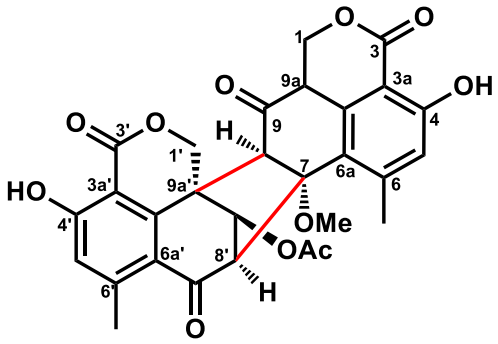
Table S2. Deduced functions of the ORFs in *dux* gene cluster

Gene	putative function	coverage/identity	Organism	Accession no.
duxA	aldo/keto reductase, putative	100/79	*Talaromyces marneffei* ATCC 18224	XP_002145784.1
duxB	FAD dependent oxidoreductase, putative	80/35	*Aspergillus flavus* NRRL3357	XP_002383905.1
duxC	C6 transcription factor, putative	100/72	*Talaromyces marneffei* ATCC 18224	XP_002145786.1
duxD	benzoate 4-monooxygenase cytochrome P450	99/83	*Talaromyces marneffei* ATCC 18224	XP_002145787.1
duxE	monooxygenase, putative (PhnB)	93/68	*Penicillium herquei* NRRL1040	AMP46752.1
duxF	conserved hypothetical protein (PhnD)	96/42	*Penicillium herquei* NRRL1040	AMP46754.1
duxG	putative isoflavone reductase family protein	99/83	*Talaromyces marneffei* ATCC 18224	XP_002145790.1
duxH	dienelactone hydrolase, putative	97/72	*Talaromyces marneffei* ATCC 18224	XP_002145791.1
duxI	polyketide synthase, putative (PhnA)	98/49	*Penicillium herquei* NRRL1040	AMP46751.1
duxJ	Maleylacetate reductase, putative	94/80	*Talaromyces marneffei* ATCC 18224	XP_002145793.1
duxK	O-methyltransferase, putative (PhnC)	94/38	*Penicillium herquei* NRRL1040	AMP46753.1
duxL	cytochrome P450, putative	99/76	*Talaromyces marneffei* ATCC 18224	XP_002145795.1
duxM	conserved hypothetical protein (cupin-2 family)	100/92	*Talaromyces marneffei* ATCC 18224	XP_002145796.1
duxN	MFS multidrug transporter	99/78	*Talaromyces marneffei* PM1	XP_002145783.1
duxA'	aldo/keto reductase, putative	100/86	*Talaromyces marneffei* ATCC 18224	XP_002145784.1
duxB'	NAD/FAD dependent oxidoreductase, putative	97/85	*Talaromyces stipitatus* ATCC 10500	XP_002478054.1
duxC'	C6 transcription factor, putative	94/81	*Talaromyces stipitatus* ATCC 10500	XP_002478055.1
duxD'	benzoate 4-monooxygenase cytochrome P450	99/76	*Talaromyces stipitatus* ATCC 10500	XP_002478056.1
duxG'	isoflavone reductase family protein (CipA)	98/90	*Talaromyces stipitatus* ATCC 10500	XP_002478059.1
duxH'	dienelactone hydrolase	100/70	*Talaromyces stipitatus* ATCC 10500	XP_002478061.1
duxI'	polyketide synthase, putative	16/69	*Talaromyces stipitatus* ATCC 10500	XP_002478062.1
duxL'	cytochrome P450, putative	100/87	*Talaromyces stipitatus* ATCC 10500	XP_002478065.1
duxM'	conserved hypothetical protein (cupin-2 family)	100/90	*Talaromyces stipitatus* ATCC 10500	XP_002478066.1
duxO	trichothecene 3-O-acetyltransferase, putative	94/25	*Talaromyces marneffei* ATCC 18224	XP_002149771.1

Table S3. *Saccharomyces cerevisiae* and *Aspergillus nidulans* strains used in this study

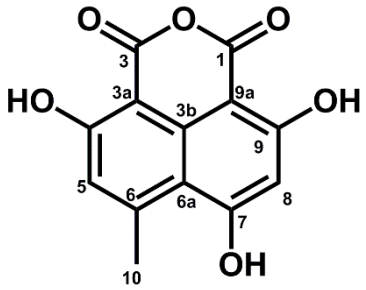
Genotype Description	**Description**
Yeast+PhnA+PhnB	*S. cerevisiae* BJ5464−NpgA + pGSS1 + pGSS4
Yeast+PhnA+PhnB+DuxM	*S. cerevisiae* BJ5464−NpgA + pGSS1 + pGSS4 + pGSS41
Yeast+PhnA+PhnB+DuxM+DuxJ	*S. cerevisiae* BJ5464−NpgA + pGSS14 + pGSS49 + pGSS40
Yeast+DuxL	*S. cerevisiae* BJ5464−NpgA + pGSS92
Yeast+DuxB′	*S. cerevisiae* BJ5464−NpgA + pGSS117
Yeast+DuxI	*S. cerevisiae* BJ5464−NpgA +*duxI*
A. nidulans +DuxD	*A. nidulans* A1145 ΔSTΔEM + pGSS119
A. nidulans +DuxI+DuxE+DuxM+DuxJ+DuxD	*A. nidulans* A1145 ΔSTΔEM + pGSS100 + pGSS101
A. nidulans +DuxI+DuxE+DuxM+DuxJ+DuxD+DuxG	*A. nidulans* A1145 ΔSTΔEM + pGSS100 + pGSS101 + pGSS102

Table S4. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound **12** in acetone-d_6.



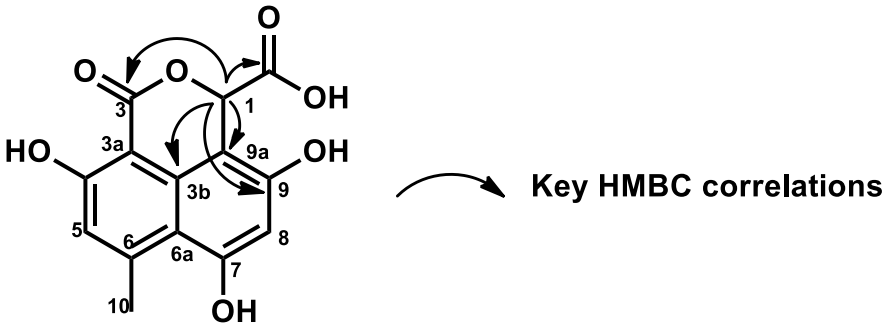
12					
	δ_H (J in Hz)	δ_C		δ_H (J in Hz)	δ_C
1	5.05 (1H, d, 13.7) 4.79 (1H, d, 13.7)	64.52	1’	5.21 (1H, d, 12.3) 5.06 (1H, d, 12.3)	70.66
2			2’		
3		169.63	3’		168.08
3a		100.64	3a’		105.08
3b		137.05	3b’		144.48
4		161.75	4’		164.10
5	6.46 (1H, s)	116.44	5’	6.65 (1H, s)	119.76
6		148.15	6’		150.44
6a		117.78	6a’		121.53
7		86.83	7’		191.35
8	4.00 (1H, brs)	56.66	8’	4.13 (1H, brs)	68.23
9		149.79	9’	5.16 (1H, brs)	76.76
9a		99.83	9a’		52.03
10	2.55 (3H, s)	20.56	10	2.03 (3H, s)	21.07
OCH3	2.94 (3H, s)	50.30	OCO*CH3* O*CO*CH3	2.16 (3H, s)	20.01 169.41

Table S5. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound **10** in DMSO-d_6



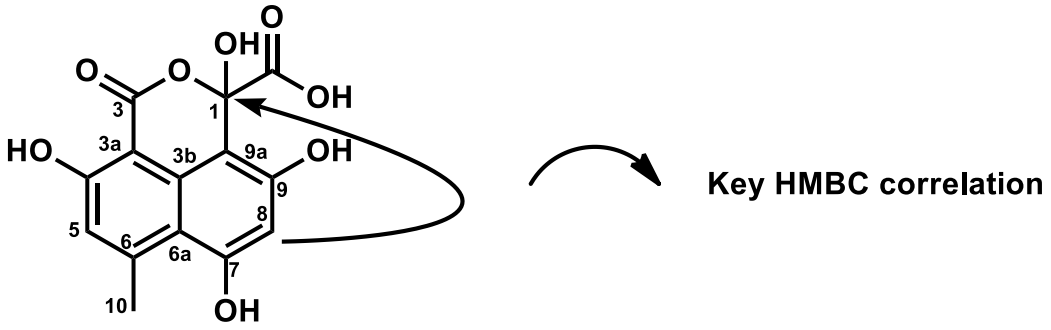
Position	**10**	
	δ_H (J in Hz)	δ_C
1		167.63
2		
3		164.49
3a		98.19
3b		136.47
4		164.73
5	6.90 (1H, s)	117.75
6		150.48
6a		112.06
7		163.82
8	6.44 (1H, s)	99.94
9		166.87
9a		92.38
10	2.79 (3H, s)	25.41

Table S6. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound **13** in DMSO-d_6



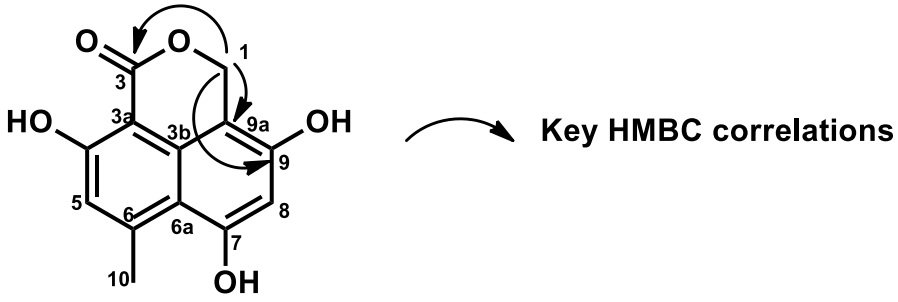
Position	**13**	
	δ_H (J in Hz)	δ_C
1	6.18 (1H, brs)	74.52
2		
3		170.79
3a		97.63
3b		132.77
4		162.34
5	6.63 (1H, s)	116.36
6		148.09
6a		112.01
7		158.56
8	6.48 (1H, s)	100.24
9		155.09
9a		97.13
10	2.72 (3H, brs)	25.26
*CO*OH		170.11

Table S7. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound **14** in DMSO-d_6



Position	**14**	
	δ_H (J in Hz)	δ_C
1		100.27
2		
3		168.88
3a		95.88
3b		132.41
4		163.22
5	6.66 (1H, s)	116.00
6		148.76
6a		111.44
7		159.12
8	6.45 (1H, s)	99.85
9		156.09
9a		101.81
10	2.77 (3H, s)	25.61
*CO*OH		169.29
4-OH	11.92 (1H, s)	
7-OH	10.51 (1H, s)	

Table S8. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound **11** in DMSO-d_6



	11	
Position	δ_H (J in Hz)	δ_C
1	4.75 (2H, s)	58.93
2		
3		162.84
3a		88.02
3b		124.66
4		154.68
5	5.73 (1H, s)	107.71
6		140.08
6a		103.75
7		147.26
8	5.55 (1H, s)	90.73
9		144.67
9a		89.91
10	1.96 (3H, s)	16.04

Table S9. ICP-MS measurement results of FLAG-tagged DuxM.

	Li	Mg	Al	Mn	Co	Ni	Cu	Zn	Ba	Pb	Fe	Ti	unit
Sample	3.829	14.78	21.38	24.39	2.961	22.95	16.19	896.8	2.766	7.648	1809	4.767	ng/mL

* The DuxM concentration is ~48 μM.

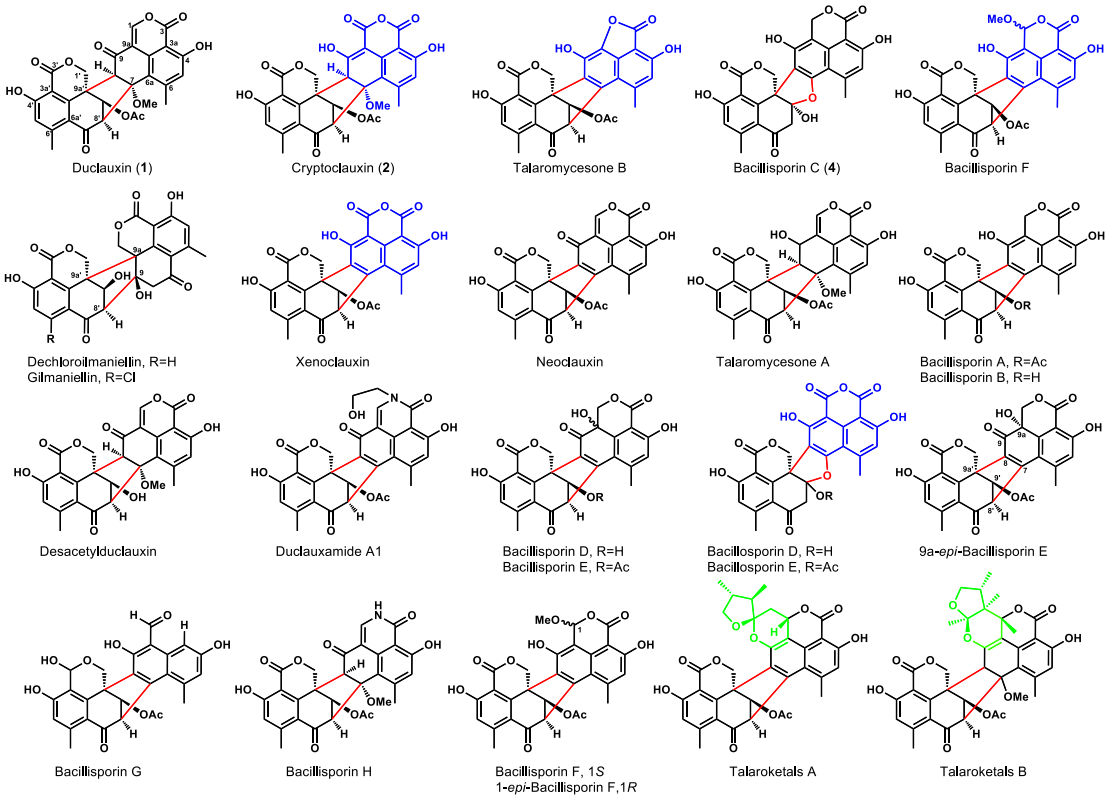


Figure S1. Natural products of duclauxins family from different fungi.

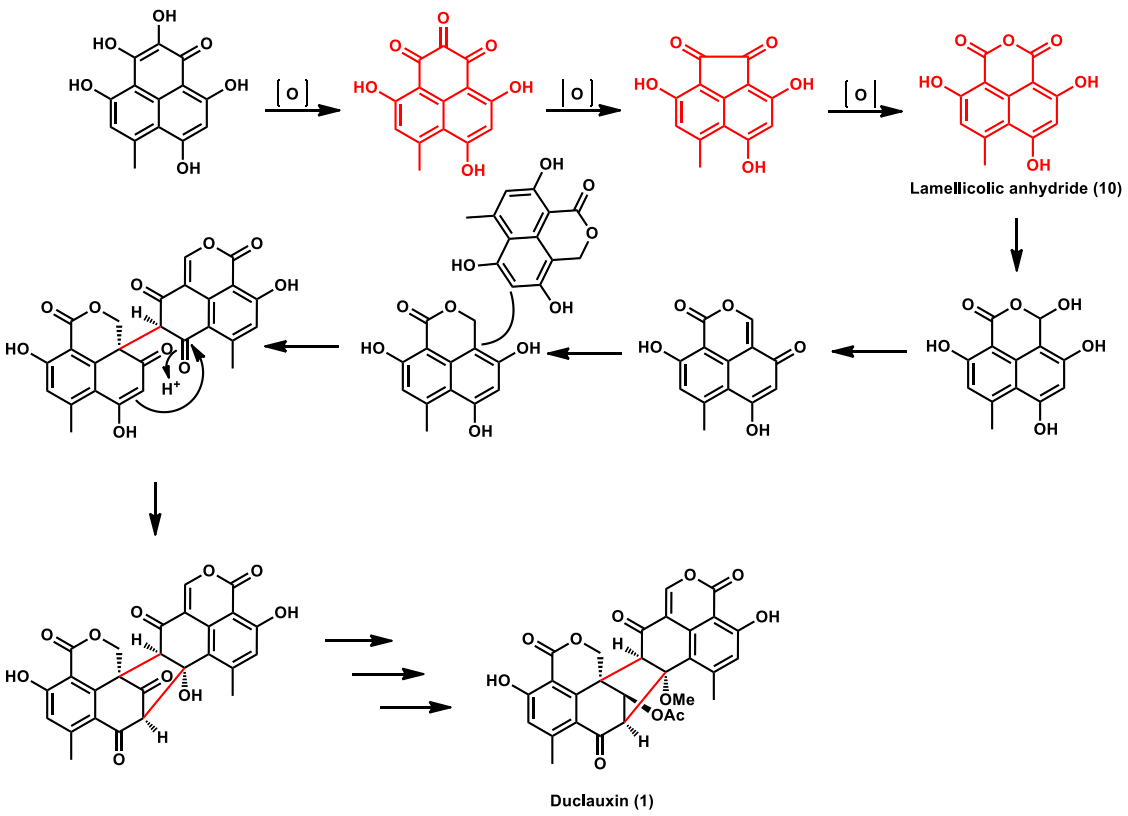


Figure S2. Proposed biosynthetic pathway for duclauxin **1**: *via* a tri-keto, a di-keto and a hydride intermediates (marked in red).

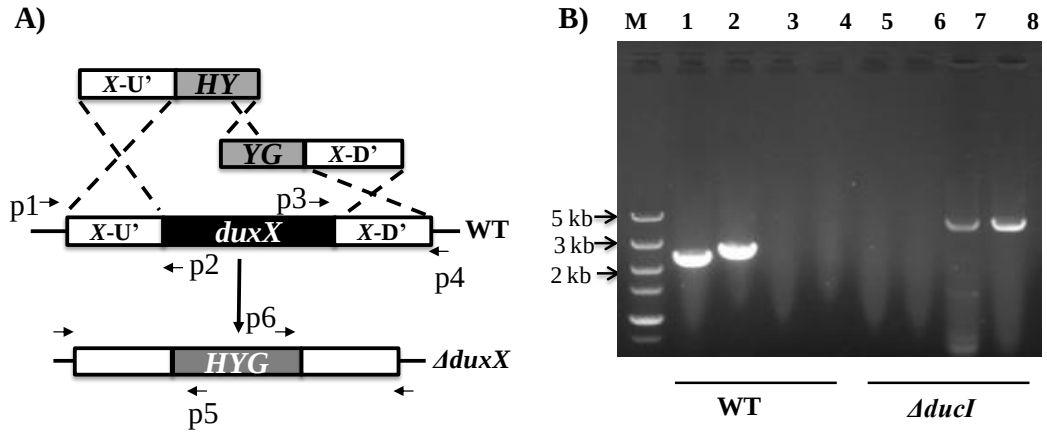


Figure S3. Gene knock-out of *dux* gene cluster in *T. stipitatus* and PCR verification of mutant. A) The method for PCR check of the mutant used in this study. *duxX* represents the target gene; 1 represents the fragment amplified by the primer pair of 1 (check X for) and p2 (check inX rev); 2 represents the fragment amplified by the primer pair of p3 (check X rev) and p4 (check inX for); 7 represents the fragment amplified by the primer pair of p1 (check X for) and p5 (check hph rev); 8 represents the fragment amplified by the primer pair of p4 (check X rev) and p6 (check hph for). The primers are shown in Table S1. B) PCR check the *ΔduxI* obtained in this study.

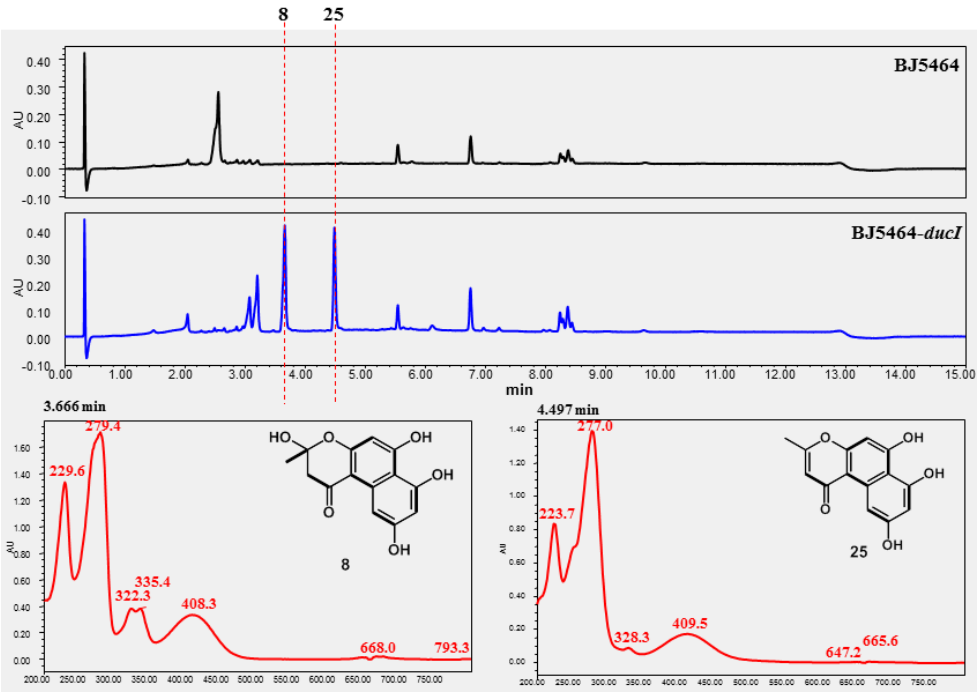


Figure S4. LC-MS analysis of heterologous expression of DuxI in *Saccharomyces cerevisiae* BJ5464. Compounds **8** (MW=276) and **25** (MW=258) are the same products to those characterized by expressing PhnA of *phn* gene cluster in *Pencillium herquei* NRRL 1040 in yeast.

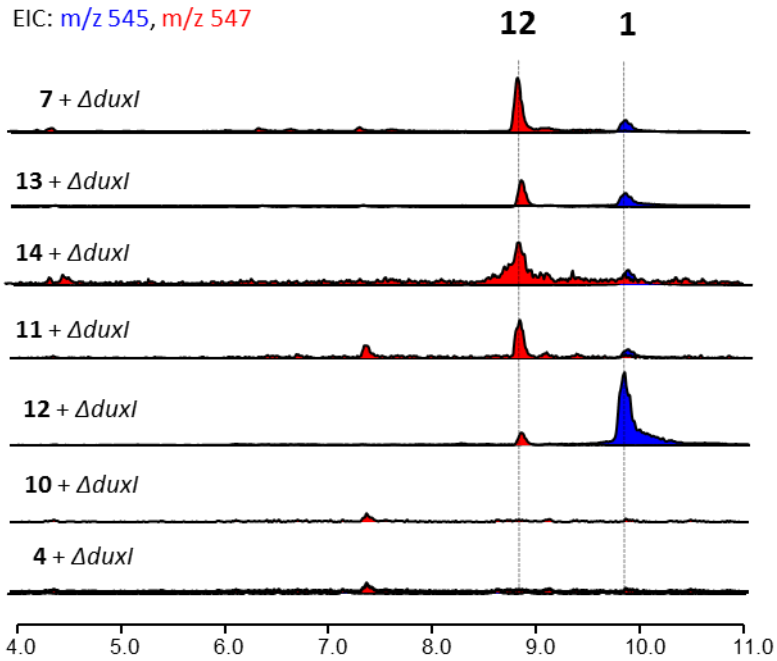


Figure S5. Product profiles of chemical complementation studies to *ΔduxI of T. stipitatus*.

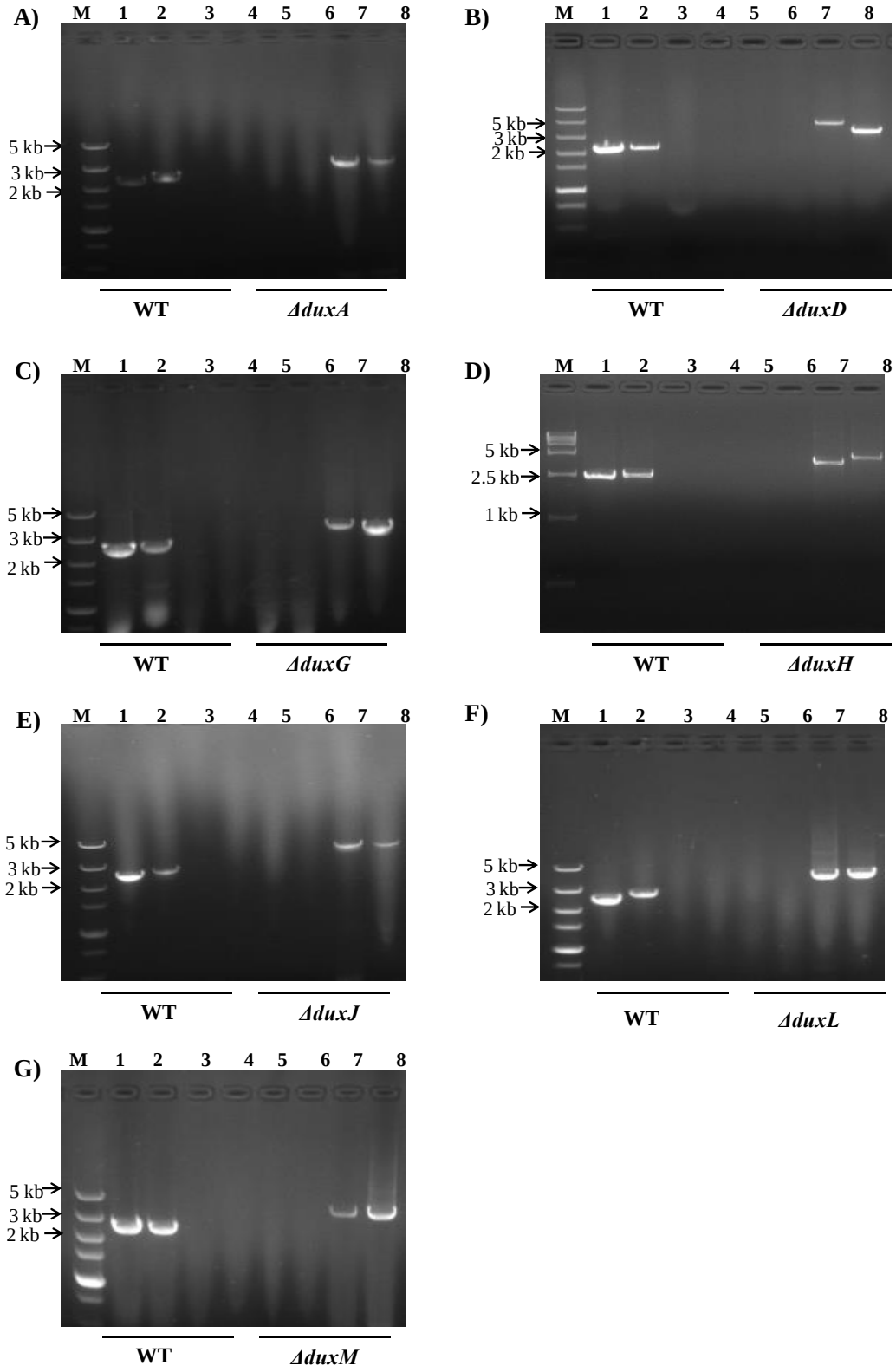


Figure S6. Gene knock-out of *duxA*, *duxD*, *duxG*, *duxH*, *duxJ*, *duxL*, and *duxM* in *T. stipitatus* and PCR verification of the mutants. A) PCR check the *ΔduxA*; B) PCR check the *ΔduxD*; C) PCR check the *ΔduxG*; D) PCR check the *ΔduxH*; E) PCR check the *ΔduxJ*; F) PCR check the *ΔduxL*; G) PCR check the *ΔduxM*.

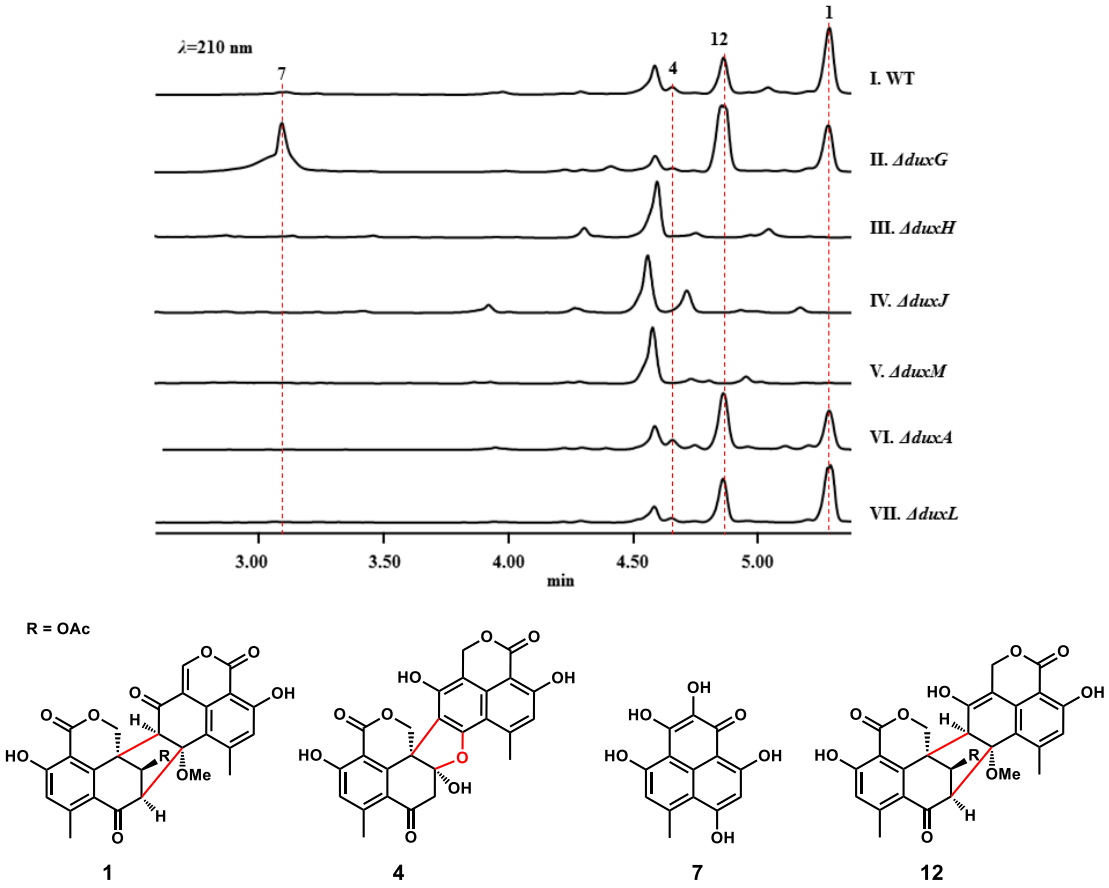


Figure S7. LC-MS analysis of organic extract obtained from the wild type and *Δdux* mutants including *ΔduxG*, *ΔduxH*, *ΔduxJ*, *ΔduxM*, *ΔduxA*, and *ΔduxL*.

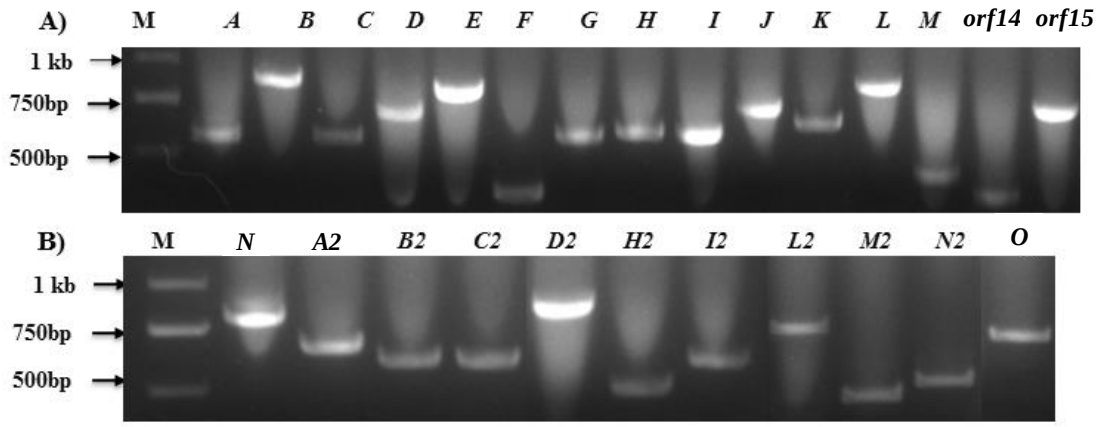


Figure S8 RT-PCR analysis of genes in *dux* gene clusters in *T. stipitatus*. A) M: marker. Lanes *A*-o*rf15* PCR reaction of the gene cluster *dux*1 using cDNA template. B) M: marker. Lanes N, A2-O, PCR reaction of the gene cluster *dux2* using cDNA template.

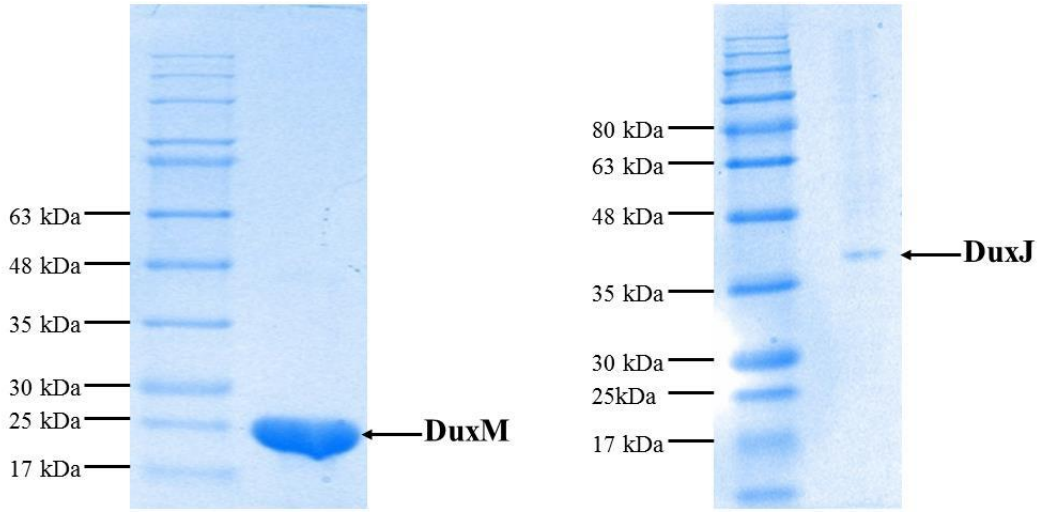


Figure S9. SDS-PAGE of heterogeneously expressed DuxM from *E. coli* BL21(DE3) and DuxJ from *S. cerevisiae* BJ5464-NpgA. DuxM contains an *N*-terminal His6-Tag (~23 kDa) and DuxJ contains an *N*-terminal Flag-Tag (~42 kDa). DuxM was purified using Ni-NTA agarose affinity chromatography and DuxJ was purified using anti-FLAG affinity chromatography.

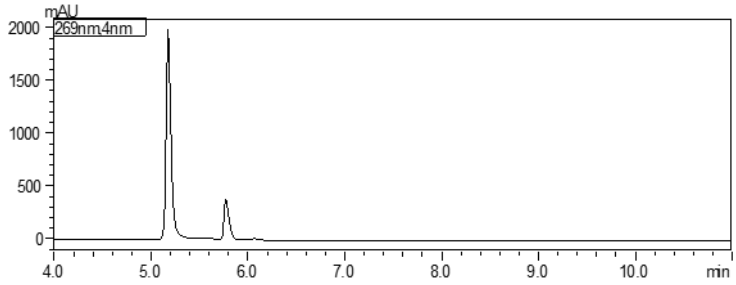
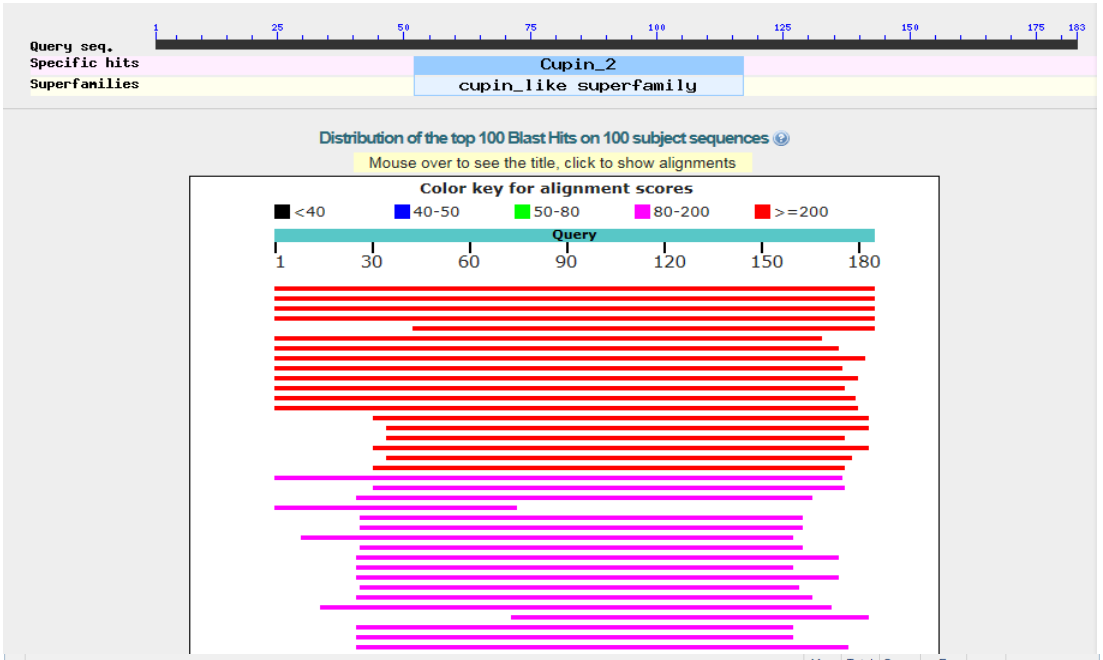


Figure S10. LC-MS analysis result of **14** stored in methanol for 30 min.



Description	Max score	Total score	Query cover	E value	Ident	Accession
conserved hypothetical protein [Talaromyces stipitatus ATCC 10500]	378	378	100%	5e-133	100%	XP_002478066.1
conserved hypothetical protein [Talaromyces marneffei ATCC 18224]	355	355	100%	6e-124	92%	XP_002145796.1
hypothetical protein ZTR_10695 [Talaromyces verruculosus]	352	352	100%	8e-123	92%	KUL82141.1
conserved hypothetical protein [Talaromyces stipitatus ATCC 10500]	351	351	100%	4e-122	90%	XP_002483093.1
conserved hypothetical protein [Talaromyces stipitatus ATCC 10500]	270	270	77%	7e-91	92%	XP_002483094.1
hypothetical protein SNOG_08269 [Parastagonospora nodorum SN15]	254	254	91%	6e-84	69%	XP_001798588.1
hypothetical protein ASPCADRAFT_55781 [Aspergillus carbonarius ITEM 5010]	252	252	93%	1e-82	66%	OOF92047.1
hypothetical protein NECHADRAFT_76237 [Nectria haematococca mpVI 77-13-4]	248	248	98%	1e-81	63%	XP_003045914.1
RmlC-like jelly roll fold protein [Metarhizium album ARSEF 1941]	248	248	94%	2e-81	65%	KHN94884.1
O-methyltransferase [Rasamsonia emersonii CBS 393.64]	260	260	97%	2e-79	63%	XP_013329850.1
hypothetical protein OIDMADRAFT_58553 [Oidiodendron maius Zn]	237	237	95%	4e-77	64%	KIM97008.1
hypothetical protein AK830_g6526 [Neonectria ditissima]	235	235	96%	4e-76	56%	KPM40037.1
hypothetical protein PV09_06926 [Verruconis gallopava]	219	219	97%	3e-69	54%	XP_016211619.1
unnamed protein product [Aspergillus niger]	214	214	82%	8e-68	64%	CAK38309.1
hypothetical protein AKAW_09059 [Aspergillus kawachii IFO 4308]	206	206	80%	6e-65	66%	GAA90945.1
hypothetical protein ASPTUDRAFT_182911 [Aspergillus tubingensis CBS 134.48]	204	204	76%	2e-64	68%	OJI89103.1
O-methyltransferase family protein [Aspergillus niger]	216	216	82%	4e-64	64%	OWW34971.1
hypothetical protein ASPBRDRAFT_185837 [Aspergillus brasiliensis CBS 101740]	203	203	77%	5e-64	68%	OJJ68005.1
unnamed protein product [Aspergillus niger]	214	214	78%	6e-61	69%	GAQ44224.1
AraC-like regulator [Metarhizium acridum CQMa 102]	163	163	94%	2e-48	47%	XP_007806524.1
hypothetical protein ASPNIDRAFT_44530 [Aspergillus niger ATCC 1015]	159	159	78%	1e-46	57%	EHA21305.1
hypothetical protein PV09_01702 [Verruconis gallopava]	157	157	75%	2e-45	48%	XP_016217645.1
conserved hypothetical protein [Talaromyces stipitatus ATCC 10500]	154	154	40%	9e-45	86%	XP_002483095.1
hypothetical protein PMG11_00782 [Penicillium brasilianum]	151	151	73%	8e-44	53%	CEJ54474.1
hypothetical protein PEBR_32170 [Penicillium brasilianum]	151	151	73%	8e-44	53%	OQQ83964.1
RmlC-like cupin [Glonium stellatum]	149	149	81%	1e-42	44%	OCL01558.1
hypothetical protein UA08_02882 [Talaromyces atroroseus]	146	146	73%	1e-41	50%	XP_020122336.1
RmlC-like cupin [Alternaria alternata]	145	145	80%	3e-41	46%	OWY42382.1
hypothetical protein SETTUDRAFT_164062 [Setosphaeria turcica Et28A]	145	145	72%	3e-41	48%	XP_008027825.1
RmlC-like cupin [Alternaria alternata]	145	145	80%	5e-41	46%	XP_018383916.1
hypothetical protein W97_02301 [Coniosporium apollinis CBS 100218]	144	144	73%	1e-40	49%	XP_007778391.1
RmlC-like cupin [Hypoxylon sp. EC38]	142	142	75%	5e-40	47%	OTA63370.1
hypothetical protein AB675_8547 [Phialophora attae]	144	144	85%	7e-40	47%	XP_018004657.1
hypothetical protein ASPFODRAFT_337769 [Aspergillus luchuensis CBS 106.47]	140	140	59%	1e-39	61%	OJZ82879.1
hypothetical protein IQ07DRAFT_593761 [Pyrenochaeta sp. DS3sAY3a]	142	142	72%	1e-39	48%	OAL56216.1
RmlC-like cupin [Paraphaeosphaeria sporulosa]	142	142	72%	1e-39	47%	XP_018034258.1
hypothetical protein PV06_03670 [Exophiala oligosperma]	141	141	81%	1e-39	45%	XP_016265487.1

Figure S11. BLASTP alignment of DuxM in *T. stipitatus* (https://www.ncbi.nlm.nih.gov/).

A)

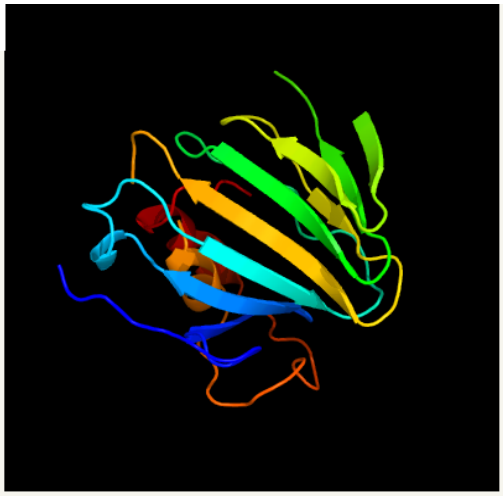


Image coloured by rainbow N → C terminus

Model dimensions (Å): **X**:41.019 **Y**:36.064 **Z**:42.501

Model (left) based on template c5fljK_

Top template information

PDB header:oxidoreductase
Chain: K: **PDB Molecule:**quercetinase qued;
PDBTitle: enzyme-substrate-dioxygen complex of ni-quercetinase

Confidence and coverage

Confidence: **100.0%** Coverage: **83%**

152 residues (83% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

3D viewing

Interactive 3D view in JSmol

For other options to view your downloaded structure offline see the FAQ

B)

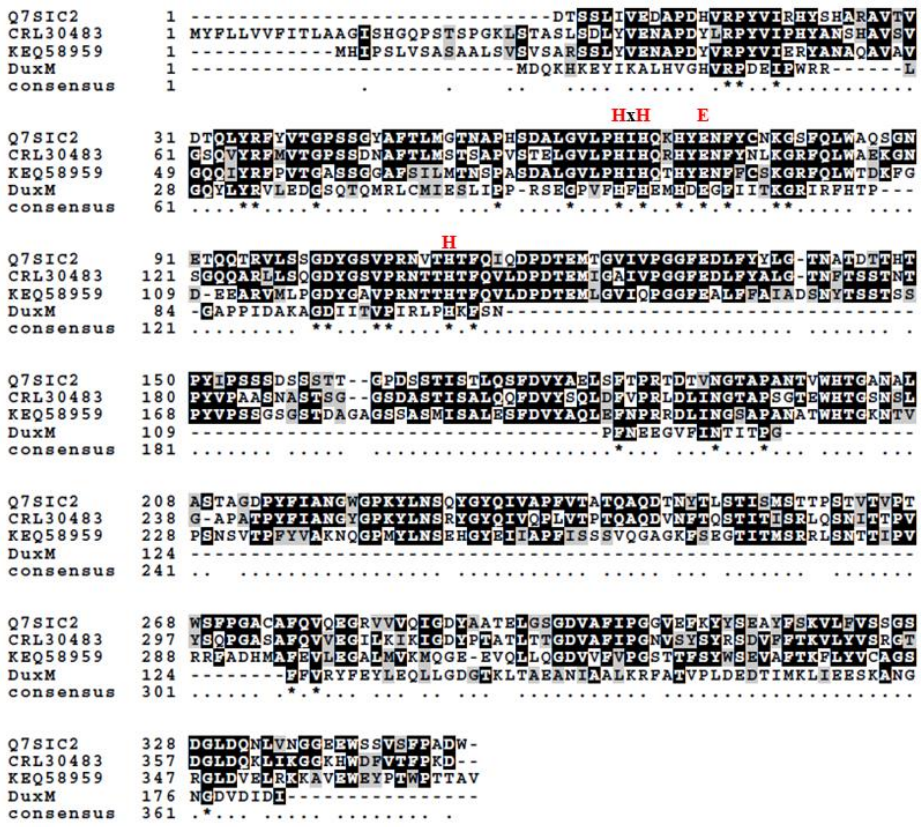


Figure S12. A) Structure analysis of DuxM by Phyre2 showed DuxM is close to quercetinase (quercetin 2, 3-dioxygenase). B) Multiple sequence alignment of DuxM and other fungal cupin family enzymes by ClustalW, a conserved characteristic HxHxxxxEx(n)H motif coordinated to a divalent metal ions centre. The amino acids corresponding to characteristic motif are numbered as $\mathbf{H_{62}xH_{64}xxxxE_{69}x(n)H_{104}}$. Q7SIC2, quercetin 2, 3-dioxygenase from *Aspergillus japonicas*; CRL30483, cupin family enzyme from *Penicillium camemberti* FM013; KEQ58959, cupin family enzyme from *Aureobasidium melanogenum* CBS 110374.

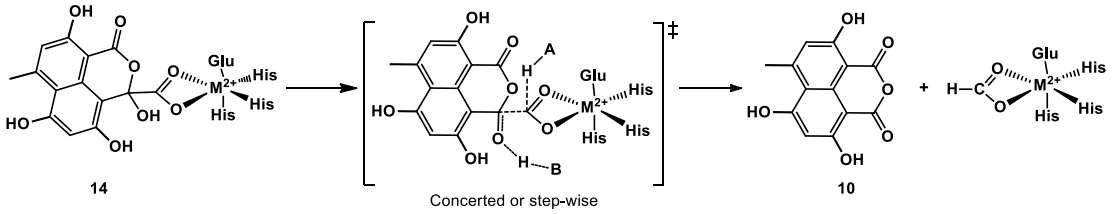


Figure S13. Proposed biosynthetic pathway for decarboxylation of **14** to form **10** catalyzed by DuxM.

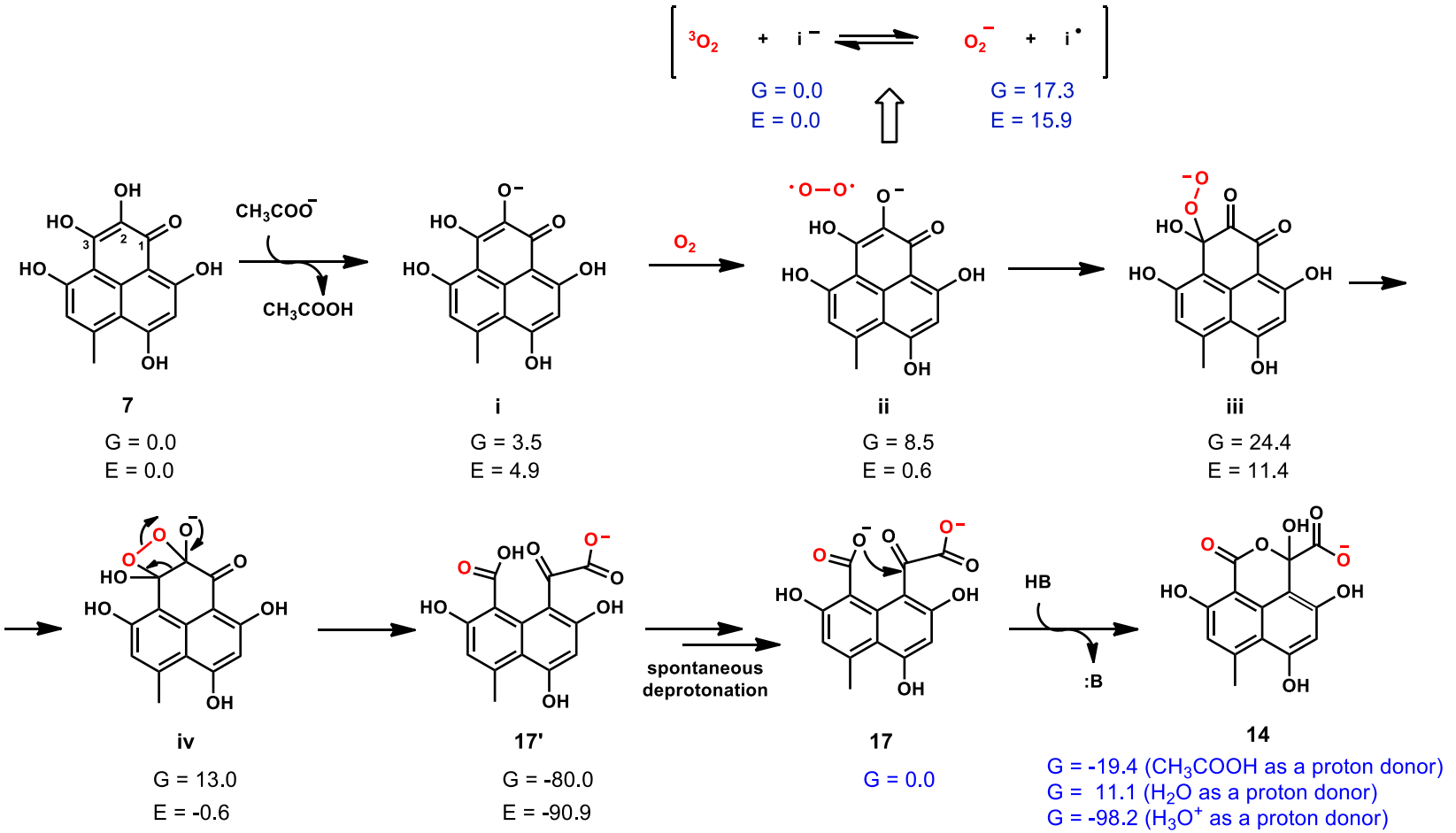


Figure S14. Computed reaction mechanism for the conversion of **7** to **14** and **15** in the absence of a metal counterion at B3LYP-D3BJ/6-311++G(d,p)–PCM(diethylether) // B3LYP/6-31G(d)–PCM(diethylether) level. All electronic (E) and Gibbs (G) energy values are given in kcal mol^{-1}

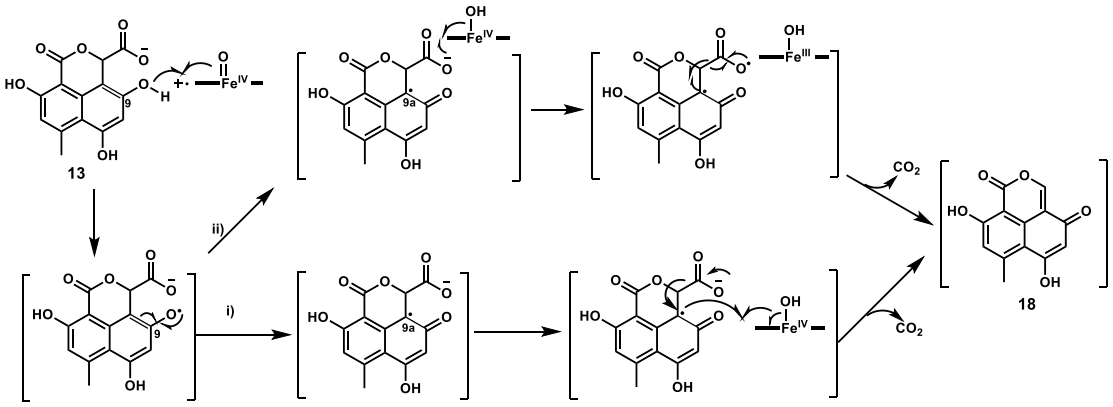


Figure S15. Two proposed routes for the DuxD catalyzed decarboxylation.

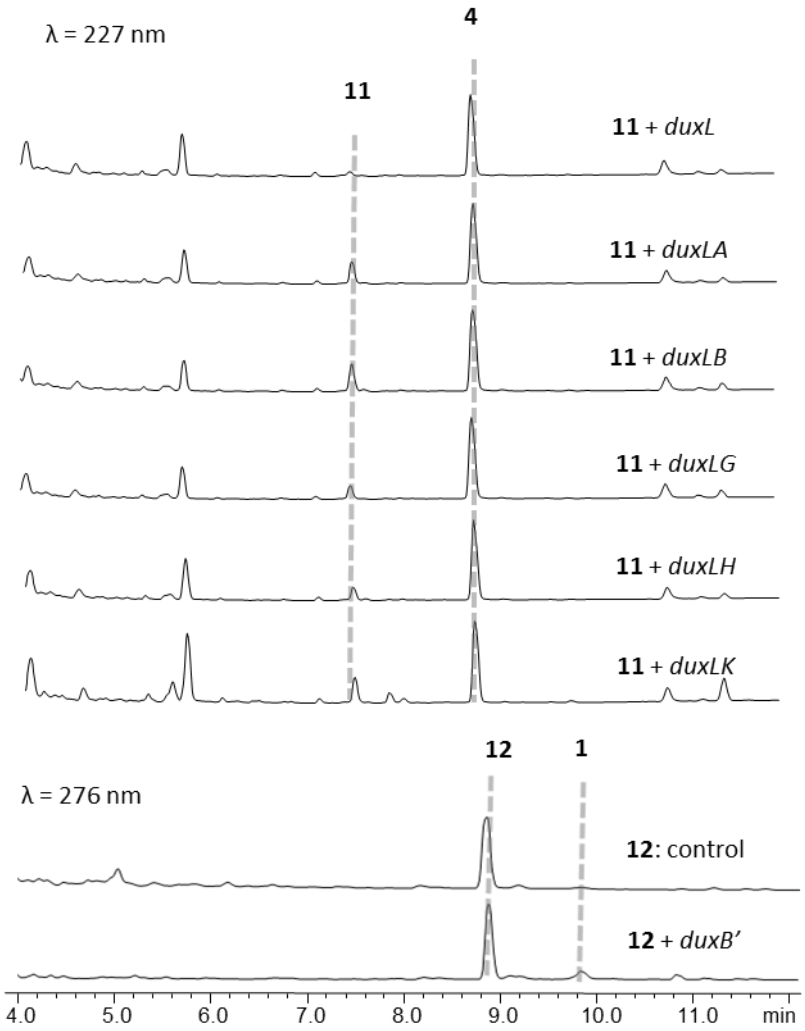


Figure S16. LC-MS analysis results of chemical complementation studies in yeast.

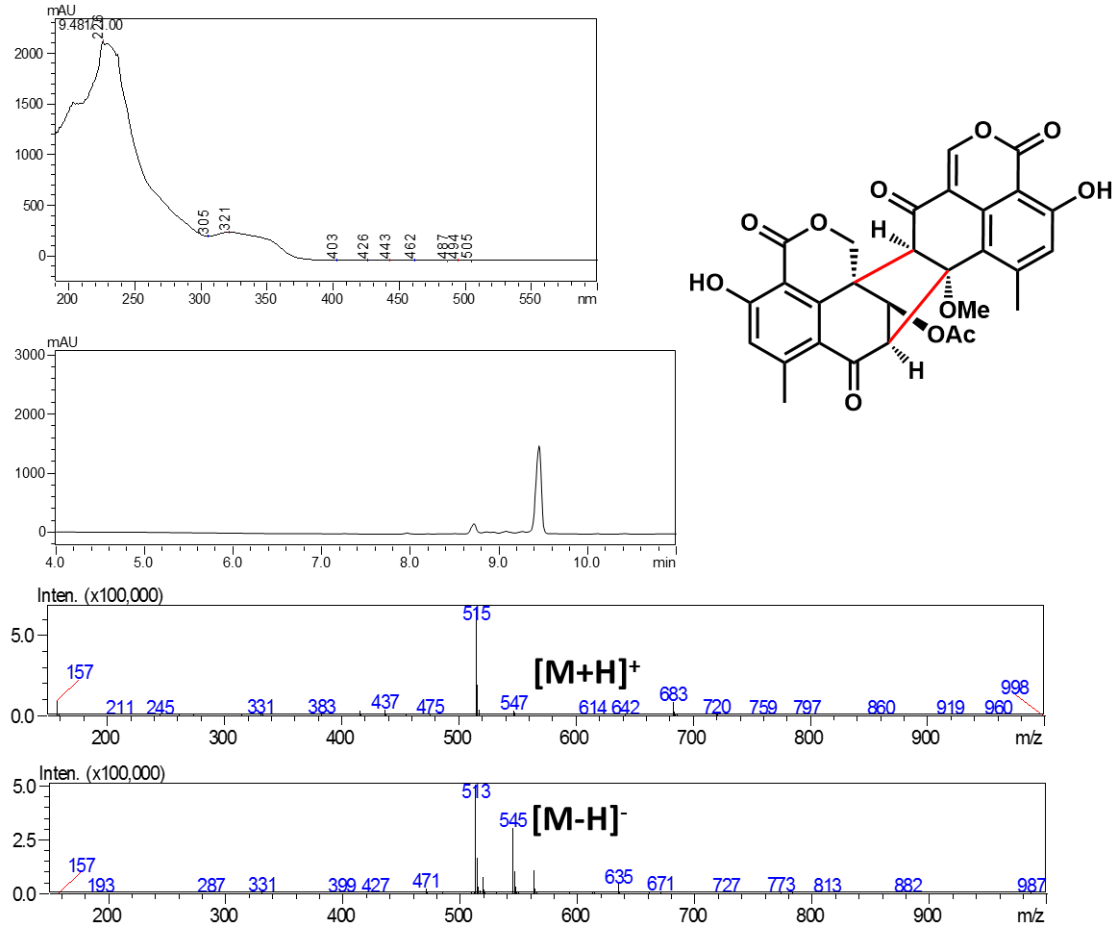


Figure S17. UV and MS spectra of **1**.

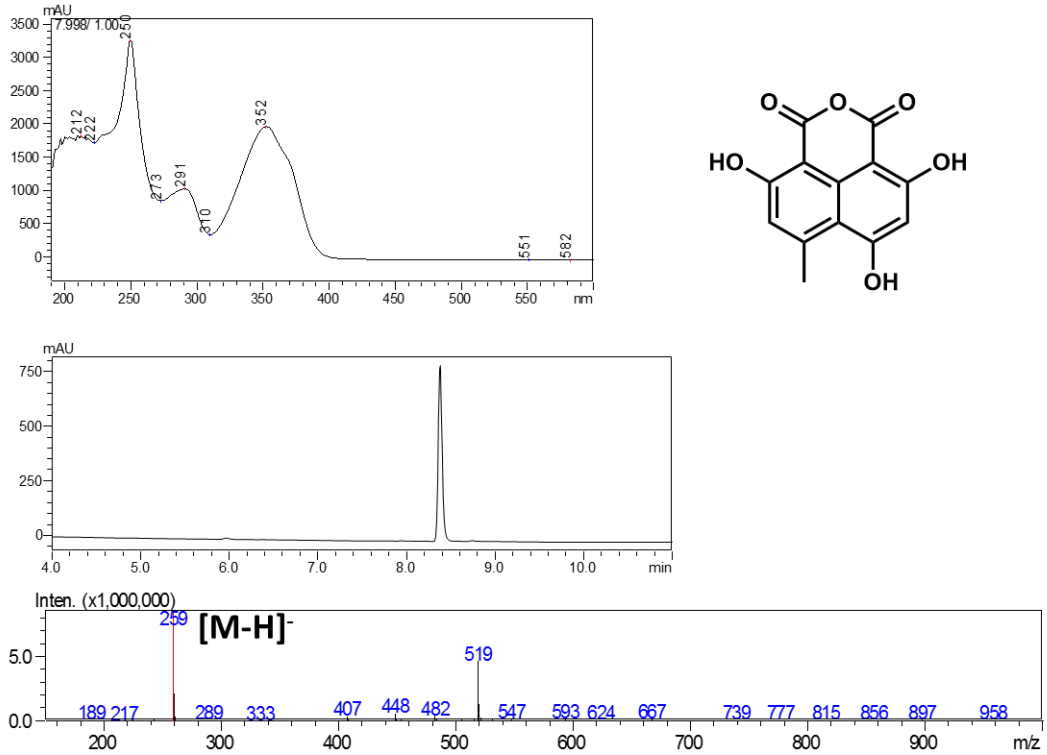


Figure S18. UV and MS spectra of **10**.

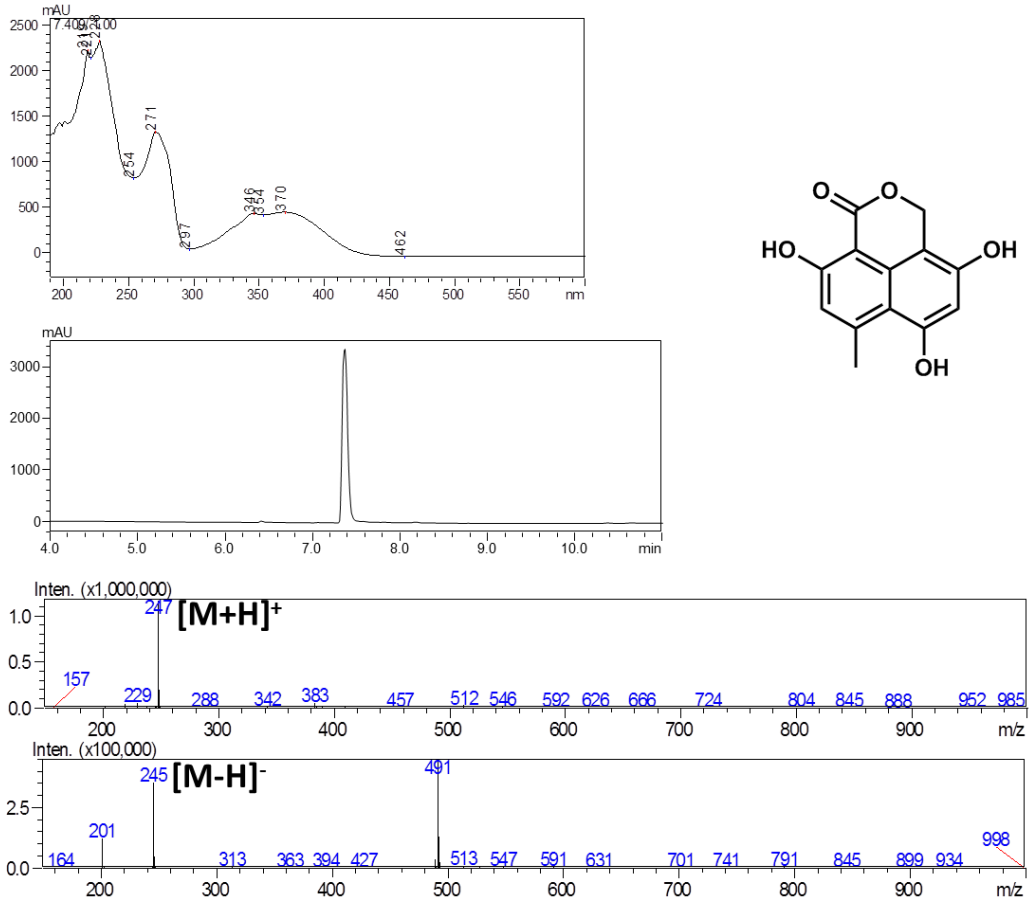


Figure S19. UV and MS spectra of **11**.

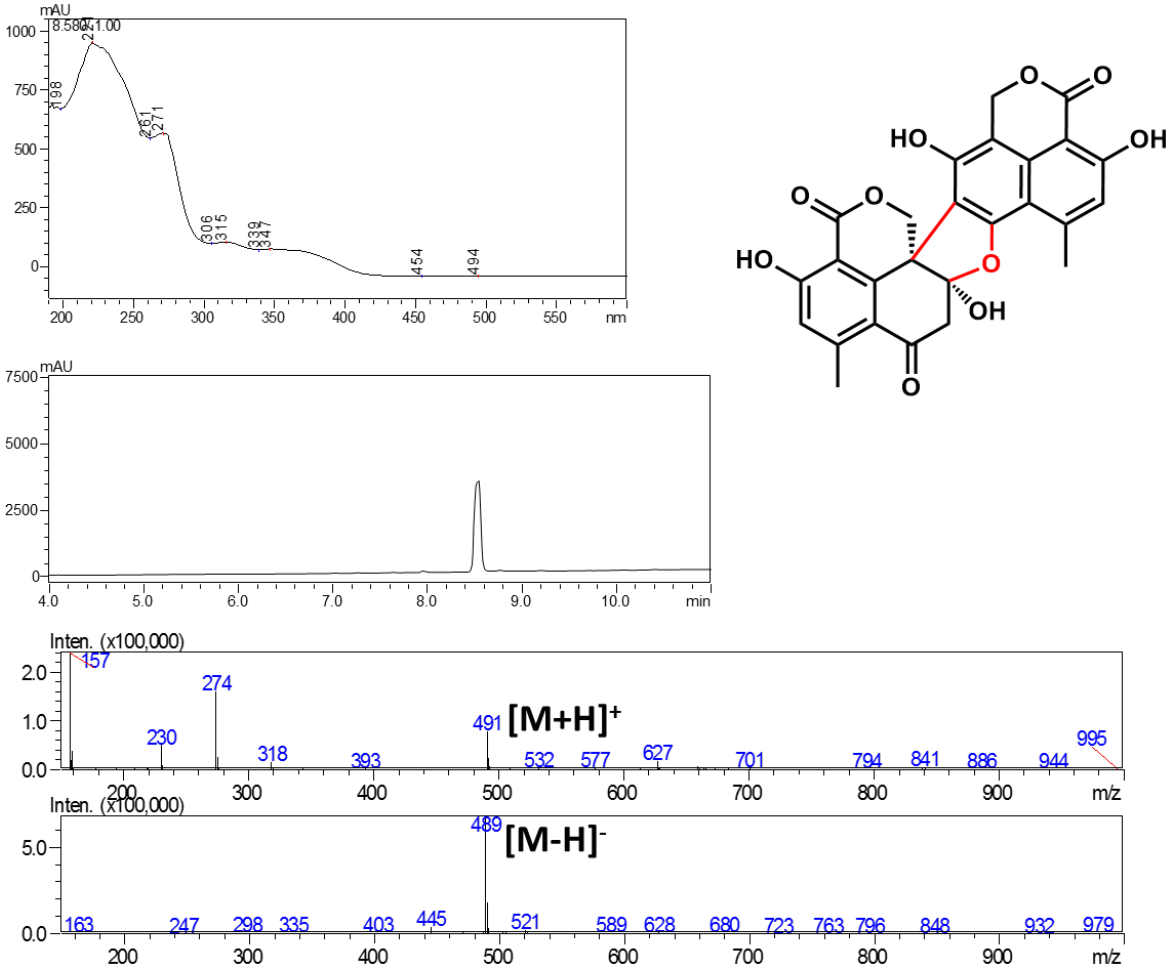


Figure S20. UV and MS spectra of **4**.

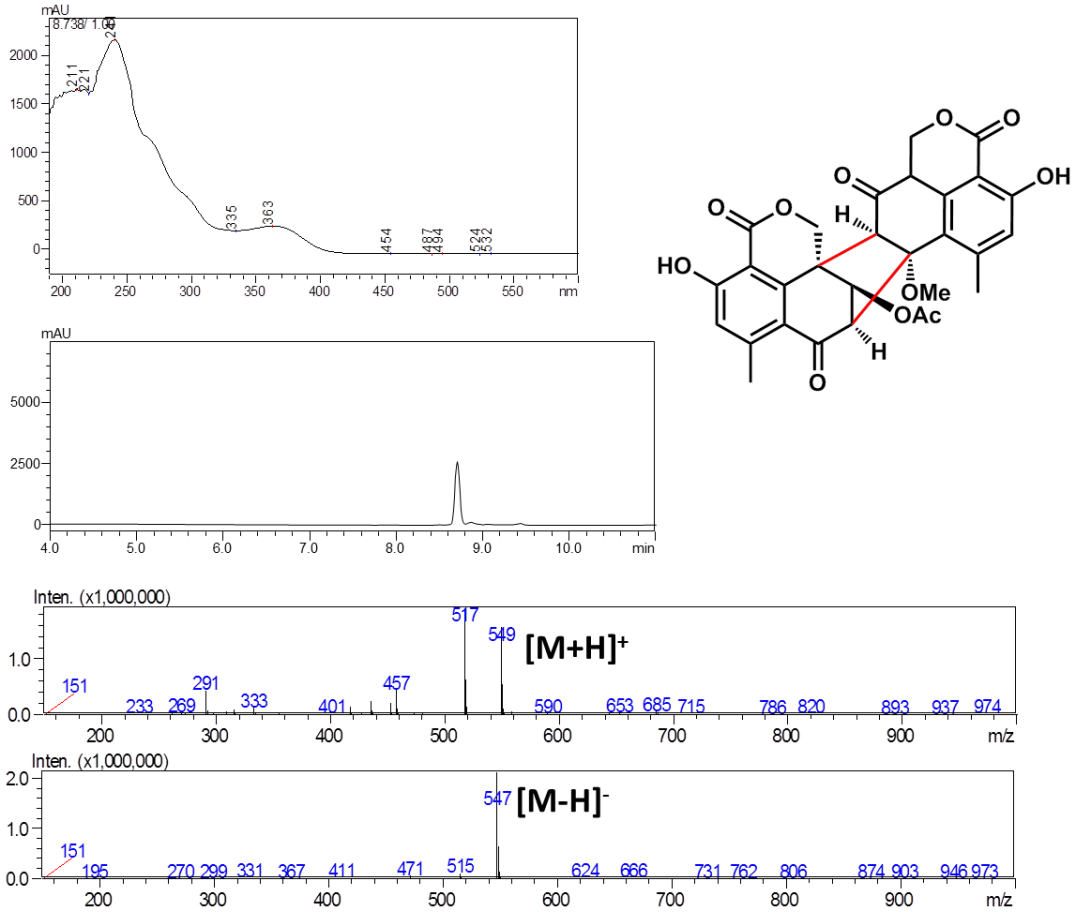


Figure S21. UV and MS spectra of **12**.

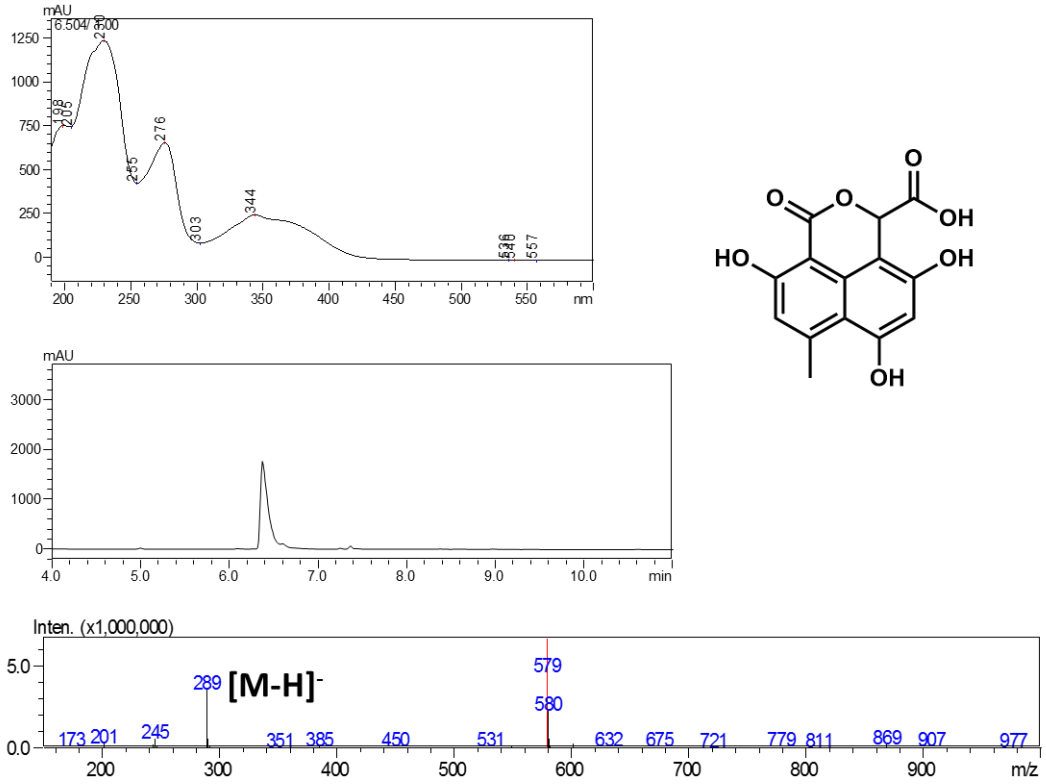


Figure S22. UV and MS spectra of **13**.

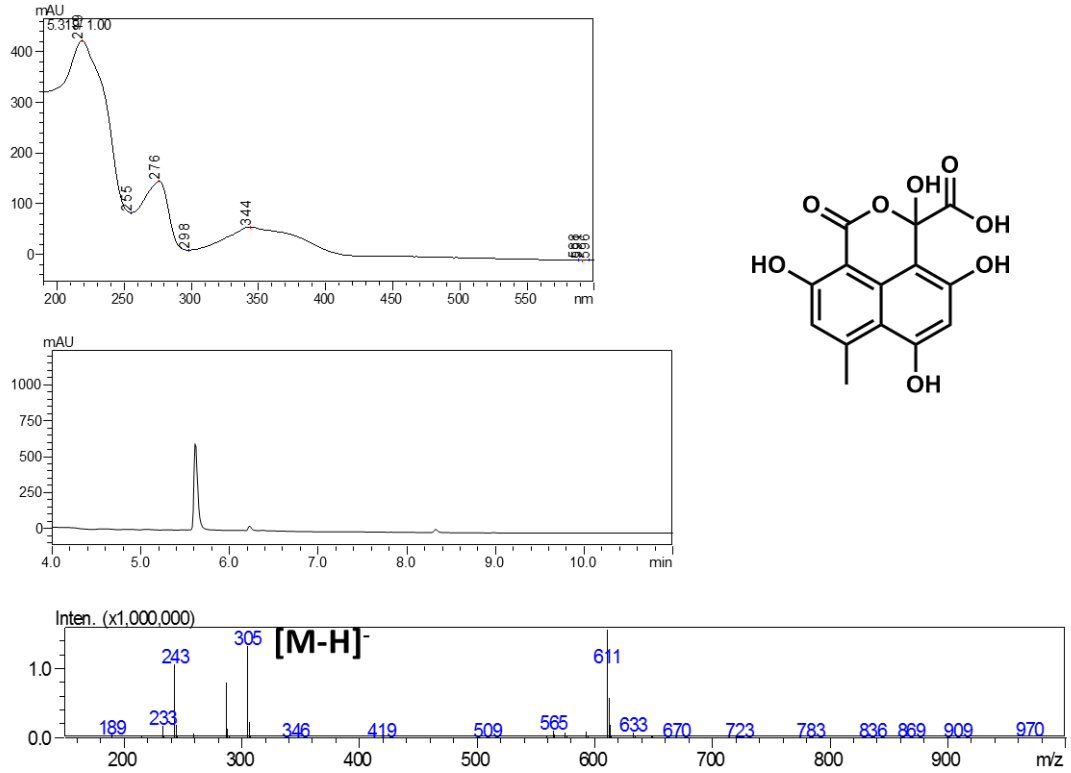


Figure S23. UV and MS spectra of **14**.

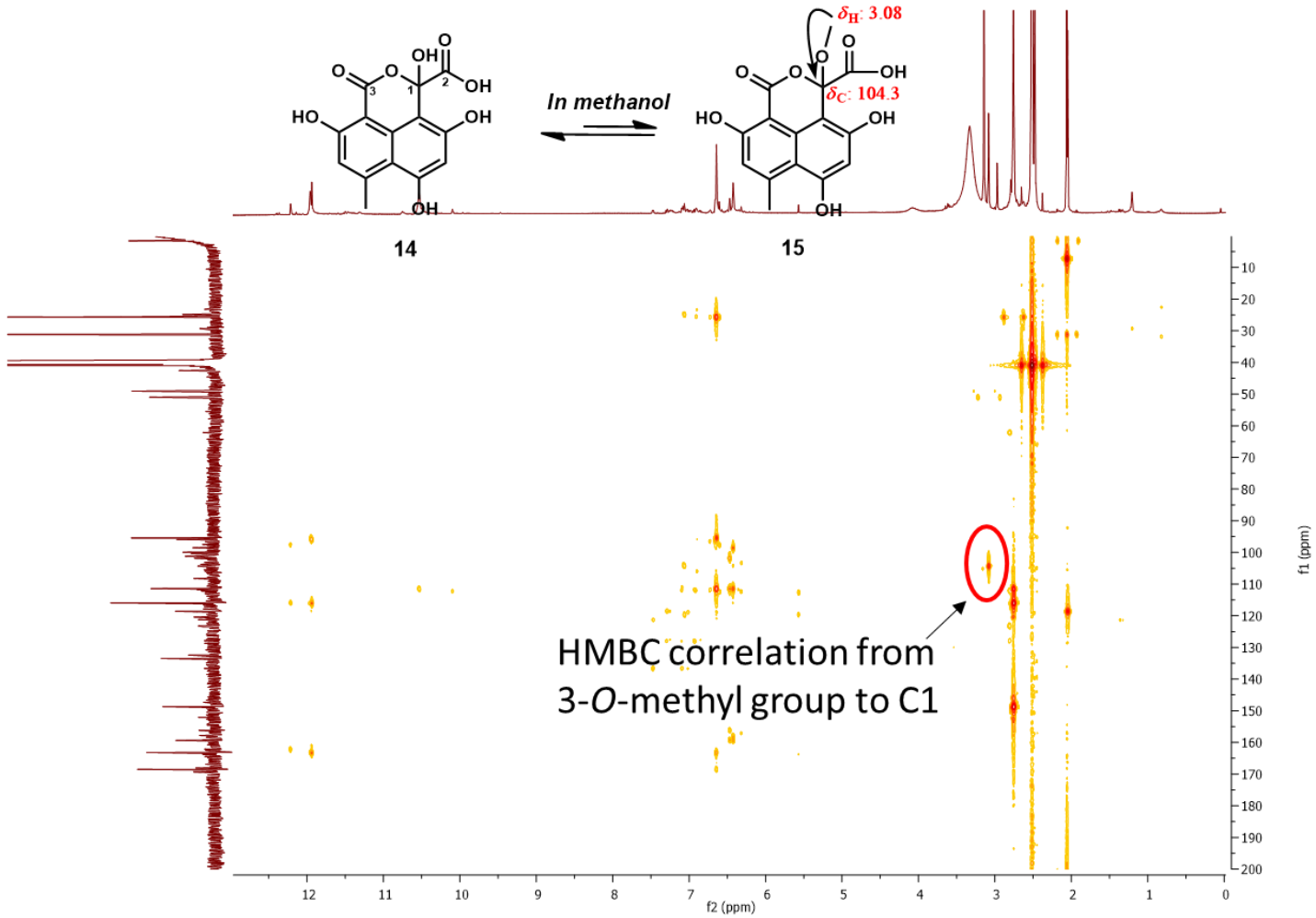


Figure S24. The HMBC spectrum of the mixture of **14** and **15**, which indicates the correlation from 3-*O*-methyl group to C1 in **15**.

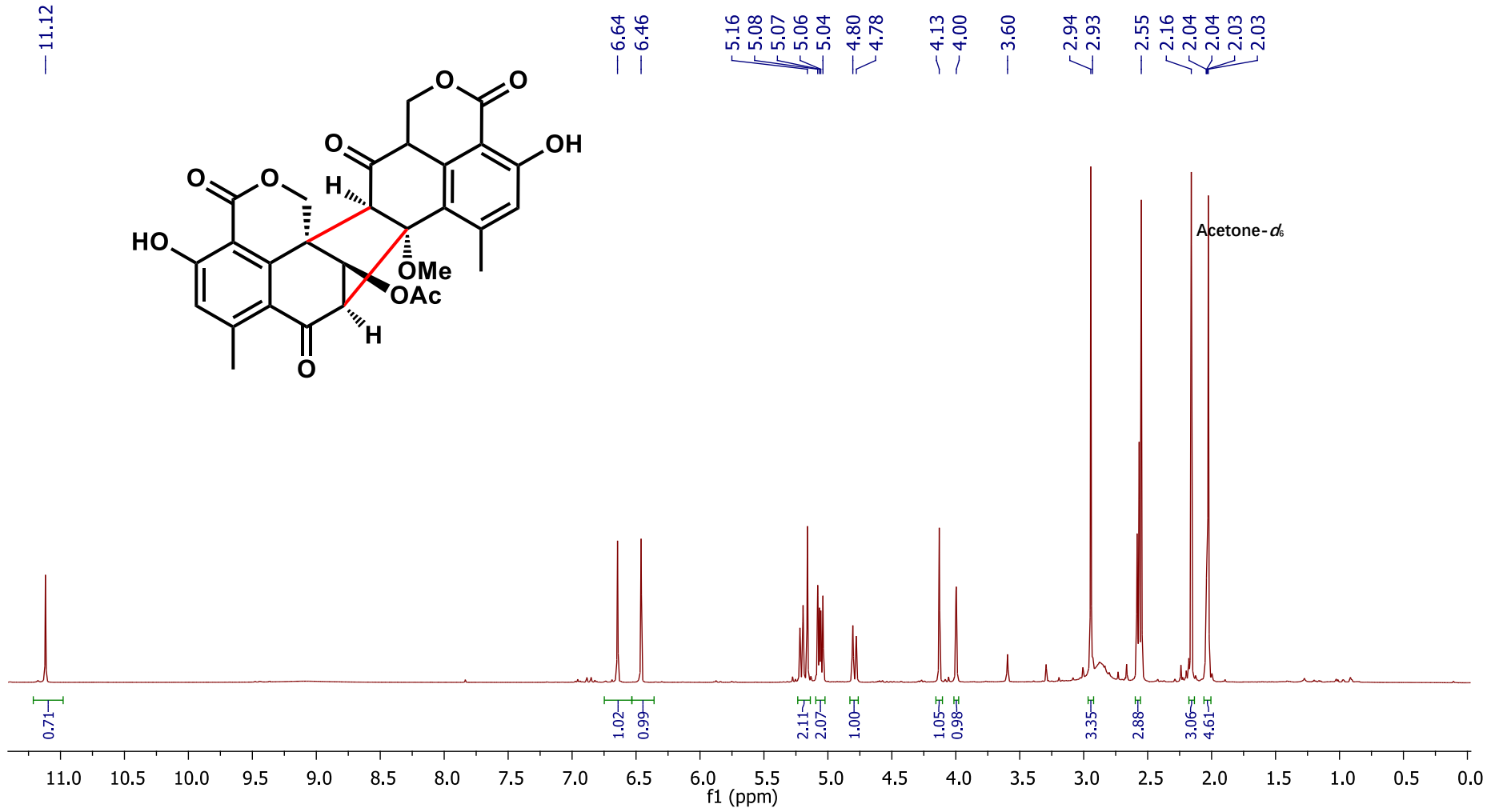


Figure S25. ^{1}H-NMR of **12** in acetone-d_6 (500 MHz).

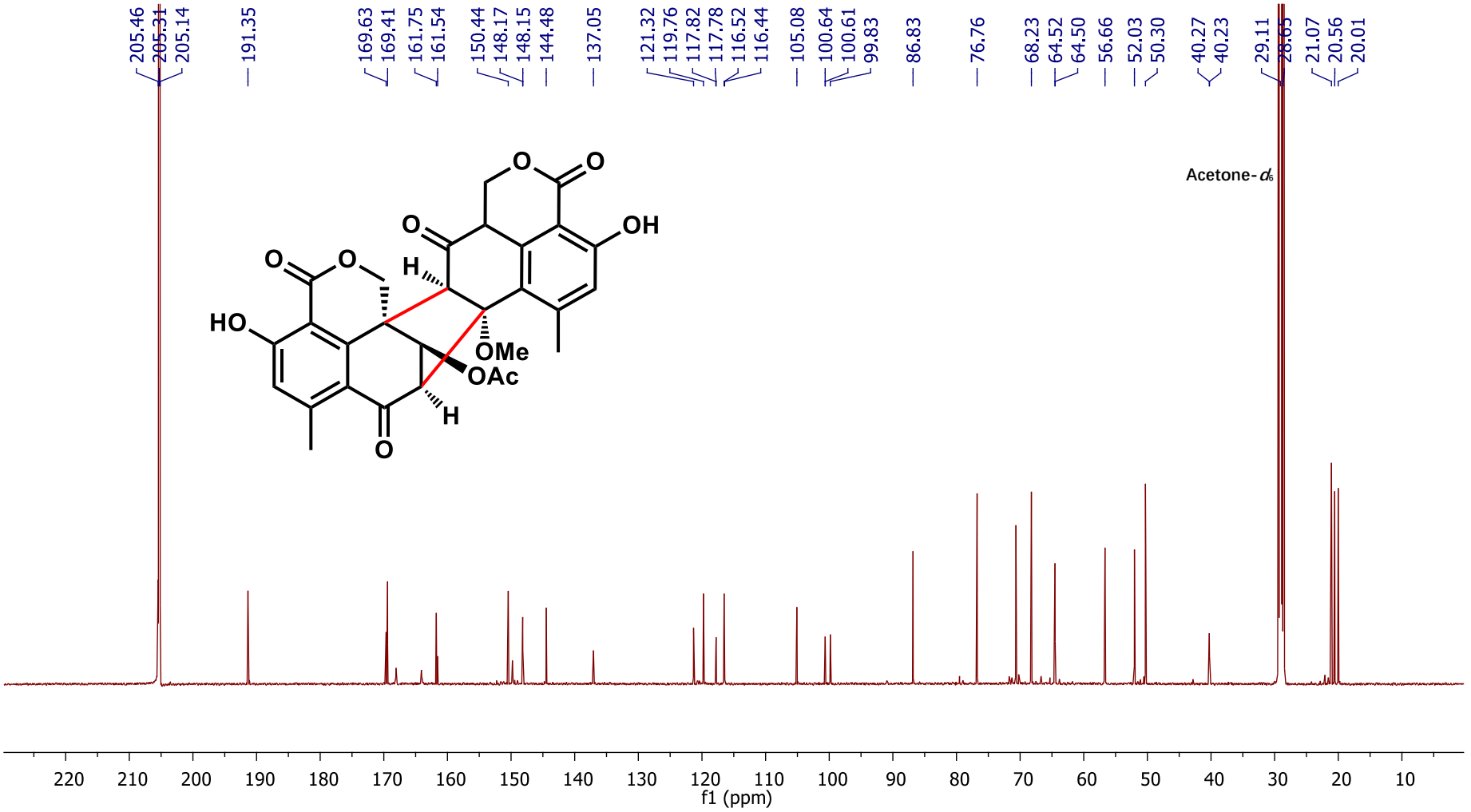


Figure S26. ^{13}C-NMR of **12** in acetone-d_6 (125 MHz).

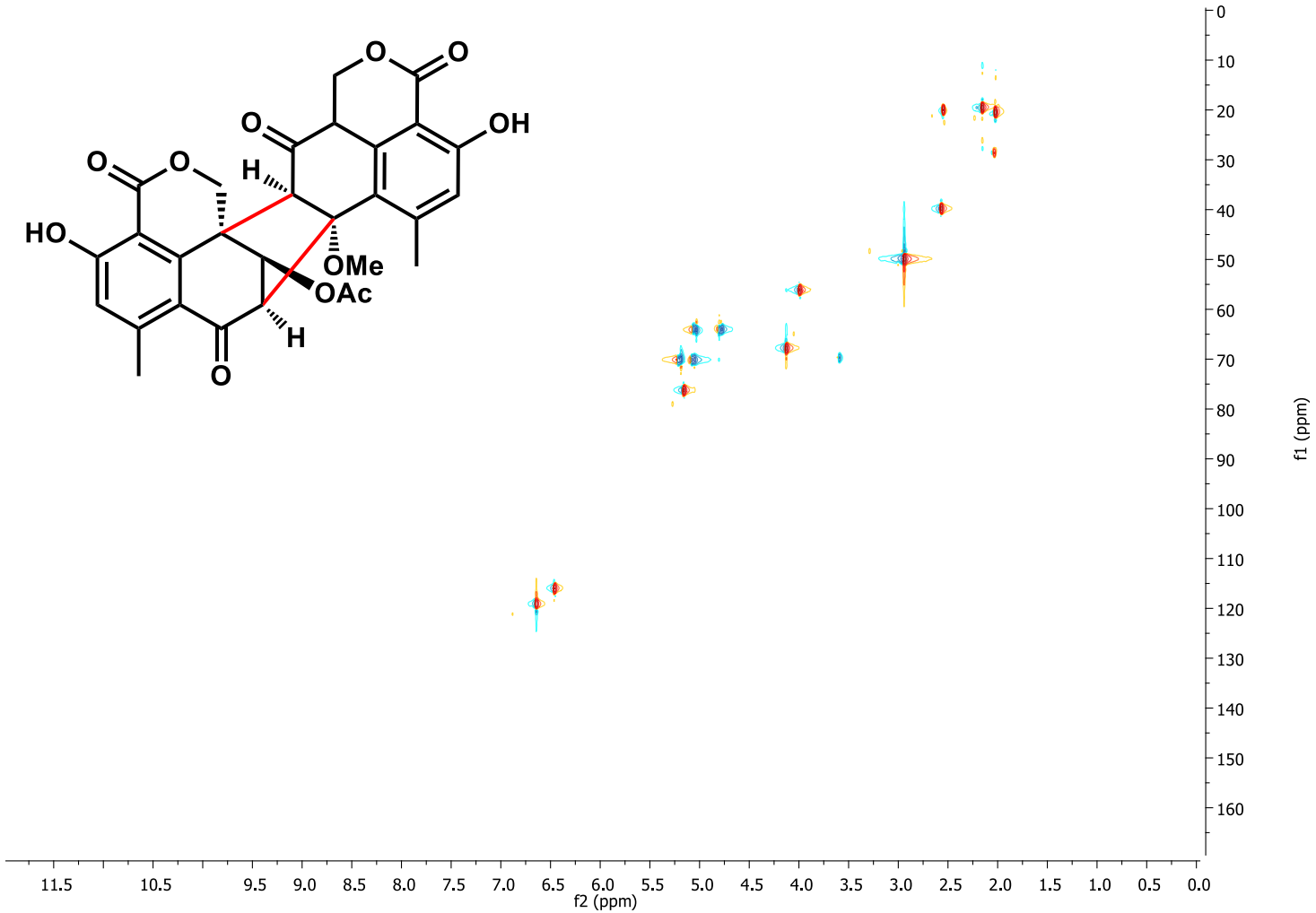


Figure S27. HSQC spectrum of **12** in acetone-d_6.

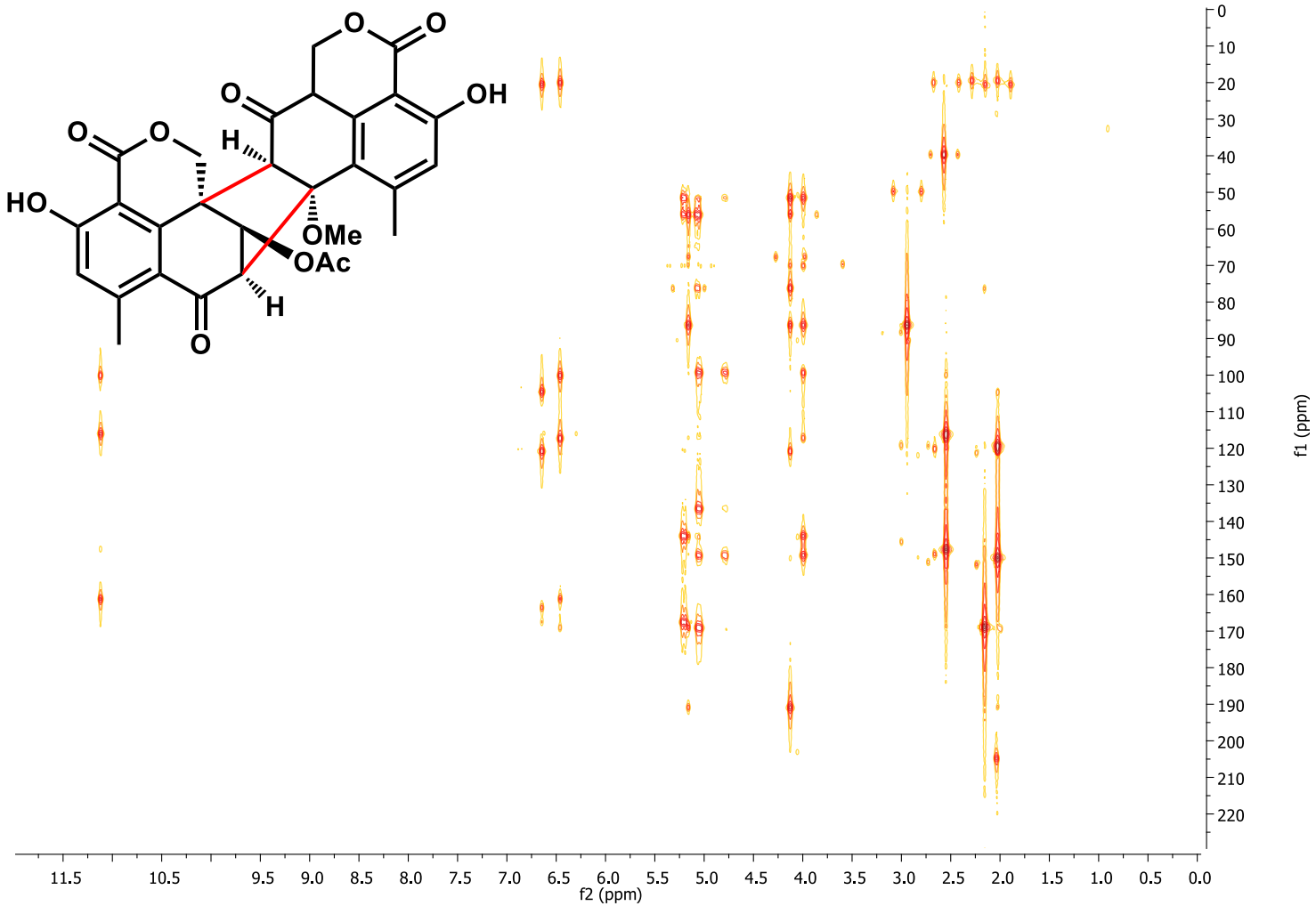


Figure S28. HMBC spectrum of **12** in acetone-d_6.

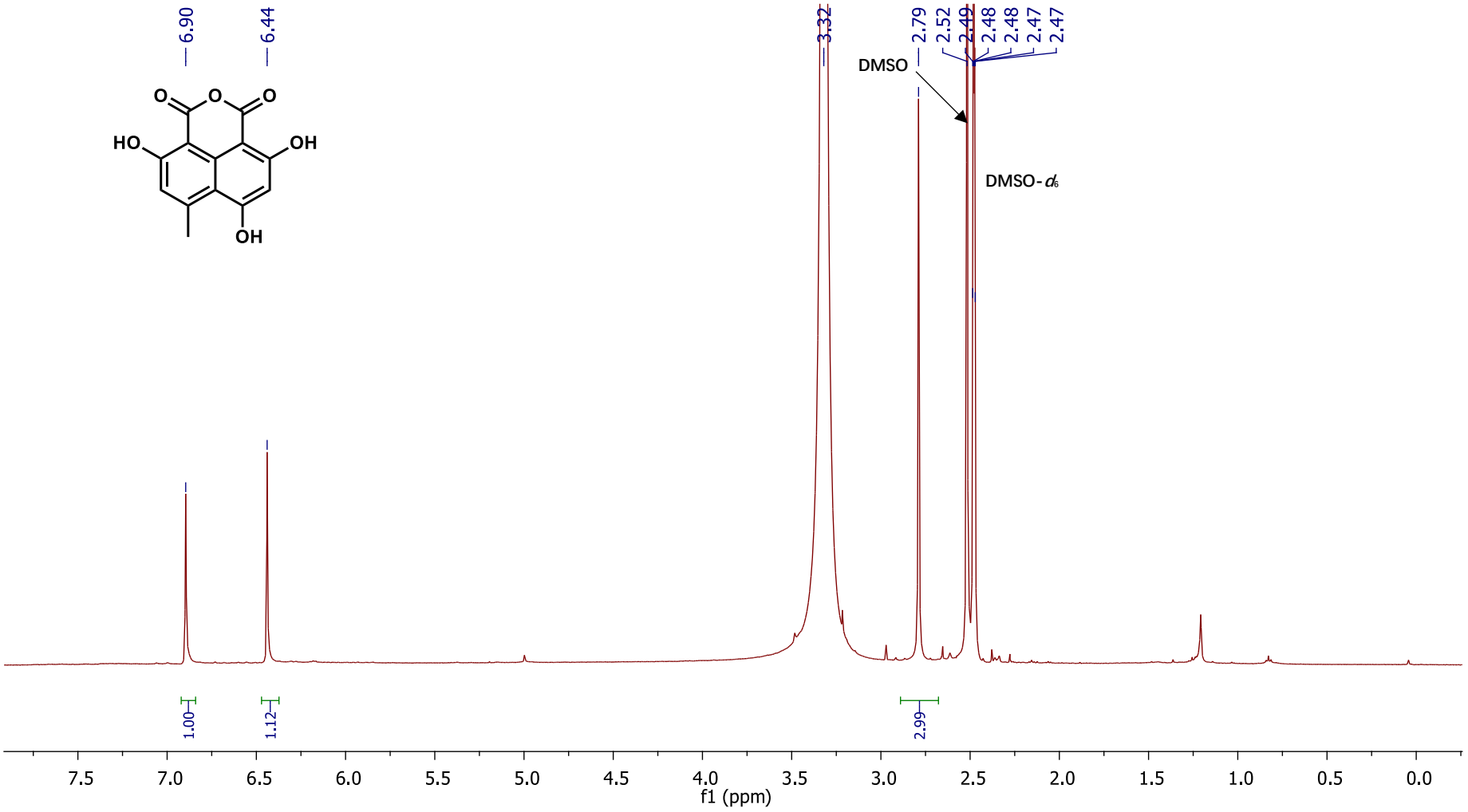


Figure S29. ^{1}H-NMR of **10** in DMSO-d_6 (500 MHz).

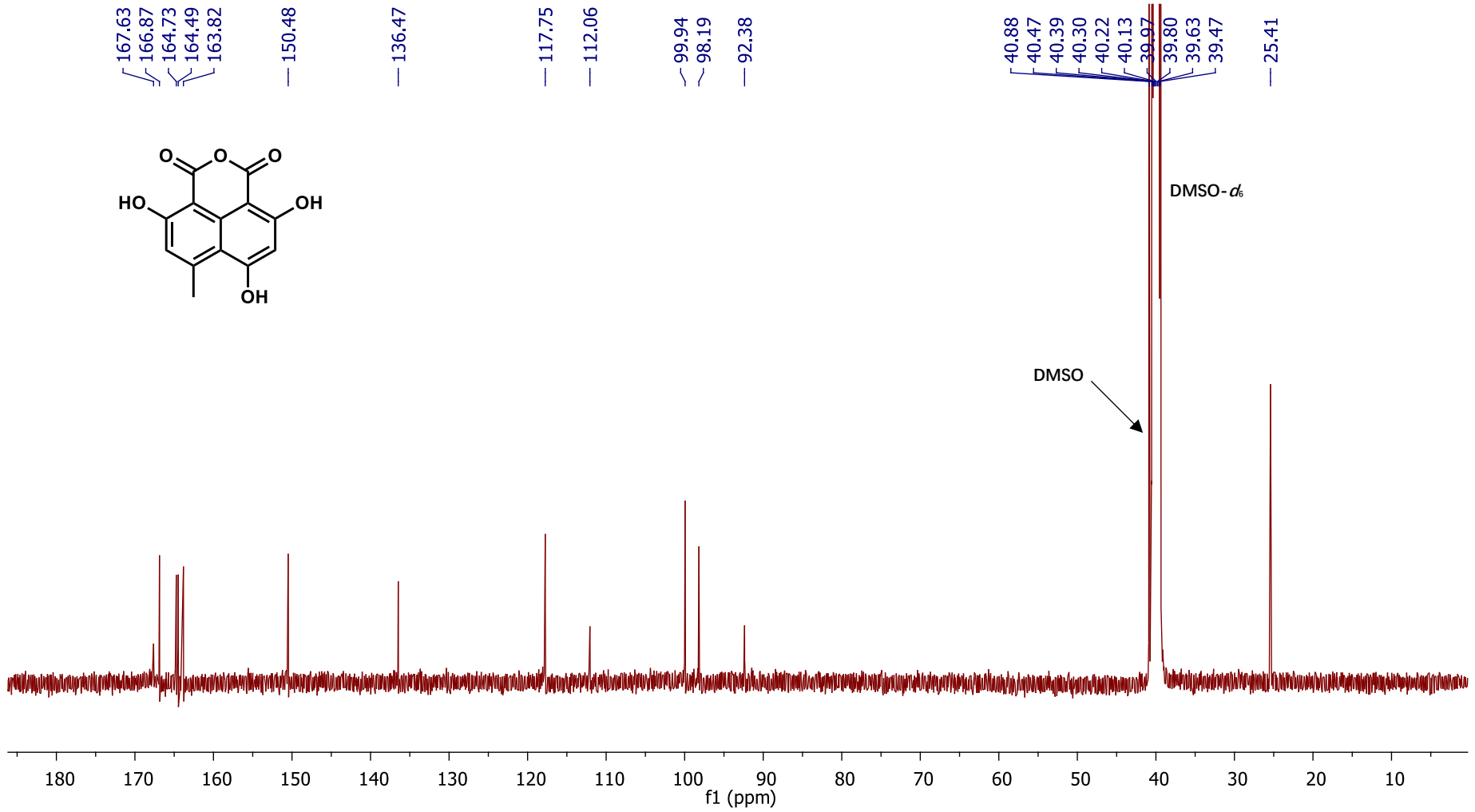


Figure S30. ^{13}C-NMR of **10** in DMSO-d_6 (125 MHz).

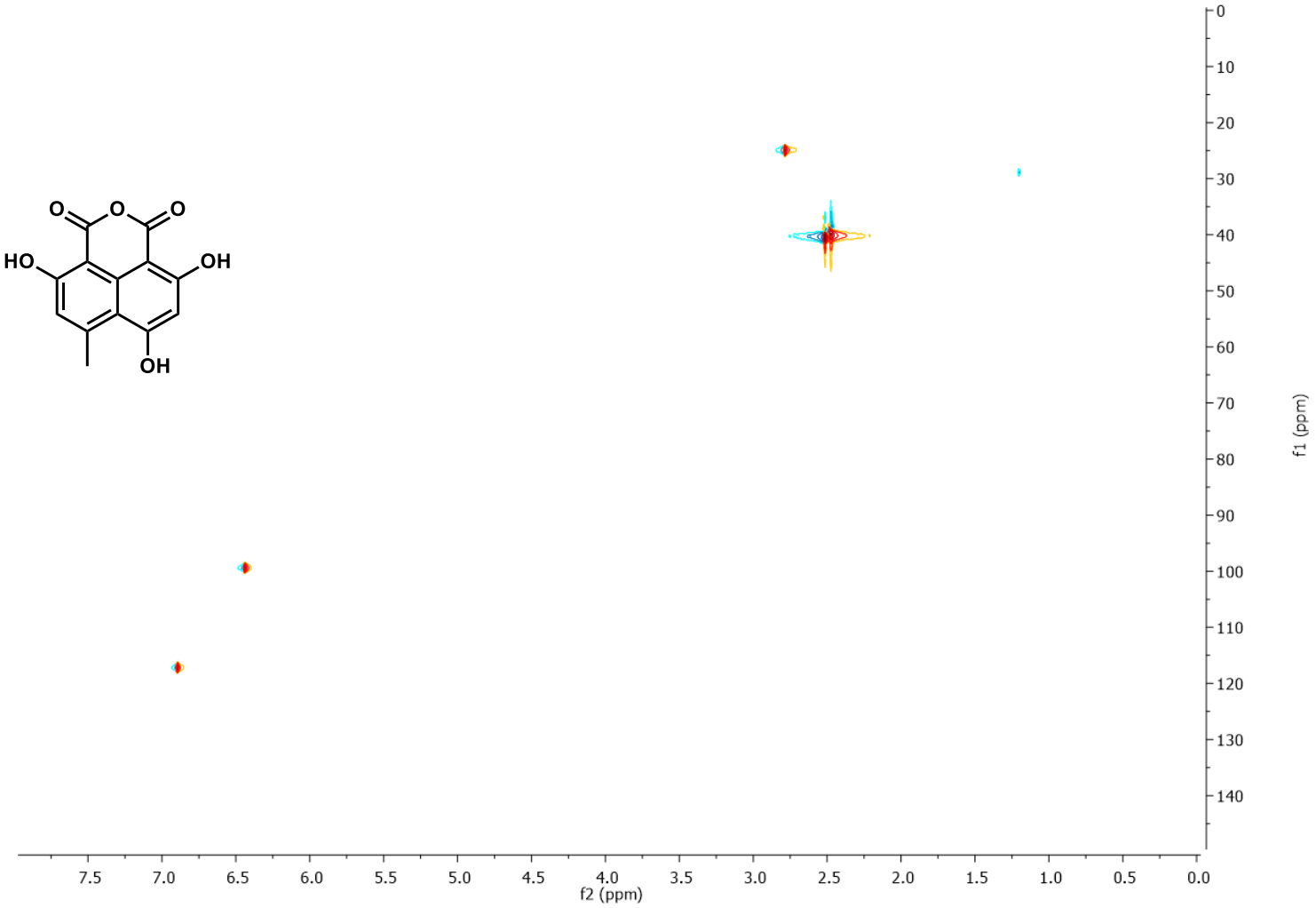


Figure S31. HSQC spectrum of **10** in DMSO-d_6.

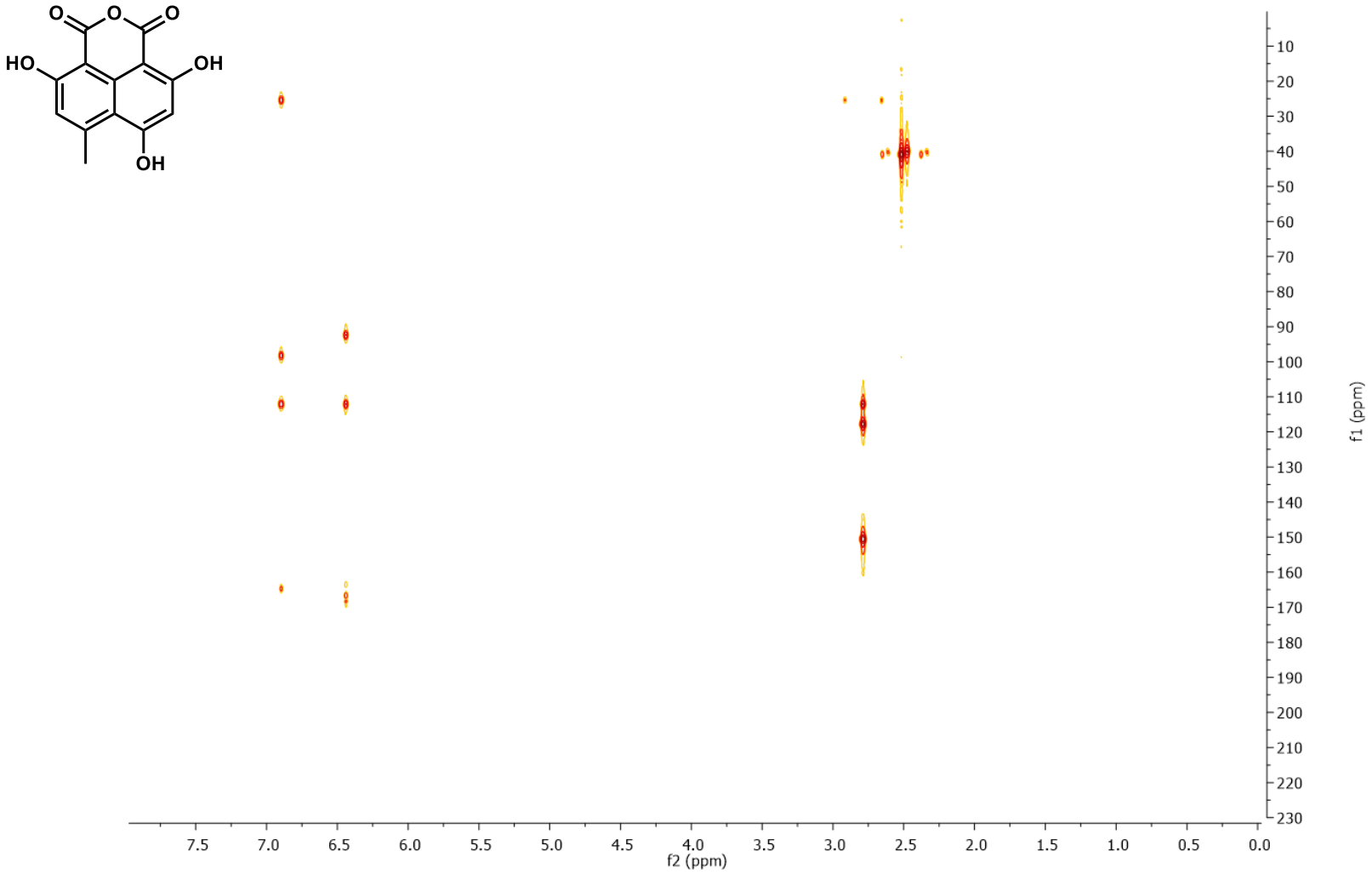


Figure S32. HMBC spectrum of **10** in DMSO-d_6.

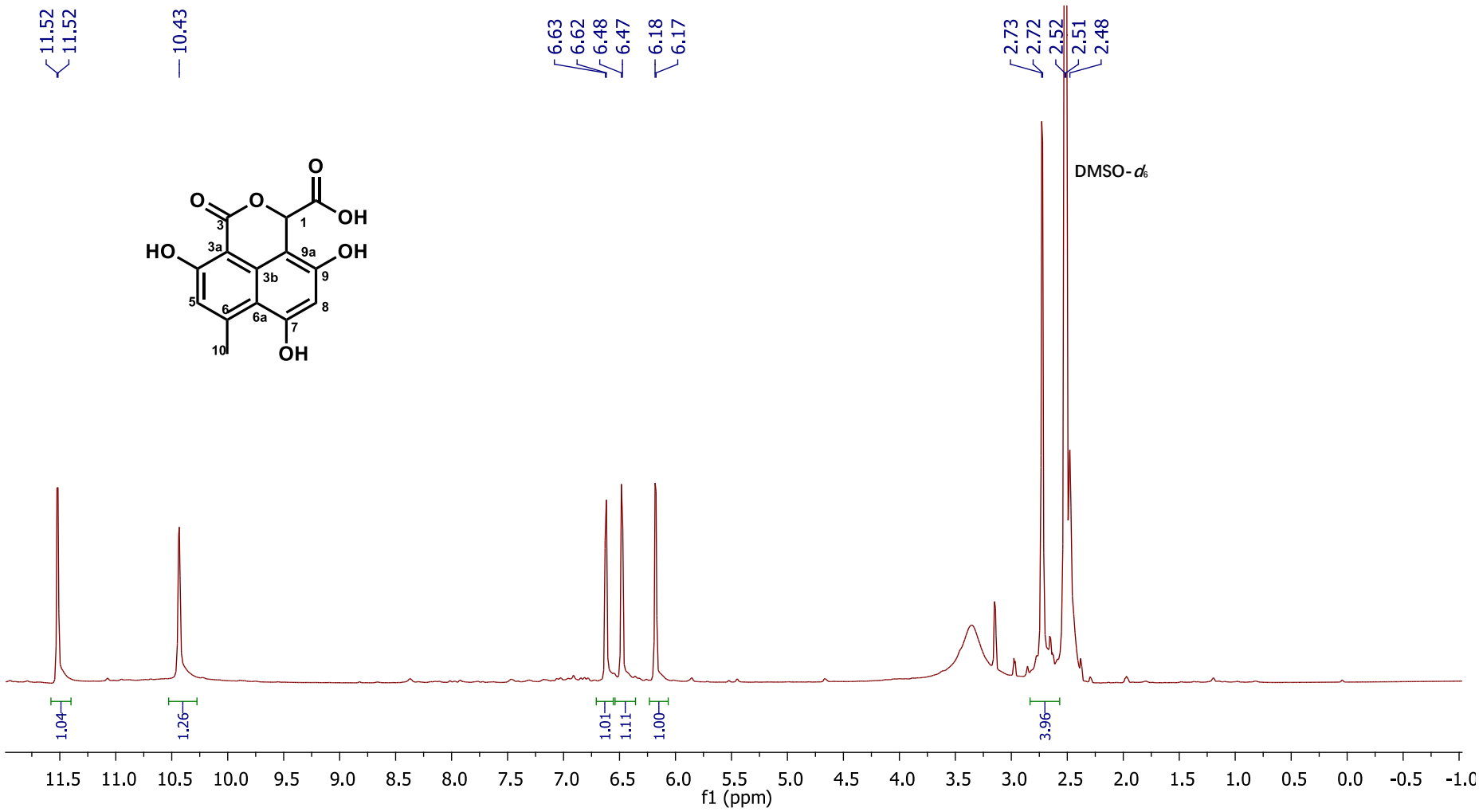


Figure S33. ^{1}H-NMR of **13** in DMSO-d_6 (500 MHz).

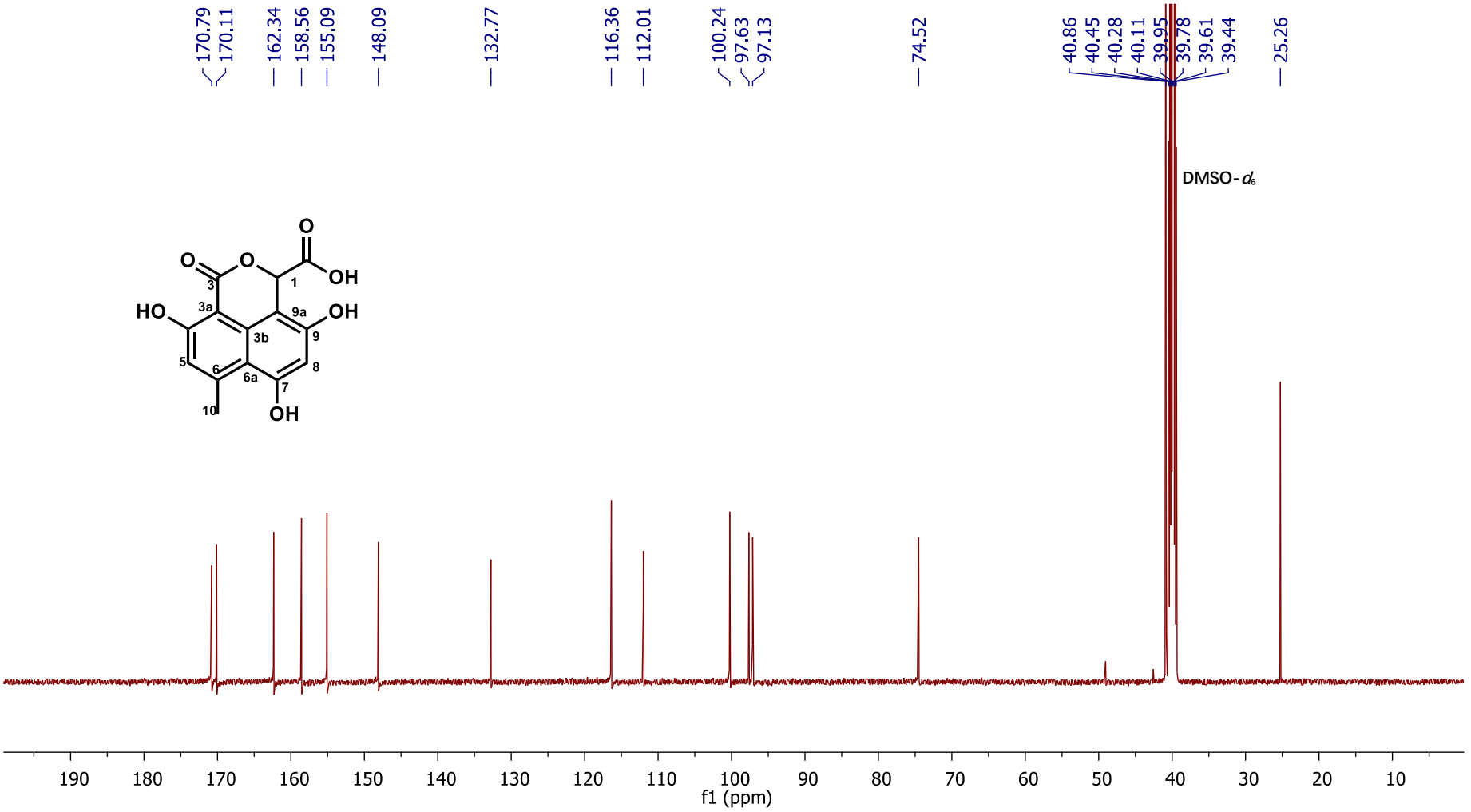


Figure S34. ^{13}C-NMR of **13** in DMSO-d_6 (125 MHz).

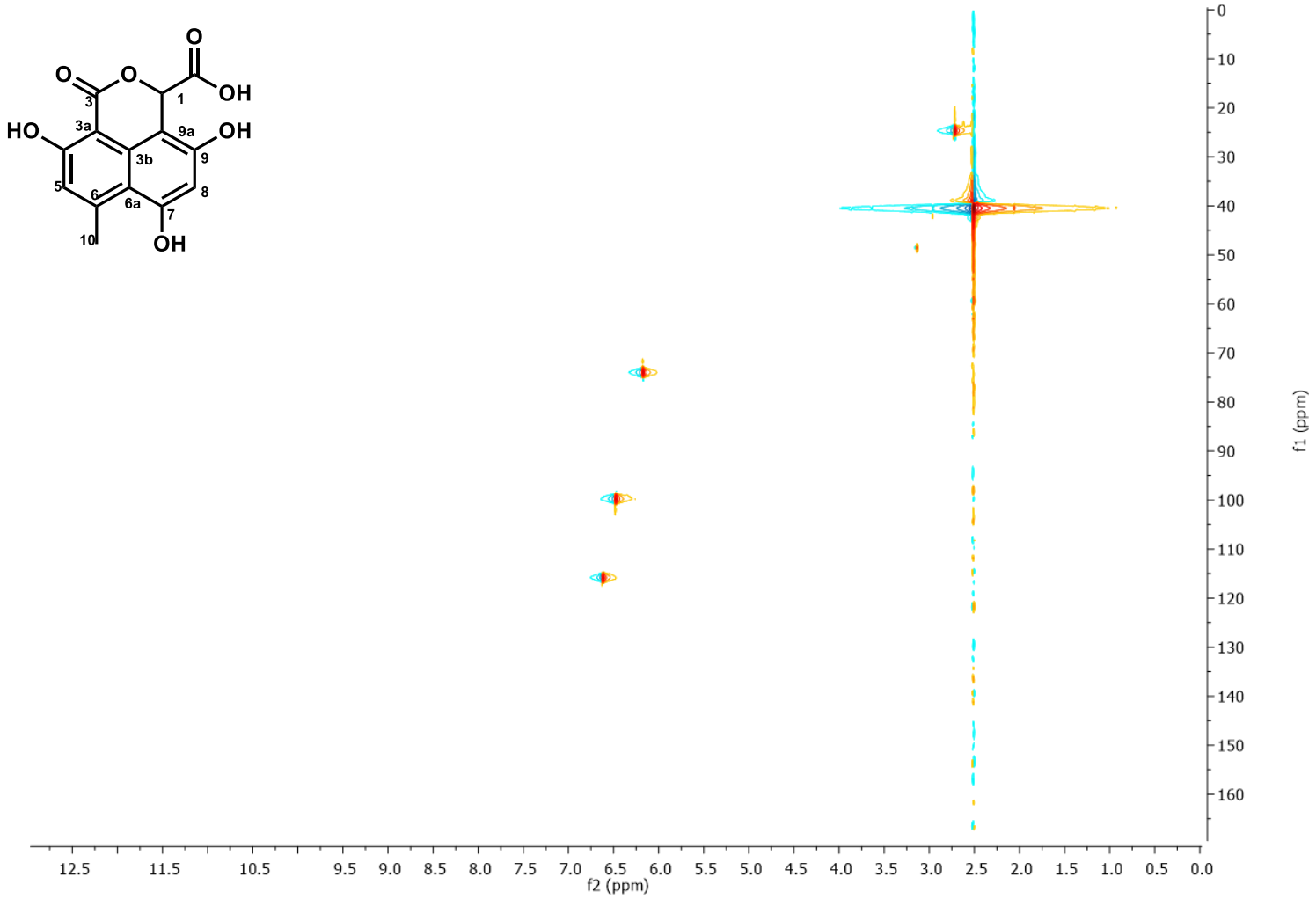


Figure S35. HSQC spectrum of **13** in DMSO-d_6.

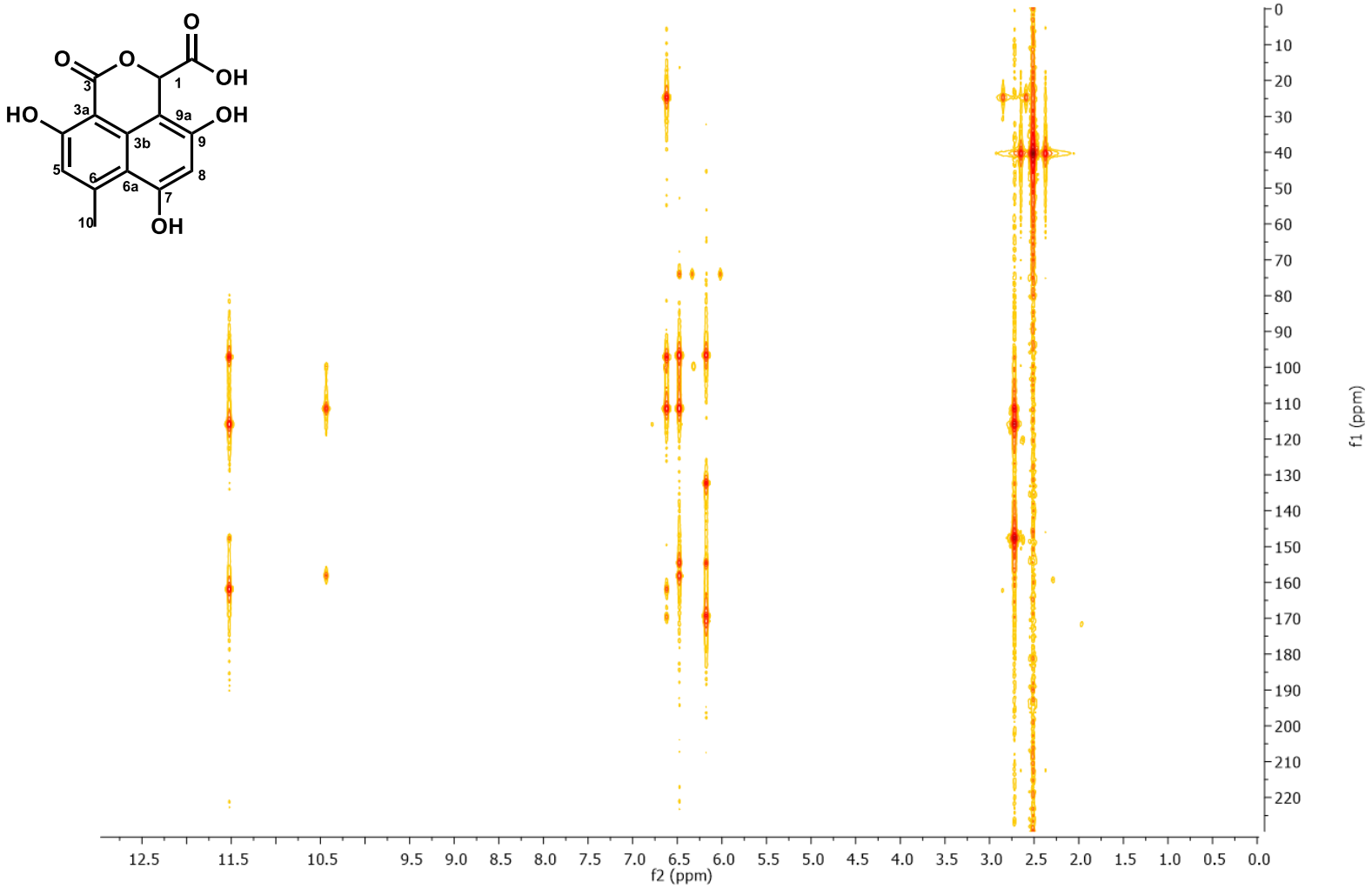


Figure S36. HMBC spectrum of **13** in DMSO-d_6.

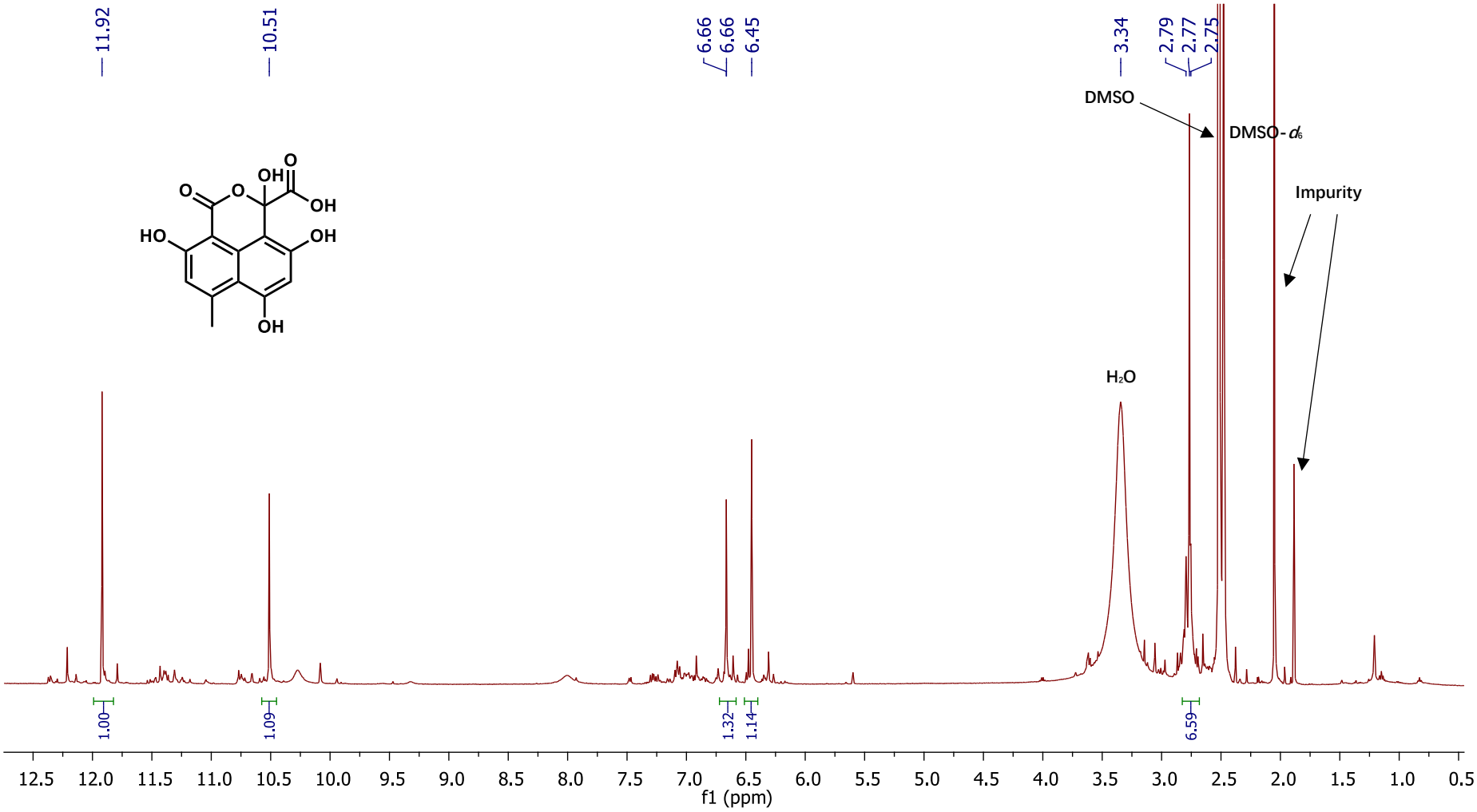


Figure S37. ^{1}H-NMR of **14** in DMSO-d_6 (500 MHz).

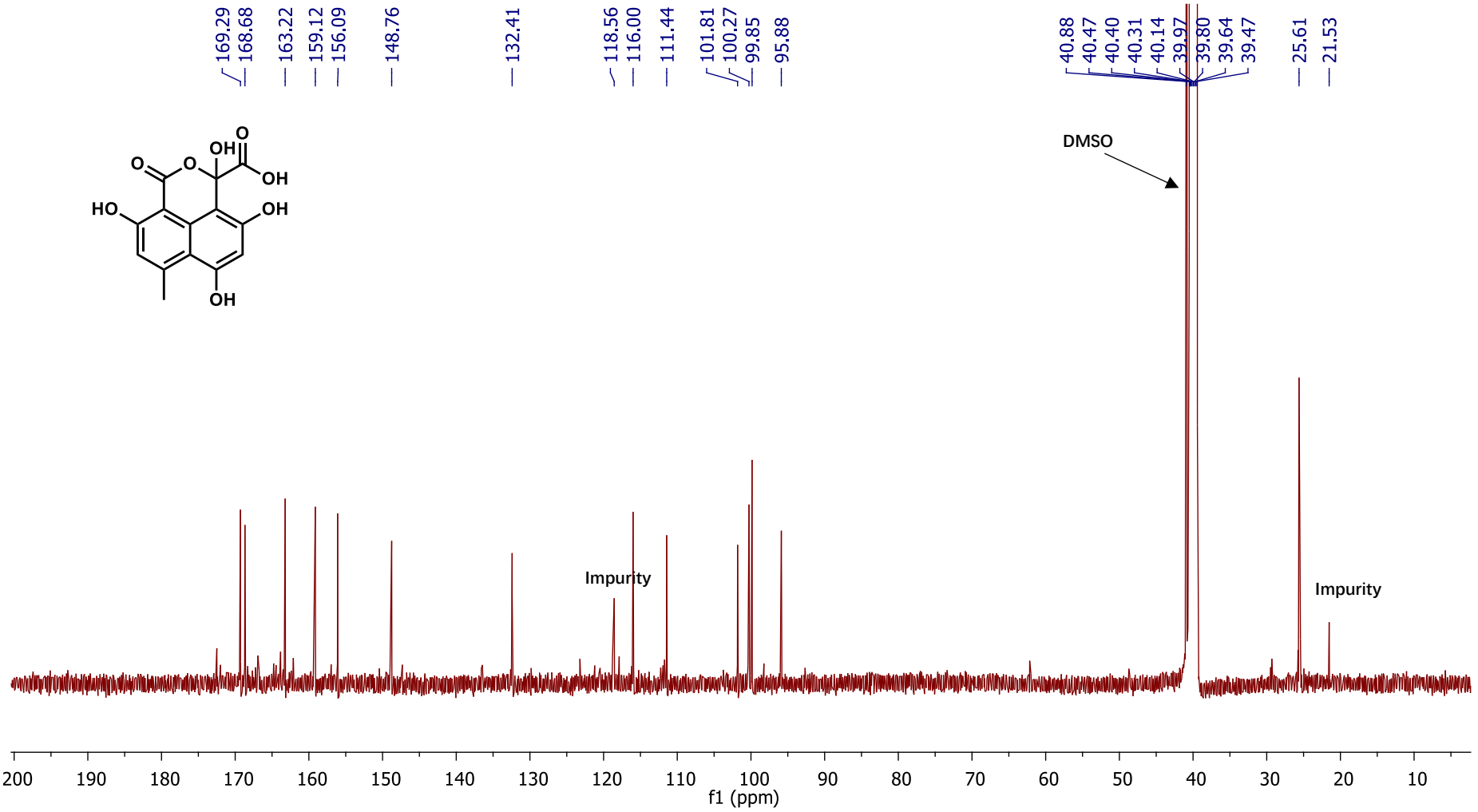


Figure S38. ^{13}C-NMR of **14** in DMSO-d_6 (125 MHz).

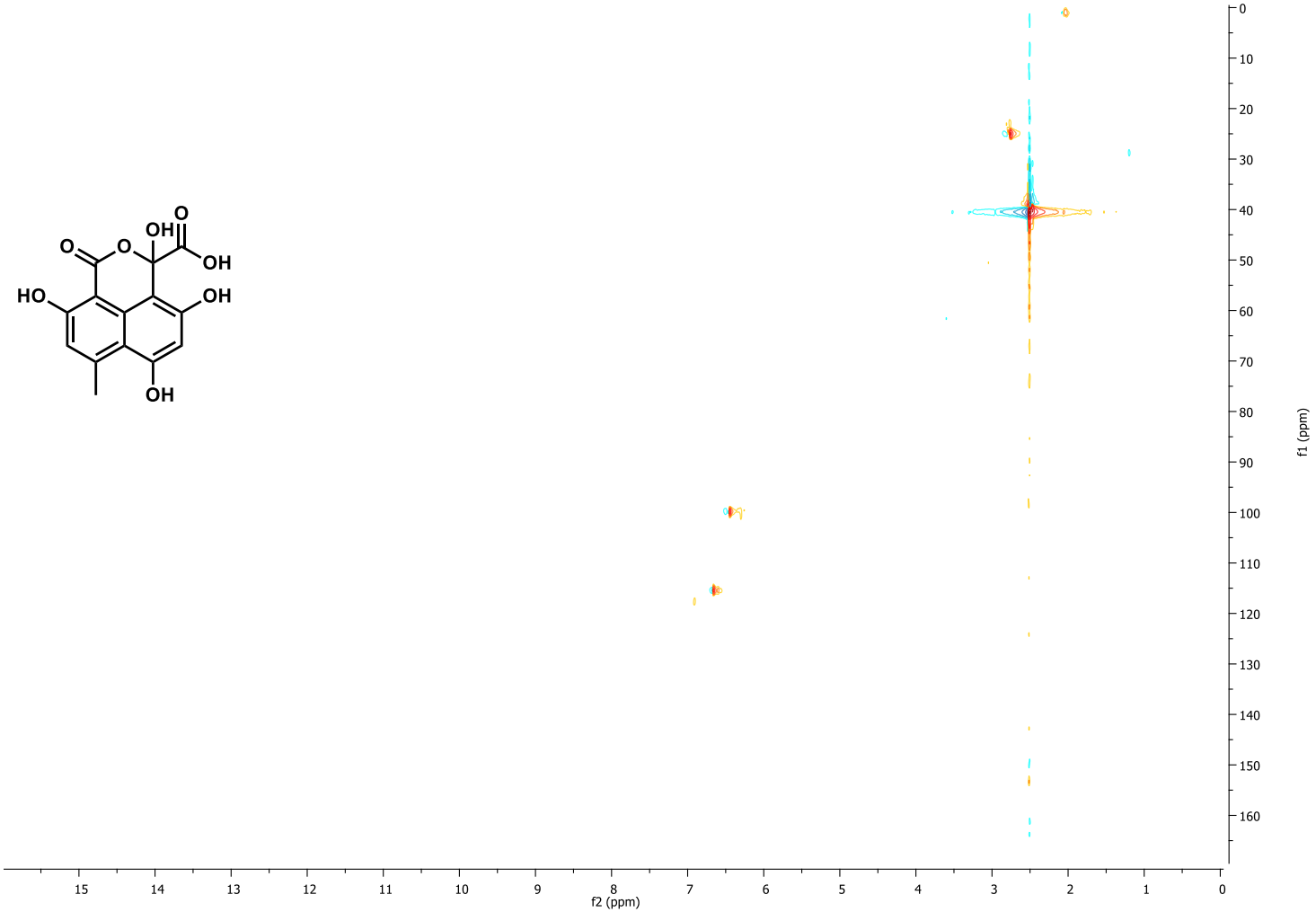


Figure S39. HSQC spectrum of **14** in DMSO-d_6.

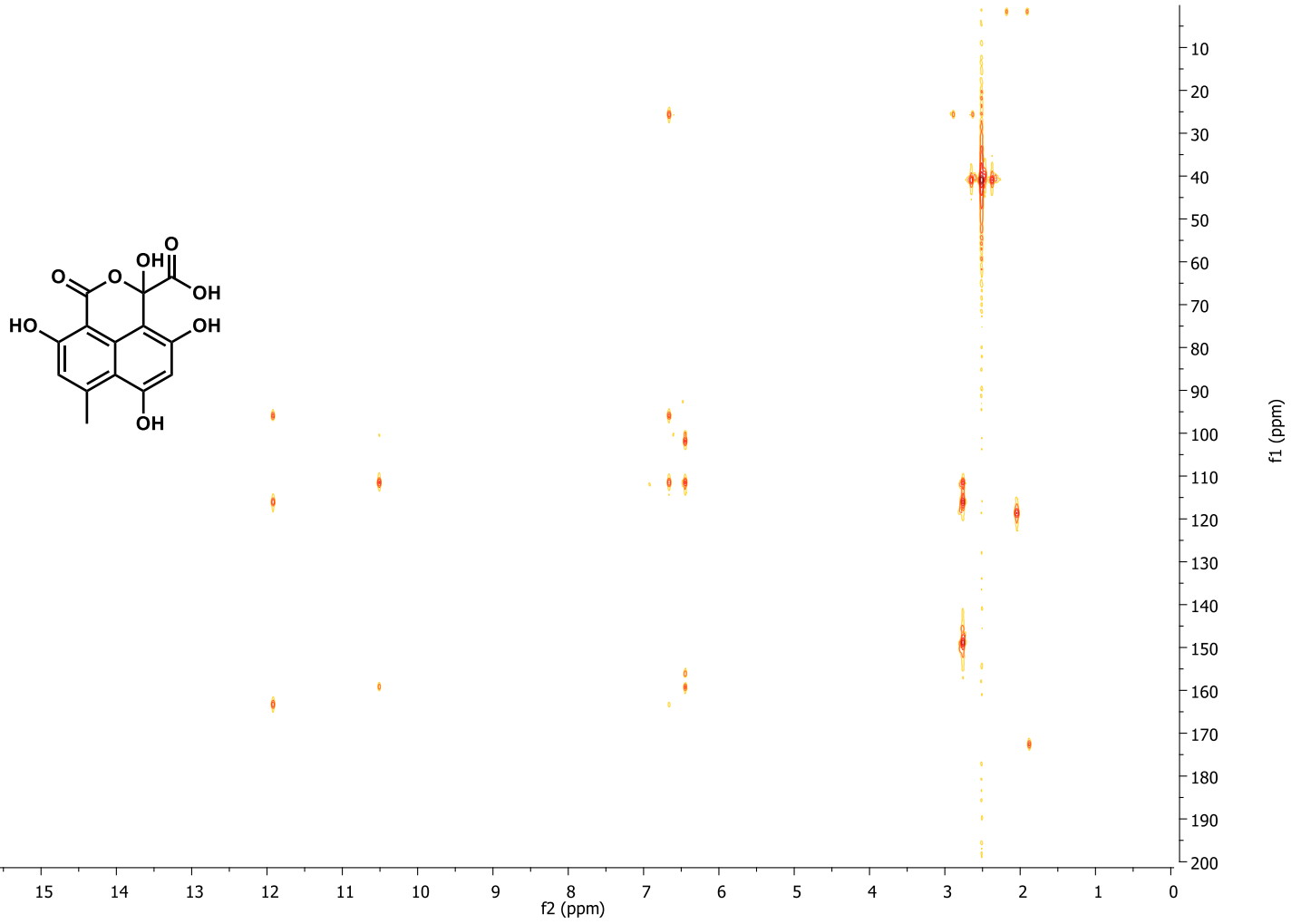


Figure S40. HMBC spectrum of **14** in DMSO-d_6.

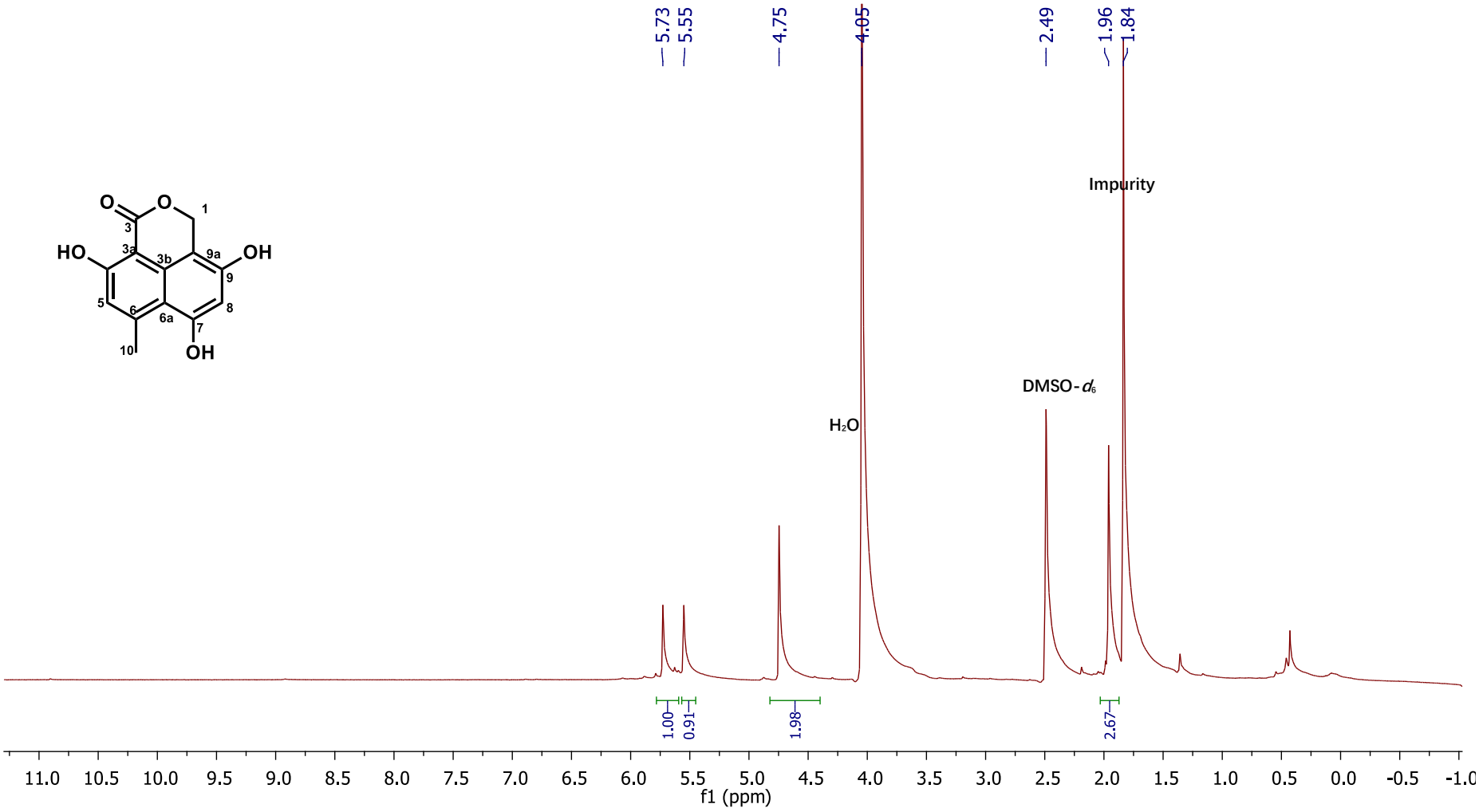


Figure S41. ^{1}H-NMR of **11** in DMSO-d_6 (500 MHz).

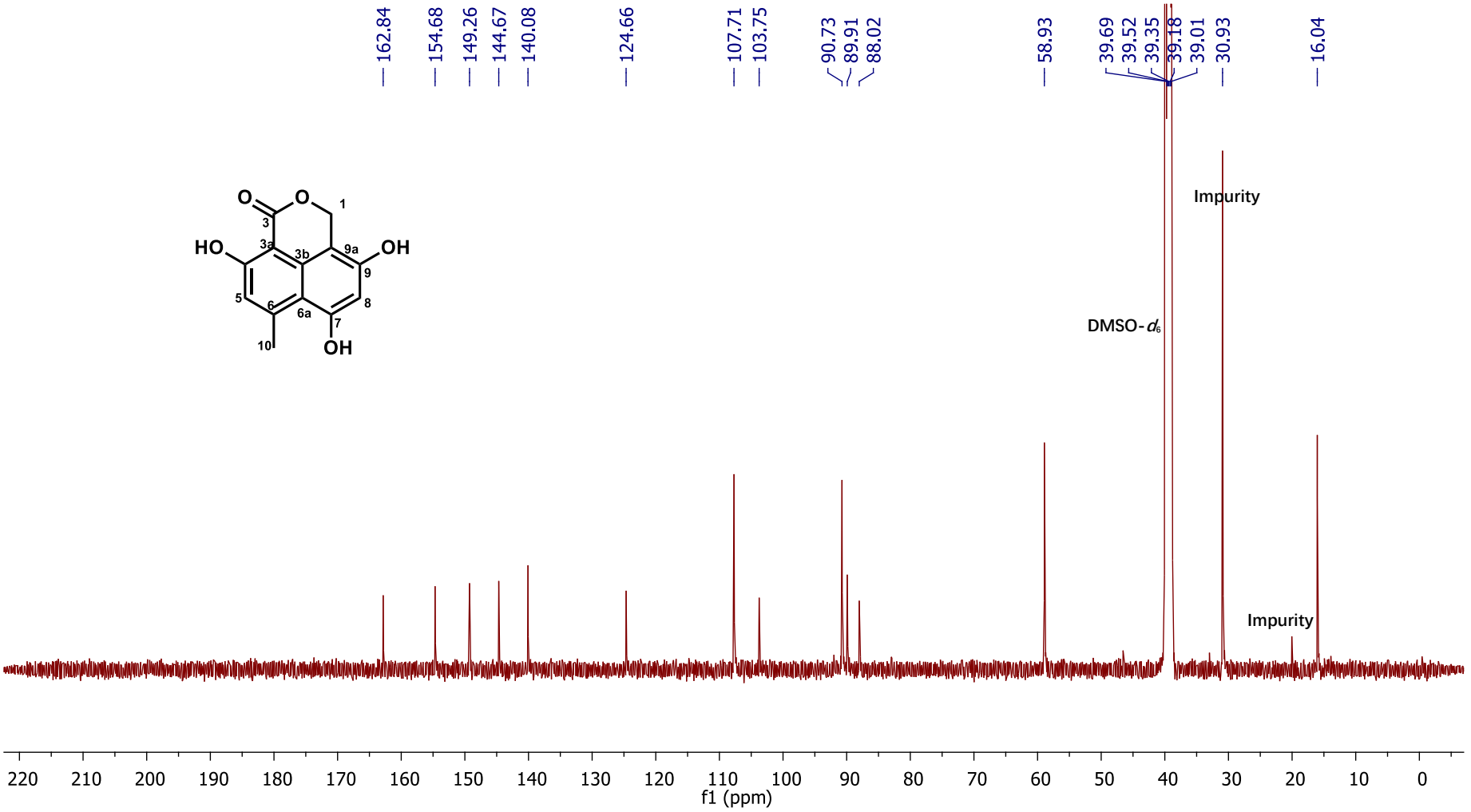


Figure S42. ^{13}C-NMR of **11** in DMSO-d_6 (125 MHz).

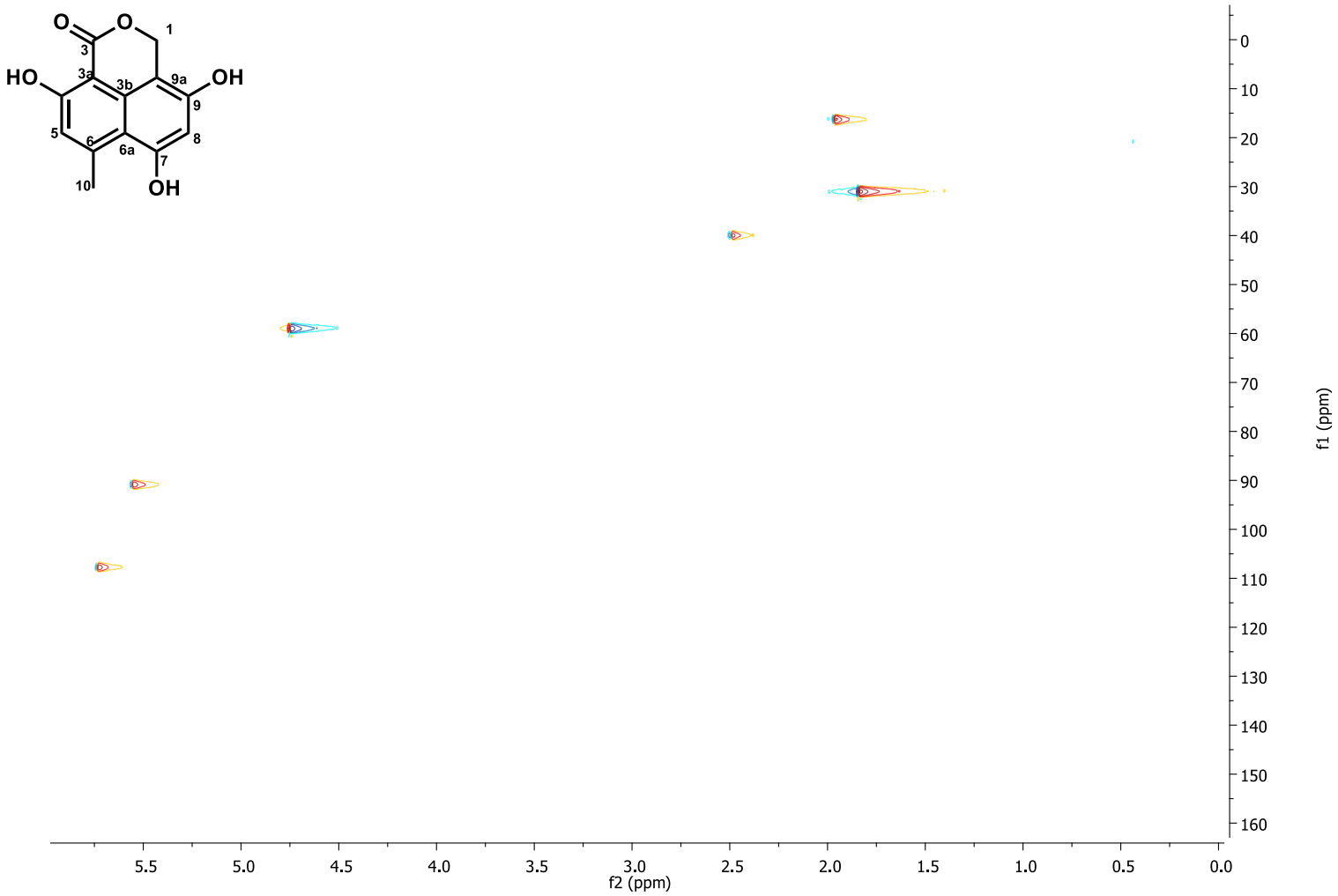


Figure S43. HSQC spectrum of **11** in DMSO-d_6.

Figure S44. HMBC spectrum of **11** in DMSO-d_6.

Supplementary computational data

Cartesian coordinates, electronic energies (in a.u.) and free energies (in a.u.) of all stationary points.

Structure	B3LYP/6-31G(d)−PCM(diethylether)			B3LYP-D3BJ/6-311++G(d,p)−PCM(diethylether) // B3LYP/6-31G(d)−PCM(diethylether)
	Energy (au)	**Gibbs correction (au)**	**Gibbs Energy**	**Energy (au)**
O_2 triplet	-150.3201015	-0.0161960	-150.3871002	-94359.0957261
O_2^-	-150.3912337	-0.0170010	-150.4974462	-94427.8336569
acetate	-228.5772789	0.0215800	-228.6661837	-143503.6299330
acetic acid	-229.0863915	0.0347560	-229.1422853	-143810.6559916
7	-990.8804782	0.1775240	-991.0792470	-622022.5451059
i	-990.3712924	0.1622100	-990.5975322	-621710.6550776
i - radical	-990.2590469	0.1652900	-990.4595499	-621626.0026552
ii complex	-1140.6973078	0.1608580	-1140.9766304	-716074.0441833
iii	-1140.6737737	0.1688040	-1140.9513488	-716063.1659697
iv	-1140.7030957	0.1697480	-1140.9695936	-716075.2070830
15'	-1140.8374258	0.1655560	-1141.1177625	-716165.5539086
15	-1140.3034811	0.1545750	-1140.6126470	-715841.6986849
12	-1140.8475409	0.1697450	-1141.1196707	-716169.3799377
H_2O	-76.4140002	0.0028200	-76.4616122	-47982.1193930
H_3O^+	-76.7849471	0.0156030	-76.8121073	-48210.0796483
OH^-	-75.8254004	-0.0082780	-75.9368789	-47645.8804021

Cartesian coordinates of optimized geometries:

12

C	-0.34560	-2.78371	-0.15751
C	-1.57762	-2.18204	-0.05854
C	-1.71008	-0.75792	0.00146
C	-0.49537	0.01514	-0.10992
C	0.76897	-0.62010	-0.22385
C	0.84570	-2.02421	-0.19701
H	-0.25608	-3.86727	-0.16274
C	-2.95979	-0.07898	0.17241
C	-0.55857	1.45201	-0.09714
C	-1.79550	2.07394	0.08068
C	-2.96668	1.29974	0.21855
H	-3.91246	1.82004	0.35847
C	0.65740	2.24999	-0.33825
O	0.69512	3.46658	-0.40852
O	1.81203	1.58584	-0.57523
C	2.04782	0.18145	-0.37967
O	2.78117	-0.23970	-1.51008
C	3.00748	0.01345	0.86459
O	3.55926	-1.13706	0.93407
O	3.11436	0.95881	1.65187
C	-4.29291	-0.77951	0.32211
H	-4.53577	-1.38196	-0.55787
H	-4.29786	-1.46242	1.17629
H	-5.08653	-0.03979	0.46509
O	-1.89833	3.42701	0.11907
H	-2.83294	3.65327	0.25876
O	1.98791	-2.71491	-0.20984
O	-2.71217	-2.94598	0.00020
H	-2.44858	-3.88025	-0.02860
H	2.14910	-0.30606	-2.24653

```
H    2.75484   -2.09571    0.13740

7

C   -1.51878   -2.53311   -0.00026
C   -2.26693   -1.36985    0.00002
C   -1.66312   -0.06453    0.00030
C   -0.22609   -0.00914    0.00015
C    0.53655   -1.21657    0.00030
C   -0.11945   -2.47036   -0.00018
H   -2.00124   -3.50615   -0.00060
C   -2.41269    1.15231    0.00020
C    0.46252    1.25317    0.00023
C   -0.31733    2.42526   -0.00014
C   -1.71845    2.35099   -0.00005
H   -2.27882    3.28339   -0.00021
C    1.90820    1.25712    0.00013
O    2.58710    2.43159   -0.00033
C    1.97844   -1.19360    0.00019
C   -3.92243    1.23188    0.00033
H   -4.35407    0.73924   -0.87563
H   -4.35387    0.74057    0.87713
H   -4.23844    2.27887   -0.00044
O    0.29018    3.63416   -0.00054
H   -0.39121    4.32718   -0.00073
O    0.56902   -3.61218   -0.00048
O   -3.62028   -1.44165   -0.00006
H   -3.88888   -2.37591   -0.00027
H    1.54608   -3.34784   -0.00047
C    2.61407    0.08093    0.00064
H    3.53624    2.20701   -0.00092
O    2.71132   -2.24188   -0.00002
O    3.98309    0.11872    0.00031
```

```
H    4.25372   -0.82344   -0.00001


acetate

C    0.20525    0.00256   -0.00631
O    0.81317   -1.10056   -0.02086
O    0.69970    1.16194    0.02508
C   -1.35302   -0.05533    0.00146
H   -1.72978    0.27111    0.98085
H   -1.76628    0.63916   -0.74105
H   -1.73348   -1.06375   -0.19579


acetic acid

C    0.09184    0.12337   -0.00000
O    0.64388    1.20387    0.00000
O    0.77956   -1.04402    0.00000
H    1.72764   -0.81089   -0.00001
C   -1.39664   -0.11132    0.00000
H   -1.68348   -0.69272   -0.88230
H   -1.68346   -0.69265    0.88234
H   -1.91935    0.84522   -0.00004
3


H2O

O   -0.90905    0.76634    0.00000
H    0.05877    0.81950    0.00000
H   -1.18199    1.69638    0.00000
```

H3O+

O	-0.89030	0.79236	-0.01270
H	0.03890	0.70724	-0.33172
H	-1.28139	1.63970	-0.33186
H	-1.42962	0.03040	-0.33172

i

C	-1.55296	-2.48857	0.00001
C	-2.27764	-1.31522	0.00019
C	-1.64554	-0.02485	0.00004
C	-0.20802	-0.00819	-0.00002
C	0.54413	-1.22290	-0.00006
C	-0.14394	-2.45944	-0.00011
H	-2.05424	-3.45341	0.00015
C	-2.36767	1.20890	-0.00016
C	0.52426	1.23483	0.00006
C	-0.23281	2.42187	0.00003
C	-1.63471	2.38730	-0.00017
H	-2.16861	3.33701	-0.00039
C	1.95116	1.18189	0.00009
O	2.73052	2.30230	0.00013
C	2.00301	-1.25478	-0.00009
C	-3.87623	1.32909	-0.00044
H	-4.32858	0.85323	-0.87647
H	-4.32886	0.85378	0.87575
H	-4.16174	2.38623	-0.00081
O	0.40915	3.62720	0.00011
H	-0.26545	4.32575	0.00004
O	0.51794	-3.61315	-0.00022
O	-3.64224	-1.36425	0.00063
H	-3.91280	-2.29722	0.00075

H	1.53054	-3.30721	-0.00022
C	2.72253	0.00040	0.00007
H	3.62820	1.84922	0.00013
O	2.62791	-2.38080	-0.00019
O	4.01024	0.11751	0.00012

i - radical

C	-1.57506	-2.48461	0.00035
C	-2.27973	-1.29809	0.00026
C	-1.62332	-0.01593	-0.00009
C	-0.18760	-0.01538	-0.00003
C	0.53922	-1.24856	-0.00012
C	-0.17390	-2.47573	0.00029
H	-2.08981	-3.44067	0.00053
C	-2.33350	1.22790	-0.00038
C	0.53175	1.23774	-0.00015
C	-0.21155	2.44089	0.00021
C	-1.60981	2.41096	-0.00012
H	-2.14506	3.35726	-0.00015
C	1.95245	1.20558	-0.00031
O	2.69886	2.30735	0.00044
C	1.98899	-1.30239	-0.00025
C	-3.83922	1.35373	-0.00089
H	-4.28385	0.87389	-0.87724
H	-4.28442	0.87410	0.87529
H	-4.12321	2.40941	-0.00109
O	0.44822	3.61583	0.00093
H	-0.19575	4.34455	0.00127
O	0.45404	-3.64845	0.00028
O	-3.63259	-1.31652	0.00033
H	-3.94060	-2.23880	0.00041
H	1.44276	-3.43234	0.00041

C	2.71735	-0.01702	-0.00042
H	3.62467	1.94568	0.00021
O	2.63429	-2.38311	-0.00004
O	3.97453	0.07394	-0.00066

ii

C	2.30595	2.24341	0.16176
C	2.82319	0.96554	0.19859
C	1.99813	-0.19913	0.03373
C	0.59314	0.02416	-0.17583
C	0.05821	1.34911	-0.22108
C	0.92835	2.45003	-0.04135
H	2.94886	3.11047	0.29038
C	2.50403	-1.53600	0.07433
C	-0.31388	-1.08120	-0.35994
C	0.23153	-2.37716	-0.30817
C	1.60392	-2.57656	-0.09763
H	1.97291	-3.60087	-0.06857
C	-1.69879	-0.79584	-0.57395
O	-2.62583	-1.76604	-0.76476
C	-1.35876	1.62537	-0.43323
C	3.95545	-1.90455	0.29206
H	4.60731	-1.48507	-0.48051
H	4.33462	-1.53418	1.24965
H	4.06544	-2.99368	0.27858
O	-0.58624	-3.45483	-0.46985
H	-0.04557	-4.25868	-0.40117
O	0.47343	3.70122	-0.06579
O	4.16070	0.78368	0.39485
H	4.57758	1.65613	0.48834
H	-0.55170	3.58468	-0.22786
C	-2.25307	0.50397	-0.65719

```
H   -3.43754   -1.18728   -0.86948
O   -1.79314    2.82976   -0.45553
O   -3.51387    0.60620   -0.88569
O   -2.89697   -0.38166    2.10167
O   -4.06012   -0.49813    1.69359
```

iii

```
C    2.05757    2.32547    0.09853
C    2.60850    1.06234    0.02347
C    1.80674   -0.12080   -0.10267
C    0.37508    0.04743   -0.11482
C   -0.19136    1.37986   -0.08981
C    0.66763    2.49705    0.02684
H    2.68774    3.20612    0.18944
C    2.36128   -1.43870   -0.19908
C   -0.48317   -1.08719   -0.19007
C    0.08957   -2.35098   -0.24354
C    1.49137   -2.50418   -0.28276
H    1.89539   -3.51287   -0.35380
C   -1.95823   -0.86857   -0.02468
O   -2.76379   -1.88382   -0.51773
C   -1.60349    1.63428   -0.27053
C    3.84280   -1.75137   -0.22471
H    4.35379   -1.25056   -1.05226
H    4.34456   -1.42942    0.69237
H    3.98808   -2.83069   -0.33431
O   -0.69220   -3.46658   -0.25355
H   -0.11012   -4.24316   -0.23025
O    0.19678    3.74938    0.04913
O    3.96196    0.91874    0.06786
H    4.36290    1.79871    0.16107
H   -0.80724    3.65343   -0.06990
```

C	-2.48009	0.46342	-0.50811
H	-3.57923	-1.40024	-0.77953
O	-2.09390	2.79944	-0.32214
O	-3.64626	0.54607	-0.92021
O	-2.29171	-0.59013	1.39424
O	-1.25100	-0.78805	2.30100

iv

C	2.06580	2.37428	-0.00552
C	2.66265	1.13394	-0.03137
C	1.90360	-0.08654	-0.03321
C	0.46266	0.02920	-0.03728
C	-0.15581	1.34243	-0.02077
C	0.66553	2.48984	0.01488
H	2.66179	3.28294	0.00820
C	2.51361	-1.38331	-0.01520
C	-0.33815	-1.14783	-0.04304
C	0.28941	-2.38597	0.01843
C	1.69234	-2.49210	0.01920
H	2.13944	-3.48436	0.04942
C	-1.84408	-1.05612	-0.06341
O	-2.41882	-1.88861	-1.00394
C	-1.59844	1.54174	-0.03517
C	4.00767	-1.63549	-0.02240
H	4.49050	-1.21417	-0.90878
H	4.50446	-1.19021	0.84432
H	4.19652	-2.71350	-0.00936
O	-0.47720	-3.51136	0.09042
H	0.10916	-4.28232	0.15273
O	0.16098	3.72817	0.06141
O	4.02122	1.04409	-0.04803

```
H    4.38945    1.94313   -0.03840
H   -0.84502    3.59716    0.07593
C   -2.52033    0.34639   -0.20639
H   -3.10900   -1.25834   -1.38153
O   -2.10649    2.68620    0.03879
O   -3.38299    0.43674   -1.15751
O   -2.37734   -1.22020    1.27886
O   -3.09135    0.08708    1.23687


O2-

O    0.00000    0.00000    0.67492
O    0.00000    0.00000   -0.67492



O2 triplet

O    0.00000    0.00000    0.60710
O    0.00000    0.00000   -0.60710


OH-

O   -0.88630    0.78309    0.00000
H   -1.29682    1.67358    0.00000


15

C    0.57155   -2.80921   -0.07695
C    1.74619   -2.10780    0.00049
C    1.76606   -0.67540    0.03815
```

```
C    0.48408    0.00638    0.04702
C   -0.74422   -0.76042    0.10312
C   -0.68378   -2.15430   -0.03180
H    0.57382   -3.89529   -0.14564
C    2.98337    0.08198    0.03794
C    0.44731    1.43973   -0.05321
C    1.65298    2.12511   -0.04776
C    2.89231    1.45708    0.01877
H    3.80809    2.04953    0.01693
C   -0.84598    2.24304   -0.33995
O   -1.07364    3.23578    0.38564
C   -2.01968   -0.12173    0.60344
C    4.37698   -0.51614    0.04261
H    4.55451   -1.15634    0.91222
H    4.56553   -1.13557   -0.84016
H    5.11885    0.29026    0.05603
O    1.65815    3.49884   -0.16513
H    2.58473    3.77841   -0.23422
O   -1.75412   -2.96133   -0.06775
O    2.94370   -2.78774    0.01380
H    2.73753   -3.73610   -0.01025
H   -2.57459   -2.36612   -0.37383
C   -3.41913   -0.37198   -0.06097
O   -1.98445    0.56933    1.61421
O   -4.29899    0.46225    0.17326
O   -1.51328    1.81622   -1.32163
O   -3.55445   -1.43920   -0.76571
```

15'

```
C   -0.62884   -2.82993    0.13584
C   -1.78918   -2.10679    0.05522
C   -1.77916   -0.67165   -0.01829
```

C	-0.48465	-0.02947	-0.02786
C	0.73089	-0.81068	-0.08904
C	0.64467	-2.21134	0.05998
H	-0.65437	-3.91371	0.21860
C	-2.97719	0.11419	-0.06846
C	-0.44506	1.40495	0.05903
C	-1.62485	2.13436	0.01844
C	-2.87081	1.48985	-0.08068
H	-3.77243	2.09773	-0.12313
C	0.80901	2.13262	0.42568
O	1.10949	3.16125	-0.39131
C	1.99228	-0.18391	-0.61286
C	-4.37812	-0.46092	-0.11305
H	-4.51895	-1.12955	-0.96661
H	-4.60768	-1.04657	0.78157
H	-5.10641	0.35271	-0.18803
O	-1.56987	3.49561	0.13759
H	-2.47738	3.83735	0.17555
O	1.67963	-3.03909	0.07165
O	-2.99709	-2.74816	0.07147
H	-2.82863	-3.70379	0.11506
H	2.58585	-2.47078	0.21521
C	3.40813	-0.46708	-0.00326
H	1.94291	3.53718	-0.04748
O	1.92682	0.61002	-1.54702
O	4.19585	0.48318	-0.00581
O	1.48597	1.86482	1.40119
O	3.64349	-1.65087	0.43411