Supporting Information for:

Expanding the Natural Products Heterologous Expression Repertoire in the Model Cyanobacterium *Anabaena* sp. Strain PCC 7120: Production of Pendolmycin and Teleocidin B-4.

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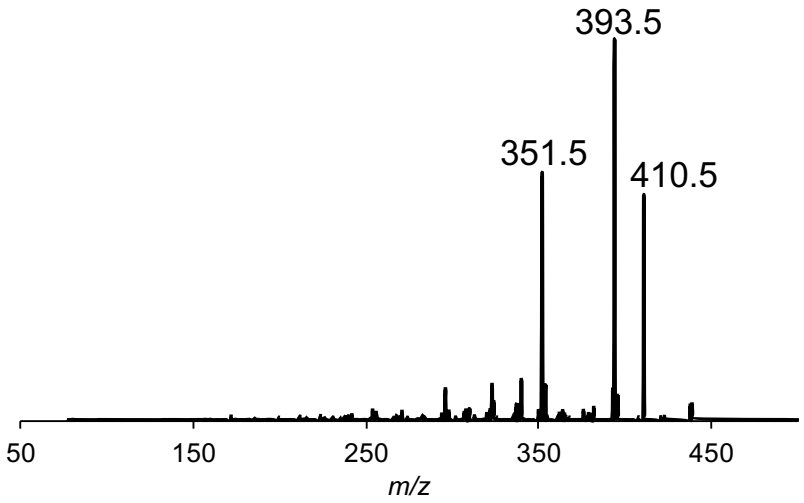

Figure S1: MS/MS spectra (positive ionization mode, 438.3, collision energy, 45.0) of lyngbyatoxin A produced by *Anabaena* 7120 containing pPJAV642.

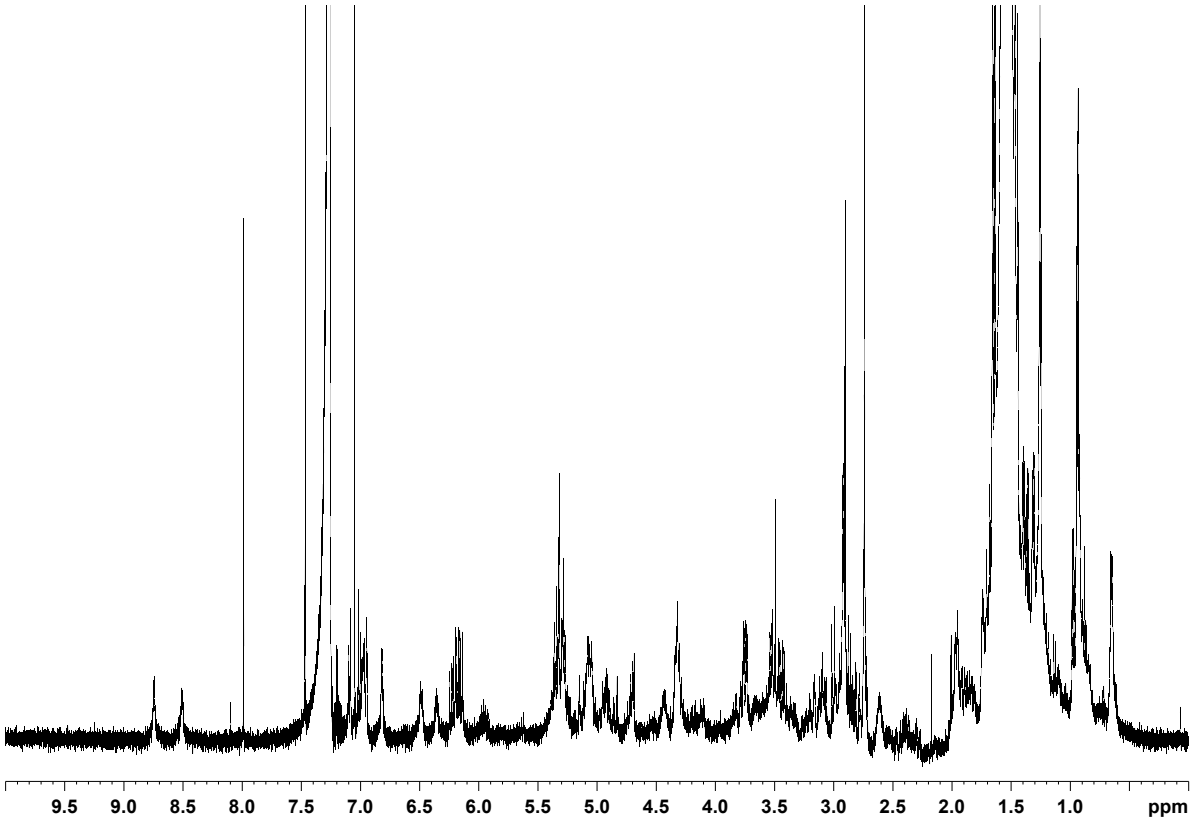

Figure S2: ^{1}H NMR spectrum (500 MHz) of lyngbyatoxin A isolated from *Anabaena* 7120 containing pPJAV647 (P_{glnA}-*ltxABtleC*).

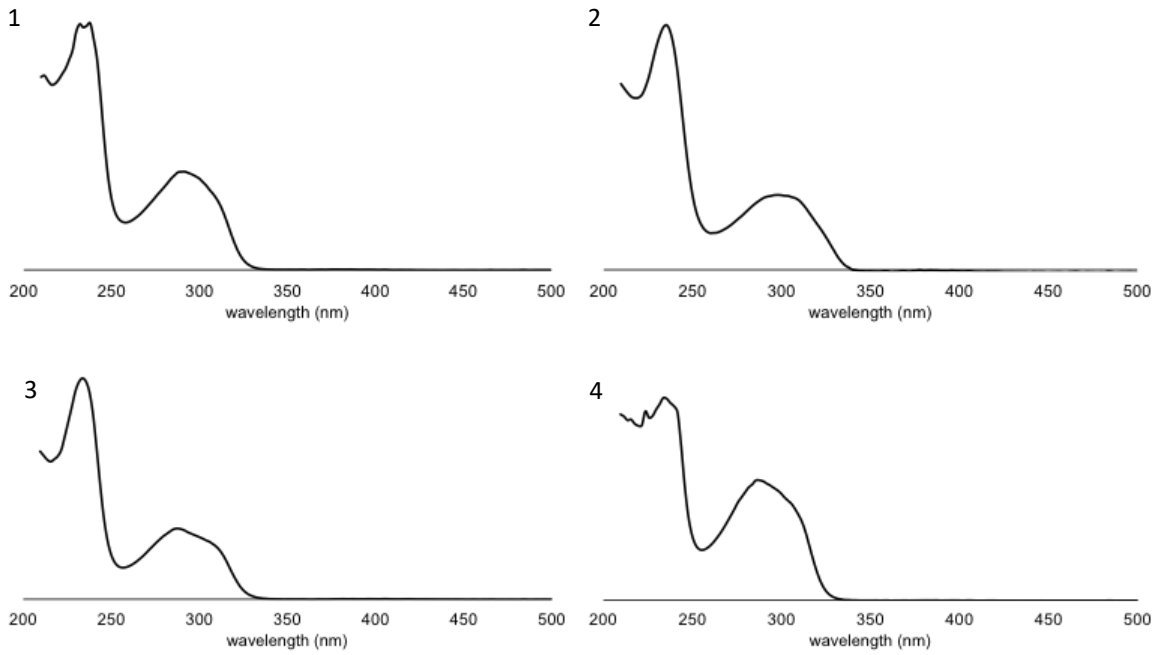

Figure S3: UV spectra of the four compounds produced by *Anabaena* 7120 containing pPJAV644 (P_{ltxA}-*ltxABC-tleD*). The numbers indicate the order of elution seen in Figure S7.

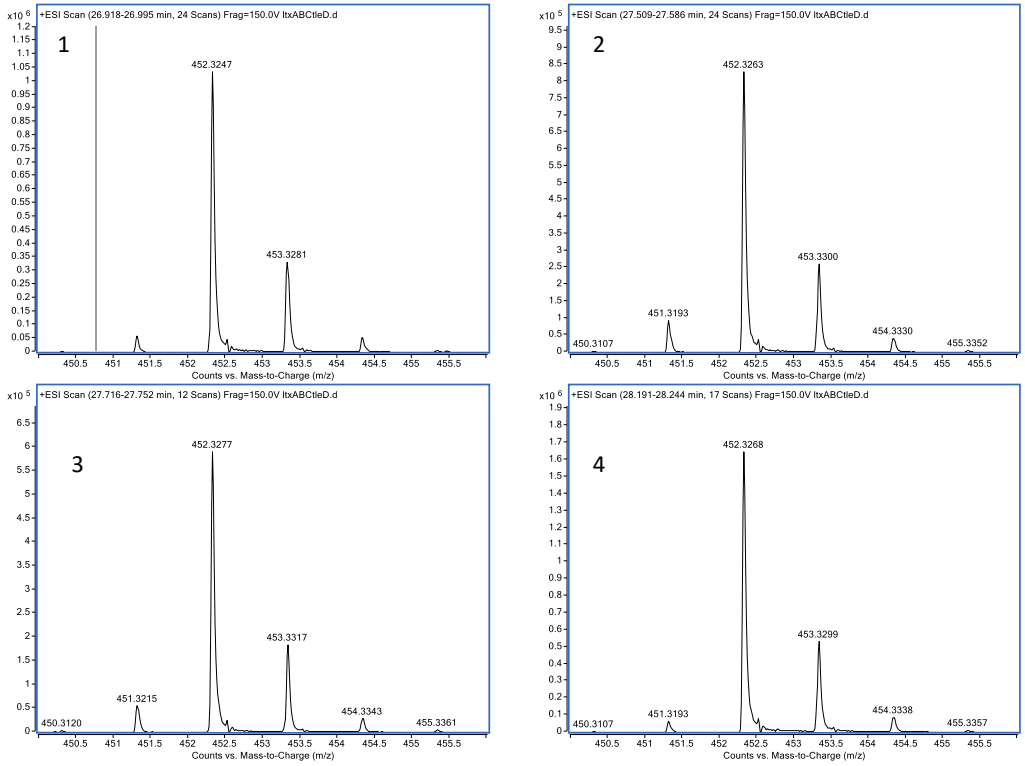

Figure S4: High resolution ESIMS spectra (positive ionization mode) for the four compounds produced by *Anabaena* 7120 containing pPJAV644 (P_{ltxA}-*ltxABC*-*tleD*). The numbers indicate the order of elution seen in Figure S7.

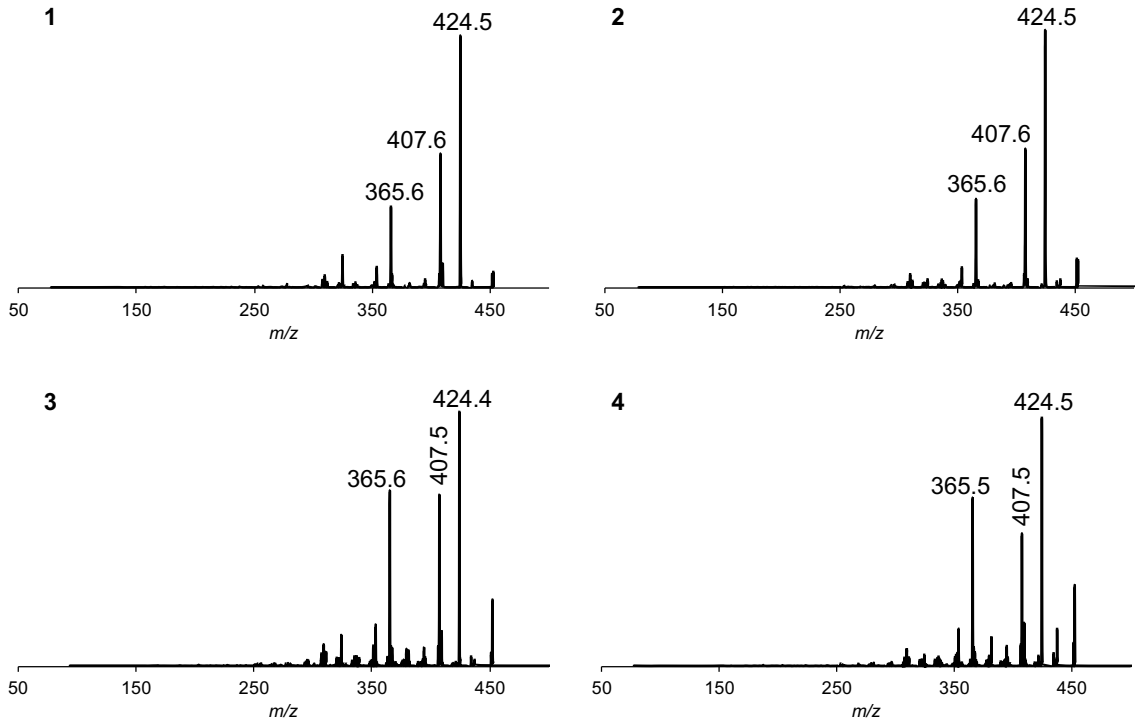

Figure S5: MS/MS spectra (positive ionization mode, 452.3, collision energy, 45.0) for the four compounds produced by *Anabaena* 7120 containing pPJAV644 (P_{ltxA}-*ltxABC-tleD*). The numbers indicate the order of elution seen in Figure S7.

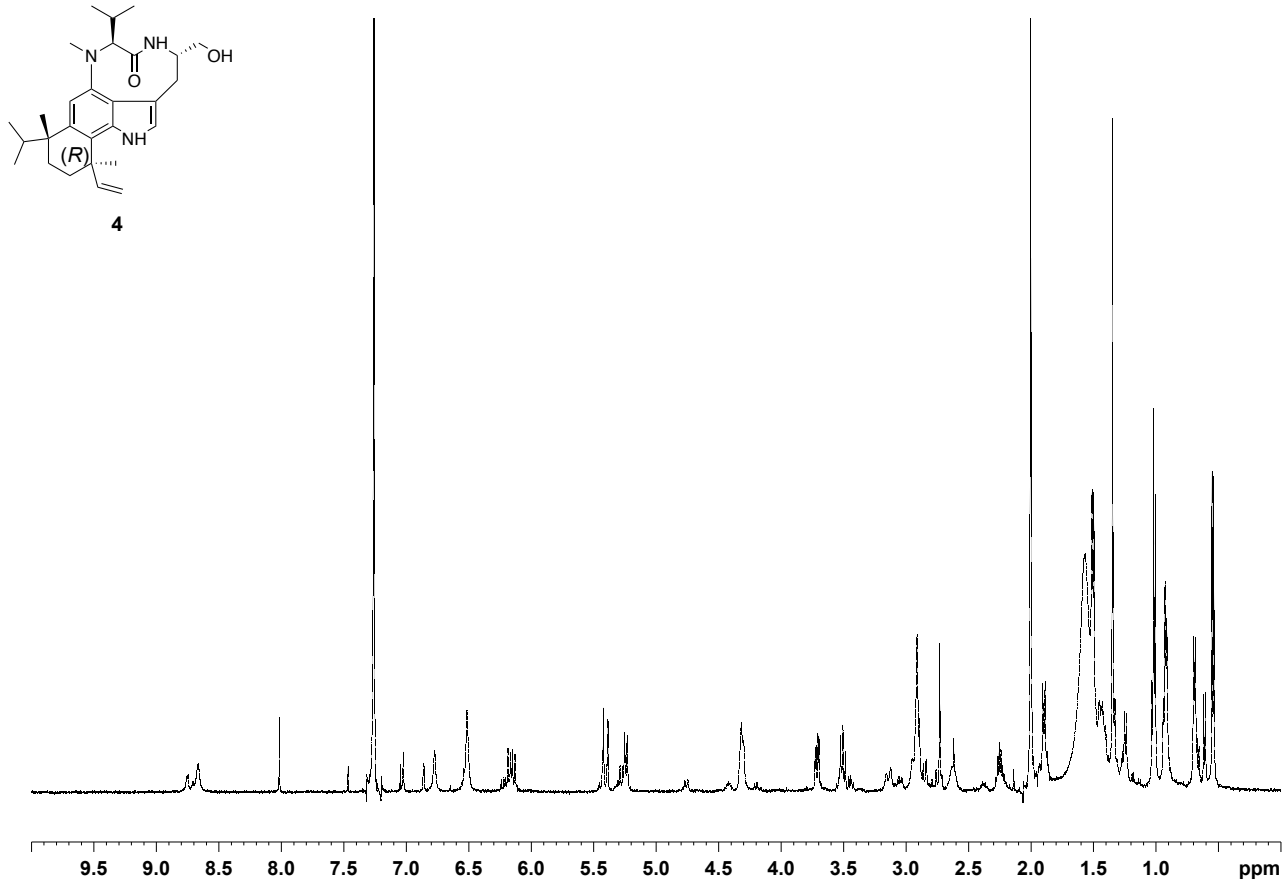

Figure S6: ^{1}H NMR spectrum (500 MHz) of teleocidin B-4 (**4**) isolated from *Anabaena* 7120 containing pPJAV647 (P_{glnA}-*ltxABC*-*tleD*).

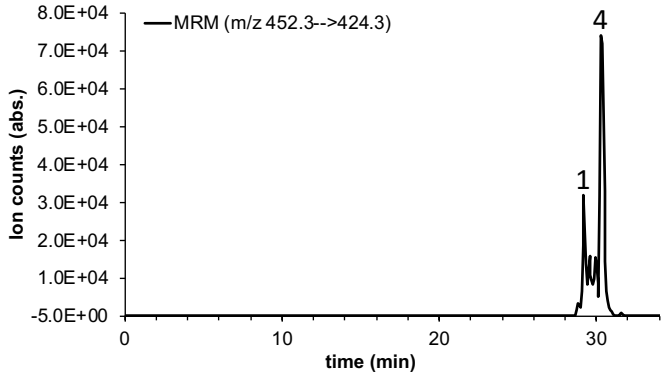

Figure S7: Representative LCMS chromatogram demonstrating the ratio of compounds produced in *Anabaena* 7120 containing pPJAV644 (P_{ltxA}-*ltxABC-tleD*). The extracted ion chromatogram was generated from the MS/MS transition established for teleocidin B (*m/z* 452.3→ 424.3). This is representative chromatogram from a single analysis, but the additional replicate runs appear similar.

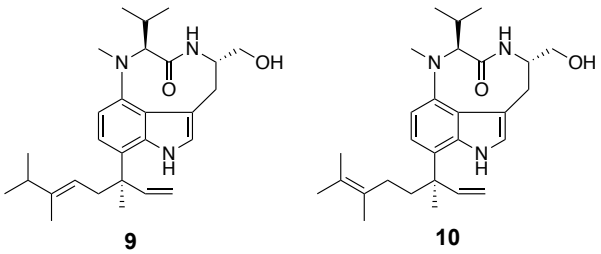

Figure S8: Potential structures of the shunt metabolites resulting from methylation by TleD followed by deprotonation.

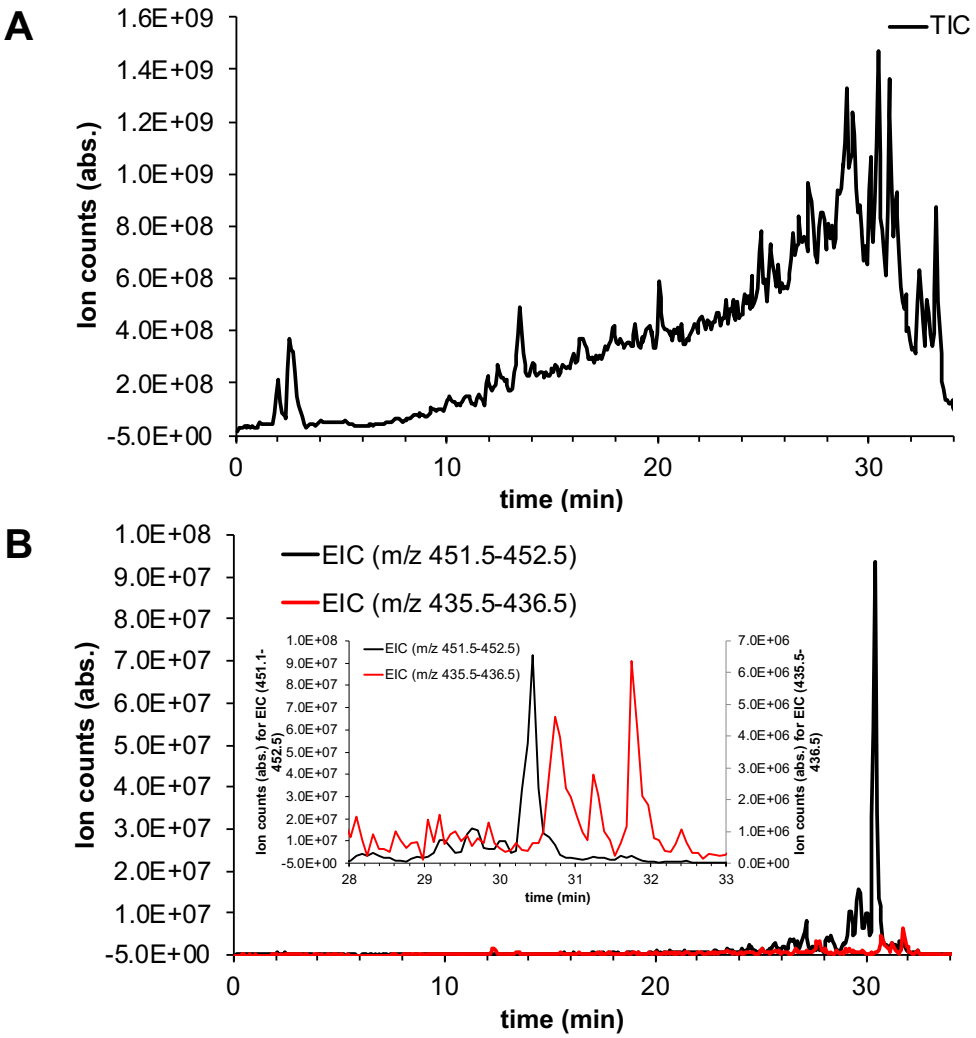

Figure S9: Representative LCMS chromatogram demonstrating the production of compounds with a *m/z* of 452 compared to *m/z* 436 produced by *Anabaena* 7120 containing pPJAV644 (P_{ltxA}-*ltxABC-tleD*). A. Total ion current chromatogram. B. Extracted ion chromatogram for "teleocidin B" (*m/z* 451.5-452.5), black trace; and "imido-teleocidin B-4" (*m/z* 435.5-436.5), red trace. Insert is the zoomed in region of panel B from 28-33 min.

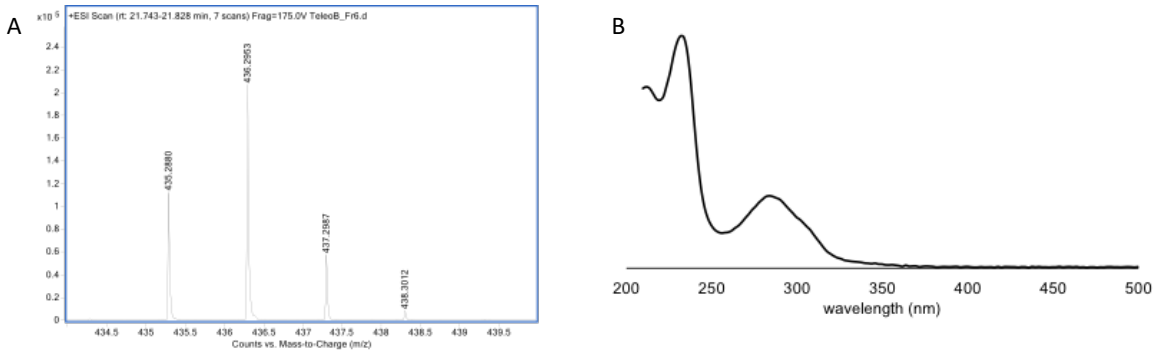

Figure S10: A. High resolution ESIMS spectra (positive ionization mode) for compound **8**; B. UV-vis spectrum of compound **8**.

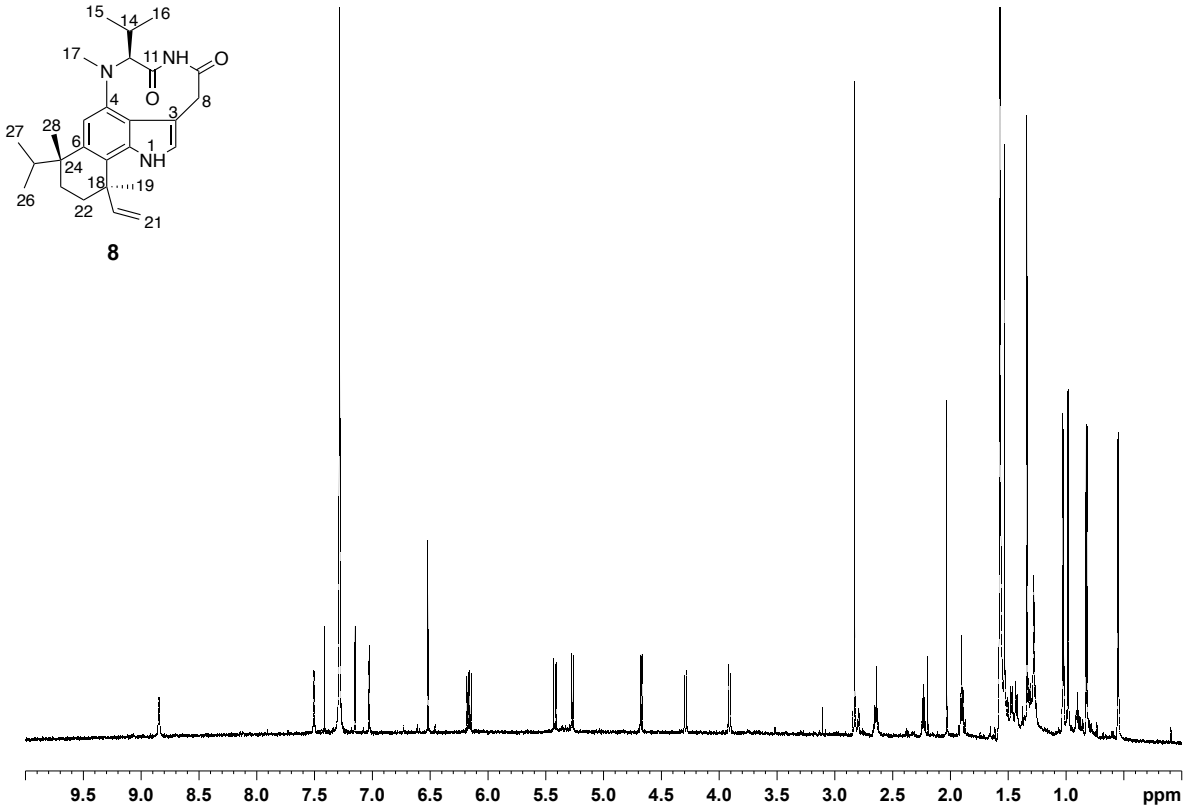

Figure S11: ^{1}H NMR (800 MHz) spectra of compound **8**.

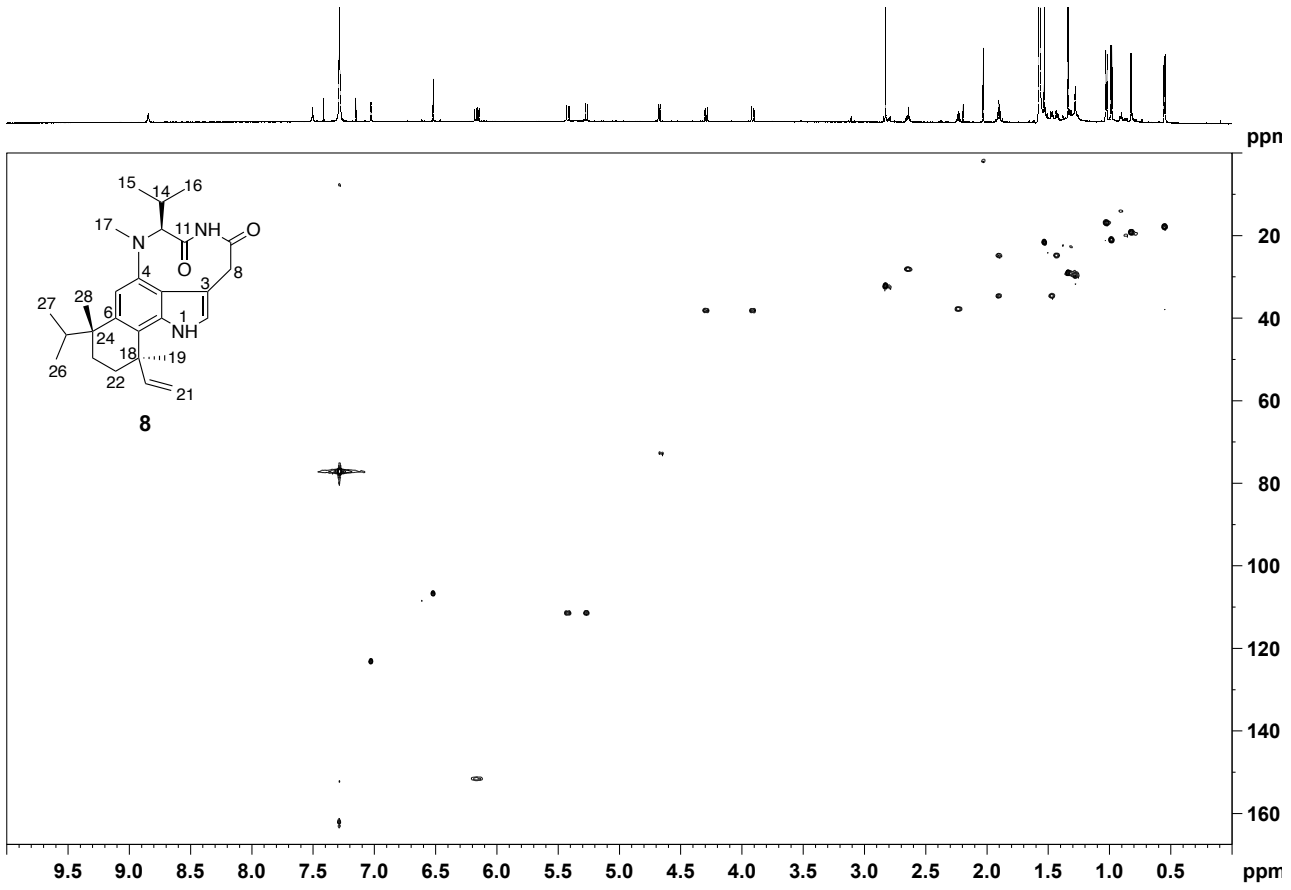

Figure S12: ^{1}H-^{13}C HSQC spectra of compound **8**.

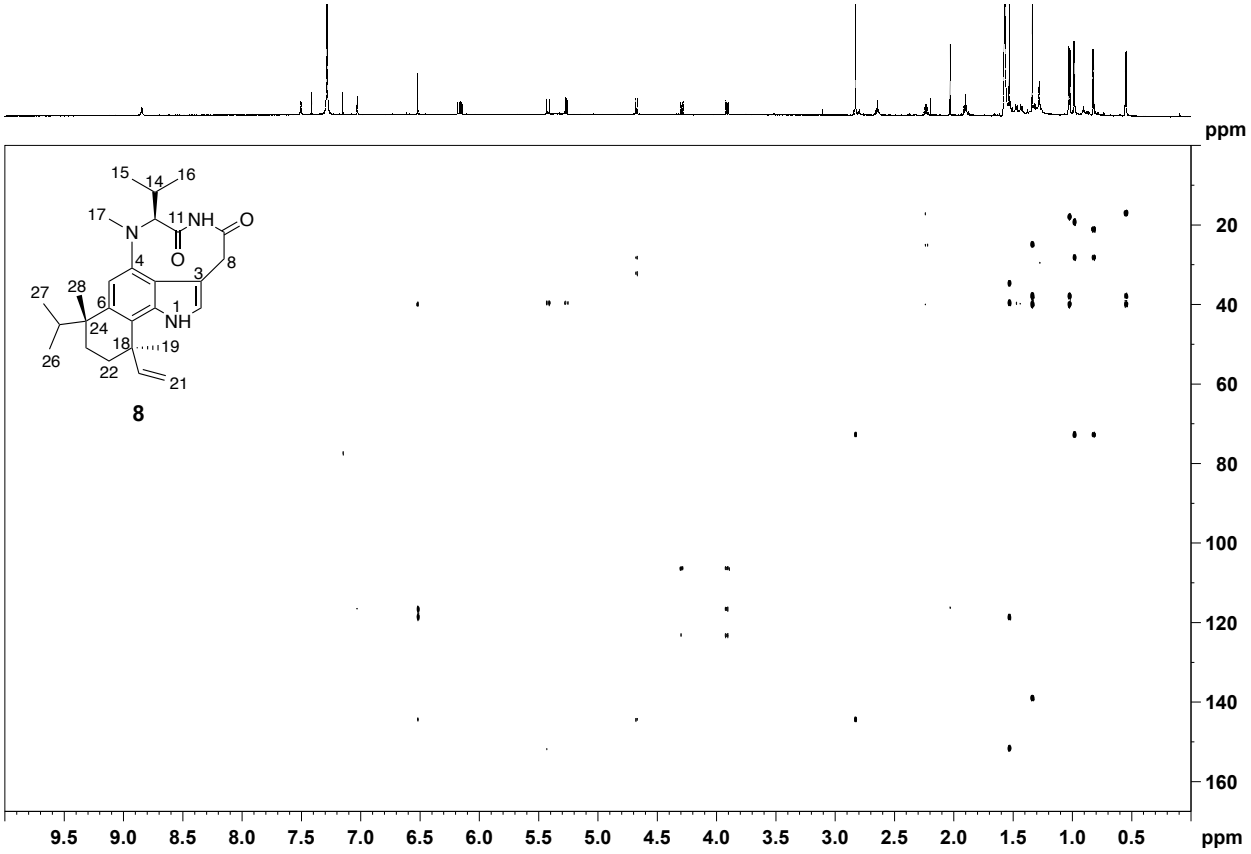

Figure S13: ^{1}H-^{13}C HMBC spectra of compound **8**.

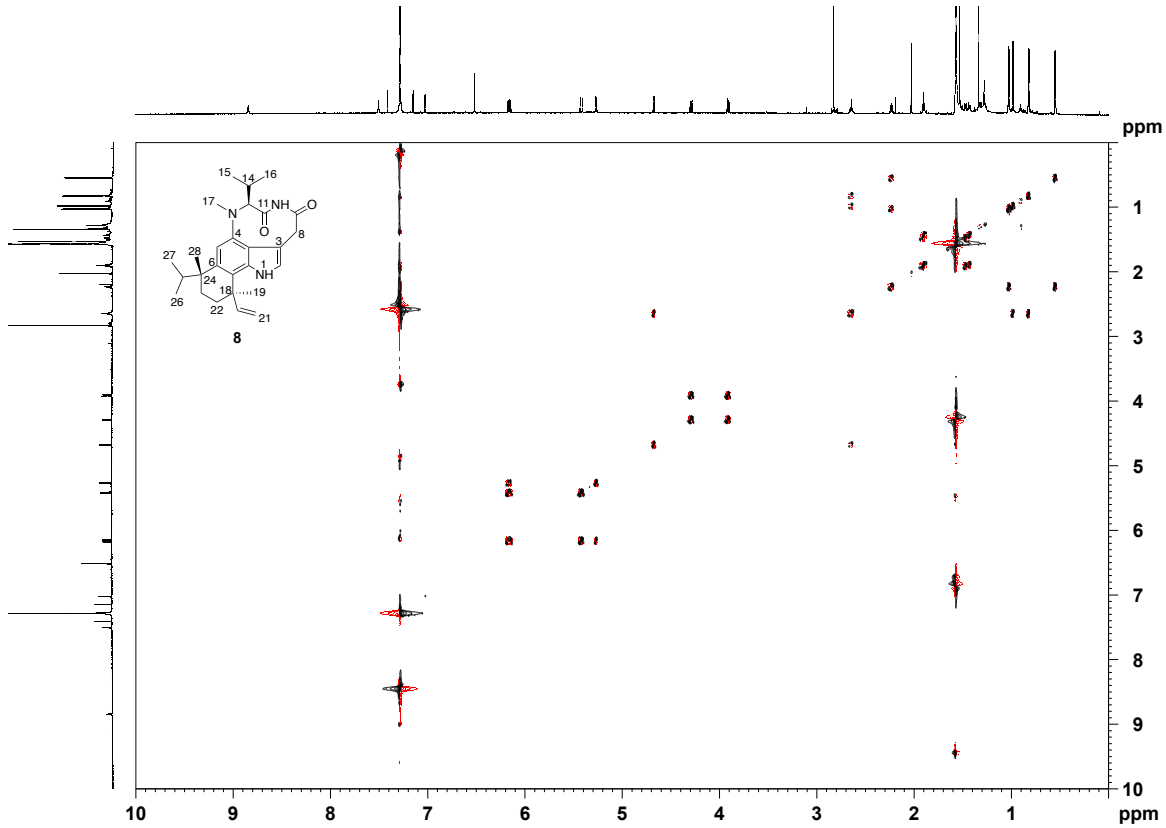

Figure S14: ^{1}H-^{1}H COSY spectra of compound **8**.

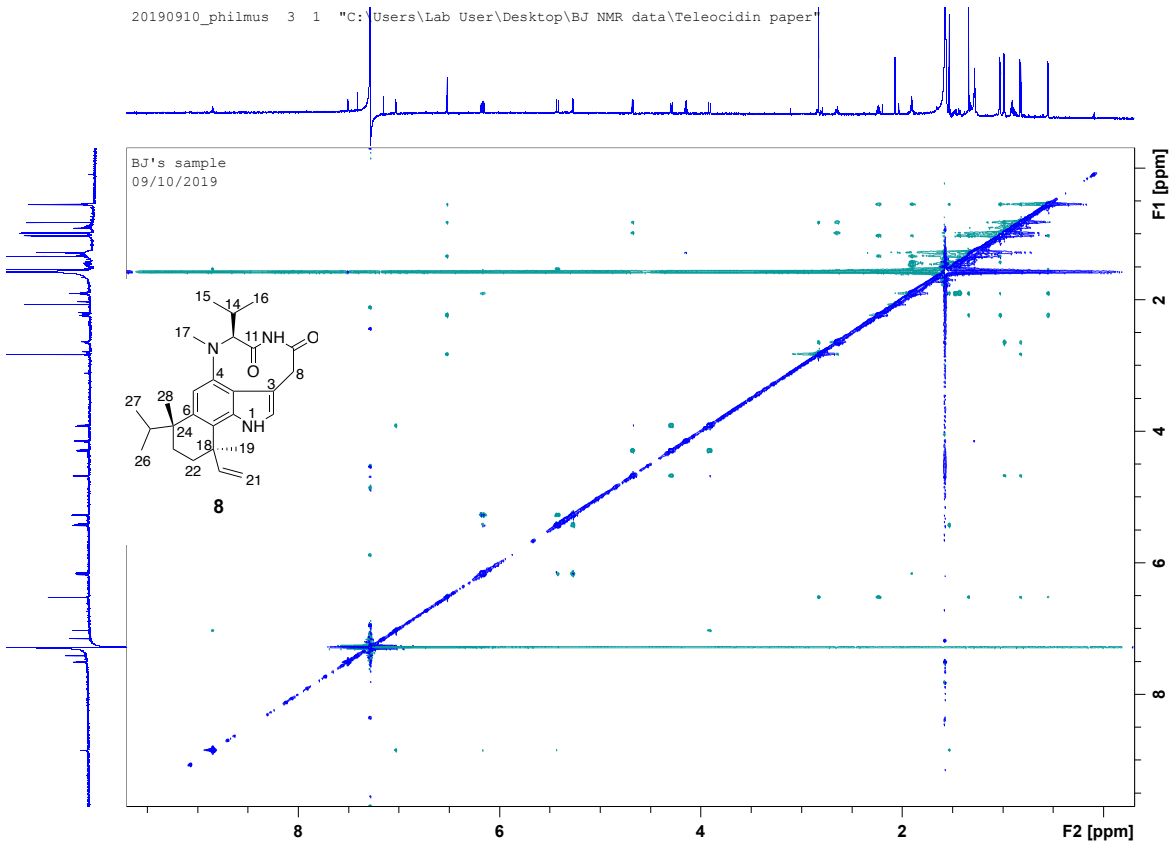

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Figure S15: ^{1}H-^{1}H NOESY spectra (mixing time, 600 ms) of compound **8**.

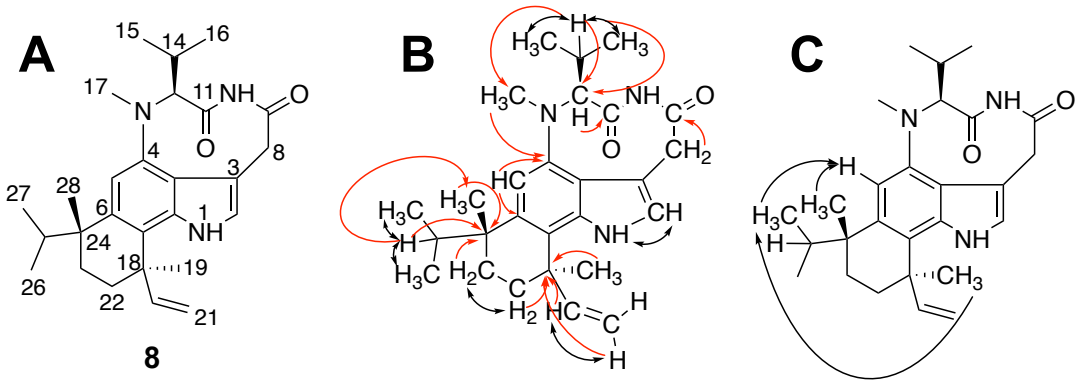

Figure S16: Structure of the isolated degradation product (**8**) derived from teleocidin B-4 with atom numbering (panel A); major HMBC (red) and COSY (black) correlations observed (panel B); and key NOESY correlations observed (panel C).

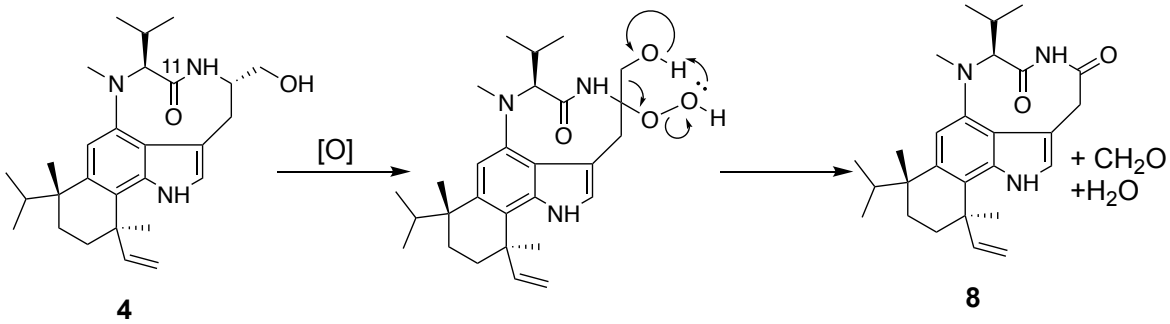

Figure S17: Proposed mechanism for the degradation of teleocidin B-4 (**4**) to compound **8**.

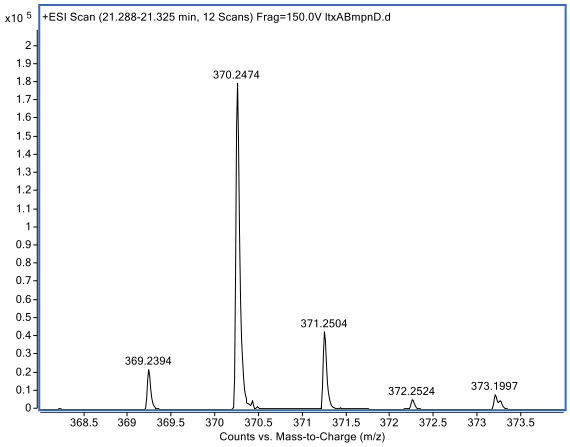

Figure S18: High resolution ESIMS spectra (positive ionization mode) for pendolmycin produced by *Anabaena* 7120 containing pPJAV643 (P_{ltxA}-*ltxAB-mpnD*).

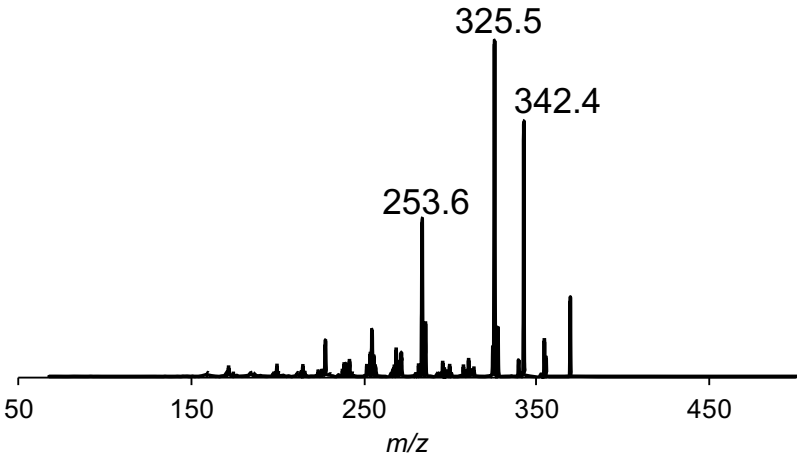

Figure S19: A. MS/MS spectra of ESIMS spectra (positive ionization mode, 370.3, CE 45.0) for pendolmycin (**3**) produced by *Anabaena* 7120 containing pPJAV643 (P_{ltxA}-*ltxAB-mpnD*).

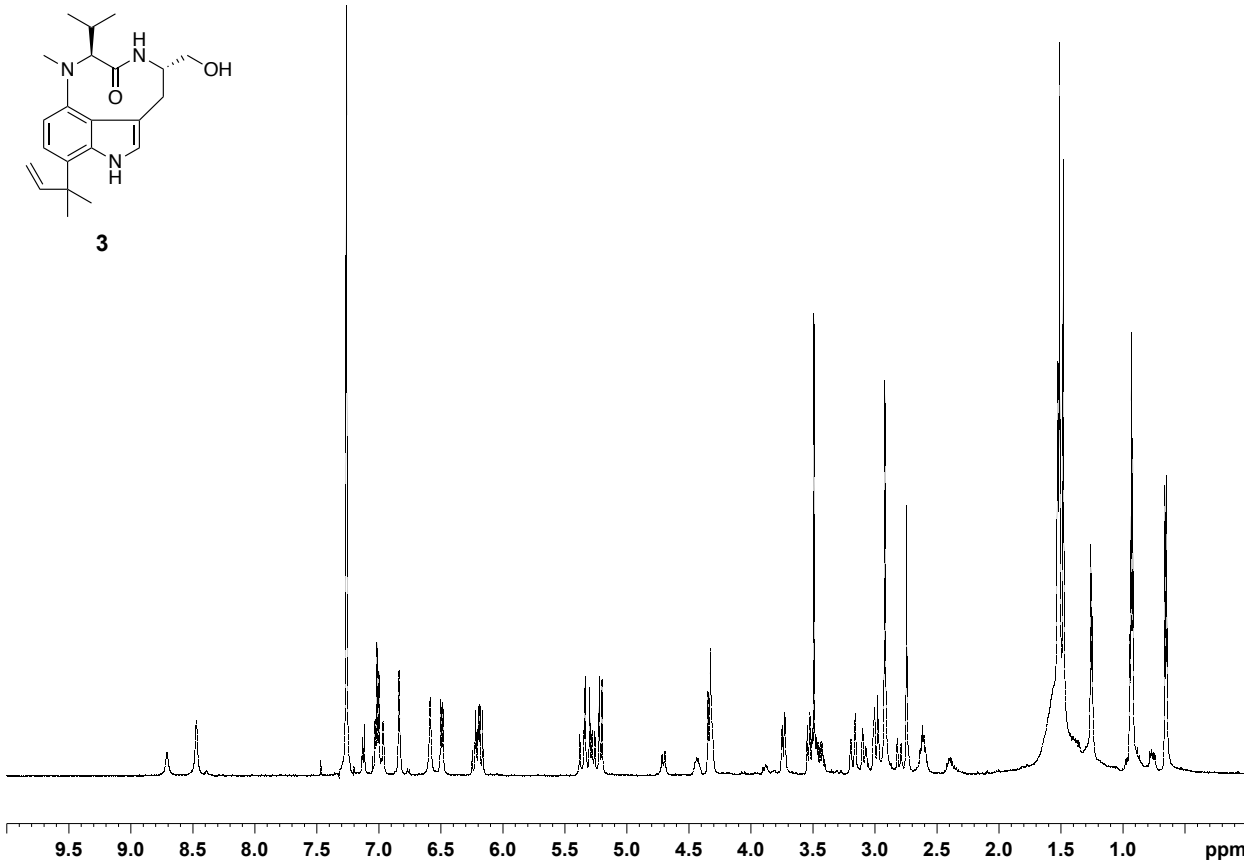

Figure S20: ^{1}H NMR spectrum (500 MHz) of pendolmycin (**3**) isolated from *Anabaena* 7120 containing pPJAV659 (P_{glnA}-*ltxAB-mpnD*).

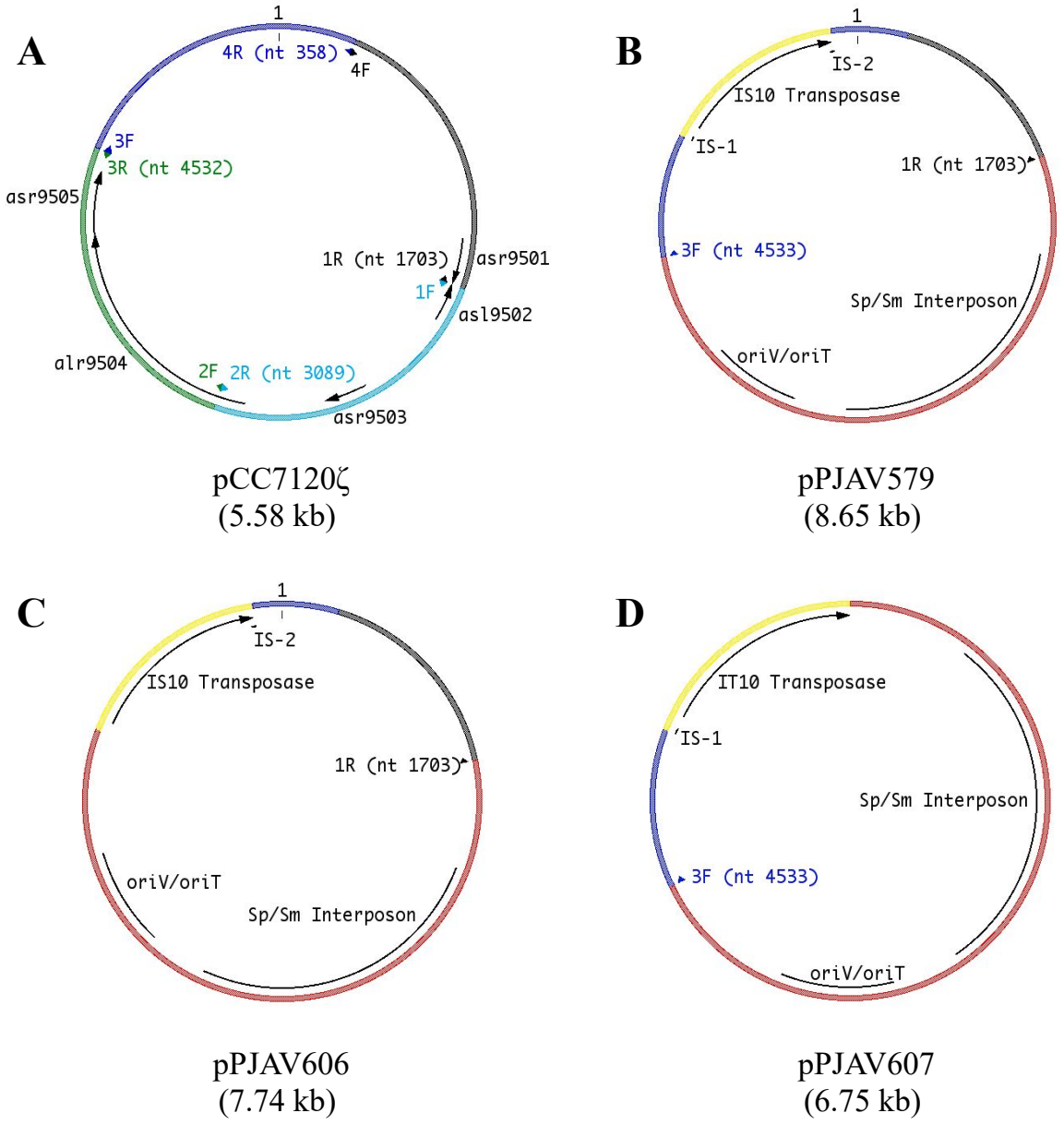

Figure S21: Diagram of plasmids created to define the pCC7120ζ origin of replication (oriV). pCC7120ζ is presented with the primers (small arrows labeled 1F/R through 4F/R; the primer names begin with zeta-SmaI followed by the number in Table S9) utilized to cut the pCC7120ζ into sections (A). Reverse primer names include the last nucleotide they amplify. The colors of sections correspond with the colors of the primers and are maintained throughout the figure for the portions capable of replication in *Anabaena* 7120. The starting point of the nucleic acid numbering on pCC7120ζ is denoted by a 1. Annotated gene names are given outside the plasmid. pPJAV579 was reisolated from *Anabaena* 7120 based on its ability to replicate in the host (B). pPJAV579 was subcloned into pPJAV606 (C) and pPJAV607 (D) in which part of the pCC7120ζ DNA was removed along with the proximal insertion sequence (IS-1 or IS-2). Origin of transfer (oriT); Sp/Sm Interposon (spectinomycin and streptomycin resistance from pDW9).

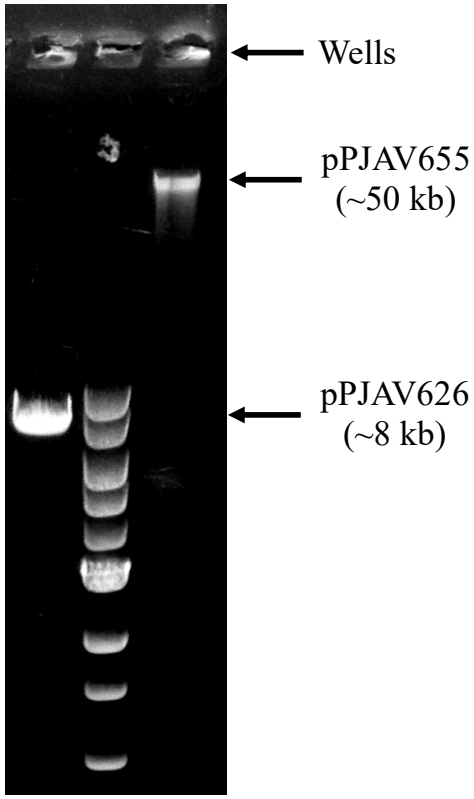

Figure S22: Depiction of pPJAV655 (pPJAV606 with the entire *Moorea producens* fragment from fos-DE3-86; lane 3) and pPJAV626 (the vector used for cloning; lane 1) as compared to the Quick-Load 1 kb DNA Ladder (New England Biolabs; lane 2) run on a 0.6% agarose gel. The bands of the ladder from top to bottom: 10 kb, 8 kb, 6 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1.5 kb, 1 kb. The plasmid DNA was isolated from *E. coli* NEB10beta.

Table S1: NMR data (500 MHz) for teleocidin B-4 (**4**) collected from *Anabaena* 7120 containing pPJAV657 (P_{glnA}-*ltxABC-tleD*) in $CDCl_3$ (δ in ppm, J in Hz).

Atom number	δH (this work)	δC (this work)[a]	δH (from ref. 1)	δC (from ref. 1)
1	8.67 (s)	-	8.65 (s)	-
2	6.77 (s)	120.8	6.76 (s)	120.8
5	6.52 (s)	106.2	6.49 (s)	106.2
8a	3.05 (dd, 14.6, 5.0)	33.3	3.11 (br d, 17.2)	33.8
8b	2.98 (dd, 18.0, 8.7)		2.94 (dd, 17.7, 4.0)	
9	4.34 (m)	55.7	4.30 (m)	55.8
10	7.02 (s)	-	7.00 (s)	-
12	4.22 (m)	70.5	4.28 (m)	70.7
14a	3.72 (11.3, 3.9)	64.3	3.70 (dd, 11.45, 4.0)	65.1
14b	3.54 (10.7, 8.8)		3.50 (dd, 11.45, 8.6)	
15	2.63 (m)	28.1	2.60 (m)	28.4
16	0.69 (d, 6.83)	19.5	0.67 (d, 6.85)	19.6
17	0.92 (6.56)	21.4	0.89 (d, 6.3)	21.6
18	2.75 (s)	35.2	2.88 (s)	32.9
20	1.50 (s)	21.4	1.49 (s)	21.5
21	6.15 (dd, 10.4, 17.5)	151.5	6.14 (dd, 10.8, 18.0)	151.8
22a	5.41(d, 17.5)	111.3	5.39 (d, 18.0)	111.3
22b	5.25 (d, 10.6)		5.22 (d, 10.8)	
23	1.90 (m), 1.45 (m)	34.6	1.90 (m), 1.47 (m)	34.7
24	1.90 (m), 1.42 (m)	24.7	1.90 (m), 1.42 (m)	24.9
26	2.25 (m)	37.5	2.23 (m)	37.8
27	0.55 (d, 6.8)	17.7	0.52 (d, 6.85)	17.9
28	1.02 (d, 6.7)	16.7	0.99 (d, 6.85)	16.9
29	1.35 (s)	29.0	1.33 (s)	29.1

[a] ^{13}C NMR shifts determined through use of an ^{1}H-^{13}C HSQC experiment; -, no HSQC signal observed due to the fact that this is a nitrogen bound hydrogen.

Table S2: Production of lyngbyatoxin A (**2**) in *Anabaena* 7120. Values are given as ng/mg dried cell mass ± standard deviation. Each replicate culture was grown on BG-11 agar media containing 1.5% agar (1 plate = approximately 40 mL).

	BG-11(NH4+)	**BG-11(Nit)**
361	0	0
361 fruc	0	0
Zeta	0	0
Zeta fruc	0	0
LtxABC	49.0 ± 12.1	7.2 ± 2.0
LtxABC fruc	98.1 ± 13.1	8.5 ± 6.5
LtxAB-TleC	47.8 ± 21.1	9.2 ± 3.2
LtxAB-TleC fruc	88.0 ± 28.0	n.o.
PglnA LtxABC	252.5 ± 34.7	203.1 ± 176.6
PglnA LtxABC fruc	181.9 ± 15.7	96.8 ± 53.4
PglnA LtxAB-TleC	71.0 ± 35.8	240.6 ± 104.6
PglnA LtxAB-TleC fruc	313.3 ± 64.1	174.3 ± 114.3
PglnA LtxABC-TleD	47.8 ± 15.2	74.9 ± 8.1
PglnA LtxABC-TleD fruc	20.3 ± 5.5	143.8 ± 52.5
LtxABC zeta-TleD	—	7.4 ± 4.7

fruc[a], denotes media containing 50 mM fructose; —, none observed. BG-11(NH4+), BG-11 media with ammonium chloride as the nitrogen source; BG-11(Nit), BG-11 media with sodium nitrate as the nitrogen source.

Table S3: Production of the individual teleocidin B-like compounds in *Anabaena* 7120. Values are given as ng/mg dried cell mass ± standard deviation. Each replicate culture was grown on BG-11 agar media containing 1.5% agar (1 plate = approximately 40 mL) with the nitrogen source listed in the table column.

	BG-11(NH4+)				**BG-11(Nit)**			
Plasmid introduced	Peak 1	Peak 2	Peak 3	Peak 4	Peak 1	Peak 2	Peak 3	Peak 4
361	0	0	0	0	0	0	0	0
361 fruc[a]	0	0	0	0	0	0	0	0
Zeta	0	0	0	0	0	0	0	0
Zeta fruc	0	0	0	0	0	0	0	0
LtxABC-TleD	9.8 ± 7.3	10.1 ± 4.1	14.1 ± 12.3	22.3 ± 8.8	12.2 ± 1.2	8.3 ± 2.0	7.6 ± 1.2	11.1 ± 2.2
LtxABC-TleD fruc[a]	19.6 ± 4.0	17.2 ± 7.5	1.3 ± 2.3	39.8 ± 10.2	18.9 ± 20.3	17.0 ± 8.8	16.5 ± 7.0	18.5 ± 6.9
LtxABC zeta-TleD	32.6 ± 15.5	7.9 ± 10.8	1.9 ± 3.3	59.0 ± 10.0	13.2 ± 22.9	0	25.2 ± 15.1	5.1 ± 8.8
LtxABC zeta-TleD fruc[a]	55.4 ± 21.8	4.6 ± 8.0	8.5 ± 2.5	69.2 ± 11.2	0	0	0	2.7 ± 2.6
PglnA LtxABC-TleD	249.0 ± 68.6	141.8 ± 45.8	98.4 ± 54.5	546.5 ± 23.3	133.9 ±116.5	84.6 ± 29.6	76.3 ± 19.3	453.0 ± 108.7
PglnA LtxABC-TleD fruc[a]	54.5 ± 8.4	21.4 ± 3.3	29.6 ± 16.0	137.7 ± 11.2	277.4 ± 47.0	102.1 ± 6.9	55.5 ± 16.9	101.0 ± 34.3

fruc[a], denotes media containing 50 mM fructose. BG-11(NH4+), BG-11 media with ammonium chloride as the nitrogen source; BG-11(Nit), BG-11 media with sodium nitrate as the nitrogen source.

Table S4: Dried cell mass values obtained during growth of *Anabaena* 7120. Each replicate culture was grown on BG-11 agar media containing 1.5% agar (1 plate = approximately 40 mL) with the nitrogen source listed in the table column.

Strain	**BG-11(NH4+)** **Dried cell mass (mg) ± S.D.**	**BG-11(Nit)** **Dried cell mass (mg) ± S.D.**
pPJAV361 (empty vector)	22.9 ± 4.0	156.2 ± 77.8
pPJAV361 (empty vector) fruc[a]	27.8 ± 3.1	149.9 ± 18.4
Zeta (empty vector)	29.5 ± 4.1	33.2 ± 4.3
Zeta (empty vector) fruc[a]	33.7 ± 6.1	87.5 ± 14.0
LtxABC	15.8 ± 5.5	108.5 ± 65.0
LtxABC fruc[a]	25.6 ± 6.6	128.6 ± 27.5
LtxABC-TleD	25.1 ± 7.5	120.8 ± 57.1
LtxABC-TleD fruc[a]	15.4 ± 6.2	71.9 ± 22.4
LtxABC zeta-TleD	25.5 ± 7.3	49.7 ± 24.9
LtxABC zeta-TleD fruc[a]	30.2 ± 3.8	122.1 ± 7.1
PglnA LtxABC-TleD	28.9 ± 11.7	51.3 ± 32.3
PglnA LtxABC-TleD fruc[a]	44.7 ± 3.9	106.8 ± 11.0
PglnA LtxAB-TleC-TleD	51.5 ± 4.1	45.1 ± 9.8
LtxAB-MpnD	11.5 ± 8.0	82.1 ± 13.3
LtxAB-MpnD fruc[a]	13.4 ± 0.8	174.6 ± 65.2
LtxAB-MpnD-TleD	13.7 ± 1.6	103.8 ± 20.7
LtxAB-MpnD-TleD fruc[a]	13.6 ± 6.0	52.3 ± 12.8
PglnA LtxAB-MpnD	19.1 ± 2.1	34.0 ± 5.2
PglnA LtxAB-MpnD fruc[a]	46.1 ± 4.1	82.9 ± 20.2

BG-11(NH4+), BG-11 media with ammonium chloride as the nitrogen source; BG-11(Nit), BG-11 media with sodium nitrate as the nitrogen source.

Table S5: NMR data (800 MHz) for compound **8** in $CDCl_3$ (δ in ppm, J in Hz) purified from collected from *Anabaena* 7120 containing pPJAV657 (P_{glnA}-*ltxABC-tleD*).

Atom number	δH	δC[a,b]	HMBC (to C#)	COSY (to H#)
1	8.85 (1H, br s)	-	-	2
2	7.03 (1H, s)	123.3[a]	3, 3a, 7a	1
3	-	106.4[b]	-	-
3a	-	116.8[b]	-	-
4	-	144.4[b]	-	-
5	6.52 (1H, s)	106.9[a]	3a, 4, 6	-
6	-	118.6.[b]	-	-
7	-	138.6[b]	-	-
7a	-	137.4[b]	-	-
8a	4.29 (1H, d, 14.6)	38.2[a]	2, 3, 9	8b
8b	3.9 (1H, d, 14.6)		2, 3, 3a, 9	8a
9	-	172.9[b]	-	-
10	7.50 (1H, br s)	-	-	-
11	-	173.8[b]	-	-
12	4.67 (1H, d, 9.9)	72.7[a]	4, 11, 14, 16, 17	14
14	2.64 (1H, m)	28.2[a]	12, 15, 16	12, 15, 16
15	0.98 (3H, d, 6.4)	21.3[a]	12, 14, 16	14
16	0.82 (3H, d, 6.4)	19.3[a]	12, 14	14
17	2.82 (3H, s)	32.2[a]	4, 12	-
18	-	39.6[b]	-	-
19	1.53 (3H, s)	21.8[a]	6, 18, 20, 22	-
20	6.16 (1H, dd, 17.6, 10.6)	151.5[a]	6, 18, 19	21a, 21b
21a	5.43 (1H, dd,17.6, 0.96)	111.4[a]	18, 20	20
21b	5.27, (1H, dd, 10.9, 0.96)		18	20
22	1.90 (m), 1.47 (1H, m)	34.6[a]	6, 18, 19	23
23	1.90 (m), 1.43 (1H, m)	24.7[a]	7, 24, 28	22
24	-	39.9[b]	-	
25	2.23 (1H, m or p)	37.6[a]	24, 26, 28	26, 27
26	1.02 (3H, d, 6.8)	17.0[a]	24, 25, 27	25
27	0.55 (3H, d, 6.4)	18.0[a]	24, 25, 26	25
28	1.34 (3, s)	29.1	7, 23, 24, 25	-

[a] ^{13}C NMR shifts determined through use of an ^{1}H-^{13}C HSQC experiment; [b] ^{13}C NMR shifts determined through use of an ^{1}H-^{13}C HMBC experiment; -, no signal observed.

Table S6: NMR data (500 MHz) for pendolmycin (**3**) in $CDCl_3$ (δ in ppm, J in Hz).

Atom number	δH (this work)	δC (this work)[a]	δH (from ref. 2)	δC (from ref. 2)
1	8.47	-[b]	8.48 (br s)	-
2	6.83	120.8	6.84 (br s)	121.1
5	6.49 (d, 8.3)	106.2	6.48 (d, 8.0)	106.4
6	7.00 (d, 7.7)	119.1	7.00 (d, 8.0)	119.1
8a	2.99 (dd, 3.7, 16.7)	33.8	3.04 (dd, 4.0, 17.5)	33.9
8b	3.17 (br d, 17.8)	33.8	3.15 (br d, 17.5)	33.9
9	4.43 (m)	54.6	4.32 (m)	55.8
10	7.47 (br s)	-	7.50 (br s)	-
12	4.34 (d, 10.0)	71.1	4.34 (d, 10.0)	71.1
14a	3.53 (dd, 9.7, 10.3)	65.0	3.56 (br dt, 12.0, 3.0)	65.1
14b	3.74 (dd, 4.0, 11.0)	65.0	3.74 (br dt, 12.0, 7.0)	65.1
14-OH	n.o.[c]	-	3.34 (br s)	-
15	2.61 (m)	28.3	2.59 (m, 7.0, 10.0)	28.6
16	0.65 (d, 6.7)	19.4	0.65 (d, 7.0)	19.6
17	0.92 (d, 6.4)	21.4	0.92 (d, 7.0)	21.6
18	2.92 (s)	32.8	2.90 (s)	33.1
20	6.19 (dd, 10.5, 17.5)	149.2	6.19 (dd, 11.0, 17.5)	149.5
21a	5.21 (br d, 10.8)	111.1	5.21 (dd, 1.5, 11.0)	111.3
21b	5.32 (br d, 17.6)	111.1	5.31 (dd, 1.5, 17.5)	111.3
22	1.48 (s)	26.9	1.47 (s)	27.2
23	1.51 (s)	26.7	1.51 (s)	26.8

[a] ^{13}C NMR shifts determined through use of an ^{1}H-^{13}C HSQC experiment; [b] -, no HSQC signal observed due to the fact that this is a nitrogen bound hydrogen, [c] n.o., not observed due to exchange with water.

Table S7: Efficiency of plasmid introduction into *Anabaena* 7120. A culture of *Anabaena* 7120 was prepared for conjugation, serial dilutions were plated and colonies counted. Conjugation mixes were prepared, serial dilutions were plated on selection, and the colonies were counted. The total colonies counted from selection divided by the possible total that could grow is presented as efficiency. Each efficiency calculation is presented as the average of three replicates.

Type of Conjugation	Plasmid 1	Plasmid 2	Efficiency
Bi-	pPJAV361	N/A	1×10^{-2}
Bi-	pPJAV500	N/A	6.9×10^{-3}
Bi-	pPJAV626	N/A	4.4×10^{-3}
Bi-	pPJAV632	N/A	5.8×10^{-5}
Tri-	pPJAV361	N/A	1.1×10^{-3}
Tri-	pPJAV500	N/A	6.1×10^{-4}
Tri-	pPJAV626	N/A	6.4×10^{-6}
Tri-	pPJAV632	N/A	6.5×10^{-6}
Tri-	pPJAV361	pPJAV626	2.3×10^{-3}
Tri-	pPJAV500	pPJAV632	8.7×10^{-7}
Quad-	pPJAV361	pPJAV626	2.4×10^{-7}
Quad-	pPJAV500	pPJAV632	5.4×10^{-8}

Bi-, biparental conjugation; Tri-, triparental conjugation; and Quad-, quadriparental conjugation; N/A, a second plasmid was not introduced in these experiments.

Supplemental Discussion: Using these types of conjugation mix, it is therefore theoretically possible to introduce two plasmids into *Anabaena* 7120 simultaneously; as a triparental mating, where both plasmids are carried by *E. coli* strain UC585, or as a quadriparental mating, where *E. coli* strain JCM113 is mixed with two *E. coli* strains each harboring only a single plasmid for transfer. To determine the relative efficiency of conjugation and selection, bi-, tri-, and quadriparental matings were carried out to introduce pPJAV361 (empty vector, SmSp resistant), pPJAV500 (harboring ltxABC, SmSp resistant),11 pPJAV626 (empty vector, Neo resistant), and pPJAV632 (harboring PltxD-tleD, Neo resistant) into *Anabaena* 7120 singly or in combinations of two plasmids with different selectable markers (Neor or Sp/Smr).

Following culture preparation, conjugation mixes were prepared, and serial dilutions were plated onto selection to determine the number of viable conjugal events. These data were then compared to colony counts from the same culture of *Anabaena* 7120 prepared but not utilized for conjugation. This comparison is presented because it provides a more realistic number of colonies produced from the protocol rather than a theoretical conjugal efficiency. In every case, plasmids based on pPJAV361 were singly introduced with a higher efficiency than those based on pPJAV626 (Table S7). When the introduction efficiency of the same plasmids was compared between bi- and triparental conjugations, biparental conjugations displayed higher efficiencies than did triparental conjugations by at least an order of magnitude. The lowest frequency of single introduction was observed from pPJAV632. Assessment of the simultaneous conjugation of two plasmids showed that triparental introduction of pPJAV361 and pPJAV626 displayed a similar efficiency to the triparental introduction of pPJAV626 singly. Because biparental conjugation of pPJAV361 displayed the highest efficiency, it is likely that co-introduction with pPJAV626 is the factor hampering the observed efficiency. Interestingly, co-conjugation of pPJAV632 with pPJAV500 displayed a greatly

decreased efficiency (>2,800-fold) in triparental mating with E. coli UC585 when compared to pPJAV361 introduced under identical conditions. We hypothesize that this is due to the larger size of pPJAV500 (22.4 kb) compared to pPJAV361 (11.2 kb). Efficiency of the simultaneous introduction of two plasmids was higher from triparental conjugations than quadraparental mixes. These results are consistent with the number of steps necessary to move all three plasmids into one E. coli cell. The bi- and triparental efficiencies are higher for single and double plasmid introductions than the analogous tri- and quadraparental mixes, respectively, because all three plasmids begin in the same cell rather than requiring a conjugal event to occur prior to introduction into *Anabaena* 7120. It is possible that the efficiencies decrease for pPJAV500 and pPJAV632 over pPJAV361 and pPJAV626 because they are larger and may cause a metabolic strain on *Anabaena* 7120, which could decrease the total number of colonies produced from each conjugation mix.

Table S8: Strains and plasmids utilized in this study.

Strain or plasmid	Characteristic(s)*	Source or reference
***Anabaena* sp.**		
PCC 7120	Wild type	Pasteur Culture Collection
E. coli		
NEB10β	Cloning strain	New England Biolabs
DH5αMCR	Cloning strain Δ*mcrBC*Δ*ecoK*	New England Biolabs
BW25113	Strain for lambda red recombination	3
JCM113	HB101 with pRL528 and pRK2013	Gift of J. C. Meeks
UC585	MC1061 with pRL528 and pRK24	4
Plasmids		
pBlueScript SK+	Cloning vector, Ap^r	Agilent
pACYC184	*E. coli* cloning vector, Cm^r Tet^r	5
pAM504	Shuttle vector for replication in *E. coli* and *Anabaena*; Km^r Nm^r	6
pDW9	Source of the Sp^r/Sm^r Ω interposon	7
fos-DE3-86	Fosmid containing *ltxA-D* from *Moorea producens*; Cm^r	8
pKD46	Temperature-sensitive □/Red recombination plasmid; Ap^r	9
pPJAV361	pAM504 with Sp^r/Sm^r Ω interposon replacing *nptII*	10
pPJAV500	pPJAV361 with P_{ltxA}-*ltxA-C*	10
pPJAV503	pPJAV361 with P_{glnA}-*ltxA-C*	10
pUC57-tleC	Source of codon optimized *tleC*; Ap^r	This study
pUC57-tleD	Source of codon optimized *tleD*; Ap^r	This study
pUC57-mpnD	Source of codon optimized *mpnD*; Ap^r	This study
pPJAV504	pPJAV361 with half of the Zeta plasmid, amplified with the primers zeta-SmaI-3F and zeta-SmaI-1R, replacing the pDU1 oriV	This study

pPJAV505	pPJAV361 with half of the Zeta plasmid, amplified with the primers zeta-SmaI-1F and zeta-SmaI-3R, replacing the pDU1 oriV	This study
pPJAV506	pPJAV361 with three-quarters of the Zeta plasmid, amplified with the primers zeta-SmaI-2F and zeta-SmaI-1R, replacing the pDU1 oriV	This study
pPJAV579	pPJAV504 reisolated from *Anabaena* 7120 with a transposon insertion in the Zeta *oriV*	This study
pPJAV580	pPJAV504 reisolated from *Anabaena* 7120 with a transposon insertion in the Zeta *oriV*	This study
pPJAV606	pPJAV579 with upstream half of the Zeta *oriV* removed	This study
pPJAV607	pPJAV579 with downstream half of the Zeta *oriV* removed	This study
pPJAV626	pAM504 with the upstream half of the Zeta *oriV* from pPJAV606 replacing the pDU1 *oriV*	This study
pPJAV631	pBlueScript SK+ carrying P_{ltxA}	This study
pPJAV632	pPJAV626 carrying P_{ltxD}-*tleD*	This study
pPJAV642	pPJAV361 carrying P_{ltxA}-*ltxAB-tleC*	This study
pPJAV643	pPJAV361 carrying P_{ltxA}-*ltxAB-mpnD*	This study
pPJAV644	pPJAV361 carrying P_{ltxA}-*ltxABC-tleD*	This study
pPJAV647	pPJAV361 carrying P_{glnA}-*ltxAB-tleC*	This study
pPJAV650	pPJAV361 carrying P_{glnA}-*ltxAB-mpnD-tleD*	This study
pPJAV653	pAM504 carrying the *hetR* coding region and a Sp^r/Sm^r Ω interposon	This study
pPJAV655	pPJAV606 carrying the entire *M. producens* portion of fos-DE3-86	This study
pPJAV657	pPJAV361 carrying P_{glnA}-*ltxABC-tleD*	This study
pPJAV659	pPJAV361 carrying P_{glnA}-*ltxAB-mpnD*	This study

* Km, kanamycin; Nm, neomycin; Sp, spectinomycin; Sm, streptomycin; Ap, ampicillin; Cm, chloramphenicol; Tet, tetracycline

Table S9: Oligonucleotide primers used in this study. Underlined bases indicate restriction enzyme recognition sequences, while italicized bases indicate additional random bases added to increase restriction enzyme efficiency.

Primer Name	Sequence (5' to 3')
505-BamHI	CTACGGGGTCTGACGCTCAGTGG
505-Sac	GTCGAACTGCGCGCTAACTATTC
aadA1-F	GCAATGACATTCTTGCAGGT
aadA1-R	ACCTACCAAGGCAACGCTAT
AMO-645	TGCTTTACTCTGGCACGGTGAC
AMO-646	TAAGTCCGCTCTTGGTCGTCTG
AMO-679	AGCTGTGCTCGACGTTGTCA
AMO-680	GCAGGAGCAAGGTGAGATGA
CAT-SacI-R	*TATAT*GAGCTCTTACGCCCCGCCCTGCCACTCATCG
fos-up-F	GGCTGCATCCGATGCAAGTGTGTCGCTG
fos-up-BamHI-R	*ATATA*GGATCCTTCGTATAATGTATGCTATACGAAGTTATTAGCG
fos-dn-BamHI-F	*ATATA*GGATCCGGTGTAACAAGGGTGAACACTATCCCATATC
fos-dn-EcoRI-R	*TATAT*GAATTCGAGCTTATCGCGAATAAATACCTGTGACGG
ltxA-int-SmaI-R	*ATATA*CCCGGGTGACATATGTGGTGGTCTCTGTAG
ltxB-int-NdeI-F	*ATCGATATCT*CATATGCTATTCCAGCTGAAGAAGTGCCATGGC
HetR-NdeI-F	CATATGAGTAACGACATCGATCTGATCAAGCG
HetR-R	TTAATCTTCTTTTCTACCAAACACCATTTGTAAAATCATGG
mpnD-ltxB-red-F	TTTTATCTGTCCTTTATCTCTGTAAATTGGAGTGTTTTCTTATGGC TGGAGATCCATTTG
mpnD-R	TTAGCGGTATAAACCTGGGGCTACATAGCATG
pAM504Ecoliup-R	GTGATGCGCCCACTGCGCATAG
pAM504-pBR-F	ATCGTCCATTCCGACAGCATCGCCAG
pAM504-pBR-R	CTGCGCGCTAACTATTCTCGACCTGC
Pcat-F	GCCGCGGCCCTCTCACTTCCCTG
PglnA-XhoI-F	*ATATA*CTCGAGCGCAGATAGTAGTCCATATCTCGTAAAC
Pltx-R	AGGGGGATAATTTTATCTAGCCCTC
PlxtA-XhoI-F	*ATATA*CTCGAGTTCACCTTCTGTCTAGAATTACAGTTTGAGG
PltxD-mpnD-red-F	CCGATTTGCATGCTATGTAGCCCCAGGTTTATACCGCTAAACTCT AGGAAAAAAACATGG
PltxD-tleC-red-F	TTAGCGTTTATTTAGCTCCCGGTGTATACCGTGAAGCATAAACTC TAGGAAAAAAACATG
PltxD-R	TAGACATCTCCAATAATAAAAAATAAAATCAATTATCCCAGAG
tleC-ltxB-red-F	TTTTATCTGTCCTTTATCTCTGTAAATTGGAGTGTTTTCTTATGGA ATCAGCTGGTCCTG
tleC-R	TTATGCTTCACGGTATACACCGGGAGC
tleD-PltxD-OEX-F	ATTATTGGAGATGTCTAATGCCACAAGAAGCCCGTACTCCTC
tleD-R	TTATACTGCTGGTTTTCTTAAAGTAGCC
Tnp-int-F	GGTGGATACACATCTTGTCATATGATC
Tnp-int-R	GAGCTAGTAGGGTTGCAGCCACGAG
Zeta2-1-walk1-F	CAACAAAGAAGGCATAGAAGC
Zeta2-1-walk1-R	CTCCATCAGCCCGTGAGGTG
Zeta-qPCR-F	CAATCTGGCAAGTATCGAGCG

Zeta-qPCR-R	GACTATCAAGCAACCTCGTGTG
zeta-SmaI-1F	*ATATA*CCCGGGGTTTAGGCTATGTTCTGCTGTTCACCTTC
zeta-SmaI-1R	*ATATA*CCCGGGGTTATCATTCACCCCCCAATCTACCTACTTG
zeta-SmaI-2F	*ATATA*CCCGGGTTATGGCATTGTCTACCAGTTTAAGC
zeta-SmaI-2R	*ATATA*CCCGGGAAGCCAGCCTACCTGATGCCCACCCAATAAAGCG
zeta-SmaI-3F	*ATATA*CCCGGGCCATAGAAACATGAGTAGCGGAGGCTTTTGACC
zeta-SmaI-3R	*ATATA*CCCGGGCATGGATAGGCACTCGTAGGCACTCCG
zeta-SmaI-4F	*ATATA*CCCGGGTCAATCAATTTTGGATTCTAGAGATAGCAG
zeta-SmaI-4R	*ATATA*CCCGGGGCAACATCATTGCATCCTTATCACCCGTG

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