Supporting Information

Biosynthesis of versipelostatin: Identification of an enzyme-catalyzed [4+2]-cycloaddition required for macrocyclization of spirotetronate-containing polyketides

Takuya Hashimoto[1], Junko Hashimoto[2], Kuniko Teruya[3], Takashi Hirano[3], Kazuo Shin-ya[4], Haruo Ikeda[5], Hung-wen Liu[6], Makoto Nishiyama[1], Tomohisa Kuzuyama[1]*

[1]Biotechnology Research Center, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, [2]Japan Biological Informatics Consortium, 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, [3]Okinawa Institute of Advanced Sciences, Okinawa Life Science Research Center 120, 5-8 Suzaki, Uruma, Okinawa 904-2234, [4]National Institute of Advanced Industrial Science and Technology, 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, [5]Kitasato Institute for Life Sciences, Kitasato University, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0373, Japan, [6]Division of Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, University of Texas at Austin, Austin, Texas 78712, United States.

Experimental Section

General

Oligonucleotides for the polymerase chain reaction (PCR) were purchased from Operon Biotechnologies (Tokyo, Japan). Draft genome sequencing was carried out using the Genome Analyzer IIx system (Illumina San Diego, CA).

Pulse field gel electrophoresis was performed with the CHEF Mapper® XA System from Bio-rad. HR-ESI-MS (negative mode) was measured using an ABSCIEX Triple TOF 5600 system equipped with a UFLC Nexera system (Shimadzu, Kyoto, Japan). NMR spectra were recorded on a JEOL ECA-600 spectrometer (JEOL, Tokyo, Japan) operating at 600 MHz for ^{1}H, 150 MHz for ^{13}C nuclei. Cells were disrupted using a Branson Sonifier 250 (Emerson Japan, Tokyo, Japan). DNA manipulation was performed according to standard protocols.

Construction of BAC library and screening of BAC clones containing *vst* biosynthetic gene cluster

The construction of the BAC library of *S. versipellis* 4083-SVS6 was performed following a previously reported protocol.[1] *S. versipellis* 4083-SVS6 cells cultured in TSB medium containing 0.5% glycine for 2 days were embedded in 0.5% InCert® agarose (Lonza, ME, USA) before digestion with 1 mg/mL lysozyme (30 °C overnight). The resulting protoplasts were lysed by the addition of 1% sodium N-lauroylsarcosinate and 1 mg ml^{-1} proteinase K at 50 °C for 24 h. The supernatant was removed and proteinase K was inactivated by 0.1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride. After removal of the detergent by repeated washing with 50 mM EDTA (pH 8.0), DNA embedded in the agarose plug was partially digested by *Bam*HI and separated with CHEF electrophoresis. DNA of around 150 kb length was cut out from the gel and purified using agarose gel. Agarose gel containing DNA fragments was melted by NaI at half the gel weight. Melted agarose and NaI were removed by dialysis (0.025 μm pore size filter) against TE buffer. The residual agarose was completely digested by DNase-free agarase. Purified DNA fragments were ligated with a large *Bam*HI segment of the BAC vector pKU503D.[2] Then, *E. coli* NEB 10-beta was transformed by electroporation with ligated DNA. The desired clone was screened with PCR using two sets of primers vst-up-F and vst-up-R, and vst-down-F and vst-down-R designed to amplify upstream and downstream of the gene cluster. The BAC clone pKU503DverP10N24 was purified with the QIAGEN Large-Construct Kit (QIAGEN) according to the manufacturer's protocol. Complete DNA sequence of pKU503DverP10N24 was obtained by PacBio sequencing (accession number: LC006086)

Inactivation of *vstJ* gene in *S. albus* J1074:: pKU503DverP10N24

For the construction of the *vstJ* (429 bp) disruption plasmid, a 2.0 kb DNA fragment containing the region upstream of *vstJ* and a 2.0 kb DNA fragment containing the region downstream of *vstJ* were

amplified with vstJ-del-pre_F and vstJ-del-pre_R, and with vstJ-del-post_F and vstJ-del-post_R, respectively. Each of the fragments was cloned into pT7blue vector to give pT7vstJpre and pT7vstJpost confirmed by DNA sequence. Each of the plasmids was digested with *Hin*dIII and *Xba*I and both fragments were simultaneously cloned into the *Hin*dIII site of pUC118apr[3] to give pUC118aprΔvstJ.

S. albus J1074::pKU503DverP10N24 was transformed with pUC118aprΔvstJ. Desired transformants were then selected on R2YE plates containing apramycin at a concentration of 25 μg ml^{-1}. The obtained transformant was cultured in R2YE medium for 2 days. Protoplasts were prepared from the transformant culture and regenerated on R2YE plates. Each of the regenerated colonies was inoculated on TSB plates with or without apramycin respectively, and an apramycin-sensitive colony was selected to obtain the *vstJ* knockout mutant, *S. albus* J1074::pKU503DverP10N24Δ*vstJ*. Successful deletion was confirmed by PCR amplification of the *vstJ* gene using vstJ-F and vstJ-R. (Fig. S9).

Bacterial culture

S. versipellis 4083-SVS6, *S. albus* J1074::pKU503DverP10N24, *S. albus* J1074:: pKU503DverP10N24Δ*vstJ* and *S. albus* J1074::pKU503D (negative control) were cultivated in K medium (2.5% soluble starch, 1.5% soybean meal, dry 0.2% yeast, and 0.4% calcium carbonate, and the pH was adjusted to 6.2). After cultivation, an equivalent volume of acetone was added to the culture broth. After the extraction, the mixture was filtered with filter paper, and then the acetone was removed by evaporation and the remaining aqueous layer was extracted with ethyl acetate.

LC/MS analytical condition

For HPLC-UV/VIS-MS analysis, 10 % MeOH containing 10 mM ammonium formate (pH3.0) and 90 % MeOH containing 10 mM ammonium formate (pH3.0) were used as eluent A and B, respectively. HPLC-UV/VIS-MS analysis was performed with a C-18 column (CAPCEL PAK C_{18} IF 2.0 × 50 mm, Shiseido) under the following conditions: a gradient from 0 to 100 % eluent B for 6.0 min, 100 % B 6.0 min to 8.0 min, and 0 % B 8.0 to 15 min.

Preparation of the intermediate (3)

S. albus J1074::pKU503DverP10N24*ΔvstJ* was inoculated into PC-1 media (1.0 % Starch, 1.0 % meat extract, 1.0 % polypeptone, 1.0 % molasses, pH 7.2) including neomycin (25 μg ml^{-1}) for 2 days, at 30 ˚C. Two ml of preculture was inoculated in K medium (4 L) including neomycin (25 μg ml^{-1}) for 3 days, at 27 ˚C on a rotary shaker at 180 rpm. Cell and broth were separated by centrifugation. Cell was extracted with acetone, and then filtered with filter paper. Acetone was removed by evaporation and the remaining aqueous layer was extracted with ethyl acetate. The ethyl acetate layer was dried by evaporation to give 480 mg of residue. The extracted residue was applied on a preparative thin layer

chromatography plate (PLC Silica gel F254, Merck, Tokyo) which was developed with ethyl acetate and hexane (v/v 3:1). The crude sample (52 mg) was then subjected to preparative HPLC using a PEGASIL ODS column (Senshu-Pak, 20 × 250 mm, Senshu, Tokyo) developed using 87.5% MeOH containing 10 mM ammonium formate (pH 3.0). Finally, **3** (12.2 mg) was obtained as a yellow oil. HR-ESI-MS: *m/z* 677.4410 $[M-H]^-$; calculated for [M ($C_{42}H_{62}O_7$)−H]$^-$, 677.4417.

Preparation of recombinant VstJ protein

The vstJ-F and vstJ-R primers were used to amplify the *vstJ* gene from the *S. versipellis* 4083 SVS6 genome by PCR. The PCR-amplified 0.4-kb DNA fragment was cloned into the pT7Blue T-vector to construct pT7-vstJ. The sequence of pT7-vstJ was confirmed by DNA sequencing. pT7-vstJ digested with *Nco*I and *Bam*HI, and cloned into the same sites of pHIS8[4] to give pHis8-vstJ. The pHis8-vstJ construct was then used to transform *E. coli* BL21 (DE3).

E. coli BL21 (DE3) harboring pHis8-vstJ was pre-cultured in Luria-Bertani (LB) medium containing 50 μg ml^{-1} kanamycin at 37 °C. The pre-culture was inoculated into Terrific Broth containing kanamycin at a concentration of 50 μg ml^{-1} and grown at 37 °C for 2 h. The broth was cooled on ice for 10 min, made 0.1 mM in isopropyl β-thiogalactopyranoside, and cultured for an additional 20 h at 18 °C. The cells were harvested by centrifugation at 3,910 × *g* at 4 °C for 10 min.

For protein extraction, cells were suspended in a buffer containing 50 mM Tris-HCl, pH 8.0, 20 mM imidazole, and 150 mM NaCl. The cell suspension was homogenized by sonication. The lysate was centrifuged at 34,700 × *g* at 4 °C for 20 min. VstJ was purified from the resulting supernatant using Ni-NTA Superflow resin (Qiagen, Tokyo, Japan). After washing with buffer containing 50 mM Tris-HCl, pH 8.0, 20 mM imidazole, and 150 mM NaCl, VstJ was eluted using the same buffer containing 250 mM imidazole. The resultant protein solution was dialyzed against 50 mM Tris-HCl, pH 8.0 and 150 mM NaCl overnight. Protein solution was concentrated to appropriate concentration with Vivaspin 10,000 MWCO (Millipore).

VstJ assay

The standard VstJ assay was performed at 30 ˚C in 500 μl reaction mixture containing 50 mM Tris-HCl pH 8.0, 0.5 mM of **3**, and 1 mg ml^{-1} VstJ. At the indicated times (1, 2, 3, 5, and 60 min), a 50 μl aliquot of the reaction mixture was removed and quenched by mixing with 50 μl of methanol. After centrifugation, the supernatant was subjected to HPLC-UV/VIS-MS analysis.

To investigate pH dependence, the VstJ assay was performed at 30 ˚C for 30 min in 100 μl reaction mixture containing 0.5 mM of **2**, 1 mg ml^{-1} VstJ and 50 mM HEPES-NaOH pH 6.7, 7.0, 7.5, or 50 mM Tris-HCl pH7.5, 8.0, 8.5, 9.0 respectively. The reaction was quenched by the addition of an equal volume of MeOH and the supernatant was subjected to HPLC-UV/VIS-MS analysis.

For determination of the kinetic parameters, the VstJ assay was performed in 0.8 ml reaction mixture containing 50 mM Tris-HCl (pH 8.0), 10 μg ml^{-1} VstJ in the presence of **3**. The concentration of **3** was varied from 5 μM to 60 μM. The enzyme-dependent decrease of **3** was monitored using a

UV-1600PC spectrophotometer (Shimadzu, Kyoto, Japan) equipped with a CPS-240A cell holder (Shimadzu) that was adjusted to 30 °C. The initial velocities were determined from the slope of a plot of the decrease of **3** as a function of the incubation time. The molar extinction coefficient (ε) of **3** was 4700 M^{-1} cm^{-1} at 308 nm. The steady-state kinetic parameters were calculated using SigmaPlot 12.3 software (Systat Software, Point Richmond, CA).

Preparation of compound 4

Standard VstJ assay was performed at 30 ˚C in 100 ml volume. After overnight reaction, the reaction mixture was extracted with ethyl acetate. Ethyl acetate was removed by evaporation. Then, the residue was subjected to preparative HPLC using a PEGASIL ODS column (Senshu-Pak, 20 × 250 mm, Senshu, Tokyo) developed with 90% MeOH containing 0.1% formic acid. A total of 1.0 mg of **4** (white powder) was obtained. HR-ESI-MS: *m/z* 677.4420 $[M-H]^-$; calculated for [M ($C_{42}H_{62}O_7$)−H]$^-$, 677.4417.

Table S1. Strains and vectors used in this study.

Vectors/Plasmids	**Characteristic(s)**	**Source/Reference**
pKU503D	BAC vector	2
pKU503DverP10N24	BAC clone including the *vst* gene cluster	This study
pUC118apr	Vector for gene inactivation	3
pUC118aprΔvstJ	Plasmid for the *vstJ* gene inactivation	This study
pHis8-vstJ	Plasmid for overexpression of the *vstJ* gene	This study
Strain		
E. coli		
NEB10β	Host for constructing BAC library	NEB
DH5α	Host for general cloning	Takara
BAA525	Methylation-deficient donor for preparation of pUC118aprΔ*vstJ*	ATCC
BL21 (DE3)	Host for protein expression	Takara
Streptomyces		
S. versipellis 4083-SVS6	VST producer (wt)	5
S. albus J1074	Host for heterologous expression	6
S. albus J1074::pKU503DverP10N24	*S. albus* J1074 transformed with the VST biosynthetic gene cluster	This study
S. albus J1074::pKU503DverP10N24*ΔvstJ*	*S. albus* J1074::pKU503DverP10N24 with the inactivated *vstJ* gene	This study

Table S2. Primers used in this study.

Primers	**Sequences (5'-3')**	
vst-up-F	5'-ACATGGTCGCGAGAATCGAA-3'	For screening of vst gene cluster from BAC library
vst-up-R	5'-ATCTTGGTACCGCACCCGAC-3'	For screening of vst gene cluster from BAC library
vst-down-F	5'-GGACAGCGTCGAGTACGTCA-3'	For screening of vst gene cluster from BAC library
vst-down-R	5'-CCAGTTCGCTCTTGAACGT-3'	For screening of vst gene cluster from BAC library
vstJ-del-pre_F:	5'-GGGAAGCTTCCTCTTCGCGTCTGAACGCG-3'	For construction of pUC118aprΔvstJ (*Hin*dIII site was indicated as underline)
vstJ-del-pre_R:	5'-GGGTCTAGACCGACGCCTTCTCGCTTGTC-3'	For construction of pUC118aprΔvstJ (*Xba*I site was indicated as underline)
vstJ-del-post_F:	5'-GGGTCTAGAAGACGAAAGGTTCCAGCTCT-3'	For construction of pUC118aprΔvstJ (HindIII site was indicated as underline)
vstJ-del-post_R:	5'-GGGAAGCTTGACGGGCCAGGCCACGACCG-3'	For construction of pUC118aprΔvstJ (*Xba*I site was indicated as underline)
vstJ-F	5'-GGGCCATGG CGCGGAAGCGAGCACCGAAG-3'	For construction of pHis8-vstJ (*Nco*I site was indicated as underline)
vstJ-R	5'-GGGGGATCC TCAGCCGCGGAGGAAGAGGG-3'	For construction of pHis8-vstJ (*Bam*HI site was indicated as underline)

Table S3. Deduced functions of ORFs in *vst* biosynthetic gene cluster.

Gene	Amino acids (aa)	Proposed function	Blast hit protein [Origin]	Identity/ Similarity (%)	Accesion number
-3	283	rRNA methyltransferase	rRNA methyltransferase [*Streptomyces albulus*]	77/80	WP_016574500
-2	136	Transcriptional regulator	MerR family transcriptional regulator [*Saccharomonospora cyanea*]	62/67	WP_005452765
-1	416	MFS transporter	MFS transporter [*Saccharomonospora cyanea*]	63/73	WP_005452767
D	405	Cytochrome P450 hydroxylase	Cytochrome P450 hydroxylase [*Streptomyces sp.* Amel2xE9]	68/76	WP_019983477
E	87	Ferredoxin	Ferredoxin [*Streptomyces sp.* 303MFCol5.2]	52/59	WP_020127680
B1	288	dTDP-sugar reductase	dTDP-4-dehydrorhamnose reductase [*Streptomyces sp.* HGB0020]	64/68	WP_016430997
B2	195	dTDP-sugar 3,5-epimerase	TDP-4-dehydrorhamnose 3,5-epimerase, partial [*Streptomyces peucetius* ATCC 27952]	64/70	ACR46366
F	349	3-Oxoacyl-(acyl carrier protein) synthase III	3-Oxoacyl-(acyl carrier protein) synthase III [*Streptomyces sp.* C]	64/68	WP_007269134
G	146	Unknown	Cyclase/dehydrase [*Frankia sp.* EuI1c]	23/27	WP_013422986
H	419	Glycosyltransferase	KijA4 [*Actinomadura kijaniata*]	45/54	ACB46466
A1	8883	Type I PKS	AmphC [*Streptomyces nodosus*]	36/40	ACB46488.1
A2	7529	Type I PKS	Polyketide synthase [*Streptomyces aizunensis*]	47/53	AAX98191
I	403	Glycosyl transferase	ChlC7 [*Streptomyces antibioticus*]	43/51	AAZ77672
B3	331	dTDP-sugar 2,3-reductase	TylCII [*Streptomyces fradiae*]	62/69	AAD41821
B4	249	NDP-sugar 4-ketoreductase	UrdR [*Streptomyces fradiae*]	50/56	AAF72551
B5	516	dTDP-sugar 2,3-dehydratase	PyrC8 [*Streptomyces rugosporus*]	50/58	AFV71305
J	142	[4+2] cycloaddition	Putative ribosomal protein L15P [*Streptomyces sp.* NRRL 11266]	18/25	BAF73716
A3	3589	Type I PKS	ChlA3 [*Streptomyces antibioticus*]	34/38	AAZ77696.1
A4	3617	Type I PKS	KijS1 [*Actinomadura kijaniata*]	29/32	ACB46488
A5	1571	Type I PKS	ChlA6 [*Streptomyces antibioticus*]	53/59	AAZ77699
K	514	FAD-dependent oxidoreductase	KijA [*Actinomadura kijaniata*]	47/52	ACB46484
B6	378	Sugar O-methyltransferase	SpnI [*Saccharopolyspora spinosa*]	40/47	AAG23270
C5	343	3-Oxoacyl-ACP synthase III	RkD [*Streptomyces sp.* 88-682]	51/60	ACZ65477
C4	362	Dehydratase	ChlD4 [*Streptomyces antibioticus*]	52/59	AAZ77706
C3	276	Acyltransferase	ChlD3 [*Streptomyces antibioticus*]	59/64	AAZ77705
C2	75	Acyl carrier protein	ChlD2 [*Streptomyces antibioticus*]	48/59	AAZ77704
C1	630	FKbH-like protein	ChlD1 [*Streptomyces antibioticus*]	59/64	AAZ77703
L	464	Crotonyl-CoA reductase	Crotonyl-CoA reductase [*Streptomyces lasaliensis*]	75/80	CAQ64684

R1	**256**	**SARP family regulator**	**ChlF2** [***Streptomyces antibioticus***]	**60/69**	AAZ77687
M	**242**	**Type II thioesterase**	**ChlK** [***Streptomyces antibioticus***]	**52/57**	AAZ77688
N	**84**	**Hypothetical protein**	**Hypothetical protein** [***Micromonospora sp.*** **L5**]	**43/55**	WP_013477259
R2	**896**	**Transcriptional regulator**	**LuxR family transcriptional regulator** [***Salinispora tropica***]	**38/46**	WP_028568330
O	**164**	**Hypothetical protein**	**YD repeat protein** [***Micromonospora aurantiaca*** **ATCC 27029**]	**5/7**	WP_018412297
P	**589**	**Dehydrogenase**	**3-hydroxyacyl-CoA dehydrogenase** [***Frankia sp.*** **EuI1c**]	**55/61**	WP_020866330
Q	**86**	**Unknown**	**Hypothetical protein** [***Streptomyces ambofaciens*** **ATCC 23877**]	**33/38**	CAI78105
B7	**355**	**Glucose-1-phosphate thymidylyltransferase**	**Glucose-1-phosphate thymidylyltransferase** [***Streptomyces peucetius*** **ATCC 27952**]	**82/88**	ACR46363
B8	**328**	**dTDP-sugar 4,6-dehydratase**	**TDP-D-glucose 4,6-dehydratase** [***Streptomyces peucetius*** **ATCC 27952**]	**81/86**	ACR46364
R3	**161**	**Regulator**	**MarR-family regulatory protein** [***Streptomyces sviceus*** **ATCC 29083**]	**78/81**	WP_007386486
+1	**147**	**Hypothetical protein**	**Hypothetical protein** [***Streptomyces hygroscopicus***]	**63/68**	WP_014670231
+2	**123**	**Hypothetical protein**	**Hypothetical protein** [***Streptomyces afghaniensis***]	**80/85**	WP_020273881
+3	**115**	**Hypothetical protein**	**Hypothetical protein** [***Chloroflexi bacterium oral taxon*** **439**]	**7/10**	WP_022848429

Table S4. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data of compound **3** measured in dimethyl sulfoxide-d_6.

no.	δ_C	δ_H (H, mult, J(Hz))	no.	δ_C	δ_H (H, mult, J(Hz))
1	170.8		22	36.2	1.35 (1H, m)
2	98.1		23	32.6	0.95 (1H, m) / 1.54 (1H, m)
3	177.1		24	25.4	1.20 (1H, m) / 1.42 (1H, m)
4	155.3		25	41.2	1.92
5	198.7		26	135.4	
6	58.5		27	129.0	5.55 (1H, s)
7	39.2	2.13 (1H, br)	28	133.7	
8	48.8	2.10 (1H, m)	29	123.1	5.22 (1H, q, 6.6)
9	214.5		30	14.0	1.59 (3H, d, 7.2)
10	49.2	2.91 (1H, t, 8-8.5) / 2.04 (1H, t, 8-8.5)	31	84.9	4.63 (1H, s) / 4.33 (1H, s)
11	69.3	3.62 (1H, dd, 8.4, 8.4)	32	24.5	1.55 (m) / 2.48 *
12	46.3	1.74 (1H, br)	33	12.4	0.69
13	121.6	5.71 (1H, s)	34	20.5	1.02 (3H, d, 5.4)
14	163.5		35	23.2	1.50 (3H, s)
15	51.6	3.66 (1H, brs)	36	24.9	1.89 (m) / 1.56 (m)
16	139.6		37	14.7	0.80
17	135.1	4.80 (1H, d, 8.4)	38	22.6	0.81
18	29.5	2.33 (1H, m)	39	14.2	0.66
19	42.7	0.89 (1H, m) / 1.28 (1H, m)	40	16.5	0.67
20	32.7	1.42 (1H, m)	41	18.1	1.66 (3H, s)
21	75.7	2.91 (1H, d)	42	17.2	1.62 (3H, s)

*peak was read from 2D-NMR chart

Table S5. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for compound **4** measured in $CDCl_3$.

no.	δ_C	δ_H (H, mult, J(Hz))	no.	δ_C	δ_H (H, mult, J(Hz))
1	167.1		22	18.3	1.48 (m) / 1.51 (m)
2	103.8		23	31.7	1.38 (m) / 1.42 (m)
3	206.3		24	41.0	
4	59.2		25	126.1	5.38 (s)
5	39.5	2.28 (m)	26	135.7	
6	49.7	2.42 (m)	27	32.0	2.40 (m)
7	211.6		28	37.7	2.50 (dd, 8.4, 14.4) / 1.61 (dd, 3.6, 14.4)
8	49.4	3.07 (dd, 7.2, 16.2) / 2.36 (m)	29	87.4	
9	70.6	3.88 (dd, 9.0, 16.8)	30	200.3	
10	47.0	2.19 (t, 12.0)	31	24.0	2.58 (m)/ 1.92 (m)
11	120.6	5.83 (s)	32	12.2	0.89 (t, 7.8)
12	135.0		33	18.3	1.06 (d, 2.4)
13	58.3	3.43 (s)	34	22.9	1.64 (s)
14	136.7		35	21.4	1.85 (m) / 1.89 (m)
15	141.2	4.97 (d, 9.6)	36	14.7	0.91 (t, 7.8)
16	29.2	2.32 (m)	37	18.2	0.79 (d, 6.6)
17	35.8	0.93 (m) / 0.77 (dd, 4.2, 12.6)	38	16.5	0.97 (d, 6.6)
18	34.7	1.47 (m)	39	16.9	0.93 (d, 6.6)
19	81.9	3.11 (dd, 1.2, 8.4)	40	21.6	1.08 (s)
20	30.4	1.63 (m)	41	21.7	1.70 (s)
21	26.3	1.23 (m) / 1.28 (m)	42	19.9	1.07 (d, 3.6)

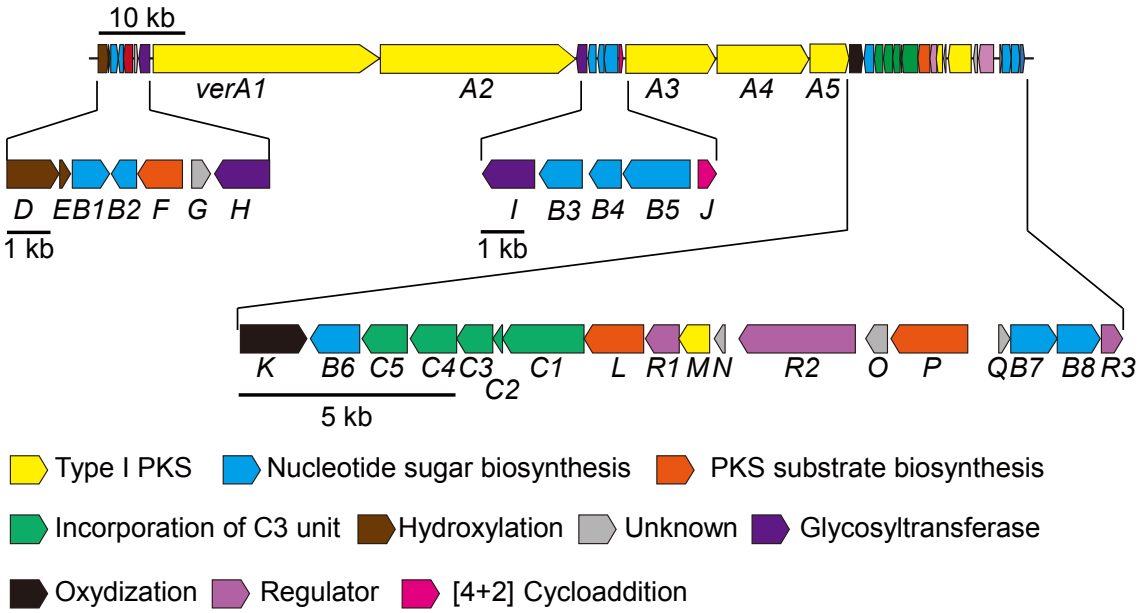


Figure S1. Organization of the biosynthetic gene cluster of versipelostatin in *S. versipellis* 4083-SVS6.

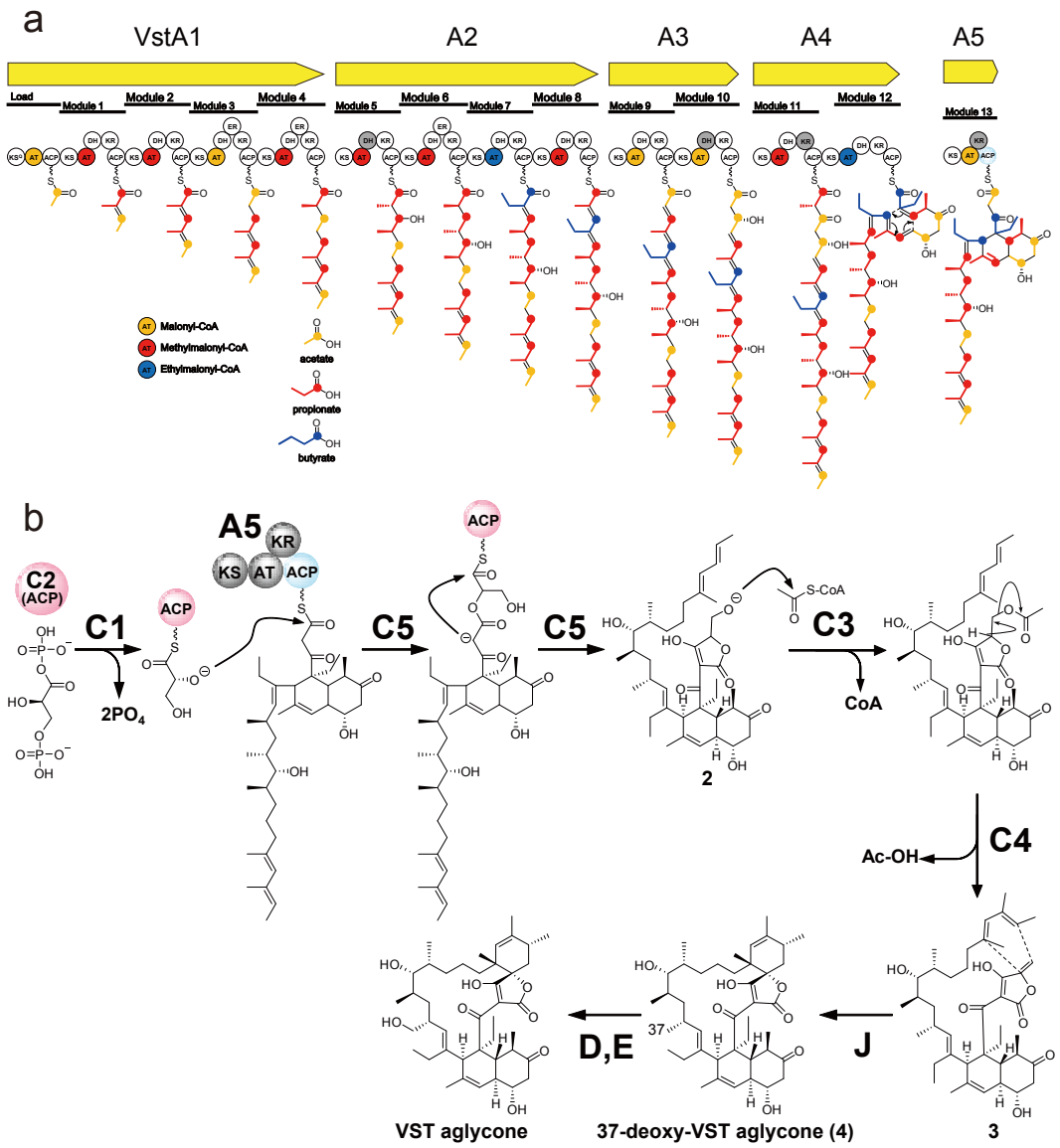


Figure S2. Putative biosynthetic pathway for the formation of VST aglycone.

(a) The deduced modules and domain organization of type I PKS encoded by *vstA1* to *A5*. The bold lines and closed circle indicate the incorporation and labeling pattern of ^{13}C-labeled acetate (yellow), propionate (red) and butyrate (blue) into VST detected in previous experiments.[5] The loading module includes a KSQ domain, which catalyzes decarboxylation of malonyl-ACP to start chain elongation. The L- or D-configuration was deduced from the ketoreductase sequence (Fig. S5). KR11, KR13, DH5, DH10 domains (filled with gray color) are predicted to be inactive from the structure of the VST. Weather DH11 is active or not is unclear since the requisite hydroxyl group is absent due to inactive KR11. (b) Putative biosynthetic pathway of incorporation of tricarbon glyceryl unit into the VST aglycone and subsequent tailoring reactions catalyzed by VstC1−C5. The [4+2]-cycloaddition is catalyzed by VstJ and the following hydroxyl at C-37 is installed by the action of VstD (a cytochrome P450 protein) and VstE (a ferredoxin protein).

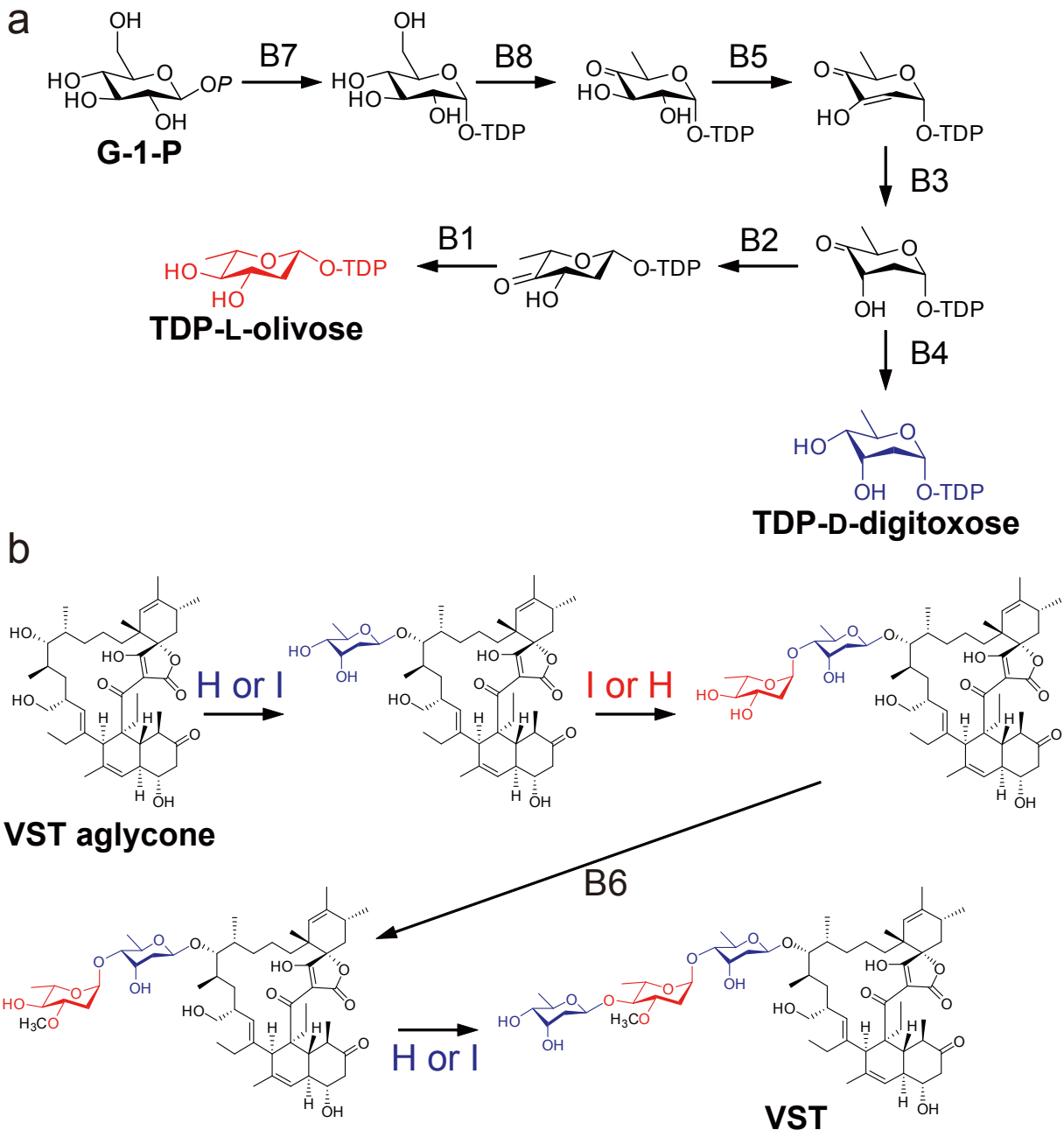


Figure S3. (a) Proposed biosynthetic pathway leading to TDP-D-digitoxose and TDP-L-olivose. (b) Proposed glycosyltransferase reactions catalyzed by VstH and VstI in VST biosynthesis.

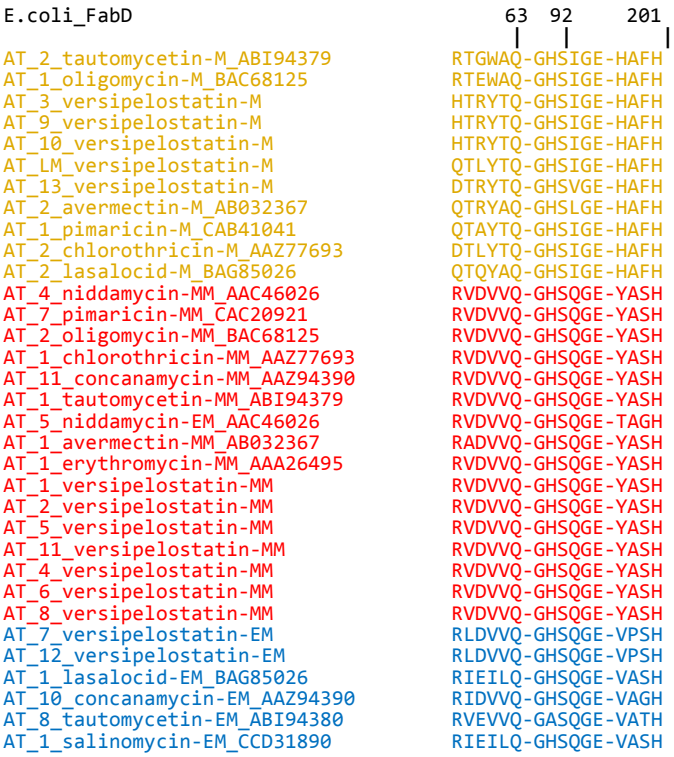


Figure S4. Amino acid sequence alignment of divergent motifs in the AT domains of PKSs for different substrate specificities.[7] Orange, malonyl-CoA; red, methylmalonyl-CoA; blue, ethylmalonyl-CoA.

```
          1                                                   50
KR1       GTVLITGGTG TLGGVVARHL VSEHGVRHLL LTGRRGPEAP GVPELRAELT
KR5       GTVLITGGTG TLGTLLARHL VGEHGVRHLL LASRRGPDAP GAVELVAELT
KR2       GTVLITGGTG TLGAAVACHL VSEHGARHLL LASRSGASAP GALELEAELT
KR3       GTILITGGTG TLATATARHL VTQHGARHLL LASRSGPNAP GAHELQTELT
KR4       GTILITGGTG TLATATARHL VTHHGARHLL LASRSGPNAP GAHELQTELT
KR6       GTILITGGTG TLATATARHL VTHHGARHLL LASRSGPNAP GAHELQTELT
KR11      GTILITGGTG TLATATARHL VTQHGARHLL LASRSGPNAP GAHELQTELT
KR7       GTILITGGTG TLGAAAARHL VHHHGARHLL LASRSGANAP GALELEAELT
KR9       GTILITGGTG TLGAAAARHL VSEHGARHLL LASRSGPNAP GALELEAELT
KR10      GTILITGGTG TLATATARHL VTHHGARHLL LASRSGPNAP GALELEAELT
KR8       GTILITGGTG TLGAAAARHL VSEHGARHLL LASRSGPNAP GALELEAELT
KR12      GTILITGGTG TLGAAAARHL VSEHGARHLL LASRSGPNAP GAHELEAELT
KR13      GTALVSGAAS VLGGQVARWL AGRGARRLLL AVGAREAEAP EVVKLSAELG

          51                                                  100
KR1       EYGAEVTVTA CDTGDPEALA ALLDAVPAEH PLTAVVHAAG VLDDAVTTSL
KR5       AHGAQVTLAS CDTGDPDQLT ALLAGVPAER RLTAVVHTAG ALDDAVIENL
KR2       AHGAHITLTA CDTGDPDQLG DLLAAVPDDH PLTAVIHTAG VLDDAVLENL
KR3       AHGAHITLTT CDTGNPDQLA DLLAAVPDDH PLTAVIHTAG TLHDATLHNL
KR4       AHGAHITLTT CDTGNPDQLA DLLAAVPDDH PLTAVIHTAG TLHDATLHNL
KR6       AHGAHITLTT CDTGNPDQLR DLLAAVPDDH PLTAVIHTAG TLHDATLDNL
KR11      AHGAHITLTT CDTGNPDQLA DLLAAVPDDH PLTAVIHTAG TLHDATLHNL
KR7       AHGAHITLTT CDTGNPTALQ ELLDAIPHDH PLTAVIHTAG TLHDATLDNL
KR9       AHGAHITLTT CDTGNPTALQ ELLDAIPHDH PLTAVIHTAG TLHDATLHNL
KR10      AHGAHITLTT CDTGNPTALQ ELLDAIPHDH PLTAVIHTAG TLHDAVLENL
KR8       AHGAHITLTT CDTGNPDQLT ALLNAIPDDH PLTAVIHTAG TLHDATLDNL
KR12      AHGAHITLTT CDTGNPDQLR DLLAAVPDDH PLTAVIHTAG TLDDAVIENL
KR13      DLGAEVTVAV CDPADRAALA GVLAGVPDGA PLTAVVHVGA AGEAGGVRAL

          101                                                 150
KR1       TAQQLDTVLH PKADAAWHLH RLTRHQDLTA FVLFSSVIGT AGGAGQANYA
KR5       TAEQLETILR SKAEAAWHLH RLTKDLDLAA FVLYSSLAGT LGDAGAASYA
KR2       TPDHLAATLH PKADAAWHLH SLTKDLDLAA FVLYSSVAGT LGSPGQAAYA
KR3       TPDHIDTVLH PKADTAWHLH HLTQNLDLAA FVLYSSAAGT LGNPGQAAYA
KR4       TPDHIDTVLH PKADTAWHLH HLTQNLDLAA FVLYSSAAGT LGNPGQAAYA
KR6       TPDHIDTVLH PKADTAWHLH HLTQHMNLAA FVLYSSAAGT LGNPGQAAYA
KR11      TPDHIDTVLH PKADTAWHLH HLTQNLDLAA FVLYSSAAGT LGNPGQAAYA
KR7       TPDHIDTVLH PKADTAWHLH HLTKNLDLAA FVLYSSVAGT LGSPGQAAYA
KR9       TPDHIDTVLH PKADTAWHLH HLTKNLDLAA FVLYSSAAGT LGNPGQAAYA
KR10      TPDHIDTVLH PKADTAWHLH HLTKNLDLAA FVLYSSAAGT LGNPGQAAYA
KR8       TPDHIDTVLH PKADTAWHLH HLTQHMNLAA FVLYSSAAGT LGSPGQAAYA
KR12      TPDRIDTVLH PKADTAWHLH HLTKNLDLAA FVLYSSVAGT LGSPGQAAYA
KR13      ..ERMDRALV RDVAAVAHLD ELTGGADLRV FTVFSSPSGL ...PGYGGGS

          151                       180
KR1       AANAFLDALA AHRHAQGLPA TSVAWGLWAT
KR5       AANSFLDALA THRHADGLPA MSLGWGFWDQ
KR2       AANTFLDALA THRHTHGLPA TSLAWGLWEE
KR3       TANTFLDALA THRHTHGQPA TSLAWGHWAQ
KR4       TANTFLDALA THRHTHGQPA TSLAWGHWAQ
KR6       AANTFLDALA THRHTHGQPA TSLAWGHWAE
KR11      TANTFLDALA THRHTHGLPA TALAWDTSAE
KR7       AANTFLDALA THRHTHGLPA TSLAWGYWAE
KR9       TANTFLDALA THRHTHGLPA TSLAWGHWAE
KR10      TANTFLDALA THRHTHGQPA TSLAWGLWEE
KR8       AANTFLDALA THRHTHGLPA TSLAWGHWAE
KR12      AANTFLDALA THRHTHGLPA TSLAWGLWEE
KR13      VAEACVEALV RGRRARGLPG LSVLSGPLEE
```

Figure S5. Amino acid sequence alignment of KR domains of VstA1-A5.

The catalytic amino acids are marked by purple shading. The yellow shading indicates the NADPH binding motif. KR13 lacks the NADPH binding motifs and the K112, Y149 and N153 residues required for the reductase activity. All of the KR domains except for KR13 contains LD(H)D residues (shown in light blue shade), a motif specific for D-configuration (B1-type).[8] Although the KR domain in Module 11 seems to be active as deduced from sequence analysis, it should be inactive as predicted from the structure of VST.

```
DH_7      PTHHGQTPTTHPLLTAAIHAADTHRTTLTGRINTTTHPYLTDHAVNGTPLLPGTAYLEMA 60
DH_12     PTHHGQTPTTHPLLTAAIHAADTHRTTLTGRINTTTHPYLTDHAVNGTPLLPATGHLDLA 60
DH_5      PTSLGLAATTHPFLGAIIDTAD-DRTLFTGRISLTTHPWLNDHAVAGTVILPGTAYLDLA 59
DH_11     PTSLGQRTTTHPLLGATIHTAD-DRTLYTGRISLTTHPWLNDHAVAGTVILPGTAYLDLA 59
DH_8      PTALGQRTTTHPLLGATIETADTDRVLFTGRISTTAHAWLNDHAVAGTVILPGTAYLDLA 60
DH_10     PTSLGQRTTTHPFLGAIVETAGTDRTLFTGRISLTTHPWLNDHAVAGTVILPGTAYLDLA 60
DH_2      PTSLGLAATTHPFLGAIIDTAD-DRTLFTGRISLTTHPWLNDHAVAGTVILPGTAYLDLA 59
DH_4      PTSLGQQSATHPLLGATIHTAD-DRTLFTGRISLTTHPWLNDHAVAGTVILPGTAYLDLA 59
DH_3      PTALGLAATTHPLLGATIHTAD-DRTLYTGRISLTTHPWLNDHAVAGTVILPGTAYLDLA 59
DH_9      PTALGLAATTHPLLGATIHTAD-DRTLYTGRISLTTHPWLNDHAVAGTVILPGTAYLDLA 59
DH_6      LAGLGLTATTHPFLGATIHTAD-DRTLYTGRISLTTHPWLNDHAVAGTTLLPGTAYLDLA 59
DH_1      VTSAGLDRSDHPMLGAAVSVAGDDGFVLTGRLSLDTHPWIADHVVLDTVLLPGSAFAELA 60

DH_7      LHAADQVGLNHVEELVIEAPLTLPENGVYDLQVTVGPADDGDRRPITVHSRPAITVSEGS 120
DH_12     LFAAAQVGLNHVEELALEAPLVLPERKDVDLQVTVGPDDGAGRRPLTIHSRPAAKPSAGG 120
DH_5      LHAATHTGHTGIQELTLHQPLVLT-DTPVDLQVTVD-----AQGQITIHSRPAPTDT--- 110
DH_11     LHTGQH-----VQELTLHHPLVLT-DTPVDLQVTVD-----AQGQITIHSRPAPTD---- 104
DH_8      LHAADHTDHTGIQELTLHQPLVLT-DTPVDLQVTVD-----AQGQITIHSRPAPTDT--- 111
DH_10     LHAADRMGLEGVEKLTIDSQLSLPEDGTVDLQVTVDPAADSGQRQITIHSRPAPSDP--- 117
DH_2      LHAATHTGHTGIQELTLHQPLTLT-DTPVDLQVTVD-----AQGQITIHSRPT------- 106
DH_4      LHAATHTDHTGIQELTLHQPLTLT-DTPVDLQLTVD-----SQGQITIHSRPAPTDT--- 110
DH_3      LHTATHTDHTGIQELTLHQPLTLT-DTPVDLQLTVD-----SQGQITIHSRPAPTDT--- 110
DH_9      LHTATHTDHTGIQELTLHQPLTLT-DTPVDLQLTVD-----SQGQITIHSRPAPTDT--- 110
DH_6      LHTGQH-----VQELTLHHPLVLT-DTPVDLQVTVD-----SQGQITIHSRPAPTDT--- 105
DH_1      LEAGAQAGCGRVEELVLEAPLALADDHAVQIQVTVGDPDEEGHRPLAVHSRPQN------ 114

DH_7      PDDAADLPWTRHATGTLTSTEEE-------PPALDGDRSWPPADSSPLDLDGFYERIAQH 173
DH_12     PDDASDIPWTRHATGTLTTSEQA-------PPAE-GDEAWPPAGTAPIDLDGFYERIAQH 172
DH_5      --DDADPAWTTHATGQLTTETVS-------VAPDT-PTTWPPTNATPISLDGFYDHLAGL 160
DH_11     --DDADPTWTTHATGQLTTDTPP-------VPHET-ATTWPPPEATPIELDDHYDRFADI 154
DH_8      --DDADPAWTTHATGQLTTTPPG-------TAPDT-PTTWPPTNATPISLDGFYTSTAER 161
DH_10     --DEPG-TWTRHATGQLTTTPPG-------TAPDT-PTTWPPTNATPISLDGFYDHLADR 166
DH_2      ---DNDAAWTTHATGQLTTDTVS-------TVPET-AAAWPPPGATPIALDGFYDHLADR 155
DH_4      --DDADPTWTTHATGQLTTDTAS-------TVPET-AAAWPPPGATPISLDGFYDHLADR 160
DH_3      --DDADPTWTTHATGQLTTDTAT-------DIPATDAAAWPPLDATPISLDGFYDHLADR 161
DH_9      --DDADPTWTTHATGQLTTDTAT-------DIPATDAAAWPPLDATPISLDGFYDHLADR 161
DH_6      --DDADPTWTTHATGQLTTDTVT-------DIPATDAAAWPPPDATPISLDGFYDHLADR 156
DH_1      --DSADRPWTRHATGTLSDGREGGGGGGGGNGAGVMESAWPPVGAVPVDPSGLYARLAEQ 172

DH_7      GYHYGPVFQGLTAAWQHEDSIYAEVTLPEGTDT--------------AGYGVHPALLDAA 219
DH_12     GYHYGPVFQGLTAAWNGDDHTCAEAALPEGTDT--------------AGYGIHPALLDAA 218
DH_5      GVDYGPVFQGLTAAWREDNELYAEVDLPEDTDT--------------TGYGIHPALLDAA 206
DH_11     GVDYGPTFQGLTAAWRHGNDLYAEVTLPEDTDT--------------TGYGIHPALLDAA 200
DH_8      GYHYGPAFQGLTAAWRHGNDLYAEVTLPEDTDA--------------TGYGIHPALLDAV 207
DH_10     GYHYGPAFQALTTAWRHGNDLYAEVTLPEDTDT--------------TGHGIHPALLDAA 212
DH_2      GYHYGPAFQGLTTAWRHGDELHAEVTLPEDTDA--------------TGFGVHPALLDAA 201
DH_4      GYHYGPAFQALTTAWRHGNELHAEVTLPEDTDT--------------TGFGIHPALLDAA 206
DH_3      GYHYGPAFQALTTAWRHGNDLYSEVTLPEDTDA--------------TGFGIHPALLDAA 207
DH_9      GYHYGPAFQALTTAWRHGNDLYSEVTLPEDTDA--------------TGFGIHPALLDAA 207
DH_6      GYHYGPAFQALTTAWRHGNDLYAEVTLPEDTDT--------------TGYGIHPALLDAA 202
DH_1      GYVYGPMFQGLRAAWQLDGELFAEVDLPQDDAEPSVLAARSDGVRAGAGFGVHPALLDAT 232

DH_7      LHATTATVG-DDVYAGKVYLPFVWSGVTLHNTA-ASGTVRVHLTRPDDERISVRLRDESG 277
DH_12     LQGSLATLG-EDAMD-QVQLPFSWRGVTLHASG-P-AALRAHLTPTGSDSIALRVLDGAG 274
DH_5      LQATTHPAFVGETGTATPVMPFSWNGITLHTPT-TPTTLRAHLTPAGDTSFAIHLADSAD 265
DH_11     LQVSAHTSPTNEANT-TPVMPFSWTGITLHTPT-TPTTLRAHLTPTNDTSVAIALTSETG 258
DH_8      FHPLLT----TDTDP-AIRLPFSWTGITLHTPT-TSTALRATITTTSDTTLTIHLTDTTG 261
DH_10     LHPLITNN--ADTDT-AIRLPFSWTGIALHATT-AATTLRVRLTPTSDTTLAIHLTDTSG 268
DH_2      LHPLLTTN---DTGT-EIRLPFSWTGITLHTTT-APTALRATITTTSDTTLTIHLTDTTG 256
DH_4      LHPLLTTNT--DTGS-EIRLPFSWTGITLHTTTTAASTLHVHLTPTSDTTFAIALTTSTG 263
DH_3      LHPLITNNTNADTDT-AIRLPFSWTGIALHATT-AATALRVHLTTTSDTTLAIHLTDTSG 265
DH_9      LHPLITNNTNADTDT-AIRLPFSWTGIALHATT-AATALRVHLTTTSDTTLAIHLTDTSG 265
DH_6      LHPLITNNTNTNTDT-AIRLPFSWTGIALHATT-AATTLRVHLTTTSDTTLAIHLTDTSG 260
DH_1      LHALAVNGLLGGEQG-EIRLPFSWTGVELHAAG--ATSVRVRITESGTDAVTVTITDTAG 289

DH_7      EAVATVRAVAVRPIDPAKLAV 298
DH_12     QPVVTVDALTVRPLDTRRLAS 295
DH_5      ESILTIDALAVRPIDIDRFRA 286
DH_11     EPVATIQTLTVRPVDPAQLTT 279
DH_8      EPVATIDALTVRPVDPAQLAT 282
DH_10     QPIATIEALTVRPVDPAQLTT 289
DH_2      EPVATIQGLTLRPIDAAQLAT 277
DH_4      EPVATIDALTVRPVDPTQLAA 284
DH_3      QPIATIEALTVRPVDPAQLAT 286
DH_9      QPIATIEALTVRPVDPAQLAT 286
DH_6      QPIATIEALTVRPVDPTQLAA 281
DH_1      VPVAVVESLTTRPVSAQRLGA 310
```

Figure S6. Amino acid sequence alignment of DH domains of VstA1-A4. The catalytic amino acids are marked by purple shading. DH domains in Module 5 and Module 10 seem to be inactive based on the fact that VST structure does not have the corresponding double bonds.

```
ER_4      TVDNLALVPHPTDTTPLPPGHVRVAVHAAGINFRDLLVTLGMVDDPRPIGGEGAGTITA 60
ER_6      TVDNLALVPHPTDTTPLPPGHVRVAVHAAGINFRDLLVTLGMVDDPRPIGGEGAGTITA 60
ER_3      TVDNLALVPHPADTTPLPPGHVRVAVHAAGINFRDLLVTLGMVDDPRPIGGEGAGTITA 60

ER_4      APDVTDYQPGDRVMGLFPHTAPHITVHQHHIAPVPHHLTTAQAATTPVAFLTAYHALHH 120
ER_6      APDVTDYQPGDRVMGLFPHTAPHITVHQHHIAPVPHHLTTAQAATTPVAFLTAYHALHH 120
ER_3      APDVTDYQPGDRVMGLFPHTAPHITVHQHHIAPVPHHLTTAQAATTPVAFLTAYHALHH 120

ER_4      AHLQPGEKVLIHAGTGGVGMAAIQIARHLGADIYATAHPTKWPTLHHLGLDQHHIASSR 180
ER_6      AHLQPGEKVLIHAGTGGVGMAAIQIARHLGADIYATAHPTKWPTLHHLGLDQHHIASSR 180
ER_3      AHLQPGEKVLIHAGTGGVGMAAIQIARHLGADIYATAHPTKWPTLHHLGLDQHHIASSR 180

ER_4      LDFEHHFRTTAPHGLDVILNSLAGEHTDASLRLLNPTTGRFIEMGKTDIREPAQLAAEH 240
ER_6      LDFEHHFRTTAPHGLDVILNSLAGEHTDASLRLLNPTTGRFIEMGKTDIREPAQLAAEH 240
ER_3      LDFEHHFRTTAPHGLDVILNSLAGEHTDASLRLLNPTTGRFIEMGKTDIREPAQLAAEH 240

ER_4      HLTYQAFDLITQTTPHHIHHMLHHLTHLLTQHHLTPLPVTTWDIRHTPHAFRHLSQARH 300
ER_6      HLTYQAFDLITQTTPHHIHHMLHHLTHLLTQHHLTPLPVTTWDIRHTPHAFRHLSQARH 300
ER_3      HLTYQAFDLITQTTPHHIHHMLHHLTHLLTQHHLTPLPVTTWDIRHTPHAFRHLSQARH 300

ER_4      GKLAL 306
ER_6      GKLAL 306
ER_3      GKLAL 306
```

Figure S7. Amino acid sequence alignment of ER domains of VstA1 and A2.

The catalytic amino acids are marked by purple shading. The yellow shading indicates the NADPH binding motif. All of the ER domains are specific for D-configuration (Y residue specific for L-configuration was not observed at V residue position shown in light blue shade)[9]

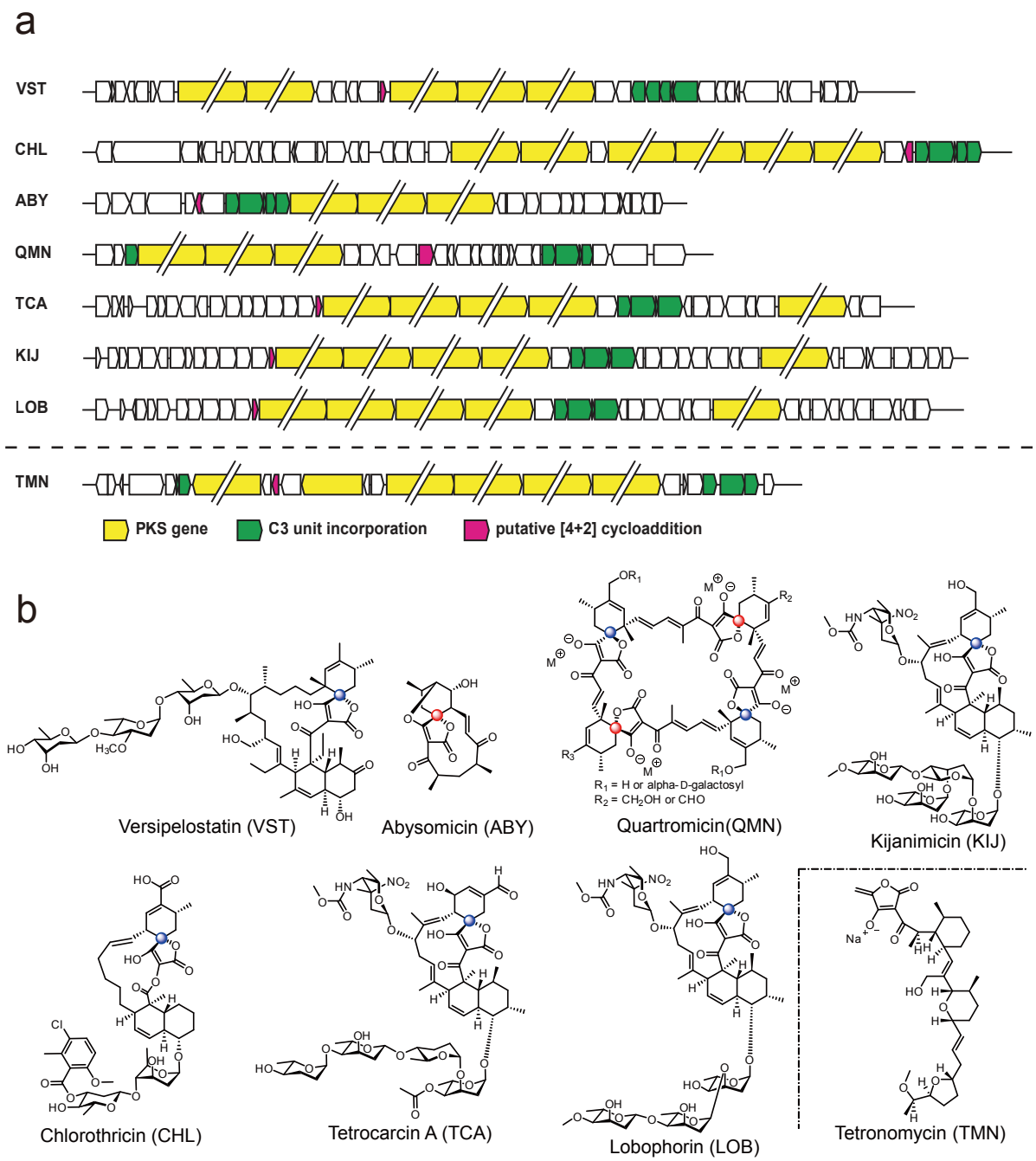
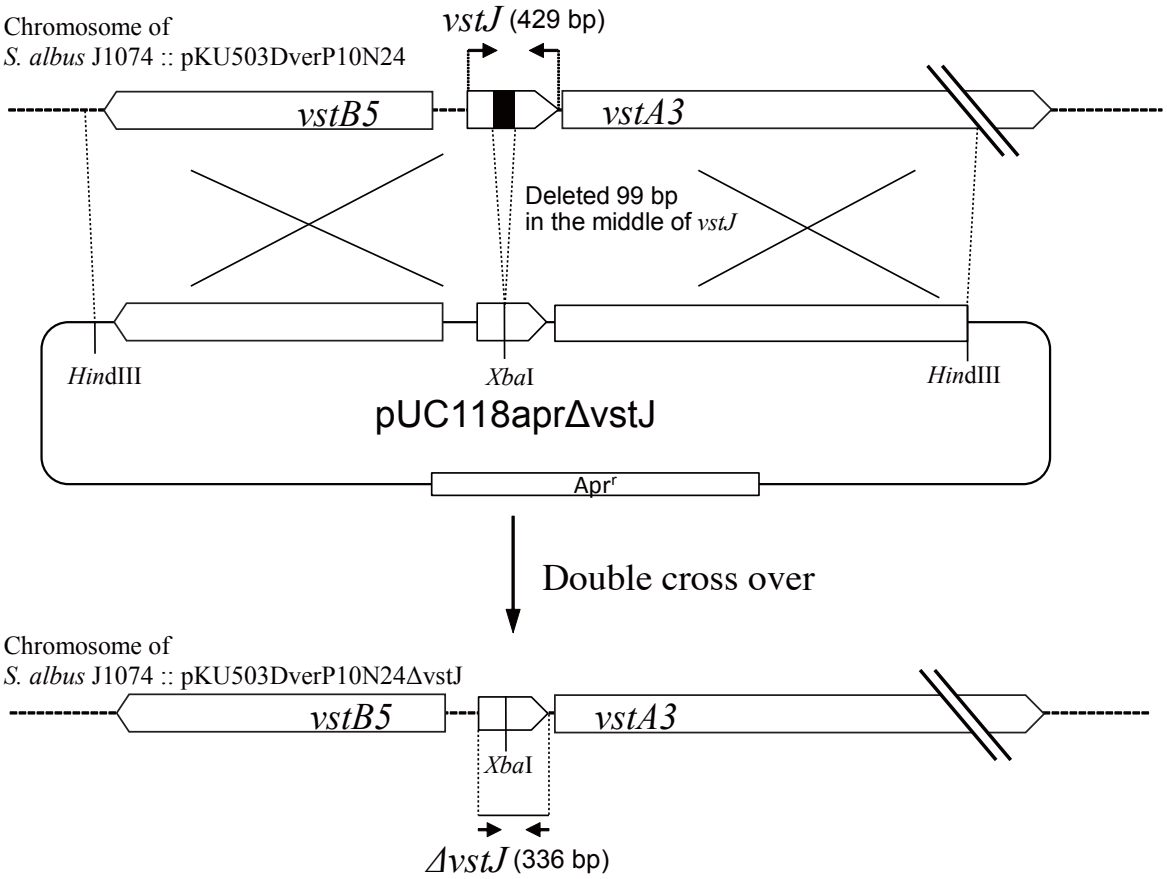
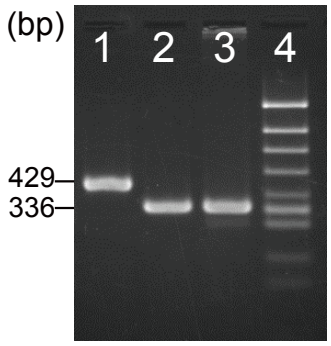


Figure S8. Biosynthetic gene clusters and structures of (spiro)tetronate antibiotics.
(a) All known biosynthetic gene clusters of spirotetronate antibiotics, VST, CHL, QMN, TCA, LOB ABY, and KIJ, contain *vstJ* homologues. Furthermore, the biosynthetic gene cluster of tetronomycin (TMN),[10] that is not a spirotetronate, but a tetronate compound, also contains a *vstJ* homologue. (b) The *R*- or *S*-configuration of the spirocarbon is presented as red or blue circle, respectively.

a



b



1: *S. albus* J1074 :: pKU503DverP10N24
2: *S. albus* J1074 :: pKU503DverP10N24ΔvstJ
3: pUC118aprΔvstJ
4: ΦX174 HincII marker

Figure S9. Disruption of *vstJ* in the genome of *S. albus* J1074:: pKU503DverP10N24.
(A) Scheme for disruption of *vstJ*. (B) Confirmation with PCR. Disruption of *vstJ* was confirmed by PCR. PCR with genomic DNA from the *S. albus* J1074:: pKU503DverP10N24 strain gave a 429-bp DNA fragment (lane 1), whereas PCR with genomic DNA from the mutant strains gave a 336-bp fragment (lanes 2 and 3).

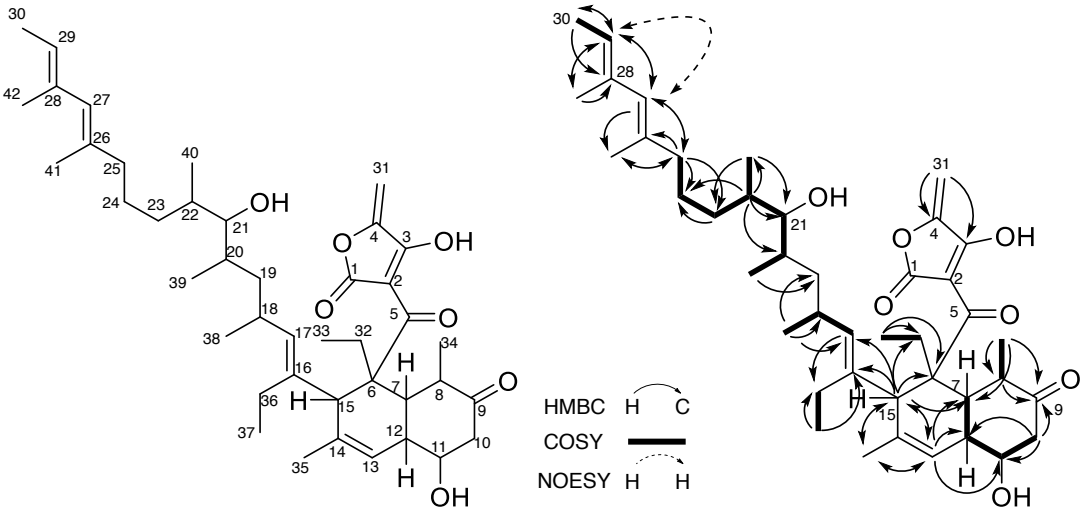


Figure S10. Structure of **3** and the correlations of HMBC, DQF-COSY, and NOESY of **3**.

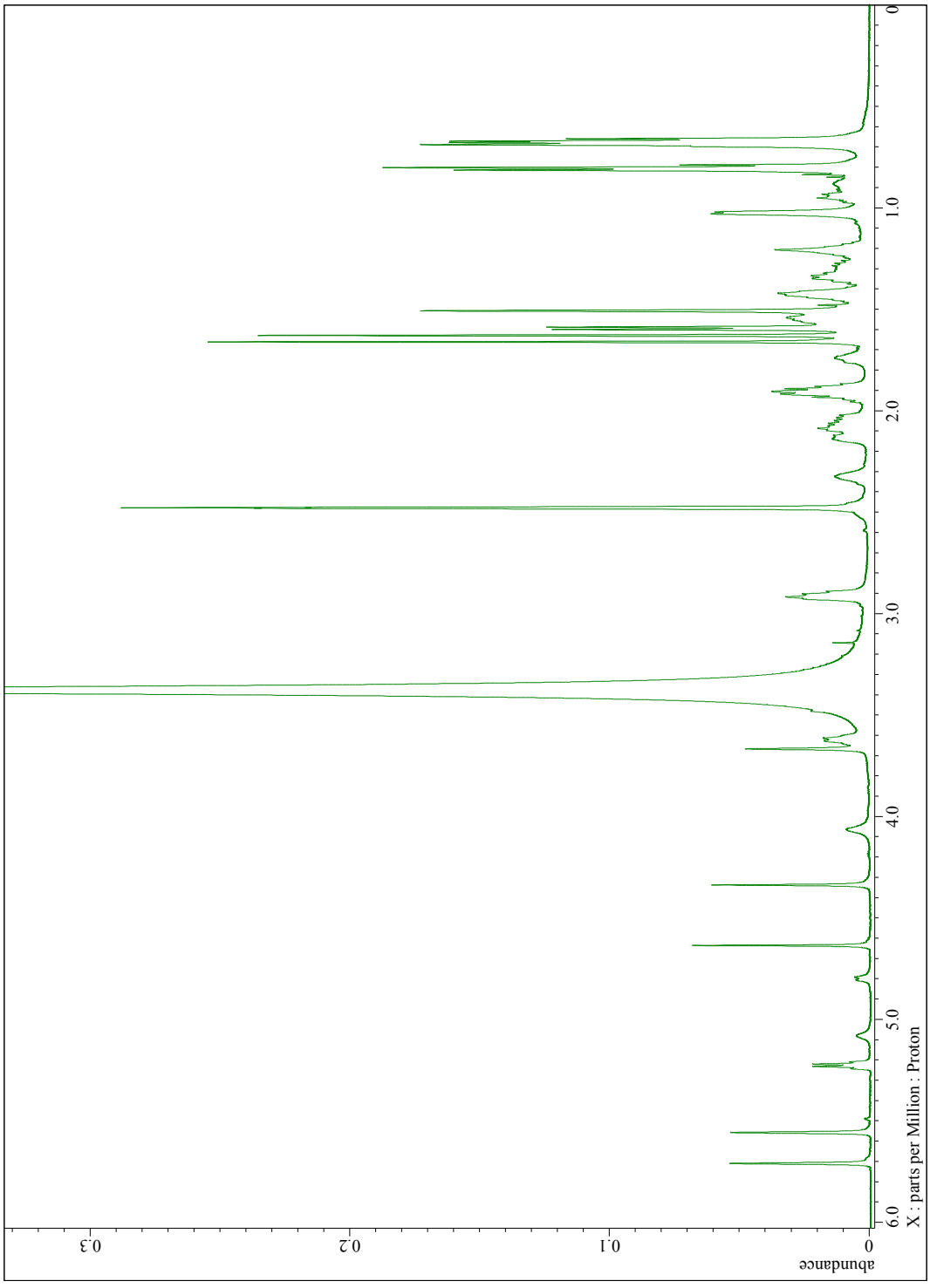


Figure S11. ^{1}H NMR (DMSO-*d6*) spectrum for **3**.

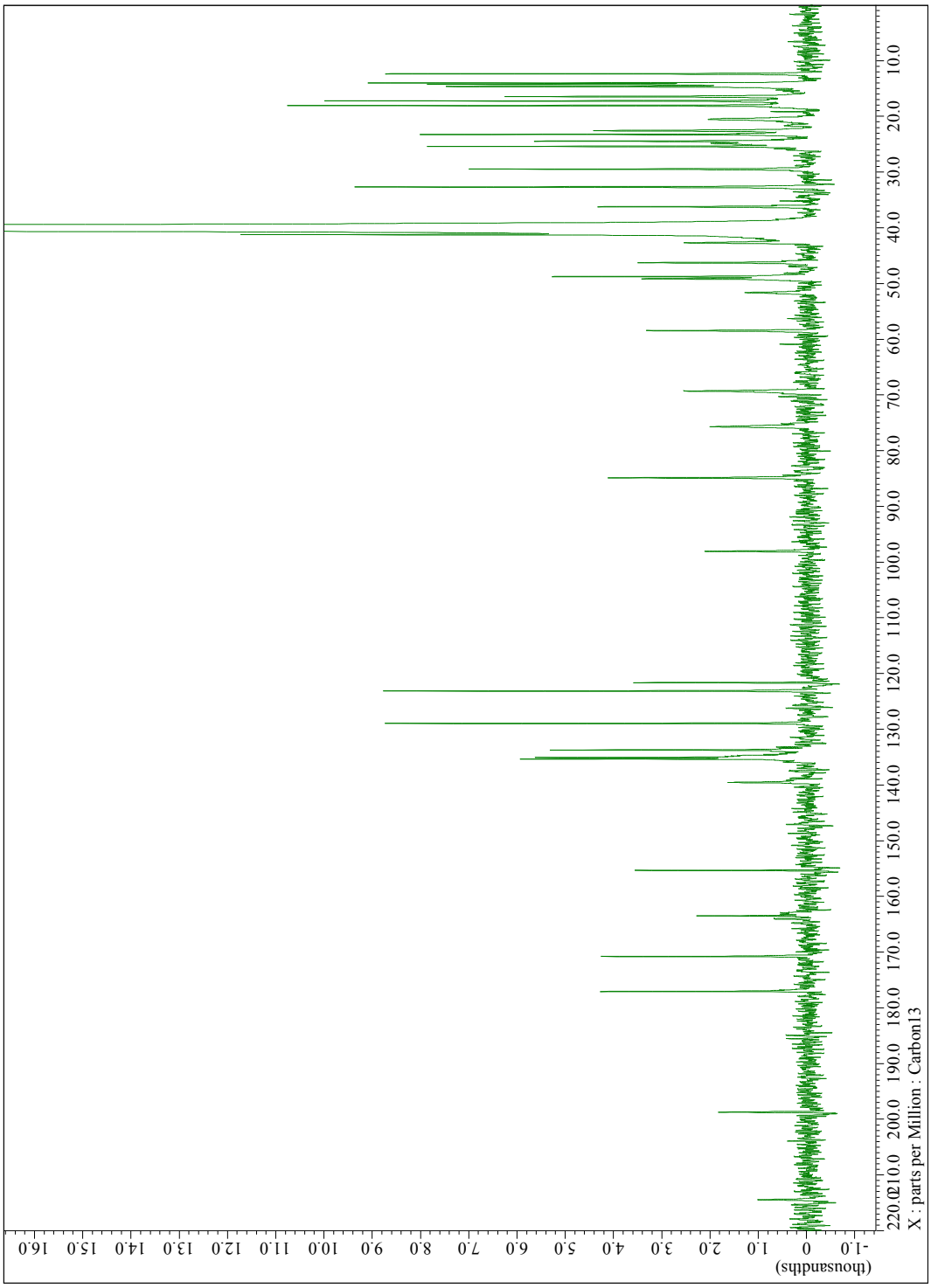


Figure S12. ^{13}C NMR (DMSO-*d6*) spectrum for **3**.

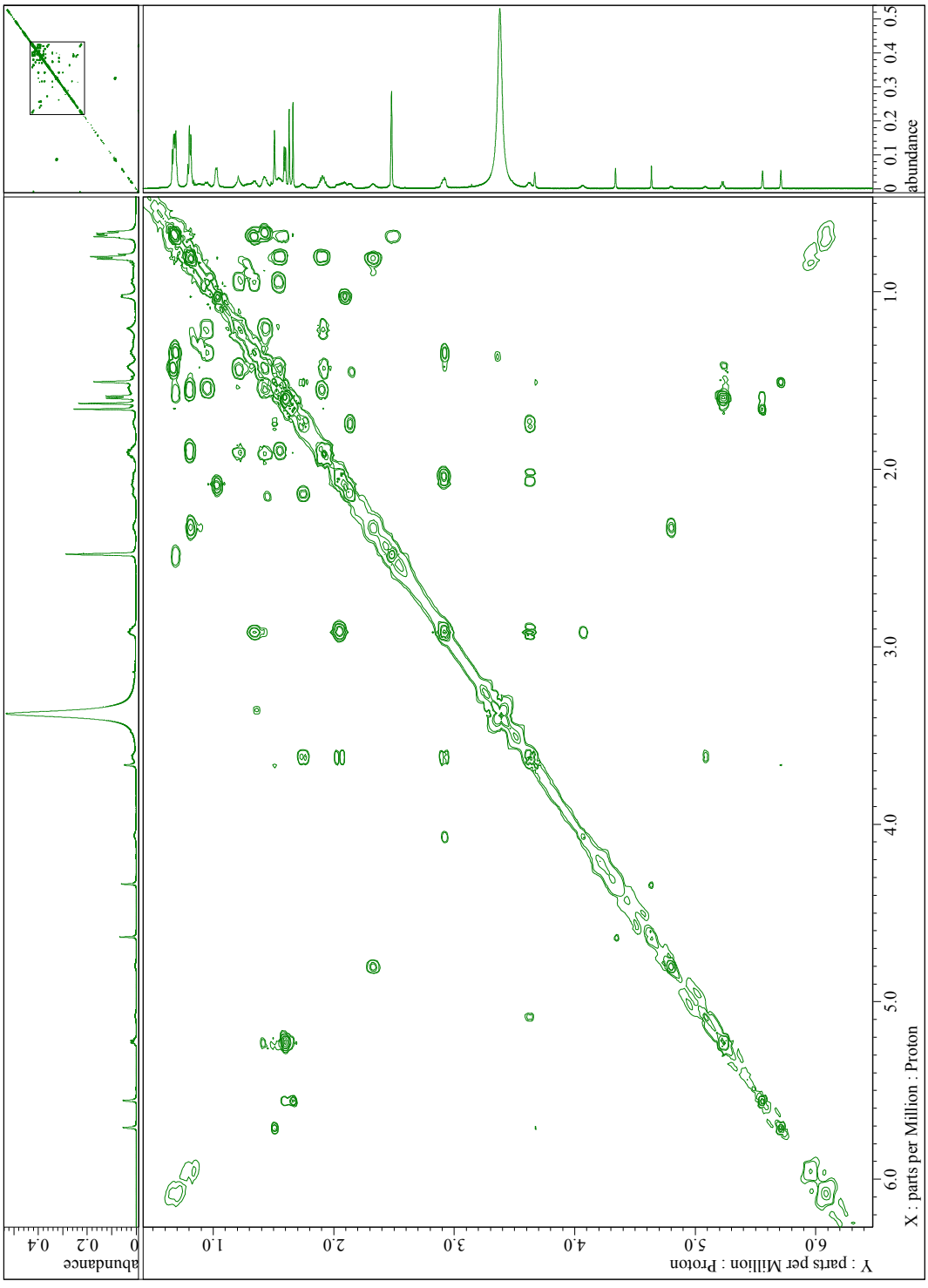


Figure S13. DQF-COSY spectrum for **3**.

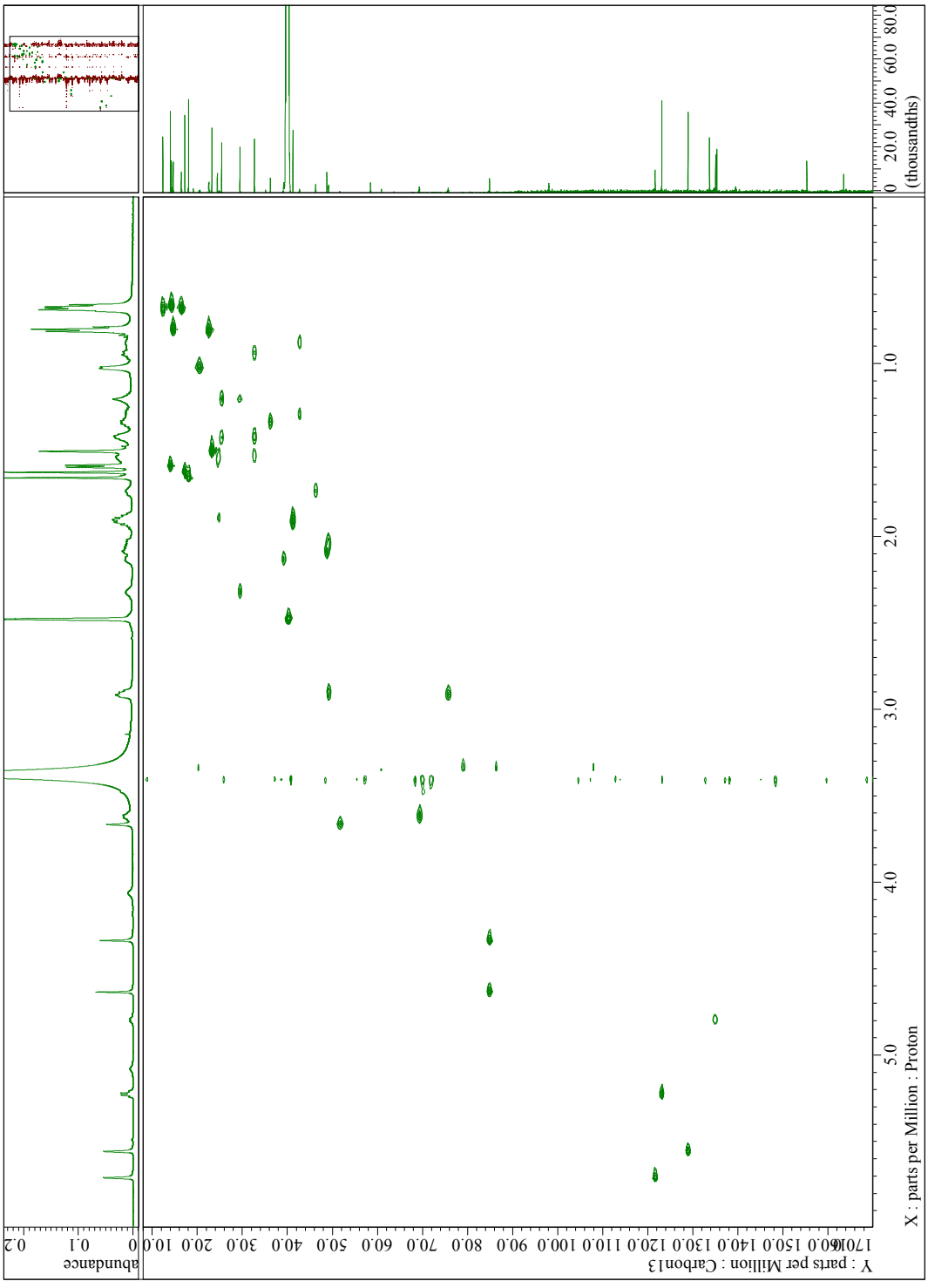


Figure S14. HSQC spectrum for **3**.

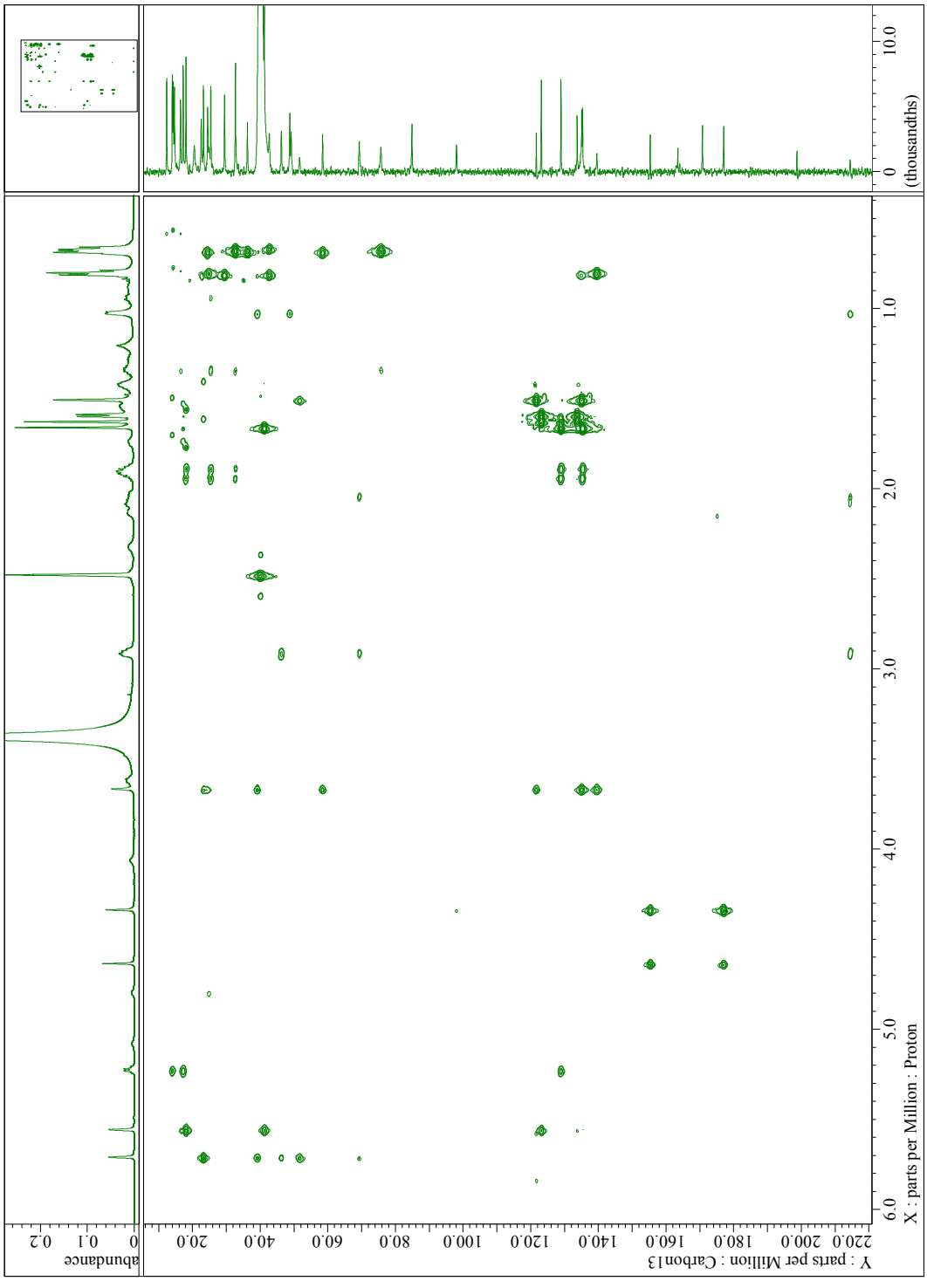


Figure S15. HMBC spectrum for **3**.

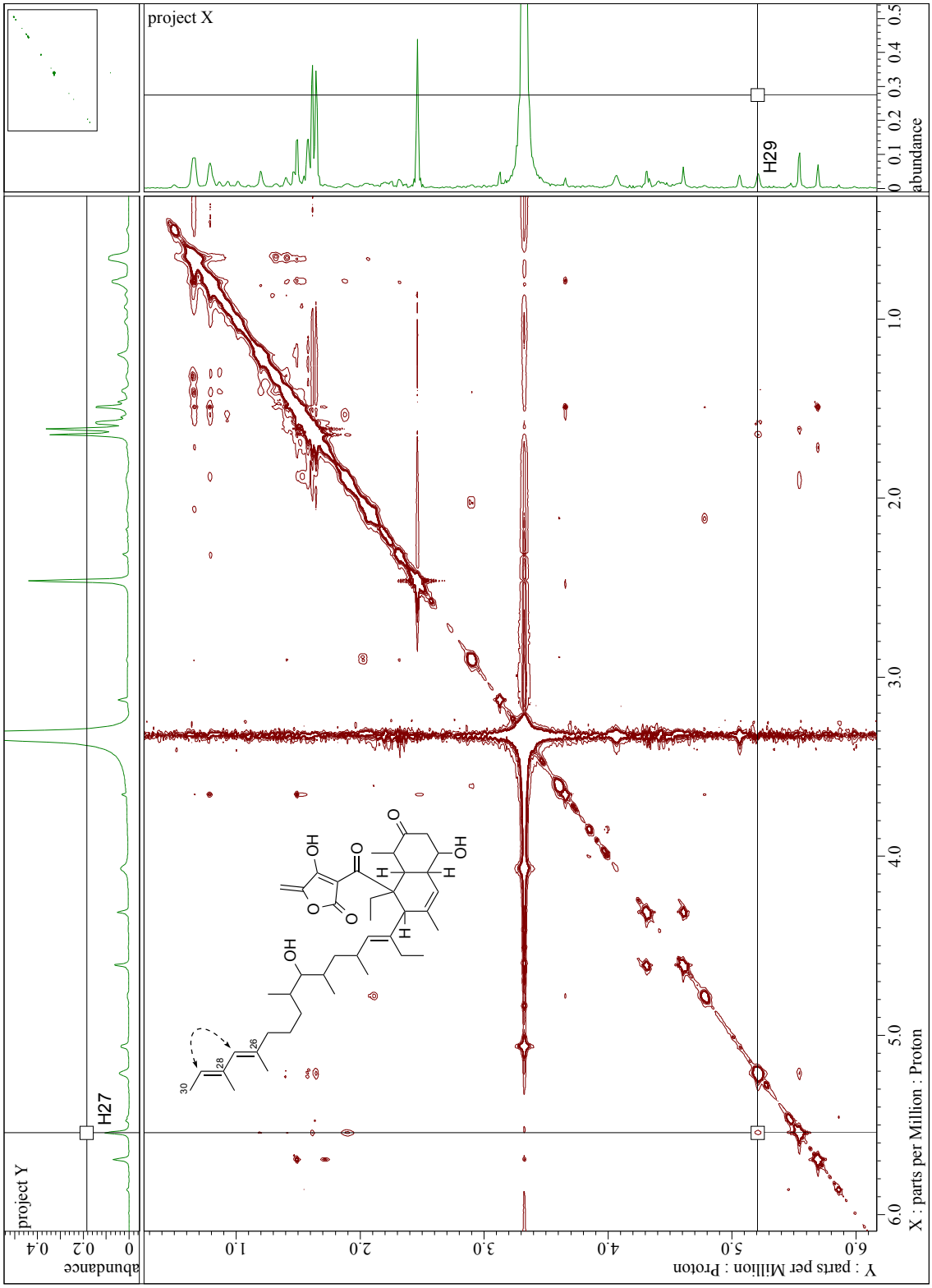


Figure S16. NOESY spectrum for **3**.

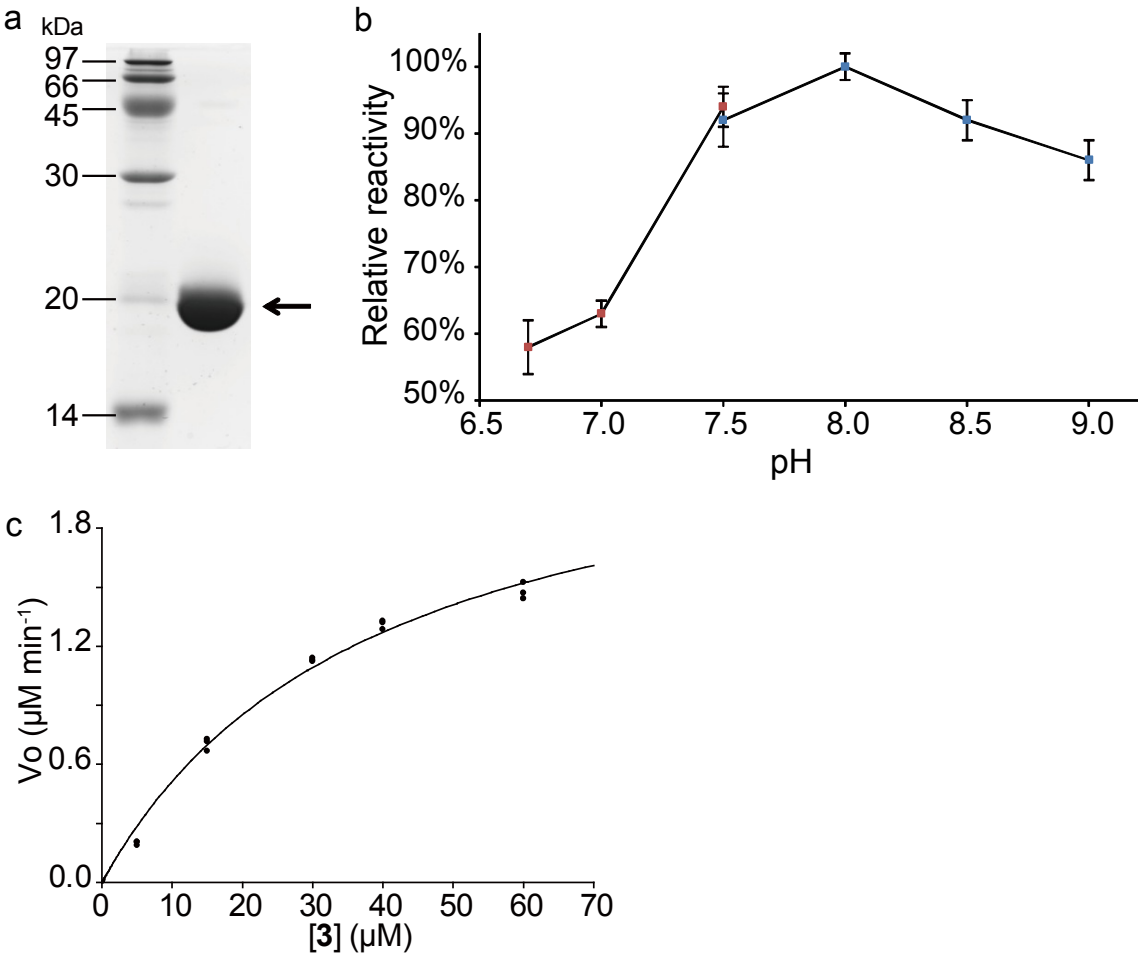


Figure S17. Characterization of VstJ.

(A) SDS-PAGE analysis. The estimated molecular weight of His8-VstJ is 17,400 Da. (B) pH dependence of the VstJ reaction. Red and blue dot represent the relative reactivities in 50 mM HEPES-NaOH or Tris-HCl buffer at each pH, respectively. (C) Michaelis-Menten plot for VstJ against **3**.

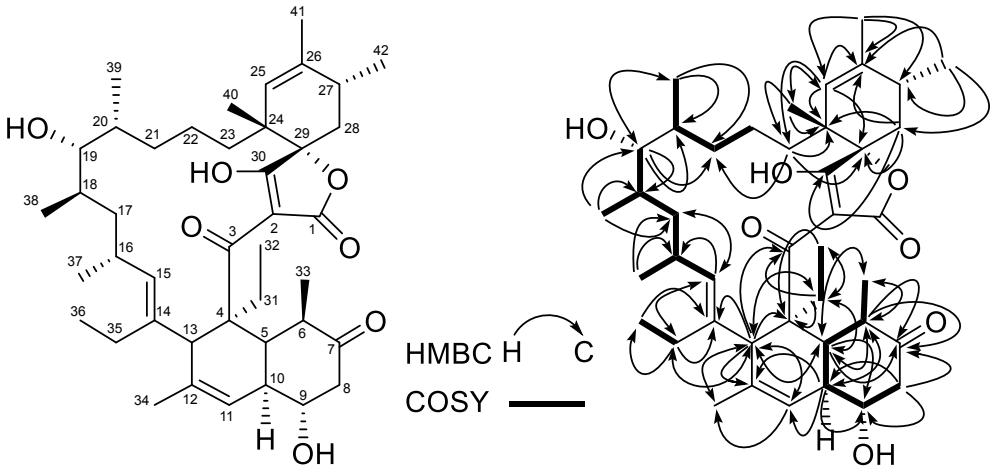


Figure S18. Structure of **4** and correlations of HMBC and DQF-COSY of **4**.

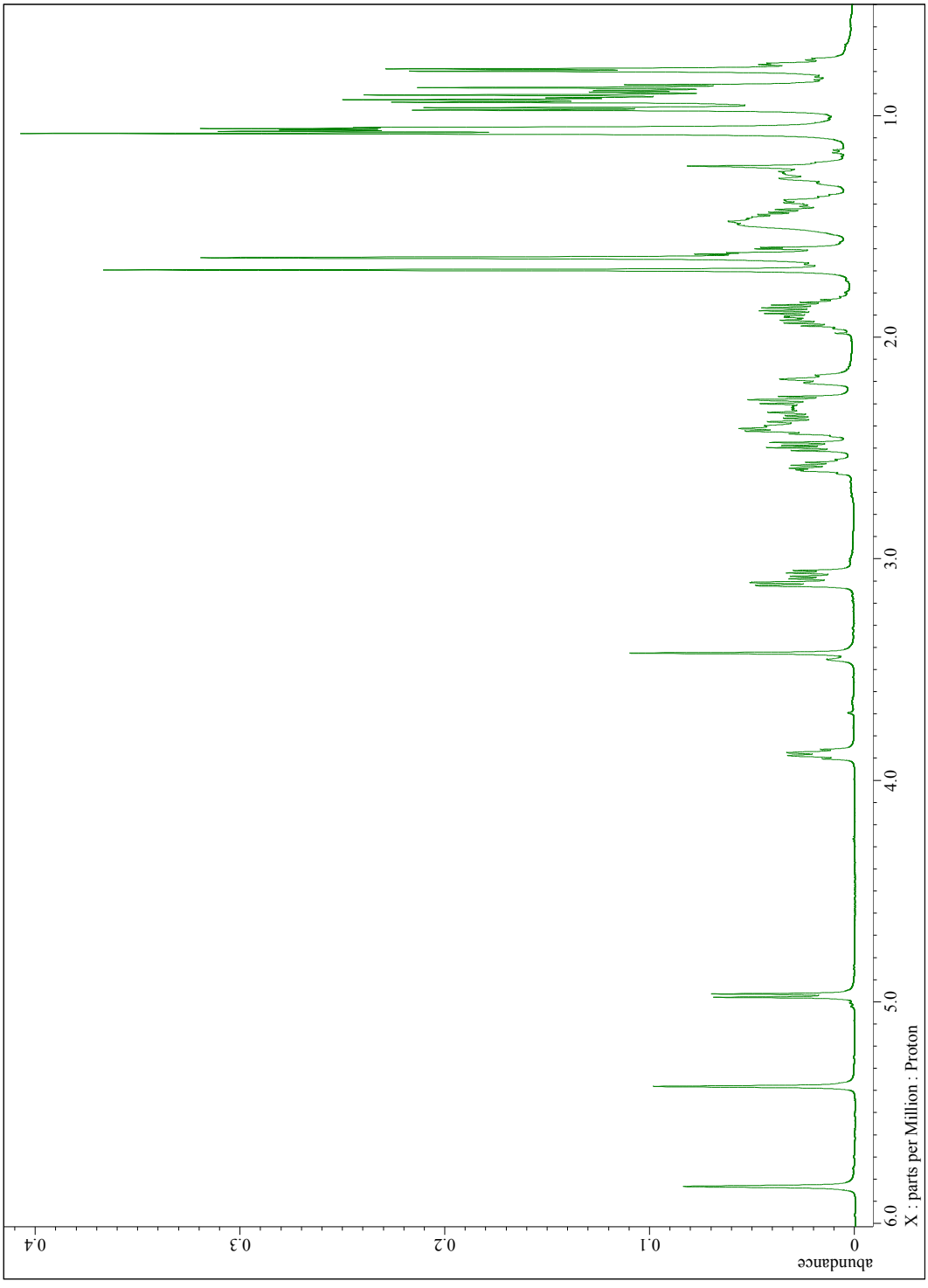


Figure S19. ^{1}H NMR ($CDCl_3$) spectrum for **4**.

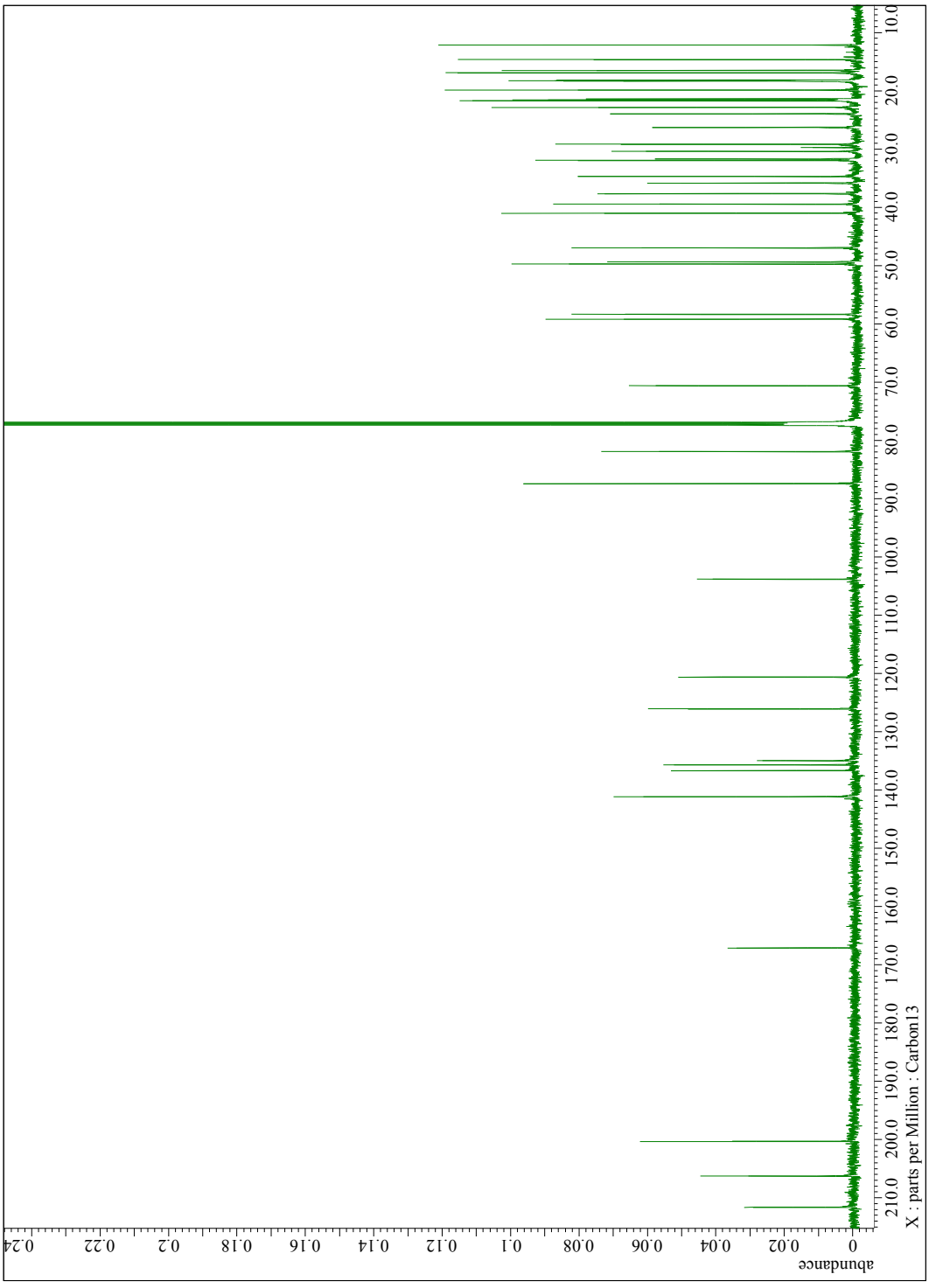


Figure S20. ^{13}C NMR ($CDCl_3$) spectrum for **4**.

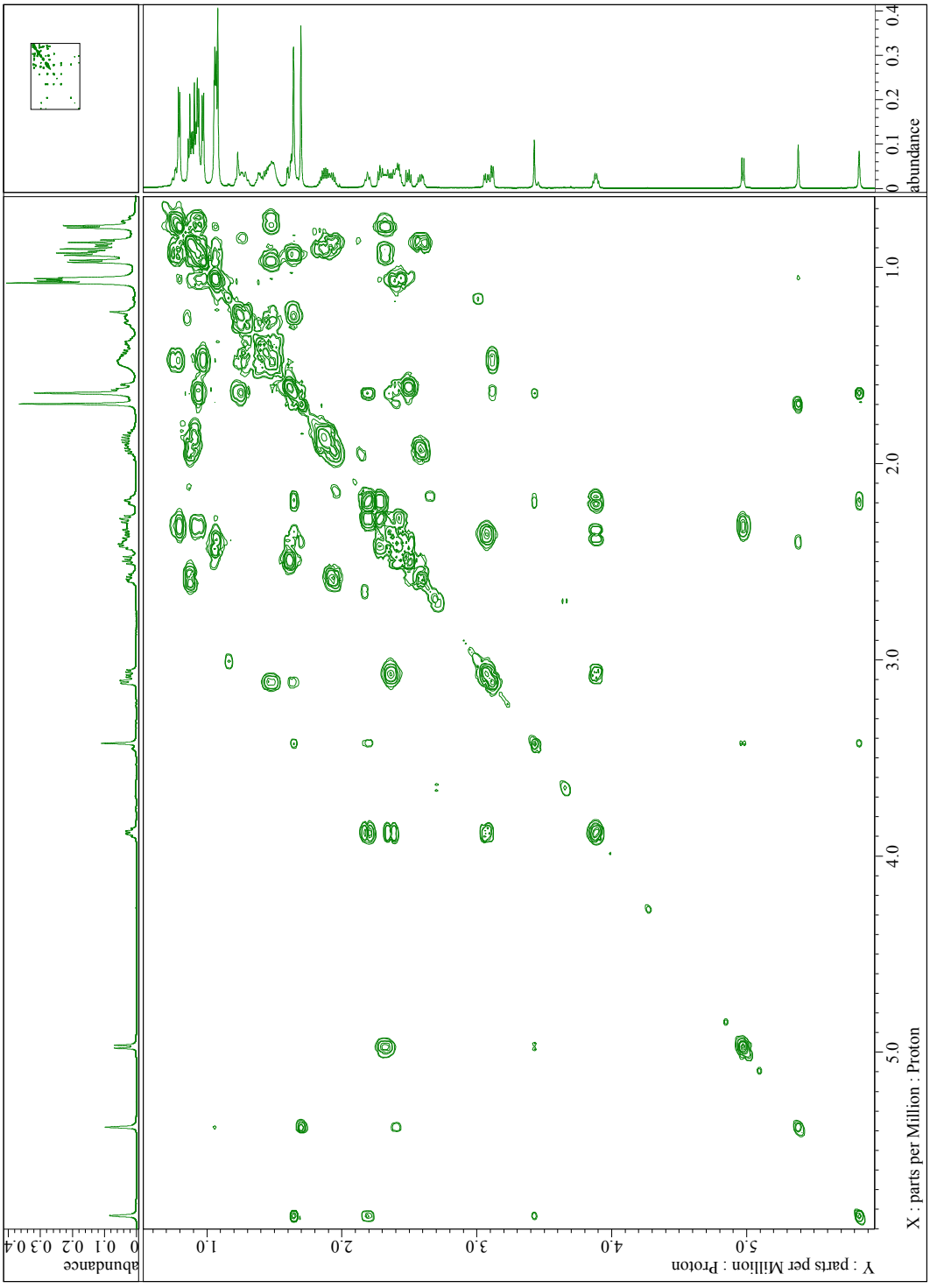


Figure S21. DQF-COSY spectrum for **4**.

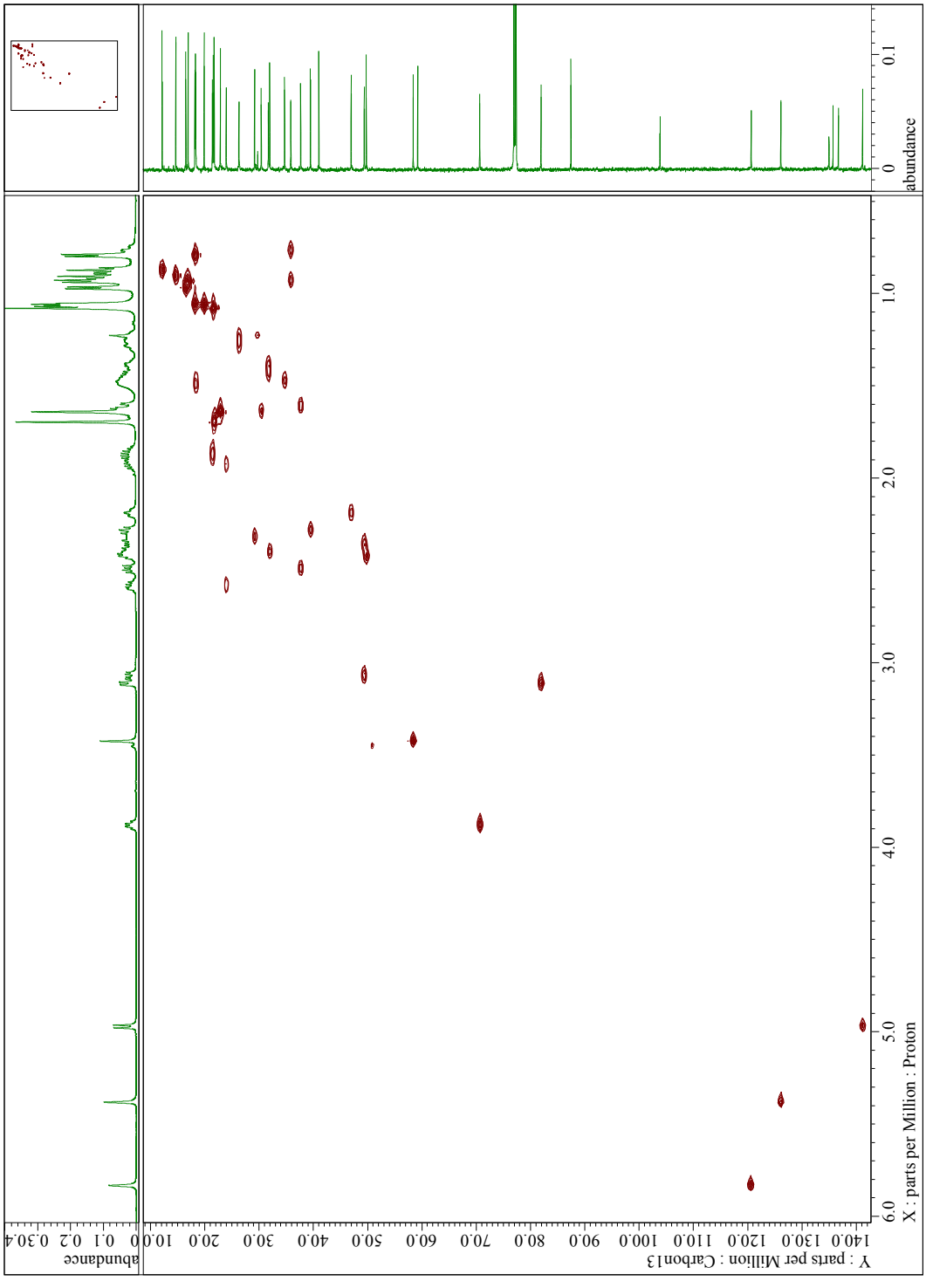


Figure S22. HSQC spectrum for **4**.

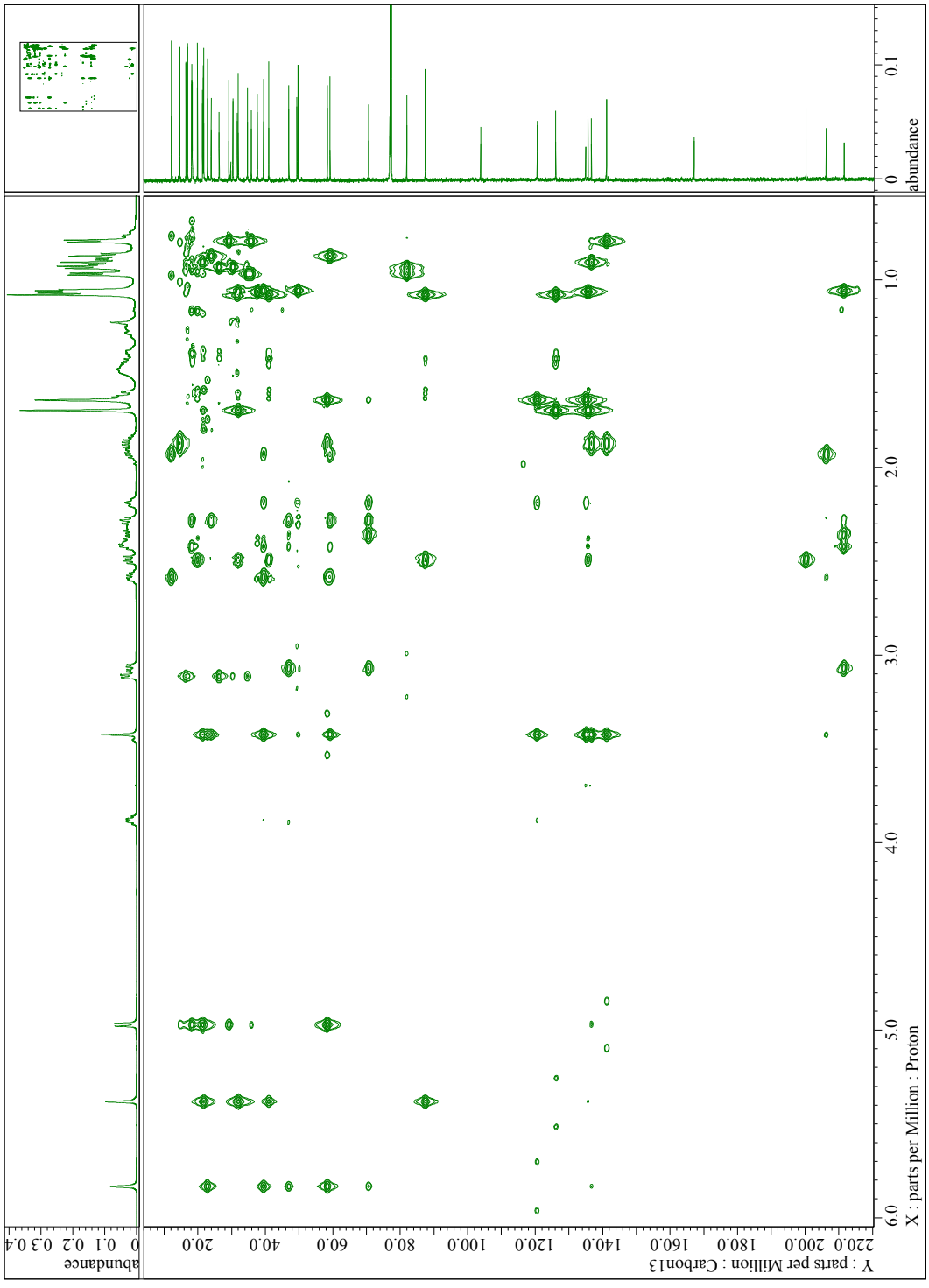


Figure S23. HMBC spectrum for **4**.

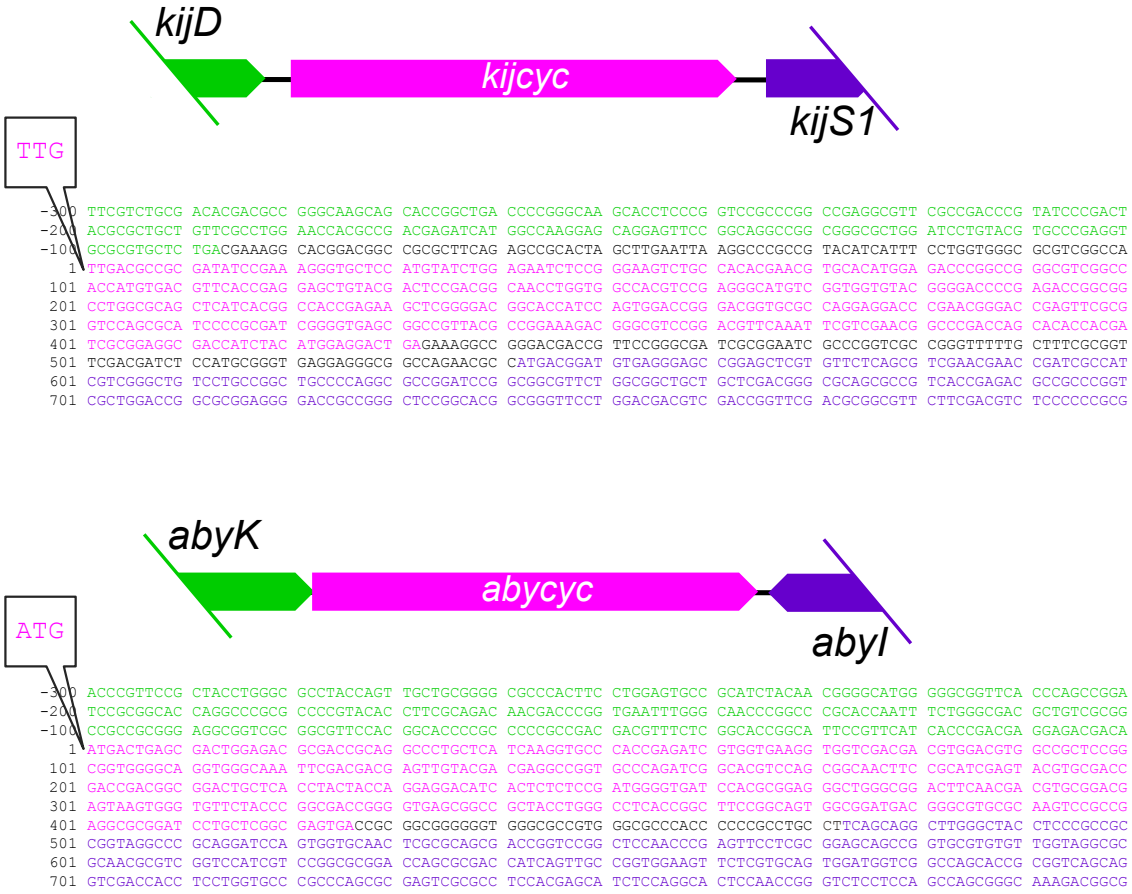


Figure S24. Annotation of VstJ homologues, KijCyc and AbyCyc in the biosynthetic gene clusters of kijanimicin and abyssomicin.

Biosynthetic gene clusters of kijanimicin (accession number: EU301739) and abyssomicin (accession number: JF752342) have been reported. VstJ homologues in biosynthetic gene clusters of Kijanimicin and Abyssomicin are not annotated as genes. Here, we name each of additional genes *kijCyc* and *abyCyc*, respectively.

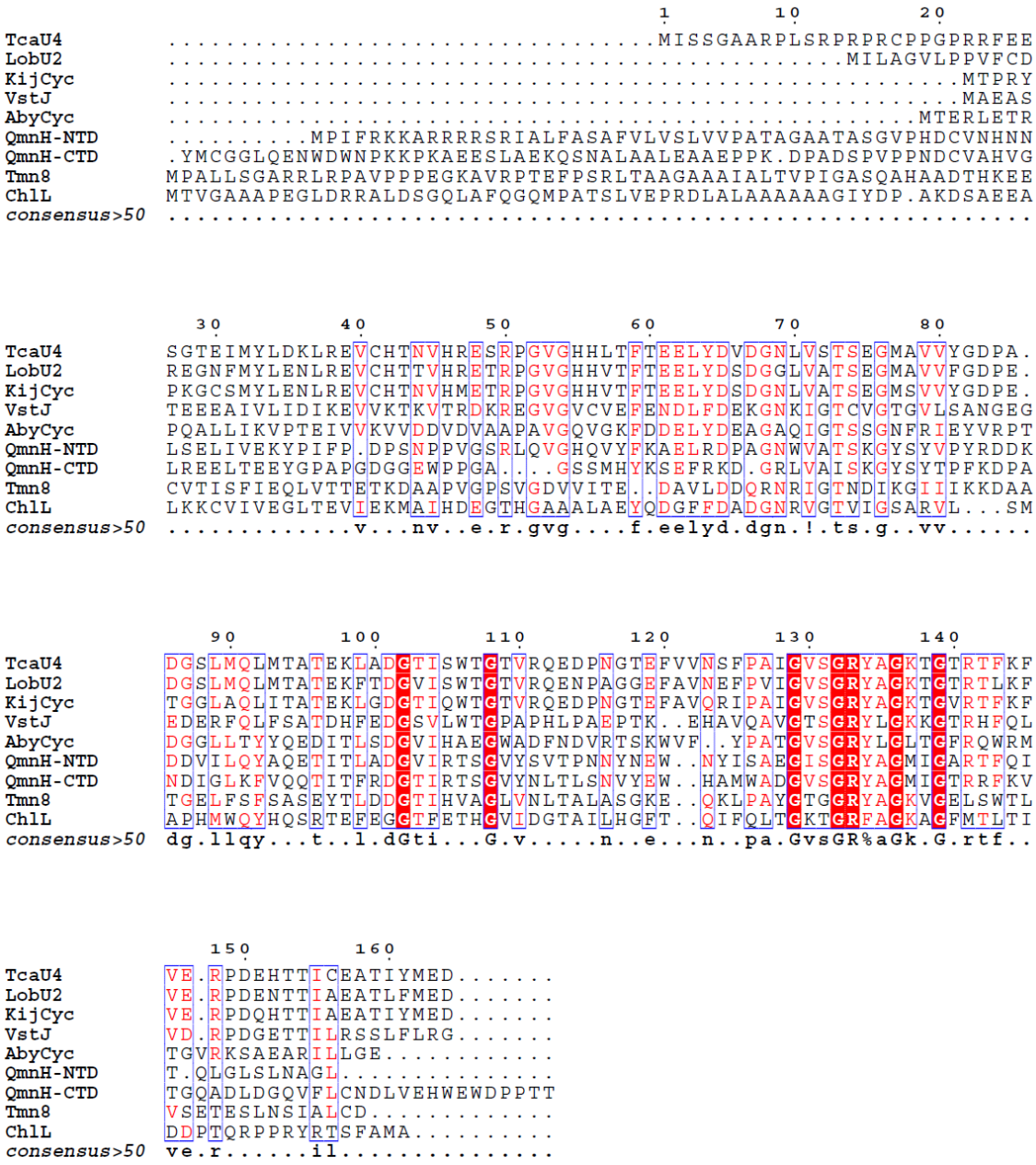


Figure S25. Multiple alignment of VstJ and its homologues.

The amino acid sequences of AbyCyc and KijCyc are deduced as shown in Fig. S18. QmnH-NTD and QmnH-CTD show N-terminal (1 aa to 178 aa) and C-terminal (179 aa to 376 aa) domains of QmnH (accession number: AFI57012). VstJ and its homologues show weak homology. However, some amino acid residues are highly conserved among the VstJ homologues.

Reference

1. Komatsu, M.; Komatsu, K.; Koiwai, H.; Yarnada, Y.; Kozone, I.; Izumikawa, M.; Hashimoto, J.; Takagi, M.; Omura, S.; Shin-ya, K.; Cane, D. E.; Ikeda, H. *ACS Synth. Biol.* **2013**, *2*, 384.
2. Komatsu, M.; Uchiyama, T.; Omura, S.; Cane, D. E.; Ikeda, H. *Proc. Nat. Acad. Sci. USA.* **2010**, *107*, 2646.
3. Hamano, Y.; Maruyama, C.; Kimoto, H. *Actinomycetologica* **2006**, *20*, 35.
4. Jez, J. M.; Ferrer, J. L.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. *Biochemistry* **2000,** 39(5), 890.
5. Chijiwa, S.; Park, H. R.; Furihata, K.; Ogata, M.; Endo, T.; Kuzuyama, T.; Hayakawa, Y.; Shin-ya, K. *Tetrahedron Lett.* **2003**, *44*, 5897.
6. https://www.broadinstitute.org/annotation/genome/streptomyces_group/MultiHome.html
7. Reeves, C. D.; Murli, S.; Ashley, G. W.; Piagentini, M.; Hutchinson, C. R.; McDaniel, R. *Biochemistry* **2001**, *40*, 15464.
8. Keatinge-Clay, A. T. *Chem. Biol.* **2007**, *14*, 898.
9. Kwan, D. H.; Sun, Y.; Schulz, F.; Hong, H.; Popovic, B.; Sim-Stark, J. C. C.; Haydock, S. F.; Leadlay, P. F. *Chem. Biol.* **2008**, *15*, 1231.
10. Demydchuk, Y.; Sun, Y.; Hong, H.; Staunton, J.; Spencer, J. B.; Leadlay, P. F., *Chembiochem* **2008**, 9 (7), 1136.