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Supporting Information

Spirocyclic cladosporicin A and cladosporiumins I and J from a *Hydractinia*-associated *Cladosporium sphaerospermum* SW67

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1 General experimental procedure

Optical rotations were recorded on a Jasco P-1020 polarimeter (Jasco, Easton, MD, USA), IR spectra were determined on a Bruker IFS-66/S FT-IR spectrometer (Bruker, Karlsruhe, Germany), and HR-ESI mass spectra were measured on a Waters UHPLC-QTOF Xevo G2-S mass spectrometer using an analytical Kinetex (2.1× 100 mm, 5 μm) (Waters Corporation, Milfod, CT, USA). The ECD spectra were recorded on a Jasco J-715 spectropolarimeter (Jasco, Easton, MD, USA), and Ultraviolet (UV) spectra were determined on an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). NMR spectra were measured on a Bruker Avance III HD 800 NMR spectrometer with a 5-mm TCI CryoProbe (Bruker, Karlsruhe, Germany). Preparative HPLC used a Waters 1525 binary HPLC pump with a Waters 996 photodiode array detector using an analytical Agilent Eclipse XDB-C18 column (250 mm × 21.2 mm i.d., 7 μm) (Waters Corporation, Milford, CT, USA), and Semi-preparative HPLC was recorded on a Shimadzu Prominence HPLC system with SPD-20A/20AV Series Prominence HPLC UV-Vis detector using a Phenomenex Luna C18(2) column (250 mm × 10 mm i.d., 10 μm) (Shimadzu, Tokyo, Japan). LC-MS analysis was attended on an Agilent 1200 series HPLC system with a diode array detector and 6130 Series ESI mass spectrometer using an analytical Kinetex (2.1× 100 mm, 5 μm) (Agilent Technologies, Santa Clara, CA, USA). Precoated silica gel F_{254} plates and reversed-phase (RP)-18 F_{254s} plates (Merck, Darmstadt, Germany) were used for thin-layer chromatography (TLC). First grade solvents (Samchun pure chemicals Co., Ltd., Pyeongtaek, Korea) were used for fractionation and isolation. HPLC grade solvents were purchased from Burdick & Jackson and Fisher Scientific. Detection of TLC spots was visualized by spraying anisaldehyde-sulfuric acid reagent and dual wavelength (254/365nm) UV lamp.

2 Cultivation procedures

2.1 Media composition

- Potato dextrose broth (PDB): 26.5 g/L potato glucose extract.
- Potato dextrose agar, (PDA): 26.5 g/L potato extract glucose, 20.0 g/L agar.
- Malt extract broth (MEB): 19 g/L malt extract broth.
- Malt extract agar (MEA): 19 g/L malt extract broth, 20.0 g/L agar.
- Glucose peptone yeast agar (GluPe) 1 g/L glucose, 0.5 g/L peptone from casein, 0.1 g/L yeast extract, 20 g/L agar, dissolved in artificial seawater (ASW)
- Wickerham's yeast malt agar (Wick):[1] 10 g glucose, 3 g/L yeast extract, 5 g peptone from casein, 20 g/L agar, dissolved in ASW
- Marine broth liquid (MBL): 40.1 g/L marine broth
- Marine broth agar (MBA): 40.1 g/L marine broth, 20.0 g/L agar;

2.2 Maintenance of *Hydractinia echinata* cultures

H. echinata colonies were obtained as single colonies on gastropod shells (common whelks (*Buccinum* spp.)) from the Marine Biological Laboratory in Woods Hole (MA 02543, USA) and Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (Helgoland, Germany). Adult polyps were kept in artificial seawater (salinity of 33.2–33.7‰, pH 8.2–8.3 and 15 °C) in aerated tanks maintained in 16 h light/8 h dark cycle and were fed daily using 3-7 day old nauplii of *Artemia salina*.

2.3 Isolation and cultivation of fungal isolates

Twenty polyps of each colony *H. echinata* were dissected and pooled. Samples were first washed with sterile sea water (300 µL) and centrifuged. The resulting supernatant was defined as SW-sample. The remaining polyp tissue was suspended in sterile sea water (100 µL) and homogenized using a sterile pestle and the resulting suspension was defined as MSW-sample. SW and MSW-samples were serially diluted from 10^{-1} – 10^{-3} using filtered sterile seawater, and 100 µL of each dilution was used to inoculate PDA and Wick agar plates supplemented with 50 mg/L streptomycin sulfate. Plates were incubated at 20 °C for up to 14 days and monitored every day. Single colonies were transferred to new agar plates and

subcultures until pure cultures were obtained. All fungal isolates were cultured on PDA (25 °C in the dark) for up to 7 days and maintained as 50% (v/v) glycerol suspensions at –80 °C (Figure S1).

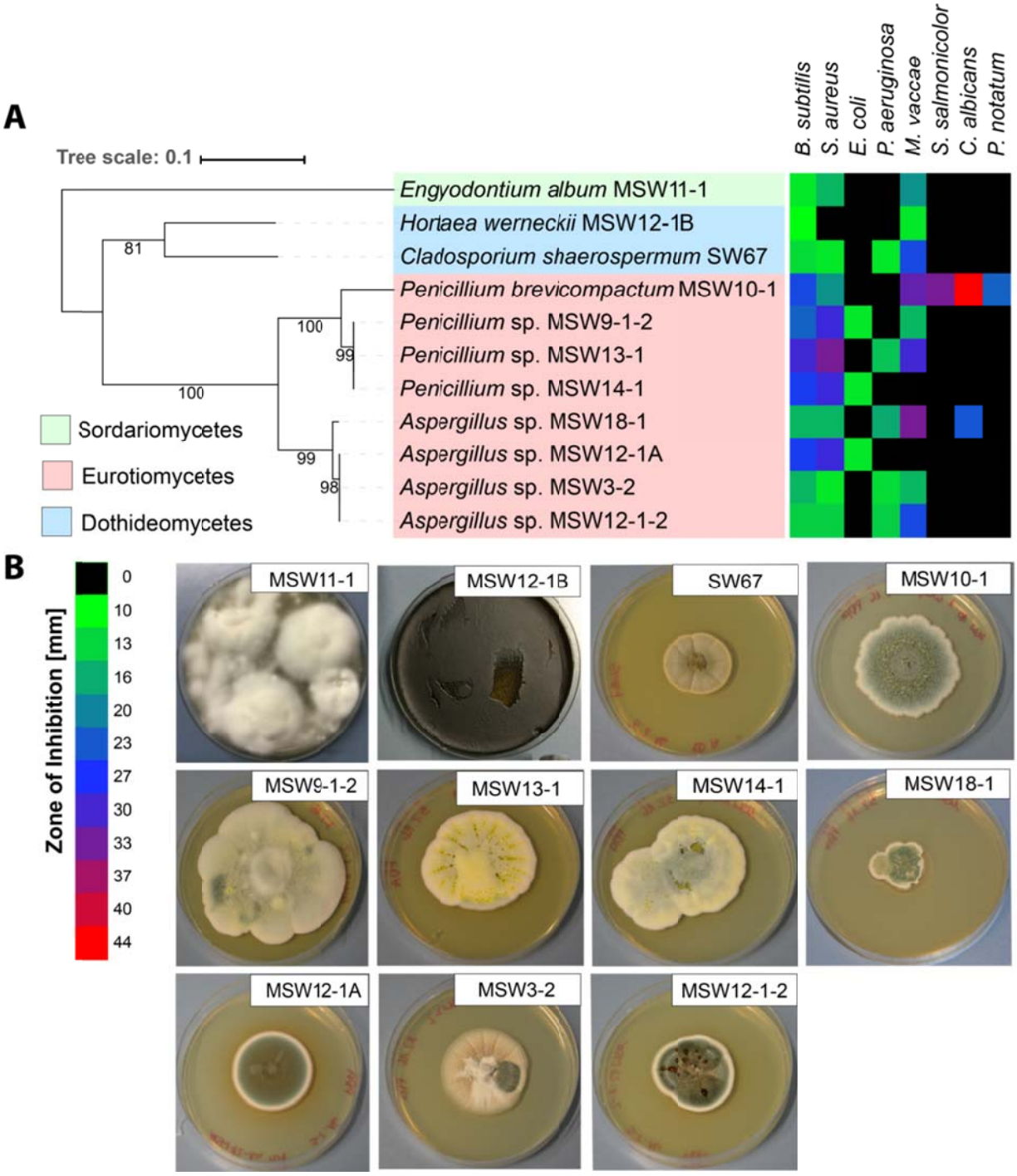

Figure S1. Overview of *H. echinata* associated fungal isolates. A) Left: phylogenetic tree based on the ITS gene sequence of isolated fungi from the surface and environment of *H. echinata*. Best DNA model was generated and robustness of interferes topologies was evaluated after 1000 bootstraps (> 50% are shown). Right: correlated heatmap showing antimicrobial activities against test strains (zone of inhibition in mm in standardized assay). B)

Representative pictures of the fungal isolates on PDA medium after 7 d of incubation at 25 °C in the dark.

3 Phylogenetic and genomic analysis

3.1 Morphological description

For microscopic identification mycelia was transferred from potato dextrose agar plates to microscope slide covered with lactophenol blue and a cover slide and examined under the microscope. Based on the ascending conidiophores the strains were morphological confirmed belonging to the genus *Cladosporium*.

3.2 DNA isolation and ITS sequencing

DNA was extracted from PDA plates using the Thermo Fischer Gene JET Genomic DNA Purification Kit according to the manual. DNA was eluted using 20 µL of deionized H2O and quantified using a NanoDrop Lite Spectrophotometer (Thermo Fisher). PCR was performed using 1 µL of DNA with primers designed to amplify ITS fragments.

ITS1: 5`TCC GTA GGT GAA CCT GCG G 3’

ITS4: 5`TCC TCC GCT TAT TGA TAT GC 3´

The amplification was performed in a 25 µL reaction volume with 5 µL of 10X HF buffer, 2.5 µM each primer, 0.25 µL Phusion polymerase, 10 mM dNTP mix and 1 µl of DNA with a concentration of 40 ng/µL. Cycling conditions were as followed: initial denaturation 98 °C for 38 sec, 35 cycles of 98 °C for 30 sec; 55 °C for 30 sec; and 72 °C for 1 min and a final extension of 1 min at 98 °C followed by 8 min of 72 °C. PCR was performed in a Peqstar Gradient cycler. PCR products were analyzed by electrophoresis on 1% agarose gel under UV light. PCR products of the right size (500 bp) were purified using the Gene JET PCR Purification Kit (Thermo Fisher) according to manual. The ITS PCR Products were sequenced separately with the ITS1 and ITS4 primer by GATC Biotech AG (Konstanz).

3.3 Phylogenetic analysis

Sequences were compared using the Basic Local Alignment Search Tool (BLAST) algorithm from NCBI to identify high degree similarity with known sequences. Partial sequences were manually compiled, assembled and trimmed using BioEdit Version 7.2.0.28 (Figure S1 and Figure S2). ITS-sequence of strain SW67 showed 100% identity with *C. parahalotolerans* Jos:Houbraken:DTO:324-B7 (MF473169) with a query cover of 100%.

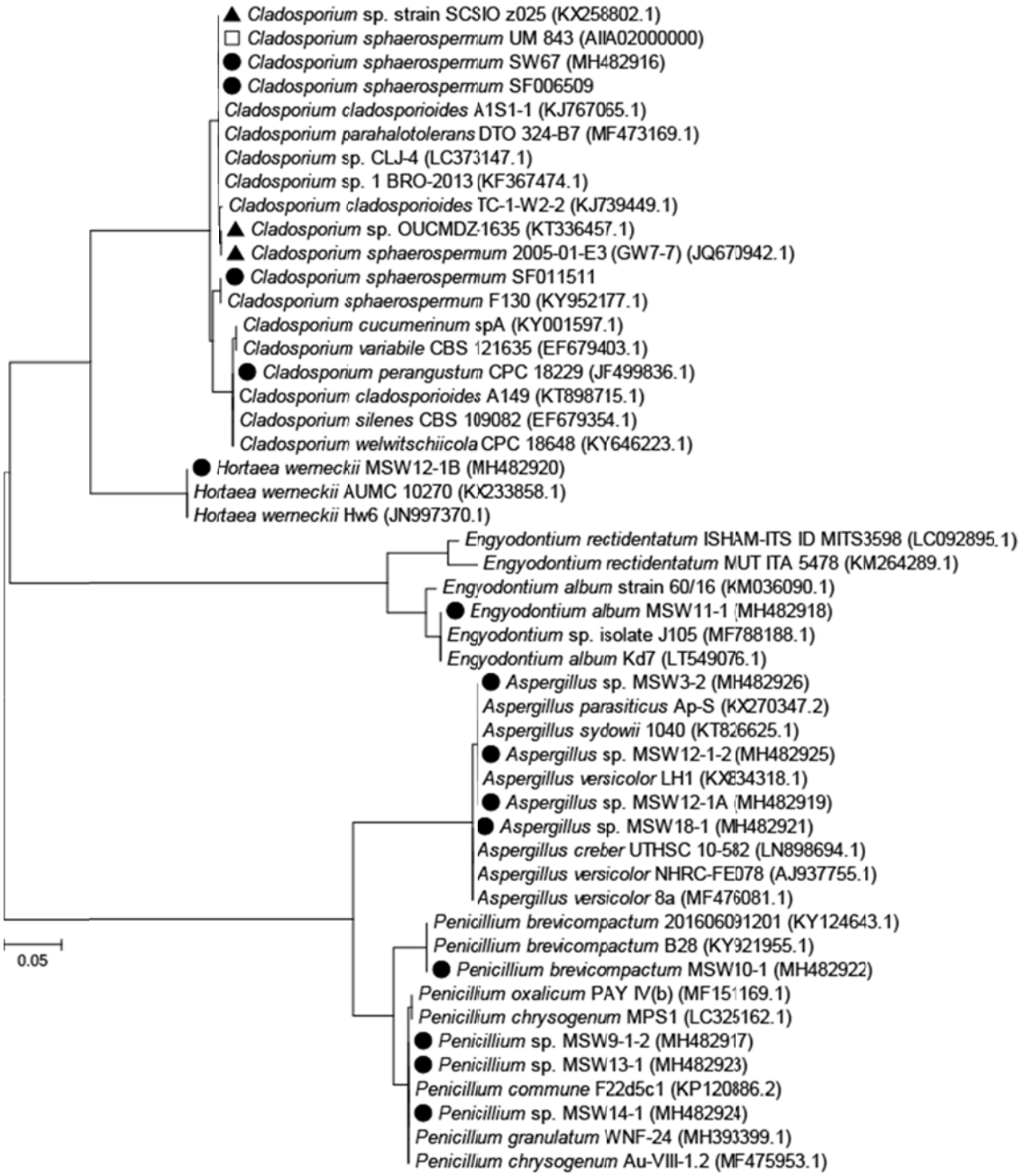

Figure S2. Maximum likelihood phylogenetic tree from analysis of available ITS sequences of isolated strains and their closest related strains retrieved from the NCBI nucleotide database. ITS sequences obtained in this work are marked with a black circle. ITS sequences from other *Cladosporium* species producing other cladosin-like compounds are marked with a black triangle. A white square indicates strain *C. sphaerospermum* UM 843. Bootstrap values were indicated at nodes of each branch based on maximum likelihood analysis of 1000 replicates. Only values > 50% were shown. Scale bar was equal to 0.1 substitutions per nucleotide position.

Gene sequences have been deposited in GenBank, accession no MH482916-MH482926.Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The robustness of branches was assessed by bootstrap analysis with 1000 replicates. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7.[2]

3.4 Genome sequencing of SW67

3.4.1 DNA isolation

PDA plates were inoculated with 100 µL of a spore suspension in sterile PBS and incubated at room temperature for 14 days in the dark. The mycelia was then scraped off with a scalpel, added to 600 µL CTAB buffer and crashed with ice cold ceramic beads (Sigma Aldrich) in a SpeedMill Plus (Analytik Jena) (3 x 1 min with 1 min cooling break in between). The supernatant was transferred to a new reaction tube and incubated with 10 µL of 20 mg/mL proteinase K solution for 30 min at 37 °C. Afterwards 20 µL of a 10 mg/mL RNase A solution was added and incubated for 15 min at room temperature. 500 µL of a $CHCl_3$/*iso*-amyl alcohol mixture (24:1) was added, inverted and centrifuged for 15 min at room temperature at 13000 rpm. The aqueous phase was transferred to a new reaction tube and 500 µL of a $CHCl_3$/*iso*-amyl alcohol mixture was added, inverted and centrifuged again. An equal volume of ice-cold isopropanol was added to the remaining aqueous phase and the DNA was precipitated for 1 h at -20 °C. Afterwards the sample was centrifuged at 4 °C for 15 min at 13000 rpm and the liquid was removed. The remaining DNA pellet was washed with 300 µL of 100% EtOH following by 5 min centrifugation at room temperature (13000 rpm). The liquid was removed and the pellet was washed with 300 µL of 70% EtOH (30% water). The liquid was removed and the pellet was dried for 3 min under reduced vacuum pressure. Finally, 50 µL of DNAse free water was added and quantified using a NanoDrop Lite Spectrophotometer (Thermo Fisher). A total of 431 ng/µL (A260/280 = 1.88) genomic DNA was send to genome sequencing.

3.4.2 Library preparation and sequencing

Genomic DNA was sheared using a Covaris S220 sonication device (Covaris Inc; Massachusetts, USA), with the following settings Duty factor = 5%, peak incident power = 175 W, cycles of burst = 200, temperature = 5 °C duration = 25 sec. Library preparation was performed with the NEBNext Utlra II kit (New England Biolabs, Frankfurt, Germany), as per the manufacturers recommendations for an insert size-range of 500-700 bp. Sequencing was performed on an Illumina MiSeq machine for 600 cycles using v3-chemistry and paired-end settings.

3.4.3 Read processing and assembly

Quality trimming and adapter clipping was performed using trimmomatic v 0.36.[3] Additional rounds of adapter clipping and filtering of low complexity reads were performed using "bbduk.sh" of the BBTools package v.36.84[4] and cutadapt v.1.13.[5] Overlapping read pairs were merged using FLASH v.1.2.11.[6]. Final assembly was performed using SPAdes v.3.10.1 with default k-mer range settings.[7]

Sequence and assembly data have been deposited at NCBI (SRR7473839; QNVK00000000)

3.4.4 ANI analysis

The G+C content of the DNA was determined with the GGDC web server available at http://ggdc.dsmz.de/ from the genome of SW67 and *C. sphaerospermum* UM 843 and in silico DDH was estimated using the same service.[8] The in silico DDH value was estimated as 99.20 % (HSP length) and the Difference in G+C was 0.1 % indicating that the two strains belong to the same species.

3.5 Biosynthetic gene cluster analysis

Putative biosynthetic gene clusters from SW67 (Acc.: QNVK00000000) was identified using antiSMASH4.[9] For comparison *C. sphaerspermum* UM 843, *Cladosporium* sp. SL-16, *C. cladosporioides* TYU and *C. cladosporioides* IMV 00236 were analyzed using the same program settings

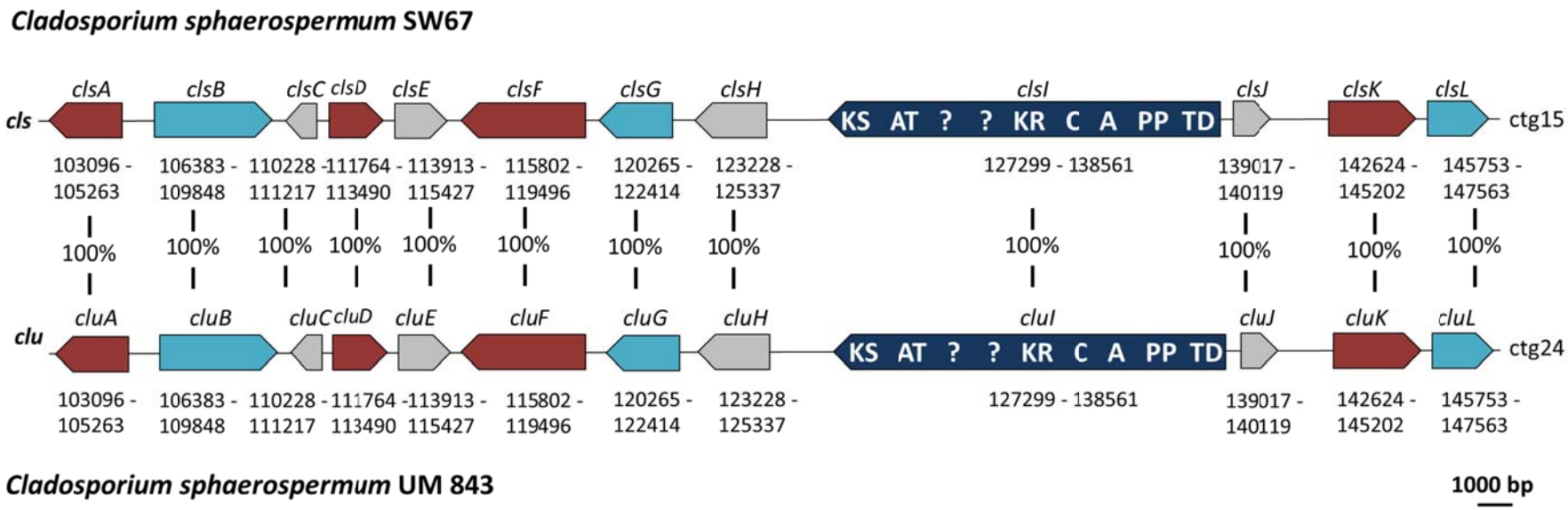

Figure S3. Comparison of biosynthetic gene cluster of SW67 and *C. sphaerospermum* UM 843. Color code: blue coding for transporter protein related genes; dark blue and red coding for biosynthetic genes. Grey coding for genes with unknown function. Percentages given between the genes are nucleotide sequence identifies between the two orthologous. Nucleotide positions within the contig are given as identifiers for the genes.

Antismash identified four terpene cluster, five PKS cluster, four NRPS cluster and a single PKS-NRPS hybrid cluster in SW67. Comparison with other published *Cladosporium* genomes downloaded from the NCBI database (04.07.2018), indicated the presence of a highly homologous NRPS-PKS gene cluster in the strain *C. sphaerspermum* UM 843 but not in the others.

The putative PKS-NRPS cluster (*clsA* to *clsL*) encodes for five genes: a siderophore esterase (*clsA*), an AMP dependend synthetase/ligase (*clsD*), a γ-glutamyl transferase (*clsF*), a cytochrome P450 (*clsK*) and a PKS-NRPS hybrid gene (*clsI*). Furthermore, three genes encoding for transport related proteins (*clsB* and *clsG*) as well as four putative genes coding for proteins with unknown functions (*clsC*, *clsE*, *clsH*, *clsJ*) (Figure S3). The domains of the putative PKS-NRPS gene were predicted using PKS/NRPS analysis predictor (KS-AT-?-?-KR-C-A-PCP-TD.[10] We performed an additional search using NaPDoS indicating that gene *clsI* (45% identity) only shows moderate similarity to gene *epoD* (*Sorangium cellulosum* So ce90 a, Acc. No. EU414841, natural product epothilone) indicating that the missing domains of *clsI* could be a dehydratase and an enoylreductase.

Table S1. Secondary metabolite biosynthesis gene clusters in the genome of SW67 as predicted from antiSMASH 4.0 fungal version.[11]

Cluster position	Cluster type	Putative annotation of involved biosynthetic enzymes	Domains involved
Ctg1_orf8 – orf16	Terpene	Crotonyl-CoA reductase/alcohol dehydrogenase; squalene synthase	-
Ctg3_orf226 – orf229	Terpene	Polyprenyl synthetase	-
Ctg3_orf246 – orf262	other	Cytochrome P450; carboxymuconolactone decarboxylase; cytochrome P450; AMP-dependent synthetase/ligase	ACP, TD
Ctg3_orf295 – orf311	Type-III PKS	Chalcone and stilbene synthase domain protein/ alpha/beta hydrolase domain-containing protein; metallo-beta-lactamase protein; phenylalanine-specific permease	-
Ctg5_458 – orf471	Other	AMP-dependent synthetase/ligase	A, PCP
Ctg6_orf228 – orf242	NRPS	NRPS	A; C
Ctg9_orf111 – orf118	Type-I PKS	PKS	KS; AT, ACP;ACP;TE
Ctg9_orf307 – orf 323	Other	AMP-dependent synthetase/ligase	A
Ctg10_orf40 – orf59	Other	FAD dependent oxidoreductase; AMP-dependent synthetase/ligase; cytochrome P450; acyl-CoA dehydrogenase type 2; alpha/beta hydrolase fold protein	A; ACP; TD; KR
Ctg11_orf80 – orf91	Terpene	Terpenoid synthase; dehydrogenase	
Ctg15_orf33 – orf 52	Type-I PKS/NRPS	Acyltransferase; AMP-dependent synthetase/ligase; gamma-glutamyltranspeptidase; PKS/NRPS; cytochrome P450	A; KS; AT; ?; ?; KR; C; A; PCP; TD
Ctg15_orf58 – orf74	NRPS	Phenylalanine specific permease; AMP-dependent synthetase/ligase; lysine/ornithine N-monooxygenase; NRPS; enoyl-CoA hydratase; siderophore biosynthesis protein	A; A; PCP; C; PCP; C
Ctg15_orf94 –	PKS	PKS; crotonyl-CoA reductase/alcohol dehydrogenase; FAD	KS; AT; DH; ER; KR; ACP;

orf110		linked oxidase domain protein	ER
Ctg18_orf14 – orf22	Terpene	Terpene synthase, short chain dehydrogenase/reductase; transketolase;	
Ctg18_orf105 – orf119	Other	NAD(P)-binding protein, glucose-1-phosphate adenylyl/thymydylyltransferase; methionine aminopeptidase, AMP-dependent synthetase/ligase, cytochrome P450	A
Ctg18_orf154-orf167	NRPS	Serine/threonine protein kinase; monooxygenase FAD-binding; NRPS; cysteine synthase; beta-lactamase; phenylalanine-specific permease	A; PCP; E; C; A;PCP; C; A, PCP; C; PCP; C; PCP
Ctg21_orf124 – orf140	Other	FAD dependent oxidoreductase; AMP-dependent synthetase/ligase; alpha/beta hydrolase; short-chain dehydrogenase/reductase; short-chain dehydrogenase/reductase;	A
Ctg22_orf12 – orf26	Other	AMP-dependent synthetase/ligase; FAD dependent oxidoreductase; short-chain dehydrogenase/reductase	A; PCP; TD; KR
Ctg22_orf92 – orf109	NRPS	short-chain dehydrogenase/reductase; 3-hydroxyisobutyrate dehydrogenase; NRPS; lysine/ornithine N-monooxigenase; 3-hydroxybutyryl-CoA dehydrogenase	A; C, A; PCP; C; PCP; C; A; C; C; C
Ctg23_orf35 – orf51	Other	AMP-dependent synthetase/ligase	A; ACP; TD
Ctg_orf17 – orf33	Other	Oxidoreductase; AMP-dependent synthetase/ligase; short-chain dehydrogenase/reductase; aldo/keto reductase family oxidoreductase	A
Ctg36_orf 0 – orf8	Type-I PKS	PKS, AMP-dependent synthetase/ligase	KS; AT; DH; ER; KR; A; ACP; TD
Ctg56_orf0 – orf3	Type-I PKS	PKS	KS; AT; ACP; cMT

Other: cluster contains a secondary metabolite-related protein that does not fit into any other category
ACP: Acyl carrier protein, TD: Terminal reductase, A: Adenylation, PCP: Peptidyl carrier protein, C: condensation , KS: ketosynthase, AT: Acyltransferase, TE: Thioesterase, KR: Ketoreductase, ER: Enoylreductase, DH: Dehydratase, E: Epiperization. cMT: C-methyltransferase

Table S2. Detailed information of the putative PKS-NRPS hybrid gene cluster of SW67 and *C. sphaerospermum* UM843 including top BLAST hits from the UniProtKB sequence database sorted after BLAST score.

Identifier UM 843	Identifier SW67	Size (AA)	Gene name	Annotation	Closest homolog (origin)	Putative annotation	Identity [%]/ score	Acc No.
Ctg24_orf 36	Ctg15_or f36	2167	*clsA*	N-acetyltransferase	*Penicillium brasilianum* ATCC 12072	Putative siderophore esterase	66.2/1014	A0A1S9RZT7
					Aspergillus clavatus ATCC 1007	Ferri-bacillibactin esterase BesA	66.9/1012	A1C4M6
					Penicillium subrubescens CBS 132785		66.2/1005	A0A1Q5UJV9
Ctg24_orf 37	Ctg15_or f37	3465	*clsB*	ABC transporter	*Aspergillus novofumigatus* IBT 16806	Putative ABC multidrug transporter	73.4/4191	A0A2I1C1G8
					Penicillium brasilianum ATCC 12072	Putative Multidrug/pheromone exporter, ABC superfamily	73.7/4187	A0A0F7VIH0
					Aspergillus lentulus CBS 117885	Leptomycin B resistance protein pmd1	72.9/4166	A0A0S7DHX8
Ctg24_orf 38	Ctg15_or f38	989	*clsC*	Uncharacterized protein	*Rachicladosporium antarcticum* CCFEE 5527	Uncharacterized proteins	77.2/1337	A0A1V8T847
							75.7/1306	A0A1V8UD24
							75.4/1299	A0A1V8TCT0
Ctg24_orf 39	Ctg15_or f39	1672	*clsD*	AMP-dependent synthetase and ligase	*Rachicladosporium* sp. CCFEE 5018	Uncharacterized protein	77.5/2338	A0A1V8UD69
						Putative 4-coumarate--CoA ligase 1		
					Cercospora beticola CBS 116456	Acetyl-CoA synthetase-like protein	73.7/2212	A0A2G5HD40
					Sphaerulina musiva. SO2202		73.2/2206	M3CCD9

Ctg24_orf40	Ctg15_orf40	1514	*clsE*	Uncharacterized protein	*Rachicladosporium* sp. CCFEE 5018	Uncharacterized proteins	64.8/1608 64.3/1599 64.2/1579	A0A1V8UP25 A0A1V8URL1 A0A1V8T8L7
Ctg24_orf41	Ctg15_orf41	3694	*clsF*	Gamma-glutamyltranspeptidase	*Quercus suber* *Exophiala dermatitidis* ATCC 34100 *Macrophomina phaseolina* MS6	γ-Glutamyltransferase/ γ-Glutamyltranspeptidase	66/1974 69.4/1964 67.0/1953	A0A2P4I3Z8 H6BKZ8 K2RPI9
Ctg24_orf42	Ctg15_orf42	2149	*clsG*	Drug resistance transporter,	*Beauveria bassiana* strain ARSEF 2860 *Beauveria bassiana* ARSEF 1564 *Beauveria bassiana* D1-5	MFS drug efflux transporter Efflux pump roqT Putative HC-toxin efflux carrier TOXA	71.1/1840 71.1/1814 72.2/1806	J4UG41 A0A2N6P2Z4 A0A0A2VQU0
Ctg24_orf43	Ctg15_orf43	2109	*clsH*	Uncharacterized protein	*Beauveria bassiana* D1-5	Uncharacterized protein	61.1/1038 61.2/1008 60.5/990	A0A0A2VAT7 A0A2S7YHY1 A0A2N6P2Z6
Ctg24_orf44	Ctg15_orf44	11262	*clsI*	NRPS/PKS	*Elaphomyces granulatus* LIP 0001132 *Aspergillus carbonarius* ITEM 5010 *Talaromyces stipitatus* ATCC 10500	Putative polyketide synthase	41.1/5469 41.5/5357 39.9/5284	A0A232LUQ5 A0A1R3RKP6 B8M2A8
Ctg24_orf45	Ctg15_orf45	1102	*clsJ*	Uncharacterized protein	*Verticillium longisporum* CBS 124.64 *Aspergillus steynii* IBT 23096 *Aspergillus campestris* IBT	Uncharacterized protein	40.2/359 39.9/353 37.4/333	A0A0G4MDE0 A0A2I2FWU0 A0A2I1D470

					28561			
Ctg24_orf46	Ctg15_orf46	2578	*clsK*	Cytochrome P450	*Aspergillus steynii* IBT 23096 *Meliniomyces variabilis* MAR *Penicillium occitanis* 290292	Cytochrome P450	41.9/379 40.4/310 34.7/309	A0A2I2FWM4 A0A2J6S9G9 A0A2H3IRR7
Ctg24_orf47	Ctg15_orf47	1810	*clsL*	Sugar transport protein	*Cercospora beticola* CBS 116456 *Cercospora zeina* CBS 118820 *Aureobasidium pullulans* EXF-150	Lactose permease MFS sugar transporter-like protein	74.6/2159 74.3/2156 72.6/2021	A0A2G5HEM8 A0A2I0RTH1 A0A074XF51

a) Homologs of biosynthetic gene clusters encoding for characterized compounds were considered; b) percent identity and positive alignments were determined using BLASTp of the UniProt server, following default parameters and using the UniProtKB database. Percent of positives is the proportion of the query sequence that aligns to each homolog residues for which the alignment scores have positive values.

4 Cultivation of SW67 and co-cultivates

4.1 Cultivation of bacterial isolates

H. echinata associated bacteria were isolated as described before.[12]

4.2 Co-cultivation assays with *H. echinata* associated bacteria and fungi

Method A:

For co-culture with bacteria, large agar plates (150 mm × 20 mm) were filled half with MEA (for fungal cultivation) and half with MBA (for bacterial cultivation).

For co-culture with other fungi agar plates were completely filled with MEA.

For each isolate three plates (150 mm × 15 mm) were inoculated with 10 µL of a fungal spore suspension of SW67 in triplicate (approx. 8×10^5 spores/mL). SW67 was then incubated at 25 °C in the dark for 3 days. After three days 10 µL of a fungal spore suspension in PBS or 10 µL of a bacterial culture (pre-culture incubated for 3 d at 30 °C in MBL) was inoculated in proximity to SW67 (Figure S4). Plates were then again incubated for additional 12 d at 25 °C in the dark.

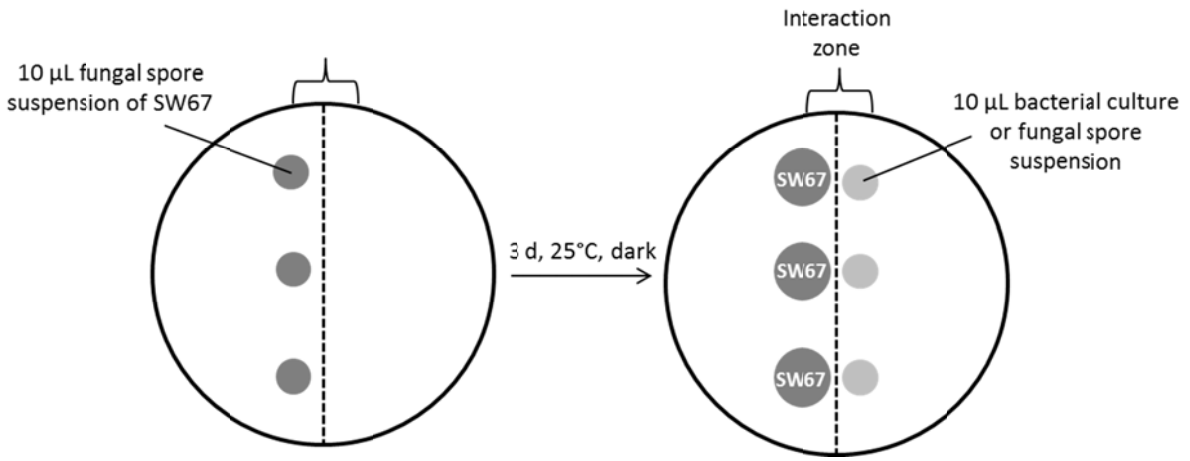

Figure S4. Scheme of co-cultivation assay.

For chemical analysis: fungus/bacterium or fungus/fungus interaction zones were cut in small squares, pooled and then soaked in MeOH overnight. The solvent was filtered and removed under reduced pressure. The residue was dissolved in 10% MeOH, centrifuged and subjected to SPE on a pre-activated C18 column (Waters, 10 g). After loading, the C18 column was washed with 10%, and then eluted with 50% and 100% MeOH. Fractions (50% and 100%) were pooled and solvent was removed under reduced pressure and the crude extract was

submitted to UHPLC-MS analysis under the concentration of 1.0 mg/mL in 100% MeOH solution (Figure S11 and Figure S5 and Table S4).

Method B: Axenic cultures of bacteria and fungi isolated from *H. echinata* were cultivated as control.

Bacteria: three MBA plates were inoculated with 100 µL of a bacterial culture in MBL and incubated for 12 d at room temperature in the dark.

Fungi: three plates of MEA were inoculated with 100 µL of a spore suspension in sterile PBS. Plates were incubated for 12 d at room temperature in the dark. Plates were then extracted like described in method A (Figure S11).

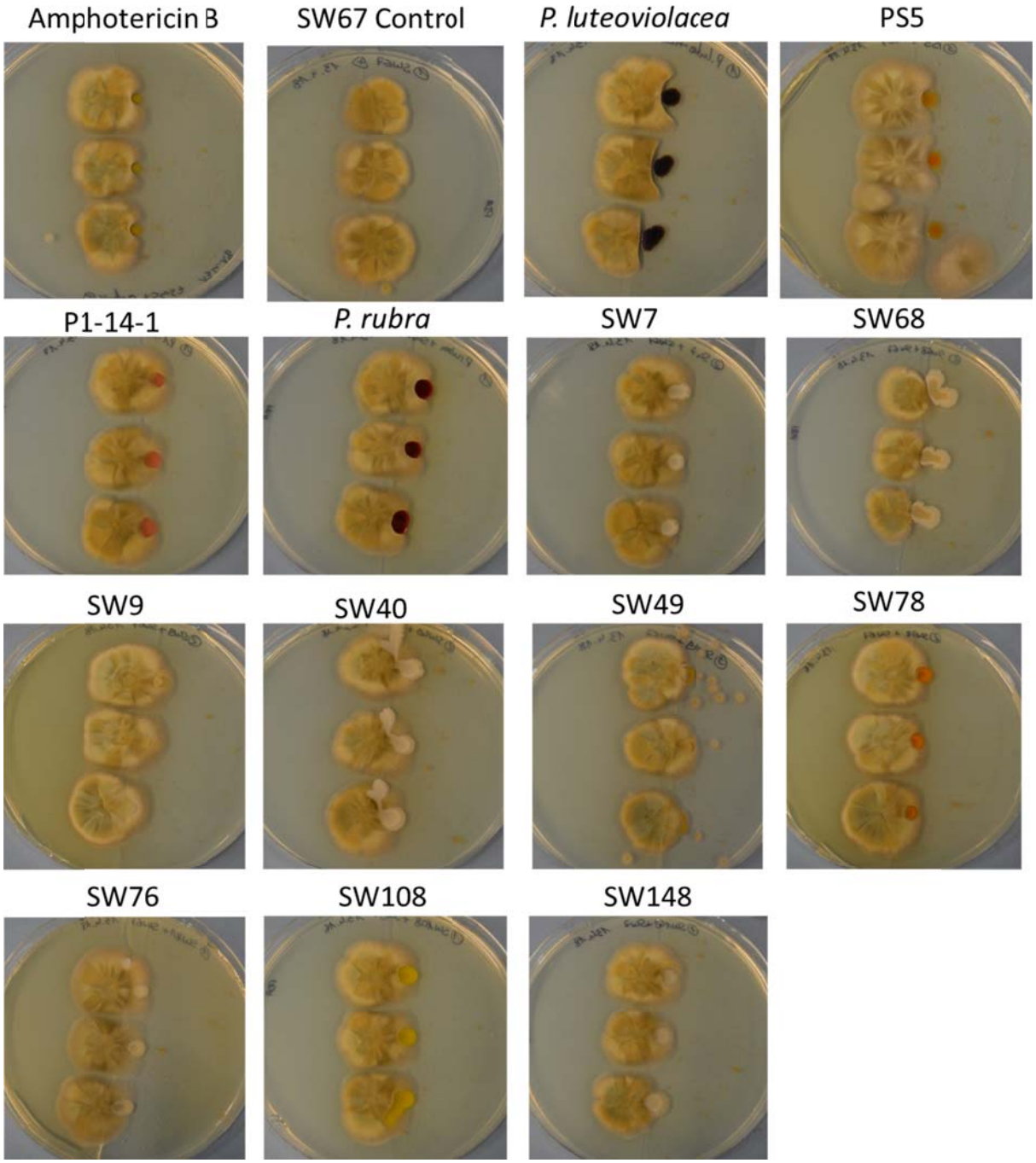

Figure S5. Representative pictures of co-cultivation of SW67 with bacteria isolated from *H. echinata* or other cnidarians (*P. luteoviolacea*, *P. rubra* and *Pseudoalteromonas* sp. PS5). Pictures were taken after 7 d of incubation at 25 °C in the dark (method A).

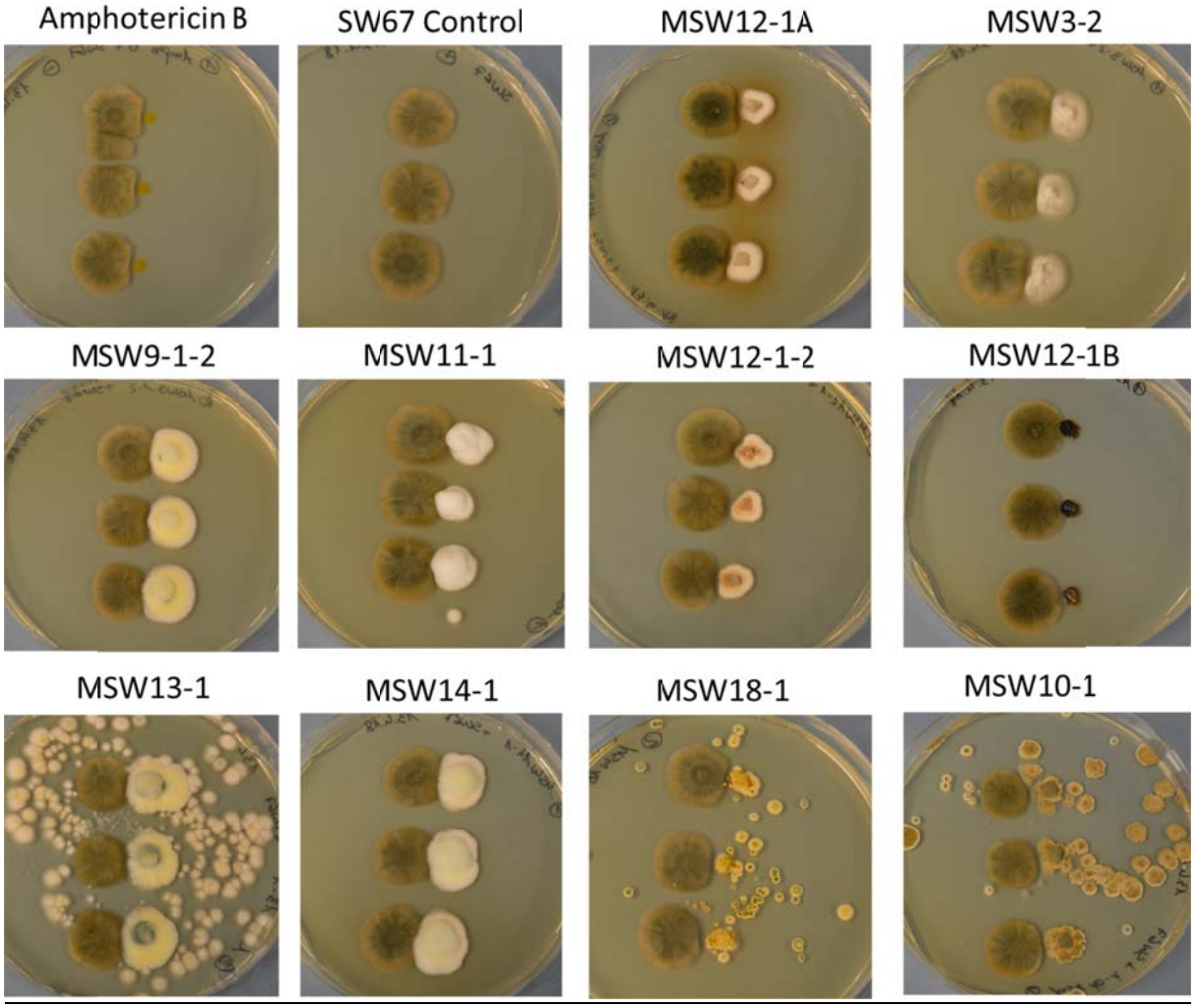

Figure S6. Representative pictures of co-cultivation of SW67 with fungi isolated from *H. echinata*. Pictures were taken after 7 d of incubation at 25 °C in the dark (method A).

Table S3. Bacterial and fungal strains used for co-cultivation with SW67 and their related species names.

Organism	Strain name	Species
Bacteria	PS5	*Pseudoalteromonas* sp.
	P1-14-1	*Pseudoalteromonas piscicida*
	P. rubra	*Pseudoalteromonas rubra* DSM 6842
	P. luteoviolaceae	*P. luteoviolaceae* DSM 6061
	SW7	*Bacillus* sp.
	SW68	*Bacillus cereus*
	SW9	*Exiguobacterium* sp.
	SW40	*Cobetia* sp.
	SW49	*Exiguobacterium oxidotolerans*
	SW78	*Planococcus* sp.
	SW76	*Pseudomonas* sp.
	SW108	*Paracoccus* sp.
	SW148	*Cobetia* sp.
Fungi	MSW12-1A	*Aspergillus* sp.
	MSW9-1-2	*Penicillium* sp.
	MSW11-1	*Engyodontium album*
	MSW12-1-2	*Aspergillus* sp.
	MSW12-1B	*Hortaea werneckii*
	MSW13-1	*Penicillium* sp.
	MSW14-1	*Penicillium* sp.
	MSW18-1	*Aspergillus* sp.
	MSW10-1	*Penicillium brevicompactum*
	MSW3-2	*Aspergillus* sp.

4.3 Time and media studies

4.3.1 Analytical scale plate cultivation of SW67

Method C: 40 plates (92 mm x 16 mm) of each medium (MEA, PDA, GluPe or Wick) were inoculated with a 100 µL aliquot of a turbid fungal spore suspension of SW67 in sterile PBS. The suspension was evenly distributed over the agar surface and dried. Plates were incubated at 25 °C in the dark for 7 d, 14 d, 21 d and 28 d. The densely covered agar plates were cut into squares, consolidated, and soaked in MeOH overnight. The solvent was filtered and removed under reduced pressure. The residue was dissolved in 10% MeOH, centrifuged and subjected to SPE on a pre-activated C18 column (Waters, 1 g). After loading, the C18 column was washed with 10%, and then eluted with 100% MeOH. The solvent of the 100% MeOH fraction was removed under reduced pressure and the extract was submitted to UHPLC-MS analysis under the concentration of 1.0 mg/mL in 100% MeOH solution (Figure S8 - Figure S10).

4.3.2 Analytical scale plate cultivation of other *Cladosporium* strains

Method D: To validate the production of derivatives **2, 3** and **4** in other *Cladosporium* strains, we analyzed *C. sphaerospermum* (JMRC, SF006509 and SF011511) and *C. perangustum* strain (CPC 18648). Three plates of MEA were inoculated with 100 µL of a spore suspension in sterile PBS and then cultivated for 14 d at 25 °C.

Extraction and LC-MS measurements was performed as described for method C. Only SW 67 showed peaks for derivatives **2** and **3** at their corresponding mass (*m/z* = 350.15 $[M+H]^+$) in the selected ion chromatogram (Figure S7).

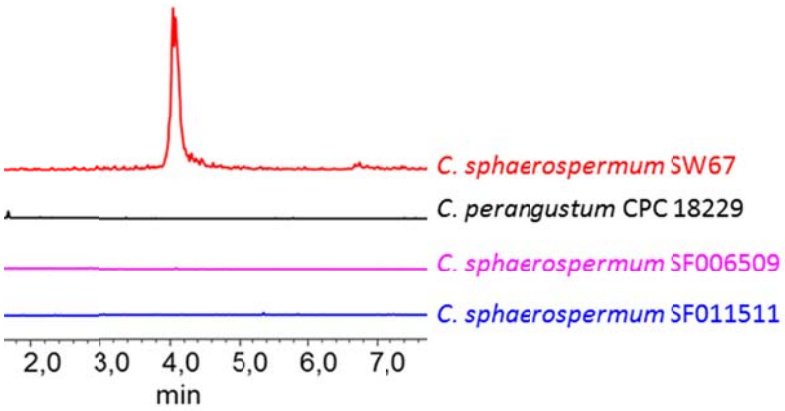

Figure S7. Comparative selected ion chromatogram (*m/z* = 350.15 $[M+H]^+$) of culture extracts obtained from SW67, *C. sphaerospermum* SF006509, *C. sphaerospermum* SF011511, *C. perangustum* CPC 18648. Plate cultures were incubated on MEA for two weeks at 25 °C in the dark.

4.4 LC-MS analysis

UHPLC-MS analysis was performed using the following procedure: 2 µL of sample was injected and analyzed using the following gradient: 0 - 1 min: 10% D, 1 - 7 min: 70% D, 7-10 min: 100% D, 10 – 13.5 min: 10% D (B: ddH_2O with 0.1% formic acid; D: MeCN with 0.1% formic acid) with a flow rate of 0.7 mL/min

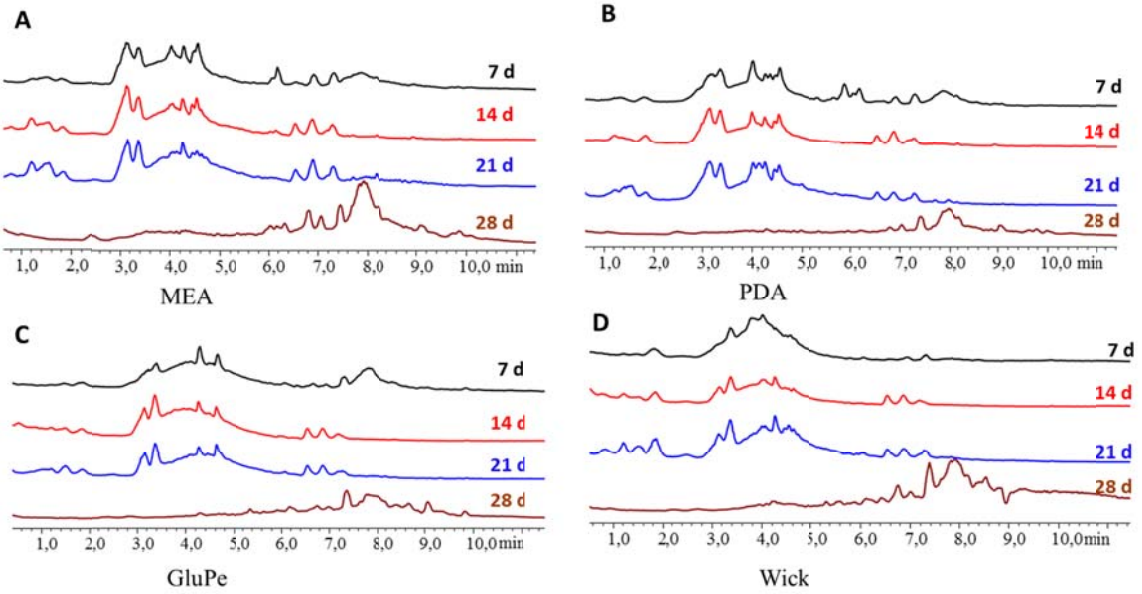

Figure S8. Comparative total ion chromatogram (positive mode) of culture extracts obtained from SW67 cultured on A) MEA; B) PDA, C) GluPe and D) Wick medium. Incubation time: 7 d, 14 d, 21 d, and 28 d at 25 °C in the dark.

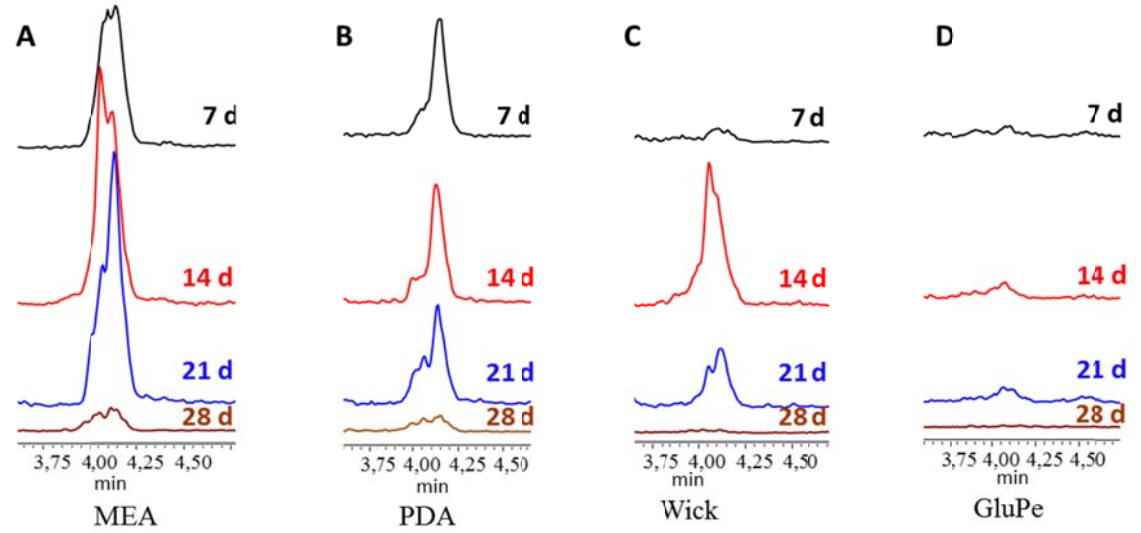

Figure S9. Comparative selected ion chromatogram (*m/z* = 350.15 $[M+H]^+$) of culture extracts obtained from SW67 incubated on: A) MEA; B) PDA, C) GluPe and D) Wick mediumfor 7 d, 14 d, 21 d, and 28 d at 25 °C in the dark.

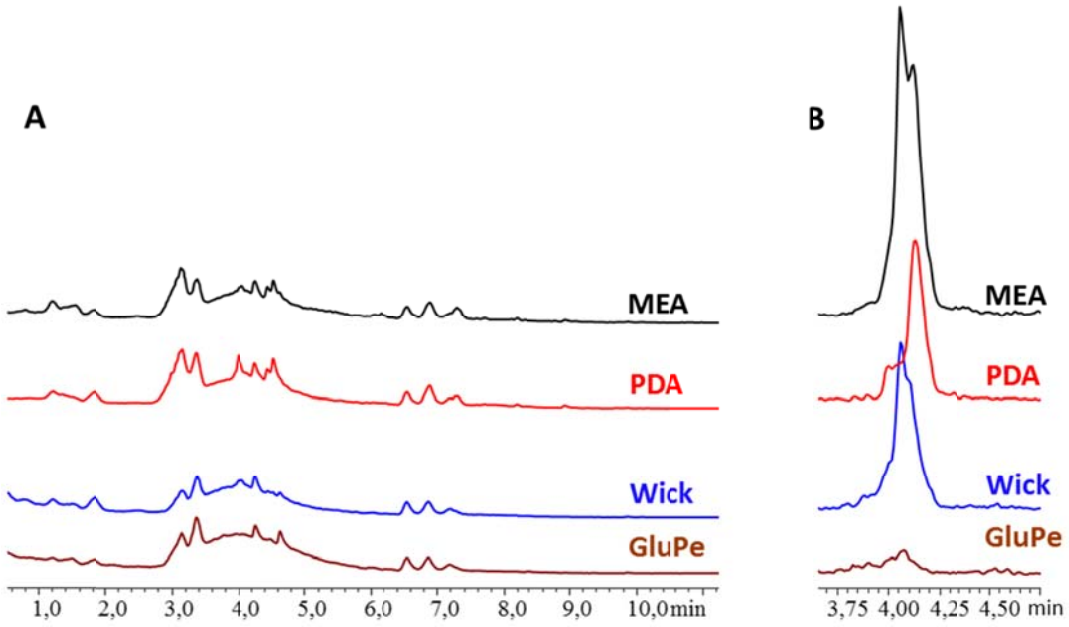

Figure S10. Comparative A) total ion chromatogram and B) selected ion chromatogram (*m/z* = 350.15 $[M+H]^+$) of SW67 culture extracts obtained from strain cultivation on MEA, PDA, Wick, GluPe medium after two weeks at 25 °C in the dark.

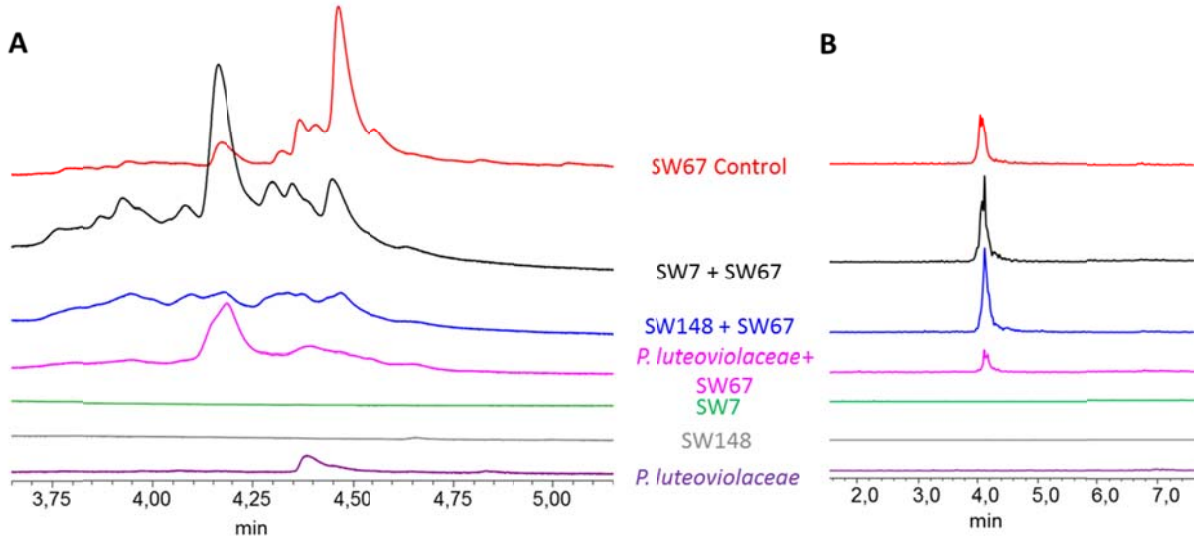

Figure S11. A) Comparative HPLC-based analysis of representative co-cultivation extracts of SW67 against co-isolated bacteria. B) Corresponding selected ion chromatogram (*m/z* = 350.15 $[M+H]^+$) of co-cultures (representative UV trace at 298 nm).

Table S4. Analysis of bacterial and fungal co-cultivation compared to axenic control cultures. Relative up- and downregulation of *m/z* = 350.15 $[M+H]^+$ signal (peak height), which is attributed to compound **2** and **3** and was automatically integrated with the Postrun analysis tool of LabSolution 5.57SP (Shimadzu). Concentration of each crude extract was 1 mg/ml (injection volume: 2 µL). Colour code represents estimate of up and downregulation in %.

Co-cultivate	Species	Relative peak height difference
SW67 control	*C. sphaerospermum* SW67 [MH482916]	0%
SW7	*Bacillus* sp. [KY382801.1]	76%
SW148	*Cobetia* sp. [KY382829.1]	76%
Amphotericin B	*C. sphaerospermum* SW67 [MH482916]	-1%
P1-14-1	*Pseudoalteromonas piscicida* [KY382776.1]	-2%
SW9	*Exiguobacterium* sp. [KY382790.1]	-5%
SW78	*Planococcus* sp. [KY382807.1]	-7%
MSW12-1-2	*Aspergillus* sp. [MH482925]	-13%
P. rubra	*Pseudoalteromonas rubra* DSM 6842	-17%
MSW18-1	*Aspergillus* sp. [MH482921]	-17%
MSW10-1	*Penicillium brevicompactum* [MH482922]	-29%
SW40	*Cobetia* sp. [KY382797.1]	-31%
MSW3-2	*Aspergillus* sp. [MH482926]	-32%
MSW12-1B	*Hortaea werneckii* [MH482920]	-32%
SW108	*Paracoccus* sp. [KY382817.1]	-41%
SW81	*Cobetia marina* [KY382809.1]	-42%
SW68	*Bacillus cereus* [KY382804.1]	-43%
SW49	*Exiguobacterium oxidotolerans* [KY382800.1]	-47%
MSW12-1A	*Aspergillus* sp. [MH482919]	-50%
PS5	*Pseudoalteromonas* sp. [KF733524.1]	-51%
MSW9-1-2	*Penicillium* sp. [MH482917]	-57%
P. luteoviolaceae	*Pseudoalteromonas luteoviolaceae* DSM 6842	-58%
MSW13-1	*Penicillium* sp. [MH482923]	-62%
MSW14-1	*Penicillium* sp. [MH482924]	-68%
MSW11-1	*Engyodonitum album* [MH482918]	-70%

Up-regulation [%]	>70	
	60- 70	
	51 - 60	
	38 - 50	
Down-regulation [%]	- (38 -50)	
	- (50 – 60)	
	- (60 – 70)	
	>70	
Standard deviation	+/- 0 - 38	

4.5 Isotope labeling

For ^{13}C labeling experiments, 2 g/L of sodium [1-^{13}C] acetate, sodium [2-^{13}C] acetate or [1-^{13}C] valine were each added separately to 1 L of PDA* medium (6.6 g/L).

Each plate was inoculated with 100 µL of a spore suspension (SW67) in sterile PBS and plates were incubated for 14 d at room temperature in the dark. Plates were cut into pieces and extracted with 100% MeOH (if not mentioned otherwise, mixtures refer to MeOH in ddH_2O) at 4 °C over night. Extracts were filtrated and the solvent was evaporated under reduced pressure. The remaining extract was re-dissolved in 10% MeOH and loaded on a pre-activated and equilibrated C18 cartridge (100 mg C18, 10% MeOH). The loaded SPE column was washed with 20% MeOH, and then metabolites eluted using 100% MeOH. Extracts were then concentrated under reduced pressure. Finally, organic extract was dissolved with 100% MeOH to yield a 1.0 mg/mL stock solution for UHPLC-MS analysis.

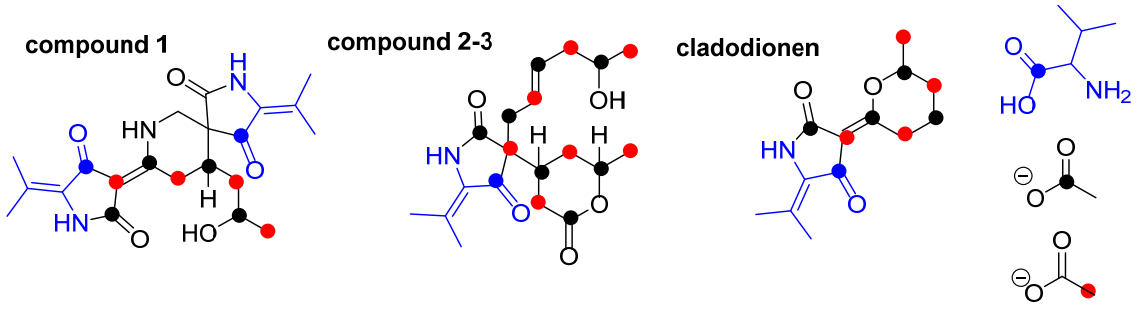

Figure S12. Possible ^{13}C-labeling pattern of compounds **1-3**.

For compound **1** (*m/z* = 400.25 $[M-H]^-$), feeding with [1-^{13}C] sodium acetate and [2-^{13}C] sodium acetate did not show any clear results due to the low production rate of compound **1** (Figure S13); only feeding with [1-^{13}C] valine resulted in the detection of the corresponding mass shift of up to +2 *m/z* (Figure S13).

For compound **2** and **3** (*m/z* = 350.15 $[M+H]^+$), feeding with [1-^{13}C] sodium acetate and [2-^{13}C] sodium acetate resulted in a mass shift of up to + 10 *m/z* for *m/z* feeding with [1-^{13}C] valine resulted in a mass shift of + 1 *m/z* (Figure S14). For cladodionen, feeding with [1-^{13}C] sodium acetate and [2-^{13}C] sodium acetate resulted in a mass shift of up to + 5 *m/z*. Feeding with [1-^{13}C] valine resulted in a mass shift of + 1 *m/z* (Figure S15).

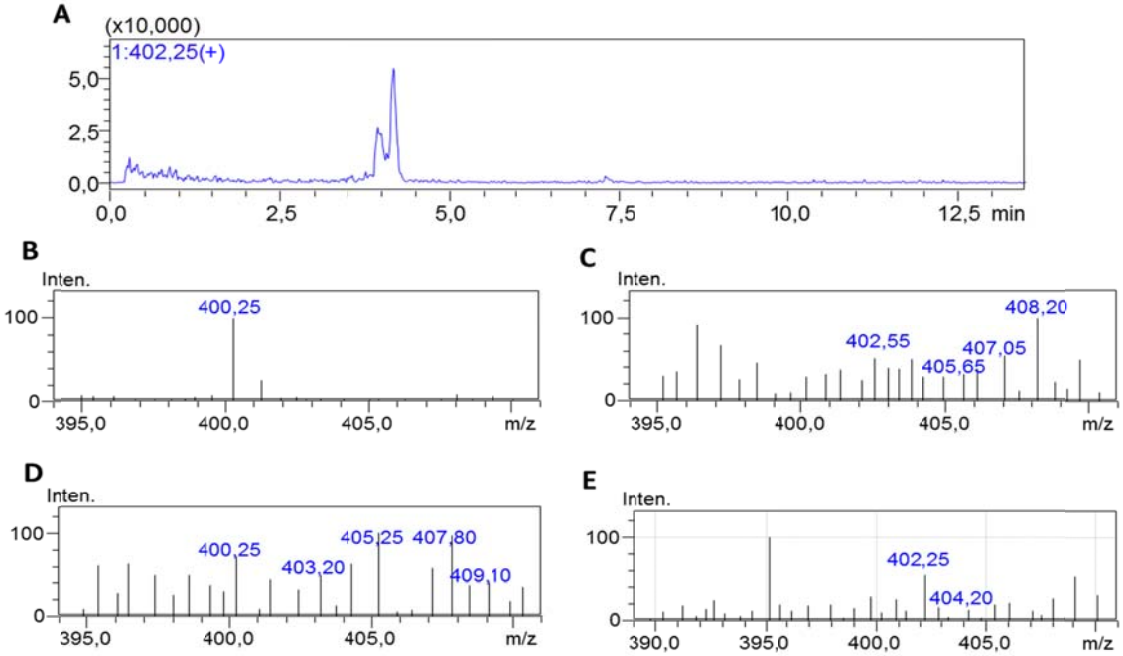

Figure S13. LC-MS analysis for compound **1** of ^{13}C-labeled culture extracts. A) Selected MS ion chromatogram (*m/z* = 402.25 $[M+H]^+$) of SW67 control without labeled precursor; B) selected ion peak analysis of compound **1** with *m/z* = 400.25 $[M-H]^-$ (SW67 control without labeled precursor); C) selected ion peak analysis of [1-^{13}C]-acetate labeling experiment; D) selected ion peak analysis of [2-^{13}C]-acetate labeling experiment; E) selected ion peak analysis of L-[1-^{13}C]-valine labeling experiment.

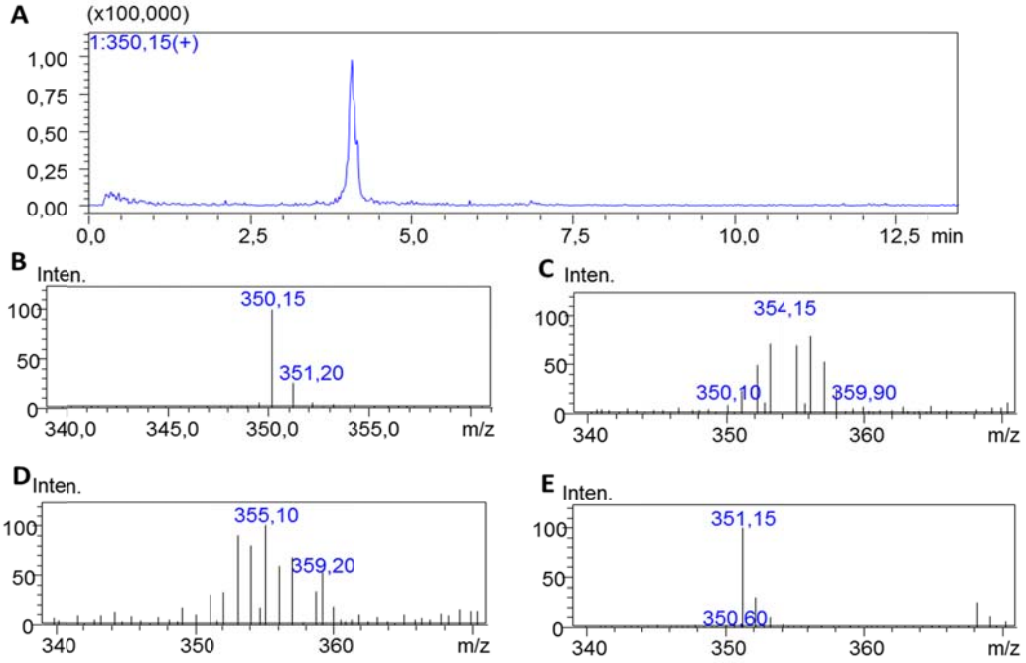

Figure S14. LC-MS analysis for compounds **2** and **3** of ^{13}C-labeled culture extracts. A) Selected MS ion chromatogram (*m/z* = 350.15 $[M+H]^+$) of SW67 control without labeled precursor; B) peak analysis of compound **2** and **3** with *m/z* = 350.15 $[M+H]^+$ (SW67 control without labeled precursor), C) peak analysis of [1-^{13}C]-acetate; D) [2-^{13}C]-acetate; E) L-[1-^{13}C]-valine labeled culture extracts.

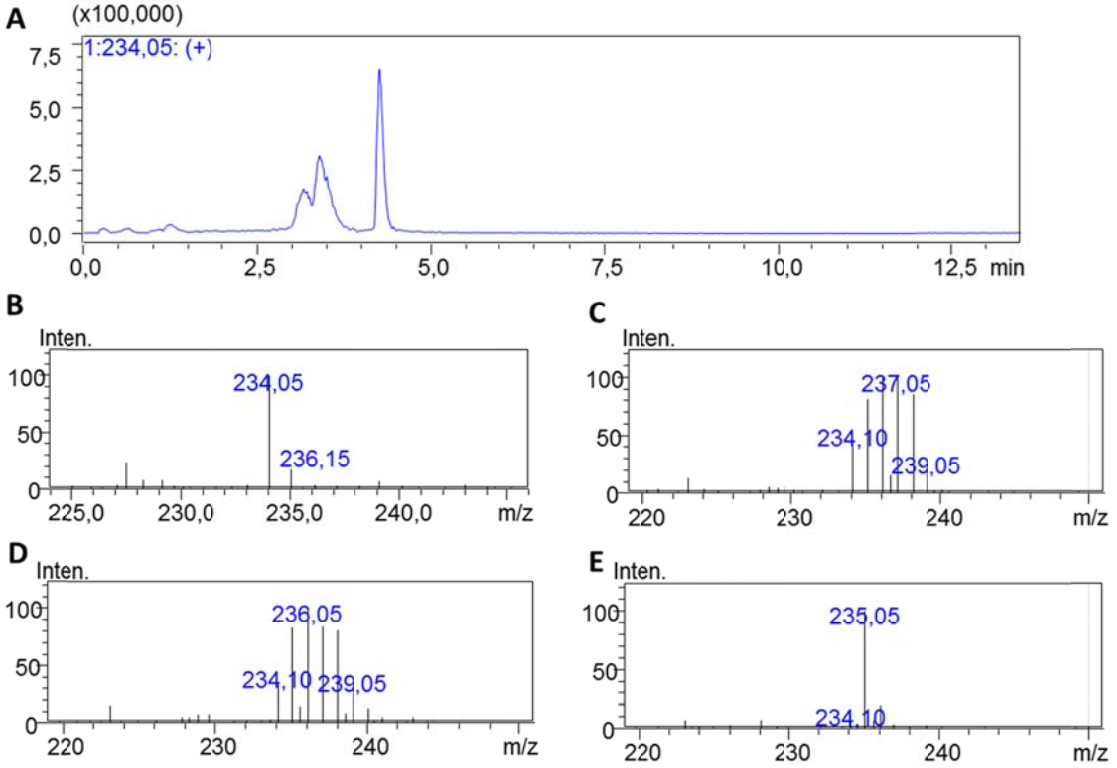

Figure S15. LC-MS analysis for cladodionen of ^{13}C-labeled culture extracts. A) Selected MS ion chromatogram (*m/z* = 234.05 $[M+H]^+$) of SW67 control without labeled precursor; B) peak analysis of cladodionen with *m/z* = 234.05 $[M+H]^+$ (SW67 control without labeled precursor), C) peak analysis of [1-^{13}C]-acetate D) [2-^{13}C]-acetate; E) L-[1-^{13}C]-valine labeling experiment.

4.6 Expression studies

4.6.1 Strain cultivation

For plate cultivation 100 µL of a spore suspension of SW67 were spread on 10 plates containing MEA and then incubated at 25 °C in the dark without shaking. For liquid cultivation 8 mL of MEB were inoculated with 80 µL of the same SW67 spore suspension. After 7 d, 11 d and 14 d incubation, mycelium was transferred to a sterile cooled reaction tube using a scalpel and immediately frozen in liquid nitrogen.

Mycelium from liquid cultures was centrifuged in a falcon tube at 8000 rpm and the liquid was removed. The remaining pellet was frozen in liquid nitrogen. All samples were kept at -80 °C until further usage or were directly submitted to extraction procedure.

4.6.2 RNA isolation and reverse transcriptase PCR

RNA was isolated according to the instructions supplied with RNeasy Plant Mini Kit (Qiagen) with an additional genomic DNA removal step. For amplification, 1 µg of RNA was added to 1 µL of 10x reaction buffer supplemented with $MgCl_2$, 1 µL of RNase free DNase 1 (1 U) (Epicentre, Madison) and filled up to 10 µL with DEPEC-treated water. The reaction mixture was incubated at 37 °C for 30 min (130 rpm shaking) and purified using the GeneJET RNA Purification Kit according to the manufacturer's instructions. The concentration and purity of RNA samples were determined using a NanoDrop Lite Spectrophotometer (Thermo Fisher). After more than 7 d of incubation no RNA could be extracted anymore from the fungi. Total RNA (1 µg) of 7 d of incubation samples was reverse transcribed (RT) with QuantiNova Reverse Transcription Kit (QIAGEN) according to the supplied protocol. Furthermore, negative controls were performed using RNA but without reverse transcriptase (RT- control) to check for complete DNAse digest. PCR was performed using 1 µL of 40 ng/µL of DNA or 1 µL of reverse transcribed RNA (cDNA) with primers designed to amplify part of the *cls* genes. The housekeeping gene beta-actin was used as a control. The amplification was performed in a 25 µL reaction volume with 5 µL of 10x HF buffer, 2.5 pM/µl each primer, 0.25µL Phusion polymerase (1 U), 10 mM dNTP mix and 1 µL of DNA with a concentration of 40 ng/µL or 1 µL of transcribed cDNA. A positive control (gDNA control) was performed with gDNA and a second negative control (C-) was performed without DNA or RNA to check for contaminations in the PCR mastermix. Cycling conditions were as followed: initial denaturation 98 °C for 38 sec, 35 cycles of 98 °C for 30 sec; 55 °C for 30 sec; and 72 °C for 1

min and a final extension of 1 min at 98 °C followed by 8 min of 72°C. PCR was performed in a Peqstar Gradient cycler. PCR products were analyzed by electrophoresis in a 2.5% agarose gel under UV light. If more than one band showed up on the gel the expected right size band was cut out of the gel and the DNA was re-extracted using the Zymoclean DNA Recovery kit (Zymo Research) according to manufacturer instructions and the product was sequenced and checked for correct base pattern.

Primer:

SW67_Sider_for: 5'- AGTCCACACATGGCTCATGG- 3'
SW67_Sider_rev: 5'- CTTCGCCTTCCTCGATACCC- 3'
Product size: 395 bp
SW67_AMP_for: 5'- TCAAGACTGGAGACGTTGGC
SW67_AMP_rev: 5'- CTTCAGCACTCGCCTCAGAA
Product size: 365 bp
SW67_Gamma3_F: 5'- CTTCTTCGCTGGCCTCCT- 3'
SW67_Gamma3_R: 5'- CCGACGCAGAACACAGTAGC- 3'
Product size: 210 bp
SW67_NRPS3_F: 5'- ATCGCACTTGTTCTGCACTG- 3'
SW67_NRPS3_R: 5'- CGCGCGTTATAGTGATCGTA- 3'
Product size: 226 bp
SW67_P450_3_F: 5'- GCTCAATCCAAGATCCCAGA- 3'
SW67_P450_3_R: 5'- ACCTAACAGCCGCTCTTGAA- 3'
Product size: 216
ACTE_for_: 5'-CGGCTTTCTACGTCTCCATC-3'
ACTE_rev: 5'-GGAGATGCCGAATCTTACCA-3'
Product size: 527

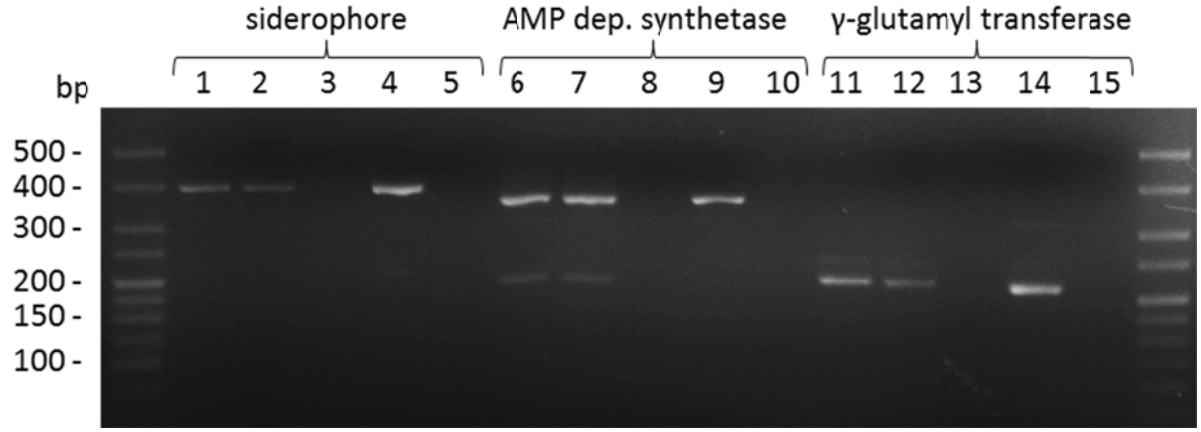

Figure S16. Detection of the *cls* biosynthetic genes and corresponding controls of the housekeeping gene β-actin by conventional reverse transcriptase-PCR, cultivated on agar plates and liquid culture for 7 d in the dark (25 °C). Amplification of *clsA* (expected product size: 395 bp) after 7 d of incubation on 1) MEA plates and 2) MEL standing liquid cultures of incubation, 3) RT-control (without reverse transcriptase enzyme), 5) gDNA control (with DNA of SW67); 5) control – (without DNA or RNA template. Amplification of *clsD* (expected product size: 365 bp) after 7d of incubation on 6) MEA plates and 7) MEL standing liquid cultures of incubation, 8) RT-control (without reverse transcriptase enzyme), 9) gDNA control (with DNA of SW67); 10) control – (without DNA or RNA template. Amplification of *clsF* (expected product size: 211 bp) after 7d of incubation on 11) MEA plates and 12) MEL standing liquid cultures of incubation, 13) RT-control (without reverse transcriptase enzyme), 14) gDNA control (with DNA of SW67); 15) control – (without DNA or RNA template).

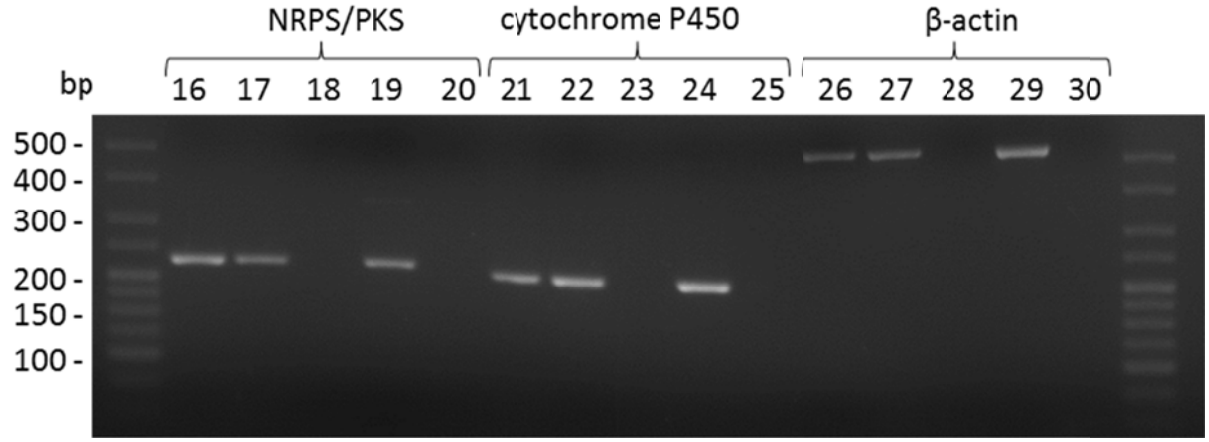

Figure S17. Detection of the *cls* biosynthetic genes and corresponding controls of the housekeeping gene β-actin by conventional reverse transcriptase-PCR, cultivated on agar plates and liquid culture for 7 d in the dark (25 °C). Amplification of *clsI* (expected product size: 226 bp) after 7 d of incubation on 16) MEA plates and 17) MEL standing liquid cultures of incubation, 18) RT-control (without reverse transcriptase enzyme), 19) gDNA control (with DNA of SW67); 20) control – (without DNA or RNA template). Amplification of *clsK* (expected product size: 216 bp) after 7d of incubation on 21) MEA plates and 22) MEL standing liquid cultures of incubation, 23) RT-control (without reverse transcriptase enzyme), 24) gDNA control (with DNA of SW67); 25) control – (without DNA or RNA template). Amplification of the housekeeping gene β-actin (expected product size: 527 bp) after 7d of incubation on 26) MEA plates and 27) MEL standing liquid cultures of incubation, 28) RT-control (without reverse transcriptase enzyme), 29) gDNA control (with DNA of SW67); 30) control – (without DNA or RNA template).

4.7 Semi preparative scale cultivation and extraction

4.7.1 Liquid cultivation

500 mL cultures (in 2 L flasks) were inoculated each with a 100 µL aliquot of a turbid fungal spore suspension of SW67 in PD broth. Cultures were left standing in the dark for 14 days. The culture was centrifuged under 6000 rpm with 10 min at 4 °C to separate the cell pellet from supernatant. The cell pellet was extracted using 50 mL MeOH in the ultrasonic bath for 10 min, and corresponding MeOH extract was obtained by centrifugation (6000 rpm, with 10 min at 4 ^{o}C), and mixed with supernatant, and loaded onto conditioned SPE C18 cartridge (10 g) by 10% MeOH. The SPE cartridge was washed by 10% MeOH, and eluted by 50% MeOH and 100% MeOH. After removing the solvent under reduced pressure, the elution was dried completely by SpeedVac. The fraction eluted by 100% MeOH was submitted to UHPLC-MS analysis under the concentration of 1.0 mg/mL in 100% MeOH solution.

4.7.2 Plate cultivation

Over all approximately 400 PDA and 400 MEA plates were inoculated with a 100 µL aliquot of a turbid fungal spore suspension of SW67 in sterile PBS. The suspension was evenly distributed over the agar surface, and the plates were incubated at 25 °C in the dark for 14d. The agar was then cut into squares, consolidated, and soaked overnight in MeOH. The MeOH phase was filtered. Then, the solvent was removed under reduced pressure to obtain the crude MeOH extract.

4.8 Extraction and isolation of metabolites

Methanolic extracts (6 g) were suspended in distilled water (250 mL) and the successively partitioned with hexane (A), dichloromethane (MC, CH_2Cl_2), and ethyl acetate (EtOAc) yielding 0.11, 0.21, and 0.14 g of organic extracts, respectively. Extracts were analyzed using LC-MS to identify the fractions containing the target ions.

4.9 Isolation of compounds from MC extract

MC extracts were found to contain the major amount of the respective target ion. Organic extracts (0.21 g) were dissolved in MeOH and separated by preparative reversed-phase HPLC with a gradient solvent system of MeOH:H_2O (4:6→8:2, flow rate: 5 mL/min), using an Agilent Eclipse XDB-C18 column (250 mm × 21.2 mm i.d., 7 µm) to yield five fractions (G1 – G5). Fraction G4 (24 mg) was purified by semi-preparative reversed-phase HPLC (45%

MeOH, flow rate 2 mL/min), using a Phenomenex Luna C18(2) column (250 mm × 10 mm i.d., 10 μm) to furnish compounds **1** (0.8 mg, t_R = 36.4 min), **2** (2.6 mg, t_R = 40.6 min) and **3** (1.8 mg, t_R = 50.8 min).

5 Characterization of compounds

5.1 Overview

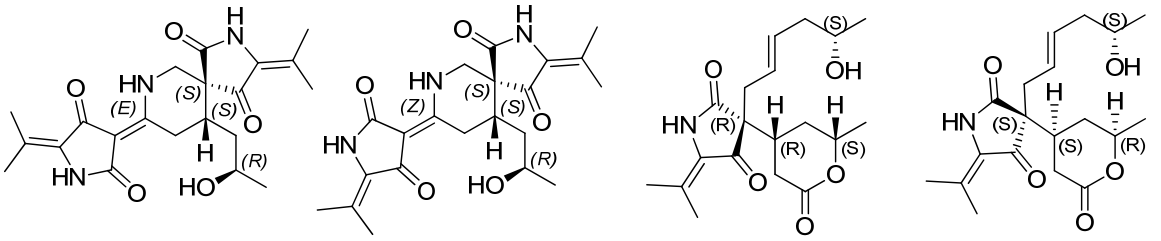

Figure S18. Structures of new compounds isolated from SW67.

Cladosporicin A (**1**): yellowish oil; $[\alpha]^{25,D}$ -15.8 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3371, 2893, 1641, 1512, 1031 cm^{-1}; UV (MeOH) λ_{max} (log ε) 200 (1.8), 245 (3.1), 285 (4.0) nm; ECD (MeOH) λ (Δε) 218 (-1.5), 253 (-3.1), 318 (1.1) nm ^{1}H (800 MHz) and ^{13}C NMR (200 MHz), see Table S7; negative HR-ESI-MS *m/z* 400.1873 $[M-H]^-$ (calcd for $C_{21}H_{26}N_3O_5$, 400.1872)

Cladosporiumin I (**2**): yellowish oil; $[\alpha]^{25,D}$ -25.4 (*c* 0.13, MeOH); IR (KBr) ν_{max} 3471, 2973, 2862, 1638, 1515, 1054 cm^{-1}; UV (MeOH) λ_{max} (log ε) 200 (2.8), 234 (4.0), 298 (2.9) nm; ECD (MeOH) λ (Δε) 209 (1.4), 230 (-3.8) nm ^{1}H (800 MHz) and ^{13}C NMR (200 MHz), see Table S8; negative HR-ESI-MS *m/z* 348.1810 $[M-H]^-$ (calcd. for $C_{19}H_{26}NO_5$, 348.1811)

Cladosporiumin J (**3**): yellowish oil; $[\alpha]^{25,D}$ -35.0 (*c* 0.13, MeOH); IR (KBr) ν_{max} 3460, 2977, 2864, 1636, 1508, 1054 cm^{-1}; UV (MeOH) λ_{max} (log ε) 198 (3.3), 234 (4.0), 298 (2.9) nm; ECD (MeOH) λ (Δε) 207 (-3.2), 234 (2.7) nm ^{1}H (800 MHz) and ^{13}C NMR (200 MHz), see Table S8; negative HR-ESI-MS *m/z* 348.1820 $[M-H]^-$ (calcd for $C_{19}H_{26}NO_5$ 348.1811)

5.1.1 Cladosin B, cinnamic acid and daidzein

The known compounds were identified as cladosin B,[27] cinnamic acid,[30] and daidzein[31] (Figure S19) by comparing the spectroscopic data with previously reported values and ESI-MS data. Daidzein was compared with our house-built UV library, the molecular ion detected in MS, and ^{1}H NMR spectrum.[31] which belongs to the isoflavonoid class.

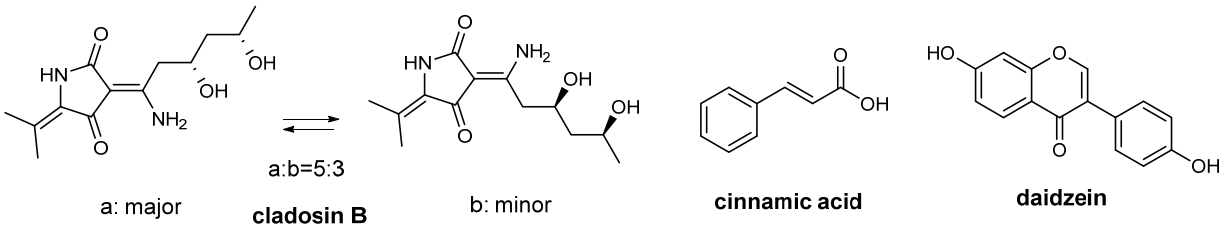

Figure S19. Structures of known compounds isolated from SW67.

5.2 Preparation of Mosher ester derivatives from compounds 2 and 3

Compounds **2** and **3** (each 0.4 mg) dissolved in deuterated pyridine (0.25 mL) were transferred into a clean NMR tube and then a small quantity of 4-(dimethylamino)pyridine was added. (*S*)-(+)-*α*-Methoxy-*α*-(trifluoromethyl) phenylacetyl (MTPA) chloride (8 μL) was transferred into the NMR tubes under a N_2 gas stream and the NMR tubes were shaken carefully to mix the sample with added reagents. The NMR tubes were stored at room temperature for overnight, affording to the (*R*)-MTPA ester derivatives of **2** and **3**. The (*S*)-MTPA ester derivatives of **2** and **3** were also acquired using (*R*)-MTPA chloride according to above described procedure. The ^{1}H NMR and TOCSY spectra were directly obtained from the Mosher ester derivatives of **2** and **3** in the NMR tubes.

(*R*)-MTPA ester of 2:

^{1}H NMR (Pyridine-d_5, 800 MHz) δ 1.21 (H-11), 2.12 (H-9a), 2.23 (H-9b), 2.62 (H-6), 5.56 (H-7); ESIMS *m/z* 588.2 $[M + Na]^+$.

(*S*)-MTPA ester of 2:

^{1}H NMR (Pyridine-d_5, 800 MHz) δ 1.14 (H-11), 2.19 (H-9a), 2.30 (H-9b), 2.68 (H-6), 5.66 (H-7); ESIMS *m/z* 588.2 $[M + Na]^+$.

(*R*)-MTPA ester of 3:

^{1}H NMR (Pyridine-d_5, 800 MHz) δ 1.17 (H-11), 2.07 (H-9a), 2.21 (H-9b), 2.57 (H-6), 5.51 (H-7); ESIMS *m/z* 588.2 $[M + Na]^+$.

(*S*)-MTPA ester of 3:

^{1}H NMR (Pyridine-d_5, 800 MHz) δ 1.10 (H-11), 2.13 (H-9a), 2.27 (H-9b), 2.62 (H-6), 5.60 (H-7); ESIMS *m/z* 588.2 $[M + Na]^+$.

5.3 ECD calculation

To obtain the conformational differences of **1a-1d**, **2a-2d**, and **3a-3d**, computational DFT calculations were performed. The first structural energy minimization of above possible enantiomers was carried out by using Avogadro 1.2.0 with the UFF force field. And then, the ground-state geometries were acquired by Tmolex 4.3.1 with the DFT settings (B3-LYP functional/M3 grid size), geometry optimization settings (energy 10^{-6} hartree, gradient norm $|dE/dxyz| = 10^{-3}$ hartree/bohr), and the basis set def-SV(P) for all atoms. The calculated ECD data of above optimized structures were obtained at the B3LYP/DFT functional settings with the basis set def2-TZVPP for all atoms. The obtained CD spectra were simulated by overlying each transition, where σ is the width of the band at 1/e height. ΔE_i and R_i are the excitation energies and rotatory strengths for transition *i*, respectively. In the current study, the value of σ was 0.10 eV.

$$\Delta\epsilon(E) = \frac{1}{2.297 \times 10^{-39}} \frac{1}{\sqrt{2\pi\sigma}} \sum_{A}^{i} \Delta E_i R_i e^{[-(E-\Delta E_i)^2/(2\sigma)^2]}$$

5.4 Computational NMR chemical shift calculations for DP4 analysis

Conformational searches were performed using the Tmolex 4.3.1 with the DFT settings (B3-LYP functional/M3 grid size), geometry optimization settings (energy 10^{-6} hartree, gradient norm $|dE/dxyz| = 10^{-3}$ hartree/bohr), and the basis set def-SV(P) for all atoms. NMR shielding constants calculations were performed on the optimized ground state geometries at the DFT B3LYP/def2-TZVPP level of theory.[13] The NMR chemical shifts of the isomers were obtained by Boltzmann averaging the ^{1}H and ^{13}C NMR chemical shifts of the stable conformers at 298.15 K. Chemical shift values were calculated using the equation below where δ_{calc}^{x} is the calculated NMR chemical shift for nucleus *x*, and σ^{o} is the shielding tensor for the proton and carbon nuclei in tetra methylsilane calculated at the DFT B3LYP/def2-TZVPP basis set (Table S9, Figure S53, Figure S54 and Figure S59):[13]

$$\delta_{calc}^{x} = \frac{\sigma^{o} - \sigma^{x}}{1 - \sigma^{o}/10^{6}}$$

The DP4 probability analysis was conducted using an applet available at http://www-jmg.ch.cam.ac.uk/tools/nmr/DP4/.

Table S5. Compounds isolated in this study and their corresponding mass as well as all cladosin-like compounds from other *Cladosporium* sp. strains.

Compound	Producing strain	Mass
Cladosporicin A	*C. sphaerospermum* SW67	400.1951 $[M-H]^-$
Cladosporiumin I	*C. sphaerospermum* SW67	348.1810 $[M–H]^-$
Cladosporiumin J	*C. sphaerospermum* SW67	348.1813 $[M–H]^-$
Cladosin A[14]	*C. sphaerospermum* 2005-01-E3	305.1475$[M+Na]^+$
Cladosin B[14]	*C. sphaerospermum* 2005-01-E3, *C. sphaerospermum* SW67	291.1316 $[M+Na]^+$
Cladosin C and Cladosin D[14]	*C. sphaerospermum* 2005-01-E3	273.1316 $[M+Na]^+$
Cladosin E[14]	*C. sphaerospermum* 2005-01-E3	257.1627 $[M+H]^+$
Cladosin F[15]	*C. sphaerospermum* 2005-01-E3	271.1658 $[M+H]^+$
Cladosin G[15]	*C. sphaerospermum* 2005-01-E3,	269.1494 $[M+H]^+$
Cladosporiumin A[16]	*Cladosporium* sp. SCSIO z0025	350.1969 $[M+H]^+$
Cladosporiumin B[16]	*Cladosporium* sp. SCSIO z0025	350.1969 $[M+H]^+$
Cladosporiumin C[16]	*Cladosporium* sp. SCSIO z0025	350.1969 $[M+H]^+$
Cladosporiumin D[16]	*Cladosporium* sp. SCSIO z0025	276.1210 $[M+Na]^+$
Cladosporiumin E[16]	*Cladosporium* sp. SCSIO z0025	274.1051 $[M+Na]^+$
Cladosporiumin F[16]	*Cladosporium* sp. SCSIO z0025	292.1155 $[M+Na]^+$
Cladosporiumin G[16]	*Cladosporium* sp. SCSIO z0025	276.1205 $[M+Na]^+$
Cladosporiumin H[16]	*Cladosporium* sp. SCSIO z0025	308.1464 $[M+Na]^+$
Cladodionen[17]	*Cladosporium* sp. OUCMDZ-1635	234.1131 $[M+H]^+$
Daidzein	*C. sphaerospermum* SW67	253.05 $[M-H]^-$
Cinnamic acid	*C. sphaerospermum* SW67	147.0 $[M-H]^-$

6 Biological Activities

6.1 Antimicrobial activity testing of crude extracts

A total of eleven isolates were evaluated for their ability to inhibit growth of standard human pathogenic microorganisms (Jena Microbial Resource Collection, Jena, Germany). Isolated fungi were grown in the dark on PDA agar plates for 7 days at room temperature (25 °C). Then, plates were cut into pieces and extracted using 100 % MeOH at 4 °C over night. Extracts were filtered and concentrated under reduced pressure, redissolved in 10% MeOH and loaded on a pre-activated and equilibrated C18 cartridge (100 mg C18, 10% MeOH, mixtures refer to MeOH in ddH_2O). The loaded SPE column was washed with 20% MeOH and metabolites were eluted using 50% MeOH and 100% MeOH. Eluted fractions were combined and dried under reduced pressure

The crude extracts (1 mg/mL) were tested for their ability to inhibit growth of standard human pathogenic microorganisms by the Jena Microbial Resource Collection (Jena, Germany). Indicator strains *Bacillus subtilis* 6633; *Staphylococcus aureus* SG511, *E. coli* SG458, *Pseudomonas aeruginosa* K799/61, *Mycobacterium vaccae* 10670, *Sporobolomyces salmonicolor* 549, *Candida albicans* C.A., *Penicillium notatum* JP36 were used (Figure S1).

SW67 showed particularly high antibacterial activity against *Mycobacterium vaccae* 10670, and was therefore selected for further analysis.

6.2 Antimicrobial activity testing of pure compounds

A total of 1 mg of compounds **2** and **3** were tested to inhibit growth of standard human pathogenic microorganisms as described above. None of the compounds showed antimicrobial activity against the tested pathogens.

6.3 *In Vitro* cytotoxicity test

A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each isolated compound against four human breast cancer cell lines (Bt549, HCC70, MDA-MB-231 and MDA-MB-468).[18] Each cell line was inoculated into standard 96-well, flat-bottom microplates and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. The attached cells were then incubated with serially diluted isolates. After continuous exposure to the compounds for 72 h, the culture medium was removed from each well and the cells were fixed with 10% cold trichloroacetic acid at 4°C for 1 h. After washing with tap

water, the cells were stained with 0.4% SRB dye and incubated for 30 min at room temperature. The cells were washed with a 1% acetic acid solution, solubilized with 10 mM unbuffered Tris base solution (pH 10.5), and the absorbance was measured at 520 nm using a microtiter plate reader. Etoposide (purity ≥98%, Sigma, USA) was used as a positive control. The half maximal inhibitory concentrations (IC_{50}) of cancer cell growth are expressed as the mean from three independent experiments.

7 Pharmacological Profiling

Table S6. Cytotoxicity of compounds **1-3** against four cultured human breast cancer cell lines using the SRB bioassay in vitro.

Compound	**IC_{50} (μM)[a]**			
	Bt549	HCC70	MDA-MB-231	MDA-MB-468
1	70.88	74.48	75.54	79.36
2	76.18	85.29	82.37	81.44
3	78.96	76.41	79.27	74.64
Etoposide[b]	1.82	1.76	2.27	2.08

[a]IC_{50} value of compounds against each tumor cell line, defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*.

[b]Etoposide served as a positive control.

7.1 Protease inhibition assay

For protease inhibition assay, 20 plates of MEA were inoculated with a 100 µL aliquot of a turbid fungal spore suspension of SW67 in sterile PBS. The suspension was evenly distributed over the agar surface and dried. Plates were incubated at 25 °C in the dark for 14 d. The densely covered agar plates were cut into squares, consolidated, and soaked in MeOH overnight. The solvent was filtered and removed under reduced pressure. The residue was dissolved in 10% MeOH, centrifuged and subjected to SPE on a pre-activated C18 column (Waters, 1 g). After loading, the C18 column was washed with 10%, and then eluted with 30, 40, 50, 60, 70, 80, 90 and 100% MeOH. The solvent of the fractions was removed under reduced pressure and the extract was submitted to UHPLC-MS analysis indicating the target ions m/z = 350.15 $[M+H]^+$ are predominant in the 50, 60 and 70% MeOH fractions.

Protease inhibition assays were performed for different protease classes (papain, trypsin, ficin, thermolysin, pepsin) with azocasein as the substrate. Inhibition assays were performed

according to the protocol of García-Carreño[19] in a reaction buffer containing 25 mM TRIS-HCl, 0.15 M NaCl, pH 7.2 (or pH 3.5 for aspartic protease). The total assay volume was 47 µL buffer with 2 µL of the protease as well as 1 µL of suitable inhibitor or 1 µL of 10 mg/mL stock solution (in 100% MeOH) of the 50, 60 and 70% MeOH fractions from SW67. End concentration of proteases were as follow: 2 mg/mL for papain (Sigma-Aldrich, P4762), 600 µg/mL for ficin (Sigma-Aldrich, F6008), 80 µg/mL for trypsin (Thermo Fisher, 23266), 2.4 mg/ml for pepsin (Sigma-Aldrich, P6887, buffer pH at 3.5) and 2 µg/mL for thermolysin (Sigma-Aldrich, P1512).

Inhibitors were dissolved in 100% MeOH and added to the assay (1 µL) resulting in an end concentration of less than 2% MeOH to avoid false positive inhibition. Concentrations of control inhibitors were prepared according to the protocol: serine protease (trypsin): PMFS (2 mM); cysteine proteases (papain und ficin): iodacetamide (2 mM), asparagine protease (pepsin): pepstatin A (1 µg/mL) (Sigma-Aldrich, P4265), metalloprotease (thermolysin): EDTA (10 mM). In all cases the given concentration of the controls inhibited enzyme activity >95%. Negative controls were performed with protease alone (without inhibitor) and 2 % end concentration MeOH resulting in a reference activity of 100%. Extract absorbance controls were performed using inhibitor (1 µL in MeOH) without protease. Blanks were performed using 49 µL buffer and 1 µL MeOH. Samples were incubated with buffer and suitable inhibitor (or without for negative controls) for 1 h at r.t. and the reaction was started by adding 50 µL of 1 % azocasein substrate and incubated for 1 h at 37 °C. The reaction was quenched with 50 µL of 10% trichloracetic acid (TCA), the separation of the precipitate was accomplished by centrifugation at 6500 g for 5 min. The hydrolyzed substrate supernatant was incubated in an equal volume of 0.5 N NaOH for 5 min and the absorbance was measured at 440 nm. Unfortunately, no protease inhibition could be observed for the purified SW67 fractions.

8 Spectroscopic data

Table S7. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data of **1** in CD_3OD.[a]

Position	(*E*)-1		(*Z*)-1	
	δ_H	δ_C	δ_H	δ_C
2		171.4, s		173.2, s
3		97.5. s		96.4, s
4		187.2, s		185.2, s
5		122.4, s		122.7, s
6		169.2, s		169.7, s
7α[b]	2.86 dd (18.0, 11.0)	28.8, t	2.87 dd (18.0, 11.0)	29.7, t
7β[b]	3.74 dd (18.0, 5.5)		3.78 dd (18.0, 5.5)	
8	2.56 m	32.2, d	2.56 m	32.2, d
9a	1.12 m	41.1, t	1.12 m	41.1, t
9b	1.50 m		1.50 m	
10	3.90 m	64.4, d	3.94 m	64.4, d
11	1.14 d (6.0)	24.5, q	1.15 d (6.0)	24.5, q
12		131.2, s		131.8, s
13	2.24 s	18.7, q	2.23 s	18.7, q
14	1.83 s	21.1, q	1.83 s	21.1, q
16		173.4, s		173.4, s
17		52.3, s		52.2, s
18		199.2, s		199.2, s
19		127.6, s		127.6, s
20	3.64 m	45.5, t	3.64 m	45.5, t
22		130.5, s		130.5, s
23	2.16 s	19.2, q	2.16 s	19.2, q
24	1.89 s	21.5, q	1.89 s	21.5, q

[a] Coupling constants (in parentheses) are in Hz. [b] Coupling contants could only be resolved by the homonuclear J-resolved spectroscopy (JRES) and HSQC-HECADE(see manuscript).

Table S8. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data of **2** and **3** in CD_3OD.[a]

Position	**2**		**3**[b]	
	δ_H	δ_C	δ_H	δ_C
2		175.9, s		176.0, s
3		59.2, s		59.2, s
4		202.1, s		202.6, s
5		127.2, s		127.5, s
6	2.42 m;	37.1, t	2.45 m	37.6, t
7	5.24 dt (15.0, 7.5)	126.7, d	5.27 dt (15.0, 7.5)	126.7, d
8	5.53 dt (15.0, 7.5)	134.3, d	5.53 dt (15.0, 7.5)	134.4, d
9a	1.99 m	44.0, t	2.01 m	44.1, t
9b	2.10 m		2.11 m	
10	3.63 m	69.1, d	3.63 m	69.0, d
11	1.04 d (6.0)	23.3, q	1.04 d (6.0)	23.2, q
12		132.0, s		132.0, s
13	2.17 s	19.8, q	2.18 s	19.8, q
14	1.84 s	22.0, q	1.85 s	21.9, q
15		174.0, s		175.3, s
16a	2.62 dd (18.0, 10.5)	32.0, t	2.42 dd (15.5, 5.4)	31.7, t
16b	2.69 dd (18.0, 7.0)		2.70 dd (15.5, 11.5)	
17	2.46 m	37.9, d	2.48 m	35.1, d
18a	1.46 ddd (13.5, 12.0, 12.0)	33.4, t	1.71 m	31.8, t
18b	1.81 ddd (13.5, 4.0, 2.0)		1.98 m	
19	4.39 m	78.6, d	4.48 m	76.2, d
20	1.31 d (6.0)	22.6, q	1.31 d (6.0)	21.5, q

[a] Coupling constants (in parentheses) are in Hz. [b] ^{13}C NMR data were assigned based on HSQC and HMBC experiments.

Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1148 formula(e) evaluated with 11 results within limits (up to 50 closest results for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
400.1873	400.1872	0.1	0.2	10.5	C21 H26 N3 O5	685.5	0.873	41.77	21	26	3	5
	400.1877	-0.4	-1.0	3.5	C6 H22 N15 O6	699.2	14.535	0.00	6	22	15	6
	400.1864	0.9	2.2	-1.5	C5 H26 N11 O10	699.0	14.312	0.00	5	26	11	10
	400.1886	-1.3	-3.2	15.5	C22 H22 N7 O	685.3	0.601	54.80	22	22	7	1
	400.1891	-1.8	-4.5	8.5	C7 H18 N19 O2	699.9	15.241	0.00	7	18	19	2
	400.1846	2.7	6.7	11.5	C17 H22 N9 O3	692.1	7.408	0.06	17	22	9	3
	400.1904	-3.1	-7.7	2.5	C10 H26 N9 O8	696.2	11.574	0.00	10	26	9	8
	400.1837	3.6	9.0	-0.5	C H22 N17 O8	702.3	17.667	0.00	1	22	17	8

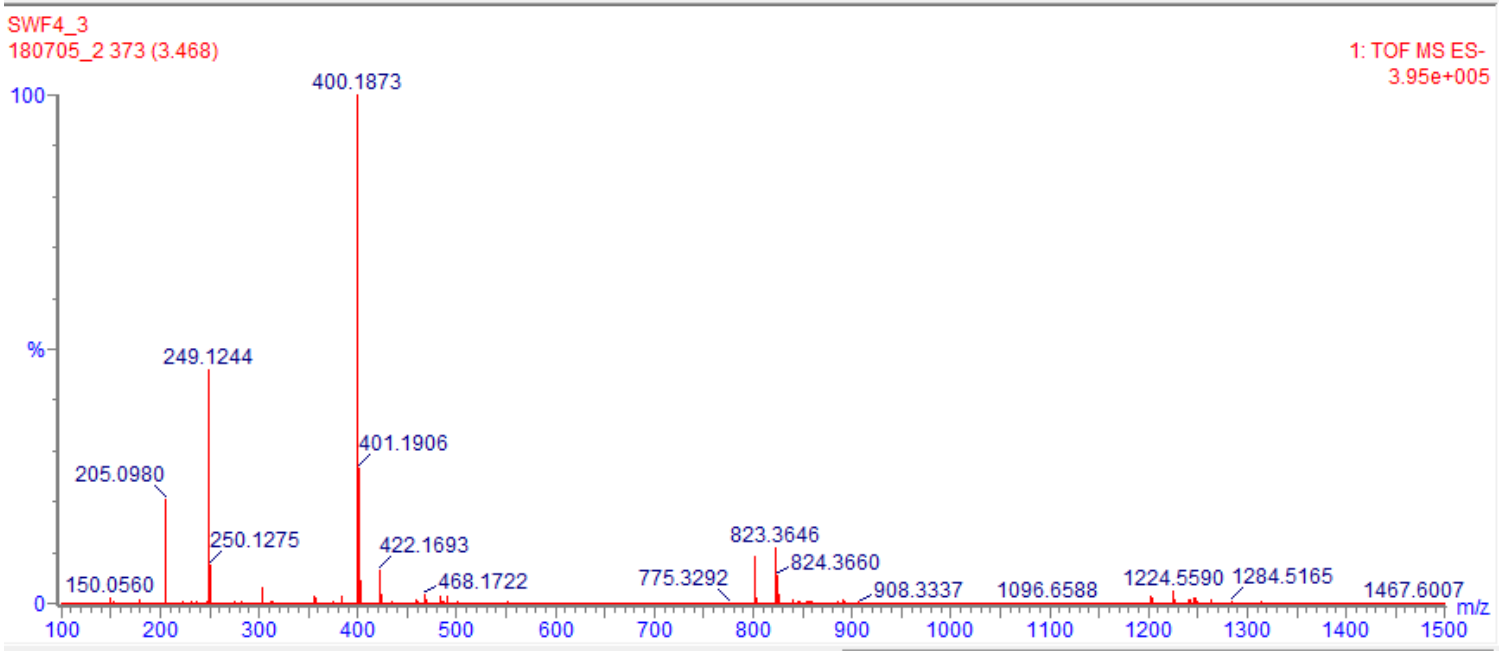

Figure S20. HR-ESIMS data of compound **1**.

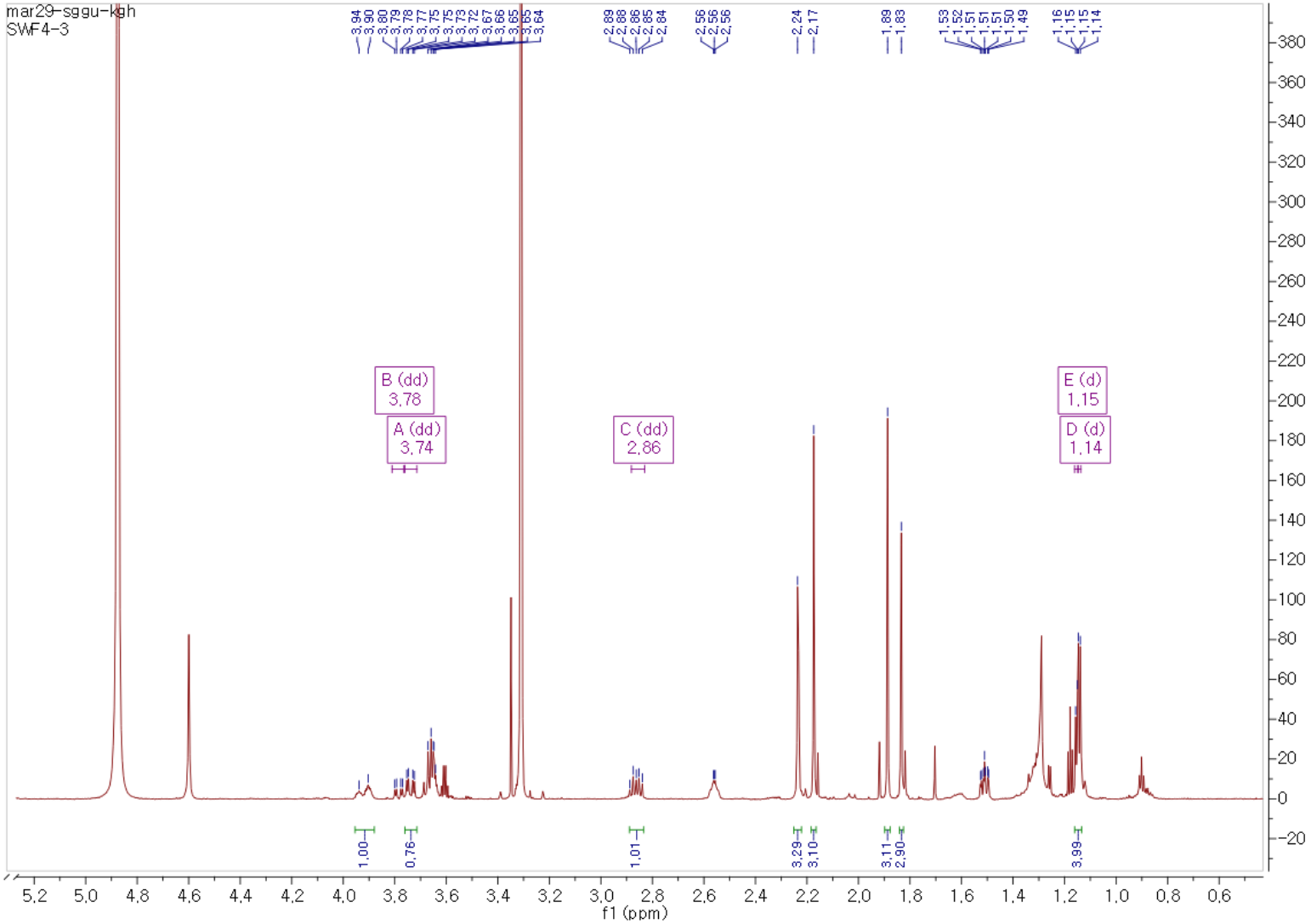

Figure S21. ^{1}H NMR spectrum of compound **1** (CD_3OD, 800 MHz).

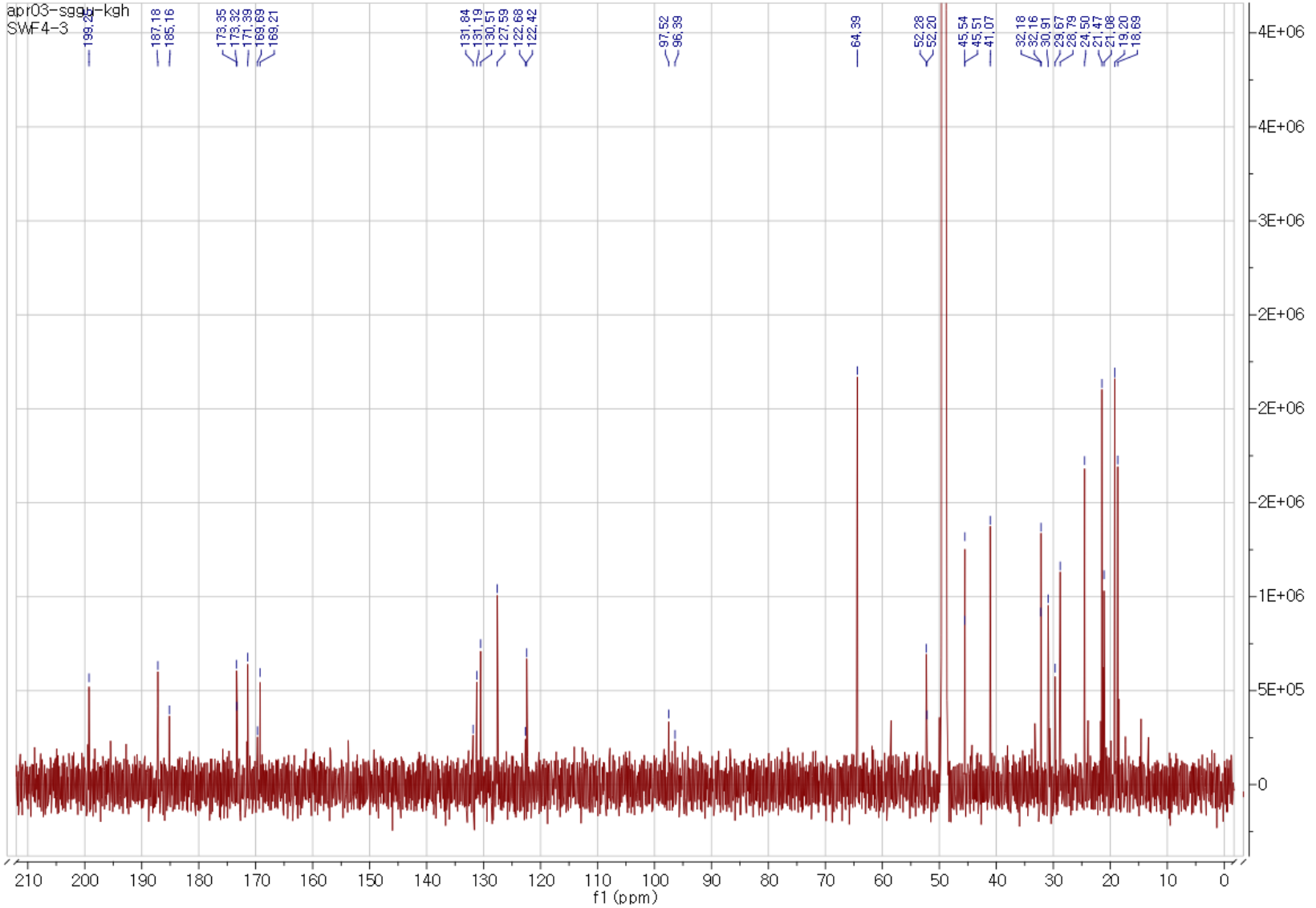

Figure S22. ^{13}C NMR spectrum of compound **1** (CD_3OD, 200 MHz).

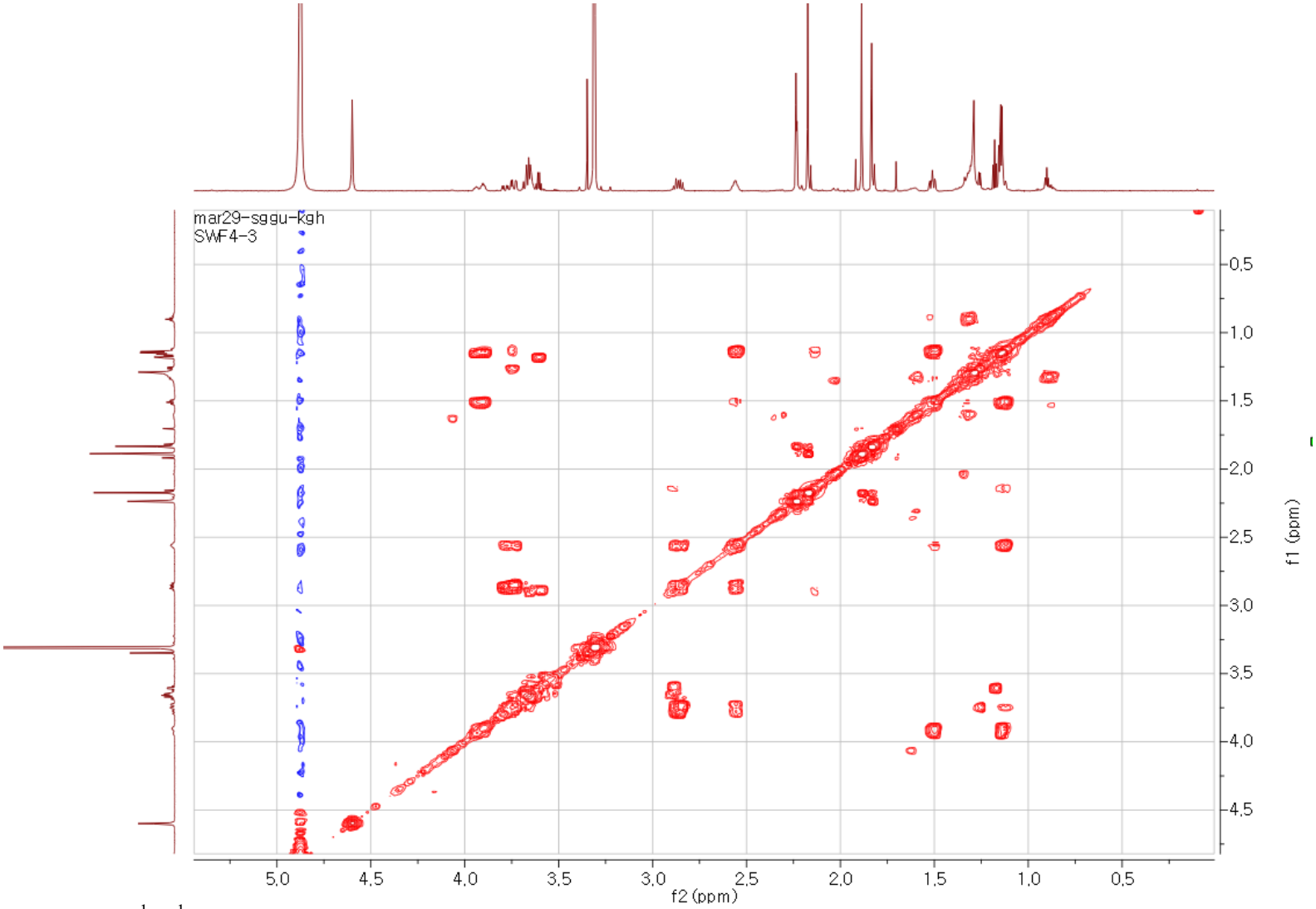

Figure S23. ^{1}H-^{1}H COSY spectrum of compound **1** (CD_3OD).

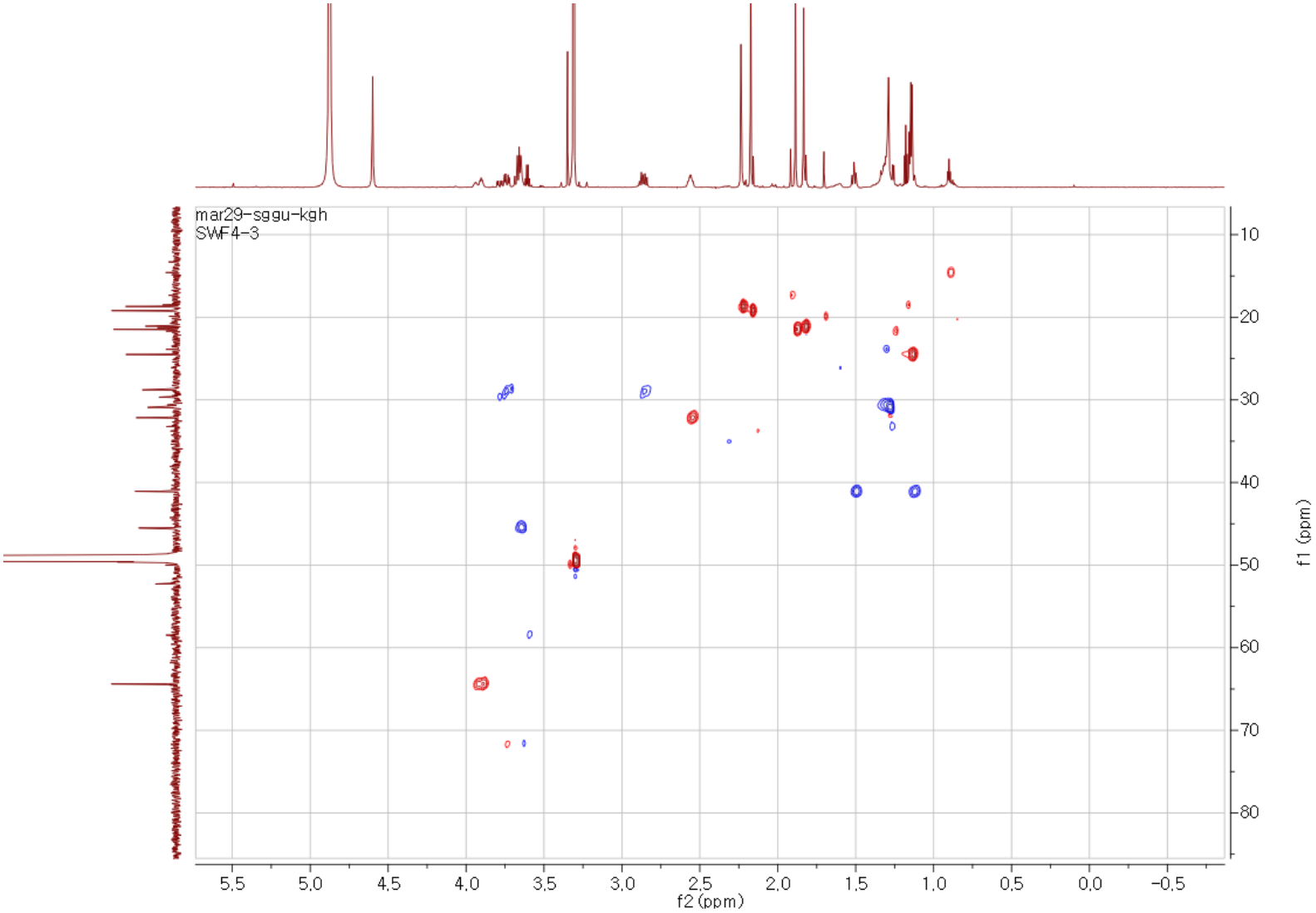

Figure S24. HSQC spectrum of compound **1** (CD_3OD).

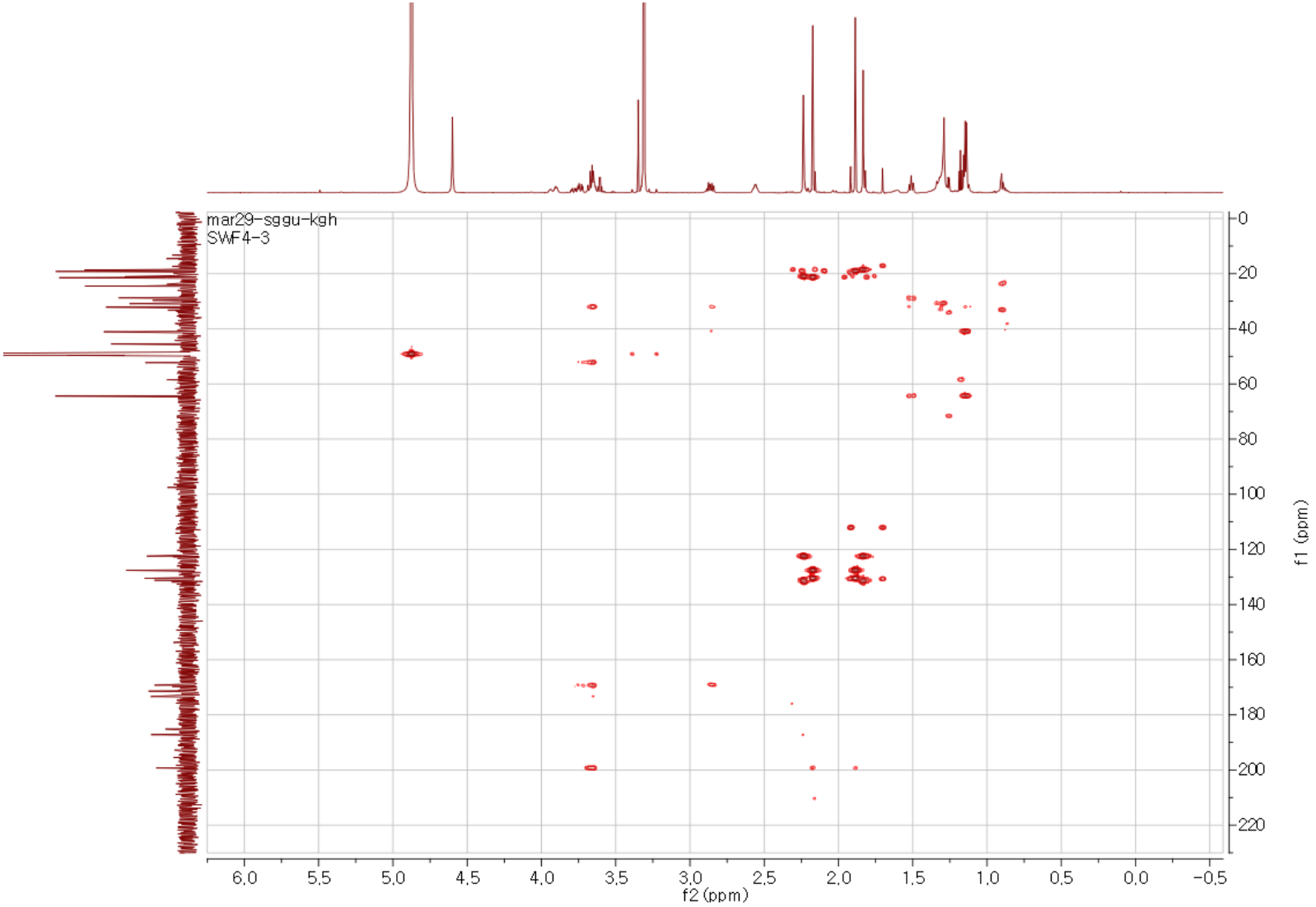

Figure S25. HMBC spectrum of compound **1** (CD_3OD).

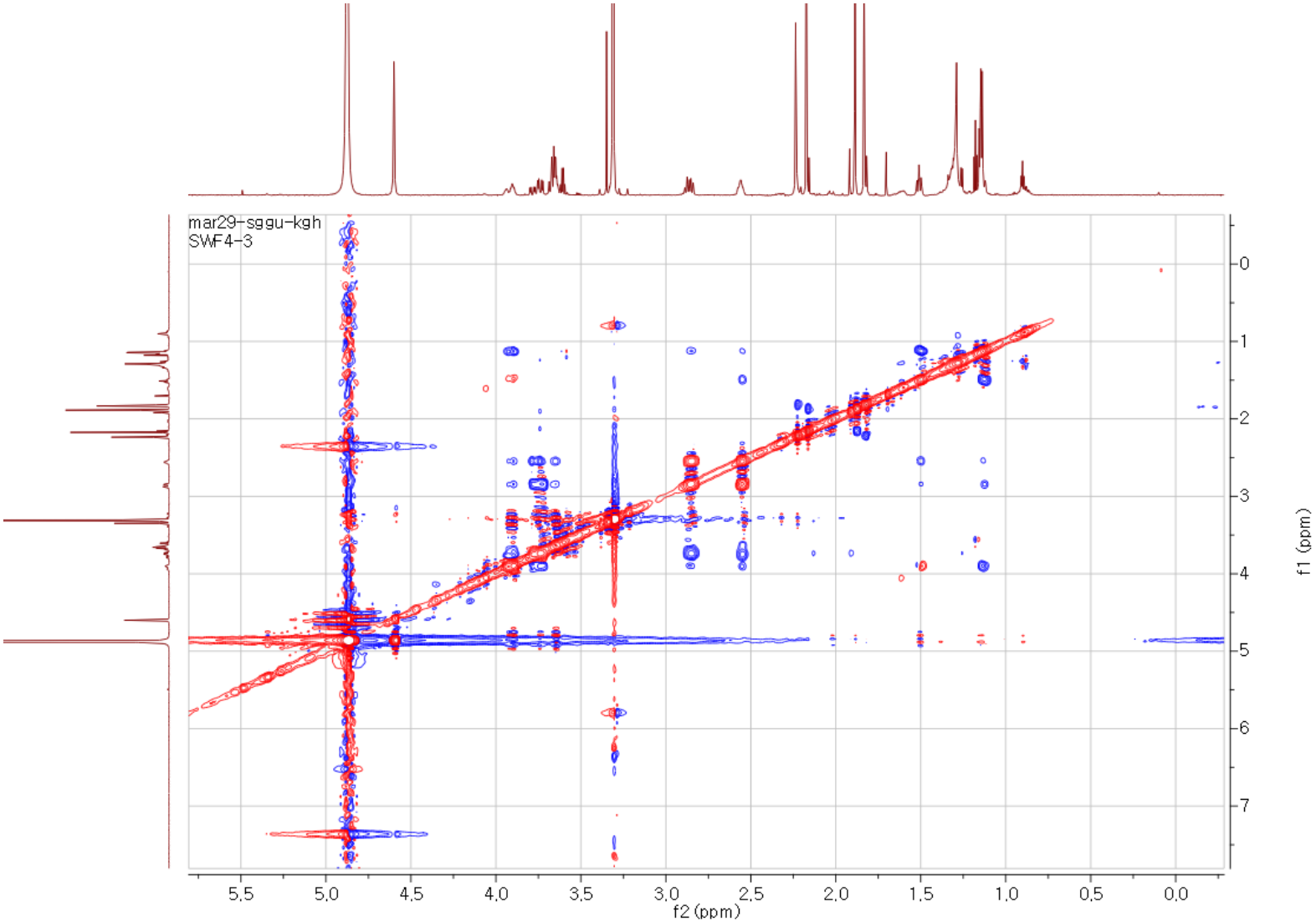

Figure S26. ROESY spectrum of compound **1** (CD_3OD).

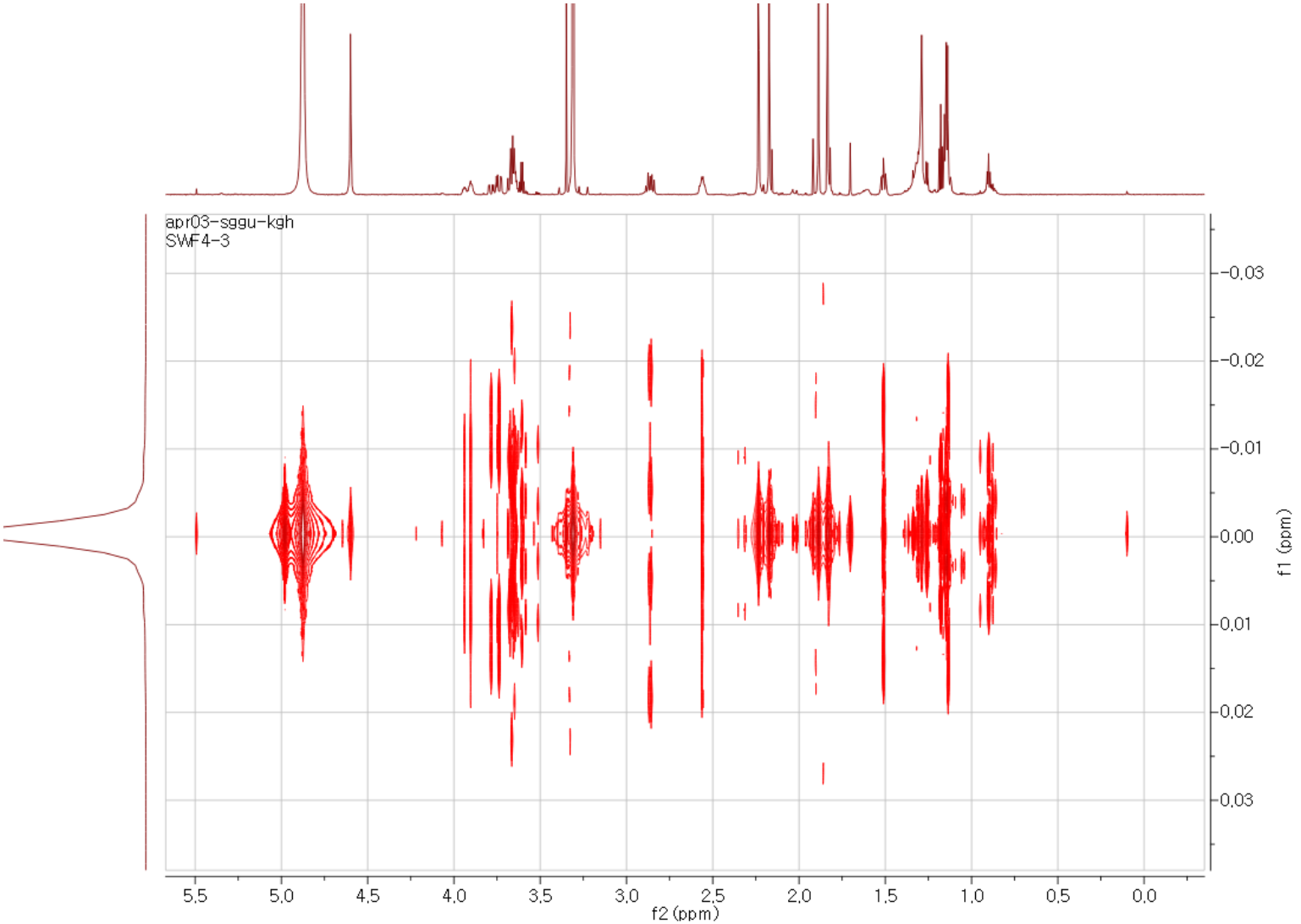

Figure S27. Homonuclear *J*-resolved spectroscopy (JRES) of compound **1**.

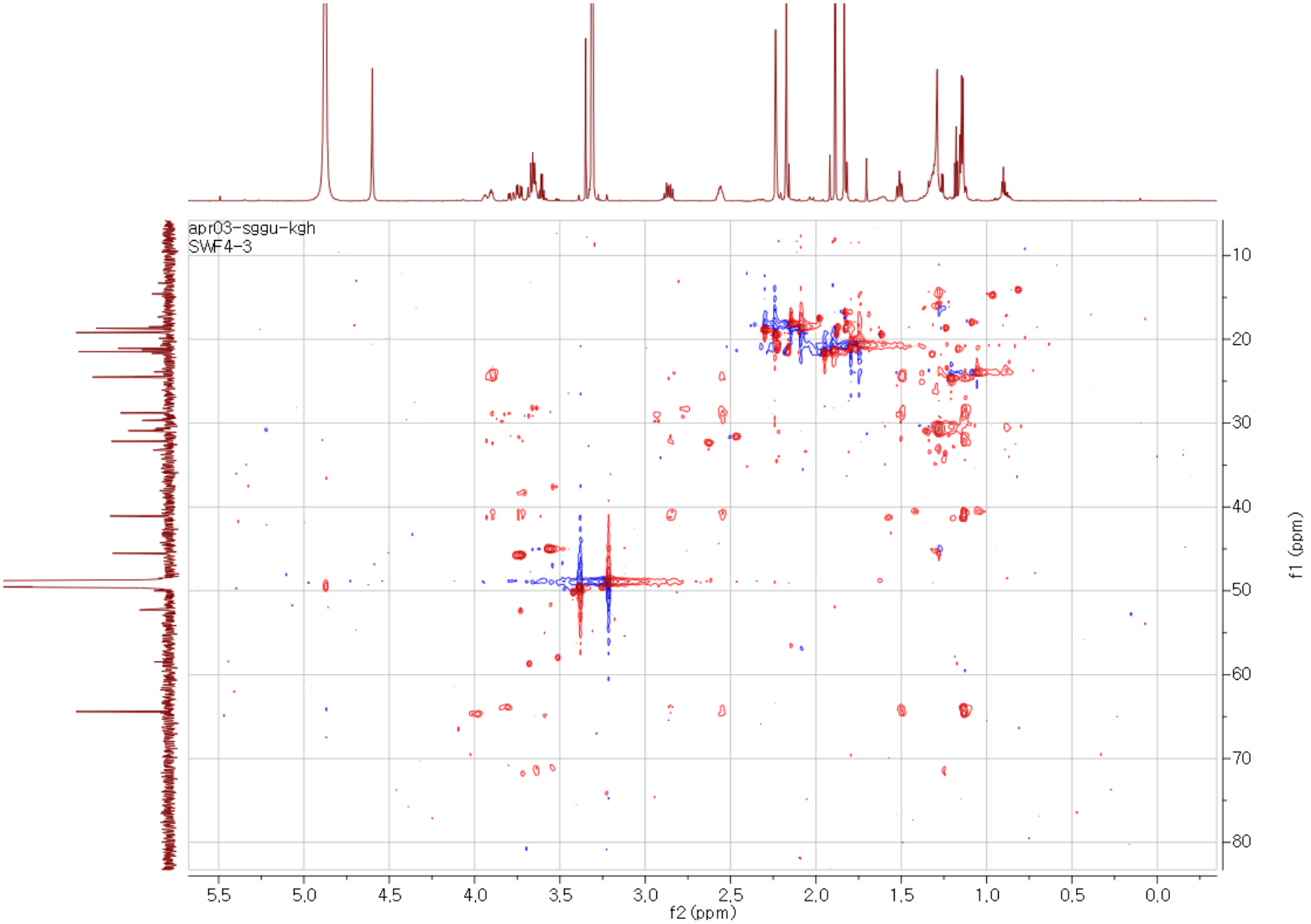

Figure S28. HSQC-HECADE data of compound **1.**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
855 formula(e) evaluated with 8 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
348.1810	348.1811	-0.1	-0.3	7.5	C19 H26 N O5	835.7	0.115	89.15	19	26	1	5
	348.1816	-0.6	-1.7	0.5	C4 H22 N13 O6	848.5	12.912	0.00	4	22	13	6
	348.1803	0.7	2.0	6.5	C H14 N23	853.2	17.602	0.00	1	14	23	
	348.1824	-1.4	-4.0	12.5	C20 H22 N5 O	837.8	2.252	10.52	20	22	5	1
	348.1829	-1.9	-5.5	5.5	C5 H18 N17 O2	849.0	13.370	0.00	5	18	17	2
	348.1789	2.1	6.0	1.5	H18 N19 O4	851.8	16.231	0.00		18	19	4
	348.1784	2.6	7.5	8.5	C15 H22 N7 O3	841.3	5.750	0.32	15	22	7	3
	348.1843	-3.3	-9.5	-0.5	C8 H26 N7 O8	845.2	9.621	0.01	8	26	7	8

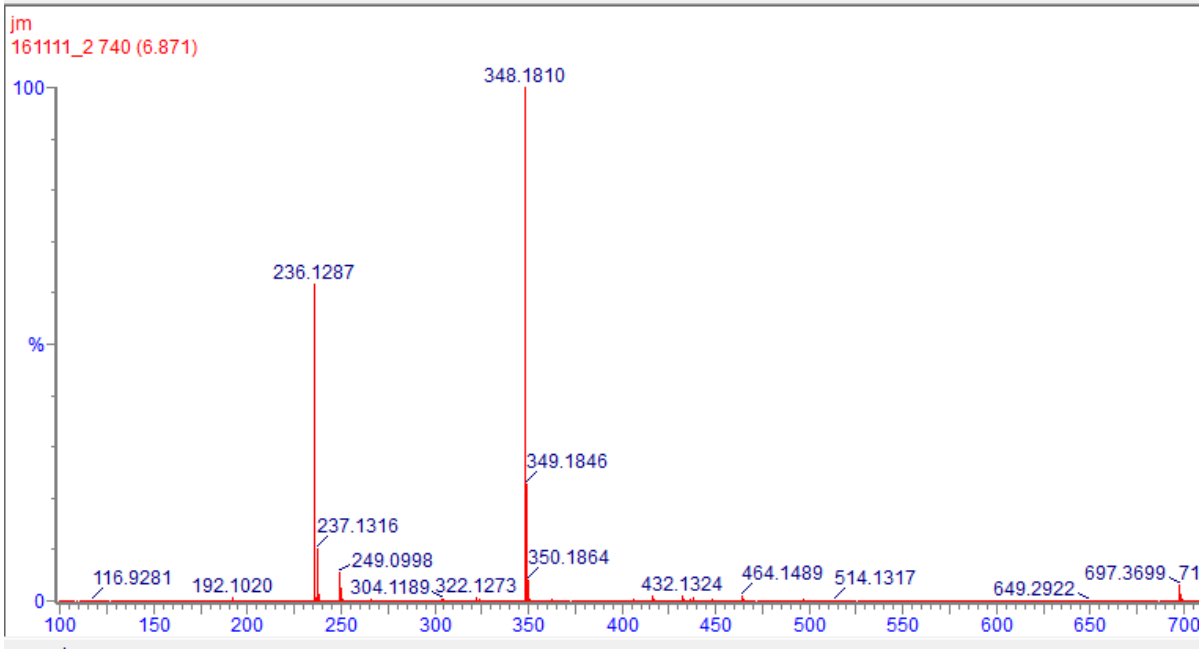

Figure S29. HR-ESIMS of compound **2**.

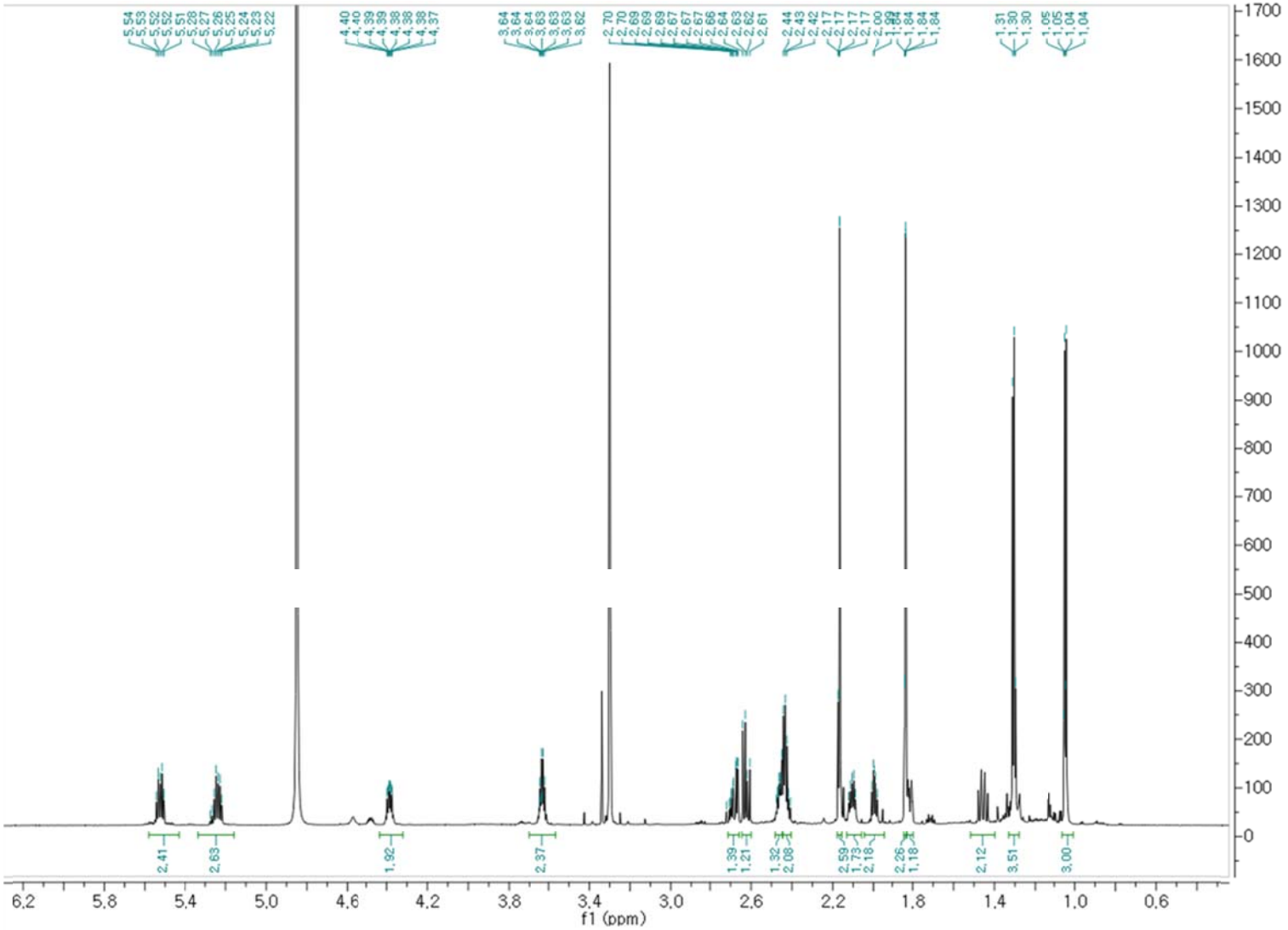

Figure S30. ^{1}H NMR spectrum of compound **2** (CD_3OD, 800 MHz).

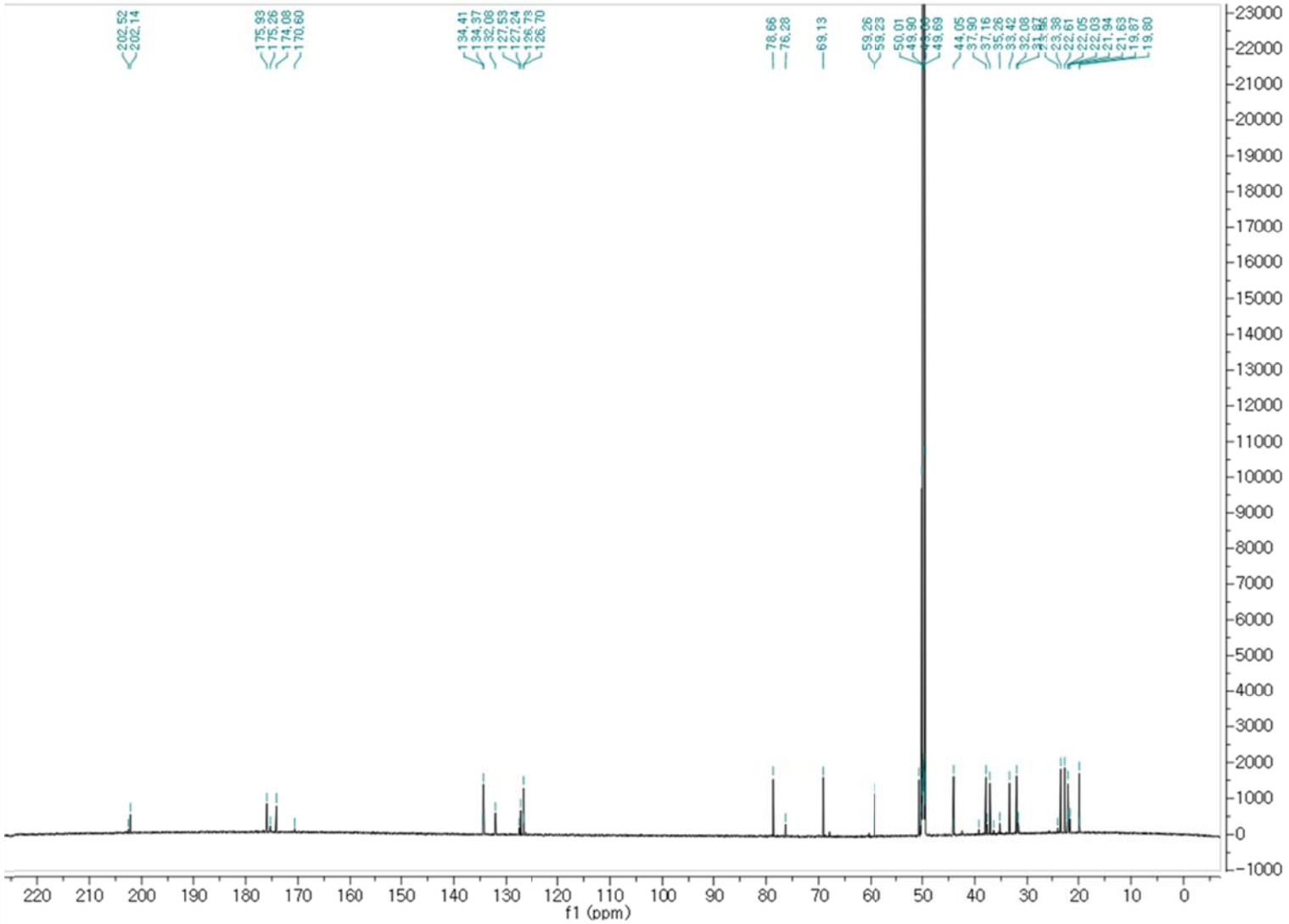

Figure S31. ^{13}C NMR spectrum of compound **2** (CD_3OD, 200 MHz).

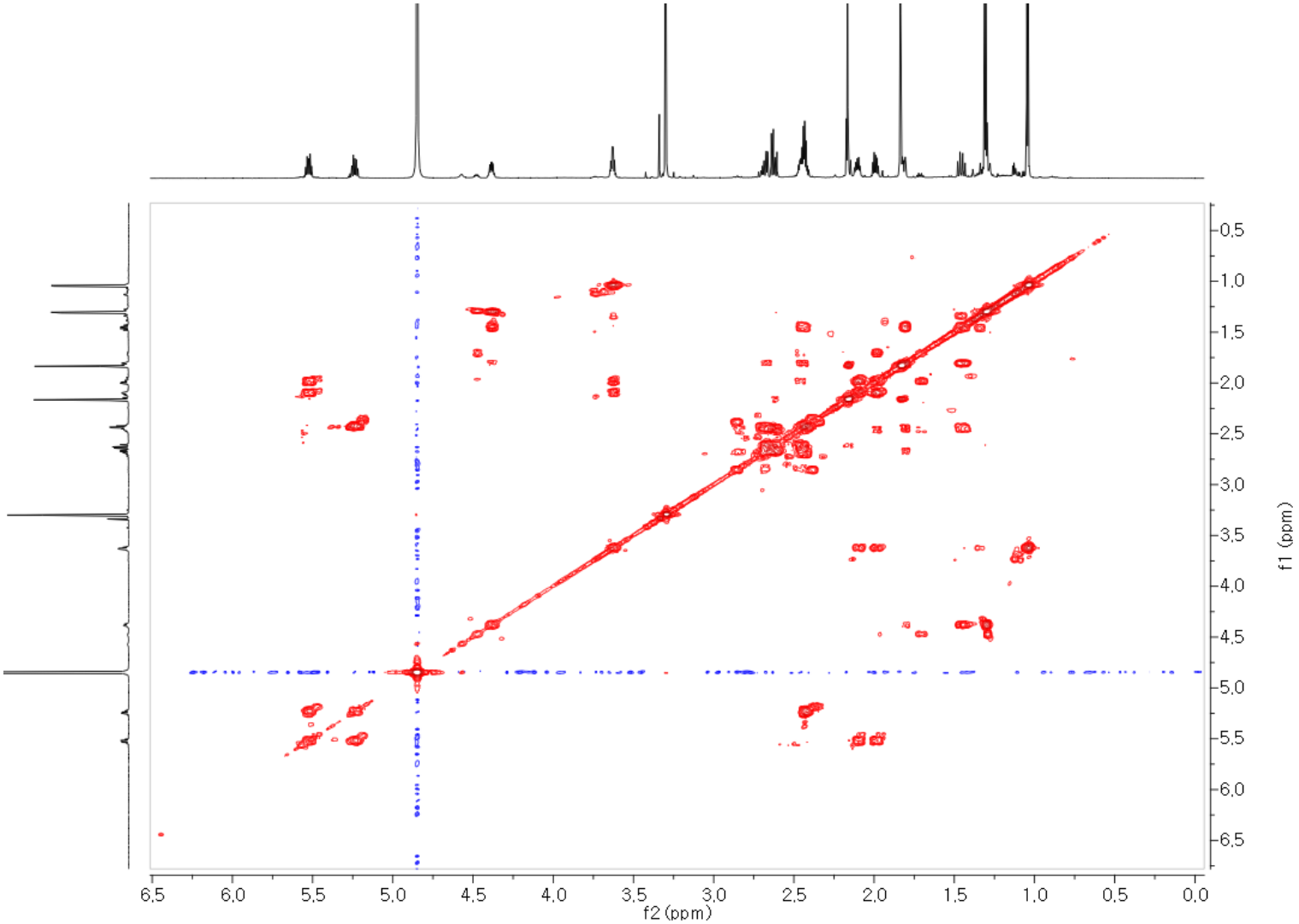

Figure S32. ^{1}H-^{1}H COSY spectrum of compound **2** (CD_3OD).

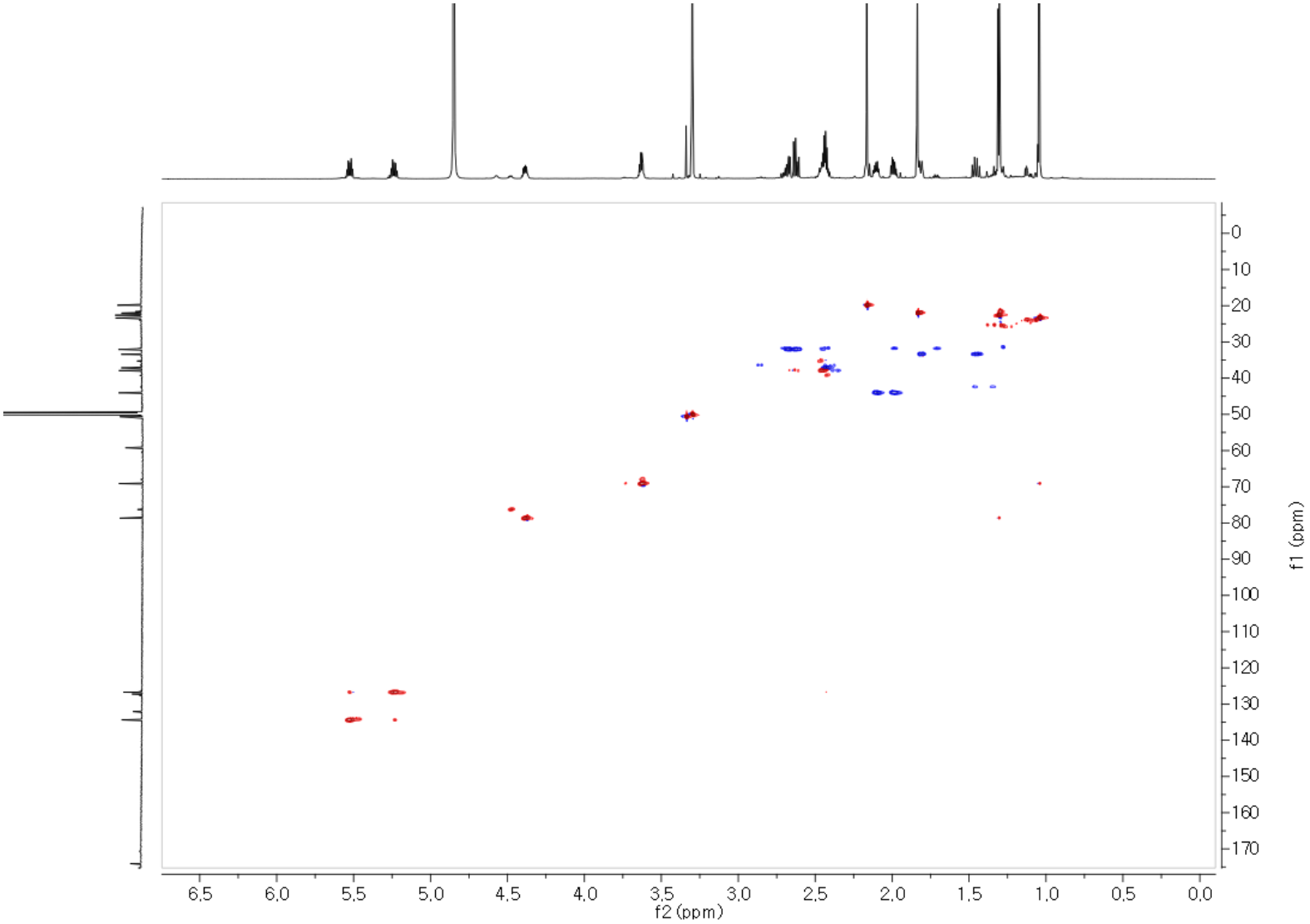

Figure S33. HSQC spectrum of compound **2** (CD_3OD).

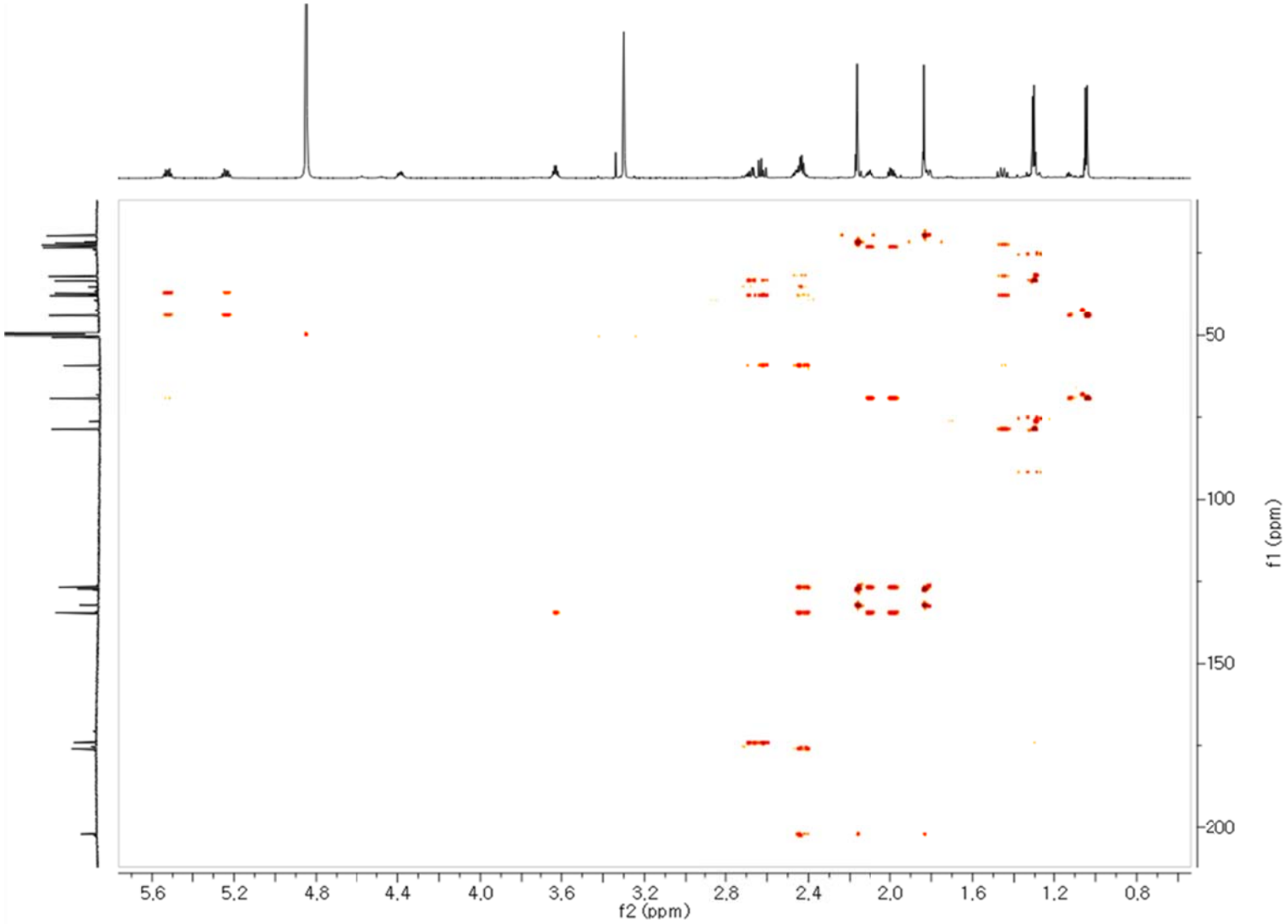

Figure S34. HMBC spectrum of compound **2** (CD_3OD).

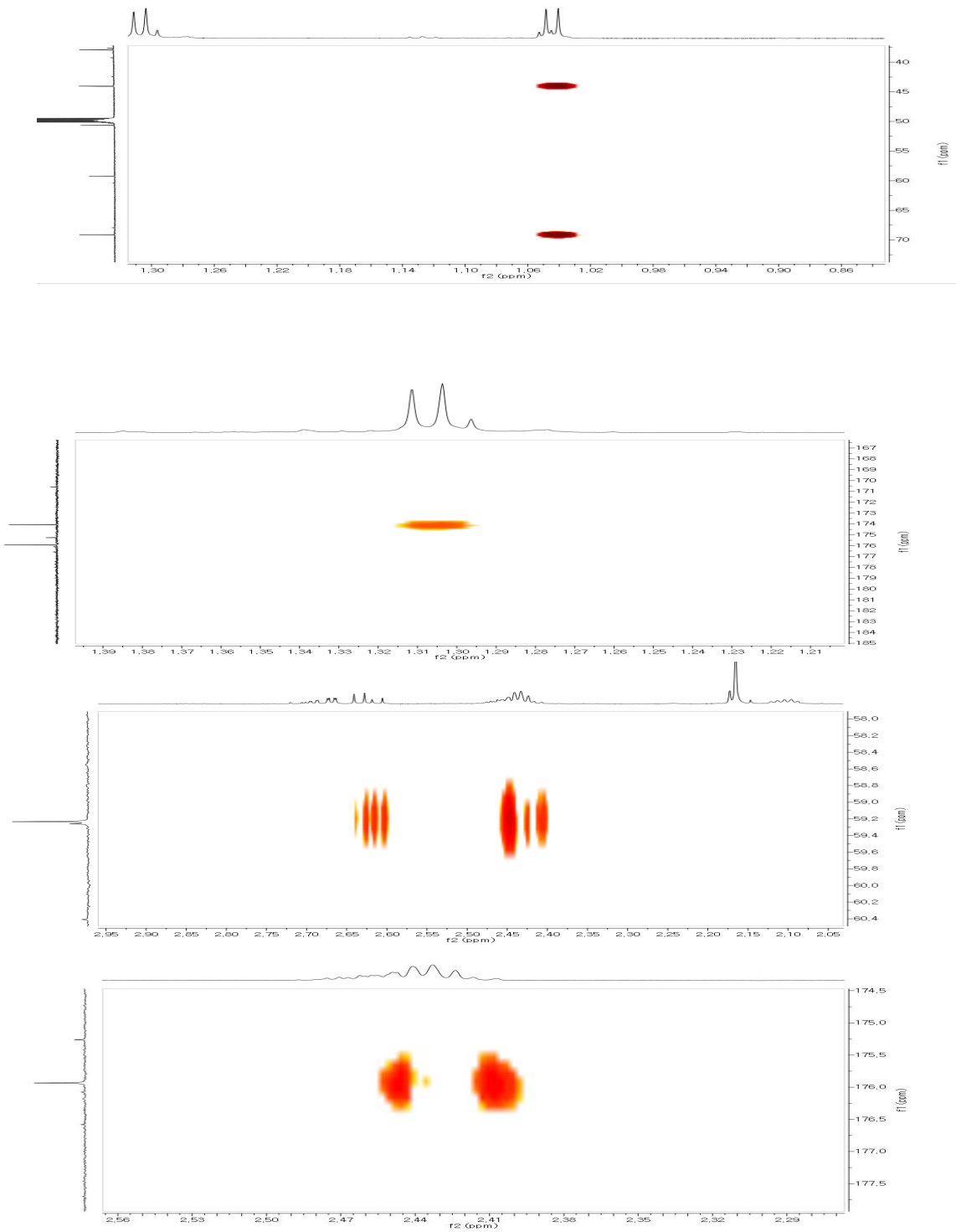

Figure S35. Expanded HMBC spectrum of compound **2**.

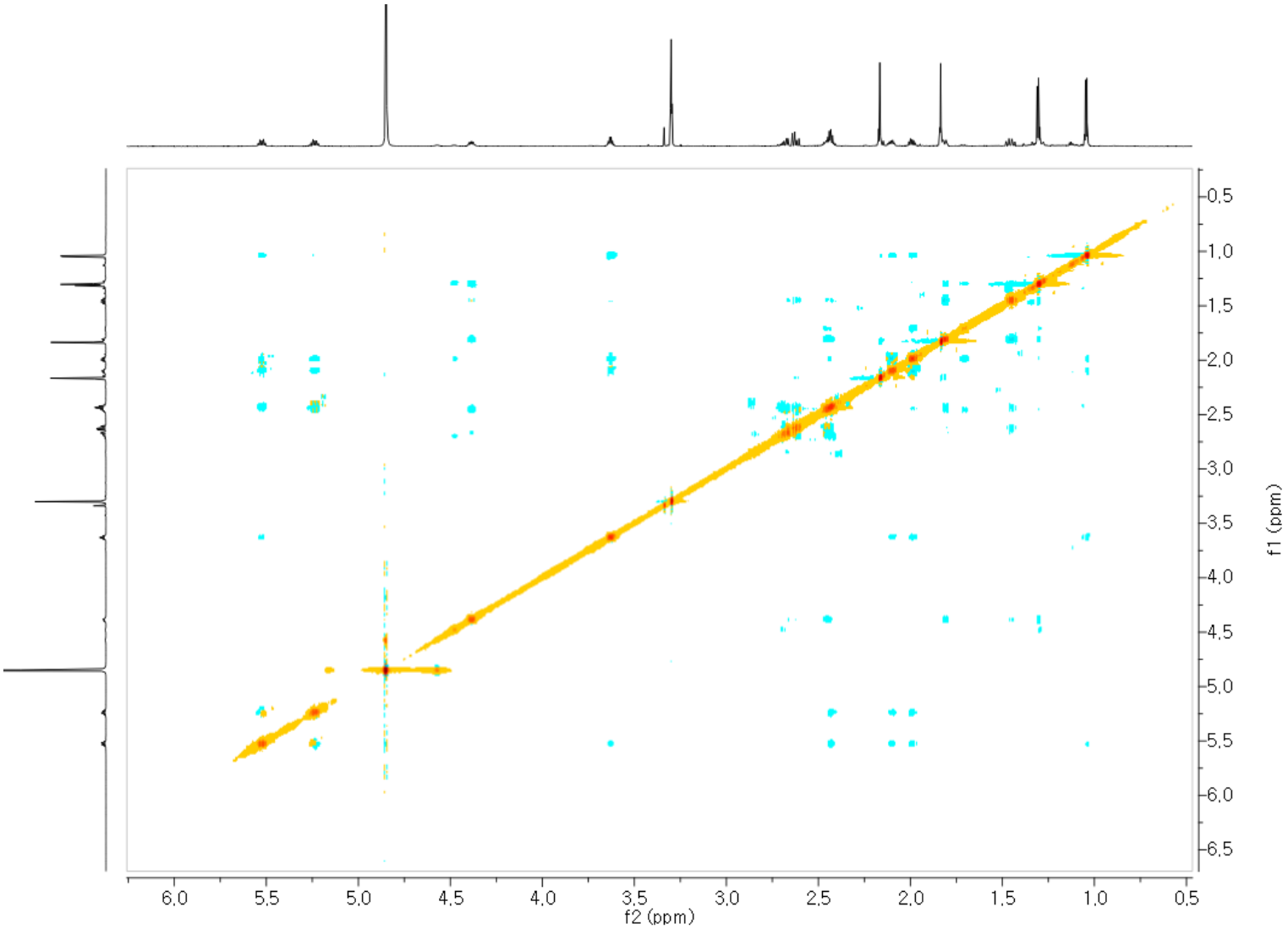

Figure S36. NOESY spectrum of compound **2** (CD_3OD).

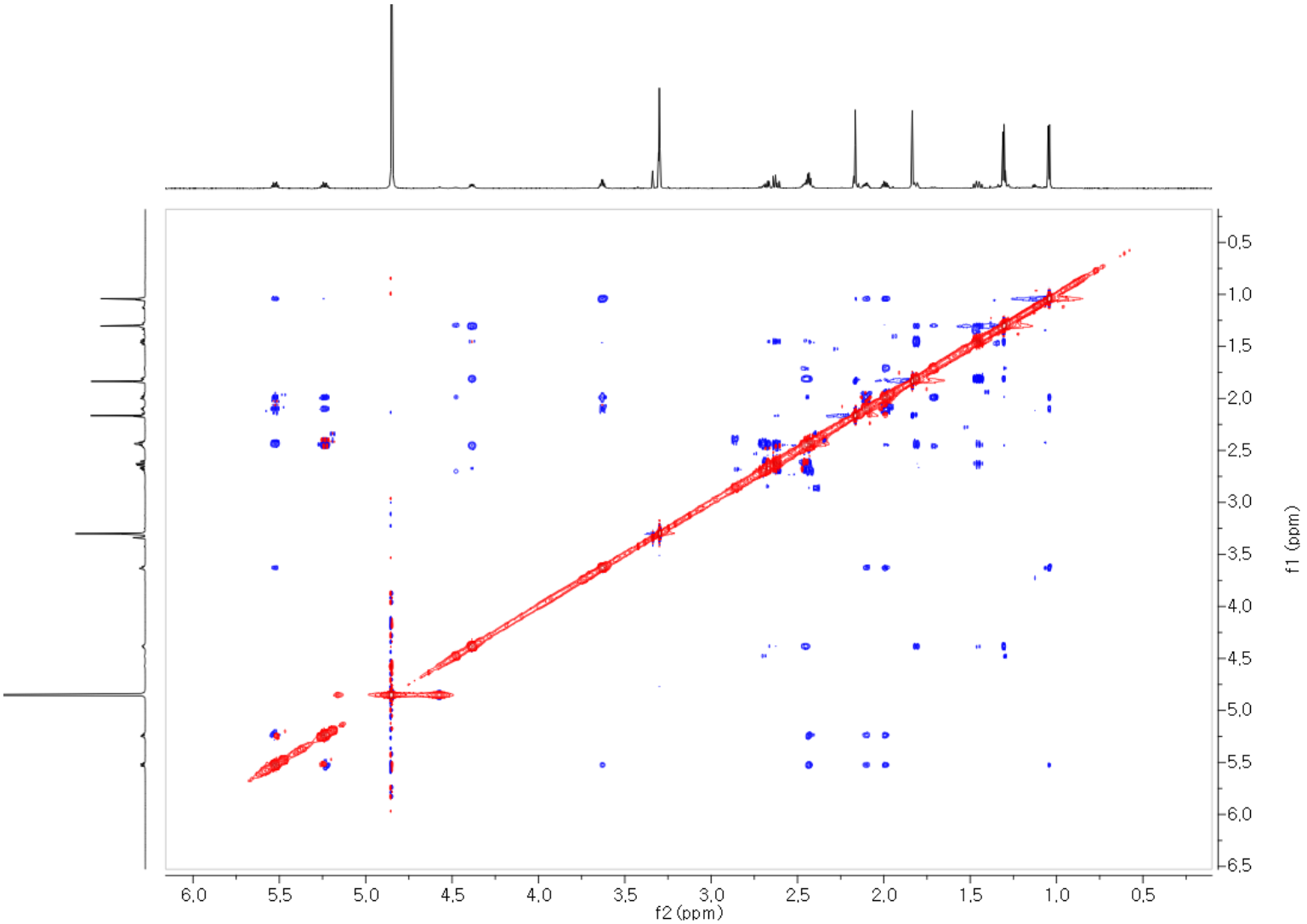

Figure S37. ROESY spectrum of compound **2** (CD_3OD).

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
833 formula(e) evaluated with 11 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
348.1820	348.1824	-0.4	-1.1	12.5	C20 H22 N5 O	1079.7	0.268	76.46	20	22	5	1
	348.1816	0.4	1.1	0.5	C4 H22 N13 O6	1090.2	10.796	0.00	4	22	13	6
	348.1811	0.9	2.6	7.5	C19 H26 N O5	1081.0	1.579	20.61	19	26	1	5
	348.1829	-0.9	-2.6	5.5	C5 H18 N17 O2	1091.1	11.629	0.00	5	18	17	2
	348.1843	-2.3	-6.6	-0.5	C8 H26 N7 O8	1085.9	6.422	0.16	8	26	7	8
	348.1789	3.1	8.9	1.5	H18 N19 O4	1093.8	14.372	0.00		18	19	4
	348.1784	3.6	10.3	8.5	C15 H22 N7 O3	1083.3	3.857	2.11	15	22	7	3
	348.1856	-3.6	-10.3	4.5	C9 H22 N11 O4	1087.2	7.806	0.04	9	22	11	4

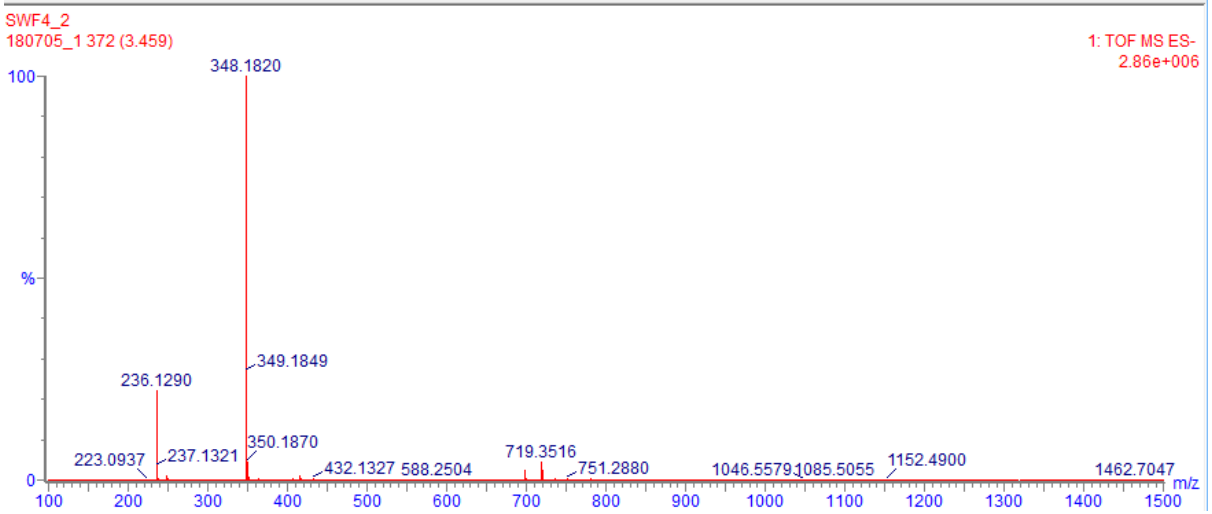

Figure S38. HR-ESIMS of compound **3**.

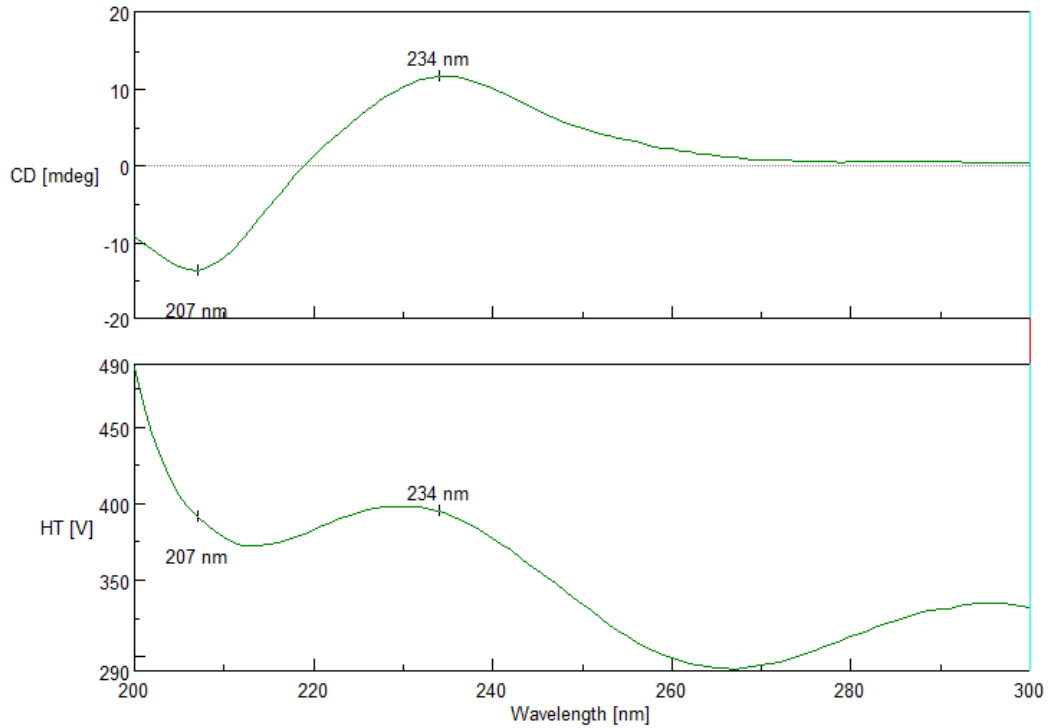

Figure S39. ECD spectrum of compound **3**.

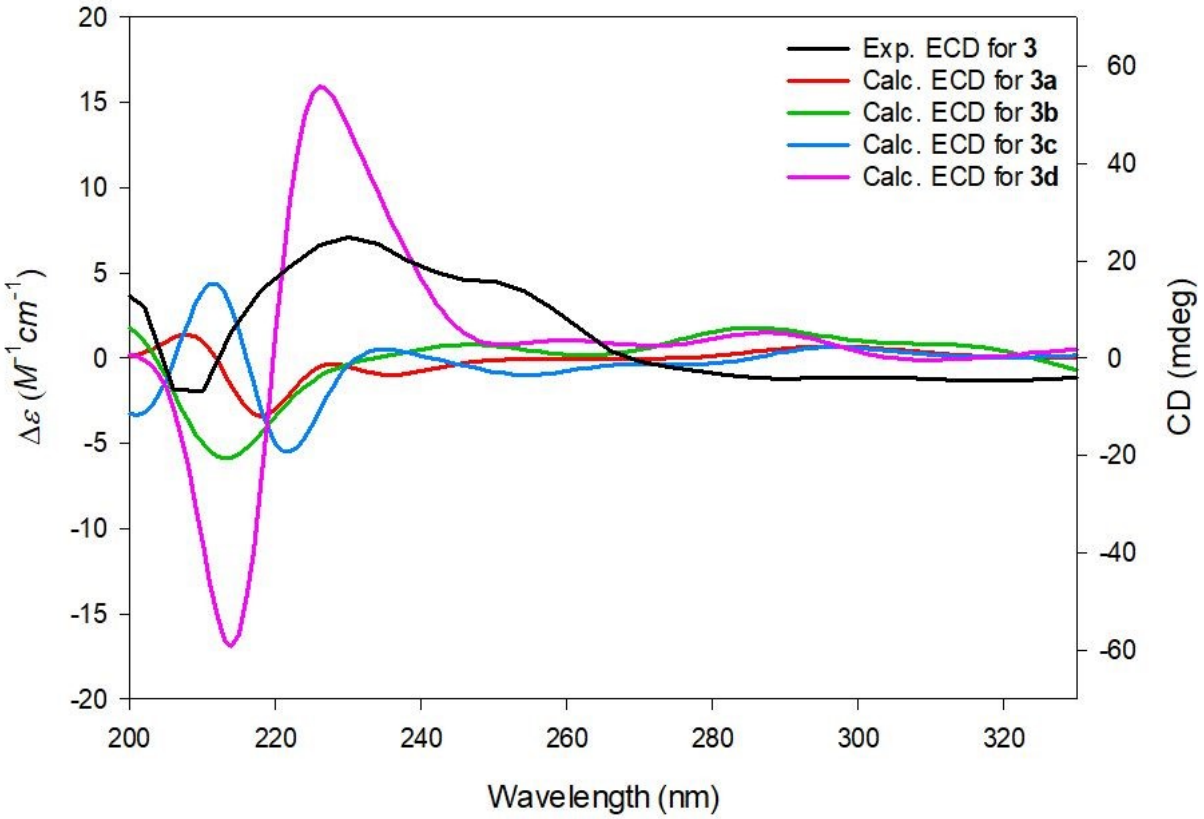

Figure S40. Experimental (black) and calculated ECD spectra of compound **3** [**3a** (3*R*,17*R*,19*S*), **3b** (3*S*,17*R*,19*S*), **3c** (3*R*,17*S*,19*R*), and **3d** (3*S*,17*S*,19*R*)].

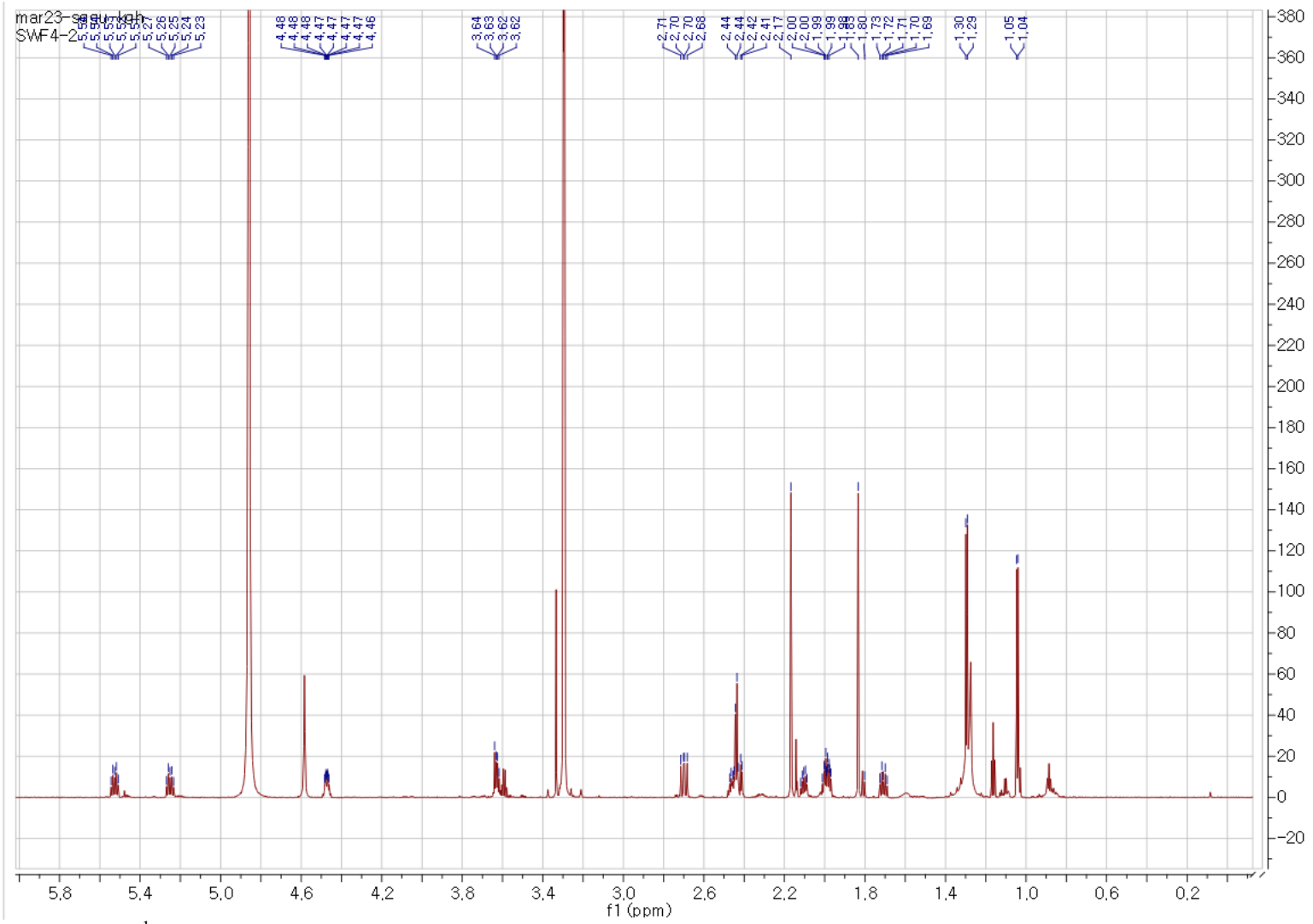

Figure S41. ^{1}H NMR spectrum of compound **3 (**CD_3OD, 800 MHz).

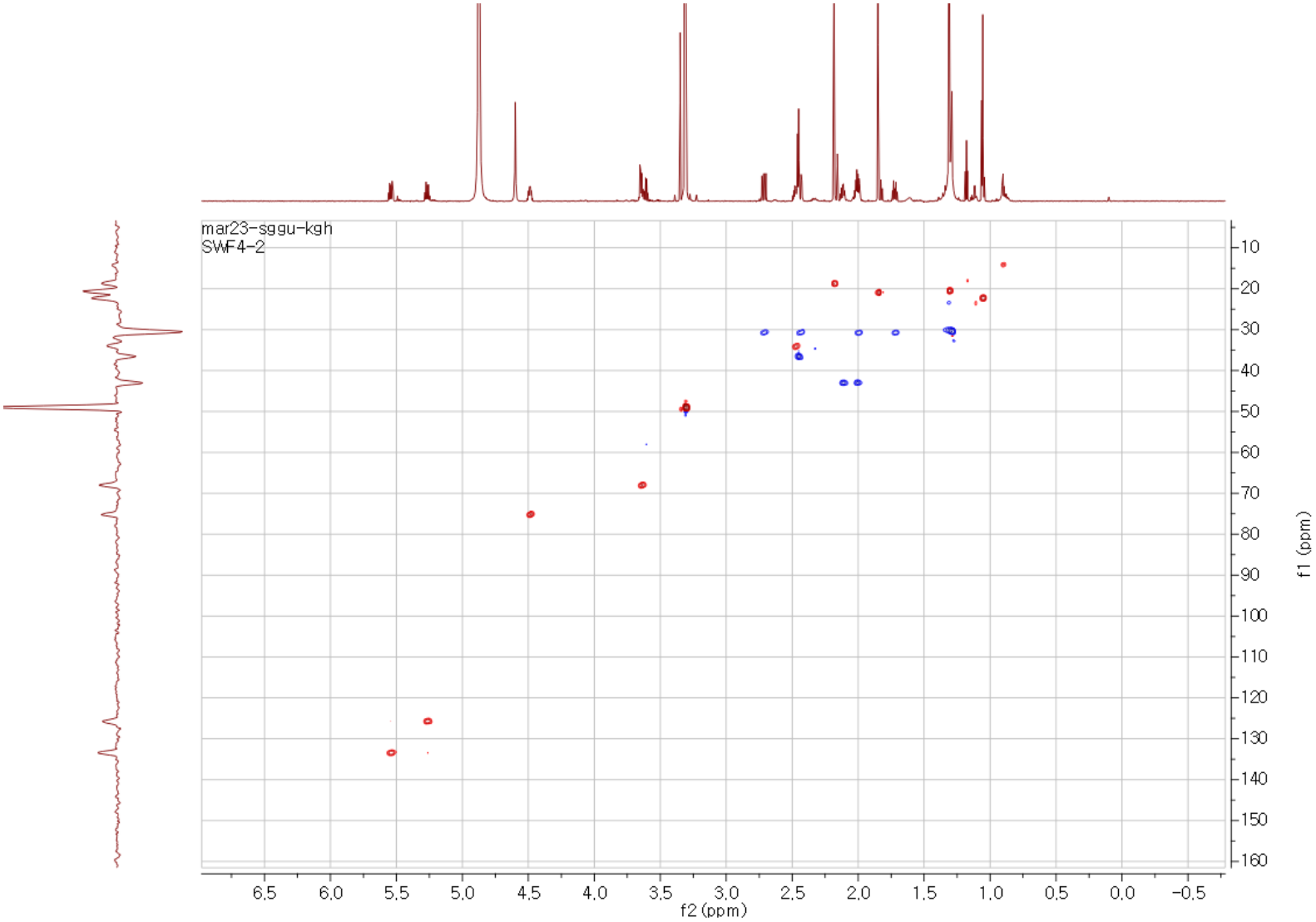

Figure S42. HSQC spectrum of compound **3 (**CD_3OD).

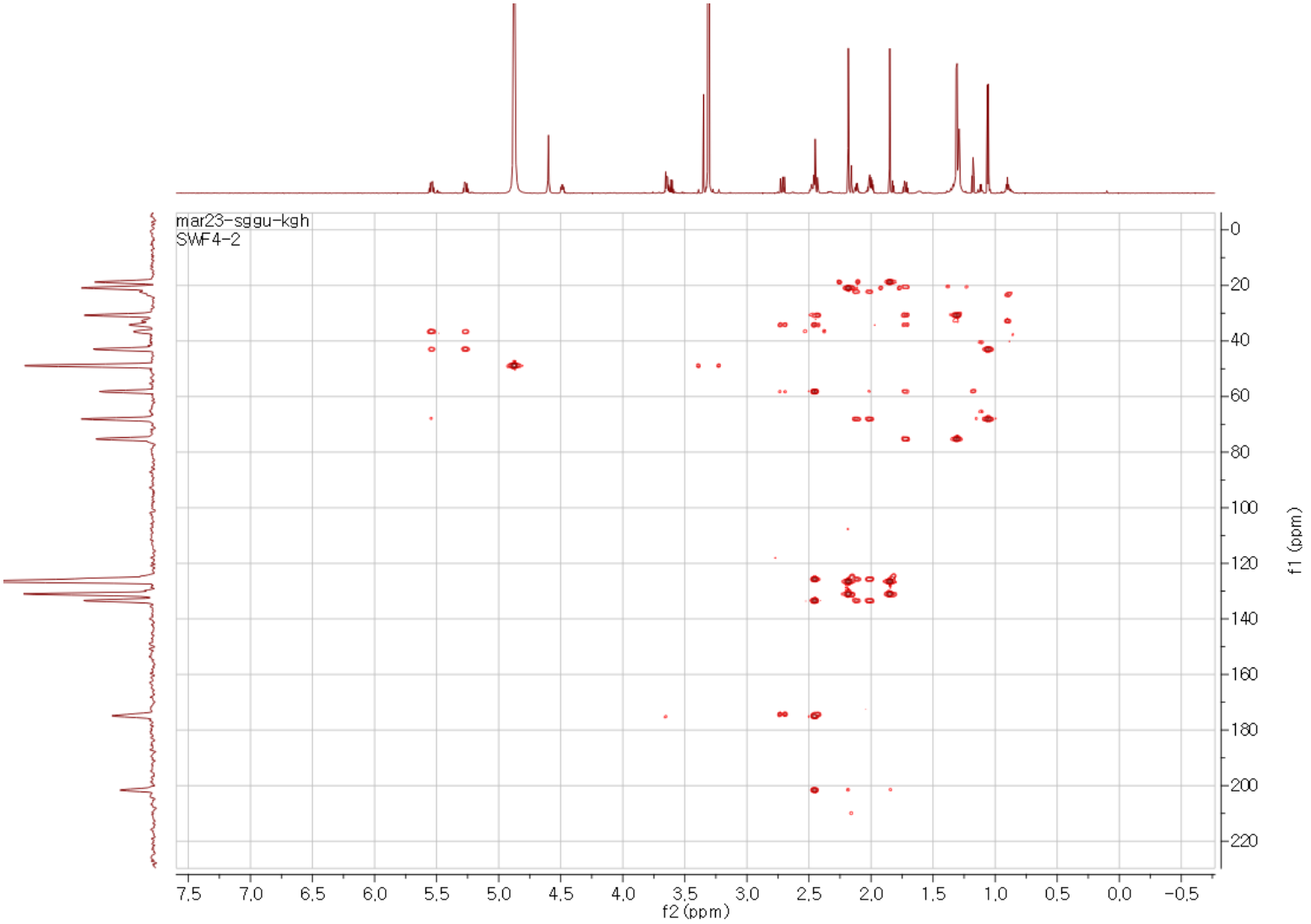

Figure S43. HMBC spectrum of compound **3 (**CD_3OD).

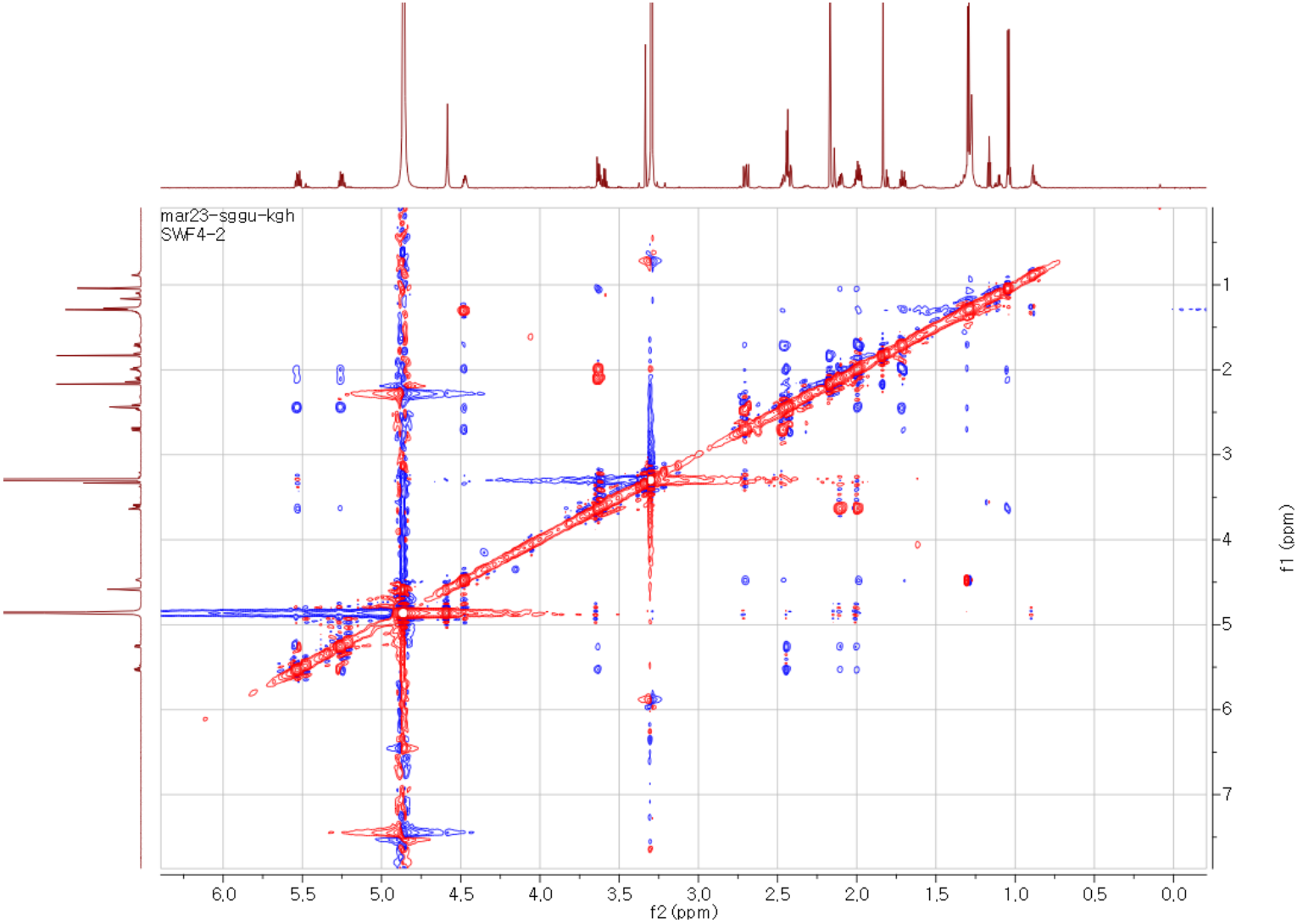

Figure S44. ROESY spectrum of compound **3** (CD_3OD).

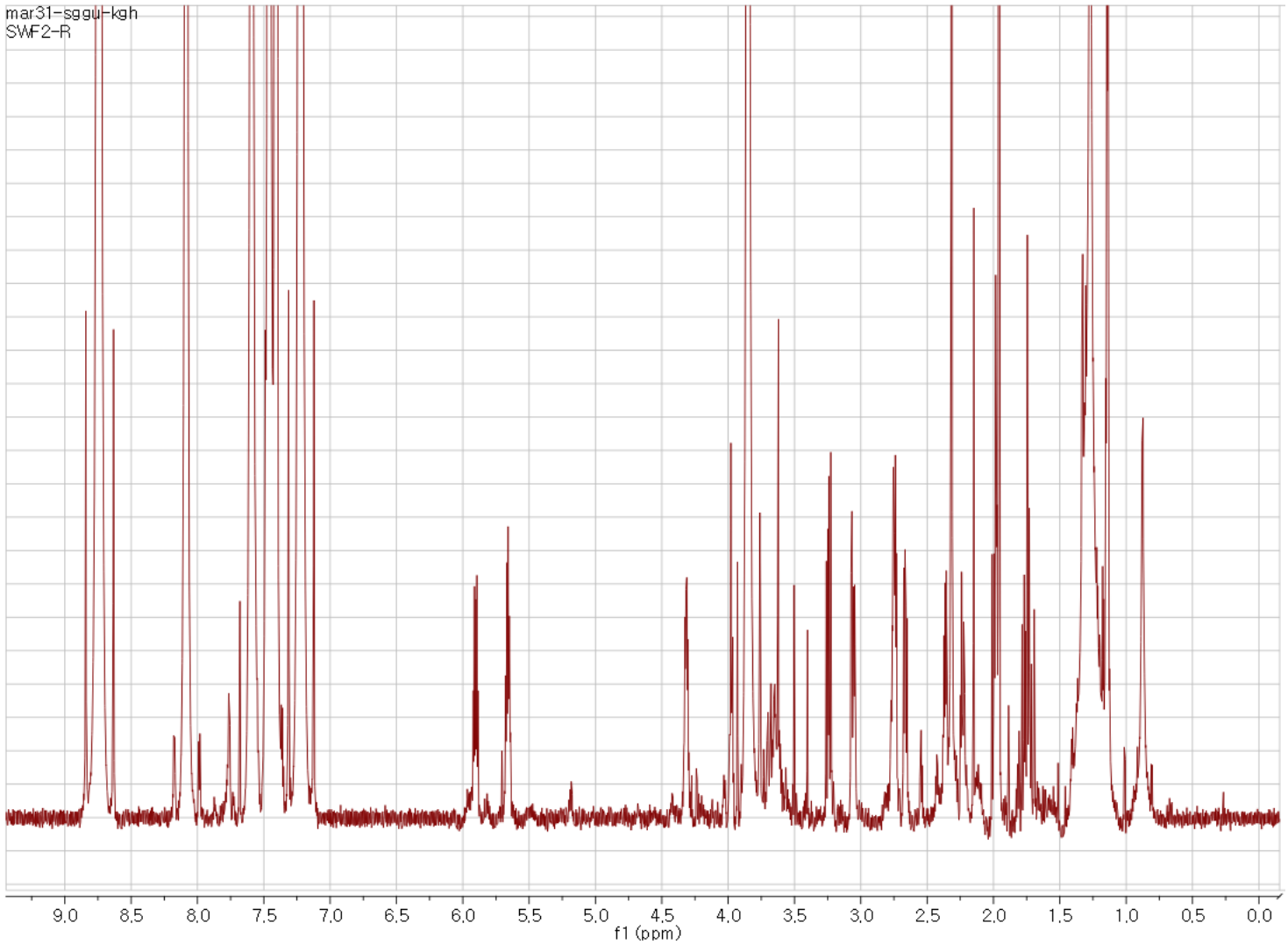

Figure S45. ^{1}H NMR spectrum of the (*R*)-MTPA esterification of compound **2.**

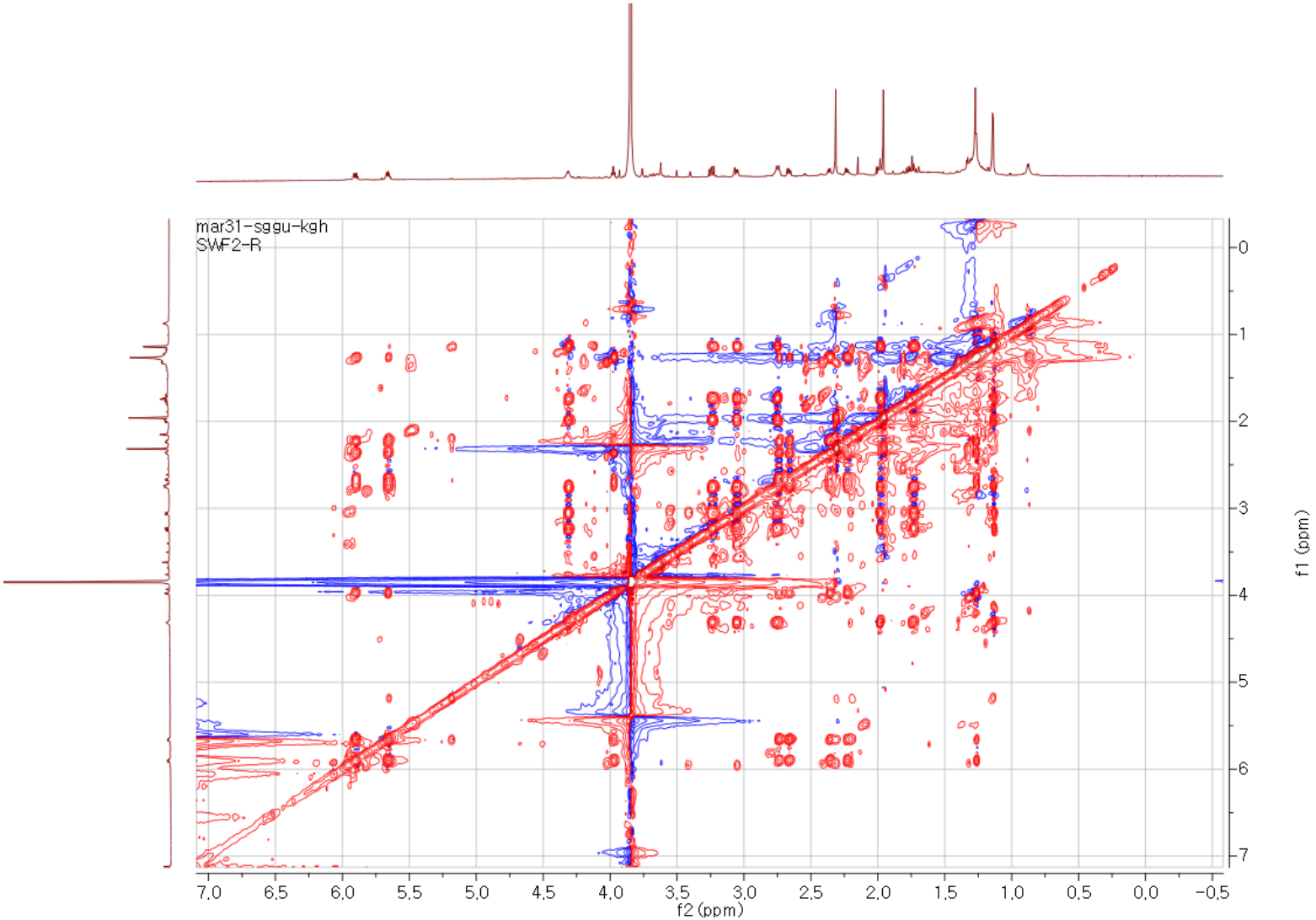

Figure S46. TOCSY spectrum of the (*R*)-MTPA esterification of compound **2.**

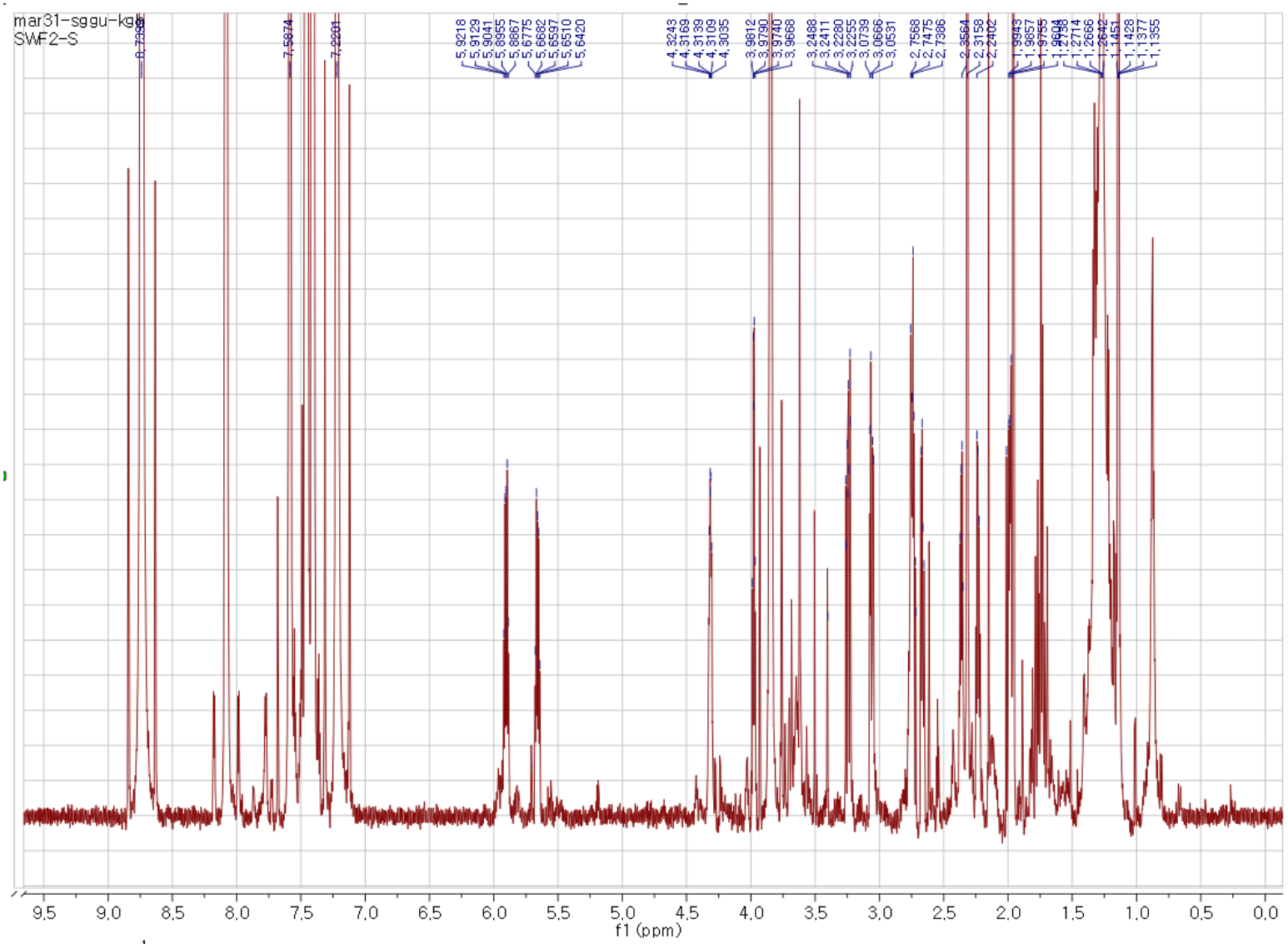

Figure S47. ^{1}H NMR spectrum of the (*S*)-MTPA esterification of compound **2.**

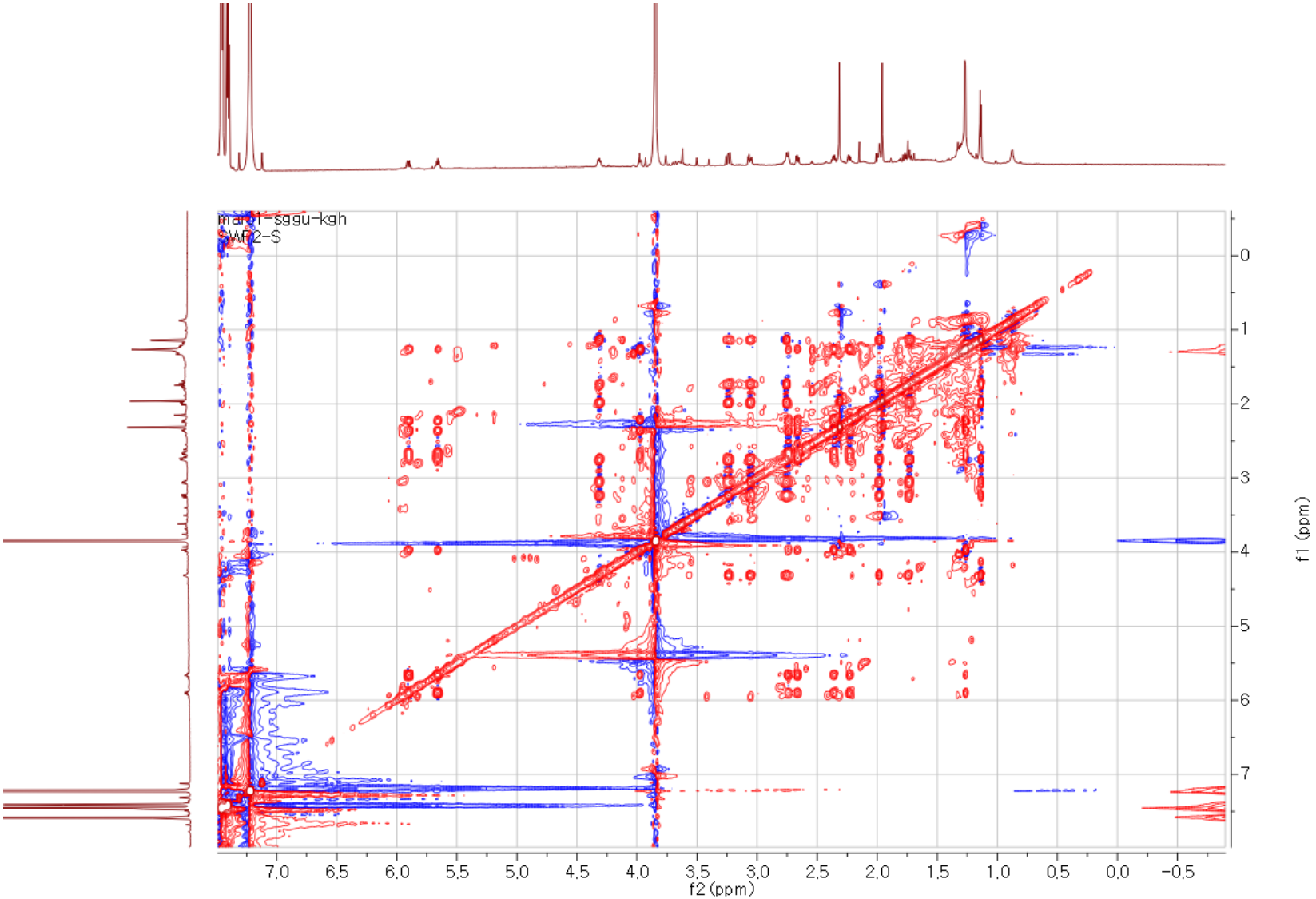

Figure S48. TOCSY spectrum of the (*S*)-MTPA esterification of compound **2.**

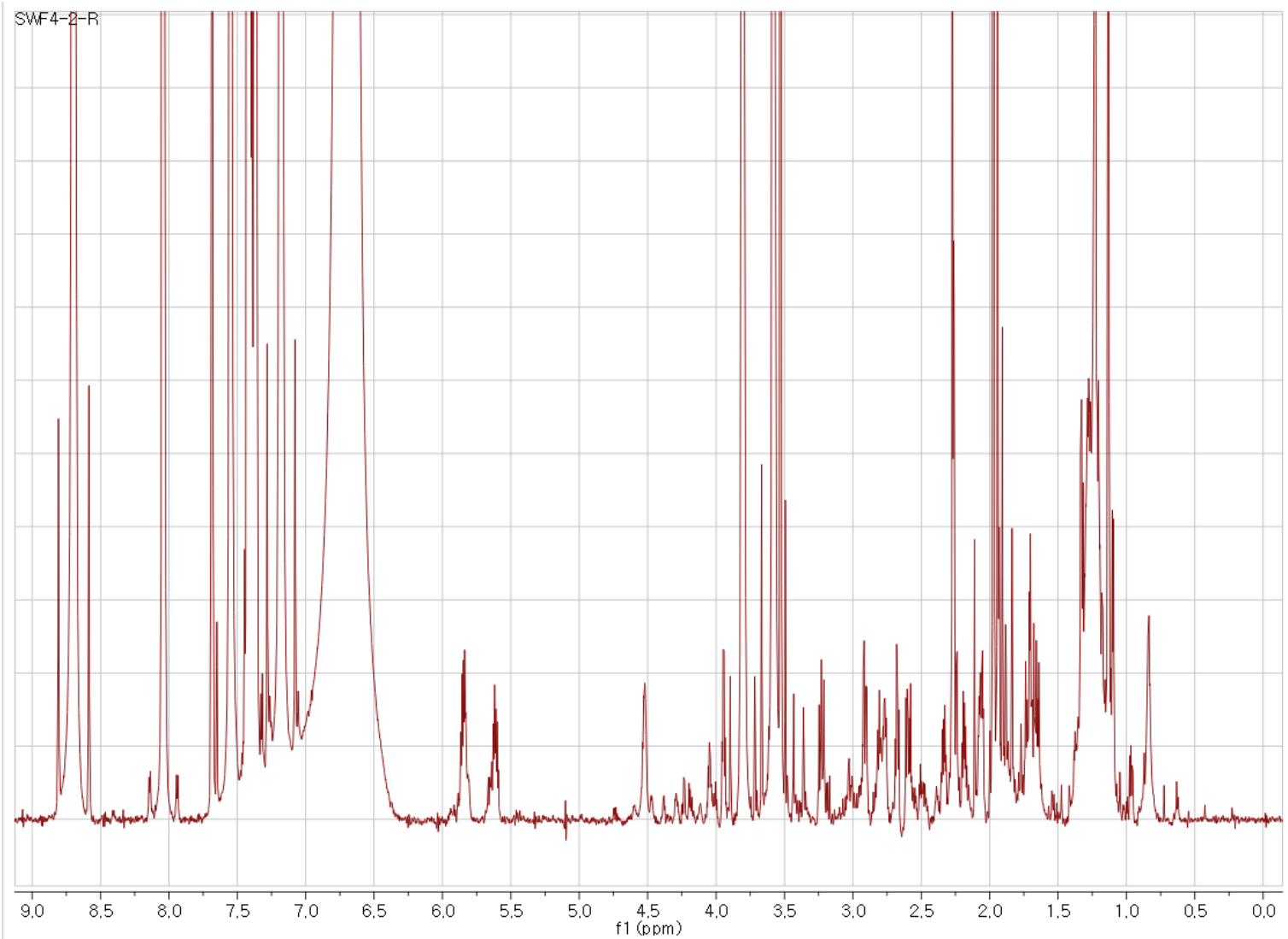

Figure S49. ^{1}H NMR spectrum of the (*R*)-MTPA esterification of compound **3.**

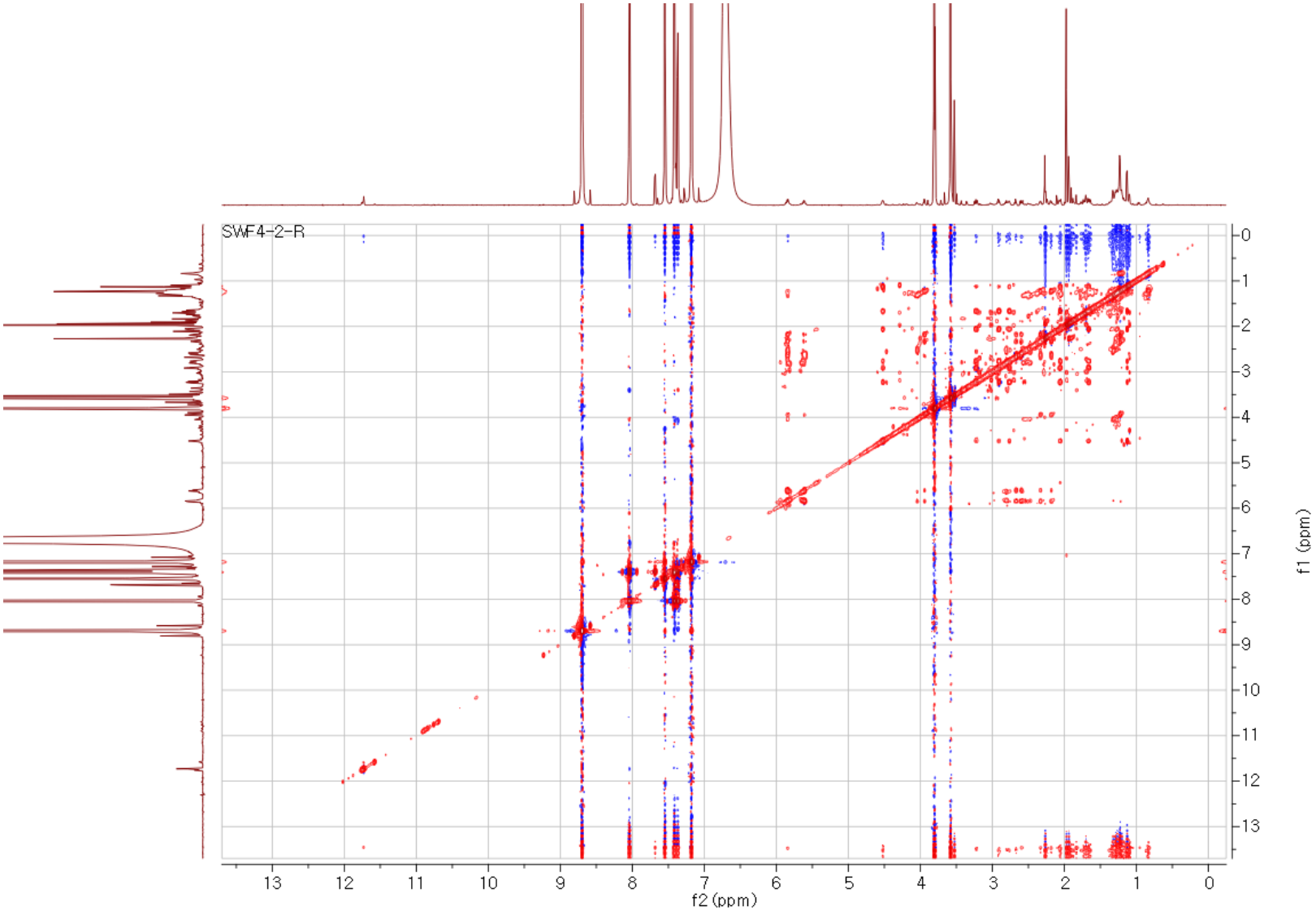

Figure S50. TOCSY spectrum of the (*R*)-MTPA esterification of compound **3.**

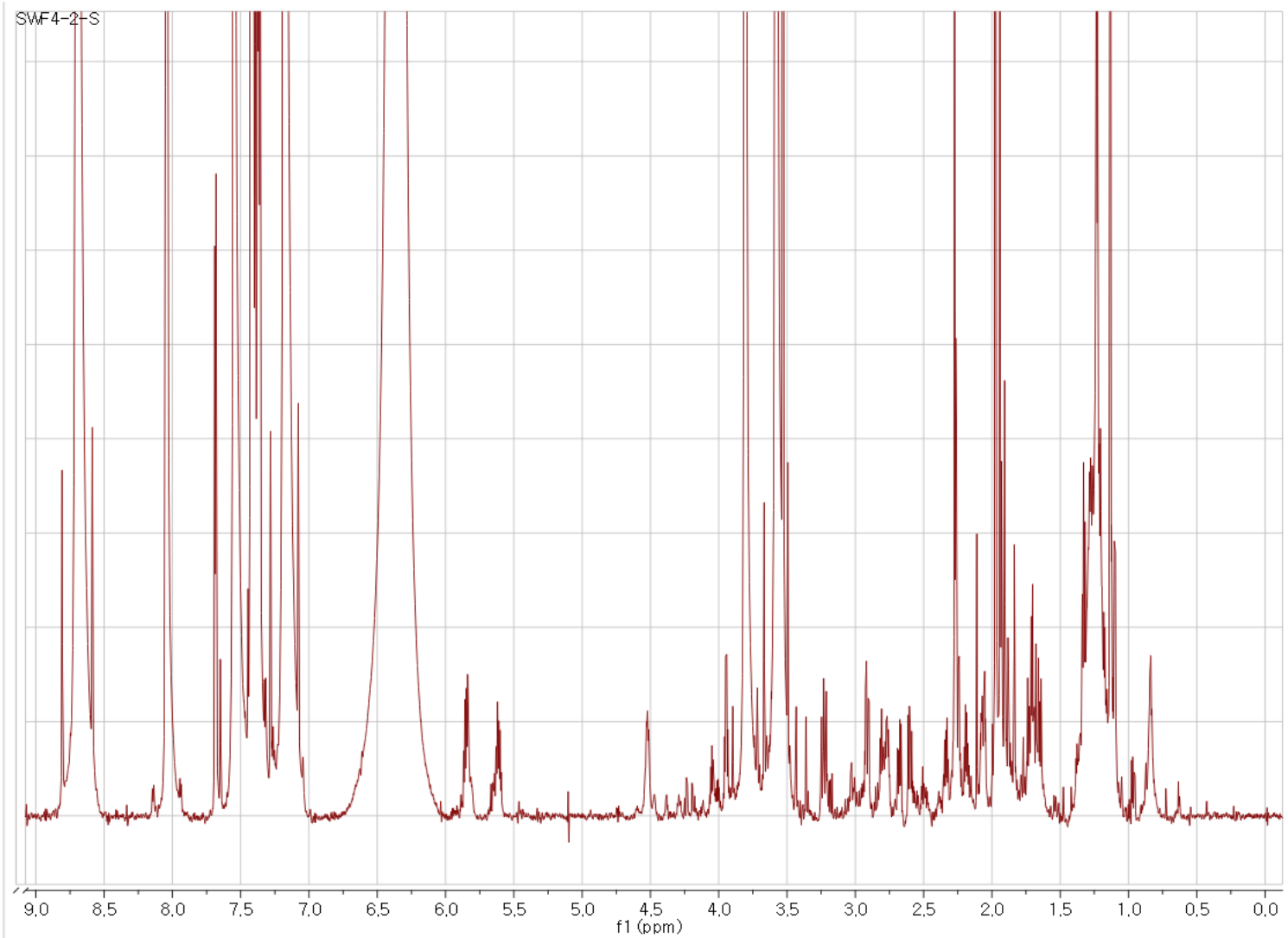

Figure S51. ^{1}H NMR spectrum of the (*S*)-MTPA esterification of compound **3.**

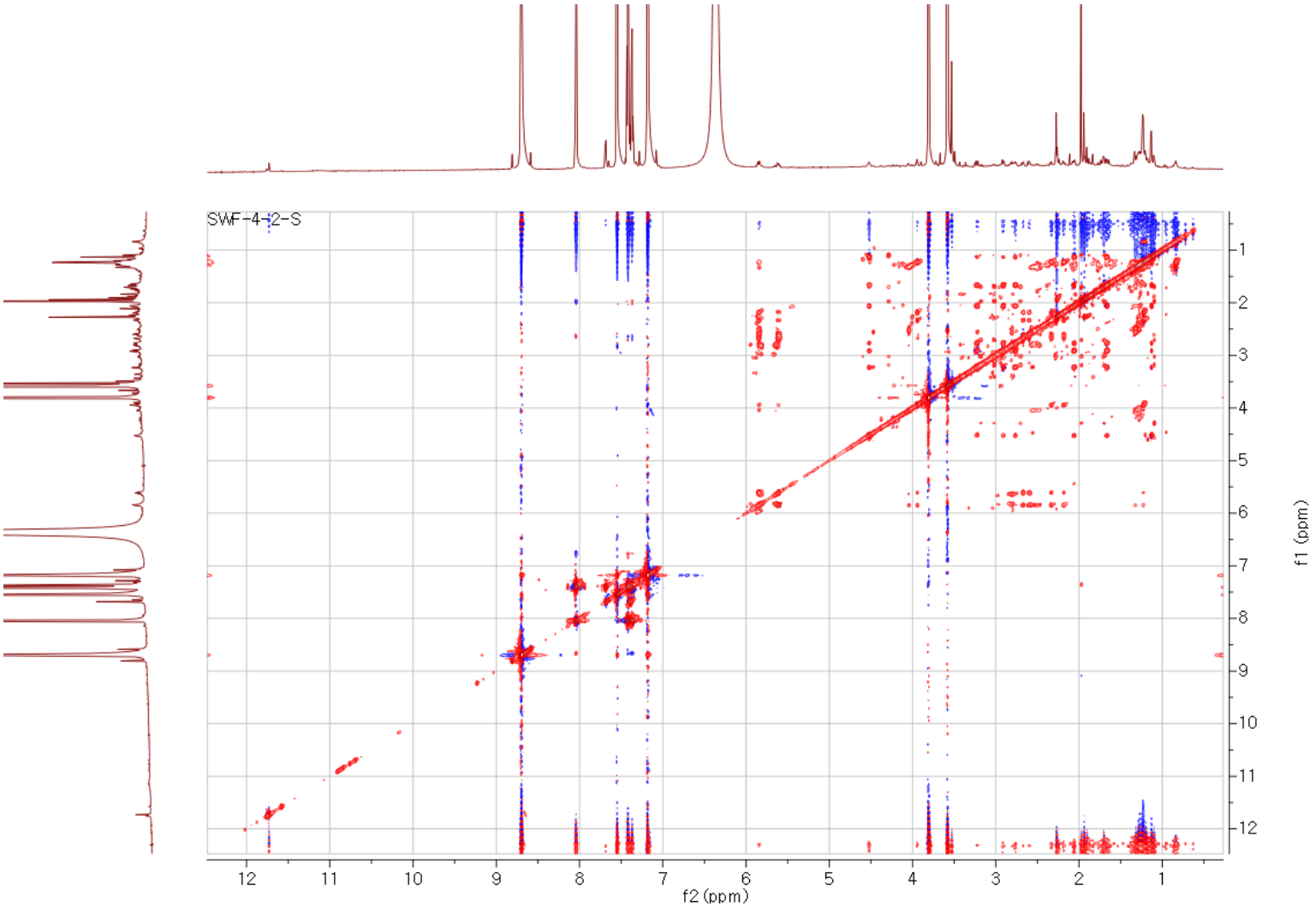

Figure S52. TOCSY spectrum of the (*S*)-MTPA esterification of compound **3.**

Table S9. Experimental (Exp.) and calculated (Cal.) NMR chemical shift values of compounds **1-3.**

Compound 1				Compound 2				Compound 3			
Position	**Exp.**	**Cal. 1**	**Cal. 2**	**Position**	**Exp.**	**Cal. 1**	**Cal. 2**	**Position**	**Exp.**	**Cal. 1**	**Cal. 2**
C1	52.28	57.12	55.49	C1	59.20	58.52	61.54	C1	58.21	63.48	59.97
C2	199.20	206.68	206.67	C2	37.90	41.99	43.34	C2	34.12	44.98	42.80
C3	173.35	173.72	177.06	C3	202.10	203.95	209.14	C3	201.64	206.77	206.97
C4	32.16	36.15	36.97	C4	175.90	176.71	174.70	C4	174.96	174.07	177.35
C5	45.52	48.77	41.72	C5	127.20	133.95	135.48	C5	126.52	135.16	134.64
C6	127.60	134.60	133.73	C6	69.10	73.31	72.13	C6	68.03	73.48	70.20
C7	64.40	68.74	74.28	C7	33.40	34.67	36.22	C7	30.76	35.16	30.74
C8	28.79	29.65	31.00	C8	32.00	37.23	34.89	C8	30.65	35.89	31.82
C9	169.21	171.57	170.32	C9	78.60	80.14	76.63	C9	75.19	80.50	78.39
C10	97.52	101.79	101.82	C10	44.00	45.49	46.06	C10	43.07	41.46	48.10
C11	21.09	21.91	21.91	C11	174.00	169.65	172.53	C11	174.32	169.28	172.48
C12	171.39	167.97	168.03	C12	132.00	130.34	131.40	C12	126.52	131.09	134.40
C13	187.18	191.80	191.87	C13	22.00	21.14	22.04	C13	20.94	21.90	21.69
C14	18.69	17.71	17.12	C14	19.80	18.79	19.13	C14	18.78	18.50	19.12
C15	122.42	136.43	136.22	C15	22.60	24.07	22.30	C15	20.53	23.73	25.08
C16	41.07	45.41	41.57	C16	134.30	141.76	135.33	C16	133.43	138.89	143.66
C17	127.60	132.33	134.46	C17	126.70	132.59	137.51	C17	125.72	137.24	134.01
C18	19.20	18.30	18.84	C18	37.10	39.71	45.44	C18	36.63	44.58	36.39
C19	21.47	21.47	21.40	C19	23.30	27.24	27.19	C19	22.24	28.13	24.27
C20	131.19	123.21	123.75	H20	2.62	1.60	2.40	H20	2.71	1.96	3.23
C21	24.50	27.03	25.98	H21	2.69	2.20	2.58	H21	2.43	1.93	1.90
H22	1.12	0.94	1.16	H22	1.46	1.66	1.37	H22	1.71	2.14	1.74
H23	1.49	1.08	2.15	H23	1.84	1.76	1.72	H23	1.85	1.83	1.75
H24	2.17	2.39	2.39	H24	1.84	1.76	1.72	H24	1.85	1.62	1.75
H25	2.17	2.39	2.39	H25	1.84	1.76	1.72	H25	1.85	1.70	1.75
H26	3.64	3.06	3.01	H26	2.46	2.62	1.75	H26	2.47	2.37	1.81
H27	2.86	2.62	2.82	H27	NH			H27	NH		

H28	3.75	4.13	4.12	H28	4.39	3.94	3.99	H28	4.49	4.07	4.58
H29	3.64	3.63	3.75	H29	OH			H29	OH		
H30	NH			H30	1.04	1.12	1.14	H30	1.06	0.96	1.09
H31	NH			H31	1.04	1.12	1.14	H31	1.06	1.05	1.09
H32	NH			H32	1.04	1.12	1.14	H32	1.06	1.18	1.09
H33	2.56	2.63	2.05	H33	3.63	3.97	3.95	H33	3.64	4.20	3.87
H34	OH			H34	2.10	2.20	2.20	H34	2.11	1.73	2.17
H35	1.14	1.05	1.06	H35	1.99	1.71	1.77	H35	2.01	2.08	1.53
H36	1.14	1.05	1.06	H36	5.53	5.56	5.46	H36	5.53	5.55	5.88
H37	1.14	1.05	1.06	H37	5.24	6.74	6.71	H37	5.27	6.87	4.96
H38	3.91	3.75	3.30	H38	2.42	2.23	1.66	H38	2.45	1.97	2.95
H39	1.83	1.65	1.66	H39	2.43	2.23	2.83	H39	2.45	2.57	2.28
H40	1.83	1.65	1.66	H40	1.31	1.19	1.28	H40	1.31	1.61	1.58
H41	1.83	1.65	1.66	H41	1.31	1.19	1.28	H41	1.31	1.14	1.58
H42	2.24	2.45	2.45	H42	1.31	1.19	1.28	H42	1.31	1.11	1.58
H43	2.24	2.45	2.45	H43	2.17	2.39	2.40	H43	2.18	1.47	2.41
H44	2.24	2.45	2.45	H44	2.17	2.39	2.40	H44	2.18	4.33	2.41
H45	1.89	1.73	1.77	H45	2.17	2.39	2.40	H45	2.18	1.38	2.41
H46	1.89	1.73	1.77	H46	1.81	2.59	2.97	H46	1.99	1.94	2.38
H47	1.89	1.73	1.77								
H48	2.17	2.39	2.39								

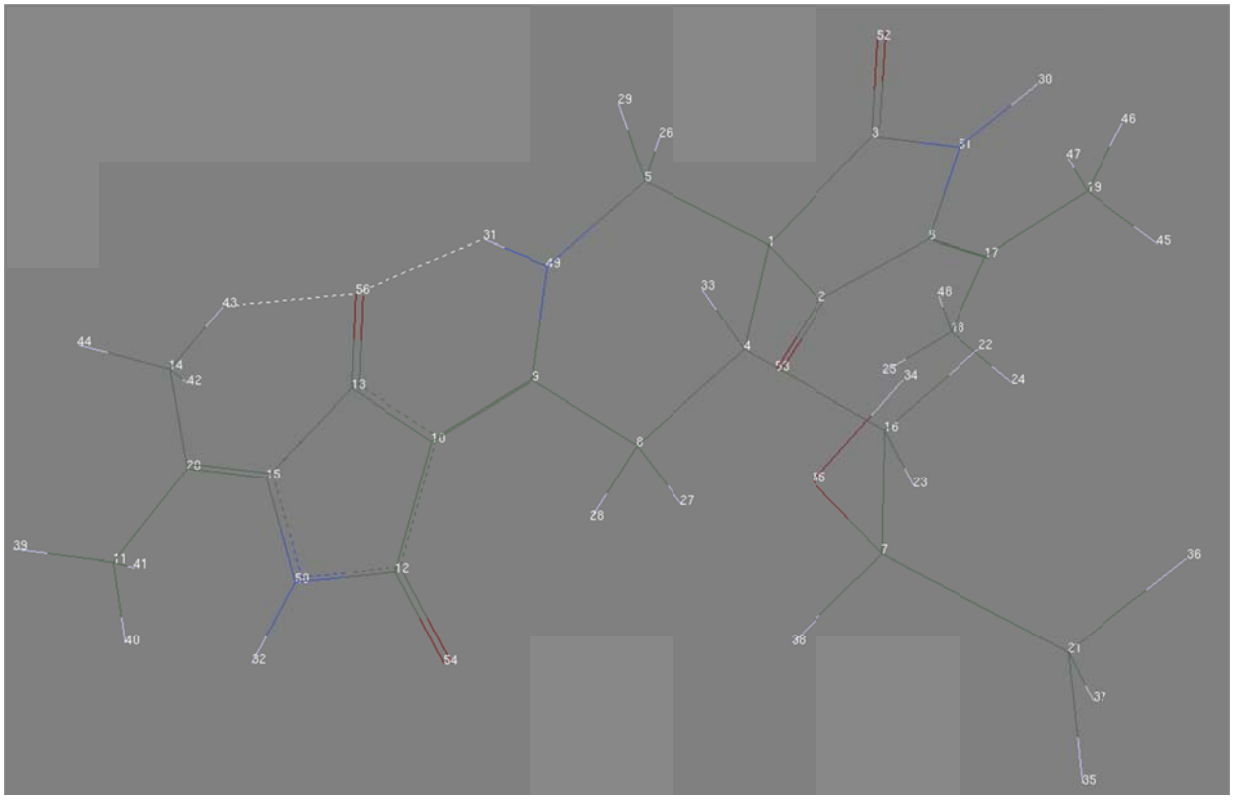

Figure S53. Optimized ground state structure and numbering for NMR chemical shift calculation of compound **1**.

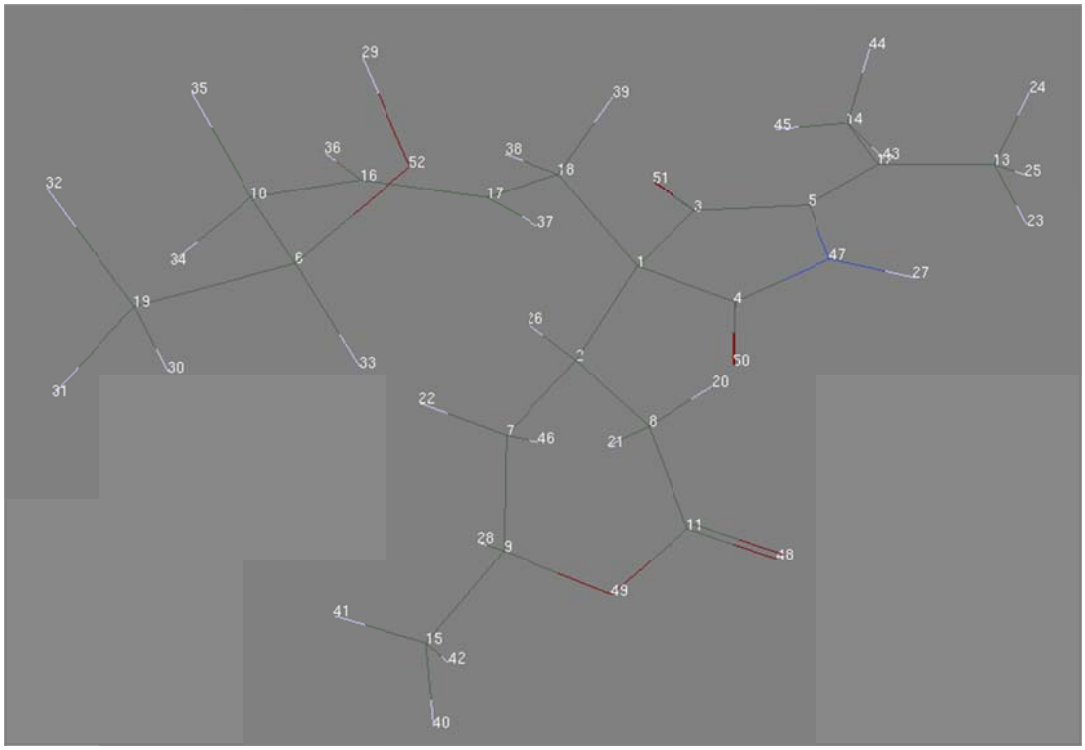

Figure S54. Optimized ground state structure and numbering for NMR chemical shift calculation of compound **2**.

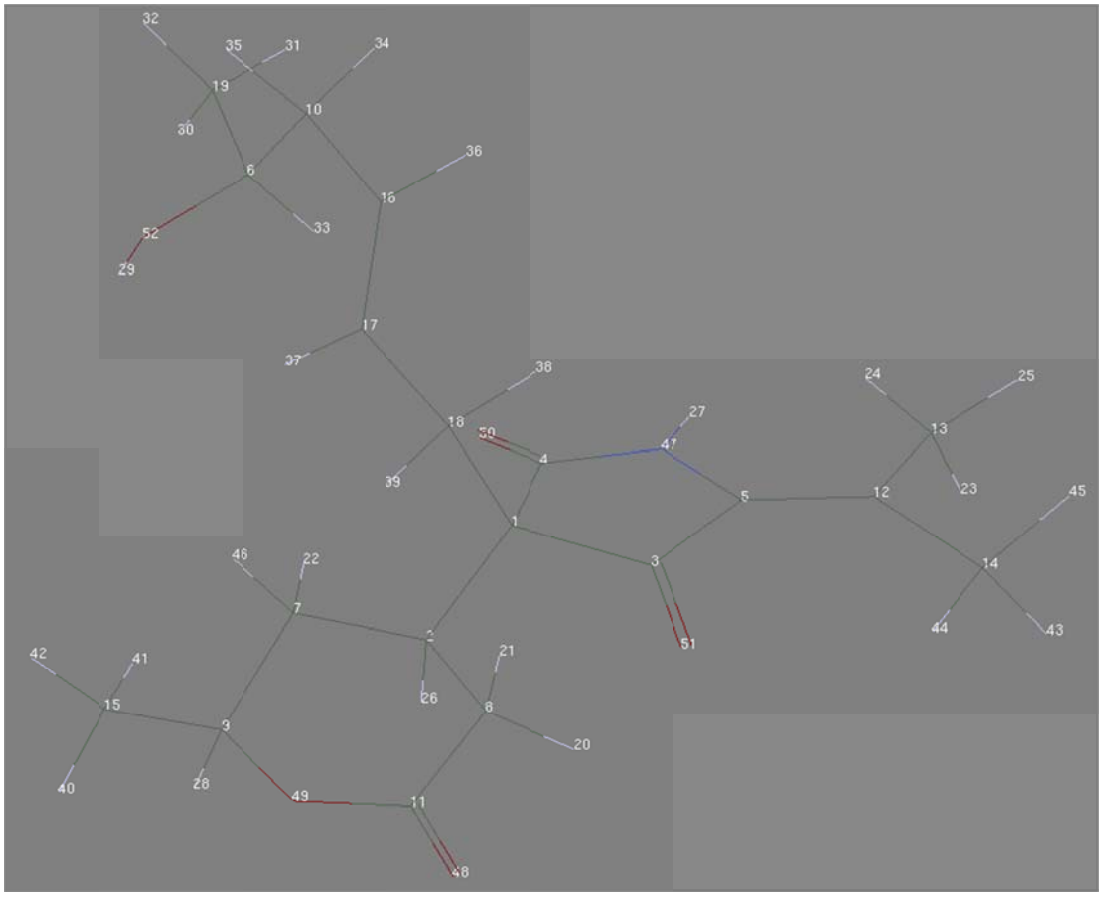

Figure S55. Optimized ground state structure and numbering for NMR chemical shift calculation of compound **3**.

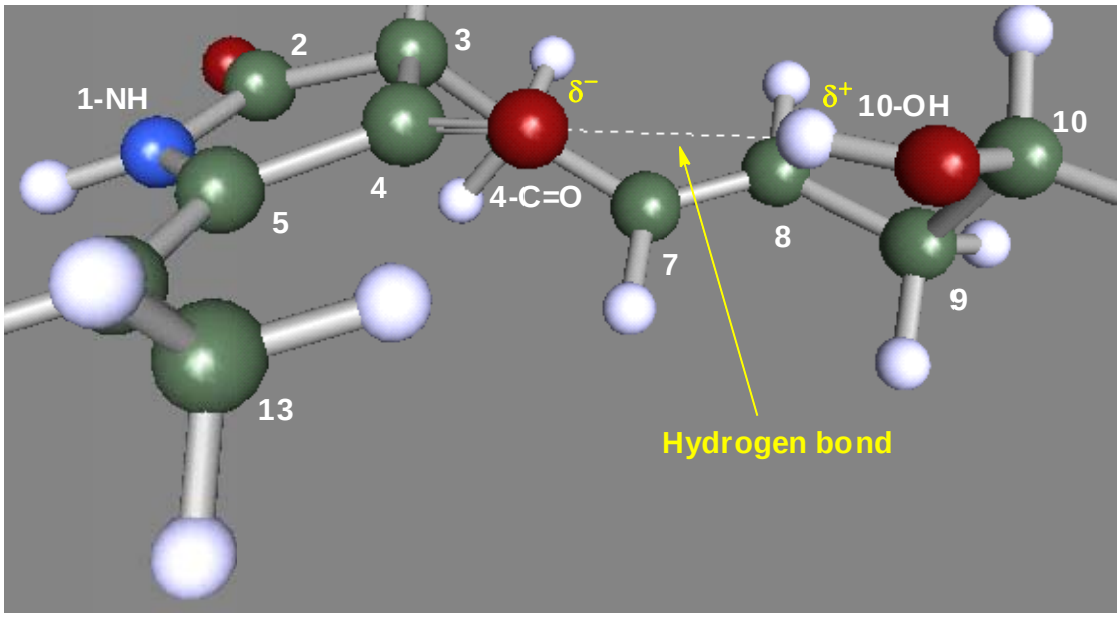

Figure S56. The anticipation of existence of hydrogen bonding in compounds **2** and **3** between hydroxyl group at C-10 and ester groups in the 2,4-pyrrolidinedione skeleton.

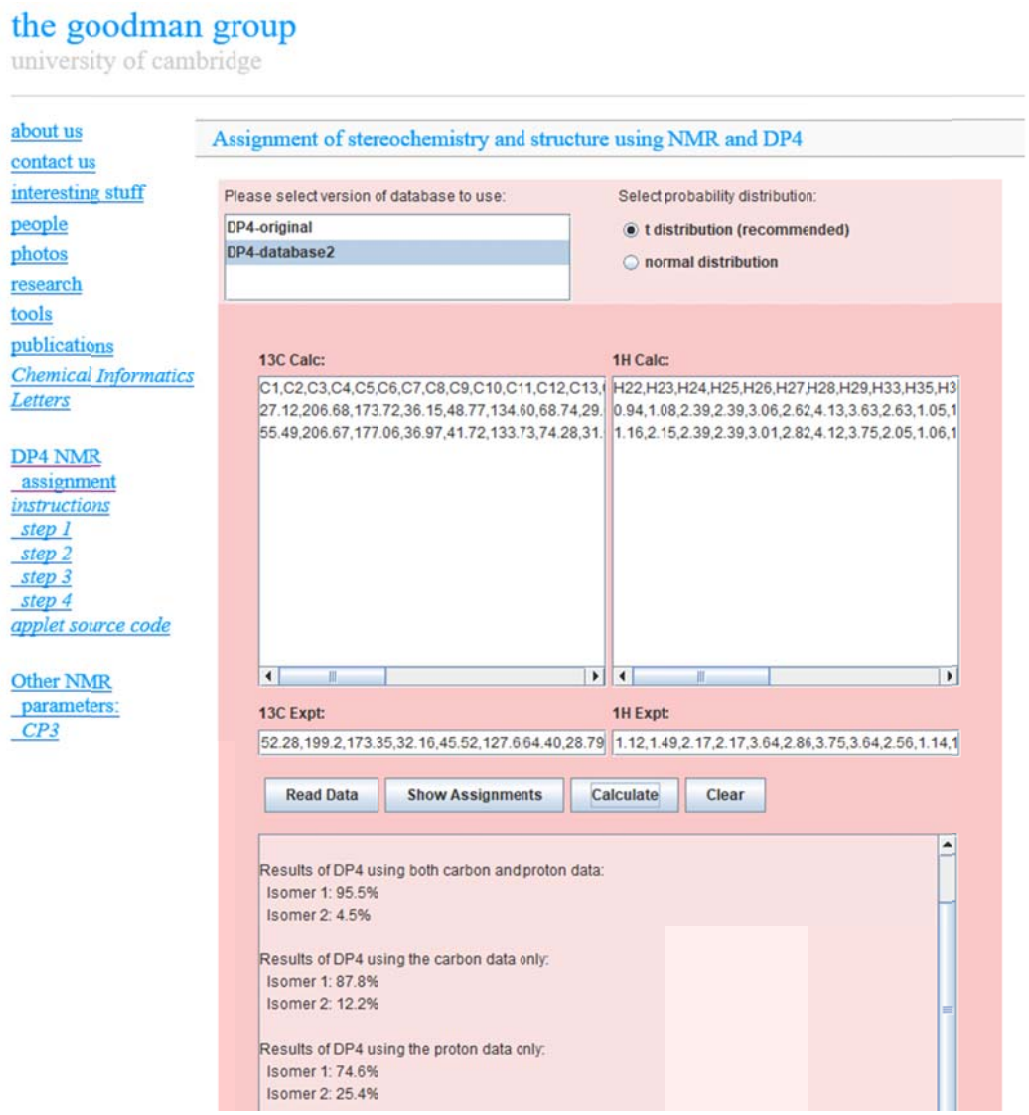

Figure S57. DP4 analysis of compound **1** (diastereomers **1a** and **1c** correspond to isomers 1 and 2)

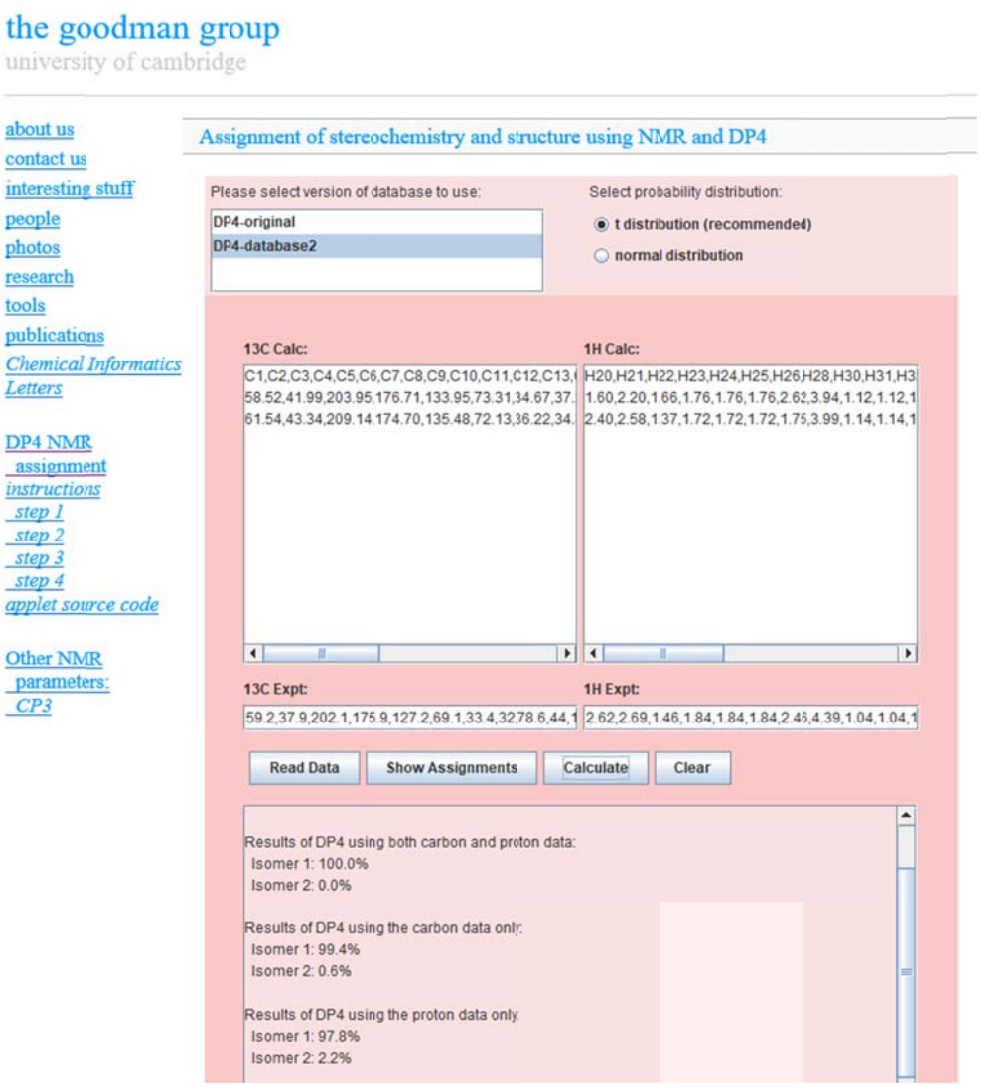

Figure S58. DP4 analysis of compound **2** with diastereomers **2a** and **2c** corresponding to isomers 1 and 2.

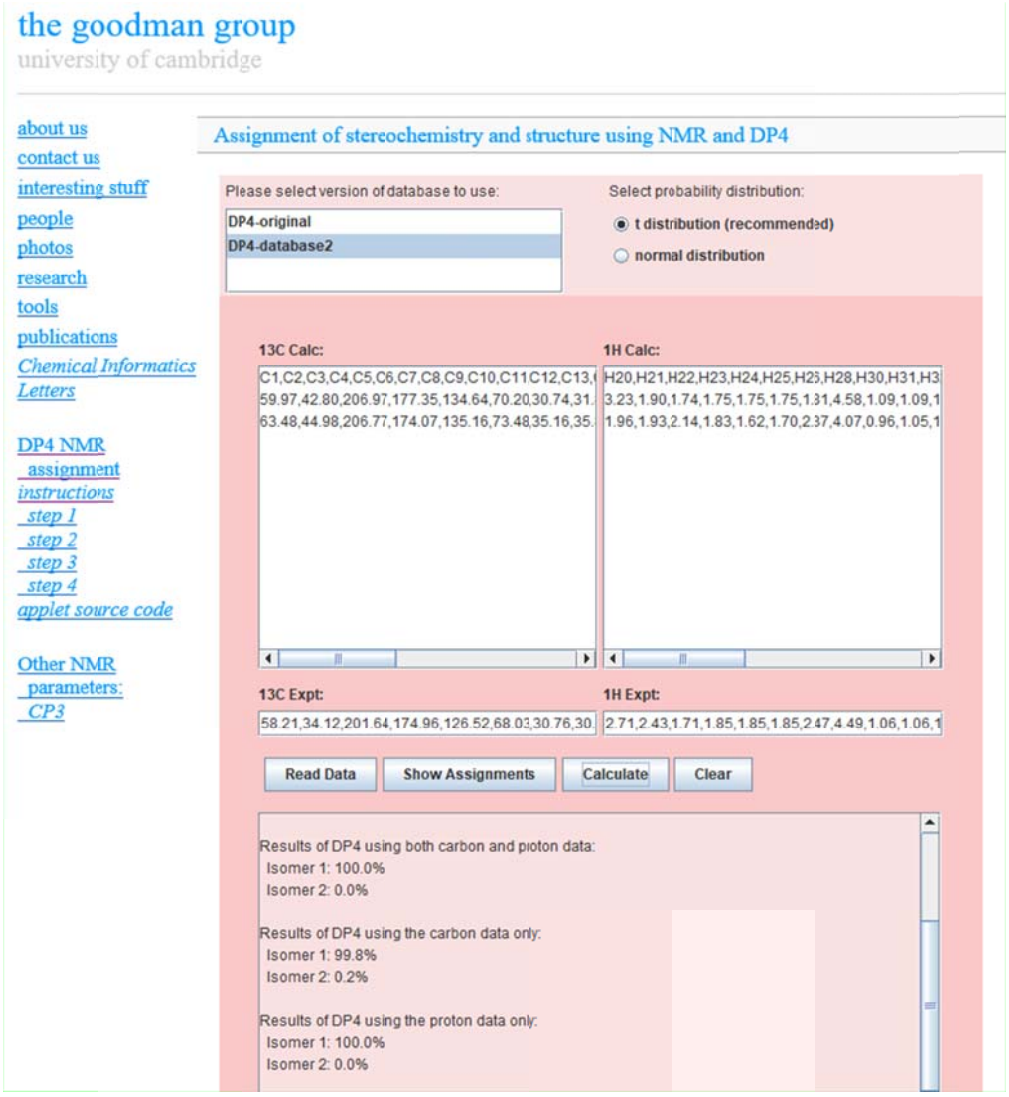

Figure S59. DP4 analysis of compound **3** with diastereomers **3d** and **3b** corresponding to isomers 1 and 2.

9 References

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