Electronic Supporting Information

Generation of two novel furanosteroids via heterologous expression of demethoxyviridin biosynthetic genes in a wortmannin-producing strain

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Supplementary Methods

General materials and experimental procedures

Oligonucleotide primers were synthesized by Tsingke Biotech Co., Ltd. (China). 2×Phanta Flash Master Mix (Vazyme, China) was used for gene amplification. PCR was conducted using an A100 thermal cycler (LongGene, China) and 2×Phanta Flash Master Mix (Vazyme, China). Plasmids are digested by FastDigest^{TM} enzymes (Thermo Scientific, USA), and then ligated with PCR products using the ClonExpress® II One Step Cloning Kit (Vazyme, China) or the ClonExpress® Multis One Step Cloning Kit.

HPLC-MS analysis was carried out using a Dionex UltiMate 3000 HPLC system (Thermo Scientific, USA) and an amaZon SL ion trap mass spectrometer coupled with an electrospray ionization (ESI) source (Bruker, USA). A Cheetah Pro System (Agela Technologies, China) with ODS (50 μm, YMC, Japan) was employed for medium-pressure liquid chromatography (MPLC). The semi-preparative HPLC was performed on a Dionex UltiMate 3000 HPLC system. The HRESIMS data were recorded by SYNAPT G2 high definition mass spectrometer (Waters, USA). NMR measurements were conducted using the Bruker Avance 400/600 spectrometers (Bruker, USA), and the solvent signals ($CDCl_3$: δ_H 7.26/δ_C 77.0) were used as the internal references.

Strains and media

Talaromyces wortmannii ATCC 26942 purchased from American Type Culture Collection was grown in the liquid maltose medium (3% maltose, 0.15% yeast extract, 0.2% KH_2PO_4, 0.4% $CaCO_3$, 0.1% $MgSO_4$, 0.25% malt extract) for production of wortmannin and its analogues. *Nodulisporium* sp. (no. 65-12-7-1) deposited in our lab was cultured in the liquid ME medium (2% malt extract, 0.1% polypeptone, 2% glucose) for genomic DNA extraction. *Escherichia coli* DH5α (Takara, Japan) for construction of recombinant plasmids was cultured in LB medium with 100 mg/L ampicillin.

Construction of recombinant plasmids

The GAPDH promoter was amplified from the genomic DNA of *T. wortmannii* ATCC 26942, and then inserted into the EcoRV-linearized pBlue, together with the *trpC* terminator amplified from pBSKII-PtrPC-EcoRV-TtrPC to construct the plasmid pBSKII-Pro(*Tw*GAPDH)-EcoRV-TtrpC. The hygromycin B phosphotransferase gene was amplified from pBSKII-*toCas9-hph*, and then ligated with EcoRV-digested pBSKII-Pro(*Tw*GAPDH)-EcoRV-TtrpC, resulting in the plasmid pBSKII-Pro(*Tw*GAPDH)-Hyg. Similarly, *vidD* and *vidA*, which were obtained through PCR amplification from the genomic DNA of *Nodulisporium* sp., were introduced into pBSKII-Pro(*Tw*GAPDH)-EcoRV-TtrpC, respectively. The resulting plasmids pBSKII-Pro(*Tw*GAPDH)-VidD and pBSKII-Pro(*Tw*GAPDH)-VidA were then utilized as the templates for the individual amplification of the *vidD* expression cassette and the *vidA* expression cassette. Finally, the *vidD* expression cassette was cloned into pBSKII-Pro(*Tw*GAPDH)-Hyg to give pBSKII-Pro(*Tw*GAPDH)-Hyg-VidD, and the two expression cassettes were co-inserted into pBSKII-

Pro(*Tw*GAPDH)-Hyg to give pBSKII-Pro(*Tw*GAPDH)-Hyg-VidD-VidA. The primers and plasmids used in the study are individually listed in Tables S1 and S2.

Transformation of *T. wortmannii* ATCC 26942

PEG-mediated protoplast transformation was utilized for transformation of *T. wortmannii*. The mycelia of *T. wortmannii* were inoculated into 10 mL of PDB medium and cultured at 220 rpm and 28 °C for two days. Subsequently, the resulting seed broth was transferred into 100 mL of PDB medium and cultured for one more day under the same condition. After that, the mycelia were harvested and subjected to digestion of cell walls using the lyase solution (0.5% Yatalase, 0.5% Driselase, 4.1% NaCl, pH 5.5). After incubation at 30 °C with shaking for three hours, the lysate was filtered to remove the cell debris. The resulting filtrate was subjected to centrifugation at 1500 rpm for 10 min, and the supernatant was then discarded. Subsequently, the pellet was washed once with TF Solution 2 (1.2 M sorbitol, 50 mM $CaCl_2 \cdot 2H_2O$, 35 mM NaCl, 10 mM Tris-HCl, pH 7.5), and the protoplast concentration was adjusted to around 1.0×10^7 cells mL^{-1} with TF Solution 2. Approximately 10 ng of plasmids (~ 10-20 μL) was added to a 200 μL protoplast suspension and gently mixed by pipetting. After the mixture was incubated on ice for 30 min, a total of 1.35 mL of TF Solution 3 (60% PEG4000, 50 mM $CaCl_2 \cdot 2H_2O$, 10 mM Tris-HCl, pH 7.5) was added in three times, followed by incubation at room temperature for 20 min. With addition of 5 mL of TF Solution 2, the mixture was then subjected to centrifugation at 1500 rpm for 10 min. The resulting precipitate was resuspended in 200 μL of TF Solution 2 and spread on the under-layer selective medium (0.2% NH_4Cl, 0.1% $(NH_4)_2SO_4$, 0.05% KCl, 0.05% NaCl, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.002% $FeSO_4 \cdot 7H_2O$, 2% glucose, 1.2 M sorbitol, 0.1% hygromycin B, 1.5% agar), followed by being covered with the upper-layer selective medium (0.2% NH_4Cl, 0.1% $(NH_4)_2SO_4$, 0.05% KCl, 0.05% NaCl, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.002% $FeSO_4 \cdot 7H_2O$, 2% glucose, 1.2 M sorbitol, 0.05% hygromycin B, 0.8% agar). And the transformants could be obtained after incubation at 30 °C for 7-10 days.

HPLC analysis of the metabolites from *T. wortmannii* ATCC 26942 and its transformants

After the strain growing in the Maltose medium for 4 days, the culture broth (100 mL) was extracted with ethyl acetate, and then concentrated under vacuum. The crude extract was resuspended in 1 mL of methanol for HPLC-MS analysis. A Cosmosil $5C_{18}$-MS-II column (4.6 mm i.d. × 250 mm, 5 μm) was employed, and the elution was subjected to a linear gradient [H_2O containing 0.1% formic acid (A) and CH_3OH (B); 1 mL min^{-1}; 35%-80% B (0-40 min), 100% B (40-50 min)].

Isolation and purification of 1-4

1 and **2**: 8 L of *T. wortmannii* ATCC 26942 culture broth was harvested and extracted with ethyl acetate. The resulting crude extract was subjected to MPLC with stepwise elution of MeOH-H_2O. The fraction containing compounds **1** and **2** was purified by semi-preparative HPLC using a YMC-Pack ODS-A column (10.0 mm i.d. × 250 mm, 5 μm) with isocratic elution of 40% MeOH-H_2O at the rate of 3 mL min^{-1}, resulting in the successful isolation of compounds **1** and **2**.

3 and **4**: 8 L of the *T. wortmannii* transformant possessing *vidD* culture broth was harvested and extracted with ethyl acetate. The resulting crude extract was subjected to MPLC with stepwise elution of MeOH-H_2O. The fraction containing compound **3** was purified by semi-preparative HPLC using a YMC-Pack ODS-A column (10.0 mm i.d. × 250 mm, 5 μm) with isocratic elution of 40% MeOH-H_2O at the rate of 3 mL min^{-1}, resulting in isolation of **3**. The fraction containing compound **4** was purified by semi-preparative HPLC using a YMC-Pack ODS-A column (10.0 mm i.d. × 250 mm, 5 μm) with isocratic elution of 40% MeOH-H_2O at the rate of 3 mL min^{-1}, resulting in isolation of **4**.

Structural characterization

Wortmannin (**1**): Yellow amorphous crystal; The ^{13}C NMR spectrum, see Fig. S1; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 216.2, 172.7, 169.5, 157.6, 150.0, 149.6, 144.8, 142.9, 140.4, 114.3, 88.5, 72.9, 70.1, 59.5, 49.2, 44.1, 40.8, 36.1, 35.7, 26.5, 22.9, 21.1, 14.6. The NMR data are in good agreement with the reported values.[1]

11-Desacetoxywortmannin (**2**): Yellow amorphous crystal; The ^{13}C NMR spectrum, see Fig. S2; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 218.4, 173.5, 157.9, 155.3, 149.2, 145.4, 142.3, 135.9, 113.5, 86.4, 71.8, 59.5, 47.8, 44.1, 40.2, 36.5, 27.7, 26.8, 25.0, 22.7, 14.0. The NMR data are in good agreement with the reported values.[2]

11-Dehydro-11-desacetoxywortmannin (**3**): Yellowish crystal; HRESIMS (positive): *m/z* 369.1353 $[M + H]^+$ (calcd. for $C_{21}H_{21}O_6$, 369.1338); NMR data, see Table S3; NMR spectra, see Figs. S3-S8.

(11*S*,12*R*)-11,12-epoxy-11-desacetoxywortmannin (**4**): Yellowish powder; HRESIMS (positive): *m/z* 385.1300 $[M + H]^+$ (calcd. for $C_{21}H_{21}O_7$, 385.1287); NMR data, see Table S4; NMR spectra, see Figs. S9-S14.

X-ray crystallographic analysis

11-Dehydro-11-desacetoxywortmannin (**3**): Upon crystallization from MeOH using the vapor diffusion method, yellowish crystals of **3** were obtained. Data were collected using a Bruker D8 Venture with Cu Kα radiation, λ = 1.54184 Å at 153.15 K. Crystal data: $C_{21}H_{22}O_7$, M = 386.38, space group monoclinic, P 2_1; unit cell dimensions were determined to be a = 9.6221 (2) Å, b = 8.1595 (2) Å, c = 11.5469 (3) Å, α = 90 °, β = 91.6252 (9) °, γ = 90 °, V = 906.20 (4) $Å^3$, Z = 2, Dx = 1.416 g/cm^3, F (000) = 408.0, μ (Cu Kα) = 0.889 mm^{-1}. 34765 reflections were collected to θ_{max} = 68.248 °, in which 3292 independent unique reflections (R_{int} = 0.0471 R_{sigma} = 0.0224) were used in all calculations. The structure was solved by direct methods using the SHELXS program, and refined by the SHELXL program and full-matrix least-squares calculations.[3] In the structure refinements, hydrogen atoms were placed on the geometrically ideal positions by the "ride on" method. The final refinement gave R_1 = 0.0355 ($I > 2\sigma(I)$), $_wR_2$ = 0.0944 (all data), S = 1.102, Flack = 0.08 (4) and Hooft = 0.10 (4). Crystallographic data for 11-dehydro-11-desacetoxywortmannin (**3**) have been deposited in the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 2450214. Copies of the data can be obtained, free of charge, on application

to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

^{13}C chemical shift calculation of (1*S*,10*R*,11*S*,12*R*,13*R*,14*S*)-4 and (1*S*,10*R*,11*R*,12*S*,13*R*,14*S*)-4

The SMILES codes of the molecules (1*S*,10*R*,11*S*,12*R*,13*R*,14*S*)-**4** and (1*S*,10*R*,11*R*,12*S*,13*R*,14*S*)-**4** were generated prior to the creation of their initial 3D structures using CORINA version 3.4. Subsequently, conformer databases were constructed with CONFLEX version 7.0, employing the MMFF94s force field. The parameters for conformer generation were set as follows: an energy window (ewindow) of 5 kcal/mol above the ground state, a maximum number of conformations per molecule (maxconfs) of 100, and an RMSD cutoff (rmsd) of 0.5 Å. Each acceptable conformer was subsequently optimized using the HF/6-31G(d) method in Gaussian 09.[4] Further refinement at the wB97XD/6-31G(d) level was conducted to determine the dihedral angles. As a result, stable conformers were identified [seven for (1*S*,10*R*,11*S*,12*R*,13*R*,14*S*)-**4** and five for (1*S*,10*R*,11*R*,12*S*,13*R*,14*S*)-**4**] (Fig. S15). These conformers were then utilized for ^{13}C NMR calculations in Gaussian 09 at the mPW1PW91/6-31+G(d) level. Deuterated chloroform was employed as the solvent, and solvent effects were accounted for using the IEFPCM solvent model. Tetramethylsilane (TMS) served as the reference standard. The comparison between calculated and experimental values was evaluated using R square (R^2) analysis, mean absolute error (MAE), and DP4+ probability (Table S5).[5]

Antifungal activity assay

Antifungal activity was evaluated in 96-well microtiter plates using a two-fold dilution method. *Candida albicans* FIM709 and *Aspergillus niger* R330 were cultured on Sabouraud dextrose agar medium (2% glucose, 1% peptone, and 1.8% agar) at 32 °C for 4–7 days. Spores were harvested and suspended in 0.9% saline to achieve a concentration of 10^7-10^9 cells/mL. Subsequently, 100 μL of the spore suspension was added to 200 mL of Sabouraud dextrose broth to prepare the spore solution. Test compounds were dissolved in dimethyl sulfoxide (DMSO) to a stock concentration of 12.5 mg/mL. A volume of 2 μL of the sample solution was mixed with 200 μL of the spore solution, followed by stepwise two-fold serial dilutions with the spore solution until the final concentration reached 0.06 μg/mL. Amphotericin B served as the positive control, while DMSO acted as the negative control. The microtiter plates were incubated at 32 °C for 48 hours. The minimum inhibitory concentration (MIC) was determined as the lowest concentration at which no visible growth of fungi was observed.

The DNA sequence of the GAPDH promoter from *T. wortmannii*

GATATGCACTGGACTGTCTGTATTCTTACATTGCATTCTTCCAACGCATACATTAC
CGGAATAGTAATTTGATAATCTCACTGAAGTAATTGTTCAATGTTAAAAGAGTAGTGT
GTCAGCTGGTGATCAGAGGCTGCCGGTGAGACATTGCGGAGAAGATCAATGAACAAG
AAATATGTGTAGGTGAATTGTGATATTAATAATAATTGGCAGCGCAATCATCCAATTA
TGCACGCTCTAGCCACATCGATCGTTCAAACGTTGTCCATGGCCAATTGATCCAGGAA
CGGCGATGCAATTTGCTCGCCGGTGAGAGCATCGATCAGCGACTAGTCGCATTCAAT

CTTACGTGTATCGTATGCGTACAGACGCTGGTACGTATGATAATTAGTGAGCATCTGT
CGGATGCAGCGTGCCGTCCATGTACATATGTATCAAATGATCTCTACAACGTGGCACA
CATGGAACACATGTATATCAATTGATGATATATGTATACGGACATGCACGGAACGCA
TGGCTCTTCGCCTCGGCAATTCTGCGACTACATAGCCTAGGCTATACACAGTATACAC
GAGTCTTTATCGTCGCGTCGGAAGCGCAGCACAAATGCTATTGGTCTCTGCCCGCGCG
CTTCCTGTTTTGCCCGGTGTATGAAACCGGAAAGCGTCGCTGGCGGATGCGGCAATTC
GCACTTCGGGTCGCCCGCCTGAGGGTAGCTCTGTTTTGGTATTACCGTATTTATGACC
TCACCGAGTCTGCCTCTTTGGGTCGACCCCGCCACCTGCTGCCCAGCTTCTCTTTTTAA
GCCTGCCGTGTCCTCTGCCTTTTTCTCTCTCTCTCTTCTCTCTTTTTCTGCTAGCTCCAT
CATCACCTCCCAGCCTATTCTATCCCAGCTGTAATTCCAAGTGAGTGTTCTCCTGGAT
GCAATGCTCCGCCCATTGCTGTGTCTGTACCCCGCTGACAGTGCCCCGCGCAGAGCTA
GCTCATCACCGCAAAT

Supplementary Tables

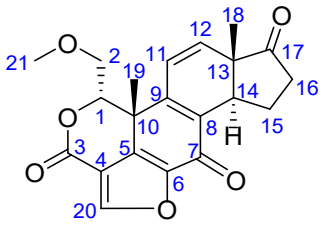
Table S1 The primers used in this study

Primer	Sequence (5' to 3')	Usage
Pro(WTMN)-F	ATCGATAAGCTTGATGATATGCACTGGACTGTCTG	Cloning the GAPDH promoter from the genomic DNA of *T. wortmannii* ATCC 26942 to construct pBSKII-Pro(*Tw*GAPDH)-EcoRV-TtrpC
Pro(WTMN)-R	CGGTCGGCATCTACTGATATCATTTGCGGTGATGAGCTAGC	
TtrPC-F	AGTAGATGCCGACCGGGATC	Cloning the *trpC* terminator from pBSKII-PtrPC-EcoRV-TtrPC to construct pBSKII-Pro(*Tw*GAPDH)-EcoRV-TtrpC
TtrPC-R2	CTGCAGGAATTCGATCAGGGCTGGTGACGGAATTT	
EcoRV-Hyg-F1	ACCGCAAATGATATCATGAAAAAGCCTGAACTCAC	Cloning *hyg* from pBSKII-*toCas9-hph* to construct pBSKII-Pro(*Tw*GAPDH)-Hyg
EcoRV-Hyg-R1	GCATCTACTGATATCCTATTCCTTTGCCCTCGGAC	
pBSK2-Pro-vidD-F	ACCGCAAATGATATCATGGCTCTTCAGGATAACAC	Cloning *vidD* from the genomic DNA of *Nodulisporium* sp. 65-12-7-1 to construct pBSKII-Pro(*Tw*GAPDH)-VidD
pBSK2-Pro-vidD-R	GCATCTACTGATATCTCACGAGGTGACCTTTACAAG	
EcoRV-vidA-F	ACCGCAAATGATATCATGGCTTTCCCTTCGCTCTC	Cloning *vidA* from the genomic DNA of *Nodulisporium* sp. 65-12-7-1 to construct pBSKII-Pro(*Tw*GAPDH)-VidA
EcoRV-vidA-R	GCATCTACTGATATCCTATATCAACTTGACCGGCC	
pBSK2-HindIII-Pro-F	GGTATCGATAAGCTTTGATGATATGCACTGGACTGTCTG	Cloning the *vidD* expression cassette from pBSKII-Pro(*Tw*GAPDH)-VidD to construct pBSKII-Pro(*Tw*GAPDH)-Hyg-VidD
pBSK2-HindIII-Ttrpc-R	CATATCATCAAGCTTCAGGGCTGGTGACGGAATTT	
SmaI-vidA-F	TTCCTGCAGCCCGGGGATATGCACTGGACTGTCTG	Cloning *vidA* expression cassette from pBSKII-Pro(*Tw*GAPDH)-VidA to construct pBSKII-Pro(*Tw*GAPDH)-Hyg-VidD-VidA
SmaI-vidA-R	AGTGGATCCCCCGGGCAGGGCTGGTGACGGAATTTTC	

Table S2 The plasmids used in this study

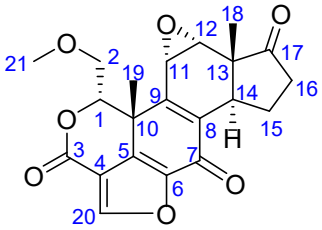
Plasmid	Characteristic	Source
pBSKII-PtrPC-EcoRV-TtrPC	The plasmid containing the *trpC* promoter and terminator	Y.-M. Zheng, et al.[6]
pBSKII-Pro(*Tw*GAPDH)-EcoRV-TtrpC	The plasmid containing the *GAPDH* promoter and the *trpC* terminator	This work
pBSKII-Pro(*Tw*GAPDH)-Hyg	The plasmid containing the *hyg* marker gene, which is under the control of the *GAPDH* promoter	This work
pBSKII-Pro(*Tw*GAPDH)-VidD	The plasmid containing *vidD*, which is under the control of the *GAPDH* promoter	This work
pBSKII-Pro(*Tw*GAPDH)-VidA	The plasmid containing *vidA*, which is under the control of the *GAPDH* promoter	This work
pBSKII-Pro(*Tw*GAPDH)-Hyg-VidD	The plasmid containing the *hyg* marker gene and *vidD*, both of which are individually under the control of the *GAPDH* promoter	This work
pBSKII-Pro(*Tw*GAPDH)-Hyg-VidD-VidA	The plasmid containing the *hyg* marker gene, *vidD*, and *vidA*, all of which are individually under the control of the *GAPDH* promoter	This work

Table S3. The NMR data of 3 in $CDCl_3$ (^1H at 400 MHz and ^{13}C at 100 MHz)



No.	δ_C, type	δ_H (J in Hz)[a]	^1H–^1H COSY	HMBC	NOESY
1	86.0, CH	4.91, br s	2	3, 5	11, 19
2	71.9, CH_2	3.18, br s	1		11
3	157.9, C				
4	113.7, C				
5	141.5, C				
6	145.6, C				
7	173.3, C				
8	133.4, C				
9	150.9, C				
10	38.7, C				
11	123.7, CH	6.12, d (9.2)	12	8, 9, 10, 13	1, 2, 19
12	141.4, CH	6.79, d (9.2)	11	9, 11, 13, 14, 17	
13	49.7, C				
14	43.7, CH	2.89	15a, 15b	8, 9, 12, 15, 17, 18	
15	21.2, CH_2	a: 2.93	14, 15b, 16a, 16b	13, 14, 17	
		b: 2.37	14, 15a, 16a, 16b	13, 14, 16, 17	
16	37.2, CH_2	a: 2.62	15a, 15b, 16b	14, 17	
		b: 2.37	15a, 15b, 16a	15, 17	
17	213.5, C				
18	12.6, CH_3	0.89, s		12, 13, 14, 17	19
19	27.8, CH_3	1.67, s		1, 5, 9, 10	1, 11, 18
20	149.1, CH	8.19, s		4, 5, 6	
21	59.3, CH_3	3.05, s		2	

Table S4. The NMR data of 4 in $CDCl_3$ (^1H at 600 MHz and ^{13}C at 150 MHz)



No.	δ_C, type	δ_H (J in Hz)[a]	^1H–^1H COSY	HMBC	NOESY
1	85.9, CH	5.02, br s	2a, 2b	2, 3, 5, 10, 19	11, 19
2	71.9, CH_2	3.44, br d (9.6)	1, 2b	10, 21	
		3.38, br d (9.6)	1, 2a	1, 10, 21	
3	157.7, C				
4	113.7, C				
5	142.5, C				
6	145.0, C				
7	172.5, C				
8	137.5, C				
9	150.6, C				
10	39.7, C				
11	49.2, CH	3.57, br s	12	8, 9, 10, 12	1, 19
12	57.7, CH	3.84, br s	11	13, 14	18
13	50.2, C				
14	38.1, CH	3.04	15a, 15b	8, 9, 12, 13, 15, 17, 18	
15	21.5, CH_2	a: 3.00	14, 15b, 16a, 16b	13, 14, 17	
		b: 2.29	14, 15a, 16a, 16b	14	18
16	37.3, CH_2	a: 2.60, br dd (19.8, 7.2)	15a, 15b, 16b	14, 15, 17	
		b: 2.36	15a, 15b, 16a	15, 17	
17	215.0, C				
18	12.4, CH_3	0.87, s		12, 13, 14, 17	12, 15b, 19
19	26.4, CH_3	1.74, s		1, 5, 9, 10	1, 11, 18
20	149.5, CH	8.21, s		4, 5, 6	
21	59.5, CH_3	3.11, s		2	

Table S5. Comparison of the ^{13}C NMR data of 4 with those calculated values of two putative stereoisomers

No.	**4**	(1*S*,10*R*,11*S*,12*R*,13*R*,14*S*) –**4**	AE	(1*S*,10*R*,11*R*,12*S*,13*R*,14*S*) –**4**	AE
C-1	85.9	84.0	1.9	83.9	2.0
C-2	71.9	70.0	1.9	69.6	2.3
C-3	157.7	156.2	1.5	156.0	1.7
C-4	113.7	112.2	1.5	111.9	1.8
C-5	142.5	141.9	0.6	142.0	0.5
C-6	145.0	142.9	2.1	143.4	1.6
C-7	172.5	170.4	2.1	170.9	1.6
C-8	137.5	138.3	0.8	138.5	1.0
C-9	150.6	154.6	4.0	153.3	2.7
C-10	39.7	42.0	2.3	42.8	3.1
C-11	49.2	50.8	1.6	48.4	0.8
C-12	57.7	58.2	0.5	55.8	1.9
C-13	50.2	53.5	3.3	52.6	2.4
C-14	38.1	39.2	1.1	45.1	7.0
C-15	21.5	22.7	1.2	22.9	1.4
C-16	37.3	38.5	1.2	37.2	0.1
C-17	215.0	218.5	3.5	219.0	4.0
C-18	12.4	11.2	1.2	11.6	0.8
C-19	26.4	24.7	1.7	24.8	1.6
C-20	149.5	147.1	2.4	147.4	2.1
C-21	59.5	56.9	2.6	56.6	2.9
MAE		1.85		2.06	
R^2		0.9987		0.9981	
DP4+		100%		0%	

Supplementary Figures

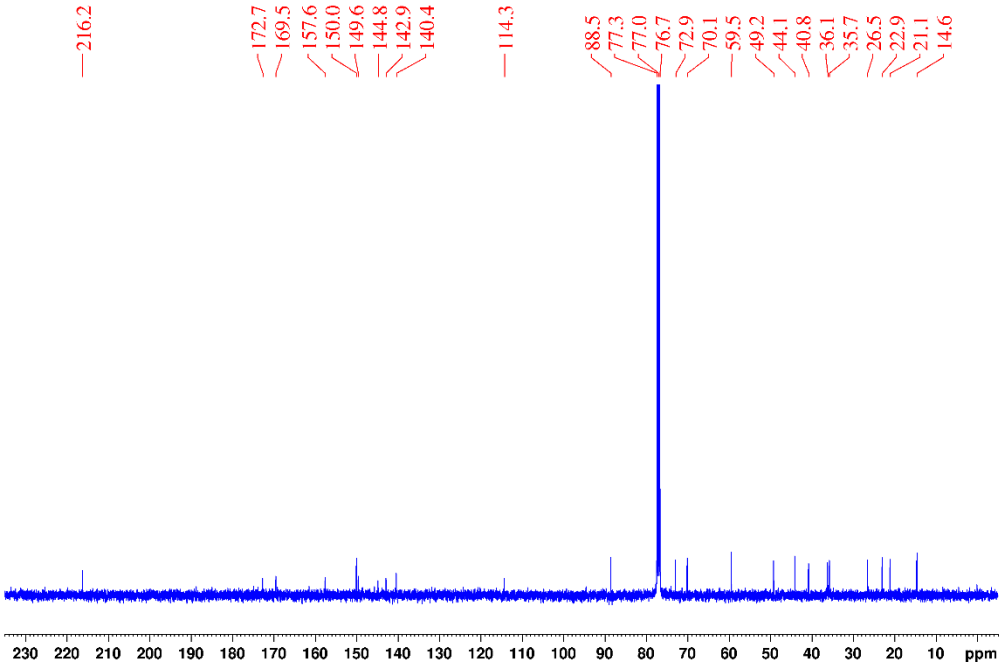


Figure S1. The ^{13}C NMR spectrum of 1 in $CDCl_3$ at 400 MHz

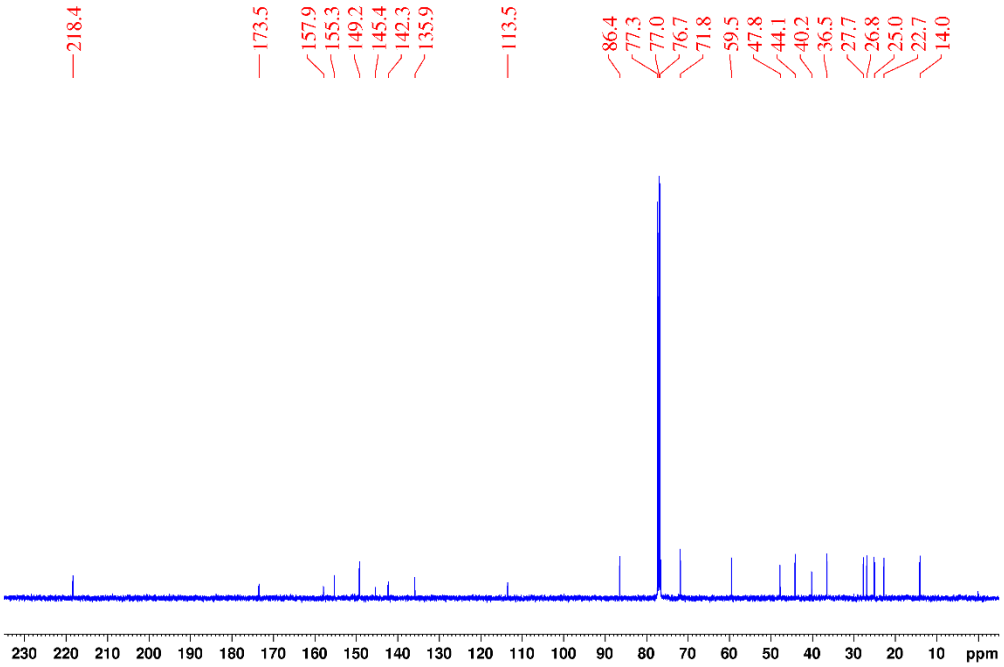


Figure S2. The ^{13}C NMR spectrum of 2 in $CDCl_3$ at 400 MHz

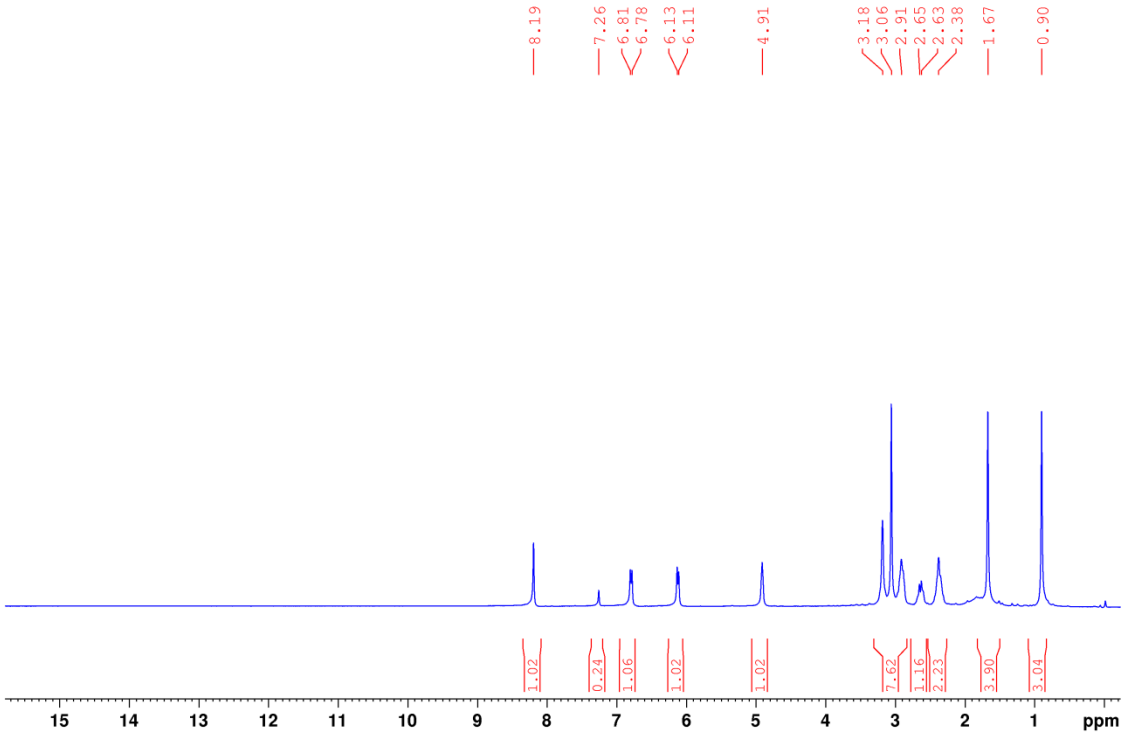


Figure S3. The ^{1}H NMR spectrum of 3 in $CDCl_3$ at 400 MHz

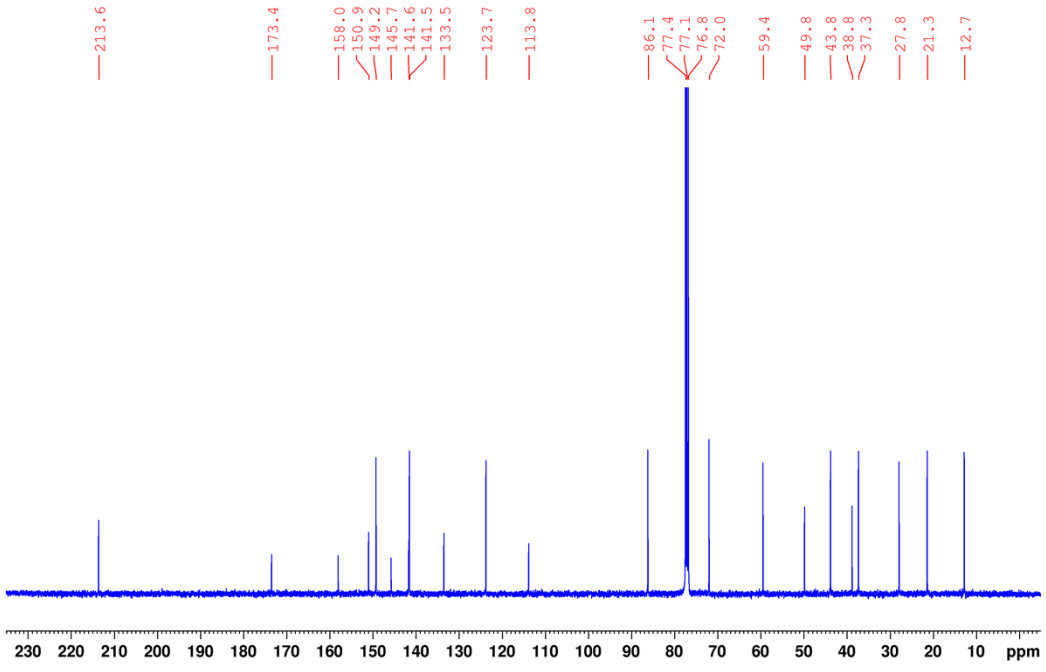


Figure S4. The ^{13}C NMR spectrum of 3 in $CDCl_3$ at 100 MHz

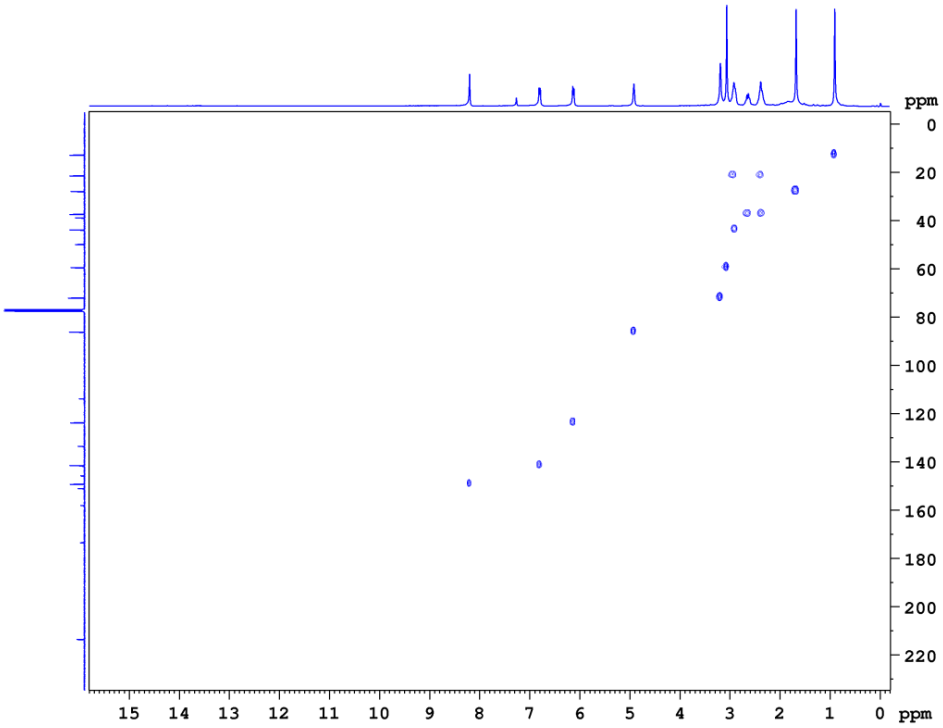


Figure S5. The HSQC spectrum of 3 in $CDCl_3$ at 400 MHz

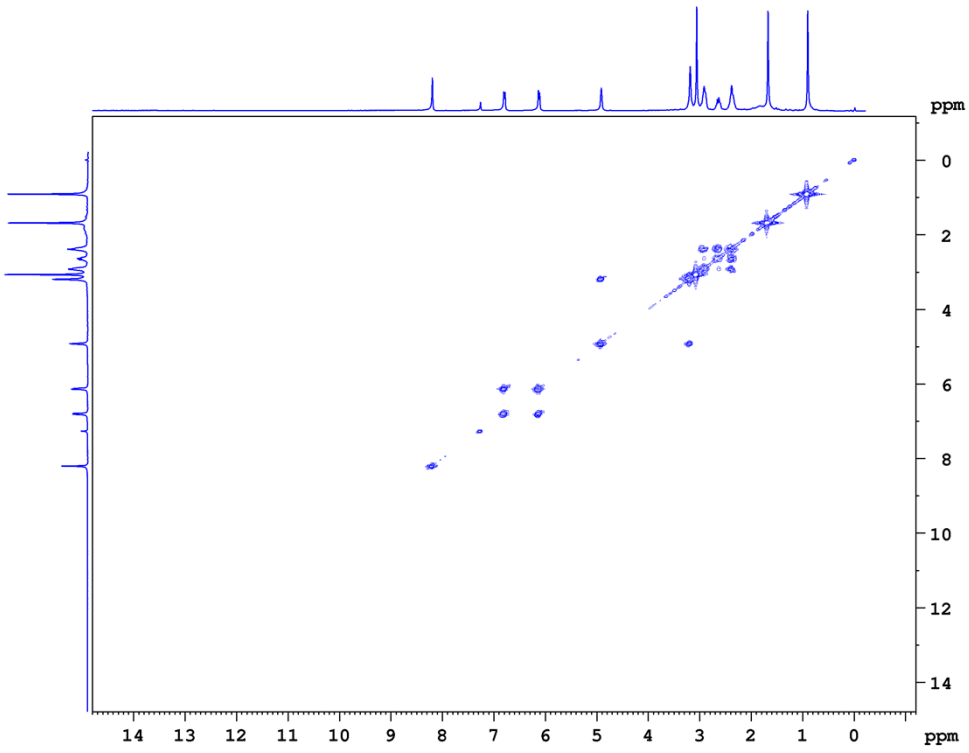


Figure S6. The ^{1}H-^{1}H COSY spectrum of 3 in $CDCl_3$ at 400 MHz

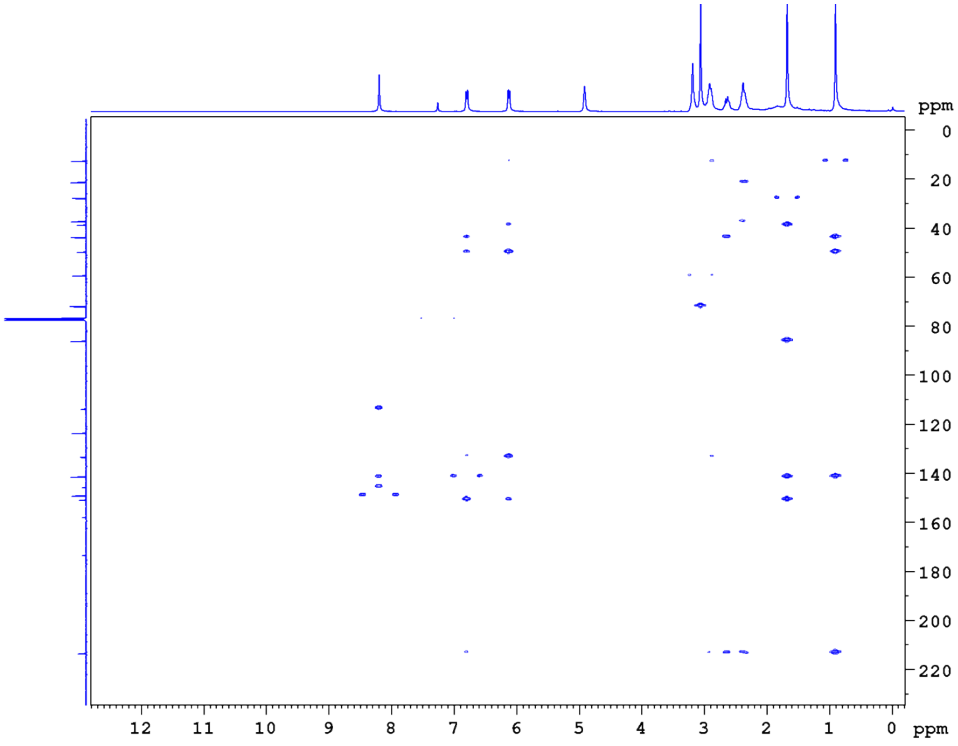


Figure S7. The HMBC spectrum of 3 in $CDCl_3$ at 400 MHz

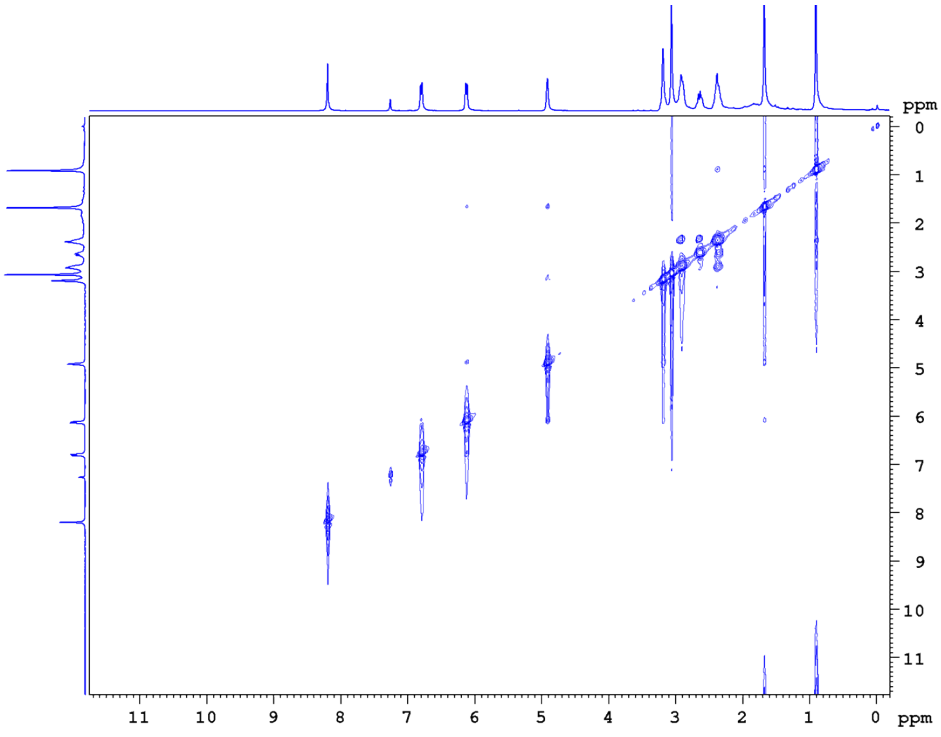


Figure S8. The NOESY spectrum of 3 in $CDCl_3$ at 400 MHz

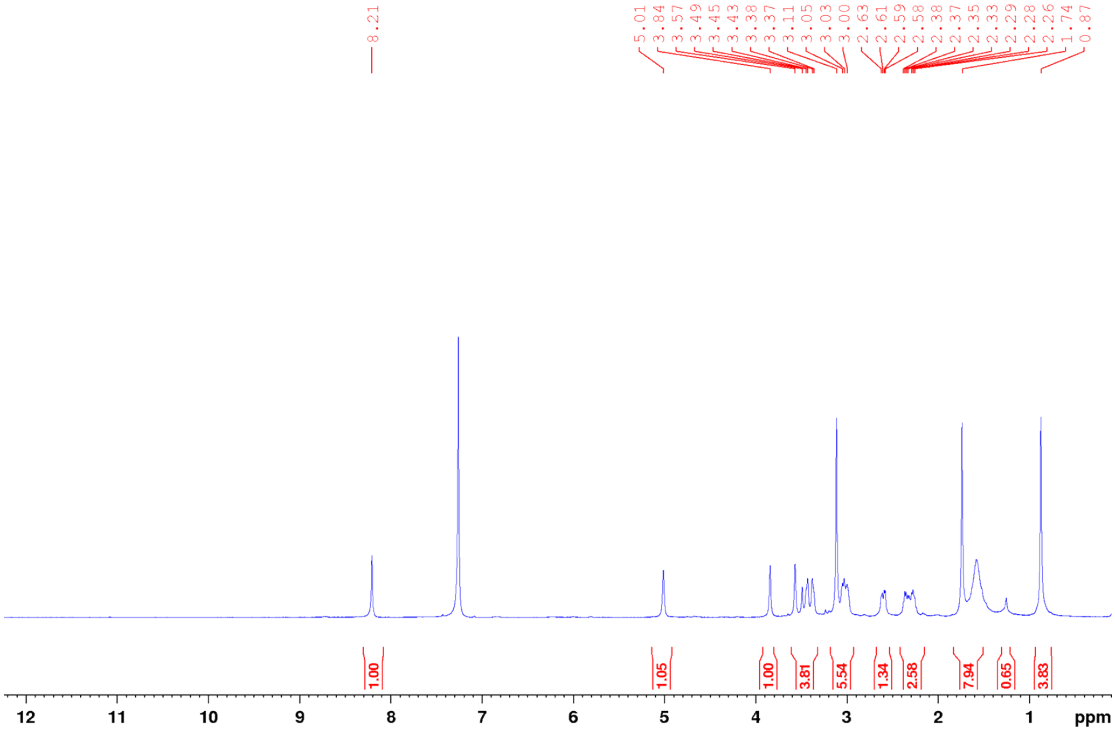


Figure S9. The ^{1}H NMR spectrum of 4 in $CDCl_3$ at 600 MHz

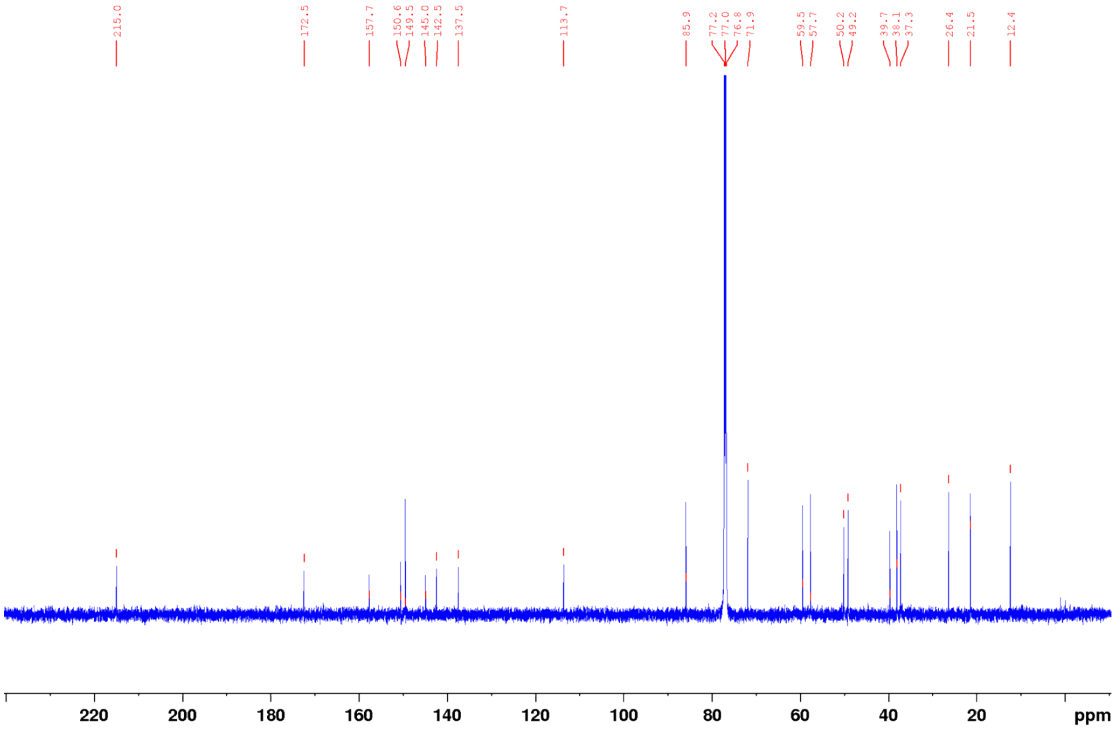


Figure S10. The ^{13}C NMR spectrum of 4 in $CDCl_3$ at 150 MHz

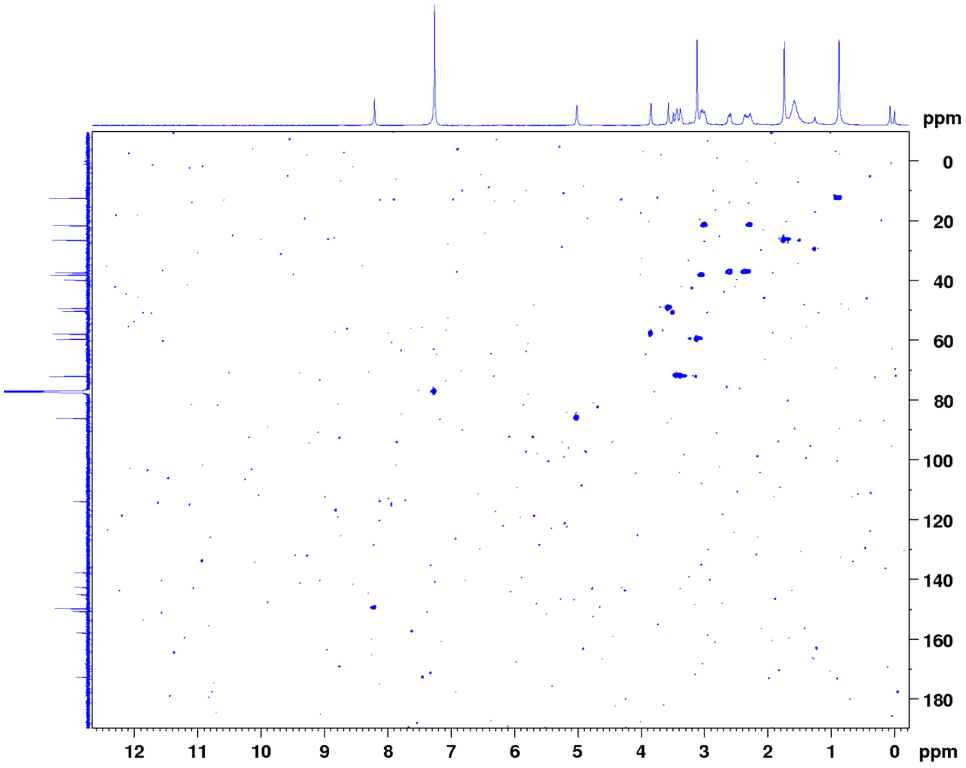


Figure S11. The HSQC spectrum of 4 in $CDCl_3$ at 600 MHz

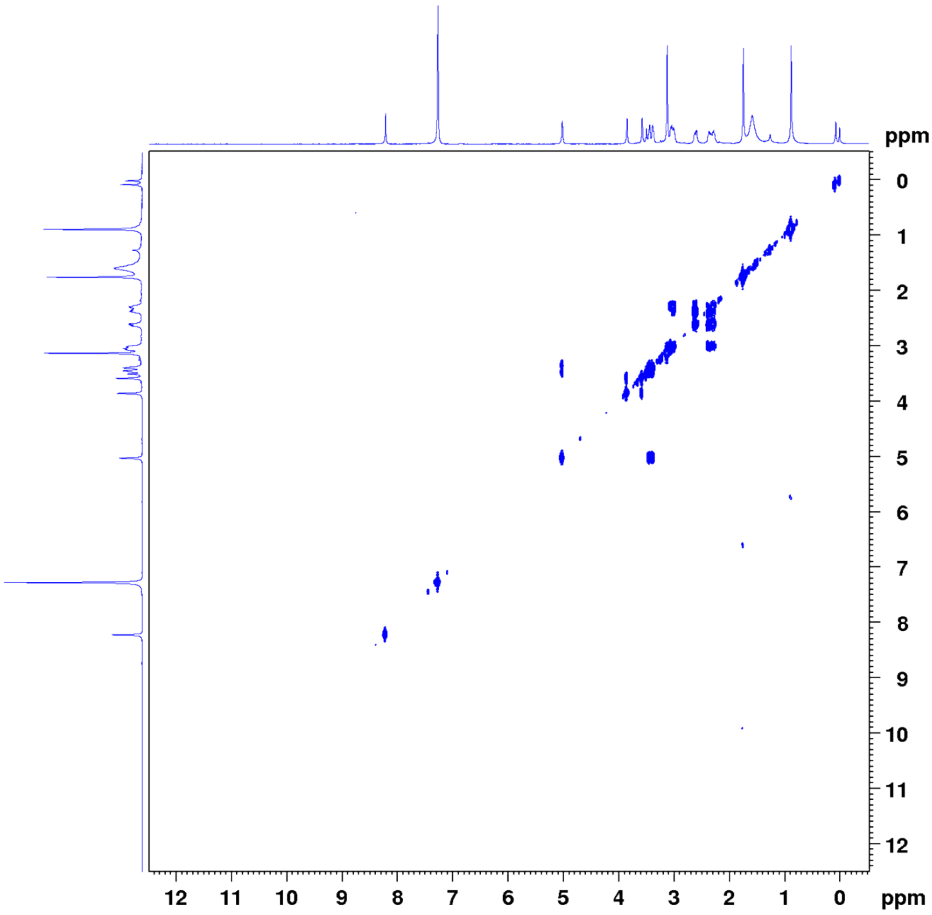


Figure S12. The ^{1}H-^{1}H COSY spectrum of 4 in $CDCl_3$ at 600 MHz

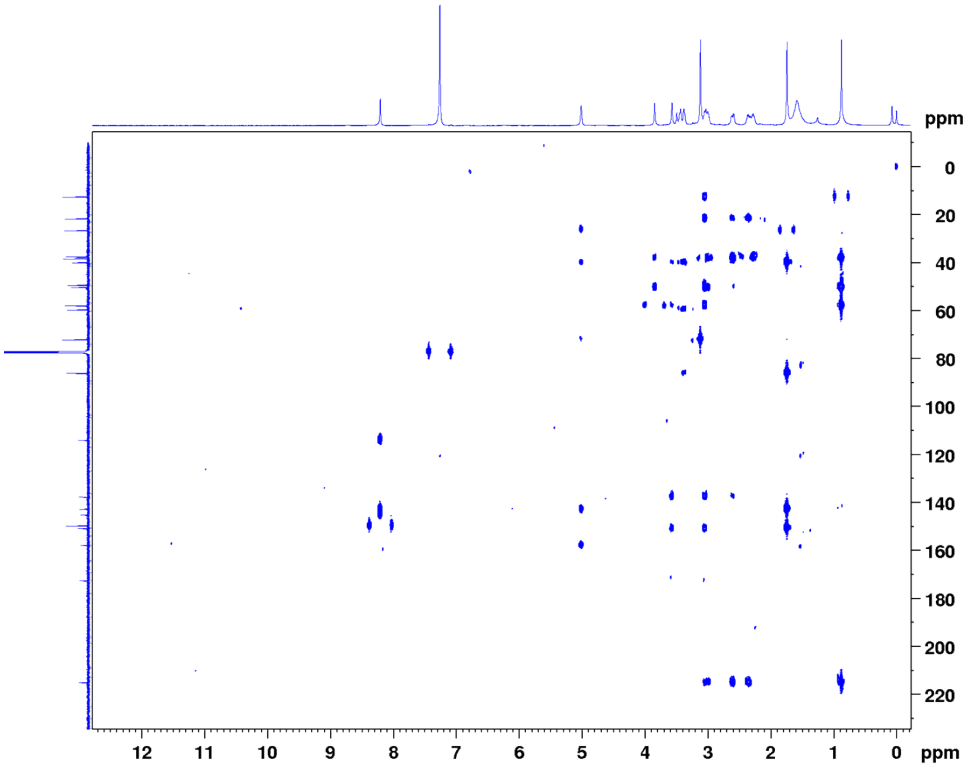


Figure S13. The HMBC spectrum of 4 in $CDCl_3$ at 600 MHz

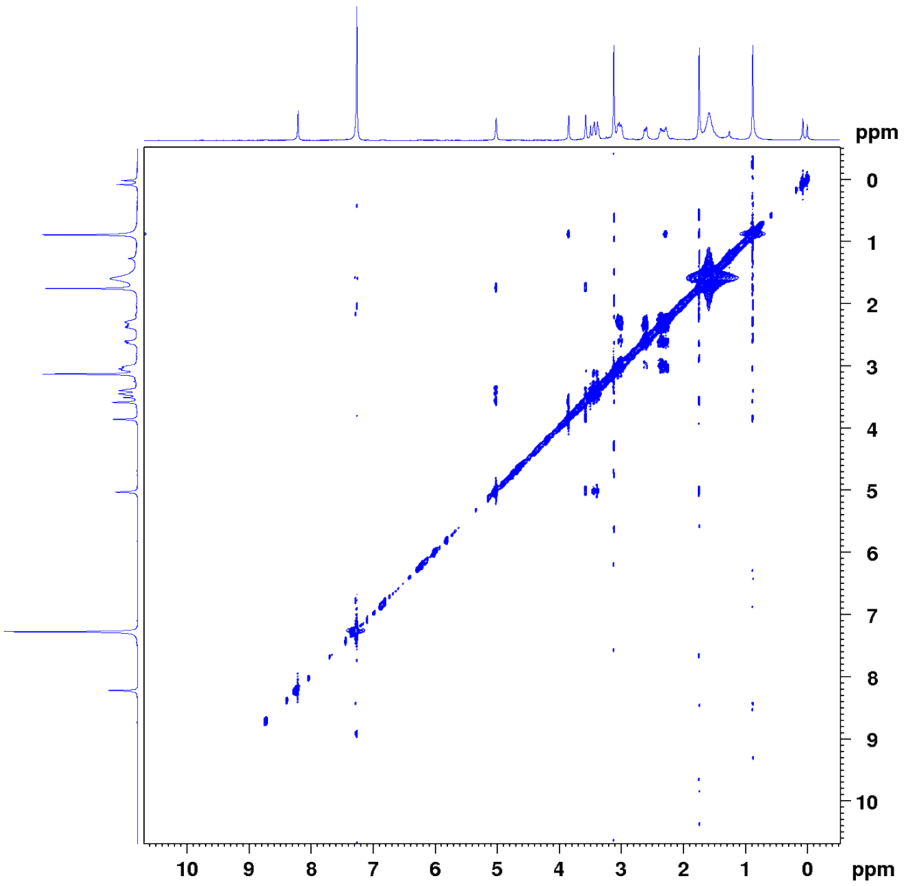


Figure S14. The NOESY spectrum of 4 in $CDCl_3$ at 600 MHz

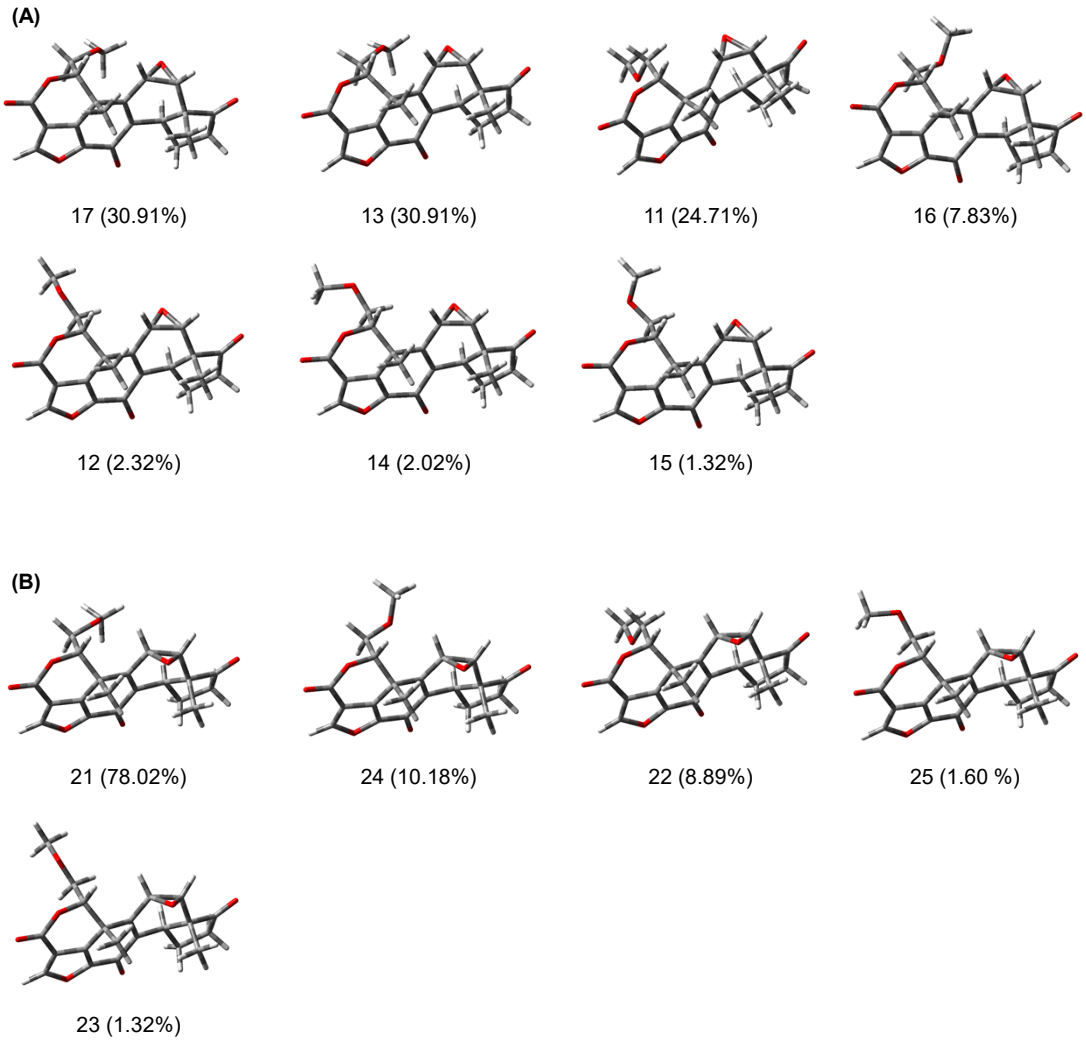
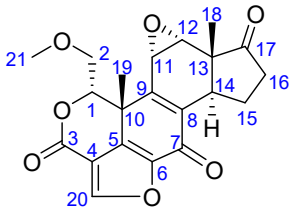
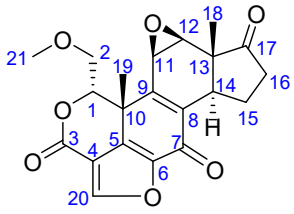


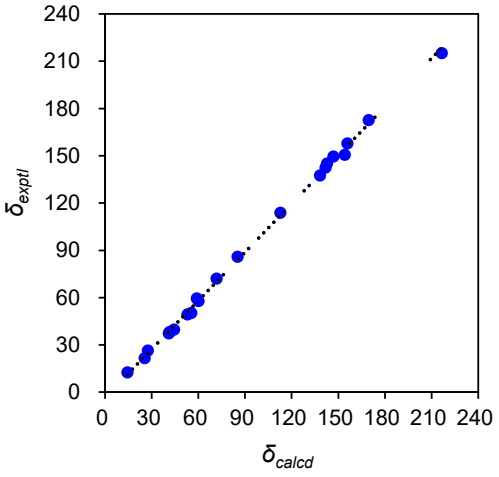
Figure S15. Most stable conformers of (1*S*,10*R*,11*S*,12*R*,13*R*,14*S*)-4 and (1*S*,10*R*,11*R*,12*S*,13*R*,14*S*)-4

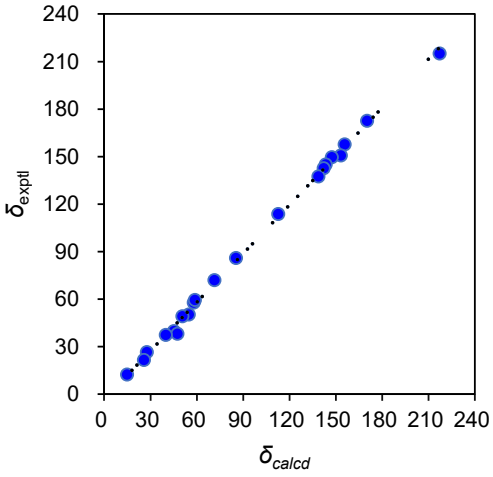


(1*S*,10*R*,11*S*,12*R*,13*R*,14*S*) –**4**



(1*S*,10*R*,11*R*,12*S*,13*R*,14*S*) –**4**





	(1*S*,10*R*,11*S*,12*R*,13*R*,14*S*) –4	(1*S*,10*R*,11*R*,12*S*,13*R*,14*S*) –4
Mean absolute error	1.8483	2.0637
DP4+	100%	0%

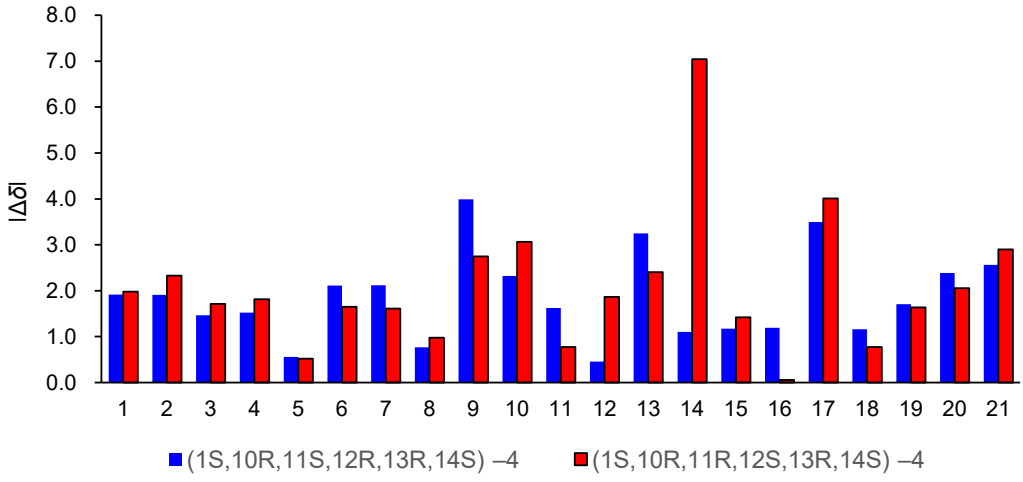


Figure S16. ^{13}C chemical shift calculation of 4

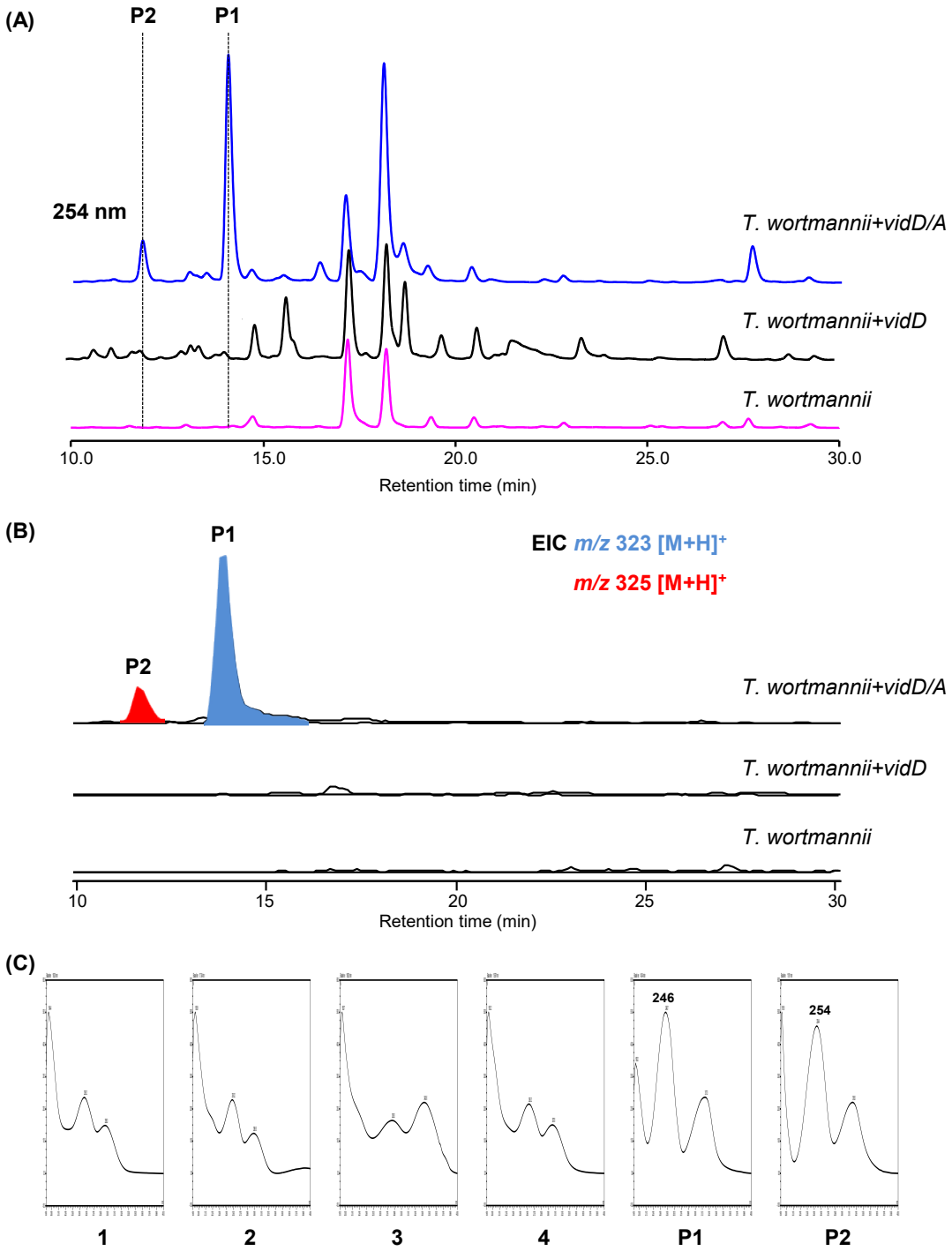


Figure S17. Combinatorial biosynthesis by introduction of *vidD* and *vidA* into *T. wortmannii*

(A) HPLC profiles of the metabolites from *T. wortmannii* and its transformants; (B) Extracted ion chromatograms of the metabolites from *T. wortmannii* and its transformants; (C) The UV absorption spectra of **1**-**4**, **P1** and **P2**

Supplementary References

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