

The discovery of actinospene, a new polyene macrolide with broad activity against plant fungal pathogens and pathogenic yeasts

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Table S1. Gene annotation for the cryptic Actn BGC based on antiSMASH 6.0 prediction.

ORF	nt (bp)	Proposed function (domain organization)	Accession number
ActnS2	7474	TI PKS, KS-AT-DH-KR-PP-KS-AT	WP_035287518.1
ActnS0	8148	TI PKS, KS-AT-PP-KS-AT-DH-KR-PP	WP_035287521.1
ActnK	1431	Glycosyltransferase family 1 protein	WP_052021789.1
ActnC	1059	DegT/DnrJ/EryC1/StrS family aminotransferase	WP_035287523.1
ActnG	1179	Cytochrome P450	WP_035287525.1
ActnF	192	Ferredoxin	WP_035287527.1
ActnS4	6012	TI PKS, KS-AT-DH-KR-PP-TE	WP_035287529.1
ActnS1	5385	TI PKS, KS-AT-DH-KR-PP	WP_052021790.1
ActnS3	27906	TI PKS, KS-AT-DH-KR-PP-KS-AT -KR-PP-KS-AT-KR-PP-KS-AT-KR-PP-KS-AT- KR-PP-KS-AT- KR-PP	WP_035287531.1
ActnA	1839	ABC transporter ATP-binding protein	WP_035287533.1
ActnB	1737	ABC transporter ATP-binding protein	WP_084176275.1
ActnD3	1176	Cytochrome P450	WP_152552277.1
ActnD2	1182	Cytochrome P450	WP_035287537.1
ActnU	1740	Acyl-CoA ligase	WP_035287549.1
ActnV	2202	Acyl-CoA dehydrogenase	WP_035287553.1
ActnR1	693	TetR/AcrR family transcriptional regulator	WP_035287558.1
ActnD1	1206	Cytochrome P450	WP_035287568.1
ActnR2	603	TetR/AcrR family transcriptional regulator	WP_052021798.1
ActnR3	1174	Sensor histidine kinase	WP_152552284.1
ActnJ	1032	GDP-mannose 4,6-dehydratase	WP_035288993.1

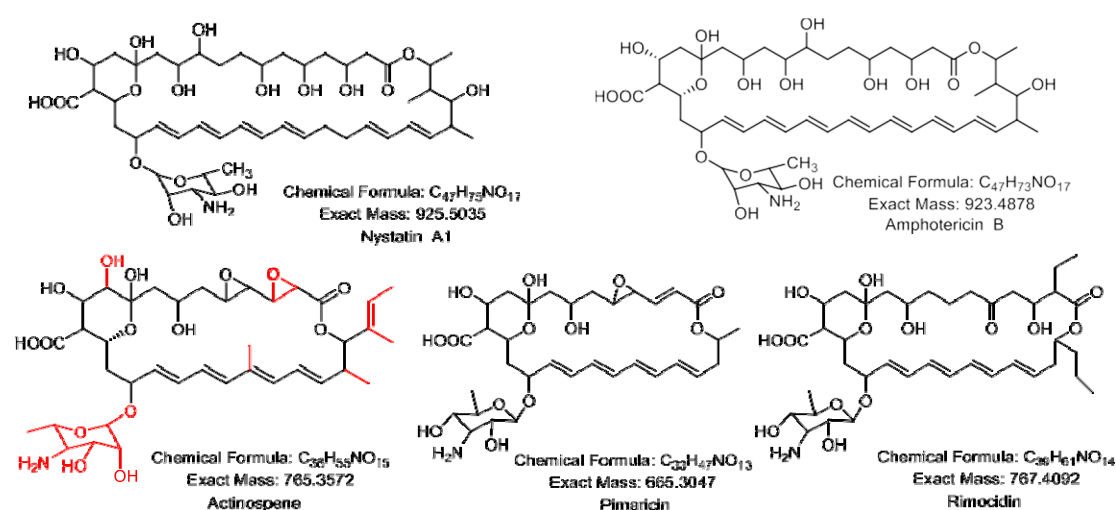


Figure S1. The planar structures of actinospene and previously reported polyene antifungal antibiotics. Differences between actinospene and pimaricin are indicated in red.

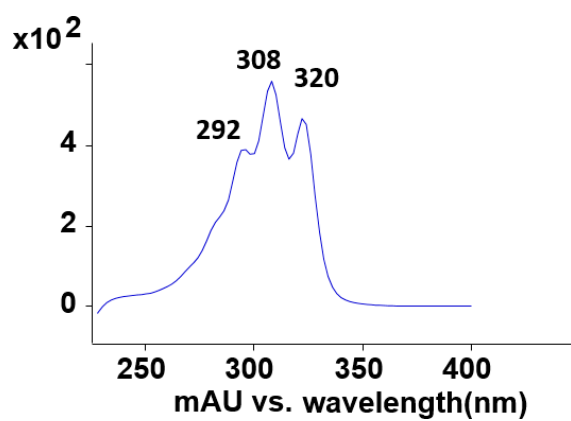


Figure S2. UV/Vis spectrum of actinospene (1).

UV/Vis spectrum of the main HPLC peak at *t_R* 5.27 min showing absorption maxima at 292, 308, and 320 nm.

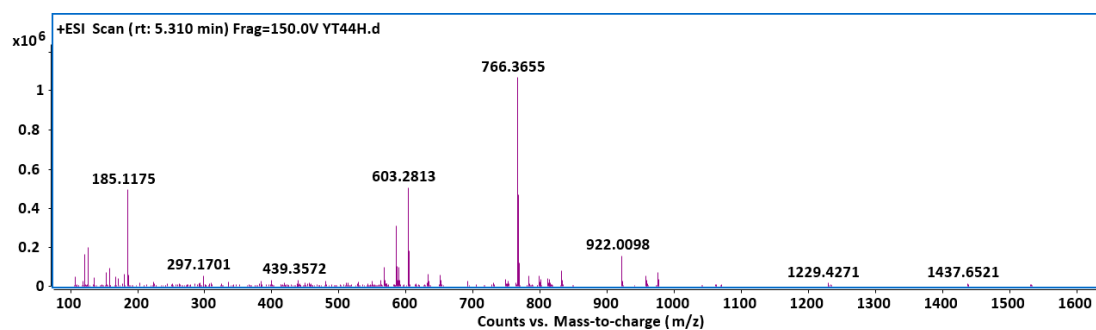


Figure S3. High resolution mass spectrum of actinospene (1).



Figure S4. Schematic representation for the cryptic Actn BGC.

ActnS0KS0	AESHEYGPGLLNA-DGAEGLHHTGTAAGVTSGRIAYALGLRGPVLTVDTA	SSGSLVALHL
AmphAKS0	TNGQDYATVTNASREDLTGHALTGLSPSIASGRLAYFLGLECPAVTLDTA	SSSSLVALHY
NysAKS0	ASGQDYAAVTHASPDLDLGHALTGLAPGVASGRLAYVFLGLECPAVTVDTT	SSSSLVALHW
ActnS3KS5	AMPQEYRVGAG---DNVDGFRLTGNTSSVVSGRLAYFLGLVGPATVDTA	CSSSLVSLHL
TetrAKS0	AMDRGYCAQASAVPRAWESMLITGASASAIISGRIAYTYGLECPALTVDTA	SSSSLVALHL
PimS0KS0	AMDRGYGTSASAAPSAWESMLITGASAVSGRIAYTYGLECPALTVDTA	SSSSLVALHL
ActnS4KS12	TDGQDYASLVFNSVADSEGHACTGIAASAIAGRLAYTFGLECPAVTVDTA	CSSSLVALHL
ActnS1KS2-4-11	TNGQDYAYLVVRSAAESTGEVGTGIAASAMSGRLAYTLGLECPAVTVDTA	CSSSLVALHS
ActnS3KS9	TNGQDYLSVLRTAAEDVRCHAATGVTASVLSGRVSYALGLECPAVTVDTA	CSSSLVALHW
ActnS3KS10	GISGDYRPSADGR-----DWQTAQSASLLSGRLAYTFGLECPVSDTA	CSSSLVAVHL
ActnS3KS7	SSFQDYAAAR---VDDLEPHVVTGSIPSVLSGRLAYVFLGLECPAVTVDTA	CSSSLVAMHL
ActnS3KS8	ASYQDYTASVSRTAESABGHMITGSLGSILSGRVAYLLGLECPAVTLDTA	CSSSLVALHL
ActnS0KS1	TFGGCYGSLDGR-GDARCFIMTGTTPSVLSGRLSYVLGLVGPALTVDTA	CSSSLVALHL
ActnS2KS3	VMYQDYADLLC-D-AEYEGFRISGSSASVASGRVAYSLGFEGPAVTVDTA	CSSSLVSLHL
ActnS3KS6	VMYSDYANALC-G-GEFEGHQGTGTSASVVSGRVSYTLGLECPAVTVDTA	CSSSLVAMHL
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Figure S5. Partial amino acid sequence comparison for the KS domains.

The KS domains of the loading module of amphotericin, pimaricin, tetramycin, nystatin PKS all contain a serine instead of a cysteine in the active site as indicated by the cross region of the red and blue boxes. Abbreviations: Nys: Nystatin; Amph: Amphotericin; Pim: pimaricin; Tetr: tetramycin.

EQPWLAEHVWGCRTLVF
THPWLADHTVACRVLF
AQPWLAVYEADGRTLVF
THPWLTDHAIGNVFFF
AYPWLTDHTMGGSVLF
AQPWLADHTVGRGTVLF
AQPWLADHTAFGQVLLF
EQPWLADHVVAGRVLFF
TLPWLADHVISGRVLFF
THPWLADHVVAGGVALF
RLSYLSEHRHVGEAVLF
RLPWLGEHRAQGEVFFF

*: * * *

WPP motif

YAFHPVLL	DAVAG
HGLHPALL	DAARR
FALHPALL	DTAVR
GGLHPALL	DAVLQ
GGLHPVLL	DAVLQ
FGVHPALL	DAALH
FTLHPALL	DAALH
GGLHPALL	DAAVQ
LG IHPALL	DAVLH
FGAHPALL	DSVVQ
YRIHPALL	DACFQ
YQLHPVLL	DACVQ
**. **:	:

LPFXW motifs

6

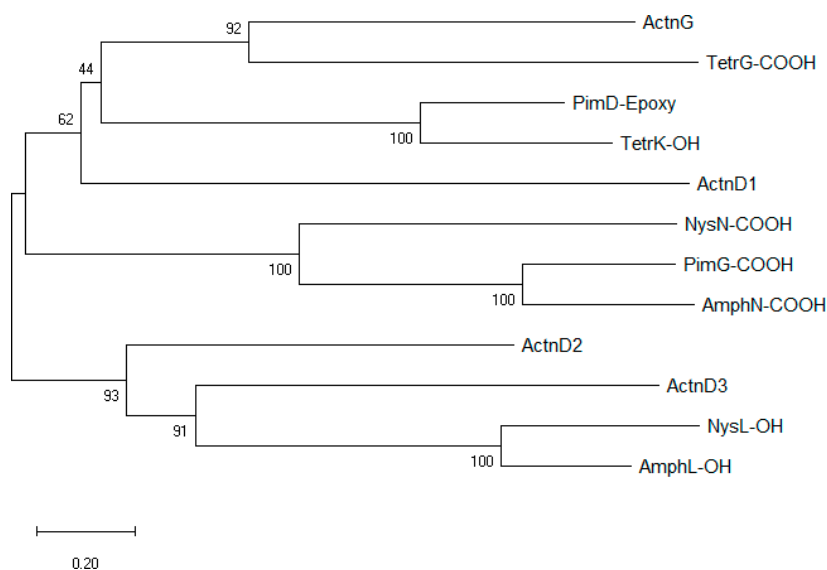


Figure S8. Phylogenetic analysis of the P450 by neighbor-Joining method.

Bootstrap values (expressed as percentage of 1000 replications) are shown at the branch points. Bar 0.2 substitutions per nucleotide position.

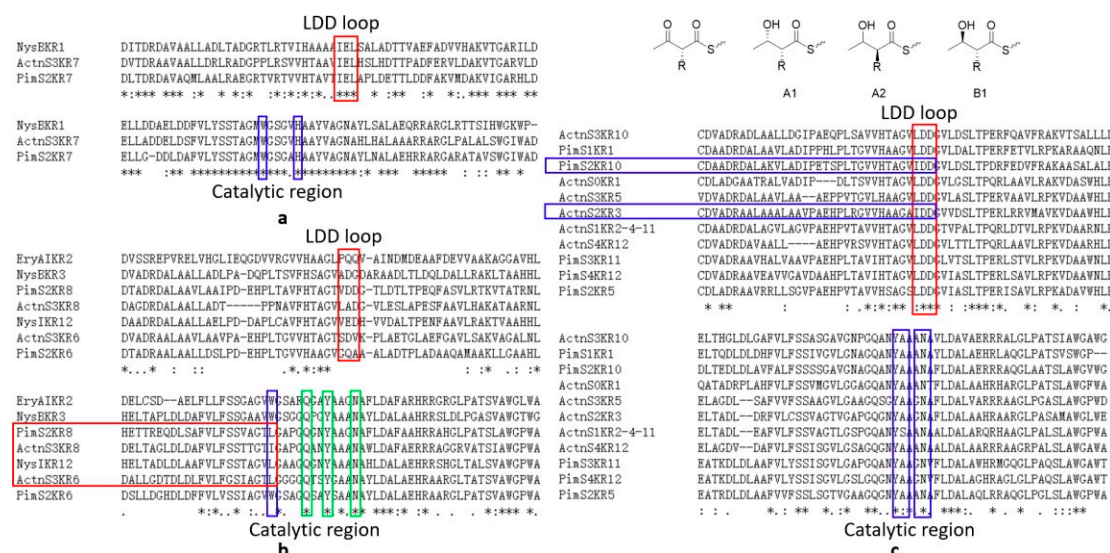


Figure S9. Partial sequence comparison for the KR domains.

Fingerprints featured for A1, A2, B1-type are highlighted by red and blue boxes [3].

Abbreviations: Ery: erythromycin.

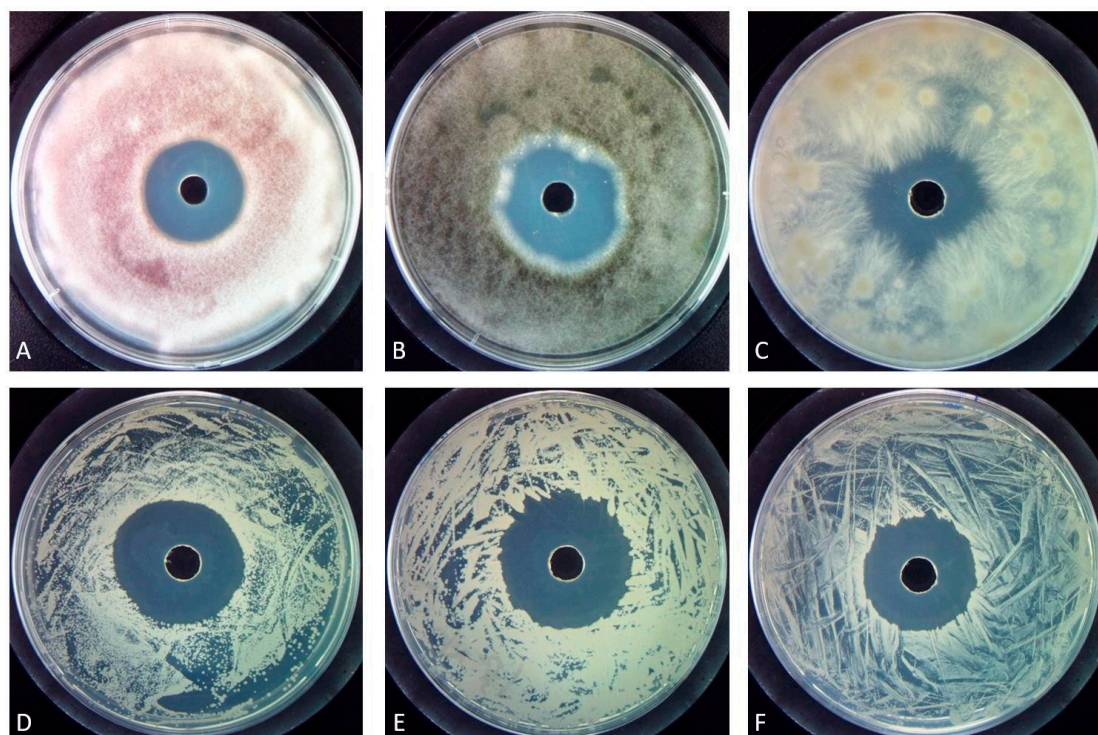


Figure S10. Antifungal activity of crude extracts from *A. spheciospongiae* against 6 pathogenic fungi.

(A) the conidial suspension of *Fusarium oxysporum*; (B) *Alternaria alternate*; (C) *Sclerotium rolfsi*; (D) the diluted broth suspension of *Candida albicans*; (E) *Cryptococcus neoformans*; (F) *Saccharomyces cerevisiae*.

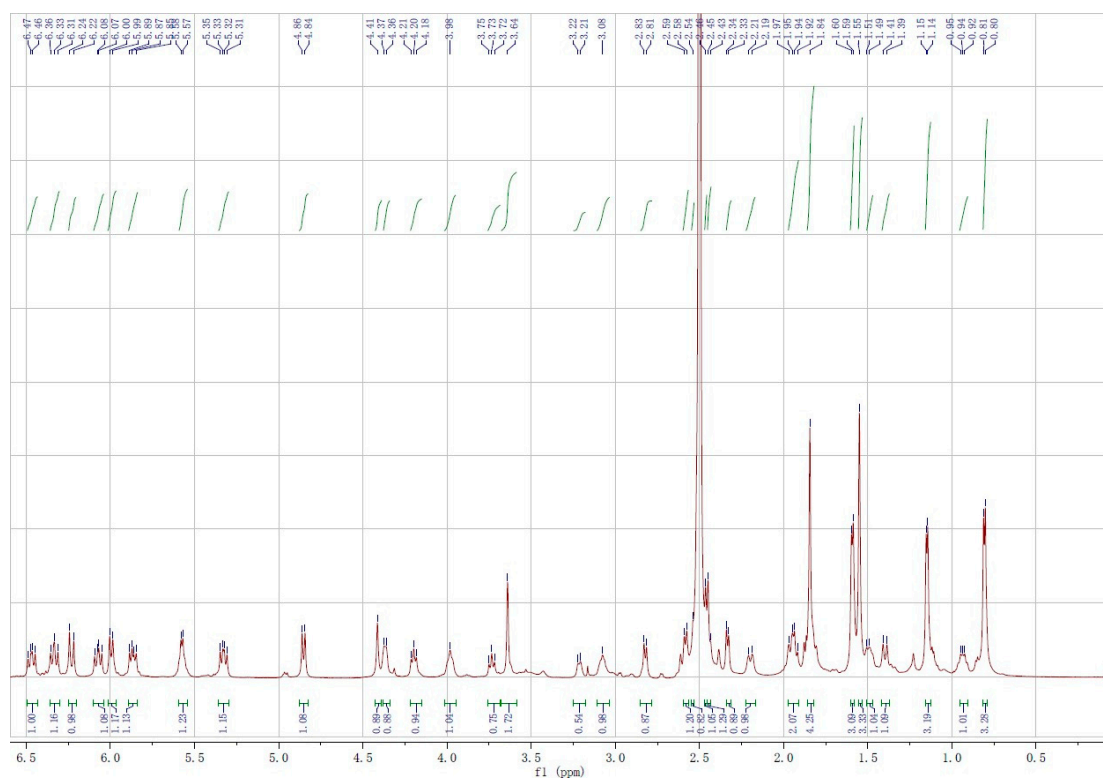


Figure S11. ^1H spectrum of actinospene (**1**) in DMSO-d_6 .

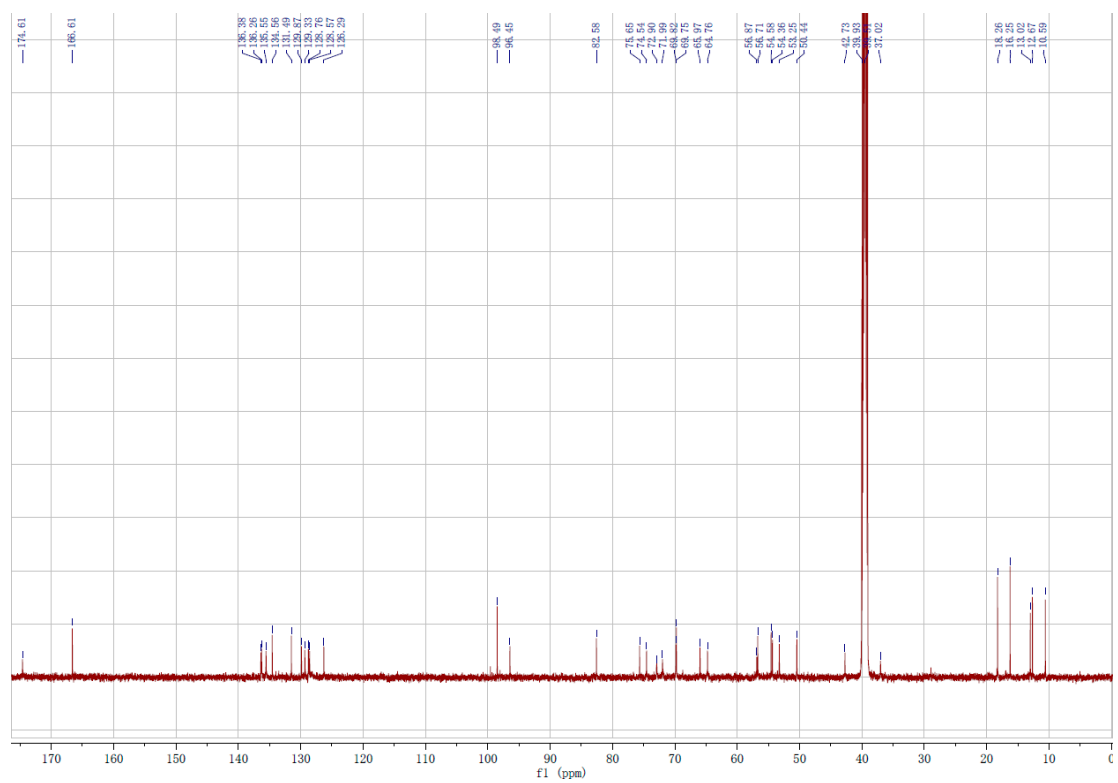


Figure S12. ^{13}C spectrum of actinospene (**1**) in DMSO-d_6 .

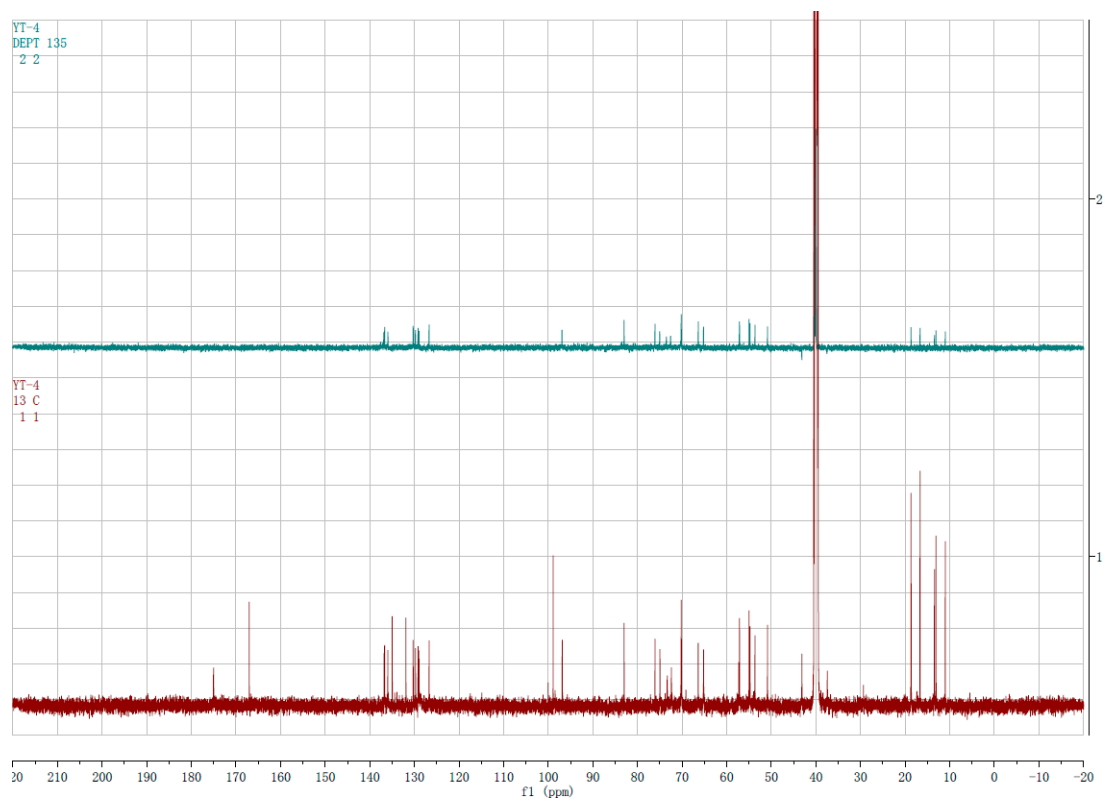


Figure S13. DEPT spectrum of actinospene (**1**) in DMSO-d_6 .

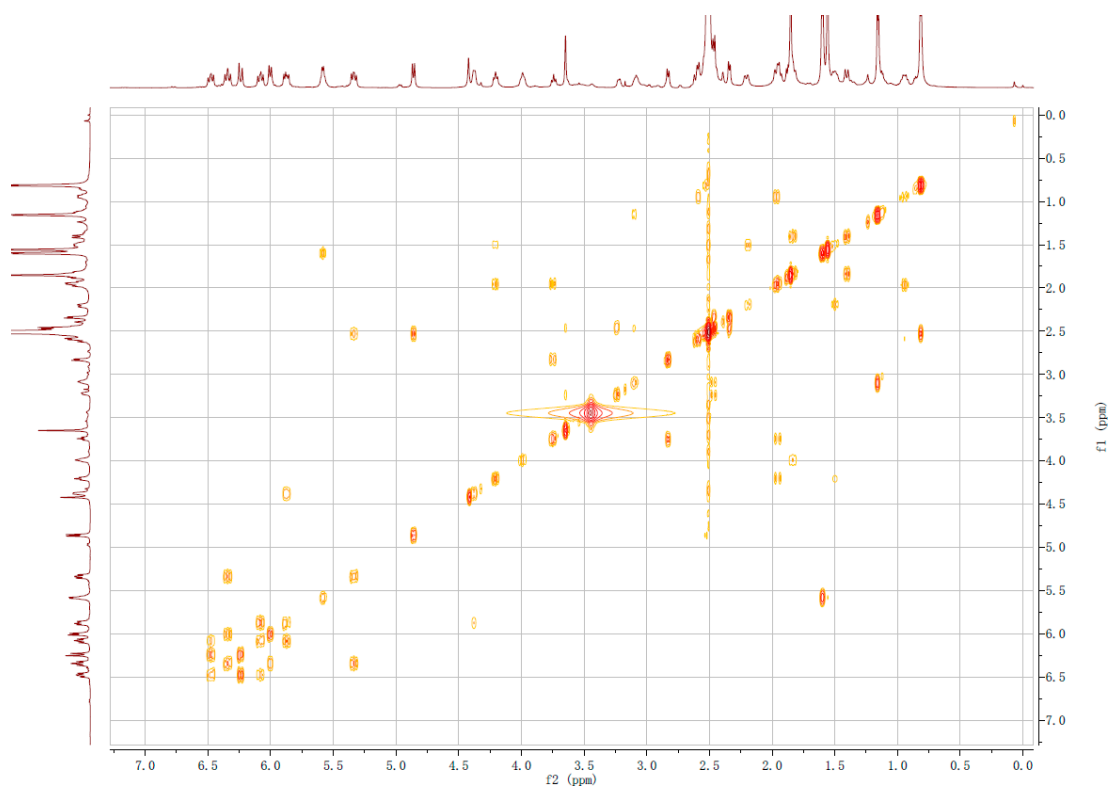


Figure S14. ^1H - ^1H COSY spectrum of actinospene (**1**) in DMSO-d_6 .

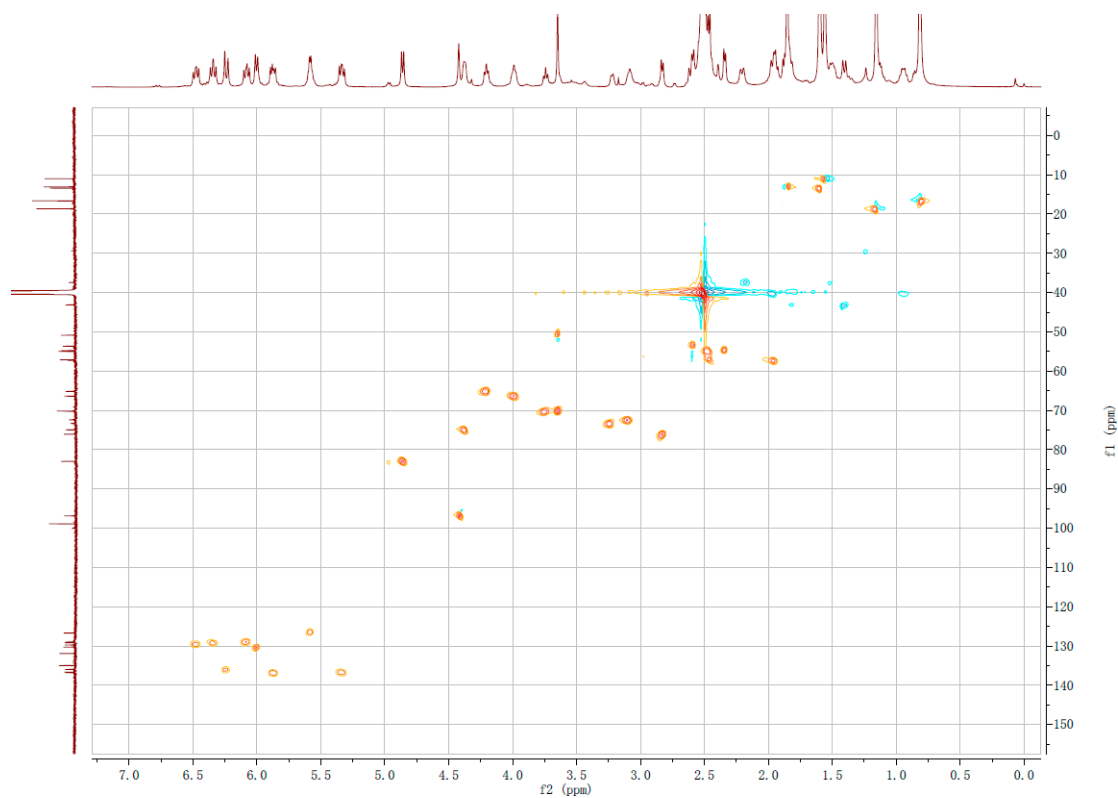


Figure S15. HSQC spectrum of actinospene (**1**) in DMSO-d_6 .

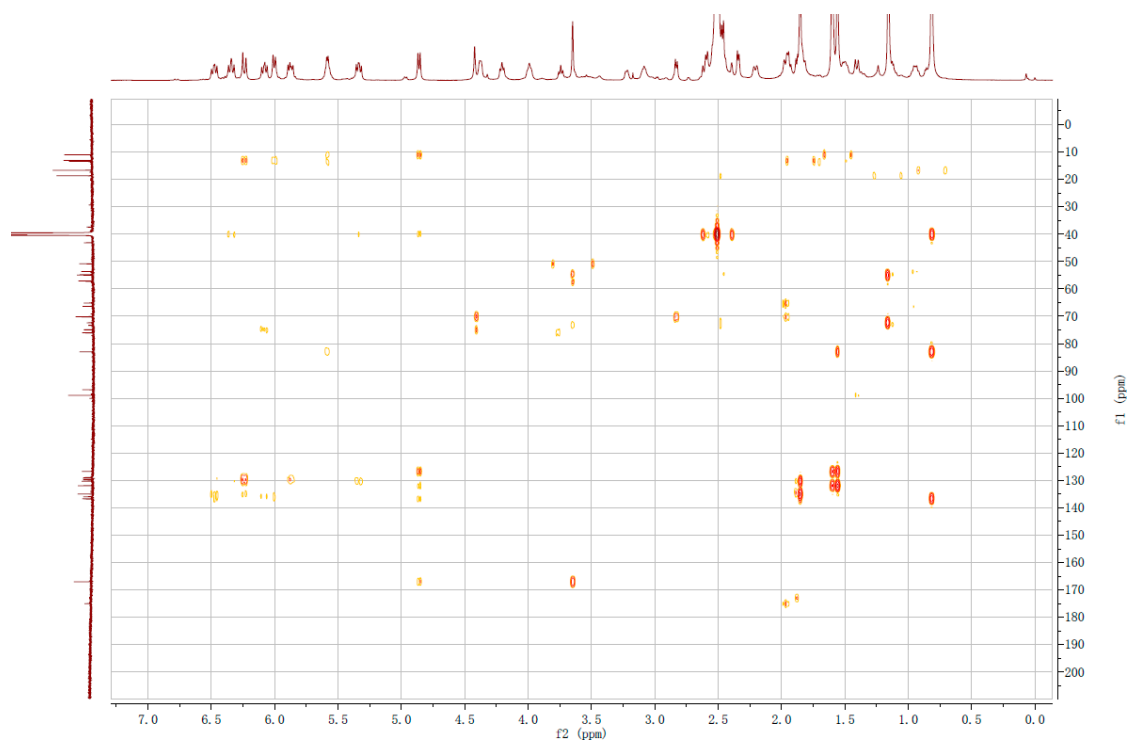


Figure S16. HMBC spectrum of actinospene (**1**) in DMSO-d₆.

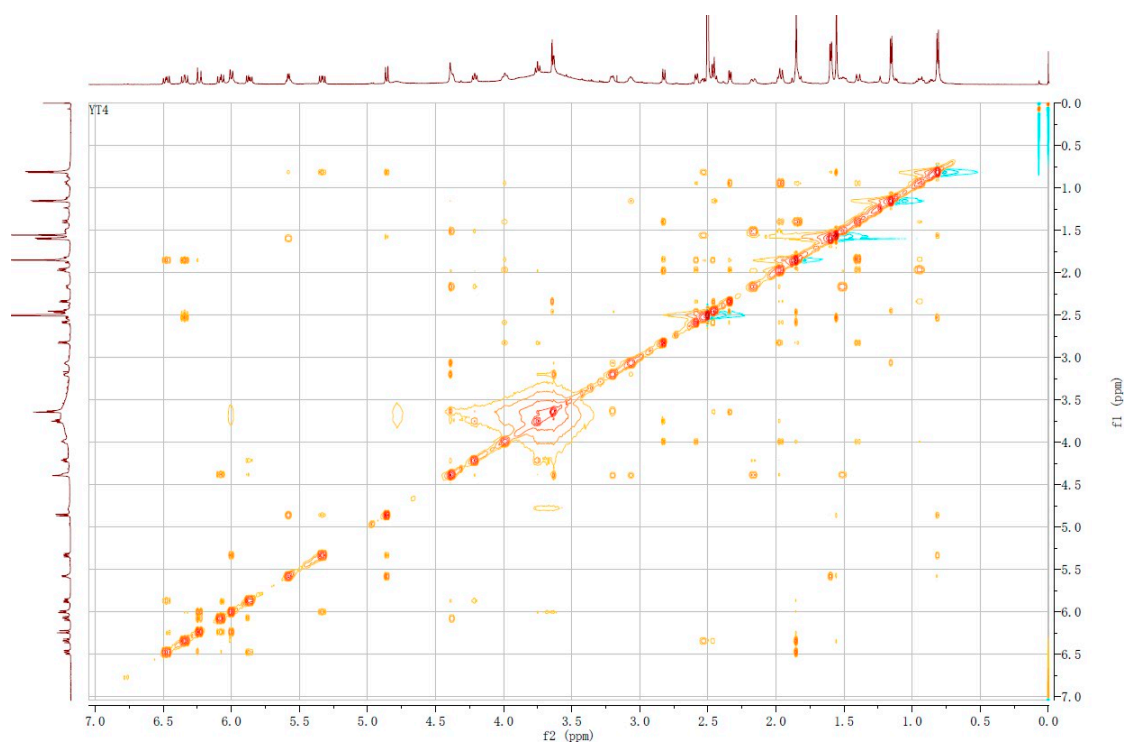


Figure S17. NOESY spectrum of actinospene (**1**) in DMSO-d₆.

References

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2. Keatinge-Clay, A., Crystal structure of the erythromycin polyketide synthase dehydratase. *Journal of molecular biology* **2008**, 384 (4), 941-953.
3. Keatinge-Clay, A. T., A tylosin ketoreductase reveals how chirality is determined in polyketides. *Chemistry & biology* **2007**, 14 (8), 898-908.