SYNTHESIS, BIOSYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF THE MACROLIDE ANTIBIOTIC LEINAMYCIN

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INTRODUCTION

In 1989, researchers at the Japanese pharmaceutical company Kyowa Hakko Kogyo isolated the potent macrolide antibiotic leinamycin, **1**, produced by *Streptomyces atroolivaceous* S-140.[1,2] Spectroscopic[1] and X-ray crystallographic analysis[3] of leinamycin revealed an 18-membered hybrid peptide-polyketide macrolactam spiro-fused to a 1,3-dioxo-1,2-dithiolane ring, a structural feature unique to this natural product. In addition to broad-spectrum antibiotic activity, the natural product exhibited significant antitumor activity in murine tumor models,[1] and nanomolar potency in several multidrug resistant (MDR) cancer cell lines.[4] Most importantly, the potency of this natural product is derived from its unique 1,3-dioxo-1,2-dithiolane ring, that exerts cytotoxicity through an unprecedented mechanism of DNA damage.[5] The challenging structure, potent biological properties, and novel mode of action of this antitumor agent make it a very attractive target from both a synthetic and medicinal standpoint. This report will discuss the efforts directed toward the synthesis of **1** and its derivatives, progress toward the understanding of its biosynthetic pathway, and the interesting structural features of **1** responsible for its biological mode of action.

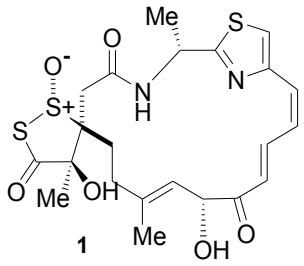
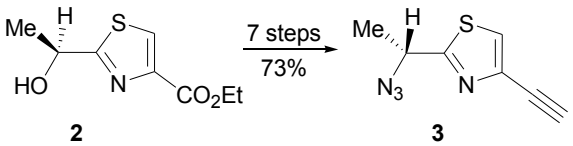


Figure 1. Leinamycin.

TOTAL SYNTHESIS OF LEINAMYCIN

Leinamycin contains several interesting structural features, including a conjugated (*Z,E*)-dienone, the chiral 1,3-dioxo-1,2-dithiolane ring and the spiro fusion of the thiolane heterocycle to an 18-membered ring. The first and only total synthesis of leinamycin was reported in 1993 by Fukuyama and Kanda.[6] Alternate routes to structural fragments of **1** have been reported. Pattenden has reported the construction of a model macrolactam using Stille vinyl-vinyl coupling as a key step.[7] Several routes to the dithiolanone ring have been published, involving ring formation by [2+2] cycloaddition or epoxidation followed by ring opening with sulfur and disulfide oxidation.[8]

Scheme 1. Synthesis of azidoacetylene 3.



step procedure by addition of *p*-TolSO_2CH_2Li, which produced methyl ketone after sulfone cleavage with Al-Hg amalgam (89%, two steps). α-Bromination, followed by thiol substitution**,** lead to spontaneous intramolecular sulfo-Michael addition to C-3. Repulsion between the methyl group of the keto-thiol side chain and C-5 directs thiol attack to the *Re* (top) face of the diene, generating the desired epimer **11** in a 10:1 ratio (80% yield of desired epimer), consistent with MM2-level transition-state modeling.[15] Oximination and activation with 2,6-dimethylbenzoyl chloride set the stage for a Beckmann fragmentation induced by excess KSEt, which generated thioester **13.** After trans-thioesterification with NaSH, oxidation to the 1,2-dithiolan-3-one core structure of leinamycin was accomplished with I_2. After deprotection, the dithiolanone ring was regio- and stereoselectively oxidized with *m*-chloroperbenzoic acid in 82% yield, completing the first total synthesis of (+)-leinamycin.

MODE OF ACTION

The interest in **1** has been driven by its potential application in novel anticancer therapies. In their seminal work, Hara and coworkers[16] concluded that the 1,3-dioxolan-3-one ring was essential for cytotoxicity, and that **1** bound to and caused single-strand breaks in plasmid DNA, inhibiting DNA synthesis in a previously unobserved thiol-dependent manner. Thus, it was postulated that **1** inhibited tumor cell growth through a similar mode of DNA binding and damage. In preliminary experiments, **1** led to DNA strand cleavage upon addition of thiols, but not other reducing agents, suggesting that nucleophilic attack converted **1** into its active form. Since **1** cleaved DNA in the presence of radical inhibitors, it was initially believed that **1** cleaves DNA by alkylation, leading to depurination/depyrimidation of the DNA, followed by strand cleavage.[16] This hypothesis was later confirmed when a covalently linked leinamycin- (*N*-7)-guanine adduct was isolated (**21**, Scheme 5).[17] Thiol activation of **1** and subsequent trapping with H_2O or MeOH resulted in **18** and **19**, implicating a common electrophilic intermediate for purination or hydrolysis/methanolysis.[17] The yield of **20** increased as [$HO(CH_2)_2SH$] (the activating thiol) increased in the reaction mixture, implying that $HO(CH_2)_2SS^-$ was generated during activation. During the course of conversion of **1** to **21**, a second product, epoxide **17**, was observed at early time points and gradually disappeared over the course of the reaction.

In 1996, Asai and coworkers proposed a mechanism for the activation of **1** (Scheme 5).[17] Episulfonium ion **16** was proposed to be the "activated" electrophilic intermediate necessary for DNA alkylation. Epoxide **17** was not the active species, as modifications to the C-8 hydroxyl (which prevent epoxide formation) *increase* potency.[18] Isolated epoxide **17** was, however, spontaneously converted into

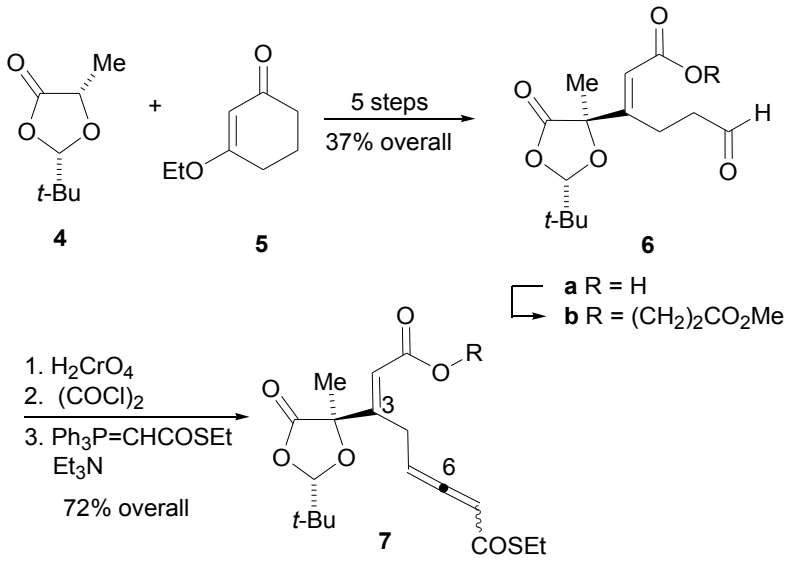
Synthesis of Acetylene Azide 3

Construction of key fragment **3** began from the known hydroxy thiazole **2**, derived from L-lactic acid (Scheme 1).[9] Conversion of **2** into acetylene azide **3** was accomplished in a seven-step sequence involving a Corey-Fuchs reaction and treatment with HN_3 under Mitsunobu conditions to introduce alkynyl and azido functionalities into **3** in 73% overall yield from **2**.

Vinyl Iodide 4

Construction of vinyl iodide **4**, began with alkylation of 3-ethoxy-2-cyclohexen-1-one with Seebach's optically pure dioxolanone **4**,[10] to afford a chiral enone as a single enantiomer (Scheme 2). α-Hydroxylation, followed by oxidative ring cleavage with periodic acid provided aldehyde **6b** after protection of the acid functionality as the 2-carboxymethyl ethyl ester in 37% yield over 5 steps. Conversion of **6b** to the acyl chloride followed by dehydrochlorination generated a ketene intermediate, which underwent Wittig olefination with Ph_3P=CHCOSEt to form a 1:1 diastereomeric mixture of allenic thioesters **7**.

Scheme 2. Synthesis of allenic thioester 7.

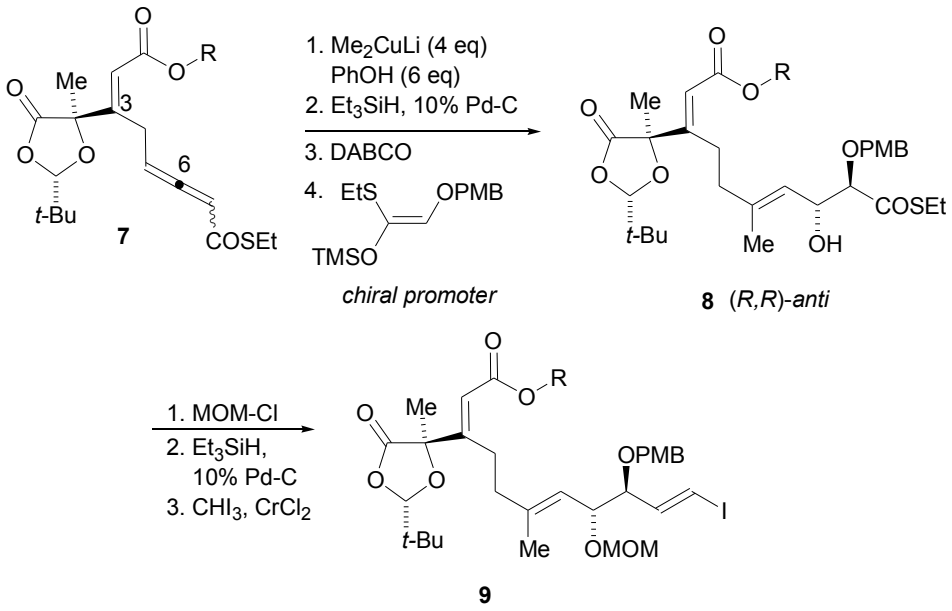


Conjugate addition of Me_2CuLi to **7** was accomplished by adding the cuprate to a solution of **7** and six equivalents of phenol, resulting in the formation of a thioester in 87% yield (Scheme 3). The thioester was efficiently reduced to the homoallylic aldehyde with Et_3SiH and Pd/C using the method developed in the Fukuyama group.[11] Isomerization to the conjugated (*E*)-enal was effected with DABCO at 23 °C. Stereochemistry of the C-8 hydroxyl group of **1** was set using a highly selective asymmetric aldol reaction developed by Mukaiyama and Kobayashi.[12] Use of a chiral promoter formed from the chelation of Sn $(OTf)_2$ by a chiral diamine ligand and association of an additional tin(IV) species provided the desired (*R,R)-anti* diastereomer of thioester **8** in 92% yield. Reduction to the aldehyde, followed by (*E*)-selective olefination[13] provided the (*E*)-vinyl iodide **9** in 66% yield.

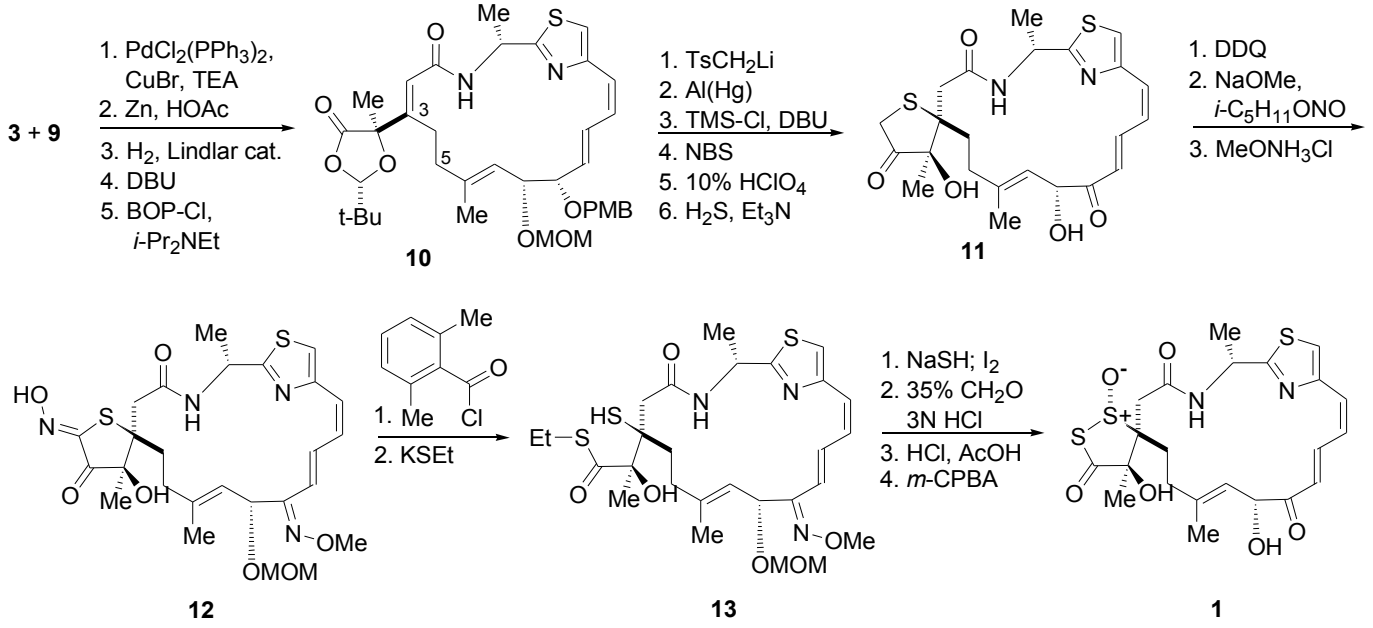
Macrocyclization and Dithiolanone Ring Construction

With fragments **3** and **9** in hand, Kanda and Fukuyama formed the 18-membered core of **1** in five steps (Scheme 4). A Sonogashira coupling reaction followed by hydrogenation with Lindlar's catalyst joined the two fragments (88%) and set the (*Z,E*)-diene geometry of the backbone of **1**. The amide coupling reagent BOP-Cl was used to cyclize the amino acid to the 18-membered lactam in 91% yield after deprotection of amino and acid functional groups. Since attempts to perform an intermolecular conjugate addition of sulfur to the C-3 position of the macrolactam ring failed due to steric congestion, an intramolecular approach was developed to install the critical 1,2-dithiolan-3-one ring.[14] Alkylation of the dioxolanone ring in **10** was accomplished in a two-

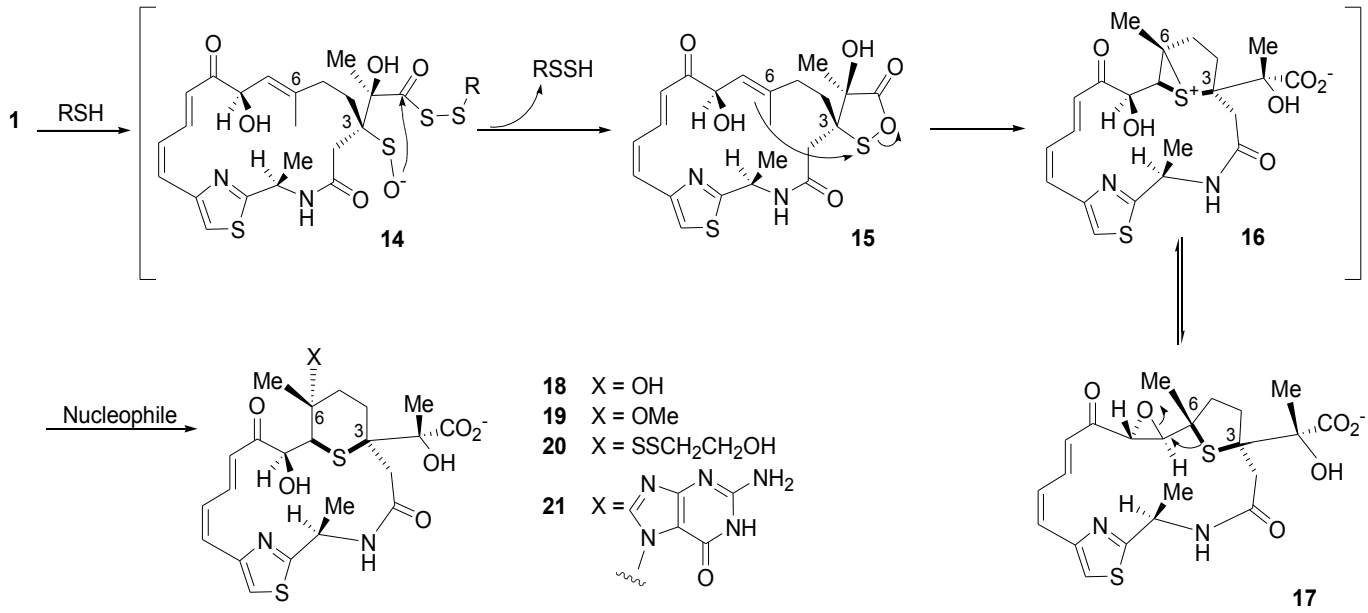
Scheme 3. Synthesis of vinyl iodide 4.



Scheme 4. Ring closing and dithiolanone ring construction.

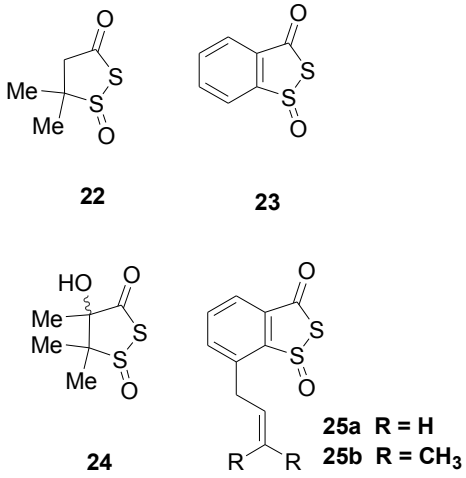


Scheme 5. Proposed mechanism of activation of 1.[17]



18 in the presence of water, implying reversibility between **17** and the reactive species. In contradiction to Hara and coworkers, the Gates research group concluded that 1,2-dithiolan-3-one 1-oxides[19] **22**-**25**, *and* **1**,[20] were capable of mediating oxidative DNA damage *in vitro*, as evidenced by partial inhibition of DNA cleavage **1** by radical scavengers, rigorous degassing cycles, or the metal chelator diethylenetriaminepentaacetic acid (DETAPAC) (Chart 1). Hydrodisulfides, byproducts of dithiolanone activation, were observed to form polydisulfides in the presence of molecular oxygen, a process which generates radical oxygen species.[21] The DNA-damaging ability of compounds **22**-**24**, which cannot be alkylated through an episulfonium ion intermediate and therefore should possess only oxidative DNA-damaging properties, is inhibited much more efficiently by radical inhibitors than the corresponding inhibition of **1**, which potentiates DNA damage through both oxidative and alkylative pathways.

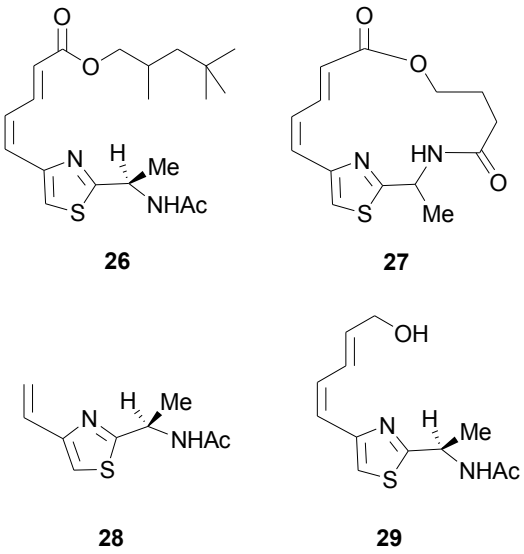
Chart 1. Dithiolanone small molecules.



Furthermore, the potential to form an episulfonium ion is not sufficient to alkylate DNA efficiently, as Chatterji showed with **25a** and **25b**,[22] implying that a noncovalent interaction recruits leinamycin to the DNA strand. Zang's observation that **1** does not bind to single-stranded DNA[23] implies that a structural feature of **1** binds to duplex DNA, presumably in the major groove, as the *N*-

7 of guanidine residues faces outward into the major groove of DNA and binding here would position the episulfonium ion close to the nucleophilic guanine *N*-7. Breydo's observation that the 5-(thiazol-4-yl)-penta-2,4-dienone backbone in **1** is required for intercalative binding to DNA, as determined through competitive intercalation of **1** and structural analogs into duplex DNA in the presence of ethidium bromide (Figure 2), indicates its role as an unconventional intercalating motif.[24]

Figure 2. DNA binding efficiencies for analogs of 1.



Compound	K_B (M^{-1})
1	1000
2	1500
25	1000
26	900
27	<100
28	<100

The DNA-cleaving ability of **1** was subsequently confirmed in living cells for the first time in 2004 by incubating **1** with MiaPaCa (human pancreatic cancer) cells.[25] Again, **1** showed potent cyctotoxicity (IC_{50} = 50 nM). More importantly MiaPaCa cells were effectively arrested in the DNA synthesis phase (S-Phase) of the cell cycle at a concentration of 100 nM, consistent with the DNA-damaging properties of **1**. If a cell's DNA is damaged when it attempts to synthesize another copy of its DNA in preparation for mitosis, checkpoint proteins responsible for verifying DNA fidelity will halt the cell in this phase, resulting in growth inhibition. In mammalian cells this condition will ultimately result in death of the malfunctioning cell through activation of the apoptotic death cascade.

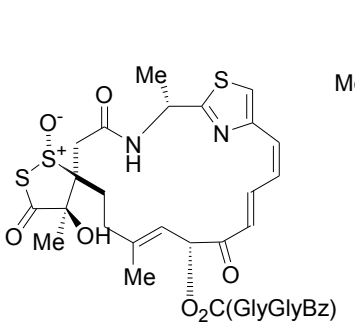
The high potency of **1** may be explained by its dual mode of action; oxidative inactivation of DNA repair enzymes such as the Ape1/Ref-1 complex may further sensitize cells to cytotoxic and genotoxic alkylative damage.[26] Furthermore, there is evidence to suggest that thiol-*independent* activation of 1 is possible, as treatment of **1** with hydroxide, cyanide or phosphines also activates **1** for DNA alkylation, albeit less efficiently than thiols.[27,28]

DERIVATIZATION OF 1

Given the observed potency of **1** in various cancer cell lines and mouse tumor models,[1,8] it should come as no surprise that derivatives of **1** are being evaluated as potential drug candidates. Of the more than 40 derivatives and analogs reported to date, several are even more potent than **1** (Figure 3).[29]

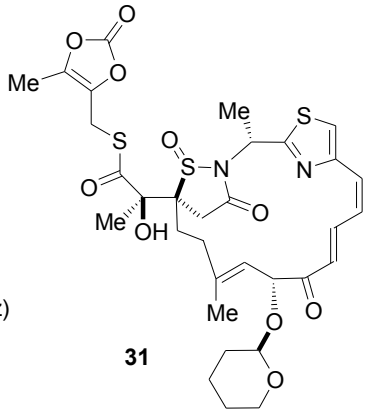
Structural modifications at the C-8 hydroxyl are well-tolerated, and represent the most common derivatization strategy reported. Analog **31**, a prodrug of the 1,2-dithiolan-3-one 1-oxide ring of **1** shows the most potent cytotoxicity of any leinamycin-based molecule to date.

Figure 3. Most potent derivative and analog of 1.



30

IC_{50} = 8.7 nM HeLa S3



31

IC_{50} = 0.67 nM HeLa S3

(**1** IC_{50} = 28 nM HeLa S3)

BIOSYNTHESIS

Shen and coworkers' recent identification of the gene cluster responsible for biosynthesis of **1**[30] is certain to have very important implications for the development of leinamycin-based chemotherapeutics. It appears that leinamycin's biosynthesis is carried out by a hybrid Polyketide (PKS)/Non-Ribosomal Peptide (NRPS) synthetase. These two classes of proteins (NRPS and PKS, respectively) biosynthesize polyketide and polypeptide natural products through a modular technique which uses a small set of highly conserved protein domains to catalyze the addition and sequential modification of a simple building block to a growing biomolecule polymer to generate products with high structural diversity.[31] The modularity of this machinery makes it amenable to genetic modification, whereby variations of the carbon skeleton of **1** may be created more easily than with chemical techniques. Elucidation of the individual steps in the biosynthetic pathway may enable access to highly diverse structural variants of **1**.

CONCLUSION

The 1,2-dithiolan-3-one 1-oxide ring and potent biological activity of leinamycin have attracted significant interest in the 16 years since identification of the antitumor antibiotic. The contributions by synthetic chemists have aided the efforts to understand the peculiar mechanism of action of this compound. The methods developed to access leinamycin's structure, as well as the work currently in progress to harness the biosynthesis of leinamycin, may enable access to new anticancer therapeutics, as well as help elucidate the complex pathways involved in the control of cell proliferation.

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