

## Supplementary Materials

**Table S1.** NMR spectroscopic data of kendomycin E (1), acquired in CD<sub>3</sub>OD (500 MHz).

Position	$\delta_C^a$	$\delta_H^b$	COSY	HMBC
1	168.5, C	-	-	-
2	104.7, C	-	-	-
3	182.6, C	-	-	-
4	150.1, C	-	-	-
4a	110.8, C	-	-	-
5	76.1, CH	5.41, d (10.6)	2.81-0.94-3.48	14.1-38.9-70.9-80.4-110.8-127.2-150.1-182.6
6	39.0, CH	2.81, m	0.94-3.48-5.41	76.1-14.1
7	80.4, CH	3.48, m	2.00-2.81	57.9-70.9-76.1-80.4-14.1-38.9
8	37.5, CH <sub>2</sub>	2.00-1.34, m	3.48-3.96	39.0-80.4
9	70.9, CH	3.96, t (10.9)	2.00/1.34-1.52/1.41	32.6
10	32.6, CH <sub>2</sub>	1.52-1.41, m	3.96	19.3-29.0-43.1-70.9
11	33.2, CH <sub>2</sub>	1.53-1.01, m	1.84	19.3-29.0
12	29.0, CH	1.84, m	0.89	-
13	43.1, CH <sub>2</sub>	1.30-1.23, m	-	19.3-29.0-33.2
14	46.3, CH	2.23, quint (8.1)	1.35	29.0-33.7-42.8-181.5
15	33.7, CH <sub>2</sub>	1.35, m	-	181.5
16	42.8, CH	2.09, m	1.25	14.5-33.7-46.3-118.3
17	118.3, C	-	-	-
18	157.1, C	-	-	-
18a	127.2, C	-	-	-
19	14.5, CH <sub>3</sub>	0.95, d (6.8)	2.81	38.9-76.1-80.4
20	57.8, CH <sub>3</sub>	3.40, s	-	80.4
21	19.3, CH <sub>3</sub>	0.89, d (6.4)	1.84	29.0-33.2-43.1
22	181.5, C	-	-	-
23	14.5, CH <sub>3</sub>	1.25, d (6.7)	2.09	33.7-42.8-118.3
24	16.2, CH <sub>3</sub>	2.78, s	-	127.2-157.1
25	7.9, CH <sub>3</sub>	1.88, s	-	104.7-127.2-168.5-182.6

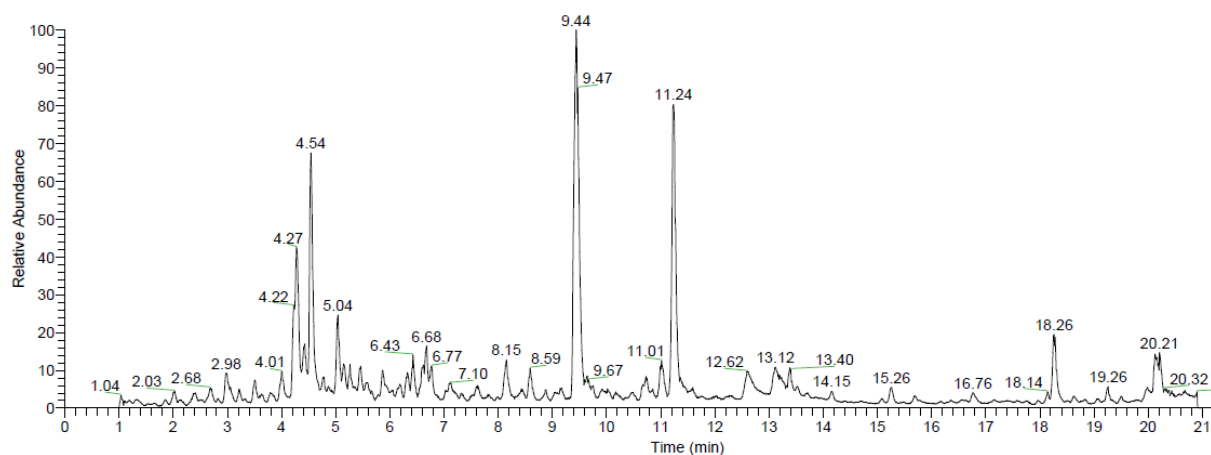
<sup>a</sup> followed by multiplicity; <sup>b</sup> followed by multiplicity and coupling constant J in Hz.

**Table S2.** Genes in kendomycin biosynthetic gene cluster from *Streptomyces* sp. CL 58-27 and their closest similar proteins.

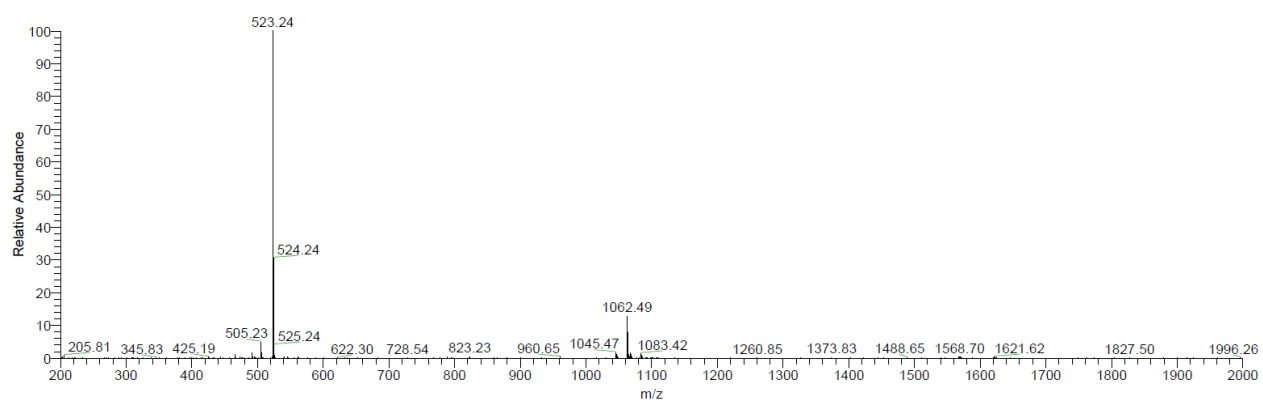
Gene #	Function	Closest Similar Protein, Identity (%)
1	methyltransferase	O-methyltransferase, 45% (migrostatin, <i>Streptomyces platensis</i> subsp. <i>rosaceus</i> )
2	unknown	-
3	methyltransferase	methyltransferase <i>ken9</i> , 54% (kendomycin, <i>Streptomyces violaceoruber</i> )
4	unknown	-
5	cytochrome P450	Cytochrome P450 <i>ncmO</i> , 51% (nocamycin, <i>Saccharothrix syringae</i> )
6	<i>nmrA</i> family protein	Hypothetical protein, 31% (dynemicin A, <i>Micromonospora chersina</i> )

7	LuxR family transcriptional regulator	Regulatory protein, 40% (salinilactam, <i>Salinispora tropica</i> CNB-440)
8	short-chain dehydrogenase/reductase SDR	Oxidoreductase, 47% (lugdunomycin, <i>Streptomyces</i> sp. QL37)
9	transcriptional regulator	Hx1R transcriptional regulator, 51% (microansamycin, <i>Micromonospora</i> sp. HK160111)
10	unknown	-
11	FAD linked oxidase domain protein	Oxidase, 57% (microansamycin, <i>Micromonospora</i> sp. HK160111)
12-15	Binding proteins	-
16	short-chain dehydrogenase/reductase SDR	2,3-dihydroxybenzoate-2,3-dehydrogenase, 37% (carboxamycin, <i>Streptomyces</i> sp. NTK 937)
17	short-chain dehydrogenase/reductase SDR	Oxidoreductase, 45% (lugdunomycin, <i>Streptomyces</i> sp. QL37)
18	unknown	-
19	thioesterase	Thioesterase, 64% (caniferolide A, <i>Streptomyces caniferus</i> )
20	chalcone and stilbene synthase domain protein (T3PKS)	Type III PKS <i>ken2</i> , 77% (kendomycin, <i>Streptomyces violaceoruber</i> )
21	unknown	-
22	enoyl-CoA hydratase	Dioxygenase <i>ken4</i> , 61% (kendomycin, <i>Streptomyces violaceoruber</i> )
23	pyruvate oxidase/decarboxylase	Benzoyl formate decarboxylase <i>ken5</i> , 73% (kendomycin, <i>Streptomyces violaceoruber</i> )
24	aldehyde dehydrogenase	Benzaldehyde dehydrogenase <i>ken6</i> , 75% (kendomycin, <i>Streptomyces violaceoruber</i> )
25	enoyl-CoA hydratase	enoyl-CoA hydratase <i>ken3/7</i> , 80% (kendomycin, <i>Streptomyces violaceoruber</i> )
26	unknown	-
27	Beta-ketoacyl synthase	Polyketidesynthase <i>merC</i> , 75% (meridamycin, <i>Streptomyces violaceusniger</i> )
28	Short chain dehydrogenase/reductase	Polyketidesynthase <i>sceO</i> , 61% (sceliphrolactam, <i>Streptomyces</i> sp. SD85)
29	Transcriptional regulator	Transcriptional regulator <i>sceP</i> , 58% (Sceliphrolactam, <i>Streptomyces</i> sp. SD85)
30	unknown	-
31	$\beta$ ketoacylsynthase (KS-AT-DH-KR/KS-AT-DH-KR/KS-AT-DH-KR)	Polyketidesynthase <i>SceQ</i> , 67% (sceliphrolactam, <i>Streptomyces</i> sp. SD85)
32	$\beta$ ketoacylsynthase (KS-AT-KR)	Polyketidesynthase <i>sceR</i> , 73% (sceliphrolactam, <i>Streptomyces</i> sp. SD85)
33	$\beta$ ketoacylsynthase (KS-AT-DH-KR)	Polyketidesynthase <i>sceS</i> , 70% (sceliphrolactam, <i>Streptomyces</i> sp. SD85)
34	unknown	-
35	unknown	-
36	cytochrome P450	cytochrome P450 <i>sceD</i> , 80% (sceliphrolactam, <i>Streptomyces</i> sp. SD85)

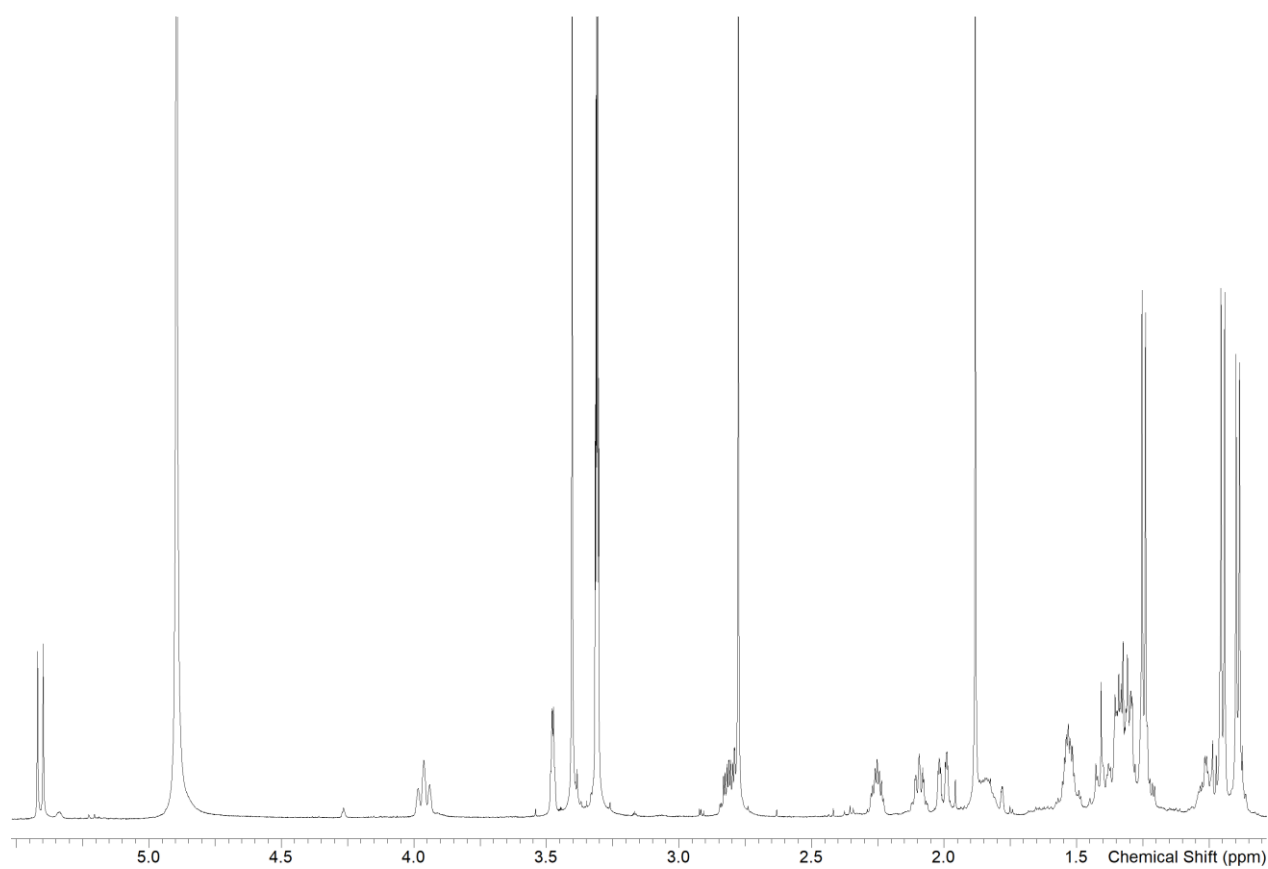
37	cytochrome P450	cytochrome P450 <i>sceE</i> , 76% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
38	dehalogenase	Haloalkane dehalogenase <i>sceF</i> , 74% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
39	acyltransferase	Transacylase <i>SceG</i> , 70% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
40	Binding protein	Binding protein <i>sceH</i> , 64% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
41	AMP dependent synthetase and ligase	AMP dependent synthetase and ligase <i>sceI</i> , 73% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
42	AMP dependent synthetase and ligase	AMP dependent synthetase and ligase <i>sceJ</i> , 70% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
43	Diaminopemellate decarboxylase	Diaminopemellate decarboxylase <i>MacK</i> , 72% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
44	unknown	-
45	unknown	-
46	T1PKS (KS-AT-DH-KR, KS-AT-DH-KR)	Polyketidesynthase <i>sceN</i> , 68% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
47	T1PKS (KS-AT-DH-KR)	Polyketidesynthase <i>sceO</i> , 74% (sceliphrolactam, <i>Streptomyces sp. SD85</i> )
48	thioesterase	53% kendomycin type I PKS (module 7-8)
49	unknown	-
50	unknown	-
51	modular PKS	56% mycotrienine



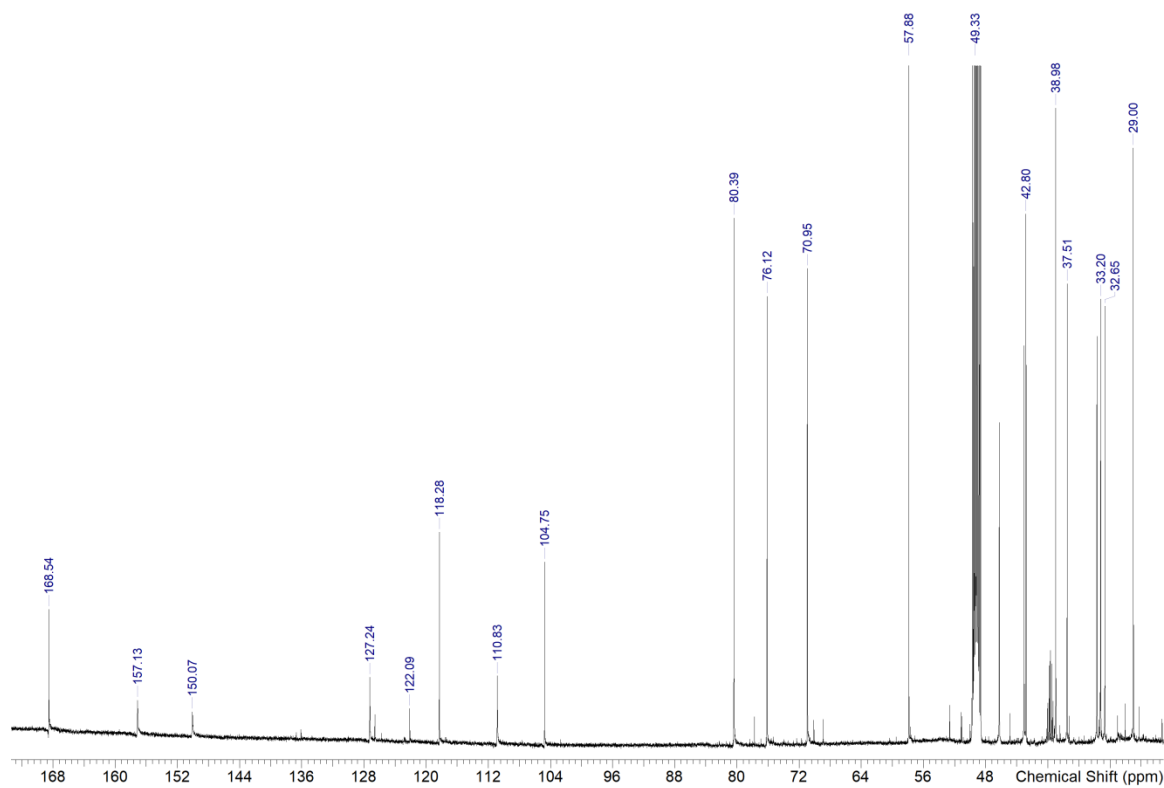
**Figure S1.** LC-HRMS chromatogram of crude extract of CL 58-27. Peak at RT 11.24 min coincides with the targeted compound kendomycin E.



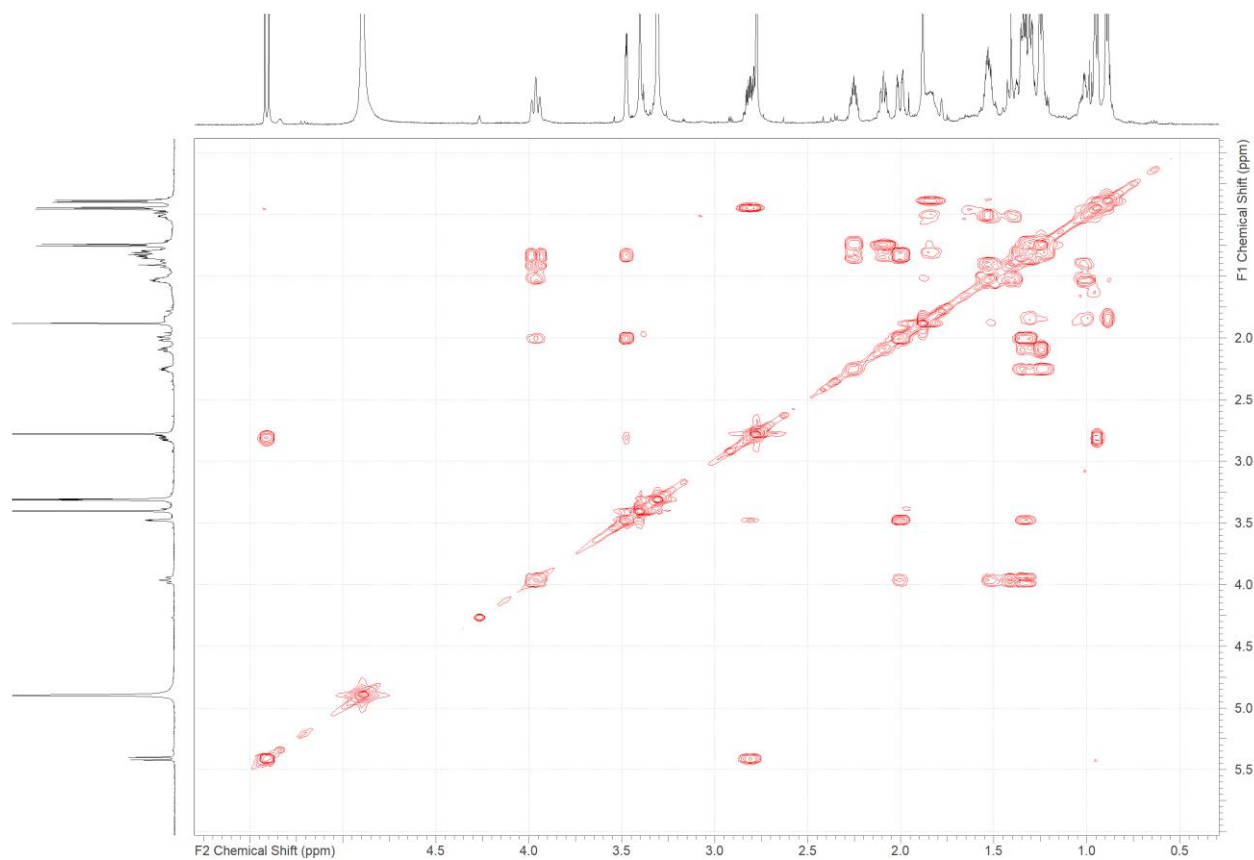
**Figure S2.** Mass chromatogram of kendomycin E showing the  $[M+H]^+$  peak with a mass of 523.24 da.



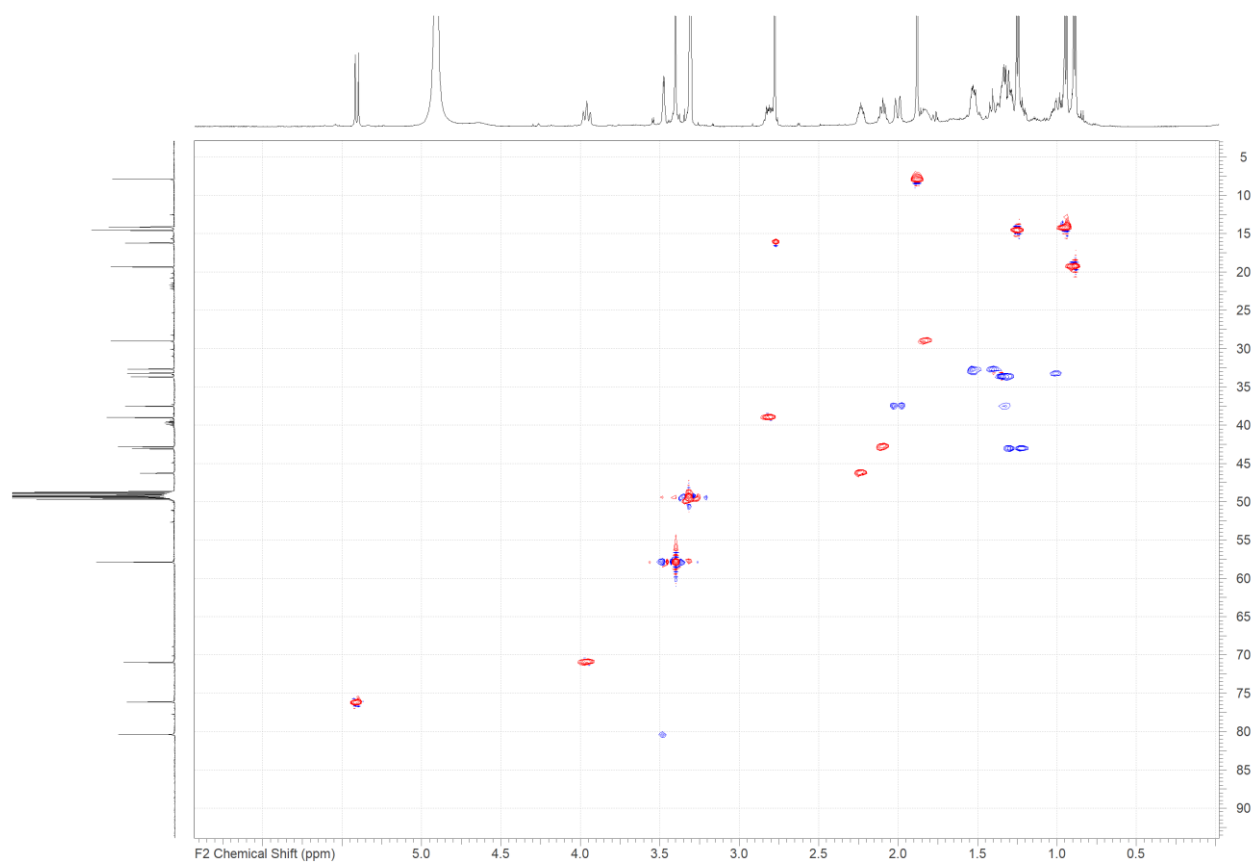
**Figure S3.**  $^1H$  NMR spectrum of kendomycin E measured in  $CD_3OD$  (500 MHz).



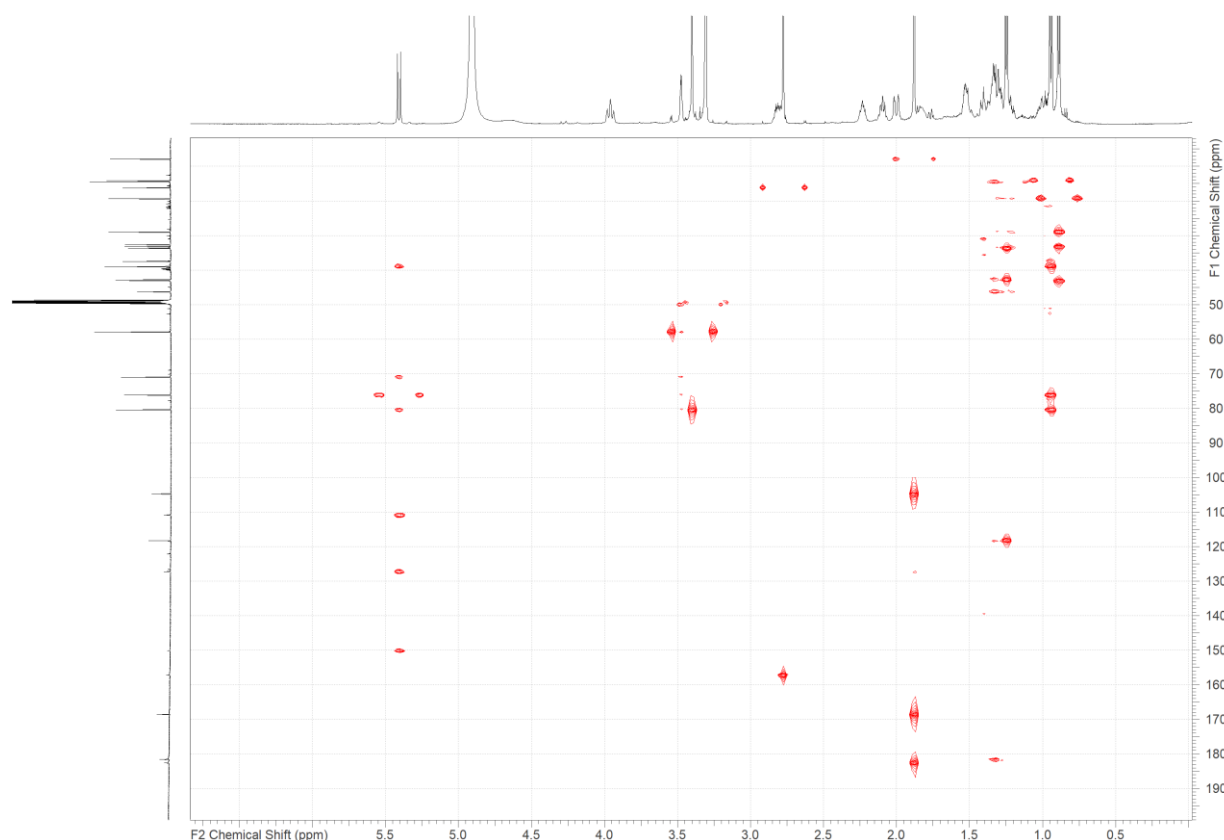
**Figure S4.** <sup>13</sup>C NMR spectrum of kendomycin E measured in CD<sub>3</sub>OD (500 MHz).



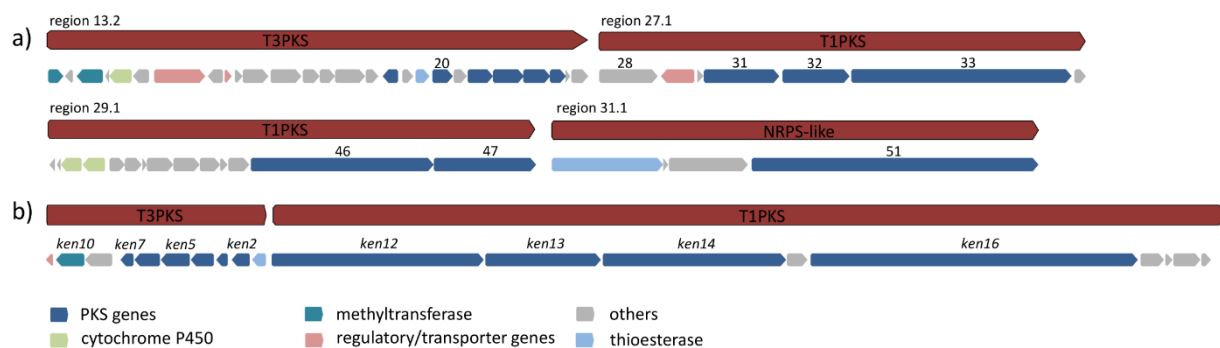
**Figure S5.** <sup>1</sup>H-<sup>1</sup>H COSY NMR spectrum of kendomycin E measured in CD<sub>3</sub>OD (500 MHz).



**Figure S6.** HSQC spectrum of kandomycin E measured in  $\text{CD}_3\text{OD}$  (500 MHz).



**Figure S7.** HMBC spectrum of kendomycin E measured in CD<sub>3</sub>OD (500 MHz).



**Figure S8.** (a) Kendomycin gene cluster in *Streptomyces* sp. Cl58-27. (b) Kendomycin gene cluster in *Streptomyces violaceruber* (strain 3844-33C) identified by Wenzel et al. [1].

## References

1. Wenzel, S.C.; Bode, H.B.; Kochems, I.; Müller, R. A type I/type III polyketide synthase hybrid biosynthetic pathway for the structurally unique ansa compound kendomycin. *ChemBiochem* **2008**, *9*, 2711–2721, doi:10.1002/cbic.200800456.