Supporting Information

Molecular Genetic Characterization of a Cluster in *A. terreus* for Biosynthesis of the Meroterpenoid Terretonin

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Supplemental Results
Detailed structural characterization

Compound **10** from *trt3* deletant strain was isolated as an amorphous colorless solid. The molecular formula was established to be $C_{26}H_{36}O_5$ by its ^{13}C NMR, DEPT and HRESIMS spectral data, indicating nine indices of hydrogen deficiency (IHD). The IR spectrum showed hydroxyl (3587 cm^{-1}) and ester or ketone functionalities (1733 and 1716 cm^{-1}). The ^1H NMR spectrum of compound **10** (Table S4) exhibited signals for six methyl groups [δ_H 0.97, 1.01, 1.03, 1.06, 1.32, and 1.74 (each 3H, s)], one methoxyl group [δ_H 3.79 (3H, s)], one vinylidene moiety [δ_H 5.13 and 5.19 (each 1H, d, J = 2.0 Hz)], and one deshielded hydroxyl proton [δ_H 9.68 (1H, s)]. The ^1H, ^{13}C, gHMQC, and gHMBC NMR spectroscopic data of compound **10** (Table S4) including the 3−carbonyl group (δ_C 217.3), the CH-5 methine group (δ_H 1.22 and δ_C 55.2), and four methyl groups (δ_H 1.06 and δ_C 27.2, CH_3-18; δ_H 1.01 and δ_C 21.7, CH_3-19; δ_H 0.97 and δ_C 19.6, CH_3-20; and δ_H 1.03 and δ_C 16.0, CH_3-21) exhibit a typical 3-carbonyl-4,4,8,10-tetramethyl-decalin partial structure that was observed in andrastin D.[1] This suggested that compound **10** was an intermediate biosynthesized before the C6 and C7 of ring B is oxidized as in terretonin (**1**) or terretonin C (**2**). Next we were able to confirm the partial structure of the D ring by identifying the key long-range ^1H–^{13}C correlations between CH_3-24 (δ_H 1.74) and two carbonyl like carbons (δ_C 177.6, C-15; δ_C 205.6, C-17) as well as one olefinic carbon (δ_C 113.2, C-16) in the gHMBC spectrum. The established structure of D ring was also comparable to that of andrastin D.[1] Besides, we identified the presence of a carboxylic methyl ester by analyzing the gHMBC spectrum that shows a ^1H–^{13}C correlation between H_3-1' (δ_H 3.79) and C-25 (δ_C 175.4). We confirmed that this methyl ester group was attached to C-14 (δ_C 68.1) by comparison to the spectroscopic data of terretonin E and F.[2] Thus, the structure of this meroterpenoid, named preterrenoid, was assigned as shown in Table S4.

Compound **11** was isolated from *trt6*Δ strain as an amorphous colorless solid. Its molecular formula was established to be $C_{26}H_{36}O_6$ by its ^{13}C NMR, DEPT and HRESIMS spectral data. The ^1H and ^{13}C NMR spectral data of the A, B and C rings of compound **11** were similar to those of compound **10** (Tables S4 and S5). The major spectral differences of compound **11** compared to **10** were the chemical shift of C-15 (δ_C 209.5) and C-16 (δ_C 74.5) in **11**. The gHMBC spectrum of compound **11** showed that there is ^1H–^{13}C correlation between H_3-24 (δ_H 1.32) with C-15 (δ_C 209.5), C-16 (δ_C 74.5) and C-17 (δ_C 211.0). This indicated that the partial structure of the ring D has been changed to 2-hydroxyl-2-methyl-cyclopentane-1,3-dione. The NOESY correlation between H_3-23 and H_3-24 cannot be observed suggesting that the methyl group at C-16 is located on the β face of D ring. Therefore, compound **11** was established to be the structure as shown in Table S5. We named it terrenoid.

Supplemental methods

Fermentation and LC-MS analysis:

All *A. terreus* strains were cultivated at 30 °C on LCMM agar plates (6 g/l $NaNO_3$, 0.52 g/l KCl, 0.52 g/l $MgSO_4 \cdot 7H_2O$, 1.52 g/l KH_2PO_4, 10 g/l D-glucose，20 g/l lactose, 15 g/l agar supplemented with 1 ml/l of a trace element solution) at 10 $\times 10^6$ spores per plate (d=10 cm). After 5 days, agar was chopped into small pieces and extracted with 50 ml MeOH followed by 60 ml 1:1 CH_2Cl_2/MeOH. The extract was evaporated *in vacuo* to yield a water residue, which was suspended in 25 ml H_2O and partitioned with 25 ml ethyl acetate (EA). The pH of the water crude was then adjusted to around 2 and the crude was extracted with 25 ml EA for a second time. The combined EA layer was evaporated *in vacuo*, re-dissolved in 1 ml of 20% DMSO in MeOH and a portion (10µl) was examined by high performance liquid chromatography–photodiode array detection–mass spectrometry (HPLC–DAD–MS) analysis. HPLC–MS was carried out using a ThermoFinnigan LCQ Advantage ion trap mass spectrometer with an RP C_{18} column (Alltech Prevail C18 2.1 mm by 100 mm with a 3µm particle size) at a flow rate of 125µl/min. The solvent gradient system for HPLC is 0-5 min 100 %-80 % A, 5-35 min 80 %-40 % A, 35-50 min 40 % A, 50-55 min 40 %-0 % A, 55-60 min 0 %-100 % A, 60-65 min 100 % A. (A: 5 % MeCN/H_2O with 0.05 % formic acid; B: MeCN with 0.05 % formic acid) The condition for MS analysis was carried out as described previously.[3]

Isolation of secondary metabolites

A. terreus wild type and mutant strains were grown at 30 °C on 1 liter LCMM agar plates (D=15 cm) for 5 days. Agar was chopped into small pieces and then soaked in 800 ml of 1:1 CH_2Cl_2/MeOH for 24 hrs. After filtration, the crude extract was evaporated *in vacuo* to yield a residue, which was then suspended in 500 ml water and partitioned with EA (500 ml) three times. The pH of the water crude was then adjusted to around 2 and the crude was extracted with EA (500 ml). The combined EA layers were evaporated *in vacuo* to a crude extract. The extract was applied to a silica gel column (Merck, 230 to 400 mesh, ASTM, 20 × 200 mm) and eluted with 250 ml CH_2Cl_2/MeOH mixtures of increasing polarity (fraction A, 1:0; fraction B, 19:1; fraction C, 9:1; fraction D, 7:3).

Further purification of the fractions with targeted compounds was carried out by gradient HPLC on a C18 reverse phase column (Phenomenex Luna 5µm C18 (2), 250 × 10 mm) with a flow rate of 5.0 ml/min and measured by a UV detector at 254 nm. The gradient system was MeCN (solvent B) and 5 % MeCN/H_2O (solvent A) both containing 0.05 % TFA.

The LC-MS profiles of each fraction of *A. terreus* NIH2624 indicated that fraction B (246 mg) contained compounds **1**-**6**. The gradient condition for semi-preparative HPLC analysis was 0-2 min 100 % A, 2-32 min 100 % -0 % A, 32-34 min 0 % A,

34-38 min 100 % A. Compound **3** (5.35 mg/L of medium), **4** (5.21 mg/L of medium), **2** (8.12 mg/L of medium), **5** (9.19 mg/L of medium) was eluted at 19.1 min, 19.6 min, 23.2 min, and 22.7 min. Compounds **1** and **6** were eluted in the same fraction at 25.1 min, which was further purified using a different gradient system (0-2 min 100 %-70 % A, 2-32 min 70 % A, 32-34 min 70 %-0% A, 34-36 min 0 %-100 % A, 36-38 min 100 % A) to yield pure compound **1** (7.46 mg/L of medium) and **6** (5.41 mg/L of medium) at retention times of 29.3 min and 30.8 min, respectively.

The LC-MS profiles of each fraction of *trt3*Δ indicated that fraction B (250 mg) contained compound **10**. The gradient condition for this deletant strain was 0-2 min 100 % A, 2-25 min 40 % -32.3 % A, 25-27 min 32.3 %-0 % A, 29-34 min 100 % A. Compound **10** (3.96 mg/L of medium) was eluted at 10.94 min.

The LC-MS profiles of each fraction of *trt5*Δ indicated that fraction B (203 mg) contained compound **7** (3.34 mg/L of medium) and the daggered intermediate (Figure 1). The gradient condition was 0-2 min 100 %-80 % A, 2-17 min 80 % -60 % A, 17-19 min 60 %-0 % A, 19-21 min 0 %-100 % A, 21-23 min 100 % A. However, the daggered intermediate was unstable and the increasing accumulation of compound **7** was observed upon isolation.

For strain *trt6*Δ, the LC-MS profiles of each fraction showed that fraction B (181 mg) had compound **11**. The HPLC gradient system was 0-2 min 100 % A, 2-32 min 100 % -0 % A, 32-34 min 0 % A, 34-36 min 0 %-100 % A, 36-38 min 100 % A. And compound **11** (5.82 mg/L of medium) was eluted at 25.7 min.

For strain *trt8*Δ, the LC-MS profiles of each fraction showed that fraction B (124 mg) had target intermediates. The gradient system was 0-2 min 100 %-80 % A, 2-32 min 80 %-40 % A, 32-44 min 40 %-0 % A, 44-46 min 0 %-100 % A, 46-48 min 100 % A. One new intermediate was eluted at 26.7 min. However, this intermediate was unstable and was further purified using a different gradient system (0-2 min 100 %-47 % A, 2-17 min 40 % A, 17-19 min 40 %-100% A, 19-21 min 100 % A) to give compound **8** (6.59 mg/L of medium).

The LC-MS profiles of each fraction of *trt9*Δ indicated that fraction B (190 mg) contained compound **9**. The gradient condition for this mutant strain was 0-2 min 100 %-60 % A, 2-30 min 60 % A, 30-32 min 60 %-0 % A, 32-34 min 0 % A, 34-36 min 0 % A-100 % A, 36-38 min 0 % A). Compound **9** (6.52 mg/L of medium) was eluted at 28.4 min.

Compound spectral data

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a GlobalWorks Cary 14 Spectrophotometer. Optical rotations were measured on a JASCO P-200 digital polarimeter. NMR spectra were collected on a Varian Mercury plus 400 spectrometer.

Terretonin (**1**). Colorless solid, mp 242-243 °C; $[\alpha]_D^{24}$ -107.9 ($CHCl_3$, *c* 0.6); IR ν_{max}^{ZnSe} 3425, 3328, 2966, 2950, 2364, 1776, 1747, 1733, 1710, 1683, 1646, 1432, 1349, 1265, 1180, 1103 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S6. The NMR data were in good agreement with the published data.[4]

Terretonin C (**2**). Colorless solid, mp 240-241 °C; $[\alpha]_D^{24}$ -106.6 ($CHCl_3$, *c* 0.5); IR ν_{max}^{ZnSe} 3747, 3635, 3444, 2983, 1762, 1736, 1707, 1684, 1641, 1554, 1452, 1191, 1176 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S6; The NMR data were in good agreement with the published data.[5]

Asterrelenin (3). Colorless needles, mp 209-210 °C; $[\alpha]_D^{24}$ 150.9 (CH_3OH, *c* 0.1); IR ν_{max}^{ZnSe} 3284, 2370, 1716, 1699, 1673, 1646, 1627, 1542, 1506, 1481, 1405, 1375, 1340, 1207, 1178, 1164, 1135 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S7; The NMR data were in good agreement with the published data.[5]

Butyrolactone III (**4**). Colorless amorphous solid; $[\alpha]_D^{24}$ 32.3 (CH_3OH, *c* 0.2); IR ν_{max}^{ZnSe} 3853, 3801, 3675, 3567, 1749, 1733, 1652, 1608, 1542, 1508, 1396, 1270, 1255, 1205, 1182, 1143 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S8; The NMR data were in good agreement with the published data.[6,7]

Epi-aszonalenin A (**5**). Colorless solid, mp 251-252 °C; $[\alpha]_D^{24}$ 321.9 ($CHCl_3$, *c* 0.1); IRν_{max}^{ZnSe} 2360, 2337, 1695, 1683, 1672, 1658, 1645, 1479, 1437, 1406, 1385, 1211, 1176, 1167, 1135 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S7; The NMR data were in good agreement with the published data.[8]

Butyrolactone I (6). Colorless amorphous solid; $[\alpha]_D^{24}$ 74.9 (CH_3OH, *c* 0.4); IR ν_{max}^{ZnSe} 3735, 3654, 3392, 2368, 1768, 1754, 1745, 1733, 1610, 1558, 1538, 1519, 1276, 1255, 1172 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S8. The NMR data were in good agreement with the published data.[7]

3,5-Dimethylorsellinic acid (7). Colorless needles, mp 195-198 °C; IR ν_{max}^{ZnSe} 3837,

2950, 1953, 1627, 1456, 1259, 1157, 1025 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; ^1H NMR (acetone-d_6, 400 MHz) δ 2.08 (3H, s), 2.15 (3H, s), 2.50 (3H, s). The NMR data were in good agreement with the published data.[9]

3,5-Dimethylorsellinate (8). Colorless needle, mp 100-102 °C; IR ν_{max}^{ZnSe} 3307, 3255, 2985, 1675, 1646, 1335, 1219, 1105, 972 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; ^1H NMR (CD_3OD, 400 MHz) δ 2.06 (3H, s), 2.11 (3H, s), 2.38 (3H, s), 3.90 (3H, s). The NMR data were in good agreement with the published data.[10]

Preterretonin A (9). Colorless amorphous solid; $[\alpha]_D^{24}$ -185.9 ($CHCl_3$, *c* 0.1); IRν_{max}^{ZnSe} 3674, 3649, 2987, 2937, 2362, 2330, 1736, 1684, 1653, 1558, 1473, 1456, 1039, 1018 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S3. The NMR data were in good agreement with the published data.[11]

Preterrenoid (10). Colorless amorphous solid; $[\alpha]_D^{24}$ -77.4 ($CHCl_3$, *c* 0.1); IRν_{max}^{ZnSe} 3853, 3743, 3629, 3587, 2937, 2364, 2343, 1733, 1716, 1699, 1686, 1620, 1456, 1404, 1250, 1207, 1138 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S4.

Terrenoid (11). Colorless amorphous solid; $[\alpha]_D^{24}$ -145.1 (CH_3OH, *c* 0.1); IRν_{max}^{ZnSe} 3726, 3631, 2360, 2341, 1623, 1558, 1541, 1209, 1099 cm^{-1}; For UV-Vis and ESIMS spectra, see Figure S2; For NMR spectra, see Table S5.

Supplementary Reference

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Table S1. Primers used in this study

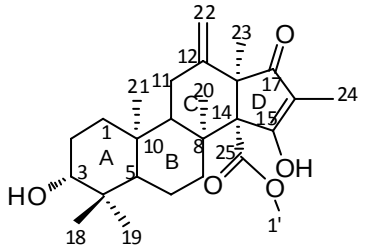
primer	Sequence (5′→3′)
ATEG_10075.1F1	GTGGAGCACGTACGCATTT
ATEG_10075.1F2	GTCCGGAAAGGGACTGTGTA
ATEG_10075.1R3	TGACCTCCACTAGCTCCAGCGAACAGGCAATGGAATAGGG
ATEG_10075.1F4	AATAGAGTAGATGCCGACCGTTGCAGCTCCAAGTGACAAC
ATEG_10075.1F5	CGCGACTCTATGTCATTTGC
ATEG_10075.1R6	CGTTCCTATCCGCGACTCTA
ATEG_10076.1F1	AGCTCCGTCAGCAAAACATC
ATEG_10076.1F2	GGGTAAGAACCATAAGGGGGTA
ATEG_10076.1R3	TGACCTCCACTAGCTCCAGCACAATGGTGGCTACCTGGAG
ATEG_10076.1F4	AATAGAGTAGATGCCGACCGGGAAGAAACGGAGCGAACTT
ATEG_10076.1F5	TTGTCCGCTGTGTACGTTTC
ATEG_10076.1R6	TCCTTCCATCAGCTCTCTGG
ATEG_10077.1F1	TGTTTCTCCAGCTGCCAGATGG
ATEG_10077.1F2	GACGGCACAAAGCCTAGAAG
ATEG_10077.1R3	TGACCTCCACTAGCTCCAGCATACGATTCACAGGGGATGC
ATEG_10077.1F4	AATAGAGTAGATGCCGACCGATTTAGAGCGGGATGCAGAG
ATEG_10077.1F5	ATCCGCTCGATTTTCCCTAC
ATEG_10077.1R6	AAACGGTTCTCCGATTACCC
ATEG_10078.1F1	TCCGATGGAATCTGGGATTG
ATEG_10078.1F2	TTGGAGGGACTTTTGCGTGA
ATEG_10078.1R3	TGACCTCCACTAGCTCCAGCACGAGTCCTACCTCCATACC
ATEG_10078.1F4	AATAGAGTAGATGCCGACCGTCCTAGGAGCCAGGAGGTTC
ATEG_10078.1F5	CCGAATGATTGGGACCATGT
ATEG_10078.1R6	TCTACCCCATAGCAAGCTCG
ATEG_10079.1F1	AGCATCGTGTCTTCCATTCC
ATEG_10079.1F2	CCGGAGCCACAATCTAACAG
ATEG_10079.1R3	TGACCTCCACTAGCTCCAGCCGGATGTAGCATCATTCACG
ATEG_10079.1F4	AATAGAGTAGATGCCGACCGATTAATTCCGATCCCAGACG
ATEG_10079.1F5	ACGCCCACATTTCTCATCC
ATEG_10079.1R6	TGAGCGAGGTGGAGAAGG
ATEG_10080.1F1	TGGAGAAATATGCTGCGATG
ATEG_10080.1F2	GAAGAGCTGACGGGTCGTAG
ATEG_10080.1R3	TGACCTCCACTAGCTCCAGCAGAGGAACATGAACGGCTTG
ATEG_10080.1F4	AATAGAGTAGATGCCGACCGCCTCATTTTTGCCATTTTGG
ATEG_10080.1F5	ACGGGGTACGACATAGGAAG
ATEG_10080.1R6	TCCTGCTTGATGGAAAGGAG
ATEG_10081.1F1	TCAGTCTTGGGGCATTTAGG
ATEG_10081.1F2	AACCTGACAGGGAGTGGATG
ATEG_10081.1R3	TGACCTCCACTAGCTCCAGCGATGGCGGATCTAGAAGACG
ATEG_10081.1F4	AATAGAGTAGATGCCGACCGGAAGGCTTTCACGATGAAC
ATEG_10081.1F5	CGTCTCGATGGGATCACTTT
ATEG_10081.1R6	TGGATGAGAGAACACGCAAG

ATEG_10082.1F1	GTTGCAGGTACCAATGCTTT
ATEG_10082.1F2	GGTTGCTACGGATCTCTTGG
ATEG_10082.1R3	TGACCTCCACTAGCTCCAGCGAGGCGAGAGGCATACAAAC
ATEG_10082.1F4	AATAGAGTAGATGCCGACCGGAGGCATATCGGCTTCTTGA
ATEG_10082.1F5	GCAGACCGCTGGTTTCTTAG
ATEG_10082.1R6	CTTTGGGAGGAGGTGGAGAG
ATEG_10083.1F1	AAAGTGATCCCATCGAGACG
ATEG_10083.1F2	GCCCATATTCTGTCCACCAG
ATEG_10083.1R3	TGACCTCCACTAGCTCCAGCCCTGGAGAGCAGATCAAAGC
ATEG_10083.1F4	AATAGAGTAGATGCCGACCGCAGGATGGTTGGATAGTCTCG
ATEG_10083.1F5	GGTTCCTAGCGTCGGTATCA
ATEG_10083.1R6	CTCGGGTGTTGTCTCAAGC
ATEG_10084.1F1	CAACGTCGGTAACCCTCTGT
ATEG_10084.1F2	TTGATAGAGGGTCTCCAGGA
ATEG_10084.1R3	TGACCTCCACTAGCTCCAGCATCCGAAGTGGTCCAATGAC
ATEG_10084.1F4	AATAGAGTAGATGCCGACCGTCGACCGAGTTTTCTTCAGG
ATEG_10084.1F5	CGACGCTAGGAACCTGATCT
ATEG_10084.1R6	AGACAAAGTCCCATCCAACG
ATEG_10085.1F1	GATGACTGTGAAAGCGTTGG
ATEG_10085.1F2	CATCCACGTTGAAGGCTAGG
ATEG_10085.1R3	TGACCTCCACTAGCTCCAGCCGCCTTTGTGAGTTTTGACC
ATEG_10085.1F4	AATAGAGTAGATGCCGACCGACTGGGGTGGAAAGATCCTC
ATEG_10085.1F5	ACTGATGCGGGAGGTAATTG
ATEG_10085.1R6	ATCGCTGGGATCATGGATAG
ATEG_10086.1F1	GAAGATCGCACCGTTGCTT
ATEG_10086.1F2	GCAAACTGACGGGCTTAGAA
ATEG_10086.1R3	TGACCTCCACTAGCTCCAGCGATACCCGATGCACTTCCAG
ATEG_10086.1F4	AATAGAGTAGATGCCGACCGTTCACTCCCCTAGATCCGTAGA
ATEG_10086.1F5	TTGGCATTCATGTCGGTCT
ATEG_10086.1R6	GTGATCGTTCCCGTATTTGG
ATEG_10087.1F1	ATTGCCAGACGGAGCTTCTA
ATEG_10087.1F2	AGCTTCTAGCAAGCATCATCC
ATEG_10087.1R3	TGACCTCCACTAGCTCCAGCATCTTGCCTGGGTTGGAGTA
ATEG_10087.1F4	AATAGAGTAGATGCCGACCGCGGTATTTAGTCAAGCTGTGG
ATEG_10087.1F5	CATATATCTGCCGGGATTGG
ATEG_10087.1R6	AGCGTATCCGTCTGTAGCAT
ATEG_10088.1F1	CGGACCACTTGAGGAAAGAA
ATEG_10088.1F2	TGGGTCTCTTCCAGACAGTG
ATEG_10088.1R3	TGACCTCCACTAGCTCCAGCAGGCTTTGCTCGGCTATTTT
ATEG_10088.1F4	AATAGAGTAGATGCCGACCGCCGTACGAGGTGATGTCGTT
ATEG_10088.1F5	GCACCATATCAGCCCACTTT
ATEG_10088.1R6	ACCATGCGCTATCTCTCGAT
ATEG_10089.1F1	CCACCAAGAAGCATAGGAGGT
ATEG_10089.1F2	GGGACTAGGGAAACACTTCCA

ATEG_10089.1R3	TGACCTCCACTAGCTCCAGCGAGGTGCCAAGCACTTCAAC
ATEG_10089.1F4	AATAGAGTAGATGCCGACCGCGGGTGGGATATGAACAAGA
ATEG_10089.1F5	ACTCTGCTGCAACAGCTGGA
ATEG_10089.1R6	TCCGGACCTTTCAAGAAGTG
Hscr	TGACCTCCACTAGCTCCAGC

Table S2. *A. terreus* strains used in this study

Fungal strain or transformants	Gene mutation(s)	Genotype
A. terreus NIH2624	-	Wildtype
CW6001.4	ATEG_10075.1Δ	ATEG_10075.1::*hph*
CW6002.8	ATEG_10076.1Δ	ATEG_10076.1::*hph*
CW6003.3	ATEG_10077.1Δ	ATEG_10077.1::*hph*
CW6004.3	ATEG_10078.1Δ	ATEG_10078.1::*hph*
CW6005.4	ATEG_10079.1Δ	ATEG_10079.1::*hph*
CW6006.5	ATEG_10080.1Δ	ATEG_10080.1::*hph*
CW6007.5	ATEG_10081.1Δ	ATEG_10081.1::*hph*
CW6008.4	ATEG_10082.1Δ	ATEG_10082.1::*hph*
CW6009.2	ATEG_10083.1Δ	ATEG_10083.1::*hph*
CW6010.3	ATEG_10084.1Δ	ATEG_10084.1::*hph*
CW6011.1	ATEG_10085.1Δ	ATEG_10085.1::*hph*
CW6012.5	ATEG_10086.1Δ	ATEG_10086.1::*hph*
CW6013.3	ATEG_10087.1Δ	ATEG_10087.1::*hph*
CW6014.3	ATEG_10088.1Δ	ATEG_10088.1::*hph*
CW6015.1	ATEG_10089.1Δ	ATEG_10089.1::*hph*

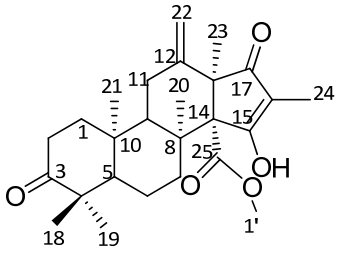


Preterretonin A (**9**)

Table S3. NMR data for compound **9** (400 and 100 MHz in $CDCl_3$)

Position	δH (*J* in Hz)	δC
1	H_α: 1.36, m H_β: 0.88, m	38.7, CH_2
2	1.58, m	27.0, CH_2
3	3.22, m	79.0, CH
4	—	38.4, C
5	0.59, d (12.0)	55.6, CH
6	H_α: 1.54, m H_β: 1.33, m	18.7, CH_2
7	H_α: 1.74, m H_β: 1.55, m	38.7, CH_2
8	—	45.2, C
9	1.26, m	45.7, CH
10	—	38.4, C
11	H_α: 2.19, dd (16.8, 8.0) H_β: 2.43, dd (16.8, 12.0)	29.0, CH_2
12	—	143.8, C
13	—	57.6, C
14	—	68.5, C
15	—	179.0, C[a]
16	—	113.2, C
17	—	205.7, C[a]
18	0.96, s	28.2, CH_3
19	0.76, s	16.0, CH_3
20	0.94, s	19.9, CH_3
21	0.94, s	16.2, CH_3
22	a: 5.12,d (2.0) b: 5.18, brs	114.0, CH_2
23	1.32, s	23.7, CH_3
24	1.75, s	6.15, CH_3
25	—	175.3, C
-OCH_3	3.79, s	52.1, CH_3
-OH		

[a]: This carbon is identified in the HMBC spectrum.

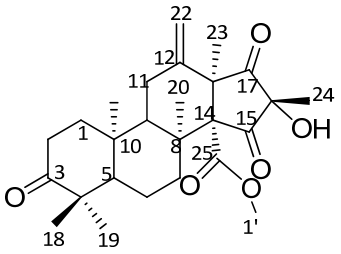


Preterrenoid (**10**)

Table S4. NMR data for compound **10** (400 and 100 MHz in $CDCl_3$)

Position	δH (*J* in Hz)	δC	HMBC[a]	COSY	NOESY
1	H_β: 1.37, m	39.3, CH_2	2, 3, 5, 10	H_α-1, H_2-2	H-9
	H_α: 1.79, m			H_β-1, H_2-2	H_3-21
2	2.40, m	33.9, CH_2	1, 3	H_2-1	
3	—	217.3, C			
4	—	47.3, C			
5	1.22, br t (10.4)	55.2, CH	4, 10, 18, 19	H_2-6	H_β-7, H_3-18
6	1.51, m	20.0, CH_2	5, 7, 8	H-5, H_2-7	H_3-19
7	H_α: 1.72, m	38.2, CH_2	5, 6	H_2-6, H_β-7	
	H_β: 1.53, m			H_2-6, H_α-7	H-5
8	—	38.2, C			
9	1.35, m	44.7, CH	1, 8, 10, 11	H_2-11	H_β-1
10	—	42.9, C			
11	H_β: 2.47, m	28.9, CH_2	9, 12, 13, 22	H-9, H_α-11	H_α-11
	H_α: 2.25, dd (16.0, 12.0)			H-9, H_β-11,	H_β-11, H_3-21
12	—	143.8, C			
13	—	57.8, C			
14	—	68.1, C			
15	—	177.6, C			
16	—	113.2, C			
17	—	205.6, C			
18	1.06, s	27.2, CH_3	3, 4, 5, 19		H-5
19	1.01, s	21.7, CH_3	3, 4, 5, 18		H_3-21, H-6
20	0.97, s	19.6, CH_3	7, 8, 9, 14		H_3-21, H_3-23
21	1.03, s	16.0, CH_3	1, 9, 10		H_α-1, H_α-11, H_3-19, H_3-20
22	a: 5.13,brd (2.0)	114.0, CH_2	11, 13		H_b-22, H_3-23
	b: 5.19,brd (2.0)				H_a-22
23	1.32, s	23.7, CH_3	12, 13, 14, 17		H_3-20, H_a-22
24	1.74, s	6.25, CH_3	15, 16, 17		
25	—	175.4, C			
-OCH_3	3.79, s	52.1, CH_3	25		
-OH	9.68, s		14, 15, 16		

[a]: HMBC correlations are from proton(s) to the indicated carbon.

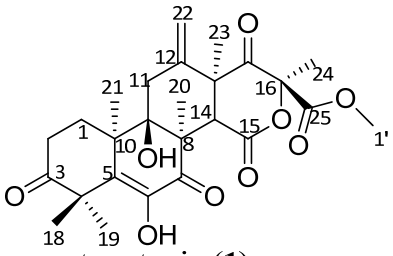


Terrenoid (**11**)

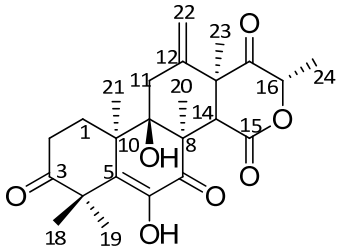
Table S5. NMR data for compound **11** (400 and 100 MHz in CD_3OD)

Position	δH (*J* in Hz)	δC	HMBC[a]	COSY	NOESY
1	H_β: 2.02, m	38.9, CH_2	2, 3, 10	H_α-1, H_2-2	H_α-1, H_β-11
	H_α: 1.40, m			H_β-1, H_2-2	H_β-1, H_3-21
2	H_α: 2.06, m	33.3, CH_2	1, 3	H_β-2, H_2-1	H_β-2
	H_β: 2.54, m			H_α-2, H_2-1	H_α-2
3	—	218.7, C			
4	—	48.3, C			
5	1.53, dd (9.6, 2.0)	54.0, CH	4, 10, 18, 19	H_2-6	H-9, H_3-18
6	H_β: 1.52, m	18.9, CH_2	5, 7	H-5, H_α-6, H_2-7	H_α-6
	H_α: 1.66,td (12.8, 2.8)			H-5, H_β-6, H_2-7	H_β-6, H_3-19
7	H_α: 2.96, td (13.2, 4.0)	33.7, CH_2	6, 8, 20	H_2-6, H_β-7	H_β-7
	H_β: 2.04, m			H_2-6, H_α-7	H_α-7
8	—	40.5, C			
9	1.44, dd (10.8, 4.0)	50.5, CH	7, 10, 11	H_2-11	H-5
10	—	37.1, C			
11	H_β: 2.43, dd (14.0, 3.6)	27.91, CH_2	9, 12	H-9, H_α-11	H_β-1, H_α-11, H_b-22
	H_α: 2.66, br t (14.0)			H-9, H_β-11,	H_β-11, H_3-20, H_3-21
12	—	145.5, C			
13	—	58.1, C			
14	—	72.9, C			
15	—	209.5, C			
16	—	74.5, C			
17	—	211.0, C			
18	1.12, s	25.9, CH_3	3, 4, 5, 19		H-5
19	1.06, s	20.5, CH_3	3, 4, 5, 18		H_α-6, H_3-21
20	1.35, s	17.5, CH_3	7, 8, 9, 14		H_α-11, H_3-21, H_3-23
21	1.07, s	14.6, CH_3	1, 9, 10		H_α-1, H_α-11, H_3-19, H_3-20
22	H_a: 4.74, d (2.0)	114.4, CH_2	11, 13	H_β-11	H_b-22
	H_b: 5.05, d (2.0)			H_β-11	H_β-11, H_a-22
23	1.61, s	22.1, CH_3	12, 13, 14, 17		H_α-11, H_3-20
24	1.32, s	24.1, CH_3	15, 16, 17		
25	—	168.0, C			
-OCH_3	3.58, s	51.1, CH_3	25		

[a]: HMBC correlations are from proton(s) to the indicated carbon.



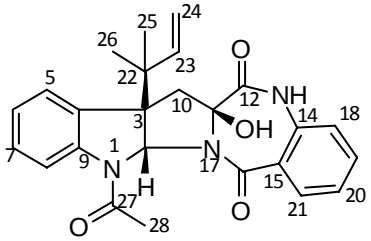
terretonin (**1**)



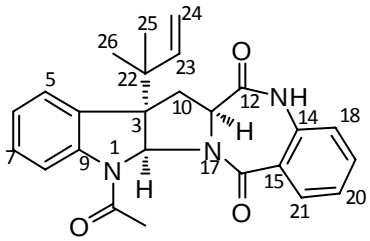
terretonin C (**2**)

Table S6. ^{1}H and ^{13}C NMR data for compound **1** (400 and 100 MHz in $CDCl_3$), and compound **2** (400 and 100 MHz in $CDCl_3$)

	δH (*J* in Hz)		δ C	
Position	**1**	**2**	**1**	**2**
1	1.76, m	1.79, dd (16.4, 8.8)	32.8, CH_2	28.4, CH_2
	2.36, m	2.39, m		
2	2.71, dd (19.2, 8.4)	2.55, m	35.1, CH_2	32.8, CH_2
	2.53 m	2.73, dd (18.8, 8.8)		
3			214.5, C	214.2, C
4			52.5, C	48.1, C
5			131.8, C	132.1, C
6			140.0, C	138.9, C
7			197.2, C	197.6, C
8			43.3, C	52.3, C
9			77.8, C	78.1, C
10			48.1, C	43.4, C
11	2.27, br d (14.4)	2.29, d (14.4)	28.3, CH_2	35.3, CH_2
	2.97, br d (14.4)	3.01, d (14.4)		
12			138.9, C	140.5, C
13			49.6, C	49.5, C
14	3.54, s	3.83, s	44.8, CH	45.2, CH
15			168.8, C	169.5, C
16		5.07, q (6.8)	85.7, CH	77.7, CH
17			201.6, C	206.7, C
18	1.21, s	1.49, s	21.4, CH_3	21.4, CH_3
19	1.44, s	1.49, s	23.8, CH_3	23.8, CH_3
20	1.47, s	1.97, s	21.4, CH_3	18.9, CH_3
21	1.47, s	1.23, s	20.0, CH_3	20.2, CH_3
22	5.09, br s	5.07, br s	117.3, CH_2	116.8, CH_2
	5.46, br s	5.39, br s		
23	1.71, s	1.42, s	18.8, CH_3	23.1, CH_3
24	1.93, s	1.50, d (6.4)	23.6, CH_3	14.82, CH_3
25			168.0, C	
1'	3.79, s		54.0, CH_3	
9-OH				
6-OH				



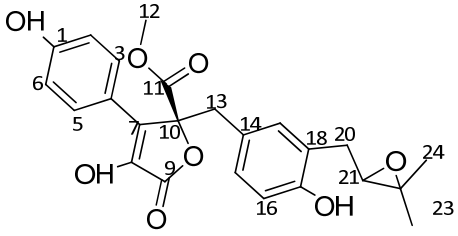
asterrelenin (**3**)



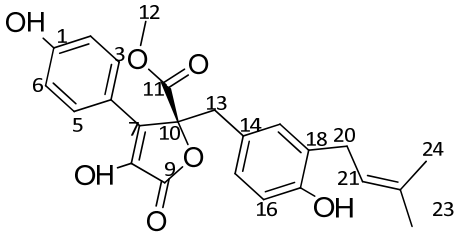
epi-aszonalenin A (**5**)

Table S7. ^{1}H and ^{13}C NMR data for compound **3** (400 and 100 MHz in CD_3OD), and compound **5** (400 and 100 MHz in DMSO-d_6)

	δH (*J* in Hz)		δ C	
Position	**3**	**5**	**3**	**5**
1			-	-
2	6.34, s	6.05, s	83.4, CH_2	81.0, CH_2
3			59.3, CH	50.5, CH
4			134.2, C	133.0, C
5	7.90, dd (8.0, 1.6)	7.46, dd (7.6, 1.2)	131.7, CH	127.3, CH
6	7.11, d (7.6)	7.09, dt (7.6, 1.2)	125.4, CH	123.4, CH
7	7.73, br d (8.0)	7.19, dt (7.6, 1.2)	134.1, CH	127.8, CH
8	7.44, d (7.2)	7.66, br d (7.6)	120.3, CH	117.9, CH
9			138.3, C	141.6, C
10	H_a: 3.73, d (12.0)	H_a: 2.99, d (13.2)	41.5, CH_2	28.9, CH_2
	H_β: 2.49, d (14.0)	H_β: 2.56, dd (13.2, 8.8)		
11		4.27, d (8.8)	89.8, C	56.7, CH
12			170.4, C	170.7, C
13		10.12, s		
14			143.1, C	137.1, C
15			126.2, C	124.6, C
16			168.8, C	166.3, C
17				
18	6.93, d (8.4)	6.97, d (8.4)	121.2, CH	120.9, CH
19	7.24, t (7.6)	7.50, dt (8.4, 1.6)	129.4, CH	132.7, CH
20	7.18, t (8.4)	7.20, br t (8.4)	124.7, CH	123.6, CH
21	7.41, dd (6.8, 1.6)	7.87, dd (8.4, 1.6)	128.6, CH	130.8, CH
22			41.8, C	40.4, C
23	5.89, dd (17.2, 10.8)	5.91, dd (17.2,10.8)	145.0, CH	143.9, CH
24	H_a: 5.15, d (18.8)	H_a: 5.11, dd (17.2, 1.2)	114.8, CH_2	114.0, CH_2
	H_b: 5.12, d (11.2)	H_b: 5.08, d (10.8, 1.2)		
25	1.20, s	0.87, s	22.9, CH_3	22.8, CH_3
26	0.97, s	1.11, s	23.8, CH_3	22.1, CH_3
27			170.4, C	169.6, C
28	2.69, s	2.61, s	24.1, CH_3	23.4, CH_3



butyrolactone III (**4**)



butyrolactone I (**6**)

Table S8. ^{1}H and ^{13}C NMR data for compound **4** (400 and 100 MHz in CD_3OD), and compound **6** (400 and 100 MHz in CD_3OD)

	δH (*J* in Hz)		δ C	
Position	**4**	**6**	**4**	**6**
1			157.9, C	159.5, C
2	6.87, dd (7.2, 2.0)	6.87, d (9.2)	115.8, CH	116.8, CH
3	7.55, dd (7.2, 2.0)	7.59, d (8.8)	128.8, CH	130.5, CH
4			121.0, C	123.3, C
5	7.55, dd (7.2, 2.0)	7.59, d (8.8)	128.8, CH	130.5, CH
6	6.87, dd (7.2, 2.0)	6.87, d (9.2)	115.7, CH	116.8, CH
7			127.6, C	129.3, C
8			138.1, C	139.9, C
9			168.0, C	170.5, C
10			84.7, C	86.9, C
11			169.7, C	171.8, C
12	3.79, s	3.78, s	53.5, CH_3	54.0, CH_3
13	3.40, s	3.46, d (14.8)	38.0, CH_2	39.8, CH_2
		3.42, d (14.8)		
14			119.6, C	125.2, C
15	6.52, dd (8.0, 2.0)	6.54, dd (8.0, 1.6)	128.8, C	129.9, C
16	6.50, d (8.0)	6.49, d (8.0)	116.8, CH	115.2, CH
17			151.7, C	155.3, C
18			124.3, C	128.6, C
19	6.46, br s	6.41, d (2.1)	131.6, CH	132.5, CH
20		3.07, d (6.8)	31.0, CH_2	28.8 CH_2
21	3.66, td (7.6, 2.0)	5.06, td (6.4, 1.2)	67.9, CH	123.7, CH
22			77.0, C	133.1, C
23	1.17, s	1.67, s	25.7, CH_3	26.1, CH_3
24	1.26, s	1.58, s	20.1, CH_3	17.9, CH_3

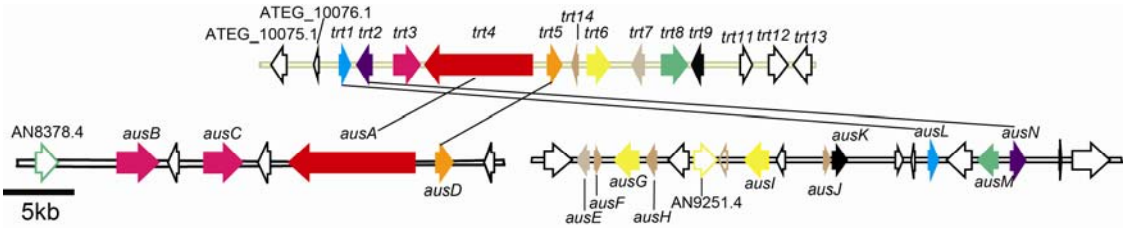


Figure S1. Comparison of the *trt* cluster and the *aus* clusters. Filled arrows represent genes that are involved in either terretonin or austinol biosynthesis. Genes in open arrows are not involved. Orthologous genes identified by homology BLAST analysis of their putative protein sequence are shown in the same color (except black). Conserved genes within the *trt* cluster and *aus* clusters are connected.

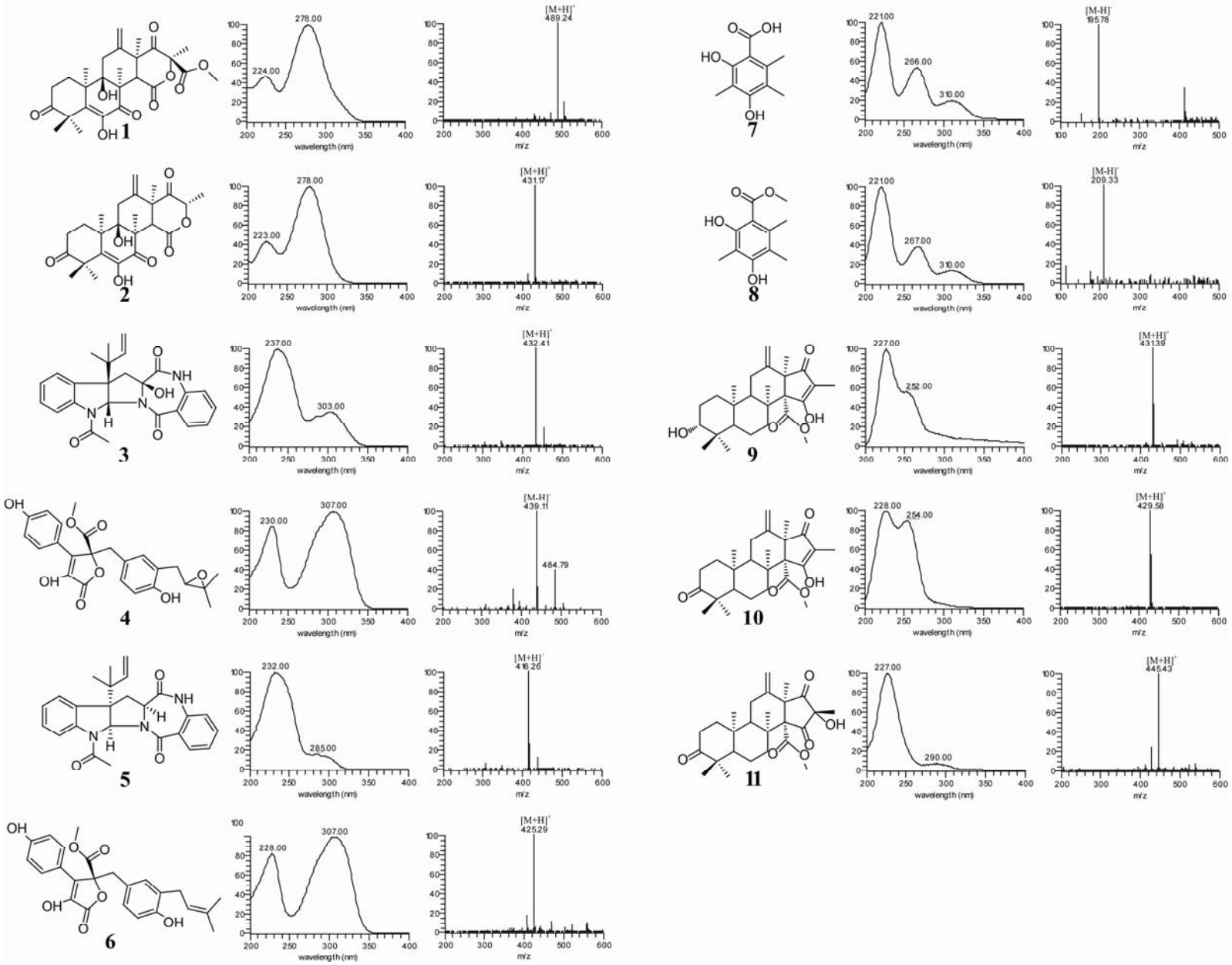


Figure S2. UV-Vis and ESIMS spectra of compounds isolated in this study

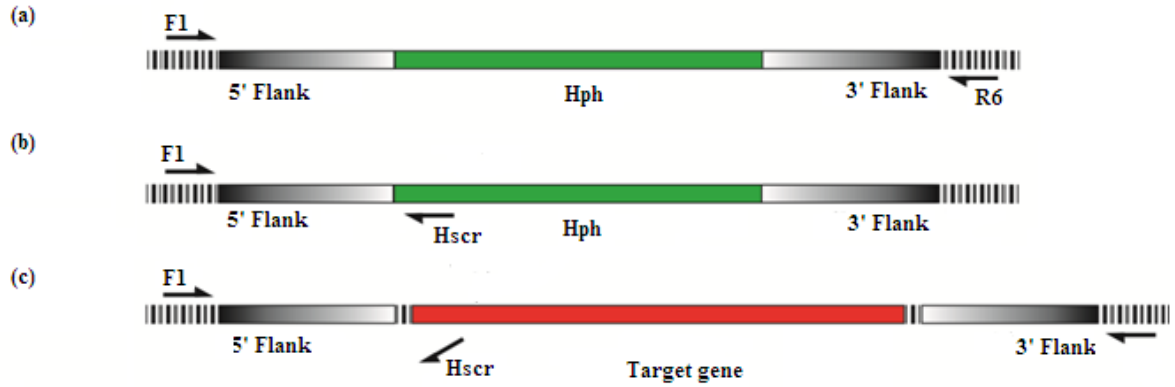


Figure S3. Schematic of the diagnostic PCR screening strategy. Two primer pairs are used for confirming homologous integration of the knockout construct at the target locus. Primers F1 and R6 anneal beyond the 5' and 3' flanks of the transforming DNA construct, respectively, and Hscr anneals within the *Hph* gene. In the knockout strain primers F1 and R6 amplify a fragment of approximately 3 kb (a) while primers F1 and Hscr produce a fragment of approximately 1 kb (b). If the target gene has not been replaced, primer Hscr will not anneal and there will be no specific amplification (c).

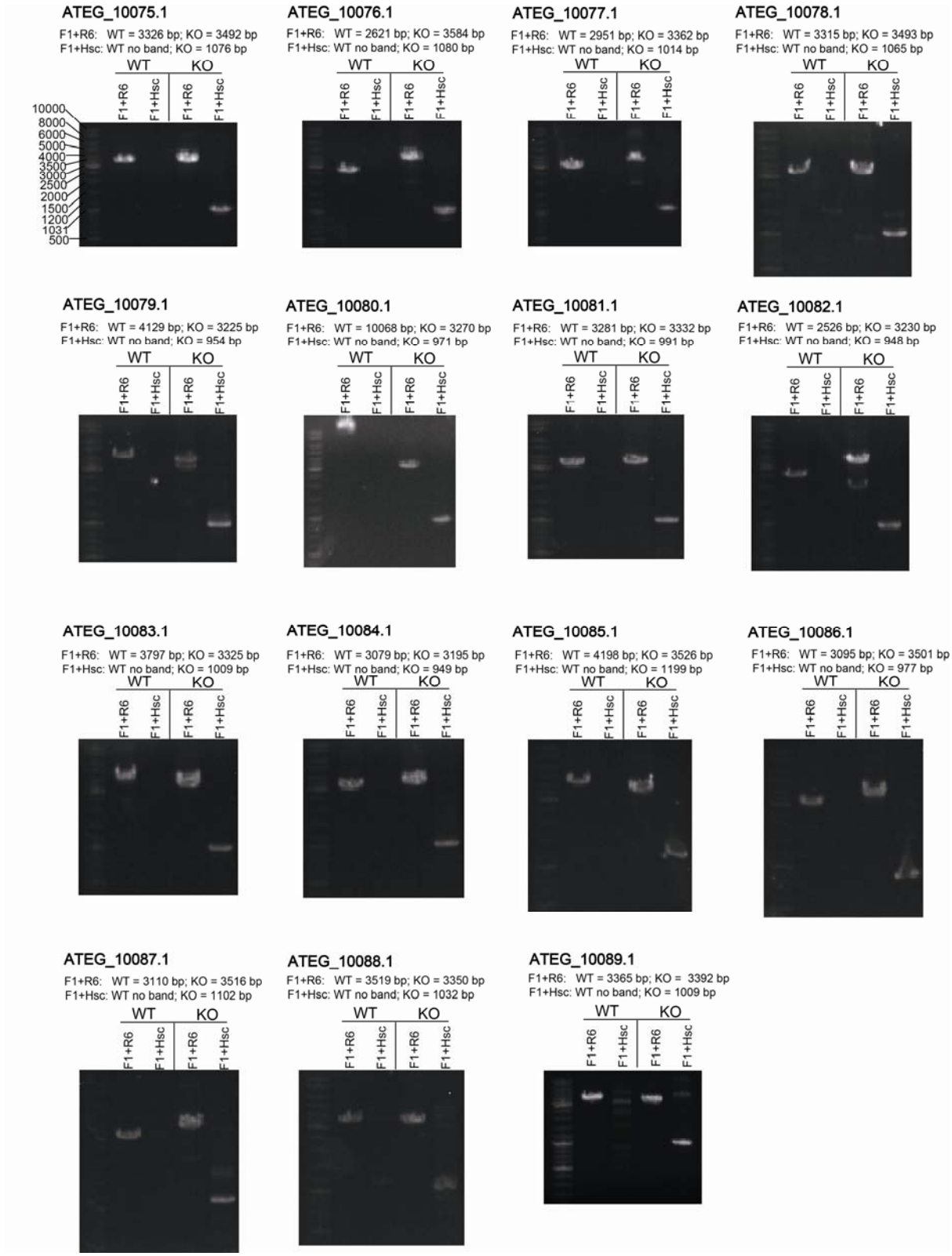


Figure S4. Diagnostic PCR for the gene deletant strains.

Figures S5-S14. Total genomic DNA was isolated from wild type and mutant strains and one microgram from each strain was digested with the restriction enzymes specified in each figure. Digests were electrophoresed on 1% agarose gels and blotted using standard methods. Biotin labeled DNA probes were generated from PCR products amplified using primers F1 and R6 specific to each locus.

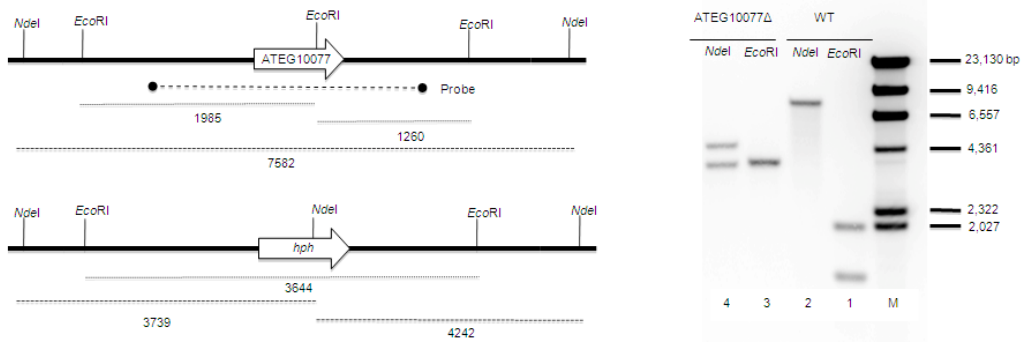


Figure S5. Southern blot confirming gene deletion of ATEG_10077.1. Replacement of the target gene with the hygromycin resistance marker removed an *EcoRI* site causing the 1985 bp and 1260 bp wild-type bands (lane 1) to become a single 3644bp band (lane 3) in the mutant strain.

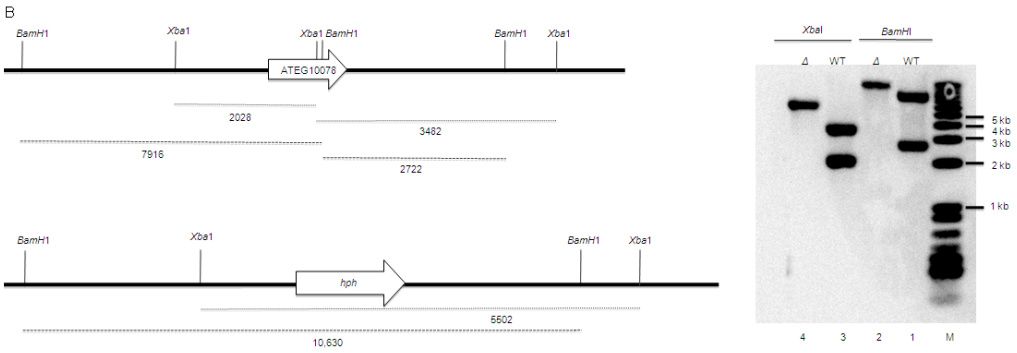


Figure S6. Southern blot confirming gene deletion of ATEG_10078.1. Replacement of the target gene with the hygromycin resistance marker removed a *BamHI* site causing the 7916 bp and 2722 bp wild-type bands (lane 1) to become a single 10,630 bp band (lane 2) in the mutant strain.

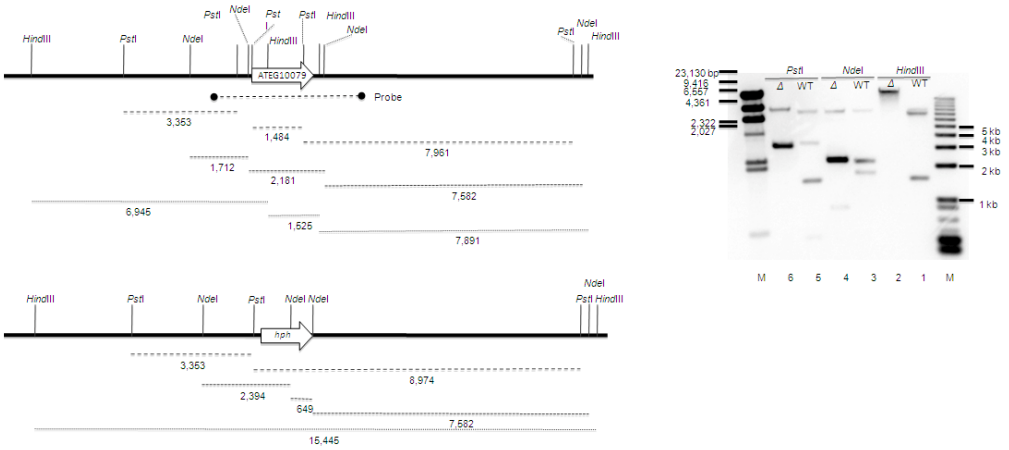


Figure S7. Southern blot confirming gene deletion of ATEG_10079.1. Replacement of the target gene with the hygromycin resistance marker removed two *HindIII* sites causing the 6945 bp, 1525 bp, and 7891 bp wild-type bands (lane 1) to become a single 15,445 bp band (lane 2) in the mutant strain.

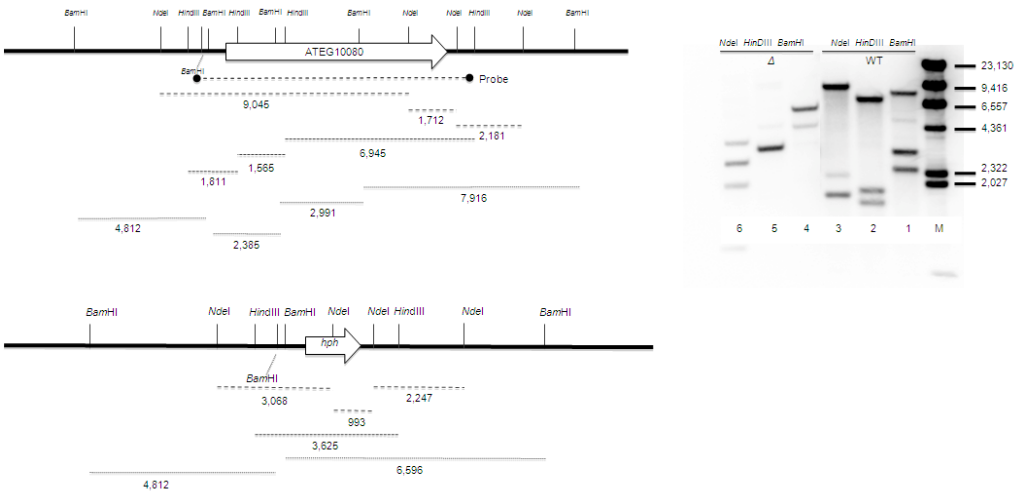


Figure S8. Southern blot confirming gene deletion of ATEG_10080.1. Replacement of the target gene with the hygromycin resistance marker removed two *HindIII* sites causing the 1811 bp, 1565 bp, and 6945 bp wild-type bands (lane 2) to become a single 3625 bp band (lane 5) in the mutant strain.

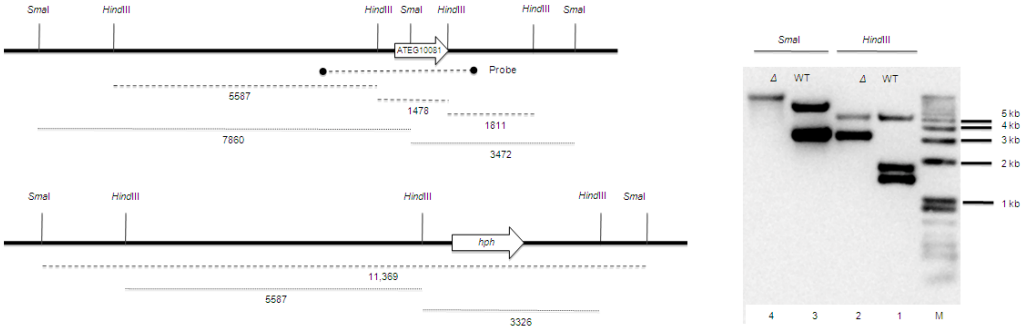


Figure S9. Southern blot confirming gene deletion of ATEG_10081.1. Replacement of the target gene with the hygromycin resistance marker removed a *SmaI* site causing the 7860 bp and 3472 bp wild-type bands (lane 3) to become a single 11,369 bp band (lane 4) in the mutant strain.

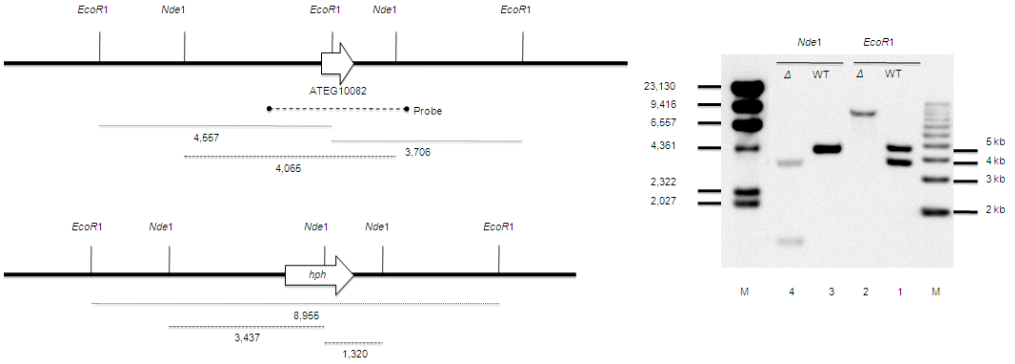


Figure S10. Southern blot confirming gene deletion of ATEG_10082.1. Replacement of the target gene with the hygromycin resistance marker removed an *EcoRI* site causing the 4557 bp and 3706 bp wild-type bands (lane 1) to become a single 8955 bp band (lane 2) in the mutant strain.

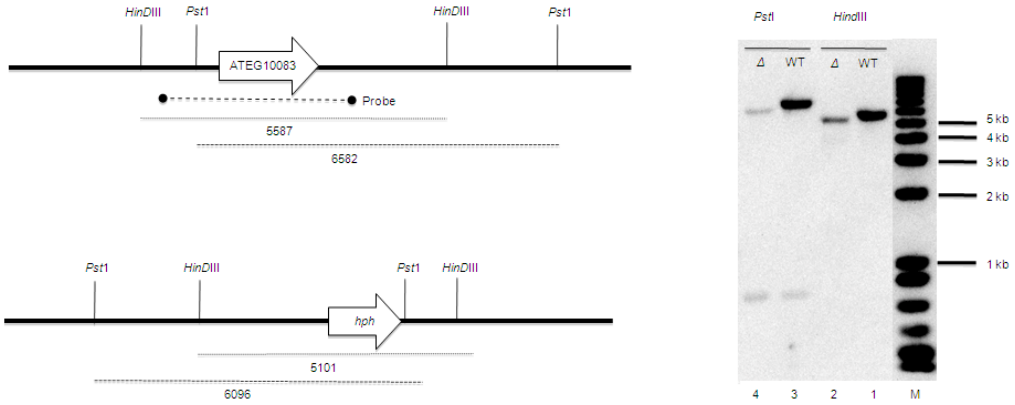


Figure S11. Southern blot confirming gene deletion of ATEG_10083.1. Replacement of the target gene with the smaller hygromycin resistance marker reduced the distance between two *HinDIII* sites by 486 bp causing the 5587 bp wild-type band (lane 1) to become a 5101 bp band (lane 2) in the mutant strain.

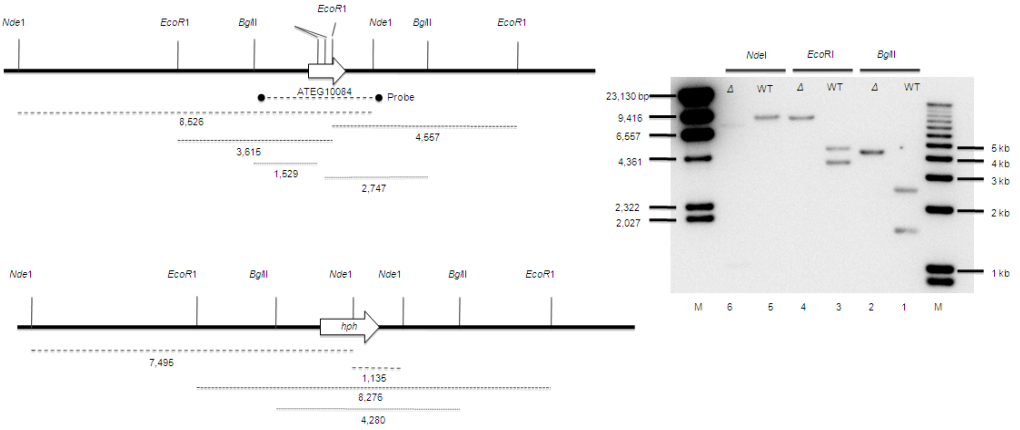


Figure S12. Southern blot confirming gene deletion of ATEG_10084.1. Replacement of the target gene with the hygromycin resistance marker removed two closely spaced *BglII* sites causing the 1529 bp and 2747 bp wild-type bands (lane 1) to become a single 4280 bp band (lane 2) in the mutant strain.

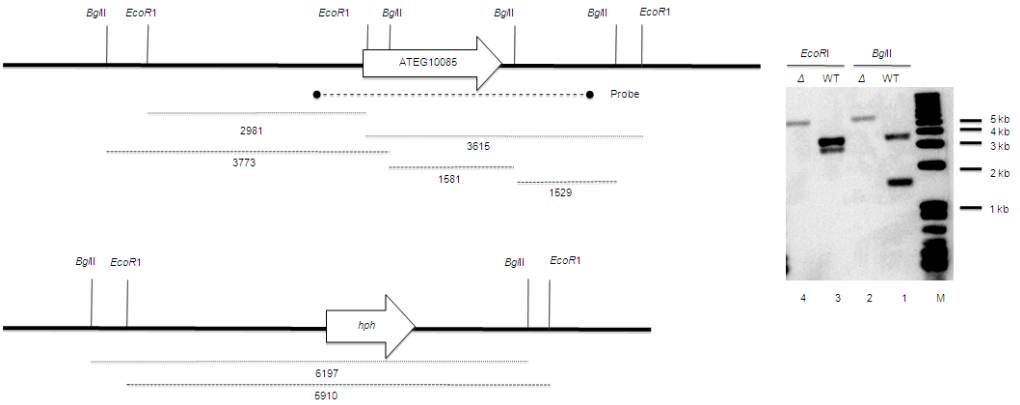


Figure S13. Southern blot confirming gene deletion of ATEG_10085.1. Replacement of the target gene with the hygromycin resistance marker removed two *BglII* sites causing the 3733 bp, and overlapping 1581 bp and 1529 bp wild-type bands (lane 1) to become a single 5910 bp band (lane 2) in the mutant strain.

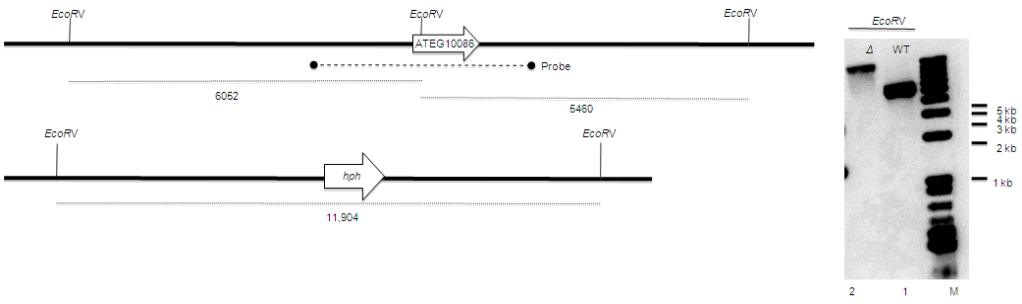
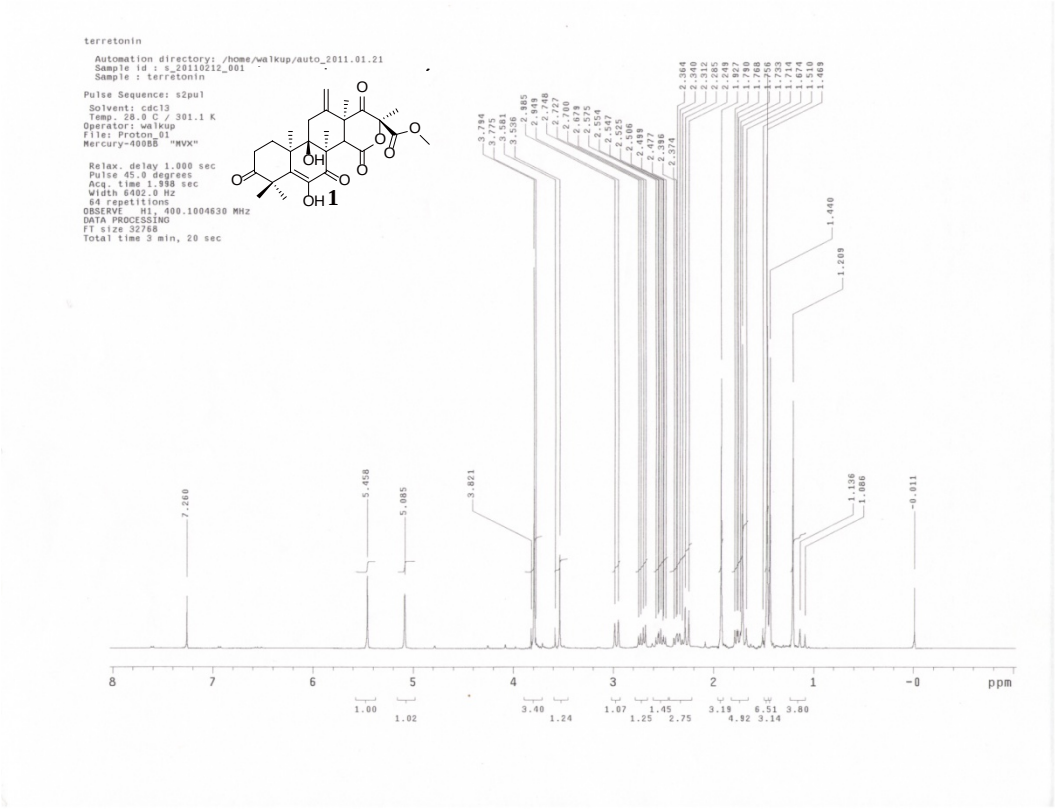
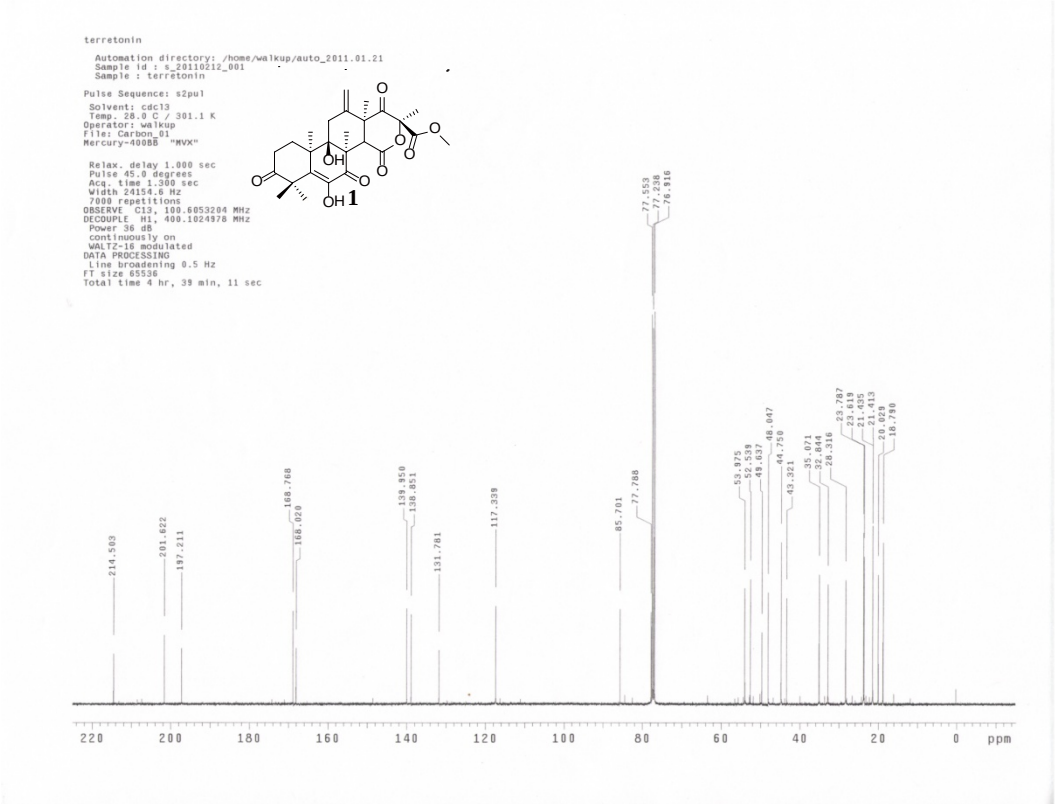


Figure S14. Southern blot confirming gene deletion of ATEG_10086.1. Replacement of the target gene with the hygromycin resistance marker removed an *EcoRV* site causing the 6052 bp and 5460 bp wild-type bands (lane 1) to become a single 11,904 bp band (lane 2) in the mutant strain.

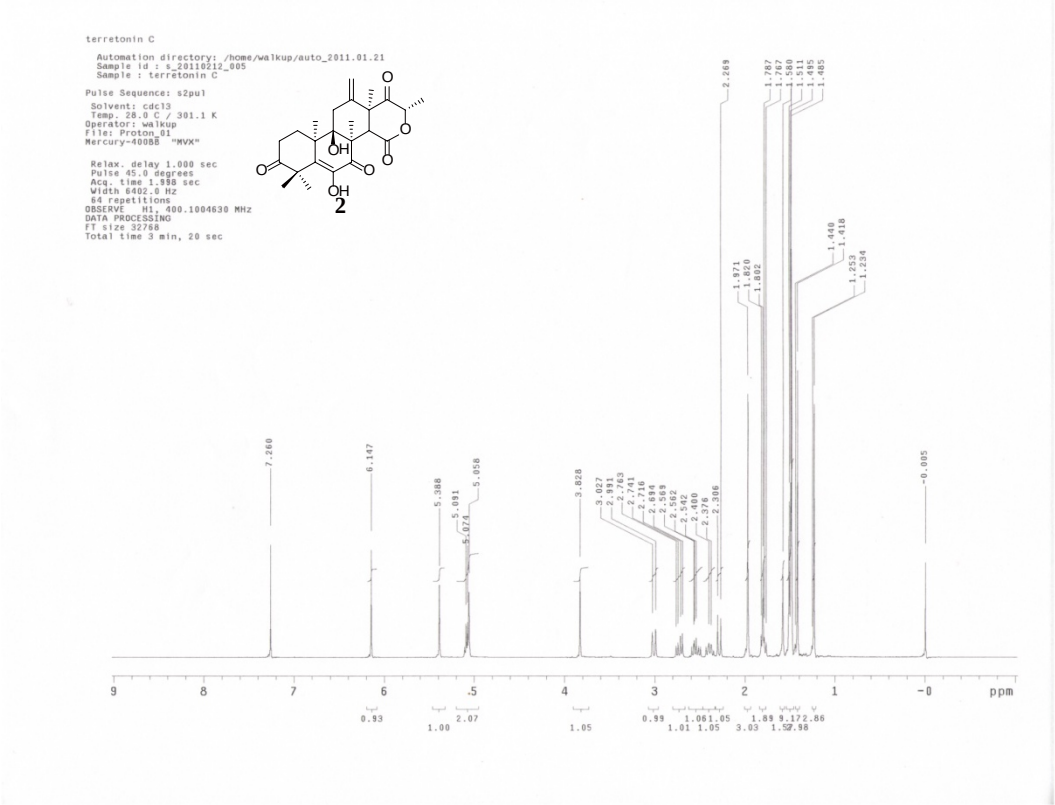


a. ^{1}H NMR spectrum of compound **1**

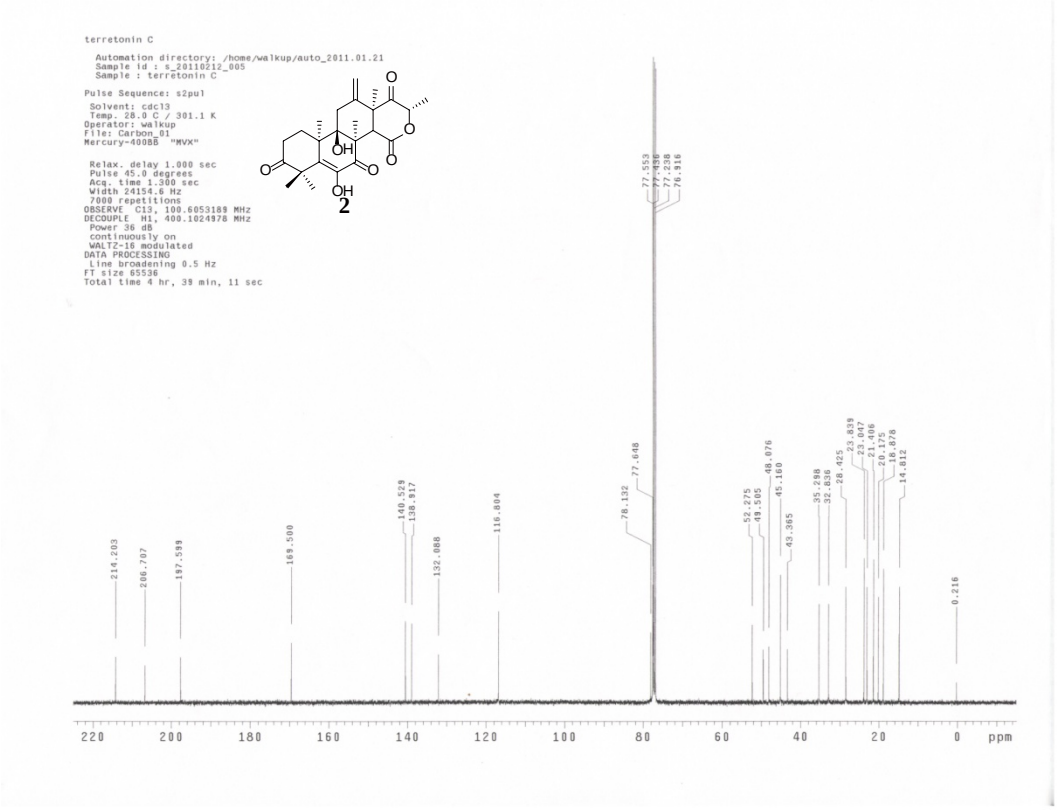


b. ^{13}C NMR spectrum of compound **1**

Figure S15. ^{1}H and ^{13}C NMR spectrum of compound **1**

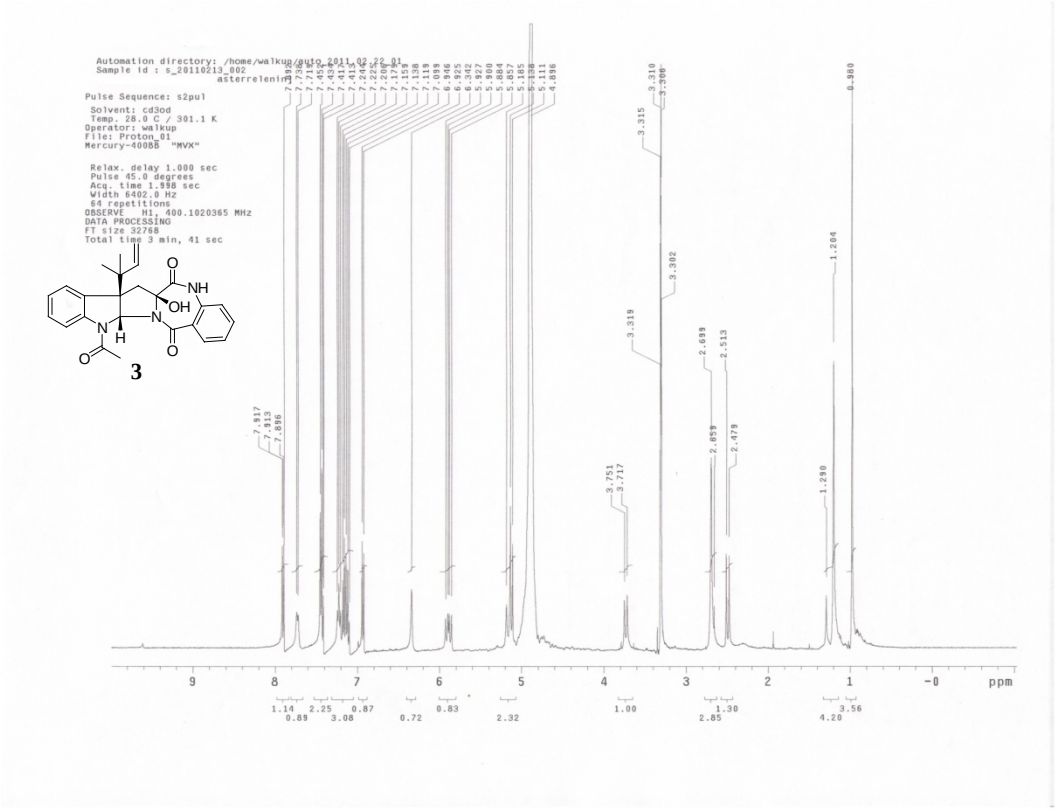


a. ^{1}H NMR spectrum of compound **2**

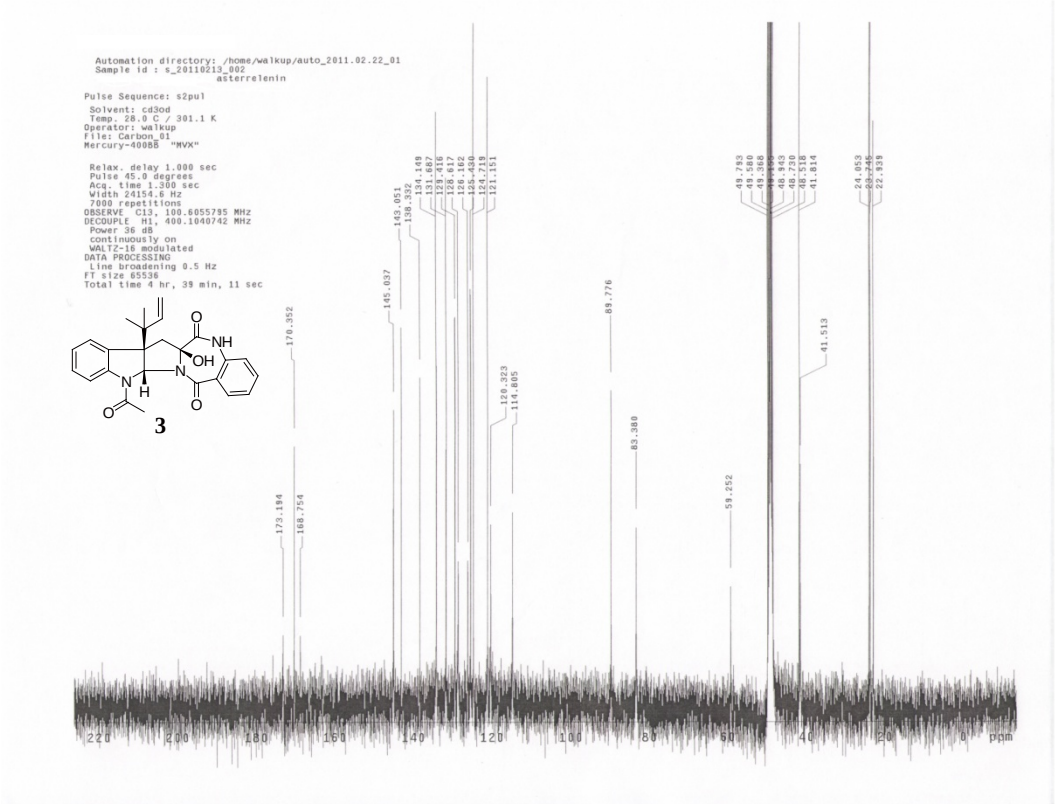


b. ^{13}C NMR spectrum of compound **2**

Figure S16. ^{1}H and ^{13}C NMR spectra of compound **2**

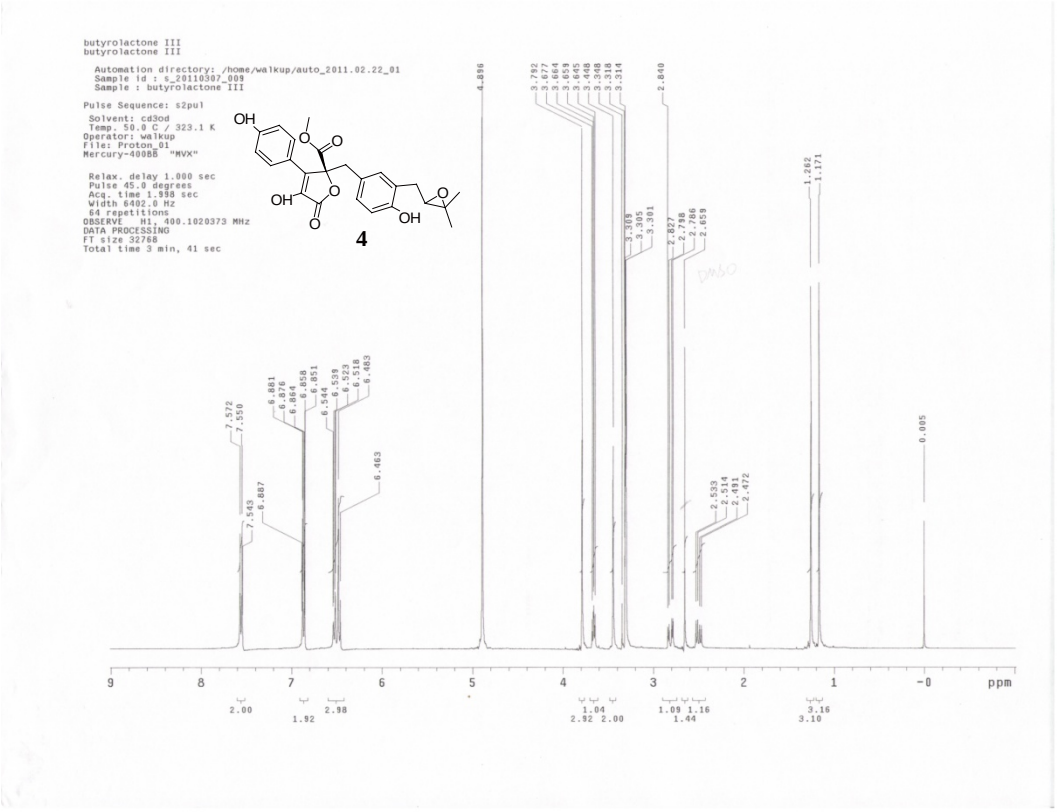


a. ^{1}H NMR spectrum of compound **3**

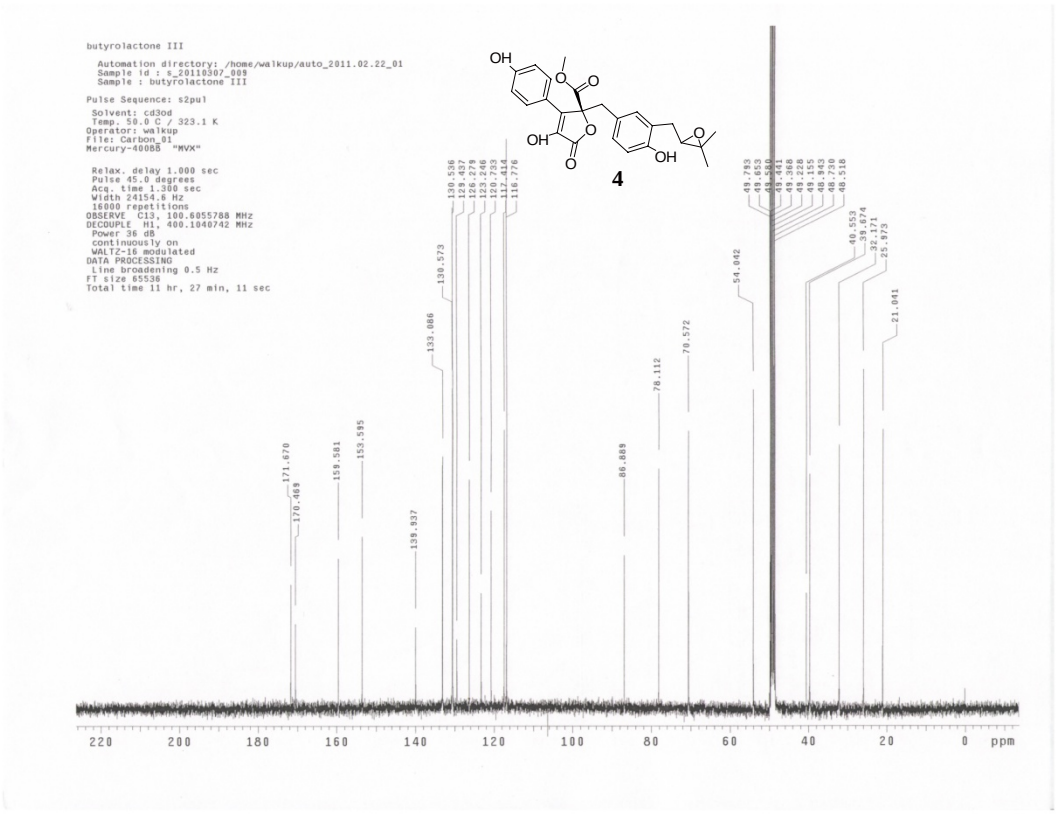


b. ^{13}C NMR spectrum of compound **3**

Figure S17. ^{1}H and ^{13}C NMR spectra of compound **3**

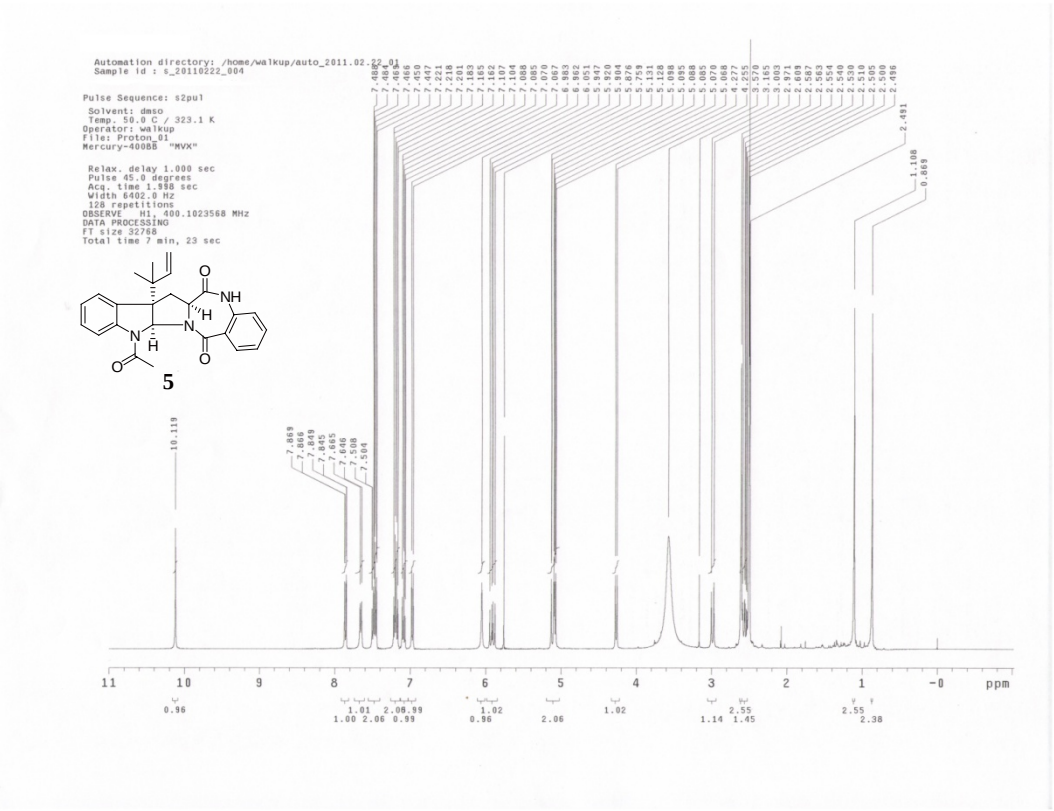


a. ^{1}H NMR spectrum of compound **4**

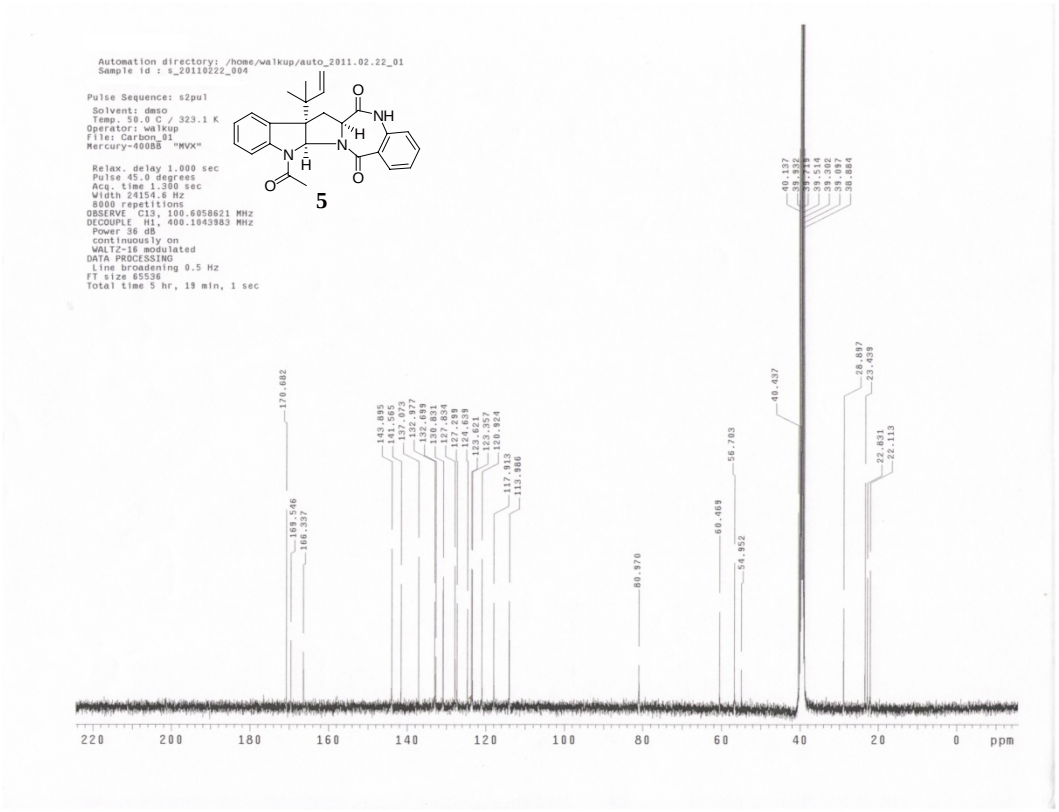


b. ^{13}C NMR spectrum of compound **4**

Figure S18. ^{1}H and ^{13}C NMR spectra of compound **4**

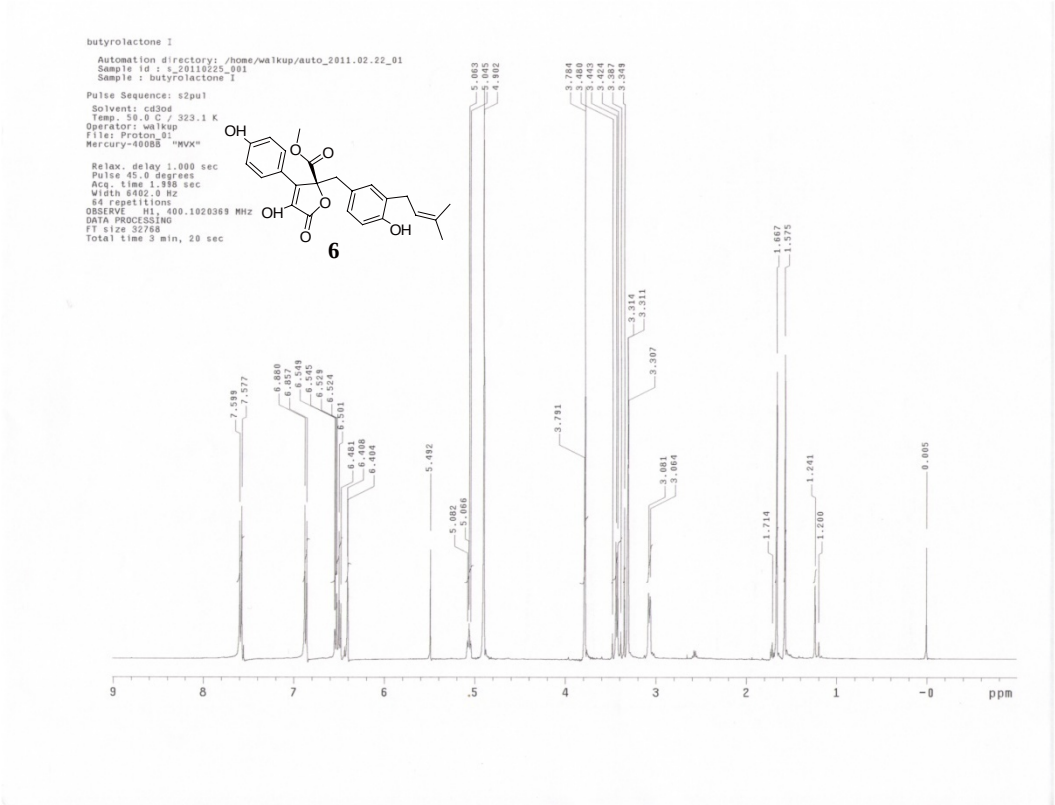


a. ^{1}H NMR spectrum of compound **5**

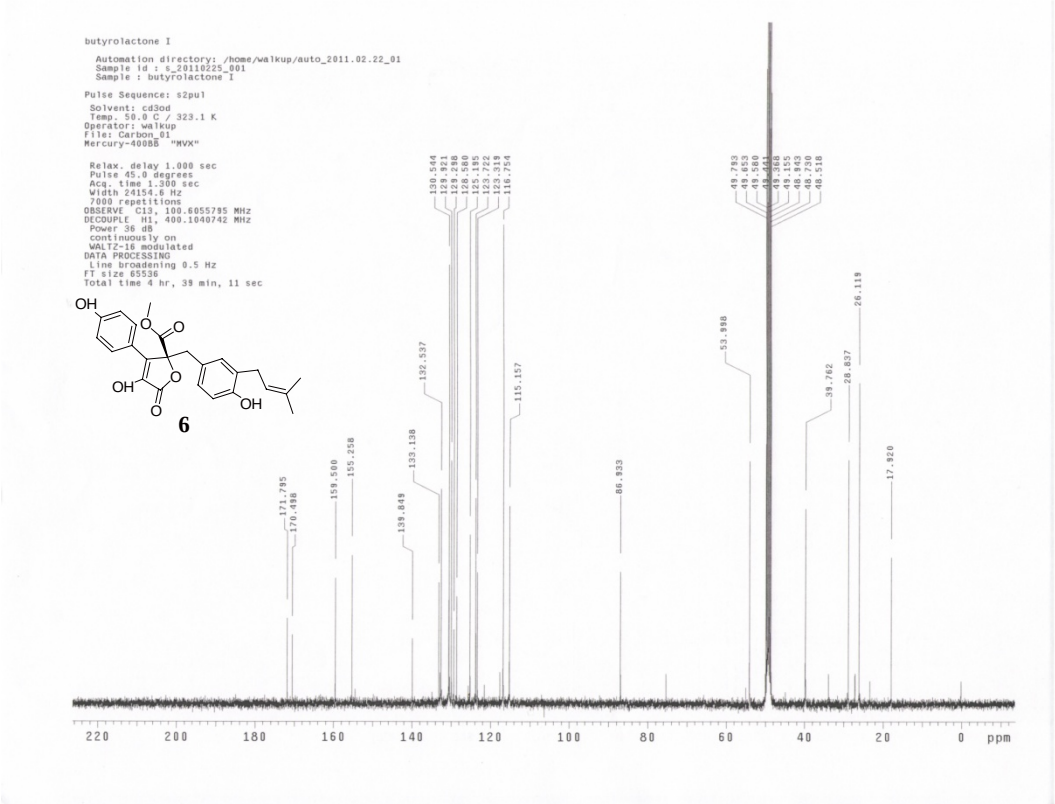


b. ^{13}C NMR spectrum of compound **5**

Figure S19. ^{1}H and ^{13}C NMR spectra of compound **5**



a. ^{1}H NMR spectrum of compound **6**



b. ^{13}C NMR spectrum of compound **6**

Figure S20. ^{1}H and ^{13}C NMR spectra of compound **6**

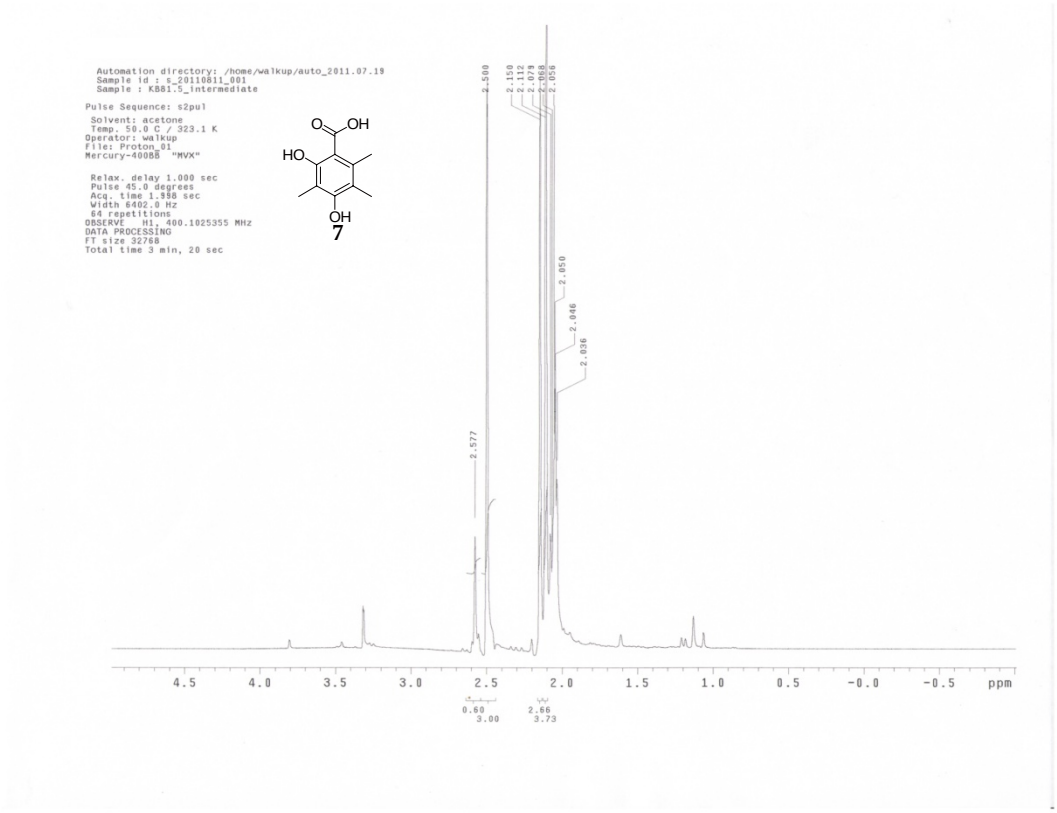


Figure S21. ^{1}H NMR spectrum of compound **7**

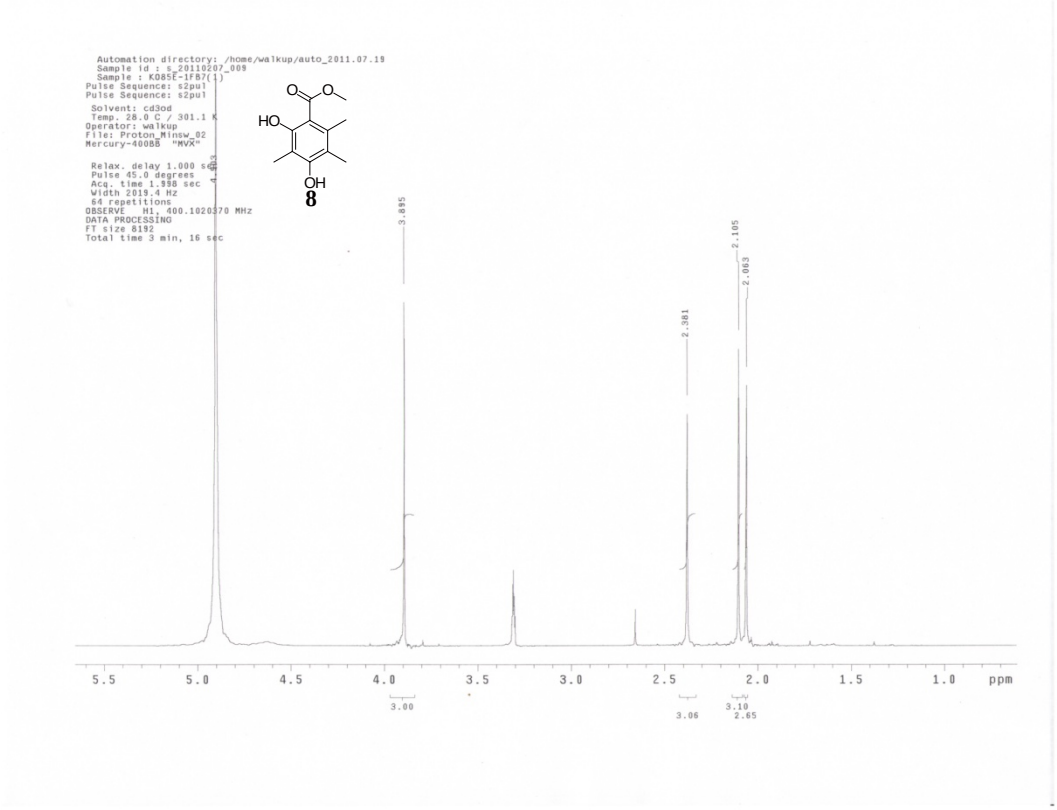
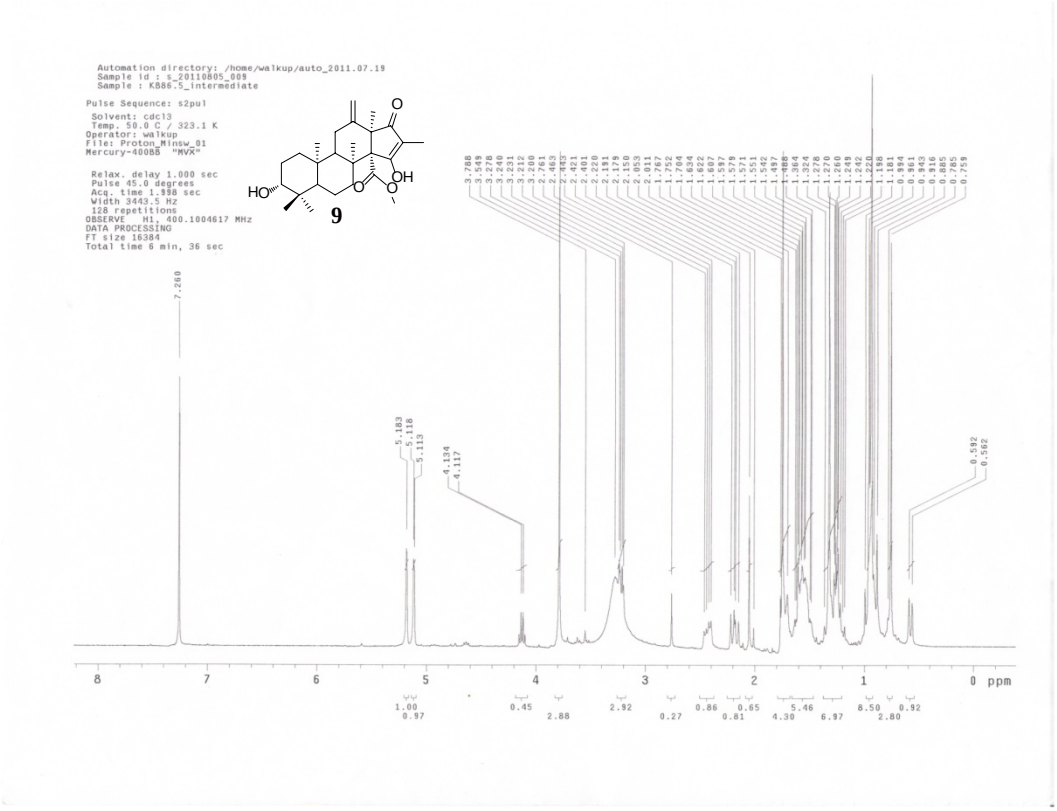
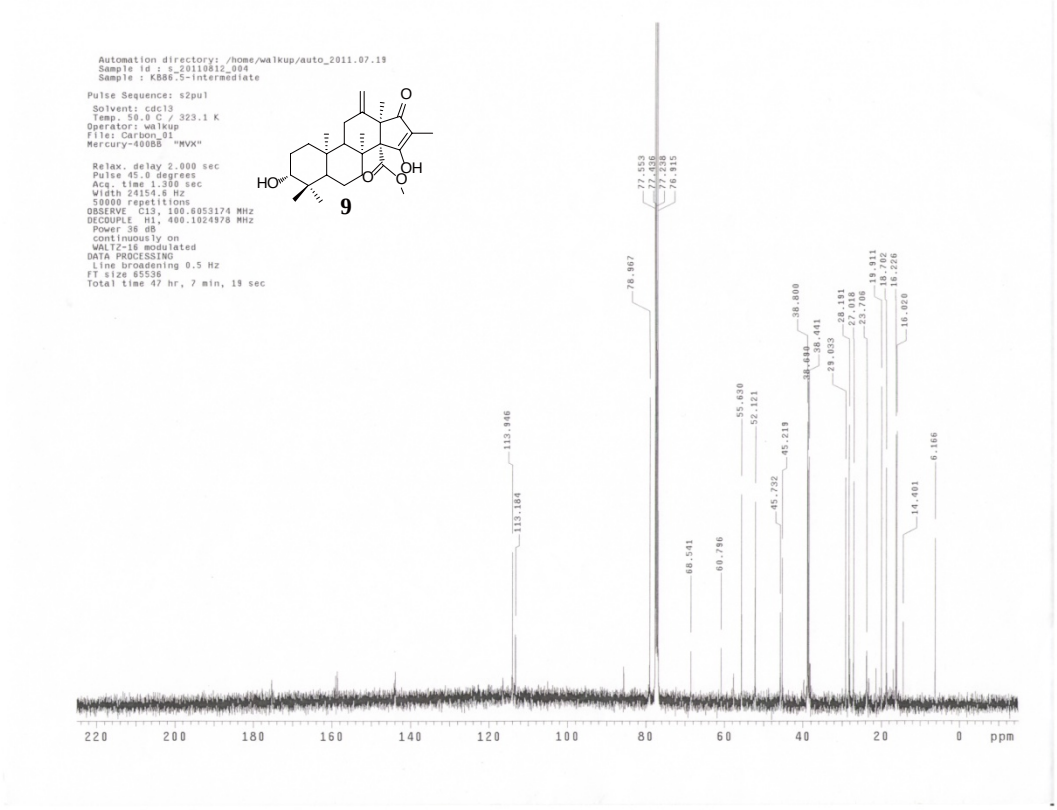


Figure S22. ^{1}H NMR spectrum of compound **8**

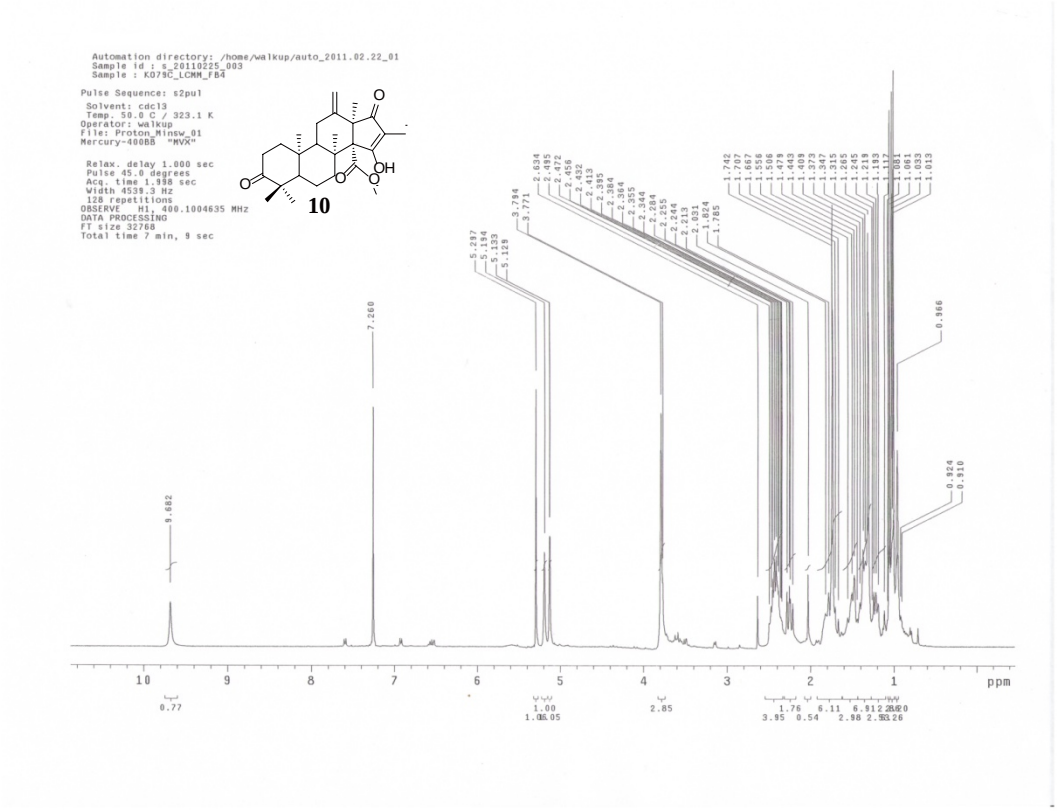


a. ^{1}H NMR spectrum of compound **9**

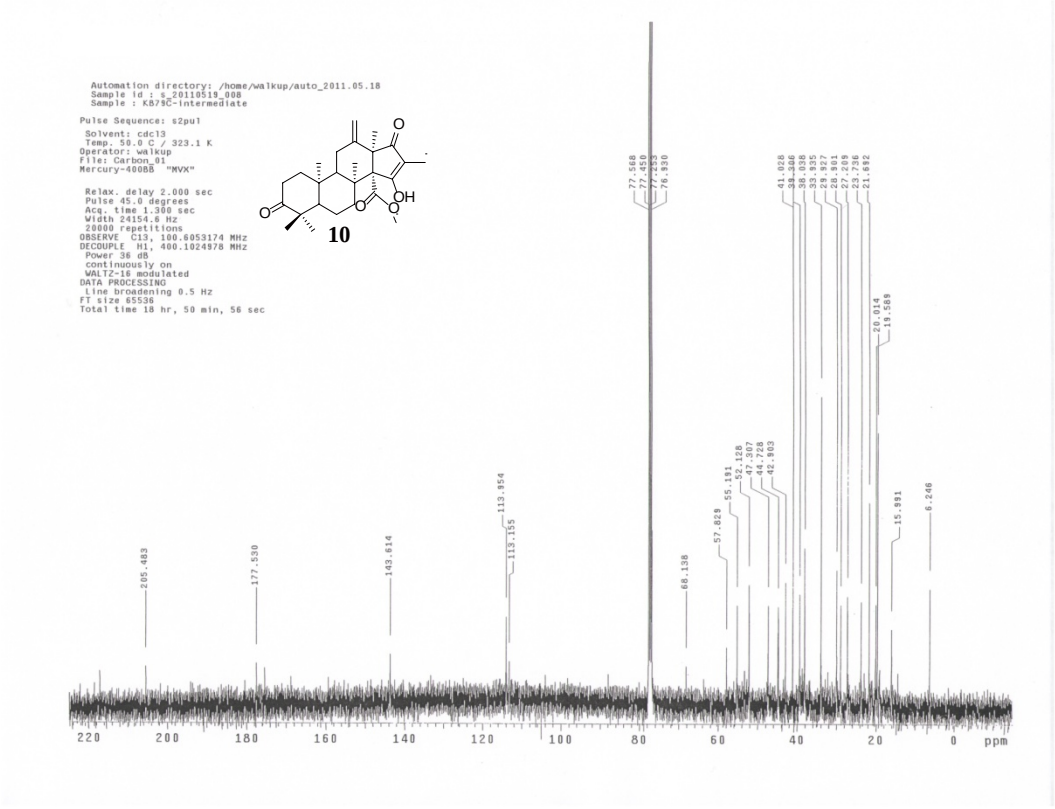


b. ^{13}C NMR spectrum of compound **9**

Figure S23. ^{1}H and ^{13}C NMR spectra of compound **9**



a. ^{1}H NMR spectrum of compound **10**



b. ^{13}C NMR spectrum of compound **10**

Figure S24. ^{1}H and ^{13}C NMR spectra of compound **10**

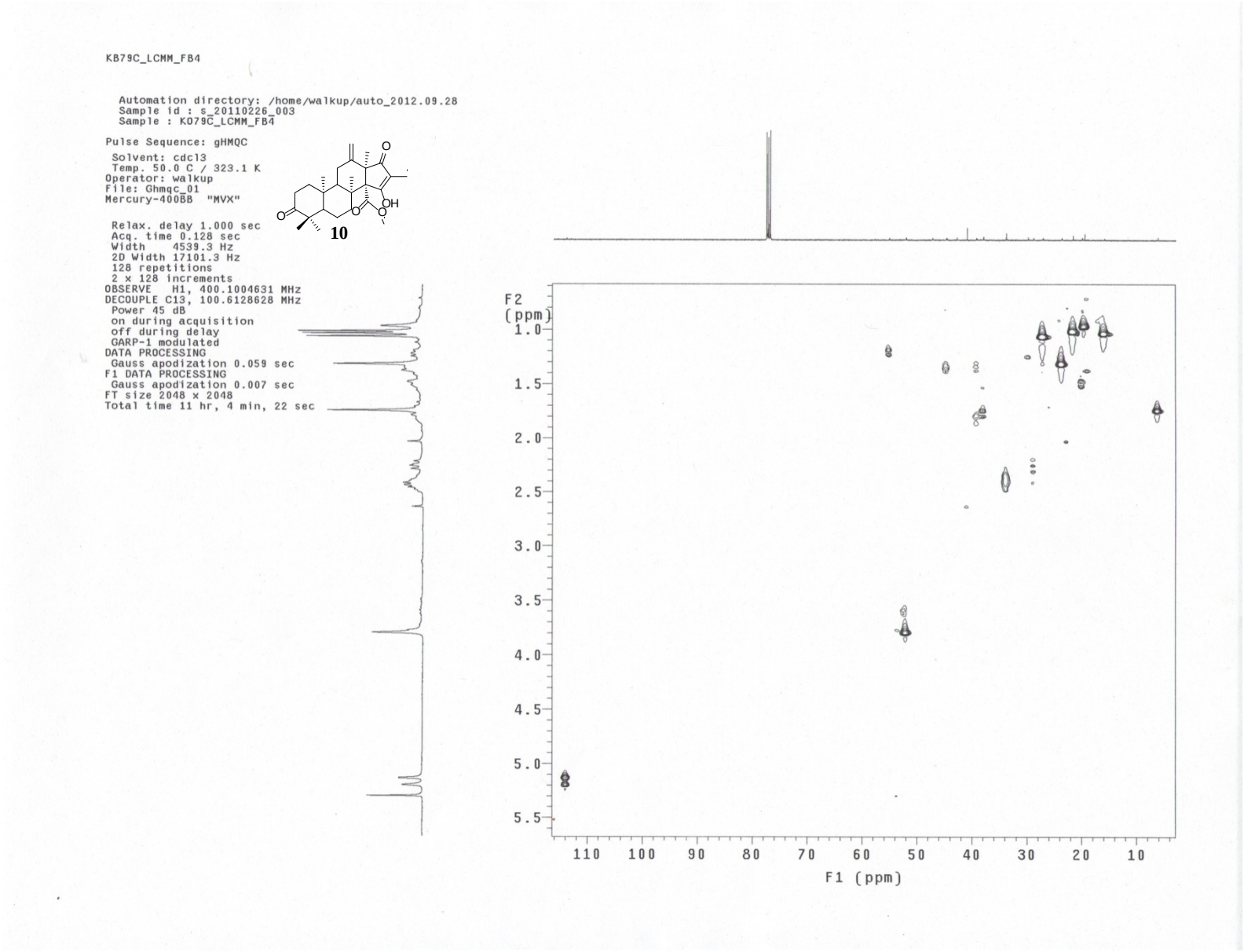


Figure S25. HMQC NMR spectrum of compound **10**

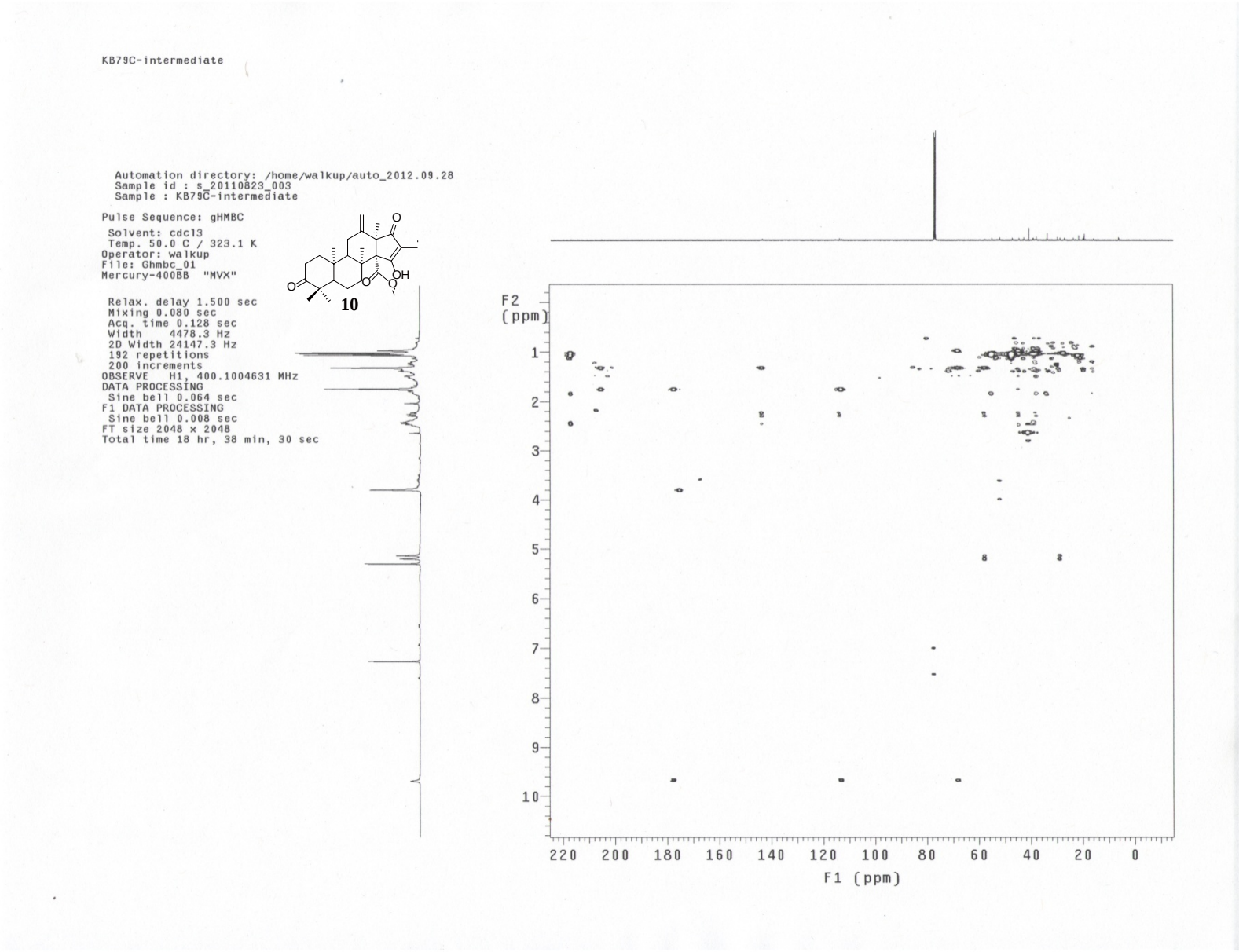


Figure S26. HMBC spectrum of compound **10**

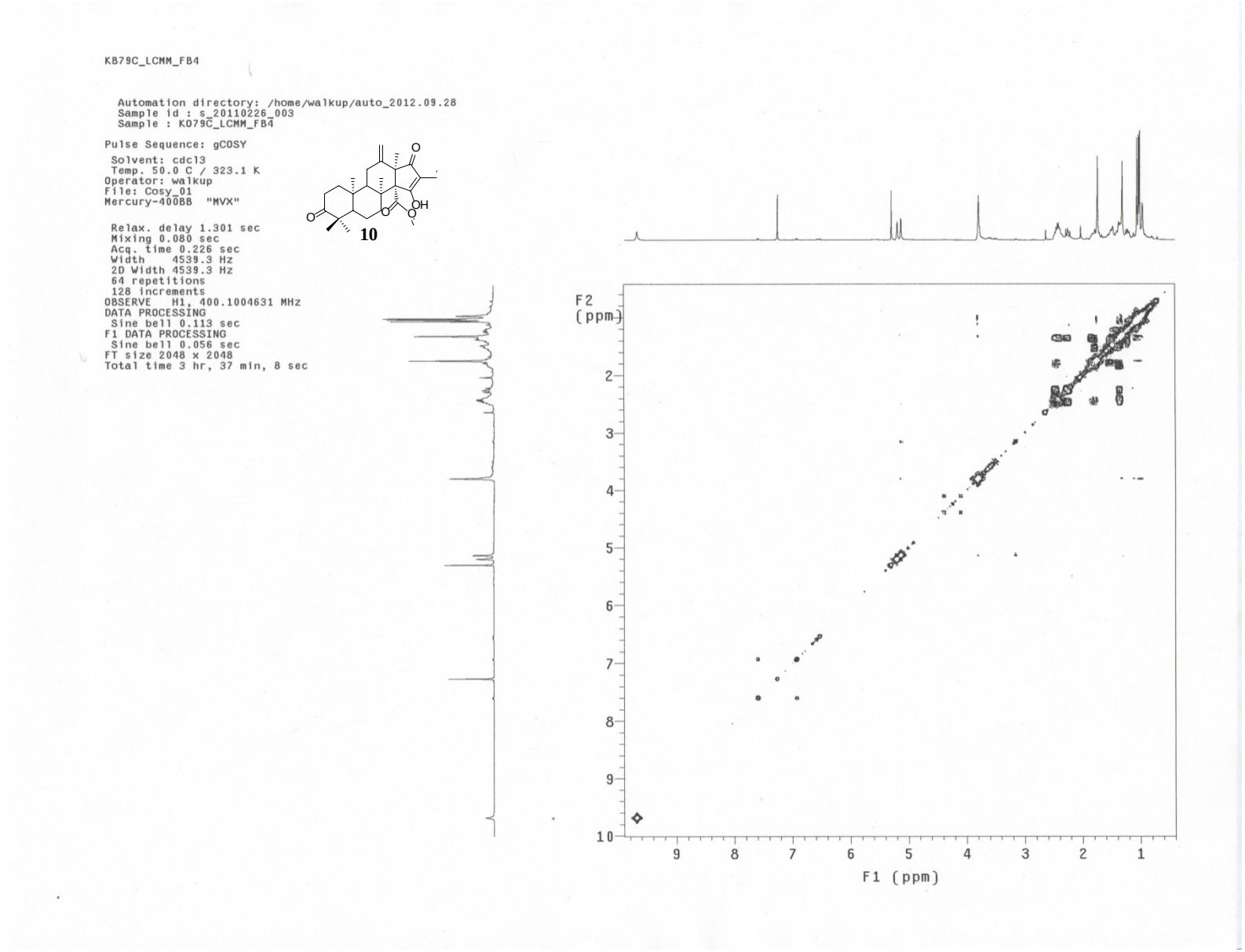


Figure S27. COSY spectrum of compound **10**

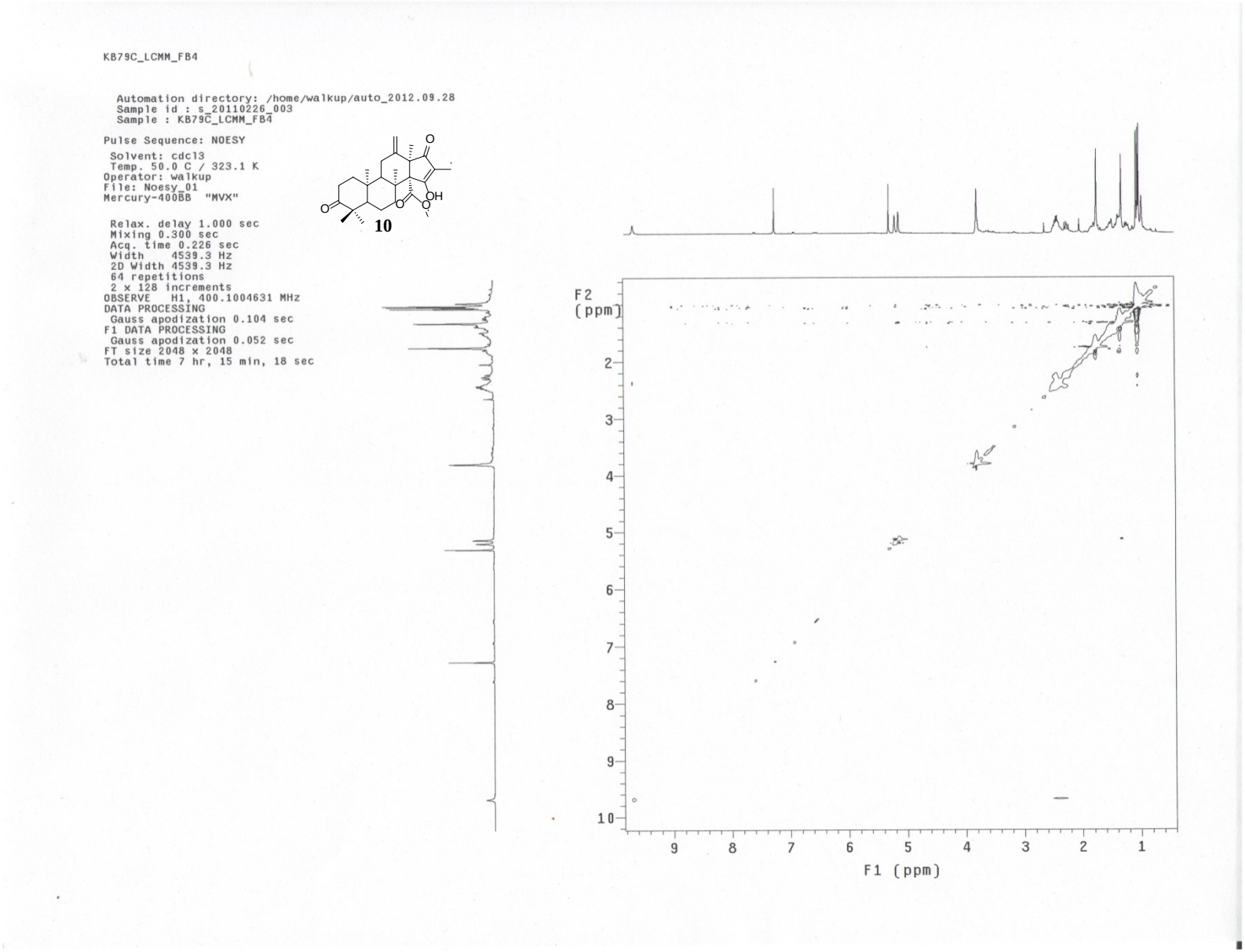
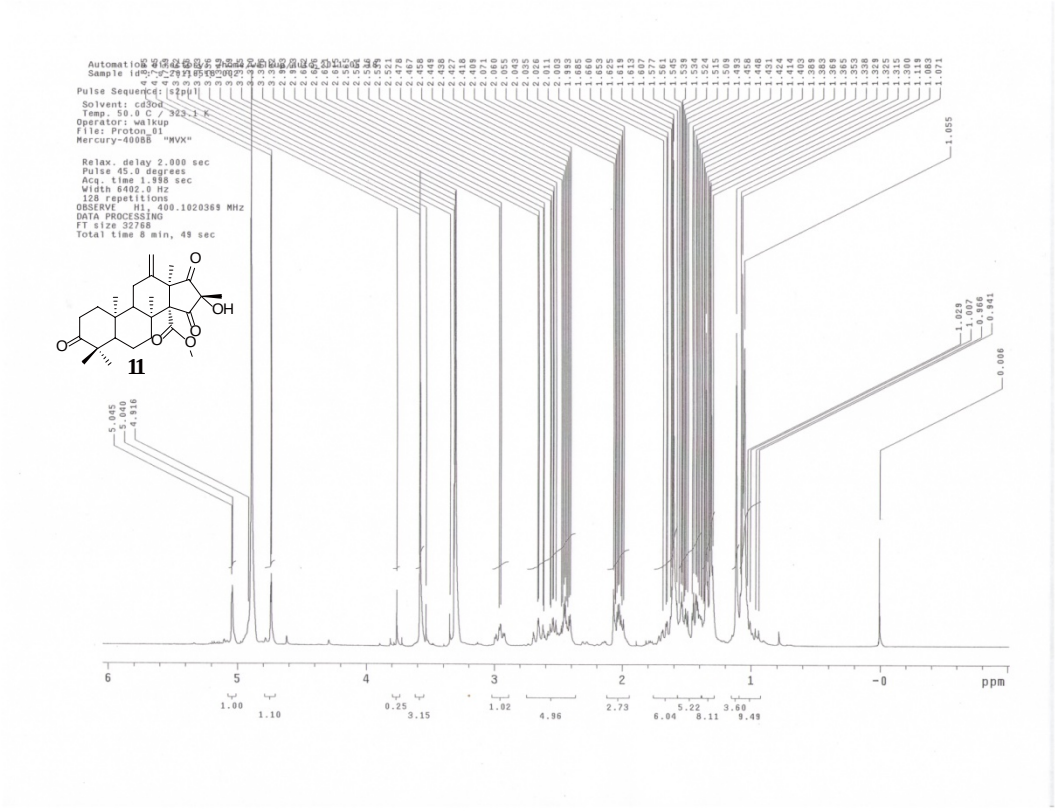
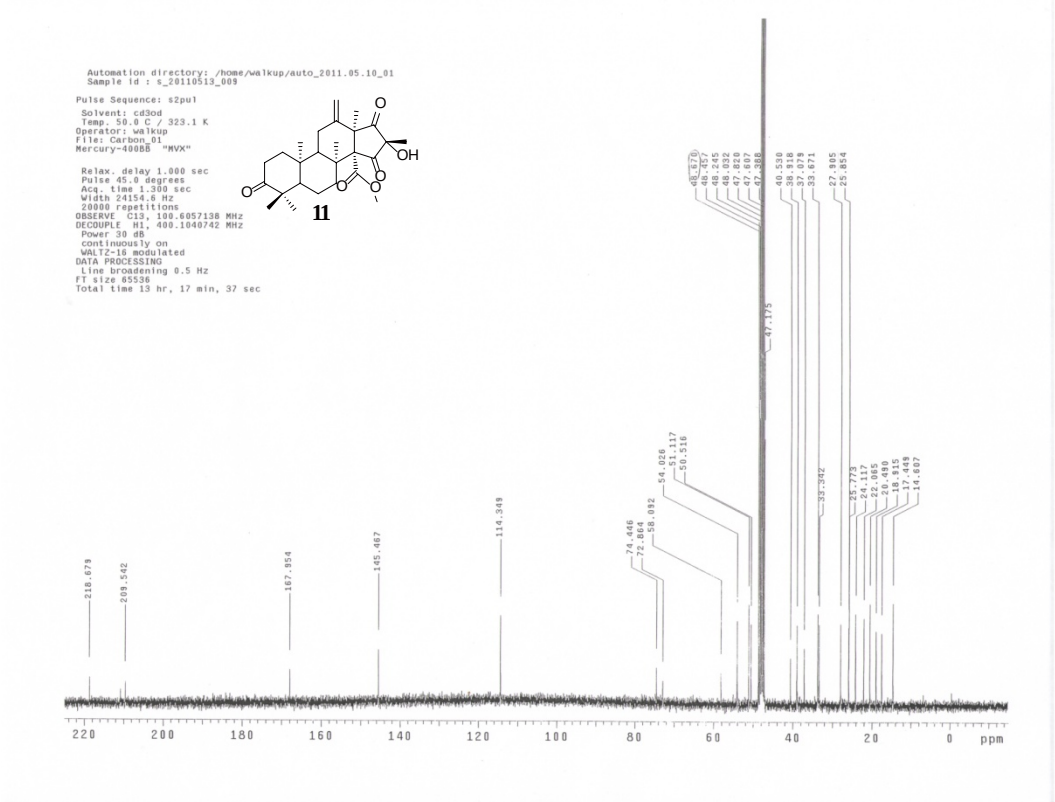


Figure S28. NOESY spectrum of compound **10**



a. ^{1}H NMR spectrum of compound **11**



b. ^{13}C NMR spectrum of compound **11**

Figure S29. ^{1}H and ^{13}C NMR spectra of compound **11**

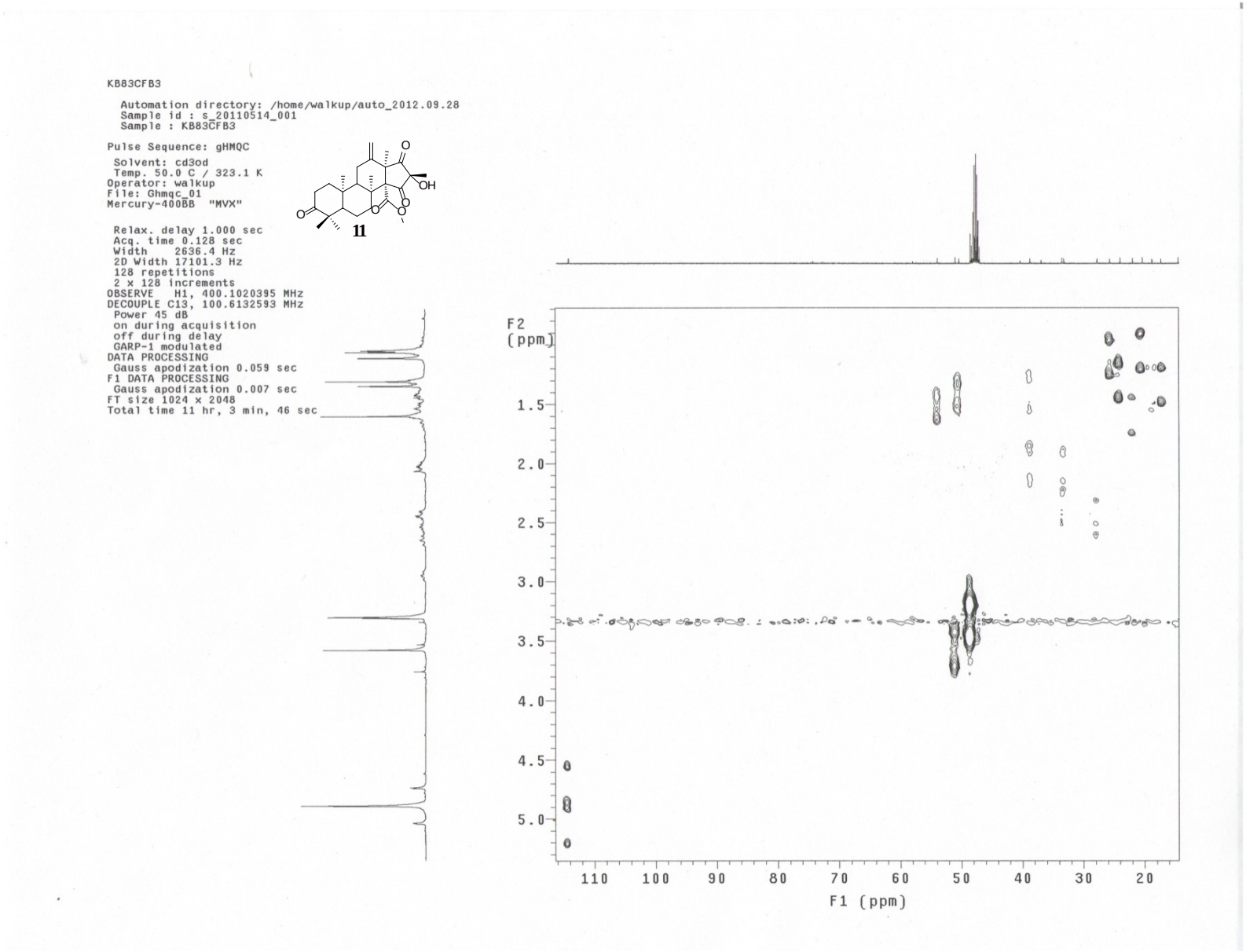


Figure S30. HMQC spectrum of compound **11**

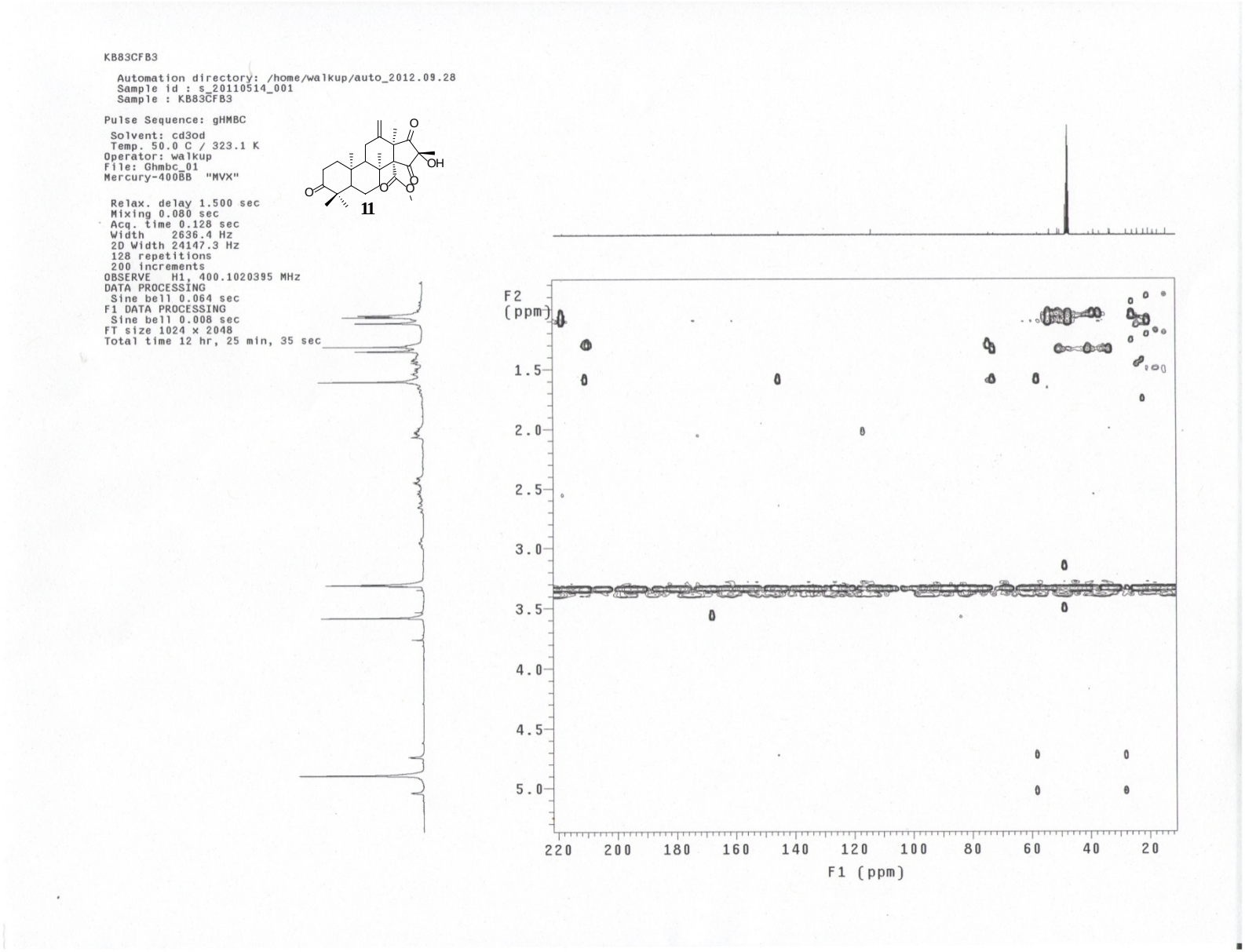


Figure S31. HMBC spectrum of compound **11**

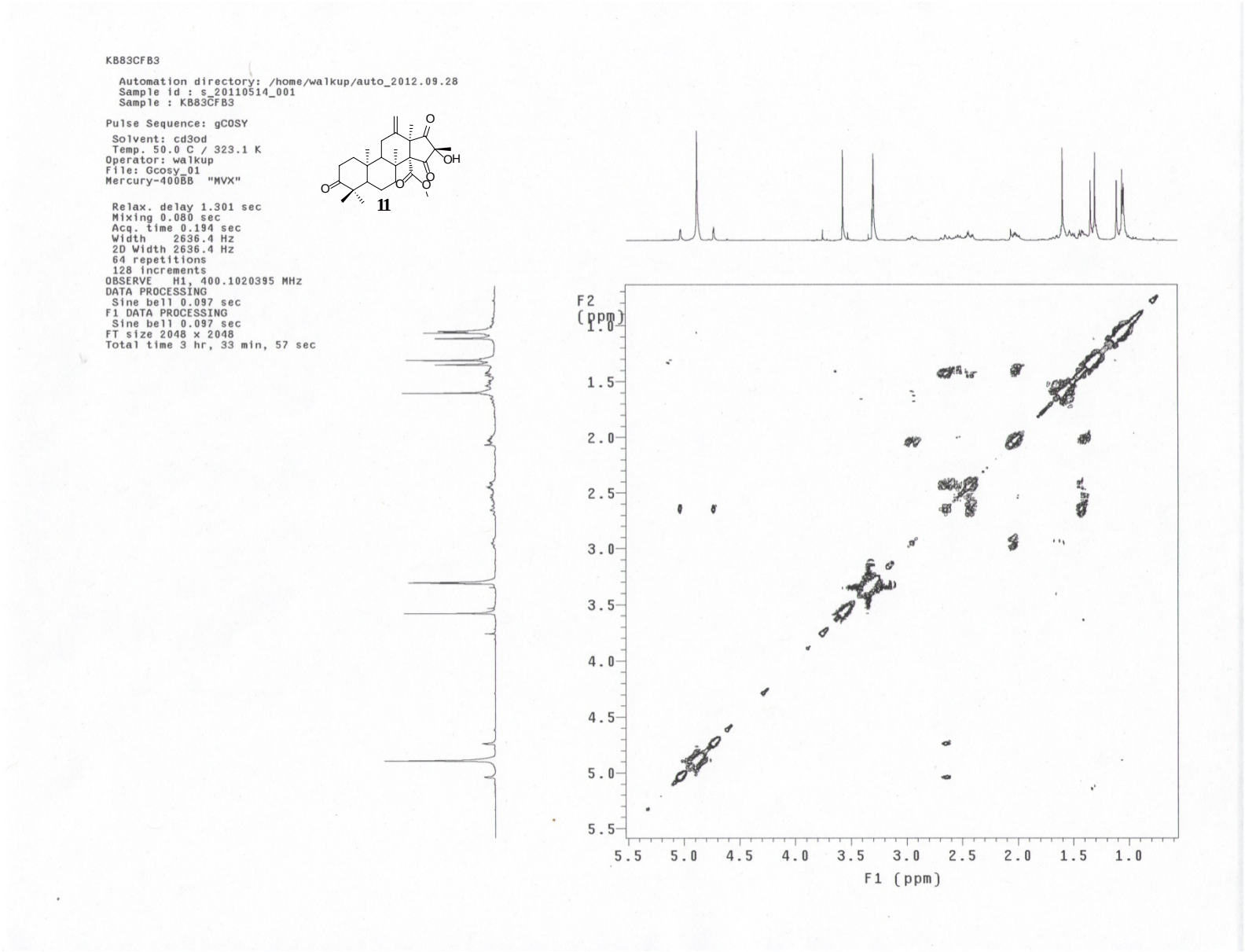


Figure S32. COSY spectrum of compound **11**

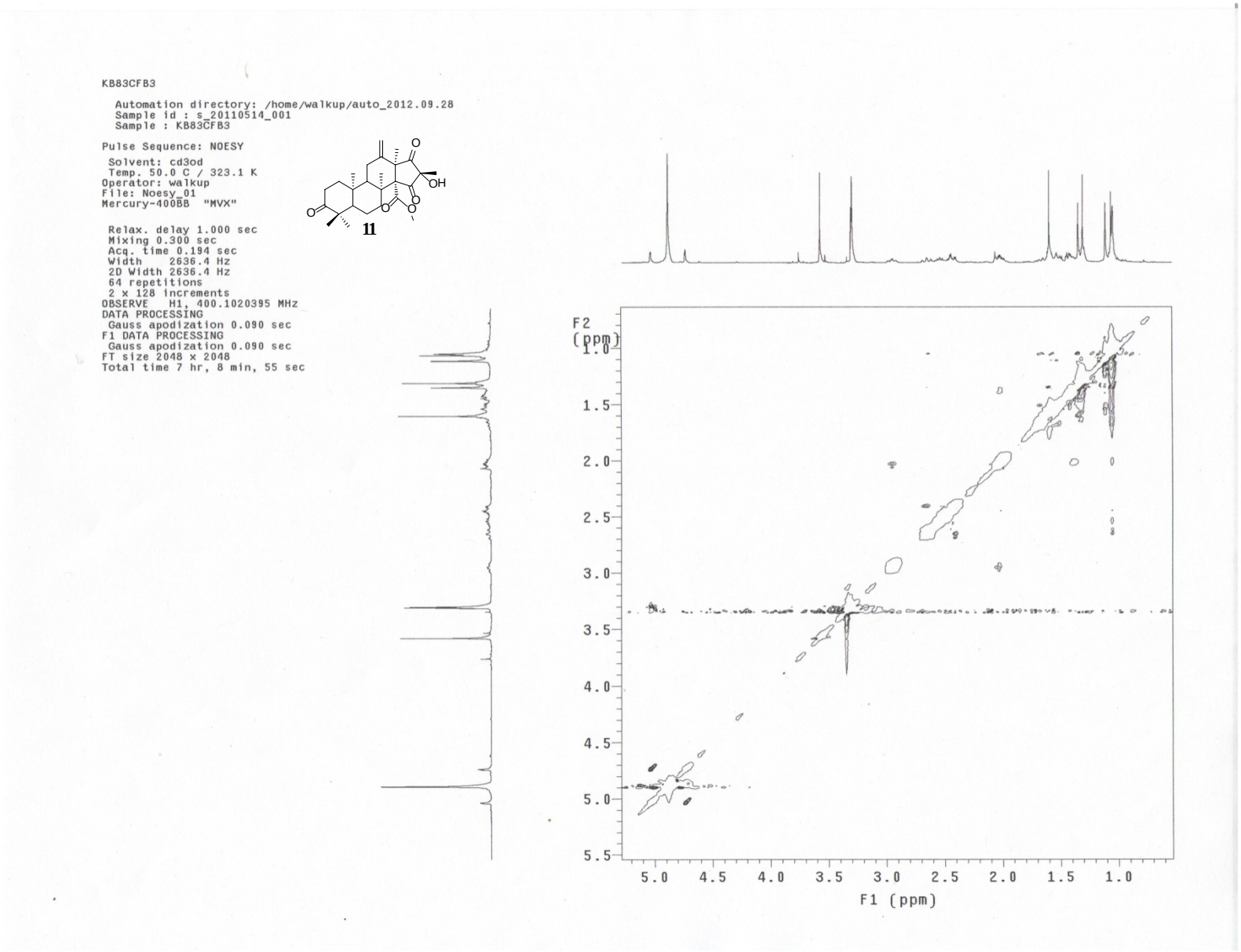


Figure S33. NOESY spectrum of compound **11**