Eight-Membered Ring-Containing Jadomycins: Implications for Non-enzymatic Natural Products Biosynthesis

Andrew W. Robertson,[†] Camilo F. Martinez-Farina,[†] Deborah A. Smithen,[†] Huimin Yin,[‡] Susan Monro,[‡] Alison Thompson,[†] Sherri A. McFarland,[‡] Raymond T. Syvitski,[§] and David L. Jakeman*,[†,⊥]

[†]Department of Chemistry and [⊥]College of Pharmacy, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada
[‡]Department of Chemistry, Acadia University, Wolfville, Nova Scotia B4P 2R6, Canada
[§]Institute for Marine Biosciences, National Research Council of Canada, Halifax, Nova Scotia B3H 3Z1, Canada

S *Supporting Information*

ABSTRACT: Jadomycin Oct (**1**) was isolated from *Streptomyces venezuelae* ISP5230 and characterized as a structurally unique eight-membered L-ornithine ring-containing jadomycin. The structure was elucidated through the semisynthetic derivatization of starting material via chemoselective acylation

of the L-ornithine α-amino group using activated succinimidyl esters. Incorporation of 5-aminovaleric acid led to jadomycin AVA, a second eight-membered ring-containing jadomycin. These natural products illustrate the structural diversity permissible from a non-enzymatic step within a biosynthetic pathway and exemplifies the potential for discovery of novel scaffolds.
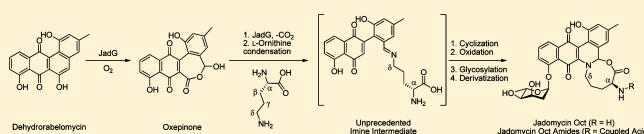
■ INTRODUCTION

The Actinobacteria genus *Streptomyces* is one of the largest sources of structurally diverse bioactive natural products. *Streptomyces* species account for the production of 32% of all identified bioactive metabolites.[1,2] The isolation of new scaffolds and the generation of diverse libraries remain one of the major goals associated with natural products research in an effort to identify unique or enhanced bioactivity for clinical treatment of disease. *Streptomyces venezuelae* ISP5230 (ATCC 10712) has been studied for its ability to produce the secondary metabolites chloramphenicol,[3] jadomycin B (Figure 1),[4−6] and
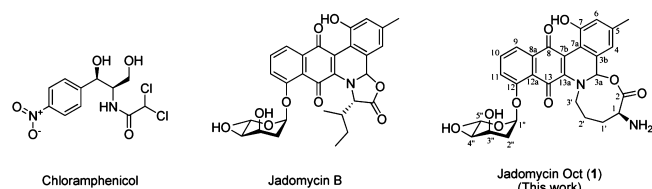

Figure 1. Structures of natural products isolated from *S. venezuelae* ISP5230.

venezuelin (structure not yet reported).[7] The jadomycins are a family of type II polyketide synthase (PKS)-derived natural products, characterized by a biosynthetically derivatized benz[*a*]anthracene scaffold. This angled polyaromatic backbone is a unique framework of the angucycline group of natural products; the largest group of type II PKS produced natural products.[8] Diversification of the biosynthetic machinery within each angucycline cluster is responsible for an array of unique structural moieties and consequently a wide variety of bioactivity.[9,10] Interestingly, Yang and co-workers have recently shown jadomycin to be involved in a complex modulation of

other exogenous natural product biosynthetic gene clusters, specifically in *Streptomyces coelicolor*.[11] In the jadomycin cluster, JadG has been identified as the enzyme responsible for the B ring opening.[9,12] The C−C cleavage proceeds via a Baeyer−Villiger oxidation producing an oxepinone intermediate.[13] The resulting reactive aldehyde couples in a non-enzymatic fashion to a singular amino acid in the culture medium, forming an imine intermediate that cyclizes into the jadomycin backbone (Scheme 1).[14−18] Glycosylation with the 2,6-dideoxy sugar L-

Scheme 1. Discovery of Alternative Amino Acid Incorporation into the Jadomycin Backbone
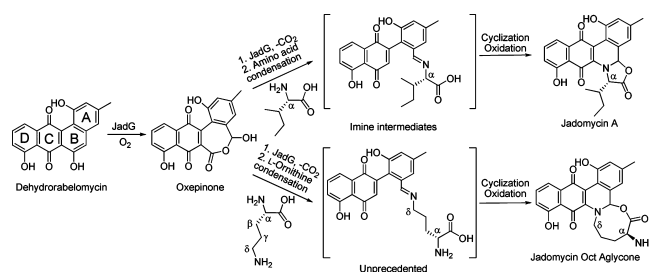
digitoxose is accomplished by the glycosyltransferase JadS.[19] The substrate specificity of natural product glycosyltransferases is an alluring approach to structural diversification.[20]

Compelling evidence for the cyclization process has been demonstrated through the chemical synthesis of jadomycin A by O'Doherty and later by Yu in the total syntheses of a key series of fully glycosylated jadomycins.[21,22] If this biosynthetic step could be exploited to react with an amine other than the α-

amine of an amino acid, it could yield a unique opportunity to expand the structural diversity of these natural products. Since L-ornithine contains α- and δ-amino groups, we hypothesized that either could act as a nucleophile forming imine intermediates and potentially undergo spontaneous cyclization with the free carboxylic acid forming either a five-membered, or eight-membered heterocyclic ring (Scheme 1). Initial cultures of *S. venezuelae* ISP5230 VS1099 were, therefore, grown with L-ornithine and our characterization of the major structural isomer commenced. We report the characterization of jadomycin Oct (**1**), a new eight-membered ring-containing jadomycin (Figure 1), providing insight into the specificity of the spontaneous amino acid incorporation and potentially the substrate promiscuity of the glycosyltransferase JadS in the biosynthetic pathway.

■ RESULTS AND DISCUSSION

Isolation and Derivatization of 1. Purification of **1** proved difficult with significant breakdown occurring during workup, necessitating derivatization for structural characterization purposes.[18] Chemo-selective derivatization of the free amino functionality using N-hydroxy succinimide activated carboxylic acids was chosen to create a small library of jadomycin amides (Scheme 2). Growths of *S. venezuelae*

Scheme 2. Semi-Synthetic Derivatization of 1
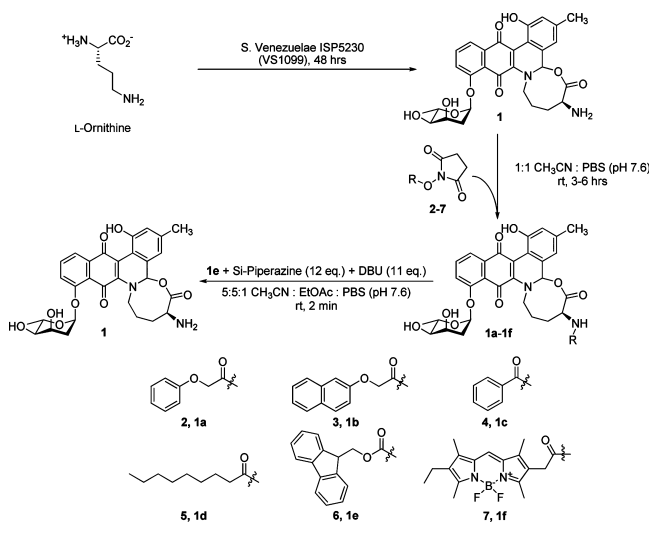
ISP5230 VS1099 in the presence of L-ornithine (60 mM) as the sole nitrogen source[23] were extracted using solid-phase methods (see Supporting Information (SI)) to yield ~100 mg L^{-1} crude extract used for all derivatizations.

Bioconjugation techniques using succinimidyl esters are well established in the literature and allow for very selective amine reactivity under mild conditions.[24] A series of activated succinimidyl esters **2–7** (Scheme 2) were prepared to protect the free amine of L-ornithine. Solvent selection proved very important for the coupling reactions. The growth extract containing **1** was found to be very water soluble with little to no solubility in most organic solvents, whereas succinimidyl esters **2–7** were organic soluble. It has been shown that aprotic organic solvents in the presence of a basic buffer can be effective in facilitating reactions involving succinimidyl esters[25] and that as pH is increased amine reactivity also increases.[26] Unfortunately, under basic conditions jadomycins undergo a

ring opening event producing an aldehyde intermediate that could potentially react with amines forming undesired imine side products; as such, a near physiological pH was selected.[27] It was found that a 1:1 mixture of CH_3CN and phosphate buffered saline (PBS, pH 7.6) allowed for the best solubility and stability of both the crude extract of **1** and the activated acids. Crude extracts were treated with excess succinimidyl esters (see SI). Production of the desired mono-derivatized compounds (**1a–1f**) was confirmed by LC-MS/MS analysis of reaction mixtures, showing characteristic jadomycin fragmentation patterns in each case (Figure 2).

Compounds **1a–1f**, with the exception of **1e**, proved more stable compared to **1** and thus were more amenable to purification. Isolated yields of the derivatives ranged from 1.5 to 14 mg L^{-1} (Table 1). Compounds **1a–1f** were isolated using liquid–liquid extractions and solid-phase methodologies (see SI) as dark-purple or red solids consisting of two diastereomers using NMR analysis, in a ratio of ~5:4 in all cases (Table 1). Many jadomycins exist as a mixture of two diastereomers in a dynamic equilibrium as a result of the ring opening event previously described.[27] HRMS analysis of the purified compounds confirmed the presence of the appropriate m/z for each analogue (Table 1).

Structural Characterization of 1a–1f. In order to identify the major structural isomer as either an eight-membered or five-membered ring system, characterization of derivatives **1a–1f** was carried out using NMR spectroscopy. ^{1}H NMR spectra and ^{1}H–^{1}H COSY analysis of **1a–1f** identified the expected jadomycin spin systems (C4 to C6, C9 to C11, and C1″ to C5″-CH_3) associated with the intact A, D, and sugar rings, respectively (Figure 3). HMBC correlation between the anomeric H1″ (δ$_H$ = ~6 ppm) and C12 (δ$_C$ = ~155 ppm) confirmed glycosylation at the 12-position of the D ring. HMBC correlations between the protons of the A and D rings identified the intact C ring quinone and the appropriate connectivity of the polyaromatic backbone (Figure 3). The ^{1}H–^{1}H COSY experiment successfully identified the C1 to C3′ spin system of the incorporated L-ornithine (Figure 3); an edited-HSQC was analyzed to assign the L-ornithine methylene linkers. The ^{1}H NMR spectra were confounded by two sets of signals, each associated with a diastereomer at the 3a position. The H1′-H3′ protons were particularly challenging to identify as they were found to be diastereotopic leading to four signals for each methylene group, denoted either H1′-3′$_{Mj}$ for the major diastereomer or H1′-3′$_{Mn}$ for the minor diastereomer.

The edited HSQC gave ^1J [1]H–[13]C correlations and also provided multiplicity information in which −CH− and −CH_3 groups are phased opposite to −CH_2− groups. These data alongside the ^{1}H–^{1}H COSY experiment allowed for identification of the appropriate [1]H–[1]H geminal coupling and ^3J coupling of the C1 to C3′ spin system, allowing us to assign connectivity within the ornithine side chain. ^{1}H NMR spectra and ^{1}H–^{1}H COSY analyses of **1a–1f** identified the appropriate signals and spin systems associated with the presence of each R functionality. The ^{1}H NMR (integration) and HSQC (phasing) identified H3a as a −CH− group. The ^{1}H–^{1}H COSY also showed the H3a proton in its own spin system, consistent with other jadomycin H3a protons. The ^{13}C NMR chemical shifts of the C3a atoms were within the expected range for most jadomycins (δ$_C$ ~ 90–96 ppm) in which the 3a position is surrounded by nitrogen, an aromatic ring carbon, and an oxygen atom. The masses identified by HRMS are also consistent with cyclized products. The HMBC experiments
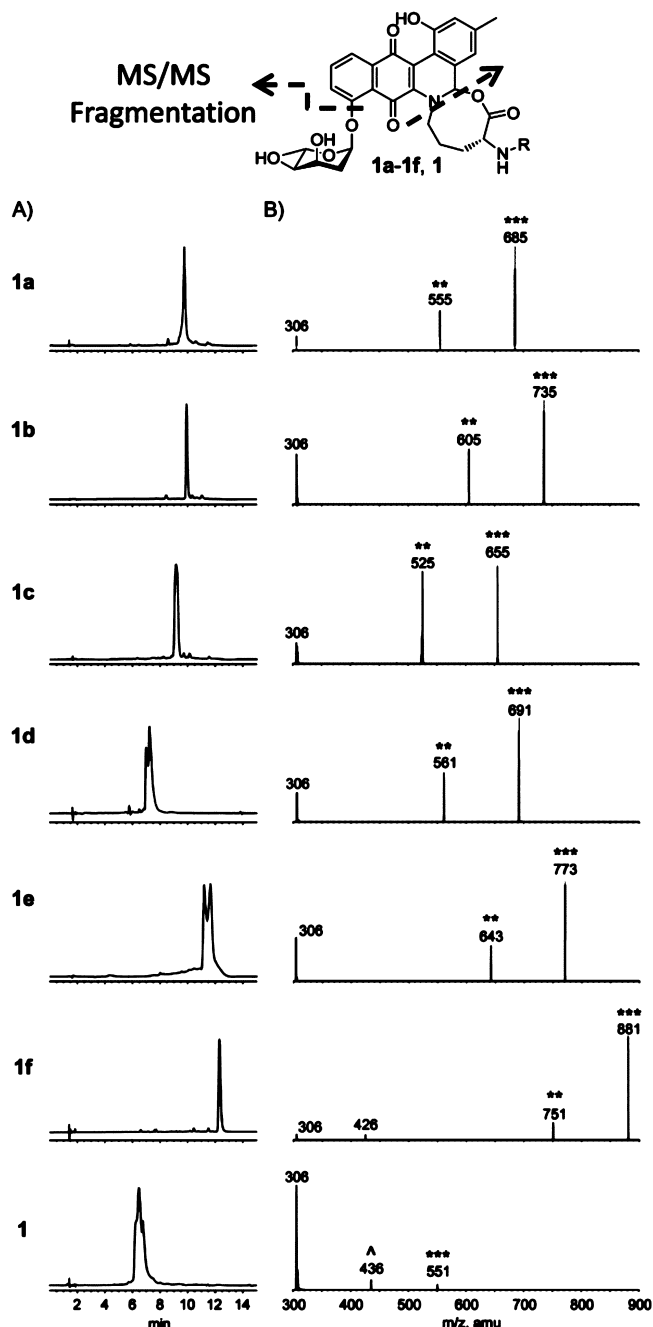
Figure 2. (A) HPLC traces of **1a**−**1f** and **1**. Compound **1d** required alternative HPLC conditions (see SI). Diastereomers are readily observed for **1d** and **1e** by HPLC. (B) LC-MS/MS fragmentation of **1a**−**1f** and **1**, illustrating $[M + H]^+$ (***), cleavage of the sugar $[M + H - digitoxose]^+$ (**) and the amino acid groups $[M + H - digitoxose - R]^+$ (m/z 306). Compound **1** showed loss of amino acid group first $[M + H - R]^+$ (∧).

enabled us to unambiguously assign the connectivity of ornithine within the structures. HMBC correlations were observed from H3a to C3′ in all cases (Figure 3). If the configurations of **1a**−**1f** contained five-membered oxazolone rings then the observed HMBC correlations between H3a and C3′ would represent unlikely ^6J couplings. In contrast, these correlations would represent much more likely ^3J couplings within eight-membered heterocycles. The H3′ protons also showed HMBC correlations back to C3a as well as C13a of the quinone ring system, identifying the close through bond

Table 1. Diastereomeric Ratios, Isolated Yields, and HRMS m/z of Jadomycin Amides 1a−1f

product	Mj:Mn[a]	yield (mg L^{-1})	HRMS[b]
1a	100:80	14	685.2392
1b	100:74	11	735.2517
1c	100:81	13	707.2202[c]
1d	100:81[d]	9	691.3194
1e	100:75	1.5	803.2838[e]
1f	100:70	12	881.3698
1	100:82	1.5	573.1834[f]

[a]Ratios of diastereomers [major diastereomer (Mj), minor diastereomer (Mn)] were determined by ^{1}H NMR integrations. [b]All HRMS m/z values reported are $[M + H]^+$ unless otherwise stated. [c]m/z of **1c** corresponds to $[M + Na]^+$. [d]Ratio was difficult to measure accurately due to overlap of ^{1}H NMR signals. [e]m/z of **1e** corresponds to $[M + MeOH - H]^-$. [f]m/z of **1** corresponds to $[M + Na]^+$.
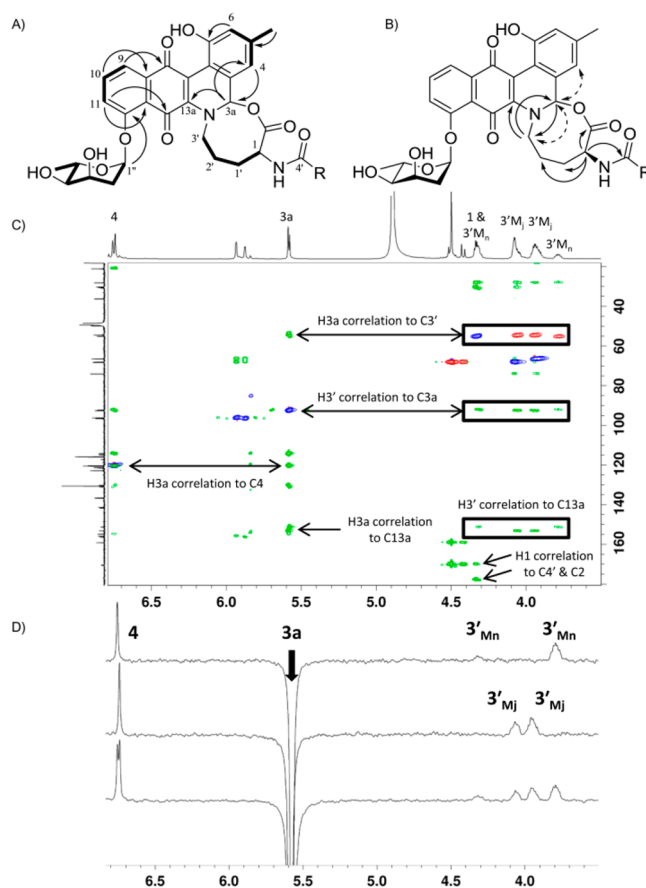
Figure 3. (A) Observed ^{1}H−^{1}H COSY (bold) and HMBC (solid arrows) correlations of compounds **1a**−**1f** illustrating intact A, B, C, D, and sugar rings. (B) Observed HMBC (solid arrows) and ROESY (dashed arrows) correlations of **1a**−**1f** identifying the configuration of the incorporated L-ornithine. (C) Overlaid edited-HSQC and HMBC spectra of **1a**. Edited HSQC data are represented in blue (−CH− or −CH$_3$) and red (−CH$_2$−), and HMBC correlations are represented in green (^2J−^4J coupling). (D) Selective ROESY spectra of **1a**, selectively irradiating H3a of the minor diastereomer (top), H3a of the major diastereomer (middle), and both simultaneously (bottom).

proximity of the 3′ position to the B ring. These correlations were readily identified as all four H3′ signals showed these correlations (Figure 3). The unique diastereotopic nature of the 3′ position also aided in ROESY analysis. Selective ROESY spectra identified NOE correlations between the H3a and H3′

protons, further substantiating the structures as the eight-membered ring-containing configuration (Figure 3). ROESY experiments were also performed irradiating each of the H3a$_{Mj}$ or H3a$_{Mn}$ separately. Correlations were observed between the appropriate H4 and H3′ of the major and minor diastereomers. The NOE correlations of H3a$_{Mj}$ to the H3′$_{Mj}$ protons were of approximate equal intensity suggesting a similar distance through space between the protons. The NOE correlations of H3a$_{Mn}$ to the H3′$_{Mn}$ protons showed different intensities also suggesting differences in the 3a stereochemistry between the major and minor diastereomers. As a final confirmation of the structure, HMBC correlations between H1 and C4′ of the amide side chain (^3J) were observed indicating selective coupling of the α-amino group of L-ornithine to the succinimidyl esters confirming the proposed eight-membered ring configuration.

Characterization of 1. With the structural characterization of **1a−1f** confirmed, investigation shifted back to the isolation and characterization of **1** to ensure L-ornithine configuration was conserved throughout the chemo-selective derivatization. Compound **1** was produced and isolated using liquid−liquid extractions and solid-phase methodologies in a time-sensitive manner (see SI) giving a dark-purple solid (1.5 mg L^{-1}), consisting of a mixture of two diastereomers in a ratio of 100:82. The diastereomeric ratios of **1a−1f** were consistent with **1**, suggesting reaction conditions did not facilitate ring opening. LC-MS/MS and HRMS data identified the appropriate fragmentation pattern and m/z expected for **1** (Figure 2 and Table 1). Structural characterization using NMR analysis showed many of the same correlations as **1a−1f**, excluding the chemo-selectively derivatized groups (see SI), confirming **1** as an eight-membered heterocyclic jadomycin. Concurrently with our investigation, Yang and co-workers described the growth and isolation of a natural product from *S. venezuelae* ISP5230 growths with L-ornithine.[17] They proposed the five-membered oxazolone ring containing structure in contrast to our eight-membered derivative.[17] Interestingly, in our hands, **1** was identified as the major product in the growth media by HPLC, with no direct evidence of an alternative five-membered oxazolone ring-containing jadomycin.[17] The isolation of **1** may provide insight into the substrate specificity of the glycosyltransferase, JadS, involved in jadomycin biosynthesis. If glycosylation occurs after amino acid incorporation, it illustrates that JadS is able to accommodate structural divergence of the glycosyl-acceptor beyond the hydroxy-quinone functionality, not only accepting five-membered ring-containing oxazolones[28] but also eight-membered ring containing derivatives.

Probing Structural Diversity. To expand the scope of this study, *S. venezuelae* ISP5230 VS1099 was grown in the presence of either 5-aminovaleric acid (60 mM) or D-ornithine (60 mM). Given that 5-aminovaleric acid only has a δ-amino group, incorporation into the jadomycin backbone was anticipated to produce the eight-membered ring containing derivative, jadomycin AVA (**8**). Likewise, based on the results obtained with L-ornithine, incorporation of D-ornithine was also predicted to produce the eight-membered ring containing compound jadomycin DOct (**9**) (Figure 4).

When *S. venezuelae* ISP5230 VS1099 was grown in the presence of 5-aminovaleric acid, bacterial growth and production of colored material were comparable to fermentations with L-ornithine (Figure S1). The major natural product, compound **8**, was isolated in a yield of 10 mg L^{-1} as a mixture
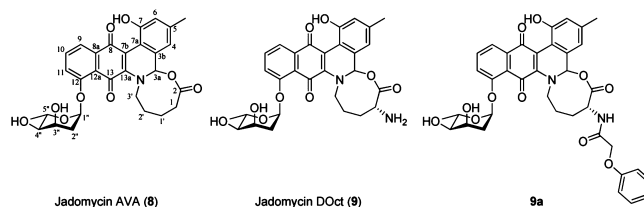
Figure 4. Structures of additional eight-membered ring-containing jadomycins isolated from growths of 5-aminovaleric acid (**8**), D-ornithine (**9**), and a semisynthetic derivative of **9** (**9a**).

of diastereomers (Mj:Mn 100:67). LC-MS/MS analysis found the expected fragmentation pattern (Figure S9), and HRMS confirmed the presence of the expected m/z (see SI). NMR analysis showed patterns and correlations similar to those observed for **1** and its derivatives, specifically the HMBC correlations from H3a to C3′ and H3′ to C3a (see Figure 4 for numbering scheme) were identified. In addition, correlations from both the H3a and H3′ to C13a were observed illustrating the close through bond proximity of these positions to the quinone B ring. Finally, NOE correlation between the H3a and H3′ protons was observed, thereby confirming the expected eight-membered ring system (Figure S62).

S. venezuelae ISP5230 VS1099 fermentation with D-ornithine was conducted on a reduced scale (1/10 the volume; 200 mL) compared to growths with L-ornithine (2 L) due to limited amino acid availability. Bacterial growth and production of colored compounds were significantly reduced compared to fermentations with L-ornithine or 5-aminovaleric acid (Figure S1). Only 5 mg of crude material was isolated from the growth; nevertheless, compound **9** was identified via LC-MS/MS analysis (Figure S11). Due to the instability and low yield of **9**, purification and characterization were not attempted. Instead we opted to use our developed methodology to derivatize **9** using succinimidyl ester **2**. Production of **9a** was confirmed via LC-MS/MS analysis of the reaction mixture (Figure S12), and the material was then purified. Compound **9a** was isolated in limited quantity (<1 mg) with significant impurities. MS/MS fragmentation of **9a** was entirely consistent with the data for **1a**, and the ^{1}H NMR spectra of both compounds were also comparable (Figure S63), suggesting the presence of an eight-membered ring containing derivative.

Biological Activity. Compounds **1a**, **1c**, **1d**, and **1f** were selected by the National Cancer Institute for testing against their 60 DTP human tumor cell line one-dose screen. In contrast to the previous jadomycins bearing five-membered oxazolone rings, cytotoxicity of the compounds was limited despite their ability to invoke Cu(II)-mediated DNA damage (Figure S15). This suggests the importance of the five-membered oxazolone ring for antitumor bioactivity over the new eight-membered ring system.[15] The expanded ring system also leads to an attenuation of the antimicrobial properties normally associated with the jadomycins. Lack of appreciable cytotoxicity under ambient conditions is desirable for photo-dynamic applications that employ light-responsive agents, so representative compounds **1a−1d** were tested for their light-induced antibacterial activity against *Streptococcus mutans* as a model system (Figure S16). All four compounds exhibited photodynamic inactivation (PDI) of bacteria, in the order **1a** > **1b** ∼ **1c** > **1d**. However, they do not appear to act via a DNA photodamaging pathway, given that gel electrophoretic mobility-shift assays revealed minimal DNA photocleavage

under comparable conditions (Figure S14). This unexpected PDI activity is a unique feature of the eight-membered ring system.

CONCLUSION

We have successfully isolated and characterized the amine-containing jadomycin Oct (**1**) and identified it as having L-ornithine incorporated as a unique eight-membered ring system. This sheds light on the potential promiscuity of the spontaneous amino acid incorporation step of jadomycin biosynthesis and how it could be further exploited. Additionally, we successfully synthesized and utilized a group of activated carboxylic acids to semisynthetically derivatize the free amine of **1**, producing a small library of jadomycin amides that act as antimicrobial phototoxins (**1a–1f**), all containing the unique eight-membered ring scaffold. These compounds are the first examples of jadomycins containing eight-membered hetero-cyclic rings. Additionally, the isolation of jadomycin AVA (**8**) unequivocally confirms the presence of the eight-membered ring. The ability to form fully cyclized compounds using amino groups other than the α-amino group of amino acids suggests the structural diversity associated with the jadomycins may be much larger than previously established. By utilizing a unique non-enzymatic process within the jadomycin biosynthetic pathway, *S. venezuelae* likely produces many jadomycin analogues under stress conditions in an ecological setting.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, material, supporting figures, NMR spectra, and additional data are described. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*david.jakeman@dal.ca

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

REFERENCES

(1) Berdy, J. *J. Antibiot.* **2005**, *58*, 1–26.
(2) Scherlach, K.; Hertweck, C. *Org. Biomol. Chem.* **2009**, *7*, 1753–1760.
(3) He, J.; Magarvey, N. F.; Piraee, M.; Vining, L. C. *Microbiology* **2001**, *147*, 2817–2829.
(4) 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.
(5) Doull, J. L.; Ayer, S. W.; Singh, A. K.; Thibault, P. *J. Antibiot.* **1993**, *46*, 869–871.
(6) Doull, J. L.; Singh, A. K.; Hoare, M.; Ayer, S. W. *J. Ind. Microbiol.* **1994**, *13*, 120–125.
(7) Goto, Y.; Li, B.; Claesen, J.; Shi, Y.; Bibb, M. J.; van der Donk, W. *PLoS Biol. [Online]* **2010**, *8*, e1000339.
(8) Kharel, M. K.; Pahari, P.; Shepherd, M. D.; Tibrewal, N.; Nybo, S. E.; Shaaban, K. A.; Rohr, J. *Nat. Prod. Rep.* **2012**, *29*, 264–325.
(9) Kharel, M. K.; Rohr, J. *Curr. Opin. Chem. Biol.* **2012**, *16*, 150–161.
(10) Janso, J. E.; Haltli, B. A.; Eustaquio, A. S.; Kulowski, K.; Waldman, A. J.; Zha, L.; Nakamura, H.; Bernan, V. S.; He, H.; Carter, G. T.; Koehn, F. E.; Balskus, E. P. *Tetrahedron* **2014**, *70*, 4156–4164.
(11) Wang, W.; Ji, J.; Li, X.; Wang, J.; Li, S.; Pan, G.; Fan, K.; Yang, K. *Proc. Nat. Acad. Sci. U.S.A.* **2014**, *111*, 5688–5693.
(12) Fan, K.; Pan, G.; Peng, X.; Zheng, J.; Gao, W.; Wang, J.; Wang, W.; Li, Y.; Yang, K. *Chem. Biol.* **2012**, *19*, 1381–1390.
(13) 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.
(14) Rix, U.; Zheng, J.; Remsing Rix, L. L.; Greenwell, L.; Yang, K.; Rohr, J. *J. Am. Chem. Soc.* **2004**, *126*, 4496–4497.
(15) Jakeman, D. L.; Dupuis, S. N.; Graham, C. L. *Pure Appl. Chem.* **2009**, *81*, 1041–1049.
(16) Jakeman, D. L.; Bandi, S.; Graham, C. L.; Reid, T. R.; Wentzell, J. R.; Douglas, S. E. *Antimicrob. Agents Chemother.* **2009**, *53*, 1245–1247.
(17) Fan, K.; Zhang, X.; Liu, H.; Han, H.; Luo, Y.; Wang, Q.; Geng, M.; Yang, K. *J. Antibiot.* **2012**, *65*, 449–452.
(18) Dupuis, S. N.; Robertson, A. W.; Veinot, T.; Monro, S. M. A.; Douglas, S. E.; Syvitski, R. T.; Goralski, K. B.; McFarland, S. A.; Jakeman, D. L. *Chem. Sci.* **2012**, *3*, 1640–1644.
(19) Wang, L.; White, R. L.; Vining, L. C. *Microbiology* **2002**, *148*, 1091–1103.
(20) Gantt, R. W.; Peltier-Pain, P.; Thorson, J. S. *Nat. Prod. Rep.* **2011**, *28*, 1811–1853.
(21) Shan, M.; Sharif, E. U.; O'Doherty, G. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 9492–9495.
(22) Yang, X.; Yu, B. *Chem.—Eur. J.* **2013**, *19*, 8431–8434.
(23) Jakeman, D. L.; Graham, C. L.; Young, W.; Vining, L. C. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 767–772.
(24) Hermanson, G. In *Bioconjugate techniques*, 3rd ed. [online]; Audet, J., Preap, M., Eds.; Elservier/Academic Press: San Diego, 2013; Chapter 3, pp 229–258. http://www.sciencedirect.com/science/book/9780123822390.
(25) Abad, S.; Nolis, P.; Gispert, J. D.; Spengler, J.; Albericio, F.; Rojas, S.; Herance, J. R. *Chem. Commun.* **2012**, *48*, 6118–6120.
(26) Kalkhof, S.; Sinz, A. *Anal. Bioanal. Chem.* **2008**, *392*, 305–312.
(27) Syvitski, R. T.; Borissow, C. N.; Graham, C. L.; Jakeman, D. L. *Org. Lett.* **2006**, *8*, 697–700.
(28) Borissow, C. N.; Graham, C. L.; Syvitski, R. T.; Reid, T. R.; Blay, J.; Jakeman, D. L. *ChemBioChem* **2007**, *8*, 1198–1203.