Supporting Information

Late-Stage Terpene Cyclization by an Integral Membrane Cyclase in the Biosynthesis of Isoprenoid Epoxycyclohexenone Natural Products

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Experimental procedures

1. General DNA Manipulation Techniques.

DNA restriction enzymes were used as recommended by the manufacturer (New England Biolabs, NEB). PCR was performed using Phusion® High-Fidelity DNA Polymerase (NEB). PCR products were confirmed by DNA sequencing. Genomic DNA samples were prepared using the CTAB isolation buffer at pH 8.0 (20 g/L cetyl trimethylammonium bromide, 1.4 M sodium chloride and 20 mM EDTA). The gene-specific primers are listed in Table S1. RNA extraction was followed the standard protocol for RiboPure™ RNA Purification Kit (yeast). The cDNA samples were prepared by using the SuperScript™ First-Strand Synthesis System with oligo-dT primers following the standard protocol.

2. Strain and Culture conditions.

E. coli TOPO10 and *E. coli* DH10b were used for cloning, following standard recombinant DNA techniques. *Saccharomyces cerevisiae* strain BJ5464-NpgA (*MATα ura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*) was used for plasmid assemble and as protein expression host.

Penicillium terrestris LM2 and the mutants were maintained on PDA (potato dextrose agar, BD) at 28℃, 7 days for sporulation. For compound production, the strains were cultured on TGA (tryptone 1%, glucose 4%, agar 2%, pH 5.6) at 28℃ for 7 days.

3. Preparation of the *macR* overexpression strain.

The overexpression of *macR* in *Penicillium terrestris* LM2 was carried out by integration of an expression cassette containing *macR* which was under control of the constitutive promoter *PgpdA*. The cassette comprised of *bar* gene, which encodes phosphinothricin acetyltransferase, as a selection marker and *macR* was introduced into *Penicillium terrestris* LM2 yielding the *macR* overexpression strain DH1 by using the PEG-mediated protoplast transformation of *Penicillium terrestris* LM2. The experiments for protoplast preparation and transformation were carried out following the protocol as described for *Penicillium oxalicum*[1].

4. Preparation of the deletion strains based on the *macR* overexpression strain DH1.

The deletion of the target gene in strain DH1 was carried out by homologous recombination. A disruption cassette comprised of selection markers *ble* (zeocin resistant gene) flanked on both sides by a 2 kb fragment that is homologous to the site of recombination in the *Penicillium terrestris* LM2 genome was introduced to DH1 to replace the target gene with the selection marker. The disruption cassette was introduced into *Penicillium terrestris* LM2 by PEG-mediated protoplast transformation as described previously. The gene knock-out of *macA* and *macJ* in strain DH1 yielded the deletion strain DH1/*ΔmacA* and DH1/*ΔmacJ*, respectively. The primers used for disruption cassette construction were listed in Table S1. The genotype of the deletion strains were verified by PCR.

5. Chemical analysis and compound isolation.

For small scale analysis, the *Penicillium terrestris* LM2 wild type and mutant strains were grown on TGA plates for 7 days at 28℃. 1 cm × 1 cm agar was extracted with 1 mL acetone, and then concentrated by evaporation. The residues were dissolved in 300 μL methanol, and 20 μL of the

solution was injected to the LC-MS system for analysis. LC-MS analysis was performed on a Shimadzu 2020 LC–MS (Phenomenex kinetex, 1.7 µm, 2.0 x 100 mm, C18 column) using positive and negative mode electrospray ionization with a linear gradient of 5−95% MeCN−H_2O supplemented with 0.1% (v/v) formic acid in 15 min followed by 95% MeCN for 3 min with a flow rate of 0.3 mL/min.

For compound isolation, the strains were cultured on TGA plates and incubated at 28℃ for 7 days. Then the agar plates were extracted with acetone. After filtration, the solvent was removed by evaporation, and the crude extracts were separated by silica chromatography. The fractions containing the target compound were combined and concentrated. For further purification, two rounds of semi-preparative HPLC were carried out. 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 deuterated solvent on Bruker AV500 spectrometer with a 5 mm dual cryo-probe at the UCLA Molecular Instrumentation Center.

For the compounds isolated, compounds **2**-**7** are known compounds[2-5]. Compound **1** is a new natural product. From 4 liters TGA culture of strain DH1, 1.5 mg compound **1**, 0.8 mg compound **2**, 5.0 mg compound **3**, and 12.0 mg compound **4** were purified as white powder. From 15 liters TGA culture of strain DH1/*ΔmacJ*, 10.0 mg compound **5**, 3.0 mg compound **6**, and 2 mg compound **7** were purified as colorless oil.

The HR-MS data of **1** is: HRMS-ESI (m/z) $[M+Na]^+$ cacld for $C_{25}H_{32}O_7Na$ 467.2046, found 467.2029.

Optical rotation data was collected on a Jasco P-1020 system. For compound **1**, $[\alpha]_D^{25}$ = +17 (*c*=0.1, MeOH); for compound **2**, $[\alpha]_D^{25}$ = +31 (*c*=0.1, MeOH); for compound **3**, $[\alpha]_D^{25}$ = +15 (*c*=0.1, MeOH); for compound **4**, $[\alpha]_D^{25}$ = +44 (*c*=0.1, MeOH).

For 6-methylsalicyclic acid (6-MSA) production, the yeast strain harboring the expression plasmid of MacA was cultured in SDCt (-Ura) medium at 28℃ for two days as the seed culture. Then 500 µL of the seed culture was inoculated into 2 mL of YPD medium at 28℃. After three days, the culture was extracted with equal volume of ethyl acetate (1% acetic acid). The organic phase was dried by evaporation and the residue was dissolved in 500 µL MeOH, of which 3 µL was injected into the LC-MS for analysis.

6. Construction of plasmids for expression of MacJ and its mutants in yeast.

For expression of MacJ, the primers MacJ-For and MacJ-Rev listed in Table S1 were used to amplify the intron-free *macJ* from the cDNA of strain DH1. The intron-free *macJ* fragment was inserted into the digested plasmid pXW02 (*Nde*I and *Pme*I) by using yeast *in vivo* homologs recombination to create the expression plasmid pTMC1-182-4, which was further confirmed by sequencing.

For mutation studies, the primer pairs MacJ-D31A-For/Rev, MacJ-D55A-For/Rev, MacJ-E72A-For/Rev, MacJ-D96A-For/Rev, and MacJ-D229A-For/Rev, were used to construct the expression plasmids for MacJ D31A, MacJ D55A, MacJ E72A, MacJ D96A, and MacJ D229A, respectively. The *macJ* mutants were amplified by PCR from pTMC1-182-4 using the QuikChange® Site-Directed Mutagenesis Kit (Stratagene) with the corresponding primer pairs. The mutated genes were confirmed by DNA sequencing.

7. Biotransformation of compound 7 to compound 2.

The yeast strain harboring the expression plasmid of MacJ was cultured in SDCt (-Leu) medium

for 2 days at 28℃ as the seed culture. Then 0.5 mL of the seed culture was inoculated into 4.5 mL of YPD medium and cultured at 28℃, 250 rpm. After 24 hrs, the culture was span down by centrifugation (4℃, 1600 rpm, 5 min). And the cell pellets were collected and resuspended in 0.5 mL of fresh YPD. Then compound **7** was added into the YPD medium to a final concentration of 0.1 mg/mL. After two days cultured at 28℃, 250 rpm, the culture was extracted with 1 mL of EtOAc. After concentrated, the crude extract was dissolved in 200 μL MeOH and 10 μL was injected into the LC-MS for analysis.

8. Microsomal fraction preparation and *in vitro* assay test.

S. cerevisiae BJ5464-NpgA was transformed with the corresponding expression plasmid of the MacJ and its mutants. Selected cells were grown in 2 mL of SDCt (-Leu) at 30℃, 250 rpm for 2 days. The culture was transferred into 20 mL of YPD medium and incubated at 30℃, 250 rpm for another 2 days. The cells were harvested by centrifugation at 1600 rpm for 5 min. All subsequent procedures were performed at 4℃ or on ice. Harvested cells were resuspended in 2 ml of TEGM buffer [50 mM Tris-HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), 1.5 mM mercaptoethanol, 20% glycerol] with 0.5% protease inhibitor. 300 μL of the mixture was transferred into a 1.5 mL tube filled with 0.5 mm beads. Then the cells were broken by vortex. Then the liquid was transferred into fresh 1.5 mL tube. After span down by centrifugation at 4000 rpm for 5 min, the supernatant was transferred to another new 1.5 mL tube and was fractioned by centrifugation at 15000 rpm for 30 min. Then, the pellet was resuspended in 100 μL of TEG buffer [20 % (v/v) glycerol, 50 mM Tris-HCl (pH 7.4) and 1 mM EDTA] to yield a microsomal fraction.

For in vitro assays, the reaction mixture was combined in a total volume of 100 μL and incubated at 30℃. The final concentration of each component within the reaction mixture is: 2 mg/mL microsomal fraction, 0.2 mg/mL substrate, 100 mM Tris-HCl (pH 7.4). After 12 hrs, the reaction mixture was extracted with 150 μL ethyl acetate twice. The organic phase was dried and dissolved in 160 μL MeOH and 20 μL was injected into the LC-MS for analysis.

9. Construction of MacA expression plasmid.

For expression of MacA, the primers MacA-p1-For/Rev and MacA-p2-For/Rev listed in Table S1 were used to amplify the intron-free *macA* from the cDNA of strain DH1. The intron-free *macA* fragments were inserted into the digested plasmid pXW55 (*Spe*I and *Pml*I) by using yeast *in vivo* homologs recombination to create the expression plasmid pTMC1-181-19, which was further confirmed by sequencing.

Table S1. DNA primers used in this study.

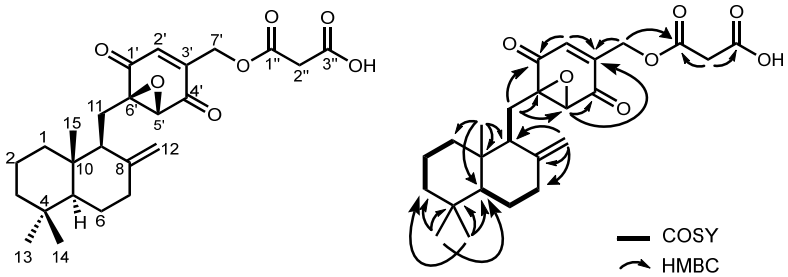
Primer Name	Sequence of Primer (5' to 3')
macA-up-For	ccttctctgaacaataaaccccacagaaggcatttttaatAACAAGGCCGATCATACTCG
macA-up-Rev	actcagcccttctctctgcgtccgtccgtctctccgcatgaAATCTCTCGCGCAGTGAATC
macA-dn-For	ttctagaactatcaccatcactcgtcttggccaaccgctcCCTCTATCCATGAGCGTGCC
macA-dn-Rev	acggtatcgataagcttgatatcgaattcctgcagcccggTTCGACTTGGACCTCGTAGG
macA-zeo-For	AGCTCTATTTGTTCGGATTCACTGCGCGAGAGATTTcatgcggagagacggacgg
macA-zeo-Rev	GGAGCGGTTGGCCAAGACGAGTGATGGTGATAGTtctagaaagaaggattacctctaaacaag
macJ-up-For	ccttctctgaacaataaaccccacagaaggcatttttaatAAGGTACCGCTATAGGAGAC
macJ-up-Rev	cagcccttctctctgcgtccgtccgtctctccgcatgTTTATTCAGATTGCTTAATGGCG
macJ-dn-For	ttctagaactatcaccatcactcgtcttggccaaccgctcATATGGGTACACGGTCATAAG
macJ-dn-Rev	acggtatcgataagcttgatatcgaattcctgcagcccggAATCGTCCACATCAAGTTCC
macJ-zeo-For	CTCTTTCCCATTTCGCCATTAAGCAATCTGAATAAAcatgcggagagacggacgg
macJ-zeo-Rev	CTTTCACCATCACCATCTTATGACCGTGTACCCATAtgagcggttggccaagacg
MacA-p1-For	CTAGCGATTATAAGGATGATGATGATAAGACTAGTATGATTACTTCAACAAGCAGCAC
MacA-p1-Rev	GGCACAGTCGGGTCGAGG
MacA-p2-For	GTGGGCCGCCTGCATTG
MacA-p2-Rev	TGTCATTTAAATTAGTGATGGTGATGGTGATGCACTTCATCCAACACCTGGCAG
MacJ-For	ATCAACTATCAACTATTAACTATATCGTAATACCATATGTGCTTCTTCGCGCTCG
MacJ-Rev	TGATAATGAAAACTATAAATCGTGAAGGCATGTTTTCAGCGAGCCTTCTGTGCC
MacJ-D31A-For	GTCTGTACCC**GCT**GGGTTCAC
MacJ-D31A-Rev	GTGAACCC**AGC**GGGTACAGAC
MacJ-D55A-For	GGCTTTCAAA**GCT**CGTTCCTACGC
MacJ-D55A-Rev	GGAACG**AGC**TTTGAAAGCCTTTTTG
MacJ-E72A-For	CATTACCTGG**GCA**GCAGTCTACG
MacJ-E72A-Rev	GACTGC**TGC**CCAGGTAATGTTCAAG
MacJ-D96A-For	GGATGATTGTC**GCT**GTGGTCC
MacJ-D96A-Rev	GGACCAC**AGC**GACAATCATCC
MacJ-D229A-For	GGATCT**GCC**ATGGTGTATGCTG
MacJ-D229A-Rev	CACCAT**GGC**AGATCCAATACTAC

Note: In the primers used for mutation studies, the mutation sites are shown in bold and are colored.

Table S2. Related to Figure 2A and S1. Overview of the Mac proteins and the proposed activities.

Protein name	Homologs in *Penicillium chrysogenum*	homologs (identity)	Predicted functional domains	proposed activity
MacR	-	YanR(58)	transcription factor	transcription factor
MacC	Pc16g00400	YanC(81)	Cytochrome P450	hydroxylase
MacB	Pc16g00390	YanB(80)	metallo-dependent hydrolase	6-MSA decarboxylase
MacG	Pc16g00380	YanG(73)	UbiA-like prenyltransferase	prenyltransferase
MacA	Pc16g00370	YanA(74)	polyketide synthase	6-MSA synthase
MacH	Pc16g00360	YanH(91)	Cytochrome P450	monooxygenase
MacI	Pc16g00350	YanI(62)	membrane-bound *O*-acyltransferase	*O*-acyl transferase
MacD	Pc16g00340	YanD(77)	short-chain dehydrogenase	dehydrogenase
MacE	Pc16g00330	YanE(82)	cupin-like family protein	oxygenase
MacJ	Pc16g00320	AtmB(35)	no conserved domain	terpene cyclase
MacF	Pc16g00310	YanF(65)	flavin-dependent dehydrogenase	oxidase

Table S3. ^{1}H NMR (500 MHz) Data and ^{13}C NMR (125 MHz) Data for Compound **1** in $CDCl_3$.

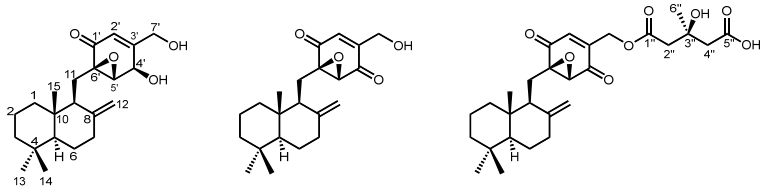


4'-oxomacrophorin E
1

No.	δ_C	δ_H (*J* in Hz)	HMBC
1	38.9, CH_2	1.14 (m), 1.77 (m)	2/3/5/10
2	19.3, CH_2	1.56 (2H, m)	1/3/4
3	42.0, CH_2	1.12 (m), 1.38 (m)	1/2/4
4	33.6, C	-	-
5	55.5, CH	1.10 (br d, 14.0)	3/4/6/7/10
6	24.4, CH_2	1.31 (m), 1.74 (m)	4/5/8/10
7	38.0, CH_2	1.95 (m), 2.34 (br d,12.3)	6/8/9/12
8	148.7, C	-	-
9	51.4, CH	1.67 (d, 11.0)	11/12/15/6′
10	39.8, C	-	-
11	20.1, CH_2	2.03 (m), 2.43 (d, 14.7)	8/9/1′/5′/6′
12	106.9, CH_2	4.50 (s), 4.81 (s)	7/8/9
13	33.6, CH_3	0.86 (3H, s)	3/4/5/14
14	21.7, CH_3	0.79 (3H, s)	3/5/13
15	14.5, CH_3	0.70 (3H, s)	1/5/9/10
1′	192.1, C	-	-
2′	132.9, CH	6.59 (s)	1′/3′/4′
3′	141.8, C	-	-
4′	191.7, C	-	-
5′	58.8, CH	3.76 (s)	3′/4′/6′
6′	62.6, C	-	-
7′	60.3, CH_2	4.88 (d, 16.7), 5.02 (d, 16.7)	2′/3′/4′/1′′
1′′	165.7, C	-	-
2′′	40.7, CH_2	3.50 (s)	1′′/3′′
3′′	170.6, C	-	-

HRMS-ESI (m/z) $[M+Na]^+$ cacld for $C_{25}H_{32}O_7Na$ 467.2046, found 467.2029.

Table S4. ^{1}H NMR (500 MHz) Data and ^{13}C NMR (125 MHz) Data for Compounds **2-4** in $CDCl_3$.



macrophorin A
2

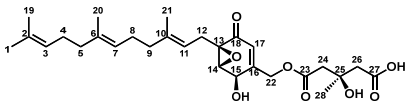
4'-oxomacrophorin A
3

4'-oxomacrophorin D
4

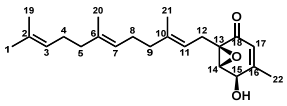
No.	**2**		**3**		**4**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	38.8, CH_2	1.12 (m), 1.74 (m)	38.9, CH_2	1.13 (m), 1.76 (m)	38.9, CH_2	1.12 (m), 1.73 (m)
2	19.3, CH_2	1.51 (2H, m)	19.3, CH_2	1.54 (2H, m)	19.3, CH_2	1.52 (2H, m)
3	42.0, CH_2	1.12 (m), 1.38 (m)	42.0, CH_2	1.13 (m), 1.39 (m)	41.9, CH_2	1.14 (m), 1.39 (m)
4	33.6, C	-	33.6, C	-	33.6, C	-
5	55.5, CH	1.11 (br d, 14.2)	55.5, CH	1.11 (br d, 14.0)	55.5, CH	1.11 (br d, 14.0)
6	24.4, CH_2	1.29 (m), 1.70 (m)	24.3, CH_2	1.30 (m), 1.71 (m)	24.3, CH_2	1.29 (m), 1.71 (m)
7	38.1, CH_2	1.93 (m), 2.34 (br d,12.4)	38.0, CH_2	1.94 (m), 2.35 (br d,12.3)	38.0, CH_2	1.93 (m), 2.35 (br d,12.3)
8	149.3, C		148.7, C	-	148.7, C	-
9	51.5, CH	1.67 (d, 11.0)	51.4, CH	1.68 (d, 11.0)	51.3, CH	1.67 (d, 11.0)
10	39.8, C	-	39.8, C	-	39.7, C	-
11	20.9, CH_2	1.98 (m), 2.47 (d, 14.7)	20.2, CH_2	1.98 (m), 2.47 (d, 14.7)	20.1, CH_2	1.98 (m), 2.46 (d, 14.7)
12	106.7, CH_2	4.55 (s), 4.82 (s)	106.9, CH_2	4.51 (s), 4.82 (s)	106.9, CH_2	4.51 (s), 4.82 (s)
13	33.5, CH_3	0.86 (3H, s)	33.5, CH_3	0.86 (3H, s)	33.5, CH_3	0.86 (3H, s)
14	21.6, CH_3	0.80 (3H, s)	21.6, CH_3	0.80 (3H, s)	21.6, CH_3	0.80 (3H, s)
15	14.5, CH_3	0.70 (3H, s)	14.4, CH_3	0.70 (3H, s)	14.4, CH_3	0.70 (3H, s)
1′	192.8, C	-	194.0, C	-	192.2, C	-
2′	121.3, CH	5.59 (s)	132.1, CH	6.64 (s)	133.0, CH	6.65 (s)
3′	155.7, C	-	146.7, C	-	142.0, C	-
4′	65.7, CH	4.68 (d, 2.5)	192.0, C	-	191.6, C	-
5′	60.6, CH	3.80 (d, 2.5)	59.1, CH	3.74 (s)	58.9, CH	3.77 (s)
6′	63.1, C	-	62.5, C	-	62.6, C	-
7′	61.1, CH_2	4.39 (d, 16.7), 4.42 (d, 16.7)	59.2, CH_2	4.37 (d, 16.7), 4.53 (d, 16.7)	59.7, CH_2	4.82 (d, 16.5), 4.96 (d, 16.5)
1″	-	-	-	-	170.9, C	-
2″	-	-	-	-	44.7, CH_2	2.70 (d, 14.5), 2.74 (d, 14.5)
3″	-	-	-	-	69.7, C	-
4″	-	-	-	-	44.4, CH_2	2.69 (d, 16.5), 2.73 (d, 16.5)
5″	-	-	-	-	172.0, C	-
6″	-	-	-	-	27.2, CH_3	1.40 (s)

All spectral data for **2-4** are consistent with those previously reported in the literature.[2,3]

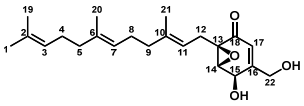
Table S5. ^{1}H NMR (500 MHz) Data and ^{13}C NMR (125 MHz) Data for Compounds **5-7**.



yanuthone E
5



7-deacetoxyyanuthone A
6



22-deacetylyanuthone A
7

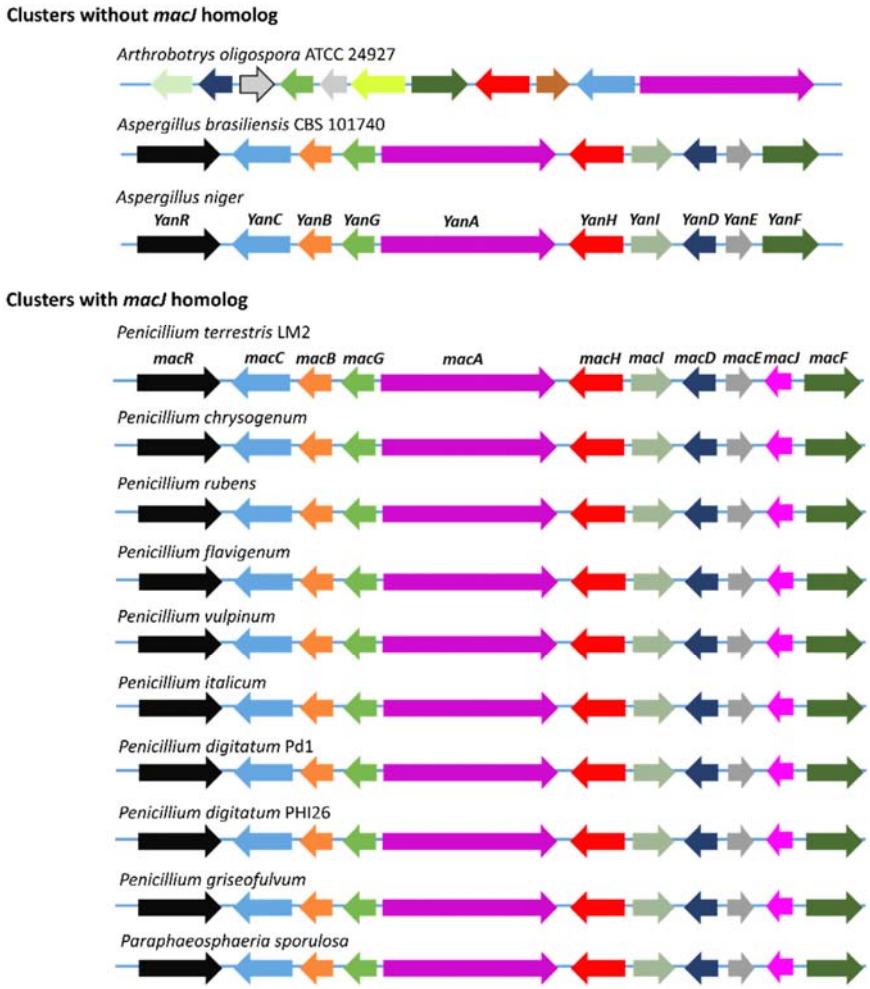
No.	**5**[a]		**6**[b]		**7**[a]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	25.8, CH_3	1.67, s	25.9, CH_3	1.61, s	25.8, CH_3	1.68, s
2	131.5, C	-	131.1, C	-	131.6, C	-
3	124.5, CH	5.07, dd (6.9, 13.9)	124.6, CH	5.03, dd (5.9, 6.2)	124.5, CH	5.05-5.10, m
4	26.6, CH_2	1.95-2.09, m	26.6, CH_2	1.88-2.02, m	26.9, CH_2	1.95-2.10, m
5	39.9, CH_2	1.95-2.09, m	39.6, CH_2	1.88-2.02, m	39.9, CH_2	1.95-2.10, m
6	135.4, C	-	134.9, C	-	135.4, C	-
7	124.0, CH	5.07, dd (6.9, 13.9)	124.1, CH	5.03, dd (5.9, 6.2)	124.0, CH	5.05-5.10, m
8	26.6, CH_2	1.95-2.09, m	26.5, CH_2	1.88-2.02, m	26.5, CH_2	1.95-2.10, m
9	39.8, CH_2	1.95-2.09, m	39.6, CH_2	1.88-2.02, m	39.8, CH_2	1.95-2.10, m
10	140.3, C	-	138.4, C	-	140.2, C	-
11	115.9, CH	5.00, t (7.2)	117.7, CH	4.98, t (7.2)	116.1, CH	5.01, t (7.0)
12	26.0, CH_2	2.50, dd (6.7, 15.5)	26.3, CH_2	2.28, dd (7.0, 15.0)	26.1, CH_2	2.51, dd (6.6, 15.3)
		2.76-2.81, m		2.62, dd (7.0, 15.0)		2.81, dd (7.8, 15.3)
13	61.4, C	-	60.5, C	-	61.5, C	-
14	59.0, CH	3.72, d (2.6)	59.7, CH	3.57, d (2.5)	59.1, CH	3.72, s
15	65.6, CH	4.66, s	66.5, CH	4.45, s	66.1, CH	4.69, s
16	151.8, C	-	159.2, C	-	156.6, C	-
17	122.8, CH	5.94, s	122.3, CH	5.66, s	121.2, CH	5.97, s
18	193.6, C	-	194.1, C	-	193.7, C	-
19	17.8, CH_3	1.59, s	18.0, CH_3	1.53, s	17.9, CH_3	1.60, s
20	16.2, CH_3	1.58, s	16.2, CH_3	1.53, s	16.2, CH_3	1.59, s
21	16.5, CH_3	1.63, s	16.5, CH_3	1.56, s	16.5, CH_3	1.64, s
22	63.7, CH_2	4.77, d (15.4)	20.3, CH_3	1.88, s	63.2, CH_2	4.42, m
		4.95, d (15.4)				
23	171.1, C	-	-	-	-	-
24	44.9, CH_2	2.64-2.79, m	-	-	-	-
25	70.2, C	-	-	-	-	-
26	44.9, CH_2	2.64-2.79, m	-	-	-	-
27	175.0, C	-	-	-	-	-
28	27.6, CH_3	1.41, s	-	-	-	-

[a] $CDCl_3$

[b] DMSO-d_6

All spectral data for **5**-**7** are consistent with those previously reported in the literature.[4,5]

Figure S1. The *yan* biosynthetic gene cluster and its homolog gene clusters identified from genome sequenced fungal species.



Note: The homolog genes are labeled with the same color. For some of the gene clusters, such as the clusters from *Penicillium italicum*, *Penicillium griseofulvum*, *Penicillium digitatum*, and *Penicillium vulpinum*, the genes within the clusters were manually re-annotated.

Figure S2. Bioinformatic analysis of MacJ. (A) Phylogenetic tree analysis of MacJ and its homologs[6]. (B) Transmembrane helices prediction of MacJ by using the TMHMM server.

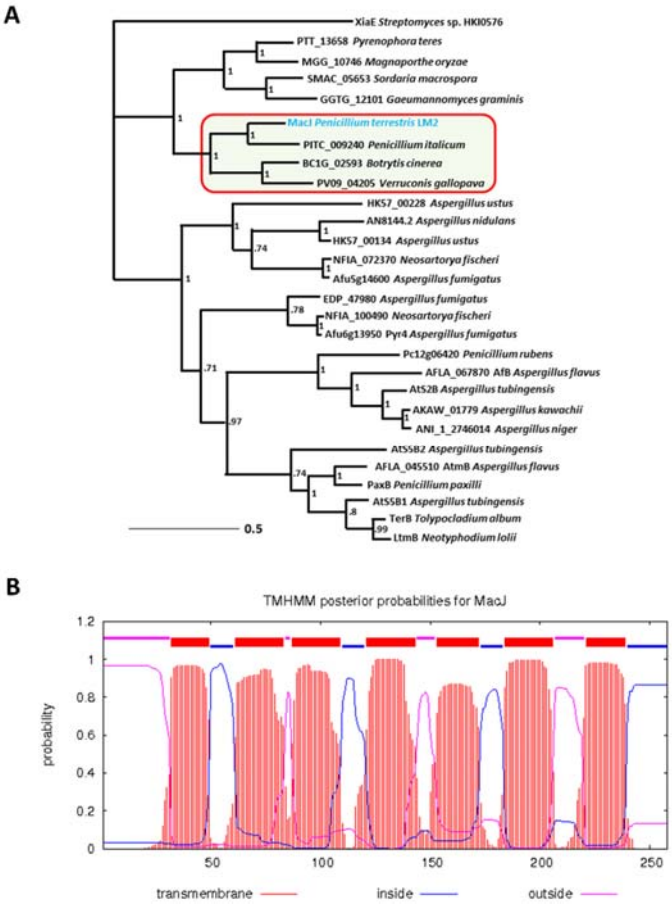


Figure S3. Production of 6-MSA by expressing MacA in yeast.

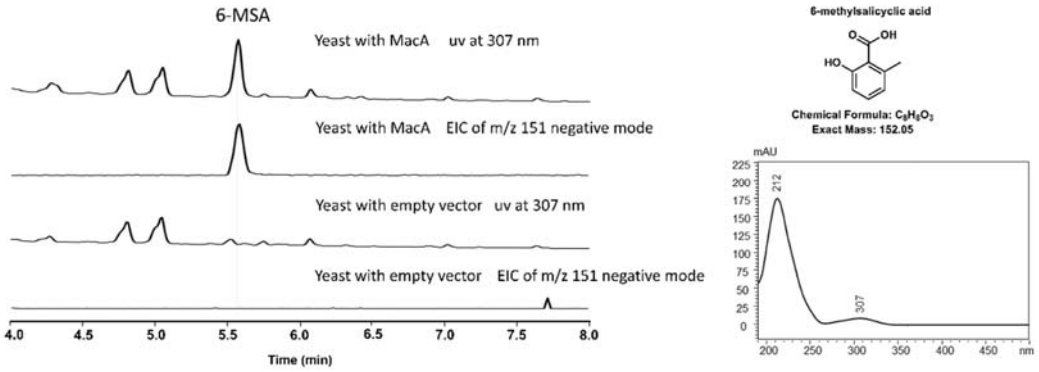


Figure S4. The in vitro assays of MacJ using extracted microsomal fractions. (A) compound **5** used as the substrate; (B) compound **6** used as the substrate; (C) farnesylhydroquinone used as the substrate. UV was detected at 220 nm.

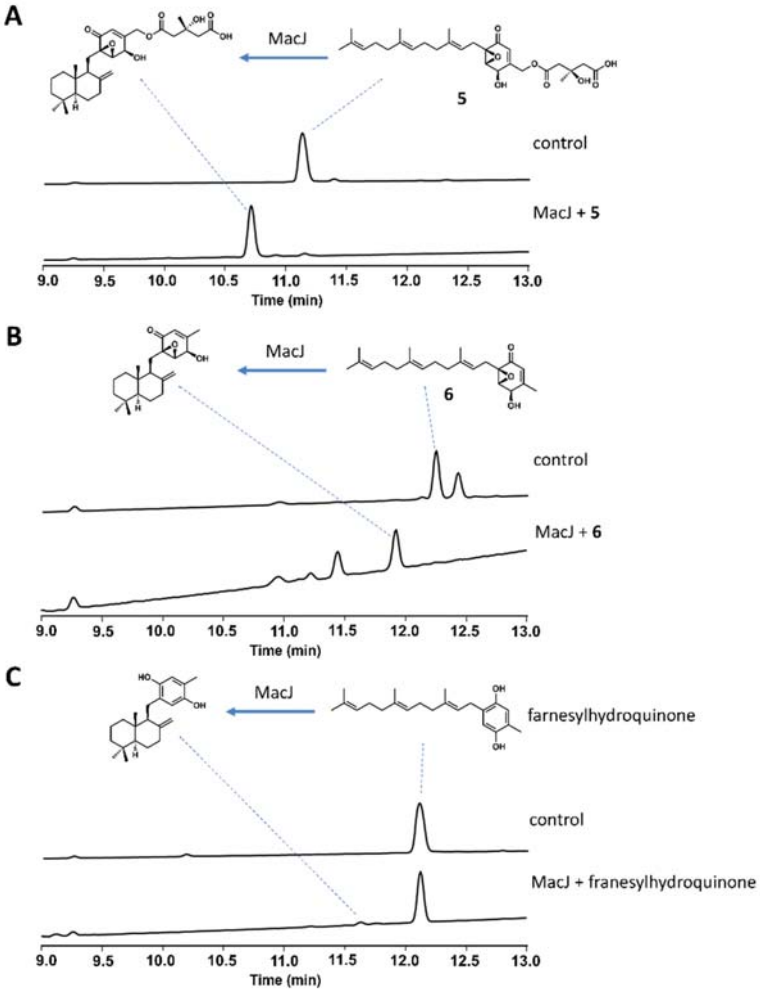


Figure S5. Proposed biosynthetic pathway of the macrophorins.

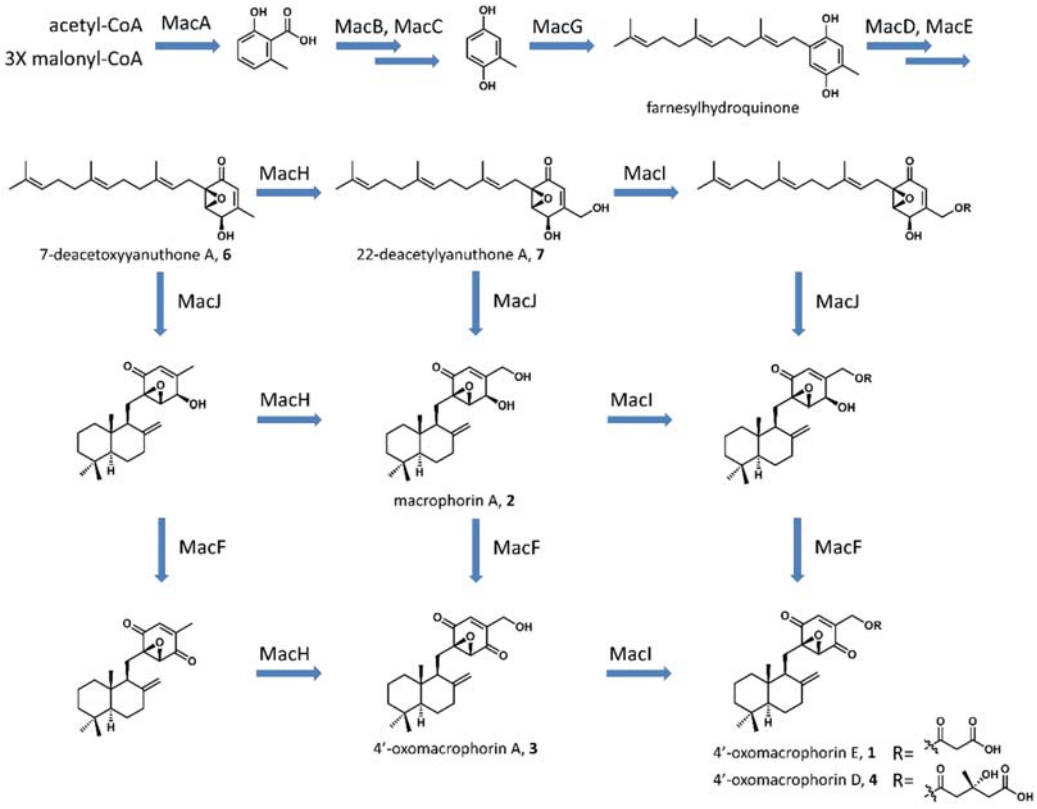
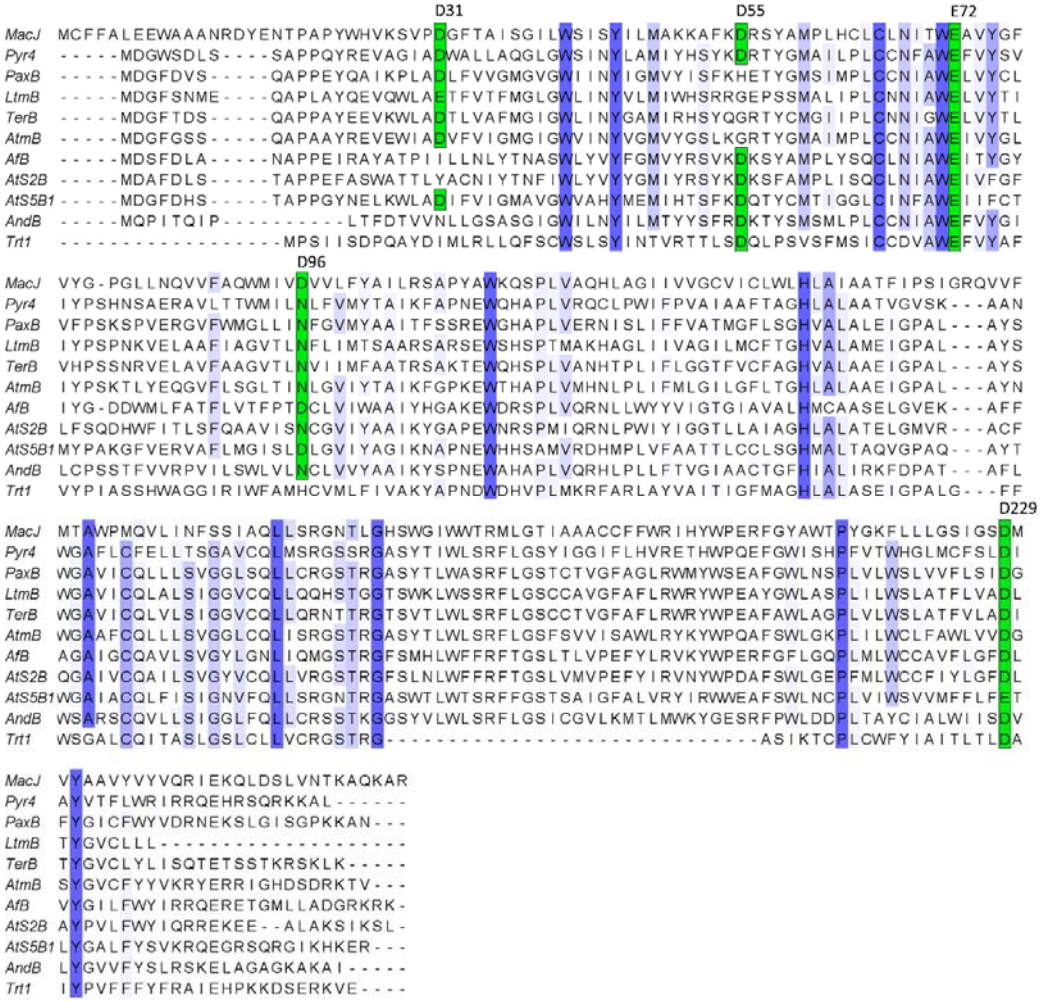


Figure S6. Sequence alignment of MacJ and its homologs.



Note: The conserved acidic residues are labeled with different colors using the amino acid sequence numbers from MacJ.

Figure S7. PCR analysis for confirming the gene deletion.

(A) Scheme of zeocin-resistance marker approach for gene knock-out. (B) Genotypical verification of each mutant by PCR. The fragments A_wt and J_wt only can be amplified from the wild type strain gDNA. The fragments A_zeo and J_zeo only can be amplified from the mutant strain gDNA. Note: Lane a, DNA marker; Lane b, using wild type strain gDNA as the PCR template; Lane c, using the mutant strain gDNA as the PCR template.

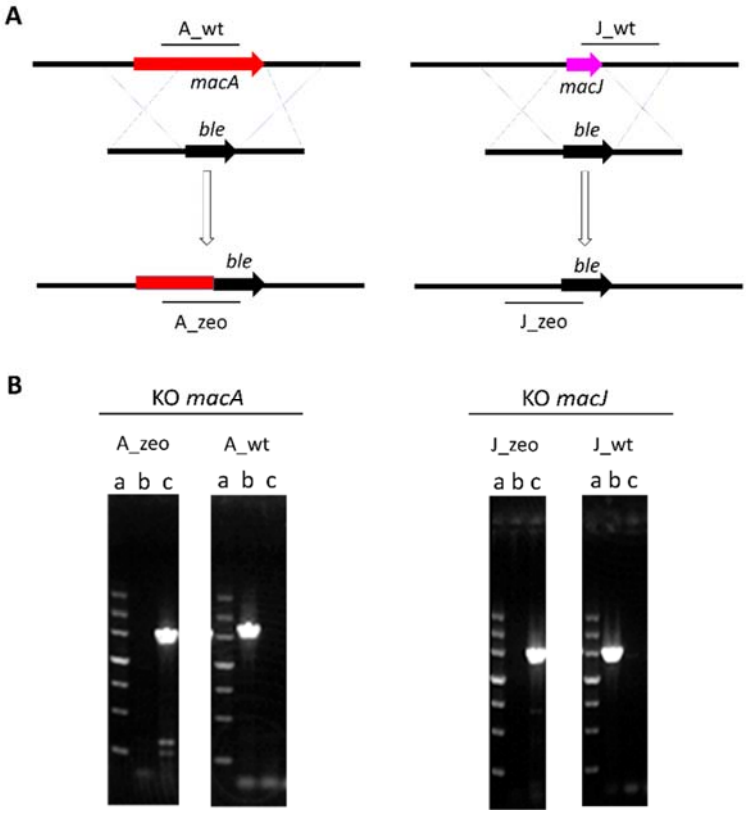


Figure S8. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **1**.

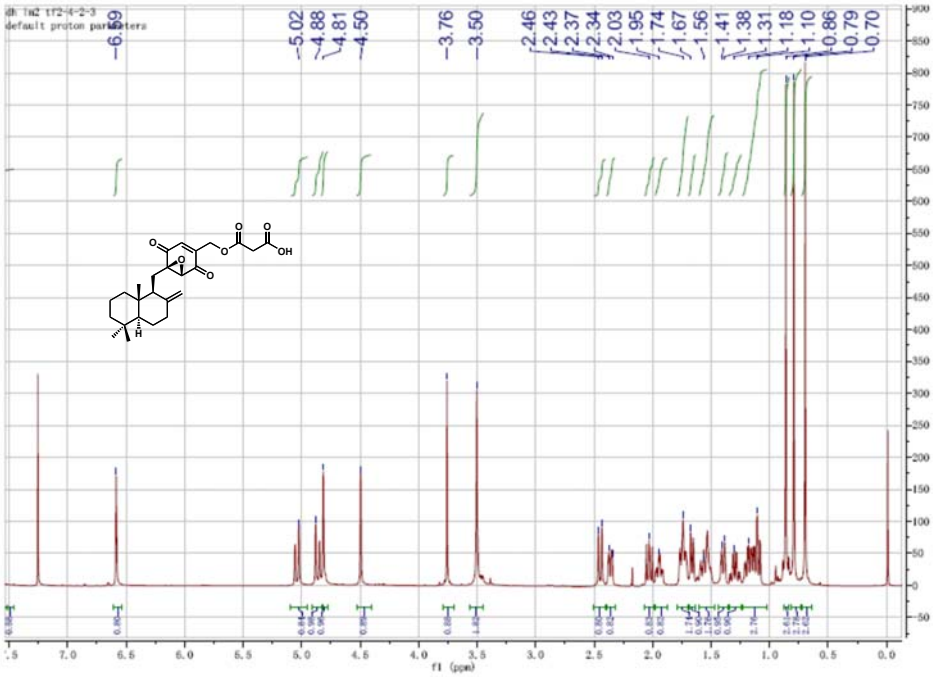


Figure S9. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **1**.

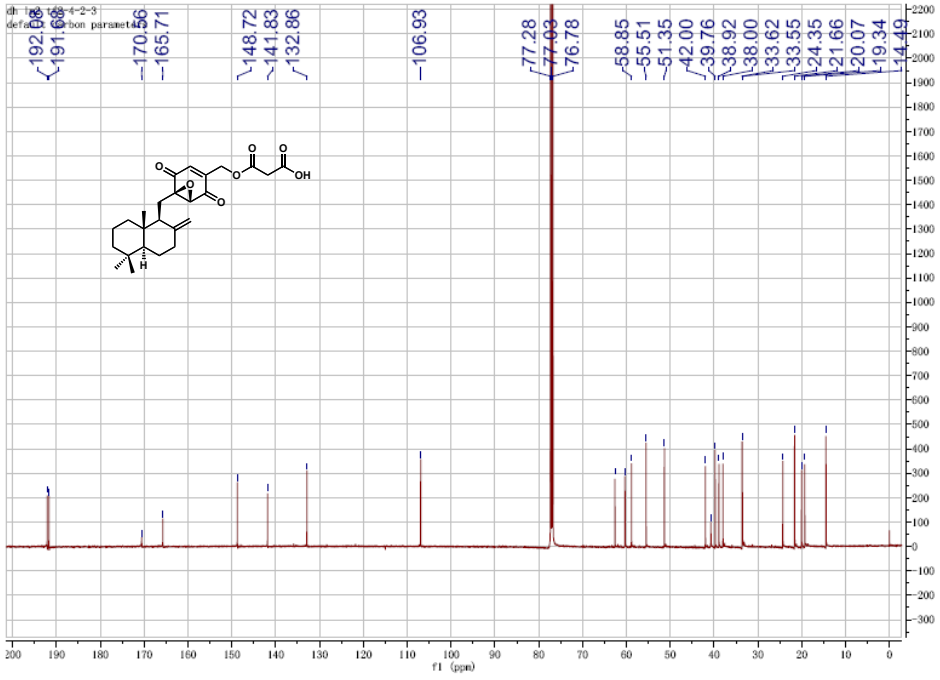


Figure S10. HSQC spectrum of **1** in $CDCl_3$.

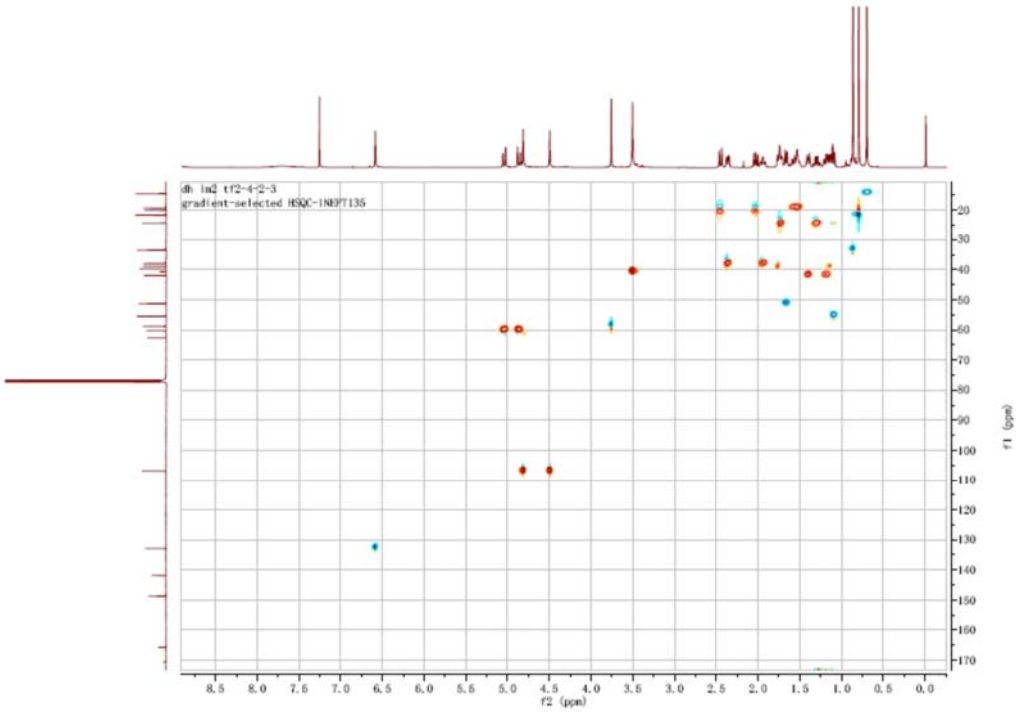


Figure S11. ^{1}H-^{1}H COSY spectrum of **1** in $CDCl_3$.

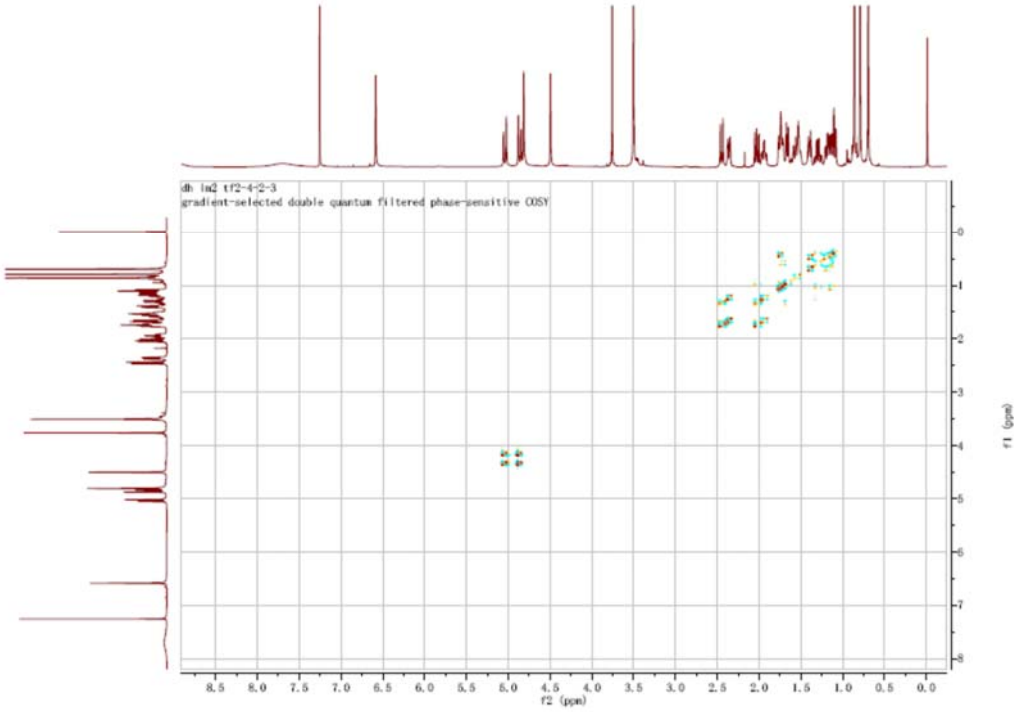


Figure S12. HMBC spectrum of **1** in $CDCl_3$.

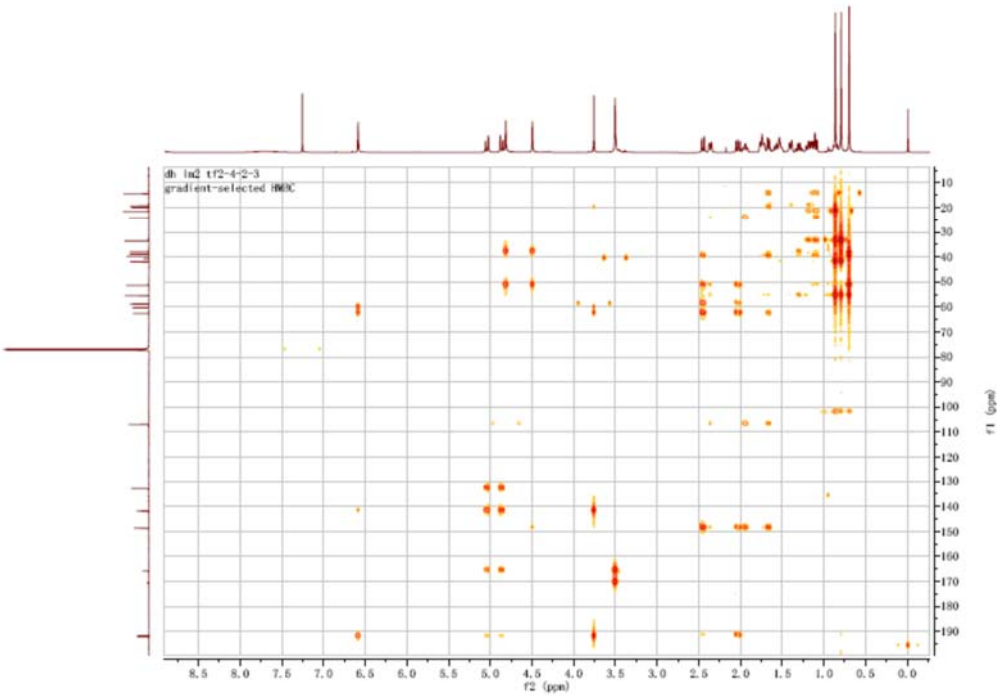


Figure S13. ^1H NMR (500 MHz, $CDCl_3$) spectrum of **2**.

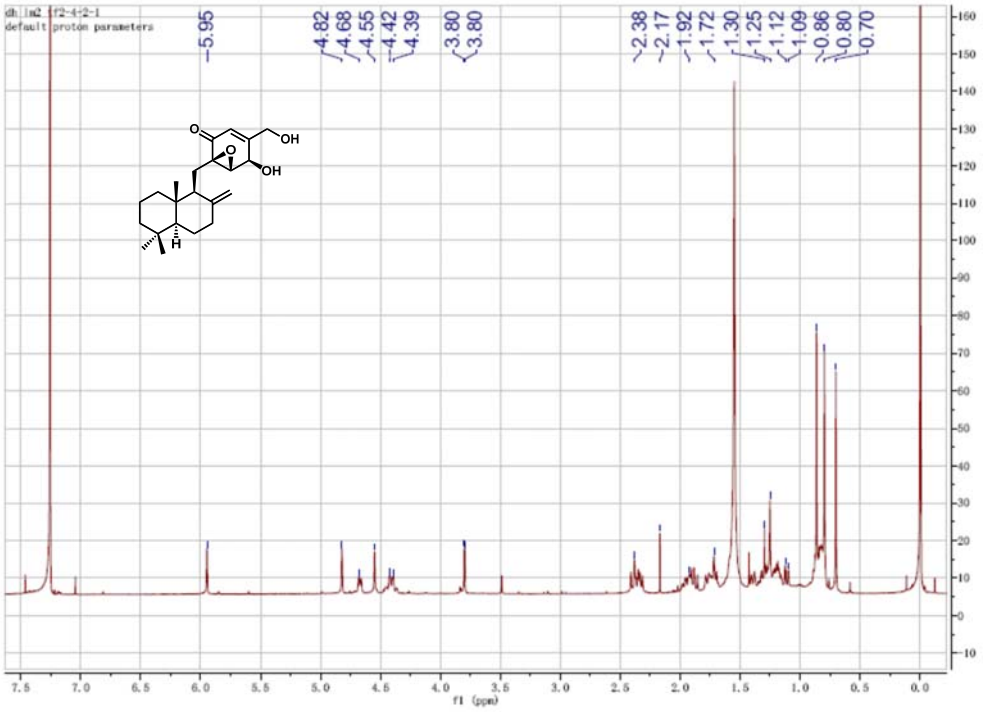


Figure S14. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **2**.

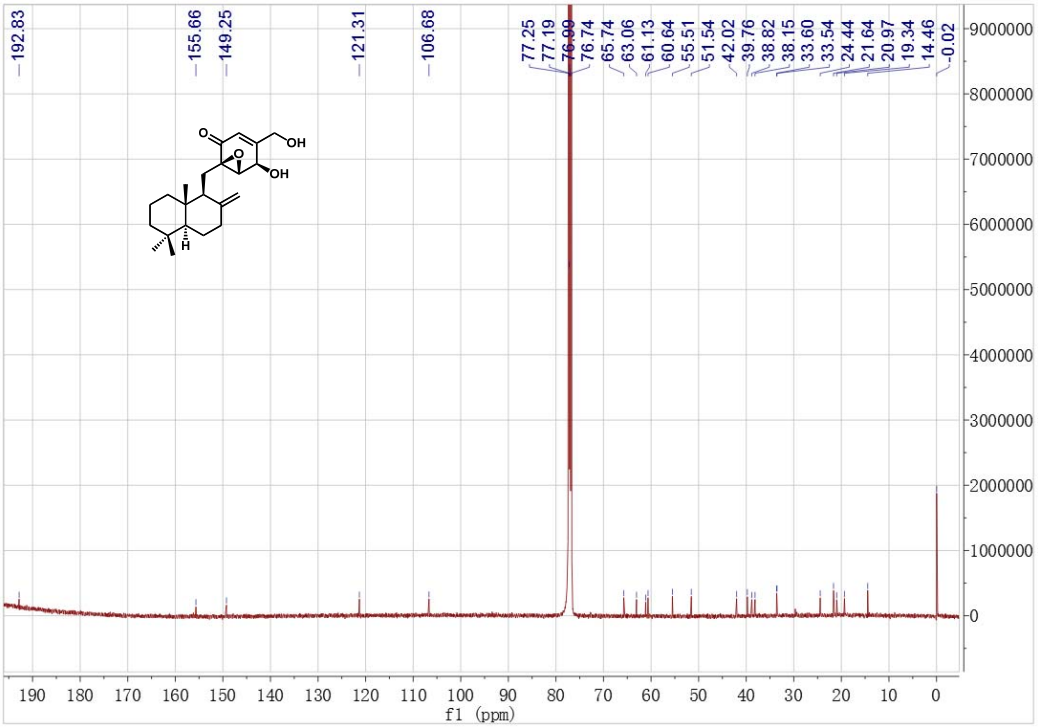


Figure S15. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **3**.

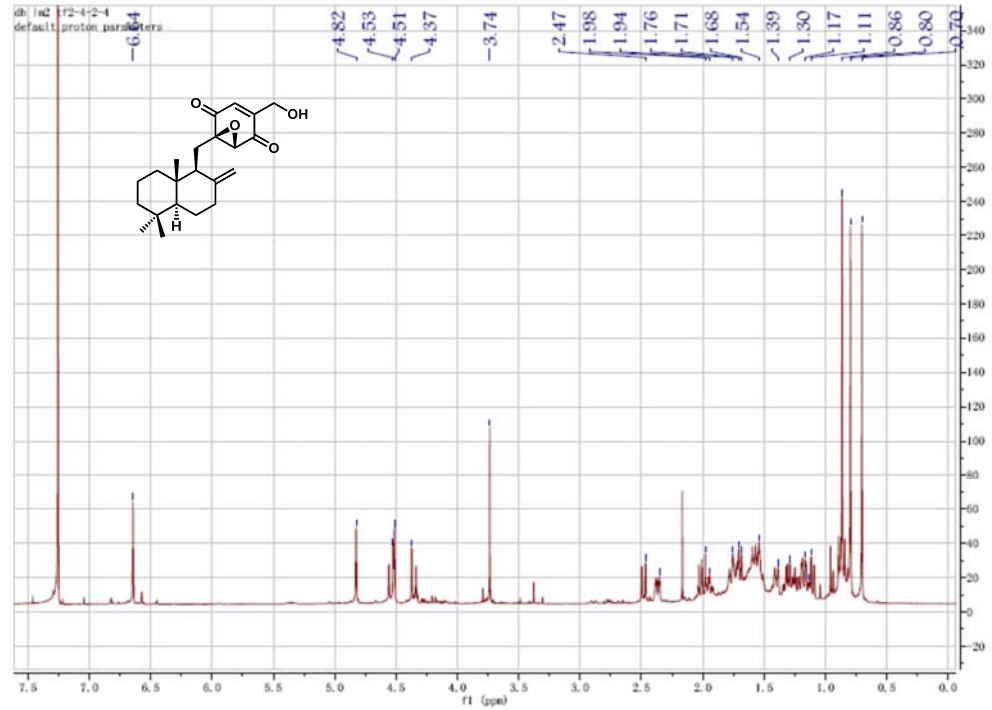


Figure S16. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **3**.

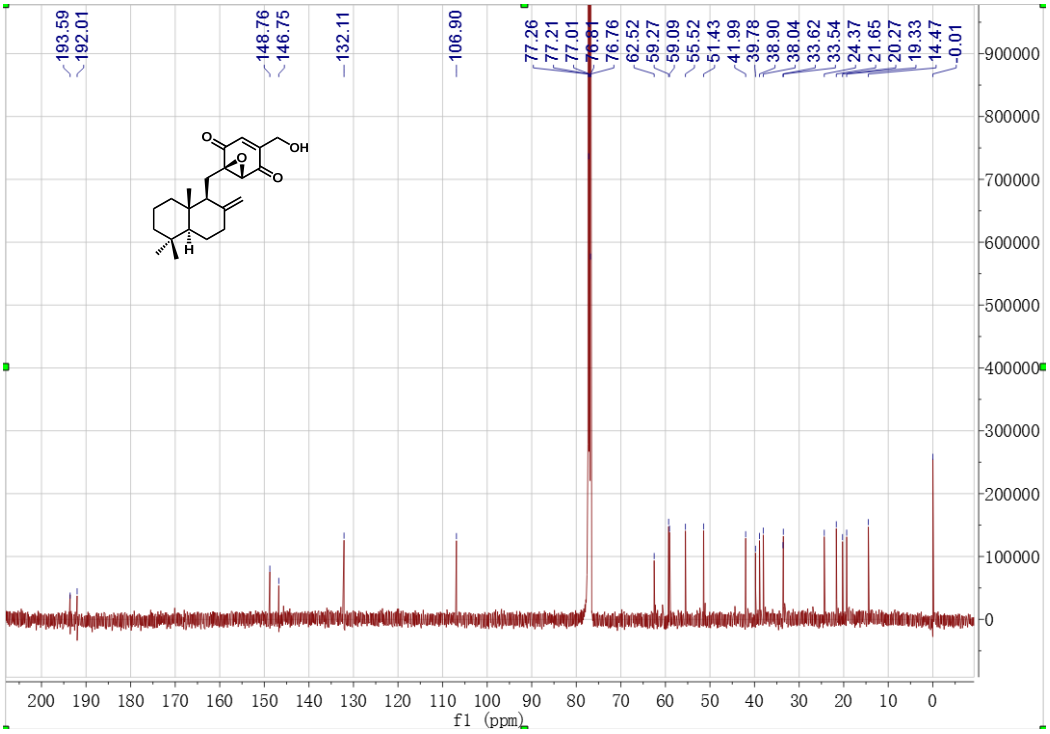


Figure S17. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **4**.

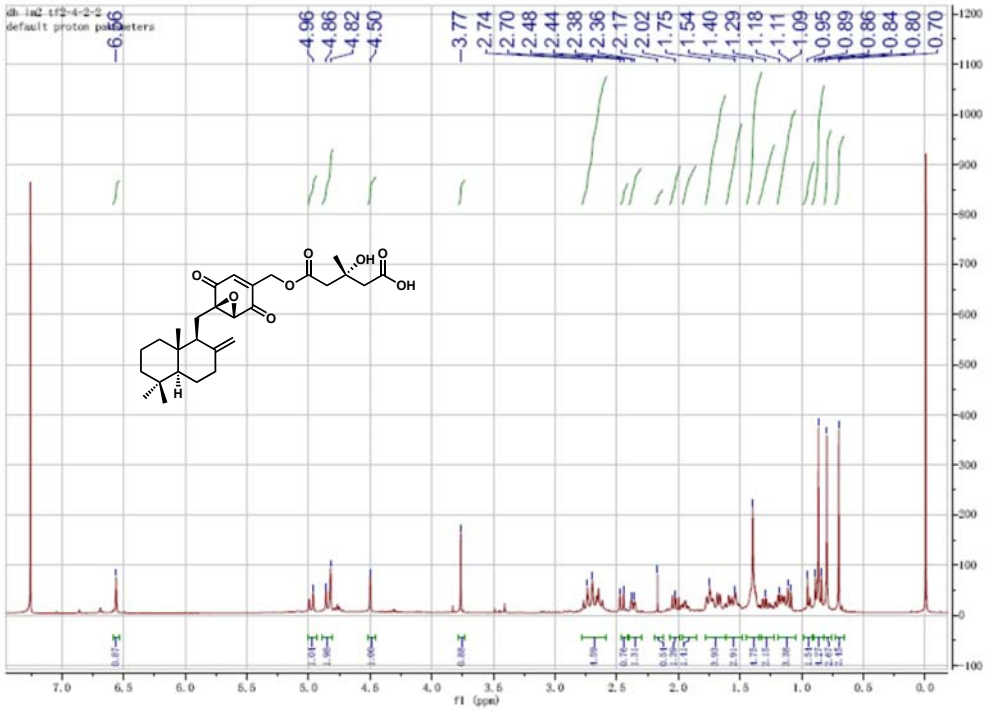


Figure S18. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **4**.

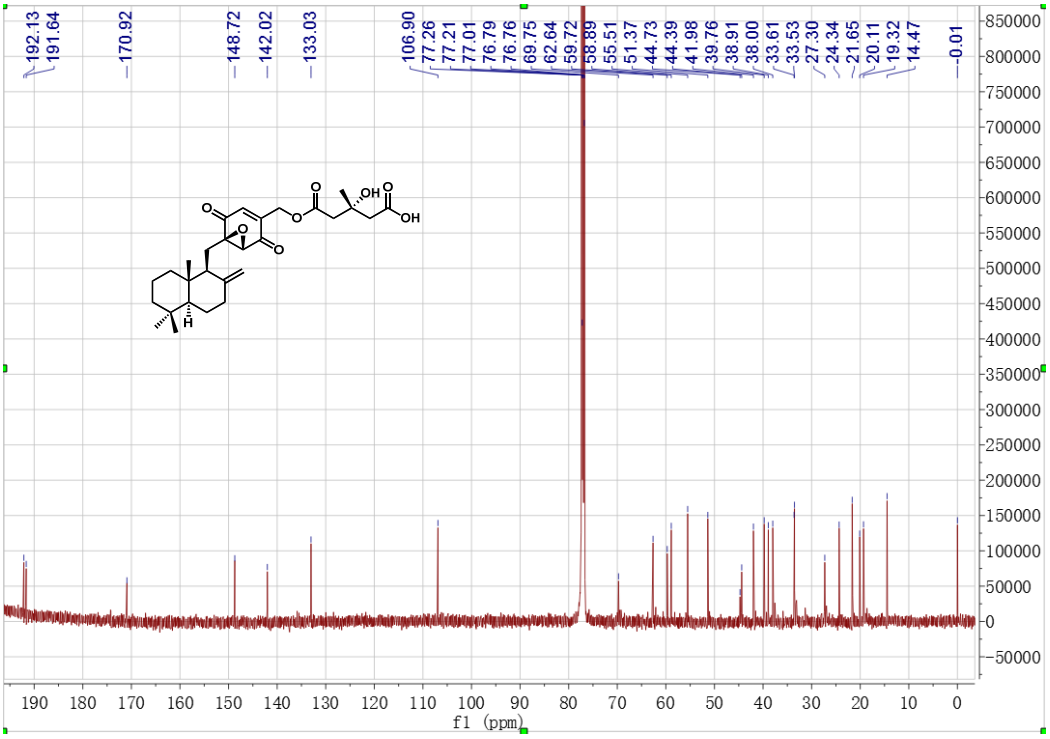


Figure S19. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **5**.

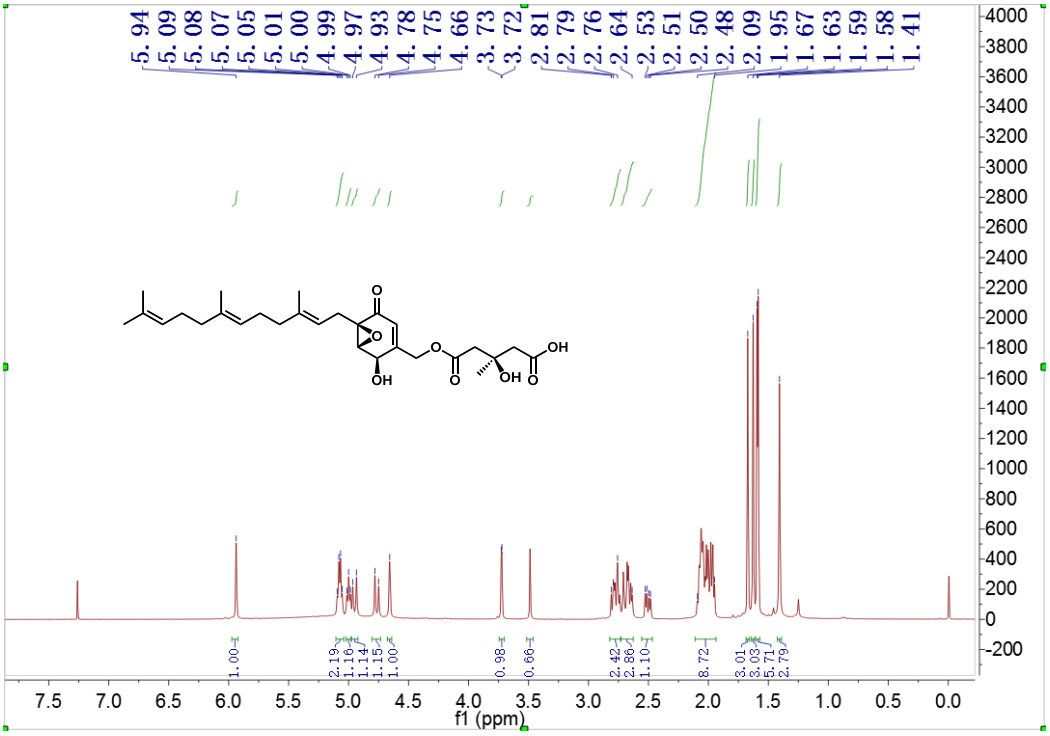


Figure S20. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **5**.

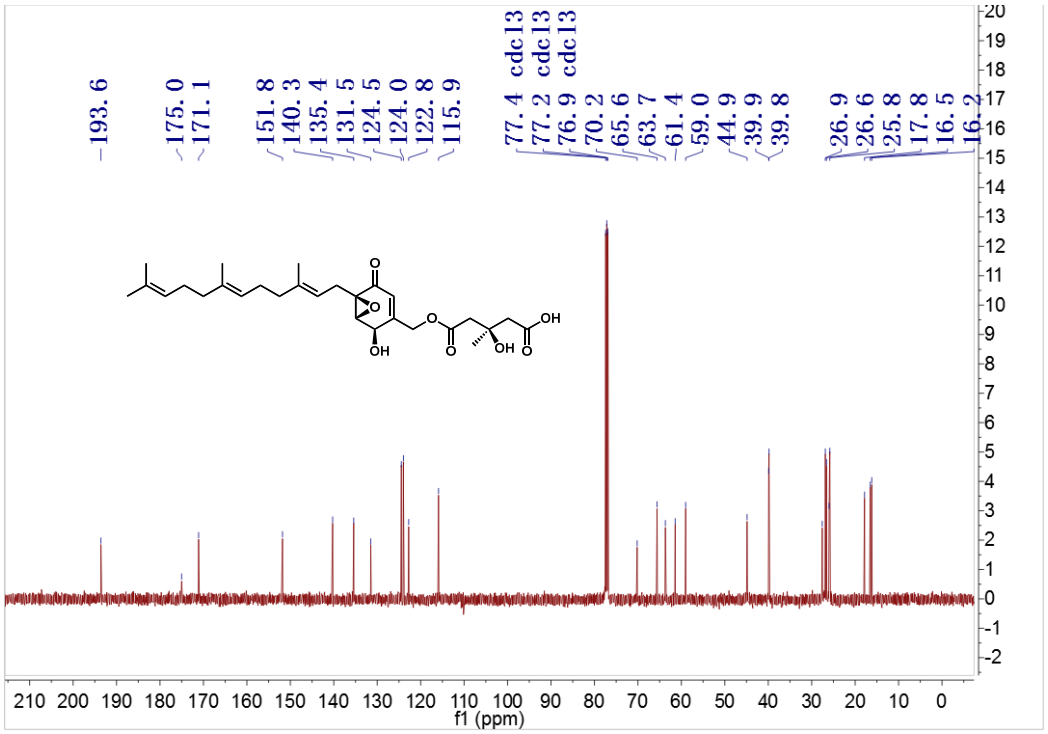


Figure S21. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **6**.

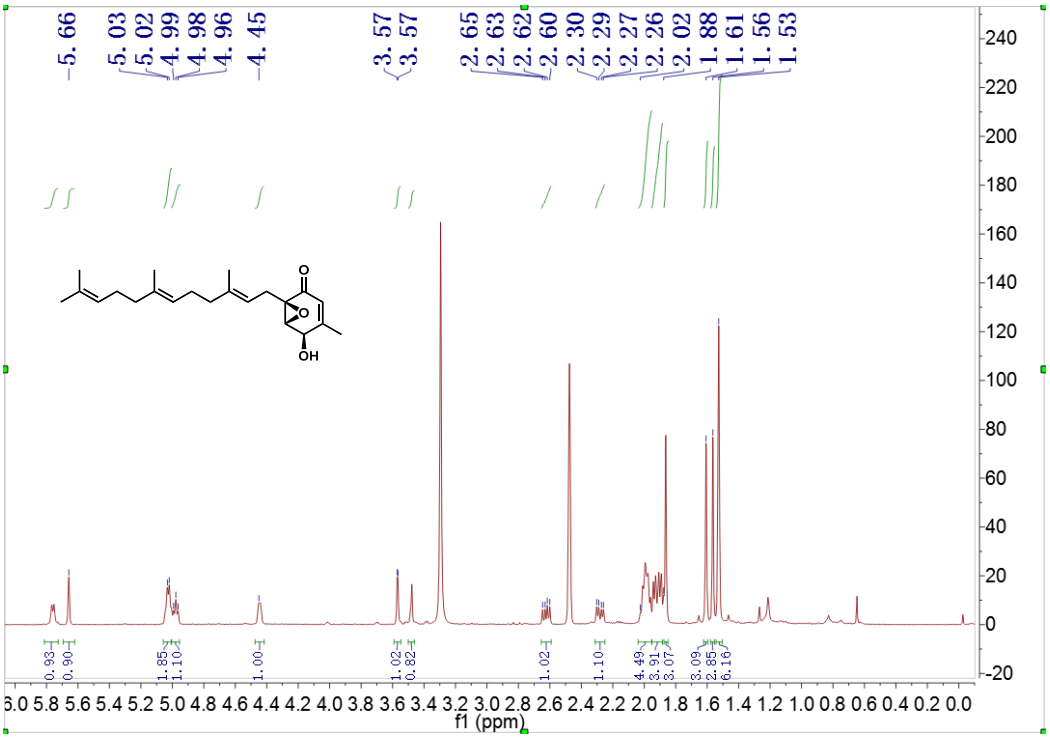


Figure S22. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of **6**.

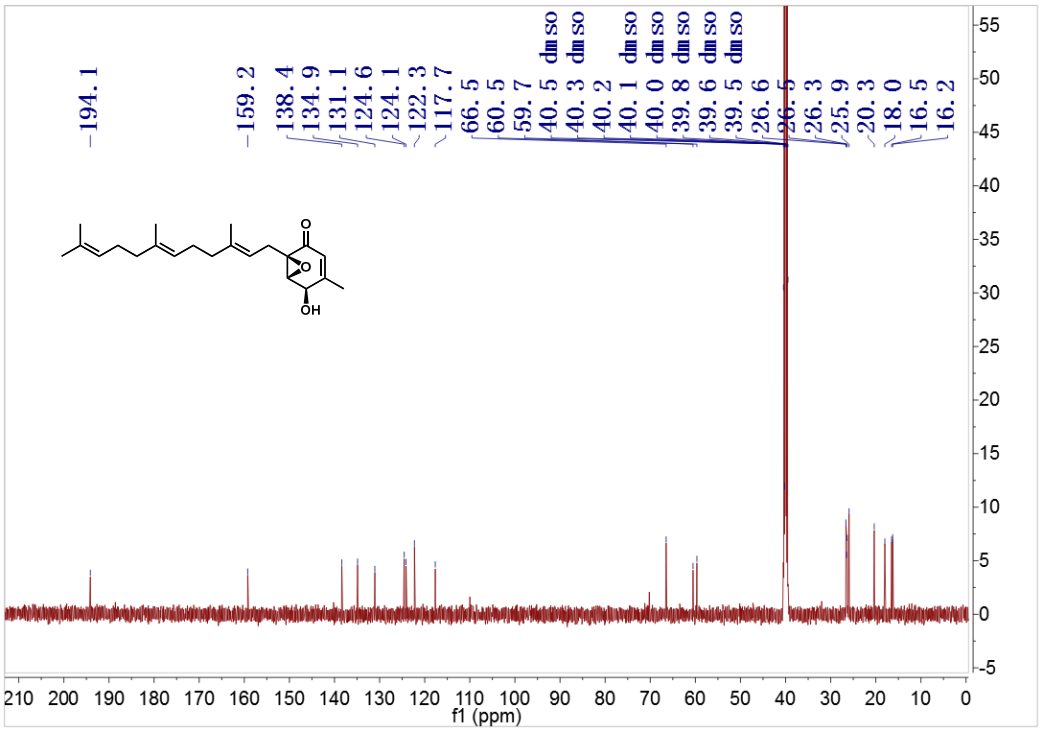


Figure S23. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **7**.

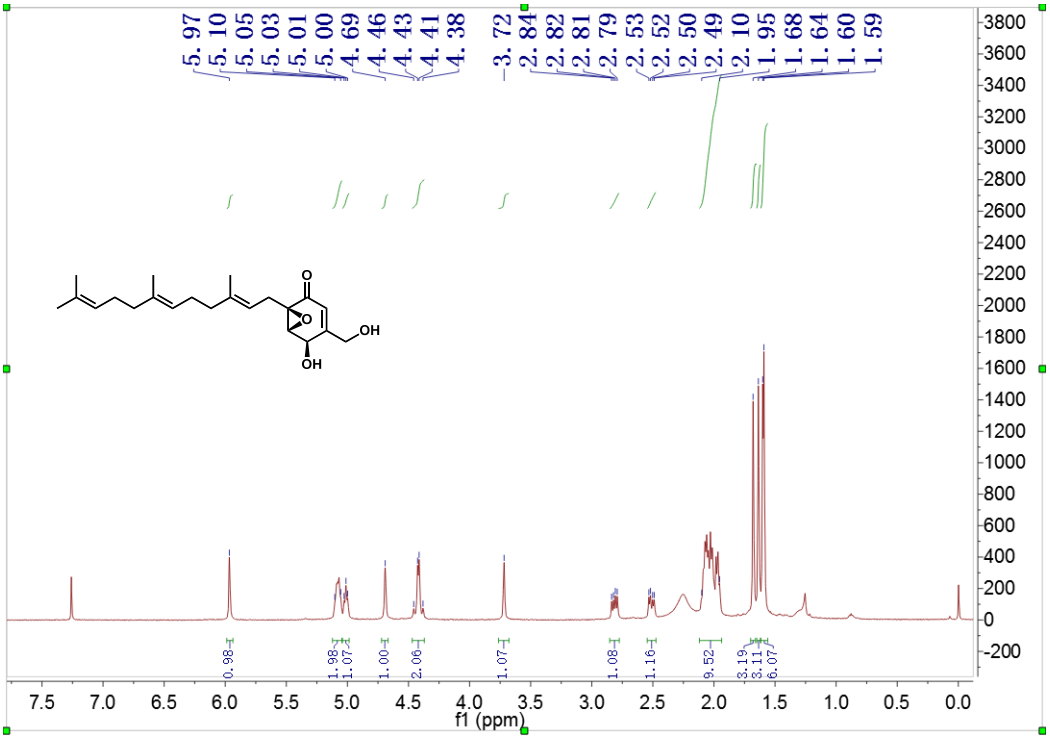
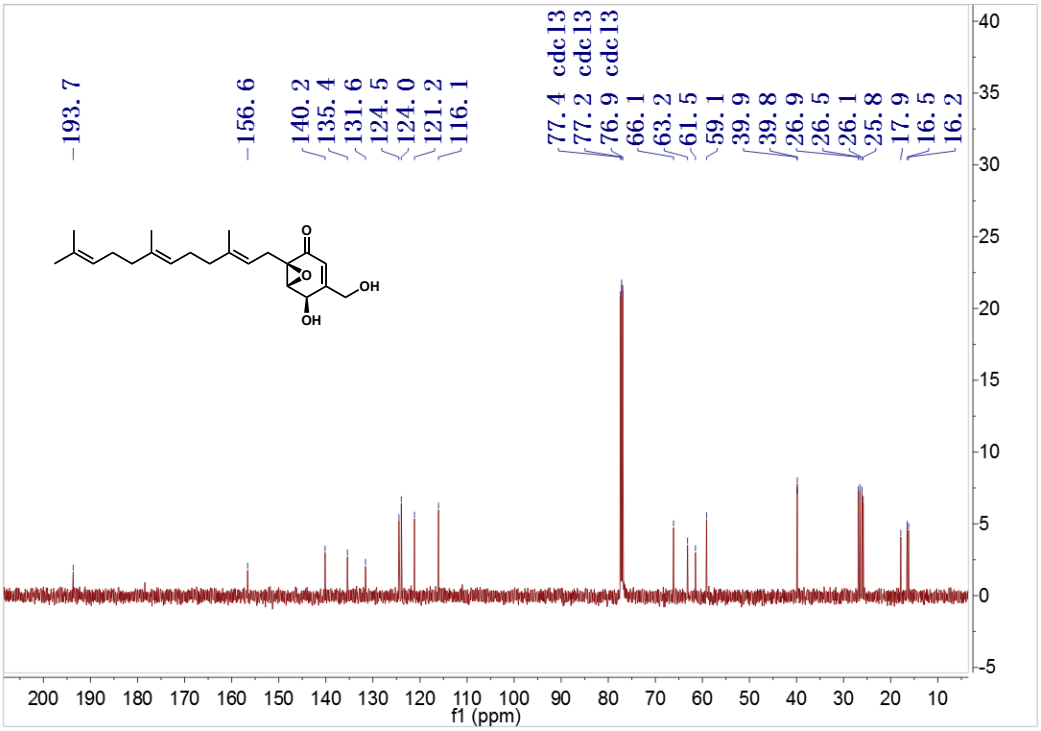


Figure S24. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **7**.



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(6) Tang, M.-C., Lin, H.-C., Li, D., Zou, Y., Li, J., Xu, W., Cacho, R. A., Hillenmeyer, M., Garg, N. K., Tang, Y. *J. Am. Chem. Soc.* **2015**, *137*, 13724–13727.