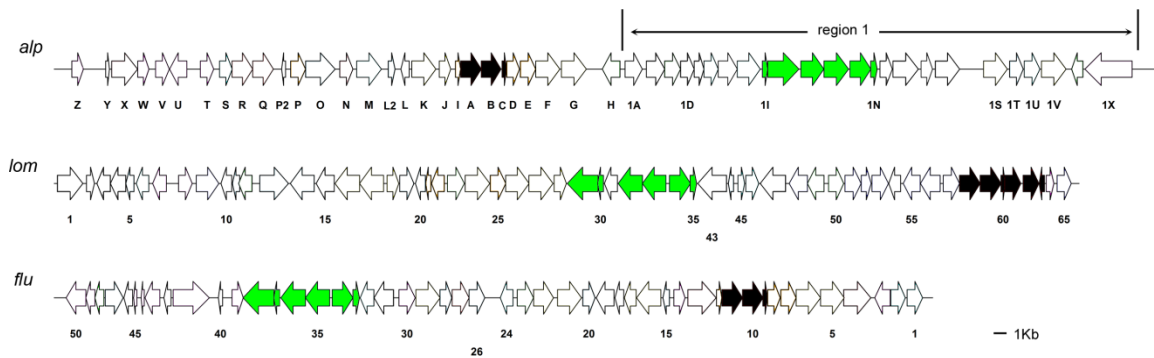
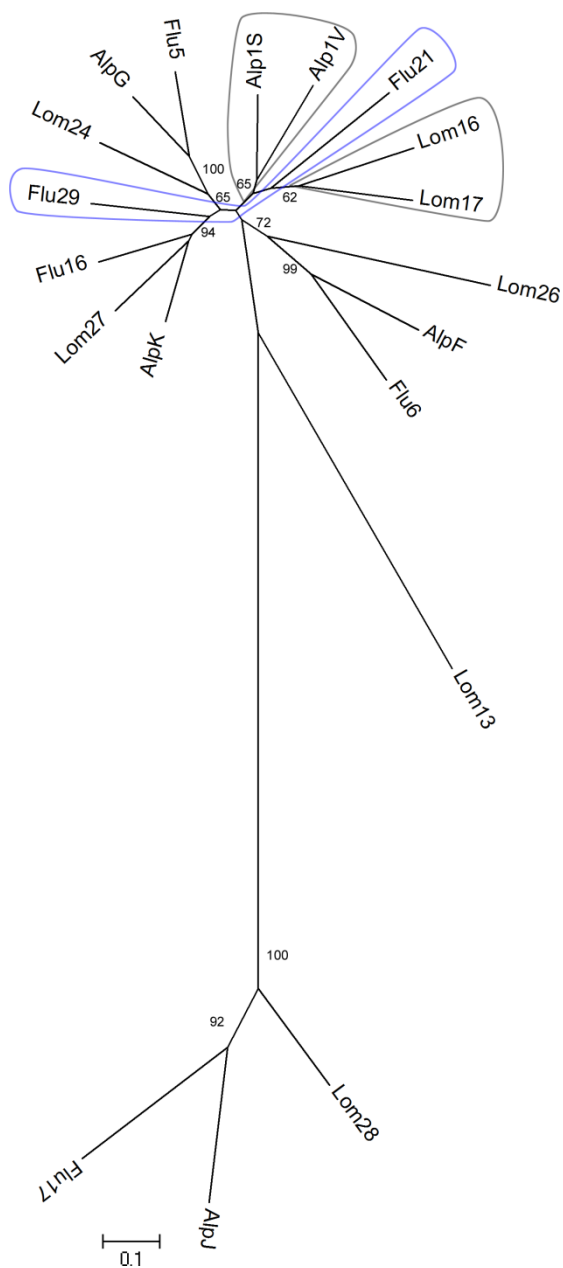


Supplementary Figure 1. The proposed biosynthetic pathways for the A-ring transformation of the kinamycin and lomaiviticin antibiotics. A, by Steven J. Gould; B, by Emily P. Balskus; C, by Bradley S. Moore.

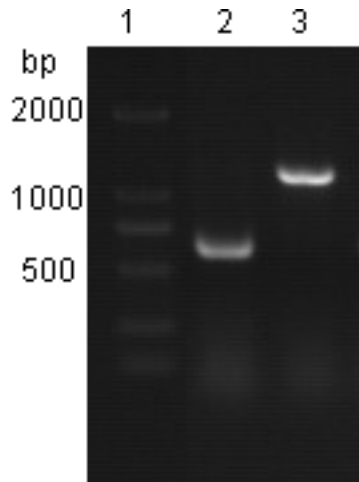


Supplementary Figure 2. Comparison of the *alp*, *lom*, and *flu* gene clusters. The three gene clusters are responsible for the biosynthesis of kinamycin, lomaiviticin and fluostatin, respectively. The DNA region labeled as “region 1” is extended in this study. The genes in black are indicated as mini-PKS, in green as the conserved six-gene subregion responsible for the diazo assembly.

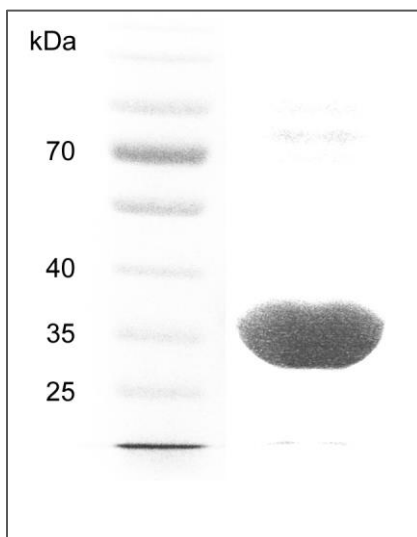


Supplementary Figure 3. Phylogenetic analysis of the oxygenases in the *alp*, *lom* and *flu* clusters.

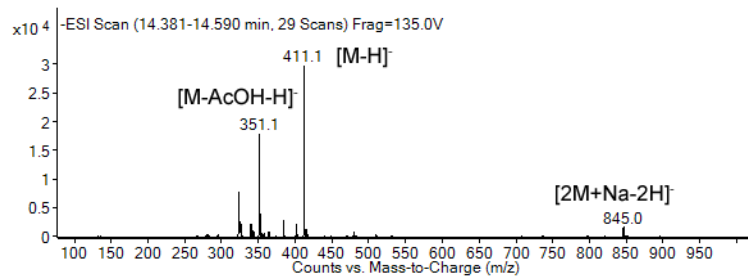
The tree was constructed by Neighbor-Joining method and the bootstrap (500 replicates) values for the main clades are shown. Scale bar represents 10% dissimilarity. The oxygenases labeled in black or blue are the candidates discussed in this study for A-ring transformation in the kinamycin, fluostatin, and lomaiviticin biosynthesis.



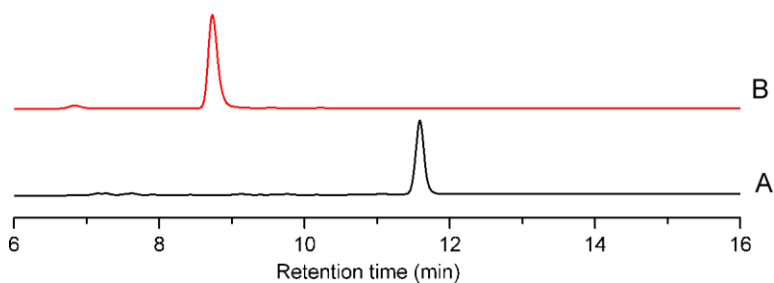
Supplementary Figure 4. PCR confirmation of *alp1U* double-deletion mutant. Lane 1, DNA ladder; lane 2, $\Delta\Delta alp1U$ giving a band 631 bp. lane 3, the starting strain giving a band corresponding to a fragment of 1,204 bp in length. Both detected bands were correct in size.



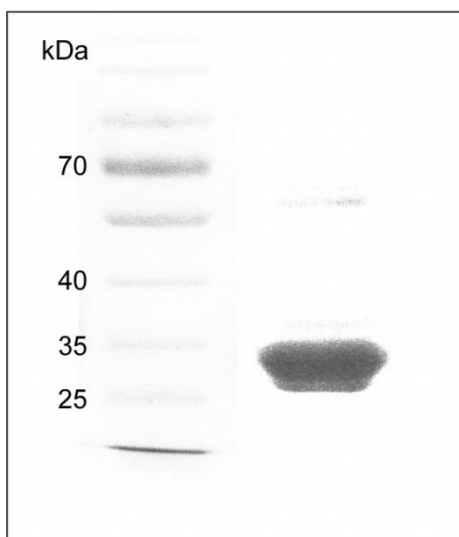
Supplementary Figure 5. SDS-PAGE analysis of the purified Alp1U. Lane 1, protein marker; lane 2, Alp1U giving a band corresponding to a protein of 37 kDa. The detected band was correct in size.



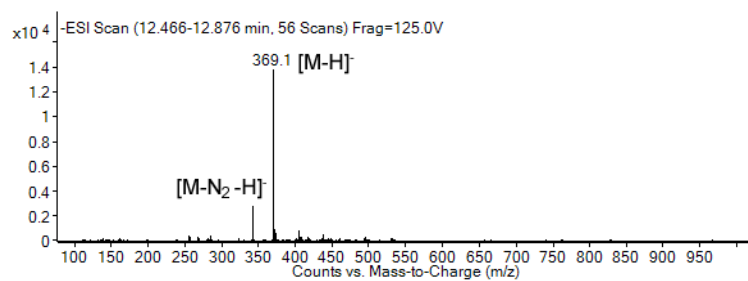
Supplementary Figure 6. LC-MS spectrum of kinamycin E (**5**) under ESI negative mode.



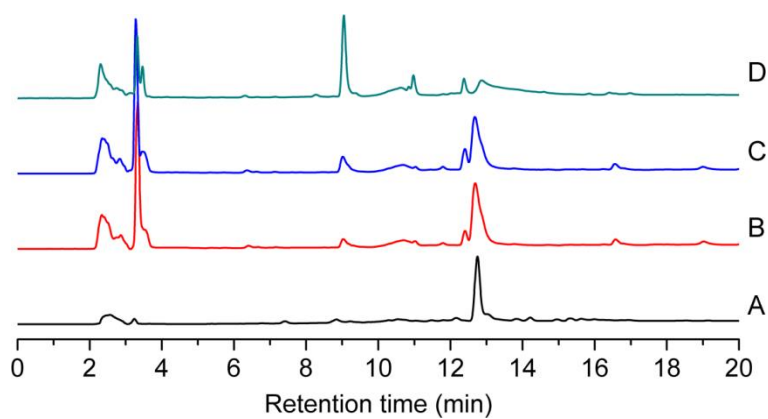
Supplementary Figure 7. HPLC analysis of kinamycin E (**5**) dissolved in $(\text{CD}_3)_2\text{SO}$. (A) before NMR measurement; (B) after NMR measurement.



Supplementary Figure 8. SDS-PAGE analysis of the purified Lom6. Lane 1, protein marker; lane 2, Lom6 giving a band corresponding to a protein of 30 kDa. The detected band was correct in size.

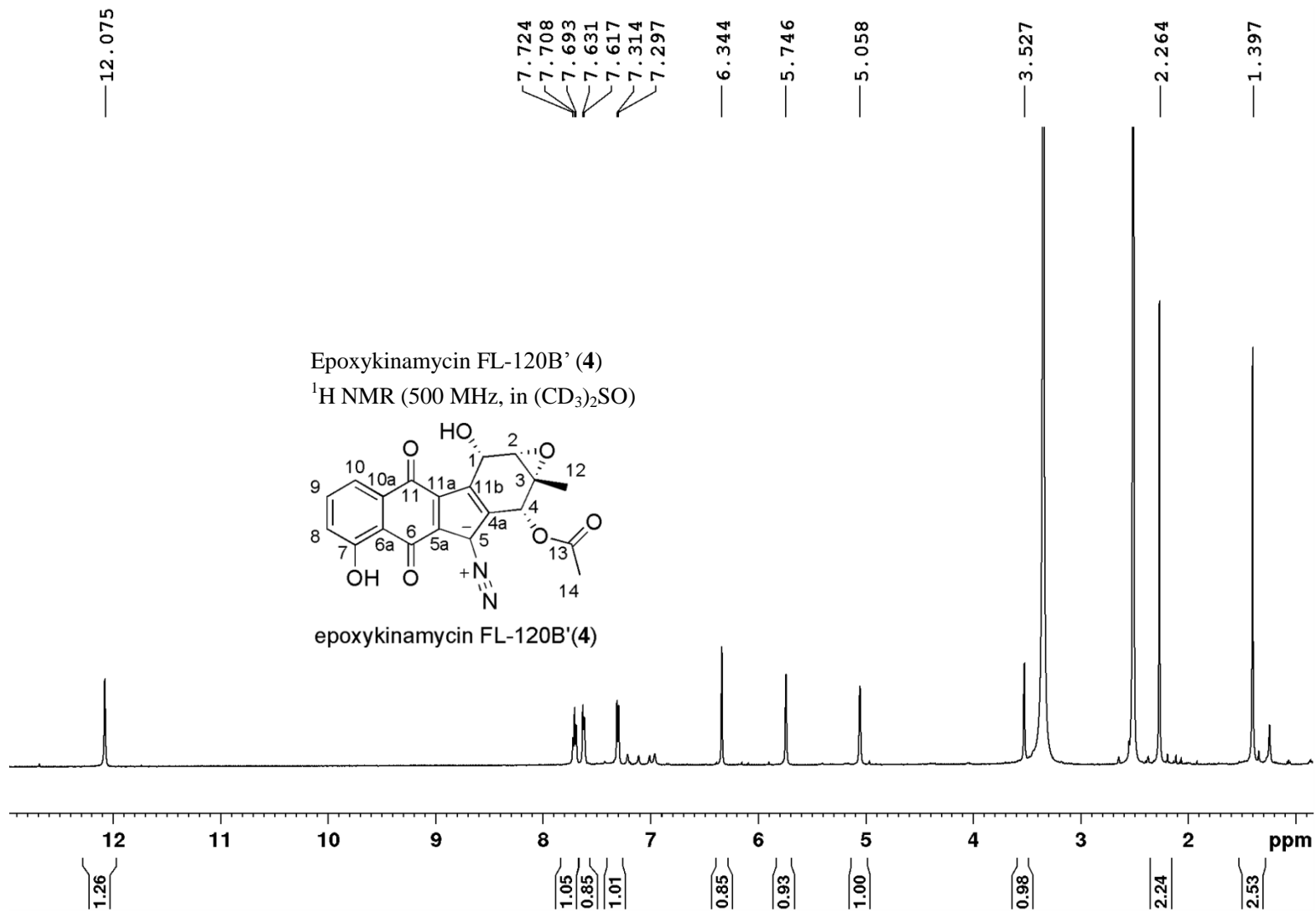


Supplementary Figure 9. LC-MS spectrum of kinamycin F (**2**) under ESI negative mode.

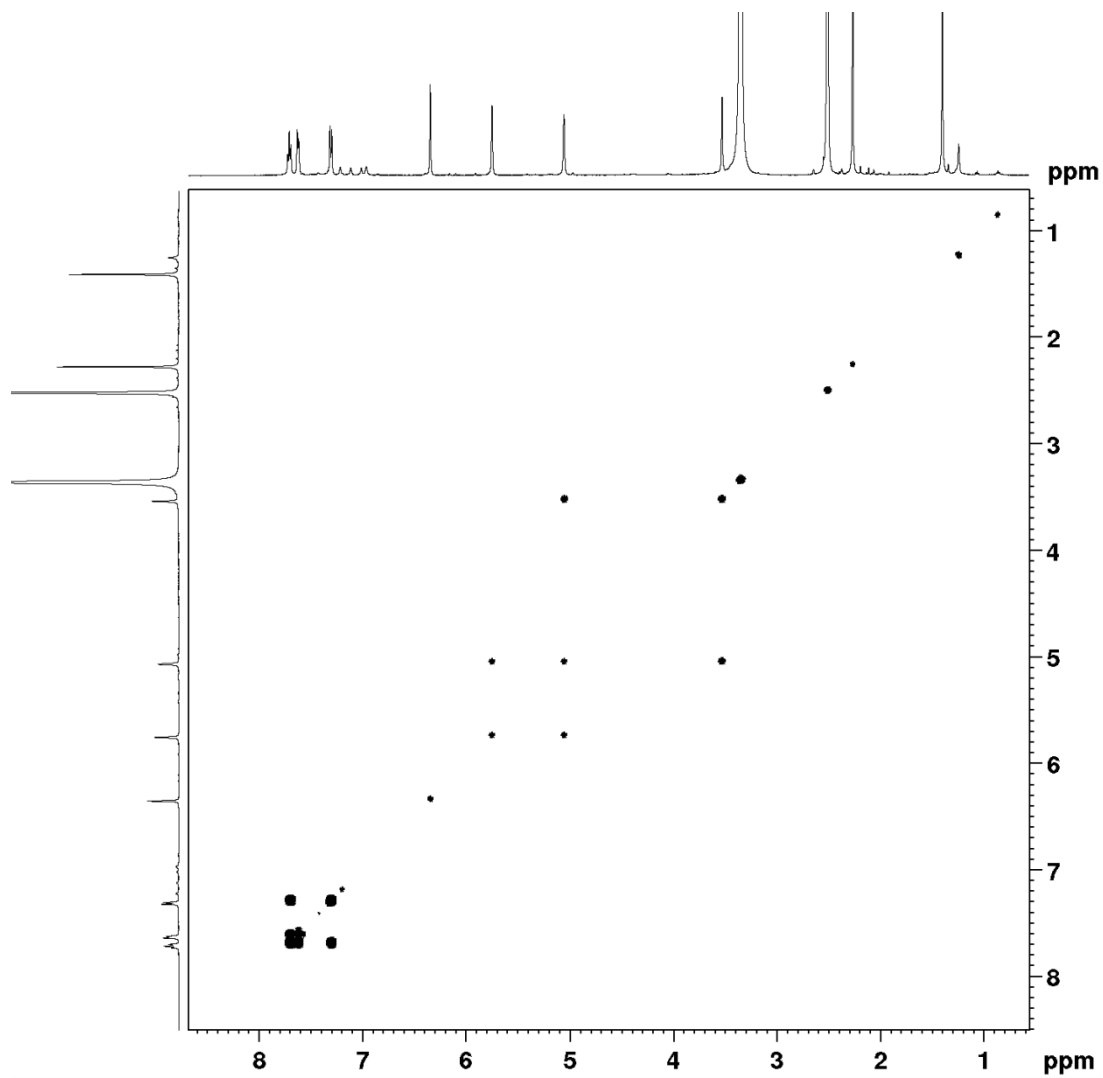


Supplementary Figure 10. HPLC profiles of Lom6 reaction with longer incubation time. (A)

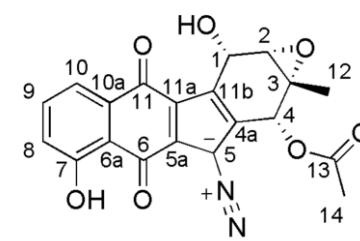
standard epoxykinamycin (**1**); (B) 10 min; (C) 20 min; (D) 50 min



Supplementary Figure 11. ¹H NMR spectrum for epoxykinamycin FL-120B' (4).

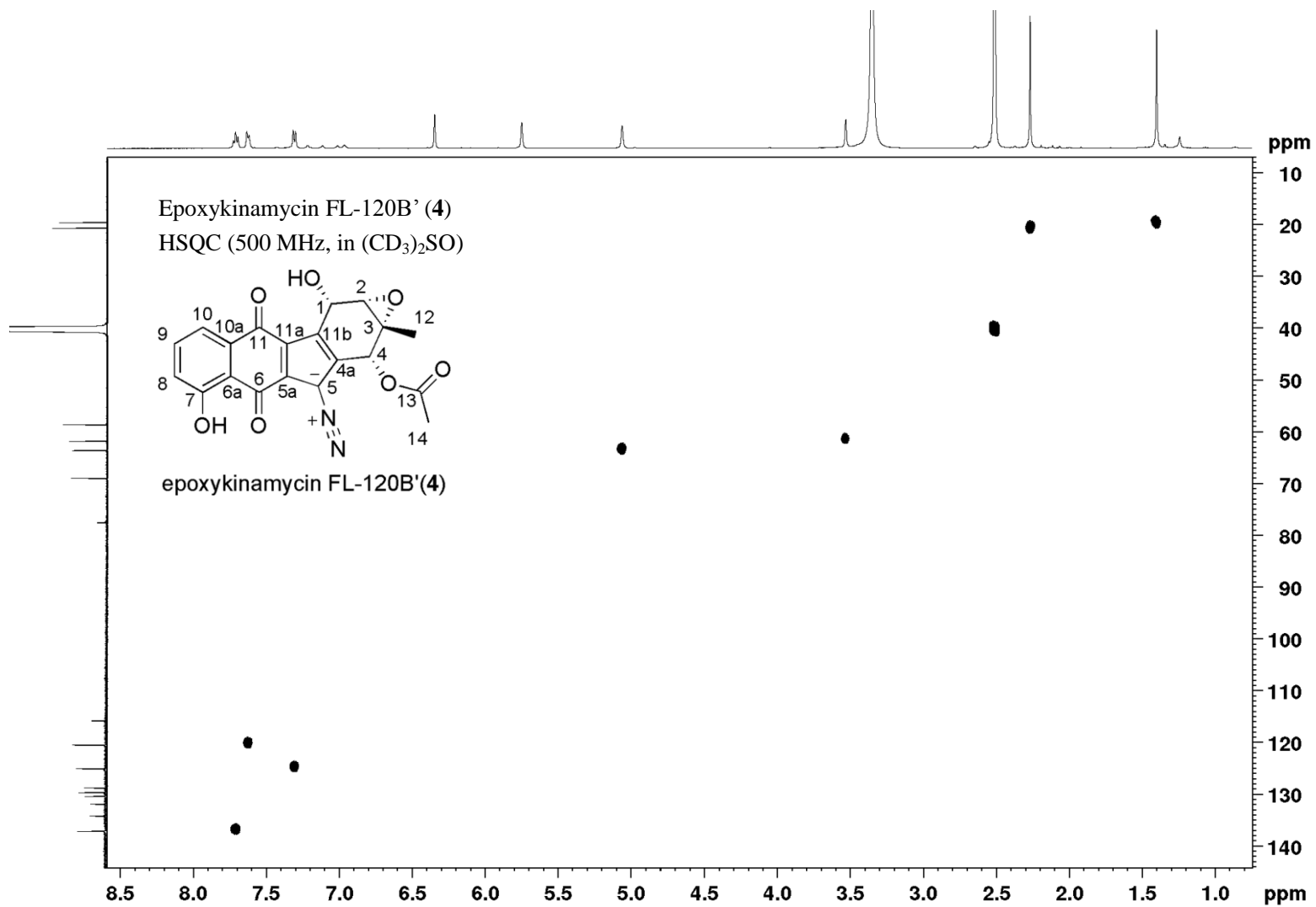


Epoxykinamycin FL-120B' (4)
 COSY (500 MHz, in $(\text{CD}_3)_2\text{SO}$)

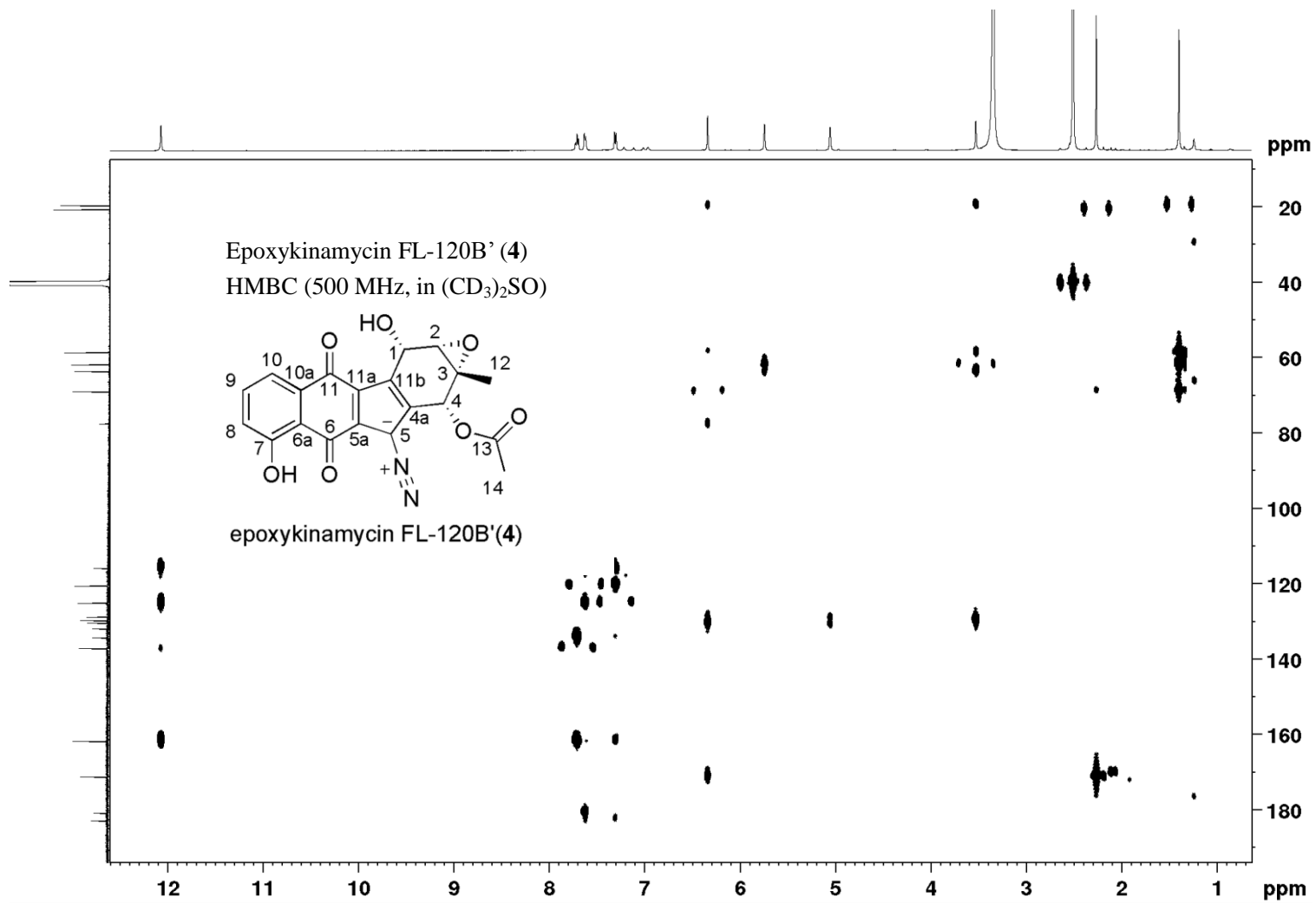


epoxykinamycin FL-120B'(4)

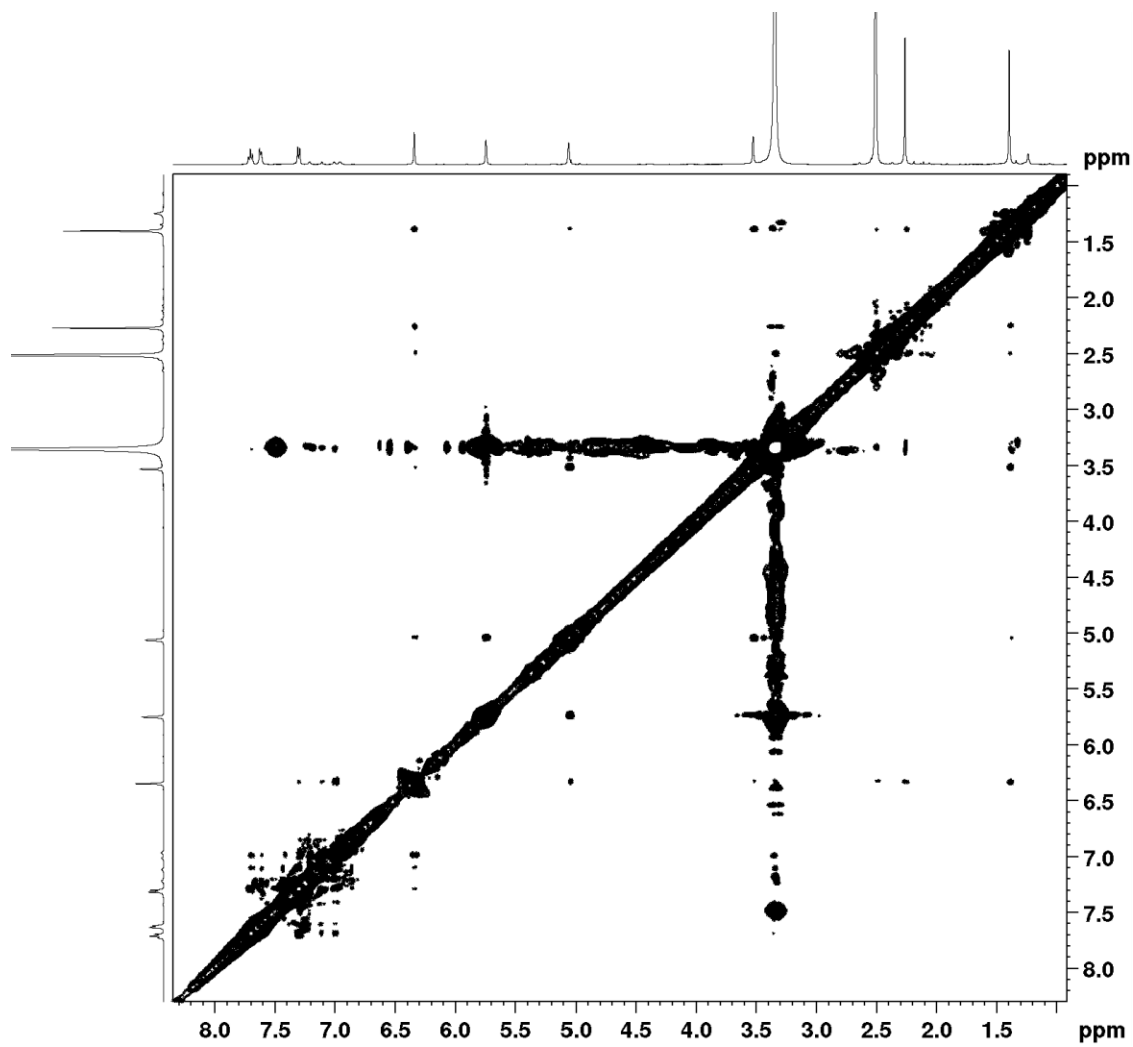
Supplementary Figure 13. COSY spectrum for epoxykinamycin FL-120B' (4).



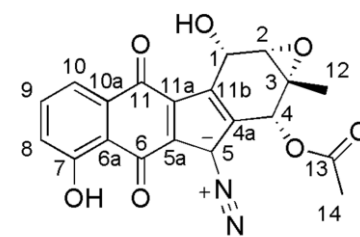
Supplementary Figure 14. HSQC spectrum for epoxykinamycin FL-120B' (4).



Supplementary Figure 15. HMBC spectrum for epoxykinamycin FL-120B' (4).

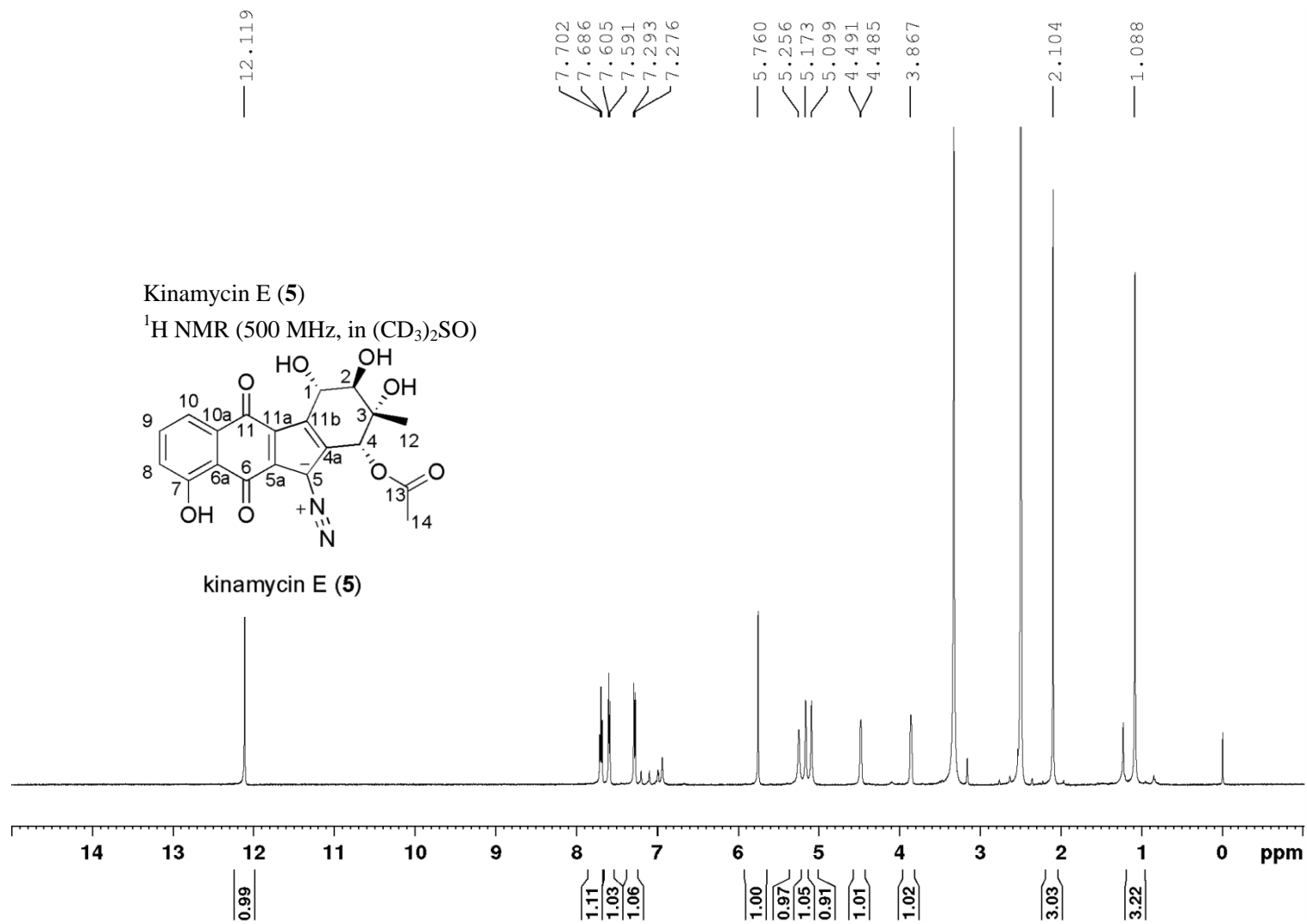


Epoxykinamycin FL-120B' (4)
 NOESY (500 MHz, in (CD₃)₂SO)

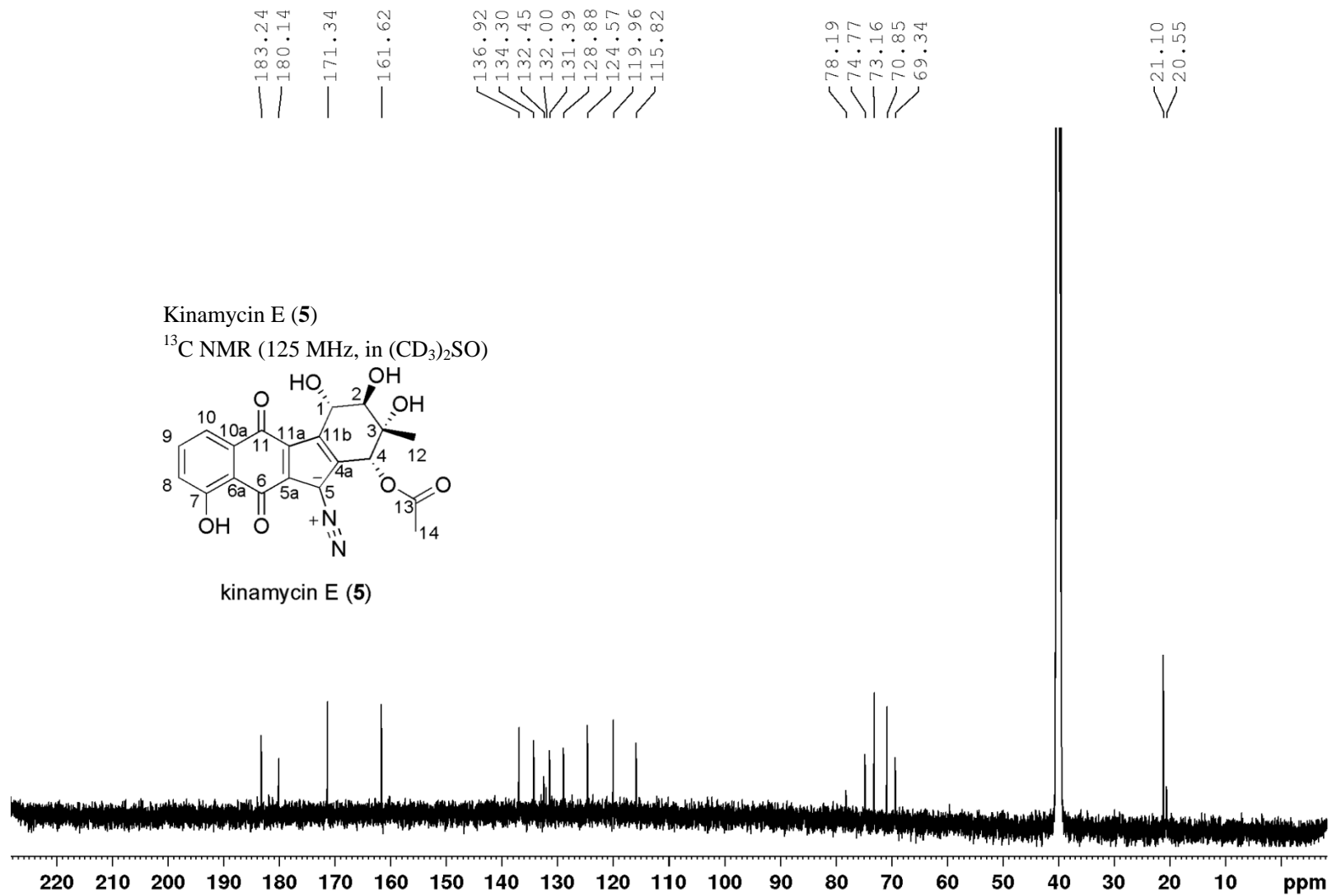


epoxykinamycin FL-120B' (4)

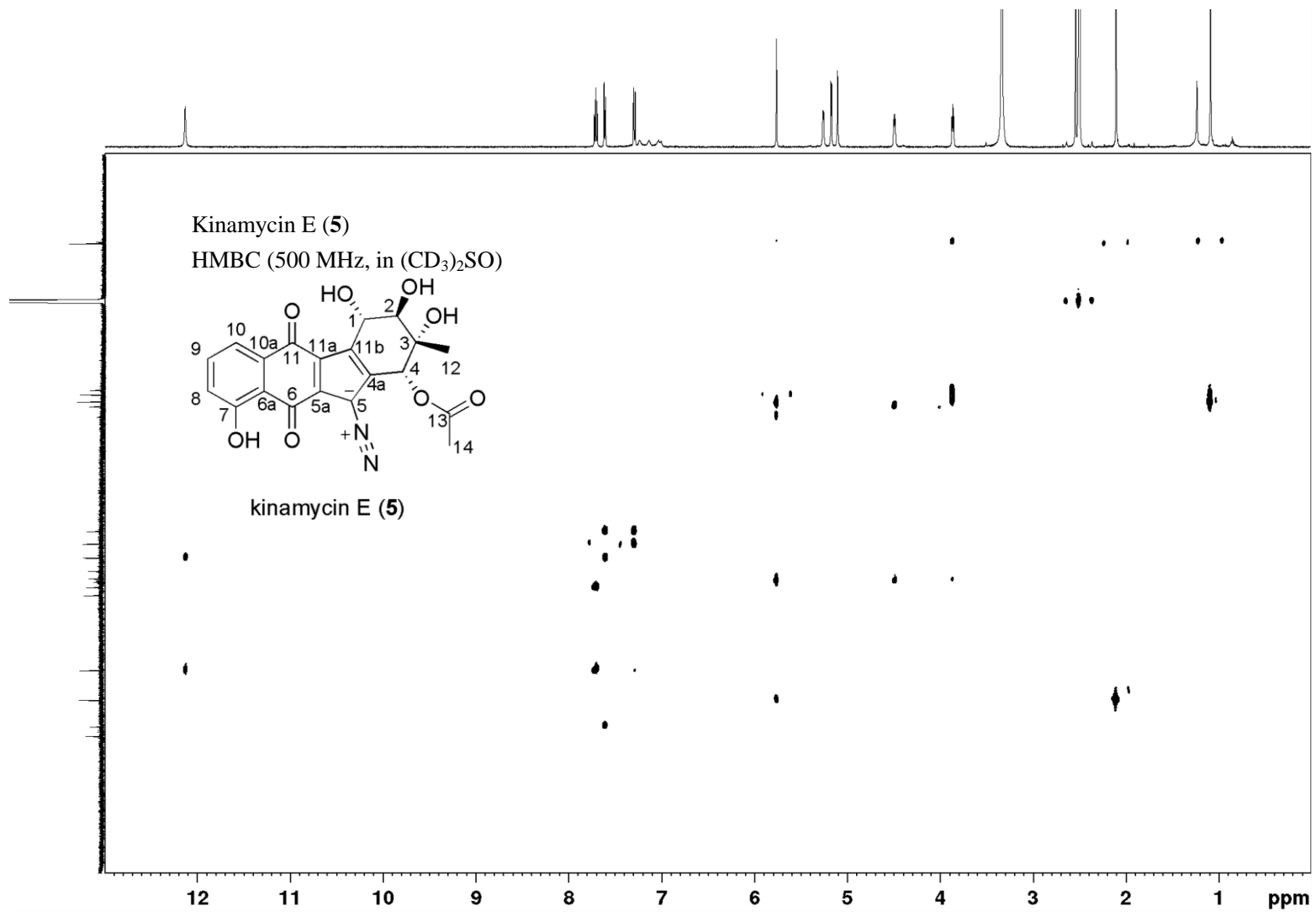
Supplementary Figure 16. NOESY spectrum for epoxykinamycin FL-120B' (4).



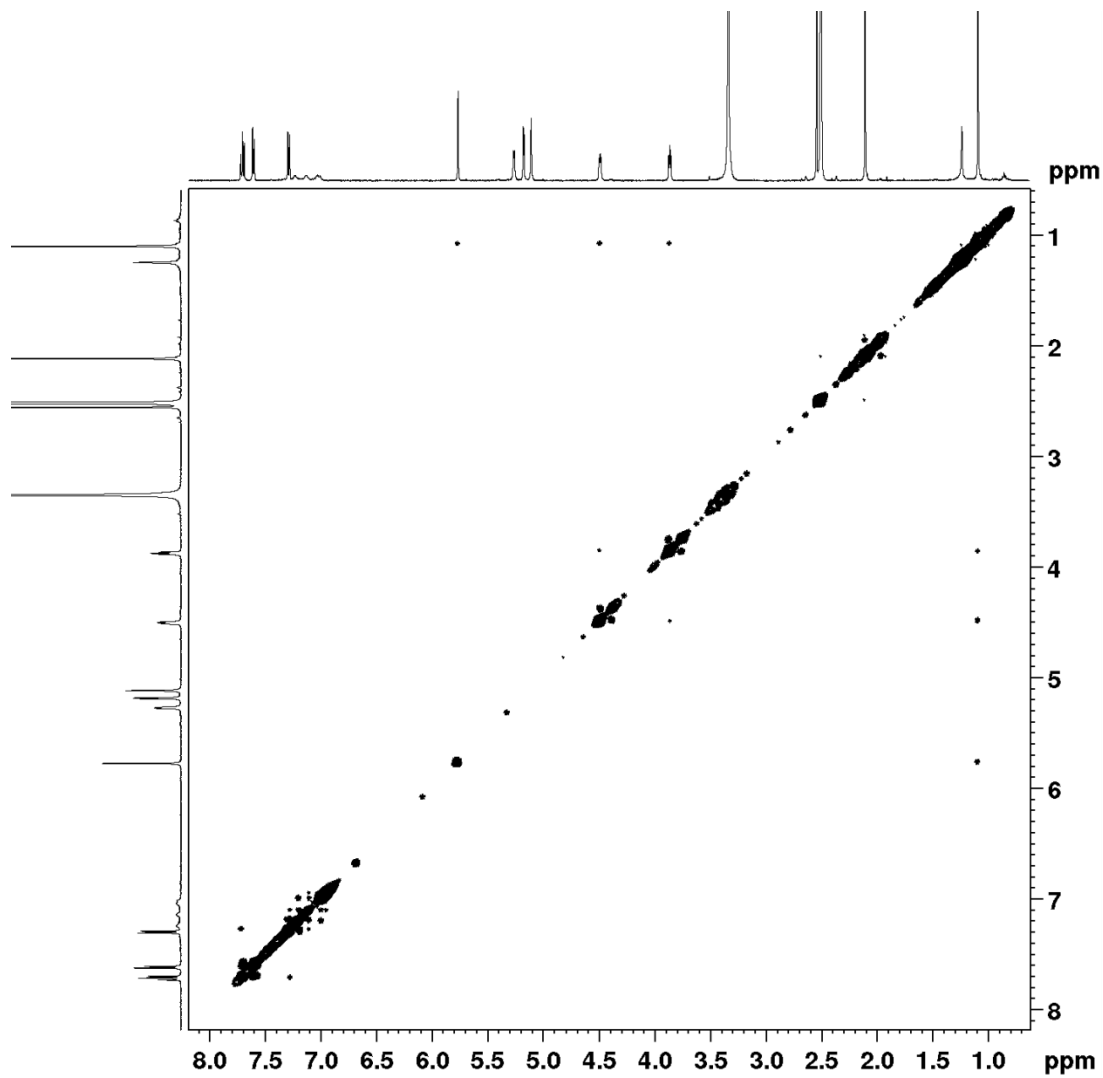
Supplementary Figure 17. ¹H NMR spectrum for kinamycin E (**5**).



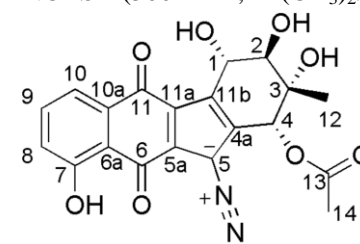
Supplementary Figure 18. ^{13}C NMR spectrum for kinamycin E (**5**).



Supplementary Figure 19. HMBC spectrum for kinamycin E (5).



Kinamycin E (5)
 NOESY (500 MHz, in (CD₃)₂SO)



kinamycin E (5)

Supplementary Figure 20. NOESY spectrum for kinamycin E (5).

Supplementary Table 1. Predicted functions of the gene products in the *alp* cluster.

gene	predicted function	note
<i>alpZ</i>	gamma-butyrolactone binding protein	
<i>alpY</i>	hypothetical protein	
<i>alpX</i>	putative carboxyl transferase	
<i>alpW</i>	TetR-family transcriptional regulator	
<i>alpV</i>	SARP family pathway specific regulator	
<i>alpU</i>	SARP family pathway specific regulator	
<i>alpT</i>	SARP family pathway specific regulator	
<i>alpS</i>	putative thioesterase	
<i>alpR</i>	putative polyketide synthase	
<i>alpQ</i>	putative chain length factor protein	
<i>alpP2</i>	hypothetical protein	
<i>alpP</i>	putative cyclase	
<i>alpO</i>	putative acyl-CoA dehydrogenase	
<i>alpN</i>	putative phosphopantetheinyl transferase	
<i>alpM</i>	peptide synthase condensation domain	
<i>alpL2</i>	conserved hypothetical protein	
<i>alpL</i>	JadX-like protein	
<i>alpK</i>	oxygenase	
<i>alpJ</i>	oxidase	
<i>alpI</i>	polyketide cyclase	
<i>alpA</i>	ketoacyl synthase	
<i>alpB</i>	chain length determinant	
<i>alpC</i>	acyl carrier protein	
<i>alpD</i>	polyketide ketoreductase	
<i>alpE</i>	cyclase/dehydratase	
<i>alpF</i>	oxygenase	
<i>alpG</i>	oxygenase	
<i>alpH</i>	putative O-methyltransferase	

the previously proposed *alp* cluster

Continued

gene	predicted function	note	
<i>alp1A</i>	conserved hypothetical protein	detoxification and DNA damage-repair	region 1
<i>alp1B</i>	putative myo-inositol phosphate synthase		
<i>alp1C</i>	putative transferase		
<i>alp1D</i>	putative abasic site repairing enzyme		
<i>alp1E</i>	conserved hypothetical protein		
<i>alp1F</i>	putative metal-dependent hydrolase		
<i>alp1G</i>	conserved hypothetical protein		
<i>alp1H</i>	putative type I phosphodiesterase		
<i>alp1I</i>	putative ferredoxin	diazo assembly machinery	
<i>alp1J</i>	hypothetical protein		
<i>alp1K</i>	putative amidase		
<i>alp1L</i>	putative glutamine synthetase		
<i>alp1M</i>	putative adenylosuccinate lyase		
<i>alp1N</i>	putative acetyltransferase		
<i>alp1O</i>	two-component system regulator	export and regulation	
<i>alp1P</i>	putative efflux protein		
<i>alp1Q</i>	conserved hypothetical protein		
<i>alp1R</i>	putative antibiotic antiporter		
<i>alp1S</i>	putative oxygenase	A-ring transformation	
<i>alp1T</i>	NDP-sugar epimerase		
<i>alp1U</i>	epoxy hydrolase		
<i>alp1V</i>	putative oxygenase		
<i>alp1W</i>	putative glutamine amidotransferase	diazo assembly	
<i>alp1X</i>	putative transcriptional regulator	machinery	

Supplementary Table 2. The NMR data of epoxykinamycin FL-120B' (4)

Position	¹³ C-NMR ^a	¹ H-NMR ^b	HMBC ^b	NOESY
1	63.4	5.06 (s, 1H)	C-4a	H-2, H-4, H ₃ -12
1-OH		5.69 (s, 1H)	C-1, C-2	
2	61.6	3.52 (t, 1H, <i>J</i> = 6.0 Hz)	C-1, C-3, C-11b, C-12	H ₃ -12
3	58.4			
4	68.8	6.34 (s, 1H)	C-3, C-5, C-11b, C-12	H ₃ -12
4a	129.5			
5	77.4			
5a	128.6			
6	182.8			
6a	115.6			
7	161.6			
7-OH		12.07 (s, 1H)	C-6a, C-7, C-8	
8	124.9	7.31 (d, 1H, <i>J</i> = 7.5 Hz)	C-6a, C-7, C-10	
9	137.0	7.72 (t, 1H, <i>J</i> = 7.5 Hz)	C-7, C-10a	
10	120.3	7.63(d, 1H, <i>J</i> = 7.5 Hz)	C-6a, C-8	
10a	134.1			
11	180.7			
11a	131.8			
11b	130.2			
12	19.4	1.40 (s, 3H)	C-2, C-3, C-4	
13	171.0			
14	20.4	2.26 (s, 3H)	C-13	

^a Recorded at 125 MHz in (CD₃)₂SO. ^b Recorded at 500 MHz in (CD₃)₂SO.

Supplementary Table 3. The NMR data of kinamycin E (**5**)

Position	¹³ C-NMR ^a	¹ H-NMR ^b	HMBC ^b	NOESY
1	69.3	4.50 (dd, 1H, <i>J</i> = 3.5, 6.0 Hz)	C-3, C-4a	H-2, H-12
1-OH		5.18 (d, 1H, <i>J</i> = 3.5 Hz)		
2	74.8	3.87 (t, 1H, <i>J</i> = 6.0 Hz)	C-1, C-3, C-11b, C-12	H-1
2-OH		5.26 (d, 1H, <i>J</i> = 6.0 Hz)		
3	73.2			
3-OH		5.10 (s, 1H)		
4	70.8	5.76 (s, 1H)	C-2, C-5, C-11b, C-12	H-12
4a	132.4			
5	78.2			
5a	128.9			
6	183.2			
6a	115.8			
7	161.6			
7-OH		12.11 (s, 1H)	C-6a, C-7, C-8	
8	124.6	7.29 (d, 1H, <i>J</i> = 8.5 Hz)	C-6a, C-7, C-10	
9	136.9	7.70 (t, 1H, <i>J</i> = 8.5 Hz)	C-7, C-10a	
10	119.9	7.61 (d, 1H, <i>J</i> = 8.5 Hz)	C-6a, C-8	
10a	134.3			
11	180.2			
11a	131.4			
11b	132.0			
12	20.6	1.09 (s, 3H)	C-2, C-3, C-4	
13	171.3			
14	21.1	2.10 (s, 3H)	C-13	

^a Recorded at 125 MHz in (CD₃)₂SO. ^b Recorded at 500 MHz in (CD₃)₂SO.

Supplementary Table 4. List of primers used in this study.

Primers	Restriction sites	Sequences
1U-upF	<i>Hind</i> III	5' CCCAAGCTTGTGCAGATCCCCGACGCC 3'
1U-upR	<i>Xba</i> I	5' GCTCTAGAGGCCAGTTCCCGGTCCGA 3'
1U-dnF	<i>Xba</i> I	5' GCTCTAGATACGCCAACGCCTACAACAGC 3'
1U-dnR	<i>Eco</i> RI	5' GGAATTCCCCATGTCGTAGCGGATGTC 3'
CK-1U-F		5' CGAGGACATGGGTAGATGACGC 3'
CK-1U-R		5' GGCCGGGCAGTTTGTCTAGTA 3'
1U-F	<i>Nde</i> I	5' GGAATTCATATGCATGGGTAGATGACGCTGAAC 3'
1U-R	<i>Eco</i> RI	5' GGAATTCGAGGTGGCGTCAGCCGA 3'
Ealp1U-F	<i>Nde</i> I	5' GGAATTCATATGCTCGCCCTGGGCACGG 3'
Ealp1U-R	<i>Hind</i> III	5' CCCAAGCTTGGAGGTGGCGTCAGCCGAAGAAG 3'