Synthetic Zippers as an Enabling Tool for Engineering of Non-Ribosomal Peptide Synthetases

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Supplementary Information - Table of Contents

1 Material and methods

1.1 Cultivation of strains

All *E. coli* DH10B::*mtaA*, *Xenorhabdus szentirmaii, Xenorhabdus nematophila* and *Photorhabdus luminescens* cells were cultivated in liquid or on solid LB-medium (pH 7.5, 10 g/L tryptone, 5 g/L yeast extract and 5 g/L NaCl). Solid media contained 1% (w/v) agar. Kanamycin (50 µg/ml), chloramphenicol (34 µg/ml) and spectinomycin (50 µg/ml) were used as selection markers. All *E. coli* cells cultures were cultivated at 37 °C and at 22 °C for peptide production purposes. *Xenorhabdus* and *Photorhabdus* strains were grown at 30 °C.

1.2 Cloning of biosynthetic gene clusters

Genomic DNA of selected *Xenorhabdus* and *Photorhabdus* strains were isolated using the Qiagen Gentra Puregene Yeast/Bact Kit. All PCRs were performed with oligonucleotides obtained from Eurofins Genomics (Supplementary Table 4). NRPS fragments for Hot Fusion cloning (*1*) were amplified with primers coding for the respective homology arms (20-30 bp) in a two-step PCR program. The coding sequences for the SYNZIPs were also attached upstream or downstream to the NRPS genes by PCR. In the following, the cloning procedure for the basic vectors is explained. pJW61/62 was obtained by the following steps: First, the SYNZIP17/18 coding sequences (pENTR-SYNZIP17/18 (*2*) were a gift from Amy Keating, Addgene plasmids #80671/80672; RRID:Addgene_80671/80672) were inserted into the plasmids pCOLA_ara/tacI and pCK_0402 by oligonucleotides KB-pACYC-FW/RV or KB-pCOLA-FW/RV in two-step polymerase chain reactions (PCRs) combined with Hot Fusion Cloning (*1*). Second, these plasmids were linearized by single-step PCRs with the help of the oligonucleotides KB-pCOLA-II-FW/RV or KB-pACYC-II-FW/RV, which further allowed us to introduce NRPS fragments by Hot Fusion cloning. Therefore, the respective NRPS coding sequences were amplified again in two-step PCRs, using oligonucleotides with additional coding regions for homology arms (20-30 bp). pJW63/64 coding for subunits of the XtpS without attached SYNZIPs were generated by amplifying pJW61/62 with a single phosphorylated [phos.] oligonucleotide pair excluding the SYNZIP coding region followed by T4 DNA ligation (following Thermo Fisher manufacturers' instructions). The control plasmids pCOLA_ara_xtpS/gxpS_tacI_JW coding for the native single protein xtpS/ gxpS were created by Hot Fusion Cloning. Therefore, the plasmid pCOLA_ara/tacI was linearized by PCR using the oligonucleotides AL-XtpS-2-1 and AD64 and the insert *xtpS* was PCR amplified with the oligonucleotides jw0136_FW and jw0137_RV.

The plasmids pJW101/102 coding for NRPSs with two attached SNYZIPs were created by Hot Fusion cloning. Before this final cloning step an pCOLA_ara/tacI plasmid carrying the SYNZIP18 sequence downstream the P_{BAD} promoter was linearized. This linearization step by PCR was done twice and allowed us to incorporate the SYNZIP1 coding region (na28_FW, na29_FW) upstream the stop codon (pQLinkHD-SYNZIP1 was a gift from Amy Keating (*2*), Addgene plasmid #80647; RRID:Addgene_80647).

The starting point for plasmid pJW103/106 was vector pCDF_ara/tacI. This vector was generated by digesting the plasmids pCOLA_ara/tacI and pCDFDuet™-1 (Novagen) with the enzymes XbaI and NdeI. The fragment of pCDFDuet™-1 carrying the pCloDF13 replicon and streptomycin/spectinomycin resistance marker was then T4 ligated with the compatible pCOLA_ara/tacI fragment. Then, this plasmid pCDF_ara/tacI was linearized in two cycles including in parallel the sequence of SYNZIP2 (na32_RV, na33_RV) (pQLinkHD-SYNZIP2 was a gift from Amy Keating (*2*), Addgene plasmid #80658; RRID:Addgene_80658) downstream the P_{BAD} promoter, followed by the incorporation of the respective NRPS coding regions by Hot Fusion Cloning.

The plasmid pCOLA_ara_ gxpS_tacI_JW was generated in two Hot Fusion Cloning steps. First, the pCOLA_ara/tacI was linearized by PCR using the primers JW_tacI_PstI_FW2 and jw0064_RV and second the first part of *gxpS* was amplified using the oligonucleotides jw0124_FW/jw0160_RV. This intermediate plasmid was then opened with PstI and the second *gxpS* part, amplified with jw0151_FW/ jw0161_RV by PCR, was then integrated into the cleaving site by Hot Fusion Cloning.

In all PCRs the S7 Fusion High-Fidelity DNA Polymerase (Mobidiag) was used according to the manufacturers' instructions. The amplified DNA was purified with the Invisorb Fragment CleanUp or MSB Spin PCRapace Kits (stratec molecular). The basic cloning of all new generated plasmids (Supplementary Table 3) was performed in *E. coli* DH10B. Each NRPS (subunit) was under the control of a P_{BAD} promotor. Plasmid isolation from *E. coli* was achieved with the Invisorb Spin Plasmid Mini Two Kit (stratec molecular). Restriction enzyme digests and the partial sequencing of essential plasmid regions, especially upstream and downstream of the NRPS genes, where the SYNZIP coding sequences were located, confirmed the correct plasmid construction.

1.3 Heterologous expression of NRPS templates and LC-MS analysis

Constructed plasmids were transformed into *E. coli* DH10B::*mtaA*. Cells were grown overnight in LB medium containing the necessary antibiotics (50 µg/ml kanamycin, 34 µg/ml chloramphenicol, 50 µg/ml spectinomycin). 100 µl of an overnight culture were used for inoculation of 10 ml LB-cultures supplemented with the respective antibiotics as selection markers and additionally containing 0.002 mg/ml L-arabinose and 2 % (v/v) XAD-16. After incubation for 72 h at 22 °C the XAD-16 was harvested. One culture volume methanol was added and incubated for 60 min at 22 °C. The organic phase was filtrated and a sample was taken of the cleared extract. After centrifugation (17,000 x *g*, 20 min) the methanol extracts were used for LC-MS analysis. All measurements were performed by using an Ultimate 3000 LC system (Dionex) with an ACQUITY UPLC BEH C18 column (130 Å, 2.1 x 50 mm, 1.7 µm particle size; Waters) at a flow rate of 0.4 ml min^{-1} using acetonitrile (ACN) and water containing 0.1% formic acid (v/v) in a gradient ranging from 5-95% of ACN over 16 min (40 °C) coupled to an AmaZonX (Bruker) electron spray ionization mass spectrometer. The BPC spectra were recorded in positive ion mode with the range from 100-1200 *m/z* and ultraviolet (UV) at 200-600 nm. The software Compass DataAnalysis 4.3 (Bruker) was used to evaluate the measurements.

1.4 Peptide quantification

The absolute production titers of selected peptides were calculated with calibration curves based on pure synthetic **1** (for quantification of **1**, **10**, **11**, **22**, **23**, **27**), **2** (for quantification of **2-5**, **7-9, 25**, **26**), **6** (for quantification of **6** and **12**), **13** (for quantification of **13** and **14**), **15** (for quantification of **15** and **16**), **17**, **19** (for quantification of **18-21**), **24** (for quantification of **24**, **30**, **31**, **32**), **28** (for quantification of **28**, **29**) and **35** (for quantification of **33/34** and **35/36**). Therefore, the pure compounds were prepared at different concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195 and 0.0195 µg/mL) and measured by LC-MS using HPLC/MS measurements as described above. The peak area for each compound at different concentrations was calculated using Compass Data Analysis and used for the calculation of a standard curve passing through the origin. Triplicates of all *in vivo* experiments were measured. The pure peptide standards **1**, **2**, **6, 13**, **17** and **35** were synthesized in-house (*3, 4*) and the further pure synthetic **15**, **19**, **24** and **28** were produced by Synpeptide.

1.5 Chemical synthesis

Chemical synthesis of all peptides was performed as described previously (*4*).

2 Supplementary Tables

Table S1. ESI-MS data of all produced peptides.

Peptide (#)	theoretical mass-to-charge ratio (*m/z*) $[M+H]^+$	Molecular formula	Reference
1	410.29	$C_{21}H_{38}N_4O_4$	(*5*)
2	586.40	$C_{32}H_{51}O_5N_5$	(*6*)
3	600.41	$C_{33}H_{53}O_5N_5$	(*6*)
4	552.41	$C_{29}H_{53}O_5N_5$	(*6*)
5	566.43	$C_{30}H_{55}O_5N_5$	(*6*)
6	556.35	$C_{27}H_{49}N_5O_5S$	-
7	556.41	$C_{28}H_{53}N_5O_6$	-
8	570.42	$C_{29}H_{55}N_5O_6$	-
9	584.44	$C_{30}H_{57}N_5O_6$	-
10	457.34	$C_{23}H_{44}N_4O_5$	-
11	471.35	$C_{24}H_{46}N_4O_5$	-
12	556.35	$C_{27}H_{49}N_5O_5S$	-
13	589.33	$C_{29}H_{44}N_6O_7$	-
14	555.35	$C_{26}H_{46}N_6O_7$	-
15	634.38	$C_{32}H_{51}N_5O_8$	(*4*)
16	600.40	$C_{29}H_{53}N_5O_8$	(*4*)
17	643.43	$C_{33}H_{54}N_8O_5$	-
18	830.54	$C_{43}H_{71}N_7O_9$	-
19	844.55	$C_{44}H_{73}N_7O_9$	-
20	858.57	$C_{45}H_{75}N_7O_9$	-
21	810.57	$C_{41}H_{75}N_7O_9$	-
22	459,30	$C_{25}H_{38}N_4O_4$	-
23	425,31	$C_{22}H_{40}N_4O_4$	-
24	778,45	$C_{41}H_{59}N_7O_8$	-
25	538.40	$C_{28}H_{51}N_5O_5$	-
26	552.41	$C_{29}H_{53}N_5O_5$	-
27	425.31	$C_{22}H_{40}N_4O_4$	-
28	826.45	$C_{45}H_{59}N_7O_8$	-
29	840.47	$C_{46}H_{61}N_7O_8$	-
30	792.47	$C_{42}H_{61}N_7O_8$	-
31	806.48	$C_{43}H_{63}N_7O_8$	-
32	792.47	$C_{42}H_{61}N_7O_8$	-
33	358.27	$C_{18}H_{35}N_3O_4$	-
34	358.27	$C_{18}H_{35}N_3O_4$	-
35	392.25	$C_{21}H_{33}N_3O_4$	-
36	392.25	$C_{21}H_{33}N_3O_4$	-
37	314.27	$C_{18}H_{35}NO_3$	-
38	328.29	$C_{19}H_{37}NO_3$	-
39	342.20	$C_{20}H_{39}NO_3$	-
40	455.38	$C_{26}H_{50}N_2O_4$	-
41	510.39	$C_{28}H_{51}N_3O_5$	(*7*)
42	496.37	$C_{27}H_{49}N_3O_5$	(*7*)
43	500.20	$C_{26}H_{49}N_3O_6$	-

Table S2. Strains used in this work.

Strain	Genotype / NRPS	Reference
E. coli DH10B	F_mcrA (*mrr-hsd*RMS-*mcr*BC), 80*lac*ZΔ, M15, Δ*lac*X74 *rec*A1 *end*A1 *ara*D 139Δ(*ara*, *leu*)7697 *gal*U *gal*K λ *rps*L (*Strr*) *nup*G / -	(*8*)
E. coli DH10B::*mtaA*	DH10B with *mtaA* from pCK_*mtaA*Δ*entD* / -	(*9*)
P. luminescens TTO1	- / *gxpS* (*6*)	DSMZ
X. bovienii SS-2004	- / *garS*/ *xfpS (7)*	(*10*)
X. nematophila ATCC 19061	- / *xtpS (5)*	ATCC
X. budapestensis DSM 16342	- / *bicA* (*11*)	DSMZ
X. miraniensis DSM 17902	- / *ambS* (*9*)	DSMZ
X. szentirmaii DSM16338	- / *szeS*	DSMZ
X. indica DSM 17382	- / *xldS* (*9*)	DSMZ
B. licheniformis ATCC 10716	- / *bacA (12)*	M. A. Marahiel / ATCC
B. subitlis MR 168	- / *srfA (13)*	ATCC

Table S3. Plasmids used in this work.

Plasmids	Genotype	Reference
pFF1_22A_szeS_gxpS	ori 2µ, kanMX4, ori ColA, kan^R, *P_{BAD}* *szeS*_Ft$A_1T_1C/E_2A_2T_2C_3$-*gxpS*_$A_3T_3C/E_4A_4T_4C/E_5A_5T_5$TE, Ypet-Flag	(*4*)
pFF1_NRPS_6	ori 2µ, kanMX4, *araC-P_{BAD}*, ori ColA, Ypet-Flag, kan^R, *bacA*-A1T1CyA2T2C3A3T3CD$_{sub4}$_*sfrA-BC*-C$_{Asub6}$A6T6E6C7A7T7TE	(*3*)
pCOLA_ara/tacI	ori ColA, kan^R, *araC-P_{BAD}* and *tac*I	(*14*)
pCK_0402	ori p15A, cm^R, *araC-P_{BAD}* and *tac*I-*araE*	unpublished
pCDF_ara/tacI	ori CloDF13, spec^R, *araC-P_{BAD}* and *tac*I	this study
pCOLA_ara_xtpS_tacI_JW	ori ColA, kan^R, *araC-P_{BAD} xtpS* and *tac*I	this study
pCOLA_ara_gxpS_tacI_JW	ori ColA, kan^R, *araC-P_{BAD} gxpS* and *tac*I	this study
pJW61	ori p15A, cm^R, *araC-P_{BAD} xtpS*_$A_1T_1C/E_2A_2T_2C_3$-SYNZIP17 and *tac*I-*araE*	this study
pJW62	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*xtpS*_$A_3T_3C/E_4A_4T_4$TE and *tac*I	this study
pJW63	ori p15A, cm^R, *araC-P_{BAD} xtpS*_$A_1T_1C/E_2A_2T_2C_3$ and *tac*I-*araE*	this study
pJW64	ori ColA, kan^R, *araC-P_{BAD} xtpS*_$A_3T_3C/E_4A_4T_4$TE, *tac*I	this study
pJW75	ori p15A, cm^R, *araC-P_{BAD} gxpS*_$A_1T_1C/E_2A_2T_2C_3$-SYNZIP17 and *tac*I-*araE*	this study
pJW76	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*gxpS*_$A_3T_3C/E_4A_4T_4C/E_5A_5T_5$TE and *tac*I	this study
pJW77	ori p15A, cm^R, *araC-P_{BAD} bicA*_$A_1T_1C/E_2A_2T_2C_3$-SYNZIP17 and *tac*I-*araE*	this study
pJW91	ori p15A, cm^R, *araC-P_{BAD} ambS*_ $A_1T_1C/E_2A_2T_2C_3$-SYNZIP17 and *tac*I-*araE*	this study
pJW92	ori p15A, cm^R, *araC-P_{BAD} szeS*_Ft$A_1T_1C/E_2A_2T_2C_3$-SYNZIP17 and *tac*I-*araE*	this study
pJW93	ori p15A, cm^R, *araC-P_{BAD} xldS*_$C_1A_1T_1C/E_2A_2T_2C_3$-SYNZIP17 and *tac*I-*araE*	this study
pJW100	ori p15A, cm^R, *araC-P_{BAD} xtpS*_A_1T_1C/E_2-(GS)$_2$-SYNZIP17 and *tac*I-*araE*	this study
pJW102	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*xtpS*_$A_2T_2C_3$-(GS)$_2$-SYNZIP1 and *tac*I	this study
pJW103	ori CloDF13, spec^R, *araC-P_{BAD} xtpS*_SYNZIP2-$A_3T_3C/E_4A_4T_4$TE and *tac*I	this study
pJW114	ori p15A, cm^R, *araC-P_{BAD} bacA*_A1T1CyA2T2C3-SYNZIP17 and *tac*I-*araE*	this study
pJW116	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*bacA*_A3T3C$_{Dsub}$4-*sfrA-BC*_C$_{Asub}$6A6T6E6C7A7T7TE and *tac*I	this study
pJW118	ori p15A, cm^R, *araC-P_{BAD} bacA*_A1T1CyA2T2C3A3-SYNZIP17 and *tac*I-*araE*	this study
pJW120	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*bacA*_T3C$_{Dsub}$4-*sfrA-BC*_C$_{Asub}$6A6T6E6C7A7T7TE and *tac*I	this study
pJW122	ori p15A, cm^R, *araC-P_{BAD} bacA*_A1T1CyA2T2C3A3T3-SYNZIP17 and *tac*I-*araE*	this study
pJW124	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*bacA*_C$_{Dsub}$4-*sfrA-BC*_C$_{Asub}$6A6T6E6C7A7T7TE and *tac*I	this study
pJW126	ori p15A, cm^R, *araC-P_{BAD} bacA*_A1T1CyA2T2C3A3T3 C$_{Dsub}$4-SYNZIP17 and *tac*I-*araE*	this study
pJW128	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*sfrA-BC*_C$_{Asub}$6A6T6E6C7A7T7TE and *tac*I	this study
pJW141	ori p15A, cm^R, *araC-P_{BAD} xldS*_C_1-SYNZIP17 and *tac*I-*araE*	this study
pNA1	ori p15A, cm^R, *araC-P_{BAD} xtpS*_ $A_1T_1C/E_2A_2T_2C_3$-SYNZIP19 and *tac*I-*araE*	this study
pNA2	ori p15A, cm^R, *araC-P_{BAD} xtpS*_ $A_1T_1C/E_2A_2T_2$-SYNZIP17 und *tac*I-*araE*	this study
pNA3	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*xtpS*_ $C_3A_3T_3C/E_4A_4T_4$TE und *tac*I	this study
pNA4	ori p15A, cm^R, *araC-P_{BAD} xtpS*_ A_1T_1C/E_2A_2-SYNZIP17 und *tac*I-*araE*	this study

pNA5	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*xtpS*_ $T_2C_3A_3T_3C/E_4A_4T_4$TE und *tac*I	this study
pNA8	ori p15A, cm^R, *araC-P_{BAD} xtpS*_$A_1T_1C/E_2A_2T_2C_3$-$(GS)_5$-SYNZIP17 and *tac*I-*araE*	this study
pNA9	ori p15A, cm^R, *araC-P_{BAD} xtpS*_$A_1T_1C/E_2A_2T_2C_3$-$(GS)_4$-SYNZIP17 and *tac*I-*araE*	this study
pNA10	ori p15A, cm^R, *araC-P_{BAD} xtpS*_$A_1T_1C/E_2A_2T_2C_3$-$(GS)_2$-SYNZIP17 and *tac*I-*araE*	this study
pNA15	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*xtpS*_$T_2C_3A_3$-SYNZIP1 and *tac*I	this study
pNA16	ori CloDF13, spec^R, *araC-P_{BAD}* SYNZIP2-*xtpS*_$T_3C/E_4A_4T_4$TE and *tac*I	this study
pNA17	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*xtpS*_$C_3A_3T_4$-SYNZIP1 and *tac*I	this study
pNA18	ori CloDF13, spec^R, *araC-P_{BAD}* SYNZIP2-*xtpS*_$C/E_4A_4T_4$TE and *tac*I	this study
pNA26	ori p15A, cm^R, *araC-P_{BAD} gxpS*_A_1T_1C/E_2A_2-SYNZIP17 and *tac*I-*araE*	this study
pNA27	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*gxpS*_$T_2C_3A_3$-SYNZIP1 and *tac*I	this study
pNA28	ori CloDF13, spec^R, *araC-P_{BAD}* SYNZIP2-*gxpS*_$T_3C/E_4A_4T_4$ $C/E_5A_5T_5$TE and *tac*I	this study
pNA29	ori p15A, cm^R, *araC-P_{BAD} sze*S_ $C_1A_1T_1C/E_2A_2$-SYNZIP17 and *tac*I-*araE*	this study
pNA30	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*szeS*_$T_2C_3A_3$-SYNZIP1 and *tac*I	this study
pNA31	ori CloDF13, spec^R, *araC-P_{BAD}* SYNZIP2-*szeS*_$T_3C/E_4A_4T_4$ $C/E_5A_5T_5C_6A_6T_6$TE and *tac*I	this study
pNA35	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*garS*_$T_2C_3A_3$-SYNZIP1 and *tac*I	this study
pNA59	ori p15A, cm^R, *araC-P_{BAD} xfpS*_C_1A_1-SYNZIP17 and *tac*I-*araE*	this study
pNA60	ori ColA, kan^R, *araC-P_{BAD}* SYNZIP18-*xfpS*_$T_1E_1C_2A_2T_2C_3A_3T_3$TE and *tac*I	this study

Table S4. Oligonucleotides used in this work. Correlations of plasmids to figures from the main text and supplementary informationt are represented in brackets.

Plasmids	Oligo-nucleotide	Sequence (5´→3´; overlapping ends)	Template
pJW61 (NRPS-1, NRPS-2, NRPS-8, NRPS-9)	KB-pACYC-FW	GAACAGTTAAAACAGAAGCGTGAACAATTAAAGCAAAAGATCGCCAATCTGCGTAAGGAGATCGAAGCCTACAAGTGACAATTAATCATCGGCTCG	pCK_0402
	KB-pACYC-RV	TTCACGCTTCTGTTTTAACTGTTCGATGCGATTACGCAATTCAGCCTTTTTCGATTTTAATTCCTCCTTCTCGTTCATGGAATTCCTCCTGTTAGC	pCK_0402
	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	-
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	-
	KB-P1-FW	TGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGG	*X. nematophila* ATCC 19061
	KB-P1-RV	CGATTTTAATTCCTCCTTCTCGTTCCAGGTTTTTAACAACAATGTGC	*X. nematophila* ATCC 19061
pJW62 (NRPS-1, NRPS-3, NRPS-7, NRPS-12, NRPS-17, NRPS-18, NRPS-19)	KB-pCOLA-FW	CATTGACAAAGAGCTGCGTGCCAACGAAAACGAACTTCGCGCCCTTGATAACGAGCTGACTGCAGCTATCTCATGACAATTAATCATCGGCTCG	pCOLA_ara/tacI
	KB-pCOLA-RV	TTGGCACGCAGCTCTTTGTCAATGGCATTTAACTCGCGGTCCAAGGCTTTCAGTTCACGCTCTTCAGCATAGAACATGGAATTCCTCCTGTTAGC	pCOLA_ara/tacI
	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	-
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	-
	KB-P2-FW	AACGAGCTGACTGCAGCTATCTCATTATGTATTCATCAACTTTTTGAACAGC	*X. nematophila* ATCC 19061
	KB-P2-RV	ATACGAGCCGATGATTAATTGTCACAGCGCCTCCACTTCG	*X. nematophila* ATCC 19061
pJW63 (NRPS-3, NRPS-4)	jw0061_FW	[phos.] TGACAATTAATCATCGGCTCG	pJW61
	jw0062_RV	CCAGGTTTTTAACAACAATGTGC	pJW61
pJW64 (NRPS-2, NRPS-4)	jw0063_FW	[phos.] TTATGTATTCATCAACTTTTTGAACAGC	pJW62
	jw0064_RV	CATGGAATTCCTCCTGTTAGCC	pJW62
pJW75 (NRPS-5, NRPS-7, NRPS-10)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw0124_FW	GGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGAAATTATC	*P. luminescens* TTO1
	jw0125_RV	TCGATTTTAATTCCTCCTTCTCGTTCCAATTTTCCAGTAATAACTCCCG	*P. luminescens* TTO1
pJW76 (NRPS-5, NRPS-8, NRPS-11, NRPS-13, NRPS-14, NRPS-15, NRPS-16)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	jw0172_FW	GGCTAACAGGAGGAATTCCATGTTCTATGCTGAAGAGCGTGAAC	*P. luminescens* TTO1
	jw0127_RV	CGAGCCGATGATTAATTGTCACAGCGCCTCCGCTTC	*P. luminescens* TTO1
pJW114 (NRPS-6, NRPS-11, NRPS-12)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw208_FW	GCTAACAGGAGGAATTCCATGGTTGCTAAACATTCATTAGAAAATGGG	pFF1_NRPS_6 (*3*)
	jw209_RV	CGATTTTAATTCCTCCTTCTCGTTCTTTGTATGGTTAAAGGACTCTAAAAGTGTC	pFF1_NRPS_6 (*3*)
pJW116 (NRPS-6, NRPS-9, NRPS-10, NRPS-43)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	jw0211_FW	CGAGCTGACTGCAGCTATCTCAAAAGCAATCCACCAGCTGTTT	pFF1_NRPS_6 (*3*)
	jw0212_RV	CGAGCCGATGATTAATTGTCATGAAACCGTTACGGTTTGTGTATTA	pFF1_NRPS_6 (*3*)
pJW77 (NRPS-15)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw0128_FW	GGGCTAACAGGAGGAATTCCATGAAAGATAACATTGCTACAGTGGCAAATAG	*X. budapestensis* DSM 16342
	jw0129_RV	CGATTTTAATTCCTCCTTCTCGTTCCAAGTTTTCAGCAACAACTGG	*X. budapestensis* DSM 16342
pJW91 (NRPS-13, NRPS-17)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw0162_FW	GCTAACAGGAGGAATTCCATGAAAAATGATAAGGTGATGACTCTG	*X. miraniensis* DSM 17902
	jw0163_RV	TCGATTTTAATTCCTCCTTCTCGTTCCACGTTTCCAGCAATAACC	*X. miraniensis* DSM 17902
pJW92 (NRPS-14, NRPS-19)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw0164_FW	GCTAACAGGAGGAATTCCATGAAAGGTAGTATTGCTAAAAAGGGAG	*X. szentirmaii* DSM16338
	jw0165_RV	TCGATTTTAATTCCTCCTTCTCGTTCCAGCTTTCCAGCAATAACC	*X. szentirmaii* DSM16338
pJW93 (NRPS-16, NRPS-18)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61

	jw0166_FW	GCTAACAGGAGGAATTCCATGAAACTTTGGAACTATAAAATGAATATGAC	*X. indica* DSM 17382
	jw0167_RV	TCGATTTTAATTCCTCCTTCTCGTTGAAATCCACCAACAGTTGTTGAC	*X. indica* DSM 17382
pJW100 (NRPS-26)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	KB-P1-FW	TGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGG	*X. nematophila* ATCC 19061
	jw0179_RV	CGATTTTAATTCCTCCTTCTCGTTGGATCCAGACCCCCAGGTTTTCAGCAATAACGTG	*X. nematophila* ATCC 19061
pJW102 (NRPS-26)	na29_FW	AACCTGGTTGCGCAGCTCGAAAACGAAGTTGCGTCTCTGGAAAATGAGAACGAAACCCTGAAGAAAAAGAACCTGCACAAAAAAGACCTGATCGCGTAC	pJW101
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW101
	jw0180_FW	AACGAGCTGACTGCAGCTATCTCACTGTGTATCCATCAGTTAATTGAACAACAG	*X. nematophila* ATCC 19061
	jw0182_RV	CGTTTTCGAGCTGCGCAACCAGGTTGGATCCAGACCCCCAGGTTTTTAACAACAATGTGCG	*X. nematophila* ATCC 19061
pJW103 (NRPS-26)	na34_FW	TGACAATTAATCATCGGCTCG	pCDF_ara/tacI
	na32_RV	GTTCTGTTCATCACGTTCCAGCTGCAGGTTGTCTTTTTTCAGACGTGCGATTTTCTTACGCAGATACGCGTTACGCGCCATGGAATTCCTCCTGTTAGCC	pCDF_ara/tacI
	na33_RV	CTGTTCGTGAGACGCAACTTCGTTTTCGAGACGCGCGATTTCGTCACGCAGGTTCGCGATGATTTTTTCCAGGTTCTGTTCATCACGTTCCAGC	pCDF_ara/tacI
	na84_RV	CTGTTCGTGAGACGCAACTTC	-
	jw0183_FW	AAACGAAGTTGCGTCTCACGAACAGTTATGTATTCATCAACTTTTTGAACAGC	*X. nematophila* ATCC 19061
	jw0188_RV	GCCTAAACCAATACGCCGT	*X. nematophila* ATCC 19061
	jw0189_FW	CGGCGTATTGGTTTAGGCCTGT	*X. nematophila* ATCC 19061
	na07_RV	CGAGCCGATGATTAATTGTCACAGCGCCTCCACTTCG	*X. nematophila* ATCC 19061
pJW118 (NRPS-44)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw208_FW	GCTAACAGGAGGAATTCCATGGTTGCTAAACATTCATTAGAAAATGGG	pFF1_NRPS_6 (*3*)
	jw0214_RV	CGATTTTAATTCCTCCTTCTCGTTGTAGCGGCGATCCATTGT	pFF1_NRPS_6 (*3*)
pJW120 (NRPS-44)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	jw0216_FW	CGAGCTGACTGCAGCTATCTCAGAAGCGCCGCGGG	pFF1_NRPS_6 (*3*)
	jw0212_RV	CGAGCCGATGATTAATTGTCATGAAACCGTTACGGTTTGTGTATTA	pFF1_NRPS_6 (*3*)
pJW122 (NRPS-45)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw208_FW	GCTAACAGGAGGAATTCCATGGTTGCTAAACATTCATTAGAAAATGGG	pFF1_NRPS_6 (*3*)
	jw0218_RV	CGATTTTAATTCCTCCTTCTCGTTTCGGTAATATGGTTTTTCTTCGG	pFF1_NRPS_6 (*3*)
pJW124 (NRPS-45)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	jw0220_FW	CGAGCTGACTGCAGCTATCTCA TTGTCTTCAGCGCAAAAAAGG	pFF1_NRPS_6 (*3*)
	jw0212_RV	CGAGCCGATGATTAATTGTCA TGAAACCGTTACGGTTTGTGTATTA	pFF1_NRPS_6 (*3*)
pJW126 (NRPS-46)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw208_FW	GCTAACAGGAGGAATTCCATGGTTGCTAAACATTCATTAGAAAATGGG	pFF1_NRPS_6 (*3*)
	jw0222_RV	CGATTTTAATTCCTCCTTCTCGTTGGCATGGCTATTTTCCCATT	pFF1_NRPS_6 (*3*)
pJW128 (NRPS-46)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	jw0224_FW	CGAGCTGACTGCAGCTATCTCACAAAAAGAACGGATGAAGGAGC	pFF1_NRPS_6 (*3*)
	jw0212_RV	CGAGCCGATGATTAATTGTCATGAAACCGTTACGGTTTGTGTATTA	pFF1_NRPS_6 (*3*)
pJW141 (NRPS-43)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	jw0166_FW	GCTAACAGGAGGAATTCCATGAAACTTTGGAACTATAAAATGAATATGAC	*X. indica* DSM 17382
	jw0254_RV	CGATTTTAATTCCTCCTTCTCGTTAAAATCTACCAATAGTTTCTGGCGC	*X. indica* DSM 17382
pNA1 (NRPS-20)	na01_FW	CGTGAACAGCTGAAACAGAAACTGGCGGCTCTGCGTAACAAACTGGACGCGTACAAAAACCGTCTG TGACAATTAATCATCGGCTCG	pCK_0402
	na02_FW	AACGAACTGGAATCTCTGGAGAACAAAAAAGAAGAACTGAAGAACCGTAACGAAGAGCTGAAGCAGAAA CGTGAACAGCTGAAACAGAAAC	pCK_0402
	jw0064_RV	CATGGAATTCCTCCTGTTAGCC	pCK_0402
	na03_FW	TGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGG	*X. nematophila* ATCC 19061

	na04_RV	CTCCAGAGATTCCAGTTCGTTCCAGGTTTTTAACAACAATGTGC	*X. nematophila* ATCC 19061
pNA2 (NRPS-24, NRPS-27)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	na03_FW	TGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGG	*X. nematophila* ATCC 19061
	na05_RV	CGATTTTAATTCCTCCTTCTCGTTAACACGATCACGGGATATTG	*X. nematophila* ATCC 19061
pNA3 (NRPS-24)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	na06	AACGAGCTGACTGCAGCTATCTCATTGCCTTTATCGTTTGGTCAAC	*X. nematophila* ATCC 19061
	na07	CGAGCCGATGATTAATTGTCACAGCGCCTCCACTTCG	*X. nematophila* ATCC 19061
pNA4 (NRPS-25, NRPS-28, NRPS-29, NRPS-30, NRPS-31, NRPS-32, NRPS-33, NRPS-42)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	na03_FW	TGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGG	*X. nematophila* ATCC 19061
	na13_RV	CGATTTTAATTCCTCCTTCTCGTTATAAATCTGGCGGGCGAA	*X. nematophila* ATCC 19061
pNA5 (NRPS-25, NRPS-48)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	na14_FW	AACGAGCTGACTGCAGCTATCTCAGTTGCGCCACAAGGAGAA	*X. nematophila* ATCC 19061
	na07_RV	CGAGCCGATGATTAATTGTCACAGCGCCTCCACTTCG	*X. nematophila* ATCC 19061
pNA8 (NRPS-23)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	na3_FW	TGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGG	*X. nematophila* ATCC 19061
	na17_RV	CGATTTTAATTCCTCCTTCTCGTTTGATCCCGAACCTGAGCCGGATCCAGACCCCCAGGTTTTTAACAACAATGTGC	*X. nematophila* ATCC 19061
pNA9 (NRPS-22)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	na3_FW	TGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGG	*X. nematophila* ATCC 19061
	na19_RV	CGATTTTAATTCCTCCTTCTCGTTCGAACCTGAGCCGGATCCAGACCCCCAGGTTTTTAACAACAATGTGC	*X. nematophila* ATCC 19061
pNA10 (NRPS-21)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	na3_FW	TGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGG	*X. nematophila* ATCC 19061
	na20_RV	CGATTTTAATTCCTCCTTCTCGTTGGATCCAGACCCCCAGGTTTTTAACAACAATGTG	*X. nematophila* ATCC 19061
pNA15 (NRPS-28, NRPS-29, NRPS-32, NRPS-37, NRPS-38, NRPS-40)	na29_FW	AACCTGGTTGCGCAGCTCGAAAACGAAGTTGCGTCTCTGGAAAATGAGAACGAAACCCTGAAGAAAAAGAACCTGCACAAAAAAGACCTGATCGCGTAC	pJW61
	na30_RV	TGAGATAGCTGCAGTCAGCTCG	pJW61
	na22_FW	GGCTAACAGGAGGAATTCCATGGTTGCGCCACAAGGAGAA	*X. nematophila* ATCC 19061
	na41_RV	CGAGCCGATGATTAATTGTCAATAGACCTGCCGGGCAAAC	*X. nematophila* ATCC 19061
pNA16 (NRPS-28, NRPS-30, NRPS-31, NRPS-35, NRPS-38)	na33_RV	CTGTTCGTGAGACGCAACTTCGTTTTCGAGACGCGCGATTTCGTCACGCAGGTTCGCGATGATTTTTTCCAGGTTCT GTTCATCACGTTCCAGC	pCDF_ara/tacI
	na34_FW	TGACAATTAATCATCGGCTCG	pCDF_ara/tacI
	na42_FW	TTGGGCTAACAGGAGGAATTCC ATGGCGGCTCCGCAGGG	*X. nematophila* ATCC 19061
	na7_RV	CGAGCCGATGATTAATTGTCA CAGCGCCTCCACTTCG	*X. nematophila* ATCC 19061
pNA17 (NRPS-27)	na28_FW	CACAAAAAAGACCTGATCGCGTACCTGGAGAAAGAAATCGCGAATCTGCGTAAGAAAATCGAAGAATGACAATTAATCATCGGCTCG	pJW61
	na29_FW	AACCTGGTTGCGCAGCTCGAAAACGAAGTTGCGTCTCTGGAAAATGAGAACGAAACCCTGAAGAAAAAGAACCTGCACAAAAAAGACCTGATCGCGTAC	pJW61
	na30_RV	TGAGATAGCTGCAGTCAGCTCG	pJW61
	na06_FW	AACGAGCTGACTGCAGCTATCTCATTGCCTTTATCGTTTGGTCAAC	*X. nematophila* ATCC 19061
	na37_RV	CGTTTTCGAGCTGCGCAACCAGGTTCATGGCTGGCGTTAGTACCG	*X. nematophila* ATCC 19061
pNA18 (NRPS-27)	na32_RV	GTTCTGTTCATCACGTTCCAGCTGCAGGTTGTCTTTTTTCAGACGTGCGATTTTCTTACGCAGATACGCGTTACGCGCCATGGAATTCCTCCTGTTAGCC	pCDF_ara/tacI
	na33_RV	CTGTTCGTGAGACGCAACTTCGTTTTCGAGACGCGCGATTTCGTCACGCAGGTTCGCGATGATTTTTTCCAGGTTCTGTTCATCACGTTCCAGC	pCDF_ara/tacI
	na34_FW	TGACAATTAATCATCGGCTCG	pCDF_ara/tacI
	na38_FW	AAACGAAGTTGCGTCTCACGAACAGTTGCCGCTGATTGATCTCAC	*X. nematophila* ATCC 19061
	na7_RV	CGAGCCGATGATTAATTGTCACAGCGCCTCCACTTCG	*X. nematophila* ATCC 19061
pNA26 (NRPS-34, NRPS-35, NRPS-36, NRPS-37, NRPS-38, NRPS-39, NRPS-40, NRPS-41)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	na51	GCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGAAAT	*P. luminescens* TTO1

	na52	CGATTTTAATTCCTCCTTCTCGTTATAAATTTGGCGAGCAAAAGC	*P. luminescens* TTO1
pNA27 (NRPS-31, NRPS-33, NRPS-34, NRPS-35, NRPS-39)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	na53	CGAGCTGACTGCAGCTATCTCAGTCGCGCCACAGGGAG	*P. luminescens* TTO1
	na54	CGTTTTCGAGCTGCGCAACCAGGTTGTAAGCTTGGCGAGCAAAGG	*P. luminescens* TTO1
pNA28 (NRPS-29, NRPS-33, NRPS-34, NRPS-36, NRPS-37)	na33_RV	CTGTTCGTGAGACGCAACTTCGTTTTCGAGACGCGCGATTTCGTCACGCAGGTTCGCGATGATTTTTTCCAGGTTCTGTTCATCACGTTCCAGC	pCDF_ara/tacI
	na34_FW	TGACAATTAATCATCGGCTCG	pCDF_ara/tacI
	na55	GAAGTTGCGTCTCACGAACAGCAAGCGCCACAAGGGGA	*P. luminescens* TTO1
	na56	CGAGCCGATGATTAATTGTCACAGCGCCTCCGCTTCAC	*P. luminescens* TTO1
pNA29	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	na57	GCTAACAGGAGGAATTCCATGAAAGGTAGTATTGCTAAAAAGGGAGATG	*X. szentirmaii* DSM16338
	na58	CGATTTTAATTCCTCCTTCTCGTTATAATGCTGACGGGCAAATG	*X. szentirmaii* DSM16338
pNA30 (NRPS-30, NRPS-36, NRPS-41, NRPS-42)	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	na59	CGAGCTGACTGCAGCTATCTCAGAATCCCCACAAGGGGAGA	*X. szentirmaii* DSM16338
	na60	TCGAGCTGCGCAACCAGGTTATAATGCTGACGGGCAAACG	*X. szentirmaii* DSM16338
pNA31 (NRPS-32, NRPS-39, NRPS-40, NRPS-41, NRPS-42)	KB-pACYC-II-FW	AACGAGAAGGAGGAATTAAAATCG	pJW61
	KB-pACYC-II-RV	CATGGAATTCCTCCTGTTAGC	pJW61
	na61	AAAACGAAGTTGCGTCTCACGAACAGGAGTTGCCACAAGGTGAAA	*X. szentirmaii* DSM16338
	na62	CGAGCCGATGATTAATTGTCAAATATTATTTACTATATTGTATTCCTCTGTACCA	*X. szentirmaii* DSM16338
pNA35	KB-pCOLA-II-FW	TGACAATTAATCATCGGCTCG	pJW62
	KB-pCOLA-II-RV	TGAGATAGCTGCAGTCAGCTCG	pJW62
	na68	TAACGAGCTGACTGCAGCTATCTCAGAAACTCCGGTCGGTAAAGTAG	*X. bovienii* SS2004
	na69	TTCGTTTTCGAGCTGCGCAACCAGGTTATAGCCGCGCACCACTAC	*X. bovienii* SS2004
pNA59 (NRPS-47, NRPS-48)	KB-pACYC-FW	GAACAGTTAAAACAGAAGCGTGAACAATTAAAGCAAAAGATCGCCAATCTGCGTAAGGAGATCGAAGCCTACAAGTGACAATTAATCATCGGCTCG	pCK_0402
	KB-pACYC-RV	TTCACGCTTCTGTTTTAACTGTTCGATGCGATTACGCAATTCAGCCTTTTTCGATTTTAATTCCTCCTTCTCGTTCATGGAATTCCTCCTGTTAGC	pCK_0402
	na125_FW	GCTAACAGGAGGAATTCCATGGATAACATTCTGGCCTCG	*X. bovienii* SS2004
	na126_RV	CAGATTTTAAACAGAGCCGCTATGTTTATAACGAGAAGGAGGAATTAAAATCG	*X. bovienii* SS2004
pNA60 (NRPS-47)	KB-pCOLA-FW	CATTGACAAAGAGCTGCGTGCCAACGAAAACGAACTTCGCGCCCTTGATAACGAGCTGACTGCAGCTATCTCATGACAATTAATCATCGGCTCG	pCOLA_ara/tacI
	KB-pCOLA-RV	TTGGCACGCAGCTCTTTGTCAATGGCATTTAACTCGCGGTCCAAGGCTTTCAGTTCACGCTCTTCAGCATAGAACATGGAATTCCTCCTGTTAGC	pCOLA_ara/tacI
	na127_FW	AACGAGCTGACTGCAGCTATCTCACGTGCCGCAGAAACGG	*X. bovienii* SS2004
	na128_RV	ATACGAGCCGATGATTAATTGTCAATCCACCAGCTCCAACAC	*X. bovienii* SS2004
pCOLA_ara_xtpS_tacI_JW	jw0136_FW	CGCTGCTGGTTCTGGCGATTGACAATTAATCATCGGCTCG	pCOLA_ara/tacI
	jw0137_RV	AACGGGTATGGAGAAACAGTAGAGAGTTGCGATAAAAAGCG	pCOLA_ara/tacI
	AL-GxpS-2-1	ACTGTTTCTCCATACCCGTTTTTTTGGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGG	*X. nematophila* ATCC 19061
	AD64	TCGCCAGAACCAGCAGCGGAGCCAGCGGATCCGGCGCGCCTTACAGCGCCTCCAC	*X. nematophila* ATCC 19061
pCOLA_ara_gxpS_tacI_JW	JW_tacI_PstI_FW2	CTGCAGGAGCTGTTGACAAT	pCOLA_ara/tacI
	jw0064_RV	CATGGAATTCCTCCTGTTAGCC	pCOLA_ara/tacI
	jw0124_FW	GGGCTAACAGGAGGAATTCCATGAAAGATAGCATGGCTAAAAAGGAAATTATC	*P. luminescens* TTO1
	jw0160_RW	GATTAATTGTCAACAGCTCCTGCAGGCAGATAGAGACGTTTGTTGGC	*P. luminescens* TTO1
	jw0151_FW/	GCCAACAAACGTCTCTATCTGCTGGATGAACACCG	*P. luminescens* TTO1
	jw0161_RV	GATTAATTGTCAACAGCTCCTGCAGTCACAGCGCCTCCGCTTCAC	*P. luminescens* TTO1

3 Supplementary Figures

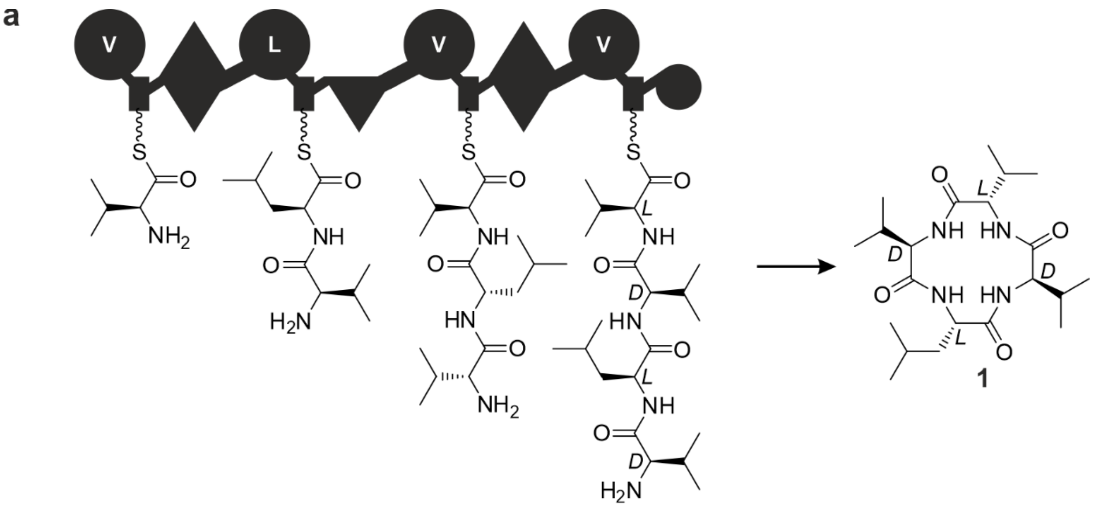


Figure S1. A schematic representation of the xenotetrapeptide (**1**) producing NRPS (XtpS).

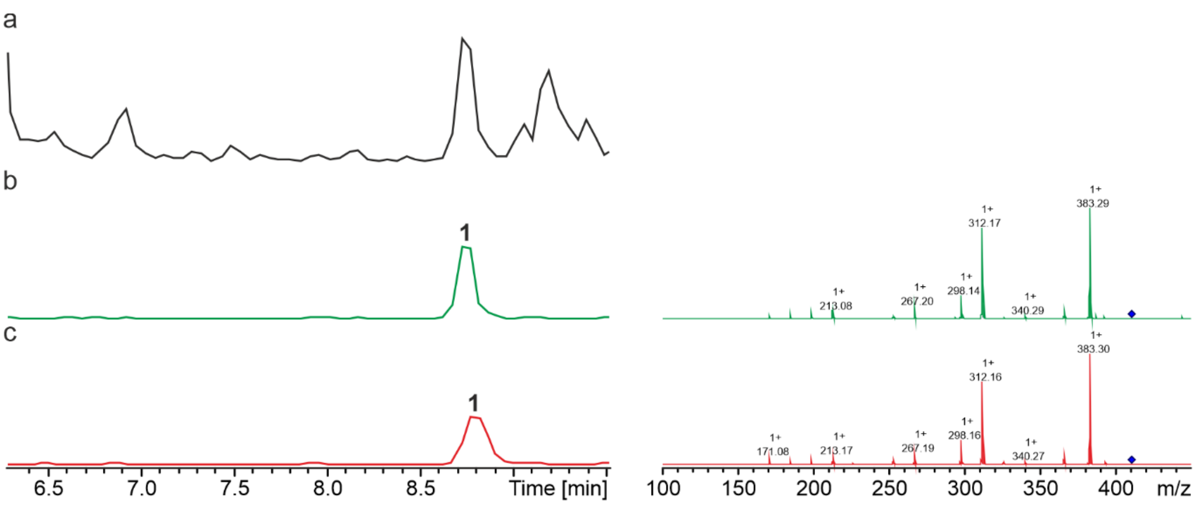


Figure S2. HPLC/MS data refers to Figure 2a (NRPS-1-4) of compound **1** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 of **1** (*m/z* $[M+H]^+$ = 411.30). (c) EIC/MS^2 data of synthetic **1** (*m/z* $[M+H]^+$ = 411.30).

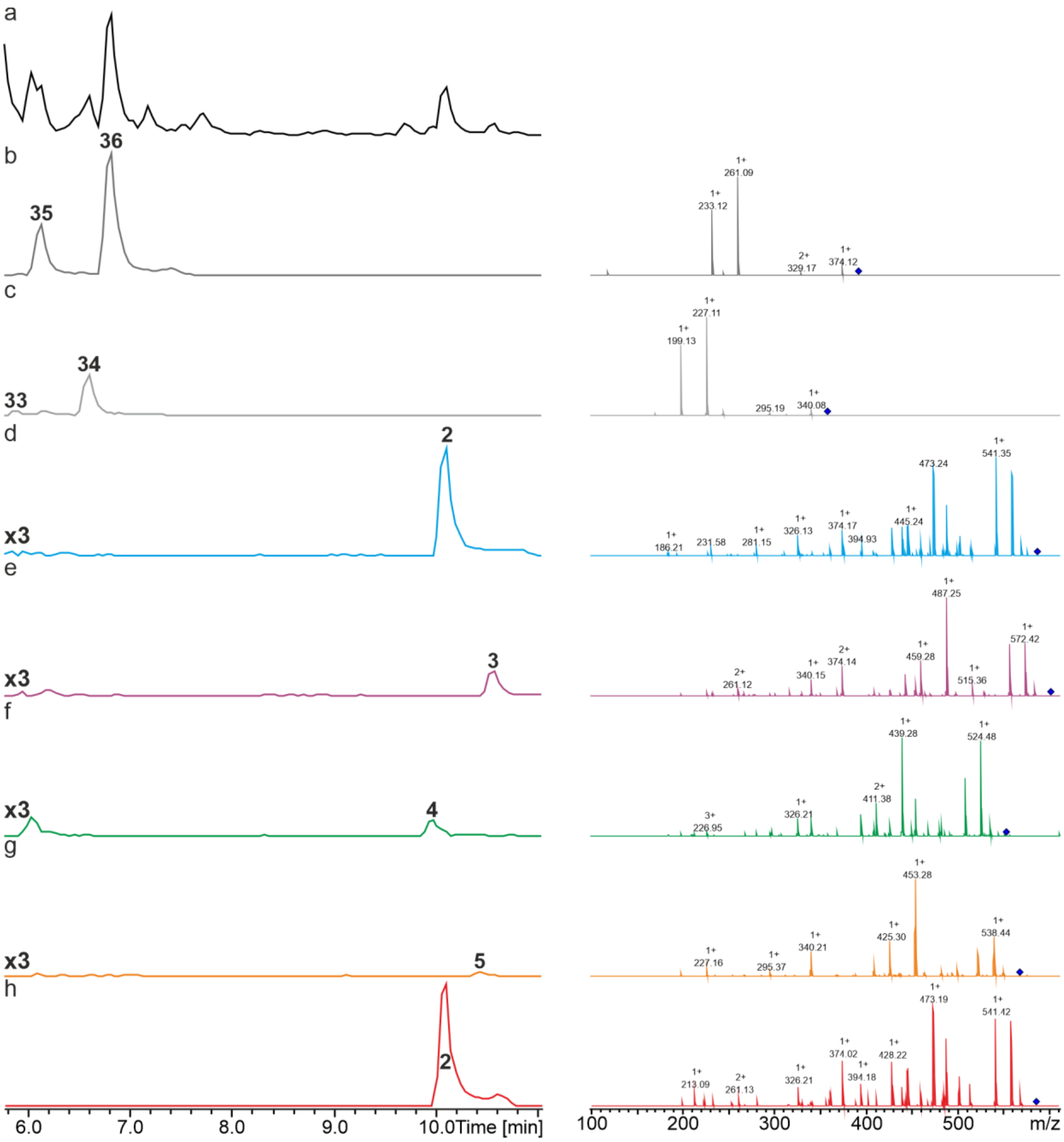


Figure S3. HPLC/MS data refers to Figure 2b (NRPS-5) of compounds **2-5**, **33**/**34** and **35**/**36** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) Extracted ion chromatogram (EIC)/MS^2 of **35/36** (*m/z* $[M+H]^+$ = 392.25). (c) EIC/MS^2 of **33/34** (*m/z* $[M+H]^+$ = 358.27). (d) EIC/MS^2 of **2** (*m/z* $[M+H]^+$ = 586.40). (e) EIC/MS^2 of **3** (*m/z* $[M+H]^+$ = 600.41). (f) EIC/MS^2 of **4** (*m/z* $[M+H]^+$ = 552.41). (g) EIC/MS^2 **5** (*m/z* $[M+H]^+$ = 566.43). EICs of **2-5** are depicted with threefold increased intensity. (h) EIC/MS^2 of synthetic **2** (*m/z* $[M+H]^+$ = 586.40).

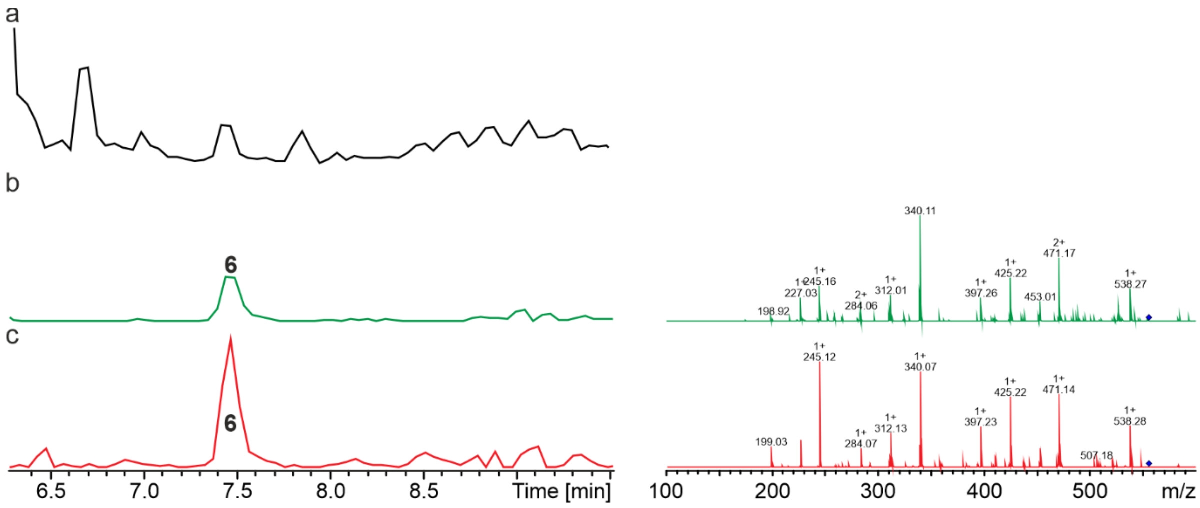


Figure S4. HPLC/MS data refers to Figure 2b (NRPS-6) of compound **6** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) Extracted ion chromatogram (EIC)/MS^2 of **6** (m/z [M+H]$^+$ = 556.35). (c) EIC/MS^2 of synthetic **6** (m/z [M+H]$^+$ = 556.35).

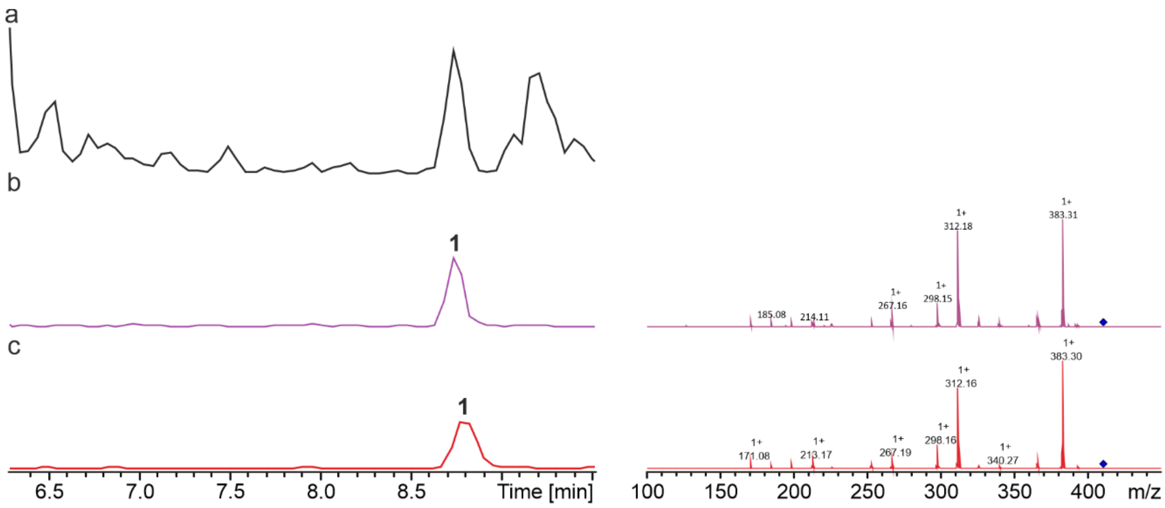


Figure S5. HPLC/MS data refers to Figure 3a (NRPS-7) of compound **1** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) Extracted ion chromatogram (EIC)/MS^2 of **1** (m/z [M+H]$^+$ = 411.30). (c) EIC/MS^2 of synthetic **1** (m/z [M+H]$^+$ = 411.30).

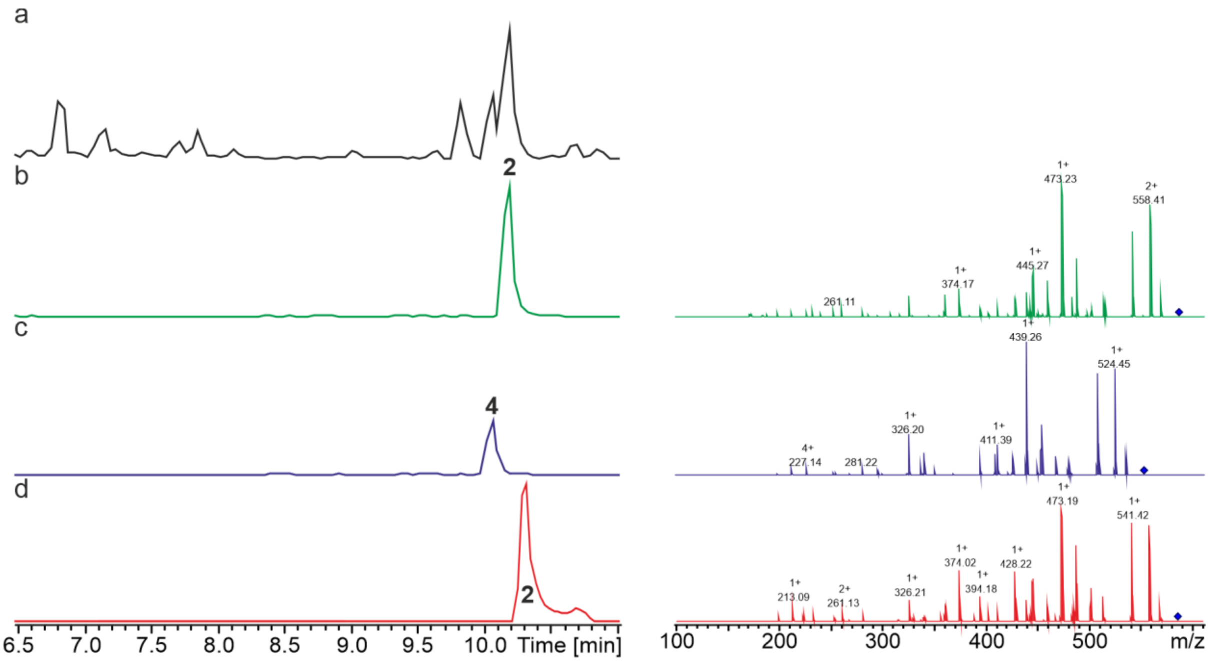


Figure S6. HPLC/MS data refers to Figure 3a (NRPS-8) of compounds **2** and **4** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) Extracted ion chromatogram (EIC)/MS^2 of **2** (m/z $[M+H]^+$ = 586.40). (c) Extracted ion chromatogram (EIC)/MS^2 of **4** (m/z $[M+H]^+$ = 552.41). (d) EIC/MS^2 of synthetic **2** (m/z $[M+H]^+$ = 586.40).

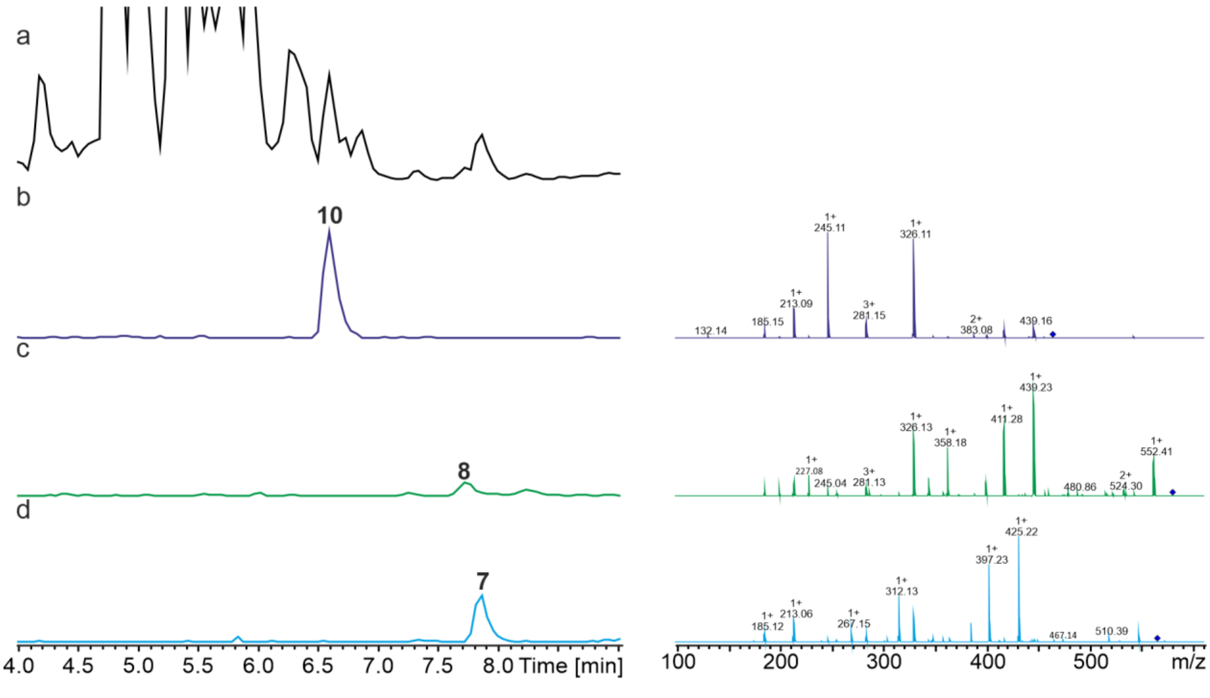


Figure S7. HPLC/MS data refers to Figure 3b (NRPS-9) of compounds **7**, **8** and **10** produced in *E. coli* DH10B::*mtaA.* (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) Extracted ion chromatogram (EIC)/MS^2 of **10** (m/z $[M+H]^+$ = 457.34). (c) Extracted ion chromatogram (EIC)/MS^2 of **8** (m/z $[M+H]^+$ = 570.42). (d) Extracted ion chromatogram (EIC)/MS^2 of **7** (m/z $[M+H]^+$ = 556.41).

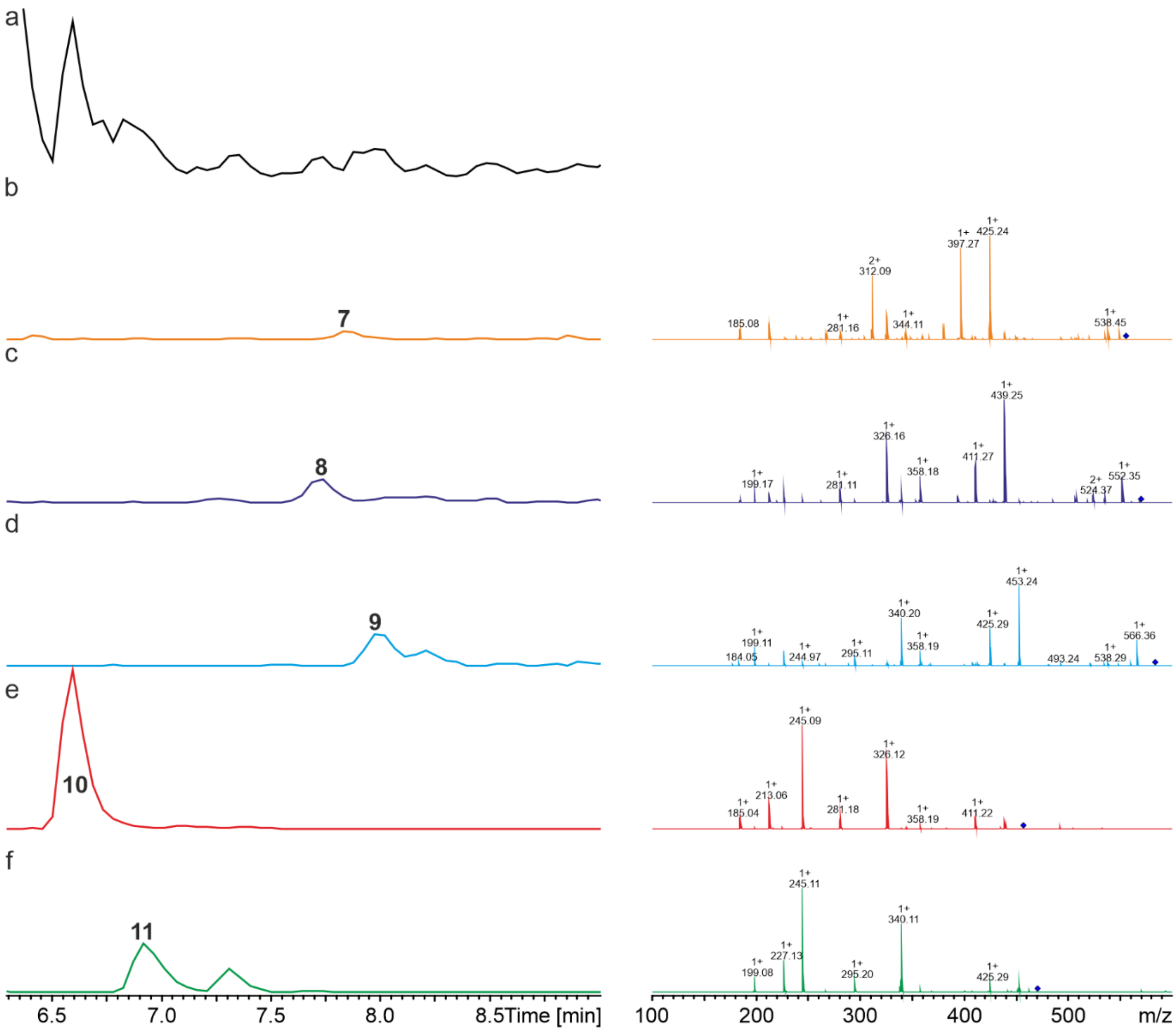


Figure S8. HPLC/MS data refers to Figure 3b (NRPS-10) of compounds **7**-**11** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) Extracted ion chromatogram (EIC)/MS^2 of **7** (*m/z* $[M+H]^+$ = 556.41). (c) Extracted ion chromatogram (EIC)/MS^2 of **8** (*m/z* $[M+H]^+$ = 570.42). (d) Extracted ion chromatogram (EIC)/MS^2 of **9** (*m/z* $[M+H]^+$ = 584.44). (e) Extracted ion chromatogram (EIC)/MS^2 of **10** (*m/z* $[M+H]^+$ = 457.34). (f) Extracted ion chromatogram (EIC)/MS^2 of **11** (*m/z* $[M+H]^+$ = 471.35).

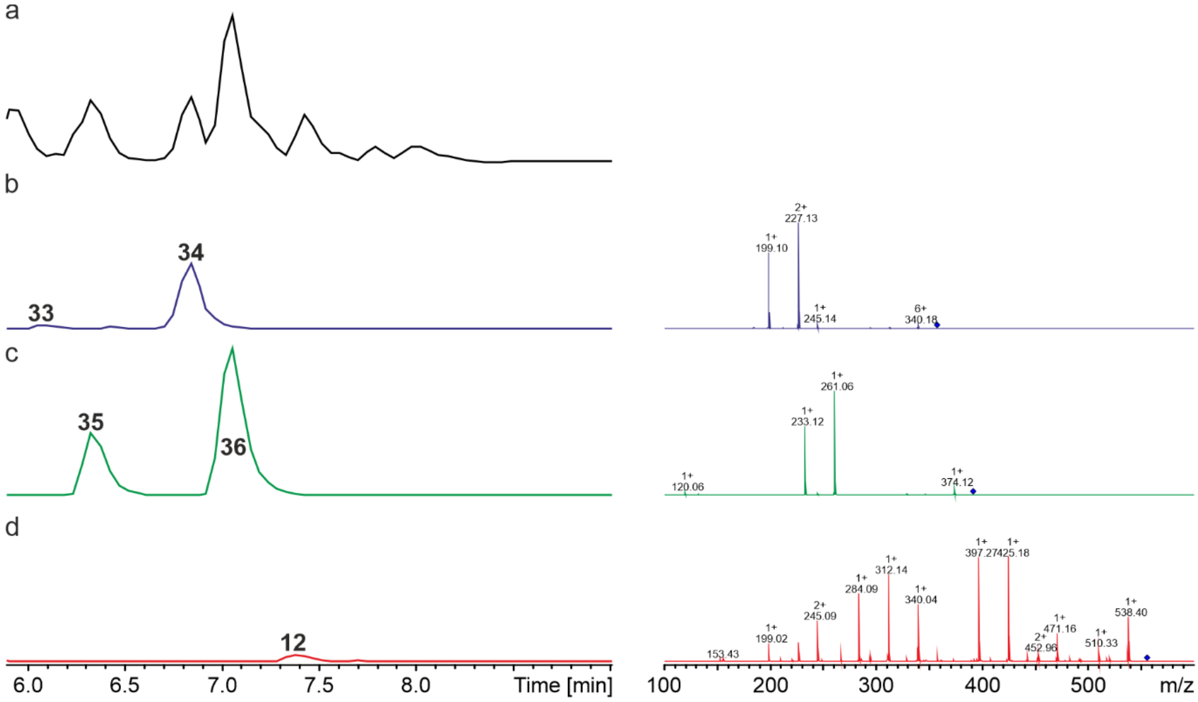


Figure S9. HPLC/MS data refers to Figure 3b (NRPS-11) of compounds **33**/**34**, **35**/**36** and **12** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data data of **33**/**34** (*m/z* $[M+H]^+$ = 358.27). (c) EIC/MS^2 data of **35/36** (*m/z* $[M+H]^+$ = 392.25). (d) Extracted ion chromatogram (EIC)/MS^2 of **12** (*m/z* $[M+H]^+$ = 556.35).

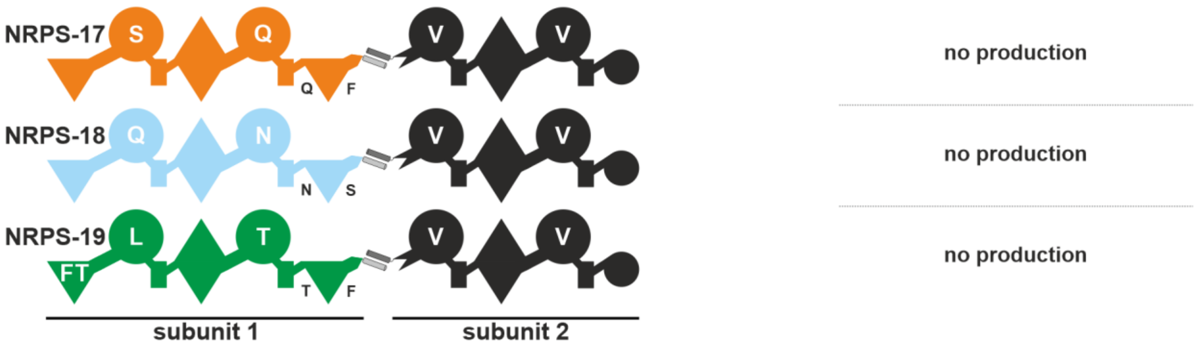


Figure S10. A schematic representation of non-functional recombinant type S NRPSs using subunit 1 building blocks from AmbS XldS and SzeS combined with XtpS subunit 2.

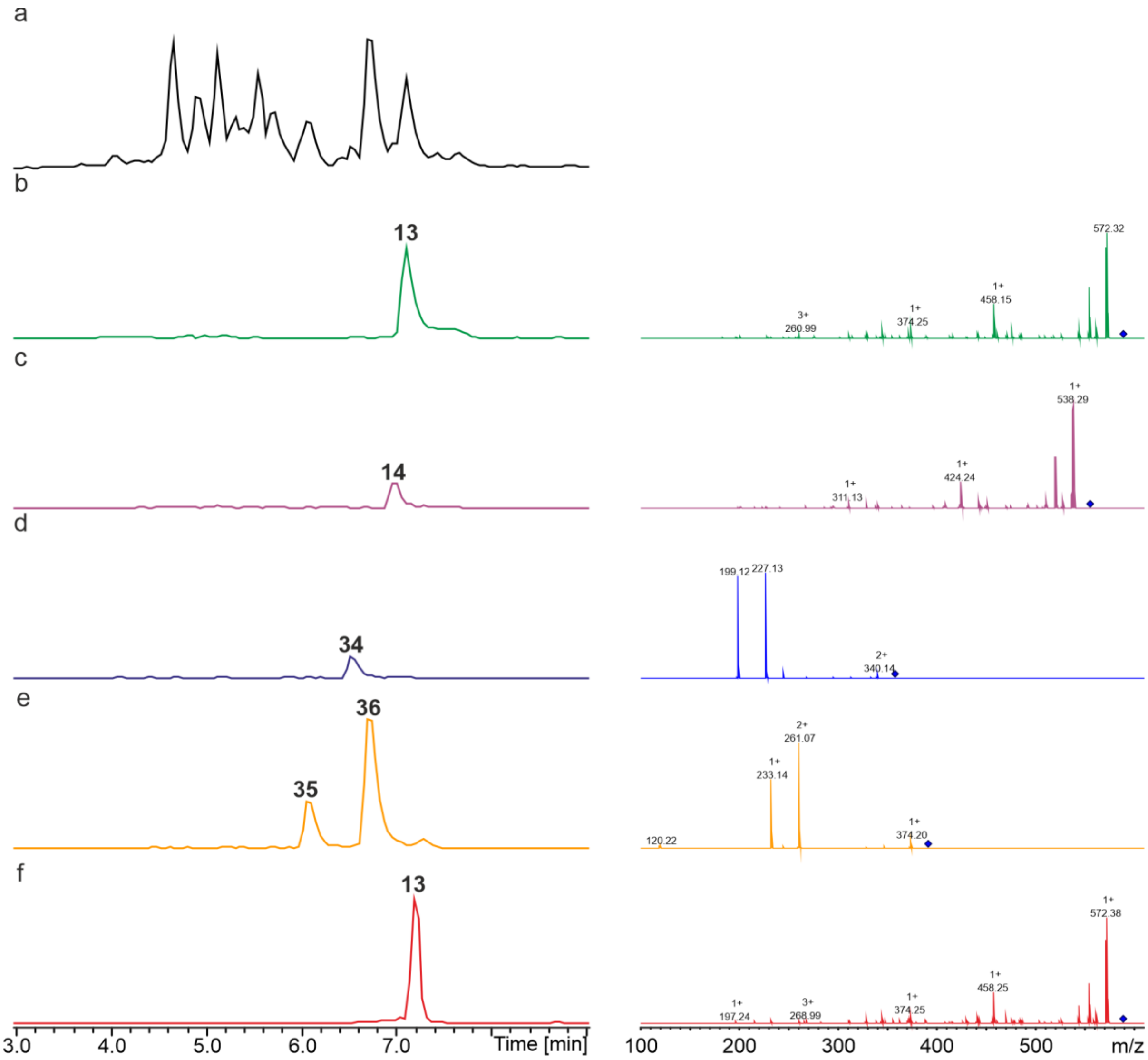


Figure S11. HPLC/MS data refers to Figure 3c (NRPS-13) of compounds **13**, **14**, **33**/**34** and **35**/**36** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogramm (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **13** (*m/z* $[M+H]^+$ = 589.33). (c) EIC/MS^2 data of **14** (*m/z* $[M+H]^+$ = 555.35). (d) EIC/MS^2 data data of **34** (*m/z* $[M+H]^+$ = 358.27). (e) EIC/MS^2 data of **35/36** (*m/z* $[M+H]^+$ = 392.25). (f) EIC/MS^2 data of synthetic **13** (*m/z* $[M+H]^+$ = 589.33).

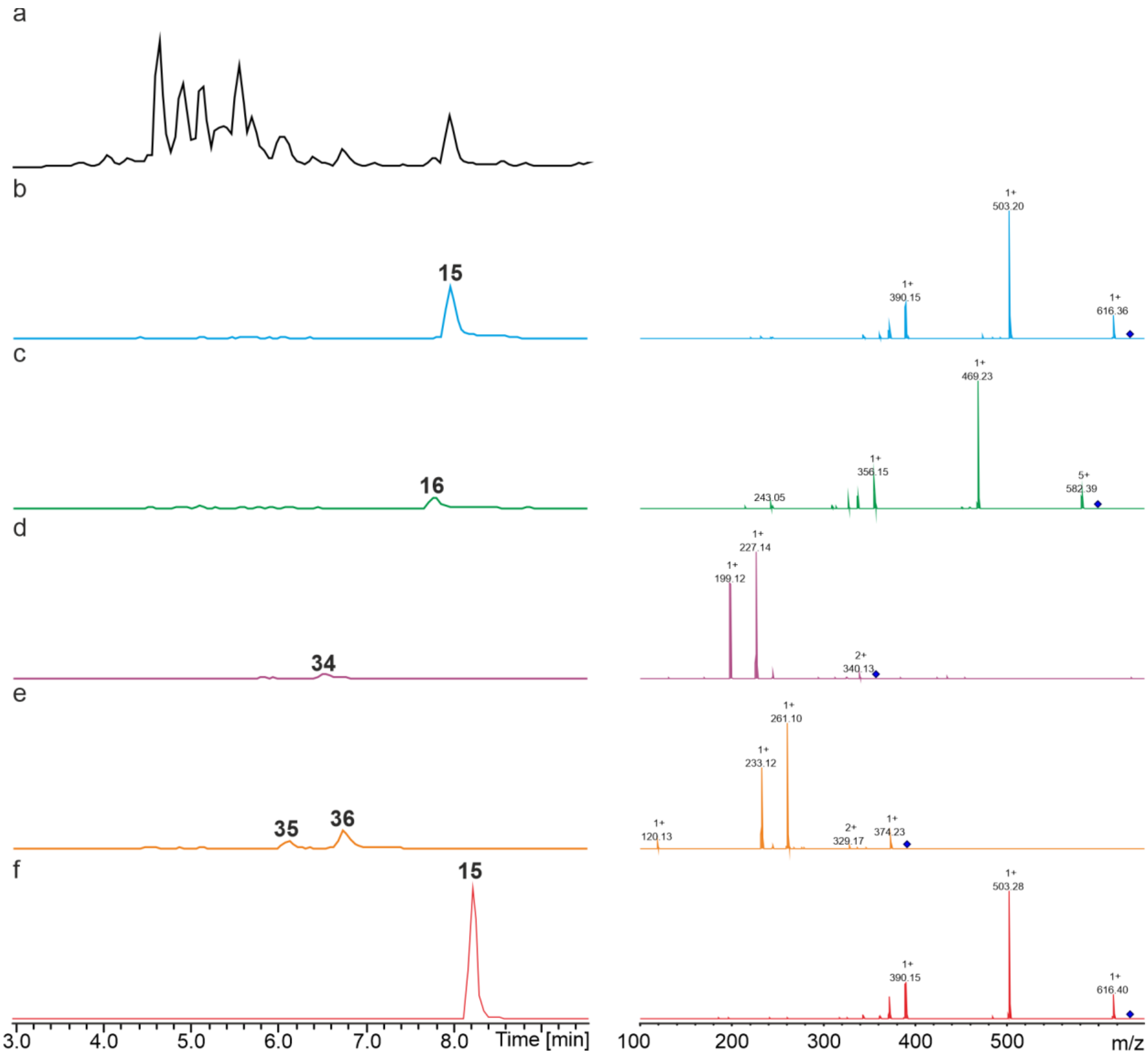
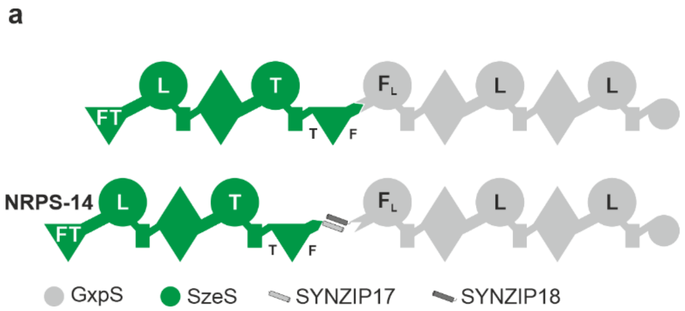


Figure S12. HPLC/MS data refers to Figure 3c (NRPS-14) of compounds **15**, **16**, **34** and **35**/**36** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogramm (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **15** (*m/z* $[M+H]^+$ = 634.38). (c) EIC/MS^2 data of **16** (*m/z* $[M+H]^+$ = 600.40). (d) EIC/MS^2 data of **34** (*m/z* $[M+H]^+$ = 358.27). (e) EIC/MS^2 data of **35/36** (*m/z* $[M+H]^+$ = 392.25). (f) EIC/MS^2 data of synthetic **15** (*m/z* $[M+H]^+$ = 634.38).



Peptide	Production (mg l^{-1})
15	14.73±0.45
16	1.68±0.71
15	23.82±2.64
16	4.07±1.10

Figure S13. Comparison of production yields of a homologous *in cis* and *trans* NRPS-14. The colour code of the NRPS subunits is depicted at the bottom of the figure. The domain assignment is as in Figure 1 plus FT (formyltransferase, N-terminal triangle) and specificities are assigned for the entire A domains.

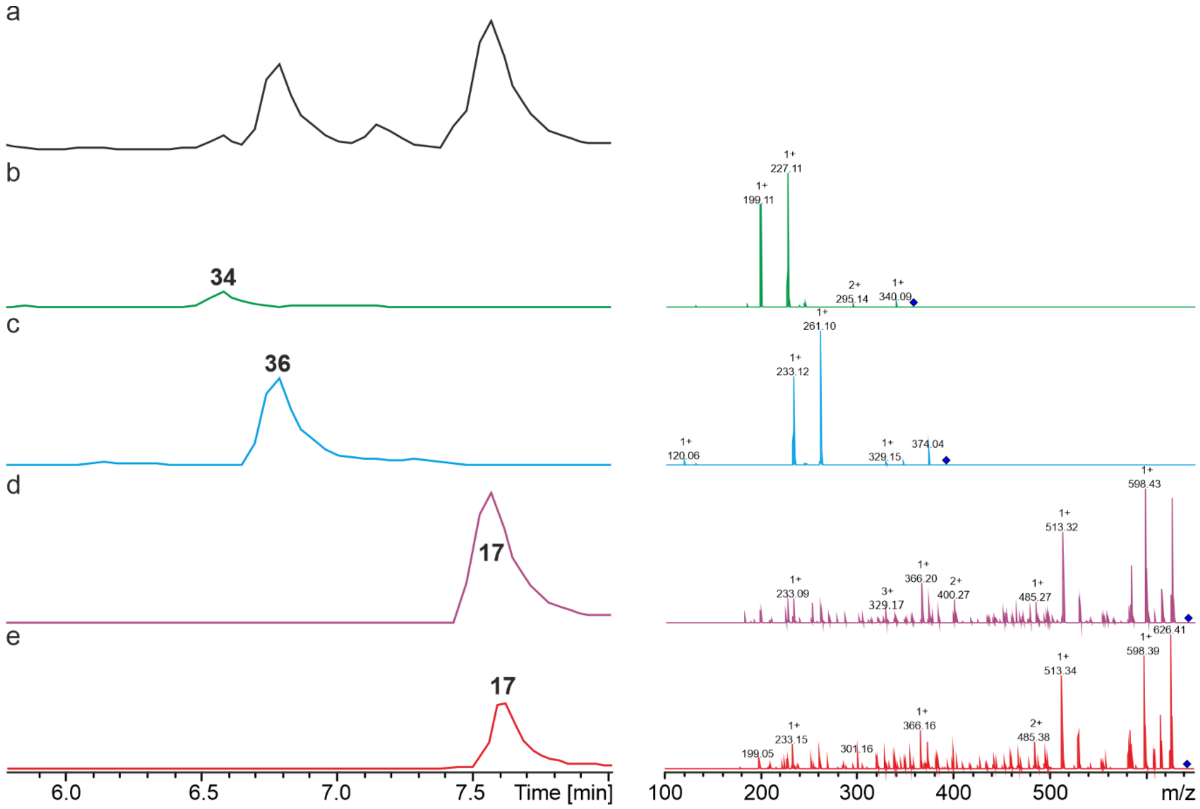


Figure S14. HPLC/MS data refers to Figure 3d (NRPS-15) of compounds **34**, **36** and **17** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogramm (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **34** (*m/z* $[M+H]^+$ = 358.27). (c) EIC/MS^2 data of **36** (*m/z* $[M+H]^+$ = 392.25). (d) EIC/MS^2 data of **17** (*m/z* $[M+H]^+$ = 643.43). (e) EIC/MS^2 data of synthetic **17** (*m/z* $[M+H]^+$ = 643.43).

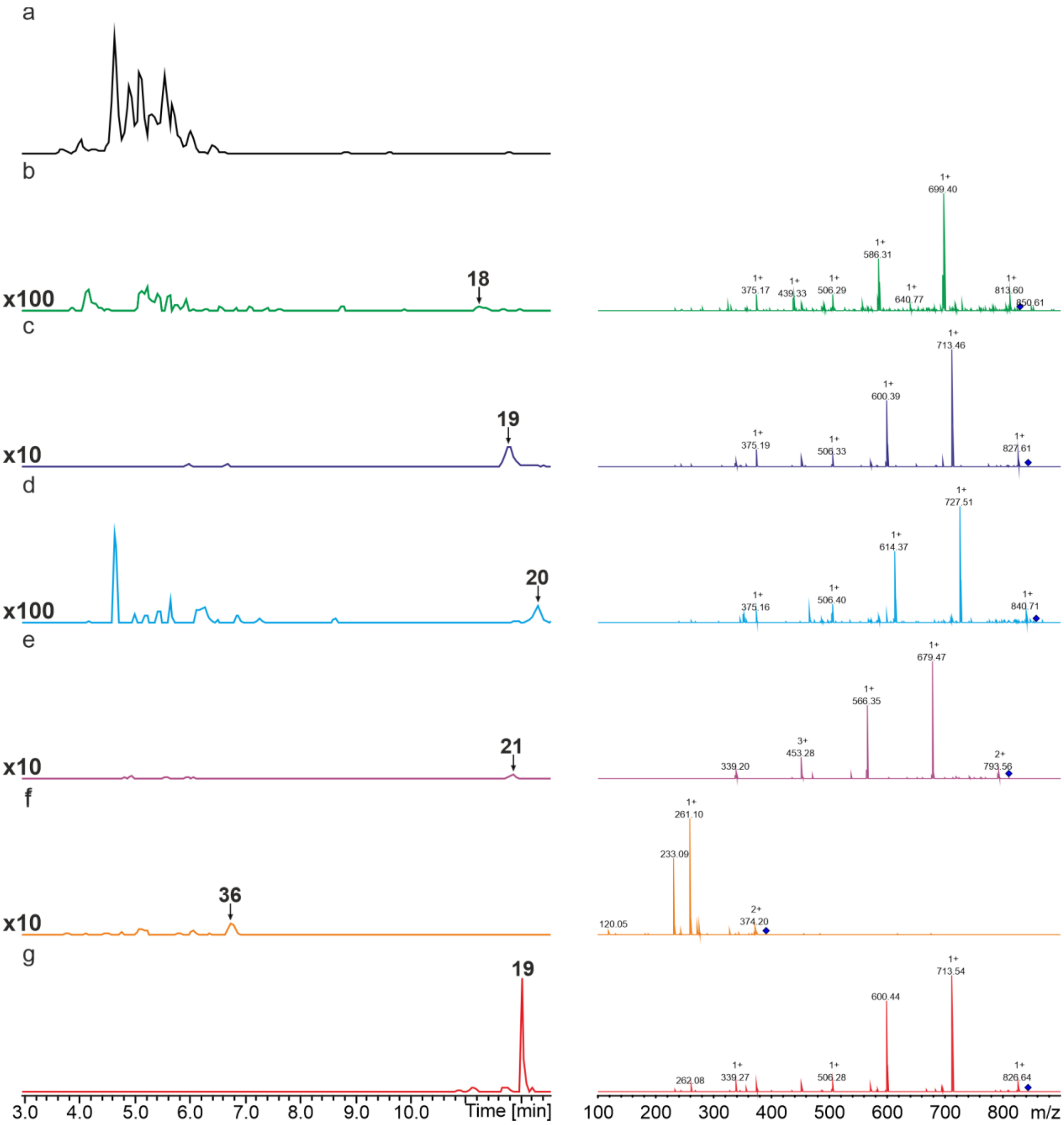
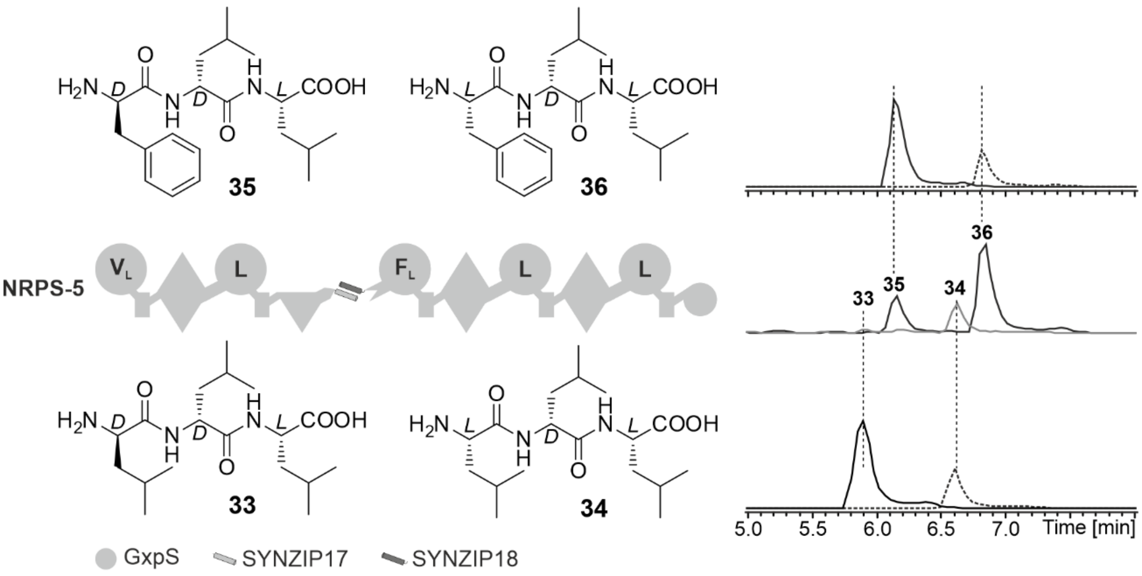
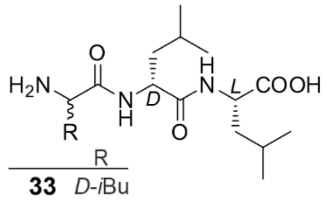


Figure S15. HPLC/MS data refers to Figure 3d (NRPS-16) of compounds **18-21** and **36** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogramm (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **18** (*m/z* $[M+H]^+$ = 830.54). (c) EIC/MS^2 data of **19** (*m/z* $[M+H]^+$ = 844.55). (d) EIC/MS^2 data of **20** (*m/z* $[M+H]^+$ = 858.57). (e) EIC/MS^2 data of **21** (*m/z* $[M+H]^+$ = 810.57). (f) EIC/MS^2 data of **36** (*m/z* $[M+H]^+$ = 392.25). (g) EIC/MS^2 data of synthetic **19** (*m/z* $[M+H]^+$ = 844.55).

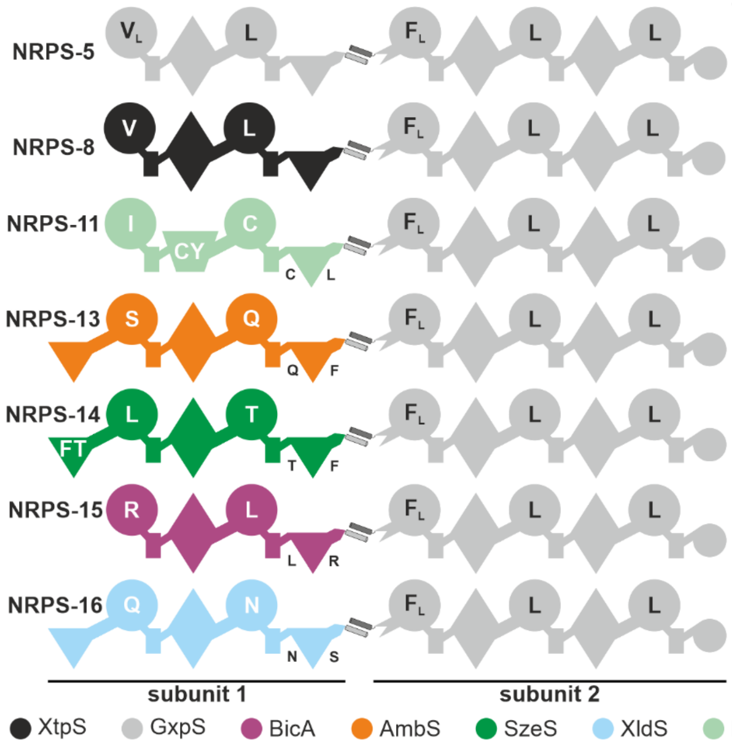
a



b



	R
33	*D*-*i*Bu
34	*L*-*i*Bu
35	*D*-Ph
36	*L*-Ph



Peptide	Production (mg l^{-1})
33	-
34	3.59±0.11
35	5.31±0.06
36	16.08±0.19
33	0.06±0.01
34	0.72±0.05
35	1.44±0.09
36	4.92±0.46
33	1.08±0.28
34	20.00±1.18
35	18.25±0.53
36	47.52±2.08
33	0.23±0.06
34	5.11±0.55
35	12.94±0.73
36	37.66±2.36
33	0.10±0.00
34	1.39±0.17
35	1.95±0.28
36	5.62±1.22
33	0.20±0.06
34	10.24±0.56
35	2.31±0.04
36	86.23±2.80
33	-
34	-
35	0.15±0.02
36	0.44±0.03

Figure S16. (a) Production of D/L-tripeptides exemplary of NRPS-5. The tripeptide production is related to the unpaired activity of GxpS subunit 2 resulted in the production of peptides **33/34** and **35/36**. The different epimers could be identified by their retention times. (b) Tripeptide **33/34** and **35/36** amounts and yields (determined in triplicates (n=3)) are given. The colour code of the NRPS subunits is depicted at the bottom of the figures. The domain assignment is as in Figure 1.

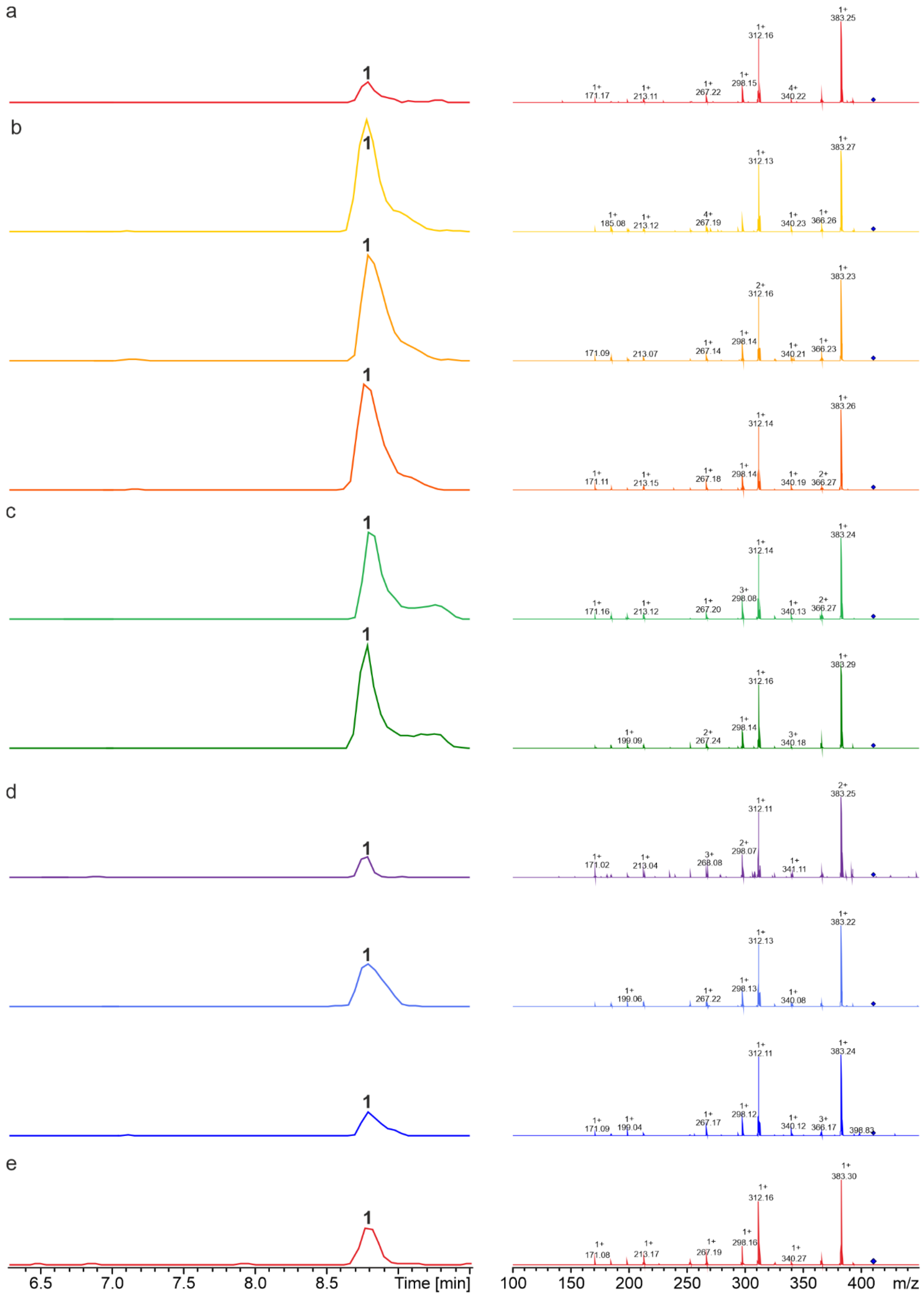


Figure S17. HPLC/MS data refers to Figure 4a (NRPS-20), Figure 4b (NRPS-21-23), Figure 4c (NRPS-24 and NRPS-25) and Figure 5 (NRPS-26-28) of compound **1** produced in *E. coli* DH10B::*mtaA*. (a) EIC/MS^2 (NRPS-20) of **1** (m/z $[M+H]^+$ = 411.30). (b) EIC/MS^2 (NRPS-21-23) of **1** (m/z $[M+H]^+$ = 411.30). (c) EIC/MS^2 (NRPS-24 and NRPS-25) of **1** (m/z $[M+H]^+$ = 411.30). (d) EIC/MS^2 (NRPS-26-28) of **1** (m/z $[M+H]^+$ = 411.30). (e) EIC/MS^2 data of synthetic **1** (m/z $[M+H]^+$ = 411.30).

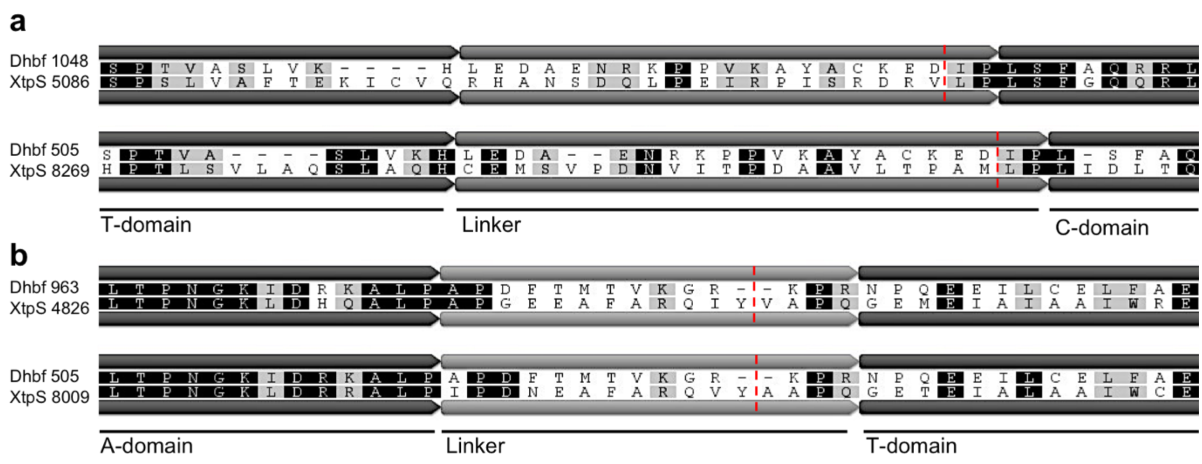
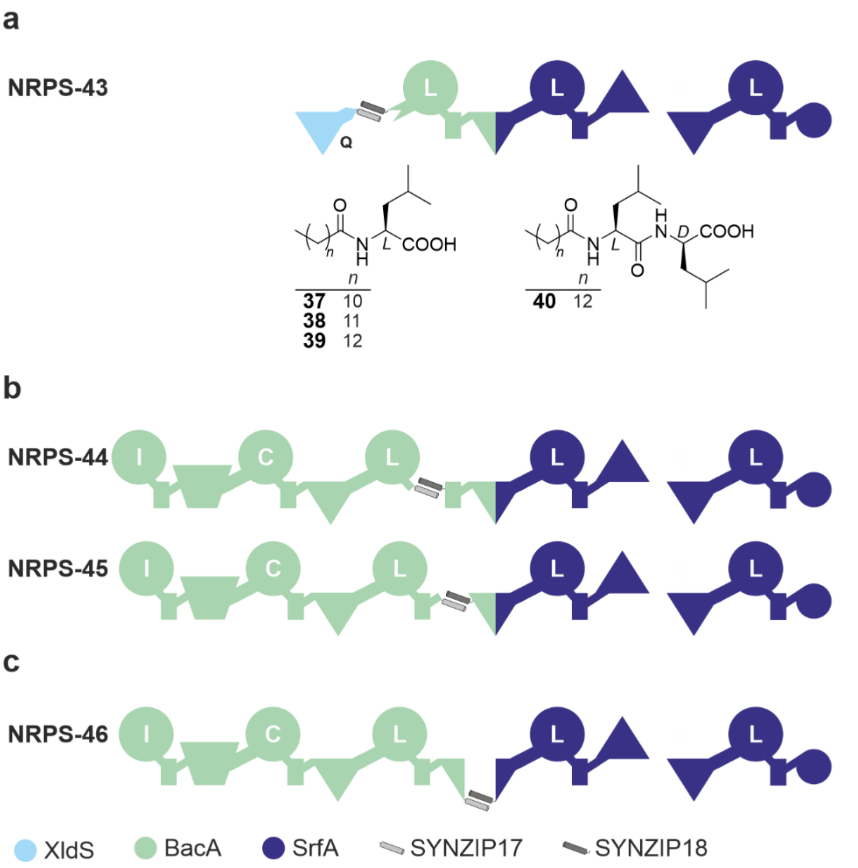


Figure S18. Sequence alignments of XtpS linker sequences. (a) XtpS T_2-C_2 (XtpS 5086) and T_3-C_3 (XtpS 8269) linker were aligned to the linker region excised from Dhbf crystal structure (Protein Database ID: 5u89). (b) XtpS A_2-T_2 (XtpS 4826) and A_3-T_3 (XtpS 8009) linker were aligned to the linker region excised from Dhbf crystal structure. Red dashed lines indicate SYNZIP insertion points.

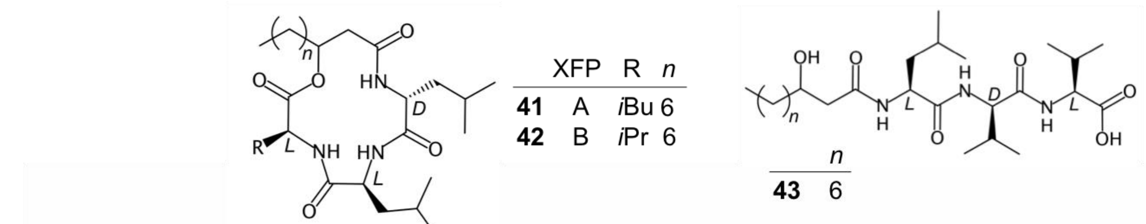


Peptide	Production (mg l^{-1})	% of WT level
(12:0)-L 37	-	-
(13:0)-L 38	-	-
(14:0)-L 39	-	-
(14:0)-LI 40	-	-

Peptide	Production (mg l^{-1})	% of WT level
IC*LIL	20.42±2.02	127.9
no production		

Peptide	Production (mg l^{-1})	% of WT level
no production		

Figure S19. Further examples of two component type S NRPS split in between and within RtpS modules.



Peptide	Production (mg l^{-1})	% of WT level
10:0(β-hydroxy)-LLL 41	0.32±0.03	26.69±2.70
10:0(β-hydroxy)-LLV 42	0.35±0.08	73.00±16.78
10:0(β-hydroxy)-LVV 43	0.06±0.02	13.70±5.07

Figure S20. Further examples of two component type S NRPS split within modules.

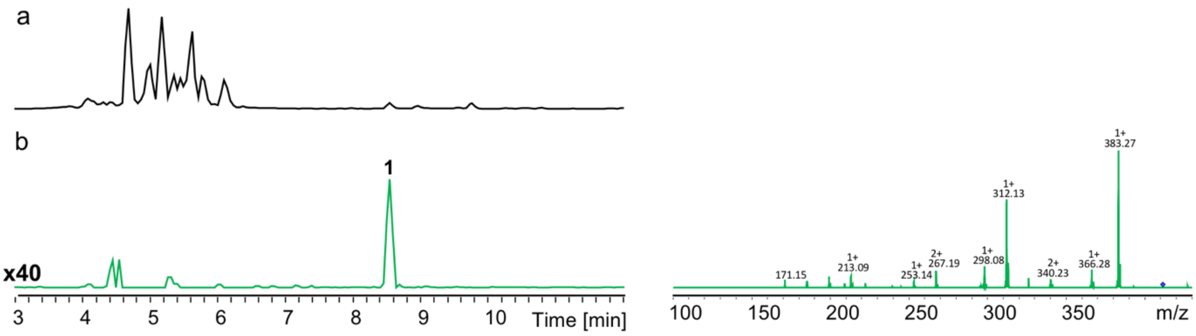


Figure S21. HPLC/MS data refers to Figure 6 (NRPS-28) of compound **1** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **1** (m/z $[M+H]^+$ = 411.30).

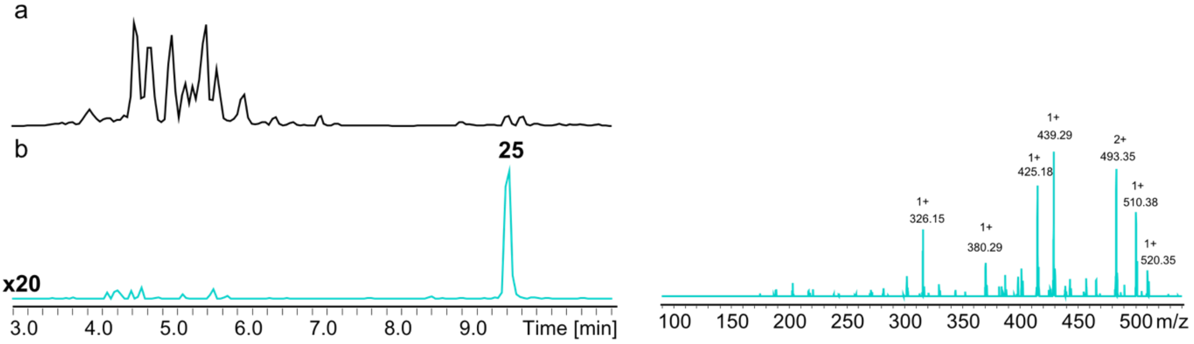


Figure S22. HPLC/MS data refers to Figure 6 (NRPS-29) of compound **25** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **25** (m/z $[M+H]^+$ = 538.40).

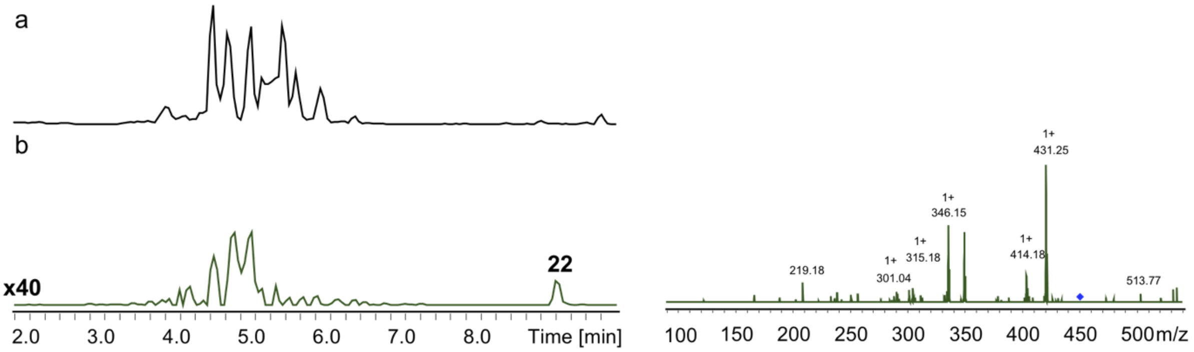


Figure S23. HPLC/MS data refers to Figure 6 (NRPS-30) of compound **22** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **22** (m/z $[M+H]^+$ = 459.30).

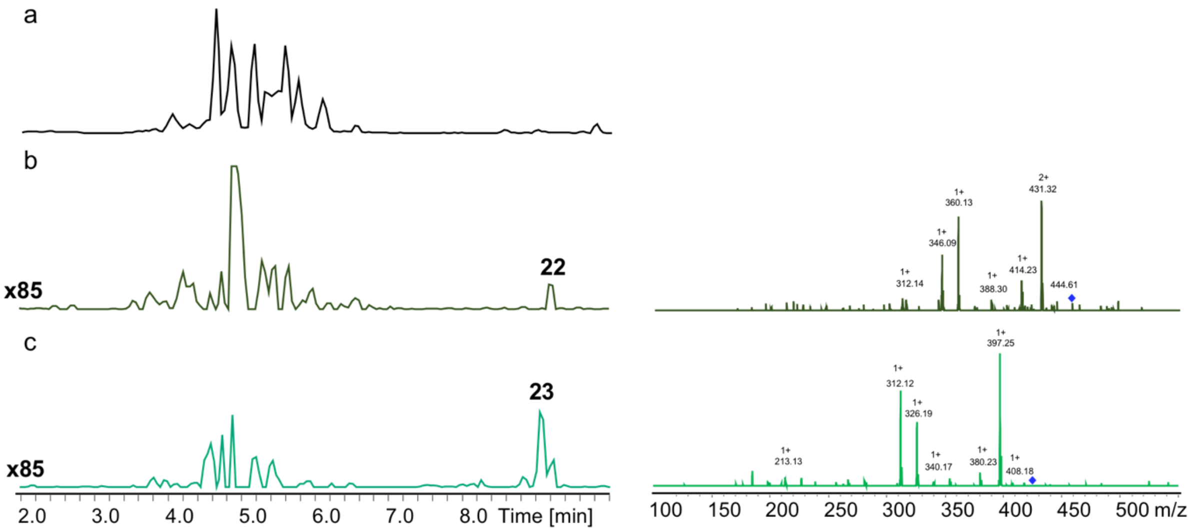


Figure S24. HPLC/MS data refers to Figure 6 (NRPS-31) and (NRPS-34) of compounds **22** and **23** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **22** (m/z [M+H]$^+$ = 459.30). (c) EIC/MS^2 data of **23** (m/z [M+H]$^+$ = 425.31).

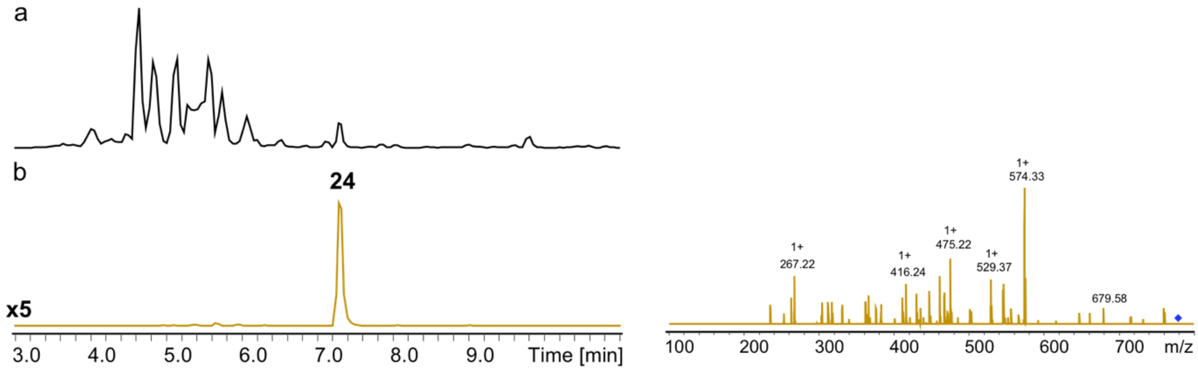


Figure S25. HPLC/MS data refers to Figure 6 (NRPS-32) of compound **24** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **24** (*m/z* [M+H]$^+$ = 778.45).

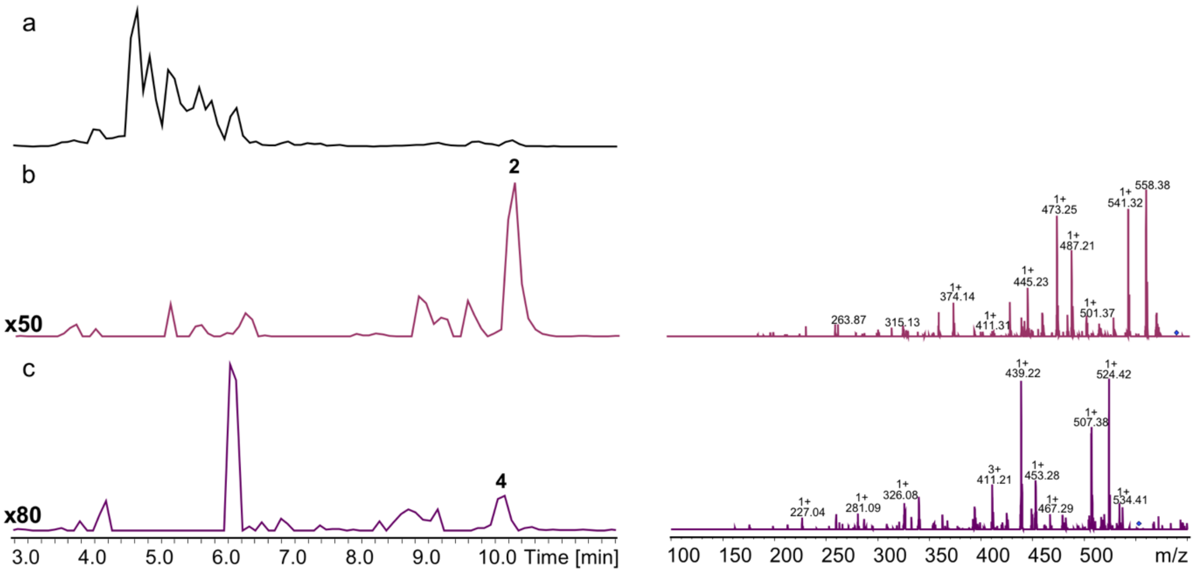


Figure S26. HPLC/MS data refers to Figure 6 (NRPS-33) of compounds **2** and **4** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **2** (*m/z* $[M+H]^+$ = 586.40). (c) EIC/MS^2 data of **4** (*m/z* $[M+H]^+$ = 552.41).

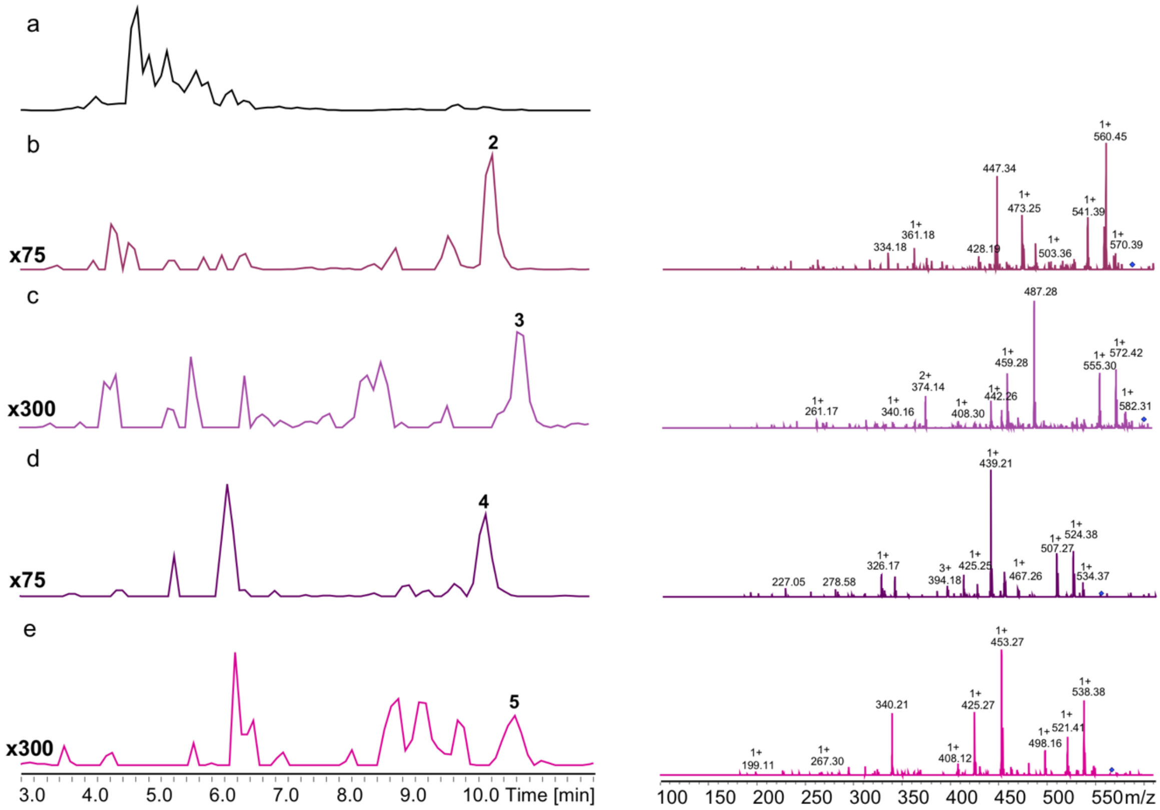


Figure S27. HPLC/MS data refers to Figure 6 (NRPS-34) of compounds **2**, **3**, **4** and **5** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **2** (m/z $[M+H]^+$ = 586.40). (c) EIC/MS^2 data of **3** (m/z $[M+H]^+$ = 600.41). (d) EIC/MS^2 data of **3** (m/z $[M+H]^+$ = 552.41). (e) EIC/MS^2 data of **3** (m/z $[M+H]^+$ = 566.43).

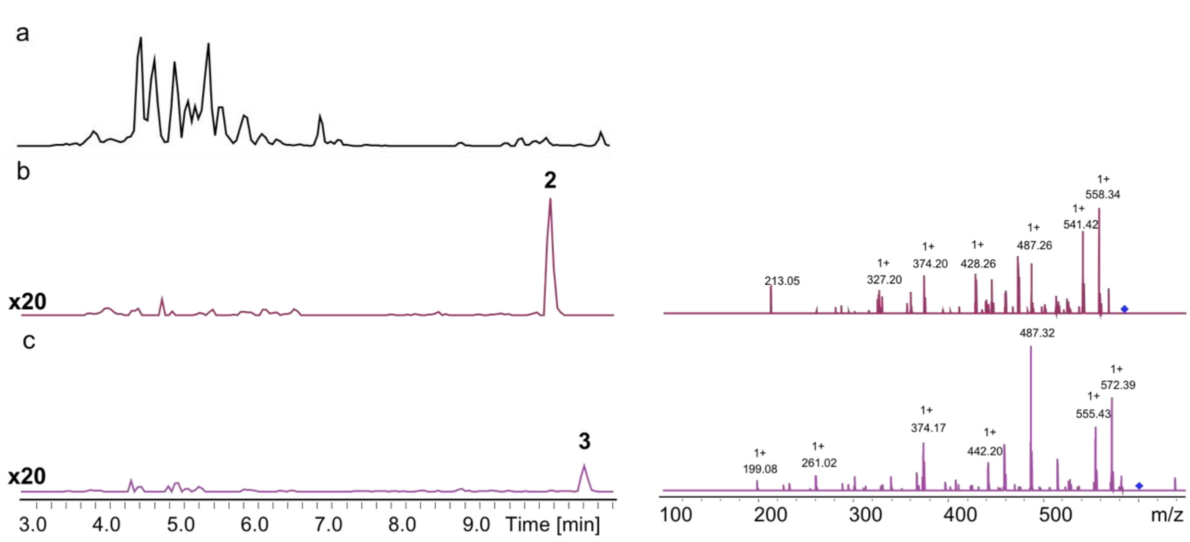


Figure S28. HPLC/MS data refers to Figure 6 (NRPS-36) of compounds **2** and **3** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **2** (m/z $[M+H]^+$ = 586.40). (c) EIC/MS^2 data of **3** (m/z $[M+H]^+$ = 600.41).

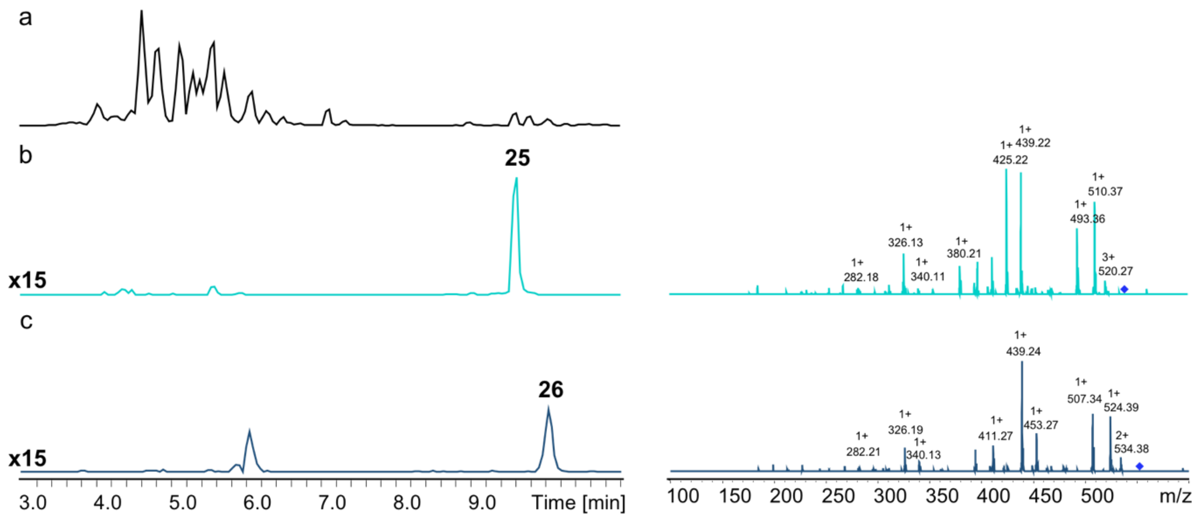


Figure S29. HPLC/MS data refers to Figure 6 (NRPS-37) of compounds **25** and **26** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **25** (m/z $[M+H]^+$ = 588.40). (c) EIC/MS^2 data of **26** (m/z $[M+H]^+$ = 552.41).

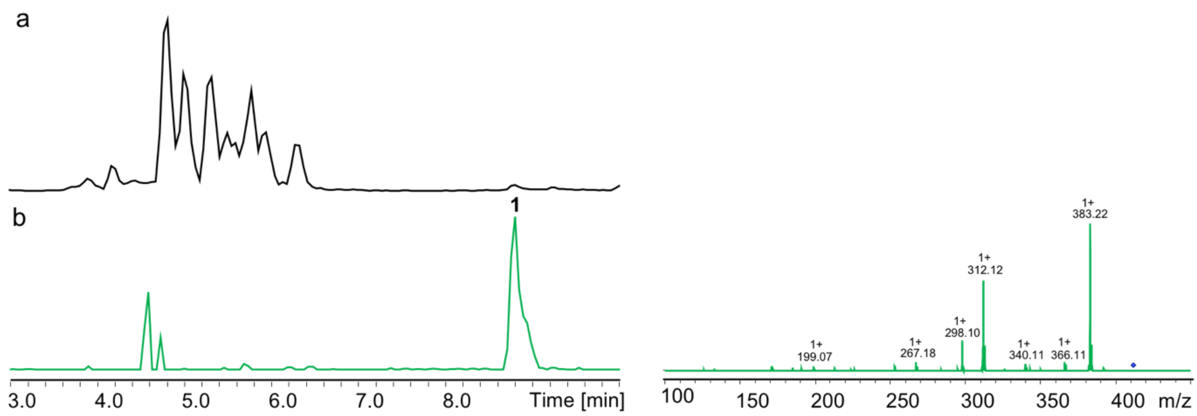


Figure S30. HPLC/MS data refers to Figure 6 (NRPS-38) of compound **1** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **1** (m/z $[M+H]^+$ = 411.29).

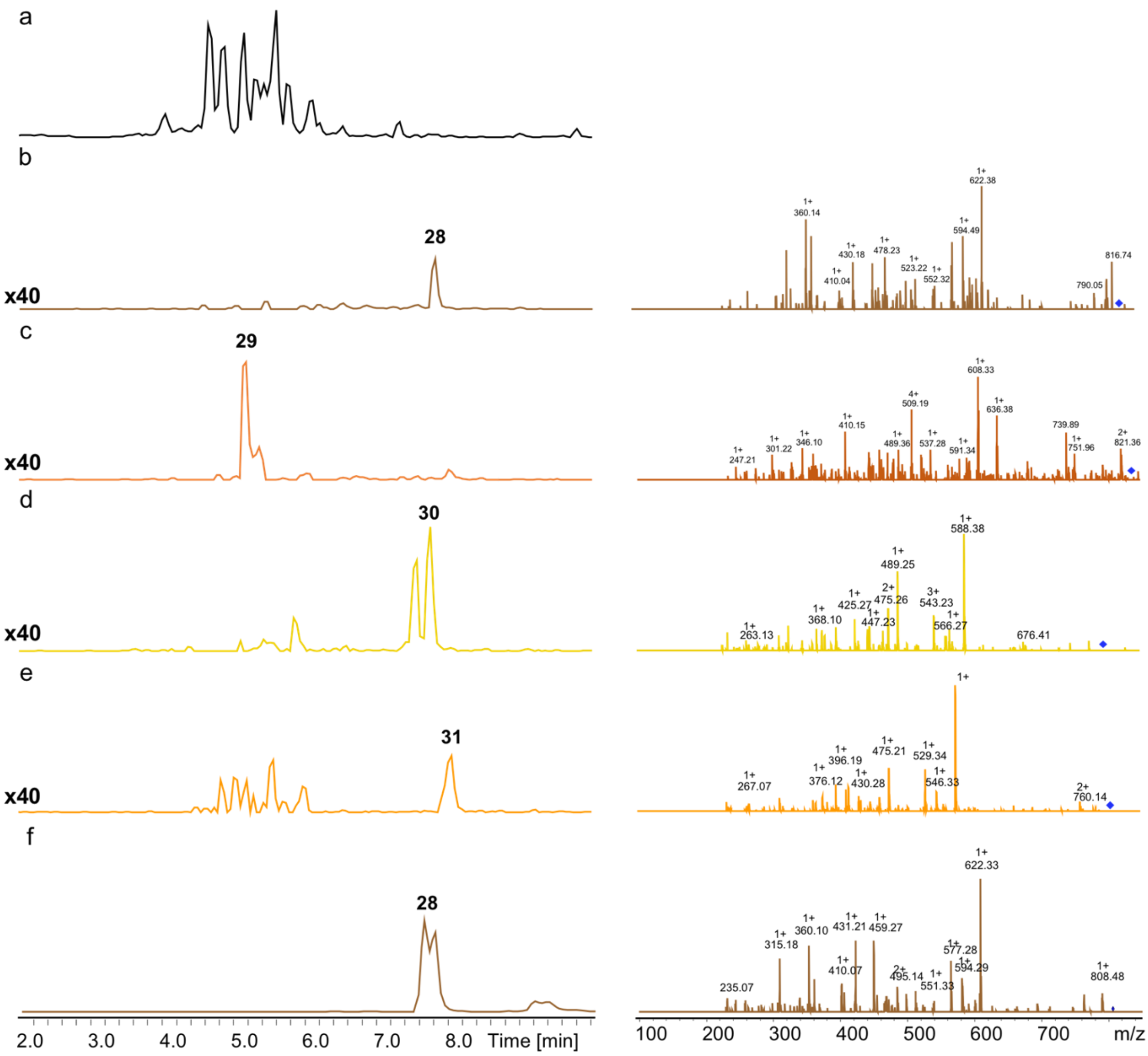


Figure S31. HPLC/MS data refers to Figure 6 (NRPS-39) of compounds **28**, **29**, **30** and **31** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **28** (m/z $[M+H]^+$ = 826.45). (c) EIC/MS^2 data of **29** (m/z $[M+H]^+$ = 840.47). (d) EIC/MS^2 data of **30** (m/z $[M+H]^+$ = 792.47). (e) EIC/MS^2 data of **31** (m/z $[M+H]^+$ = 806.48). (f) EIC/MS^2 data of synthetic **28** (m/z $[M+H]^+$ = 826.45).

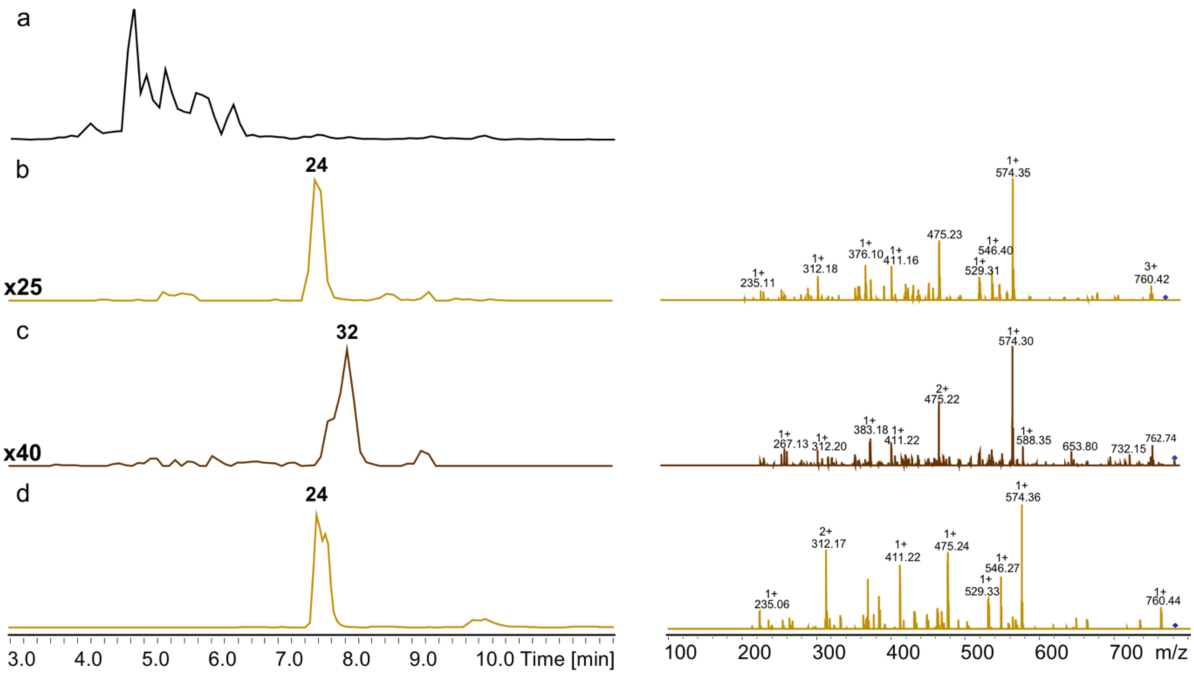


Figure S32. HPLC/MS data refers to Figure 6 (NRPS-40) of compounds **24** and **32** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **24** (m/z $[M+H]^+$ = 778.45). (c) EIC/MS^2 data of **32** (m/z $[M+H]^+$ = 792.47). (d) EIC/MS^2 data of synthetic **24** (m/z $[M+H]^+$ = 778.45).

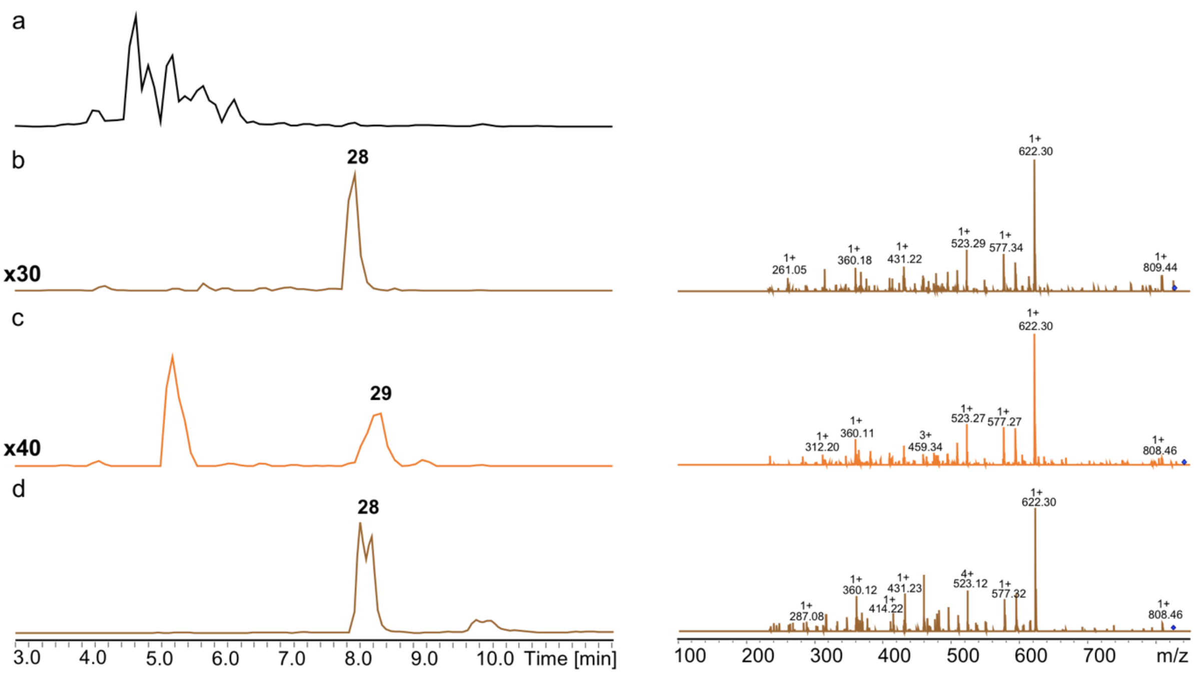


Figure S33. HPLC/MS data refers to Figure 6 (NRPS-41) of compounds **28** and **29** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **28** (m/z $[M+H]^+$ = 826.45). (c) EIC/MS^2 data of **29** (m/z $[M+H]^+$ = 840.47). (d) EIC/MS^2 data of synthetic **28** (m/z $[M+H]^+$ = 826.45).

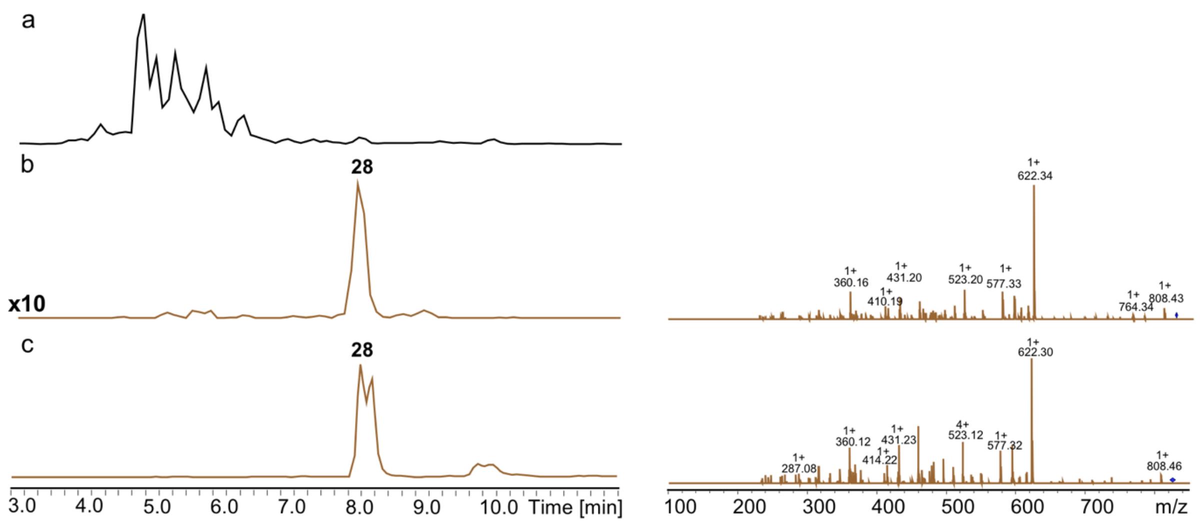


Figure S34. HPLC/MS data refers to Figure 6 (NRPS-42) of compound **28** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **28** (m/z $[M+H]^+$ = 826.45). (c) E EIC/MS^2 data of synthetic **28** (m/z $[M+H]^+$ = 826.45).

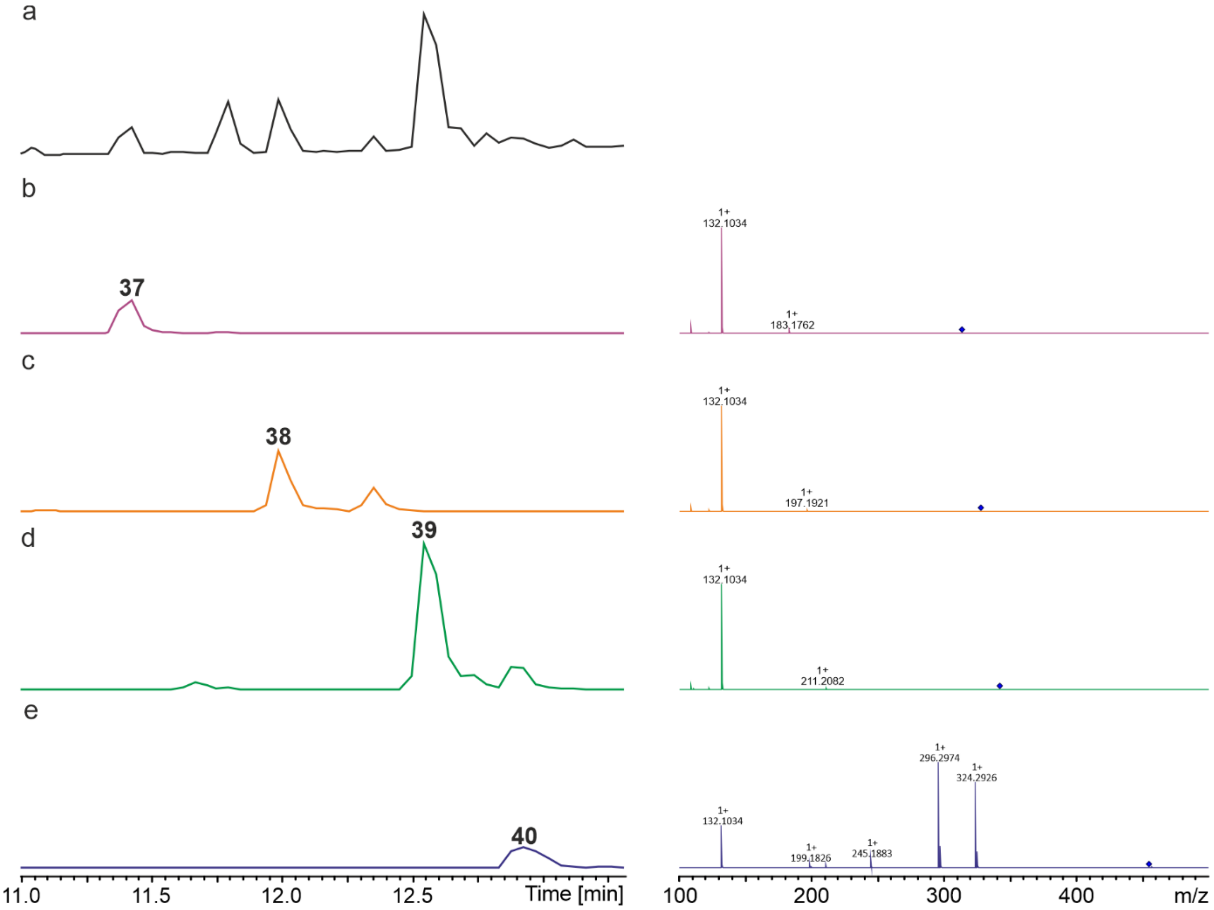


Figure S35. HPLC/MS data refers to Supplementary Figure 16 (NRPS-43) of compounds **37**, **38**, **39** and **40** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **37** (m/z $[M+H]^+$ = 314.27). (c) E EIC/MS^2 data of **38** (m/z $[M+H]^+$ = 328.29). (d) E EIC/MS^2 data of **39** (m/z $[M+H]^+$ = 342.20). (e) E EIC/MS^2 data of **40** (m/z $[M+H]^+$ = 455.38).

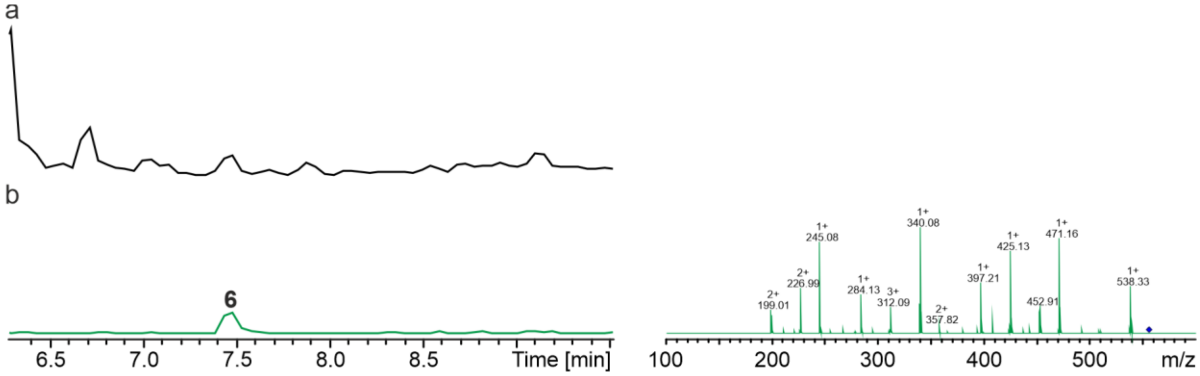


Figure S36. HPLC/MS data refers to Supplementary Figure 16 (NRPS-44) of compound **6** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **6** (m/z $[M+H]^+$ = 556.35).

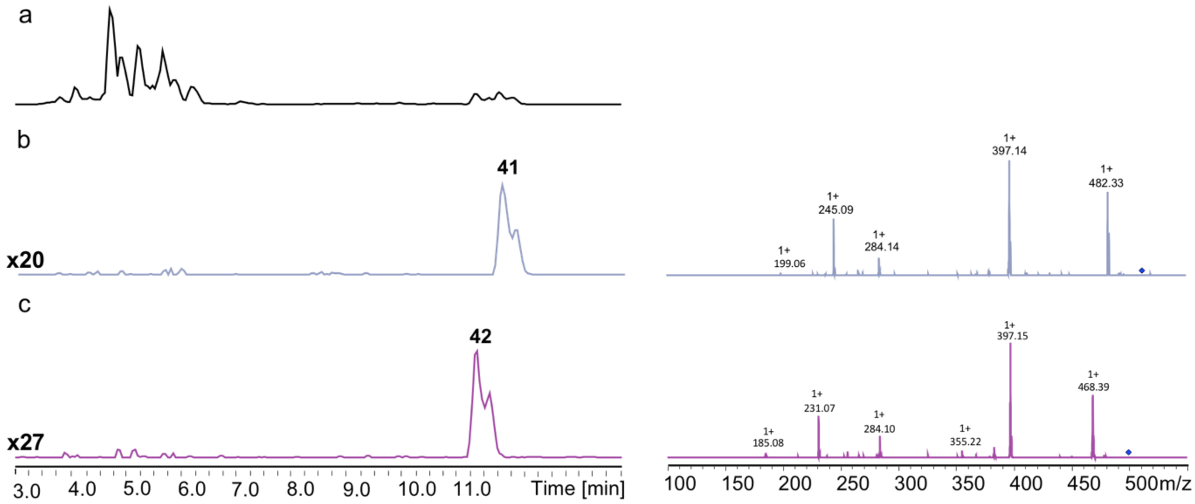


Figure S37. HPLC/MS data refers to Supplementary Figure 17 (NRPS-47) of compounds **41** and **42** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **41** (m/z $[M+H]^+$ = 510.39). (c) E EIC/MS^2 data of **42** (m/z $[M+H]^+$ = 496.37).

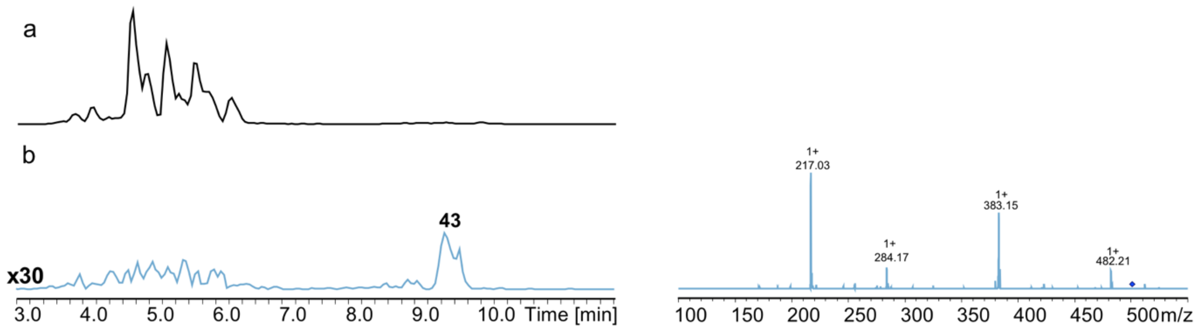


Figure S38. HPLC/MS data refers to Supplementary Figure 17 (NRPS-48) of compound **43** produced in *E. coli* DH10B::*mtaA*. (a) Base Peak Chromatogram (BPC) of an exemplary culture extract. (b) EIC/MS^2 data of **43** (m/z $[M+H]^+$ = 500.37).

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