microorganisms

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Article

Dudomycins: new secondary metabolites produced after heterologous expression of an NRPS cluster from *Streptomyces albus* ssp. *chlorinus* NRRL B-24108

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Supplementary.

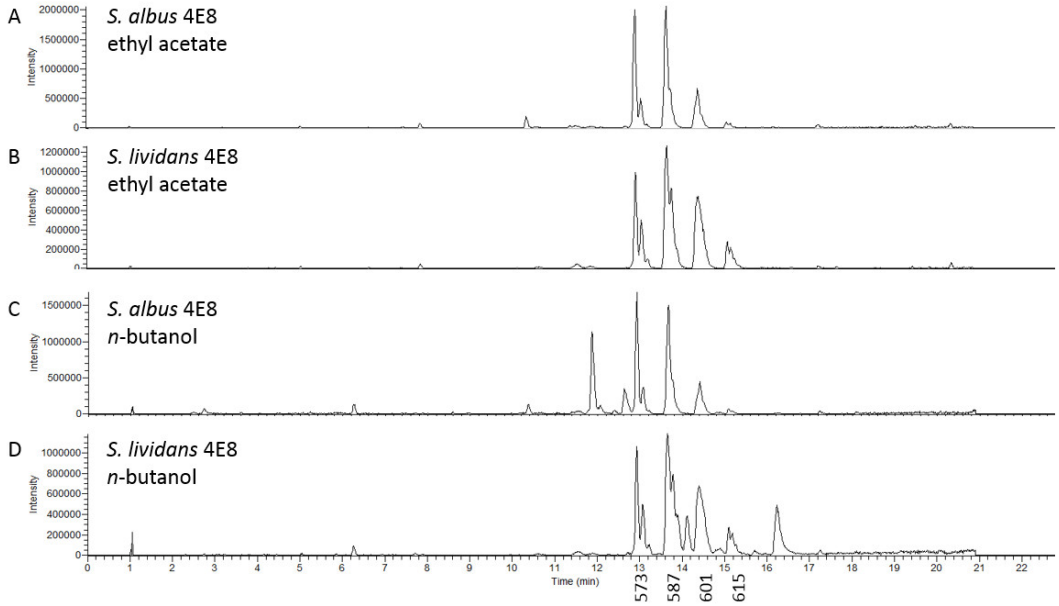

Figure S1. Production of dudomycins by different hosts using different organic solvents for extraction. Base Peak Chromatogram (BPC) extracted for masses M+H+ [573-574], [587-588], [601-602], [615-616]. Butanolic extractions led to more background peaks in crude extracts. *S. albus* 4E8 showed higher production levels compared to *S. lividans* 4E8.

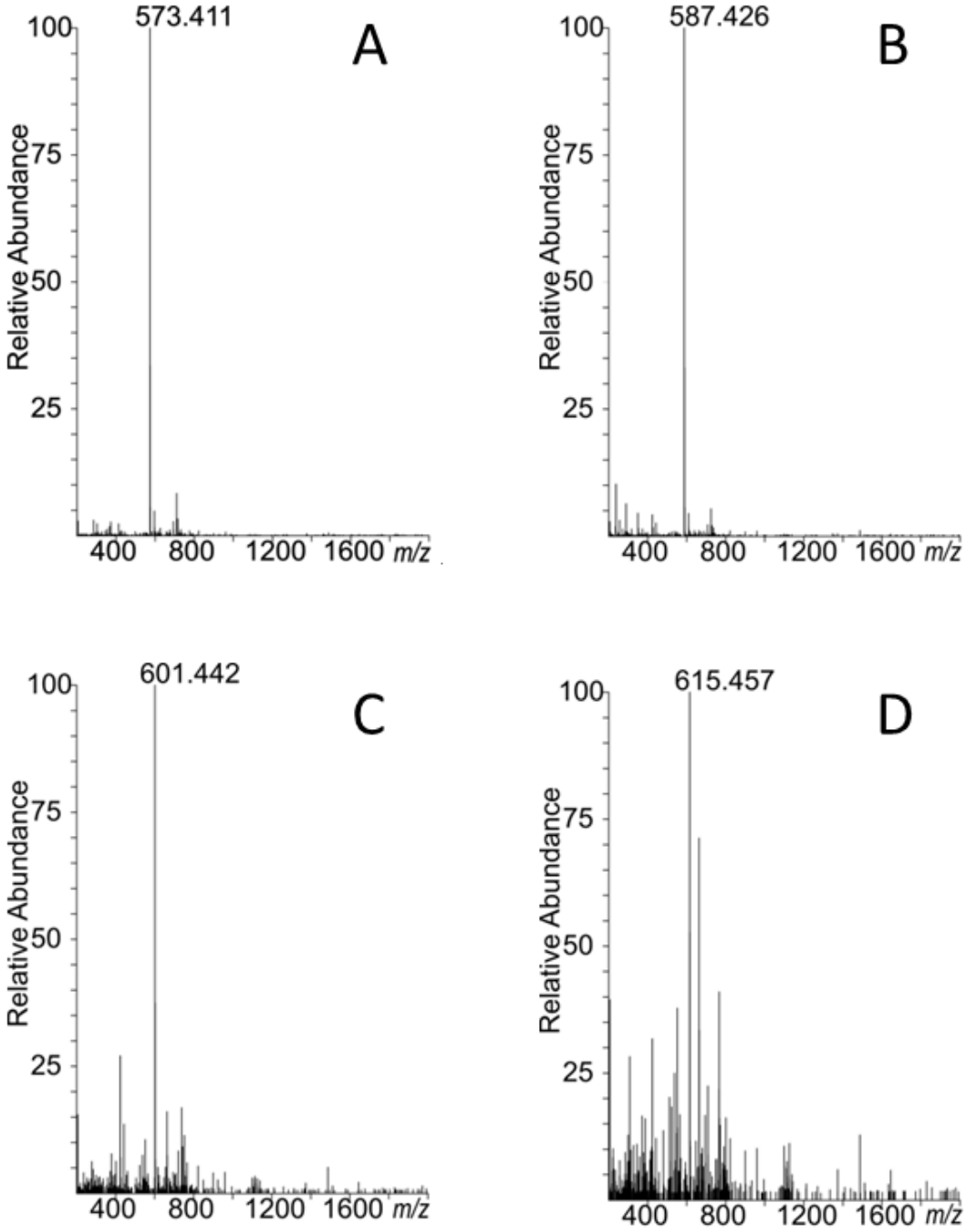

Figure S2. A to D: Mass spectra of dudomycins A to D.

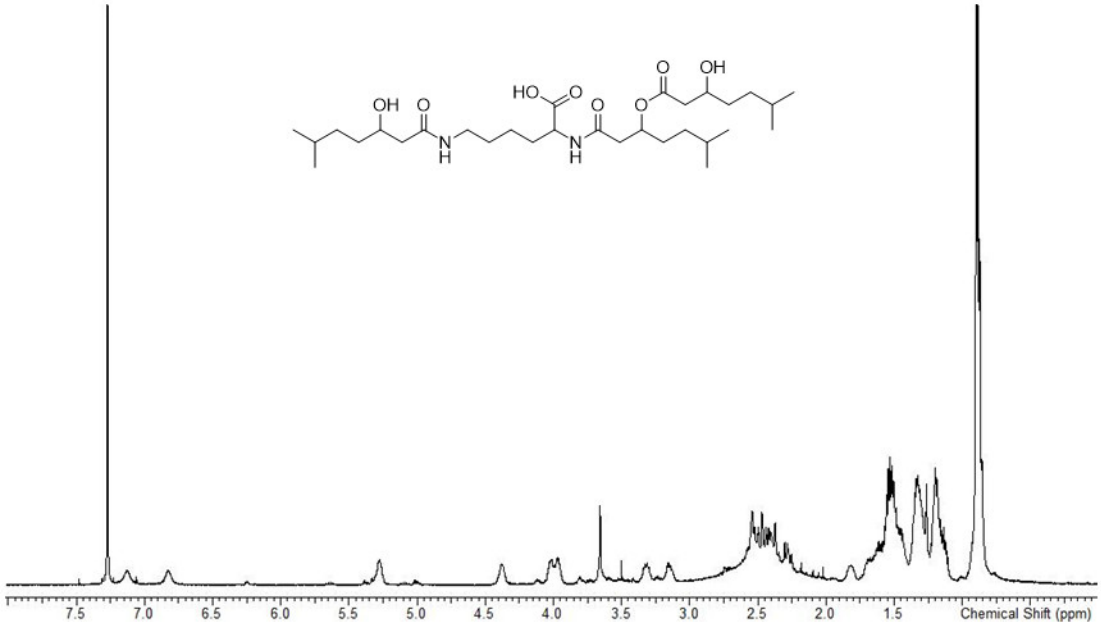

Figure S3. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of dudomycin A.

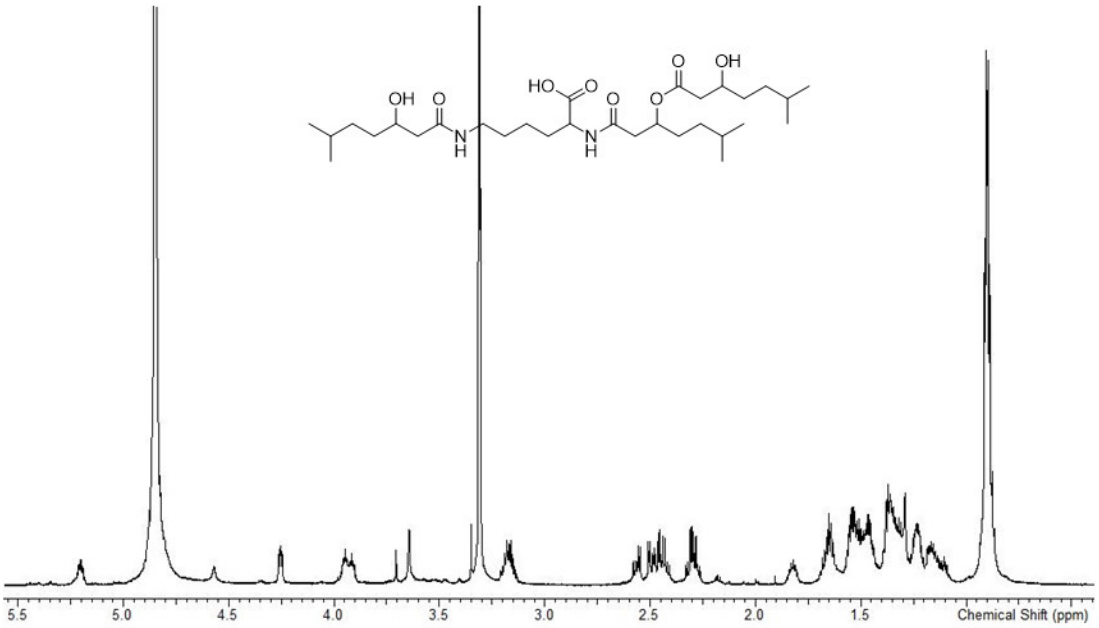

Figure S4. ^{1}H NMR (500 MHz, CD_3OD) spectrum of dudomycin A.

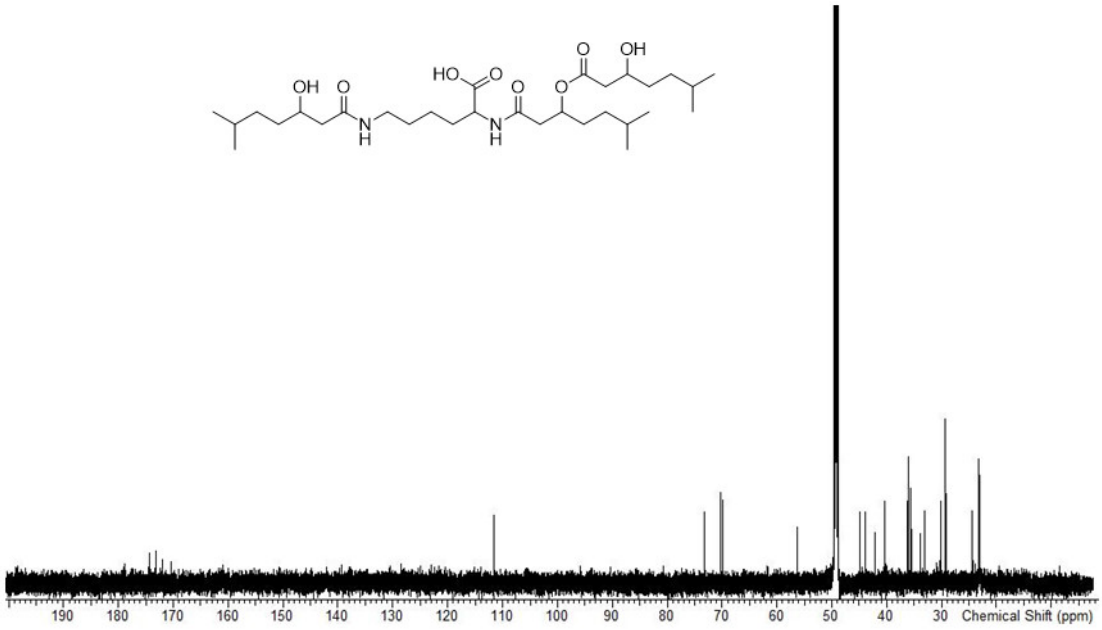

Figure S5. ^{13}C NMR (176 MHz, CD_3OD) spectrum of dudomycin A.

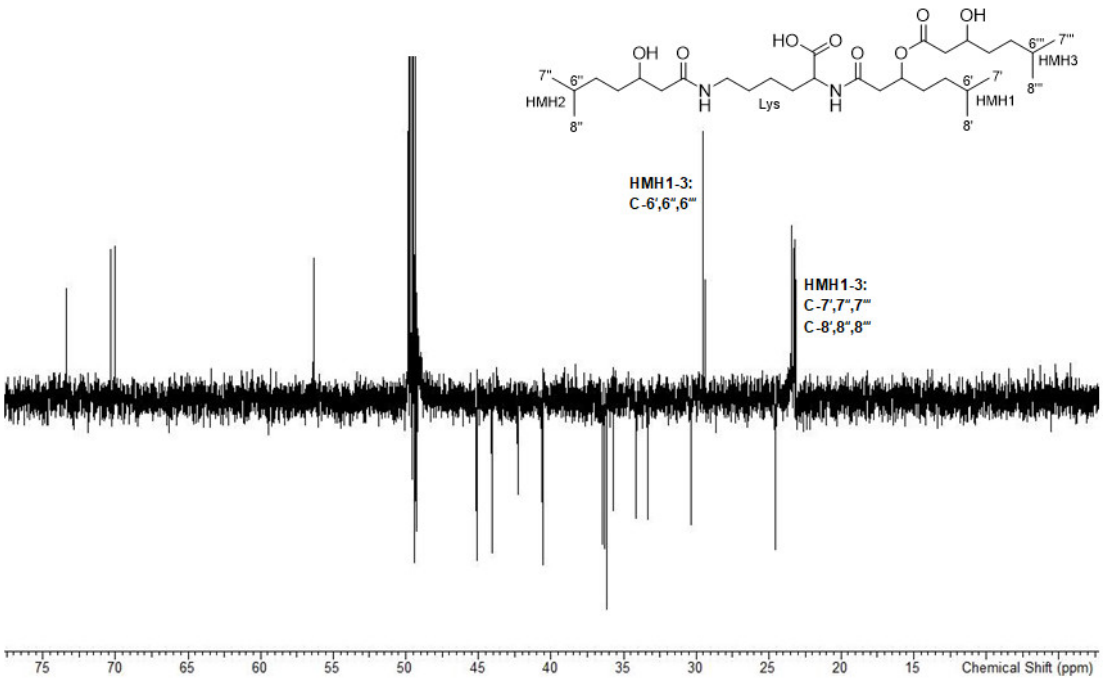

Figure S6. DEPT-135 (176 MHz, CD_3OD) spectrum of dudomycin A.

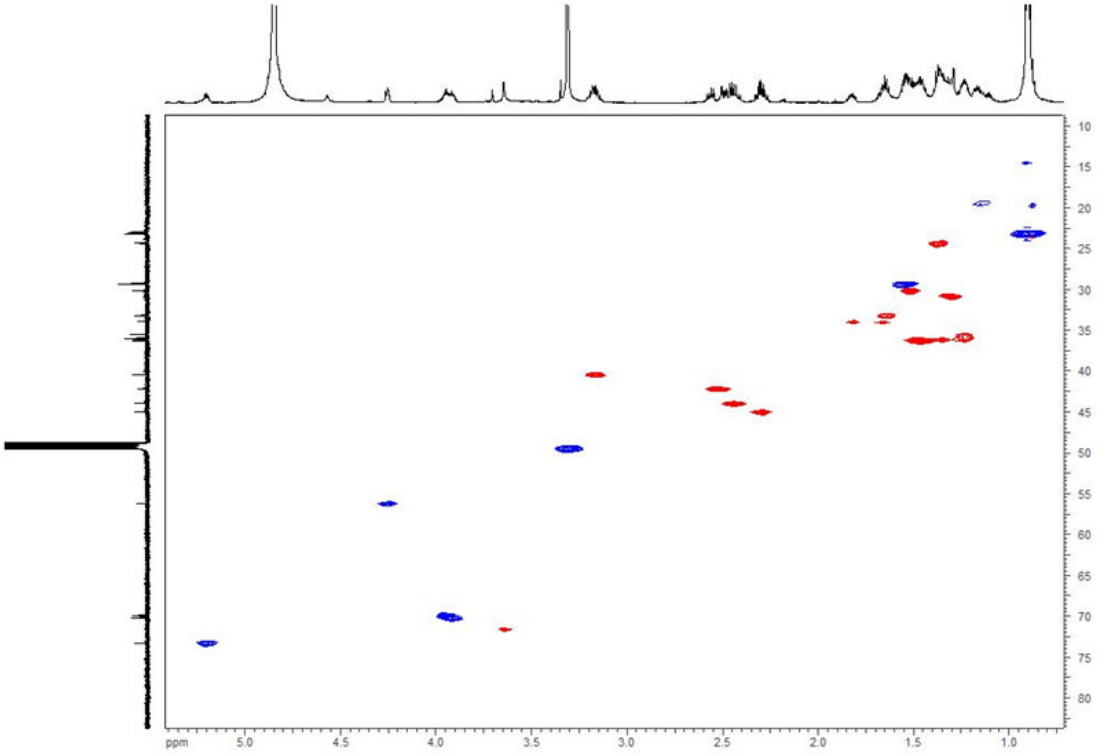

Figure S7. HSQC (700 MHz, CD_3OD) spectrum of dudomycin A.

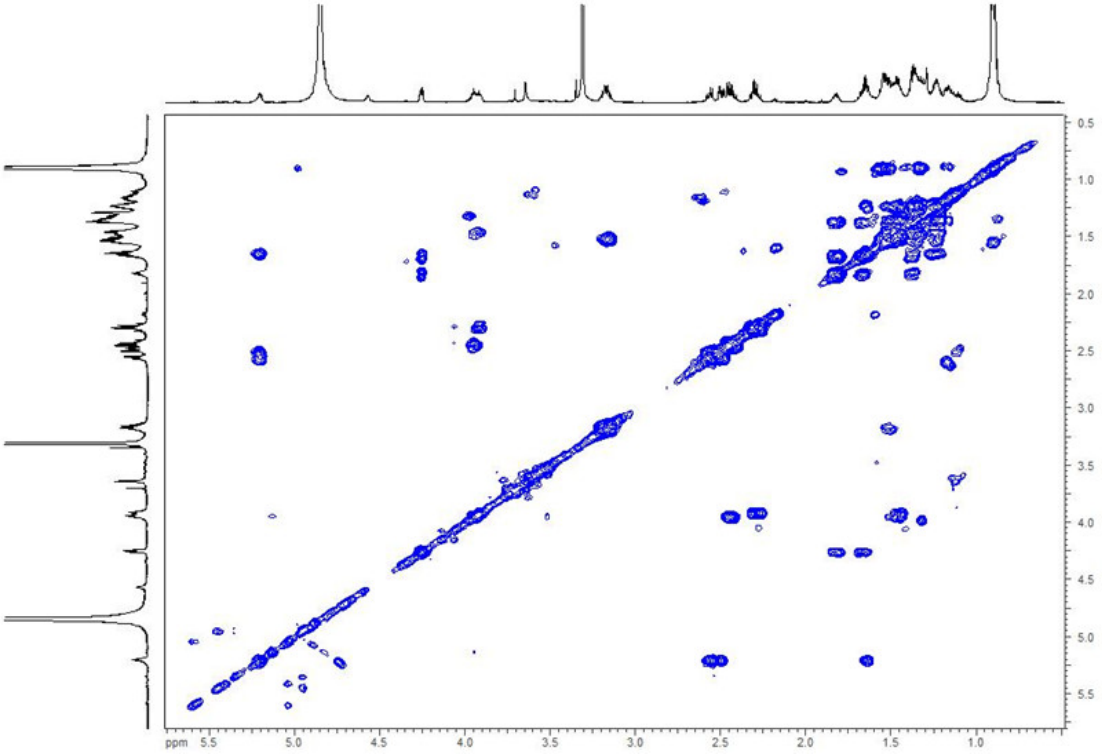

Figure S8. COSY (700 MHz, CD_3OD) spectrum of dudomycin A.

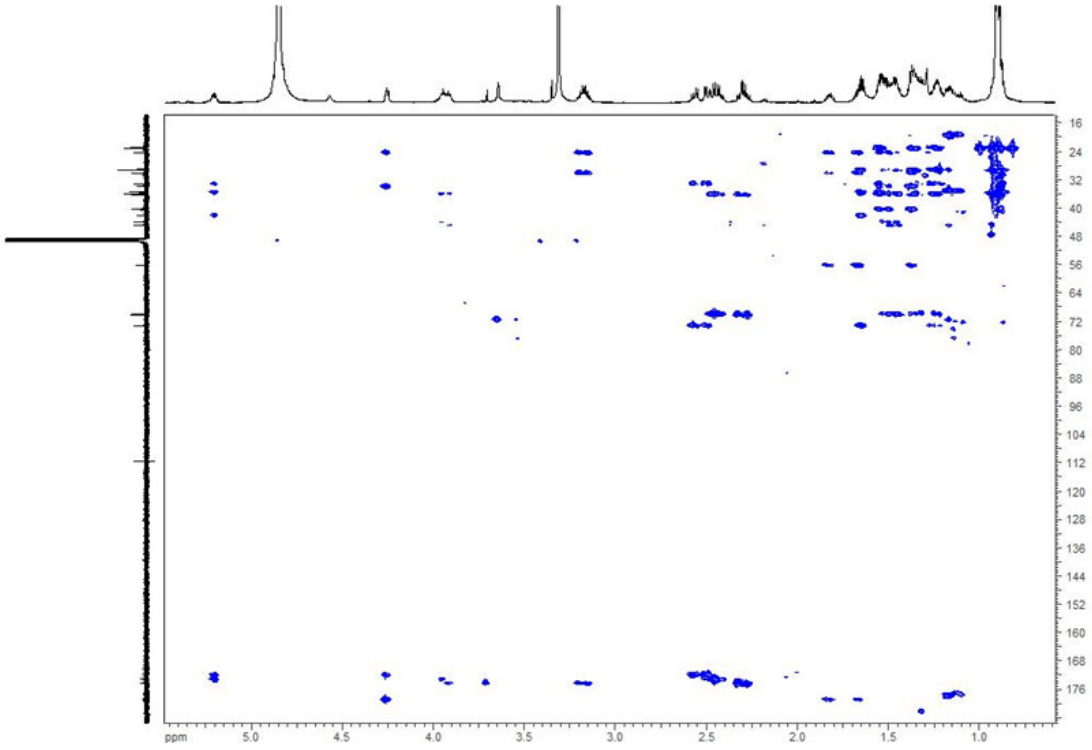

Figure S9. HMBC (700 MHz, CD_3OD) spectrum of dudomycin A.

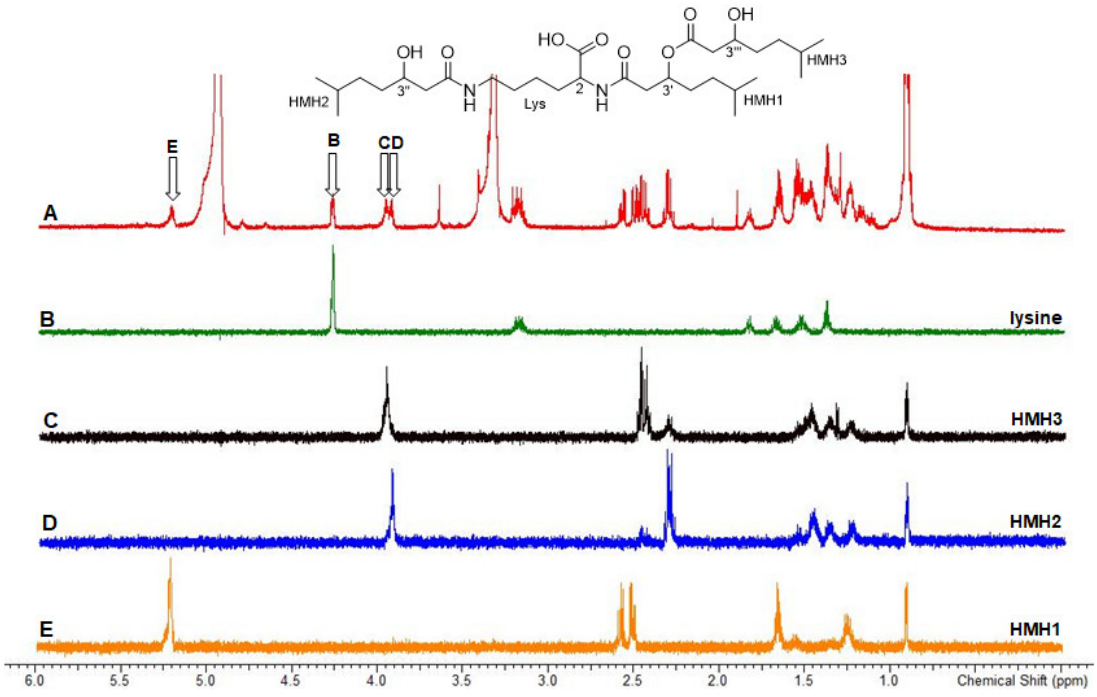

Figure S10. A. ^{1}H NMR spectrum of dudomycin A showing the irradiation points B (Lys, H-2), C (HMH3, H-3‴), D (HMH2, H-3″) and E (HMH1, H 3′). B-E. Selective 1D TOCSY (700 MHz, CD_3OD) spectra of the corresponding irradiation points.

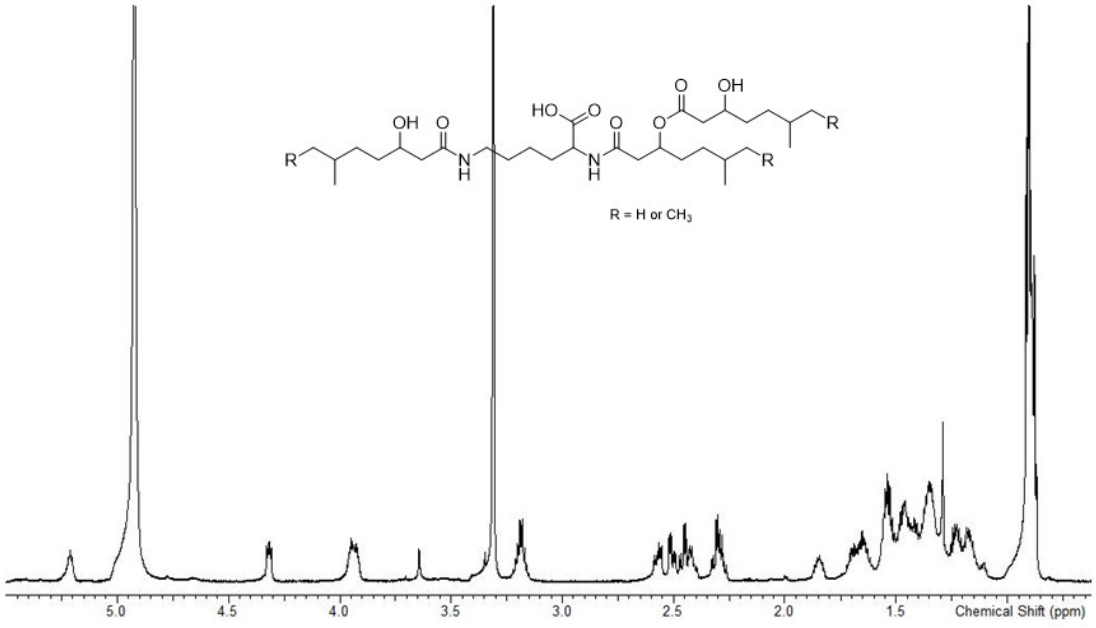

Figure S11. ^{1}H NMR (700 MHz, CD_3OD) spectrum of dudomycin B.

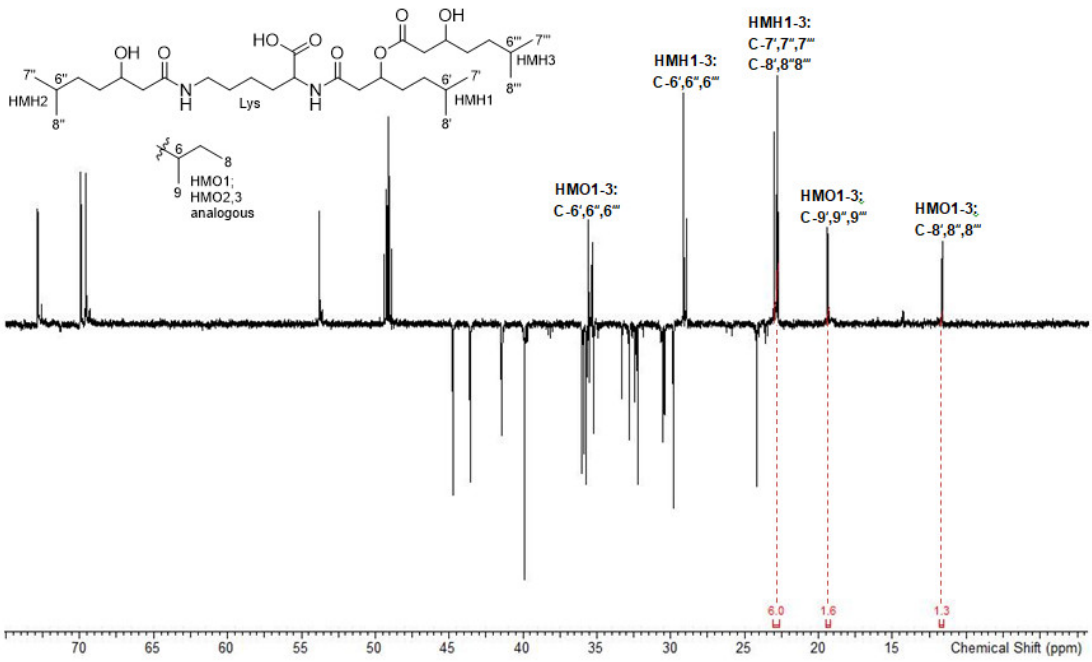

Figure S12. DEPT-135 (176 MHz, CD_3OD) spectrum of dudomycin B showing a 2/1 ratio of the methyl signals of HMH and HMO.

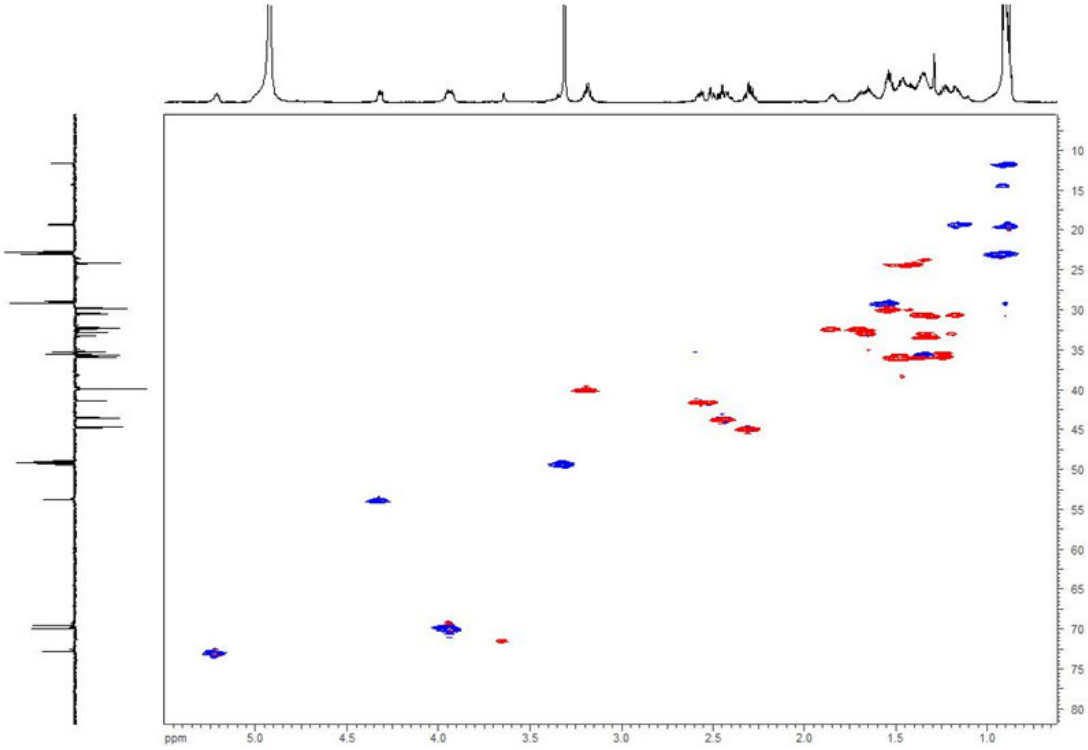

Figure S13. HSQC (700 MHz, CD_3OD) spectrum of dudomycin B.

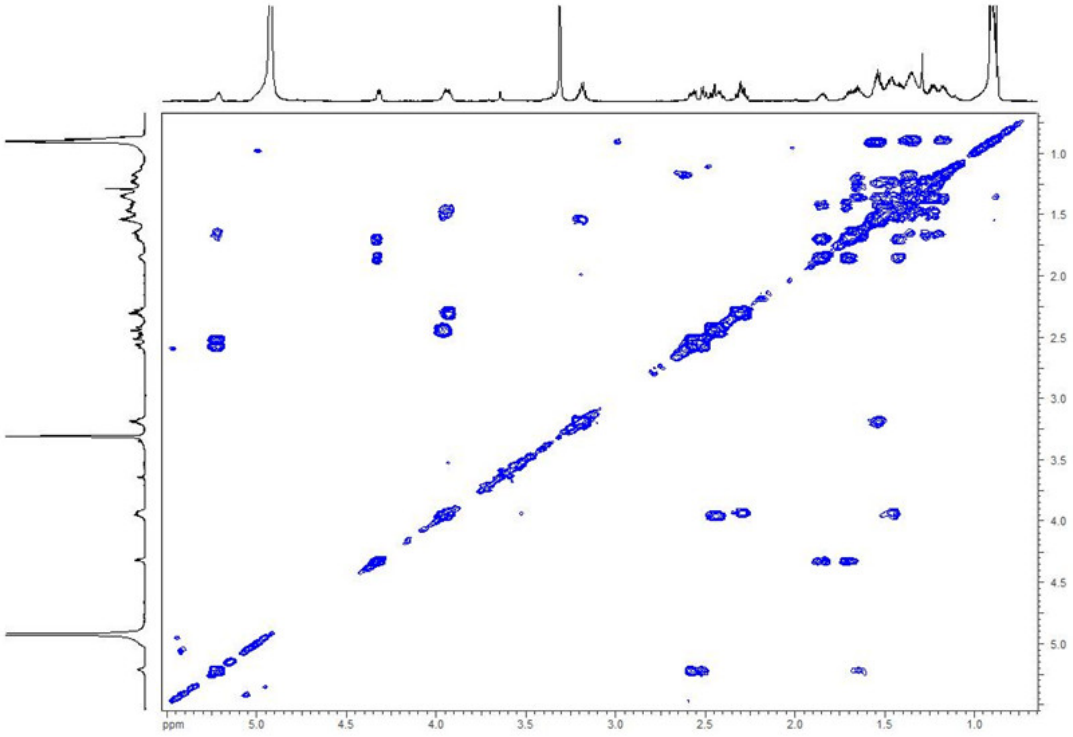

Figure S14. COSY (700 MHz, CD_3OD) spectrum of dudomycin B.

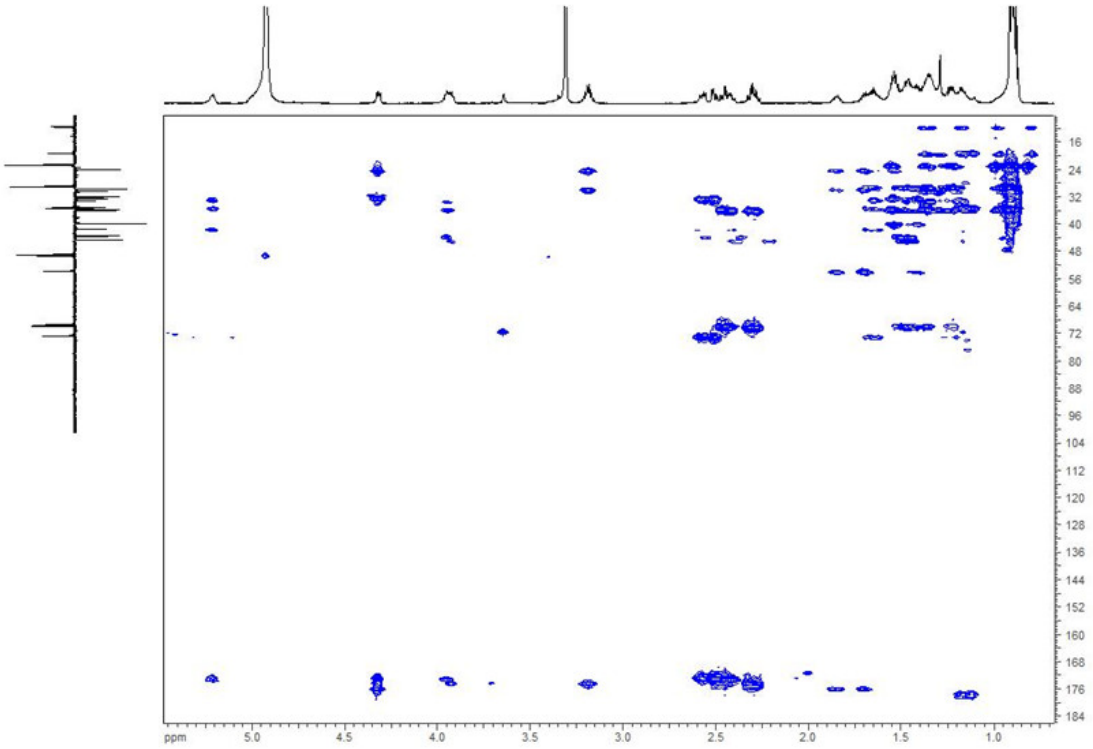

Figure S15. HMBC (700 MHz, CD_3OD) spectrum of dudomycin B.

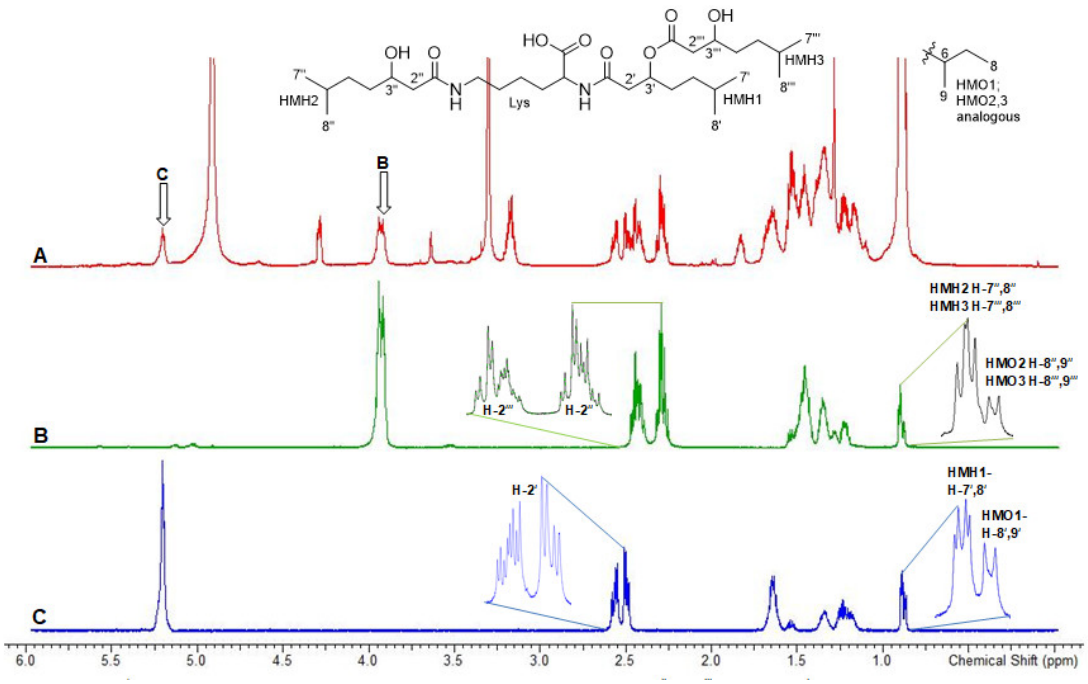

Figure S16. A. ^{1}H NMR spectrum of dudomycin B showing the irradiation points B (H-3′′/ H-3′′′) and C (H-3′). B-D. Selective 1D TOCSY (700 MHz, CD_3OD) spectra of the corresponding irradiation points.

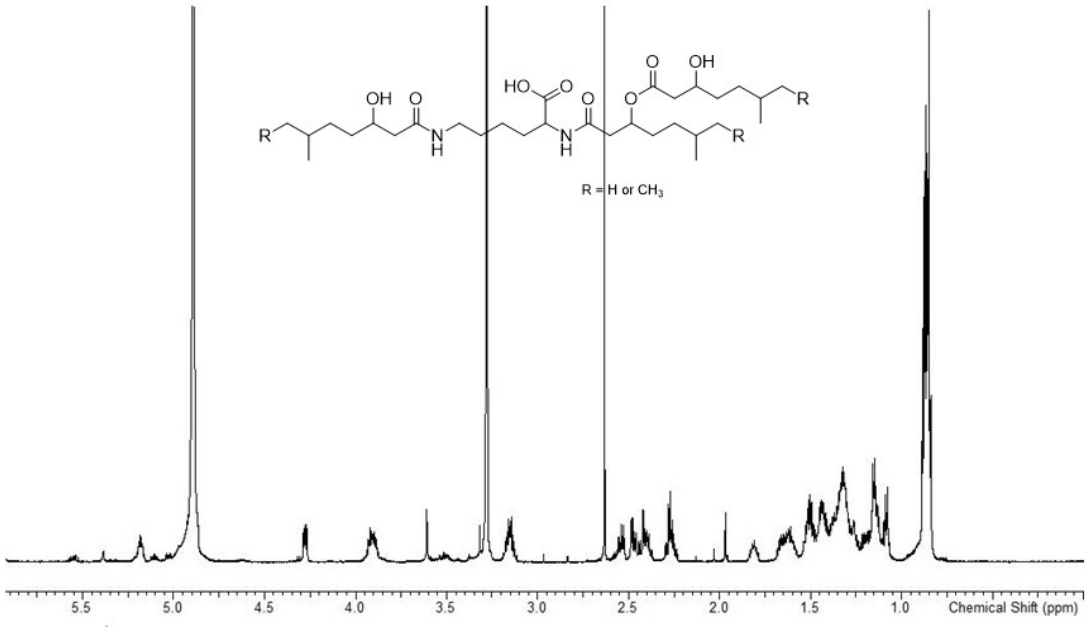

Figure S17. ^{1}H NMR (700 MHz, CD_3OD) spectrum of dudomycin C.

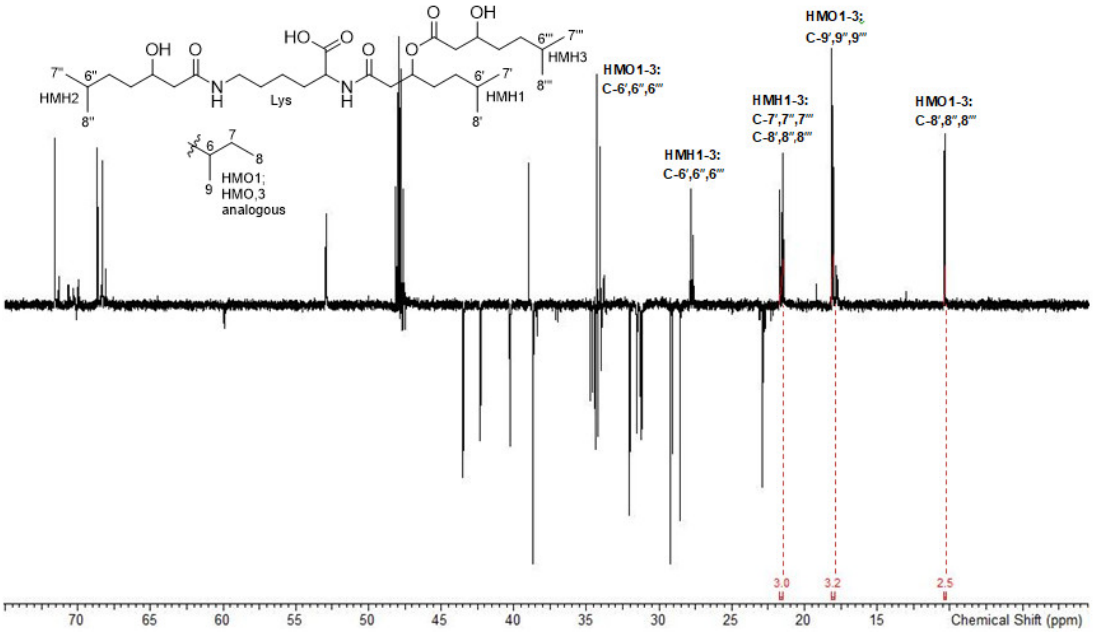

Figure S18. DEPT-135 (176 MHz, CD_3OD) spectrum of dudomycin C showing an approximate 1/2 ratio of the methyl signals of HMH and HMO.

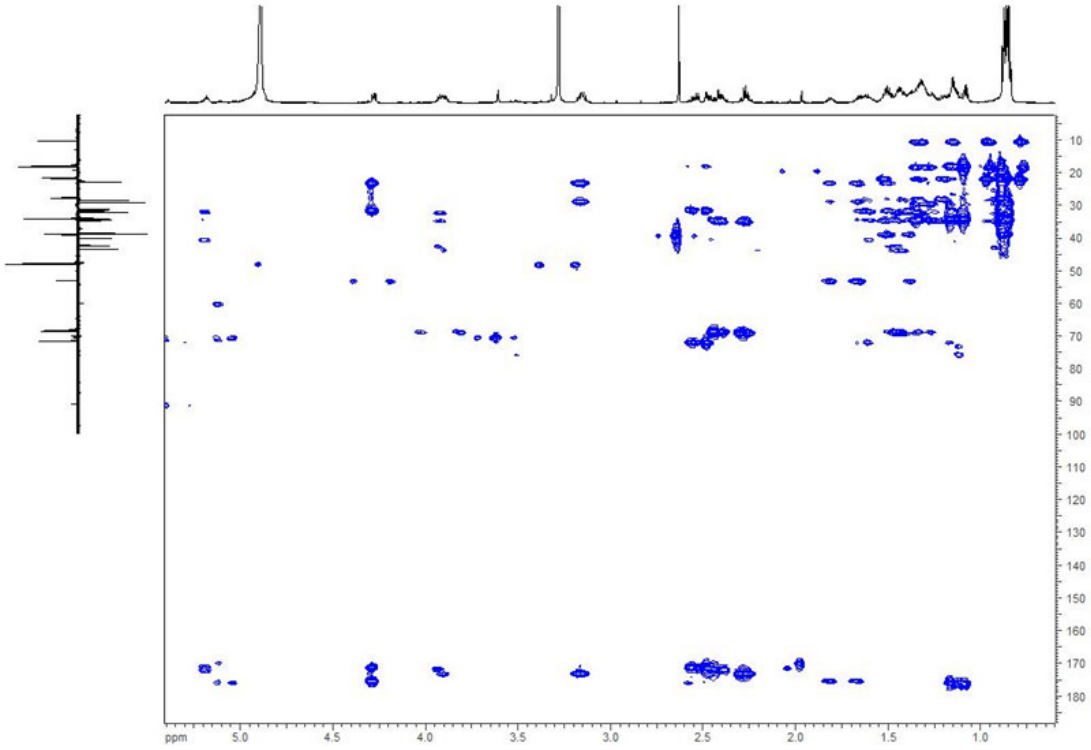

Figure S19. HMBC (700 MHz, CD_3OD) spectrum of dudomycin C.

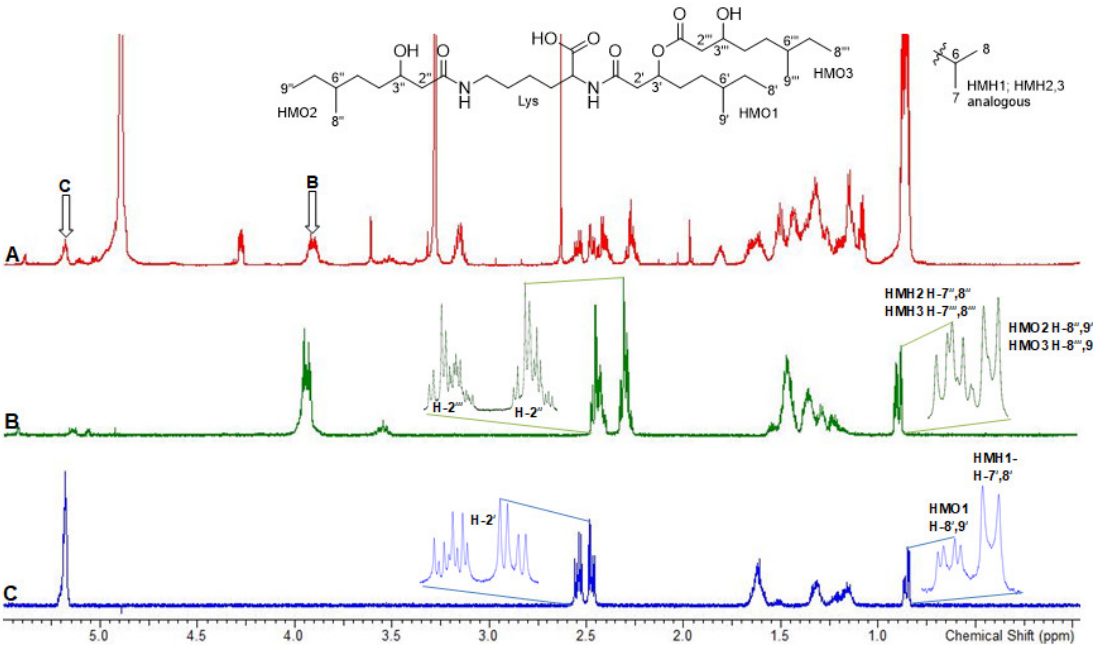

Figure S20. A. ^{1}H NMR spectrum of dudomycin C showing the irradiation points B (H-3′′/ H-3′′′) and C (H-3′). B-D. Selective 1D TOCSY (700 MHz, CD_3OD) spectra of the corresponding irradiation points.

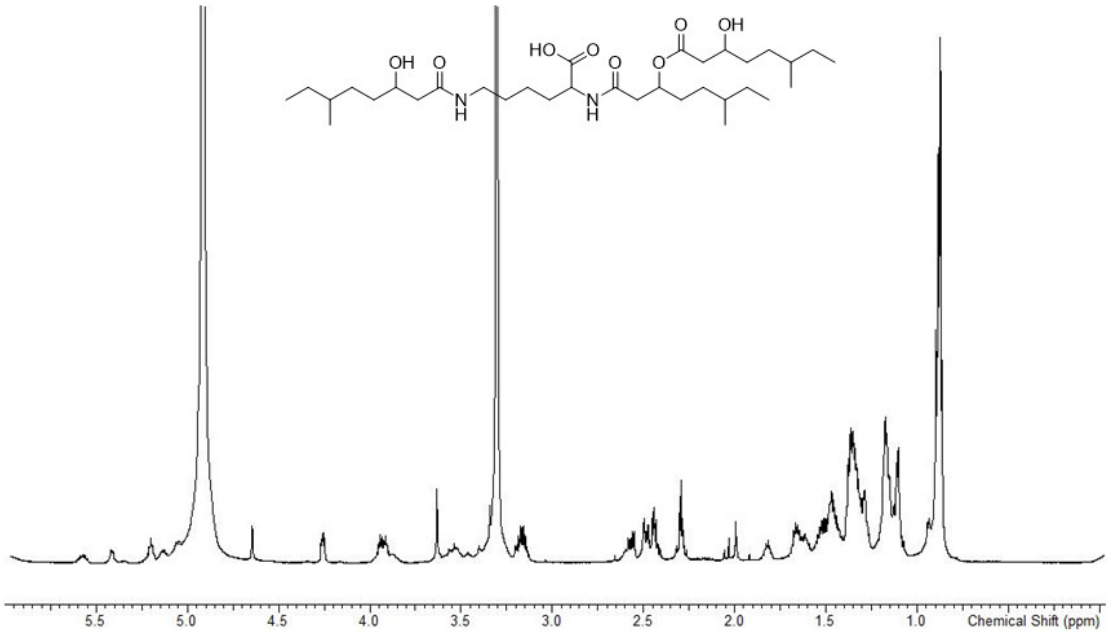

Figure S21. ^{1}H NMR (700 MHz, CD_3OD) spectrum of dudomycin D.

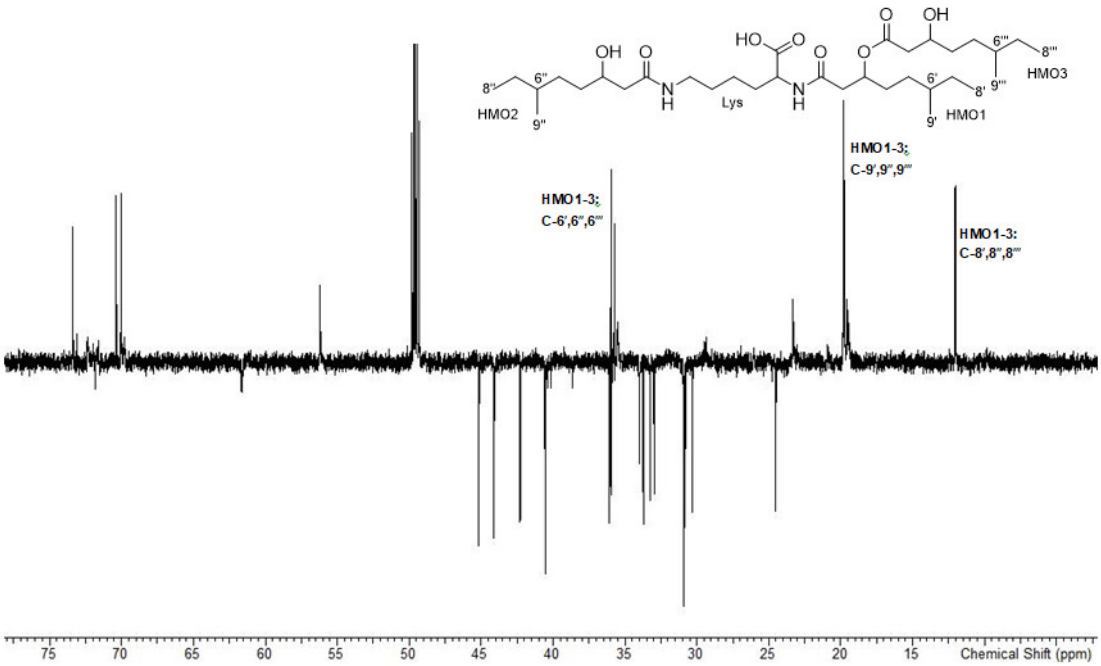

Figure S22. DEPT-135 (176 MHz, CD_3OD) spectrum of dudomycin D.

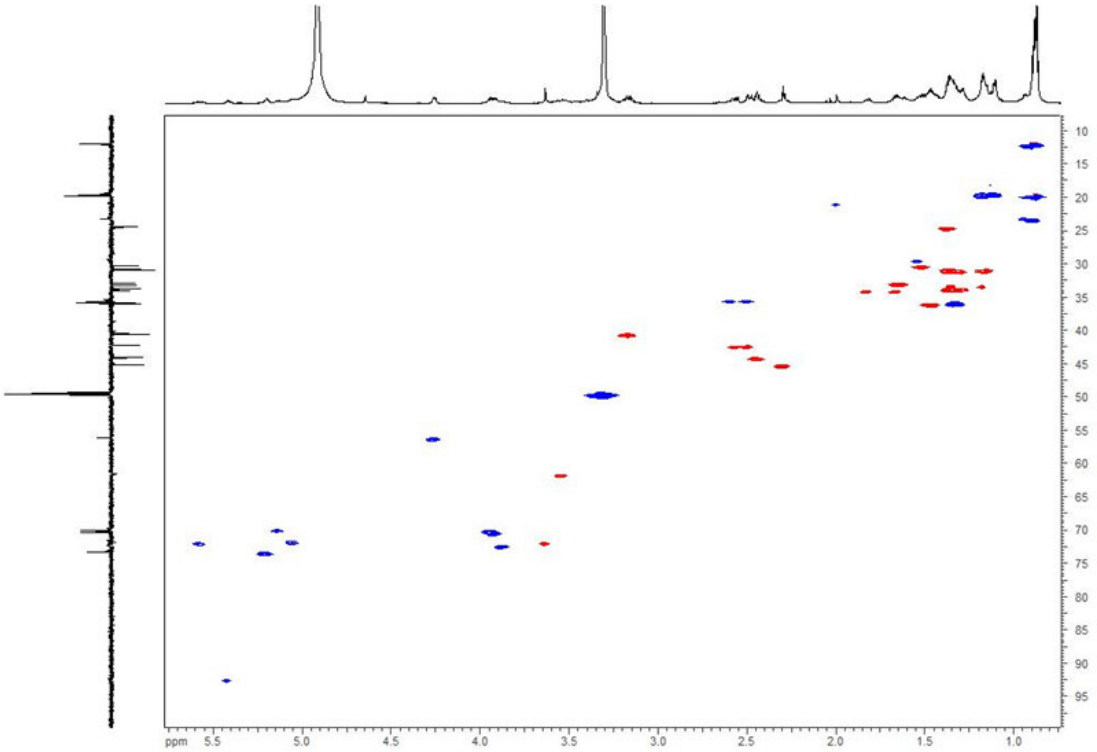

Figure S23. HSQC (700 MHz, CD_3OD) spectrum of dudomycin D.

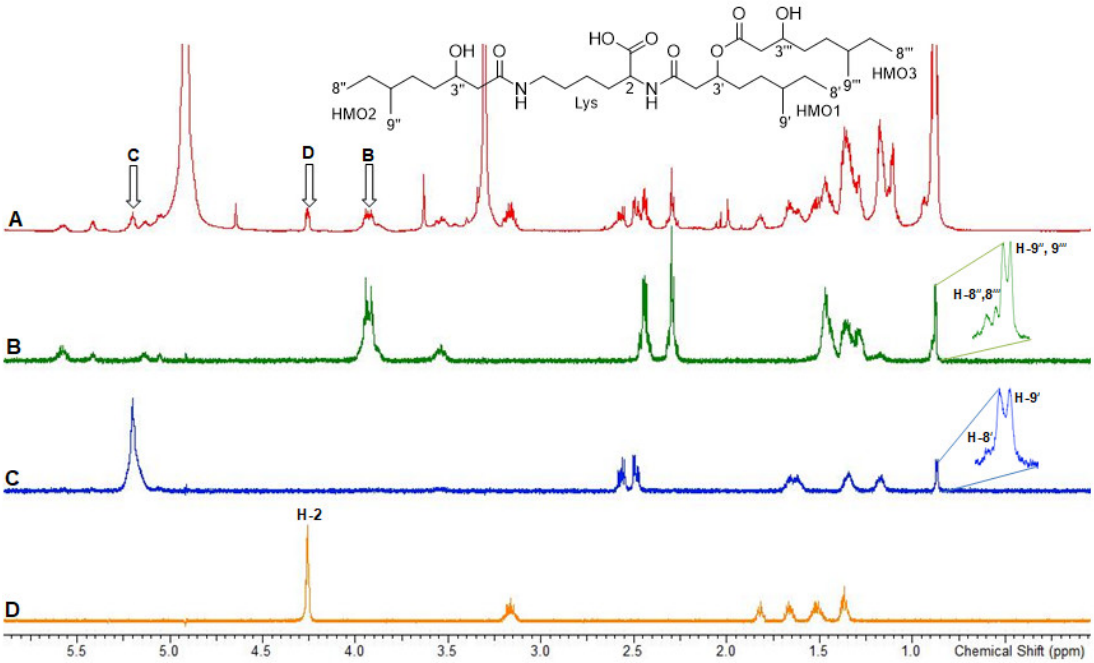

Figure S24. A. ^{1}H NMR spectrum of dudomycin D showing the irradiation points B (HMO2/3, H-3''/ H-3'''), C (HMO1, H-3') and D (Lys, H-2). B D. Selective 1D TOCSY (700 MHz, CD_3OD) spectra of the corresponding irradiation points.

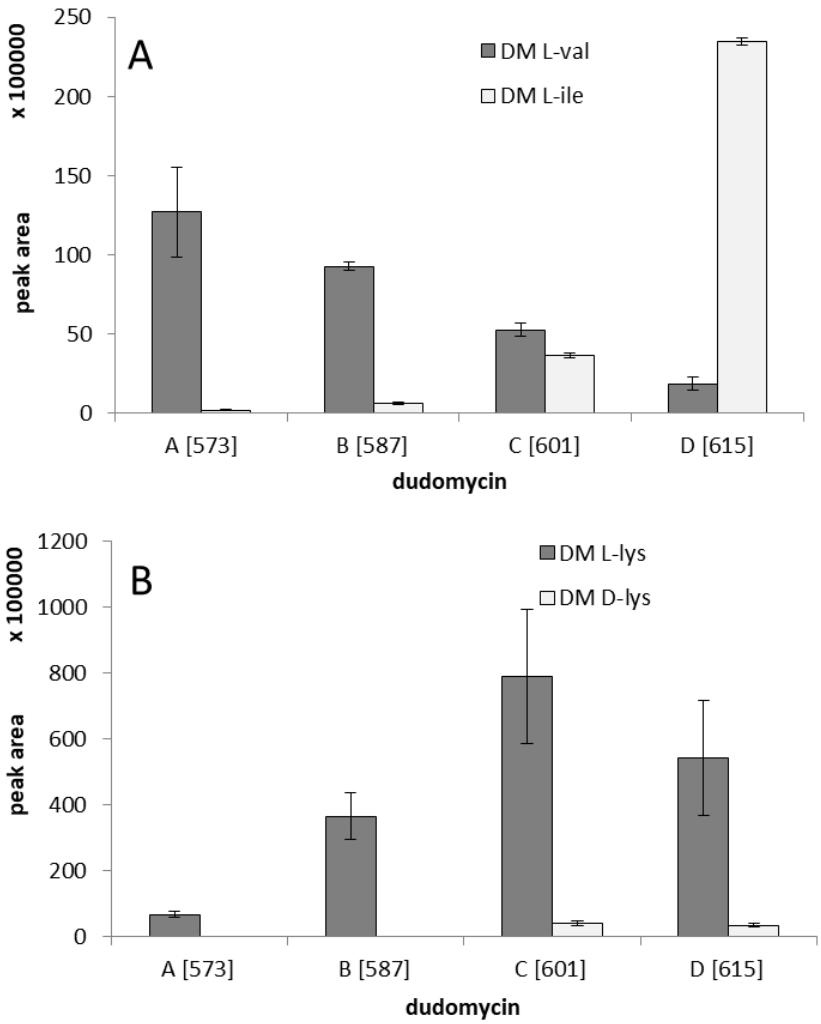

Figure S25. Production of dudomycin derivatives in defined medium (DM) with single sources of nitrogen.

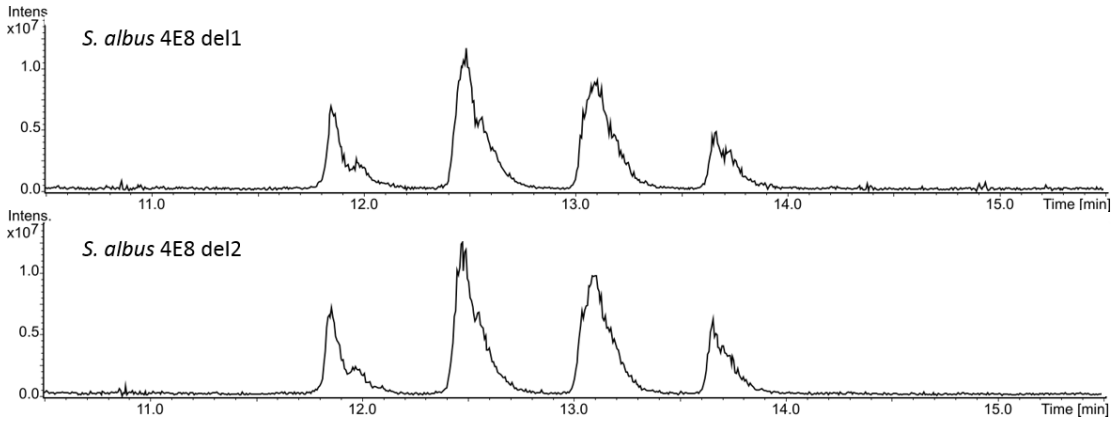

Figure S26. Production of dudomycins after gene deletion experiments. BPC extracted for masses [573-574], [587-588], [601-602], [615-616].

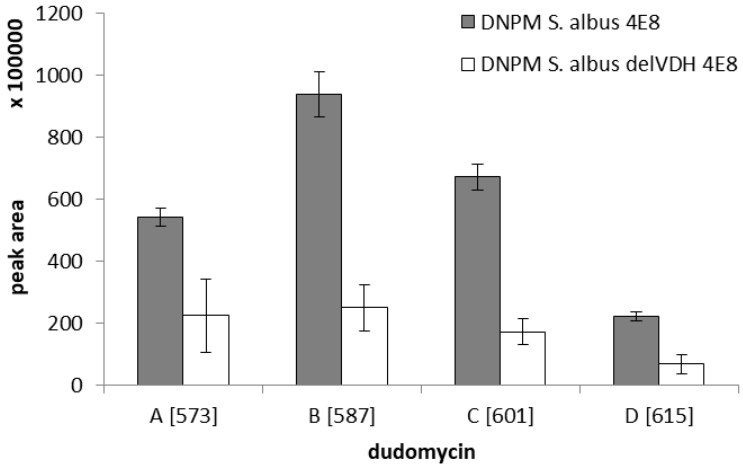

Figure S27. Decreased production of dudomycin derivatives by *S. albus* delVDH 4E8.

Table S1. NMR data of dudomycin A in CD_3OD.

unit	no.	δ_C, type	δH, (J in Hz)
Lys	1	178.9, C	-
	2	56.2, CH	4.26, dd (4.8, 7.5)
	3	34.0, CH_2	1.83, m; 1.677, m
	4	24.3, CH_2	1.38, m
	5	30.2, CH_2	1.51, m
	6	40.4, CH_2	3.17, m
	NH-1	-	7.12, bs[1]
	NH-2	-	6.81, bs[1]
HMH1	1'	171.9, C	-
	2'	42.2, CH_2	2.56, dd (7.7, 14.1); 2.50, dd (5.1, 14.4)
	3'	73.3, CH	5.20, dd (4.94, 7.5)
	4'	33.2, CH_2	1.64, m
	5'	35.6, CH_2	1.23, m
	6'	29.2, CH	1.54, m
	7'	23.1, CH_3	0.88, d (6.5)
	8'	23.0, CH_3	0.89, d (6.5)
HMH2	1''	174.3	-
	2''	44.9, CH_2	2.32, dd (4.5, 13.9); 2.28, dd (8.0, 13.9)
	3''	70.2, CH	3.92, m
	4''	36.3, CH_2	1.47, m
	5''	36.0, CH_2	1.23, m
	6''	29.4, CH	1.56, m
	7''	23.1, CH_3	0.90, d (4.4)
	8''	23.1, CH_3	0.89, d (4.4)
HMH3	1'''	173.2	-
	2'''	43.9, CH_2	2.47, dd (5.4, 14.8); 2.42, d (7.6, 14.9)
	3'''	69.9, CH	3.95, m
	4'''	36.2, CH_2	1.49, m; 1.348, m
	5'''	36.0, CH_2	1.23, m
	6'''	29.4, CH	1.56, m
	7'''	23.3, CH_3	0.91, d (4.8)
	8'''	23.3, CH_3	0.90, d (4.8)

[1]NH Signals measured in $CDCl_3$

Table S2. NMR data of dudomycin D in CD_3OD.

unit	no.	δC‡, type	δH, (J in Hz)
Lys	2	55.8, CH	4.26, dd (4.5, 6.8)
	3	33.6, CH_2	1.82, m; 1.66, m
	4	24.1, CH_2	1.38, m
	5	29.9, CH_2	1.51, m
	6	40.1, CH_2	3.17, m
HMO1	2'	41.9, CH_2	2.57, dd (8.5, 14.4); 2.49, dd (4.9, 14.4)
	3'	72.9, CH	5.20, m
	4'	32.8, CH_2	1.64, m
	5'	32.5, CH_2	1.36, m; 1.18, m
	6'	35.3, CH	1.34, m
	7'	30.4, CH_2	1.33, m; 1,17, m
	8'	11.6, CH_3	0.88, ovl
	9'	19.3, CH_3	0.87, d (6.0)
HMO2	2"	44.7, CH_2	2.31, dd (4.9, 13.5); 2.28, dd (8.2, 13.6)
	3"	69.9, CH	3.92, m
	4"	35.6, CH_2	1.47, m
	5"	33.3, CH_2	1.30, m; 1.37, m
	6"	35.5, CH	1.34, m
	7"	30.5, CH_2	1.35, m; 1.37, m
	8"	11.6, CH_3	0.89, ovl
	9"	19.4, CH_3	0.88, d (5.4)
HMO3	2"'	43.7, CH_2	2.46, dd (5.5, 15.1); 2.424, d (8.2, 15.1)
	3"'	69.6, CH	3.94, m
	4"'	35.5, CH_2	1.47, m
	5"'	33.3, CH_2	1.30, m; 1.37, m
	6"'	35.5, CH	1.34, m
	7"'	30.5, CH_2	1.35, m; 1.37, m
	8"'	11.6 CH_3	0.89, ovl
	9"'	19.4, CH_3	0.88, d (5.4)

‡from DEPT-135 measurement

Table S3. Strains, BACs, plasmids and primers used in this work.

Material	Purpose
A. Bacterial strains	
Streptomyces albus Del14	optimized heterologous host [1]
Streptomyces lividans Del8	optimized heterologous host [2]
Streptomyces albus J1074 delVDH	heterologous host [3]
Escherichia coli GB05 RedCC	cloning host [Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS)]
Escherichia coli ET12567 pUB307	alternate host intergeneric conjugation [4]
B. BACs	
4E8	heterologous expression of NRPS cluster
4E8 del1	determination left border of NRPS cluster
4E8 del2	determination right border of NRPS cluster
C. Plasmids	
pUC19	ampicillin resistance marker
pXCM hygformax	hygromycin resistance marker
D. PCR primer	
20190429_1_fw [4E8 del1]	CAGGGAGGAGGCCACCCCGCGCCGGTAGAAGACGCCCAGCAGGCCGACCGCG TCAGGTGGCACTTTTCG
20190429_1_rev [4E8 del1]	GATCGCGTGCATGCCCAGATGGTCGCGCGCATACCGCCGTCGGAACGGGCGCG GATATCCTACTATGCCGAGGTATAATGTAGCCAGCGTGTTACCAATGCTTAATC AGTG
20190429_2_fw [4E8 del2]	GGCGACGGCTCCCCGGGCCCGCGAGCCCACGACTCCCGTCCCCACGGCCATCA GGCGCCGGGGGCGGTGT
20190429_2_rev [4E8 del2]	GCGATGAGTATCCGTACTCATGTCCGGGCCGCGGGTGCCGCCTAGCGTGAAAT ACTTGACATATCACTGT

Table S4. Proposed functions of the genes in the DNA fragment containing the dudomycin gene cluster

gene #	**locus tag**	**putative function**
1	SACHL_42600	glycosyltransferase
2	SACHL_42610	hypothetical protein
3	SACHL_42620	phosphatase
4	SACHL_42630	N,N'-diacetyllegionaminic acid synthase
5	SACHL_42640	hypothetical protein
6	SACHL_42650	hydrolase
7	SACHL_42660	membrane lipoprotein precursor
8	SACHL_42670	galactose/methyl galactoside import ATP-binding protein
9	SACHL_42680	ribose transport system permease protein
10	SACHL_42690	branched-chain amino acid transport system / permease component
11	SACHL_42700	cytidine deaminase
12	SACHL_42710	pyrimidine-nucleoside phosphorylase
13	SACHL_42720	hypothetical protein
14	SACHL_42730	hypothetical protein
15	SACHL_42740	hypothetical protein
16	SACHL_42750	hypothetical protein
17	SACHL_42760	hypothetical protein
18	SACHL_42770	hypothetical protein
19	SACHL_42780	ubiquinone biosynthesis O-methyltransferase
20	SACHL_42790	zinc metallo-peptidase
21	SACHL_42800	hypothetical protein
22 [*dudA*]	SACHL_42810	dimodular nonribosomal peptide synthase
23 [*dudB*]	SACHL_42820	inner membrane transport protein
24 [*dudC*]	SACHL_42830	transcriptional regulator
25	SACHL_42840	demethylrebeccamycin-D-glucose O-methyltransferase
26	SACHL_42850	hypothetical protein

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[3] N. Manderscheid, "Strain development for heterologous expression of secondary metabolite clusters in actinobacteria," Universität des Saarlandes, 2015.

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