Electronic supplementary materials

Activation of anthrachamycin biosynthesis in *Streptomyces chattanoogensis* L10 by site-directed mutagenesis of *rpoB*

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Table S1 Strains and plasmids used in this study

Strains	Discription	Reference
S.chattanoogensis L10	Wild type	(Du *et al*. 2011)
S.chattanoogensis ZJUY1	The R*poB* (H437Y) mutant strain	This study
S.chattanoogensis ZJUY2	The RpoB (R440C) mutant strain	This study
S.chattanoogensis ZJUY3	The RpoB (S433P) mutant strain	This study
S.chattanoogensis ZJUY4	The RpoB (D427N) mutant strain	This study
S.chattanoogensis ZJUY5	The RpsL (R86L) mutant strain	This study
S.chattanoogensis ZJUY6	The RpsL (V87K) mutant strain	This study
S.chattanoogensis ZJUY7	The RpsL (K88E) mutant strain	This study
S.chattanoogensis ZJUY8	The RpsL (D89R)mutant strain	This study
S.chattanoogensis ZJUY9	The RpsL (L90K)mutant strain	This study
S.chattanoogensis ZJUY10	The RpsL (G92D)mutant strain	This study
S.chattanoogensis ZJUY11	The KS α subunit gene *chaA* disrupted in ZJUY1	This study
S.chattanoogensis ZJUY12	The gene *chaI* disrupted in ZJUY1	This study
E.coli DH5α	General cloning strain	This study
E.coli BL21(DE3)	Strain for recombinant protein expression	Invitrogen, USA
E.coli ET12567/PUZ8002	Methylation-deficient *E. coli* for conjugation with the helper plasmid	(Mao *et al*. 2015)
Plasmids	Discription	Reference
pKC1139	Temperature-sensitive shuttle vector for gene disruption	(Muth *et al*. 1989)
pKC1139-*rpsL*	*rpsL* and flanking regions cloned in pKC1139 vector	This study
pKC1139-*rpoB*	*rpoB* and flanking regions cloned in pKC1139 vector	This study
pKCmu1	The RpoB (H437Y) mutant plasmid	This study
pKCmu2	The R*poB* (R440C) mutant plasmid	This study
pKCmu3	The *RpoB* (S433P) mutant plasmid	This study
pKCmu4	The R*poB* (D427N) mutant plasmid	This study
pKCmu5	The RpsL (R86L) mutant plasmid	This study
pKCmu6	The RpsL (V87K) mutant plasmid	This study
pKCmu7	The RpsL(K88E) mutant plasmid	This study
pKCmu8	The RpsL(D89R) mutant plasmid	This study
pKCmu9	The *RpsL*(L90K) mutant plasmid	This study
pKCmu10	The RpsL(G92D) mutant plasmid	This study
pKC1139-Δ*chaA*	*chaA* disruption plasmid based on pKC1139	This study
pKC1139-Δ*chaI*	*chaI* disruption plasmid based on pKC1139	This study
pET32a	Expression vector	This study
pET32a-$adpA_{ch}$	$adpA_{ch}$ ORF cloned in *Eco*RI/*Hin*dIII site of pET32a	This study
pClone 007 Blunt Simple Vector	General clone vector	TsingKe, China
pClone1	*chaI* promoter (*chaIp*) in pClone 007 Blunt Simple Vector	This study

Table S2 Oligonucleotide primers used in this study

Primers	Sequence (5'→3')	Description
F1	GATATCCGTCTGTGATGAGAAGATGG	For amplification of flanking regions of *rpsL*
R1	CGGAATTCCGGCGGACCAGACCAGGG	
F2	GATATCTCTTGGCCGCCTCGCGCAAC	For amplification of flanking regions of *rpoB*
R2	CGGAATTCAAGTTGACGTCGAGCACTAT	
M13F	GTAAAACGACGGCCAGT	M13 universal primers
M13R	CAGGAAACAGCTATGACAT	
F3	AGGTCCTTCACAAGGCCACCACGC	For amplification of changed Arg-86 to Leu in RpsL
F4	GCAGGTCCTTCTTACGGCCACC	For amplification of changed Val-87 to Lys in RpsL
F5	CACCCGGCAGGTCCCCCACACGGCC	For amplification of changed Lys-88 to Glu in RpsL
F6	GAACACCCGGCAGGCGCTTCACACG	For amplification of changed Asp-89 to Arg in RpsL
F7	CGAACACCCGGCTTGTCCTTCACAC	For amplification of changed Leu-90 to Lys in RpsL
F8	GTAGCGAACATCCGGCAGGTCC	For amplification of changed Gly-92 to Asp in RpsL
F9	GGGTCTGACCTACAAGCGCCGT	For amplification of changed His-437 to Tyr in RpoB
F10	CCACAAGCGCTGTCTGTCGGCG	For amplification of changed Arg-440 to Cys in RpoB
F11	CAACCCGCTGCCGGGTCTGACC	For amplification of changed Ser-433 to Phe in RpoB
F12	CCAGTTCATGAACCAGACCAAC	For amplification of changed Asp-427 to Asn in RpoB
Rmu1	GGCCAGTGCCTAGCTTGGGCT	For amplification fragments without *Hin*d III restriction site
F13	AAGCTTCACCGAACCGCCCGCCTACG	For amplification of 5’ flanking region of
R13	TCTAGAGACCCGTCGCCCGGTCACTG	*chaA*
F14	TCTAGACCCGAGAGGAGGACGGCATG	For amplification of 3’ flanking region of
R14	GATATCGCCCGGGTCACCAGGTCGAT	*chaA*
F15	CCGGAATTCCGGATGAGCCAGGACTCCGCT	For amplification of $adpA_{ch}$
R15	CCAAGCTTGGGTCACCCTACGGGGCGTTC	
F16	FAM-GTAAAACGACGGCCAGTGTC	For amplification of 5’-FAM labeled probe
R16	CACAGGAAACAGCTATGACCAT	
F17	CCATATGGGCGCATGGAGATAGCGGATA	For amplification of *chaI* promoter
R17	CCATATGGCGCAGGTGAAATGCGACAC	
F18	TGGCTGCGGCAGTTGCTC	gene expression of *relA* for qRT PCR
R18	GAAGTCGACGGGCGTGGC	
F19	TCGTCACCAAGACTCCGC	gene expression of *relC* for qRT PCR

R19	CGTTCAGGTCGGGCATCT	
F20	CTGCAACGCCTTCCACATG	gene expression of *chaA* for qRT PCR
R20	GCAGGTCGTTCTGCTTGGTG	
F21	CCTGGTCTACAACGGGGTGC	gene expression of *chaGT1* for qRT PCR
R21	AGCAGATGGAGCGGGACA	
F22	CCACCTGGCGGGCATGAT	gene expression of *chaM* for qRT PCR
R22	ACCGCAGCAACCCGAGGG	
F23	CGTTCAGTACACCCCGGAGA	gene expression of *chaS* for qRT PCR
R23	CCTCGATGACCAGGTAGCG	
F24	GCACGACACTCAGGTCAAGG	gene expression of *chaI* for qRT PCR
R24	GGTGCGGGCGATATTGGA	
F25	CAGAATCGTCGGGCTGTGC	gene expression of $adpA_{ch}$ for qRT PCR
R25	GACGAAGAGCTCACGGGGAT	
F26	AGGGCAACCTCGGTCTGA	gene expression of *hrdB* for qRT PCR
R26	ATGTGGACGGGGATACGG	
F27	GCTCTAGAGCGAATGGATCATCACCGTG	For amplification of 5' flanking region of *chaI*
R27	CCAAGCTTGGGAACGCGGTTCACCTGAC	
F28	GCTCTAGAGCCCCCGCGAGAGAACAGCA	For amplification of 3' flanking region of *chaI*
R28	CCGGAATTCCGGGAACCCCGCGGCGCGGAG	

Table S3 ^{1}H and ^{13}C NMR data of anthrachamycin in DMSO-d_6 (600 MHz)

Anthrachamycin		
Position	^{1}H NMR (*J* Hz)	^{13}C NMR
1	—	154.4, qC
2	7.01 (1H, s)	112.5, CH
3	—	141.6, qC
4	7.31 (1H, s)	119.5, CH
4a	—	139.7, qC
5	7.50 (1H, s)	116.25, CH
6	—	155.61, qC
6a	—	119.2, qC
7	—	191.5, qC
7a	—	115.5, qC
8	—	160.3, qC
9	7.29(1H, d, 7.9)	122.8, CH
10	7.80 (1H, t, 7.8)	137.6 CH
11	7.46 (1H, d, 7.2)	117.9,CH
11a	—	137.2, qC
12	—	185,3 qC
12a	—	137.8, qC
12b	—	115.8, qC
13	2.43 (3H, s)	21.9, CH_3
1’	4.92 (1H, d, 5.9)	100.6, CH
2’	3.44, (1H, m)	72.0, CH
3’	3.44, (1H, m)	87.8, CH
4’	3.26, (1H, m)	68.2, CH
5’	3.41, (1H, m)	76.6, CH
6’	3.68, 3.37, m	60.6, CH_2
1’’	4.35 (1H, d, 7.8)	104.0, CH
2’’	3.08 (1H, m)	73.7, CH
3’’	3.17 (1H, m)	76.0, CH
4’’	3.05 (1H, m)	70.1, CH
5’’	3.19 (1H, m)	76.9, CH
6’’	3.69, 3.39, m	61.1, CH_2

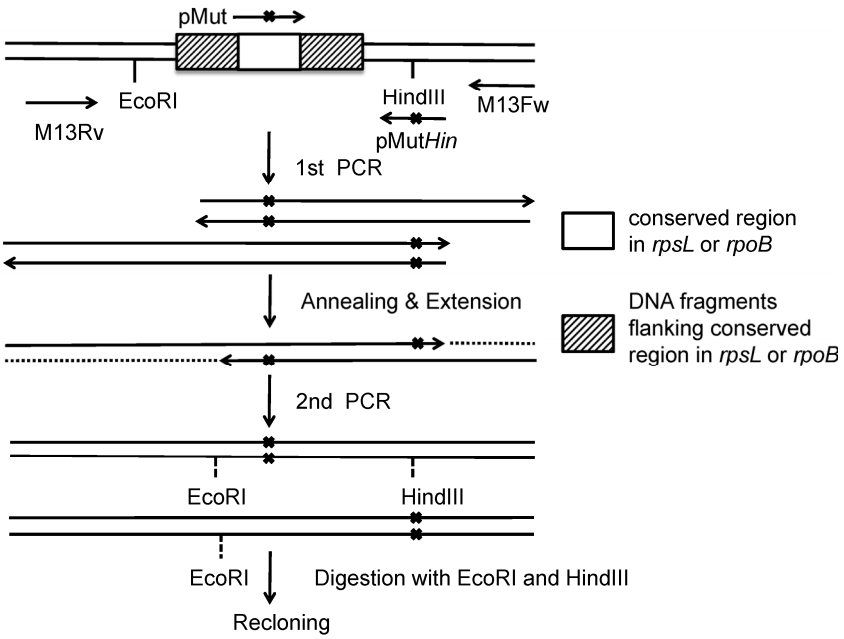

Fig. S1 Schematic representation of overlapping PCR strategy for introducing mutations into *rpsL* or *rpoB*

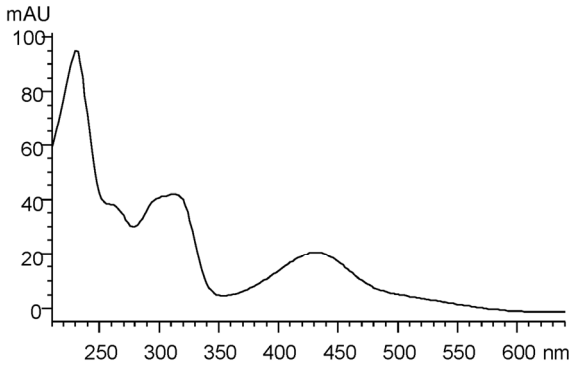

Fig. S2 UV absorption of anthrachamycin

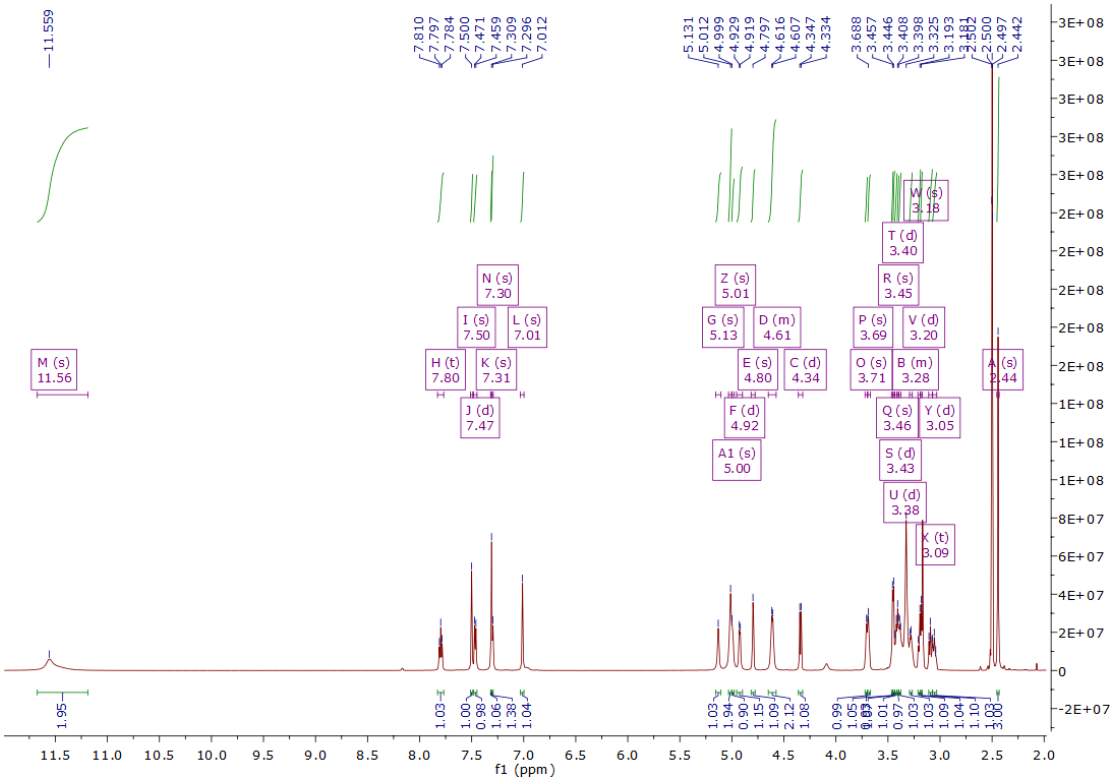

Fig. S3 ^{1}H NMR spectrum of anthrachamycin

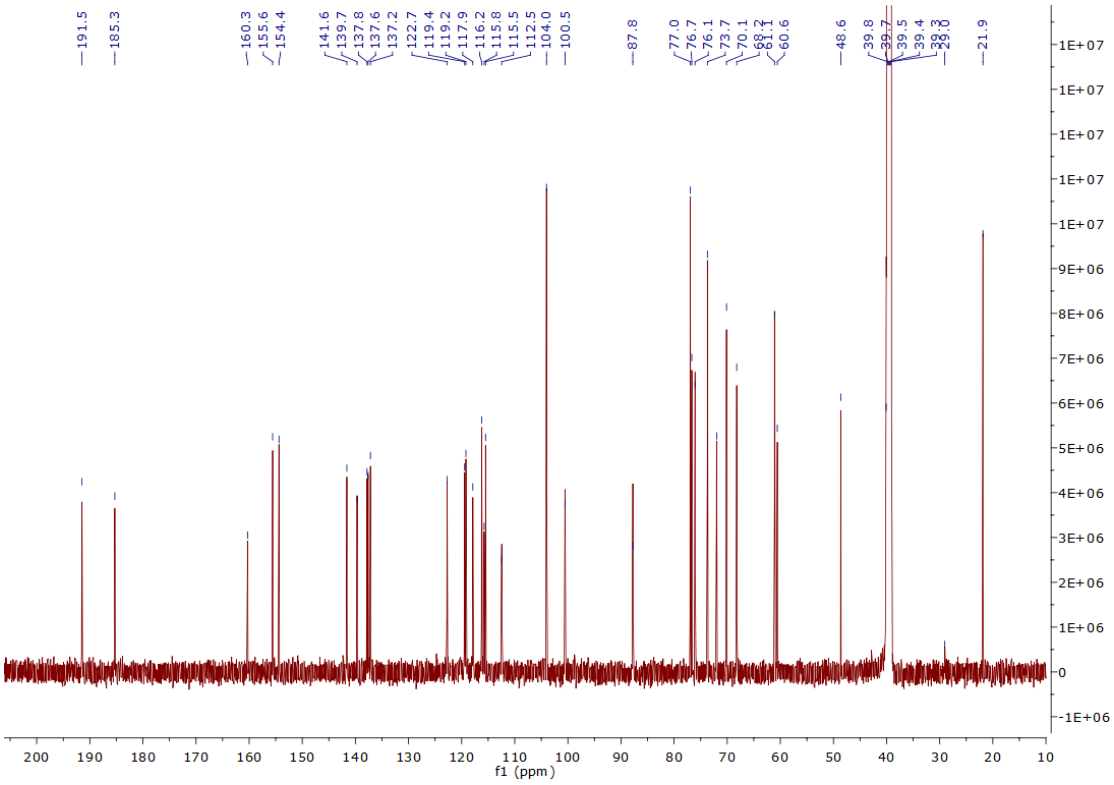

Fig. S4 ^{13}C NMR spectrum of anthrachamycin

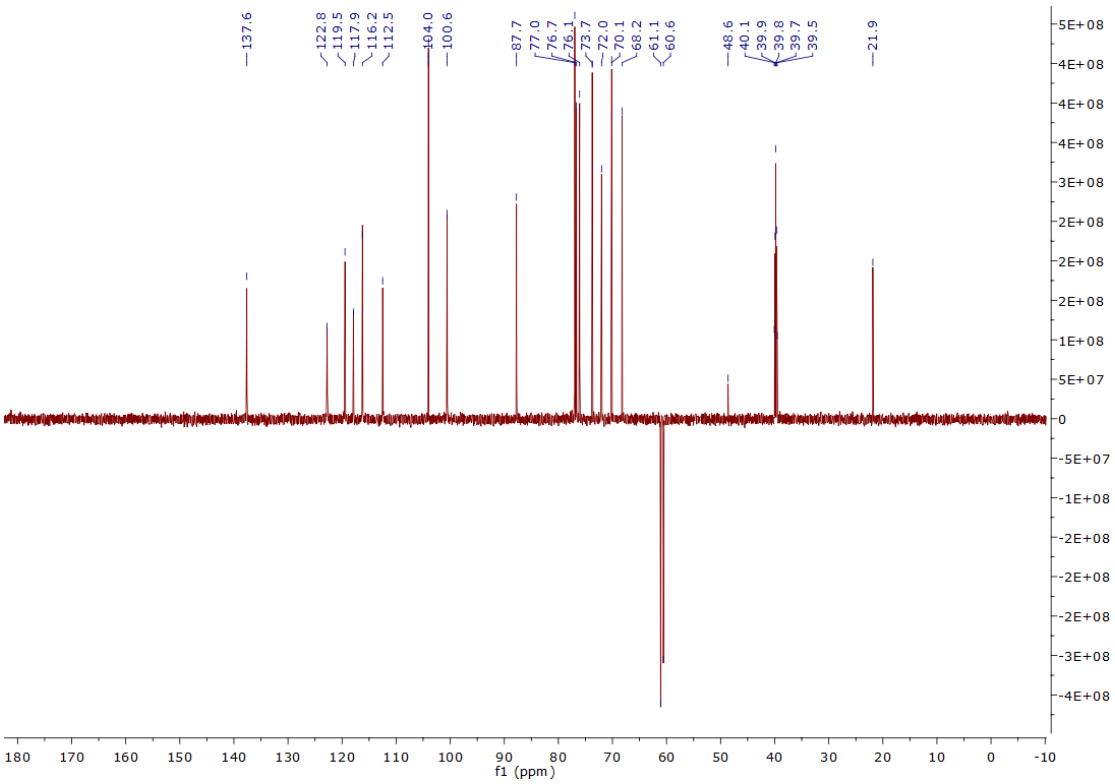

Fig. S5 Distortionless enhancement by polarization transfer (DEPT135) spectrum of anthrachamycin

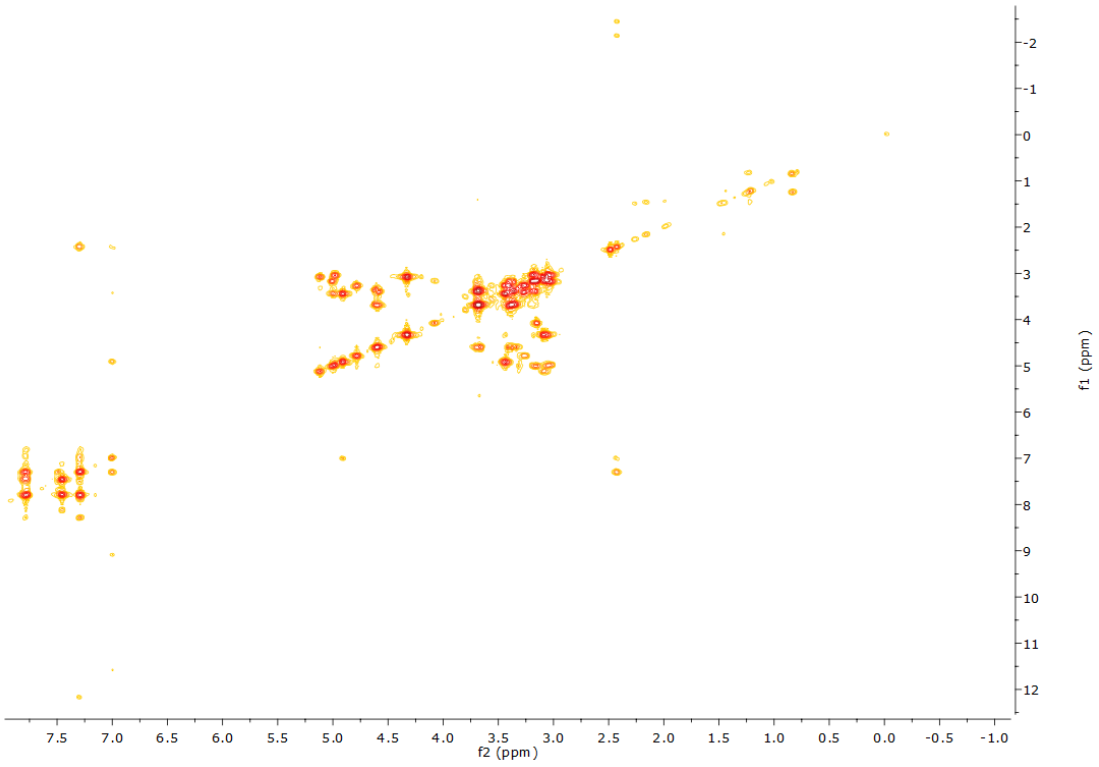

Fig. S6 Correlated spectroscopy (COSY) spectrum of anthrachamycin

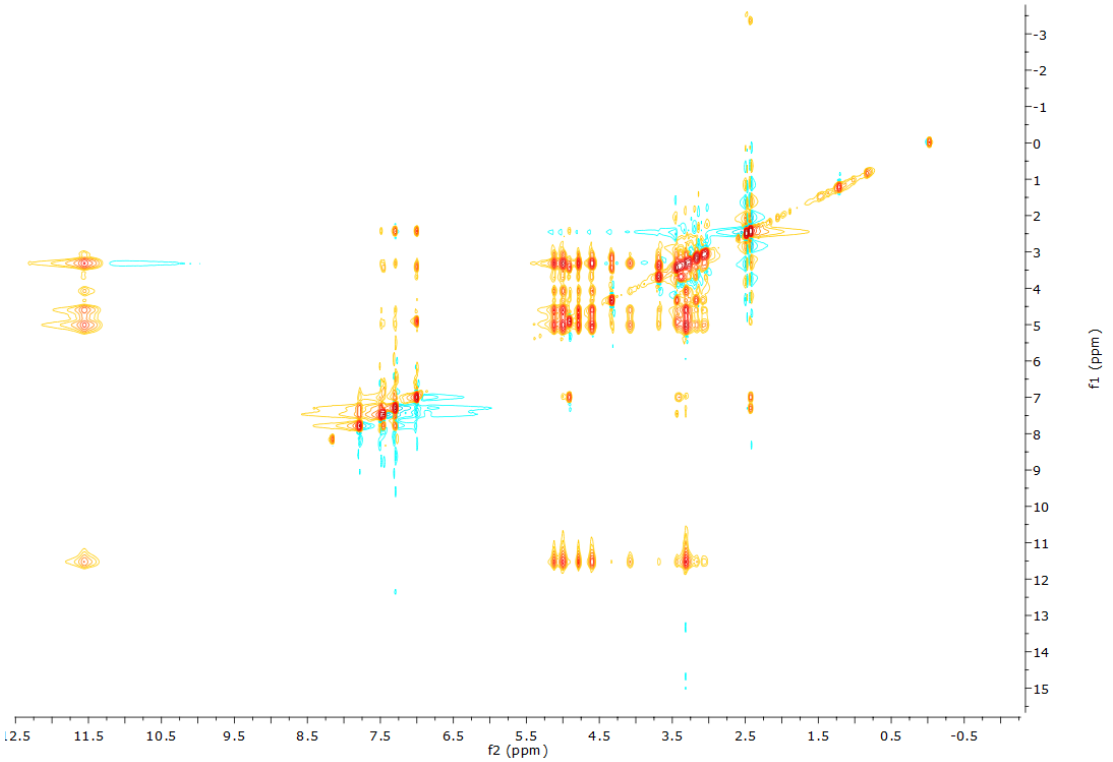

Fig. S7 Nuclear overhauser enhancement spectroscopy (NOESY) spectrum of anthrachamycin

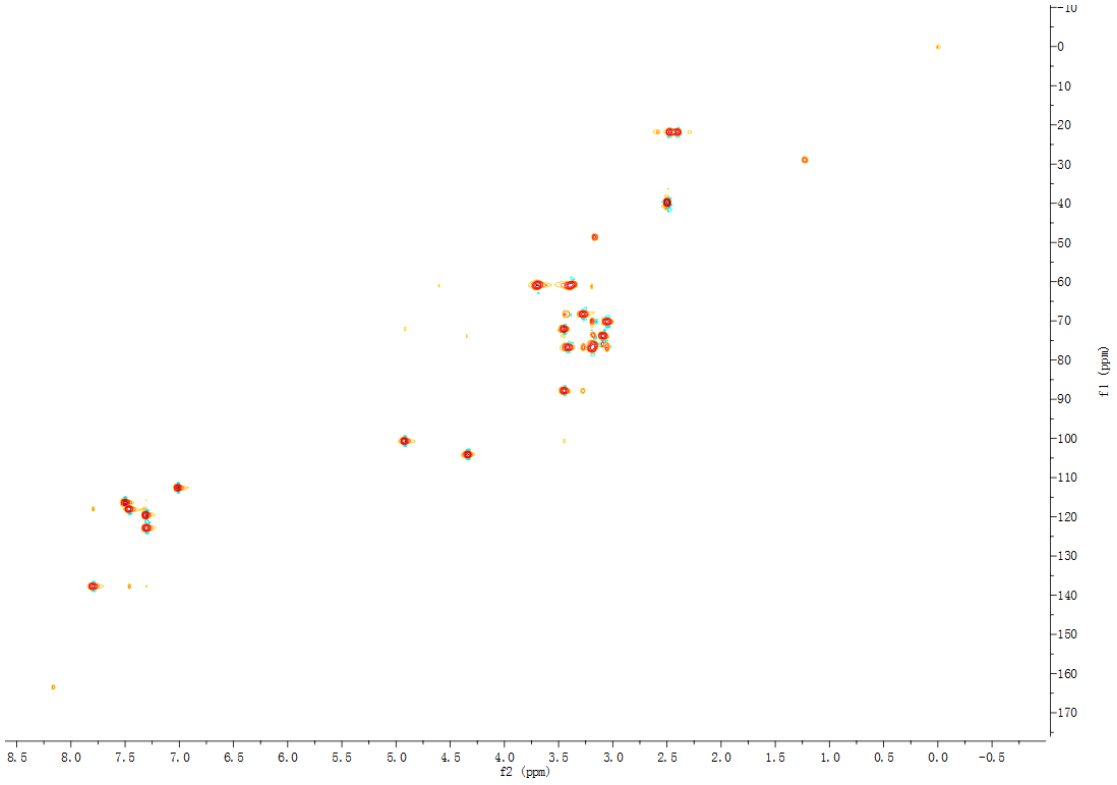

Fig. S8 Heteronuclear single quantum coherence (HSQC) spectrum of anthrachamycin

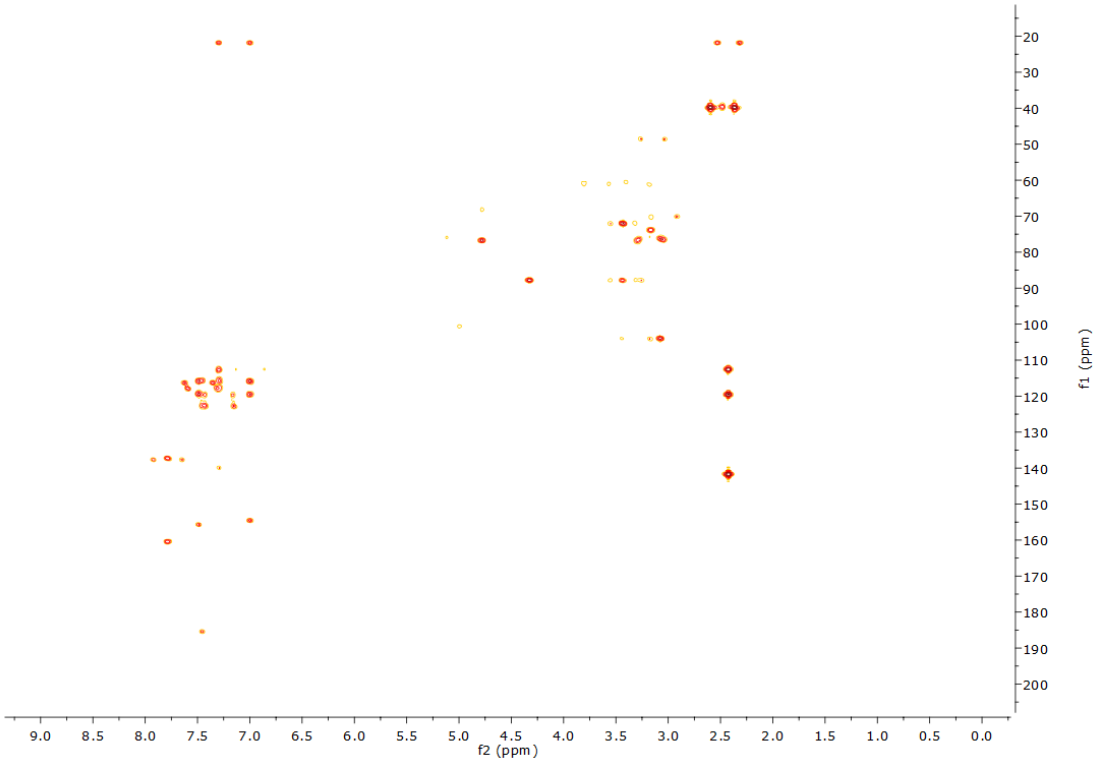

Fig. S9 Heavy mental binding capacity (HMBC) spectrum of anthrachamycin

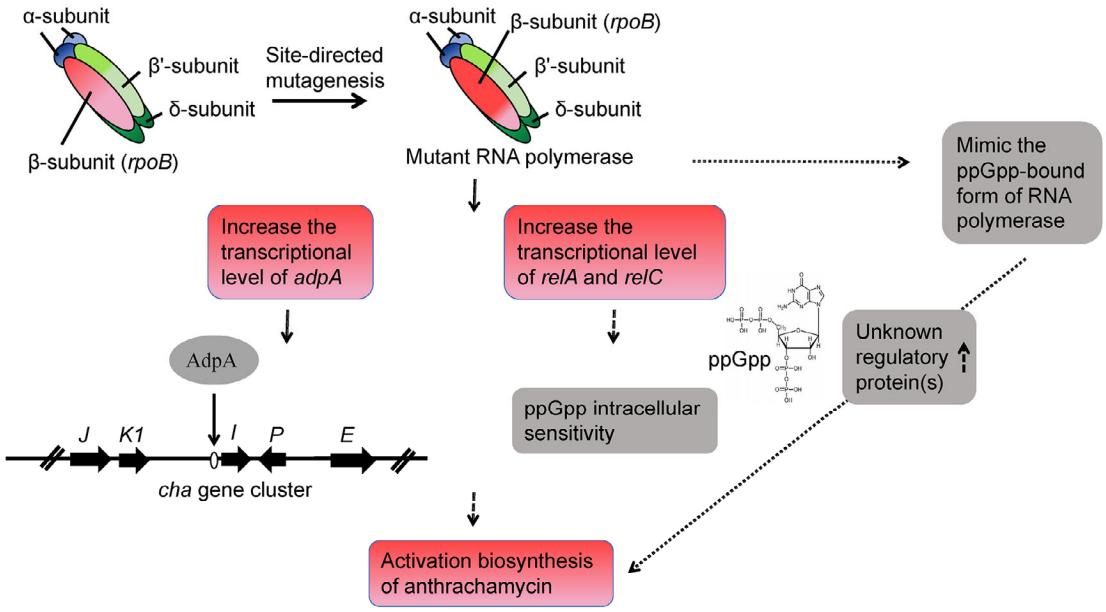

Fig. S10 Speculated mechanism for activating anthrachamycin biosynthesis

Arrows indicate activation and dashed lines denote that the precise mechanism is under investigation

References

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