

# The diiron monooxygenase CmlA from chloramphenicol biosynthesis allows reconstitution of $\beta$ -hydroxylation during glycopeptide antibiotic biosynthesis

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## Supporting Information

**Supplementary Table 1.** Primer sequences and template DNA used to clone Tcp11 module 6 hybrid constructs

Final construct	Primers	Insert template DNA	Vector template DNA
Tcp11 module 6 hybrid (A <sub>tei</sub> -PCP <sub>sky7</sub> )	FrM6_AT_1.FOR AGAAGGAGATATACCATGAAACATCACCATC FrM6_AT_1.REV TCGCGGCCTGCAGACATCCCGG	Plasmid encoding Tcp11 module 6 (A-PCP architecture)	-
	Fr2_Sky7.FOR GTCTGCAGGCCGCGAGCCGC Fr2_Sky7.REV CCAAGCACTCTCGAGGGACTCCCGGGC	Plasmid encoding Sky30 module 7 PCP	
	Vector.FOR CTCGAGAGTGCTTGGAGTCATCC Vector.REV GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTCTAGAG GG	-	pET-GB1-1d series
Tcp11 module 6 hybrid (A <sub>tei</sub> -PCP <sub>sky10</sub> )	Fr1_Sky10.FOR AGAAGGAGATATACCATGAAACATCACCATC Fr1_Sky10.REV GCGCGGCTTGCAGACATCCCGGCGAAATC	Plasmid encoding Tcp11 module 6 (A-PCP architecture)	-
	Fr2_Sky10.FOR GTCTGCAAGCCGCGCCGCGggaac Fr2_Sky10.REV CCAAGCACTCTCGAGGGCAGTGGACTTCTCGCCGC	Plasmid encoding Sky31 module 10 PCP	
	Vector.REV GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTCTAGAG GG Vector.FOR CTCGAGAGTGCTTGGAGTCATCC	-	pET-GB1-1d series
Tcp11 module 6 hybrid (A <sub>tei</sub> -PCP <sub>kis6</sub> )	F1_M6_AT.FOR AGAAGGAGATATACCATGAAACATCACCATC F1_M6_AT.REV TCAGCATCTGCAGACATCCCGGCGAAATC	Plasmid encoding Tcp11 module 6 (A-PCP architecture)	-
	F2-Kis-PCP.FOR GTCTGCAGATGCTGAACGGGCCG F2-Kis-PCP.REV GCAGCCGGATCAAGCTTACTTCTCGAAC	Plasmid encoding KisK module 6 PCP	
	V-M6AT(h).REV GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTCTAGAG GG V-M6AT(h).FOR GCTTGATCCGGCTGCTAACAAAG	-	pET-GB1-1d series

**Supplementary Table 2.** Summary of HRMS data for all hexapeptide species reported in this work for both methylamide (M) and hydrolyzed (H) forms.

Hexapeptide product		[M + H] <sup>+</sup> Theoretical	[M + H] <sup>+</sup> Observed	RT (Min)	Δ(ppm)
Bht	M	986.35668	986.35535	27.10	1.33
	H	973.32504	973.32452	28.82	0.52
Cl-Bht	M	1020.31770	1020.31525	29.70	2.45
	H	1007.28610	1007.28546	31.69	0.64
F-Bht	M	1004.34725	1004.34601	27.86	1.24
	H	991.31562	991.31537	29.64	0.25
Br-Bht	M	1064.26720	1064.26685	30.62	0.35
	H	1051.23560	1051.23621	32.62	0.61
I-Bht	M	1112.25332	1112.25574	31.97	2.42
	H	1099.22168	1099.2250	34.11	3.30
di-Cl-Bht	M	1054.27873	1054.27734	31.79	1.39
	H	1041.24710	1041.24707	34.29	0.03
meta-Bht	M	986.35668	986.35742	24.96	0.75
	H	973.32504	973.32593	26.64	0.91
β-OH Phe	M	970.36176	970.36200	31.57	0.25
	H	957.33013	957.33118	34.26	1.10

**Supplementary Table 3.** Summary of MS<sup>2</sup> data for all hexapeptide species indicating amino acid specific ammonium ions that originate from the alternate amino acid residues present at the C-terminus of the peptides.

Fragment	Fragment Mass	Hexapeptide Product (% intensity)*							
		Bht	F-Bht	Cl-Bht	Di-Cl-Bht	Br-Bht	I-Bht	β-OH Phe	meta-Bht
Bht/ Meta-Bht	152.07061	0.20	-	-	-	-	-	-	4.17
(-H <sub>2</sub> O)	134.06056	0.21	-	-	-	-	-	-	0.41
F-Bht	170.06119	-	1.45	-	-	-	-	-	-
(-H <sub>2</sub> O)	152.05114	-	0.32	-	-	-	-	-	-
Cl-Bht	186.03164	-	-	1.76	-	-	-	-	-
(-H <sub>2</sub> O)	168.02158	-	-	0.12	-	-	-	-	-
Di-Cl-Bht	219.99267	-	-	-	2.85	-	-	-	-
(-H <sub>2</sub> O)	201.98261	-	-	-	0.31	-	-	-	-
Br-Bht	229.98112	-	-	-	-	1.34	-	-	-
(-H <sub>2</sub> O)	211.97107	-	-	-	-	0.19	-	-	-
I-Bht	277.96726	-	-	-	-	-	1.35	-	-
(-H <sub>2</sub> O)	259.95720	-	-	-	-	-	0.29	-	-
β-OH Phe/ meta- Tyr/ Tyr	136.07570	3.36	3.26	3.80	4.21	3.42	3.23	8.54	6.16
(-H <sub>2</sub> O)	118.06564	-	-	-	-	-	-	0.81	-
Hpg	122.06005	100	100	100	100	100	100	100	100
Dpg	138.05496	16.0	22.7	27.6	31.4	23.1	22.2	24.1	26.3

\* Fragments indicate the relative ion intensity for the specific fragments relative to the base peak in the MS<sup>2</sup> spectra (Hpg ammonium ion, set to 100%) conform to within 0.001 Dalton of the exact masses expected for the residue-specific losses. Expected fragments shown in gray boxes, with the only fragments not specific to the product peptide for the peptide containing β-OH Phe, as this is also the mass of the Tyr-2 residue present in all hexapeptides; eliminated fragment for β-OH Phe is only found in the MS<sup>2</sup> fragments for the correct hexapeptide.