

Supporting information

Rediscovery of tetronomycin as a broad-spectrum and potent antibiotic against drug-resistant gram-positive bacteria

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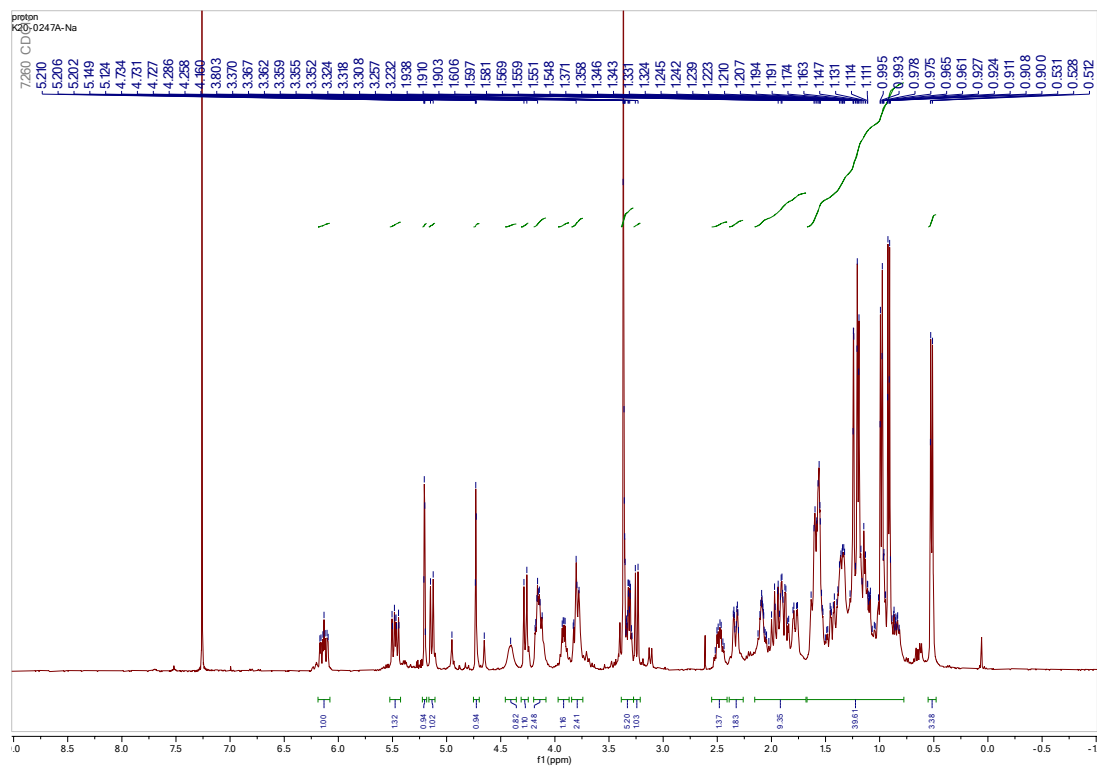
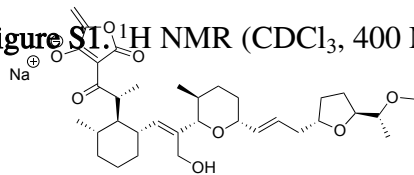


Figure S1. ¹H NMR (CDCl₃, 400 MHz) spectrum of tetronomycin sodium salt (2).



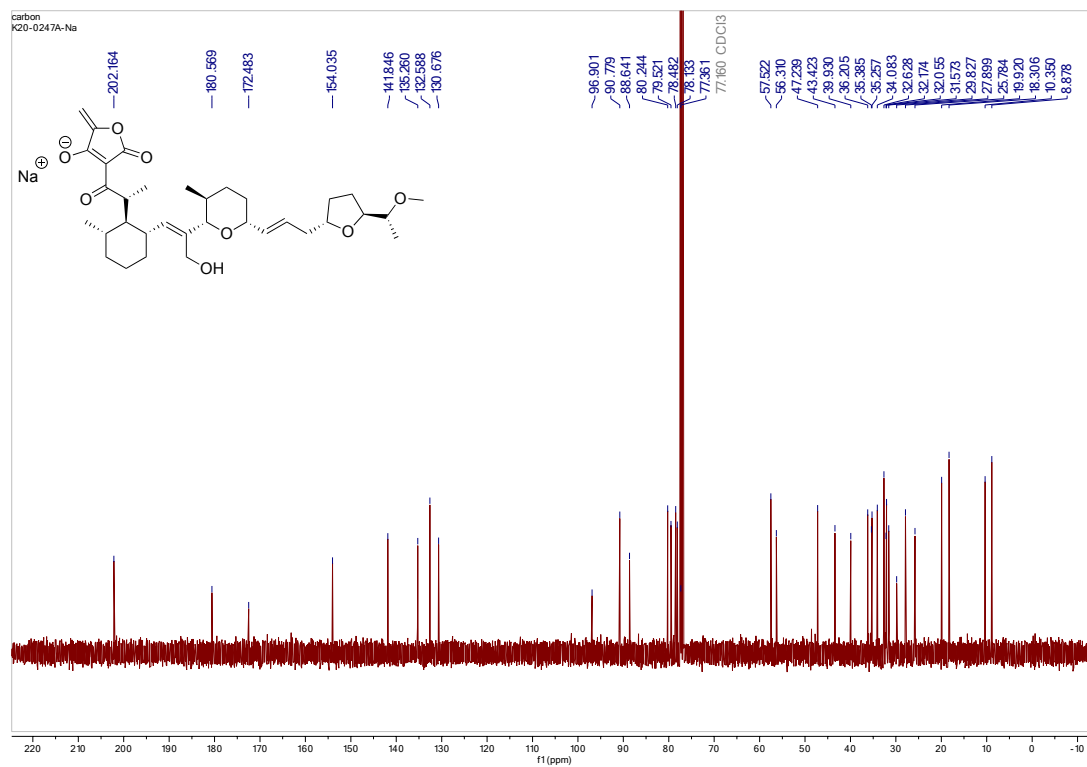


Figure S2. ¹³C NMR (CDCl₃, 100 MHz) spectrum of tetronomycin sodium salt (2).

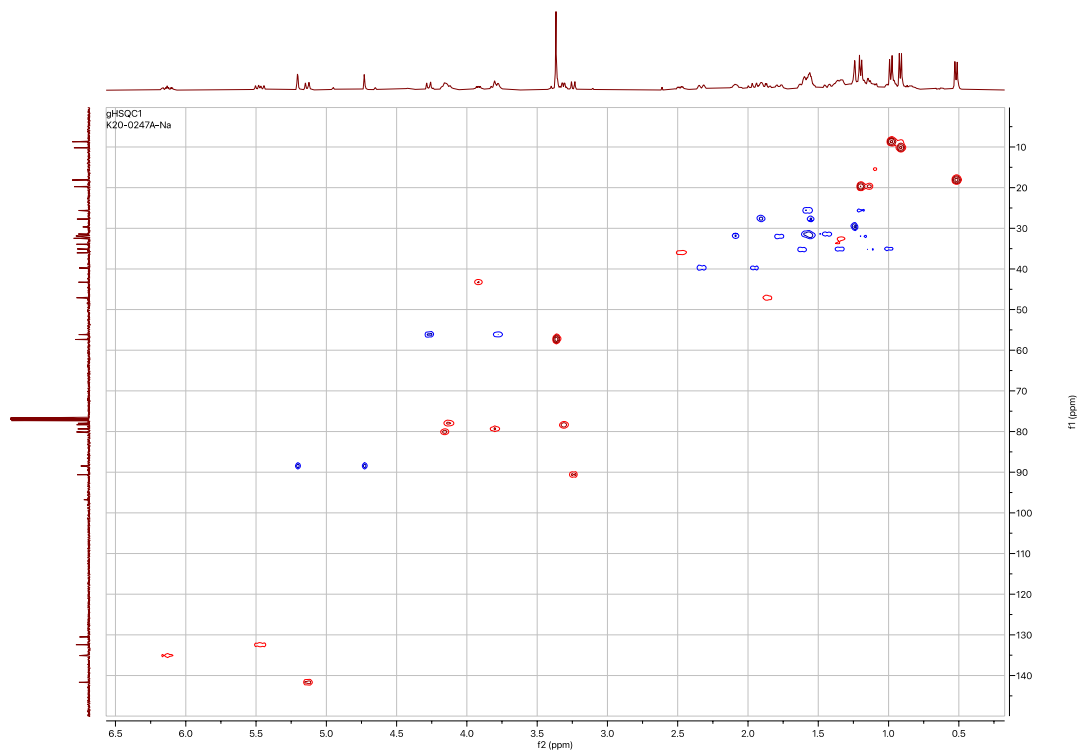


Figure S3. HSQC (CDCl₃, 400 MHz) spectrum of tetronomycin sodium salt (**2**).

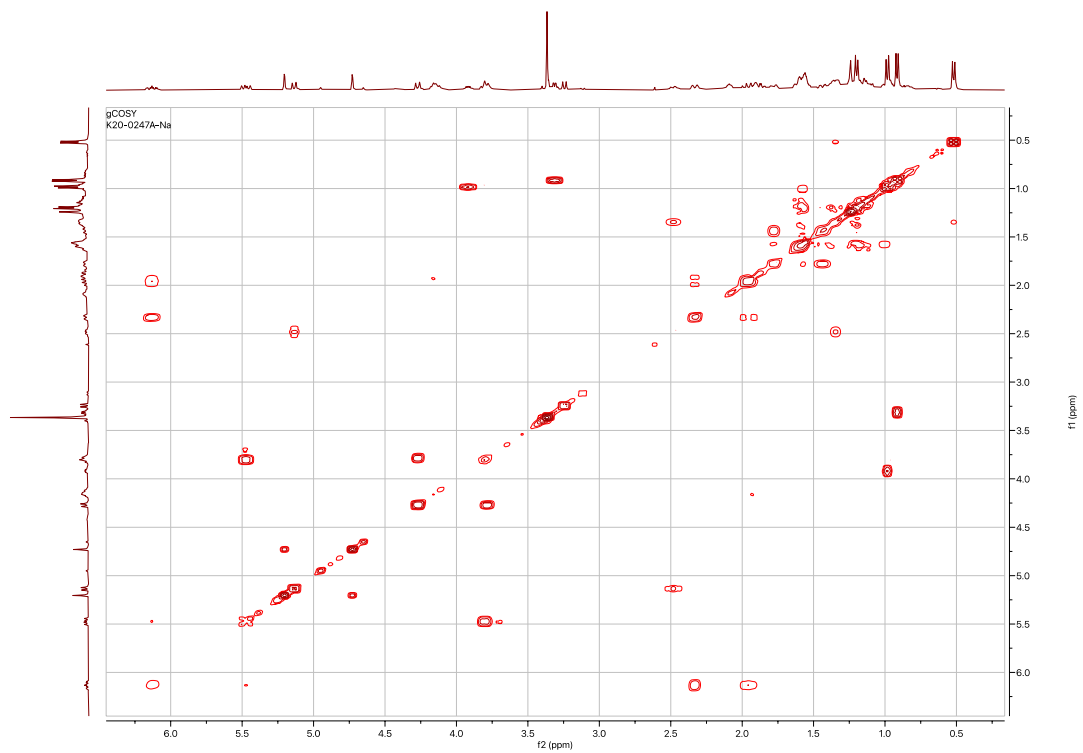


Figure S4. COSY (CDCl_3 , 400 MHz) spectrum of tetronomycin sodium salt (**2**).

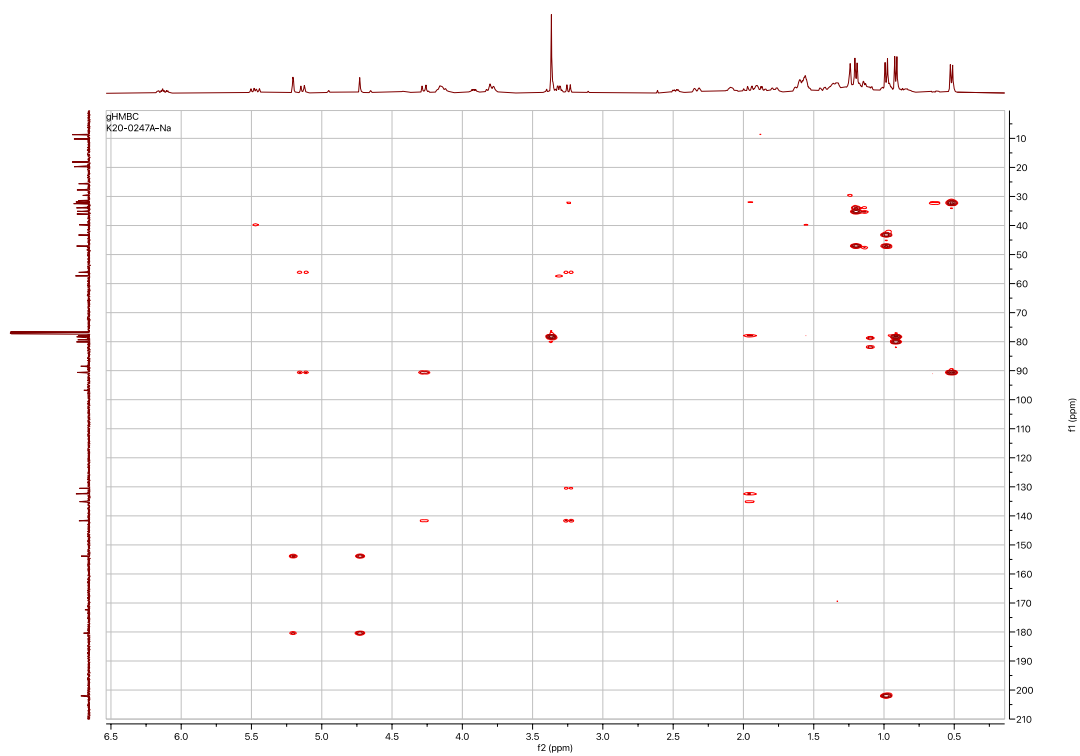


Figure S5. HMBC (CDCl₃, 400 MHz) spectrum of tetronomycin sodium salt (2).

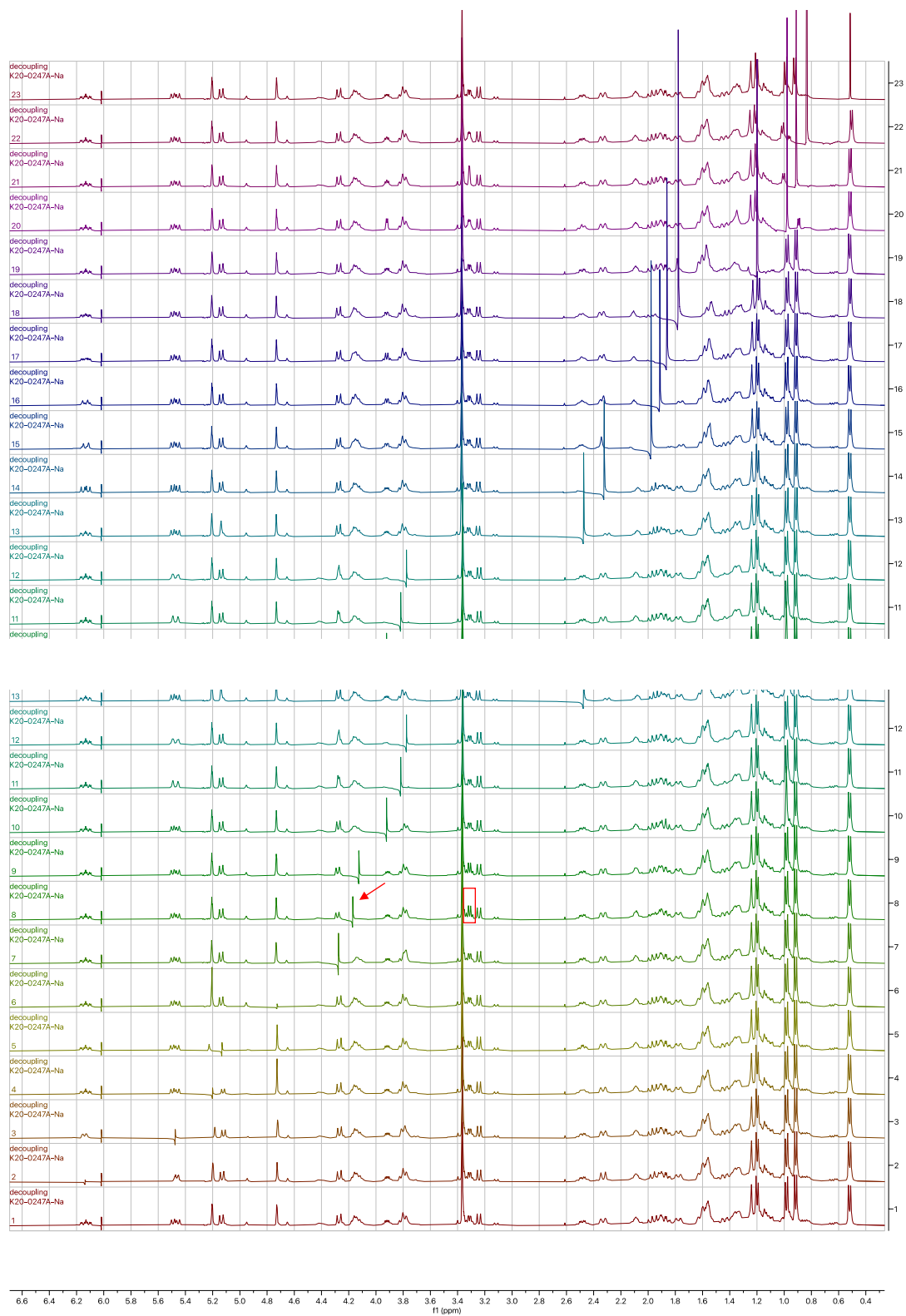


Figure S6. Decoupling experiments of tetromycin sodium salt (2).

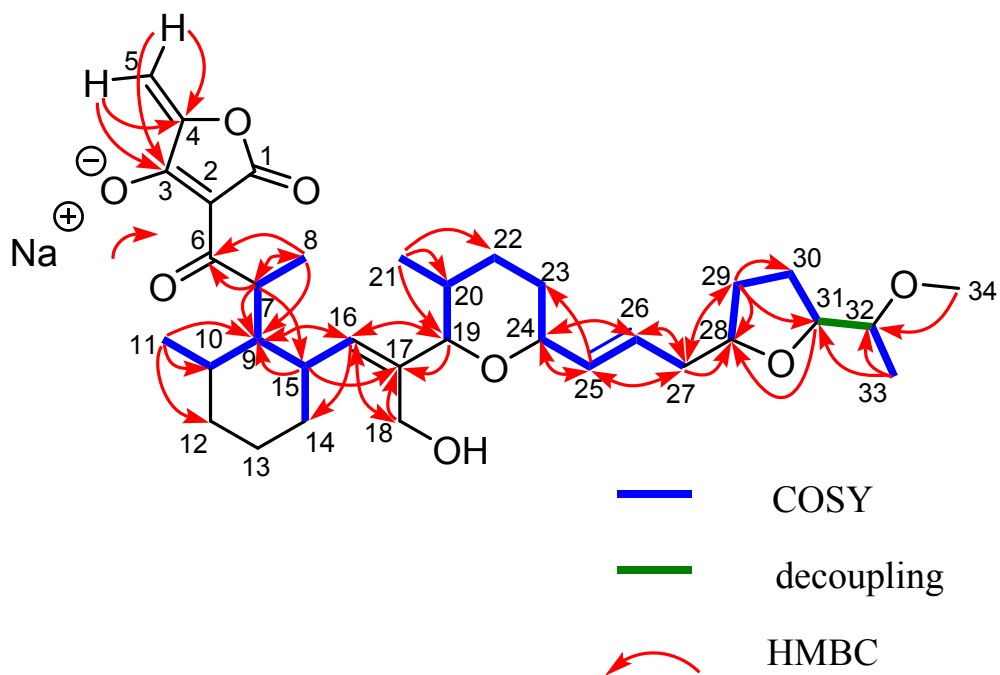


Figure S7. COSY, decoupling, and HMBC analyses of tetronomycin sodium salt (2).

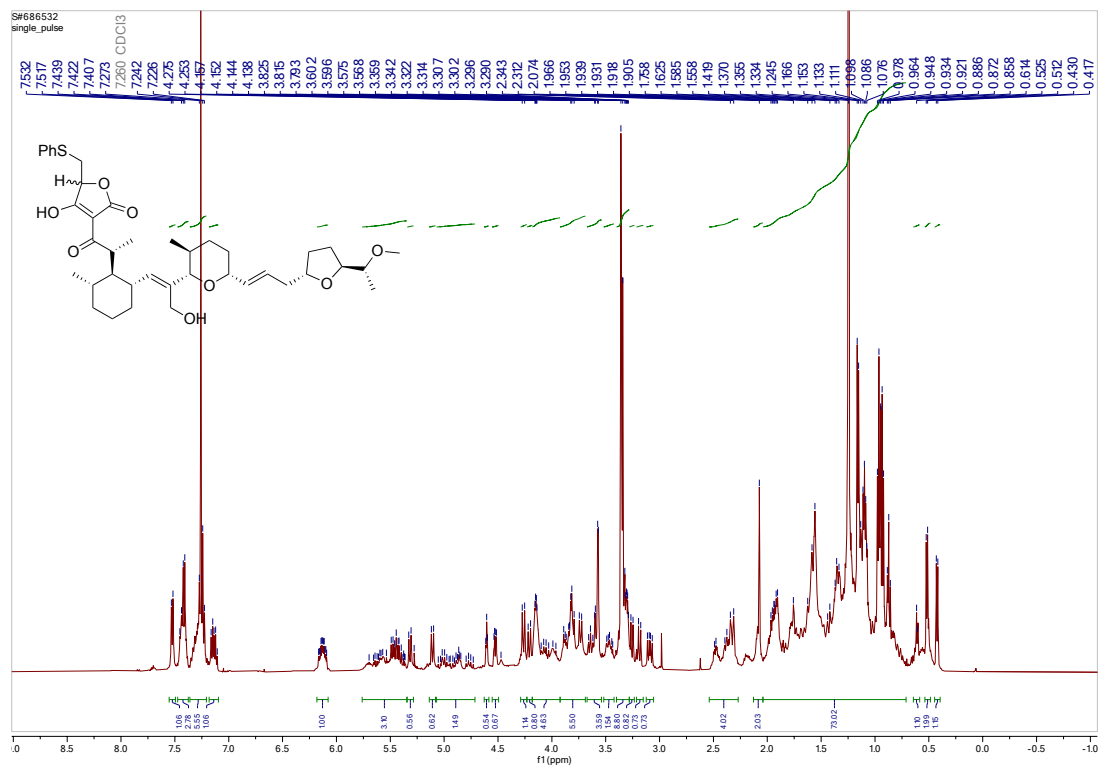


Figure S8. ¹H NMR (CDCl₃, 500 MHz) spectrum of tetronomycin thioacetal derivative

(3).

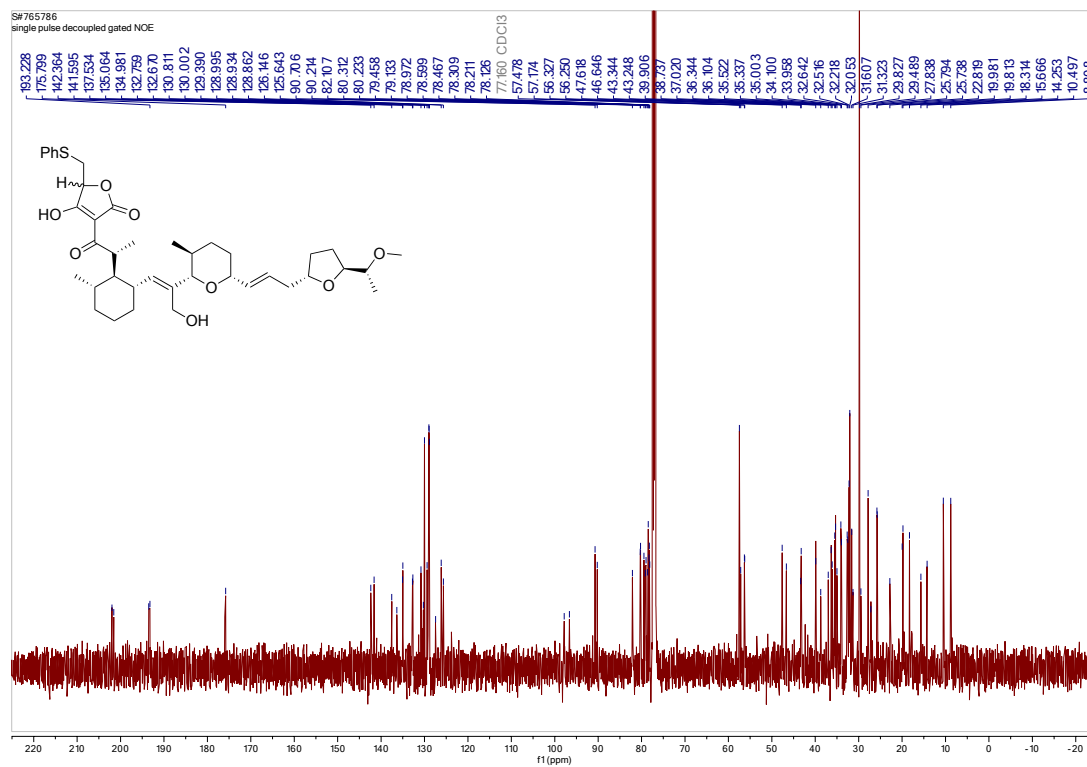


Figure S9. ^{13}C NMR (CDCl_3 , 125 MHz) spectrum of tetronomycin thioacetal derivative

(3).

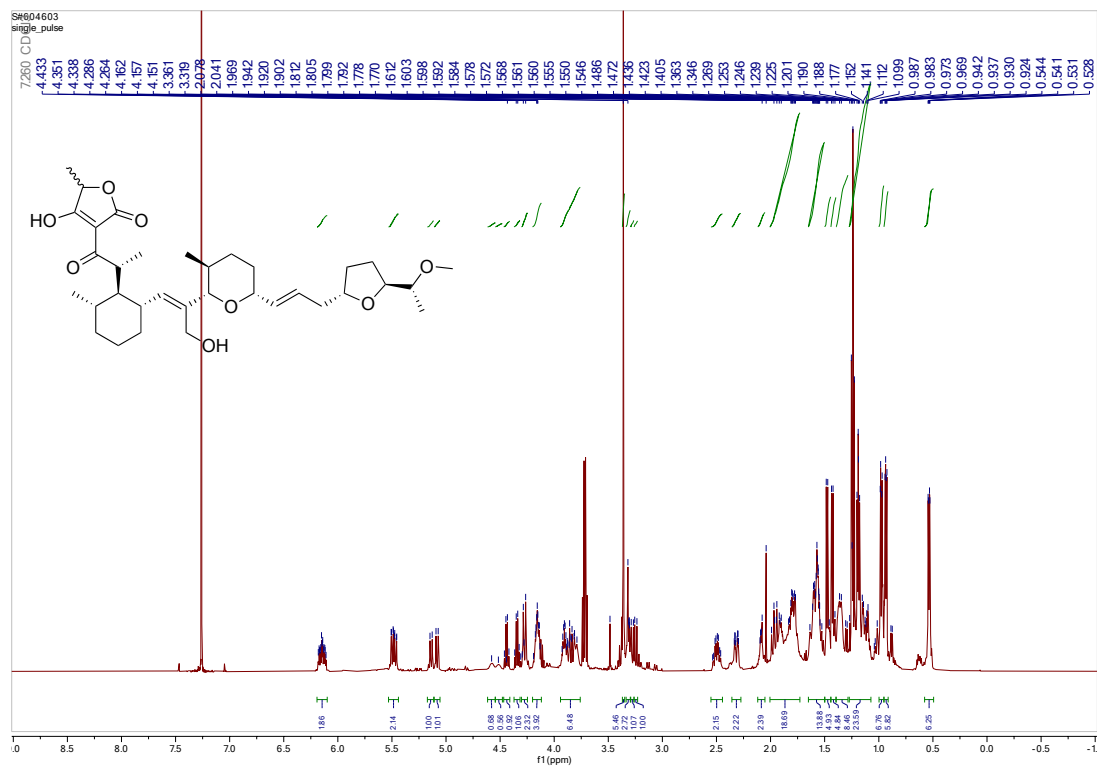


Figure S10. ¹H NMR (CDCl₃, 500 MHz) spectrum of reduced tetronomycin (4).

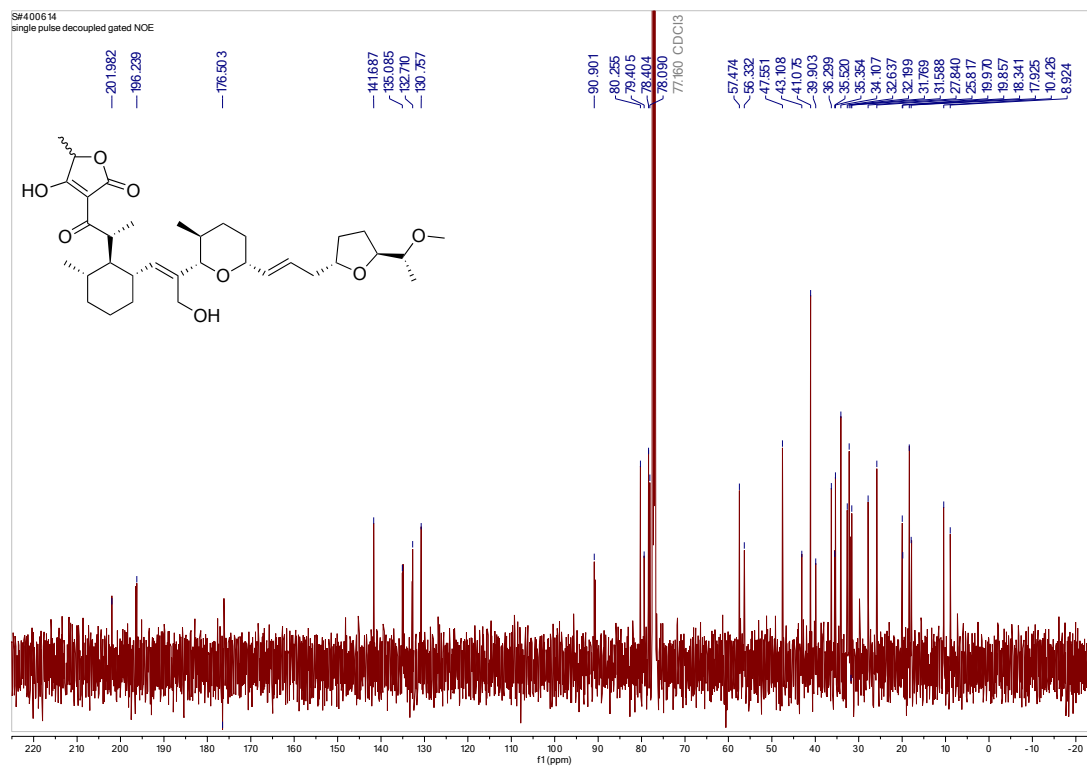


Figure S11. ¹³C NMR (CDCl₃, 125 MHz) spectrum of reduced tetromycin (**4**).

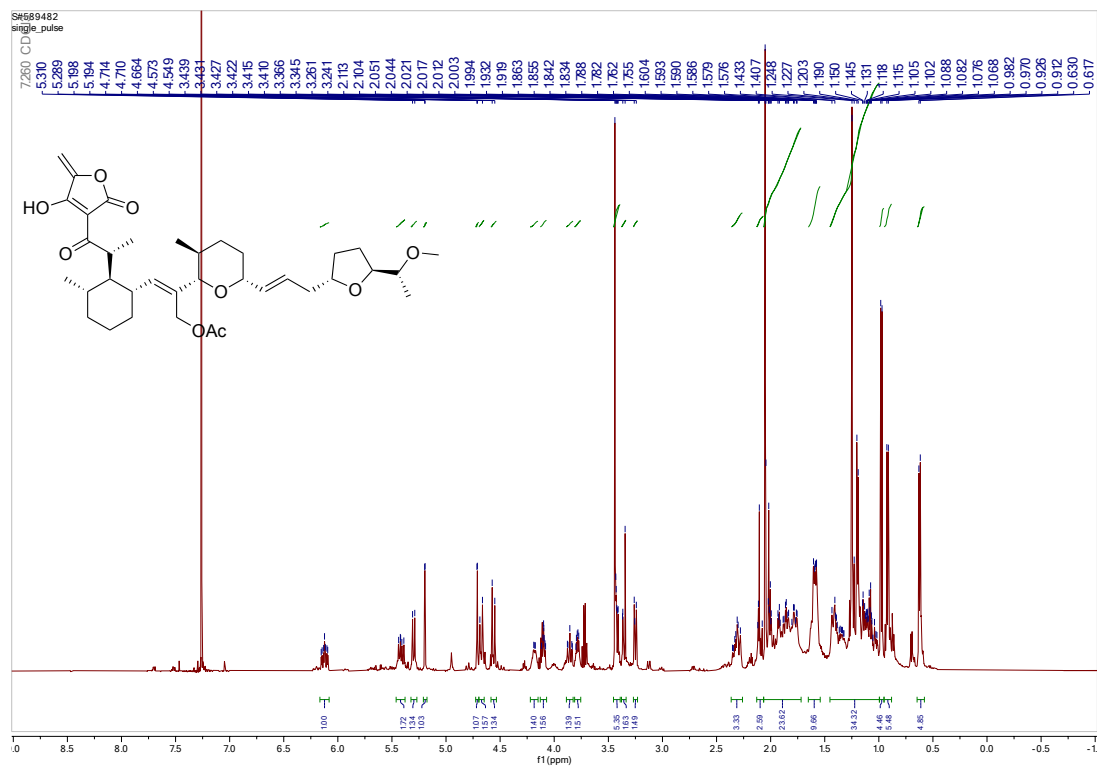


Figure S12. ^1H NMR (CDCl_3 , 500 MHz) spectrum of acetyl tetronomycin (5).

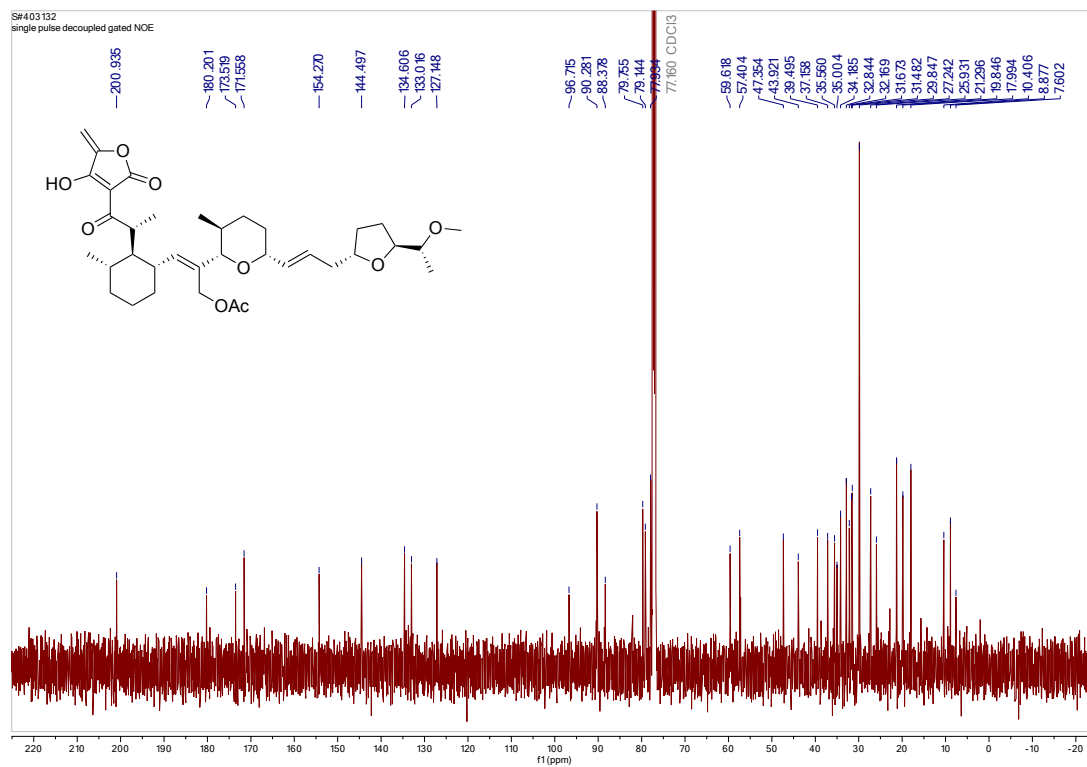


Figure S13. ^{13}C NMR (CDCl_3 , 125 MHz) spectrum of acetyl tetromycin (**5**).

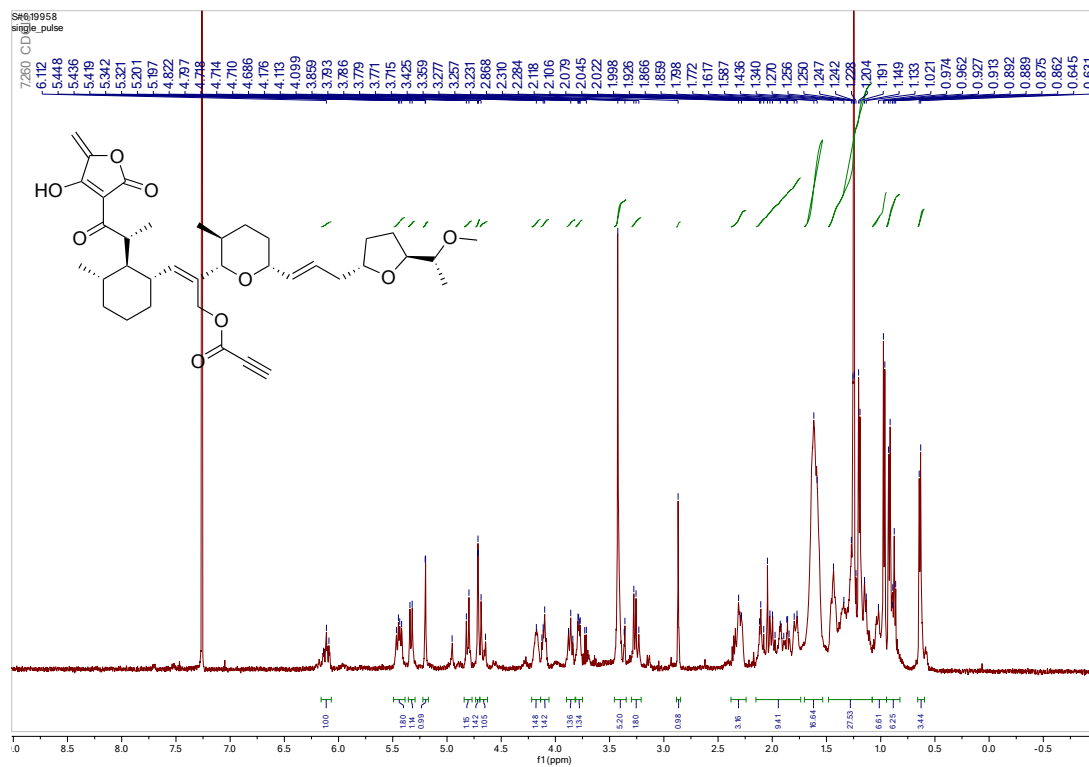


Figure S14. ¹H NMR (CDCl₃, 500 MHz) spectrum of propynoic tetronomycin (6).

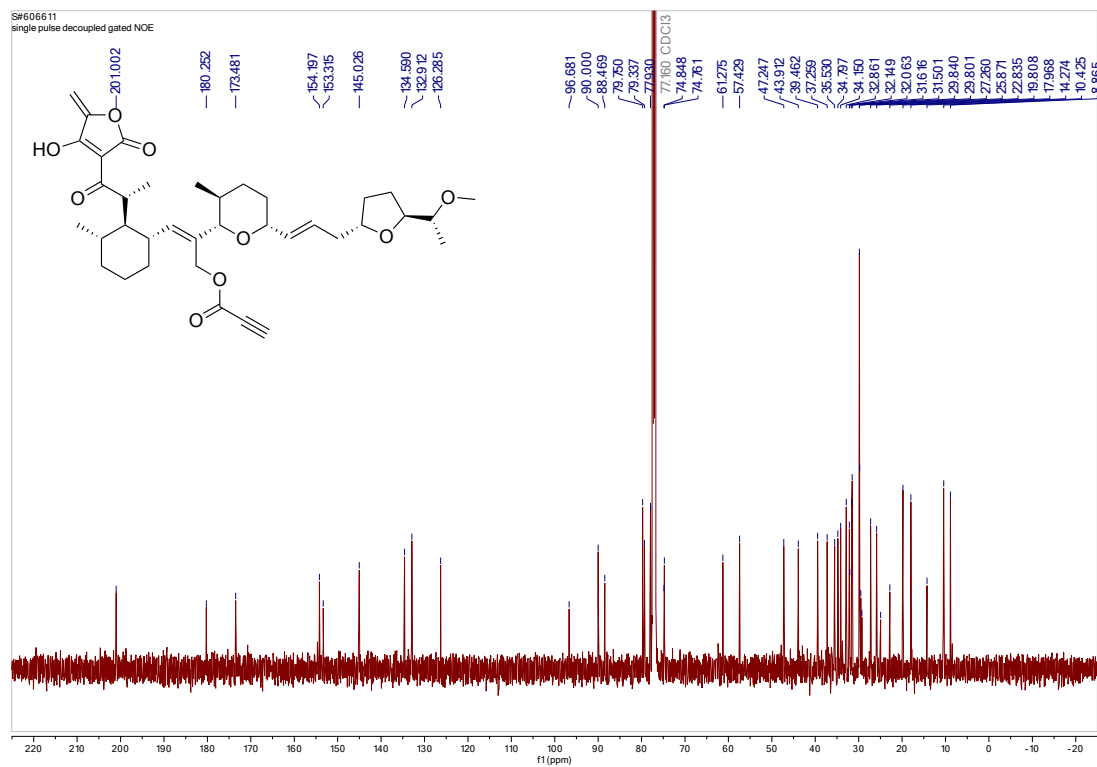


Figure S15. ^{13}C NMR (CDCl_3 , 125 MHz) spectrum of propynoic tetronomicin (6).

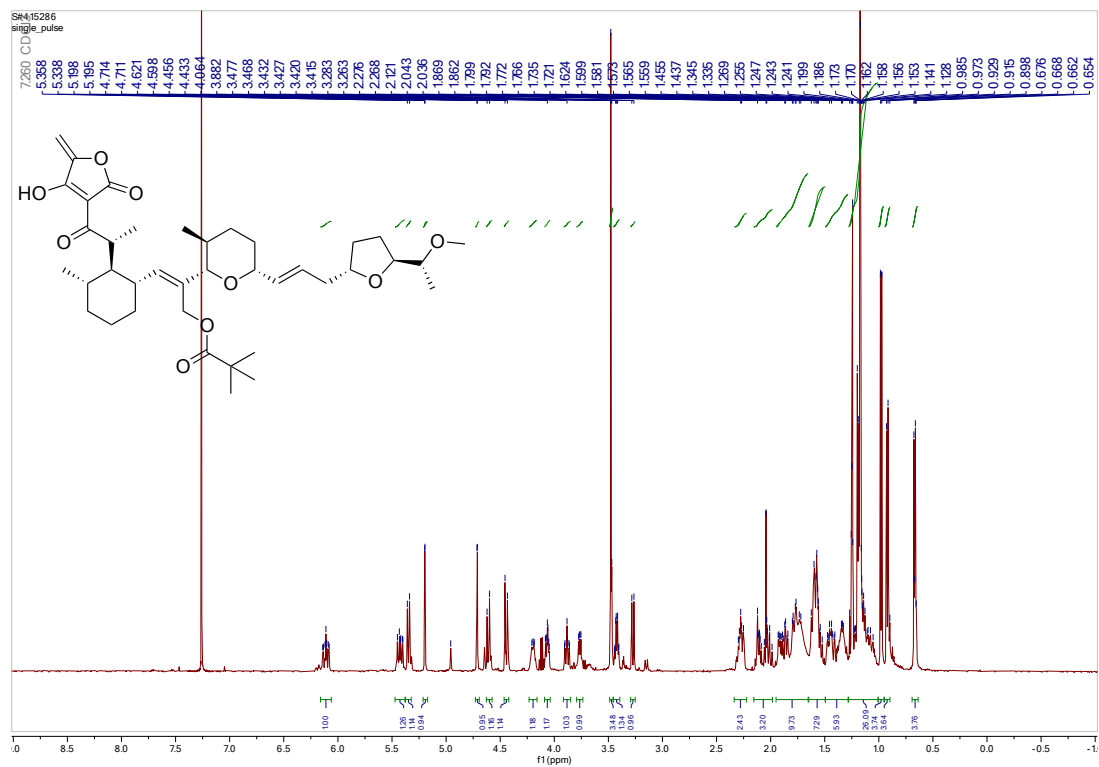


Figure S16. ¹H NMR (CDCl₃, 500 MHz) spectrum of pivaloyl tetronomycin (7).

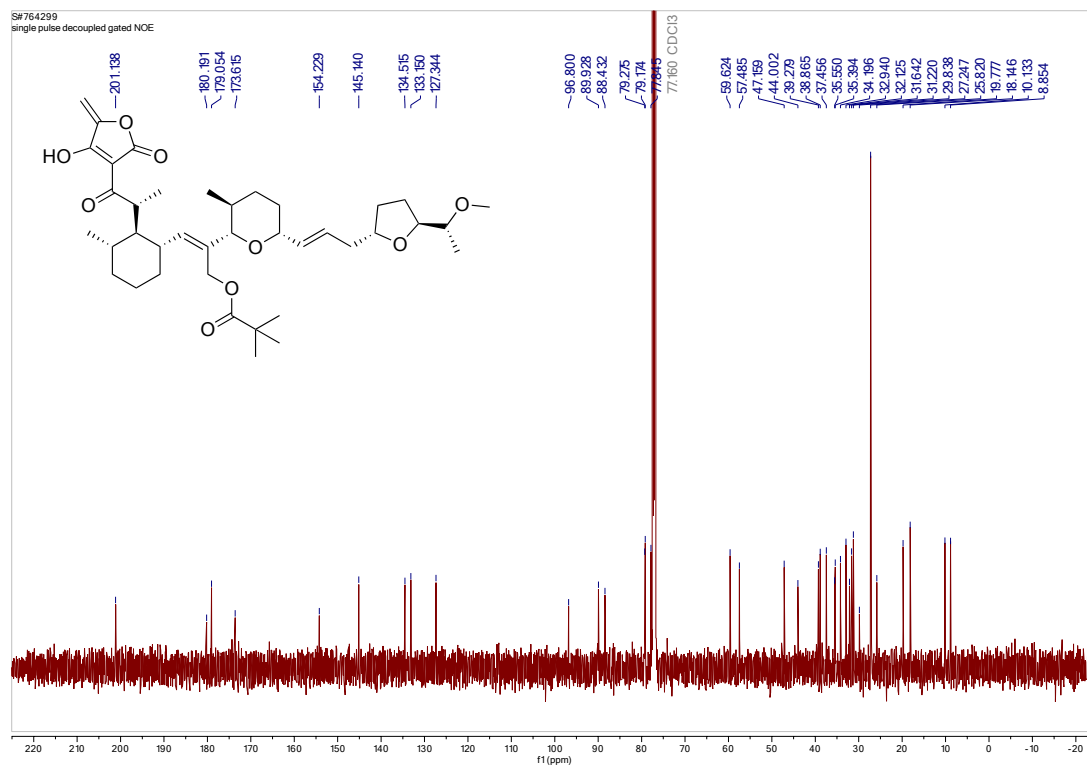


Figure S17. ^{13}C NMR (CDCl_3 , 125 MHz) spectrum of pivaloyl tetronomycin (7).

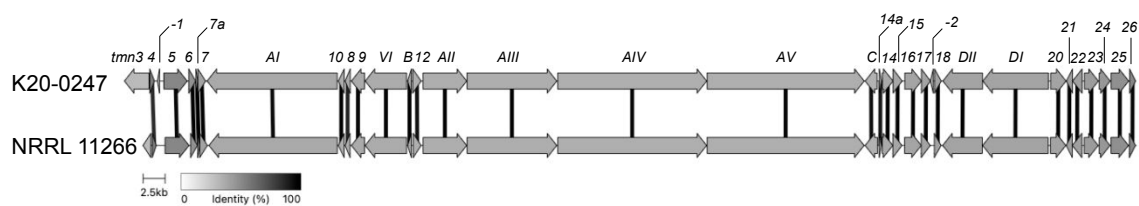


Figure S18. Comparison of biosynthetic gene clusters of tetronomycin between *Streptomyces* sp. K20-0247 and *Streptomyces* sp. NRRL 11266

Similar genes are connected by the line and line color indicates the identity between translation products

Table S1. Annotation of biosynthetic gene cluster of tetronomycin in *Streptomyces* sp.

K20-0247 (The GenBank accession number: OP903461)

gene	annotation	Amino acids	identity (%)
<i>tmn3</i>	LuxR family transcriptional regulator	935	99.3*
<i>tmn4</i>	PLP-dependent oxidase	180	100
<i>tmn-1</i>	hypothetical protein	82	98.8**
<i>tmn5</i>	LuxR family transcriptional regulator	887	99.7
<i>tmn6</i>	a/b hydrolase	263	99.2
<i>tmn7a</i>	acyl carrier protein	75	100
<i>tmn7</i>	2-oxo acid dehydrogenase subunit E2	292	99.3
<i>tmnAI</i>	type I polyketide synthase	4943	97.7
<i>tmn10</i>	DedA family membrane protein	223	100
<i>tmn8</i>	putative ribosomal protein L15P	196	100
<i>tmn9</i>	FAD-dependent monooxygenase	500	97.6
<i>tmnVI</i>	type I polyketide synthase	1574	97.5
<i>tmnB</i>	NTF2 family protein	141	99.3
<i>tmn12</i>	methyltransferase	300	100
<i>tmnAII</i>	type I polyketide synthase	1658	98.8
<i>tmnAIII</i>	type I polyketide synthase	3408	98.9
<i>tmnAIV</i>	type I polyketide synthase	5657	97.3
<i>tmnAV</i>	type I polyketide synthase	5963	97.9
<i>tmnC</i>	FAD-dependent epoxidase	473	99.3
<i>tmn14a</i>	ferredoxin	80	97.5
<i>tmn14</i>	cytochrome P450	400	100
<i>tmn15</i>	3-oxoacyl-(acyl-carrier-protein) synthase III	346	99.4
<i>tmn16</i>	HAD-IIIC family phosphatase	633	99.5
<i>tmn17</i>	a/b hydrolase	345	99.7
<i>tmn-2</i>	no hit	109	-

<i>tmn18</i>	SARP family transcriptional regulator	256	99.6
<i>tmnDII</i>	type I polyketide synthase	1508	99.5
<i>tmnDI</i>	type I polyketide synthase	2488	98.9
<i>tmn20</i>	acyltransferase	584	100
<i>tmn21</i>	TetR family transcriptional regulator	232	100
<i>tmn22</i>	membrane protein	367	98.1
<i>tmn23</i>	phosphopantetheinyl transferase	530	99.6
<i>tmn24</i>	ATP-grasp domain-containing protein	415	98.8
<i>tmn25</i>	hypothetical protein	663	98.8
<i>tmn26</i>	hypothetical protein	235	99.1

*Because an amino acid sequence of Tmn3 deposited in MiBiG was shortened, alignment was conducted using a short Tmn3 sequence.

**Because *tmn-1* shows no similarity to known genes in *tmn* cluster of *Streptomyces* sp. NRRL 11266, similarity to WP_220189144.1 derived from *Streptomyces* sp. PIP175 was shown in this table.

Figure S19. Determination of Ca²⁺ ionophobic activity of **1** and **4**.

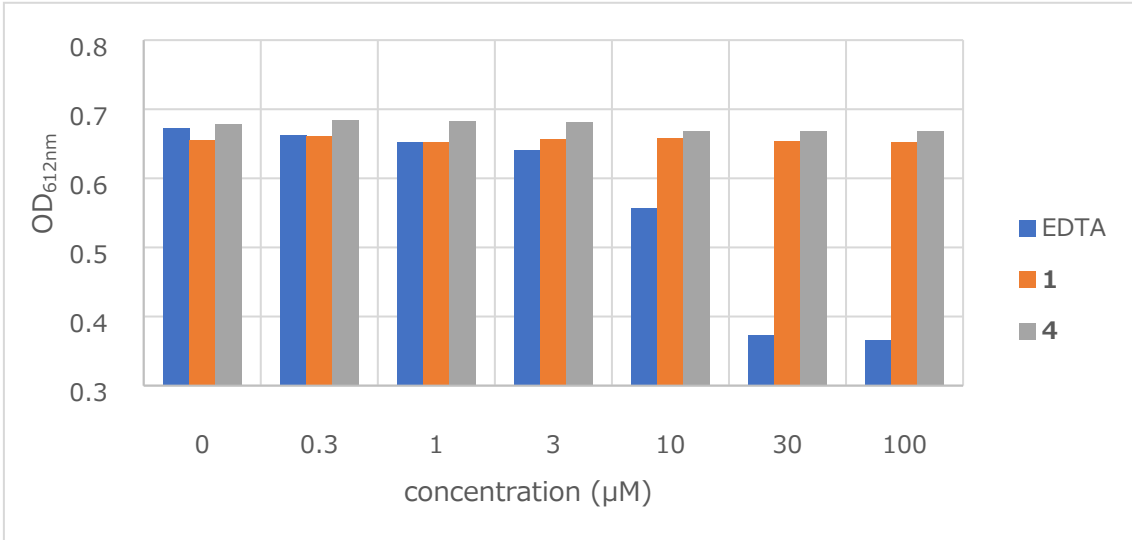


Table S2. Results of activity evaluation of tetrnomycin and its derivatives using 6 mm paper-discs (stopping circle diameter: mm)

Test strain: MRSA (ATCC43300)								
Medium: Mueller hinton agar								
Compound ($\mu\text{g}/\text{disc}$)	3	1	0.3	0.1	0.03	0.01	0.003	0.001
Compound 3	N.T.	11.86	10.38	8.43	7.57	7.25	∅	∅
Tetronomycin	N.T.	26.72	23.73	19.47	12.09	7.62	unclear	∅
VCM	12.00							
Compound 4	18.77	15.98	10.23	∅	∅	∅	∅	∅
Tetronomycin	25.91	24.78	21.65	19.93	15.47	12.73	6.78	∅
VCM	10.23							
Compound 5	21.93	20.95	18.16	15.39	12.21	8.69	∅	∅
Tetronomycin	27.18	25.78	22.15	20.60	14.98	12.32	10.42	unclear
VCM	10.63							
Compound 6	N.T.	19.09	15.14	10.15	7.22	∅	∅	∅
Tetronomycin	N.T.	26.16	22.91	18.58	15.03	11.35	8.11	∅
VCM	10.55							
Compound 7	25.03	20.06	17.38	11.27	9.97	7.83	∅	∅
Tetronomycin	25.30	23.26	20.45	17.68	15.11	12.32	11.86	∅
VCM	10.47							

VCM: Vancomycin

N.T. : not tested ∅ : No inhibition

Table S3. Information of strains used in this study.

No	Strains	Source	Characteristic	Reference No.
1	<i>Staphylococcus aureus</i>	ATCC 6538P	MSSA	
2	<i>Staphylococcus aureus</i>	ATCC 19636	MSSA	
3	<i>Staphylococcus aureus</i> ISP217	Clinical isolate, laboratory stock	MSSA, macrolide resistant	1
4	<i>Staphylococcus aureus</i> KUB854	Clinical isolate, laboratory stock	MRSA (N315-derived strain)	
5	<i>Staphylococcus aureus</i> KUB855	Clinical isolate, laboratory stock	MRSA	
6	<i>Staphylococcus aureus</i> KUB856	Clinical isolate, laboratory stock	MRSA	
7	<i>Staphylococcus aureus</i>	ATCC700699	VISA	
8	<i>Staphylococcus aureus</i> KUB877	Clinical isolate, laboratory stock	LZD resistant	2
9	<i>Staphylococcus epidermidis</i> KUB795	Clinical isolate, laboratory stock		3
10	<i>Kocuria rhizophila</i>	ATCC9341		
11	<i>Enterococcus faecalis</i>	ATCC29212	Vancomycin-susceptible	
12	<i>Enterococcus faecalis</i>	NCTC12201	VRE, vanA	4
13	<i>Enterococcus faecalis</i> KUB7012	Clinical isolate, laboratory stock	VRE, vanB	
14	<i>Enterococcus faecium</i>	NCTC12204	VRE, vanA	
15	<i>Enterococcus faecium</i> KUB7013	Clinical isolate, laboratory stock	VRE, vanB	
16	<i>Enterococcus gallinarum</i> KUB 7014	Clinical isolate, laboratory stock	VRE, vanC	
17	<i>Escherichia coli</i>	NIHJ JC-2		
18	<i>Citrobacter freundii</i>	ATCC8090		
19	<i>Klebsiella pneumoniae</i>	NCTC9632		
20	<i>Proteus vulgaris</i>	ATCC8427		
21	<i>Morganella morganii</i>	IID 602		
22	<i>Serratia marcescens</i>	IFO12648		
23	<i>Enterobacter cloacae</i>	IFO13535		
24	<i>Klebsiella aerogenes</i>	NCTC10006		
25	<i>Pseudomonas aeruginosa</i> 46001	Clinical isolate, laboratory stock		4
26	<i>Pseudomonas aeruginosa</i> E-2	Clinical isolate, laboratory stock		5
27	<i>Acinetobacter calcoaceticus</i>	IFO12552		

The laboratory stock strains of KUB854, 855, 856, 877, 7012, 7013, and 7014 were clinically isolated at the hospitals in Japan.

References

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5. Saito M, et al. Studies on the combination action of sisomicin, gentamicin and piperacillin, cefmetazole against *Pseudomonas aeruginosa* and *Serratia marcescens*. *Jpn. J. Antibiot.* 1983, **36**, 37-46.