SUPPORTING INFORMATION

β-Terrecyclene synthase constructs the quadrane backbone in terrecyclic acid biosynthesis

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Supplementary Methods

1. General materials and methods

Biological reagents, chemicals, media, and enzymes were purchased from standard commercial sources unless s stated. Fungal, yeast, and bacterial strains, plasmids, and primers used in this study are summarized in Tables S2, S3, and S4, respectively. DNA and RNA manipulations were carried out using the Zymo ZR Fungal/Bacterial DNA Microprep™ kit and Invitrogen Ribopure™ kit, respectively. DNA sequencing was performed at Laragen, Inc. Transcriptome sequencing was performed at UCLA Technology Center for Genomics & Bioinformatics Core Facility. The primers were synthesized by IDT, Inc and Beijing Tsingke Biotech Co. Ltd.

2. Construction of *Saccharomyces cerevisiae* strains

Plasmid pXW55[1] (*URA3* marker) digested with *Spe*I and *Pml*I was used to introduce the *terA* gene. A 1.1 kb fragment containing cDNA of *terA* obtained from PCR using primers TerA-xw55-recomb-F and TerA-xw55-recomb-R was cloned into pXW55 using yeast homologous recombination to afford pTerA-xw55. The plasmid pTerA-xw55 was then transformed into *Saccharomyces cerevisiae* RC01[1] to generate strain TY301 (Table S2). Yeast transformation was performed using Frozen-EZ Yeast Transformation II Kit™ (Zymo Research).

Plasmid pXW06[1] (*TRP1* marker) digested with *Nde*I and *Pme*I was used to introduce the *terB* gene. A 1.5 kb fragment containing cDNA of *terB* obtained from PCR using primers TerB-xw06-recomb-F and TerB-xw06-recomb-R was cloned into pXW06 using yeast homologous recombination to afford pTerB-xw06. The plasmid pTerA-xw55 and pTerB-xw06 were then transformed into *Saccharomyces cerevisiae* RC01 to generate strain TY325 (Table S2). Yeast transformation was performed using Frozen-EZ Yeast Transformation II Kit™ (Zymo Research).

Plasmid pXW02[1] (*LEU2* marker) digested with *Nde*I and *Pme*I was used to introduce the *terC* gene. A 0.8 kb fragment containing cDNA of *terC* obtained from PCR using primers TerC-xw02-recomb-F and TerC-xw02-recomb-R was cloned into pXW02 using yeast homologous recombination to afford pTerC-xw02. The plasmid pTerA-xw55, pTerB-xw06, and pTerC-xw02 were then transformed into *Saccharomyces cerevisiae* RC01 to generate strain TY326 (Table S2). Yeast transformation was performed using Frozen-EZ Yeast Transformation II Kit™ (Zymo Research).

The mutant pTerA-C111A-xw55 was generated from pTerA-xw55 by PCR using the forward primer

TerA-C111A-F and reverse primer TerA-C111A-R. The resulting plasmid pTerA-C111A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY310 (Table S2).

The mutant pTerA-V114A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-V114A-F and reverse primer TerA-V114A-R. The resulting plasmid pTerA-V114A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY311 (Table S2).

The mutant pTerA-F115A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-F115A-F and reverse primer TerA-F115A-R. The resulting plasmid pTerA-F115A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY312 (Table S2).

The mutant pTerA-D116A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-D116A-F and reverse primer TerA-D116A-R. The resulting plasmid pTerA-D116A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY313 (Table S2).

The mutant pTerA-L120A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-L120A-F and reverse primer TerA-L120A-R. The resulting plasmid pTerA-L120A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY314 (Table S2).

The mutant pTerA-F189A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-F189A-F and reverse primer TerA-F189A-R. The resulting plasmid pTerA-F189A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY315 (Table S2).

The mutant pTerA-I219A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-I219A-F and reverse primer TerA-I219A-R. The resulting plasmid pTerA-I219A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY316 (Table S2).

The mutant pTerA-Q260A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-Q260A-F and reverse primer TerA-Q260A-R. The resulting plasmid pTerA-Q260A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY317 (Table S2).

The mutant pTerA-W342A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-W342A-F and reverse primer TerA-W342A-R. The resulting plasmid pTerA-W342A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY319 (Table S2).

The mutant pTerA-S343A-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-S343A-F and reverse primer TerA-S343A-R. The resulting plasmid pTerA-S343A-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY320 (Table S2).

The mutant pTerA-C111S-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-C111S-F and reverse primer TerA-C111S-R. The resulting plasmid pTerA-C111S-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY330 (Table S2).

The mutant pTerA-C111Y-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-C111Y-F and reverse primer TerA-C111Y-R. The resulting plasmid pTerA-C111Y-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY331 (Table S2).

The mutant pTerA-C111D-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-C111D-F and reverse primer TerA-C111D-R. The resulting plasmid pTerA-C111D-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY332 (Table S2).

The mutant pTerA-V114L-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-V114L-F and reverse primer TerA-V114L-R. The resulting plasmid pTerA-V114L-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY333 (Table S2).

The mutant pTerA-V114F-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-V114F-F and reverse primer TerA-V114F-R. The resulting plasmid pTerA-V114F-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY334 (Table S2).

The mutant pTerA-V114T-xw55 was generated from pTerA-xw55 by PCR using the forward primer

TerA-V114T-F and reverse primer TerA-V114T-R. The resulting plasmid pTerA-V114T-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY335 (Table S2).

The mutant pTerA-V114D-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-V114D-F and reverse primer TerA-V114D-R. The resulting plasmid pTerA-V114D-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY336 (Table S2).

The mutant pTerA-F115Y-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-F115Y-F and reverse primer TerA-F115Y-R. The resulting plasmid pTerA-F115Y-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY337 (Table S2).

The mutant pTerA-F115W-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-F115W-F and reverse primer TerA-F115W-R. The resulting plasmid pTerA-F115W-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY338 (Table S2).

The mutant pTerA-D116E-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-D116E-F and reverse primer TerA-D116E-R. The resulting plasmid pTerA-D116E-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY339 (Table S2).

The mutant pTerA-D116N-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-D116N-F and reverse primer TerA-D116N-R. The resulting plasmid pTerA-D116N-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY340 (Table S2).

The mutant pTerA-L120I-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-L120I-F and reverse primer TerA-L120I-R. The resulting plasmid pTerA-L120I-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY341 (Table S2).

The mutant pTerA-L120V-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-L120V-F and reverse primer TerA-L120V-R. The resulting plasmid pTerA-L120V-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY342 (Table S2).

The mutant pTerA-F189Y-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-F189Y-F and reverse primer TerA-F189Y-R. The resulting plasmid pTerA-F189Y-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY343 (Table S2).

The mutant pTerA-F189W-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-F189W-F and reverse primer TerA-F189W-R. The resulting plasmid pTerA-F189W-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY344 (Table S2).

The mutant pTerA-Q260N-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-Q260N-F and reverse primer TerA-Q260N-R. The resulting plasmid pTerA-Q260N-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY345 (Table S2).

The mutant pTerA-Q260E-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-Q260N-F and reverse primer TerA-Q260E-R. The resulting plasmid pTerA-Q260E-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY346 (Table S2).

The mutant pTerA-W342F-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-W342F-F and reverse primer TerA-W342F-R. The resulting plasmid pTerA-W342F-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY351 (Table S2).

The mutant pTerA-W342Y-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-W342Y-F and reverse primer TerA-W342Y-R. The resulting plasmid pTerA-W342Y-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY352 (Table S2).

The mutant pTerA-S343T-xw55 was generated from pTerA-xw55 by PCR using the forward primer TerA-S343T-F and reverse primer TerA-S343T-R. The resulting plasmid pTerA-S343T-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY353 (Table S2).

The mutant pTerA-S343F-xw55 was generated from pTerA-xw55 by PCR using the forward primer

TerA-S343F-F and reverse primer TerA-S343F-R. The resulting plasmid pTerA-S343F-xw55 was verified by DNA sequencing and then transformed into *S. cerevisiae* RC01 to give TY354 (Table S2).

3. Construction of *Aspergillus nidulans* strains

Plasmid pYTU (*pyrG* marker)[2], pYTR (*riboB* marker)[2], and pYTP (*pyroA* marker)[2] digested with *Pac*I and *Swa*I were used as vectors to insert genes. Genes to be expressed were amplified through PCR using the cDNA of *Aspergillus terreus* ATCC 20516 as a template. A 1.1 kb fragment obtained using primers TerA-pYTU-recomb-F and TerA-pYTU-recomb-R was cloned into pYTU by yeast homologous recombination to obtain pTerA-pYTU. A 1.5 kb fragment obtained using primers TerB-pYTR-recomb-F and TerB-pYTR-recomb-R was cloned into pYTR by yeast homologous recombination to obtain pTerB-pYTR. A 0.8 kb fragment obtained using primers TerC-pYTP-recomb-F and TerC-pYTP-recomb-R was cloned into pYTP by yeast homologous recombination to obtain pTerC-pYTP. Both plasmids of pTerA-pYTU and pTerB-pYTR were transformed into *A. nidulans* A1145 following standard protocols[2] to result in the *A. nidulans* strain TY302. Briefly, the recombinant plasmids were dissolved in STC buffer (1.2 M sorbitol, 10 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.5), and added to the protoplasts of *A. nidulans*. The mixture was grown on the regeneration dropout solid medium (CD medium with 1.2 mM sorbitol and appropriate supplements) at 37 °C for about 2 days. All three plasmids (pTerA-pYTU, pTerB-pYTR, and pTerC-pYTP) were transformed into *A. nidulans* to result in the *A. nidulans* strain TY303. All three empty vectors pYTU, pYTR, and pYTP were transformed into *A. nidulans* to result in the *A. nidulans* strain TY305 as the control strain in this study.

4. Characterization of TerA

Heterologous expression and purification of TerA. To express TerA, TY301 was first grown in 2 mL uracil drop-out medium (Bacto technical grade casamino acids 5 g/L, Difco yeast nitrogen base without amino acid, with ammonium sulfate 6.7 g/L, tryptophan 0.02 g/L, adenine 0.02 g/L, dextrose 20 g/L) at 28 °C for 1 d, the culture of TY301 was then transferred into 1 L fresh YPD medium (yeast extract 10 g/L, peptone 20 g/L) supplemented with 2% dextrose, and the culture was shaken at 28 °C at 250 rpm for 2 d. The cells were harvested by centrifugation (3,800 rpm, 30 min, 4 °C) and resuspended in TBS buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl). After sonication and centrifugation (12,000 rpm, 60 min, 4 °C), the supernatant was subjected to His-tag affinity purification. Purified proteins in buffer (50 mM Tris-HCl, pH 7.2, 50 mM NaCl, and 8% glycerol) were aliquoted,

flash-frozen in liquid nitrogen, and stored at -80 °C.

Biochemical analysis of TerA. The *in vitro* biochemical assay was performed in the 100 μL reaction mixture (50 mM Tris-HCl pH 7.5, 1 μM TerA, 1 mM FPP or [12-$^2H_3$]-FPP and 5 mM $MgCl_2$). The reaction was incubated at 28 °C overnight and extracted twice with 50 μL hexanes. The organic phase was subjected to GC-MS analysis. GC-MS analyses were performed using SHIMADZU GCMS-QP2010 equipped with a DB-5 ms column. An inlet temperature of 250 °C and constant pressure of 68.1 KPa were used. The oven temperature was initially at 50 °C for 3 min and then ramped at 10 °C/min to 320 °C, followed by a hold at 300 °C for 5 min. To further separate compounds **25** and **34** in Figure 3B, another GC method with a longer running time was used: the oven temperature was initially at 50 °C for 3 min and then ramped at 10 °C/min to 120 °C, 1 °C /min to 185 °C, 15 °C /min to 300 °C, followed by a hold at 300 °C for 3 min.

Determination of product distributions and deuterium kinetic isotope effect. Triplicate assays were performed on the TerA-V114A mutant using FPP and deuterium-labeled substrate [12-$^2H_3$]-FPP, respectively. The enzymatic products of TerA were quantified by GC-MS analysis. When FPP was used as the substrate, the relative abundances of β-terrecyclene (**25**) and β-caryophyllene (**34**) were determined by the normalized intensity of the extracted ion with *m/z* 204, which were 850 ± 30 and 3400 ± 120, respectively. Consequently, the ratio of relative abundance of **25** to **34** was found to be 1:4. On the other hand, when [12-$^2H_3$]-FPP was employed as the substrate, the relative abundances of deuterium-labeled products [12-$^2H_2$]-**25** and [12-$^2H_2$]-**34** were quantified using the normalized intensity of the extracted ion with *m/z* 206, which were 400 ± 28 and 4000 ± 95, respectively. Therefore, the ratio of relative abundance of **25** to **34** was determined to be 1:10. As a result, when [12-$^2H_3$]-FPP was utilized as the substrate, there was a significant shift in the distribution of sesquiterpene products **25** and **34**, with the intensity of **25** being reduced by approximately 53%, and the intensity of **34** increasing by around 18%. The observed value of the primary kinetic isotope effect (KIE) on the deprotonation of cation intermediate **26** was calculated based on the altered distribution of the products **25** and **34** after the utilization of [12-$^2H_3$]-FPP as the substrate, using a previously reported equation[3], as follows:

$$\frac{k_H}{k_D} = \frac{\left(\frac{\text{abundance of } \mathbf{25}}{\text{abundance of } \mathbf{34}}\right)_{FPP}}{\left(\frac{\text{abundance of } [12\text{-}^2H_3]\text{-}\mathbf{25}}{\text{abundance of } [12\text{-}^2H_3]\text{-}\mathbf{34}}\right)_{[12\text{-}^2H_3]\text{-FPP}}} = \frac{\left(\frac{850}{3400}\right)}{\left(\frac{400}{4000}\right)} = 2.5$$

5. Fermentation, bioconversion, compound analyses, and isolation

The fermentation of *A. nidulans* strains expressing *ter* genes was carried out in liquid CD-ST medium (20 g/L starch, 20 g/L casein hydrolysate, 6 g/L $NaNO_3$, 1.52 g/L KH_2PO_4, 0.52 g/L $MgSO_4 \cdot 7H_2O$, 0.52 g/L KCl, and 1 mL/L trace elements solution) at 28 °C, 220 rpm, 3d. To analyze and isolate the metabolites, fermentation broth was centrifuged at 3800 rpm for 10 min, and supernatant was extracted using ethyl acetate three times. The organic phase was combined and dried over sodium sulfate, and concentrated to oil form, which was subjected to HPLC analysis and purification.

Fermentation of the *S. cerevisiae* expressing *ter* genes was carried out using YPD medium supplemented with 2% dextrose for 3 days at 28 °C, 250 rpm. Then the fermentation broth was centrifuged at 3800 rpm for 10 min, and the supernatant was extracted using ethyl acetate three times. The organic phase was combined and dried over sodium sulfate, and concentrated to oil form, which was subjected to GC or HPLC analysis.

GC-MS analyses of the metabolites were performed using SHIMADZU GCMS-QP2010 equipped with a DB-5 ms column. An inlet temperature of 250 °C and constant pressure of 68.1 KPa were used. The oven temperature was initially at 50 °C for 3 min and then ramped at 10 °C/min to 320 °C, followed by a hold at 300 °C for 5 min.

HPLC-MS analyses were performed using a Shimadzu LC-MS 2020 (Phenomenex® Kinetex, 1.7 μm, 2.0 × 100 mm, C18 column) using positive and negative mode electrospray ionization. The elution was performed using water and acetonitrile with gradient as follows: 0-2.0 min, 5% acetonitrile (*v/v*); 2.0-18.0 min, 5-95% acetonitrile (*v/v*); 18.0-22.0 min, 95% acetonitrile (*v/v*); 22.0-22.1 min, 95-5% acetonitrile (*v/v*); 22.1-25.0 min, 5% acetonitrile (*v/v*) at flow rate of 0.3 mL/min. The HPLC buffers were supplemented with 0.1% formic acid (*v/v*). However, this general analytical method used in Figures S5 and S12 was not able to separate compounds **32** and **33**. Therefore, the LC-MS method was optimized to analyze the samples in Figures 2C, 2D, 2E, and S6 as follows. To separate compounds **32** and **33**, HPLC-MS analyses were performed using a Shimadzu LC-MS 2020 (Waters ACQUITY UPLC®BEH C18, 1.7 μm, 2.1 × 100 mm column) using positive and negative mode electrospray ionization. The elution was performed using water and acetonitrile with gradient as follows: 0-2.0 min, 5% acetonitrile (v/v); 2.0-41.0 min, 5-55% acetonitrile (v/v); 41.0-41.1 min, 55-100% acetonitrile (v/v); 41.1-47.0 min, 100% acetonitrile (v/v); 47.0-47.1 min, 95-5% acetonitrile (v/v); 47.1-50.0 min, 5% acetonitrile (v/v) at flow rate of 0.3 mL/min. The HPLC buffers were supplemented with 0.1% formic

acid (v/v).

HPLC purifications were performed using a Shimadzu Prominence HPLC (Phenomenex® Kinetex, 5 μm, 10.0 × 250 mm, C18 column). The elution method was a linear gradient of 65-100% (*v/v*) acetonitrile/water in 25 min, with a flow rate of 2.5 mL/min.

Isolation of compound 25. A seed culture of *S. cerevisae* strain TY301, heterologously expressing *terA*, was cultivated in 200 mL of uracil drop-out medium for 2 days at 28 ℃ and 200 rpm. Subsequently, a 5 L fermentation of the yeast was conducted in YPD medium for 3 days at 28 ℃ and 200 rpm. To isolate **25**, the fermentation broth of TY301 was centrifuged (3600 rpm, 10 min), and the resulting cell pellet was collected and immersed in 1L of acetone. The organic phase was then dried over sodium sulfate, concentrated to oil form, and subjected to silica column purification using hexane. The compound **25** (60.5 mg) was obtained as a colorless oil that readily dissolved in hexanes and chloroform.

Isolation of compounds 32, 2, and 33. A seed culture of *A. nidulans* strain TY302, heterologously expressing *terAB*, was cultivated on CD agar medium (glucose 10 g/L, $NaNO_3$ 6 g/L, KH_2PO_4 1.52 g/L, $MgSO_4 \cdot 7H_2O$ 0.52 g/L, KCl 0.52 g/L, trace elements solution 1 mL/L, agar 20 g/L, pH 6.5) at 28 ℃ for 3 days. The spores were then harvested and transferred to CD-ST liquid medium to produce compounds **32**, **2**, and **33** for an additional 3 days at 28°C. The fermentation broth of TY302 was collected and extracted three times using ethyl acetate. The organic phase was concentrated under reduced pressure and subjected to silica gel chromatography, eluting with $CHCl_3$/MeOH to obtain crude fractions containing **32**, **2**, and **33**, respectively. Subsequently, the crude fractions underwent further purification using HPLC on a Shimadzu Prominence HPLC (CAPCELL PAK, 5 μm, 10.0 × 250 mm, ADME-HR column). Elution was performed using a linear gradient of 20-80% (v/v) acetonitrile/water containing 0.1% acetic acid over 30 min at a flow rate of 2.0 mL/min to obtain 8.2 mg **32**, 6.5 mg **2**, and 20.7 mg **33**, respectively.

Characterization of compound 34. A seed culture of *S. cerevisae* strain TY311, heterologously expressing mutant *terA*-V114A were cultivated in 5 mL of uracil drop-out medium for 2 days at 28 ℃

and 200 rpm. Subsequently, a 50 mL fermentation of the yeast was conducted using YPD medium for 3 days at 28 ℃ and 200 rpm. To extract the sesquiterpene metabolites, the fermentation broth of TY311 was centrifuged (3600 rpm, 10 min), and the cell pellet was collected and immersed in 3 mL acetone. The organic phase was then concentrated to oily form and subjected to GC-MS analysis. Compound **34** exhibited a molecular formula of $C_{15}H_{24}$, as deduced by EI-MS $[M]^+$ *m/z* 204, and displayed an identical mass spectrum to that of a known compound, β-caryophyllene, as shown in Figure S12B. In addition, an authentic standard of β-caryophyllene was analyzed using GC-MS analysis, which exhibited the same retention time as compound **34** (Figure S3A). These findings led to the identification of **34** as β-caryophyllene.

Characterization of compound 35. A seed culture of *S. cerevisae* strain TY335 and TY336, heterologously expressing mutants *terA*-V114T and *terA*-V114D, respectively, were grown in 5 mL of uracil drop-out medium for 2 days at 28 ℃ and 200 rpm. Subsequently, a 50 mL fermentation of the yeast was conducted using YPD medium for 3 days at 28 ℃ and 200 rpm. To extract the sesquiterpene metabolites, the fermentation broth of TY335 and TY336 was centrifuged (3600 rpm, 10 min), and the cell pellet was collected and immersed in 3 mL of acetone. The organic phase was concentrated to an oily form and subjected to GC-MS analysis. Compound **35** exhibited a molecular formula of $C_{15}H_{24}$, as determined by EI-MS $[M]^+$ *m/z* 204, and displayed an identical mass spectrum to that of a known compound, α-humulene, as shown in Figure S12C. These findings led to the identification of **35** as α-humulene.

Validation of the modifications by endogenous metabolic enzymes in *Aspergillus nidulans*. The control strain of *A. nidulans*, TY305, carrying three empty vectors, pYTU, pYTP, and pYTR, was cultivated in 15 mL of CD-ST liquid medium at 28 ℃ for 2 days. Subsequently, the culture was supplemented with compounds **2**, **32**, and **33** individually, at a final concentration of 0.2 mM. After an additional 4 h of incubation to allow for bioconversion, the resulting broth was extracted twice using ethyl acetate. The organic phase was concentrated under reduced pressure and subjected to LC-MS analysis (Figure S7).

6. Differential gene expression analysis of *ter* gene cluster

Aspergillus terreus ATCC 20516 was cultivated on PDA medium (potato dextrose agar, BD) for 7 d for sporulation. For RNA extraction, the strain was grown in liquid PDB medium (potato dextrose broth, BD) before and after terrecyclic acid production. Fermentation broths of *A. terreus* ATCC

20516 were collected on days 2, 3, 4, 5, 6, and 7, and extracted three times using ethyl acetate. The resulting organic phase was concentrated under reduced pressure and subjected to LC-MS analysis (Figure S13). HPLC-MS analyses were performed using a Shimadzu LC-MS 2020 (Phenomenex® Kinetex, 1.7 μm, 2.0 × 100 mm, C18 column). Electrospray ionization in positive and negative modes was utilized. The elution gradient consisted of water and acetonitrile as follows: 0-2.0 min, 5% acetonitrile (*v/v*); 2.0-18.0 min, 5-95% acetonitrile (*v/v*); 18.0-22.0 min, 95% acetonitrile (*v/v*); 22.0-22.1 min, 95-5% acetonitrile (*v/v*); 22.1-25.0 min, 5% acetonitrile (*v/v*), with a flow rate of 0.3 mL/min. The HPLC buffers were supplemented with 0.1% formic acid (*v/v*). The result indicated that terrecyclic acid production commenced on the fifth day of fermentation (Figure S13). Therefore, the transcriptome datasets of *A. terreus* ATCC 20516 fermented for 2, 3, and 4 days were considered as terrecyclic acid non-producing conditions, while the transcriptome datasets of *A. terreus* ATCC 20516 fermented for 5, 6, and 7 days were considered as terrecyclic acid producing conditions.

Transcriptome analysis was carried out as described. In brief, 20 million sequence reads for each of three replicates before and after terrecyclic acid production was generated, which was quality-filtered (> Q30) using the FASTX tool kit (http://hannonlab.cshl.edu/fastx_toolkit/). Data of all reads of three replicates under each condition were aligned to the *Aspergillus terreus* ATCC 20516 genome using hisat2[4] with default settings. On average 87% of the reads were mapped to the A. *terreus* ATCC 20516 genome. Then the short read mapping of each sample was assembled and quantified as reads per kilobase per million reads (RPKM) values using stringtie[5], which was followed by differential gene expression analysis of non-producing and producing conditions using DESeq2[6]. The mean expression (meanA or meanB) of non-producing (A) and producing (B) conditions were calculated from the normalized read counts of three biological replicates. The normalized read count is then used to calculate the log2-fold change (Table S5). The expression of the *ter* gene cluster was analyzed by comparing the log2-fold change of genes g10006~g10015 including the *ter* gene cluster (Table S5). The log2-fold change of genes within the *ter* cluster is between 3.3 and 8.1. And the reads of *ter* genes under producing conditions (meanB) is around 10000 to 60000, which is significantly up-regulated compared to the meanB values of the nearby genes beyond the cluster. Although the log2-fold change of g10007 was positive infinite due to no reads under non-producing conditions mapped (meanA = 0), its value of meanB is much lower than the *ter* biosynthetic genes (Table S5).

7. Structure determination of compounds

Compound **25**, colorless oil readily dissolved in hexanes and chloroform, had a molecular formula

$C_{15}H_{24}$, as deduced from GC-MS $[M]^+$ *m/z* 204. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.83 (dt, *J* = 2.6, 1.3 Hz, 1H), 4.56 (dt, *J* = 2.9, 1.4 Hz, 1H), 2.49 (ddd, *J* = 15.9, 7.6, 1.7 Hz, 1H), 2.16 (m, 1H), 2.06 (dd, *J* = 11.4, 7.5 Hz, 1H), 2.02~1.99 (m, 1H), 1.99~1.93 (m, 1H), 1.88~1.79 (m, 1H), 1.79~1.75 (m, 1H), 1.74~1.68 (m, 1H), 1.65 (d, *J* = 14.0 Hz, 1H), 1.63~1.57 (m, 2H), 1.51~1.41 (m, 1H), 1.26~1.21 (m, 1H), 1.19 (s, 3H), 1.09 (s, 3H), 0.91 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.2, 102.9, 59.8, 54.7, 52.1, 50.2, 39.5, 37.7, 35.6, 35.2, 28.8, 28.1, 27.1, 26.8, 17.5 (Figure S2 and Table S6).

Compound **32**, colorless needles readily dissolved in acetone and chloroform, had a molecular formula $C_{15}H_{22}O_3$, as deduced from LC-MS $[M-H]^-$ *m/z* 249. ^{1}H NMR (700 MHz, $CDCl_3$): δ 5.21 (s, 1H), 4.89 (s, 1H), 4.47 (d, *J* = 4.8 Hz, 1H), 2.92 (d, *J* = 8.9 Hz, 1H), 2.79 (dd, *J* = 13.0, 6.7 Hz, 1H), 2.20~2.12 (m, 1H), 1.98 (td, *J* = 13.2, 4.9 Hz, 1H), 1.94~1.88 (m, 1H), 1.87~1.84 (m, 1H), 1.78~1.74 (m, 1H), 1.65 (d, *J* = 14.5 Hz, 1H), 1.62 (d, *J* = 14.5 Hz, 1H), 1.58~1.53 (m, 1H), 1.17 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (175 MHz, $CDCl_3$): δ 179.9, 160.6, 109.8, 77.0, 55.5, 54.3, 51.3, 48.9, 48.6, 40.0, 38.6, 34.9, 29.3, 27.5, 23.1. (Figure S2 and Table S6). Crystal data: monoclinic, space group $P2_1$ (no. 4), *a* = 6.36910(10) Å, *b* = 12.0175(2) Å, *c* = 9.0830(2) Å, β = 108.989(2)°, *V* = 657.39(2) Å^3, *Z* = 2, *T* = 106(2) K, μ(Cu Kα) = 0.692 mm^{-1}, D_{calc} = 1.265 g/cm^3, 6277 reflections measured (10.3° ≤ 2Θ ≤ 148.74°), 2560 unique (R_{int} = 0.0208, R_{sigma} = 0.0230) which were used in all calculations. The final R_1 was 0.0277 (I > 2σ(I)) and wR_2 was 0.0737 (all data).

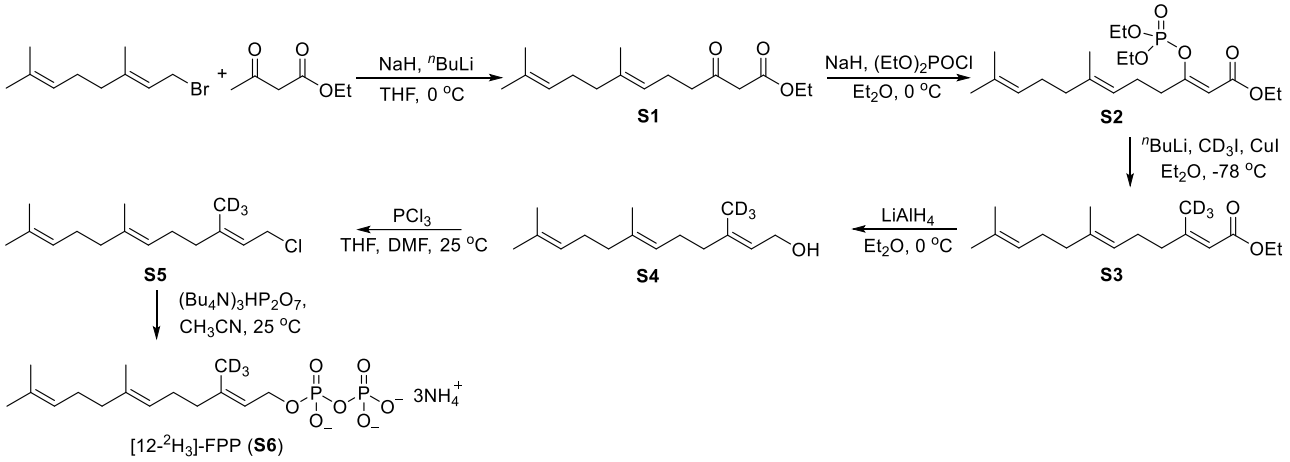
Compound **33**, colorless needles readily dissolved in acetone and chloroform, had a molecular formula $C_{15}H_{22}O_3$, as deduced from LC-MS $[M-H]^-$ *m/z* 249. ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.78~2.76 (overlaped, 2H), 2.55~2.48 (m, 1H), 2.46 (q, *J* = 6.8 Hz, 1H), 2.36 (dd, *J* = 20.1, 7.6, 1H), 1.96~1.91 (m, 1H), 1.91~1.86 (m, 2H), 1.86~1.84 (m, 1H), 1.80~1.74 (m, 1H), 1.41 (d, *J* = 15.2 Hz, 1H), 1.39 (d, *J* = 15.2 Hz, 1H), 1.13 (s, 3H), 1.10 (s, 3H), 0.99 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 219.3, 181.4, 55.0, 51.7, 49.4, 48.1, 47.7, 45.1, 40.6, 39.6, 34.3, 28.9, 27.3, 22.2, 8.7. (Figure S2 and Table S6). Crystal data: orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 6.45480(10) Å, *b* = 17.2854(3) Å, *c* = 12.2879(2) Å, *V* = 1371.01(4) Å^3, *Z* = 4, *T* = 106(5) K, μ(Cu Kα) = 0.664 mm^{-1}, D_{calc} = 1.213 g/cm^3, 12582 reflections measured (8.83° ≤ 2Θ ≤ 133.158°), 2425 unique (R_{int} = 0.0569, R_{sigma} = 0.0281) which were used in all calculations. The final R_1 was 0.0910 (I > 2σ(I)) and wR_2 was 0.2585 (all data).

Compound **2**, colorless needles readily dissolved in acetone and chloroform, had a molecular formula $C_{15}H_{20}O_3$, as deduced from LC-MS $[M-H]^-$ *m/z* 247. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.96 (s, 1H), 5.20

(s, 1H), 3.00 (d, *J* = 8.0 Hz, 1H), 2.95 (dd, *J* = 11.3, 9.7 Hz, 1H), 2.63 (dd, *J* = 19.3, 11.3 Hz, 1H), 2.52 (dd, *J* = 19.3, 9.6 Hz, 1H), 2.19~2.07 (m, 1H), 1.96 (t, *J* = 3.1 Hz, 1H), 1.92~1.86 (m, 1H), 1.84~1.79 (m, 2H), 1.76 (s, 2H), 1.23 (s, 3H), 1.18 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.5, 180.2, 150.4, 116.1, 54.8, 53.9, 48.7, 47.9, 46.3, 41.5, 40.4, 34.7, 28.8, 27.3, 22.5. (Figure S2) Compound **2** is identical to terrecyclic acid A as reported[7].

8. Synthetic procedures of [12-$^2H_3$]-FPP

[12-$^2H_3$]-FPP (**S6**) as (2*E*,6*E*,10*E*)-3-[$^2H_3$]methyl-7,11-dimethyl-undeca-2,6,10-trienyl diphosphate trisammonium salt was prepared generally according to the previously reported method[8]. The synthetic procedure is outlined in Scheme S1.



Scheme S1. Preparation of [12-$^2H_3$]-FPP

Ethyl (*E*)-7,11-dimethyl-3-oxododeca-6,10-dienoate (**S1**). NaH (22 mmol, 60% suspension in mineral oil) was dispersed in anhydrous THF (20 mL) and the mixture was cooled to 0 °C. To the stirring suspension was added ethyl acetoacetate dropwise. After 10 min, the monosodium salt of ethyl acetoacetate was treated with n-butyllithium (1.6 M in hexane, 13.2 mL, 21 mmol). After 20 min, neryl bromide (6.6 g, 22 mmol) was added to the resulting solution slowly, and stirring was continued for an additional 30 min at 0 °C. Then the reaction was quenched by NH_4Cl (aq), and extracted with ether (3 × 50 mL). The organic layers were combined, washed with saturated NaCl (aq), and dried over $MgSO_4$. After purification using flash chromatography on silica gel (hexanes/ethyl acetate 9:1), 4.27 g (80%) of the product **S1** was obtained as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.11~5.01 (m, 2H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.43 (s, 2H), 2.56 (t, *J* = 7.4 Hz, 2H), 2.33~2.22 (m, 2H), 2.09~2.00 (m, 2H), 2.00~1.92 (m, 2H), 1.67 (s, 3H), 1.60 (s, 3H), 1.59 (s, 3H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 202.8, 167.3, 136.9, 131.6, 124.3, 122.2, 61.5, 49.5, 43.2, 39.8, 26.7, 25.8, 22.3,

17.8, 16.1, 14.2.

Ethyl (2*Z*,6*E*)-3-((diethoxyphosphoryl)oxy)-7,11-dimethyldodeca-2,6,10-trienoate (**S2**). To a stirred solution of β-ketoester **S1** (1.06 g, 3.98 mmol) in anhydrous diethyl ether (50 mL) at 0 °C under nitrogen was added sodium hydride (60% dispersion in mineral oil, 175 mg, 4.38 mmol) and the mixture was stirred for 15 min at 0 °C before the addition of diethyl chlorophosphate (0.86 mL, 5.97 mmol). This resulting mixture was stirred at 0 °C for 2 h before being quenched by the addition of NH_4Cl (aq). The quenched mixture was extracted with ether (3 x 20 mL). Then the organic extracts were combined and washed with brine before drying ($MgSO_4$), filtration, and concentration under reduced pressure. After purification by flash chromatography on silica gel (1:1 hexane/ethyl acetate). 1.04 g (65%) of the title compound **S2** was prepared as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.33 (s, 1H), 5.16~4.96 (m, 2H), 4.25 (overlapped, q, J = 7.1 Hz, 4H), 4.13 (q, J = 7.1 Hz, 2H), 2.43 (t, J = 7.4 Hz, 2H), 2.32~2.21 (m, 2H), 2.08~2.00 (m, 2H), 1.99~1.89 (m, 2H), 1.66 (s, 3H), 1.59 (s, 3H), 1.58 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H), 1.34 (t, J = 7.1 Hz, 3H), 1.25 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.9 (d, J = 1.9 Hz), 161.5 (d, J = 7.1 Hz), 137.1, 131.6, 124.2, 121.9, 105.5 (d, J = 7.5 Hz), 64.9 (d, J = 6.3 Hz), 60.0, 39.8, 35.4, 26.7, 25.8, 25.0, 17.8, 16.3, 16.2 (d, J = 0.8 Hz), 14.4.

Ethyl (2*E*,6*E*)-7,11-dimethyl-3-[$^2H_3$]methyldodeca-2,6,10-trienoate (S3). CD_3I (0.72 g, 5 mmol) was added to anhydrous diethyl ether (10 mL) under an inert atmosphere and was cooled to 0 °C. Under stirring, n-butyllithium (1.6 M in hexane, 6.2 mL, 10 mmol) was added dropwise. After 30 min, CuI (0.48 g, 2.5 mmol) was added and the mixture was stirred until the precipitate was completely dissolved. Then the mixture was cooled to -78 °C, and a solution of the enol phosphate **S2** (0.48 g, 1.25 mmol) in anhydrous diethyl ether (10 mL) was added dropwise. The resulting solution was stirred at -78 °C for 1.5 h and quenched by the addition of iodomethane (2.5 mL), which was followed by adding a 1:1 ice-cold mixture of concentrated ammonia and brine (20 mL). After warming to room temperature, the mixture was extracted with ether and purified using flash chromatography on silica gel (19:1 hexane/ethyl acetate) to obtain the title compound **S3** as a colorless oil (0.17 g, 50%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.67 (s, 1H), 5.13~5.05 (m, 2H), 4.14 (q, J = 7.1 Hz, 2H), 2.19~2.14 (m, 4H), 2.09~2.02 (m, 2H), 2.00~1.95 (m, 2H), 1.68 (s, 3H), 1.60 (s, 6H), 1.28 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.0, 159.8, 136.3, 131.5, 124.4, 123.1, 115.9, 59.6, 41.0, 39.8, 26.8, 26.1, 25.8, 17.8, 16.2, 14.5.

(2*E*,6*E*)-7,11-dimethyl-3-[$^2H_3$]methyldodeca-2,6,10-trien-1-ol (S4). To a stirred solution of the

deuterated ester **S3** (97.4 mg, 0.36 mmol) in diethyl ether (10 mL) at 0 °C was added $LiAlH_4$ (1.0 M in THF, 0.32 mL, 0.32 mmol) and the mixture was stirred at 0 °C for 30 min. The reaction was quenched by the addition of saturated potassium sodium tartrate solution (10 mL), and then the mixture was separated after standing for 2 h. The aqueous layer from the reaction mixture was extracted with diethyl ether (3 x 10 mL), and the organic layers from the reaction mixture and extraction were combined, washed with brine before drying (Na_2SO_4), filtration and concentration under reduced pressure. The crude alcohol **S4** (28 mg, 74%) was purified by flash chromatography on silica gel (7:1 hexane/ethyl acetate), and 46.0 mg (56%) of the title compound **S4** was obtained as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.40 (t, J = 6.9 Hz, 1H), 5.14~5.04 (m, 2H), 4.12 (d, J = 7.0 Hz, 2H), 2.16~2.07 (m, 2H), 2.06~2.00 (m, 4H), 1.98~1.95 (m, 2H), 1.66 (s, 3H), 1.58 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.6, 135.4, 131.4, 124.4, 123.9, 123.5, 59.4, 39.8, 39.6, 26.8, 26.4, 25.8, 17.8, 16.1.

(2*E*,6*E*)-1-chloro-7,11-dimethyl-3-[$^2H_3$]methyl dodeca-2,6,10-triene (S5). To a stirred solution of PCl_3 (24.4 mg, 0.18 mmol) in DMF (0.015 mL) and THF (0.150 mL) at room temperature was slowly added deuterated farnesol **S4** dropwise (68.7 mg, 0.30 mmol). After being stirred for 30 min, ice water was added to the mixture to quench the reaction. The resulting mixture was centrifuged at 12000 rpm for 1 min to obtain supernatant, which was then washed with saturated $NaHCO_3$ solution and brine to give deuterated farnesyl chloride **S5** (45.8 mg, 64%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.47~5.40 (m, 1H), 5.15~5.03 (m, 2H), 4.10 (d, J = 8.0 Hz, 2H), 2.14~2.09 (m, 2H), 2.08~2.02 (m, 4H), 1.99~1.95 (m, 2H), 1.68 (s, 3H), 1.60 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 143.0, 135.8, 131.5, 124.5, 124.0, 123.6, 41.3, 39.8, 39.5, 26.8, 26.2, 25.8, 17.8, 16.2.

(2*E*,6*E*)-3-[$^2H_3$]methyl-7,11-dimethyl-undeca-2,6,10-trienyl diphosphate trisammonium salt，[12-2H3]-FPP (S6). To a stirred solution of tris(tetra-*n*-butyl)ammonium hydrogen pyrophosphate (27.0 mg, 0.03 mmol) in 0.5 mL CH_3CN at room temperature was slowly added the solution of the deuterated farnesyl chloride **S5** (4.6 mg, 0.02 mmol) in 0.5 mL CH_3CN. After the mixture was stirred for 2.5 h, it was concentrated under reduced pressure. The residue was dissolved in 1 mL mixture of 2-propanol and 25 mM NH_4HCO_3 (1:49). The resulting solution was then loaded on the column of Dowex 50WX8 (200-400 mesh, NH_4^+ form), and eluted with a mixed solution of 2-propanol and NH_4HCO_3. The fractions of 0-50 mL were combined and lyophilized to give product **S6** (5.3 mg, 60%). ^{1}H NMR (700 MHz, D_2O): δ 5.45 (t, J = 7.1 Hz, 1H), 5.24~5.15 (m, 2H), 4.53~4.42 (m, 2H), 2.18~2.14 (m, 2H), 2.13~2.077 (m, 4H), 2.03~2.00 (m, 2H), 1.68 (s, 3H), 1.61 (s, 6H); ^{31}P NMR (202 MHz, D_2O) δ -9.89 (d, J = 19.6 Hz), -10.63 (d, J = 19.6 Hz).

9. Preparation of microsomal fraction containing TerB and biochemical analysis

The *A. nidulans* strain TY304 harboring pTerB-pYTR was cultured in 30 mL CD-ST medium at 28°C and 200 rpm for 3 d. Then the mycelia were harvested and resuspended in Buffer A (0.6 M sorbitol, 0.1 M KCl, 1.0 mM EDTA, 1.0 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 50 mM Tris-HCl, pH 7.5). The mixture was homogenized using zirconium silicate beads at 4°C for 5 min. The homogenate was then centrifuged at 14000 rpm and 4°C for 10 min to remove the precipitation. The supernatant was transferred into new tubes and centrifuged at 14000 rpm and 4°C for 8 h. The supernatant was discarded and the microsomal fraction in the precipitate was resuspended in Buffer B (20% glycerol, 1.0 mM EDTA, 1.0 mM DTT, 50 mM Tris-HCl, pH 7.5). *In vitro* biochemical analysis of TerB was performed on a 100 μL scale with microsomal cytochrome P450 in the presence of reducing partners (0.2 mM NADPH, 0.2 mM FAD, and 0.2 mM FMN) and 0.04 mM substrate **25** at 28 °C overnight.

10. Preparation and biochemical analysis of TerC

To express and purify TerC, primers TerC -pET-F and TerC-pET-R were used to amplify a 0.8 kb DNA fragment containing *terC*. The PCR product was cloned into pET28a using *Nde*I and *Not*I restriction sites. The resulting plasmid pTerC-pET28a was transformed into *E. coli* BL21 (DE3) to obtain TY004. TerC fused a 6 × His-tag with a molecular weight of ~29 kD were expressed at 16 °C 220 rpm for 20 h after 100 μM β-D-1-thiogalactopyranoside IPTG induction (IPTG was added when OD_{600} = 0.8). Cells of 1 L culture were then harvested by centrifugation at 5000 rpm and 4 °C. Cell pellet was resuspended in 15 mL Buffer A10 (20 mM Tris-HCl pH 7.5, 50 mM NaCl, 8% glycerol, 10 mM imidazole). The cells were lysed by sonication, and the insoluble material was sedimented by centrifugation at 12000 rpm at 4 °C for 60 min. The protein supernatant was then incubated with 1 mL Ni-NTA resin for 4 h with slow, constant rotation at 4 °C. Subsequently the Ni-NTA resin was washed with 10 column volumes of Buffer A50 (Buffer A + 50 mM imidazole). For elution of the target protein, the Ni-NTA resin was incubated for 10 min with 10 mL Buffer A250 (Buffer A + 250 mM imidazole). The supernatant from the elution step was then analyzed by SDS-PAGE. The elution fraction containing the recombinant protein was buffer exchanged into storage buffer (50 mM Tris-HCl pH 8.5, 50 mM NaCl, 10% glycerol, 5 mM dithiothreitol, 5 mM GSH). *In vitro* activity assays of TerC on **32** or **33** were carried out in a 100 μL reaction mixture containing 50 mM Tris-HCl pH 7.5, 0.2 mM

$NADP^+$, and 1 μM TerC in the presence of 0.04 mM **32** or **33** at 28 °C for 10 min. The reducing ability of TerC upon **2** was verified by incubating 0.04 mM **2** with 1 μM TerC in the presence of 0.2 mM NADPH in a 100 μL reaction mixture containing 50 mM Tris-HCl pH 7.5 at 28 °C for 10 min (Figure S6).The reaction was quenched by adding an equal volume of methanol and centrifuged at 14000 for 5 min. The supernatant was subjected to LC-MS analysis.

Supplementary Figures

Figure S1. Putative terpenoids gene clusters in *Aspergillus terreus* ATCC 20516. Abbreviations: terpene cyclase (TC); methyltransferase (MT); short-chain dehydrogenase/reductase (SDR), FAD-dependent monooxygenases (FMO); major facilitator superfamily (MFS); (transcription factor) TF; hypothetical protein (HP).

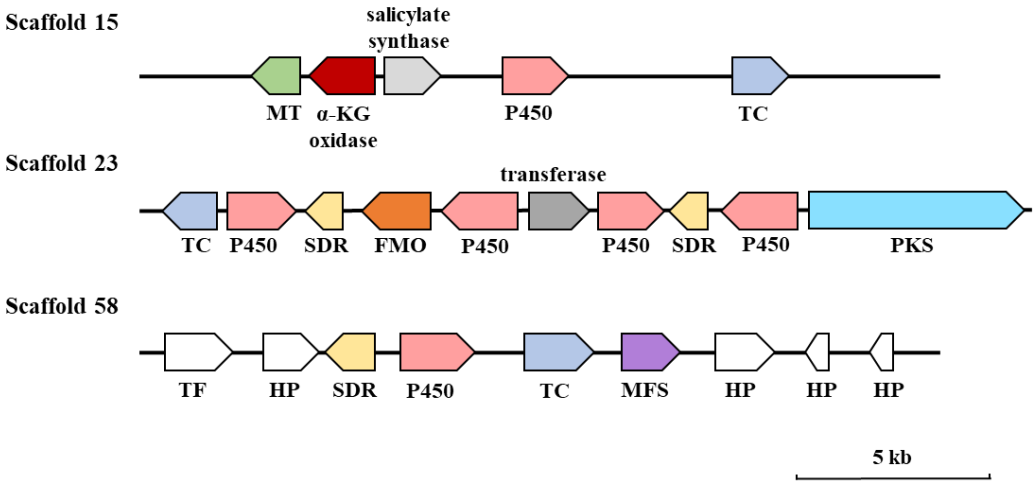
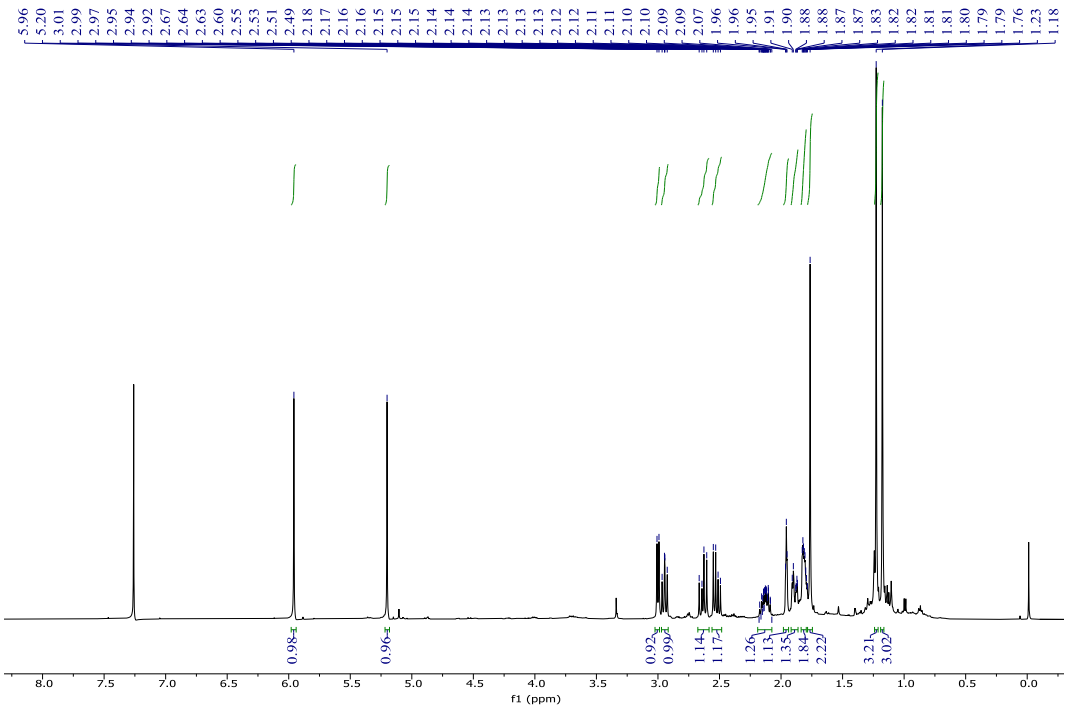
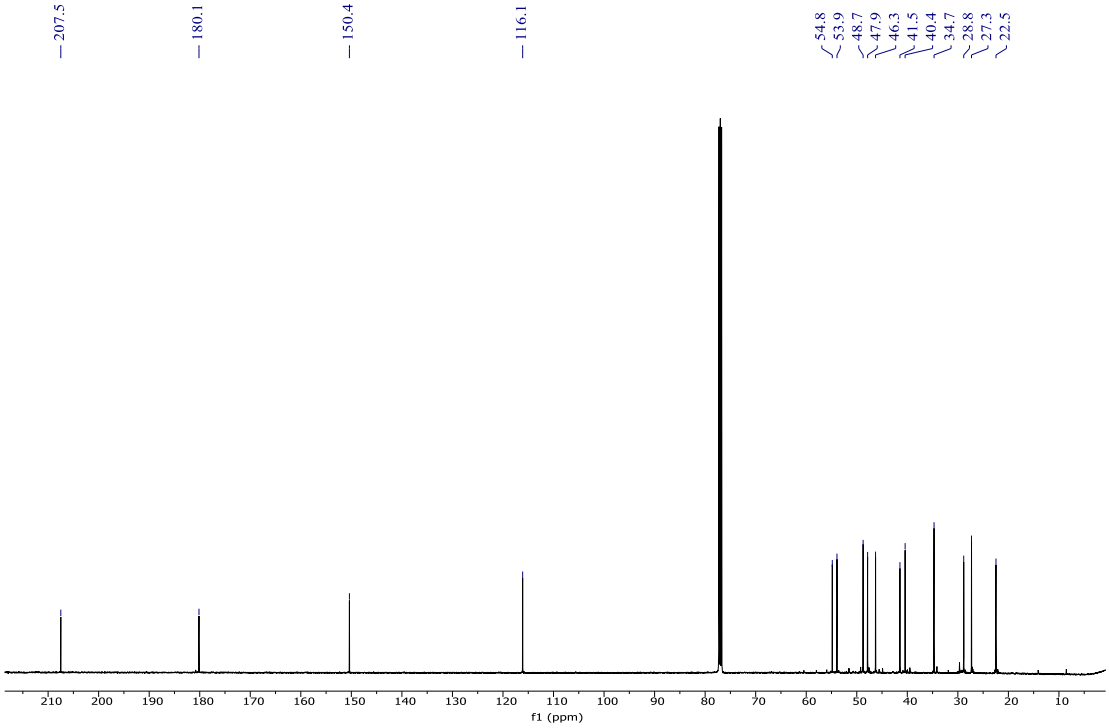


Figure S2. NMR analyses of the compounds in this study

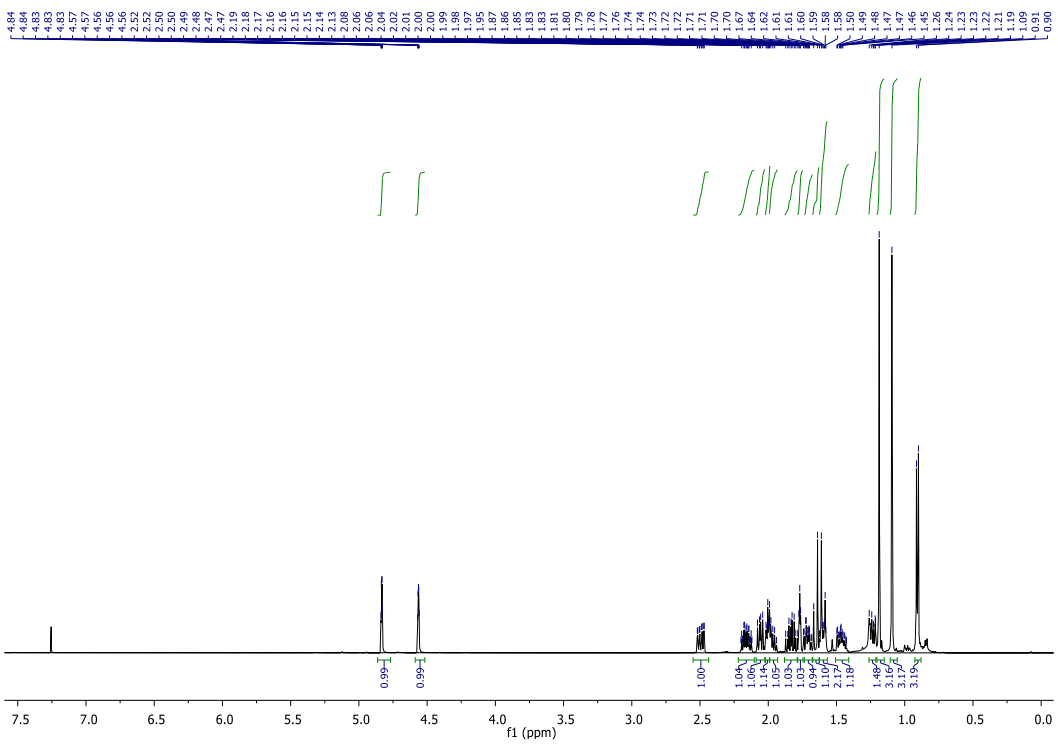
^{1}H NMR of compound 2 (500 MHz, $CDCl_3$):



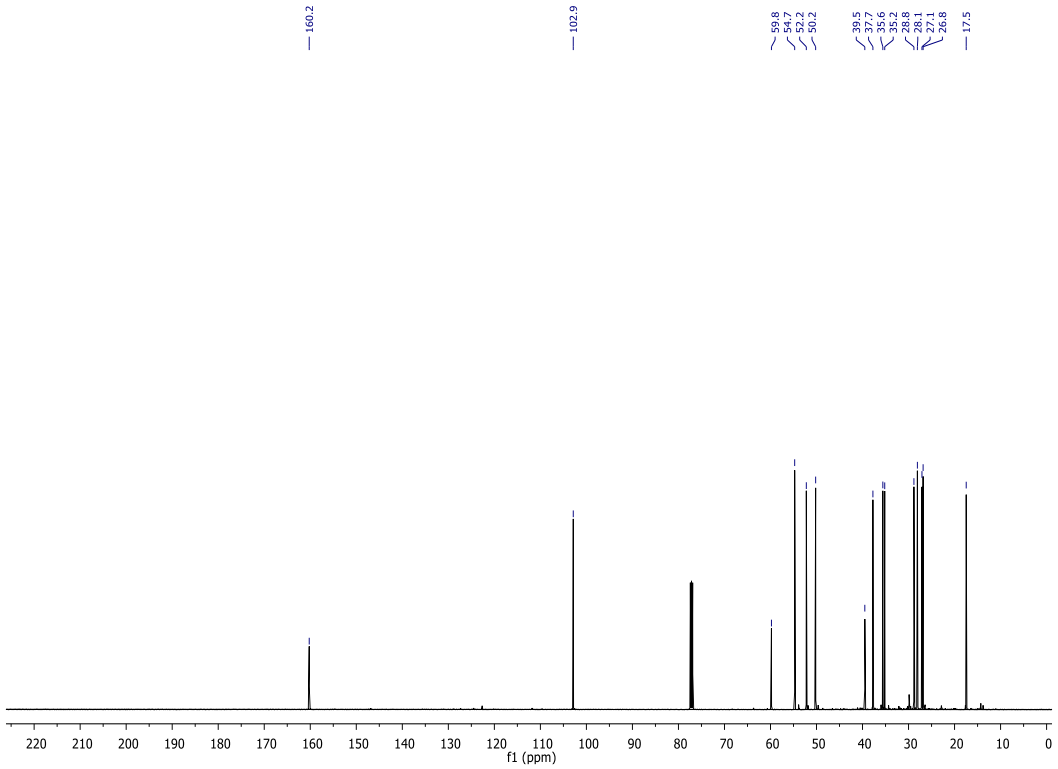
^{13}C NMR of compound 2 (126 MHz, $CDCl_3$):



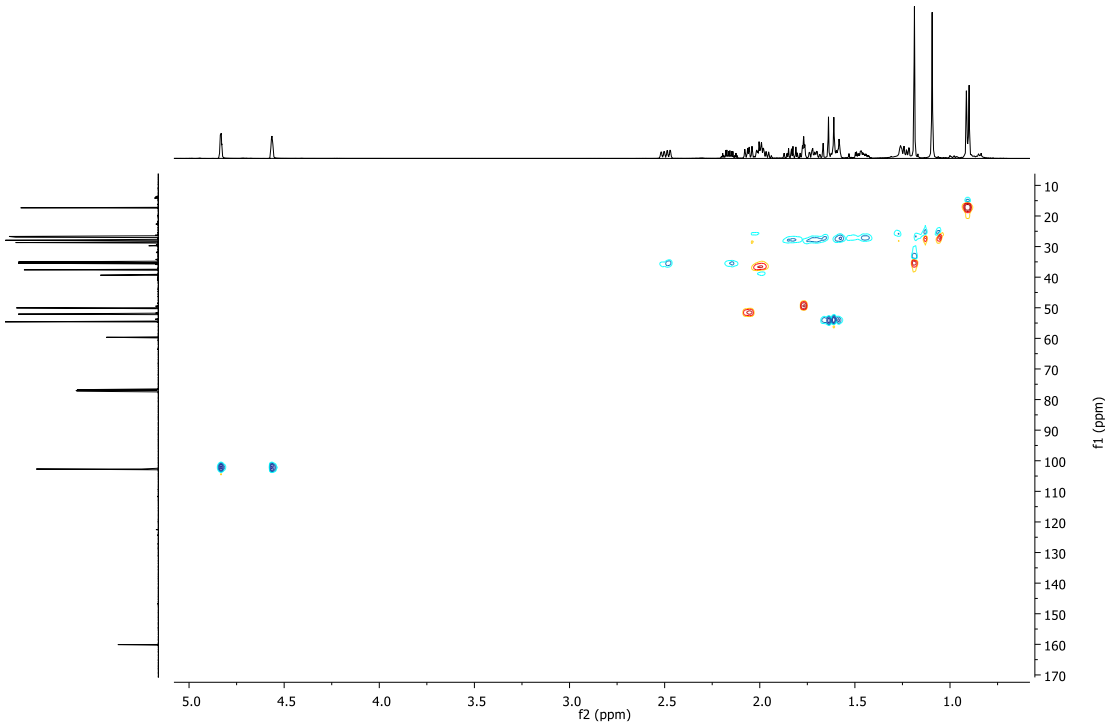
^{1}H NMR of compound 25 (500 MHz, $CDCl_3$):



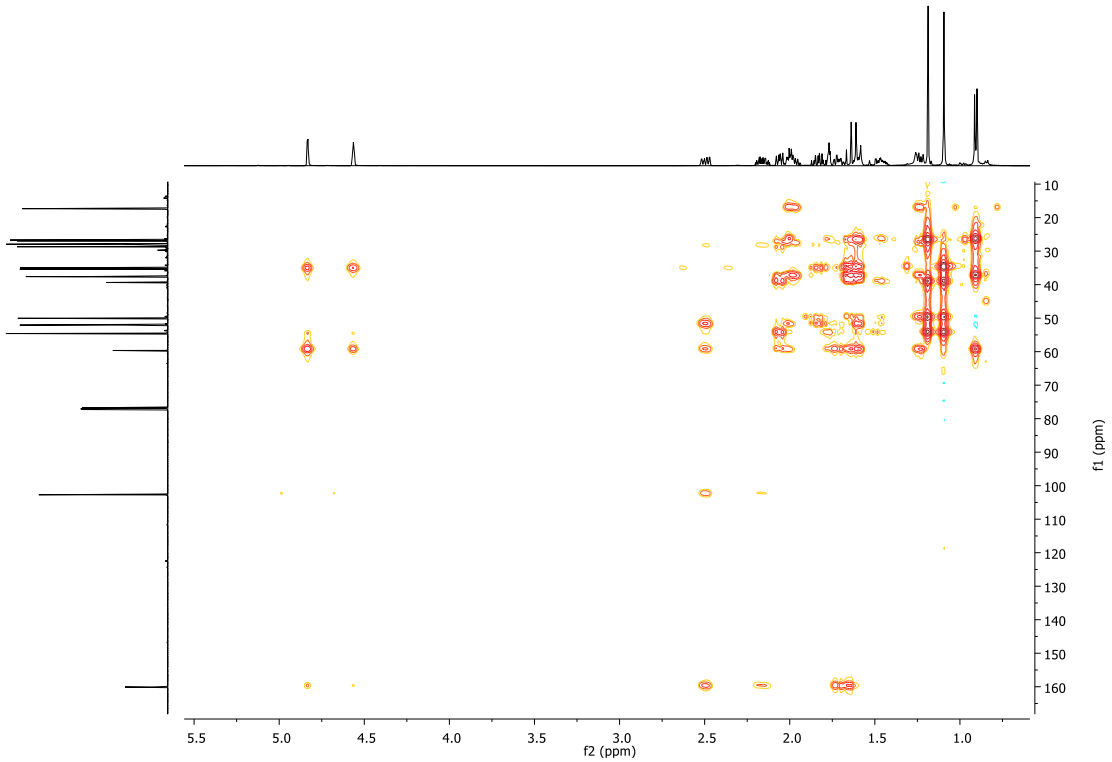
^{13}C NMR of compound 25 (126 MHz, $CDCl_3$):



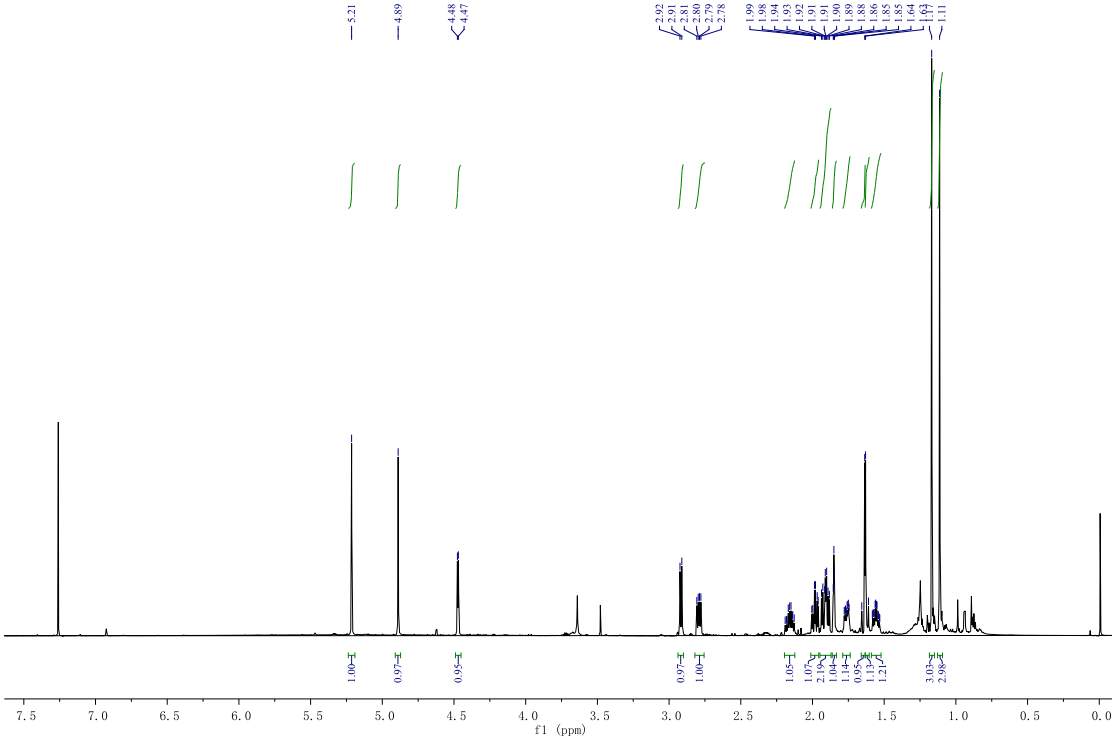
HSQC of compound 25 (500 MHz, $CDCl_3$):



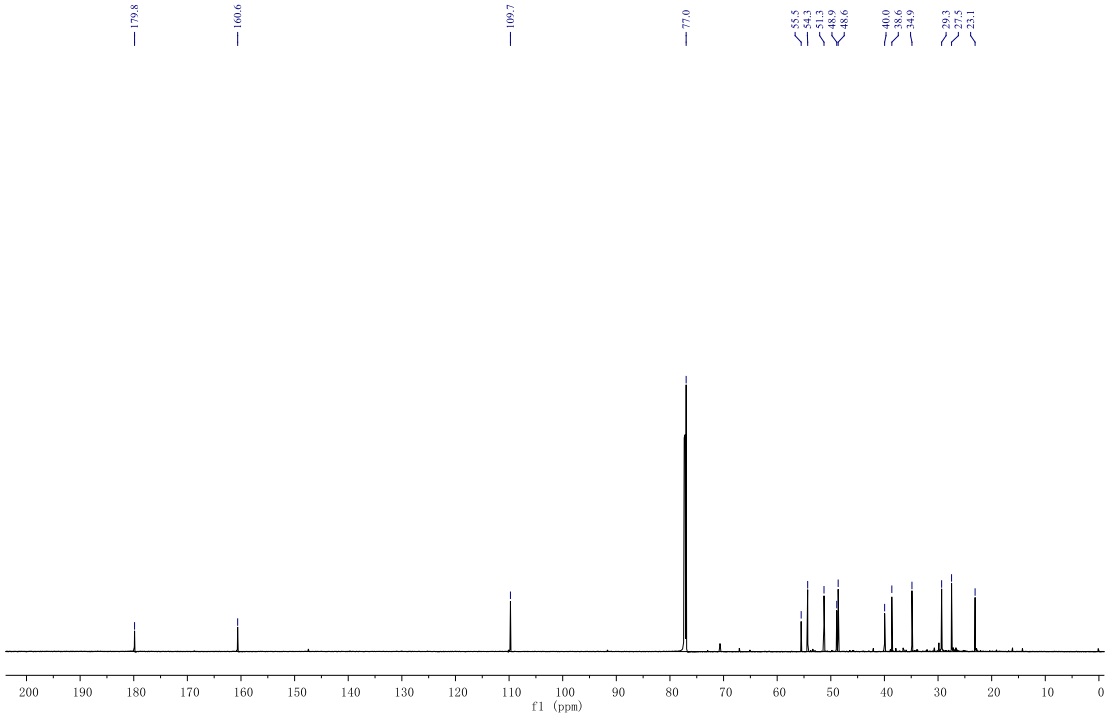
HMBC of compound 25 (500 MHz, $CDCl_3$):



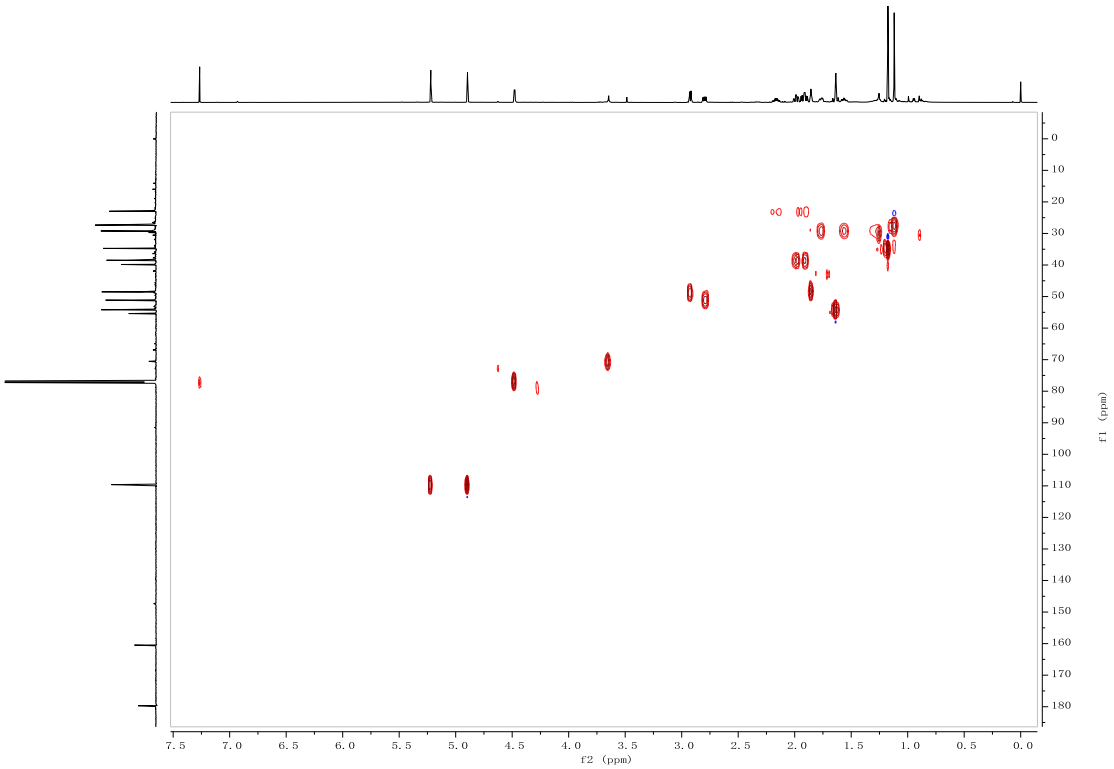
^{1}H NMR of compound 32 (700 MHz, $CDCl_3$):



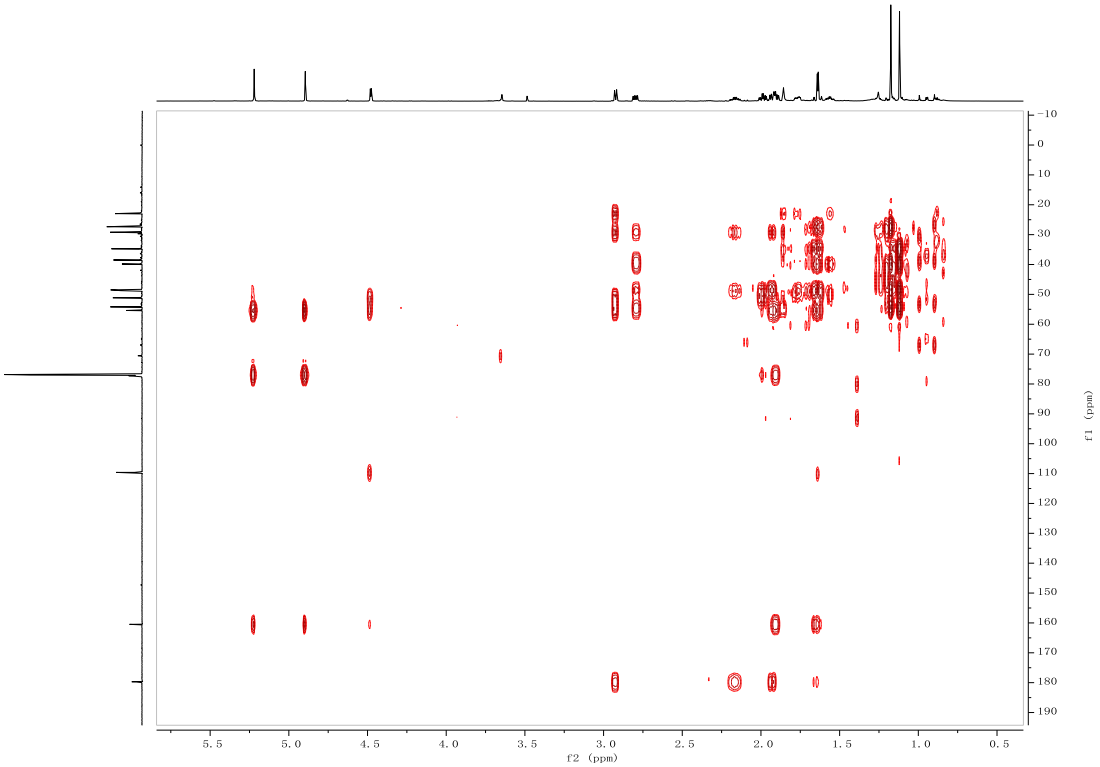
^{13}C NMR of compound 32 (176 MHz, $CDCl_3$):



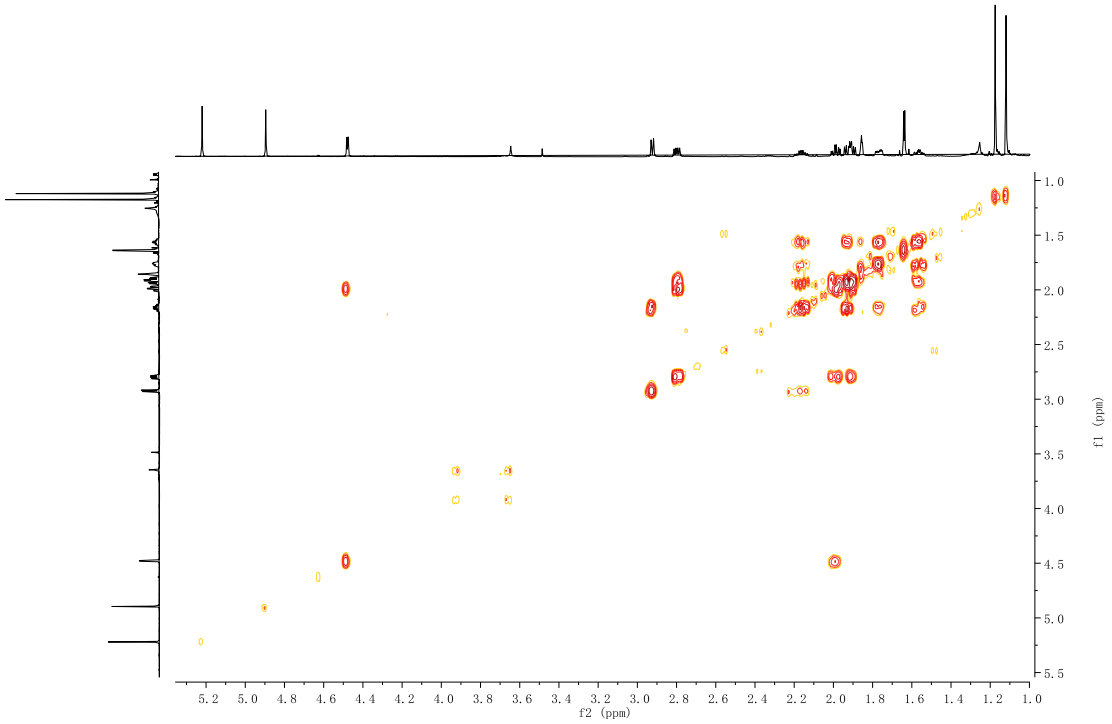
HSQC of compound 32 (700 MHz, $CDCl_3$):



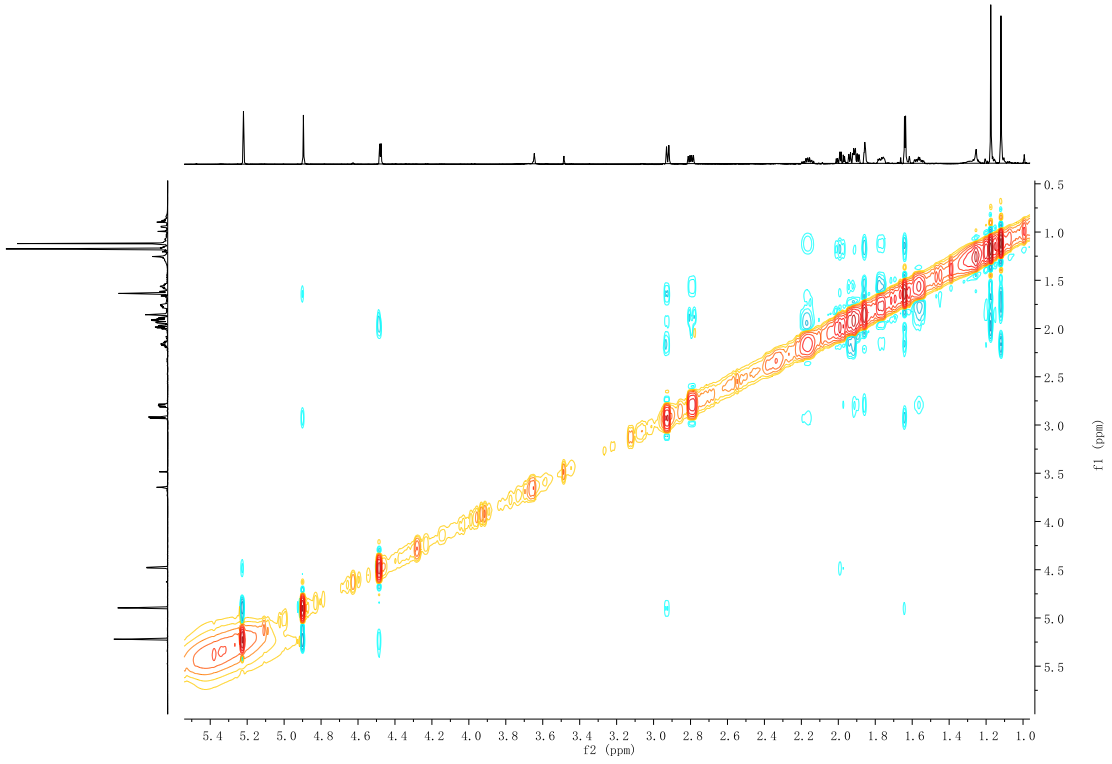
HMBC of compound 32 (700 MHz, $CDCl_3$):



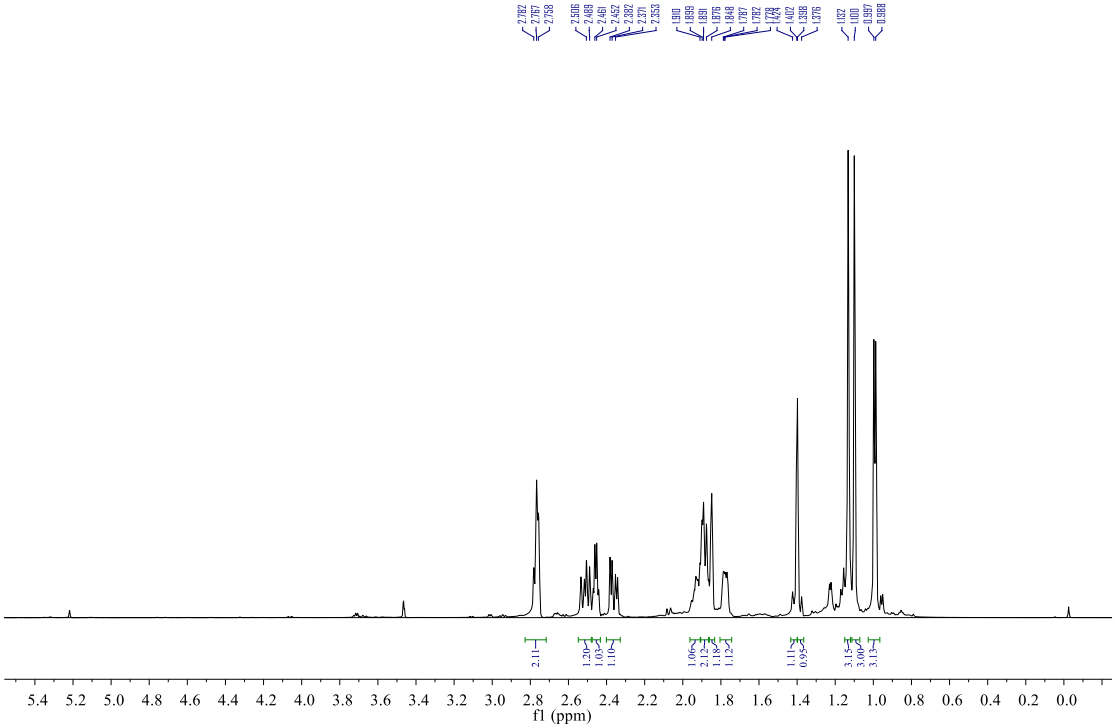
^{1}H-^{1}H COSY of compound 32 (700 MHz, $CDCl_3$):



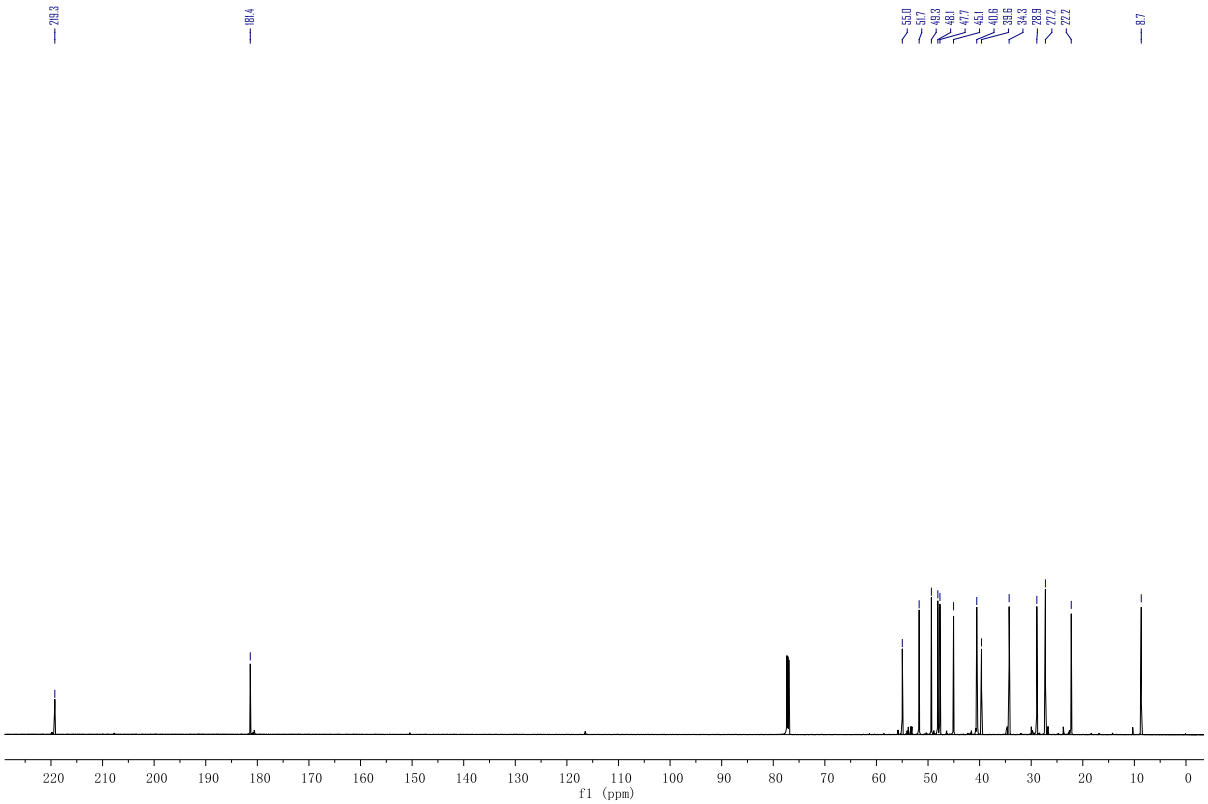
NOESY of compound 32 (700 MHz, $CDCl_3$):



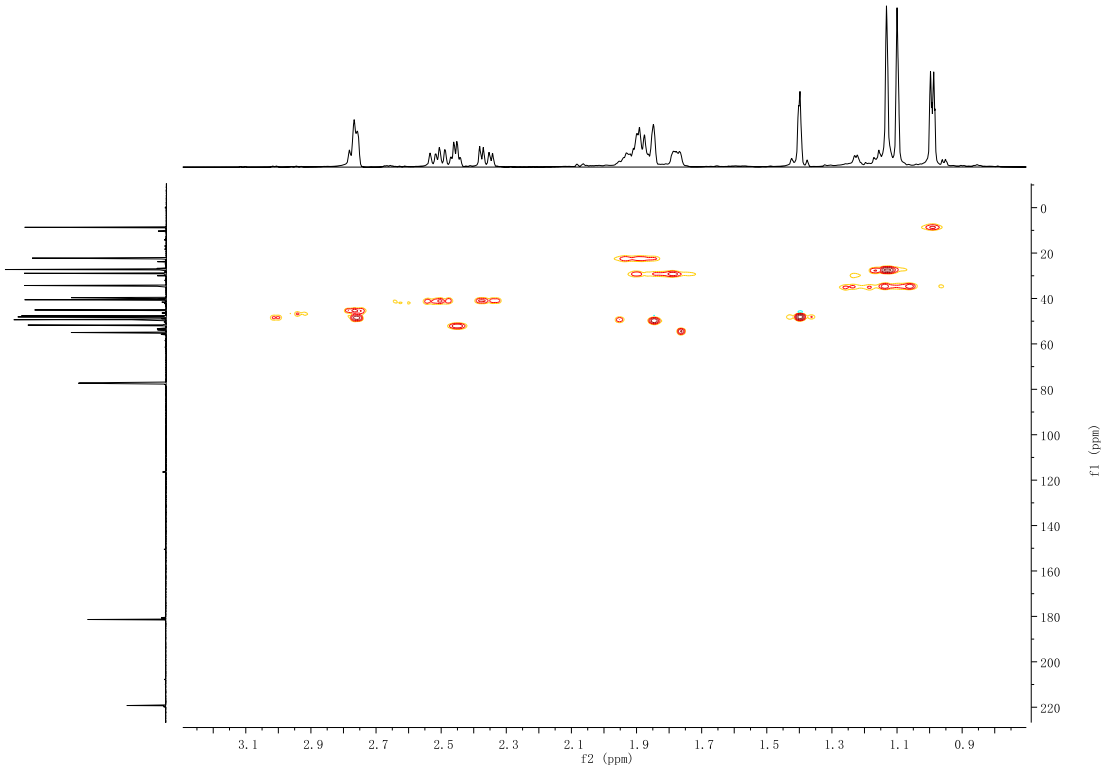
^{1}H NMR of compound 33 (700 MHz, $CDCl_3$):



^{13}C NMR of compound 33 (176 MHz, $CDCl_3$):



HSQC of compound 33 (500 MHz, $CDCl_3$):



HMBC of compound 33 (500 MHz, $CDCl_3$):

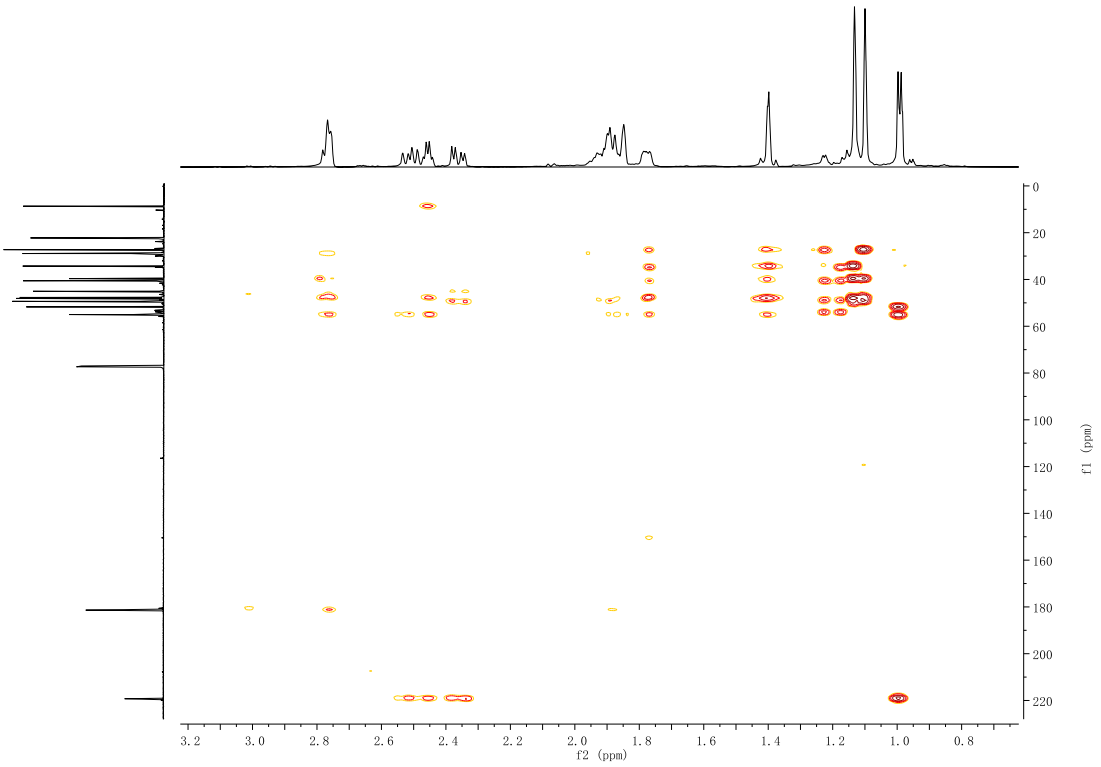


Figure S3. X-ray crystallographic structure of the compounds in this study. Structure of compound **32** (A, CCDC number: 2271713) and **33** (B, CCDC number: 2271714) with 30% probability displacement ellipsoids.

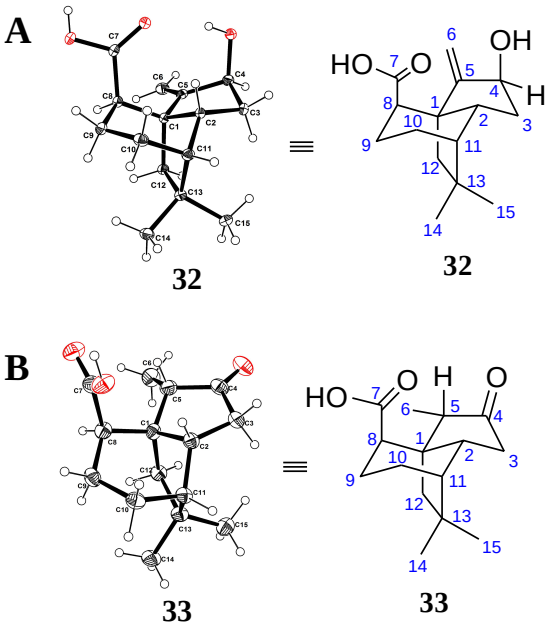


Figure S4. SDS-PAGE analysis of purified proteins. SDS-PAGE analysis of purified TerA (**A**), TerC (**B**), TerA-V114A (**C**) from *S. cerevisiae* RC01.

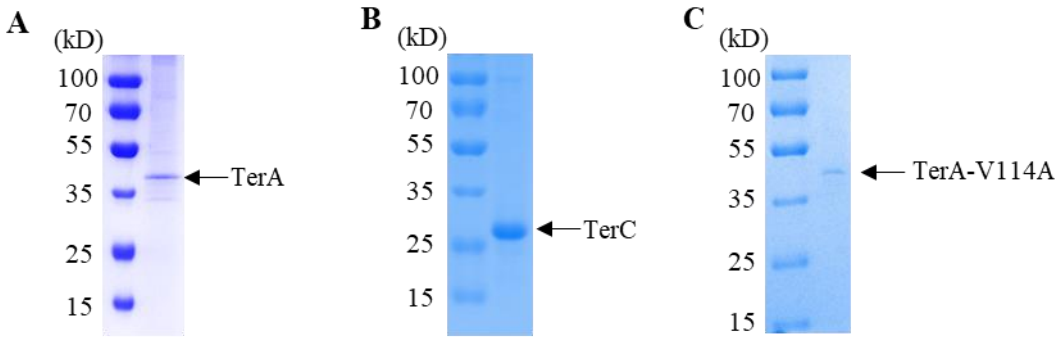


Figure S5. Heterologous expression of *ter* biosynthetic genes in *S. cerevisiae*. (i) *S. cerevisiae* RC01 carrying the empty vectors; (ii) *S. cerevisiae* RC01 carrying *terA* and *terB*; (iii) *S. cerevisiae* RC01 carrying *terA*, *terB*, and *terC*. The EICs were obtained using LC-MS. The Y-axes of the chromatograms for each experiments are shown in the same scale, and the quantities of the compounds are comparable.

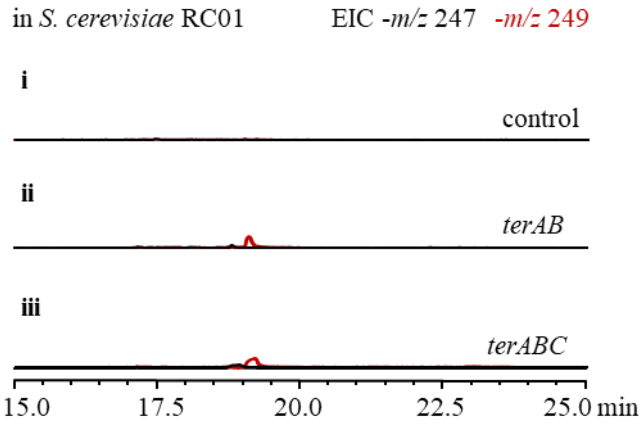


Figure S6. The reducing reaction of 2 catalyzed by TerC. (A) LC-MS analysis of the metabolites: i. standard compound **2**; ii. standard compound **33**; iii. standard compound **32**; iv. conversion of **2** into **32** catalyzed by TerC. The EICs were obtained using LC-MS. The Y-axes of the chromatograms for each experiments are shown in the same scale, and the quantities of the compounds are comparable. (B) TerC catalyzes the conversion of **2** into **32**, while the shunt product **33** is not generated.

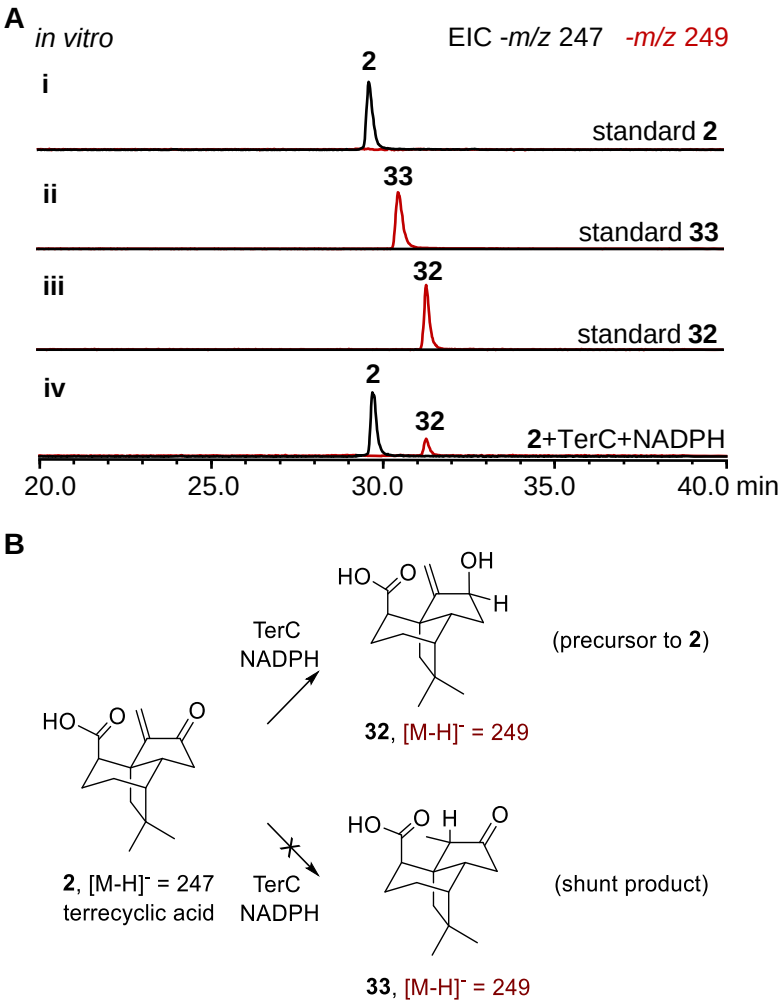


Figure S7. Modifications of 32 and 2 by endogenous metabolic enzymes in *Aspergillus nidulans*. (A) LC-MS analysis of the metabolites: i. standard compound **2**; ii. standard compound **32**; iii. standard compound **33**; iv. metabolites of *A. nidulans*; v. metabolites of *A. nidulans* fed with **2**; vi. metabolites of *A. nidulans* fed with **32**; v. metabolites of *A. nidulans* fed with **33**. The EICs were obtained using LC-MS. The Y-axes of the chromatograms for each experiments are shown in the same scale, and the quantities of the compounds are comparable. (B) Proposed modification reactions of **32** and **2** by endogenous metabolic enzymes in *A. nidulans*.

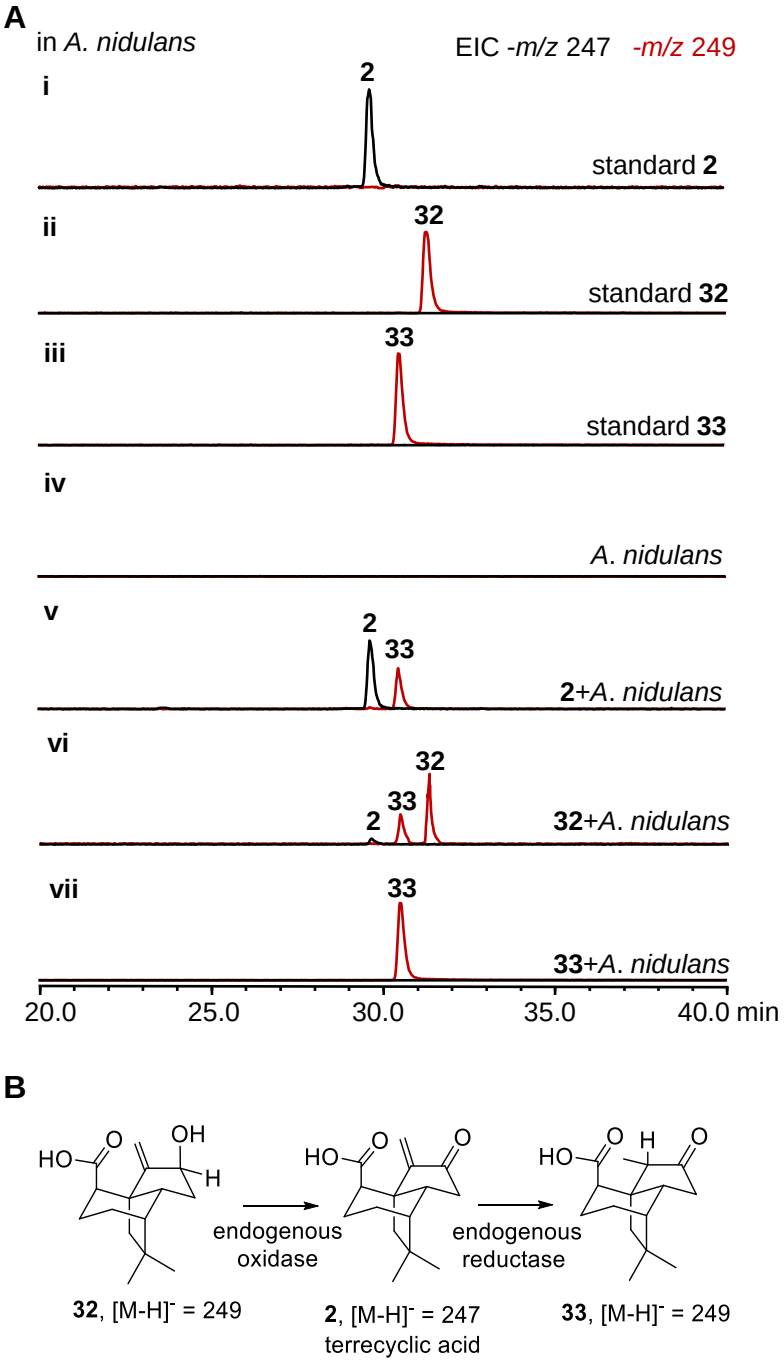


Figure S8. Alignment of TerA to representative sesquiterpene cyclases

The amino acid sequence identity between TerA and epi-isozizaene synthase is 19.5%； the amino acid sequence identity between TerA and selinadiene synthase is 21.9%； the amino acid sequence identity between TerA and pentalenene synthase is 23.8%.

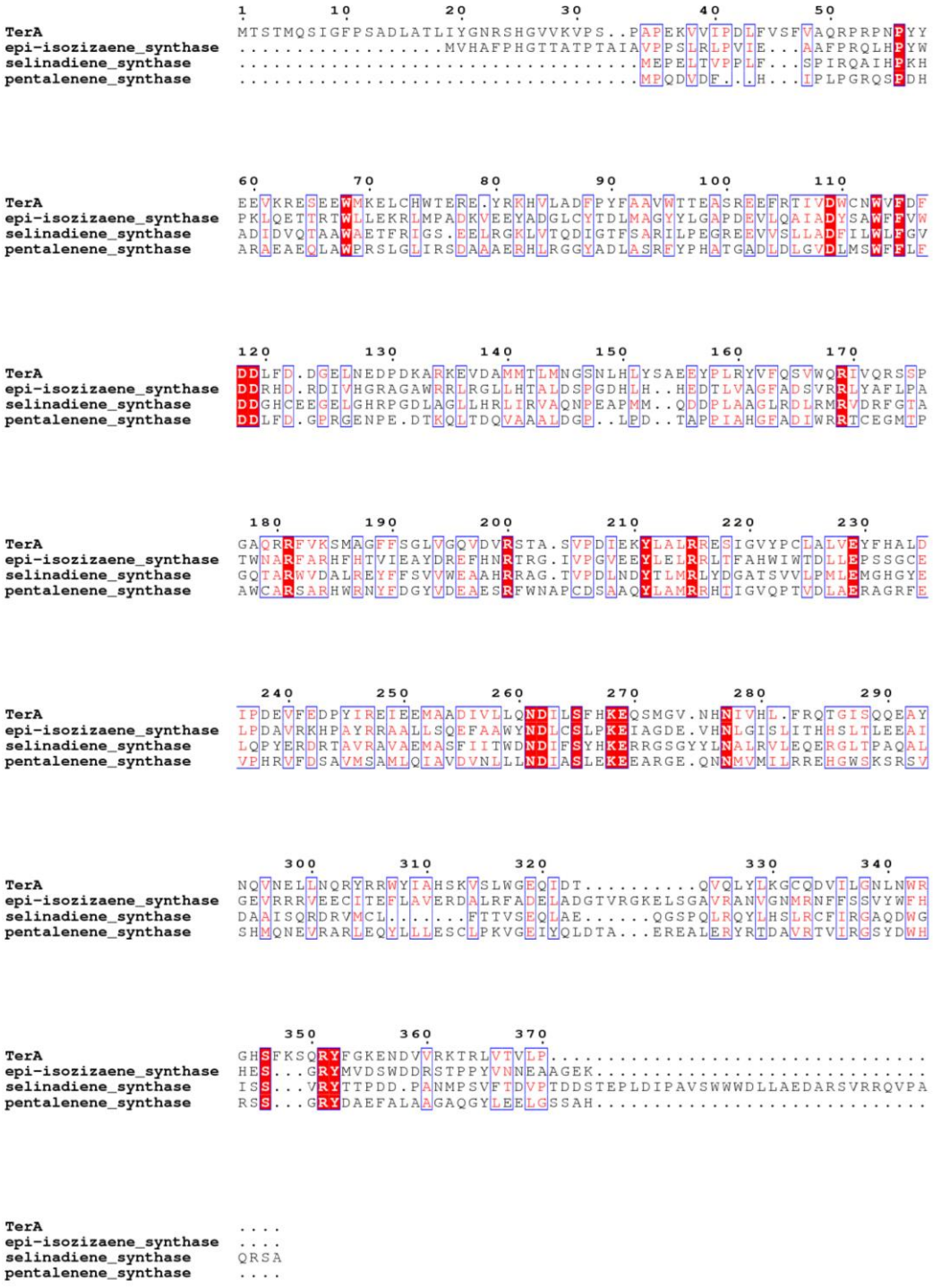


Figure S9. Alignment of the predicted three-dimensional structure of TerA to representative sesquiterpene cyclases. (A) Superimposing TerA (cyan) to epi-isozizaene synthase[9] (PDB: 4LTV, yellow, RMSD = 1.686 Å for 204 Cα atoms) with amino acid sequence identity of 19.5%. (B) Superimposing TerA (cyan) to selinadiene synthase[10] (PDB: 4OKZ, orange, RMSD = 0.685 Å for 211 Cα atoms) with an amino acid sequence identity of 21.9%. (C) Superimposing TerA (cyan) to pentalenene synthase[11] (PDB: 1PS1, purple, RMSD = 1.421 Å for 221 Cα atoms) with an amino acid sequence identity of 23.8%.

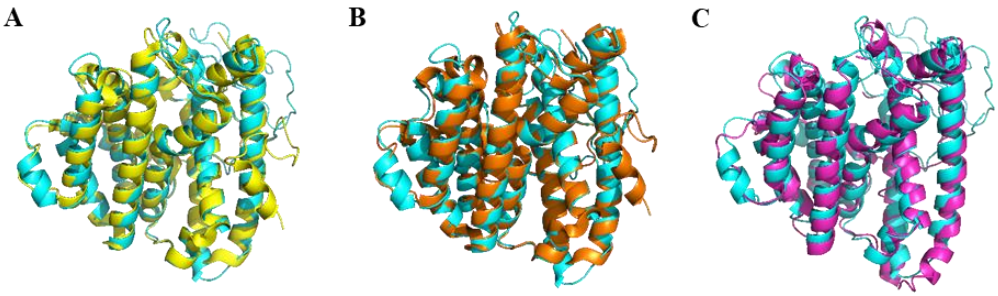


Figure S10. Prediction of the key residues in the active site that are responsible for β-terrecyclene formation. Left, superimposing TerA (cyan) to selinadiene synthase (PDB: 4OKZ, orange, RMSD = 0.685 Å for 211 Cα atoms) complexed with 2,3-dihydro-FPP (yellow) and Mg^{2+} (green sphere). Right, a zoomed-in view of the active site. Side chains of the key residues of TerA proposed to be involved in FPP folding are shown in cyan.

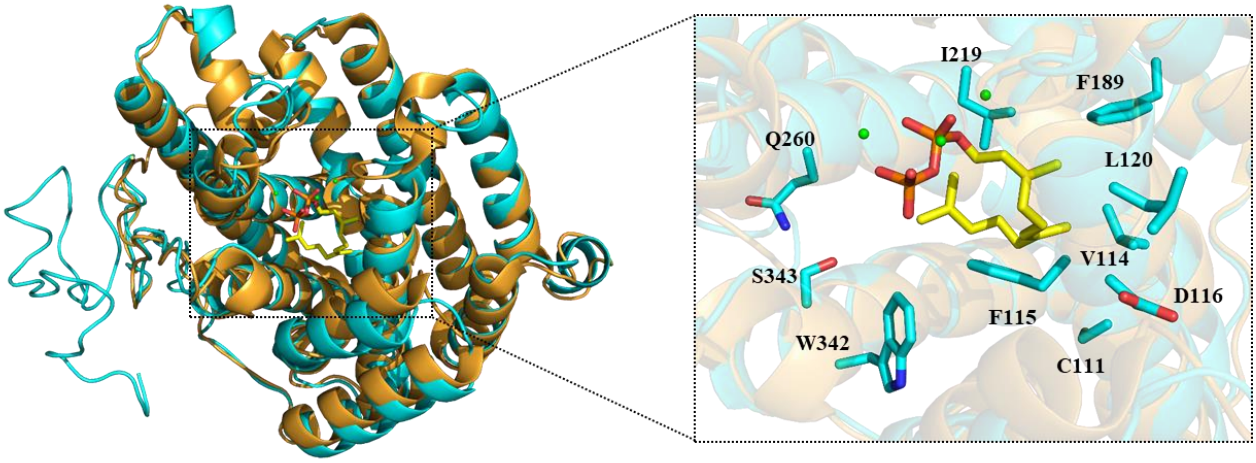
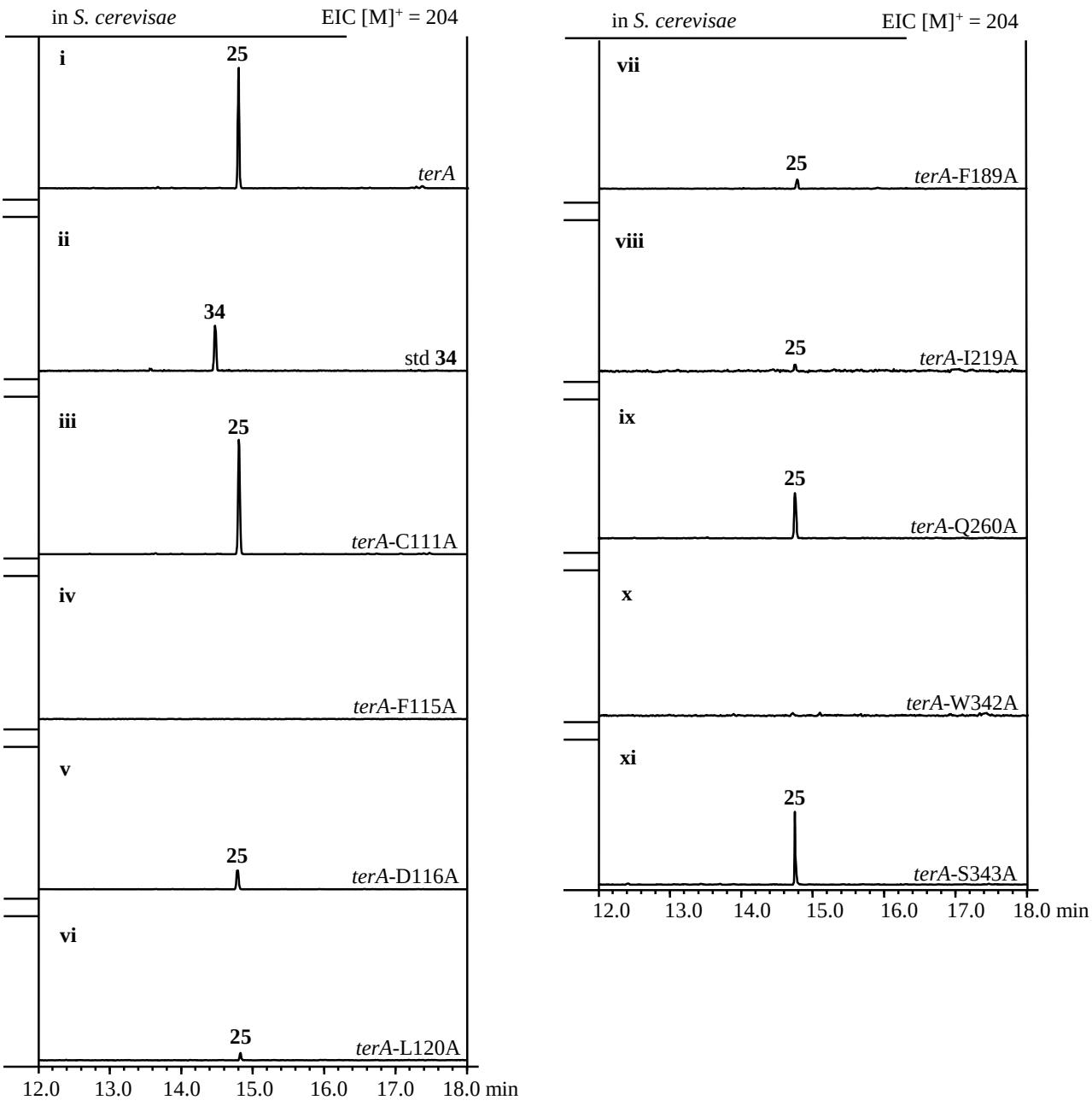
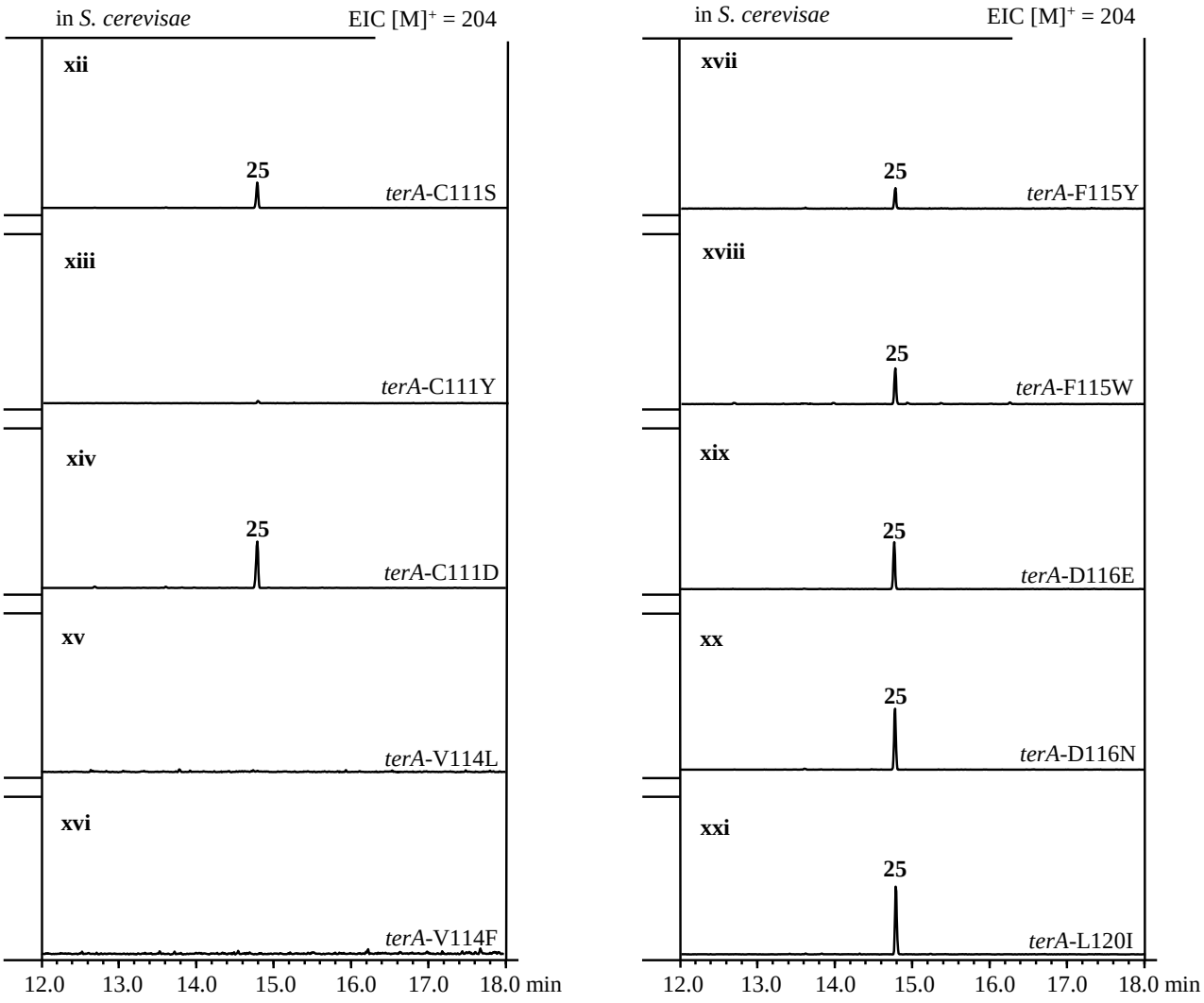
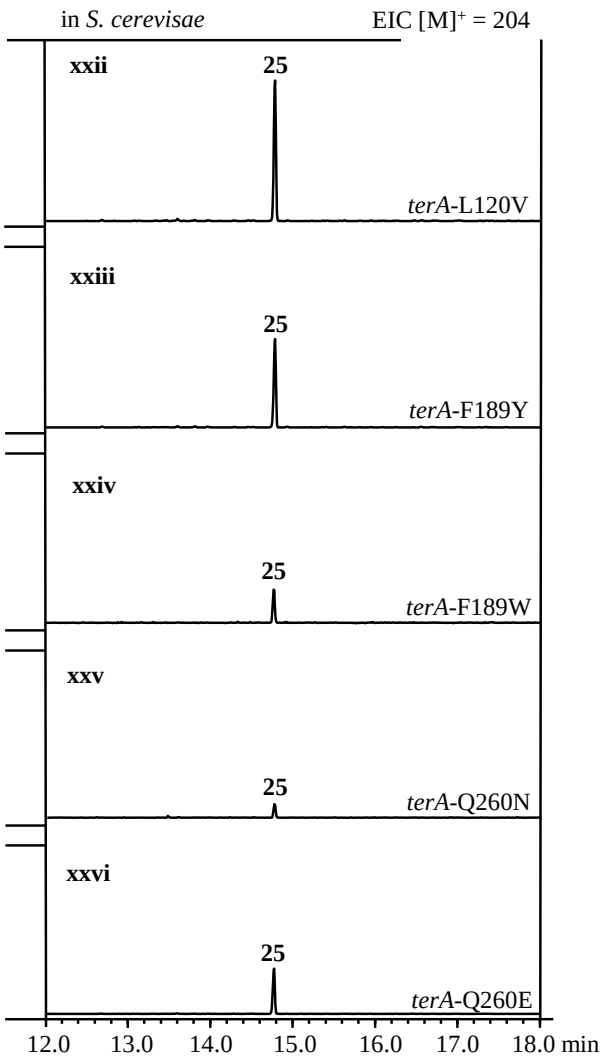


Figure S11. Sesquiterpene products profile of *S. cerevisiae* expressing *terA* mutants. i. *S. cerevisiae* expressing wildtype *terA*; ii. Standard **34**; iii. *S. cerevisiae* expressing *terA*-C111A; iv. *S. cerevisiae* expressing *terA*-F115A; v. *S. cerevisiae* expressing *terA*-D116A; vi. *S. cerevisiae* expressing *terA*-L120A; vii. *S. cerevisiae* expressing *terA*-F189A; viii. *S. cerevisiae* expressing *terA*-I219A; ix. *S. cerevisiae* expressing *terA*-Q260A; x. *S. cerevisiae* expressing *terA*-W342A; xi. *S. cerevisiae* expressing *terA*-S343A; xii. *S. cerevisiae* expressing *terA*- C111S; xiii. *S. cerevisiae* expressing *terA*-C111Y; xiv. *S. cerevisiae* expressing *terA*-C111D; xv. *S. cerevisiae* expressing *terA*-V114L; xvi. *S. cerevisiae* expressing *terA*-V114F; xvii. *S. cerevisiae* expressing *terA*-F115Y; xviii. *S. cerevisiae* expressing *terA*-F115W; xix. *S. cerevisiae* expressing *terA*-D116E; xx. *S. cerevisiae* expressing *terA*-D116N; xxi. *S. cerevisiae* expressing *terA*-L120I; xxii. *S. cerevisiae* expressing *terA*-L120V; xxiii. *S. cerevisiae* expressing *terA*-F189Y; xxiv. *S. cerevisiae* expressing *terA*-F189W; xxv. *S. cerevisiae* expressing *terA*-Q260N; xxvi. *S. cerevisiae* expressing *terA*-Q260E; xxvii. *S. cerevisiae* expressing *terA*-W342F; xxviii. *S. cerevisiae* expressing *terA*-W342Y; xxix. *S. cerevisiae* expressing *terA*-S343T; xxx. *S. cerevisiae* expressing *terA*-S343F.


in S. cerevisae
EIC [M]+ = 204
i
25
terA
ii
34
std 34
iii
25
terA-C111A
iv
terA-F115A
v
25
terA-D116A
vi
25
terA-L120A
12.0
13.0
14.0
15.0
16.0
17.0
18.0 min
in S. cerevisae
EIC [M]+ = 204
vii
25
terA-F189A
viii
25
terA-I219A
ix
25
terA-Q260A
x
terA-W342A
xi
25
terA-S343A
12.0
13.0
14.0
15.0
16.0
17.0
18.0 min


in S. cerevisae
EIC [M]+ = 204
xii
25
terA-C111S
xiii
terA-C111Y
xiv
25
terA-C111D
xv
terA-V114L
xvi
terA-V114F
12.0
13.0
14.0
15.0
16.0
17.0
18.0 min
in S. cerevisae
EIC [M]+ = 204
xvii
25
terA-F115Y
xviii
25
terA-F115W
xix
25
terA-D116E
xx
25
terA-D116N
xxi
25
terA-L120I
12.0
13.0
14.0
15.0
16.0
17.0
18.0 min


in S. cerevisae
EIC [M]+ = 204
xxii
25
terA-L120V
xxiii
25
terA-F189Y
xxiv
25
terA-F189W
xxv
25
terA-Q260N
xxvi
25
terA-Q260E
12.0
13.0
14.0
15.0
16.0
17.0
18.0 min

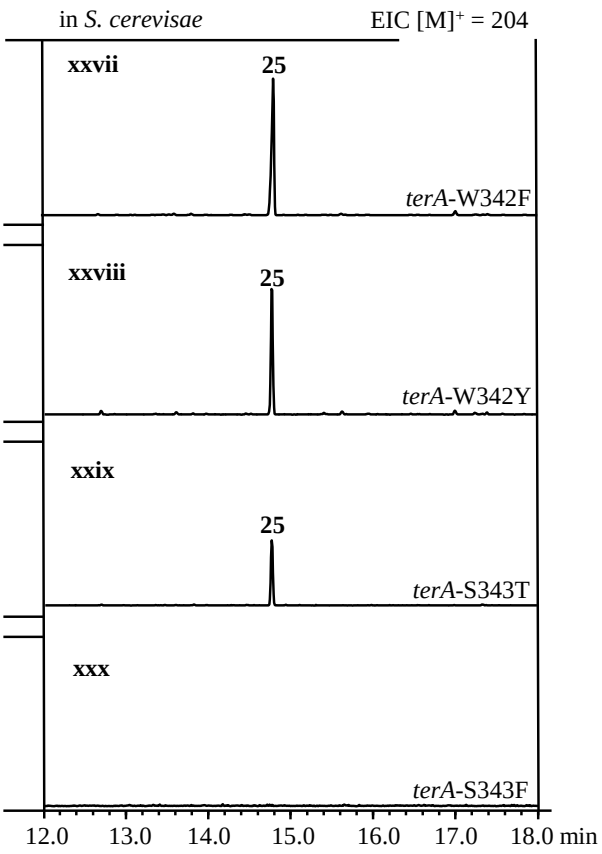

in S. cerevisae
EIC [M]+ = 204
xxvii
25
terA-W342F
xxviii
25
terA-W342Y
xxix
25
terA-S343T
xxx
terA-S343F
12.0
13.0
14.0
15.0
16.0
17.0
18.0 min

Figure S12. The GC-MS spectrum of sesquiterpenes in this study

(A) GC-MS spectrum of **25** (top) and [12-$^{2}H_2$]-**25** (bottom); (B) GC-MS spectrum of **34** (top), standard β-caryophyllene (middle), and [12-$^{2}H_2$]-**34** (bottom); (C) GC-MS spectrum of **35** (top) and standard α-humulene (bottom).

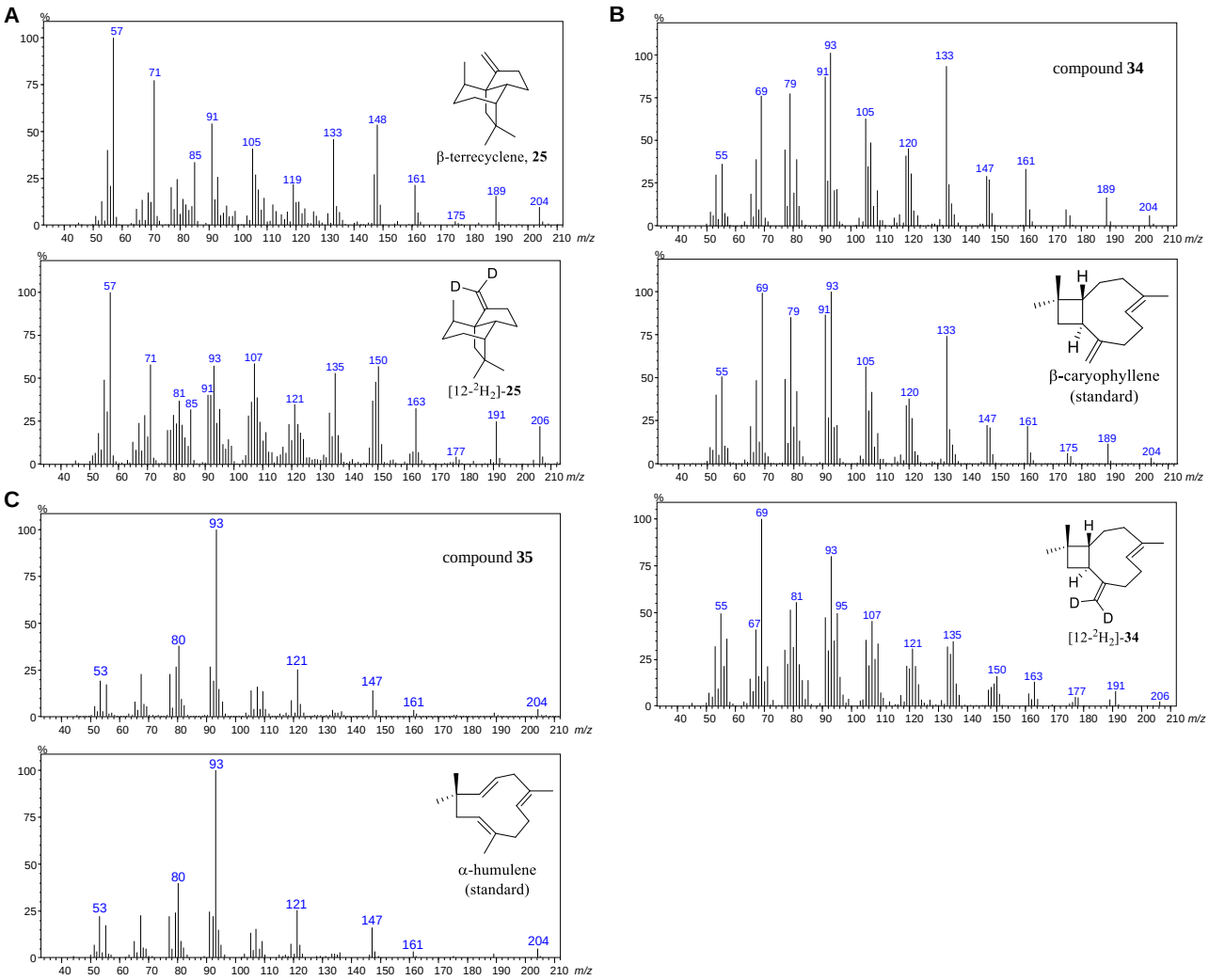
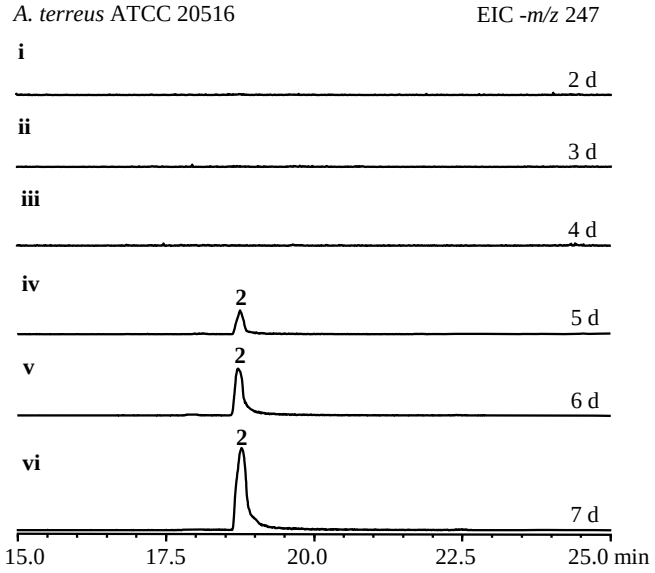


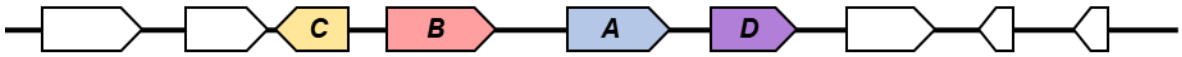
Figure S13. Time-course of terrecyclic acid produced by *Aspergillus terreus* ATCC 20516

i. LC-MS analysis of terrecyclic acid (**2**) produced by *A. terreus* ATCC 20516 in 2 d; ii. LC-MS analysis of **2** produced by *A. terreus* ATCC 20516 in 3 d; iii. LC-MS analysis of **2** produced by *A. terreus* ATCC 20516 in 4 d; iv. LC-MS analysis of **2** produced by *A. terreus* ATCC 20516 in 5 d; v. LC-MS analysis of **2** produced by *A. terreus* ATCC 20516 in 6 d; vi. LC-MS analysis of **2** produced by *A. terreus* ATCC 20516 in 7 d.



Supplementary Tables

Table S1. Terrecyclic acid biosynthetic gene cluster of *Aspergillus terreus* ATCC 20516



Aspergillus terreus ATCC 20516, *ter* gene cluster (accession: OR168753), 20.0 kb

gene	Size (gene/protein)	BLASTP homologs	identity/similarity (%)	proposed function
terA	1574/367	QDO73502.1	49/68	terpene synthase
terB	1952/510	EZF29424.1	51/67	cytochrome P450
terC	1149/274	TKA59789.1	46/64	short-chain dehydrogenase/reductase
terD	1530/510	XP_001211049.1	92/96	major facilitator superfamily transporter

Table S2. Microbial strains used in this study

strain	genotype	source
Aspergillus		
Aspergillus terreus ATCC 20516	wild type	ATCC
Aspergillus nidulans A1145	*ΔpyrG, ΔpyroA, ΔriboB, ΔEM*	[2]
TY302	*Aspergillus nidulans* carrying pTerA-pYTU and pTerB-pYTR	this study
TY303	*Aspergillus nidulans* carrying pTerA-pYTU, pTerB-pYTR, and pTerC-pYTP	this study
TY304	*Aspergillus nidulans* carrying pTerB-pYTR	this study
TY305	*Aspergillus nidulans* carrying pYTU, pYTP, and pYTR as control strain	this study
Saccharomyces cerevisiae		
RC01	*MATα ura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 ura3-52::atCPR prb1 Δ1.6R can1 GAL*	[1]
TY301	RC01 carrying pTerA-xw55	this study
TY310	RC01 carrying pTerA-xw55-C111A	this study
TY311	RC01 carrying pTerA-xw55-V114A	this study
TY312	RC01 carrying pTerA-xw55-F115A	this study
TY313	RC01 carrying pTerA-xw55-D116A	this study
TY314	RC01 carrying pTerA-xw55-L120A	this study
TY315	RC01 carrying pTerA-xw55-F189A	this study
TY316	RC01 carrying pTerA-xw55-I219A	this study
TY317	RC01 carrying pTerA-xw55-Q260A	this study
TY319	RC01 carrying pTerA-xw55-W342A	this study
TY320	RC01 carrying pTerA-xw55-S343A	this study
TY325	RC01 carrying pTerA-xw55 and pTerB-xw02	this study
TY326	RC01 carrying pTerA-xw55, pTerB-xw02 and pTerC-xw06	this study
TY330	RC01 carrying pTerA-xw55-C111S	this study
TY331	RC01 carrying pTerA-xw55-C111Y	this study
TY332	RC01 carrying pTerA-xw55-C111D	this study
TY333	RC01 carrying pTerA-xw55-V114L	this study
TY334	RC01 carrying pTerA-xw55-V114F	this study
TY335	RC01 carrying pTerA-xw55-V114T	this study
TY336	RC01 carrying pTerA-xw55-V114D	this study
TY337	RC01 carrying pTerA-xw55-F115Y	this study
TY338	RC01 carrying pTerA-xw55-F115W	this study
TY339	RC01 carrying pTerA-xw55-D116E	this study
TY340	RC01 carrying pTerA-xw55-D116N	this study
TY341	RC01 carrying pTerA-xw55-L120I	this study
TY342	RC01 carrying pTerA-xw55-L120V	this study
TY343	RC01 carrying pTerA-xw55-F189Y	this study
TY344	RC01 carrying pTerA-xw55-F189W	this study

TY345	RC01 carrying pTerA-xw55-Q260N	this study
TY346	RC01 carrying pTerA-xw55-Q260E	this study
TY351	RC01 carrying pTerA-xw55-W342F	this study
TY352	RC01 carrying pTerA-xw55-W342Y	this study
TY353	RC01 carrying pTerA-xw55-S343T	this study
TY354	RC01 carrying pTerA-xw55-S343F	this study
Escherichia coli		
DH10β		NEB
BL21(DE3)		NEB
TY004	BL21 (DE3) carrying pTerC-pET28a	this study

Table S3. Primers for PCR amplification in this study

primer	sequences of primer (5'→3')
TerA-xw55-recomb-F	aatcaactatcaactattaactatatcgtaataccatatggattataaggatgatgatgataagatg acgtcc
TerA-xw55-recomb-R	gataatgaaaactataaatcgtgaaggcattcagtgatggtgatggtgatggggcaacacggt caccag
TerA-pYTU-recomb-F	ctgagcttcatccccagcatcattacacctcagcaatgacgtccaccatgcaaagtatcg
TerA-pYTU-recomb-R	gacttcaacacagtggaggacataccgtaattttctgtcagggcaacacggtcaccagc
TerB-pYTR-recomb-F	gactaaccattaccccgccacatagacacatctaaacaatggccaactttggtgacattg
TerB-pYTR-recomb-R	acacttctgctaaagggtatcatcgaaagggagtcatccactactccctcagcggagtcg
TerC-pYTP-recomb-F	ctcccttctctgaacaataaaccccacagaaggcatttatgacggcaacatcgacggaag
TerC-pYTP-recomb-R	agtaggagtgatgagacccaacaaccatgataccaggggttacagatgcccctgaccacc
TerC-pET-F	tatcatatgacggcaacatcgacggaag
TerC-pET-R	atagcggccgcttacagatgcccctgaccacc
TerA-C111A-F	actgggccaactgggtctttgactttgatgatttgttc
TerA-C111A-R	agttggcccagtccacaatagtgcgaaactc
TerA-V114A-F	actgggcctttgactttgatgatttgttcgacgacg
TerA-V114A-R	tcaaaggcccagttgcaccagtccacaatag
TerA-F115A-F	tgggtcgctgactttgatgatttgttcgacgacg
TerA-F115A-R	aagtcagcgacccagttgcaccagtcc
TerA-D116A-F	tctttgcctttgatgatttgttcgacgacggc
TerA-D116A-R	tcaaaggcaaagacccagttgcaccagtc
TerA-L120A-F	atgatgcgttcgacgacggcgagc
TerA-L120A-R	tcgaacgcatcatcaaagtcaaagacccagttgc
TerA-F189A-F	tggccggggctttctcgggtctggtgggc
TerA-F189A-R	agaaagccccggccatgctcttgac
TerA-I219A-F	agtccgctggagtatacccatgcttggcc
TerA-I219A-R	actccagcggactcccgacgaaggg
TerA-Q260A-F	ttctggcgaacgatatcctgtcgttccacaaag
TerA-Q260A-R	tcgttcgccagaaggacaatgtccgccg
TerA-W342A-F	tgaacgcgagcttcaagtcgcagcgatac
TerA-W342A-R	aagctcgcgttcaggttccctaggatcacatcc
TerA-S343A-F	actgggccttcaagtcgcagcgatactttgg
TerA-S343A-R	ttgaaggcccagttcaggttccctaggatcac
TerB-xw06-recomb-F	caactatcaactattaactatatcgtaataccatcatatggccaactttggtgacattgc
TerB-xw06-recomb-R	atacttgataatggaaactataaatcgtgaaggcatgtttctactccctcagcggagtcg
TerC-xw02-recomb-F	caatcaactatcaactattaactatatcgtaataccatatgacggcaacatcgacggaag
TerC-xw02-recomb-R	actaatataggcatacttgataatgaaaactataaatcgttacagatgcccctgaccacc
TerA-C111S-F	actggtccaactgggtctttgactttgatgatttgttc
TerA-C111S-R	ccagttggaccagtccacaatagtgcgaaactc
TerA-C111Y-F	actggtacaactgggtctttgactttgatgatttgttc
TerA-C111Y-R	acccagttgtaccagtccacaatagtgcgaaactc
TerA-C111D-F	actgggacaactgggtctttgactttgatgatttgttc
TerA-C111D-R	agttgtcccagtccacaatagtgcgaaactc

TerA-V114L-F	actggctgtttgactttgatgatttgttcgacgacg
TerA-V114L-R	tcaaacagccagttgcaccagtccacaatag
TerA-V114F-F	ctggtgcaactggttttttgactttgatgatttgttcgacg
TerA-V114F-R	atcatcaaagtcaaaaaaccagttgcaccagtccac
TerA-V114T-F	actggacctttgactttgatgatttgttcgacgacg
TerA-V114T-R	tcaaaggtccagttgcaccagtccacaatag
TerA-V114D-F	gtgcaactgggactttgactttgatgatttgttcgacgacg
TerA-V114D-R	gtcaaagtcccagttgcaccagtccacaatag
TerA-F115Y-F	tgggtctatgactttgatgatttgttcgacgacg
TerA-F115Y-R	aagtcatagacccagttgcaccagtcc
TerA-F115W-F	caactgggtctgggactttgatgatttgttcgacgacg
TerA-F115W-R	aagtcccagacccagttgcaccagtcc
TerA-D116E-F	tctttgaatttgatgatttgttcgacgacggc
TerA-D116E-R	tcaaattcaaagacccagttgcaccagtcc
TerA-D116N-F	gtctttaactttgatgatttgttcgacgacggcgag
TerA-D116N-R	tcaaagttaaagacccagttgcaccagtcc
TerA-L120I-F	atgatatattcgacgacggcgagc
TerA-L120I-R	tcgaatatatcatcaaagtcaaagacccagttgc
TerA-L120V-F	atgatgtgttcgacgacggcgagc
TerA-L120V-R	tcgaacacatcatcaaagtcaaagacccagttgc
TerA-F189Y-F	tggccgggtatttctcgggtctggtgggccag
TerA-F189Y-R	agaaatacccggccatgctcttgac
TerA-F189W-F	tggccgggtggttctcgggtctggtgggc
TerA-F189W-R	agaaccacccggccatgctcttgac
TerA-Q260N-F	ccttctgaacaacgatatcctgtcgttccacaaag
TerA-Q260N-R	tcgttgttcagaaggacaatgtccgccg
TerA-Q260E-F	ttctggaaaacgatatcctgtcgttccacaaag
TerA-Q260E-R	tcgttttccagaaggacaatgtccgccg
TerA-W342F-F	tgaactttagcttcaagtcgcagcgatac
TerA-W342F-R	aagctaaagttcaggttccctaggatcacatcc
TerA-W342Y-F	tgaactatagcttcaagtcgcagcgatac
TerA-W342Y-R	aagctatagttcaggttccctaggatcacatcc
TerA-S343T-F	actggactttcaagtcgcagcgatactttgg
TerA-S343T-R	ttgaaagtccagttcaggttccctaggatcac
TerA-S343F-F	actggttcttcaagtcgcagcgatactttgg
TerA-S343F-R	ttgaagaaccagttcaggttccctaggatcac

Table S4. Plasmids used in this study

plasmids	features	source
pXW55	protein expression vector in *S. cerevisiae* RC01 (*URA3* marker)	[1]
pXW06	protein expression vector in *S. cerevisiae* RC01 (*TRP1* marker)	[1]
pXW02	protein expression vector in *S. cerevisiae* RC01 (*LEU2* marker)	[1]
pYTU	protein expression vector in *A. nidulans A1145* (*pyrG* marker)	[2]
pYTR	protein expression vector in *A. nidulans A1145* (*riboB* marker)	[2]
pYTP	protein expression vector in *A. nidulans A1145* (*pyroA* marker)	[2]
pET28a	protein expression vector in *E. coli* BL21 (DE3)	Addgene
pTerA-xw55	pXW55 expressing TerA	this study
pTerA-pYTU	pYTU expressing TerA	this study
pTerB-pYTR	pYTR expressing TerB	this study
pTerC-pYTP	pYTP expressing TerC	this study
pTerC-pET28a	pET28a expressing TerC	this study
pTerA-C111A-xw55	pXW55 expressing TerA with C111A mutation	this study
pTerA-V114A-xw55	pXW55 expressing TerA with V114A mutation	this study
pTerA-F115A-xw55	pXW55 expressing TerA with F115A mutation	this study
pTerA-D116A-xw55	pXW55 expressing TerA with D116A mutation	this study
pTerA-L120A-xw55	pXW55 expressing TerA with L120A mutation	this study
pTerA-F189A-xw55	pXW55 expressing TerA with F189A mutation	this study
pTerA-I219A-xw55	pXW55 expressing TerA with I219A mutation	this study
pTerA-Q260A-xw55	pXW55 expressing TerA with Q260A mutation	this study
pTerA-W342A-xw55	pXW55 expressing TerA with W342A mutation	this study
pTerA-S343A-xw55	pXW55 expressing TerA with S343A mutation	this study
pTerB-xw06	pXW06 expressing TerB	this study
pTerC-xw02	pXW02 expressing TerC	this study
pTerA-C111S-xw55	pXW55 expressing TerA with C111S mutation	this study
pTerA-C111Y-xw55	pXW55 expressing TerA with C111Y mutation	this study
pTerA-C111D-xw55	pXW55 expressing TerA with C111D mutation	this study
pTerA-V114L-xw55	pXW55 expressing TerA with V114L mutation	this study
pTerA-V114F-xw55	pXW55 expressing TerA with V114F mutation	this study
pTerA-V114T-xw55	pXW55 expressing TerA with V114T mutation	this study
pTerA-V114D-xw55	pXW55 expressing TerA with V114D mutation	this study
pTerA-F115Y-xw55	pXW55 expressing TerA with F115Y mutation	this study
pTerA-F115W-xw55	pXW55 expressing TerA with F115W mutation	this study
pTerA-D116E-xw55	pXW55 expressing TerA with D116E mutation	this study
pTerA-D116N-xw55	pXW55 expressing TerA with D116N mutation	this study
pTerA-L120I-xw55	pXW55 expressing TerA with L120I mutation	this study
pTerA-L120V-xw55	pXW55 expressing TerA with L120V mutation	this study
pTerA-F189Y-xw55	pXW55 expressing TerA with F189Y mutation	this study
pTerA-F189W-xw55	pXW55 expressing TerA with F189W mutation	this study

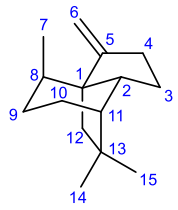
pTerA-Q260N-xw55	pXW55 expressing TerA with Q260N mutation	this study
pTerA-Q260E-xw55	pXW55 expressing TerA with Q260E mutation	this study
pTerA-W342F-xw55	pXW55 expressing TerA with W342F mutation	this study
pTerA-W342Y-xw55	pXW55 expressing TerA with W342Y mutation	this study
pTerA-S343T-xw55	pXW55 expressing TerA with S343T mutation	this study
pTerA-S343F-xw55	pXW55 expressing TerA with S343F mutation	this study

Table S5. Differential gene expression analysis of *ter* gene cluster

gene	protein	proposed function	meanA (non-producing)	meanB (producing)	log_2-fold change (B/A)
g10006	ORF-3	fungal-specific transcription factor domain-containing protein	285.85	261.35	-0.13
g10007	ORF-2	major facilitator superfamily domain-containing protein	0	19.46	∞
g10008	ORF-1	transcriptional regulatory protein	2941.73	2964.95	0.01
g10009	TerC	short chain dehydrogenase	2609.77	61651.55	4.56
g10010	TerB	cytochrome P450	3880.67	38491.72	3.31
g10011	TerA	terpene synthase	38.97	10538.59	8.08
g10012	TerD	major facilitator superfamily transporter	627.19	19582.59	4.96
g10013	ORF+1	hypothetical protein	99.03	318.25	1.68
g10014	ORF+2	hypothetical protein	10513.28	4365.83	-1.27
g10015	ORF+3	hypothetical protein	7.97	98.96	3.63

Table S6. NMR data and structure

^{1}H (500 MHz, $CDCl_3$) and ^{13}C NMR (126 MHz, $CDCl_3$) of compound 25:



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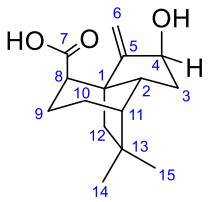
no.	δ_H, mult. (J in Hz)	δ_C	type	HMBC
1	-	59.8	C	-
2	2.06, dd (11.4, 7.5)	52.1	CH	59.8, 54.7, 39.5, 28.1
3	1.84, m	28.8	CH_2	52.1, 50.2, 35.6
3’	1.71, m			160.2, 59.8
4	2.49 ddd (15.9, 7.6, 1.7)	35.6	CH_2	160.2, 102.9, 59.8, 52.1, 28.1
4’	2.16, m			160.2, 102.9, 28.1
5	-	160.2	C	-
6	4.83, dt (2.6, 1.3)	102.9	CH_2	160.2, 59.8, 35.6
6’	4.56, dt (2.9, 1.4)			160.2, 59.8, 35.6
7	0.91, d (7.2)	17.5	CH_3	59.8, 37.7, 27.1
8	1.97, m	37.7	CH	59.8, 52.1, 28.1, 27.1, 17.5
9	2.00, m	27.1	CH_2	59.8, 50.2, 37.7, 28.1, 17.5
9’	1.23, m			59.8, 50.2, 37.7, 28.1, 17.5
10	1.59, m	28.1	CH_2	52.1, 37.7, 27.1
10’	1.46, m			54.7, 52.1, 39.5, 27.1
11	1.77, m	50.2	CH	59.8, 54.7, 52.1, 35.2
12	1.65, d (14.0)	54.7	CH_2	160.2, 59.8, 50.2, 39.5, 37.7, 35.2, 26.8
12’	1.60, m			160.2, 52.1, 50.2, 39.5, 37.7, 35.2, 26.8
13	-	39.5	C	-
14	1.09, s	26.8	CH_3	54.7, 50.2, 39.5, 35.2
15	1.19, s	35.2	CH_3	54.7, 50.2, 39.5, 26.8

^{1}H (700 MHz, $CDCl_3$) and ^{13}C NMR (176 MHz, $CDCl_3$) of compound 32:



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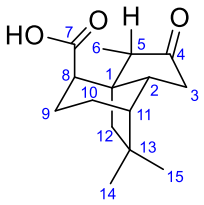
no.	δ_H, mult. (J in Hz)	δ_C	type	HMBC
1	-	55.5	C	-
2	2.79, dd (13.0, 6.7)	51.3	CH	55.5, 54.3, 48.9, 48.6, 38.6, 40.0, 29.3
3	1.98, td (13.2, 4.9)	38.6	CH_2	77.0, 51.3, 48.6
3’	1.91, m			160.6, 77.0, 55.5
4	4.47, d (4.8)	77.0	CH	160.6, 109.8, 55.5, 51.3
5	-	160.6	C	-
6	5.21, s	109.8	CH_2	160.6, 77.0, 55.5
6’	4.89, s			160.6, 77.0, 55.5
7	-	179.9	C	-
8	2.92, d (8.9)	48.9	CH	179.9, 55.5, 54.3, 51.3, 29.3, 23.1
9	2.17, m	23.1	CH_2	179.9, 48.6, 29.3
9’	1.91, m			179.9, 55.5, 48.6, 29.3
10	1.76, m	29.3	CH_2	48.9, 48.6, 23.1
10’	1.56, m			51.3, 40.0, 23.1
11	1.85, m	48.6	CH	55.5, 34.9, 29.3, 27.5
12	1.65, d (14.5)	54.3	CH_2	160.6, 55.5, 48.9, 48.6, 40.0, 34.9, 27.5
12’	1.62, d (14.5)			55.5, 48.9, 48.6, 40.0, 34.9, 27.5
13	-	40.0	C	-
14	1.17, s	34.9	CH_3	54.3, 48.6, 40.0, 27.5
15	1.11, s	27.5	CH_3	54.3, 48.6, 40.0, 34.9

^{1}H (700 MHz, $CDCl_3$) and ^{13}C NMR (175 MHz, $CDCl_3$) of compound 33:



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no.	δ_H, mult. (*J* in Hz)	δ_C	type	HMBC
1	-	55.0	C	-
2	2.77, m	45.1	CH	55.0, 47.7, 39.6, 28.9
3	2.51, m	40.6	CH_2	219.3, 55.0, 49.4, 45.1
3’	2.36, dd (20.1, 7.6)			219.3, 49.4, 45.1
4	-	219.3	C	-
5	2.46, q (6.8)	51.7	CH	219.3, 55.0, 48.1, 8.7
6	0.99, d (6.7)	8.7	CH_3	219.3, 55.0, 51.7
7	-	180.4	C	-
8	2.77, m	48.1	CH	180.4, 55.0, 47.7, 28.9
9	1.93, m	22.2	CH_2	180.4, 48.1, 28.9
9’	1.89, m			180.4, 55.0, 49.4, 28.9
10	1.89, m	28.9	CH_2	48.1, 39.6
10’	1.78, m			48.1
11	1.85, m	49.4	CH	55.0, 47.7, 22.2
12	1.41, d (15.2)	47.7	CH_2	55.0, 48.1, 39.6, 34.3, 27.3
12’	1.39, d (15.2)			55.0, 48.1, 39.6, 34.3, 27.3
13	-	39.6	C	-
14	1.10, s	34.3	CH_3	49.4, 47.7, 39.6, 27.3
15	1.13, s	27.3	CH_3	49.4, 47.7, 39.6, 34.3

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