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Enzyme-catalysed [6+4] cycloadditions in the biosynthesis of natural products

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This PDF file includes:

Supplementary Text

General experimental procedures. All NMR spectra were acquired on Bruker Avance 600 at 600 MHz for ^{1}H and 150 MHz for ^{13}C or AvanceIII 400 at 400 MHz for ^{1}H and 100 MHz for ^{13}C. High resolution LC-MS analysis was conducted on an Agilent 6530 TOF LC-MS spectrometer with a Porshell 120 (EC-C18 column, 4.5×50 mm, 2.7 µm, Agilent Techonologies). X-ray crystallographic data was obtained on a Bruker Smart APEXIII single crystal diffractometer. Analytical HPLC was performed on an Agilent 1260 HPLC system with a DAD detector equipped with a Porshell 120 (EC-C18, 4.5×50 mm, 2.7 µm, Agilent Techonologies). UV absorbance was measured on a NanoDrop 2000c spectrometer (Thermo Technology). Biochemicals and media were purchased from Sinopharm Chemical (China), Oxoid (UK) or Sigma-Aldrich (USA). Chemical reagents were purchased from standard commercial sources including J&K (China), Sigma- Aldrich (USA), Merck (Germany).

Physical data of isolated compounds

Compound **4**, yellow amorphous oil, UV(MeOH): λmax (log*ε*) = 229 nm (0.759), 296 nm (0.866); IR (KBr) ν_{max} 3303, 2962, 2932, 2874, 1622, 1589, 1532, 1395, 1007, 973 cm^{-1}; HR-ESIMS (positive mode): *m*/*z* calcd. for $C_{21}H_{31}O_5Na_2$: 409.1967, found 409.1965 $[M-H+2Na]^+$. ^{1}H and ^{13}C NMR data see **Supplementary Table 4.**

Compound **5**, yellow amorphous oil, UV(MeOH): λmax (log*ε*) = 234 nm (0.298), 293 nm (0.417); IR (KBr) ν_{max} 3359, 2927, 1690, 1615, 1396, 1303, 1242, 1140, 1061, 1007, 969 cm^{-1}; HR-ESIMS (positive mode): *m*/*z* calcd. for $C_{21}H_{32}O_6Na$: 403.2097, found 403.2096 $[M+Na]^+$. ^{1}H and ^{13}C NMR data see **Supplementary Table 5.**

Compound **6**, colorless needle, UV(MeOH): λmax (log*ε*) = 218 nm (0.96), 222 nm (0.7); IR (KBr) ν_{max} 3380, 2944, 2832, 1449, 1115, 1033, 670 cm^{-1}; HR-ESIMS (positive mode): *m*/*z* calcd. for $C_{21}H_{30}O_4Na$: 369.2042, found 369.2041 $[M+Na]^+$. ^{1}H and ^{13}C NMR data see **Supplementary Table 6.**

Compound **7**, HR-ESIMS (positive mode): *m*/*z* calcd. for $C_{21}H_{30}O_4Na$: 369.2042, found 369.2037 $[M+Na]^+$. ^{1}H and ^{13}C NMR data see **Supplementary Table 7.**

Compound **8**, colorless needle, UV(MeOH): λmax (log*ε*) = 206 nm (0.25), 218 nm (0.75); HR-ESIMS (positive mode): *m*/*z* calcd. for $C_{21}H_{30}O_4Na$: 369.2042, found 369.2043 $[M+Na]^+$. ^{1}H and ^{13}C NMR data see **Supplementary Table 9.**

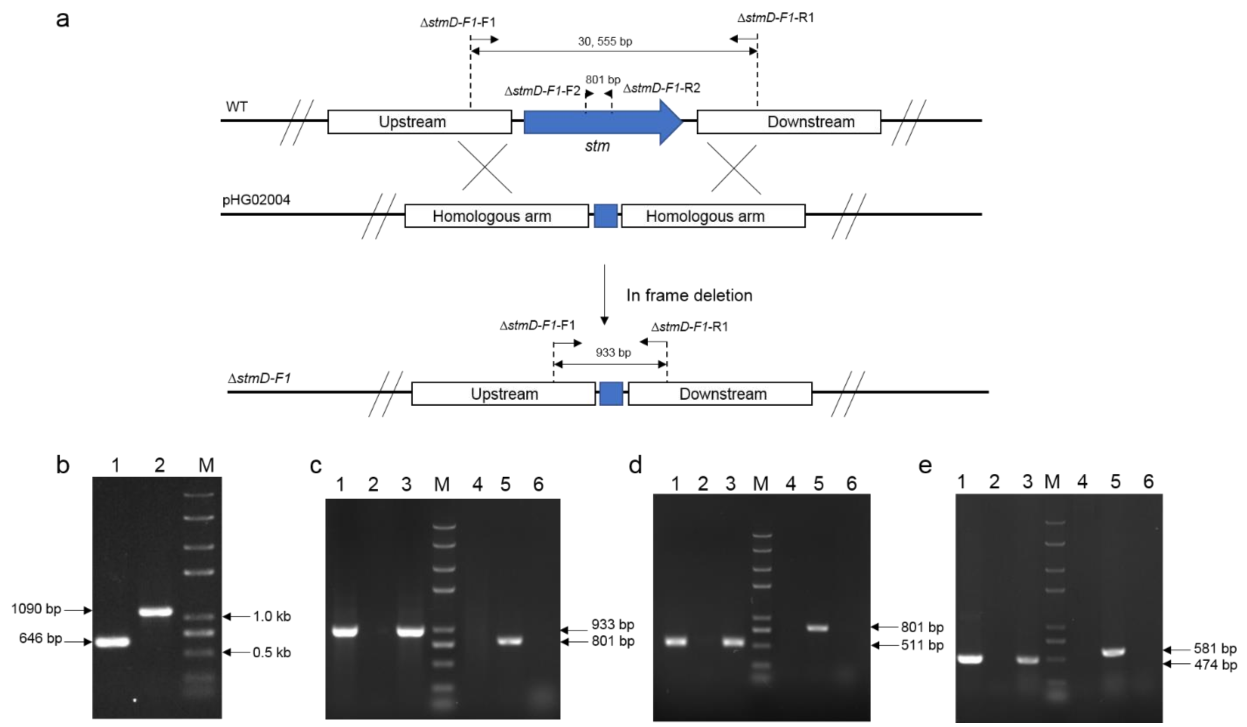
Compound **10**, yellow amorphous oil, UV(MeOH): λmax (log*ε*) = 234 nm (0.21), 275 nm (0.31), 327 nm (0.22); IR (KBr) ν_{max} 3385, 3181, 2970, 2937, 2881, 1729, 1675, 1531, 1457, 1386, 1255, 1203 cm^{-1}; HR-ESIMS (positive mode): *m*/*z* calcd. for $C_{25}H_{39}NO_5SNa$: 488.2447, found 488.2440 $[M+Na]^+$. ^{1}H and ^{13}C NMR data see **Supplementary Table 10.**

Crystal data of compounds 6 and 8

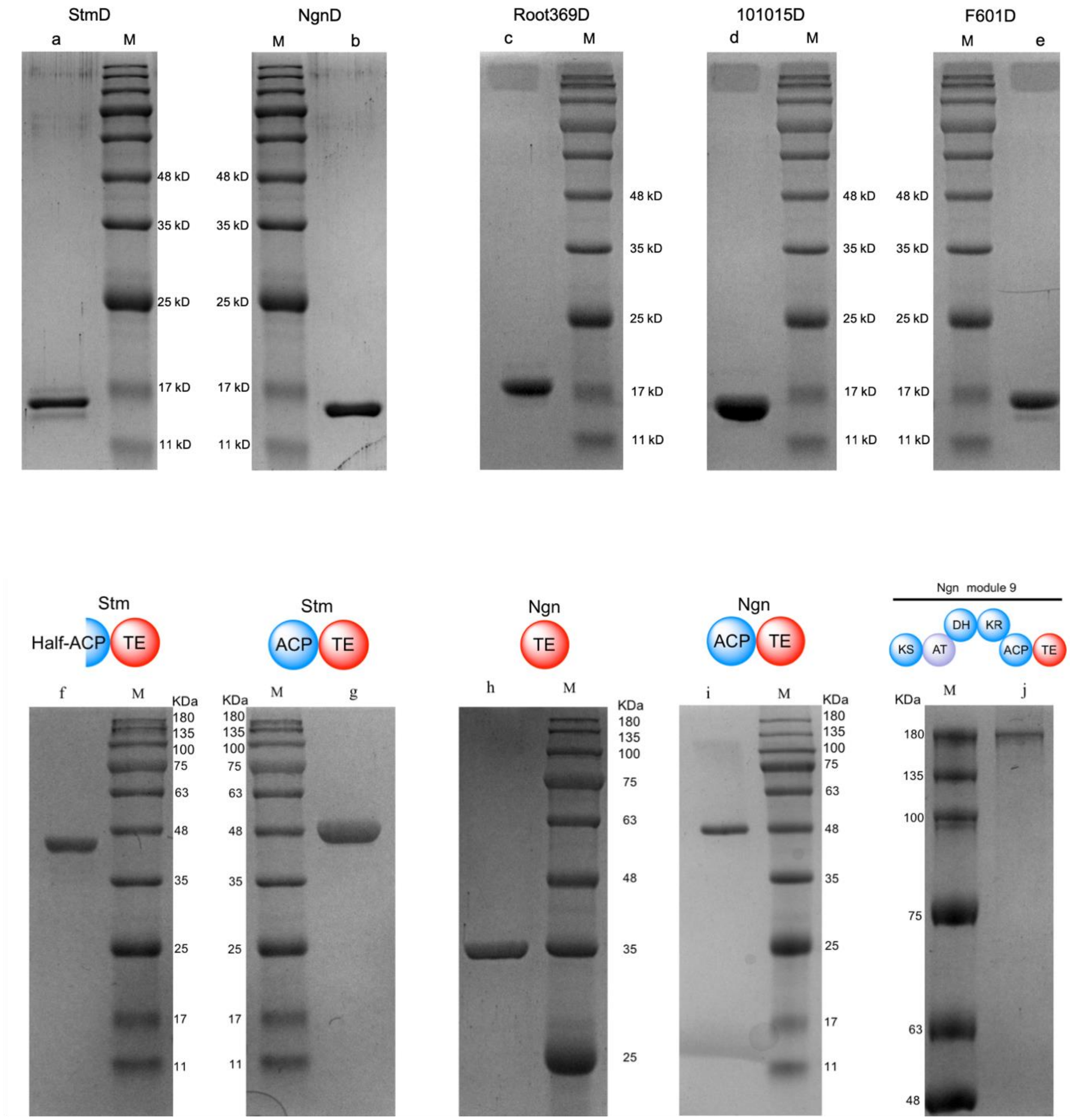
Crystal Data for compound **6**, $C_{21}H_{30}O_4$ (M = 346.45 g/mol): monoclinic, space group P21 (no. 4), a = 7.1821(12) Å, b = 10.1451(17) Å, c = 12.471(2) Å, α = 90°, β = 97.763(5)°, γ = 90°, V = 900.4(3) Å3, Z = 2, T = 153 K, μ(CuKα) = 0.087 mm^{-1}, *Dcalc* = 1.278 g/cm^3, F(000) = 376; 5600 reflections measured (5.196° ≤ 2Θ ≤ 50.014°), 2845 unique (R_{int} = 0.051, R_{sigma} = 0.0644) which were used in all calculations. The final R_1 was 0.0391 (I > 2σ(I)) and wR_2 was 0.1137 (all data); The crystallographic data have been deposited in the Cambridge Crystallographic Data Centre as CCDC 1843311.

Crystal Data for compound **8**, $C_{21}H_{30}O_4$ (M = 346.45 g/mol): triclinic, space group P1 (no. 1), a = 6.9357(11) Å, b = 8.1669(15) Å, c = 8.4498(13) Å, α = 84.343(5)°, β = 87.877(4)°, γ = 79.868(3)°, V = 468.77(13) Å3, Z = 1, T = 153 K, μ(CuKα) = 0.083 mm^{-1}, $Dcalc$ = 1.227 g/cm^3, F(000) = 188; 3277 reflections measured (4.846° ≤ 2Θ ≤ 50.012°), 2263 unique (R_{int} = 0.0434, R_{sigma} = 0.0739) which were used in all calculations. The final R_1 was 0.0457 (I > 2σ(I)) and wR_2 was 0.1226 (all data); The crystallographic data have been deposited in the Cambridge Crystallographic Data Centre as CCDC 1843314.

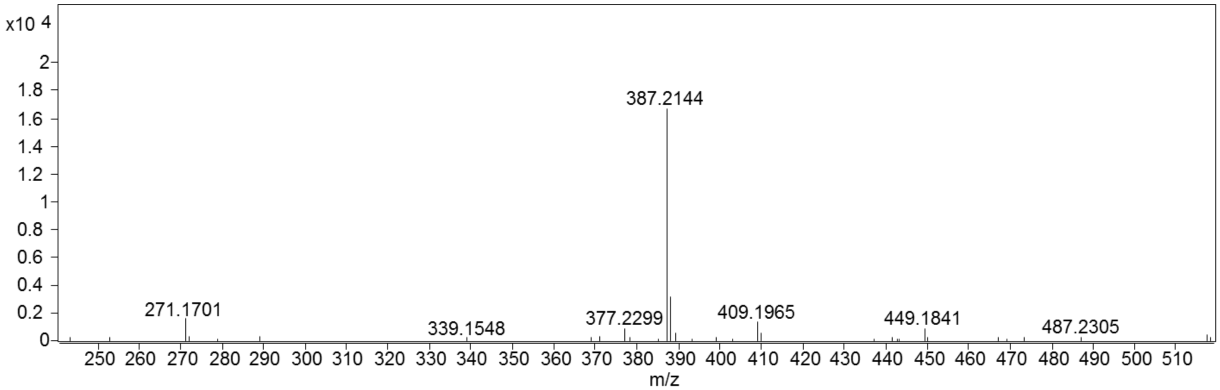
Computational details. The DFT calculations were performed with the Gaussian 09 program package[37]. The procedure starts with a preliminary conformation search with molecular mechanics (MM). Schrödinger Maestro 9.8 and AMBER were used for MM calculations. We used the force field OPLS_2005, GB/SA solvent model for water, and the mixed torsional/low-mode sampling method that was built in the program. For the transition structures, we constrained the forming C-C bonds to around 2.2 Å by setting corresponding very high bond stretching force constants. The low-energy conformers that are within 5 kcal/mol of the global minimum were saved and then optimized with DFT in Gaussian 09. The geometry optimizations of minima and transition states involved were carried out at the B3LYP-D3 level of theory[38] with the 6-31G(d) basis set. The vibrational frequencies were computed at the same level to check whether each optimized structure is an energy minimum or a transition state and to evaluate its zero-point vibration energy (ZPVE) and thermal corrections at 298 K. The conformer with lowest energy was presented in the text. For a transition structure, we consider the lowest conformer with one negative normal-mode force constant for the desired vibration is the transition structure that we describe in more detail. Solvation energies were computed at the M06-2X level of theory[39] with the 6-311+G(d,p) basis set using the gas-phase optimized structures and the CPCM model[40,41] in water. NMR chemical shift calculations were performed with B3LYP/6-311+G(2d,p) in acetonitrile using the CPCM model on the QM-optimized structures. Quasi-classical molecular dynamics simulations on a B3LYP-D3/6-31G(d) energy surface were initiated in the region of the potential energy surface near **TS-1** or **TS-3**. Each real normal mode of **TS-1** or **TS-3** was given its zero-point energy (ZPE) plus a Boltzmann sampling of the thermal energy available at 298 K with a random phase. The trajectories were integrated in time (the step length for integration was 1 fs) in both the forward and backward directions until either the [6+4] or [4+2] adduct was formed or reactant **3** was reformed. We started with the monomer structure of NgnD and performed molecular dynamics (MD) simulations on the protein in water for 15 ns, which is long enough for equilibration. We found that the size of the pocket (potential active site) reaches its largest at 11 ns, so we took the structure at the end of 11 ns and docked in the DFT-optimized structures of reactant **3** or transition state **TS-1**. For the reaction in enzyme, the substrate or TS were docked into the enzyme active site using AutoDock Vina[42]. The binding mode that ranks best in each case was then used as a starting point for subsequent MD simulations. The docking structures were solvated in a water box using AmberTools 16[43]. Separate ensembles of substrates were constructed by carrying out classical MD equilibrations in NgnD using MM for environment. Classical MD was performed using Amber 16 on the reactant and the transition state for 11 ns in NgnD. The molecular models constructed in the first step (water boxes and docked protein complexes) were used for MD simulations with RESP[44] charges assigned on substrate and TS. The FF99SBildn[45] force field was used for protein residues. General Amber Force Field (GAFF)[46] was used for reactant and transition structures. In the enzyme, the system was minimized for 20000 steps, and gradually heated to 300 K and then equilibrated for 100 ps under constant T and P. Production run was conducted for 11 ns under constant volume and temperature. During the classical MD on the TS, restraining potentials of 200 kcal/mol/Å^2 were applied to the reaction coordinates in the TS. In each case, 10 residues are found to be close to the substrate/TS (**Fig. 3c**). Then, we analyzed the distances between these residues and the center of mass of the substrate/TS for the time between 10th and 11th ns (**Supplementary Fig. 10**). Snapshots (typically 2500) of reactant or TS were sampled from last 1 ns production MD runs at 400 fs intervals in NgnD.



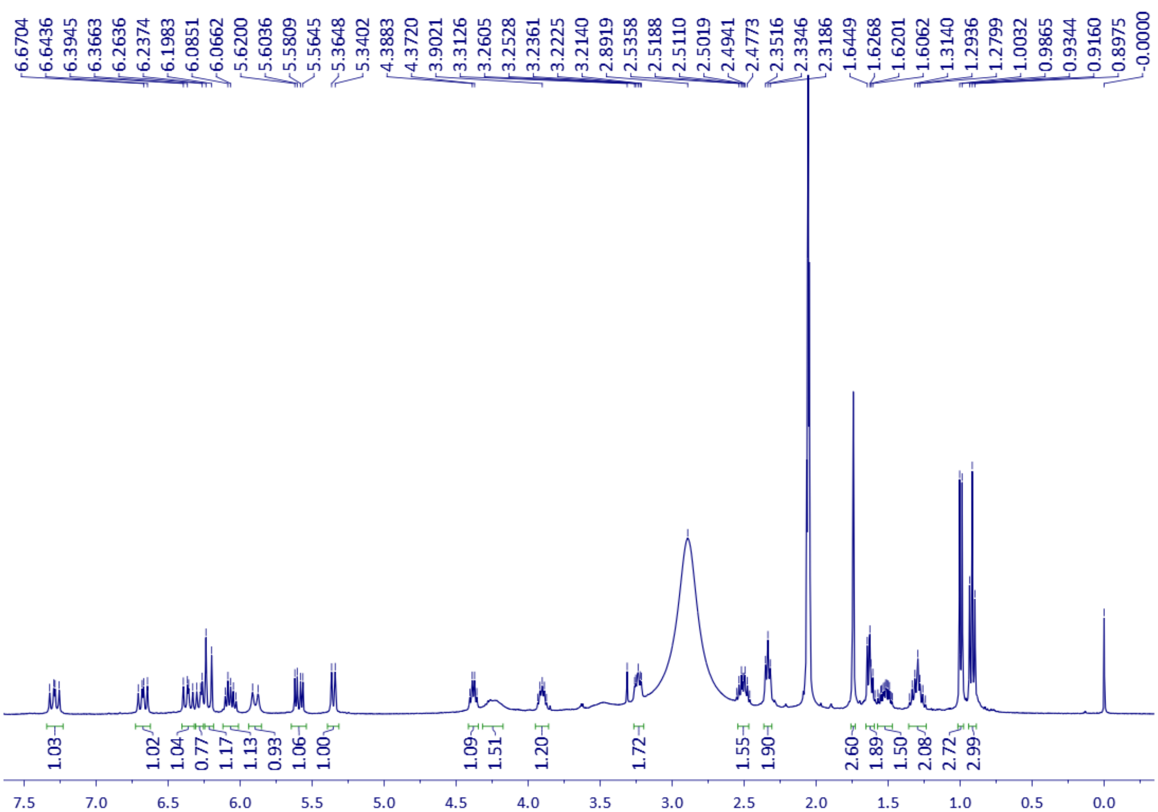
Supplementary Fig. 1. Generation of the *S. seoulensis* A01 mutant strains. (a) Construction of in frame deletion of *stmD-stmF1* in *S. seoulensis* A01 strain. Two homologous are jointed together through a scar of target gene. The genotype of each mutant is confirmed by PCR amplification using the genomic DNA from the mutant or wild type strain as the template and the size of predicted fragments are indicated. For large fragments deletion, two pair of primers were used for genotype confirmation. (b) The *S. seoulensis* HG02001 mutant (Δ*stmD*). Lane 1, HG02001 mutant; Lane 2, *S. seoulensis* A01; Lane M, *Trans*2K® Plus II DNA marker; (c) The *S. seoulensis* HG02005 mutant (Δ*stmD-stmF1*). (d) The *S. seoulensis* HG02006 mutant (Δ*stmY-stmF1*). (e) The *S. seoulensis* HG02004 mutant (Δ*stmA*). Lane 1 and 4, each mutant strain*;* Lane 2 and 5, *S. seoulensis* A01 as negative control; Lane 3 and 6, each plasmid used for gene deletion as positive control, Lane M, *Trans*2K® Plus II DNA marker.; Lane 1-3 all amplificated by the screen-F1/R1 primers, product of which is too large to be amplified in WT strain. Lane 4-6 all amplificated by the screen-F2/R2 primers, product of which is located in the deletion region and cannot be amplified in mutant strain and plasmid. All these screening experiments were repeated independently to identify at least three desired mutants and the fermentation results of each individual mutant are similar.



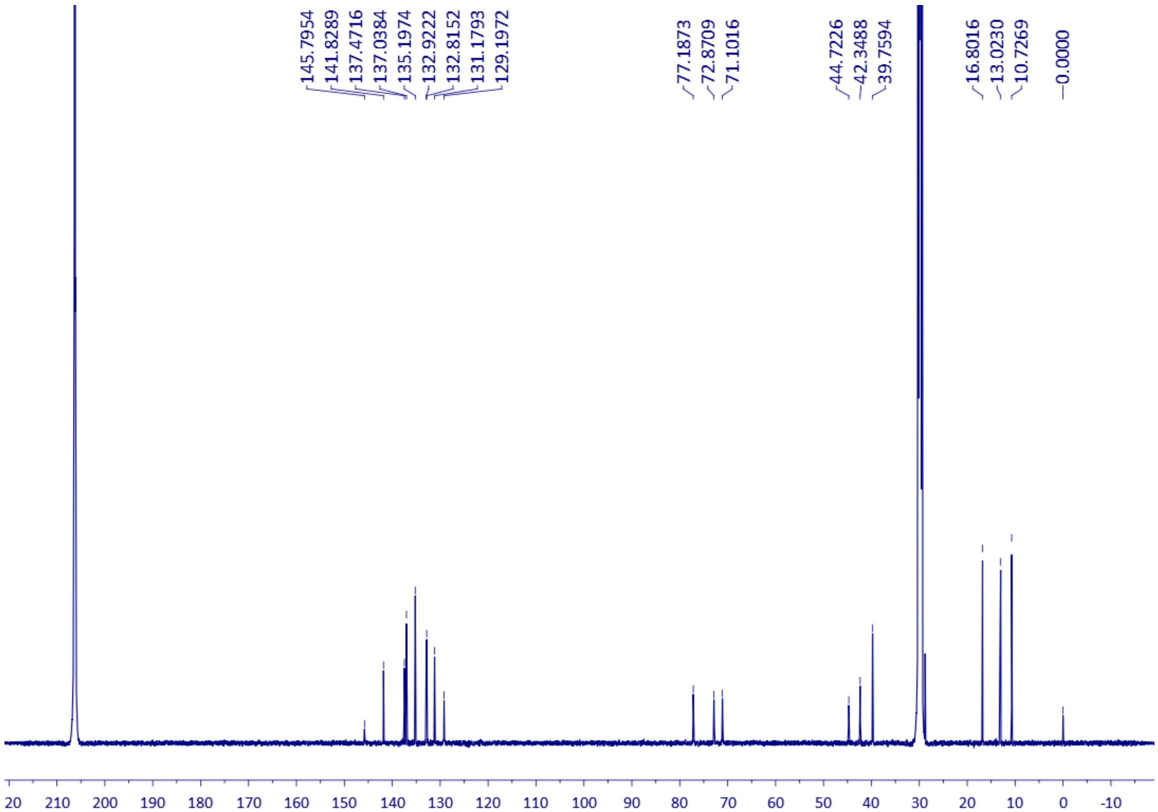
Supplementary Fig. 2. SDS-PAGEs of the purified proteins. (a) StmD (16.6 kDa). (b) NgnD (16.6 kDa). (c) Root369D (16.6 kDa), (d) 101015D (16.7 kDa), (e) F601D (16.9 kDa), (f) StmC/half-ACP-TE (36.1 kDa), (g) StmC/ACP-TE (38.1 kDa), (h) NgnC/TE (33.9 kDa), (i) NgnC/ACP-TE (39.4 kDa), (j) NgnC/module9-TE (208.5 kDa). M, protein ladder. The expression and purification of these proteins were repeated independently at least twice with similar results.



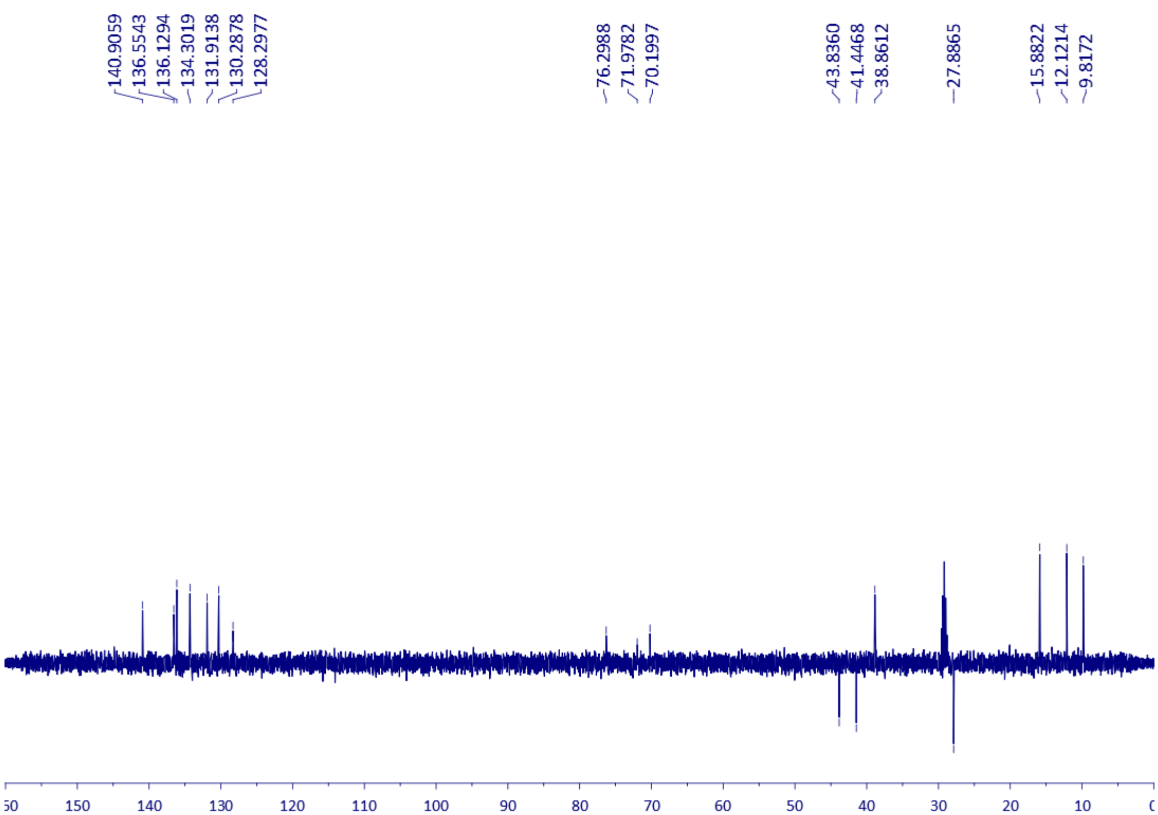
Supplementary Fig. 3a. HR-ESIMS spectrum of **4**.



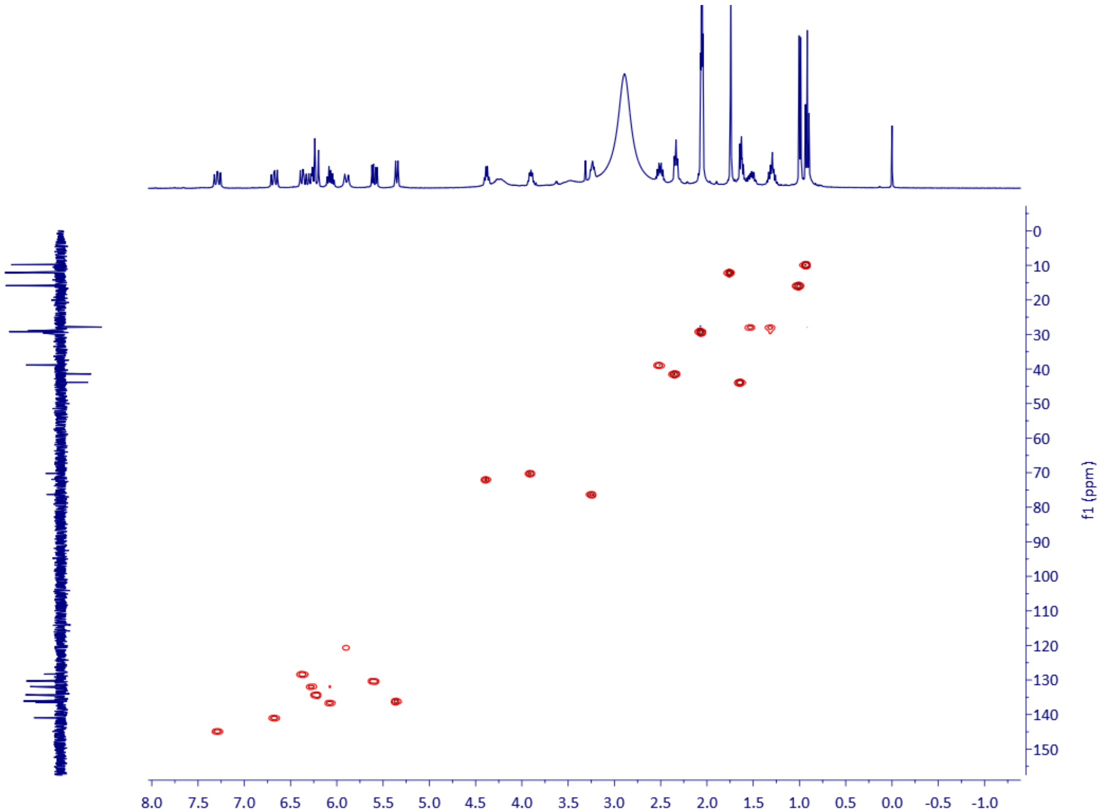
Supplementary Fig. 3b. ^{1}H NMR spectrum of **4** in acetone-d_6 (400 MHz).



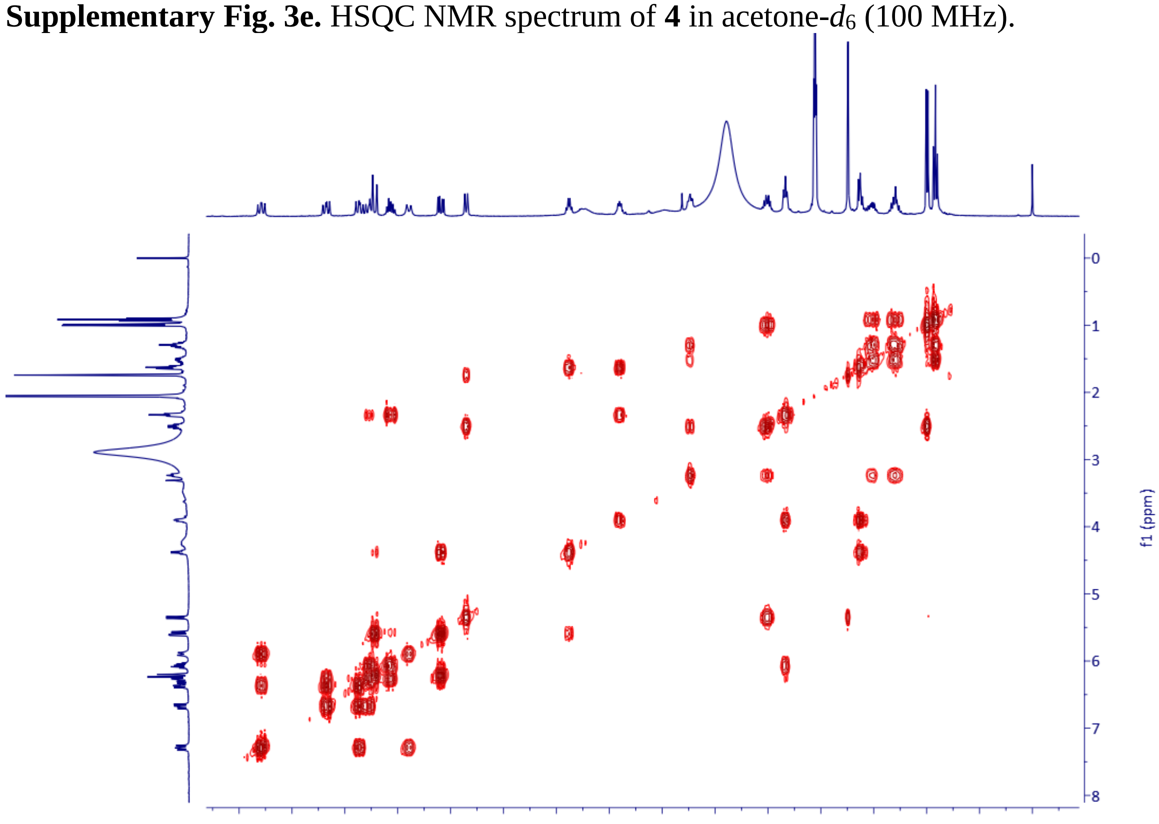
Supplementary Fig. 3c. ^{13}C NMR spectrum of **4** in acetone-d_6 (150 MHz).



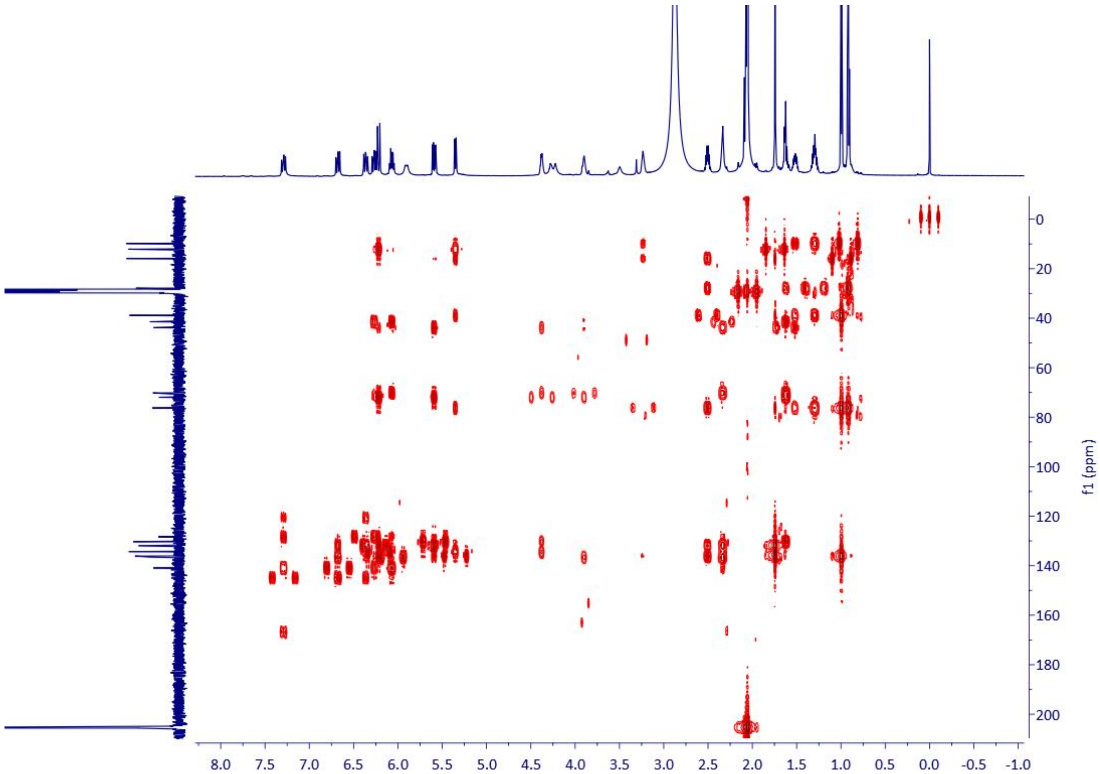
Supplementary Fig. 3d. DEPT-135 NMR spectrum of **4** in acetone-d_6 (100 MHz).



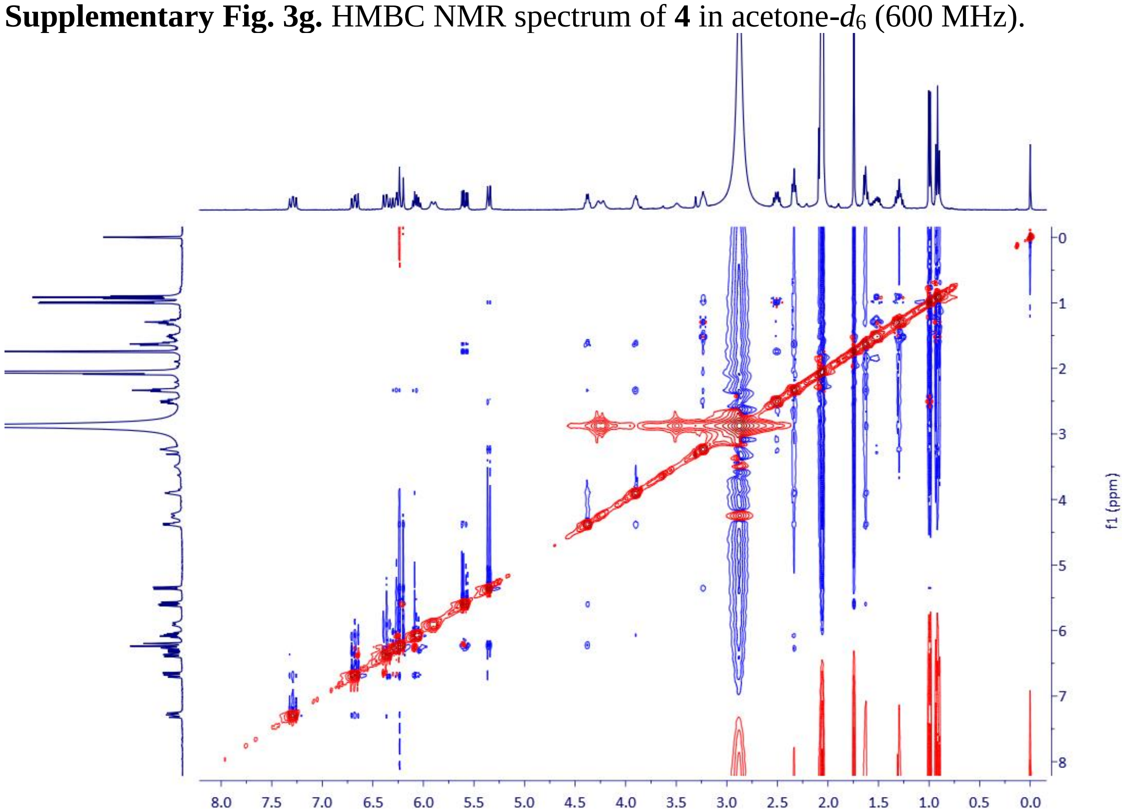
Supplementary Fig. 3e. HSQC NMR spectrum of **4** in acetone-d_6 (100 MHz).



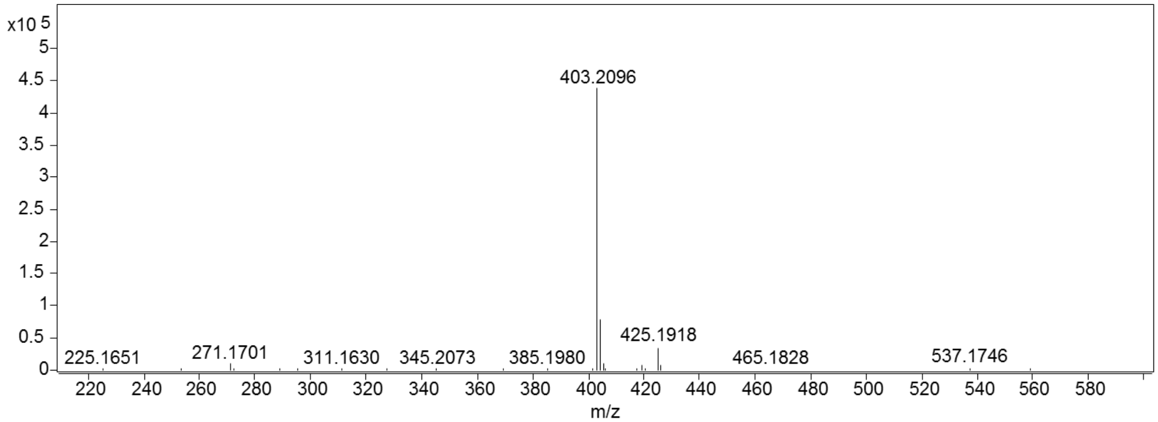
Supplementary Fig. 3f. ^{1}H-^{1}H COSY NMR spectrum of **4** in acetone-d_6 (400 MHz).



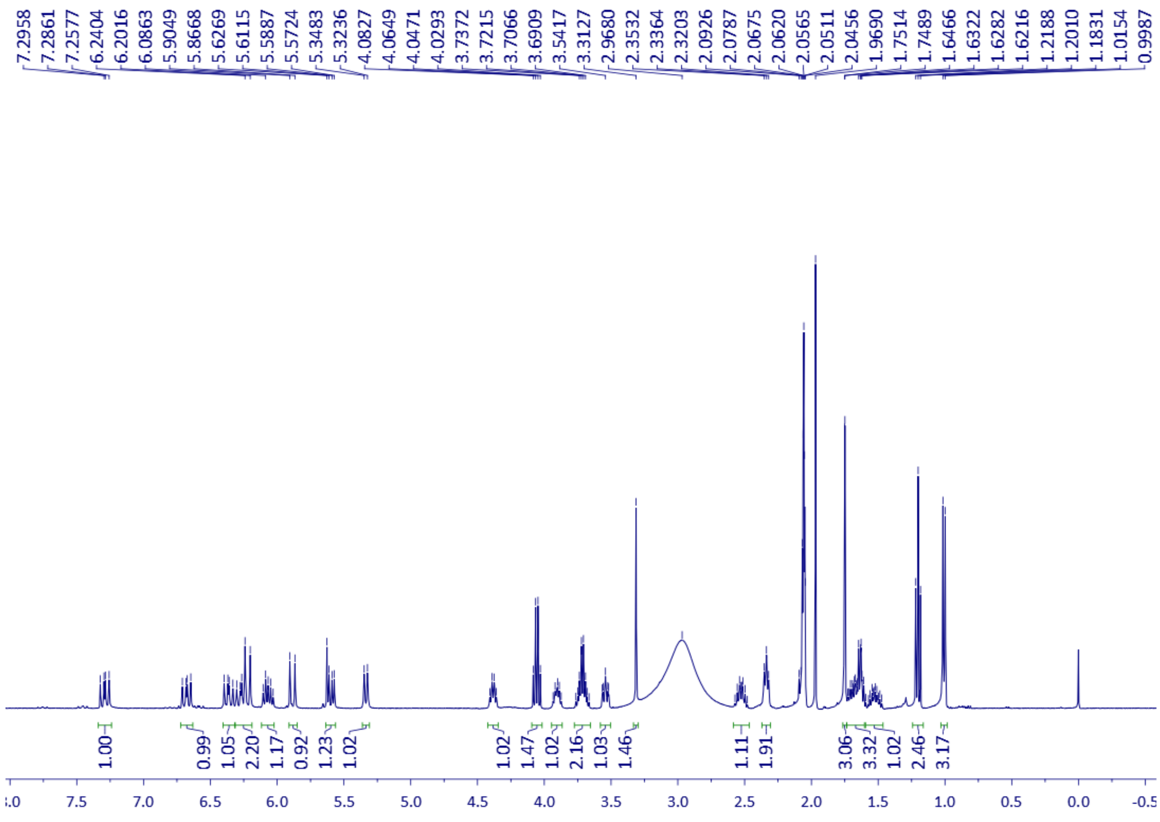
Supplementary Fig. 3g. HMBC NMR spectrum of **4** in acetone-d_6 (600 MHz).



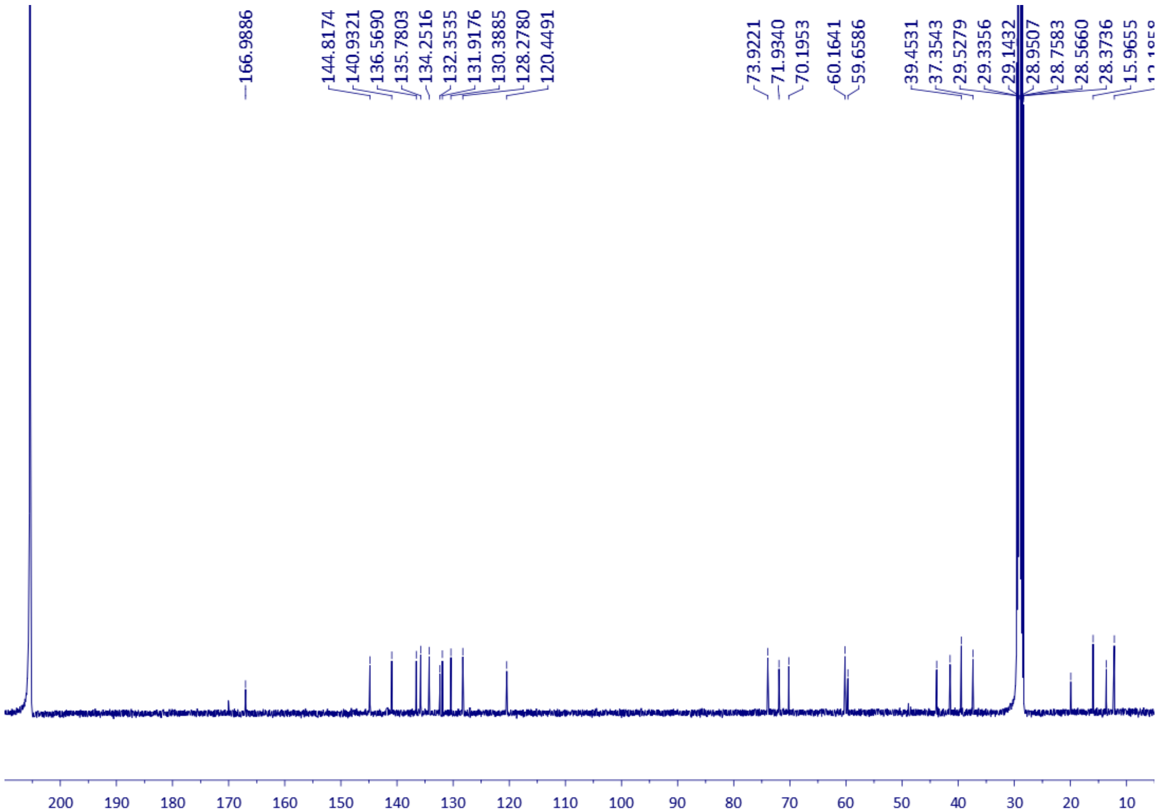
Supplementary Fig. 3h. NOESY spectrum of **4** in acetone-d_6 (600 MHz).



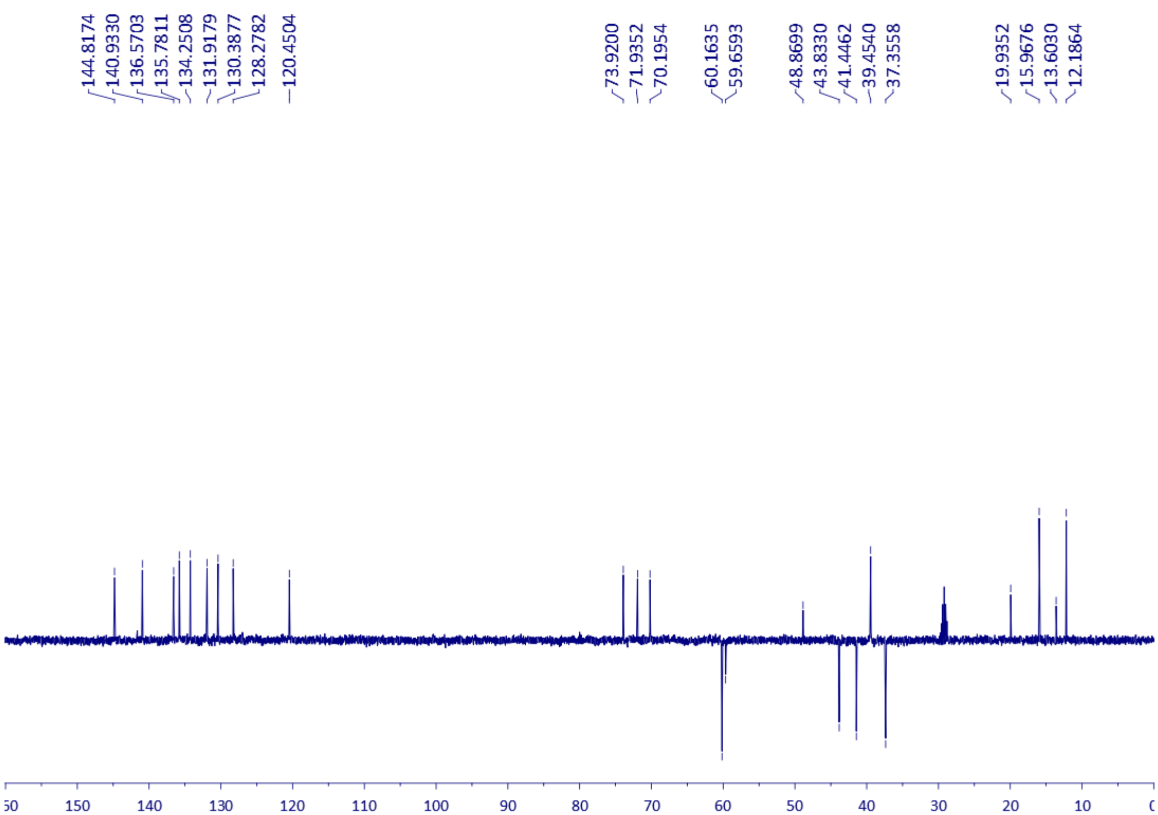
Supplementary Fig. 4a. HR-ESIMS of spectrum of **5**.



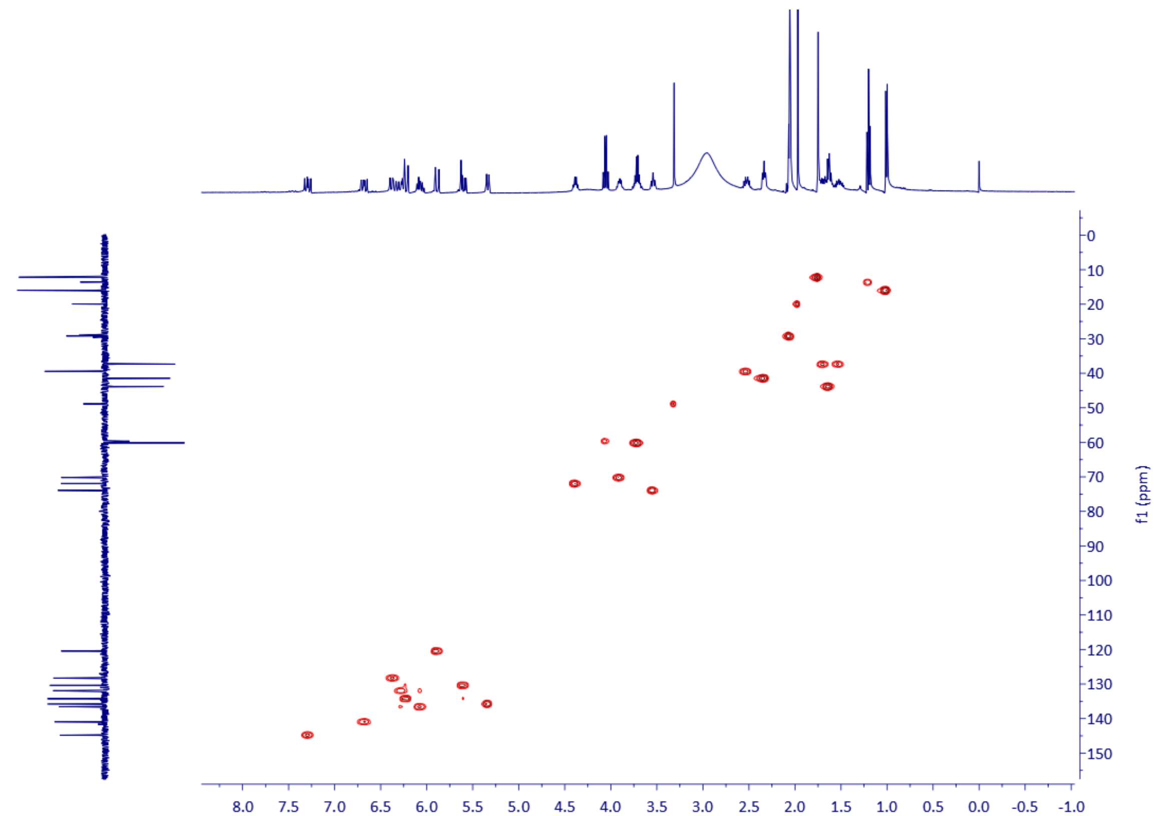
Supplementary Fig. 4b. [1]H NMR spectrum of **5** in acetone-d_6 (400 MHz).



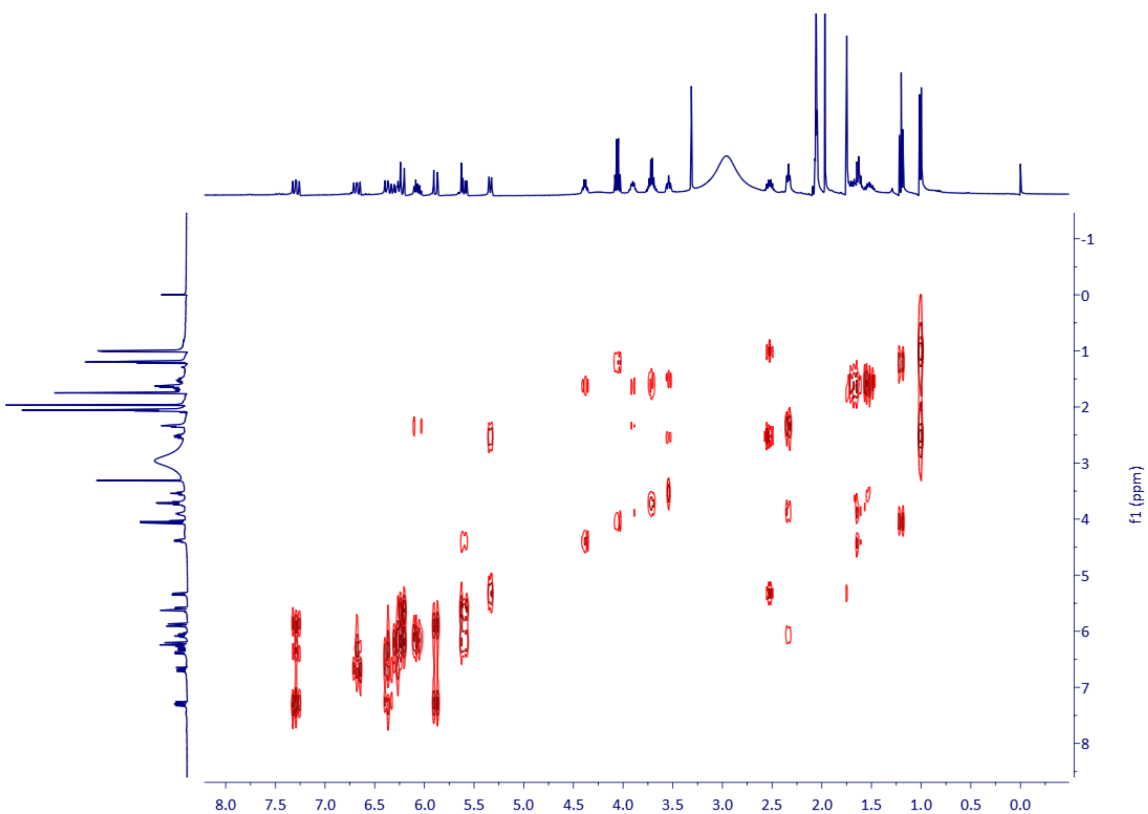
Supplementary Fig. 4c. ^{13}C NMR spectrum of **5** in acetone-d_6 (100 MHz).



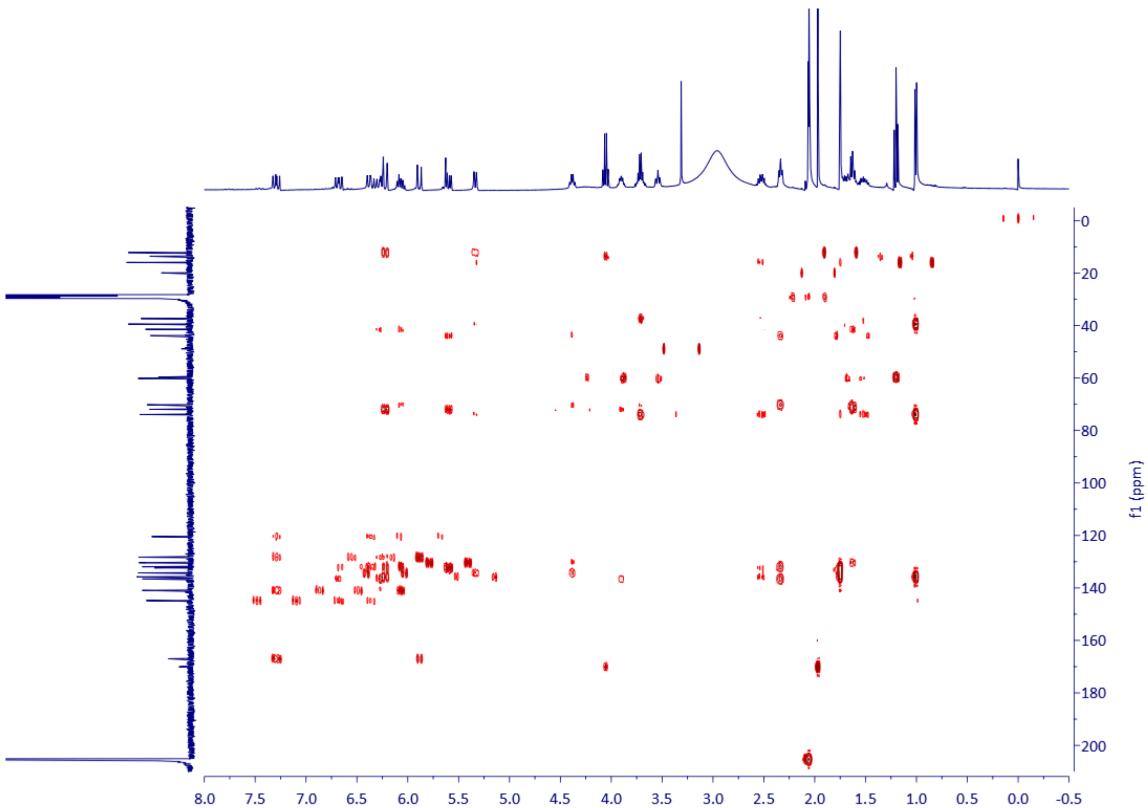
Supplementary Fig. 4d. DEPT-135 NMR spectrum of **5** in acetone-d_6 (100 MHz).



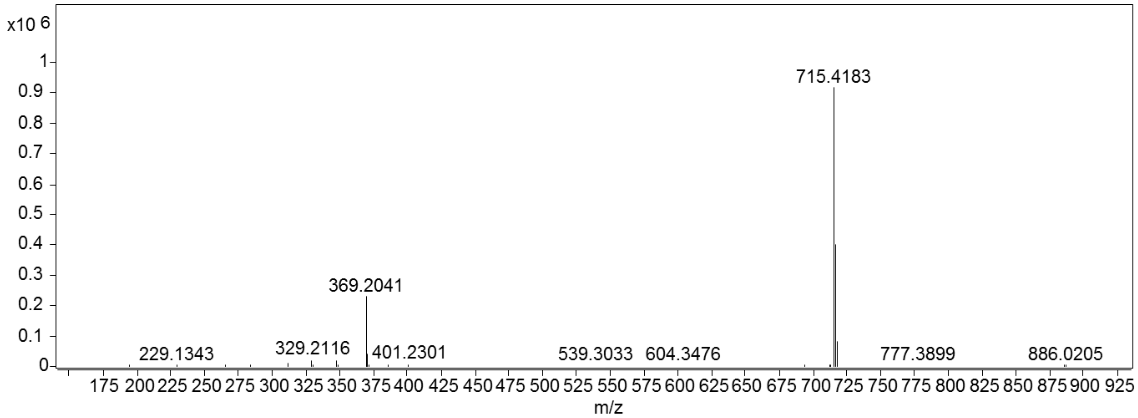
Supplementary Fig. 4e. HSQC NMR spectrum of **5** in acetone-d_6 (400 MHz).



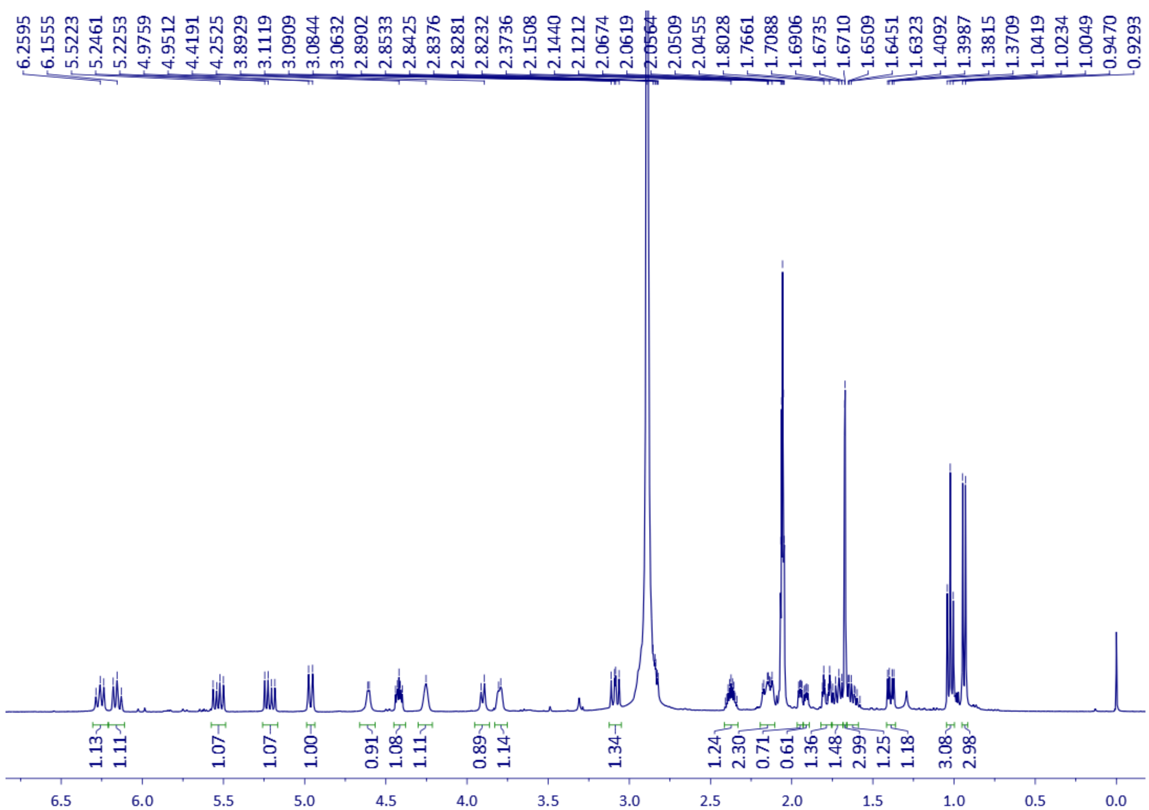
Supplementary Fig. 4f. ^1H-^1H COSY NMR spectrum of **5** in acetone-d_6 (400 MHz).



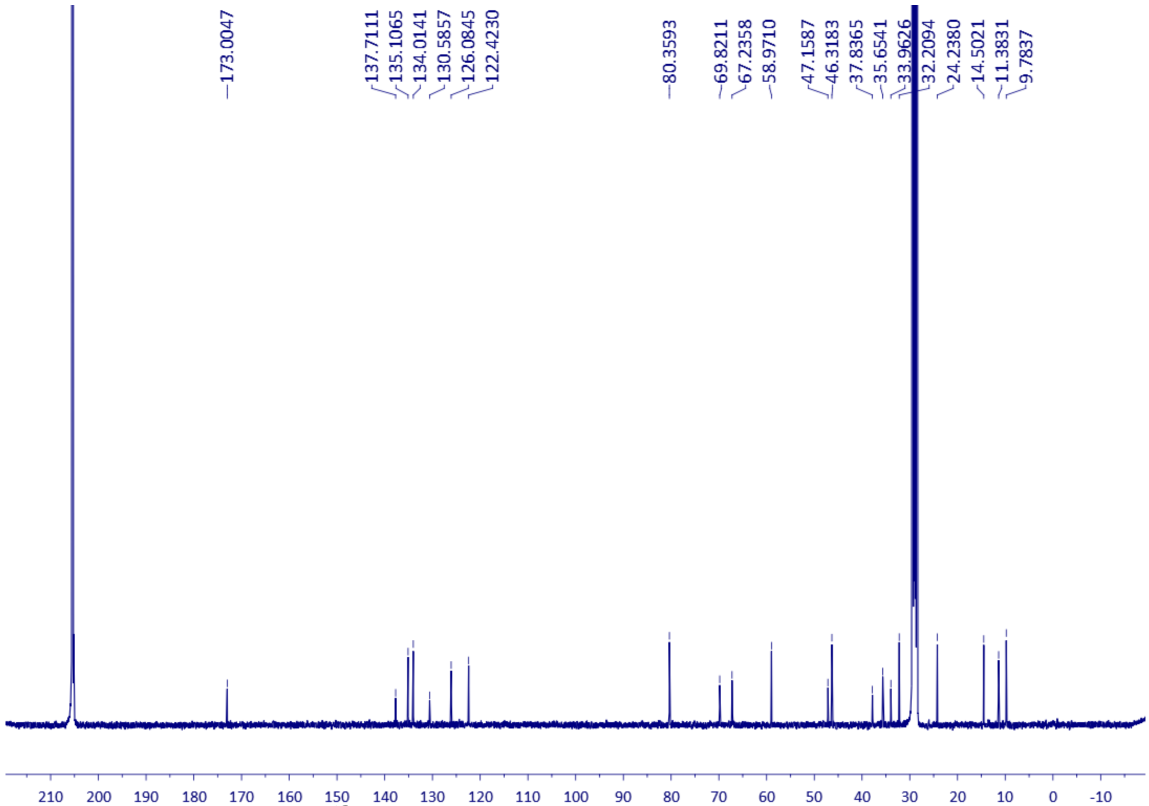
Supplementary Fig. 4g. HMBC NMR spectrum of **5** in acetone-d_6 (400 MHz).



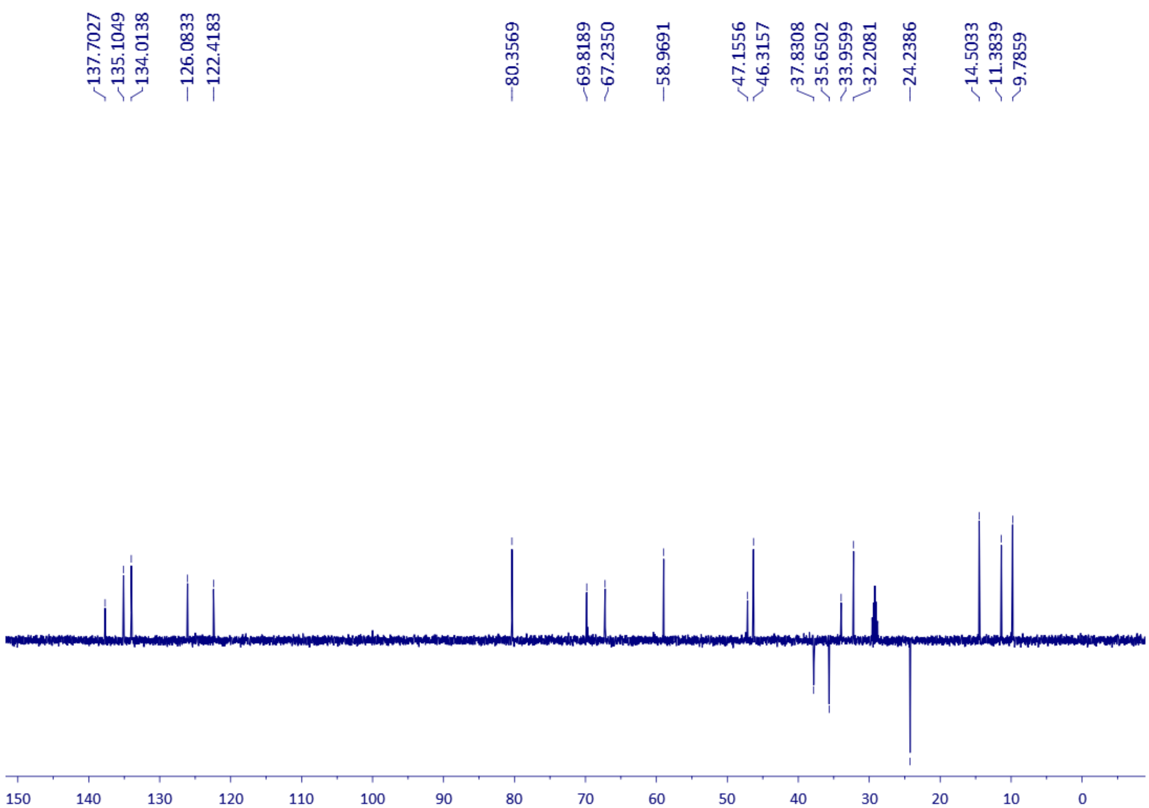
Supplementary Fig. 5a. HR-ESIMS spectrum of **6**.



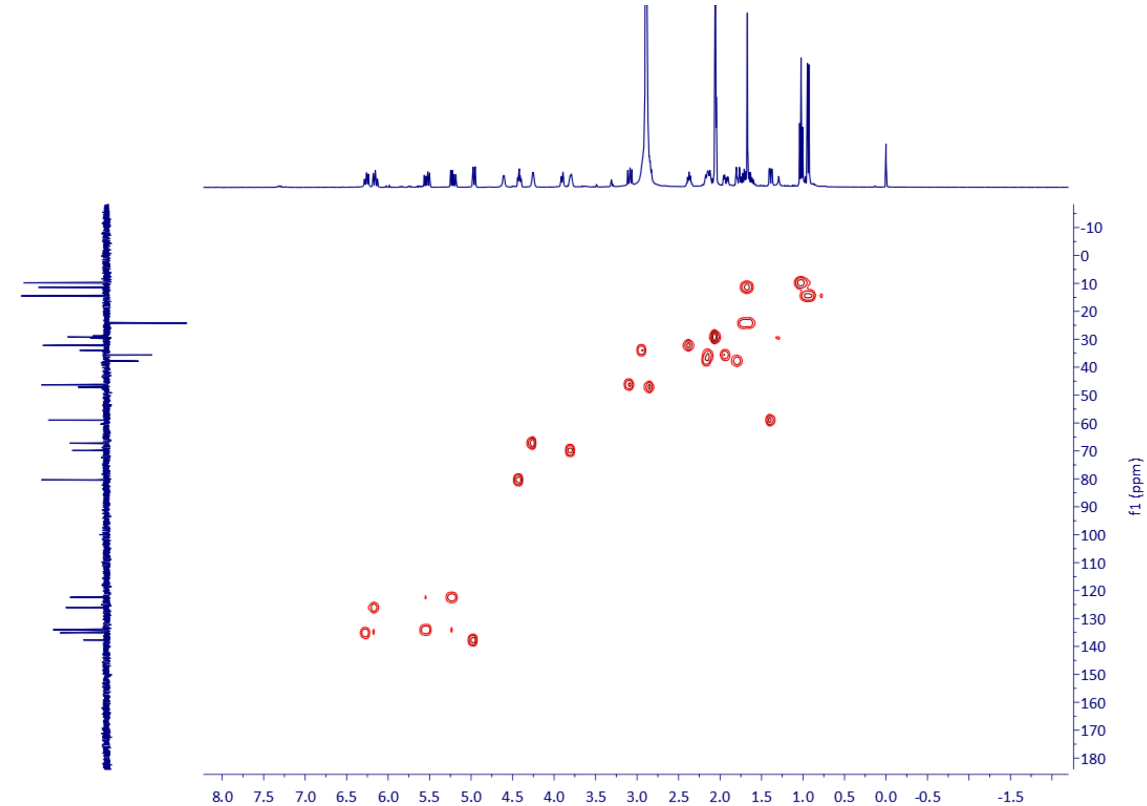
Supplementary Fig. 5b. ^{1}H NMR spectrum of **6** in acetone-d_6 (400 MHz).



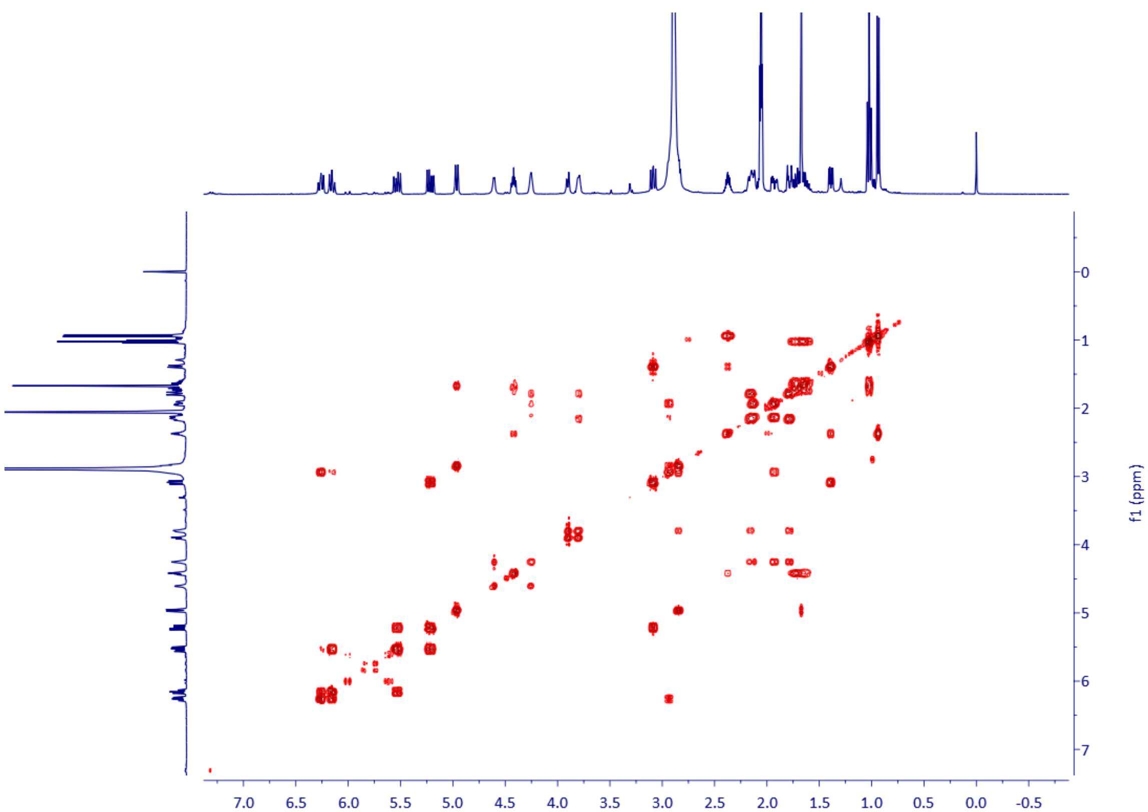
Supplementary Fig. 5c. ^{13}C NMR spectrum of **6** in acetone-d_6 (100 MHz).



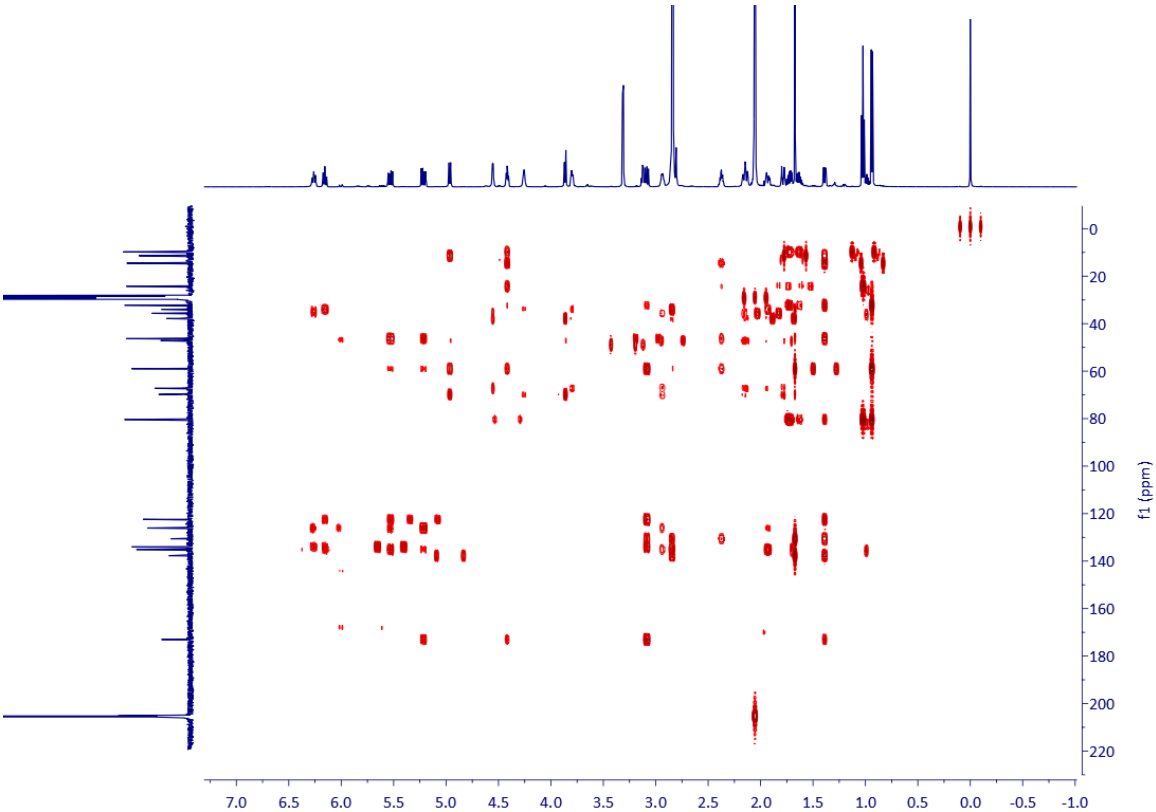
Supplementary Fig. 5d. DEPT-135 NMR spectrum of **6** in acetone-d_6 (100 MHz).



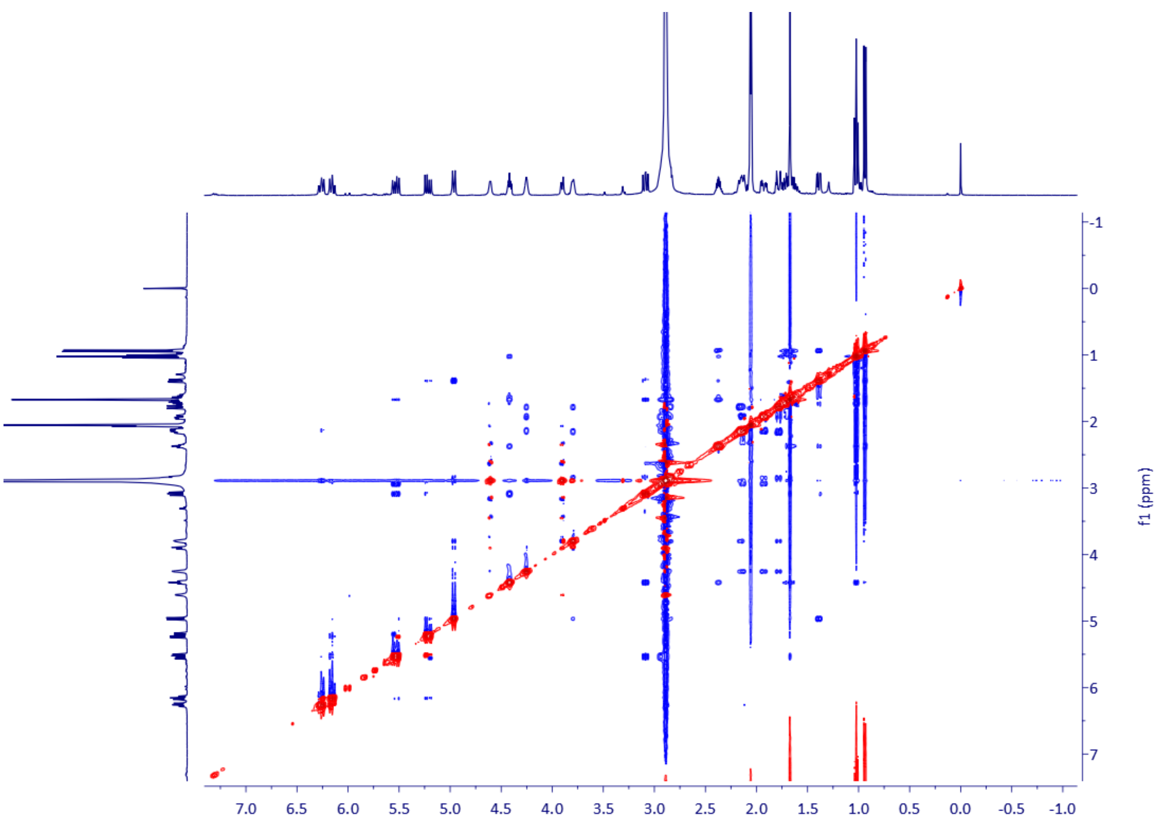
Supplementary Fig. 5e. HSQC NMR spectrum of **6** in acetone-d_6 (400 MHz).



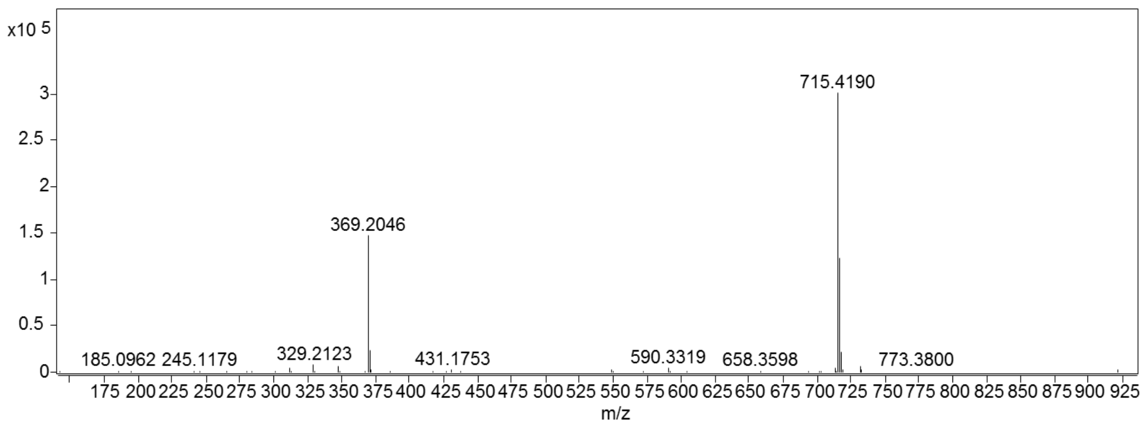
Supplementary Fig. 5f. ^{1}H-^{1}H COSY NMR spectrum of **6** in acetone-d_6 (400 MHz).



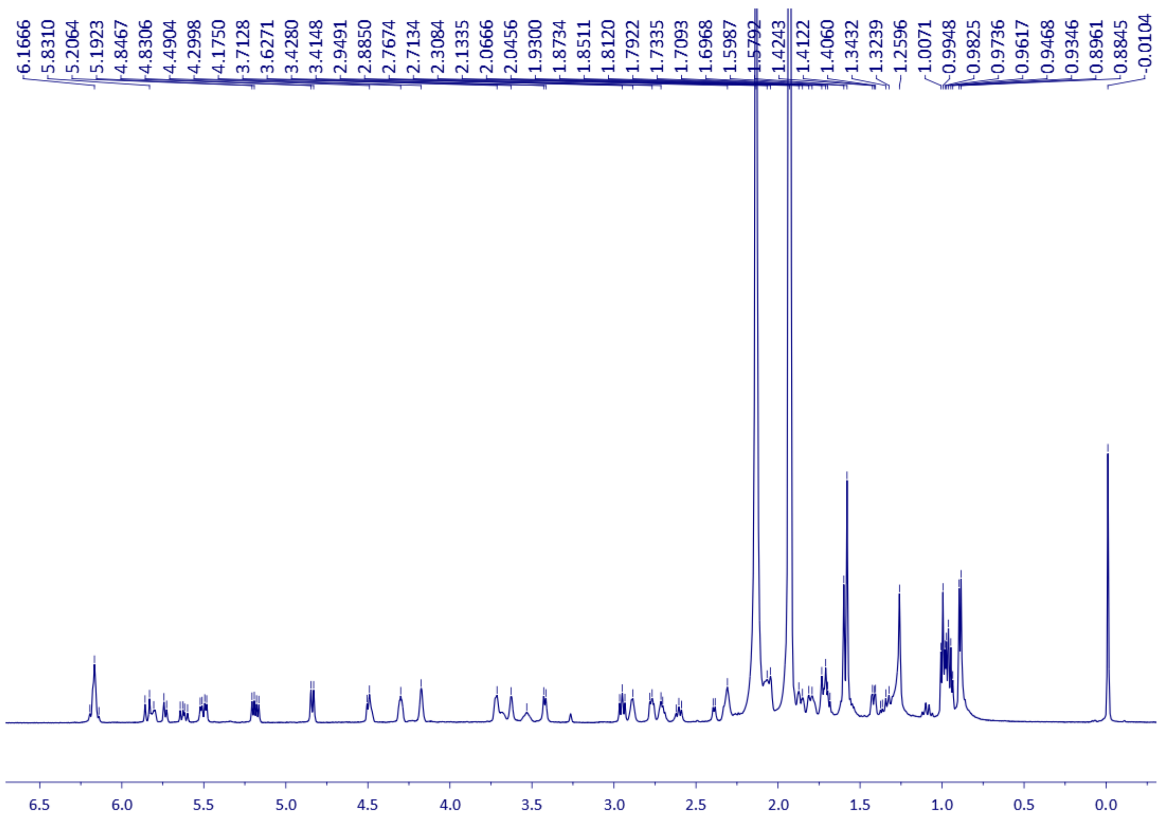
Supplementary Fig. 5g. HMBC NMR spectrum of **6** in acetone-d_6 (600 MHz).



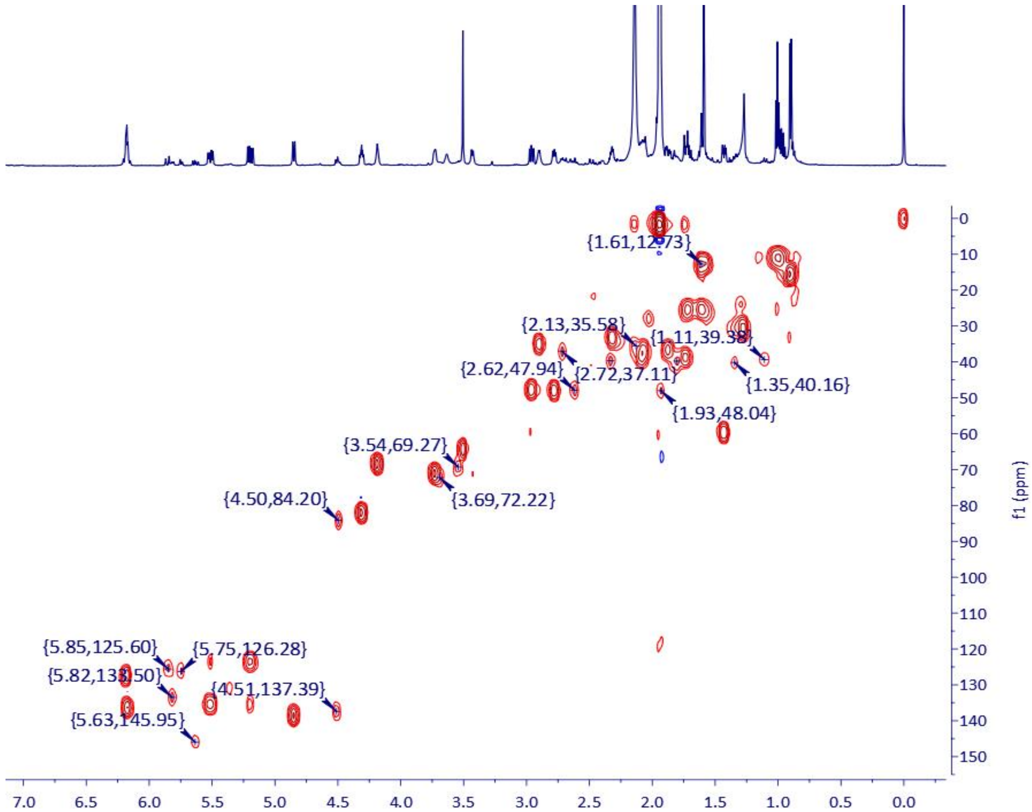
Supplementary Fig. 5h. NOESY spectrum of **6** in acetone-d_6 (600 MHz).



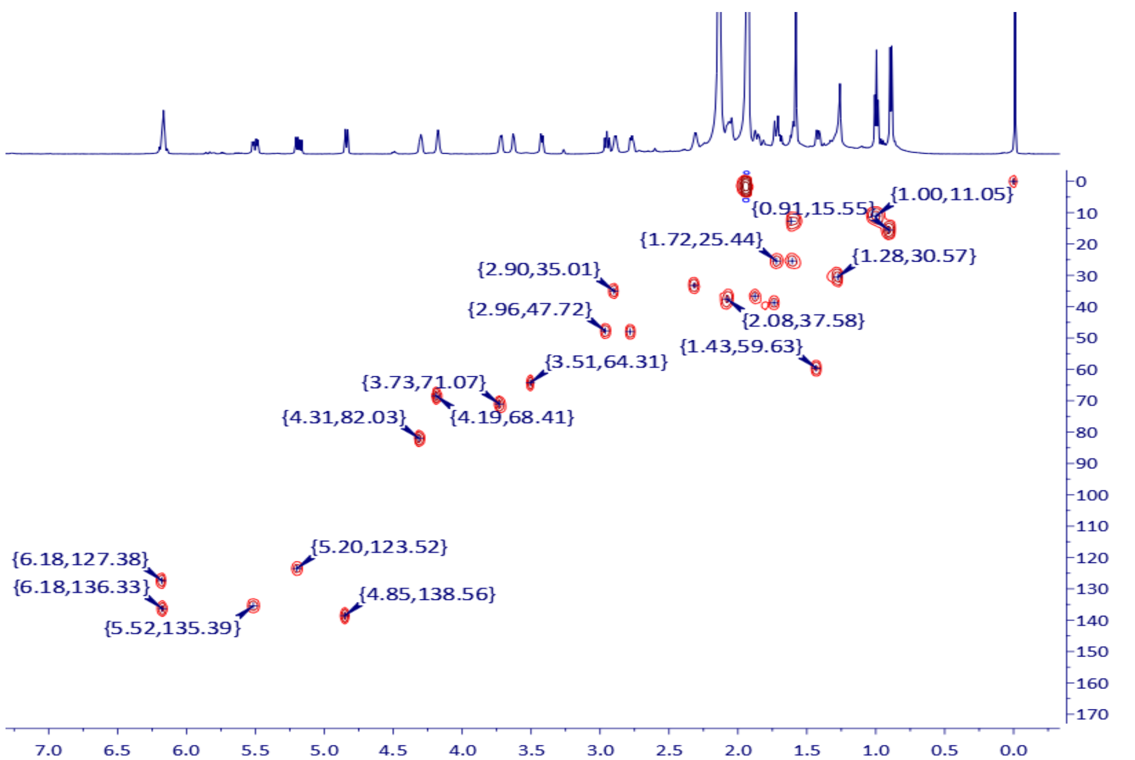
Supplementary Fig. 6a. HR-ESIMS spectrum of **7**.



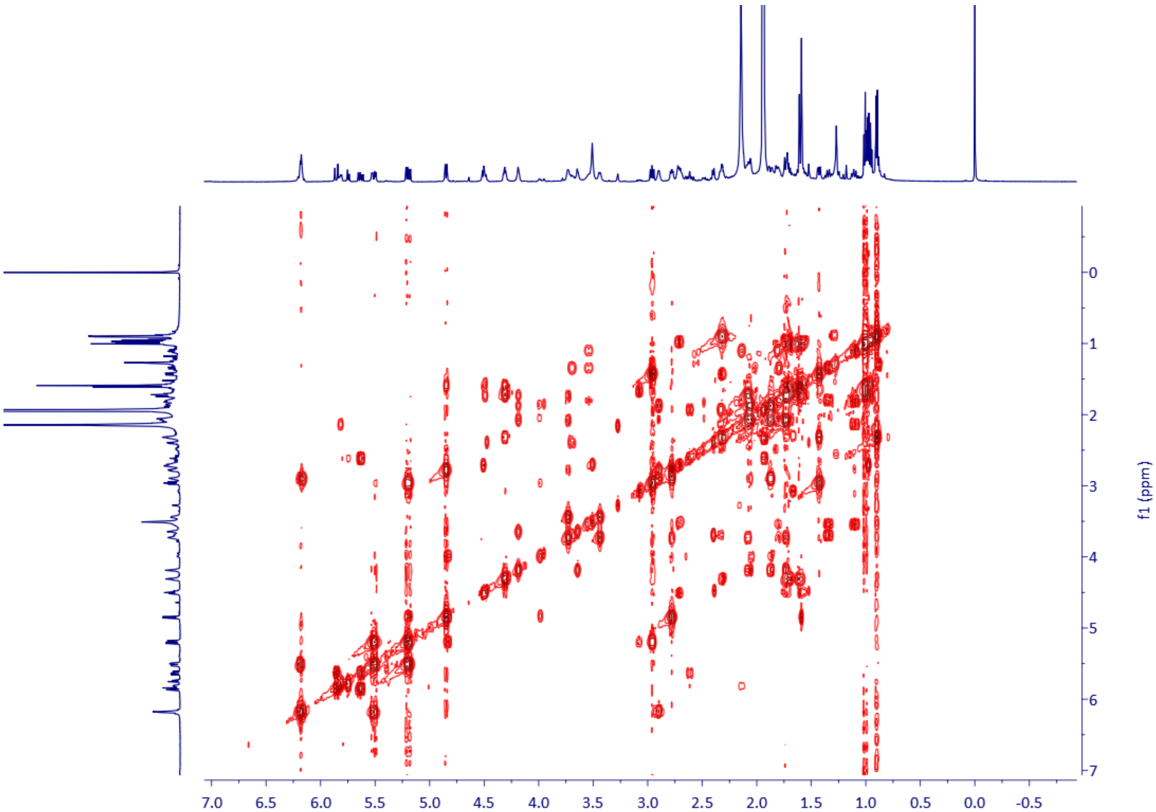
Supplementary Fig. 6b. ^{1}H NMR spectrum of mixture of **6** and **7** in acetonitrile-d_3 (600 MHz).



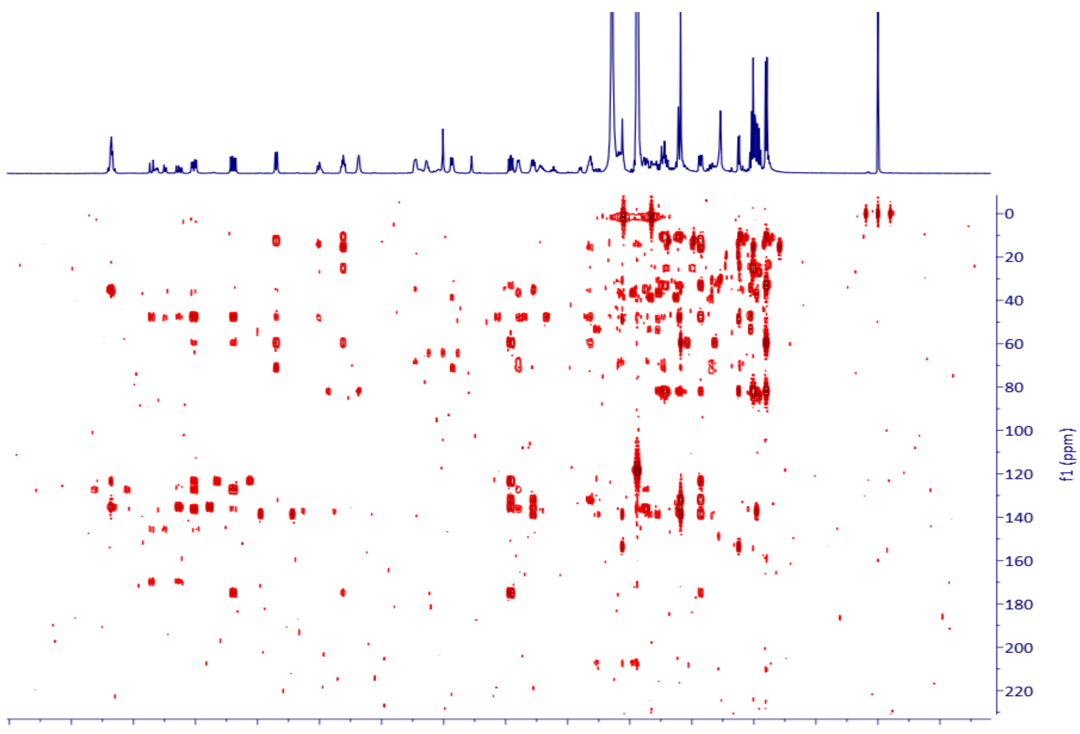
Supplementary Fig. 6c. HSQC NMR spectrum of mixture of **6** and **7** in acetonitrile-d_3 (600 MHz). The signals for **7** were pointed.



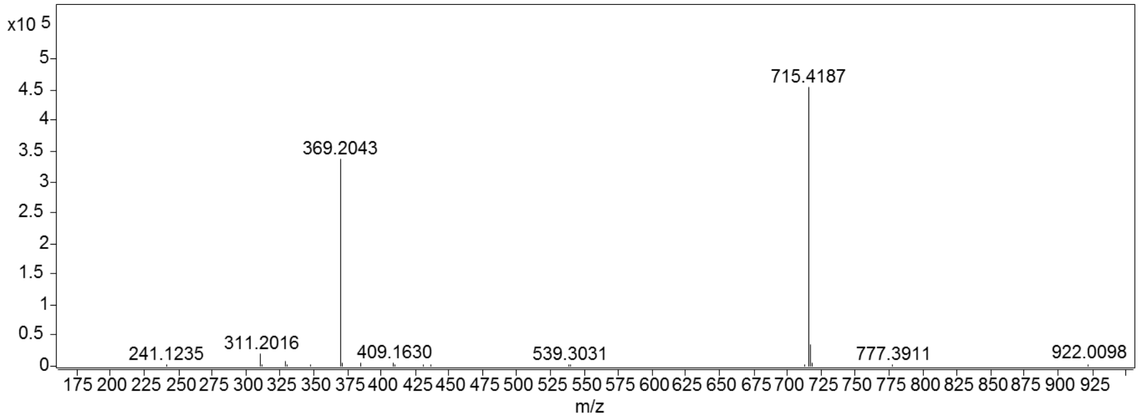
Supplementary Fig. 6d. HSQC NMR spectrum of **6** in acetonitrile-d_3 (600 MHz).



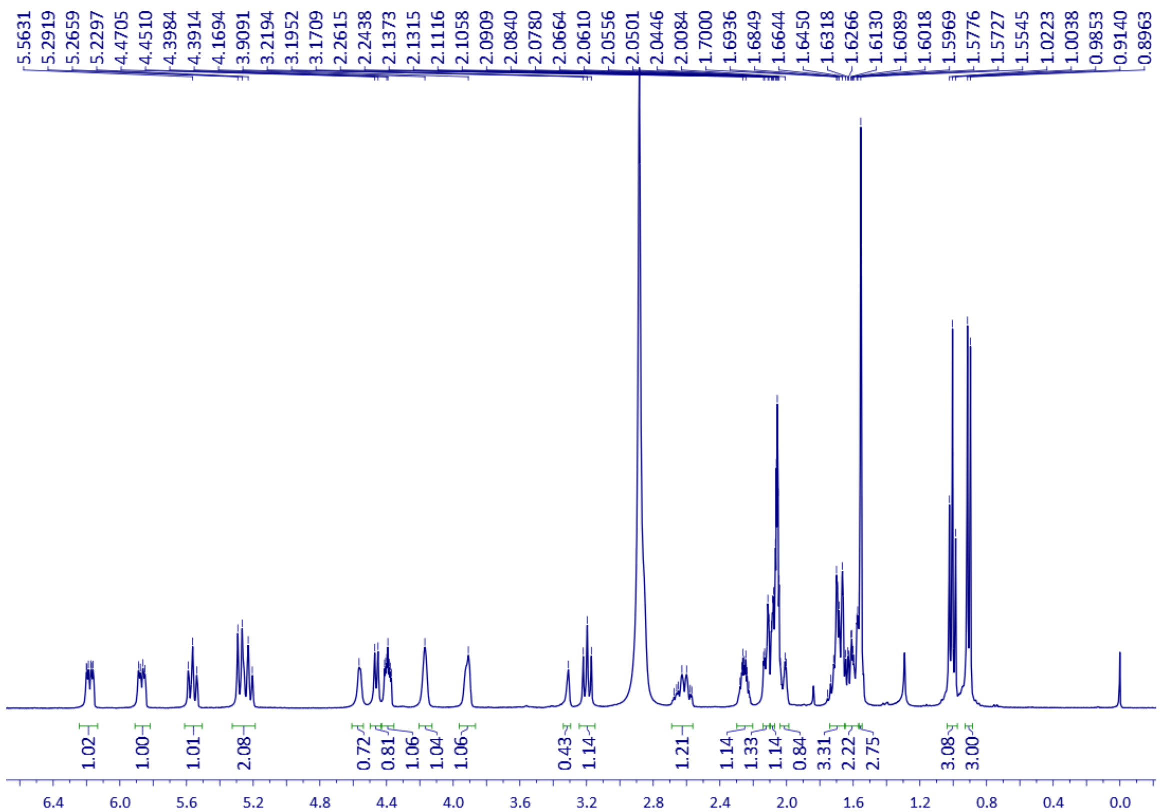
Supplementary Fig. 6e. ^{1}H-^{1}H COSY NMR spectrum of mixture of **6** and **7** in acetonitrile-d_3 (600 MHz).



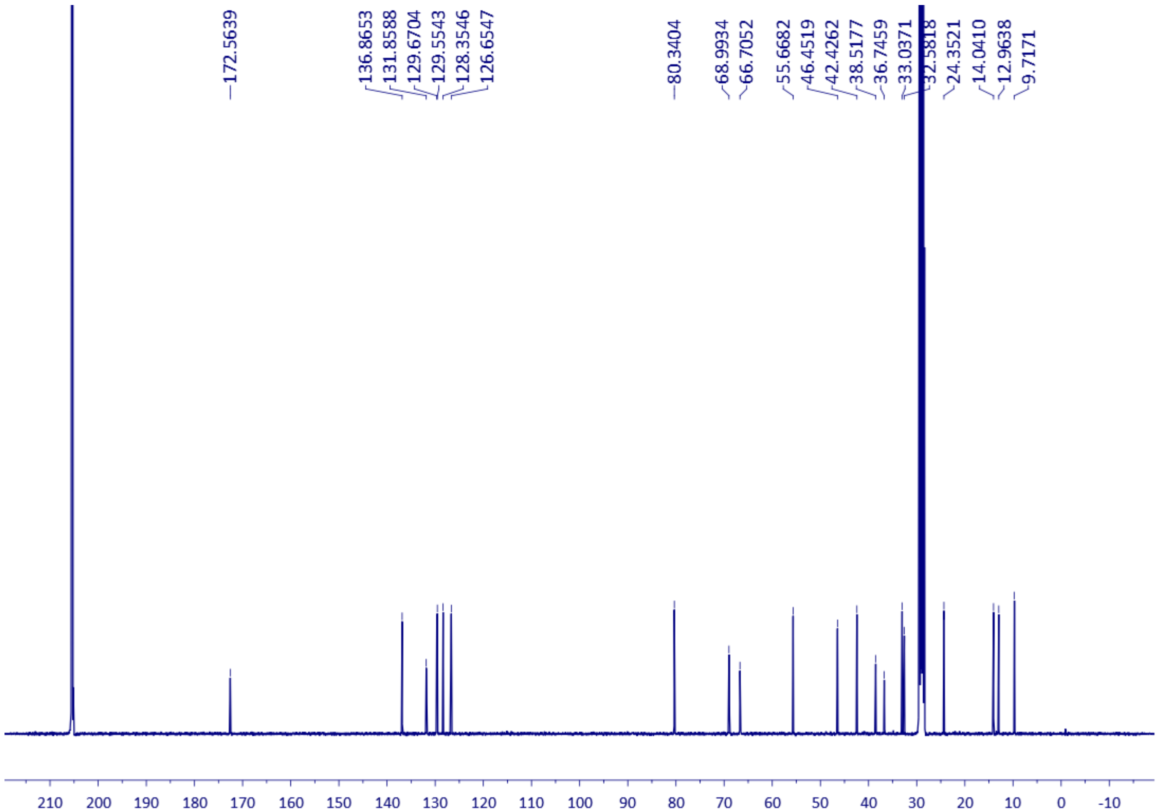
Supplementary Fig. 6f. HMBC NMR spectrum of mixture of **6** and **7** in acetonitrile-d_3 (600 MHz).



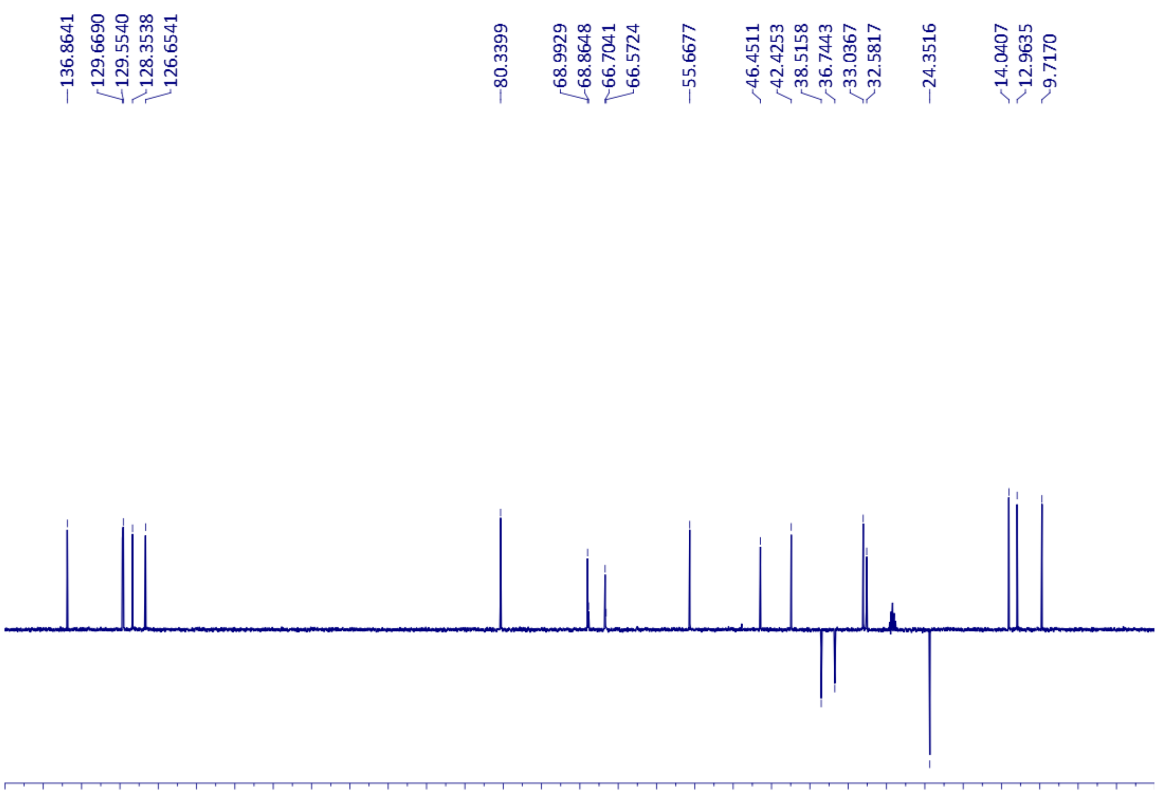
Supplementary Fig. 7a. HR-ESIMS spectrum of **8**.



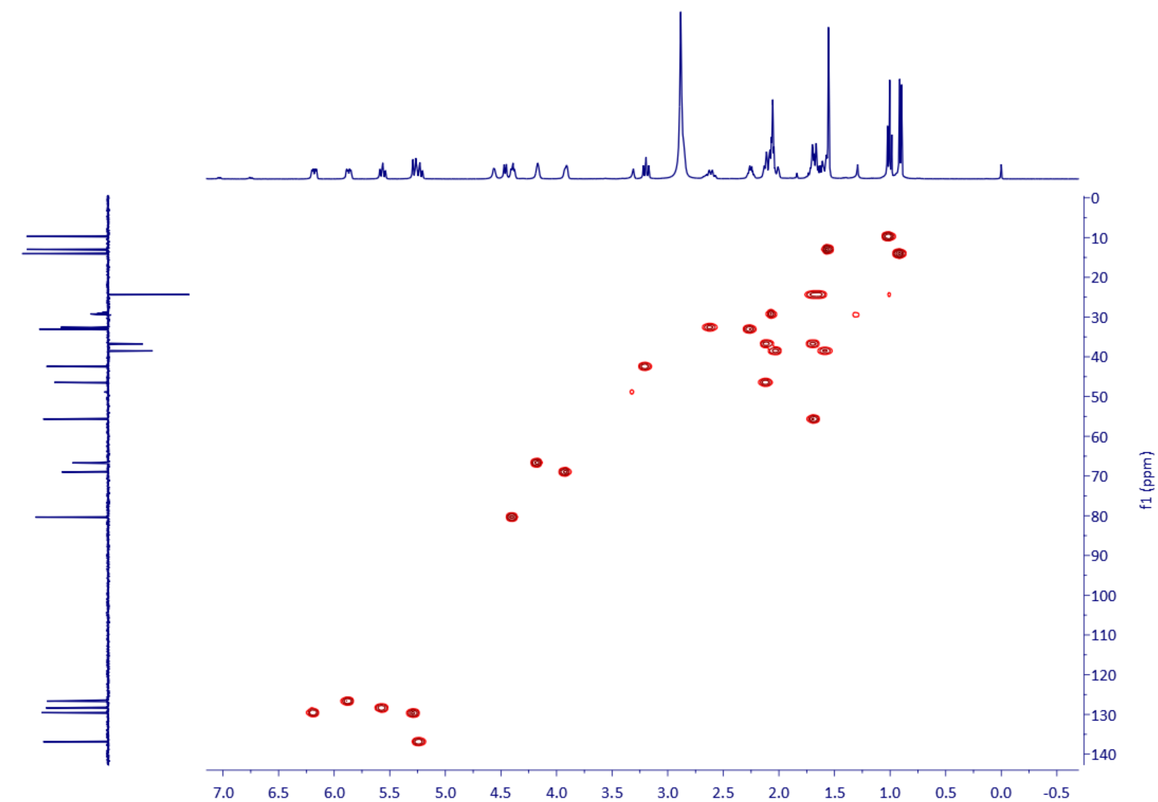
Supplementary Fig. 7b. [1]H NMR spectrum of **8** in acetone-d_6 (400 MHz).



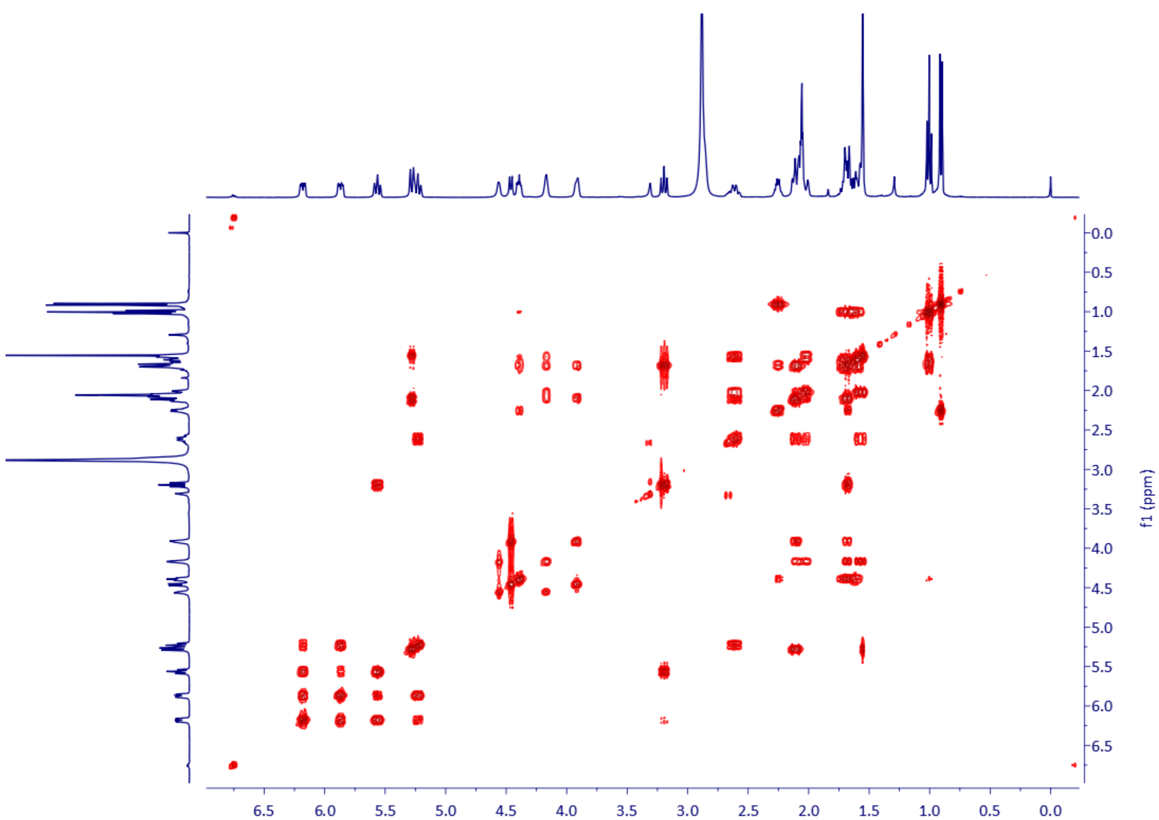
Supplementary Fig. 7c. ^{13}C NMR spectrum of **8** in acetone-d_6 (100 MHz).



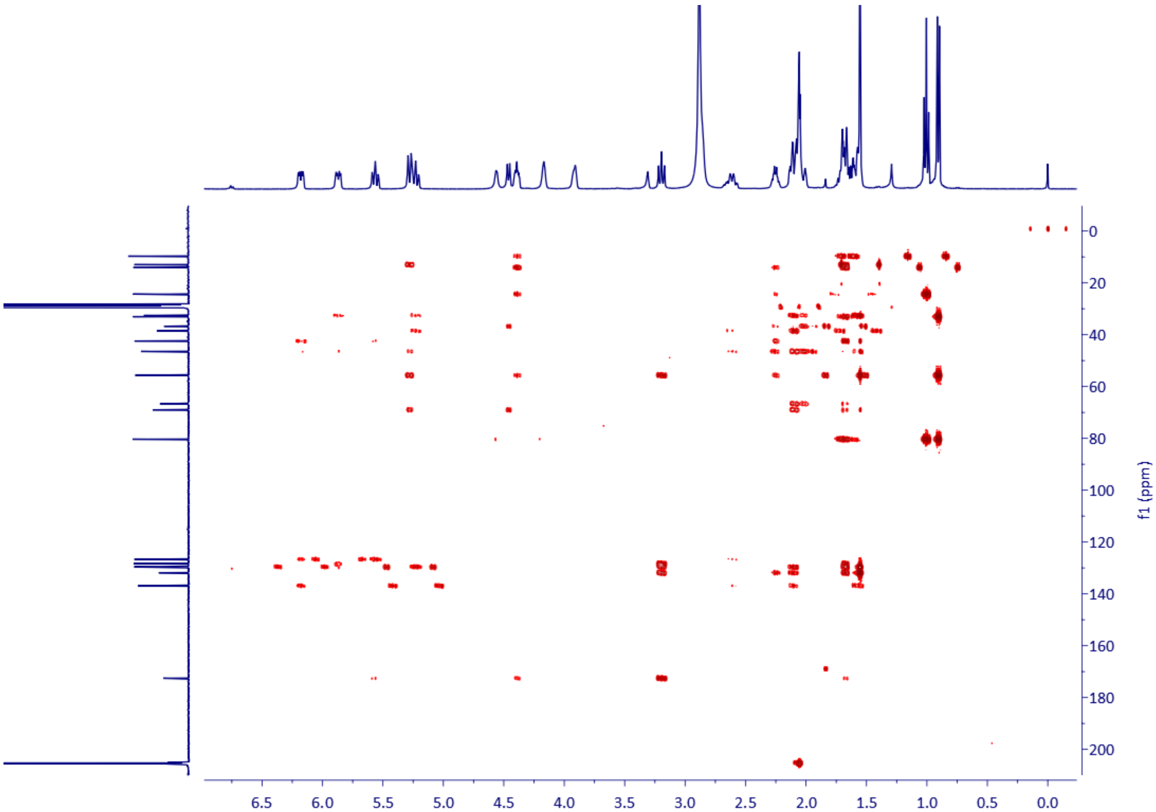
Supplementary Fig. 7d. DEPT-135 NMR spectrum of **8** in acetone-d_6 (100 MHz).



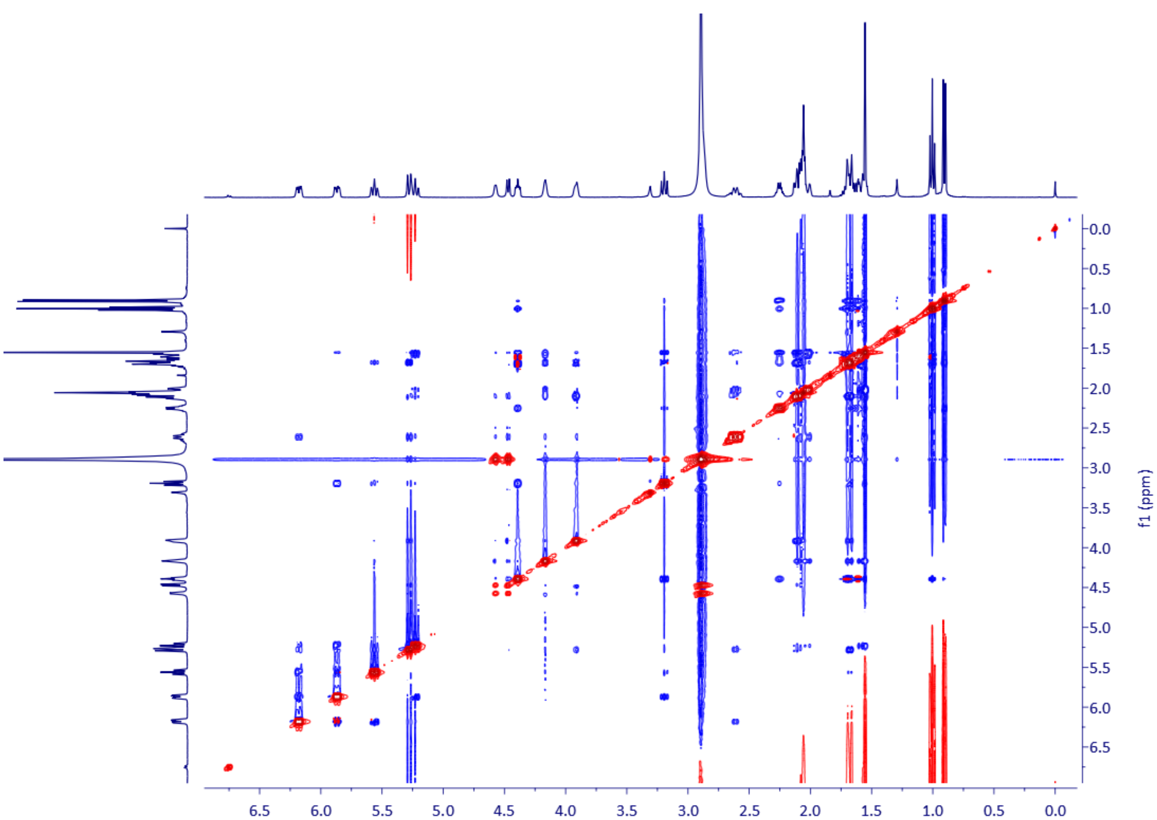
Supplementary Fig. 7e. HSQC NMR spectrum of **8** in acetone-d_6 (400 MHz).



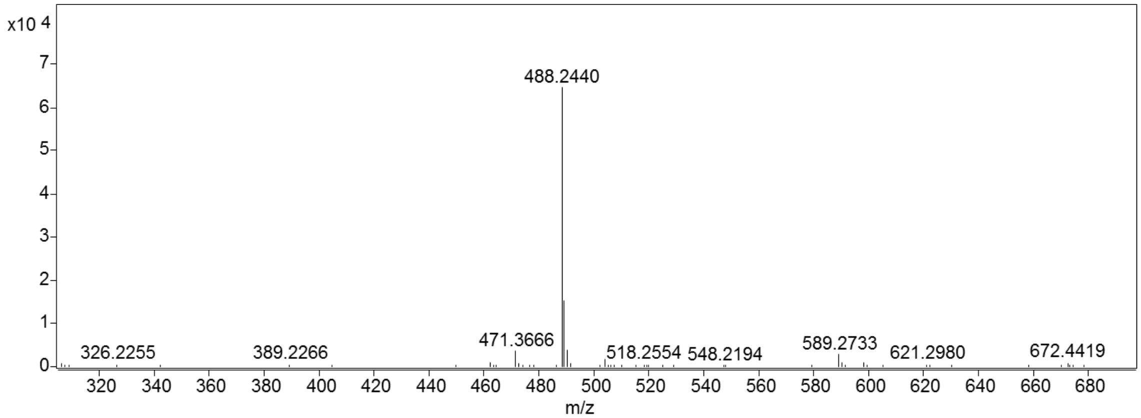
Supplementary Fig. 7f. ^1H-^1H COSY NMR spectrum of **8** in acetone-d_6 (400 MHz).



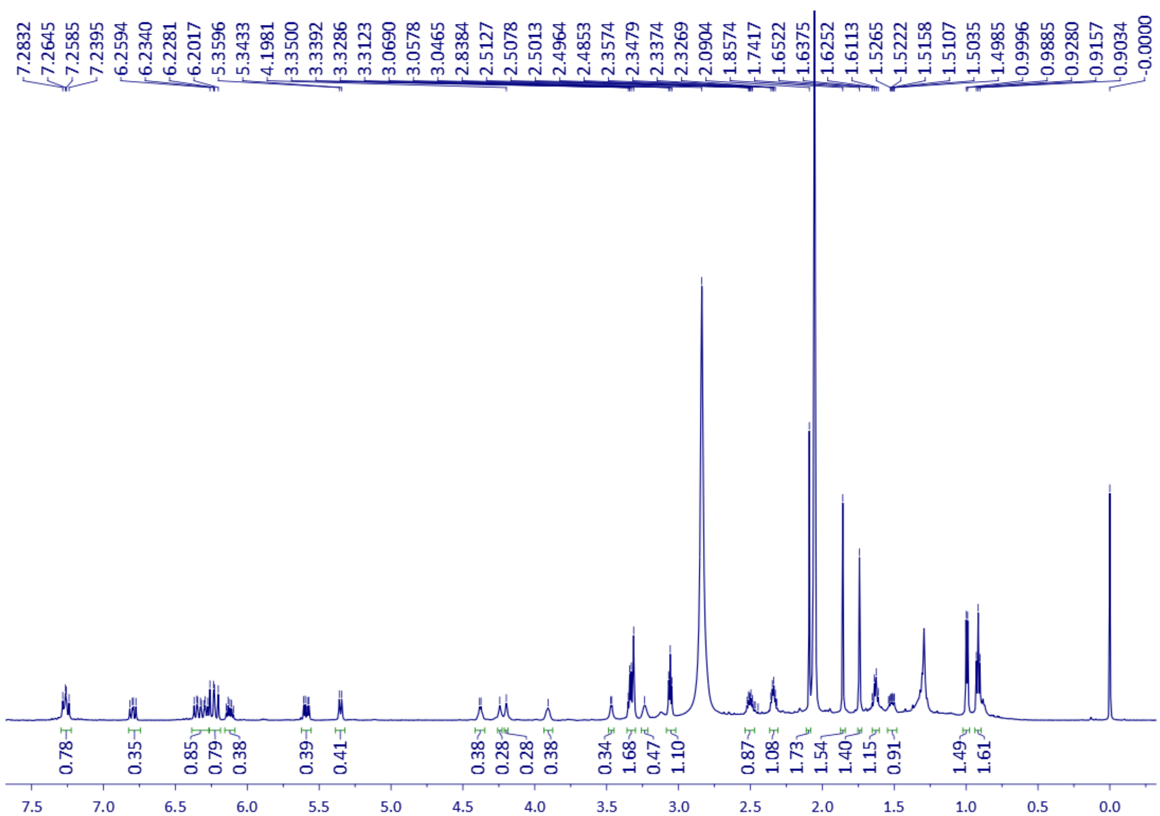
Supplementary Fig. 7g. HMBC NMR spectrum of **8** in acetone-d_6 (400 MHz).



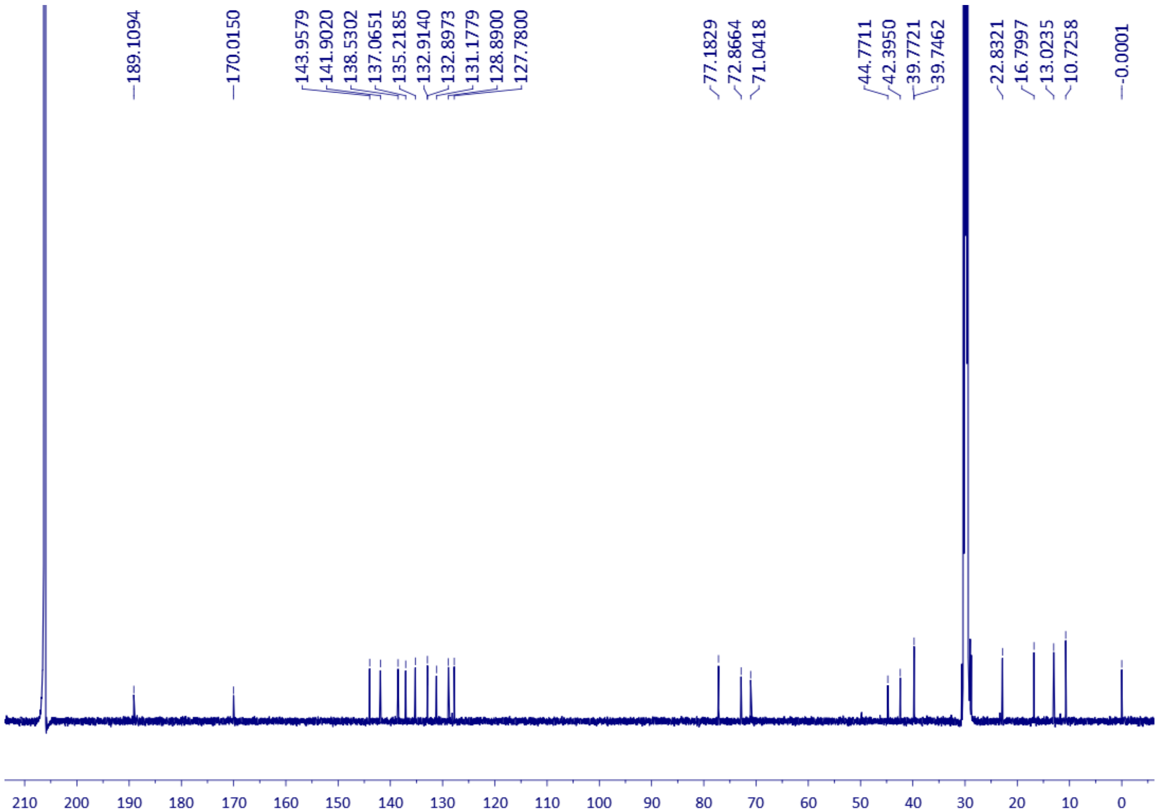
Supplementary Fig. 7h. NOESY spectrum of **8** in acetone-d_6 (600 MHz).



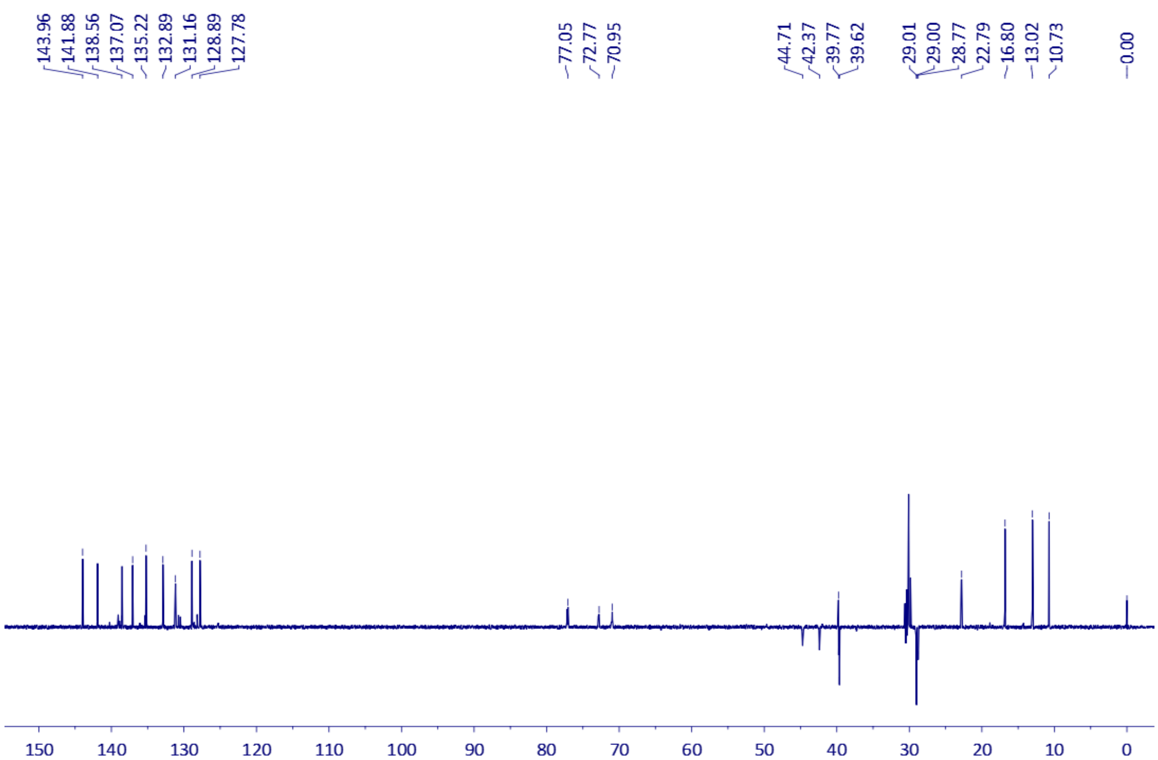
Supplementary Fig. 8a. HR-ESIMS spectrum of **10**.



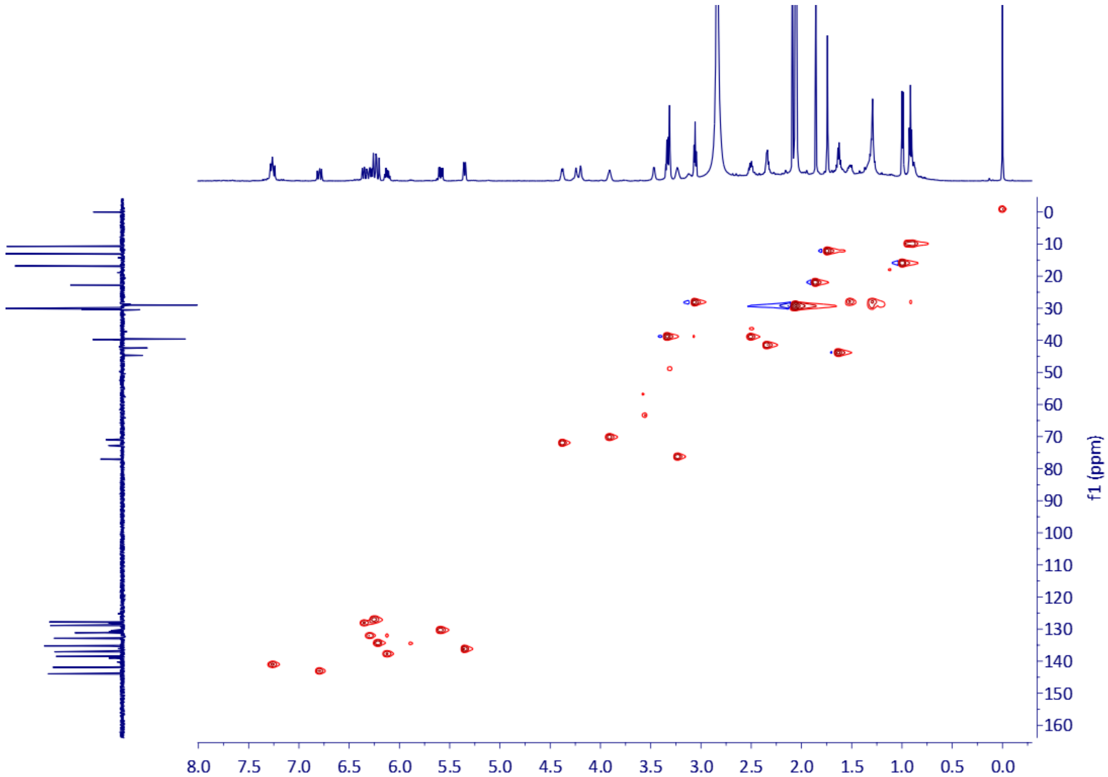
Supplementary Fig. 8b. ^{1}H NMR spectrum of **10** in acetone-d_6 (600 MHz).



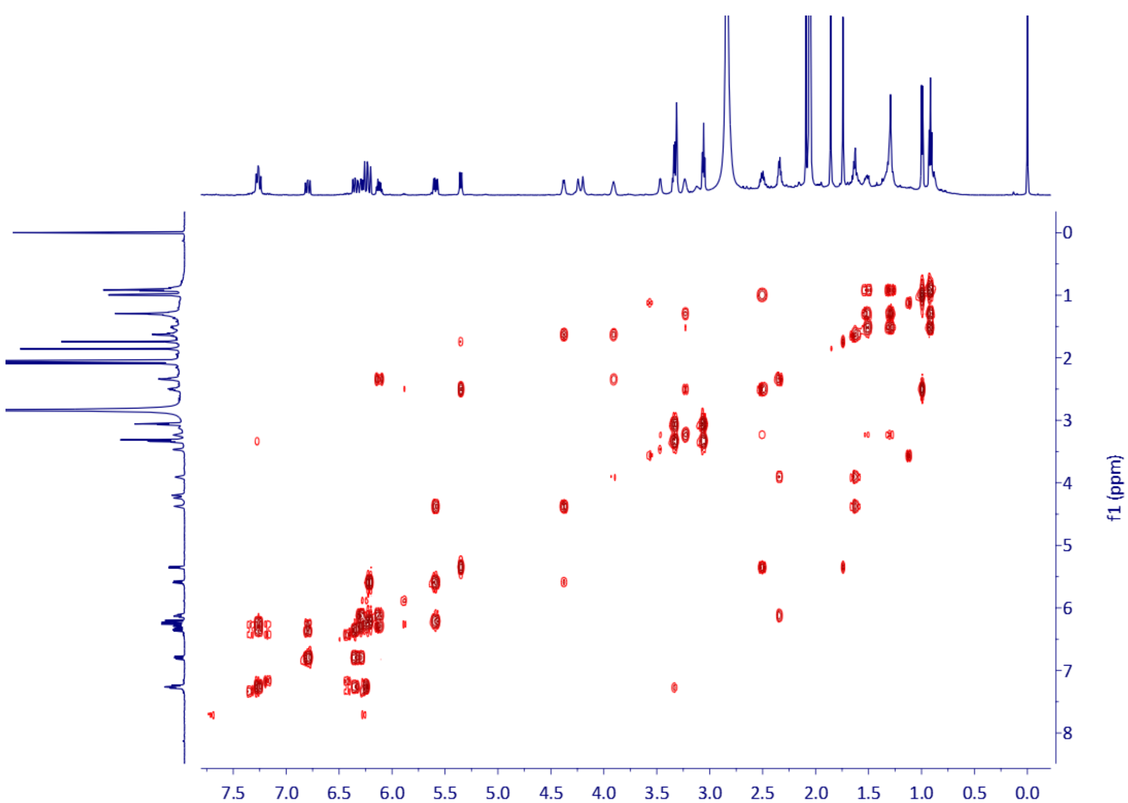
Supplementary Fig. 8c. ^{13}C NMR spectrum of **10** in acetone-d_6 (150 MHz).



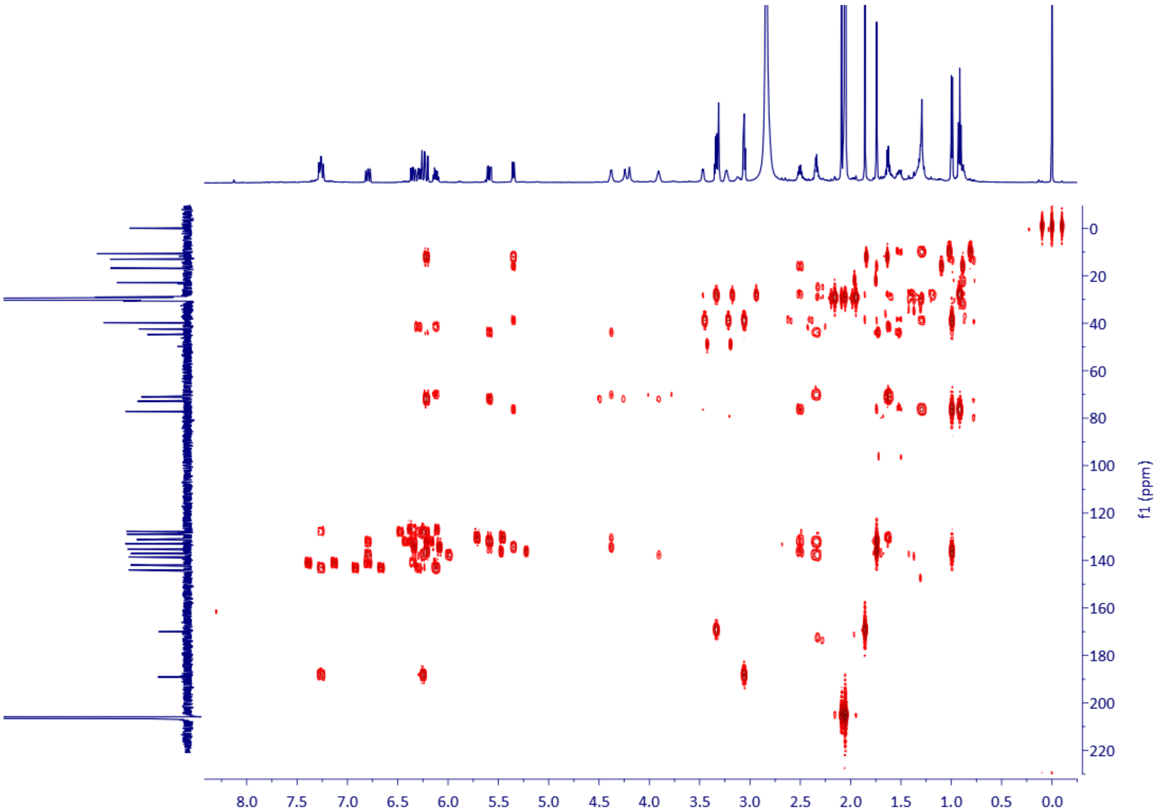
Supplementary Fig. 8d. DEPT-135 NMR spectrum of **10** in acetone-d_6 (150 MHz).



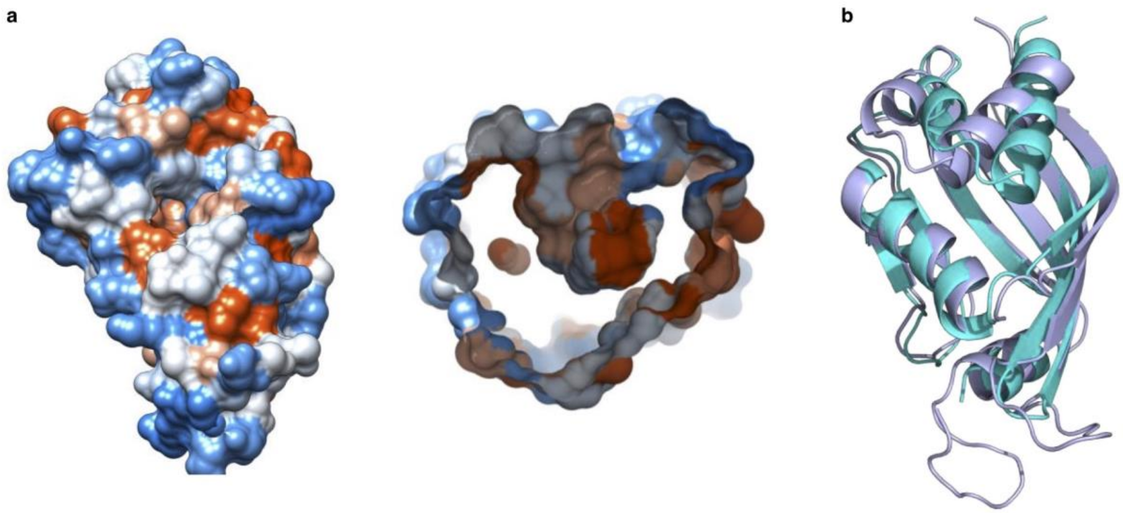
Supplementary Fig. 8e. HSQC NMR spectrum of **10** in acetone-d_6 (600 MHz).



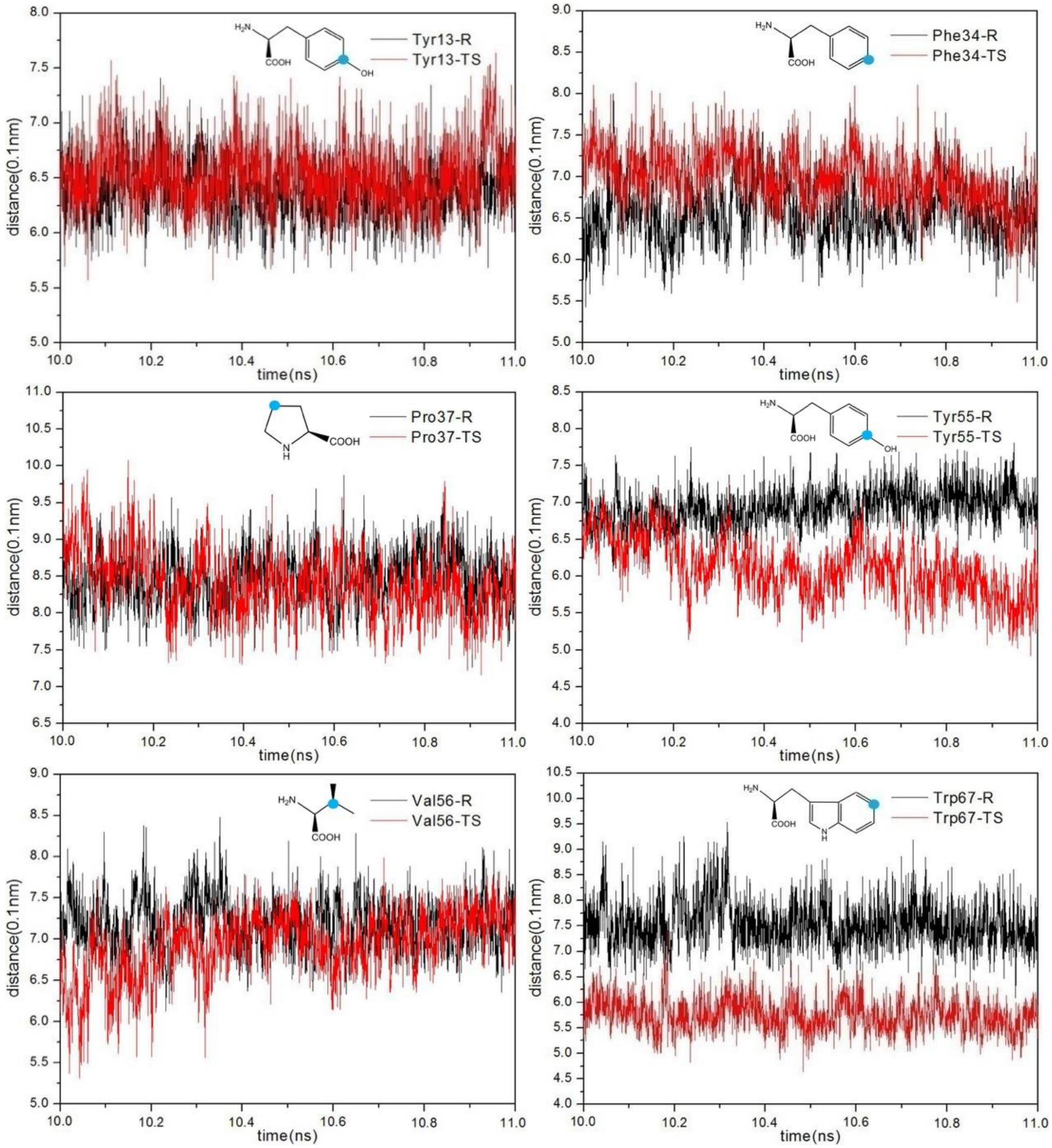
Supplementary Fig. 8f. ^{1}H-^{1}H COSY NMR spectrum of **10** in acetone-d_6 (600 MHz).



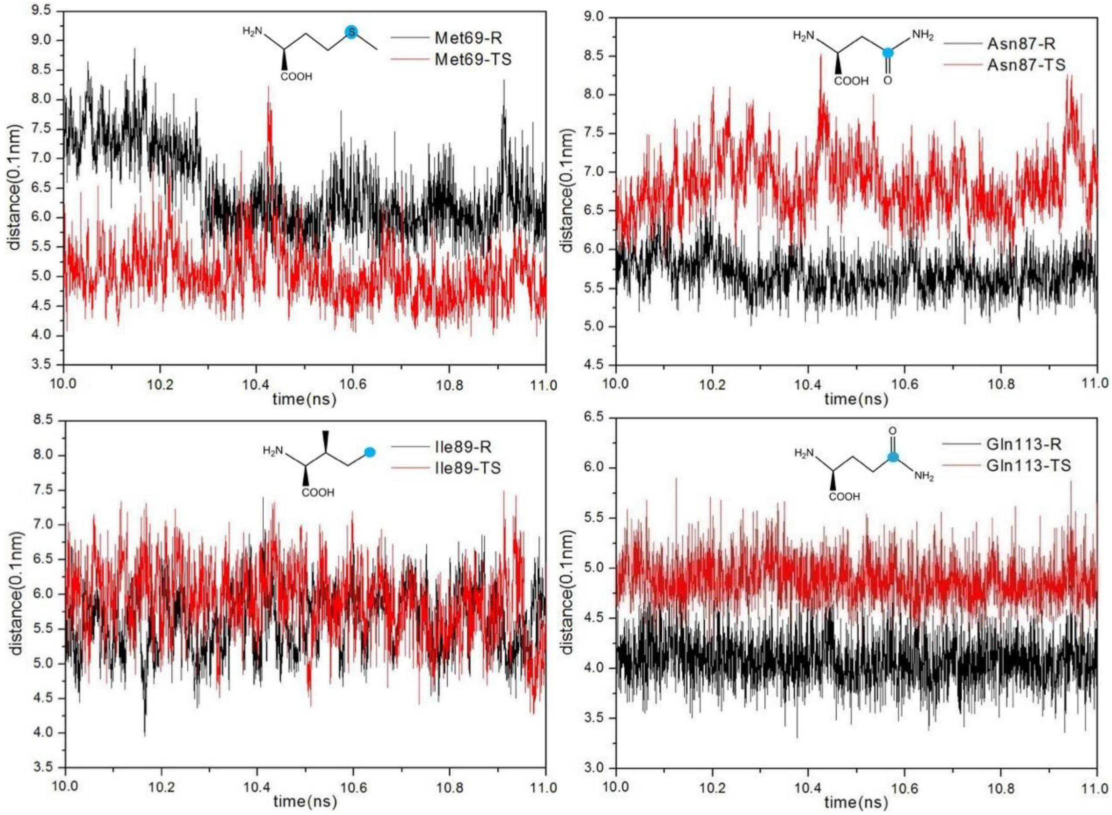
Supplementary Fig. 8g. HMBC NMR spectrum of **10** in acetone-d_6 (600 MHz).



Supplementary Fig. 9. Structures of NgnD and comparison with structure of α + β barrel superfamily protein. (**a**) Colored surface of NgnD and transpancy NgnD viewed from bottom to reveal the active cavity. The molecular surface was colored by amino acid hydrophobicity according to the Kyte-Doolittle scale, the minimal hydrophobicity was colored dodger blue, while the most hydrophobicity was colored orange red and the zero hydrophobicity was colored as white. (**b**) Superimposed cartoon structure of NgnD and Bal32a (PDB: 1TUH). Chains of NgnD were colored as light blue, and Bal32a was colored as cyan. Proteins of dimeric α+β barrel superfamily are evolutionarily conserved with diverse functions, including monooxygenase in antibiotic biosynthesis[26], meroterpenoid isomerase[27], limonene-1,2-epoxide hydrolases[28], type II polyketide cyclase SnoL[29], and scytalone dehydratase[30], all of which share similarities to NgnD with r.m.s. deviations of 1.82−3.33 Å, albeit no sequences similarities.



Supplementary Fig. 10. Distances between residues (blue dot shown on the structure) and the center of mass of the substrate/TS for the time between 10th and 11th ns. The black line and the red line represent the reactant and the transition state, respectively.



Supplementary Fig. 10 (cont.). Distances between residues (blue dot shown on the structure) and the center of mass of the substrate/TS for the time between 10^{th} and 11^{th} ns. The black line and the red line represent the reactant and the transition state, respectively.

Supplementary Table 1. Bacterial strains and plasmids.

Plasmid/Strain	Relevant characteristics	Reference
Plasmid		
pKC1139	*E. coli*-*Streptomyces* shuttle plasmid used for gene disruption, temperature sensitive	47
pSET152-*kasO*p*	pSET152 derived plasmid containing the promoter *kasO*p*	48
pET-28a(+)	Protein expression vector used in *E. coli*, encoding N-terminal His-tag, kanamycin resistance	Novagen
pET-22b(+)	Protein expression vector used in *E. coli*, encoding C-terminal His-tag, ampicillin resistance	Novagen
pHG02001	pKC1139 derived plasmid for disruption of *stmA*	This study
pHG02002	pKC1139 derived plasmid for disruption of *stmD*	This study
pHG02003	pKC1139 derived plasmid for disruption of *stmD*-*stmF1*	This study
pHG02004	pKC1139 derived plasmid for disruption of *stmY*-*stmF1*	This study
pHG02005	pSET152-*kasO*p* derived plasmid for complementation of *stmD* in HG02001	This study
pHG02006	pSET152-*kasO*p* derived plasmid for complementation of *ngnD* in HG02001	This study
pHG02007	pET-28a(+) derived plasmid for expressing N-terminal His-tag StmD	This study
pHG02008	pET-28a(+) derived plasmid for expressing N-terminal His-tag NgnD	This study
pHG02009	pET-28a(+) derived plasmid for expressing N-terminal His-tag 101015D	This study
pHG02010	pET-28a(+) derived plasmid for expressing N-terminal His-tag F601D	This study
pHG02011	pET-28a(+) derived plasmid for expressing N-terminal His-tag Root369D	This study
pHG02012	pET-22b(+) derived plasmid for expressing C-terminal His-tag StmD	This study
pHG02013	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD	This study
pHG02014	pET-22b(+) derived plasmid for expressing C-terminal His-tag 101015D	This study
pHG02015	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (Y13A)	This study
pHG02016	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (F34Y)	This study
pHG02017	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (P37A)	This study
pHG02018	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (Y55A)	This study
pHG02019	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (Y55F)	This study
pHG02020	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (V56A)	This study
pHG02021	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (V56S)	This study
pHG02022	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (W67A)	This study
pHG02023	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (M69A)	This study
pHG02024	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (N87A)	This study
pHG02025	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (I89A)	This study

pHG02026	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (Q113A)	This study
pHG02027	pET-28a(+) derived plasmid for expressing N-terminal His-tag StmC-HACP-TE	This study
pHG02028	pET-28a(+) derived plasmid for expressing N-terminal His-tag StmC-ACP-TE	This study
pHG02029	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnC-TE	This study
pHG02030	pET-28a(+) derived plasmid for expressing N-terminal His-tag NgnC-ACP-TE	This study
pHG02031	pET-28a(+) derived plasmid for expressing N-terminal His-tag NgnC-M9-TE	This study
pHG02032	pSET152-*kasO*p* derived plasmid for complementation of *ngnD (F34Y/W67A)* in HG02001	This study
pHG02033	pET-22b(+) derived plasmid for expressing C-terminal His-tag NgnD variant (F34A/W67A)	This study
***E. coli* strains**		
DH5α	General cloning host	49
BL21 (DE3)	Heterologous host for protein expression	NEB
ET12567 (pUZ8002)	Methylation-deficient host used for *E. coli-Streptomyces* intergeneric conjugation	47
***S. seoulensis* strains**		
A01	Wild type strain for streptoseomycin (STM) production	
HG02001	Δ*stmD,* in-frame deletion mutant strain in WT, STM non-producing	This study
HG02002	complementation of *ΔstmD* mutant by *stmD*, STM producing	This study
HG02003	complementation of *ΔstmD* mutant by *ngnD*, STM producing	This study
HG02004	Δ*stmA,* in-frame deletion mutant strain in WT, STM non-producing	This study
HG02005	Δ*stmD-stmF1,* in-frame deletion mutant strain in WT, STM non-producing	This study
HG02006	Δ*stmY-stmF1,* in-frame deletion mutant strain in WT, STM non-producing	This study
HG02007	complementation of *ΔstmD-stmF1*mutant by *ngnD-F34Y/W67A*, STM non-producing	This study

Supplementary Table 2. Oligonucleotide primers used in this study.

Oligonucleotide	Sequence[a]	Enzyme sites
a. for amplification of homologous arms from genomic DNA for gene disruption (5'-3')		
Δ*stmA*-UP-F	**AACGACGGCCAGTGCCAAGCTT**CAGTTCCATCGGCCAGAAGAT	*Hin*dIII
Δ*stmA*-UP-R	**AACGACGGCCAGTGCCAAGCTT**CAGTTCCATCGGCCAGAAGAT	
Δ*stmA*-DOWN-F	ATGGCGACGCCTCATATG	
Δ*stmA*-DOWN-R	**AGCTATGACATGATTACGAATTC**GGTTCGATGAAGGTGGTGT	*Eco*RI
Δ*stmD*-UP-F	**AACGACGGCCAGTGCCAAGCTT**CTGGATGACATCGACCGAAAC	*Hin*dIII
Δ*stmD*-UP-R	**CGCGGATCAGTAGCGTCG**CATGTGTCCTCCGCTTGC	
Δ*stmD*-DOWN-F	CGACGCTACTGATCCGCG	
Δ*stmD*-DOWN-R	**AGCTATGACATGATTACGAATTC**GATCGGCTGCATGTACGACTA	*Eco*RI
Δ*stmD-stmF1*-UP-F	**AACGACGGCCAGTGCCAAGCTT**GTGGTCATGTCAGGTGTG	*Hin*dIII
Δ*stmD-stmF1*-UP-R	**GTGCGCATTGAACTGGAC**CTGGGTTATCTCAGCCAT	
Δ*stmD-stmF1*-DOWN-F	GTCCAGTTCAATGCGCAC	
Δ*stmD-stmF1*-DOWN-R	**AGCTATGACATGATTACGAATTC**ATCGACTCGGCGAACAC	*Eco*RI
Δ*stmY-stmF1*-UP-F	**AACGACGGCCAGTGCCAAGCTT**CGTTTCGGCCATCCTGTTT	*Hin*dIII
Δ*stmY-stmF1*-UP-R	**GTGCGCATTGAACTGGAC**GACGCCTTCGCGTTGATC	
Δ*stmY-stmF1*-DOWN-F	GTCCAGTTCAATGCGCAC	
Δ*stmY-stmF1*-DOWN-R	**AGCTATGACATGATTACGAATTC**ATCGACTCGGCGAACAC	*Eco*RI
b. for screening of the correct mutants (5'-3')		
ScreenΔ*stmA*-F	AGCCACCGCCCGTGTACC	
ScreenΔ*stmA*-R	GTCCTGGTTGACGGCCGAAC	
ScreenΔ*stmD*-F	AAATAGCTCCGATGGAATCCG	
ScreenΔ*stmD*-R	ATCGAACACCTGGCACTG	
ScreenΔ*stmD-stmF1*-F1	AATGCTTGAACGCGCGAA	
ScreenΔ*stmD-stmF1*-R1	CGCCTTCAGGAAGTAGGAGAC	
ScreenΔ*stmY-stmF1*-F1	TACGGTGCAGCCACTGCT	
ScreenΔ*stmY-stmF1*-R1	AGGTTGACTTTCCGCACTACTC	
ScreenΔ*stmD-stmF1*-F2	GCATTTCCCATATTCGAGAGAGA	
ScreenΔ*stmD-stmF1*-R2	CGGAGAACAGATAGGTGAAGAAC	
ScreenΔ*stmY-stmF1*-F2	GCATTTCCCATATTCGAGAGAGA	
ScreenΔ*stmY-stmF1*-R2	CGGAGAACAGATAGGTGAAGAAC	
c. for genes complementation (5'-3')		
152-StmD-F	**TGCTGCATGCATACGTACTAGT**CATTCTCACGGTGTGACC	*Spe*I
152-StmD-R	**CTATGACATGATTACGAATTC**CCTGGGAACCACACGAC	*Eco*RI

152-NgnD-F	**TGCTGCATGCATACGTACTAGT**GTCCTGGAAGCTCTGCGA	*Spe*I
152-NgnD-R	**CTATGACATGATTACGAATTC**GGCATTGTCCACGGTAGATT	*Eco*RI

d. for protein expression (5'-3')

28a-StmD-F	**GTGCCGCGCGGCAGCCATATG**GCTGAGATAACCCAGGAA	*Nde*I
28a-StmD-R	**CTCGAGTGCGGCCGCAAGCTT**TCAGTAGCGTCGCTCTCC	*Hin*dIII
22b-StmD-F	**GTGCCGCGCGGCAGCCATATG**GCTGAGATAACCCAGGAA	*Nde*I
22b-StmD-R	**CTCGAGTGCGGCCGCAAGCTT**GTAGCGTCGCTCTCCGGC	*Hin*dIII
22b-NgnD-F	**GTGCCGCGCGGCAGCCATATG**ACCGAGATCACCCCC	*Nde*I
22b-NgnD-R	**CTCGAGTGCGGCCGCAAGCTT**GTAGCGGCGTTCGCCGTC	*Hin*dIII
28a-101015D-F	**GTGCCGCGCGGCAGCCATATG**ACCGAGATCACCCCCGAG	*Nde*I
28a-101015D-R	**CTCGAGTGCGGCCGCAAGCTT**TCAGTAGCGGCGTTCGCC	*Hin*dIII
22b-101015D-F	**GTGCCGCGCGGCAGCCATATG**ACCGAGATCACCCCCGAG	*Nde*I
22b-101015D-R	**CTCGAGTGCGGCCGCAAGCTT**GTAGCGGCGTTCGCCGTC	*Hin*dIII
28a-F601D-F	**GTGCCGCGCGGCAGCCATATG**ACTGAGATCACCCCCGAG	*Nde*I
28a-F601D-R	**CTCGAGTGCGGCCGCAAGCTT**TCAGAGGTAGCGACGTTC	*Hin*dIII
28a-Root369D-F	**GTGCCGCGCGGCAGCCATATG**ACCGCGATCACCCCCGAG	*Nde*I
28a-Root369D-R	**CTCGAGTGCGGCCGCAAGCTT**GCGGTTCCTGCGTGTTCC	*Hin*dIII
28a-StmC-HACPTE-F	**GTGCCGCGCGGCAGCCATATG**GATCTGCGCAACCGGCTG	*Nde*I
28a-StmC-HACPTE-R	**CTCGAGTGCGGCCGCAAGCTT**TCACAGGGTCCCCAGCCA	*Hin*dIII
28a-StmC-ACPTE-F	**GTGCCGCGCGGCAGCCATATG**GTGGAGCGGGACACGCCG	*Nde*I
28a-StmC-ACPTE-R	**CTCGAGTGCGGCCGCAAGCTT**TCACAGGGTCCCCAGCCA	*Hin*dIII
22b-NgnC-TE-F	**GTGCCGCGCGGCAGCCATATG**GAATTGGCACAGACGGCC	*Nde*I
22b-NgnC-TE-R	**CTCGAGTGCGGCCGCAAGCTT**TTTCGTCCTCTCTTGTTCGGT	*Hin*dIII
28a-NgnC-ACPTE-F	**GTGCCGCGCGGCAGCCATATG**CTGTCCACCCGGCTCGCC	*Nde*I
28a-NgnC-ACPTE-R	**CTCGAGTGCGGCCGCAAGCTT**TCATTTCGTCCTCTCTTGTTC	*Hin*dIII
28a-NgnC-M9TE-F	**GTGCCGCGCGGCAGCCATATG**GACATTGCCGTAGCTGCC	*Nde*I
28a-NgnC-M9TE-R	**CTCGAGTGCGGCCGCAAGCTT**TCATTTCGTCCTCTCTTGTTC	*Hin*dIII

d. for NgnD site mutation (5'-3')

NgnD-M69A-F	CACGTGGAGCgcaCAGCCCGTCACGCTG
NgnD-M69A-R	CCGCCGGAAGCCTCCAGC
NgnD-F34Y-F	GGACCTGCGCtatCTGGCGCCGG
NgnD-F34Y-R	TCCGACCAGTACAGCGCG
NgnD-V56S-F	CATGTCCTATagtCAGGGAATGCTGGAGGCTTC
NgnD-V56S-R	AAGTCGTCGATGCCGACC
NgnD-Y13A-F	CGCCGCCGCTgcaCAGGCGGTCA

NgnD-Y13A-R	ACCAGTTCGGGGGTGATC
NgnD-P37A-F	CTTCCTGGCGgcaGGTAGCCACG
NgnD-P37A-R	CGCAGGTCCTCCGACCAG
NgnD-Y55A-F	CTTCATGTCCgcaGTGCAGGGAATGCTGGAGGCTTCC
NgnD-Y55A-R	TCGTCGATGCCGACCCGC
NgnD-Y55F-F	CTTCATGTCCttcGTGCAGGGAATGCTGGAGG
NgnD-Y55F-R	TCGTCGATGCCGACCCGC
NgnD-V56A-F	CATGTCCTATgcaCAGGGAATGCTGG
NgnD-V56A-R	AAGTCGTCGATGCCGACC
NgnD-W67A-F	CGGCGGCACGgcaAGCATGCAGC
NgnD-W67A-R	GAAGCCTCCAGCATTCCC
NgnD-N87A-F	GATCGACGCCgcaCGGATCCACG
NgnD-N87A-R	GAGTAGCCGTCCTCGTTG
NgnD-I89A-F	CGCCAACCGGgcaCACGCGGTAC
NgnD-I89A-R	TCGATCGAGTAGCCGTCC
NgnD-Q113A-F	CTCCGGCGTGgcaATGCTCAAGTG
NgnD-Q113A-R	ATATCGAGCACGTCGAAC

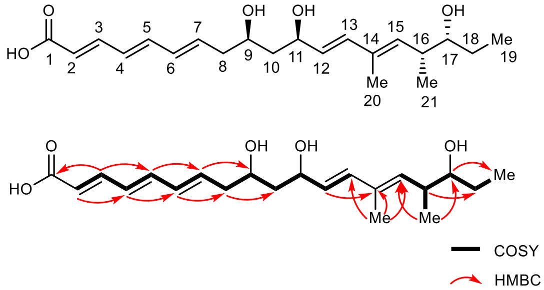
[a] Letters highlighted in bold are sequences used for ligation independent cloning and the enzyme sites are indicated by underline. Lowercase letter indicated mutated nucleotide.

Supplementary Table 3. Annotation of the *ngn* cluster[a].

Gene	Size[b]	Proposed function	homologs[c]	Identity/Similarity (%/%)
orf1	180	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	AccB WP_107660550.1	89/93
orf2	363	GGDEF domain-containing protein	ABR67765.1	84/88
orf3	479	peptidase M14	(WP_107657111.1)	96/98
ngnN2	325	keto-acyl ACP-synthase	NgnN2 (AEI59693.1)	97/93
ngnN3	700	prolyl carrier protein dehydrogenase	NgnN3 (AEI59692.1)	96/93
ngnN4	520	proline adenyltransferase	NgnN4 (AEI59690.1)	92/91
ngnN5	91	proline carrier protein	NgnN5 (AEI59691.1)	93/90
ngnJ	315	LysR family transcriptional regulator	WP_040706732.1	90/94
ngnI	308	retinol dehydrogenase	AGC42222.1	35/49
ngnO3	265	Phytanoyl-CoA dioxygenase	StmO3 (AWH12930.1)	51/66
ngnP2	405	Cytochrome P450	StmP2 (AWH12921.1)	41/58
ngnF	65	ferredoxin	StmF (AWH12920.1)	41/55
ngnM	278	methyltransferase	StmM2 (AWH12917.1)	55/68
ngnG	280	short-chain dehydrogenase	DC31_16040 (KDA05327.1)	50/65
ngnO1	310	LLM class flavin-dependent oxidoreductase	StmO1 (AWH12914.1)	56/66
ngnO2	421	pristinamycin IIA synthase subunit A	StmO2 (AWH12915.1)	82/90
ngnP1	420	cytochrome P450	StmP1 (AWH12916.1)	78/89
ngnK	348	L-glyceraldehyde 3-phosphate reductase	StmK (AWH12918.1)	70/82
ngnA	5961	type I polyketide synthase	StmA (AWH12936.1)	68/77
ngnB	4762	type I polyketide synthase	StmB (AWH12937.1)	64/73
ngnC	3663	type I polyketide synthase	StmC (AWH12938.1)	64/74
ngnD	152	nuclear transport factor 2 family protein	StmD (AWH12907.1)	86/91
ngnV	259	thioesterase	StmV (AWH12931.1)	54/67
orf4	1187	DNA polymerase III subunit alpha	DnaE (GAJ85646.1)	85/92
orf5	104	hypothetical protein	WP_107657113.1	89/92
orf6	60	hypothetical protein	WP_040742977.1	66/86

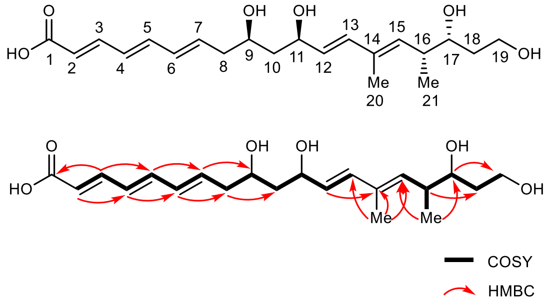
[a]The sequence has been deposited in GenBank with accession number MH544245. [b]Numbers are in amino acids. [c]Given in numbers are NCBI accession numbers.

Supplementary Table 4. NMR spectroscopic data of compound **4** (acetone-d_6).



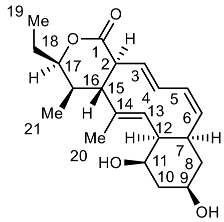
No.	δ_C	δ_H (J in Hz)	COSY	HMBC
1	166.7, C			
2	120.6, CH	5.91, d (15.1)	H3	
3	144.9, CH	7.29, dd (15.1, 11.4)	H2, H4	1, 2, 4, 5
4	128.3, CH	6.36, dd (14.9, 11.4)	H3, H5	2, 3
5	140.9, CH	6.68, dd (14.9, 10.8)	H4, H6	3, 6, 7
6	131.9, CH	6.27, dd (15.1, 10.8)	H5, H7	8
7	136.6, CH	6.07, dt (15.1, 7.4)	H6, H8	5, 8, 9
8	41.5, CH2	2.33, m	H7, H9	6, 7, 9, 10
9	70.2, CH	3.90, m	H8, H10	7
10	43.8, CH2	1.62, m	H9, H11	8, 9, 11, 12
11	72.0, CH2	4.38, dd (12.4, 6.6)	H10, H12	9, 10, 12, 13
12	130.3, CH	5.59, dd (15.7, 6.6)	H11, H13	10, 11, 13
13	134.3, CH	6.22, d (15.7)	H12	11, 15
14	132.0, C			
15	136.1, CH	5.35, d (9.9)	H16	13
16	38.9, CH	2.50, ddt (13.5, 9.9, 6.7)	H15, H17, H21	14, 15, 17
17	76.3, CH	3.23, m	H16, H18	
18	27.9, CH2	1.28, m	H17, H19	16, 17, 19
		1.50, m		
19	9.8, CH3	0.91, t (7.4)	H18	17, 18
20	12.1 CH3	1.74, d (0.9)		14, 15,
21	15.9, CH3	0.99, d (6.7)	H16	16, 17

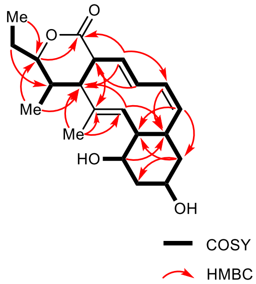
Supplementary Table 5. NMR spectroscopic data of compound **5** (acetone-d_6).



No.	δ_C	δ_H (J in Hz)	COSY	HMBC
1	167.0, C			
2	120.5, CH	5.88, d (15.1)	H3	
3	144.8, CH	7.29, dd (15.1, 11.3)	H2, H4	1, 2, 4, 5
4	128.3, CH	6.35, dd (14.9, 11.3)	H3, H5	2, 3
5	140.9, CH	6.67, dd (14.9, 10.6)	H4, H6	3, 6
6	131.9, CH	6.26, dd (15.0, 10.6)	H5, H7	8
7	136.6, CH	6.05, dt (15.0, 7.4)	H6, H8	5
8	41.5, CH2	2.31, t (6.6)	H7, H9	6, 7, 9, 10
9	70.2, CH	3.89, m	H8, H10	7
10	43.8, CH2	1.62, m	H9, H11	8, 9, 11, 12
11	72.0, CH2	4.38, dd (12.9, 6.6)	H10, H12	9, 10, 12, 13
12	130.4, CH	5.58, dd (15.5, 6.6)	H11, H13	10, 11, 13
13	134.3, CH	6.22, d (15.5)	H12	11
14	132.3, C			
15	135.8, CH	5.34, d (9.9)	H16	13, 16, 17
16	39.5, CH	2.50, m	H15, H17	14, 15, 17, 18
17	74.0, CH	3.54, ddd (9.5, 7.3, 2.4)	H16, H18	
18	37.4, CH2	1.51, m	H17, H19	16, 17, 19
		1.66, m		
19	60.2, CH2	3.70, m	H18	17, 18
20	12.3, CH3	1.74, d (1.0)		14, 15
21	15.9, CH3	1.0, d (6.7)	H16	16, 17

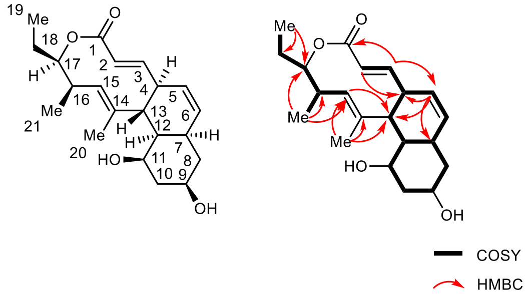
Supplementary Table 6. NMR spectroscopic data of **6** (acetone-d_6).





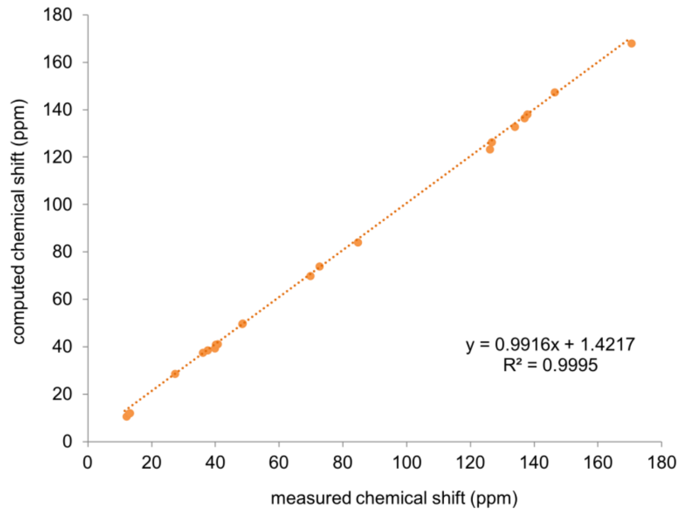
No.	δ_C	δ_H (J in Hz)	COSY	HMBC
1	173.0, C			
2	46.3, CH	3.09, dd (11.0, 8.5)	H3, H15	1, 3, 4, 14, 15
3	122.4, CH	5.23, dd (16.7, 8.5)	H2, H4	1, 2, 5
4	134.0, CH	5.53, dd (16.7, 8.7)	H3, H5	2, 3, 6
5	126.1, CH	6.16, t (10.2)	H4, H6	3, 4, 7
6	135.1, CH	6.26, m	H5, H7	4, 5, 7
7	33.9, CH	2.94, m	H6, H8, H12	4, 5, 8, 9, 11, 12
8	35.6, CH2	2.14, m	H7, H9	6, 9, 12
		1.95, ddd (14.4, 5.5, 3.1)		5, 6, 7, 9, 12,
9	67.2, CH	4.25, m	H8,H10, 9OH	7, 10, 11
10	37.8, CH2	2.18, m	H9, H11	8, 9, 12
		1.79, dt (14.3, 2.8)		9, 11, 12,
11	69.8, CH	3.80, m	H10, H12, 11OH	7, 9, 10, 12,
12	47.1, CH	2.85, m	H11, H13	7, 11, 6, 13
13	137.7, CH	4.97, d (10)	H12	11, 15, 20
14	130.6, C			
15	58.9, CH	1.40, dd (11.1, 4.2)	H2, H16	2, 3, 13, 16, 17, 21
16	32.2, CH	2.38, m	H15, H17	2, 15, 21
17	80.3, CH	4.41, ddd (8.4, 4.7, 3.5)	H16, H18	15, 18, 19, 21
18	24.3, CH2	1.71, m	H17, H19	16, 17, 19
		1.63, m		17, 19
19	9.8, CH3	1.02, t (7.4)	H18	17, 18
20	11.4, CH3	1.67, br s		13, 14, 15
21	14.5, CH3	0.93, d (7.1)	H16	15, 16, 17
	9-OH	4.59, d (4.8)	H9	8, 9, 10
	11-OH	3.88, d (8.3)	H11	10, 11, 12

Supplementary Table 7. NMR spectroscopic data of compound **7** (acetonitrile-d_3).



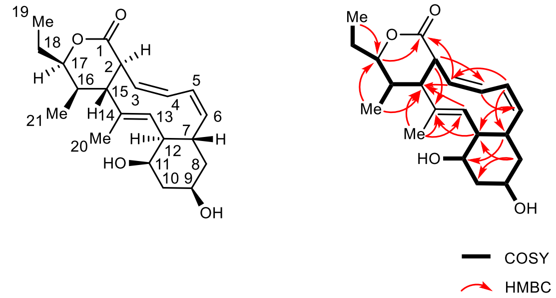
No.	δ_C	δ_H (J in Hz)	COSY	HMBC
1	170.0, C			
2	125.60, CH	5.85, d (16.2)	H3	1, 3, 4
3	145.95, CH	5.63, dd (16.2, 10.0)	H2, H4	1
4	47.94, CH	2.62, t (10.0)	H3, H5, H13	
5	126.28, CH	5.75, br d (10.0)	H4, H6	3,4, 7, 13
6	133.50, CH	5.82, ddd (10.0, 5.2, 2.2)	H5, H7	
7	35.58, CH	2.13, m	H6, H8, H12	
8	39.38, CH2	1.11, m	H7, H9	
9	69.27, CH	3.54, m	H8, H10	
10	40.16, CH2	1.80, m	H9, H11	
		1.34, m		
11	72.22, CH	3.69, m	H10, H12	
12	39.64, CH	2.33, m	H7, H11, H13	
13	48.04, CH	1.93, m	H4, H12	
14	136.4, C			
15	137.39, CH	4.51, d (8.1)	H16	13
16	37.11, CH	2.72, m	H15, H17, H21	
17	84.20, CH	4.50, m	H16, H18	
18	26.9, CH2	1.56, m	H19	
		1.70, m		
19	11.6, CH3	0.95, t (7.4)	H18	17, 18
20	12.73, CH3	1.61, br s		13
21	12.4, CH3	0.97, d (7.0)	H16	15, 16, 17

Supplementary Table 8. Experimental and calculated ^{13}C NMR data for compound **7** (acetonitrile-d_3). Regression line is indicated for experimental measured ^{13}C NMR data (x axis) correlated with computed chemical shift (y axis) with correlation coefficient R^2 = 0.9995, which is caculated in Excel.



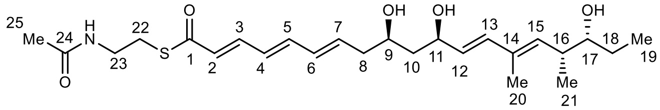
No.	Observed δ_C	Calculated δ_C
1	170.0, C	168.4
2	125.60, CH	123.8
3	145.95, CH	147.8
4	47.94, CH	50.1
5	126.28, CH	126.8
6	133.50, CH	133.2
7	35.58, CH	37.9
8	39.38, CH2	39.8
9	69.27, CH	70.4
10	40.16, CH2	41.6
11	72.22, CH	74.3
12	39.64, CH	41.4
13	48.04, CH	50.3
14	136.4, C	136.8
15	137.39, CH	138.5
16	37.11, CH	38.9
17	84.20, CH	84.4
18	26.9, CH2	29.0
19	11.6, CH3	11.0
20	12.73, CH3	12.4
21	12.4, CH3	12.3

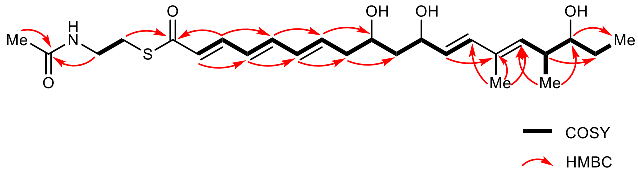
Supplementary Table 9. NMR spectroscopic data of compound **8** (acetone-d_6).



No.	δC	δH (*J* in Hz)	COSY	HMBC
1	172.6, C			
2	42.4, CH	3.19, t (9.7)	H3, H15	1, 3, 4, 14
3	128.4, CH	5.57, ddd (11.1, 8.3, 1.4)	H2, H4	1, 2, 5
4	129.5, CH	6.19, ddd (11.1, 4.3, 1.4)	H3, H5	2, 5, 6
5	126.7, CH	5.88, dd (11.0, 4.3)	H4, H6	3, 6, 7, 12
6	136.8, CH	5.23, ddd (11.0, 8.4, 1.3)	H5, H7	4, 7, 8
7	32.6, CH	2.61, m	H6, H8, H12	5, 6, 8, 12
8	38.5, CH2	2.02, m	H7, H9	6, 7, 9, 10, 12
		1.58, m		7, 12
9	66.7, CH	4.17, br s	H8,H10, 9OH	
10	36.7, CH2	2.10, m	H9, H11	7, 8, 9, 11, 12
		1.68, m		8
11	68.9, CH	3.92, m	H10, H12, 11OH	
12	46.5, CH	2.11, m	H11, H13	6, 14
13	129.7, CH	5.29, d (10.4)	H12	11, 15, 20
14	131.9 C			
15	55.7, CH	1.69, m	H2, H16	
16	33.0, CH	2.25, m	H15, H17	2, 12, 14, 15, 18, 21
17	80.3, CH	4.39, ddd (8.1, 5.1, 2.6)	H16, H18	1, 15, 18, 19, 21
18	24.3, CH2	1.63, m	H17, H19	16, 17
		1.68, m		16, 17, 19
19	9.73, CH3	1.00, t (7.4)	H18	17, 18
20	12.9, CH3	1.55, d (0.9)		2, 12, 13, 14, 15
21	14.0, CH3	0.91, d (7.1)	H16	15, 16, 17
	9-OH	4.55, d (3.8)	H9	
	11-OH	4.45, d (7.8)	H11	10, 11

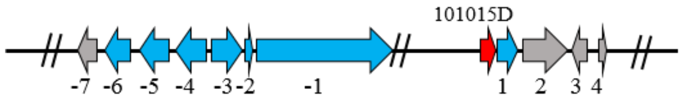
Supplementary Table 10. NMR spectroscopic data of compound **10** (acetone-d_6).





No.	δC	δH (*J* in Hz)	HMBC
1	189.1, C		
2	127.8, CH	6.24, d (15.3)	
3	141.8, CH	7.25, dd (15.3, 11.3)	1, 2, 4, 5
4	128.8, CH	6.34, dd (14.9, 11.3)	2, 3
5	143.9, CH	6.79, dd (14.9, 11.0)	3
6	132.9, CH	6.29, dd (15.2, 11.0)	8
7	138.5, CH	6.12, m	5, 8, 9
8	42.4, CH2	2.34, m	6, 7, 9, 10
9	71.1, CH	3.90, m	7
10	44.7, CH2	1.65, m	8, 9, 11, 12
11	72.9, CH	4.37, dd (12.4, 6.4)	9, 10, 12, 13
12	131.2, CH	5.58, dd (15.6, 6.4)	10, 11, 14
13	135.2, CH	6.21, d (15.8)	11, 15
14	132.9, C		
15	137.1, CH	5.34, d (9.8)	13, 16, 17
16	39.7, CH	2.49, m	14, 17, 18
17	77.2, CH	3.22, m	
18	22.3, CH2	1.29, m 1.50, m	16, 17
19	10.7, CH3	0.91, t (7.4)	18, 19
20	13.0, CH3	1.74, s	14, 15
21	16.8, CH3	0.99, d (6.7)	16, 17
22	39.7, CH2	3.06, t(6.8)	1, 23
23	39.7, CH2	3.33, m	22，24
24	169.9, C		
25	22.8, CH3	1.86, s	24
9-OH		4.24, br s	
11-OH		4.19, br s	

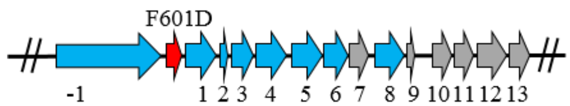
Supplementary Table 11. Annotation of the *stmD* homologue and its neighboring genes in *N. tenerifensis* NBRC 101015.



Protein	Size (aa)	Proposed function	Homolog	Identity/similarity, (%/%)
Orf_-7	283	hypothetical protein	TU76_02335 (KMN02617.1)	53/72
Orf_-6	324	LLM class flavin-dependent oxidoreductase	StmO1 (AWH12914.1)	53/64
Orf_-5	421	pristinamycin IIA synthase subunit A	StmO2 (AWH12915.1)	82/90
Orf_-4	423	cytochrome P450	StmP1 (AWH12916.1)	75/87
Orf_-3	394	cytochrome P450	StmP3 (AWH12925.1)	76/86
Orf_-2	63	ferredoxin	StmF1 (AWH12935.1)	76/87
Orf_-1	2771	type I polyketide synthase	StmC (AWH12938.1)	47/58
101015D	152	nuclear transport factor 2 family protein	StmD (AWH12907.1)	82/90
Orf_1	256	thioesterase	StmV (AWH12931.1)	50/63
Orf_2	1214	DNA polymerase III subunit alpha	WP_081869253.1	86/93
Orf_3	112	hypothetical protein	CLV40_101308 (PPK71119.1)	58/67
Orf_4	59	hypothetical protein	WP_043660705.1	58/72

[a]Given in numbers are NCBI accession numbers. Gene marked in red is the homolog of *stmD*; genes marked in blue is those conserved in *stm* or *ngn* gene cluster.

Supplementary Table 12. Annotation of the *stmD* homologue and its neighboring genes in *S. tsukubaensis* F601.



Protein	Size (aa)	Proposed function	Homolog	Identity/similarity, (%/%)
Orf_-1	1229	type I polyketide synthase	StmC (AWH12938.1)	61/50
F601D	153	nuclear transport factor 2 family protein	StmD (AWH12907.1)	78/88
Orf_1	398	cytochrome P450	StmP3 (AWH12925.1)	73/83
Orf_2	47	ferredoxin	StmF1 (AWH12935.1)	53/70
Orf_3	276	SAM-dependent methyltransferase	StmM2 (AWH12917.1)	55/69
Orf_4	439	cytochrome P450	StmP1 (AWH12916.1)	74/87
Orf_5	436	pristinamycin IIA synthase subunit A	StmO2 (AWH12915.1)	78/87
Orf_6	351	LLM class flavin-dependent oxidoreductase	StmO1 (AWH12914.1)	48/56
Orf_7	246	SDR family oxidoreductase	WP_040742805.1	76/86
Orf_8	401	cytochrome P450	StmP4 (AWH12932.1)	40/55
Orf_9	75	ferredoxin	WP_091433785.1	61/77
Orf_10	228	TetR family transcriptional regulator	WP_098010282.1	48/62
Orf_11	277	hypothetical protein	WP_058940059.1	54/68
Orf_12	425	Cytochrome P450	WP_083895055.1	63/75
Orf_13	280	*O*-methyltransferase	SnogM (AAG42853.1)	43/60

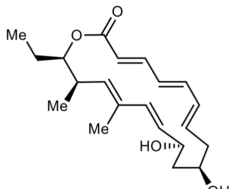
[a]Given in numbers are NCBI accession numbers. Gene marked in red is the homolog of *stmD*; genes marked in blue is those conserved in *stm* or *ngn* gene cluster.

Supplementary Table 13. Annotation of the *stmD* homologue and its neighboring genes in *S.* sp. Root369.

Protein	Size (aa)	Proposed function	Homolog	Identity/similarity, (%/%)
Orf_-2	3606	type I polyketide synthase	StmB (AWH12937.1)	47/59
Orf_-1	3858	type I polyketide synthase	StmC (AWH12938.1)	52/63
root369D	151	nuclear transport factor 2 family protein	StmD (AWH12907.1)	48/66
Orf_1	278	thioesterase	StmV (AWH12931.1)	49/64
Orf_2	642	monooxygenase	ASD17_03920 (KQX23592.1)	51/68
Orf_3	507	aldehyde dehydrogenase	QR97_20565 (AKN71864.1)	94/97
Orf_4	426	transcriptional regulator	AQI94_17755 (KUM87198.1)	93/95
Orf_5	199	N-acetylmuramoyl-L-alanine amidase	CLW08_0629 (PKB34464.1)	75/85
Orf_6	337	acetamidase	SZN_09603 (EGX60014.1)	94/97
Orf_7	402	ROK domain-containing protein	SSEG_09798 (EDY59080.1)	95/96
Orf_8	481	xylulose kinase	ABB07_32890 (AKJ14687.1)	92/95
Orf_9	388	xylose isomerase	DF19_25540 (KDN75320.1)	95/97
Orf_10	149	hypothetical protein	WP_079163039.1	96/99
Orf_11	446	esterase-like activity of phytase family protein	SLINC_1292 (ANS63516.1)	87/92

[a]Given in numbers are NCBI accession numbers. Gene marked in red is the homolog of *stmD*; genes marked in blue is those conserved in *stm* or *ngn* gene cluster.

Supplementary Data 1. DFT-computed energies and Cartesian coordinates.



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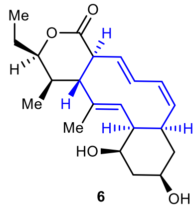

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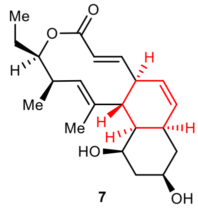

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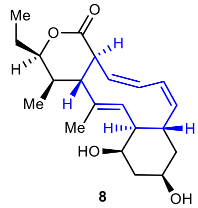

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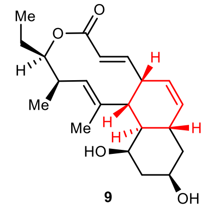

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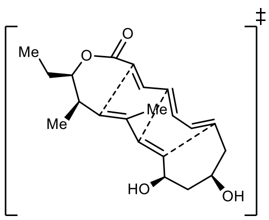
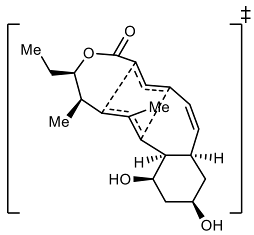
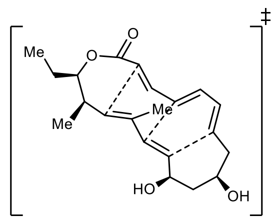


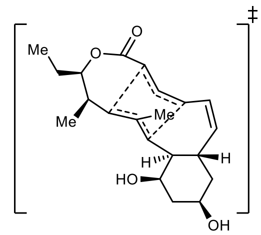
TS-1



TS-2



TS-3



TS-4

Gibbs free energies (in kcal/mol)	B3LYP-D3/ 6-31G(d)	CPCM(water)-B3LYP-D3/6-311+G(d,p)// B3LYP-D3/6-31G(d)	**CPCM(water)-M06-2X/6-311+G(d,p)// B3LYP-D3/6-31G(d)**	CPCM(water)-ωB97XD/6-311+G(d,p)// B3LYP-D3/6-31G(d)
TS-1	21.1	21.1	**23.1**	24.1
TS-2	9.5	11.3	**5.6**	6.1
TS-3	21.4	21.5	**23.0**	24.0
TS-4	9.4	11.5	**5.9**	6.3
6	-9.4	-6.5	**-19.1**	-20.9
7	-5.2	-4.2	**-16.7**	-19.1
8	-15.1	-12.2	**-25.5**	-27.4
9	-4.0	-1.1	**-13.7**	-15.9

3

G_{water} = -1118.553272 Hartree

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C	-1.734781	-1.680686	-0.110544
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H	-5.848847	0.452113	2.162697
H	-3.047120	-0.834391	2.241062
H	-4.546816	-1.689202	1.882139
H	-4.877548	-0.193716	-0.126755
H	-3.163732	-1.013569	-1.560944
H	-1.642943	-2.253284	0.814440
H	0.618266	-2.347839	0.775192
H	2.045538	-1.028818	-1.592556
H	3.247344	1.570755	2.077318
H	3.042175	2.975531	-0.642481
H	1.023579	2.180881	1.581915
H	0.579132	3.445880	-1.187267
H	-1.173760	1.917417	0.873572
H	-1.907248	3.020897	-1.903069
H	-3.914840	-3.279096	0.361707
H	-3.519878	-3.417027	-1.355139
H	-4.997222	-2.572677	-0.854315
H	3.855393	-2.480512	-0.846806
H	4.763564	-1.516138	1.303708
H	3.265068	-0.586863	1.449472
H	3.909277	0.310952	-0.964069
H	5.392667	2.061762	-0.074690
H	5.595597	1.155938	1.436947
H	0.081657	0.360890	-1.868230
H	-0.031281	-1.128969	-2.810509
H	-1.497722	-0.231239	-2.380316
H	2.429883	-3.832659	0.457052
H	6.142018	0.074965	-1.357615

6

G_{water} = -1118.583634 Hartree

C	6.245788	-0.829626	1.306347
C	5.218194	-1.403899	0.326158
C	3.912978	-0.614137	0.316659
C	2.769749	-1.215527	-0.518809
C	1.516632	-0.282396	-0.466468
C	0.289124	-0.865113	0.212368
C	-0.913717	-0.669209	-0.357368
C	-2.280709	-0.912494	0.222192
C	-2.829901	0.340727	1.018027
C	-2.785650	1.670281	0.281151
C	-1.693012	2.441890	0.124438
C	-0.373955	2.023884	0.587178
C	0.770928	2.106516	-0.107654
C	1.879585	1.143103	0.179477
C	3.231525	1.602004	-0.339476
C	3.179885	-1.481370	-1.975110
C	-3.272094	-1.381744	-0.872188
C	-4.645207	-1.705679	-0.261910
C	-5.231544	-0.526680	0.518284
C	-4.241204	0.012999	1.561406
C	0.474875	-1.435383	1.597929
O	3.450885	2.669882	-0.859672
O	4.241197	0.703125	-0.192773
O	-3.373097	-0.453098	-1.945214
O	-5.591271	0.465745	-0.467437
H	6.476940	0.209283	1.052530
H	7.177179	-1.405220	1.281209
H	5.865712	-0.850219	2.335452
H	5.635422	-1.409464	-0.686538
H	4.983337	-2.445282	0.584274
H	3.571358	-0.506827	1.357517
H	2.522835	-2.178745	-0.056601
H	1.237619	-0.048354	-1.499026
H	-0.945711	-0.185669	-1.331299
H	-2.236600	-1.723916	0.963511
H	-2.172193	0.427812	1.892082
H	-3.710974	2.000326	-0.180372
H	-1.763670	3.343671	-0.482493
H	-0.365085	1.415260	1.489925
H	0.834500	2.665781	-1.038385
H	1.984018	0.993895	1.262774
H	3.523248	-0.560193	-2.458703
H	2.323817	-1.863844	-2.541306
H	3.983683	-2.221030	-2.045549
H	-2.861659	-2.297828	-1.316150
H	-4.548778	-2.559593	0.420750

H	-5.339768	-1.992138	-1.058933
H	-6.146258	-0.854249	1.036264
H	-4.150333	-0.753603	2.343394
H	-4.668372	0.899502	2.051595
H	-0.453303	-1.837479	2.010945
H	0.835045	-0.666691	2.297757
H	1.218659	-2.241373	1.616592
H	-4.050223	0.198918	-1.693124
H	-5.915759	1.253146	-0.003114

7

G_{water} = -1118.579852 Hartree

O	1.964748	-2.324614	-1.500148
O	4.538944	-1.109038	2.398866
O	-2.901642	2.324491	1.935865
C	2.892810	-1.398307	-0.945877
C	3.229219	-1.720605	0.513434
C	4.311514	-0.777245	1.031465
C	3.874892	0.680977	0.860433
C	3.442051	1.015131	-0.590859
C	2.988213	2.447847	-0.672911
C	1.707539	2.807003	-0.770367
C	0.554321	1.836124	-0.825480
C	0.992033	0.344132	-0.456093
C	2.357309	0.037807	-1.122700
C	-0.563314	2.190793	0.114250
C	-1.827736	1.832468	-0.140816
C	-2.791750	1.614050	0.962196
C	-0.168077	-0.598494	-0.737161
C	-0.529172	-0.873740	-2.176923
C	-0.955480	-0.947781	0.294838
C	-2.318652	-1.604686	0.262470
C	-2.601832	-2.316526	1.594611
C	-3.427538	-0.571637	-0.102976
C	-4.828223	-1.175578	-0.221210
C	-5.847330	-0.184275	-0.790114
O	-3.549441	0.474104	0.898778
H	2.255356	-3.216736	-1.256219
H	5.216111	-0.504608	2.741411
H	3.833621	-1.445343	-1.525522
H	2.333005	-1.633211	1.138037
H	3.584992	-2.756259	0.599911
H	5.231159	-0.944839	0.441497
H	4.690438	1.355093	1.159021

H	3.046350	0.869367	1.552851
H	4.330855	0.897255	-1.230081
H	3.762350	3.212325	-0.618083
H	1.442769	3.862422	-0.818527
H	0.162592	1.821975	-1.853509
H	1.146458	0.345889	0.628517
H	2.255401	0.212145	-2.201976
H	-0.290801	2.499733	1.122895
H	-2.084789	1.476937	-1.132529
H	-1.001606	0.003548	-2.643403
H	-1.234390	-1.704851	-2.266280
H	0.352307	-1.128564	-2.767887
H	-0.641600	-0.636037	1.291741
H	-2.350746	-2.346751	-0.547734
H	-2.701031	-1.583598	2.402731
H	-3.522017	-2.907383	1.557223
H	-1.777551	-2.992845	1.845020
H	-3.167595	-0.131004	-1.071114
H	-5.153453	-1.512211	0.769087
H	-4.764510	-2.066184	-0.860514
H	-5.561548	0.143442	-1.797831
H	-6.843051	-0.635990	-0.855022
H	-5.914726	0.701990	-0.151861

8

G_{water} = -1118.593862 Hartree

C	-3.383753	-1.572376	-0.548189
C	-4.782891	-1.560937	0.088163
C	-5.261990	-0.142499	0.405193
C	-4.239861	0.610418	1.261441
C	-2.828535	0.632174	0.643688
C	-2.345673	-0.838167	0.345869
H	-5.493260	-2.043360	-0.592048
H	-4.769342	-2.137377	1.022059
H	-3.049739	-2.612109	-0.654107
H	-4.588385	1.639124	1.436183
H	-4.198219	0.130924	2.250297
H	-2.874932	1.140717	-0.327466
H	-2.304946	-1.371111	1.305897
H	-6.224486	-0.188021	0.937430
O	-5.463663	0.515939	-0.864397
H	-5.609806	1.460492	-0.698802
O	-3.410992	-1.041207	-1.869586
H	-4.049267	-0.304504	-1.861006

C	-1.001041	-0.800701	-0.320954
H	-1.062503	-0.563454	-1.380656
C	-1.866998	1.363383	1.542978
H	-2.018778	1.219798	2.614068
C	0.213657	-0.855181	0.246694
C	0.458450	-1.149189	1.707629
H	1.160797	-1.982465	1.840226
H	-0.460026	-1.408471	2.237978
H	0.892269	-0.283428	2.225001
C	1.412033	-0.429967	-0.583349
C	1.735408	1.094753	-0.260777
C	2.685436	-1.321999	-0.443060
H	1.108767	-0.446408	-1.635347
H	1.740999	1.199662	0.832292
C	3.743231	-0.588239	0.397279
H	2.421140	-2.234456	0.104681
H	3.299735	-0.317331	1.366956
C	-0.805031	2.078074	1.144523
H	-0.137718	2.474121	1.912114
C	3.214902	-1.737343	-1.824378
H	2.424485	-2.240592	-2.391459
H	3.544152	-0.866179	-2.400911
H	4.060034	-2.428518	-1.746456
C	5.036292	-1.352491	0.662287
H	4.769897	-2.336820	1.070072
H	5.547973	-1.526251	-0.290461
C	5.970424	-0.611396	1.623940
H	5.494879	-0.463365	2.601640
H	6.897135	-1.172953	1.783005
H	6.229561	0.372757	1.222087
O	4.126235	0.633407	-0.285504
C	3.140286	1.458433	-0.724230
O	3.419405	2.405398	-1.421094
C	0.702439	2.005389	-0.859498
H	0.888956	2.306052	-1.887362
C	-0.437633	2.373453	-0.259698
H	-1.162165	2.941689	-0.844553

9

G_{water} = -1118.575181 Hartree

C	-3.089137	-1.296908	-0.702882
C	-4.405084	-1.690792	-0.007788
C	-5.181752	-0.474818	0.508784
C	-4.304807	0.429112	1.387305

C	-3.022796	0.847499	0.657542
C	-2.225334	-0.418169	0.228264
H	-5.034655	-2.250607	-0.707966
H	-4.185689	-2.348432	0.842825
H	-2.524322	-2.209496	-0.933686
H	-4.879238	1.316495	1.693317
H	-4.053691	-0.110257	2.311596
H	-3.327703	1.358666	-0.269081
H	-2.075020	-0.993853	1.148652
H	-6.056376	-0.813964	1.084295
O	-5.639512	0.231453	-0.665240
H	-6.021260	1.078383	-0.385747
O	-3.319374	-0.659265	-1.957113
H	-4.122829	-0.118153	-1.851706
C	-0.838605	-0.110991	-0.404489
H	-0.883274	-0.391756	-1.460999
C	-2.132848	1.782447	1.434056
H	-2.466994	2.179571	2.391022
C	0.328430	-0.849782	0.237815
C	0.347366	-0.997915	1.742451
H	-0.404625	-1.717727	2.091280
H	0.109352	-0.044587	2.229313
H	1.313797	-1.342378	2.117396
C	1.377081	-1.190175	-0.532443
C	1.886897	1.664030	-0.284100
C	2.744466	-1.682836	-0.100015
H	1.289738	-1.005292	-1.603453
H	1.839291	1.474614	0.783200
C	3.618143	-0.520022	0.463954
H	2.631651	-2.382677	0.739453
H	3.051339	-0.022308	1.257930
C	-0.939431	2.086920	0.912393
H	-0.234434	2.750548	1.407173
C	3.438564	-2.418613	-1.256830
H	2.785681	-3.203525	-1.653182
H	3.667245	-1.719881	-2.068484
H	4.374673	-2.887967	-0.939977
C	4.953073	-0.978130	1.056297
H	4.756063	-1.811061	1.744268
H	5.584175	-1.367925	0.250406
C	5.686673	0.151233	1.785542
H	5.089115	0.536100	2.621826
H	6.643323	-0.196530	2.189923
H	5.885566	0.981213	1.100923
O	3.971639	0.443933	-0.560004
C	3.173828	1.469370	-0.993459

O	3.527385	2.099392	-1.964292
C	0.756361	1.799979	-0.988723
H	0.838220	1.916671	-2.067886
C	-0.597582	1.495317	-0.432934
H	-1.351260	1.841486	-1.153885

TS-1

G_{water} = -1118.516518 Hartree

Imaginary frequency: -372.3 cm^{-1}

C	-6.01009900	-0.37007400	-1.62776500
C	-5.19958800	-1.17886400	-0.61061700
C	-3.83469100	-0.54999000	-0.32202600
C	-2.89993000	-1.43805100	0.55202500
C	-1.51059700	-0.86353900	0.72980100
C	-0.38187100	-1.14622300	0.00098100
C	0.88671100	-0.69130500	0.48406000
C	2.14781100	-1.03061400	-0.02224800
C	2.94217000	0.54122400	-1.24705800
C	2.87172600	1.75272600	-0.53439500
C	1.67351200	2.37900600	-0.17979200
C	0.42318600	1.92236500	-0.57503700
C	-0.80759500	2.25276400	0.03188100
C	-1.95223200	1.58883500	-0.31309900
C	-3.18292100	1.69900700	0.47666900
C	-3.51233600	-1.71927700	1.93669000
C	3.28781800	-1.22894300	0.99050100
C	4.63336300	-1.57378300	0.32951100
C	5.22704800	-0.48866300	-0.56771100
C	4.27729500	-0.03473300	-1.68995300
C	-0.43320800	-1.91319400	-1.30324900
O	-3.42020100	2.58914500	1.26804600
O	-4.11896800	0.70598200	0.32039700
O	3.38818100	-0.16564500	1.92202100
O	5.60848900	0.59490400	0.30058100
H	-6.17348600	0.64779000	-1.26143300
H	-6.98673900	-0.83165800	-1.80910800
H	-5.48611100	-0.30419200	-2.58980600
H	-5.76008200	-1.25824400	0.32688700
H	-5.03726400	-2.20074700	-0.97892200
H	-3.34021900	-0.37671000	-1.28652400
H	-2.82383200	-2.38910600	0.00795800
H	-1.36827500	-0.29273800	1.64483300
H	0.88632700	-0.14667700	1.42486900
H	2.16534900	-1.76370600	-0.83123000

H	2.14096500	0.35018500	-1.95510800
H	3.78366900	2.14013200	-0.09200600
H	1.71715500	3.19359100	0.54213500
H	0.37018900	1.21749400	-1.40082200
H	-0.82745600	2.91967600	0.89193100
H	-1.94211100	0.95709500	-1.19113000
H	-3.71258600	-0.77978800	2.46210000
H	-2.82149800	-2.31327600	2.54405400
H	-4.45298100	-2.27266400	1.85914800
H	3.00758900	-2.10530500	1.59274900
H	4.51112700	-2.48529300	-0.26998800
H	5.35725900	-1.79594800	1.12099300
H	6.13281500	-0.89723200	-1.04323300
H	4.10311100	-0.90292500	-2.33812400
H	4.80490200	0.70390300	-2.31417700
H	0.11407700	-2.86206100	-1.23618200
H	0.03451800	-1.33419800	-2.11025700
H	-1.45504200	-2.13880700	-1.61639200
H	3.97499800	0.50325900	1.53014100
H	5.99620900	1.29923300	-0.24237600

TS-2

G_{water} = -1118.544415 Hartree

Imaginary frequency: -269.5 cm^{-1}

C	-6.08939000	-0.48829600	-1.54669400
C	-5.21959600	-1.22154000	-0.52099300
C	-3.85736000	-0.55404300	-0.33118200
C	-2.86043200	-1.36406000	0.54718100
C	-1.50063000	-0.69755400	0.66247800
C	-0.35787700	-0.98794800	-0.07907000
C	0.84947000	-0.35564200	0.27778300
C	2.20319000	-0.74121200	-0.25984500
C	2.93342000	0.35146700	-1.15054900
C	2.92435000	1.71450600	-0.51799200
C	1.76478700	2.34520100	-0.25370600
C	0.48413800	1.75961900	-0.61208600
C	-0.72669300	2.10601000	-0.00470000
C	-1.90188600	1.45675900	-0.33408000
C	-3.13992300	1.66540400	0.42938900
C	-3.42203900	-1.62585000	1.95619100
C	3.10707500	-1.19557200	0.92621100
C	4.47459800	-1.69277400	0.43018300
C	5.20907800	-0.64787500	-0.41012600
C	4.33144700	-0.15136300	-1.56668600

C	-0.44019600	-1.76421400	-1.37887300
O	-3.33310300	2.57208200	1.21254000
O	-4.13159000	0.73121700	0.25393800
O	3.23866200	-0.19375600	1.92288900
O	5.57685600	0.41549700	0.49247800
H	-6.25543000	0.54688400	-1.23388400
H	-7.06415500	-0.97560300	-1.65653800
H	-5.60928000	-0.47121300	-2.53334500
H	-5.73655900	-1.25391300	0.44386300
H	-5.05323800	-2.26078900	-0.83515900
H	-3.40936200	-0.42284300	-1.32561700
H	-2.74334600	-2.32877000	0.03571100
H	-1.33711800	-0.18047900	1.60447200
H	0.89397200	0.10126500	1.26081200
H	2.09594500	-1.61397200	-0.91850800
H	2.33852200	0.40168500	-2.07335700
H	3.86553500	2.15099900	-0.20014400
H	1.76148600	3.27538900	0.31272500
H	0.42654000	1.25003400	-1.57237800
H	-0.72028800	2.72421700	0.89164900
H	-1.96119300	0.93178600	-1.27977000
H	-3.66509600	-0.68018000	2.45186100
H	-2.68340900	-2.15456300	2.56778800
H	-4.32941400	-2.23628300	1.92302500
H	2.59477500	-2.03026900	1.42053500
H	4.33687000	-2.59363400	-0.18156500
H	5.09086600	-1.96538800	1.29349900
H	6.12629100	-1.09418100	-0.82471900
H	4.21538600	-0.98913300	-2.26792600
H	4.85859500	0.63912200	-2.11969800
H	0.12358300	-2.70456800	-1.33026800
H	-0.01756900	-1.18292300	-2.20891200
H	-1.46820100	-2.01437500	-1.65144700
H	3.94138400	0.41180500	1.62767100
H	6.00758700	1.11621800	-0.02139700

TS-3

G_{water} = -1118.516568 Hartree

Imaginary frequency: -378.5 cm^{-1}

C	-3.41351700	-1.29077100	-0.88130800
C	-4.75838800	-1.43550000	-0.14962200
C	-5.26631300	-0.16666600	0.53590700
C	-4.27531300	0.43582200	1.53917200
C	-2.95833400	0.85015500	0.91992500

C	-2.24077300	-0.98571400	0.06229400
H	-5.51387700	-1.75889400	-0.87458400
H	-4.67070400	-2.22610800	0.60630200
H	-3.19921300	-2.26972000	-1.33488100
H	-4.75232800	1.31502600	2.00380200
H	-4.11115700	-0.27787900	2.35781300
H	-3.04487000	1.24275300	-0.08816200
H	-2.27846400	-1.53962900	1.00004000
H	-6.20074100	-0.40600100	1.06651600
O	-5.55884500	0.76925100	-0.52365600
H	-5.77519400	1.62884200	-0.12931200
O	-3.47485900	-0.37365200	-1.96863700
H	-4.15672700	0.28586600	-1.74638800
C	-0.97868400	-0.79043300	-0.50885400
H	-0.95974300	-0.48835300	-1.55323900
C	-1.93016100	1.36754100	1.71661000
H	-1.98748900	1.21792400	2.79472600
C	0.27400400	-1.09278100	0.11123200
C	0.27932300	-1.55421000	1.55525300
H	-0.15745600	-2.55710000	1.65570700
H	-0.31484800	-0.87842400	2.17853100
H	1.28704500	-1.58749800	1.97644100
C	1.43122300	-0.99012700	-0.62594700
C	1.90601100	1.59550200	-0.21965000
C	2.79952700	-1.53339100	-0.26955000
H	1.32600300	-0.66448400	-1.65834300
H	1.88182200	1.24590200	0.80250800
C	3.73256700	-0.48534200	0.40576800
H	2.67890000	-2.32904500	0.47799600
H	3.20861600	-0.07330700	1.27783200
C	-0.73555200	1.85845500	1.18502200
H	0.09312100	2.01403400	1.87443400
C	3.44674400	-2.14219500	-1.52731700
H	2.76030200	-2.85031500	-2.00315400
H	3.68730300	-1.35629000	-2.25065600
H	4.37021500	-2.67822500	-1.28839200
C	5.06484800	-1.05699300	0.89427300
H	4.85635200	-1.95538200	1.49065400
H	5.65554200	-1.37355800	0.02812000
C	5.86424900	-0.04490400	1.72057900
H	5.30859800	0.26249500	2.61564500
H	6.81885800	-0.47037800	2.04838300
H	6.07226900	0.85084500	1.12784900
O	4.07944200	0.57122400	-0.50818200
C	3.16116500	1.48303500	-0.97095500
O	3.43306300	2.11246700	-1.97307900

C	0.77669000	2.12146000	-0.78697900
H	0.84099000	2.43011400	-1.82814100
C	-0.49914200	2.06242500	-0.17621600
H	-1.35416800	2.17344400	-0.83869600

TS-4

G_{water} = -1118.543914 Hartree

Imaginary frequency: -276.6 cm^{-1}

C	-3.24844000	-1.28513100	-0.82531400
C	-4.59531000	-1.61598700	-0.16038800
C	-5.24654500	-0.39002700	0.48387700
C	-4.28350800	0.31509300	1.44557300
C	-2.95285100	0.68414500	0.77412200
C	-2.27337900	-0.61819200	0.18896700
H	-5.27406400	-2.03680500	-0.91034600
H	-4.44261400	-2.37631900	0.61586300
H	-2.78928200	-2.22025600	-1.16861500
H	-4.76258800	1.21927200	1.84923000
H	-4.09808700	-0.34378900	2.30596800
H	-3.18006900	1.30955000	-0.09945900
H	-2.17136500	-1.30695600	1.03699100
H	-6.15401600	-0.69582200	1.02621300
O	-5.62418500	0.48169000	-0.60435000
H	-5.90533600	1.33284900	-0.23338000
O	-3.41231100	-0.48321800	-1.98943500
H	-4.16055100	0.11612000	-1.81322500
C	-0.92707000	-0.42111600	-0.45844300
H	-0.96569300	-0.25851700	-1.53060800
C	-2.01903600	1.41822300	1.68496400
H	-2.24462600	1.45026300	2.74960300
C	0.27117500	-0.93118500	0.08185800
C	0.30904100	-1.40277100	1.52390500
H	1.32667900	-1.59096600	1.87350500
H	-0.25753900	-2.33353900	1.65863600
H	-0.13486700	-0.65416800	2.18823500
C	1.44287500	-0.82828900	-0.66514900
C	1.85066000	1.49352600	-0.16596100
C	2.78497300	-1.46180700	-0.34305500
H	1.32411400	-0.53721600	-1.70586300
H	1.82652300	1.24322800	0.88827000
C	3.72539200	-0.50477200	0.44525300
H	2.62094700	-2.32051600	0.32191600
H	3.19100600	-0.16026900	1.34090500
C	-0.86173500	1.91892700	1.21111900

H	-0.12105500	2.33973300	1.89010400
C	3.44119000	-1.96805600	-1.63965500
H	2.74672800	-2.61155300	-2.19012200
H	3.71509500	-1.12665100	-2.28441100
H	4.34581900	-2.54863900	-1.43598100
C	5.03807900	-1.14172400	0.90064600
H	4.80147900	-2.08118800	1.41804900
H	5.63393300	-1.39954500	0.01860600
C	5.84720700	-0.21918700	1.81728300
H	5.28455100	0.02861000	2.72628100
H	6.78619500	-0.69315300	2.12281000
H	6.08548000	0.71604400	1.30200900
O	4.09771000	0.62410900	-0.36560000
C	3.15008000	1.48944200	-0.85449300
O	3.42836600	2.16385000	-1.82425700
C	0.72035700	2.00685800	-0.77492300
H	0.80844900	2.26753200	-1.82743600
C	-0.55714800	1.90007000	-0.21683600
H	-1.38986200	2.13818200	-0.87643800

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