Supporting information for:

Functional and structural insights into a novel promiscuous ketoreductase of the lugdunomycin biosynthetic pathway

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SUPPLEMENTAL METHODS

Bacterial strains and culture conditions.

Bacterial strains used in this study are summarized in Table S1. *Escherichia coli* JM109 was the host for plasmid propagation, *E. coli* ET12567/pUZ8002 for conjugation, and *E. coli* strains BL21(DE3) pLysS Star and TOP10 for protein expression and purification. All *E. coli* strains were cultured in Luria Bertani broth (LB) or on agar plates supplemented), except for TOP10, which was cultured in 2×YE liquid medium. Antibiotics were added where appropriate (ampicillin 100 μg ml^{-1}; kanamycin 50 μg ml^{-1}; chloramphenicol 25 μg ml^{-1}). Media for growth of *Streptomyces* are described in the *Streptomyces* laboratory manual (*1*). *Streptomyces* sp. QL37 and its derivatives were grown on SFM agar plates for sporulation, on SFM agar supplemented with 60 mM MgCl2 and 60 mM $CaCl_2$ for conjugation, on R5 agar for protoplast transformation, and in YEME liquid medium for genomic DNA preparation. For the analysis of LugOII proteins, *lugOII* was cloned behind the constitutive *ermE* promoter and inserted into the conjugative plasmid pWHM3-*oriT*; this construct was introduced in *Streptomyces* sp. QL37 Δ*lugOII* by conjugation. For studies on angucycline and limamycin production, *Streptomyces* sp. QL37 was grown on R5 agar supplemented with 0.8% peptone and 1% mannitol for seven days at 30 °C. Whereas, MM agar supplemented with 0.5% glycerol and 1% mannitol was used for lugdunomycin production studies, with antibiotics (thiostrepton 20 μg ml^{-1} and/or apramycin 50 μg ml^{-1}) where relevant.

Metabolite extraction, isolation and characterization.

HPLC purifications were performed on a Waters preparative HPLC system equipped with a photodiode array detector (PDA). The absorption was monitored at 220 nm, 290 nm and 350 nm. LC-MS analysis was performed on Shimadzu LC-MS 9030 system comprised of a UPLC with an attached PDA, coupled to a QTOF HRMS, which uses ESI as an ionization source. NMR spectra were acquired on a Bruker AVIII-600 NMR spectrometer (Bruker BioSpin GmbH).

Compound **5** (containing a smaller amount of **4**) was isolated and identified previously **(*2*)**. Compound **4** was isolated from a *lugOI* null mutant of *Streptomyces* sp. QL37 (our unpublished data). TO do so, 100 μL fresh spores (1×10^7) of the mutant strain were inoculated onto R5 agar plates (7.5 L; 25 mL × 300 plates) supplemented with 0.8% peptone and 1%

mannitol, and then incubated at 30 °C for seven days. The agar was cut into small blocks and homogenized with a pestle. The resultant lysate was extracted three times with an equal volume of ethyl acetate by soaking for 24 h at room temperature. The extract was centrifuged and evaporated under reduced pressure at 40 °C to obtain a dry mass of 7.8 g. The crude extract was adsorbed on silica gel and loaded on a silica gel column (VLC, 125 × 50 mm, 60 Å pore size, 230-400 mesh, Sigma). One column volume of *n*-hexane was applied to de-fat the solution. A gradient elution was employed from ethyl acetate to methanol, which generated 15 fractions. Fractions containing **4** (eluted with ethyl acetate–methanol 3:1) were confirmed by LC-MS and UV spectrum analysis, and were then pooled and concentrated to obtain 1.4 g dry mass. After dissolving in methanol, the pooled fractions were subjected to a preparative scale HPLC for the purification of **4**. A linear gradient of water and acetonitrile (solvent A = H_2O; solvent B = acetonitrile; 0–30 min, 20% to 80% B) at a flowrate of 12 mL/min was used to purify the compound on a SunFire C_{18} column (10 µm, 100 Å, 19 × 150 mm). The UV chromatogram was monitored at 220, 290 and 355 nm. HPLC peaks were manually collected and dried to yield pure **4** (2 mg). The compound was identified by matching its retention time, UV, and MS data to 8-*O*-methylrabelomycin which was previously identified by NMR from the wild type strain **(*2*)**.

8-*O*-methylrabelomycin (4): bright yellow solid; UV λ_{max} (LC-MS): 221, 266, 417 nm; HRESIMS *m/z* 353.1023 $[M+H]^+$ (calcd for $C_{20}H_{16}O_6$, 353.1020).

1-deoxo-1-hydroxy-8-*O*-methylrabelomycin (7) (*2*): yellow solid; UV λ_{max} (LC-MS): 220, 260, 285, 433 nm; HRESIMS *m/z* 355.1173 $[M+H]^+$ (calcd for $C_{20}H_{19}O_6$, 355.1176); ^1H and ^{13}C NMR data (Table S4, Data S2).

SM 196B (8) (*3*): yellow solid; UV λ_{max} (LC-MS): 218, 259, 381 nm; HRESIMS *m/z* 339.1229 $[M+H]^+$ (calcd for $C_{20}H_{19}O_5$, 339.1227); ^1H and ^{13}C NMR data (Table S4, Data S3).

LC-MS/MS-based peptide identification.

For identification of LugOII proteins, *Streptomyces* sp. QL37 Δ*lugOII* harbouring plasmid pWHM3-*ermE*p-*lugOII* was incubated in 250 ml TSB medium in 1 L Erlenmayer flasks at 30°C for 5 days. Cells were centrifugated and lysed by sonication, followed by removing of the cell debris. The soluble supernatant was dialyzed in 20 mM Tris, pH 7.5 and loaded on a monoQ ion-exchange chromatography column. Elution was performed with a gradient 20 mM Tris, 1

M NaCl, pH 7.5. The protein fractions were detected by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). The sequences of the targeted bands were identified by LC-MS/MS peptide analysis.

The semi-purified crude extract of LugOII overexpression strain was process by SDS-PAGE and stained with Coomassie. The target bands were excised into small cubes and destained with 0.1 M ammonium bicarbonate/acetonitrile (1:1, v/v). Saturated gel pieces were digested with trypsin (0.1 µg, recombinant, proteomics grade, Roche) at 37°C overnight. The digestion solution was withdrew and further desalted using STAGE-Tipping (*4*). Briefly, peptide was loaded on a conditioned StageTip with 2 pieces of 1 mm diameter SDB-XC plug (Empore), washed twice with 0.5% formic acid solution, and eluted with elution solution (80% acetonitrile, 0.5% formic acid). Acetonitrile was then evaporated in a SpeedVac. Sample solution (3% acetonitrile, 0.5% formic acid) was added for analysis.

Peptides were injected and analysed by reversed-phase liquid chromatography on a nanoAcquity UPLC system (Waters) equipped with HSS-T3 C18 1.8 µm, 75 µm X 250 mm column (Waters). A gradient from 1% to 40% acetonitrile in 60 min (ending with a brief regeneration step to 90% for 3 min) was applied. [Glu^1]-fibrinopeptide B was used as lock mass compound and sampled every 30 s. Online MS/MS analysis was done using Synapt G2-Si HDMS mass spectrometer (Waters) with an $UDMS^E$ method set up as described in (*5*).

Raw data from all samples were first analysed using the vender software ProteinLynx Global SERVER (PLGS) version 3.0.3. Generally, mass spectrum data were generated using an MS^E processing parameter with charge 2 lock mass 785.8426, and default energy thresholds. For protein identification, default workflow parameters except an additional acetyl in N-terminal variable modification were used. Reference protein database was downloaded from GenBank with the accession number NC_003888.3.

LC-MS analysis of metabolites.

LC-MS/MS acquisition was performed using Shimadzu Nexera X2 UHPLC system, with attached PDA, coupled to Shimadzu 9030 QTOF mass spectrometer, equipped with a standard ESI source unit, in which a calibrant delivery system (CDS) is installed. Samples were dissolved in MeOH to a final concentration of 1 mg/mL, and 2 µL were injected into a Waters Acquity HSS C_{18} column (1.8 µm, 100 Å, 2.1 × 100 mm). The column was maintained at 30 °C, and run at a

flow rate of 0.5 mL/min, using 0.1% formic acid in H_2O as solvent A, and 0.1% formic acid in acetonitrile as solvent B. A gradient was employed for chromatographic separation starting at 5% B for 1 min, then 5 – 85% B for 9 min, 85 – 100% B for 1 min, and finally held at 100% B for 4 min. The column was re-equilibrated to 5% B for 3 min before the next run was started. The PDA acquisition was performed in the range 200 – 600 nm, at 4.2 Hz, with 1.2 nm slit width. The flow cell was maintained at 40 °C.

All samples were analyzed in positive polarity, using data dependent acquisition mode. In this regard, full scan MS spectra (*m/z* 100–2000, scan rate 20 Hz) were followed by three data dependent MS/MS spectra (*m/z* 100–2000, scan rate 20 Hz) for the three most intense ions per scan. The parameters used for the ESI source were: interface voltage 4 kV, interface temperature 300 °C, nebulizing gas flow 3 L/min, and drying gas flow 10 L/min. The instrument was calibrated using standard NaI solution (Shimadzu) before each run sequence. Additionally, a calibrant solution consisting of the Agilent API-TOF reference mass solution kit was introduced through the CDS system, the first 0.5 min of each run, and the masses detected were used for post-run mass correction of the file, ensuring stable accurate mass measurements.

SUPPLEMENTAL FIGURES

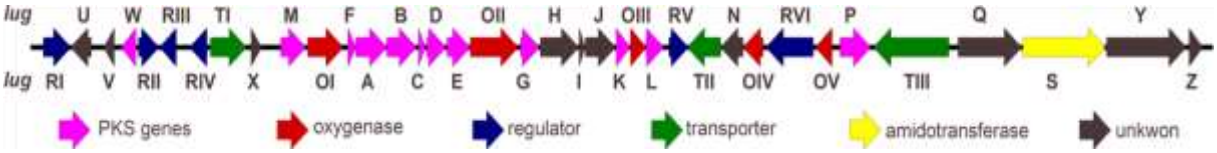


Figure S1. Graphical representation of the *lug* gene cluster of *Streptomyces* sp. QL37. The *lug* cluster, which is responsible for the biosynthesis of lugdunomycin (*2*), contains genes encoding polyketide synthases (pink), tailoring enzymes (red), regulators (blue) and transporters (green). The gene *lugOII* that is located downstream of *lugA-F* (the minimal PKS genes) encodes a two-domain oxidoreductase and is the subject of this study. Hypothetical genes are labeled in black.

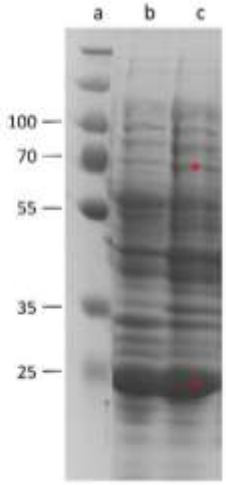


Figure S2. SDS-PAGE analysis of semi-purified LugOII isoforms. Lane a, protein marker (in kDa). Lane b and c, LugOII full-length gives a band corresponding to a protein of 70-kDa, while the reductase domain of LugOII generates a band at 27-kDa. LugOII was overexpressed in *Streptomyces* sp. QL37 (OII^-) and purified by employing ion exchange chromatography (IEX) using a monoQ column. Both bands were analysed by in-gel digestion followed by LC-MS/MS analysis, confirming the presence of two isoforms of LugOII (LugOII full-length and the separated reductase domain, labeled by asterisks).

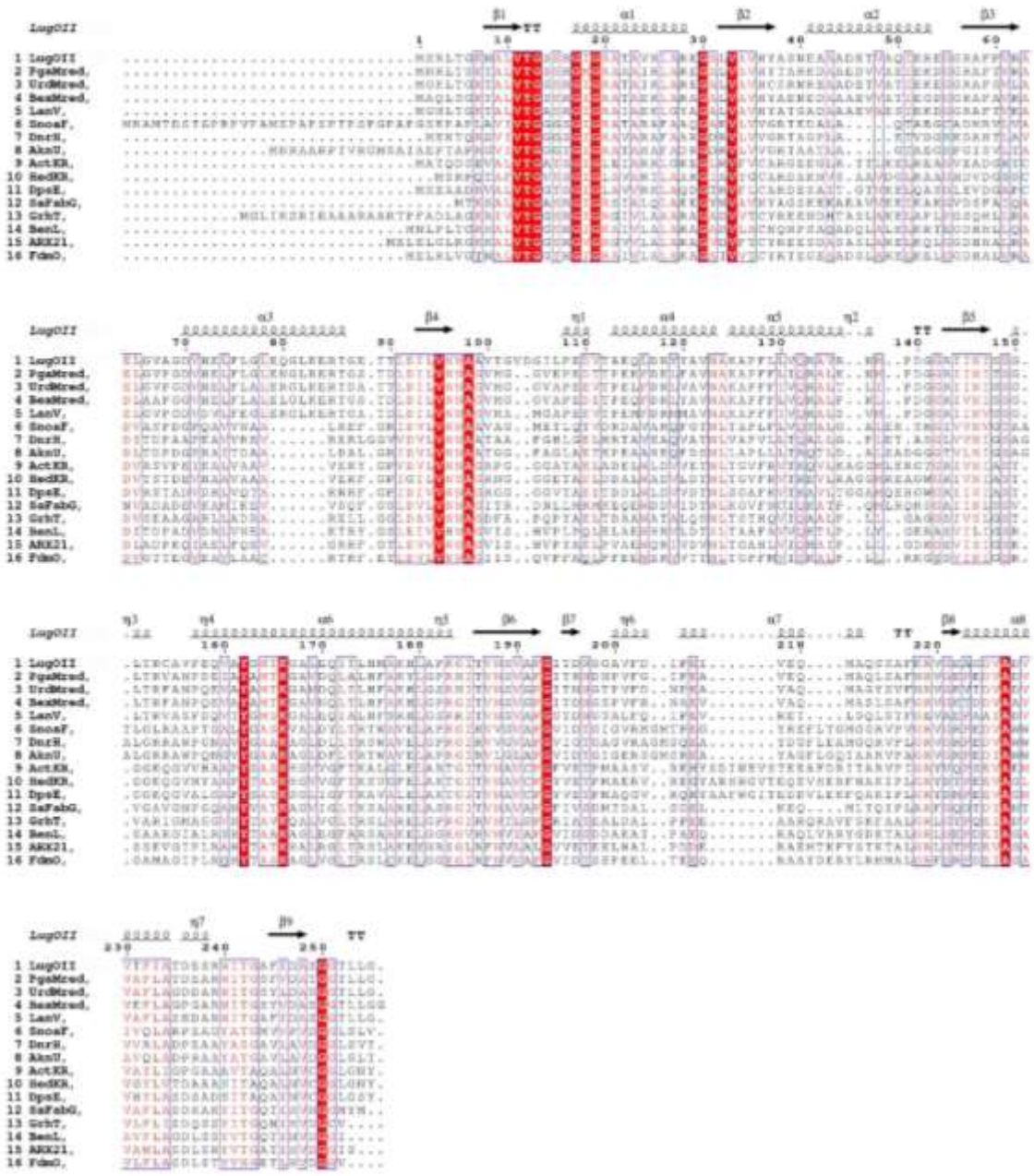


Figure S3. Multi-sequence alignment of LugOII and the functionally characterized keto-reductase (KRs). Sequences include PgaMred from *Streptomyces* sp. PGA64, UrdMred from *Streptomyces fradiae* Tu 2717, BexMred from *Streptomyces fulvissimus* DSM 40593, SnoaF from *Streptomyces nogalater*, AknU from *Streptomyces galilaeus*, DnrH from *Streptomyces peucetius*, DpsE from *Streptomyces peucetius*, HedKR from *Streptomyces griseoruber*, ActKR from *Streptomyces coelicolor* (Muller 1908), ARX21 from uncultured bacterium, BenL from *Streptomyces* sp. A2991200, GrhT from *Streptomyces* sp. CN48+, and FdmO from *Streptomyces griseus*. The secondary structural features are labeled on the top of the sequence based on the crystal structure of LugOII.

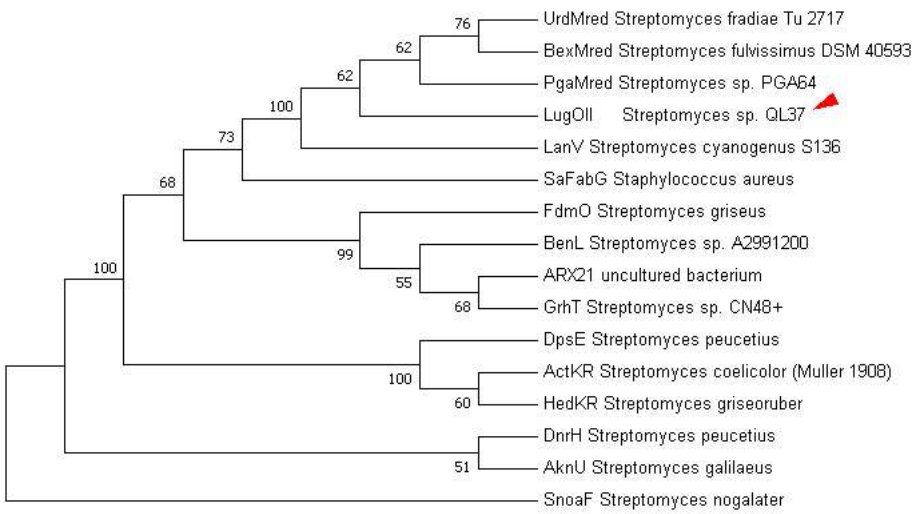


Figure S4. An unrooted maximum likelihood tree of the KRs that are related to LugOII. The phylogenetic tree was drawn as a consensus model. Bootstrap values are shown at each node as percentage of 1000 replicates.

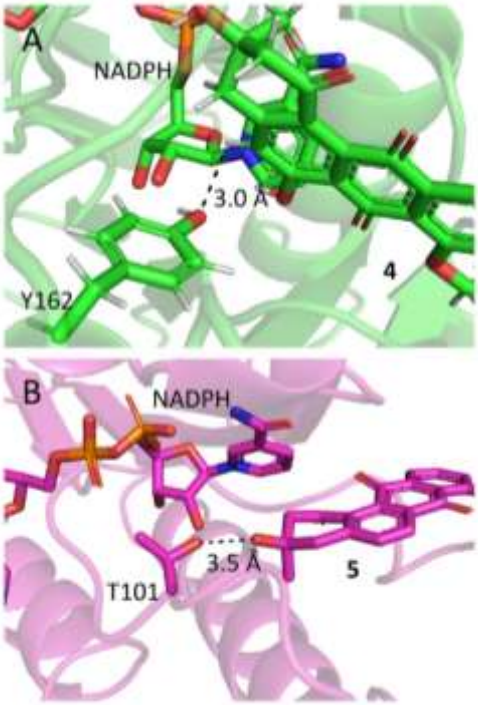


Figure S5. The interactions between the ligands and the key residues of LugOII structure. The residues and ligands are shown in sticks. (A) The distance between the OH group of Y162 and C5 of ligand **4** is around 3.0 Å. (B) The hydrogen bond between T101 and ligand **5** is around 3.3 Å.

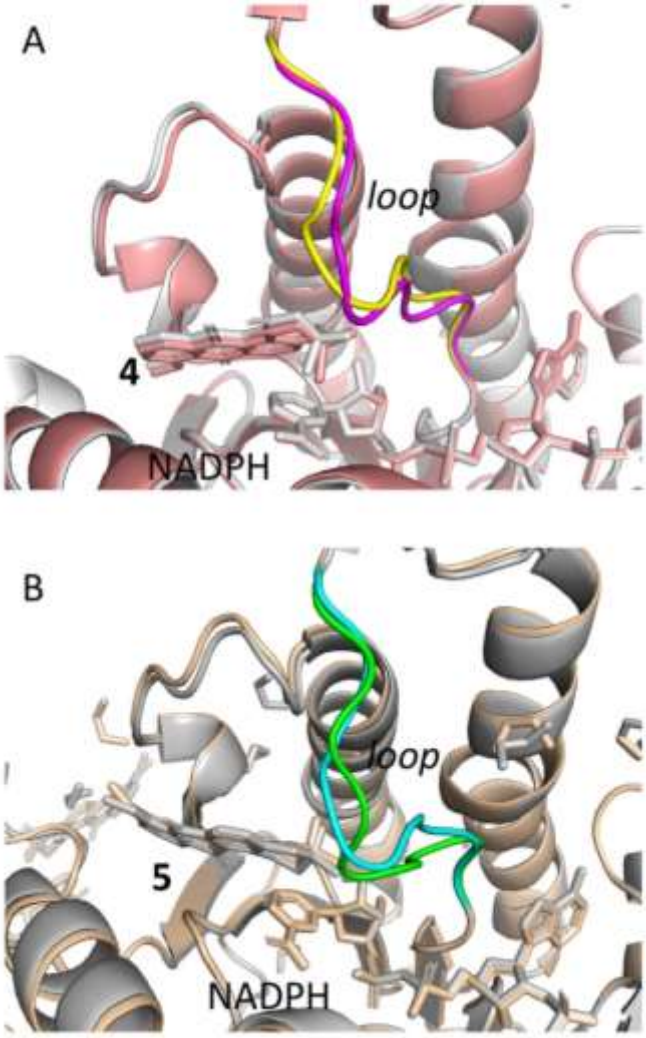
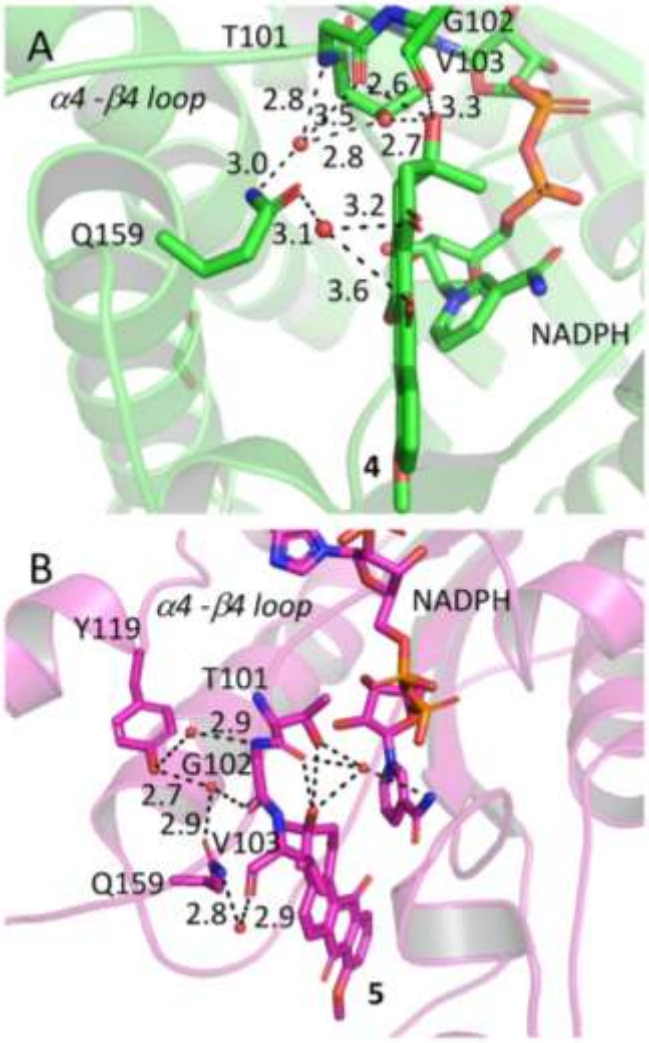


Figure S6. Alignment of the different chains from LugOII complex structures. Ligands are shown in sticks. (A) Superimposition of chain A and B in the ligand **4** bonded LugOII structure. The loop region of chain A is highlighted in yellow and that of chain B in magenta. (B) Superimposition of chain A and B in ligand **5** bonded LugOII structure. The loop region of chain A is highlighted in green and that of chain B in cyan. Related to Figure 4.



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214 **Figure S7. Interaction network of Q159 in LugOII complex structures.** The ligands and
215 residues are shown in sticks. Interactions of Q159 with T101, G102, V103, and either
216 compound **4** (A) or **5** (B), via water molecules that stabilize the α4-β4 loop.

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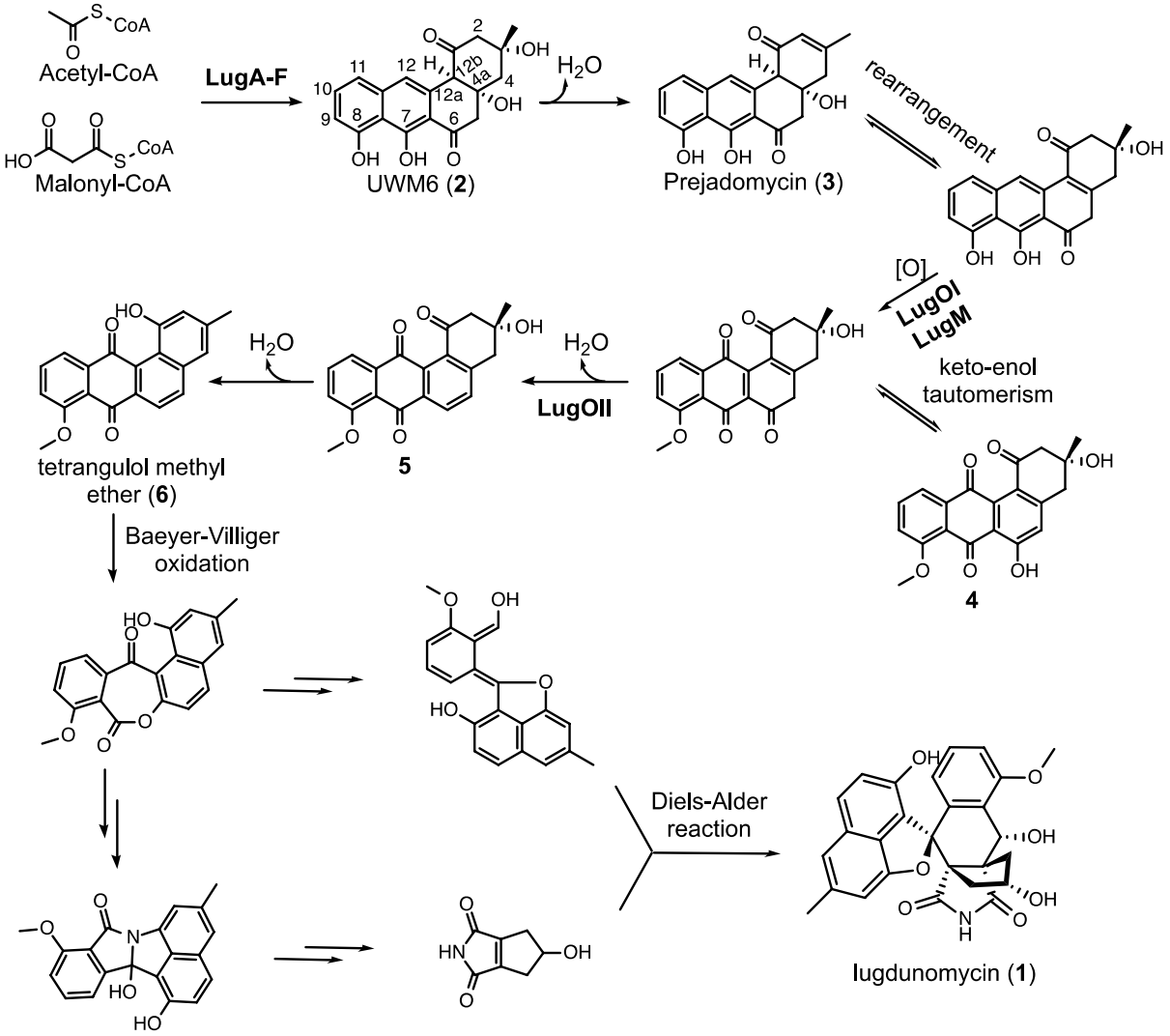
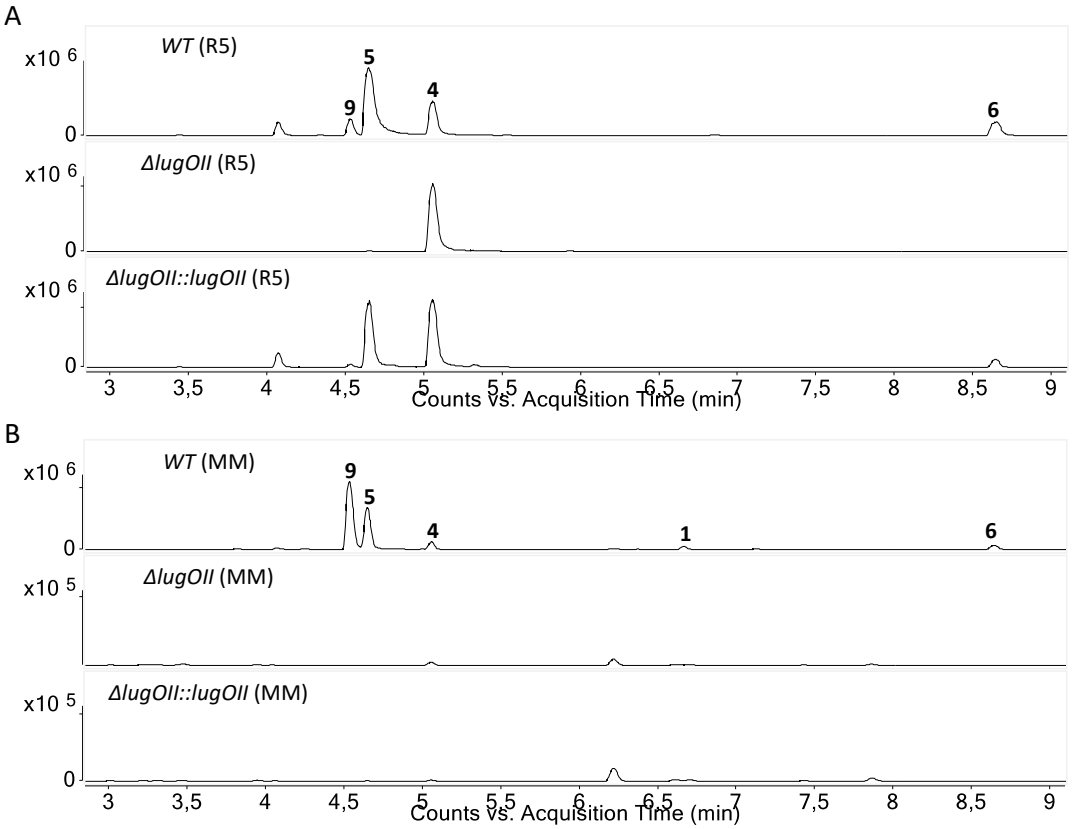
SUPPLEMENTAL DATA

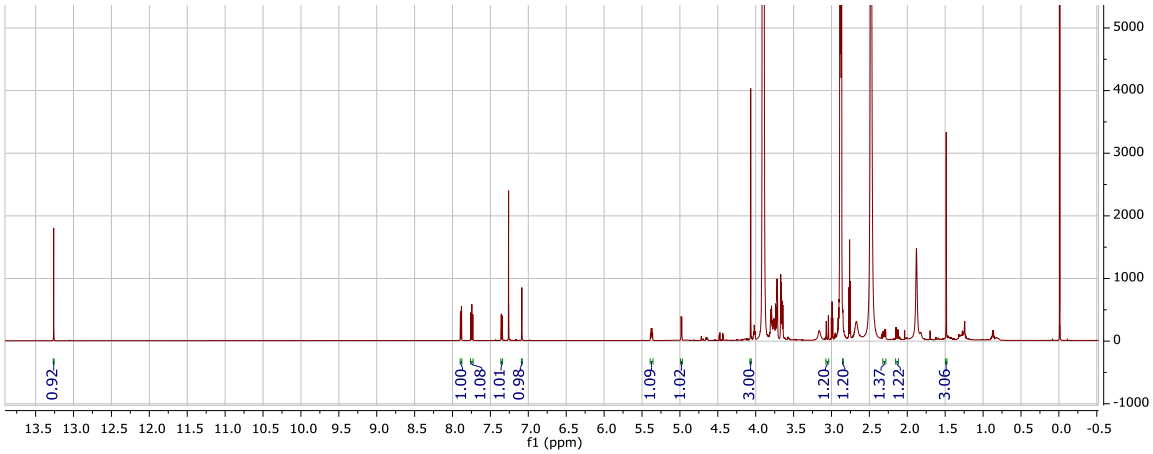
Scheme S1. Postulated lugdunomycin biosynthetic pathway (*2*)**.** Flavoenzyme (LugOI), methyl transferase (LugM) and keto-reductase (LugOII) are proposed to react sequentially in the early steps of angucycline biosynthesis, as part of the lugdunomycin biosynthetic pathway.



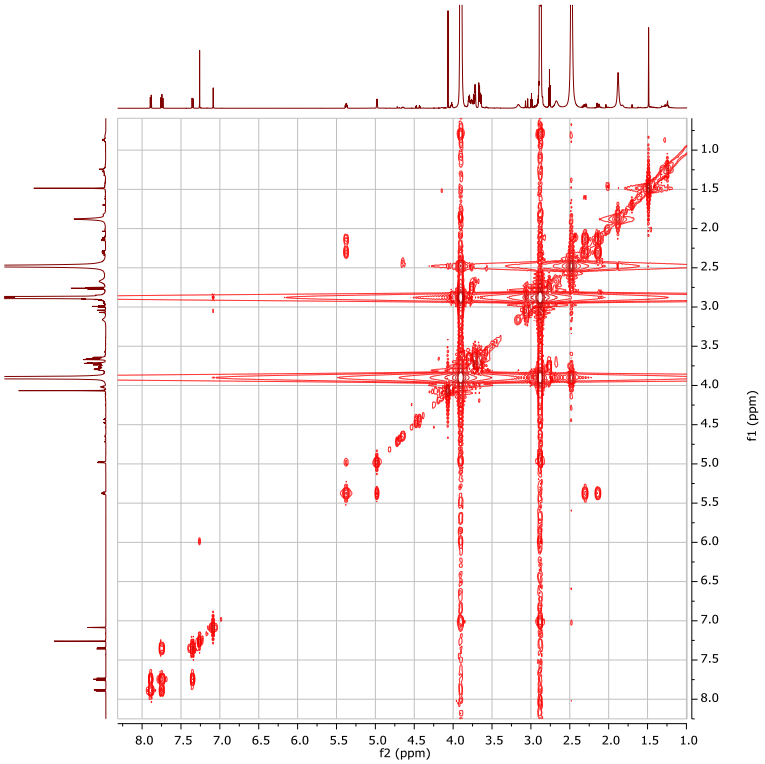
Data S1. LC-MS analysis of the metabolite profiles from the extracts derived from wild-type S sp. QL37, ΔlugOII and ΔlugOII::lugOII. (A) Extracted ion chromatogram of the wild-type, *ΔlugOII* and *ΔlugOII::lugOII* strains grown on R5 medium (containing 0.8% peptone and 1% mannitol). Deletion of *lugOII* blocks the production of **5**, **6** and **9**, while its introduction under the control of *ermE* promoter restores their production. (B) Extracted ion chromatogram of the wild-type and *ΔlugOII* and *ΔlugOII::lugOII* strains grown on MM medium (containing 0.5% glycerol and 1% mannitol). Deletion of *lugOII* blocks the production of **1**, **5**, **6** and **9**. The complementation of *lugOII* didn't work. Note that **1** is only produced in MM medium.



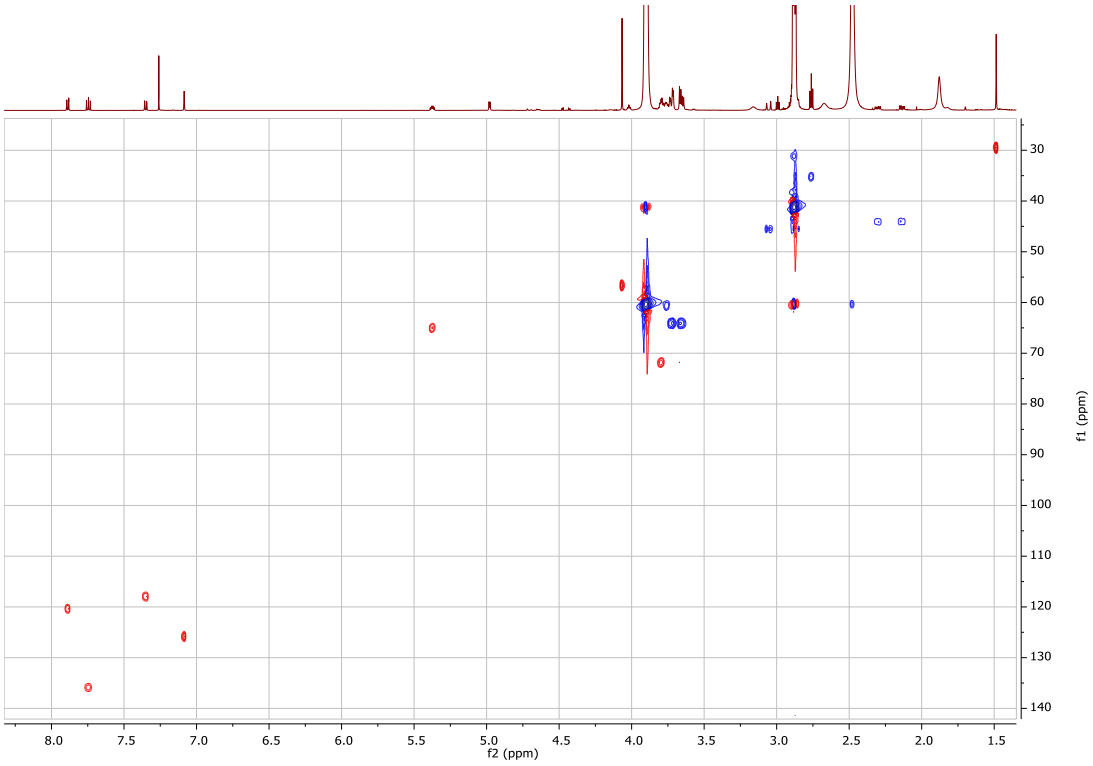
Data S2. Spectroscopic data of compound 7.



1H NMR spectrum of **7** in CDCl3 (600 MHz, 298 K).



COSY spectrum of **7** in $CDCl_3$ (600 MHz, 298 K).



241

242 Multiplicity-edited HSQC spectrum of **7** in $CDCl_3$ (600 MHz, 298 K).

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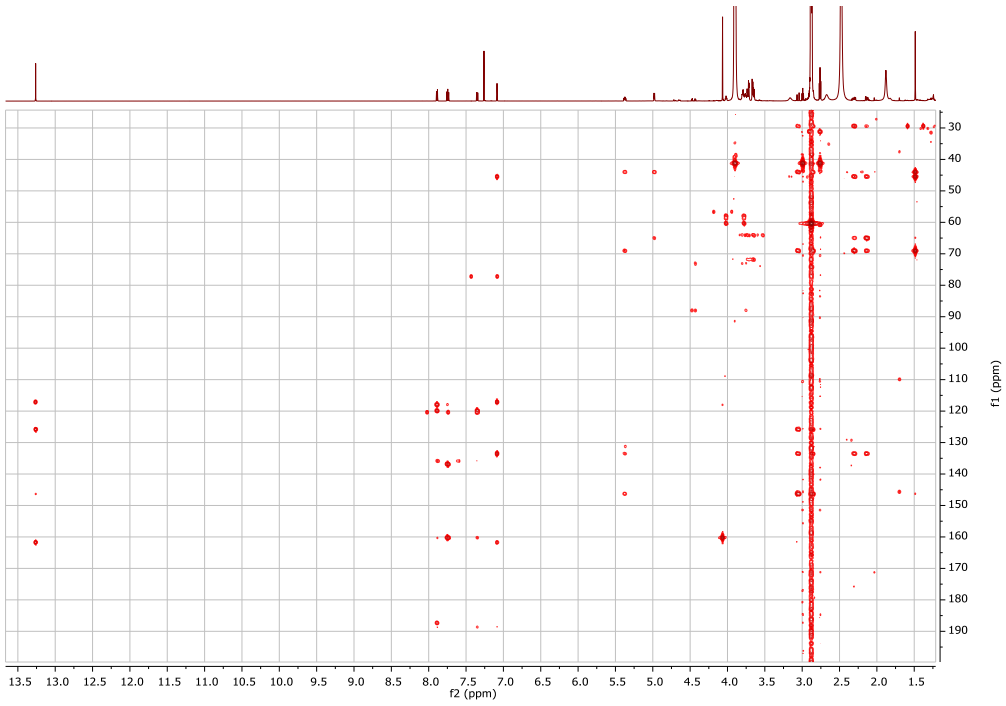

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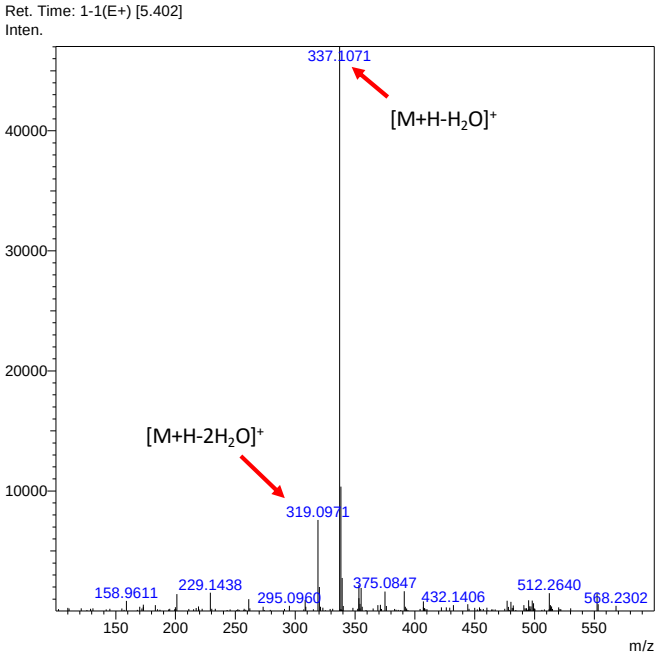
246 HMBC spectrum of **7** in $CDCl_3$ (600 MHz, 298 K).



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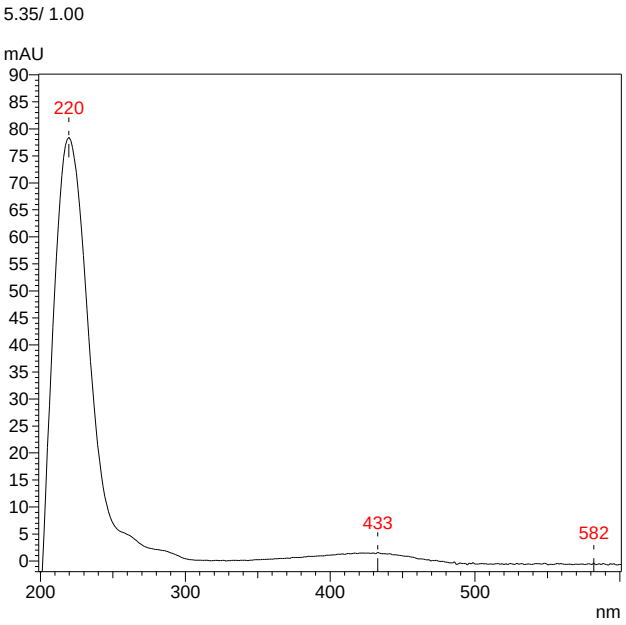
248 (+)-HR-ESI-MS spectrum of **7.**

249



250

251 UV spectrum of **7.**

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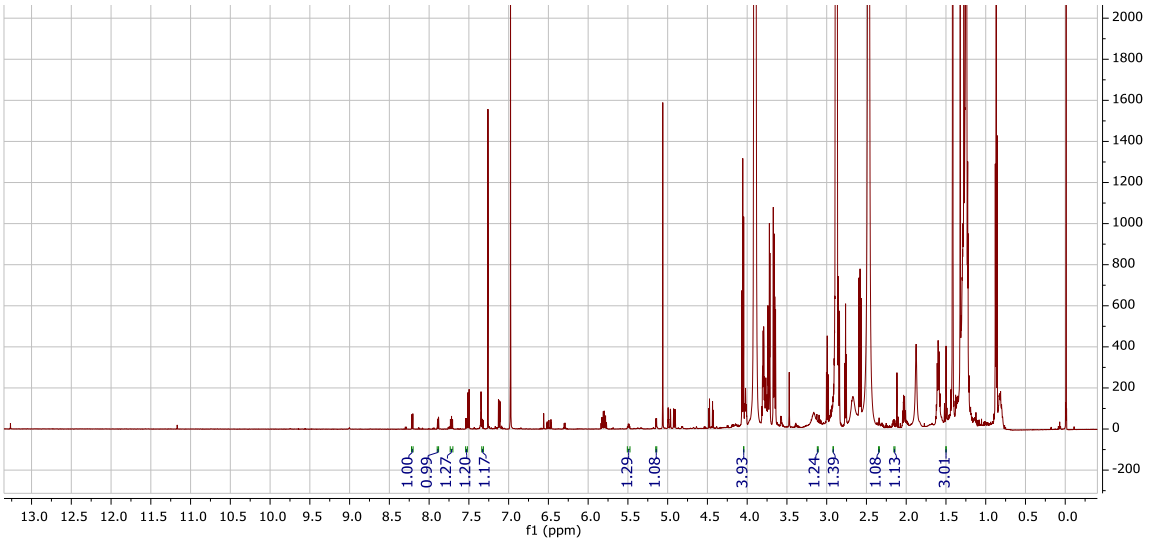
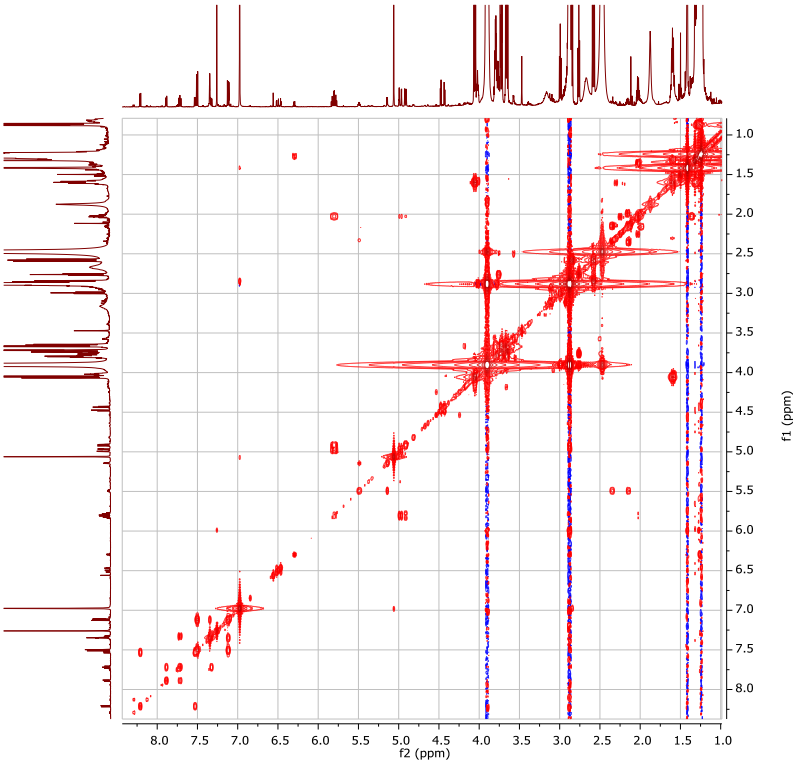
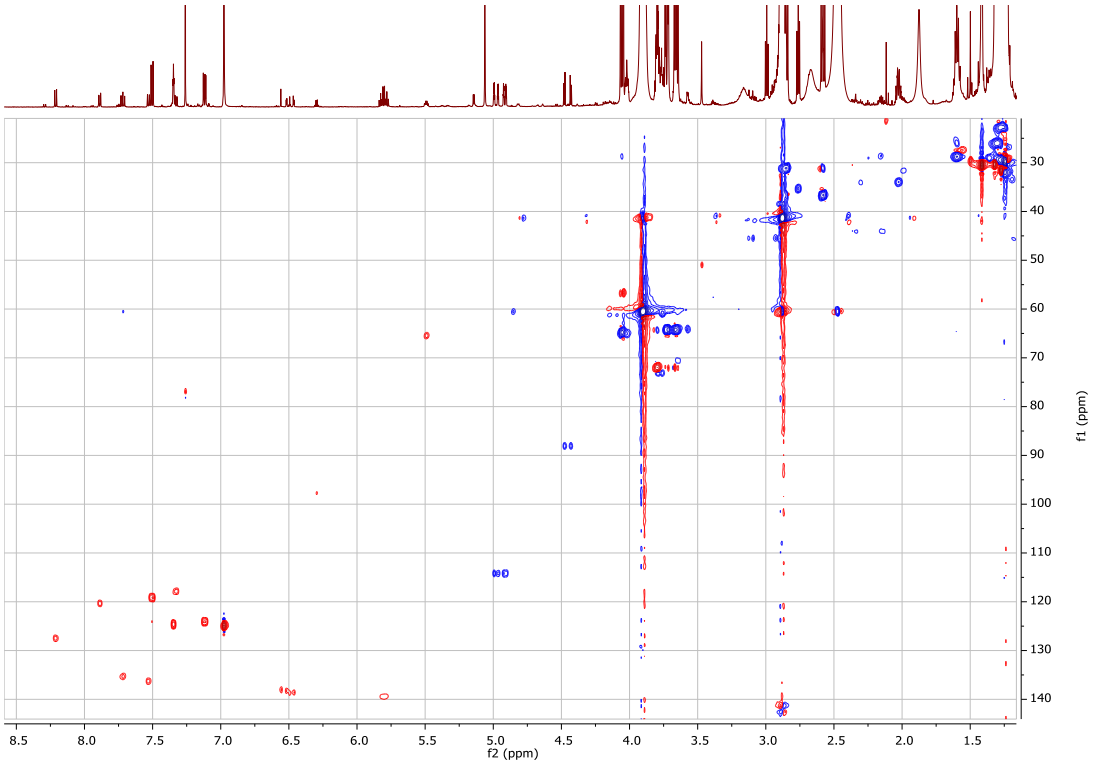
Data S3. Spectroscopic data of compound 8.



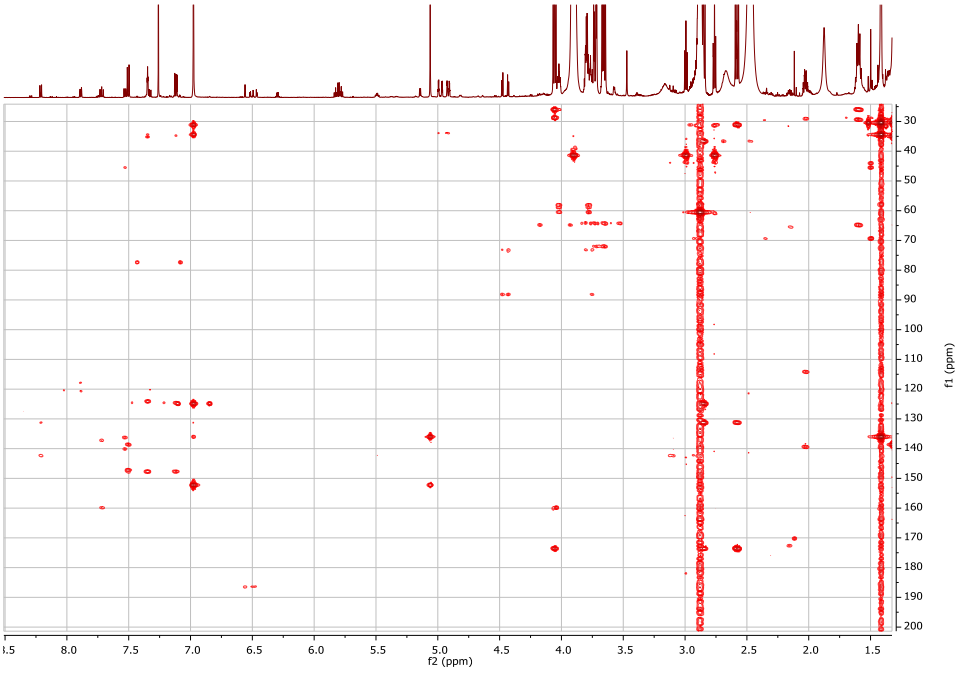
^{1}H NMR spectrum of **8** in $CDCl_3$ (600 MHz, 298 K).



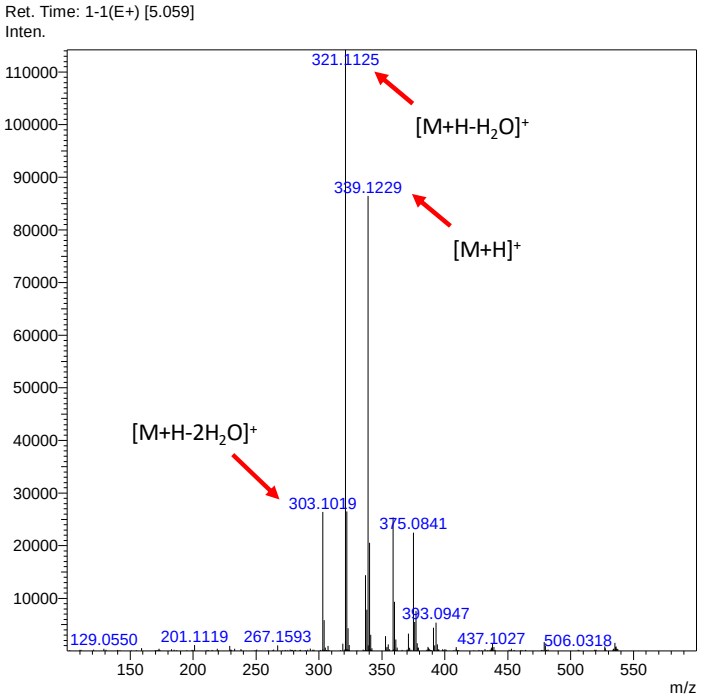
COSY spectrum of **8** in $CDCl_3$ (600 MHz, 298 K).



Multiplicity-edited HSQC spectrum of **8** in $CDCl_3$ (600 MHz, 298 K).

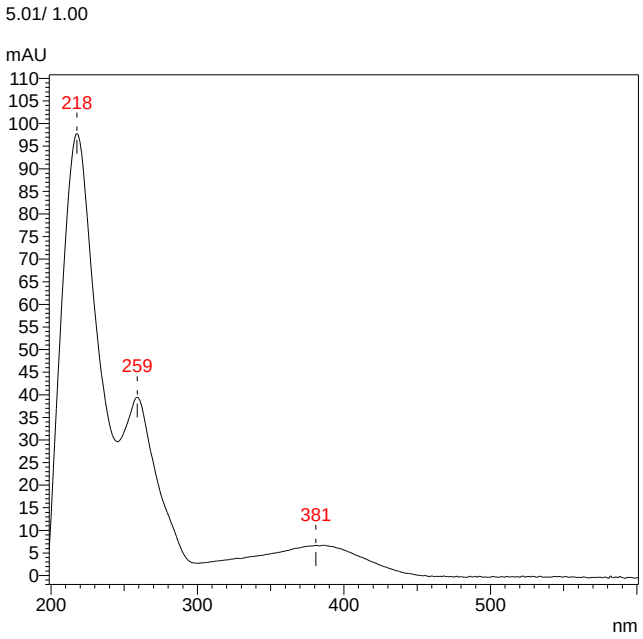


HMBC spectrum of **8** in $CDCl_3$ (600 MHz, 298 K).



264

265 (+)-HR-ESI-MS spectrum of **8**.



266

267 UV spectrum of **8**.

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SUPPLEMENTAL TABLES

Table S1. Bacterial strains used in this study. For all the primers used, see table S2 (below).

Bacterial strains	Relevant characteristics	Metabolites produced	References
Streptomyces sp. QL37	wild-type strain	**1**, **4**, **5**, **6**, **9**	This study
Streptomyces sp. QL37 (OI^-)	*lugOI* deletion mutant	**4**	This study
Streptomyces sp. QL37 (OII^-)	*lugOII* deletion mutant	**4**	This study
Streptomyces sp. QL37 (OII^-1)	*lugOII* deletion mutant harbouring pWHM3-*oriT*-p*ermE*-lugOII*	**4**, **5**, **6**, **9**	This study
Streptomyces sp. QL37 (OII^+1)	wild-type harbouring pWHM3-*oriT*-p*ermE*-lugOII*	**4**, **5**, **6**, **9**	This study
E. coli JM109	Strain for plasmid propagation	-	(*6*)
E. coli ET12567/pUZ8002	*E. coli* host for conjugative transfer of plasmids to *Streptomyces*	-	(*7*)
E.coli BL21(DE3) pLysS Star	*E. coli* host for over-expression of proteins	-	(*8*)

Table S2. Primers used for amplifying the DNA sequences in this study.

Name of primers	Oligonucleotide sequence (5’ -> 3’)
LugOII_fw	gtcaCATATGagcaggctcacgggcaagaacgcac
LugOII_rv	gtcaAAGCTTtcatccgaggagcgtcccgccgctcgcgtc
lugM_fw	gtcaCATATGactgcgcagcacccccctgttgtggaag
lugM_rv	gtcaAAGCTTtcagcggtccttgtggcccagcaccagc
LugOII_V156A_fw	ctgcgccGCAccggagcaggtc
LugOII_V156A_rv	cgggtgagaccggacgagatg
LugOII_S214Y_fw	ggcgcagTACtccgccttcaagcgc
LugOII_S214Y_rv	atctgctccacgatctcggggatg
LugOII_C154F_fw	tcacccgctTcgccgtgccggag
LugOII_C154F_rv	gcacggcgAagcgggtgagaccg
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LugOII_T101M_fw	cgtccactccCAtgacggccgcgttg
LugOII_T101M_rv	gcggccgtcaTGggagtggacggc
LugOII_I194S_fw	tccgccgttgtcggtTGAtcccggtgccacgctgtt
LugOII_I194S_rv	agcgtggcaccgggaTCAaccgacaacggcggagcg
dLugOII_check_Fw	tcgacccacaccacggaatc
dlugOII check_RV	ttcctcgccgatgtgcttgg
lugOII_LF_Fw	cgataagcttggtaccgagctgtggctggacgtgatcaac
lugOII_LF_Rv	cgattctagacgtggtgcccacgggtcagc
lugOII_RF_Fw	cgattctagaggcgggacgctcctcggatg
lugOII_RF_Rv	cgatgaattcggtgagggccggcgagtagg
lugOI_KO_check_Fw	cgagtgccatgccctgatgaag
lugOI_KO_check_Rv	gcgacgatcagagtgcttgg
lugOI-LF-Fw	cgataagcttagatcttccatcccgccttctgaagac
lugOI-LF-Rv	cgattctagagactacgactgatgcgtccatgtg
lugOI-RF-Fw	cgattctagaggaccggccaggtgagattc
lugOI-RF-Rv	cgatggtaccgccggtcgttctgcttggtc
lugOII_OE_Fw	cgatGGATCCATATGGGCACCACGCGCGACGCGGAC
lugOII_OE_Rv	cgatGGATCCGTGCACCGAGGACGGCTCATC

275 **Table S3. X-Ray data collection, processing and refinement.**

	Apo LugOII	NADPH binary complex	NADPH plus **4** ternary complex	NADPH plus **5** ternary complex
Data collection				
PDB accession code	6YQ6	6YPZ	6YQ3	6YQ0
Beamline	X06DA	X06DA	BL13B1	X06DA
Wavelength (Å)	1.0	1.0	1.0	1.0
Detector	PILATUS 2MF	PILATUS 2MF	ADSC Quantum-315 CCD	PILATUS 2MF

Resolution range (Å)[a]	47.47-2.08 (2.13-2.08)	49.3-1.08 (1.108-1.08)	24.70-1.57 (1.61-1.57)	49.49-1.08 (1.108-1.08)
Space group	$P6_122$	I121	I121	I121
A, b, c (Å)	185.99, 185.99, 75.64	110.31, 59.97, 87.83	87.97, 60.21, 88.31	110.47, 60.38, 88.1
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 128.5, 90.0	90.0, 102.5, 90.0	90.0, 128.7, 90.0
Total observations[a]	1837762 (285625)	1326582 (201219)	695227 (166021)	1330599 (198024)
No. unique reflections[a]	87905 (14133)	428985 (67732)	60525 (6124)	431114 (68021)
Redundancy[a]	20.9 (20.2)	3.1 (3.0)	2.9 (2.8)	3.1 (2.9)
Completeness (%)[a]	99.8 (99.0)	95.4 (99.1)	98.1 (91.9)	95.4 (99.0)
Mean $I/\sigma(I)$[a]	12.5 (1.7)	13.4 (2.1)	22.5 (2.7)	16.3 (3.2)
R_{merge}[a, b]	0.399 (2.839)	0.054 (0.679)	0.053 (0.464)	0.049 (0.435)
R_{meas}[a, c]	0.409 (2.912)	0.064 (0.827)	0.064 (0.566)	0.058 (0.532)
$CC_{½}$[a, d]	0.994 (0.431)	0.999 (0.648)	0.999 (0.818)	0.999 (0.801)
Wilson *B* value ($Å^2$)	33.82	12.94	16.88	11.77
Refinement				
No. of reflections[b]	44261/3168	181839/13457	57497/4253	183000/13494
Final R_{work}[a, c]	0.168 (0.273)	0.146 (0.270)	0.142 (0.218)	0.155 (0.248)
Final R_{free}[a, c]	0.204 (0.287)	0.165 (0.266)	0.166 (0.239)	0.170 (0.249)
R.m.s. bond deviations (Å)	0.015	0.022	0.017	0.022
R.m.s. angle deviations (°)	1.830	2.215	2.114	2.206
Number of atoms	4027	4481	4421	4583
Number of water molecules	363	507	484	551
Number of other molecules	16 EDO, 4 PEG, 3 SO_4^{2-}	2 NADPH, 12 EDO, 2 PEG	2 compound **4**, 2 NADPH, 2 EDO	2 compound **5**, 2 NADPH, 14 EDO
Mean *B*-factor ($Å^2$)				
protein	29.74	12.04	17.00	10.56
water	38.82	24.87	28.60	23.10
ligands		9.42	24.76	11.59

others	54.25	22.72	26.63	17.16
Ramachandran plot (%)[e]				
Favored regions	97.63	97.80	97.40	97.22
Allowed regions	2.37	2.20	2.60	2.78
Outliers	0	0	0	0

[a] Values for the outer resolution shell are given in parentheses.
[b] The data set was split into "working" and "free" sets consisting of 95 and 5% of the data, respectively. The free set was not used for refinement.
[c] The R-factors R_{work} and R_{free} are calculated as follows: R = Σ (| Fobs - Fcalc |)/Σ| Fobs |, where Fobs and Fcalc are the observed and calculated structure factor amplitudes, respectively.
[d] Diffraction precision indicator based on R_{free} based as calculated by REFMAC5(*9*).
[e] As calculated using MOLPROBITY(*10*).

Table S4. ^{1}H and ^{13}C NMR data of 7 and 8 at 298 K in CDCl3 with added TMS [a].

Position	7		8	
	δ_C, type	**δ_H, mult. (*J* in Hz)**	**δ_C, type**	**δ_H, mult. (*J* in Hz)**
1	65.0, CH	5.37, ddd (6.9, 5.5, 4.3)	65.5, CH	5.49, m
2	44.1, CH_2	a: 2.3, ddd (13.8, 6.9, 1.8)	44.0, CH_2	a: 2.35, m
		b: 2.14, ddd (13.8, 5.5, 1.4)		b: 2.15, m
3	69.1, C		69.3, C	
4	45.5, CH_2	a: 3.05, dt (16.6, 1.4)	45.5, CH_2	a: 3.11, m
		b: 2.85, m		b: 2.91, m
4a	146.3, C		142.3, C	
5	125.8, CH	7.08, d (1.1)	136.3, CH	7.53, dt (8.0, 0.9)
6	161.7, C		127.5, CH	8.21, d (8.0)
6a	117.1, C		136.3, C	
7	188.6, C		ND[c], C	
7a	119.9, C		120.7, C	
8	160.2, C		159.9, C	
9	118.0, CH	7.35, dd (8.5, 1.1)	117.9, CH	7.3, dd (8.5, 1.1)
10	135.9, CH	7.75, dd (8.5, 7.7)	135.3, CH	7.72, dd (8.5, 7.8)
11	120.4, CH	7.89, dd (7.7, 1.1)	120.3, CH	7.89, dd (7.8, 1.1)
11a	136.9, C		137.2, C	

12	187.3, C		ND[c], C	
12a	ND[c], C		131.2, C	
12b	133.5, C		140.1, C	
1-OH		4.98, d (4.3)		5.14, d (4.5)
3-CH_3	29.5, CH_3	1.49, s	29.6, CH_3	1.50, s
6-OH		13.26, s		
8-OH				
8-OCH_3	56.7, CH_3	4.07, s	56.7, CH_3	4.04, s

[a] ^{1}H 600 MHz, ^{13}C chemical shifts inferred from multiplicity-edited HSQC and HMBC spectra.
[b] Chemical shifts assignments were interchanged in literature(*11*). Re-assignments were based on the observed HMBC correlations.
[c] ND: Not determined due to lack of HMBC correlations to them.

REFERENCES

1. Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F., and Hopwood, D. A. (2000) *Practical Streptomyces genetics*, John Innes Foundation, Norwich, U.K.
2. Wu, C. S., van der Heul, H. U., Melnik, A. V., Lubben, J., Dorrestein, P. C., Minnaard, A. J., Choi, Y. H., and van Wezel, G. P. (2019) Lugdunomycin, an angucycline-derived molecule with unprecedented chemical architecture, *Angew Chem Int Edit 58*, 2809-2814.
3. Grabley, S., Hammann, P., Hutter, K., Kluge, H., Thiericke, R., Wink, J., and Zeeck, A. (1991) Secondary metabolites by chemical-screening .19. Sm-196-a and Sm-196-B, novel biologically-active angucyclinones from Streptomyces Sp, *J Antibiot 44*, 670-673.
4. Rappsilber, J., Mann, M., and Ishihama, Y. (2007) Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips, *Nat. Protoc. 2*, 1896-1906.
5. Distler, U., Kuharev, J., Navarro, P., Levin, Y., Schild, H., and Tenzer, S. (2014) Drift time-specific collision energies enable deep-coverage data-independent acquisition proteomics, *Nat. Methods 11*, 167-170.
6. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors, *Gene 33*, 103-119.
7. MacNeil, D. J., Occi, J. L., Gewain, K. M., MacNeil, T., Gibbons, P. H., Ruby, C. L., and Danis, S. J. (1992) Complex organization of the Streptomyces avermitilis genes encoding the avermectin polyketide synthase, *Gene 115*, 119-125.
8. Zhang, X., and Studier, F. W. (1997) Mechanism of inhibition of bacteriophage T7 RNA polymerase by T7 lysozyme, *J Mol Biol 269*, 10-27.

9. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Refinement of macromolecular structures by the maximum-likelihood method, *Acta Crystallogr D 53*, 240-255.
10. Davis, I. W., Leaver-Fay, A., Chen, V. B., Block, J. N., Kapral, G. J., Wang, X., Murray, L. W., Arendall, W. B., 3rd, Snoeyink, J., Richardson, J. S., and Richardson, D. C. (2007) MolProbity: all-atom contacts and structure validation for proteins and nucleic acids, *Nucleic Acids Res 35*, W375-383.
11. Shaaban, K. A., Srinivasan, S., Kumar, R., Damodaran, C., and Rohr, J. (2011) Landomycins P-W, cytotoxic angucyclines from Streptomyces cyanogenus S-136, *J Nat Prod 74*, 2-11.