Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supplementary Information

Isolation and Biosynthesis of Labdanmycins: New Labdane Diterpenes from Endophytic *Streptomyces*

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Contents of Supporting Information

Tab. S1. Homologous genes and proposed function of genes in labdanmycins biosynthetic gene cluster of *Streptomyces* sp. KIB 015

NO.	Size aa	Proposed function by a BLAST search (blastp)	Identity/ positive	Accession number
orf1	471	AraC family transcriptional regulator [*Streptomyces niveus*]	90%/92%	WP_069625743.1
orf2	125	pyridoxamine 5'-phosphate oxidase [*Streptomyces* sp. AcH505]	91%/95%	KIF71030.1
orf3	465	ATP-binding protein [*Streptomyces niveus*]	85%/90%	WP_023536773.1
orf4	139	dynein regulation protein LC7 [*Streptomyces* sp. 1]	57%/70%	WP_099897893.1
orf5	128	DUF742 domain-containing protein [*Streptomyces niveus*]	97%/97%	WP_078074393.1
orf6	194	GTPase [*Streptomyces* sp. Ncost-T10-10d]	81%/89%	SCF65553.1
labE	436	cytochrome P450 [*Streptomyces niveus*]	96%/97%	WP_078074391.1
labA	348	geranylgeranyl diphosphate synthase [*Streptomyces niveus*]	94%/96%	WP_078074390.1
labB	553	labdadienyl diphosphate synthase [*Streptomyces anulatus*]	76%/82%	BAR97461.1
labC	453	cytochrome P450 [*Streptomyces niveus*]	93%/97%	WP_078074388.1
labD	321	labdatriene synthase [*Streptomyces anulatus*]	76%/83%	BAR97463.1
orf1	334	transcriptional regulator [*Streptomyces niveus*]	97%/98%	WP_078074386.1
orf1	469	N-acetylmuramoyl-L-alanine amidase [*Streptomyces niveus*]	95%/97%	WP_078079387.1
orf1	258	M23 family peptidase [*Streptomyces niveus*]	98%/100%	WP_078074385.1
orf1	161	DUF456 domain-containing protein [*Streptomyces anulatus*]	69%/80%	WP_057666569.1
orf1	334	luxR-family transcriptional regulator [*Streptomyces laurentii*]	81%/88%	BAU86910.1

Tab. S2. Strains and plasmids used and generated in this study.

Strains/Plasmids	Characteristic(s)	Sources
E. coli		
DH5α	Host strain for cloning	Invitrogen
BL21(DE3)	Host strain for protein expression	Novagen
XL1-Blue MR	Host for constructing genomic library	Agilent Technologies
BW25113/pIJ790	Host strain for PCR targeting	Ref. 1
ET12567/pUZ8002	Donor strain for conjugation	Ref. 1
Streptomyces		
S. sp. KIB 015	Wild type, Diterpene producer	This study
S. sp. KIB 015 Δ*labB*	The *labB* gene disrupted mutant of KIB 015	This study
S. sp. KIB 015 Δ*labE*	The *labE* gene disrupted mutant of KIB 015	This study
S. albus J1074	Host strain for heterologous expression	Ref. 2
S. coelicolor M1154	Host strain for heterologous expression	Ref. 3
S. albus 9B5	*S. albus* J1074 integrated with pJTU2554-9B5 which contains labdanmycins biosynthetic gene cluster	This study
S. coelicolor 9B5	*S. coelicolor* M1154 integrated with pJTU2554-9B5 which contains labdanmycins biosynthetic gene cluster	This study
Plasmids		
pSupercos-1	Kan^r, Cosmid vector for genomic library construction	Stratagene
19A10	Kan^r, Cosmid pSupercos-1 which contains labdanmycins biosynthetic gene cluster	This study
19A10 Δ*labB*	Kan^r, gene inactivation clone used for *labB* mutant	This study
19A10 Δ*labE*	Kan^r, gene inactivation clone used for *labE* mutant	This study
pJTU2554	Apr^r, Cosmid vector for genomic library construction	Ref. 4
9B5	Apr^r, Cosmid pJTU2554 which contains labdanmycins biosynthetic gene cluster	This study

Tab. S3. Primers used in this study

Primers	Targeted genes	Sequences
for screening genomic library		
SG-F	*labD*	5'-TGAGTGCGTCCACCTCCTCG-3'
SG-R		5'-GCAGTGACGGCAGGTTGAGC-3'
For PCR targeting		
Tar-labE -F	*labE*	5'-GTGAGTATCACACCACCGGACGTCACCGCTGTGCCCCTGttccggggatccgtcgacc-3'
Tar-labE -R		5'-CTAGGAAGTCGTCACCGACATACGGTCGCCTGCCGTCGAtgtaggctggagctgcttc-3'
Tar-labB -F	*labB*	5'-ATGACCGCCCGCCCGACCCCCGTCACCGCAACCGTGAGGttccggggatccgtcgacc-3'
Tar-labB -R		5'-TCATGCGTCCGTCCTACCTTCCGCGTCTGTGGGTGCGTTtgtaggctggagctgcttc-3'
For gene validation		
Che-labE -F	*labE*	5'- GTGAGTATCACACCACCG -3'
Che-labE -R		5'- CTAGGAAGTCGTCACCGAC -3'
Che-labB -F	*labB*	5'- ATGACCGCCCGCCCGACC -3'
Che-labB -R		5'- TCATGCGTCCGTCCTACC -3'

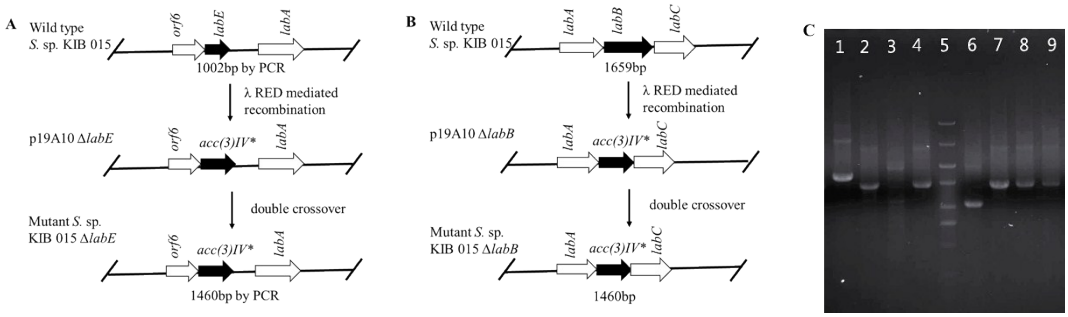


Fig. S1. Gene replacement of *labB* and *labE* using the PCR-targeting method. (A) Scheme for the construction of *labE* replacement mutant. (*acc(3)IV** included *acc(3)IV* and *OriT*.) (B) Scheme for the construction of *labB* replacement mutant. (*acc(3)IV** included *acc(3)IV* and *OriT*.) (C) Gel electrophoresis of PCR products. Lane 1: *labB* (*S.* sp. KIB 015), 1659 bp; Lane 2-4: *ΔlabB*, 1460 bp; Lane 5: DNA molecular ladder (5000 bp, 3000 bp, 2000 bp, 1500 bp, 1000 bp, 750 bp, 500 bp, 250 bp). Lane 6: *labE* (*S.* sp. KIB 015), 1002 bp; Lane 2-4: *ΔlabE*, 1460 bp;

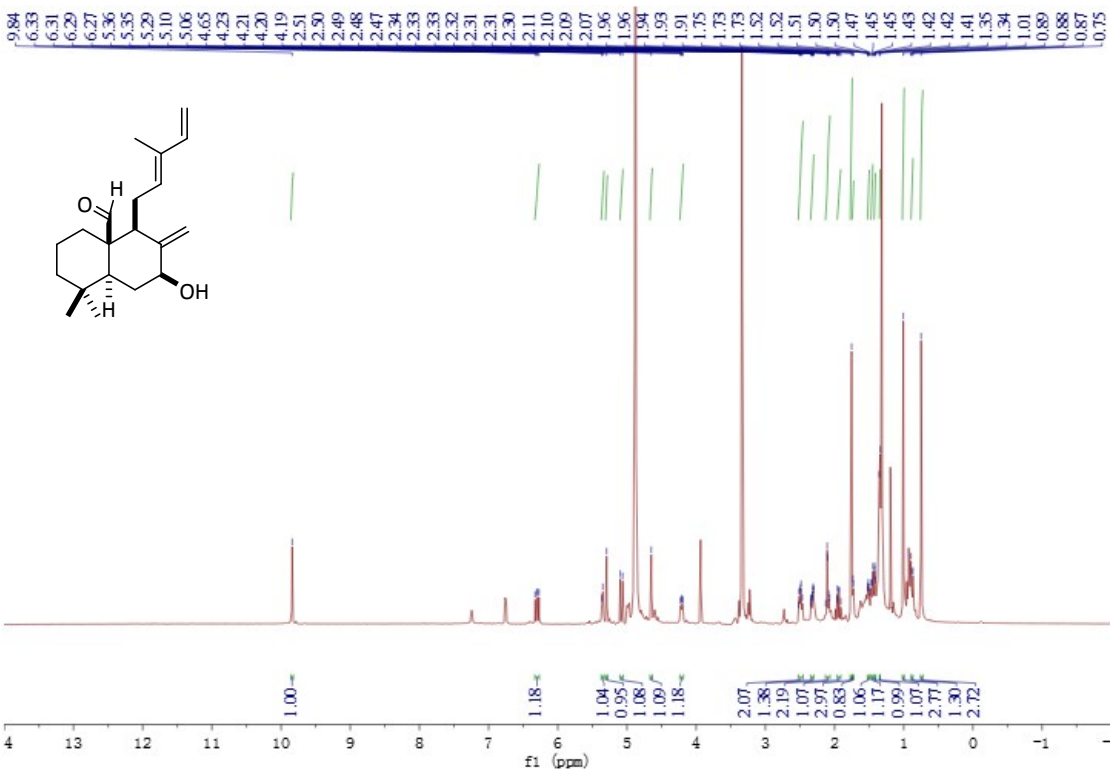


Fig. S2. ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **1**

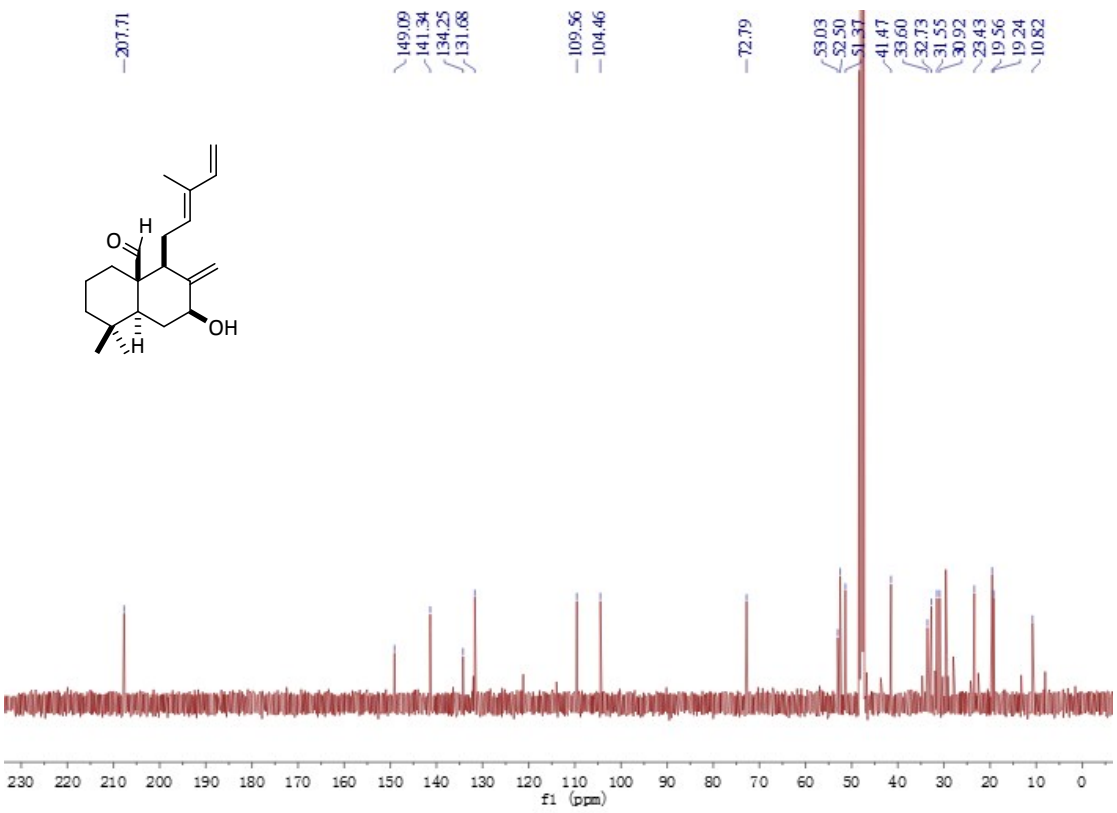


Fig. S3. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **1**

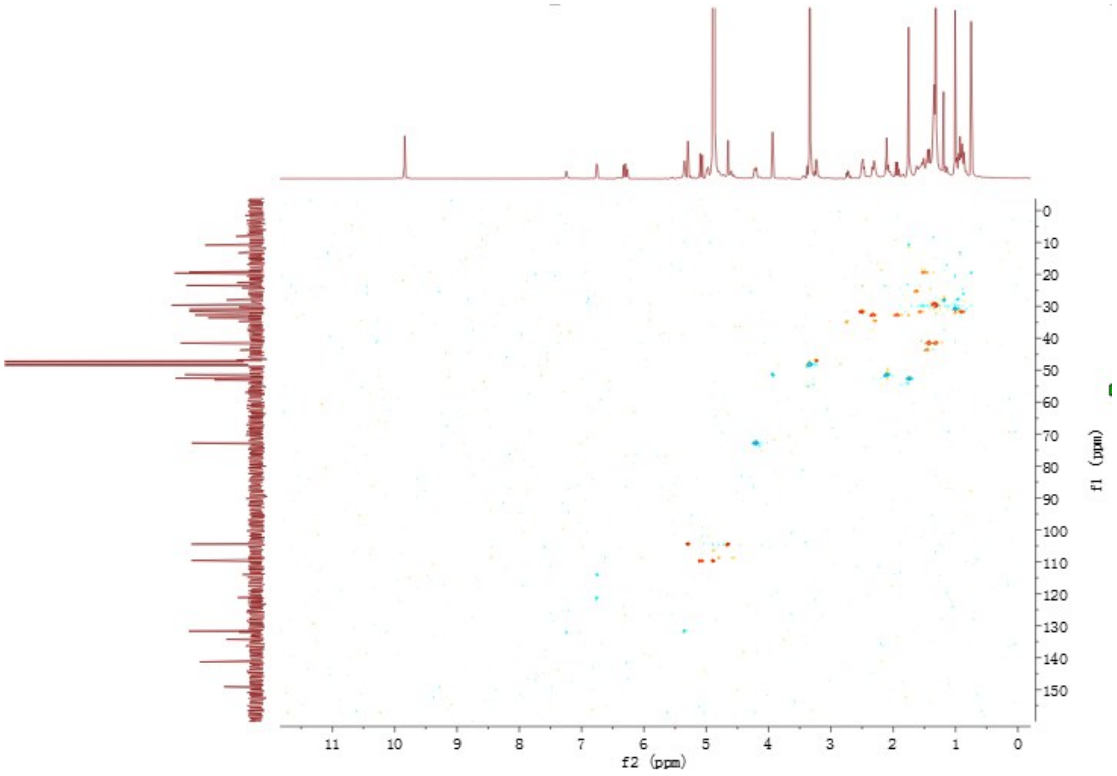


Fig. S4. HMQC spectrum of compound **1** (in CD_3OD)

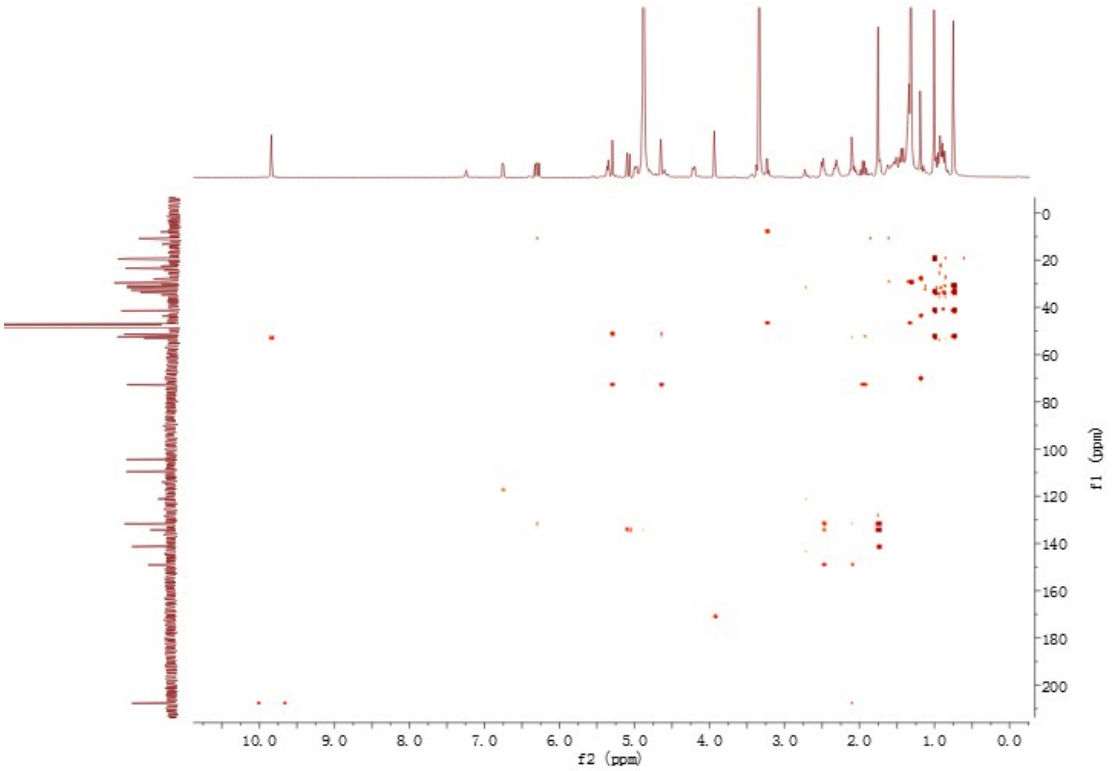


Fig. S5. HMBC spectrum of compound **1** (in CD_3OD)

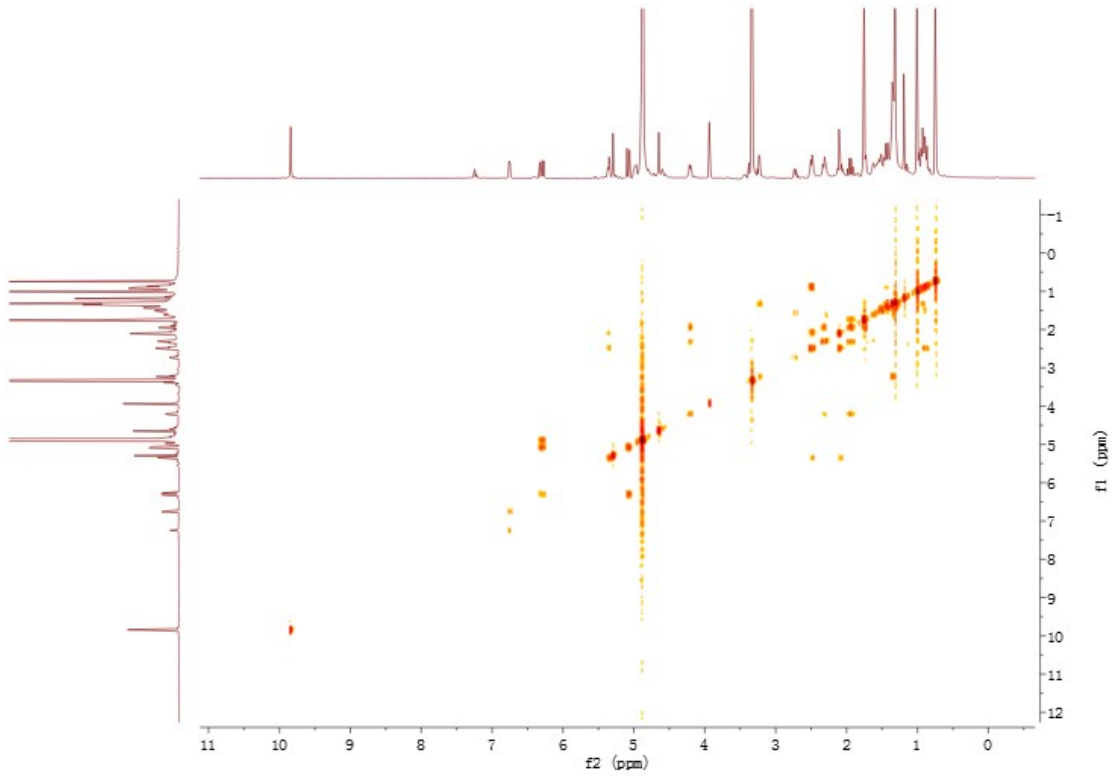


Fig. S6. ^1H-^1H COSY spectrum of compound **1** (in CD_3OD)

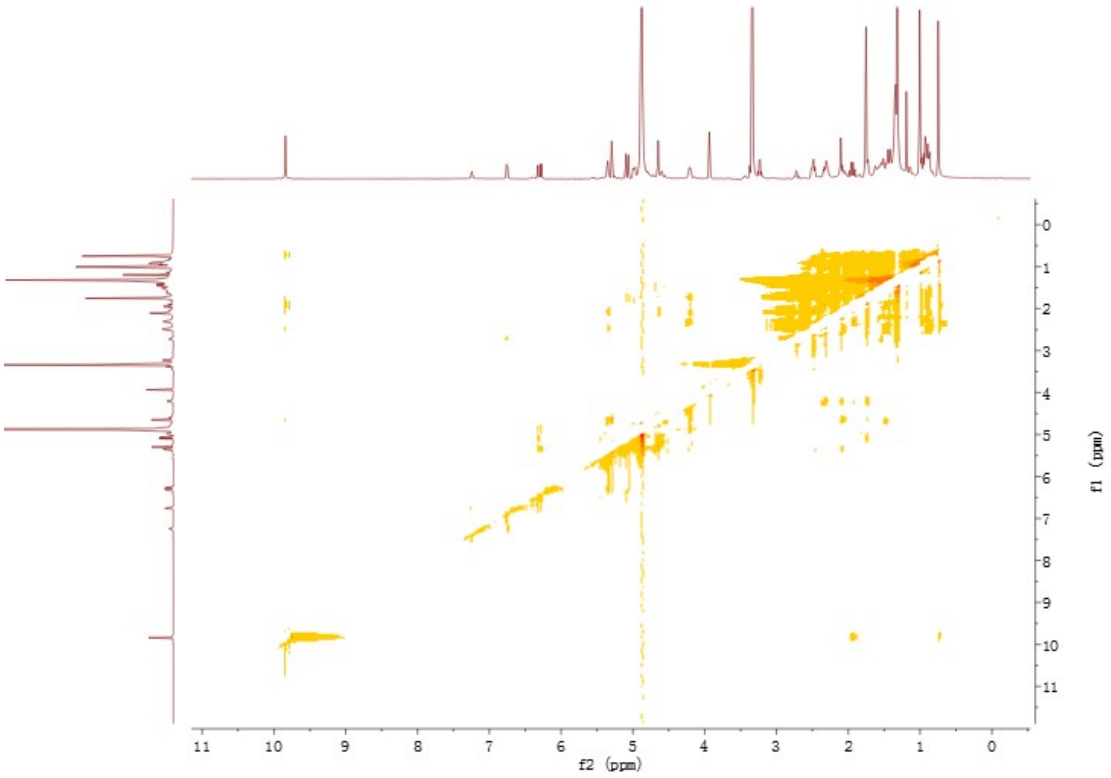


Fig. S7. ROESY spectrum of compound **1** (in CD_3OD)

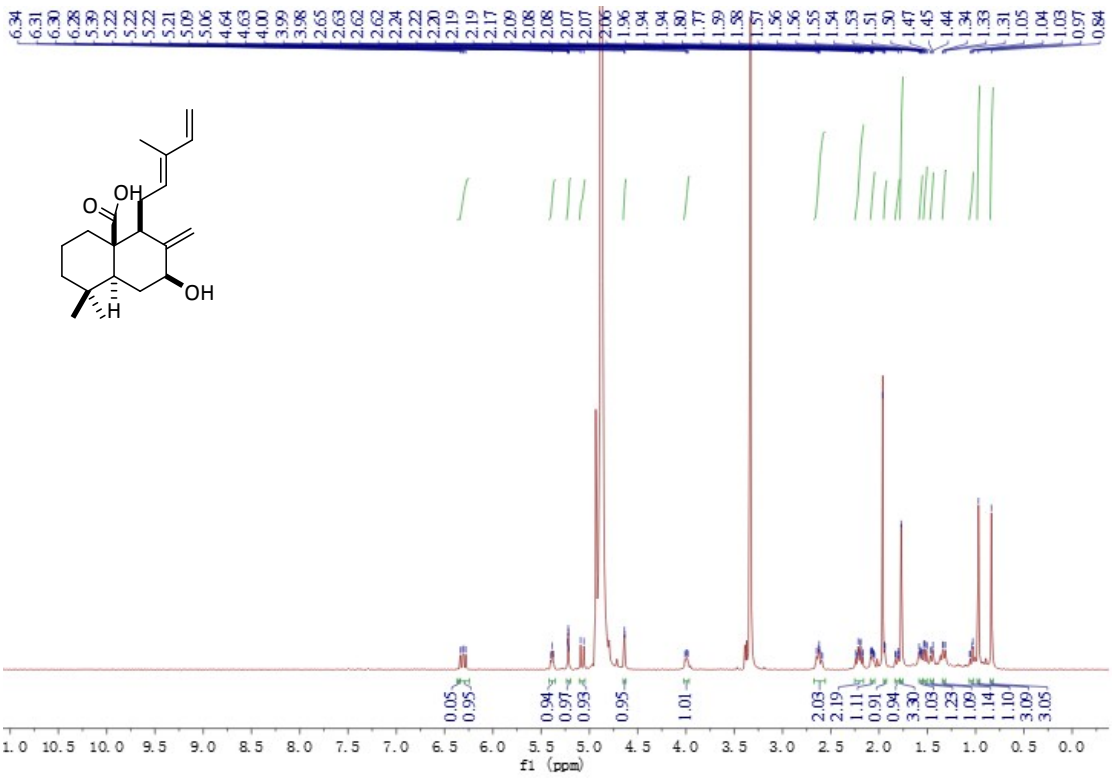


Fig. S8. ^1H NMR spectrum (CD_3OD, 500 MHz) of compound **2**

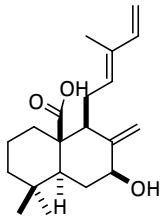


Fig. S9. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **2**

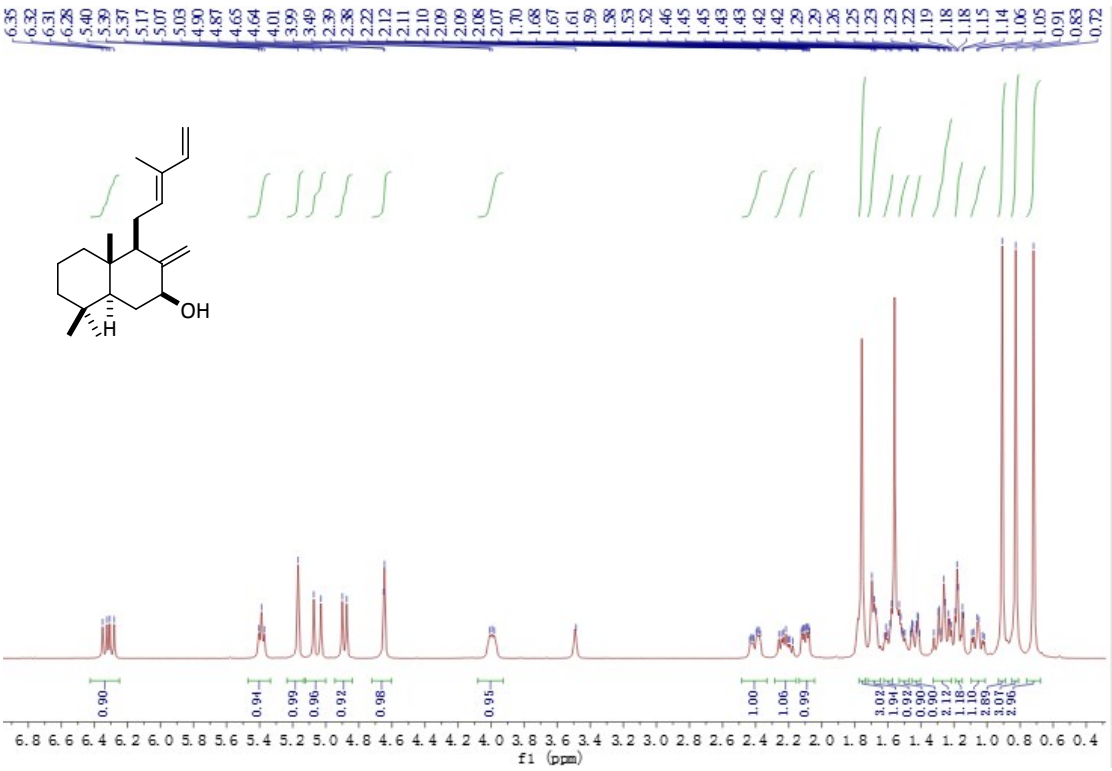
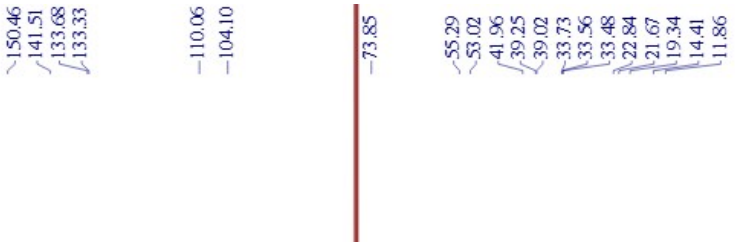


Fig. S10. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **3**



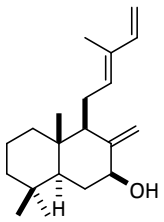


Fig. S11. ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of compound **3**

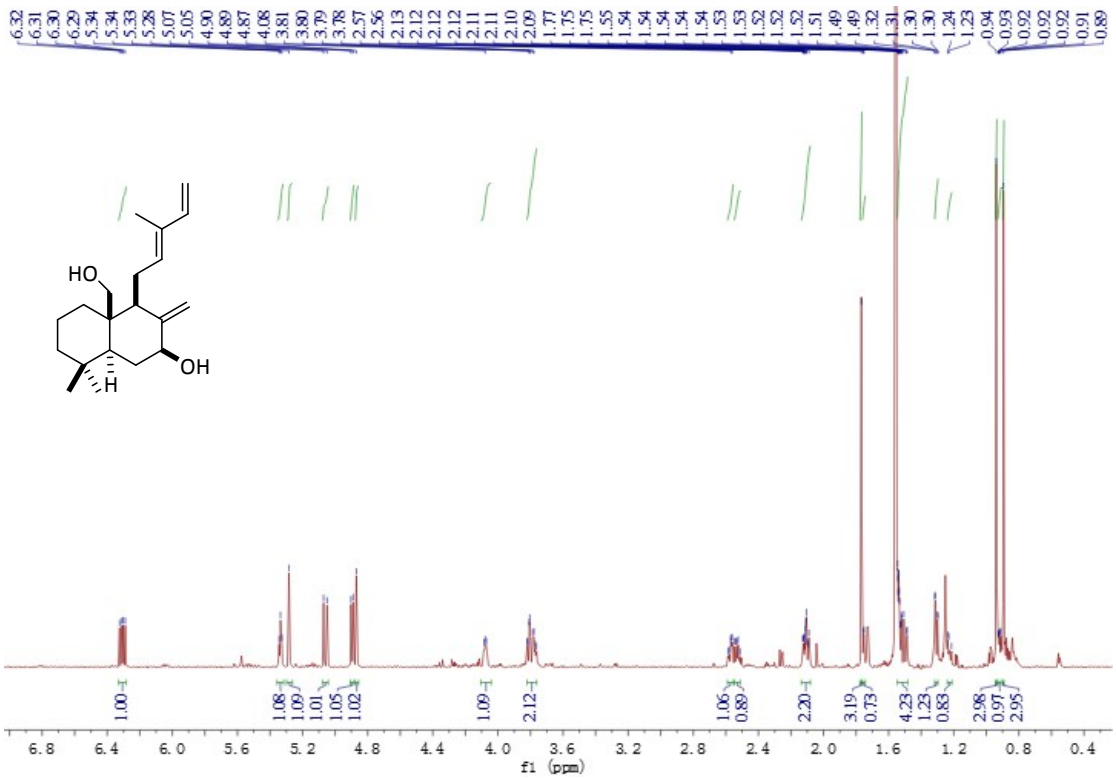
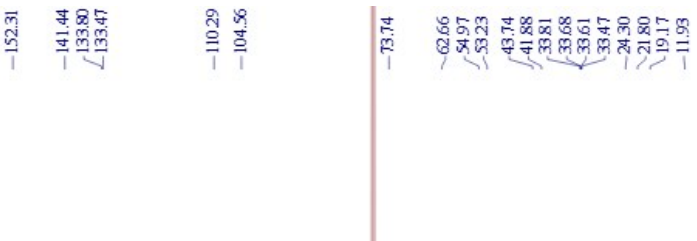


Fig. S12. ^{1}H NMR spectrum ($CDCl_3$, 800 MHz) of compound **4**



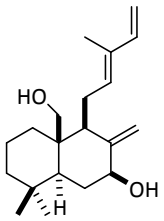


Fig. S13. ^{13}C NMR spectrum ($CDCl_3$, 200 MHz) of compound **4**

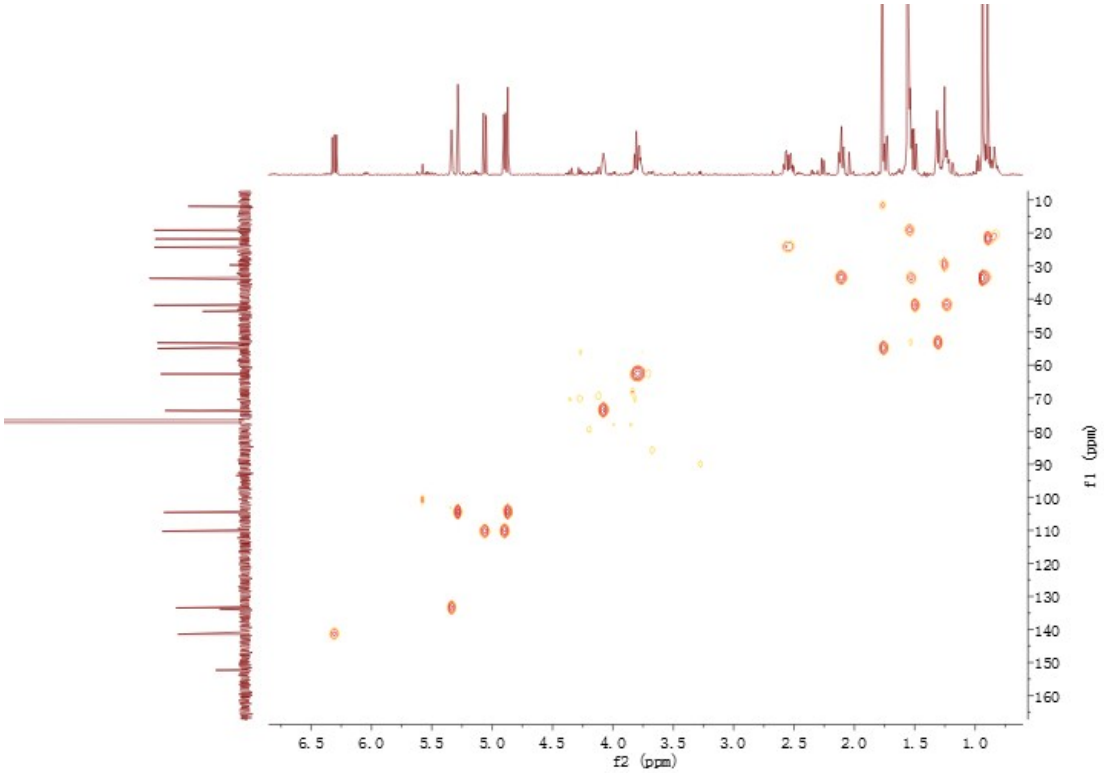


Fig. S14. HSQC spectrum of compound **4** (in $CDCl_3$)

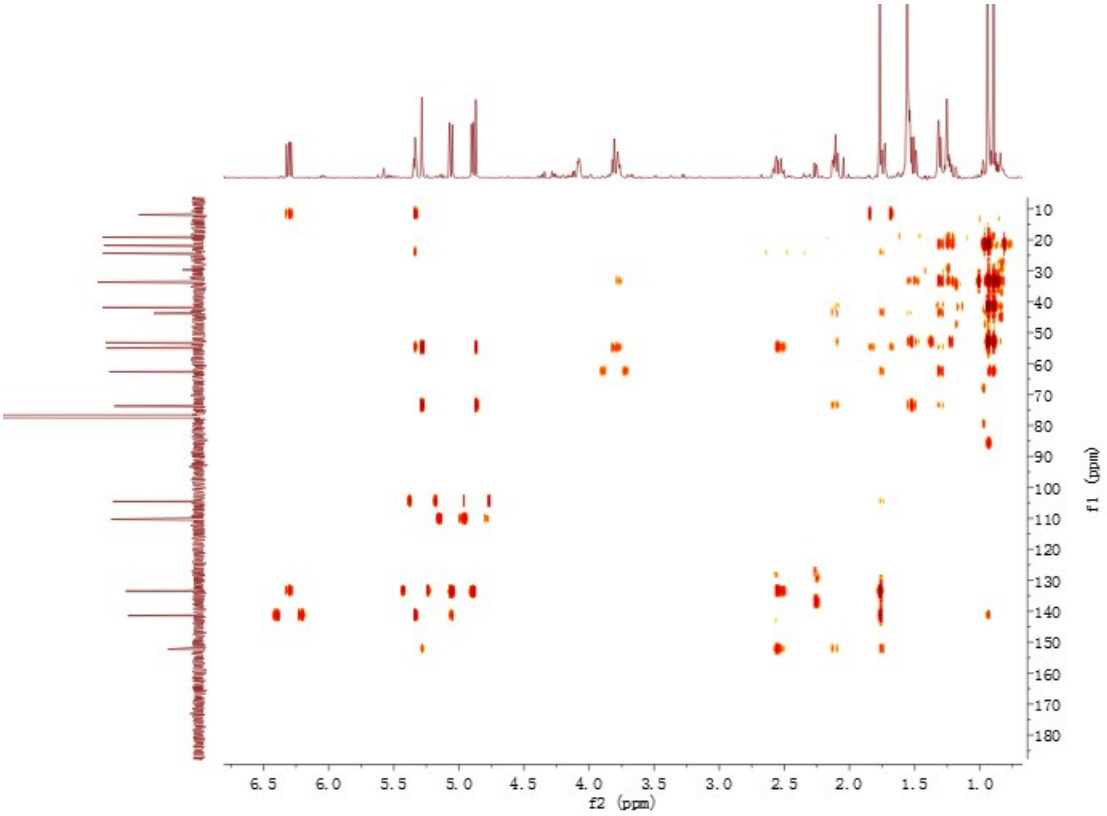


Fig. S15. HMBC spectrum of compound **4** (in $CDCl_3$)

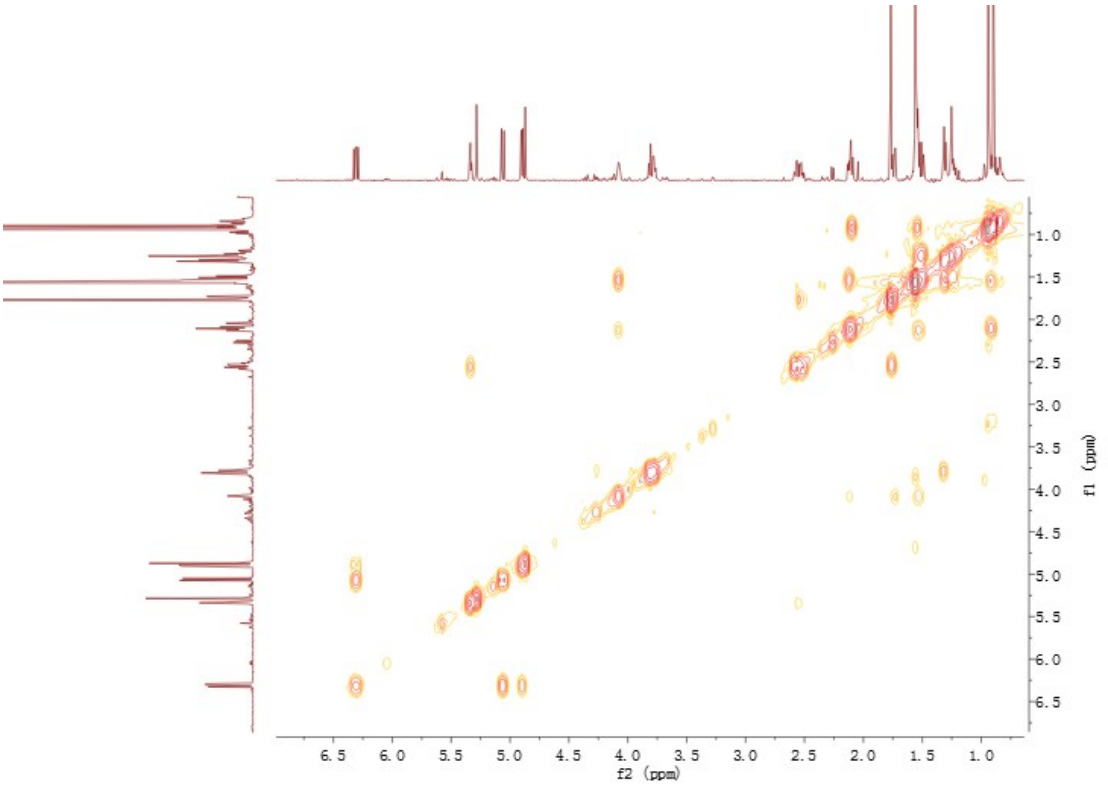


Fig. S16. ^{1} H-^{1}H COSY spectrum of compound **4** (in $CDCl_3$)

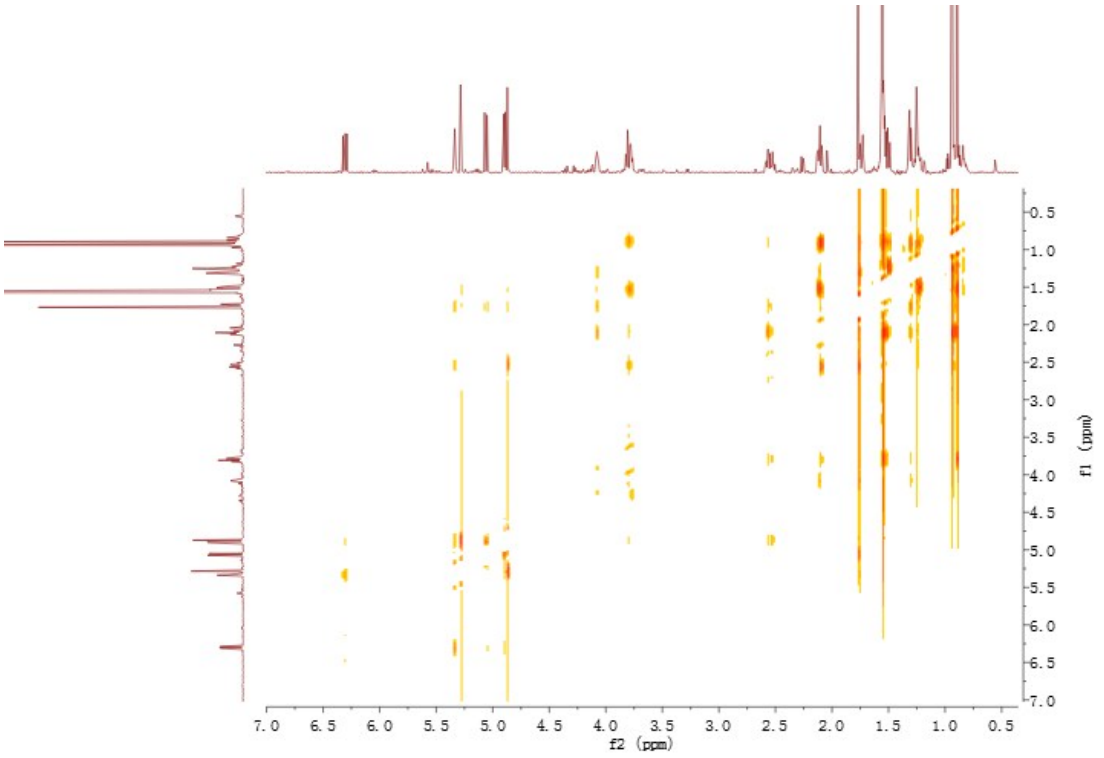


Fig. S17. ROESY spectrum of compound **4** (in $CDCl_3$)

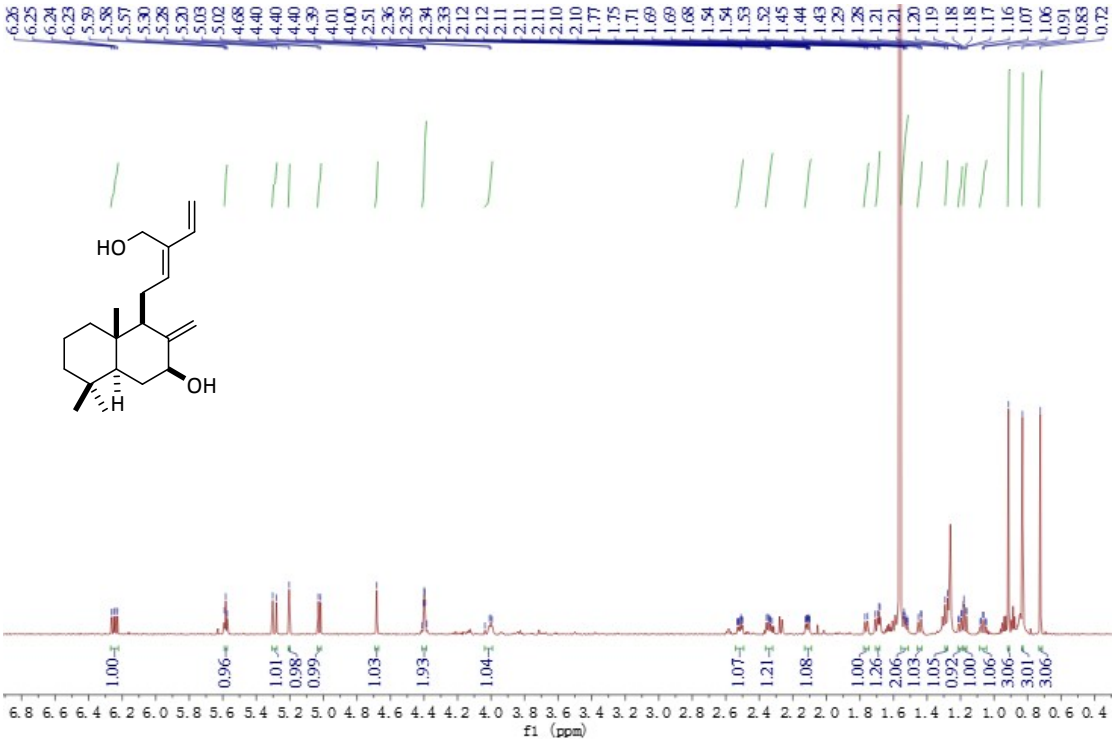


Fig. S18. ^1H NMR spectrum ($CDCl_3$, 800 MHz) of compound **5**

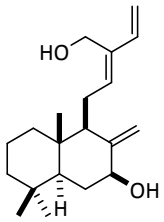


Fig. S19. ^{13}C NMR spectrum ($CDCl_3$, 200 MHz) of compound **5**

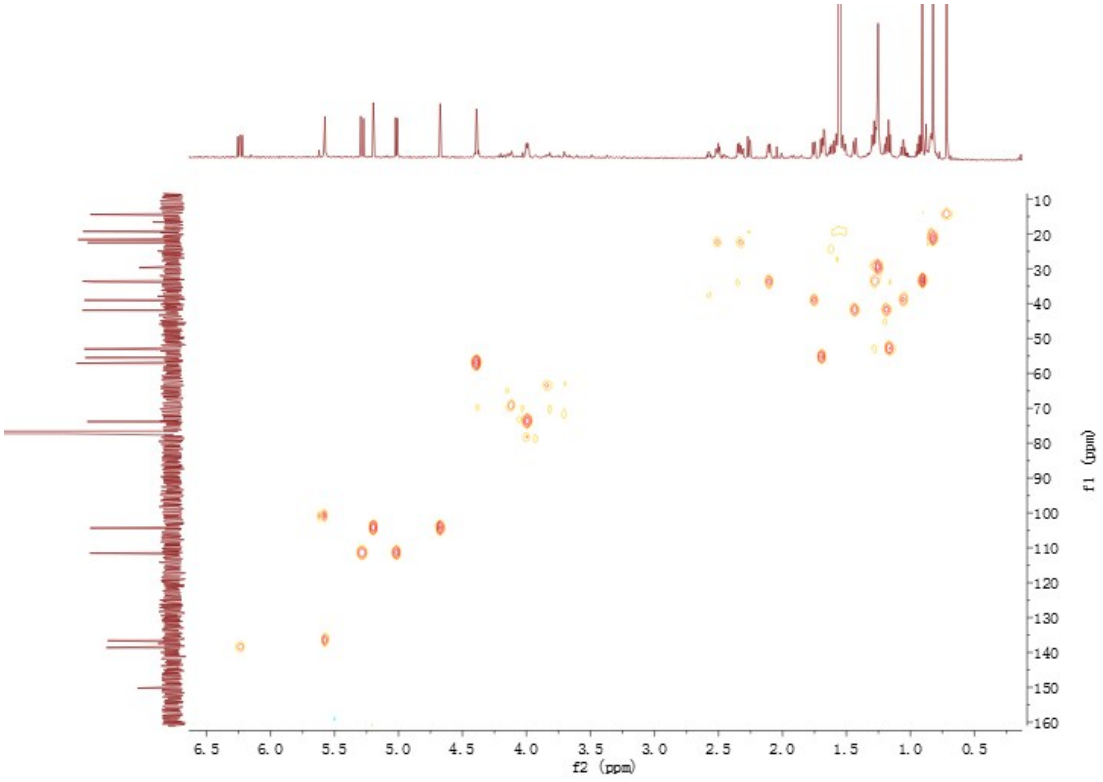


Fig. S20. HSQC spectrum of compound **5** (in $CDCl_3$)

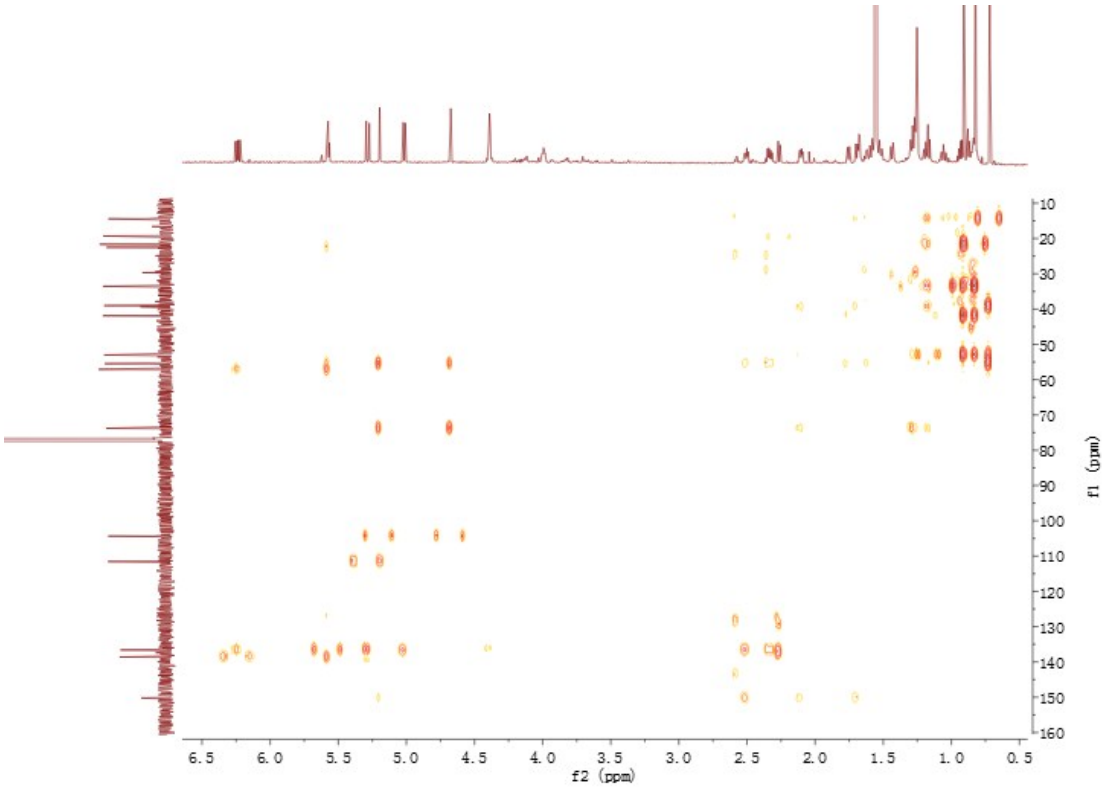


Fig. S21. HMBC spectrum of compound **5** (in $CDCl_3$)

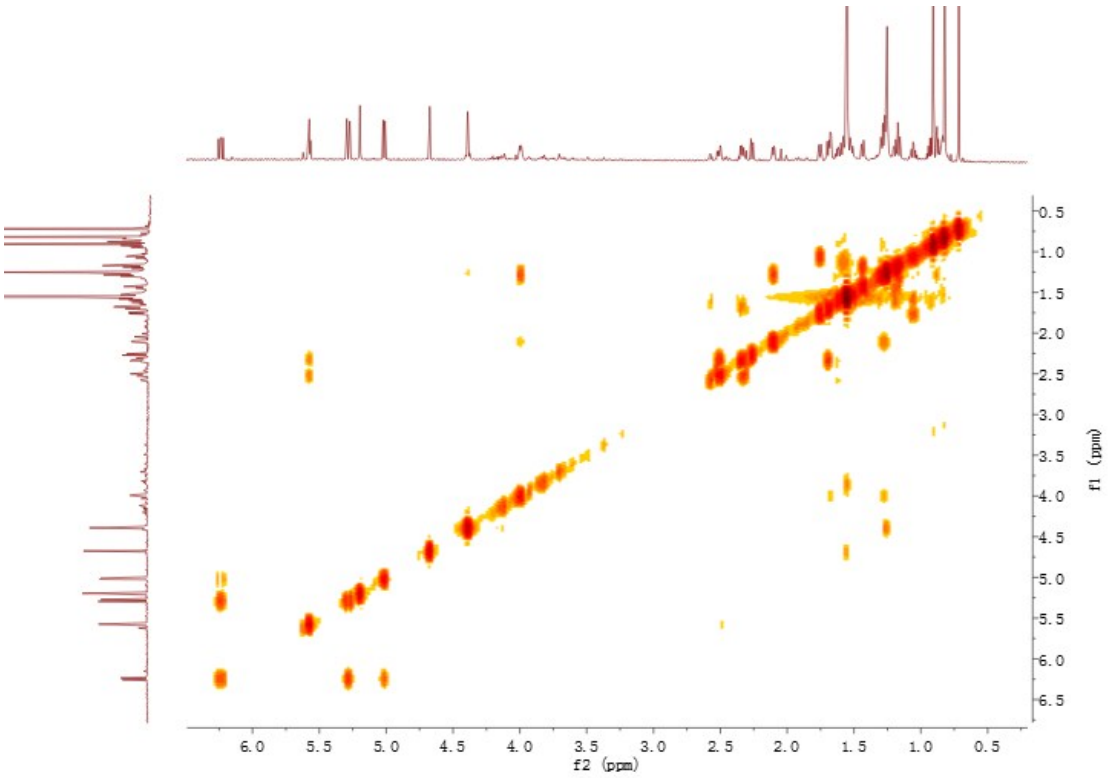


Fig. S22. ^{1} H-^{1}H COSY spectrum of compound **5** (in $CDCl_3$)

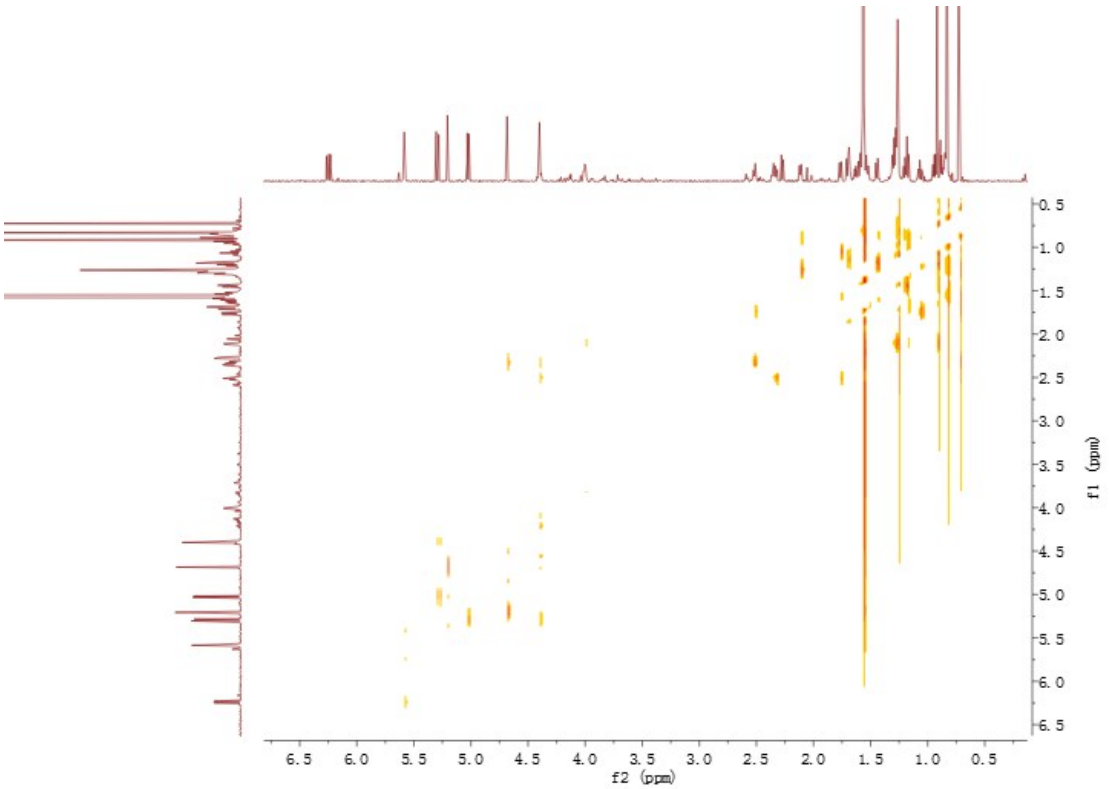


Fig. S23. ROESY spectrum of compound **5** (in $CDCl_3$)

Supplementary References

1. M. S. B. Paget, L. Chamberlin, A. Atrih, S. J. Foster and M. J. Buttner, 1999, *J. Bacteriol.*, **181**, 204–211.
2. K. F. Chater and L. C. Wilde, *Microbiology*, 1980, **116**, 323–334.
3. J. P. Gomez-Escribano and M. J. Bibb, *Microb. Biotechnol.*, 2011, **4**, 207–215.
4. L. Li, Z. Xu, X. Xu, J. Wu, Y. Zhang, X. He, T. Zabriskie and Z. Deng, *ChemBioChem*, 2008, **9**, 1286–1294.