1 Supplementary Data

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3 Isolation of a Post-PKS C-C Branching Jadomycin from *S. venezuelae* ISP5230 in
4 the presence of 8-aminooctanoic acid

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31 **1 General**

32 All chemical and biological reagents were purchased from commercial sources and used without further
33 purification. All solvents used in the described chromatographic methods were HPLC grade, unless
34 otherwise indicated. The presence and purity of the jadomycin of interest was monitored by thin layer
35 chromatography (TLC) throughout the purification. TLC plates were normal phase silica (250 μ m
36 thickness) and glass-backed (Silicycle).

2 Jadomycin Production Methods

Bacterial Growth Conditions

S. venezuelae VS1099 cultures were grown on MYM-apramycin agar [maltose (0.4%, w/v), yeast extract (0.4%, w/v), malt extract (1%, w/v), agar (1.5%, w/v), apramycin (0.005% w/v)] incubated at 30°C until sporulation was observed. MYM growth media [maltose (0.4%, w/v), yeast extract (0.4%, w/v), malt extract (1%, w/v), pH 7] were inoculated with a patch of mycelia and spores and incubated at 30°C with shaking (250 rpm) for 16 – 20 hours. The cultures were monitored by microscope until the mycelia were dense and fibrous. At that point, the cells were pelleted (3750 rpm, 4°C) and the supernatant was discarded. The cell pellet was re-suspended and washed twice with MSM media [MgSO₄ (0.04% w/v), MOPS (0.377% w/v), salt solution (0.9% v/v), trace mineral solution (0.45% v/v), 0.2% w/v FeSO₄ solution (0.45% v/v), pH 7.5] to remove the rich MYM media. The salt solution is NaCl (1% w/v) and CaCl₂ (1% w/v) in ddH₂O and the trace mineral solution is ZnSO₄ (0.088% w/v), CuSO₄ (0.0039% w/v), MnSO₄ (0.00061% w/v), H₃BO₃ (0.00057% w/v), and (NH₄)₆Mo₇O₂₄ (0.00037% w/v) in ddH₂O. The cells were then re-suspended in minimal MSM solution as an inoculum slurry.

The MSM production media was separately prepared and included 8-aminooctanoic acid (60 mM) and D-serine (15 mM) and the pH was corrected to 7.5 using NaOH (5 M) before autoclaving. After autoclaving, filter sterilized 30% glucose solution (2% v/v) and separately autoclaved 9 mM phosphate solution (0.54% v/v) were added. The re-suspended *S. venezuelae* slurry was then added to the media to an optical density of 0.4-0.6 at 600 nm (OD₆₀₀) as measured with a SpectraMax Plus Microplate Reader (Molecular Devices). The inoculated media was ethanol shocked (3% v/v) to induce jadomycin production and incubated at 30°C with shaking (250 rpm). Culture pH was corrected daily to 7.5 using NaOH (5 M) or HCl (5 M) for the duration of the production period.

Monitoring Jadomycin Productions

Jadomycin productions were monitored daily by analysing aliquots at 600 nm (OD₆₀₀) to assess cell growth and clarified aliquots at 526 nm (Abs₅₂₆) to assess production of colored compounds.

Figure S1. Monitoring cell growth at 600 nm (●) and production of colored compounds at 526 nm (■) in the presence of (A) 8-aminooctanoic acid (60 mM) and D-serine (15 mM), (B) D-serine (15 mM). Error bars correspond to standard deviation between replicates.

As seen in **Figure S1**, *S. venezuelae* VS1099 was capable of growth and jadomycin production in the presence of 8-aminooctanoic acid with D-serine supplementation. The productions were stopped after 72 hours.

3 Jadomycin Purification Methods

Filtration

Upon completion of the jadomycin production, the bacteria were removed from the solution by filtration through Whatman #5 filters, followed by 0.45 μ m and 0.22 μ m Millipore filters.

Flash chromatography

Flash chromatography was performed using a Silisep™ prepacked phenyl column (12 g, 70 mL) purchased from Silicycle® that had been preconditioned with ddH₂O (3 column volumes, CV), methanol (4 CV), and again with ddH₂O (4 CV). The production media filtrate was passed through

the column followed by a ddH₂O wash (a minimum of 1 CV) to remove all water-soluble material. The crude natural product was eluted using 100% methanol (0.5-1 CV). The solvent was removed *in vacuo*. Following the initial phenyl column, 122.4 mg of crude natural product were isolated.

Preparative thin layer chromatography

The crude material was then purified using preparative TLC. Preparative TLC was performed using four 20 × 20 cm glass backed, normal phase silica TLC plates (Silicycle® 1000 µm thickness). The crude natural product was brought up in minimal methanol and spotted onto the plate. The plate was eluted in 5% methanol in dichloromethane solvent system. When the plate had been completely developed, it was removed from the solvent to allow air-drying. Several bands, including the violet band of the jadomycin of interest were removed by scraping the silica from the plate. The compounds were eluted from the silica using methanol, which was subsequently removed *in vacuo*. Following preparative TLC, 5.5 mg of violet material was isolated, in addition to 2.3 mg of red material and 2.0 mg of the secondary violet material.

Preparative high-performance liquid chromatography

The semi-pure Jad 8AA Ethanol (**1**, violet material from the previous step) was purified using preparative HPLC. The material was brought up in methanol and injected in 200 µL volumes. A Millipore Waters preparative-HPLC (717 Plus Autosampler, 486 Tunable Absorbance Detector, 600E Controller) with an Altex Ultrasphere-ODS 5 µm C18 column (10 mm x 25 cm) was used. The jadomycin separation was monitored by UV detection at 254 nm. Prep-HPLC was conducted using a flow rate of 5.0 mLmin⁻¹ and a linear gradient of water (A) and methanol (B) from 95:5 to 5:95 A:B over 30.0 minutes, followed by a plateau at 5:95 A:B for 30.0 minutes, a linear gradient from 5:95 to 95:5 A:B over 10.0 minutes, and finally a re-equilibration at 95:5 A:B for 5.0 minutes.

4 Compound Characterization Methods

Ultra-violet-visible spectroscopy

UV-Vis spectroscopy on **1a** and **1b** was carried out on a SpectraMax Plus Microplate Reader (Molecular Devices) and analyzed using SoftMax® Pro Version 4.8 Software. Samples were dissolved in methanol, placed in a quartz cuvette (1 cm path length), and scanned over a 280 nm – 700 nm range (1 nm intervals). Two dilutions were used to calculate extinction coefficients (ϵ) from several maximal absorbance wavelengths (λ_{max}).

High-performance liquid chromatography

HPLC of purified jadomycin were performed using a Hewlett Packard Series 1050 instrument equipped with an Agilent Zorbax 5 μm Rx-C18 column (15.0 cm $\times$ 4.6 mm). Injections were monitored at an absorbance of 254 nm. HPLC was conducted using a flow rate of 1.0 mLmin⁻¹ and a linear gradient from 90:10 A:B to 40:60 A:B over 8.0 min, followed by a plateau at 40:60 A:B from 8.0 to 10.0 min, and finally a linear gradient from 40:60 A:B to 90:10 A:B over 5.0 min. Buffer A is an aqueous solution containing 12 mM *n*-Bu₄NBr, 10 mM KH₂PO₄, and 5% acetonitrile at pH 4.0 and B is 100% acetonitrile. Samples were analyzed by injecting 20 μL aliquots with 10-100 μM concentrations.

High-resolution mass spectrometry

High resolution MS/MS/MS of Jad Undec was performed on a Thermo Scientific Orbitrap LTQ Velos High Resolution Mass Spectrometer using an ESI+ source with fragmentation observed using 35.00 v CID. HRMS of **1a**, **1b**, Jad 8AA Methoxy (and deuteromethoxy) and Jad 8AA Acetate were performed on a Bruker Daltonics MicroTOF Focus Mass Spectrometer using an ESI⁺ source.

Nuclear magnetic resonance spectroscopy

NMR analyses of jadomycins were recorded using a Bruker AVIII 700 MHz Spectrometer (^1H : 700 MHz, ^{13}C : 176 MHz) equipped with a 1.7 mm probe located at the Canadian National Research Council Institute for Marine Biosciences (NRC-IMB) in Halifax, Nova Scotia. Spectra were recorded in CD_3OD with chemical shifts given in ppm after calibrating to the residual solvent peak (CD_3OD : 3.31 ppm), unless otherwise noted.

Structural characterization and signal assignments were accomplished using ^1H -NMR chemical shifts and multiplicities, ^{13}C -NMR chemical shifts, ^1H - ^1H correlated spectroscopy (COSY), ^1H - ^{13}C heteronuclear single quantum coherence (HSQC) spectroscopy, ^1H - ^{13}C heteronuclear multiple bond correlation (HMBC) spectroscopy, and ^1H - ^1H rotating frame nuclear Overhauser effect spectroscopy (ROESY).

5 Compound Characterization Data

Jadomycin 8AA Ethanol Major (**1a**)

UV-Vis (8.67×10^{-4} M and 1.73×10^{-4} M, MeOH), λ_{max} (296 (9589), 326 (10305), 382 (1990), 553 (1347)); TLC R_f : 0.61 (9:1 DCM: MeOH); HPLC R_t = 8.279 min.

Figure S2. Analytical HPLC trace of **2a**.

143 **Table S1:** ¹H-NMR Data of **1a** in CD₃OD (1H: 700 MHz)

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Position	δ ¹ H (ppm)	Multiplicity (<i>J</i> (Hz))	δ ¹³ C (ppm)	COSY	HMBC
1a	3.51	m	58.4	1b, 2(a,b)	2, 3a
1b	3.42	m	58.4	1a, 2(a,b)	2, 3a
2a	1.92	m	35.6	1(a,b), 2b	2, 3a, 3b
2b	1.79	m	35.6	1(a,b), 2a, 3a	2, 3a, 3b
3a	4.75	dd (5.7, 9.3)	64.2	2b	1, 1', 2, 3b, 4, 7a, 13a
3b			133.0		
4	6.60	s	119.2	6, 5-CH ₃	3a, 3b, 5-CH ₃ , 6, 7a
5			140.8		
5-CH ₃	2.34	s	21.0	4, 6	4, 5, 6, 7a
6	6.72	s	119.4	4, 5-CH ₃	4, 5-CH ₃ , 7, 7a
7			154.6		
7-OH					
7a			114.1		
7b			111.7		
8			179.6		
8a			119.7		
9	7.80	d (7.5)	120.3	10	8, 11
10	7.72	t (8.3)	136.6	9, 11	12, 12a
11	7.51	d (8.4)	120.9	10	9, 12, 13
12			155.6		
12a			137.6		
13			185.9		
13a			156.8		
1'a	3.64	m	54.4	1'b, 2'	2', 3a, 13a
1'b	4.17	m	54.4	1'a	2', 3', 3a, 13a
2'	1.79	m	31.4	1'a	None
3'	1.41	bm	27.6	4'	4'
4'	1.41	bm	30.2	3', 5'	3'
5'	1.38	bm	30.7	4', 6'	4', 6'
6'	1.62	m	27.7	5', 7'	5', 7'
7'	2.18	t (7.6)	39.2	6'	5', 6', 8'
8'			182.9		
1''	5.96	d (3.1)	96.2	2''(a,b)	3'', 5'', 12
2''(a)	2.38	dd (2.6, 15)	36.3	1'', 2''(b), 3''	1'', 3'', 4''
2''(b)	2.25	dt (3.5, 15)	36.3	1'', 2''(a), 3''	None
3''	4.09	d (3.0)	68.3	2''(a,b), 4''	None
3''-OH					
4''	3.30	dd (3.6, 10.2)	74.0	3'', 5''	3'', 5'', 5''-CH ₃
4''-OH					
5''	3.94	m	66.3	4'', 5''-CH ₃	3'', 5''-CH ₃
5''-CH ₃	1.23	d (6.6)	18.1	5''	4'', 5''

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Figure S3. ¹H NMR of **1a** in CD₃OD (1H: 700 MHz).

Figure S4. ¹³C NMR of **1a** in CD₃OD (13C: 176 MHz).

Figure S5. ^1H - ^1H COSY of **1a** in CD_3OD .

Figure S6. ^1H - ^{13}C Edited HSQC of **1a** in CD_3OD . CH_3/CH phased up (blue), CH_2 phased down (red).

Figure S7. ^1H - ^{13}C HMBC spectrum of **1a** in CD_3OD .

Figure S8. ^1H - ^1H ROESY spectrum of **1a** in CD_3OD ($D_1 = 2.0\text{s}$, $P_{15} = 250000\text{ }\mu\text{s}$).

Figure S9. High resolution mass spectrum of **1a**. $[M-H]^-$ 622.2639 found, 622.2658 calculated.

Jadomycin 8AA Ethanol Minor (1b)

UV-Vis (8.67×10^{-4} M, 1.73×10^{-4} M, MeOH), $\lambda_{\max}$ (298 (16421), 320 (4550), 555 (2436)); TLC

R_f : 0.60 (9:1 DCM: MeOH); HPLC R_t = 8.367 min.

Figure S10. Analytical HPLC trace of **1b**.

169 **Table S2:** ¹H-NMR Data of **1b** in CD₃OD (1H: 700 MHz)

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Position	δ ¹ H (ppm)	Multiplicity (<i>J</i> (Hz))	δ ¹³ C (ppm)	COSY	HMBC
1a	3.43	m	58.3	1b, 2(a,b)	2, 3a
1b	3.35	m	58.3	1a, 2(a,b)	2, 3a
2a	1.82	m	35.8	1(a,b), 2b, 3a	1, 3a, 3b
2b	1.69	m	35.8	1(a,b), 2a, 3a	1, 3a, 3b
3a	4.63	dd (6.9, 9.3)	63.9	2(a,b)	1, 1', 2, 3b, 4, 7a, 13a
3b			134.0		
4	6.50	s	119.3	5-CH ₃ , 6	3a, 3b, 5-CH ₃ , 6, 7, 7a
5			140.9		
5-CH ₃	2.24	s	20.9	4, 6	4, 5, 6, 7a
6	6.62	s	119.5	4, 5-CH ₃	4, 5-CH ₃ , 7, 7a
7			154.6		
7-OH					
7a			114.0		
7b			111.7*		
8			180.5		
8a			119.7*		
9	7.74	d (8.0)	121.4	10	8, 11
10	7.63	t (8.7)	136.7	9, 11	12, 12a
11	7.40	d (8.3)	121.1	10	9, 10, 12, 13
12			156.4		
12a			137.5		
13			184.5		
13a			155.4		
1'a	3.41	m	55.1	1'b	2', 3a, 3', 13a
1'b	4.21	m	55.1	1'a, 2'	2', 3a, 3', 13a
2'	1.69	m	31.2	1'b	3'
3'	1.23	bm	27.5	4'	4'
4'	1.28	bm	30.1	3', 5'	3', 5'
5'	1.23	bm	30.6	4', 6'	4'
6'	1.51	m	27.6	5', 7'	5', 7', 8'
7'	2.07	2.07	39.1	6'	5', 6', 8'
8'			183.4		
1''	5.83	d (3.3)	97.0	2''(a,b)	3'', 5'', 12
2''(a)	2.37	dd (2.4, 14.8)	36.2	1'', 2''(b), 3''	1'', 3'', 4''
2''(b)	2.14	dt (3.6, 14.8)	36.2	1'', 2''(a), 3''	None
3''	4.01	d (3.0)	67.9	2''(a, b), 4''	4''
3''-OH					
4''	3.21	dd (3.2, 9.8)	74.0	3'', 5''	3'', 5'', 5''-CH ₃
4''-OH					
5''	3.84	m	66.4	4'', 5''-CH ₃	1''
5''-CH ₃	1.14	d (6.7)	18.0	5''	4'', 5''

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*Chemical shifts from NMR data of **1a**, ¹³C spectra of **1b** not collected due to limited material

Figure S11. ^1H NMR of **1b** in CD_3OD (1H: 700 MHz).

Figure S12. ^1H - ^1H COSY of **1b** in CD_3OD .

177
178 **Figure S13.** ^1H - ^{13}C Edited HSQC of **1b** in CD_3OD . CH_3/CH phased up (blue), CH_2 phased down (red).

179
180 **Figure S14.** ^1H - ^{13}C HMBC spectrum of **1b** in CD_3OD .

Figure S15. High resolution mass spectrum of **1b**. $[M-H]^-$ 622.2658 found, 622.2658 calculated.

Figure S16. High resolution MS/MS/MS fragmentation of Jad Undec (not isolated). (A) HRMS (ESI+) for $C_{32}H_{35}NO_9$ $[M+H]^+$, 578.2385 found, 578.23846 calculated; (B) HRMS (ESI+): MS/MS 448.1760 $[M+H-\text{digitoxose}]^+$ 306.0763 $[M+H-\text{digitoxose}-C_8H_{14}O_2]^+$; (C) HRMS (ESI+): MS/MS/MS 306.0763 $[M+H-\text{digitoxose}-C_8H_{14}O_2]^+$.

Jadomycin 8AA Methoxy

TLC R_f : 0.67 (9:1 DCM: MeOH); HPLC R_t = 8.464 min.

Figure S17. Structures of Jad 8AA Methoxy and deuteromethoxy analogues.

Figure S18. High resolution mass spectrum of Jad 8AA Methoxy. $[M-H]^-$ 608.2491 found, 608.2496 calculated.

Figure S19. High resolution mass spectrum of Jad 8AA Deuteromethoxy. $[M-H]^-$ 611.2678 found, 611.2690 calculated.

207 *Jadomycin 8AA Acetate*
208 TLC R_f : 0.54 (9:1 DCM: MeOH); HPLC R_t = 8.859 min.

209
210 **Figure S20.** Structure of Jad 8AA Acetate.
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213 **Figure S21.** High resolution mass spectrum of Jad 8AA Acetate. $[M-H]^-$ 636.2468 found,
214 636.2450 calculated.
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Table S3: Numerical data for NCI DTP Single Dose Screen of jadomycins. Results given as growth % where positive values indicate growth inhibition and negative values indicate cytotoxicity.

Cell Name	Jad G	Jad Hep	Jad AVA	Jad Non	Jad 7AA
A549/ATCC	NT	64.0	81.9	59.9	74.5
EKVX	77.5	91.0	99.8	97.0	96.3
HOP-62	8.5	132.4	77.9	122.7	81.5
HOP-92	NT	118.4	118.4	133.7	112.2
NCI-H226	149.8	105.7	98.4	118.3	107.3
NCI-H23	-6.2	89.0	94.4	79.8	99.6
NCI-H322M	8.4	105.0	102.9	94.7	106.9
NCI-H460	7.4	77.9	107.9	65.3	109.7
NCI-H522	-62.5	68.3	91.5	34.1	71.8
COLO 205	40.2	94.5	99.1	53.3	101.5
HCC-2998	28.1	90.2	93.6	73.0	109.6
HCT-116	5.5	64.9	71.2	24.5	79.7
HCT-15	9.4	73.6	96.3	46.1	95.2
HT29	15.4	100.3	96.9	93.7	87.3
KM12	-9.1	93.2	106.2	83.4	102.4
SW-620	6.1	121.9	121.7	99.9	123.4
BT-549	23.1	85.9	80.8	83.6	91.0
HS 578T	-7.2	92.8	105.1	71.5	115.4
MCF7	-24.8	91.9	100.7	17.7	99.6
MDA-MB-2	28.8	46.5	93.7	32.7	89.5
MDA-MB-4	8.7	95.3	110.3	58.3	109.6
T-47D	90.5	110.6	76.1	85.6	78.2
IGROV1	NT	92.5	95.8	96.4	106.8
NCI/ADR-R	40.8	93.1	101.0	91.0	101.5
OVCAR-3	-36.1	17.1	100.0	7.5	100.9
OVCAR-4	43.7	80.4	104.7	90.2	102.2
OVCAR-5	99.6	118.5	104.4	130.5	109.8
OVCAR-8	-7.0	87.9	95.4	52.9	92.8
SK-OV-3	171.7	93.3	77.7	89.8	76.1
CCRF-CEM	-26.7	91.7	83.7	57.8	100.8
HL-60(TB)	-29.6	97.4	102.7	79.2	103.0
K-562	11.2	94.8	110.7	28.3	103.7
MOLT-4	-28.3	93.7	86.4	63.3	93.3
RPMI-8226	-3.9	94.1	115.9	73.3	106.3
SR	NT	71.8	103.4	54.2	105.0
786-0	49.5	109.8	97.5	101.2	97.3
A498	53.0	NT	NT	NT	NT
ACHN	38.7	87.4	98.6	69.1	98.9
CAKI-1	60.1	97.0	98.0	96.9	98.2
RXF 393	-59.9	NT	NT	NT	NT
SN12C	14.6	90.8	105.4	66.3	100.1
TK-10	27.1	148.8	112.5	142.4	110.3
UO-31	20.7	97.6	97.1	99.9	100.2
LOX IMVI	8.0	71.4	100.3	34.3	98.3
M14	-83.5	79.1	89.6	66.6	103.9
MALME-3M	-80.1	42.9	116.6	40.6	118.8
MDA-MB-4	-77.9	30.8	108.0	13.1	106.9
SK-MEL-2	-53.6	84.9	106.4	46.4	94.7
SK-MEL-28	10.8	114.9	116.4	75.8	121.8
SK-MEL-5	-3.5	97.4	103.8	86.9	97.4
UACC-257	-46.7	NT	NT	NT	NT
UACC-62	43.0	105.5	109.4	89.5	109.5
DU-145	-100.0	73.5	111.3	57.2	107.9
PC-3	-4.6	75.7	75.0	87.2	84.1
SF-268	6.0	101.4	107.1	90.8	101.6
SF-295	11.0	115.7	98.2	93.9	102.0
SF-539	0.2	95.2	92.7	83.5	96.8
SNB-19	43.7	90.1	104.3	97.1	112.2
SNB-75	73.7	106.8	115.3	117.5	86.6
U251	7.1	90.5	95.7	63.1	95.2