

Supplementary

Bonsecamin: A New Cyclic Pentapeptide Discovered through Heterologous Expression of a Cryptic Gene Cluster

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Supplementary.

Table S1. Strains, BACs, plasmids and primers used in this work.

Material	Purpose
A. Bacterial strains	
<i>Streptomyces albus</i> Del14	heterologous host [1]
<i>Escherichia coli</i> GB05 RedCC	cloning host [Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS)]
<i>Escherichia coli</i> ET12567 pUB307	alternate host intergeneric conjugation [2]
B. BACs	
2O18	heterologous expression of NRPS cluster
2O18_del1	determination downstream border of NRPS cluster
2O18_del2	determination upstream border of NRPS cluster
2O18_delKR_delbla	single gene inactivation
2O18_delPCP_delbla	single gene inactivation
2O18_delTE2_delbla	single gene inactivation
C. Plasmids	
pUC19	ampicillin resistance marker
D. PCR primer Red/ET	
20200815_1_fw [2O18_del1]	TAGTCCAGCGTCATCAGCGGGCGTCCGAGGCACTGCGGACCACGAGGCGCGTCAGGTGGCAC TTTTCG
20200815_1_rev [2O18_del1]	TCCGACGGCGGGCGGCCCGCACTAGGCTCGCCGCATGACGGACGTCGACTTTTCTACGGGGT CTGAC
20210315_1_fw [2O18_del2]	CTATCGTCGCCACGCCTTGGTGCACGGGAAATCCGGTGTGATGCCGGTGCCGTCAGGTGGCACT TTTTCG
20200815_2_rev [2O18_del2]	CACTGGATGCCAGGCAGGGGGTACGCAGCATGACCGAGGAGGACGCGGCCTTTTCTACGGGG TCTGAC
20201217_1_fw [2O18_delKR_delbla]	GCTGGTGAACCCGCCGTCGACGGTGACCGTGGAGCCGGTCACCTGGCGGGAGTTTAAACCGTC AGGTGGCACTTTTTCG

20201217_1_rev [2O18_delKR_delbla]	GGAGTGCTCACCGCGGGCGCCGCTCGCGGGCAAGGCCGCCGTCATCACGGGTTTAAACGACTTT CTACGGGGTCTGAC
20201217_2_fw [2O18_delPCP_delbla]	GGCCAGGGCGGCCAGTTCGCCAGCCGCGGGATGCGGGTGAGGTCGGTGAAGTTTAAACCGTC AGGTGGCACTTTTCG
20201217_2_rev [2O18_delPCP_delbla]	CGCGCGGTCTGGCAGCAGATCCTGGGGCTGACGGCGGAGGAGATCGGTGGTTTAAACGACTTT TCTACGGGGTCTGAC
20201217_4_fw [2O18_delTE2_delbla]	CAGTTCCGCGGTGCGGGCCGATTGCCGCGCACGAAGTAGTGGCCGCCGAGTTTAAACCGTC AGGTGGCACTTTTCG
20201217_4_rev [2O18_delTE2_delbla]	TGCGTGCCGTATCCGTGCGGGCACCCGGTCAACTTCAAACCGCTGGCCGGTTTAAACGACTTT CTACGGGGTCTGAC

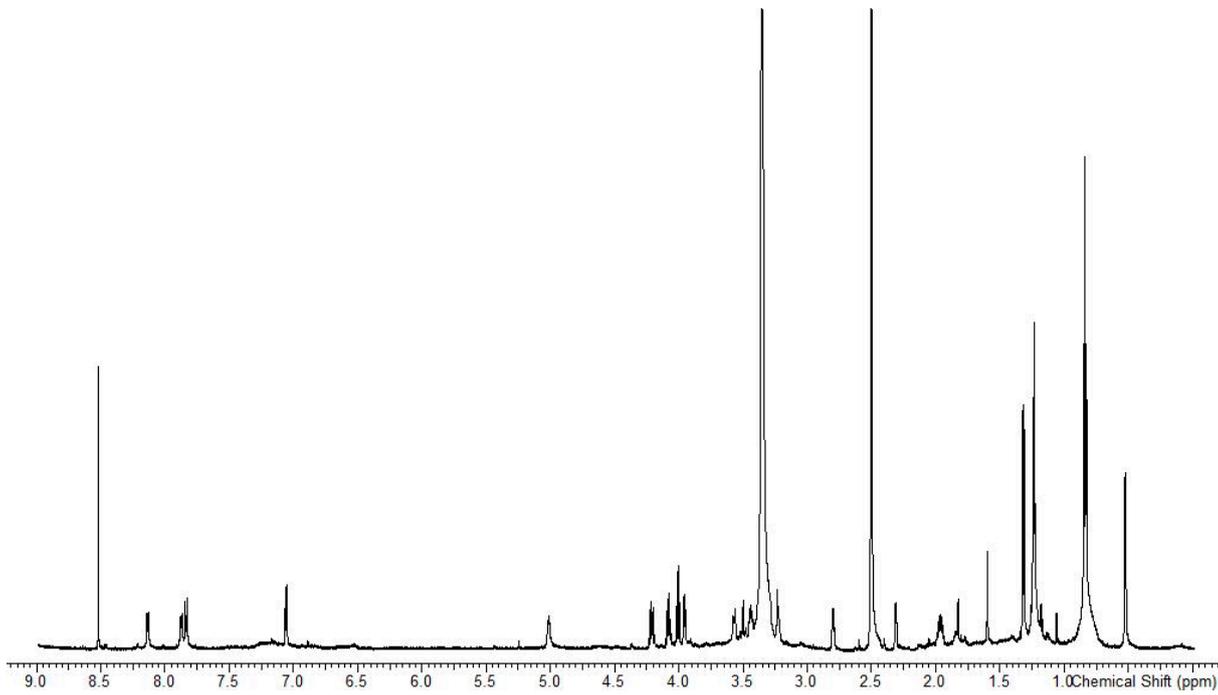


Figure S1: ¹H NMR spectrum (700 MHz, DMSO-*d*₆) of bonsecamin.

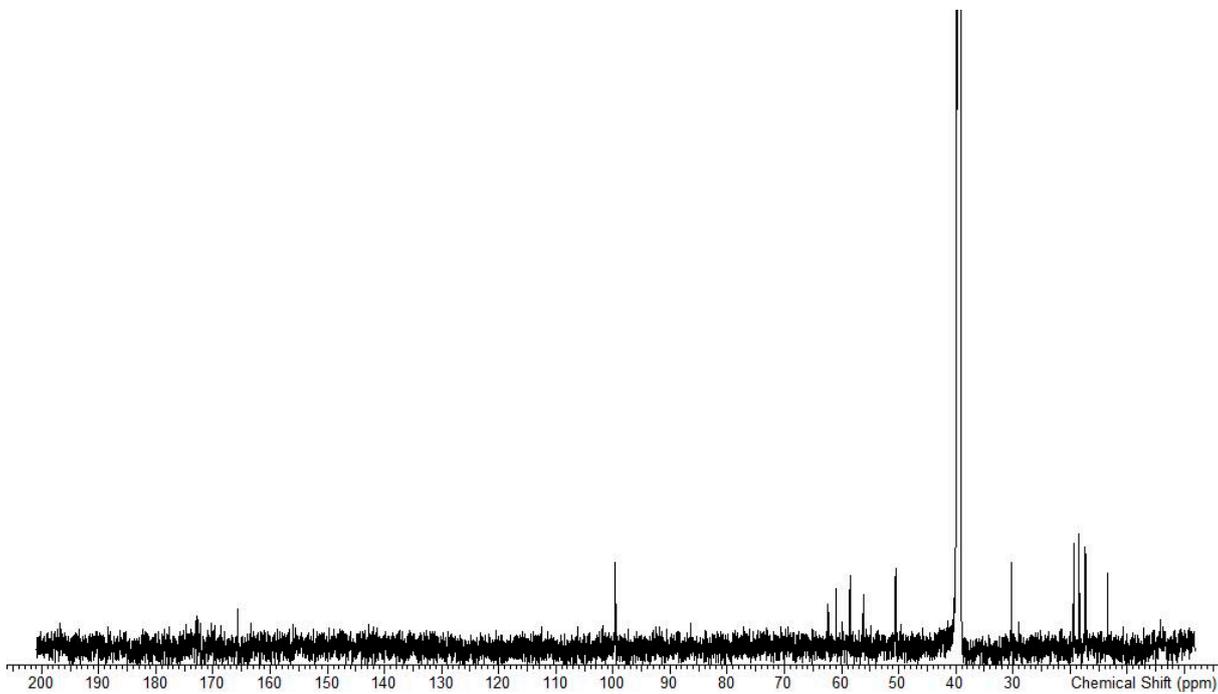


Figure S2: ¹³C NMR spectrum (700 MHz, DMSO-*d*₆) of bonsecamin.

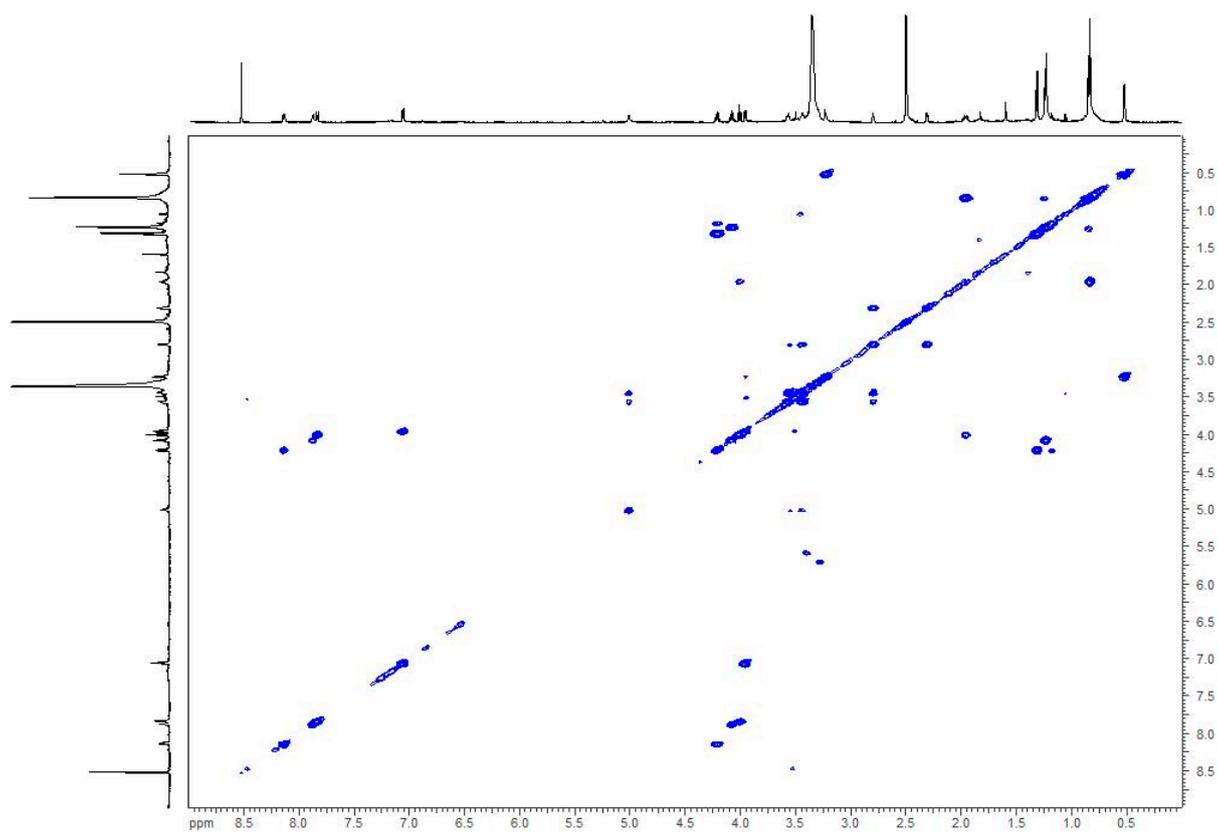


Figure S3: ^1H - ^1H COSY spectrum (700 MHz, $\text{DMSO-}d_6$) of bonsecamin.

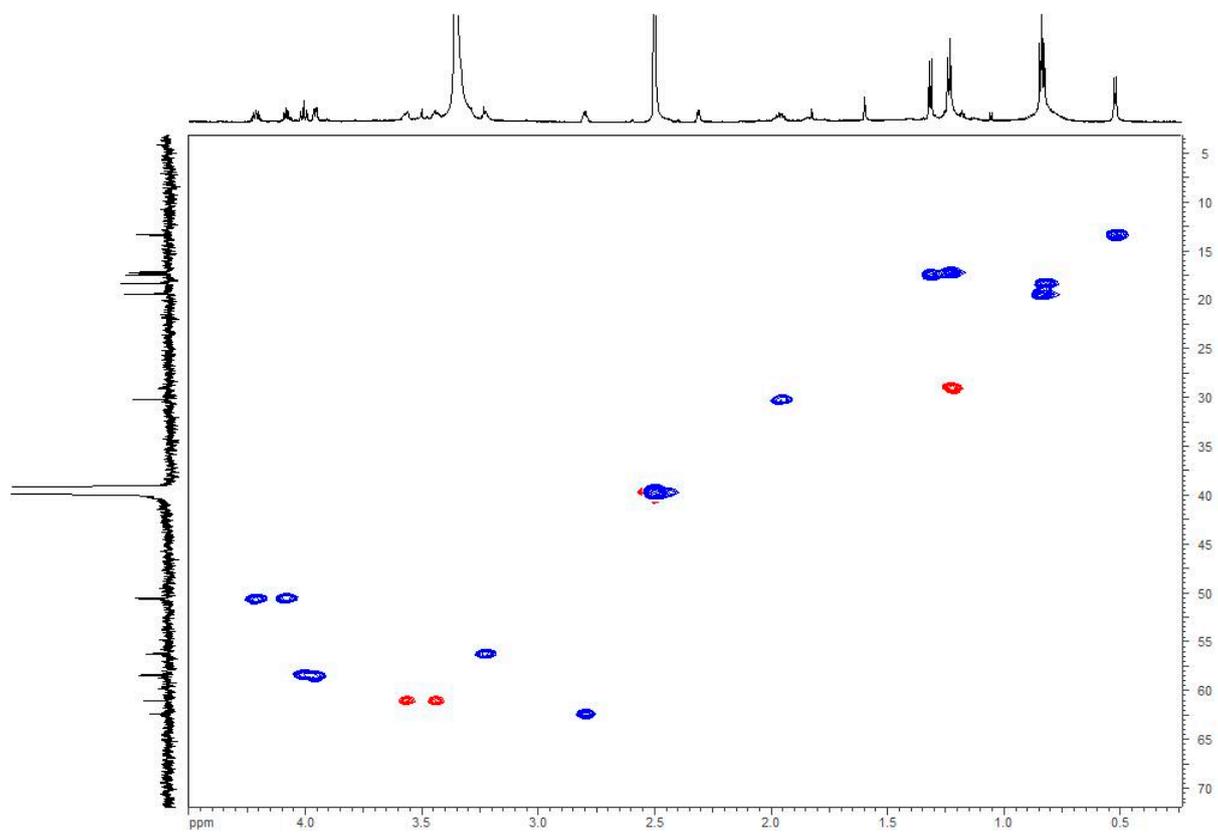


Figure S4: Edited-HSQC spectrum (700 MHz, $\text{DMSO-}d_6$) of bonsecamin.

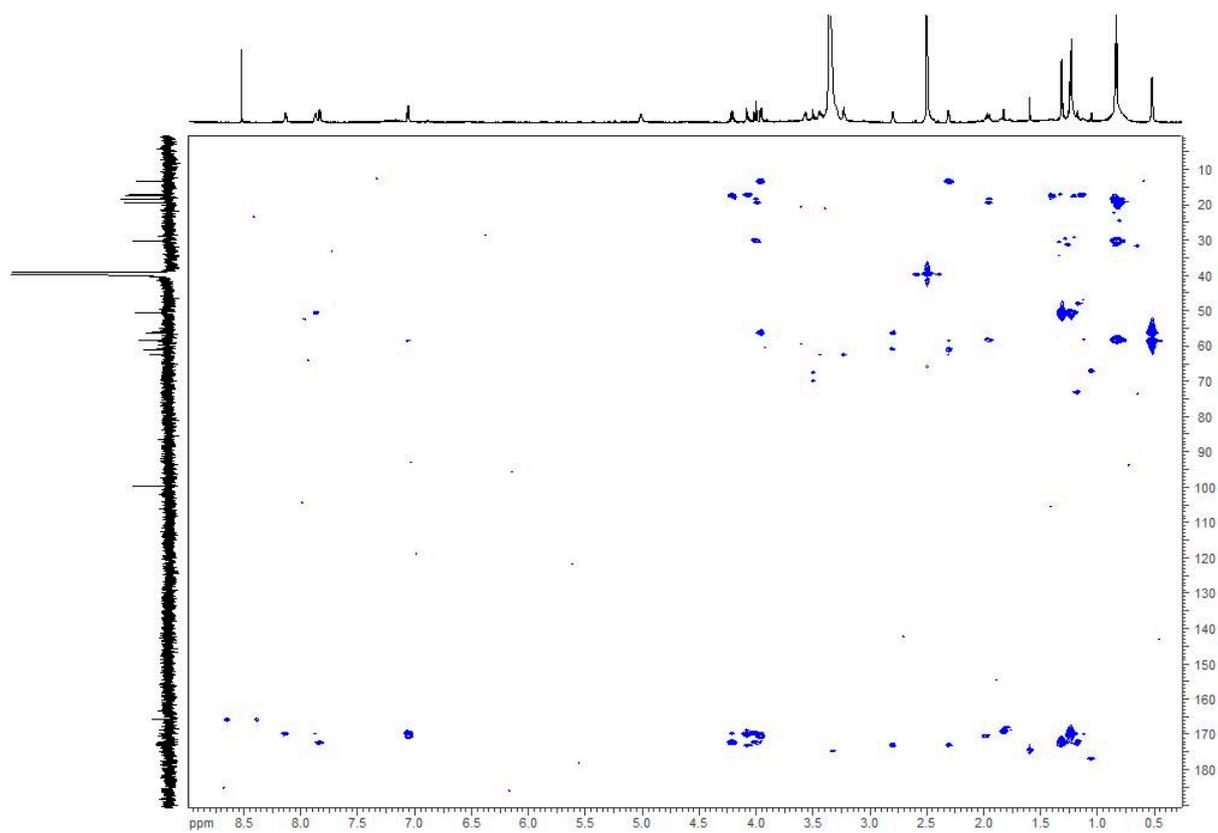


Figure S5: HMBC spectrum (700 MHz, DMSO-*d*₆) of bonsecamin.

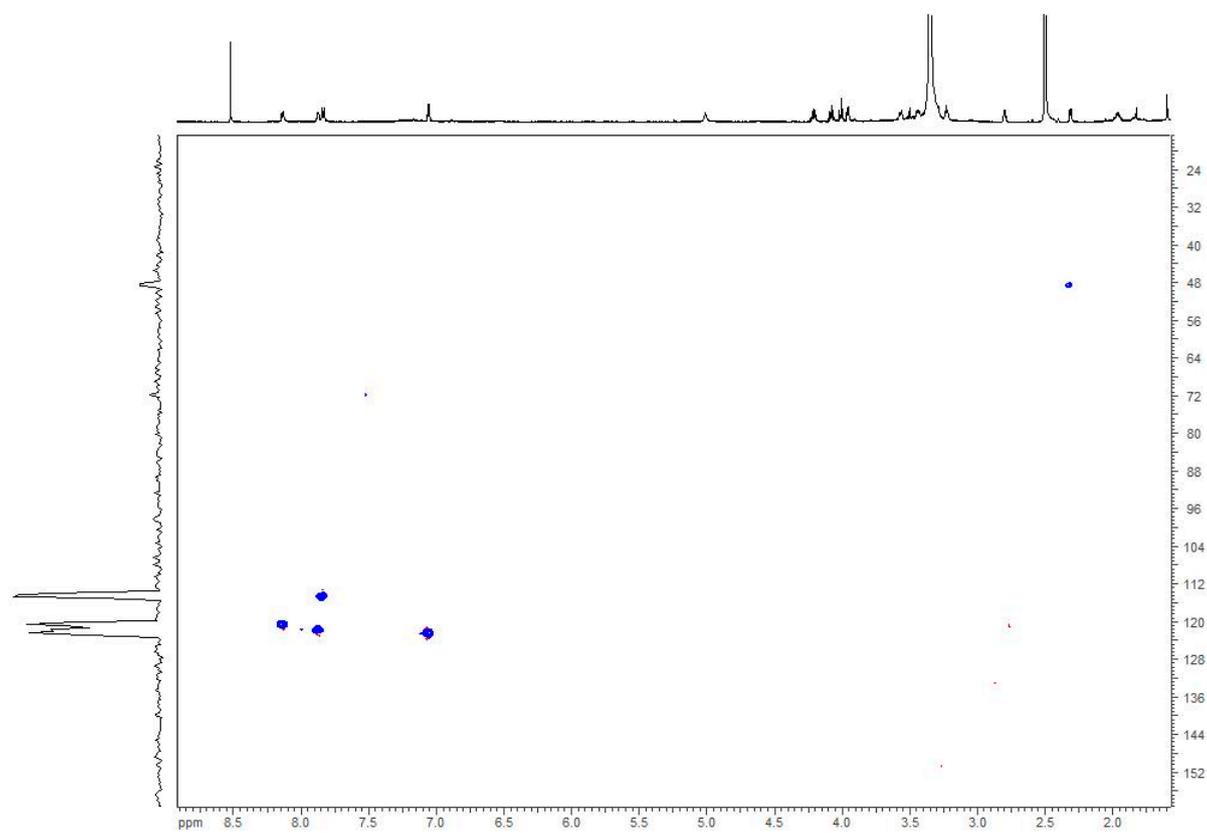


Figure S6: ¹⁵N-HSQC spectrum (700 MHz, DMSO-*d*₆) of bonsecamin.

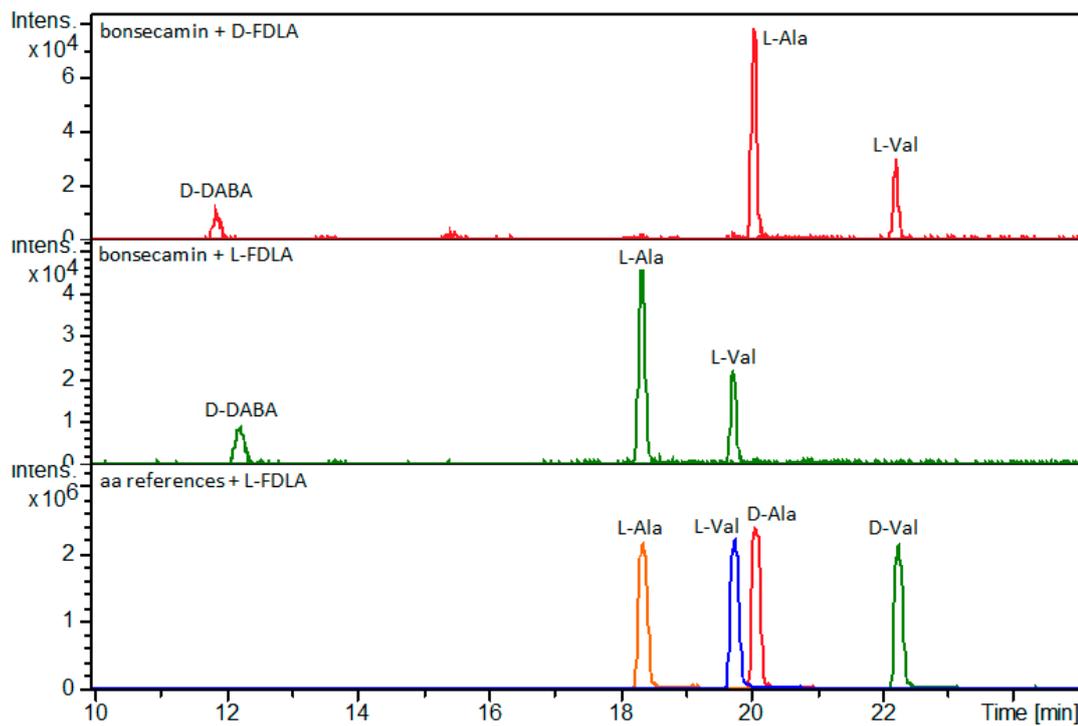


Figure S7: LC-MS chromatograms of hydrolyzed bonsecamin derivatized with D- or L-FDLA and the amino acid (aa) references derivatized with L-FDLA.

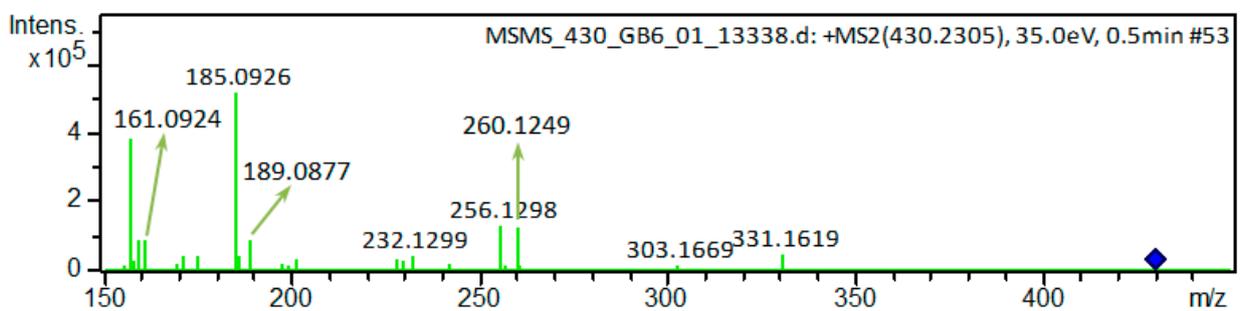
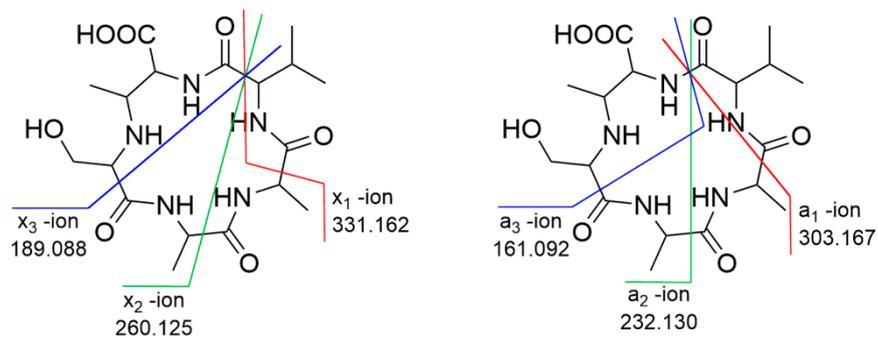


Figure S8: MS/MS fragmentation of bonsecamin.

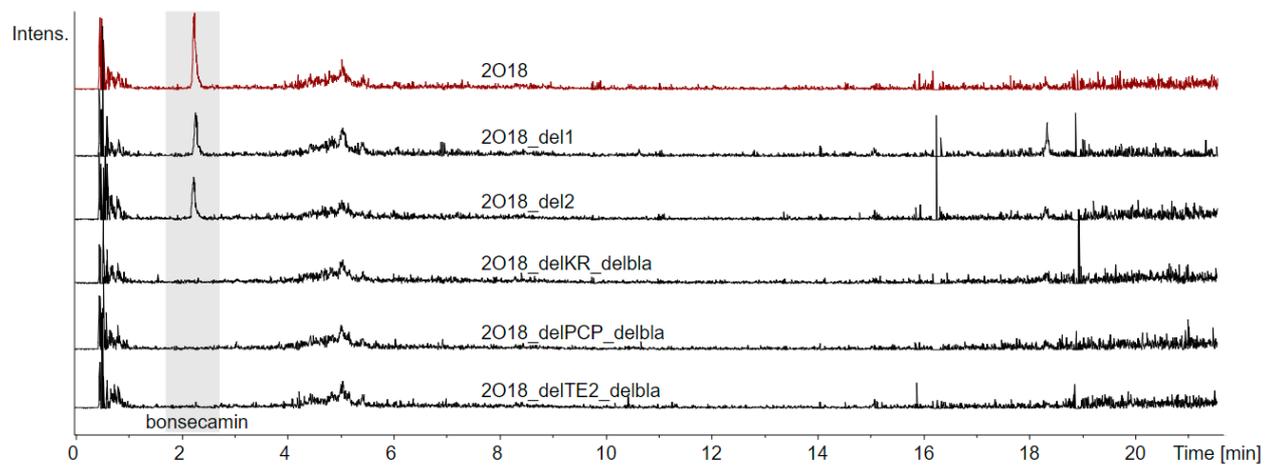


Figure S9. Production of bonsecamin in *S. albus* Del14 mutant after gene deletion experiments. EIC extracted for masses [430-431].

Table S2. Putative products of the genes in the DNA fragment encoding bonsecamin production.

gene #	locus tag	putative product
1	SACHL_05130	Catalase
2	SACHL_05120	hypothetical protein
3	SACHL_05110	Cobalt import ATP-binding protein CbiO
4	SACHL_05100	Cobalt transport protein CbiQ
5	SACHL_05090	Cobalt transport protein CbiN
6	SACHL_05080	Cobalt transport protein CbiM
7	SACHL_05070	-
8 [<i>bonA</i>]	SACHL_05060	enterobactin exporter EntS
9 [<i>bonB</i>]	SACHL_05050	Tyrocidine synthase 3 - val
10 [<i>bonC</i>]	SACHL_05040	Tyrocidine synthase 3 - ser
11 [<i>bonD</i>]	SACHL_05030	Linear gramicidin dehydrogenase LgrE
12 [<i>bonE</i>]	SACHL_05020	(-)-trans-carveol dehydrogenase
13 [<i>bonF</i>]	SACHL_05010	Dimodular nonribosomal peptide synthase - thr
14 [<i>bonG</i>]	SACHL_05000	Alanine-anticapsin ligase BacD
15	SACHL_04990	hypothetical protein
16	SACHL_04980	hypothetical protein
17	SACHL_04970	-
18	SACHL_04960	P-aminobenzoate N-oxygenase AurF
19	SACHL_04950	hypothetical protein
20	SACHL_04940	hypothetical protein
21	SACHL_04930	hypothetical protein
22	SACHL_04920	CGNR zinc finger
23	SACHL_04910	(S)-2-haloacid dehalogenase
24	SACHL_04900	Putative phenylalanine aminotransferase
25	SACHL_04890	All-trans-nonaprenyl-diphosphate synthase (geranyl-diphosphate specific)
26	SACHL_04880	prenyltransferase
27	SACHL_04870	Squalene-hopene cyclase
28	SACHL_04860	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase

- [1] M. Myronovskiy, B. Rosenkränzer, S. Nadmid, P. Pujic, P. Normand, A. Luzhetskyy, Generation of a cluster-free *Streptomyces albus* chassis strains for improved heterologous expression of secondary metabolite clusters, *Metab. Eng.* 49 (2018) 316–324. <https://doi.org/10.1016/j.ymben.2018.09.004>.
- [2] F. Flett, V. Mersinias, C.P. Smith, High efficiency intergeneric conjugal transfer of plasmid DNA from *Escherichia coli* to methyl DNA-restricting streptomycetes, *FEMS Microbiol. Lett.* 155 (2006) 223–229. <https://doi.org/10.1111/j.1574-6968.1997.tb13882.x>.