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Biosynthesis and Total Synthesis Studies on the Jadomycin Family of Natural Products

Ehesan U. Sharif[a] and George A. O'Doherty*[a]

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Jadomycins are unique angucycline polyketides produced by *Streptomyces venezuelae* soil bacteria under specific nutrient and environmental conditions. Their unique structural complexity and biological activities have engendered extensive study of the jadomycin class of natural compounds in terms of biological activity, biosynthesis, and synthesis. This review outlines recent developments in the study of the synthesis and biosynthesis of jadomycins.

1. Introduction

Nature provides us with ample sources of therapeutically useful molecules. Often the natural products are derivatized to modulate their activities in order to achieve greater specificity and selectivity. In fact, more than half of the drugs on the market today are derived from natural products.[1] The necessity to discover and develop new and potent therapeutic agents, to address a wide array of disease and health-related conditions, has encouraged scientists to discover versatile and efficient routes for the biosynthesis and synthesis of new biologically active natural products.

[a] Department of Chemistry and Chemical Biology, Northeastern University,
Boston, MA 02115, USA
E-mail: G.O'Doherty@neu.edu
http://nuweb9.neu.edu/odoherty

Jadomycins are novel angucycline polyketide natural products each containing an unusual 8*H*-benzo[*b*]phenanthridine backbone with variously fused nitrogen- and oxygen-containing heterocycles (e.g., oxazolidine, pyrimidine, oxazinane). With the exception of jadomycin A (aka jadomycin B aglycon), all the jadomycins are glycosylated with a L-digitoxose sugar unit (Figure 1).[2] This sugar moiety plays a key role in the bioactivity exhibited by these compounds (e.g., jadomycin B exhibits anti-yeast activity whereas jadomycin A is inactive).[3] Jadomycins are active against Gram-positive and Gram-negative bacteria and show anticancer activity, which has tentatively been attributed to aurora-B kinase inhibition and/or DNA cleaving capacity.[4] These secondary metabolites are produced by Gram-positive soil bacteria *Streptomyces venezuelae* ISP5230 (ATCC10712) under nutrient limitation conditions along with additional stress (e.g., heat shock, phase infec-

Ehesan Ul Sharif was born in Assam, India. He received his B.Sc. degree (Chemistry Hons.) from Cotton College in Gauhati, Assam, India in 2004 and M.Sc. (Organic Chemistry Hons.) from Delhi University, Delhi, India in 2006. He began his doctoral studies in the spring of 2007 at West Virginia University in Morgantown, WV and moved to Northeastern University in Boston, MA in the fall of 2010. At Northeastern he is working on the synthesis and testing of DNA-cleaving anticancer angucycline natural products under the supervision of Prof. George O'Doherty.

Prof. George O'Doherty was born in Kilkenny, Ireland, He received his B.S. from Rensselaer Polytechnic Institute in Troy, New York, working with Alan R. Cutler. He got his Ph.D. from The Ohio State University in Columbus, Ohio working with Leo A. Paquette. He spend two postdoctoral stints, the first with Barry M. Trost (Stanford University) and the second with Anthony G. M. Barrett (Imperial College) before he began his independent career at the University of Minnesota and later West Virginia University. Currently his is a professor in Chemistry and Chemical Biology at Northeastern University, Boston, where his research is focused on the de novo asymmetric synthesis of biologically important natural products and carbohydrates for SAR-type studies with the goal of discovering new anticancer and antimicrobial compounds.

tion, or exposure to toxic concentration of ethanol).[5,6] In contrast, chloramphenicol is the only antibiotic currently characterized from *Streptomyces venezuelae* under normal growth conditions.[7]

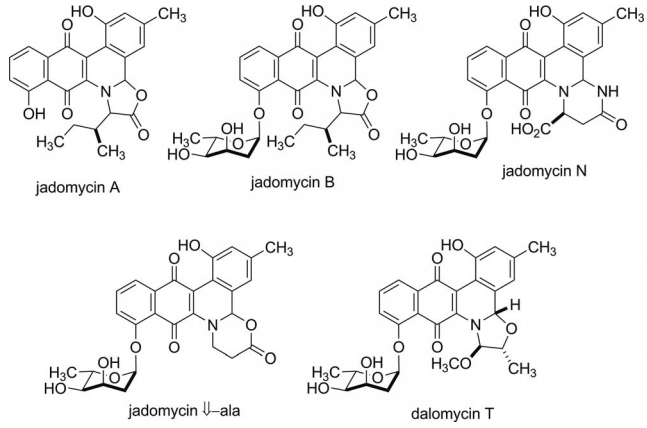


Figure 1. Jadomycin A and its diverse structural congeners.

2. Jadomycin Biosynthesis

2.1. Regulatory Genes for Jadomycin Biosynthesis

Since the early isolation work of Ayer,[5] the unique structure of the jadomycins has inspired extensive study of their biosynthesis. These initial studies ultimately led to the discovery of related jadomycin secondary metabolites. In 1994, Vining was the first to clone and characterize the polyketide synthase (PKS) genes responsible for jadomycin B biosynthesis in *Streptomyces venezuelae* ISP5230.[8] Later, more detailed investigations of the gene clusters were carried out by Yang.[9] A schematic diagram depicting an organizational map of the jadomycin gene cluster is shown in Figure 2. The genome sequence of *Streptomyces venezuelae* ISP5230 (ATCC10712) was recently reported.[6b] AntiSMASH analysis of the genome sequence identified 26 natural product biosynthetic gene clusters along with four additional polyketide synthase clusters and an independent lantibiotic biosynthetic cluster, although no production of the lantibiotic was observed in *Streptomyces venezuelae*.[10]

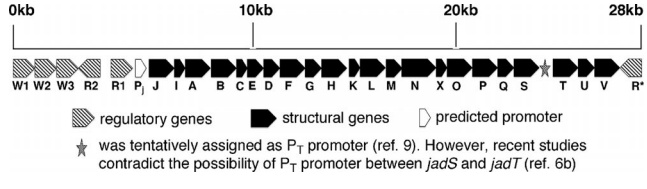


Figure 2. Organizational map of jadomycin gene cluster.

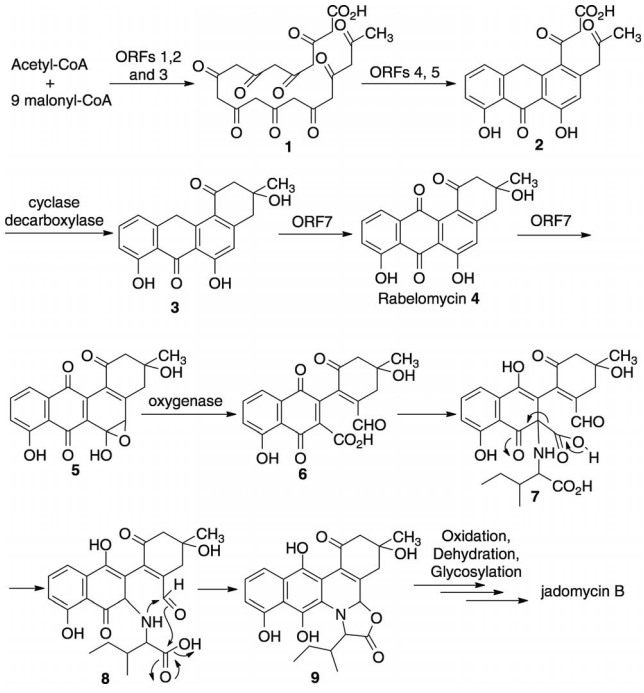
2.2. Sequencing Region Upstream of PKS-II

The need for both nutrient depletion and environmental stress on *S. venezuelae* to induce jadomycin production is quite unique, suggesting the existence of a control mechanism.[11] In contrast, most other streptomycetes produce type-II PKS antibiotics under normal growth conditions[7] and modulate the antibiotic production upon physiological imbalance.[12] Evidence for this control mechanism can be found in the genetic code for jadomycin. An Open Reading Frame (ORF) upstream of type-II PKS encodes for a 196 amino acid sequence (*jadR*$_2$). This amino acid sequence resembles known proteins (e.g., MtrR, AcrR, TetC, and TcmR) that regulate resistance to xenobiotic exposure. Thus, when the nucleotide sequence of the *jadR*$_2$ repressor gene in *S. venezuelae* was disrupted,[13] jadomycin B was produced under normal growth conditions, and overproduction was observed when these mutants were exposed to ethanol stress. This findings point to the conclusion that *jadR*$_2$ negatively regulates the expression of biosynthetic genes of jadomycin B.[11]

In 2001, a second regulatory gene (*jadR*$_1$) encoding for 234 amino acids was identified by Vining[14] The gene was located immediately upstream of the repressor gene (*jadR*$_2$) and resembles sequences for two component regulator systems.[15] The mutants generated by disruption or deletion of *jadR*$_1$ did not produce jadomycin B, implying that the gene functions as a positive regulator in the antibiotic biosynthesis. When a disrupted chromosomal copy of *jadR*$_1$ was complemented with a cloned gene, recovery of the wild-type phenotype was observed. In addition, a negative effect on the growth of *S. venezuelae* occurred upon increasing the copy number of *jadR*$_1$ in the plasmid. This investigation by Vining led to the conclusion that both *jadR*$_1$ and *jadR*$_2$ form an interacting stress-responsive regulatory system for the biosynthesis of jadomycin B.[14]

2.3. Sequencing Region Downstream of PKS-II

In 1996, Vining and co-workers cloned and sequenced DNA from a region downstream of an overlapping PKS gene cluster in *S. venezuelae* (ISP5230), which is responsible for jadomycin B biosynthesis.[6a] Their analysis of the nucleotide sequence located one complete ORF (ORF6) and two incomplete ORFs (ORF4 and ORF7). The amino acid sequences for ORF6 and ORF7 resemble those of oxygenases. Transformation of *S. venezuelae* with an ORF6 disruption vector[16] gave a mutant that blocked jadomycin B biosynthesis. Instead, the mutant accumulated a non-nitrogenous secondary metabolite (rabelomycin, **4**), which is also produced by *Streptomyces olivaceus* (Scheme 1).[17] This suggested that rabelomycin (**4**) is an intermediate[18] in the biosynthesis of jadomycin, where ORF4, ORF6, and ORF7 encode for oxygenases that are involved in the oxidative cleavage of the B ring in the angucycline **4** to afford aldehyde **6** All that remains for the biosynthesis of jadomycin B is A ring dehydration/aromatization, isoleucine incorporation, oxazoline ring formation, and glycosylation. However, the specific order of these sequences is still in doubt. Left of *jadR*$_2$, a cluster of three other regulatory genes (*jadW*$_1$, *jadW*$_2$, and *jadW*$_3$) were identified by Vining et al. (2003).[19]

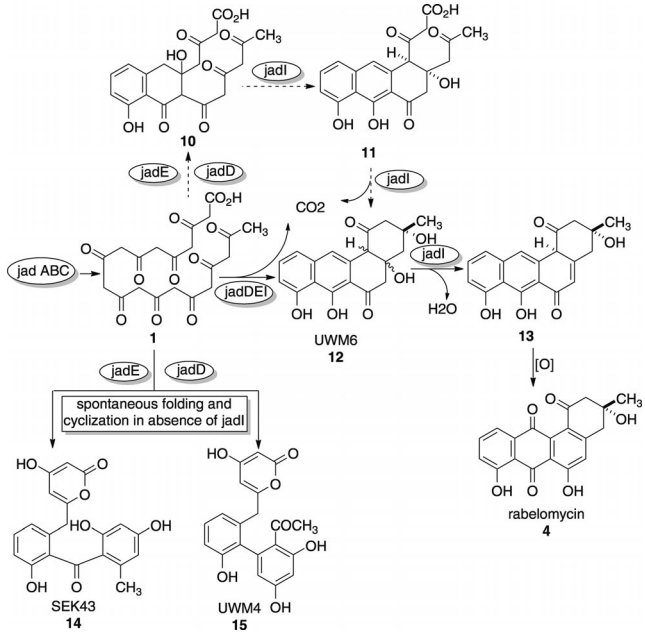


Scheme 1. First hypothesized jadomycin biosynthetic pathway.

Subsequently, another ORF (*jadJ*) was found between $jadR_1$ and *jadI*.[20] The gene *jadJ* encodes for proteins that are homologous to the biotin carboxylase (BC) and biotin carboxyl carrier protein (BCCP) components of acyl-coenzyme A carboxylase. The mutants produced by disruption of *jadJ* significantly reduced jadomycin production, without negative effects on the growth or morphology of the mutants. Vining et al. thus concluded that *jadJ* is not essential for fatty acid biosynthesis, but rather is responsible for supplying malonyl-coenzyme A for the synthesis of polyketide intermediates. Later, several more genes for the biosynthesis of jadomycin were identified by Vining.[21] Three complete ORFs (*jadM*, -*N*, and -*X*) and one incomplete ORF (*jadO*) were found. The last of these (*jadO*) relates to the biosynthesis of the sugar portion of jadomycin (Scheme 7). Although the role of *jadX* could not be identified, the roles of *jadM*, -*N* and, -*O* were assigned by homology to known proteins. The other two complete ORFs were assigned as a holo-ACP synthase (*jadM*) and an acyl-coenzyme A decarboxylase (*jadN*). The incomplete ORF (*jadO*) showed strong similarities to NDP-hexose-2,3-dihydratase, indicating its role in the synthesis of the L-digitoxose sugar unit in jadomycin B.

2.4. UWM6 – a New Intermediate in Jadomycin Biosynthesis

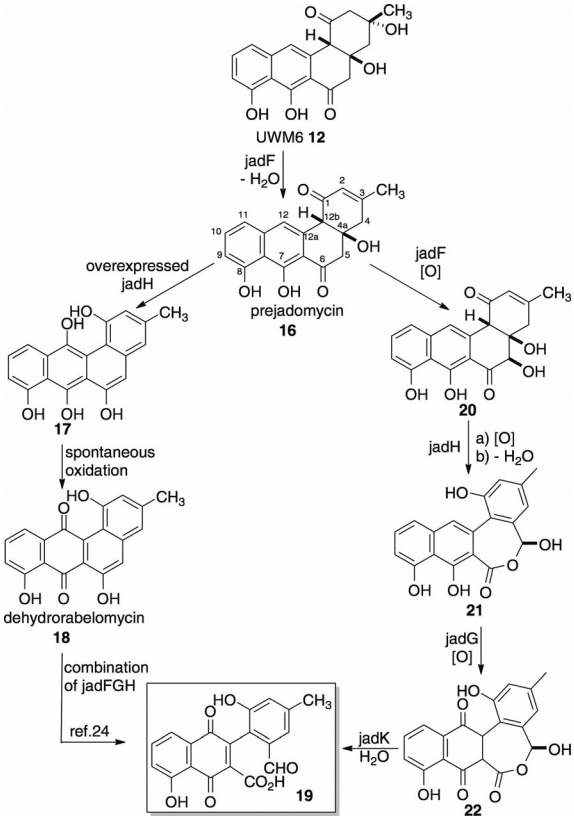
A new gene (*jadI*) upstream of *jadA* was characterized by Hutchinson et al.[22] *JadI* has homology to a related cyclase responsible for the biosynthesis of the ABCD rings of jadomycin in combination with the minimal PKS genes (*jadABC*), a cyclase (*jadD*), and a ketoreductase (*jadE*). When these genes are expressed in *Streptomyces lividans*, rabelomycin (**4**) is produced along with a new angucycline – UWM6 (**12**, Scheme 2). When this experiment was run with an incomplete *jadI* shunt, products without the C ring [SEK43 (**14**) and UWM4 (**15**)] were obtained), thus suggesting the role of *jadI* in ABCD ring construction and the potential of UWM6 (**12**) and/or rabelomycin (**4**) as intermediates in the biosynthesis of jadomycin B.[23]



Scheme 2. Polyketides produced from *jad* PKS gene cassettes in *S. lividans*.

2.5. Proposed Ring Opening Mechanism of UWM6

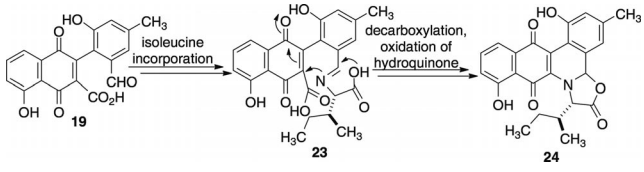
Initial biosynthetic studies had suggested that rabelomycin is the intermediate in jadomycin biosynthesis and that *jadF* catalyzes the oxygenation of rabelomycin leading to B ring opening.[6a] However, subsequent work discovered other intermediates in the pathway, including UWM6 (**12**) and prejadomycin (**16**, Scheme 3), which suggested that rabelomycin is not an actual intermediate in the pathway of jadomycin B biosynthesis.[24,25] The most recent studies by Yang et al.[26] suggests that *jadF* is a bifunctional enzyme that catalyzes the dehydration of UWM6 (**12**) to prejadomycin (**16**) and oxygenation at C-12 to form **17**, which spontaneously air oxidizes to form dehydrorabelomycin (**18**). Although the exact identity of the enzyme responsible for the B ring opening of dehydrorabelomycin (**18**) is still unknown, it is assumed that some combination of *jadF*, *jadH*, and *jadG* is responsible for this reaction (**18** to **19**).[24] Rohr et al. have proposed a mechanism for the C–C bond cleavage in the B ring by a Baeyer–Villiger-type mechanism (**20** to **21**). Subsequent hydrolysis of lactone intermediate **22** by *jadK* would give aldehyde **19**. However, at that time, the C-12 oxygenation was incorrectly assigned as performed by *jadG* (Scheme 3).[25]



Scheme 3. Proposed ring opening of UWM6.

3. Amino Acid Incorporation in Jadomycin Biosynthesis

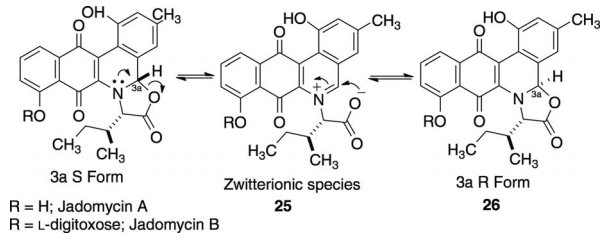
Regardless of the pathway of its formation, aldehyde **19** is believed to be the intermediate that incorporates the amino acids. Initial amino acid condensation is believed to be followed by a series of decarboxylation and ring-closing sequences to form the pentacyclic skeleton of jadomycin. Early on, Vining et al.[6a] suggested that isoleucine incorporation begins with an intermolecular 1,4-addition across the quinone enone (**6** to **7**), which is followed by a decarboxylation (**7** to **8**). A subsequent intramolecular addition to the aldehyde and lactonization should give the pentacyclic core (i.e., **8** to **9**, Scheme 1). In view of the presence of excess amino acid nucleophile (e.g., isoleucine) and the relative reactivity of the functional groups, Rohr et al. proposed an alternative mechanism. Their mechanism begins with the amine of isoleucine condensing with the aldehyde to form intermediate imine **23** (Scheme 4). A subsequent cascade



Scheme 4. Proposed non-enzymatic incorporation of isoleucine.

cyclization (carboxylate addition to the imine and amine addition to the quinone)[27] and decarboxylation leads to the pentacyclic core of jadomycin.

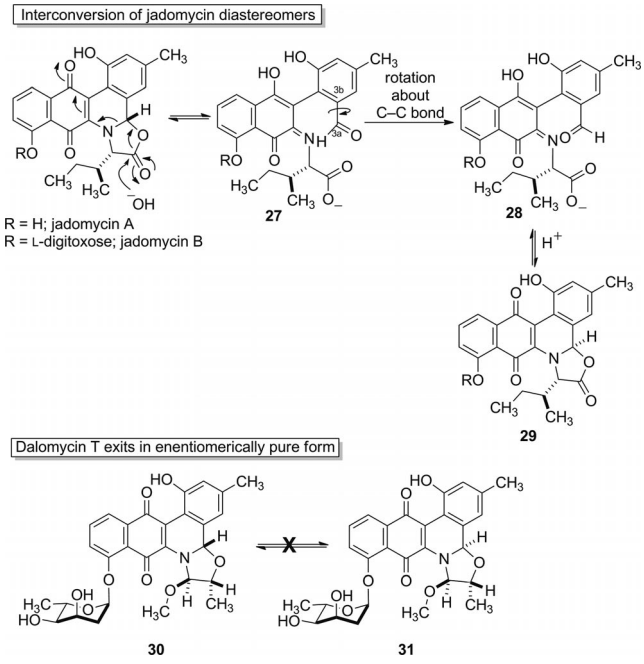
It is still an open question as to whether the amino acid incorporation proceeds through an enzymatic or a spontaneous process. To date, no enzyme responsible for the process has been isolated in the jadomycin gene cluster. Rohr et al. suggested the possibility of a non-enzymatic incorporation of amino acid based on the fact that jadomycin occurs as an inseparable mixture of diastereomers.[27] They produced a range of jadomycin B analogues with different amino acids present in the culture medium. A careful NMR analysis of the jadomycin analogues and molecular modeling studies suggested that the jadomycin is a mixture of diastereomers at the aminal position. They proposed that the mixture of diastereomers equilibrate via an undetected zwitterionic intermediate (Scheme 5). A recent total synthesis of jadomycin A and a carbasugar analogue of jadomycin B by O'Doherty et al. (see synthetic approach to jadomycin) supports the proposal that amino acid incorporation is indeed a non-enzymatic process.[28]



Scheme 5. Proposed mechanism of diastereomeric interconversion.

3.1. Alternative Mechanism of Ring Opening in Jadomycin B

Jakeman et al. proposed an alternative mechanism for the interconversion of the jadomycin diastereomers.[29] Their proposal was based on the isolation of an aldehyde intermediate (pH ≈ 11) as a mixture of conformational isomers – **27** and **28** (Scheme 6) – about the aldehyde C–C bond. They suggested that these two conformational isomers can selectively cyclize into the two corresponding diastereomeric forms of jadomycin B upon acidification (pH ≈ 4). Interestingly, dalomycin T (**30**, with an oxazolidine ring and the same angucycline framework as jadomycin B) was not responsive to the same acid-base treatment. This led to the conclusion that the carbonyl group of the oxazolone ring is essential for ring opening. They suggest that the aldehyde intermediate **27** (Scheme 6) is formed by nucleophilic attack of hydroxide ion at the carbonyl carbon of the oxazolone ring, with subsequent opening of the oxazolone giving aldehydes **27**/**28**. Not surprisingly, dalomycin T (**30**), which lacks the required carbonyl group, does not undergo a similar isomerization and exists as a single diastereomer.

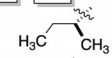
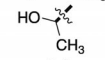
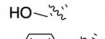
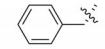
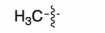
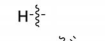
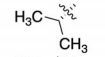
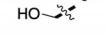
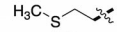
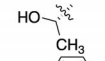
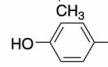
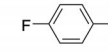
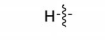
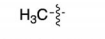
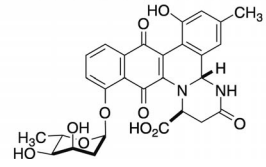


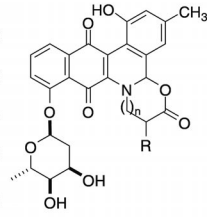
Scheme 6. Role of the carbonyl group in interconversation of the stereocenter at 3a.

3.2. Incorporation of Non-natural/Non-proteogenic Amino Acids

Jadomycin analogues can be obtained by use of different amino acids in the growth medium. Doull et al. were the first to observe this phenomenon.[4a] Differently colored pigments were observed in the culture medium when L-isoleucine was replaced with other nitrogen sources, but no effort was made to isolate or characterize these compounds. Rohr and Jakeman were the first to characterize and report novel jadomycin analogues by the fermentation of *Streptomyces venezuelae* in the presence of excess amino acids.[4d,27,30] The new jadomycin analogues that were produced in the presence of different amino acids are listed in Table 1. Jadomycin N, with a unique six-membered ring containing two nitrogen atoms, was obtained when L-asparagine was used as a nitrogen source. It is probably formed by the cyclization of the primary amine side chain of L-asparagine rather than ring closure through a carboxylic acid.[30b] Jakeman et al. have reported spectroscopic evidence suggesting that jadomycin analogues can also be obtained from the non-natural D-amino acids and non-proteogenic/synthetic amino acids. The size of the oxazolone ring in jadomycin analogues can also be expanded by use of β-amino acids: thus, if either β-alanine or racemic 3-aminoisobutyric acid are used as sole nitrogen sources in *Streptomyces venezuelae* culture, derivatives containing six-membered oxazinanone rings are produced.[30a,30c,30d] These efforts led to the first systematic study of structure–activity relationships associated with jadomycins. Substitution of, for instance, the alkyl side chain on the oxazolone ring of jadomycin B resulted in changes to the bioactivity (antibacterial and DNA cleaving properties), whereas the two analogues with no alkyl side chain on the oxazolone ring were significantly less active.[2,30b,30d]

Table 1. Jadomycin analogues produced by use of different amino acids.

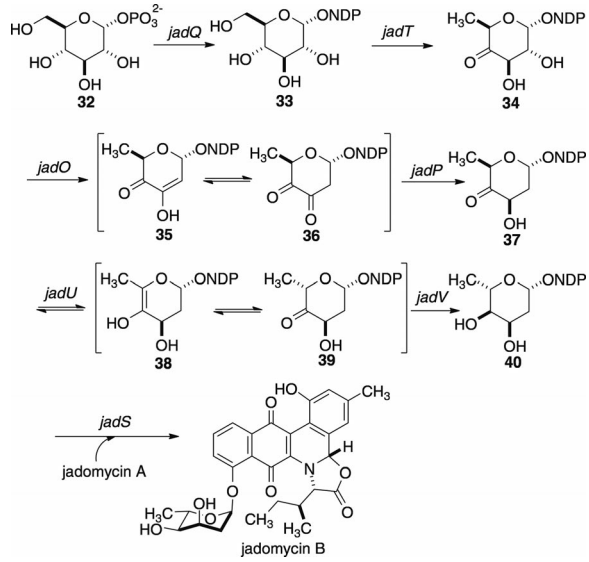
Amino acids used	Analogues produced	R =	n =
L-isoleucine	jadomycin B		0
D-threonine	jadomycin DT		0
L-serine	jadomycin S		0
L-phenylalanine	jadomycin F		0
Alanine	jadomycin Ala		0
Glycine	jadomycin G		0
L-leucine	jadomycin L		0
D-serine	jadomycin DS		0
D-methionine	jadomycin DM		0
L-threonine	jadomycin T		0
L-tyrosine	jadomycin T		0
DL-4-fluoro-phenylalanine			0
⇓-alanine			1
DL-aminoiso-butyric acid			1
L-asparagine	jadomycin N		



4. Biosynthesis of the L-Digitoxose Sugar Unit

The biosynthesis of the sugar portion of jadomycin has also attracted significant attention. In the jadomycin gene cluster, immediately downstream of *jadN*, eight structural genes involved in the biosynthesis of the carbohydrate portion of jadomycin B (*jadX*, *-O*, *-P*, *-Q*, *-S*, *-T*, *-U* and *-V*) are located. The specific functions of the corresponding proteins from seven of these genes (*jadO*, *-P*, *-Q*, *-S*, *-T*, *-U*, and *-V*) were assigned by sequence analysis and comparisons to the related genes involved in deoxy sugar biosynthesis (2,6-dideoxy sugar L-digitoxose). To carry out sequence analysis, the genes were cloned and propagated in *Escherichia coli*.[31] Analysis of the intermediates produced by the mutants and the sequence similarities to genes described in other species producing deoxysugar derivatives confirmed that these seven genes (*JadO*, *-P*, *-Q*, *-S*, *-T*, *-U*, and *-V*) were responsible for the dideoxy sugar biosynthesis and attachment to the aglycon (jadomycin A). In contrast to the other seven, *jadX* is not essential for jadomycin B biosynthesis, although the presence of *jadX* improves the yield for the production of jadomycin B. Vining et al. proposed a

biosynthetic pathway for the production of the L-digitoxose sugar unit from glucose-1-phosphate (**32**, Scheme 7) based on these results.[3]

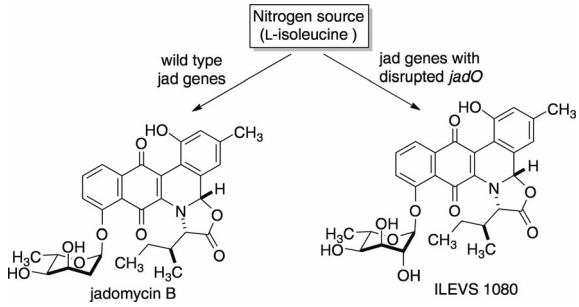


Scheme 7. Proposed biosynthesis of L-digitoxose sugar unit.

jadQ encodes glucose-1-phosphate nucleotidyltransferase, which activates glucose to afford the nucleotide diphosphate (NDP) derivative **33**. A 4,6-dehydratase encoded by *jadT* converts this NDP-glucose **33** into NDP-4-keto-6-deoxy-D-glucose **34**. An NDP-hexose 2,3-dehydratase encoded by *jadO* converts **34** into a keto-enol intermediate **35**, which exists in equilibrium with the diketone intermediate **36**. This diketone intermediate is transformed into NDP-2,6-dideoxy-D-*threo*-4-hexulose **37** by an oxidoreductase encoded by *jadP*. Isomerization of this D-*threo* hexulose **37** to the L-*erythro* form **39** occurs through the *jadU* product (NDP-4-keto-2,6-dideoxy-5-epimerase) via intermediate **38**. Finally, the ketone group of NDP-4-keto hexulose **39** is reduced by NDP-4-keto-2,6-dideoxyhexose 4-ketoreductase, encoded by *jadV*, to give the L-digitoxose moiety **40** in jadomycin B biosynthesis. It is unclear whether glycosylation occurs on jadomycin A or other intermediates prior to the formation of the oxazolone ring by incorporation of amino acid. Comparison of the amino acid sequence encoded by *jadS* shows a resemblance to a deoxy-glycosyltransferase, so it was proposed that *jadS* is involved in installing the L-digitoxose sugar unit onto the jadomycin B.

4.1. Substrate Flexibility of 2,6-Dideoxyglycosyl Transferase

The function of *jadO* as a nucleoside diphosphate hexose (NDP-hexose) 2,6-dehydratase was confirmed by Jakeman et al.[32] The jadomycin analogue ILEVS. 1080 was reported after a careful analysis of the natural products formed with use of a previously developed *S. venezuelae* strain (VS. 1080).[3] NMR and MS/MS analysis showed that the sugar unit in ILEVS. 1080 differs from the L-digitoxose in the case of jadomycin B. In the new sugar the axial hydrogen at the C-2 position is replaced with an axial hydroxy group (Scheme 8). The disrupted *jadO* could not dehydrate at the C-2 position of NDP-4-keto-6-deoxy-D-glucose **34** (Scheme 7), so the biosynthetic mechanism produced the sugar unit with the C-2-hydroxy functionality. This unnatural 6-deoxy sugar is then transferred to phenanthroviridin by *jadS* (a 2,6-dideoxyhexosyltransferase) to produce ILEVS. 1080 (Scheme 8). It is worth noting that this is the first report of a 2,6-dideoxysugar-*O*-glycosyltransferase with substrate flexibility at the C-2 position. The yield of ILEVS. 1080 production (2.6 mg/3 L)[32] is significantly lower than jadomycin B (12 mg L^{-1}).[27] The low yield of ILEVS. 1080 is likely the result of alteration in the kinetic parameters of the enzymes downstream of *jadO* as they process a non-natural substrate.



Scheme 8. Effect of *jadO* on the sugar unit.

5. Biosynthetic Improvemments for Jadomycin B Production

Effects of heat shock, ethanol treatment, and phase infection on the production of jadomycin B by *Streptomyces venezuelae* have been studied extensively by the groups of Doull,[4a] Rohr,[27] and Jakeman.[33] Initial studies on *S. venezuelae* ISP5230 in galactose/isoleucine medium and corresponding media with different amino acids replacing isoleucine led to isolation and characterization of jadomycin B analogues with modified oxazolone ring substituents.[2,3,4a] Rohr's fermentation procedure differs from that of Doull et al. only in the starting culture volume and the volume and time of ethanol shock.[4a,27] A more robust and efficient set of culture conditions was developed by Vining and Jakeman, in which glucose was used as the carbon source instead of galactose and MOPS was employed as the buffer along with low concentrations of phosphate and immediate ethanol shock. This altered media can effect jadomycin B/analogue production in a time-efficient and cost-effective manner.[32]

Yang et al.[9] reported a genetically modified strain of *Streptomyces venezuelae* that exhibits a twofold increase in the production of jadomycin B relative to the wild type. Four regulatory genes (3′ end 272 bp of *jadW2, jadW3, jadR2*, and *jadR1*) and the negative promoter upstream of *jadJ* (P_J) were replaced with promoter sequence ermEP*. The promoter sequence ermEP* can effect the expression

of jadomycin biosynthetic genes from *jadJ* and is able, in contrast with the wild type, to produce jadomycin without ethanol shock.

6. Synthetic Approaches

6.1. Benzo[*b*]phenanthridine Synthesis

Benzo[*b*]phenanthridine-containing natural products (Figure 3) are structurally unique angucyclines isolated from different species of *Streptomyces*.[34] Despite many synthetic efforts, there has only been a limited number of successful total syntheses of angucycline antibiotics of this type. This lack of successful syntheses can be attributed both to the structural complexity and to the instability associated with these structures, particularly with the glycosidic bond. In contrast, there have been several successful approaches to the phenanthridine core.

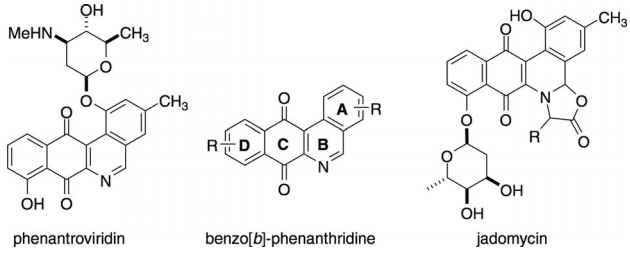
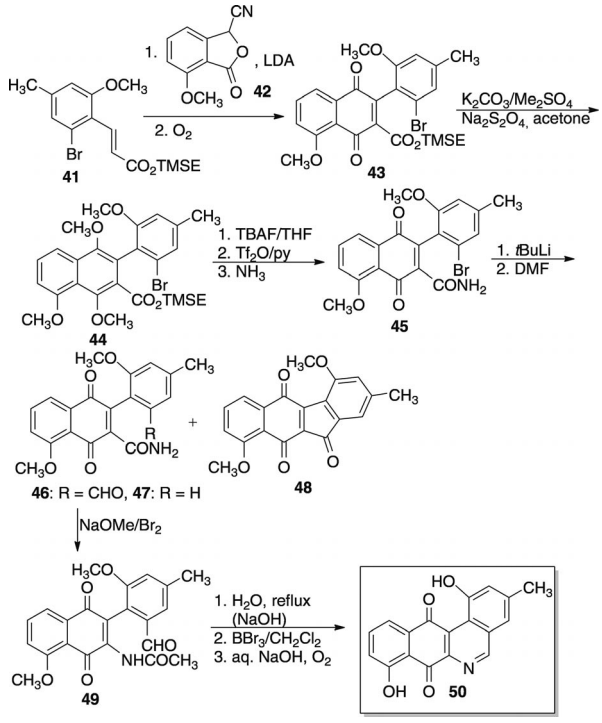


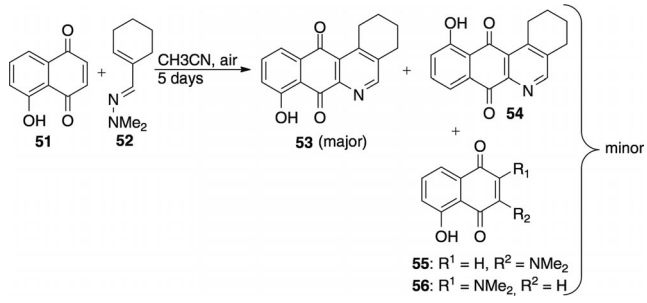
Figure 3. The phenanthridine core in natural products.

Gould[35] reported the first synthesis of the naturally occurring benzo[*b*]phenanthridine aglycon in 1991 (Scheme 9). The A, C, and D rings were constructed by coupling of cyanophthalide **42** with substituted bromocinnamate **41**, followed by air oxidation to give quinone **43**. This was then converted into amide **45** by reduction/methylation of quinone **43**, followed by removal of TMSE. The C-5 carbon was installed by formylation to give the desired product **46** along with dehalogenated product **47** and fluorenone **48**. Finally the B ring was installed in phenanthridine **50** by a Hoffmann reaction followed by hydrolysis/imine formation.



Scheme 9. Synthesis of the phenanthridine ring system.

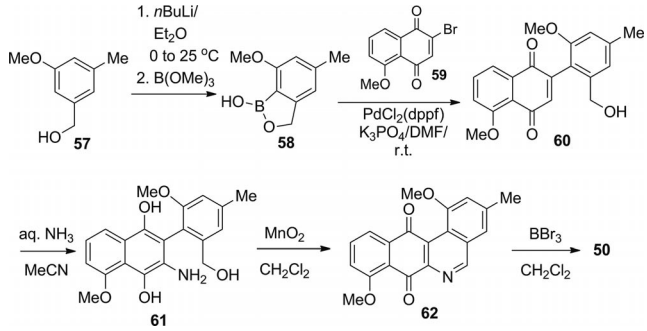
In 1997, Valderrama et al. reported a synthesis of the benzo[*b*]phenanthridine framework by employing a hetero Diels–Alder reaction (Scheme 10).[36] The angular tetracyclic system was synthesized by an A plus CD to ABCD strategy. The B ring was constructed by a regioselective [4+2] cycloaddition of 5-hydroxy-1,4-naphthoquinone (**51**) and *N*,*N*-dimethylhydrazone **52** followed by spontaneous air oxidation/aromatization. The high regioselectivity of the cycloaddition can be attributed to polarization of the azadiene **52** and the directing effect of the C5 hydroxy group on juglone (**51**). The regioselectivity of cycloaddition of juglone and azadienes was previously established by Potts[37] and Fillion.[38] Although a higher degree of regioselectivity was achieved, the reaction was slow at room temperature and was associated with the formation of minor byproducts (e.g., regioisomer **54** and addition/oxidation products **55** and **56**).



Scheme 10. DA approach to the phenanthridine ring.

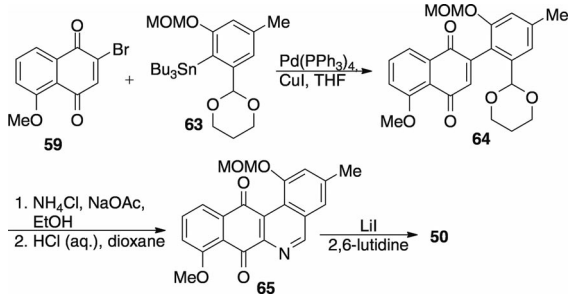
The most common method for constructing the phenanthridine B ring has been through the condensation of 3-aminoquinones with aryl aldehydes at the quinone 3-position.[39,40,41] In 1997, Snieckus reported the synthesis of phenanthrovirdin aglycon **50**. The ACD rings were constructed by Suzuki–Miyaura coupling of bromojuglone **59** (Scheme 11) and oxaborole **58**. The 3-amino functionality was introduced through a 1,4-addition to the quinone **60**. The B ring was then constructed by condensation of the amine and the aldehyde after MnO_2 oxidation of allylic alcohol **61**. Subsequent removal of methyl protection gave the desired phenanthrovirdine aglycon **50**.[39]

Echavarren et al. have also reported the synthesis of phenanthrovirdine aglycon **50** by the same cyclization procedure. They constructed the ACD ring system by Stille coupling of bromo juglone **59** with arylstannane **63** (Scheme 12). A 1,4-addition of ammonia onto the quinone,



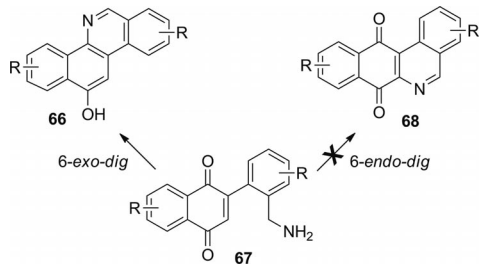
Scheme 11. Construction of B ring by condensation of amine and aldehyde.

followed by subsequent deprotection of the acetal and condensation, gave the B ring of the tetracycline **65**. MOM and methyl protecting groups were removed to provide **50**.[40]



Scheme 12. Construction of B ring by condensation of amine and aldehyde.

Construction of the B ring of phenanthrovirdine by amine condensation with the aldehyde has also been achieved by Ishikawa (see total synthesis of dimethylated jadomycin A).[41] Although the B ring has been successfully constructed by the intramolecular condensation of an amine to the C3 aryl aldehyde of juglone, 1,4-addition of the arylamine/imine at the C3 position of juglone does not give the desired B ring of benzo[*b*]phenanthridine **68** (Scheme 13). Instead, it undergoes a 6-*exo*-*dig* cyclization by condensing with C1 carbonyl of juglone **67** to form benzo[*c*]phenanthridine **66**.[40]

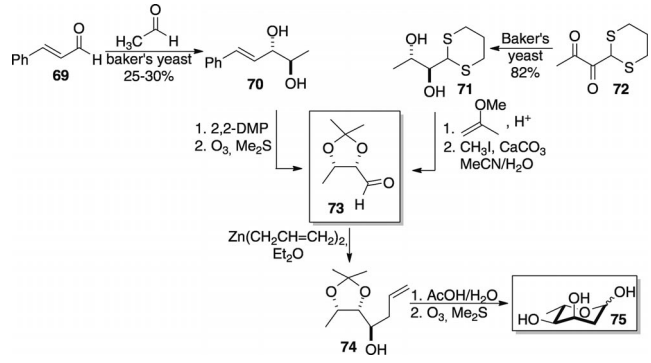


Scheme 13. Regioselectivity of B ring formation.

A solution to this problem came in 2010 from the synthesis of jadomycin A by O'Doherty. They showed that the B ring in the benzo[*b*]phenanthridine skeleton could be constructed regioselectively by changing the mechanism to circumvent the issues associated with a 6-*endo*-dig cyclization. Specifically, they decided to use a 6π-electrocyclic ring closure instead of 1,4-addition of an amine to the quinone (see total synthesis of jadomycin A and carbasugar analogue of jadomycin B).[28]

6.2. Synthesis of the L-Digitoxose Sugar Unit

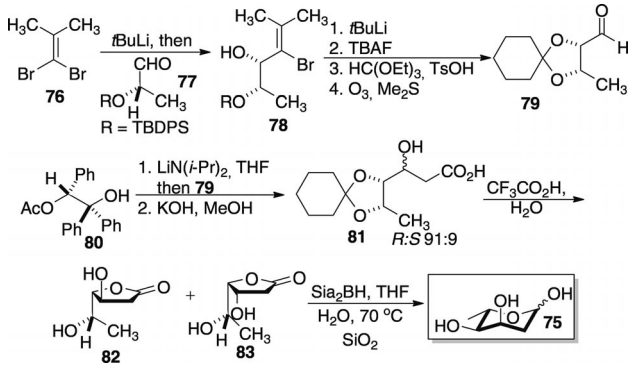
In contrast with the syntheses of the aglycon, there have been many approaches to the synthesis of the L-digitoxose sugar. In 1982, Fronza et al. reported a stereospecific synthesis of L-digitoxose by addition of diallylzinc onto chiral aldehyde **73** (Scheme 14). The stereochemistry of addition is controlled by steric interaction of the chiral α,β-dialkoxy aldehyde. Deprotection and ozonolysis gave L-digitoxose (**75**).[42] Three years later, Fujisawa et al. discovered a highly stereospecific and scalable synthesis of the key intermediate (glyceraldehyde derivative **73**) by diastereoselective reduction of α,β-diketodithiane with baker's yeast and subsequent protection of the diol.[43] Other related approaches to the sugar have been achieved by isoxazoline anion chemistry.[44,45]



Scheme 14. Synthesis of L-digitoxose employing baker's yeast reduction.

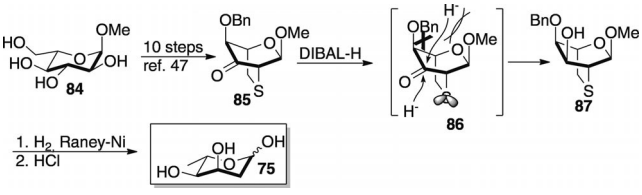
Braun et al. (1991) synthesized L-digitoxose from *O*-silylated lactal **77** (Scheme 15). Lactal **77** can be obtained from optically pure and commercially available (*S*)-lactate. The sugar C3 and C4 stereocenters were installed by the sequential diastereoselective addition of anions (**78** to **79** and **79** to **81**). Finally, L-digitoxose (**75**) was obtained by reducing a mixture of lactones **82** and **83** with disiamylborane followed by hydrolysis and silica gel chromatography (Scheme 15).[46]

In the same year, Tatsuta et al. reported the synthesis of L-digitoxose (**75**) from glucose sugar **84** (Scheme 16). Key to their approach was the use of a highly stereocontrolled hydride addition to a carbonyl group at the C-3 position of 2,6-anhydro-2-thiosugar **85**. The structure of the 2,6-anhydro-2-thiosugar and the configuration of the anomeric methoxy group direct the incoming nucleophile in a stereo-



Scheme 15. Synthesis of L-digitoxose from (*S*)-lactate.

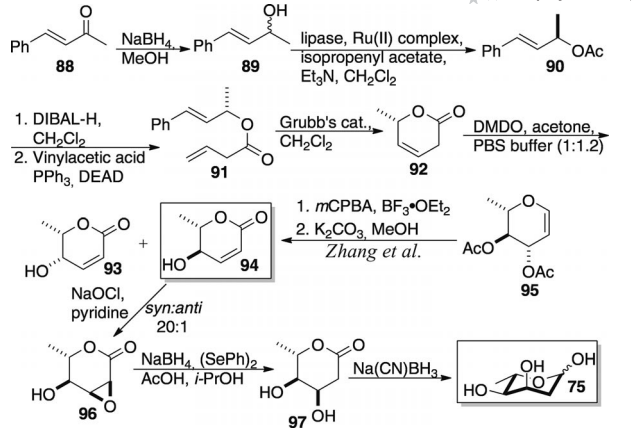
selective manner. The unfavorable 1,3-diaxial interaction overrides the lone-pair repulsion from the sulfur and so the hydride is delivered from the bottom face. The key intermediate **85** was prepared in 10 steps starting from methyl α-L-glucopyranoside (**84**).[47]



Scheme 16. Synthesis of L-digitoxose from L-glucose.

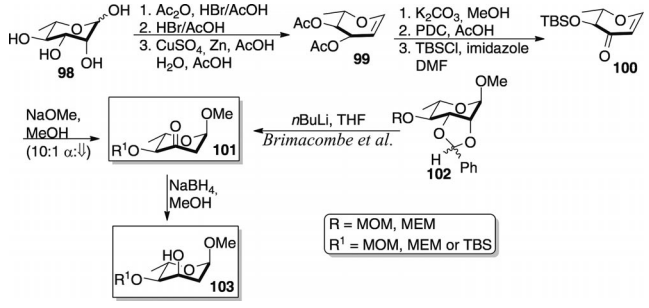
In 2005, Wang et al. reported a synthetic strategy for the optically pure L-digitoxose sugar unit by employing kinetic resolution followed by ring closing metathesis. The synthesis began with commercially available acyclic *trans*-4-phenylbut-3-en-2-one (**88**, Scheme 17). Reduction of the α,β-unsaturated carbonyl was followed by enzyme/metal-catalyzed dynamic kinetic resolution to obtain optically pure allylic alcohol **90**. Mitsunobu esterification of **90** followed by Grubbs' ring closing metathesis and subsequent epoxidation/epoxide opening gave the key intermediate **94**. This α,β-unsaturated lactone **94** (Osmundalactone) can be transformed into L-digotoxose (**75**) by a hydroxy-directed epoxidation/reductive epoxide opening strategy.[48] Zhang et al. also reported the synthesis of the L-digitoxose sugar unit from Osmundalactone by Wang's procedure in 2007. However lactone **94** was synthesized from commercially available peracetyl L-rhamnal (**95**) by $BF_3{\cdot}OEt_2$-induced epoxidation.[49]

In 2007, Jakeman reported synthesis of the L-digotoxose sugar unit based on a method developed by Brimacombe and co-workers (1982). Their approach utilized a Klemer–Rodemeyer[50] elimination with fully protected L-rhamnopyranoside **102** (Scheme 18).[51] The stereoselectivity was based on preferential removal of the quasi-axial hydrogen, which cleaves the benzylidene acetal protection to form 2-deoxy-3-ketosugar **101**. The ketone of **101** was stereoselectively reduced with $NaBH_4$ to give the desired L-digitoxose



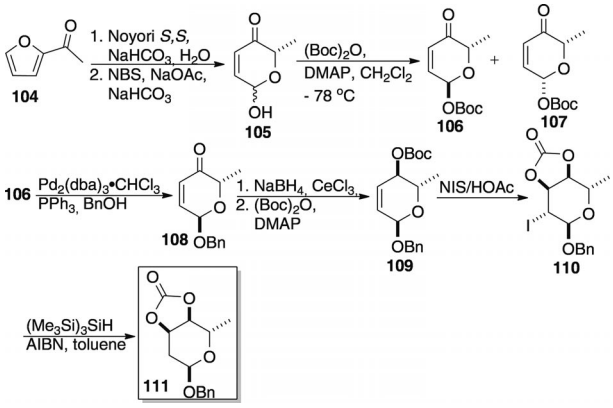
Scheme 17. Synthesis of L-digitoxose via Osmundalactone.

stereochemistry. The major drawbacks to this method were the formation of side products from the S_N2 reaction of *n*BuLi at the anomeric position and deprotonation at C-2 and the benzylidene ring. Jakeman and Timmons improved the yield of the Klemer–Rodemeyer elimination reaction by changing the base to *s*BuLi and switching to an isopropylidene protecting group.[52] They also reported a new method with improved yield to obtain the key intermediate, 2-deoxy-3-ketosugar **101**, from L-rhamnose (**98**).



Scheme 18. Synthesis of L-digitoxose from from L-rhamnose.

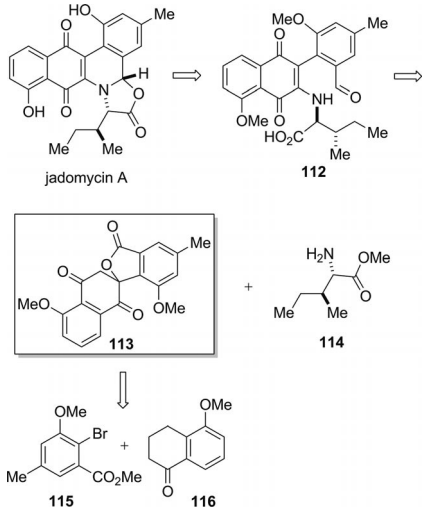
In contrast to traditional approaches to carbohydrates from carbohydrates with pre-existing stereocenters, the O'Doherty group has been interested in the synthesis of carbohydrates from achiral starting materials.[53] These de novo asymmetric approaches have been developed from both achiral furans[54] and dienoates.[55] In 2010, they reported a de novo synthesis of L-digitoxose from the achiral and inexpensive acyl furan **104** (Scheme 19). Asymmetric Noyori reduction and Achmatowicz rearrangement gave pyranoside **105**. The diastereomers **106** and **107** were separated after Boc protection. Stereoselective palladium glycosylation of **106**, followed by Luche reduction and NIS-mediated iodocarbonate formation, installed the digitoxose stereochemistry in **110**. Radical deiodination by use of tris(trimethylsilyl)silane (TTMSS) gave the desired L-digitoxose glycosyl donor **111**.[28]



Scheme 19. De novo synthesis of L-digitoxose sugar.

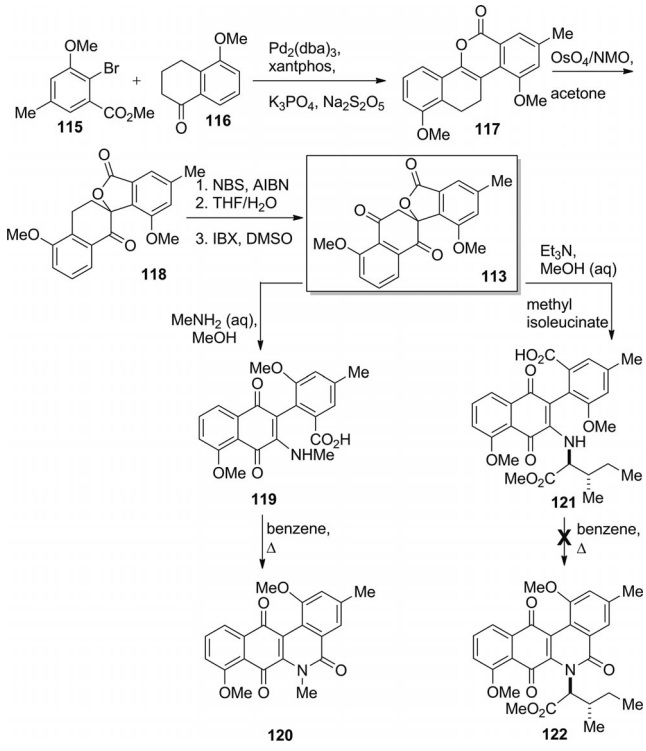
7. Total Synthesis of Jadomycin

To date, there have been only two reported synthetic efforts directed towards the total synthesis of jadomycins. In 2010, Ishikawa et al.[41] reported the synthesis of dimethyl-protected jadomycin A (Scheme 20). Their route used α-spirolactonedihydroquinone **113** as the key intermediate for construction of the jadomycin ring system. Retrosynthetically, they envisioned the construction of the jadomycin skeleton by imine cyclization followed by oxazolone formation of amino-aldehyde **112**. This cyclization precursor was obtained from the spirolactone intermediate **113**, which was in turn prepared from bromobenzene derivative **115** and tetralone **116**.



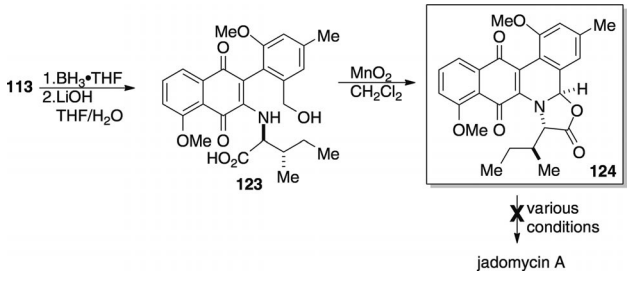
Scheme 20. Retrosynthetic analysis of jadomycin A.

Palladium-catalyzed coupling of *O*-bromobenzoate **115** (Scheme 21) and 5-methoxytetralone **116** gave enol-lactone **117**. Subsequent dihydroxylation and rearrangement produced the spiro[lactone-tetralone]-type compound **118**. Benzylic bromination and S_N2 displacement was followed by allylic oxidation to give the masked naphthoquinone **113**. As a model study to form the phenanthroviridin skeleton, **113** was treated with methylamine to form 2-aminoquinone **119**, which upon thermolysis generated **120**. Unfortunately, when methyl isoleucinate was used as a nitrogen source instead of methylamine under identical conditions, the desired dehydrative cyclization did not proceed. The most likely reason for this is unfavorable steric interaction between the carboxy and the amino acid fragments in **121**. Because of the free rotation around the C–C bond, **121** adopts an energetically more stable conformation in which the carboxy and the amino functional groups are not in close proximity to undergo dehydration/cyclization (Scheme 21).



Scheme 21. Synthesis of key intermediate spiro[lactone-tetralone]-type compound **113**.

Ishikawa next decided to investigate the use of Snieckus's oxidative cyclization procedure[39] involving the intramolecular condensation of an amine and aldehyde to construct the B ring. The carboxylic acid group on the masked naphthoquinone **113** was reduced with BH_3·THF complex and the isoleucine methyl ester was hydrolyzed with lithium hydroxide to give **123** (Scheme 22). Allylic oxidation of **123** to an aldehyde with MnO_2 promoted condensation of the secondary amine with the aldehyde to form an iminium sys-



Scheme 22. Synthesis of dimethylated jadomycin A.

tem. This iminium intermediate was then trapped by carboxylate to form the pentacyclic ring system in jadomycin (**124**). Unfortunately, all attempts to remove the methyl protecting groups were unsuccessful.

That same year, O'Doherty et al. reported a successful synthesis of jadomycin A and the carbasugar analogue of jadomycin B.[28] The key transformation utilized to construct the B ring in jadomycin was a 6π-electrocyclic ring closure. Retrosynthetically it was envisioned that the B ring in the phenanthridine core could be obtained by a 6π-electrocyclic ring closure and that the oxazolone ring could be constructed through an acid-promoted cyclization reaction (Figure 4). The connection between C-7a and C-7b could be formed by Stille coupling between arylstannane **127** (Scheme 23) and bromojuglone **128**. The stannane and the bromojuglone should be obtainable from the commercially available starting materials **125** and 5-hydroxy-bromojuglone, respectively.

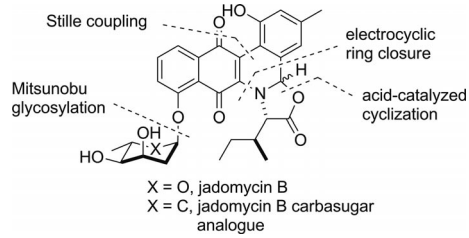
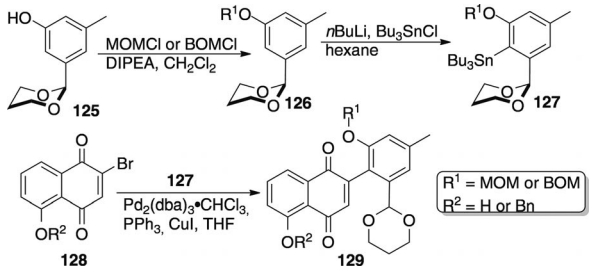


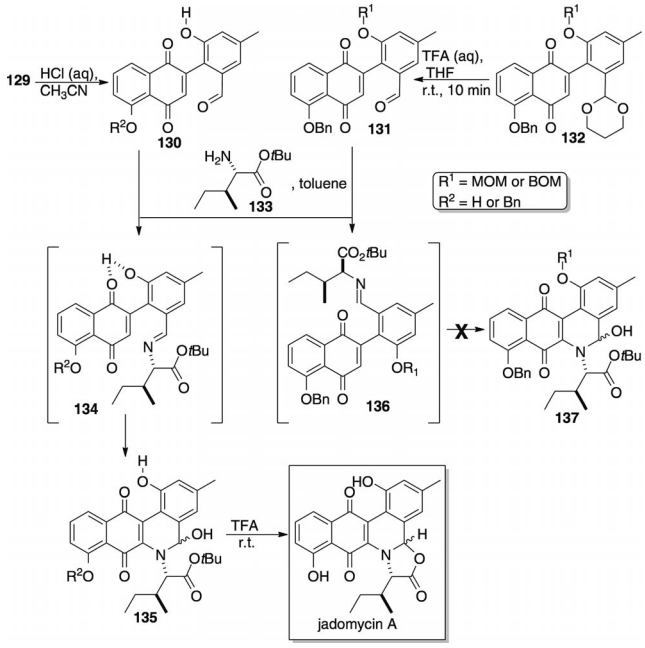
Figure 4. Retrosynthetic analysis of jadomycin.



Scheme 23. Stille coupling: synthesis of intermediates **129**.

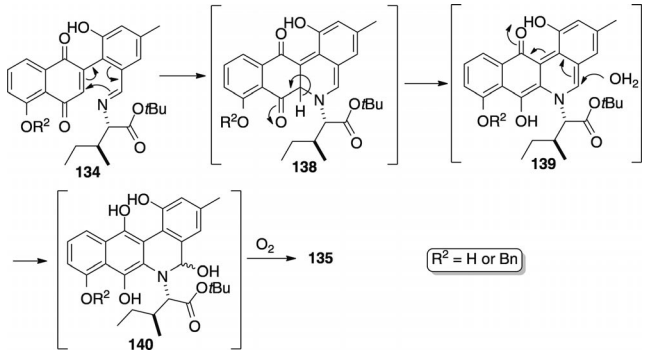
The stannane **127** (Scheme 23) was prepared by directed *ortho*-metalation of **126** with use of MOM or BOM ethers as *O*-directing groups. These metalation precursors were prepared from commercially available phenol **125**. The other coupling partner **128** was prepared by benzylation of commercially available bromojuglone. Stille coupling was carried out with stannane **127** and unprotected or protected bromojuglones **128** to give tricyclic adducts **129**.

The acetal protection on **129** was selectively removed with aqueous THF to generate aldehyde **131** (Scheme 24). This aldehyde was then converted into 6π-electrocyclic ring closure precursor **136** by condensation with *tert*-butyl isoleucinate (**133**). Unfortunately, **136** did not undergo the expected electrocyclic ring closure. This unexpected result was attributed to an unfavorable alignment of the π-electrons due to steric congestion from the nitrogen substituent, which could lead to an unfavorable alignment for the electrocyclic ring closure. Fortunately, when the MOM or BOM group was removed, the 6π-electrocyclic ring closure readily occurred to give jadomycin precursor **135**. Finally, acid-promoted formation of the oxazolone ring with simultaneous debenzylation yielded jadomycin A as a 6:1 diastereomeric mixture.



Scheme 24. Synthesis of jadomycin A.

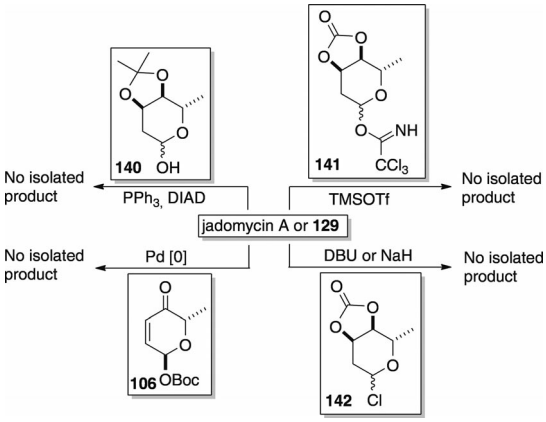
The authors assert that the removal of the offending protecting group benefited the cyclization beyond the removal of a negative steric interaction. They suggest that the presence of H-bonding between the C-7 hydroxy group and the C-8 carbonyl group of **134** preferentially aligned the cyclization precursor in the correct orientation, thus promoting electrocyclic ring closure to **138**. Once the B ring in **138** is formed, a subsequent 1,3-proton transfer forms intermediate **139**, which undergoes hydration under the reaction condition to form quinol **140**. Quinol **140** undergoes air oxidation to give **135** (Scheme 25).



Scheme 25. Plausible mechanism of ring closure.

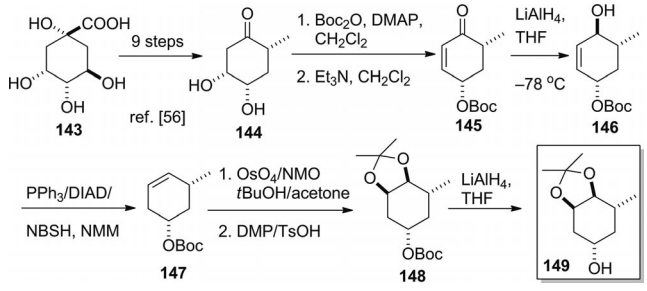
After the successful synthesis of jadomycin A, a total synthesis of jadomycin B was attempted by the direct installation (Schmidt glycosylation, S_N2 alkylation, Pd-catalyzed glycosylation, and Mitsunobu conditions) of a suitably pro-

tected sugar moiety (**106**, **140**, **141**, or **142**, Scheme 26). The required L-digitoxose sugar units were synthesized by the route given in Scheme 19. Despite numerous attempts with jadomycin A and **129**, it was never possible to isolate a glycosylation product. The failure to glycosylate the jadomycin aglycon successfully was attributed to the acid sensitivity of the glycosylated products, both to the reaction conditions and to silica gel chromatography. This acid sensitivity has also been seen in jadomycin B. In fact, only tangential signs (a distinctive wine-red color) of glycosylation between **129** and **140** could be seen under the Mitsunobu glycosylation conditions.



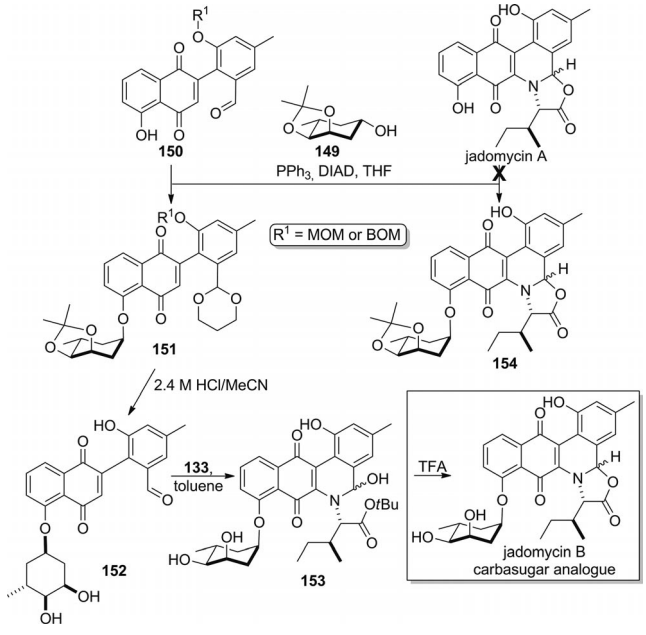
Scheme 26. Attempted glycosylations.

With the acid sensitivity precluding the total synthesis of jadomycin B, it was decided to investigate the synthesis of a less acid-labile cyclitol (5a-carbasugar) analogue of jadomycin B. Quinic acid (**143**, Scheme 27) was converted into dihydroxy ketone **144** by a previously reported method[56] in nine steps. Enone **145** was obtained by Boc protection of **144** followed by base-mediated elimination. This enone was selectively reduced to **146** by treatment with $LiAlH_4$ at low temperature. Myers rearrangement of the allylic alcohol gave alkene **147**, and stereoselective dihydroxylation and diol protection gave **148**. Removal of the Boc protection with $LiAlH_4$ gave the Mitsunobu glycosylation precursor **149**.



Scheme 27. Synthesis of cyclitol equivalent of L-digitoxose.

Mitsunobu glycosylation was first attempted with cyclitol donor **149** and jadomycin A as the acceptor (Scheme 28). Unfortunately, only a complex mixture was obtained, which was attributed to the sensitive oxazolone ring. Successful glycosylation occurred when cyclitol acceptor **150** was used, forming stable acetal **151**. Global deprotection with aqueous HCl removed all the protecting groups to afford **152**. This set the platform for introduction of amino acid and successive ring closure. Condensation of *tert*-butyl isoleucinate (**133**) with aldehyde **152** promoted 6π-electrocyclic ring closure forming the B ring **153**. Finally an acid-catalyzed lactonization was used to install the oxazolone ring and the carbasugar analogue of jadomycin B was synthesized as 2.5:1 diastereomeric mixture.



Scheme 28. Synthesis of carbasugar analogue of jadomycin B.

8. Conclusions

Jadomycin biosynthesis and synthetic studies have been reviewed in detail. These biosynthetic studies have led to a better understanding of the biosynthetic pathway, which has led to the discovery of new analogues and improved production of various jadomycins for further biological evaluation. Similarly, the synthetic contributions have shed light on the reactivity and stability of the jadomycin ring system and the associated natural products. In this regard, the access to the cyclitol analogue of jadomycin B allows access to more stable analogues for further structure–activity relationship (SAR) studies.

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