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Isolation and Synthetic Diversification of Jadomycin 4-Amino-L-phenylalanine

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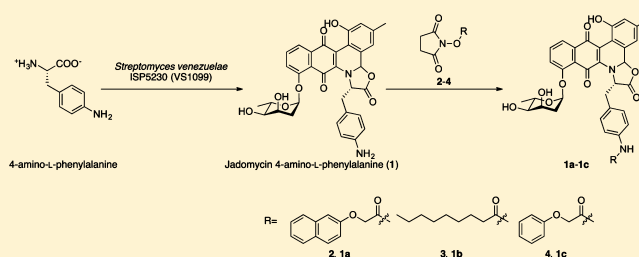
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Supporting Information

ABSTRACT: *Streptomyces venezuelae* ISP5230 was grown in the presence of phenylalanine analogues to observe whether they could be incorporated into novel jadomycin structures. It was found that the bacteria successfully produced jadomyicins incorporating 4-aminophenylalanine enantiomers. Upon isolation and characterization of jadomycin 4-amino-L-phenylalanine (1), it was synthetically derivatized, using activated succinimidyl esters, to yield a small jadomycin amide library. These are the first examples of oxazolone-ring-containing jadomyicins that have incorporated an amino functionality subsequently used for derivatization.



The isolation and characterization of novel natural products with unique bioactivity remains a major goal in natural product chemistry. Current natural product research aims to discover novel natural products through the use of genome mining in an effort to identify cryptic biosynthetic gene clusters whose products are unknown.¹ Analysis of these clusters and their natural products may result in unique structural diversity or bioactivity. The soil bacteria *Streptomyces venezuelae* ISP5230 (ATCC 10712) has been shown to contain over 30 secondary metabolite gene clusters,¹ where only those of chloramphenicol² and jadomycin³ have been studied. The jadomyicins are a family of antibiotics discovered serendipitously while exploring culture conditions for the production of chloramphenicol.³ Jadomycin A³ and subsequently the glycosylated analogue, jadomycin B,⁴ were discovered when the bacteria was subjected to heat, phage, or ethanol shock. Since this initial discovery, the jadomycin scaffold has been shown to be amenable to alteration through the incorporation of both proteinogenic and non-proteinogenic amino acids.^{5,6} This is accomplished through the exploitation of a unique nonenzymatic step within the biosynthetic pathway.

Jadomyicins are produced via a type II polyketide synthase biosynthetic pathway (Scheme 1) involving the nonenzymatic incorporation of the amino acid to form an imine intermediate, cyclization and decarboxylation of the imine to form the five-membered oxazolone ring, and then glycosylation to furnish the fully formed jadomycin.^{7–10} The fact that the incorporation of the amino acid is nonenzymatic has allowed for the derivatization of the jadomyicins through the introduction of varying amino acids in the production media.^{5,6} This methodology has been utilized to afford upward of 25 novel

jadomyicins (Scheme 1).⁵ Some of these amino acids have introduced specific chemical handles into the jadomycin structure that can be used to selectively alter the natural product, as was shown with the jadomycin triazoles⁶ and jadomycin Oct.¹¹ The introduction of new amino acids and subsequent derivatization will lead to novel structural diversity and potentially improved bioactivity.¹² It is known that within *S. venezuelae* ISP5230 4-aminophenylalanine is an intermediate in the chloramphenicol biosynthetic pathway.^{13,14} Our hypothesis was, therefore, that the bacteria may be able to take up and incorporate phenylalanine analogues into new jadomyicins.

RESULTS AND DISCUSSION

To this end, phenylalanine and 11 differentially substituted phenylalanine analogues (Figure 1) were evaluated for biosynthetic incorporation into the jadomycin scaffold. They were selected in an effort to introduce a chemical handle to allow for further derivatization of the natural products. Fermentations were carried out using *S. venezuelae* ISP5230 VS1099 under defined culture conditions with the amino acids being the sole nitrogen source for the bacteria.¹⁶ Cell growth and jadomycin production were monitored at 600 and 526 nm, respectively.¹⁶ The *S. venezuelae* grew comparably with all of the 11 nonproteinogenic phenylalanine analogues (Figures S1–S4). However, on the basis of the absorbance at 526 nm (Figure 1), production of each jadomycin varied significantly.

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Scheme 1. Jadomycin Biosynthetic Pathway, Where R Is an Amino Acid Side Chain and R¹ Is a Proton¹⁵ or a Carboxylate Group⁹

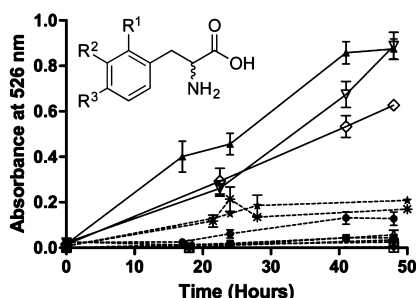
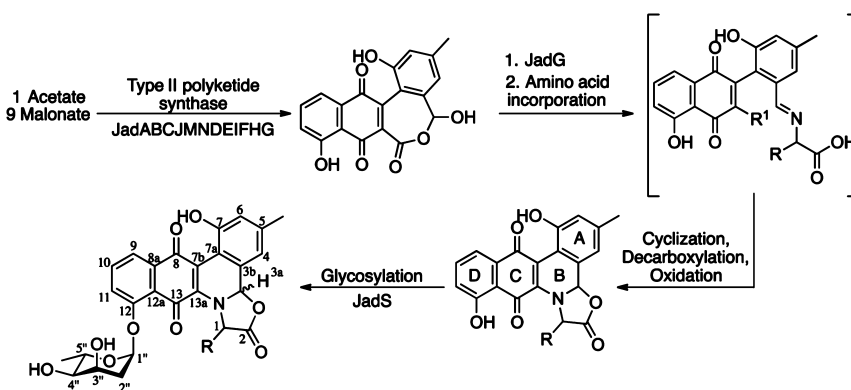


Figure 1. Jadomycin production curves monitored at 526 nm and the structure of phenylalanine analogues screened. R¹ = R² = R³ = H for L-phenylalanine (\blacktriangle); R¹ = R² = H and R³ = NH₂ for 4-amino-D-phenylalanine (\diamond) and 4-amino-L-phenylalanine (∇); R¹ = R² = H and R³ = Br for 4-bromo-D-phenylalanine (\blacksquare) and 4-bromo-L-phenylalanine (\bullet); R¹ = R² = H and R³ = Cl for 4-chloro-D-phenylalanine (\blacktriangledown) and 4-chloro-L-phenylalanine (Δ); R¹ = R² = H and R³ = CN for 4-cyano-D-phenylalanine ($*$) and 4-cyano-L-phenylalanine (\star); R² = H and R¹ = R³ = Cl for 2,4-dichloro-D-phenylalanine (\circ); R¹ = H and R² = R³ = Cl for 3,4-dichloro-D-phenylalanine (\blacklozenge) and 3,4-dichloro-L-phenylalanine (\square). Error bars correspond to standard deviation between triplicates.

The initial absorbance results indicated that the 4-amino-phenylalanine enantiomers were most successfully incorporated by the bacteria into secondary metabolites. This was also corroborated by HPLC and LC-MS/MS analysis that showed appreciable production of the jadomycin derivatives. Surprisingly, however, no significant incorporation for any of the other nine nonproteinogenic amino acids was observed based on absorbance, HPLC, and LC-MS/MS analysis (data not shown). Although 4-aminophenylalanine is known to be an intermediate in the chloramphenicol biosynthetic pathway,^{13,14} higher concentrations of chloramphenicol were not observed when *S. venezuelae* ISP5230 was grown with 4-amino-L-phenylalanine. This may explain why the other nine nonproteinogenic phenylalanine analogues were not incorporated. As a result of the higher absorbance values observed for growth with 4-amino-L-phenylalanine (Figure 1), these cultures were selected for further investigation.

Jadomycin 4-amino-L-phenylalanine (**1**) was isolated from 2 L of liquid culture using solid phase methods for initial extraction of the crude natural product, purified by chromatography, and subsequently characterized (Figure 2), yielding 17.8 mg L⁻¹ of natural product (Table 1). Characterization was carried out using high-resolution mass spectrometry (HRMS,

Table 1) and 1D/2D NMR spectroscopy experiments (see the SI). Compound **1** was isolated as a mixture of diastereomers (~2:1, Table 1) as determined by ¹H NMR spectroscopy. Diastereomers arise from the oxazolone ring H3a stereochemistry (Scheme 1). Once **1** had been isolated, chemical derivatization was pursued by chemoselectively reacting the free amino functional group to furnish analogues **1a–1c**.

Compound **1** was derivatized by selective acylation of the free amino group according to previously established methodology, using a series of *N*-hydroxy succinimidyl esters, **2–4**, to yield three novel derivatives:¹¹ jadomycin 4-amino-L-phenylalanine naphthoxyacetylamide (**1a**), jadomycin 4-amino-L-phenylalanine nonoylamide (**1b**), and jadomycin 4-amino-L-phenylalanine phenoxyacetylamide (**1c**) (Scheme 2). It was found that it was beneficial to derivatize the crude jadomycin extract, as has been previously described,^{6,11} to reduce breakdown of the derivatives during the purification process. Reaction progress was monitored by thin layer chromatography (TLC), observing the disappearance of **1** (*R_f* = 0.25, 5:5:1 CH₃CN/EtOAc/H₂O) with the formation of a new band at a higher *R_f* (see SI). These derivatives were then isolated as mixtures of diastereomers (Table 1) after workup and chromatographic separation and fully characterized spectroscopically (Figure 2, Table 1, and SI).

One-dimensional ¹H NMR spectroscopy and two-dimensional correlation (COSY) NMR spectroscopy experiments were used to confirm the presence of the A, D, and sugar rings in compounds **1** and **1a–1c** (SI). These chemical shifts were consistent with those of recently isolated jadomyces.¹¹ Heteronuclear multiple-bond correlation (HMBC) NMR spectroscopy established the remaining connectivity, including glycosylation of the jadomycin backbone at position 12 through the correlation of H1'' to C12 and the presence of the quinone functionality on the C-ring through the correlation of H3a to C13. Having established the typical jadomycin connectivity, we turned our attention to determine which amino group, the aniline or the α -amino group, had been incorporated into the jadomycin backbone. HMBC correlations were observed from H3a to a signal at approximately 55 ppm, which was assigned as C1. The chemical shift for H1 (3.34 ppm) is different from those of recently isolated eight-membered-ring structures (~4.5 ppm)¹¹ and past oxazolone-ring-containing jadomyces (~5.0 ppm),⁵ and the origin of this difference remains unclear. Rotating frame Overhauser effect spectroscopy (ROESY) NMR was also conducted to confirm through-space interactions. Irradiating H3a resulted in correlations to H4 for compounds **1**

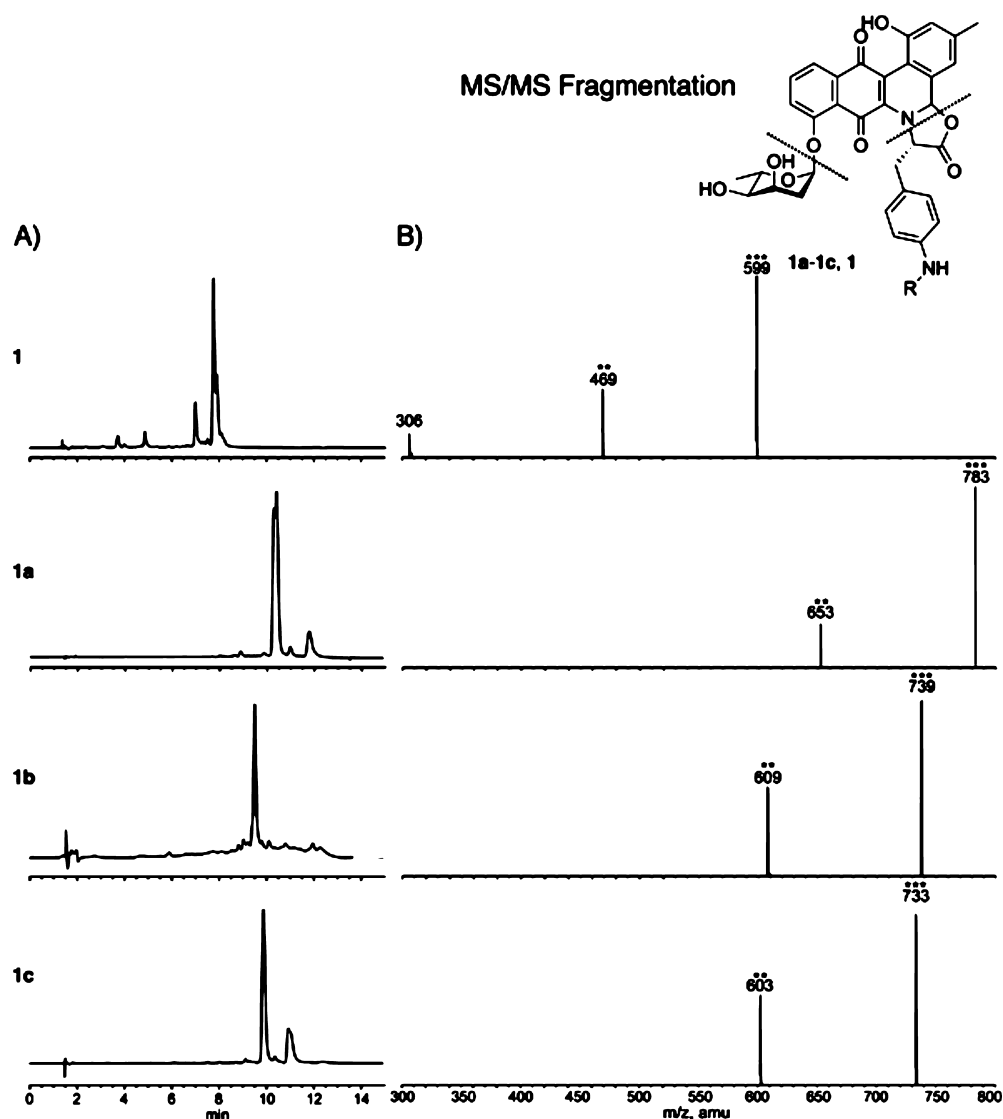


Figure 2. (A) HPLC traces of **1** and **1a–1c**. (B) LC-MS/MS spectra of **1** and **1a–1c** showing $[M + H]^+$ (***) and cleavage of the sugar $[M + H - \text{digitoxose}]^+$ (***) for all compounds and the further cleavage of the amino acid $[M + H - \text{digitoxose} - R]^+$ (m/z 306) for **1**.

Table 1. Diastereomeric Ratios, Isolated Yield, and HRMS m/z of **1** and **1a–1c**

product	$M_j:M_n^a$	yield (mg L ⁻¹)	HRMS	calculated mass
1	100:45	17.8	599.2040 ^b	599.2024
1a	100:84	22.4	813.2678 ^c	813.2665
1b	100:74	14.0	769.3356 ^c	769.3342
1c	100:50	14.0	763.2517 ^c	763.2508

^aRatios of diastereomers were determined by ¹H NMR integrations.

^bHRMS is $[M + H]^+$. ^cHRMS is $[M + \text{MeOH} - H]^+$.

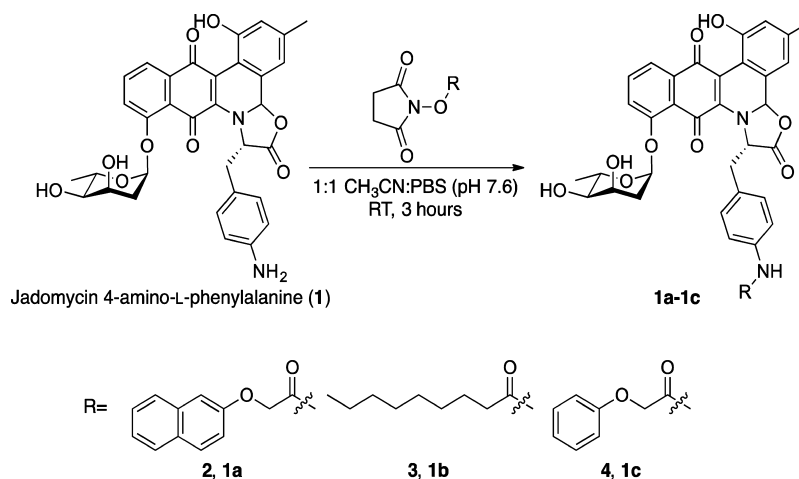
and **1a–1c**, but no correlations were observed from H3a to H1. This is in contrast to recently isolated eight-membered-ring congeners¹¹ and previous oxazolone-ring-containing jadomycins;⁵ again the origin of this absence of correlations remains unclear. However, the line-widths of the ¹H NMR spectra presented (see SI) are consistent with the formation of a five-membered-ring structure because broader ring signals were observed for the eight-membered-ring congeners.¹¹ Finally, there were no additional HMBC correlations from H3a to an additional aromatic carbon, as would occur if the aniline had been incorporated into the jadomycin backbone. Therefore, all

of the NMR evidence is consistent with the isolation of a five-membered oxazolone-ring-containing derivative. The mass spectra (Figure 2) also indicate cyclization occurred. If the aniline had been incorporated into the jadomycin backbone and had not cyclized, another mass would be observed. The connectivity for the naphthoxy, phenoxy, and nonoyl substituents was also established through the use of both COSY and HMBC NMR (see SI), and their chemical shifts and diastereomeric ratios (Table 1) were found to be similar to recently isolated compounds.¹¹

Compounds **1b** and **1c** were selected by the NCI to be tested against their 60 DTP (developmental therapeutics program) human tumor cell line one-dose screen. The resulting evaluation showed no appreciable cytotoxic activity (Tables S11 and S12). Compounds **1a–1c** were also tested for their copper-induced DNA cleavage capability and photodynamic inactivation of bacteria. No appreciable activity was found (Figures S8–S12), in contrast to other derivatized jadomycins.¹¹

The jadomycin library has been successfully expanded through the incorporation of 4-amino-L-phenylalanine into

Scheme 2. Derivatization of Jadomycin 4-Amino-L-phenylalanine



the jadomycin structure, along with the three novel derivatives prepared through acylation of the amino group. The fact that the amino acid was incorporated is significant, as the bacteria failed to incorporate the other phenylalanine analogues. Experiments to try and ascertain why the incorporation of the other phenylalanine analogues was unsuccessful are in progress and will be reported in due course. The incorporation of an amino functionality into a jadomycin offers a structural feature that is present within several clinically used drugs including aminoglycosides,¹⁷ and doxorubicin,¹⁸ that provides key electrostatic and hydrogen-bonding opportunities with the drug target.¹⁹ Additionally, amino substituents modify the biological activities of these types of compounds by acting as weak bases, enhancing cellular absorption. The spectroscopic data that we observed are consistent with 4-amino-L-phenylalanine incorporated as a five-membered ring containing oxazolone jadomycin. Given our recent discovery of eight-membered ring containing jadomycins, resulting from the incorporation of ornithine and 5-aminovaleric acid,¹¹ we investigated whether a novel E-ring backbone resulting from incorporation of the aniline in preference over the α -amino group had occurred, but found no evidence to that effect. Nevertheless, this has become an ongoing line of inquiry in our laboratory, and results will be published in due course.

EXPERIMENTAL SECTION

General Experimental Procedures. All reagents used were purchased from commercial sources and used without further purification. All solvents used were HPLC grade. Compounds 2–4 were prepared following literature protocol.¹ Flash chromatography was performed on a Biotage SP1 unit (Biotage) using prepacked normal phase silica columns (25 or 40 g) from Silicycle. Thin layer chromatography plates used for monitoring reactions and calculating R_f values were glass backed, normal phase silica plates (250 μm thickness) purchased from Silicycle. Compounds 1 and 1a–1c did not require chemical or ultraviolet visualization, as they are strongly colored. Preparative TLC plates used for purification were glass backed (20 cm \times 20 cm) normal phase silica plates (1000 μm thickness) purchased from Silicycle. Preparative TLC was performed using a specified solvent system to develop the plate, the plate was removed from the solvent to allow to fully air-dry, and then TLC was rerun in the same solvent system. This was repeated in order to achieve better separation. Once sufficient separation had been achieved, the silica was scraped off of the plate, and the band of interest eluted using the same solvent system used for development. Size exclusion chromatography was carried out using Sephadex LH-20 resin (GE Healthcare). NMR

analyses of 1 and 1a–1c were recorded using a Bruker AV-III 700 MHz spectrometer (^1H : 700 MHz; ^{13}C : 176 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. All spectra were recorded in $\text{MeOD-}d_4$. Chemical shifts are given in ppm and have been calibrated to residual solvent peaks (MeOD : 3.31 ppm). Structural characterization and signal assignments were accomplished using ^1H NMR chemical shifts and multiplicities and ^{13}C NMR chemical shifts. In addition, ^1H – ^1H COSY, ^1H – ^{13}C heteronuclear single quantum coherence (HSQC), and ^1H – ^{13}C HMBC NMR experiments were used in the NMR analyses. HPLC of purified jadomycin and crude extracts was performed using a Hewlett-Packard Series 1050 instrument equipped with an Agilent Zorbax 5 μm Rx-C₁₈ column (150 cm \times 4.6 mm). Jadomycins' presence was monitored at an absorbance of 254 nm, using 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 the remaining 5.0 min with a flow rate of 1.0 mL/min. Buffer A is an aqueous buffer containing 12 mM *n*-Bu₄NBr, 10 mM KH₂PO₄, and 5% HPLC grade acetonitrile (CH_3CN) at pH 4.0, and B is 100% HPLC grade CH_3CN . Samples were analyzed by injecting 20 μL aliquots. Low-resolution mass spectra were obtained using an Applied Biosystems hybrid triple quadrupole linear ion trap (2000 Qtrap) mass spectrometer equipped with an electrospray ionization (ESI) source coupled to an Agilent 1100 HPLC system fitted with a Phenomenex Kinetex 2.6u (150 \times 2.10 mm) Hilic column. Samples were prepared in 100% HPLC grade methanol prior to injection of 5 μL . The samples were run using an isocratic gradient of 30% 5 mM ammonium acetate (pH 5.5) and 70% CH_3CN with a flow rate of 120 $\mu\text{L}/\text{min}$. In order to analyze for jadomycin production, an enhanced product ion (EPI) scan was used, in positive mode (ESI+), in order to detect jadomycin ions along with their characteristic fragments, jadomycin aglycone and phenanthroviridin, over a range of 300–900 m/z . EPI scans were run using a capillary voltage of +4500 kV, declustering potential of +80 V, and curtain gas of 10 (arbitrary units). Scans were conducted using two steps, 300.0 to 320 amu (0.005 s) and 300.0 to 900.0 amu (0.150 s). Spectra were analyzed using Analyst software version 1.4.1 (Applied Biosystems). HRMS traces of all jadomycins were recorded on a Bruker Daltonics MicroTOF Focus mass spectrometer. Jadomycin 4-amino-L-phenylalanine used an ESI+ source, while jadomycin 4-amino-L-phenylalanine naphthoxyacetylamide, jadomycin 4-amino-L-phenylalanine nonoylamide, and jadomycin 4-amino-L-phenylalanine phenox-yacetylamide required ESI-. All ultraviolet–visible (UV–vis) spectroscopy 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 280–700 nm range using 1 nm intervals. Two separate dilutions were used in each case

(concentrations are listed with the appropriate characterization data) to calculate a series of extinction coefficients (ϵ) from several maximal absorbance wavelengths (λ_{\max}).

Culturing *S. venezuelae* ISP5230 VS1099 for Jadomycin Production. MYM growth media was prepared by addition of maltose (0.4%, w/v), yeast extract (0.4%, w/v), and malt extract (1%, w/v) to distilled, deionized water (ddH₂O). The pH was adjusted to 7.0 with NaOH (5 M) and HCl (5 M) as necessary. MSM production media was prepared by addition of MgSO₄ (0.04%, w/v), MOPS (0.377%, w/v), salt solution (0.9%, v/v), trace mineral solution (0.45%, v/v), and 0.2% w/v FeSO₄·7H₂O solution (0.45%, v/v) to ddH₂O. The pH was adjusted to 7.5 by NaOH (5 M) and HCl (5 M) as necessary. The salt solution was made by addition of NaCl (1%, w/v) and CaCl₂ (1%, w/v) to ddH₂O. The trace mineral solution was made by addition of ZnSO₄·7H₂O (0.088%, w/v), CuSO₄·5H₂O (0.0039%, w/v), MnSO₄·4H₂O (0.00061%, w/v), H₃BO₃ (0.00057%, w/v), and (NH₄)Mo₇O₂₄·4H₂O (0.00037%, w/v) to ddH₂O. *Streptomyces venezuelae* ISP5230 VS1099 cultures were grown and stored on MYM agar [maltose (0.4%, w/v), yeast extract (0.4%, w/v), malt extract (1%, w/v), agar (1.5%, w/v), aparmycin (0.005%, w/v)] incubated at 30 °C for a period of 2 to 4 weeks. MYM growth media was inoculated with a loop of cells (1 cm × 1 cm) and incubated for a period of 16–24 h with shaking (250 rpm) at 30 °C. The growth was stopped when cells reached this point. Once the initial growth period was complete, the bacteria were pelleted (8500 rpm, 4 °C) and the supernatant was decanted. The cell pellet from the 250 mL MYM cultures was resuspended and washed with approximately 50 mL of MSM solution. This process was repeated twice to ensure removal of all traces of MYM. The cells were then resuspended in minimal MSM solution (~15 mL). The production media was prepared by addition of 5–60 mM (determined by solubility limitations) of the corresponding amino acid to the MSM solution, then adjusting the pH to 7.5 using NaOH (5 M) and HCl (5 M) as required, and autoclaving the solution. The amino acids investigated included L-phenylalanine (20 mM), 4-amino-D-phenylalanine (30 mM), 4-amino-L-phenylalanine (30 mM), 4-bromo-D-phenylalanine (20 mM), 4-bromo-L-phenylalanine (20 mM), 4-chloro-D-phenylalanine (5 mM), 4-chloro-L-phenylalanine (5 mM), 4-cyano-D-phenylalanine (60 mM), 4-cyano-L-phenylalanine (60 mM), 2,4-dichloro-D-phenylalanine (5 mM), 3,4-dichloro-D-phenylalanine (5 mM), and 3,4-dichloro-L-phenylalanine (5 mM). After sterilization, a separately filtered sterilized 30% glucose solution (2%, v/v) and a separately autoclaved 9 mM phosphate solution (0.54%, v/v) were added to the MSM production media. The resuspended *S. venezuelae* was then inoculated into the production media to an absorbance at 600 nm (OD₆₀₀) of 0.6. The production media was immediately ethanol shocked (3%, v/v) to induce jadomycin production. The media was incubated with shaking (250 rpm) at 30 °C while being monitored via OD₆₀₀, Abs₅₂₆, and high-performance liquid chromatography (HPLC). At 24 h the pH was readjusted to 7.5 using NaOH (5 M) or HCl (5 M) as required. Once the production period had finished, the cells were removed via filtration using Whatman #5 filter paper, followed by 0.45 and 0.22 μm Millipore filters. The production media was then passed through a phenyl column (Silicycle) that had been preconditioned using 100% methanol followed by ddH₂O. The material was loaded and washed with ddH₂O (6–8 L for a 2 L production) with the crude natural product being eluted off of the column using 100% methanol (~250 mL for a 2 L production). The solvent was then removed *in vacuo*. The presence of the natural product was checked by HPLC and LC-MS/MS. Purification was continued as outlined in the respective section.

Jadomycin Productions. Productions using L-phenylalanine for jadomycin incorporation were done solely as standards, as this jadomycin has been previously isolated.⁵ Cell growth and jadomycin production were successful (Figure S1), and purification was not pursued. Productions using separate 4-bromophenylalanine enantiomers, 4-chlorophenylalanine enantiomers, 2,4-dichloro-D-phenylalanine, and 3,4-dichlorophenylalanine enantiomers demonstrated that the cells were able to grow in the presence of these phenylalanine analogues, but jadomycin production was poor (Figure S2). The productions were allowed to proceed until 336 h due to low Abs₅₂₆

readings to investigate whether improved jadomycin production would occur. These jadomycin productions did not work, as determined by HPLC and LC-MS/MS (data not shown), and purification was not pursued. Productions using separate 4-cyanophenylalanine enantiomers demonstrated that the cells were able to grow in the presence of both 4-cyanophenylalanine enantiomers, but jadomycin production was poor (Figure S3). The productions were stopped after the typical 48 h production period. These jadomycin productions did not work as determined by HPLC and LC-MS/MS (data not shown), and purification was not pursued.

Jadomycin 4-Amino-L-phenylalanine (1). Productions using separate 4-aminophenylalanine enantiomers demonstrated that the cells appeared to be able to grow in the presence of both enantiomers and appear to produce jadomycin (Figure S4). The productions were stopped after the typical 48 h production period. HPLC and LC-MS/MS analysis was carried out on the crude material, confirming the presence of jadomycin 4-aminophenylalanine. Production of jadomycin was more favorable using 4-amino-L-phenylalanine as opposed to the 4-amino-D-phenylalanine, based on higher Abs₅₂₆ (Figure S4), as such large-scale fermentations were carried out with 4-amino-L-phenylalanine. The initial 70 g phenyl column (Silicycle) yielded 206 mg of crude material. Previous work had demonstrated that a water/ethyl acetate extraction was beneficial,¹¹ and as such was attempted. The extraction removed some impurities, but the natural product was found in both phases (as layers). As such, these fractions were pooled together and the solvent was removed *in vacuo*, yielding 93.1 mg of crude natural product. This material was then applied to a 25 g silica column preconditioned with 20% CH₃OH in EtOAc. The material was eluted using a 20 mL/min flow rate, collecting 9 mL fractions. Purification was accomplished using a gradient system with solvent A (20% CH₃OH in EtOAc) and solvent B (7:2:1 EtOAc/CH₃OH/H₂O). The column was run with an initial isocratic step of 100% solvent A (2 CV), followed by a linear increasing gradient of 0% to 100% solvent B (5 CV), with a final isocratic step of 100% solvent B (2 CV). Fractions were analyzed using TLC, and the appropriate fractions combined and dried *in vacuo*, yielding 23.5 mg of crude material. Final purification was accomplished using a Sephadex LH-20 size exclusion column eluted with methanol. The fractions were combined using TLC analysis. The solvent was removed *in vacuo*, yielding 1 (12.6 mg) as a mixture of diastereomers ($M_f:M_n$ 20:9) by ¹H NMR. TLC R_f 0.25 (5:5:1 CH₃CN/EtOAc/H₂O); HPLC t_R = 7.76 min; UV-vis (3.0 × 10⁻⁴ and 3.8 × 10⁻⁵ M, MeOH) λ_{\max} (ϵ) = 294 (24 194), 539 (2057); LRMS (ESI⁺) MS/MS (599) found 599 [M + H]⁺, 469 [M + H - digitoxose]⁺, 306 [M + H - digitoxose - C₉H₁₁NO₂]⁺; HRMS (ESI⁺) for C₃₃H₃₁N₂O₉ [M + H]⁺ 599.2040 found, 599.2024 calcd; NMR spectra to follow (see SI); see characterization tables (Tables S2 and S3) for numbering.

Derivatization of Jadomycin 4-Amino-L-Phenylalanine. For derivatization purposes, jadomycin 4-amino-L-phenylalanine was not fully purified. This is beneficial to avoid breakdown of the natural product during the purification procedures. The material was first passed down a 70 g phenyl column (Silicycle), eluted, and separated into aliquots, yielding ~100 mg L⁻¹.

Synthesis of Jadomycin 4-Amino-L-phenylalanine Naphthoxycetylamide (1a). Crude 1 (62.4 mg) was dissolved in 20 mL of 1:1 acetonitrile/phosphate-buffered saline (PBS, 50 mM phosphate, 145 mM NaCl, pH 7.6) and added to (2-naphthoxy)acetic acid N-hydroxysuccinimide ester (48.3 mg) in a 25 mL round-bottom flask dropwise with gentle stirring. The flask was corked and protected from light. The reaction was allowed to proceed for 3 h until complete, as determined by TLC (5:5:1 CH₃CN/EtOAc/H₂O) (Figure S5). Once the reaction was complete, the mixture was extracted with dichloromethane (DCM, 3 × 15 mL). The derivative was determined to be in the organic layer by TLC, and the DCM was removed *in vacuo*. The crude material was brought up in minimal methanol in DCM and run on a preparative TLC (5:5:1 CH₃CN/EtOAc/H₂O) as previously described. Solvent was then removed *in vacuo*, yielding 26.7 mg of product. Final purification was accomplished using two consecutive Sephadex LH-20 size-exclusion columns eluting with 5:5:1 CH₃CN/EtOAc/H₂O. The solvent was removed *in vacuo*, yielding 1a (14 mg)

as a mixture of diastereomers ($M_f:M_n$ 25:21) by ^1H NMR. TLC R_f 0.45 (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$); HPLC t_R = 10.34 min; UV-vis (3.8×10^{-4} and 4.8×10^{-5} M, MeOH) λ_{max} (ϵ) = 299 (15 916), 396 (2175), 546 (1635); LRMS (ESI⁺) MS/MS (783) found 783 $[\text{M} + \text{H}]^+$, 653 $[\text{M} + \text{H} - \text{digitoxose}]^+$; HRMS (ESI⁻) for $\text{C}_{46}\text{H}_{41}\text{N}_2\text{O}_{12}$ $[\text{M} + \text{MeOH} - \text{H}]^-$ 813.2678 found, 813.2665 calcd; NMR spectra to follow (see SI); see characterization tables (Tables S4 and S5) for numbering.

Synthesis of Jadomycin 4-Amino-L-phenylalanine Nonoylamide (1b). Crude **1** (83.0 mg) was dissolved in 20 mL of 1:1 acetonitrile/PBS (pH 7.6) and added to nonoic acid *N*-hydroxysuccinimide ester (76.0 mg) in a 50 mL round-bottom flask dropwise with gentle stirring. The flask was corked and protected from light. After 4 h the reaction had not proceeded to completion, as observed by the presence of **1** by TLC analysis. Another 36 mg of nonoic acid *N*-hydroxysuccinimide ester was dissolved in 5 mL of a 1:1 $\text{CH}_3\text{CN}/\text{PBS}$ solution and added to the reaction flask. The reaction was allowed to proceed for an additional 6 h until complete, as determined by TLC (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$) (Figure S6). Upon completion, the mixture was diluted with 20 mL of PBS and extracted with DCM (3×50 mL). The derivative was determined to be in the organic layer by TLC, and DCM was removed *in vacuo*. The reaction mixture was brought up in minimal DCM (~ 2 mL) and filtered. The filtrate was purified using a 40 g silica column preconditioned with 1:1 $\text{CH}_3\text{CN}/\text{EtOAc}$. The material was eluted using a 35 mL/min flow rate, collecting 9 mL fractions. Purification was accomplished using a gradient system with solvent A (1:1 $\text{CH}_3\text{CN}/\text{EtOAc}$) and solvent B (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$). The column was run with an initial isocratic step of 100% solvent A (1 CV), followed by a linear increasing gradient of 0% to 100% solvent B (10 CV), with a final isocratic step of 100% solvent B (5 CV). Fractions were analyzed by TLC, and those containing the compound of interest were combined and dried *in vacuo*, yielding 20.0 mg of material. The crude material was brought up in minimal methanol in DCM and run on preparative TLC (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$) as previously described. Solvent was then removed *in vacuo*, yielding 17.0 mg of product. Final purification was accomplished using a Sephadex LH-20 size-exclusion column, eluting with 5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$. **1b** was then dried *in vacuo*, redissolved in 10 mL of 1:1 $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, and extracted with hexanes (3×10 mL). The $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ layer was dried, yielding **1b** (14 mg) as a mixture of diastereomers ($M_f:M_n$ 100:74) by ^1H NMR. TLC R_f 0.70 (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$); HPLC t_R = 9.50 min; UV-vis (3.4×10^{-4} and 4.2×10^{-5} M, MeOH) λ_{max} (ϵ) = 303 (13 830), 391 (3230), 547 (2297); LRMS (ESI⁺) MS/MS (739) found 739 $[\text{M} + \text{H}]^+$, 609 $[\text{M} + \text{H} - \text{digitoxose}]^+$; HRMS (ESI⁻) for $\text{C}_{43}\text{H}_{49}\text{N}_2\text{O}_{11}$ $[\text{M} + \text{MeOH} - \text{H}]^-$ 769.3356 found, 769.3342 calcd; NMR spectra to follow (see SI); see characterization tables (Tables S6 and S7) for numbering.

Synthesis of Jadomycin 4-Amino-L-phenylalanine Phenoxyacetylamine (1c). Crude **1** (107.0 mg) was dissolved in 20 mL of 1:1 acetonitrile/PBS (pH 7.6) and added to phenoxyacetic acid *N*-hydroxysuccinimide ester (66.9 mg) in a 25 mL round-bottom flask dropwise with gentle stirring. The flask was corked and protected from light. The reaction was allowed to proceed for 1 h until completion, as determined by TLC (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$) (Figure S7). Upon completion, the reaction mixture was extracted with DCM (4×20 mL). The derivative was determined to be in the organic layer by TLC, and DCM was removed *in vacuo*, yielding 88.6 mg of crude product. This material was then purified using a 40 g silica column preconditioned with 1:1 $\text{CH}_3\text{CN}/\text{EtOAc}$. The material was eluted using a 20 mL/min flow rate, collecting 9 mL fractions. Purification was accomplished using a gradient system with solvent A (1:1 $\text{CH}_3\text{CN}/\text{EtOAc}$) and solvent B (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$). The column was run with an initial isocratic step of 100% solvent A (1 CV), followed by a linear increasing gradient of 0% to 100% solvent B (10 CV), with a final isocratic step of 100% solvent B (10 CV). Fractions were analyzed by TLC, and those containing the compound of interest were combined and dried *in vacuo*, yielding 22.4 mg of material. The crude material was brought up in minimal methanol in DCM and run on preparative TLC (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$) as previously described. Solvent was then removed *in vacuo*, yielding 17.8

mg of product. Final purification was accomplished using two consecutive Sephadex LH-20 size-exclusion columns eluting with 5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$. The solvent was removed *in vacuo*, yielding **1c** (14 mg) as a mixture of diastereomers ($M_f:M_n$ 2:1) by ^1H NMR. TLC R_f 0.70 (5:5:1 $\text{CH}_3\text{CN}/\text{EtOAc}/\text{H}_2\text{O}$); HPLC t_R = 9.87 min; UV-vis (1.2×10^{-3} and 1.5×10^{-4} M, MeOH) λ_{max} (ϵ) = 300 (4530), 392 (746), 548 (565); LRMS (ESI⁺) MS/MS (733) found 733 $[\text{M} + \text{H}]^+$, 603 $[\text{M} + \text{H} - \text{digitoxose}]^+$; HRMS (ESI⁻) for $\text{C}_{42}\text{H}_{39}\text{N}_2\text{O}_{12}$ $[\text{M} + \text{MeOH} - \text{H}]^-$ 763.2517 found, 763.2508 calcd; NMR spectra to follow (see SI); see characterization tables (Tables S8 and S9) for numbering.

Biological Evaluation of Jadomycin Derivatives. All biological evaluation was carried out as previously reported and can be found in the SI.¹¹

■ ASSOCIATED CONTENT

📄 Supporting Information

Bacterial growth curves, biological evaluation, characterization tables, and NMR spectra for compounds **1** and **1a–1c**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/np5009398.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Blin, K.; Medema, M. H.; Kazempour, D.; Fischbach, M. A.; Breitling, R.; Takano, E.; Weber, T. *Nucleic Acids Res.* **2013**, *41*, W204–W212.
- (2) He, J.; Magarvey, N.; Pirae, M.; Vining, L. C. *Microbiology* **2001**, *147*, 2817–2829.
- (3) Ayer, S. W.; McInnes, A. G.; Thibault, P.; Walter, J. A.; Doull, J. L.; Parnell, T.; Vining, L. C. *Tetrahedron Lett.* **1991**, *32*, 6301–6304.
- (4) Doull, J. L.; Ayer, S. W.; Singh, A. K.; Thibault, P. *J. Antibiot.* **1993**, *46*, 869–871.
- (5) Borrisow, C. N.; Graham, C. L.; Syvitski, R. T.; Reid, T. R.; Blay, J.; Jakeman, D. L. *ChemBioChem* **2007**, *8*, 1198–1203.
- (6) Dupuis, S. N.; Robertson, A. W.; Veinot, T.; Monroe, S. M. A.; Douglas, S. E.; Syvitski, R. T.; Goraliski, K. B.; McFarland, S. A.; Jakeman, D. L. *Chem. Sci.* **2012**, *3*, 1640–1644.
- (7) Rix, U.; Zheng, J.; Rix, L. L. R.; Greenwell, L.; Yang, K.; Rohr, J. *J. Am. Chem. Soc.* **2004**, *126*, 4496–4497.
- (8) Shan, M.; Sharif, E. U.; Odoherly, G. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 9492–9495.
- (9) Rohr, J.; Kharel, M. K. *Curr. Opin. Chem. Biol.* **2012**, *16*, 150–161.
- (10) Tibrewal, N.; Pahari, P.; Wang, G.; Kharel, M. K.; Morris, C.; Downey, T.; Hou, Y.; Bugni, T. S.; Rohr, J. *J. Am. Chem. Soc.* **2012**, *134*, 18181–18184.
- (11) Robertson, A. W.; Martinez-Farina, C. M.; Smithen, D. A.; Yin, H.; Monroe, S.; Thompson, A.; McFarland, S. A.; Syvitski, R. T.; Jakeman, D. L. *J. Am. Chem. Soc.* **2015**, *137*, 3271–3275.
- (12) Jakeman, D. L.; Dupuis, S. N.; Graham, C. L. *Pure Appl. Chem.* **2009**, *81*, 1041–1049.
- (13) Fernández-Martínez, L. T.; Borsetto, C.; Gomez-Escribano, J. P.; Bibb, M. J.; Al-Bassam, M. M.; Chandra, G.; Bibb, M. J. *Antimicrob.*

Agents Chemother. **2014**, published online, doi: 10.1128/AAC.04272-14.

(14) He, J.; Magarvey, N.; Pirae, M.; Vining, L. C. *Microbiology* **2001**, *147*, 2817–2829.

(15) Yang, X.; Yu, B. *Chem.—Eur. J.* **2013**, *19*, 8431–8434.

(16) Jakeman, D. L.; Graham, C. L.; Young, W.; Vining, L. C. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 767–772.

(17) Padilla, I. M. G.; Burgos, L. *Plant Cell Rep.* **2010**, *29*, 1203–1213.

(18) Tacar, O.; Sriamornsak, P.; Dass, C. R. *J. Pharm. Pharmacol.* **2013**, *65*, 157–170.

(19) Kaufmann, A. M.; Krise, J. P. *J. Pharm. Sci.* **2007**, *96*, 729–746.