Supporting Information

Discovery of pentaene polyols by activation of an enediyne gene cluster: biosynthetic implications for 9-membered enediyne core structures

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General Experimental Procedures

All chemical and biological regents used in this study were from commercial sources, unless otherwise specified. High-resolution electrospray ionization mass data (HR-ESI-MS) were recorded on an LTQ-ORBITRAP-ETD instrument. HR-APCI-MS/MS were recorded on TOF-x500r-AB SCIEX. UV spectra were measured on a Waters 2998 photo-diode array (PDA) Detector. CD spectra were recorded on J-815 from JASCO. NMR spectra were acquired using 500 or 600 MHz Bruker spectrometers. The chemical shifts in ^{1}H NMR and ^{13}C NMR spectra were referenced to the solvents for methanol-d_4 (δ_H 3.31 and δ_C 49.0), and DMSO-d_6 (δ_H 2.50 and δ_C 39.6).

Column chromatography was performed using silica gel (200–300 mesh, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China), RP-C18 (AAG12S50, YMC Co., Ltd.), and Sephadex LH-20 (GE Healthcare). Fermentation crude extracts of *Streptomyces* sp. CB02130 and its mutants and purified natural products were analyzed on the ACQUITY UPLC (Ultra Performance Liquid Chromatography) system (Waters), equipped with a PDA detector and a C-18 column (1.7 *µ*m, 2.1 × 100 mm, ACQUITY UPLC BEH C18). The flow rate was applied at 0.3 mL min^{-1}. Alternatively, the Waters E2695 HPLC (High Performance Liquid Chromatography) system, equipped with a PDA detector and a Welch AQ-C18 column (5 *µ*m, 250 × 4.6 mm). The flow rate was applied at 1.0 mL min^{-1}. The mobile phase consisted of buffer A (ultrapure H_2O containing 0.1 % HCO_2H) and buffer B (chromatographic grade CH_3CN containing 0.1 % HCO_2H). Semipreparative reversed phase-HPLC (RP-HPLC) was performed using a Waters 1525 binary HPLC pump equipped with a Waters 2489 UV/visible detector and a Welch Ultimate AQ-C18 column (250 × 10 mm, 5 *µ*m).

Oligonucleotide primer synthesis and DNA sequencing were performed by Tsingke (Changsha, China). Trelief™ SoSoo cloning kit and Taq polymerase (Tsingke), restriction endonucleases, T4 DNA ligase (NEB), DNA gel extraction kit, and plasmid preparation kit (Sangon Biotech, Shanghai, China) were from commercial sources.

Strains and culture conditions.

The *S.* sp. CB02130 strain was previously isolated from Wuliang Mountain, at an altitude of 1900—2700 m in Yunnan province, China.[1] *Escherichia coli* DH5α was used for cloning and *E. coli* S17-1 was used for conjugation. (see **Table S3** for all plasmids and strains used). *E. coli* carrying plasmids were grown in Luria-Bertani (LB) medium at 37 °C for general cloning, and were selected with appropriate antibiotics.

S. sp*.* CB02130 and its mutants were cultured in 250-mL baffled flasks containing 50 mL tryptic soy broth (TSB) liquid medium for 48 h (30 °C, 220 rpm. Then the seed culture (3 mL) was transferred to the production medium (50 mL) containing (per liter) glucose 40 g, casamino acids 5 g, NaCl 5 g, $MgSO_4$ 2.5 g, K_2HPO_3 1 g, $CaCO_3$ 2 g. After fermentation for 7 days, the resulting culture supernatants were extracted by ethyl acetate (EtOAc) (50 mL × 3). The EtOAc fractions were concentrated to 2 mL in vacuum and analyzed by HPLC using a linear gradient of CH_3CN in H_2O with 0.1% formic acid (0—1 min, 20%; 1—20 min, 20%—60%; 20—26 min, 60%—80%; 26—30 min,80%; 30—35 min, 80%-20%; 35—38 min, 20%). Alternatively, 2% of macroporous resins (XAD-16N) was added in the production medium to absorb expected metabolites; the resins were subsequently extracted with MeOH (50 mL × 2) and concentrated to 2 mL. A aliquot of ~20 µL analyte was injected for HPLC analysis.

Genome neighborhood network (GNN) analysis of biosynthetic gene clusters (BGCs) homologous to neocarzinostatin BGC and annotation of *wls* BGC

A total of 15 known enediyne BGCs, including, calicheamicin (*cal*), dynemycin (*dyn*), esperamicin (*esp*), sungeidine (*sgd*), tiancimycin (*tnm*), uncialamycin (*ucm*), yangpumicin (*ypm*), kedarcidin (*ked*), maduropeptin (*mdp*), C-1027 (*sgc*), neocarzinosatin (*ncs*), amycolamycin (*acm*), cyanosporaside C (*cya*), cyanosporaside F (*cyn*), sporolides (*spo*), and seven putative enediyne BGCs from CB02130, CB02261, CB00455, CB03578, CB02400, CB02009, and CB01883, which are homologous to neocarzinostatin BGC, were analyzed by GNN.[1] The resulting proteins (1172) were all used in an *all versus all* BLAST search by BLAST^{+} with an *E*-value limit of 10^{-6}. The determination of the *E*-value limit was based on the lowest similarity between apoproteins for 9-membered enediyne biosynthessi. Next, self-loops and undirected duplicates were deleted. Cytoscape v3.0 was then used for GNN generation, visualization, and analysis. All GNNs were displayed using the “organic” layout with edge widths corresponding to the *E*-value between proteins.

The proteins belonging to *wls* BGC from *S*. sp. CB02130 were also identified based the comparison with the known enediyne BGCs and GNN analysis. If no homologous proteins were identified, a BlastP search against GenBank was then performed to deduce the putative function.

Distribution of the WlsPDH homologs

We performed a Blastp search against the nonredundant protein sequence database from NCBI (March 2, 2022) using WlsPDH as an in silico probe. A total of 67 proteins with over 50% sequence identity were identified, encoding putative prephenate dehydrogenases. Further antiSMASH 5.0 analyses of neighboring genes of these putative prephenate dehydrogenases led to the discovery of 67 putative enediyne BGCs. Since many of these 67 putative enediyne BGCs are highly homologous, we next used PKSE sequence identity cutoff (≥ 93.86%) to filter them to 31 representative BGCs using CD-HIT. The 93.86% PKSE sequence identity threshold was selected by the same sequence identity between two PKSEs from previously identified C-1027 producers: *Streptomyces globisporus* and *Streptomyces* sp. CB02366.[2] Finally, all the proteins in the remaining 31 BGCs were used in an *all versus all* BLAST search using BLAST$^+$ and an *E*-value limit of 10^{-6}. Cytoscape v3.4 was used for GNN visualization and analysis.

Gene replacement

For gene replacement of *wls*R3, two 2-kb fragments of the up-stream and down-stream of *wls*R3 were amplified from the genomic DNA of CB02130, with the primers wlsR3 UpF and wlsR3 UpR, or wlsR3 DnF and wlsR3 DnR. The resulting DNA fragments and a 919-bp thiostrepton-resistant gene (*tsr*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4001. The plasmid pYX4001 was next conjugated to C02130 from *E. coli* S17-1 to obtain thiostrepton-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and thiostrepton or thiostrepton only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and thiostrepton-resistance phenotype were obtained, yielding YX4001 (Δ*wls*R3). The gene replacement of *wlsR3* was further confirmed by PCR using primers wlsR3_KOtest_F and wlsR3_KOtest_R.

For gene replacement of *wls*R2, two 2-kb fragments of the up-stream and down-stream of *wls*R3 were amplified from the genomic DNA of CB02130, with the primers wlsR2 UpF and wlsR2 UpR, or wlsR2 DnF and wlsR2 DnR. The resulting DNA fragments and a 919-bp thiostrepton-resistant gene (*tsr*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4010. The plasmid pYX4001 was next conjugated to C02130 from *E. coli* S17-1 to obtain thiostrepton-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and thiostrepton or thiostrepton only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and thiostrepton-resistance phenotype were obtained, yielding YX4011 (Δ*wls*R2). The gene replacement of *wlsR2* was further confirmed by PCR using primers wlsR2_KOtest_F and wlsR2_KOtest_R.

For gene replacement of *wls*E, two 2-kb fragments of the up-stream and down-stream of *wls*E were amplified from the genomic DNA of CB02130, with the primers wlsE UpF and wlsE UpR, or wlsE DnF and wlsE DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4010. The plasmid pYX4001 was next conjugated toYX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4003 (Δ*wls*R3/Δ*wls*E). The gene replacement of *wlsE* was further confirmed by PCR using primers wlsE_KOtest_F and wlsE_KOtest_R.

For gene replacement of *wls*C4, two 2-kb fragments of the up-stream and down-stream of *wls*C4 were amplified from the genomic DNA of CB02130, with the primers wlsC4 UpF and wlsC4 UpR, or wlsC4 DnF and wlsC4 DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4005. The plasmid pYX4005 was next conjugated to YX4001 from *E. coli*

S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4005 (Δ*wls*R3/Δ*wls*C4). The gene replacement of *wls*C4 was further confirmed by PCR using primers wlsC4_KOtest_F and wlsC4_KOtest_R.

For gene replacement of *wls*ORF3, two 2-kb fragments of the up-stream and down-stream of *wls*ORF3 were amplified from the genomic DNA of CB02130, with the primers *wls*ORF3 UpF and *wls*ORF3 UpR, or *wls*ORF3 DnF and *wls*ORF3 DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4006. The plasmid pYX4006 was next conjugated to YX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4006 (Δ*wls*R3/Δ*wls*ORF3). The gene replacement of *wls*orf3 was further confirmed by PCR using primers wlsprf3_KOtest_F and wlsorf3_KOtest_R.

For gene replacement of *wls*ORF30, two 2-kb fragments of the up-stream and down-stream of *wlsORF30* were amplified from the genomic DNA of CB02130, with the primers wlsORF30 UpF and wlsORF30 UpR, or wlsORF30 DnF and wlsORF30 DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4007. The plasmid pYX4007 was next conjugated to YX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4007 (Δ*wls*R3/Δ*wls*ORF30). The gene replacement of *wls*ORF30 was further confirmed by PCR using primers wlsorf30_KOtest_F and wlsorf30_KOtest_R.

For gene replacement of *wls*PDH, two 2-kb fragments of the up-stream and down-stream of *wls*PDH were amplified from the genomic DNA of CB02130, with the primers wlsPDH UpF and wlsPDH UpR, or wlsPDH DnF and wlsPDH DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4008. The plasmid pYX4008 was next conjugated to YX4001 or CB02130 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants, yielding YX4008 (Δ*wls*R3/Δ*wls*PDH) or YX4009 (*Δwls*PDH). The gene replacement of *wls*PDH was further confirmed by PCR using primers wlsPDH_KOtest_F and wlsPDH_KOtest_R.

For gene replacement of *wls*F, two 2-kb fragments of the up-stream and down-stream of *wls*F were amplified from the genomic DNA of CB02130, with the primers wlsF UpF and wlsF UpR, or wlsF DnF and wlsF DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4009. The plasmid pYX4009 was next conjugated to YX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4010 (*Δwls*F/*Δwls*R3). The gene replacement of *wls*F was further confirmed by PCR using primers wlsF_KOtest_F and wlsF_KOtest_R.

For gene replacement of *wls*ORF8, two 2-kb fragments of the up-stream and down-stream of *wls*ORF8 were amplified from the genomic DNA of CB02130, with the primers wlsorf8_UpF and wlsorf8_UpR, or wlsorf8_ DnF and wlsorf8_DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4018. The plasmid pYX4018 was next conjugated to YX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4018 (*Δwls*R3/*Δwls*ORF8). The gene replacement of *wls*ORF8 was further confirmed by PCR using primers wlsorf8_KOtest_F and wlsorf8_KOtest_R.

For gene replacement of *wls*ORF24, two 2-kb fragments of the up-stream and down-stream of *wls*ORF24 were amplified from the genomic DNA of CB02130, with the primers wlsorf24_UpF and wlsorf24_UpR, or wlsorf24_DnF and wlsorf24_DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4019. The plasmid pYX4019 was next conjugated to YX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4019 (*Δwls*R3/*Δwls*ORF24). The gene replacement of *wls*ORF24 was further confirmed by PCR using primers wlsorf24_KOtest_F and wlsorf24_KOtest_R.

For gene replacement of *wls*L, two 2-kb fragments of the up-stream and down-stream of *wls*L were amplified from the genomic DNA of CB02130, with the primers wlsL_UpF and wlsL_UpR, or wlsL_DnF and wlsL_DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4020. The plasmid pYX4020 was next conjugated to YX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4020 (*Δwls*R3/*Δwls*L). The gene replacement of *wls*L was further confirmed by PCR using primers wlsL_KOtest_F and wlsL_KOtest_R.

For gene replacement of *wls*E7, two 2-kb fragments of the up-stream and down-stream of *wls*E7 were amplified from the genomic DNA of CB02130, with the primers wlsE7_UpF and wlsE7_UpR, or wlsE7_DnF and wlsE7_DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4021. The plasmid pYX4021 was next conjugated to YX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4021 (*Δwls*R3/*Δwls*E7). The gene replacement of *wls*E7 was further confirmed by PCR using primers wlsE7_KOtest_F and wlsE7_KOtest_R.

For gene replacement of *wls*E9, two 2-kb fragments of the up-stream and down-stream of *wls*E9 were amplified from the genomic DNA of CB02130, with the primers wlsE9 UpF and wlsE9_UpR, or wlsE9_DnF and wlsE9_DnR. The resulting DNA fragments and a 1490-bp kanamycin-resistant gene (*kana*) were cloned into pOJ260 using the Trelief™ SoSoo cloning kit to obtain pYX4022. The plasmid pYX4022 was next conjugated to YX4001 from *E. coli* S17-1 to obtain kanamycin-resistant ex-conjugants. The exconjugants were picked onto two types of MS plates containing apramycin and kanamycin or kanamycin only. After 4−5 days of growth at 30 °C, ex-conjugants with apramycin-sensitive and kanamycin-resistance phenotype were obtained, yielding YX4022 (*Δwls*R3/*Δwls*E9). The gene replacement of *wls*E9 was further confirmed by PCR using primers wlsE9_KOtest_F and wlsE9_KOtest_R.

Overexpression of genes belonging to *wls* BGC in CB02130

The putative regulatory genes*,* *wls*R1, *wls*R3, *wls*R5, *wls*R6, *wls*R7 were amplified from the genomic DNA of CB02130, using primers wlsR1_F/R, wlsR3_F/R, wlsR5_ F/R, wlsR6_F/R, wlsR7_F/R. The resulting DNA fragments were purified and ligated to a modified plasmid-based on pSET152 to place them under the control of *ermE* promoter, [3] resulting in gene overexpressing plasmids pYX4011, pYX4002, pYX4012, pYX4013, and pYX4014. These plasmids were similarly conjugated to CB02130 to result in strains YX4012, YX4013, YX4014, YX4015. In addition, pYX4002 was also introduced to YX4001 (Δ*wls*R3) to afford YX4002. For investigation of regulator genes in combination to produce the mature enediyne product, pYX4013 and pYX4011 were also introduced to YX4001 (Δ*wls*R3) to afford YX4023 and YX4024, respectively.

Co-expression of wlsE/wlsE10 and *PKSE*$_{CB02366}$/*WlsE10* for complementation of Δ*wls*E/Δ*wls*R3

*Wls*E and *Wls*E10 were amplified by PCR from the genomic DNA of CB02130 with the primers C-wlsEE10_F and C-wlsEE10_R. The resulting DNA fragments were purified and ligated to a pSET152 plasmid containing an *ermE** promoter, digested with *XbaI* and *Hind*III to yield pYX4015. *PKSE*$_{CB02366}$ was amplified by PCR from genomic DNA of CB02366 with the primers C-2366E_F and C-2366E_R.The PCR product was purified and ligated directly into pSET152, yielding pYX4016. The DNA fragment of *ermE*-PKSE*$_{CB02366}$ was subcloned from pYX4016 with the primers C-2366EwlsE10_F and C-2366EwlsE10_R. *Wls*E10 was amplified by PCR from genomic DNA of CB02130 with the primers C-wlsE10_F and C-wlsE10_R. The two DNA fragments *ermE*-PKSE*$_{CB02366}$ and *wls*E10 were cloned into pSET152 using the Trelief™ SoSoo cloning kit to obtain pYX4017.The plasmids pYX4015 and pYX4017 were separately conjugated to Δ*wls*E/Δ*wls*R3 to result in strains YX4016 (*wls*E*wls*E10/Δ*wls*E/Δ*wls*R3) and YX4017 (PKSE$_{CB02366}$ *wls*E10/Δ*wls*E/Δ*wls*R3).

Large-scale fermentation, isolation of 5–9

To isolate compounds **8** – **9**, YX4001(Δ*wls*R3) was cultured in two 1.5-L flasks containing 500 mL of TSB. After growth at 30 ºC for 2 days, 50 mL of seed culture were inoculated into fifty 2.5-L baffled flasks containing 500 mL of production medium. After fermentation for 7 days (30 ºC, 220 rpm), The resulting fermentation supernatant was acidified by addition of HCl to pH = 4 and extracted with EtOAc (25 L × 3). The extract (15 g) was fractionated by silica-gel column chromatography eluted with petroleum ether (PE)/EtOAC (v/v, 10:1, 6:1, 4:1, 2:1, 1:1, 1:2) to give 18 fractions (Fr1–Fr18). Further isolation was guided by HPLC analysis, which showed that **9** was mainly in Fr13 (3.3 g) and **8** was mainly in Fr16 (1.66 g). Fr13 was further fractionated by silica-gel column chromatography eluted with CH_2Cl_2/MeOH (v/v, 100:1, 100:2, 100:3, 100:4, 100:5, 100:6, 100:7, 100:8) to give 24 fractions (C1–C24). Fraction C22 (237 mg) was subjected to a Sephadex LH-20 chromatography and eluted by methanol to yield 12 fractions (M1-M12). The fractions of M8 and M9 were pooled and further purified by semi-preparative RP-HPLC, using a linear gradient of CH_3CN in H_2O with 0.1% formic acid (0–2 min, 20%; 2–12 min, 20%-30%; 12–14 min, 30%; 14–16min, 30%–70%; 16–20 min, 70%–20%; 20–22 min, 20%) at a flow rate of 3.0 mL/min flow rate to give **9** (5 mg). Fr16 (1.66 g) were similarly purified from Fr13 to afford **8** (9 mg).

To isolate compounds **5** – **7**, YX4008 (Δ*wls*PDH/Δ*wls*R3) was cultured in 60 2.5-L baffled flask containing 500 mL production medium and 10 g (wet) XAD-16N resin. After fermentation for 7 days, the resins containing **5** – **7** were separated by filtering through a metal sieve (60 mesh), washed with H_2O, and dried in air at room temperature. The resins were then extracted by PE (2 × 2 L) and MeOH (4 × 2 L). The methanol extract was concentrated in vacuum under 37 ºC. The crude extract (33.55 g) was dissolved EtOAC : H_2O (1 : 1) (2 L) and further extracted with EtOAC (1 L × 3). Next, the organic phase was dried in vacuum (8.9 g) and subjected to ODS column to give 21 fractions (O1–O21) by stepwise elution with 20%, 25%, 30%, 35%, 40%, 45%, 50% MeOH in H_2O. Fraction O15 (105 mg) containing **5** was next subjected to a Sephadex LH-20 chromatography eluted by methanol to yield 16 fractions (Y1–Y16). Fraction Y9 (15 mg) was further purified for two times by semi-preparative RP-HPLC to give **5** (1.8 mg), using a gradient of CH_3CN in H_2O (0–14 min, 25%–30%; 14–18 min, 30%–70%; 18–23 min, 70%–25%; 23–25 min, 25%) at a flow rate of 3.0 mL/min.

Fraction O14 (56 mg) containing **6** and **7** were similarly purified by Sephadex LH-20 chromatography and semi-preparative RP-HPLC to afford **6** (0.3 mg) and **7** (0.5 mg), using a gradient of CH_3CN in H_2O (0–18 min, 20%; 18–20 min, 20%–50%; 20–25 min, 50%; 25–30 min, 50%–20%; 30–33 min, 20%) at a flow rate of 3.0 mL/min flow rate

Compound **5**: white amorphous powder. $[\alpha]_D^{25}$ 0 (c 0.09, MeOH); UV (MeOH): λ_{max} = 302.1, 316.5, 331.4, 348.2 nm. HR-ESI-MS m/z: 289.1416 $[M + Na]^+$. *Calcd* for $C_{15}H_{22}NaO_4$; Found 289.1403.

Compound **6**: white amorphous powder. $[\alpha]_D^{25}$ +149 (c 0.0428, MeOH); UV (MeOH): λ_{max} =301.9, 316.2, 331.1, 348.2 nm. HR-ESI-MS m/z: 289.1416 $[M + Na]^+$. *Calcd* for $C_{15}H_{22}NaO_4$; Found 289.1407.

Compound **7**: white amorphous powder. UV (MeOH): λ_{max} = 302.1, 316.2, 331.4, 348.2. APCI-HR-MS m/z: 249.1491 [M - $C_3H_6O_2$ + H]$^+$. *Calcd* for $C_{15}H_{20}O_3$; Found 249.1474. Please refer to a mechanistic proposal for the fragment ion in **Figure S6**.

Compound **8**: white amorphous powder. UV (MeOH): λ max =219.7, 241.0, 295.5, 323.0 nm. HR-ESI-MS m/z: 181.0493 [M + H]$^+$. *Calcd* for $C_9H_9O_4$; Found 181.0501.

Compound **9**: white amorphous powder. UV (MeOH): λ max =219.7, 237.5, 296.35, 323.0 nm. HR-ESI-MS m/z: 195.0650 [M + H]$^+$. *Calcd* for $C_{10}H_{11}O_4$; Found 195.0657.

Co-expression of WlsE/WlsE10 for heptaene production in *E. coli.*

The genes for *Wls*E and *Wls*E10 were amplified by PCR from genomic DNA of CB02130 with the primers wlsEE10_F and wlsEE10_R. The resulting DNA fragments were purified and ligated to a pET28a plasmid digested with *Nde*I and *Hind*III, yielding pET28a-*wls*EE10. pET28a-*wls*E/E10 was introduced into *E. coli* BL21(DE3) to result in a strain expression WlsE/E10. This strain was grown in LB media supplemented with kanamycin (50 *µ*g/mL) at 37 °C to OD_{600} = 0.6. Protein expression was induced with isopropyl thiogalactoside (IPTG) at a final concentration of 0.1 mM. After 6 hours at 16 °C, cells were pelleted (4 000 rpm, 10 min) and extracted with MeOH (50 mL × 2). The obtained extract was concentrated to 2 mL and subjected to HPLC analysis.

Expression and purification of WlsC4 from *E. coli* BL21 (DE3).

The *wls*C4 gene was amplified by PCR from genomic DNA of CB02130 with the primers wlsC4_F and wlsC4_R. The 1633-bp PCR product was digested with *Nde*I and *Hind*III, and ligated directly into pET28a (Novagen) expression vector cleaved with the same enzymes. The resultant ligation product was transferred into *E. coli* DH5a and plated onto LB plates containing 50 µg/mL kanamycin. After overnight incubation at 37 °C, positive white colonies were picked for isolation of recombinant expression plasmid and confirmed by DNA sequencing, which was subsequently introduced into *E. coli* BL21(DE3) to express the target protein.

E. coli BL21(DE3) harboring pET28b-*wls*C4 was cultivated in 500 mL LB supplemented with 50 µg/mL kanamycin (37 °C, 220 rpm). After the optical density of the fermentation culture reached to OD_{600} = 0.4 ~ 0.6, it was first placed at a shaker under 16 °C for 30 min. Next, IPTG was added to the final concentration of 0.1 mM and the culture was futher cultivated for 22 hrs at 16 °C. The resulting *E. coli* cells were collected by centrifugation, resuspended in phosphate buffered saline (PBS) (100 mM, pH = 7.4), and lysed by sonication on ice. The cellular debris were removed through centrifugation for 10 min (4 000 rpm, 4 °C). WlsC4 was then purified from the resulting supernatant using Ni-NTA agarose resin-based chromatography, and the recombinant His_6-tagged WlsC4 was obtained by elution with the elution buffer (PBS, pH = 7.4, 250 mM imidazole). WlsC4 was then desalted by dialysis with PBS (pH = 7.4) on ice for 8 hours and concentrated using protein ultrafiltration tube (Millipore, 10000 MWCO). The purity of the isolated protein was determined by SDS-PAGE, and its concentration was determined by BCA Protein quantification kit (Beijing Dingguo Changsheng Biotechnology Co., Ltd).

In vitro enzyme assay of WlsC4.

The purified His_6-tagged WlsC4 (40 µM) was incubated with L-phenylalanine (2 mM) or L-tyrosine (2 mM) as a substrate to test its enzymatic activity in Tris-HCl (100 mM, pH 8.8) at 25 °C for 4 h. The reaction was terminated by addition of HCl to pH < 2. The quenched mixture (100 µL) was centrifuged for 15 min (15 000 rpm, 4 °C) and the supernatant was analyzed by LC-MS (1.7 *µ*m, 2.1 × 100 mm, Waters) using a linear gradient of CH_3CN in H_2O with 0.1% formic acid (0–5 min, 1%–5%; 5–15 min, 5%–25%; 15–18 min, 25%; 18–24 min, 25%–1%; 24–27 min, 1%) at a flow rate of 0.4 mL/min.

Table S1. List of genes in *wls* BGC and their proposed functions. The genes that are not found in known enediyne BGCs are shaded in light blue, whereas the genes from the minimal enediyne PKS gene cassette are shaded in light green.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)	Proposed roles in *wls*
*wls*orf1	495	Transmembrane efflux protein	SgcB (AAL06672)	32.42	Resistance
*wls*orf2	431	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	33.41	Unknown
*wls*orf3	344	Chorismatase	FkbO (Q9KID9.1)	45.43	Unknown
*wls*C1	494	A-domain type II peptide synthetase	SgcC1 (AAL06681)	30.19	β-tyrosine biosynthesis
*wls*C4	537	Ammonia lyases	SgcC4 (AAL06680)	80.45	β-tyrosine biosynthesis
*wls*B	538	Transmembrane efflux protein	SgcB (AAL06672)	46.56	Resistance
*wls*Apo	147	Apoprotein	CagA (AAL06658)	37.67	Apoprotein
*wls*orf8	466	Monooxygenase	SpoT4 (ABP55175)	31.71	Unknown
*wls*orf9	510	C-methyltransferase	SpoT6 (ABP55177)	48.62	Unknown
*wls*F	389	Epoxide hydrolase	SgcF (AAL06662)	69.09	Core biosynthesis
*wls*B1	469	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	49.66	Resistance
*wls*H	459	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	81.99	Naphthoic acid biosynthesis
*wls*D1	211	Anthranilate synthase II	SgcD1 (AAL06663)	77.08	Naphthoic acid biosynthesis
*wls*D	493	2-Amino-4-deoxychorismate synthase	SgcD (AAL06664)	71.46	Naphthoic acid biosynthesis
*wls*D5	478	Phenylacetyl-CoA ligase	SgcD5 (AAL06665)	77.27	Naphthoic acid biosynthesis
*wls*G	223	2-Amino-4-deoxychorismate dehydrogenase	SgcG (AAL06666)	85.59	Naphthoic acid biosynthesis
*wls*D3	412	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	70.66	Naphthoic acid biosynthesis
*wls*D4	335	O-methyltransferase	SgcD4 (AAL06683)	74.32	Naphthoic acid biosynthesis
*wls*B4	702	Antibiotic transporter	SgcB4 (AAL06682)	68.09	Unknown
*wls*I	262	Hydrolase	SgcI (AAL06675)	45.86	Naphthoic acid biosynthesis
*wls*D6	432	3-O-Acyltransferase	SgcD6 (AAL06667)	40.24	Naphthoic acid coupling
*wls*T2	605	NRPS A-PCP didomain	SpoT2 (ABP55169.1)	59.05	Unknown
*wls*C5	456	C-domain type II peptide synthetase	SgcC5 (AAL06678)	43.60	β-tyrosine coupling
*wls*orf24	411	Hydroxylase	Spoorf17	68.41	Unknown
*wls*orf25	416	Cytochrome P450 hydroxylase	NcsB3 (AAM77997)	52.49	Naphthoic acid biosynthesis
*wls*D2	448	FAD-binding monooxygenase	SgcD2 (AAL06669)	58.44	Naphthoic acid biosynthesis
*wls*J	140	NTF2-Like Superfamily Protein	SgcJ (AAL06676)	62.28	Core biosynthesis
*wls*L	416	Oxidoreductase	SgcL (AAL06685)	65.74	Unknown
*wls*C	526	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	74.56	β-tyrosine biosynthesis
wlsORF30	553	2-hydroxyacyl-CoA lyase 2	Ilvbl (Q6DDK5.1)	35.96	Unknown
*wls*orf31	332	O-Methyltransferase domain-containing protein	NcsB1 (AAM77984)	27.04	β-tyrosine biosynthesis
*wls*PDH	298	Probable prephenate dehydrogenase NovF	NovF(Q9L9G2.1)	39.22	Unknown
*wls*E11	265	Unknown	NcsE11 (AAM78004)	69.08	Core biosynthesis
*wls*M	344	Unknown	SgcM (AAL06686)	56.01	Core biosynthesis
*wls*E9	555	Oxidoreductase	NcsE9 (AAM78005)	80.99	Core biosynthesis
*wls*E8	195	Unknown	NcsE8 (AAM78006)	74.87	Core biosynthesis
*wls*R6	357	Regulatory protein	NcsR6 (AAM78007)	57.27	Regulation
*wls*R5	260	AraC family transcriptional regulator	NcsR5 (AAM78008)	66.29	Regulation
*wls*E7	447	Cytochrome P450	NcsE7 (AAM78009)	77.70	Core biosynthesis
*wls*E6	182	Flavin reductase	NcsE6 (AAM78010)	70.56	β-tyrosine biosynthesis

*wls*E10	152	Type II thioesterase	NcsE10 (AAM78011)	84.31	Core biosynthesis
*wls*E	1957	Enediyne polyketide synthase	NcsE (AAM78012)	74.89	Core biosynthesis
*wls*E5	350	Unknown	NcsE5 (AAM78013)	75.56	Core biosynthesis
*wls*E4	616	Unknown	NcsE4 (AAM78014)	77.60	Core biosynthesis
*wls*E3	307	Unknown	NcsE3 (AAM78015)	72.64	Core biosynthesis
*wls*E2	325	Unknown	NcsE2 (AAM78016)	64.83	Core biosynthesis
*wls*E1	147	HxlR family transcriptional regulator	NcsE1 (AAM78017)	71.43	Regulation
*wls*R7	404	Transcriptional regulator	NcsR7 (AAM78019)	58.90	Regulation
*wls*R3	216	TetR-like transcriptional regulator	NcsR3 (AAM78020)	47.57	Regulation
*wls*C2	316	Autoregulator biosynthesis protein	NcsC2 (AAM78021)	54.38	Regulation
*wls*R2	208	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	61.58	Regulation
*wls*R1	293	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	61.30	Regulation

[a] Given in parentheses are NCBI accession numbers. Homologue proteins from the C-1027 and neocarzinostatin pathway were selected for comparison. If no homologue protein was found within the C-1027 and neocarzinostatin gene clusters, homologues from other known enediyne gene clusters, including calicheamicin (*cal*), dynemycin (*dyn*), esperamicin (*esp*), sungeidine (*sgd)*, tiancimycin (*tnm*), uncialamycin (*ucm*), yangpumicin (*ypm*), kedarcidin (*ked*), maduropeptin (*mdp*), amycolamycin (*acm*), cyanosporaside C (*cya*), cyanosporaside F (*cyn*), sporolides (*spo*), were firstly selected over others genes in GenBank.

Table S2. List of Primers used in this study.

Primer name	Sequence (5'–3 ')	Function
wlsR2 UpF	cgaatgtgaacagttccatgatcttgctgatgctgg	Amplifying upstream DNA fragment of *wlsR2*
wlsR2 UpR	gcggccgcggatcctctagagcaggacgtcgttttgttct	
wlsR2 DnF	acgacggccagtgccaagcttagcatggtgagacaggagagg	Amplifying downstream DNA fragment of *wlsR2*
wlsR2 DnR	caaccgataatcgatgtggcggatcatgc	
wlsR2_tsrF	ccgccacatcgattatcggttggccgcga	Amplifying thiostrepton resistance gene for pYX4010
wlsR2_tsrR	tcatggaactgttcacattcgaacggtctctg	
wlsR2_KOtest_F	agagcgttcaacagttctgc	Comfirming *wlsR2* deletion in CB02130
wlsR2_KOtest_R	cctggggtgagatcgagtac	
wlsR3 UpF	cgaatgtgaacagctgtctcctgaagagcgc	Amplifying upstream DNA fragment of *wlsR3*
wlsR3 UpR	gcggccgcggatcctctagaagcgcagctcgacgtg	
wlsR3 DnF	gacggccagtgccaagcttatctccgcaggggagctg	Amplifying downstream DNA fragment of *wlsR3*
wlsR3 DnR	caaccgataaagtgcctccgcgtcag	
wlsR3_tsrF	cgcggaggcactttatcggttggccgcgagattc	Amplifying thiostrepton resistance gene for pX4001
wlsR3_tsrR	caggagacagctgttcacattcgaacggtctctgc	
wlsR3_KOtest_F	agcctggcagtggaagg	Comfirming *wlsR3* deletion in CB02130
wlsR3_KOtest_R	gacttctggtacgacagccg	
wlsE UpF	cacattccacagggtgaagggcacctcgg	Amplifying upstream DNA fragment of *wls*E
wlsE UpR	gcggccgcggatcctctagaggcttcgccaagaccg	
wlsE DnF	cgacggccagtgccaagcttctcctcgaagccgaccg	Amplifying downstream DNA fragment of *wls*E
wlsE DnR	acgagttcttctgacgcctcatcgaggatccg	
wlsE_kanaF	cgatgaggcgtcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4003
wlsE_kanaR	gtgcccttcaccctgtggaatgtgtgtcagttagggt	
wlsE_KOtest_F	caggttgccgtcgagcagatg	Confirming *wls*E deletion in Δ*wls*R3
wlsE_KOtest_R	catcgccggtctctccgtc	
wlsorf3_UpF	cgacggccagtgccaagcttccggtgctcccggatttctg	Amplifying upstream DNA fragment of *wls*orf3
wlsorf3_UpR	acgagttcttctgagccgctatggccgtacgtc	
wlsorf3_DnF	cacattccacaggtggagatcgaaggagtctgcc	Amplifying downstream DNA fragment of *wls*orf3
wlsorf3_DnR	gcggccgcggatcctctagacagctcctccagcatggcc	
wlsorf3_kanaF	ccatagcggctcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4006
wlsorf3_kanaR	cttcgatctccacctgtggaatgtgtgtcagttagggt	
wlsorf3_KOtest_F	caccctggactgcctcttcg	Confirming *wls*orf3 deletion in Δ*wls*R3
wlsorf3_KOtest_R	cgaggaaggcccagacgaac	
wlsORF30 UpF	cgacggccagtgccaagcttacccaccggttcttcaccacac	Amplifying upstream DNA fragment of *wlsORF30*
wlsORF30 UpR	acgagttcttctgacggtcgaaacgtccggc	
wlsORF30 DnF	cacattccacagcgtcacgaggatgttcccgc	Amplifying downstream of *wlsORF30*
wlsORF30 DnR	gcggccgcggatcctctagaccggaactgagcgacaccg	
wlsORF30_kanaF	gtttcgaccgtcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4007
wlsORF30_kanaR	atcctcgtgacgctgtggaatgtgtgtcagttagggt	
wlsORF30_KOtest_F	tgtcggcgaaggacatggtcg	Confirming *wlsORF30* deletion in Δ*wls*R3
wlsORF30_KOtest_R	cgaagccgtgggcaggac	
wlsPDH UpF	cgacggccagtgccaagcttgccgatcaggtctcccggtag	Amplifying upstream DNA fragment of *wls*PDH
wlsPDH UpR	acgagttcttctgaacggtgcgagatgcgtgc	

wlsPDH DnF	cacattccacagcagagccacttcgacgctcg	Amplifying downstream DNA fragment of *wls*PDH
wlsPDH DnR	gcggccgcggatcctctagaaagctcgccaccctgacc	
wlsPDH _kanaF	ctcgcaccgttcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance cassette for pYX4008
wlsPDH _kanaR	gaagtggctctgctgtggaatgtgtgtcagttagggt	
wlsPDH _KOtest_F	acccagccgaagtcggagag	Confirming *wls*PDH deletion in Δ*wls*R3
wlsPDH _KOtest_R	gacctcgctcaggcctaccg	
wlsC4 UpF	cgacggccagtgccaagcttacttcgccaggaagctggc	Amplifying upstream DNA fragment of *wls*C4
wlsC4 UpR	acgagttcttctgagacgccgtagatgggaacgt	
wlsC4 DnF	cacattccacaggcctccaaggccacgtacg	Amplifying downstream DNA fragment of *wls*C4
wlsC4 DnR	gcggccgcggatcctctagaacagcaaccagcagctgtgg	
wlsC4 _kanaF	ctacggcgtctcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4005
wlsC4_kanaR	gccttggaggcctgtggaatgtgtgtcagttagggt	
wlsC4 _KOtest_F	tcacctcctgccgcacg	Confirming *wls*C4 deletion in Δ*wls*R3
wlsC4 _KOtest_R	tcgacgtcggtgtacctggc	
wlsF UpF	cgacggccagtgccaagcttacatcgtggtgattcatggcgg	Amplifying upstream DNA fragment of *wls*F
wlsF UpR	gttcttctgaacgccagtcgaacgaggtg	
wlsF DnF	cacattccacagatcgtccactggacggagttcg	Amplifying downstream DNA fragment of *wls*F
wlsF DnR	gcggccgcggatcctctagaatcgccgaggacttcaagcgc	
wlsF_kanaF	ctcgttcgactggcgttcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4009
wlsF_kanaR	gtccagtggacgatctgtggaatgtgtgtcagttagggt	
wlsF_KOtest_F	agagcggcggacgatacg	Confirming *wls*F deletion in Δ*wls*R3
wlsF_KOtest_R	accgcgaagtagtactccgc	
wlsR1_F	cttgggctgcaggtcgactctagagcggtgtgtcggttcg	Amplifying *wls*R1 gene
wlsR1_R	tcgtgccggttggtaggatccccacaccactgataggtccg	
wlsR5_F	tcgtgccggttggtaggatccactggccctgcattgttc	Amplifying *wls*R5 gene
wlsR5_R	tgggctgcaggtcgactctagaagggaaggaagagcggc	
wlsR6_F	tgggctgcaggtcgactctagatgtctttgagatcatgctccagg	Amplifying *wls*R6 gene
wlsR6_R	tcgtgccggttggtaggatcccctgtgaagcaactagccg	
wlsR7_F	tgggctgcaggtcgactctagaagtcgtcccgggttcc	Amplifying *wls*R7 gene
wlsR7_R	tcgtgccggttggtaggatccacttggcgaagtgcatacg	
wlsR3_F	cttgggctgcaggtcgactctagaacgcggaggcactcccg	Amplifying *wls*R3 gene
wlsR3_R	aatcgtgccggttggtaggatccaactcgcccttgccatcgg	
wlsC4_F	aataagctttcagcgcaactcgacgtc	Amplifying *wls*C4 gene for pET28a-*wls*C4
wlsC4_R	tgaattccatatgacctcagtcgagaccacc	
wlsEE10_F	tcgagtgcggccgcaagcttagggtgctcacgccgc	Amplifying *wls*EE10 gene for pET28a-*wls*E/E10
wlsEE10_R	gtgccgcgcggcagccatatgagcgaagagaccgtgaccgac	
C-wlsEE10_F	cttgggctgcaggtcgactctagaagggtgctcacgccgc	Amplifying *wlsEE10* gene for pYX4015
C-wlsEE10_R	aatcgtgccggttggtaggatccagcgaagagaccgtgaccgac	
C-2366E_F	cttgggctgcaggtcgactctagacgtgctcatgtgcgggc	Amplifying *2366E* gene for pYX4016
C-2366E_R	aatcgtgccggttggtaggatccacgagccacgaagaacaaggc	
C-wlsE10_F	ggtaccgagcagggtgctcacgccgc	Amplifying *wlsE10* gene for pYX4017
C-wlsE10_R	atcgtgccggttggtaggatccttcgccttcctcactcgcga	
C-2366EwlsE10_F	cttgggctgcaggtcgactctagacgtgctcatgtgcgggc	Amplifying *2366E* gene for pYX4017
C-2366EwlsE10_R	gtgagcaccctgctcggtaccagcccgacccgag	
wlsorf8_UpF	cgacggccagtgccaagcttaggcgctgaagcgggc	Amplifying upstream DNA fragment of *wls*orf8

wlsorf8_UpR	gagttcttctgagaacgccactgcgccgtg	
wlsorf8_DnF	cacattccacagggaggcggaagcgtgtccg	Amplifying downstream DNA fragment of *wls*orf8
wlsorf8_DnR	gcggccgcggatcctctagaagttccgcacgttggtgg	
wlsorf8_KanaF	cgcagtggcgttctcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4018
wlsorf8_kanaR	cttccgcctccctgtggaatgtgtgtcagttagggt	
wlsorf8_KOtest_F	aggcggtcacgttcaagtagc	Confirming *wls*orf8 deletion in Δ*wls*R3
wlsorf8_KOtest_R	aggaggcgaccgaggtct	
Wlsorf24_UpF	cgacggccagtgccaagcttacggtggccgatgtgctcaac	Amplifying upstream DNA fragment of *wls*orf24
Wlsorf24_UpR	gagttcttctgatccgccgtacctgcgc	
Wlsorf24_DnF	attccacaggcacgccacgcagcagtg	Amplifying downstream DNA fragment of *wls*orf24
Wlsorf24_DnR	gcggccgcggatcctctagaacgacggcacggtgttcc	
Wlsorf24_KanaF	gcaggtacggcggatcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4019
Wlsorf24_kanaR	ctgcgtggcgtgcctgtggaatgtgtgtcagttagggt	
Wlsorf24_KOtest_F	accgcttcgacgagtccac	Confirming *wls*orf24 deletion in Δ*wls*R3
Wlsorf24_KOtest_R	acctcgccgttgacgaagg	
wlsL_UpF	cgacggccagtgccaagcttagggtgtcgctcggttctagg	Amplifying upstream DNA fragment of *wls*L
wlsL_UpR	gagttcttctgaaccaacaacgagctgtacggcc	
wlsL_DnF	cacattccacagacgcgtggggatgtcgg	Amplifying downstream DNA fragment of *wls*L
wlsL_DnR	gcggccgcggatcctctagaacggcttcgaggagcacatgtacg	
wlsL_KanaF	acagctcgttgttggttcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4020
wlsL_kanaR	atccccacgcgtctgtggaatgtgtgtcagttagggt	
wlsL_KOtest_F	aggcctgggccatgaagg	Confirming *wls*L deletion in Δ*wls*R3
wlsL_KOtest_R	atgcgcctcatcgtggca	
wlsE7_UpF	cgacggccagtgccaagcttaggtcgttcgctatcgtcgttg	Amplifying upstream DNA fragment of *wls*E7
wlsE7_UpR	agttcttctgaatggaggcggtcttcgtcac	
wlsE7_DnF	acacattccacagaccgagcaccttgcggga	Amplifying downstream DNA fragment of *wls*E7
wlsE7_DnR	gcggccgcggatcctctagaaaccaggtctgtgtgccggac	
wlsE7_KanaF	agaccgcctccattcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4021
wlsE7_kanaR	aggtgctcggtctgtggaatgtgtgtcagttagggt	
wlsE7_KOtest_F	actgcttcgtcggtctcttcc	Confirming *wls*E7 deletion in Δ*wls*R3
wlsE7_KOtest_R	acgagggaggtgaccacacg	
wlsE9_UpF	cgacggccagtgccaagcttactgctggagagcggagttg	Amplifying upstream DNA fragment of *wls*E9
wlsE9_UpR	cgagttcttctgaacgattctggcccaggcac	
wlsE9_DnF	cacattccacagacgctccaggacggtcac	Amplifying downstream DNA fragment of *wls*E9
wlsE9_DnR	gcggccgcggatcctctagaacgctgctgtatccgacctgg	
wlsE9_KanaF	ggccagaatcgttcagaagaactcgtcaagaaggcg	Amplifying kanamycin resistance gene for pYX4022
wlsE9_kanaR	gtcctggagcgtctgtggaatgtgtgtcagttagggt	
wlsE9_KOtest_F	acgtgccgtatctgccgg	Confirming *wls*E9 deletion in Δ*wls*R3
wlsE9_KOtest_R	acgttctcgtcatcgggagc	

Table S3. List of bacterial strains and plasmids used in this study.

Name	**Description**	**Reference or Sources**
E. coli DH5α	General cloning host	Invitrogen
E. coli S17-1	Donor strain for conjugation	Invitrogen
E. coli BL21 (DE3)	Host strain for protein expression	Invitrogen
S. sp. CB02130	wild-type strain isolated from Wuliangshan Mountain.	1
YX4001	a mutant where *wls*R3 was replaced by thiostrepton resistance cassette (Δ*wls*R3)	This study
YX4002	YX4001 derivative where *wls*R3 was overexpressed using pYX4002 (*wls*R3/Δ*wls*R3)	This study
YX4003	YX4001 derivative where *wls*E was replaced by kanamycin resistance cassette (Δ*wls*E/Δ*wls*R3)	This study
YX4004	YX4001 derivative where *wls*E10 was replaced by kanamycin resistance cassette (Δ*wls*E10/Δ*wls*R3)	This study
YX4005	YX4001 derivative where *wls*C4 was replaced by kanamycin resistance cassette (Δ*wls*C4/Δ*wls*R3)	This study
YX4006	YX4001 derivative where *wls*orf3 was replaced by kanamycin resistance cassette (Δ*wls*orf3/Δ*wls*R3)	This study
YX4007	YX4001 derivative where *wlsORF30* was replaced by kanamycin resistance cassette (Δ*wls*orf3/Δ*wls*R3)	This study
YX4008	YX4001 derivative where *wls*PDH was replaced by kanamycin resistance cassette (Δ*wls*PDH/Δ*wls*R3)	This study
YX4009	a mutant where *wls*PDH was disrupted by kanamycin resistance cassette (Δ*wls*PDH)	This study
YX4010	YX4001 derivative where *wls*F was replaced by kanamycin resistance cassette (Δ*wls*F/Δ*wls*R3)	This study
YX4011	a mutant where *wls*R2 was replaced by thiostrepton resistance cassette (Δ*wls*R2)	This study
YX4012	a mutant where *wls*R1 was overexpressed using pYX4011 (*wls*R1)	This study
YX4013	a mutant where *wls*R5 was overexpressed using pYX4012 (*wls*R5)	This study
YX4014	a mutant where *wls*R6 was overexpressed using pYX4013 (*wls*R6)	This study
YX4015	a mutant where *wls*R7 was overexpressed using pYX4014 (*wls*R7)	This study
YX4016	YX4003 derivative where *wls*E and *wls*E10 was overexpressed using pYX4015 (*wls*E/Δ*wls*R3)	This study
YX4017	YX4003 derivative where PKSE$_{CB02366}$ and *wls*E10 was overexpressed using pYX4016 (*2366*E/Δ*wls*R3)	This study
YX4018	YX4001 derivative where *wls*ORF8 was replaced by kanamycin resistance cassette (Δ*wls*ORF8/Δ*wls*R3)	This study
YX4019	YX4001 derivative where *wls*ORF24 was replaced by kanamycin resistance cassette (Δ*wls*ORF24/Δ*wls*R3)	This study
YX4020	YX4001 derivative where *wls*L was replaced by kanamycin resistance cassette (Δ*wl*LΔ*wls*R3)	This study
YX4021	YX4001 derivative where *wls*E7 was replaced by kanamycin resistance cassette (Δ*wls*E7/Δ*wls*R3)	This study
YX4022	YX4001 derivative where *wls*E9 was replaced by kanamycin resistance cassette (Δ*wls*E9/Δ*wls*R3)	This study
YX4023	YX4001 derivative where *wls*R6 was overexpressed using pYX4013 (Δ*wls*R6/Δ*wls*R3)	This study
YX4024	YX4001 derivative where *wls*R1 was overexpressed using pYX4011 (Δ*wls*R1/Δ*wls*R3)	This study
WlsC4-BL21(DE3)	Expressing WlsC4 in *E. coli* BL21(DE3) for in vitro assay	This study
WlsE/E10-BL21(DE3)	Co-expression of WlsE/WlsE10 in *E. coli* BL21(DE3) for heptaene production in *E. coli.*	This study

pOJ260	*E. coli-Streptomyces* shuttle vector, *oriT*, Apra^r	Commercial
pSET152	*E. coli-Streptomyces* shuttle vector, *attB*, *lacZa*, Apra^r, *oriT*	Commercial
pET28a	Protein expression in *E. coli.*	Commercial
pYX4001	pOJ260 plasmid derivative for generating Δ*wls*R3, Th^r	This study
pYX4002	pSET152 plasmid derivative with *wls*R3 under control of constitutive promoter *ermE**, Apra^r	This study
pYX4003	pOJ260 plasmid derivative for generating Δ*wls*E/Δ*wls*R3, Kana^r	This study
pYX4004	pOJ260 plasmid derivative for generating Δ*wls*E10/Δ*wls*R3, Kana^r	This study
pYX4005	pOJ260 plasmid derivative for generating Δ*wls*C4/Δ*wls*R3, Kana^r	This study
pYX4006	pOJ260 plasmid derivative for generating Δ*wls*orf3/Δ*wls*R3, Kana^r	This study
pYX4007	pOJ260 plasmid derivative for generating Δ*wlsORF30*/Δ*wls*R3, Kana^r	This study
pYX4008	pOJ260 plasmid derivative for generating Δ*wls*PDH and Δ*wls*PDH/Δ*wls*R3, Kana^r	This study
pYX4009	pOJ260 plasmid derivative for generating Δ*wls*F/Δ*wls*R3, Kana^r	This study
pYX4010	pOJ260 plasmid derivative for generating Δ*wls*R2, Th^r	This study
pYX4011	pSET152 plasmid derivative with *wls*R1 under control of constitutive promoter *ermE**, Apra^r	This study
pYX4012	pSET152 plasmid derivative with *wls*R5 under control of constitutive promoter *ermE**, Apra^r	This study
pYX4013	pSET152 plasmid derivative with *wls*R6 under control of constitutive promoter *ermE**, Apra^r	This study
pYX4014	pSET152 plasmid derivative with *wls*R7 under control of constitutive promoter *ermE** Apra^r	This study
pYX4015	pSET152 plasmid derivative with *wls*E and *wls*E10 under control of constitutive promoter *ermE** Apra$^{r\ r}$	This study
pYX4016	pSET152 plasmid derivative with 2366E under control of constitutive promoter *ermE** Apra^r	This study
pYX4017	pSET152 plasmid derivative with *PKSE*$_{CB02366}$ and *wlsE10* under control of constitutive promoter *ermE** Apra^r	This study
pET28a-*wls*C4	The overexpression plasmid for *wls*C4 in BL21(DE3)	This study
pET28a-*wls*E/E10	The overexpression plasmid for heptaene in BL21(DE3)	This study
pYX4018	pOJ260 plasmid derivative for generating Δ*wls*ORF8/Δ*wls*R3, Kana^r	This study
pYX4019	pOJ260 plasmid derivative for generating Δ*wls*ORF24/Δ*wls*R3, Kana^r	This study
pYX4020	pOJ260 plasmid derivative for generating Δ*wls*L/Δ*wls*R3, Kana^r	This study
pYX4021	pOJ260 plasmid derivative for generating Δ*wls*E7/Δ*wls*R3, Kana^r	This study
pYX4022	pOJ260 plasmid derivative for generating Δ*wls*E9/Δ*wls*R3, Kana^r	This study

*Abbreviations: Apra^r, apramycin resistance; Th^r, thiostrepton resistance; Kana^r, kanamycin resistance

Table S4. ^{1}H and ^{13}C{^{1}H} NMR data of compounds **5**－**7**. (**5**－**7** in CD_3OD-d_4, ^{1}H NMR at 600 MHz, ^{13}C{^{1}H} NMR at 125 MHz)

	5		**6**		**7**	
Position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	67.12, CH_2	3.49 (m, 2H)	67.20, CH_2	3.49 (m, 2H)	67.11, CH_2	3.48 (m, 2H)
2	77.90, CH	4.17 (m, 1H)	77.56, CH	4.16 (m, 1H)	77.56, CH	4.16 (m, 1H)
3	132.90~134.40, CH	5.73(dd, J = 15.0, 2.4 Hz, 1H)	132.90~134.40, CH	5.73 (dd, J = 15, 6.6 Hz, 1H)	132.91~134.41, CH	5.73 (dd, J = 15, 6.6 Hz, 1H)
4	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.91~134.41, CH	6.28~6.39 (m, 1H)
5	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.91~134.41, CH	6.28~6.39 (m, 1H)
6	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.91~134.41, CH	6.28~6.39 (m, 1H)
7	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.91~134.41, CH	6.28~6.39 (m, 1H)
8	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.91~134.41, CH	6.28~6.39 (m, 1H)
9	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.91~134.41, CH	6.28~6.39 (m, 1H)
10	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.91~134.41, CH	6.28~6.39 (m, 1H)
11	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.90~134.40, CH	6.30~6.39 (m, 1H)	132.91~134.41, CH	6.28~6.39 (m, 1H)
12	132.90~134.40, CH	5.74 (dd, J = 15.0, 3 Hz, 1H)	132.90~134.40, CH	5.80 (dd, J = 13.8, 6.6 Hz, 1H)	132.91~134.41, CH	5.80 (dd, J = 14.4, 6.6 Hz, 1H)
13	71.74, CH	3.62 (t, J = 6.6 Hz, 1H)	71.68, CH	3.67 (t, J = 6.6 Hz, 1H)	71.67, CH	3.68, (m, 1H)
14	74.04, CH	3.90 (m, 1H)	74.05,CH	3.94 (m, 1H)	73.86, CH	3.65 (m, 1H)
15	18.86, CH_3	1.10 (d, J = 6.6 Hz, 3H)	18.70, CH_3	1.14 (d, J = 5.4 Hz, 3H)	18.71, CH_3	1.14 (d, J = 6.4 Hz, 3H)
1’					64.38, CH_2	3.59, (m,2H)
2’					74.05, CH	3.65 (m, 1H)
3’					64.38, CH_2	3.48, (m, 2H)

Table S5. Distribution of the 67 WlsPDH homologous proteins from GenBank and the respective co-localized PKSE gene. The sequence identity and similarity of each WlsPDH homolog with WlsPDH was obtained by Blastp.

Accession	**e-value**	**Organism**	**Identities (%)**	**Positives (%)**	**PKSE**
WP_103513060.1	1e-154	*Streptomyces* sp. SM13	91	94	WP_103513069.1
WP_123459559.1	3e-128	*Streptomyces* sp. PanSC19	81	85	WP_123459568.1
WP_221747881.1	3e-133	*Streptomyces lateritius* Z1-26	81	84	WP_221747872.1
WP_073918736.1	8e-132	*Streptomyces* sp. CB02009	78	82	WP_073918718.1
WP_105870312.1	2e-101	*Streptomyces* sp. ST5x	75	82	WP_105867409.1
PKW00348.1	6e-126	*Streptomyces* sp. 5112.2	75	82	PKW00358.1
WP_093699552.1	3e-126	*Streptomyces* sp. 2231.1	74	82	WP_093699548.1
OKJ71875.1	3e-125	*Streptomyces* sp. CB01883	74	81	WP_073901596.1
AWI32822.1	2e-125	*Streptomyces tirandamycinicus* HNM0039	74	82	AWI33274.1
WP_189835466.1	5e-124	*Streptomyces zaomyceticus* JCM 4864	73	81	WP_189835917.1
WP_073819485.1	1e-123	*Streptomyces* sp. CB02261	73	81	WP_073819478.1
GGY73077.1	9e-122	*Streptomyces omiyaensis* JCM 4806	72	81	GGY73156.1
WP_229883849.1	1e-110	*Streptomyces omiyaensis* JCM 4806	72	80	WP_189852606.1
WP_200428877.1	5e-118	*Streptomyces* sp. NE5-10	71	80	WP_200428886.1
WP_061929045.1	1e-117	*Streptomyces bungoensis* DSM 41781	71	79	WP_061929060.1
WP_093804280.1	2e-117	*Streptomyces* sp. Wb2n-11	70	79	WP_093804271.1
WP_155074227.1	6e-93	*Streptomyces taklimakanensis* TRM43335	67	76	WP_155074302.1
WP_109541238.1	1e-95	*Streptomyces* sp. NWU49	66	74	WP_109541294.1
WP_004994139.1	3e-95	*Streptomyces viridosporus* T7A	66	74	WP_026085636.1
WP_123083521.1	3e-92	*Streptomyces* sp. ADI95-16	64	74	WP_123083512.1
WP_073797075.1	2e-81	*Streptomyces* sp. CB03578	64	72	WP_073797070.1
WP_073776212.1	7e-79	*Streptomyces* sp. MJM1172	64	72	WP_073776205.1
WP_209520460.1	8e-79	*Streptomyces* sp. KCTC 0041BP	64	72	WP_209520461.1
WP_190032669.1	8e-79	*Streptomyces goshikiensis* JCM 4640	64	72	WP_190032671.1
WP_037800482.1	1e-78	*Streptomyces* sp. Mg1	63	71	WP_047961333.1
WP_073936185.1	3e-83	*Streptomyces* sp. CB02400	62	71	WP_073937997.1
WP_081241542.1	1e-70	*Streptomyces viridosporus* NRRL 2414	62	70	WP_081237698.1
WP_228969408.1	1e-81	*Streptomyces* sp. DH5	61	70	WP_228969415.1
WP_190099772.1	3e-81	*Streptomyces griseoflavus* JCM 4479	61	70	WP_190099780.1

WP_182904357.1	3e-57	*Microbispora* sp. H13382	57	63	WP_182904364.1
WP_169979400.1	4e-57	*Microbispora* sp. H10836	57	63	WP_169979379.1
WP_225800890.1	2e-59	*Streptomyces* sp. NK15101	57	65	WP_225800882.1
WP_153526004.1	8e-77	*Streptomyces jumonjinensis* NRRL 5741	56	65	WP_153526009.1
WP_169944057.1	4e-56	*Microbispora* sp. H11081	56	63	WP_169944073.1
WP_153480251.1	3e-72	*Streptomyces katsurahamanus* T-272	56	65	WP_153480900.1
GIH75461.1	5e-62	*Planobispora longispora* NBRC 13918	56	64	GIH75452.1
WP_239316055.1	7e-55	*Planobispora longispora* NBRC 13918	55	64	WP_203890119.1
WP_196441346.1	1e-64	*Planomonospora* sp. ID67723	55	65	WP_196441279.1
WP_223178679.1	4e-75	*Streptomyces boluensis* YC537	55	66	WP_161696329.1
WP_073721047.1	9e-73	*Streptomyces* sp. TSRI0281	55	64	WP_073721055.1
WP_204057437.1	1e-64	*Microbispora corallina* NBRC 16416	54	62	WP_204057426.1
WP_051457722.1	2e-64	*Microbispora* sp. ATCC PTA-5024	54	62	WP_063794622.1
WP_173267136.1	5e-65	*Streptomyces* sp. CWH03	54	63	WP_173267119.1
WP_184879857.1	2e-62	*Actinomadura livida* JCM 3387 sequence12	53	62	WP_184879841.1
WP_073753614.1	4e-65	*Streptomyces* sp. CB02058	53	60	WP_073753628.1
WP_221474752.1	2e-57	*Sphaerisporangium rubeum* DSM 44936	53	60	WP_184979809.1
WP_093256825.1	2e-57	*Thermostaphylospora chromogena* DSM 43794	53	62	WP_093256734.1
TDB92362.1	2e-60	*Actinomadura* sp. 7K534	52	62	TDB96418.1
WP_204052624.1	5e-62	*Microbispora siamensis* NBRC 104113	52	60	WP_204052369.1
WP_116428710.1	9e-52	*Streptomyces spongiicola* 531S	52	62	WP_116428706.1
WP_189650259.1	1e-55	*Nonomuraea spiralis* JCM 3286	52	60	WP_189650279.1
WP_093891684.1	8e-63	*Streptosporangium canum* CGMCC 4.2126	52	62	WP_093891671.1
WP_086577273.1	2e-62	*Streptosporangium minutum* M26	52	62	WP_086578318.1
WP_184882159.1	2e-60	*Sphaerisporangium siamense* NBRC 107570	52	60	WP_184882142.1
WP_184616680.1	3e-60	*Sphaerisporangium krabiense* NBRC 107571	52	61	WP_184616671.1
WP_237104053.1	7e-54	*Nonomuraea* sp. MG754425	52	61	WP_237109081.1
WP_189164291.1	2e-60	*Sphaerisporangium melleum* NBRC 107356	52	61	WP_189164301.1
GGK91151.1	5e-60	*Sphaerisporangium melleum* JCM 13064	52	61	GGK91218.1
WP_051865492.1	4e-62	*Streptosporangium roseum* NRRL B-2638	52	62	WP_031168554.1
WP_012893415.1	5e-62	*Streptosporangium roseum* DSM 43021	52	62	WP_012893430.1
WP_148432627.1	3e-57	*Nonomuraea* sp. PA05	51	59	WP_148432609.1
WP_238498517.1	8e-54	*Streptosporangium* sp. KLBMP 9127	51	61	WP_238498573.1
WP_214410496.1	5e-58	*Sphaerisporangium* sp. H8589	50	63	WP_214410487.1

NYF38201.1	6e-56	*Streptosporangium sandarakinum* DSM 45763	50	60	NYF38184.1
WP_125637902.1	2e-56	*Nonomuraea* sp. WAC 01424	50	58	WP_125637924.1
WP_204066954.1	2e-59	*Planobispora siamensis* NBRC 107568	50	60	WP_204067024.1
WP_197093687.1	7e-59	*Nonomuraea* sp. SBT364	50	59	WP_049565860.1

Table S6. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Nonomuraea* sp. PA05.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
PA05_orf1	430	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	53
PA05_orf2	394	Alkylhalidase	SgcC3 (AAL06656)	36
PA05_orf3	586	A-domain type II peptide synthetase	SgcC1 (AAL06681)	31
PA05_orf4	70	Unknown (MbtH-like protein)	Spoorf19(ABP55170)	58
PA05_orf5	353	p-hydroxymandelate synthase	AcmP1 (ATV95608)	54
PA05_orf6	450	C-domain type II peptide synthetase	SgcC5 (AAL06678)	45
PA05_orf7	450	Glycosyl transferase	SgcA6 (AAL06670)	65
PA05_orf8	327	dNTP-glucose dehydratase	SgcA (AAL06671)	77
PA05_orf9	423	C-methyltransferase	SgcA3 (AAL06661)	63
PA05_orf10	246	N-methyl transferase	SgcA5 (AAL06660)	70
PA05_orf11	385	Amino transferase	SgcA4 (AAL06659)	66
PA05_orf12	1013	SARP family regulator	Spoorf26 (ABP55181)	30
PA05_orf13	63	Homeobox protein TGIF2LY	TGIF2LY(Q8IUE0)	54
PA05_orf14	114	5-hydroxyisourate hydrolase	hiuH (P76341.1)	64
PA05_orf15	577	2-isopropylmalate synthase	leuA (D2ATJ4)	63
PA05_orf16	924	Oxygen regulatory protein	nreC(Q7WZY4.1)	43
PA05_orf17	69	Glucoside xylosyltransferase 2	gxylt2(Q6DE37.1)	37
PA05_orf18	305	Unknown	Ncs56 (AAM78025)	61
PA05_orf19	321	Unknown	SgcE2 (AAL06703)	64
PA05_orf20	302	Unknown	SgcE3 (AAL06702)	56
PA05_orf21	647	Unknown	NcsE4 (AAM78014)	56
PA05_orf22	324	Unknown	NcsE5 (AAM78013)	72
PA05_orf23	**1905**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**61**
PA05_orf24	144	Thioesterase	NcsE10 (AAM78011)	85
PA05_orf25	176	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	59
PA05_orf26	457	Cytochrome P450	SgcE7 (AAL06697)	64
PA05_orf27	245	AraC family transcriptional regulator	SgcR2 (AAL06696)	48
PA05_orf28	167	RNA polymerase sigma-E factor	sigE (Q82EA9.1)	35
PA05_orf29	189	Unknown	NcsE8 (AAM78006)	62
PA05_orf30	545	FDA-dependent oxidoreductase	NcsE9 (AAM78005)	76
PA05_orf31	379	Unknown	Ncs14 (AAM77983)	46
PA05_orf32	268	Unknown	SgcE11 (AAL06691)	63
PA05_orf33	72	Calmodulin-like protein 12	CML12(P25071.3)	46
PA05_orf34	498	CoA ligase	NcsB2 (AAM77987)	27
PA05_orf35	343	Conserved oligomeric Golgi complex subunit 6	cog6 (A1DNX2.1)	35
PA05_orf36	328	Bialaphos biosynthetic pathway regulatory protein	brpA (Q01108.1)	34
PA05_orf37	536	Methylmalonyl-CoA decarboxylase	mdporf-2 (ABY66032.1)	75
PA05_orf38	93	Alanine--tRNA ligase	ALATS (P36428.3)	34
PA05_orf39	255	4'-phosphopantetheinyl transferase HetI	hetI (P37695.2)	34
PA05_orf40	152	Unknown	SgcT (AAL06706)	40

PA05_orf41	180	Unknown	SgcS (AAL06705)	70
PA05_orf42	215	Phenazine biosynthesis protein PhzD2	PhzD2 (P0DPC1.1)	48
PA05_orf43	446	Oxidoreductase	SgcL (AAL06685)	42
PA05_orf44	**316**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**38**

Table S7. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Planobispora longispora* NBRC 13918

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
NBRC13918_orf1	742	Polysulfide reductase chain A	psrA (P31075.1)	26
NBRC13918_orf2	960	Transcriptional regulator	AcmR4 (ATV95651)	36
NBRC13918_orf3	86	ABC transporter D family member 2	ABCC2 (Q6NLC1.1)	33
NBRC13918_orf4	59	Nidogen	Ndg (A1Z877.1)	56
NBRC13918_orf5	103	Protein furry homolog [Homo sapiens]	FRY (Q5TBA9.1)	35
NBRC13918_orf6	112	5-hydroxyisourate hydrolase	hiuH (Q4VYA5.1)	30
NBRC13918_orf7	917	HTH-type transcriptional regulator MalT	malT (Q8D4P3.1)	23
NBRC13918_orf8	57	Sodium- and chloride-dependent GABA transporter 2	SLC6A13 (Q9NSD5.3)	42
NBRC13918_orf9	317	Unknown	Ncs56 (AAM78025)	64
NBRC13918_orf10	327	Unknown	SgcE2 (AAL06703)	65
NBRC13918_orf11	306	Unknown	SgcE3 (AAL06702)	56
NBRC13918_orf12	642	Unknown	SgcE4 (AAL06701)	58
NBRC13918_orf13	338	Unknown	NcsE5 (AAM78013)	69
NBRC13918_orf14	**1913**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**63**
NBRC13918_orf15	142	Thioesterase	NcsE10 (AAM78011)	84
NBRC13918_orf16	178	Flavin reductase	SgcE6 (AAL06698)	57
NBRC13918_orf17	457	Cytochrome P450	SgcE7 (AAL06697)	64
NBRC13918_orf18	214	AraC family transcriptional regulator	SgcR2 (AAL06696)	50
NBRC13918_orf19	187	Unknown	NcsE8 (AAM78006)	65
NBRC13918_orf20	550	FDA-dependent oxidoreductase	NcsE9 (AAM78005)	78
NBRC13918_orf21	338	Unknown	Ncs14 (AAM77983)	47
NBRC13918_orf22	267	Unknown	SgcE11 (AAL06691)	66
NBRC13918_orf23	**320**	**Probable prephenate dehydrogenase NovF**	**novF(Q9L9G2.1)**	**40**
NBRC13918_orf24	111	Unknown	Spoorf12 (ABP55159)	30
NBRC13918_orf25	372	O-acyltransferase	cyaA4 (AGO97197)	38
NBRC13918_orf26	67	No hit	no	no
NBRC13918_orf27	359	DUF2855 family protein	WP_203890131.1	100
NBRC13918_orf28	226	Unknown	Spoorf22 (ABP55176)	34
NBRC13918_orf29	448	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	49
NBRC13918_orf30	474	Oxidoreductase	spoT4 (ABP55175)	28
NBRC13918_orf31	413	P-450 hydroxylase	NcsB3 (AAM77997)	48
NBRC13918_orf32	282	Monooxygenase	KedU11 (FV52167.1)	29
NBRC13918_orf33	460	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	31

Table S8. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Actinomadura livida strain* JCM 3387

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
JCM3387_orf1	130	epoxide hydrolase-like protein	Kedorf-2 (AFV52129)	68
JCM3387_orf2	321	2-acylphloroglucinol 4-prenyltransferase	PT1L (A0A0B5A051.1)	30
JCM3387_orf3	558	2-isopropylmalate synthase	leuA (D2ATJ4.1)	58
JCM3387_orf4	**311**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
JCM3387_orf5	266	Unknown	NcsE11 (AAM78004)	59
JCM3387_orf6	367	Unknown	Ncs14 (AAM77983)	47
JCM3387_orf7	551	Oxidoreductase	SgcE9 (AAL06693)	76
JCM3387_orf8	189	Unknown	SgcE8 (AAL06694)	67
JCM3387_orf9	351	Unknown	SgcE2 (AAL06703)	64
JCM3387_orf10	326	Unknown	SgcE3 (AAL06702)	55
JCM3387_orf11	664	Unknown	SgcE4 (AAL06701)	58
JCM3387_orf12	346	Unknown	SgcE5 (AAL06700)	65
JCM3387_orf13	**1929**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**63**
JCM3387_orf14	146	Thioesterase	NcsE10 (AAM78011)	80
JCM3387_orf15	183	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	61
JCM3387_orf16	454	Cytochrome P450	SgcE7 (AAL06697)	63
JCM3387_orf17	295	AraC family transcriptional regulator	SgcR2 (AAL06696)	43
JCM3387_orf18	526	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	76
JCM3387_orf19	158	Unknown	SgcJ (AAL06676)	59
JCM3387_orf20	151	Unknown	SgcJ (AAL06676)	39
JCM3387_orf21	530	Oxidoreductase	cyaN2 (AGO97205)	66
JCM3387_orf22	393	Oxidoreductase	SgcL (AAL06685)	60
JCM3387_orf23	154	thiol peroxidase	AcmU6 (ATV95618)	33
JCM3387_orf24	264	Tryptophan 2,3-dioxygenase	kynA (B4UMQ6.1)	54
JCM3387_orf25	270	Oxidoreductase	ypmI(SCL57600.1)	31
JCM3387_orf26	281	Kynurenine formamidase	afmid (Q566U4.2)	33
JCM3387_orf27	254	Macrocin O-methyltransferase	tylF (Q9S4D5.1)	56
JCM3387_orf28	442	O-acyltransferase	cyaA4 (AGO97197.1)	28
JCM3387_orf29	140	ATP phosphoribosyltransferase regulatory subunit	hisZ (B3PDB6.1)	31
JCM3387_orf30	390	Amino transferase	SgcA4 (AAL06659)	29
JCM3387_orf31	236	N-methyl transferase	SgcA5 (AAL06660)	45
JCM3387_orf32	328	NDP-hexose reductase	CalS12 (AAM70349)	61

Table S9. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Microbispora corallina strain* NBRC 16416

Gene	AA a	Putative Function	Protein homologs^a	Identity (%)
NBRC16416_orf1	216	HTH-type transcriptional repressor KstR2	kstR2 (P9WMB8.1)	32
NBRC16416_orf2	397	Uncharacterized MFS-type transporter YkuC	ykuC (O31695.2)	22
NBRC16416_orf3	260	Demethylmenaquinone methyltransferase	menG (Q72HI4.1)	29
NBRC16416_orf4	330	Unknown	SgcE2 (AAL06703)	65
NBRC16416_orf5	315	Unknown	NcsE3 (AAM78015)	58
NBRC16416_orf6	655	Unknown	SgcE4 (AAL06701)	58
NBRC16416_orf7	342	Unknown	NcsE5 (AAM78013)	68
NBRC16416_orf8	**1933**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**65**
NBRC16416_orf9	146	Thioesterase	NcsE10 (AAM78011)	80
NBRC16416_orf10	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	58
NBRC16416_orf11	466	Cytochrome P450	SgcE7 (AAL06697)	67
NBRC16416_orf12	115	Terephthalate 1,2-dioxygenase, reductase component 1	tphA1I(Q3C1E0.1)	33
NBRC16416_orf13	258	AraC family transcriptional regulator	SgcR2 (AAL06696)	52
NBRC16416_orf14	98	DNA-directed RNA polymerase II subunit RPB1	RPB1 (Q75A34.2)	33
NBRC16416_orf15	284	[Acyl-carrier-protein] phosphodiesterase PptH	pptH (I6YEE1.1)	57
NBRC16416_orf16	233	RNA polymerase sigma-E factor	sigE (Q82EA9.1)	40
NBRC16416_orf17	189	Unknown	NcsE8 (AAM78006)	73
NBRC16416_orf18	551	FDA-dependent oxidoreductase	NcsE9 (AAM78005)	79
NBRC16416_orf19	368	Unknown	SgcM (AAL06686)	48
NBRC16416_orf20	267	Unknown	NcsE11 (AAM78004)	62
NBRC16416_orf21	341	DAHP synthase	Kedorf-11 (AFV52120.1)	50
NBRC16416_orf22	**285**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
NBRC16416_orf23	384	Endoglucanase B	cenB (P26225.1)	39
NBRC16416_orf24	56	tRNA N6-adenosine threonylcarbamoyltransferase	tsaD (Q2VNJ2.1)	47
NBRC16416_orf25	422	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	69
NBRC16416_orf26	400	3-O-Acyltransferase	SgcD6 (AAL06667)	46
NBRC16416_orf27	223	2-Amino-4-deoxychorismate dehydrogenase	SgcG (AAL06666)	82
NBRC16416_orf28	492	Phenylacetyl-CoA ligase	SgcD5 (AAL06665)	74
NBRC16416_orf29	528	2-Amino-4-deoxychorismate synthase	SgcD (AAL06664)	63
NBRC16416_orf30	335	Alcohol dehydrogenase	mdpC8 (ABY66028)	35
NBRC16416_orf31	115	Transposase for insertion sequence element IS6120	P35883.1	62
NBRC16416_orf32	80	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG	mnmG (A4YJT4)	45
NBRC16416_orf33	282	Esterase/hydrolase	Ncs27 (AAM77996)	34
NBRC16416_orf34	414	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	55
NBRC16416_orf35	205	Anthranilate synthase II	SgcD1 (AAL06663)	69
NBRC16416_orf36	150	DNA-directed RNA polymerase subunit alpha	rpoA (Q7V9Y5.1)	32
NBRC16416_orf37	77	Beta-galactosidase-1-like protein 3	Glb1l3 (Q5XIL5.1)	29
NBRC16416_orf38	242	Unknown	Spoorf22 (ABP55176)	54
NBRC16416_orf39	445	Quinoprotein glucose dehydrogenase B	gdhB (P13650.1)	22
NBRC16416_orf40	386	Inositol-3-phosphate synthase	ino1 (Q54N49.1)	28

NBRC16416_orf41	539	Quinohemoprotein alcohol dehydrogenase ADH IIB	qbdA (Q8GR64)	26
NBRC16416_orf42	385	Epoxide hydrolase	SgcF (AAL06662)	74
NBRC16416_orf43	499	FAD-binding monooxygenase	SgcD2 (AAL06669)	59
NBRC16416_orf44	139	Unknown	SgcJ (AAL06676)	52
NBRC16416_orf45	86	Flagellar hook-basal body complex protein FliE	fliE (Q52945.2)	37
NBRC16416_orf46	517	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	28
NBRC16416_orf47	527	Transmembrane efflux protein	SgcB (AAL06672)	52
NBRC16416_orf48	374	Oxidoreductase	SgcL (AAL06685)	63
NBRC16416_orf49	416	P-450 hydroxylase	NcsB3 (AAM77997)	52
NBRC16416_orf50	339	Zinc-containing alcohol dehydrogenase	spoT5 (ABP55167)	72
NBRC16416_orf51	275	Methyltransferase	SgdA5 (QIJ31495.1)	37
NBRC16416_orf52	120	Hypothetical protein	Spoorf16 (ABP55163)	58
NBRC16416_orf53	454	C-domain type II peptide synthetase	SgcC5 (AAL06678)	48
NBRC16416_orf54	96	Type II PCP	SgcC2 (AAL06679)	50
NBRC16416_orf55	530	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	77
NBRC16416_orf56	454	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	51
NBRC16416_orf57	507	Methyltransferase type 11	spoT6 (ABP55177)	57
NBRC16416_orf58	260	NAD(P)-dependent dehydrogenase	AcmU3 (ATV95612)	30
NBRC16416_orf59	237	Oxidoreductase	SgcN (AAL06687)	32
NBRC16416_orf60	453	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	39
NBRC16416_orf61	408	Oxidoreductase	SgcL (AAL06685)	31
NBRC16416_orf62	459	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	32
NBRC16416_orf63	120	DGPFAETKE family protein	Spoorf12 (ABP55159)	38
NBRC16416_orf64	1069	A-domain type II peptide synthetase	SgcC1 (AAL06681)	45
NBRC16416_orf65	534	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	78

Table S10. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Microbispora* sp. H13382

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
H13382_orf1	538	Methylmalonyl-CoA decarboxylase	mdporf-2 (ABY66032.1)	76
H13382_orf2	76	D-inositol 3-phosphate glycosyltransferase	mshA (Q4JSW2.1)	39
H13382_orf3	328	Bialaphos biosynthetic pathway regulatory protein	brpA (Q01108)	31
H13382_orf4	367	Trigger factor	tig (B6JGU6.1)	38
H13382_orf5	267	Unknown	SgcE11 (AAL06691)	68
H13382_orf6	363	Unknown	SgcM (AAL06686)	53
H13382_orf7	553	Oxidoreductase	SgcE9 (AAL06693)	78
H13382_orf8	189	Unknown	NcsE8 (AAM78006)	71
H13382_orf9	187	RNA polymerase sigma-E factor	sigE (P38133.2)	41
H13382_orf10	281	[Acyl-carrier-protein] phosphodiesterase PptH	PptH (I6YEE1.1)	58
H13382_orf11	256	AraC family transcriptional regulator	SgcR2 (AAL06696)	53
H13382_orf12	460	Cytochrome P450	SgcE7 (AAL06697)	62
H13382_orf13	183	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	60
H13382_orf14	132	Thioesterase	NcsE10 (AAM78011)	83
H13382_orf15	**1942**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**66**
H13382_orf16	336	Unknown	NcsE5 (AAM78013)	70
H13382_orf17	655	Unknown	NcsE4 (AAM78014)	58
H13382_orf18	314	Unknown	NcsE3 (AAM78015)	57
H13382_orf19	328	Unknown	NcsE2 (AAM78016)	65
H13382_orf20	83	Biotin-dependent acetyl-/propionyl-coenzyme A carboxylase epsilon subunit	accE5 (P96886)	39
H13382_orf21	955	Putative HTH-type transcriptional regulator Mb0914c	MB0914C (P59969.1)	45
H13382_orf22	598	2-isopropylmalate synthase	leuA (D2ATJ4.1)	71
H13382_orf23	112	5-hydroxyisourate hydrolase	hiuH (P76341.1)	35
H13382_orf24	69	Serine/threonine-protein kinase PrkC	PrkC (O34507.1)	48
H13382_orf25	1051	Transcriptional regulator	AcmR4 (ATV95651)	34
H13382_orf26	91	Type II PCP	SgcC2 (AAL06679)	44
H13382_orf27	393	Oxidoreductase	SgcL (AAL06685)	69
H13382_orf28	**295**	**Probable prephenate dehydrogenase**	**NovF(Q9L9G2.1)**	**41**
H13382_orf29	253	Oxidoreductase	SgcN (AAL06687)	30

H13382_orf30	226	Phenazine biosynthesis protein PhzD1	PhzD1 (P0DPB9.1)	61
H13382_orf31	70	Peptide chain release factor 3	prfC (Q8XPH8.1)	32

Table S11. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Nonomuraea* sp. WAC 01424

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
WAC01424_orf1	**297**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
WAC01424_orf2	83	Type II PCP	SgcC2 (AAL06679)	37
WAC01424_orf3	87	Type II PCP	SgcC2 (AAL06679)	45
WAC01424_orf4	275	Purine nucleoside phosphorylase	novF (Q9KYV7.1)	39
WAC01424_orf5	395	S-adenosylmethionine synthase	metK (B1W470.1)	78
WAC01424_orf6	215	Phenazine biosynthesis protein PhzD1	phzD1 (P0DPB9.1)	50
WAC01424_orf7	185	Unknown	SgcS (AAL06705)	66
WAC01424_orf8	160	Unknown	SgcT (AAL06706)	42
WAC01424_orf9	241	hypothetical protein	Cya-4 (AGO97186)	26
WAC01424_orf10	256	4'-phosphopantetheinyl transferase HetI	HetI(P37695)	32
WAC01424_orf11	111	Phosphoglucosamine mutase	glmM (Q11DI7)	48
WAC01424_orf12	470	Efflux pump transporter	NcsA1 (AAM77999)	35
WAC01424_orf13	527	Methylmalonyl-CoA decarboxylase	mdporf-2 (ABY66032.1)	76
WAC01424_orf14	71	E3 ubiquitin-protein ligase DZIP3	DZIP3(Q7TPV2.2)	41
WAC01424_orf15	328	Bialaphos biosynthetic pathway regulatory protein	brpA(Q01108)	32
WAC01424_orf16	376	30S ribosomal protein S2	rps2 (A8A8X0.1)	28
WAC01424_orf17	500	A-domain type II peptide synthetase	SgcC1 (AAL06681)	27
WAC01424_orf18	267	Unknown	SgcE11(AAL06691)	66
WAC01424_orf19	345	Unknown	SgcM (AAL06686)	50
WAC01424_orf20	550	Oxidoreductase	SgcE9 (AAL06693)	79
WAC01424_orf21	186	Unknown	SgcE8 (AAL06694)	64
WAC01424_orf22	157	RNA polymerase sigma-E factor	sigE (Q82EA9)	38
WAC01424_orf23	283	[Acyl-carrier-protein] phosphodiesterase PptH	PptH (I6YEE1)	57
WAC01424_orf24	252	AraC family transcriptional regulator	SgcR2 (AAL06696)	50
WAC01424_orf25	448	Cytochrome P450	SgcE7 (AAL06697)	64
WAC01424_orf26	177	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	62
WAC01424_orf27	142	Thioesterase	NcsE10 (AAM78011)	78
WAC01424_orf28	**1904**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**62**
WAC01424_orf29	323	Unknown	NcsE5 (AAM78013)	72
WAC01424_orf30	648	Unknown	SgcE4 (AAL06701)	57
WAC01424_orf31	312	Unknown	SgcE3 (AAL06702)	55
WAC01424_orf32	330	Unknown	NcsE2 (AAM78016)	62
WAC01424_orf33	62	Uronate isomerase	uxaC (Q3BMC5.1)	43
WAC01424_orf34	923	Oxygen regulatory protein NreC	NreC (Q7WZY4.1)	41
WAC01424_orf35	623	2-isopropylmalate synthase	leuA (D2ATJ4.1)	62
WAC01424_orf36	112	5-hydroxyisourate hydrolase	hiuH (P76341.1)	31
WAC01424_orf37	66	Replicase large subunit	P89659.2	41
WAC01424_orf38	64	Taste receptor type 1 member 2	TAS1R2 (A3QP07.1)	37
WAC01424_orf39	1025	Transcriptional regulator	AcmR4 (ATV95651)	32

Table S12. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Planomonospora* sp. ID67723

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
ID67723_orf1	316	Unknown	Ncs56 (AAM78025)	64
ID67723_orf2	331	Unknown	NcsE2 (AAM78016)	66
ID67723_orf3	309	Unknown	NcsE3 (AAM78015)	56
ID67723_orf4	658	Unknown	SgcE4 (AAL06701)	57
ID67723_orf5	323	Unknown	NcsE5 (AAM78013)	71
ID67723_orf6	**1927**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**65**
ID67723_orf7	142	Thioesterase	NcsE10 (AAM78011)	82
ID67723_orf8	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	60
ID67723_orf9	464	Cytochrome P450	SgcE7 (AAL06697)	67
ID67723_orf10	240	AraC family transcriptional regulator	SgcR2 (AAL06696)	49
ID67723_orf11	284	Acyl-carrier-protein] phosphodiesterase PptH	PptH (I6YEE1)	55
ID67723_orf12	192	RNA polymerase sigma-E factor	sigE (P38133.2)	40
ID67723_orf13	188	Unknown	SgcE8 (AAL06694)	71
ID67723_orf14	549	FDA-dependent oxidoreductase	NcsE9 (AAM78005)	81
ID67723_orf15	349	Unknown	SgcM (AAL06686)	52
ID67723_orf16	267	Unknown	NcsE11 (AAM78004)	65
ID67723_orf17	318	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase	TPS21 (Q84UU4)	32
ID67723_orf18	328	Bialaphos biosynthetic pathway regulatory protein	brpA (Q01108)	31
ID67723_orf19	92	DNA-directed RNA polymerase subunit beta'	rpoC2 (P73334.1)	43
ID67723_orf20	551	Methylmalonyl-CoA decarboxylase	mdporf-2 (ABY66032.1)	75
ID67723_orf21	89	Formin-F	forF (Q5TJ56.1)	41
ID67723_orf22	252	Putative 4'-phosphopantetheinyl transferase slr0495	slr0495 (Q55185.1)	28
ID67723_orf23	241	Methylesterase 8	MES8 (O80475.1)	36
ID67723_orf24	166	Unknown	SgcT (AAL06706)	42
ID67723_orf25	188	Unknown	SgcS (AAL06705)	70
ID67723_orf26	348	Epoxide hydrolase	SgcF (AAL06662)	30
ID67723_orf27	454	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	34
ID67723_orf28	615	Asparagine synthetase	asnO (O05272)	46
ID67723_orf29	404	FAD-dependent oxidoreductase	dynE13(ACB47064)	33
ID67723_orf30	632	Anthranilate synthase II	SgcD1 (AAL06663)	31
ID67723_orf31	217	Phenazine biosynthesis protein PhzD1	PhzD1(P0DPB9.1)	55
ID67723_orf32	254	Short chain dehydrogenase	KedU16 (AFV52172)	40
ID67723_orf33	1059	A-domain type II peptide synthetase	SgcC1 (AAL06681)	40
ID67723_orf34	552	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	72
ID67723_orf35	525	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	77
ID67723_orf36	406	Oxidoreductase	SgcL (AAL06685)	26
ID67723_orf37	268	N-methyl transferase	SgcA5 (AAL06660)	45
ID67723_orf38	369	Amino transferase	SgcA4 (AAL06659)	31
ID67723_orf39	711	Formate dehydrogenase subunit alpha	fdhA (P61159)	23
ID67723_orf40	360	1,2-phenylacetyl-CoA epoxidase, subunit E	paaE (P76081.1)	40

ID67723_orf41	145	Putative 1,2-phenylacetyl-CoA epoxidase, subunit D	paaD (P76080)	45
ID67723_orf42	243	1,2-phenylacetyl-CoA epoxidase, subunit C	paaC (P76079.1)	48
ID67723_orf43	411	1,2-phenylacetyl-CoA epoxidase, subunit A	paa A(P76077.1)	60
ID67723_orf44	550	A-domain type II peptide synthetase	SgcC1 (AAL06681)	28
ID67723_orf45	263	Arylamine N-acetyltransferase	nat (O86309.3)	33
ID67723_orf46	551	A-domain type II peptide synthetase	SgcC1 (AAL06681)	34
ID67723_orf47	631	Radical SAM C-methyltransferase	KedN5 (AFV52163)	31
ID67723_orf48	374	Enoyl reductase	KedU32 (AFV52194)	30
ID67723_orf49	263	Type II thioesterase	KedU43 (AFV52205)	38
ID67723_orf50	409	3-O-Acyltransferase	SgcD6 (AAL06667)	37
ID67723_orf51	293	Dioxygenase swnH1	swnH1(D4AU26.1)	32
ID67723_orf52	151	transport protein	CynR5 (AGO97175.1)	32
ID67723_orf53	143	Probable zinc-binding alcohol dehydrogenase Rv1895	Rv1895 (O07737)	61
ID67723_orf54	403	C-methyltransferase	SgcA3 (AAL06661)	36
ID67723_orf55	452	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	28
ID67723_orf56	514	Methyltransferase	spoT6 (ABP55177)	45
ID67723_orf57	475	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	46
ID67723_orf58	431	Glycosyl transferase	SgcA6 (AAL06670)	52
ID67723_orf59	326	dNDP-hexose 4, 6-dehydratase	NcsC1 (AAM77990)	33
ID67723_orf60	411	P-450 hydroxylase	NcsB3 (AAM77997)	47
ID67723_orf61	523	Transmembrane efflux protein	SgcB (AAL06672)	51
ID67723_orf62	391	O-acyltransferase	cyaA4 (AGO97197.1)	44
ID67723_orf63	250	Short chain dehydrogenase	KedU16 (AFV52172)	40
ID67723_orf64	467	FAD-binding monooxygenase	SgcD2 (AAL06669)	52
ID67723_orf65	444	C-domain type II peptide synthetase	SgcC5 (AAL06678)	45
ID67723_orf66	261	Hydrolase	SgcI (AAL06675)	48
ID67723_orf67	429	Acyl-CoA N-acyltransferase	KedN4 (AFV52164.1)	31
ID67723_orf68	244	Probable N-octanoylanthranilate hydrolase AqdA1	AqdA1 (A0A0G3FWY4.1)	33
ID67723_orf69	500	A-domain type II peptide synthetase	SgcC1 (AAL06681)	26
ID67723_orf70	265	Tryptophan 2,3-dioxygenase	kynA (B4UMQ6.1)	53
ID67723_orf71	401	Uncharacterized aminotransferase YcbU	YcbU (P42253.3)	23
ID67723_orf72	437	NDP glucose dehydrogenase	AcmA2 (ATV95598)	69
ID67723_orf73	434	Amino transferase	SgcA4 (AAL06659)	29
ID67723_orf74	136	Unknown	SgcJ (AAL06676)	55
ID67723_orf75	91	Type II PCP	SgcC2 (AAL06679)	45
ID67723_orf76	393	Oxidoreductase	SgcL (AAL06685)	69
ID67723_orf77	**348**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**40**

Table S13. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Sphaerisporangium melleum* NBRC 107356

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
NBRC107356_orf1	187	Unknown	SgcS (AAL06705)	72
NBRC107356_orf2	186	Alkylhydroperoxidase	SgcO (WP_010056303)	55
NBRC107356_orf3	131	Uncharacterized protein Mb0035	Mb0035(P64674)	33
NBRC107356_orf4	263	Alkylhydroperoxidase	SgcO (WP_010056303)	53
NBRC107356_orf5	383	Acyltransferase	cynA4 (AGO97170)	37
NBRC107356_orf6	535	Transmembrane efflux protein	SgcB (AAL06672)	53
NBRC107356_orf7	87	Uncharacterized protein F44E2.8	F44E2.8(P34438.2)	27
NBRC107356_orf8	327	Unknown	SgcE2 (AAL06703)	65
NBRC107356_orf9	297	Unknown	NcsE3 (AAM78015)	56
NBRC107356_orf10	655	Unknown	NcsE4 (AAM78014)	59
NBRC107356_orf11	339	Unknown	SgcE5 (AAL06700)	69
NBRC107356_orf12	**1930**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**65**
NBRC107356_orf13	142	Thioesterase	NcsE10 (AAM78011)	78
NBRC107356_orf14	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	57
NBRC107356_orf15	478	Cytochrome P450	SgcE7 (AAL06697)	65
NBRC107356_orf16	243	AraC family transcriptional regulator	SgcR2 (AAL06696)	52
NBRC107356_orf17	189	Unknown	SgcE8 (AAL06694)	67
NBRC107356_orf18	551	Oxidoreductase	SgcE9 (AAL06693)	79
NBRC107356_orf19	373	Unknown	Ncs14 (AAM77983)	49
NBRC107356_orf20	267	Unknown	SgcE11 (AAL06691)	64
NBRC107356_orf21	266	Probable methyltransferase PMT5	PMT5(Q3EC77.2)	25
NBRC107356_orf22	89	tRNA pseudouridine synthase A	truA(P70973.2)	29
NBRC107356_orf23	**362**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**38**
NBRC107356_orf24	543	Transmembrane efflux protein	SgcB (AAL06672)	50
NBRC107356_orf25	109	DGPFAETKE family protein	Spoorf12 (ABP55159)	41
NBRC107356_orf26	157	D-ribose pyranase	rbsD (B7IGD0)	30
NBRC107356_orf27	414	3-O-Acyltransferase	SgcD6 (AAL06667)	25
NBRC107356_orf28	391	Mycoketide-CoA synthase	pks12 (I6XD69.1)	27
NBRC107356_orf29	244	Phosphoribosylaminoimidazole-succinocarboxamide synthase	purC (A0R4I0.1)	44
NBRC107356_orf30	448	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	49

Table S14. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Sphaerisporangium rubeum* DSM 44936

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
DSM44936_orf1	313	Unknown	SgcE2 (AAL06703)	64
DSM44936_orf2	314	Unknown	SgcE3 (AAL06702)	58
DSM44936_orf3	653	Unknown	NcsE4 (AAM78014)	57
DSM44936_orf4	327	Unknown	NcsE5 (AAM78013)	68
DSM44936_orf5	**1926**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**64**
DSM44936_orf6	145	Thioesterase	NcsE10 (AAM78011)	84
DSM44936_orf7	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	60
DSM44936_orf8	466	Cytochrome P450	SgcE7 (AAL06697)	64
DSM44936_orf9	255	AraC family transcriptional regulator	SgcR2 (AAL06696)	55
DSM44936_orf10	176	Unknown	NcsE8 (AAM78006)	66
DSM44936_orf11	551	Oxidoreductase	SgcE9 (AAL06693)	79
DSM44936_orf12	346	Unknown	SgcM (AAL06686)	50
DSM44936_orf13	267	Unknown	SgcE11 (AAL06691)	61
DSM44936_orf14	344	DAHP synthase	Kedorf-11 (AFV52120.1)	50
DSM44936_orf15	**293**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
DSM44936_orf16	326	Reticulon-4-interacting protein 1, mitochondrial	RTN4IP1 (Q8WWV3)	32
DSM44936_orf17	78	Uncharacterized HTH-type transcriptional regulator MJ0272	MJ0272 (Q57720.2)	49
DSM44936_orf18	141	p-hydroxybenzoic acid efflux pump subunit AaeB	AaeB (Q6DAH4.1)	32
DSM44936_orf19	415	P-450 hydroxylase	NcsB3 (AAM77997)	47
DSM44936_orf20	537	Transmembrane efflux protein	SgcB (AAL06672)	54
DSM44936_orf21	239	Fructose-1-phosphate phosphatase YqaB	YqaB (P77475.1)	36
DSM44936_orf22	376	Amino transferase	SgcA4 (AAL06659)	28
DSM44936_orf23	250	N-methyl transferase	SgcA5 (AAL06660)	51

Table S15. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces boluensis* YC537

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
YC537_orf1	267	Unknown	SgcM (AAL06686)	59
YC537_orf2	**485**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**37**
YC537_orf3	273	Unknown	SgcE11 (AAL06691)	76
YC537_orf4	152	Thioesterase	NcsE10 (AAM78011)	85
YC537_orf5	551	Oxidoreductase	SgcE9 (AAL06693)	86
YC537_orf6	196	Unknown	NcsE8 (AAM78006)	72
YC537_orf7	363	Regulatory protein	SgcR1 (AAL06695)	65
YC537_orf8	255	AraC family transcriptional regulator	SgcR2 (AAL06696)	68
YC537_orf9	448	Cytochrome P450	SgcE7 (AAL06697)	78
YC537_orf10	150	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	64
YC537_orf11	153	Thioesterase	NcsE10 (AAM78011)	62
YC537_orf12	**1936**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**75**
YC537_orf13	386	Unknown	SgcE5 (AAL06700)	75
YC537_orf14	640	Unknown	SgcE4 (AAL06701)	77
YC537_orf15	328	Unknown	SgcE3 (AAL06702)	73
YC537_orf16	359	Unknown	SgcE2 (AAL06703)	69
YC537_orf17	147	HxlR family transcriptional regulator	SgcE1 (AAL06704)	69
YC537_orf18	188	Unknown	SgcS (AAL06705)	69
YC537_orf19	156	Unknown	SgcT (AAL06706)	54
YC537_orf20	393	Transcriptional regulator	SgcR3 (AAL06707)	62
YC537_orf21	213	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	40
YC537_orf22	326	Unknown	Ncs56 (AAM78025)	71

Table S16. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces jumonjinensis* NRRL 5741.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
NRRL5741_orf1	344	Oxidase	SgcQ (AAL06690)	58
NRRL5741_orf2	138	Guanylate cyclase	GC (S7VVK4.1)	38
NRRL5741_orf3	241	4'-phosphopantetheinyl transferase HetI	HetI (P37695.2)	30
NRRL5741_orf4	392	S-adenosylmethionine synthase	metK (Q9X4Q2.1)	83
NRRL5741_orf5	518	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	58
NRRL5741_orf6	205	Unknown	Ncs13 (AAM77982)	39
NRRL5741_orf7	334	Unknown	SgcM (AAL06686)	61
NRRL5741_orf8	291	Fibronectin	FN1 (Q28749.2)	28
NRRL5741_orf9	**351**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**40**
NRRL5741_orf10	266	Unknown	SgcE11 (AAL06691)	82
NRRL5741_orf11	155	Type II thioesterase	SgcE10 (AAL06692)	86
NRRL5741_orf12	541	Oxidoreductase	SgcE9 (AAL06693)	91
NRRL5741_orf13	176	Unknown	SgcE8 (AAL06694)	80
NRRL5741_orf14	351	Regulatory protein	SgcR1 (AAL06695)	70
NRRL5741_orf15	241	AraC family transcriptional regulator	SgcR2 (AAL06696)	74
NRRL5741_orf16	449	Cytochrome P450	SgcE7 (AAL06697)	82
NRRL5741_orf17	179	Flavin reductase	SgcE6 (AAL06698)	70
NRRL5741_orf18	153	Type II thioesterase	SgcE10 (AAL06692)	51
NRRL5741_orf19	**1948**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**79**
NRRL5741_orf20	298	Unknown	SgcE5 (AAL06700)	76
NRRL5741_orf21	460	Unknown	SgcE4 (AAL06701)	80
NRRL5741_orf22	328	Unknown	SgcE3 (AAL06702)	80
NRRL5741_orf23	330	Unknown	SgcE2 (AAL06703)	75
NRRL5741_orf24	147	HxlR family transcriptional regulator	SgcE1 (AAL06704)	78
NRRL5741_orf25	187	Unknown	SgcS (AAL06705)	79
NRRL5741_orf26	160	Unknown	SgcT (AAL06706)	65
NRRL5741_orf27	400	Transcriptional regulator	SgcR3 (AAL06707)	68

Table S17. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces* sp. DH5.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
DH5_orf1	415	P-450 hydroxylase	NcsB3 (AAM77997)	51
DH5_orf2	412	Oxidoreductase	SgcL (AAL06685)	30
DH5_orf3	460	C-domain type II peptide synthetase	SgcC5 (AAL06678)	41
DH5_orf4	584	A-domain type II peptide synthetase	SgcC1 (AAL06681)	32
DH5_orf5	410	L-arginine-specific L-amino acid ligase	rizA (B5UAT8.1)	26
DH5_orf6	317	O-acetylserine sulfhydrylase	cysK (P0A535.1)	52
DH5_orf7	212	Serine acetyltransferase	cysE (Q9ZK14.1)	48
DH5_orf8	421	Uncharacterized protein y4rH	y4rH (P55641.1)	28
DH5_orf9	414	Uncharacterized protein y4rH	y4rH (P55641.1)	31
DH5_orf10	64	Serine/threonine-protein kinase CLA4	CLA4 (Q9HFW2.1)	41
DH5_orf11	**295**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
DH5_orf12	268	Unknown	SgcE11 (AAL06691)	73
DH5_orf13	353	Unknown	SgcM (AAL06686)	54
DH5_orf14	557	Oxidoreductase	SgcE9 (AAL06693)	83
DH5_orf15	195	Unknown	NcsE8 (AAM78006)	77
DH5_orf16	357	StrR-like transcriptional regulator	NcsR6 (AAM78007)	60
DH5_orf17	255	AraC family, transcriptional regulator	NcsR5 (AAM78008)	71
DH5_orf18	449	P-450 hydroxylase	NcsE7 (AAM78009)	78
DH5_orf19	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	69
DH5_orf20	152	Thioesterase	NcsE10 (AAM78011)	83
DH5_orf21	**1969**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**74**
DH5_orf22	357	Unknown	NcsE5 (AAM78013)	75
DH5_orf23	616	Unknown	NcsE4 (AAM78014)	79
DH5_orf24	308	Unknown	NcsE3 (AAM78015)	75
DH5_orf25	329	Unknown	NcsE2 (AAM78016)	68
DH5_orf26	147	Transcription regulator	NcsE1 (AAM78017)	71
DH5_orf27	395	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	60
DH5_orf28	225	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	49
DH5_orf29	320	dNDP-hexose dehydratase	NcsC2 (AAM78021)	57
DH5_orf30	208	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	66
DH5_orf31	315	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	59
DH5_orf32	501	2-amino-4-deoxychorismate synthase	sgcD (Q8GMH4.1)	72
DH5_orf33	276	Unknown	Ncs55 (AAM78024)	72

Table S18. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces* sp. NK15101.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
NK15101_orf1	316	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	60
NK15101_orf2	209	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	66
NK15101_orf3	319	dNDP-hexose dehydratase	NcsC2 (AAM78021)	58
NK15101_orf4	213	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	50
NK15101_orf5	403	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	62
NK15101_orf6	147	Transcription regulator	NcsE1 (AAM78017)	73
NK15101_orf7	342	Unknown	SgcE2 (AAL06703)	70
NK15101_orf8	307	Unknown	NcsE3 (AAM78015)	75
NK15101_orf9	636	Unknown	NcsE4 (AAM78014)	79
NK15101_orf10	390	Unknown	NcsE5 (AAM78013)	77
NK15101_orf11	**1960**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**75**
NK15101_orf12	152	Thioesterase	NcsE10 (AAM78011)	84
NK15101_orf13	181	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	71
NK15101_orf14	423	P-450 hydroxylase	NcsE7 (AAM78009)	78
NK15101_orf15	256	AraC family, transcriptional regulator	NcsR5 (AAM78008)	67
NK15101_orf16	372	StrR-like transcriptional regulator	NcsR6 (AAM78007)	60
NK15101_orf17	195	Unknown	NcsE8 (AAM78006)	78
NK15101_orf18	556	Oxidoreductase	SgcE9 (AAL06693)	81
NK15101_orf19	268	Unknown	NcsE11 (AAM78004)	75
NK15101_orf20	**299**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**41**
NK15101_orf21	86	Type II PCP	SgcC2 (AAL06679)	41
NK15101_orf22	288	Dioxygenase swnH1	swnH1 (E9F8L9.1)	34
NK15101_orf23	418	P-450 hydroxylase	NcsB3 (AAM77997)	48
NK15101_orf24	142	Unknown	SgcJ (AAL06676)	61
NK15101_orf25	396	Oxidoreductase	SgcL (AAL06685)	65
NK15101_orf26	528	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	75
NK15101_orf27	445	C-domain type II peptide synthetase	SgcC5 (AAL06678)	52
NK15101_orf28	544	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	78
NK15101_orf29	849	A-domain type II peptide synthetase	SgcC1 (AAL06681)	43
NK15101_orf30	482	FAD-binding monooxygenase	SgcD2 (AAL06669)	54
NK15101_orf31	412	Oxidoreductase	SgcL (AAL06685)	27

Table S19. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptosporangium canum* CGMCC 4.2126.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
CGMCC4.2126_orf1	331	Unknown	SgcE2 (AAL06703)	66
CGMCC4.2126_orf2	300	Unknown	NcsE3 (AAM78015)	56
CGMCC4.2126_orf3	658	Unknown	NcsE4 (AAM78014)	58
CGMCC4.2126_orf4	329	Unknown	NcsE5 (AAM78013)	69
CGMCC4.2126_orf5	**1930**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**67**
CGMCC4.2126_orf6	142	Thioesterase	NcsE10 (AAM78011)	84
CGMCC4.2126_orf7	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	59
CGMCC4.2126_orf8	482	Cytochrome P450	SgcE7 (AAL06697)	66
CGMCC4.2126_orf9	255	AraC family transcriptional regulator	SgcR2 (AAL06696)	53
CGMCC4.2126_orf10	164	RNA polymerase sigma-E factor	sigE (P38133.2)	41
CGMCC4.2126_orf11	176	Unknown	SgcE8 (AAL06694)	71
CGMCC4.2126_orf12	551	Oxidoreductase	SgcE9 (AAL06693)	81
CGMCC4.2126_orf13	374	Unknown	SgcM (AAL06686)	48
CGMCC4.2126_orf14	267	Unknown	NcsE11 (AAM78004)	65
CGMCC4.2126_orf15	395	Acyltransferase	cynA4 (AGO97170)	40
CGMCC4.2126_orf16	311	Arylamine N-acetyltransferase	nat (P0A5L9.1)	32
CGMCC4.2126_orf17	407	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	56
CGMCC4.2126_orf18	334	O-methyltransferase	SgcD4 (AAL06683)	58
CGMCC4.2126_orf19	342	DAHP synthase	Kedorf-11 (AFV52120.1)	50
CGMCC4.2126_orf20	**283**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
CGMCC4.2126_orf21	416	P-450 hydroxylase	NcsB3 (AAM77997)	49
CGMCC4.2126_orf22	183	Pyrimidine reductase	Cyaorf7 (AGO97196)	32
CGMCC4.2126_orf23	122	Acid phosphatase	gpm2 (Q6MWZ7.1)	28
CGMCC4.2126_orf24	110	30S ribosomal protein S18	rpsR (A5EXN0.1)	45
CGMCC4.2126_orf25	148	50S ribosomal protein L3	rplC (A8IAS6.2)	24
CGMCC4.2126_orf26	118	ATPase	Cynorf32 (AGO97175)	44
CGMCC4.2126_orf27	451	Efflux pump transporter	NcsA1 (AAM77999)	33
CGMCC4.2126_orf28	411	Oxidoreductase	SgcL (AAL06685)	29
CGMCC4.2126_orf29	459	C-domain type II peptide synthetase	SgcC5 (AAL06678)	47
CGMCC4.2126_orf30	143	Unknown	SgcJ (AAL06676)	64
CGMCC4.2126_orf31	393	Oxidoreductase	SgcL (AAL06685)	67
CGMCC4.2126_orf32	526	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	78
CGMCC4.2126_orf33	251	Oxidoreductase	SgcN (AAL06687)	31
CGMCC4.2126_orf34	468	FAD-binding monooxygenase	SgcD2 (AAL06669)	53
CGMCC4.2126_orf35	362	p-hydroxymandelate synthase	AcmP1 (ATV95608)	60
CGMCC4.2126_orf36	58	Unknown	Spoorf19 (ABP55170)	56
CGMCC4.2126_orf37	592	A-domain type II peptide synthetase	SgcC1 (AAL06681)	31
CGMCC4.2126_orf38	460	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	29
CGMCC4.2126_orf39	475	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	50
CGMCC4.2126_orf40	550	Transmembrane efflux protein	SgcB (AAL06672)	47

CGMCC4.2126_orf41	282	Esterase/hydrolase	Ncs27 (AAM77996)	36
CGMCC4.2126_orf42	336	O-methyltransferase	SgcD4 (AAL06683)	61

Table S20. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptosporangium sandarakinum* DSM 45763.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
DSM45763_orf1	562	CoA ligase	NcsB2 (AAM77987)	27
DSM45763_orf2	263	Tryptophan 2,3-dioxygenase	kynA (Q2IEC3.1)	43
DSM45763_orf3	432	Kynureninase	kynU (P83788.1)	26
DSM45763_orf4	165	Redox-sensitive transcriptional activator SoxR	SoxR(Q51506.1)	51
DSM45763_orf5	500	Transmembrane efflux protein	SgcB (AAL06672)	30
DSM45763_orf6	396	Uncharacterized protein YahJ	YahJ (P77554.1)	36
DSM45763_orf7	243	Nta operon transcriptional regulator	ntaR (P54988.2)	32
DSM45763_orf8	369	Calmodulin-interacting protein 111	CIP111 (Q9LET7.1)	35
DSM45763_orf9	152	HTH-type transcriptional regulator MhqR	MhqR (O31672.1)	28
DSM45763_orf10	267	Unknown	SgcE11 (AAL06691)	67
DSM45763_orf11	409	Unknown	SgcM (AAL06686)	45
DSM45763_orf12	550	FDA-dependent oxidoreductase	NcsE9 (AAM78005)	80
DSM45763_orf13	176	Unknown	SgcE8 (AAL06694)	67
DSM45763_orf14	187	RNA polymerase sigma-E factor	SigE (P38133.2)	39
DSM45763_orf15	284	AraC family transcriptional regulator	SgcR2 (AAL06696)	52
DSM45763_orf16	474	Cytochrome P450	SgcE7 (AAL06697)	65
DSM45763_orf17	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	60
DSM45763_orf18	144	Thioesterase	NcsE10 (AAM78011)	83
DSM45763_orf19	**1961**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**65**
DSM45763_orf20	323	Unknown	NcsE5 (AAM78013)	69
DSM45763_orf21	652	Unknown	NcsE4 (AAM78014)	59
DSM45763_orf22	298	Unknown	NcsE3 (AAM78015)	57
DSM45763_orf23	329	Unknown	NcsE2 (AAM78016)	64
DSM45763_orf24	286	UPF0182 protein MAV_4137	MAV_4137(A0QK47.1)	36
DSM45763_orf25	89	Biotin-dependent acetyl-/propionyl-coenzyme A carboxylase epsilon subunit	accE5 (P96886.1)	55
DSM45763_orf26	927	HTH-type transcriptional regulator MalT	MalT (Q8D4P3.1)	23
DSM45763_orf27	665	2-isopropylmalate synthase	leuA (D2ATJ4.1)	65
DSM45763_orf28	112	5-hydroxyisourate hydrolase	hiuH (Q8ZQ52.1)	32
DSM45763_orf29	52	Legumain	LGMN (Q99538.1)	33
DSM45763_orf30	1029	Transcriptional regulator	AcmR4 (ATV95651)	33
DSM45763_orf31	90	Type II PCP	SgcC2 (AAL06679)	42
DSM45763_orf32	164	ECF RNA polymerase sigma factor SigJ	SigJ (L0TCG5.1)	53
DSM45763_orf33	394	Oxidoreductase	SgcL (AAL06685)	69
DSM45763_orf34	**315**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**

Table S21. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptosporangium* sp. KLBMP 9127.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
KLBMP9127_orf1	**305**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**36**
KLBMP9127_orf2	191	Putative carboxymethylenebutenolidase	(P95862.1)	23
KLBMP9127_orf3	436	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	29
KLBMP9127_orf4	483	Efflux pump transporter	NcsA1 (AAM77999)	36
KLBMP9127_orf5	169	Uncharacterized protein Mb2934c	Mb2934c (P65054.1)	24
KLBMP9127_orf6	298	LysR transcriptional regulator	Kedorf-15 (AFV52116)	27
KLBMP9127_orf7	394	Oxidoreductase	SgcL (AAL06685)	66
KLBMP9127_orf8	91	Type II PCP	SgcC2 (AAL06679)	45
KLBMP9127_orf9	84	Type II PCP	SgcC2 (AAL06679)	52
KLBMP9127_orf10	259	Argininosuccinate lyase 2	argH2 (Q981V0.1)	25
KLBMP9127_orf11	261	Hydrolase	SgcI (AAL06675)	47
KLBMP9127_orf12	136	Unknown	Ncs16 (AAM77985)	50
KLBMP9127_orf13	416	Acyl-CoA N-acyltransferase	KedN4 (AFV52164.1)	33
KLBMP9127_orf14	459	C-domain type II peptide synthetase	SgcC5 (AAL06678)	50
KLBMP9127_orf15	404	FAD-binding monooxygenase	SgcD2 (AAL06669)	54
KLBMP9127_orf16	250	Short chain dehydrogenase	KedU16 (AFV52172)	35
KLBMP9127_orf17	467	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	29
KLBMP9127_orf18	528	Methyltransferase type 11	spoT6 (ABP55177)	45
KLBMP9127_orf19	483	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	46
KLBMP9127_orf20	150	transport protein	CynR5 (AGO97175.1)	29
KLBMP9127_orf21	412	Oxidoreductase	SgcL (AAL06685)	26
KLBMP9127_orf22	527	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	74
KLBMP9127_orf23	296	Dioxygenase swnH1	swnH1(E9F8L9.1)	36
KLBMP9127_orf24	555	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	74
KLBMP9127_orf25	1060	A-domain type II peptide synthetase	SgcC1 (AAL06681)	42
KLBMP9127_orf26	310	epoxide hydrolase-like protein	Kedorf-2 (AFV52129)	27
KLBMP9127_orf27	422	3-O-Acyltransferase	SgcD6 (AAL06667)	34
KLBMP9127_orf28	271	Type II thioesterase	KedU43 (AFV52205)	40
KLBMP9127_orf29	379	Acyl-CoA dehydrogenase	KedU41 (AFV52203)	31
KLBMP9127_orf30	643	Radical SAM C-methyltransferase	KedN5 (AFV52163)	29
KLBMP9127_orf31	555	A-domain type II peptide synthetase	SgcC1 (AAL06681)	35
KLBMP9127_orf32	265	Arylamine N-acetyltransferase 2	Nat2 (P50295.1)	29
KLBMP9127_orf33	526	CoA ligase	NcsB2 (AAM77987)	27
KLBMP9127_orf34	490	1,2-phenylacetyl-CoA epoxidase, subunit A	paaA (P76077.1)	63
KLBMP9127_orf35	249	1,2-phenylacetyl-CoA epoxidase, subunit C	paaC (P76079.1)	48
KLBMP9127_orf36	163	1,2-phenylacetyl-CoA epoxidase, subunit D	paaD (P76080.2)	43
KLBMP9127_orf37	368	1,2-phenylacetyl-CoA epoxidase, subunit E	PaaE(P76081.1)	41
KLBMP9127_orf38	723	Polysulfide reductase chain A	psrA (P31075.1)	27
KLBMP9127_orf39	187	Unknown	SgcS (AAL06705)	71
KLBMP9127_orf40	159	Unknown	SgcT (AAL06706)	46

KLBMP9127_orf41	253	hypothetical protein	Cya-4 (AGO97186)	27
KLBMP9127_orf42	252	4'-phosphopantetheinyl transferase HetI	HetI (P37695.2)	36
KLBMP9127_orf43	163	RNA polymerase II elongation factor ELL3	ELL3(Q9HB65.2)	32
KLBMP9127_orf44	540	Methylmalonyl-CoA decarboxylase	mdporf-2 (ABY66032.1)	74
KLBMP9127_orf45	99	Levansucrase; Flags: Precursor	levS (Q70XJ9.1)	48
KLBMP9127_orf46	328	Bialaphos biosynthetic pathway regulatory protein	brpA (Q01108.1)	30
KLBMP9127_orf47	339	Probable D-serine dehydratase	dsdA (A6UB95.1)	38
KLBMP9127_orf48	541	A-domain type II peptide synthetase	SgcC1 (AAL06681)	28
KLBMP9127_orf49	294	Tryptophan 2,3-dioxygenase	kynA (B4UMQ6.1)	55
KLBMP9127_orf50	405	Isopenicillin N epimerase	cefD (P18549.3)	28
KLBMP9127_orf51	267	Unknown	SgcE11 (AAL06691)	66
KLBMP9127_orf52	350	Unknown	SgcM (AAL06686)	49
KLBMP9127_orf53	550	FDA-dependent oxidoreductase	NcsE9 (AAM78005)	78
KLBMP9127_orf54	189	Unknown	SgcE8 (AAL06694)	67
KLBMP9127_orf55	188	RNA polymerase sigma-E factor	sigE (P38133.2)	40
KLBMP9127_orf56	283	[Acyl-carrier-protein] phosphodiesterase	PptH (I6YEE1.1)	59
KLBMP9127_orf57	240	AraC family, transcriptional regulator	NcsR5 (AAM78008)	49
KLBMP9127_orf58	458	Cytochrome P450	SgcE7 (AAL06697)	65
KLBMP9127_orf59	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	59
KLBMP9127_orf60	142	Thioesterase	NcsE10 (AAM78011)	83
KLBMP9127_orf61	**1925**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**65**
KLBMP9127_orf62	323	Unknown	SgcE5 (AAL06700)	68
KLBMP9127_orf63	650	Unknown	NcsE4 (AAM78014)	55
KLBMP9127_orf64	290	Unknown	SgcE3 (AAL06702)	60
KLBMP9127_orf65	329	Unknown	NcsE2 (AAM78016)	63
KLBMP9127_orf66	313	Unknown	Ncs56 (AAM78025)	65

Table S22. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Planobispora siamensis strain* NBRC 107568.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
NBRC107568_orf1	**357**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**40**
NBRC107568_orf2	394	Oxidoreductase	SgcL (AAL06685)	70
NBRC107568_orf3	91	Type II PCP	SgcC2 (AAL06679)	47
NBRC107568_orf4	337	Acyl-CoA synthetase/P-450 monooxygenase	KedU28 (AFV52184)	53
NBRC107568_orf5	136	Unknown	Ncs16 (AAM77985)	53
NBRC107568_orf6	343	dNTP-glucose synthase	SgcA1 (AAL06657)	61
NBRC107568_orf7	447	Amino transferase	SgcA4 (AAL06659)	28
NBRC107568_orf8	448	NDP glucose dehydrogenase	AcmA2 (ATV95598)	70
NBRC107568_orf9	418	Kynureninase	kynU (A7GPY3.1)	30
NBRC107568_orf10	266	Tryptophan 2,3-dioxygenase	kynA (A9B4J6.1)	51
NBRC107568_orf11	500	CoA ligase	NcsB2 (AAM77987)	27
NBRC107568_orf12	241	Probable N-octanoylanthranilate hydrolase AqdA1	AqdA1 (A0A0G3FWY4.1)	34
NBRC107568_orf13	412	Acyl-CoA N-acyltransferase	KedN4 (AFV52164)	32
NBRC107568_orf14	261	Hydrolase	SgcI (AAL06675)	47
NBRC107568_orf15	444	C-domain type II peptide synthetase	SgcC5 (AAL06678)	45
NBRC107568_orf16	468	FAD-binding monooxygenase	SgcD2 (AAL06669)	53
NBRC107568_orf17	250	Oxidoreductase	SgcN (AAL06687)	32
NBRC107568_orf18	391	O-acyltransferase	cyaA4 (AGO97197.1)	43
NBRC107568_orf19	535	Transmembrane efflux protein	SgcB (AAL06672)	51
NBRC107568_orf20	417	P-450 hydroxylase	NcsB3 (AAM77997)	48
NBRC107568_orf21	324	dNDP-hexose 4, 6-dehydratase	NcsC1 (AAM77990)	33
NBRC107568_orf22	432	Glycosyl transferase	SgcA6 (AAL06670)	49
NBRC107568_orf23	475	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	47
NBRC107568_orf24	504	Methyltransferase type 11	spoT6 (ABP55177)	44
NBRC107568_orf25	484	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	30
NBRC107568_orf26	353	C-methyltransferase	SgcA3 (AAL06661)	39
NBRC107568_orf27	147	transport protein	CynR5 (AGO97175.1)	33
NBRC107568_orf28	299	Dioxygenase swnH1	swnH1(E9F8L9.1)	35
NBRC107568_orf29	384	3-O-Acyltransferase	SgcD6 (AAL06667)	34
NBRC107568_orf30	263	Type II thioesterase	KedU43 (AFV52205)	38
NBRC107568_orf31	385	Enoyl reductase	KedU32 (AFV52194)	31
NBRC107568_orf32	633	Radical SAM C-methyltransferase	KedN5 (AFV52163)	31
NBRC107568_orf33	550	A-domain type II peptide synthetase	SgcC1 (AAL06681)	33
NBRC107568_orf34	267	Arylamine N-acetyltransferase 2	Nat2 (P50295.1)	28
NBRC107568_orf35	511	A-domain type II peptide synthetase	SgcC1 (AAL06681)	28
NBRC107568_orf36	432	1,2-phenylacetyl-CoA epoxidase, subunit A	paaA (P76077.1)	61
NBRC107568_orf37	243	1,2-phenylacetyl-CoA epoxidase, subunit C	paaC (P76079.1)	47
NBRC107568_orf38	154	Putative 1,2-phenylacetyl-CoA epoxidase, subunit D	paaD (P76080.2)	43
NBRC107568_orf39	384	1,2-phenylacetyl-CoA epoxidase, subunit E	PaaE(P76081.1)	38
NBRC107568_orf40	726	Formate dehydrogenase subunit alpha	fdhA (P61159.2)	23

NBRC107568_orf41	370	Amino transferase	SgcA4 (AAL06659)	31
NBRC107568_orf42	261	N-methyl transferase	SgcA5 (AAL06660)	45
NBRC107568_orf43	411	Oxidoreductase	SgcL (AAL06685)	28
NBRC107568_orf44	506	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	77
NBRC107568_orf45	567	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	71
NBRC107568_orf46	1030	A-domain type II peptide synthetase	SgcC1 (AAL06681)	41
NBRC107568_orf47	254	Type I PKS: KS, AT , (ACP), DH, KR, TD	NcsE (AAM78012)	35
NBRC107568_orf48	217	Phenazine biosynthesis protein PhzD1	PhzD1(P0DPB9.1)	60
NBRC107568_orf49	639	Anthranilate synthase II	SgcD1 (AAL06663)	32
NBRC107568_orf50	395	FAD-dependent oxidoreductase	dynE13(ACB47064)	33
NBRC107568_orf51	617	Asparagine synthetase [glutamine-hydrolyzing] 3	asnO(O05272.3)	44
NBRC107568_orf52	453	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	29
NBRC107568_orf53	351	epoxide hydrolase-like protein	Kedorf-2 (AFV52129)	30
NBRC107568_orf54	187	Unknown	SgcS (AAL06705)	72
NBRC107568_orf55	159	Unknown	SgcT (AAL06706)	45
NBRC107568_orf56	241	hypothetical protein	Cya-4 (AGO97186)	27
NBRC107568_orf57	252	4'-phosphopantetheinyl transferase HetI	HetI (P37695.2)	34
NBRC107568_orf58	101	Nipped-B-like protein B [Danio rerio]	nipblb (F1QBY1.1)	27
NBRC107568_orf59	115	Exodeoxyribonuclease 7 large subunit	xseA (Q5F8V5.1)	32
NBRC107568_orf60	250	etracycline repressor protein class A from transposon 1721	tetR (P03038)	28
NBRC107568_orf61	224	Putative acetyltransferase OgpAT	OgpAT (Q2CEE2.1)	35
NBRC107568_orf62	170	Aminoglycoside N(6')-acetyltransferase type 1	aacA7 (P50858.1)	31
NBRC107568_orf63	551	Methylmalonyl-CoA decarboxylase	mdporf-2 (ABY66032.1)	75
NBRC107568_orf64	90	Threonine--tRNA ligase	thrS (B9JWF4.1)	30
NBRC107568_orf65	136	Inner membrane protein YmfA	YmfA (P75962.2)	24
NBRC107568_orf66	328	Bialaphos biosynthetic pathway regulatory protein	brpA(Q01108.1)	30
NBRC107568_orf67	327	Isoleucine--tRNA ligase	ileS (B0UV12.1)	26
NBRC107568_orf68	267	Unknown	SgcE11 (AAL06691)	68
NBRC107568_orf69	384	Unknown	SgcM (AAL06686)	48
NBRC107568_orf70	550	FDA-dependent oxidoreductase	NcsE9 (AAM78005)	81
NBRC107568_orf71	154	Unknown	SgcE8 (AAL06694)	68
NBRC107568_orf72	242	RNA polymerase sigma-E factor	sigE (P38133.2)	41
NBRC107568_orf73	285	[Acyl-carrier-protein] phosphodiesterase	PptH(I6YEE1.1)	56
NBRC107568_orf74	256	AraC family transcriptional regulator	SgcR2 (AAL06696)	49
NBRC107568_orf75	424	Cytochrome P450	SgcE7 (AAL06697)	67
NBRC107568_orf76	67	Lysylphosphatidylglycerol biosynthesis bifunctional protein	LysX (A1UHB3.1)	34
NBRC107568_orf77	186	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	60
NBRC107568_orf78	142	Thioesterase	NcsE10 (AAM78011)	82
NBRC107568_orf79	**1948**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**65**
NBRC107568_orf80	323	Unknown	NcsE5 (AAM78013)	70
NBRC107568_orf81	660	Unknown	SgcE4 (AAL06701)	57
NBRC107568_orf82	298	Unknown	NcsE3 (AAM78015)	56
NBRC107568_orf83	331	Unknown	NcsE2 (AAM78016)	64

Table S23. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces* sp. SM13.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
SM13_orf1	537	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	80
SM13_orf2	534	Transmembrane efflux protein	SgcB (AAL06672)	46
SM13_orf3	147	Apo-protein	NcsA (AAM77994)	42
SM13_orf4	478	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	26
SM13_orf5	510	Methyltransferase type 11	spoT6 (ABP55177)	49
SM13_orf6	389	Epoxide hydrolase	SgcF (AAL06662)	69
SM13_orf7	469	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	49
SM13_orf8	69	Acetate kinase	ackA(A8AZH2.1)	39
SM13_orf9	459	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	82
SM13_orf10	211	Anthranilate synthase II	SgcD1 (AAL06663)	74
SM13_orf11	493	2-Amino-4-deoxychorismate synthase	SgcD (AAL06664)	71
SM13_orf12	478	Phenylacetyl-CoA ligase	SgcD5 (AAL06665)	78
SM13_orf13	223	2-Amino-4-deoxychorismate dehydrogenase	SgcG (AAL06666)	86
SM13_orf14	412	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	71
SM13_orf15	335	O-methyltransferase	SgcD4 (AAL06683)	73
SM13_orf16	700	Antibiotic transporter	SgcB4 (AAL06682)	67
SM13_orf17	262	Hydrolase	SgcI (AAL06675)	46
SM13_orf18	432	3-O-Acyltransferase	SgcD6 (AAL06667)	40
SM13_orf19	605	A-domain type II peptide synthetase	SgcC1 (AAL06681)	31
SM13_orf20	494	C-domain type II peptide synthetase	SgcC5 (AAL06678)	43
SM13_orf21	411	Oxidoreductase	SgcL (AAL06685)	29
SM13_orf22	416	P-450 hydroxylase	NcsB3 (AAM77997)	53
SM13_orf23	448	FAD-binding monooxygenase	SgcD2 (AAL06669)	58
SM13_orf24	140	Unknown	SgcJ (AAL06676)	58
SM13_orf25	393	Oxidoreductase	SgcL (AAL06685)	67
SM13_orf26	526	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	74
SM13_orf27	553	Acetolactate synthase isozyme 3 large subunit	ilvI(P40811.3)	27
SM13_orf28	332	O-methyltransferase	SgcD4 (AAL06683)	27
SM13_orf29	81	Heat-inducible transcription repressor	HrcA (Q3B2T3.1)	65
SM13_orf30	**298**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**38**
SM13_orf31	265	Unknown	SgcE11 (AAL06691)	70
SM13_orf32	343	Unknown	SgcM (AAL06686)	56
SM13_orf33	555	Oxidoreductase	SgcE9 (AAL06693)	83
SM13_orf34	195	Unknown	NcsE8 (AAM78006)	74
SM13_orf35	348	Regulatory protein	SgcR1 (AAL06695)	60
SM13_orf36	260	AraC family, transcriptional regulator	NcsR5 (AAM78008)	67
SM13_orf37	447	P-450 hydroxylase	NcsE7 (AAM78009)	78
SM13_orf38	182	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	72
SM13_orf39	152	Thioesterase	NcsE10 (AAM78011)	85
SM13_orf40	**1958**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**75**

SM13_orf41	351	Unknown	NcsE5 (AAM78013)	76
SM13_orf42	636	Unknown	NcsE4 (AAM78014)	78
SM13_orf43	307	Unknown	NcsE3 (AAM78015)	73
SM13_orf44	325	Unknown	SgcE2 (AAL06703)	66
SM13_orf45	147	Transcription regulator	NcsE1 (AAM78017)	71
SM13_orf46	403	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	58
SM13_orf47	216	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	50
SM13_orf48	314	dNDP-hexose dehydratase	NcsC2 (AAM78021)	55
SM13_orf49	208	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	61
SM13_orf50	293	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	62
SM13_orf51	461	Argininosuccinate lyase	argH (B1X082.1)	27
SM13_orf52	257	Unknown	Ncs55 (AAM78024)	69
SM13_orf53	367	Probable phosphoenolpyruvate synthase	ppsA (O29548.1)	23
SM13_orf54	634	Kynurenine 3-monooxygenase	kmo (A5IG23.1)	44
SM13_orf55	355	Methionine synthase	(Q975N4.1)	23
SM13_orf56	99	Uncharacterized transporter C4B3.13	C4B3.13 (Q9USK3.1)	31
SM13_orf57	114	Tyrosine--tRNA ligase	tyrS (Q7TUT0.2)	32

Table S24. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Sphaerisporangium siamense strain* NBRC 107570.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
NBRC107570_orf1	276	N-methyl transferase	SgcA5 (AAL06660)	50
NBRC107570_orf2	382	Amino transferase	SgcA4 (AAL06659)	28
NBRC107570_orf3	915	Imidazole glycerol phosphate synthase subunit HisF	HisF(A1AV74.1)	25
NBRC107570_orf4	234	Fructose-1-phosphate phosphatase YqaB	YqaB (P77475.1)	33
NBRC107570_orf5	539	Transmembrane efflux protein	SgcB (AAL06672)	56
NBRC107570_orf6	416	P-450 hydroxylase	NcsB3 (AAM77997)	47
NBRC107570_orf7	**290**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**40**
NBRC107570_orf8	267	Unknown	SgcE11 (AAL06691)	67
NBRC107570_orf9	367	Unknown	SgcM (AAL06686)	49
NBRC107570_orf10	551	Oxidoreductase	SgcE9 (AAL06693)	80
NBRC107570_orf11	176	Unknown	SgcE8 (AAL06694)	70
NBRC107570_orf12	256	AraC family transcriptional regulator	SgcR2 (AAL06696)	55
NBRC107570_orf13	464	Cytochrome P450	SgcE7 (AAL06697)	63
NBRC107570_orf14	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	57
NBRC107570_orf15	142	Thioesterase	NcsE10 (AAM78011)	81
NBRC107570_orf16	**1930**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**64**
NBRC107570_orf17	330	Unknown	NcsE5 (AAM78013)	70
NBRC107570_orf18	638	Unknown	SgcE4 (AAL06701)	58
NBRC107570_orf19	299	Unknown	SgcE3 (AAL06702)	57
NBRC107570_orf20	330	Unknown	SgcE2 (AAL06703)	66
NBRC107570_orf21	77	acetyl-CoA carboxylase	AcmU19 (ATV95633)	43
NBRC107570_orf22	231	Alkylhydroperoxidase	SgcO (WP_010056303)	50
NBRC107570_orf23	131	Uncharacterized protein Mb0035	Mb0035(P64674.1)	32
NBRC107570_orf24	185	Alkylhydroperoxidase	SgcO (WP_010056303)	54
NBRC107570_orf25	187	Unknown	SgcS (AAL06705)	67

Table S25. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Sphaerisporangium* sp. H8589.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
H8589_orf1	425	RNA polymerase sigma factor	AcmU22 (ATV95653)	50
H8589_orf2	424	GTP pyrophosphokinase	relA (P52560.1)	50
H8589_orf3	109	Putative DNA-binding protein YizB	YizB (C0H3Y4.1)	36
H8589_orf4	382	Putative glutamate--cysteine ligase 2-1	Q5YW64.1	48
H8589_orf5	522	FAD-dependent oxidoreductase domain-containing protein 2	Foxred2 (Q3USW5.1)	35
H8589_orf6	187	Unknown	SgcS (AAL06705)	68
H8589_orf7	186	Alkylhydroperoxidase	SgcO (WP_010056303)	55
H8589_orf8	93	Sulfur carrier protein FdhD	FdhD (Q7WBA1.1)	29
H8589_orf9	130	Uncharacterized protein Mb0035	Mb0035 (P64674.1)	30
H8589_orf10	230	Alkylhydroperoxidase	SgcO (WP_010056303)	51
H8589_orf11	73	Semaphorin-4D	Sema4d (O09126.2)	33
H8589_orf12	324	Unknown	SgcE2 (AAL06703)	65
H8589_orf13	327	Unknown	SgcE3 (AAL06702)	55
H8589_orf14	652	Unknown	SgcE4 (AAL06701)	59
H8589_orf15	331	Unknown	SgcE5 (AAL06700)	70
H8589_orf16	**1915**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**65**
H8589_orf17	142	Thioesterase	NcsE10 (AAM78011)	81
H8589_orf18	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	58
H8589_orf19	463	Cytochrome P450	SgcE7 (AAL06697)	64
H8589_orf20	253	AraC family transcriptional regulator	SgcR2 (AAL06696)	52
H8589_orf21	176	Unknown	NcsE8 (AAM78006)	68
H8589_orf22	551	Oxidoreductase	SgcE9 (AAL06693)	79
H8589_orf23	369	Unknown	Ncs14 (AAM77983)	51
H8589_orf24	267	Unknown	SgcE11 (AAL06691)	64
H8589_orf25	**324**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
H8589_orf26	540	Transmembrane efflux protein	SgcB (AAL06672)	50
H8589_orf27	109	DGPFAETKE family protein	Spoorf12 (ABP55159)	31

Table S26. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces bungoensis* DSM 41781.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
DSM41781_orf1	615	Asparagine synthetase	asnB (P54420.2)	25
DSM41781_orf2	353	Putative isomerase YraM	YraM (O07931.2)	27
DSM41781_orf3	374	Acyl-CoA dehydrogenase	KedU41 (AFV52203)	32
DSM41781_orf4	472	Allantoinase	allB (Q1J391.1)	36
DSM41781_orf5	608	FAD-dependent oxidoreductase	tnmP(AME18017)	37
DSM41781_orf6	110	Macrophage metalloelastase	MMP12 (P39900.1)	29
DSM41781_orf7	61	Bifunctional purine biosynthesis protein	PurH (A9WEK8.1)	38
DSM41781_orf8	325	R2-like ligand binding oxidase	A4F7B2.1	29
DSM41781_orf9	470	Macrolide efflux protein A	mefA (P86889.1)	26
DSM41781_orf10	338	Cytoplasmic tRNA 2-thiolation protein 1	B4NN33.1	31
DSM41781_orf11	260	Uncharacterized glycosyltransferase MJ1057	MJ1057 (Q58457.2)	20
DSM41781_orf12	378	Type I PKS: KS, AT , KR, DH, ACP	NcsB (AAM77986)	34
DSM41781_orf13	456	CoA ligase	NcsB2 (AAM77987)	27
DSM41781_orf14	567	Acetolactate synthase large subunit	ilvI (O85293.1)	25
DSM41781_orf15	231	GTPase Obg	obg (A9IFF9.1)	31
DSM41781_orf16	433	Gamma-aminobutyraldehyde dehydrogenase	patD (A4WAR9.1)	33
DSM41781_orf17	709	Unknown	KedU42 (AFV52204.1)	34
DSM41781_orf18	370	dNDP-hexose 4, 6-dehydratase	NcsC1 (AAM77990)	34
DSM41781_orf19	307	Mycothiol acetyltransferase	mshD (D6ZEJ5.1)	31
DSM41781_orf20	416	Uncharacterized protein y4rH	y4rH (P55641.1)	32
DSM41781_orf21	422	Uncharacterized protein y4rH	y4rH (P55641.1)	29
DSM41781_orf22	219	Serine acetyltransferase	cysE (Q06750.1)	42
DSM41781_orf23	320	O-acetylserine sulfhydrylase	cysK (P0A535.1)	50
DSM41781_orf24	410	L-arginine-specific L-amino acid ligase	rizA (B5UAT8.1)	25
DSM41781_orf25	492	Transmembrane efflux protein	SgcB (AAL06672)	30
DSM41781_orf26	429	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	34
DSM41781_orf27	343	3-hydroxybenzoate synthase	O30478.1	46
DSM41781_orf28	494	A-domain type II peptide synthetase	SgcC1 (AAL06681)	29
DSM41781_orf29	537	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	79
DSM41781_orf30	576	Transmembrane efflux protein	SgcB (AAL06672)	46
DSM41781_orf31	151	Apo-protein	NcsA (AAM77994)	42
DSM41781_orf32	461	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	30
DSM41781_orf33	512	Methyltransferase type 11	spoT6 (ABP55177)	49
DSM41781_orf34	389	Epoxide hydrolase	SgcF (AAL06662)	68
DSM41781_orf35	468	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	52
DSM41781_orf36	54	Type-2 serine--tRNA ligase	serS2 (Q46AN5.1)	42
DSM41781_orf37	77	CTP synthase	pyrG (Q11S24.1)	40
DSM41781_orf38	435	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	82
DSM41781_orf39	217	Anthranilate synthase II	SgcD1 (AAL06663)	77
DSM41781_orf40	492	2-Amino-4-deoxychorismate synthase	SgcD (AAL06664)	71

DSM41781_orf41	480	Phenylacetyl-CoA ligase	SgcD5 (AAL06665)	74
DSM41781_orf42	223	2-Amino-4-deoxychorismate dehydrogenase	SgcG (AAL06666)	85
DSM41781_orf43	413	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	69
DSM41781_orf44	335	O-methyltransferase	SgcD4 (AAL06683)	74
DSM41781_orf45	701	Antibiotic transporter	SgcB4 (AAL06682)	67
DSM41781_orf46	262	Hydrolase	SgcI (AAL06675)	46
DSM41781_orf47	453	3-O-Acyltransferase	SgcD6 (AAL06667)	39
DSM41781_orf48	603	A-domain type II peptide synthetase	SgcC1 (AAL06681)	30
DSM41781_orf49	69	Unknown	Spoorf19 (ABP55170)	50
DSM41781_orf50	456	C-domain type II peptide synthetase	SgcC5 (AAL06678)	44
DSM41781_orf51	410	Oxidoreductase	SgcL (AAL06685)	30
DSM41781_orf52	416	P-450 hydroxylase	NcsB3 (AAM77997)	52
DSM41781_orf53	448	FAD-binding monooxygenase	SgcD2 (AAL06669)	58
DSM41781_orf54	141	Unknown	SgcJ (AAL06676)	59
DSM41781_orf55	393	Oxidoreductase	SgcL (AAL06685)	67
DSM41781_orf56	526	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	76
DSM41781_orf57	555	Sulfoacetaldehyde acetyltransferase	xsc (Q93PS3.1)	27
DSM41781_orf58	332	O-methyl transferase	NcsB1 (AAM77984)	28
DSM41781_orf59	96	Coiled-coil and C2 domain-containing protein 1-like	Q29M42.2	38
DSM41781_orf60	**298**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**38**
DSM41781_orf61	269	Unknown	SgcE11 (AAL06691)	73
DSM41781_orf62	346	Unknown	SgcM (AAL06686)	56
DSM41781_orf63	555	Oxidoreductase	SgcE9 (AAL06693)	84
DSM41781_orf64	192	Unknown	NcsE8 (AAM78006)	75
DSM41781_orf65	358	StrR-like transcriptional regulator	NcsR6 (AAM78007)	62
DSM41781_orf66	265	AraC family, transcriptional regulator	NcsR5 (AAM78008)	68
DSM41781_orf67	440	P-450 hydroxylase	NcsE7 (AAM78009)	78
DSM41781_orf68	181	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	73
DSM41781_orf69	152	Thioesterase	NcsE10 (AAM78011)	85
DSM41781_orf70	**1959**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**74**
DSM41781_orf71	372	Unknown	NcsE5 (AAM78013)	76
DSM41781_orf72	616	Unknown	NcsE4 (AAM78014)	77
DSM41781_orf73	308	Unknown	NcsE3 (AAM78015)	74
DSM41781_orf74	325	Unknown	SgcE2 (AAL06703)	68
DSM41781_orf75	147	Transcription regulator	NcsE1 (AAM78017)	71
DSM41781_orf76	401	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	62
DSM41781_orf77	100	NAD kinase	nadK(Q1ISV1.1)	36
DSM41781_orf78	215	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	49
DSM41781_orf79	260	Oxidoreductase	SgcN (AAL06687)	32
DSM41781_orf80	241	Phosphoglycolate phosphatase	Q3SGR5.1	22
DSM41781_orf81	205	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	48
DSM41781_orf82	317	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	57
DSM41781_orf83	189	Breakpoint cluster region protein	BCR (P11274.2)	27

Table S27. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces lateritius* Z1-26.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
Z1-26_orf1	285	Unknown	Ncs55 (AAM78024)	70
Z1-26_orf2	217	MerR family transcriptional regulator	Spoorf2 (ABP55136)	35
Z1-26_orf3	507	Adenine deaminase	ade (Q92AR6.1)	28
Z1-26_orf4	289	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	60
Z1-26_orf5	209	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	68
Z1-26_orf6	321	dNDP-hexose dehydratase	NcsC2 (AAM78021)	57
Z1-26_orf7	214	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	51
Z1-26_orf8	403	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	60
Z1-26_orf9	147	Transcription regulator	NcsE1 (AAM78017)	73
Z1-26_orf10	325	Unknown	SgcE2 (AAL06703)	71
Z1-26_orf11	307	Unknown	NcsE3 (AAM78015)	75
Z1-26_orf12	616	Unknown	NcsE4 (AAM78014)	80
Z1-26_orf13	392	Unknown	NcsE5 (AAM78013)	78
Z1-26_orf14	**1960**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**76**
Z1-26_orf15	152	Thioesterase	NcsE10 (AAM78011)	82
Z1-26_orf16	151	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	75
Z1-26_orf17	423	P-450 hydroxylase	NcsE7 (AAM78009)	78
Z1-26_orf18	260	AraC family, transcriptional regulator	NcsR5 (AAM78008)	66
Z1-26_orf19	352	Regulatory protein	SgcR1 (AAL06695)	63
Z1-26_orf20	195	Unknown	NcsE8 (AAM78006)	76
Z1-26_orf21	555	Oxidoreductase	SgcE9 (AAL06693)	85
Z1-26_orf22	346	Unknown	SgcM (AAL06686)	56
Z1-26_orf23	269	Unknown	SgcE11 (AAL06691)	73
Z1-26_orf24	**298**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**38**
Z1-26_orf25	87	E3 ubiquitin-protein ligase RNF126	RNF126(Q91YL2.1)	37
Z1-26_orf26	332	O-methyltransferase	SgcD4 (AAL06683)	27
Z1-26_orf27	549	Acetolactate synthase isozyme 3 large subunit	ilvI (P40811.3)	26
Z1-26_orf28	526	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	74
Z1-26_orf29	393	Oxidoreductase	SgcL (AAL06685)	68
Z1-26_orf30	142	Unknown	SgcJ (AAL06676)	60
Z1-26_orf31	448	FAD-binding monooxygenase	SgcD2 (AAL06669)	58
Z1-26_orf32	172	HTH-type transcriptional regulator PapX	PapX (P42193.1)	26
Z1-26_orf33	440	P-450 hydroxylase	NcsB3 (AAM77997)	51
Z1-26_orf34	410	Oxidoreductase	SgcL (AAL06685)	30
Z1-26_orf35	457	C-domain type II peptide synthetase	SgcC5 (AAL06678)	43
Z1-26_orf36	72	Unknown	Spoorf19 (ABP55170)	48
Z1-26_orf37	614	A-domain type II peptide synthetase	SgcC1 (AAL06681)	30
Z1-26_orf38	432	3-O-Acyltransferase	SgcD6 (AAL06667)	41
Z1-26_orf39	262	Hydrolase	SgcI (AAL06675)	46
Z1-26_orf40	702	Antibiotic transporter	SgcB4 (AAL06682)	69

Z1-26_orf41	334	O-methyltransferase	SgcD4 (AAL06683)	75
Z1-26_orf42	414	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	71
Z1-26_orf43	223	2-Amino-4-deoxychorismate dehydrogenase	SgcG (AAL06666)	83
Z1-26_orf44	478	Phenylacetyl-CoA ligase	SgcD5 (AAL06665)	78
Z1-26_orf45	492	2-Amino-4-deoxychorismate synthase	SgcD (AAL06664)	72
Z1-26_orf46	215	Anthranilate synthase II	SgcD1 (AAL06663)	77
Z1-26_orf47	435	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	81
Z1-26_orf48	69	Quinolinate synthase A	nadA (Q7V7S6.1)	52
Z1-26_orf49	471	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	51
Z1-26_orf50	389	Epoxide hydrolase	NcsF2 (AAM78002)	68
Z1-26_orf51	511	Methyltransferase type 11	spoT6 (ABP55177)	51
Z1-26_orf52	461	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	33
Z1-26_orf53	148	Apo-protein	NcsA (AAM77994)	39
Z1-26_orf54	524	Transmembrane efflux protein	SgcB (AAL06672)	46
Z1-26_orf55	537	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	82

Table S28. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces* sp. CWH03.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
CWH03_orf1	254	Unknown	Ncs55 (AAM78024)	67
CWH03_orf2	605	Putative zinc finger and SCAN domain-containing protein 5D	ZSCAN5DP (P0CG00.1)	30
CWH03_orf3	317	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	58
CWH03_orf4	205	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	51
CWH03_orf5	231	Lipoyl synthase	lipA (Q9Y9E3.2)	36
CWH03_orf6	262	Methyltransferase	ucmG(AMK92579)	37
CWH03_orf7	212	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	50
CWH03_orf8	401	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	60
CWH03_orf9	147	Transcription regulator	NcsE1 (AAM78017)	72
CWH03_orf10	336	Unknown	NcsE2 (AAM78016)	67
CWH03_orf11	328	Unknown	NcsE3 (AAM78015)	73
CWH03_orf12	636	Unknown	NcsE4 (AAM78014)	78
CWH03_orf13	387	Unknown	NcsE5 (AAM78013)	79
CWH03_orf14	**1961**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**75**
CWH03_orf15	152	Thioesterase	NcsE10 (AAM78011)	82
CWH03_orf16	180	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	74
CWH03_orf17	440	P-450 hydroxylase	NcsE7 (AAM78009)	81
CWH03_orf18	255	AraC family, transcriptional regulator	NcsR5 (AAM78008)	69
CWH03_orf19	361	StrR-like transcriptional regulator	NcsR6 (AAM78007)	59
CWH03_orf20	195	Unknown	NcsE8 (AAM78006)	76
CWH03_orf21	555	Oxidoreductase	SgcE9 (AAL06693)	84
CWH03_orf22	268	Unknown	SgcE11 (AAL06691)	73
CWH03_orf23	**298**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**30**
CWH03_orf24	87	Type II PCP	SgcC2 (AAL06679)	44
CWH03_orf25	287	Dioxygenase swnH1	swnH1 (D4AU26.1)	31
CWH03_orf26	416	P-450 hydroxylase	NcsB3 (AAM77997)	49
CWH03_orf27	143	Unknown	SgcJ (AAL06676)	59
CWH03_orf28	375	Oxidoreductase	SgcL (AAL06685)	65
CWH03_orf29	528	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	76
CWH03_orf30	446	C-domain type II peptide synthetase	SgcC5 (AAL06678)	52
CWH03_orf31	541	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	78

Table S29. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces* sp. Mg1.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
Mg1_orf1	330	Hypothetical protein	KedE1 (AFV52144)	59
Mg1_orf2	121	TetR/AcrR family transcriptional regulator	WP_234358425.1	49
Mg1_orf3	406	S-adenosylmethionine synthase	metK (B1W470.1)	93
Mg1_orf4	424	Na(+), Li(+), K(+)/H(+) antiporter	mdrP (A0A1C7E424.1)	28
Mg1_orf5	70	Probable tautomerase RSp0893	RS01664 (Q8XRG1.3)	38
Mg1_orf6	287	Alpha-ketoglutarate-dependent sulfate ester dioxygenase	atsK (Q9WWU5.1)	46
Mg1_orf7	257	4-hydroxy-2-oxovalerate aldolase	bphF (O86013.1)	43
Mg1_orf8	319	Bifunctional L-3-cyanoalanine synthase/cysteine synthase D2	CYSD2(Q9SXS7.1)	49
Mg1_orf9	611	Asparagine synthetase	asnO (O05272.3)	30
Mg1_orf10	353	Putative isomerase	YraM (O07931.2)	28
Mg1_orf11	367	Acyl-CoA dehydrogenase	KedU41 (AFV52203)	33
Mg1_orf12	474	Allantoinase	allB(Q82LL4.1)	37
Mg1_orf13	612	FAD-dependent oxidoreductase	dynE13(ACB47064)	38
Mg1_orf14	102	FAD-dependent monooxygenase	WP_161692844	77
Mg1_orf15	61	unknown	WP_061928956.1	78
Mg1_orf16	329	R2-like ligand binding oxidase	MRA_0241 (A5TYV8.1)	29
Mg1_orf17	449	MFS transporter	WP_209520506.1	99
Mg1_orf18	351	unknown	WP_209520507.1	99
Mg1_orf19	264	glycosyltransferase family 2 protein	WP_123083558.1	100
Mg1_orf20	381	Type I PKS: KS, AT , KR, DH, ACP	NcsB (AAM77986)	31
Mg1_orf21	456	A-domain type II peptide synthetase	SgcC1 (AAL06681)	28
Mg1_orf22	582	Acetolactate synthase large subunit	ilvB(P42463.1)	33
Mg1_orf23	218	3-oxoacyl-ACP synthase	WP_010360138.1	45
Mg1_orf24	422	Aldehyde dehydrogenase	mdporf1 (ABY66031.1)	27
Mg1_orf25	713	Unknown	KedU42 (AFV52204.1)	34
Mg1_orf26	325	dNDP-hexose 4, 6-dehydratase	NcsC1 (AAM77990)	33
Mg1_orf27	307	GNAT family N-acetyltransferase	WP_123083552.1	100
Mg1_orf28	417	Argininosuccinate lyase 2	argH2(Q981V0.1)	35
Mg1_orf29	423	Argininosuccinate lyase 2	argH2(Q981V0.1)	27
Mg1_orf30	214	Serine acetyltransferase	cysE(P71405.2)	47
Mg1_orf31	318	O-acetylserine sulfhydrylase	cysK(P0A535.1)	51
Mg1_orf32	410	L-arginine-specific L-amino acid ligase	rizA(B5UAT8.1)	27
Mg1_orf33	497	Efflux pump transporter	NcsA1 (AAM77999)	32
Mg1_orf34	343	3-hydroxybenzoate synthase	(O30478.1)	49
Mg1_orf35	494	A-domain type II peptide synthetase	SgcC1 (AAL06681)	27
Mg1_orf36	541	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	80
Mg1_orf37	523	Transmembrane efflux protein	SgcB (AAL06672)	46
Mg1_orf38	399	aminotransferase	ycbU(P42253.3)	25
Mg1_orf39	263	Tryptophan 2,3-dioxygenase	kynA(B4UMQ6.1)	52
Mg1_orf40	514	A-domain type II peptide synthetase	SgcC1 (AAL06681)	29

Mg1_orf41	148	Apo-protein	NcsA (AAM77994)	44
Mg1_orf42	456	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	28
Mg1_orf43	515	Methyltransferase type 11	spoT6 (ABP55177)	51
Mg1_orf44	70	no	no	no
Mg1_orf45	404	Epoxide hydrolase	SgcF (AAL06662)	67
Mg1_orf46	468	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	50
Mg1_orf47	261	Hydrolase	SgcI (AAL06675)	47
Mg1_orf48	444	3-O-Acyltransferase	SgcD6 (AAL06667)	41
Mg1_orf49	303	Arylamine N-acetyltransferase 2	Nat2(P50298.1)	25
Mg1_orf50	602	A-domain type II peptide synthetase	SgcC1 (AAL06681)	30
Mg1_orf51	73	Unknown (MbtH-like protein)	AcmP7 (ATV95609)	48
Mg1_orf52	459	C-domain type II peptide synthetase	SgcC5 (AAL06678)	41
Mg1_orf53	411	Oxidoreductase	SgcL (AAL06685)	28
Mg1_orf54	415	P-450 hydroxylase	NcsB3 (AAM77997)	50
Mg1_orf55	447	FAD-binding monooxygenase	SgcD2 (AAL06669)	59
Mg1_orf56	142	Unknown	SgcJ (AAL06676)	64
Mg1_orf57	393	Oxidoreductase	SgcL (AAL06685)	68
Mg1_orf58	526	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	76
Mg1_orf59	572	acetolactate synthase	Q57725.1	31
Mg1_orf60	332	SAM-dependent methyltransferase	ucmJ (AMK92576)	38
Mg1_orf61	409	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	53
Mg1_orf62	337	O-methyl transferase	NcsB1 (AAM77984)	56
Mg1_orf63	297	acetolactate synthase	ilvB (Q57725.1)	31
Mg1_orf64	104	Unknown	OKI38084.1	99
Mg1_orf65	**279**	**Probable prephenate dehydrogenase**	**NovF(Q9L9G2.1)**	**36**
Mg1_orf66	272	Unknown	SgcE11 (AAL06691)	71
Mg1_orf67	357	Unknown	SgcM (AAL06686)	56
Mg1_orf68	555	Oxidoreductase	SgcE9 (AAL06693)	85
Mg1_orf69	199	Unknown	NcsE8 (AAM78006)	78
Mg1_orf70	374	Regulatory protein	SgcR1 (AAL06695)	57
Mg1_orf71	258	AraC family, transcriptional regulator	NcsR5 (AAM78008)	70
Mg1_orf72	423	P-450 hydroxylase	NcsE7 (AAM78009)	77
Mg1_orf73	181	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	73
Mg1_orf74	152	Thioesterase	NcsE10 (AAM78011)	84
Mg1_orf75	**1965**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**75**
Mg1_orf76	352	Unknown	NcsE5 (AAM78013)	76
Mg1_orf77	616	Unknown	NcsE4 (AAM78014)	74
Mg1_orf78	307	Unknown	NcsE3 (AAM78015)	71
Mg1_orf79	326	Unknown	SgcE2 (AAL06703)	72
Mg1_orf80	147	Transcription regulator	NcsE1 (AAM78017)	73
Mg1_orf81	401	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	63
Mg1_orf82	105	Unknown	GHD82081.1	99
Mg1_orf83	212	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	51

Mg1_orf84	315	dNDP-hexose dehydratase	NcsC2 (AAM78021)	58
Mg1_orf85	208	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	66
Mg1_orf86	314	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	62
Mg1_orf87	557	SMC-Scp complex subunit ScpB	WP_234388934	46
Mg1_orf88	287	Unknown	Ncs55 (AAM78024)	63
Mg1_orf89	60	Unknown	GHD82097.1	93
Mg1_orf90	124	transcription factor	KJY47981.1	82
Mg1_orf91	505	helicase L207/L206	Q5UQ22.2	28

Table S30. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces* sp. NE5-10.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
NE5-10_orf1	216	Anthranilate synthase II	SgcD1 (AAL06663)	73
NE5-10_orf2	338	Alcohol dehydrogenase	mdpC8 (ABY66028)	31
NE5-10_orf3	436	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	79
NE5-10_orf4	63	Histidine--tRNA ligase	hisS(A4YJ03.1)	39
NE5-10_orf5	446	3-O-Acyltransferase	SgcD6 (AAL06667)	43
NE5-10_orf6	279	Epoxide hydrolase	SgcF (AAL06662)	73
NE5-10_orf7	466	FAD-binding monooxygenase	SgcD2 (AAL06669)	61
NE5-10_orf8	139	Unknown	SgcJ (AAL06676)	57
NE5-10_orf9	502	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	27
NE5-10_orf10	108	Alanine--tRNA ligase	alaS (Q3ILF3.1)	33
NE5-10_orf11	**298**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**37**
NE5-10_orf12	269	Unknown	SgcE11 (AAL06691)	70
NE5-10_orf13	347	Unknown	SgcM (AAL06686)	55
NE5-10_orf14	555	Oxidoreductase	SgcE9 (AAL06693)	84
NE5-10_orf15	213	Unknown	SgcE8 (AAL06694)	74
NE5-10_orf16	369	StrR-like transcriptional regulator	NcsR6 (AAM78007)	57
NE5-10_orf17	255	AraC family, transcriptional regulator	NcsR5 (AAM78008)	67
NE5-10_orf18	449	P-450 hydroxylase	NcsE7 (AAM78009)	80
NE5-10_orf19	181	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	73
NE5-10_orf20	152	Transcription regulator	NcsE1 (AAM78017)	84
NE5-10_orf21	**1964**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**75**
NE5-10_orf22	361	Unknown	NcsE5 (AAM78013)	78
NE5-10_orf23	635	Unknown	NcsE4 (AAM78014)	77
NE5-10_orf24	307	Unknown	NcsE3 (AAM78015)	75
NE5-10_orf25	325	Unknown	NcsE2 (AAM78016)	66
NE5-10_orf26	147	Transcription regulator	NcsE1 (AAM78017)	72
NE5-10_orf27	404	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	62
NE5-10_orf28	62	Polyketide synthase-nonribosomal peptide synthetase	phmA (Q0V6Q6.2)	46
NE5-10_orf29	204	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	53
NE5-10_orf30	331	dNDP-hexose dehydratase	NcsC2 (AAM78021)	54
NE5-10_orf31	209	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	65
NE5-10_orf32	307	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	62
NE5-10_orf33	494	hypothetical protein	WP_241845776	80
NE5-10_orf34	221	MerR family transcriptional regulator	CyaR5 (AGO97221)	39

Table S31. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces* sp. TSRI0281.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
TSRI0281_orf1	354	Methionine synthase	metE (Q980A9.1)	25
TSRI0281_orf2	190	Pyrimidine reductase	Cyaorf7 (AGO97196)	33
TSRI0281_orf3	97	ADP-ribose glycohydrolase	ARH3 (Q28FQ6.1)	59
TSRI0281_orf4	433	Citrate/shikimate transporter	SgcK (AAL06677)	63
TSRI0281_orf5	179	Alkylhydroperoxidase	SgcO (WP_010056303)	65
TSRI0281_orf6	115	Cysteine--tRNA ligase	cysS (A5N4M6.1)	30
TSRI0281_orf7	146	Apo-protein	NcsA (AAM77994)	44
TSRI0281_orf8	554	Transmembrane efflux protein	SgcB (AAL06672)	46
TSRI0281_orf9	460	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	29
TSRI0281_orf10	515	Methyltransferase type 11	spoT6 (ABP55177)	49
TSRI0281_orf11	390	Epoxide hydrolase	SgcF (AAL06662)	67
TSRI0281_orf12	469	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	51
TSRI0281_orf13	89	Probable polyketide synthase 27	pks27 (Q54G30.1)	33
TSRI0281_orf14	446	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	83
TSRI0281_orf15	218	Anthranilate synthase II	SgcD1 (AAL06663)	77
TSRI0281_orf16	493	2-Amino-4-deoxychorismate synthase	SgcD (AAL06664)	70
TSRI0281_orf17	474	Phenylacetyl-CoA ligase	SgcD5 (AAL06665)	76
TSRI0281_orf18	254	2-Amino-4-deoxychorismate dehydrogenase	SgcG (AAL06666)	84
TSRI0281_orf19	411	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	70
TSRI0281_orf20	350	O-methyltransferase	SgcD4 (AAL06683)	74
TSRI0281_orf21	701	Antibiotic transporter	SgcB4 (AAL06682)	67
TSRI0281_orf22	260	Hydrolase	SgcI (AAL06675)	47
TSRI0281_orf23	439	3-O-Acyltransferase	SgcD6 (AAL06667)	38
TSRI0281_orf24	108	C4-dicarboxylate transport protein	dctA (C1D8S5.1)	34
TSRI0281_orf25	662	FAD-dependent oxidoreductase	dynE13(ACB47064)	36
TSRI0281_orf26	474	Allantoinase	allB (Q1J391.1)	35
TSRI0281_orf27	367	Acyl-CoA dehydrogenase	KedU41 (AFV52203)	32
TSRI0281_orf28	354	Putative isomerase YraM	YraM (O07931.2)	24
TSRI0281_orf29	606	Asparagine synthetase	asnB (P54420.2)	26
TSRI0281_orf30	313	Cysteine synthase	Q00834.1	46
TSRI0281_orf31	265	5-keto-4-deoxy-D-glucarate aldolase	garL (A8AQ14.1)	34
TSRI0281_orf32	286	Alkylsulfatase	atsK (Q6FBW1.1)	45
TSRI0281_orf33	422	Efflux pump transporter	NcsA1 (AAM77999)	29
TSRI0281_orf34	407	Argininosuccinate lyase 2	argH2 (Q981V0.1)	33
TSRI0281_orf35	427	Uncharacterized protein y4rH	y4rH (P55641.1)	26
TSRI0281_orf36	212	Serine acetyltransferase	cysE(Q06750.1)	41
TSRI0281_orf37	317	O-acetylserine sulfhydrylase	cysK (P0A535.1)	52
TSRI0281_orf38	413	L-arginine-specific L-amino acid ligase	rizA (B5UAT8.1)	25
TSRI0281_orf39	590	A-domain type II peptide synthetase	SgcC1 (AAL06681)	31
TSRI0281_orf40	457	C-domain type II peptide synthetase	SgcC5 (AAL06678)	41

TSRI0281_orf41	413	Oxidoreductase	SgcL (AAL06685)	31
TSRI0281_orf42	414	P-450 hydroxylase	NcsB3 (AAM77997)	50
TSRI0281_orf43	449	FAD-binding monooxygenase	SgcD2 (AAL06669)	60
TSRI0281_orf44	143	Unknown	SgcJ (AAL06676)	57
TSRI0281_orf45	393	Oxidoreductase	SgcL (AAL06685)	67
TSRI0281_orf46	91	Glycine cleavage system H protein	gcvH (A1RFY7.1)	34
TSRI0281_orf47	511	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	75
TSRI0281_orf48	225	50S ribosomal protein L2	rplB (Q839G1.1)	27
TSRI0281_orf49	343	Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating	NSDHL (Q3ZBE9.1)	26
TSRI0281_orf50	386	Ferredoxin--NADP reductase	Cpar_1603 (B3QPZ8.1)	32
TSRI0281_orf51	157	Serine-protein kinase RsbW	RsbW (A7Z1M9.1)	26
TSRI0281_orf52	140	Leucine--tRNA ligase	leuS (Q65VR5.1)	27
TSRI0281_orf53	311	Protein nfe2	nfe2 (Q52994.1)	30
TSRI0281_orf54	478	Uncharacterized protein Cbei_0202	Cbei_0202 (Q05627.2)	22
TSRI0281_orf55	259	Putative protein adenylyltransferase MJ1305	MJ1305 (Q58701.1)	48
TSRI0281_orf56	372	radical SAM-domain containing protein	Cyaorf36 (AGO97225.1)	24
TSRI0281_orf57	365	radical SAM-domain containing protein	Cyaorf36 (AGO97225.1)	23
TSRI0281_orf58	431	Glutamate--tRNA ligase	gltX (Q47SA6.1)	67
TSRI0281_orf59	556	Probable acetolactate synthase large subunit	ilvB (Q57725.1)	26
TSRI0281_orf60	332	O-methyl transferase	NcsB1 (AAM77984)	29
TSRI0281_orf61	60	Protein maph-9	maph-9 (Q18452.2)	50
TSRI0281_orf62	192	Alkylhydroperoxidase	SgcO (WP_010056303)	76
TSRI0281_orf63	216	Type II beta-Tyr adenylation domain protein	mdpC1 (ABY66004)	41
TSRI0281_orf64	411	Regulator	SgcR (AAL06689)	84
TSRI0281_orf65	324	Oxidase	SgcQ (AAL06690)	64
TSRI0281_orf66	256	4'-phosphopantetheinyl transferase HetI	HetI (P37695.2)	29
TSRI0281_orf67	126	Cysteine--tRNA ligase	cysS (Q2SX78.1)	37
TSRI0281_orf68	392	S-adenosylmethionine synthase	metK (B1W470.1)	87
TSRI0281_orf69	136	Unknown	Ncs13 (AAM77982)	33
TSRI0281_orf70	348	Unknown	SgcM (AAL06686)	62
TSRI0281_orf71	**328**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**42**
TSRI0281_orf72	267	Unknown	SgcE11 (AAL06691)	82
TSRI0281_orf73	156	Type II thioesterase	SgcE10 (AAL06692)	86
TSRI0281_orf74	552	Oxidoreductase	SgcE9 (AAL06693)	93
TSRI0281_orf75	185	Unknown	SgcE8 (AAL06694)	82
TSRI0281_orf76	370	Regulatory protein	SgcR1 (AAL06695)	74
TSRI0281_orf77	258	AraC family transcriptional regulator	SgcR2 (AAL06696)	72
TSRI0281_orf78	449	Cytochrome P450	SgcE7 (AAL06697)	85
TSRI0281_orf79	182	Flavin reductase	SgcE6 (AAL06698)	69
TSRI0281_orf80	154	Transcription regulator	NcsE1 (AAM78017)	53
TSRI0281_orf81	**1937**	**Enediyne polyketide synthase**	**SgcE (AAL06699)**	**82**
TSRI0281_orf82	313	Unknown	SgcE5 (AAL06700)	81
TSRI0281_orf83	642	Unknown	SgcE4 (AAL06701)	86

TSRI0281_orf84	325	Unknown	SgcE3 (AAL06702)	83
TSRI0281_orf85	327	Unknown	NcsE2 (AAM78016)	66
TSRI0281_orf86	195	HxlR family transcriptional regulator	SgcE1 (AAL06704)	80
TSRI0281_orf87	187	Unknown	SgcS (AAL06705)	83
TSRI0281_orf88	143	Unknown	SgcT (AAL06706)	68
TSRI0281_orf89	413	Transcriptional regulator	SgcR3 (AAL06707)	75
TSRI0281_orf90	201	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	43
TSRI0281_orf91	254	Methyltransferase	ucmG(AMK92579)	37
TSRI0281_orf92	224	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	41
TSRI0281_orf93	305	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	39
TSRI0281_orf94	557	Putative helicase L207/L206	alcA (Q5UQ22.2)	37
TSRI0281_orf95	133	Alcohol dehydrogenase 1	alcA (P08843.2)	37
TSRI0281_orf96	319	Unknown	Ncs56 (AAM78025)	68
TSRI0281_orf97	196	Alkylhydroperoxidase	SgcO (WP_010056303)	65
TSRI0281_orf98	133	Alcohol dehydrogenase 1	alcA (P08843.2)	36
TSRI0281_orf99	69	D-aminoacyl-tRNA deacylase	dtd (Q3ZAH5.1)	29

Table S32. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces spongiicola* 531S.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
531S_orf1	255	Unknown	Ncs55 (AAM78024)	72
531S_orf2	544	Cypemycin cysteine dehydrogenase (decarboxylating)	cypD(E5KIB9.1)	38
531S_orf3	317	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	60
531S_orf4	205	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	49
531S_orf5	231	Hydroxymethylpyrimidine/phosphomethylpyrimidine kinase	ThiD (Q9ZL00.1)	32
531S_orf6	262	Oxidoreductase	Ypml (SCL57600.1)	37
531S_orf7	213	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	51
531S_orf8	407	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	60
531S_orf9	147	Transcription regulator	NcsE1 (AAM78017)	71
531S_orf10	341	Unknown	NcsE2 (AAM78016)	67
531S_orf11	300	Unknown	NcsE3 (AAM78015)	74
531S_orf12	616	Unknown	NcsE4 (AAM78014)	78
531S_orf13	374	Unknown	NcsE5 (AAM78013)	75
531S_orf14	**1963**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**76**
531S_orf15	152	Transcription regulator	NcsE1 (AAM78017)	84
531S_orf16	181	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	73
531S_orf17	440	P-450 hydroxylase	NcsE7 (AAM78009)	80
531S_orf18	256	AraC family, transcriptional regulator	NcsR5 (AAM78008)	69
531S_orf19	366	StrR-like transcriptional regulator	NcsR6 (AAM78007)	59
531S_orf20	195	Unknown	NcsE8 (AAM78006)	74
531S_orf21	555	Oxidoreductase	SgcE9 (AAL06693)	84
531S_orf22	268	Transcription regulator	NcsE1 (AAM78017)	72
531S_orf23	**298**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**36**
531S_orf24	114	Type II PCP	SgcC2 (AAL06679)	44
531S_orf25	288	Dioxygenase swnH1	swnH1 (D4AU26.1)	31
531S_orf26	418	P-450 hydroxylase	NcsB3 (AAM77997)	49
531S_orf27	143	Unknown	SgcJ (AAL06676)	62
531S_orf28	375	Oxidoreductase	SgcL (AAL06685)	66
531S_orf29	528	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	76
531S_orf30	446	C-domain type II peptide synthetase	SgcC5 (AAL06678)	52
531S_orf31	541	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	78
531S_orf32	894	A-domain type II peptide synthetase	SgcC1 (AAL06681)	42
531S_orf33	486	FAD-binding monooxygenase	SgcD2 (AAL06669)	55
531S_orf34	412	Oxidoreductase	SgcL (AAL06685)	26
531S_orf35	808	Formate dehydrogenase subunit alpha	FdhA (P61159.2)	23
531S_orf36	447	Glycerol phosphate ABC transporter	SgcH (AAL06653)	51

Table S33. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces taklimakanensis* TRM43335.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
TRM43335_orf1	67	Tyrosine-protein phosphatase non-receptor type 1	PTPN1(P18031.1)	35
TRM43335_orf2	268	Unknown	Ncs55 (AAM78024)	66
TRM43335_orf3	311	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	65
TRM43335_orf4	208	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	65
TRM43335_orf5	325	dNDP-hexose dehydratase	NcsC2 (AAM78021)	58
TRM43335_orf6	213	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	52
TRM43335_orf7	398	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	66
TRM43335_orf8	147	Transcription regulator	NcsE1 (AAM78017)	71
TRM43335_orf9	326	Unknown	NcsE2 (AAM78016)	72
TRM43335_orf10	328	Unknown	SgcE3 (AAL06702)	66
TRM43335_orf11	635	Unknown	NcsE4 (AAM78014)	76
TRM43335_orf12	359	Unknown	NcsE5 (AAM78013)	78
TRM43335_orf13	**1964**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**76**
TRM43335_orf14	152	Transcription regulator	NcsE1 (AAM78017)	86
TRM43335_orf15	188	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	75
TRM43335_orf16	467	P-450 hydroxylase	NcsE7 (AAM78009)	78
TRM43335_orf17	258	AraC family, transcriptional regulator	NcsR5 (AAM78008)	66
TRM43335_orf18	360	Regulatory protein	SgcR1 (AAL06695)	61
TRM43335_orf19	194	Unknown	NcsE8 (AAM78006)	75
TRM43335_orf20	555	Oxidoreductase	SgcE9 (AAL06693)	84
TRM43335_orf21	348	Unknown	SgcM (AAL06686)	58
TRM43335_orf22	269	Unknown	SgcE11 (AAL06691)	71
TRM43335_orf23	**298**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
TRM43335_orf24	500	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	26
TRM43335_orf25	141	Unknown	SgcJ (AAL06676)	55
TRM43335_orf26	461	FAD-binding monooxygenase	SgcD2 (AAL06669)	59
TRM43335_orf27	386	Epoxide hydrolase	SgcF (AAL06662)	72
TRM43335_orf28	437	3-O-Acyltransferase	SgcD6 (AAL06667)	46
TRM43335_orf29	65	Triosephosphate isomerase	tpiA (Q1D8I9.1)	48
TRM43335_orf30	432	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	63

Table S34. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces viridosporus* T7A.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
T7A_orf1	271	Unknown	Ncs55 (AAM78024)	74
T7A_orf2	244	FeMo cofactor biosynthesis protein NifB	NifB (P10390.1)	33
T7A_orf3	133	Histone-lysine N-methyltransferase SETD5	SETD5 (Q5XJV7.2)	31
T7A_orf4	305	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	57
T7A_orf5	205	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	48
T7A_orf6	240	Formate-dependent phosphoribosylglycinamide formyltransferase	purT (A4Y447.1)	27
T7A_orf7	263	Oxidoreductase	tnmI(AME18007)	37
T7A_orf8	229	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	50
T7A_orf9	398	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	61
T7A_orf10	147	Transcription regulator	NcsE1 (AAM78017)	71
T7A_orf11	325	Unknown	NcsE2 (AAM78016)	67
T7A_orf12	308	Unknown	NcsE3 (AAM78015)	76
T7A_orf13	621	Unknown	NcsE4 (AAM78014)	78
T7A_orf14	354	Unknown	NcsE5 (AAM78013)	75
T7A_orf15	**1956**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**74**
T7A_orf16	152	Transcription regulator	NcsE1 (AAM78017)	85
T7A_orf17	181	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	72
T7A_orf18	440	P-450 hydroxylase	NcsE7 (AAM78009)	80
T7A_orf19	266	AraC family, transcriptional regulator	NcsR5 (AAM78008)	67
T7A_orf20	354	StrR-like transcriptional regulator	NcsR6 (AAM78007)	60
T7A_orf21	195	Unknown	NcsE8 (AAM78006)	76
T7A_orf22	555	Oxidoreductase	SgcE9 (AAL06693)	84
T7A_orf23	346	Unknown	SgcM (AAL06686)	58
T7A_orf24	269	Unknown	SgcE11 (AAL06691)	71
T7A_orf25	**288**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
T7A_orf26	500	Oxidoreductase	spoT4 (ABP55175)	29
T7A_orf27	141	Unknown	SgcJ (AAL06676)	56
T7A_orf28	463	FAD-binding monooxygenase	SgcD2 (AAL06669)	60
T7A_orf29	386	Epoxide hydrolase	SgcF (AAL06662)	72
T7A_orf30	446	3-O-Acyltransferase	SgcD6 (AAL06667)	43
T7A_orf31	62	Asparagine--tRNA ligase, cytoplasmic	nars-1 (Q19722.1)	27
T7A_orf32	436	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	78
T7A_orf33	332	Zinc-containing alcohol dehydrogenase	AcmP5 (ATV95592)	29
T7A_orf34	217	Anthranilate synthase II	SgcD1 (AAL06663)	70
T7A_orf35	491	2-Amino-4-deoxychorismate synthase	SgcD (AAL06664)	65
T7A_orf36	222	2-Amino-4-deoxychorismate dehydrogenase	SgcG (AAL06666)	80
T7A_orf37	536	Transmembrane efflux protein	SgcB (AAL06672)	48
T7A_orf38	371	Beta-lactamase domain-containing protein 2	lact-2(Q09621.1)	32
T7A_orf39	460	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	30
T7A_orf40	415	Oxidoreductase	SgcL (AAL06685)	27

T7A_orf41	448	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	33
T7A_orf42	260	Oxidoreductase	SgcN (AAL06687)	29
T7A_orf43	238	Unknown	Spoorf22 (ABP55176)	39
T7A_orf44	453	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	49
T7A_orf45	517	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	77
T7A_orf46	84	Type II PCP	SgcC2 (AAL06679)	58
T7A_orf47	471	C-domain type II peptide synthetase	SgcC5 (AAL06678)	50
T7A_orf48	119	Hypothetical protein	Spoorf16 (ABP55163)	57
T7A_orf49	277	Methyltransferase	CalE5 (AAM94788)	34
T7A_orf50	749	P-450 hydroxylase	NcsB3 (AAM77997)	53
T7A_orf51	393	Oxidoreductase	SgcL (AAL06685)	62
T7A_orf52	512	Methyltransferase type 11	spoT6 (ABP55177)	52
T7A_orf53	537	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	87
T7A_orf54	878	A-domain type II peptide synthetase	SgcC1 (AAL06681)	48
T7A_orf55	410	L-arginine-specific L-amino acid ligase	rizA (B5UAT8.1)	25
T7A_orf56	320	O-acetylserine sulfhydrylase	cysK (P0A535.1)	50
T7A_orf57	219	Serine acetyltransferase	cysE (Q06750.1)	41
T7A_orf58	443	Uncharacterized protein y4rH	y4rH (P55641.1)	28
T7A_orf59	416	Argininosuccinate lyase 2	argH2 (Q981V0.1)	33
T7A_orf60	125	Eukaryotic peptide chain release factor GTP-binding subunit	sup35 (O74718.2)	28
T7A_orf61	59	Putative NrdI-like protein	P0DC72.1	44
T7A_orf62	146	Apo-protein	NcsA (AAM77994)	43
T7A_orf63	70	DNA-directed RNA polymerase subunit beta	rpoC (B1I1M9.1)	29
T7A_orf64	609	FAD-dependent oxidoreductase	tnmP (AME18017)	38
T7A_orf65	473	Allantoinase	allB (Q1J391.1)	35
T7A_orf66	365	Acyl-CoA dehydrogenase	KedU41 (AFV52203)	35
T7A_orf67	353	Putative isomerase YraM	YraM (O07931.2)	27
T7A_orf68	612	Asparagine synthetase [glutamine-hydrolyzing] 1	asnB (P54420.2)	27
T7A_orf69	135	Cysteine synthase 3	cysl-3 (O01592.1)	42
T7A_orf70	68	Probable tautomerase SERP0934	SERP0934 (Q5HPH8.3)	36
T7A_orf71	421	Na(+), Li(+), K(+)/H(+) antiporter	mdrP (A0A1C7E424.1)	29
T7A_orf72	335	O-methyltransferase	SgcD4 (AAL06683)	58
T7A_orf73	446	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	57
T7A_orf74	479	Phenylacetyl-CoA ligase	SgcD5 (AAL06665)	75
T7A_orf75	91	Glucan 1,3-beta-glucosidase D	exgD (Q5AVZ7.1)	40
T7A_orf76	306	Uncharacterized N-acetyltransferase AF_0521	AF_0521 (O29729.1)	31
T7A_orf77	66	dTDP-glucose 4,6-dehydratase	novT (Q9L9E8.1)	39
T7A_orf78	77	FMN-dependent NADH:quinone oxidoreductase 2	azoR2 (O32224.1)	47
T7A_orf79	141	DNA mismatch repair protein MutL	MutL (B2II71.1)	54
T7A_orf80	332	Unknown	Ncs56 (AAM78025)	58
T7A_orf81	320	Oxidoreductase	SgcN (AAL06687)	51

Table S35. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Thermostaphylospora chromogena* DSM 43794.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
DSM43794_orf1	1035	Transcriptional regulator	AcmR4 (ATV95651)	32
DSM43794_orf2	59	Membrane protein insertase YidC	YidC (Q21DG0.1)	44
DSM43794_orf3	112	5-hydroxyisourate hydrolase	hiuH (Q8ZQ52.1)	35
DSM43794_orf4	575	2-isopropylmalate synthase	leuA (D2ATJ4.1)	63
DSM43794_orf5	921	Oxygen regulatory protein NreC	NreC (Q7WZY4.1)	44
DSM43794_orf6	63	Ribose-phosphate pyrophosphokinase	prs (Q8NRU9.1)	31
DSM43794_orf7	342	Unknown	Ncs56 (AAM78025)	62
DSM43794_orf8	331	Unknown	NcsE2 (AAM78016)	64
DSM43794_orf9	318	Unknown	SgcE3 (AAL06702)	58
DSM43794_orf10	647	Unknown	SgcE4 (AAL06701)	56
DSM43794_orf11	324	Unknown	NcsE5 (AAM78013)	70
DSM43794_orf12	**1892**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**62**
DSM43794_orf13	142	Transcription regulator	NcsE1 (AAM78017)	83
DSM43794_orf14	179	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	59
DSM43794_orf15	457	Cytochrome P450	SgcE7 (AAL06697)	64
DSM43794_orf16	255	AraC family transcriptional regulator	SgcR2 (AAL06696)	50
DSM43794_orf17	56	Glutamyl-tRNA reductase	hemA (B1JEP7.1)	40
DSM43794_orf18	145	RNA polymerase sigma-E factor	sigE (Q82EA9.1)	38
DSM43794_orf19	183	Unknown	SgcE8 (AAL06694)	68
DSM43794_orf20	550	FDA-dependent oxidoreductase	NcsE9 (AAM78005)	79
DSM43794_orf21	357	Unknown	SgcM (AAL06686)	48
DSM43794_orf22	267	Unknown	SgcE11 (AAL06691)	65
DSM43794_orf23	328	Bialaphos biosynthetic pathway regulatory protein	brpA (Q01108.1)	32
DSM43794_orf24	61	Basal body-orientation factor 1	bbof1 (Q08C53.2)	30
DSM43794_orf25	536	methylmalonyl-CoA carboxyltransferase	AcmU20 (ATV95634)	73
DSM43794_orf26	123	Methionyl-tRNA formyltransferase	fmt (A9B2Z9.1)	32
DSM43794_orf27	249	Putative 4'-phosphopantetheinyl transferase slr0495	slr0495(Q55185.1)	31
DSM43794_orf28	241	hypothetical protein	Cya-4 (AGO97186)	27
DSM43794_orf29	150	Unknown	SgcT (AAL06706)	45
DSM43794_orf30	181	Unknown	SgcS (AAL06705)	66
DSM43794_orf31	413	C-methyltransferase	SgcA3 (AAL06661)	32
DSM43794_orf32	329	NDP-hexose-3-ketoreductase	KedS3 (AFV52211)	49
DSM43794_orf33	151	transport protein	CynR5 (AGO97175.1)	31
DSM43794_orf34	546	Transmembrane efflux protein	SgcB (AAL06672)	50
DSM43794_orf35	739	Polysulfide reductase chain A	psrA (P31075.1)	28
DSM43794_orf36	284	HxlR family transcriptional regulator	SgcE1 (AAL06704)	35
DSM43794_orf37	368	Oxidoreductase	SgcL (AAL06685)	28
DSM43794_orf38	465	FAD-binding monooxygenase	SgcD2 (AAL06669)	54
DSM43794_orf39	452	C-domain type II peptide synthetase	SgcC5 (AAL06678)	49
DSM43794_orf40	415	P-450 hydroxylase	NcsB3 (AAM77997)	49

DSM43794_orf41	472	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	48
DSM43794_orf42	250	Oxidoreductase	SgcN (AAL06687)	33
DSM43794_orf43	451	Glycosyl transferase	SgcA6 (AAL06670)	48
DSM43794_orf44	465	NDP-hexose 2,3-dehydratase	CalS14 (AAM70359)	52
DSM43794_orf45	433	NDP glucose dehydrogenase	AcmA2 (ATV95598)	61
DSM43794_orf46	333	dNDP-hexose 4, 6-dehydratase	NcsC1 (AAM77990)	34
DSM43794_orf47	189	NUDIX domain-containing protein	AcmU1 (ATV95597)	69
DSM43794_orf48	399	Amino transferase	SgcA4 (AAL06659)	28
DSM43794_orf49	218	Unknown	Spoorf22 (ABP55176)	41
DSM43794_orf50	246	N-methyl transferase	SgcA5 (AAL06660)	43
DSM43794_orf51	436	Glycosyl transferase	SgcA6 (AAL06670)	34
DSM43794_orf52	291	dNTP-glucose synthase	SgcA1 (AAL06657)	37
DSM43794_orf53	284	Dioxygenase swnH1	swnH1 (D4AU26.1)	33
DSM43794_orf54	90	Type II PCP	SgcC2 (AAL06679)	45
DSM43794_orf55	528	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	77
DSM43794_orf56	498	A-domain type II peptide synthetase	SgcC1 (AAL06681)	51
DSM43794_orf57	519	Methyltransferase type 11	spoT6 (ABP55177)	48
DSM43794_orf58	274	Methyltransferase	CalE5 (AAM94788)	40
DSM43794_orf59	135	Unknown	Ncs16 (AAM77985)	58
DSM43794_orf60	522	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	76
DSM43794_orf61	117	Phosphopentomutase	deoB (A8FET4.1)	32
DSM43794_orf62	460	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	30
DSM43794_orf63	394	Oxidoreductase	SgcL (AAL06685)	64
DSM43794_orf64	**315**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**39**
DSM43794_orf65	180	Uncharacterized protein YyaP	YyaP (P37508.1)	33

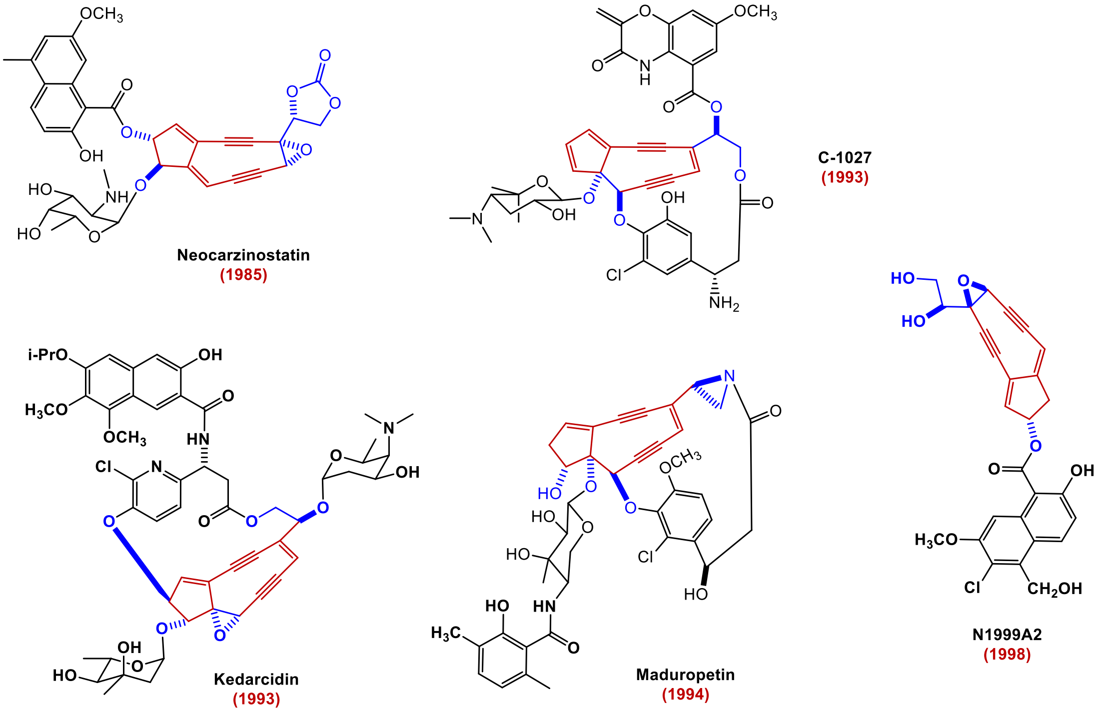
Table S36. Predicted functions of ORFs in the enediyne biosynthetic gene cluster from *Streptomyces tirandamycinicus* HNM0039.

Gene	AA a	Putative Function	Protein homologs[a]	Identity (%)
HNM0039_orf1	536	MIO-dependent tyrosine 2,3-aminomutase	SgcC4 (AAL06680)	80
HNM0039_orf2	524	Transmembrane efflux protein	SgcB (AAL06672)	48
HNM0039_orf3	148	Apo-protein	NcsA (AAM77994)	39
HNM0039_orf4	461	Flavin-dependent oxidoreductase	Ncs32 (AAM78001)	32
HNM0039_orf5	512	Methyltransferase type 11	spoT6 (ABP55177)	49
HNM0039_orf6	389	Epoxide hydrolase	SgcF (AAL06662)	69
HNM0039_orf7	469	Glycerol phosphate ABC transporter	SgcB1 (AAL06653)	51
HNM0039_orf8	94	Hypothetical protein	Kedorf-14 (AFV52117.1)	34
HNM0039_orf9	188	Uncharacterized HTH-type transcriptional regulator in lacX 3'region	P42097.1	26
HNM0039_orf10	363	N-ethylmaleimide reductase	NemA (P77258.1)	43
HNM0039_orf11	161	Kyphoscoliosis peptidase	KY (Q8NBH2.3)	25
HNM0039_orf12	71	Histidine decarboxylase	hdc (P05034.2)	47
HNM0039_orf13	435	Coenzyme F390 synthase-like protein	SgcH (AAL06673)	82
HNM0039_orf14	214	Anthranilate synthase II	SgcD1 (AAL06663)	74
HNM0039_orf15	492	2-Amino-4-deoxychorismate synthase	SgcD (AAL06664)	72
HNM0039_orf16	478	Phenylacetyl-CoA ligase	SgcD5 (AAL06665)	76
HNM0039_orf17	243	2-Amino-4-deoxychorismate dehydrogenase	SgcG (AAL06666)	84
HNM0039_orf18	414	Cytochrome P450 hydroxylase	SgcD3 (AAL06684)	73
HNM0039_orf19	334	O-methyltransferase	SgcD4 (AAL06683)	75
HNM0039_orf20	701	Antibiotic transporter	SgcB4 (AAL06682)	67
HNM0039_orf21	262	Hydrolase	SgcI (AAL06675)	48
HNM0039_orf22	431	3-O-Acyltransferase	SgcD6 (AAL06667)	41
HNM0039_orf23	590	A-domain type II peptide synthetase	SgcC1 (AAL06681)	31
HNM0039_orf24	84	Unknown	Spoorf19 (ABP55170)	46
HNM0039_orf25	456	C-domain type II peptide synthetase	SgcC5 (AAL06678)	44
HNM0039_orf26	410	Oxidoreductase	SgcL (AAL06685)	30
HNM0039_orf27	441	P-450 hydroxylase	NcsB3 (AAM77997)	50
HNM0039_orf28	448	FAD-binding monooxygenase	SgcD2 (AAL06669)	60
HNM0039_orf29	142	Unknown	SgcJ (AAL06676)	61
HNM0039_orf30	393	Oxidoreductase	SgcL (AAL06685)	67
HNM0039_orf31	526	Chlorophenol-4-monooxygenase	SgcC (AAL06674)	75
HNM0039_orf32	547	Probable acetolactate synthase large subunit	ilvB (O08353.1)	27
HNM0039_orf33	332	O-methyl transferase	NcsB1 (AAM77984)	27
HNM0039_orf34	65	Serine/threonine-protein kinase LATS1	LATS1(O95835.1)	57
HNM0039_orf35	**298**	**Probable prephenate dehydrogenase**	**NovF (Q9L9G2.1)**	**37**
HNM0039_orf36	269	Unknown	SgcE11 (AAL06691)	75
HNM0039_orf37	400	Unknown	SgcM (AAL06686)	60
HNM0039_orf38	555	Oxidoreductase	SgcE9 (AAL06693)	85
HNM0039_orf39	199	Unknown	NcsE8 (AAM78006)	76
HNM0039_orf40	389	StrR-like transcriptional regulator	NcsR6 (AAM78007)	62

HNM0039_orf41	258	AraC family, transcriptional regulator	NcsR5 (AAM78008)	67
HNM0039_orf42	440	P-450 hydroxylase	NcsE7 (AAM78009)	78
HNM0039_orf43	181	Flavin-dependent oxidoreductase	NcsE6 (AAM78010)	75
HNM0039_orf44	151	Transcription regulator	NcsE1 (AAM78017)	84
HNM0039_orf45	**1962**	**Enediyne polyketide synthase**	**NcsE (AAM78012)**	**75**
HNM0039_orf46	380	Unknown	NcsE5 (AAM78013)	73
HNM0039_orf47	636	Unknown	NcsE4 (AAM78014)	79
HNM0039_orf48	300	Unknown	NcsE3 (AAM78015)	75
HNM0039_orf49	325	Unknown	NcsE2 (AAM78016)	69
HNM0039_orf50	147	Transcription regulator	NcsE1 (AAM78017)	74
HNM0039_orf51	389	SgcR3 like transcriptional regulator	NcsR7 (AAM78019)	62
HNM0039_orf52	61	ATP synthase subunit alpha 1	atpA1 (Q07YM0.1)	36
HNM0039_orf53	206	γ-Butyrolactone receptor protein	NcsR3 (AAM78020)	52
HNM0039_orf54	261	Oxidoreductase	SgcN (AAL06687)	36
HNM0039_orf55	245	Phosphoglycolate phosphatase	Q3SGR5.1	26
HNM0039_orf56	196	γ-Butyrolactone receptor protein	NcsR2 (AAM78022)	47
HNM0039_orf57	315	γ-Butyrolactone biosynthesis enzyme	NcsR1 (AAM78023)	61
HNM0039_orf58	507	Putative ripening-related protein 5	Q8LN49.1	24
HNM0039_orf59	255	Unknown	Ncs55 (AAM78024)	72

Figure S1. Structures of known 9-membered enediynes and isolated natural products as potential aromatized products from 9-membered enediynes via Myers-Saito aromatization. The enediyne cores or aromatized cores were highlighted in red, while the hydroxyl groups and epoxides periphery to the core structure were labeled in blue color. Note that the aziridine functionality in maduropeptin and a cyanide group in cyanosporasides were also highlighted in blue color.

9-membered enediynes



Neocarzinostatin
(1985)

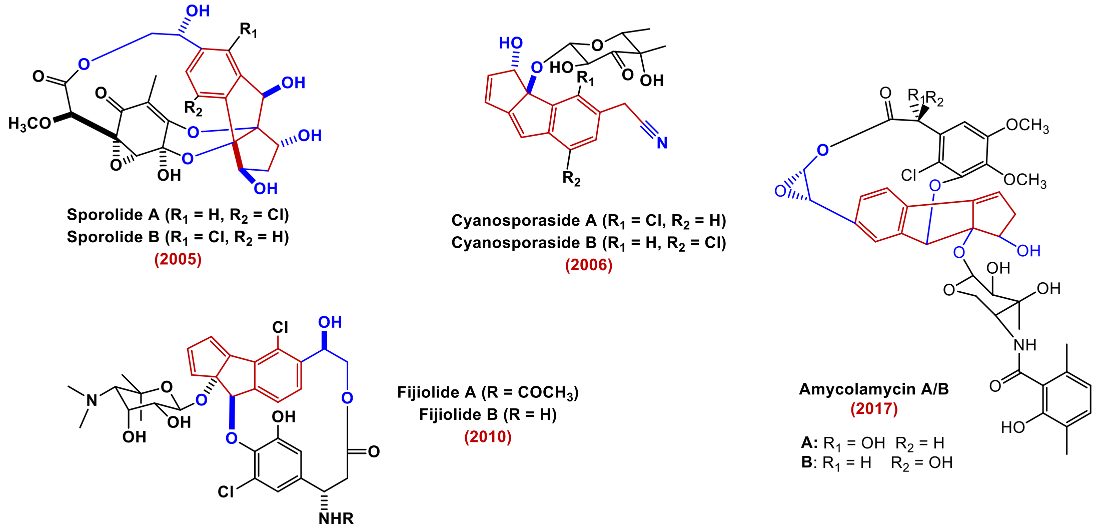
C-1027
(1993)

Kedarcidin
(1993)

Maduropetin
(1994)

N1999A2
(1998)

Compounds likely from the aromatization of 9-membered enediynes



Sporolide A (R_1 = H, R_2 = Cl)
Sporolide B (R_1 = Cl, R_2 = H)
(2005)

Cyanosporaside A (R_1 = Cl, R_2 = H)
Cyanosporaside B (R_1 = H, R_2 = Cl)
(2006)

Amycolamycin A/B
(2017)

A: R_1 = OH R_2 = H
B: R_1 = H R_2 = OH

Fijiolide A (R = $COCH_3$)
Fijiolide B (R = H)
(2010)

Figure S2. The GNN analysis of putative enediyne BGCs. They are from CB02130, CB01883, CB02400, CB02261, CB03578, and CB00455, which are co-clustered with the neocarzinostatin BGC, along with 15 known BGCs of enediyne natural products or aromatized products derived from enediynes for the biosynthesis of calicheamicin, dynemycin, esperamicin, sungeidine, tiancimycin, uncialamycin, yangpumicin, kedarcidin, maduropeptin, C-1027, amycolamycin, cyanosporaside C, cyanosporaside F, and sporolides. The putative enediyne BGC from CB02130 is named as *wls*, since the strain was originally isolated from Wuliangshan Mountin. The analysis was displayed with an *E*- value threshold of 10^{-6}. WlsPDH, WlsOrf3, and WlsORF30 (highlighted in large red rectangles) are unique to *wls* and the putative enediyne BGCs from CB01883, CB02400, CB02261, CB03578, and CB00455, which are thus tentatively named as *wls*-type BGCs.

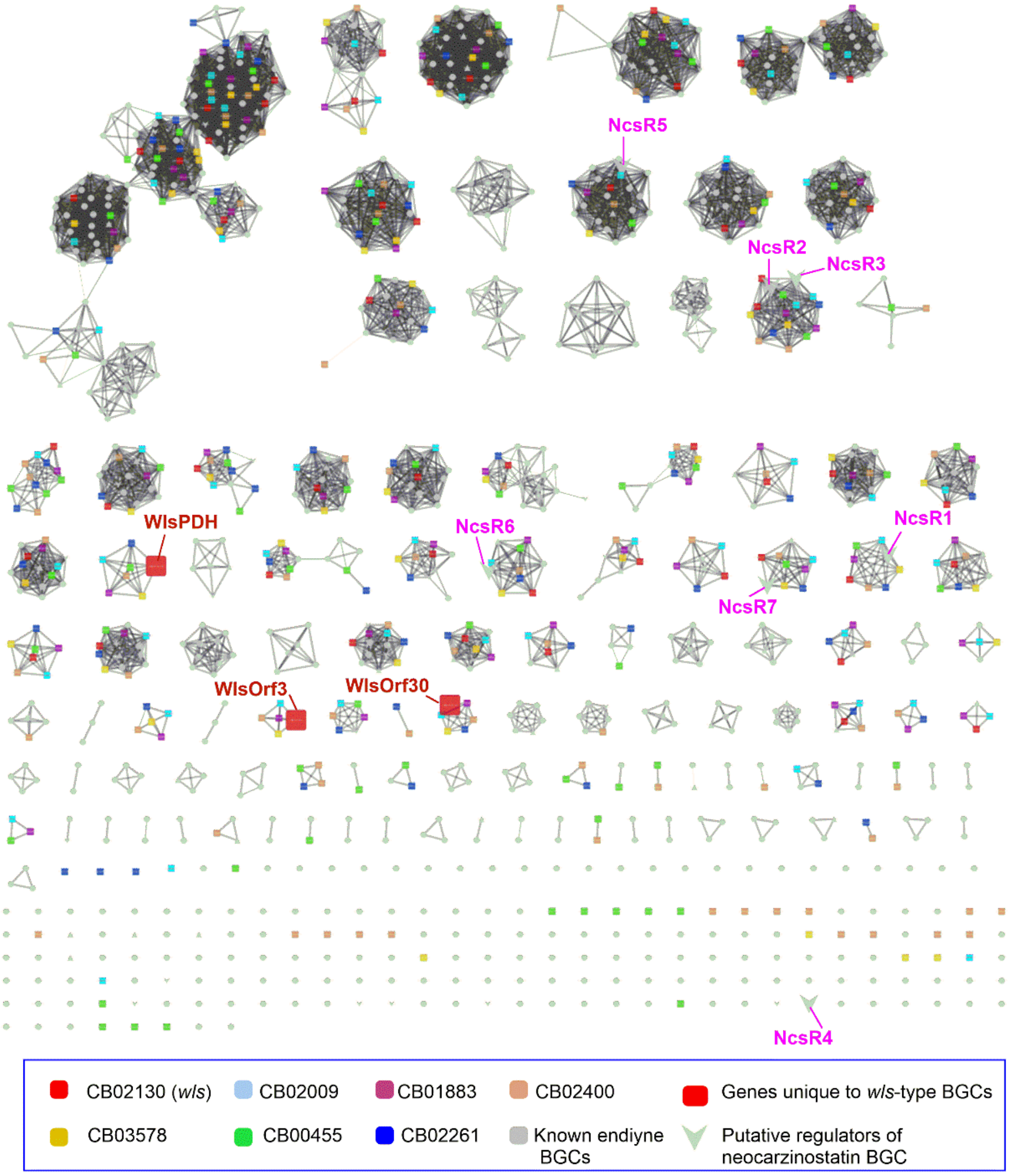


Figure S3. Production of heptaene (**1**) in *E. coli* and *S*. sp. CB02130. (A) Production of **1** from recombinant *E. coli* strains coexpressing *wls*E/E10. (B) The UV spectra of **1** from recombinant *E. coli***.** (C) The HR-APCI-LCMS spectrum of purified **1**, which was isolated from the *E. coli* strain co-expressed with *wls*E/E10 under the control of T7Lac promoter in pET28a. The appearance of a series of peaks with the expected $[M+H]^+$ molecular ion of 199.1487 suggests the presence of stereoisomers of **1** under the current chromatography condition. (D) HPLC analysis of the methanol extract of *S*. sp. CB02130 mycelium, in comparison to the standard of **1**. (E) The UV spectra of **1** from *S*. sp. CB02130.

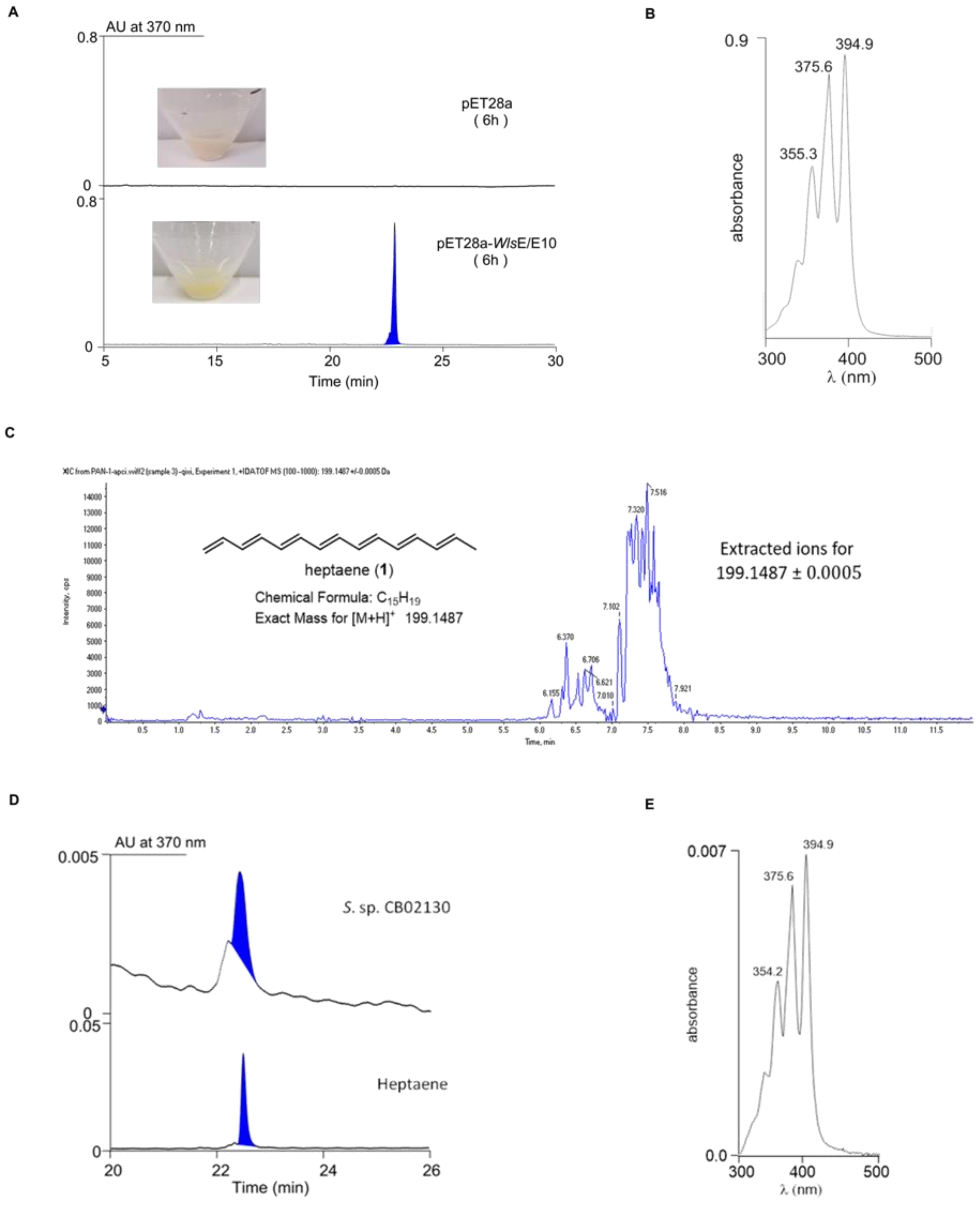
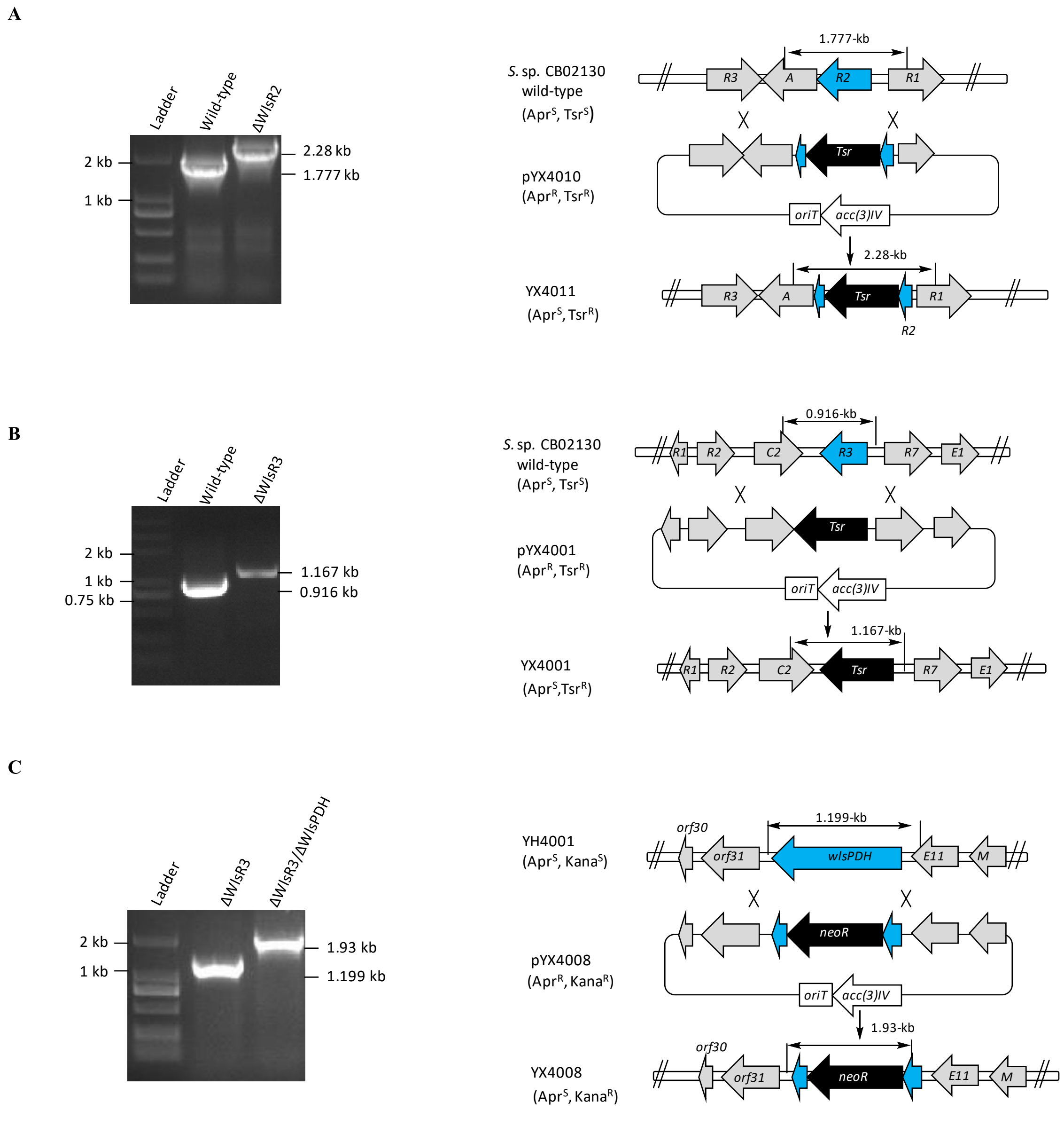


Figure S4. Confirmation of gene replacement mutants in *S*. sp. CB02130 and Δ*wlsR3* (YX4001). (A) *wls*R2 gene replacement in CB02130 leads to YX4011; (B) *wls*R3 gene replacement in CB02130 leads to YX4001; (C) *wls*PDH gene replacement in CB02130 leads to YX4008; (D) *wls*PKSE gene replacement in YX4001 leads to YX4003; (E) *wl*sorf3 gene replacement in YX4001 leads to YX4006; (F) *wlsORF30* gene replacement in YX4001 leads to YX4007; (G) *wls*PDH gene replacement in YX4001 leads to YX4009; (H) *wls*F gene replacement in YX4001 leads to YX4010; (I) *wls*C4 gene replacement in YX4001 leads to YX4005. (J) wlsorf8 gene replacement in YX4001 leads to YX4018; (K) wlsorf24 gene replacement in YX4001 leads to YX4019 ; (L) wlsL gene replacement in YX4001 leads to YX4020; (M) wlsE7 gene replacement in YX4001 leads to YX4021; (N) wlsE9 gene replacement in YX4001 leads to YX4022.



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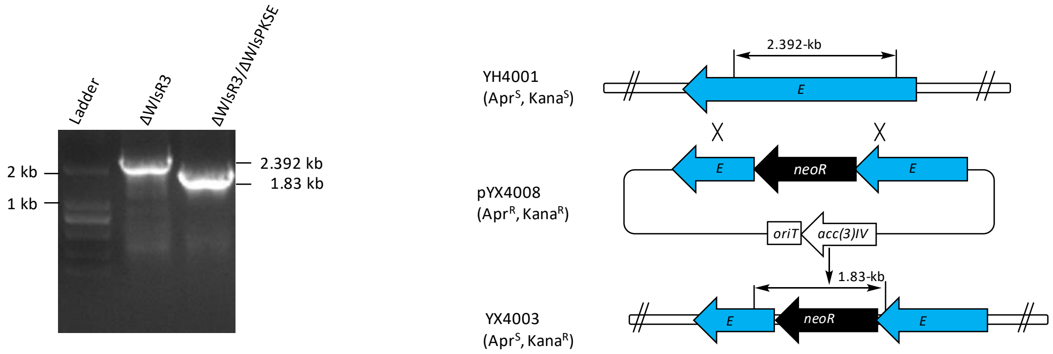
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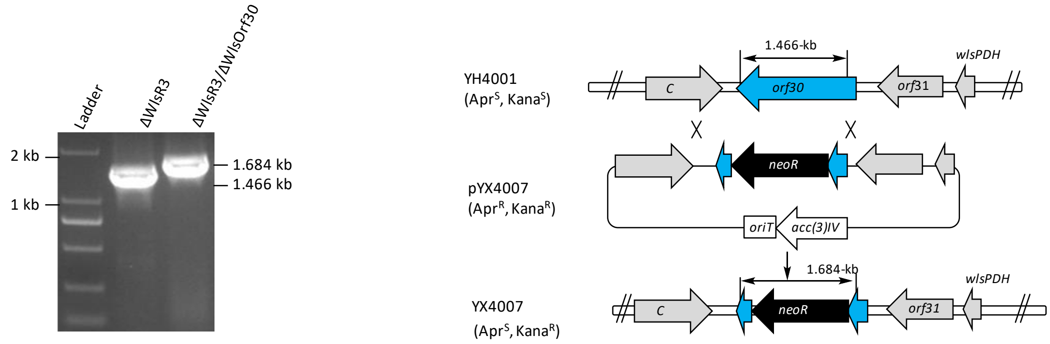
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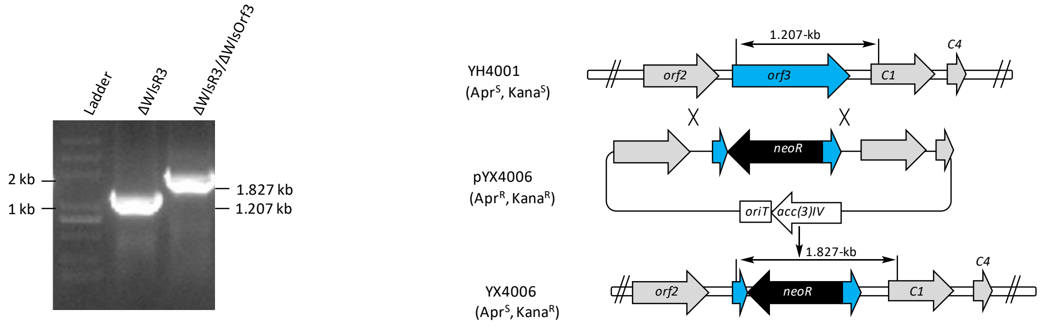
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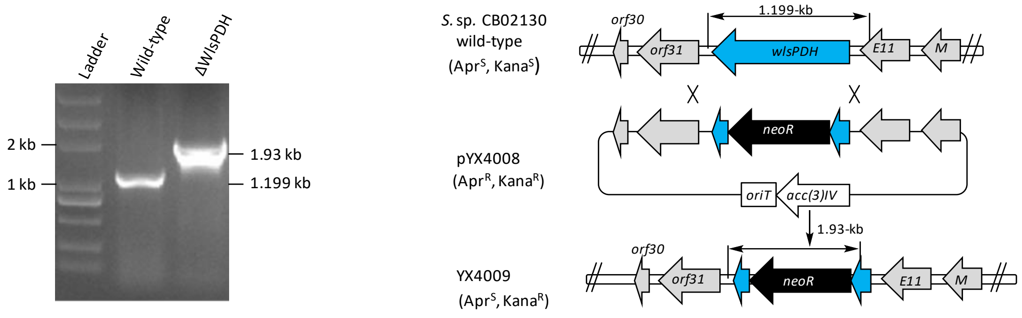
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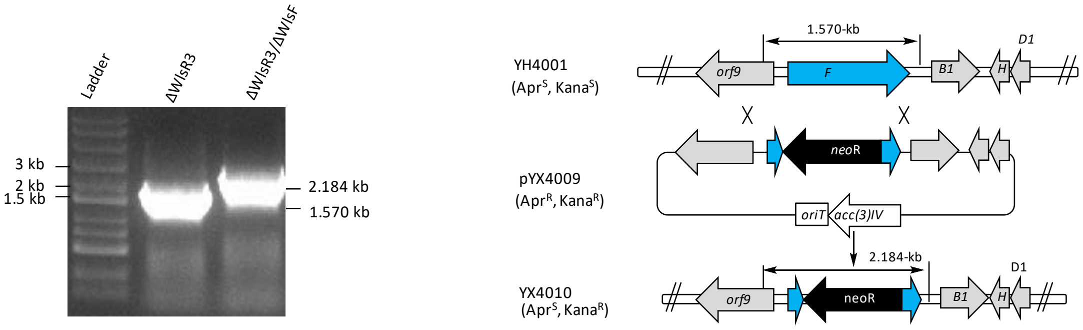
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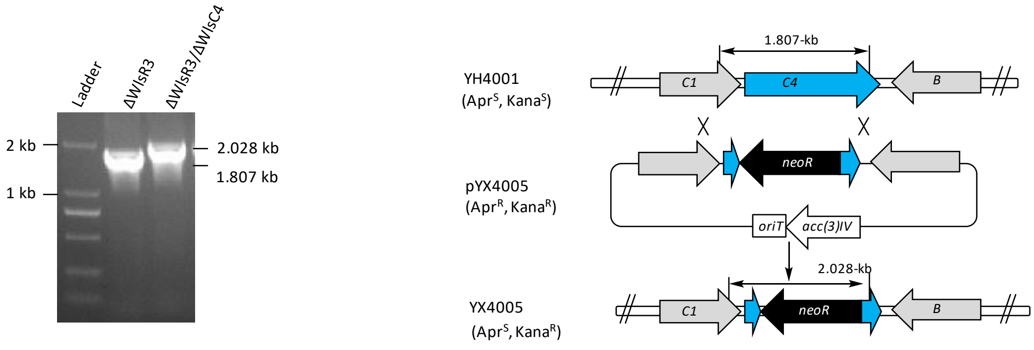
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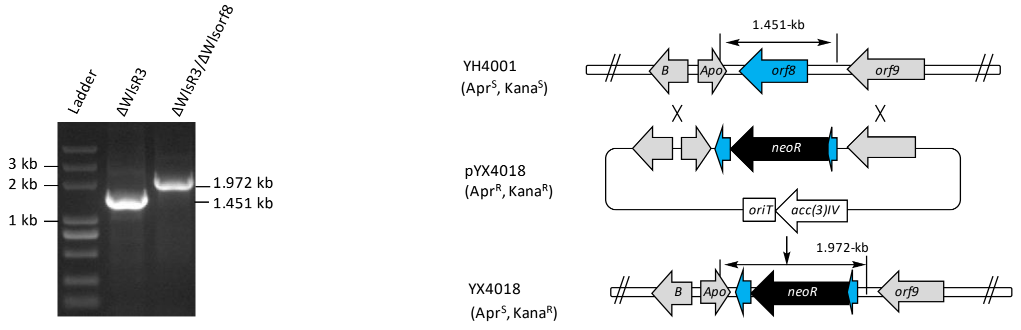
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1 kb
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B
Apo
orf8
orf9
pYX4018
(Apr^R, Kana^R)
neoR
oriT
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(Apr^S, Kana^R)
B
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neoR
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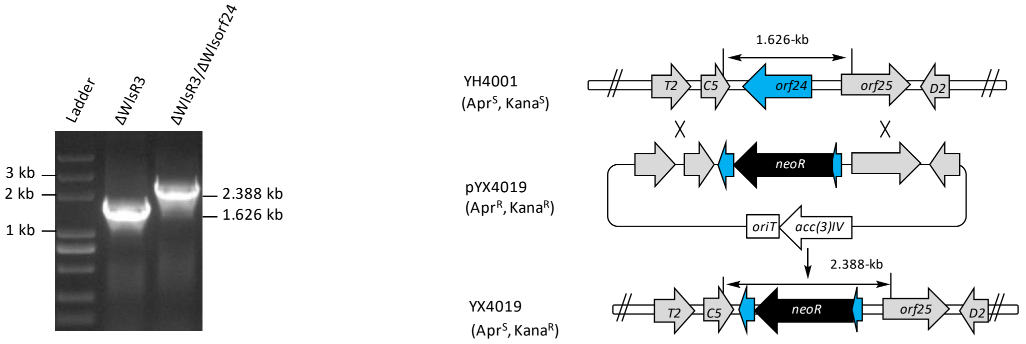
K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C5
orf24
orf25
D2
pYX4019
(Apr^R, Kana^R)
neoR
oriT
acc(3)IV
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(Apr^S, Kana^R)
T2
C5
neoR
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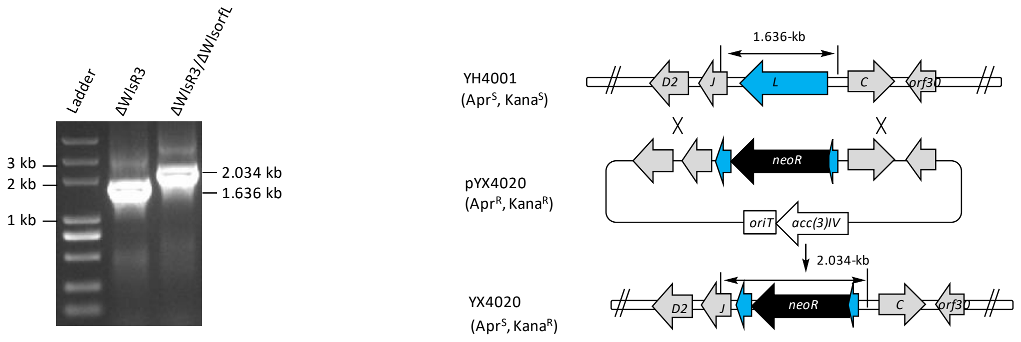
D2

L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D2
J
L
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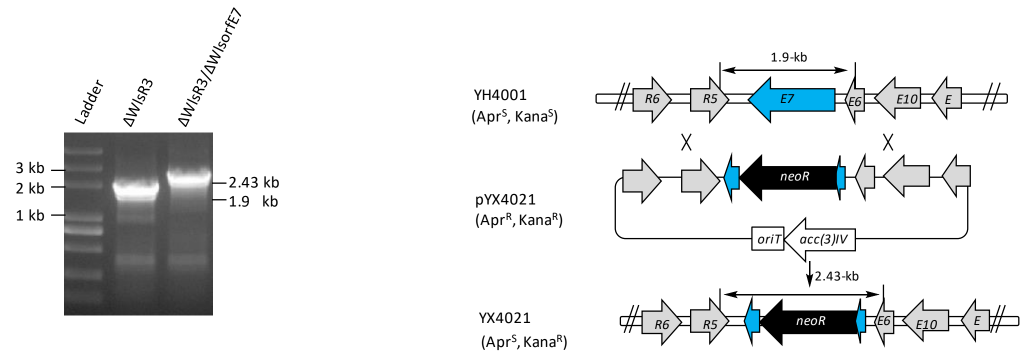

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(Apr^S, $Kana^S$)
1.9-kb
R6
R5
E7
E6
E10
E
pYX4021
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neoR
oriT
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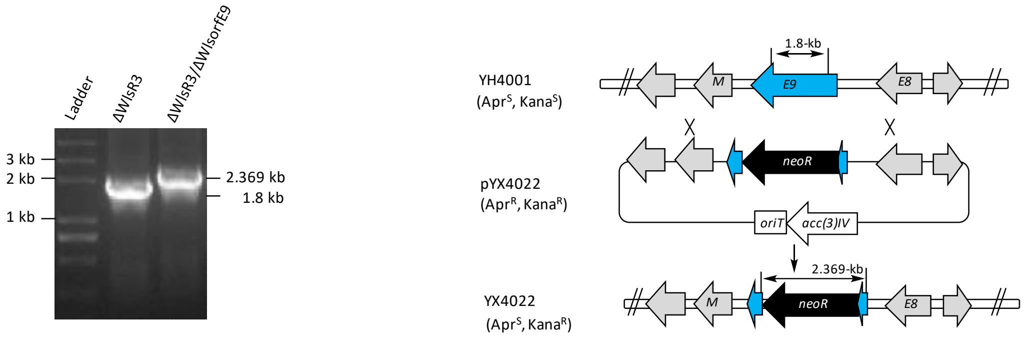
(Apr^S, $Kana^R$)

N



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ΔWlsR3/ΔWlsorfE9
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(Apr^S, $Kana^S$)
1.8-kb
M
E9
E8
pYX4022
(Apr^R, $Kana^R$)
neoR
oriT
acc(3)IV
2.369-kb
YX4022
(Apr^S, $Kana^R$)

Figure S5 HPLC profiles of _S_. sp. CB02130 wild-type strain (panel I) and its mutants, including YX4012 (CB02130::_ermE*-wlsR1_) (panel II), YX4011 (_ΔwlsR2_) (panel III), YX4001 (_ΔwlsR3_) (panel IV), YX4013 (CB02130::_ermE*-wlsR4_) (panel V), YX4014 (CB02130::_ermE*-wlsR5_) (panel VI), and YX4015 (CB02130::_ermE*-wlsR6_) (panel VII).

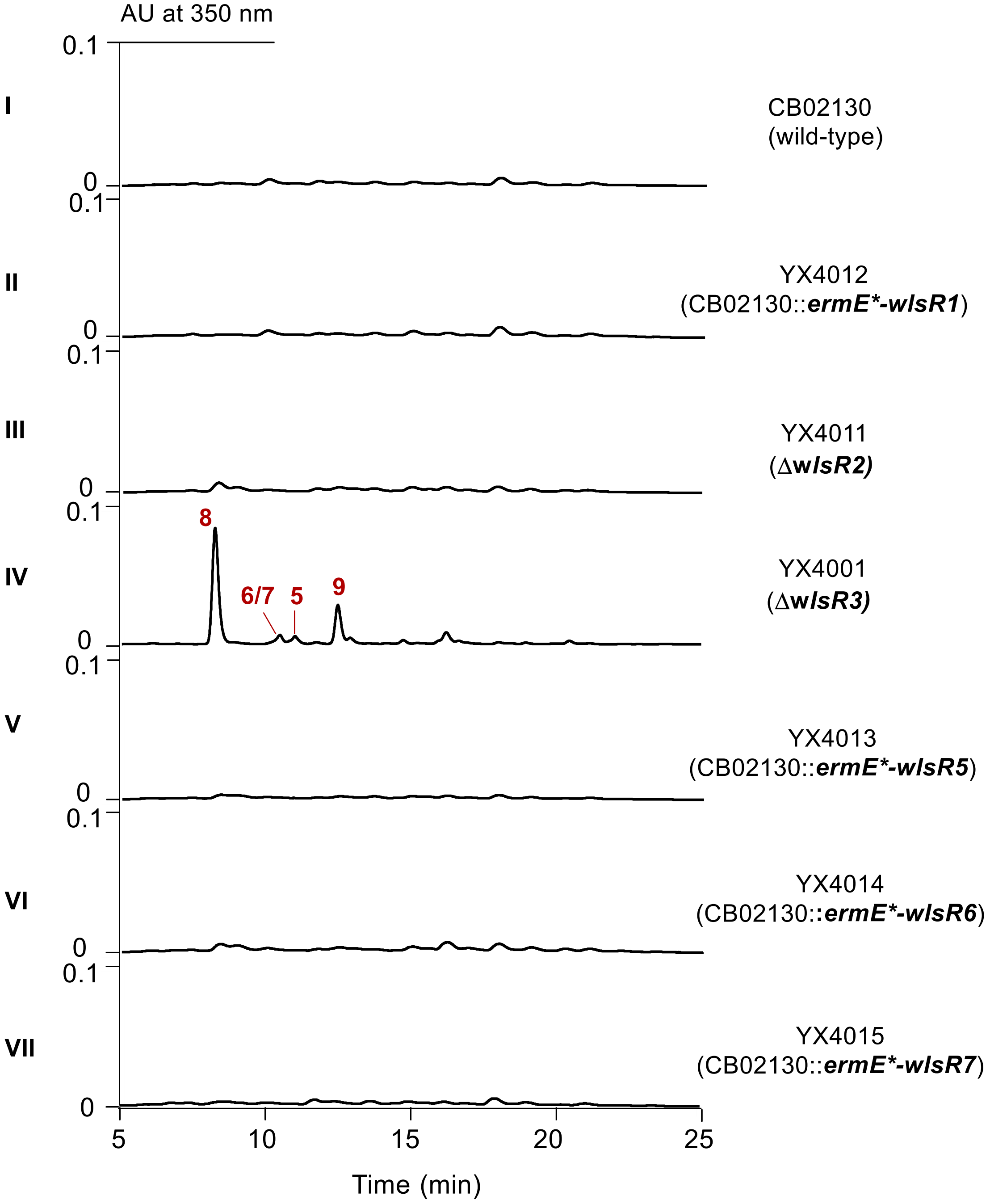
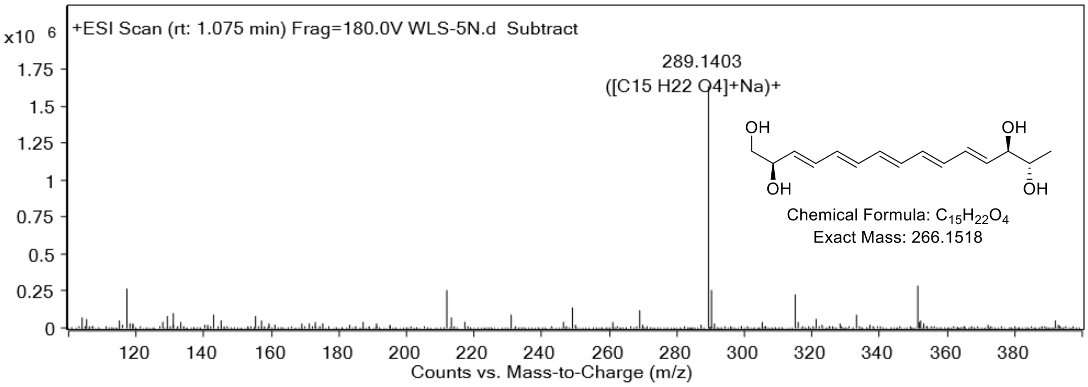


Figure S6. HR-ESI-MS spectra of **5**, **6**, **8**, **9** and APCI-HR-MS and APCI-HR-MS/MS spectra of **7**. The mechanistic proposal for the deduced MS of **7** was provided, showing the proposed fragmented ions with corresponding molecular weights.

5



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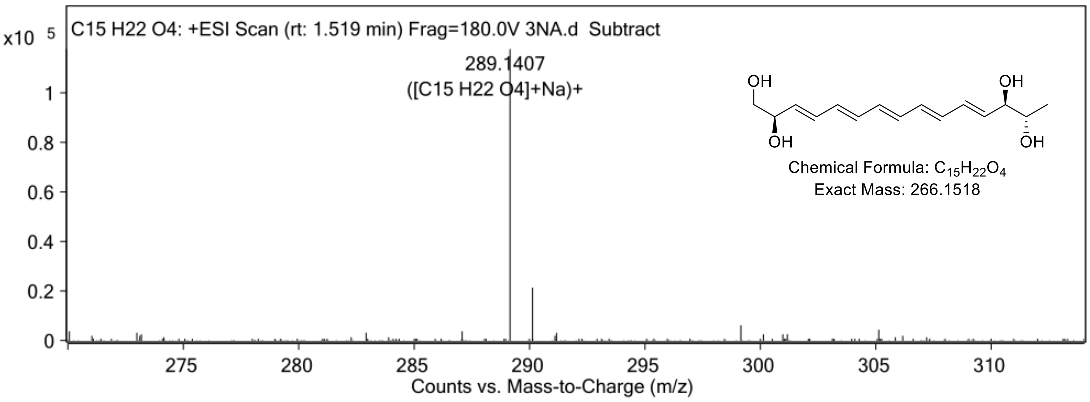

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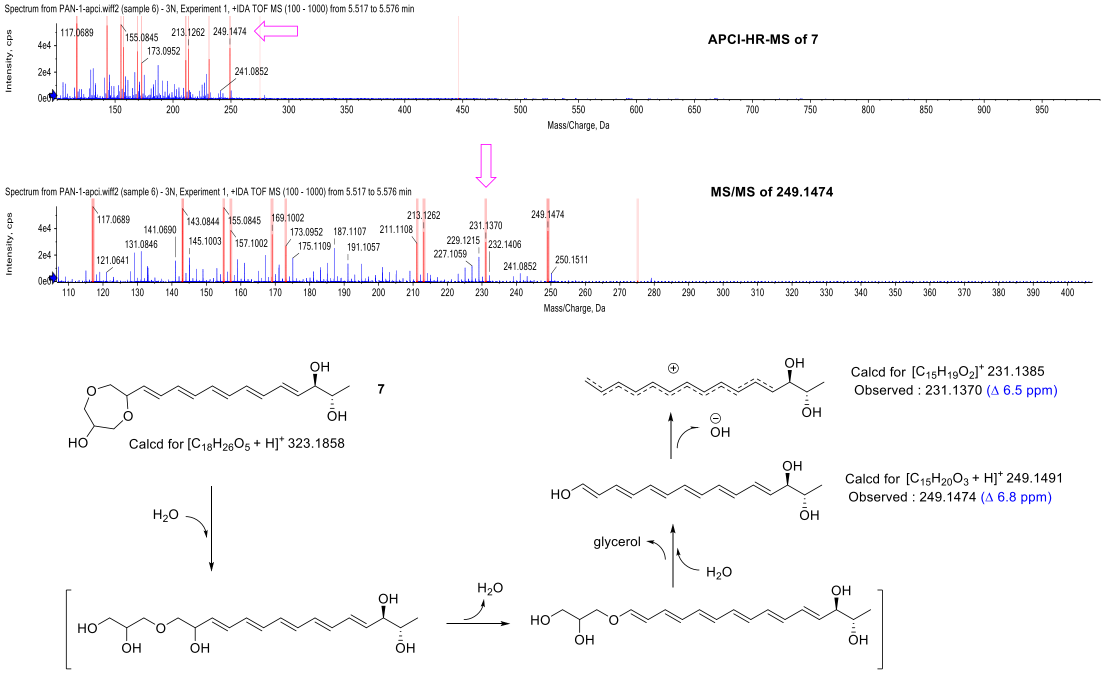

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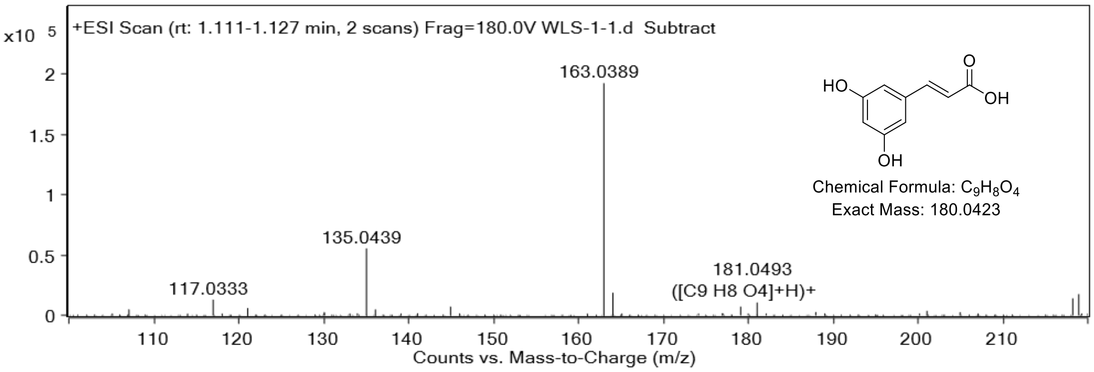
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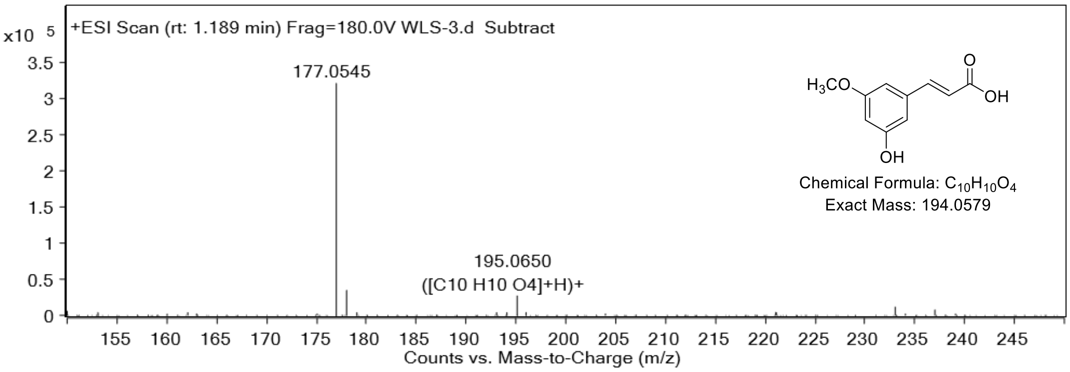
2
1.5
1
0.5
0
163.0389
135.0439
117.0333
181.0493
([C9 H8 O4]+H)+
110 120 130 140 150 160 170 180 190 200 210
Counts vs. Mass-to-Charge (m/z)
HO
O
OH
OH
Chemical Formula: $C_9H_8O_4$
Exact Mass: 180.0423

9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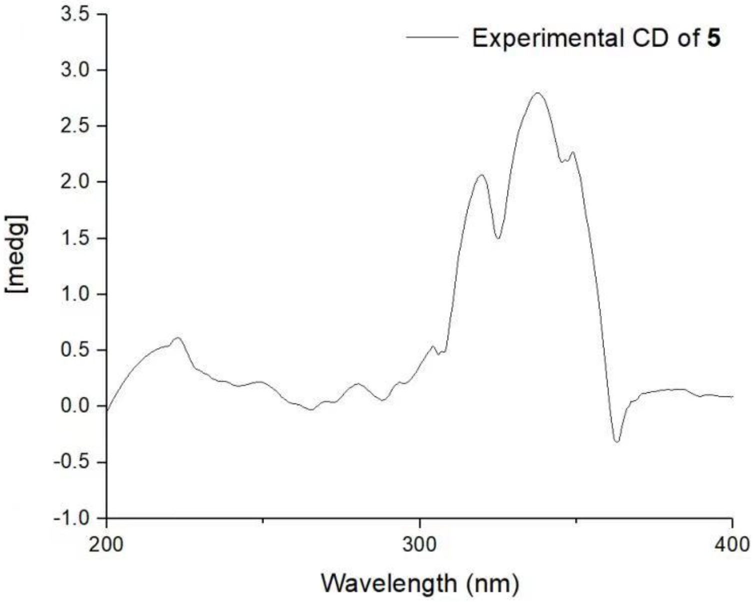
3
2.5
2
1.5
1
0.5
0
177.0545
195.0650
([C10 H10 O4]+H)+
155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245
Counts vs. Mass-to-Charge (m/z)
H_3CO
O
OH
OH
Chemical Formula: $C_{10}H_{10}O_4$
Exact Mass: 194.0579

Figure S7.CD spectra of **5** and **6**.



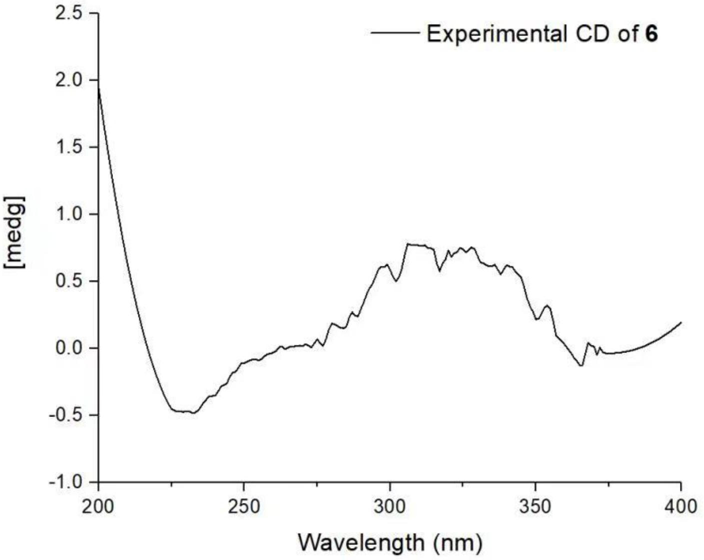
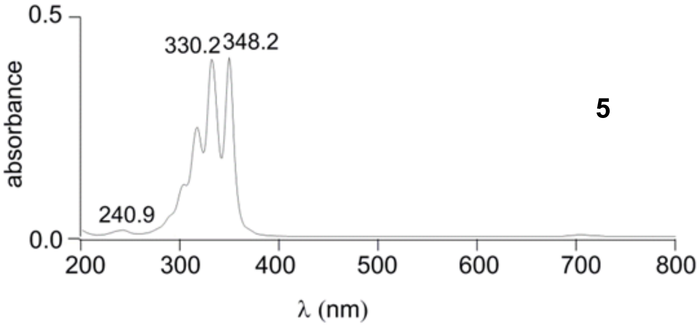


Figure S8. UV spectra of **5**–**9**.



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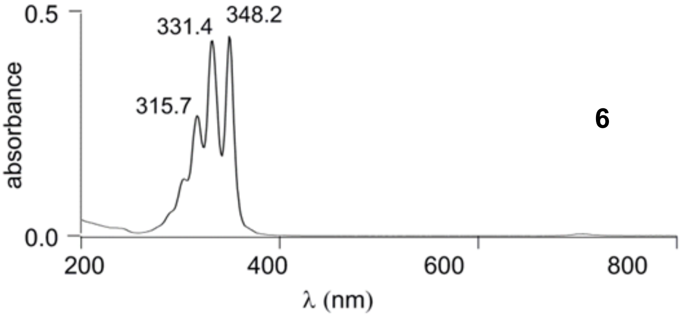

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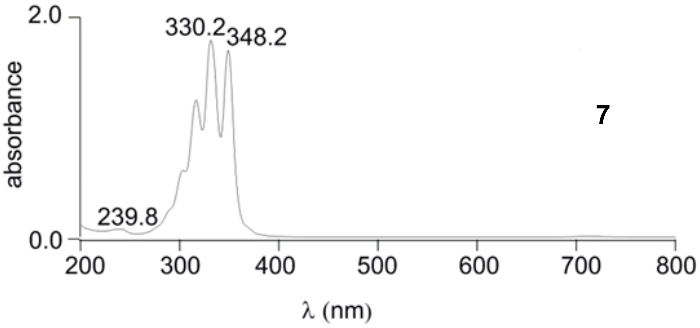

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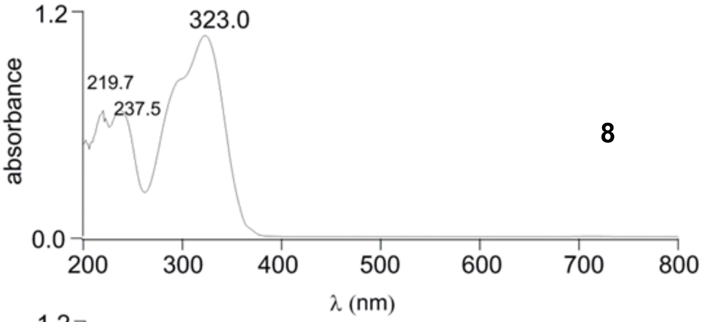

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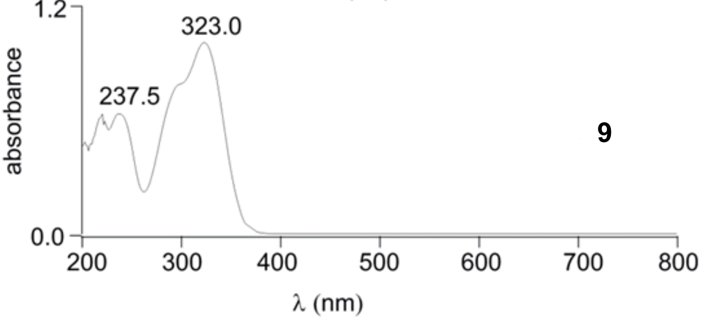


9

Figure S9 The time course analysis of crude extracts in *S*. sp. CB02130 wild-type and YX4001 (*Δwls*R3). The production of **5**–**9** was observed as early as 72 h after the start of fermentation in YHX4001 (*Δwls*R3).

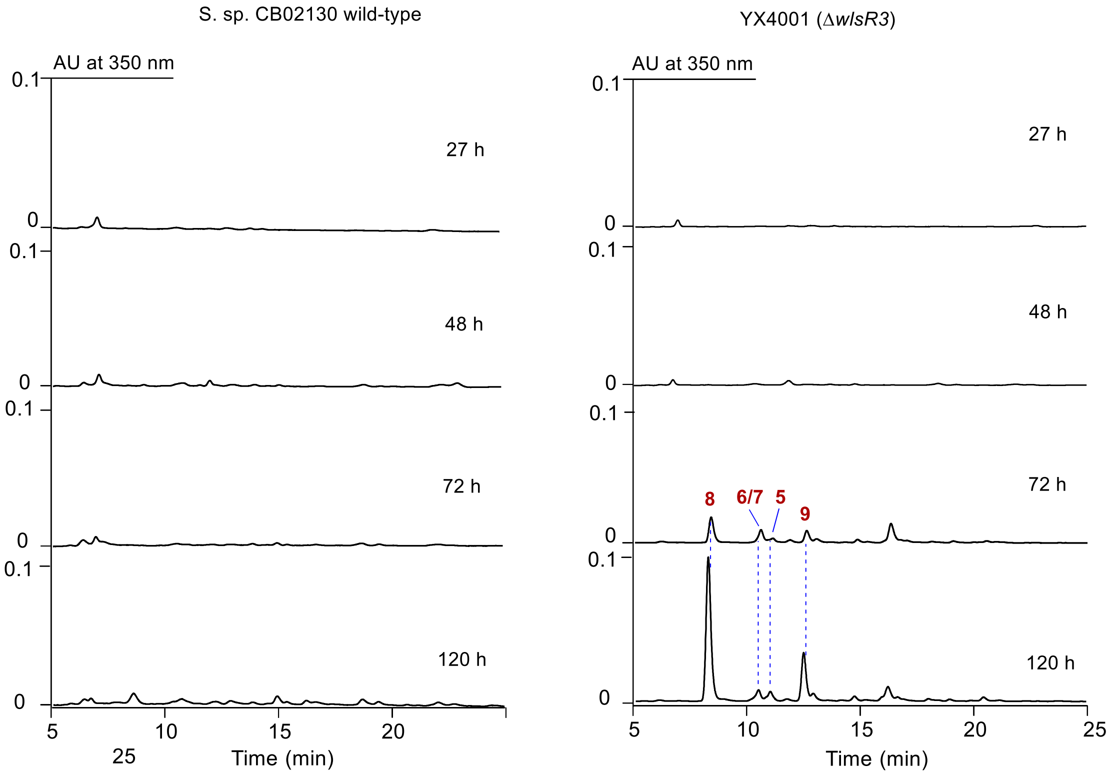


Figure S10 Characterization of WlsC4 as an ammonia lyase with L-Phe or L-Tyr as its substrates. (A) Phylogenetic analysis of WlsC4 and homologues. They include L-tyrosine ammonia mutases SgTAM and CcTAM, L-phenylalanine ammonia mutase TcPAM, L-tyrosine ammonia lyases RsTAL and RcTAL, L-phenylalanine ammonia lyases EncP and PcPAL.WlsC4, S. sp. CB02130 (OKJ20239.1); KedY4, *Streptoalloteichus* sp. ATCC 53650 (AFV52190); SgTAM (Also SgcC4), *Streptomyces globisporus* (Q8GMG0); RsTAL, *Rhodobacter sphaeroides* (Q3IWB0); RcTAL, *Rhodobacter capsulatus* (WP_023923512); PcPAL, *Petroselinum crispum* (P24481); EncP, *Streptomyces maritimus* (AAF81735); TcPAM, *Taxus canadensis* (AAT47186); CcTAM, *Chrondromyes crocatus* (Q0VZ68). Previously characterized aminomutases are labeled with a black circle and characterized ammonia lyase are labeled with a black star. (B) Purification of WlsC4 as an N-His$_6$-tagged protein. (C) UV spectrum of WlsC4. HPLC analysis (D) and ESI-LCMS (E) of WlsC4-catalyzed products cinnamic acid or p-hydroxycinnamic acid, using L-Phe or L-Tyr as substates, respectively.

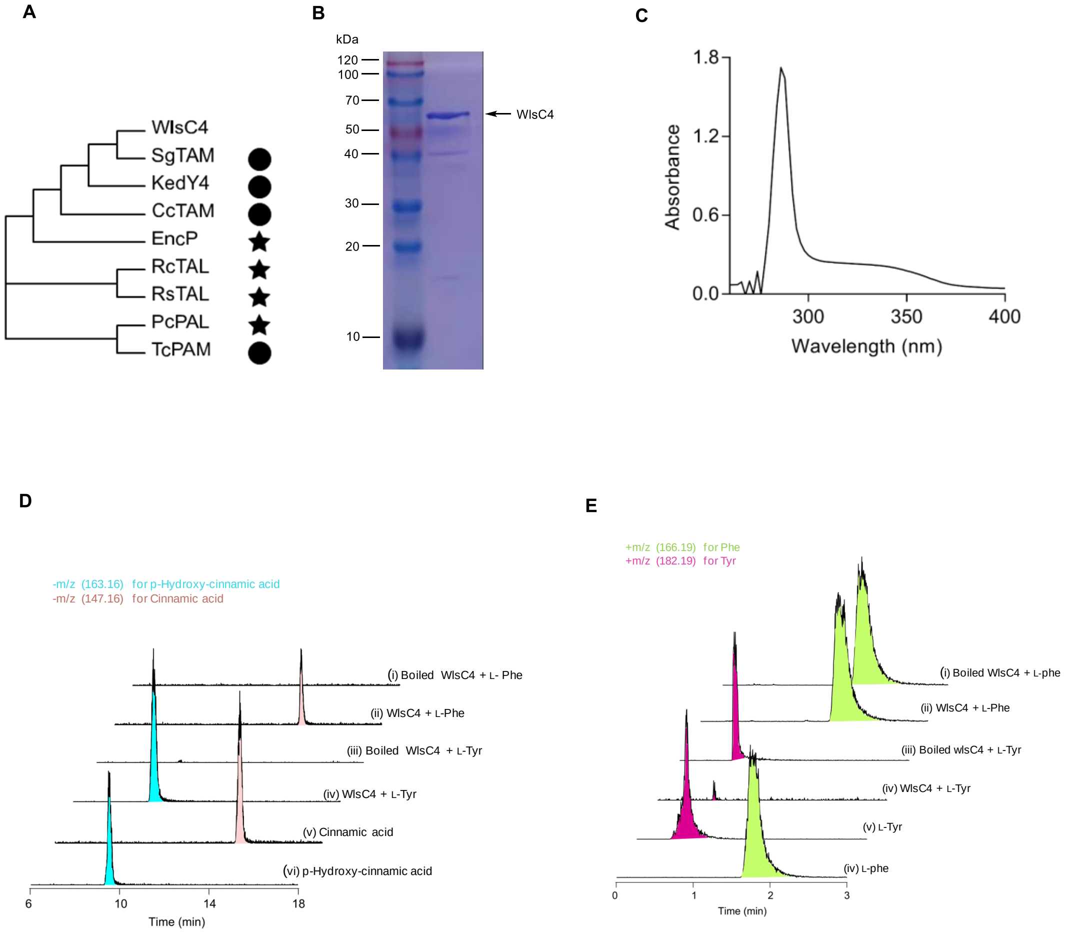


Figure S11 L-Phenylalanine (L-Phe) or L-tyrosine (L-Tyr) as the sole nitrogen source in its production medium for YX4001 (Δ*wlsR3*) growth and the production of **5**–**9** or **1**. (A) The production of **5**–**9** in the indicated production medium with L-Phe as the sole carbon source (**III**), in comparison to the original production medium (**I**) or L-Tyr (IV) as the sole nitrogen source, or with no nitrogen source (**II**). (B) The production of heptaene (**1**) in YX4001 (Δ*wlsR3*) in the indicated production media. Note that **1** was extracted from the mycelium of YX4001 in respective medium using MeOH, while **5**–**9** were extracted from the supernatant of YX4001 using EtOAc, concentrated, redissolved in MeOH, and analyzed. A typical 2-mL concentrated fermentation extract of YX4001 was obtained from each 50-mL fermentation culture, while individual sample (20~50 μL) was injected for HPLC analysis.

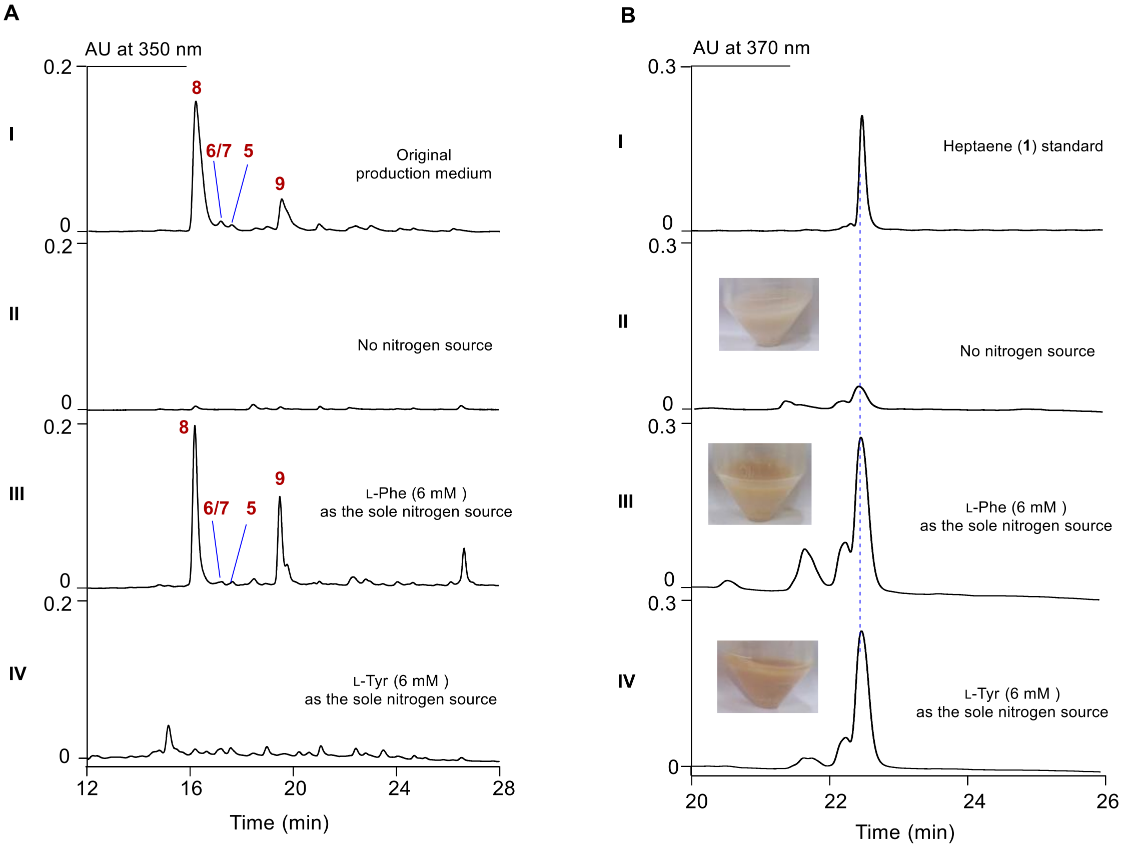


Figure S12 L-Phe contributes to the production of polyene polyols **5–7.** (A) L-Phe with different concentrations (2, 4, 6 mM) was added to production medium as the sole nitrogen source before inoculation. (B) 6 mM L-Phe was used as an extra nitrogen source into the original production medium before inoculation.

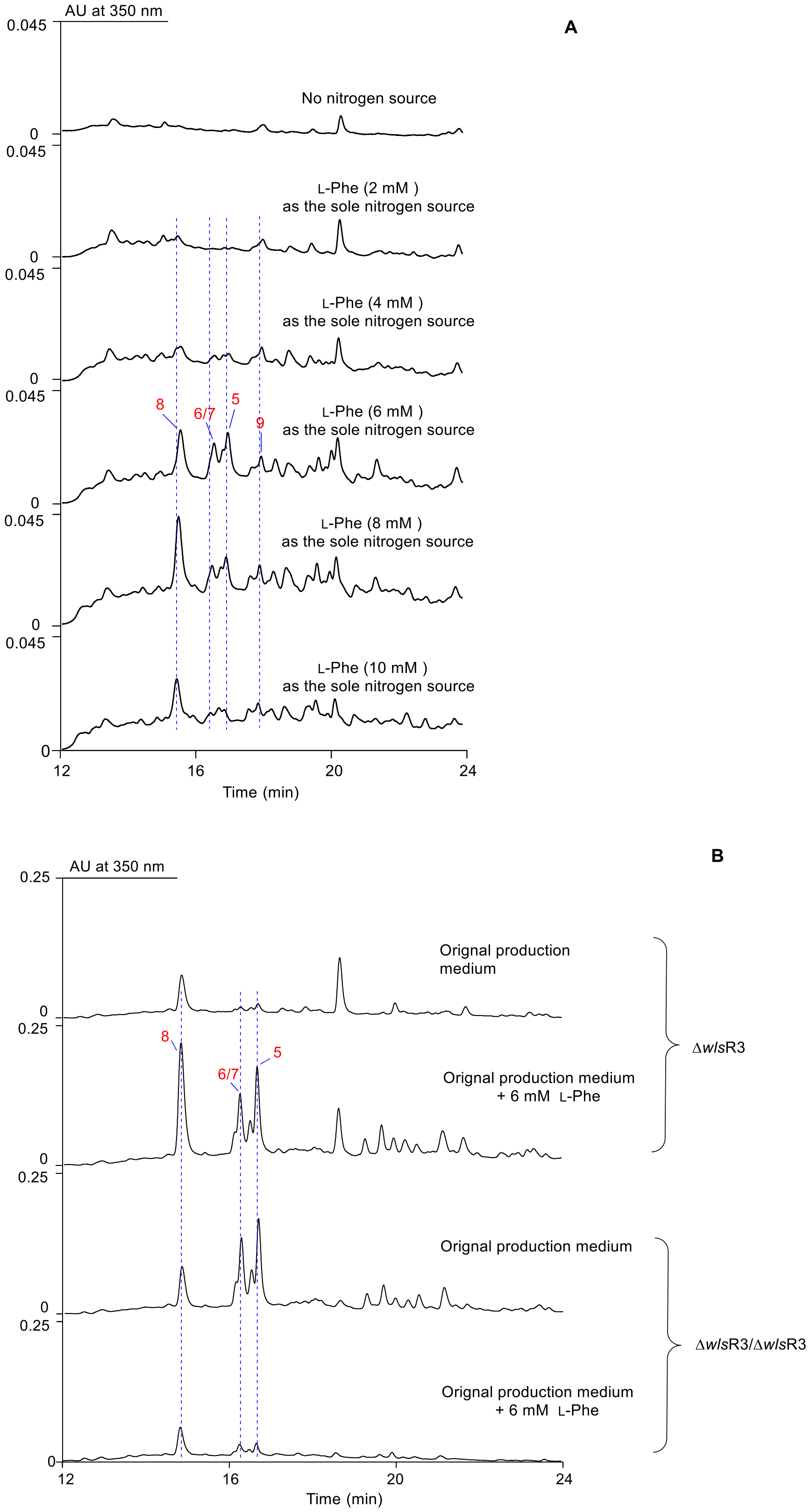


Figure S13 Gene inactivation of *wls*ORF8, *wls*ORF24, *wls*L, *wls*E7, and *wls*E9 in *ΔwlsR3* had no effects towards the production of **5**–**7**.

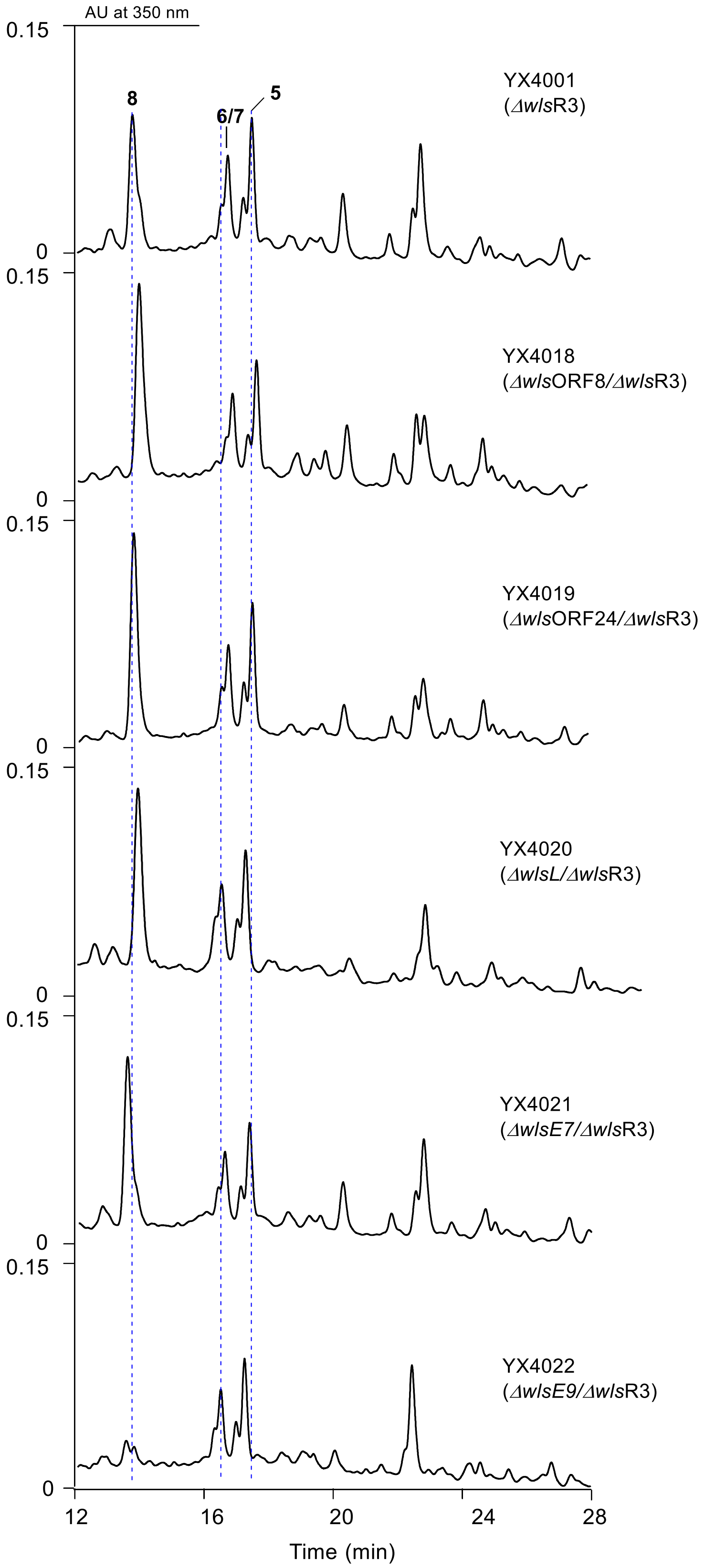


Figure S14 Overexpression of putative positive regulators *wls*R1 and *wls*R6 under the control of *ermEp** in *Δwls*R3 had no effects towards the production of **5**–**7**.

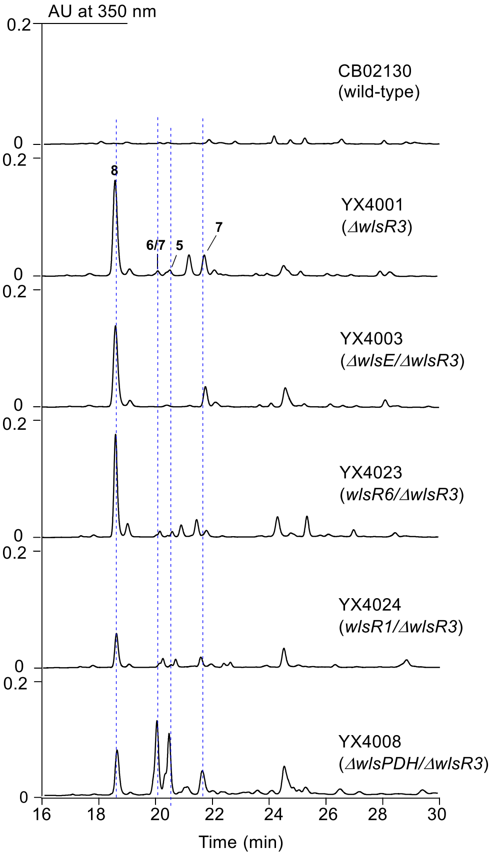


Figure S15 Yield increasement of heptaene **1** in Δ*wls*R3 and Δ*wls*PDH/Δ*wls*R3**,** while further overexpression of putative positive regulators *wls*R1 and *wls*R6 under the control of *ermEp** in *wls*R3 had no effects towards the production of **1**.

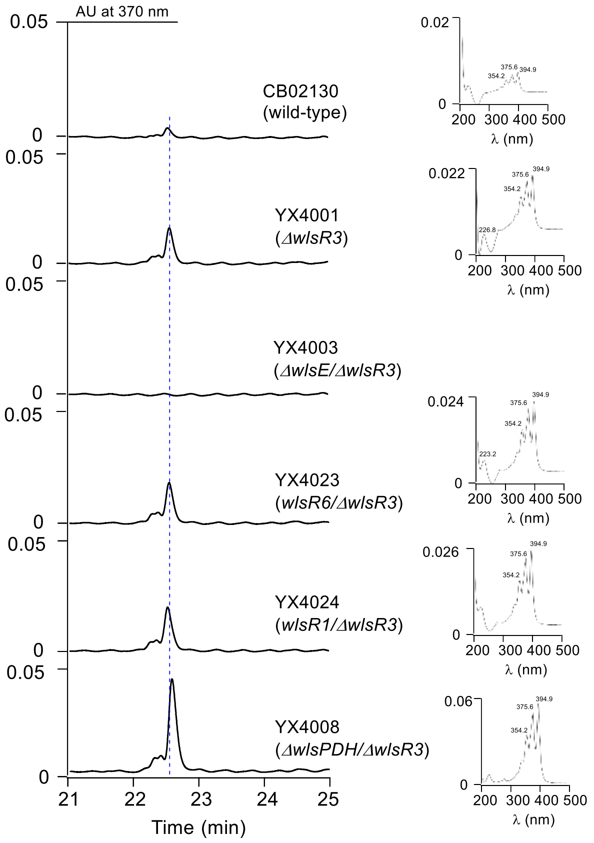


Figure S16. ^{1}H NMR spectrum of **5** in CD_3OD-d_4 (600 MHz).

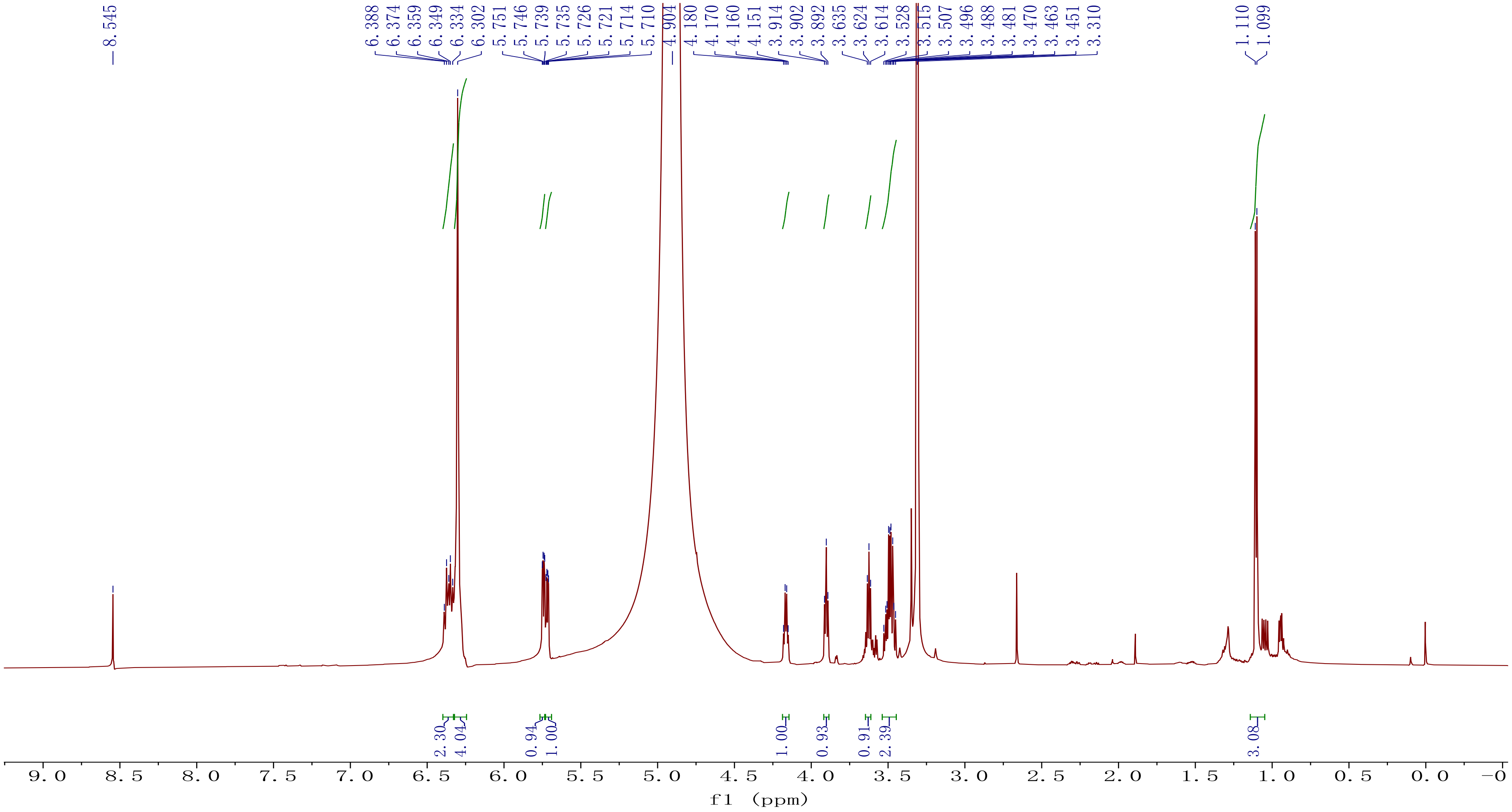


Figure S17. Expanded ^{1}H NMR spectrum of **5** in CD_3OD-*d₄* (600 MHz).

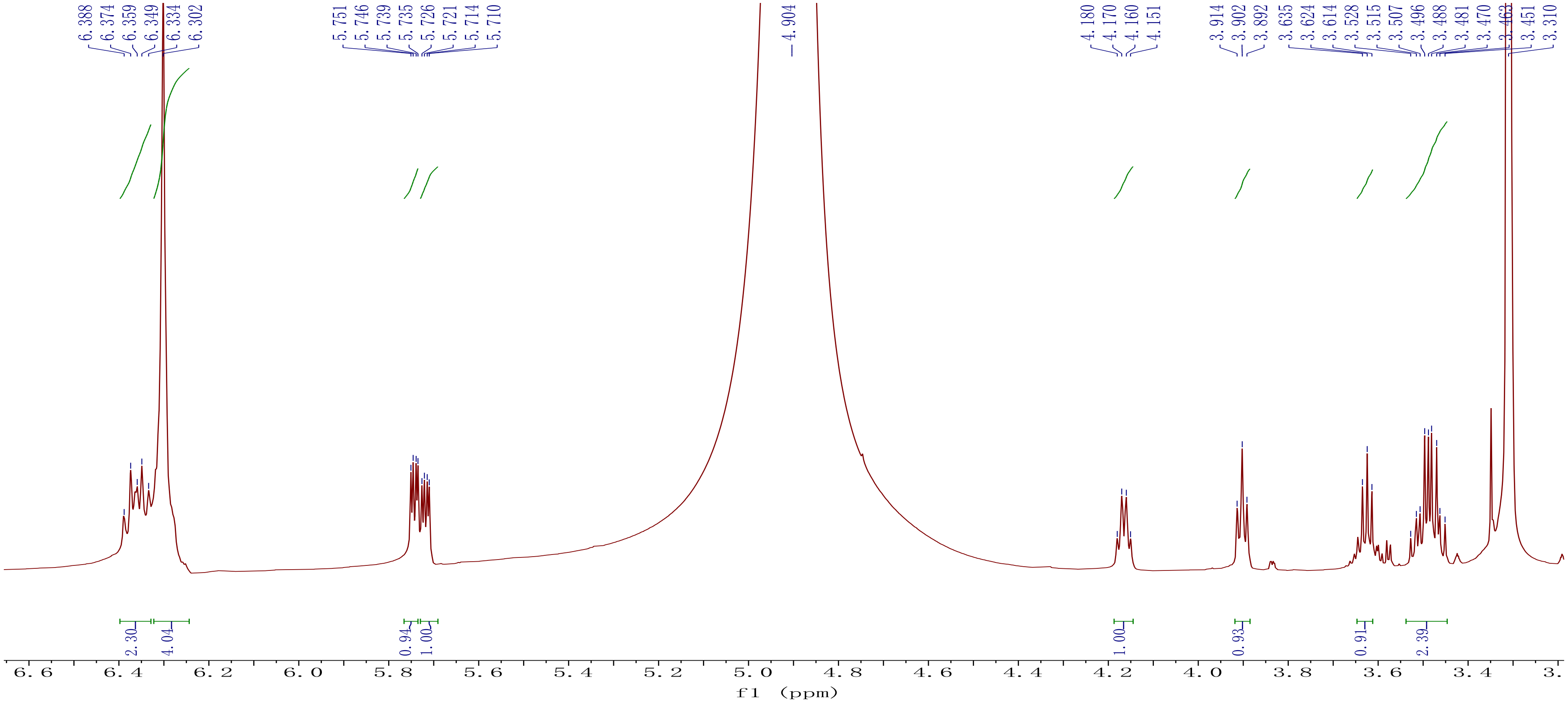


Figure S18. Expanded ^{1}H NMR spectrum of **5** (5.70 – 5.77 ppm) in CD_3OD-*d_4* (600 MHz).

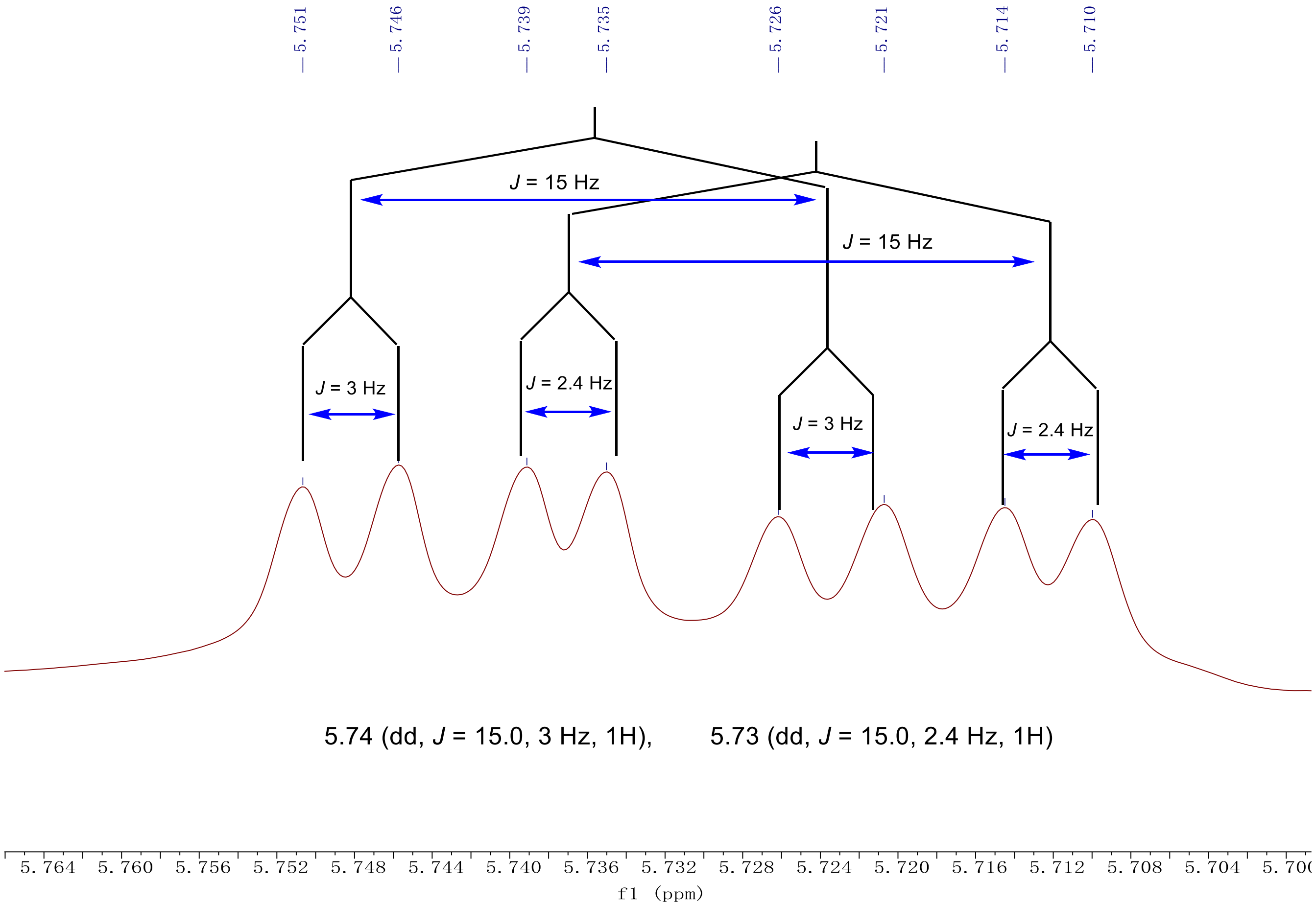


Figure S19. ^{13}C NMR spectrum of **5** in CD_3OD-*d*$_4$ (150 MHz).

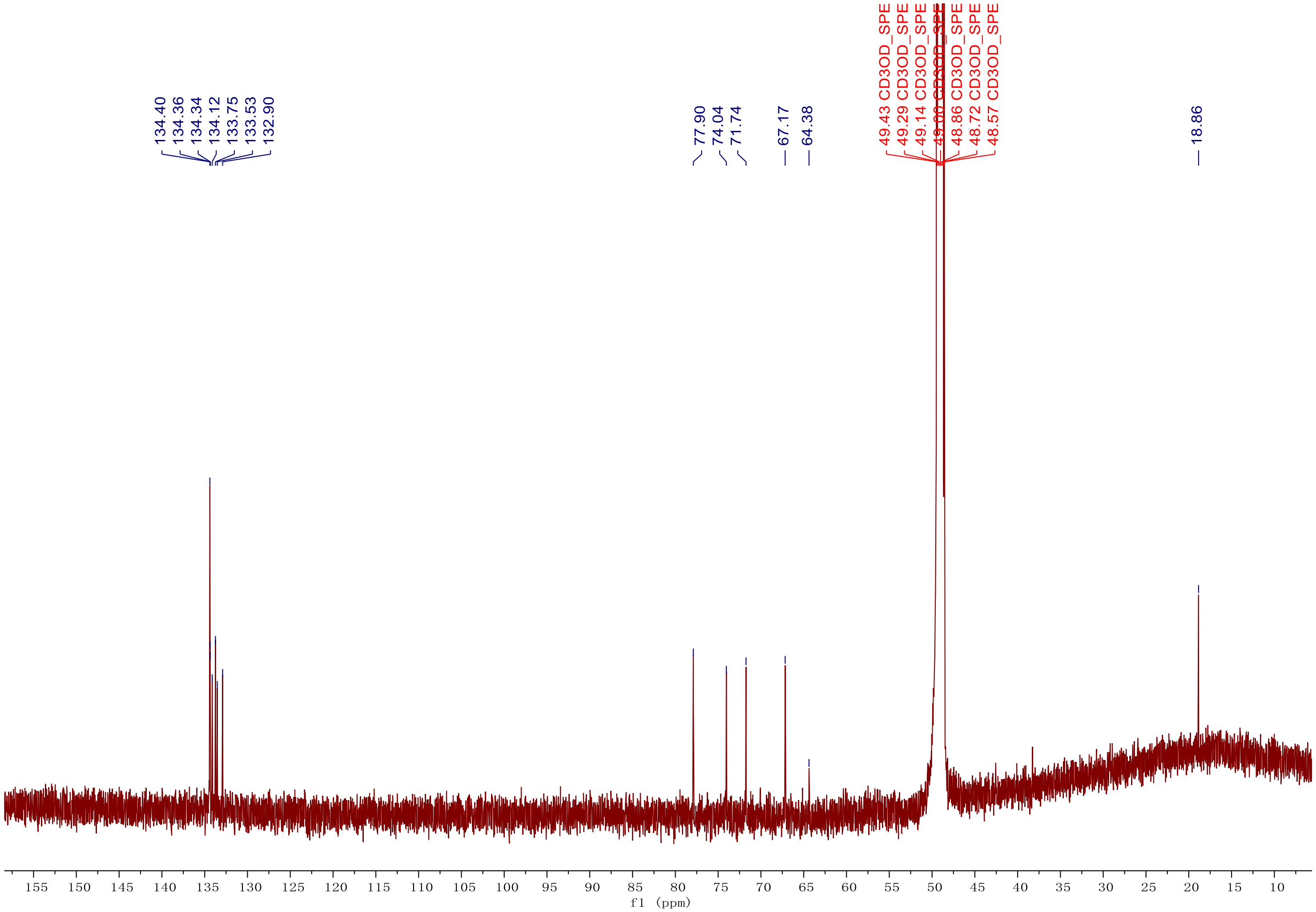


Figure S20. Expanded ^{13}C NMR spectrum of **5** in CD_3OD-*d$_4$* (150 MHz).

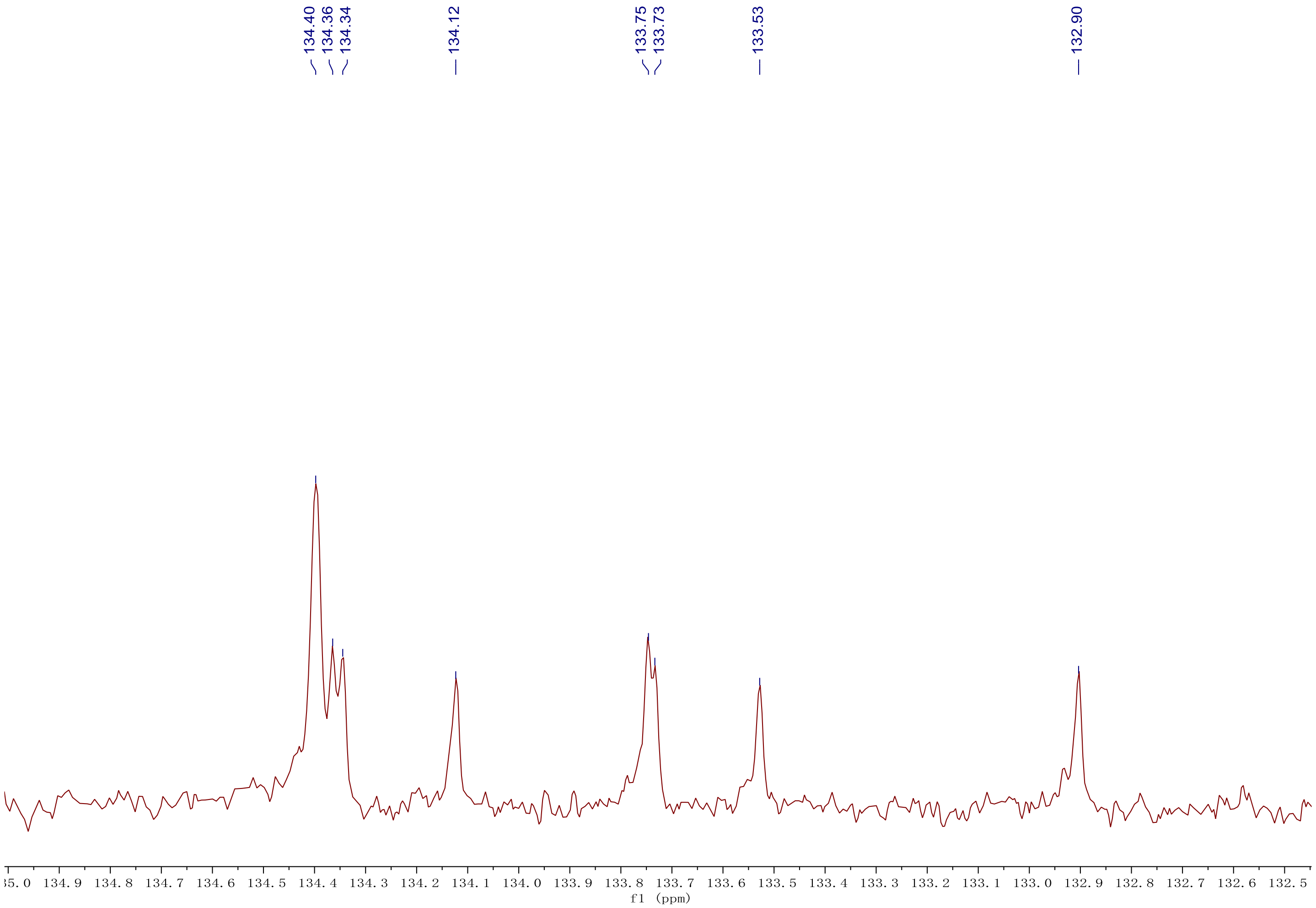


Figure S21. DEPT135 spectrum of **5** in CD$_3$OD-*d*$_4$.

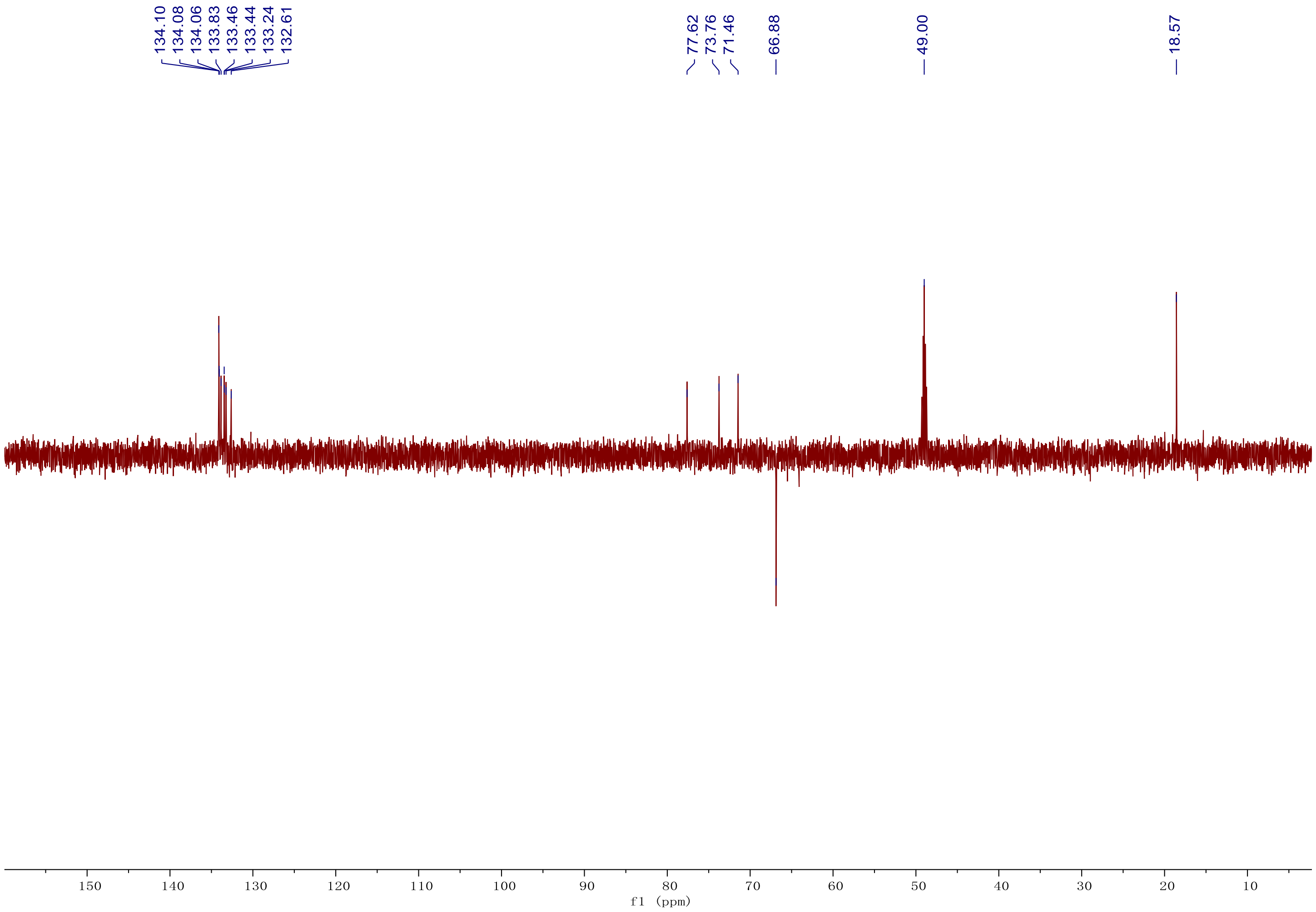


Figure S22. Expanded DEPT135 spectrum of **5** in CD_3OD-*d_4*.

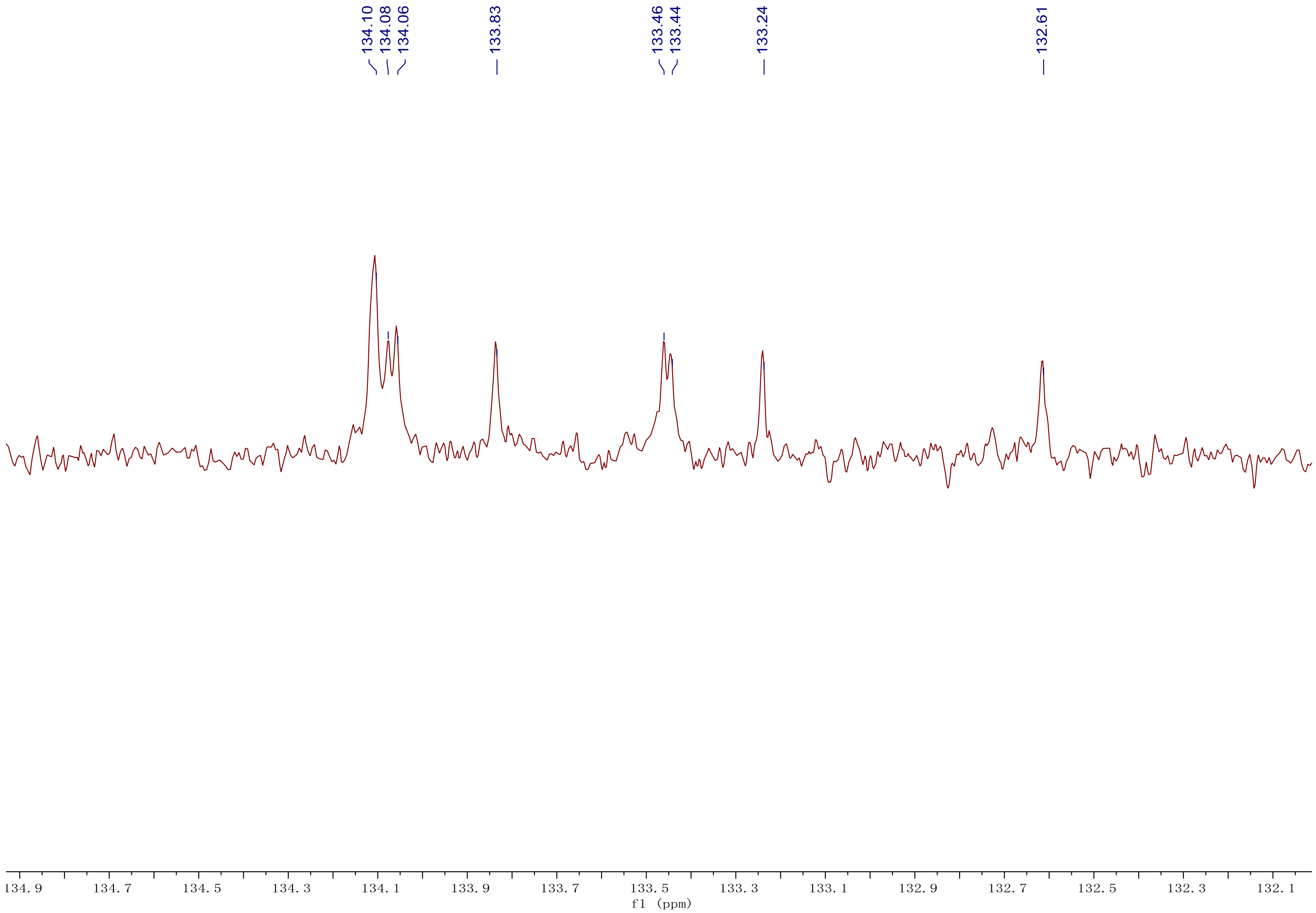


Figure S23. HSQC spectrum of **5** in CD_3OD-*d*$_4$.

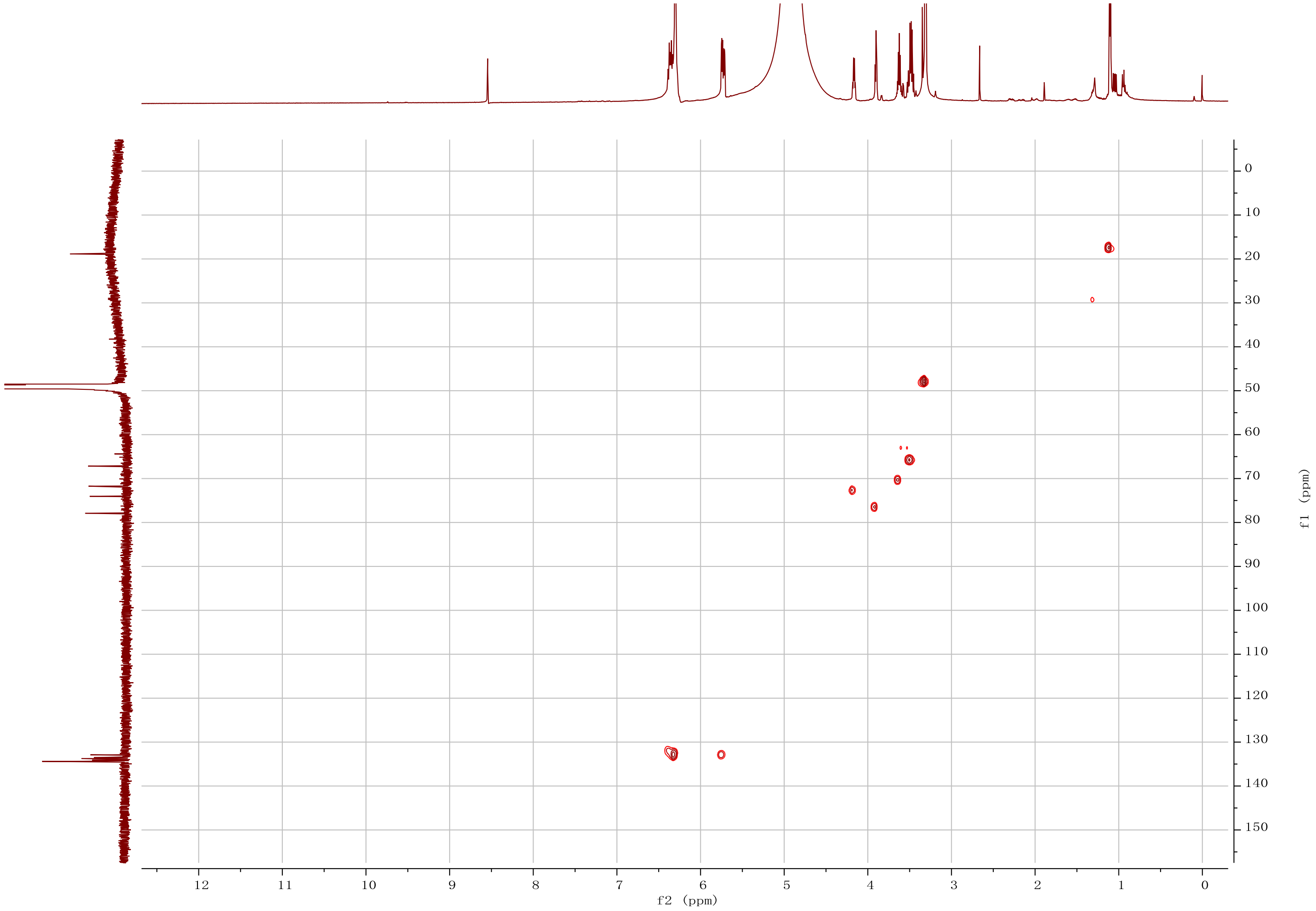


Figure S24. HMBC spectrum of **5** in CD$_3$OD-*d*$_4$.

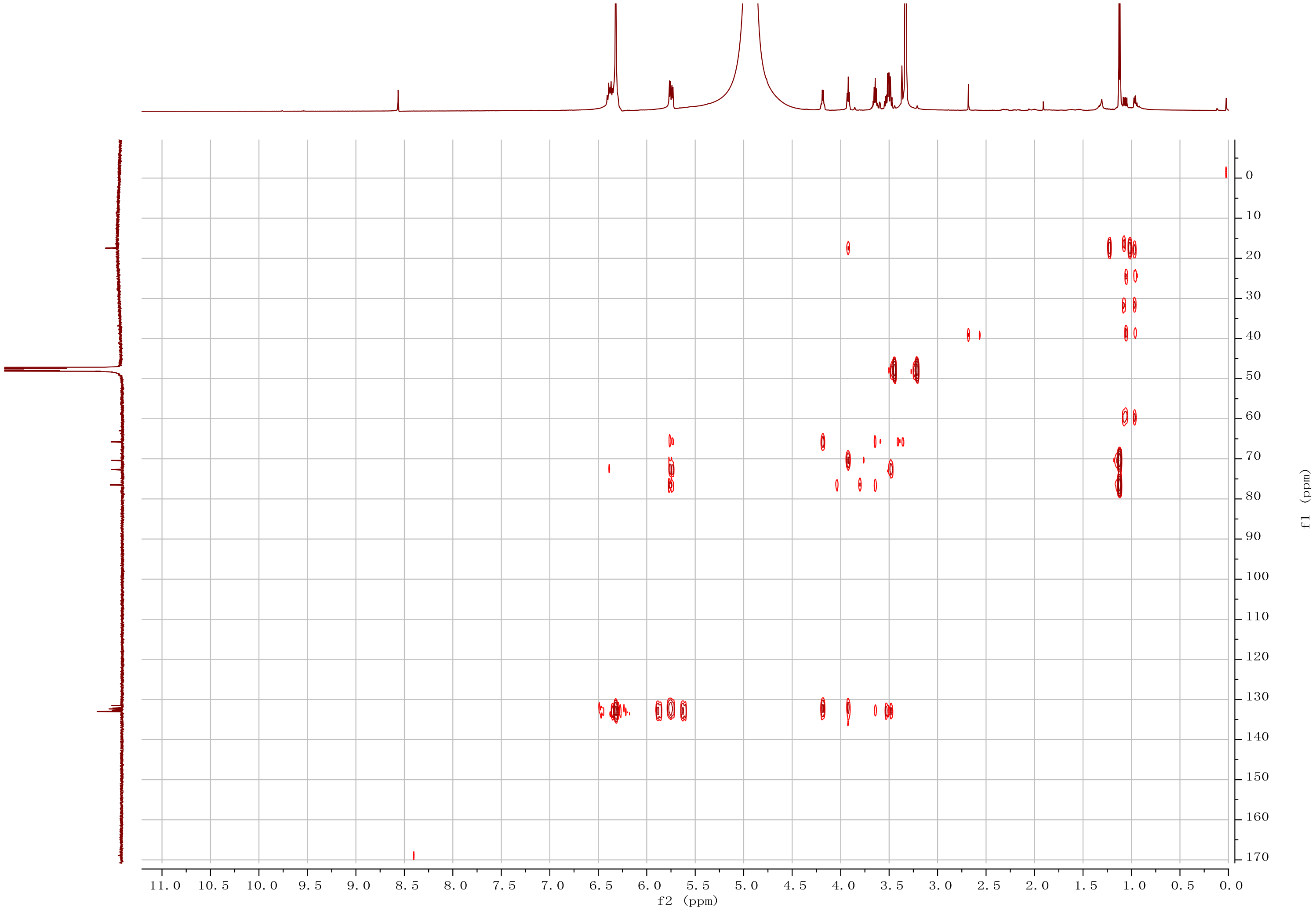


Figure S25. ^{1}H-^{1}H COSY spectrum of **5** in CD_3OD-*d*$_4$.

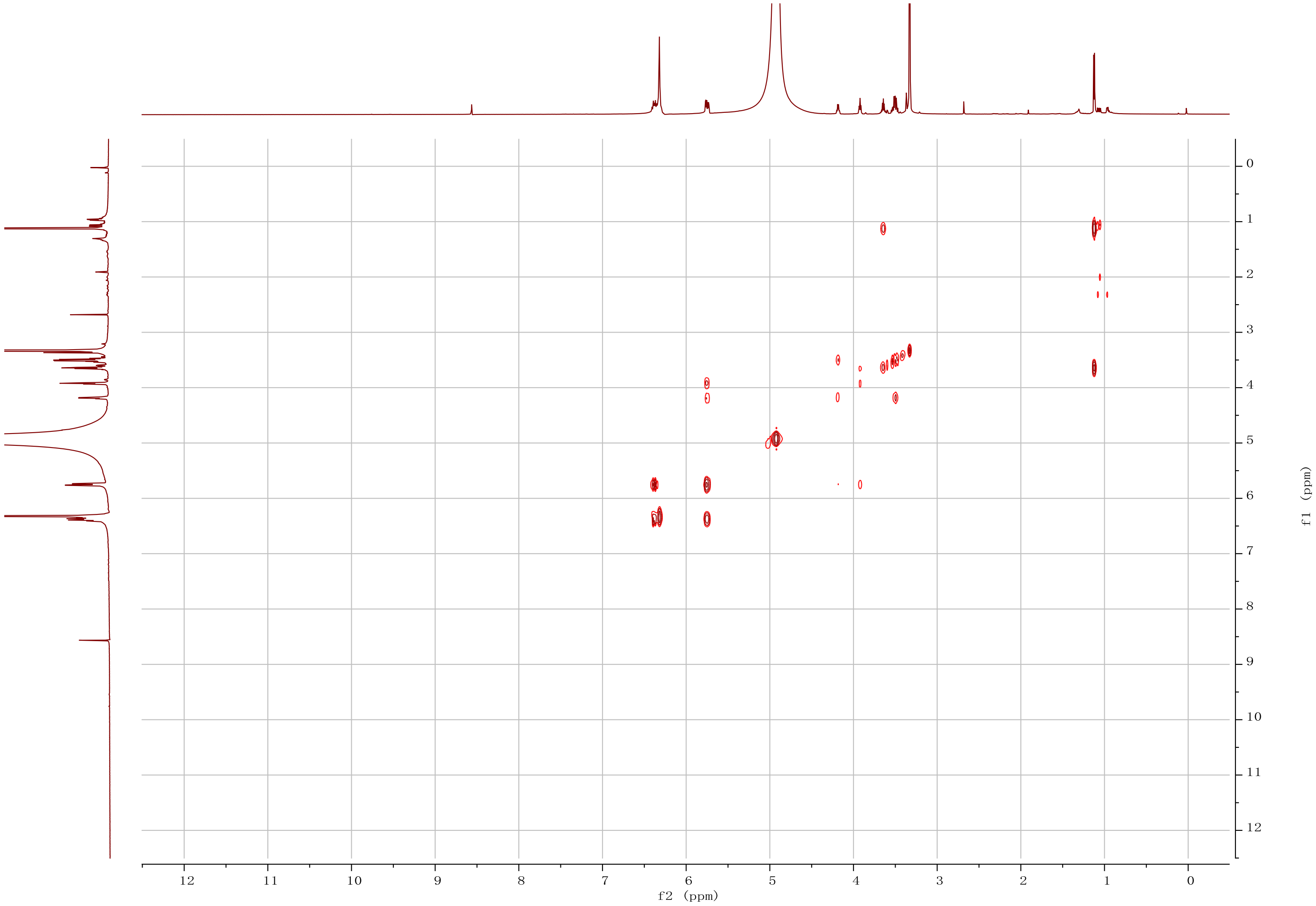


Figure S26. NOESY spectrum of **5**.

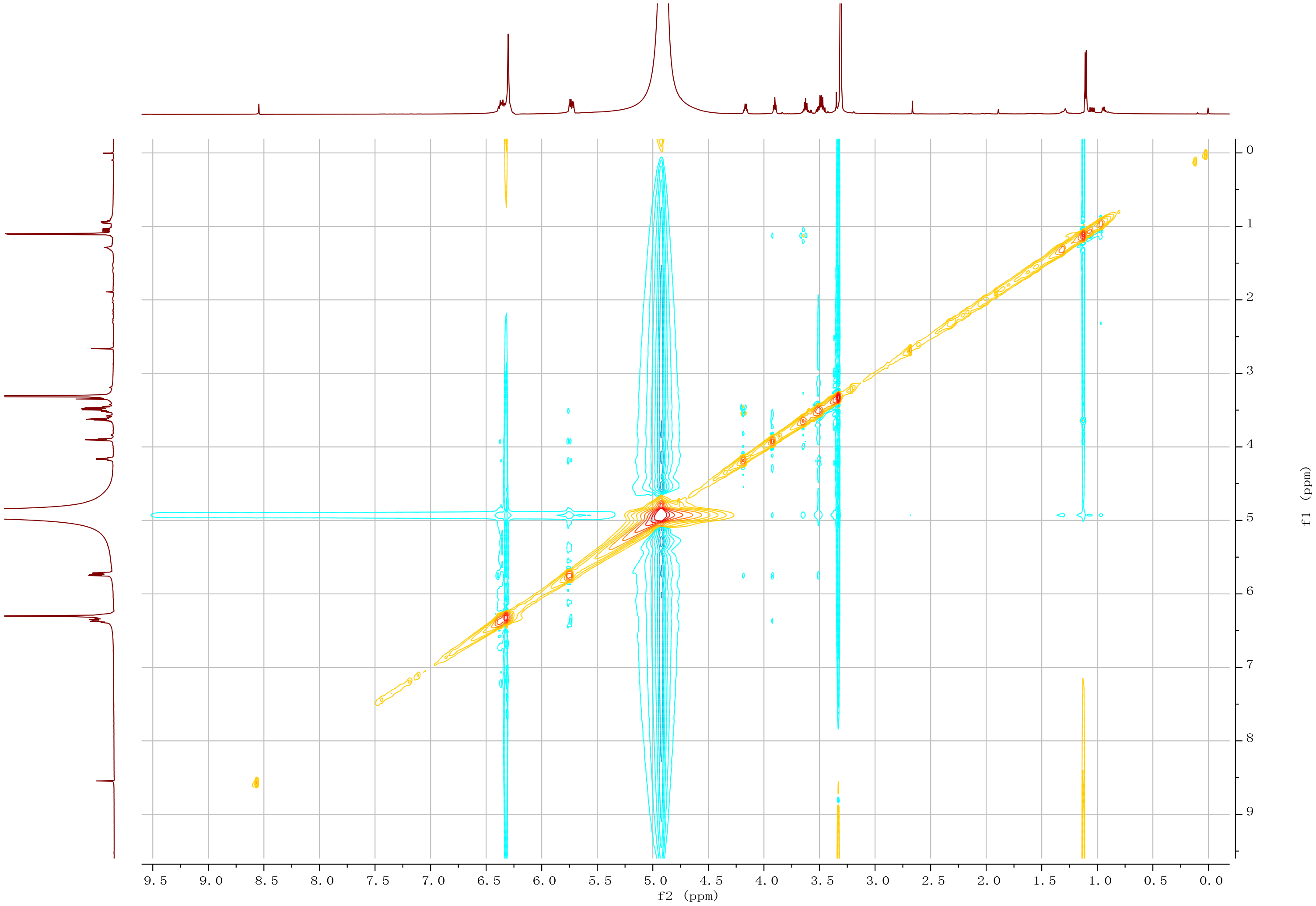


Figure S27. ^{1}H NMR spectrum of **6** in CD$_3$OD-*d*$_4$ (600 MHz).

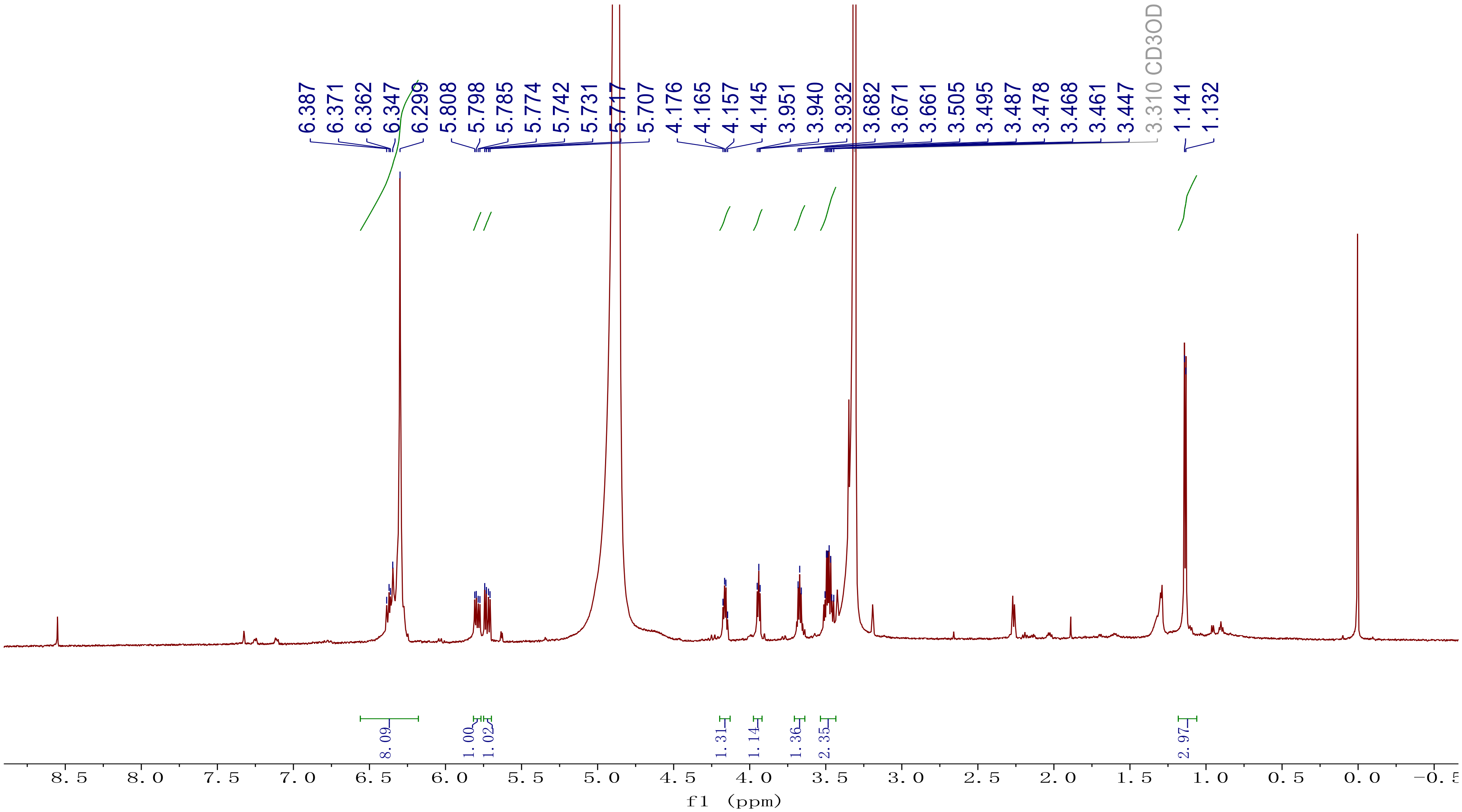


Figure S28. Expanded ^{1}H NMR spectrum of **6** in CD_3OD-*d_4* (600 MHz).

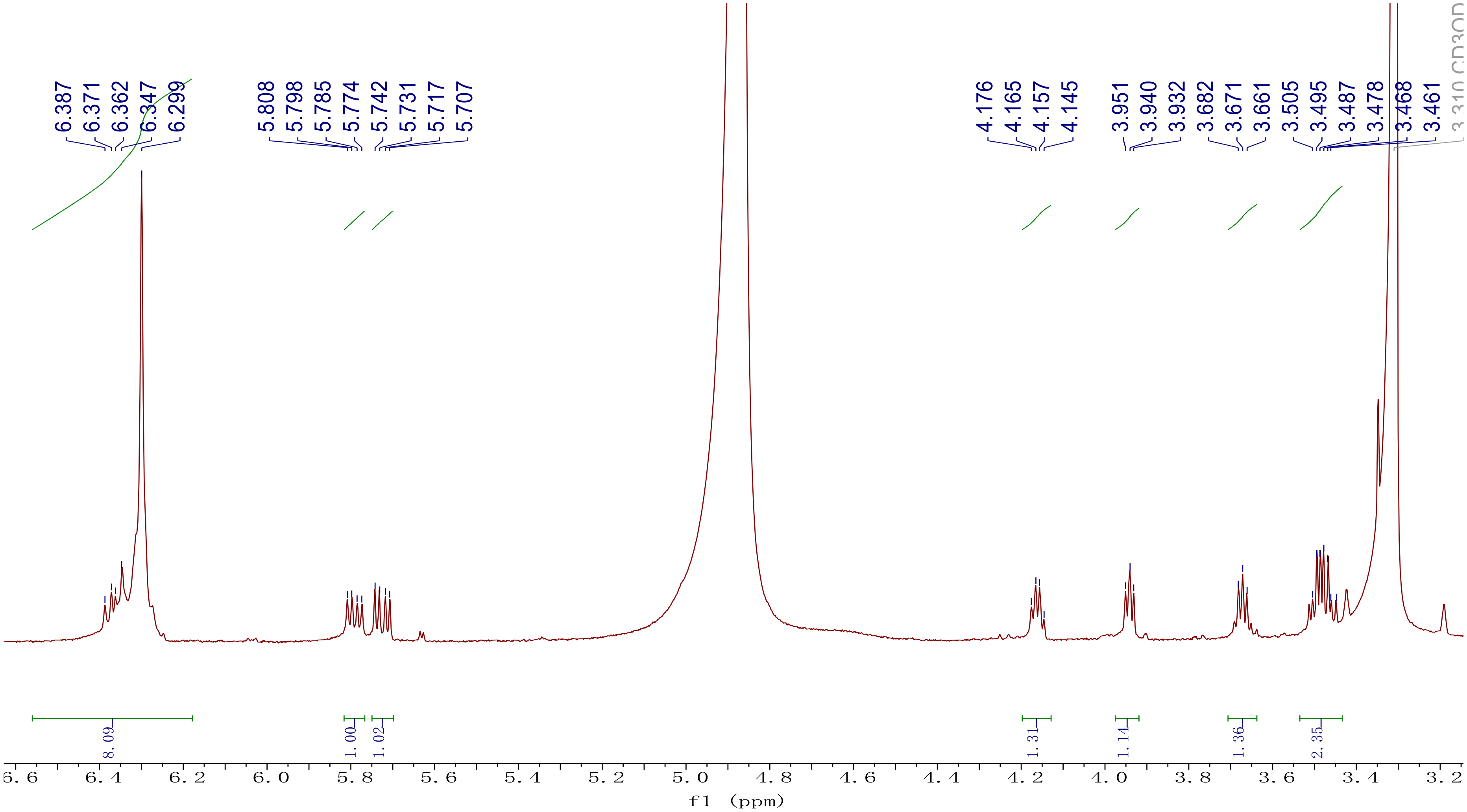
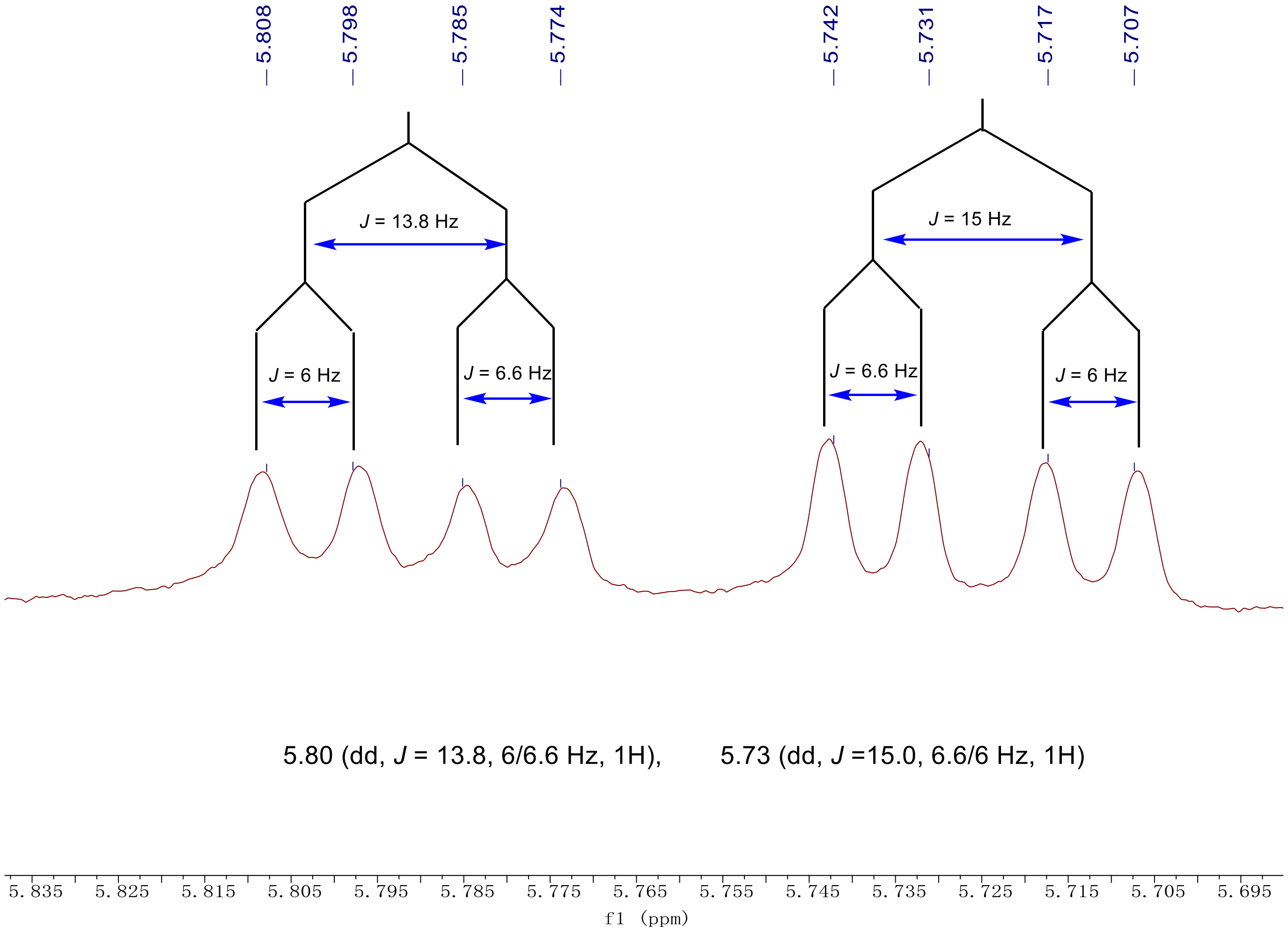


Figure S29. Expand ^{1}H NMR spectrum of **6** (5.69 – 5.85 ppm) in CD_3OD-*d_4* (600 MHz).



5.80 (dd, *J* = 13.8, 6/6.6 Hz, 1H), 5.73 (dd, *J* =15.0, 6.6/6 Hz, 1H)

Figure S30. ^{13}C NMR spectrum of **6** in CD_3OD-d_4 (150 MHz)

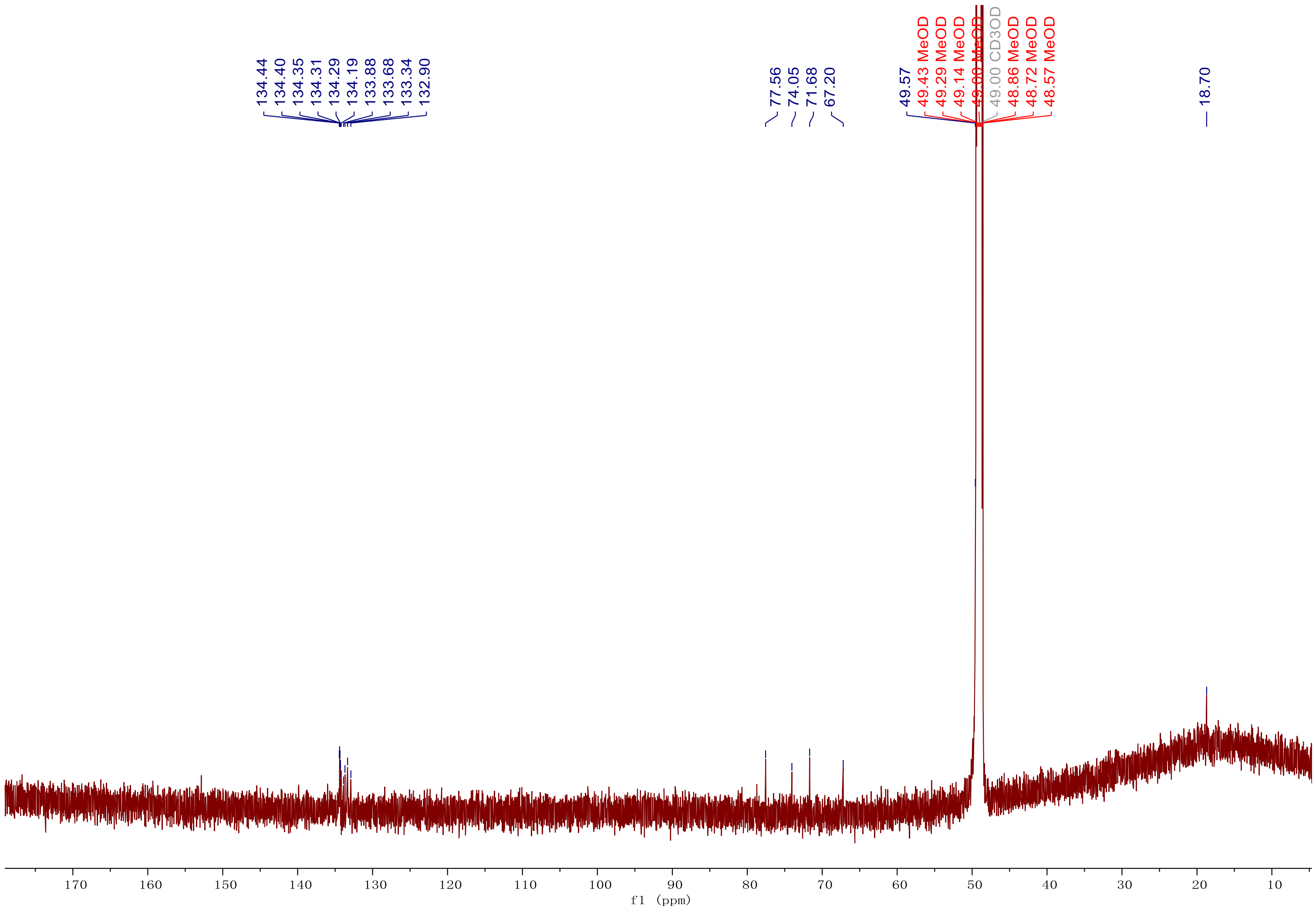


Figure S31. Expanded ^{13}C NMR spectrum of **6** in CD_3OD-*d*$_4$ (150 MHz).

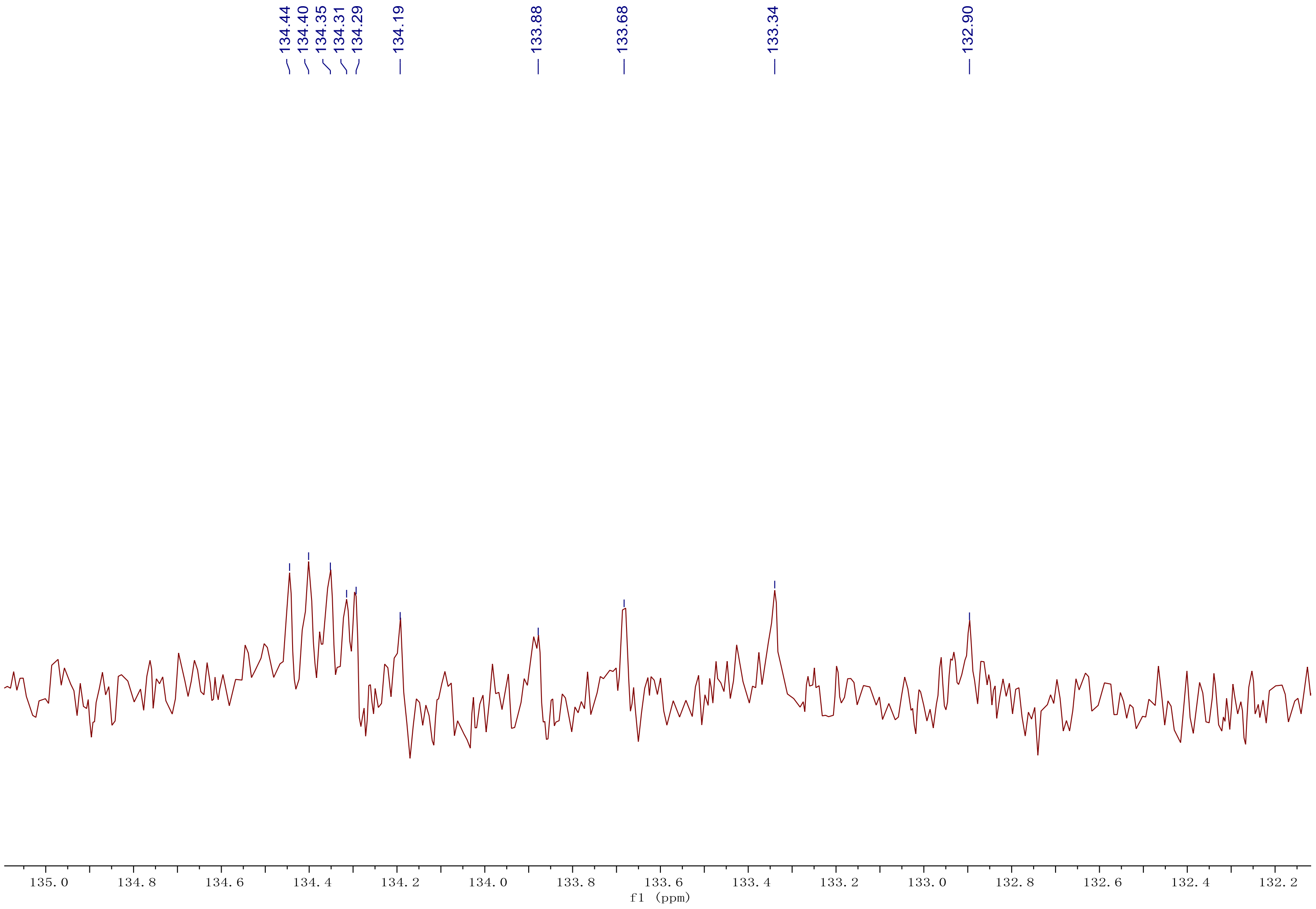


Figure S32. DEPT135 spectrum of **6** in CD_3OD-*d*$_4$.

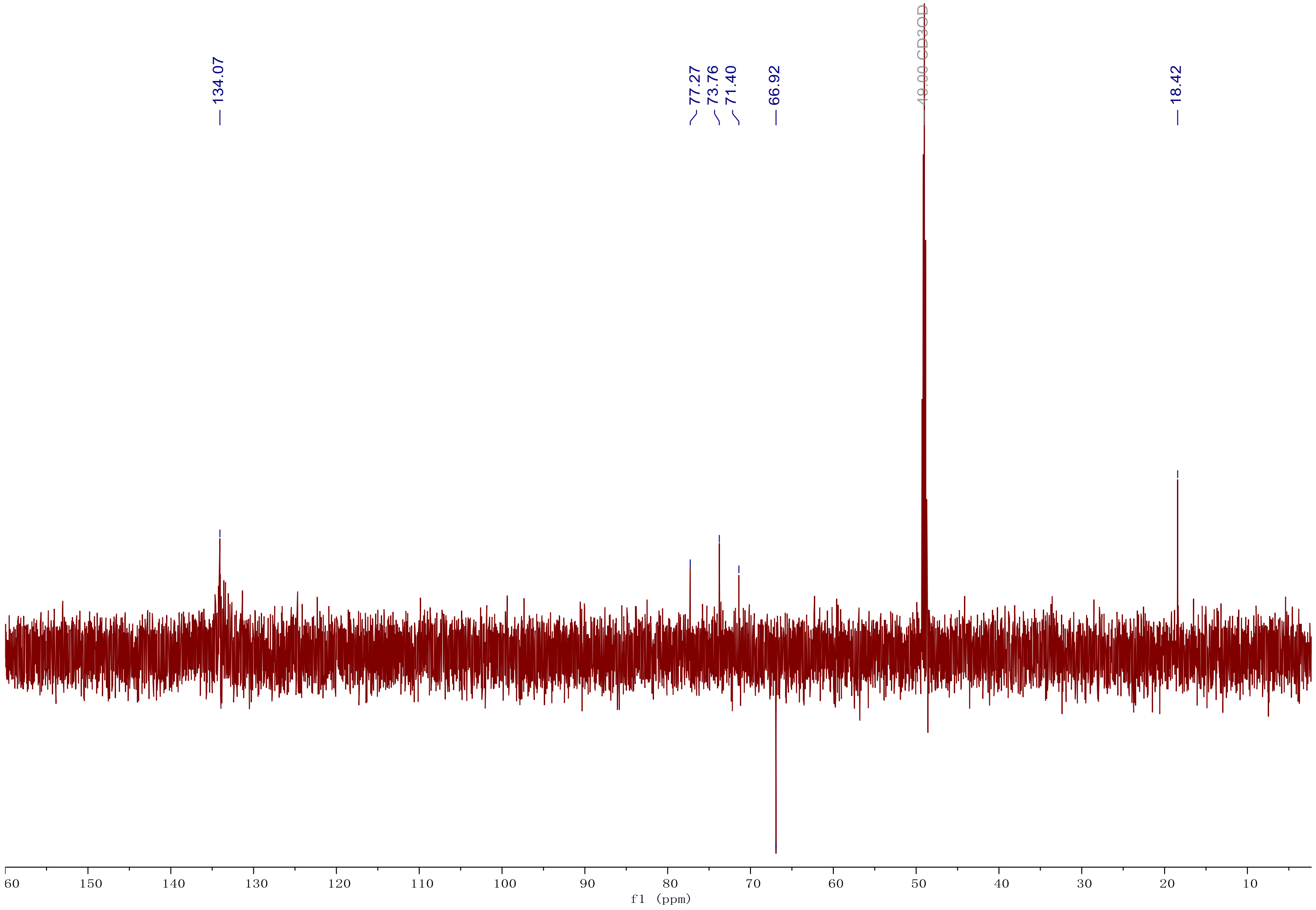


Figure S33. DEPT90 spectrum of **6** in CD_3OD-*d_4*.

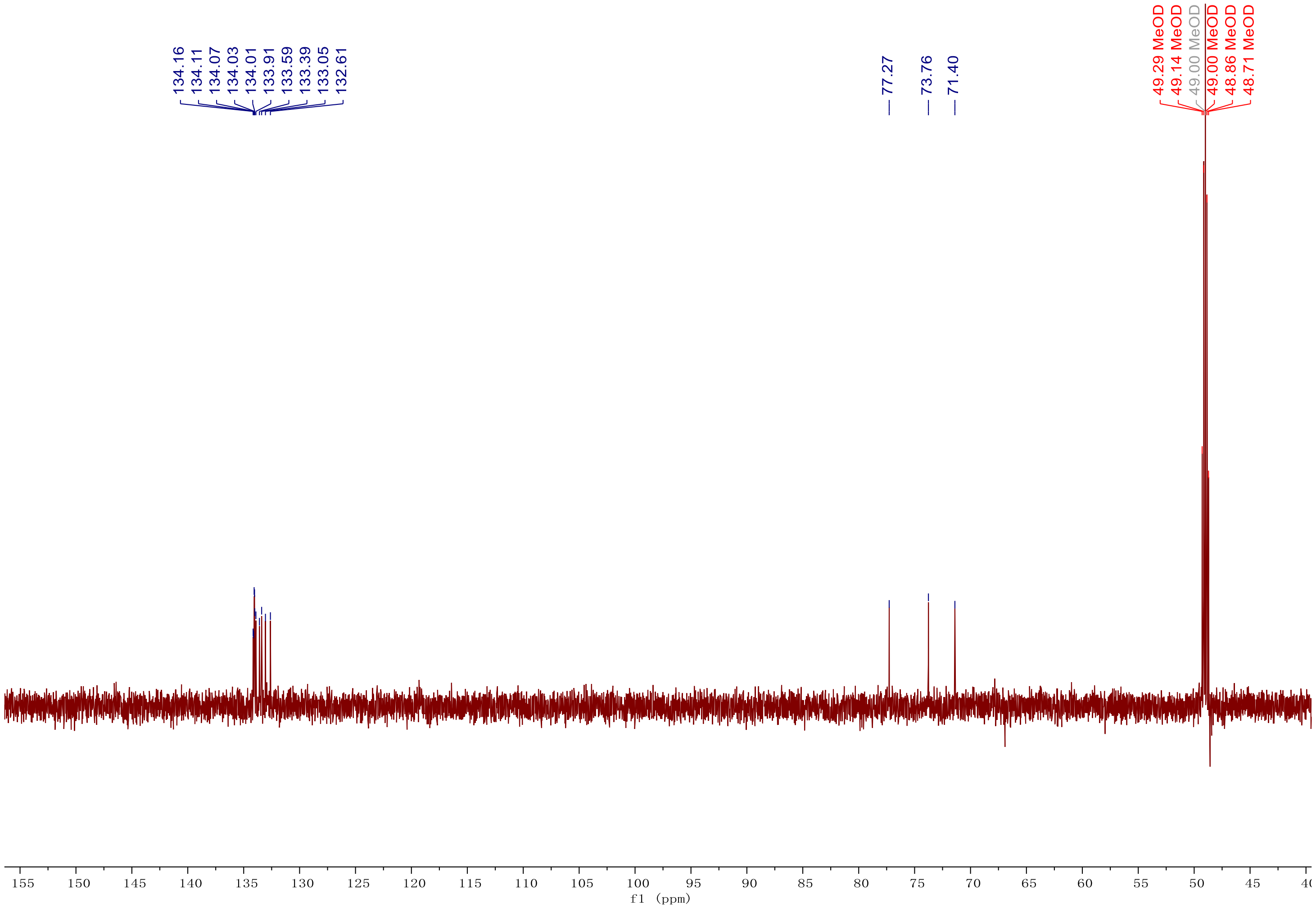


Figure S34. Expanded DEPT90 spectrum of **6** in CD_3OD-*d*$_4$.

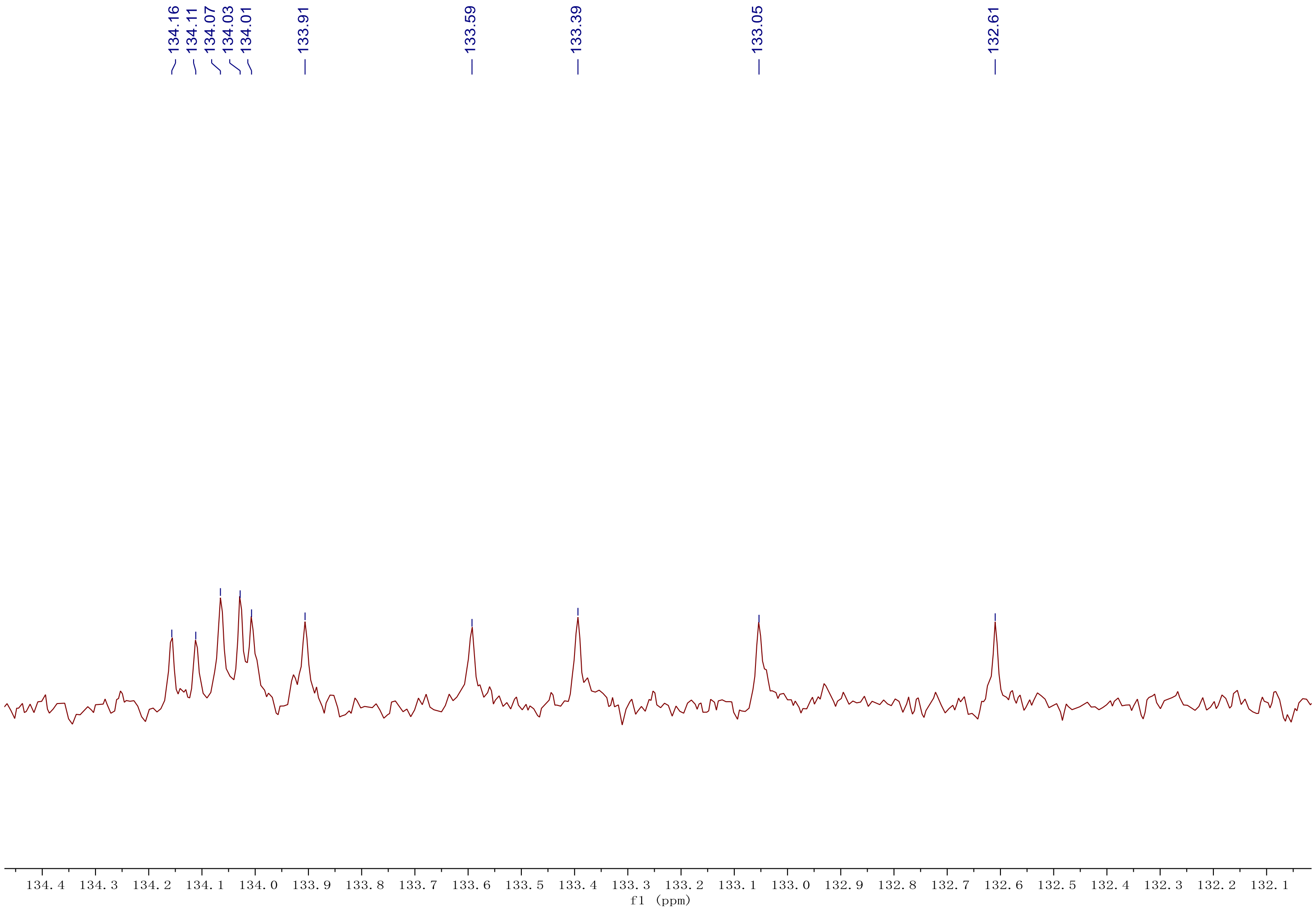


Figure S35. The homonuclear decoupling experiment for **6** using the Homodec sequence in CD_3OD-*d_4*.

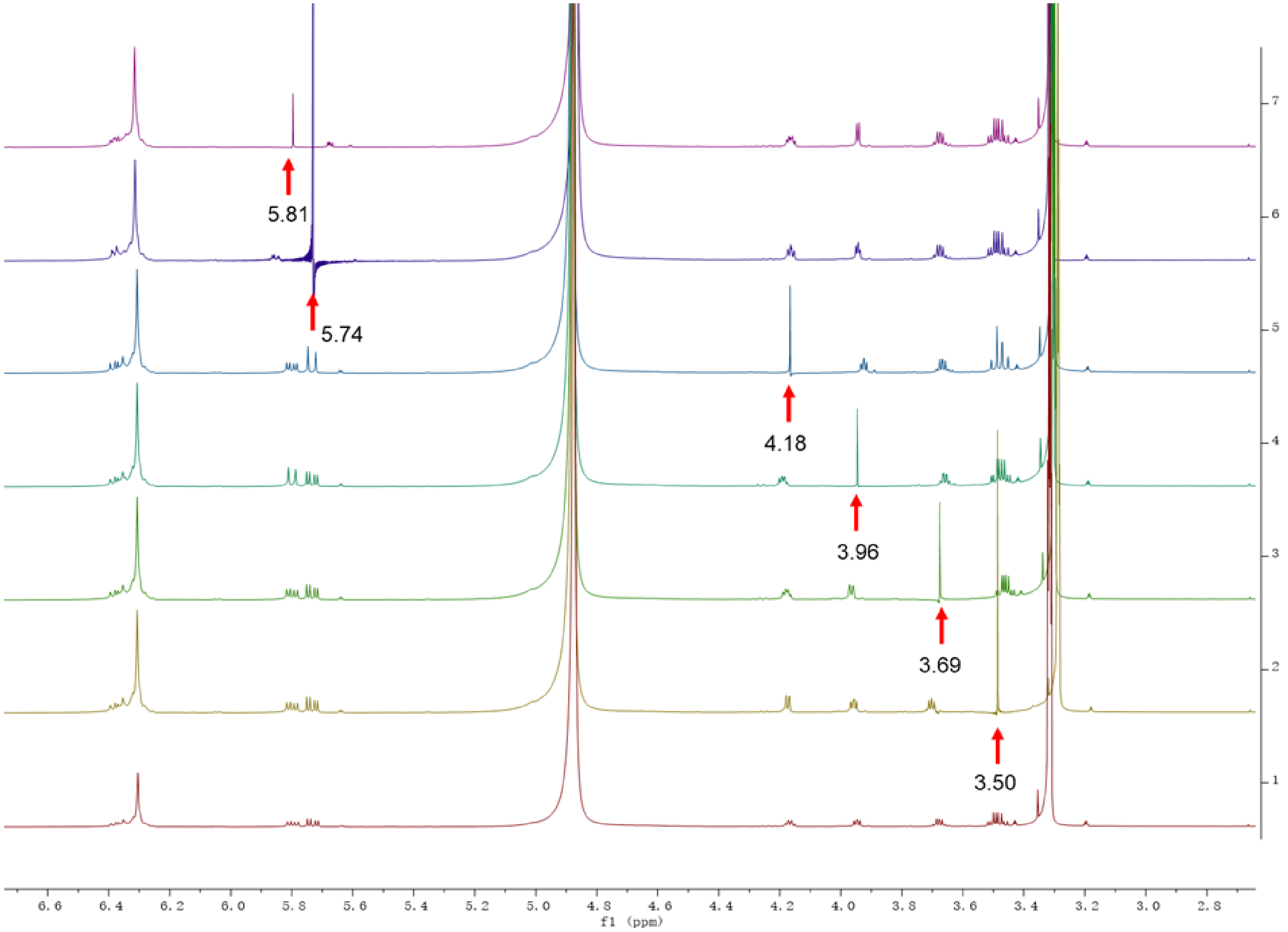


Figure S36. HSQC spectrum of **6** in CD_3OD-*d_4*.

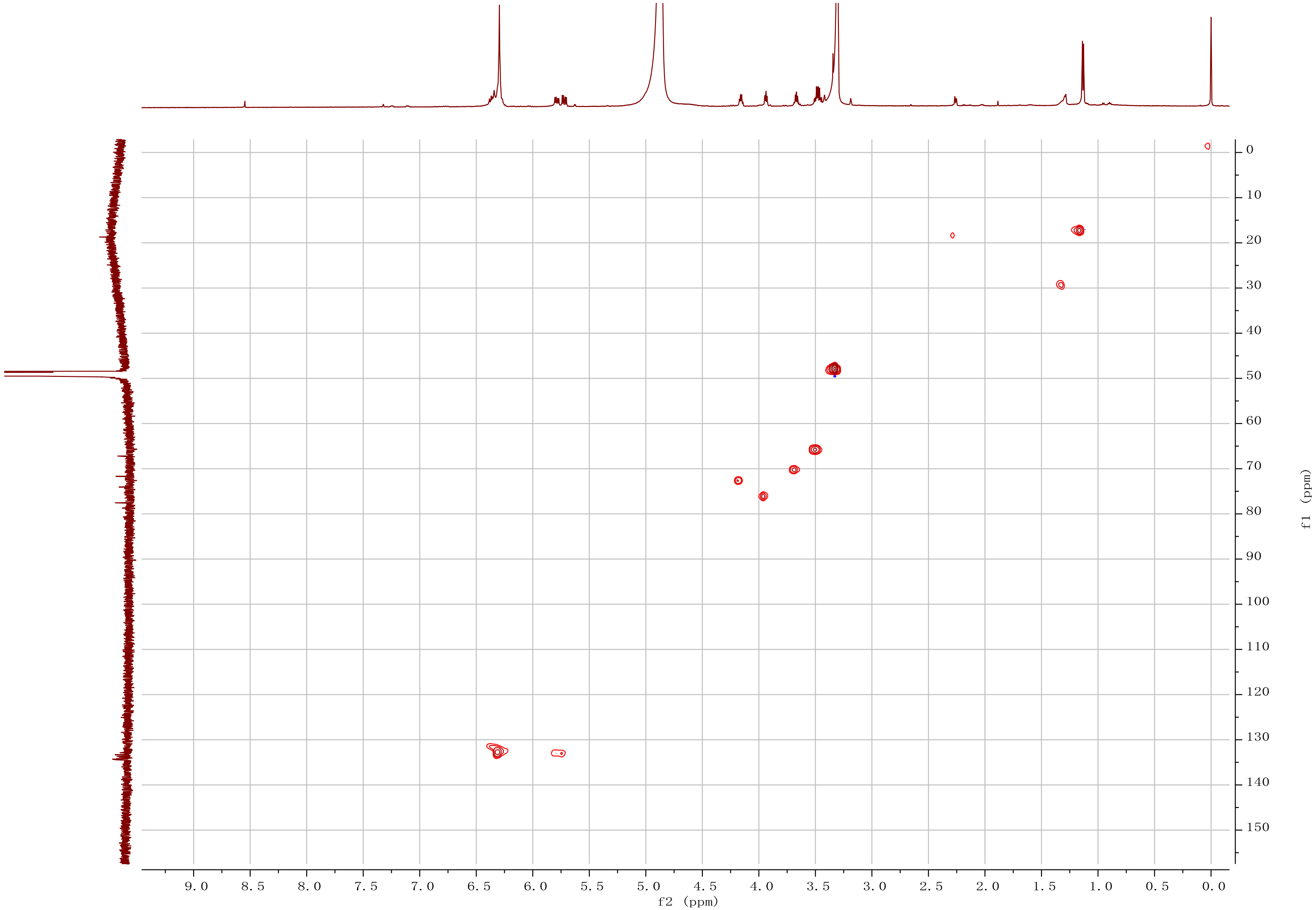


Figure S37. HMBC spectrum of **6** in CD_3OD-*d_4*.

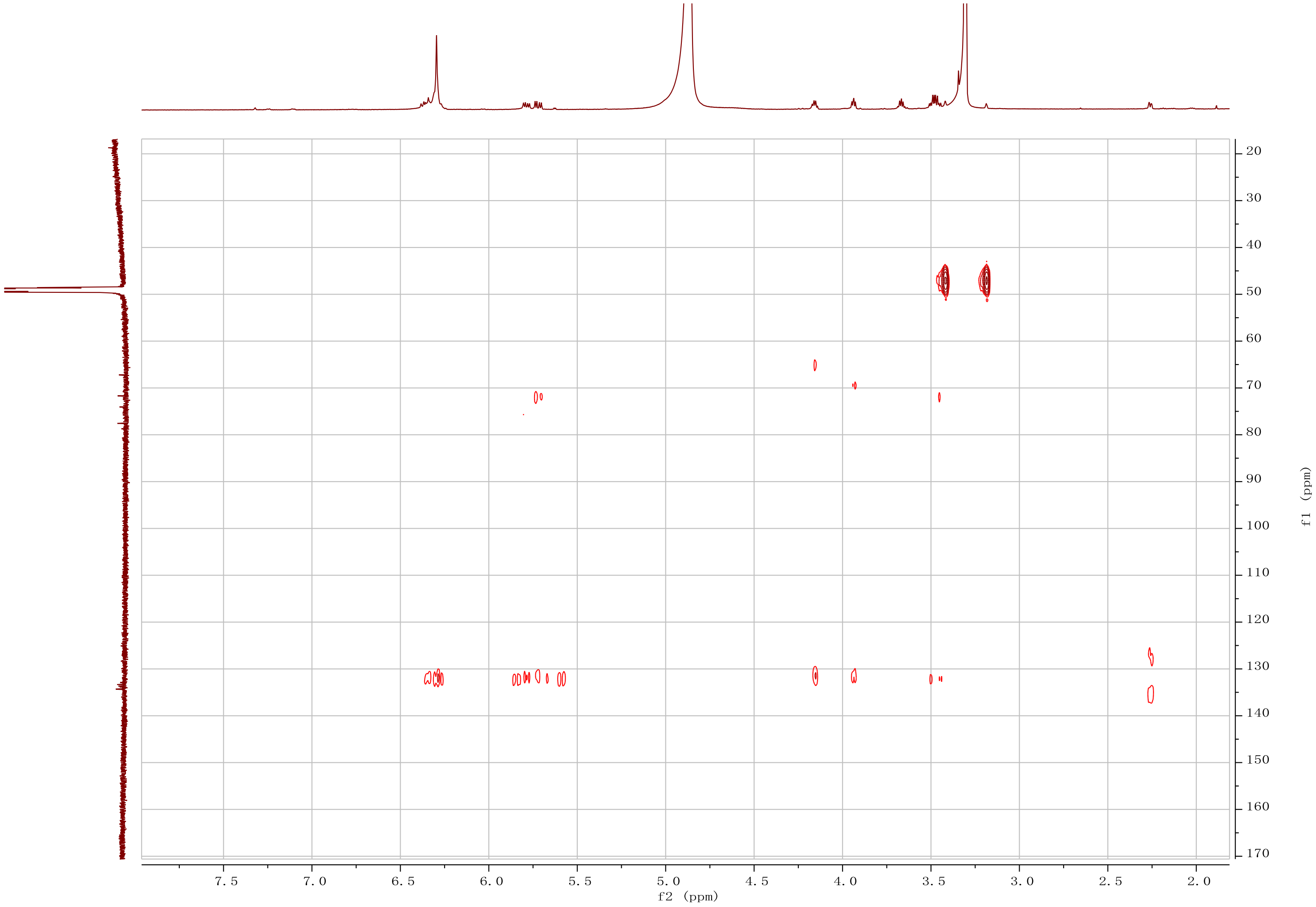


Figure S38. ^{1}H-^{1}H COSY spectrum of **6** in CD$_3$OD-*d*$_4$.

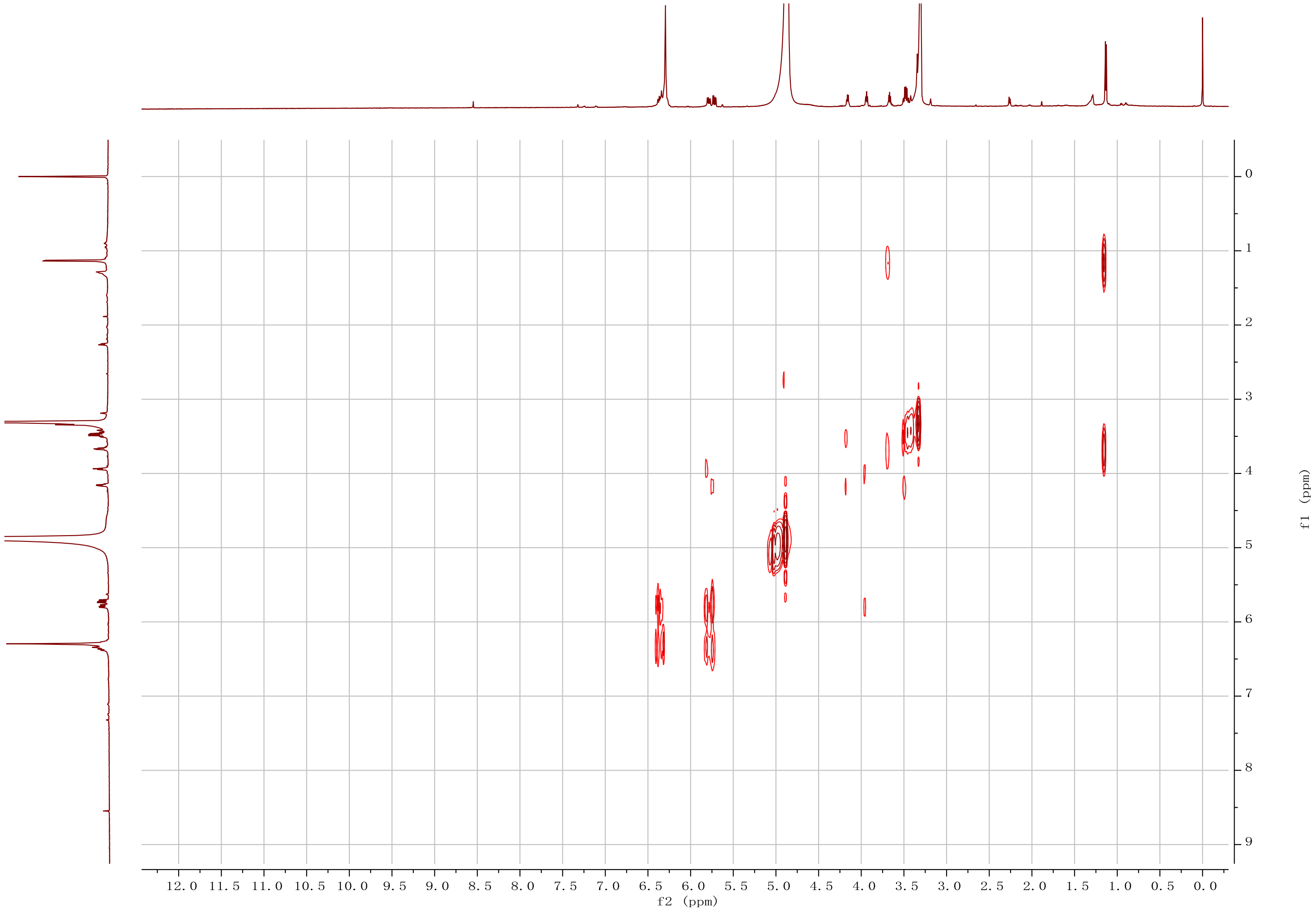


Figure S39. NOESY spectrum of **6**.

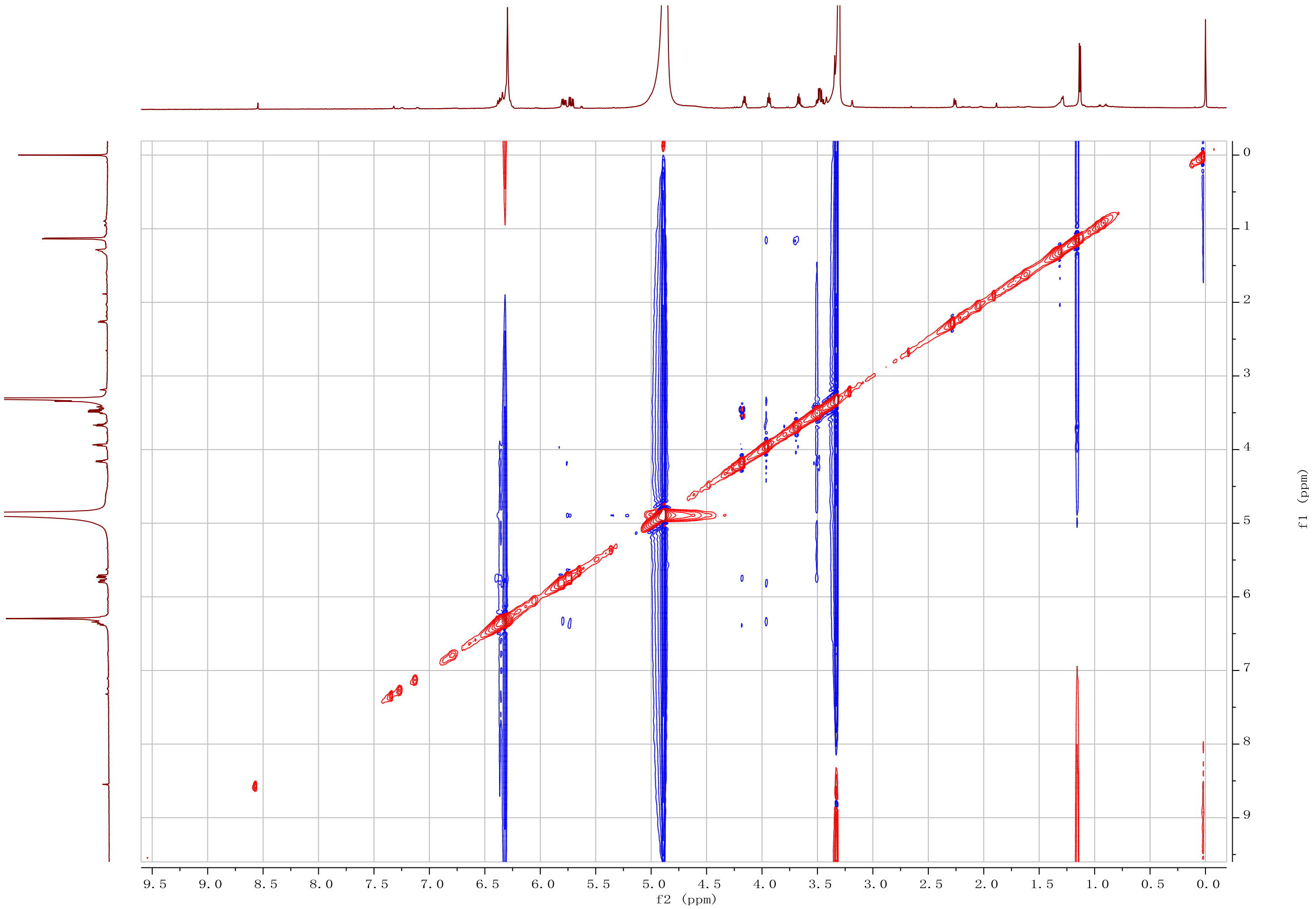


Figure S40. ^{1}H NMR spectrum of **7** in CD_3OD-d_4 (600 MHz).

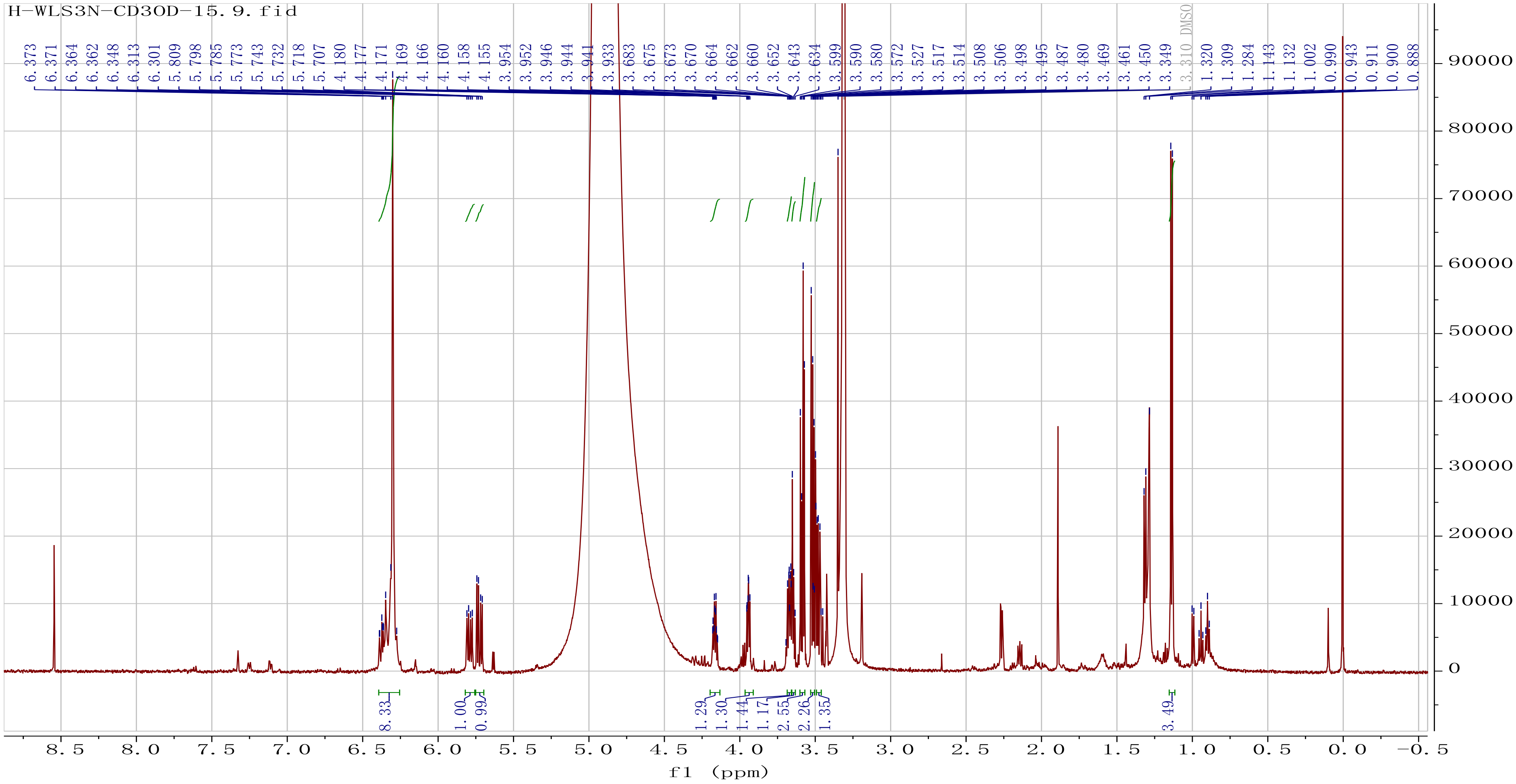


Figure S41. Expanded ^{1}H NMR spectrum of **7** in CD_3OD-*d_4* (600 MHz).

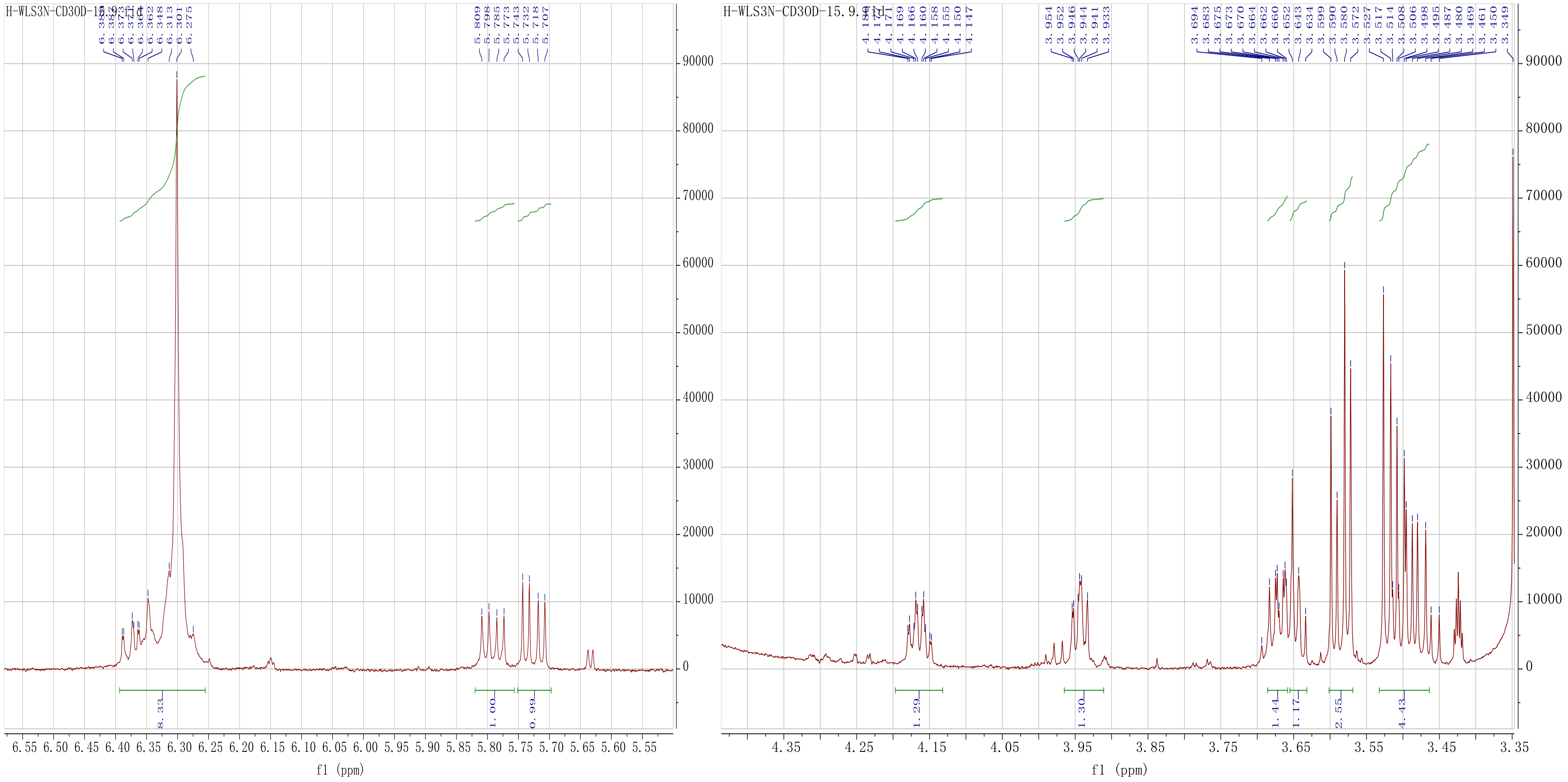


Figure S42. Expanded ^{1}H NMR spectrum of **7** (5.69–5.85 ppm) in CD_3OD-*d*$_4$ (600 MHz).

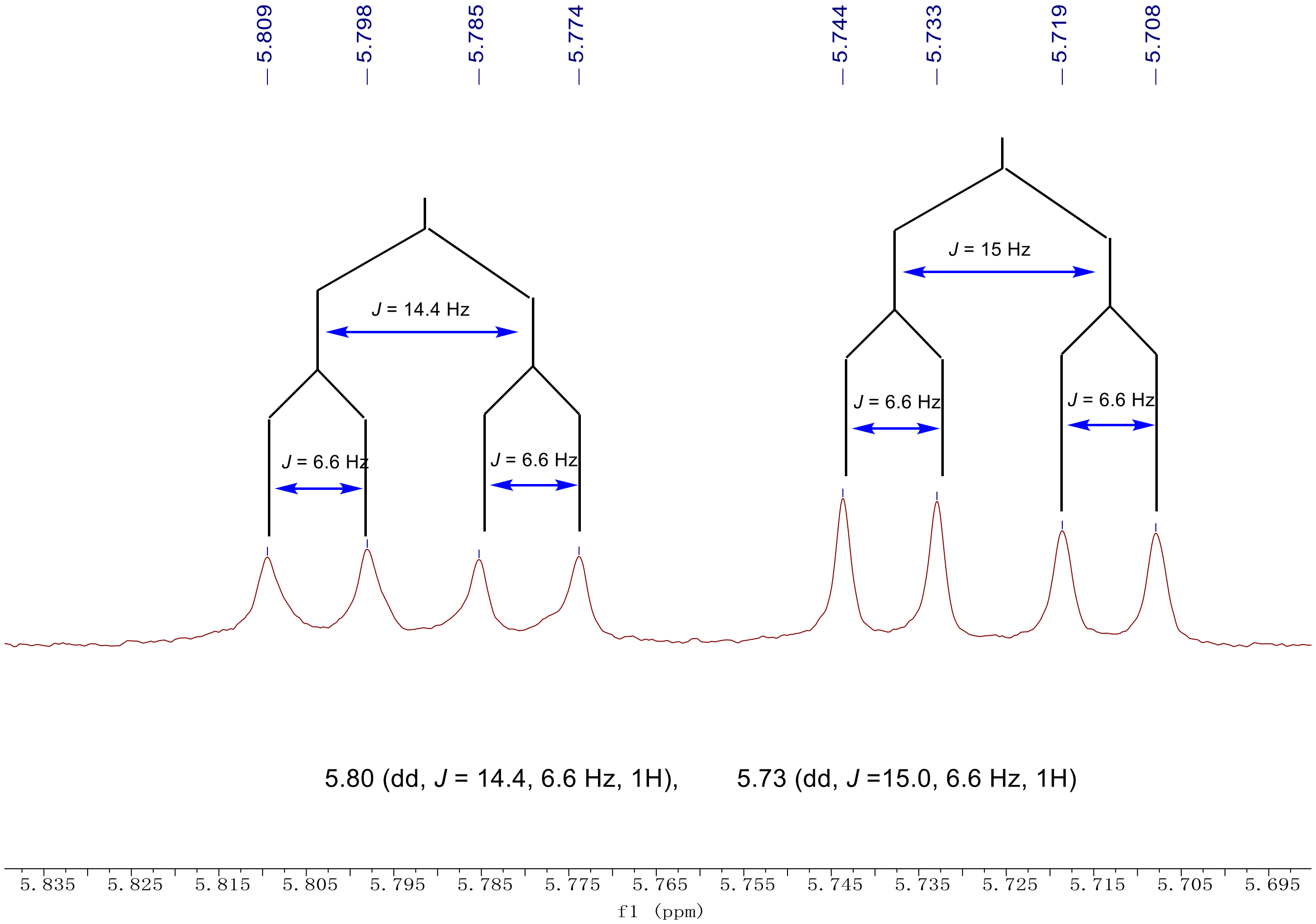


Figure S43. ^{13}C NMR spectrum of **7** in CD_3OD-*d_4* (150 MHz).

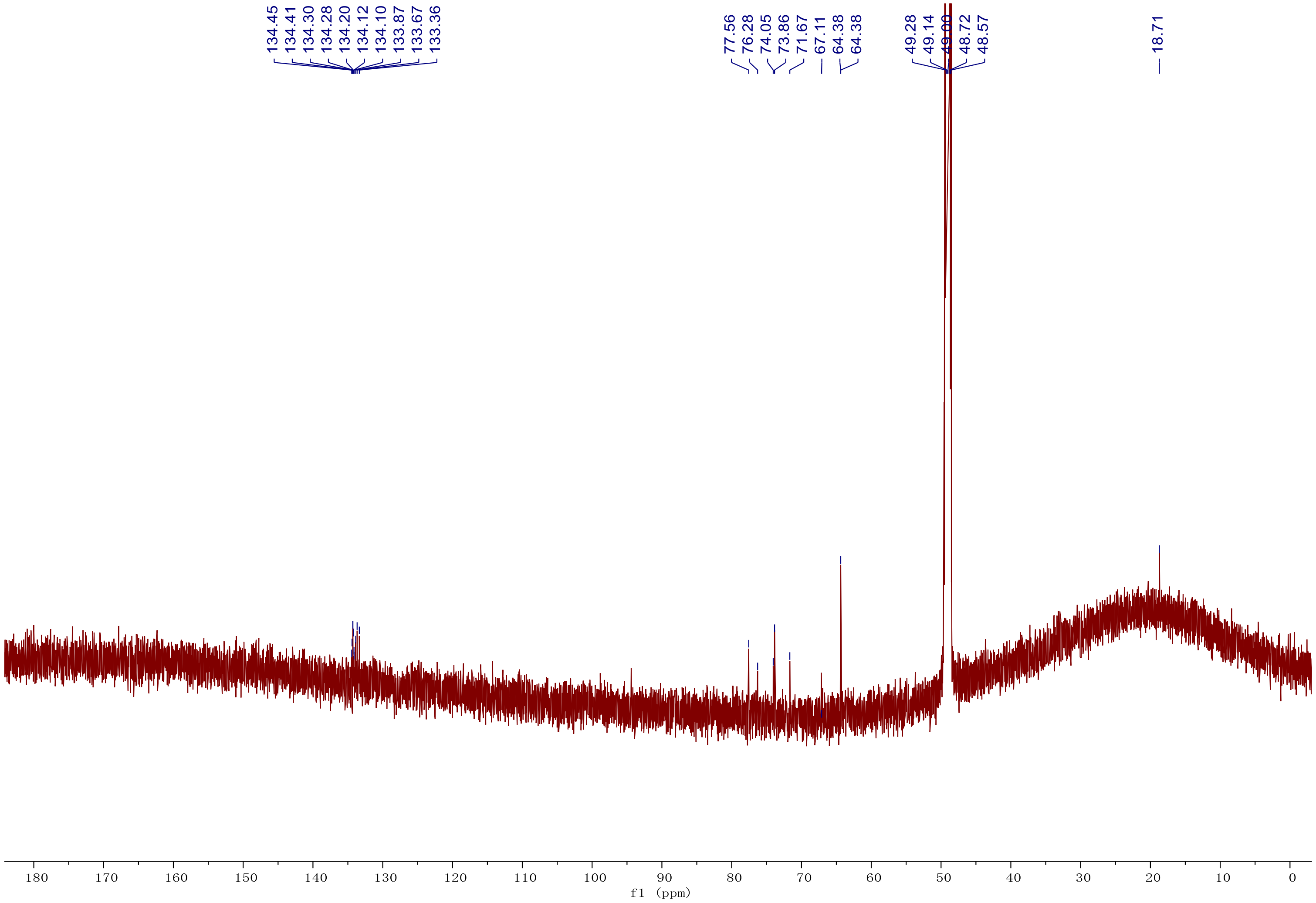


Figure S44. Expanded ^{13}C NMR spectrum of **7** in CD_3OD-*d_4* (150 MHz).

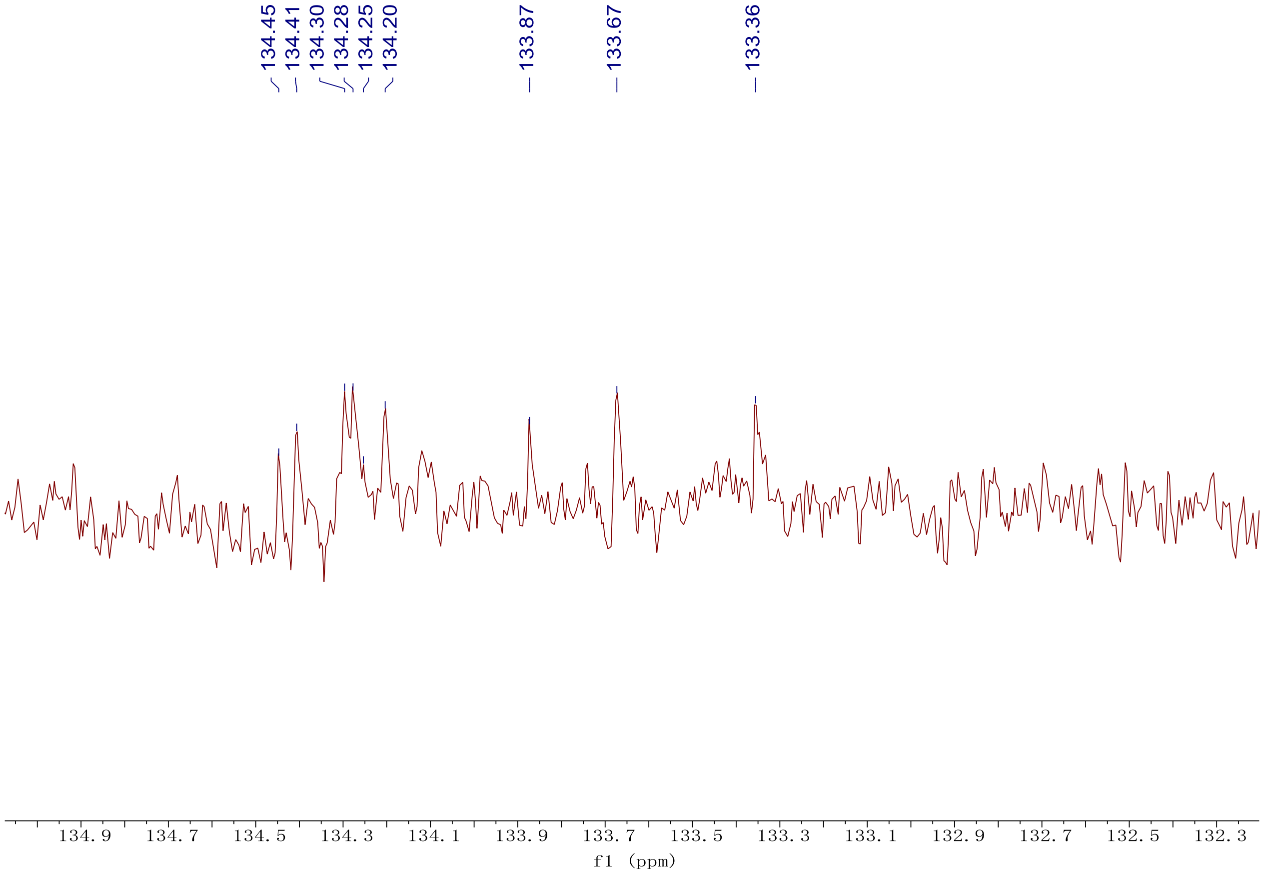


Figure S45. DEPT135 spectrum of **7** in CD$_3$OD-*d*$_4$.

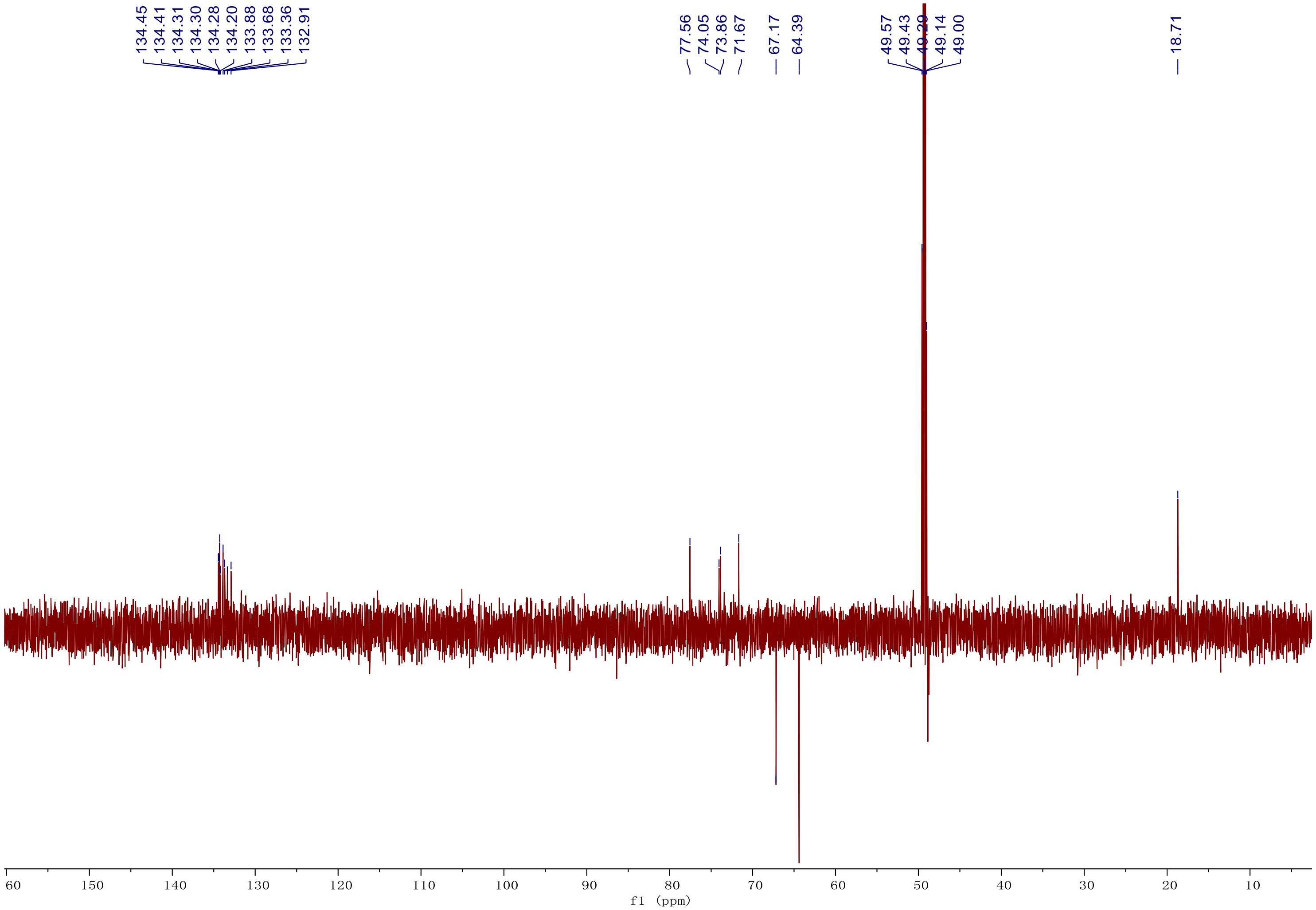


Figure S46. Expanded DEPT135 spectrum of **7** in CD_3OD-*d₄*.

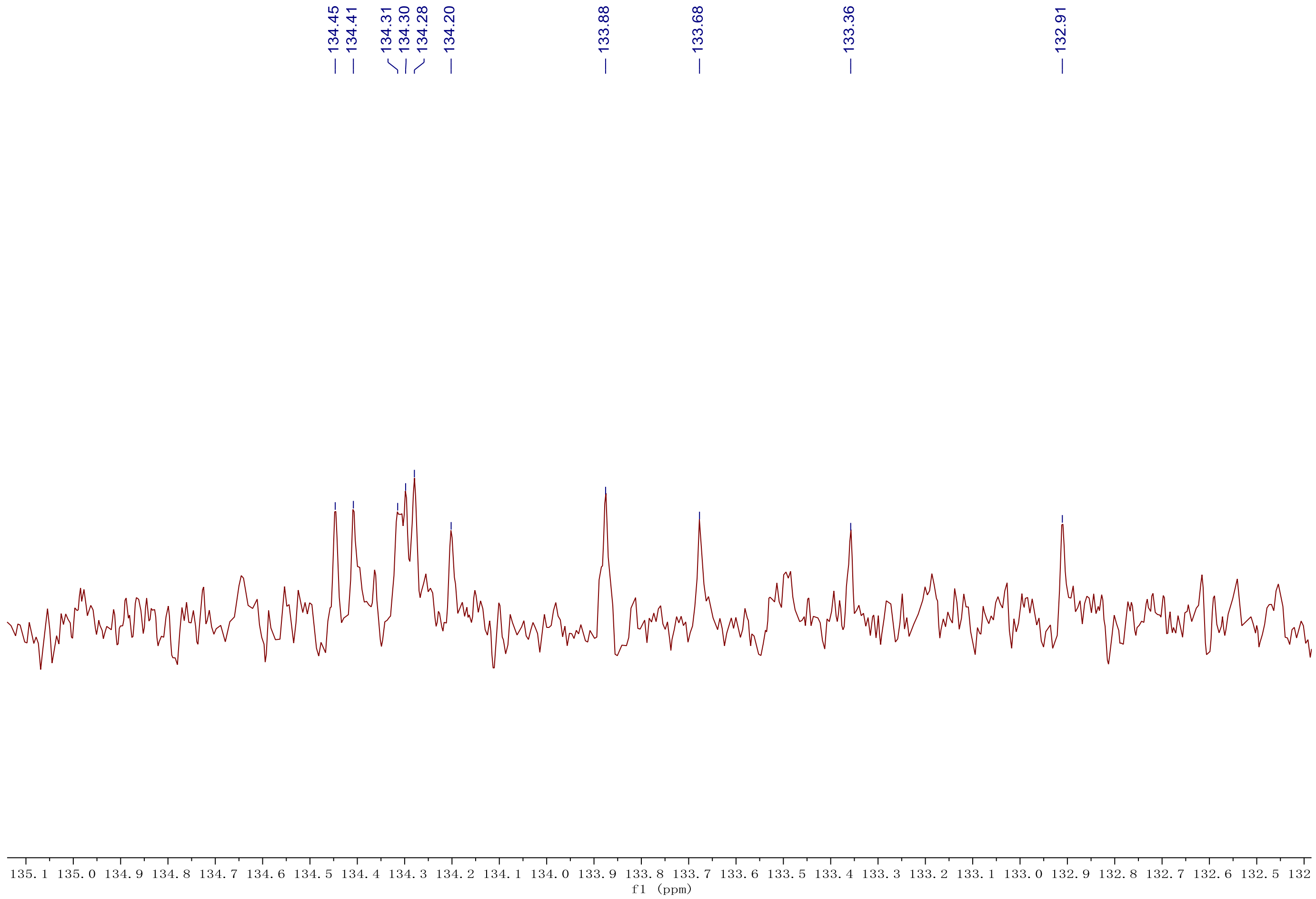


Figure S47. ^{1}H-^{1}H COSY spectrum of **7** in CD_3OD-*d*$_4$.

Figure S48. Expanded ^{1}H-^{1}H COSY spectrum of **7** in CD_3OD-d_4.

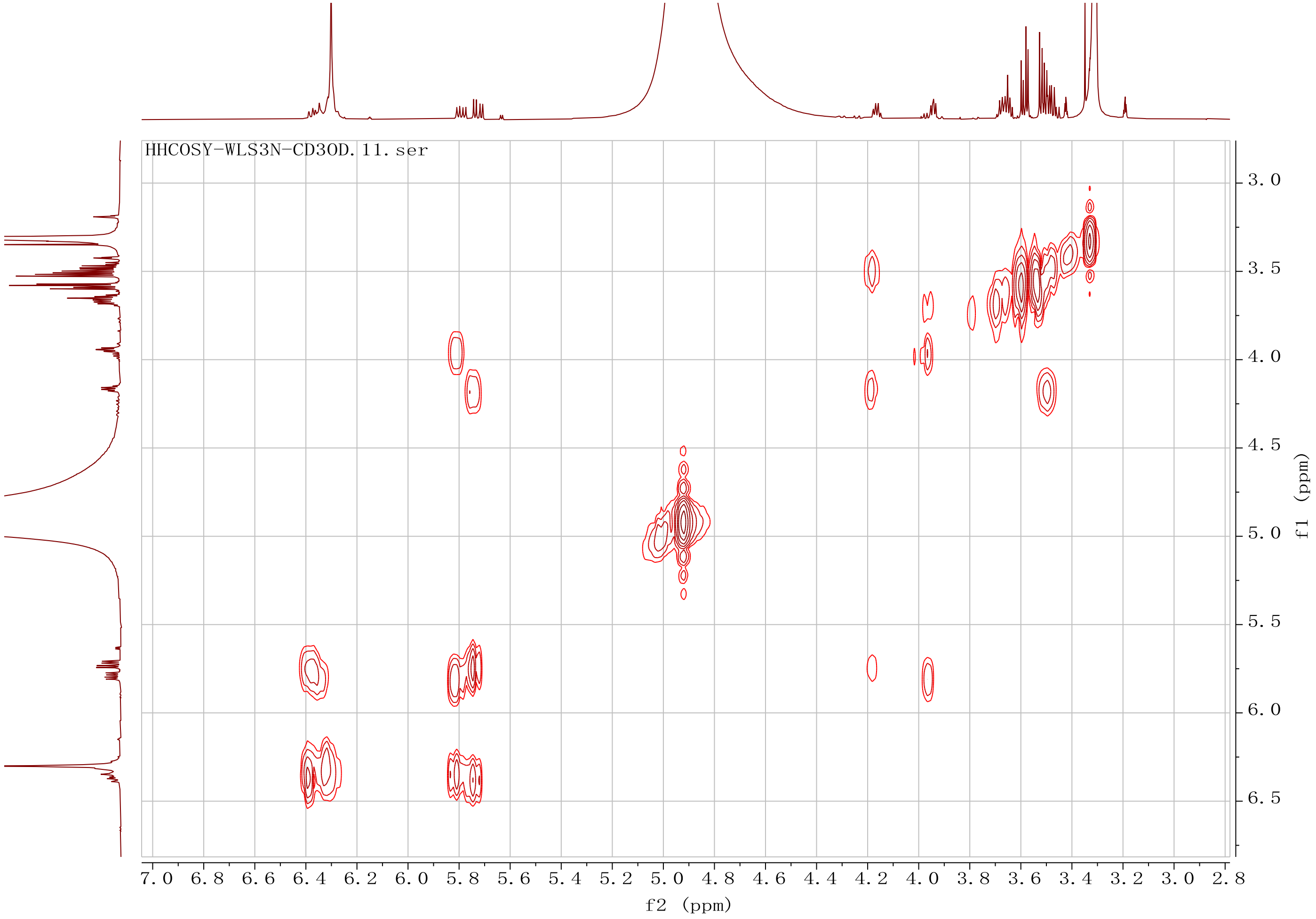


Figure S49. HSQC spectrum of **7** in CD_3OD-d_4.

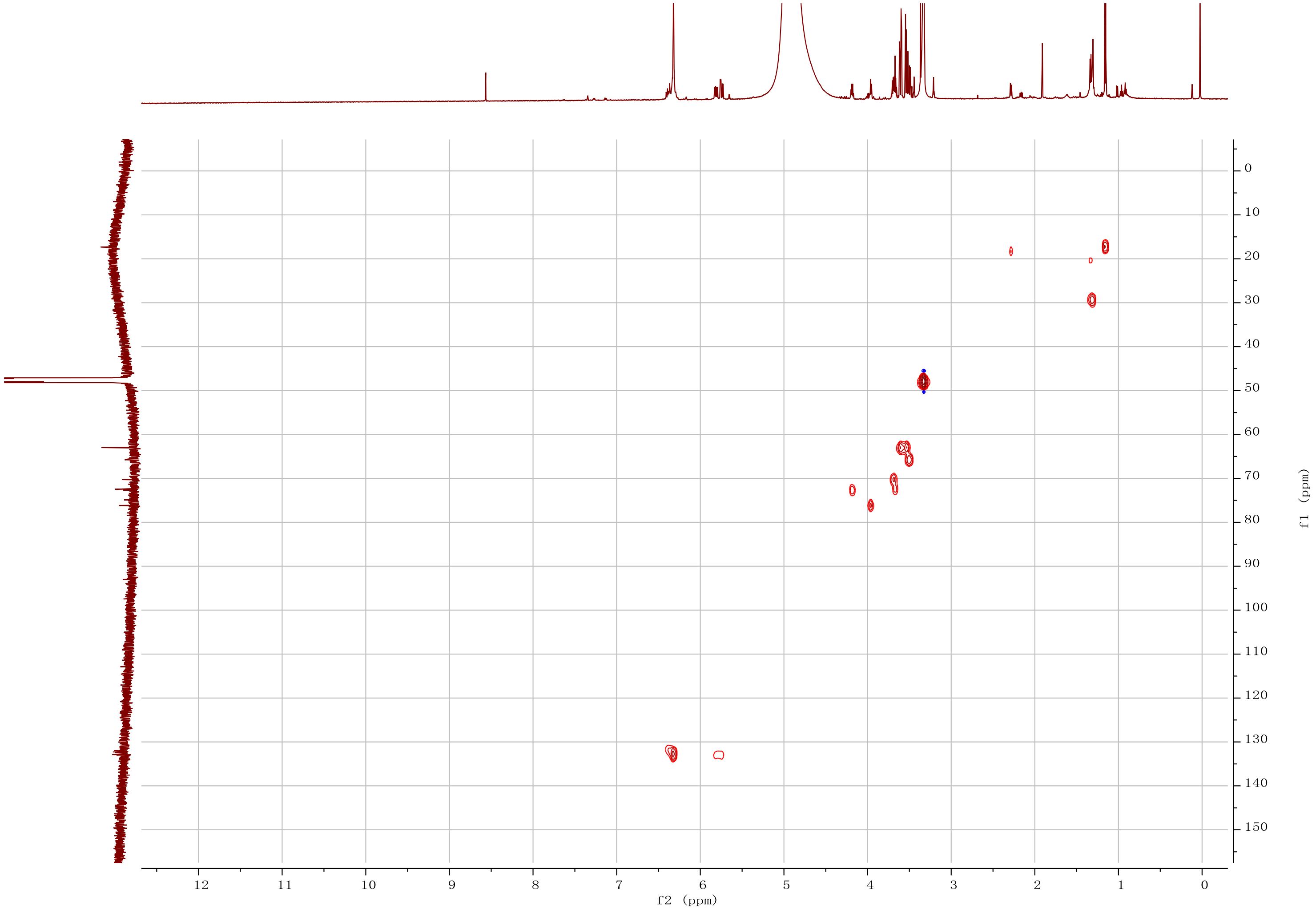


Figure S50. Expanded HSQC spectrum of **7** in CD_3OD-*d_4*.

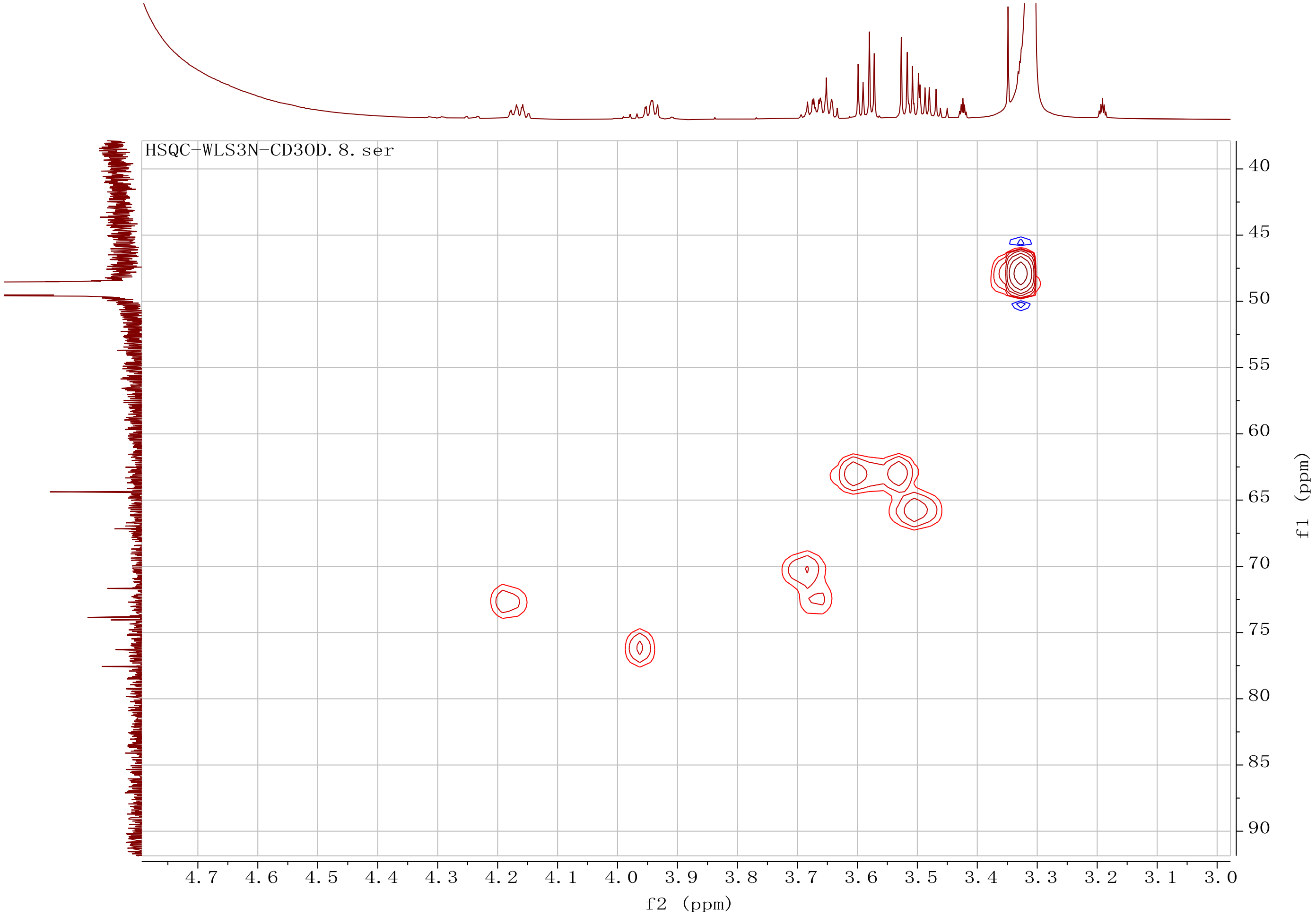


Figure S51. HMBC spectrum of **7** in CD_3OD-*d_4*.

Figure S52. Expanded HMBC spectrum of **7** in CD$_3$OD-d_4.

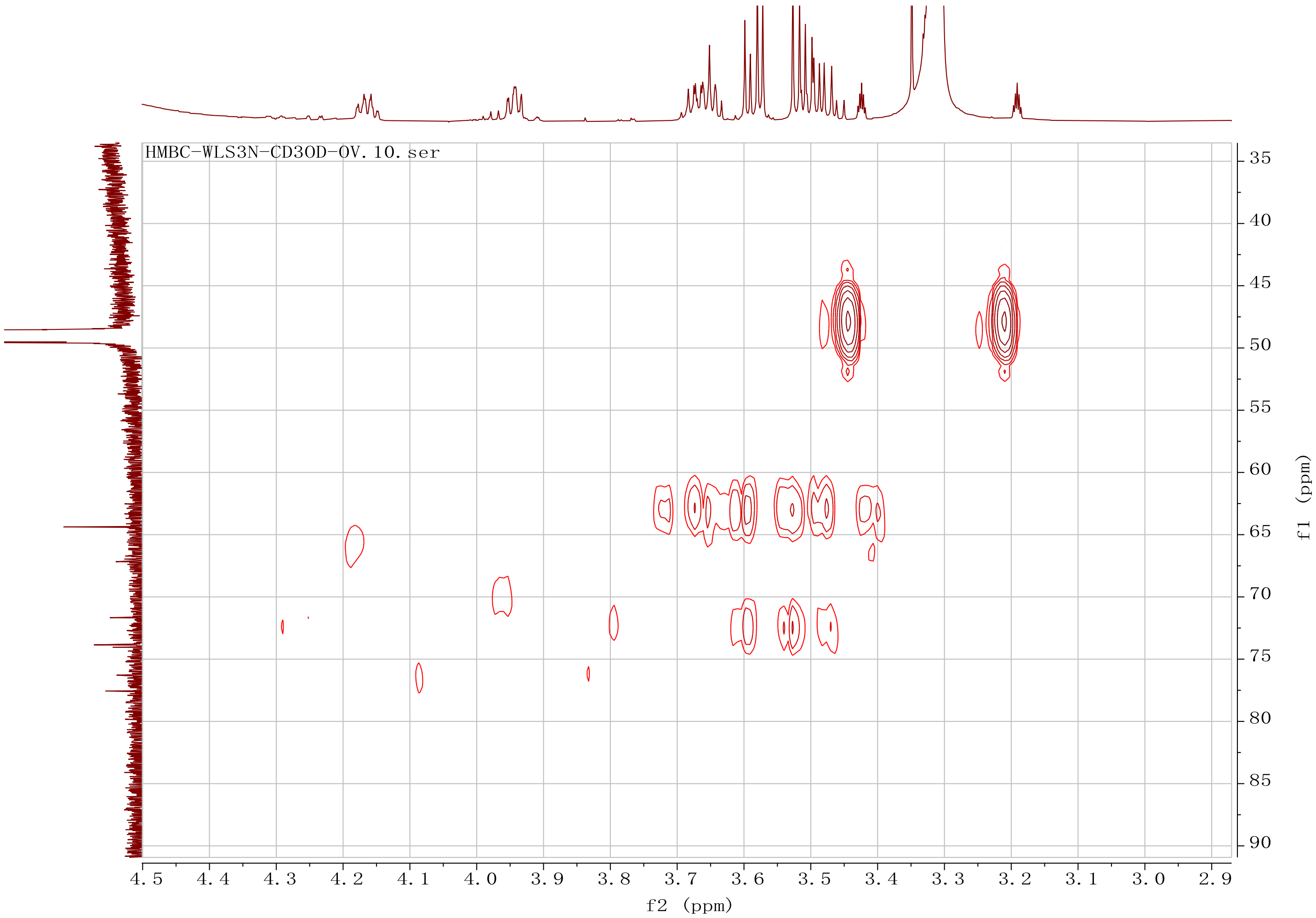


Figure S53. NOESY spectrum of **7**.

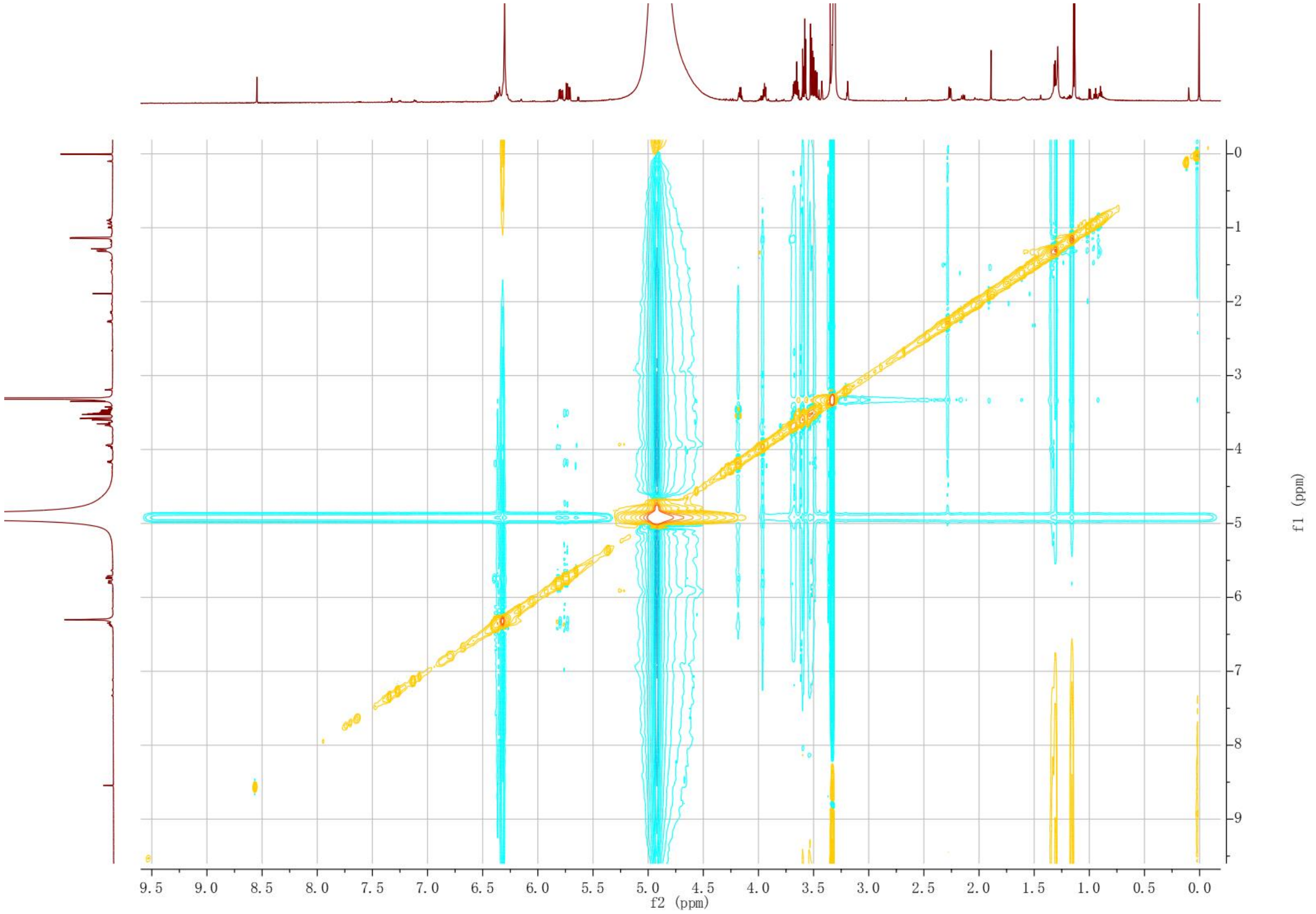


Figure S54. ^{1}H NMR (500 MHz) spectrum of **8** in DMSO-d_6.

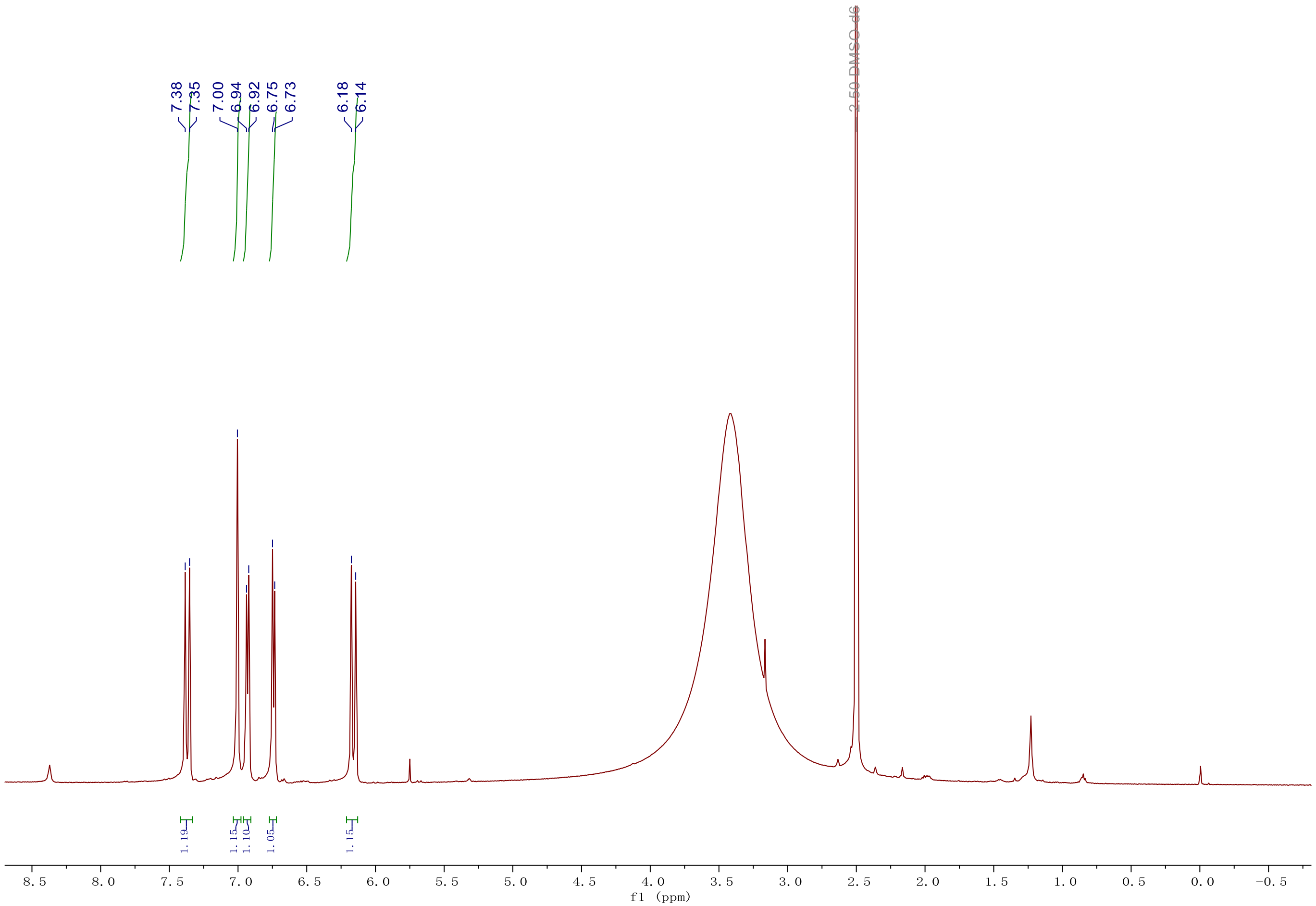


Figure S55. ^{13}C NMR (125 MHz) spectrum of **8** in DMSO-d_6.

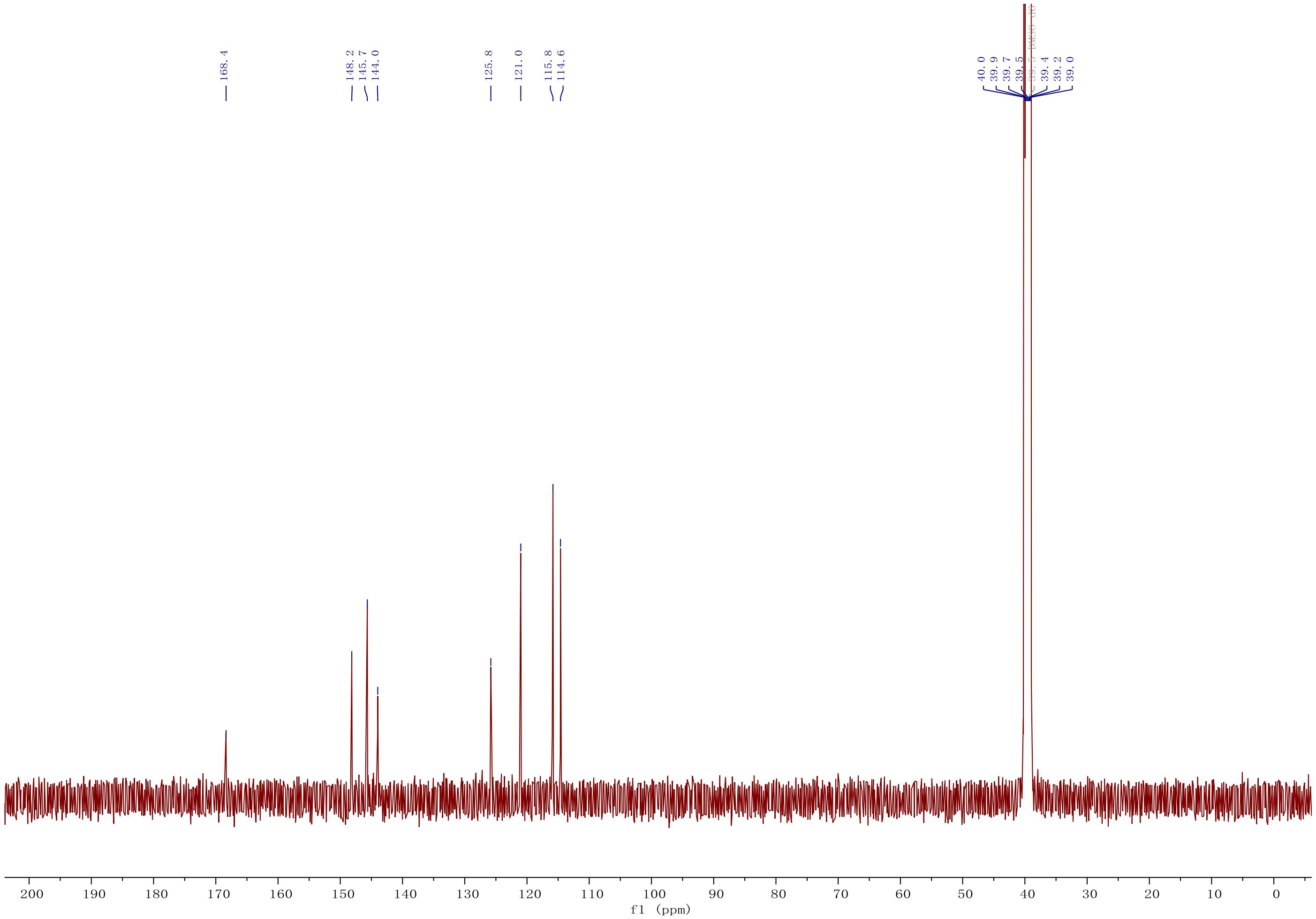


Figure S56. DEPT135 spectrum of **8** in DMSO-d_6.

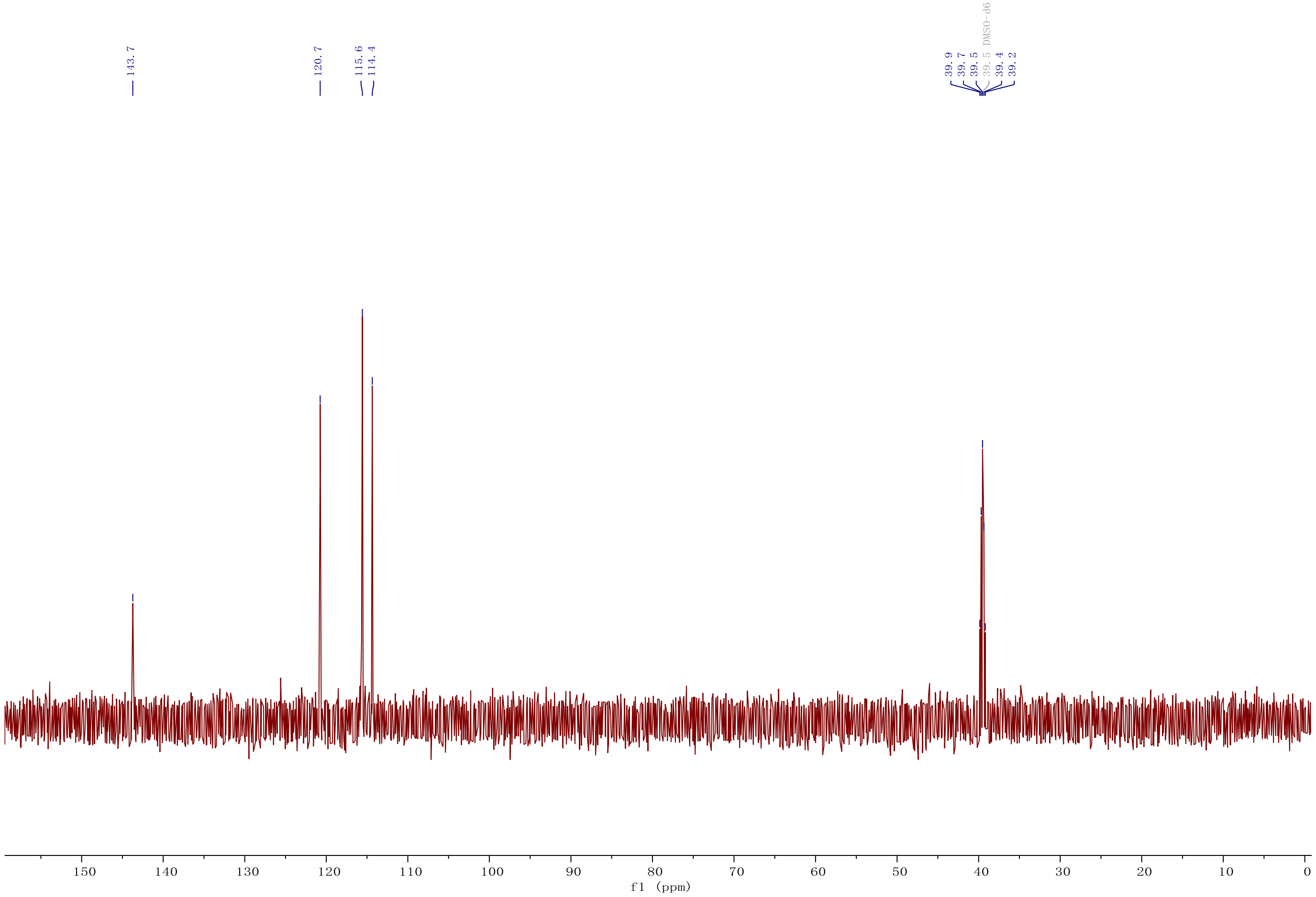


Figure S57. ^{1}H-^{1}H COSY spectrum of **8** in DMSO-*d*$_6$.

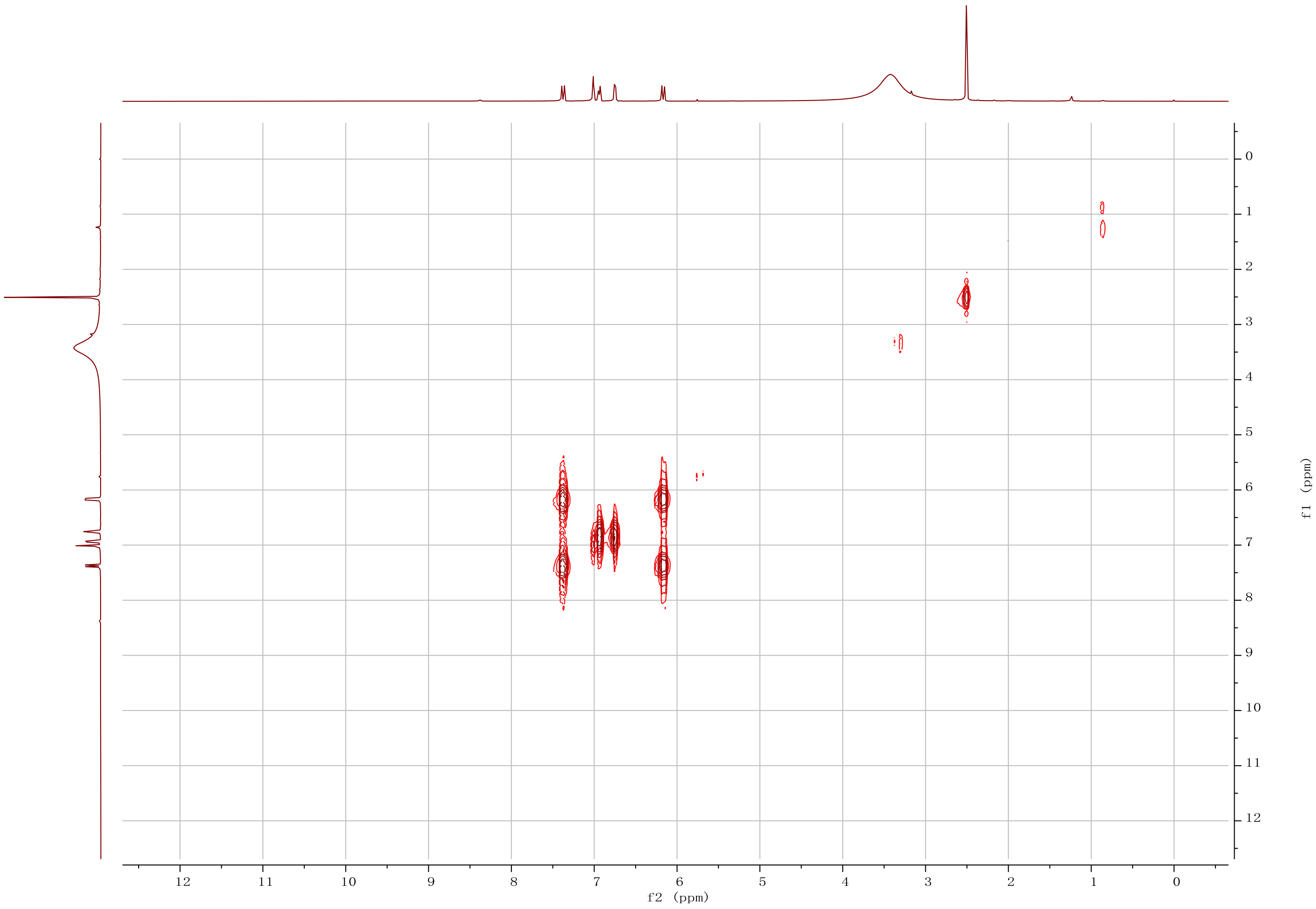


Figure S58. HSQC spectrum of **8** in DMSO-d_6.

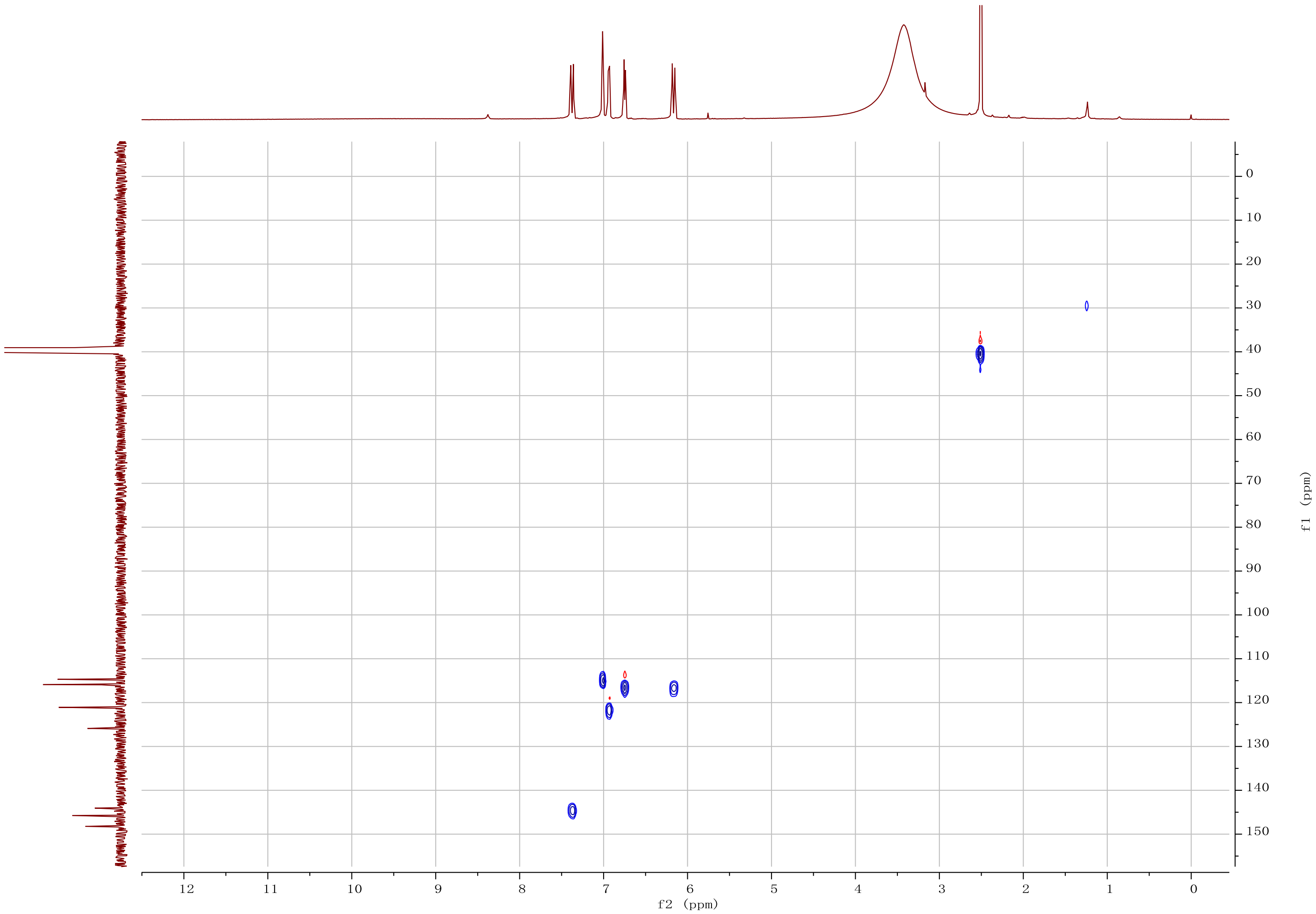


Figure S59. HMBC spectrum of **8** in DMSO-d_6.

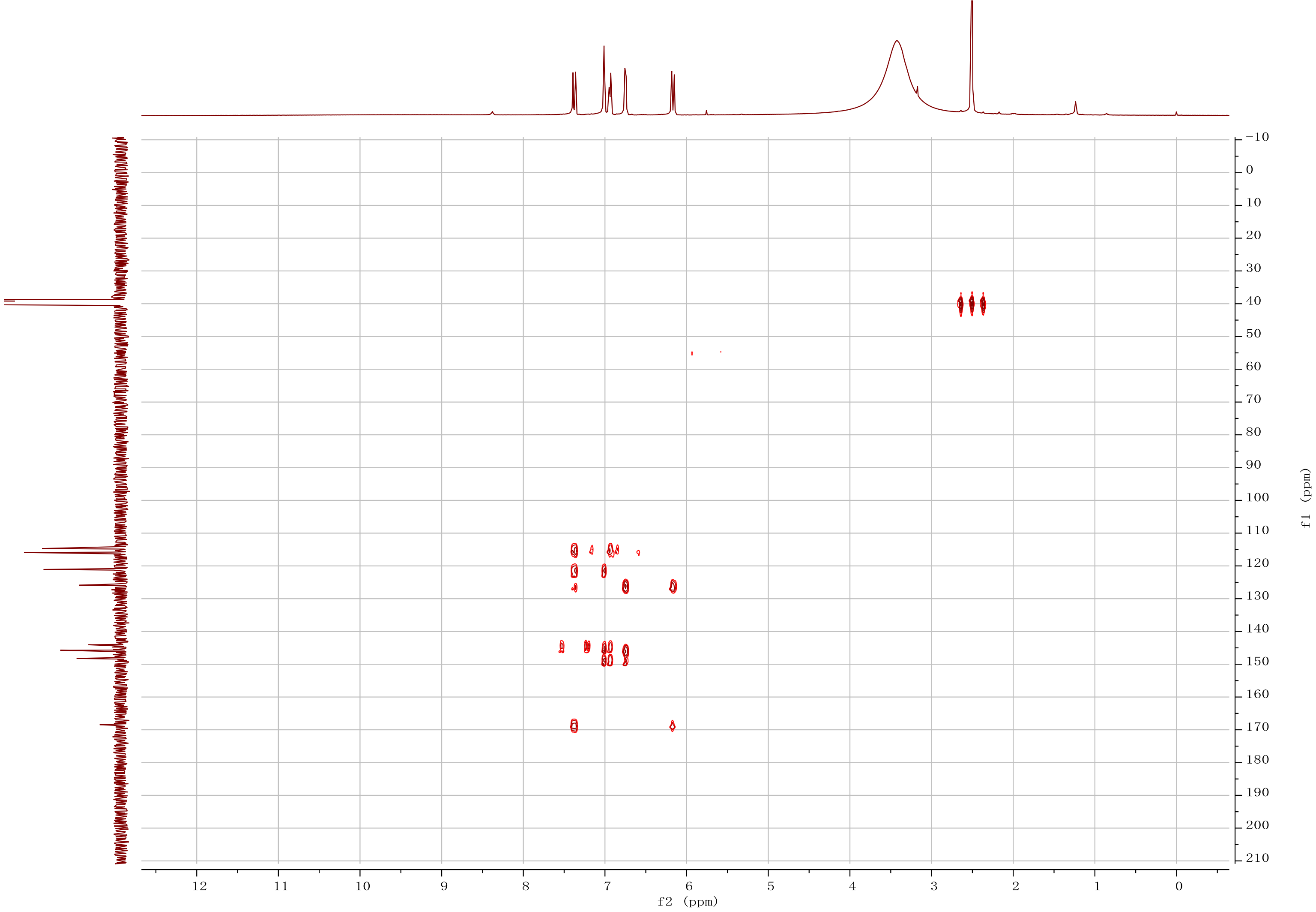


Figure S60. ^{1}H NMR (500 MHz) spectrum of **9** in DMSO-d_6.

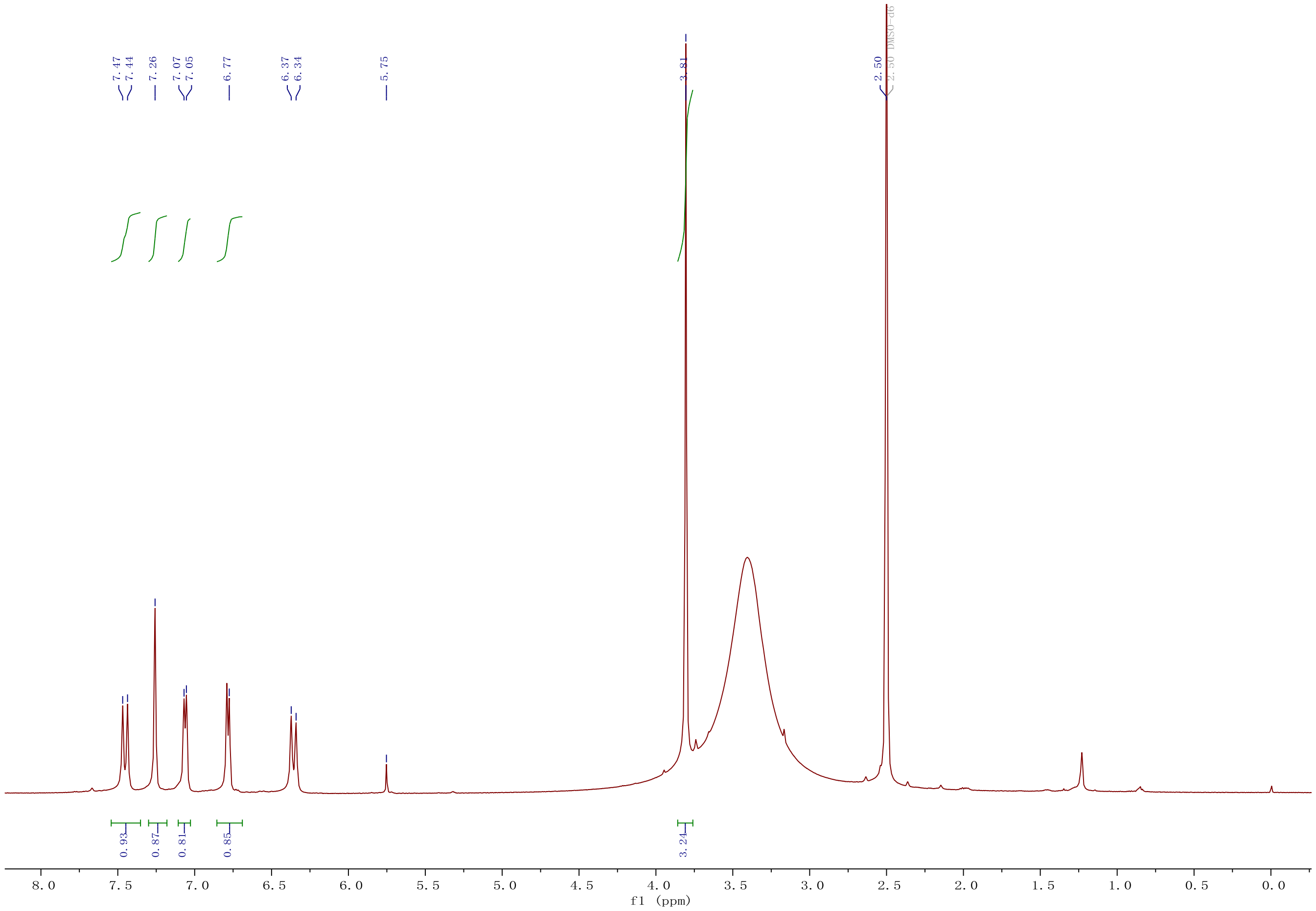
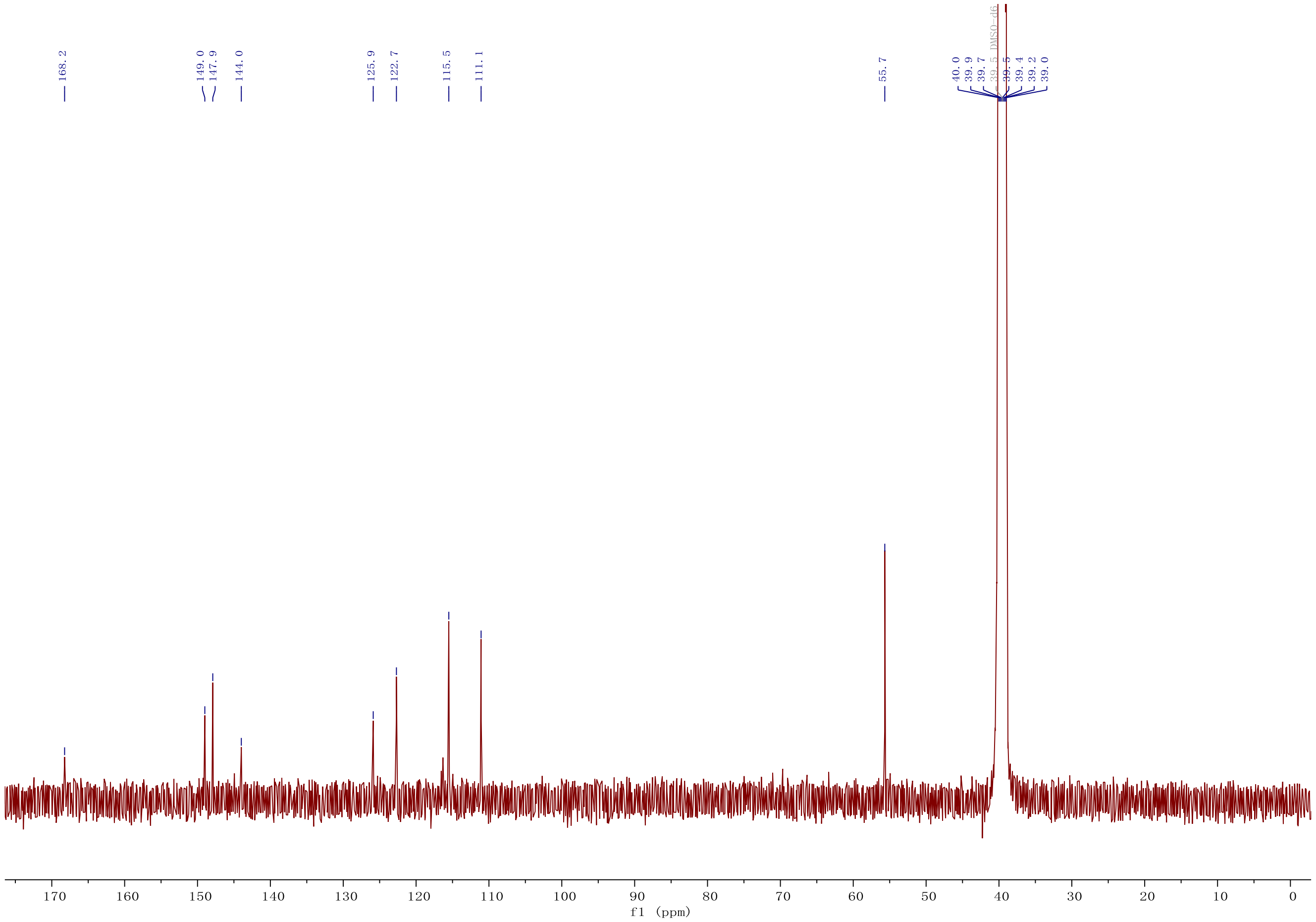


Figure S61. ^{13}C NMR (125 MHz) spectrum of **9** in DMSO-d_6.



Reference

(1) X. Yan, H. Ge, T. Huang, Hindra, D. Yang, Q. Teng, I. Crnovčić, X. Li, J. D. Rudolf, J. R. Lohman, Y. Gansemans, X. Zhu, Y. Huang, L. X. Zhao, Y. Jiang, F. Van Nieuwerburgh, C. Rader, Y. Duan, B. Shen, *mBio* **2016**, *7 e02104-16*.

(2) X. Yan, Hindra, H. Ge, D. Yang, T. Huang, I. Crnovcic, C.Y. Chang, S.M. Fang, T. Annaval, X. Zhu, Y. Huang, L. X. Zhao, Y. Jiang, Y. Duan, B. Shen. *J. Nat. Prod.* **2018**, *81*, 594-599.

(3) Liu. L, Li. S, Sun. R, Qin. X, Ju. J, Zhang. C, Duan. Y, Huang. Y, *Org. Lett.* **2020**, 22, 4614-4619..