Supporting information

Asperphenamate biosynthesis reveals a novel two-module NRPS system to synthesize amino acid esters in fungi

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Supplementary Methods

Strains, media and growth conditions

The fungal strains used in this study are summarized in Table S1. *Penicillum brevicompactum* xz118 isolated from leaves of an unknown plant growing in Heihe Forest Park, Shaanxi, China, was used as the parental strain for gene cloning and gene knock-outing experiments. The parental strain and its transformants were grown at 25 ^{o}C on Potato Dextrose Agar (PDA) medium with appropriate antibiotics as required. *Aspergillus nidulans* strains were grown at 37 ^{o}C or 25 ^{o}C on GMM medium with appropriate supplements (0.5 g L^{-1} uracil, 0.5 g L^{-1} uridine, 0.5 μM pyridoxine HCl, and/or 0.65 μM riboflavin).[1] *Escherichia coli* strain DH5α was cultured in LB medium with appropriate antibiotics for the resistance markers on the plasmid DNA. *Saccharomyces cerevisiae* strain BJ5464-NpgA (*MATα ura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*) was propagated as the yeast assembly host and the heterologous expression host, and it was grown on Yeast Extract Peptone Dextrose Medium (YPD) at 28 ^{o}C. After transformation, *S. cerevisiae* was selected on synthetic dextrose complete medium (SDCt) with appropriate supplements corresponding to the auxotrophic markers at 28 ^{o}C.[2]

Sequence analysis

Initial prediction and analysis of the asperphenamate biosynthesis gene cluster (*apm*) was carried out using antiSMASH (http://antismash.secondarymetabolites.org/). The functional prediction of the open reading frames (ORFs) encoding proteins was performed using on-line BLAST methods (http://blast.ncbi.nlm.nih.gov). Detailed prediction of NRPS domains was performed using NRPSpredictor2 (http://nrps.informatik.uni-tuebingen.de). The genes in *apm* cluster were named *apmA-D* (Table 1). The accession numbers for *apmA* and *apmB* are KX443597.1 and KX443596.1, respectively.

Gene cloning and plasmid construction

Plasmids used in this study are listed in Table S2 and the oligonucleotide sequences synthesized by Shanghai Sango Biotech are given in Table S3. PCR amplification was carried out using the TransStart® FastPfu DNA polymerase (Transgene Biotech) or Phusion® High-Fidelity DNA Polymerase from New England Biolabs (NEB) on a T100™ Thermal cycler from Bio-Rad. PCR screenings for transformants were performed by using 2 × Taq Mix kit from TIANGEN BIOTECH. All the restriction enzymes used in this study were purchased from New England Biolabs (NEB). Plasmids preparation, digestion with restriction enzyme and gel electrophoresis were performed by standard methods.[3]

To generate the NRPS gene deletion cassettes, we used Double-joint PCR strategy as described previously.[4] Briefly, hygromycin B was amplified from the plasmid pUCH2-8 (Table S2). Around 1.5 kb fragments upstream and downstream of the genes of *apmA*, *apmB, apmC* and *apmD* were amplified from *P. brevicompactum* genomic DNA using the designed primers, respectively (Table S3).Three PCR fragments were fused to one fragment each and then connected to the pGEMT vector with T4 ligase to give plasmids pYWL9, pYWL10, pYWL13, pYWL18, respectively (Table S2).

To construct the heterologous expression vectors, SOE (splicing by overlapping extension)-PCR and yeast based assembly approaches were used.[5] For construction of NRPS expression vector, we firstly created the plasmid pYWL27 with *Aspergillus nidulans gpdA* promoter integration into pYH-WA-pyrG (Table S2). *AngpdA* promoter was amplified from *A. nidulans* genomic DNA using primers pgpdA-for and pgpdA-rev (Table S3) was inserted into the corresponding sites of pYH-WA-pyrG with Quick-change method. NRPSs *apmA* and *apmB* (including four PCR fragments with overlapping regions (about 150bp) were amplified from *P. brevicompactum* cDNA or genomic DNA using the corresponding primers (Table S3). Then, cDNA fragments were assembled with SwaI digested pXW55, gDNA fragments were assembled with NheI digested pYWL27orNotI digested pYWB2 and transformed into *S. cerevisiae*

BJ5464-NpgA using the S. c. EasyComp Transformation Kit (Invitrogen). The yeast colonies obtained were analysed by PCR screening. Yeast plasmids were isolated by using the Zymoprep (D2001) Kit (Zymo Research) and transformed into *E. coli* DH5α. All plasmids including pYWL40, pYWL41, pYPZ27, pYPZ28 and pYWL52 were verified by restriction enzyme digestion.

Genetic manipulations and knock-out of *P. brevicompactum*

For the creation of deletion strains of *apmA*, *apmB*, *apmC* and *apmD* genes in *P. brevicompactum* based on the homologous recombination strategy, the deletion cassettes were amplified from the template pYWL9, pYWL10, pYWL18 andpYWL13 using corresponding primers (Table S3). Then, the resulted deletion cassette DNA fragments were transformed into *P. brevicompactum* by protoplast transformation referenced previously.[5] *P. brevicompactum* spores were collected from PDA (potato dextrose agar, BD) after 5 days incubation at 25 °C, and induced to young germ tubes in LMM at 28 °C for 12 hours with 100 rpm agitation. Cells were collected and washed twice with sterilized water and resuspended in enzyme cocktail solution containing 50 mg/ml Lysing Enzymes and 30 mg/ml Yatalase in osmotic medium (1.2 M $MgCl_2$, 10 mM sodium phosphate, pH 5.8) at 28°C for about 10 hours. Protoplasts were gathered with Trapping buffer (0.6 M Sorbitol, 0.1 M Tris-HCI, pH 7.0) and STC buffer, successively. Protoplasts were gently mixed with DNA and incubated for 50 min on ice. 1.25 ml of PEG 6000 solution (60% PEG 6000, 50 mM $CaCl_2$, 50 mMTris-HCI, pH 7.5) was added for 100 μl protoplast mixture, incubated at room temperature for 20 min and plated on regeneration selection medium (PDA, 1.2 M sorbitol, 80 μg/ml hygromycin B). Hygromycin B resistant colonies were selected after culturing on PDA at 28 °C for 4 days. The disruption mutants were inoculated to screen on PDA medium (100 μg/ml hygromycin B) and verified by using diagnostic PCR with primers inside and outside the corresponding gene (Table S3). The genotypes of all mutants were verified by PCR (Fig. S2).The spores of transformants were inoculated on LMM medium stationary incubation for about 2 days for gDNA isolation. Mycelia were

collected and grounded to disrupt cells. Cell lysate was solubilized in LETS buffer (10 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.5% SDS, 0.1 M LiCl) and extracted twice with phenol/chloroform. Genomic DNA was precipitated with ethanol, and resuspended in TE buffer.

RNA extraction and reverse transcriptase PCR (RT-PCR)

P. brevicompactum was grown on YES liquid medium at 25 °C for 2 days. Mycelia were collected and RNA was prepared with the Trizol Reagent (Invitrogen). Briefly, mycelia were grinded and suspended in 1 ml Trizol (Invitrogen). The extraction mixture was incubated at room temperature for 5 min, and 0.2 ml chloroform was added for homogenization. Shake tube vigorously for 15 seconds, incubate for 3 min and then centrifuged at 15000 rpm for 15 min at 4 °C. The supernatant was removed into a new tube. RNA was precipitated with 100 % isopropanol and resuspended in RNase-free water. The RNA integrity was confirmed by electrophoresis on TAE agarose gel. The FastQuant RT Kit (with gDNase) (TIANGEN) was used to remove the genomic DNA and to prepare the cDNA from total RNA by with FQ-RT primer as described by the manufacture book. RT-PCR was performed with 2×Taq® Mix (TIANGEN) in the presence of reverse transcribed RNA. Primers were listed in Table S3.

Phylogenetic analysis of NRPS C-terminal reductase (R) domains

NRPS domain structures were identified by using multiple alignments and Pfam analysis. NRPS R domains were selected from *P. brevicompactum*, *A. terrus* and *A. aculeatus* for the comparison with (1) C-terminal reductase domains of high identity (over 60%) known fungal tetramate synthases, (2) known redox-active C-terminal reductase domains from selected nonribosomal peptide synthetases, and (3) selected structurally characterised short-chain reductase/dehydrogenases.[6] The sequences of each C-terminal reductase (R) domains from NRPS were aligned using the program BioEdit (ver.7.0.9) and ClusterW analysis. Then, the sequence data were determined

for the most optimal substitution model. The rates among sites using the function "Find the Best DNA/Protein Models (ML)" of MEGA version 6, and Kimura-2 parameter substitution model and Gamma distributed with Invariant sites (LG+G) were found to be the best, gaps were treated as partial deletion according to the description by Hall.[7] All the sequence matrices were analysed using the ML method and subjected to 1000 bootstrap replications.

Heterologous expression of the *apmA* and *apmB* gene in *A. nidulans*

Aspergillus nidulans strain LO8030 was used as the recipient host.[1] Fungal protoplast preparation and transformation were performed according to the method described previously.[5] The NRPS *apmA* containing plasmid pYPZ27, NRPS *apmB* containing plasmid pYPZ28 and the empty vector pYWL27 were transformed into the host strain *A. nidulans* LO8030 to create the *apmA* or *apmB* expression strain TYWL6, TYWL7 and the control strain TYWL8, respectively (Table S1). The NRPS *apmA* containing plasmid pYWL52 was transformed into the mutant strainTYWL7 to create the *apmA* and *apmB* expression strain TYWL14. Transformants were verified using diagnostic PCR with appropriate primers (Table S3). GMM and LMM medium were used to cultivate the transformants for HPLC analysis of secondary metabolites.

Feeding assays

For feeding of *P. brevicompactum* strains with compounds (solubilized in DMSO), spores of *ΔapmA* or *Δ*apmB were inoculated in 10 ml of YES together with 200 μg/ml compound **3**, **4**, **5** or **6** and further cultured for 2 days at 25 ^{o}C. The mycelia and medium were extracted with 25 ml methanol: acetate ethyl: acetic acid (MEA) (10:89:1) with 1 h sonication at room temperature. After removal of the solvent under reduced pressure, the residue obtained was dissolved in 400 μl methanol for HPLC or LC-MS analysis.

For feeding assays of *A. nidulans* strains, TYWL6 (*apmA* expression), TYWL7 (*apmB* expression), TYWL8 (control) and TYWL14 (*apmA* and *apmB* expression) were

inoculated in the appropriate GMM medium with compounds (solubilized in DMSO) for 3-7 days. Then the mycelia and medium were extracted with EtOAc for HPLC or LC-MS analysis.

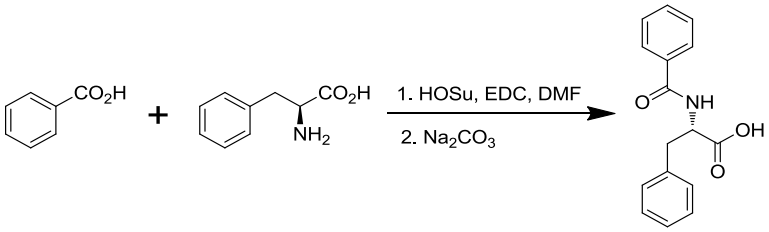
To obtain compound **3** for further experiment, TYWL6 was inoculated in GMM medium with 60 μg/ml compound **4** and **5** and cultivated for 6 days at 25 ^{o}C. Then the mycelia and medium were extracted with EtOAc and compound **3** was further purified with HPLC. The NMR data obtained for **3** corresponded well with the reported data.[8] ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 8.15 (1H, d, *J*=8.4 Hz), 7.78 (2H, d, *J*=7.7 Hz), 7.49 (1H, br t, *J*=7.3 Hz), 7.43 (1H, br t, *J*=7.4 Hz), 7.14-7.16 (5H, m), 4.83 (1H, t, *J*=5.7 Hz), 4.15 (1H, m), 3.39-3.51 (2H, m), 2.95 (1H, dd, *J*=13.7, 5.2 Hz), 2.79 (1H, dd, *J*=13.7, 9.1).

For feeding assays in yeast, *S. cerevisiae* strain BJ5464–NpgA with/without plasmid pYWL40 (*apmA* expression) were inoculated in the appropriate SDCt(A,T) medium for 3 days, and further cultured in 20 ml of YPD for 4 days. *S. cerevisiae* with plasmid pYWL41 (expressing *apmB*) were inoculated in the 500 ml YPD medium for 3 days, and then the cultures were concentrated to 60 ml and 60 μg/ml compound **4** and **5** and 30 μg/ml **3** (final concentration) were added for further cultivation for 4 days, followed by extraction with EtOAc and HPLC analysis.

Isolation of compound 1

Compound **1** was isolated from a 3-day culture of *P. brevicompactum* grown on YES medium or a 6-day culture of TYWL7 grown on GMM medium by repeated column purification and semi-preparative HPLC and characterised by NMR, which corresponded well to the published data, respectively.[9] ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.63 (2H, d, *J*=7.9 Hz), 7.59 (2H, d, *J*=8.0 Hz), 7.44 (1H, t, *J*=7.3 Hz),7.37 (1H, t, *J*=7.1 Hz), 7.32 (2H, t, *J*=7.6 Hz), 7.23 (2H, br t, *J*=6.6 Hz), 7.14-7.23 (10H), 6.60 (1H, d, *J*=8.4 Hz), 6.50 (1H, d, *J*=6.4 Hz), 4.85 (1H, q, *J*=6.7 Hz), 4.55 (1H, m), 4.47 (1H, dd, *J*=11.3, 2.9 Hz), 3.96 (1H, dd, *J*=11.3, 4.2 Hz), 3.23 (1H, dd, *J*=13.9, 6.5 Hz), 3.14 (1H, dd, *J*=13.9, 7.0 Hz), 2.93 (1H, dd, *J*=13.7, 6.4 Hz), 2.82 (1H, dd, *J*=13.7, 8.4 Hz).

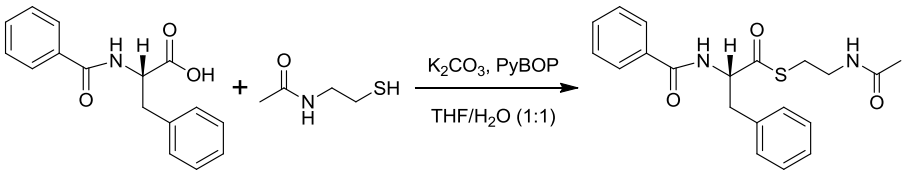
Chemical synthesis of compound 2



Compound **2** was synthesized according to the reported method.[10] The reaction substrates are L-phenylalanine / L-phenylalanine-^{15}N (meilunbio®) (**4**) and benzoic acid / benzoic acid-α-^{13}C (Sigma-Aldrich®) (**5**), respectively. At room temperature, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (5.5 mmol) was added to a solution of benzoic acid (**5**) (5.0 mmol) and 1-hydroxysuccinimide (5.5 mmol) in dimethylformamide (DMF) and the resulting mixture was stirred until the starting material disappeared. After addition of water, the resulting mixture was extracted with Et_2O, washed with brine, dried over Na_2SO_4. Evaporation of the solvent was afforded good purity of the compound for next step.

A solution of compound **4** (2.3 mmol) and Na_2CO_3 (6.88 mmol) in water (7 ml) was added to a solution of activated benzoic acid (2.28 mmol) in CH_2Cl_2 and stirred for 2 h. After removal of the solvent under reduced pressure, the residue was acidified with 1N HCl. The precipitate was extracted with EtOAc, washed with brine, then dried over Na_2SO_4. After evaporation of the solvent, the residue was purified with HPLC (MeCN: H_2O, 40 %).

Chemical synthesis of compound 2-SNAC



Compound **2**-SNAC was synthesized according to the reported method.[11] Potassium carbonate (20.7 mg, 0.15 mmol, 4.0 equiv) was added to a stirred solution of compound 2 (10.0 mg, 0.037 mmol, 1.0 equiv) and PyBOP (58.0 mg, 0.11 mmol, 3.0 equiv) in 50% THF/water (3 ml). To the resulting clear and colorless solution was

added *N*-acetylcysteamine (8.1 µl, 0.074 mmol, 2.0 equiv) and the reaction was stirred for 2 hours. The solvent was then removed and the residue was dissolved in MeOH and purified by preparative HPLC with detection at 210 nm. Solvent A (water, 0.1% HCOOH) and B (MeOH) was used and run over a constant gradient of 55% (B). The purified compound **2**-SNAC was submitted to HR-MS and ^{1}H NMR.HR-MS: *m/z* calculated for $C_{20}H_{22}N_2O_3S$: 370.4653 $[M+H]^+$. Found: 371.1475. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.69 (2H, d, *J*=7.6 Hz), 7.51 (1H, t, *J*=7.2 Hz), 7.42 (2H, t, *J*=7.6 Hz), 7.31 (2H, t, *J*=7.2 Hz), 7.26 (1H, d, *J*=7.2 Hz), 7.20 (2H, d, *J*=7.2 Hz), 6.70 (1H, d, *J*=7.5 Hz), 6.14 (1H, br s), 5.08 (1H, m), 3.39 (2H, m), 3.27 (1H, dd, *J*=14.1, 5.7), 3.19 (1H, dd, *J*=14.1, 7.5), 3.04 (2H, t, *J*=5.9 Hz), 1.92 (3H, s).

Analytical methods and equipment overview

HPLC analysis were performed on Waters 2695 Separations Module 241 with a RP C_{18} column (38020-41 COSMOSIL 5C18-MS-II Packed 242 Column; 4.6 mm I.D. × 250 mm) at a flow rate of 1 ml min^{-1}. Water and methanol were used as solvents. For analysis of the extracts, a linear gradient of 40 – 100 % (v/v) methanol in 20 min was used. The column was then washed with 100 % (v/v) methanol for 5 min and equilibrated with 30 % (v/v) methanol for 5 min. Detection was carried out with a photodiode array detector and illustrated at 254 nm in this paper. For isolation of compound **1**, **2**, and **3**, Shimadzu LC-6AD with a YMC-Pack ODS-A column (250×10 mm I.D., 5 µm) was used. Water and methanol/ acetonitrile were used as solvents. For isolation of **2** and **3**, 40 % acetonitrile was used with a flow rate at 3.0 ml/min. The column was then washed with 100 % (v/v) acetonitrile for 10 min and equilibrated with 40 % (v/v) acetonitrile for 10 min.

All MS, HR-MS and LC-MS analysis were performed on an Agilent 6520 Accurate-Mass QTOF LC-MS system (Agilent Technologies) equipped with a RP C_{18} column (Kromasil 100-5 C_{18} column; 4.6 x 250 mm; 25 ^{o}C) and an electrospray ionization (ESI) source. NMR spectra were recorded at room temperature with a Bruker Avance-500 spectrometer.

Chemical analysis and characterisation of compounds

For structural elucidation, the isolated products were subjected to NMR and MS analysis. Chemical shifts were referenced to the solvent signal at 7.26 ppm for $CDCl_3$, 2.50 ppm for DMSO-d_6 or 3.31 ppm for MeOH-d_4. All spectra were processed with MestReNova 5.2.2 (Metrelab Research, Santiago de Compostella, Spain).

Supplementary Tables

Table S1 Strains used in this study

Strains	Genotype	References
Aspergillus nidulans LO8030	*pyroA4, riboB2, pyrG89, nkuA::argB*, *sterigmatocystin cluster (AN7804-AN7825)Δ,emericellamide cluster (AN2545-AN2549)Δ,asperfuranone cluster (AN1039-AN1029)Δ,monodictyphenone cluster (AN10023-AN10021)Δ,terrequinone cluster (AN8512-AN8520)Δ,austinol cluster part 1 (AN8379-AN8384)Δ,austinol cluster part 2 (AN9246-AN9259)Δ, F9775 cluster (AN7906-AN7915)Δ, asperthecin cluster (AN6000-AN6002)Δ*.	[1]
Saccharomyces cerevisiae BJ5464-NpgA	*MATα ura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*	[5]
Wild type	*Penicillium brevicompactum* xz118	This study
ΔapmA	*ΔapmA::hph* in *P. brevicompactum*	This study
ΔapmB	*ΔapmB::hph* in *P. brevicompactum*	This study
ΔapmC	*ΔapmC::hph* in *P. brevicompactum*	This study
ΔapmD	*ΔapmD::hph* in *P. brevicompactum*	This study
TYWL6	*gpdA::apmA::AfpyrG* in *A. nidulans* LO8030	This study
TYWL7	*gpdA::apmB::AfpyrG* in *A. nidulans* LO8030	This study
TYWL8	pYH-WA-pyrG in *A. nidulans* LO8030	This study
TYWL14	*apmA::Afribo*,*apmB::AfpyrG* in *A. nidulans* LO8030	This study

Table S2 Plasmids used in this study

Plasmid name	Description	Reference
pYH-WA-pyrG	*URA3, WA flanking, AfpyrG, Amp*	[5]
pXW55	*2μ, URA3, ADH2p::ACPC, Amp*	[12]
pUCH2-8	hygromycin resistance vector	[13]
pYWB2	*AfRiboB* in pYH-WA-pyrG-KI	[14]
pYWL9	*apmA* deletion cassette in pGEMT	This study
pYWL10	*apmB* deletion cassette in pGEMT	This study
pYWL13	*apmD* deletion cassette in pGEMT	This study
pYWL18	*apmC* deletion cassette in pGEMT	This study
pYWL27	*A. nidulans* gpdA promoter in pYH-WA-pyrG	This study
pYWL40	*apmA* expression cassette in pXW55	This study
pYWL41	*apmB* expression cassette in pXW55	This study
pYWL52	*apmA* expression cassette in pYWB2	This study
pYPZ27	*apmA* expression cassette in pYWL27	This study
pYPZ28	*apmB* expression cassette in pYWL27	This study

Table S3 PCR primers used in this study

Primers name	Oligonucleotide sequences (5'-3')	Uses
KOapmA-5F-f	GCGCATTGTTAGATTTCATACACGGTGCCT GGAGGATCCACGACTAGGGAAG	*apmA* 5 flanks amplification
KOapmA-5F-r	CATATGGAGTGCATATTCGGTG	
KOapmA-3F-f	CAGCTATGCTGGTTTCTCAAAC	*apmA* 3 flanks amplification
KOapmA-3F-r	GTCAGGCCATTTTCATATGGCAATGCGCAG CTACGACACTGAGGCCATTC	
KOapmB-5F-f	GTAGTCACTTCGCAGATGCG	*apmB* 5 flanks amplification
KOapmB-5F-r	GCGCATTGTTAGATTTCATACACGGTGCCT GGAGAAGTAGGCGAGATATCGG	
KOapmB-3F-f	GTCAGGCCATTTTCATATGGCAATGCGCAG GGTTTTTACGGAGTCAACTCCG	*apmB* 3 flanks amplification
KOapmB-3F-r	CCATGGCTGGTAACTTTGTC	
KOapmC-5F-f	GTGCCGTGCCTGGGACCGAG	*apmC* 5 flanks amplification
KOapmC-5F-r	GCGCATTGTTAGATTTCATACACGGTGCCT GGATGTGGTGGCTATGATGGAATAG	
KOapmC-3F-f	GTCAGGCCATTTTCATATGGCAATGCGCAG ATGGCTTTCAATGCTTATTAGTC	*apmC* 3 flanks amplification
KOapmC-3F-r	GTGAGTAAACGTCGATCGTTGG	
KOapmD-5F-f	ATCCAGTGTCAGCGTTAGTGG	*apmD* 5 flanks amplification
KOapmD-5F-r	GCGCATTGTTAGATTTCATACACGGTGCCT GGGTGATGCACTTTTCCAAGGTTG	
KOapmD-3F-f	GTCAGGCCATTTTCATATGGCAATGCGCAG GTTGCCTGACTGGTATATGATGC	*apmD* 3 flanks amplification
KOapmD-3F-r	CTATTCGGGTTGCCGGAAAG	
hyg-FF	CCTCGAGGTCGAVAGAAGATATTGAAGGC AGGCACCGTGTATGAAATCTAAC	*Hygromycin B* amplification
hyg-RR	CGGGCTGCAGGATATCAAGCTTATCGCTGC GCATTGCCATATGAAAATGGC	
hyg-scr-5R	GGACGATATCCCGCAAGAGG	transformant screening
hyg-scr-3F	GCATTCCCATTCCCATCGTG	
apmA-scr-5F	CGGGATGTGCATTGTACCTTG	*apmA* transformant screening
apmA-scr-3R	CCACACACTCGCGTTGTCAG	
apmA-RT-F	GAATGAACCCACGATCAACC	
apmA-RT-R	CACAGACTTCGCTACCCTTG	
apmB-scr-5F	CATAGCCCAATTGCTGCAAG	*apmB* transformant screening
apmB-scr-3R	GACACACACAATGTCATCGG	
apmB-RT-F	CGATGTGGTGTATGGACAC	
apmB-RT-R	GTGTCTTCAGCATGACCTCG	
apmC-scr-5F	GGACGTCCACCTGCAGTTC	*apmC* Transformant
apmC-scr-3R	GGAATTCGATGGCGCTAACC	

		screening
apmC-RT-F	CTCCCTCACCGATCTCGGAG	
apmC-RT-R	CATGAGCCAGCGAACGCATG	
apmD-scr-5F	ATGCAGAGCCACTCACGTTC	*apmD*
apmD-scr-3R	TAACGAGGGGGTATCGGAGAC	transformant
apmD-RT-F	CGCAGAACATTGCAACGCAC	screening
apmD-RT-R	TTAGCCCGAGGGAAGGGTG	
XW-apmA1-F	CATATGGCTAGCGATTATAAGGATGATGATGATAAGAATGCCGTCTATAGTCATTGACG	*apmA* fragment amplification for
XW-apmA1-R	CGAGATCCTTGTTGGATGCGG	Yeast assembly
XW-apmA2-F	GTGTGGACCATTGTCACGATG	
XW-apmA2-r	GGTGTCATGAGCCCACACAAG	
XW-apmA3-f	CCAAGATTCCTGGCTCTTACG	
XW-apmA3-r	GACTCCATAGGACTGCTTGATG	
XW-apmA4-f	GACAAGGCCCTTGCCATCAGC	
XW-apmA4-r	TTTGTCATTTAAATTAGTGATGGTGATGGTGATGCACCGCAGTGATGGTCATTCTCTTC	
pYH-apmA1-f	GTATATTCATCTTCCCATCCAAGAACCTTTAATCGCTAGCACCCTTTCTTGCCTTGCCC	
pYH-apmA3-r	TACACAACATATTTCGTCAGACACAGAATAACTCTCCGCCATCCACATTCGTGAGAAAC	
XW-apmB1-f	CCATATGGCTAGCGATTATAAGGATGATGATGATAAGAATGTTACAAGTCGTGCCGGGC	*apmB* fragment amplification for
XW-apmB1-R	CCTCAGGTCCGACATTCTGTG	Yeast assembly
XW-apmB2-F	GATCGAGGTGTGCATGCATG	
XW-apmB2-R	GGATTTGAGGCTGCACTCGAG	
XW-apmB3-F	GCCACAGCAGCTTGTGATTTC	
XW-apmB3-R	GCGGACGAAGTCAGAGAACTG	
XW-apmB4-F	CCGATTGATCATGCGCATCTC	
XW-apmB4-r	ATTTGTCATTTAAATTAGTGATGGTGATGGTGATGCACCCGGGCTCGGGAAGGGGAATC	
pYH-apmB1-f	ATATTCATCTTCCCATCCAAGAACCTTTAATCGCTAGCATGTTACAAGTCGTGCCGGGC	
pYH-apmB3-r	TATACACAACATATTTCGTCAGACACAGAATAACTCTCCGGTATTGCACAGTAGCAAGC	
apmA-swaI-f	CATATTTAAATCATGCGGAGAGACGGACGGTC	*apmA* fragment amplification
apmA-swaI-r	CGGATTTAAATCCATCCACATTCGTGAGAAACTGC	
pgpdA-for	GGACTTGACTCTCCTTCTCCTGATCCATGCGGAGAGACGGACGGTC	create the vector pYWL27
pgpdA-rev	CAGACACAGAATAACTCTCGCTAGCGATTAAAGGTTCTTGGATGGG	

Supplementary Figures

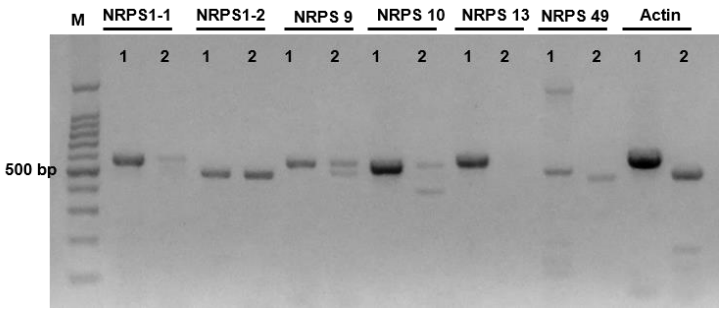


Fig. S1 Transcriptional analysis of targeted NRPS encoding genes in *P. brevicompactum* by RT-PCR. 1: gDNA is as the template; 2: cDNA is as the template.

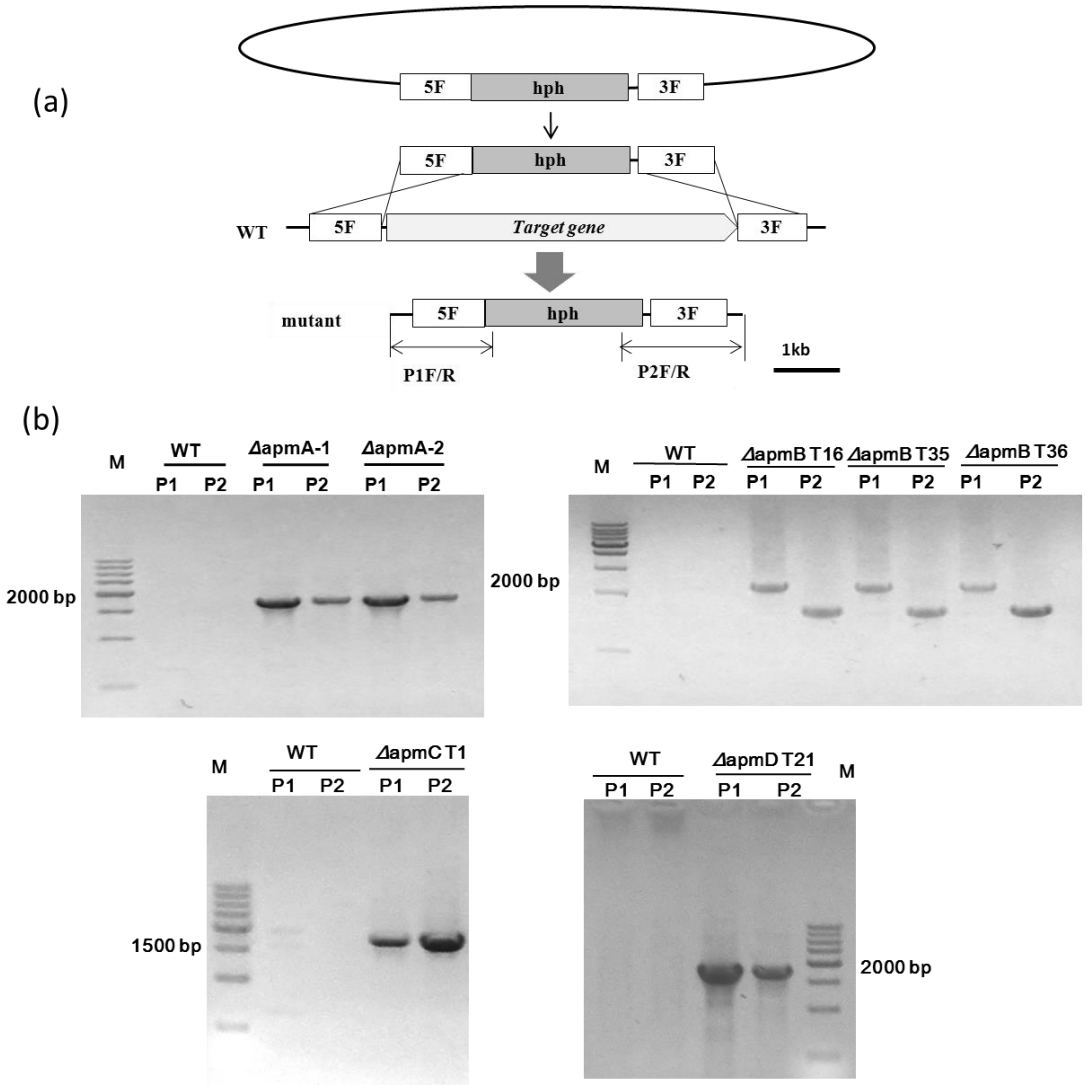


Fig. S2 Generation of *P. brevicompactum* deletion strains

(a) Schematic illustration for disruption of targeted genes. The *hph* gene in vector pUCH2-8 is used as a selection maker after transformation. Transformation was performed by homologous recombination via protoplast transformation method. Two pairs of primers including P1F/R and P2F/R were used for transformant screening. (b) Confirmation of *ΔapmA*, *ΔapmB*, *ΔapmC* and *ΔapmD* strains by diagnostic PCR, respectively. Specific bands (ca. 1.5 kb) were only found in mutants but not in WT.

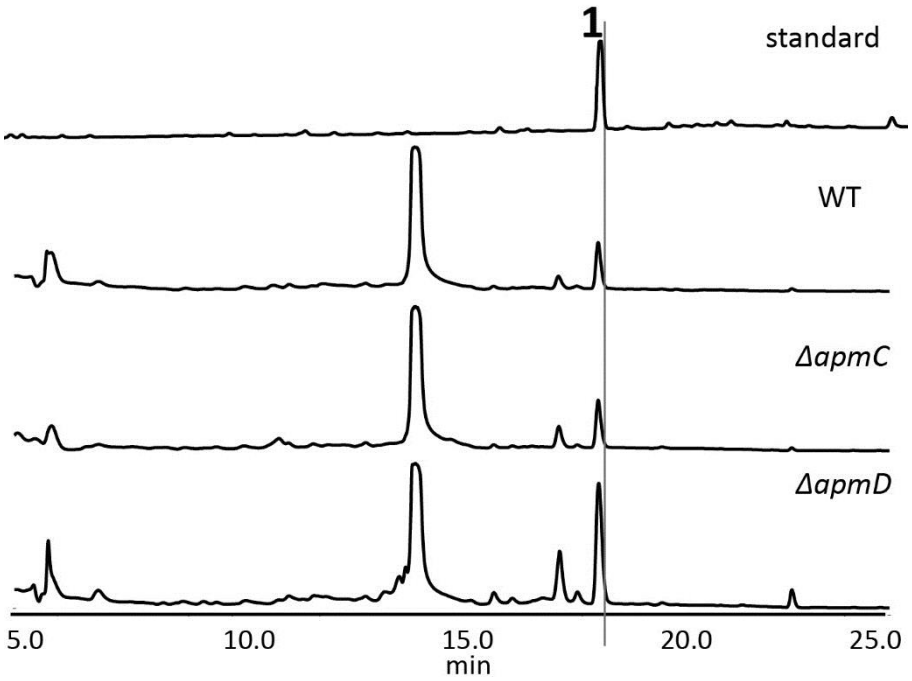


Fig. S3 HPLC analysis of *apmC* and *apmD* deletion strains

The compound **1** still exists in the deletion mutants of *apmC* and *apmD* in comparison to standard. The detection is at 254 nm.

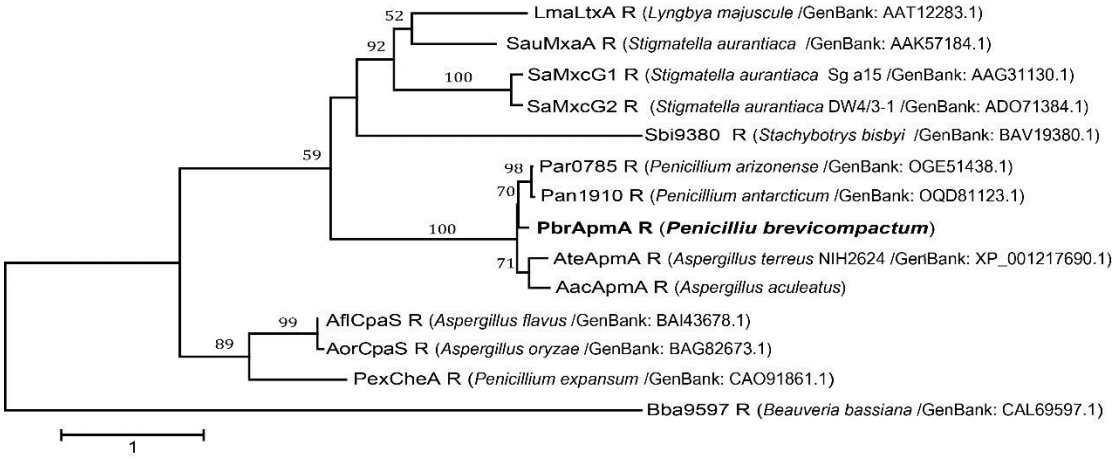


Fig. S4 Phylogenetic analysis of the C-terminal reductase (R) domains of ApmA

Maximum likelihood phylogeny was inferred from the amino acid sequences of C-terminal reductase (R) domains in certain species. The bootstraps support values above 50% are shown on the nodes. The most suitable substitution model is (LG+G) model and tested by 1000 replicates of bootstrap tests. The gaps were treated as partial deletion according to the description by Hall.[7] A domains are followed by the species names and Genbank accession number. The (R) domain of ApmA in this study is marked in bold letter. Protein name abbreviations: chaetoglobosin synthetase (CheA), polyketide synthase-nonribosomal peptide synthetase (CpaS), lyngbyatoxin synthetase (LtxA), myxalamid NRPS module (MxaA), myxochelin NRPS module (MxcG).

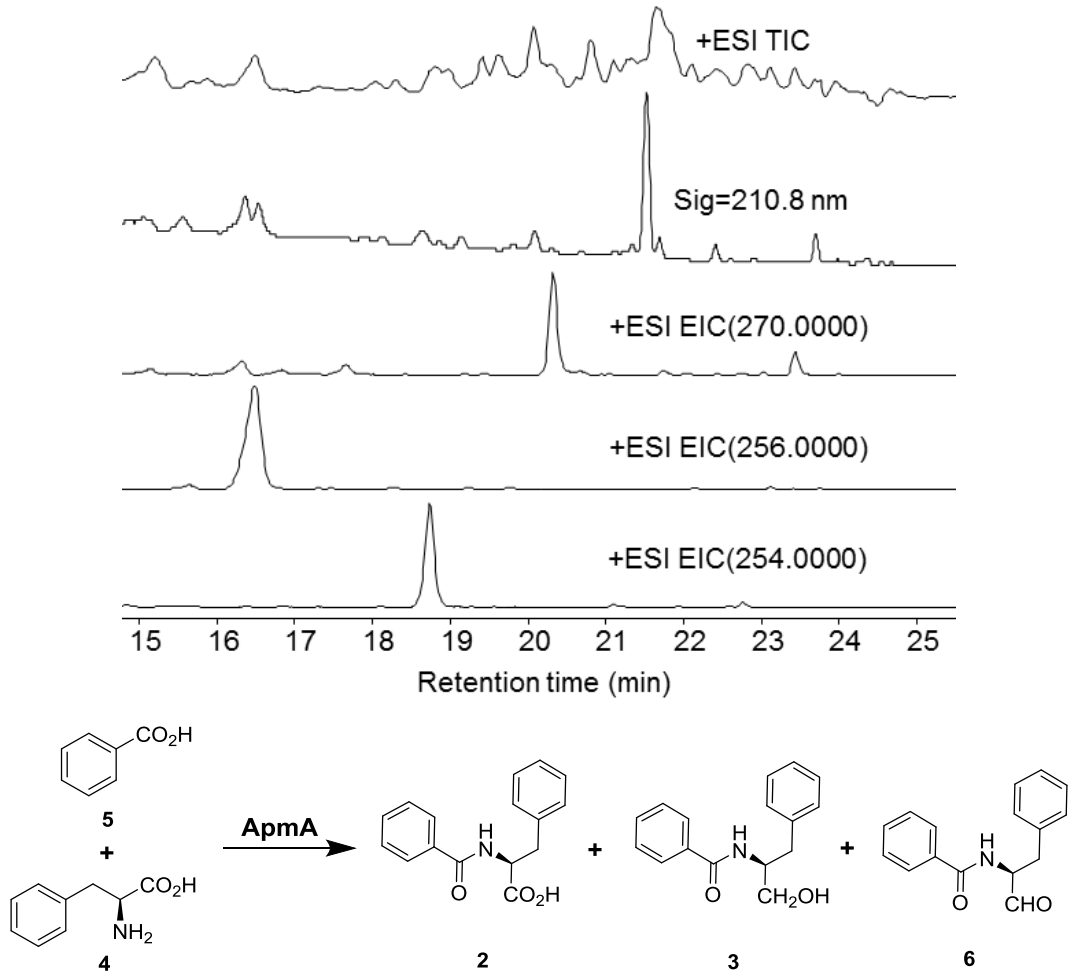


Fig. S5 LC-MS analysis of *apmA* gene expression in *S. cerevisiae*

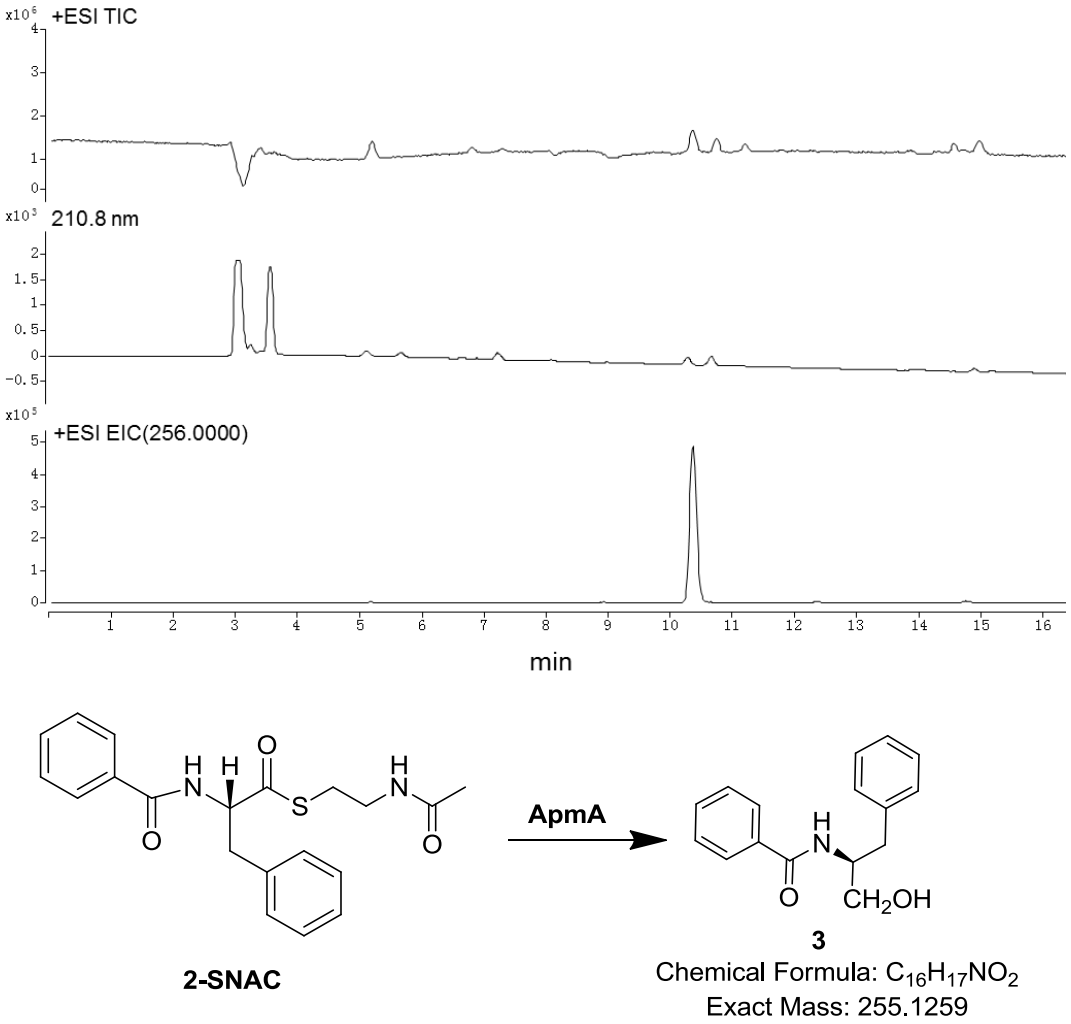


Fig. S6 LC-MS analysis of feeding experiment in *apmA* expressed *A. nidulans* with **2**-SNAC as substrate

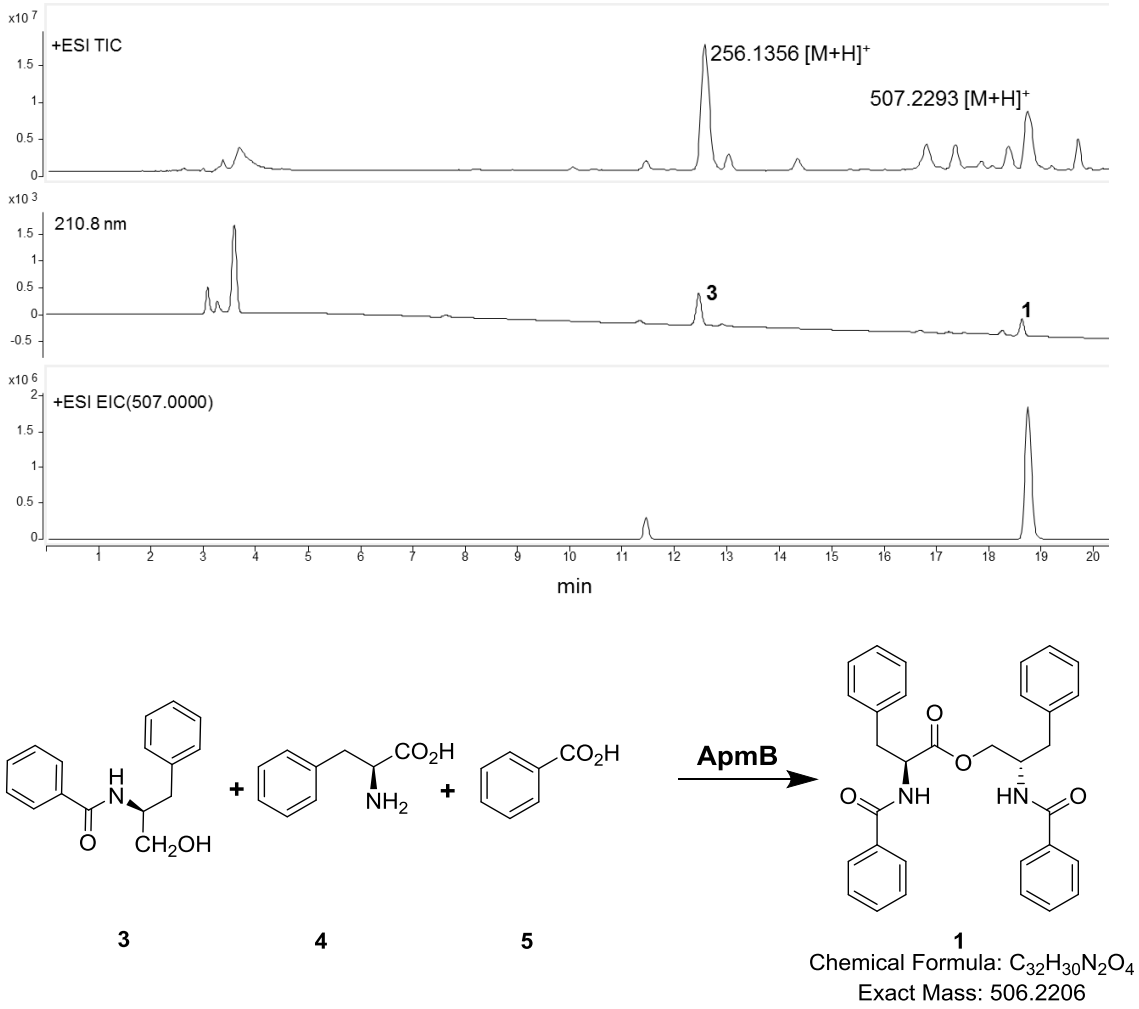


Fig. S7 LC-MS analysis of crude extracts from *apmB* expressed *A. nidulans*

Feeding experiments were performed by using **3**, **4** and **5** as substrates and **1** was detected from the crude extracts

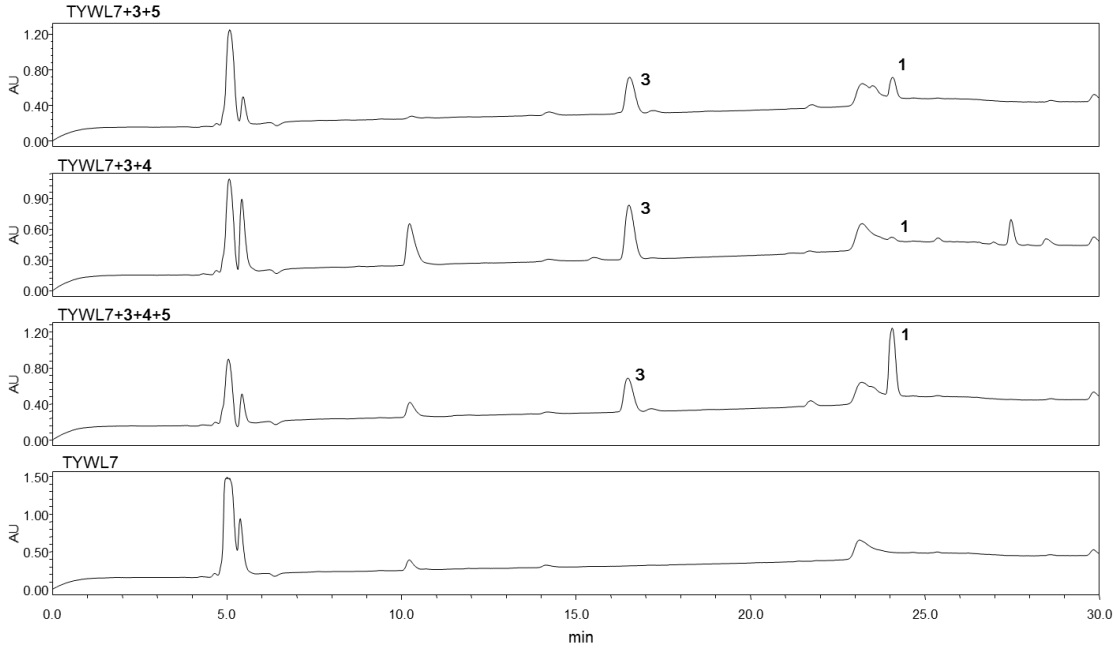


Fig. S8 HPLC analysis of feeding experiment in *apmB* expressed *A. nidulans*

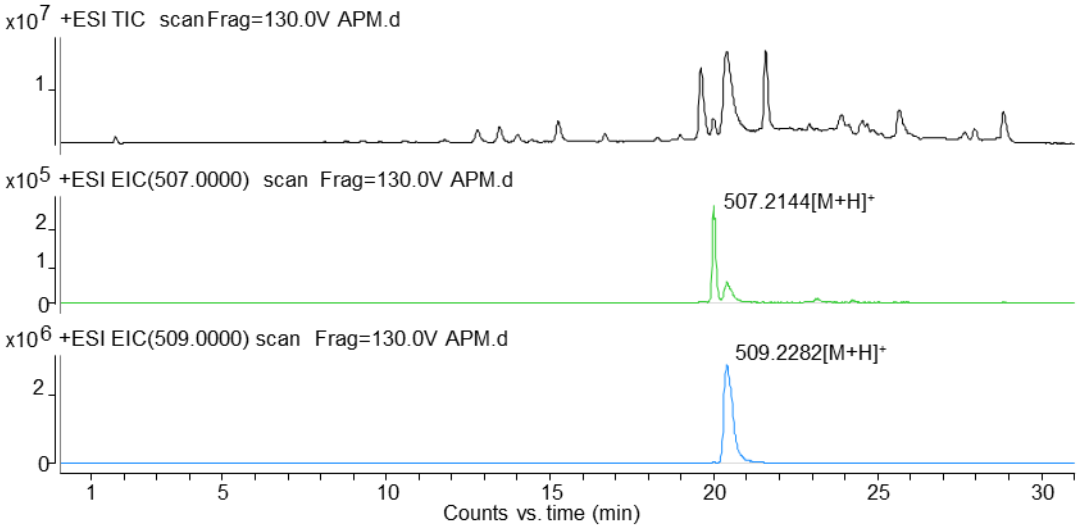


Fig. S9 LC-MS analysis of feeding experiment in *apmB* expressed *A. nidulans* with $^{13}C,^{15}N$-**2** and **3** as substrates

The molecular weight of compound **1** was detected a**s** 509.2282 $[M+H]^+$ which indicated $^{13}C,^{15}N$-**2** and **3**'s integration into **1**. Minor amount of unlabeled **1** 507.2144 $[M+H]^+$ was also detected.

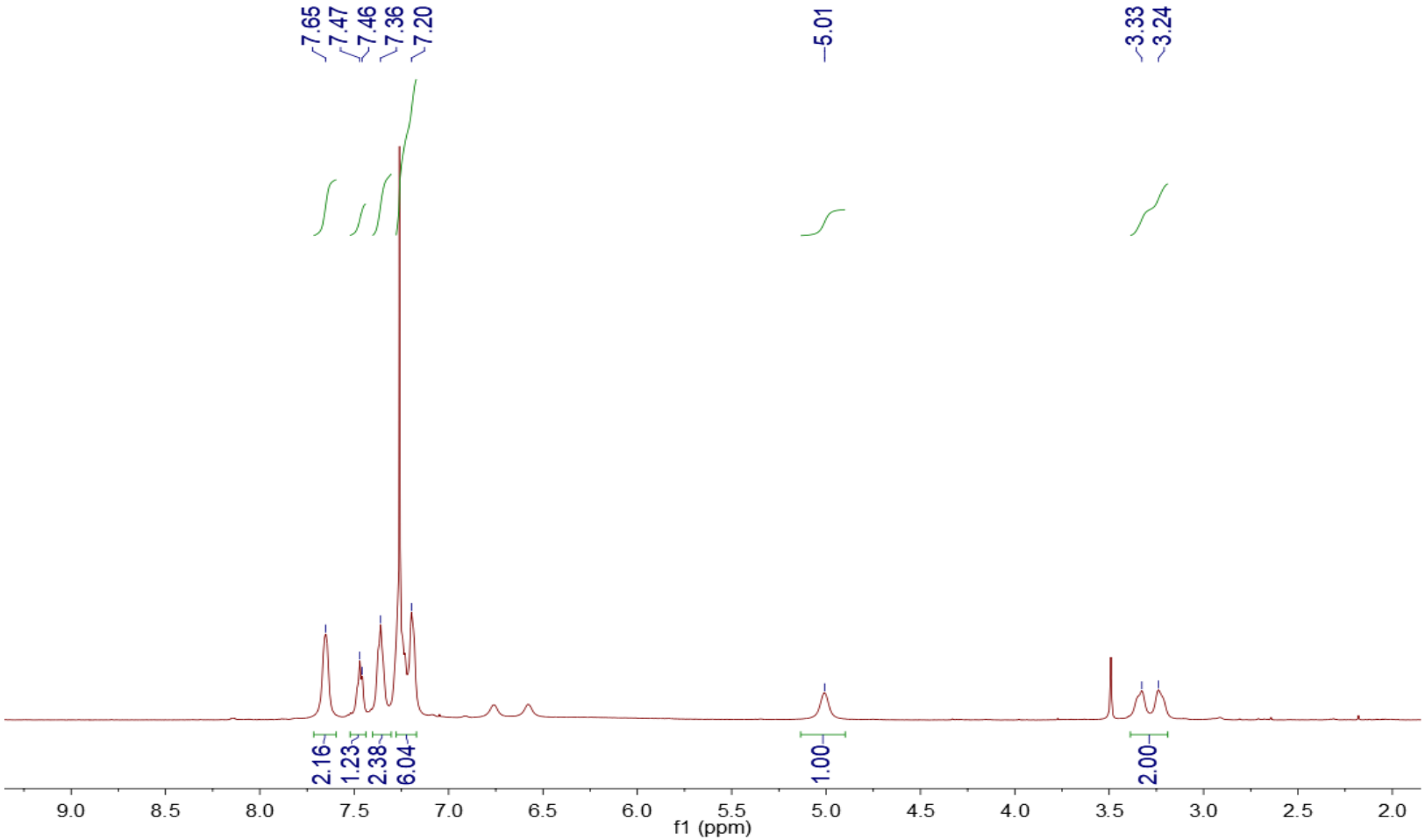


Fig. S10 ^{1}H NMR of *N*-benzoyl-L-phenylalanine (^{13}C,^{15}N-**2**) in $CDCl_3$.

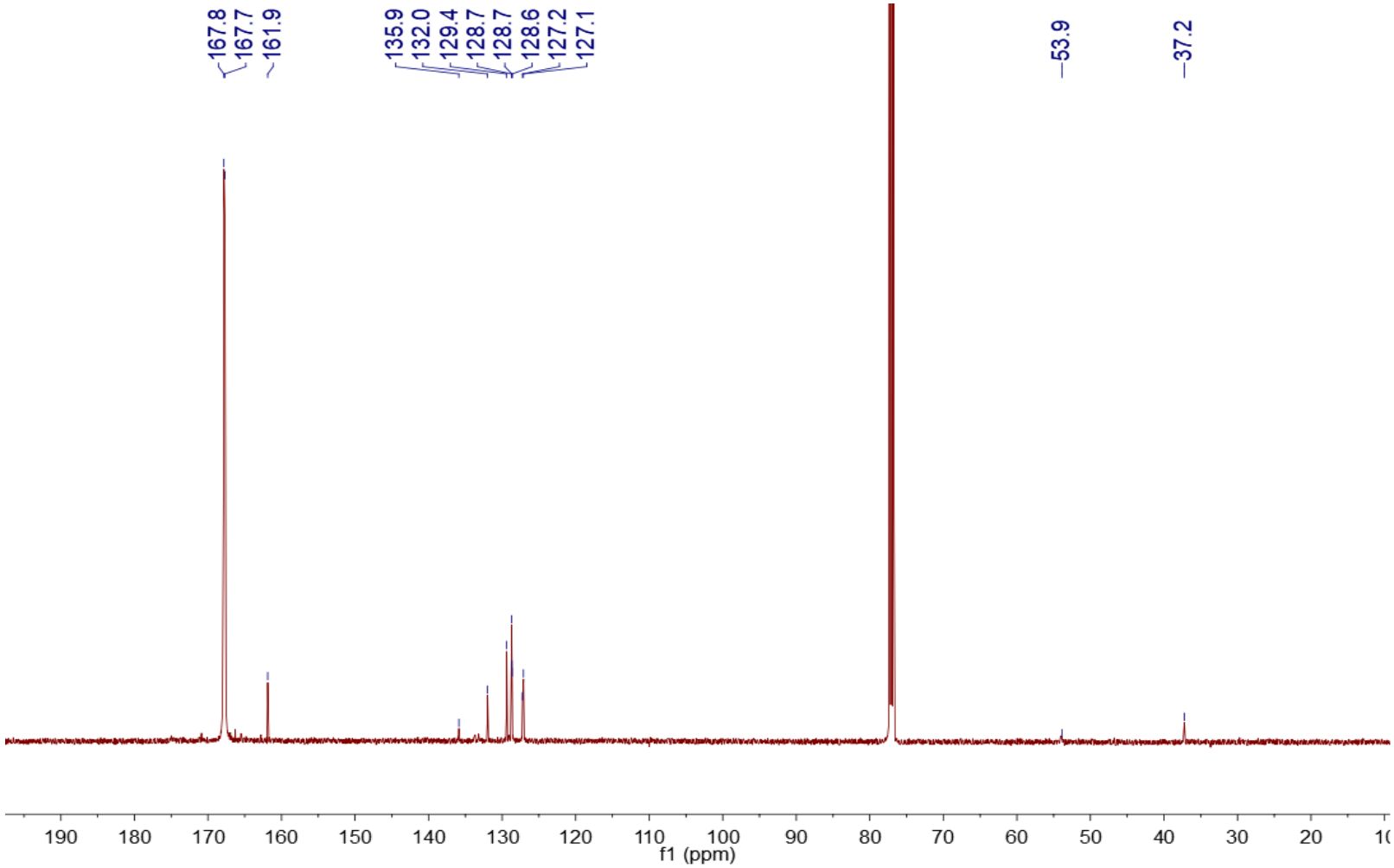


Fig. S11 ^{13}C NMR of *N*-benzoyl-L-phenylalanine (^{13}C,^{15}N-**2**) in $CDCl_3$.

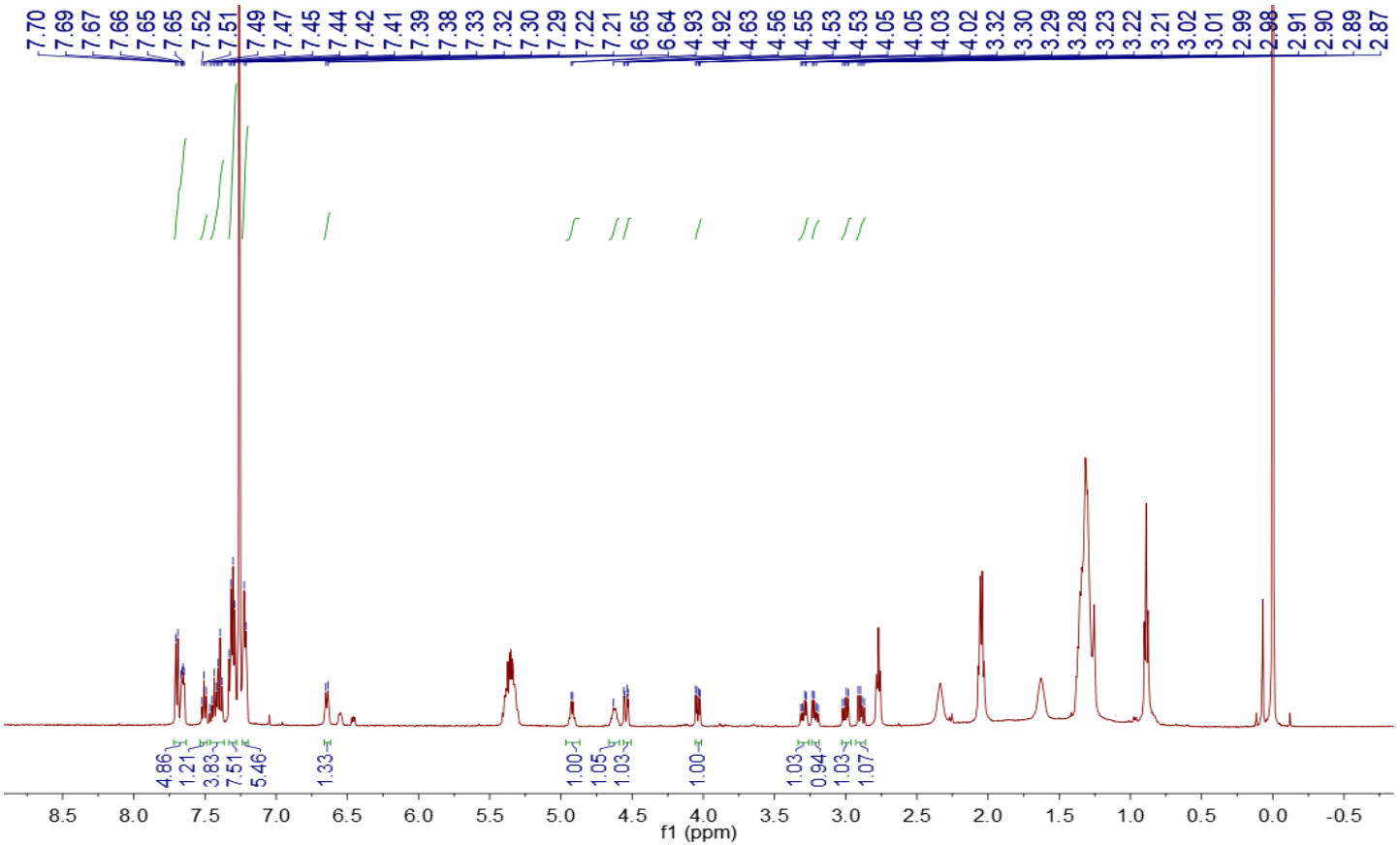


Fig. S12 ^{1}H NMR of asperphenamate (^{13}C,^{15}N-**1**) in $CDCl_3$.

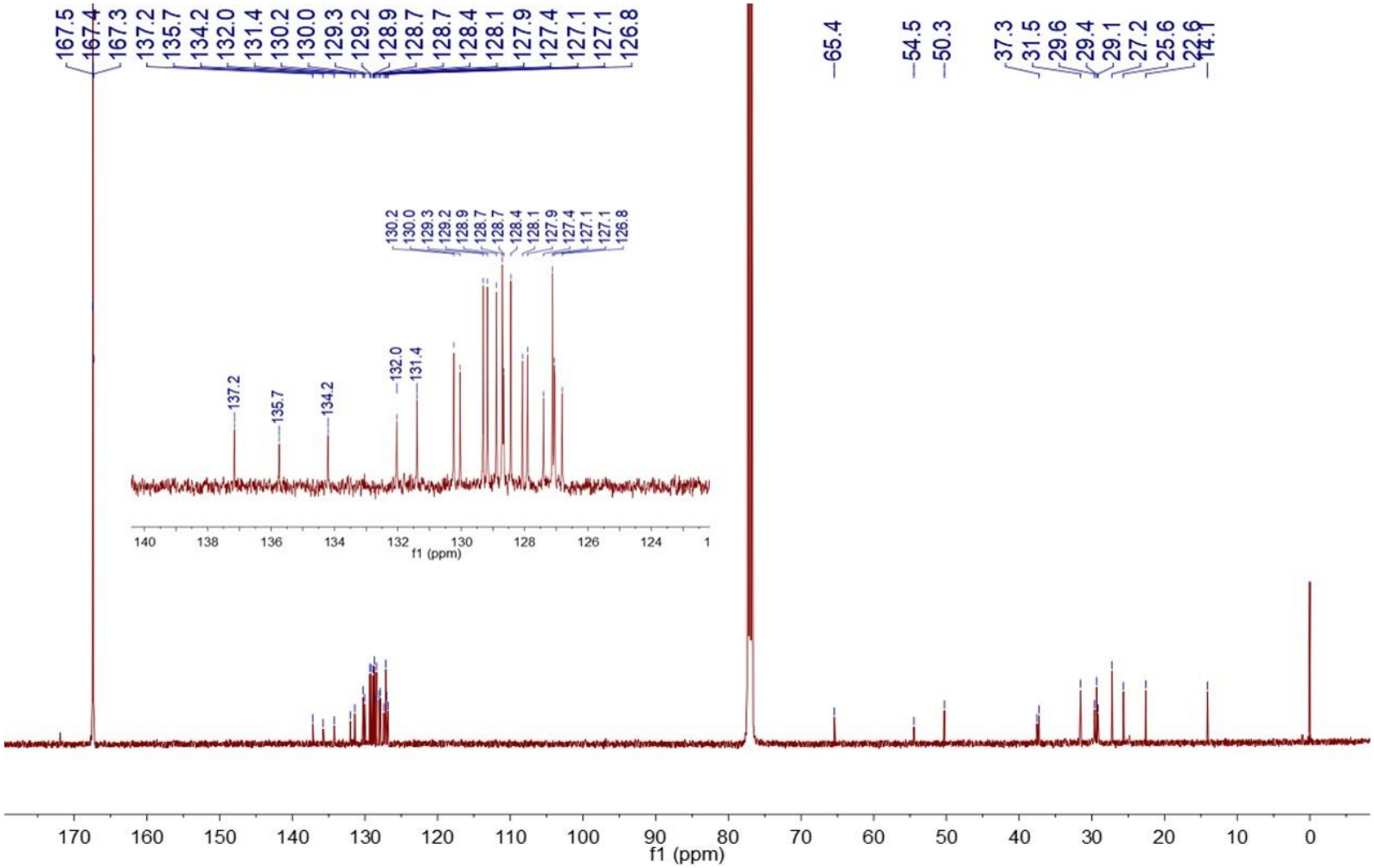


Fig. S13 ^{13}C NMR of asperphenamate (^{13}C,^{15}N-**1**) in $CDCl_3$.

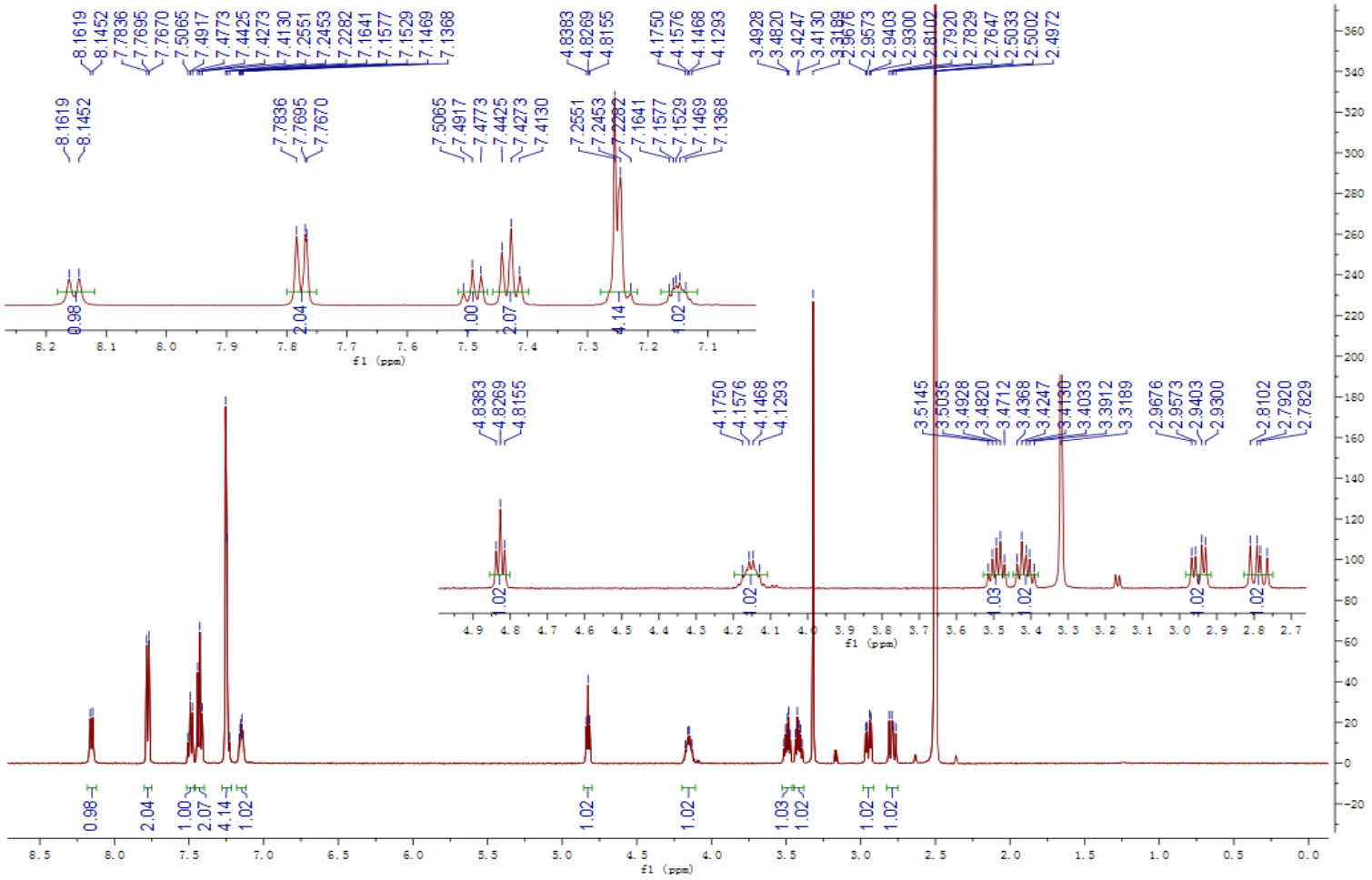


Fig. S14 ^{1}H NMR of *N*-benzoyl-L-phenylalaninol (**3**) in DMSO-d_6.

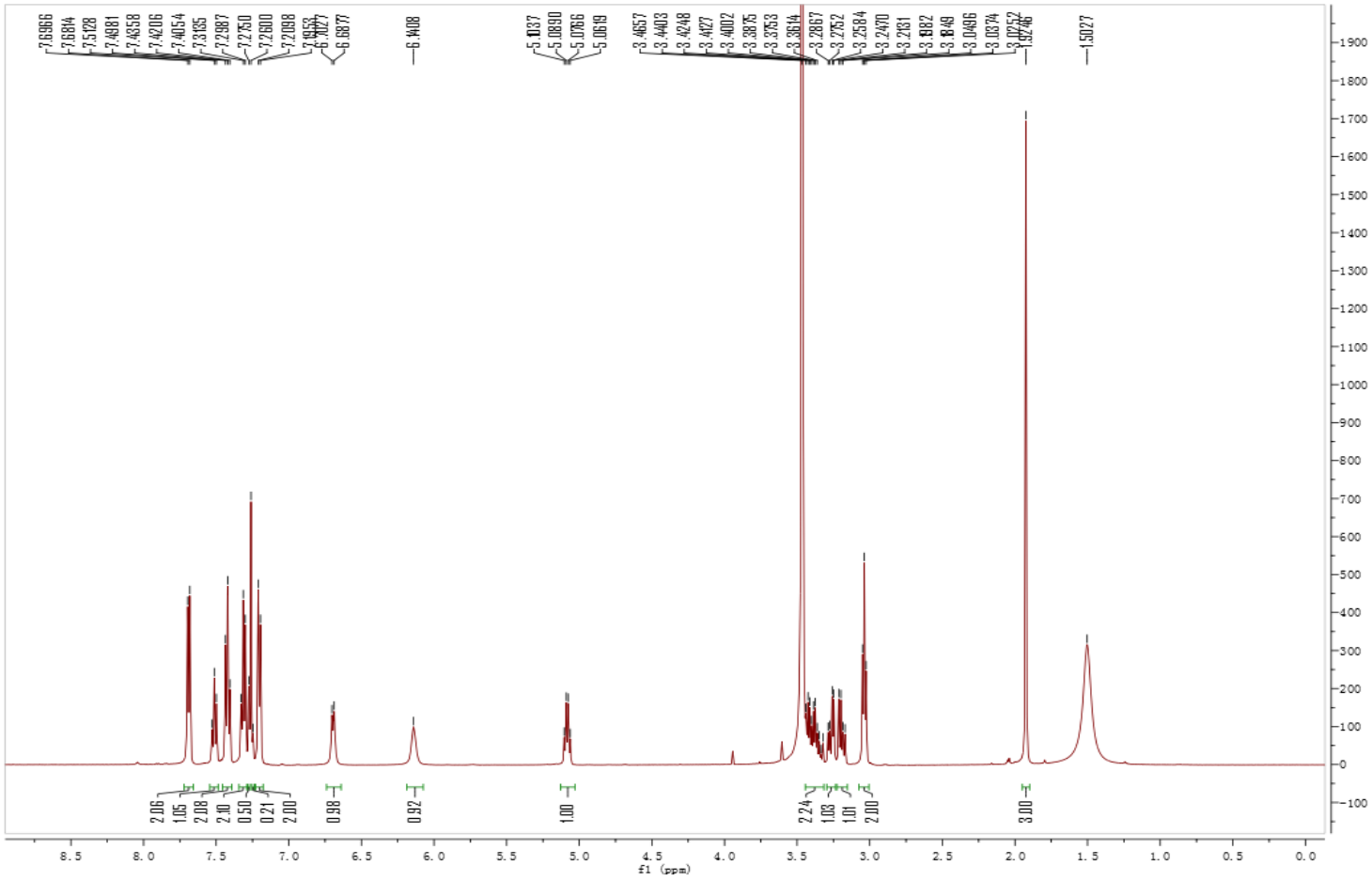


Fig. S15 ^{1}H NMR of **2**-SNAC in $CDCl_3$.

Supplementary References

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