Biosynthetic 4,6-Dehydratase Gene Deletion: Isolation of a Glucosylated Jadomycin Natural Product Provides Insight into the Substrate Specificity of Glycosyltransferase JadS

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1. Construction of deletion mutants

Methods

Escherichia coli strains were grown on Luria-Bertani/lysogeny broth (LB; tryptone 1% (w/v), NaCl 1% (w/v), and yeast extract 0.5 % (w/v)) agar and cultured in LB liquid media in a rotary shaking incubator at 250 rpm and 37˚C. *Streptomyces venezuelae* strains were grown on maltose yeast extract media (MYM; maltose 0.4% (w/v), yeast extract 0.4% (w/v) and malt extract 1% (w/v)) agar and cultured in MYM liquid media in a rotary shaking incubator at 250 rpm and 30˚C unless otherwise indicated. Kanamycin (50 μg/mL), chloramphenicol (25 μg/mL), apramycin (50 μg/mL), and naladixic acid (50 μg/ml) were added to the media when appropriate. *E. coli* ET12567 pUZ8002 was cultured with chloramphenicol and kanamycin.

Construction of pKC1139_Δ*jadT*

A 1 kb fragment immediately upstream of *jadT* was amplified by PCR from *S. venezuelae* ISP5230 genomic DNA with the following primers; Primer 1: 5' GGTGGTAAGCTTGGCCGCGGAGCCGTACGAGGAGCTGATCGC, HindIII site is underlined, and Primer 2: 5' GGTGGTTGCATGGTCGTCGTCACCATATGTCATGCCGTCCCTCCGAGGAGCGCGTTGAG, NdeI site is underlined. A 1 kb fragment immediately downstream of *jadT* was amplified by PCR with the following primers; Primer 3: 5' GGTGGTTCGGAGGGACGGCATGACATATGGTGACGACGACCATGCAGGTGACCCCCCTG, NdeI site is underlined, and Primer 4: 5' GGTGGTTCTAGATCCGGCTCCGTACCGTCGTAGACGCCCCGC, XbaI site is underlined. Primer 2 and Primer 3 each contained an identical 40 bp long sequence to facilitate overlap extension PCR (OE-PCR). The upstream and downstream PCR fragments were used as templates for OE-PCR in which the two fragments anneal along the common 40 bp sequence act as self-primers (Figure S1). Extensive non-specific amplification was observed if Primer 1 and Primer 4 were included in the reaction at the outset, however this was reduced if these primers were added after the first 15 cycles of PCR. The PCR product provided the Δ*jadT* deletion

cassette, which was digested with HindIII and XbaI, then ligated with pKC1139[2] (a gift form Dr. Russ Kerr, UPEI) and cloned into *E. coli* NovaBlue Giga competent cells. The plasmid pKC1139_Δ*jadT* was sub-cloned into *E. coli* ET12567 pUZ8002. It is necessary to clone pKC1139_Δ*jadT* into the methylation deficient strain *E. coli* ET12567 pUZ8002 due to the methylation-specific restriction systems of *Streptomyces*.

Construction of *S. venezuelae*_ Δ*jadT* strain

Conjugation between *E. coli* ET12567 pUZ8002 pKC1139_ Δ*jadT* and *S. venezuelae* ISP5230 was performed as previously described[1] with minor modifications. The conjugation plates were overlayed with 1 mL of soft nutrient agar containing 0.5 mg/mL naladixic acid and 0.5 mg/mL apramycin. Potential exconjugants were patched to MYM agar containing naladixic acid and apramycin and incubated at 39˚C. The exconjugants were incubated at 39°C to select for single crossover recombination events, as the temperature sensitive replicon in pKC1139 is inactive above 34˚C.[2] Potential exconjugants were patched to MYM agar containing apramycin and incubated at 30°C, followed by several rounds of growth on MYM agar without antibiotics. Potential exconjugants were screened for apramycin sensitivity and apramycin sensitive colonies were screened for the deletion of *jadT* by PCR using Primer 1 and Primer 4. (Figure S2).

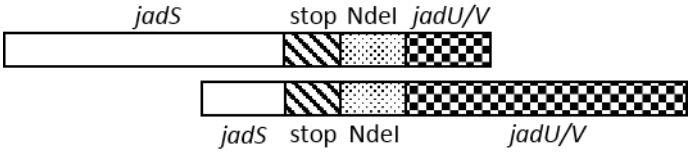
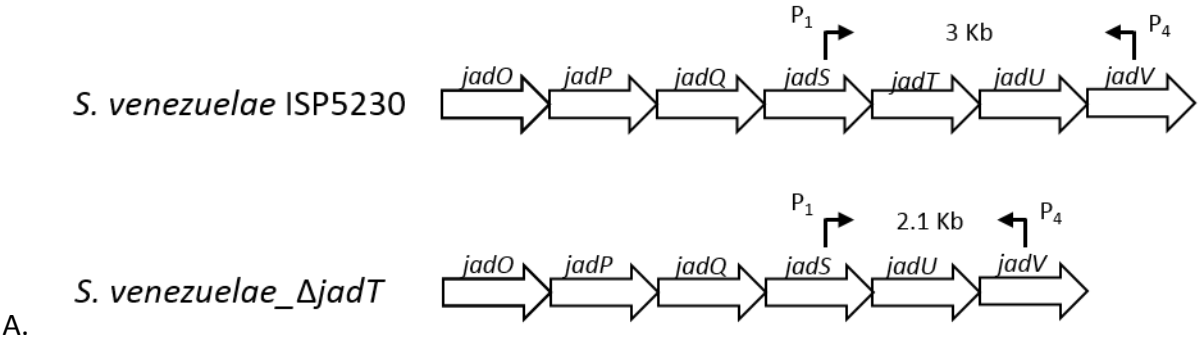
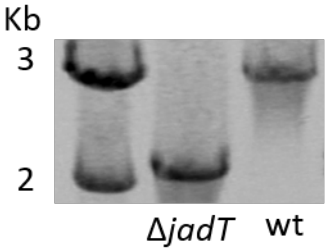


Figure S1. The 1 kb PCR fragments immediately upstream and downstream of *jadT* used for OE-PCR. The identical nucleotide sequences common to both fragments act as self-primers and include the final 17 bp of *jadS* (white), the *jadS* stop codon (diagonal stripes), an NdeI restriction site (dots), and the initial 17 bp of *jadU* (checks).



A.



B.

Figure S2. (A.) The sugar biosynthetic genes of the jadomycin gene cluster in *S. venezuelae* ISP5230 and *S. venezuelae*_Δ*jadT*. The locations of binding for Primer 1 and Primer 4 are indicated with black arrows. The expected size of the PCR product for each strain is indicated. (B.) Agarose gel of the PCR products of *S. venezuelae*_Δ*jadT* and *S. venezuelae* ISP5230 using Primer 1 and Primer 4.

Preparation of conjugation vector pKC1139_ Δ*SV0189*

A 0.9 kb region upstream of SVEN_0189(*Sv0189)* was amplified from *S. venezuelae (*ISP5230) genomic DNA using primers 5 and 6:

Primer 5: 5'-GGTGGTAAGCTTGTGTCCACGACCACCGAAAG-3' (HindIII site is underlined) Primer 6: 5'-CTGGATCTCCCGTGCGGACCACAGTAGACGAGACGATACGTATC-3' (32 bp overlap region is underlined)

A 1.3 kb downstream region was similarly amplified using primers 7 and 8:

Primer 7: 5'-CTCGTCTACTGTGGTCCGCACGGGAGATCCAGGAGTCCATG-3' (32 bp overlap region is underlined)

Primer 8: 5'-GGTGGTCTAGACGGTGAAGGGATTCGGCGTGAC-3' (XbaI site is underlined)

Q5 DNA polymerase (New England Bioscience) was used for PCR reactions, following manufacturer protocols. The upstream and downstream PCR fragments were used as the template for OE-PCR amplification with the outer primers (primers 5 and 8) to produce the 2.2 kb deletion cassette. Non-specific binding was not observed when primers were added at the outset of the reaction, so the self-annealing step described before was not necessary. Digestion of the deletion cassette with HindIII and Xbal and ligation with linearized and calf intestinal alkaline phosphatase (CIP, New England Bioscience) treated pK1139 followed by transformation to NEB 5-alpha competent *E. coli (*high efficiency) resulted in the isolation of conjugal vector pKC1139_ Δ*Sv0189*. The vector was sub-cloned to competent *E. coli* ET12567 pUZ8002.

Preparation of conjugation vector pKC1139_Δ*jadS*_Δ*jadT*

Regions upstream and downstream of the genes *jadS* and *jadT* site were amplified from *S. venezuelae (*ISP5230) genomic DNA. The 1.3 kb upstream fragment was amplified using primers 9 and 10.

Primer 9: 5'-GCAGAAGCTTGACCAGGTCCGCAACACG-3' (HindIII site is underlined)

Primer 10: 5'-CCTGCATGGTCGTCGTCACTTCTAGACATGGTTCTCTCTCCGC-3' (XbaI site is underlined)

A 1.1 kb downstream region was similarly amplified using primers 11 and 12:

Primer 11: 5'- GCGGAGAGAGAACCATGTCTAGAAGTGACGACGACCATGCAGG-3' (XbaI site is underlined)

Primer 12: 5'- GCTGATGAATTCGGTCGTACTCGCCCTGC-3' (EcoRI site is underlined)

Cloning procedures were identical to those described for the assembly of pKC1139_ Δ*Sv0189*, with the following exception: the upstream and downstream PCR fragments were successively ligated to pKC1139 due using XbaI/EcoRI and XbaI/HindIII restriction sites, respectively. pKC1139_ Δ*jadS_ΔjadT* was sub-cloned to competent *E. coli* ET12567 pUZ8002.

Intergenic conjugation and screening procedures for *S. venezuelae*_Δ*jadS*_Δ*jadT*, *S. venezuelae*_Δ*jadT*_Δ*Sv0189*, and *S. venezuelae*_Δ*Sv0189* strains

Conjugation was performed as described above, with modifications as indicated, using the strains *S. venezuelae*_Δ*jadT* or *S. venezuelae* ISP5230 as recipients and *E. coli* ET12567 pUZ8002 harbouring pKC1139_Δ*Sv0189* or pKC1139_Δ*jadS*_Δ*jadT* as the conjugal donor. After overnight incubation, a soft nutrient agar overlay containing 50 μg/mL apramycin and 25 μg/mL naladixic acid was applied to the conjugation plates. After 4 days, potential exconjugants were patched to MYM (50 μg/mL apramycin) and incubated at 37°C (rather than 39°C), enabling the bacteria to grow at a faster rate. Subsequent steps were performed at 30°C, as before; potential exconjugants were patched to MYM agar containing apramycin, followed by three rounds of sporulation on MYM agar without antibiotics. At this point, colonies identified as having acquired sensitivity to apramycin were screened using isolated genomic DNA and primer pairs 5 and 8, and 1 and 4 to identify single deletion mutants (*S. venezuelae*_Δ*Sv0189)* and double deletion mutants *(S. venezuelae*_Δ*jadT*_Δ*Sv0189),* respectively, and primers 13 (13: 5'-CTGGAGGAGAAGCCGGAGCACC-3') and 12 were used for identifying *S. venezuelae* Δ*jadS*_Δ*jadT* double deletion mutants (**Figure S3**).

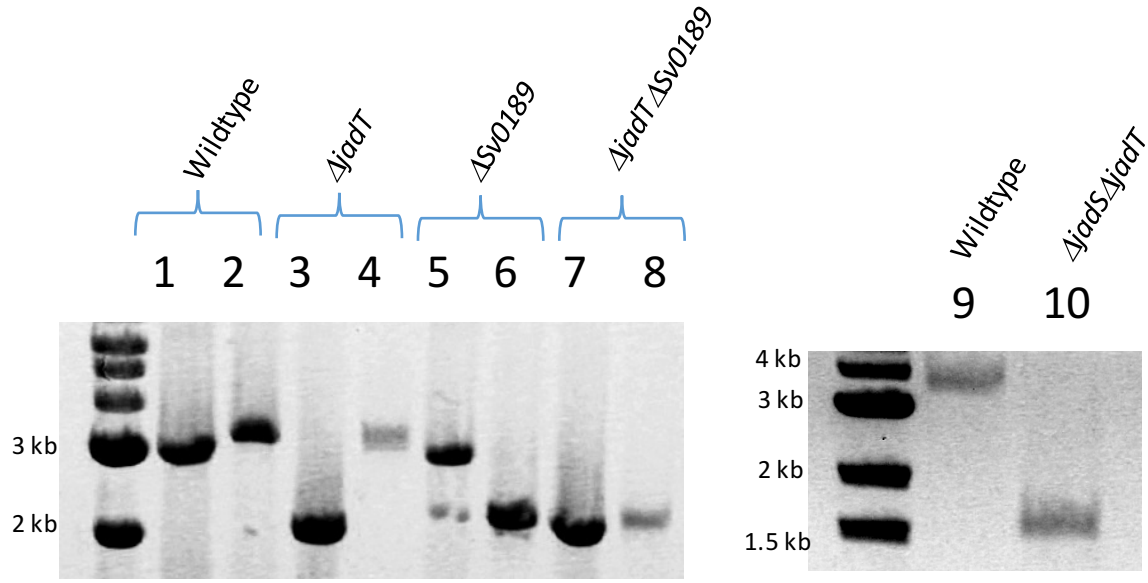


Figure S3. TAE-agarose gel (0.75 %) showing PCR amplification products with primers 1 and 4 (lanes 1,3,5,7); primers 5 and 8 (lanes 2,4,6,8); and primers 12 and 13 (lanes 9 and 10) for indicated *S.venezuelae* strains.

References

1. Kieser, T., Bibb, M., Buttner, M., Chater, K., Hopwood, D. 2000. Practical Streptomyces genetics. Norwich: The John Innes Foundation. 613 p.

2. Bierman, R., Logan, K., O'brien, E.T., Seno, R., Nagaraja, R., and Schoner. B. (1992) Plasmid cloning vectors for the conjugal transfer of DNA from Escherichia coli to Streptomyces spp. Gene. 116: 43.

Bioinformatics analyses

Protein-protein homology searches were performed using blastp (http://blast.ncbi.nlm.nih.gov/Blast.cgi). GT1 family glycosyltransferases were identified from the CAZy database (http://www.cazy.org). Sequence alignments and percent identities were determined using ClustalOmega (1.2.1, https://www.ebi.ac.uk/Tools/msa/clustalo/).

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Percent Identity  Matrix - created by Clustal2.1


   1: SVEN_0537    100.00   20.81   26.24   17.56   21.78   23.21
   2: SVEN_3444     20.81  100.00   32.43   24.70   20.47   21.21
   3: SVEN_6860     26.24   32.43  100.00   20.98   23.03   22.07
   4: SVEN_5997     17.56   24.70   20.98  100.00   23.32   23.45
   5: OleD          21.78   20.47   23.03   23.32  100.00   74.50
   6: SVEN_0189     23.21   21.21   22.07   23.45   74.50  100.00
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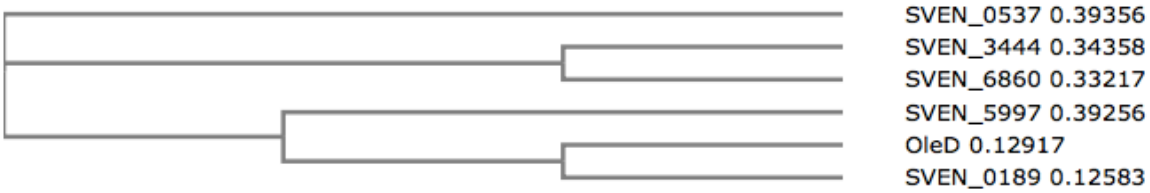

Figure S4. Identity matrix and phylogenetic tree generated by ClustalOmega (1.2.1) analysis.

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SVEN_0537       ----------------------------------MNILLCPLSDGGYLYPAIAAGRELRR
SVEN_3444       ------------------------------------------------MVGLAVRLRE
SVEN_6860       MRTGRTPRQPTPRLRITSSPPMLGRRAPVGQTGGVRIVMMTAGSRGDVAPYTGLSAGLVR
SVEN_5997       ----------------------------------MRYLFTTIPGTSHTLPLVPLAHAALA
OleD            --------------------------MTTQTTPAHIAMFSIAAHGHVNPSLEVIRELVA
SVEN_0189       ----------------------MVVMTTTASRAHIAMFSIAAHGHVNPSLEVIRELVA

SVEN_0537       RGHHVSVLA--RSSAAPVVAESGLPCLP-------AED---FGGRRAFSATWWGTTGAAQ
SVEN_3444       LGAEVRVCAPPDEEFTELLYGIGVPPVPVGAPVRPLVTTVVPG----------STEGLA-
SVEN_6860       AGHEVTLA--AHGVFEPLVTGSGVRFRA--LPVDPRAELHSPRGRRLHD----ARTGAGK
SVEN_5997       AGHEVAFAA--SGPALRAANAAGLQTIAA-------------AGD--EAAEPYE-ELIAK
OleD            RGHRVTYAI--PPVFADKVAATGARPVLYHSTL--------PGPD--ADPEAWGSTLLDN
SVEN_0189       RGHRVSYAV--PASFAEKVAATGATPVVYTSTL--------PT-D--DDPDAWGTELIDN
                 * .*                *

SVEN_0537       YRAV-----------------VRAARETRAELLVTSVLCNGALLAAEALDLPVVVVGLSV
SVEN_3444       --------KRVSDLIAAQFGAVAAAAEGCDALVAT---GPLPVTAGA--RSVAEKLGIRY
SVEN_6860       LVRLASMARSAADEMTAAL--VEVARE-GDVLLVGGALGPLGYAIAD--GLSVPAMGLHL
SVEN_5997       VTTSDLAQEFPGPKILPYV--SGIFGEVGARLV------EGVAEAARTWRADAVVFPPNH
OleD            VEPF-LN---DAIQALPQL--ADAYADDIPDLVLHDITSYPARVLARRWGVPAVSLSPNL
SVEN_0189       LEPF-LR---DAEQALPQL--AEAFDRDRPDLVLHDITSYPAPVLAHSWGVPAVSLWPNL
                                      *:           .      .  .
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Figure S5. Clustal Omega (1.2.1) alignment glycosyltransferase family 1 enzymes from *S. venezuelae* (ATCC 10712*)* and OleD. OleD and SVEN_0189 are 74% identical, whereas JadS is 21% identical with them both.

2. Analysis of natural products for mutant strains, natural products isolation and sharacterization

General Methods

All reagents were purchased from commercial sources and used without further purification unless otherwise stated. Solvents used for all reactions and chromatographic methods were HPLC grade. Biotage SP1™ unit was used to perform flash chromatography, using pre-packed normal phase silica columns (12 g, 40 g, and 80 g) from SiliCycle®. Glass-backed thin layer chromatography (TLC) plates (SiliCycle®) layered with 250 µm silica were

used to assess purity of compounds. Compounds were highly colored, thus ultraviolet (UV) light or visualization reagents were not required.

All compounds were characterized by liquid chromatography tandem-mass spectrometry (LC-MS/MS), high resolution mass spectrometry (HRMS), and 1D- and 2D-nuclear magnetic resonance (NMR) spectroscopy. Low resolution LC-MS/MS spectra were obtained on an Applied Biosystems hybrid triple quadrupole linear ion trap (2000Qtrap) mass spectrometer using an electrospray ionization (ESI) source. This was coupled to an Agilent 1100 high performance liquid chromatography (HPLC) instrument with a Phenomenex Kinetex 2.6 μm Hilic column (150 mm × 2.10 mm). Samples were prepared in methanol and 5 μL aliquots were injected onto the column. Elution of compounds was accomplished using an isocratic gradient that is composed of (7:3) CH_3CN: 2mM ammonium acetate in water (pH 5.5) with a flow rate of 120 μL min^{-1} for 10 min. For low resolution LC-MS/MS, the instrument was used in positive mode (ESI+) for acquisition of all jadomycins. Enhanced product ionization (EPI) was performed with scans that were conducted over a range of 300-900 *m/z* scanning for $[M+H]^+$ and the appropriate jadomycin fragmentation. Spectra were analyzed using Analyst software version 1.4.1 (Applied Biosysthems). HRMS traces of all jadomycins were analyzed on a Bruker Daltonics MicroTOF Focus Mass Spectrometer using an ESI- source.

All ultraviolet-visible (UV-vis) spectroscopy was carried out on a SpectraMax Plus Microplate Reader (Molecular Devices). Samples were placed in a quartz cuvette (1 cm path length) and scanned over a range of 280-700 nm using 5 nm intervals in order to determine maximal absorbance wavelengths (λmax).

^1H NMR and ^1H-^{13}C heteronuclear multiple bond correlation (HMBC) were recorded using a Bruker AV-III 700 MHz Spectrometer (^1H: 700 MHz, ^{13}C: 150 MHz) equipped with an ATMA 5 mm TCI cryoprobe located at the Canadian National Research Council Institute for Marine Biosciences (NRC-IMB) in Halifax, Nova Scotia. ^1H-^1H Correlation spectroscopy (COSY) and ^1H-^{13}C heteronuclear single quantum coherence (HSQC) experiments were recorded using a Bruker AV 500 MHz Spectrometer (^1H: 500 MHz, ^{13}C: 125 MHz) equipped with an auto-tune and match (ATMA) broadband observe (BBFO) Smartprobe located at the Nuclear Magnetic Resonance Research Resource (NMR-3) facility (Dalhousie University). All spectra were recorded using methanol-d_4. Chemical shifts (δ) were given in ppm, and calibrated to residual solvent peaks; 3.31 and 79.0 ppm for ^1H and ^{13}C NMR spectra, respectively.

Media Composition

All media was prepared with distilled and de-ionized water. **MYM broth** [maltose (4 g/L), yeast extract (4 g/L), malt extract (10 g/L), pH 7.0]; **MYM agar** [maltose (4 g/L), yeast extract (4 g/L), malt extract (10 g/L), agar (15 g/L), pH 7.0]; **MSM broth** [$MgSO_4$ (0.4 g/L), MOPS (3.77 g/L), salt solution (9 mL 1% w/v NaCl, 1% w/v $CaCl_2$), $FeSO_4 \cdot 7H_2O$ (4.5 mL 0.2% w/v), trace mineral solution (4.5 mL), pH 7.5]. **Trace mineral solution** [$ZnSO_4 \cdot 7H_2O$ (880 mg/L), $CuSO_4 \cdot 5H_2O$ (39 mg/L), $MnSO_4 \cdot 4H_2O$ (6.1 mg/L), H_3BO_3 (5.7 mg/L), $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ (3.7 mg/L). All media was adjusted to pH 7 or 7.5 with 5 M NaOH or 5 M HCl as required. All solutions were autoclaved at 120°C for 30 minutes prior to use. 250 mL broth solution were autoclaved in 1 L glass Erlenmeyer flasks, and 125 mL of MYM agar was autoclaved in 250 mL glass Erenmeyer flasks.

Bacteria Maintenance and jadomycin production

S. venezuelae strains were maintained on MYM agar plates for 1-3 weeks for use in jadomycin productions. For long term storage, spore solutions were stored in 20% glycerol at -70°C.

General procedure for small scale productions with mutant strains

A small path of cells from a well-sporulated plate (1 week of growth) was used to inoculate MYM (2 × 25 mL) and grown overnight at 30°C with agitation (250 rpm). The cells were pelleted in a 50 mL falcon tube by centrifugation at 3750 rpm (4°C) for 10-30 minutes. The cells were washed twice with MSM (~30 mL), and re-suspended in 5 mL MSM. This cell suspension was used to inoculate MSM (3 × 25 mL) supplemented with D-serine (60 mM), glucose (33 mM) and phosphate (50 μM) to an initial OD600 value of ~0.6. Immediately, ethanol (750 μL per 25 mL) was added to each flask to stimulate jadomycin production. Cultures were kept at 30°C with agitation (250 rpm) for 48 h. Bacterial growth was monitored by optical density at 600 nm (OD_{600}). Jadomycin and colored natural product production were monitored by absorbance of clarified culture media at 526 nm (**Figure S6**).

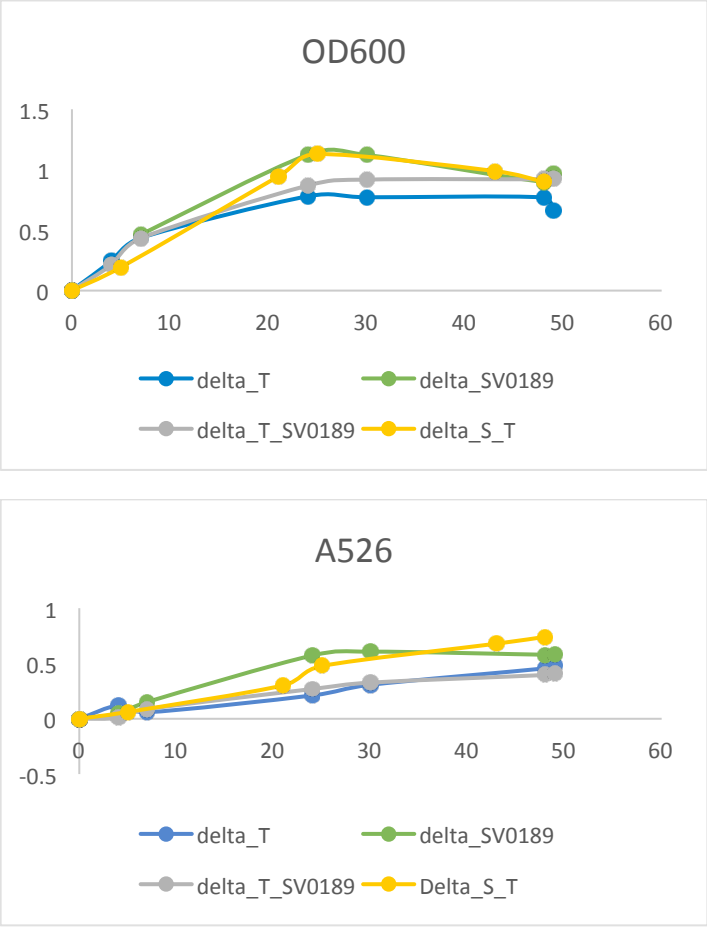

Figure S6. *S. venezuelae_ΔjadT* mutant (blue), *S. venezuelae_ ΔSv0189* (green), *S. venezuelae_ΔjadT_ΔSv0189* (grey), and *S. venezuelae_ ΔjadS ΔjadT* mutant (yellow), growth curves measured at 600 nm (OD_{600}) and measured at 526 nm (Abs_{526}) estimating production of colored compounds.

General work-up and analysis of small scale productions

For each culture condition, 50 mL of the culture media was collected at 48 h. The cells were pelleted at 8000 rpm for 10-15 minutes and the supernatant passed through 0.45 μM filters. Cells were discarded. The supernatant was applied to a 2 g silica phenyl column (silicycle) that had been preconditioned with methanol (10 mL), followed by water (30 mL). The column was then washed with water (50 mL), and subsequently the coloured material bound to the column was eluted with methanol (10 mL). The methanol extract was concentrated and the recovered mass was determined for each strain: 10.4 mg material was obtained from the *ΔjadT* strain, 5.7 mg from the *ΔjadSΔjadT* strain, 7.2 mg from the *ΔSv0189* strain, and 3.0 mg from the *ΔjadTΔSv0189* strain. The extracts were analyzed by TLC and HPLC (**Figures S7-S8**).

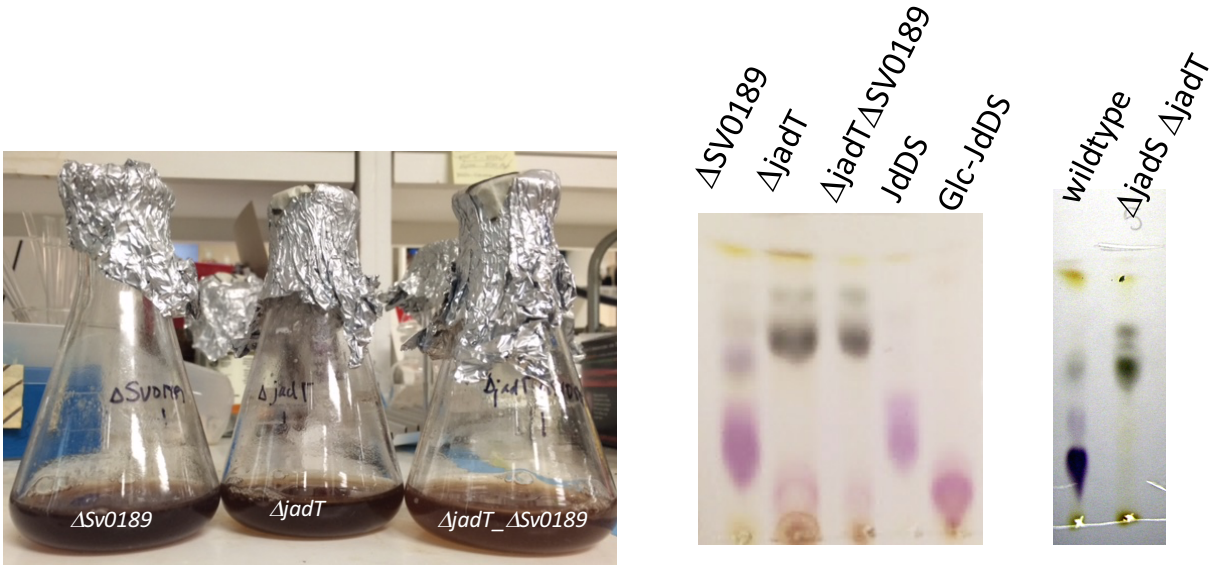

Figure S7. **Left**: Cultures producing jadomycins after 48 h incubation. **Centre**: TLC (20 % methanol/dichloromethane) of methanol extract for each strain as indicated alongside standards for jadomycin DS (JdDS) and glucosylated-JdDS (glc-JdDS); **Right**: TLC (20 % methanol/dichloromethane) showing the methanol extract for the wildtype strain which produces primarily JdDS (purple spot), and the equivalent extract for the *ΔjadSΔjadT* strain that produces jadomycin aglycone (green spots) and no visible glucosylated species (absence of polar purple spots).

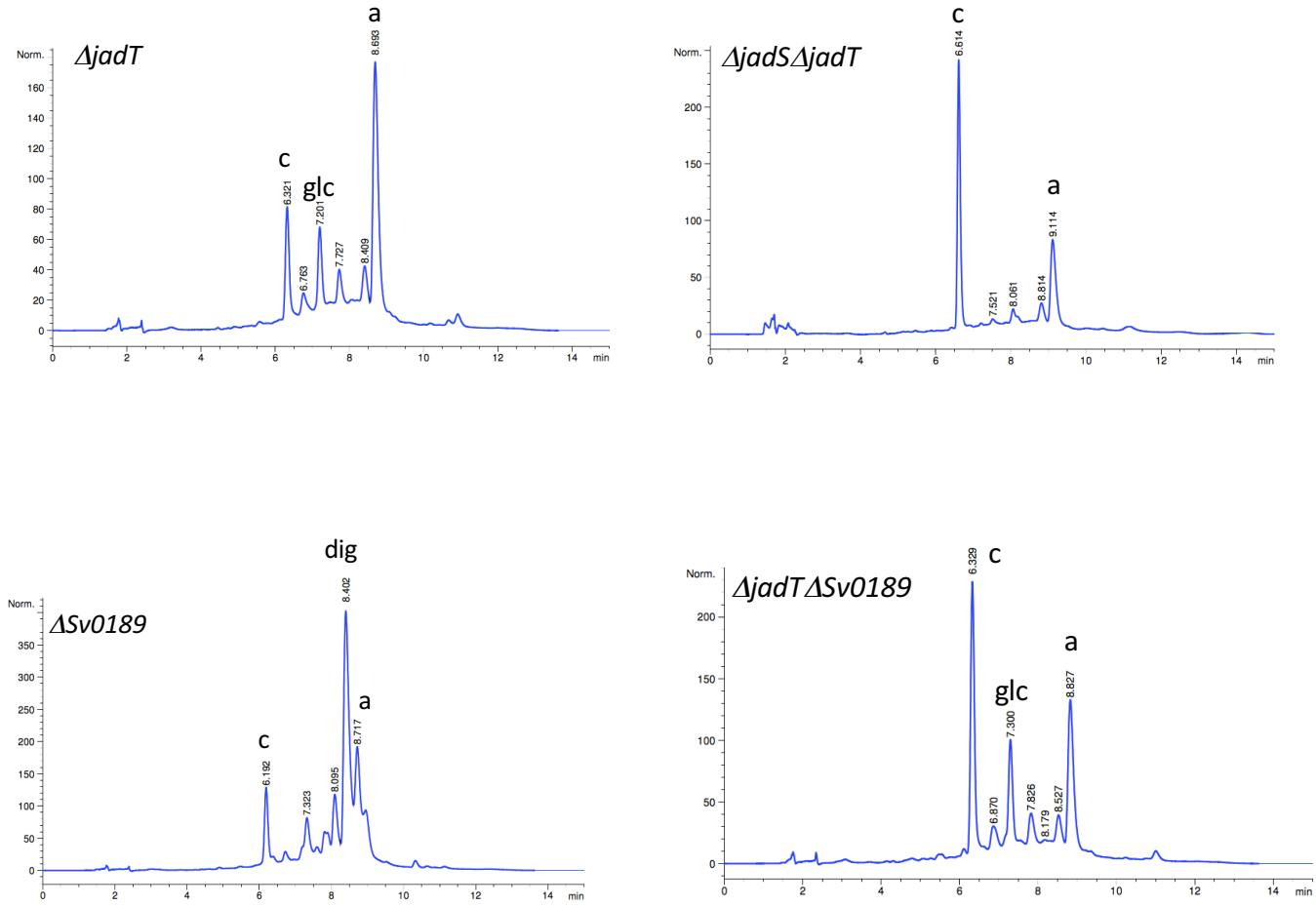

Figure S8. HPLC traces of the methanol extract from the phenyl column for indicated strains. **c**, chloramphenicol; **a**, jadomcyin aglycon; **dig**, JdDS; **glc**, glc-JdDS. For HPLC reversed-phase conditions see *J. Am. Chem. Soc.* **2015**, 137, 3271–3275.

Large scale production with *S. venezuelae*_Δ*jadT*

A 1 × 1 cm lawn of *S. venezuelae*_Δ*jadT* was used to inoculate 250 mL MYM media (2 × 250 mL in 1 L flasks). Growths were incubated at 30°C with agitation (250 rpm) for 16-24 hours. The cell suspension was centrifuged at 3750 rpm (4°C) for 30-45 minutes. The supernatant was removed and the cell pellet was washed with 60 mL MSM without amino acid. The washing step was repeated more than twice to remove all of the nutrient rich MYM. The cell pellet was re-suspended in 100 mL MSM without amino acid. Autoclaved MSM media containing D-serine (60 mM, 4 × 250 mL in 1 L flasks) were supplemented with glucose (to a final concentration of 33 mM) and phosphate (to a final concentration of 50 μM) before being inoculated with the pre-growth *S. venezuelae*_Δ*jadT* cell suspension to an initial OD_{600} of 0.6. Growths were ethanol shocked using 100% ethanol (3% v/v) and incubated at 30°C with agitation (250 rpm) for 48 hours. The pH of the media was readjusted to 7.5 after 24 hours with 5 M NaOH.

Extraction of natural products from bacterial cultures

Bacterial cells were removed via suction filtration through Whatman No. 5 filter paper, followed by 0.45 μm then 0.22 μm Millipore Durapore® membrane filters. The clear media was then passed through a reversed-phase SiliCycle® phenyl column (20 g), and washed with distilled water until flow-through was colorless (~2-4 L) to remove all water-soluble material. Material was eluted from the column with 100 % methanol and dried *in vacuo* to yield 20 mg crude material.

Purification of glucosylated jadomycin DS

A new purple compound of interest was observed by TLC analysis (R_f = 0.2, CH_2Cl_2:CH_3OH, 8:2) when compared to previously isolated jadomycin DS (R_f = 0.5). The crude extract was brought up in a minimal volume of methanol and dichloromethane, and applied to a 40 g silica column preconditioned with methanol and dichloromethane. Isolation of the compound was accomplished using a gradient system comprised of methanol and dichloromethane with a flow rate of 30 mL/min with collection of 6 mL fractions. To start, an initial isocratic gradient step using dichloromethane (4 column volumes (CV)) was performed. This was followed by a linearly increasing gradient of 0% to 20% methanol over 5 CV, which was then followed by an flat gradient of 40% methanol over 5 CV. Fractions of interest were identified visually for colour, and combined based on TLC analysis for the presence of the compound of interest. Combined fractions were concentrated to yield 3 mg of crude glucosylated jadomycin. TLC analysis (R_f = 0.2, DCM:MeOH, 8:2). NMR data can be found in Table 1; LRMS (ESI^+): MS/MS (55π6) found 556 $[M+H]^+$, 394 $[M+H\text{-}C_6H_{10}O_5]^+$; HRMS ($ESI^-$): $C_{27}H_{24}NO_{12}$ requires 554.1304, found 554.1286. UV-Vis (1.6×10^{-3} and 2.0×10^{-4} M, methanol): λ_{max}= 285, 380, 520.

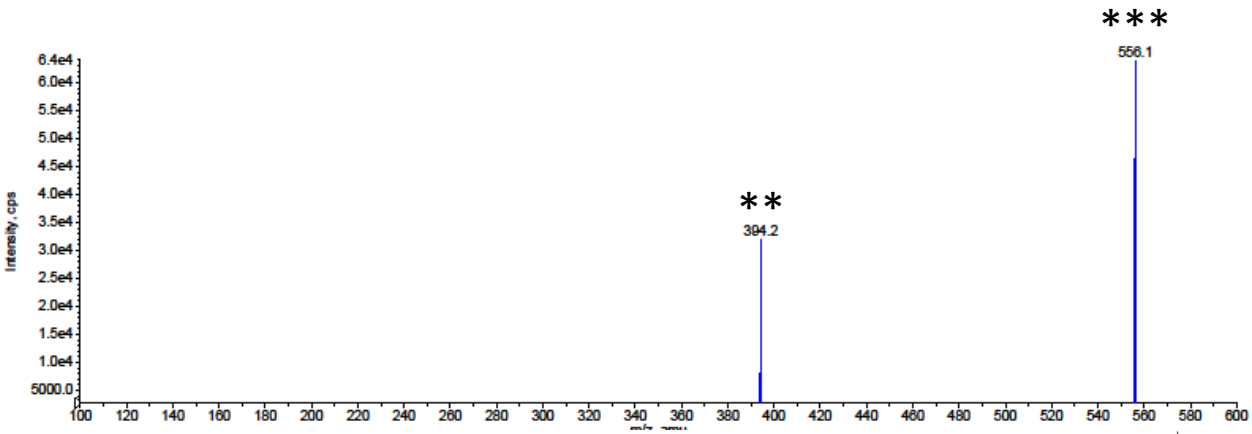

Figure S9. LC-MS/MS fragmentation of the glucosylated jadomycin (parent ion $[M+H]^+$ 556, showing $[M+H]^+$ (***) and cleavage of the sugar $[M+H\text{-}C_6H_{10}O_5]^+$ (**).

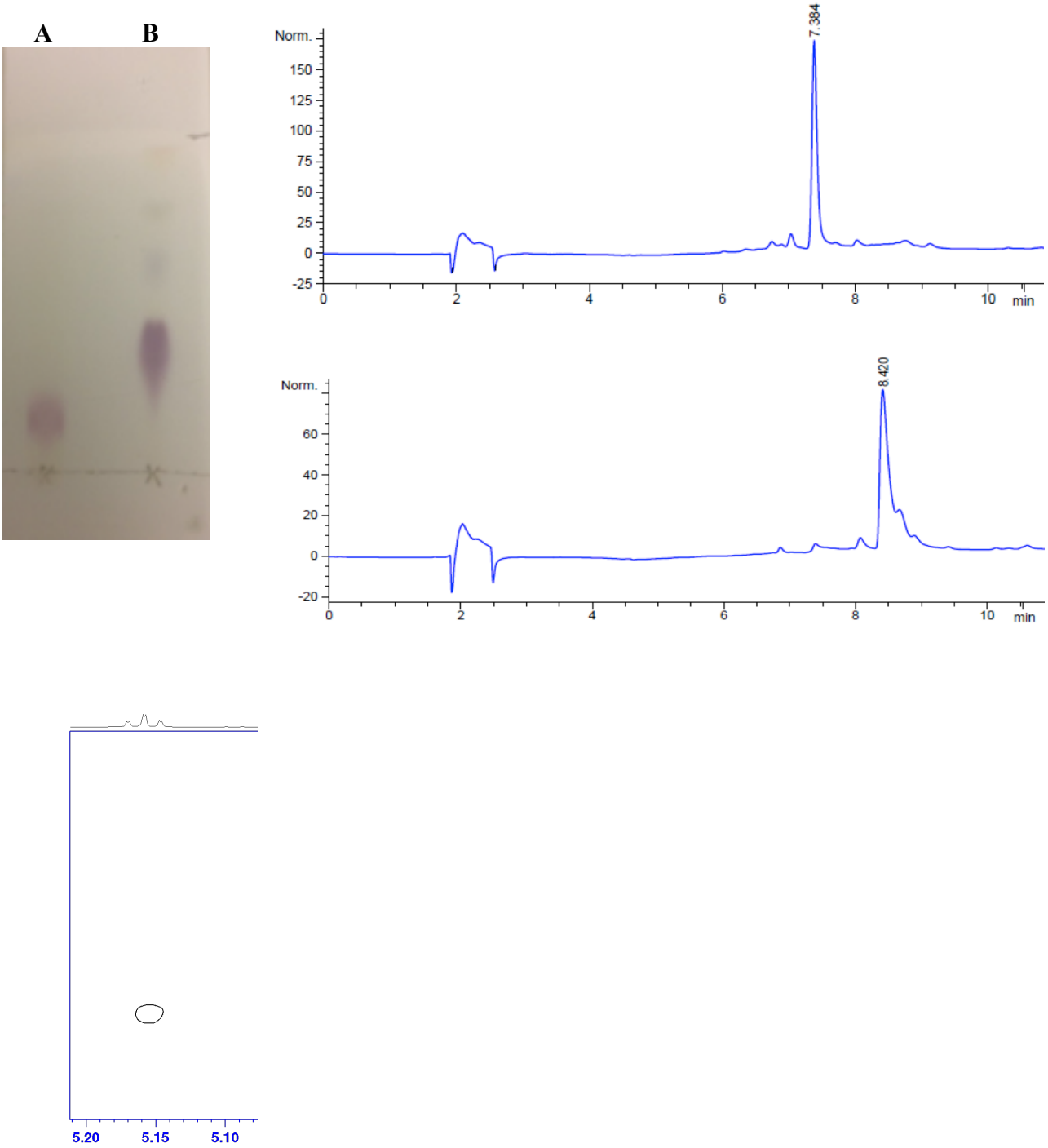

Figure S10. **Top:** Comparison of glucosylated jadomycin DS (A and top HPLC trace), and jadomycin DS (B and bottom HPLC trace). Conditions: Normal phase silica eluant (8:2 CH_2Cl_2:CH_3OH). For HPLC reversed-phase conditions see *J. Am. Chem. Soc.* **2015**, 137, 3271–3275. **Bottom:** HMBC expansion showing the correlation between the anomeric proton of the glucose (H1") and C12 of the angycyclic backbone.

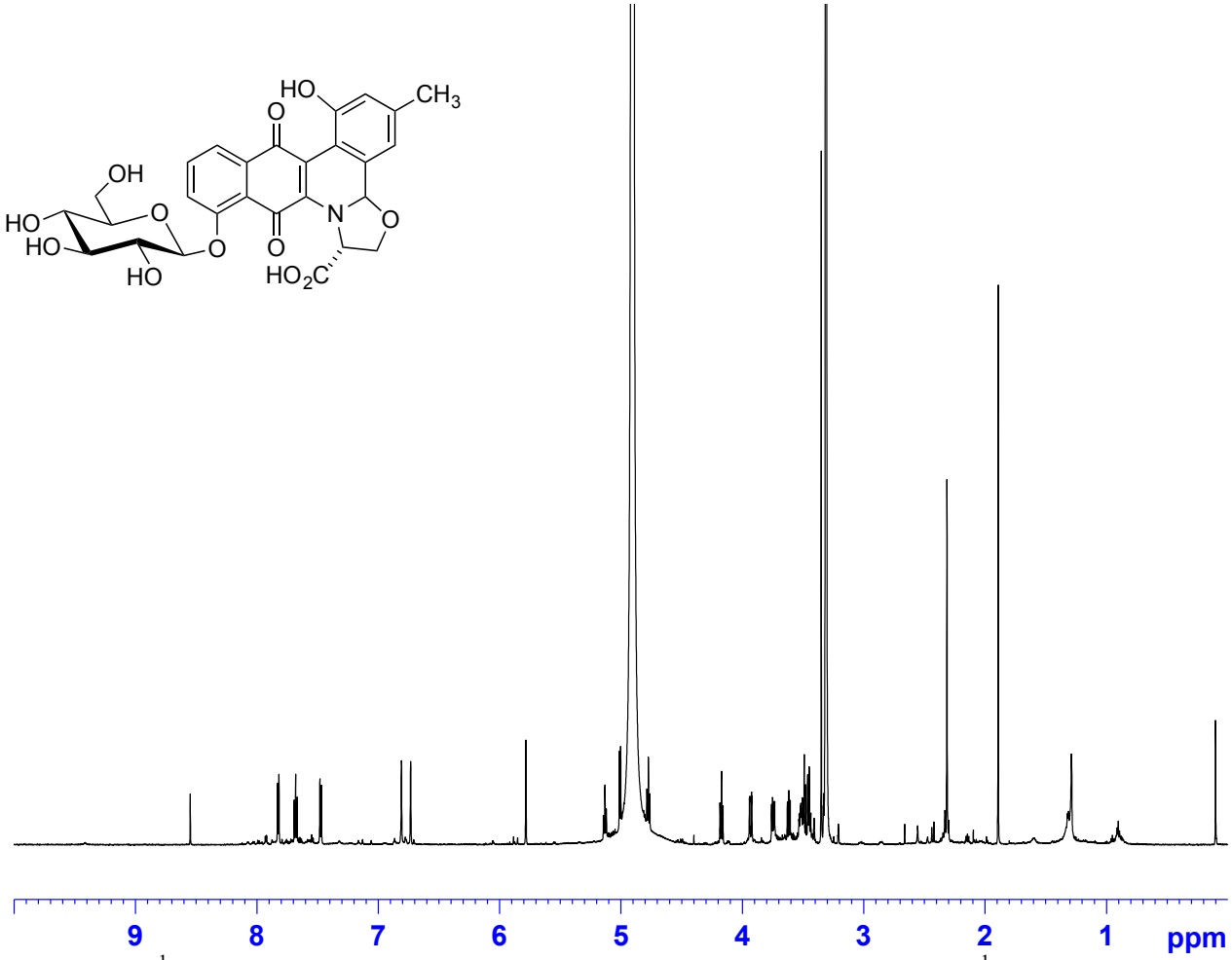

Figure S7. ^{1}H-NMR spectrum of the glucosylated jadomycn in methanol-d_4 (^{1}H: 700 MHz).

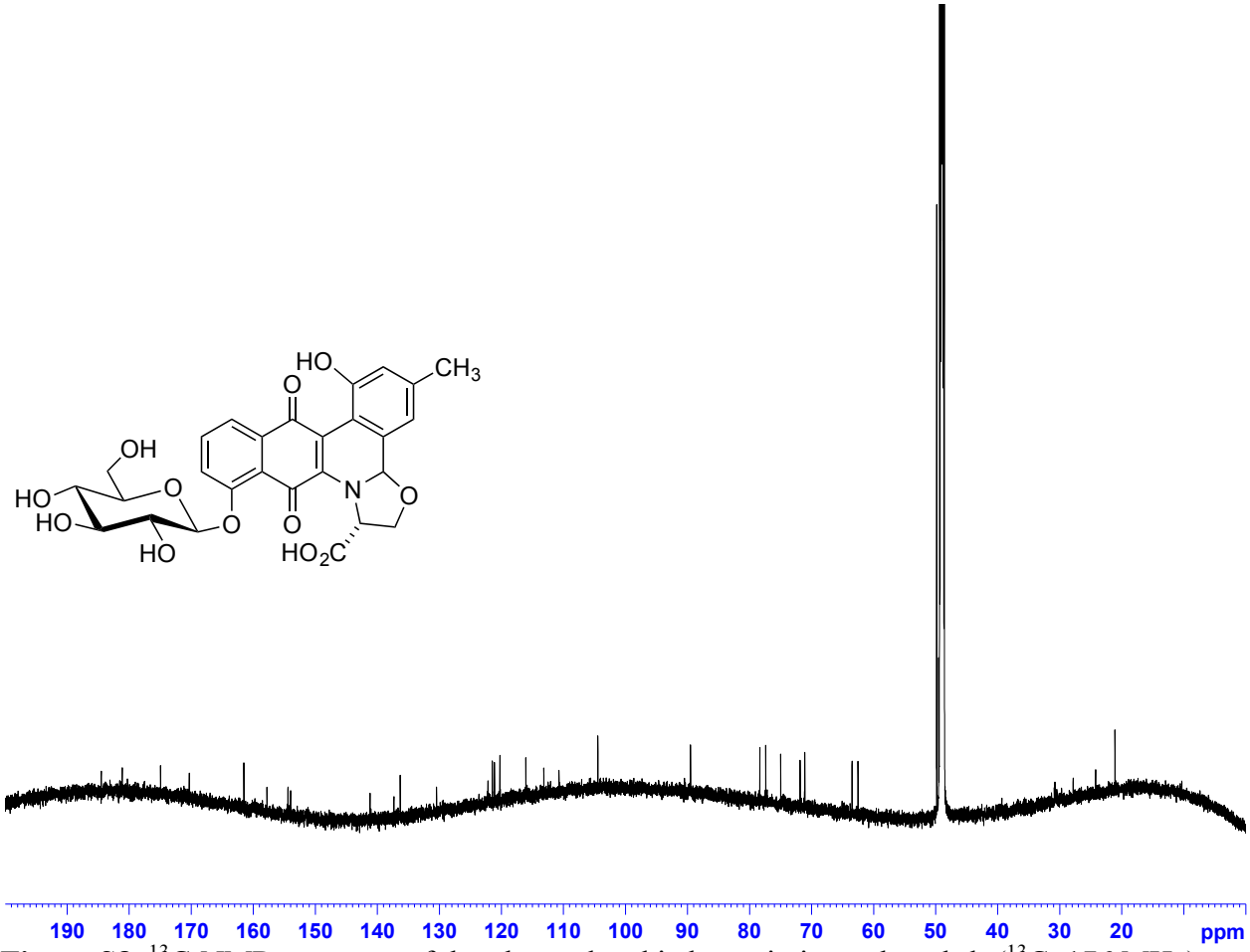

Figure S8. ^{13}C-NMR spectrum of the glucosylated jadomycin in methanol-d_4 (^{13}C: 176 MHz).

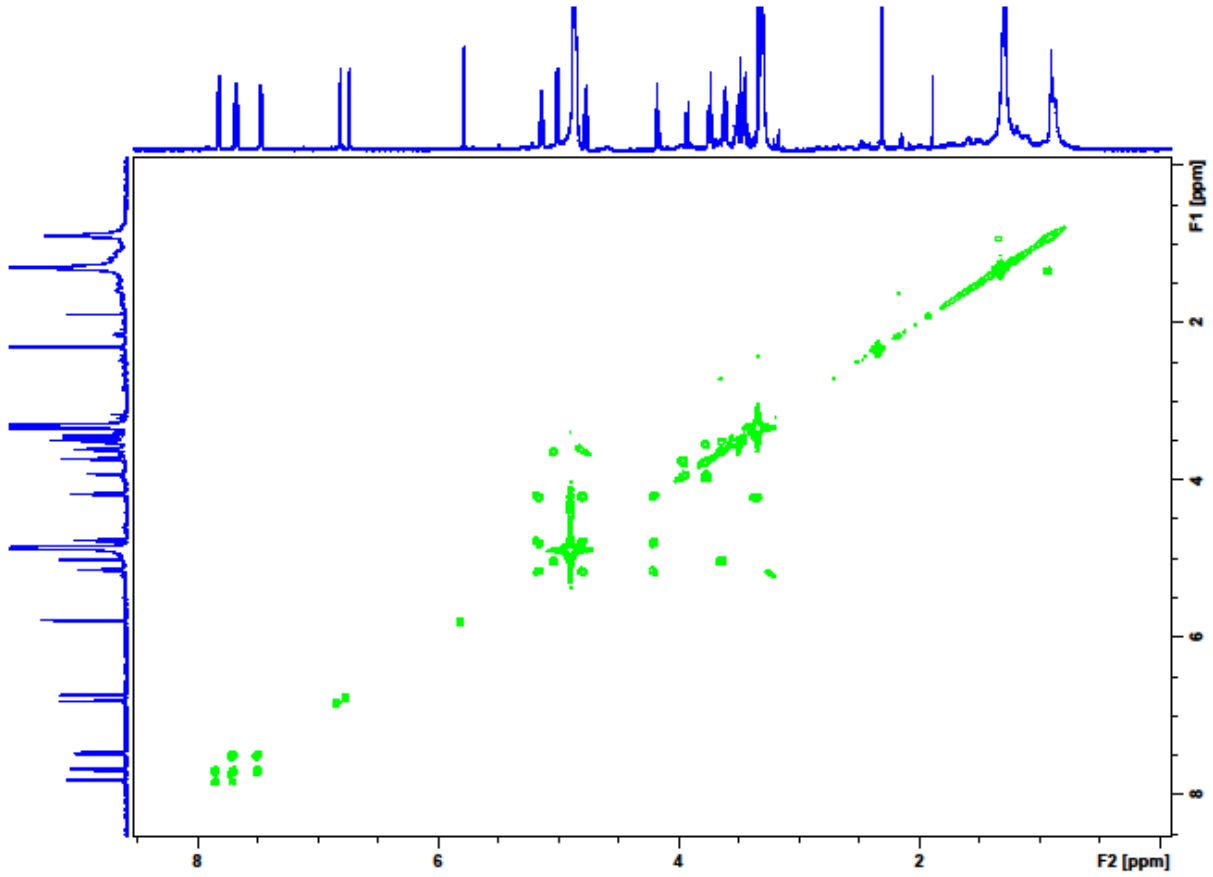

Figure S9. COSY (^{1}H-^{1}H) NMR spectrum of glucosylated jadomycin in methanol-d_4 (700 MHz).

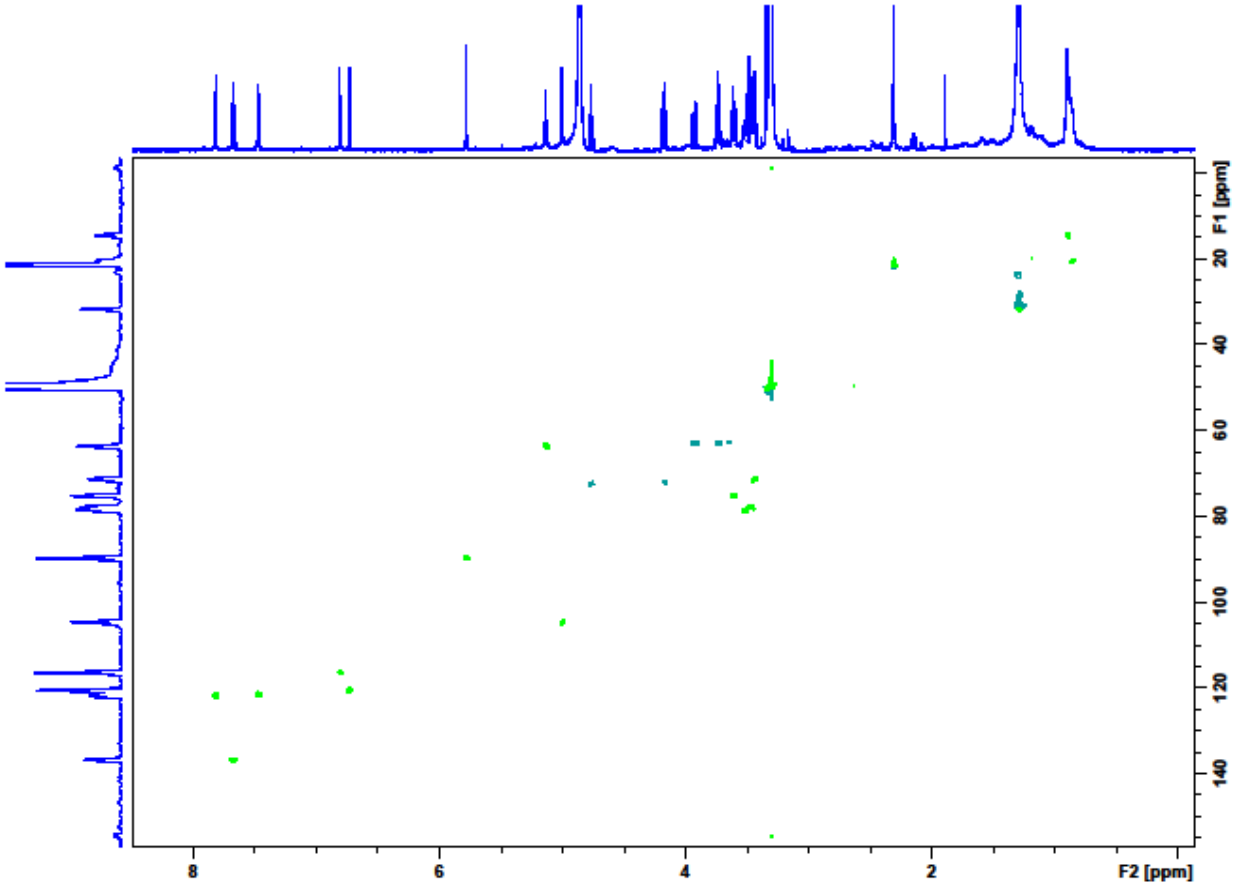

Figure S10. Edited-HSQC (^{1}H-^{13}C) NMR spectrum of the glucosylated jadomycin in methanol-d_4.

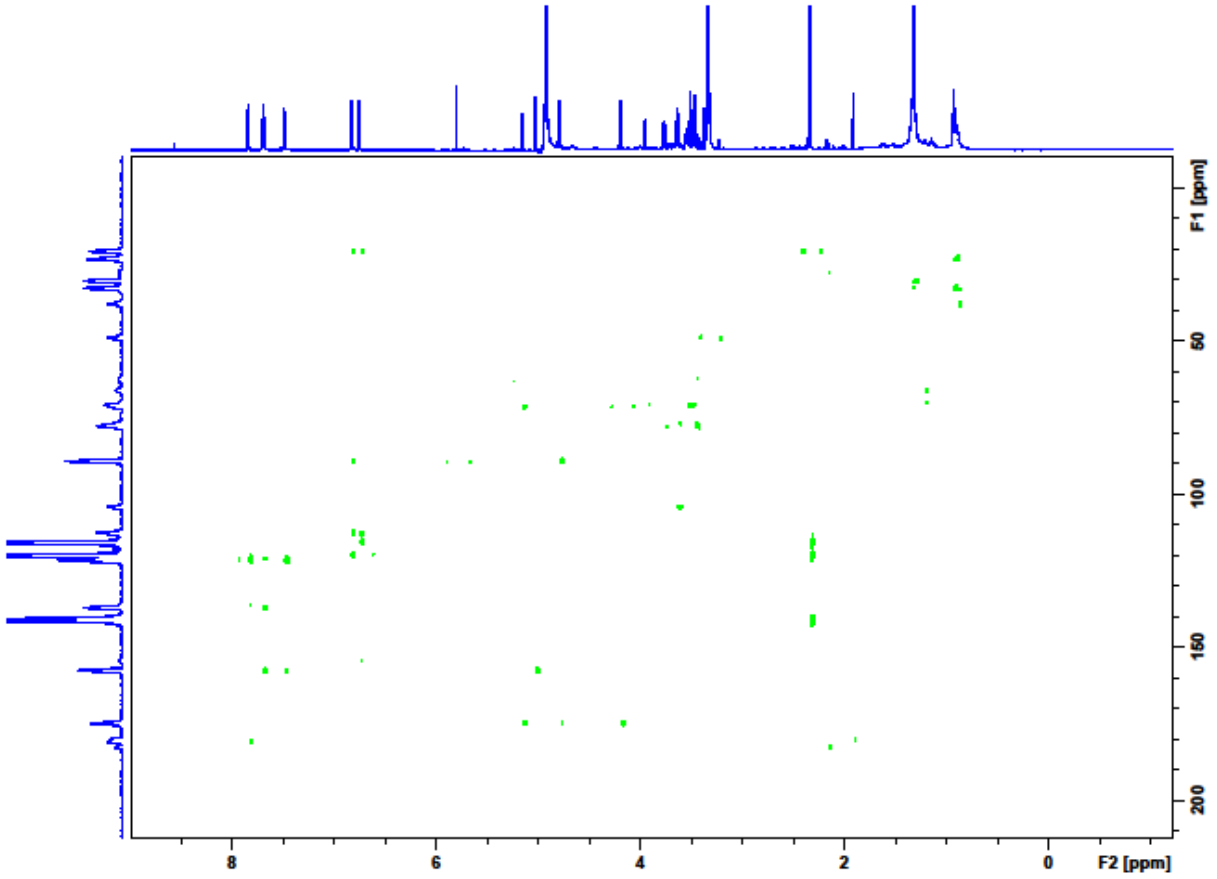

Figure S11. HMBC (^{1}H-^{13}C) NMR spectrum of the glucosylated jadomycin in methanol.