Supplementary Information

Gene editing enables rapid engineering of complex antibiotic assembly lines.

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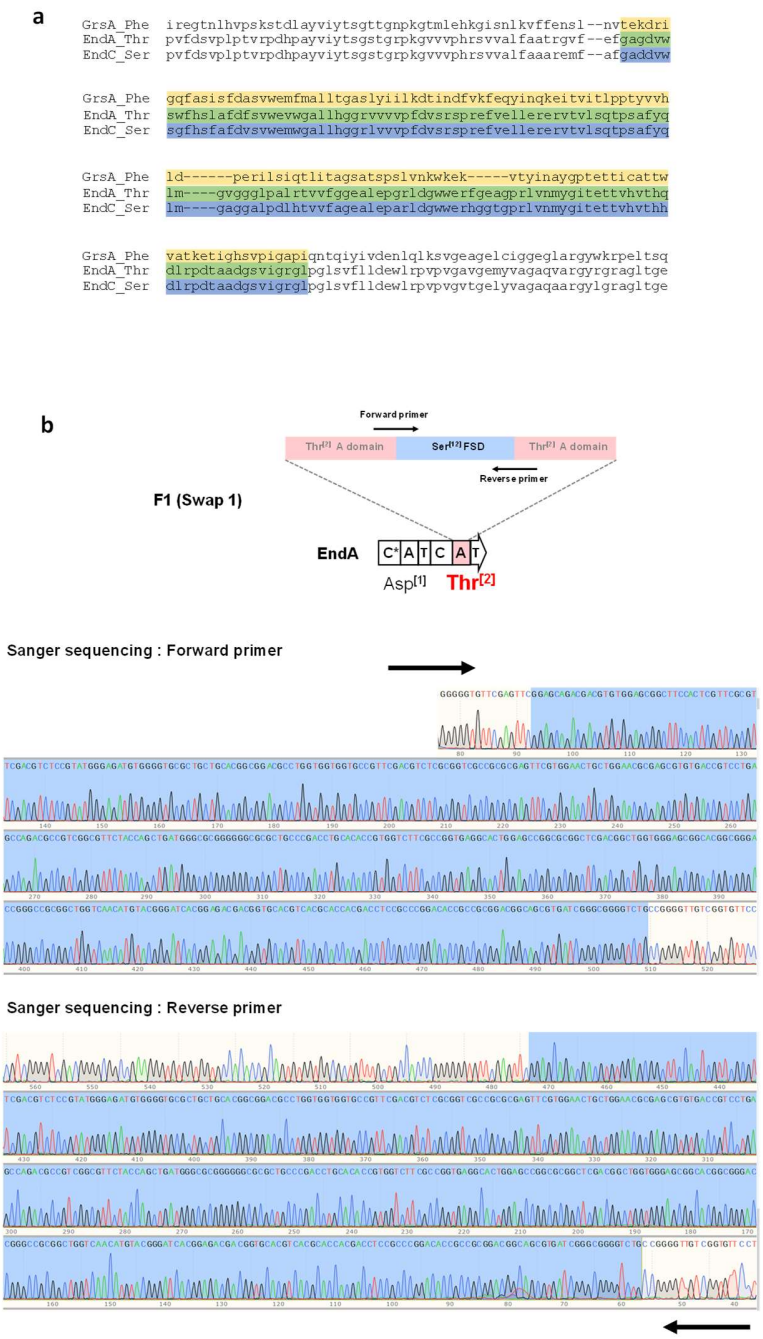
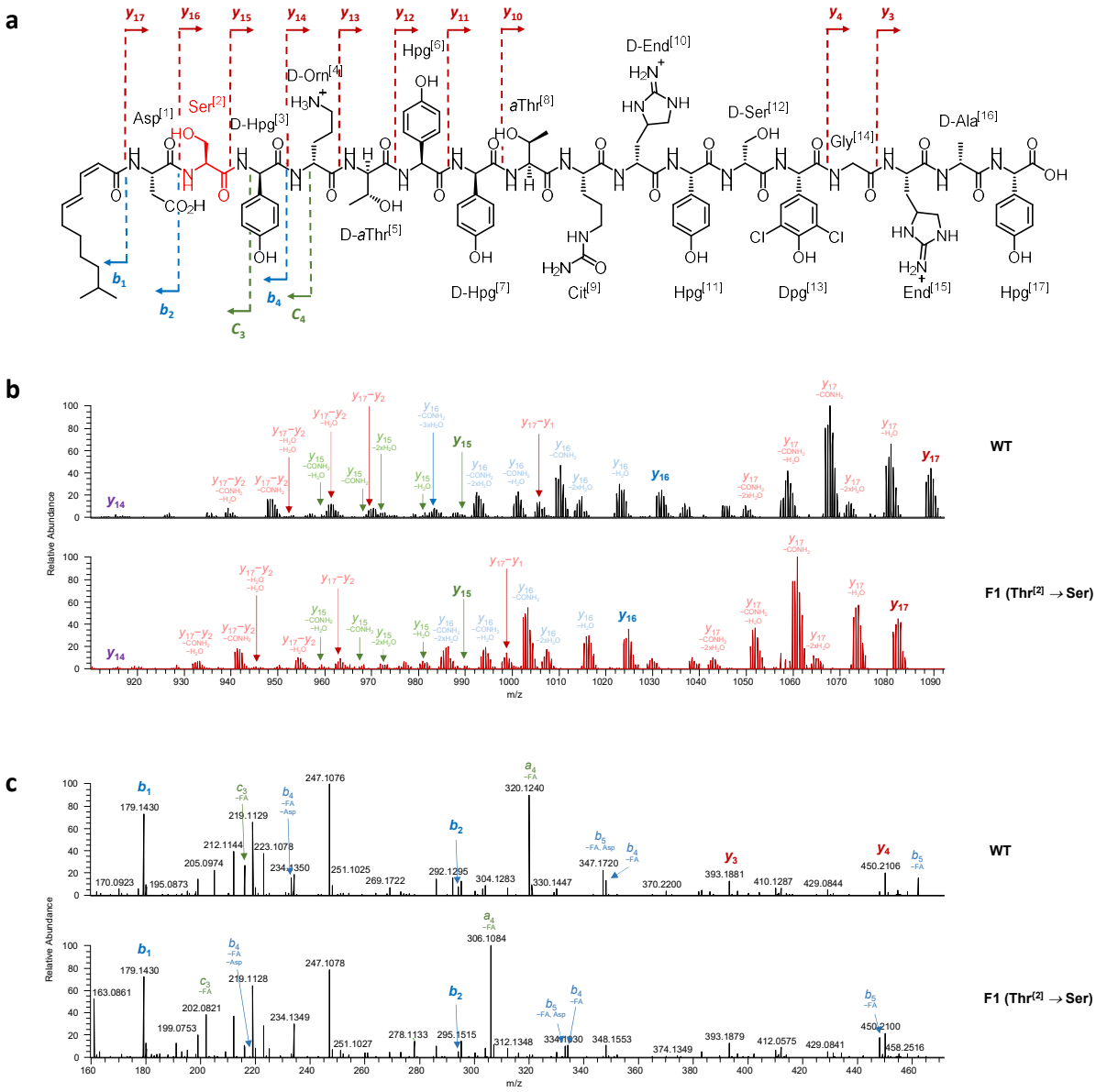
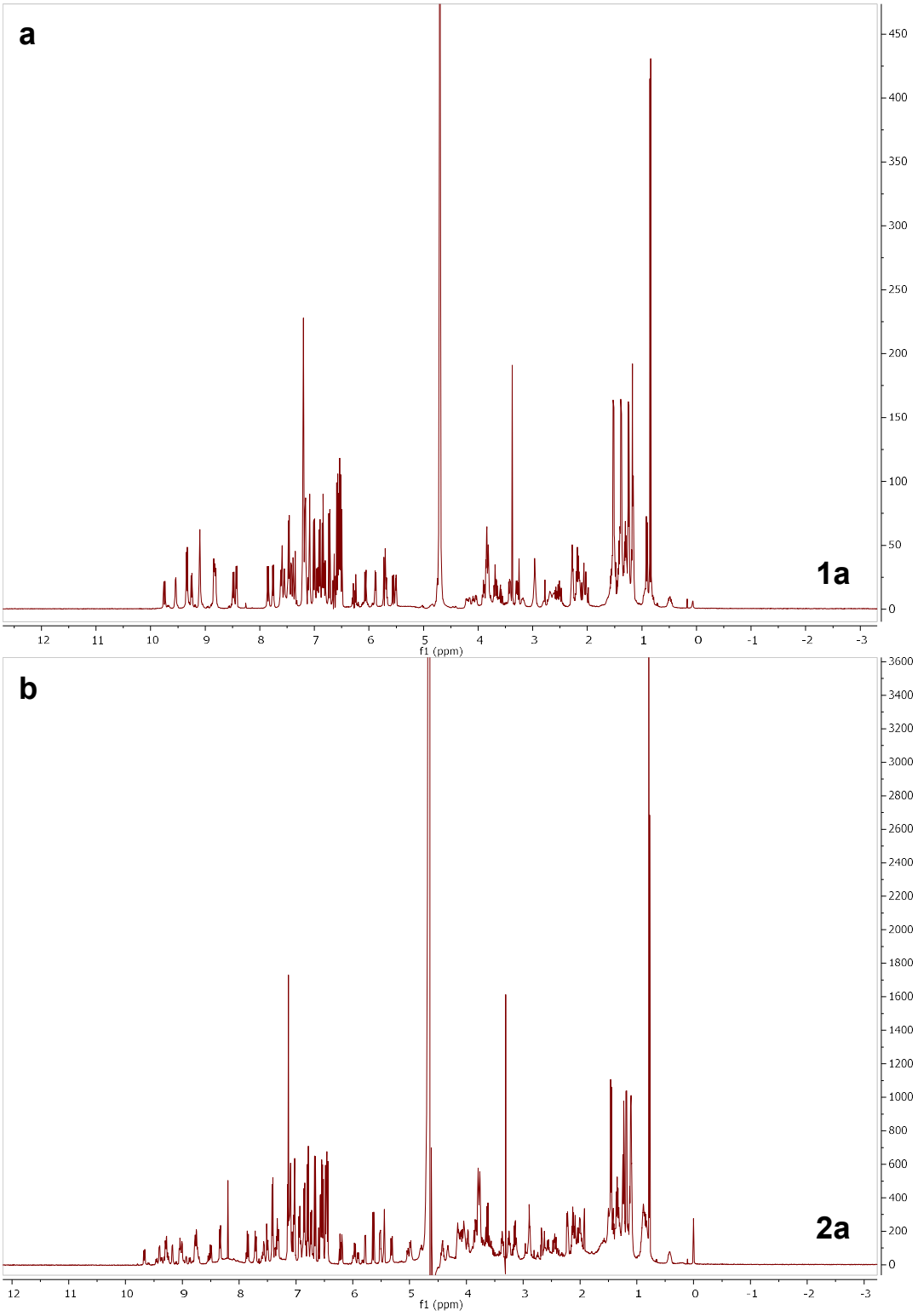


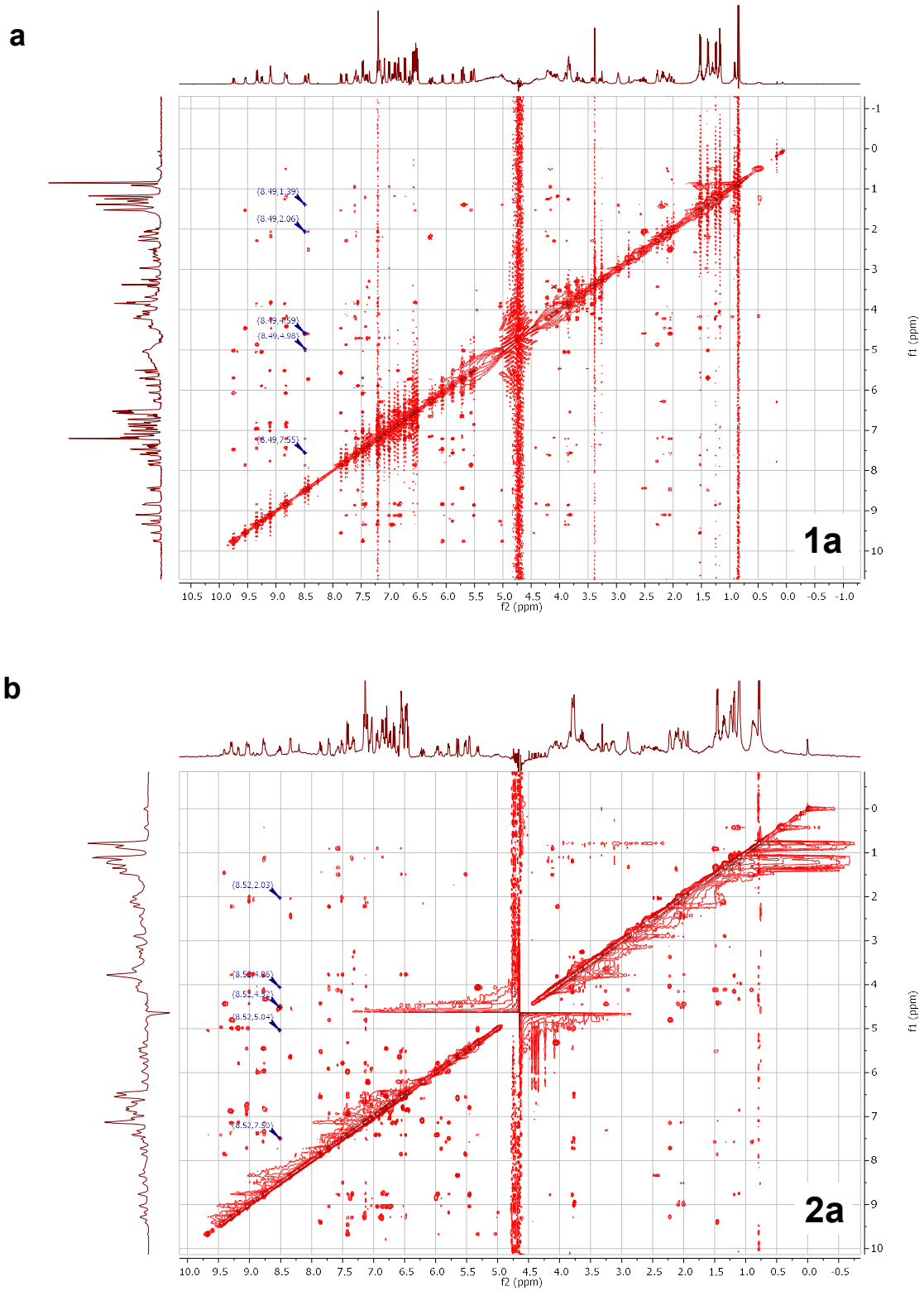
Supplementary Fig. 1. Sequence alignments of the subdomain for the swap 1 constructs. a, Alignment of the GrsA Phe-A domain with the *endA* Thr[2] and *endC* Ser[12] A domains, with the subdomain regions highlighted. **b**, An example sequencing result for the subdomain of mutant F1 showing the swap 1 has occurred.



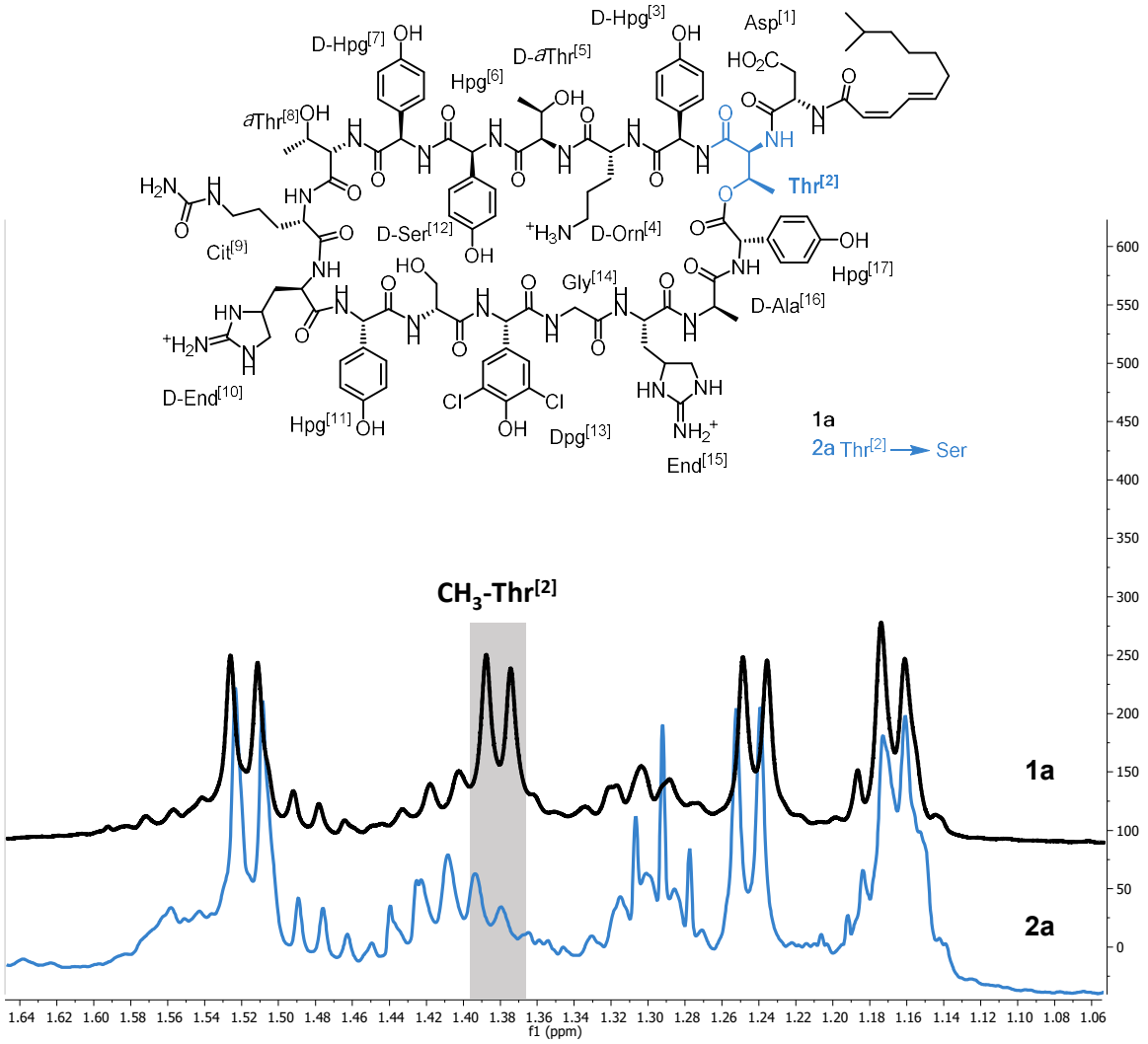
Supplementary Fig. 2. Characterization of 2a produced in this study. a, MS/MS fragmentation observed in **2a**. **b**, MS/MS fragmentation of *y* ions. **c**, MS/MS fragmentation of *b* and *c* ions.



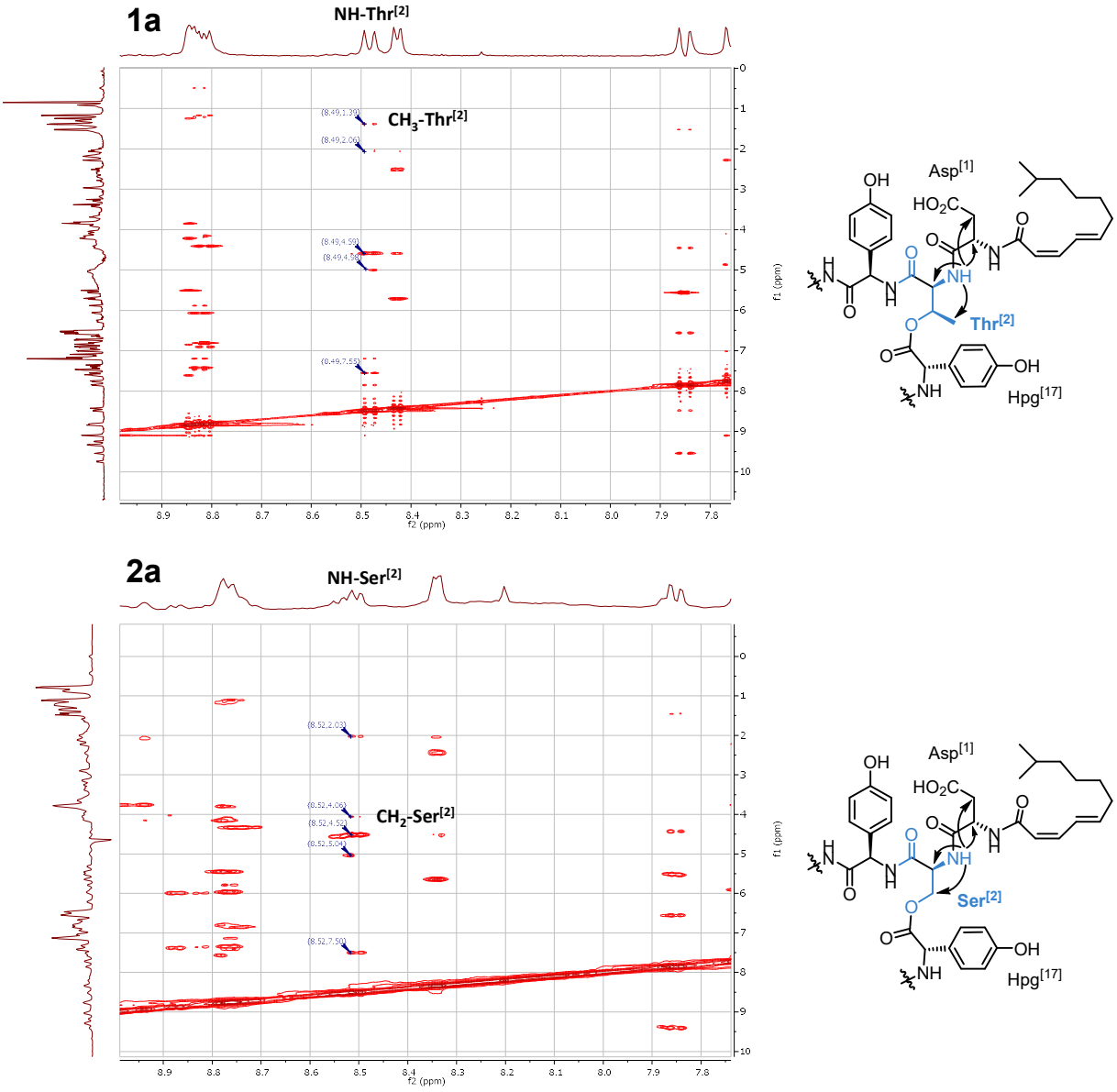
Supplementary Fig. 3. ^{1}H 1D spectra of 1a (a) and 2a (b) in H_2O-D_2O-TFA (9:1:0.05, v/v/v), acquired at 500 MHz.



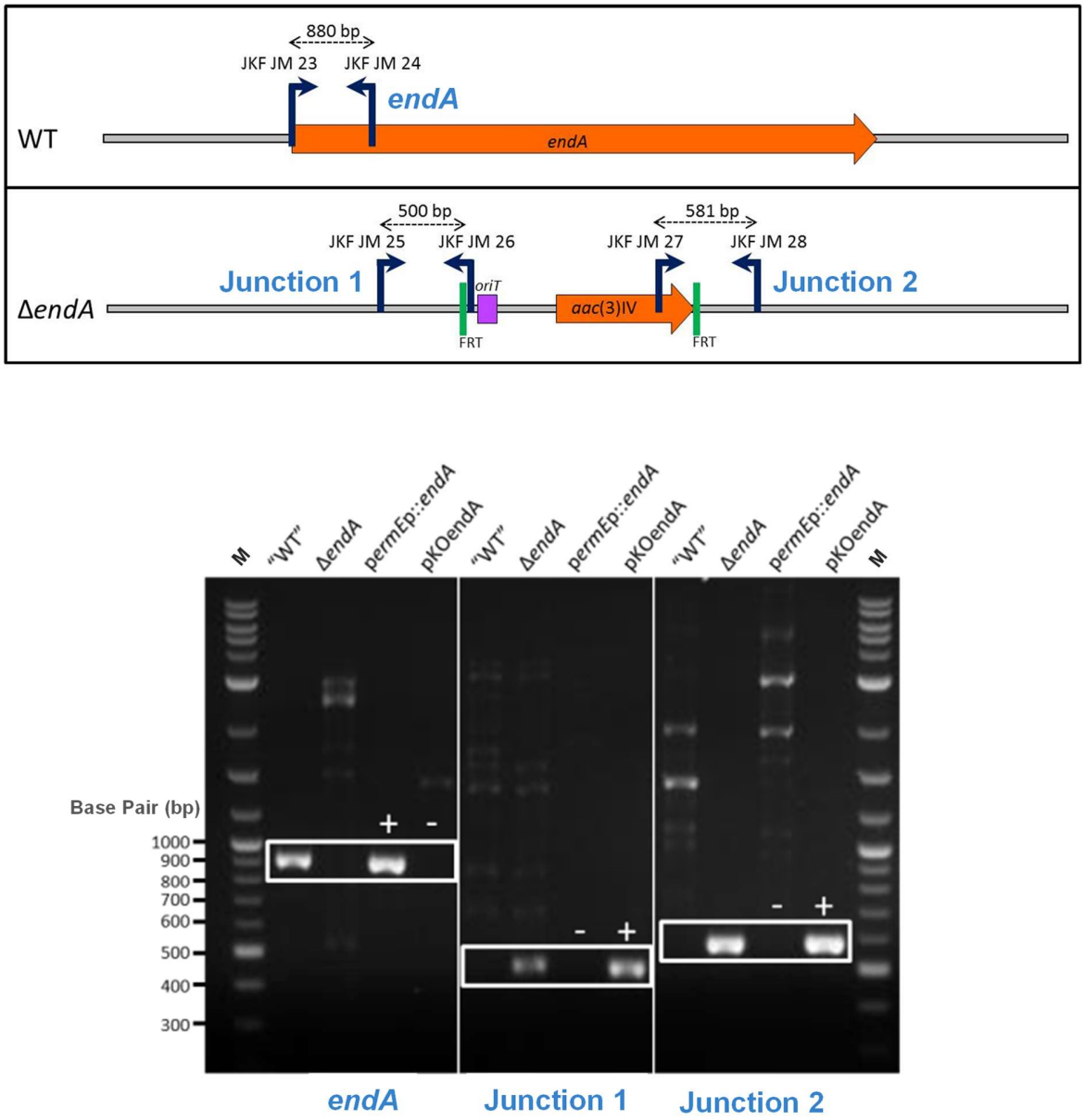
Supplementary Fig. 4. 2D NOESY spectra of 1a (a) and 2a (b) in H_2O-D_2O-TFA (9:1:0.05, v/v/v), acquired at 500 MHz.



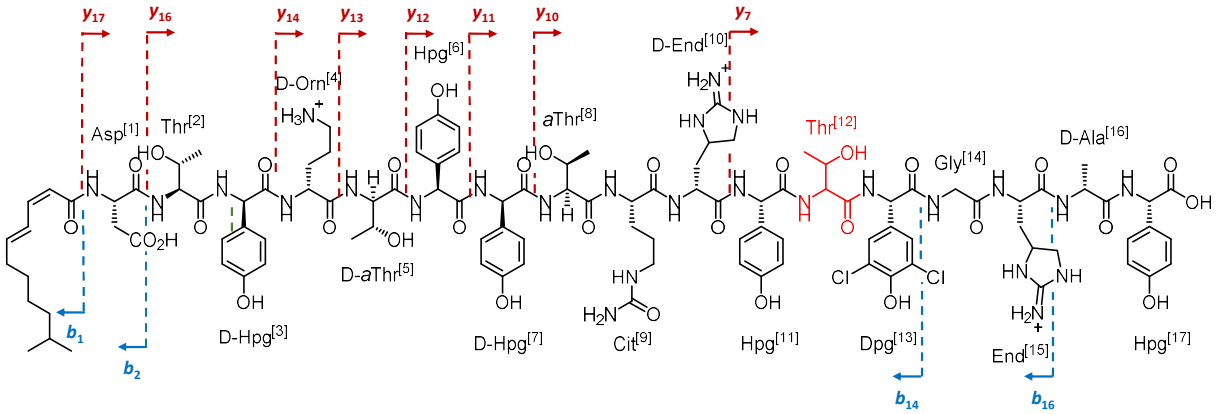
Supplementary Fig. 5. Enlarged section of the ^{1}H NMR spectra of 1a and 2a. Comparison of the proton spectra revealed the loss of a doublet at 1.39 ppm corresponding to the methyl moiety at Thr[2] in **1a**



Supplementary Fig. 6. Enlarged section of the NOESY spectra of 1a and 2a, focusing on the NH moiety of residue 2. Comparison of correlations from the NH moiety revealed the loss of a methyl signal and the appearance of a new signal at 4.06 ppm corresponding to the methylene moiety in **2a.**



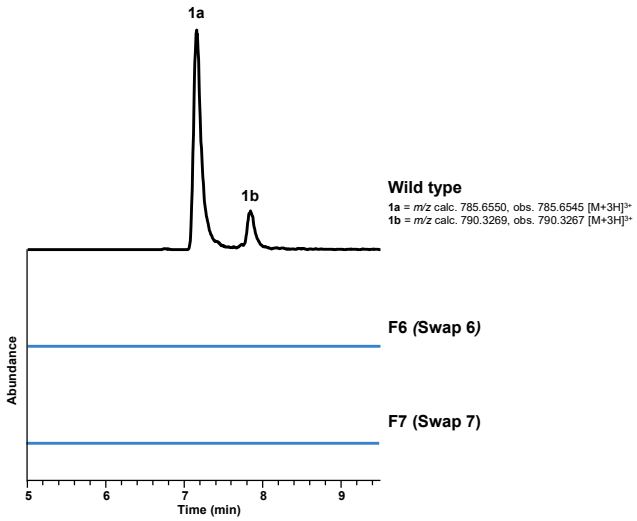
Supplementary Fig. 7. Homologous recombination mediated *endA* gene knock-out and gene complementation. Diagnostic PCR using the indicated primers to determine that *endA* has been replaced by an apramycin resistance cassette (*aac(3)IV*) in the *endA* deletion strain (Δ*endA*) and has been complemented in *endA* complementation strain (*permEp::endA*). Wild type (WT) and plasmid used for *endA* knock-out (pKOendA) were used as controls. This experiment was performed once.



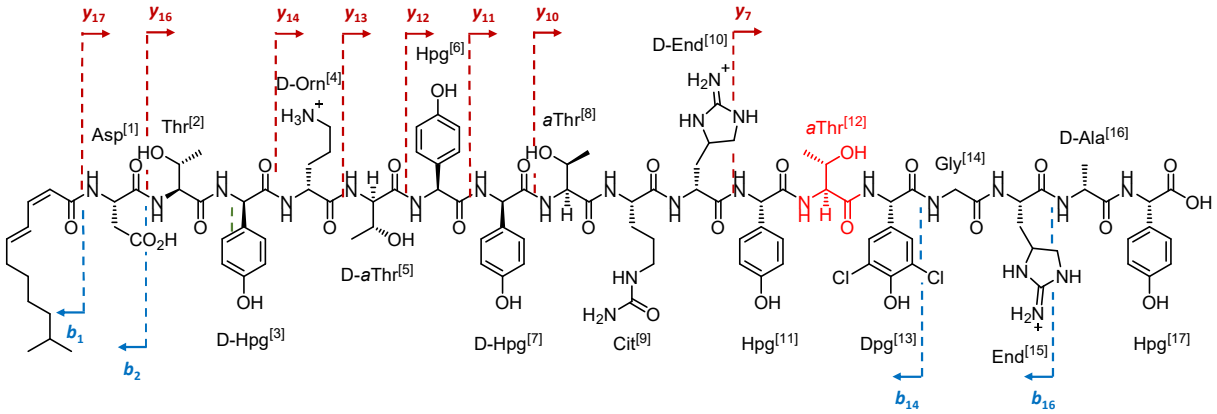
3a (Ser[12] → Thr)

	Calc' [M+H]$^+$	Obs'd [M+H]$^+$	Calc' [M+2H]$^{2+}$	Obs'd [M+2H]$^{2+}$	Calc' [M+3H]$^{3+}$	Obs'd [M+3H]$^{3+}$
Parent					790.327	790.326
y_{17}			1095.919	1095.919	731.280	731.277
y_{16}			1037.904	1137.907	692.272	692.273
y_{14}			921.861	921.862		
y_{13}			864.822	864.824		
y_{12}			814.298	814.302		
y_{11}			739.774	739.778		
y_{10}			666.249	666.257		
y_7	917.275	917.281				
$b_{14\ (-FA,\ -CONH2,\ -H2O)}$			843.329	843.322		
$b_{16\ (-FA)}$			978.887	978.861		
b_2	294.171	294.170				
b_1	179.143	179.143				

Supplementary Fig. 8. LC-HRMS/MS characterization of 3a produced by mutant F2 in this study.

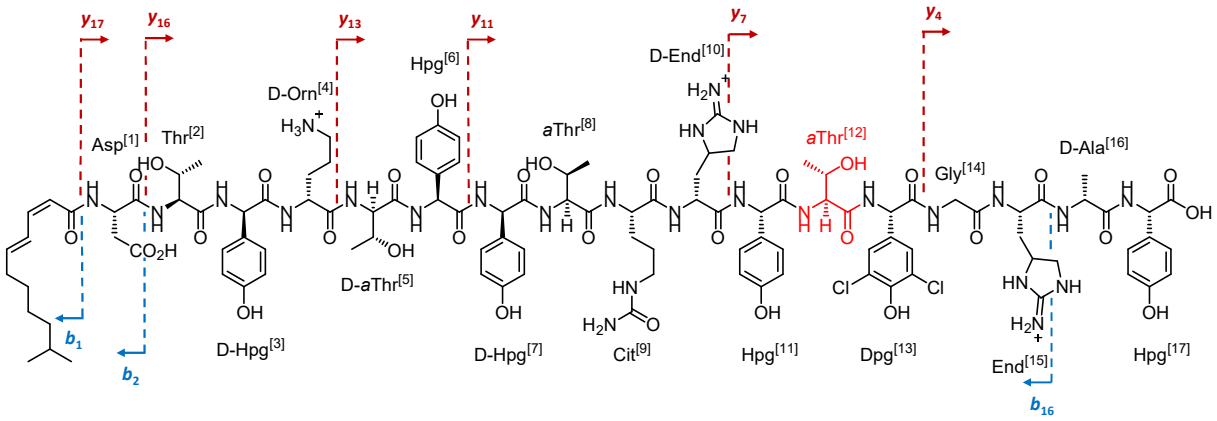


Supplementary Fig. 9. Extracted ion chromatographs from LC-HRMS analysis of extracts from *S. fungicidicus* strains used in this study. No production of enduracidin was observed in both F6 and F7 mutants.



4a (Ser[12] → aThr)						
	Calc' $[M+H]^+$	Obs'd $[M+H]^+$	Calc' $[M+2H]^{2+}$	Obs'd $[M+2H]^{2+}$	Calc' $[M+3H]^{3+}$	Obs'd $[M+3H]^{3+}$
Parent					790.327	790.326
y_{17}			1095.919	1095.920	730.948	730.950
y_{16}			1037.904	1037.906	692.272	692.273
y_{14}			921.861	921.868		
y_{13}			864.822	864.822		
y_{12}			814.298	814.282		
y_{11}			739.774	739.789		
y_{10}			666.249	666.249		
y_7	917.275	917.277				
$b_{14\ (-FA,\ -CONH2,\ -H2O)}$			842.827	842.813		
$b_{16\ (-FA)}$			978.887	978.869		
b_2	294.171	294.171				
b_1	179.144	179.143				

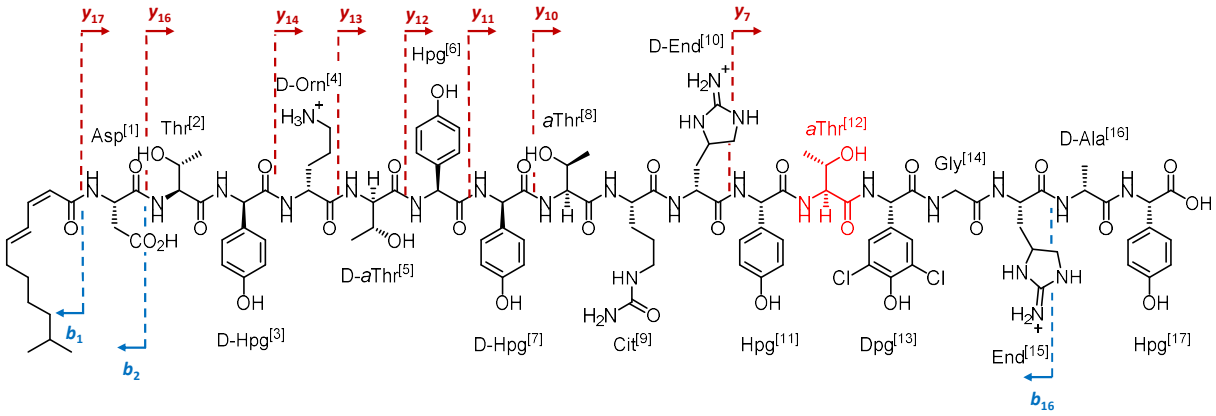
Supplementary Fig. 10. LC-HRMS/MS characterization of 4a produced by mutant F3 in this study.



4a (Ser[12] → *a*Thr)

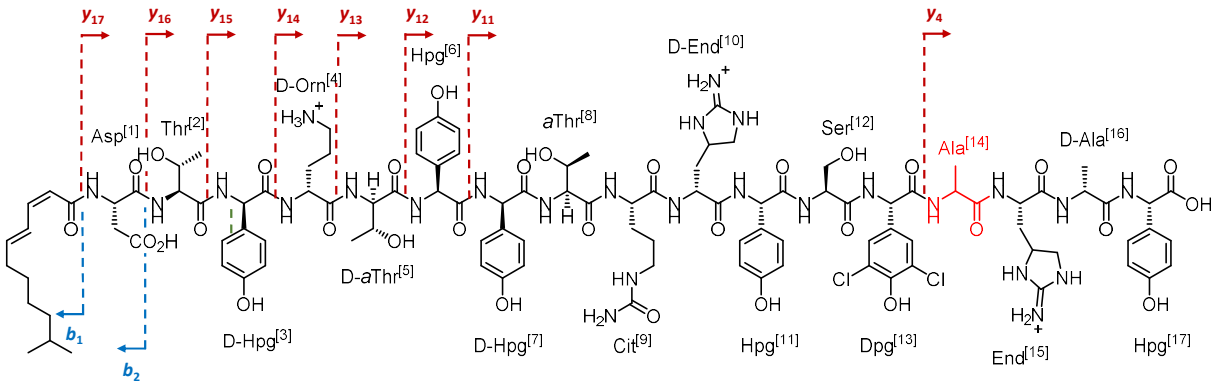
	Calc' [M+H]$^+$	Obs'd [M+H]$^+$	Calc' [M+2H]$^{2+}$	Obs'd [M+2H]$^{2+}$	Calc' [M+3H]$^{3+}$	Obs'd [M+3H]$^{3+}$
Parent					790.327	790.327
y_{17}			1095.919	1095.919		
y_{16}			1037.904	1037.906	692.272	692.271
y_{13}			864.822	864.822		
y_{11}			739.774	739.774		
y_7	917.275	917.270				
y_4	450.210	450.208				
$b_{16\ (-FA)}$			978.887	978.883		
b_2	294.171	294.1701				
b_1	179.144	179.143				

Supplementary Fig. 11. LC-HRMS/MS characterization of 4a produced by mutant F4 in this study.



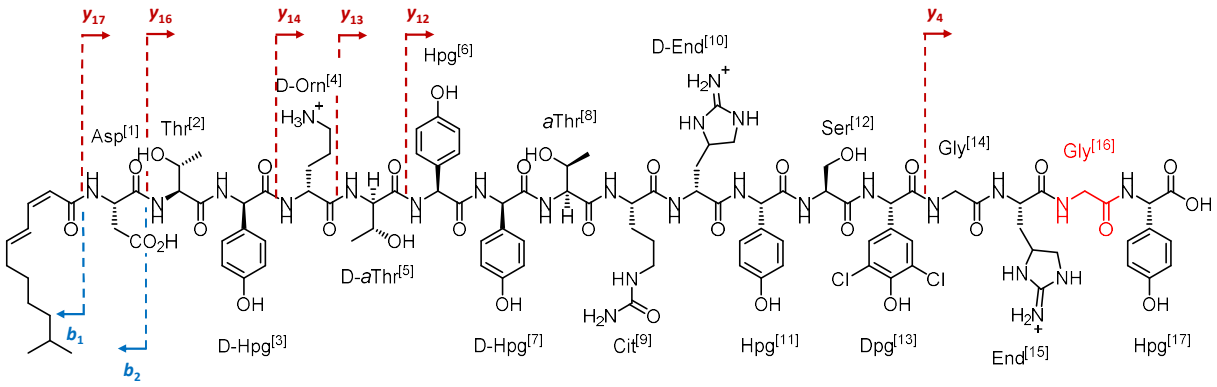
4a (Ser[12] → *a*Thr)						
	Calc' $[M+H]^+$	Obs'd $[M+H]^+$	Calc' $[M+2H]^{2+}$	Obs'd $[M+2H]^{2+}$	Calc' $[M+3H]^{3+}$	Obs'd $[M+3H]^{3+}$
Parent					790.327	790.327
y_{17}			1095.919	1095.919		
y_{16}			1037.904	1037.906	692.272	692.272
y_{14}			921.861	921.856		
y_{13}			864.822	864.823		
y_{12}			814.298	814.301		
y_{11}			739.774	739.776		
y_{10}			666.249	666.254		
y_7	917.275	917.271				
$b_{16\ (-FA)}$			978.887	978.870		
b_2	294.171	294.170				
b_1	179.144	179.143				

Supplementary Fig. 12. LC-HRMS/MS characterization of 4a produced by mutant F5 in this study.



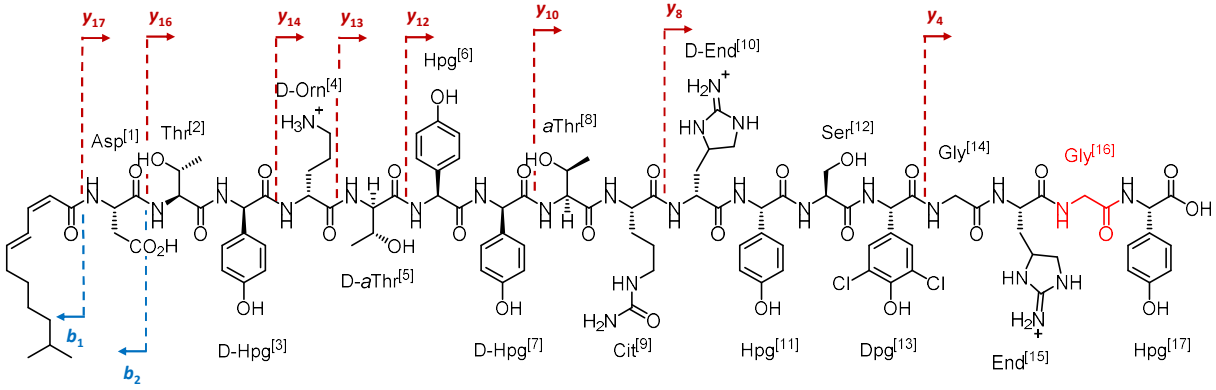
5a (Gly[14]→ Ala)						
	Calc' $[M+H]^+$	Obs'd $[M+H]^+$	Calc' $[M+2H]^{3+}$	Obs'd $[M+2H]^{3+}$	Calc' $[M+3H]^{3+}$	Obs'd $[M+3H]^{3+}$
Parent					790.327	790.328
y_{17}			1095.919	1095.919		
y_{16}			1037.904	1037.906	692.272	692.273
y_{15}			996.385	996.380		
y_{14}			921.861	921.862		
y_{13}			864.822	864.823		
y_{12}			814.298	814.303		
y_{11}			739.774	739.771		
y_4	464.2252	464.2244				
b_2	294.171	294.171				
b_1	179.144	179.143				

Supplementary Fig. 13. LC-HRMS/MS characterization of 5a produced by mutant F8 in this study.



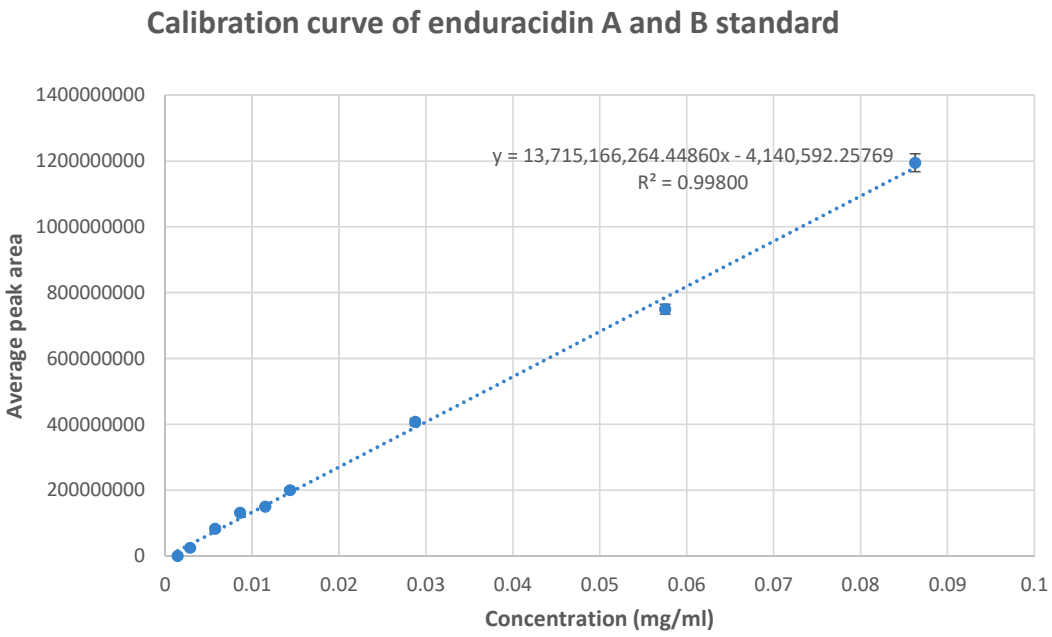
6a (Ala[16] → Gly)						
	Calc' $[M+H]^+$	Obs'd $[M+H]^+$	Calc' $[M+2H]^{3+}$	Obs'd $[M+2H]^{3+}$	Calc' $[M+3H]^{3+}$	Obs'd $[M+3H]^{3+}$
Parent					780.983	780.984
y_{17}			1081.903	1081.904	721.605	721.611
y_{16}			1023.888	1023.891	682.928	682.929
y_{14}			907.846	907.850		
y_{13}			850.806	850.806		
y_{12}			800.282	800.287		
y_4	436.194	436.196				
b_2	294.171	294.170				
b_1	179.144	179.143				

Supplementary Fig. 14. LC-HRMS/MS characterization of 6a produced by mutant F9 in this study.



6a (Ala[16] → Gly)						
	Calc' [M+H]+	Obs'd [M+H]+	Calc' [M+2H]3+	Obs'd [M+2H]3+	Calc' [M+3H]3+	Obs'd [M+3H]3+
Parent					780.983	780.984
y_{17}			1081.903	1081.904	721.605	721.602
y_{16}			1023.888	1023.891	682.928	682.929
y_{14}			907.846	907.841		
y_{13}			850.806	850.808		
y_{12}			800.282	800.289		
y_{10}			651.234	651.242		
y_{8}			522.168	522.168		
y_{4}	436.194	436.195				
b_{2}	294.171	294.170				
b_{1}	179.144	179.143				

Supplementary Fig. 15. LC-HRMS/MS characterization of 6a produced by mutant F10 in this study.



Supplementary Fig. 16. Calibration curve of commercial enduracidin a standard, plotted based on the peak area of the extracted ion chromatogram of m/z 785.6550 $[M+H]^+$. The experiment was performed in triplicate, n=3. Data are presented as mean values +/- standard error. Source data are provided with this paper.

Supplementary Table 1. Bacterial strains and plasmids used in this study.

Strain/Plasmid	Characteristic(s)	Source/ Reference
***Actinoplanes* sp. ATCC 33076**	Wild type strain, Ramoplanin-producing	ATCC
***Streptomyces coelicolor* M145**	Wild type strain, CDA-producing	ATCC
***Streptomyces* sp. DSM 40338**	Wild type strain, Pristinamycin-producing	DSMZ
***Pseudomonas syringae* DSM 10604**	Wild type strain, Syringafactin-producing	DSMZ
***Streptomyces griseolus* NRRL 3739**	Wild type strain, predicted to produce Streptobactin	NRRL
***Streptomyces rochei* NRRL B1559**	Wild type strain, predicted to produce Antimycin and Lipopeptide 8D1-1	NRRL
***Streptomyces rimosus sub. paramomycinus* NRRL 2455**	Wild type strain, predicted to produce Tyrobetaine	NRRL
Streptomyces fungicidicus		
ATCC 21013	Wild type strain, Enduracidin-producing	ATCC
F1	ATCC 21013 derivative with A domain swapping ($Thr^{[2]} \rightarrow Ser^{[12]}$)	This study
Δ*endA*	Δ*endA* deletion mutant of ATCC 21013	This study
Δ*endA*::*endA*	Δ*endA* mutant of ATCC 21013 complemented with *endA* (Δ*endA*::*endA*)	This study
F1a	Δ*endA* mutant of ATCC 21013 complemented with *endA* with A domain swapping ($Thr^{[2]} \rightarrow Ser^{[12]}$)	This study
F2	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow Thr^{[2]}$)	This study
F3	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow$ *Allo*-$Thr^{[5]\_Ramo13}$)	This study
F4	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow$ *Allo*-$Thr^{[8]\_Ramo17}$)	This study
F5	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow$ *Allo*-$Thr^{[12]\_Ramo14}$)	This study
F6	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow Ala^{[16]}$)	This study
F7	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow Ala^{[16]\_Ramo14}$)	This study
F8	ATCC 21013 derivative with A domain swapping ($Gly^{[14]} \rightarrow Ala^{[16]}$)	This study
F9	ATCC 21013 derivative with A domain swapping ($Ala^{[16]} \rightarrow Gly^{[14]}$)	This study
F10	ATCC 21013 derivative with A domain swapping ($Ala^{[16]} \rightarrow Gly^{[14]\_Ramo14}$)	This study
F11	ATCC 21013 derivative with A domain swapping ($Thr^{[2]} \rightarrow Ser^{[1]\_CDAPS1}$)	This study
F12	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow Thr^{[2]\_Streptobactin}$)	This study
F13	ATCC 21013 derivative with A domain swapping ($Gly^{[14]} \rightarrow Ala^{[4]\_Tyrobetaine}$)	This study
F14	ATCC 21013 derivative with A domain swapping ($Ala^{[16]} \rightarrow Gly^{[8]\_Lipopeptide\ 8D1\text{-}1}$)	This study
F15	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow Thr^{[1]\_Antimycin}$)	This study
F16	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow$ *Allo*_$Thr^{[2]_Pristinamycin}$)	This study
F17	ATCC 21013 derivative with A domain swapping ($Ser^{[12]} \rightarrow$ *Allo*_$Thr^{[5]_Syringafactin}$)	This study
Escherichia coli		
DH5α	Host for general cloning	NEB
ET12567 (pUZ8002)	Donor strain for conjugation between *E.coli* and *Streptomyces*	*(1)*
BW25113 (pIJ790)	Strain for λ Red recombination system	*(2)*

Plasmids		
pDWU5	pBluescript KS+ based vector used for gene inactivation and gene complementation in this study	John Innes Centre
pIJ86	Template for ermE* promoter	John Innes Centre
pIJ10700	Template for hygromycin resistance cassette	John Innes Centre
pSET152	Integrative vector for gene complementation in this study	*(3)*
pIJ773	Template for apramycin resistance cassette	*(2)*
pCRISPomyces-2	*E.coli-Streptomyces* shuttle vector for gene inactivation in Streptomyces species	*(4)*
pZY104	pCRISPomyces-1 derivative for A domain swapping ($Thr^{[2]} \longrightarrow Ser^{[12]}$)	This study
pZY150	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow Thr^{[2]}$)	This study
pZY152	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow aThr^{[5]\_Ramo13}$)	This study
pZY153	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow aThr^{[8]\_Ramo17}$)	This study
pZY154	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow aThr^{[12]\_Ramo14}$)	This study
pZY187	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow Ala^{[16]}$)	This study
pZY188	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow Ala^{[16]\_Ramo14}$)	This study
pZY167	pCRISPomyces-1 derivative for A domain swapping ($Gly^{[14]} \longrightarrow Ala^{[16]}$)	This study
pZY163	pCRISPomyces-1 derivative for A domain swapping ($Ala^{[16]} \longrightarrow Gly^{[14]}$)	This study
pZY164	pCRISPomyces-1 derivative for A domain swapping ($Ala^{[16]} \longrightarrow Gly^{[14]\_Ramo14}$)	This study
WL1	pCRISPomyces-1 derivative for A domain swapping ($Thr^{[2]} \longrightarrow Ser^{[1]\_CDAPS1}$)	This study
WL15	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow Thr^{[2]\_Streptobactin}$)	This study
KR20	pCRISPomyces-1 derivative for A domain swapping ($Gly^{[14]} \longrightarrow Ala^{[4]\_Tyrobetaine}$)	This study
AH22	pCRISPomyces-1 derivative for A domain swapping ($Ala^{[16]} \longrightarrow Gly^{[8]\_Lipopeptide\ 8D1\text{-}1}$)	This study
KR19	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow Thr^{[1]\_Antimycin}$)	This study
AH11	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow Allo\_Thr^{[2]\_SnbC}$)	This study
KR14	pCRISPomyces-1 derivative for A domain swapping ($Ser^{[12]} \longrightarrow Allo\_Thr^{[5]\_Syringafactin}$)	This study

Supplementary Table 2. Bioinformatics information for each swap mutant

Mut.	Swap	A- domain	Sd size (aa)	Replacement A-domain	Sd size (aa)	Identity/Similarity (%)	
						A-domain	Sd
F1	1*	Thr[2]	139	Ser[12]	139	92/93	88/89
F1a	1†	Thr[2]	139	Ser[12]	139	92/93	88/89
F2	2*	Ser[12]	139	Thr[2]	139	92/93	88/89
F3	3*	Ser[12]	139	*Allo*-Thr[5] (Ramo13)	138	65/75	71/75
F4	4*	Ser[12]	139	*Allo*-Thr[8] (Ramo17)	143	54/63	55/65
F5	5*	Ser[12]	139	*Allo*-Thr[12] (Ramo14)	138	59/69	65/71
F6	6*	Ser[12]	139	Ala[16]	135	47/60	28/44
F7	7*	Ser[12]	139	Ala[16] (Ramo14)	133	48/61	31/48
F8	8*	Gly[14]	135	Ala[16]	135	75/82	75/83
F9	9*	Ala[16]	135	Gly[14]	135	75/82	75/83
F10	10*	Ala[16]	135	Gly[14] (Ramo14)	134	64/73	64/74
F11	11*	Thr[2]	139	Ser[1] (CDAPS1)	141	46/56	32/40
F12	12*	Ser[12]	139	Thr[2] (Streptobactin)‡	143	60/70	64/72
F13	13*	Gly[14]	135	Ala[2] (Tyrobetaine)‡	144	34/45	29/40
F14	14*	Ala[16]	135	Gly[8] (Lipopeptide 8D1-1)‡	135	53/65	44/59
F15	15*	Ser[12]	139	Thr[1] (Antimycin)‡	146	58/67	67/73
F16	16*	Ser[12]	139	Thr[2] (SnbC)	145	59/70	62/73
F17	17*	Ser[12]	139	*Allo*-Thr[5] (SyfB)	144	50/64	57/69

* Swap *via* CRISPR-cas9 editing
† Swap *via* gene complementation
‡ Predicted natural product and gene cluster
Shaded regions show successful swaps

Supplementary Table 3. Key proton chemical shifts (ppm) that identify each residues in enduracidin **a** and its new analogue.

	Reported-1a*[5]		This study-1a**		This study-2a**	
Residue	NH	CH_α	NH	CH_α	NH	CH_α
Asp[1]	8.14	4.58	8.43	4.5	8.41	4.57
Thr[2]	8.55	4.95	8.48	4.9	8.52 (Ser)	5.05 (Ser)
Hpg[3]	9.56	5.94	9.76	6.06	9.74	6.03
Orn[4]	8.73	4.05	8.82	3.85	8.83	3.86
Thr[5]	7.4	4.39	7.59	4.19	7.58	4.20
Hpg[6]	8.96	6.81	8.82	6.81	8.81	6.81
Hpg[7]	9.05	5.43	9.10	5.88	9.09	5.97
Thr[8]	-	3.77	7.55	3.8	7.56	3.84
Cit[9]	7.64	4.18	7.61	4.2	7.63	4.18
End[10]	7.73	-	7.76	4.8	7.78	4.87
Hpg[11]	9.38	6.94	9.33	6.94	9.33	6.93
Ser[12]	9.45	5.04	9.33	5.05	9.38	5.05
Dpg[13]	8.97	5.72	9.25	5.8	9.24	5.85
Gly[14]	7.73	3.87	8.82	4.2	8.83	4.15
End[15]	-	-	9.10	5.5	9.09	5.52
Ala[16]	9.68	4.38	9.54	4.44	9.46	4.48
Hpg[17]	7.94	5.44	7.85	5.5	7.92	5.59
Side chain	-	5.67	-	5.71	-	5.71

* H_2O-DMSO-*d6* (4:1, v/v)
** H_2O-D_2O-TFA (9:1:0.05, v/v/v)

Supplementary Table 4. Yield of enduracidin (**1a** & **1b**) and engineered variants in each cultivation flasks as estimated based on the standard curve of commercial enduracidin standard.

Production of enduracidin (1a & 1b) and engineered variants (a & b) in mg/L												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Average**	**Error**
WT	4.14	0.51	3.68	1.16	0.50	0.91	2.86	3.30	0.65	2.45	2.02	± 0.45
F1	0.93	2.77	0.49	0.63	2.90	1.78	0.68	2.64	0.45	1.12	1.44	± 0.32
F2	0.65	1.54	0.91	0.95	1.31	1.00	1.87	1.21	0.89	0.80	1.11	± 0.12
F3	0.35	0.36	0.62	0.42	0.43	0.49	0.41	0.50	0.66	0.85	0.51	± 0.05
F4	0.32	0.29	0.33	0.38	0.31	0.27	0.27	0.43	0.40	0.26	0.33	± 0.02
F5	0.48	0.66	0.75	0.61	0.39	0.58	0.55	0.53	0.84	1.01	0.64	± 0.06
F8	0.24	0.32	0.24	0.28	0.36	0.32	0.38	0.39	0.35	0.39	0.33	± 0.02
F9	0.49	0.32	0.43	0.28	0.51	0.38	0.37	0.33	0.59	0.26	0.40	± 0.03
F10	14.55*	1.91	1.41	0.89	15.68*	1.70	1.77	2.97	1.62	0.88	1.64	± 0.22
WT**	1.32	2.66	2.37	-	-	-	-	-	-	-	2.11	± 0.41
F12**	0.31	0.41	0.27	-	-	-	-	-	-	-	0.33	± 0.04
F15**	0.46	0.71	1.83	-	-	-	-	-	-	-	1.00	± 0.42
F16**	0.38	0.31	0.54	-	-	-	-	-	-	-	0.41	± 0.07
F17**	0.87	0.77	0.55	-	-	-	-	-	-	-	0.73	± 0.09

* Statistical outlier

** Distinct batch of additional swap mutants

Supplementary Table 5. Mutations identified via whole genome sequencing

Strain	Position	SNV	Indel	Predicted gene affected	
				Accession number	Description
F1	3272110		C deleted	Intergenic region	
F2	*No mutations*				
F7	1696202-1696204		GAG inserted	WP_004933182.1	Insertion of Leu into iron chelate uptake ABC transporter family permease subunit CDS
	2785247	G to A		WP_121546049.1	Pro to leucine change in a helix-turn-helix domain-containing protein (transcriptional regulator)
	6732973		G inserted	WP_121547643.1	Change in reading frame of arginine deiminase CDS
F8	2862314	A to G		(1): WP_121546097.1 (2): N/A	2 overlapping genes: (1) silent mutation in a phosphatase PAP2 family protein CDS; (2) Lys to Arg in a hypothetical protein
	3299859	T to C		Intergenic region	
	3300293	A to G		WP_121546361.1	Phe to Ser, in a helix-turn-helix domain-containing protein(transcriptional regulator)
	3318273	G to A		WP_121548461.1	Leu to Phe in a hypothetical protein CDS
	3353049	A to G		WP_004930176.1	Asn to Asp in a M23 family metallopeptidase CDS
	3374078	A to G		WP_121546404.1	Glu to Gly in a NAD(P)/FAD-dependent oxidoreductase
	3380245	A to G		WP_004930124.1	Silent mutation in a sigma-70 family RNA polymerase sigma factor
	3391478	A to G		WP_163013272.1	Lys to Glu in a transporter CDS
	3432781	A to G		WP_121546444.1	Leu to Pro in a MFS transporter CDS
	3442305	G to A		WP_121546450.1	Arg to Cys in a molecular chaperone Hsp90
	3793404	G to A		WP_121546666.1	Ala to Val in a thiol reductant ABC exporter subunit CydD
	4550232		T insertion	Intergenic region	
	4550248		G insertion	Intergenic region	
	4984294		C deletion	WP_004926351.1	Change in reading frame of a PhoH family protein
F9	2442344		G inserted	Intergenic region	
	2543897	T to C		WP_121545921.1	Tyr to His in a helix-turn-helix domain-containing protein (transcriptional regulator)
	2747858	G to A		WP-163013249.1	Ala to Thr in a alpha/beta hydrolase CDS
	4097794		G deleted	N/A	Hypothetical protein coding sequence (will shift reading frame)
	4561287	C to T		WP_121547137.1	Glu to Lys in an RNA-directed DNA polymerase CDS
	4728880-4728888		Deletion of 9 bp (TGGACACCA)	WP_004926852.1	Deletion of 3 amino acids (Asp, Thr, Met) in a sucC gene
	5305688	T to C		WP_048459195.1	Start codon of a GntR family transcriptional regulator gene changed to a Thr
F10	2442339		G insertion	Intergenic region	
	5527161		C insertion	WP_121547644.1	Insertion (frame shift) in argF gene (ornithine carbamoyltransferase)

Supplementary Methods

Sequences of subdomains for Swaps 12-15

>FSD Thr[2] streptobactin*
ggggccgaggacgtctggacgctgttccactcctacgccttcgacttctcggtctgggagctgtggggtgccctgct
gcacggcggccggctggtcgtcgtcccgcacctgatcagccgcgacccggcggccttcctggagctgctcgcccgcg
agcgggtcaccgtgctcaaccagacgccgtccgccttctaccagctcagcgccgccgaccgggaccgccccggcagc
gaactcgccctgcgctacgtggtgttcggcggtgaggcgctcgaactcgcccggctggacgactggtacgaccggca
cgccgagcacgcccccacgctggtcaacatgtacggcatcaccgagaccaccgtgcacgtctcctacatcgccctgg
accgggcgagcgccgccgccgggagcagcagcaccatcggcgtc

>FSD Gly[14] tyrobetaine*
ggccccggctgccgcatgtcgcagaccttcggcctgaccttcgacccgtccgtgtacgacctgttcgtcgcgtgggg
ctccggcgccaccgtcgtcgtcccggacaaggacgagctgtaccggcccgtggactacgtggtacgccgccgcctca
cccactggttctccgtaccgtcgctggtcaccaccgccgagcggtacggcaatctgccgcctggccgcgtcaccacg
ctgcgccacagctgcttcagcgcggagccggtcaccgcgcagctgaccgcgcgctggcacgaggtcgcgcccggcag
ccgtatccacaacctgtacgggccgaccgaactgaccatcacctgcaccgaccacgaactcgccgacggcggagccg
ccgcggcgggcagcgccaacggcacggtgcctgtcggccggccgtac

>FSD Ala[16] lipopeptide 8D1-1*
accgacggcgaccggctgctcgccgtgaccacggtgggcttcgacatcgcgggtctggagatcttcctgccgctgct
gcacggggcggtgctggtgctcgcggacgaggagaccgcgaaggacccgcacgcgctgctgcgtgccgtgaccgcgt
cgggaatcaccatggtccaggcgacaccgagcctgtggcagggcgtcggggccgtggccgcggacgagctggccgga
gtacgcgtcctggtgggtggggaggcgctgccgcacgagctggcgcgcacgctgacggagcgggcccgttccgtgac
gaacctctacggtccgaccgaggtgacgatctgggccaccgccgccgacgtcgccgagtccgggccggtcatcggcg
tgccgctcgccaacacgtcg

>FSD Thr[1] antimycin*
cgggccgacgacgtgtggacgctgttccactcgtacgccttcgacttctccgtctgggagatctggggcgccctgct
gcacggcggccgggtcgtcgtcgtcccgtacgccgtcagccgctcgccccgggagttcctggagctggtgcaccgcg
agggggtgaccgtcctcagccagaccccgtccgcgttcgagcagctcgtcgacgccgacctggagcggggcggggac
gccggggcgctgcgccacgtcgtcttcggcggcgaggccctgcgcccggcacggctgcgcccctgggccgaccgcca
cggcctggaccgcccggcgctggtgaacatgtacggcatcaccgagaccaccgtgcacgtcacccaccaccgcctca
cccgcgccgacctggacgacccgcgccgaggcagcgtcatcggcacccccctc

*Natural products are predicted using anti-SMASH

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