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Electronic Supporting Information

Kinamycin Biosynthesis Employs a Conserved Pair of Oxidases for B-ring Contraction†

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Materials and Methods

Bacterial strains, culture conditions, and general remarks. All *Escherichia coli* strains were cultured in LB broth at 37°C supplemented with appropriate antibiotics when needed. *E. coli* JM109 was used for plasmid propagation, ET12567/pUZ8002 for conjugation, and BL21(DE3) for protein expression. *Streptomyces ambofaciens* ΔΔ*alpW* (the starting strain in this study), *S. lividans* TK24, and their derivatives were maintained on MYM solid medium for sporulation, on MS solid medium for conjugation, in R2 liquid medium for metabolite production, and in YEME liquid medium for genomic DNA preparation at 28°C.[1]

All HPLC analyses were performed on a Shimadzu LC-20AT system equipped with a diode array detector, using an analytical column Agilent ZORBAX SB-C18 (4.6×250 mm, 5 μm) with a flow rate of 1.0 ml/min. Solvent A was water with 0.1% trifluoroacetic acid (TFA), and Solvent B was acetonitrile with 0.1% TFA. The gradient was 25% B to 100% B in 20 min, and then kept for 5 min. The absorption was monitored at 223 nm and 276 nm.

All NMR spectra were acquired on a BrukerAvance 500 MHz ($B_0$11.74 T) spectrometer equipped with a 5 mm Prodigy probe. $CDCl_3$ and $(CD_3)_2SO$ were used as the NMR solvent for spectra recorded, and using TMS as internal standard. Accurate mass was recorded on an AGILENT 1200HPLC/6520 Q-TOF-MS mass spectrometer (Agilent Technologies Inc., USA) within 0.4 ppm errors between theoretical and measured values. LC-MS was performed on an Agilent 1260/6460 Triple-Quadrupole LC/MS system, using an Agilent ZORBAX SB-aq C18 column (2.1×100 mm, 3.5 μm).

Construction of gene disruption mutants and their complementation. For in-frame deletion of *alpK* and *alpJ*, experiments were carried out using the temperature-sensitive plasmid pKC1139. Homologous fragments were amplified from the genomic DNA template by PCR using primers K-upF, K-upR, K-dnF, and K-dnR for *alpK*, and AlpJ1F, AlpJ1R, AlpJ2F, and AlpJ2R for *alpJ*. The sequences and restriction sites are listed in Table S4. The PCR products were purified, digested with the corresponding restriction enzymes and then ligated to the predigested pKC1139 to generate the gene knockout plasmids pKC1139-DalpK and pKC1139-DalpJ. The correct construction of these plasmids was confirmed by sequencing at Invitrogen. The gene knockout plasmids were transformed into the starting strain via conjugation from ET12567/pUZ8002. The transformants were cultured at 28°C for two successive generations to enable double-crossover to occur and then transferred to 37°C for plasmid loss. The knockout mutants were confirmed by PCR using primers CK-KF and CK-KR for *alpK*, and AlpJ12-S5-F and AlpJ12-S5-R for *alpJ*. The sequences used are listed in Table S4.

For complementation experiments, the integrative plasmid pSET616 (pSET152

derivative constructed in our lab, containing SF14 promoter and *neo* reporter gene) was used. The DNA sequences were amplified by PCR from the genomic DNA using primers K-F and K-R for *alpK*, AlpJ-psf-5 andAlpJ-Psf-3 for *alpJ*, alpKJ-F and alpKJ-R for *alpKJ*, and AlpJ-P-native-5 and AlpJ-psf-3for *alpJ* with its native promoter. Sequences and restriction sites are listed in Table S4. PCR products were digested with the corresponding enzymes and then ligated to the predigested pSET616 to generate gene complementation plasmids pSET-alpK, pSET-alpJ, pSET-alpKJ, and pSET-alpJnat. pSET-CK was generated by self-ligation of the predigested pSET616. The correct construction of these plasmids was confirmed by sequencing at Invitrogen. The gene complementation plasmids were transformed to the corresponding gene knockout mutants to generate ΔΔ*alpK*::*alpK*, ΔΔ*alpJ*::*alpJ*, ΔΔ*alpJ*::*alpJnat*, ΔΔ*alpJ*::*alpKJ,* and ΔΔ*alpJ*::CK. The transformants were cultured at 28°C for several successive generations to isolate single colonies of corresponding strains.

Expression of enzymes in *Streptomyces lividans* TK24 and Feeding experiments. The complementation plasmids pSET-CK, pSET-alpJ and pSET-alpKJ were transformed to *S. lividans* TK24 to generate TK24::CK, TK24::*alpJ*, and TK24::*alpKJ* via conjugation from ET12567/pUZ8002. Transformants were cultured at 28°C for several successive rounds to isolate single colonies of corresponding strains.

For feeding experiments, the transformants were cultured in R2 liquid medium at 28°C 220 rpm, and the substrate dehydrorabelomycin (**1**) was added at 24 hr. The fermentation broths were harvested at 48 hr and extracted with equal volume of ethyl acetate after pH was adjusted to 3~4.

Expression and purification of enzymes in *Escherichia coli*. For expression of N terminal His_6-tagged AlpK, pET28a was used and for expression of C terminal His_6-tagged AlpJ, pET30a was used. DNA fragments were amplified by PCR from the genomic DNA using primers EalpK-F2, EalpK-R2, AlpJ-30C-5, and AlpJ-30C-3. PCR products were digested by corresponding enzymes listed in Table S4 and then ligated to the predigested pET vectors to generate pET28a-His-AlpK and pET30a-AlpJ. Correct construction of these plasmids was confirmed by sequencing at Invitrogen.

The expression plasmids were transformed into competent *E. coli* BL21(DE3) strain. The recombination strains were cultured in LB with 50 μg/ml kanamycin at 37°C. Protein expression procedure was according to Novagen Manual. Briefly, the recombinant strains were induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM when the OD_{600} of the culture reached 0.6. After induction, the cultures were incubated at 18°C for another 12 hr. Then cells were collected by centrifugation (8,000×g, 15 min) at 4°C. The pellets were resuspended in ice cold 1×Binding Buffer (500 mM NaCl, 20 mM Tris-HCl, 5 mM imidazole, pH 7.9) and the cells were disrupted by sonication (total time 10~20 min, 2 s cooling, 2 s burst at 250 W). Cell debris was removed by centrifugation (12,000×g, 10 min) and the supernatant was applied onto the prewashed Ni-NTA column. The crude proteins were washed firstly with 1×Wash Buffer (500 mM NaCl, 20 mM Tris-HCl, 60 mM imidazole, pH 7.9) and then eluted with 0.5×Elute Buffer (500 mM NaCl, 20 mM Tris-HCl, 1 M imidazole, pH 7.9). The purified proteins were concentrated by centrifugation (4,000×g, 30 min) in 10 kDa ultrafiltration tubes (Centriplus YM series, Millipore). Protein concentrations were

quantified by Bradford method.[2]

In vitro enzymatic assays and bioconversion product preparation. For combinational enzymatic reactions, the reaction mixture contained Tris-HCl (50 mM, pH 7.5), dehydrorabelomycin (**1**) (20 μM), FAD (2 μM), NADPH (10 mM), NADH (10 mM), N-acetyl cysteine (5 μM), AlpK (5 μM), AlpJ (10~20 μM), and Fre (1 μM). The reaction mixture were incubated at 30°C for 10~15 min, and then loaded directly onto HPLC.

For analysis of producing seongomycin (**10**), the reaction mixture was 50 μl, composed of AlpK, AlpJ, dehydrorabelomycin (**1**), FAD, NADPH, and N-acetyl cysteine. For preparation of seongomycin (**10**), the reaction volume was amplified to 200 μl, incubation time increased to 30 min, and product was purified by analytical column on HPLC without organic extraction, and the HPLC gradient was changed to 25% B to 80% B in 15 min. Finally, 2.0 mg seongomycin (**10**) was dissolved in $(CD_3)_2SO$ for NMR experiments.

For analysis of producing **9** and **12**, the reaction mixture was 50 μl, composed of AlpJ, Fre, FAD, NADH, and dehydrorabelomycin (**1**). HRMS (+ESI) of **12**: calculated for $C_{36}H_{22}O_8$: 583.1387, observed: 583.1382. For preparation, the reaction volume was amplified to 200 μl, incubation time increased to 20-60 min. The HPLC gradient was changed to 50% B to 100% B in 15 min, and the product was purified by analytical column on HPLC without organic extraction. The product **9** was extremely unstable, and would convert to **12** spontaneously. So the reaction mixture was used directly for the LC-MS experiment for analysis of **9**.

For analysis of converting **9** to **10** by AlpK, the reaction mixture composed of AlpJ,

Fre, FAD, NADH, NADPH, and dehydrorabelomycin (**1**) was incubated for 10 min, and then AlpK and N-acetyl cysteine was added into the reaction mixture for another 10 min incubation.

For analysis of the necessity of FAD, the reaction mixture was composed of AlpK, AlpJ, dehydrorabelomycin (**1**), NADPH, N-acetyl cysteine, and with or without FAD.

Metabolite extraction and preparation. For analysis of POJ1 (**4**), POJ2 (**5**), and POJ4 (**6**), 100 ml fermentation broth of the strain ΔΔ*alpJ*::*alpJ* was extracted with equal volume of ethyl acetate after pH was adjusted to 3~4. The ethyl acetate was removed by vacuum evaporation and the extract was dissolved in methanol.

For preparation of **6**, an 8-liter fermentation broth was extracted by ethyl acetate. The crude extract was dissolved in methanol and then applied to the Sephadex LH-20 chromatography using methanol as eluting solvent. The fractions containing **4** and **6** were collected and pooled, and further purified by reverse-phase semi-preparative HPLC (YMC-Pack Pro C18, 250×10 mm, 5 μm; 3 ml/min, UV detection at 223 nm and 276 nm) with a linear gradient from 50% B to 100% B in 20 min. **4** was found to convert gradually to **6** during preparation. At last, 2.0 mg **6** was dissolved in $CDCl_3$ for NMR measurements.

For preparation of PK1 (**8**), a 7-liter fermentation of ΔΔ*alpK* was performed. The mycelia were collected by filtration and extracted twice with acetone. The solvent was removed by vacuum evaporation and the residue was extracted with large quantity of ethyl acetate after pH was adjusted to 3~4. The crude extract was dried by vacuum evaporation and the residue was dissolved in methanol. The Sephadex LH-20

chromatography was applied, using methanol as eluting solvent. The fractions containing **8** were collected and pooled, and further purified by reverse-phase semi-preparative HPLC (YMC-Pack Pro C18, 250×10 mm, 5 μm; 3 ml/min, UV detection at 223 and 276 nm) with a linear gradient from 70% B to 100% B in 15 min. Finally, 4.0 mg **8** was dissolved in $(CD_3)_2SO$ for NMR measurements.

Figure S1. Comparison of the *jad, gil,* and *alp* gene clusters. The three gene clusters are responsible for biosynthesis of jadomycin, gilvocarcin, and kinamycin, respectively. The genes in black are responsible for generation of prejadomycin, i.e., *jadIABCED, gilGABCFK,* and *alpIABCDE*; then the tailoring enzymes encoded by *jadFH, gilOIOIV,* and *alpFG* (in grey color) further convert prejadomycin to dehydrorabelomycin (**1**); finally the oxidases, JadG, GilOII, and AlpJ (in yellow color) catalyze the B-ring cleavage reaction. In the jadomycin and gilvocarcin biosynthesis, *jadY* and *gilH* (in green color) encode FAD/FMN reductase supplying the cofactor for their corresponding oxidases. The gene *alpK* (in red color) which locates upstream of *alpJ* encodes an FAD-dependent monooxygenase in the *alp* cluster and was found in this study to cooperate with AlpJ in B-ring transformation reaction.

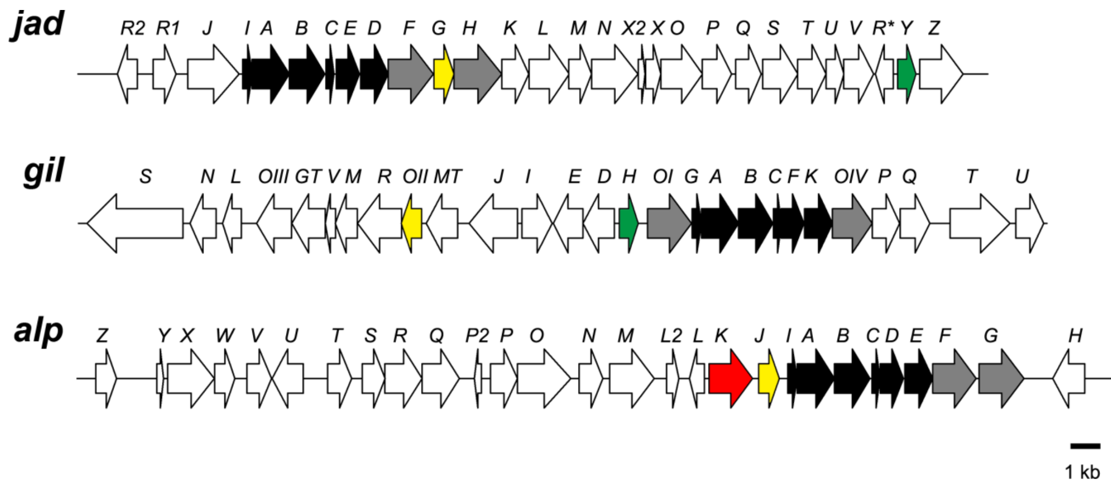


Figure S2. Phylogenetic tree of selected FAD-dependent monooxygenases involved in angucycline biosynthesis. The tree was constructed by Neighbor-Joining method and the bootstrap (500 replicates) values for the main clades are shown. Scale bar represents 5% dissimilarity. According to the function of these enzymes, M series and E series are given, (M series involved in C2,3-dehydration, and E series involved in C12-hydroxylation and C4a,12b-dehydration or C12b-hydroxylation) and AlpK is clustered to an uncharacterized branch. Accession numbers for the reference sequences are as follows: AlpF, CAT87869; AlpG, CAJ87868; AlpK, CAJ87877; KinO1, AAO65343; KinO2, AAO65352; KinOR, AAO65351; JadF, AAV52246; JadH, AAV52248; SimA7, AAK06782; SimA8, AAK06789; Sim7, AAL15585; UrdE, CAA60567; UrdMox, AAF00206; LanE, AAD13534; LanM2ox, AAT64431; PgaMox, AAK57530; PgaE, AAK57522; Aur1Iox, AAX57196; Aur1A, AAX57188; GilOIV, AAP69585; GilOI, AAP69582; PHBH, 1PDH.

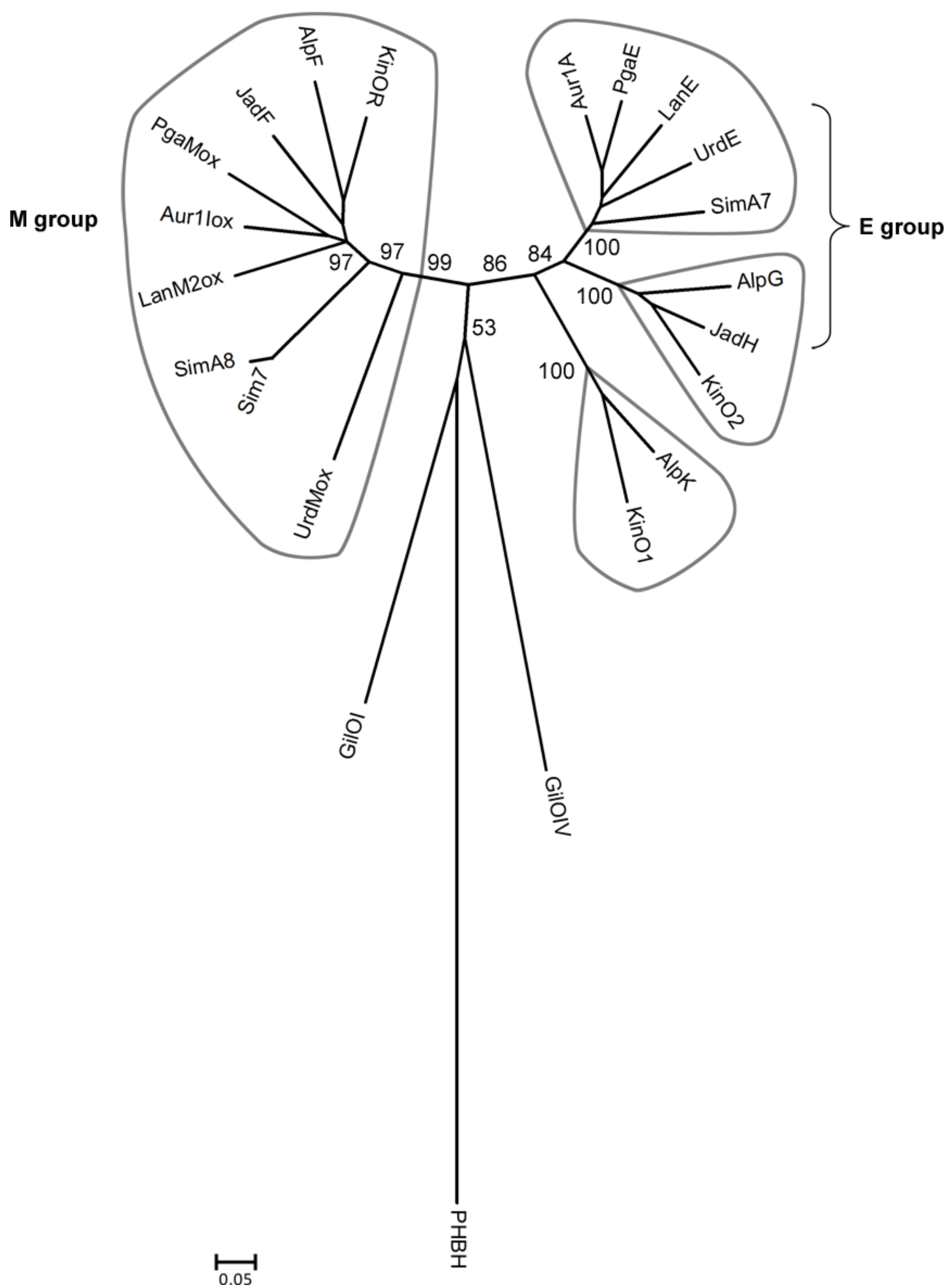

M group
E group
AlpF
KinOR
JadF
PgaMox
Aur1Iox
LanM2ox
SimA8
Sim7
UrdMox
Aur1A
PgaE
LanE
UrdE
SimA7
AlpG
JadH
KinO2
AlpK
KinO1
GilOI
GilOIV
PHBH
97
97
99
86
84
100
100
100
53
0.05

Figure S3. The partial sequences of the *alp, kin, lom,* and *flu* gene clusters revealed a conserved *alpK-alpJ* arrangement, amongst the *kin*, *lom*, and *flu* gene clusters ~~are~~ responsible for the biosynthesis of kinamycin, lomaiviticin, and fluostatin. Genes in red, i.e., *kinO1*, *lom27*, and *flu16* are *alpK* homologs; genes in yellow, i.e., *kinG*, *lom28*, and *flu17* are *alpJ* homologs. Genes in black encode the ketoacyl synthase alpha and beta subunits of corresponding polyketide synthases.

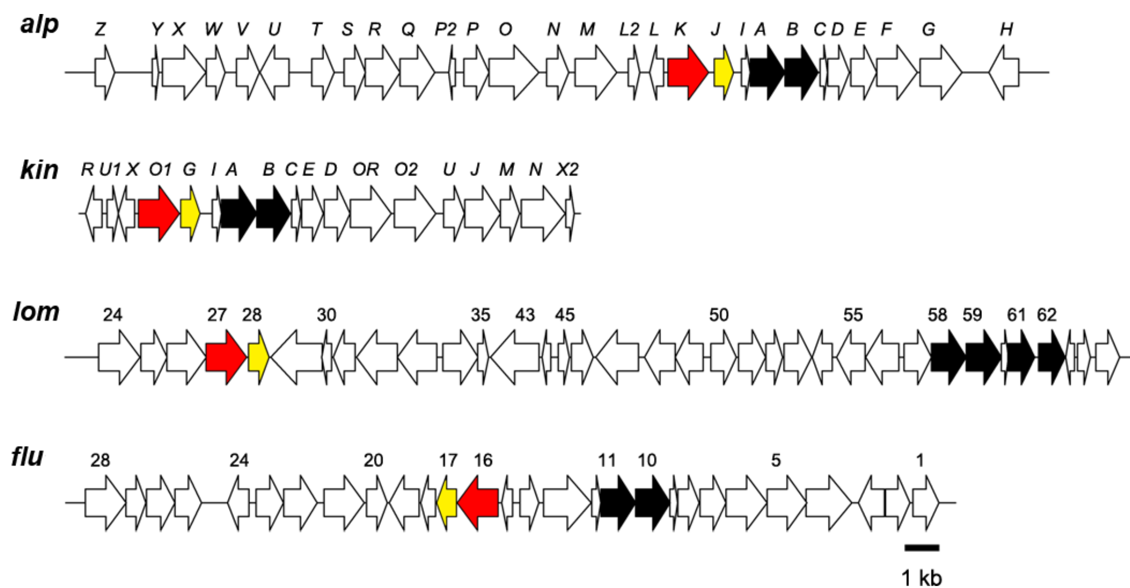


Figure S4. PCR confirmation of *alpJ* double-deletion mutant. Lane 1, DNA ladder; lane 2, the starting strain giving a band corresponding to a fragment of 1,066 bp in length; lane 3, ΔΔ*alpJ* giving a band of 355 bp. Both detected bands were correct in size.

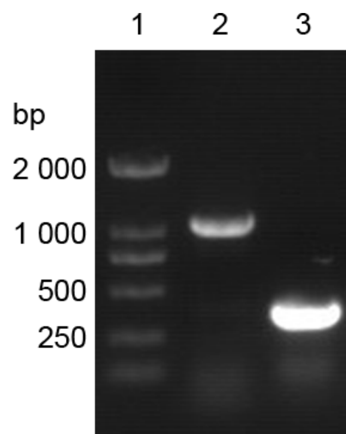


Figure S5. LC-MS ESI$^-$ analyses of POJ1 (**4**) and POJ4 (**6**). All the *m/z* signals match the calculated values, respectively.

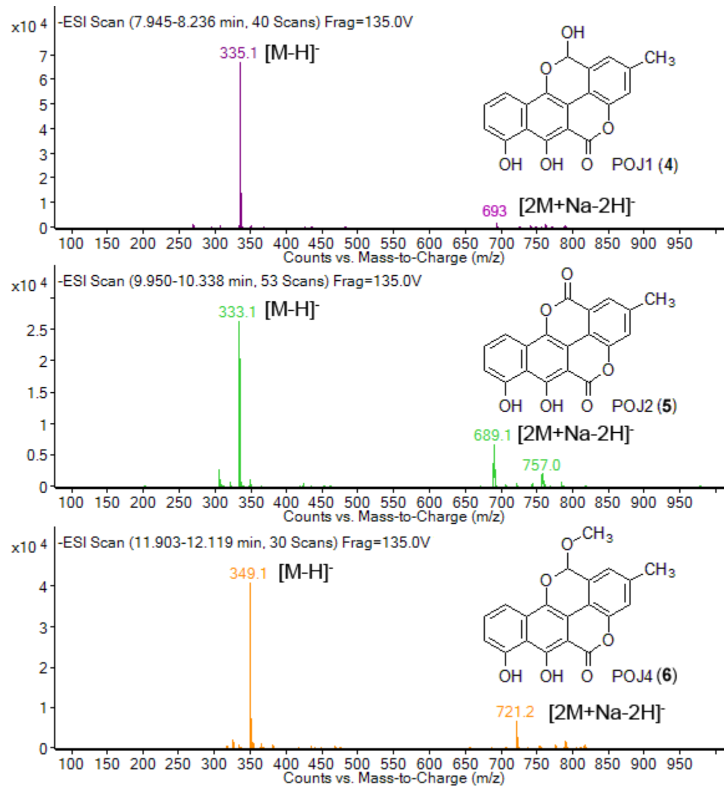


Figure S6. PCR confirmation of *alpK* double-deletion mutant. Lane 1, DNA ladder; lane 2, the starting strain giving a band corresponding to a fragment of 2,011 bp in length; lane 3, ΔΔ*alpK* giving a band 1,075 bp. Both detected bands were correct in size.

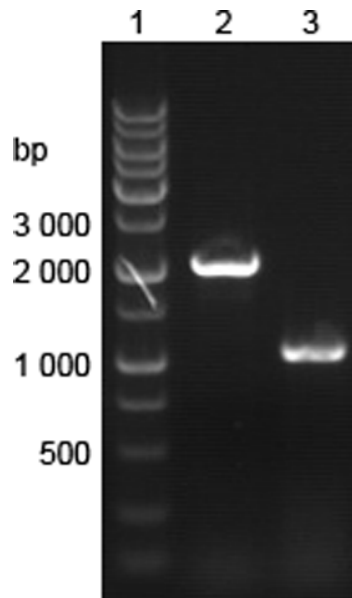
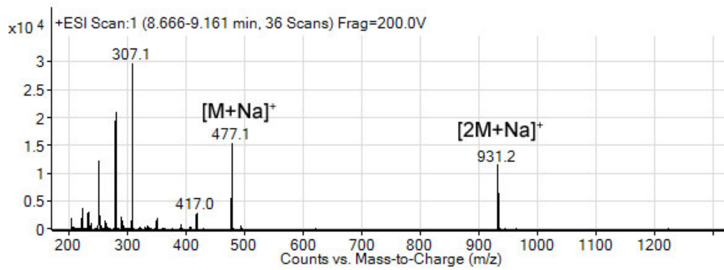


Figure S7. LC-MS ESI^{-} analyses of kinamycin D (**3**). All the *m/z* signals match the calculated values, respectively.

The starting strain



The complementation mutant ΔΔ*alpK*::*alpK*

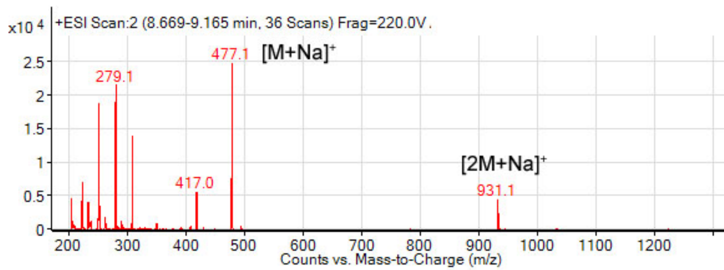


Figure S8. LC-MS ESI^{-} analyses of kinamycin D (**3**). All the *m/z* signals match the calculated values, respectively.

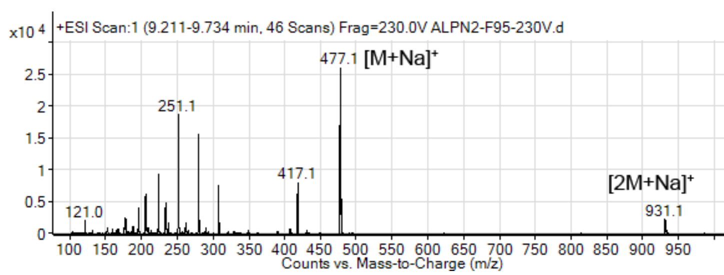


Figure S9. LC-MS spectra of PK1 (**8**) and seongomycin (**10**).

(A) is **8** under ESI positive mode and *m/z* 873.4 corresponds to $[M+H]^+$ ion;

(B) is **8** under ESI negative mode, *m/z* 871.2 is $[M-H]^-$, and *m/z* 435.0 is the double-charged ion.

(C) is **10** under ESI negative mode. *m/z* 452 is the $[M-H]^-$ ion.

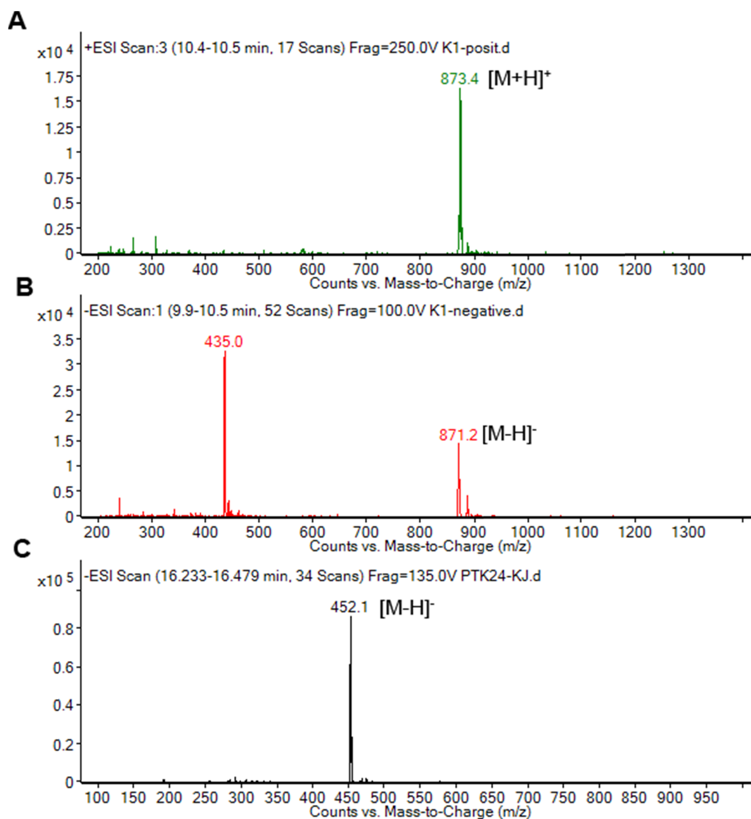


Figure S10. SDS-PAGE analysis of the purified AlpJ and AlpK. Lane 1, protein marker; lane 2, AlpJ giving a band corresponding to a protein of 27.2 kDa; lane 3, AlpK giving a band 54.5 kDa. Both detected bands were correct in size.

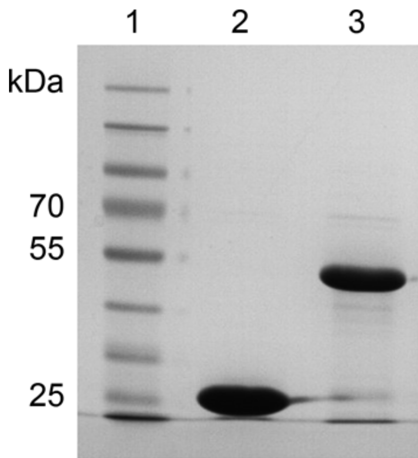


Figure S11. LC-MS spectrum of **9** under ESI negative mode. *m/z* 291 is the $[M-H]^-$ ion.

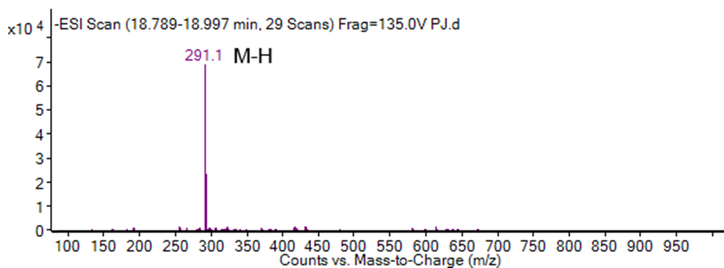


Figure S12. LC-MS spectrum of seongomycin (**10**) under ESI negative mode. *m/z* 452 is the $[M-H]^-$ ion.

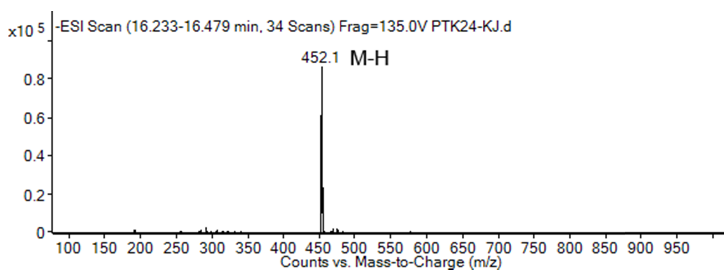


Figure S13. Fluorescent detection of NADPH consumption. The control group was colored in red, containing AlpK and NADPH. The experimental group was colored in black, containing AlpK, NADPH, and dehydrorabelomycin (**1**). The result showed that without **1**, AlpK didn't consume NADPH; and with the addition of **1,** NADPH decreased linearly.

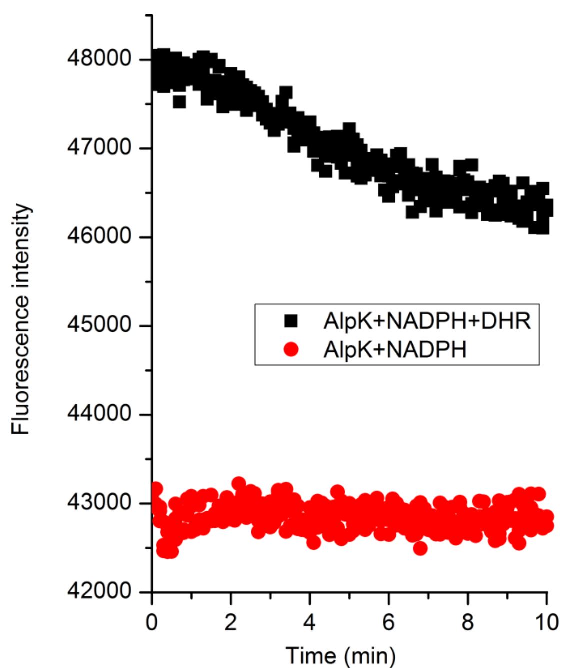


Figure S14. The ^{1}H NMR spectrum of POJ4 (**6**) in $CDCl_3$.

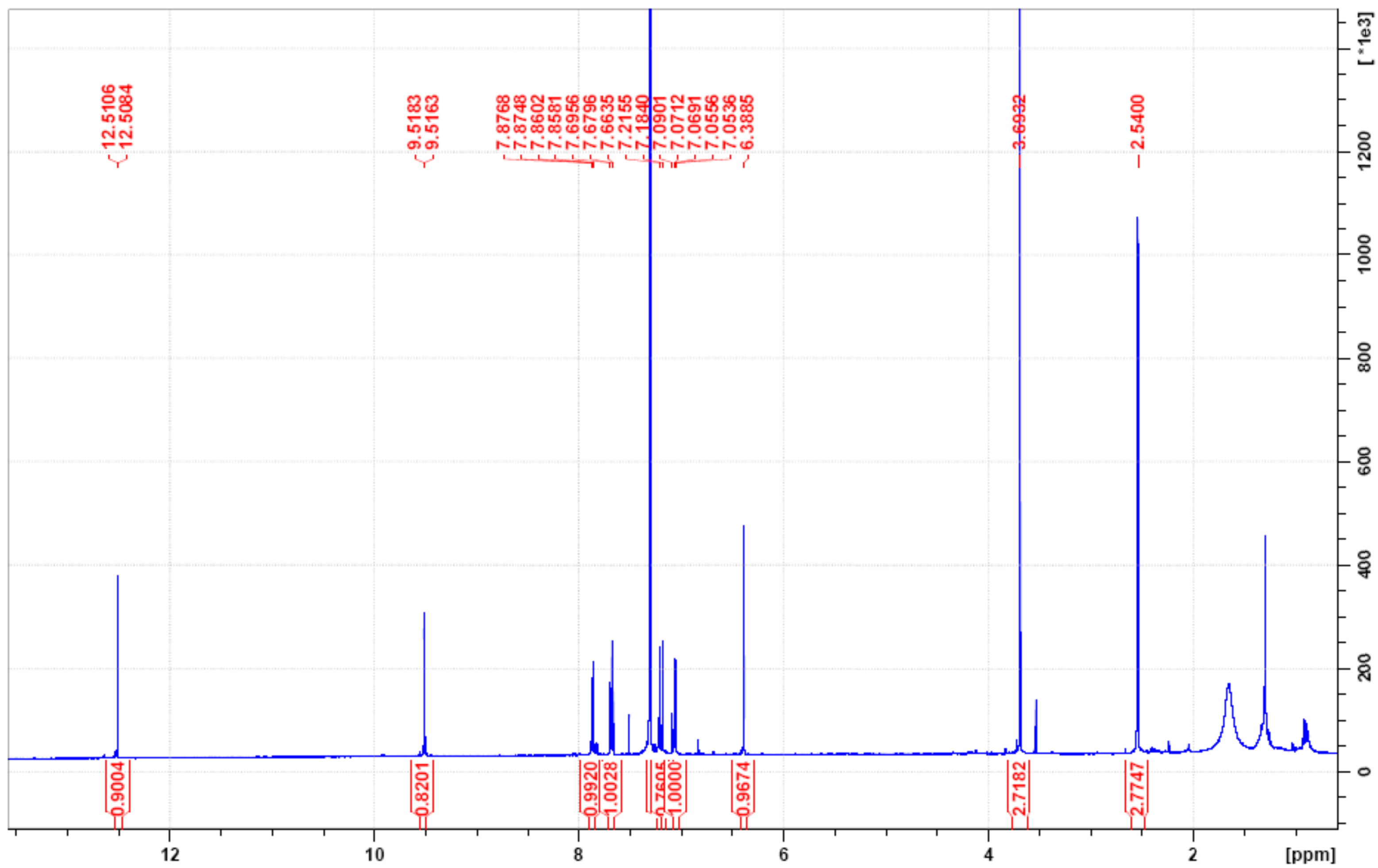


Figure S15. The ^{13}C NMR spectrum of POJ4 (**6**) in $CDCl_3$.

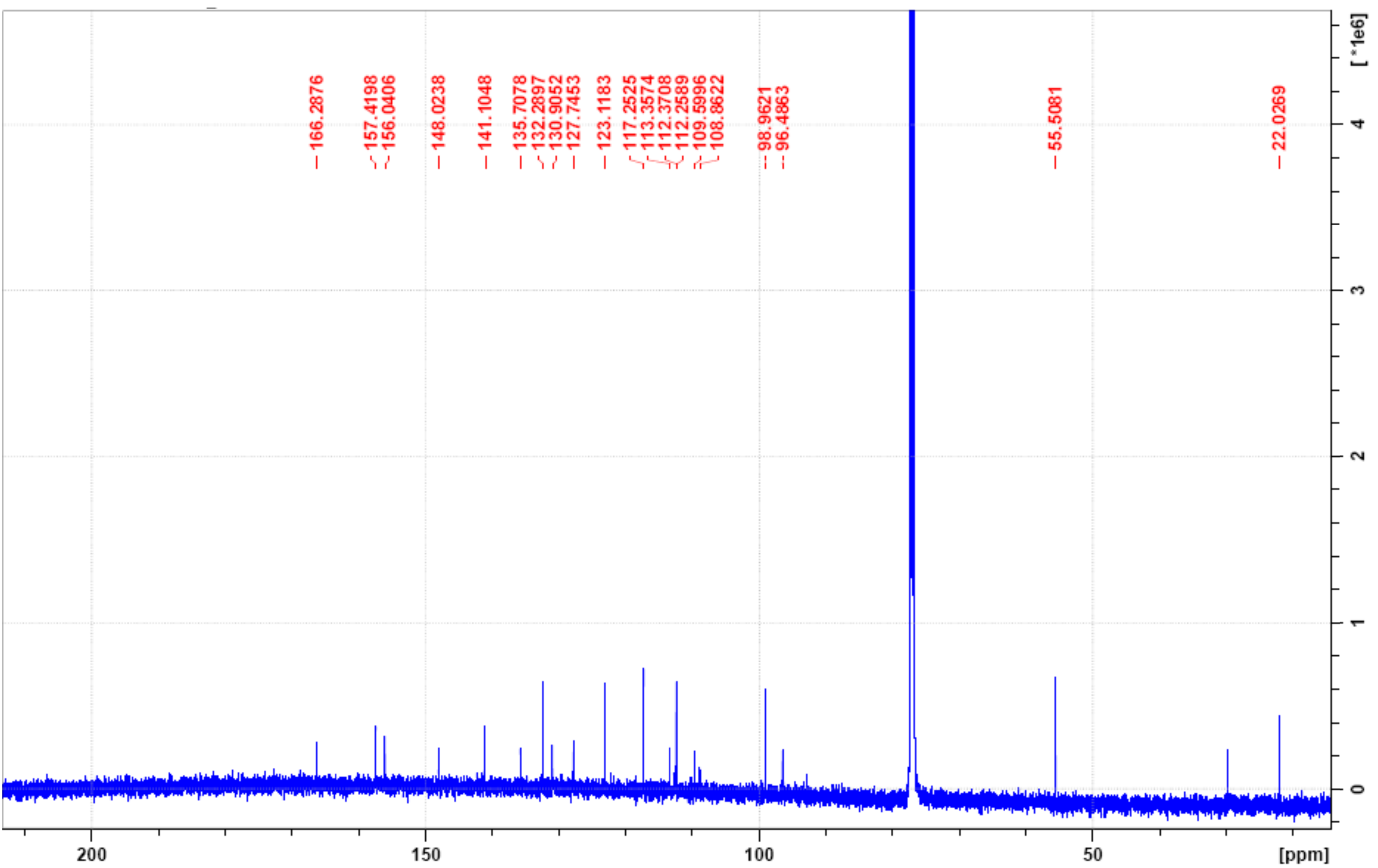


Figure S16. The HSQC spectrum of POJ4 (**6**) in $CDCl_3$.

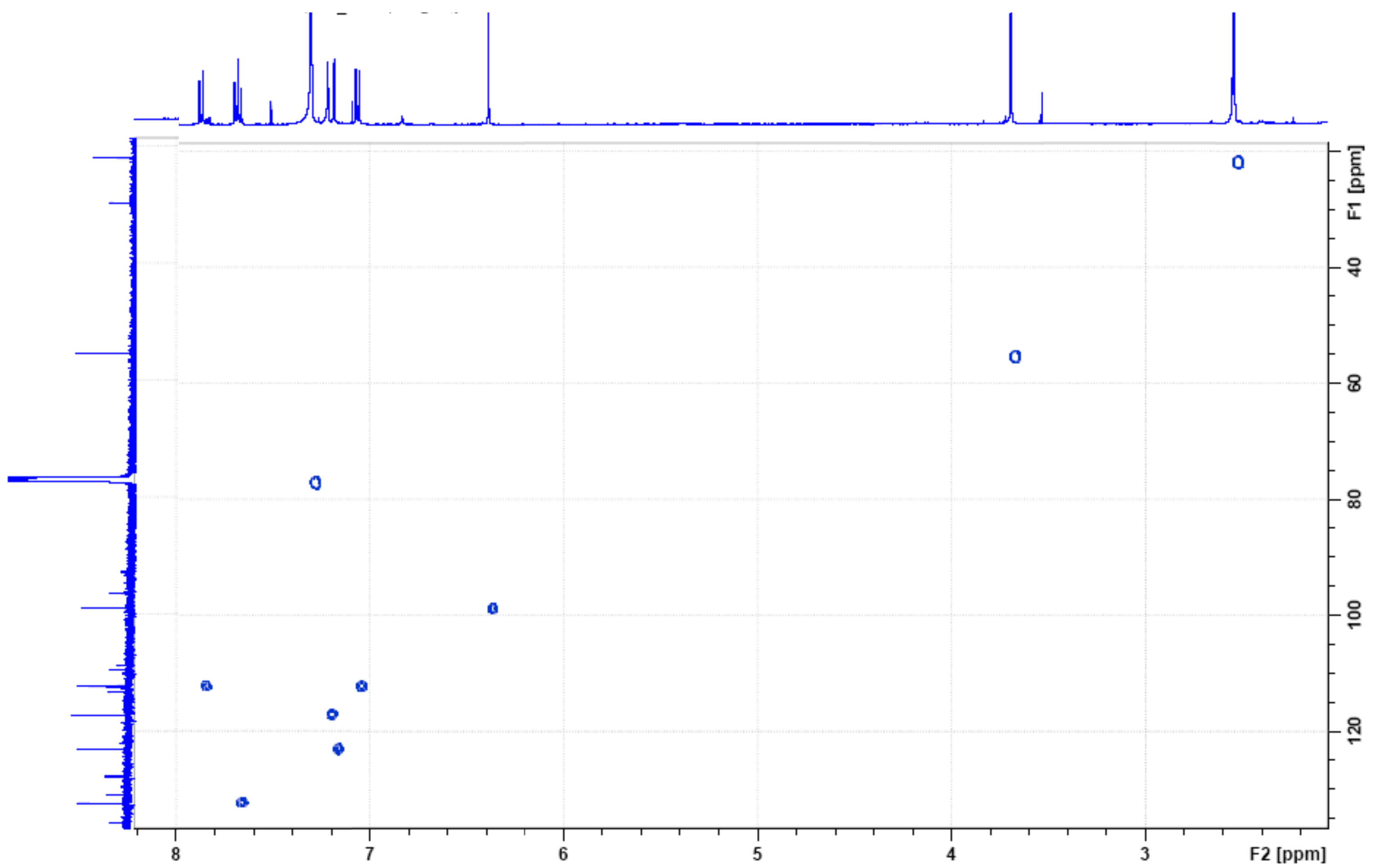


Figure S17. The HMBC spectrum of POJ4 (**6**) in $CDCl_3$.

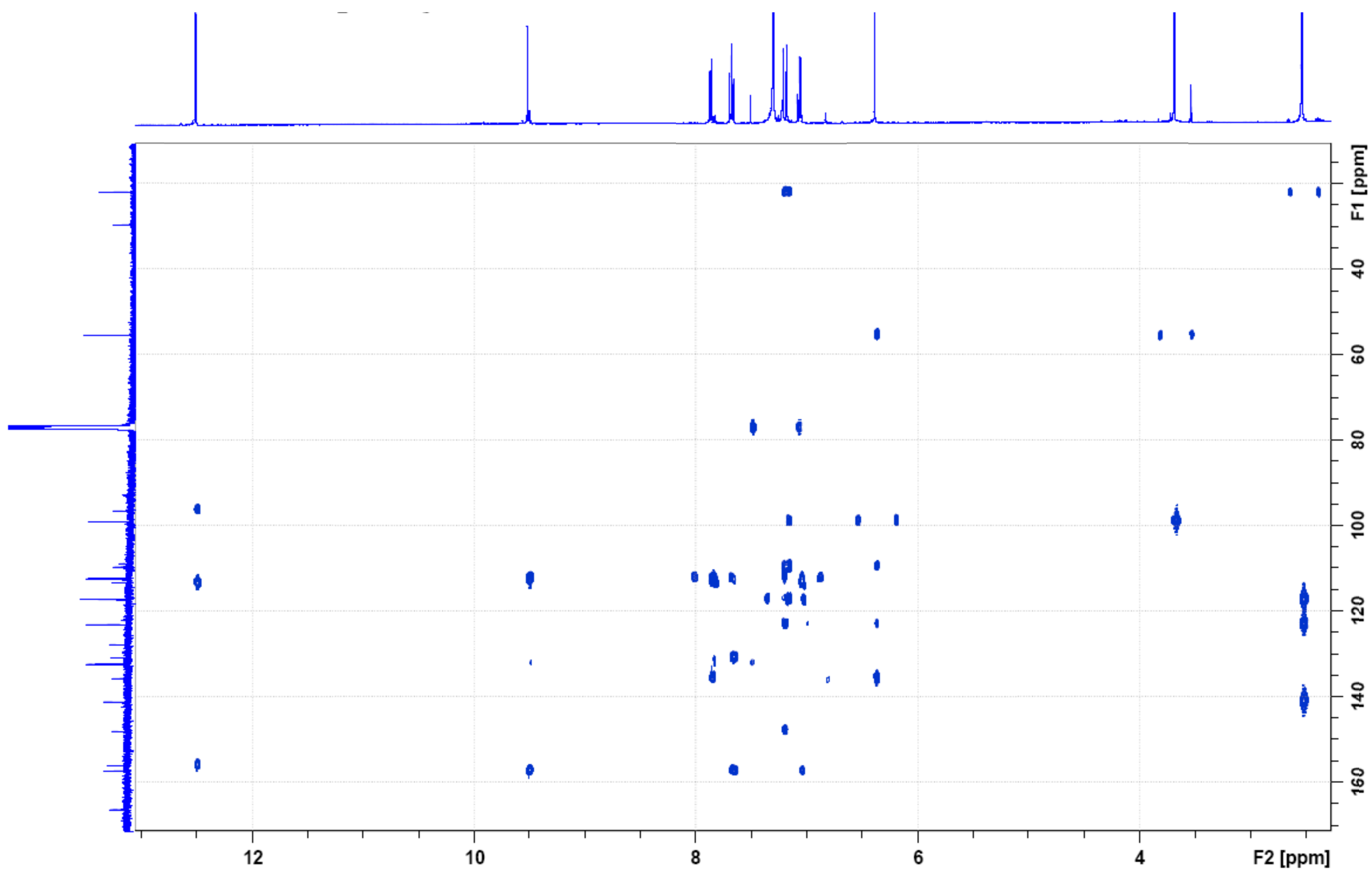


Figure S18. The ^{1}H NMR spectrum of PK1 (**8**) in $(CD_3)_2SO$.

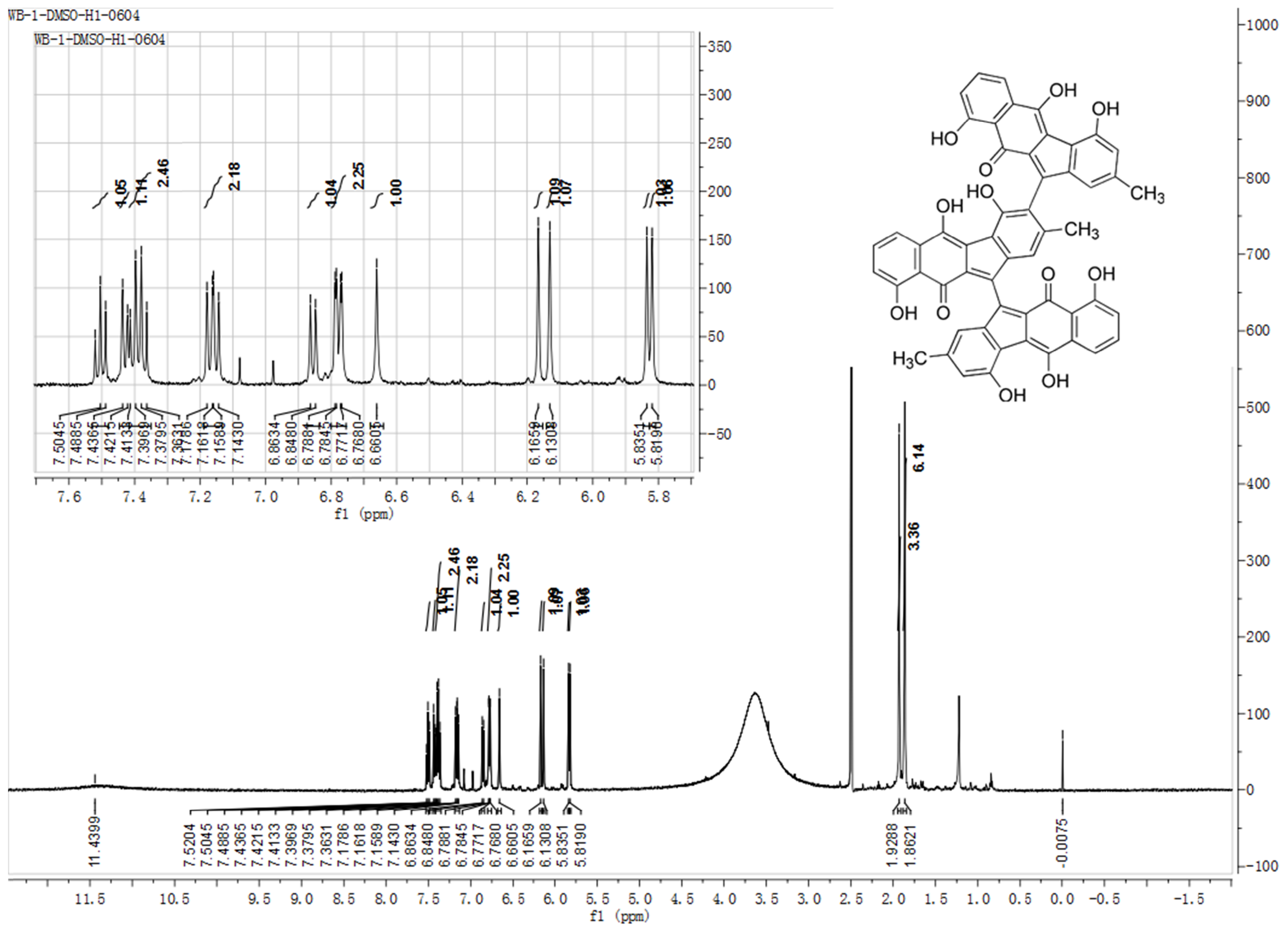


Figure S19. The ^{13}C NMR spectrum of PK1 (**8**) in $(CD_3)_2SO$.

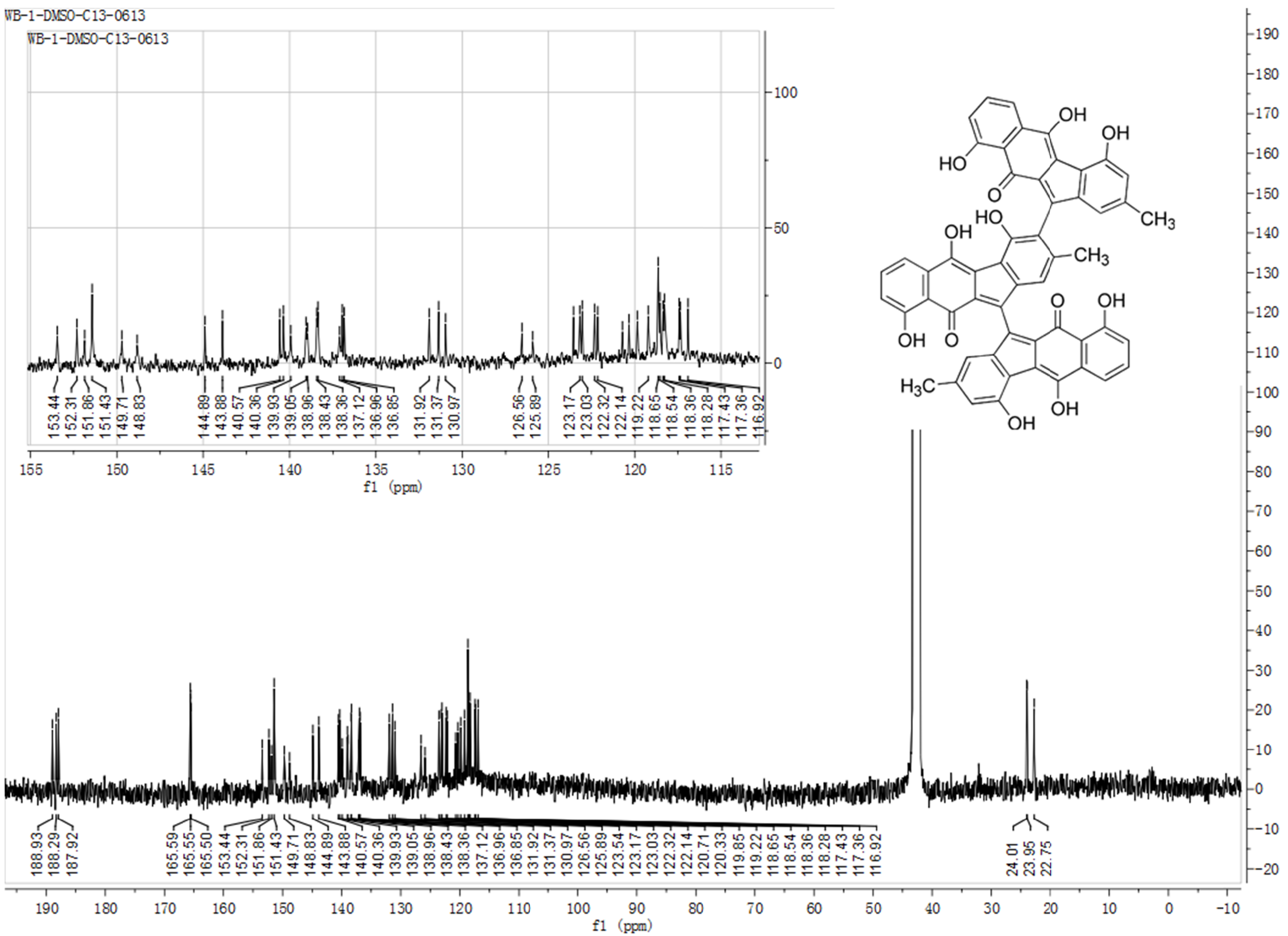


Figure S20. The HSQC spectrum of PK1 (**8**) in $(CD_3)_2SO$.

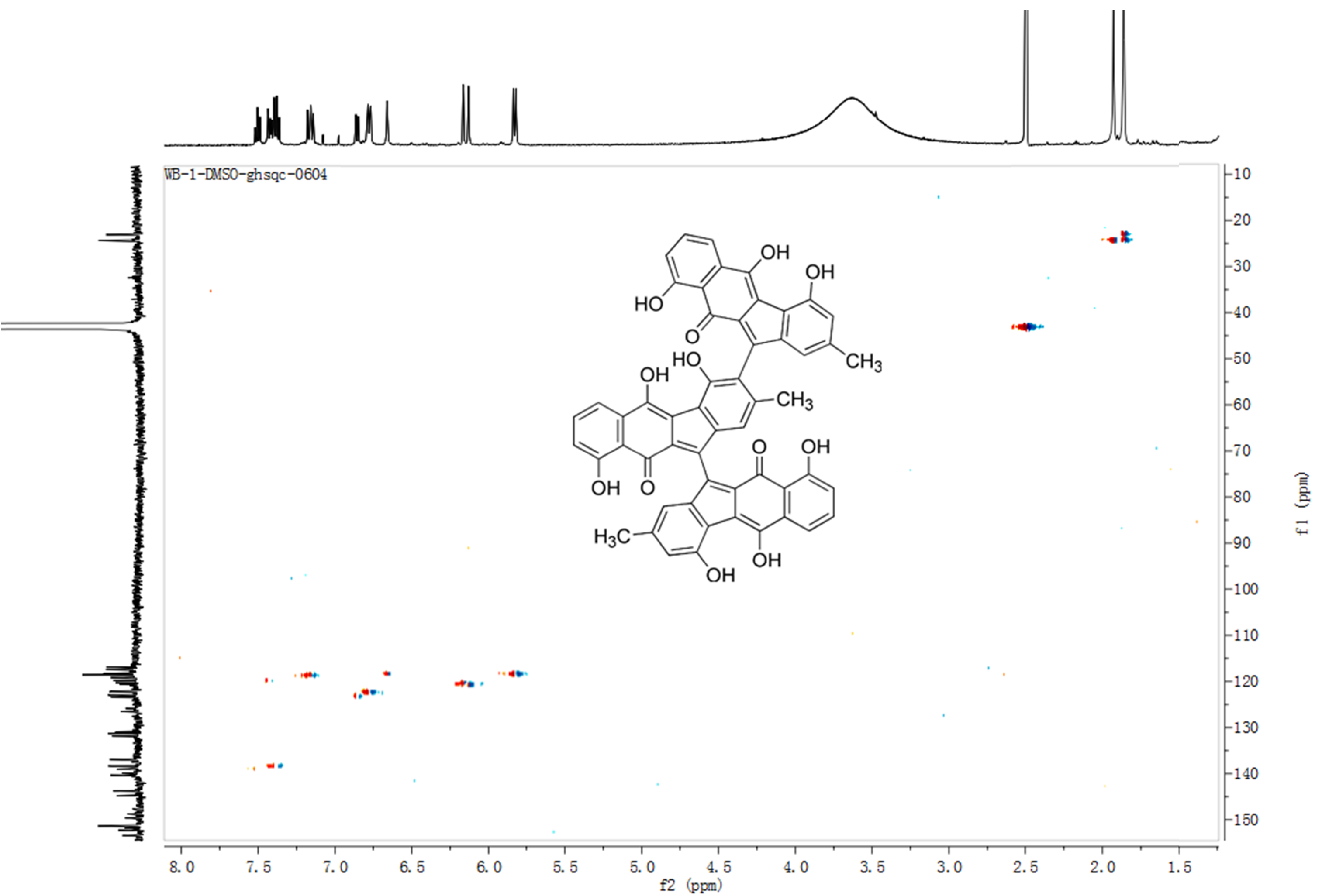


Figure S21. The COSY spectrum of PK1 (**8**) in $(CD_3)_2SO$.

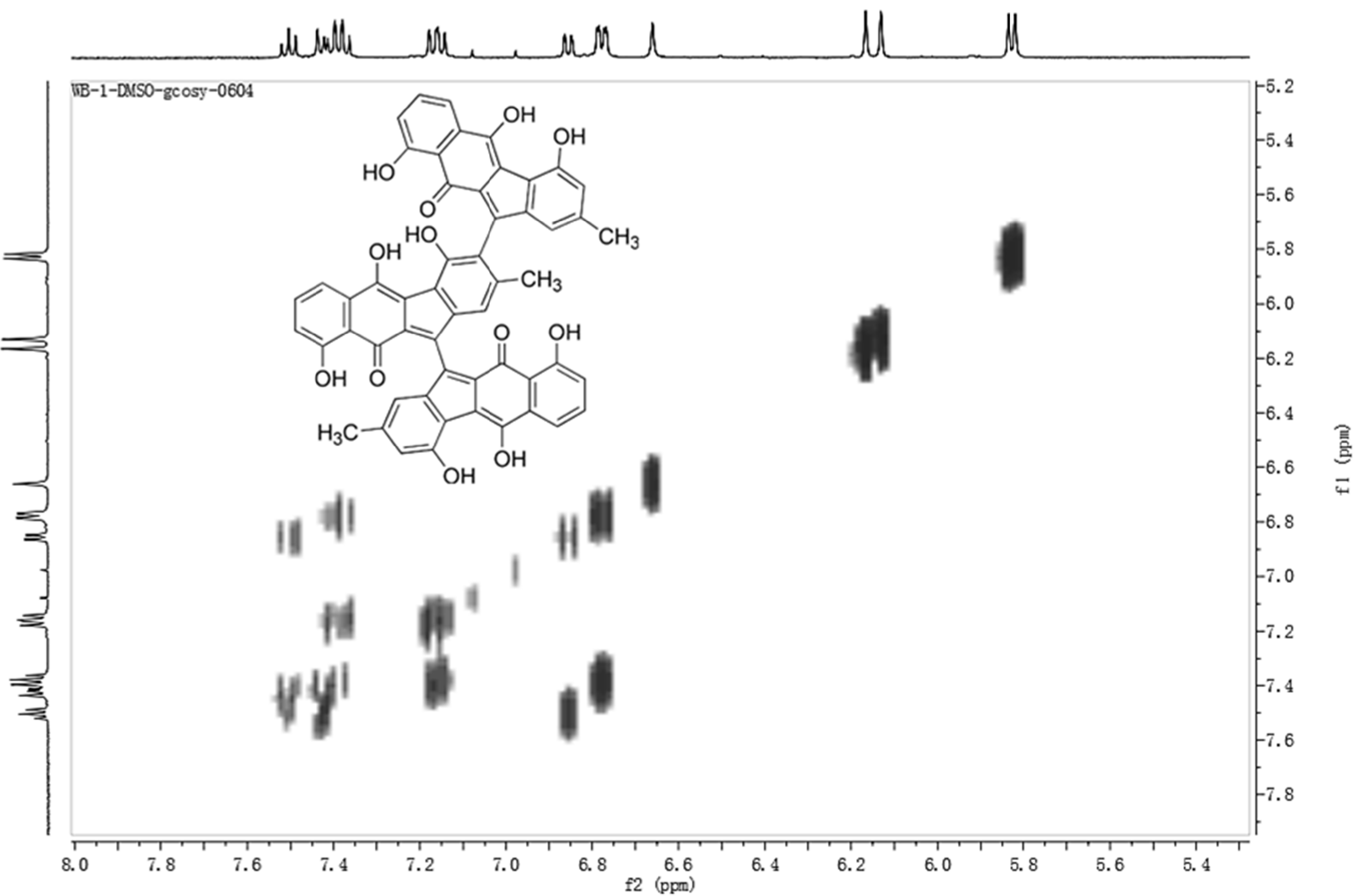


Figure S22. The HMBC spectrum of PK1 (**8**) in $(CD_3)_2SO$.

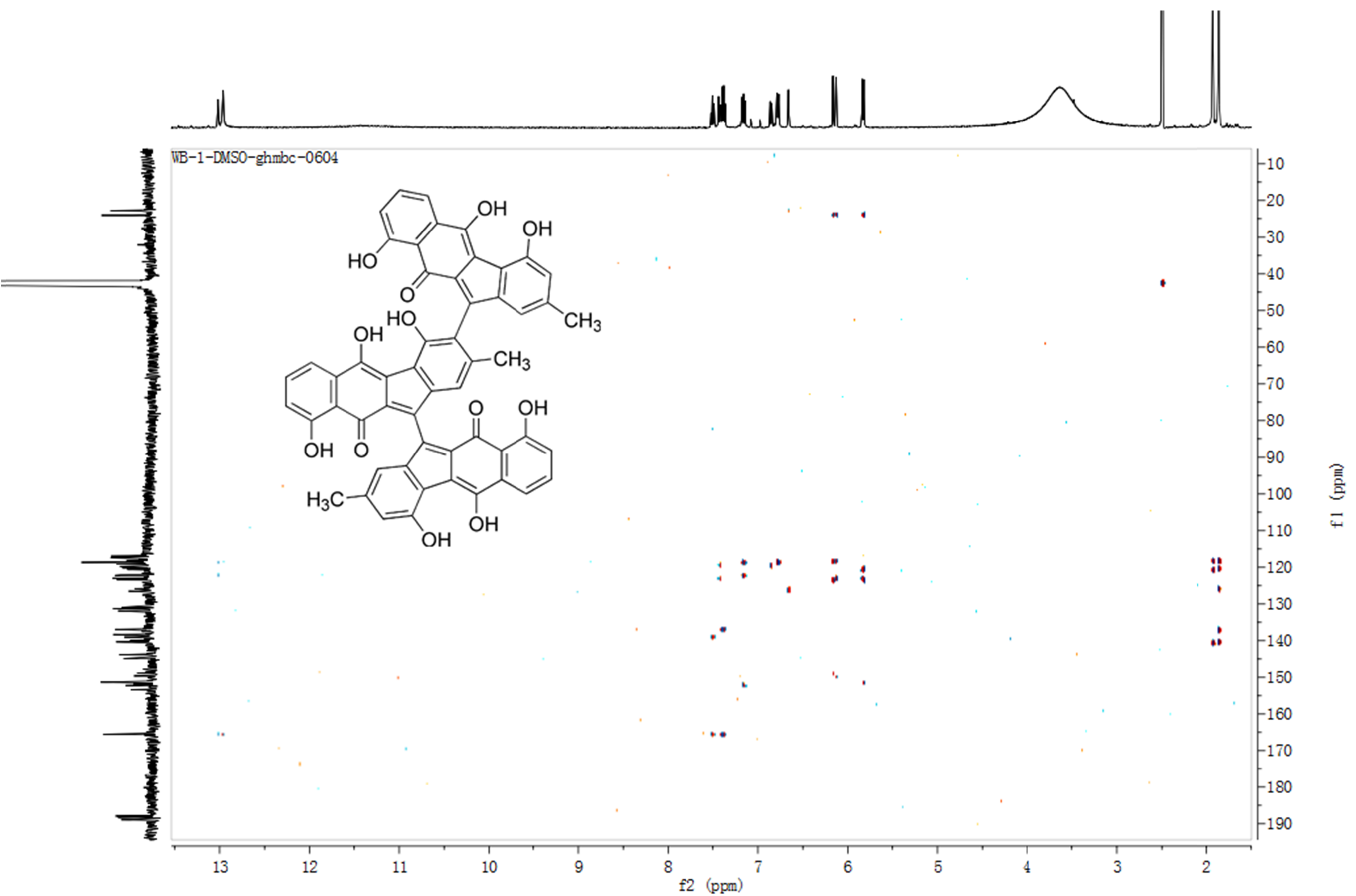


Figure S23. The ^{1}H NMR spectrum of seongomycin (**10**) in $(CD_3)_2SO$.

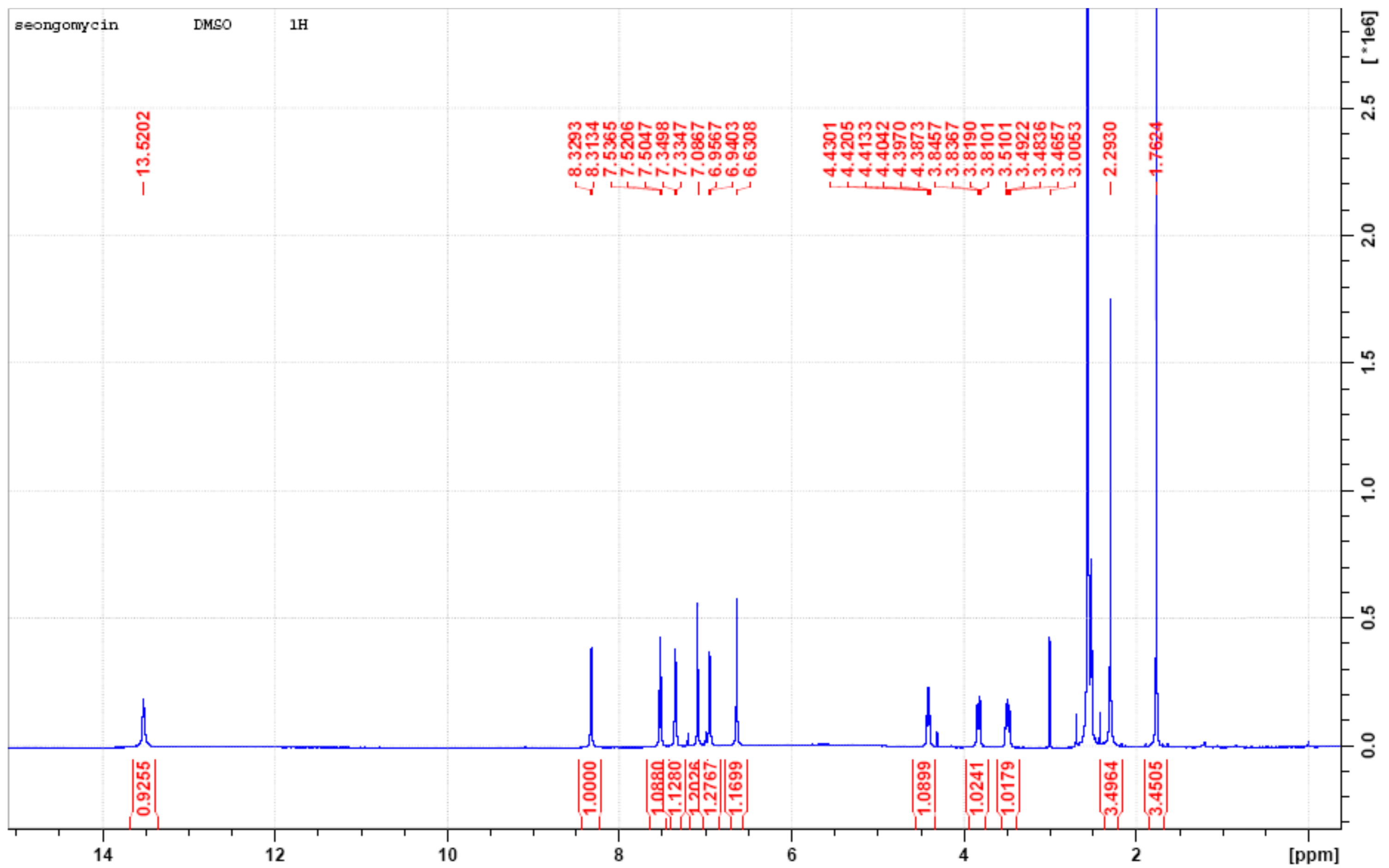


Figure S24. The ^{13}C NMR spectrum of seongomycin (**10**) in $(CD_3)_2SO$.

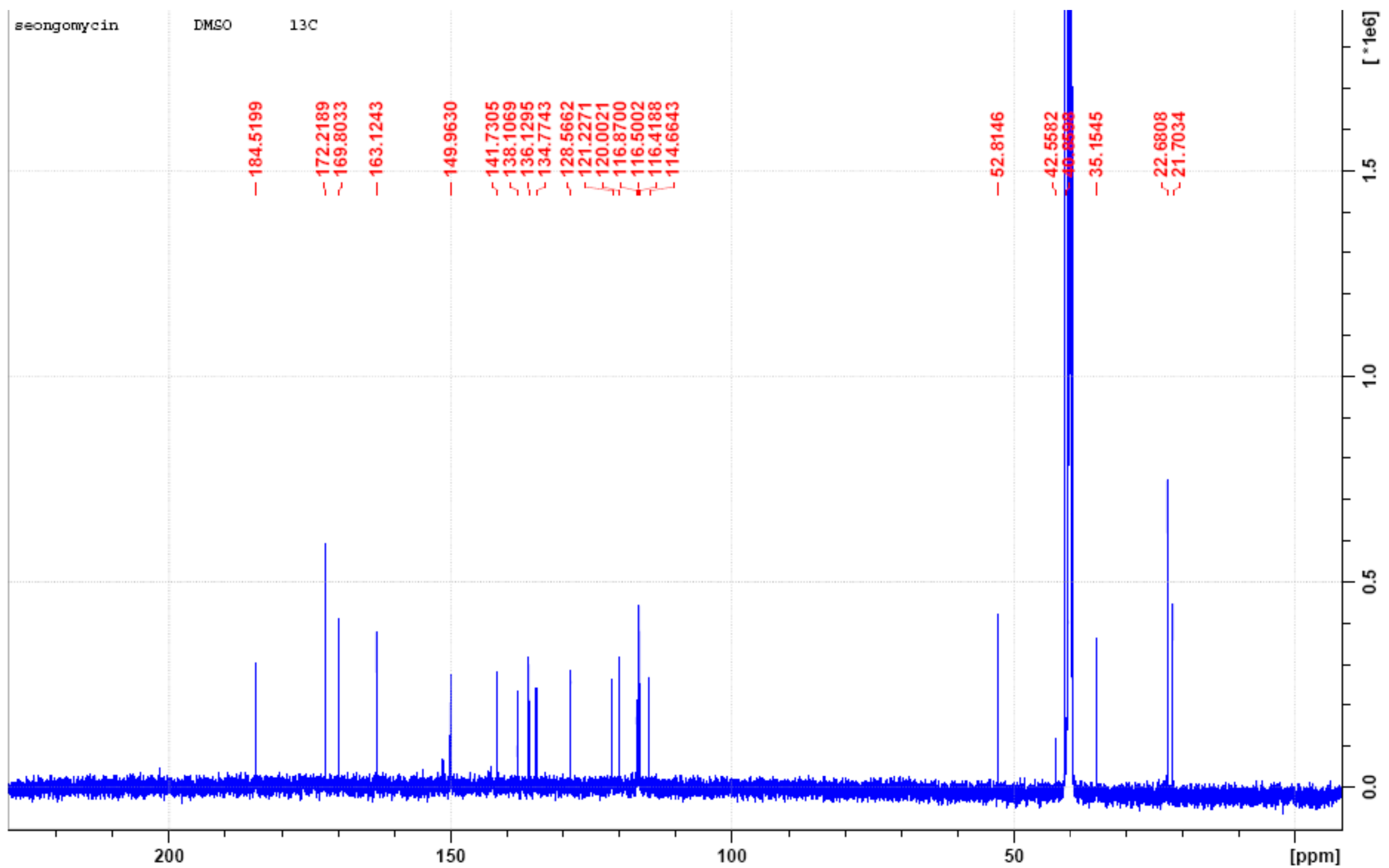


Figure S25. The COSY spectrum of seongomycin (**10**) in $(CD_3)_2SO$.

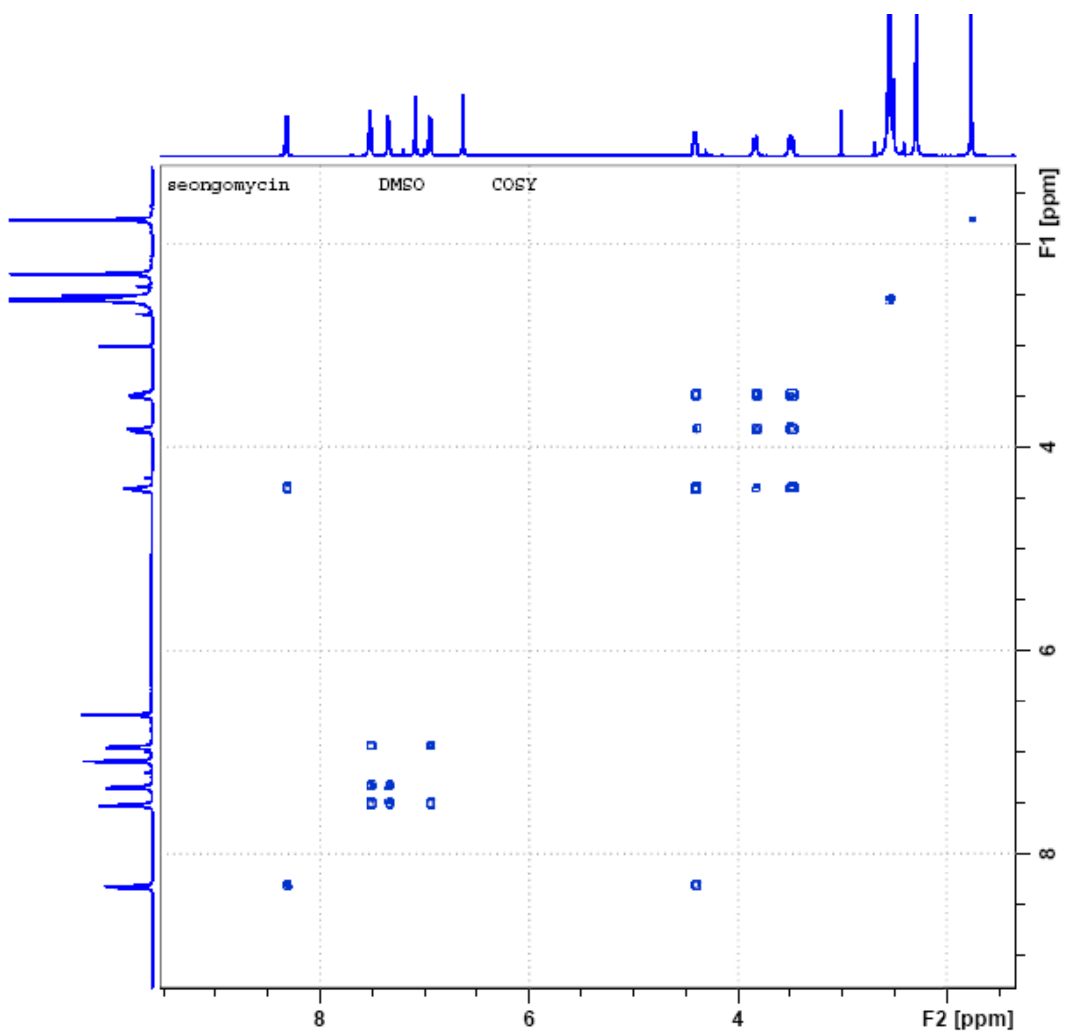


Figure S26. The HSQC spectrum of seongomycin (**10**) in $(CD_3)_2SO$.

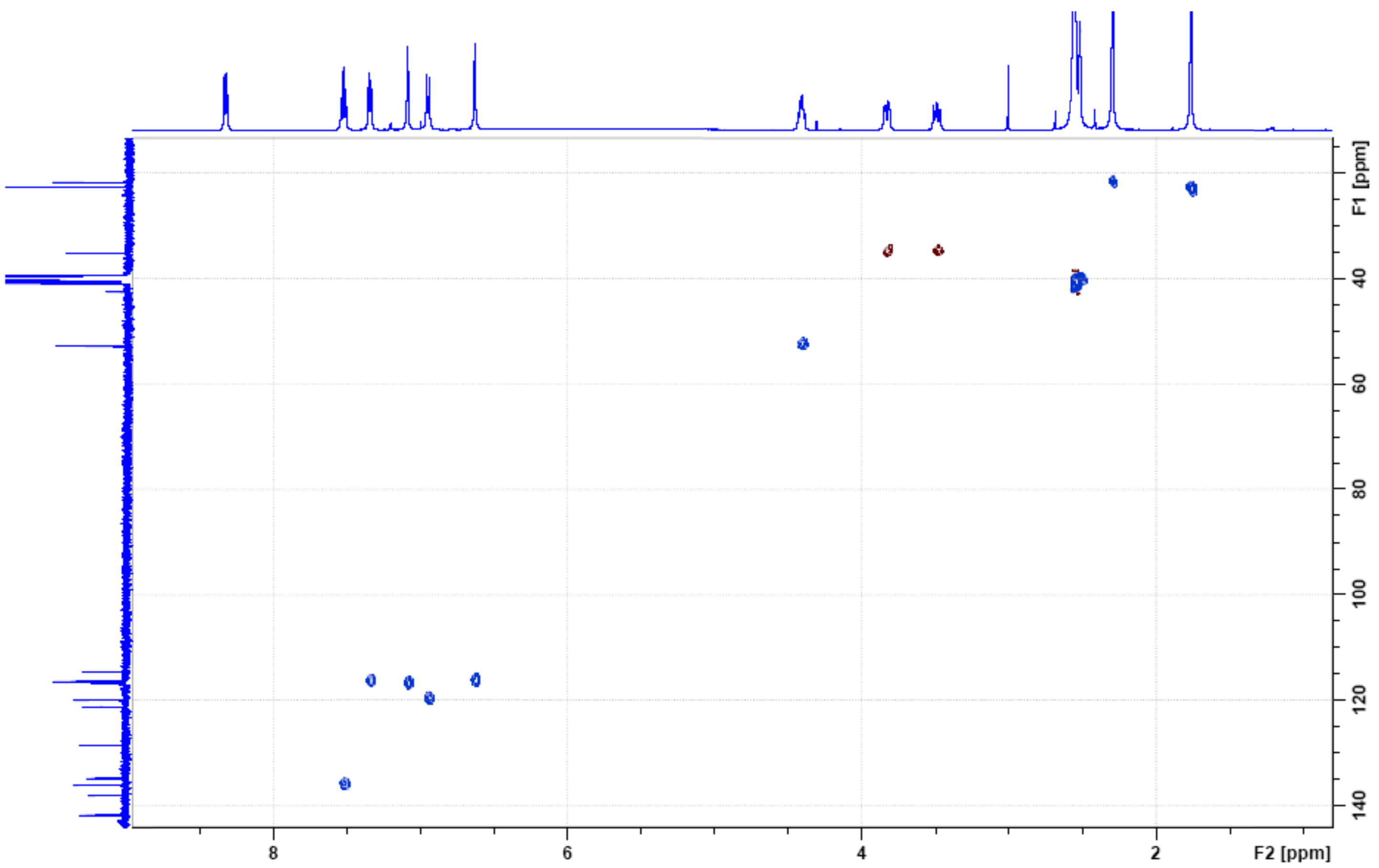


Figure S27. The HMBC spectrum of seongomycin (**10**) in $(CD_3)_2SO$.

seongomycin DMSO 1H

F1 [ppm]

50

100

150

12

10

8

6

4

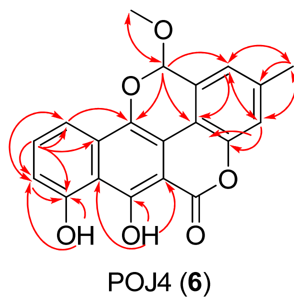
2

F2 [ppm]

Table S1. The ^{1}H NMR and ^{13}C NMR data of POJ4 (**6**) in $CDCl_3$.

Position	^{13}C-NMR[a]	^{1}H-NMR[b]	HMBC[b]
1	148.0		
2	117.2	7.21 (s, 1H)	C-1, C-4, C-5a, C-13
3	141.1		
4	123.1	7.18 (s, 1H)	C-2, C-5a, C-13, C-14
5	127.7		
5a	109.6		
6	166.3		
6a	113.4		
7	156.0		
7-OH		12.51 (s, 1H)	C-6a, C-7, C-7a
7a	96.5		
8	157.4		
8-OH		9.51 (s, 1H)	C-8, C-9, C-10
9	112.3	7.09 (dd, 1H, J =0.75, 8.0 Hz)	C-8, C-11
10	132.3	7.69 (t, 1H, J =8.0 Hz)	C-8, C-9, C-11, C-11a
11	112.4	7.87 (dd, 1H, J =0.75, 8.0 Hz)	C-9, C-12
11a	130.9		
12	135.7		
13	22.0	2.54 (s, 3H)	C-2, C-3, C-4
14	99.0		C-4, C-5a, C-12, C-15
15	55.5	3.69 (s, 3H)	C-14

[a] Recorded at 125 MHz in $CDCl_3$. [b] Recorded at 125 MHz in $CDCl_3$.



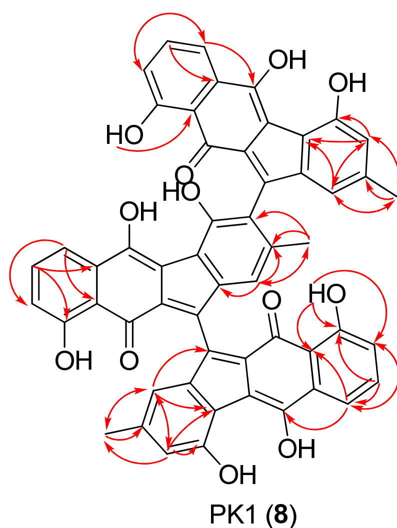
POJ4 (**6**)

Table S2. The ^{1}H NMR and ^{13}C NMR data of PK1 (**8**) in $(CD_3)_2SO$.

Position	^{13}C-NMR[a]	^{1}H-NMR[b]	HMBC[b]
1	151.4, qC		
2	118.3, CH	5.84 s	1, 4, 11b, 12
3	140.6, qC		
4	120.7, CH	6.13 s	2, 5,11b, 12
4a	144.9, qC [a]		
5	149.7, qC		
5a	131.9, qC [a]		
6	187,9, qC [a]		
6a	118.5, qC		
7	165.6, qC		
7-OH		12.96 s	6a
8	122.1, CH	6.78 d (8.2)	10
9	138.4, CH	7.40 t (8.2)	10a
10	118.7, CH	7.17 d (7.7)	8, 11
10a	137.0, qC		
11	151.9, qC		
11a	117.4, qC [a]		
11b	123.0, qC		
12	24.0, CH_3	1.93 s	2, 3, 4
1′	not found		
2′	125.9, qC		
3′	137.1, qC		
4′	118.3, CH	6.66 s	3′, 4a′, 12′
4a′	139.9, qC [a]		
5′	not found		
5a′	131.0, qC [a]		
6′	188.3, qC		
6a′	119.2, qC		
7′	165.5, qC		
7′-OH		12.97 s	
8′	123.2, CH	6.86 d (7.7)	
9′	139.1, CH	7.50 t (8.0)	7′, 10a′
10′	119.9, CH	7.43 d (7.5)	6a′, 8′
10a′	139.0, qC		
11′	153.4, qC		

11a′	116.9, qC [a]		
11b′	126.6, qC		
12′	22.8, CH_3	1.86 s	2′, 3′, 4′
1″	151.4, qC		
2″	118.4, CH	5.82 s	1″, 4″, 11b″, 12″
3″	140.4, qC		
4″	120.3, CH	6.17 s	2″, 5″, 11b″, 12″
4a″	143.9, qC [a]		
5″	148.8, qC		
5a″	131.4, qC [a]		
6″	188.9, qC [a]		
6a″	118.7, qC		
7″	165.6, qC		
7″-OH		13.02 s	6a″, 7″, 8″
8″	122.3, CH	6.78 d (8.3)	10″
9″	138.4, CH	7.38 t (8.2)	7″, 10a″
10″	118.7, CH	7.15 d (7.7)	6a″, 8″, 11″
10a″	136.9, qC		
11″	152.3, qC		
11a″	117.4, qC [a]		
11b″	123.5, qC		
12″	23.9, CH_3	1.86 s	3″, 4″

[a] Recorded at 125 MHz in $(CD_3)_2SO$. [b] Recorded at 500 MHz in $(CD_3)_2SO$.

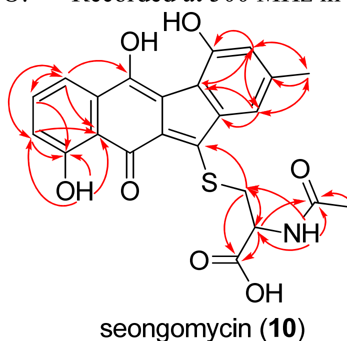


PK1 (**8**)

Table S3. The ^{1}H NMR and ^{13}C NMR data of seongomycin (**10**) in $(CD_3)_2SO$.

Position	^{13}C-NMR[a]	^{1}H-NMR[b]	HMBC[b]
1	116.9	7.08 (s, 1H)	C-3, C-4, C-4a, C-11a, C-12
2	138.1		
3	116.5	6.63 (s, 1H)	C-1, C-4, C-4a, C-12
4	150.0		
4a	121.2		
4b	114.7		
5	151.4		
5a	134.8		
6	116.4	7.34 (d, 1H, J = 7.0 Hz)	C-5, C-7, C-8, C-9a, C-9,C-10
7	136.1	8.32 (d, 1H, J = 7.0 Hz)	C-5a, C-6 C-9
8	120.0	6.95 (d, 1H, J = 7.0 Hz)	C-9, C-9a
9	163.2		
9-OH		13.52 (S, 1H)	C-8, C-9, C-9a
9a	116.4		
10	184.5		
10a	128.6		
11	141.7		
11a	142.8		
12		2.29 (s, 3H)	C-1, C-2, C-3
13	35.2	3.84 (m, 1H) 3.52 (m, 1H)	C-11, C-14, C-15
14	52.8	4.43 (m, 1H)	C-13, C-15, C-16
15	172.2		
16	169.8		
17	22.7	1.76 (s, 3H)	C-16
NH		8.32 (d, 1H, J = 7.0 Hz)	C-13, C-14, C-16

[a] Recorded at 125 MHz in $(CD_3)_2SO$. [b] Recorded at 500 MHz in $(CD_3)_2SO$.



seongomycin (**10**)

Table S4. List of primers used in this study.

primer	restriction site	sequence
K-upF	*Hind*III	5' CCCAAGCTTCGGACGCCGTCATGCTGG 3'
K-upR	*Bam*HI	5' CGGGATCCCATCTGCCCTCTTCTCACTGGT 3'
K-dnF	*Bgl*II	5' GAAGATCTCAGGGGCTGCTGGACACCTAC 3'
K-dnR	*Xba*I	5' GCTCTAGACGCCCGCGTACCGCTTTT 3'
CK-KF	/	5' TCCAGGCGTCGTCGAGTTCC 3'
CK-KR	/	5' GGCATGGGTTTCTTCGCTTCC 3'
K-F	*Bgl*II	5' GAAGATCTGAGAAGAGGGCAGATGGAAT 3'
K-R	/	5' TCAGGCGGTGGGGCCGAAC 3'
EalpK-F2	*Nde*I	5' GGAATTCCATATGGAATTCTACGATTCGGACG 3'
EalpK-R2	*Hind*III	5' CCCAAGCTTTCAGGCGGTGGGGCCGAACCAG 3'
alpKJ-F	*Kpn*I	5' GGGGTACCGCGGAACACCAGTGAGAA 3'
alpKJ-R	*Mfe*I	5' AAACAATTGAGCCCCTCTACGCCGTC 3'
AlpJ1F	*Hind*III	5' CCCAAGCTTGAGTCATCCCGGTCGGCCACTTCG 3'
AlpJ1R	*Xba*I	5' GCTCTAGACTCGGCGGAGATGATGGGCATGG 3'
AlpJ2F	*Xba*I	5' GCTCTAGAACGCGTTCGGCCGGCGAGTGAG 3'
AlpJ2R	*Eco*RI	5' GGAATTCATCGCCTCGGCCATCTCCGCGC 3'
AlpJ12-S5-F	/	5' ATGCCCATCATCTCCGCCGAG 3'
AlpJ12-S5-R	/	5' CGACGATCAGCGTGCTGTGC 3'
AlpJ-Psf-5	*Bam*HI	5' CGGGATCCCACCTTGAAGGGAAGCGAAGAAACC 3'
AlpJ-Psf-3	*Kpn*I	5' GGGGTACCTCACTCGCCGGCCGAACGCG 3'
AlpJ-30C-5	*Nde*I	5' GGAATTCCATATGCCCATCATCTCCGCCGAGG 3'
AlpJ-30C-3	*Hind*III	5' CCCAAGCTTCTCGCCGGCCGAACGCGTG 3'
AlpJ-P-native-5	*Xba*I	5' GCTCTAGACCCCCCGCCCGGCCCCC 3'

Supplementary Table 5. HRMS data in this study.

Compound	Formula	$[M+H]^+$	Calculated	Found	Delta ppm
PK1 (**10**)	$C_{54}H_{32}O_{12}$	$C_{54}H_{33}O_{12}$	873.1967	873.1966	-0.15
12	$C_{36}H_{22}O_8$	$C_{36}H_{23}O_8$	583.1387	583.1382	0.37

References

1. T. Kieser, M. J. Bibb, M. J. Buttner, K. F. Chater and D. A. Hopwood, *Practical streptomyces genetics*, John Innes Foundation Norwich, 2000.
2. M. M. Bradford, *Anal Biochem*, 1976, 72, 248-254.