

Biosynthetic allene and alkyne formation by enzymatic prenyl demethylation

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Experimental Procedures

1. Strains and culture conditions

*Penicillium polonicum*¹ was maintained in liquid PDB medium (PDA medium without agar) at 28 °C for isolation of genomic DNA. Genomic DNA of *Nemania serpens* was kindly provided by Prof. Michio Sato. *Aspergillus nidulans* A1145 ΔEMΔST host was previously developed in our lab². The *A. nidulans* strain was grown at 28 °C in CD media (1 L: 10 g Glucose, 50 mL 20 × Nitrate salts, 1 mL Trace elements, pH 6.5, and 20 g/L Agar for solid cultivation) for sporulation or in CD-ST media (1L: 20 g Starch, 20 g Casamino acids, 50 mL 20 × Nitrate salts, 1 mL Trace elements, pH 6.5)² for heterologous expression of gene clusters, compound production and RNA extraction. *Escherichia coli* strains were cultivated either on lysogeny broth (LB) agar plates or in LB liquid medium. Growth media were supplied with antibiotics as required at the following concentrations: kanamycin (50 µg mL⁻¹), and ampicillin (100 µg mL⁻¹). *Saccharomyces cerevisiae* strain BJ5464-NpgA (*MATa ura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*) was used as the yeast host for *in vivo* homologous recombination to construct the *A. nidulans* plasmids.

2. General molecular biology techniques

E. coli TOP10 cells were used for cloning, following standard recombinant DNA techniques. *E. coli* BL21(DE3) (Novagen) was used as the *E. coli* host for protein expression. DNA restriction enzymes were used as recommended by the manufacturer (New England Biolabs, NEB). Genomic DNA from all fungal strains was prepared using LETS isolation buffer (10 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.5% SDS, 0.1 M LiCl). PCR was performed using Q5 High-Fidelity DNA Polymerase (NEB). The gene-specific primers are listed in Supplementary Table 2. PCR products were confirmed by DNA sequencing. For isolation of RNA from *A. nidulans* transformants, the strains were grown on CD-ST liquid for 3 days at 28 °C. The RNA extraction steps were performed using RiboPure™ Yeast RNA Isolation Kit (Ambion) following the manufacturer's instructions. Residual genomic DNA in the extracts was digested by DNase I (2 U/mL) (Invitrogen) at 37 °C for 4 hours. SuperScript III First-Strand Synthesis System (Invitrogen) was used for cDNA synthesis with Oligo-dT primers following directions from the user manual.

3. Plasmid construction for heterologous expression in *A. nidulans*

For heterologous expression in *A. nidulans*, three plasmid vectors, pYTU, pYTP, and pYTR containing auxotrophic markers for uracil (*pyrG*), pyridoxine (*pyroA*), and riboflavin (*riboB*), respectively, were used to construct plasmids for *A. nidulans* heterologous expression. Genes in the *ppn* and *nse* cluster were amplified with PCR from the genomic DNA of *A. flavus*. The *gpdA* promoters from *Penicillium oxalicum* (constitutive *POgpdA*), *A. niger* (constitutive *gpdA*, *glaA* induced by starch) and *Penicillium expansum* (constitutive *PEgpdA*) were amplified by PCR. pYTP and pYTR were digested with PacI/PspXI. pYTU was digested with PacI/NotI. The amplified gene fragments and the corresponding vectors were co-transformed into *S. cerevisiae* strain BJ5464-NpgA for homologous recombination. The yeast plasmids were extracted using Zymoprep™ Yeast Plasmid Miniprep I (Zymo Inc. USA), and then electrically transformed into *E. coli* TOP10 to isolate single plasmids. The plasmids were extracted from *E. coli* using the Zyppy™ Plasmid Miniprep Kit (Zymo Research) and confirmed with sequencing by Laragen and Primordium Lab.

4. Preparation of protoplast of *A. nidulans* and transformation

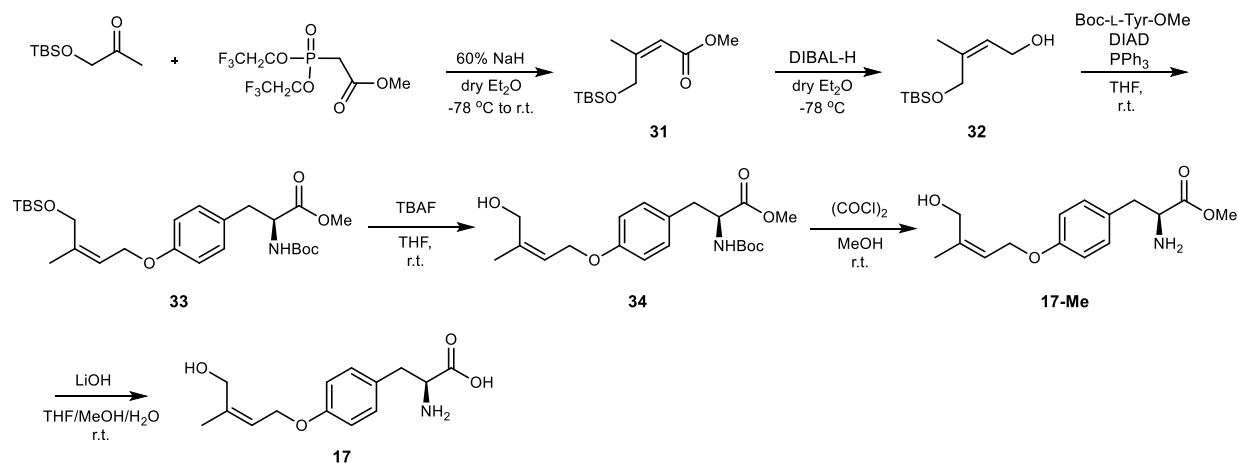
The transformation of *Aspergillus nidulans* A1145 ΔEMΔST², spores were inoculated into 50 mL liquid CD

media in a 125-mL flask and germinated at 30 °C shaking at 250 rpm for ~9 h. The 20 X Nitrate salts solution was prepared by dissolving 120 g NaNO₃, 10.4 g KCl, 10.4 g MgSO₄•7H₂O, and 30.4 g KH₂PO₄ in 1 L double distilled water. The trace elements solution (100 mL) contained 2.20 g ZnSO₄•7H₂O, 1.10 g H₃BO₃, 0.50 g MnCl₂•4H₂O, 0.16 g FeSO₄•7H₂O, 0.16 g CoCl₂•5H₂O, 0.16 g CuSO₄•5H₂O, and 0.11 g (NH₄)₆Mo₇O₂₄•4H₂O. The dropout components for selection for the three expression vectors were uracil/uridine, pyridoxine and riboflavin. *A. nidulans* A1145 ΔEM was initially grown on CD agar plates containing 10 mM uridine, 5 mM uracil, 0.5 μg/mL pyridoxine HCl and 2.5 μg/mL riboflavin at 37°C for 5 days. The germinated spores were harvested by centrifugation at 3,500 rpm for 10 min, and washed with Osmotic buffer (10 mL, 1.2 M MgSO₄, 10 mM sodium phosphate buffer, pH 5.8). The mycelia were then mixed with Osmotic buffer (10 mL, 30 mg lysing enzymes from *Trichoderma*, 20 mg Yatalase) in a 125-mL flask. Protoplasts were prepared by incubating the mixture overnight at 30 °C with gentle shaking at 80 rpm. Cells were collected in a 30-mL Corex tube and overlaid gently by 10 mL of Trapping buffer (0.6 M sorbitol, 0.1 M Tris HCl, pH 7.0). Centrifugation at 3,500 rpm for 15 min at 4 °C layered the protoplasts at the interface of the two buffers. The protoplasts were then pipetted to a sterile 15-mL falcon tube and washed with STC buffer (10 mL, 1.2 M sorbitol, 10 mM CaCl₂, 10 mM Tris-HCl pH 7.5). The protoplasts were resuspended in STC buffer (1 mL).

For each transformation, 3 μL of each plasmid (>100 ng/μL) was added to 60 μL of the *A. nidulans* A1145 ΔSTΔEM protoplast suspension prepared as above, and the mixture was incubated for 1 h on ice. 600 μL PEG solution (60% PEG, 50 mM of CaCl₂, and 50 mM of Tris-HCl, pH 7.5) was added to the protoplast mixture, followed by additional incubation at room temperature for 20 min. The mixture was spread on the CD sorbitol plate (CD solid medium with 1.2 M sorbitol and the appropriate supplements: 10 mM of uridine, 5 mM of uracil, 0.5 μg/mL of pyridoxine HCl, and/or 2.5 μg/mL of riboflavin according to the markers in the transformed plasmids) and incubated at 37 °C for 3-4 days.

5. General procedure for the synthesis of substrates³⁻⁵

5.1 Chemical synthesis of 17-Me and 17.



Under an argon atmosphere, to a solution of sodium hydride (NaH, 60 % dispersion in mineral oil, 0.96 g, 24 mmol) in 30 mL of dry diethylether (Et₂O) was added triethyl bis(2,2,2-trifluoroethyl) (methoxycarbonylmethyl)phosphonate (4.4 mL, 20 mmol) dropwise at 0 °C. After stirring for 1 hour at 0 °C, the reaction mixture was cooled down to -78 °C. 1-(tert-butyldimethylsilyloxy)-2-propanone (5.2 mL, 24 mmol) was

then added to the reaction mixture dropwise. The reaction was allowed stirring at -78 °C to r.t. overnight. The mixture was quenched by 50 mL of saturated NH₄Cl aqueous solution. The mixture was extracted with 3 x 50 mL of diethyl ether, and the combined organic layers were washed with brine, dried using anhydrous Na₂SO₄. The organic layer was filtered to remove Na₂SO₄, and carefully concentrated under reduced pressure to give 1.6 g of crude product **31**. The product was used for next reaction without further purification.

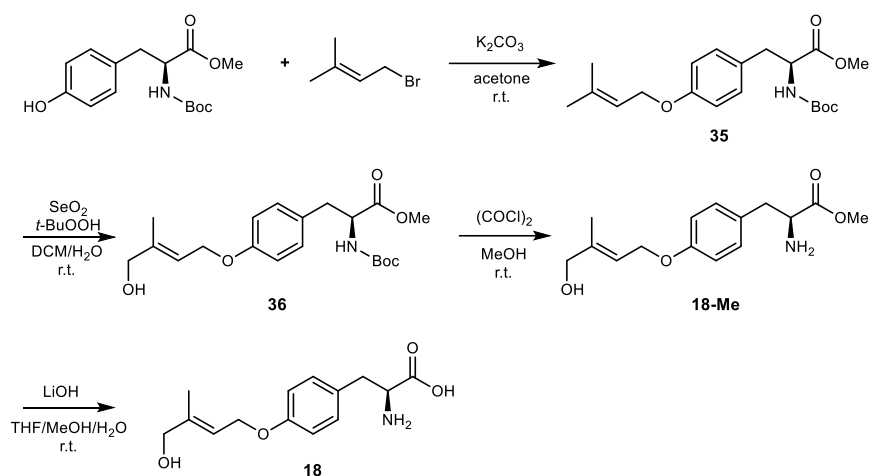
Under an argon atmosphere, to a solution of the crude (1.6 g) in 30 mL of dry Et₂O was added diisobutylaluminium hydride (1 M DIBAL-H in hexane, 16.4 mL, 16.4 mmol) dropwise at -78 °C. After addition, the reaction kept stirring at the same temperature until no starting material could be detected by TLC. The reaction mixture was quenched by a few drops of water and 4M NaOH. The resultant solid was removed by filtration and washed with Et₂O. The filtrate was carefully concentrated under reduced pressure to give 0.92 g of the corresponding alcohol **32**. The product was used for next reaction without further purification.

A mixture of alcohol (0.92 g), *N*-(*tert*-butoxycarbonyl)-L-tyrosine methyl ester (1.26 g, 4.3 mmol), triphenylphosphine (1.35 g, 5.15 mmol), DIAD (1.10 mL, 5.15 mmol) and 30 mL of THF were stirred for 24 h at room temperature under an argon atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was purified by reverse-phase column using Combi-Flash system with a linear gradient of 5-95% acetonitrile: water supplemented with 0.1% formic acid to give 1.24 g of the corresponding compound **33** as yellow oil (13% in 3 steps).

To a solution of the compound (1.24 g, 2.5 mmol) in 20 mL of THF was added 1M TBAF in THF (3.0 mL, 3.0 mmol) dropwise at r.t. After stirring the reaction mixture at r.t. overnight, 30 mL of water was added to the reaction mixture. The mixture was extracted by 3 x 30 mL of EtOAc, and the combined organic layer was washed with 50 mL of brine, and dried by anhydrous Na₂SO₄. After the filtration to remove Na₂SO₄, the organic layer was concentrated under reduced pressure to give 0.73 g of the compound **34**. The product was used for next reaction without further purification. ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.25 (d, *J* = 8.1 Hz, 1H), 7.12 (dd, *J* = 9.0, 3.0 Hz, 2H), 6.82 (d, *J* = 8.1 Hz, 2H), 5.42 (t, *J* = 6.5 Hz, 1H), 4.55 (d, *J* = 6.4 Hz, 2H), 4.10 (ddd, *J* = 9.8, 8.0, 5.1 Hz, 1H), 3.99 (s, 2H), 3.60 (s, 3H), 2.90 (dd, *J* = 13.8, 5.2 Hz, 1H), 2.77 (dd, *J* = 13.8, 9.9 Hz, 1H), 1.75 (d, *J* = 1.5 Hz, 3H), 1.33 (s, 9H). ¹³C NMR (126 MHz, DMSO) δ 21.0, 28.1, 35.6, 51.7, 55.5, 59.8, 63.6, 78.3, 114.3, 121.2, 129.3, 130.1, 140.3, 155.4, 157.0, 172.7.

Compound **34** (0.73 g, 1.82 mmol) was dissolved in MeOH (15 mL) and allowed to stir at room temperature for 5 min. oxalyl chloride (0.50 mL, 5.80 mmol) was then added to the solution *via* micropipette directly into the reaction solvent mixture dropwise. The reaction mixture was allowed to stir at r.t. for 4 h. Upon complete consumption of the *N*-Boc-protected compound, water (10 mL) was added to the flask slowly. The crude material was subsequently extracted with 3 x 30 mL of EtOAc and washed with 30 mL of water and 30 mL of brine. The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under the reduced pressure. The residue was purified by reverse-phase column using Combi-Flash system with a gradient of 5-20% (acetonitrile : water) supplemented with 0.1% formic acid to give 0.33 g of the corresponding compound **17-Me** as yellow oil (47% in 2 steps). **17-Me** further undergo hydrolysis to give **17** following the method for chemical synthesis of **7-Me** and **7**. ¹H NMR (500 MHz, D₂O) δ 7.14 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.57 (dt, *J* = 7.0, 4.3 Hz, 1H), 4.55 (d, *J* = 6.9 Hz, 2H), 4.11 (s, 2H), 3.40 (dd, *J* = 7.4, 5.4 Hz, 1H), 2.89 (dd, *J* = 13.7, 5.4 Hz, 1H), 2.72 (dd, *J* = 13.6, 7.4 Hz, 1H), 1.78 (m, 3H). ¹³C NMR (126 MHz, D₂O) δ 20.4, 39.8, 57.3, 59.9, 64.1, 115.0, 115.0, 122.0, 130.5, 130.5, 131.1, 140.8, 156.2, 182.3. HRMS (ESI, M+H⁺) calculated for C₁₄H₂₀O₄⁺ 266.1387; found 266.1387.

5.2 Chemical synthesis of 18-Me and 18.



A suspension of Boc-L-Tyr-OMe (0.89 g, 3.0 mmol, 1.0 equiv) and K_2CO_3 (0.50 g, 3.6 mmol, 1.2 equiv) in 20 mL of acetone was stirred at room temperature, and 3,3-dimethylallyl bromide (0.42 mL, 3.6 mmol, 1.2 equiv) was added dropwise. The mixture was stirred at r.t. for 18 h, and then poured into 150 mL of saturated $NaHCO_3$ aqueous solution. The mixture was extracted by 3 x 30 mL of EtOAc, and the combined organic layer was washed with brine, dried using anhydrous Na_2SO_4 , and concentrated under reduced pressure to give 1.01 g of the compound **35** as colorless oil. 1H NMR (500 MHz, $DMSO-d_6$) δ 7.25 (d, J = 8.0 Hz, 1H), 7.12 (d, J = 8.2 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 5.45 – 5.36 (m, 1H), 4.47 (d, J = 6.6 Hz, 2H), 4.10 (ddd, J = 9.9, 8.1, 5.1 Hz, 1H), 2.90 (dd, J = 13.8, 5.2 Hz, 1H), 2.77 (dd, J = 13.8, 9.9 Hz, 1H), 1.73 (s, 3H), 1.69 (s, 3H), 1.33 (s, 9H). ^{13}C NMR (126 MHz, $DMSO$) δ 18.0, 25.4, 28.1, 51.7, 55.5, 64.2, 78.2, 114.3, 120.1, 129.3, 130.0, 136.8, 155.4, 157.1, 172.7.

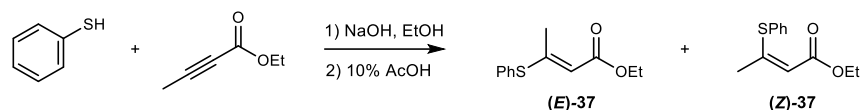
1.01 g of **35** was dissolved in DCM (18 mL), followed by the addition of 70% $t-BuOOH$ aqueous solution (2.0 mL, 9.0 mmol) and SeO_2 (66 mg, 0.60 mmol). The mixture was stirred for 3 days at r.t. The reaction mixture was poured into 300 mL of water. The mixture was extracted with 3 x 30 mL of EtOAc, and the combined organic layer was washed with brine, dried using anhydrous Na_2SO_4 , and concentrated under reduced pressure to give crude product containing alcohol **36** and aldehyde **48**. The crude mixture was further purified by reverse-phase column using Combi-Flash instrument with a gradient of 5-95% (acetonitrile : water) supplemented with 0.1% formic acid to give 0.21 g of **36** as pale yellow oil (20% yield) and 0.38 g of aldehyde **48** as colorless oil. The NMR data of synthetic **36**: 1H NMR (500 MHz, $CDCl_3$) δ 7.02 (d, J = 8.2 Hz, 2H), 6.87 – 6.77 (m, 2H), 5.75 (tq, J = 6.5, 1.5 Hz, 1H), 4.56 (d, J = 6.5 Hz, 2H), 4.53 (m, 1H), 4.08 (s, 2H), 3.71 (s, 3H), 3.08 – 2.94 (m, 2H), 1.75 (d, J = 1.2 Hz, 3H), 1.41 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.1, 28.4, 37.6, 52.4, 54.7, 64.4, 67.9, 80.4, 114.9, 120.4, 128.1, 130.4, 139.8, 155.5, 157.8, 172.6. The NMR data of synthetic **48**: 1H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.04 (d, J = 8.3 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 6.66 (tq, J = 5.4, 1.4 Hz, 1H), 5.04 (d, J = 8.4 Hz, 1H), 4.83 (dq, J = 5.5, 1.2 Hz, 2H), 4.52 (q, J = 6.6 Hz, 1H), 3.69 (s, 3H), 3.10 – 2.92 (m, 2H), 1.81 (d, J = 1.3 Hz, 3H), 1.39 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 9.7, 28.4, 37.5, 52.3, 54.6, 64.8, 80.1, 114.7, 129.0, 130.6, 139.8, 147.9, 157.2, 172.5, 194.2.

Compound **36** (0.21 g, 0.52 mmol) was dissolved in MeOH (10 mL) and allowed to stir at room temperature for 5 min. oxalyl chloride (0.14 mL, 1.60 mmol) was then added to the solution *via* micropipette directly into the reaction solvent mixture dropwise. The reaction mixture was allowed to stir at r.t. for 4 h. Upon complete consumption of the *N*-Boc-protected compound, water (10 mL) was added to the flask slowly. The crude material was subsequently extracted with 3 x 30 mL of EtOAc and washed with 30 mL of water and 30 mL of brine. The

organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under the reduced pressure. The residue was purified by reverse-phase column using Combi-Flash system with a gradient of 5-20% (acetonitrile : water) supplemented with 0.1% formic acid to give 0.10 g of the corresponding compound **18-Me** as yellow oil (69% yield). **18-Me** further undergo hydrolysis to give **18** following the method for chemical synthesis of **7-Me** and **7**. ¹H NMR (500 MHz, D₂O) δ 7.05 (d, *J* = 8.5 Hz, 2H), 6.87 – 6.77 (m, 2H), 5.54 (dp, *J* = 6.7, 1.7 Hz, 1H), 4.50 (d, *J* = 6.7 Hz, 2H), 3.87 (s, 2H), 3.30 (m, 1H), 2.78 (dd, *J* = 13.6, 5.5 Hz, 1H), 2.63 (dd, *J* = 13.7, 7.3 Hz, 1H), 1.58 (s, 3H). ¹³C NMR (126 MHz, D₂O) δ 13.1, 25.0, 39.8, 48.8, 57.3, 64.7, 66.4, 67.8, 115.0, 115.0, 119.1, 130.5, 130.5, 131.1, 141.1, 156.3, 182.4. HRMS (ESI, M+H⁺) calculated for C₁₄H₂₀O₄⁺ 266.1387; found 266.1401.

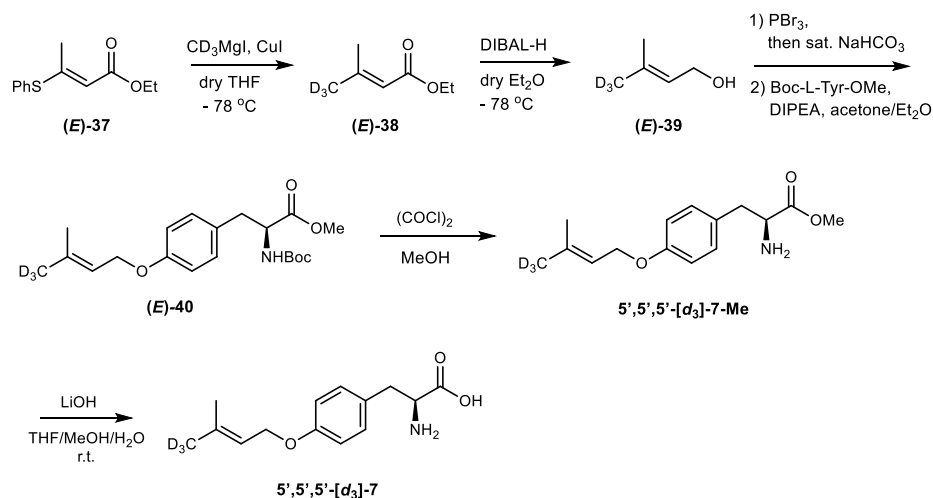
5.3 Chemical synthesis of 4',4',4'-[d₃]-7-Me and -7 and 5',5',5'-[d₃]-7-Me and -7.

5.3.1 Synthesis of (*E*)-**37** and (*Z*)-**37**.

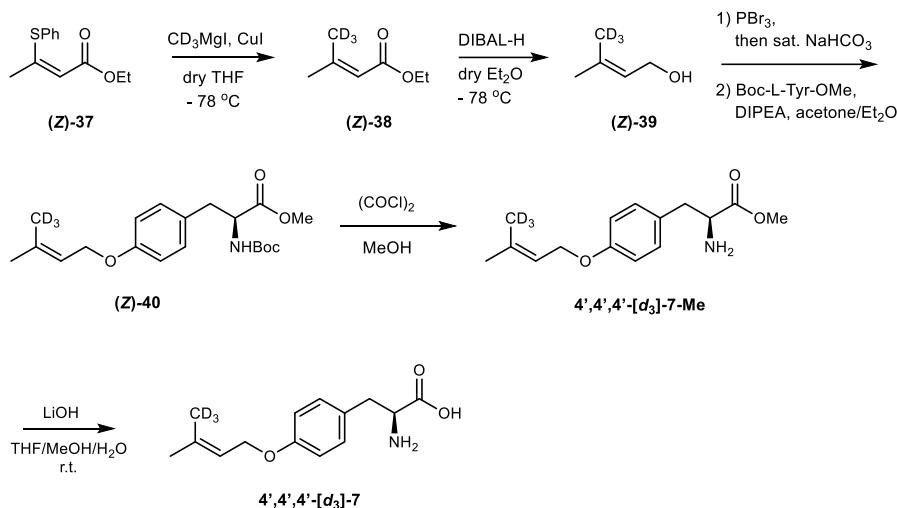


To a stirring suspension of NaOH (1.2 g, 30 mmol) in 60 mL of EtOH was added PhSH (3.5 mL, 34.2 mmol) and ethyl 2-butynoate (3 mL, 25.7 mmol). After stirring at r.t. for 4 hours, the reaction was acidified with 15 mL of 10% AcOH. The organic layer was then washed with 60 mL of water and 60 mL of brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by normal-phase silica gel chromatography using Combi-Flash instrument with a gradient of 10% (Et₂O : hexane) to give 2.94 g (*E*)-**37** (51%) and 0.92 g (*Z*)-**37** (16%) as yellow oil.

5.3.2 Synthesis of 5',5',5'-[d₃]-7-Me and -7.



5.3.3 Synthesis of 4',4',4'-[d₃]-7-Me and -7.



Compound (*E*)-38 and (*Z*)-38. Under an argon atmosphere, to a stirring suspension of CuI (3 g, 16 mmol) in 30 mL dry THF at -78 °C was added CD₃MgI (1 M solution in Et₂O, 20 mL, 20 mmol). After the mixture was stirred at -78 °C for 15 minutes, a solution of (*E*)-37 (888 mg, 4 mmol) in 12 mL of dry THF was added dropwise and stirred at -78 °C for 4 hours. The mixture was then stirred at 0 °C and 50 mL of saturated NH₄Cl was added slowly. The aqueous layer was extracted with Et₂O twice, the organic layers were combined and washed with 30 mL of water and 30 mL of brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure carefully. The residue was purified by normal-phase silica gel chromatography using Combi-Flash instrument with a gradient of 2.5% (Et₂O : pentane) to give 192 mg of (*E*)-38 (37%) as white oil. (*Z*)-38 (222 mg, 42%, white oil) was acquired through the same procedure.

Compound (*E*)-39 and (*Z*)-39. Under an argon atmosphere, to a stirring solution of (*E*)-38 (475 mg, 3.6 mmol) in 2.5 mL of dry Et₂O was added DIBAL-H (1 M DIBAL-H in hexane, 9 mL, 9 mmol) dropwise at -78 °C. After stirring for 1 hour, the mixture was then stirred at 0 °C and a few drops of water and 4 M NaOH was added. The resultant solid was removed by filtration and washed twice with 5 mL of cold Et₂O, then dried over anhydrous Na₂SO₄ at 0 °C. The product solution containing (*E*)-39 was filtered and used for next reaction without further purification.

Compound (*E*)-40 and (*Z*)-40. PBr₃ (266 µL, 2.8 mmol) in 2 mL of dry Et₂O was added dropwise into the resulted product solution upon stirring at 0 °C for 2 hours. The reaction was quenched at 0 °C by adding 15 mL of saturated NaHCO₃. The aqueous layer was extracted with 2 x 5 mL of Et₂O and the organic layers were combined. Boc-L-Tyr-OMe (1.26 g, 4.3 mmol) in 20 mL of 1:1 acetone/Et₂O and DIPEA (1.4 mL, 8 mmol) were added into the resulted organic layer and stirred for 16 hours. The reaction was concentrated under reduced pressure and the residue was purified by reverse-phase column using Combi-Flash instrument with a gradient of 30-95% (acetonitrile : water) to give 588 mg of (*E*)-40 (45%) as yellow oil. (*Z*)-40 (563 mg, 43%) was acquired through the same procedure.

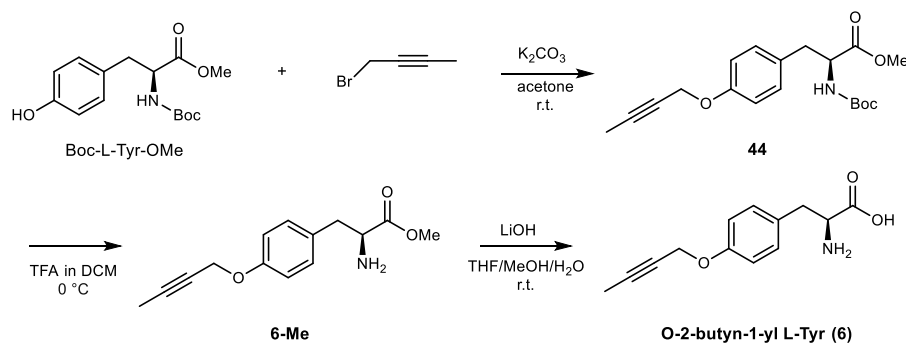
Compound 4',4',4'-[d₃]-7-Me and 5',5',5'-[d₃]-7-Me. (*E*)-40 (363 mg, 1 mmol) was dissolved in MeOH (10 mL) and allowed to stir at room temperature for 5 min. Oxalyl chloride (170 µL, 2 mmol) was then added to the solution into the reaction mixture dropwise. The reaction mixture was allowed to stir at r.t. for 4 h. Upon complete consumption of (*E*)-40, 10 mL of water was added slowly. The crude material was subsequently extracted with 3 x 10 mL of EtOAc, combined and washed with 30 mL of water and 30 mL of brine. The organic layer was dried over

3.02 (qd, $J = 14.0, 5.9$ Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 28.4, 37.6, 52.3, 54.6, 66.0, 80.0, 87.2, 115.1, 128.4, 130.4, 155.2, 157.6, 172.6, 209.6.

0.21 g of **43** was dissolved in CH_2Cl_2 (5 mL), and trifluoroacetic acid (TFA, 5 mL) was added dropwise at 0°C . The mixture was stirred at rt for 0.5 h and then concentrated under reduced pressure to give the deprotected intermediate to give 0.20 g of **1-Me** as brown solid with quantitative yield. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 7.16 – 7.06 (m, 2H), 6.96 – 6.86 (m, 2H), 5.48 (p, $J = 6.7$ Hz, 1H), 4.97 (dt, $J = 6.7, 2.6$ Hz, 2H), 4.54 (dt, $J = 6.7, 2.6$ Hz, 2H), 4.25 (t, $J = 6.6$ Hz, 1H), 3.67 (s, 3H), 3.03 (qd, $J = 14.2, 6.6$ Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 35.2, 52.6, 53.5, 65.1, 76.9, 86.9, 114.9, 126.5, 130.5, 157.3, 169.5, 208.7.

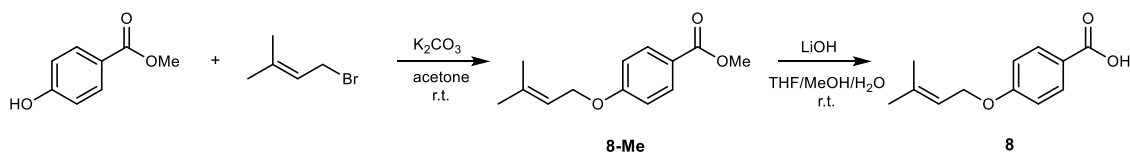
0.20 g of **1-Me** was dissolved in THF/MeOH/ H_2O (4:2:3 (v/v/v), 9.0 mL), LiOH (23 mg, 0.97 mmol, 1.2 equiv) was added, and the mixture was stirred at rt for 3 h. After completion (LCMS monitoring), the reaction mixture was directly purified by reverse-phase column using Combi-Flash instrument with a gradient of 5-30% (acetonitrile : water) supplemented with 0.1% formic acid to give 0.11 g of **1** (58% yield) as yellow solid.

5.5 Chemical synthesis of compound 6.



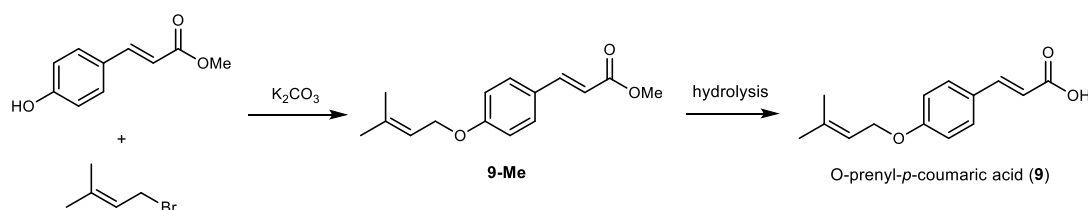
1-bromo-2-butyne (0.32 mL, 3.6 mmol) was added to a suspension of Boc-L-Tyr-OMe (0.88 g, 3.0 mmol) and K_2CO_3 (0.50 g, 3.6 mmol) in 20 mL of acetone. The mixture was stirred at r.t. for 18 h, and then poured into 100 mL of saturated NaHCO_3 aqueous solution. The mixture was extracted by 3 x 30 mL of EtOAc, and the combined organic layer was washed with brine, dried using anhydrous Na_2SO_4 , and concentrated under reduced pressure to give 0.90 g of crude **44**. The 0.20 g of crude **44** was dissolved in 5 mL of CH_2Cl_2 , and 5 mL of trifluoroacetic acid was added dropwise at 0°C . The reaction mixture was stirred at r.t. until deprotection was complete. The solvent and excess TFA were removed under reduced pressure to give crude **6-Me**. The crude **6-Me** was dissolved in THF/MeOH/ H_2O (4:2:3 (v/v/v), 9.0 mL), LiOH (24 mg, 1.0 mmol) was added, and the mixture was stirred at rt for 3 h. After completion (LCMS monitoring), the reaction mixture was directly purified by reverse-phase column using Combi-Flash instrument with a gradient of 5-30% (acetonitrile : water) supplemented with 0.1% formic acid to give 0.10 g of **6** as yellow solid.

5.6 Chemical synthesis of O-prenylbenzoic acid (8).



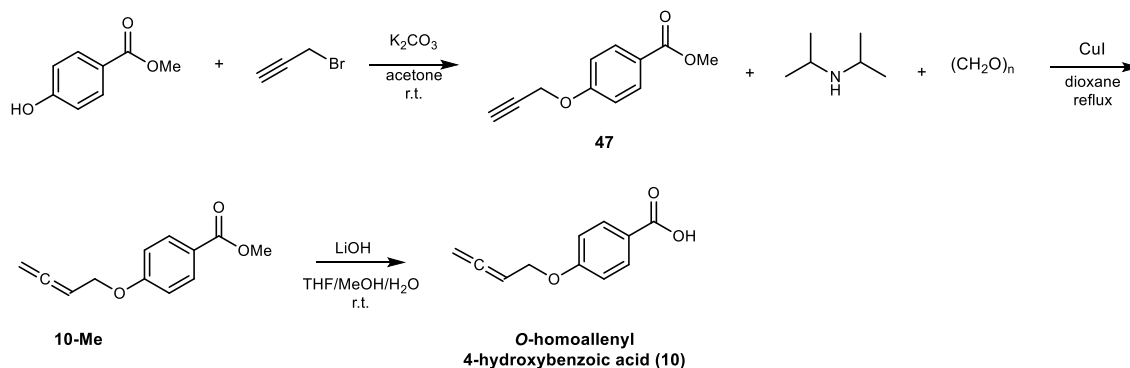
3,3-Dimethylallyl bromide (0.14 mL, 1.2 mmol, 1.2 equiv) was added to a suspension of 4-hydroxybenzoic acid methyl ester (0.15 g, 1.0 mmol, 1.0 equiv) in 15 mL of acetone. The mixture was stirred at r.t. for 18 h, and then poured into 100 mL of saturated NaHCO₃ aqueous solution. The mixture was extracted by 3 x 30 mL of EtOAc, and the combined organic layer was washed with brined, dried using anhydrous Na₂SO₄, and concentrated under reduced pressure to give 201 mg of crude **8-Me**. The crude **8-Me** was dissolved in THF/MeOH/H₂O (4:2:3 (v/v/v), 9.0 mL), LiOH (48 mg, 2.0 mmol) was added, and the mixture was stirred at rt for 1 h. After completion (LCMS monitoring), the pH of the reaction mixture was adjusted to ~ 3, and extracted with 3 x 30 mL of EtOAc. The combined organic layer was washed with brined, dried using anhydrous Na₂SO₄, and concentrated under reduced pressure to give crude **6**, which was subsequently purified by reverse-phase column using Combi-Flash instrument with a gradient of 5-95% (acetonitrile : water) supplemented with 0.1% formic acid to give 0.15g of **6** as white solid (73% yield, 2 steps).

5.7 Chemical synthesis of O-prenyl-*p*-coumaric acid (**9**).



Compound **9** was synthesized following the same procedure used for compound **8**, except that a *p*-coumaric acid ester was employed as the substrate in place of the 4-hydroxybenzoic acid ester. The NMR data of synthetic **9-Me**: ¹H NMR (500 MHz, CDCl₃) δ 7.64 (d, *J* = 15.9 Hz, 1H), 7.49 – 7.40 (m, 2H), 6.90 (d, *J* = 8.8 Hz, 2H), 6.30 (d, *J* = 15.9 Hz, 1H), 5.47 (tp, *J* = 6.6, 1.6 Hz, 1H), 4.52 (d, *J* = 6.8 Hz, 2H), 3.78 (s, 3H), 1.84 – 1.78 (m, 3H), 1.74 (d, *J* = 1.3 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 18.3, 25.9, 51.6, 65.0, 115.1, 115.2, 119.3, 127.1, 129.8, 138.7, 144.7, 160.8, 167.9.

5.8 Chemical synthesis of O-homoallenyl-4-hydroxybenzoic acid (**10**).

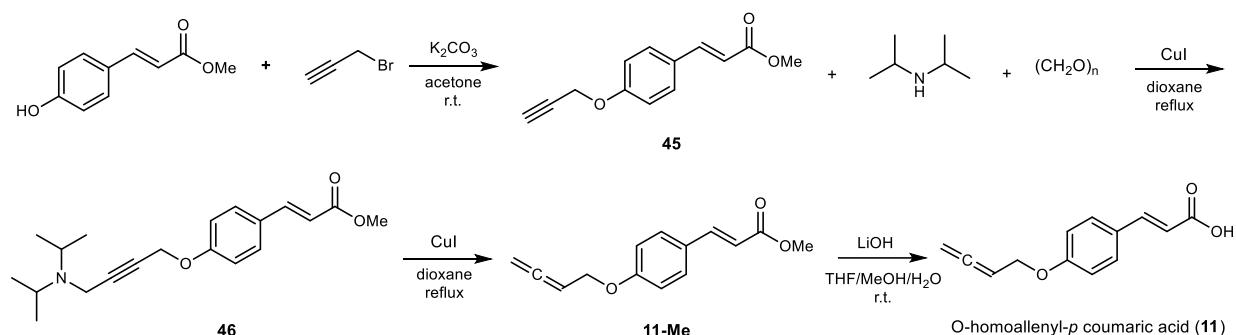


A suspension of 4-hydroxybenzoic acid ester (0.61 g, 4.0 mmol, 1.0 equiv) and K₂CO₃ (0.53 g, 4.8 mmol, 1.2 equiv) in 30 mL of acetone was stirred at room temperature, and propargyl bromide (0.52 mL, 4.8 mmol, 1.2 equiv) was added dropwise. The mixture was stirred at r.t. for 18 h, and then poured into 150 mL of saturated NaHCO₃ aqueous solution. The mixture was extracted by 3 x 50 mL of EtOAc, and the combined organic layer was washed with brined, dried using anhydrous Na₂SO₄, and concentrated under reduced pressure to give 0.70g of crude **10-Me**.

The crude residue **47** was dissolved in 30 mL of 1,4-dioxane, and CuI (0.15 g, 0.80 mmol, 0.2 equiv) diisopropylamine (1.1 mL, 8.0 mmol, 2.0 equiv), and paraformaldehyde (0.31 g, 10.0 mmol, 2.5 equiv) were added. The mixture was heated to reflux and stirred overnight. After cooling to r.t., the mixture was filtered through Celite to remove precipitates and washed with EtOAc. The combined filtrates were concentrated under reduced pressure to afford crude mixture. The mixture was further purified by reverse-phase column using Combi-Flash instrument with a gradient of 5-95% (acetonitrile : water) supplemented with 0.1% formic acid to give 0.68 g of **10-Me** (83% yield, 2 steps) as brown oil. The NMR data of synthetic **10-Me** matched the reported values⁵. ¹H NMR (500 MHz, CDCl₃) δ 7.92 (d, *J* = 8.9 Hz, 1H), 6.85 (d, *J* = 8.9 Hz, 1H), 5.33 (p, *J* = 6.7 Hz, 0H), 4.83 (dt, *J* = 6.8, 2.5 Hz, 1H), 4.52 (dt, *J* = 6.6, 2.6 Hz, 1H), 3.81 (s, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 51.5, 65.6, 76.6, 86.5, 114.2, 122.5, 131.3, 161.9, 166.4, 209.3.

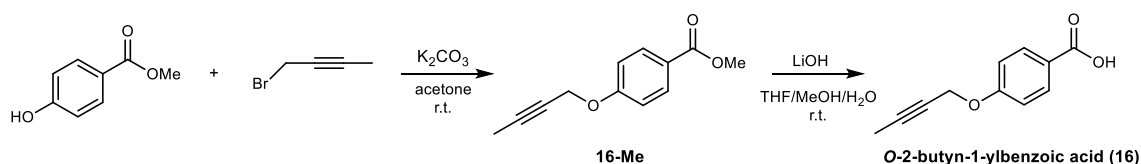
To a solution of **10-Me** (0.68 g, 3.3 mmol) in THF/MeOH/H₂O (4:2:3 (v/v/v), 18 mL), added LiOH (0.16 g, 6.6 mmol). The reaction mixture was allowed to stir at room temperature. After reaction completion, adjust the pH to 3 and extracted with 3 x 30 mL of EtOAc. The extracts were washed with water and saturated brine, and then dried over anhydrous MgSO₄ to give 0.63 g of **10** with quantitative yield.

5.9 Chemical synthesis of O-homoallenyl-*p*-coumaric acid (**11**).



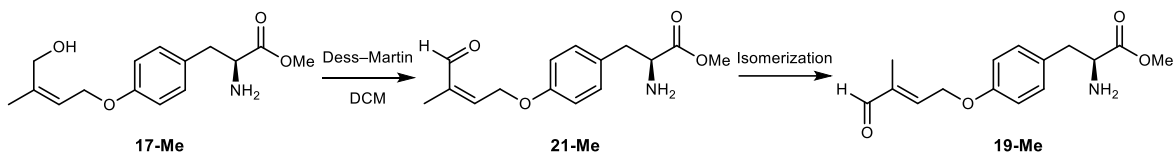
The synthesis of **11** followed the same procedure as for compound **10**, except that a *p*-coumaric acid ester was used in place of the 4-hydroxybenzoic acid ester. The NMR data of synthetic **11-Me** matched the reported values⁵. **11-Me**: ¹H NMR (500 MHz, CDCl₃) δ 7.64 (d, *J* = 15.9 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 8.3 Hz, 2H), 6.31 (d, *J* = 15.9 Hz, 1H), 5.38 (p, *J* = 6.7 Hz, 1H), 4.87 (dd, *J* = 6.3, 2.9 Hz, 2H), 4.59 (dd, *J* = 6.5, 3.0 Hz, 2H), 3.79 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 51.7, 66.0, 86.9, 115.3, 115.5, 127.4, 129.8, 144.6, 160.3, 167.9, 209.7.

5.10 Chemical synthesis of O-2-butyne-1-ylbenzoic acid (**16**).



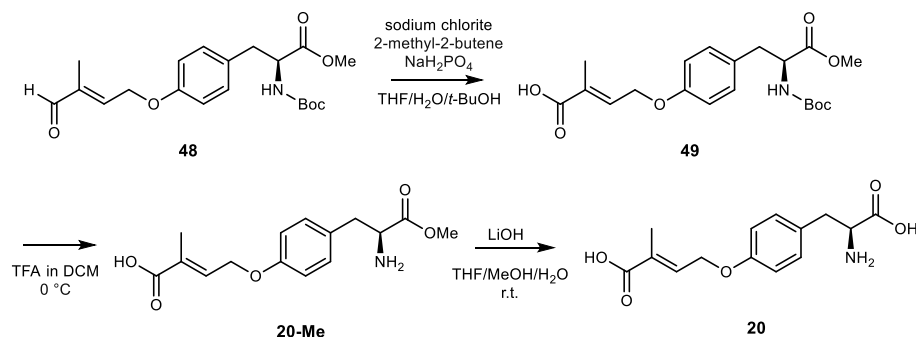
Compound **16** was synthesized following the same procedure used for compound **8**, except that a 1-bromo-2-butyne (0.36 mL, 4.06 mmol) was employed as the substrate in place of the prenyl bromide. The NMR data of synthetic **12**: ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.89 (d, *J* = 9.0 Hz, 2H), 7.04 (d, *J* = 9.0 Hz, 2H), 4.82 (s, 1H), 1.83 (d, *J* = 2.3 Hz, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 3.1, 56.1, 74.3, 84.0, 114.6, 123.4, 131.2, 160.9, 166.9.

5.11 Chemical synthesis of 19-Me.



To a stirred solution of **17-Me** (20 mg, 0.072 mmol, 1.0 eq.) in DCM was added Dess-Martin periodinane (36 mg, 0.086 mmol, 1.2 eq.) at 0°C. The reaction mixture was stirred at 0°C for 0.5 hour, then filtered through Celite and the precipitate was further washed with DCM. The filtrate was successively washed with a saturated solution of NaHCO₃ and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude residue was further purified by reverse-phase column using HPLC system with a linear gradient of 5-50% acetonitrile: water supplemented with 0.1% formic acid to afford 5.0 mg of **19-Me** as a yellow oil (25% yield). We were unable to obtain the corresponding C₄-aldehyde (**21-Me**) likely due to the spontaneous *E/Z* isomerization during the reaction and purification.

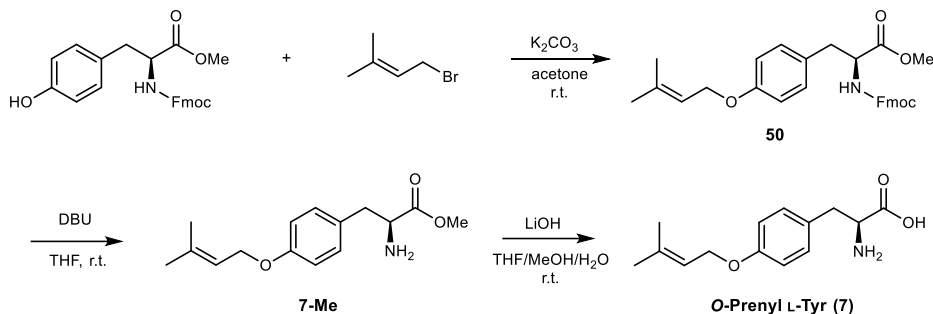
5.12 Chemical synthesis of 20.



To a stirred solution of the **48** (0.39 g, 1.0 mmol) in tetrahydrofuran/water/*tert*-butanol (22.5 mL, 4:4:1) were added 2-methyl-2-butene (1.60 mL, 15.0 mmol) at 0 °C, followed by the sequential addition of sodium dihydrogen phosphate monohydrate (0.72 g, 6.0 mmol) and sodium chlorite (80% purity, 0.34 g, 3.0 mmol). The resulting reaction mixture was stirred at r.t. overnight. The reaction mixture was diluted with brine and extracted with 3 x 30 mL of EtOAc. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated reduced pressure to give 0.40 g of the crude compound **49**, which was directly used in the next step without further purification.

To a stirred solution of **49** (70 mg, 0.18 mmol) in 10 mL of DCM were added 3 mL of TFA at 0 °C. The resulting reaction mixture was stirred at r.t. until all substrate is deprotected (checked by LC/MS). The reaction mixture was concentrated under reduced pressure to afford **20-Me**, which was subsequently subjected to hydrolysis with lithium hydroxide. The resultant residue was purified by reverse-phase column using Combi-Flash instrument with a gradient of 5-95% (acetonitrile : water) supplemented with 0.1% formic acid to give 25 mg of **20** as pale yellow oil.

5.13 Chemical synthesis of 7-Me and 7.



Compound **50** (120 mg, 0.20 mmol) was synthesized as same method as **35**, except that Fmoc-L-Tyr-OMe was employed as the substrate instead of Boc-L-Tyr-OMe. The crude **50** was dissolved in THF, followed by dropwise addition of DBU (45 μ L, 0.30 mmol, 1.5 equiv) at room temperature. The reaction mixture was stirred for 2 h. After completion, the reaction was quenched with saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (3×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (hexanes/EtOAc 3:1) to yield methyl ester **7** as a white solid (85 mg). Further hydrolysis of **7-Me** was carried out by dissolving the purified ester in THF/MeOH/ H_2O (1:1), followed by addition of LiOH (2.0 equiv). The mixture was stirred at room temperature for 2 h. Upon completion, the reaction was lyophilized to yield **7** as a white solid.

5.14 Chemical synthesis of 1',1'-[d_2]-**7** and 2'-[d_1]-**7**.

Regarding the synthesis of 1',1'-[d_2]-**7**, the same synthetic scheme from **32** to **17** in **Chemical synthesis of 17-Me and 17** without TBAF step was applied with 1,1-[d_2]-prenol as the starting material. Similarly, regarding the synthesis of 2'-[d_1]-**7**, the same synthetic scheme was used with 2-[d_2]-prenol as the starting material. 1,1-[d_2]-prenol⁶ and 2-[d_2]-prenol⁷ were synthesized by the reported procedures.

6. Sequence information

PpnA amino acid sequence

MAQTLLDESPEFQFGRYQFASNGLSTSSADLEPREHRRITRYPYYPVELSIWQQRVNSELDSFESVHHRFW
WSRHTGKALAVLLYNAQYPADLQYWNLKFFAEAVAPHLGVAPILGSDTPIWPSFMTDDGTPVELSWDW
GTKDAPPMVRYSVPIGLHAGTSVDPGNLTAGPAFQERLTRSLPTMRLEWFHHFKDFFNIPNAKEGEFHE
DTRDHNSSIFYGFDCSETEITPKVYFFPKLRAKASGQSNLDVLFQAMRTAPHVTDNRNFEAGDIFHAFCSNV
GSKSLEHEMLAIDLIDPLQSRLKIYFRSRETTFQSVINIMTLEGRIRNPKLYEGLVDLHRLWTALFGVYAVD
QPLREVEHRTSGILYNFEFRLGEALPVAKIYLPVRHYCTSDEAVIRALNDYFQGQQKGKYMPTYVRAMST
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PpnB amino acid sequence

MLESIQQALFPVGRSLIVDYIATGLTLFLVYLIRTRYFHGLNKIPGPFLASVTSWVKWDTVRREKMPFVNT
QLHERYGPLVRIGPNHISASSAESIQVVHRSKSGFTKSGIYGILQPSYEGTDLHNVFSTQDAEYHAALKRT
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DVDGICHLDHDDQMMYFAVWGQITTIERAWAKVKALATGTRKENPLNFALVLVQKREANPTDSTDMLN
LFLALHKSVPEKFTIRDVIAAAYINVVTAHDVVAITLRVVYYLAKNPAMQKKLQQEIDDAETAGKLSNP

AKYTEITPLSYVLAVTNEALRIHPSTGLILERRCPTGGVTLHGQYIPEGTIIGVNCWVVNRDKGIFGHDAH
EFRPERWIDSDPKDVSRMRTNMFTFGAGARNCIGKNLAMMQLTKIIVELYRNFDIVLENPKDWTVSGG
WLTRQTEMMDMVLTKRK

PpnC amino acid sequence

MNHSDIVLREWECELETICGKNSAKVVLTGRTLSDIPDVIAVARYNVLSIDIDLSAIKAMEMSLSILEKRLHSG
DVIYGVNTGFGGSADVTRTKKHVELQRALIRELHYGILPTGERDHRPLQLEGNLGQHRHDLSSLESGLESTY
LPWSWARASILIRINSLISGCSAVRPVIVQRMQDLLKHDIIPMIPLRGSISSSGDLSPSYICGAIQGKSTLRV
LSRDNKHVYADSAFERAGLEPVVIQAKEGLAVTNGTTVSAAVAALALHDTHSLALLAQVLTAMSVEALA
GTSESFHPFFSEVRPHPGQMESARNILNFLSGSRLTKVNDGAHSSLRQDRYSIRTAPQWLGPILEDLSLAHQ
QVFIECNSATDNPLVTPEGEFIHGGNFQAKSVTSAMEKARQGIQIGRMLFSQCTEIIINPATSRGLPPNLVA
EDPGISLIFKGFDLNIASLAAELGFLASPVNHVQTAEMGNQSLNSLALISARYTHTANEVLSQLMAAHLIA
VCQALDLRAMHAQFLEGYHPEFVQLVDAHAEETLPSDGIPKIPNPIEVSGNLTNGLADPQLKPVEPKTV
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CAHRDAYLVHGDATGLLGKASRAMYVFLRRSLGVPLLATRNLATPTVGGMNGESGTHGGVEAPTGVGSY
TGAVYRALRDGTLAKVAVDILRDSVKAEVEVGE

PpnD amino acid sequence

MDESILILVALCALCLAFQLLRTRYHRGLNAIPGPFSAFCNLWKILAVYNNDMPRRNISVHKKYGPVVRI
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DLE SKIDTCVEMFTNQLRKRTQDDGPTNVDMSLVVHLFAFDCLGELNVSKKFGYLDTGDFNGFIEGSDKVL
IKTGLFGQAPFLQVIRKLIDTRWGAEKLNPNVLKYTTAVVRQRLEKPTETPDMLNSFLALRKAQPEKLSIRD
ITGSIYINLMAGHDVLAVTLRTILYYVARSPVEDKLRGELATIITHYRPTDAIPYTETSKLPYLGAVINESLR
IHGNLGLINERVTPPEGARIDGYHIPGGTIVGINPWVIHRNTEIFGEDVETFRPERWLDGPEDSIQEMKRNL
FSFGAGPRMCIGKNIAMMQIYKFITQFYRHFTFELASPEKDWHVIGNWVTKQTEMMDMLVTQAKPRQI

NseB amino acid sequence

MKYPTTGQLQQVHLGIGPKGYEPVASYQGDQKLYTQEHEILQASILGFCPEHLWHHGSNKASCPRPILVT
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RPDFLVGDYSGGPSTETRYRLTEINARFCFNGFMHQAYGQEGLSDLGAGRNGLIHATDSSKILDGLLSLFP
DRPLHLLKGEEPGIDIHMFIDFVYRHIGIKPRLITPADRLIPDPQKKDGSKLCCLVKDQQNASLINESRLV
TSKGEVVEEVHQVGLLELHQHELFGLSREMLREISLRCFNDMRTILLVHDKRMLGIIKQEMPTLVARKVLT
HDQGEALERGIDSFIPGSSELNELIQTLDSPELRKEYLLKPIRGKGAGIIFGDEVGPDEWLSTLERLRNP
HFVSGNTMYVVQRRIWPRLYEVILNSSGDRGNYPLIGTYHTTNGQLLGLGTWRSSPDRIKAVSHGGGWIC
SVLDEYAESSE

Supplementary Tables

Supplementary Table 1. Comparative BLASTP analysis of the *ppn* gene cluster with homologous cluster from *Penicillium polonicum*, *Nemania serpens*, and *Xylaria telfairii*.

<i>Penicillium polonicum</i> (<i>ppn</i>)	<i>Xylaria telfairii</i> (% identity) (<i>xte1</i>)	<i>Xylaria telfairii</i> (% identity) (<i>xte2</i>)	<i>Nemania serpens</i> (% identity) (<i>nse</i>)	Homolog in Swiss-Prot database (% identity)
PpnA (PT) OQD61507.1	Xte1A (51%) KAI0446169.1	Xte2A (68%) KAI0441401.1	NseA (PT) (66%) KAI1163401.1	4-O-dimethylallyl-L-tyrosine synthase, M1VV66.1 (34%)
PpnB (P450) OQD61637.1	Xte1B (79%) KAI0446171.1	Xte2B (68%) KAI0441400.1	NseB (P450) (69%) KAI1163402.1	Cytochrome P450 monooxygenase, D7PI20.2 (GsfF, 33%)
PpnC (TAL) OQD61545.1	Xte1C (65%) KAI0446168.1	Xte2C (AT) KAI0441399.1	NseC (AT) (72%) KAI1163403.1	Phenylalanine ammonia-lyase, A0AAN4PAE6.1 (LenB, 40%)
PpnD (P450) OQD61524.1	Xte1D (70%) KAI0446170.1	N/A	N/A	Cytochrome P450 monooxygenase, C8V0D4.1 (CicH, 33%)
PpnE (MT) OQD61540.1	Xte1E (60%) KAI0446175.1	N/A	N/A	Methyltransferase, A0A6F8RNE2.1, (GrgD, 28%)
PpnF (BBE) OQD61464.1	Xte1F (65%) KAI0446173.1	N/A	N/A	VAO-type flavoprotein oxidase VAO615, G2QDQ9.1, (46%)
PpnG (SDR) OQD61477.1	Xte1G (65%) KAI0446172.1	N/A	N/A	Ketoreductase CTB6, A0A2G5ICG8.1, (37%)

Supplementary Table 2. Plasmids used in this study

Plasmids	Vector	Genes
pML 9001	pYTU	<i>ppnA</i> (dimethylallyltryptophan synthase)
pML 9002	pYTP	<i>ppnB</i> (cytochrome P450)
pML 9003	pYTU	<i>ppnC</i> (phenylalanine ammonia lyase)
pML 9004	pYTR	<i>ppnD</i> (cytochrome P450)- <i>ppnE</i> (methyltransferase)
pML 9005	pYTU	<i>ppnA</i> (dimethylallyltryptophan synthase)- <i>ppnB</i> (cytochrome P450)- <i>ppnC</i> (phenylalanine ammonia lyase)
pML 9006	pYTR	<i>ppnD</i> (cytochrome P450)
pML 9007	pYTP	<i>ppnF</i> (BBE-like)- <i>ppnG</i> (SDR)
pML 9008	pYTP	<i>ppnA</i> (dimethylallyltryptophan synthase)
pML 9009	pYTU	<i>nseA</i> (dimethylallyltryptophan synthase)
pML 9010	pYTU	<i>nseA</i> (dimethylallyltryptophan synthase)- <i>nseB</i> (cytochrome P450)
pML 9011	pYTR	<i>nseB</i> (cytochrome P450)
pML 9012	pYTU	<i>nseC</i> (acyltransferase)
pML 9013	pYTU	<i>ppnA</i> (dimethylallyltryptophan synthase)- <i>pfaA</i> (PKS-NRPS)

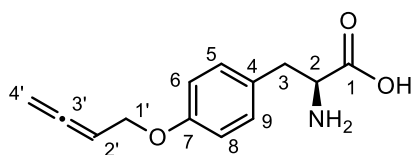
Primers	Sequence (5'-3')
pML 9001 F1	TAACCTCGCGGGTGTTCCTTGACGATGGCATCCTGCACTCCGGTGAATTGATTGTTGGGTGAC
pML 9001 R1	AATTCGGGGCTTTCATCAAGTAAAGTCTGAGCCATTGTTTAGATGTGTCTATGTGGCGGG
pML 9001 F2	ACCATTACCCCGCCACATAGACACATCTAAACAATGGCTCAGACTTTACTTGATGAAAGC
pML 9001 R2	ACAGTGGAGGACATAACCCGTAATTTCTGATTAAATCACATTCCCTCAGCTCTATGCCC
pML9002 F1	TTAGTAACCTCGCGGGTGTTCCTTGACGATGGCATCCTACTCCGGTGAATTGATTGTTGGGTG
pML9002 R1	ACGGGGAAGAGAGCTTGTGTATCGATTCAAGCATTGTTTAGATGTGTCTATGTGGCGGG
pML9002 F2	ACCATTACCCCGCCACATAGACACATCTAAACAATGCTTGAATCGATACAACAAGCTCTC
pML9002 F3	TGGACATGGTTTTGACGAAGAGGAAGTAACCACTTAACGTTACTGAAATCATCAAACAGC
pML9002 R2	AGCTGTTTGATGATTTTCAGTAACGTTAAGTGGTTACTTCCTCTTCGTCAAAACCATGTCC
pML9002 R3	ATGAGACCCAACAACCATGATACCAGGGGAAGAAGGATTACCTCTAAACAAGTGTACCTG
pML9003 F1	AGTAACCTCGCGGGTGTTCCTTGACGATGGCATCCTGCACTCCGGTGAATTGATTGTTGGGTG
pML9003 R1	TCCCACTCCCTCAGAACAATATCCGAGTGATTCAATTGTTTAGATGTGTCTATGTGGCGGG
pML9003 R2	ACACAGTGGAGGACATAACCCGTAATTTCTGATTAAATGGCGCTATGTACGCATTTCGAC
pML9003 F2	CCATTACCCCGCCACATAGACACATCTAAACAATGAATCACTCGGATATTGTTCTGAGGG
pML9004 F1	TACCCCGCCACATAGACACATCTAAACAATGGATGAGTCTTTAATATTGGTTGCACTTTG
pML9004 F2	CAAGCCCCCTACCTTCCAGCTCGCTCGAAACAGATTGATTAAAGGTGCCGAACGAGC
pML9004 F3	CCCTTCTCTGAACAATAAACCCACAGAAGGCATTTATGTCAGAAGTCAAGAATGCCCTC
pML9004 R2	AGGATATTCCTCGAGGGCATTCTTGACTTCTGACATAAATGCCTTCTGTGGGGTTTATTG
pML9004 R3	GCTAAAGGGTATCATCGAAAGGGAGTCATCCAATTTTGCCGTGGATATTCTTAGAGACTC
pML9004 R1	TTATATCATTTATAGCTCGTTCGGCACCTTTAATCAAATCTGTTTCGAGCGAGCTGGAAG
pML9005 F1	CCTCGCGGGTGTTCCTTGACGATGGCATCCTGCACTCCGGTGAATTGATTGTTGGGTG
pML9005 F2	ACCATTACCCCGCCACATAGACACATCTAAACAATGAATCACTCGGATATTGTTCTGAGG
pML9005 F3	CCGACCACGACCACCCTGTGCAATGCGTACATAGCGCCGATTTCGTCCAGGGCTTCCCAAG
pML9005 F4	ACATGATCTAACAACCTTCTAGTAAACCGCAATCATGGCTCAGACTTTACTTGATGAAAGC
pML9005 F5	TTTCTATTAGGGCATAGAGCTGAAGGAATGTGTTTGTCTCCAGGAATACATGTGAGCTTAC
pML9005 F6	AGTGCATACAGAACACTTCAAACAATCGCAAAAATGCTTGAATCGATACAACAAGCTCTC
pML9005 R1	TCCCACTCCCTCAGAACAATATCCGAGTGATTCAATTGTTTAGATGTGTCTATGTGGCGGG
pML9005 R2	CCAATATTCCAACCTTGGGAAGCCCTGGACGAATCGGCGCTATGTACGCATTTCGACAGG
pML9005 R3	GGGGCTTTCATCAAGTAAAGTCTGAGCCATGATTGCGGTTTACTAGAAAGTTGTTAGATCA
pML9005 R4	AGAATCAGTAAGCTCACATGTATTCTGGAGCAAACACATTCCTTCAGCTCTATGCCCT
pML9005 R5	GAAGAGAGCTTGTGTATCGATTCAAGCATTTTTGCATTGTTTGAAGTGTCTGTATGC
pML9005 R6	AACACAGTGGAGGACATAACCCGTAATTTCTGATTGACCCCGTGGTTCGGGTACCATAC
pML9006 F1	TACCCCGCCACATAGACACATCTAAACAATGGATGAGTCTTTAATATTGGTTGCACTTTG
pML9006 R1	CTAAAGGGTATCATCGAAAGGGAGTCATCCAATTTAAATCTGTTTCGAGCGAGCTGGAAG
pML9007 F1	TCCCTTCTCTGAACAATAAACCCACAGAAGGCATTTATGTCACCCTACTGGCAGAATCG
pML9007 F2	AGTAAGTAAGCAAGAGCGTTACGTATACCCTCTGGCATTGATTGTCGTCAGGGCTTCCCA
pML9007 F3	TATACATGATCTAACAACCTTCTAGTAAACCGCAATCATGTGTTCCGCAGACCTTCTCATC
pML9007 R1	CCCAATATTCCAACCTTGGGAAGCCCTGGACGAATCAAATGCCAGAGGGTATACGTAACG
pML9007 R2	CGGTGATGAGAAGGTCTGCGGAACACATGATTGCGGTTTACTAGAAAGTTGTTAGATCATG
pML9007 R3	GAGTGATGAGACCCAACAACCATGATACCAGGGGATTTACAGCAGTGTCTCTCGTGGTCTT
pML9008 F1	AGTAACCTCGCGGGTGTTCCTTGACGATGGCATCCTACTCCGGTGAATTGATTGTTGGGTG
pML9008 F2	ACCATTACCCCGCCACATAGACACATCTAAACAATGGCTCAGACTTTACTTGATGAAAGC
pML9008 F3	ACTTGTTTGAATCTGAGGTATATCTCTGACCACTTAACGTTACTGAAATCATCAAACAGC
pML9008 R1	TGTTTAGATGTGTCTATGTGGCGGG
pML9008 R2	GTTTGATGATTTTCAGTAACGTTAAGTGGTCAGAGATATACCTCAGATTCTGAACAAGTGTA
pML9008 R3	ATGAGACCCAACAACCATGATACCAGGGGAAGAAGGATTACCTCTAAACAAGTGTACCTG
pML9009 F1	CCTGAGCTTCATCCCCAGCATCATTACACCTCAGCAATGTTCTCTTCGTTCCGGTGAATTG
pML9009 R1	CAACACAGTGGAGGACATAACCCGTAATTTCTGGCTGAGGTAGTTCCTGATTGTTTCG
pML9010 F1	CCTGAGCTTCATCCCCAGCATCATTACACCTCAGCAATGTTCTCTTCGTTCCGGTGAATTG
pML9010 F2	GGGATGAATGAATCGAAACAATCAGGAACACTACCTCAGCGATTTCGTCAGGGCTTCCCAAG
pML9010 F3	CTTATACATGATCTAACAACCTTCTAGTAAACCGCAATCATGGAGTCCACACAGCACATCG

Supplementary Table 3. Primers used in this study

Supplementary Table 3 (continued). Primers used in this study.

Primers	Sequence (5'-3')
pML9010 R1	CCAATATTCCAACCTTGGGAAGCCCTGGACGAATCGCTGAGGTAGTTCCTGATTGTTTCG
pML9010 R2	GATTGCGGTTTACTAGAAGTTGTTAGATCATGTA
pML9010 R3	CAACACAGTGGAGGACATACCCGTAATTTTCTGGGCGCAATCCGTGCCGCAATGTC
pML9011 F1	GACTAACCATTACCCCGCCACATAGACACATCTAAACAATGGAGTCCACACAGCACATC
pML9011 F2	GCAAGAGGGGAGCCTTGCTTGGAGCATAACCACTTAACGTTACTGAAATCATCAAACAGC
pML9011 R1	CGTCAAGCTGTTTGATGATTTTCAGTAACGTTAAGTGGTTATGCTCCAAGCAAGGCTCCC
pML9011 R2	AAAGGGTATCATCGAAAGGGAGTCATCCAAAGAAGGATTACCTCTAAACAAGTGTACCTG
pML9012 F1	CCTGAGCTTCATCCCCAGCATCATTACACCTCAGCAATGGTTAGCCTGGGCCTCGCTAAG
pML9012 F2	CAGGGGACAAGCAGGCTGCTAAATTGTGACCACTTAACGTTACTGAAATCATCAAACAGC
pML9012 R1	CTGTTTGATGATTTTCAGTAACGTTAAGTGGTCACAATTTAGCAGCCTGCTTGTCCCCTGC
pML9012 R2	ACAGTGGAGGACATACCCGTAATTTTCTGAAGAAGGATTACCTCTAAACAAGTGTACCTG
pML9013 F1	GTAACCTCGCGGGTGTTCTTGACGATGGCATCCTGCACTCCGGTGAATTGATTGGGGTG
pML9013 F2	AACCATTACCCCGCCACATAGACACATCTAAACAATGGCTCAGACTTTACATGGAGAAAG
pML9013 F3	AACCTTCACCCGACAATGCAGTATCTGCATTTTTTGTCTCCAGGAATACATGTGAGCTTAC
pML9013 F4	CAAGTGCATACAGAACACTTCAAACAATCGCAAAAATGTGCGAGCGACAGCCTCTAGC
pML9013 F5	GGACGAGATTACGCCCCGACAG
pML9013 F6	CAGGTCTACTCGAAGCGCTGAAGGAATAGCCACTTAACGTTACTGAAATCATCAAACAGC
pML9013 R1	CGGGGCTTTCTCCATGTAAAGTCTGAGCCATTGTTTAGATGTGTCTATGTGGCGGG
pML9013 R2	TAGAATCAGTAAGCTCACATGTATTCCTGGAGCAAAAATGCAGATACTGCATTGTCTGGG
pML9013 R3	AACGATAGCTAGAGGCTGTCGCTGCGACATTTTTGCGATTGTTGAAGTGTCTGTATGC
pML9013 R4	GGCTATCGAGTCCGTAGTCAAGTAAAC
pML9013 R5	TGTTTGATGATTTTCAGTAACGTTAAGTGGCTATTCCTTCAGCGCTTCGAGTAGACCTGCC
pML9013 R6	TGGAGGACATACCCGTAATTTTCTGATTAAAGAAGGATTACCTCTAAACAAGTGTACCTG

Supplementary Table 4. Spectroscopic data of compound *O*-homoallenyl-L-Tyr (**1**)



O-homoallenyl-L-Tyr (**1**)

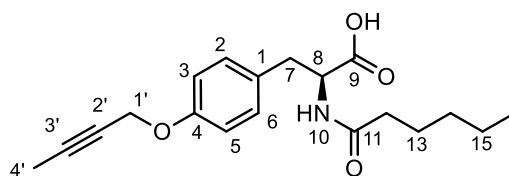
Position	<i>O</i> -homoallenyl-L-Tyr (1) in DMSO- <i>d</i> ₆	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		175.4, C
2	3.56, m	55.8, CH
3	2.70, m; 2.78, m	36.6, CH ₂
4		130.2, C
5	7.17, d (8.0)	130.4, CH
6	6.85, d (7.2)	114.7, CH
7		156.7, C
8	6.85, t (7.2)	114.7, CH
9	7.17, d (8.0)	130.4, CH
1'	4.52, d (6.6)	65.1, CH ₂
2'	5.47, m	87.1, CH
3'		208.7, C
4'	4.97, d (6.6)	76.9, CH ₂

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, DMSO-*d*₆, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₃H₁₆NO₃⁺ 234.1125; found 234.1130.

[α]_D^{24.1} – 4° (*c* 0.1, MeOH/H₂O, 70%, v/v).

Supplementary Table 5. Spectroscopic data of compound sinuxylamide B (**2**)



Sinuxylamide B (**2**)

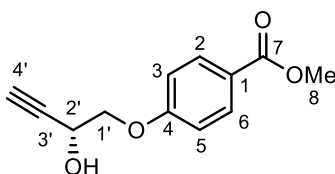
Position	Sinuxylamide B (2) in CD ₃ OD		Reported Sinuxylamide B (2) in CD ₃ OD ⁸
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type	δ_{C} , type
1		165.8, C	174.5, C
2	4.61, m	55.8, CH	54.3, CH
3	2.88, m; 3.16, m	36.6, CH ₂	36.6, CH ₂
4		130.1, C	129.8, C
5	7.13, d (8.1)	129.9, CH	129.7, CH
6	6.85, d (8.0)	114.4, CH	114.3, CH
7		156.9, C	156.7, C
8	6.85, d (8.0)	114.4, CH	114.3, CH
9	7.13, d (8.1)	129.9, CH	129.7, CH
10		174.6, C	174.4, C
11	2.15, t (7.5)	35.7, CH ₂	35.3, CH ₂
12	1.49, m	25.3, CH ₂	25.3, CH ₂
13	1.18, m	31.1, CH ₂	31.1, CH ₂
14	1.28, m	22.1, CH ₂	22.1, CH ₂
15	0.87, t (7.3)	13.0, CH ₃	13.1, CH ₃
1'	4.62, s	55.8, CH ₂	55.6, CH ₂
2'		74.0, C	73.9, C
3'		82.6, C	82.6, C
4'	1.81, s	1.8, CH ₃	1.8, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CD₃OD, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₉H₂₆NO₄⁺ 332.1856; found 332.1861.

[α]_D^{24.1} + 12° (*c* 0.1, MeOH). Compound **2** showed the same positive optical rotation as reported Sinuxylamide B⁸.

Supplementary Table 6. Spectroscopic data of penipratynolene (**5**)



Penipratynolene (**5**)

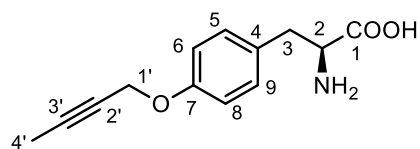
Position	penipratynolene (5) in acetone- d_6		Reported penipratynolene (5) in acetone- d_6 ⁹
	δ_H (J in Hz)	δ_C , type	δ_C , type
1		166.9, C	166.7, C
2		123.7, C	123.6, C
3	7.96, d (8.9)	132.2, CH	132.1, CH
4	7.07, d (8.9)	115.3, CH	115.1, CH
5		163.4, C	163.2, C
6	7.07, d (8.9)	115.3, CH	115.1, CH
7	7.96, d (8.9)	132.2, CH	132.1, CH
1'	4.18, m	72.7, CH ₂	72.6, CH ₂
2'	4.74, s	61.2, CH	61.0, CH
3'		83.5, C	83.3, C
4'	2.98, m	74.7, CH	74.6, CH
OMe	3.84 s	52.0, CH ₃	51.9, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, acetone- d_6 , “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₂H₁₃O₄⁺ 221.0808; found 221.0802.

$[\alpha]_D^{24.1} - 16^\circ$ (c 0.1, CDCl₃). Compound **1** showed the same negative optical rotation as reported penipratynolene⁹.

Supplementary Table 7. Spectroscopic data of compound *O*-2-butyn-1-yl L-Tyr (**6**)



O-2-butyn-1-yl L-Tyr (**6**)

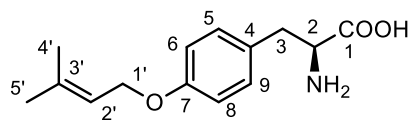
Position	<i>O</i> -2-butyn-1-yl-L-tyrosine (6) in DMSO- <i>d</i> ₆ (500MHz)	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		169.2, C
2	3.34, m	55.6, CH
3	2.78, m; 3.06, m	36.1, CH ₂
4		129.9, C
5	7.17, d (8.7)	130.3, CH
6	6.85, d (8.6)	114.5, CH
7		156.2, C
8	6.85, t (8.6)	114.5, CH
9	7.17, d (8.7)	130.3, CH
1'	4.69, q (2.4)	55.7, CH ₂
2'		74.9, C
3'		83.3, C
4'	1.82, t (2.3)	3.2, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, DMSO-*d*₆, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₃H₁₆NO₃⁺ 234.1125; found 234.1132.

$[\alpha]_{\text{D}}^{24.1} - 32$ (c 0.1, MeOH/H₂O, 70%, v/v)

Supplementary Table 8. Spectroscopic data of compound *O*-Prenyl L-Tyr (**7**)



O-Prenyl L-Tyr (**7**)

Position	<i>O</i> -Prenyl L-Tyr (7) in DMSO- <i>d</i> ₆	
	δ_{H} (<i>J</i> in Hz)	δ_{H} (<i>J</i> in Hz)
1		165.5, C
2	3.26, m	55.9, CH
3	2.70, m; 2.78, m	36.5, CH ₂
4		130.2, C
5	7.17, d (8.0)	130.3, CH
6	6.85, d (7.2)	114.4, CH
7		157.1, C
8	6.85, t (7.2)	114.4, CH
9	7.17, d (8.0)	130.3, CH
1'	4.48 (d, 6.7)	64.1, CH ₂
2'	5.41, m	120.2, CH
3'		136.8, C
4'	1.69 (d, 1.4)	18.0, CH ₃
5'	1.73 (d, 1.4)	25.4, CH ₃

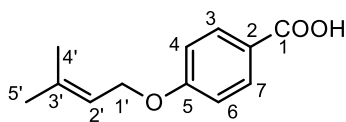
NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, DMSO-*d*₆, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₄H₂₀NO₃⁺ 250.1438; found 250.1434.

$[\alpha]_{\text{D}}^{24.1} - 22$ (c 0.1, MeOH/H₂O, 70%, v/v)

Compound **3** showed the same positive optical rotation as reported *O*-Prenyl L-Tyr¹⁰.

Supplementary Table 9. Spectroscopic data of *O*-prenyl-benzoic acid (**8**)



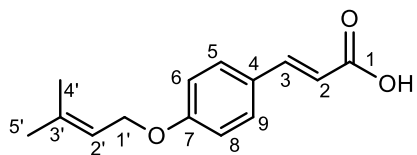
O-prenyl-benzoic acid (**8**)

Position	<i>O</i> -prenyl-benzoic acid (8) in CDCl ₃		Reported <i>O</i> -prenyl-benzoic acid (8) in CDCl ₃ ¹¹
	δ_H (<i>J</i> in Hz)	δ_C , type	δ_H (<i>J</i> in Hz)
1		172.3, C	
2		121.7, C	
3	8.06, d (8.9)	131.5, CH	8.09, d (6.8)
4	6.95, d (8.9)	114.5, CH	6.98, d (6.8)
5		163.5, C	
6	6.95, d (8.9)	114.5, CH	6.98, d (6.8)
7	8.06, d (8.9)	131.5, CH	8.09, d (6.8)
1'	4.58 (d, 6.3)	65.2, CH ₂	4.62, d (5.4)
2'	5.49, m	119.1, CH	5.52, m
3'		139.1, C	
4'	1.76, d (1.3)	18.4, CH ₃	1.79, s
5'	1.81, d (1.3)	26.0, CH ₃	1.84, s

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CDCl₃, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₂H₁₅O₃⁺ 207.1016; found 207.1006.

Supplementary Table 10. Spectroscopic data of *O*-prenyl *p*-coumaric acid (**9**)



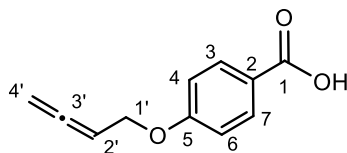
O-prenyl *p*-coumaric acid (**9**)

Position	<i>O</i> -prenyl <i>p</i> -coumaric acid (9) in CDCl ₃		Reported <i>O</i> -prenyl <i>p</i> -coumaric acid (9) in CDCl ₃ ¹²
	δ_H (<i>J</i> in Hz)	δ_C , type	δ_C , type
1		173.1, C	172.9, C
2	6.32 (d, 15.9)	114.7, CH	114.2, CH
3	7.75 (d, 15.8)	146.9, CH	146.8, CH
4		126.8, C	126.7, C
5	7.50, d (8.8)	130.2, CH	130.1, CH
6	6.93, d (8.8)	115.2, CH	115.0, CH
7		161.2, C	161.1, C
8	6.93, d (8.8)	115.2, CH	115.0, CH
9	7.50, d (8.8)	130.2, CH	130.1, CH
1'	4.55 (d, 6.7)	65.1, CH ₂	64.1, CH ₂
2'	5.49, m	119.3, CH	119.2, CH
3'		138.9, C	138.8, C
4'	1.75 (d, 1.4)	18.4, CH ₃	18.2, CH ₃
5'	1.81 (d, 1.4)	26.0, CH ₃	25.8, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CD₃CN, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₄H₁₇O₃⁺ 233.1172; found 233.1159.

Supplementary Table 11. Spectroscopic data of compound *O*-homoallenyl 4-hydroxybenzoic acid (**10**)



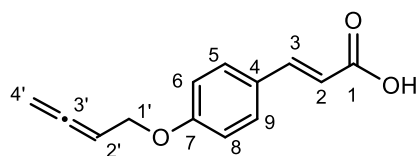
O-homoallenyl
4-hydroxybenzoic acid (**10**)

<i>O</i> -homoallenyl 4-hydroxybenzoic acid (10) in CDCl ₃ (500MHz)		
Position	δ_H (<i>J</i> in Hz)	δ_C , type
1		171.5, C
2		121.9, C
3	8.06, d (8.9)	132.5, CH
4	6.96, d (8.9)	114.7, CH
5		163.0, C
6	6.96, d (8.9)	114.7, CH
7	8.06, d (8.9)	132.5, CH
1'	4.64, dt (2.5, 6.8)	66.1, CH ₂
2'	5.40, m	86.7, CH
3'		209.8, C
4'	4.90, dt (2.5, 6.6)	77.1, CH ₂

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CDCl₃, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₁H₁₁O₃⁺ 191.0703; found 191.0696.

Supplementary Table 12. Spectroscopic data of *O*-homoallenyl *p*-coumaric acid (**11**)



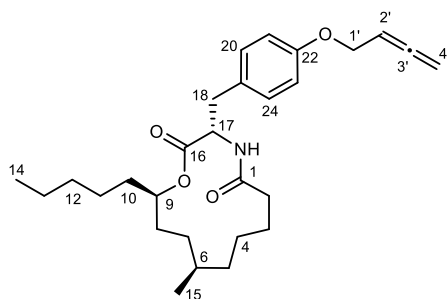
O-homoallenyl *p*-coumaric acid (**11**)

Position	<i>O</i> -homoallenyl <i>p</i> -coumaric acid (11) in CDCl ₃		Reported <i>O</i> -homoallenyl <i>p</i> -coumaric acid (11) in CDCl ₃ ⁵
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type	δ_{C} , type
1		172.8, C	171.8, C
2	6.32 (d, 15.7)	114.9, CH	114.7, CH
3	7.75 (d, 15.7)	146.8, CH	146.7, CH
4		127.1, C	127.0, C
5	7.50, d (8.2)	130.2, CH	130.1, CH
6	6.93, d (8.3)	115.4, CH	115.3, CH
7		160.6, C	160.5, C
8	6.93, d (8.3)	115.4, CH	115.3, CH
9	7.50, d (8.2)	130.2, CH	130.1, CH
1'	4.61, m	66.1, CH ₂	66.2, CH ₂
2'	5.39, m	86.8, CH	86.9, CH
3'		209.7, C	209.9, C
4'	4.89, m	77.0, CH ₂	77.1, CH ₂

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CDCl₃, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₃H₁₃O₃⁺ 217.0859; found 217.0864.

Supplementary Table 13. Spectroscopic data of compound **13**



Unnatural *N*-demethylmelearoride A with allene tag (**13**)

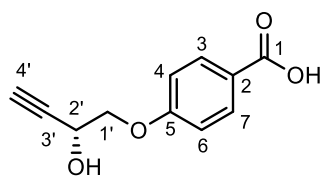
Position	13 in CDCl ₃	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1'	4.52, dt (2.5, 6.9)	66.0, CH ₂
2'	5.37, m	87.2, CH
3'		209.6, C
4'	4.85, m	76.6, CH ₂
1		173.0, C
2	2.12, m; 2.24, m	37.1, CH ₂
3	1.48, m; 1.62, m	25.4, CH ₂
4	1.33, m	23.7, CH ₂
5	1.22, m	34.0, CH ₂
6	1.31, m	29.7, CH
7	1.07, m; 1.42, m	27.4, CH ₂
8	1.50, m	28.8, CH ₂
9	4.80, m	75.8, CH
10	1.38, m; 1.52, m	33.6, CH ₂
11	1.08, m	25.1, CH ₂
12	1.18, m	31.7, CH ₂
13	1.24, m	22.6, CH ₂
14	0.85, m	14.2, CH ₃
15	0.81, m	20.6, CH ₃
16		172.1, C
17	4.80, m	53.8, CH
18	3.04, m	36.6, CH ₂
19		129.1, C
20	7.13, d (8.6)	130.4, CH
21	6.82, d (8.6)	115.0, CH
22		157.4, C
23	6.82, d (8.6)	115.0, CH
24	7.13, d (8.6)	130.4, CH
NH	5.65, d (9.6)	

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CDCl₃, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₂₈H₄₂NO₄⁺ 456.3108; found 456.3110.

[α]_D^{24.1} – 38° (*c* 0.1, MeOH).

Supplementary Table 14. Spectroscopic data of compound demethyl-5 (**14**)



demethyl-5 (**14**)

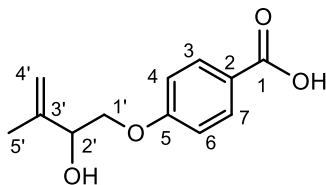
Position	demethyl-5 (14) in acetone- <i>d</i> ₆	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		167.6, C
2		124.0, C
3	7.99, d (8.9)	132.5, CH
4	7.06, d (8.9)	115.2, CH
5		163.3, C
6	7.06, d (8.9)	115.2, CH
7	7.99, d (8.9)	132.5, CH
1'	4.18, m	72.7, CH ₂
2'	4.74, m	61.2, CH
3'		83.4, C
4'	2.98, m	74.8, CH

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, acetone-*d*₆, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₁H₁₁O₄⁺ 207.0652; found 207.0651.

$[\alpha]_{\text{D}}^{24.1} - 10^{\circ}$ (*c* 0.1, MeOH).

Supplementary Table 15. Spectroscopic data of compound **15**



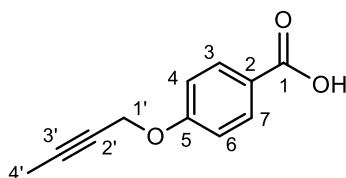
Position	15 in CDCl ₃	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		170.9, C
2		122.3, C
3	8.05, d (8.6)	132.5, CH
4	6.97, d (8.6)	114.5, CH
5		163.0, C
6	6.97, d (8.6)	114.5, CH
7	8.05, d (8.6)	132.5, CH
1'	4.01, m; 4.12, m	71.4, CH ₂
2'	4.51, m	73.7, CH
3'		143.2, C
4'	5.04, s; 5.17, s	113.3, CH ₂
5'	1.84, s	19.0, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CDCl₃, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₂H₁₅O₄⁺ 223.0965; found 223.0962.

$[\alpha]_{\text{D}}^{24.1} + 44^{\circ}$ (*c* 0.1, MeOH).

Supplementary Table 16. Spectroscopic data of *O*-but-2-ynyl benzoic acid (**16**)



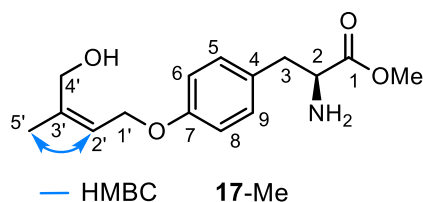
O-but-2-ynyl benzoic acid (**16**)

<i>O</i> -but-2-ynyl benzoic acid (16) in DMSO- <i>d</i> ₆		
Position	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		166.9, C
2		123.4, C
3	7.89, d (8.9)	131.2, CH
4	7.03, d (8.9)	114.6, CH
5		160.9, C
6	7.03, d (8.9)	114.6, CH
7	7.89, d (8.9)	131.2, CH
1'	4.82, m	56.1, CH ₂
2'		74.3, C
3'		84.0, C
4'	1.83, t (2.3)	3.1, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, DMSO-*d*₆, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₁H₁₁O₃⁺ 191.0703; found 191.0691.

Supplementary Table 17. Spectroscopic data of compound **17** methyl ester (**17-Me**)



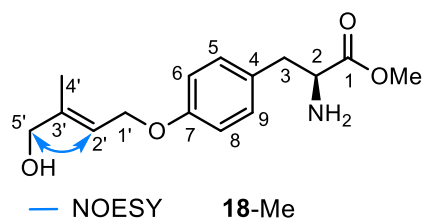
Position	17-Me in D ₂ O	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		170.0, C
2	4.39, m	54.1, CH
3	3.19, m; 3.29, m	34.7, CH ₂
4		126.4, C
5	7.22, d (8.7)	130.6, CH
6	7.00, d (8.6)	115.6, CH
7		157.3, C
8	7.00, d (8.6)	115.6, CH
9	7.22, d (8.7)	130.6, CH
1'	4.65, d, 6.6)	64.1, CH ₂
2'	5.66, m	122.0, CH
3'		141.0, C
4'	4.18, s	60.0, CH ₂
5'	1.84 (d, 1.5)	20.4, CH ₃
OMe	3.83, s	53.5, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, D₂O, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₅H₂₂NO₄⁺ 280.1543; found 280.1539.

$[\alpha]_{\text{D}}^{24.1} - 12^{\circ}$ (*c* 0.1, MeOH).

Supplementary Table 18. Spectroscopic data of compound **18** methyl ester (**18-Me**)



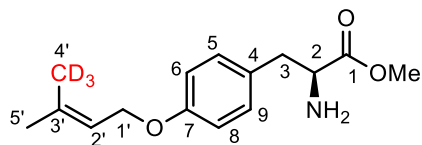
Position	18-Me in DMSO- <i>d</i> ₆ (500MHz)	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		169.5, C
2	4.24, m	53.4, CH
3	3.04, m	35.2, CH ₂
4		126.2, C
5	7.12, d (8.5)	130.5, CH
6	6.89, d (8.7)	114.8, CH
7		157.8, C
8	6.89, d (8.7)	114.8, CH
9	7.12, d (8.5)	130.5, CH
1'	4.57, d (6.5)	64.1, CH ₂
2'	5.64, m	118.0, CH
3'		140.6, C
4'	1.64, s	13.8, CH ₃
5'	3.84, s	65.5, CH ₂
OMe	3.68, s	52.6, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, DMSO-*d*₆, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₅H₂₂NO₄⁺ 280.1543; found 280.1543.

$[\alpha]_{\text{D}}^{24.1} - 14^{\circ}$ (*c* 0.1, MeOH).

Supplementary Table 19. Spectroscopic data of compound 4',4',4'-[d₃]-7-Me



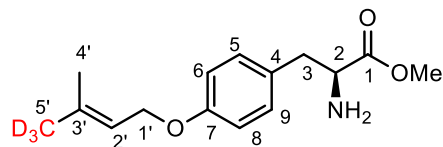
4',4',4'-[d₃]-7-Me

Position	4',4',4'-[d ₃]-7-Me in CD ₃ CN	
	δ_H (<i>J</i> in Hz)	δ_C , type
1		165.1, C
2	3.81, m	56.0, CH
3	2.90, m; 2.98, m	39.3, CH ₂
4		129.3, C
5	7.10, d (8.9)	131.4, CH
6	6.84, d (8.4)	115.5, CH
7		158.9, C
8	6.84, d (8.4)	115.5, CH
9	7.10, d (8.9)	131.4, CH
1'	4.50 (d, 7.2)	65.5, CH ₂
2'	5.44, m	120.9, CH
3'		138.5, C
4'		18.1, CD ₃
5'	1.77, s	25.7, CH ₃
OMe	3.66, s	52.7, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CD₃CN, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₅H₁₉D₃NO₃⁺ 267.1783; found 267.1770.

Supplementary Table 20. Spectroscopic data of compound 5',5',5'-[d₃]-7-Me



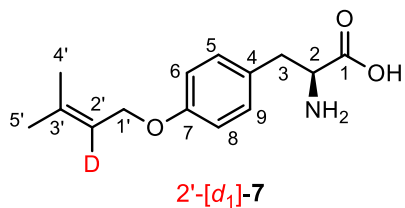
5',5',5'-[d₃]-7-Me

Position	5',5',5'-[d ₃]-7-Me in CD ₃ CN (500MHz)	
	δ_H (<i>J</i> in Hz)	δ_C , type
1		173.5, C
2	4.31, m	56.1, CH
3	2.83, m; 3.02, m	37.4, CH ₂
4		129.8, C
5	7.09, d (8.6)	131.2, CH
6	6.84, d (8.4)	115.4, CH
7		158.7, C
8	6.84, d (8.4)	115.4, CH
9	7.09, d (8.6)	131.2, CH
1'	4.50 (d, 6.7)	65.4, CH ₂
2'	5.43, m	120.9, CH
3'		138.5, C
4'	1.72, s	18.1, CH ₃
5'		25.7, CH ₃
OMe	3.66, s	52.6, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CD₃CN, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₅H₁₉D₃NO₃⁺ 267.1783; found 267.1780.

Supplementary Table 21. Spectroscopic data of compound **2'-[d₁]-7**

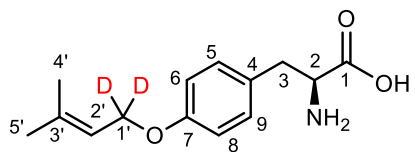


Position	2'-[d₁]-7 in DMSO- <i>d</i> ₆ (500MHz)	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		165.7, C
2	3.59, d (6.1)	67.0, CH
3	1.76, m	25.1, CH ₂
4		130.1, C
5	7.10, d (8.0)	130.1, CH
6	6.80, d (7.7)	114.1, CH
7		156.6, C
8	6.80, t (7.7)	114.1, CH
9	7.10, d (8.0)	130.1, CH
1'	4.46, s	64.1, CH ₂
2'		119.9, C
3'		136.6, C
4'	1.69, s	18.0, CH ₃
5'	1.73, s	25.4, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, DMSO-*d*₆, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₄H₁₉DNO₃⁺ 251.1500; found 251.1496.

Supplementary Table 22. Spectroscopic data of compound **1',1'-[d₂]-7**



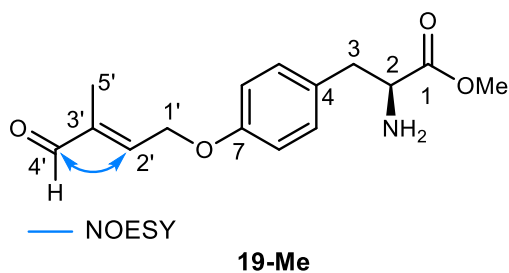
1',1'-[d₂]-7

Position	1',1'-[d₂]-7 in DMSO- <i>d</i> ₆ (500MHz)	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1		178.1, C
2	3.09, m	57.9, CH
3	2.46, m; 2.94, m	40.8, CH ₂
4		132.3, C
5	7.10, d (8.1)	130.2, CH
6	6.80, d (8.1)	114.2, CH
7		156.6, C
8	6.80, d (8.1)	114.2, CH
9	7.10, d (8.1)	130.2, CH
1'		63.5
2'	5.40, s	120.1, CH
3'		136.8, C
4'	1.69, s	18.0, CH ₃
5'	1.73, s	25.5, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, DMSO-*d*₆, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₄H₁₈D₂NO₃⁺ 252.1563; found 252.1561.

Supplementary Table 23. Spectroscopic data of compound **19-Me**

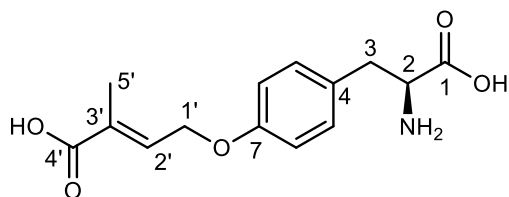


Position	19-Me in D ₂ O (500MHz)	
	δ_H (J in Hz)	δ_C , type
1		170.0, C
2	4.40, m	54.1, CH
3	3.21, m; 3.30, m	34.7, CH ₂
4		126.7, C
5	7.24, d (8.6)	130.7, CH
6	6.87, d (8.7)	115.5, CH
7		157.2, C
8	6.87, d (8.7)	115.5, CH
9	7.24, d (8.6)	130.7, CH
1'	5.04, m	65.1, CH ₂
2'	6.87, td (1.4, 5.5)	150.3, CH
3'		139.9, C
4'	9.38, s	198.2, CHO
5'	1.80, d (1.3)	8.7, CH ₃
OMe	3.83, s	53.5, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, D₂O, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

HRMS (ESI, M+H⁺) calculated for C₁₅H₂₀NO₄⁺ 278.1387; found 278.1392.

Supplementary Table 24. Spectroscopic data of compound **20**



20

Position	20 in CD ₃ OD (500MHz)	
	δ_H (J in Hz)	δ_C , type
1		176.7, C
2	3.19, brs	58.8, CH
3	2.47, m; 2.82, m	42.0, CH ₂
4		132.1, C
5	6.93, d (8.5)	131.5, CH
6	6.61, d (8.6)	115.7, CH
7		158.9, C
8	6.61, d (8.6)	115.7, CH
9	6.93, d (8.5)	131.5, CH
1'	4.41, m	66.3, CH ₂
2'	6.33, m	131.9, CH
3'		137.9, C
4'		176.7, C
5'	1.65, d (1.3)	14.4, CH ₃

NMR spectrum (500 MHz) for ¹H, NMR spectrum (125 MHz) for ¹³C, CD₃OD, “m” means overlapped or multiple with other signals. Chemical shifts are reported in ppm.

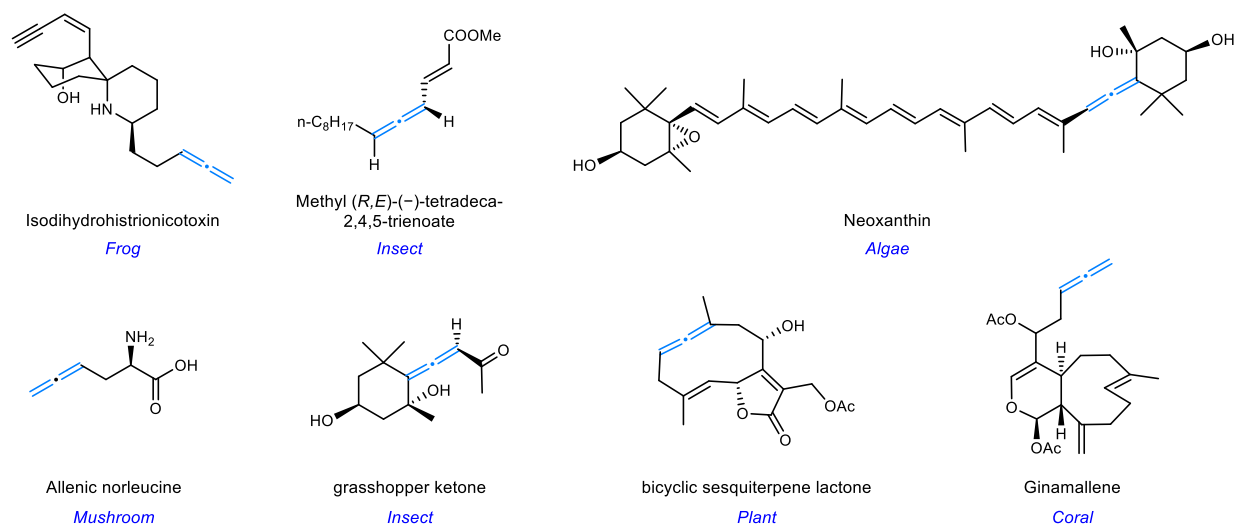
HRMS (ESI, M+H⁺) calculated for C₁₄H₁₈NO₅⁺ 280.1179; found 280.1180.

Supplementary Table 25. Computed energy components (in Hartree) for all intermediates and transition states calculated at the B3LYP-D3(BJ)/def2-TZVP/SMD(Et₂O)//B3LYP-D3(BJ)/def2-SVP/IEFPCM(Et₂O) level of theory at 298 K, 1 atm.

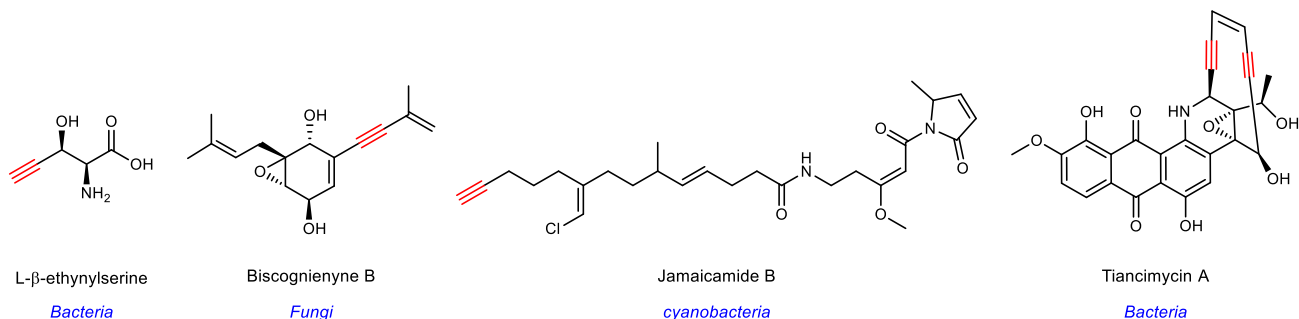
Structure	E_SPC ^a	E ^b	ZPE	H_SPC ^c	TΔS	TΔqh-S ^d	G(T)_SPC	qh-G(T)_SPC ^e
Int1	-3495.6026	-3493.2517	0.5639	-3494.9978	0.1121	0.1053	-3495.1099	-3495.1032
Int1_Q	-3495.5859	-3493.2109	0.5605	-3494.9835	0.1154	0.1085	-3495.0989	-3495.0919
Int10	-3228.5165	-3226.4850	0.4871	-3227.9940	0.1019	0.0953	-3228.0959	-3228.0893
Int10_Q	-3228.5164	-3226.4848	0.4870	-3227.9939	0.1026	0.0960	-3228.0965	-3228.0899
Int11	-537.7144	-537.1050	0.1756	-537.5269	0.0498	0.0482	-537.5767	-537.5751
Int12	-3228.5212	-3226.4975	0.4907	-3227.9964	0.0995	0.0933	-3228.0959	-3228.0898
Int13	-3228.0693	-3226.0450	0.4780	-3227.5572	0.0985	0.0930	-3227.6557	-3227.6502
Int2	-3495.6102	-3493.2629	0.5660	-3495.0044	0.1098	0.1033	-3495.1143	-3495.1077
Int2_Q	-3495.5866	-3493.2407	0.5634	-3494.9826	0.1123	0.1054	-3495.0950	-3495.0880
Int3	-652.8939	-652.1516	0.2191	-652.6606	0.0565	0.0539	-652.7171	-652.7145
Int4	-463.0313	-462.5131	0.1806	-462.8393	0.0492	0.0477	-462.8885	-462.8870
Int5	-3419.6261	-3417.3743	0.5525	-3419.0353	0.1081	0.1009	-3419.1434	-3419.1362
Int5_Q	-3419.6254	-3417.3736	0.5525	-3419.0346	0.1090	0.1016	-3419.1436	-3419.1363
Int6	-652.9179	-652.1755	0.2200	-652.6839	0.0551	0.0530	-652.7390	-652.7369
Int7	-652.7114	-651.9626	0.2202	-652.4769	0.0555	0.0533	-652.5324	-652.5302
Int8	-3419.6305	-3417.3806	0.5522	-3419.0401	0.1075	0.1005	-3419.1476	-3419.1406
Int8_Q	-3419.6301	-3417.3804	0.5523	-3419.0397	0.1077	0.1009	-3419.1474	-3419.1406
Int9	-3228.5148	-3226.4853	0.4895	-3227.9903	0.1011	0.0946	-3228.0914	-3228.0850
Int9_Q	-3228.5144	-3226.4850	0.4896	-3227.9900	0.1014	0.0951	-3228.0914	-3228.0851
Product_allene	-462.4603	-461.9409	0.1703	-462.2791	0.0475	0.0460	-462.3266	-462.3251
Product_yn	-462.4605	-461.9405	0.1706	-462.2785	0.0498	0.0475	-462.3282	-462.3260
TS1	-3495.5766	-3493.2306	0.5592	-3494.9787	0.1069	0.1010	-3495.0855	-3495.0796
TS1_Q	-3495.5527	-3493.2081	0.5564	-3494.9566	0.1111	0.1039	-3495.0677	-3495.0605
TS2	-3495.5837	-3493.2353	0.5635	-3494.9806	0.1085	0.1026	-3495.0891	-3495.0832
TS2_Q	-3228.5144	-3226.4820	0.4868	-3227.9928	0.1021	0.0951	-3228.0949	-3228.0879
TS3	-652.8781	-652.1340	0.2163	-652.6475	0.0566	0.0541	-652.7041	-652.7016
TS4	-3419.5905	-3417.3464	0.5461	-3419.0070	0.1059	0.0990	-3419.1129	-3419.1060
TS4_Q	-3419.5940	-3417.3443	0.5458	-3419.0106	0.1070	0.0999	-3419.1176	-3419.1105
TS5	-652.6825	-651.9361	0.2183	-652.4502	0.0537	0.0522	-652.5039	-652.5024
TS6	-3419.6047	-3417.3529	0.5447	-3419.0222	0.1071	0.0998	-3419.1293	-3419.1220
TS6_Q	-3419.6057	-3417.3548	0.5456	-3419.0227	0.1059	0.0994	-3419.1286	-3419.1221
TS7	-3228.4869	-3226.4563	0.4829	-3227.9698	0.0987	0.0928	-3228.0684	-3228.0626
TS7_Q	-3228.4891	-3226.4599	0.4832	-3227.9720	0.0983	0.0928	-3228.0703	-3228.0648
TS8	-3228.5128	-3226.4821	0.4877	-3227.9907	0.0990	0.0930	-3228.0897	-3228.0837
TS9	-3228.4845	-3226.4570	0.4876	-3227.9627	0.0993	0.0934	-3228.0620	-3228.0561

^aElectronic energy at single point level. ^bElectronic energy at optimization level. ^cEnthalpy after single point energy correction. ^dTΔS with quasi-harmonic correction. ^eGibbs free energy with single point energy and quasi-harmonic corrections.

a Allenic natural products



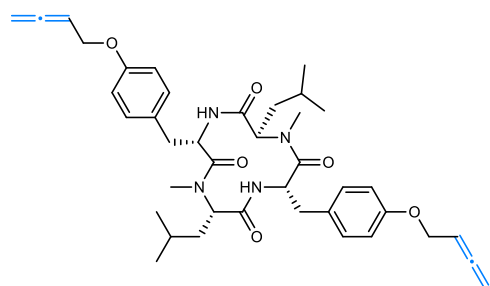
b Alkyne containing natural products



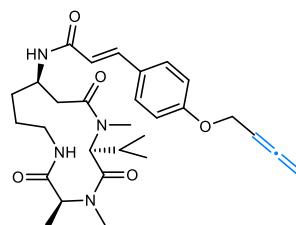
Supplementary Fig. 1 | Representative allene- and alkyne-containing natural products.^{13–17} **a**, Allene containing natural product, were isolated from diverse origins. Several exhibit notable biological activities: isotetrahydrohistrionicotoxin, from “Poison-dart frog”, interacts with ion channels of the nicotin acetylcholin receptor¹³; methyl (R,E)-(-)-tetradeca-2,4,5-trienoate functions as an insect pheromone¹³; allenic norleucine is associated with mushroom toxicity¹³; Grasshopper ketone is secreted as a defensive metabolite by *Romalea microptera*¹³; and bicyclic sesquiterpene lactones from *Vernonia* sp feature endocyclic allenes¹³. **b**, Selected internal or terminal alkyne containing natural products from fungi and bacteria^{14–17}, whose corresponding alkyne-forming enzymes have been characterized/identified.

Allenic or alkyne ether natural products in fungi

Allenic ether

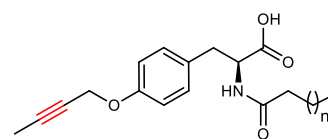


Pseudoxyllemycin B
Xylaria sp.



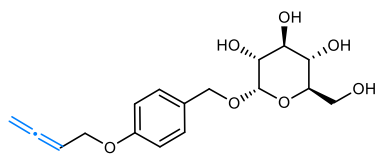
Xyloallenolide A
Xylaria sp.

Alkyne ether

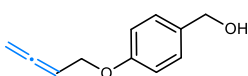


$n = 3$ Sinuxylamide B (2)
 $n = 2$ Sinuxylamide C
 $n = 1$ Sinuxylamide D

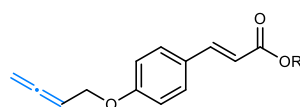
Xylaria sp.



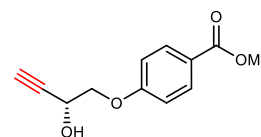
Terricollene A
Neurospora terricola



Terricolyne C
Xylaria sp.

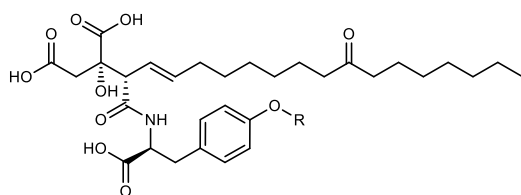


R = Me Eucalyptene A
R = H O-homoallenyl *p*-coumaric acid
Xylaria sp.

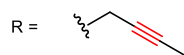


Penipratynolene (5)
Penicillium polonicum
Penicillium bilaiae

Synthetic Alkyne ether



R = H viridifungins A

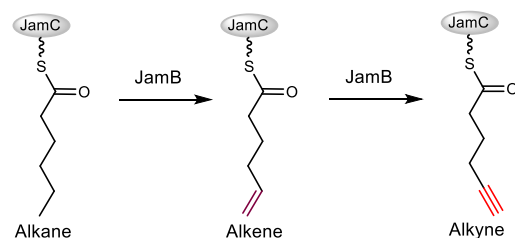


CH4630808 (NA808)

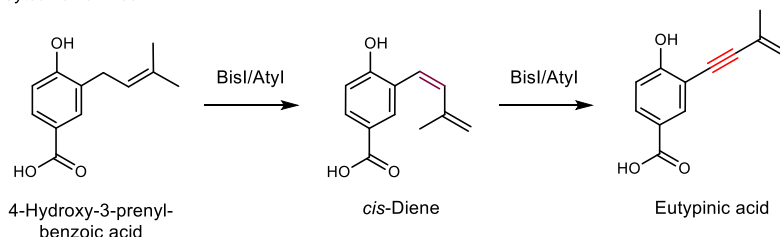
anti-hepatitis C virus agent

Supplementary Fig. 2 | Structures of synthetic and natural allenic ether or alkynyl ethers. a, Examples of natural products featuring aromatic allenic or alkynyl ether motifs, with allene and alkyne groups highlighted in light blue and red, respectively.^{1,8,18–20} Most were identified from *Xylaria* sp, with the exceptions of terricollene from *Neurospora terricola* and penipratynolene from *Penicillium polonicum*. **b,** Structure of synthetic alkyne ether CH4630808 (NA808), a novel anti-hepatitis C virus agent.²¹

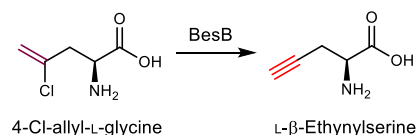
Desaturase



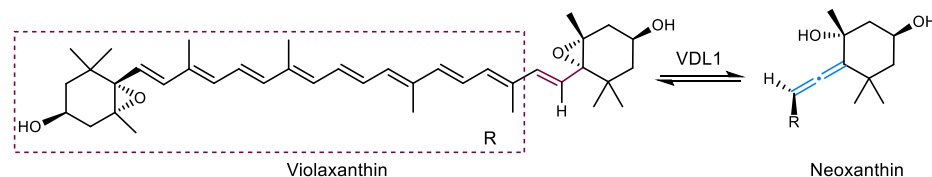
Cytochrome P450



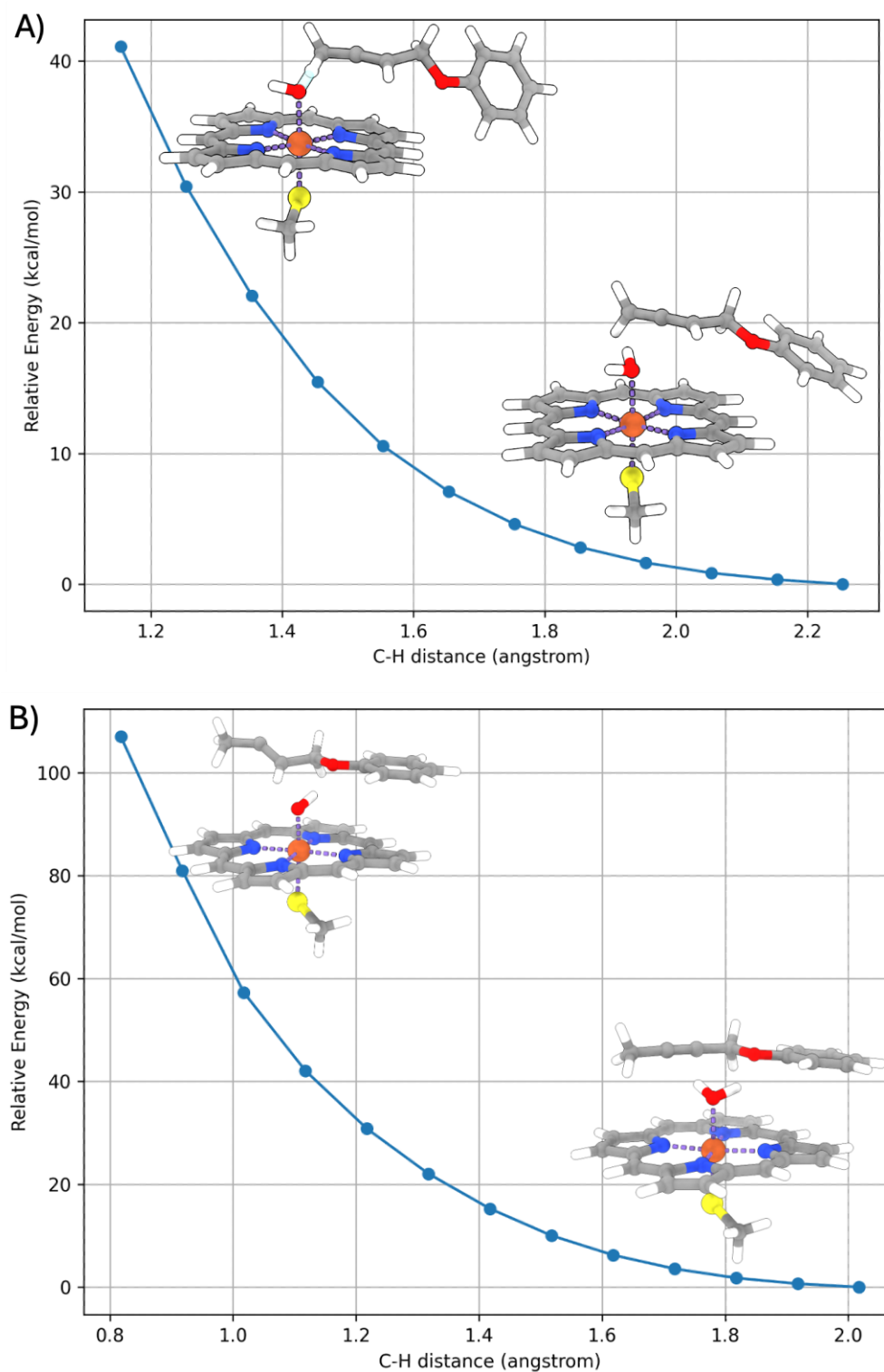
PLP-dependent enzyme



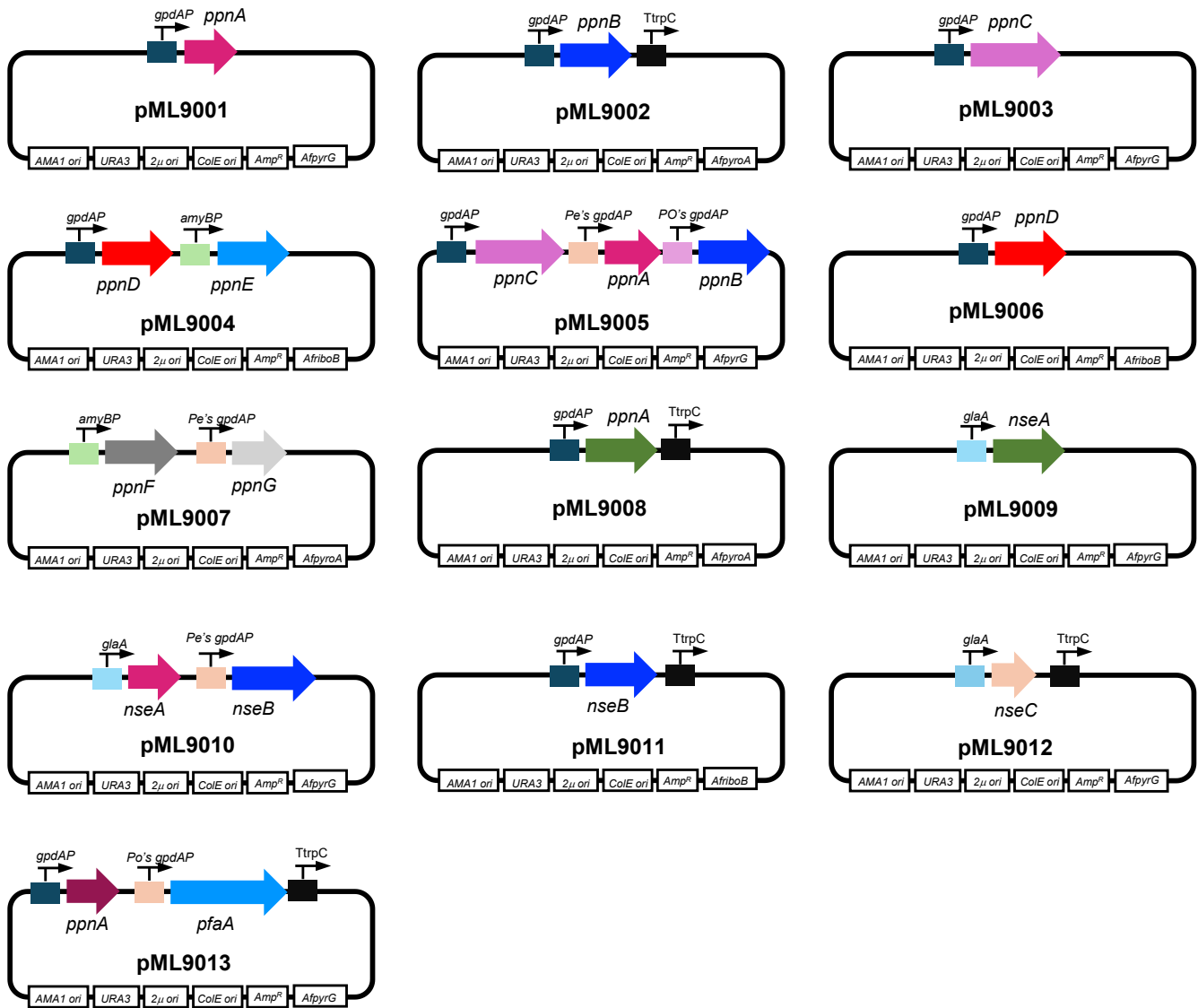
De-epoxidase like enzyme



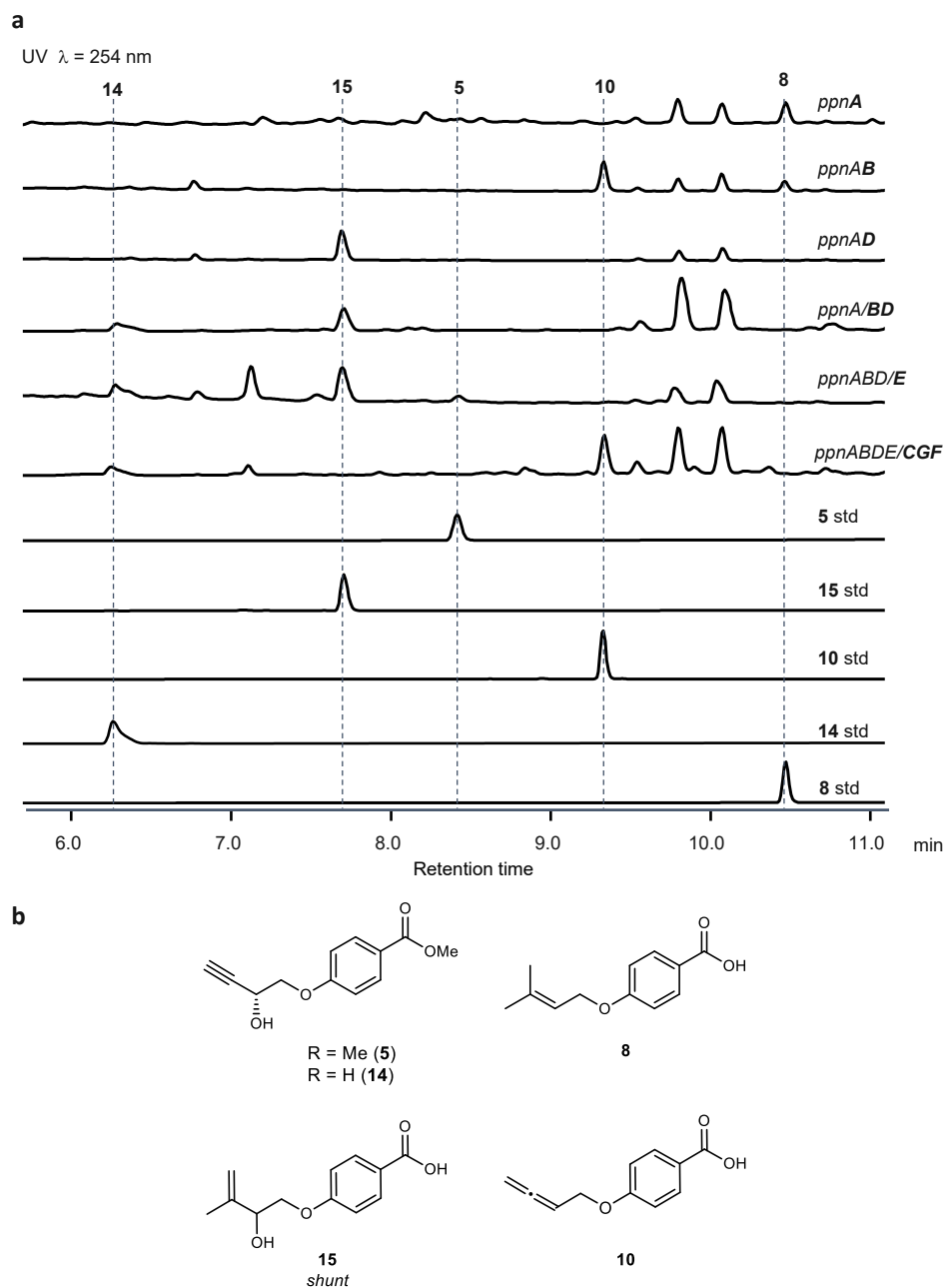
Supplementary Fig. 3 | Reported enzymes which catalyze the formation of alkyne or allene. a, For alkynes-forming enzymes, Zhang's group reported a bacterial membrane-bound di-iron-dependent desaturase, JamB¹⁴, which catalyzes the successive oxidation of JamC-tethered saturated fatty acid to generate a terminal alkyne. Both Lin's and Gao's groups independently discovered P450s AtyI²² and BisI¹⁵ that catalyze the consecutive desaturations of a prenyl group to yield 1,3-enyne moiety. Additionally, Chang's and Ryan's groups reported a PLP-dependent enzyme BesB that catalyzes the formation of L-β-ethynylserine from 4-Cl-allyl-L-glycine by a redox-neutral ionic mechanism via a PLP-tethered allene intermediate^{16,23}. **b,** Only known enzyme for formation of allenes is an algal violaxanthin de-epoxidase like enzyme VDL1 that catalyzes the conversion of violaxanthin to neoxanthin with the allene formation through 1,2-elimination²⁴.



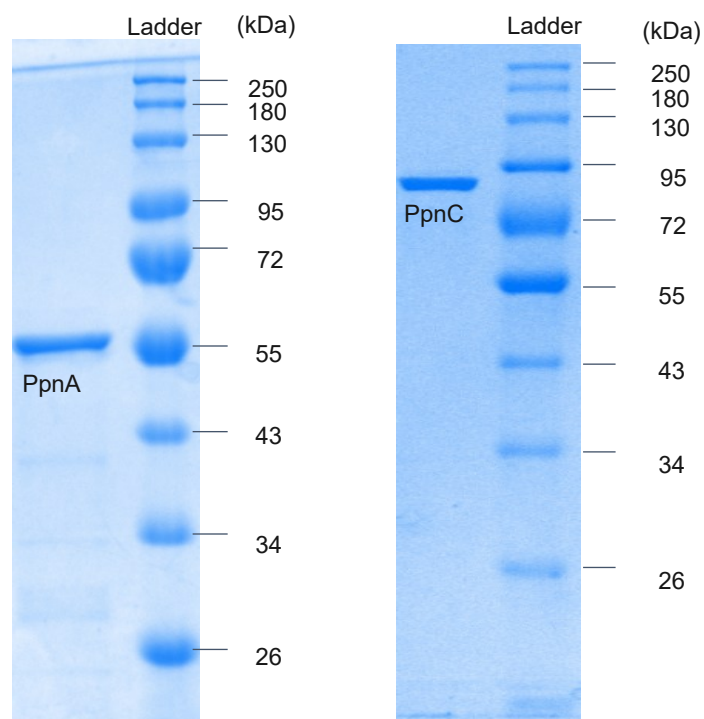
Supplementary Fig. 4 | Computational relaxed scan of the directly attack from Int4 to allene (A) and alkyne product (B). The results show a dramatic energy decrease during the HAT process. We tried TS optimization, but no saddle points can be located. Therefore, we believe these processes are barrierless on the potential energy surface, but could have very small barriers on the free energy surface.



Supplementary Fig. 5 | Plasmids used for heterologous expression of *ppn* and *nse* genes in *A. nidulans* heterologous host.

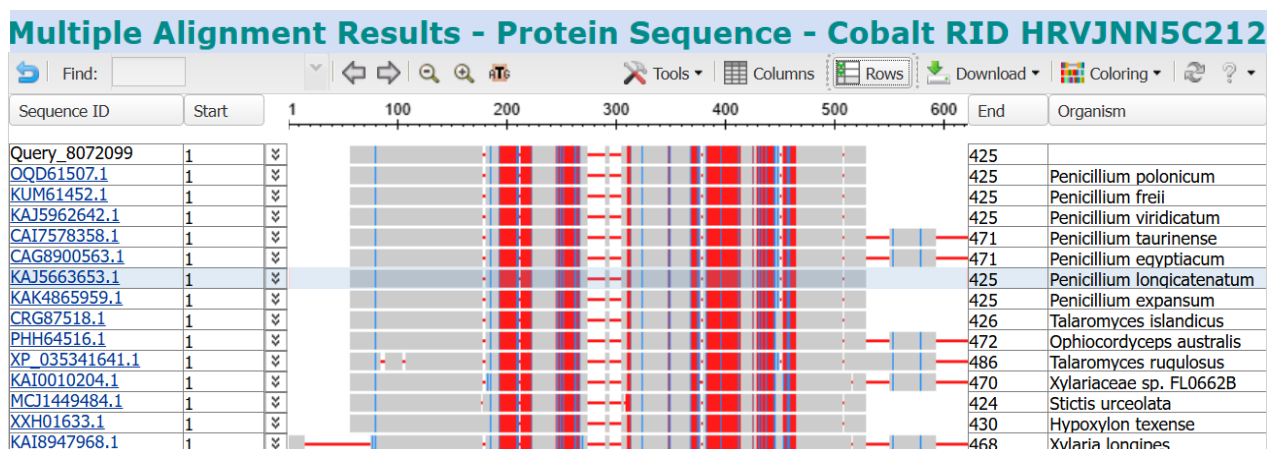


Supplementary Fig. 6 | Heterologous expression of the *ppn* cluster. a. Heterologous overexpression of *ppn* genes in *A. nidulans*. Overexpression of *ppnA* led to the formation of **8**, a shunt product. Co-expression of *ppnAB* produced **10**. Expression of *ppnAD* yielded **15**, and expression of *ppnABD* generated **15** as the major product with trace amounts of **14**. Overexpression of *ppnABDE* resulted in trace production of **5**. Introduction of the full cluster (*ppnABDECFG*) did not significantly increase the production of **5**. HPLC traces presented on the same scale are shown and were monitored at 254 nm. **b.** Structure of compounds **5**, **8**, **10**, **14** and **15**.



Supplementary Fig. 7 | SDS-PAGE gels of purified proteins used in this study. Expected molecular weights of PpnA (prenyltransferase), PpnC (aromatic amino acid ammonia lyase), 54 kDa, and 80 kDa, respectively. These experiments were repeated three times independently and representative results are shown.

a



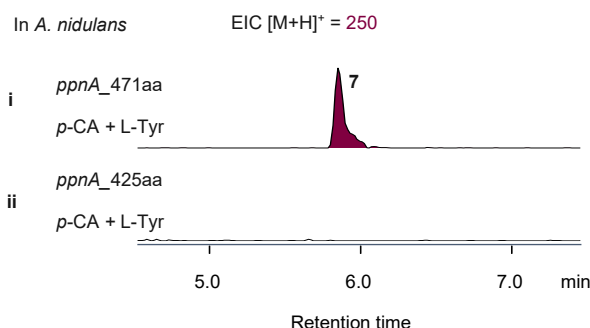
b PpnA_425aa

MAQTLDESPEFQFGRYQFASNGLSTSSADLEPREHDDRITRYPYYPVELSIWQVRVNSELDSFESVHHRFWWSRHTGKALAVLLYNAQYPADLQYWNLKFFAE
AVAPHLGVAPAILGSDTPIWPSFMTDDGTPVELSWDWGTDAPPMVRYSVIEPLHAGTSVDPGNLTAGPAFQERLTRSLPTMRLEWFHHFKDFFNIPNAKEGE
FHEDTRDHNSSIFYGDCSEITEITPKVYFFPKLRASGQSNLDVLFQAMRTAPHVTDNRNEAGDIFHAFCSVSGSKSLEHEMLAIDLIDPLQSRLLKIYFRSRETTF
QSVINIMTLEGRIRNPKLYEGLVDLHRLWTALFGVYAVDQPLREVEHRTSGILYNFEFRLGEALPVAKIYLPVRHYCTSDEAVIRALNDYFQGGQKKGKYPDYVRA
MSTLL*

PpnA_471aa

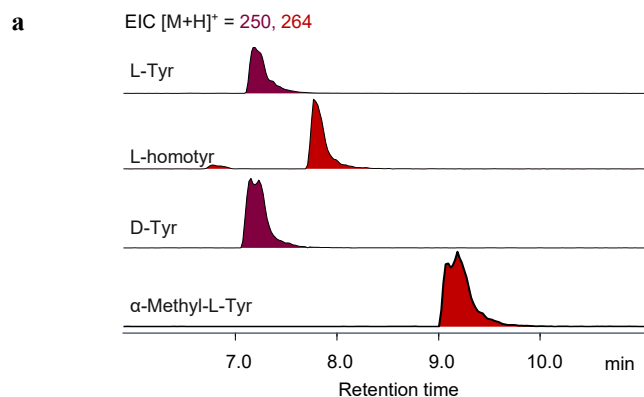
MAQTLDESPEFQFGRYQFASNGLSTSSADLEPREHDDRITRYPYYPVELSIWQVRVNSELDSFESVHHRFWWSRHTGKALAVLLYNAQYPADLQYWNLKFFAE
AVAPHLGVAPAILGSDTPIWPSFMTDDGTPVELSWDWGTDAPPMVRYSVIEPLHAGTSVDPGNLTAGPAFQERLTRSLPTMRLEWFHHFKDFFNIPNAKEGE
FHEDTRDHNSSIFYGDCSEITEITPKVYFFPKLRASGQSNLDVLFQAMRTAPHVTDNRNEAGDIFHAFCSVSGSKSLEHEMLAIDLIDPLQSRLLKIYFRSRETTF
QSVINIMTLEGRIRNPKLYEGLVDLHRLWTALFGVYAVDQPLREVEHRTSGILYNFEFRLGEALPVAKIYLPVRHYCTSDEAVIRALNDYFQGGQKKGKYPDYVRA
MSTLFTPKSMRENSGVQTYVGCAIRPDGTLRVVSYFKPQVPVHLFESEVYL*

c

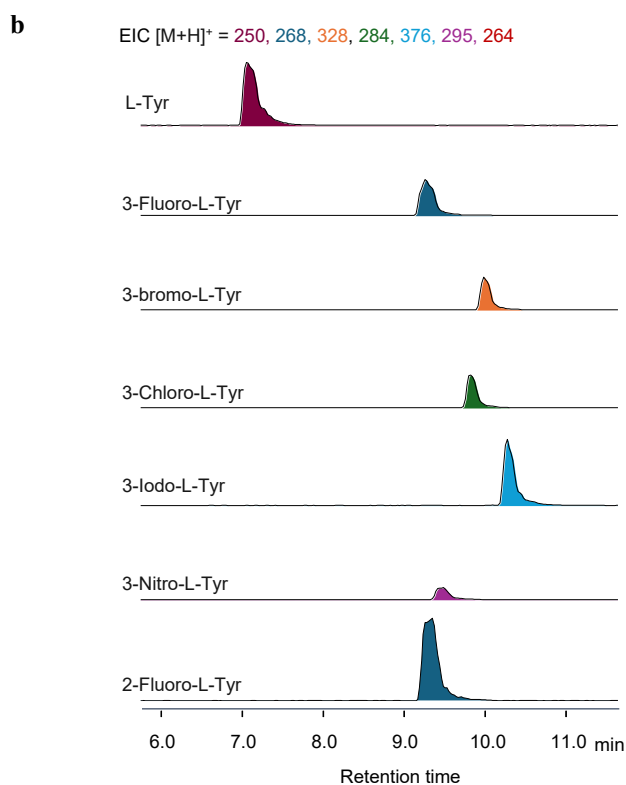
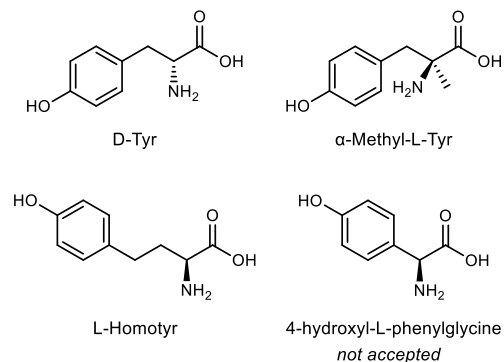


Supplementary Fig. 8 | Annotation of PpnA. a. Multiple sequence alignment of PpnA homologs retrieved from the NCBI database shows two alternative annotations for PpnA: a shorter around 425 amino acids (aa) form and a longer form of around 471aa. b. Amino-acid sequences of the two annotated PpnA variants. The 425aa form corresponds to a truncated annotation lacking the C-terminal region, whereas the 471aa variant contains the full-length sequence. c. LC–MS analysis of *A. nidulans* expressing the two PpnA variants. Only expression of the full-length PpnA_471aa supported in vivo production of compound 7 (EIC m/z +250), whereas the truncated PpnA_425aa failed to generate detectable products. These results indicate that the 471 aa variant is the catalytically active form and that the current NCBI gene annotation of PpnA from *P. polonicum* is misannotated. Selected-ion

chromatography traces presented on the same scale are shown.

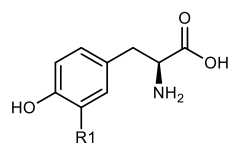


L-Tyr analogs with minimal backbone modification



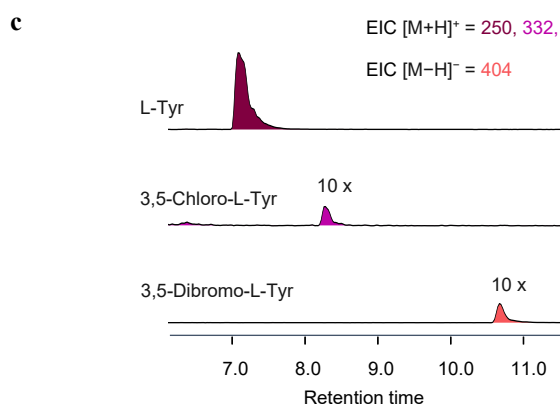
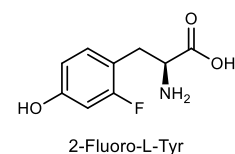
Mono-substituted L-Tyr analogs are accepted by PpnA

3-substitue L-Tyr



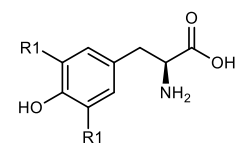
R₁ = F 3-Fluoro-L-Tyr
R₁ = Cl 3-Chloro-L-Tyr
R₁ = Br 3-Bromo-L-Tyr
R₁ = I 3-Iodo-L-Tyr
R₁ = NO₂ 3-Nitro-L-Tyr

2-disubstitue L-Tyr



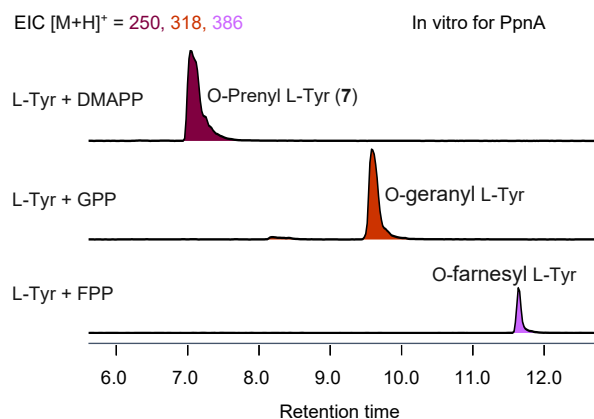
Di-substituted L-Tyr analogs are not well-accepted by PpnA

3,5-disubstitue L-Tyr

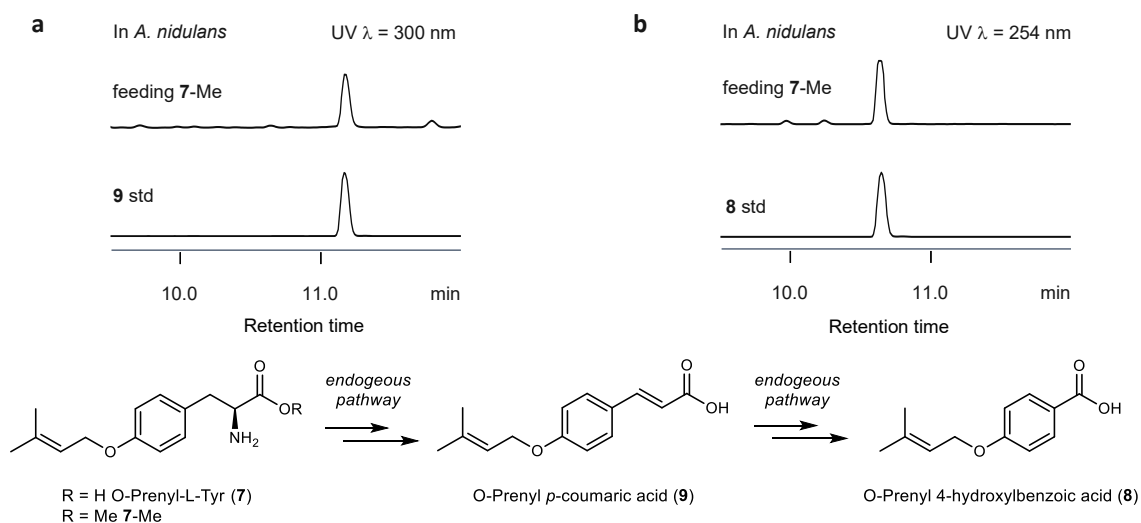


R₁ = Cl 3,5-Chloro-L-Tyr
R₁ = Br 3,5-Dibromo-L-Tyr
R₁ = I 3,5-Diiodo-L-Tyr
R₁ = NO₂ 3,5-Dinitro-L-Tyr

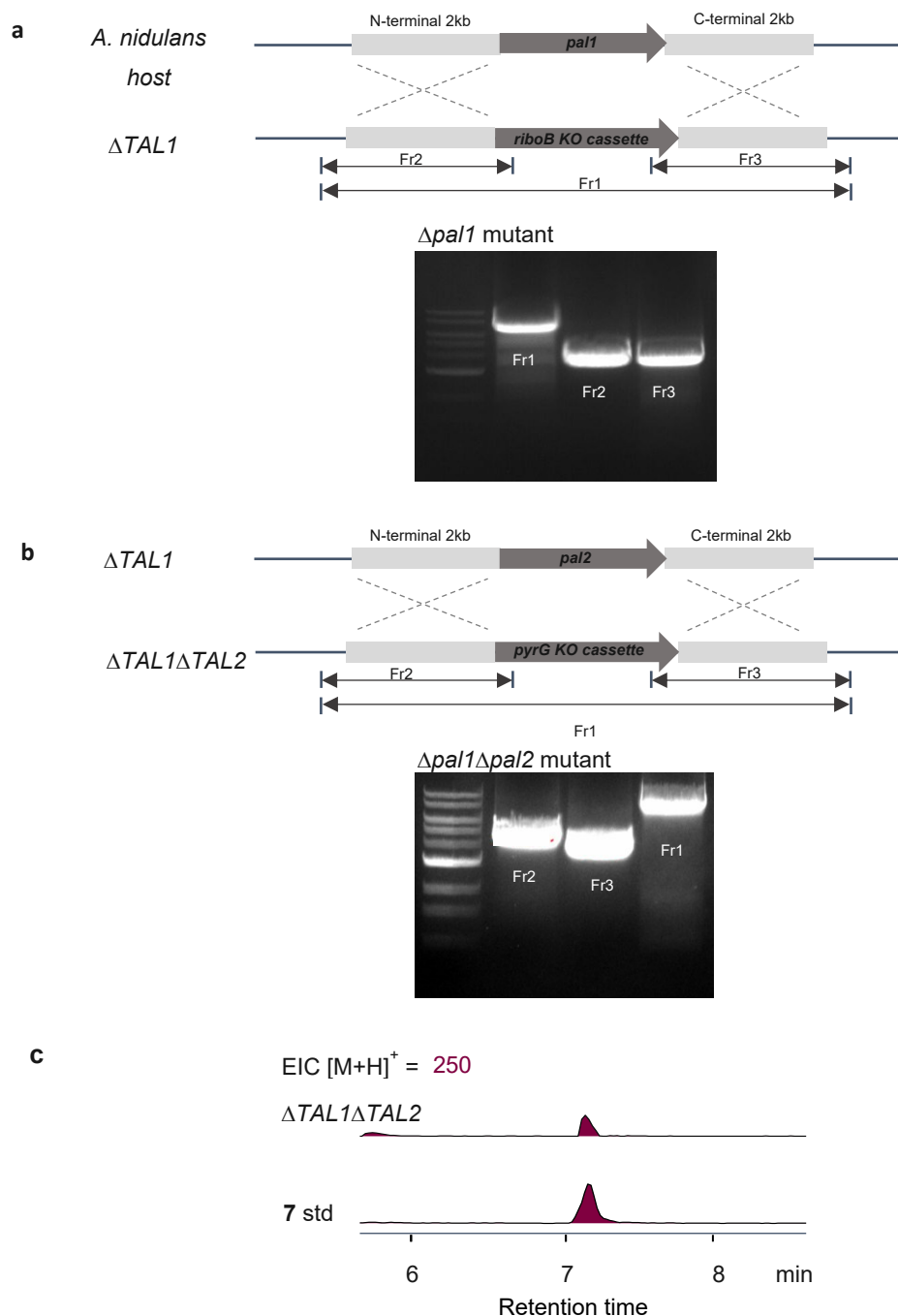
Supplementary Fig. 9 | Substrate specificity of PpnA toward L-tyrosine analogs. **a.** PpnA tolerates L-Tyr analogs with minor backbone modifications, including D-Tyr, α -Me-L-Tyr, and L-homotyrosine, yielding products with $[M + H]^+ = 250$ for L-Tyr and D-Tyr; $[M + H]^+ = 264$ for α -Me-L-Tyr and L-homotyrosine. In contrast, 4-hydroxy-L-phenylglycine is not recognized as a substrate. **b.** Substrate scope of PpnA toward mono-substitute L-tyrosine analogs. PpnA activates a range of mono-substituted L-Tyr derivatives, including 3-fluoro-, 3-chloro-, 3-bromo-, 3-iodo-, 3-nitro-, and 2-fluoro-L-Tyr, as shown by LC–MS detection of corresponding prenylated products ($[M + H]^+ = 250$ for L-Tyr; $[M + H]^+ = 268$ for 3-fluoro-, and 2-fluoro-L-Tyr; $[M + H]^+ = 284$ for 3-bromo-L-Tyr; $[M + H]^+ = 376$ for 3-Iodo-L-Tyr; $[M + H]^+ = 285$ for 3-nitro-L-Tyr). **c.** Di-substituted L-Tyr analogs are not efficiently accepted by PpnA. No product formation was observed for 3,5-diiodo-L-Tyr or 3,5-dinitro-L-Tyr, while trace levels of products were detected for 3,5-dichloro- and 3,5-dibromo-L-Tyr ($[M + H]^+ = 332$ for 3,5-dichloro-L-Tyr; $[M - H]^- = 404$ for 3,5-dibromo-L-Tyr). The y-axis represents ion counts, and all selected ion chromatograms are shown on the same scale. The colors of the traces correspond to the MS spectra of the respective prenylated products.



Supplementary Fig. 10 | Substrate specificity of PpnA toward different prenyl donors. LC–MS analysis of reactions using L-Tyr with various prenyl donors shows that PpnA accepts DMAPP, GPP, and FPP to generate distinct prenylated products with $[M + H]^+ = 250, 318$, and 386 , respectively. The chromatograms (EICs) correspond to the mono-, geranyl-, and farnesyl-tyrosine products, confirming that PpnA can utilize multiple isoprenoid diphosphates as prenyl donors. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds.

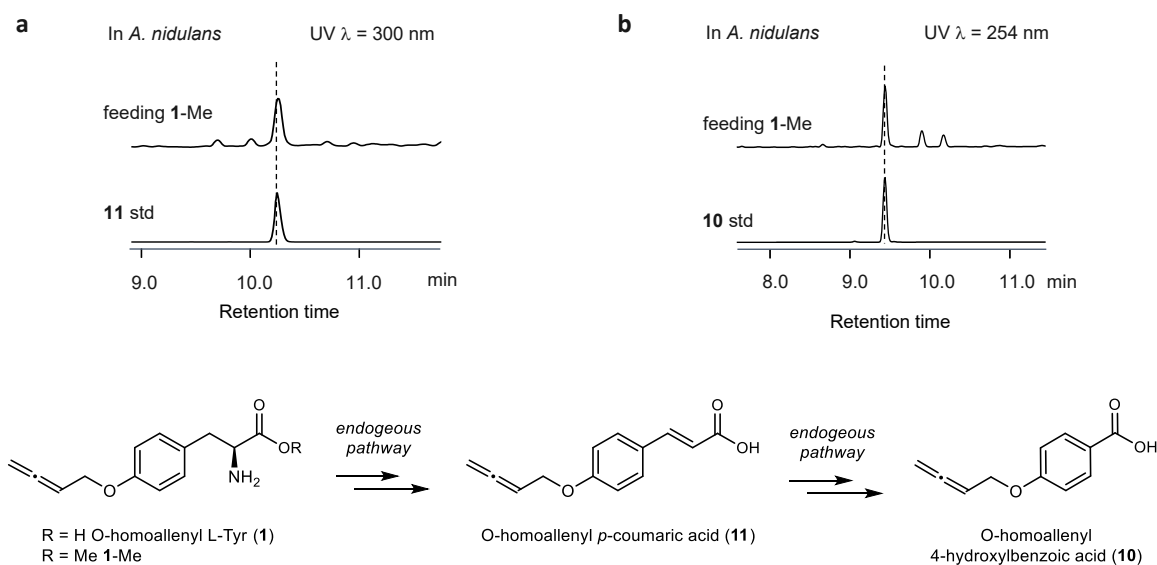


Supplementary Fig. 11 | LC-MS analysis of the metabolites extracted from *A. nidulans* feeding 7-Me. a. LC traces of *A. nidulans* extracts after feeding 7-Me, monitored at UV = 300 nm. The formation of *O*-prenyl-*p*-coumaric acid (9) was observed, suggesting conversion of 7-Me through an endogenous pathway. **b.** LC traces monitored at UV = 254 nm showing formation of *O*-prenyl-4-hydroxybenzoic acid (8) via intermediate 9 through an endogenous pathway. These results support that compound 8 is the shunt from 7 through endogenous metabolism. HPLC traces presented on the same scale are shown.

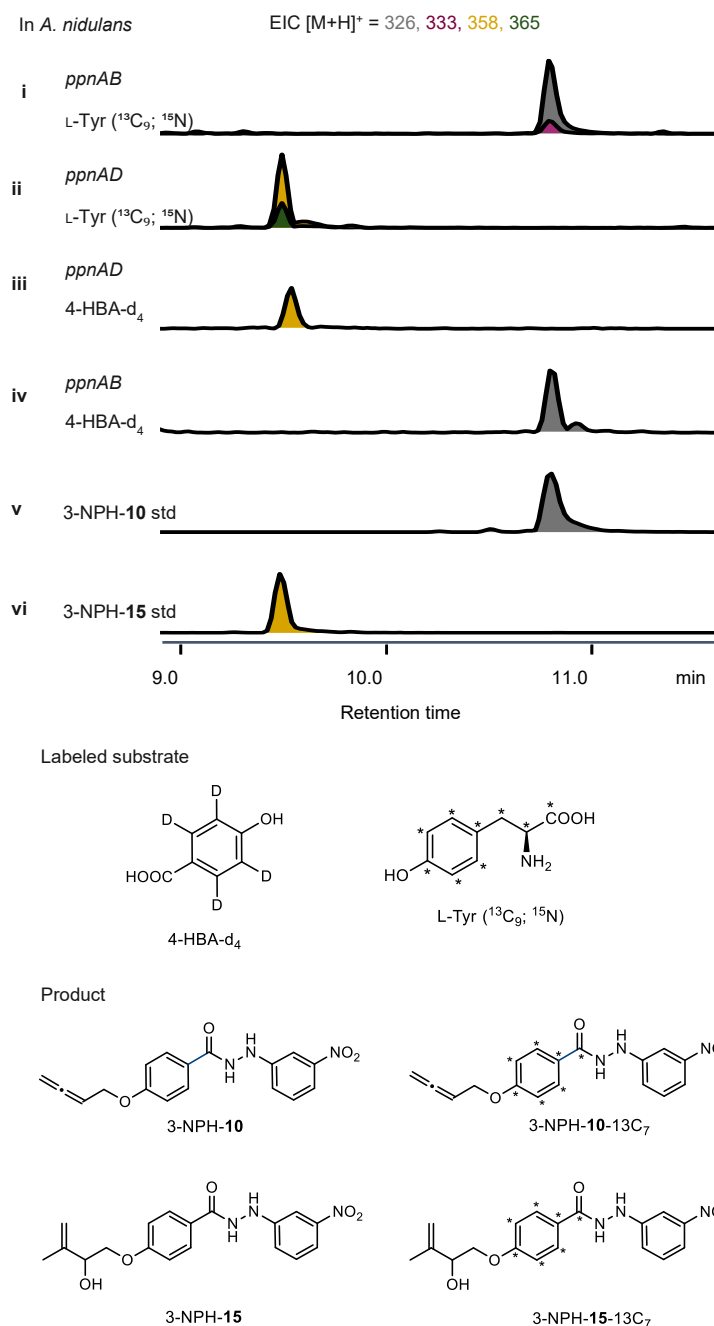


Supplementary Fig. 12 | Diagnostic PCR for *A. nidulans* A1145 Δ TAL1 Δ TAL2 mutant. Sequence identity of PpnC to *A. nidulans* endogenous tyrosine ammonia-lyases (TALs): TAL1 (XP_661501.2), 41%; TAL2, 41% (XP_663679.1). Recombinant expression and purification of TAL1 and TAL2 from *E. coli* yielded no detectable soluble protein, preventing confirmation of their proposed deamination activity in vitro. Instead, functional analysis proceeded through gene deletion in *A. nidulans*. **a.** PCR analysis confirmed knockout of TAL1 via insertion of a DNA cassette containing the *riboB* (riboflavin) selection marker. **b.** PCR confirmation of TAL2 knockout via insertion of a DNA cassette containing the *pyrG* (uracil) selection marker. **c.** Overexpression of *ppnA* in the *A. nidulans* A1145 Δ TAL1 Δ TAL2 mutant led to accumulation of compound 7 without feeding of L-Tyr. However, 7

was still slowly converted to *O*-prenyl-4-hydroxybenzoic acid (**8**). This suggests that additional endogenous pathways may contribute to processing of the tyrosine derived moiety in vivo, potentially including transaminase-mediated reaction pathways. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds.



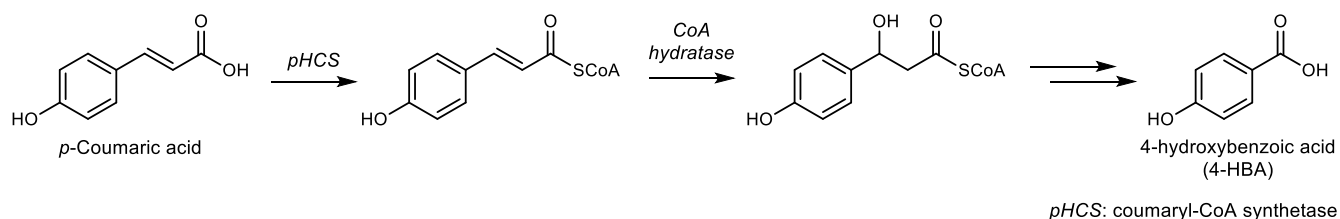
Supplementary Fig. 13 | LC-MS analysis of metabolites extracted from *A. nidulans* feeding 1-Me. a. LC traces of *A. nidulans* extracts after feeding 1-Me, monitored at 300 nm. The formation of *O*-homoallenyl *p*-coumaric acid (**11**) was observed, suggesting conversion of 1-Me through an endogenous pathway. HPLC traces presented on the same scale are shown. **b.** LC traces monitored at 254 nm showing formation of *O*-homoallenyl 4-hydroxybenzoic acid (**10**) via intermediate **11** through an endogenous pathway. HPLC traces presented on the same scale are shown.



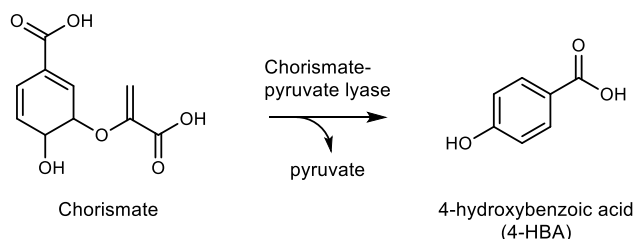
Supplementary Fig. 14 | LC-MS analysis of the metabolites extracted from *A. nidulans* expressing *ppnAB* or *ppnAD* feeding with labeled L-Tyr ($^{13}\text{C}_9$; ^{15}N) and 4-hydroxybenzoic acid- d_4 (4-HBA- d_4) after 5 days. The crude extracts were derivatized with 3-nitrophenylhydrazine (3-NPH) prior to LC-MS analysis, in order to increase the MS sensitivity. Labeled 3-NPH-10 and 3-NPH-15 were detected when feeding 0.5 mM L-Tyr ($^{13}\text{C}_9$; ^{15}N) to *A. nidulans* expressing *ppnAB* and *ppnAD* respectively. Feeding 0.5 mM 4-HBA- d_4 did not lead to deuterium incorporation in 3-NPH-10 or 3-NPH-15. Selected ion monitoring of 3-NPH-10 ($[M+H]^+ = 326$), 3-NPH-10- $^{13}\text{C}_7$

($[M + H]^+ = 333$), 3-NPH-**15** ($[M + H]^+ = 358$), and 3-NPH-**15**- $^{13}\text{C}7$ ($[M + H]^+ = 365$) is shown. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds. Note that * on the structure indicate the isotope-labeled carbon atom (^{13}C).

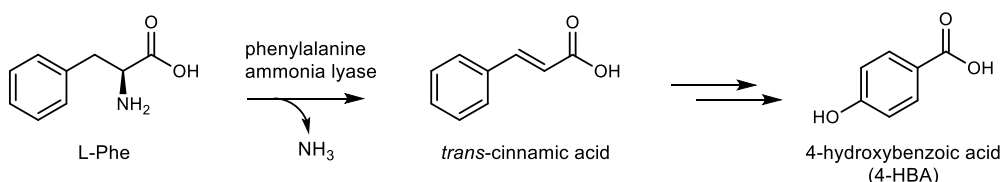
In plant



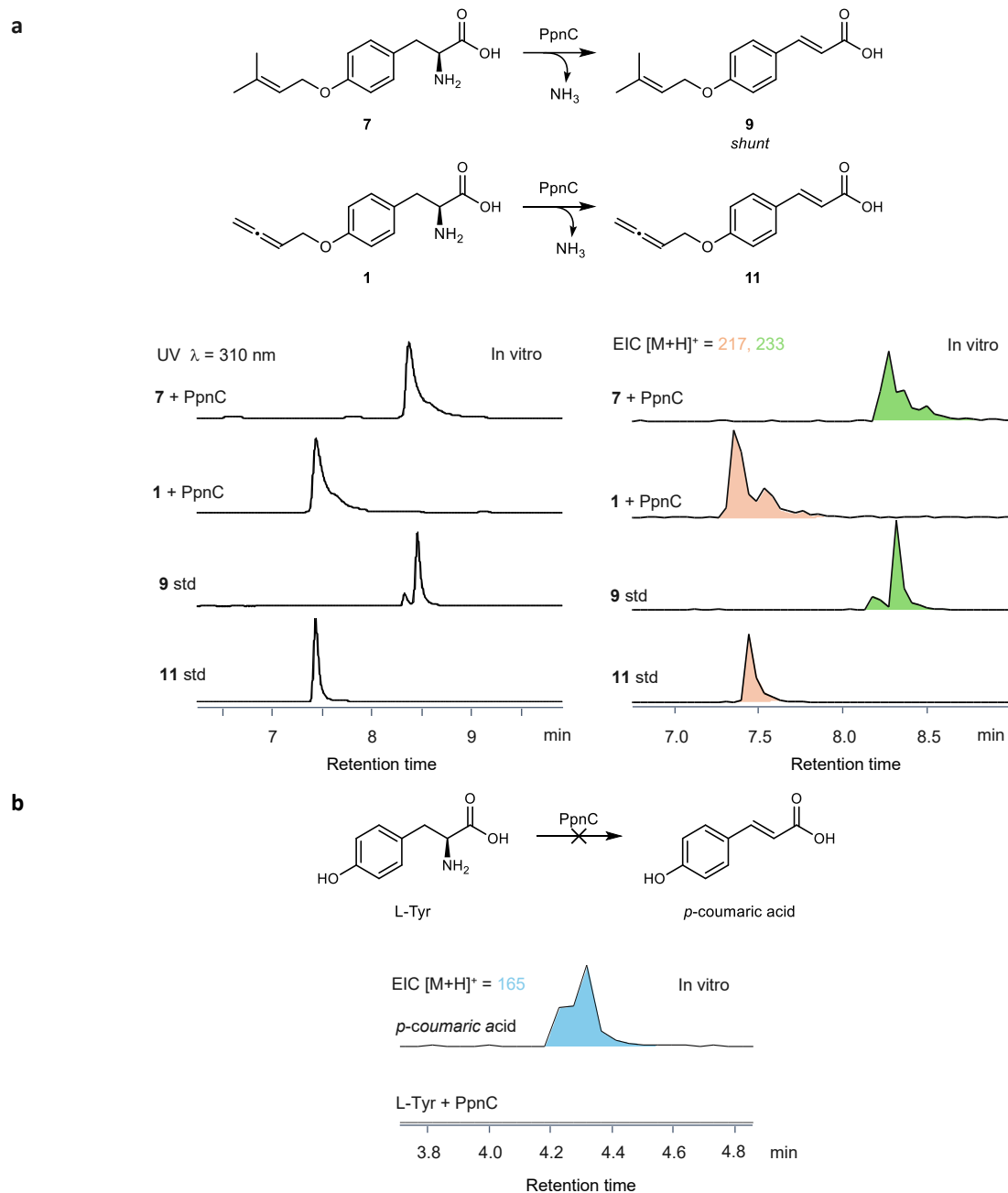
In bacteria



In fungi

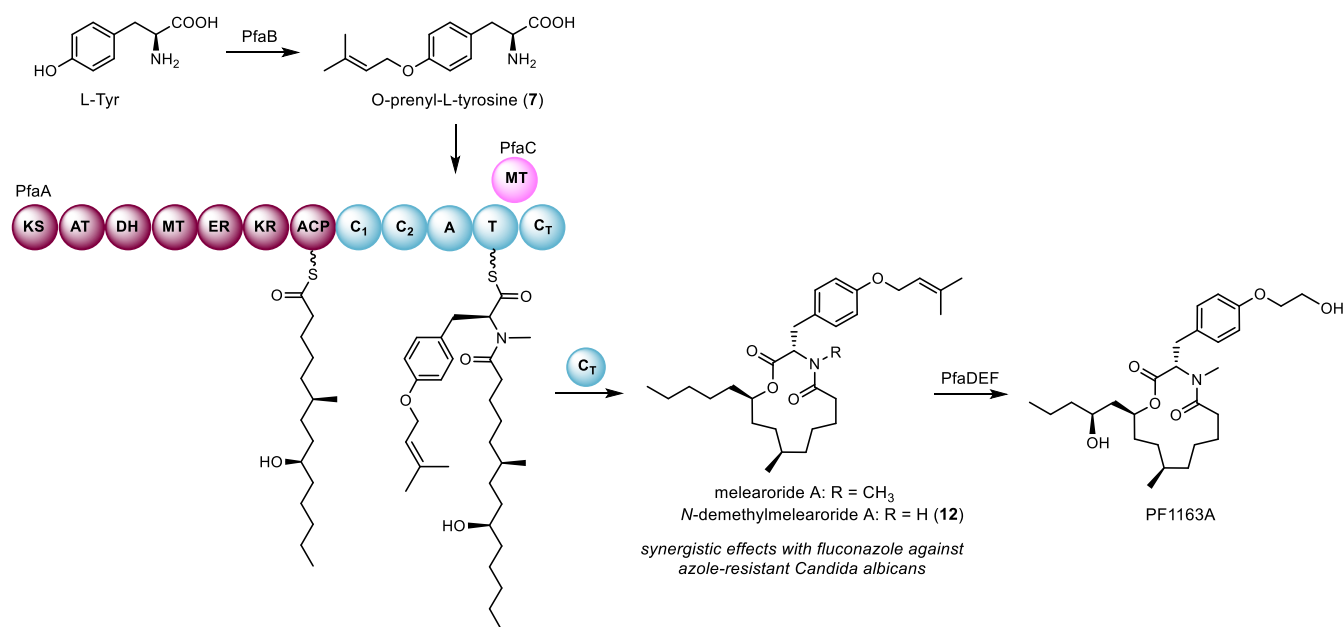


Supplementary Fig. 15 | Pathways for 4-hydroxybenzoic acid (4-HBA) formation in plants, bacteria, and fungi. In plants, 4-HBA formation has been proposed to proceed via a CoA-dependent route, in which coumaric acid is first activated to its CoA thioester by coumaroyl-CoA synthase, followed by hydration by a CoA hydratase and subsequent C–C bond cleavage and oxidation to yield 4-HBA²⁵. In bacteria, 4-HBA is generated from chorismate through the action of chorismate pyruvate lyase, which cleaves pyruvate from chorismate²⁶. In fungi, phenylalanine ammonia-lyase (PAL) catalyzes the deamination of L-Phe to form cinnamic acid, which is further converted to 4-HBA through downstream oxidative steps²². However, the 4-hydroxybenzoic acid moiety in the penipratynolene (**5**) is biosynthesized from a pathway starting from L-Tyr, which is different from the plant, bacterial, and canonical fungal catabolic pathways described above.

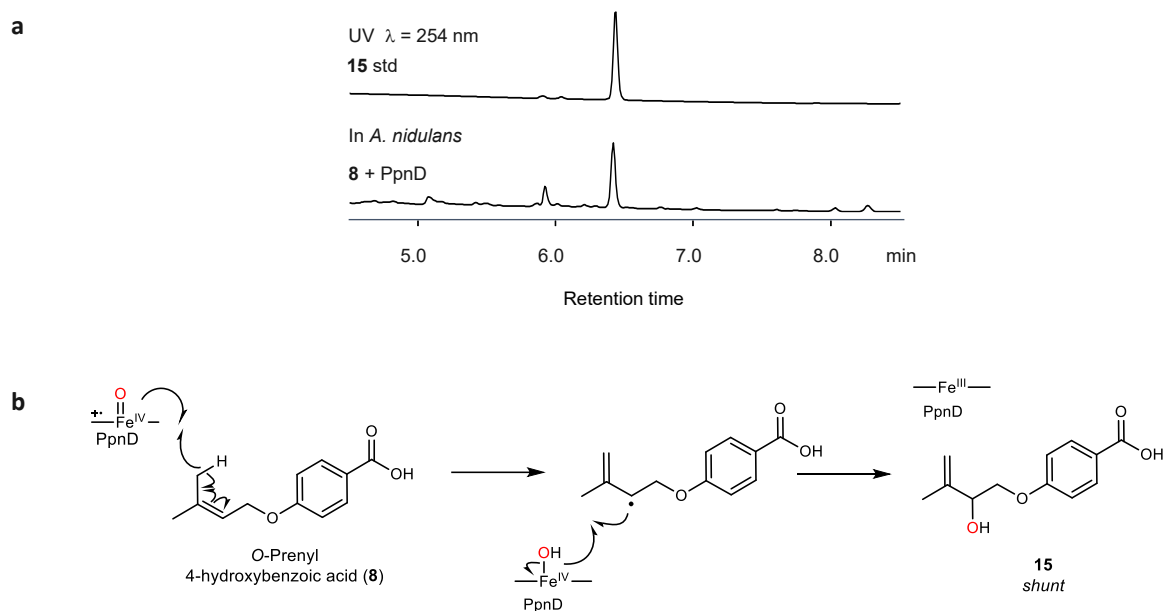


Supplementary Fig. 16 | In vitro reaction of PpnC. a. Left panel, LC-MS traces of in vitro reaction of PpnC using substrates *O*-homoallyl L-Tyr (**1**), *O*-prenyl L-Tyr (**7**), and *O*-but-2-ynyl L-Tyr (**6**). PpnC efficiently convert **1** and **7** to form **11** and **9**, respectively. Trace amount deaminated **6** is also observed. **Right panel**, EIC of in vitro reaction in **a**, showing the desired product, $[\text{M} + \text{H}]^+ = 233$ for **9** and $[\text{M} + \text{H}]^+ = 217$ for **11**. HPLC analysis ($\lambda = 310 \text{ nm}$) of enzymatic reaction of PpnC with **1** or **7** as the substrate. HPLC traces presented on the same scale are shown. Authentic standards of **9** and **11** are synthetically prepared. **b. LC-MS traces** of in vitro reaction of PpnC with L-Tyr

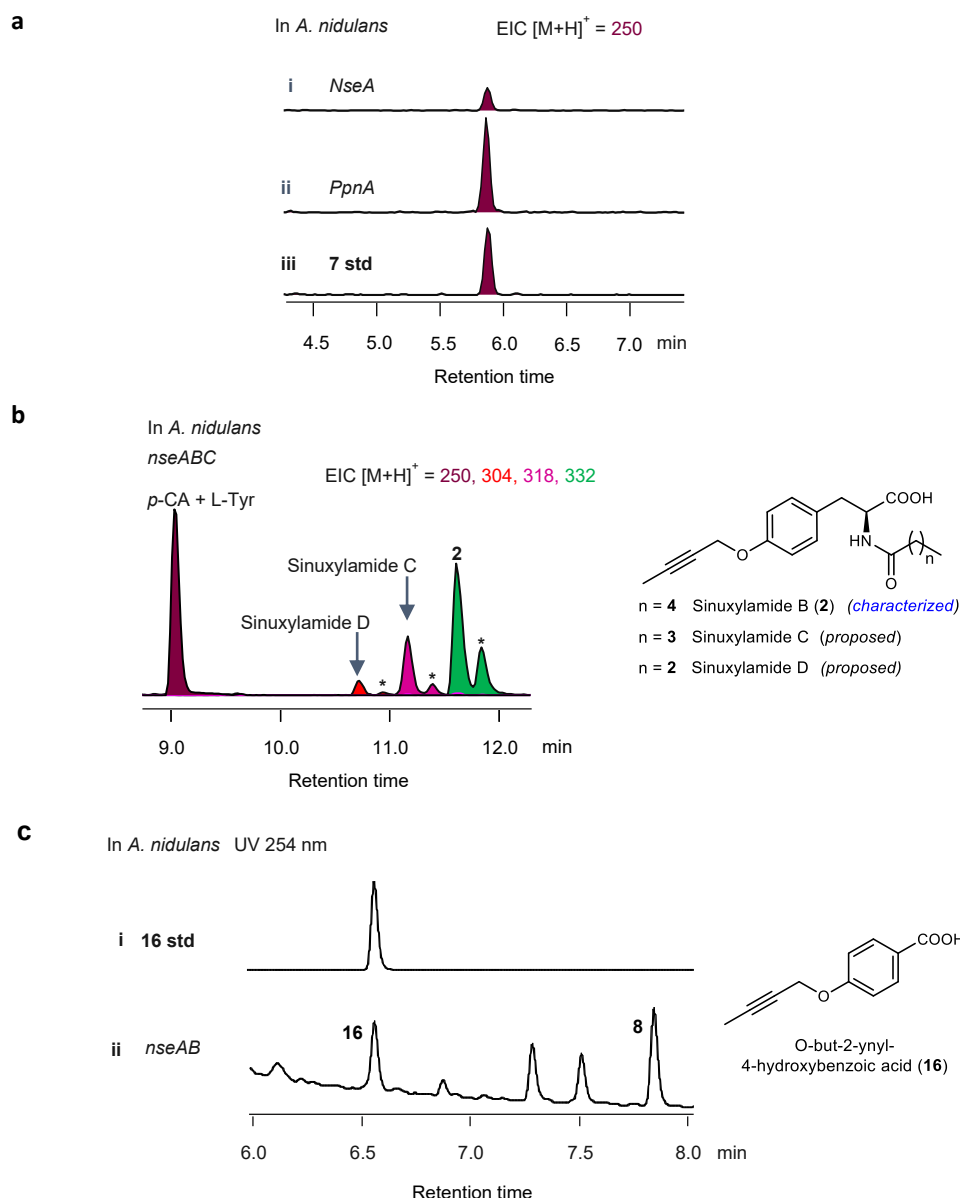
as the substrate, no deaminated product *p*-coumaric acid (*p*-CA) was observed. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds.



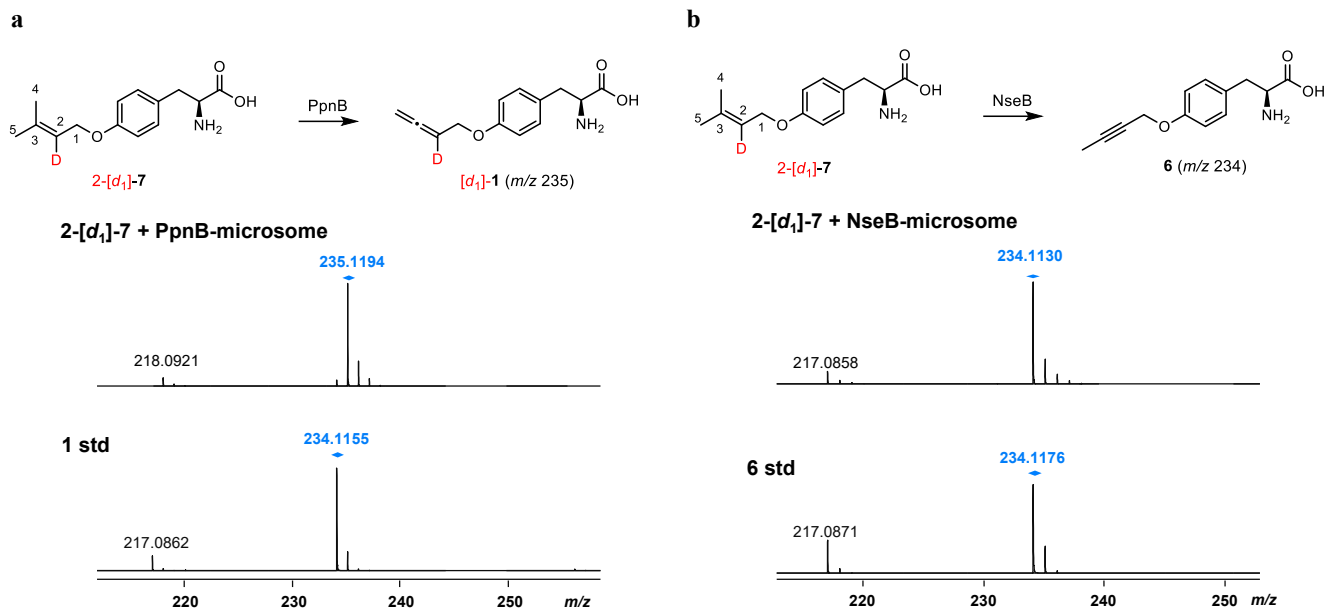
Supplementary Fig. 17 | Reported native pathway for melearolide A and PF1163A²⁷. Compound 7 is incorporated by PfaA to form *N*-demethylmelearolide A or melearolide A. Downstream tailoring enzymes further modify the side chain and prenyl group to yield PF1163A. The pathway enzymes include: PfaA (PKS–NRPS hybrid; domain architecture: KS–AT–DH–MT–ER–KR–ACP–C₁–C₂–A–T–C_T), PfaB (prenyltransferase), PfaC (methyltransferase), PfaD (short-chain reductase), PfaE (α-ketoglutarate-dependent dioxygenase), and PfaF (globin-like ER-bound oxygenase).



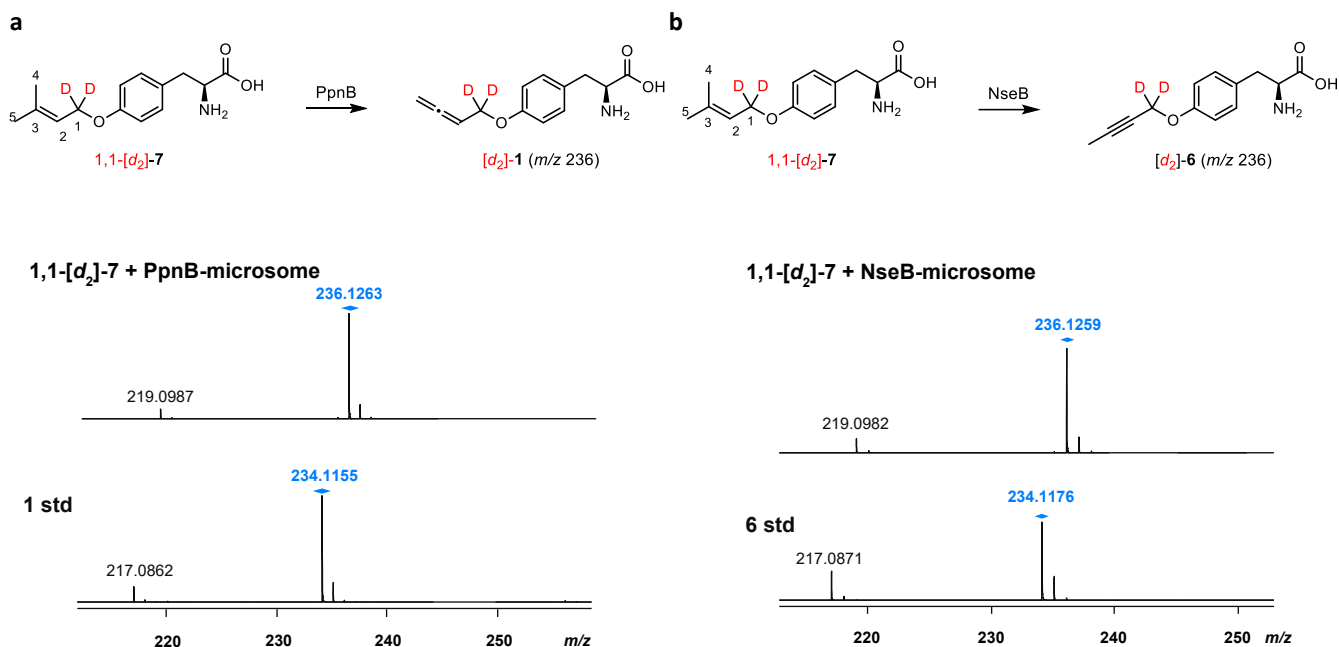
Supplementary Fig. 18 | PpnD catalyzes oxidative modification of *O*-prenyl-4-hydroxybenzoic acid (8**).** **a.** HPLC analysis showing that direct feeding of *O*-prenyl-4-hydroxybenzoic acid (**8**) to *A. nidulans* expressing PpnD resulted in the formation of shunt **15**, indicating that PpnD catalyzes an oxidative modification in the later stage of the pathway. HPLC traces presented on the same scale are shown and were monitored at 254 nm. **b.** Proposed mechanism of the PpnD-catalyzed reaction.



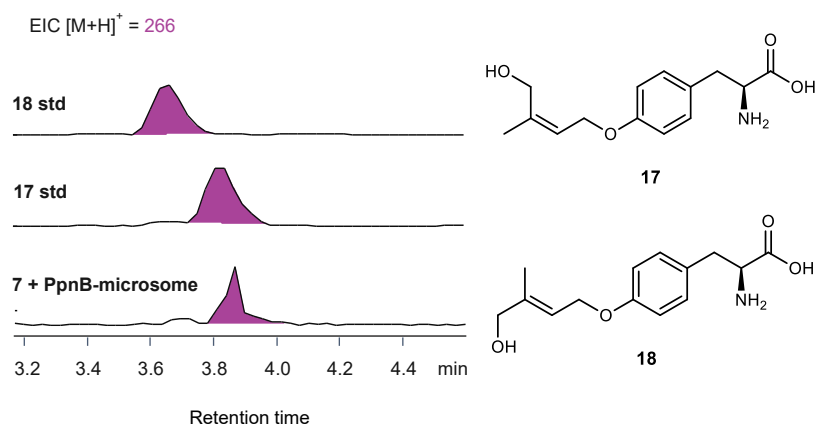
Supplementary Fig. 19 | Overexpression of NseABC leads to production of internal alkyne-containing natural products, sinuxylamides. **a.** Compound **7** was also observed when *nesA* was expressed in *A. nidulans*. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds. **b.** Overexpression of NseABC in *A. nidulans* led to the formation of Sinuxylamide B, C, and D upon feeding of L-Tyr and *p*-coumaric acid (*p*-CA). Note that * indicates the proposed allene analogs of corresponding sinuxylamides. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds. **c.** Overexpression of NseAB in *A. nidulans* without feeding of L-Tyr and *p*-CA resulted in the detection of compound **16**, suggesting that *O*-but-2-ynyl L-Tyr (**6**) was metabolized to **16** through an endogenous pathway. HPLC chromatograms were monitored at 254 nm. Authentic standard of **16** is synthetically prepared.



Supplementary Fig. 20 | LC-QTOF analysis of *A. nidulans* microsomes containing PpnB or NseB incubated with 2-[d₁]-7. +1 Da mass shift was observed for product **1** when 2-[d₁]-7 was supplied in the microsomal assays containing PpnB. The deuterium was lost for product **6** when 2-[d₁]-7 was supplied in the microsomal assays containing NseB. The x axis represents mass-to-charge ratio (*m/z*), and the y axis represents relative ion abundance.

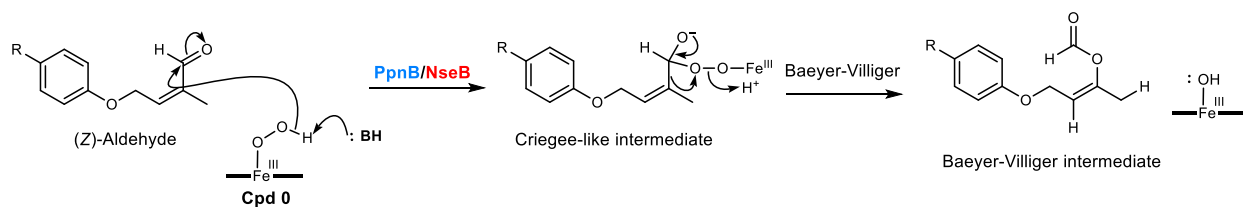


Supplementary Fig. 21 | LC-QTOF analysis of *A. nidulans* microsomes containing PpnB or NseB incubated with 1,1-[d₂]-7. +2 Da mass shift was observed for products **1** and **6** when 1,1-[d₂]-7 was supplied in the microsomal assays, supporting retention of the hydrogen atoms on the prenyl methylene group during PpnB- and NseB-catalyzed transformations. The x axis represents mass-to-charge ratio (*m/z*), and the y axis represents relative ion abundance.

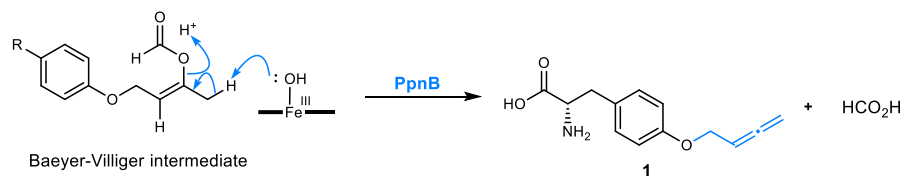


Supplementary Fig. 22 | Identification of intermediate 17 by incubating *O*-prenyl L-Tyr (7) with *ppnB*. Compound 17 was detected based on the retention time match with the synthetic standard. This further confirms that the 17 is a bona fide intermediate in the pathway. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds.

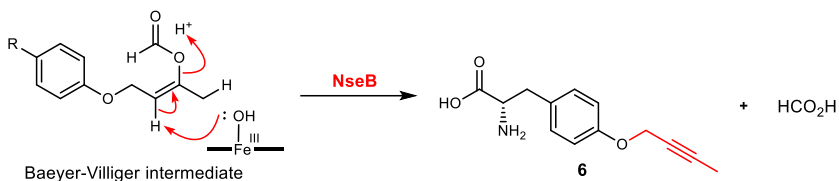
Proposed Baeyer-Villiger intermediate formation by Cpd 0



For allene formation



For alkyne formation



Supplementary Fig. 23 | Alternative proposed mechanism of allene and alkyne formation via Baeyer-Villiger (BV) intermediate. Based on the proposed mechanism of the CYP51-catalyzed reaction involving a BV intermediate, analogous reaction mechanism for PpnB- and NseB-catalyzed allene and alkyne formation can also be proposed.

a

In *A. nidulans*

EIC $[M+H]^+ = 234$

p-CA + L-Tyr

PpnB + **19**-Me

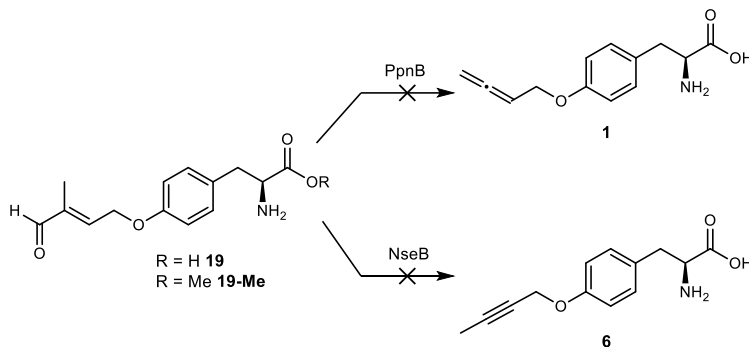
NseB + **19**-Me

1 std

6 std

3.0 4.0 5.0 min

Retention time



b

In *A. nidulans*

EIC $[M+H]^+ = 234$

p-CA + L-Tyr

PpnB + **20**

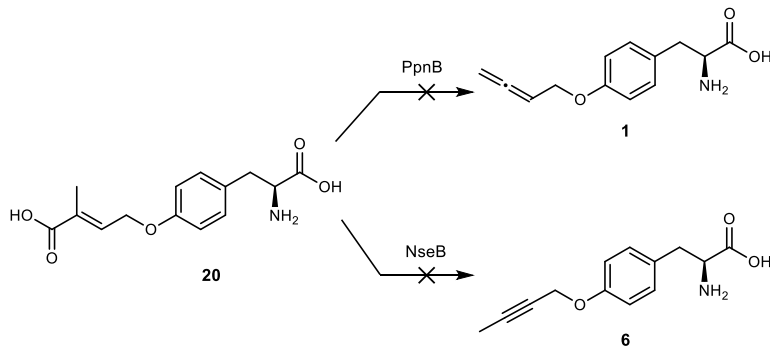
NseB + **20**

1 std

6 std

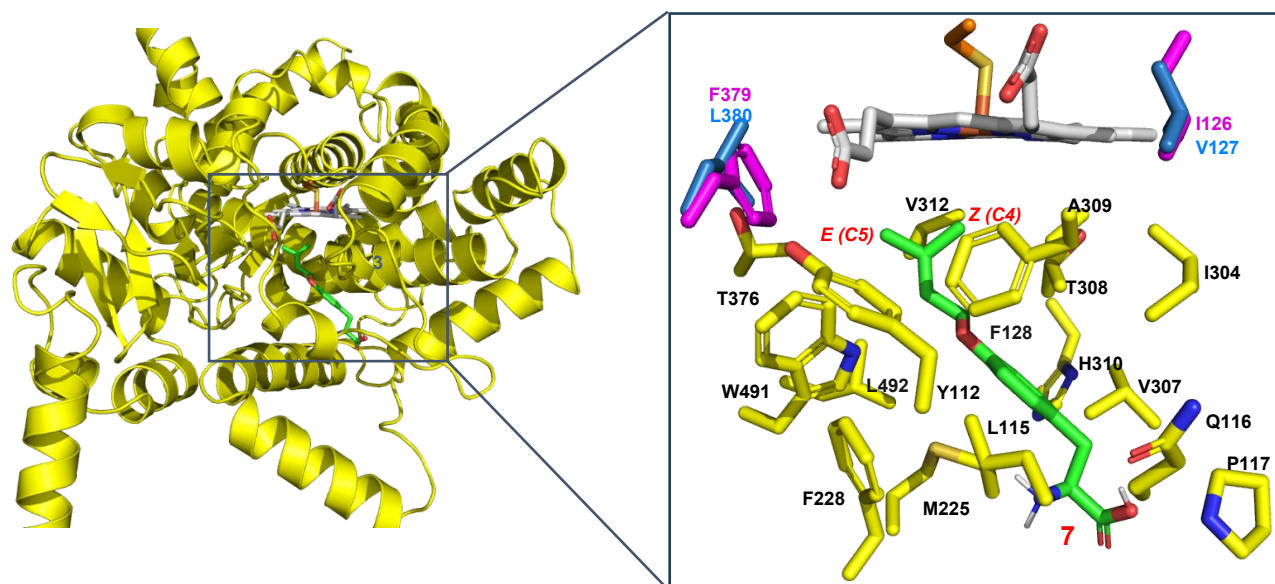
3.5 4.0 4.5 5.0 min

Retention time

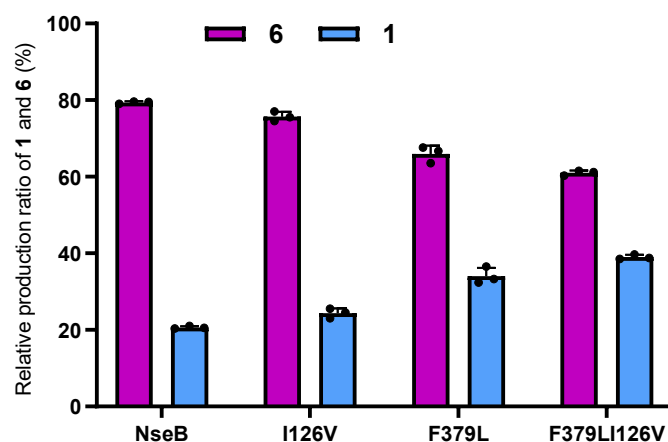


Supplementary Fig. 24 | Compounds 19 and 20 are not the intermediate toward 1 or 6. Feeding compounds **19** (a) or **20** (b) to *A. nidulans* expressing PpnB or NseB did not result in production of **1** or **6**, indicating that neither **19** nor **20** functions as a biosynthetic intermediate. These results indirectly suggest that the pathway leading to **1** and **6** must proceed through the *Z*-aldehyde or *Z*-carboxylic acid intermediates; however, chemical synthesis of these intermediates resulted in isomerization to *E* configuration, preventing direct verification. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds.

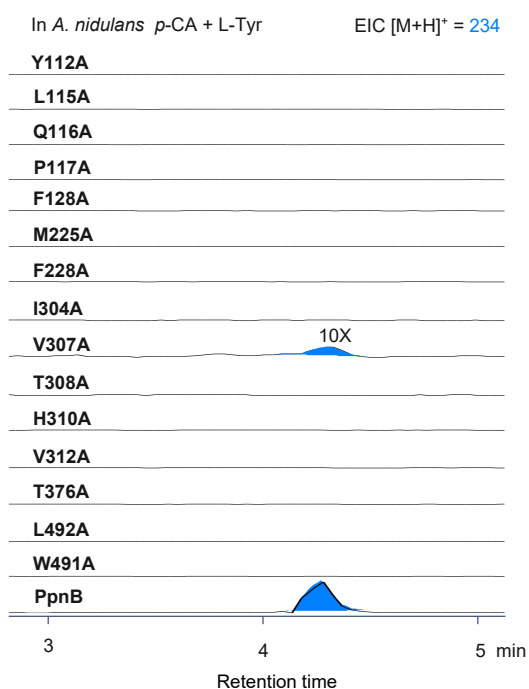
a Structure prediction of PpnB



b

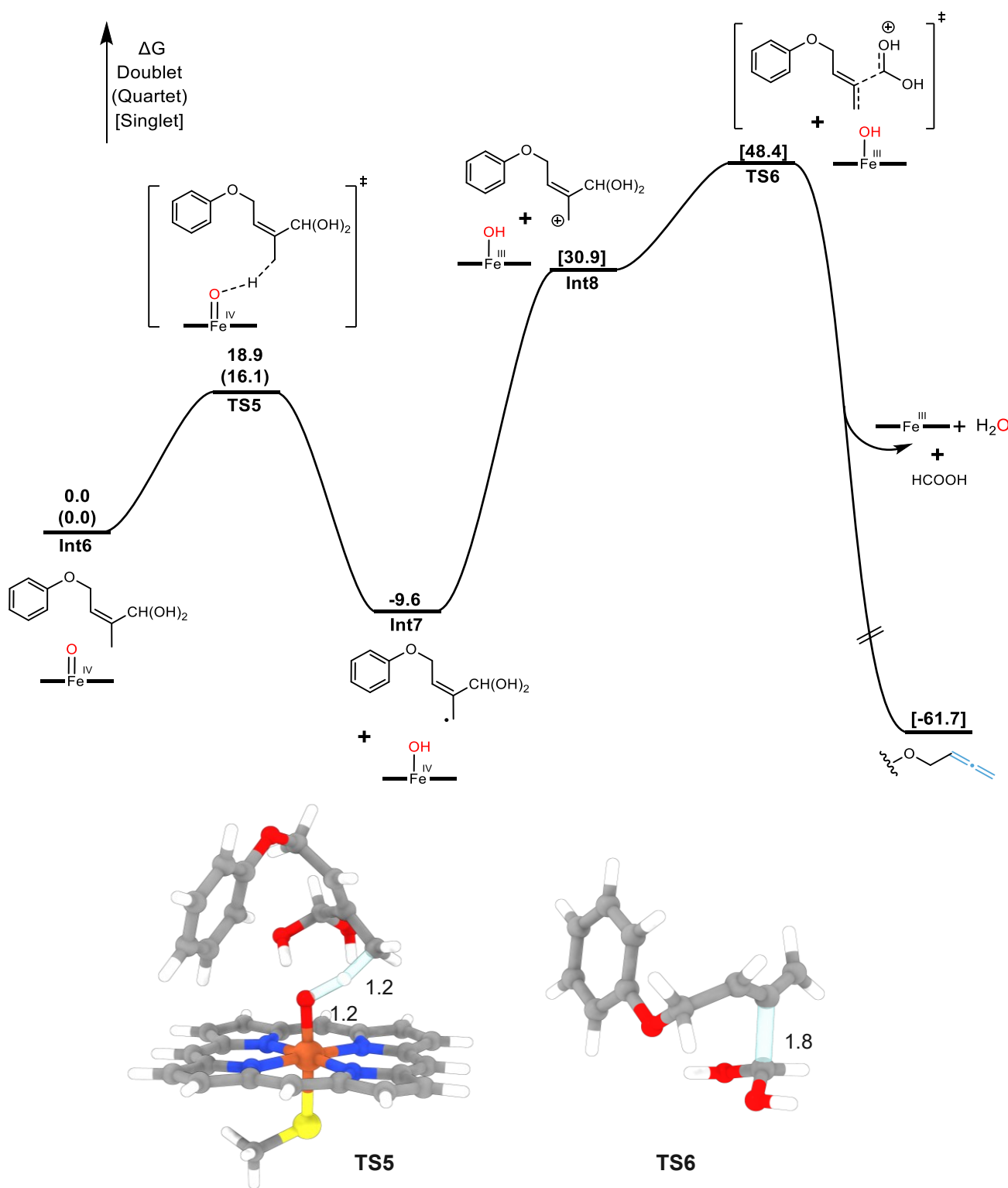


c



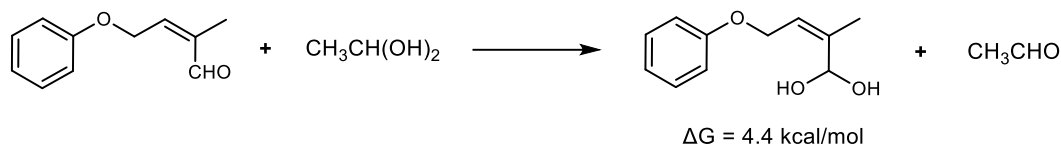
Supplementary Fig. 25 | Comparison of residues forming substrate pocket between PpnB and NseB. a. Docking simulation of **7** in the AlphaFold 3²⁸ predicted structure of PpnB containing heme cofactor. The active site was performed using Autodock Vina²⁹. One representative binding mode is shown, with **7** rendered in green and the heme cofactor in grey. PpnB and NseB share high overall sequence identity and display nearly identical substrate-binding pockets, consistent with their conserved active-site architectures. The only differences within the pocket occur at two positions: NseB contains **I126** and **F379** (magenta), whereas PpnB carries the corresponding **V127** and **L380** residues (blue). **b.** Extracted ion chromatograms (EICs) showing the production of **1** by wild-type NseB and the I126V, F379L, and I126V/F379L mutants. Values are presented as mean $\pm$ s.d. of three independent experiments

(black filled circles; $n = 3$). The relative peak area of **1** increased from 19% in wild-type NseB to 24% in I126V, 37% in F379L, and 38% in the I126V/F379L double mutant. Differences in endogenous metabolism of **1** and **6** were not considered. Although mutation at these two positions enhanced allene formation, the double mutation did not completely shift the product profile to match PpnB. This suggests that additional distal residues also contribute to shaping the active-site pocket and controlling product selectivity. **Note:** Percent values represent the relative peak area of compound **1**, calculated as a fraction of the combined peak areas of compounds **1** and **6** ($\mathbf{1} + \mathbf{6} = 100\%$). **c.** Alanine-scanning mutagenesis of the PpnB substrate-binding pocket. The mutants were coexpressed with ppnA in *A. nidulans* supplemented with *p*-CA and L-Tyr. Most alanine substitutions abolished the production of **1**, indicating that residues surrounding the substrate-binding pocket are important for substrate binding and product formation. Selected-ion chromatography traces presented on the same scale are shown and the colors of the traces show the MS of corresponding compounds.



Supplementary Fig. 26 | Computed energy profile for the potential cationic allene formation mechanism. The energies were calculated by B3LYP-D3(BJ)/def2-TZVP/SMD(Et₂O)//B3LYP-D3(BJ)/def2-SVP/IEFPCM(Et₂O) at 298

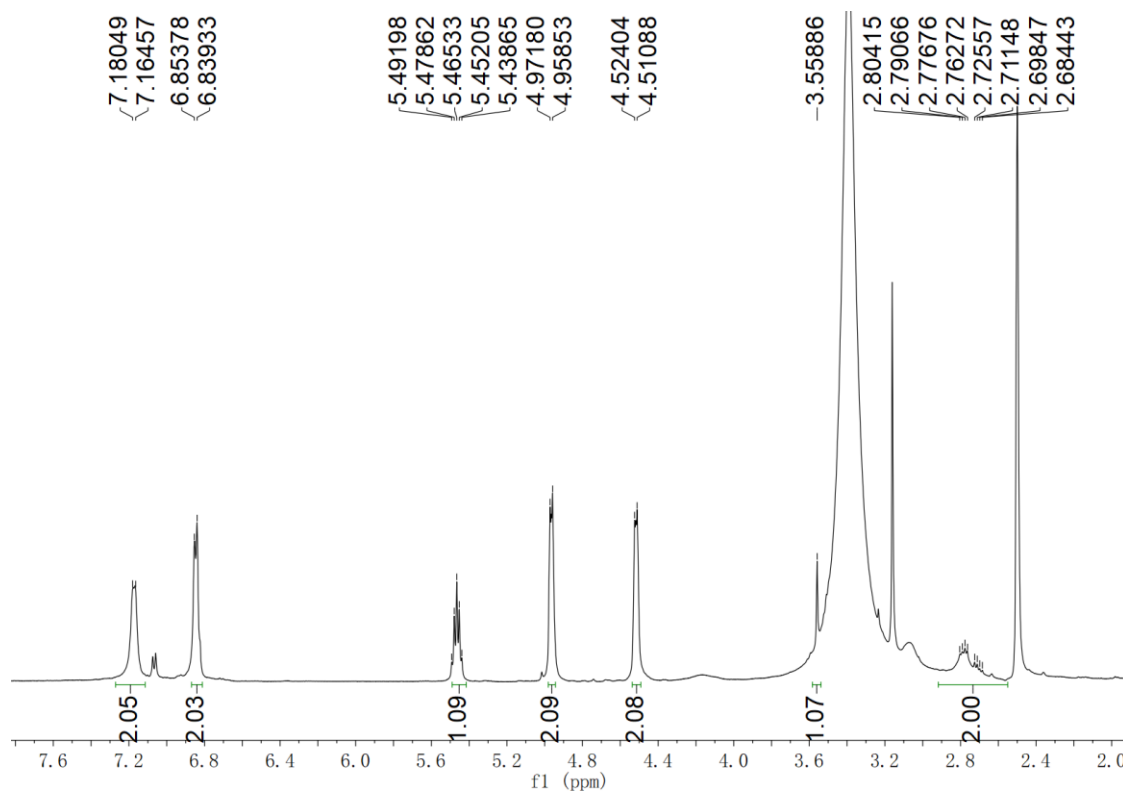
K, 1 atm and are given in kcal/mol. Bond distances are labeled in angstrom. For all iron-containing species, possible spin states with free energy are reported. As shown in the Figure, another potential Cpd I mechanism starts from the H-abstraction from the allylic C(sp³)-H by Cpd I *via* **TS5** to yield an allylic radical **Int7** and Cpd II. **Int7** is proposed to have a subsequently single electron transfer (SET) that oxidizing itself to allylic cation **Int8** and concurrently reducing Cpd II to Fe(III)-OH. **Int8** then fragments through **TS6** to give the allene product and protonated formic acid, which can react with Fe(III)-OH to restore the Fe(III) state. However, this mechanism is precluded by the highly unfavorable SET process, which is endergonic by 40.5 kcal/mol.



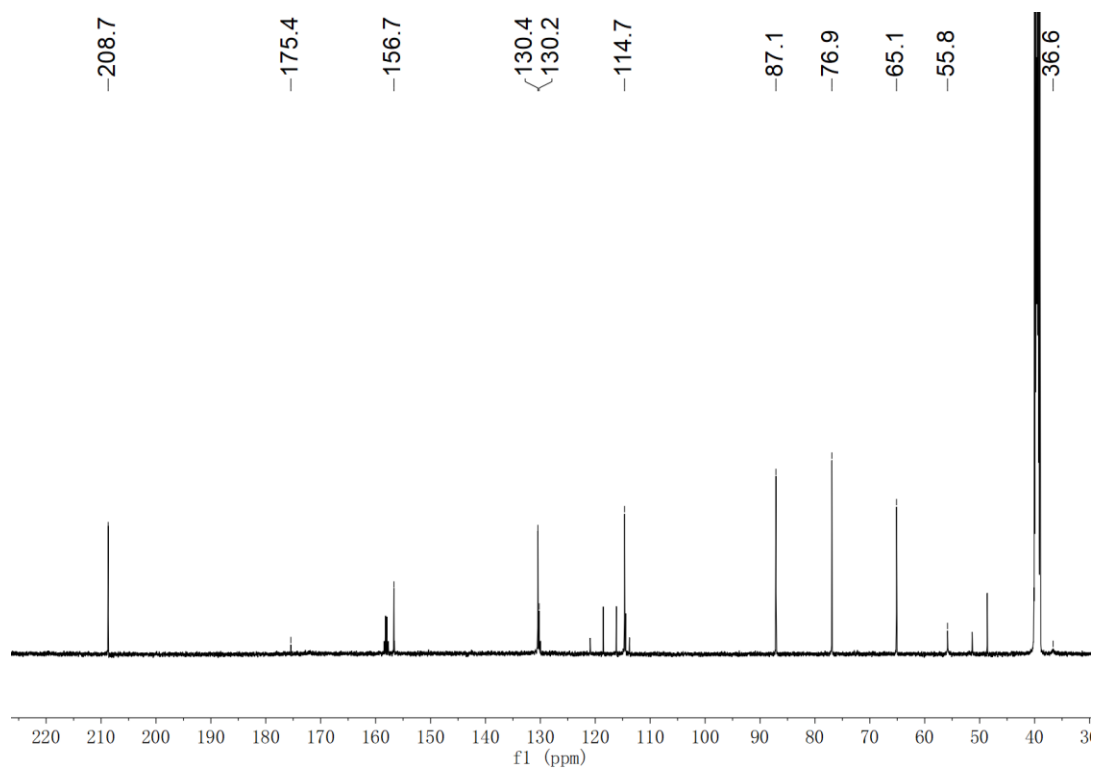
Supplementary Fig. 27 | Calculated free-energy difference between the gem-diol and aldehyde plus water. The aldehyde substrate hydration free energy is calculated using the isodesmic reaction developed by Julio Casado *et al*³⁰. The energies are calculated by B3LYP-D3(BJ)/def2-TZVP/SMD(H₂O)//B3LYP-D3(BJ)/def2-SVP/IEFPCM(H₂O) level of theory. The absolute hydration free energy of the aldehyde substrate is deduced from the experimental hydration equilibrium constant for acetaldehyde, which is measured to be 1.07 at room temperature^{31,32}:

$$\Delta G_{rxn} = \Delta G_{substate} - \Delta G_{CH_3CHO}$$

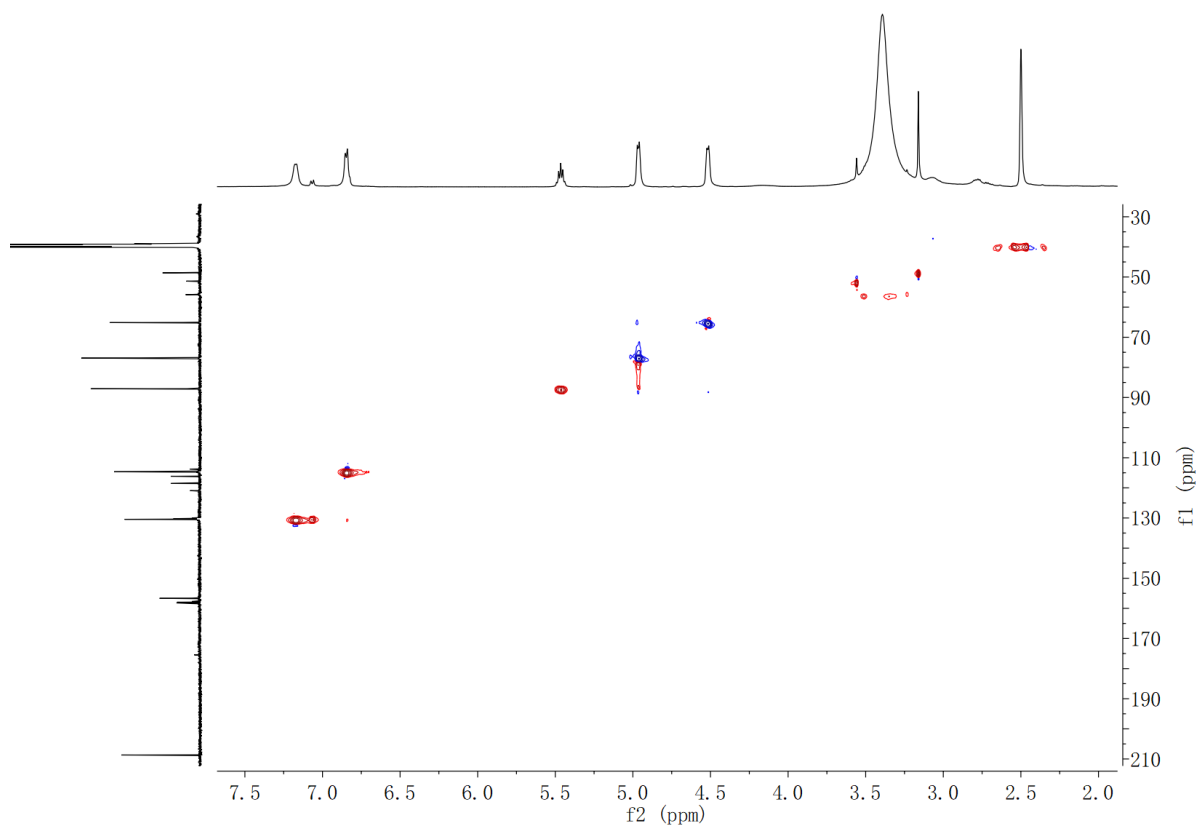
$$\Delta G_{CH_3CHO} = -RT \ln K_{hyd} = -0.03 \text{ kcal/mol}$$



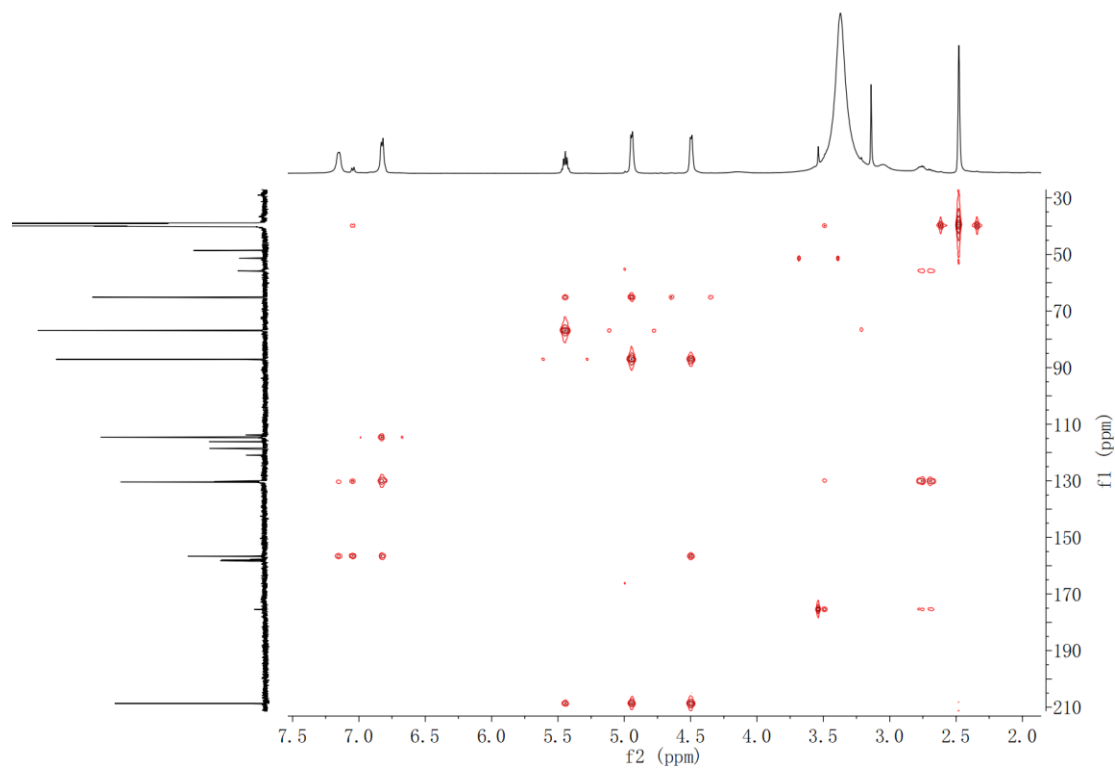
Supplementary Fig. 28. ¹H NMR spectrum of *O*-homoallenyl-L-Tyr (**1**) in DMSO-*d*₆



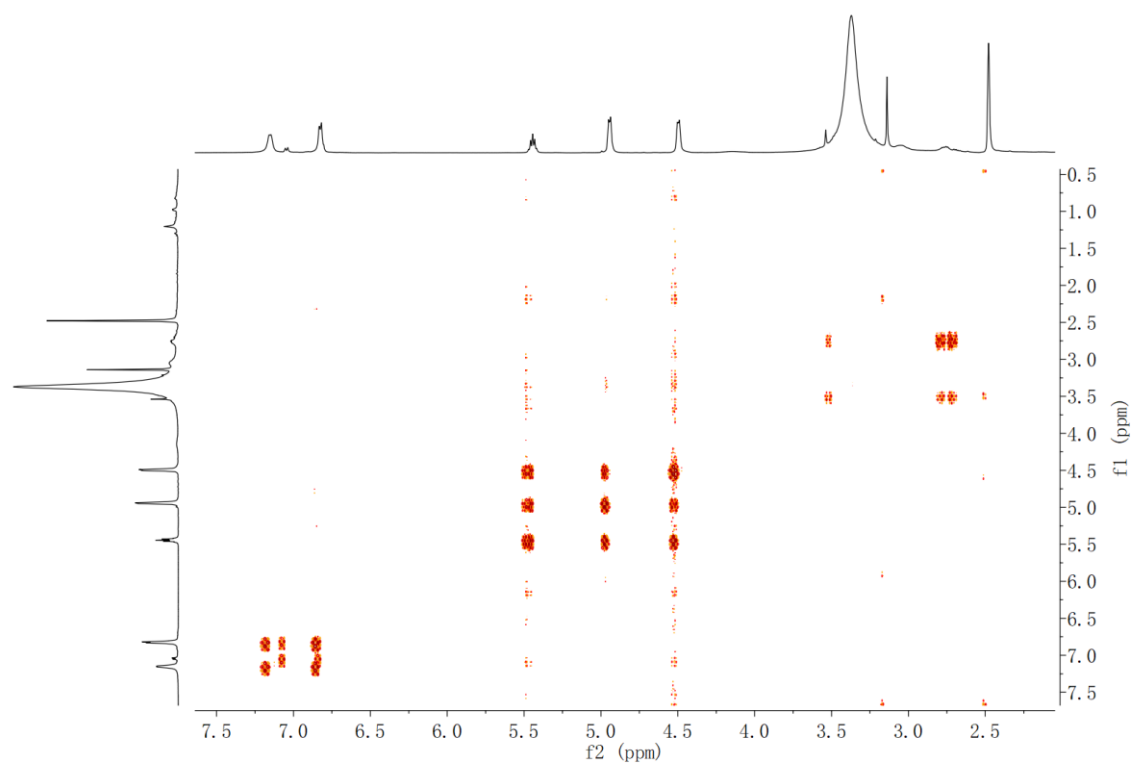
Supplementary Fig. 29. ¹³C NMR spectrum of *O*-homoallenyl-L-Tyr (**1**) in DMSO-*d*₆



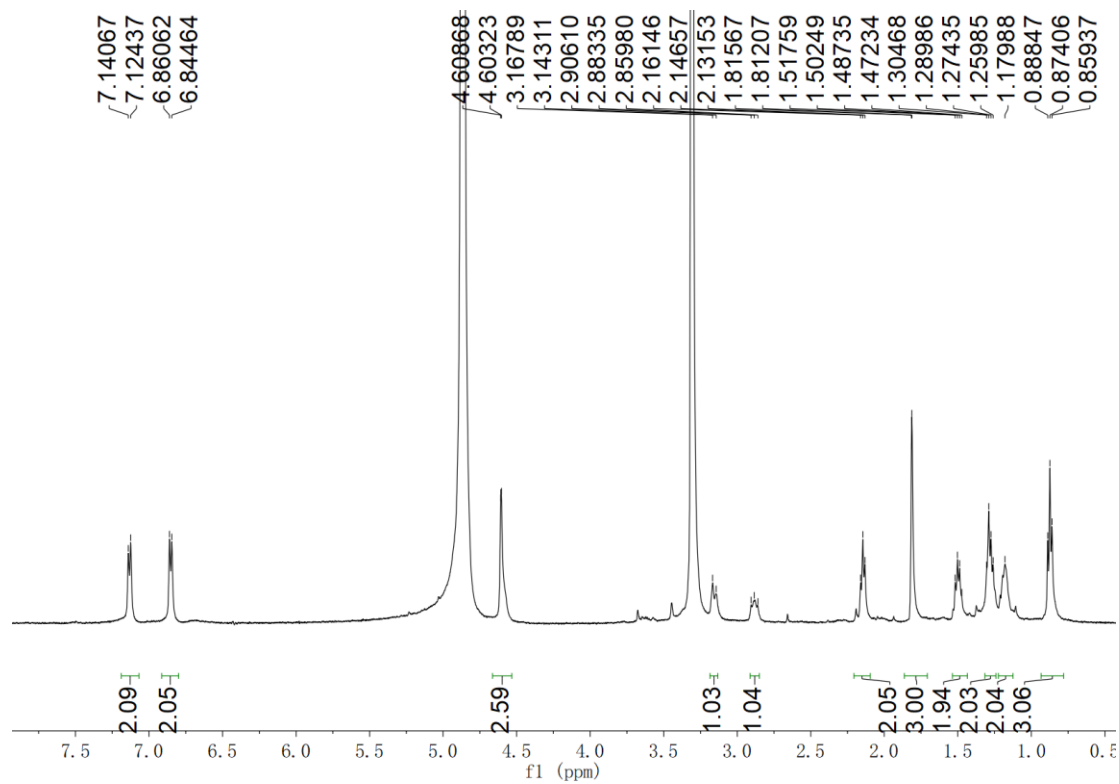
Supplementary Fig. 30. HSQC spectrum of *O*-homoallenyl- L-Tyr (**1**) in DMSO-*d*₆



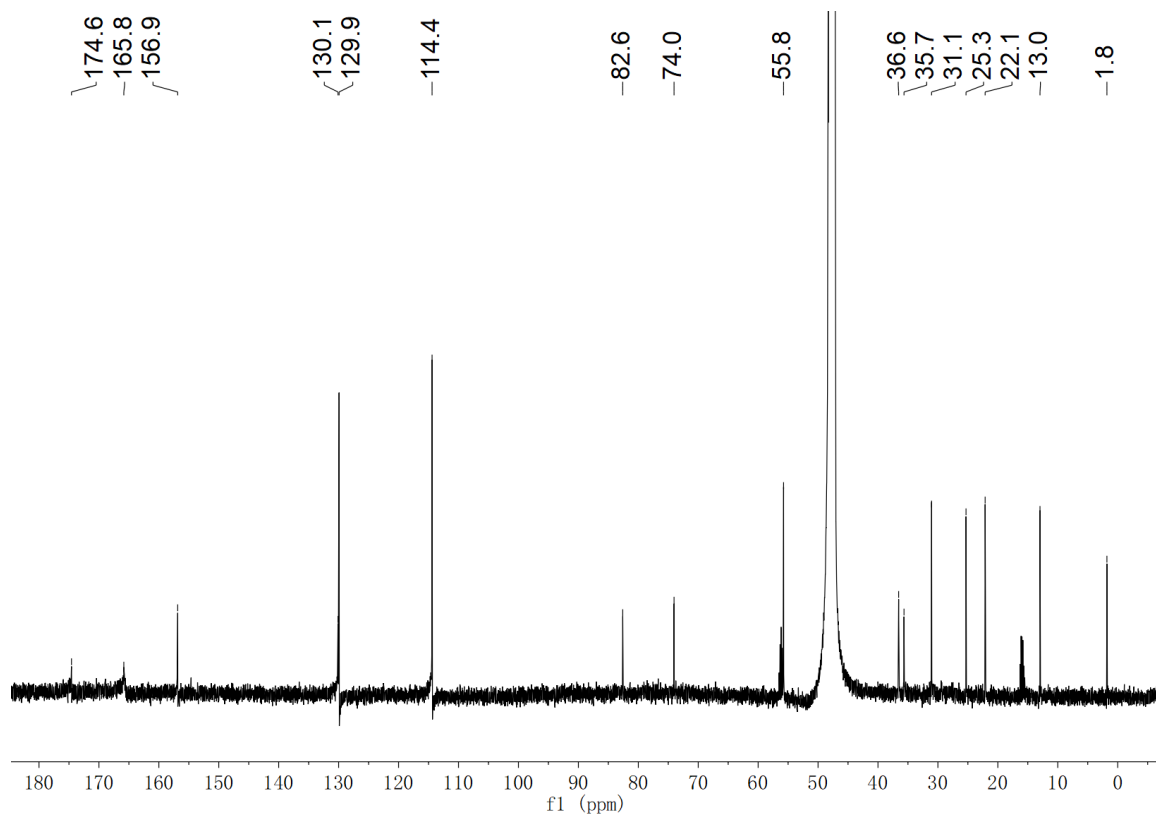
Supplementary Fig. 31. HMBC spectrum of *O*-homoallenyl- L-Tyr (**1**) in DMSO-*d*₆



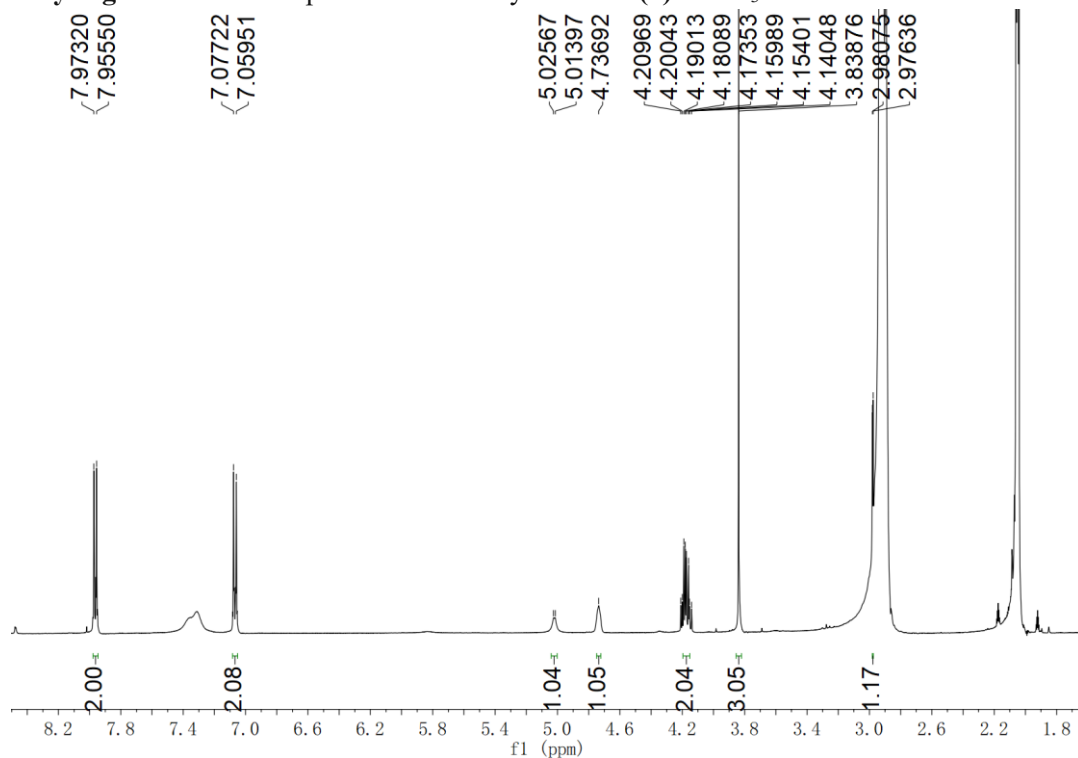
Supplementary Fig. 32. ^1H - ^1H COSY spectrum of *O*-homoallenyl- L -Tyr (**1**) in DMSO- d_6



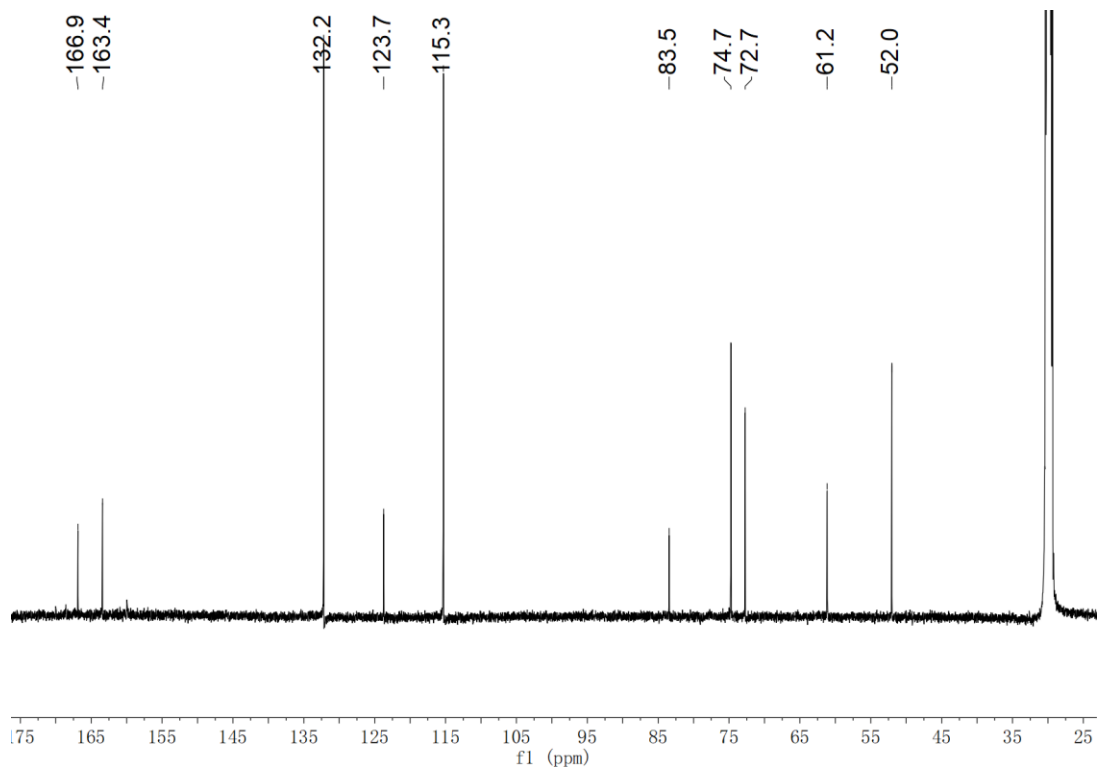
Supplementary Fig. 33. ^1H NMR spectrum of Sinuxylamide B (**2**) in CD_3OD



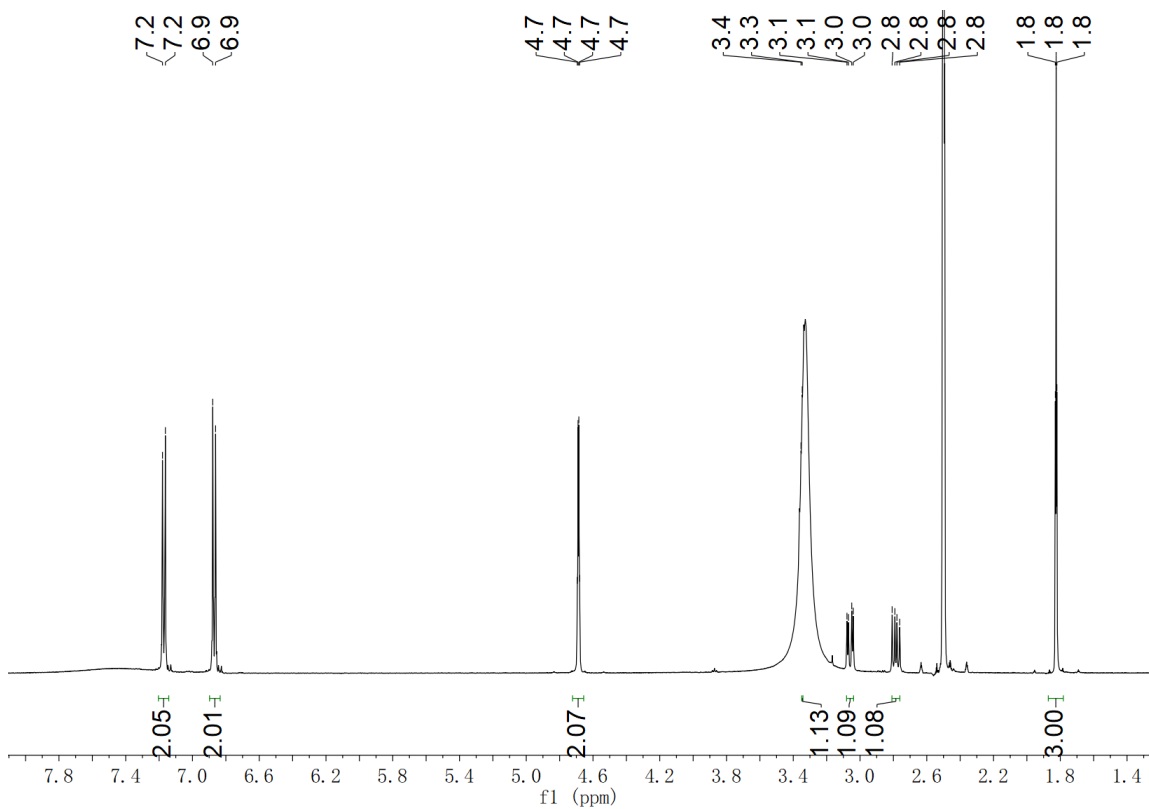
Supplementary Fig. 34. ¹³C NMR spectrum of Sinuxylamide B (**2**) in CD₃OD



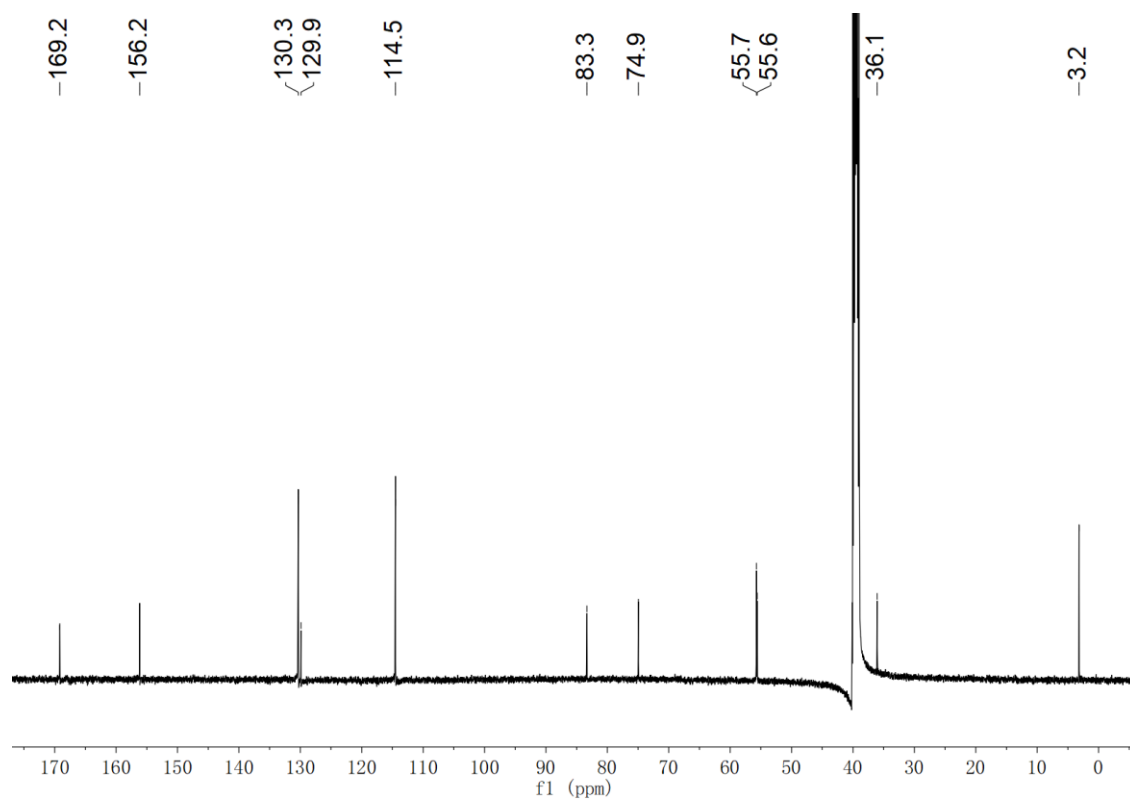
Supplementary Fig. 35. ¹H NMR spectrum of penipratynolene (**5**) in acetone-*d*₆



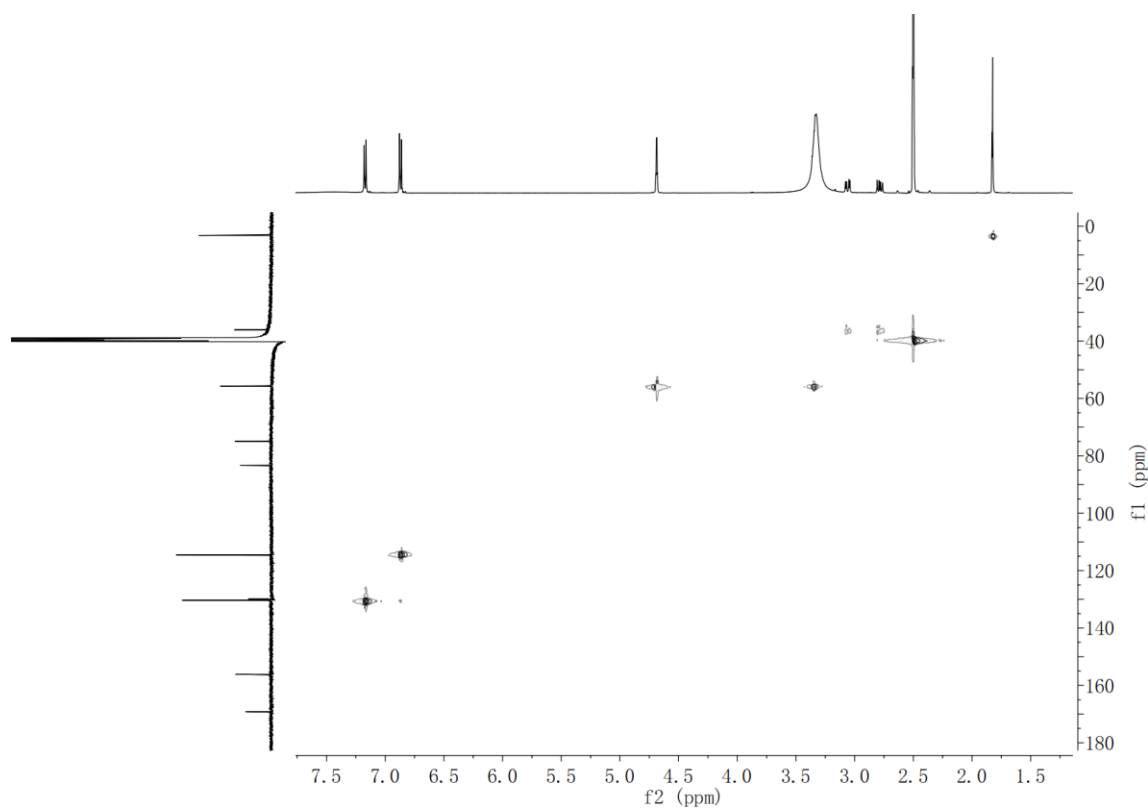
Supplementary Fig. 36. ^{13}C NMR spectrum of penipratynolene (**5**) in acetone- d_6



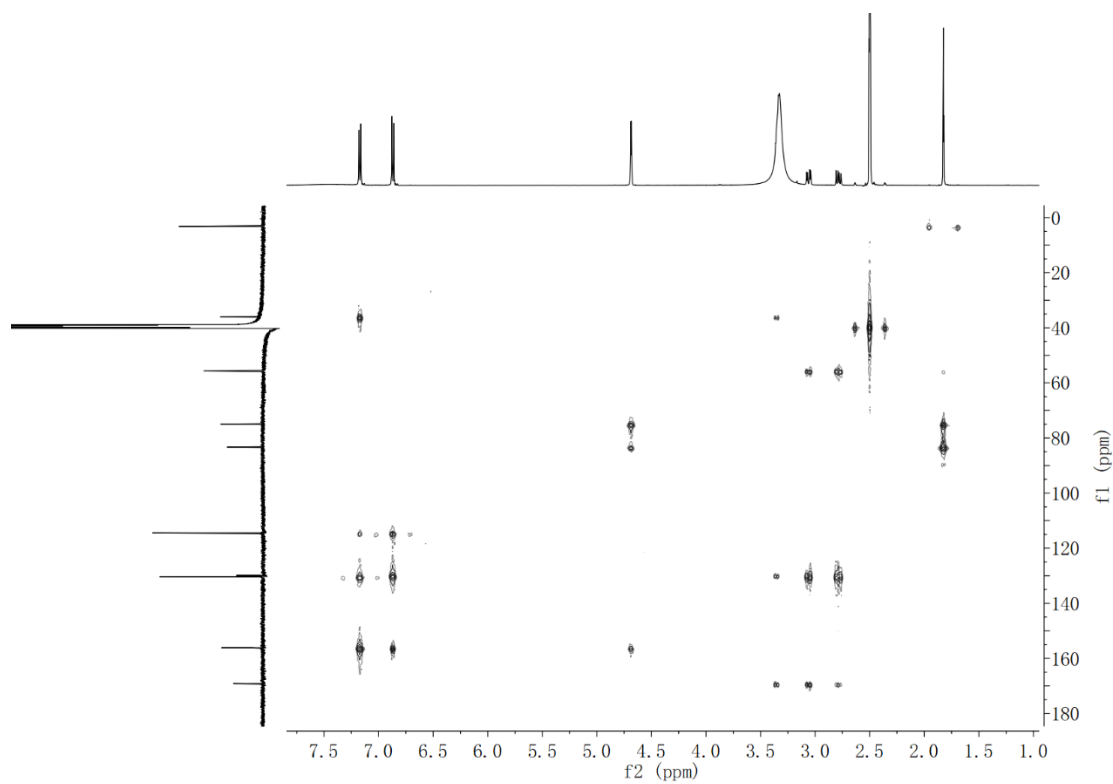
Supplementary Fig. 37. ^1H NMR spectrum of *O*-2-butyln-1-yl L-Tyr (**6**) in DMSO- d_6



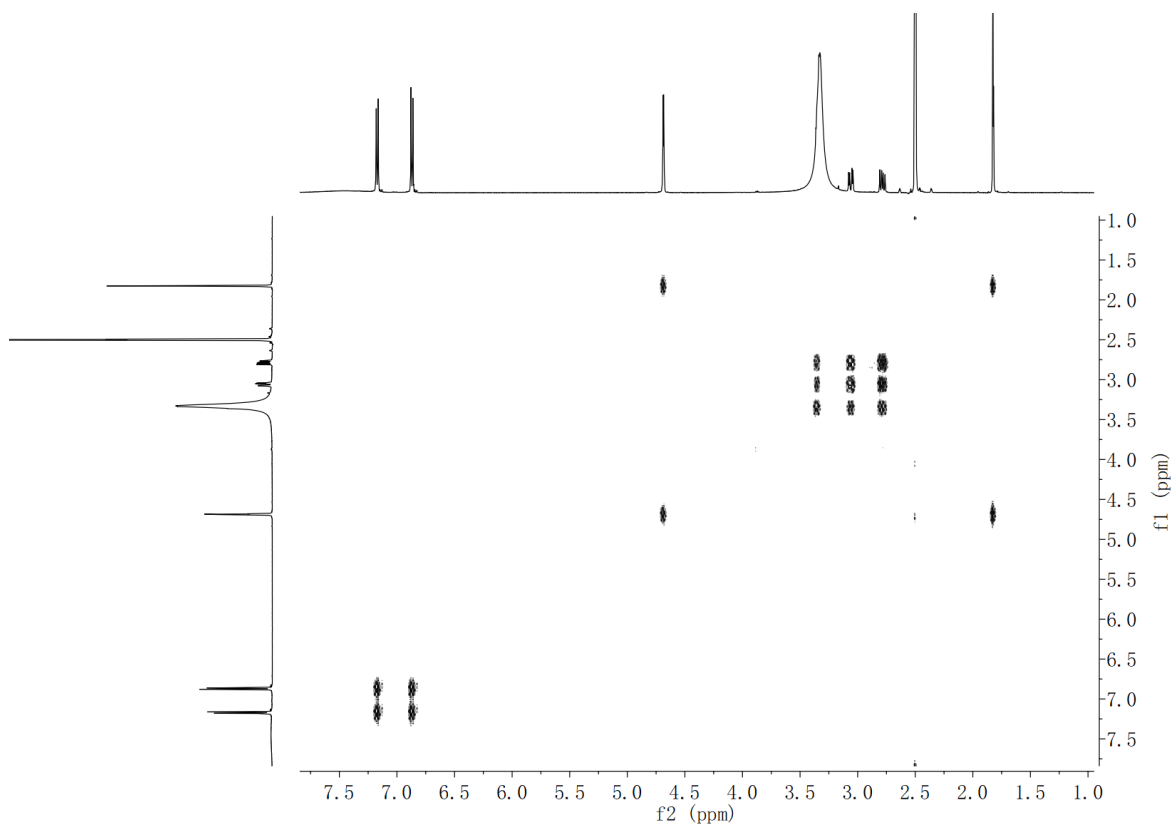
Supplementary Fig. 38. ^{13}C NMR spectrum of *O*-2-butyn-1-yl L-Tyr (**6**) in $\text{DMSO-}d_6$



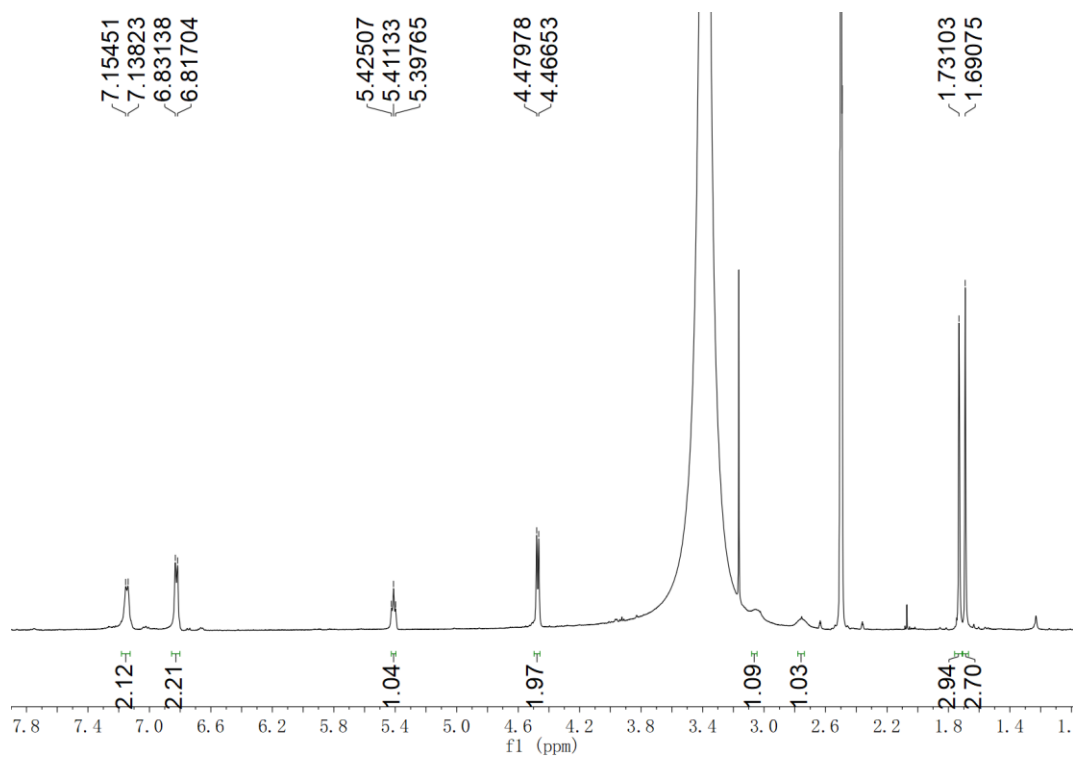
Supplementary Fig. 39. HSQC NMR spectrum of *O*-2-butyn-1-yl L-Tyr (**6**) in $\text{DMSO-}d_6$



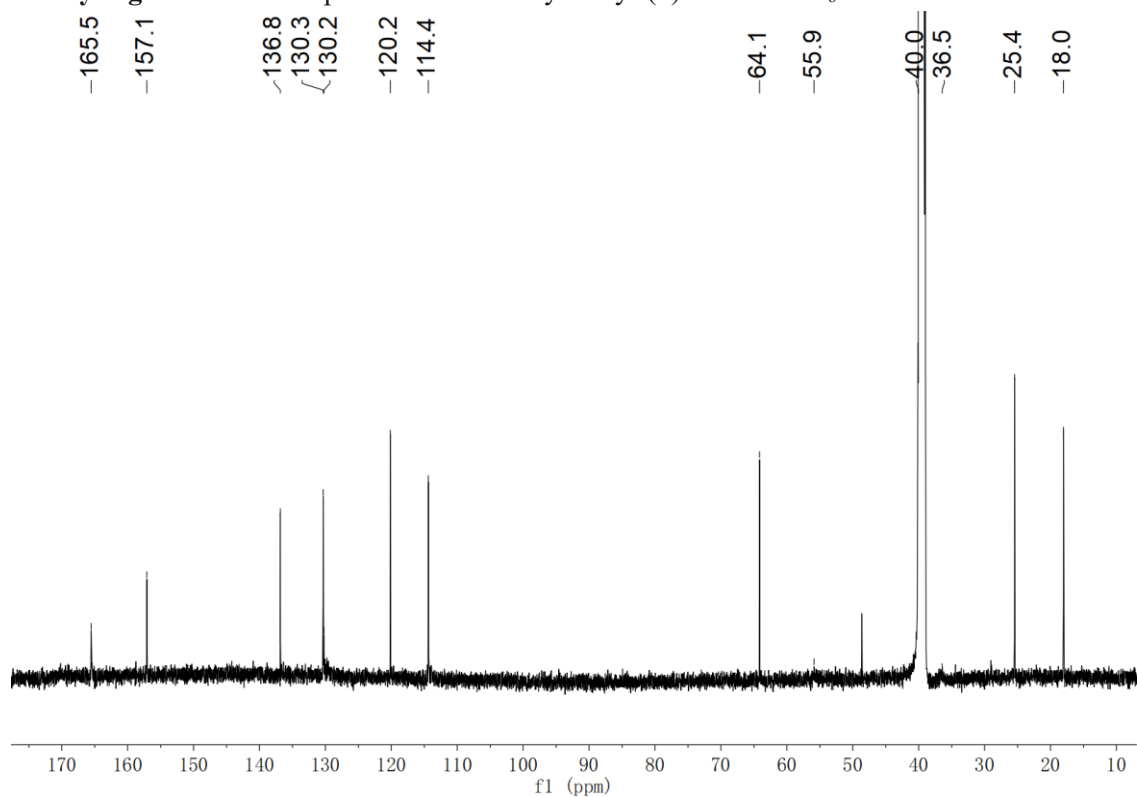
Supplementary Fig. 40. HMBC NMR spectrum of *O*-2-butyn-1-yl L-Tyr (**6**) in DMSO-*d*₆



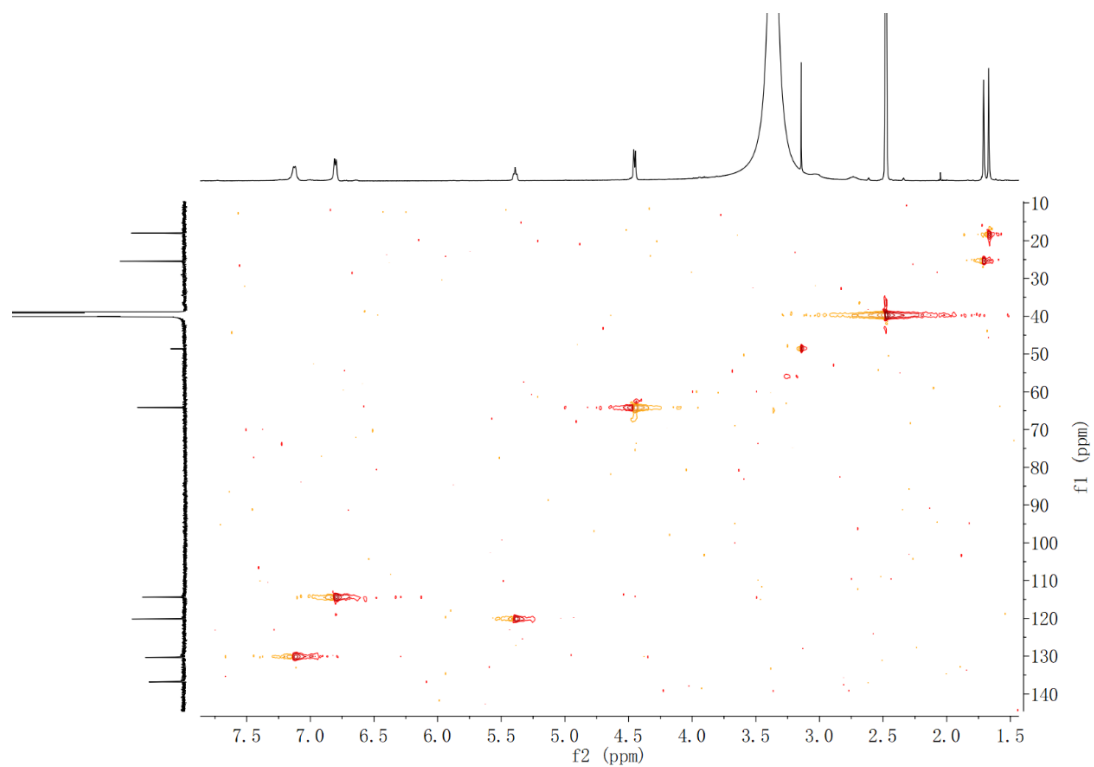
Supplementary Fig. 41. ¹H-¹H COSY NMR spectrum of *O*-2-butyn-1-yl L-Tyr (**6**) in DMSO-*d*₆



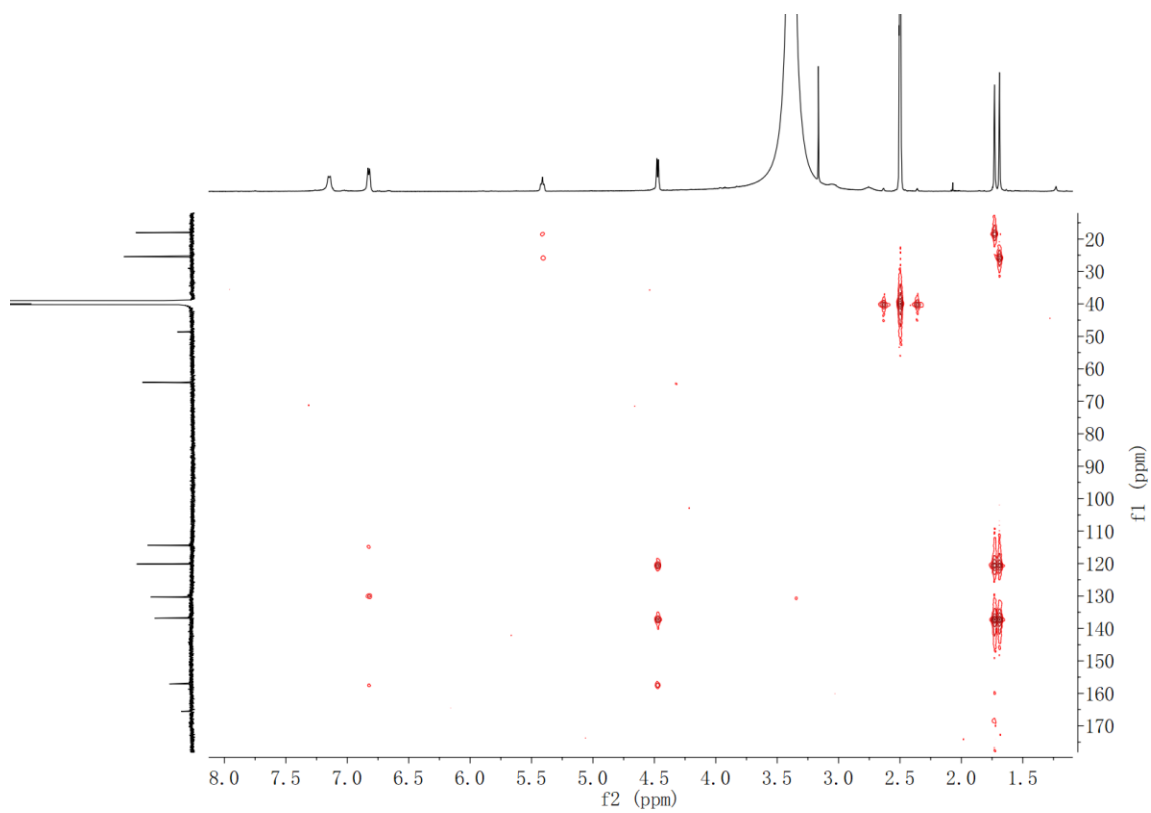
Supplementary Fig. 42. ¹H NMR spectrum of *O*-Prenyl L-Tyr (**7**) in DMSO-*d*₆



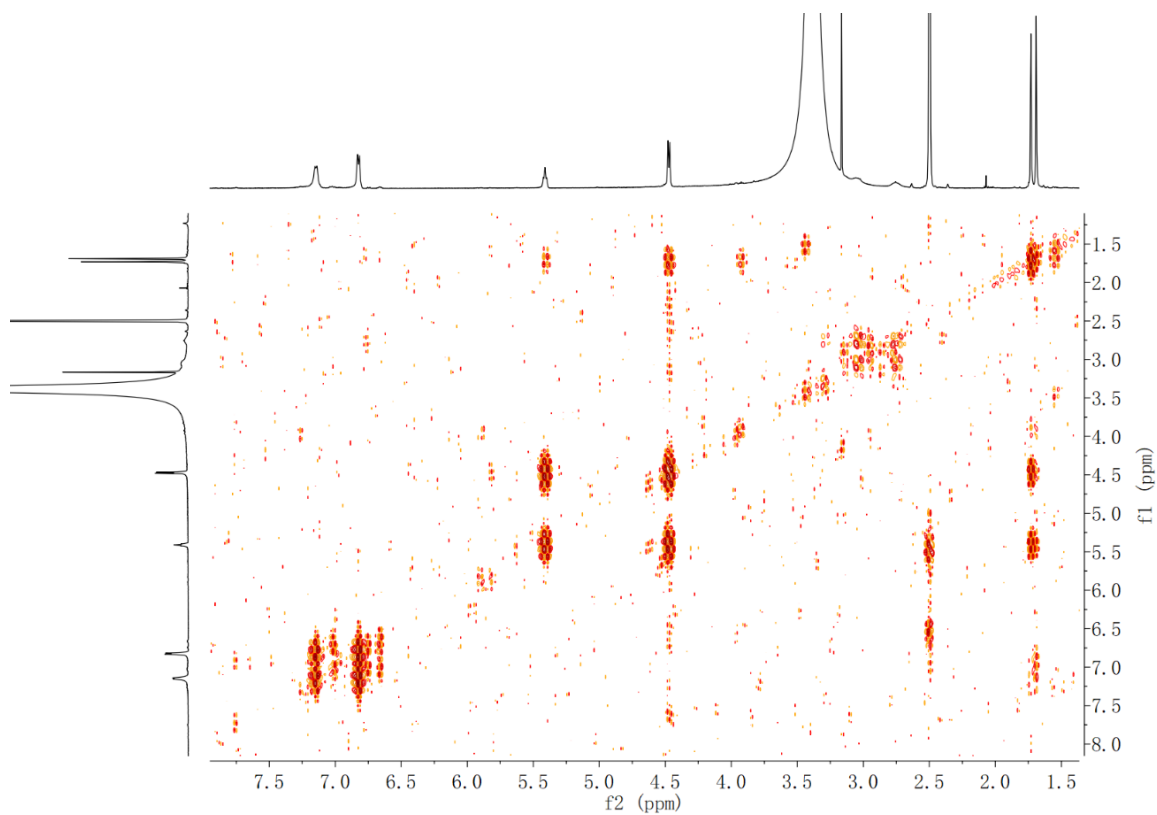
Supplementary Fig. 43. ¹³C NMR spectrum of *O*-Prenyl L-Tyr (**7**) in DMSO-*d*₆



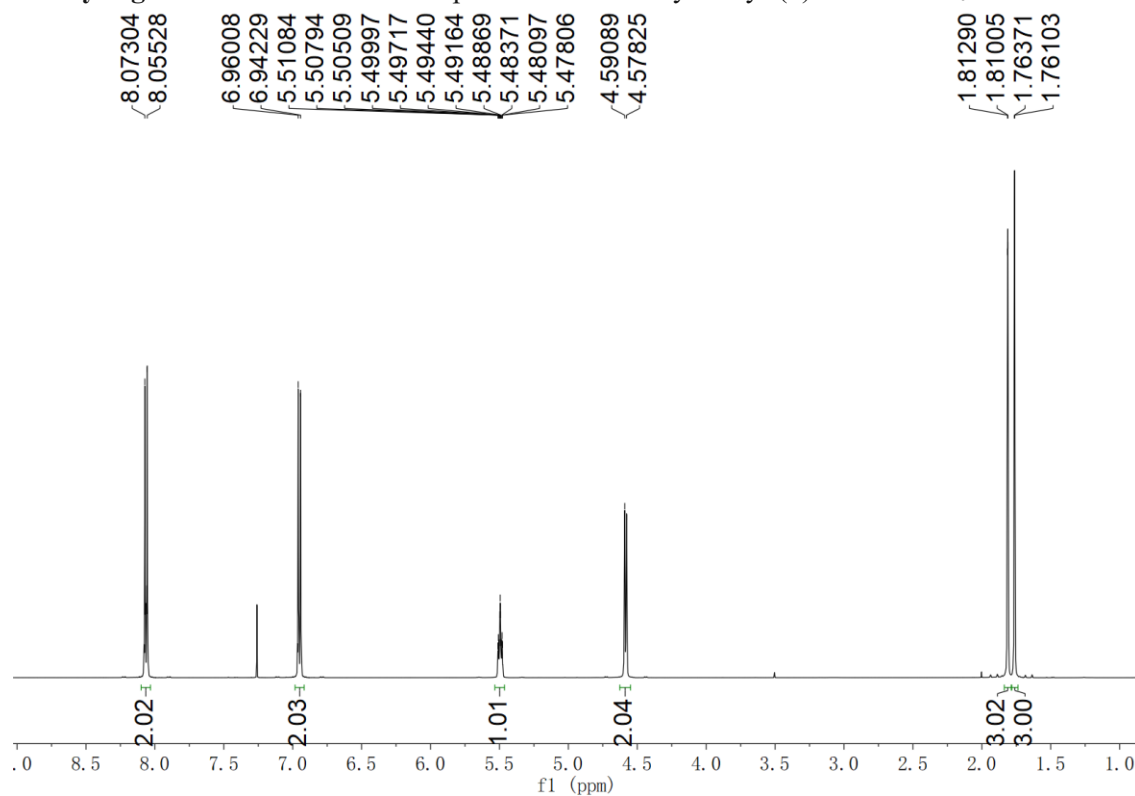
Supplementary Fig. 44. HSQC NMR spectrum of *O*-Prenyl L-Tyr (**7**) in DMSO-*d*₆



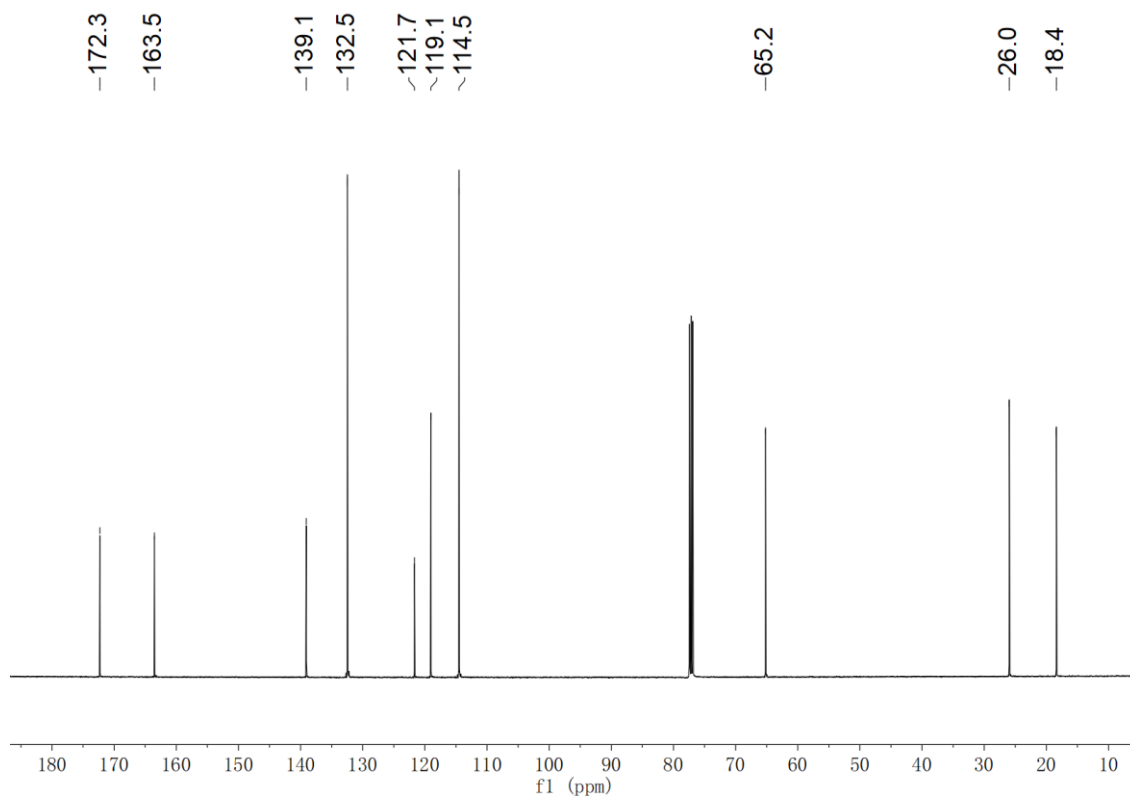
Supplementary Fig. 45. HMBC NMR spectrum of *O*-Prenyl L-Tyr (**7**) in DMSO-*d*₆



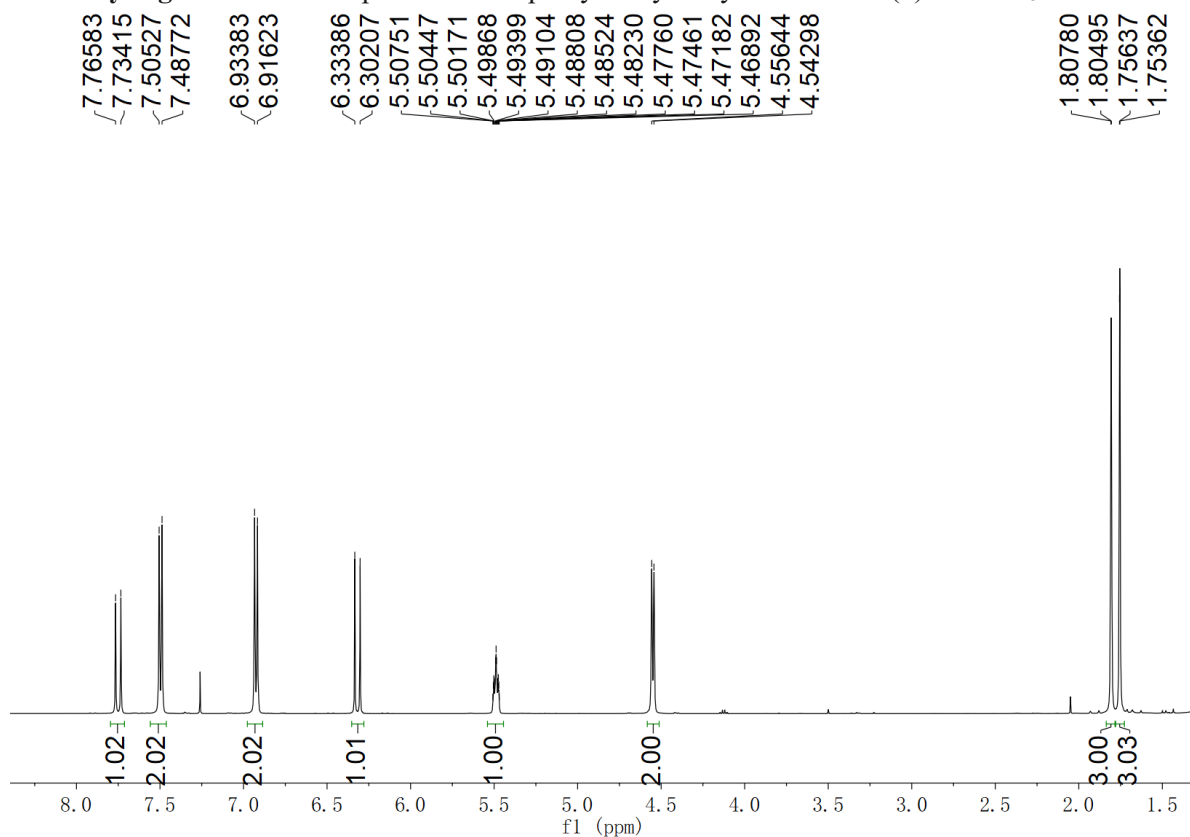
Supplementary Fig. 46. ^1H - ^1H COSY NMR spectrum of *O*-Prenyl L-Tyr (**7**) in $\text{DMSO-}d_6$



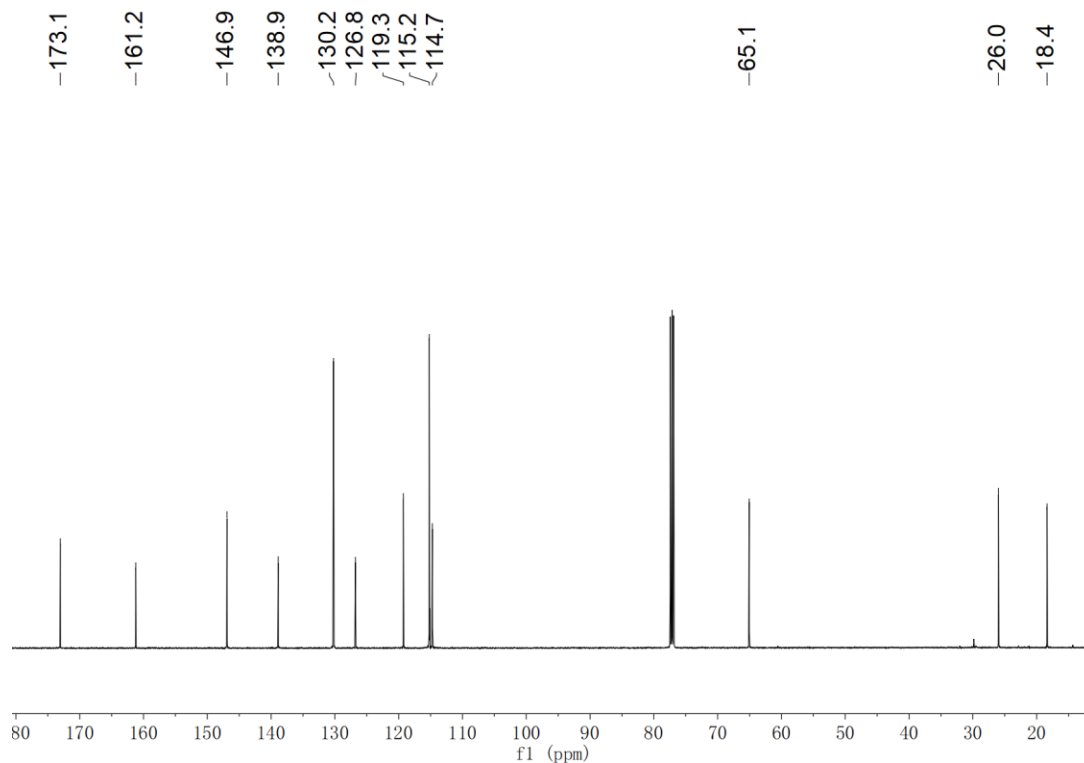
Supplementary Fig. 47. ^1H NMR spectrum of *O*-prenyl-4-hydroxybenzoic acid (**8**) in CDCl_3



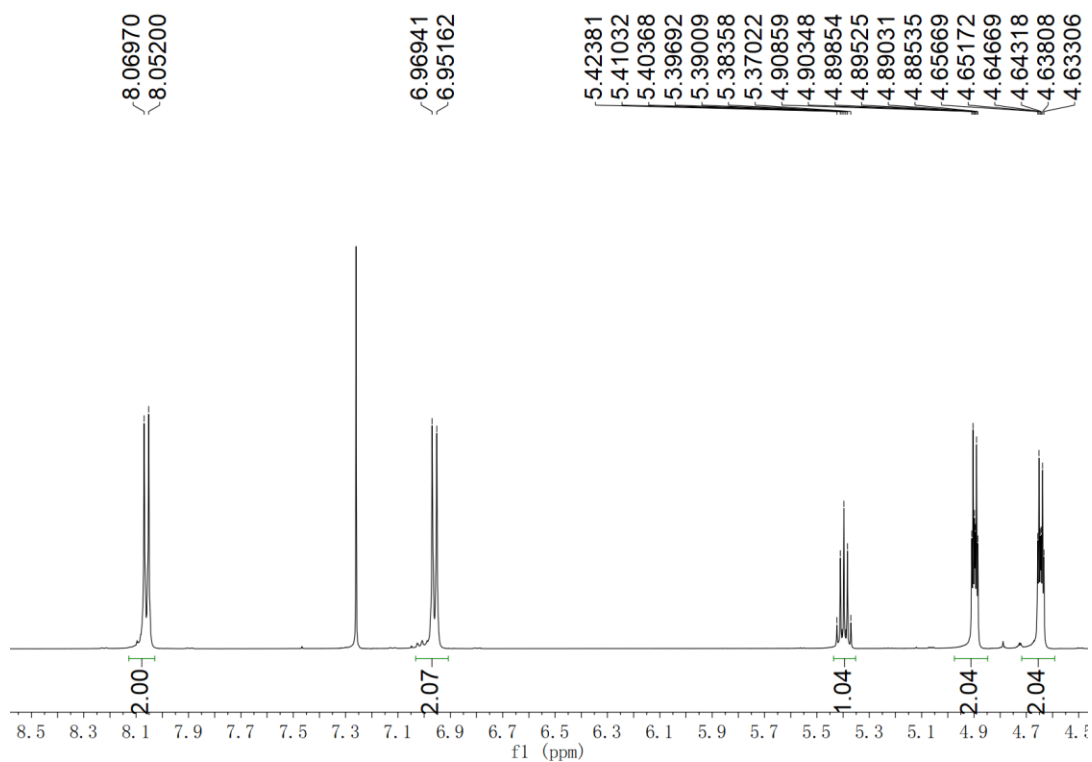
Supplementary Fig. 48. ^{13}C NMR spectrum of *O*-prenyl-4-hydroxybenzoic acid (**8**) in CDCl_3



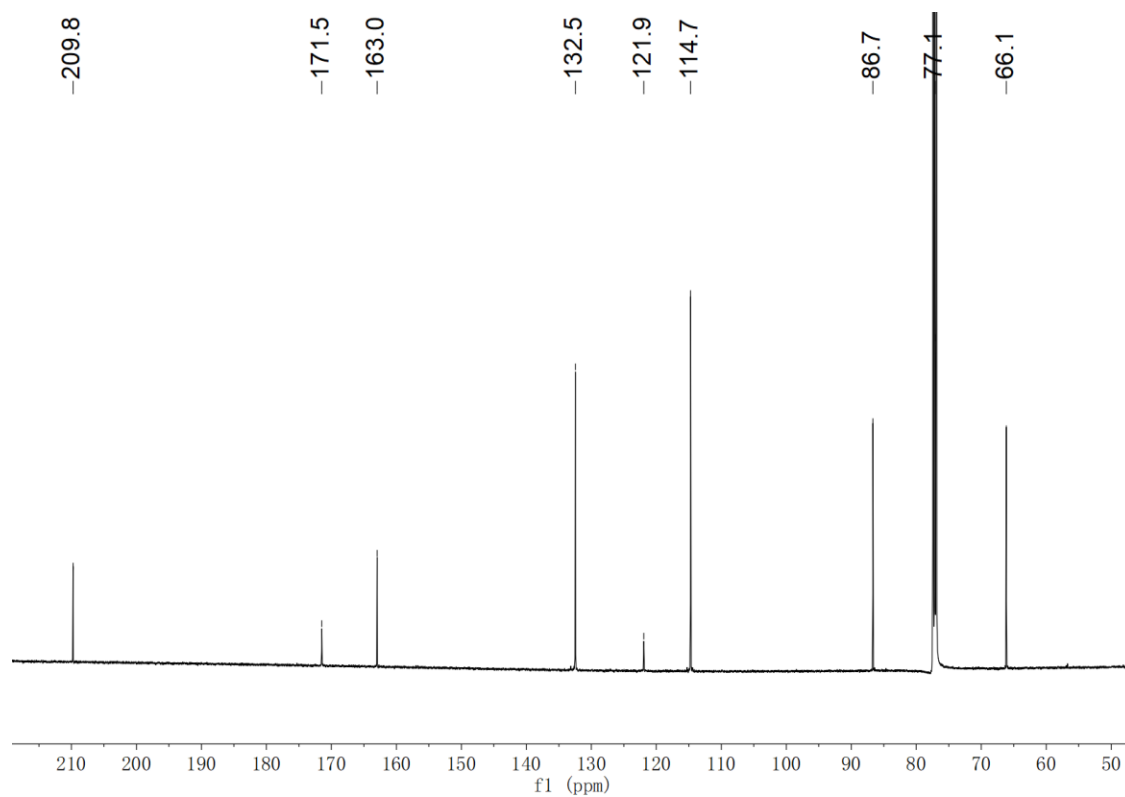
Supplementary Fig. 49. ^1H NMR spectrum of *O*-prenyl *p*-coumaric acid (**9**) in CDCl_3



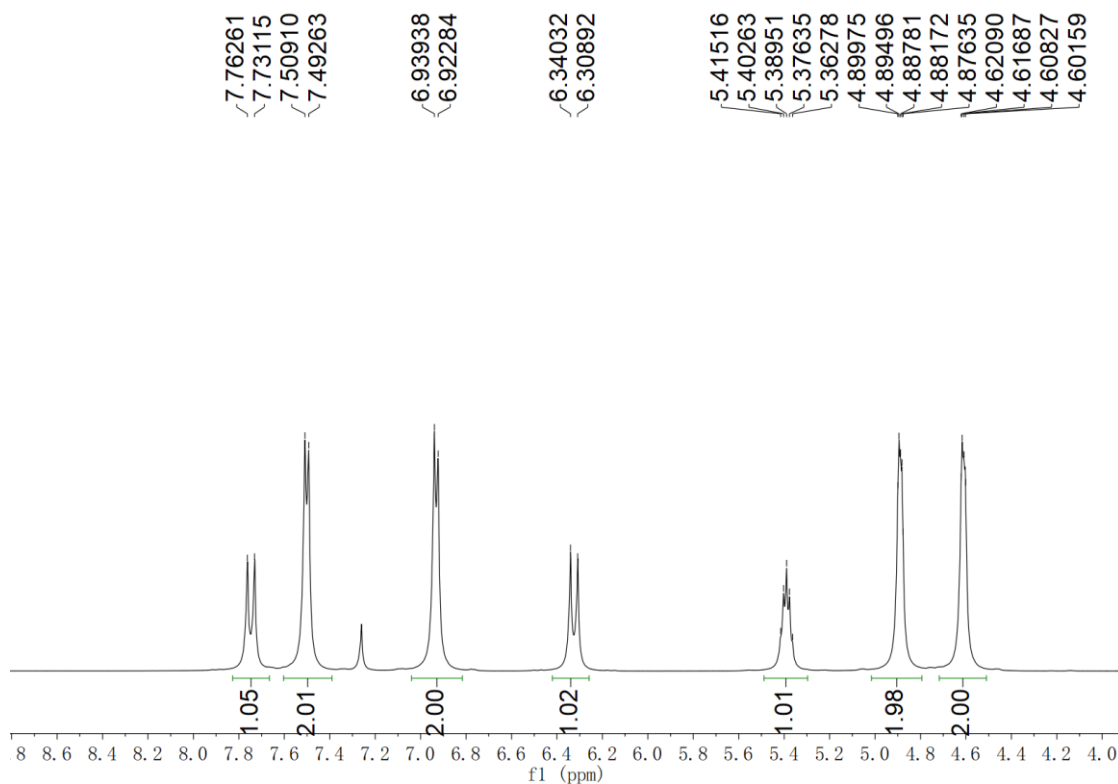
Supplementary Fig. 50. ¹³C NMR spectrum of *O*-prenyl *p*-coumaric acid (**9**) in CDCl₃



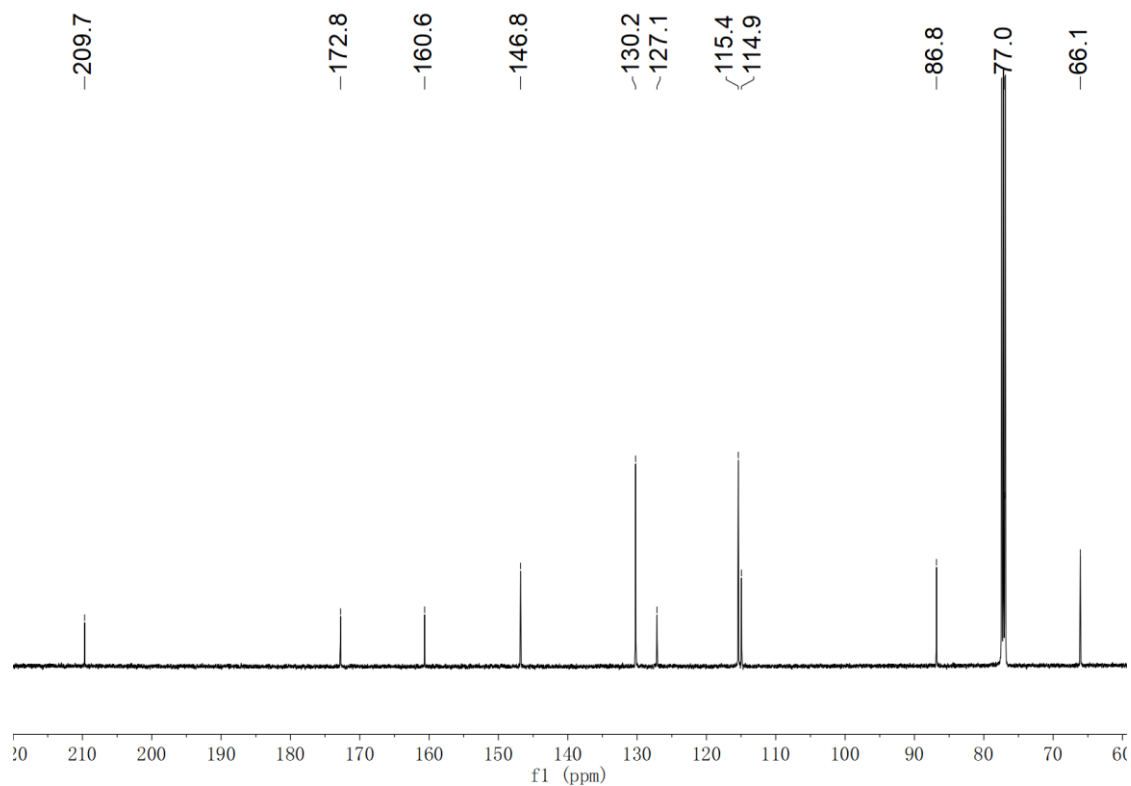
Supplementary Fig. 51. ¹H NMR spectrum of *O*-homoallenyl 4-hydroxybenzoic acid (**10**) in CDCl₃



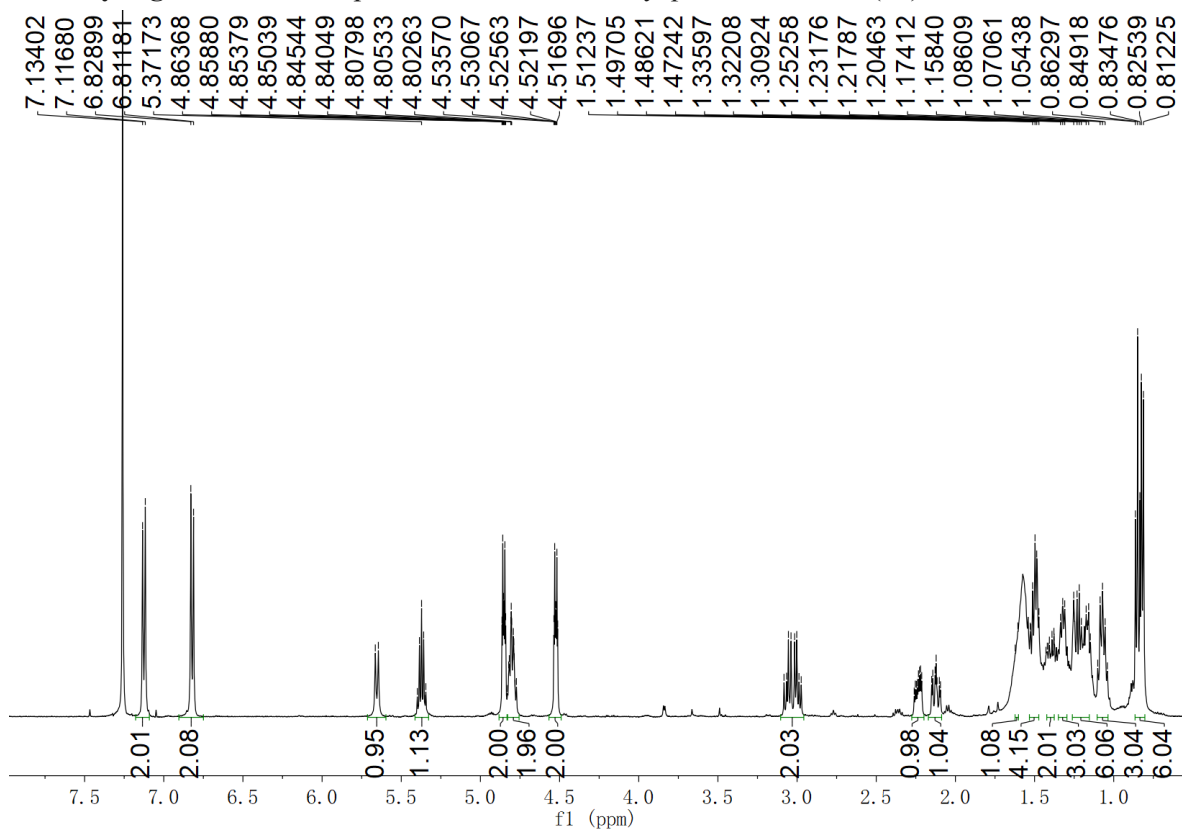
Supplementary Fig. 52. ^{13}C NMR spectrum of *O*-homoallenyl 4-hydroxybenzoic acid (**10**) in CDCl_3



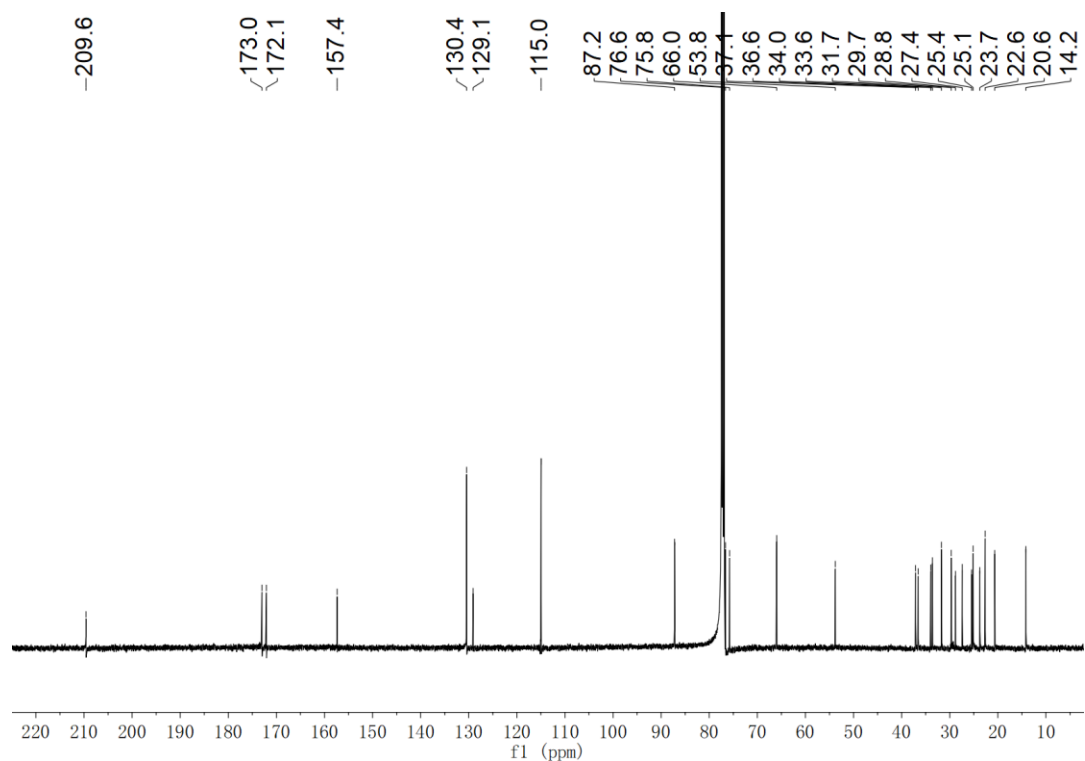
Supplementary Fig. 53. ^1H NMR spectrum of *O*-homoallenyl *p*-coumaric acid (**11**) in CDCl_3



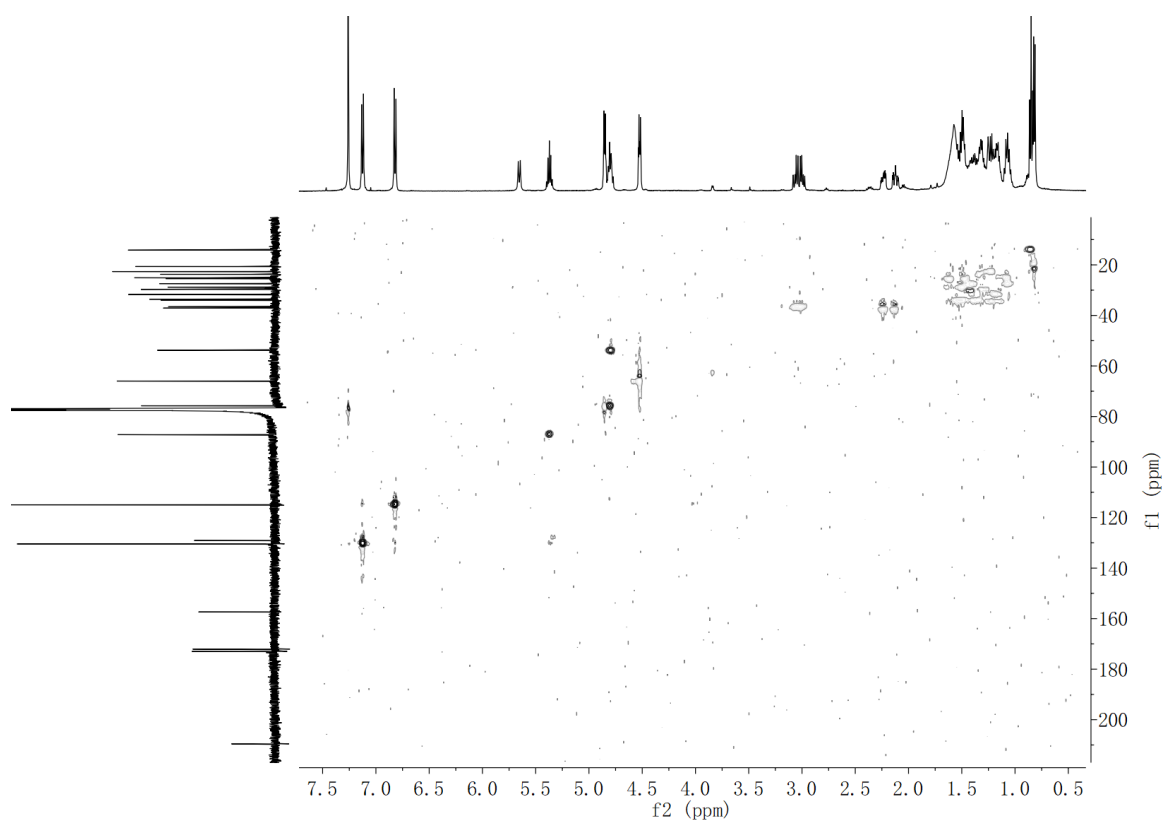
Supplementary Fig. 54. ¹³C NMR spectrum of *O*-homoallenyl *p*-coumaric acid (**11**) in CDCl₃



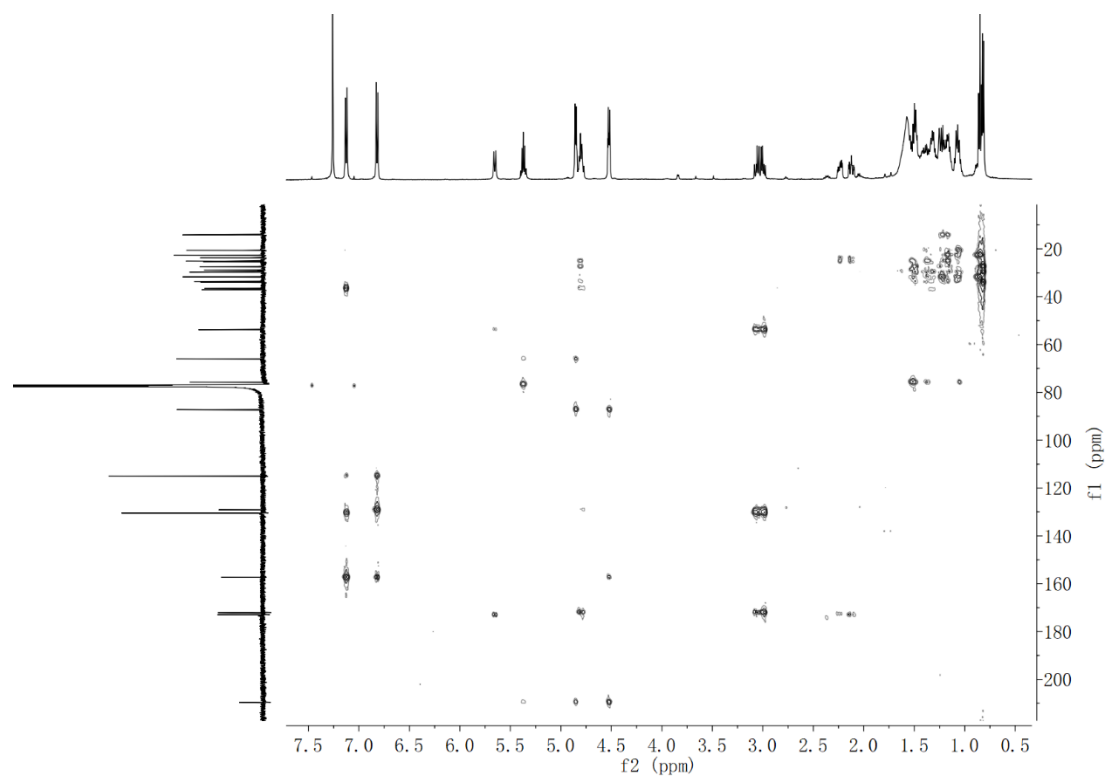
Supplementary Fig. 55. ¹H NMR spectrum of compound **13** in CDCl₃



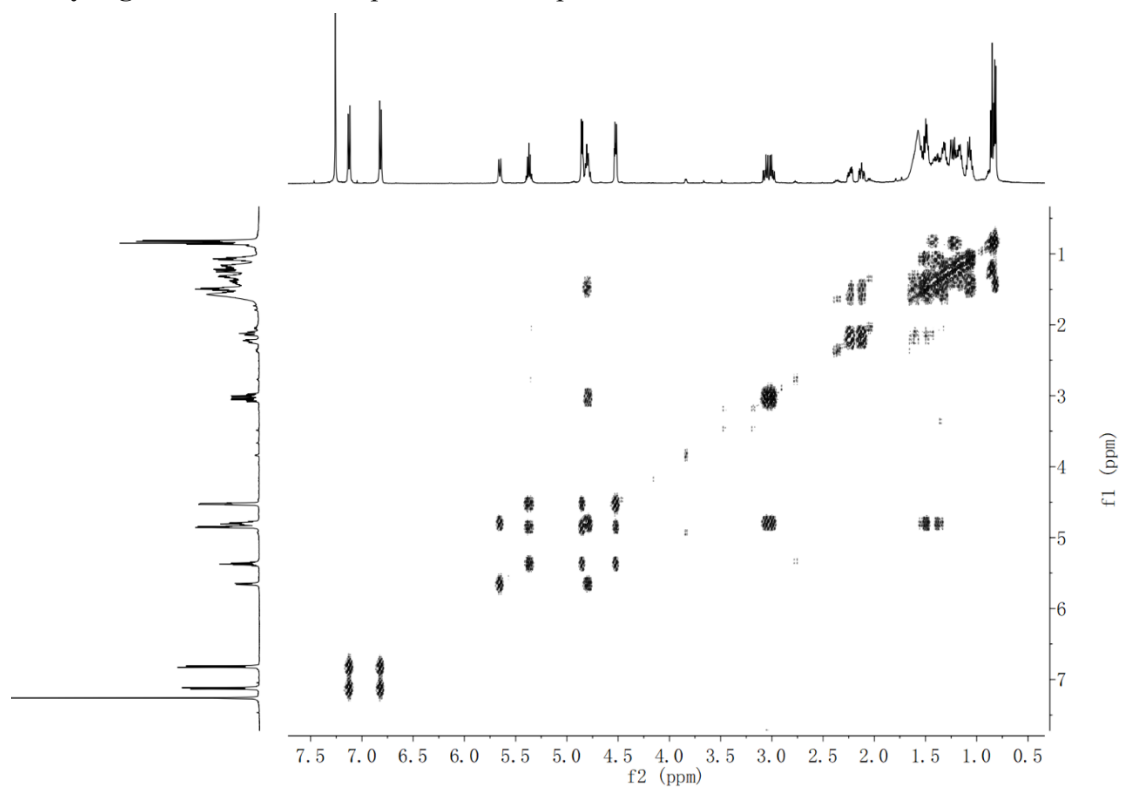
Supplementary Fig. 56. ^{13}C NMR spectrum of compound **13** in CDCl_3



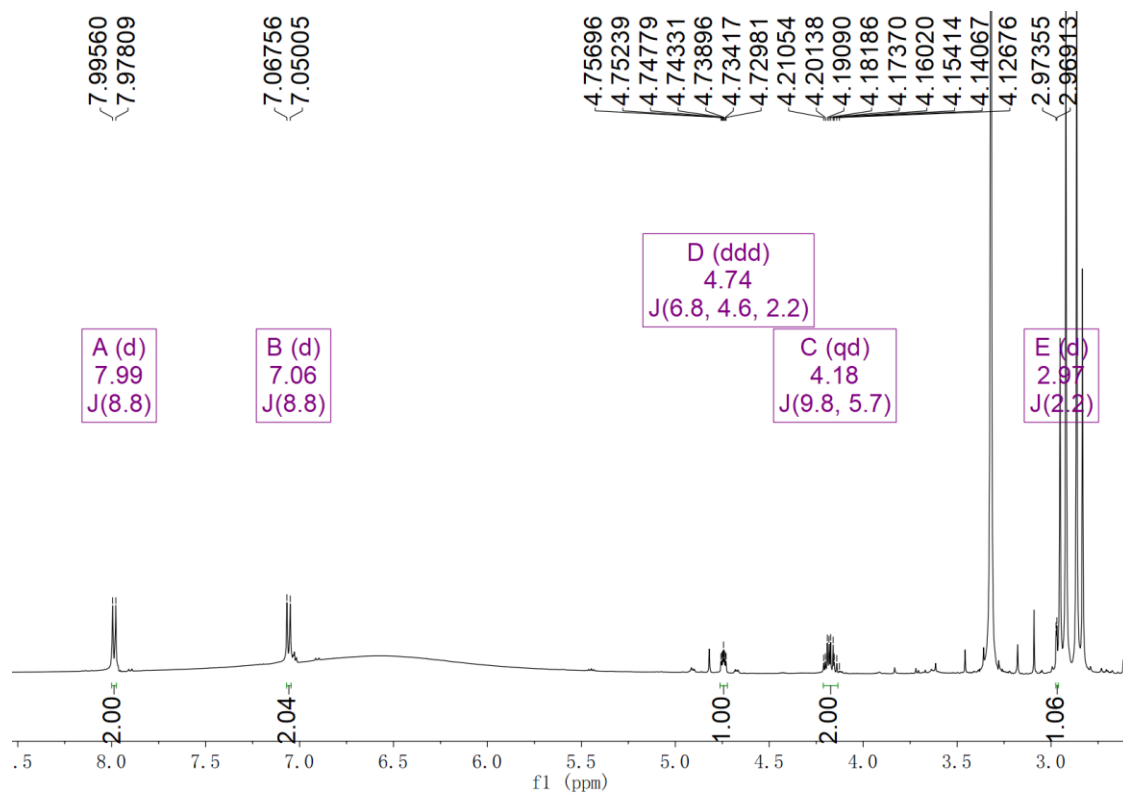
Supplementary Fig. 57. HSQC NMR spectrum of compound **13** in CDCl_3



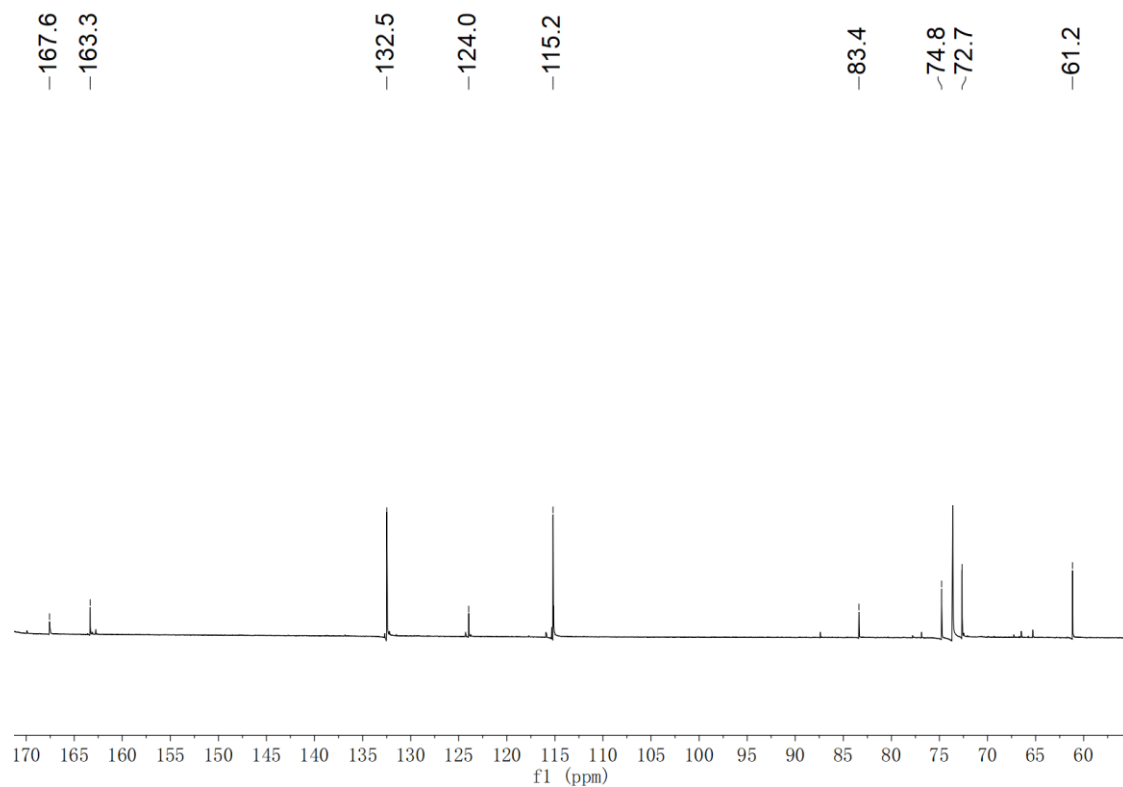
Supplementary Fig. 58. HMBC NMR spectrum of compound **13** in CDCl_3



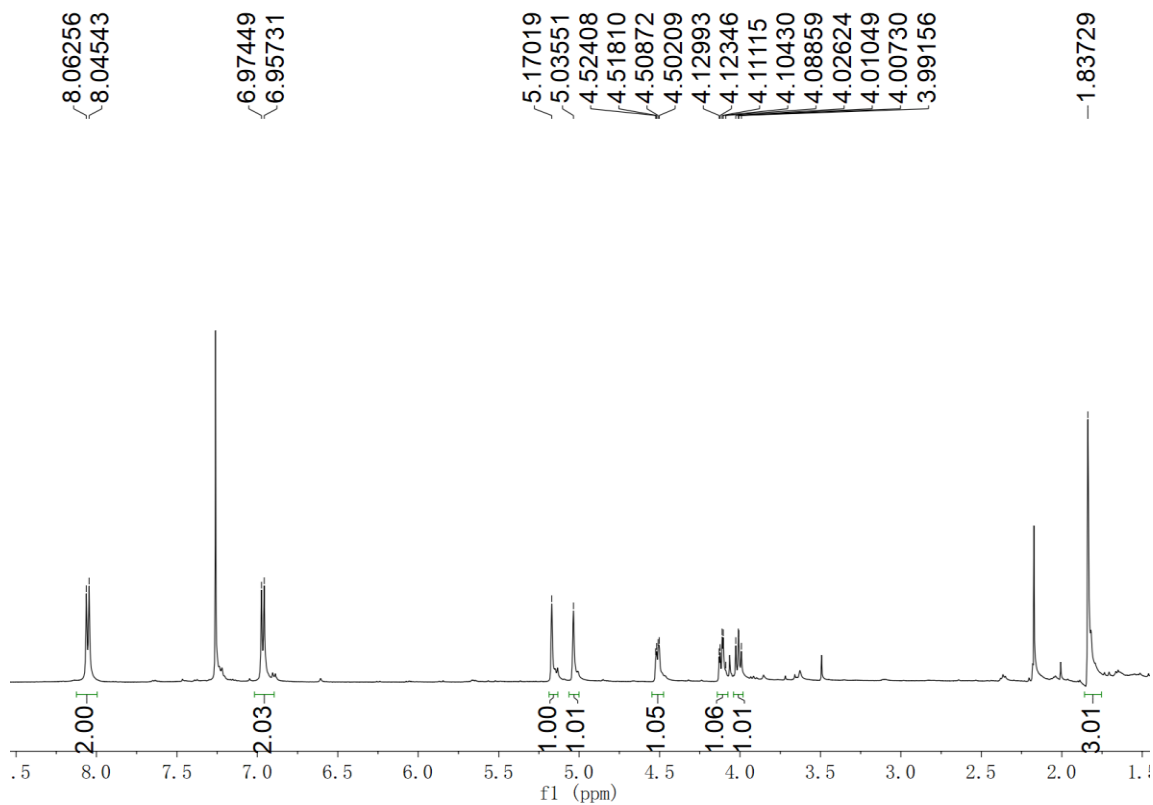
Supplementary Fig. 59. ^1H - ^1H COSY NMR spectrum of compound **13** in CDCl_3



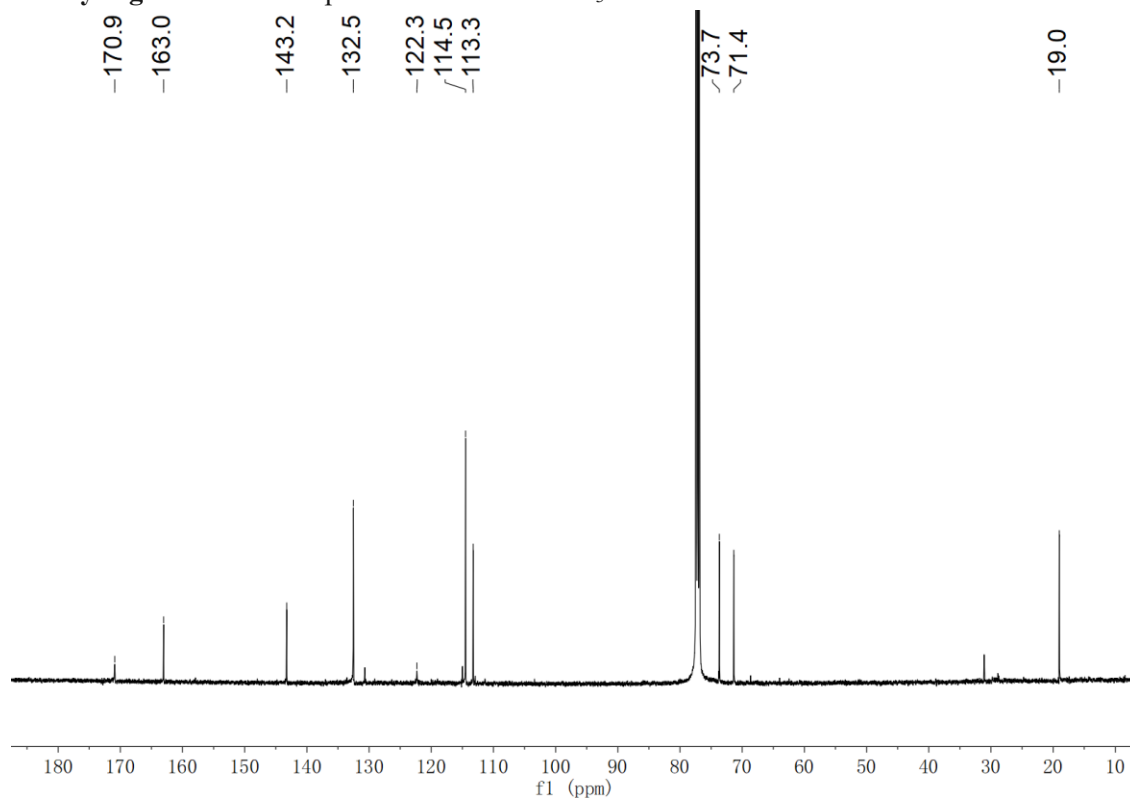
Supplementary Fig. 60. ^1H NMR spectrum of demethyl-5 (**14**) in acetone- d_6



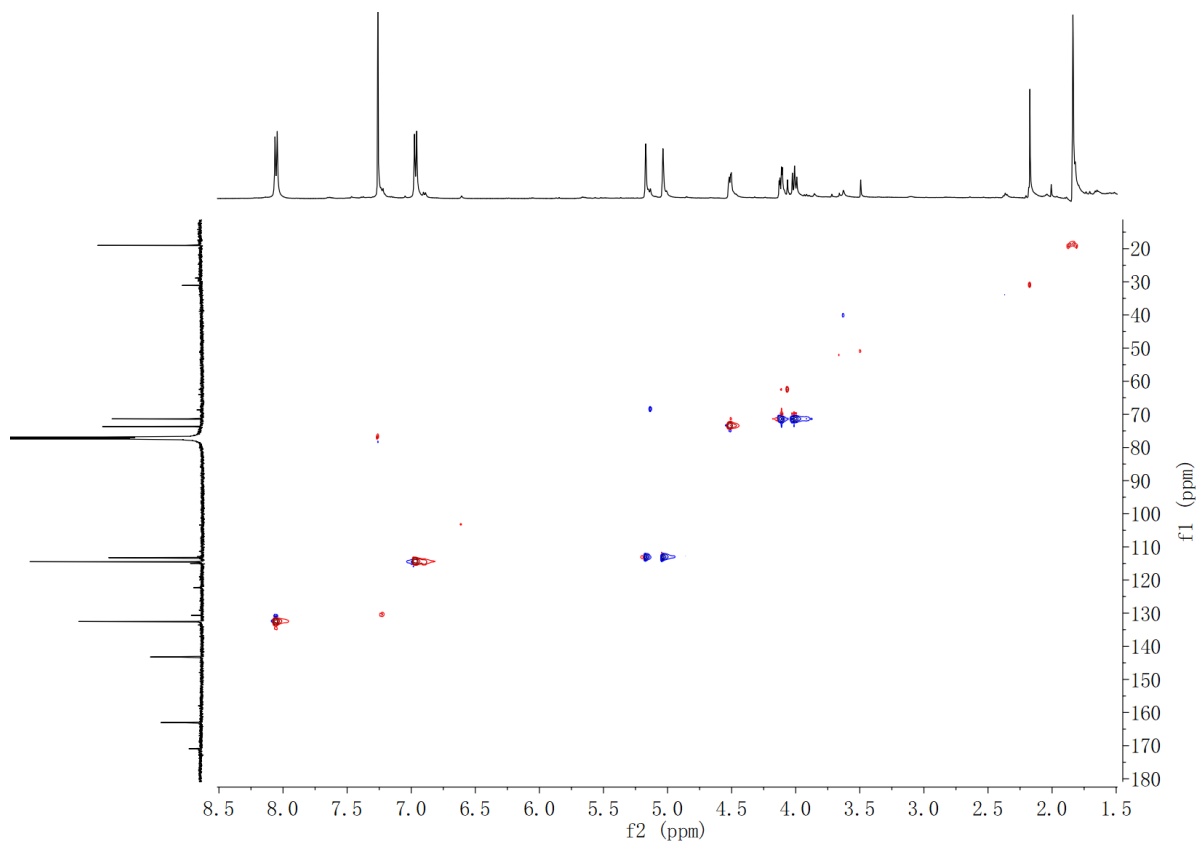
Supplementary Fig. 61. ^{13}C NMR spectrum of demethyl-5 (**14**) in acetone- d_6



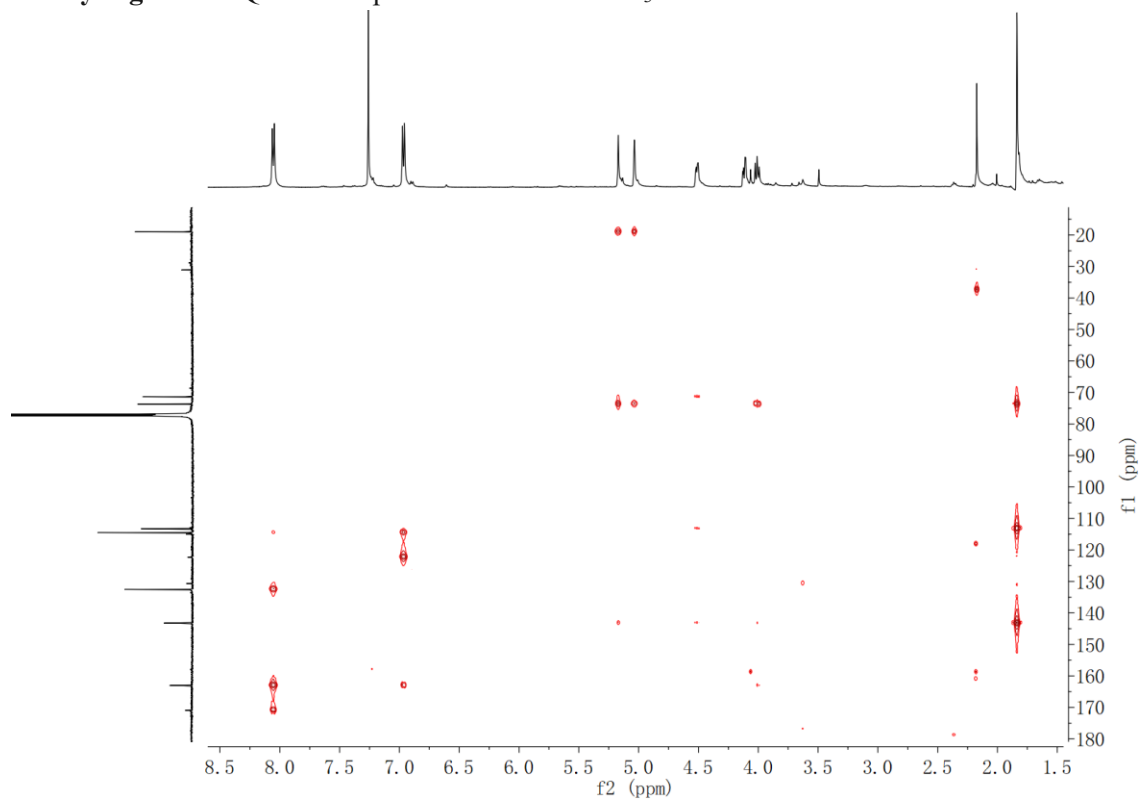
Supplementary Fig. 62. ¹H NMR spectrum of **15** in CDCl₃



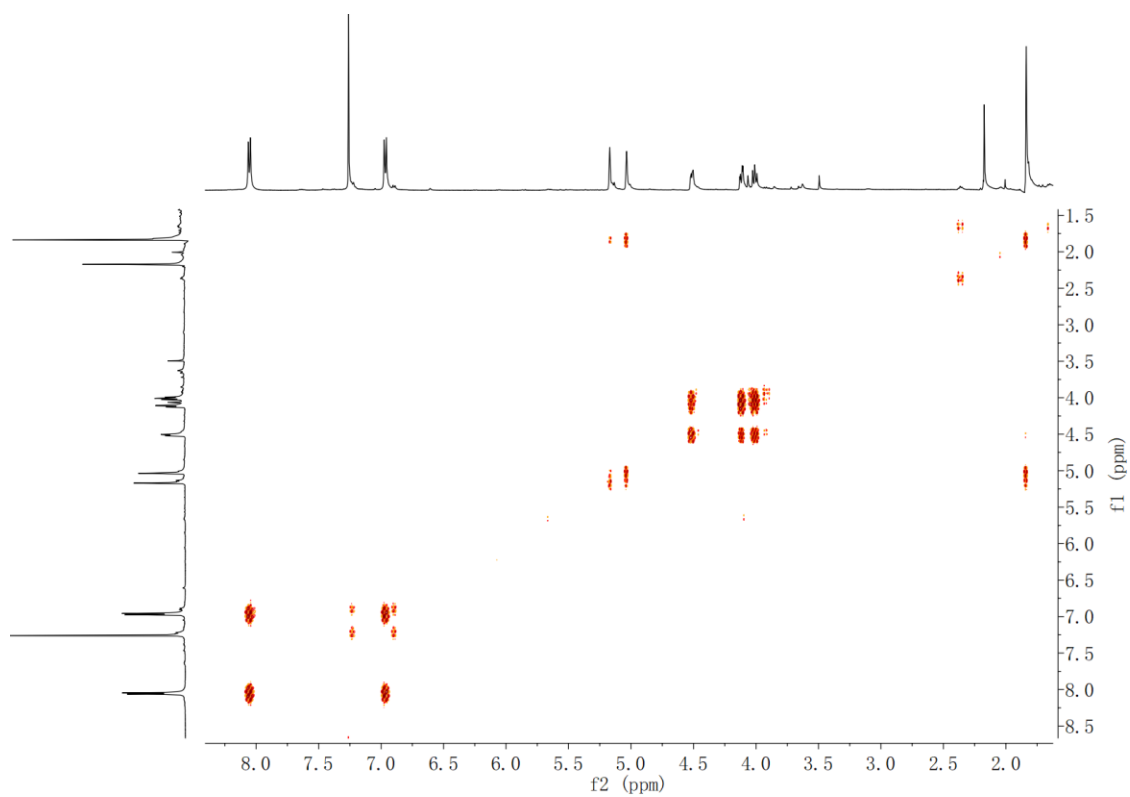
Supplementary Fig. 63. ¹³C NMR spectrum of **15** in CDCl₃



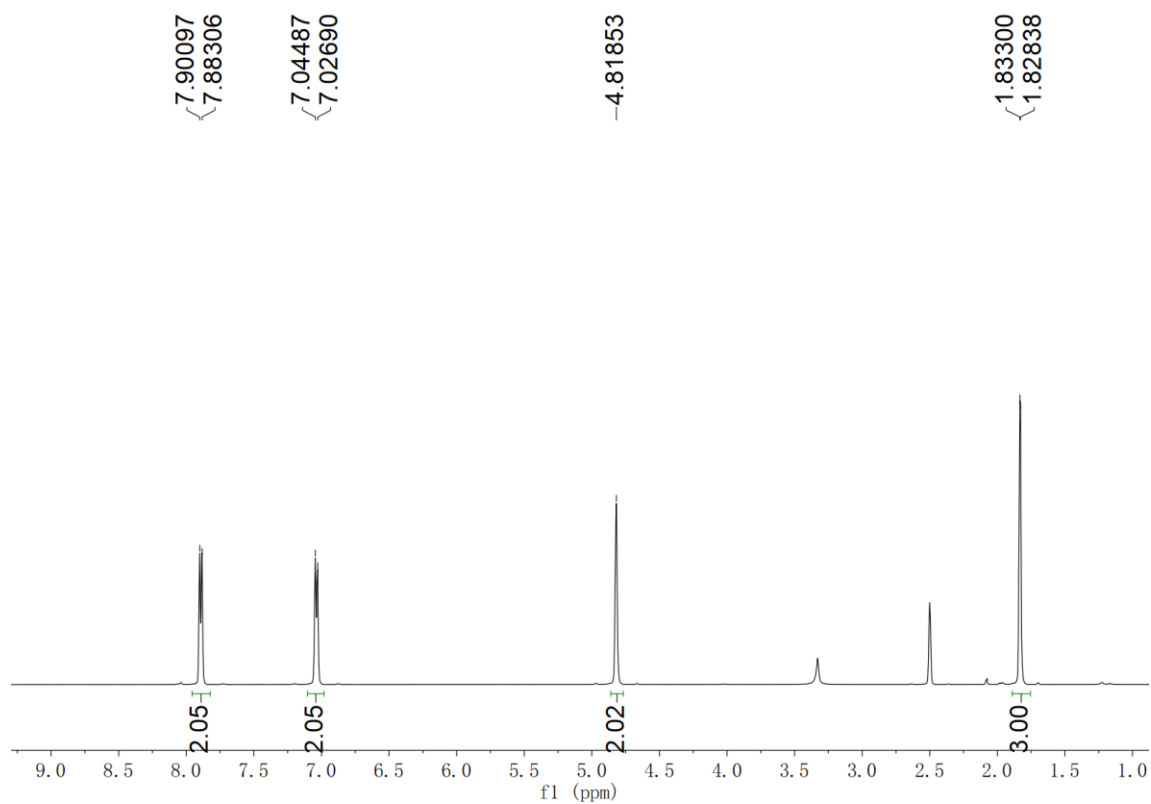
Supplementary Fig. 64. HSQC NMR spectrum of **15** in CDCl_3



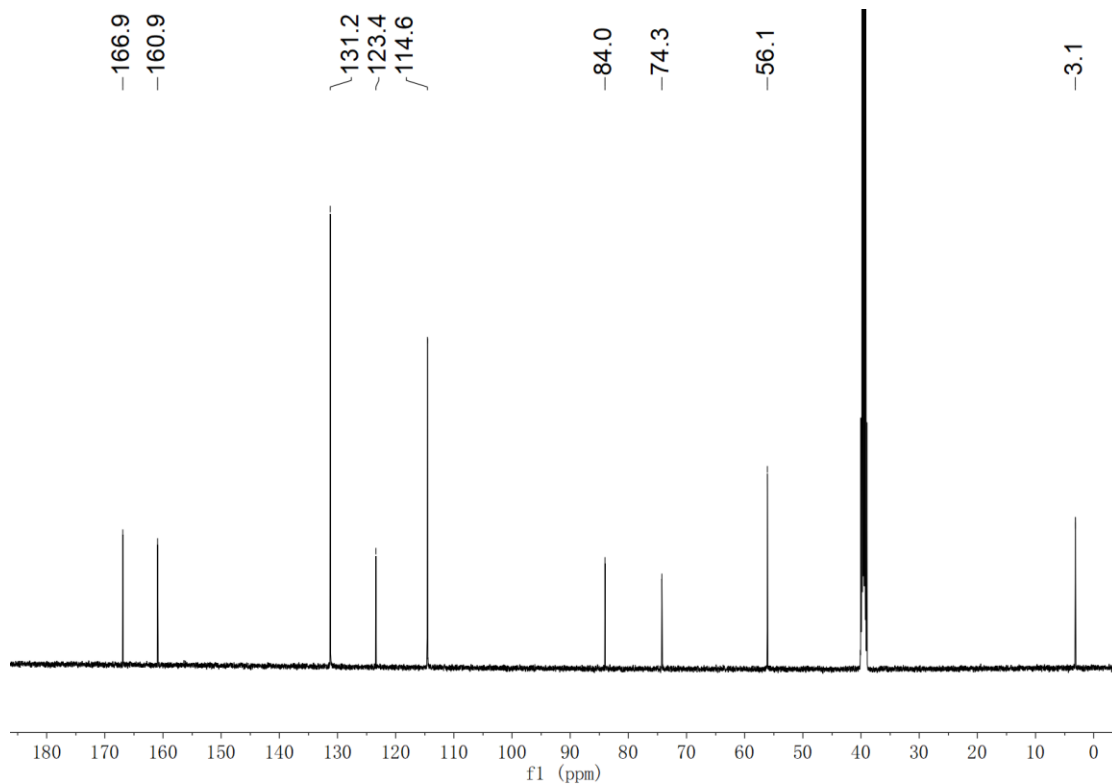
Supplementary Fig. 65. HMBC NMR spectrum of **15** in CDCl_3



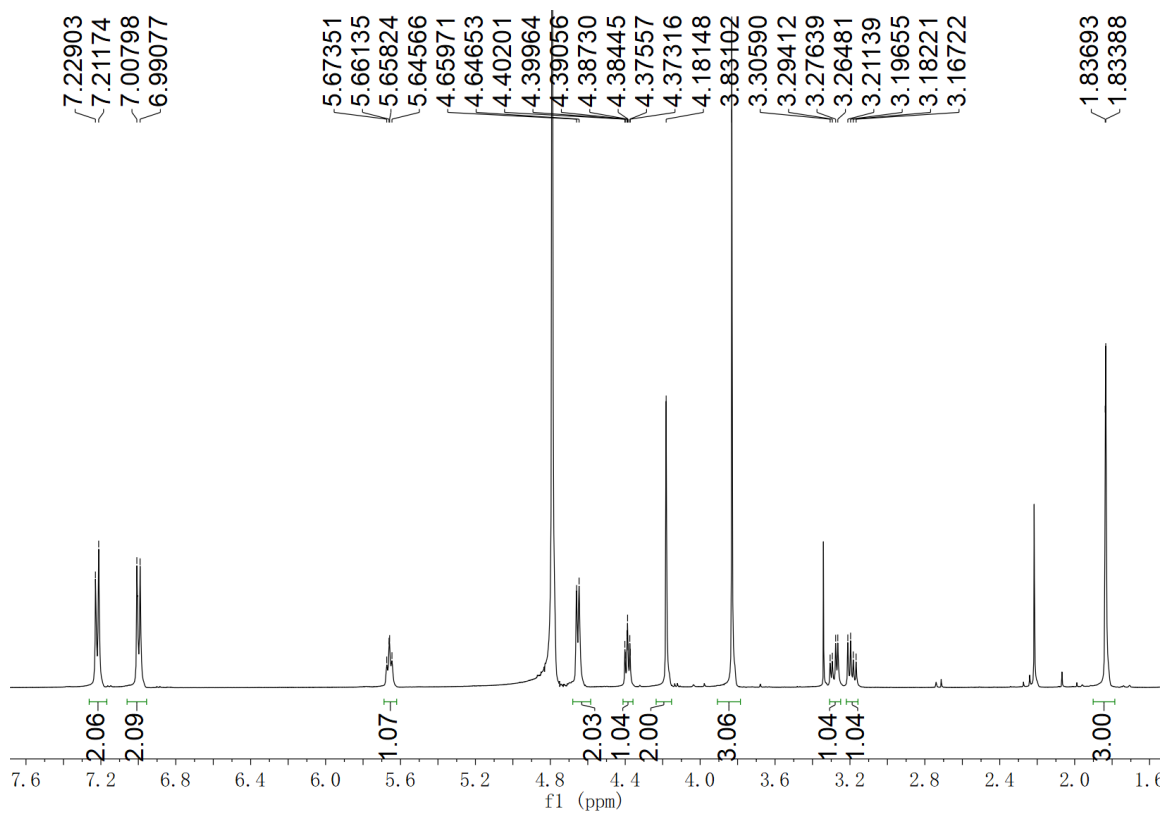
Supplementary Fig. 66. ^1H - ^1H COSY NMR spectrum of **15** in CDCl_3



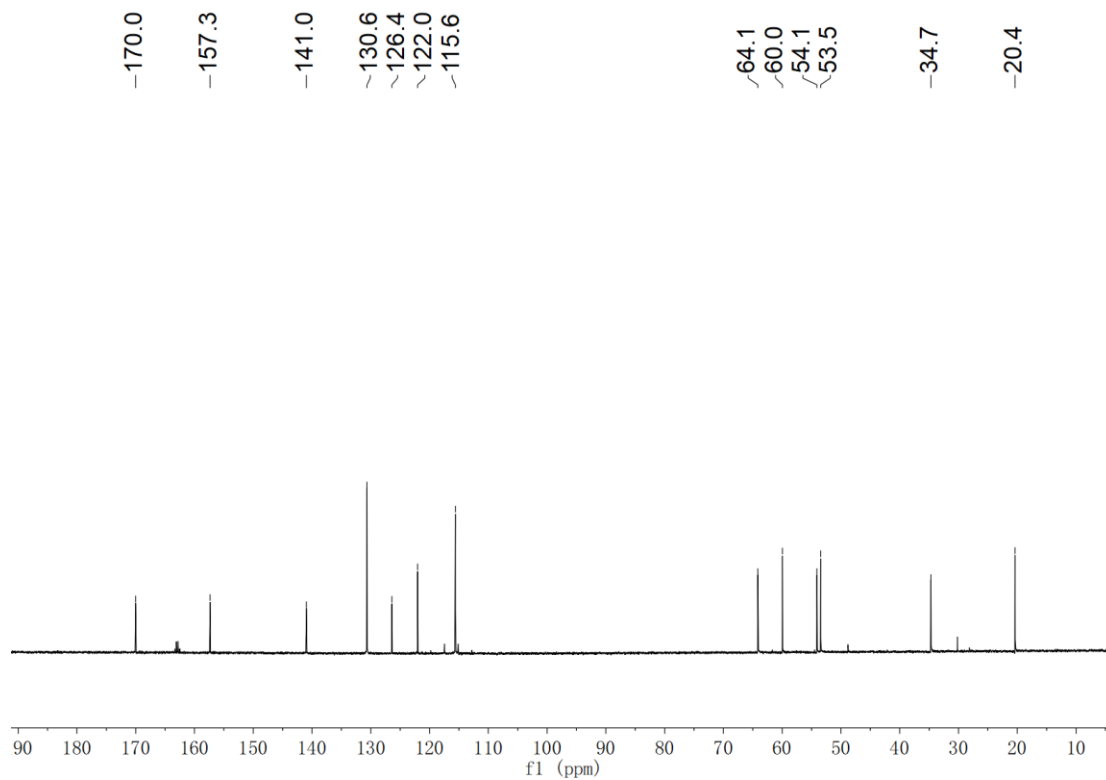
Supplementary Fig. 67. ^1H NMR spectrum of O-but-2-ynyl benzoic acid (**16**) in $\text{DMSO}-d_6$



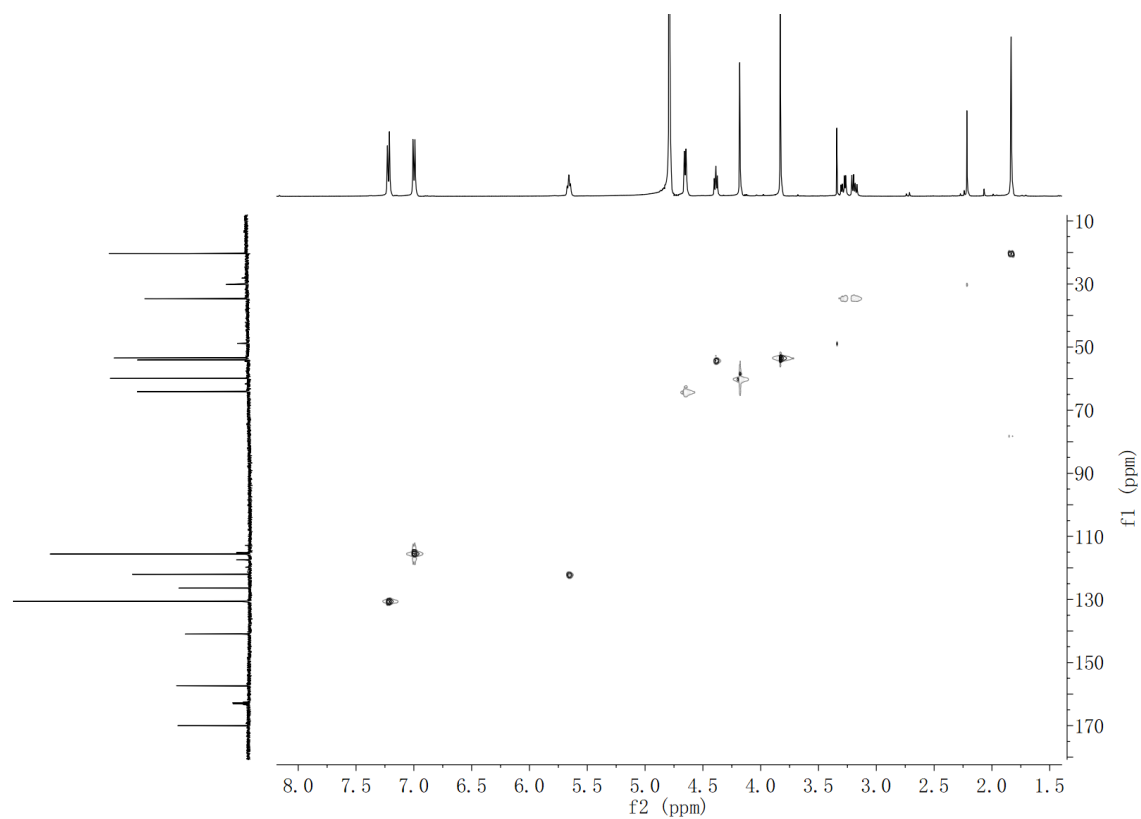
Supplementary Fig. 68. ¹³C NMR spectrum of O-but-2-ynyl benzoic acid (**16**) in DMSO-*d*₆



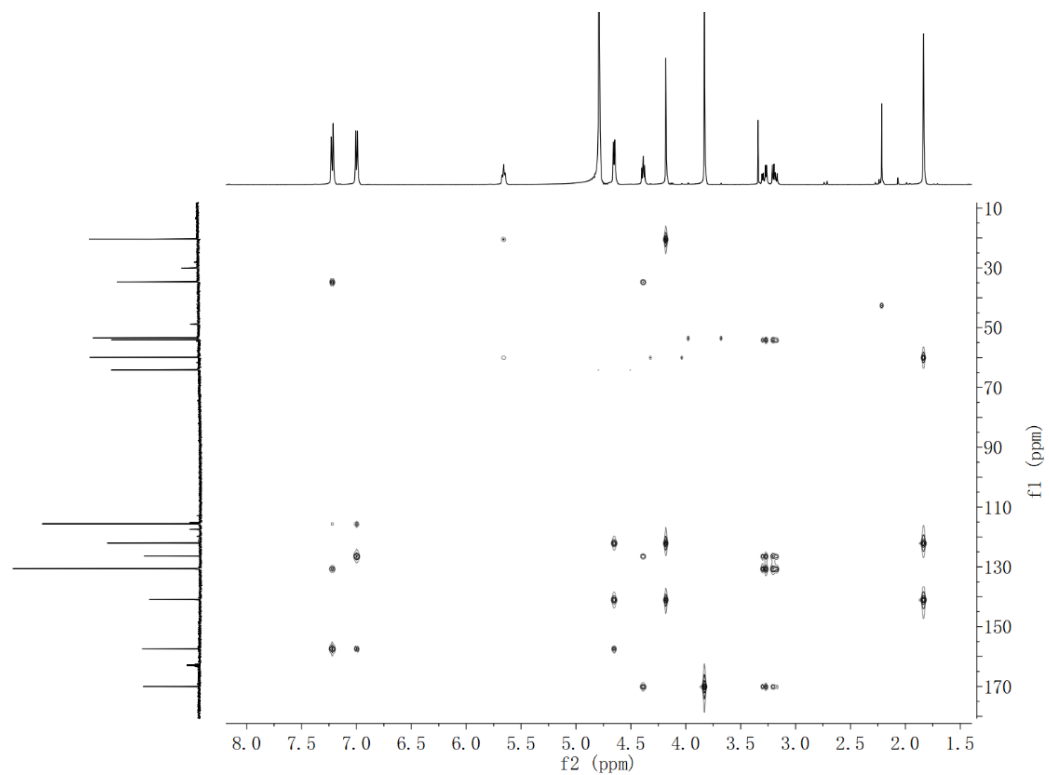
Supplementary Fig. 69. ¹H NMR spectrum of 17-Me in D₂O



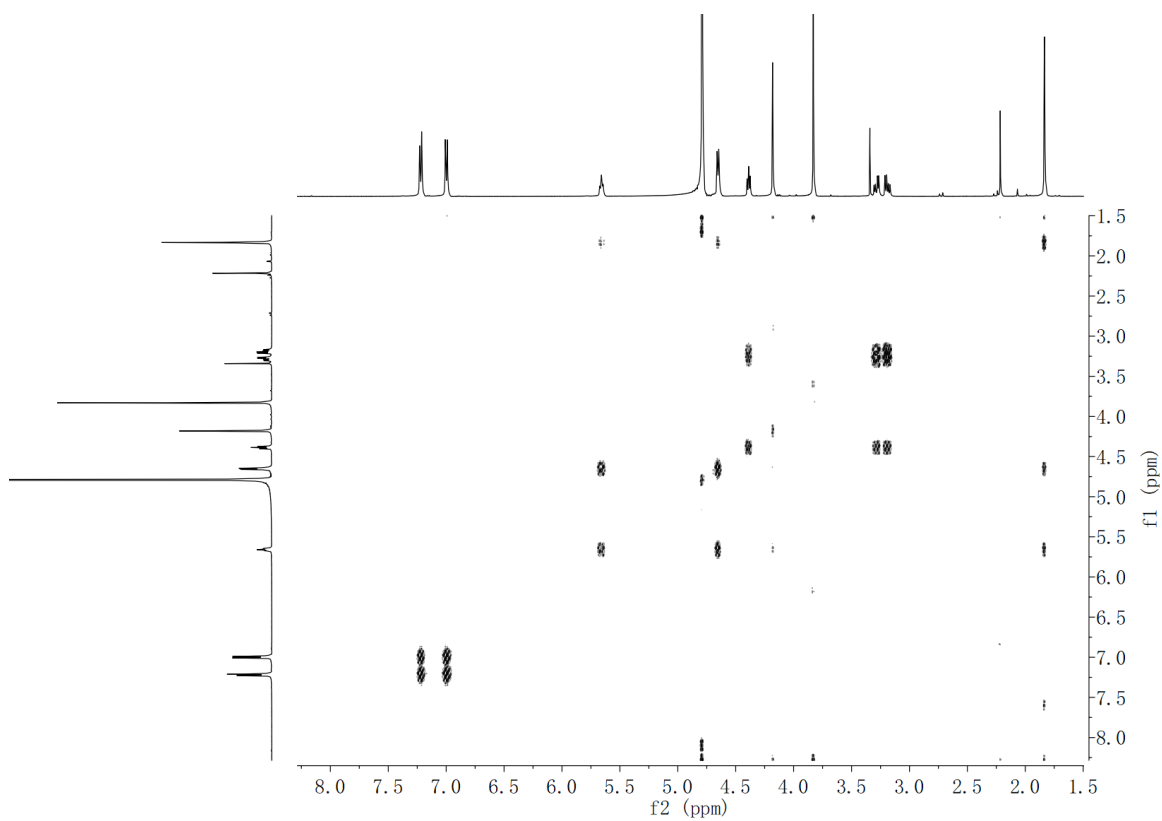
Supplementary Fig. 70. ^{13}C NMR spectrum of 17-Me in D_2O



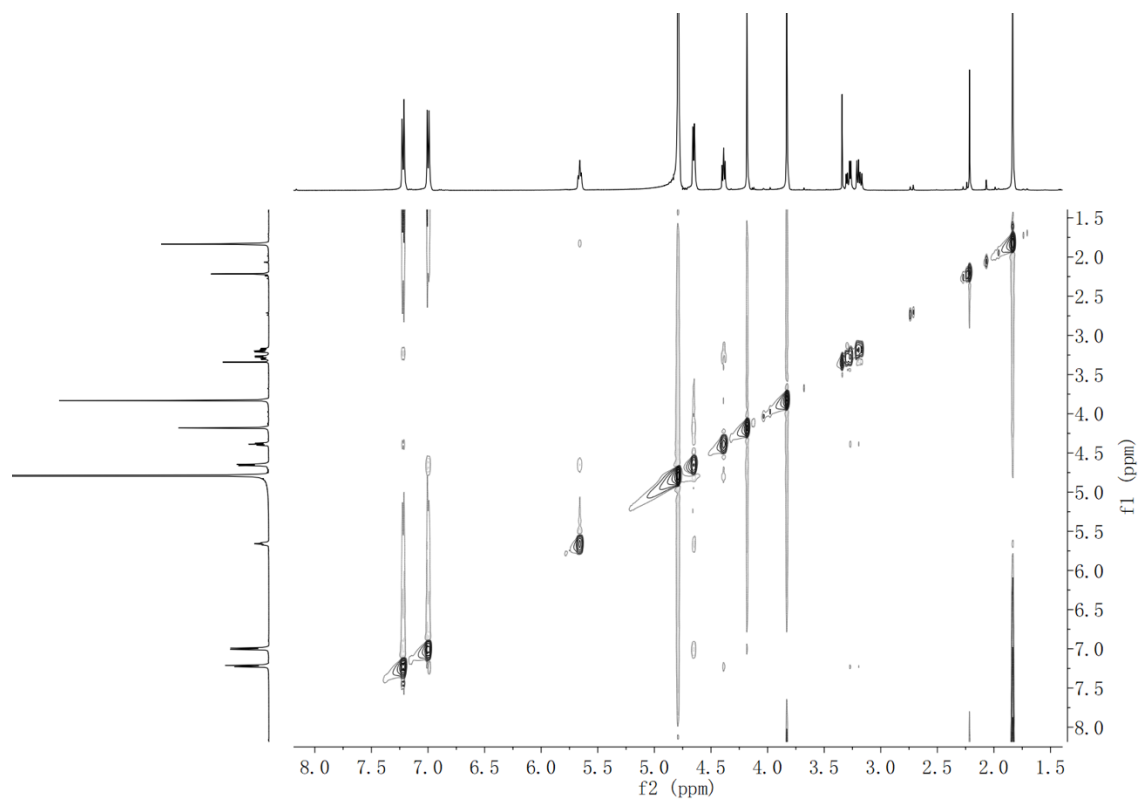
Supplementary Fig. 71. HSQC NMR spectrum of 17-Me in D_2O



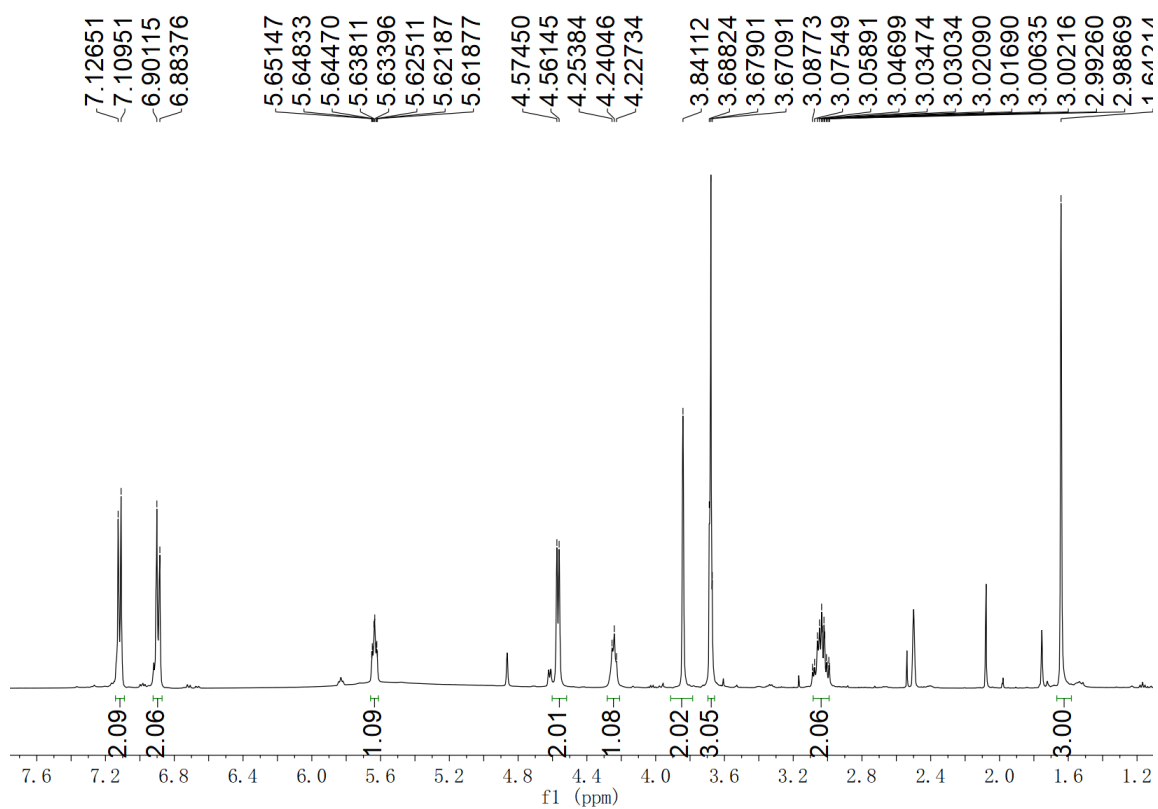
Supplementary Fig. 72. HMBC NMR spectrum of **17-Me** in D₂O



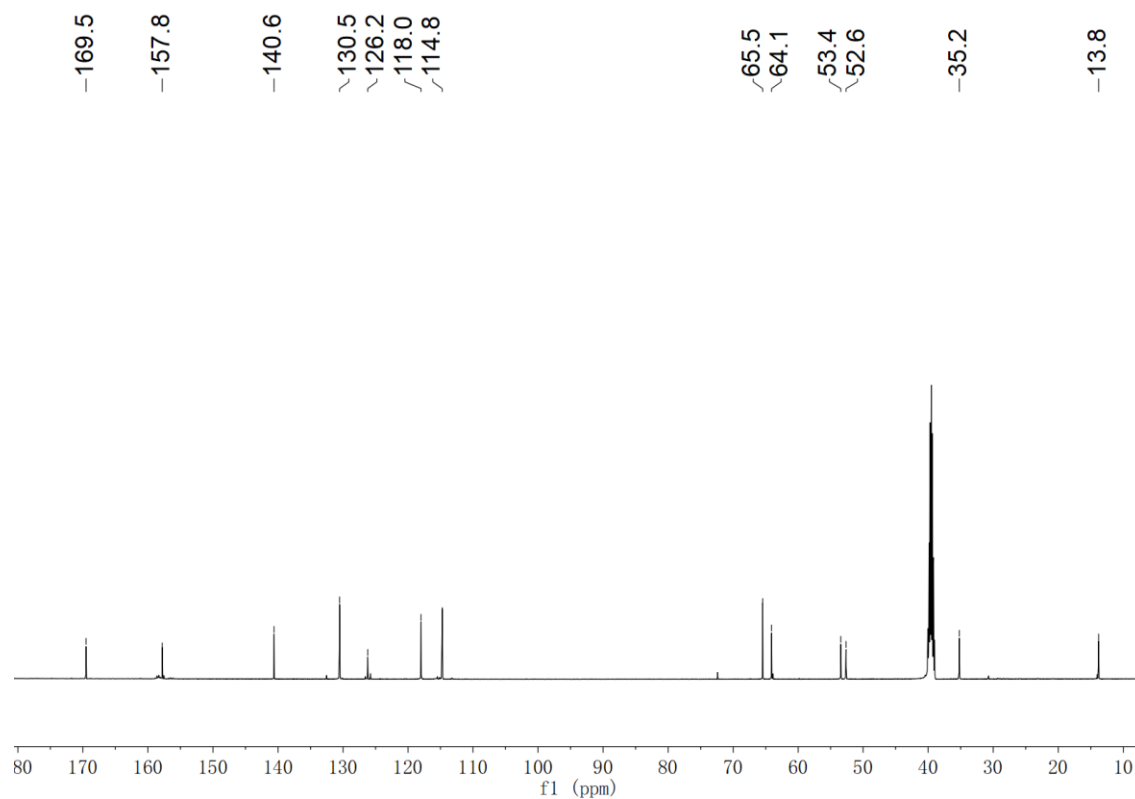
Supplementary Fig. 73. ¹H-¹H COSY NMR spectrum of **17-Me** in D₂O



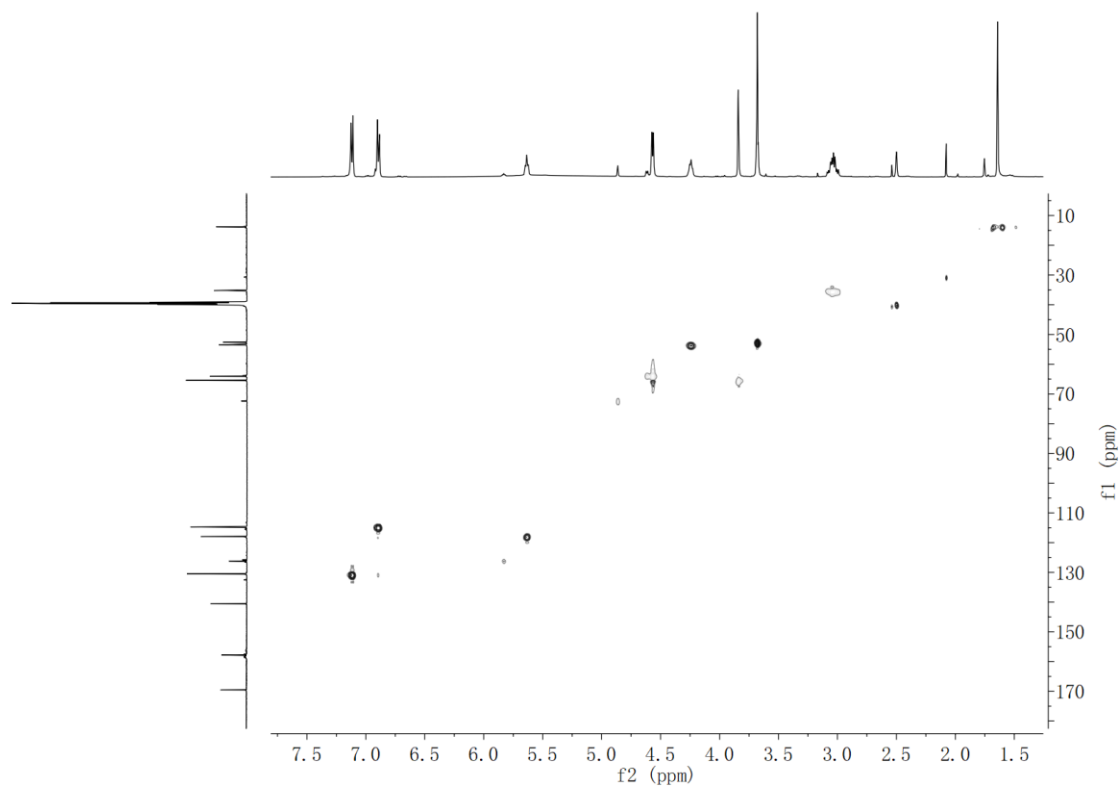
Supplementary Fig. 74. NOESY NMR spectrum of **17-Me** in D₂O



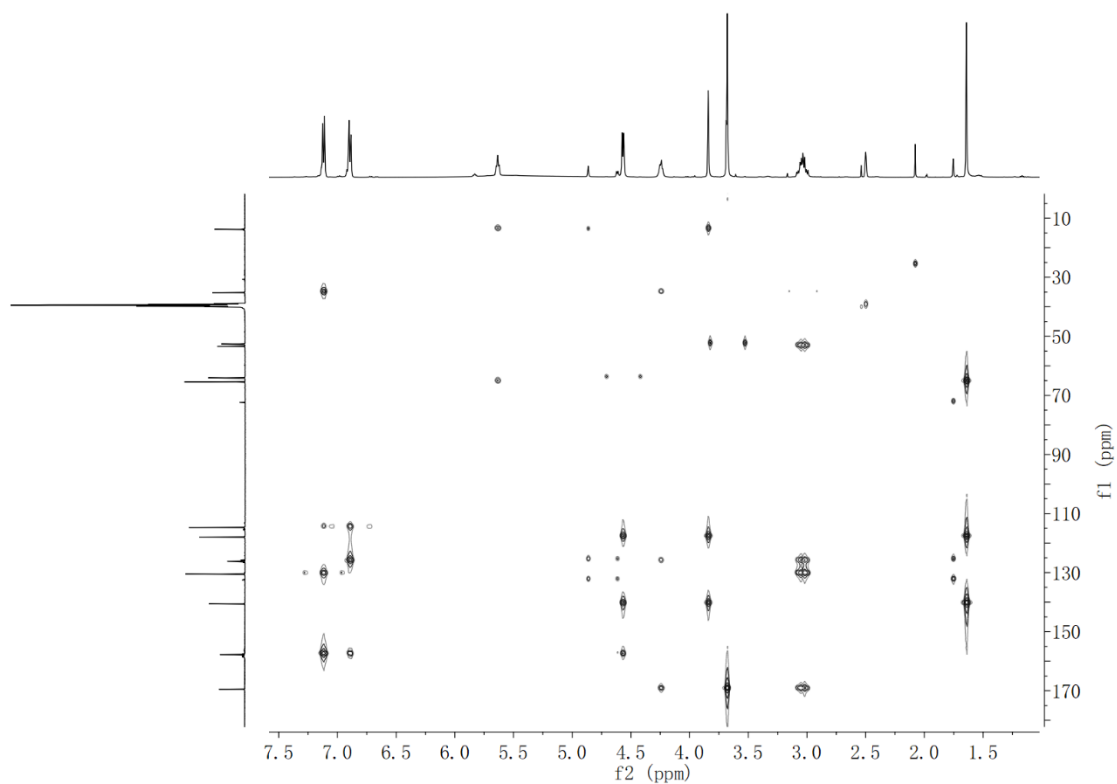
Supplementary Fig. 75. ¹H NMR spectrum of **18-Me** in DMSO-*d*₆



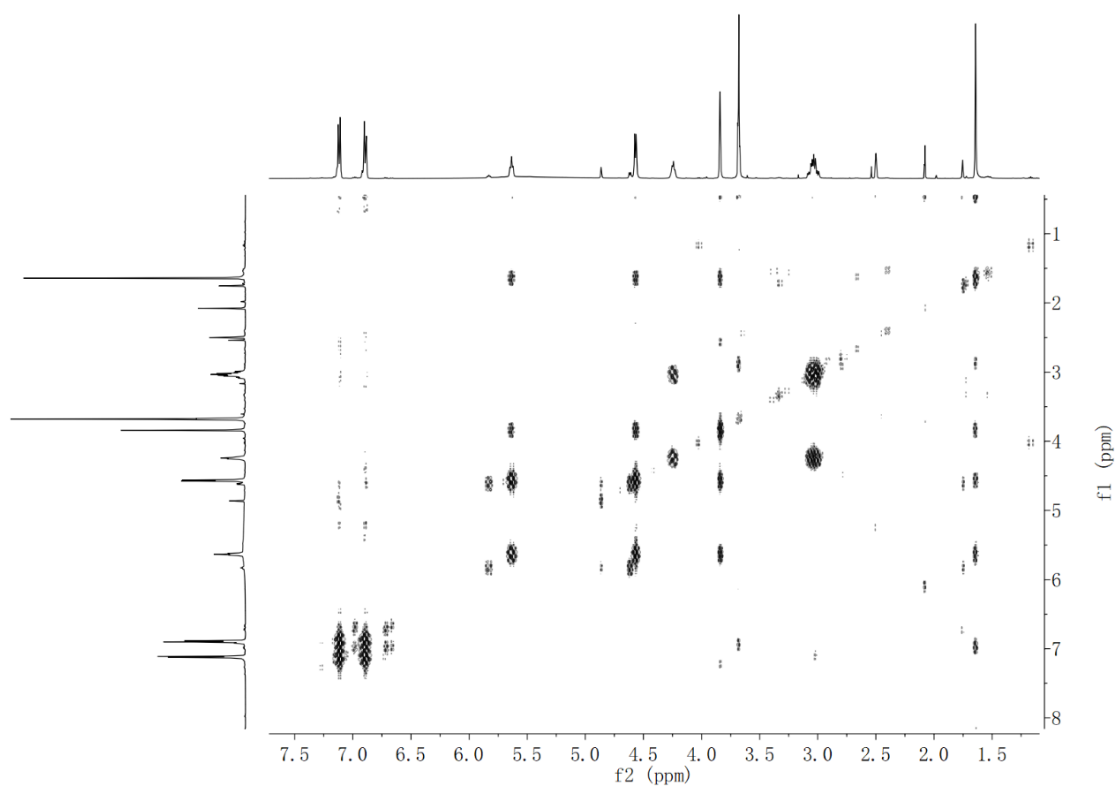
Supplementary Fig. 76. ^{13}C NMR spectrum of **18-Me** in $\text{DMSO-}d_6$



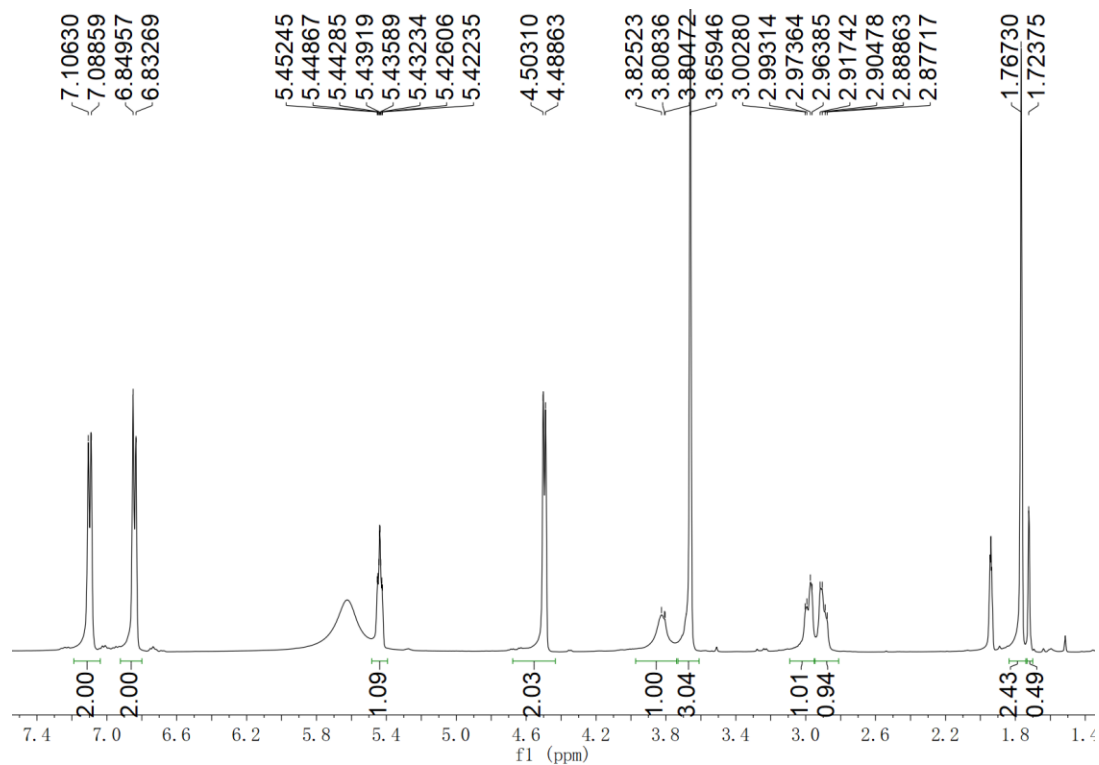
Supplementary Fig. 77. HSQC NMR spectrum of **18-Me** in $\text{DMSO-}d_6$



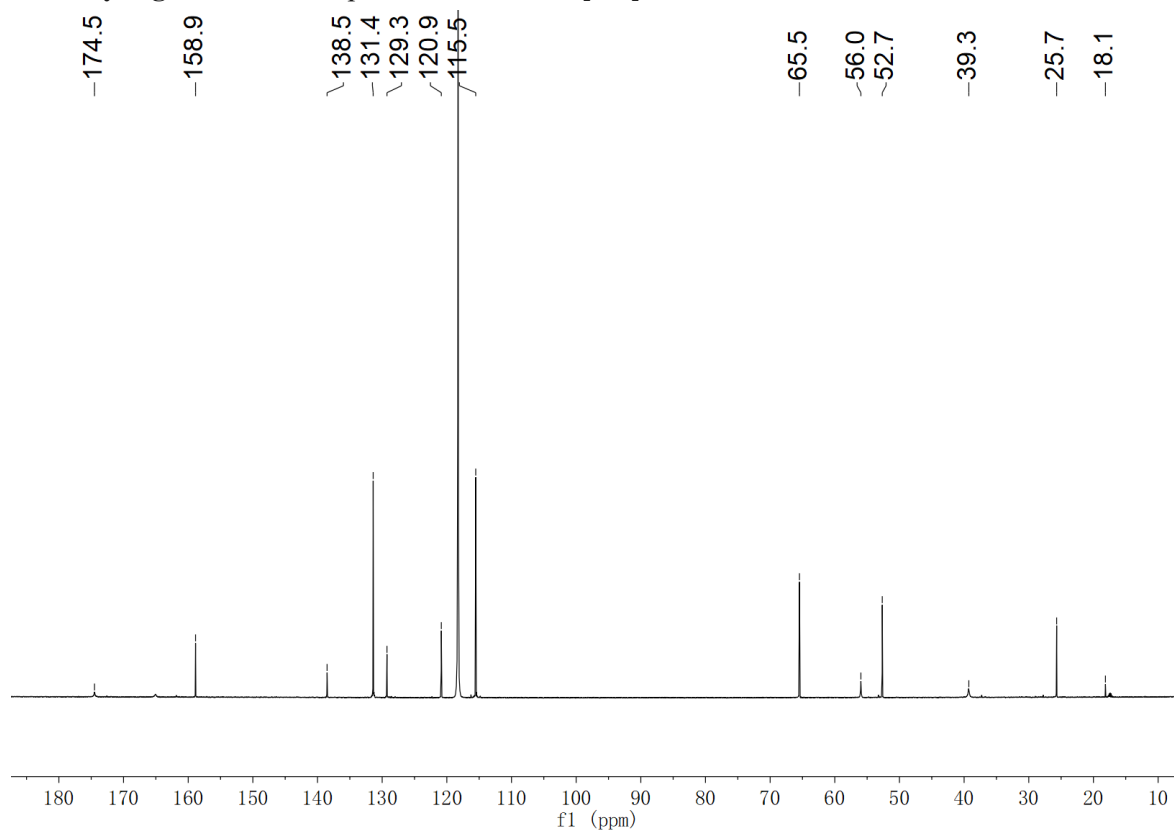
Supplementary Fig. 78. HMBC NMR spectrum of **18-Me** in DMSO-*d*₆



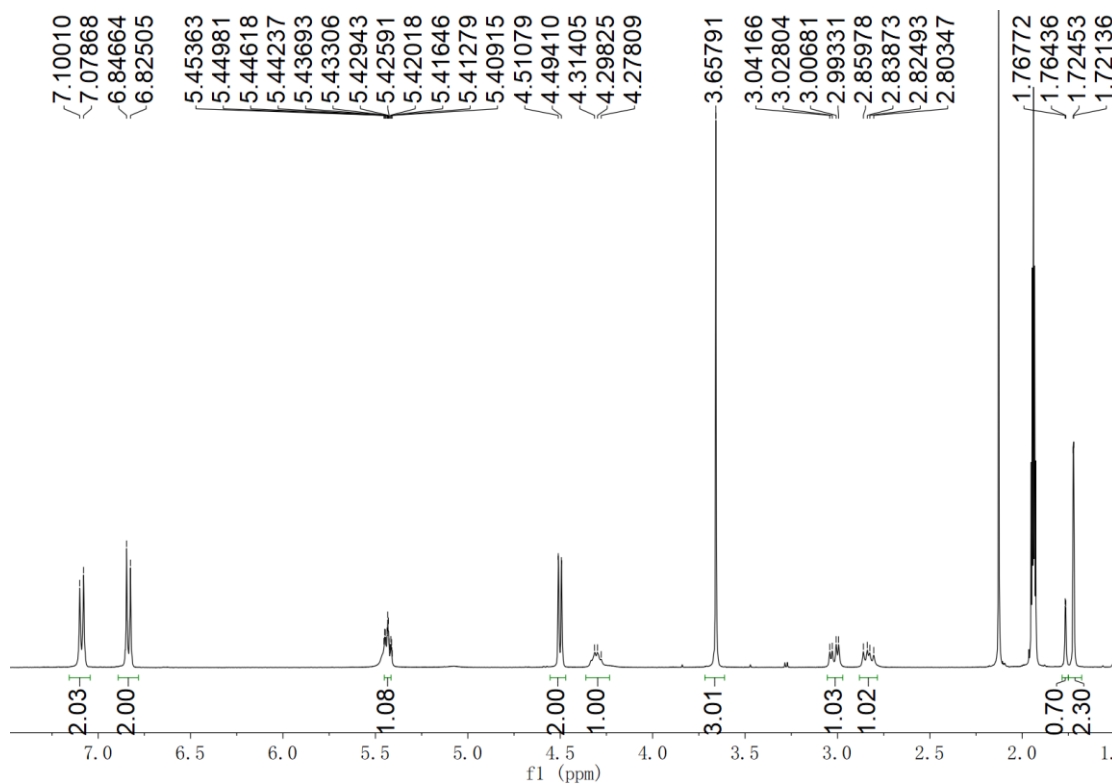
Supplementary Fig. 79. ^1H - ^1H COSY NMR spectrum of **18-Me** in DMSO-*d*₆



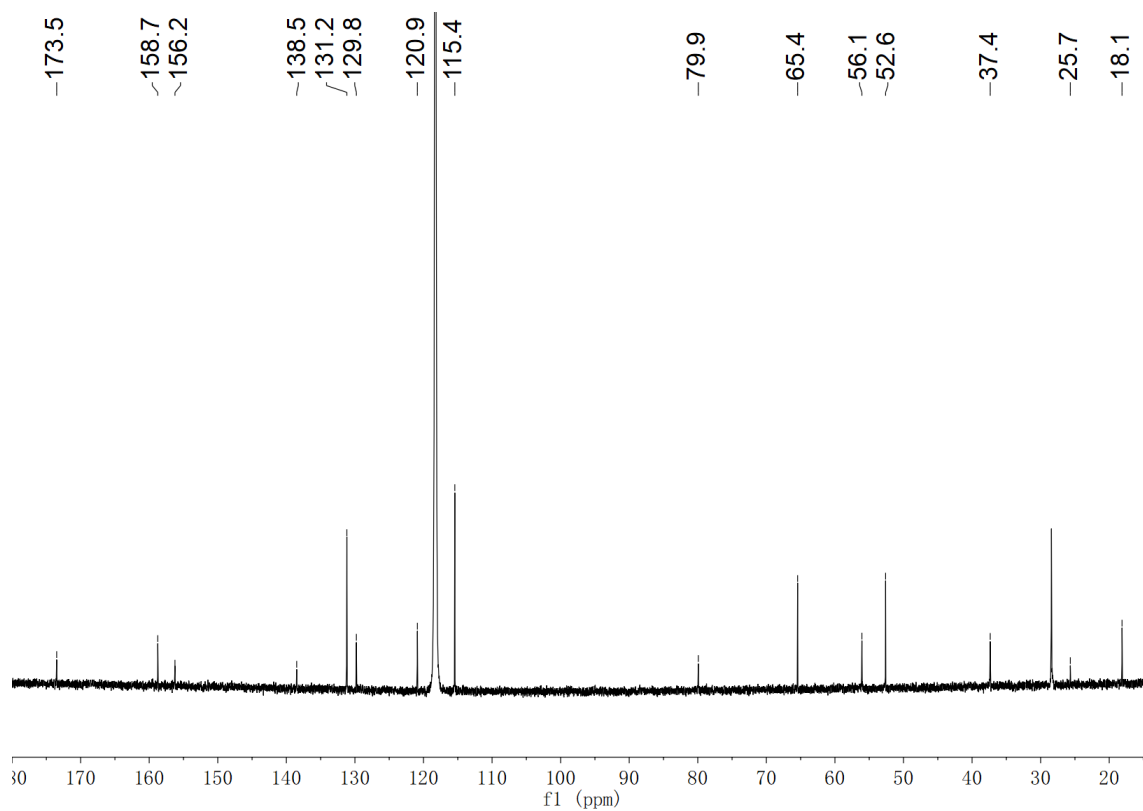
Supplementary Fig. 80. ¹H NMR spectrum of 4',4',4'-[²H₃]-7-Me in CD₃CN



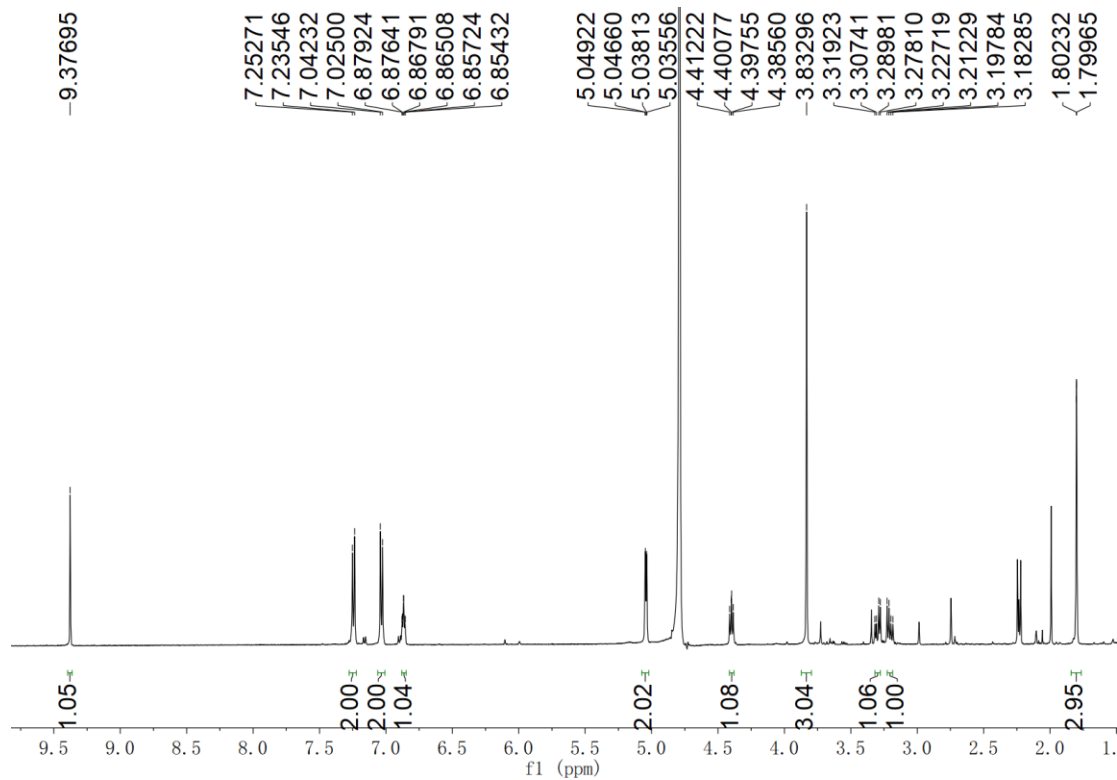
Supplementary Fig. 81. ¹³C NMR spectrum of 4',4',4'-[²H₃]-7-Me in CD₃CN



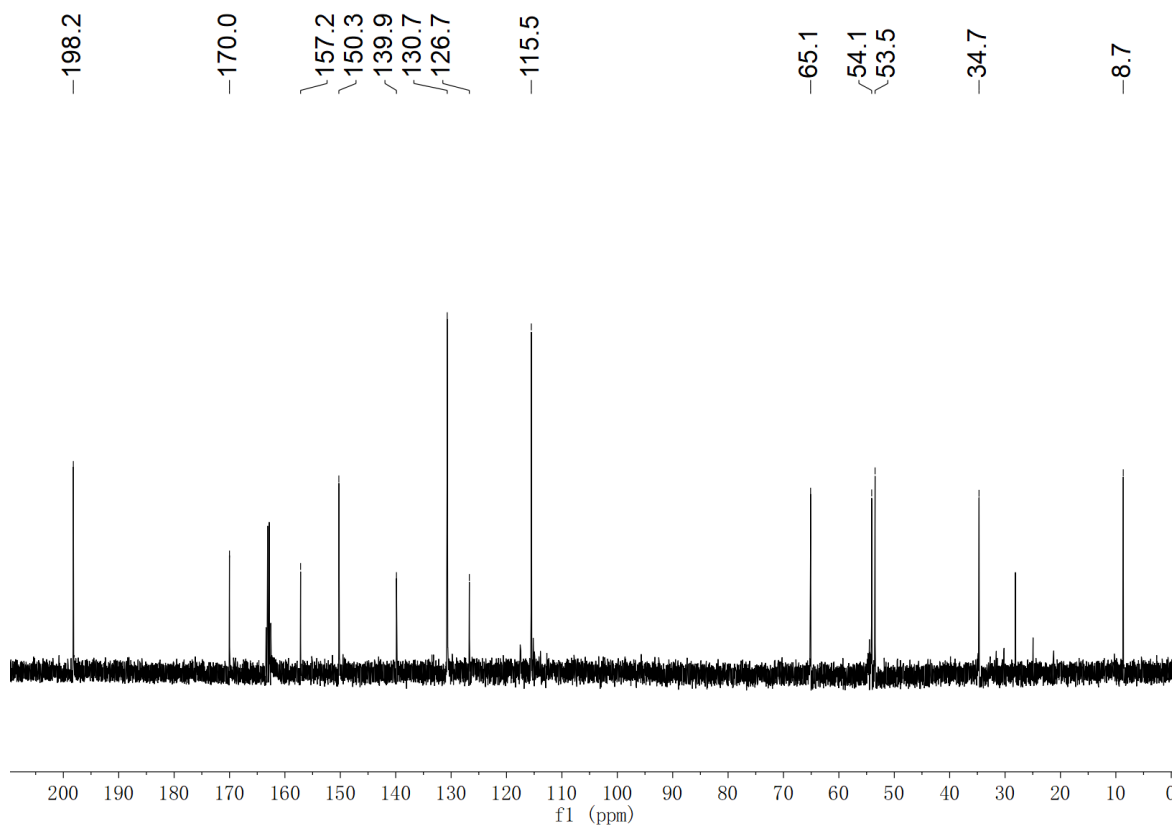
Supplementary Fig. 82. ^1H NMR spectrum of 5',5',5'-[$^2\text{H}_3$]-7-Me in CD_3CN



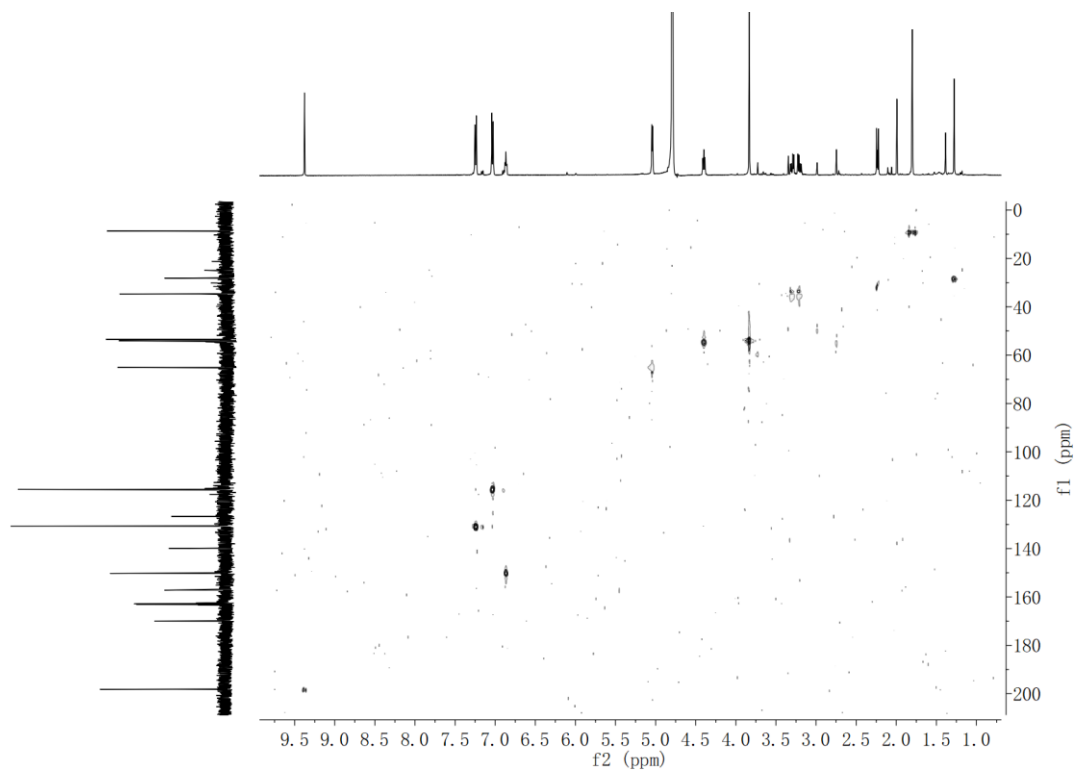
Supplementary Fig. 83. ^{13}C NMR spectrum of 5',5',5'-[$^2\text{H}_3$]-7-Me in CD_3CN



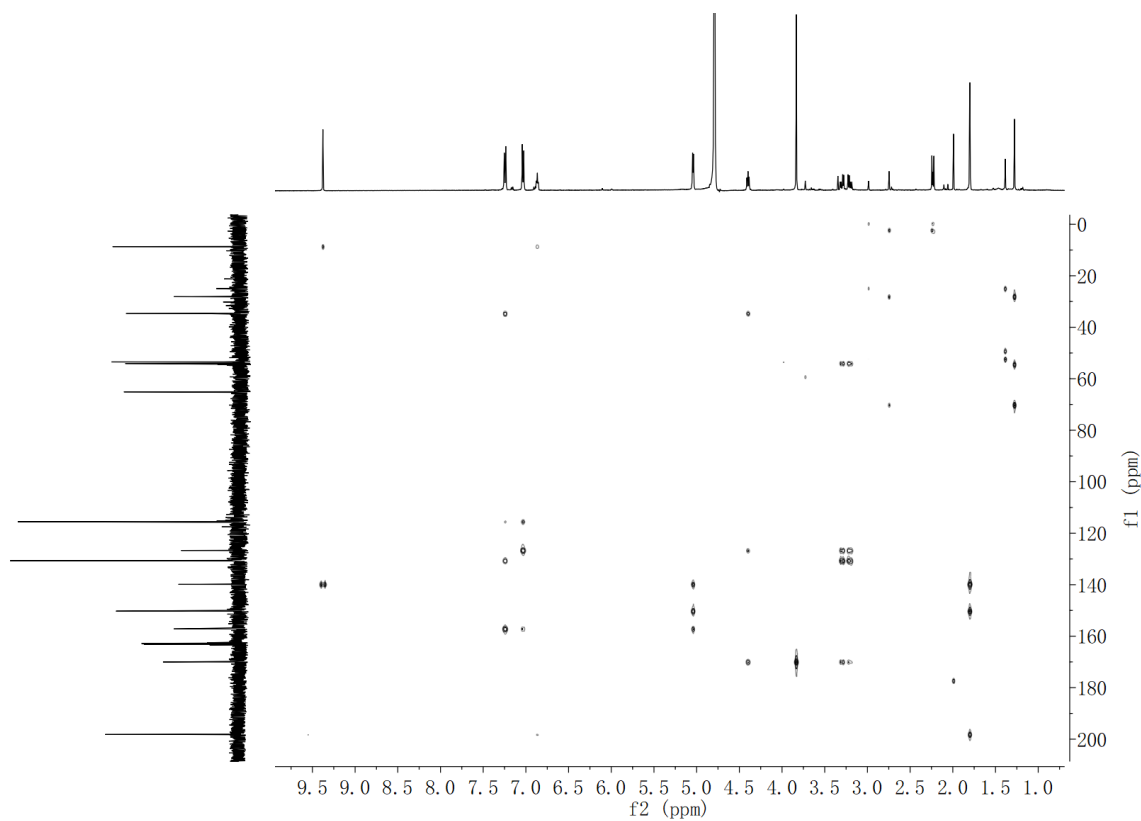
Supplementary Fig. 84. ¹H NMR spectrum of compound **19-Me** in D₂O



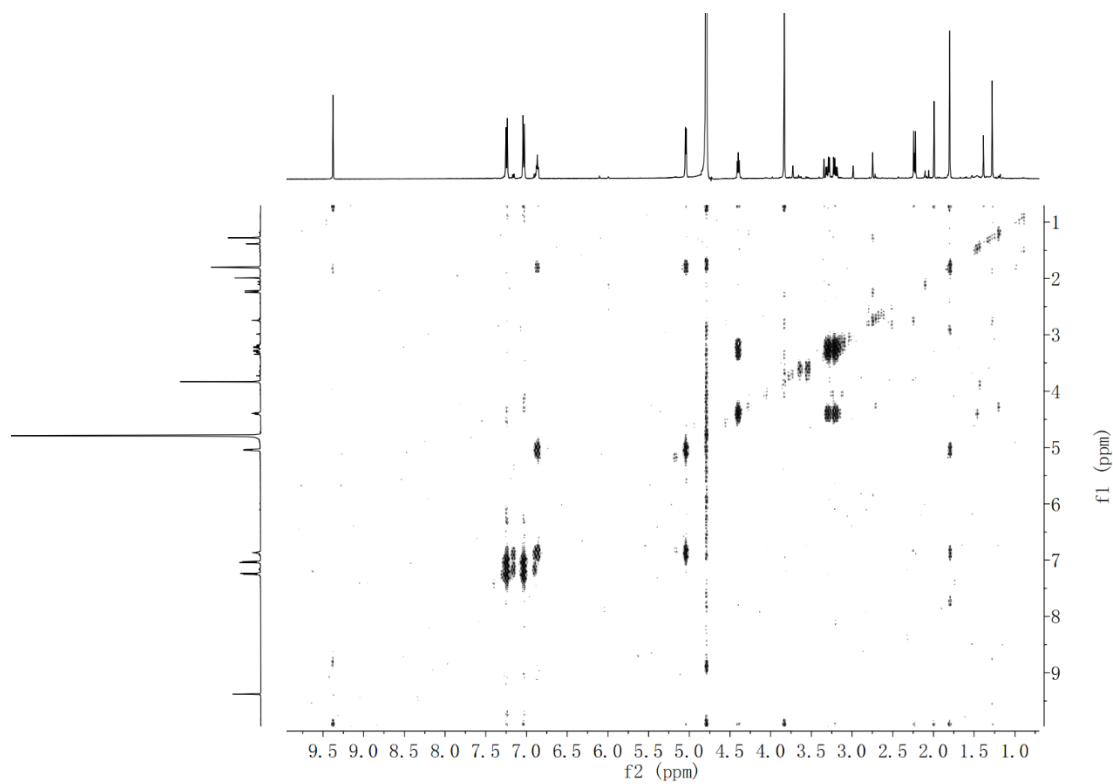
Supplementary Fig. 85. ¹³C NMR spectrum of compound **19-Me** in D₂O



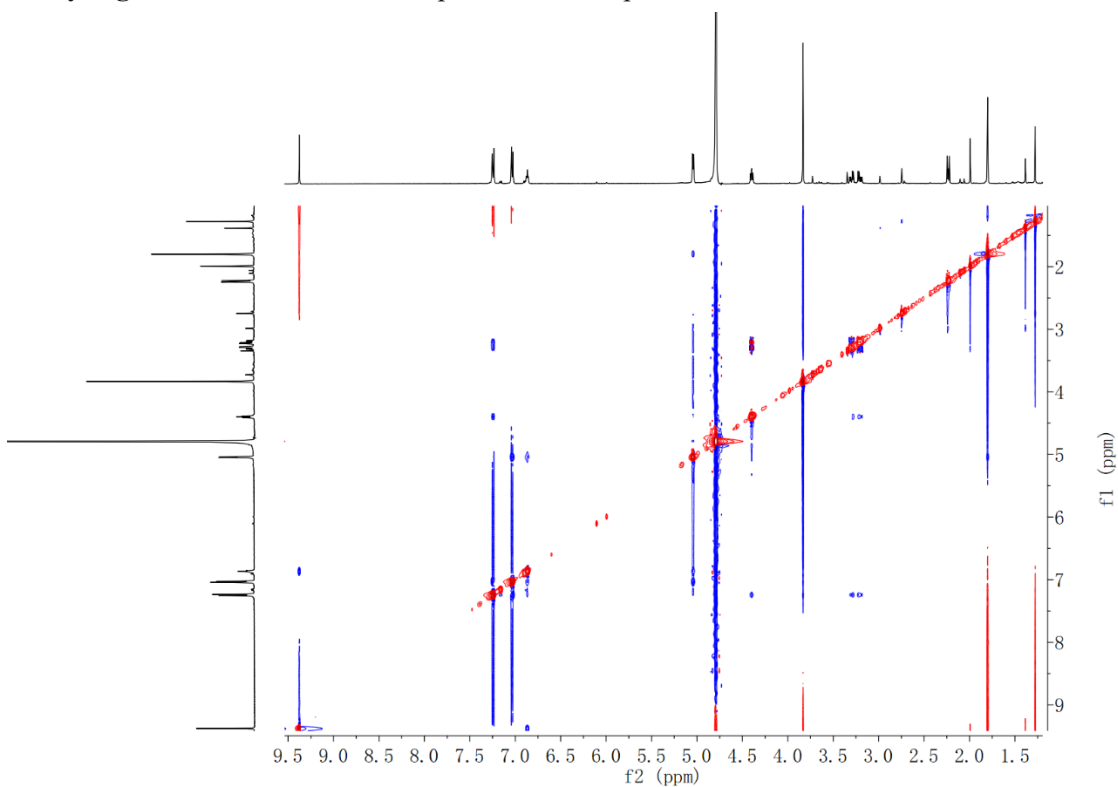
Supplementary Fig. 86. HSQC NMR spectrum of compound **19-Me** in D₂O



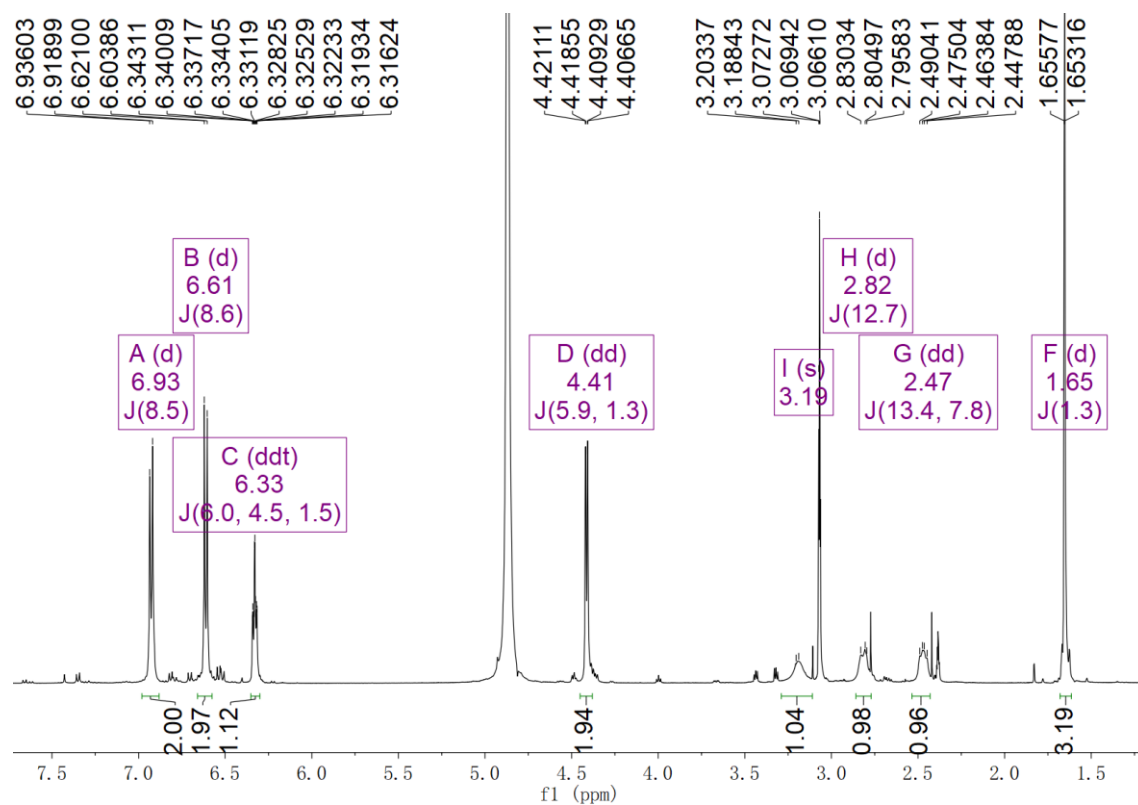
Supplementary Fig. 87. HMBC NMR spectrum of compound **19-Me** in D₂O



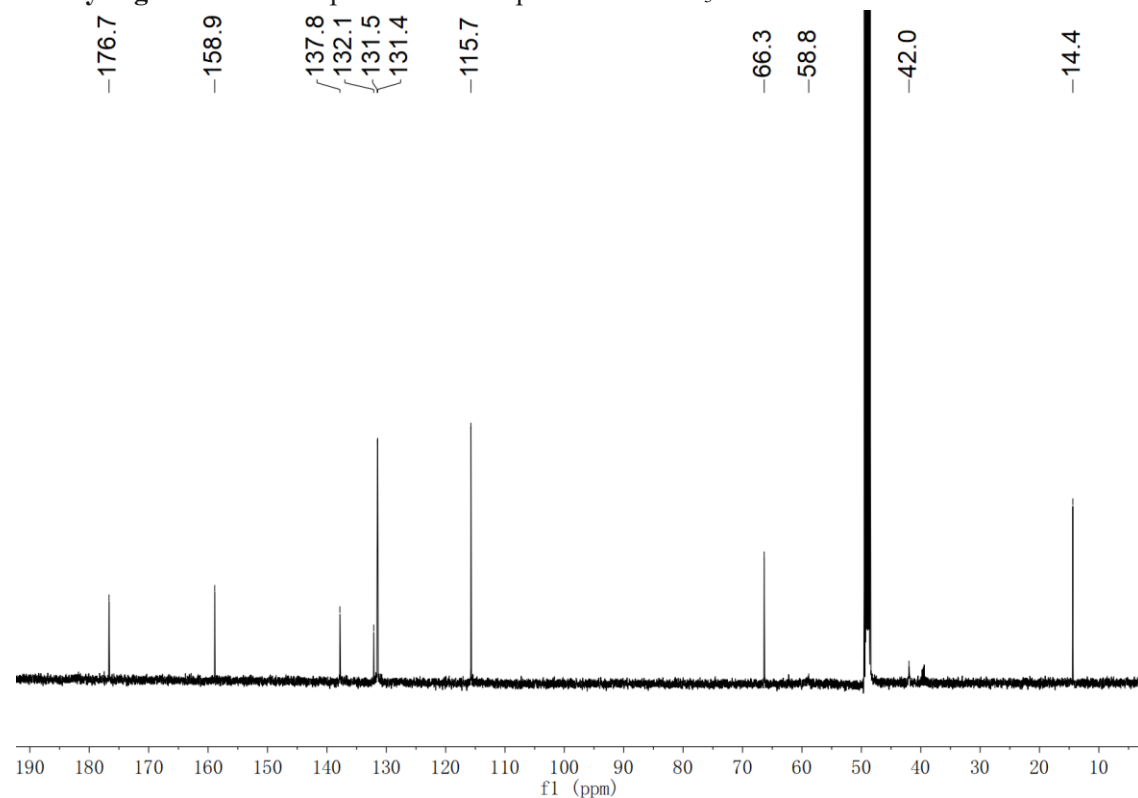
Supplementary Fig. 88. ^1H - ^1H COSY NMR spectrum of compound **19-Me** in D_2O



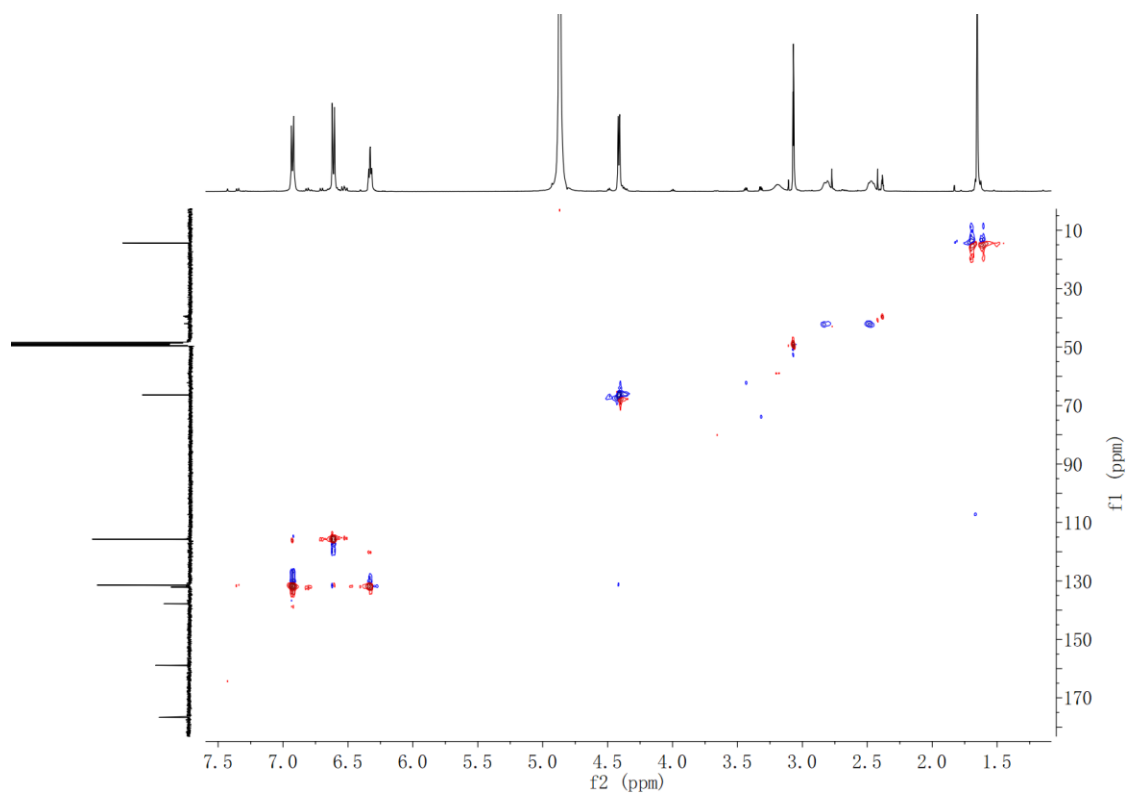
Supplementary Fig. 89. NOESY NMR spectrum of compound **19-Me** in D_2O



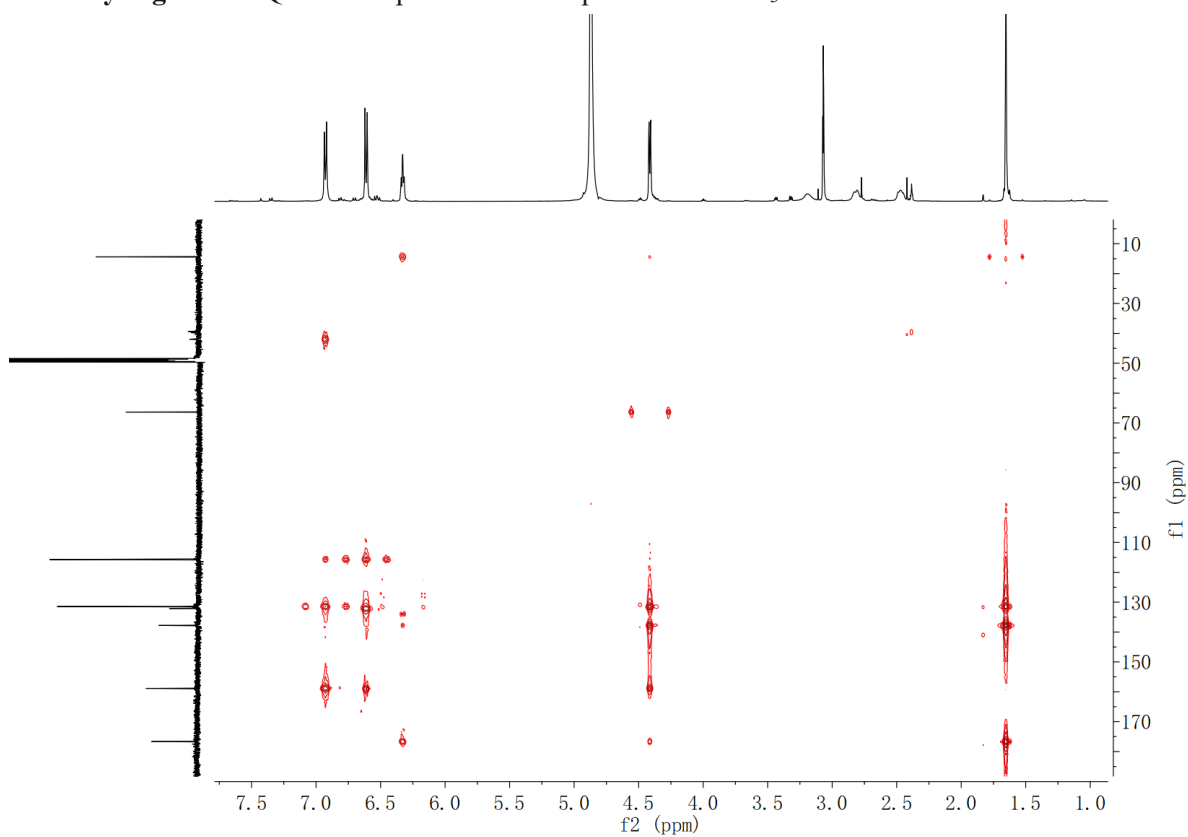
Supplementary Fig. 90. ¹H NMR spectrum of compound **20** in CD₃OD



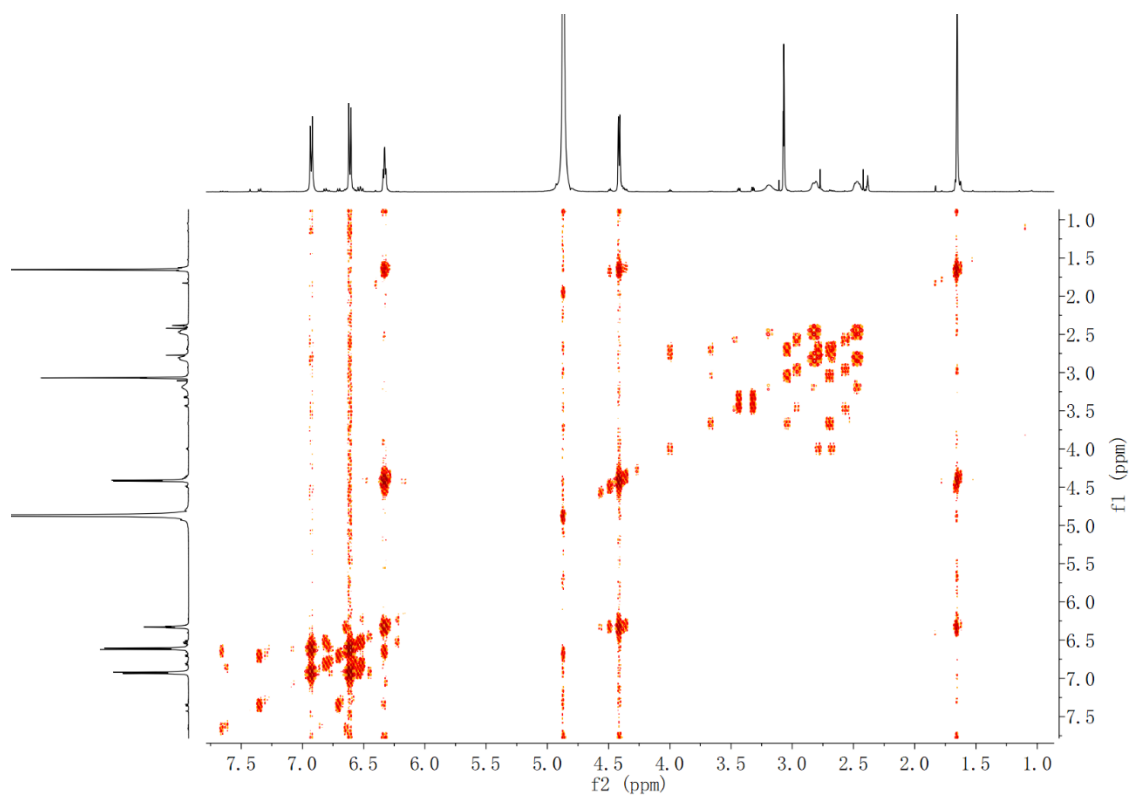
Supplementary Fig. 91. ¹³C NMR spectrum of compound **20** in CD₃OD



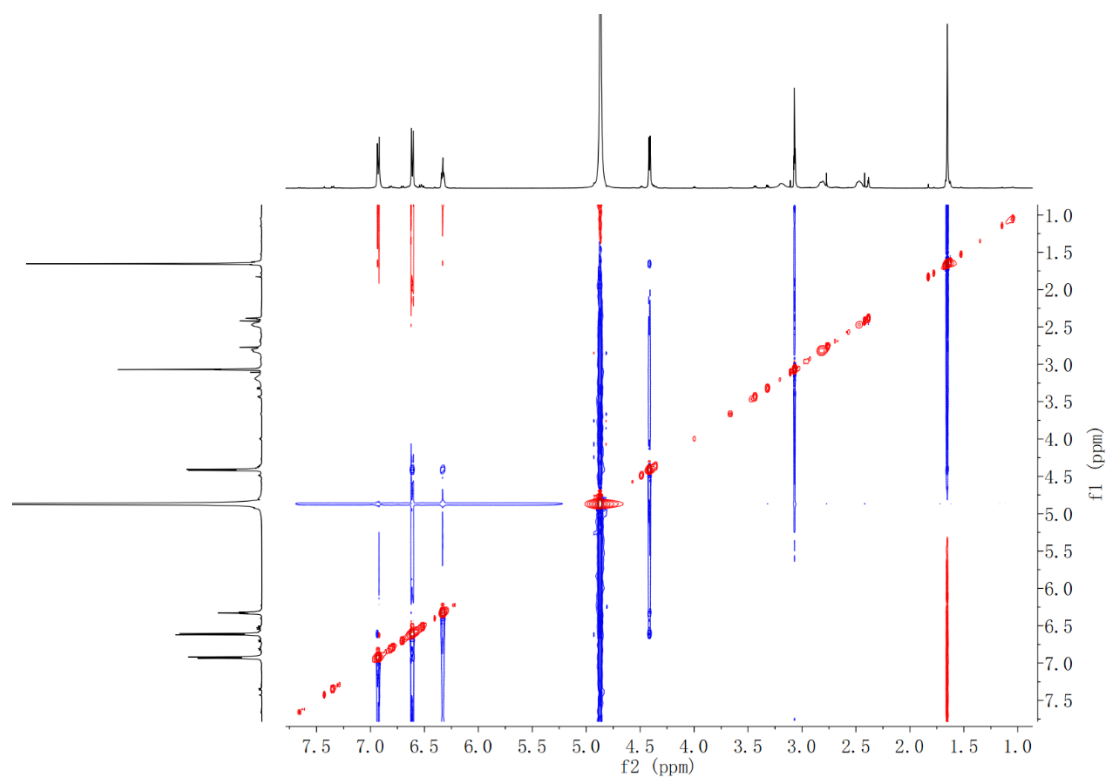
Supplementary Fig. 92. HSQC NMR spectrum of compound **20** in CD₃OD



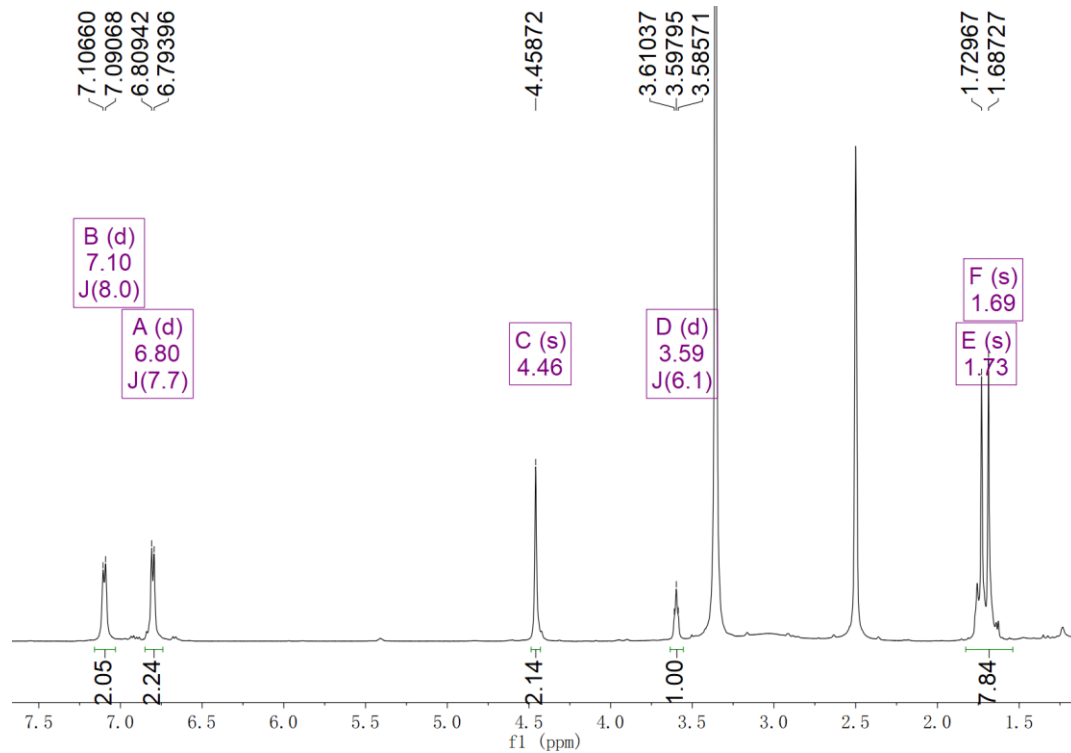
Supplementary Fig. 93. HMBC NMR spectrum of compound **20** in CD₃OD



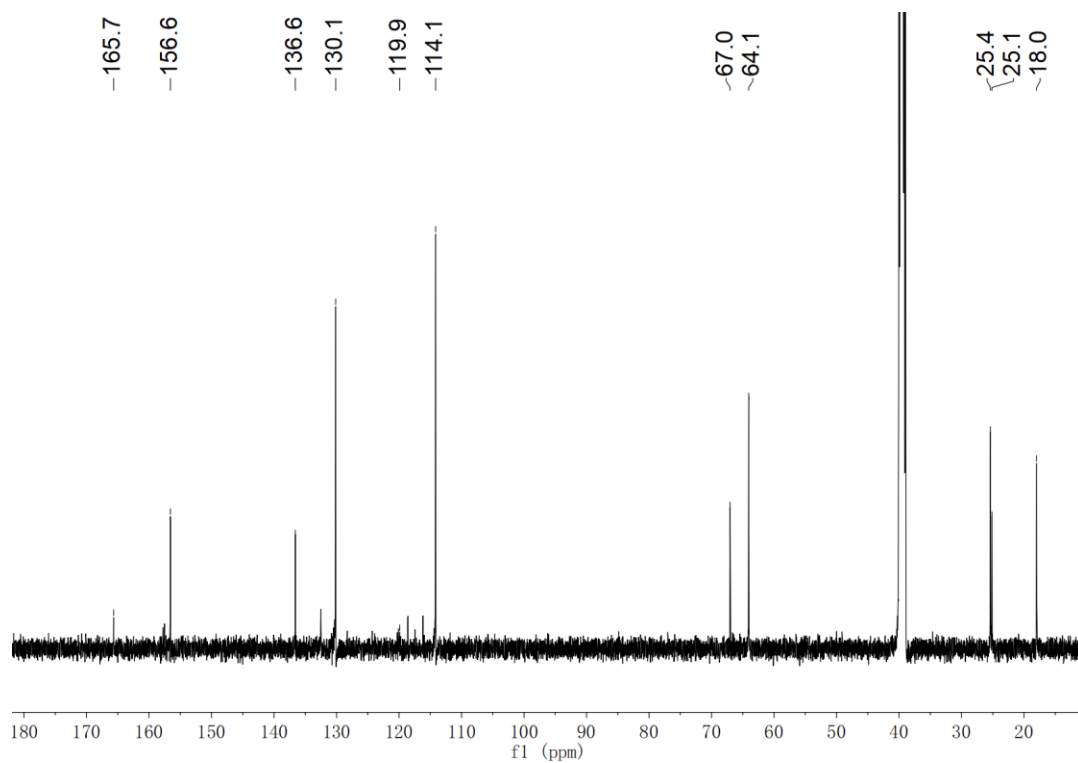
Supplementary Fig. 94. ^1H - ^1H COSY NMR spectrum of compound **20** in CD_3OD



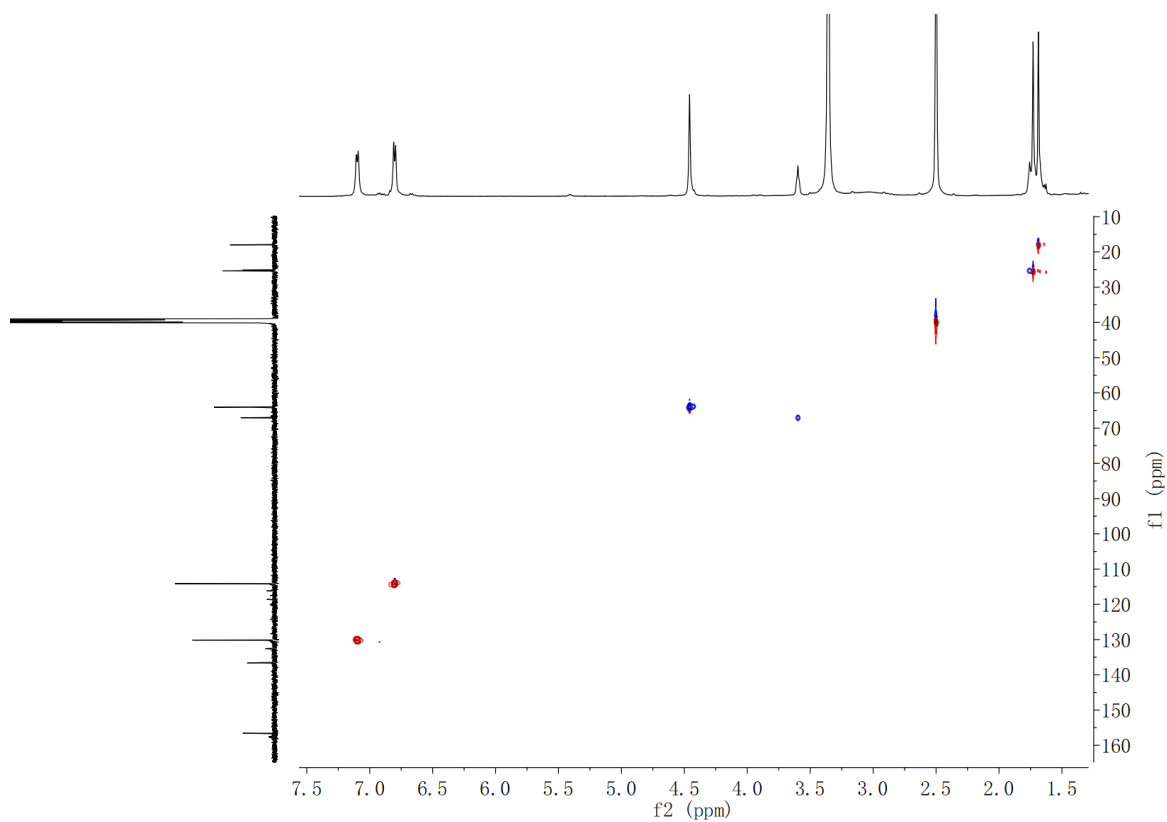
Supplementary Fig. 95. NOESY NMR spectrum of compound **20** in CD₃OD



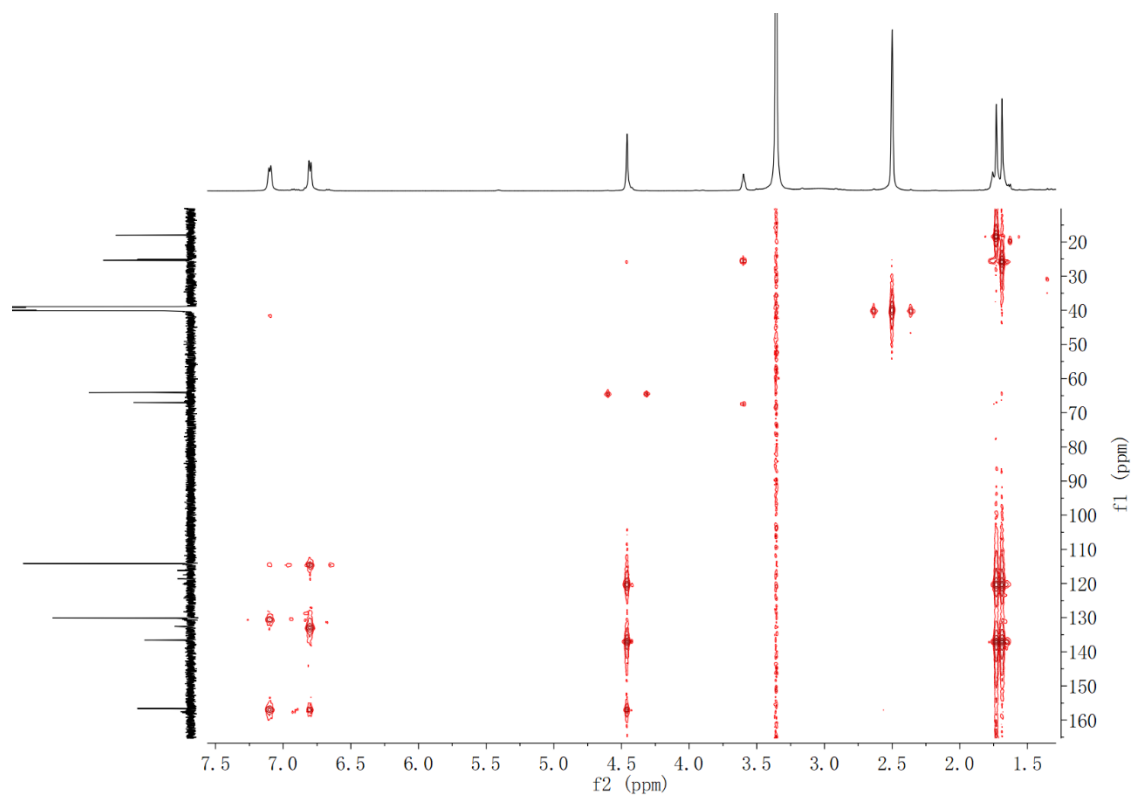
Supplementary Fig. 96. ¹H NMR spectrum of compound **2'-[d₁]-7** in DMSO-*d*₆



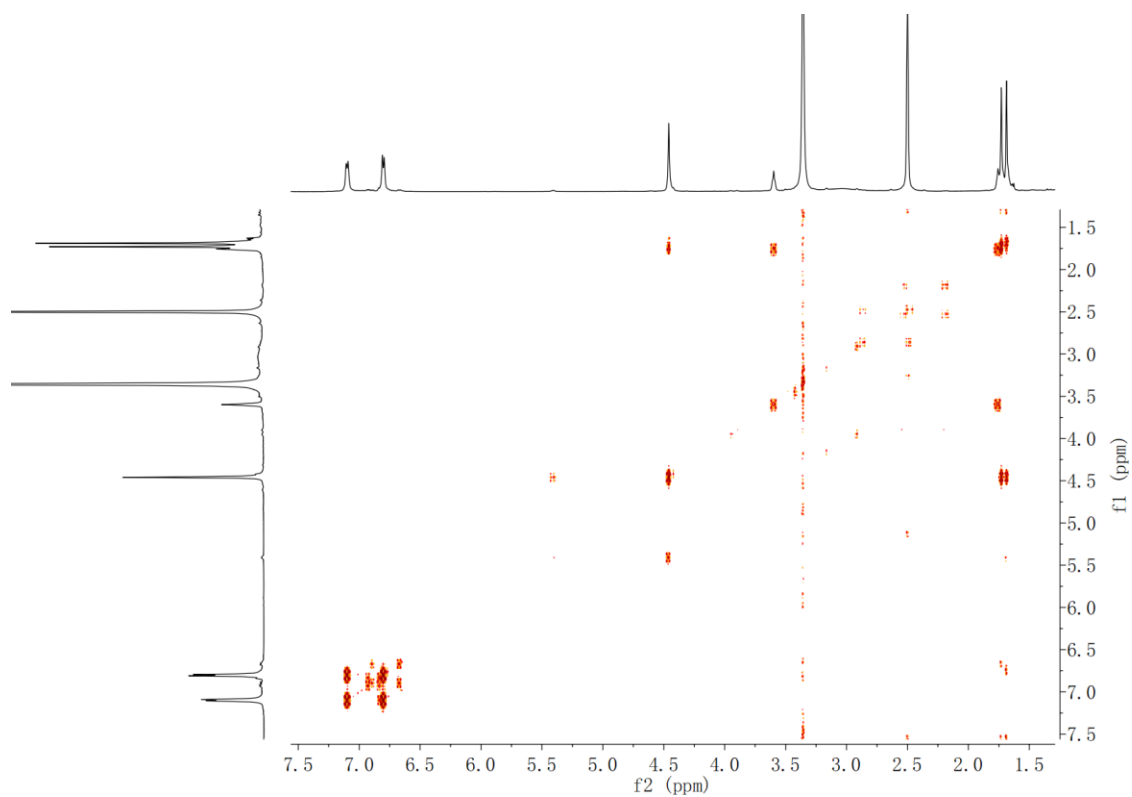
Supplementary Fig. 97. ^{13}C NMR spectrum of compound $2'-[d_1]-7$ in $\text{DMSO}-d_6$



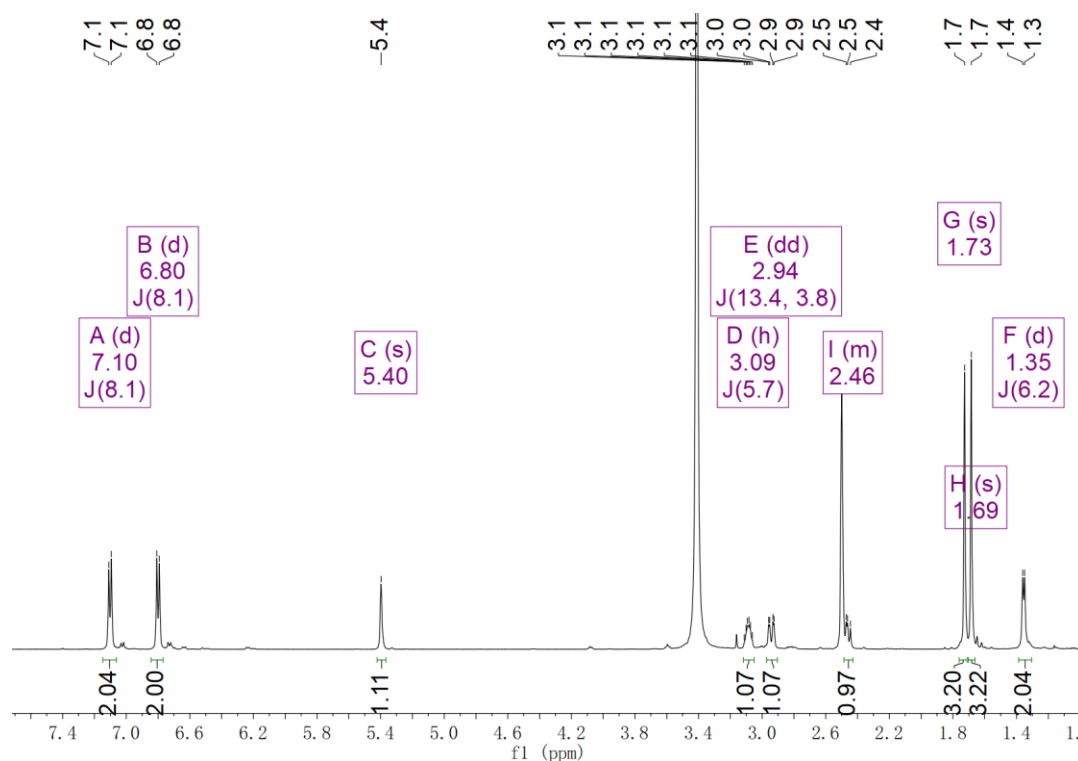
Supplementary Fig. 98. HSQC spectrum of compound $2'-[d_1]-7$ in $\text{DMSO}-d_6$



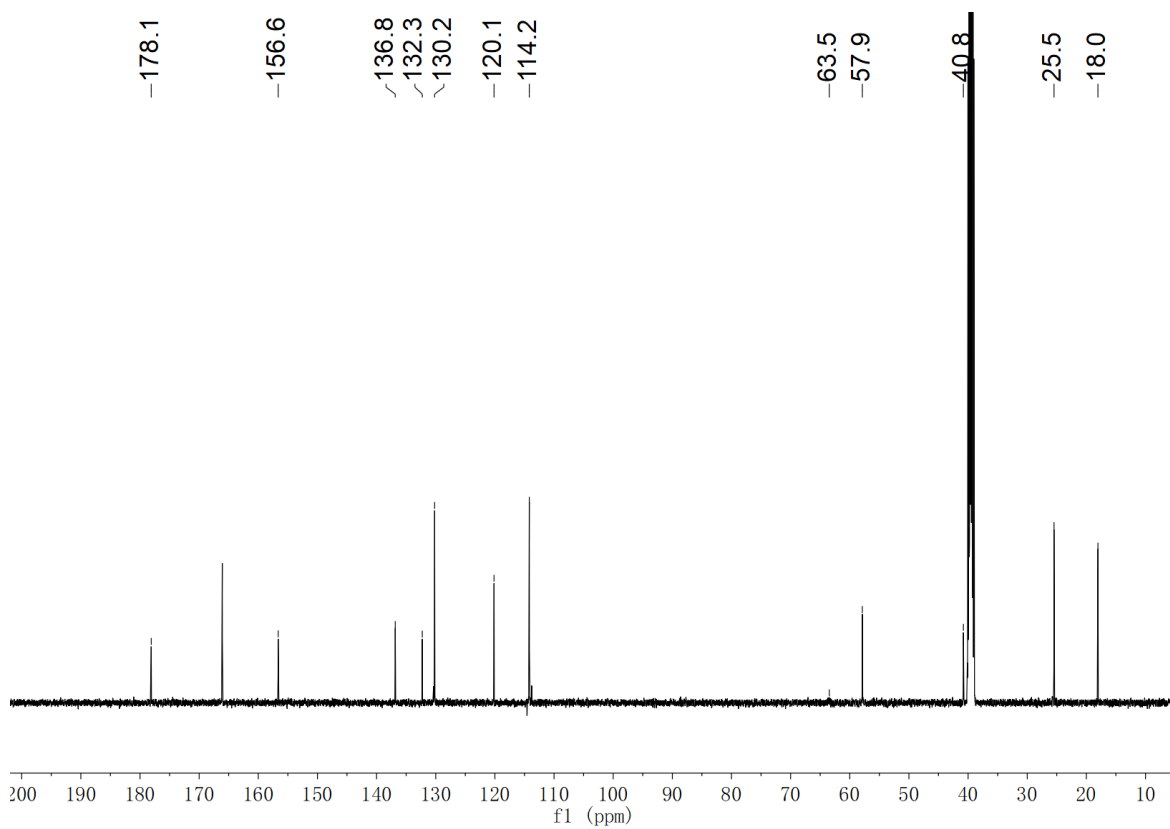
Supplementary Fig. 99. HMBC NMR spectrum of compound **2'-[d₁]-7** in DMSO-*d*₆



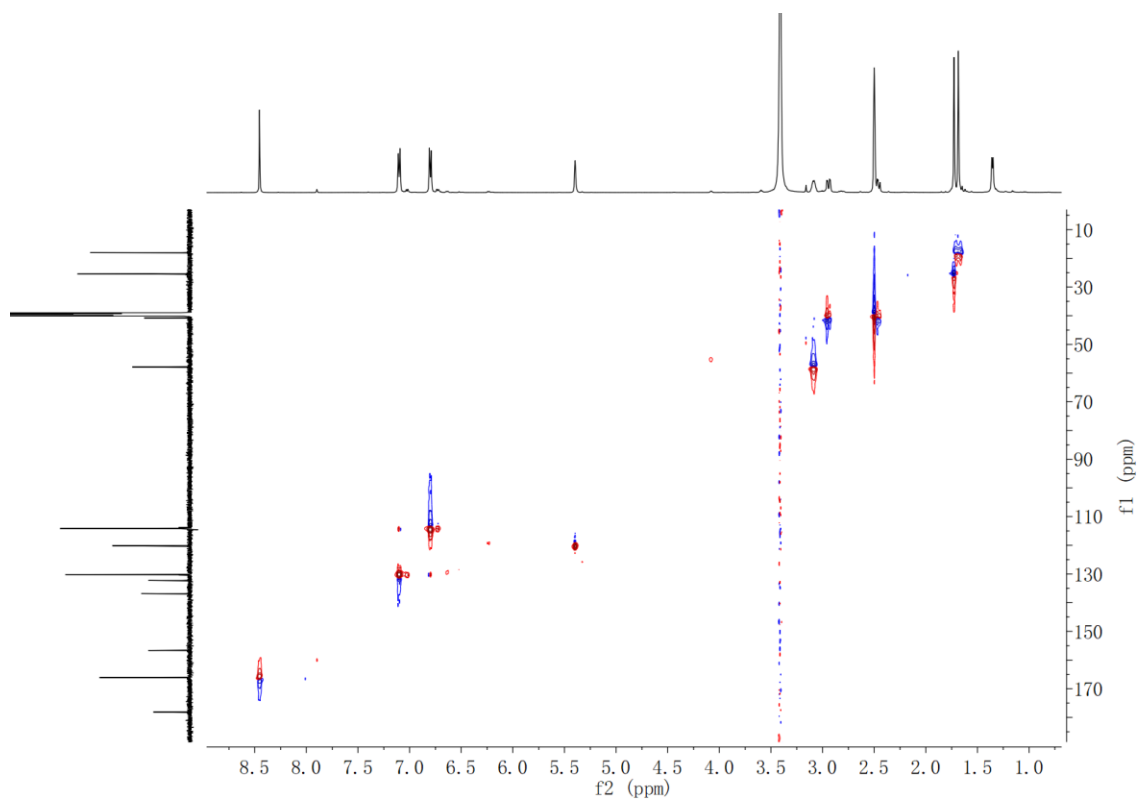
Supplementary Fig. 100. ¹H-¹H COSY spectrum of compound **2'-[d₁]-7** in DMSO-*d*₆



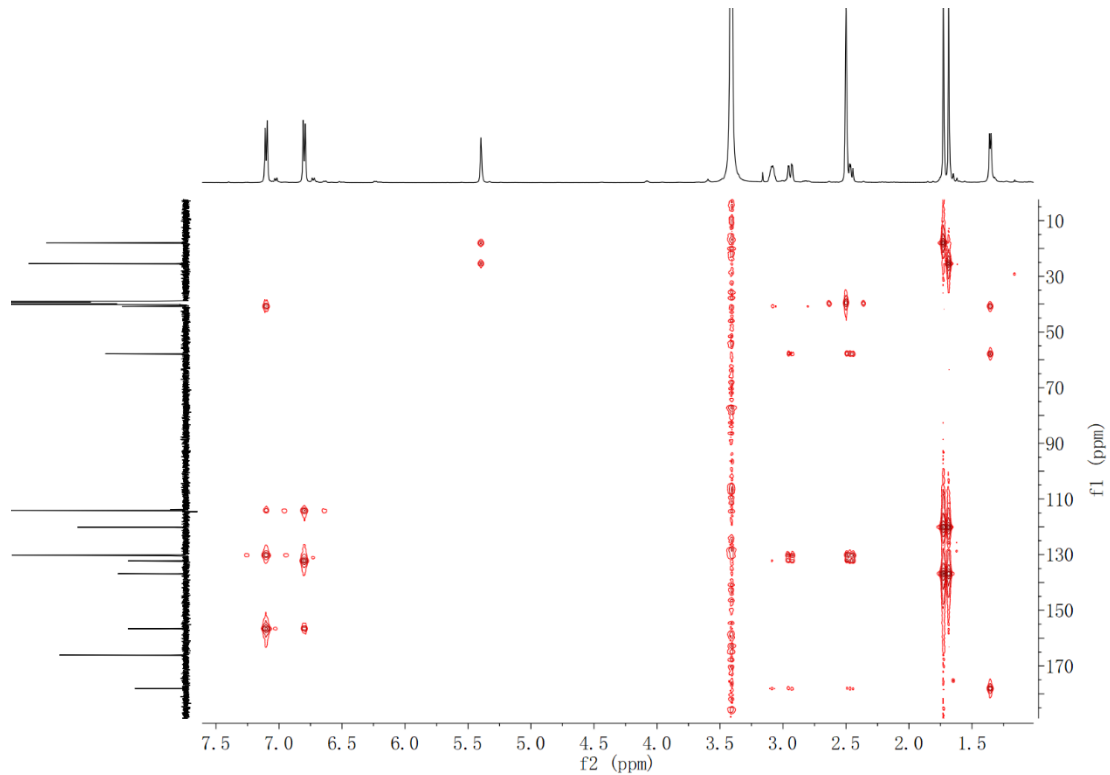
Supplementary Fig. 101. ¹H NMR spectrum of compound 1',1'-[d₂]-7 in DMSO-*d*₆



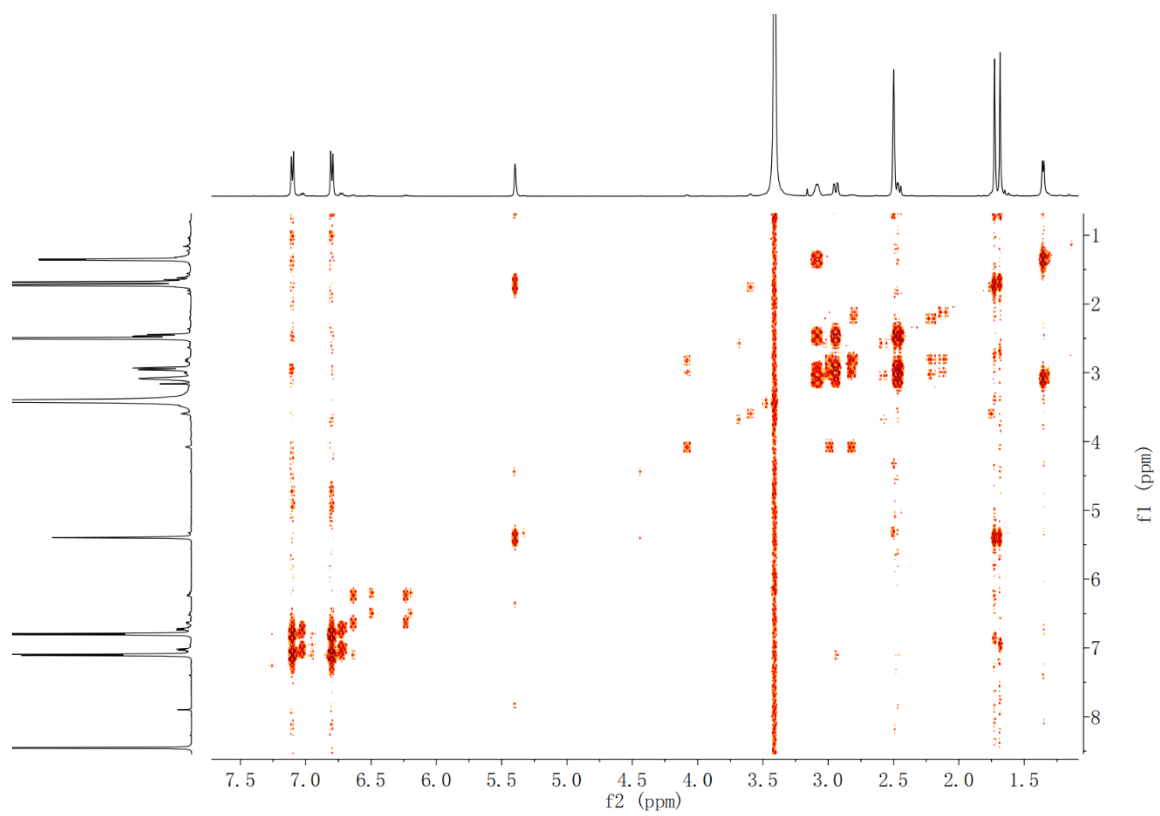
Supplementary Fig. 102. ¹³C NMR spectrum of compound 1',1'-[d₂]-7 in DMSO-*d*₆



Supplementary Fig. 103. HSQC spectrum of compound **1',1'-[d₂]-7** in DMSO-*d*₆



Supplementary Fig. 104. HMBC spectrum of compound **1',1'-[d₂]-7** in DMSO-*d*₆



Supplementary Fig. 105. ^1H - ^1H COSY spectrum of compound **1',1'-[d₂]-7** in $\text{DMSO-}d_6$

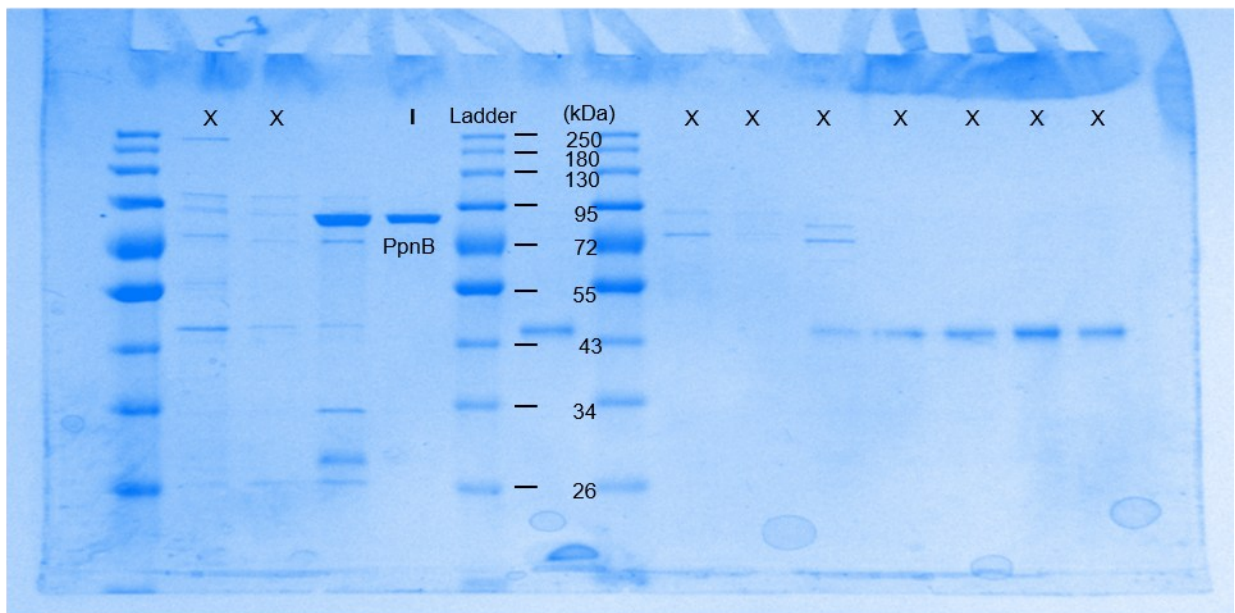
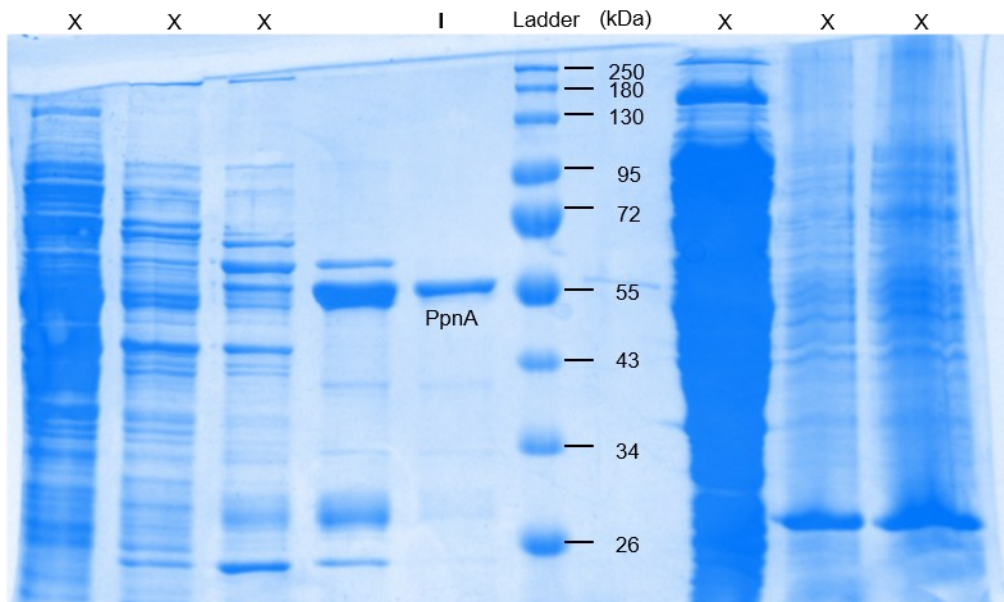
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Uncropped SDS-PAGE.

Uncropped SDS-PAGE used in Supplementary Fig. 7. **I**: the lane is used in Supplementary Fig. 7. **X**: the lane is irrelevant sample.