Direct Ligation of Carrier Protein-Bound Thioesters: A Versatile Method for the Characterization of Fatty Acid Tailoring Enzymes during Lipopeptide Biosynthesis

Table S1. Oligonucleotide pairs used for PCR amplification reactions.

Oligonucleotide Primers (5'-3')	**Restriction Site**	**Expression vector**	**target gene**
AAAAAAGGATCCATGAGTACGGACCCCA AGTCGGTTG	BamHI	pQTev	*ACP (Sco3249)*
AAAAAAAAGCTTTCACGCCGCTTCCAGA CCCG	HindIII		
AAAAAAGAATTCACGCAACGCGAAGAAG AGCTGGCC	EcoRI	pET28a(+)	*hxcO*
AAAAAACTCGAGCGGGCGTACTCCGGCC TGCA	XhoI		
AAAAAAGAATTCCCGAAGCTGCGGATCG CAGTCG	EcoRI	pET28a(+)	*hcmO*
AAAAAACTCGCTCGAGCGGCGGCGGCAG CGGTG	XhoI		

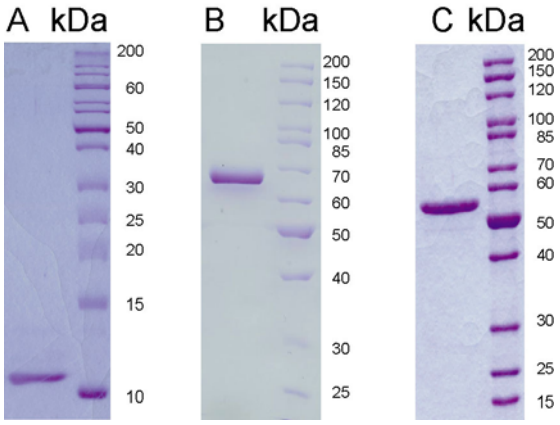

Figure S1. Coomassie stained SDS-PAGE of purified ACP (A, 11.5 kDa), HxcO (B, 66.8 kDa), and HcmO (B, 47.0 kDa).

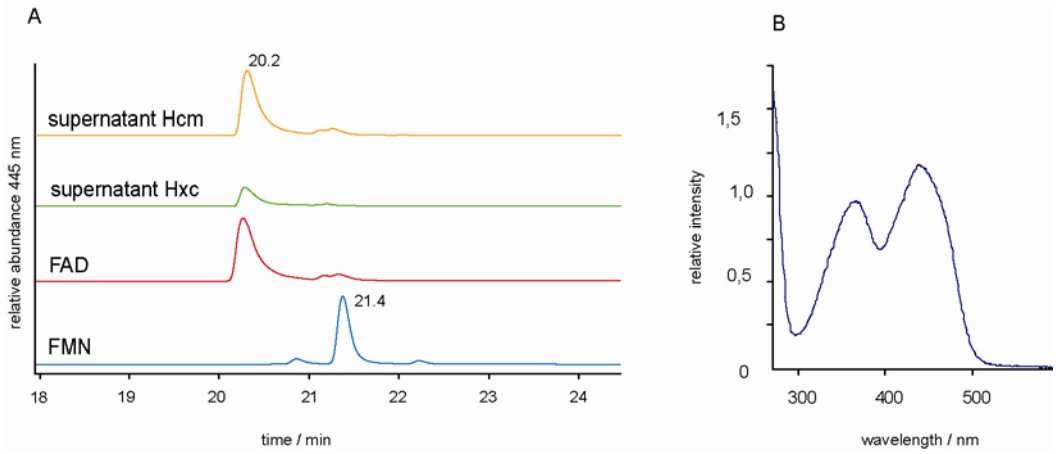

Figure S2. HPLC Analysis of HxcO and HcmO cofactor and UV-visible spectrum of HcmO. The UV-visible spectrum of HcmO highlights the λ_{max} at 377 and 450 nm.

Direct Ligation of Carrier Protein-Bound Thioesters: A Versatile Method for the Characterization of Fatty Acid Tailoring Enzymes during Lipopeptide Biosynthesis

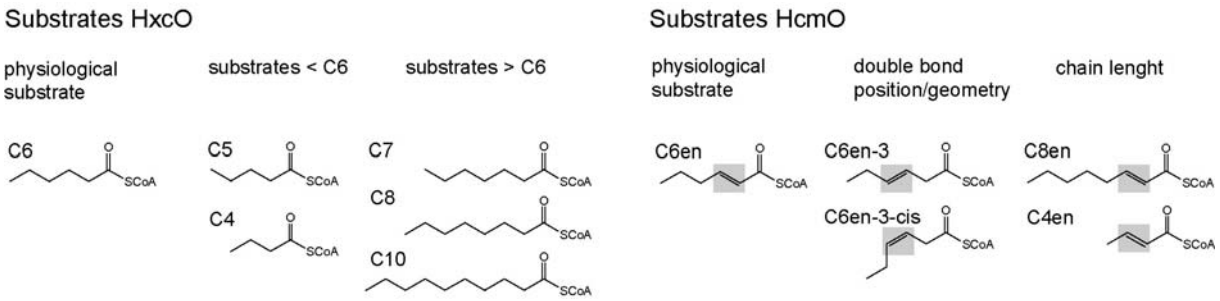

Figure S3. Acyl-CoA substrates used in this study.

Table S2. MALDI-TOF analysis of chemically synthesized fatty acid CoA derivatives.

FA-CoA	**c.m. $[M+H]^+$**	**o.m. $[M+H]^+$**
C4	838.1	838.2
C5	852.2	852.2
C6	866.2	866.1
C7	880.2	880.2
C8	894.2	894.1
C10	922.2	922.2
C6-EN2	864.2	864.1
C8-EN2	892.2	892.2
C4-EN2 (crotonyl)	836.1	836.2
C6-EN3	864.2	864.2
C6-EN3cis	864.2	864.2

Table S3. MALDI-TOF analysis of chemoenzymatically synthesized CDA derivatives.

	c.m. $[M+H]^+$	**o.m. $[M+H]^+$**
Hexanoyl-CDA	1466.6	1466.7
Hex-2-enoyl-CDA	1464.5	1464.7

Table S4. $[M+H]^{2+}$ mass fragments of fatty acid-*S*-Ppan-PCP (Lys^{57}-Arg^{71}) subjected to MS^2.

Chain length	**Saturated fatty acid**	**Unsaturated fatty acid**	**Epoxidized fatty acid**
C4	1032.5	1030.5	1038.5
C5	1039.5	1037.5	1045.5
C6	1046.5	1044.5	1052.5
C7	1053.5	1051.5	1059.5
C8	1060.5	1058.5	1068.5
C10	1074.5	1072.5	1080.5

Direct Ligation of Carrier Protein-Bound Thioesters: A Versatile Method for the Characterization of Fatty Acid Tailoring Enzymes during Lipopeptide Biosynthesis

Assays with acyl-CoA substrates

Reaction mixtures for the detection of HxcO oxidation/epoxidation products were prepared using 250 µM hexanoyl-CoA substrate, 5-50 µM HxcO and 100 µM FAD in assay buffer. Reaction mixtures for the detection of HcmO epoxidation product were prepared using 250 µM hexenoyl-CoA substrate, 10 µM HxcO, 250 µM FAD and 250 µM NAD(P)H in assay buffer. Incubations were carried out at different temperatures and several periods of time.

Assays with chemoenzymatically synthesized CDA substrates

HxcO Assay: The reaction mixture contained 100 µM hexanoyl-CDA, 20 µM enzyme and 60µM FAD in a total volume of 75 µL. After incubation at 25 °C for 30 min the reaction was quenched by the addition of 15µL formic acid and directly analyzed by LC-ESI-MS.

HcmO Assay: The reaction mixture contained 100 µM hex-2-enoyl-CDA, 20 µM enzyme, 60µM FAD, 100 µM NAD(P)H in a total volume of 75 µL. After incubation at 25 °C for 30 min the reaction was quenched by the addition of 15µL formic acid and directly analyzed by LC-ESI-MS.

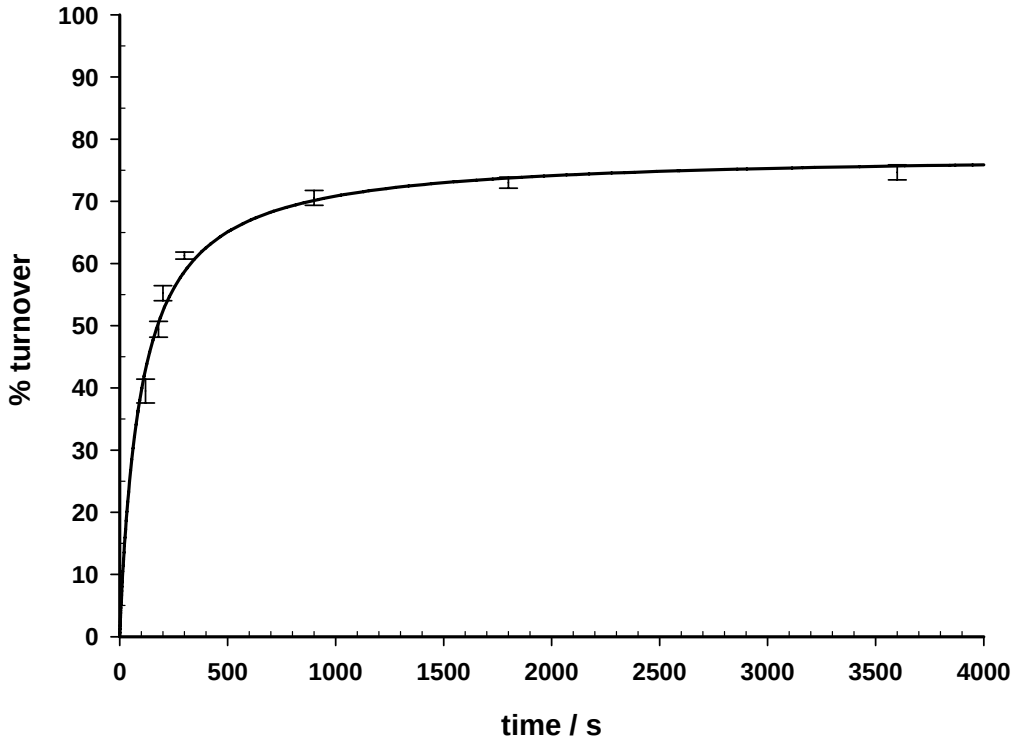

Figure S4. Kinetics of *(2R,3S)*-2,3-epoxyhexanoic-*S*-Ppan-ACP formation mediated by HxcO. Samples were trypsinized at different time points following initiation of the enzymatic reactions, and were then fractionated and analyzed by HPLC-MS^2. The percentage of HxcO reaction product tethered to the ACP is plotted as a function of time.