Supporting Information

Formation of terrestric acid in *Penicillium crustosum* requires redox-assisted decarboxylation and stereoisomerization

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Experiment Procedures

1. Computer-assisted sequence analysis

Sequence analysis of terrestric acid gene cluster was carried out by antiSMASH (http://antismash.secondarymetabolites.org/) and by comparison with known entries in database. The genomic DNA sequence of the terrestric acid cluster from *P. crustosum* PRB-2 reported in this study is available at GenBank under the accession number MK360919. Multiple sequence alignments for TraH and analogues were carried out with the program ClustalW and visualized with ESPript 3.2 (http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi) to identify strictly conserved amino acid residues.

2. Strains, media and growth conditions

The fungal strains used in this study are summarized in Table S1. *Penicillium crustosum* strain PRB-2 was isolated from a deep-sea sediment collected in Prydz Bay at a depth of -526 m.[1] The wild type strain PRB-2 and deletion mutants *ΔtraA* and *ΔtraG* were cultivated on PDA plates (potato dextrose broth, Sigma) with 1.6% agar at 25°C for sporulation and in PD surface culture at 25°C for 7 days for detection of secondary metabolites (SMs).

Aspergillus nidulans strains were grown at 37°C on GMM medium (1.0% glucose, 50 mL/L salt solution, 1 mL/L trace element solution, 1.6% agar) for sporulation and transformation with appropriate nutrition as required, and incubated at 25°C in PD medium for 7 days for SM detection.[2-4] The salt solution contains (*w/v*) 12% $NaNO_3$, 1.04% KCl, 1.04% $MgSO_4{\cdot}7H_2O$, and 3.04% KH_2PO_4. The trace element solution comprises (*w/v*) 2.2% $ZnSO_4{\cdot}7H_2O$, 1.1% H_3BO_3, 0.5% $MnCl_2{\cdot}4H_2O$, 0.16% $FeSO_4{\cdot}7H_2O$, 0.16% $CoCl_2{\cdot}5H_2O$, 0.16% $CuSO_4{\cdot}5H_2O$, 0.11% $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, and 5% Na_4EDTA.

Escherichia coli DH5α and BL21(DE3) were grown in liquid or on solid Luria-Bertani (LB) medium (1% NaCl, 1% tryptone, and 0.5% yeast extract) for standard DNA manipulation. 50 μg/mL carbenicillin or 25 μg/mL kanamycin were supplemented for cultivation of recombinant *E. coli* strains.

3. Genomic DNA isolation

The mycelia of *P. crustosum* and *A. nidulans* were collected on sterilized filter paper and then suspended in 400 μL of LETS buffer (10 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 0.5% SDS, and 0.1 M LiCl) in 2 mL Eppendorf tubes and vigorous vortexed with four glass beads (2.85 mm in diameter). 300 μL LETS buffer were added in the solution, which was subsequently treated with 700 μL phenol: chloroform: isoamylol (25:24:1). The genomic DNA (gDNA) was precipitated by addition of 900 μL absolute ethanol and centrifugation at 17,000 x *g* for 30 min. After washing with 70% ethanol and drying, the obtained DNA was dissolved in 50 μL distillated H_2O.

4. RNA isolation and cDNA synthesis

For isolation of RNA from *P. crustosum* PRB-2, the fungus was cultivated in liquid PD medium shaking at 230 rpm for 7 days and the cells were collected by centrifugation. RNA extraction was performed by using Fungal RNA Mini kit (VWR OMEGA bio-tek E.Z.N.A) according to the standard manufacturer´s instruction. The ProtoScript II First Strand cDNA Synthesis kit (BioLabs) was used for cDNA synthesis with Oligo-dT primers.

5. PCR amplification, gene cloning and plasmid construction

Plasmids used in this study are listed in Table S2. The oligonucleotide sequences for PCR amplification are given in Table S3. Genetic manipulation in *E. coli* was carried out according to the protocol by Sambrook and Russell.[5] All primers were synthesized by Seqlab GmbH (Göttingen, Germany). PCR amplification was carried out by using Phusion® High-Fidelity DNA polymerase from New England Biolabs (NEB) on a T100TM Thermal cycler from Bio-Rad. PCR reaction mixtures and thermal profiles were set as recommended by the manufacturer´s instruction.

To construct pJF80 and pJF81 for *traG* deletion, primers were designed with split-marker strategy by using p5HY and p3YG vectors (Figure S4).[6,7] To construct the plasmids for heterologous expression of *traA* and *traAG* in *A. nidulans*, an assembly approach based on the homologous recombination in *E. coli* was used (Figure S2).[8] Full length of *traA* including its terminator of 497 bp was amplified from gDNA of *P. crustosum* PRB-2 as the template by PCR with primer pairs A.n-traA-1F-For/1F-Rev and A.n-traA-2F-For/2F-Rev (Table S3) and inserted into the corresponding sites of pYH-wA-pyrG with homologous flanking sequences of the *wA* gene to create pJF27.[4] For co-expression of *traA* and *traG*, *traA* including *gpdA* promoter and its terminator of 497 bp from pJF27, and *traG* with its 778 bp promoter and 568 bp terminator from *P. crustosum* PRB-2 were cloned into pYWB2 by homologous recombination with flanking sequences of the *wA* gene to create pJF91.[9] Herein, primers A.n-traAG-1F-For/traA-1F-Rev, A.n-traA-2F-For/traAG-1F-Rev and A.n-traAG-2F-For/2F-Rev (Table S3) were used for PCR amplification.

To construct the plasmid for expressing *traD* and *traH* in *E. coli*, the coding region of *traD* and *traH* were amplified by PCR from cDNA with the primer pairs TraD-28-For/Rev and TraH-28-For/Rev (Table S3). The expression vector pET-28a (+) was digested with BamHI and EcoRI, and ligated with DNA fragments by homologous recombination yielding the expression plasmid pJF72 for TraD and pJF74 for TraH, which were confirmed by sequencing (Seqlab GmbH).

6. Deletion of *traG* in *P. crustosum* and cultivation of deletion mutants.

Fresh conidia from 7-day PDA culture of *P. crustosum* PRB-2 were inoculated into 30 mL LMM medium (1.0% glucose, 50 mL/L salt solution, 1 mL/L trace element solution, and 0.5% yeast extract) in 100 mL flask and incubated at 25°C and 230 rpm for germination. Mycelia were harvested after 11 h by centrifugation at 2,800 x *g* for 10 min, and washed with distillated H_2O. The mycelia were then transferred into a 50 mL flask with 10 mL of osmotic buffer (1.2 M $MgSO_4$ in 10 mM sodium phosphate, pH 5.8) containing 50 mg lysing enzyme from *Trichoderma harzianum* (Sigma) and 20 mg yatalase from *Corynebacterium sp.* OZ-21 (OZEKI Co., Ltd.). After shaking at 30°C and 100 rpm for 2.5 h, the cells were transferred into a 50 mL falcon tube and overlaid gently with 10 mL of trapping buffer (0.6 M sorbitol in 0.1 M Tris-HCl, pH 7.0). After centrifugation at 4°C and 2,800 x *g* for 10 min, the protoplasts were collected from the interface of the two buffer systems. The protoplasts were then transferred to a sterile 15 mL falcon tube and resuspended in 200 μL of STC buffer (1.2 M sorbitol, 10 mM $CaCl_2$, and 10 mM Tris-HCl, pH 7.5) for transformation.

The *via* PCR constructed gene deletion cassettes mentioned above were transformed into *P. crustosum* by polyethylene glycol (PEG) mediated protoplast transformation. The DNA fragments (2 μg) were incubated with 100 μL of the protoplasts for 50 min on ice. 1.25 mL of PEG solution (60% PEG 4000, 50 mM $CaCl_2$, 50 mM Tris-HCl, pH 7.5) was then added and gently mixed. After incubation at room temperature for 30 min, the mixture was transferred in 5 mL STC buffer and spread on plates with SMM bottom medium (1.0% glucose, 50 mL/L salt solution, 1 mL/L trace element solution, 1.2 M sorbitol, and 1.6% agar) containing 200 μg/mL hygromycin B. SMM top medium (1.0% glucose, 50 mL/L salt solution, 1 mL/L trace element solution, 1.2 M sorbitol, and 0.8% agar) containing 100 μg/mL hygromycin B was overlaid softly on the plates. Three days later, the transformants were transferred onto fresh PDA plates containing 200 μg/mL hygromycin B for second round selection. The obtained transformants were inoculated in PD medium for isolation of genomic DNA to verify the integrity, which was carried out by PCR amplification (Figure S4). The obtained Δ*traG* mutant was cultivated in PD liquid medium at 25 °C for 7 days, together with Δ*traA* mutant in a previous study.[6] For SM detection of the deletion mutants, cultures were extracted with ethyl acetate, dissolved in a mixture of MeOH and H_2O (8 : 2) and analyzed on LC-MS by method B (see below for methods of HPLC and LC-MS analysis).

7. Heterologous expression of *traA* and *traG* in *A. nidulans*

A. nidulans strain LO8030 was used as the recipient host.[3] Fungal protoplast preparation and transformation were performed according to the method described previously.[3] pJF27 containing the PKS-NRPS gene *traA* was transformed into LO8030 to create the *traA* expression strain JF15. pJF91 containing both *traA* and *traG* was transformed into LO8030 to create the *traAG* expression strain JF45. Potential transformants were verified by PCR using the primers traA-F/R or traG-F/R (Table S3). Differing from *P. crustosum*, germination condition was at 37°C with appropriate nutrition as supplements (0.75 μM riboflavin and 0.5 μM pyridoxine for JF15, 5% uracil, 6% uridine and 0.5 μM pyridoxine for JF45) for 6 h. Protoplastation condition was at 37°C for 2.5 h. *A. nidulans* strains were cultivated in PD liquid medium at 25°C for 7 days for LC-MS analysis (method B) of the SM production.

8. Precursor feeding in *P. crustosum* deletion mutants

For feeding experiments, the precursors (5*S*)-carboxylcrustic acid (**4**) and (5*S*)-viridicatic acid (**5**) were dissolved in DMSO to give 1 M stock solution, and added to 10 mL of PD cultures of respective deletion mutants, **4** to Δ*traA* mutant, and **5** to Δ*traA* and Δ*traG*, leading to final concentrations of 0.4 mM. After further cultivation at 25°C for 7 d in PD medium, the secondary metabolites were extracted with ethyl acetate, dissolved in a mixture of MeOH and H_2O (8 : 2) and analyzed on LC-MS by method B (see below for methods of HPLC and LC-MS analysis).

9. Overproduction and purification of TraD and TraH

The expression plasmids pJF72 and pJF74 were constructed for TraD and TraH expression in *E. coli* as mentioned above. The recombinant *E. coli* BL21(DE3) strains were cultivated in Terrific Broth (TB) medium (2.4% yeast extract, 2.0% tryptone, 0.4% glycerol, 0.1 M phosphate buffer, pH 7.4). TraH expression was induced with 0.5 mM IPTG at 20°C for 16 h and TraD at 16°C for 16 h. The recombinant His_6-tagged protein were purified on Ni-NTA affinity chromatography

(Qiagen, Hilden) using the published procedures.[10,11] The purity for TraH and TraD were confirmed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Figure S12). The protein concentration was determined on Nanodrop 2000c spectrophotometer (Thermo Scientific, Braunschweig, Germany). 4.5 mg/L of protein can be obtained for TraH, 10.6 mg/L of protein can be obtained for TraD from the bacterial culture.

10. *In vitro* assays of TraD and TraH

To determine the enzyme activity of TraH toward (5*S*, 5'*S*)-crustosic acid (**1**) or (5*S*, 5'*S*)-crustosic acid methyl ester (**7**), the enzyme assays (50 μL) contained phosphate buffer (20 mM, pH 7.4), ascorbic acid (1 mM), (5*S*, 5'*S*)-crustosic acid (**1**, 0.5 mM) or (5*S*, 5'*S*)-crustosic acid methyl ester (**7**, 0.5 mM), DTT (1 mM), $Fe[(NH_4)_2(SO_4)_2]$ (1 mM), 2-oxoglutarate (1 mM), glycerol (0.5 − 5%), DMSO (5%), and the purified recombinant TraH (5.4 μM). The enzyme assays were carried out at 37 °C for 30 min and terminated with one volume of acetonitrile. The reaction mixtures were centrifuged at 17,000 x *g f*or 30 min before further analysis on HPLC and LC-MS by method A (see below for methods of HPLC and LC-MS analysis).

To determine the enzyme activity of TraD toward dehydroterrestric acid (**3**), the enzyme assays (50 μL) contained phosphate buffer (20 mM, pH 7.4), NAD(P)H (2 mM), glycerol (0.5 − 5%), DMSO (5%), dehydroterrestric acid (**3**, 0.5 mM), and the purified recombinant TraD (0.6 μM). The enzyme assays were incubated at 30 °C for 10 min and terminated with one volume of acetonitrile. The reaction mixtures were centrifuged at 17,000 x *g* for 30 min before further analysis on HPLC and LC-MS by method A. The same reaction condition was used for the enzyme assay of TraD with (5'*S*)-dehydrocrustosic acid methyl ester (**8**).

To prove the conversion of (5*S*, 5'*S*)-crustosic acid (**1**) by TraH and TraD, the enzyme assays (50 μL) contained phosphate buffer (20 mM, pH 7.4), ascorbic acid (1 mM), (5*S*, 5'*S*)-crustosic acid (**1**, 0.5 mM), $Fe[(NH_4)_2(SO_4)_2]$ (1 mM), 2-oxoglutarate (1 mM), NADPH (2 mM), glycerol (0.5 − 5%), DMSO (5%), and the purified recombinant TraH (5.4 μM) and TraD (0.6 μM). The enzyme assays were incubated at 30 °C for 30 min and terminated with one volume of acetonitrile. The reaction mixtures were centrifuged at 17,000 x *g* for 30 min before further analysis on HPLC and LC-MS by method A.

To prove the conversion of (5*S*)-carboxylcrustic acid (**4**) to (5*R*)-crustic acid (**6**) by TraH and TraD, the enzyme assays (50 μL) containing phosphate buffer (20 mM, pH 7.4), ascorbic acid (1 mM), (5*S*)-carboxylcrustic acid (**4**, 0.5 mM), DTT (1 mM), $Fe[(NH_4)_2(SO_4)_2]$ (1 mM), 2-oxoglutarate (1 mM), NAD(P)H (2 mM), glycerol (0.5 − 5%), DMSO (5%), and the purified recombinant TraH (12 μM) and TraD (6 μM) were incubated at 30°C for 16 h and terminated with one volume of acetonitrile. The reaction mixtures were centrifuged at 17,000 x *g* for 30 min before further analysis on HPLC and LC-MS by method A. In addition, (5*S*)-carboxylcrustic acid (**4**, 0.5 mM) was incubated with TraH (12 μM) in the presence of ascorbic acid (1 mM), DTT (1 mM), $Fe[(NH_4)_2(SO_4)_2]$ (1 mM), 2-oxoglutarate (1 mM) at 37°C for 16 h. After terminated with acetonitrile and centrifugation, the enzyme assay was further analyzed on HPLC and LC-MS by method A.

11. Large-scale fermentation, extraction and isolation of secondary metabolites

To isolate **4** from *A. nidulans* carrying *traA*, the transformant JF15 was cultivated in 60 x 250 mL flasks each containing 50 mL PD liquid medium with appropriate nutrition as supplement at 25°C for 7 days. The supernatant and mycelia were separated, and extracted with ethyl acetate and acetone, separately. The acetone extract was concentrated under reduced pressure to afford an aqueous solution, and then extracted with ethyl acetate. The two ethyl acetate extracts were combined and evaporated under reduced pressure to give a crude extract (0.8 g). The crude extract was applied to Sephadex LH-20 column eluted with methanol, yielding twenty fractions (1 – 20). Fraction 8 was purified on a semi-preparative HPLC (acetonitrile / H_2O (40 : 60) with 0.1% trifluoroacetic acid) yielding compound **4** (8 mg).

To isolate **5** from *A. nidulans* carrying *traA* and *traG*, the transformant JF45 was cultivated in 10 x 1 L flasks each containing 100 g rice and 150 mL H_2O (with appropriate nutrition as supplement) at 25°C for 7 days. After extracting with 15 L ethyl acetate and concentrated under reduced pressure, the crude extract (2.5 g) obtained from JF45 cultivation was applied to silica gel column chromatography by using petroleum ether / EtOAc (1 : 1, 1 : 3, and 1 : 5, *v/v*) as elution solvents, giving fractions 1 − 10. **5** (15 mg) was obtained from fraction 3 after purification on Sephadex LH-20 column using MeOH as eluent.

To isolate **5** from *P. crustosum* PRB-2, spores were inoculated in 4 L PD liquid medium and cultivated at 25°C for 14 days. 1.0 g crude extract was obtained after extraction, and subjected to silica gel column chromatography by using petroleum ether / EtOAc (10 : 1, 3 : 1, 1 : 1, 1 : 3, 1 : 6, *v/v*) as elution solvents, giving fractions 1 − 5. **5** (3 mg) was obtained from fraction 3 by applying to Sephadex LH-20 column using MeOH as eluent.

To isolate the accumulated products **4** and **6** from Δ*traG* mutant, the strain was cultivated in 6 L PD liquid medium at 25°C for 14 days and extracted as mentioned above. The resulted crude extract (2.5 g) was subjected to silica gel column chromatography by using stepwise gradient elution with the mixtures of petroleum ether / EtOAc (10 : 1, 5 : 1, 3 : 1, and 1 : 1, *v/v*) to give 25 fractions. Subsequent purification on semi-preparative HPLC with isocratic elution using acetonitrile / H_2O (40 : 60) supplied with 0.1% trifluoroacetic acid yielded 3 mg of **4**, using acetonitrile / H_2O (70 : 30) with 0.1% trifluoroacetic acid yielded 5 mg of **6**.

To prepare the enzyme products for structural elucidation, assays were carried out in large scales (10 − 20 mL) using the reaction conditions mentioned above. The reaction mixtures were extracted with double volume of ethyl acetate for three times. The organic phases were combined and concentrated under vacuum. The resulted residues were dissolved in acetonitrile and centrifuged at 17,000 x *g* for 20 min. After isolation on semi-preparative HPLC eluted with 40% acetonitrile containing 0.1% trifluoroacetic acid, compound **2** from the incubation mixture of TraH, TraD and **1**, compound **3** from TraH and **1**, and **5** from TraH and **4**, were obtained.

12. Determination of kinetic parameters

For determination of kinetic parameters of TraH toward **1** or **7**, the enzyme assays were carried out in a similar way as mentioned above, excepting that substrates at final concentrations of

0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 mM, respectively. The enzyme assays were incubated at 37°C for 30 min and terminated with one volume of acetonitrile. The supernatants were subjected to HPLC analysis by method A after centrifuging at 17,000 *x g* for 30 min. The K_M and *kcat* values were obtained by analysis with GraphPad Prism 8.

13. HPLC and LC-MS analysis of secondary metabolites

Analysis of SMs was performed on an Agilent series 1200 HPLC (Agilent Technologies, Böblingen, Germany) with an Agilent Eclipse XDB-C18 column (150 × 4.6 mm, 5 μm). Water (A) and acetonitrile (B), both with 0.1% (*v/v*) formic acid, were used as solvents at flow rate of 0.5 mL/min. The substances were eluted with a linear gradient from 5 – 100% B in 15 min, then washed with 100% (*v/v*) solvent B for 5 min and equilibrated with 5% (*v/v*) solvent B for 5 min (method A) or with a linear gradient from 5 – 100% B in 40 min, then washed with 100% (*v/v*) solvent B for 5 min and equilibrated with 5% (*v/v*) solvent B for 10 min (method B). UV absorptions at 280 nm were illustrated in this study. Semi-preparative HPLC was performed on the same equipment with an Agilent Eclipse XDB-C18 column (9.4 × 250 mm, 5 μm) column and a flow rate of 2 mL/min.

LC-MS analysis was performed on an Agilent 1260 HPLC system equipped with a microTOF-Q III spectrometer (Bruker, Bremen, Germany) by using Multospher 120 RP18-5μ column (250 × 2 mm, 5 μm) (CS-Chromatographie Service GmbH) and method A or method B for separation at flow rate of 0.25 mL/min. Electrospray positive or negative ionization mode was selected for determination of the exact masses. The capillary voltage was set to 4.5 kV and a collision energy of 8.0 eV. Sodium formate was used in each run for mass calibration. The masses were scanned in the range of *m/z* 100 – 1500. Data were evaluated with the Compass DataAnalysis 4.2 software (Bruker Daltonik, Bremen, Germany).

14. NMR analysis

NMR spectra were recorded on a JEOL ECA-500 MHz spectrometer (JEOL, Tokyo, Japan). The spectra were processed with MestReNova 6.1.0 (Metrelab) or Delta 5.0.4 (JEOL). Chemical shifts are referenced to those of the solvent signals. NMR data are given in Tables S4 – S8 and spectra in Figures S21 – S35.

15. Circular dichroism (CD) spectroscopic analysis

CD spectra were taken on a J-815 CD spectrometer (Jasco Deutschland GmbH, Pfungstadt, Germany). The samples were dissolved in methanol and measured in the range of 200–400 nm by using a 1 mm path length quartz cuvette (Hellma Analytics, Müllheim, Germany). The CD spectra are given in Figures S36.

16. Measurement of optical rotations

The optical rotation was measured with the polarimeter Jasco DIP-370 at 20°C using the D-line of the sodium lamp at λ=589.3 nm. Prior to the measurement, the polarimeter was calibrated with methanol or ethanol as solvent.

17. Physiochemical properties of the compounds described in this study

(5*R*, 5'*S*)-terrestric acid (**2**): Yellow oil; CD (MeOH): λ_{max} ($\Delta\varepsilon$) 282 (+0.9) nm, 228 (+2.8), 212

(+7.5); HRMS (*m/z*): (ESI/$[M+H]^+$) calcd. for $C_{11}H_{15}O_4$, 211.0965, found 211.0963.

(5'*S*)-dehydroterrestric acid (**3**): White powder; HRMS (*m/z*): (ESI/$[M+H]^+$) calcd. for $C_{11}H_{13}O_4$, 209.0808, found 209.0806.

(5*S*)-carboxylcrustic acid (**4**): Yellow oil; $[\alpha]_D^{20}$ = -46.5 (*c* 0.40, MeOH); HRMS (*m/z*): (ESI/$[M+H]^+$) calcd. for $C_{12}H_{13}O_6$, 253.0707, found 253.0719
CD spectrum (MeOH) of sample isolated from a *A. nidulans* strain JF15 harboring *traA*: λ_{max} ($\Delta\varepsilon$) 332 (-1.9), 268 (-1.7), 228 (-4.1) nm .
CD spectrum (MeOH) of sample isolated from Δ*traG* mutant of *P. crustosum*: λ_{max} ($\Delta\varepsilon$) 346 (-2.8), 279 (-3.9), 223 (-6.0) nm.

(5*S*)-viridicatic acid (**5**): Yellow oil; $[\alpha]_D^{20}$ = -73.6 (*c* 1.0, EtOH); HRMS (*m/z*): (ESI/$[M+H]^+$) calcd. for $C_{12}H_{17}O_6$, 257.1020, found 257.1038.
CD spectrum (MeOH) of sample isolated from a *A. nidulans* strain JF45 harboring *traA* and *traG*: λ_{max} ($\Delta\varepsilon$) 258 (-11.4), 230 (-11.3) nm.
CD spectrum (MeOH) of sample isolated from *P. crustosum* PRB-2: λ_{max} ($\Delta\varepsilon$) 259 (-7.0), 230 (-7.6) nm.

(5*R*)-crustic acid (**6**): Yellow oil; $[\alpha]_D^{20}$ = +19.1 (*c* 0.25, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 317 (+0.2), 258 (+2.1), 228 (+5.2) nm; HRMS (*m/z*): (ESI/$[M+H]^+$) calcd. for $C_{11}H_{13}O_4$, 209.0808, found 209.0827.

(5*S*, 5'*S*)-custosic acid methyl ester (**7**): Yellow oil; HRMS (*m/z*): (ESI/$[M+H]^+$) calcd. for $C_{13}H_{17}O_6$, 269.1020, found 269.1037.

(5'*S*)-dehydrocrustosic acid methyl ester (**8**): White powder; HRMS (*m/z*): (ESI/$[M+Na]^+$) calcd. for $C_{13}H_{14}NaO_6$, 289.0683, found 289.0700.

Supplementary Tables

Table S1. Strains used in this study

Strains	Genotype	Source/Ref.
Penicillium crustosum		
PRB-2	Wild type	[1]
Δ*traA*	Δ*traA*::*hph* in *P. crustosum* PRB-2	[6]
Δ*traG*	Δ*traG*::*hph* in *P. crustosum* PRB-2	This study
Aspergillus nidulans		
LO8030	*pyroA4*, *riboB2*, *pyrG89*, *nkuA::argB*, sterigmatocystin cluster (*AN7804-AN7825*)Δ, emericellamide cluster (*AN2545-AN2549*) Δ, asperfuranone cluster (*AN1039-AN1029*) Δ, monodictyphenone cluster (*AN10023-AN10021*) Δ, terrequinone cluster (*AN8512-AN8520*) Δ, austinol cluster part 1 (*AN8379-AN8384*) Δ, austinol cluster part 2 (*AN9246-AN9259*) Δ, F9775 cluster (*AN7906-AN7915*) Δ, asperthecin cluster (*AN6000-AN6002*) Δ	[2,3]
JF15	*gpdA::traA::AfpyrG* in *A. nidulans* LO8030	This study
JF45	*gpdA::traA::traG::Ribo* in *A. nidulans* LO8030	This study

Table S2. Plasmids used and constructed in this study

Plasmids	Description	Source/Ref.
p5HY	Two-third of the *hph* resistance gene at the 5´-end, originated from the pUChph and inserted into pESC-URA. For gene replacement using *hph* as selection marker.	6
p3YG	Two-third of the *hph* resistance gene at the 3´-end, originated from the pUChph and inserted into pESC-URA. For gene replacement using *hph* as selection marker.	6
pJF80	A 1054 bp US PCR fragment of *traG* from genomic DNA of *P. crustosum* PRB-2 inserted in p5HY.	This study
pJF81	A 939 bp DS PCR fragment of *traG* from genomic DNA of *P. crustosum* PRB-2 inserted in p3YG.	This study
pYH-wA-pyrG	*URA3*, *wA flanking*, *AfpyrG*, *Amp*	4
pYWB2	*URA3*, *wA flanking*, *Afribo*, *Amp*	9
pJF27	*pYH-wA-traA*; a 12068 bp fragment of *traA* with its terminator from genomic DNA of *P. crustosum* PRB-2 inserted in *pYH-wA-gpdA*	This study
pJF91	*pYH-wA-traAG*; a 12788 bp fragment of *traA* with *gpdA* promoter and its terminator from pJF27, and a 2528 bp fragment of *traG* with its promoter and terminator from genomic DNA of *P. crustosum* PRB-2 inserted in *pYWB2*	This study
pJF72	pET-28a(+)-*traD*; a 804 bp fragment of *traD* from cDNA of *P. crustosum* PRB-2 with BamHI and EcoRI inserted in pET28a(+)	This study
pJF74	pET-28a(+)-*traH*; a 984 bp fragment of *traH* from cDNA of *P. crustosum* PRB-2 with BamHI and EcoRI inserted in pET28a(+)	This study

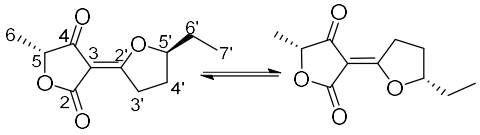
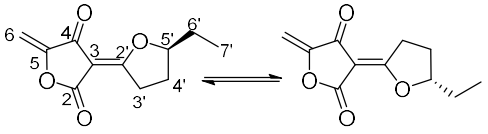
US: upstream; DS: downstream

Table S3. Primers used in this study

Primers	Sequence 5'-3'	Targeted amplification
5F-R	GCTGAAGTCGATTTGAGTCCAC	US of *hph* to verify 5F of *P. crustosum* mutant
3F-F	GCATTAATGCATTGGACCTCGC	DS of *hph* to verify 3F of *P. crustosum* mutant
traA-F	TGCATCTTGTAGAGCTCGC	1819 bp partial fragment of *traA*
traA-R	GAGGGCGGTTTTAGAATCAATTG	
traG-up-F	GAATTGTTAATTAAGAGCTCAGATCTCTAGCAGGACTCATCACAGACG	1054 bp upstream fragment of *traG* to construct pJF80
traG-up-R	CAACCCTCACTAAAGGGCGGCCGCACTAGCCGGGCTTCAGGGAAATTC	
traG-down-F	CGACTCACTATAGGGCCCGGGCGTCGACCCATGGTCCGATTGAGCTGG	939 bp downstream fragment of *traG* to construct pJF81
traG-down-R	CTAGCCGCGGTACCAAGCTTACTCGAGGCATGATTTGCCTCTAGACCCC	
traG-F	CAACACAATGTCACGGTACC	1071 bp partial fragment of *traG*
traG-R	CAGACATGGCCTGGGTACG	
traG-5F-F	CCGACAGACGAATATGGTGGC	US of *hph* to verify Δ*traG* mutant
traG-3F-R	CAGACATGCTTTCCGCAC	DS of *hph* to verify Δ*traG* mutant
A.n-traA-1F-For	CATCTTCCCATCCAAGAACCTTTAATCATGGTTCTACCCCAGCCC	DNA of 1st *traA* fragment 5442 bp from *P. crustosum* to construct pJF27
A.n-traA-1F-Rev	CTCATCAAGCCCGTGGACGAGCAAATGACTGTGAGCAACCACCATAG	
A.n-traA-2F-For	CTATGGTGGTTGCTCACAGTCATTTGCTCGTCCACGGGCTTGATGAG	DNA of 2nd *traA* fragment 6176 bp with its 497 bp terminator from *P. crustosum* to construct pJF27
A.n-traA-2F-Rev	GACACAGAATAACTCTCGCTAGCGTAGCTGGCAAATATAGTTACCT	
A.n-traAG-1F-For	CTTGACTCTCCTTCTCCTGATCGGATCCCATGCGGAGAGACGGACG	DNA of 6190 bp from pJF27 with A.n-traA-1F-Rev to construct pJF91
A.n-traAG-1R-Rev	TTAGTTTGCAAAATCGACGATTGCTGTAGCTGGCAAATATAGTTACCTA	DNA of 6673 bp from pJF27 with A.n-traA-2F-For to construct pJF91
A.n-traAG-2F-For	GATAGGTAACTATATTTGCCAGCTACAGCAATCGTCGATTTTGCAAAC	DNA of *traG* with its 788 bp promoter and 568 bp terminator from *P. crustosum* to construct pJF91
A.n-traAG-2R-Rev	CAACACCATATTTTAATCCCATGTGCATGGATACTCAGGTGGTATAATT	
TraD-28-For	GTGGACAGCAAATGGGTCGCGGATCCATGAAAGTTTTGATTATTTTTGC	804 bp fragment of *traD* to construct pJF72
TraD-28-Rev	GCAAGCTTGTCGACGGAGCTCGAATTCTCACGCTTCTTTGACGTCG	
TraH-28-For	CTGGTGGACAGCAAATGGGTCGCGGATCCATGTCTGTCGATGCGGCC	984 bp fragment of *traH* to construct pJF74
TraH-28-Rev	CAAGCTTGTCGACGGAGCTCGAATTCCTACAATGAAGTATCATCCGTCA	

US: upstream; DS: downstream

Table S4. ^{1}H NMR data of compounds **2** and **3**

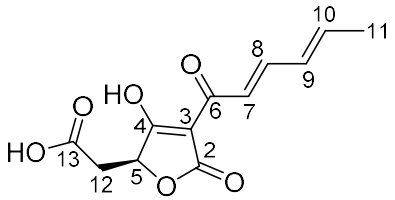
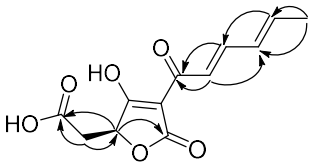
Compound	 (5*R*, 5'*S*)-terrestric acid (**2**, $CDCl_3$)[12]	 (5'*S*)-dehydroterrestric acid (**3**, DMSO-d_6)[13]
Position	δ_H, multi., *J* in Hz	δ_H, multi., *J* in Hz
5	4.62, q, 7.0, 1H	-
6	1.48, d, 7.0, 3H	5.18, d, 2.5, 1H
	-	5.01, d, 2.5, 1H
3´	3.60, ddd, 20.0, 9.6, 4.5, 1H	3.56, ddd, 19.9, 9.4, 4.2, 1H[a]
	3.27, ddd, 20.0, 9.6, 5.1, 1H	3.48, ddd, 19.9, 9.4, 4.2, 1H[a]
4´	2.36, m, 1H	2.33, m, 1H
	1.87, m, 1H	1.84, m, 1H
5´	4.93, m, 1H	5.02, m, 1H
6´	1.97, m, 1H	1.81, m, 1H
	1.79, m, 1H	1.74, m, 1H
7´	1.07, t, 7.5, 3H	0.97, t, 7.4, 3H

Note: Due to the *Z*/*E* isomerization, there were two sets of signals in a ratio of 1:1 showed in the ^{1}H NMR spectra. The corresponding signals are overlapping in most cases with each other. Therefore, only one set of the NMR data was listed in the table. The *Z*/*E* isomerization was proved by deuterium incorporation after incubation of (5*S*, 5'*S*)-crustosic acid (**1**) in D_2O-enriched milieu (Figure S10).

[a] signals overlapped with those of water.

Compounds **3** and **2** were isolated from incubation mixtures of **1** with TraH alone and with TraH and TraD, respectively. Their NMR data correspond very well to those reported previously.[12,13]

Table S5. NMR data of compound **4**

Compound		
	(5*S*)-carboxylcrustic acid (**4**, CD_3OD)	

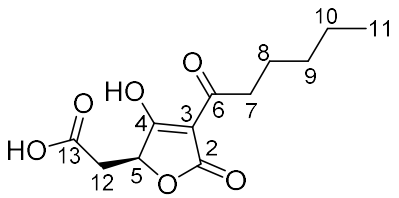
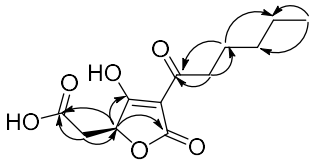
Position	δ_H, multi., *J* in Hz	δ_C	Key HMBC correlations
2	-	167.8[a]	
3	-	n.d.[b]	
4	-	n.d.[b]	
5	4.95, dd, 5.9, 4.1, 1H	80.2	C-2, C-13
6	-	178.5	
7	7.10, d, 15.3, 1H	119.0	C-6, C-9
8	7.68, dd, 15.3, 9.9, 1H	149.5	C-6, C-10
9	6.50, m, 1H	132.2	C-8, C-11
10	6.50, m, 1H	146.5	C-8, C-11
11	1.96, d, 5.5, 3H	19.3	C-9, C-10
12	2.99, dd, 17.4, 4.1, 1H	35.8	C-5, C-13
	2.88, dd, 17.4, 5.9, 1H	-	
13	-	172.2	

Note: [a] Signals acquired from HMBC correlations.

[b] Signals not detected in neither ^{13}C NMR nor HMBC spectrum.

Compound **4** was isolated from *A. nidulans* JF15 harboring pJF27 with *traA*.

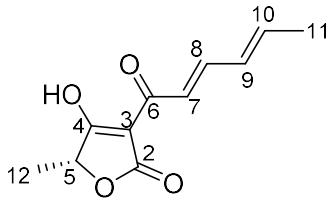
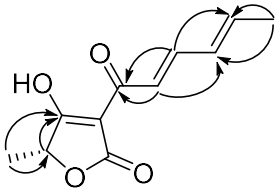
Table S6. NMR data of compound **5**

Compound	  (5*S*)-viridicatic acid (**5**, DMSO-d_6)		
Position	δ_H, multi., *J* in Hz	δ_C	Key HMBC correlations
2	-	174.2	
3	-	94.2	
4	-	171.8	
5	4.37, dd, 9.3, 3.5, 1H	75.7	C-2, C-4, C-13
6	-	194.2	
7	2.61, dd, 8.5, 6.9, 2H	40.1[a]	C-6, C-8, C-9
8	1.45, m, 2H	24.2	C-6, C-9, C-10
9	1.25, m, 2H	31.3	C-10
10	1.25, m, 2H	22.0	C-9
11	0.85, t, 7.0, 3H	13.9	C-9, C-10
12	2.69, dd, 16.0, 3.5, 1H	37.6	C-5, C-13
	2.20, dd, 16.0, 9.3, 1H	-	
13	-	193.7	
13-OH	12.44, s, 1H	-	

Note: [a] signals overlapped with those of solvents.

Compound **5** was isolated from *A. nidulans* JF45 harboring pJF91 with *traA* and *traG*.

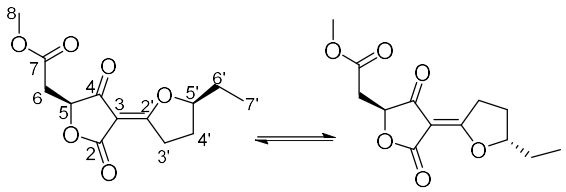
Table S7. NMR data of compound **6**

Compound	 (5*R*)-crustic acid (**6**, CD_3OD)		
Position	δ_H, multi., *J* in Hz	δ_C	Key HMBC correlations
2	-	173.7	
3	-	96.8	
4	-	201.4[a]	
5	4.79, q, 7.0, 1H	80.8	C-2, C-4
6	-	179.4	
7	7.11, d, 15.2, 1H	119.0	C-6, C-9
8	7.69, dd, 15.2, 9.9, 1H	149.9	C-6, C-10
9	6.50, m, 1H	132.2	C-7, C-11
10	6.50, m, 1H	146.9	C-8, C-11
11	1.96, d, 5.6, 3H	19.4	C-9, C-10
12	1.45, d, 7.0, 3H	17.0	C-4, C-5

Note: [a] Signal acquired from HMBC correlations.

Compound **6** was isolated from a Δ*traG*-mutant.

Table S8. NMR data of compound **7**

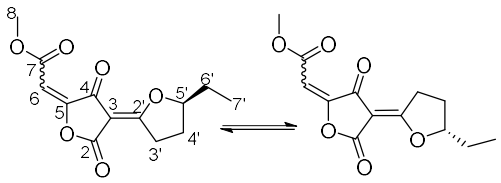
Compound	
	(5*S*, 5'*S*)-crustosic acid methyl ester (**7**, $CDCl_3$)

Position	δ_H, multi., *J* in Hz	δ_C
2	-	170.6/167.2
3	-	95.8/95.3
4	-	197.5/193.7
5	4.83, dd, 6.9, 4.1, 1H	78.8/78.6
6	3.00, dd, 9.0, 4.1, 1H	35.9/35.9
	2.85, dd, 9.0, 6.9, 1H	-
7	-	169.8/169.8
8	3.70, s, 3H	52.3/52.3
9	-	-
10	-	-
11	-	-
12	-	-
2´	-	187.0/186.3
3´	3.61, ddd, 14.0, 8.7, 4.2, 1H	34.1/33.8
	3.28, ddd, 14.0, 9.5, 8.6, 1H	-
4´	2.37, m, 1H	27.9/27.8
	1.87, m, 1H	-
5´	4.93, m, 1H	93.3/92.8
6´	1.98, m, 1H	26.6/26.6
	1.79, m, 1H	-
7´	1.06, t, 7.5, 3H	9.7/9.5

Note: due to the *Z*/*E* isomerization, there were two sets of signals in a ratio of 1:1 showed in the ^{1}H NMR spectra. The corresponding signals are overlapping in most cases with each other. Therefore, only one set of the NMR data was listed in the table.

Compound **7** was isolated from a methanol solution of **1**.

Table S9. ^{1}H NMR data of compound **8**

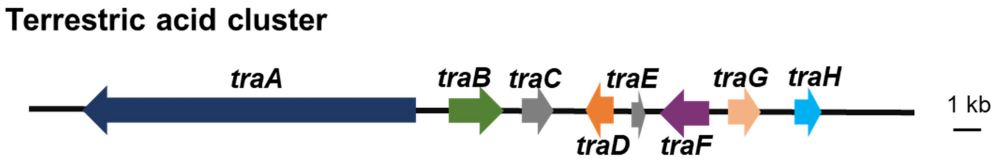
Compound	
	(5'*S*)-dehydrocrustosic acid methyl ester (**8**, $CDCl_3$)
Position	δ_H, multi., *J* in Hz
6	5.91, s, 1H
8	3.81, s, 3H
3´	3.68, ddd, 20.3, 9.4, 4.0, 1H
	3.35, m, 1H
4´	2.43, m, 1H
	2.01, m, 1H[a]
5´	5.02, m, 1H
6´	1.93, m, 1H[a]
	1.83, m, 1H
7´	1.08, t, 7.4, 3H

Note: due to the *Z*/*E* isomerization, there were two sets of signals in a ratio of 1:1 showed in the ^{1}H NMR spectra. The corresponding signals are overlapping in most cases with each other. Therefore, only one set of the NMR data was listed in the table.

[a] signals overlapped with those of water.

Compound **8** was isolated from incubation mixture of **7** with TraH.

Supplementary Figures



Protein	Putative function
TraA	carboxylcrustic acid synthase
	KS-AT-DH-MeT-KR-ACP-C-A-PCP
TraB	cytochrome P450
TraC	hypothetical protein
TraD	flavin containing dehydroterrestric acid hydrogenase
TraE	hypothetical protein
TraF	transporter
TraG	crustic acid reductase
TraH	2-oxoglutarate-dependent crustosic acid decarboxylase

The domain organization of TraA was deduced as ketosynthase (KS), acyltransferase (AT), dehydratase (DH), methyltransferase (MeT), ketoreductase (KR), and acyl carrier protein (ACP) domains of PKS, and the condensation (C), adenylation (A), peptidyl carrier protein (PCP) domains of NRPS.[14,15]

Figure S1. Deduced functions of ORFs in terrestric acid gene cluster of *P. crustosum* PRB-2[6]

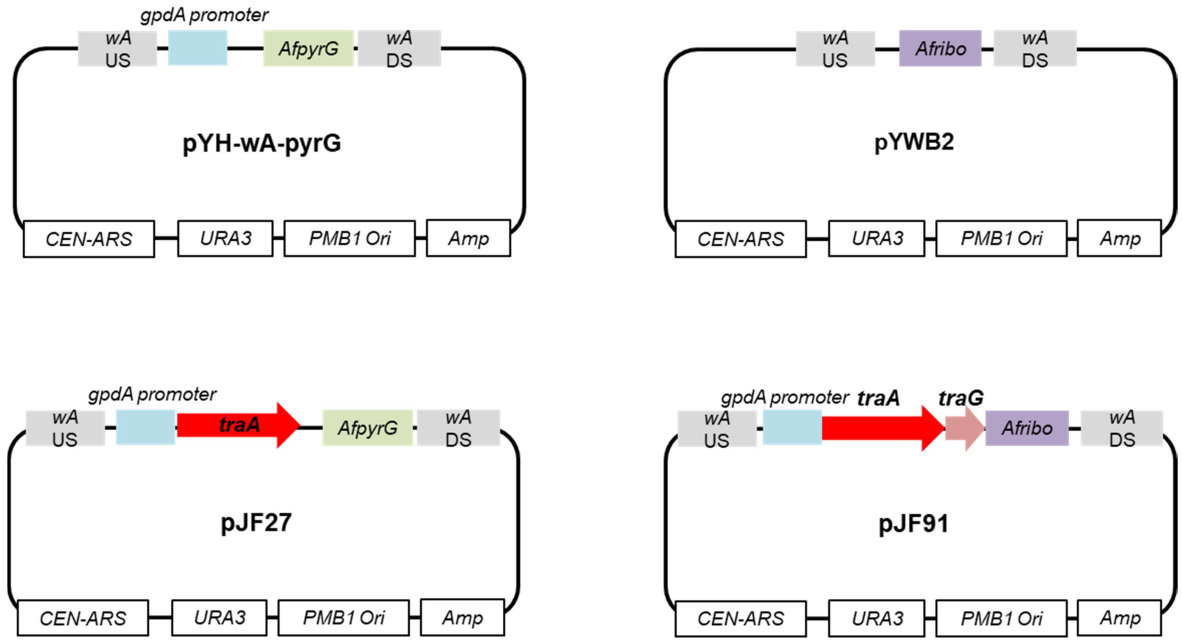


Figure S2. Constructs used for heterologous expression of *traA* and *traAG* in *A. nidulans*

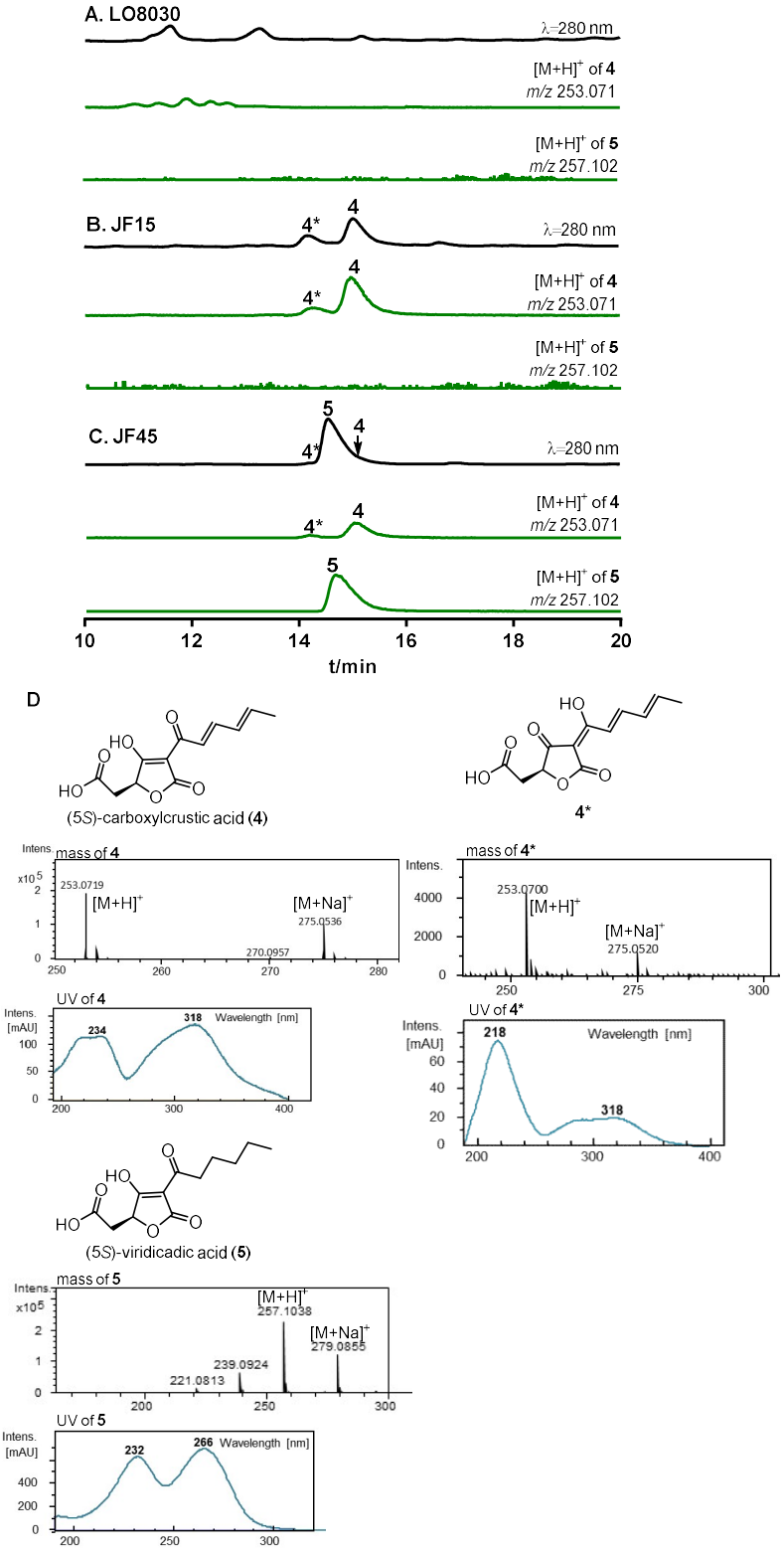


Figure S3. LC-MS analysis of the metabolite profile of different *A. nidulans* strains LO8030 as an expression host in (A), JF15 carrying the expression construct for *traA* (B), JF45 for co-expression of *traA* and *traG* (C) were cultivated in PD medium at 25 °C for 7 days. UV absorptions at 280 nm, $[M+H]^+$ of **4** at *m/z* 253.071 ± 0.005 and $[M+H]^+$ of **5** at *m/z* 257.102 ± 0.005 are illustrated. Structures, mass, and UV spectra of **4**, **4*** and **5** are shown in (D).

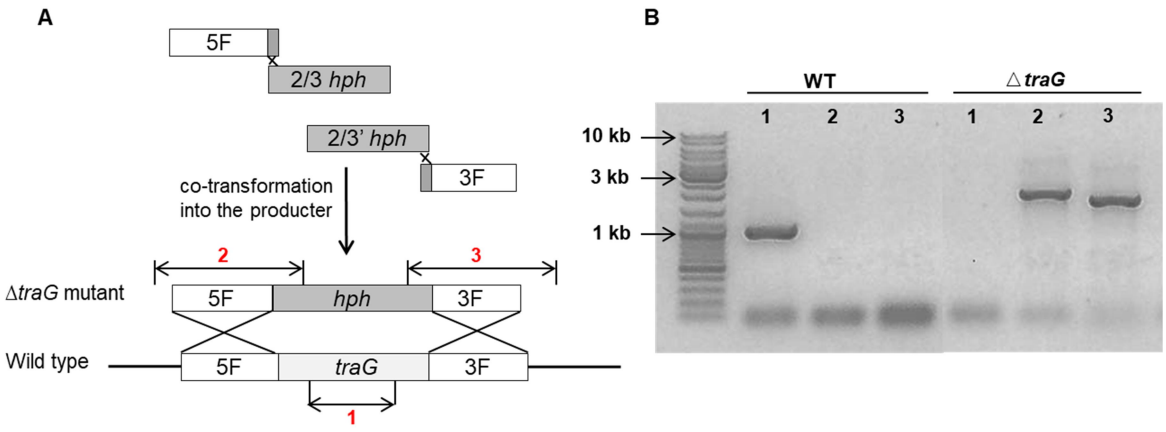


Figure S4. Verification of Δ*traG*-mutant from *P. crustosum* PRB-2
Gene deletion strategy in *P. crustosum* was schematically represented in (A). PCR amplification for three different fragments from genomic DNA of WT and Δ*traG*-mutant was used to prove the presence/absence of *traG* and its site specific integration with the help of up- and downstream regions (B). The PCR primers are given in Table S3.

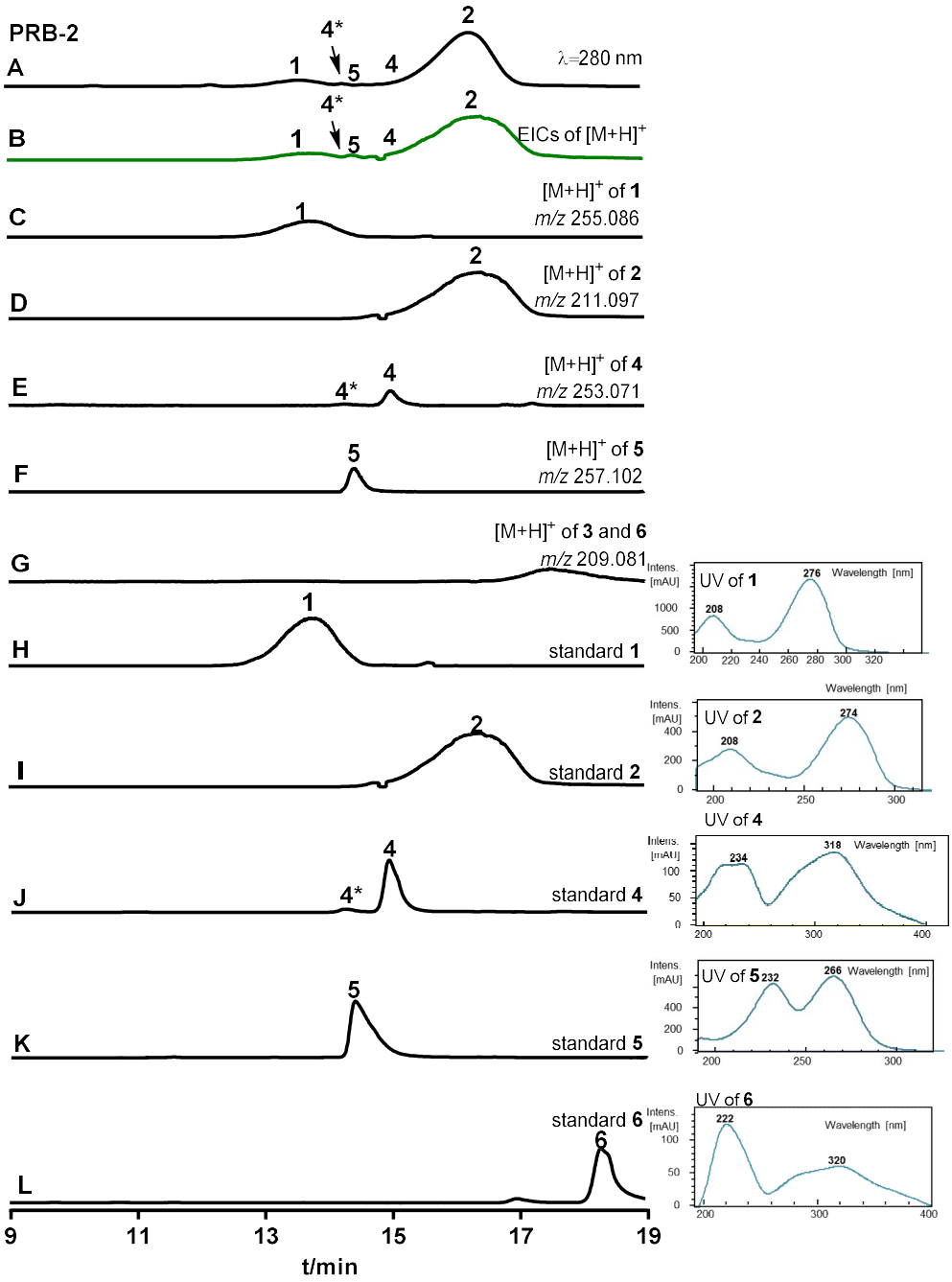


Figure S5. LC-MS detection of secondary metabolites from a 7 days-old liquid PD surface culture of *P. crustosum* PRB-2

UV absorptions at 280 nm are illustrated in (A). EICs in dark green refer total $[M+H]^+$ ions of **1** – **6** with a tolerance range of ± 0.005 (B), and in black refer $[M+H]^+$ ions of **1** – **6** (C – G), respectively. Standards of **1**, **2**, **4** – **6** are shown in H – L.

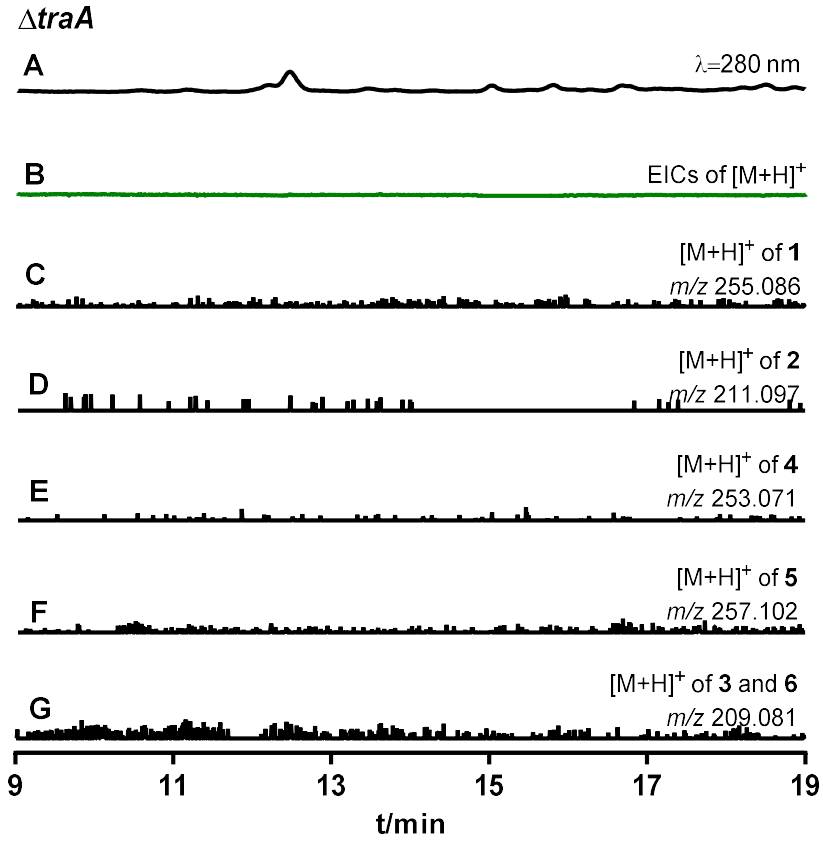


Figure S6. LC-MS detection of the metabolites in the terrestric acid biosynthesis in Δ*traA*-mutant obtained from a previous study[6]

UV absorptions at 280 nm are illustrated in (A). EICs in dark green refer total $[M+H]^+$ ions of **1** – **6** with a tolerance range of ± 0.005 (B), and in black refer $[M+H]^+$ ions of **1** – **6** (C − G), respectively.

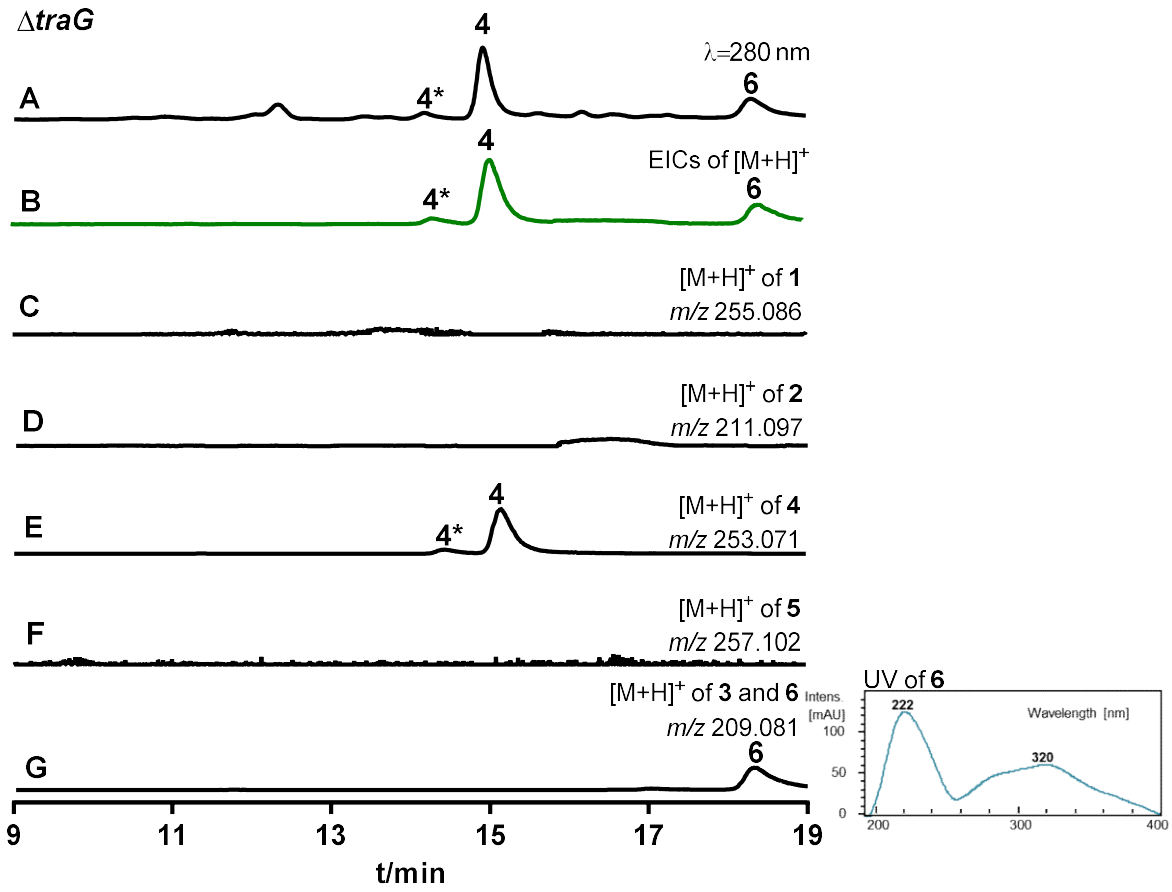


Figure S7. LC-MS detection of the metabolites in the terrestric acid biosynthesis in Δ*traG*-mutant UV absorptions at 280 nm are illustrated in (A). EICs in dark green refer total $[M+H]^+$ ions of **1** – **6** with a tolerance range of ± 0.005 (B), and in black refer $[M+H]^+$ ions of **1** – **6** (C−G), respectively.

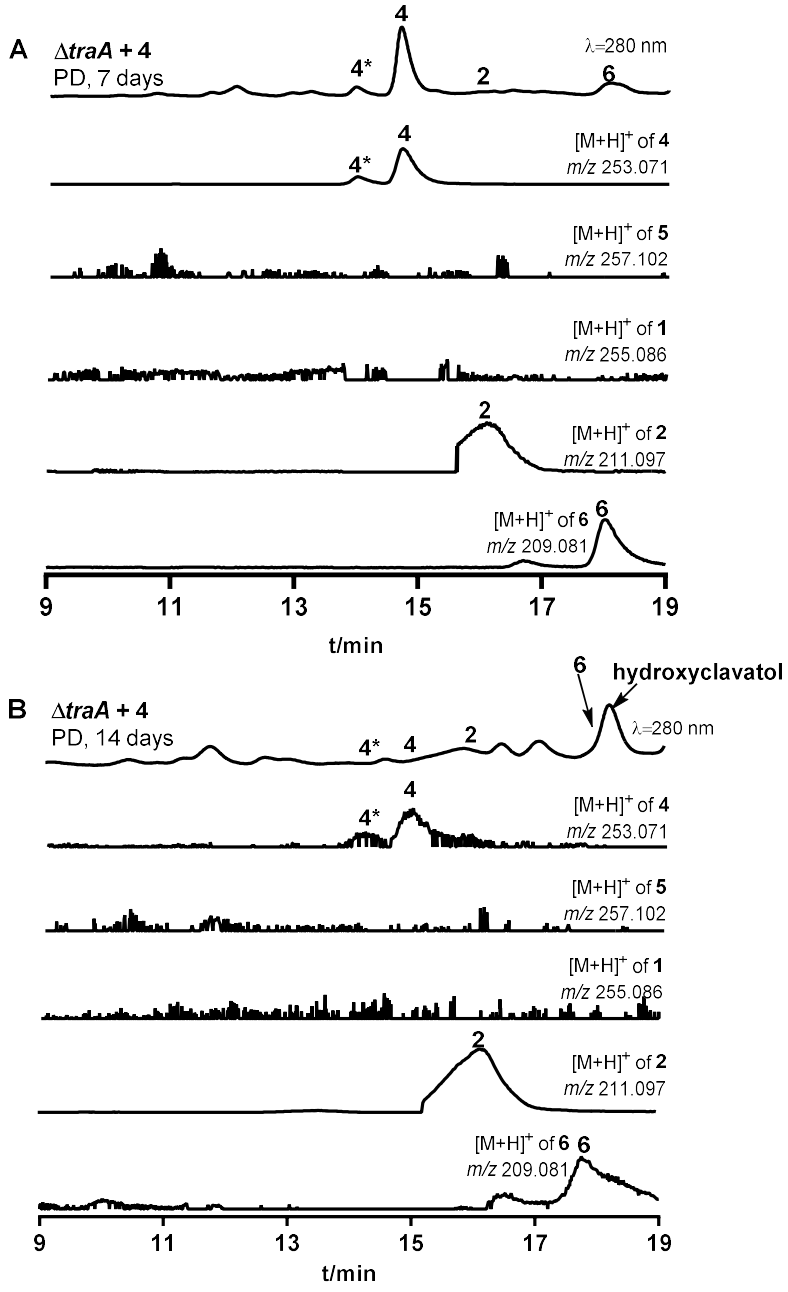


Figure S8. LC-MS detection of the metabolite profile of Δ*traA* mutant after feeding with **4** Δ*traA* culture were fed with **4** and maintained for 7 days (A) and 14 days (B). UV absorptions at 280 nm are illustrated. EICs refer $[M+H]^+$ ions of **1**, **2**, **4**, **5** and **6** with a tolerance range of ± 0.005.

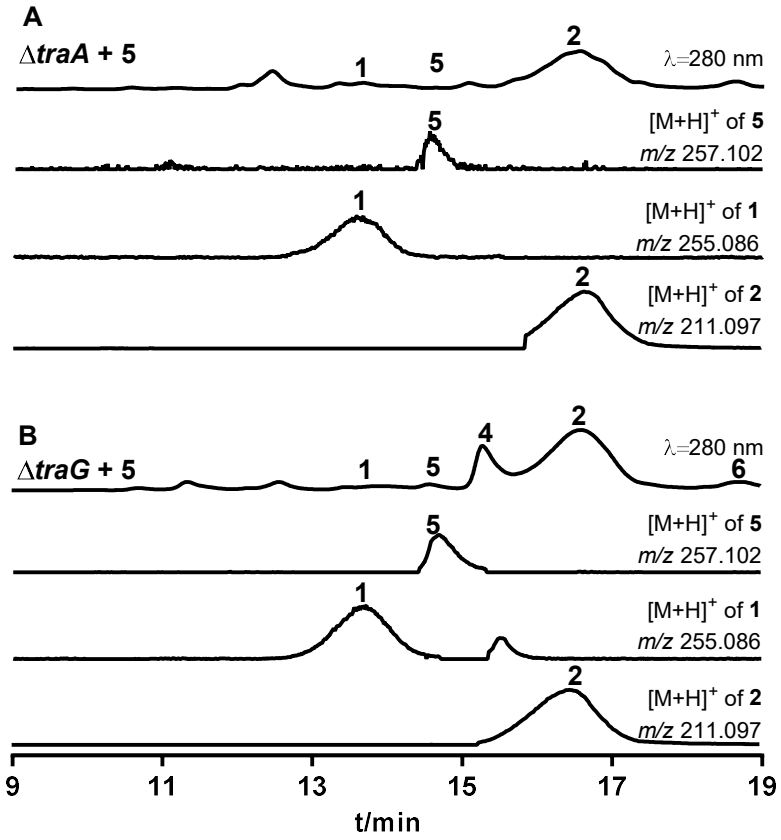


Figure S9. LC-MS detection of the metabolite profile of Δ*traA* and Δ*traG* mutants after feeding with **5**

Δ*traA* (A) and Δ*traG* (B) cultures were fed with **5** and maintained for 7 days. UV absorptions at 280 nm are illustrated. EICs refer $[M+H]^+$ ions of **1**, **2** and **5** with a tolerance range of ± 0.005.

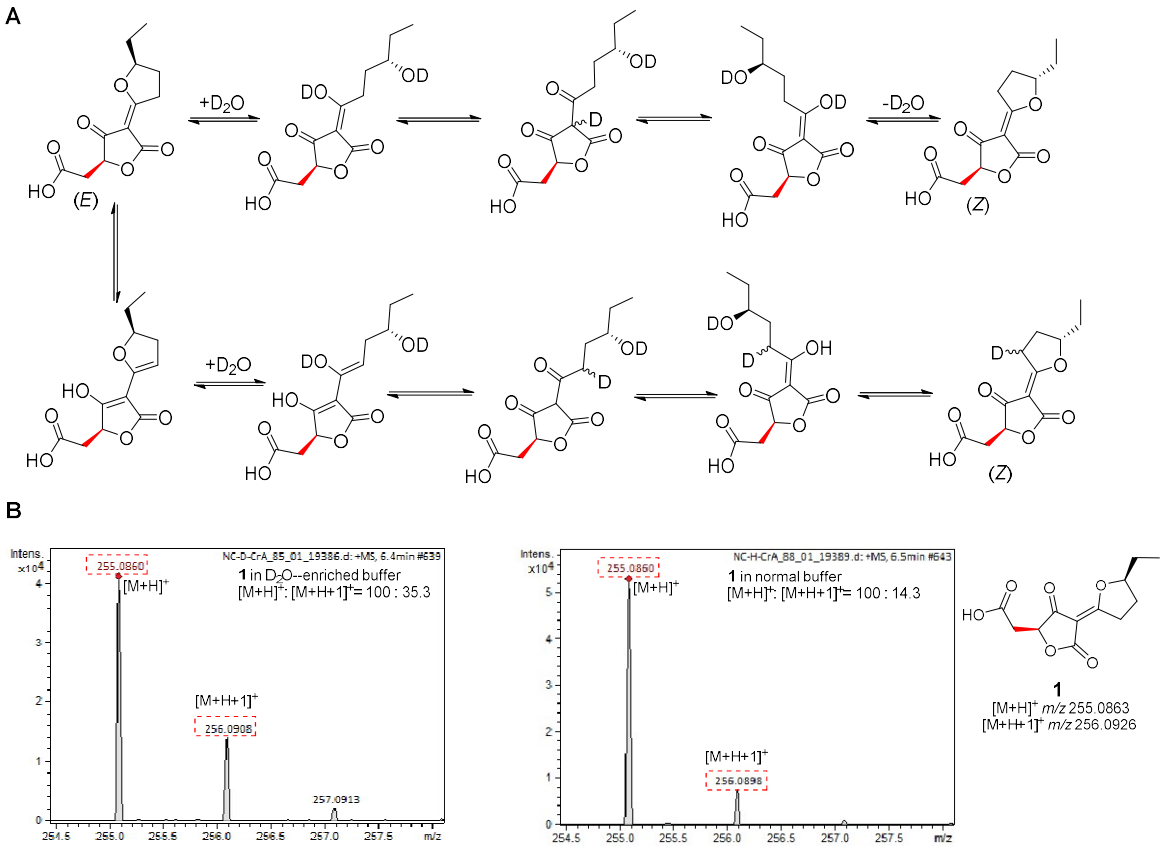


Figure S10. Incorporation of deuterium in **1** *via E/Z*-isomerization in D_2O-enriched milieu

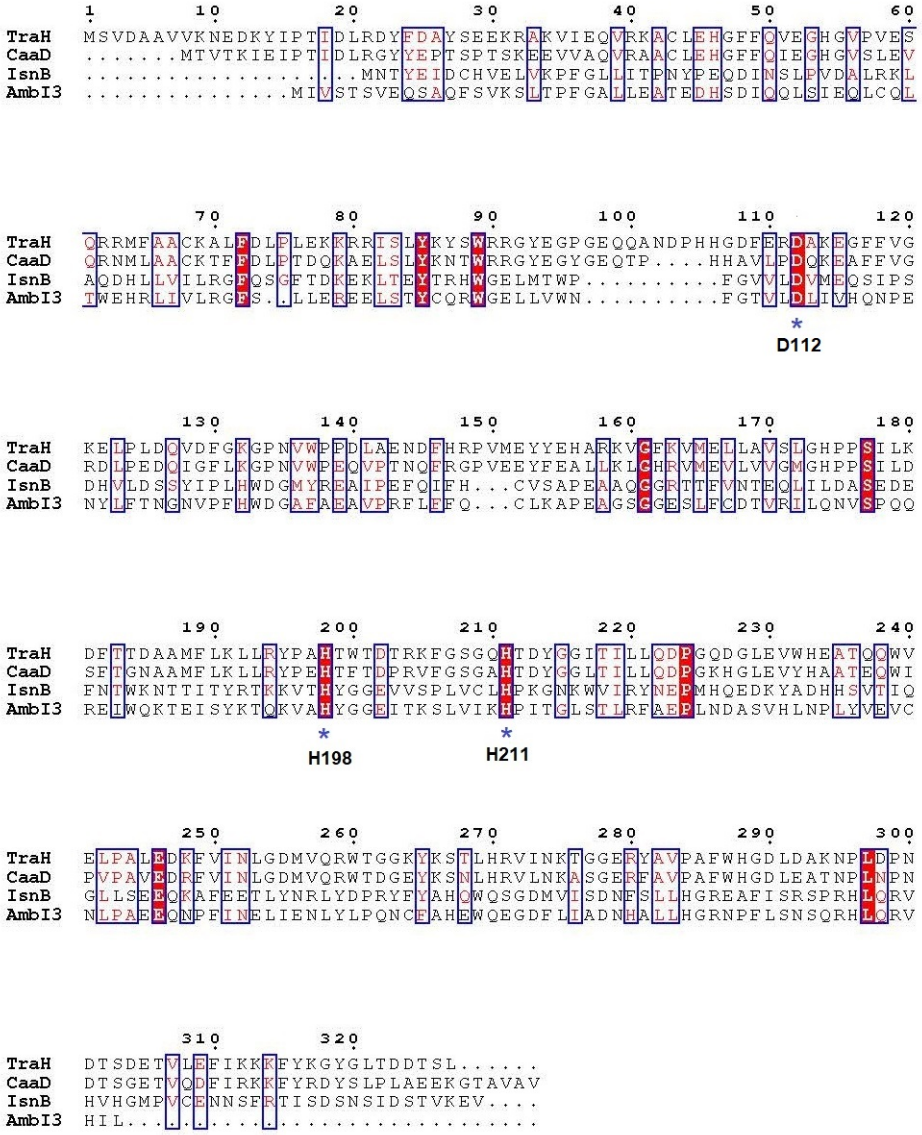


Figure S11. Sequence alignments of non-heme Fe^{II}-2OG-dependent decarboxylases CaaD (XP_001392490), IsnB (CEK22194.1), and AmbI3 (AIJ28554.1) are from *Aspergillus niger*, *Xenorhabdus nematophila,* and *Fischerella ambigua*, respectively.[16-18] TraH also contains the typical conserved 2-His-1-Asp ion-binding triad of non-heme Fe^{II}/2-oxoglutarate-dependent enzyme (His_{198}, His_{211} and Asp_{112}) (marked with *). Protein sequence alignments were carried out by using the sequence alignment function of ClustalW and visualized with ESPript 3.0 (http://espript.ibcp.fr/ESPript/ESPript/).

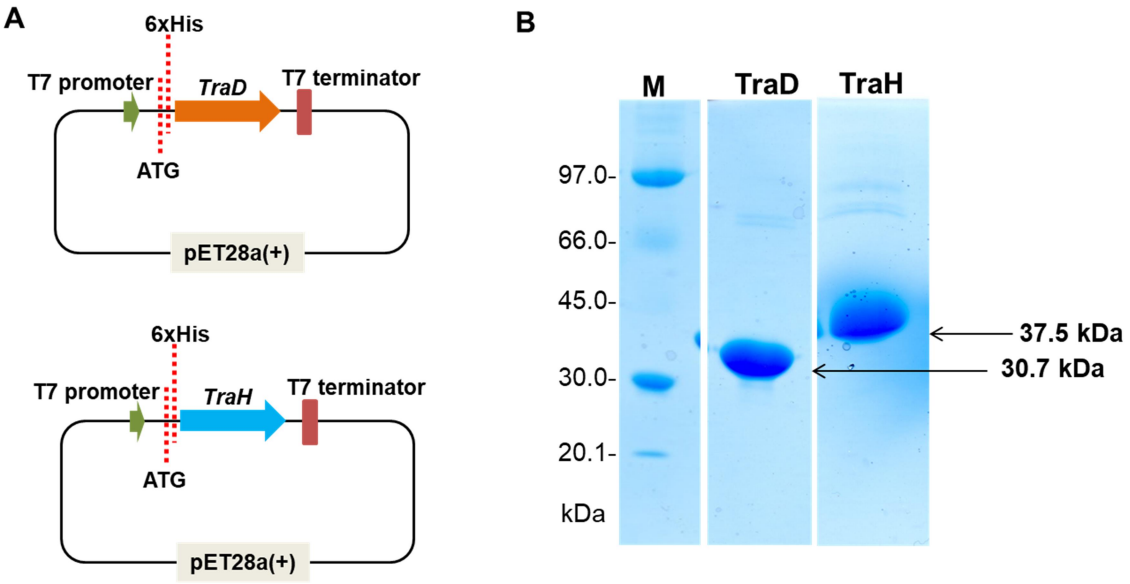


Figure S12. Analysis of recombinant TraD and TraH on SDS-PAGE

TraD and *traH* were separately inserted into pET28a(+) with 6xHis-tag at its *N*-terminal (A). The purified recombinant histidine-tagged TraD and TraH were separated on a 12% SDS-PAGE (B).

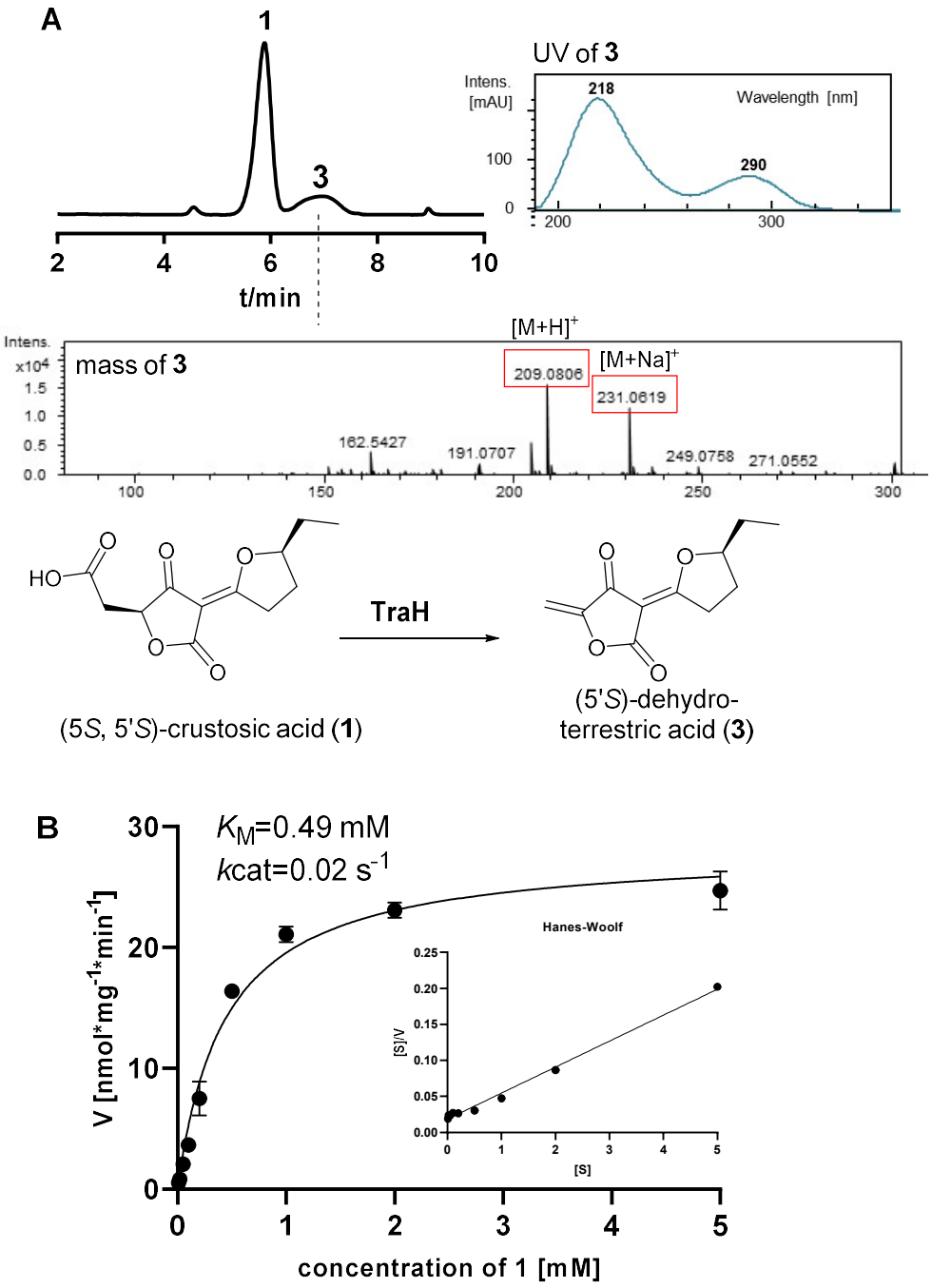


Figure S13. Oxidative decarboxylation of **1** catalyzed by TraH
LC-MS analysis of incubation mixture of **1** with TraH (A), determination of kinetic parameter of the TraH toward **1** (B).

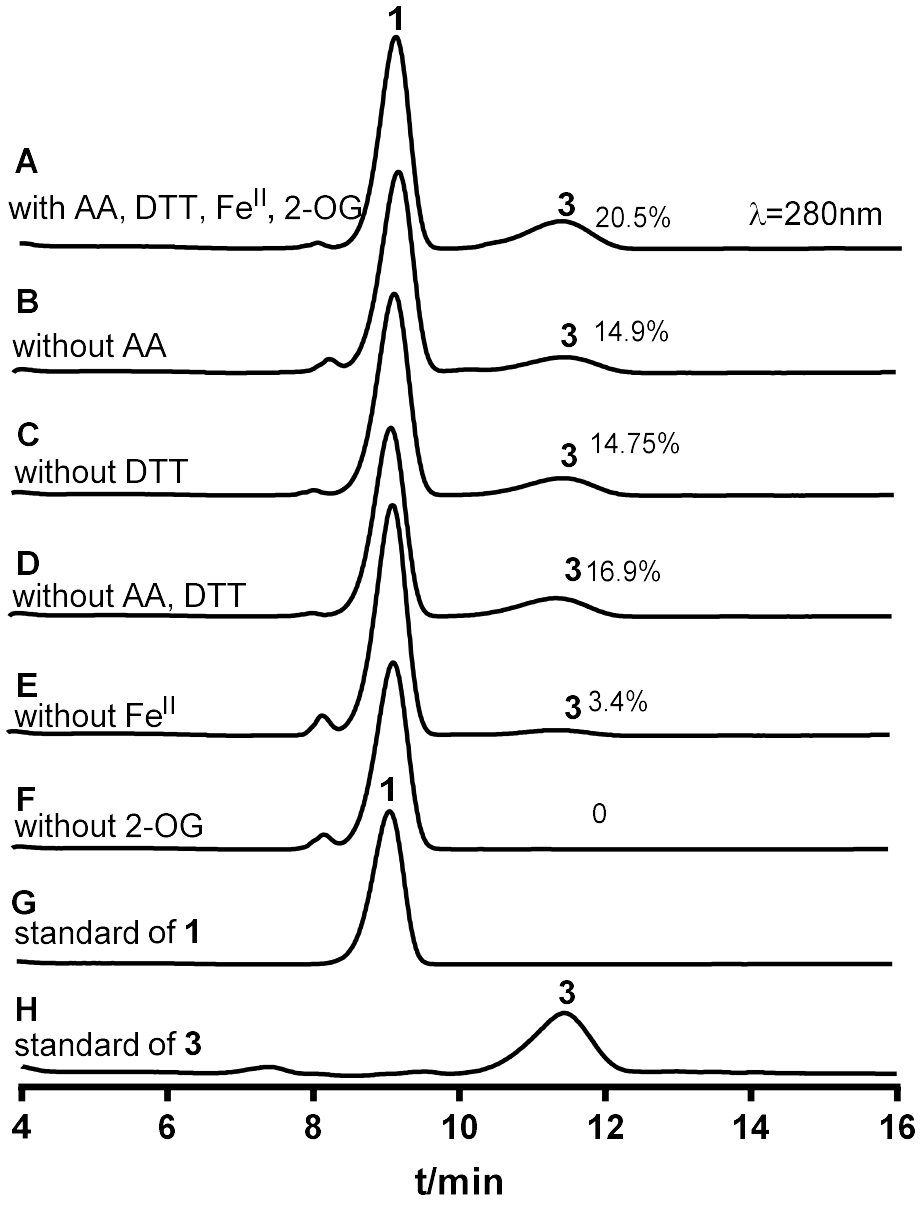


Figure S14. HPLC analysis of the incubation mixtures of **1** with TraH
5.4 μM TraH in the full assay with ascorbic acid (AA), dithiothreitol (DTT), $Fe[(NH_4)_2(SO_4)_2]$ (Fe^{II}) and 2-oxoglutarate (2OG) (A); full assay without AA (B); full assay without DTT (C); full assay without AA and DTT (D); full assay without exogenous Fe^{II} (E); full assay without 2OG (F), standards of **1** (G) and **3** (H). UV absorptions at 280 nm are illustrated.

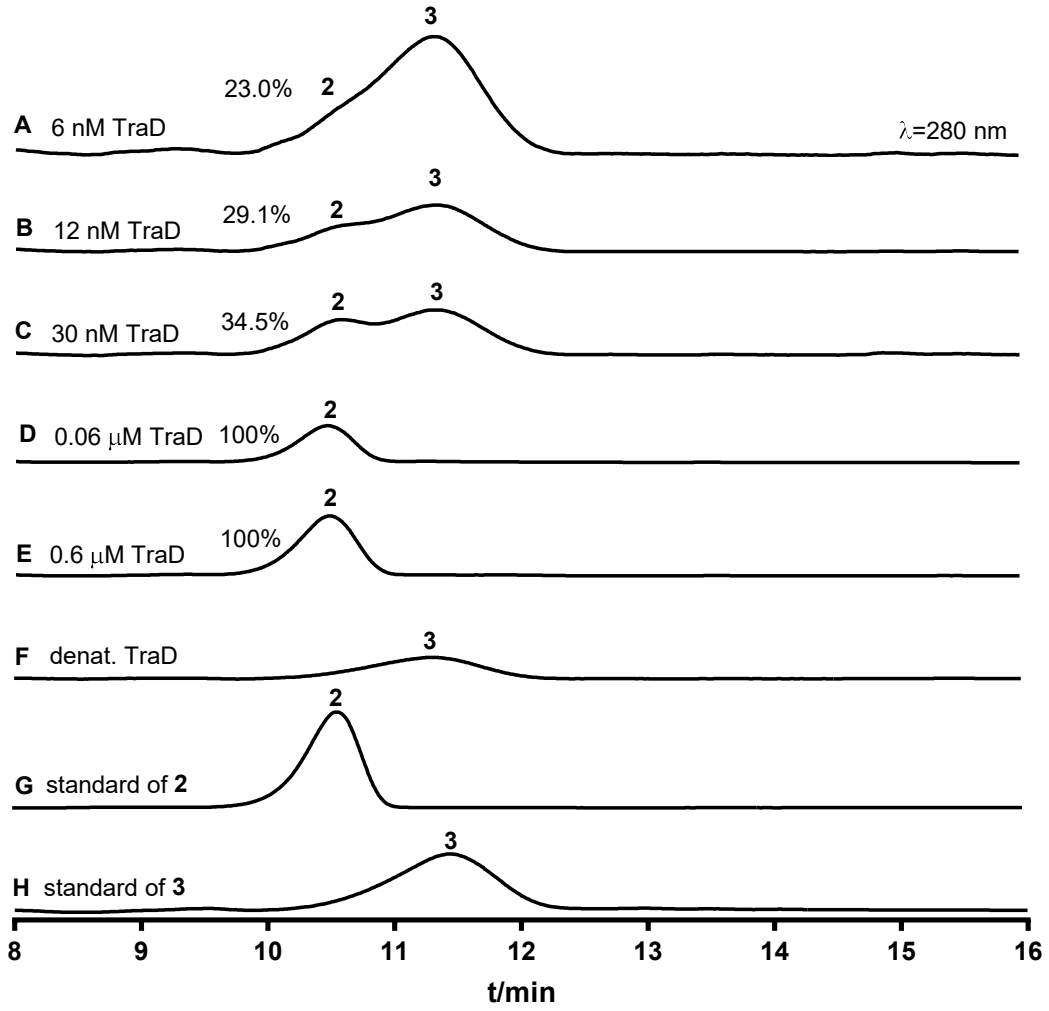


Figure S15. HPLC analysis of incubation mixtures of **3** with TraD at different concentrations

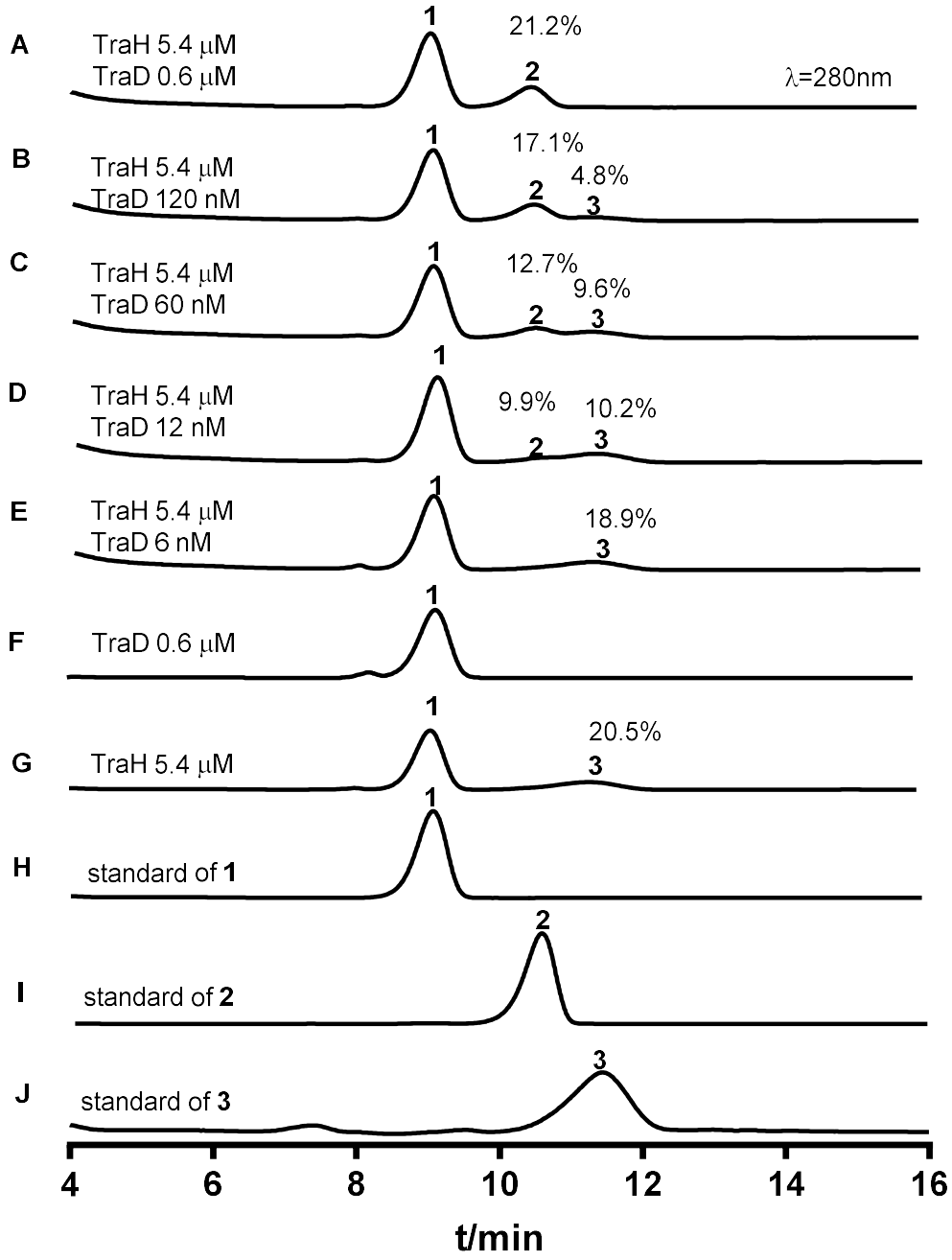
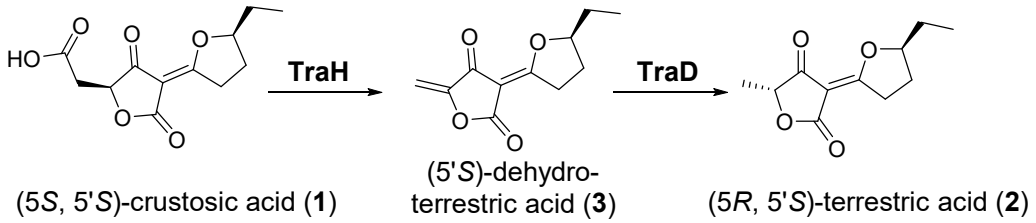


Figure S16. HPLC analysis of sequential reaction products in enzyme assays of TraH and TraD with **1**



(5*S*, 5'*S*)-crustosic acid (**1**) (5*R*, 5'*S*)-terrestric acid (**2**)

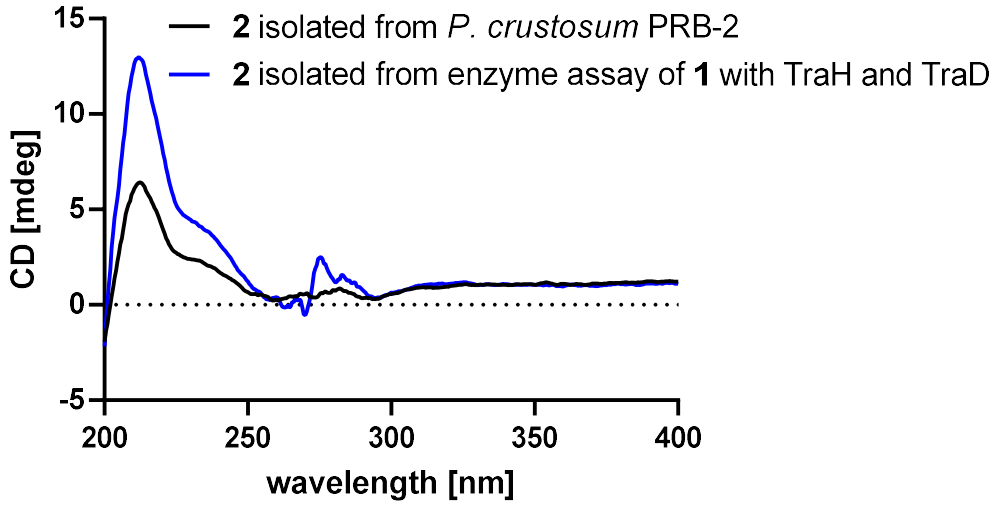


Figure S17. Comparison of CD spectra of two terrestric acid samples

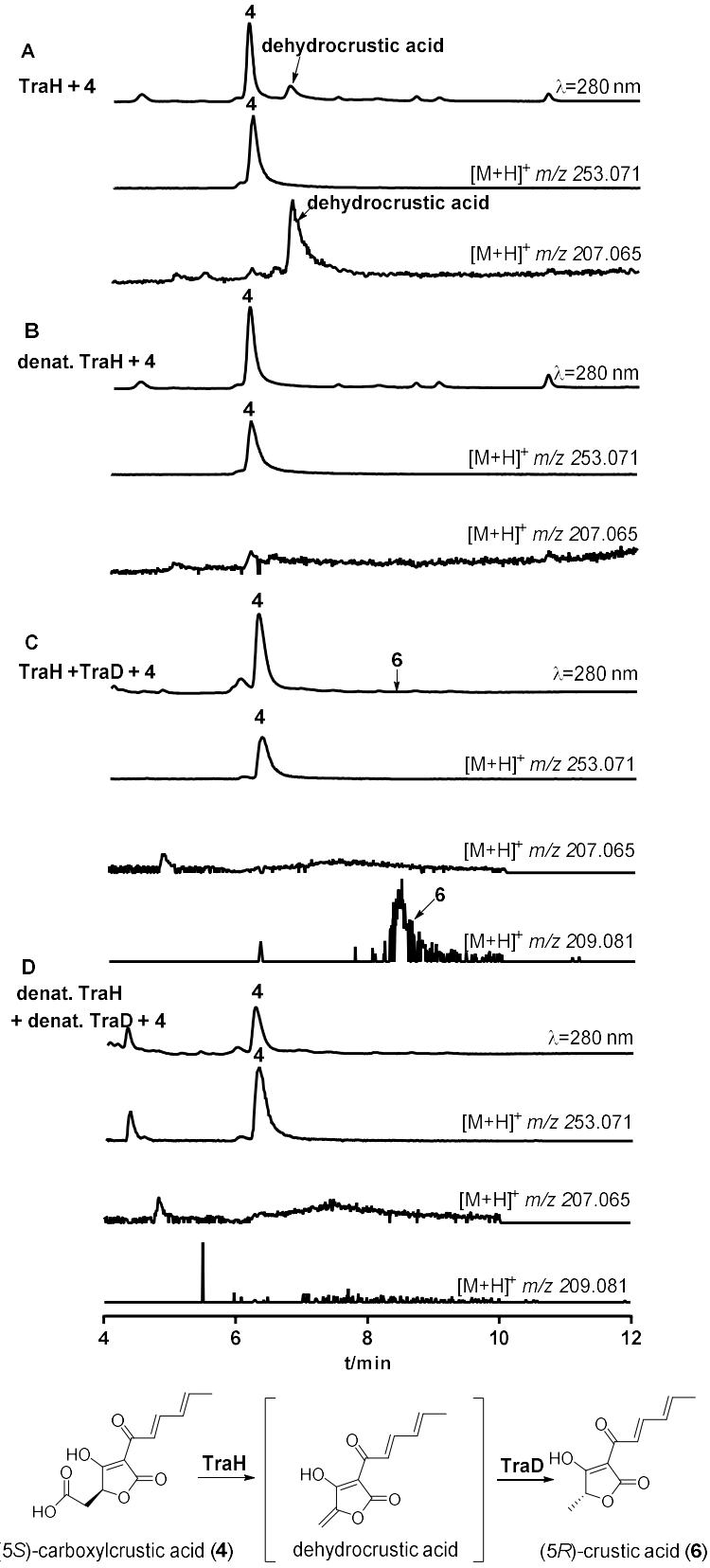


Figure S18. LC-MS analysis of enzyme assays of **4** with TraH without or together with TraD Incubation mixtures of **4** with TraH (A), **4** with denat TraH (B) at 37℃ for 16 h, **4** with TraH and TraD (C), and **4** with denat TraH and TraD (D) at 30℃ for 16 h. EICs refer $[M+H]^+$ ions of **4**, **6** and dehydrocrustic acid with a tolerance range of ± 0.005.

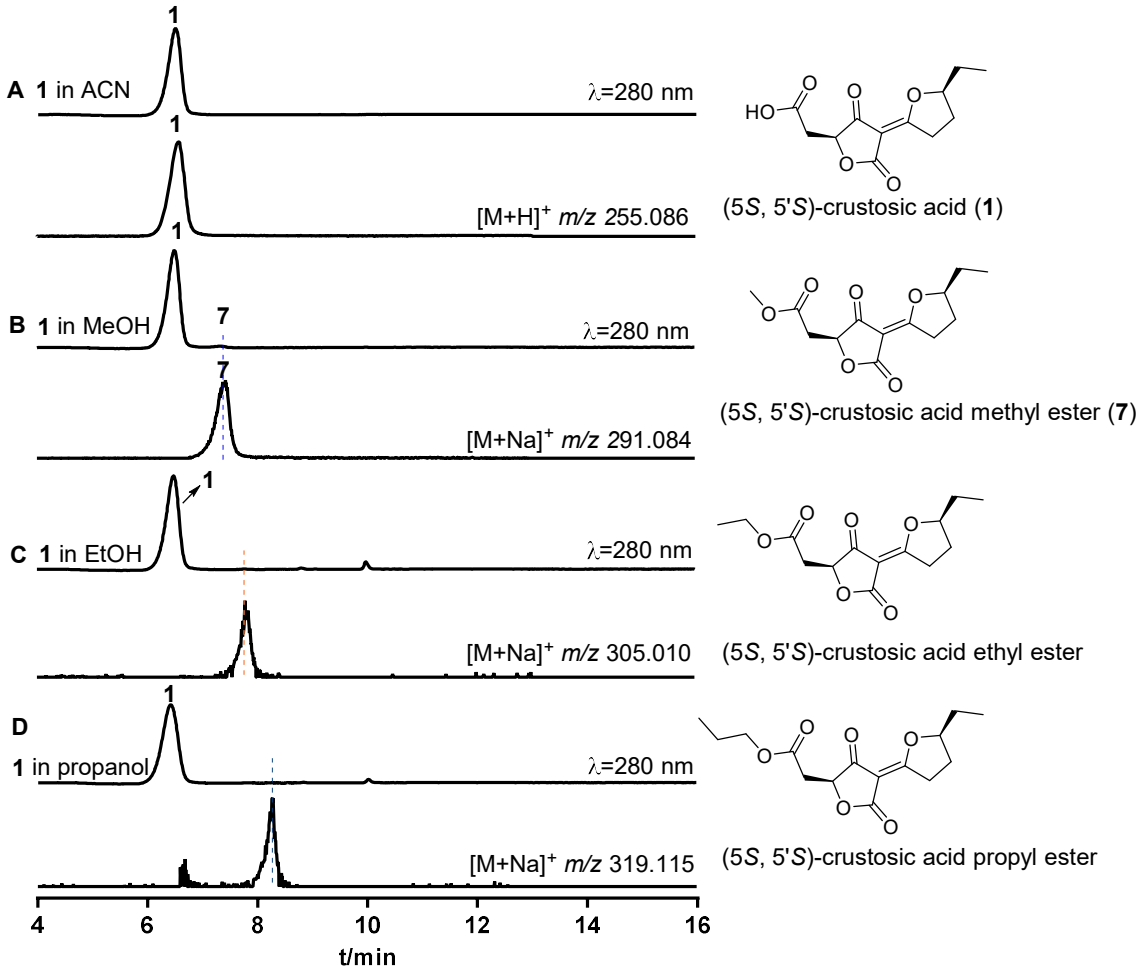
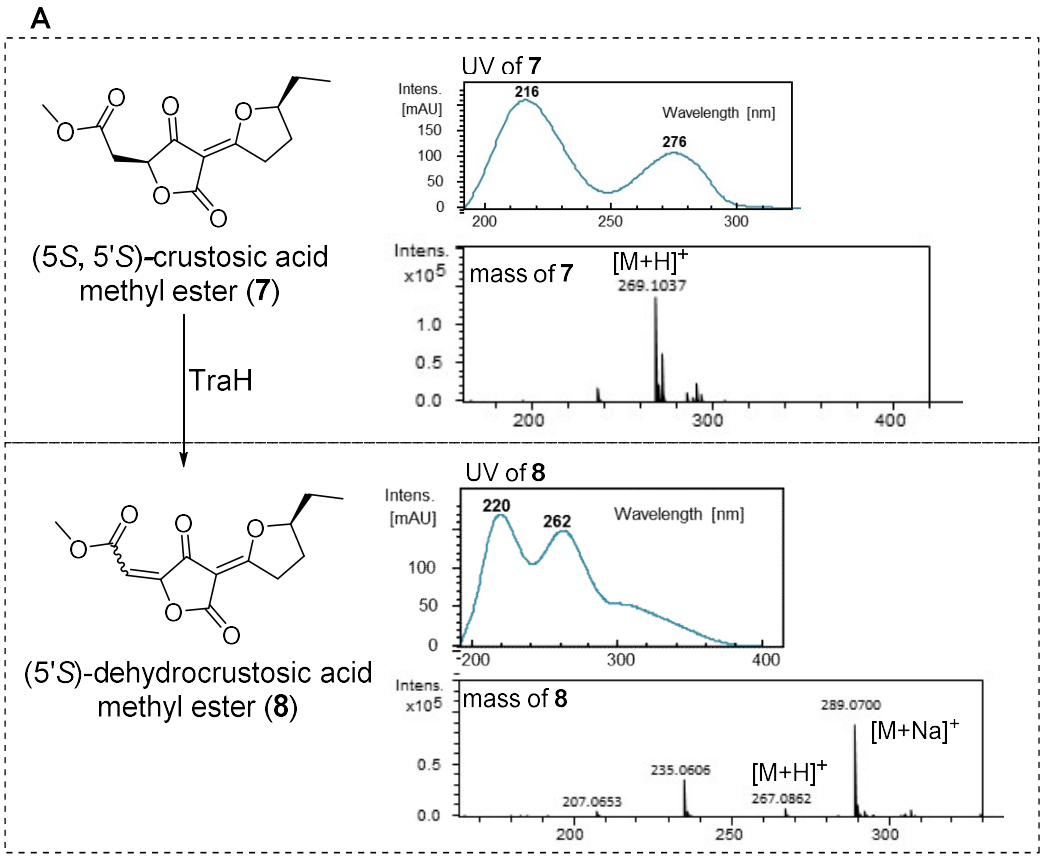


Figure S19. LC-MS analysis of spontaneous ester formation of **1** with different alcohols
0.4 mM solutions of crustosic acid (**1**) in ACN, MeOH, EtOH or n-propanol were kept at 25℃ for 24 h and subjected directly to LC-MS analysis. UV absorptions at 280 nm are illustrated. EIC at *m/z* 255.086 ± 0.005 refer $[M+H]^+$ ion of (5*S*, 5'*S*)-crustosic acid (**1**), EICs at *m/z* 291.084 ± 0.005, 305.010 ± 0.005, 319.115 ± 0.005 refer $[M+Na]^+$ ion of its methyl ester (**7**), ethyl ester, propyl ester, respectively.



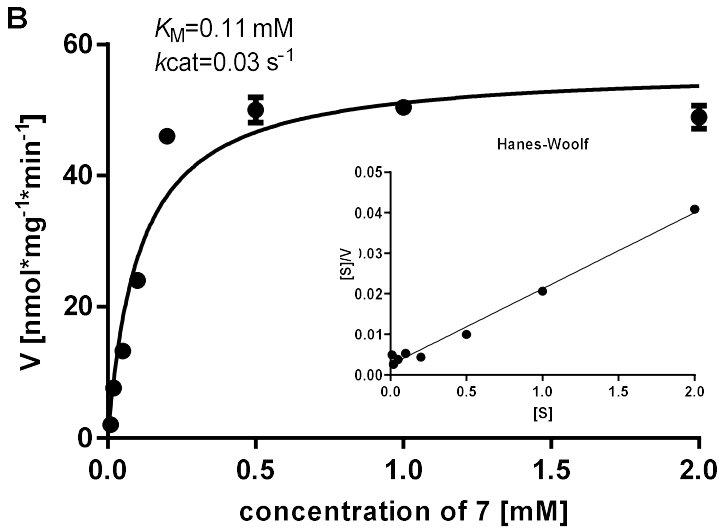


Figure S20. Conversion of **7** to **8** catalyzed by TraH
LC-MS analysis of incubation mixture of **7** with TraH (A), determination of kinetic parameter of the TraH toward **7** (B).

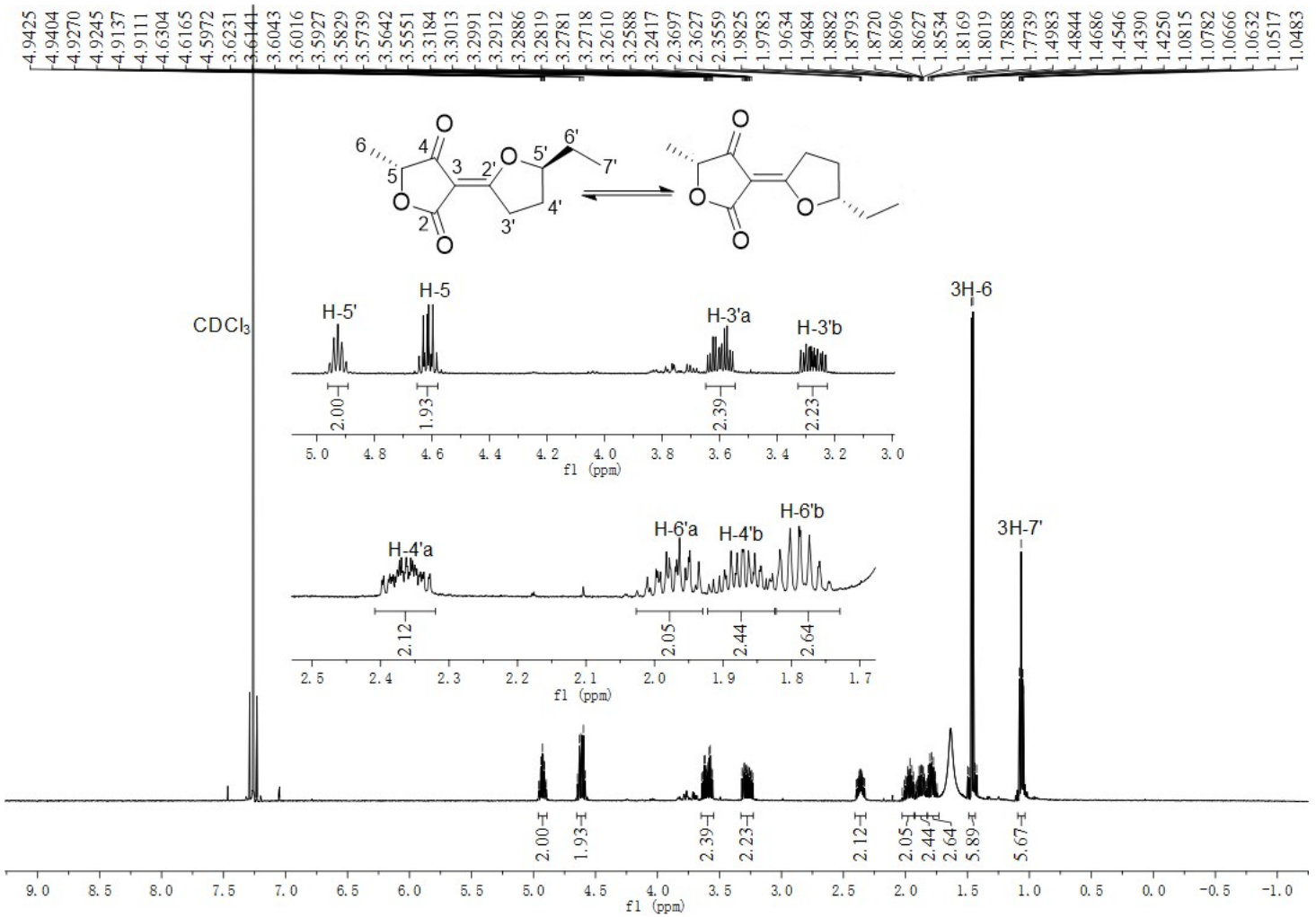


Figure S21. ^{1}H NMR spectrum of compound **2** isolated from an incubation mixture of **1** with TraH and TraD in $CDCl_3$ (500MHz)

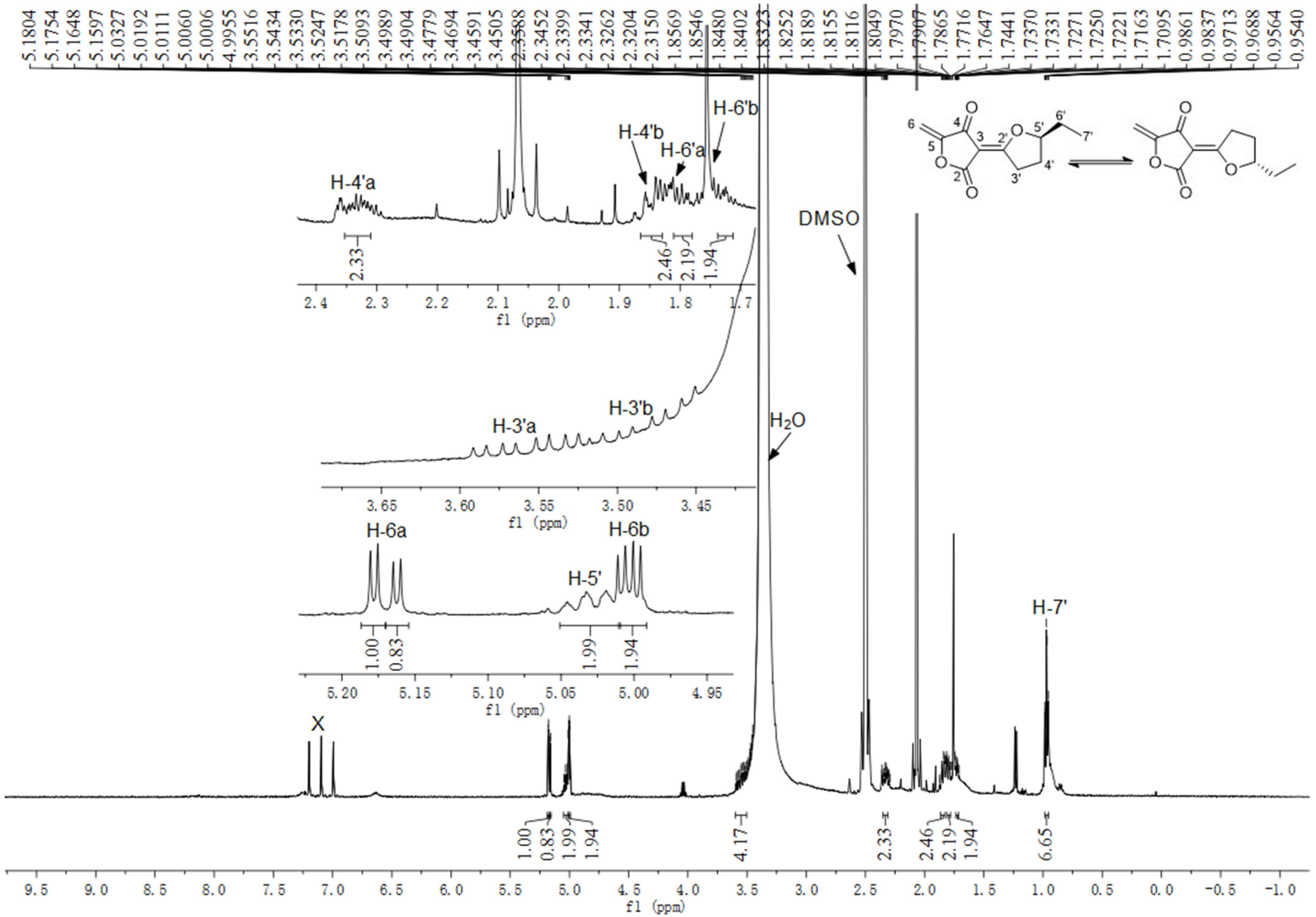


Figure S22. ^{1}H NMR spectrum of compound **3** in DMSO-d_6 (500MHz)

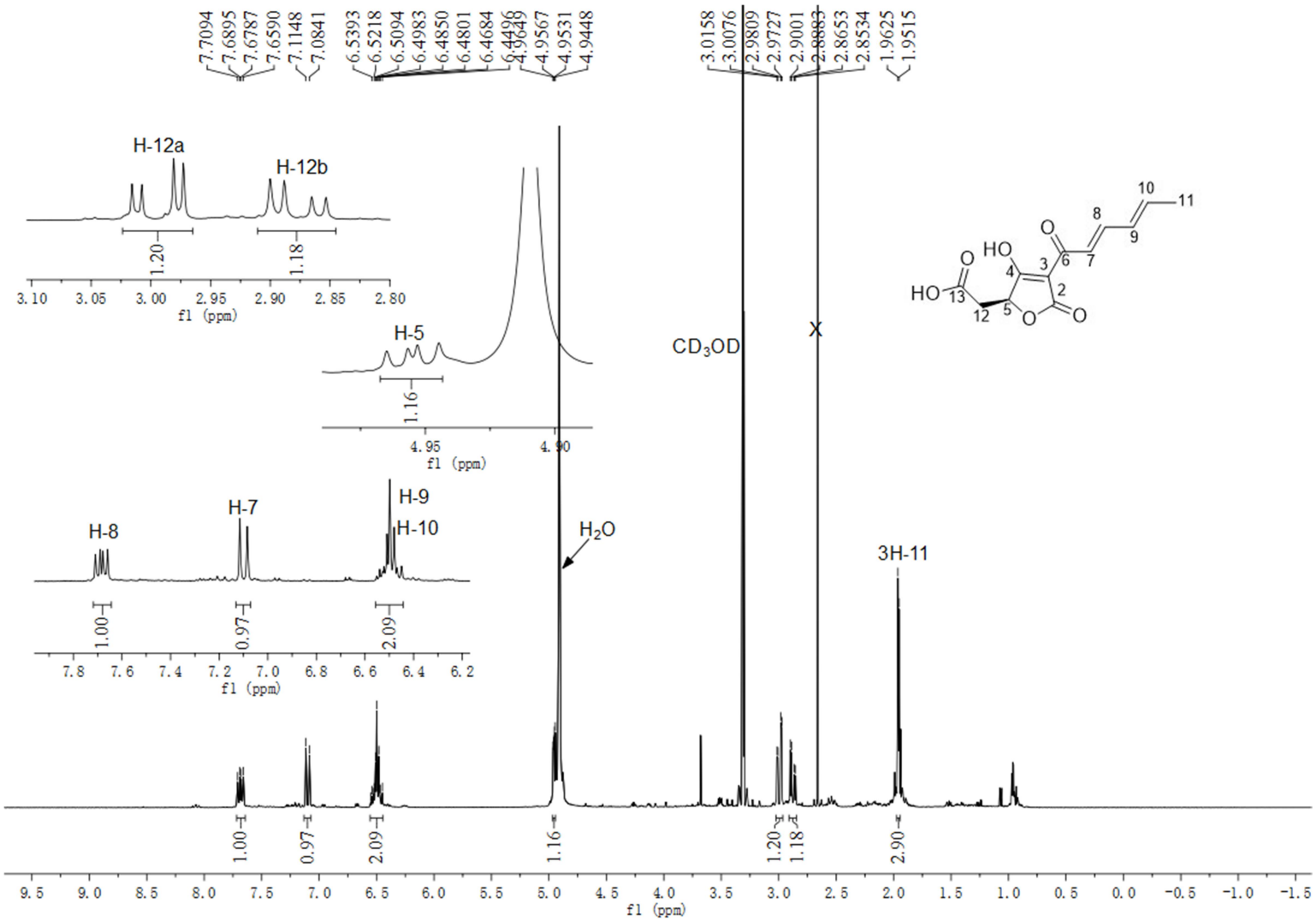


Figure S23. ^{1}H NMR spectrum of compound **4** isolated from *A. nidulans* JF15 harboring *traA* in CD_3OD (500MHz)

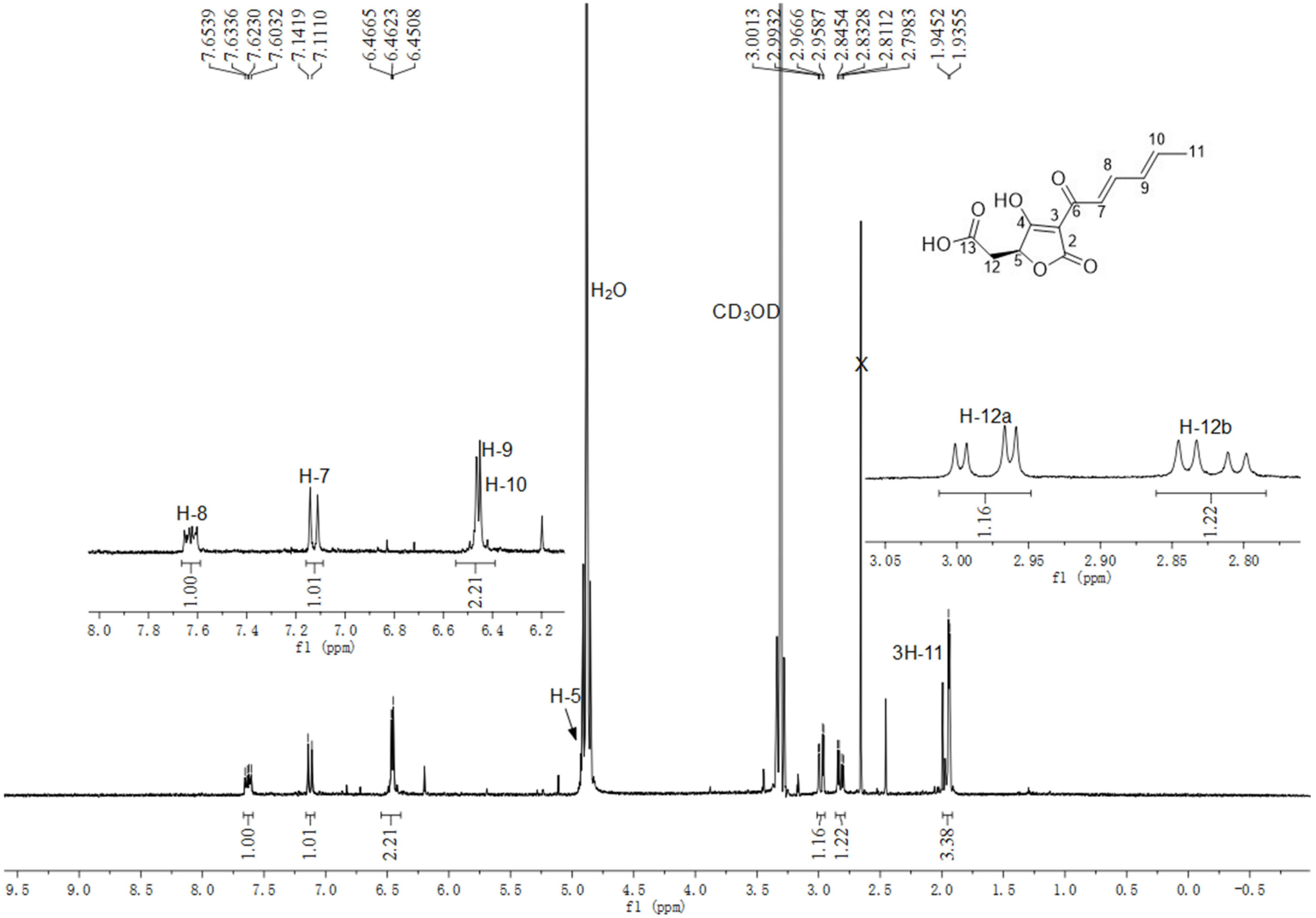


Figure S24. [1]H NMR spectrum of compound **4** isolated from Δ*traG*-mutant in CD_3OD (500MHz)

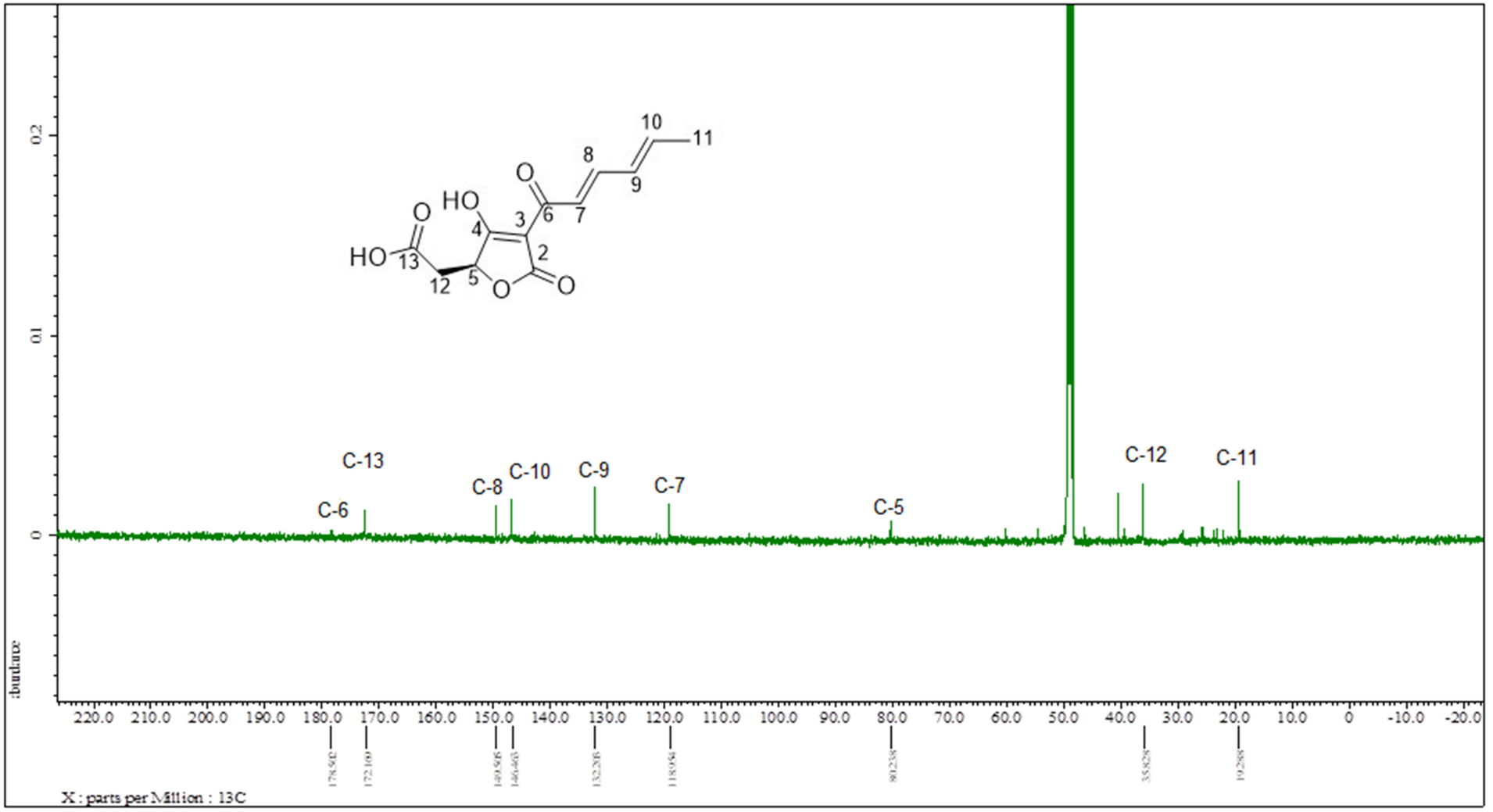


Figure S25. ^{13}C NMR spectrum of compound **4** isolated from *A. nidulans* JF15 harboring *traA* in CD_3OD (125MHz)

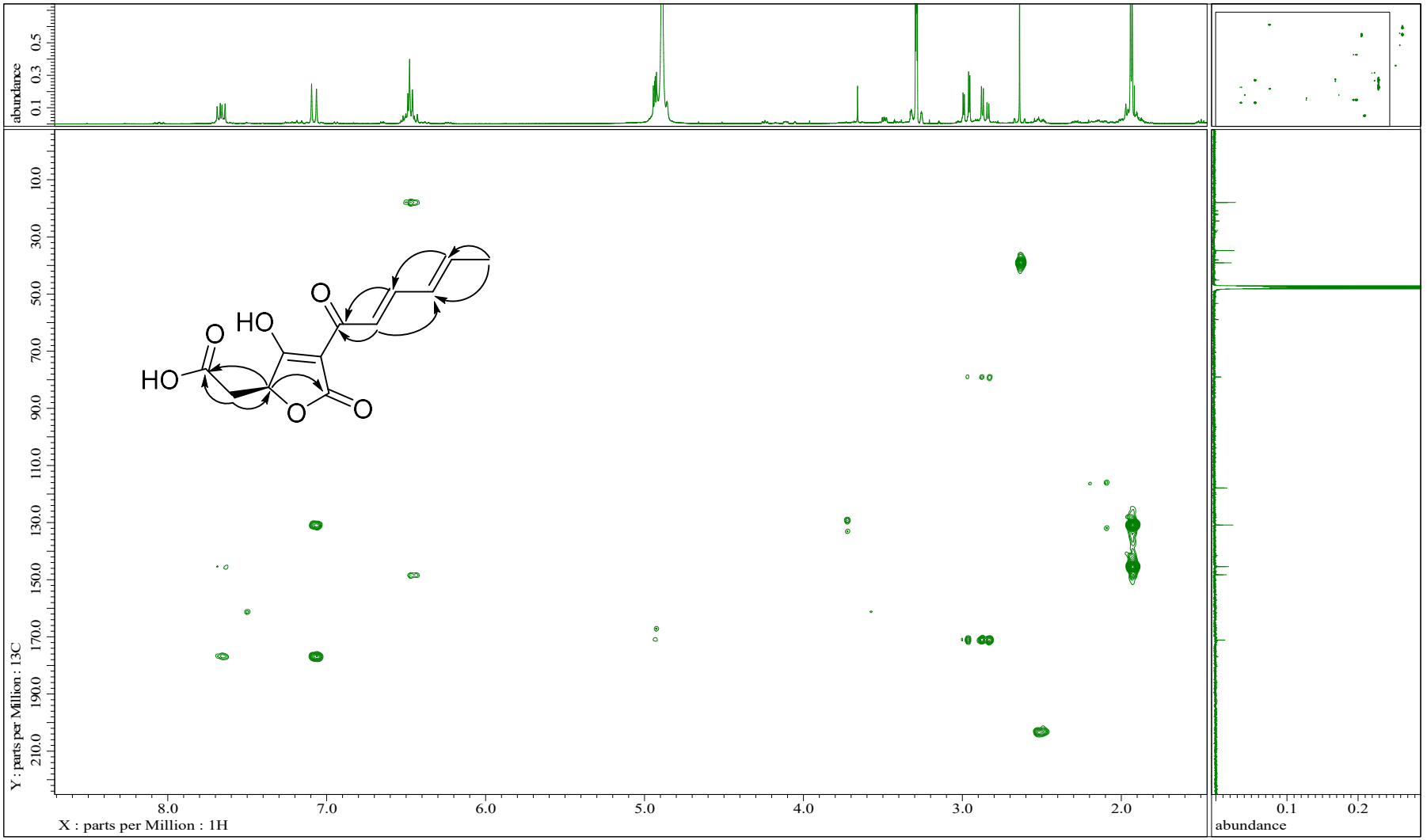


Figure S26. HMBC spectrum of compound **4** isolated from *A. nidulans* JF15 harboring *traA* in CD_3OD

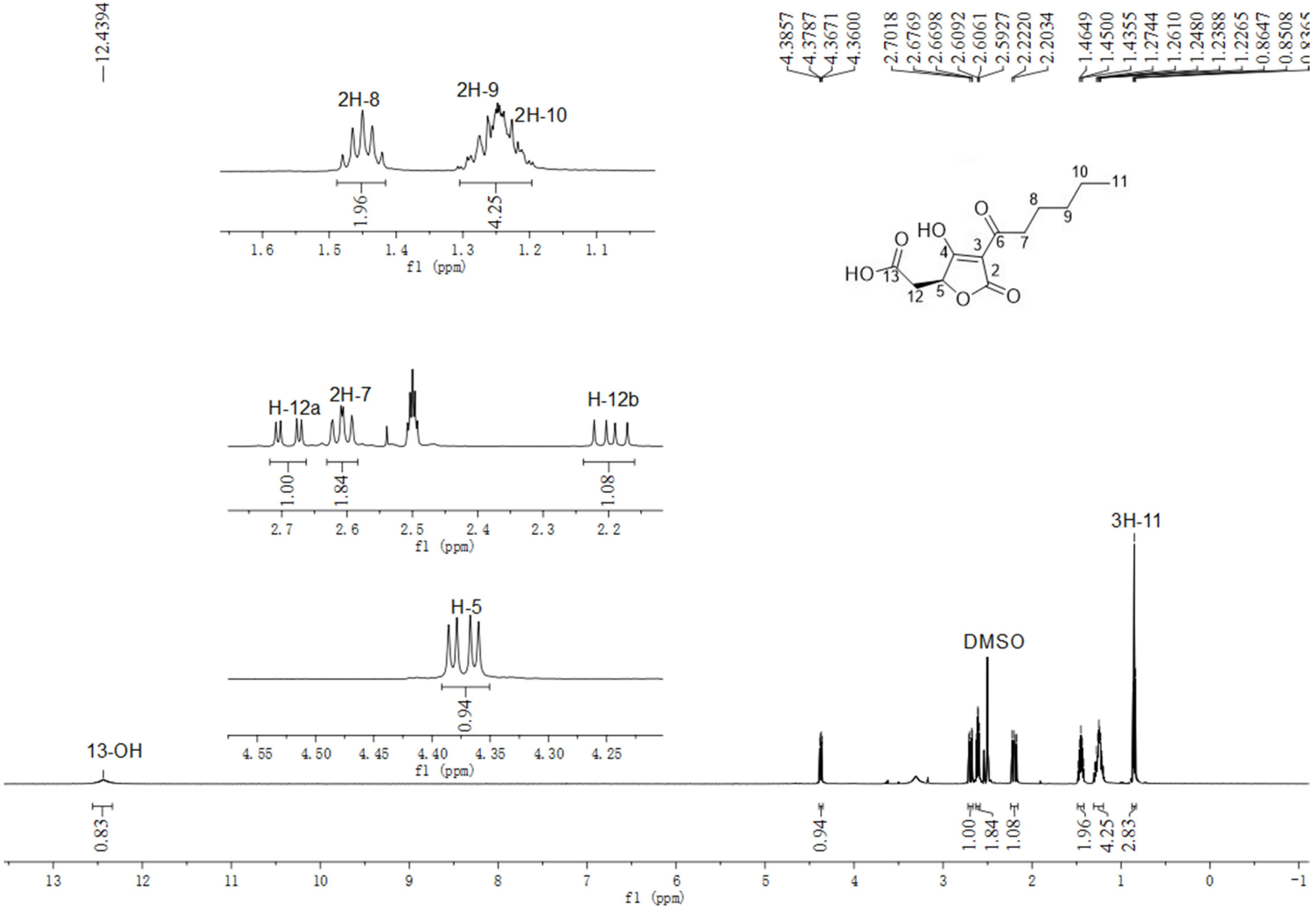


Figure S27. [1]H NMR spectrum of compound **5** isolated from *P. crustosum* PRB-2 in DMSO-d_6 (500MHz)

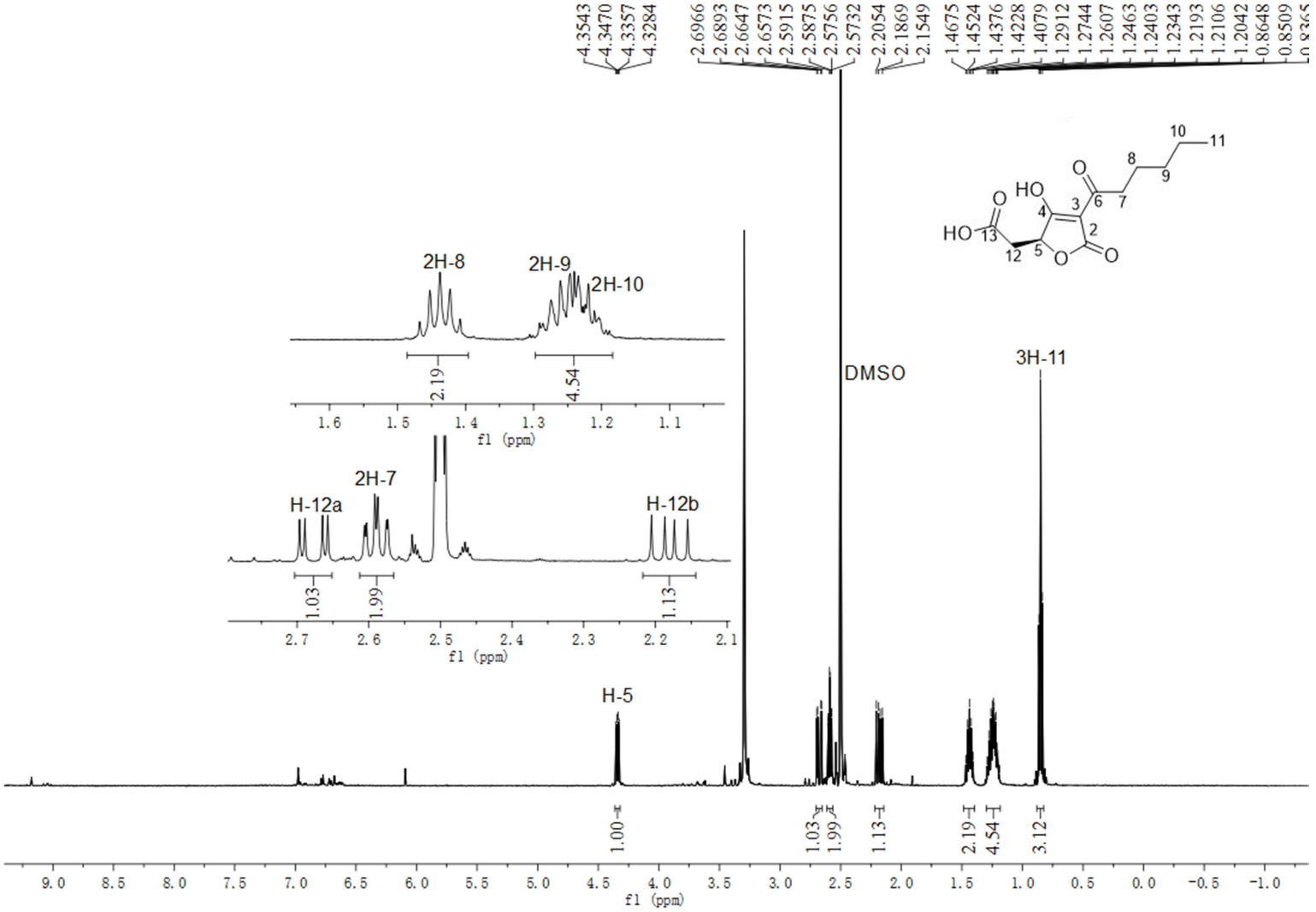


Figure S28. ^{1}H NMR spectrum of compound **5** isolated from *A. nidulans* JF45 harboring *traAG* in DMSO-d_6 (500MHz)

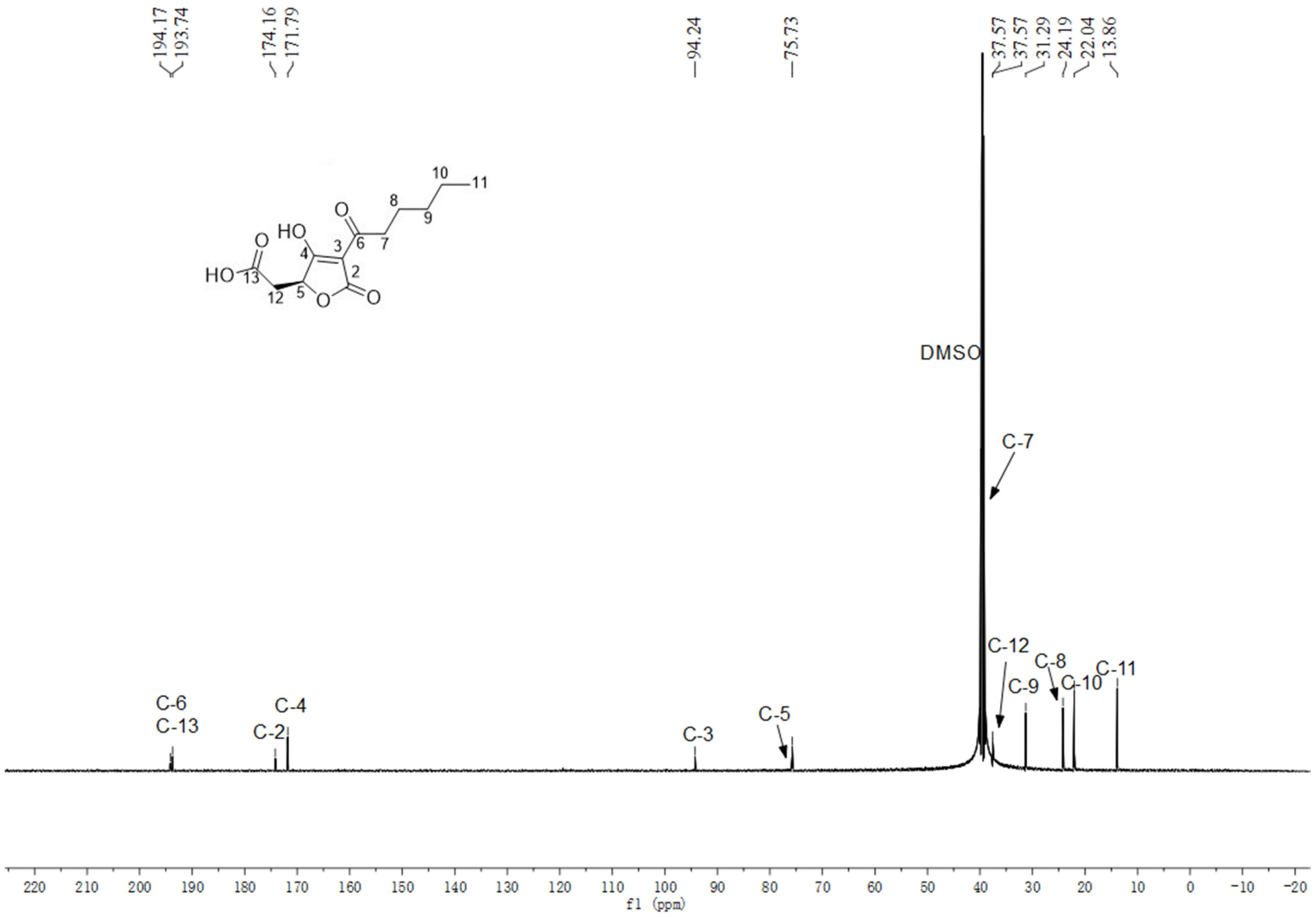


Figure S29. ^{13}C NMR spectrum of compound **5** isolated from *A. nidulans* JF45 harboring *traAG* in DMSO-d_6 (125MHz)

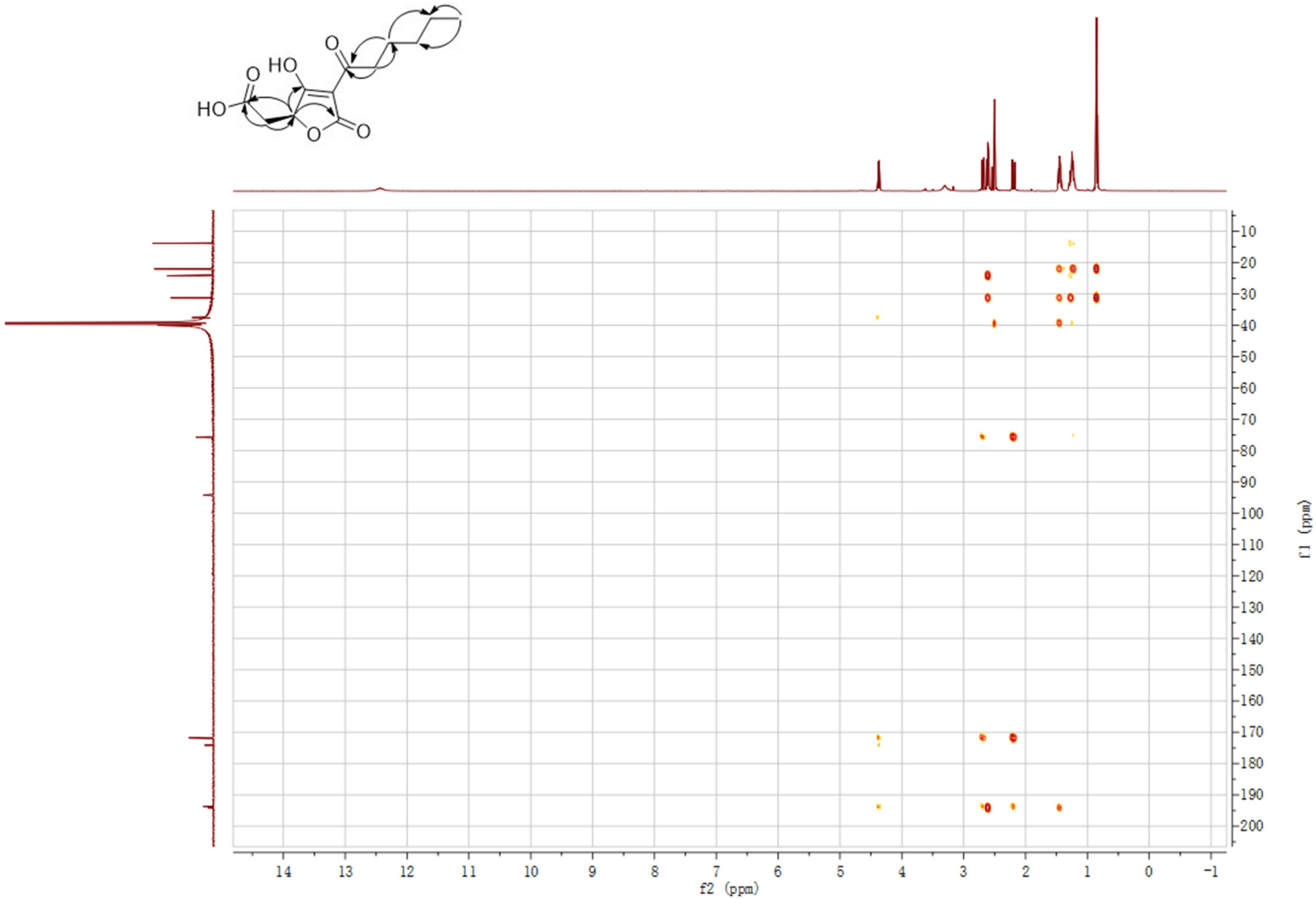


Figure S30. HMBC spectrum of compound **5** isolated from *A. nidulans* JF45 harboring *traAG* in DMSO-d_6

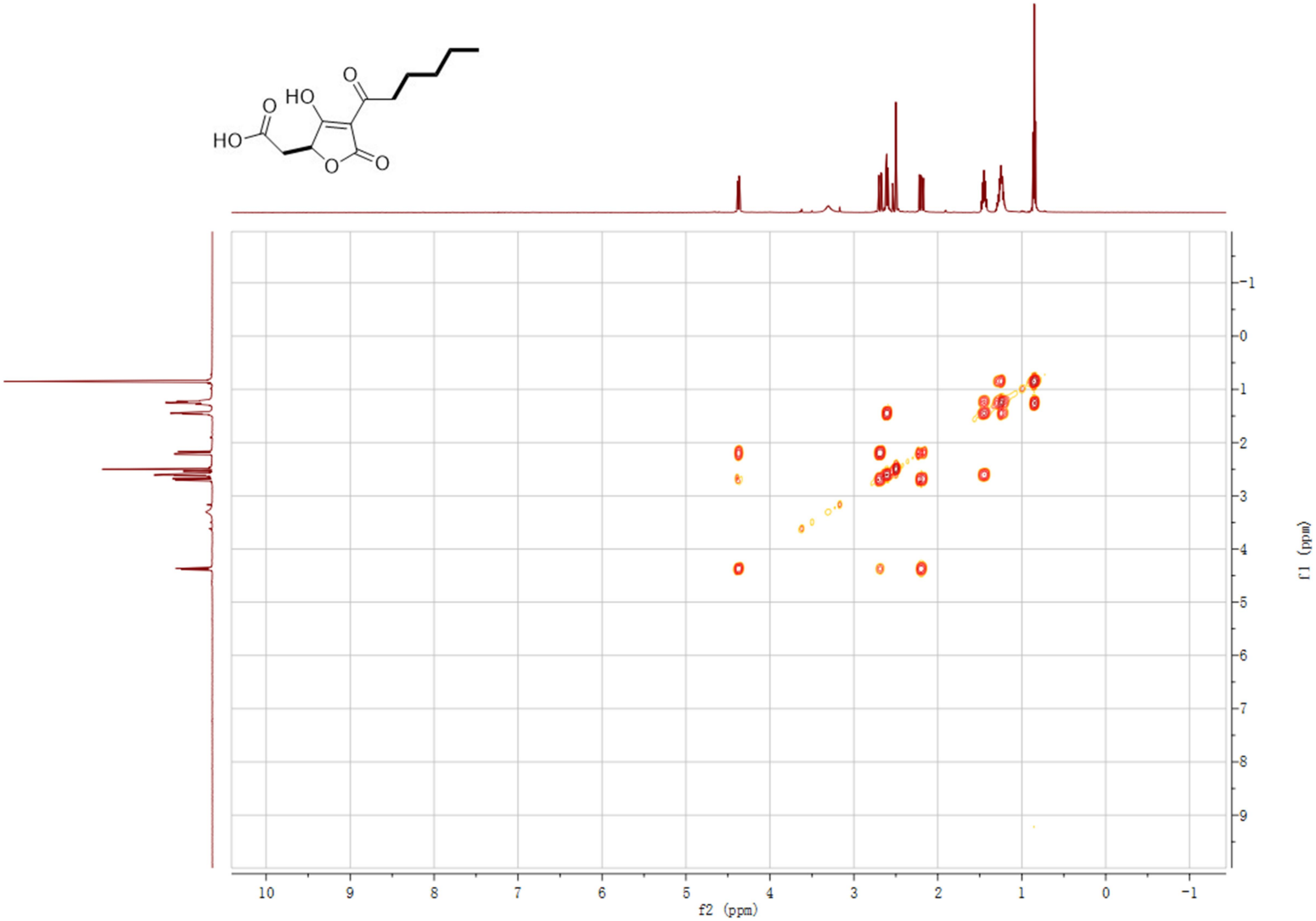


Figure S31. ^{1}H-^{1}H COSY spectrum of compound **5** isolated from *A. nidulans* JF45 harboring *traAG* in DMSO-d_6

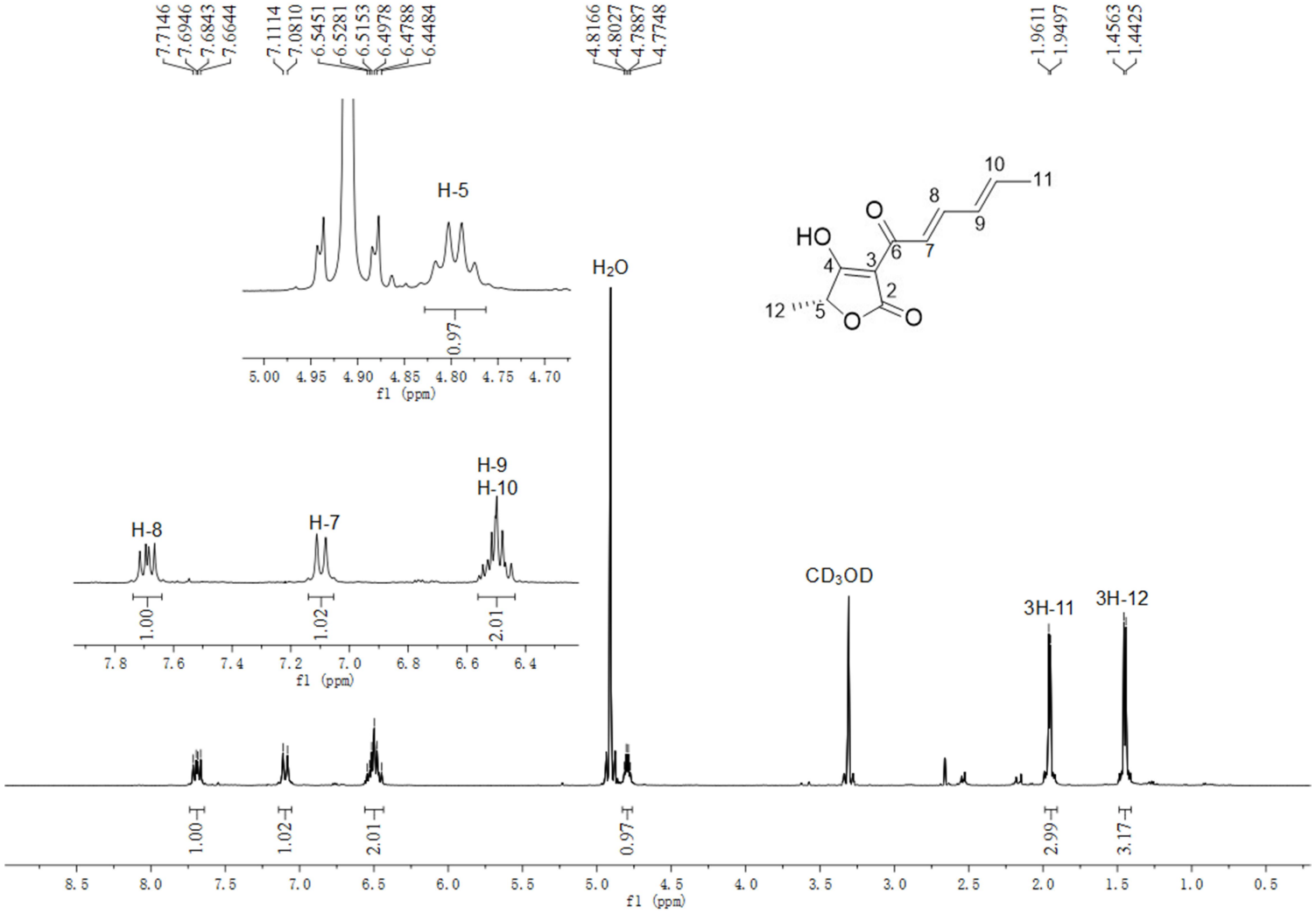


Figure S32. ^{1}H NMR spectrum of compound **6** in CD_3OD (500MHz)

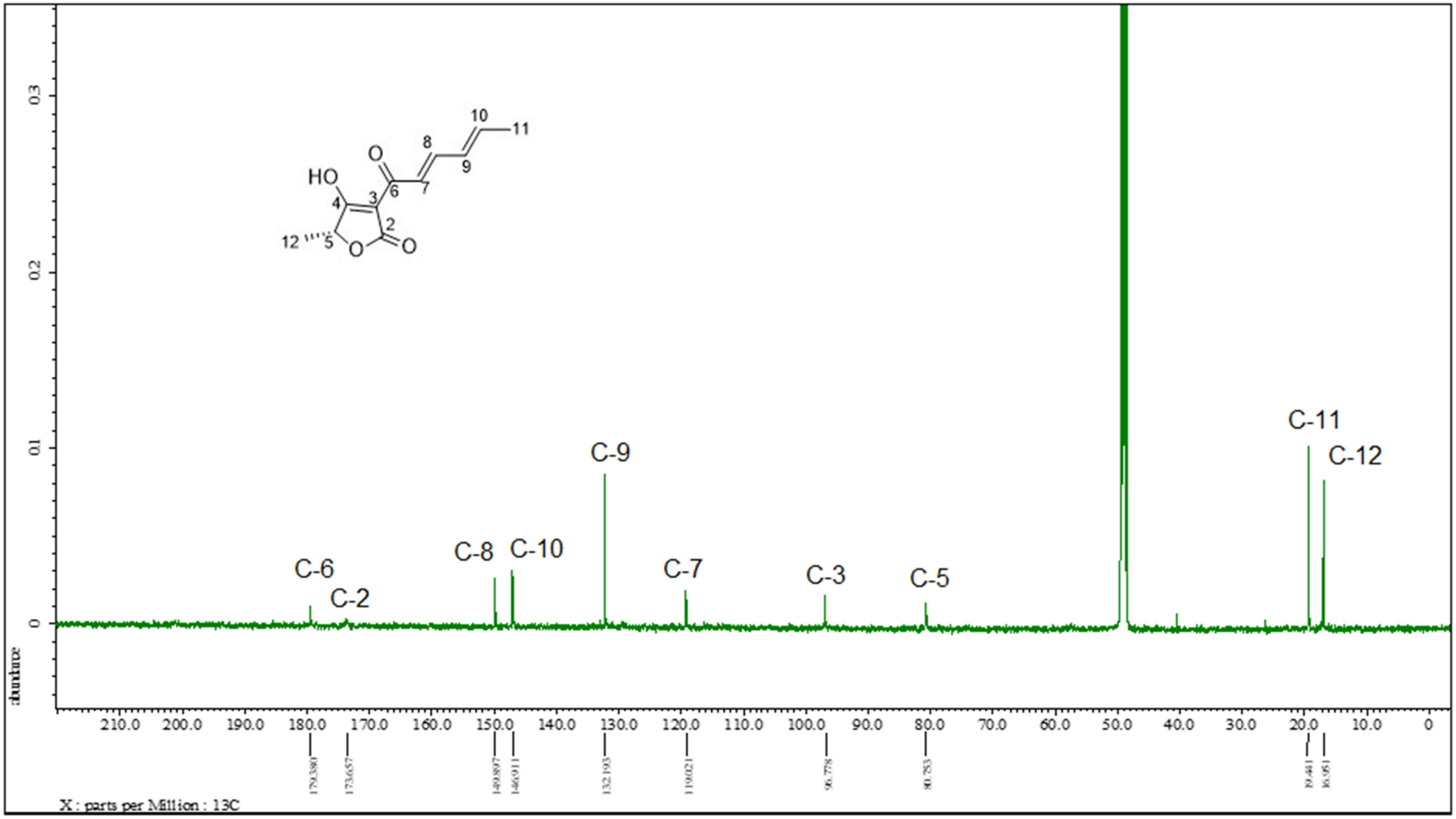


Figure S33. ^{13}C NMR spectrum of compound **6** in CD_3OD (125MHz)

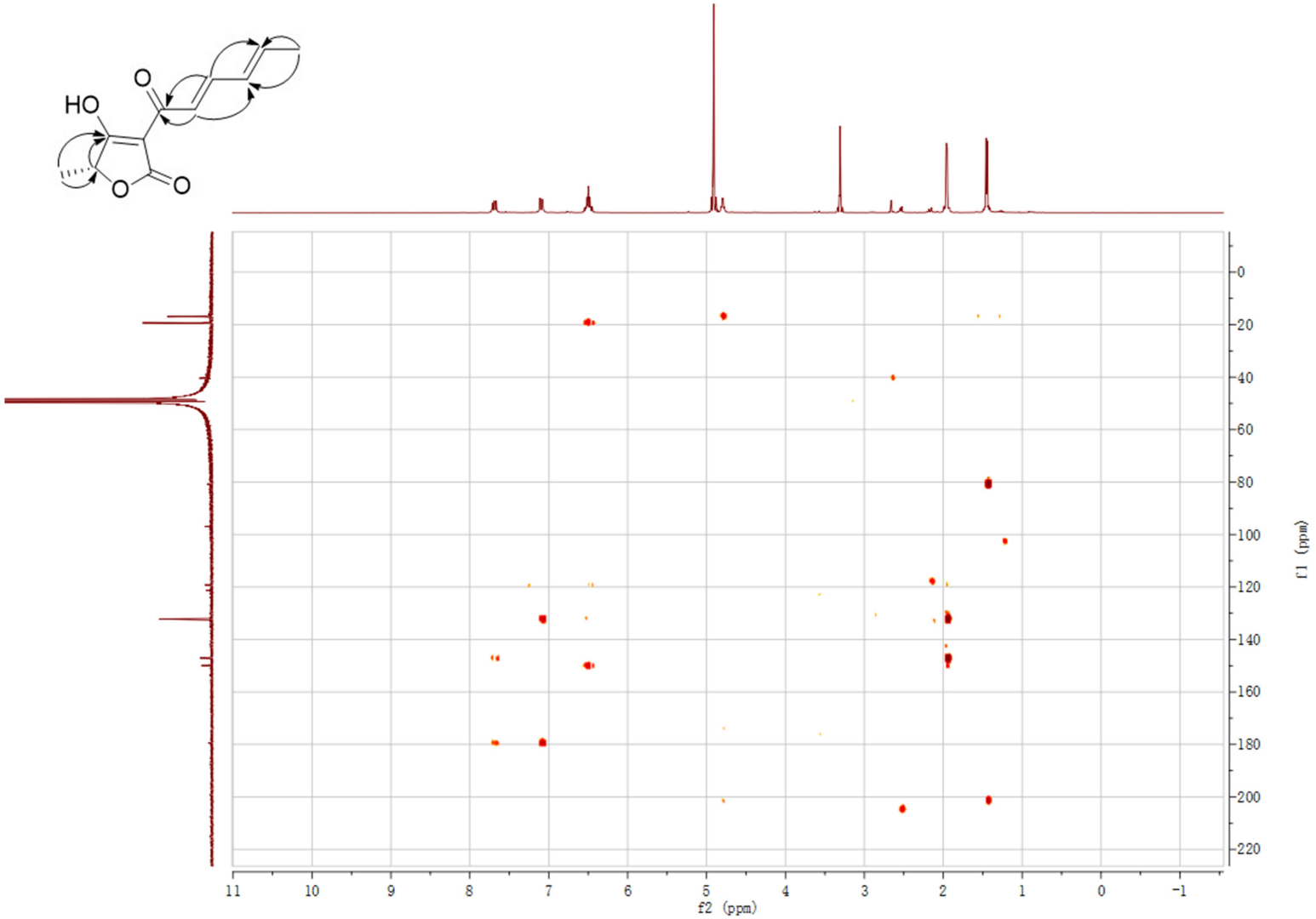


Figure S34. HMBC spectrum of compound **6** in CD_3OD

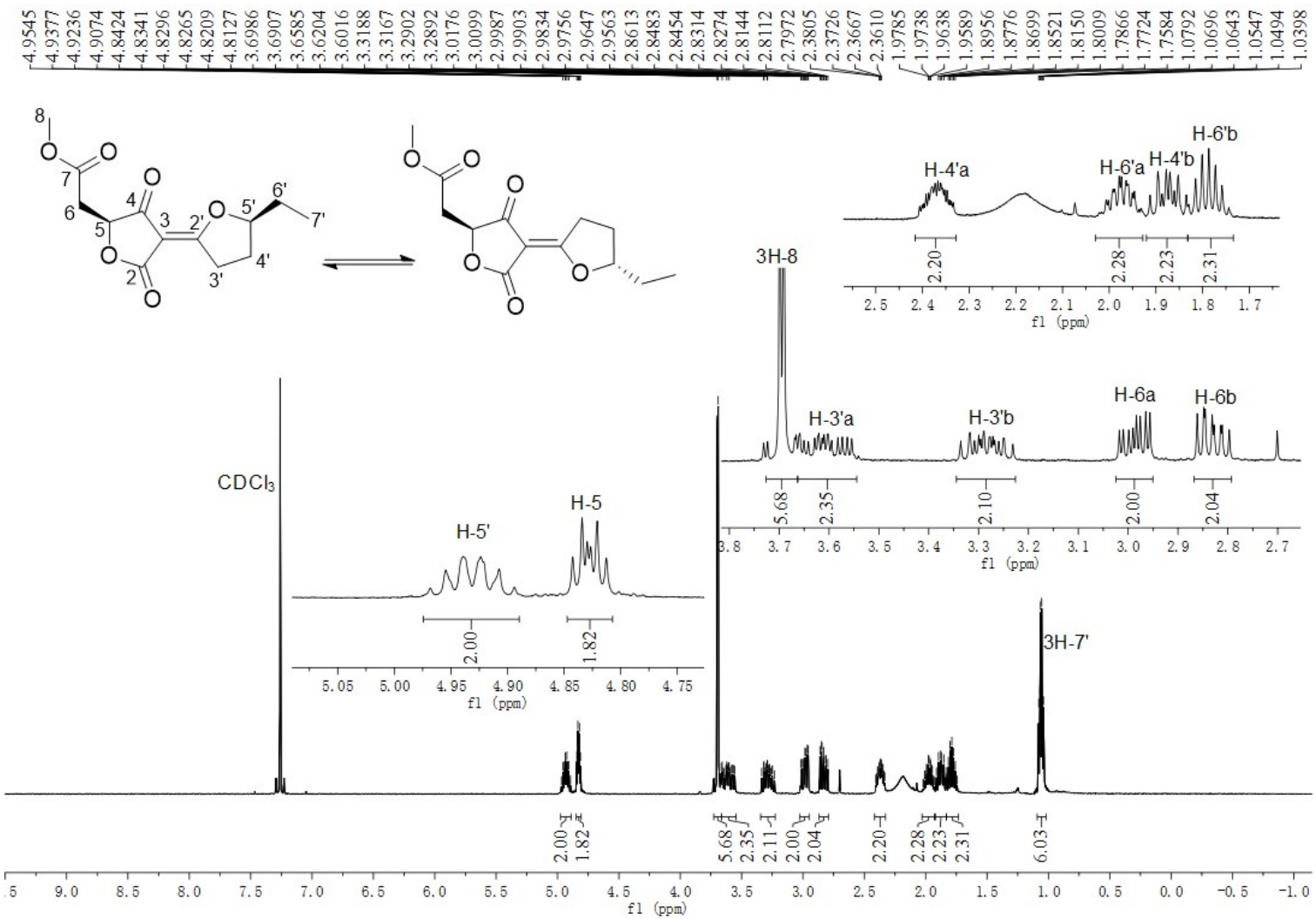


Figure S35. ^{1}H NMR spectrum of compound **7** in $CDCl_3$ (500MHz)

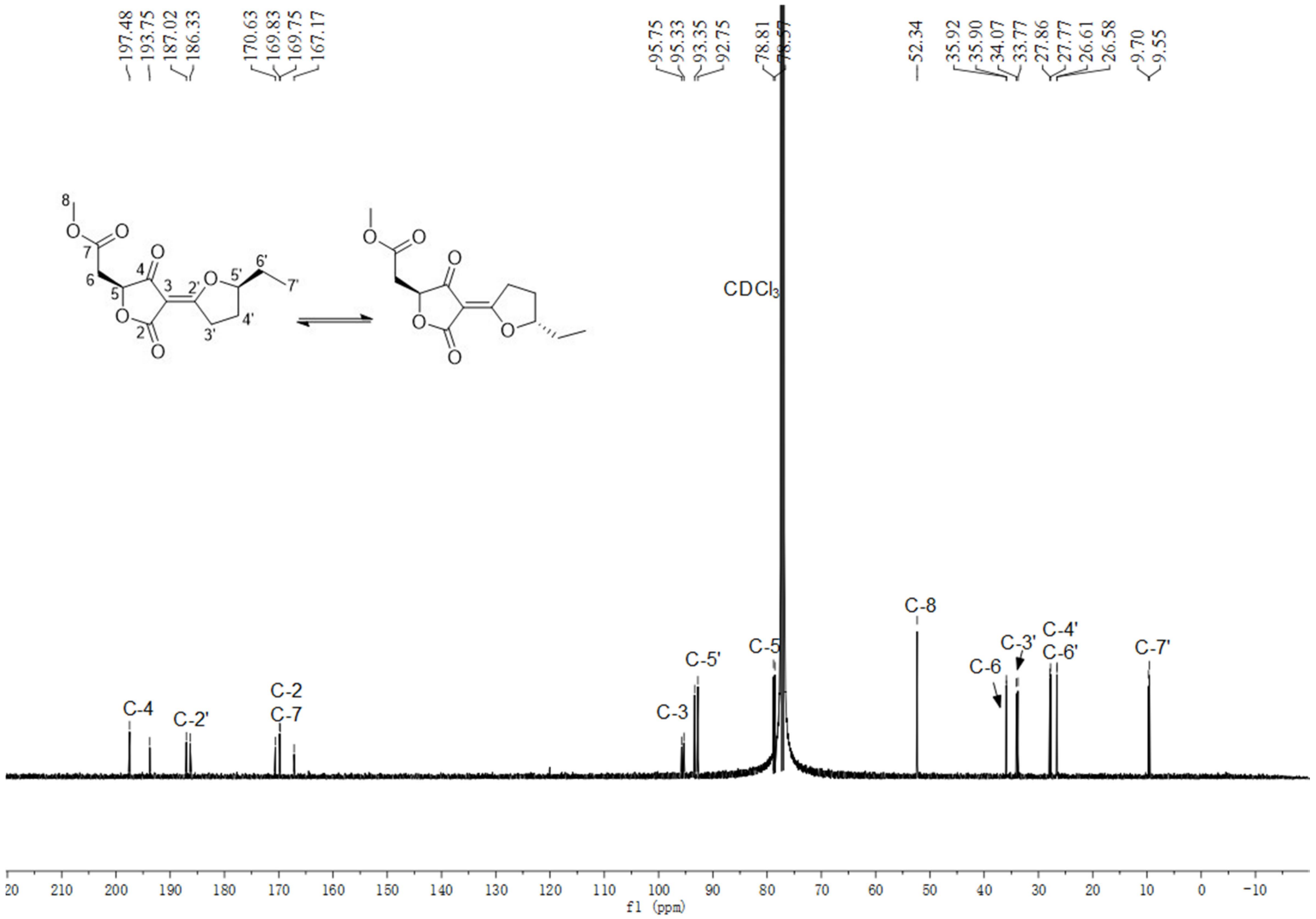


Figure S36. ^{13}C NMR spectrum of compound **7** in $CDCl_3$ (125MHz)

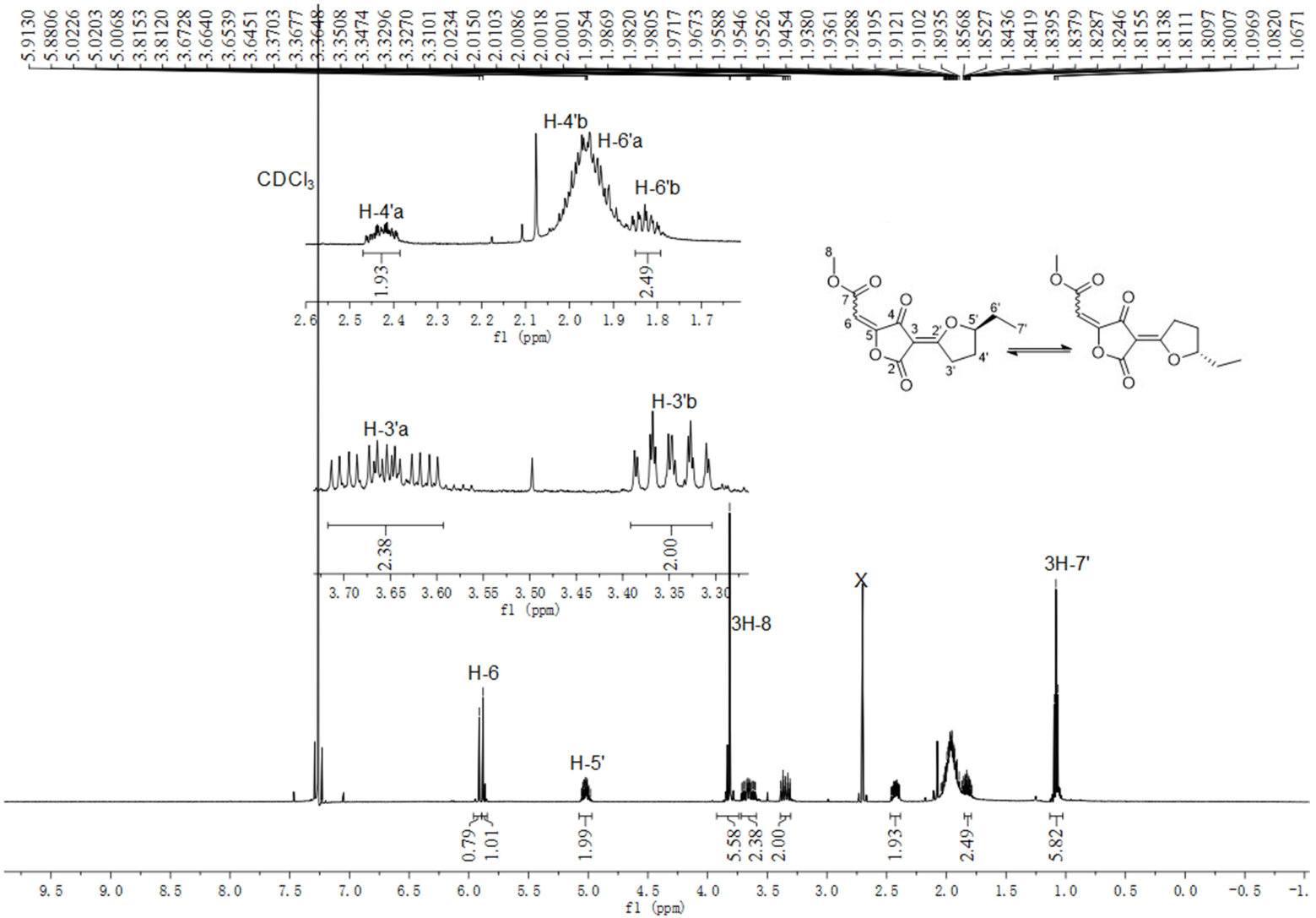


Figure S37. ^{1}H NMR spectrum of compound **8** in $CDCl_3$ (500MHz)

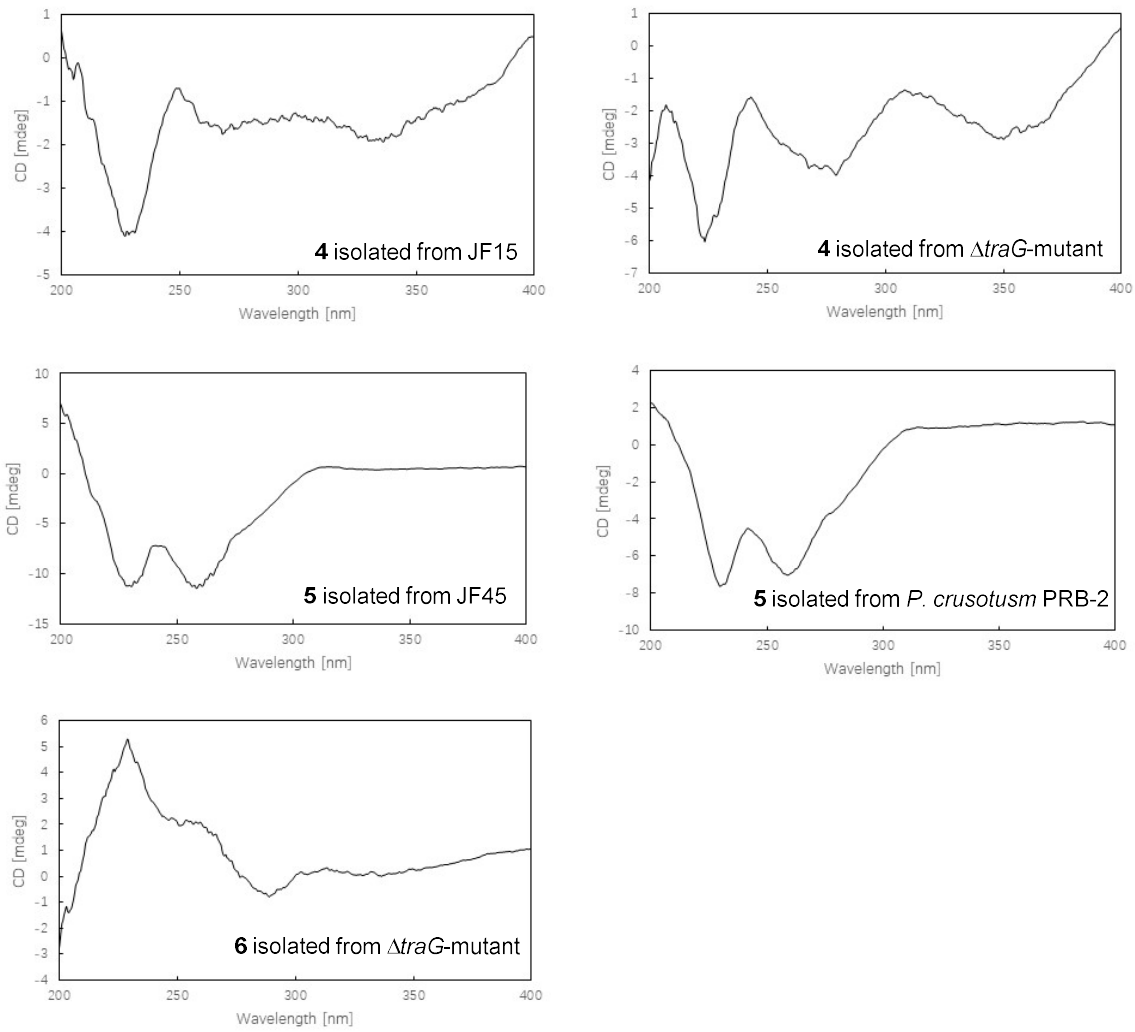


Figure S38. CD spectra of compounds **4** – **6**

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