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Modular approaches to lankacidin antibiotics

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Abstract

Lankacidins are a class of polyketide natural products isolated from Streptomyces spp. that show promising antimicrobial activity. Owing to their complex molecular architectures and chemical instability, structural assignment and derivatization of lankacidins is challenging. Herein we describe three fully synthetic approaches to lankacidins that enable access to new structural variability within the class. We use these routes to systematically generate stereochemical derivatives of both cyclic and acyclic lankacidins. Additionally, we access a new series of lankacidins bearing a methyl group at the C4 position, a modification intended to increase chemical stability. In the course of this work, we discovered that the reported structures for two natural products of the lankacidin class were incorrect, and we determine the correct structures of

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Author Contributions

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Detailed synthetic procedures, characterization data, 1D- and 2D-NMR spectra, and crystallographic data for final compounds and synthetic intermediates (PDF)

X-ray data for compound **12** (CIF)

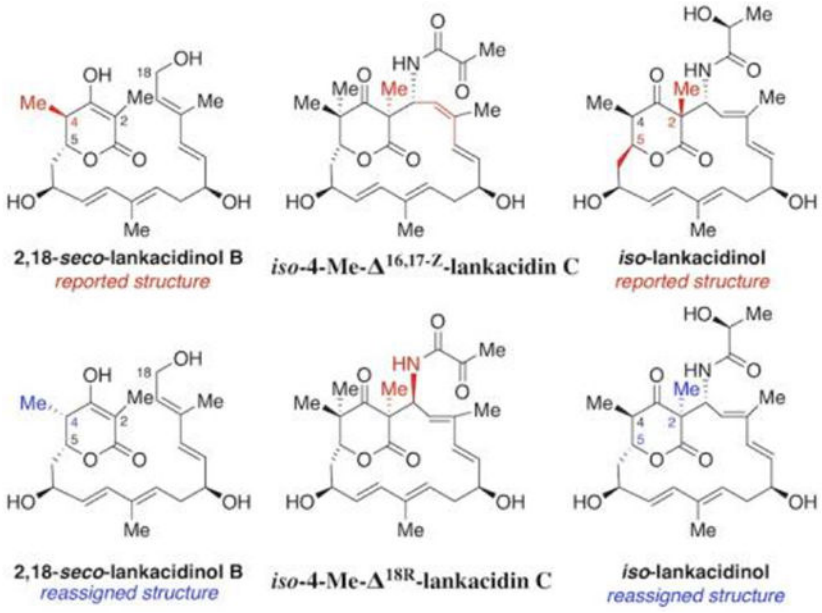
X-ray data for compound **60** (CIF)

X-ray data for compound **70** (CIF)

X-ray data for compound **87** (CIF)

2,18-*seco*-lankacidinol B and *iso*-lankacidinol. We also evaluate the ability of several *iso*- and *seco*-lankacidins to inhibit the growth of bacteria and to inhibit translation in vitro. This work grants insight into the rich chemical complexity of this class of antibiotics and provides an avenue for further structural derivatization.

Graphical Abstract



Keywords

Lankacidin antibiotics; modular synthesis; structural reassignment; Stille coupling; Julia–Kociensky olefination; Tsuji–Trost reaction

Introduction

Soil bacteria provide a rich natural source of natural products, with a variety of biological activities. Lankacidins are a class of polyketide/nonribosomal peptide natural products isolated from the soil bacterium *Streptomyces rochei* that feature a β-keto-δ-lactone core that is often contained within a 17-membered macrocycle (lankacidins **1–4** and **11**).[1] The structures of lankacidins C (**1**) and A (**2**) were assigned in part by X-ray crystallographic analysis of p-bromophenylsulfonylhydrazone derivatives.[2] The cores of the other family members were subsequently assigned by analogy.[1b, 3] More recently, Wang and co-workers isolated two new family members of lankacidins group whose structures lack a bond between the carbons at positions 2 and 18 in the macrocycle.[4] 2,18-*seco*-lankacidinol A (**7**) was proposed to contain a C18–O ether linkage instead of C2–C18 bond. 2,18-*seco*-lankacidinol B (**9**) is the first acyclic member of the lankacidin class lack of C2–C18 bond (Figure 1). Research in our group revealed the original assignment of C4 stereochemistry of 2,18-seco-lankacidinol B (**9**) was incorrect and made an amendment, whose stereochemistry

was deviated from all other family members.[5] Hong and co-workers have also recently reassigned 2,18-*seco*-lankacidionol A (**8**) to contain an oxazolidinone motif.[6]

In addition to their complex and diverse structures, lankacidins possess diverse biological properties, including promising antimicrobial activity toward many macrolide-resistant strains.[7] Lankacidin A (**2**) is commercially used in livestock industries for the treatment of porcine infected with *Serpulina* (*Treponema*) *hyodysenteriae*. Lankacidin C (**1**) and lankacidinol (**3**) have comparable activity to erythromycin as inhibitors of protein synthesis in vitro.[7c] In addition, lankacidin C (**1**) was shown to exhibit activity against L1210 leukemia, B16 melanoma, and 6C3 HED/OG lymphosarcoma cell lines.[8] 2,18-*seco*-lankacidinol A (**8**) and B (**10**) was reported to have antitumor activities against PC-3 and A549 cancer cell lines.[2]

Previously, structure-activity relationship studies on lankacidin class have mainly relied on semisynthesis, or the chemical modification of fermentation products. These approaches have primarily been restricted to modification of the alcohol functional groups. The chemical instability of the β-keto-δ-lactone core of lankacidins has impeded their therapeutic development and has complicated their structural assignments.[9] Early chemical degradation experiments showed that lankacidinol (**3**) was unstable under mild acidic conditions, resulting in opening of the macrocycle via cleavage of the C2-C18 bond (Scheme 1). Under basic conditions, the β-keto-δ-lactone motif fragments by means of decarboxylation, delivering lankacyclinol (**5**). Similar degradation experiments also showed that lankacidinol (**3**) could converted to *iso*-lankacidinol (**11**), proposed to be epimeric at C5 and result from a retro oxa-Michael/oxa-Michael process in reflux pyridine by Harada and coworkers.[4]

Due to the complexity of the scaffold and diverse bioactivity, the lankacidin class has received extensive interest from synthesis community. Over the past three decades, the fragile and congested structure has driven several groups to develop creative strategies to synthesize the scaffolds of lankacidin members (Scheme 2). In 1993, Kende and coworkers reported the first completed synthesis toward lankacidin C (**1**) in 34 steps (longest linear sequence).[10] Stereoselective acylation of the β-lactam **14** with thioester **13** to form the β-lactam followed by reduction by KEt_3BH gave an intermediate carbinol as a single isomer. Under acidic conditions, N→O acyl migration, relieving the ring strain of the β-lactam, delivered lactone **15**. Displacement of the allylic chloride with cyanide followed by exposure to LiHMDS promoted provided the macrocycle **17**. This intermediate was converted to lankacidin C (**1**) in 6 more steps. In 2000, Williams and coworkers reported a synthesis of lankacyclinol (**5**).[11] Under modified Julia olefination conditions, sulfone **18** and aldehyde **19** were coupled in 72% yield. Horner-Wadsworth-Emmons reaction provided macrocycle. After global deprotection, they reached lankacyclinol (**5**). In 2017, an elegant biomimetic approach was disclosed by Hong and colleagues in 8 steps.[12] Their route assembled all essential stereocenters in the linear carbon chain (**22** to **23**) and used a bioinspired Mannich macrocyclization to reach the 18-membered macrocycle. During the past three decades, Thomas' group made great contributions towards members of lankacidin group.[13] A model study between lactone **24** and phenylnitrone could quickly access β-keto-δ-lactone precursor **25** in 37% yield. However, their strategy was complicated by methylation with wrong C2

stereo center for lankacidinol (**3**). In their second-generation study, they adopted N→O acyl migration strategy for lactam **26**, which end up with rather unstable β-keto-δ-lactone intermediate **27** that was unstable at room temperature.

Synthesis and Structural Reassignment of 2,18-seco-Lankacidinol B

Continuing with our interest toward natural products featuring antibiotic activity,[14] we have been developing modular methods for the synthesis of lankacidins. 2,18-seco-lankacidinol B, the first acyclic member of the lankacidin class, was our first target due to the simplicity of the skeleton, which could pave the way toward syntheses of other family members. During our study, we found the original assignment of C4 stereochemistry of 2,18-seco-lankacidinol B (**9**) was incorrect.[5]

We envisioned the *seco*-lankacidins could arise from a coupling two halves of approximately equal size and complexity (Scheme 3). The synthesis of right half **34** commenced with epoxide opening by lithium acetylide,[15] followed by protection to generate protected diol **30** in 78% yield in one pot. Oxidative deprotection of the PMB group delivered a primary alcohol, which underwent Mitsunobu reaction with benzo[d]thiazole-2-thiol, followed by oxidation to form sulfone **31** in 77% yield over 2 steps. Julia–Kociensky olefination with commercially available aldehyde **32** provided dienoate **33** in 80% yield as a single stereoisomer.[16] Reduction of the ethyl ester to the primary alcohol (DIBAL-H) followed by palladium-catalyzed hydrostannylation delivered right half **34** in 84% yield.[17] The route to allylic alcohol **34** requires 6 steps from simple building blocks epoxide **28** and lithium acetylide **29**, proceeds in 40% overall yield in multi-gram scale.

With right half **34** secured, we move our attention toward the δ-lactone motif. Aldehyde **38** was prepared by an aldol reaction between aldehyde **35** and thiazolidinethione **36** followed by silyl protection and DIBAL-H reduction. The δ-lactone motif was introduced by an Evans aldol reaction between aldehyde **38** with chiral β-keto imide **39**, followed by NaOMe promoted lactonization to remove the chiral auxiliary, resulting in formation of the desired δ-lactone in one pot. This protocol closely mirrors an elegant method employed by Hong and coworkers.[12] Although moderate diastereoselectivity (**40:41** = 3:1) was observed, this protocol could secure the desired isomer **40** in decagram scale.[18]

With both right half **34** and left halves **40** in hand, various palladium source, ligands and solvent were screened for the Stille-cross coupling, resulting in either decomposition of starting material or low yield (see Supporting Information for details).[19] Fortunately, it was found that the desired product was formed at room temperature in less than 20 minutes by applying $Ph_2PO_2NBu_4$ as tin scavenger and DMSO as solvent (75% yield).[20] It is noteworthy that ice water was used to quench the reaction, as heat release during the aqueous workup was found to be detrimental to yield Desilylation with hydrogen fluoride provided the reported 2,18-seco-lankacidinol B (**9**). After careful comparison the ^{1}H- and ^{13}C-NMR spectra between authentic sample and synthetic 4(*R*), 5(*R*)-2,18-seco-lankacidinol B (**9**), we found the original assigned structure was incorrect. Most notably, the methine proton of C5 and the C20 methyl protons were significantly shifted, as were the ^{13}C signals corresponding to C3, C5, C6, and C20. Taken together, these data suggested that the

structural differences resided on the β-keto-δ-lactone ring. To further elucidate the real structure of 2,18-seco-lankacidinol B, three other diastereomers of left half was tested for the structure of 2,18-seco-lankacidinol B and isomer 4(*S*), 5(*R*) turned out be the final correct stereochemistry.[5]

Evans aldol reaction between common intermediate **38** and β-keto imide **39** followed by base promoted cyclization provided *syn* diastereomers **42** in 90% yield. Stille-cross coupling and deprotection provided 4(*S*), 5(*R*)-2,18-seco-lankacidinol B (**10**) in 63% yield over two steps. The ^{1}H- and ^{13}C-NMR spectra of 4(*S*), 5(*R*)-2,18-seco-lankacidinol B matched the reported spectra of the natural product. The reassignment makes 2,18-seco-lankacidinol B stereochemically unique among reported lankacidin-class natural products, all of which have been assigned the opposite stereochemistry at C4. This raises interesting questions about the biosynthesis pathway. The C20 methyl groups of lankacidin C (**1**) and lankacidinol (**3**) are proposed to be installed by a methyltransferase in the lankacidin polyketide synthase machinery prior to macrocyclization. It is possible that the analogous methyl transfer in the biosynthesis pathway proceeds with the opposite stereochemical preference, or that epimerization occurs during the biosynthesis. Alternatively, it is possible that epimerization of the C20 methyl group occurs independent of the biosynthetic machinery.

Synthetic Approaches towards Lankacidin C and 4-Methyl Lankacidin C

Our initial strategy towards lankacidin C (**1**) aimed to build upon our synthesis of 2,18-*seco*-lankacidinol B (**9**) by closing the macrocycle with a Tsuji–Trost reaction (Scheme 4).[21] We attempted to affect ring closure on intermediate **43** using several combinations of palladium catalysts, bases, solvents, temperatures, and concentrations. Unfortunately, we were unable to find a set of conditions that provided detectable amounts of the desired macrocycle **44**. Meanwhile, photochemistry was attempted for the macrocycle formation.[22] Azide **45** was transformed to imine smoothly by ruthenium catalyst, followed by acylation in situ delivered N-acylimine **46** in one pot. However tremendous efforts for intramolecular Mannich reaction were all met with failure.

We thought that the failure of many of these approaches could be due to instability of the products and starting materials due to the fragile β-keto-δ-lactone, a known contributor of the instability of the lankacidin class (vide supra). The signature peaks in the proton NMR spectra for this sensitive functionality were completely missing after many of our attempted reaction conditions, although the signals for the rest of the molecule were often still present. In order to improve the stability of the β-keto-δ-lactone core, we introduced methyl group at the C4 position, which we anticipated would suppress the β elimination process (Scheme 4).

The retrosynthetic analysis for 4-Me-lankacidin C (**48**) is illustrated in Figure 2. 4-Me-lankacidin C (**48**) could be derived from macrocycle compound **49** through an intermolecular C-H nitrene insertion to install the C-N bond at a late stage.[23] The key macrocycle **49** would be constructed from two halves of approximately equal complexity, left half **50** and right half **51** via two palladium-catalyzed reactions: Stille coupling reaction and Tsuji–Trost reaction. Left half **50** and right half **51** could be readily prepared from commercially available or known compounds according our previous efforts.

Our synthesis commenced with preparation of the left half **50**. First, compound **52** and aldehyde **38** were allowed to react under Gerwick's protocol to produce alcohol **53** with 68% yield.[24] Propionylaiton of the resulting alcohol, followed by cyclization with NaHMDS to generate enol anion-lactone which was trapped by Ac_2O *in situ* to form enol acetate **50** in 74% yield over 2 steps. Right half **51** was accessed by acetylation of known compound **31**. With both left half **50** and right half **51** in hand, under the condition that was developed for 2,18-seco-lankacidinol B (**10**), bis-diene compound **54** was produced in 79% yield. In our efforts to use the Tsuji-Trost reaction to construct the macrocycle, it was found Pd_2dba_3 as a palladium source and K_2CO_3 as a base in DMF at 90 °C, a pair of isomers, minor isomer **55** and major isomer **56** were obtained in 30% yield as a mixture of 1:2 Z/E ratio of C16-C17 double bond. Importantly, these conditions failed to produce any product when the C4 methyl group was absent. To our surprise, the formation of C2-C18 bond of these two isomers results in the opposite stereochemistry at the C2 position than anticipated.

We carried macrocycle **55** forward to synthesize a lankacidin analog. Reduction of ketone of **55** with $NaBH_4$ in MeOH provides a pair of inseparable alcohol **57** in 88% yield as a 3:1 diastereomeric mixture (major diastereomer shown). The resulting alcohol was trapped with trichloroacetyl isocyanate followed by hydrolysis with neutral aluminum oxide to obtain carbamate **58** in 60% yield. In the presence of silver(I) triflate, PhIO, and tris(2-pyridylmethyl)amine (TPA) carbamate **59** was furnished in 33% yield as a single stereoisomer. The stereochemistry of **59** was further unambiguously confirmed by X-ray crystallography of its desilylated derivative **60**. Acylation of **59** with **61** followed by hydrolysis of the cyclic carbamate and deacetylation provided diol **63** in 41% yield over 2 steps.

At this stage, only oxidation and deprotection were required to develop the lankacidin analog. After a extensive screening, we found that Markó's protocol for alcohol oxiation[25] converted diol **63** to ketone **64** in 50% yield. Global silyl deprotection with HF-pyridine complex provided *iso*-4-Me-$\Delta^{16,17\text{-}Z}$-lankacidin C (**65**) in 45% yield over 2 steps.

We next carried forward the major Tsuji–Trost diastereomer **56**. Surprisingly, the $NaBH_4$ condition failed to reduce the ketone **56**. Numerous approaches were evaluated (see supporting information for a summary) before an optimum condition was identified. Treatment of **56** with $LiBH_4$ in ^{i}PrOH delivers alcohol **66** in 93% yield as a 4.3:1 diastereomeric mixture (major diastereomer shown). Then through 2 steps developed previously, carbamate **68** was obtained in 31% yield over 2 steps. Next, amidation followed by hydrolysis does not give the desired diol **69**, but instead returned the carbamate **68** indicating the exocyclic amide bond is prone to hydrolysis. Treatment with LiOH, however, provided the desired product **69** in 60% yield. The structure of **69** was verified by X-ray analysis of its analog **70**. Next, we slightly optimized the previous copper mediated oxidation, using DBAD instead of DEAD, and lowing the reaction temperature from 70 °C to 50 °C, to provide 70% yield of **71**. Desilylation of **71** provided to *iso*-4-Me-Δ^{18R}-lankacidin C (**72**) in 95% yield.

The salient features of this route include Stille coupling reaction and Tsuji-Trost reaction to form the macrocycle and intermolecular C-H nitrene insertion to install the C-N

bond.Although this C–H amination route failed to deliver the natural configuration of the C4-Me-lankacidins, it did provide *iso*-4-Me-$\Delta^{16,17\text{-}Z}$-lankacidin C (**65**) and *iso*-4-Me-Δ^{18R}-lankacidin C (**72**) for biological evaluation (vide infra).

Synthesis and Structural Reassignment of iso-Lankacidinol

In 1975, a few years after isolation of lankacidin C, Harada separated a new component of lankacidin group from the culture filtrate of *Sterptomyces rochei*, named iso-lankacidinol (**11**). The stereochemistry of the iso-lankacidinol was proposed by a series of chemical degradation and NMR studies, whose C5 stereocenter was different from other family members. The unique (4*R*, 5*S*) stereochemistry of proposed iso-lankacidinol (**11**) was thought to be generated via retro-oxa-Michael addition/oxa-Michael addition process from lankacidinol (**3**) (Scheme 6).[4] We found this mechanistically unlikely because there was no reported epimerization of the C4 and C5 stereocenters and recent mechanistic studies from the Hong group,[26] showed that decarboxylation was more rapid than oxa-Michael addition. These considerations prompted us to reconsider the structure assignment of iso-lankacidinol. Instead of breaking C5-O bond, we proposed the retro-Mannich/Mannich addition between C2 and C18 bond is more likely. Here, we aimed to synthesize the reported and our proposed structure of *iso*-lankacidinol from three fragments by means of the sequential esterification, Julia–Kociensky olefination and Stille coupling. Due to the instability of the β-keto-δ-lactone core, we planned to install it at a late stage via Dieckmann cyclization. The three requisite fragments could be rapidly assembled from a collection of eight simple chemical building blocks (Figure 3).

As shown in Scheme 7, our synthesis commenced with the synthesis of the bottom fragment **77**. Oxidative deprotection of the PMB group with compound **30** provided primary alcohol in 92% yield, followed by palladium-catalyzed hydrostannylation and DMP oxidation in sequence delivered the bottom fragment **77** in 58% yield over two steps. Using the Evans aldol reaction to initiate the synthesis of the left fragment, we could predictably establish the stereochemistry at C4 and C5 on the β-keto-δ-lactone. $TiCl_4$-mediated *syn*-selective aldol reaction delivered the desired secondary alcohol **78** in 93% yield with high diastereoselectivity (>20:1).[27] Synthesis of the top fragment began with a Wittig reaction between the aldehyde **74** (available in 4 steps from *L*-aspartic acid dibenzyl ester *p*-toluenesulfonate salt)[28] and ylide **75**.[29] Reduction of the enal **79**, followed by Mitsunobu displacement of the resulting allylic alcohol with benzo[d]thiazole-2-thiol provided thioether **80** in 85% yield over two steps. Lactam opening under acidic conditions, basification, and addition of acyl chloride **76** furnished the top fragment **81** in 67% yield. It is worth noting that the syntheses of each of the fragments (**77, 78**, and **81**) are scalable and are either stereospecific from chiral starting materials (as for **77** and **81**) or are predictably stereoselective (as for **78**).

With efficient, scalable access to the three fragments completed, we turned our efforts toward their assembly. Alcohol **78** was coupled to carboxylic acid **81** with EDCI in the presence of DMAP in 60% yield. Oxidation of the resulting thioether **82**, Julia–Kociensky olefination with aldehyde **77**, and macrocycle formation by means of $Pd_2(dba)_3$ and LiCl provided macrocycle **83** in 62% over three steps. Importantly, the entire sequence to

assemble all three fragments and close the macrocyclic ring is highly convergent, requiring only four steps. The Dieckmann cyclization to close the final β-keto-δ-lactone ring required differentiation between two acidic α-protons: those adjacent to the ester within macrocycle and the methine on the amide side chan. After screening a selection of bases, we found that LiHMDS provided the desired cyclization product **84**, albeit in low yield (5%). Before exploring a larger panel of conditions to optimize this yield, we sought to take the material forward to the final product so as to compare spectral data with the reported data. Only two steps were required to complete the synthesis: K_2CO_3 with MeI provided a single C-methylation product in quantitative yield as a single diastereomer. Desilylation with hydrogen HF pyridine complex gave final product **85** in 92% yield over two steps. Not to our surprise, the ^{1}H- and ^{13}C-NMR spectra of **85** did not match the reported spectra of iso-lankacidinol. Due to the chemical instability of this compound, we were unable to obtain X-ray-quality crystal, and NOE signals in several solvents were overlapping, preventing assignment of the stereochemistry at C2. Inspection of a 3D model of enol **84** suggested that the methylation would provide the C2(S) stereochemistry, which would match the reported structure. However, without conclusive evidence for the C2 stereochemistry, we could not rule out at this stage if we had instead synthesized the C2 epimer of the reported structure. We decided to pursue a synthesis of other diastereomers of the δ-lactone ring in hopes that we could conclusively assign their structures and that one would provide spectra that matched the reported data.

We hypothesized that the stereochemistry of C4 and C5 in *iso*-lankacidinol might match that of 2,18-*seco*-lankacinol B (**10**), which we reassigned and differs from the rest of the class. To test this hypothesis, we sought to modify our synthesis to access the (4*S*, 5*R*) stereoisomer. Evans aldol reaction between aldehyde **38** and imide (*S*)-**73** provided 4(*S*), 5(*R*) *syn*-aldol product in high yield. Following the same four-step procedure developed in scheme 7, we could quickly access macrocycle **86**. NaH in DMF at 50 °C followed by the addition of MeI provided the methylated macrocycle in one pot. Desilylation with hydrogen fluoride–pyridine complex provided the final product **87**. Unfortunately, the ^{1}H- and ^{13}C-NMR spectra of **87** did not match the reported spectra of **11**. X-ray crystallographic analysis of compound **87** revealed that the stereochemistry of C2 methyl group, installed in the penultimate step of our synthesis, was inverted compared to other family members, which is consistent with 3-D models of the methylation precursor. Intriguingly, the chemical shift and splitting pattern of the C4 proton in **87** (2.74 ppm) differed by 0.32 ppm compared to the reported spectrum of **11** (3.06 ppm), but the C18 methine proton shift closely matched the spectrum of the natural product (5.59 ppm for **11**, 5.52 ppm for **87**). Although our synthesis of **87** could not rule out C4 being the cause of the misassignment, we began to suspect that C2 might be the source of the variability with respect to other family members.

With this in mind, we moved our efforts toward synthesis of compound **12**. We leveraged an asymmetric strategy utilizing Oppolzer's camphorsultam as the chiral auxiliary to access the required *anti* stereochemistry at C4 and C5.[30] Mukaiyama aldol reaction of aldehyde **38** with the TMS enol ether of *L*-camphorsultam derivative **88** proceeded with high diastereoselectivity (>20:1). Subsequent methanolysis provided methyl ester **89** in 85% yield over two steps. Esterification, oxidation, Julia–Kociensky olefination, and Stille coupling

furnished macrocycle **91** in good yield. LiHMDS-promoted Dieckmann cyclization proceeded in 35% yield, and C2-methylation was once again highly diastereoselective (>20:1). Desilylation provided the final compound **12** in 92% yield, whose structure was further confirmed by X-ray crystallography. Compared to the other crystal structures of family members, the entire β-keto-δ-lactone ring is rotated 180°, and the C5 proton sits inside the macrocyclic ring system. The ^{1}H- and ^{13}C-NMR spectra of the compound **12** perfectly matched the reported spectra of *iso*-lankacidinol, which confirmed the *iso*-lankacidinol is the C2 isomer of the lankacidinol (**3**).

Antimicrobial activity of *seco*- and *iso*-lankacidinols and biosynthetic considerations

Many lankacidin antibiotics exhibit antimicrobial activity by inhibiting the bacterial ribosome. For well-known members of the class such as lankacidin C (**1**), activities against several strains of bacteria and the binding mode to the ribosome have been reported.[8] Prior to our work in the area, the activity of 2,18-*seco*-lankacidinol B (**10**) against three strains of Gram-positive bacteria (*M. luteus*, *B. subtilis*, and *S. aureus*) were reported, and no biological data for *iso*-lankacidinol (**12**) was available. We first measured the activity of 2,18-*seco*-lankacidinols **9** (originally reported structure) and **10** (reassigned structure) against a panel of 10 Gram-positive and Gram-negative bacteria by the broth microdilution method.[5] Neither **9** nor **10** exhibited substantial activity against any of the common pathogens in our panel, although both were able to inhibit the growth of *H. influenzae* at 32 μg/mL. These data suggest that either the macrocycle or the pyruvamide sidechain are necessary for antimicrobial activity within the lankacidin class.

We next measured the ability of *iso*-lankacidinols to inhibit the ribosome by means of an in vitro translation (IVT) assay. This assay enables a direct assessment of inhibition of the ribosome without the requirement for molecules to accumulate in a live bacterial cell. This removes complications that can arise due to cell penetration, efflux, enzymatic deactivation, and several other factors. We evaluated inhibition the *E. coli* ribosome for five *iso*-lankacidinols at a single concentration of 10 μM (Figure 4). The non-natural 4-Me-*iso*-lankacidinols (**65** and **72**) and the diastereomeric *iso*-lankacidinols (**85** and **87**) did not inhibit translation at this concentration compared to DMSO control. The natural product *iso*-lankacidinol (**12**) showed small but measurable inhibition of translation. These results suggest that the stereochemistry within and around the β-keto-δ-lactone core of lankacidinolsis important for translation inhibitory activity in cyclic members of the lankacidin class.

The lack of inhibitory activity of the natural product *iso*-lankacidinol (**12**) raises interesting questions about its evolutionary and biosynthetic origins. Chagot, Weissman, and colleagues demonstrated lankacidinol A (**4**) can be obtained from acyclic precursor **92** (LC-KA05) by means of LkcE, an enzyme in the lankacidin biosynthetic gene cluster that belongs to the monoamine oxidase family (Figure 5).[31] The mechanistic details of the macrocyclization reaction remain unclear, but it was proposed that oxidation of C18 in **92** by LkcE provides an activated N-acyliminium (**93**) that serves as an electrophile for an intramolecular

Mannich reaction. Stereochemical diversity at C2 could arise from imperfect selectivity with respect to the face of the β-keto-δ-lactone precursor. Such lack of enzymatic stereochemical fidelity in secondary metabolite biosynthesis is uncommon but precedented.[32] It is also possible that the C2 stereochemical variability arises from an enzyme outside of the lankacidin biosynthetic gene cluster[33] or through a non-enzymatic pathway. *Iso*-lankacidinol may be an artifact that arose during the evolution of the amide oxidase LkcE, which obtained a second function as a macrocyclase.[31] This could explain the low activity and low natural abundance of *iso*-lankacidinol (**12**).

In conclusion, we have developed fully synthetic routes to lankacidin antibiotics that give access to the unusual *seco*- and *iso*- members of the class. Our route to *seco*-lankacidinols enabled facile variation of the β-keto-δ-lactone core of these acyclic family members, resulting in the structural reassignment of 2,18-*seco*-lankacidinol B (**10**). The inclusion of a methyl group at C4 provided enough chemical stability to enable macrocyclization with an extremely demanding Tsuji–Trost reaction followed by a selective intramolecular C–H amination to provide 4-Me-*iso*-lankacidinols. Modifaction of the route for late-stage introduction of the β-keto-δ-lactone enabled access to diastereomeric *iso*-lankadicinols, resulting in the structural reassignment of *iso*-lankacidinol (**12**). Evaluation of the antibiotic and in vitro inhibitory of these fully synthetic lankacidins provided structure–activity relationships that were previously lacking in the class. This work provides an important basis for understanding of the biosynthesis of lankacidins, and we anticipate that it will inform future work to improve the therapeutic potential of this class.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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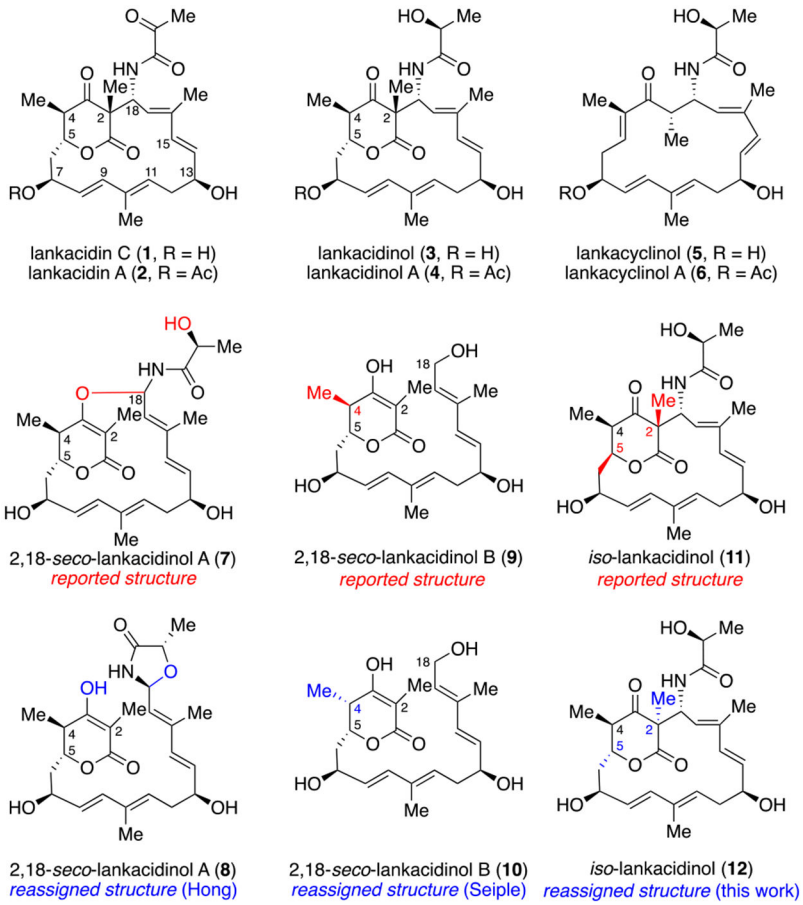


Figure 1.
Members of the lankacidin class of natural products.

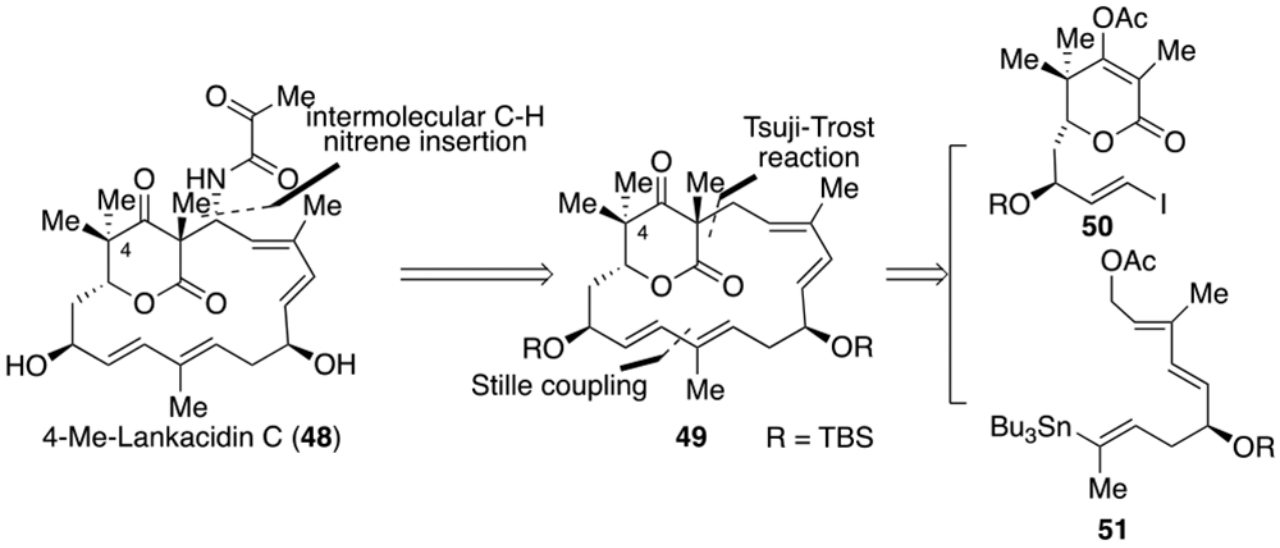


Figure 2.
Rationale for 4-methyl lankacidins.

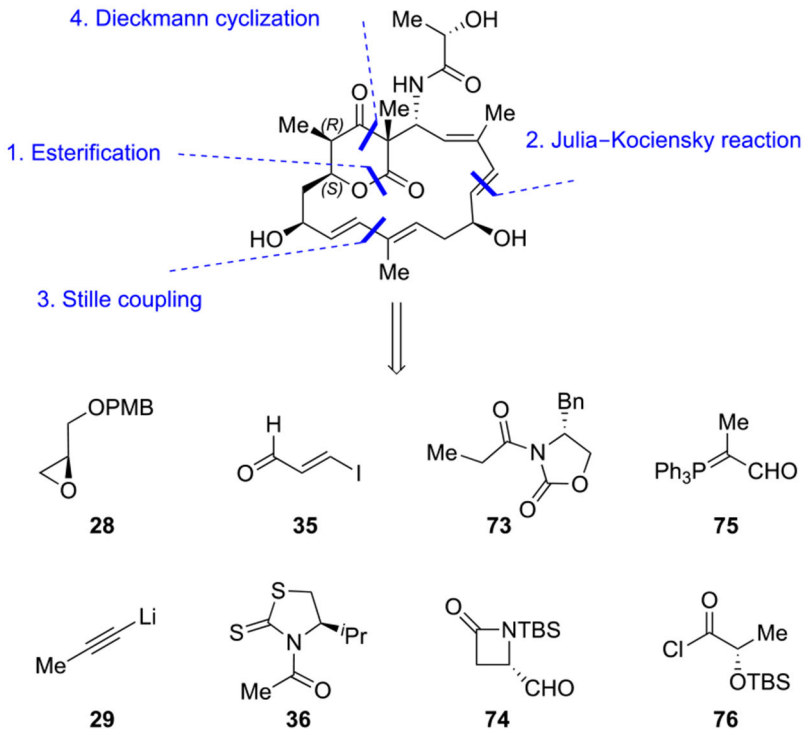


Figure 3.
General retrosynthetic analysis of *iso*-lankacidinol (**11**).

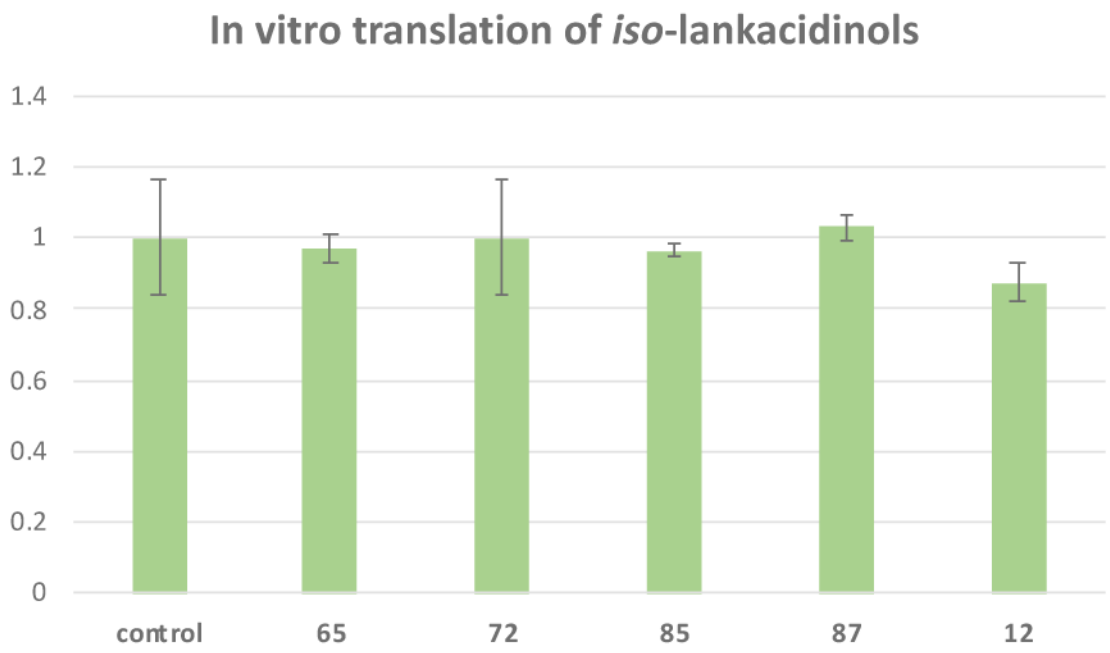


Figure 4.
Inhibition of translation by *iso*-lankacidinols. Data acquired using a PURExpress® In Vitro Protein Synthesis Kit (E6800, New England Biolabs).

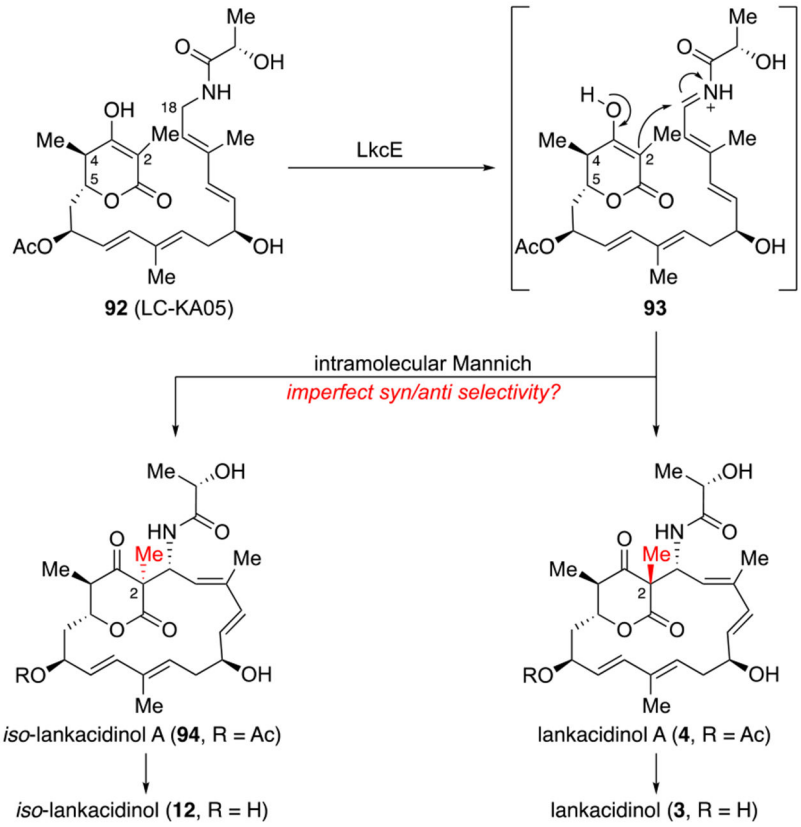
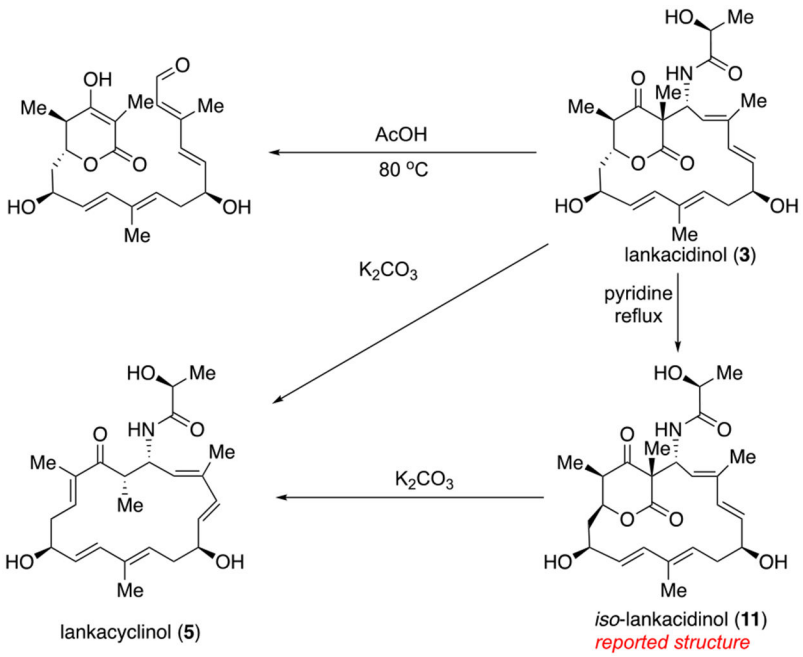
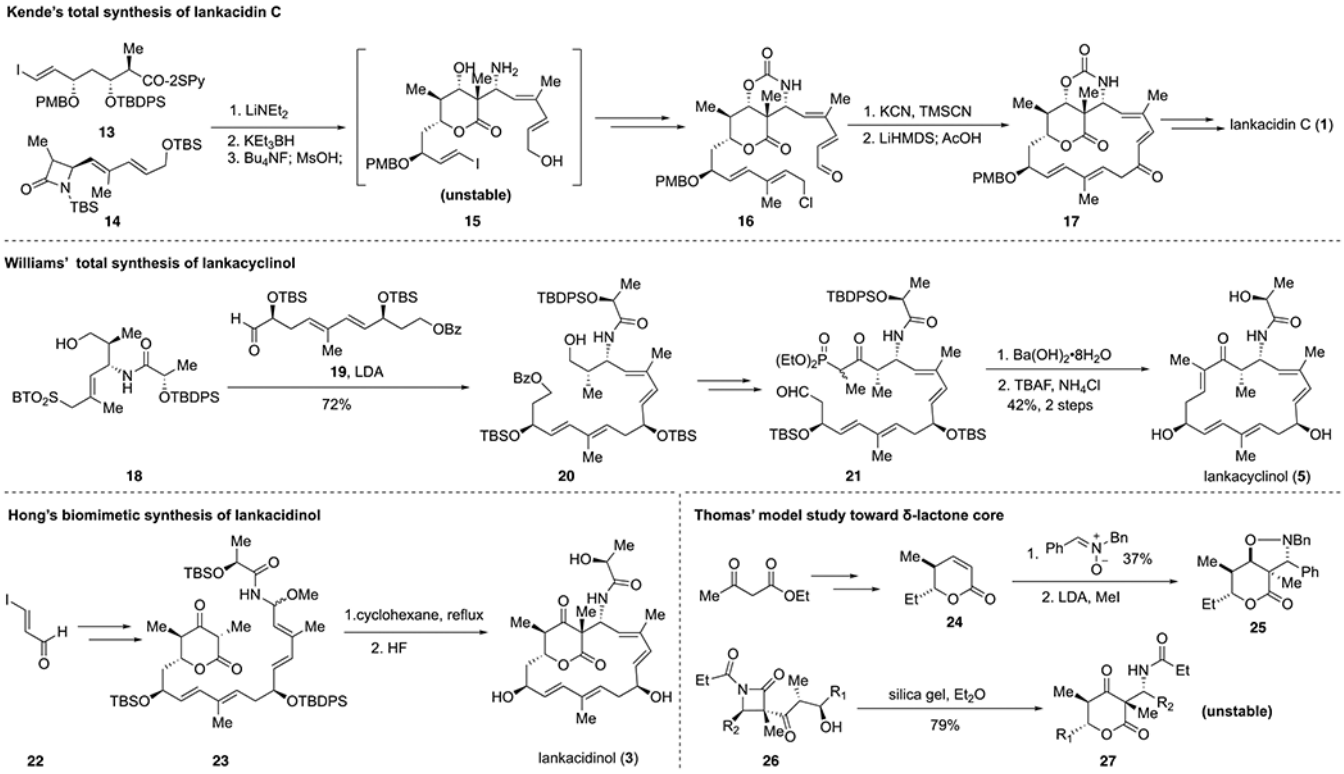


Figure 5.

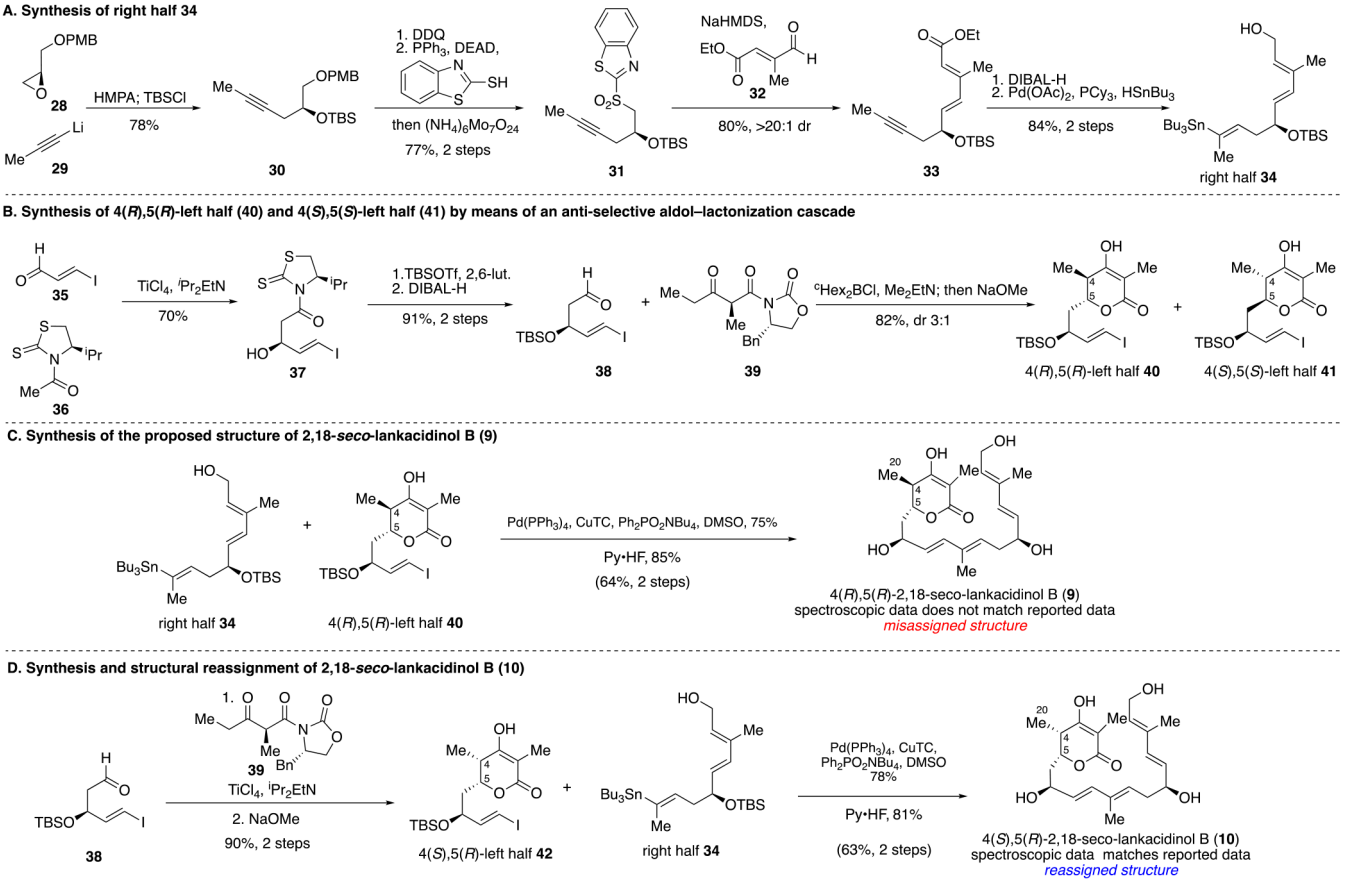
Intramolecular Mannich reaction selectivity as a potential biosynthetic origin of *iso*-lankacidinols.



Scheme 1.
Selected transformations between lankacidin family members

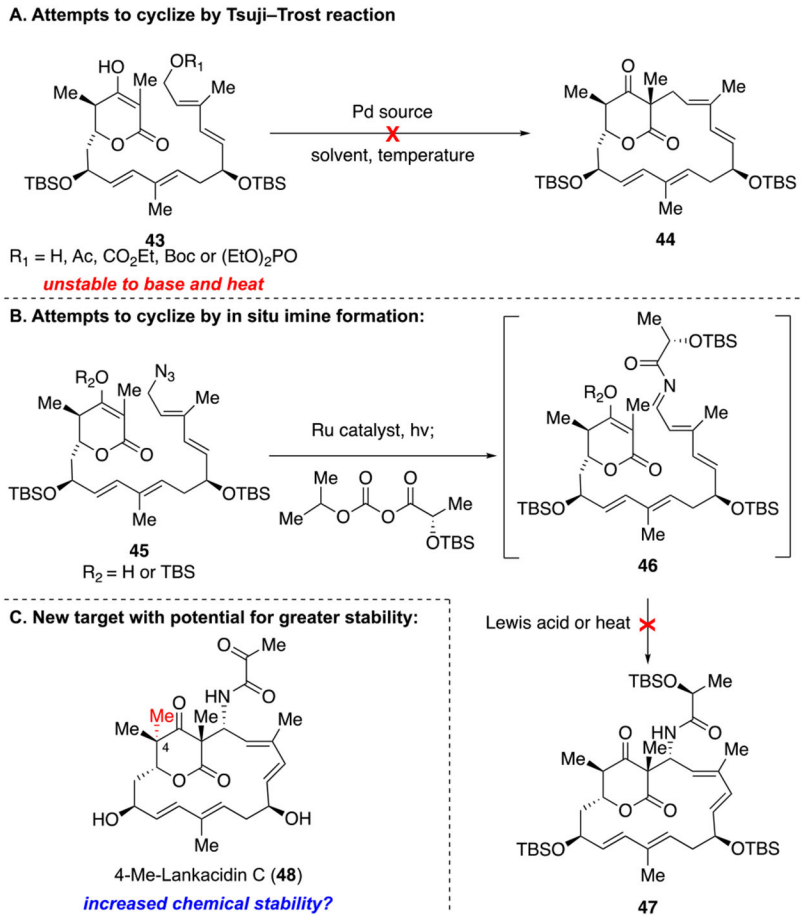


Scheme 2.
Previous syntheses toward lankacidin family members

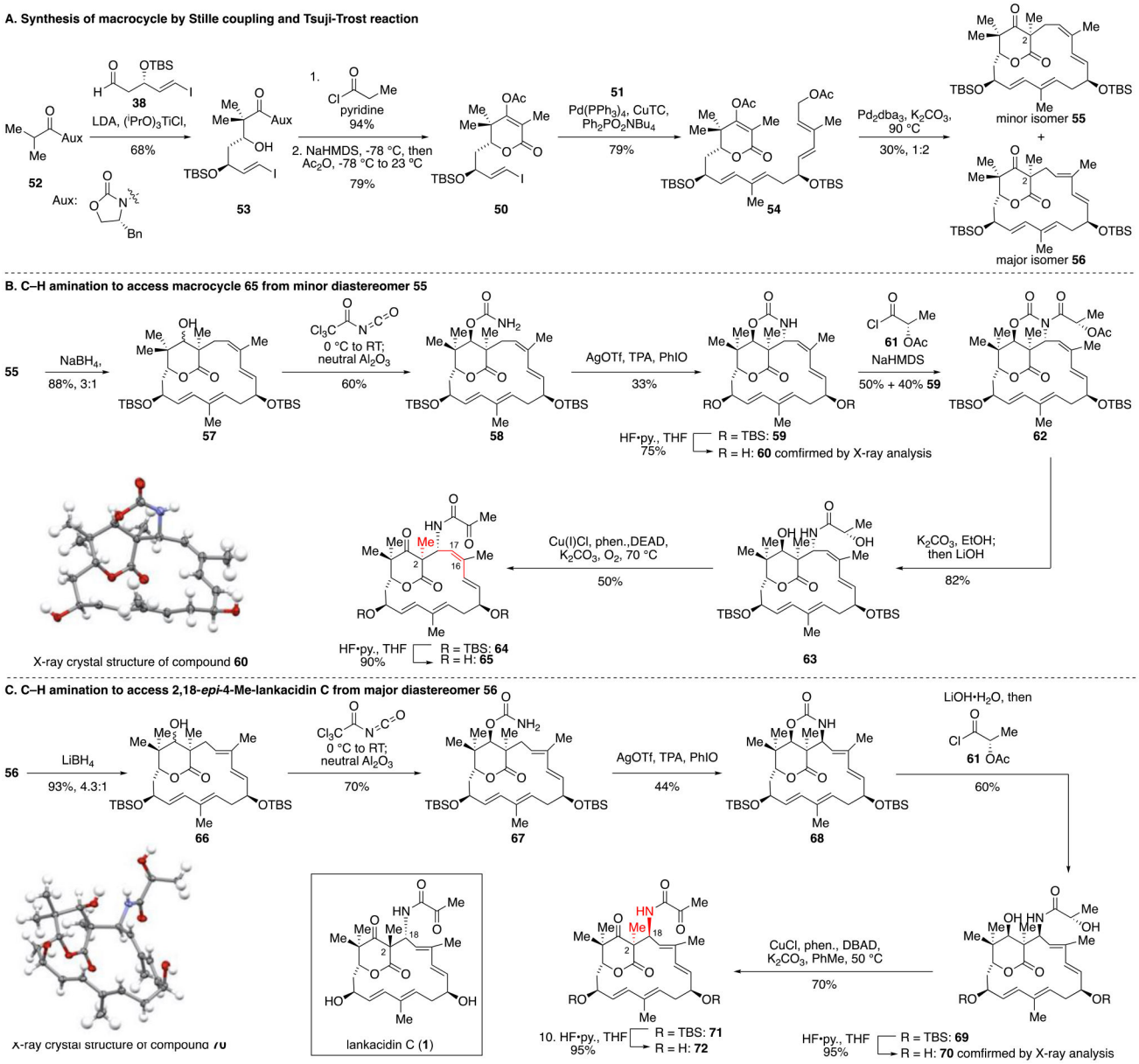


Scheme 3.

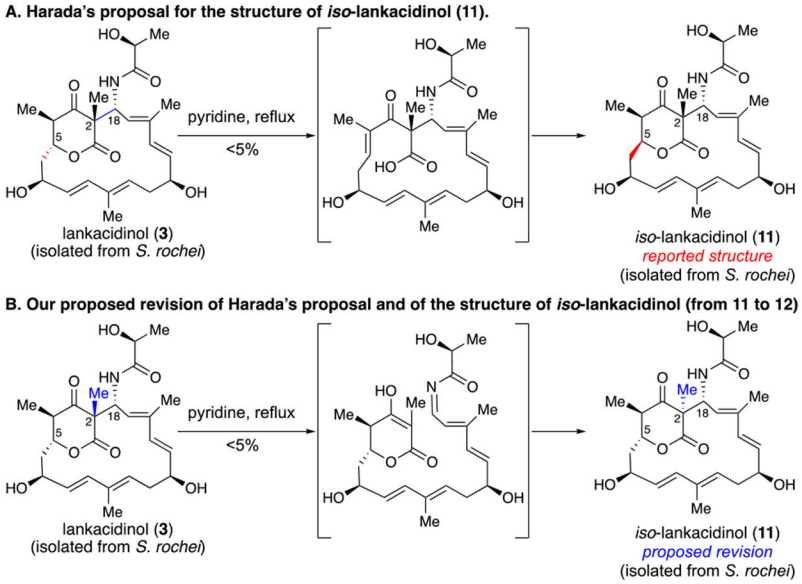
Synthesis and structural reassignment of 2,18-seco-Lankacidinol B



Scheme 4.
Failed strategies for macrocycle formation.

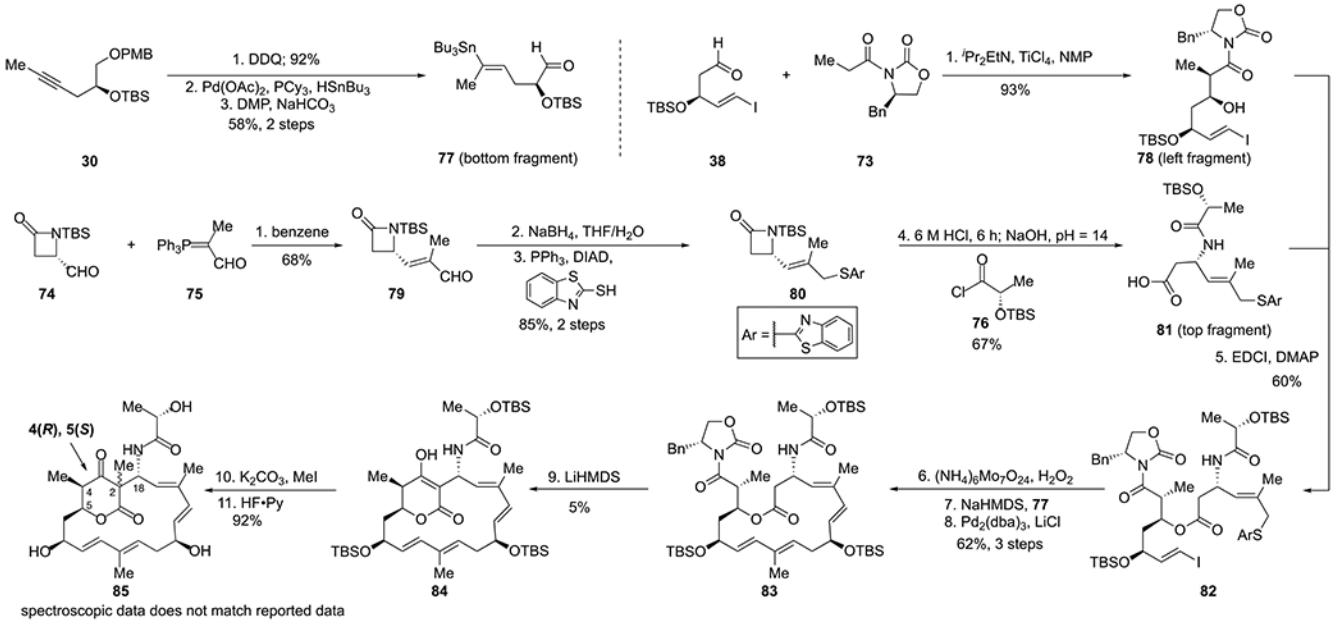


Scheme 5.
Synthesis of 4-methyl lankacidins

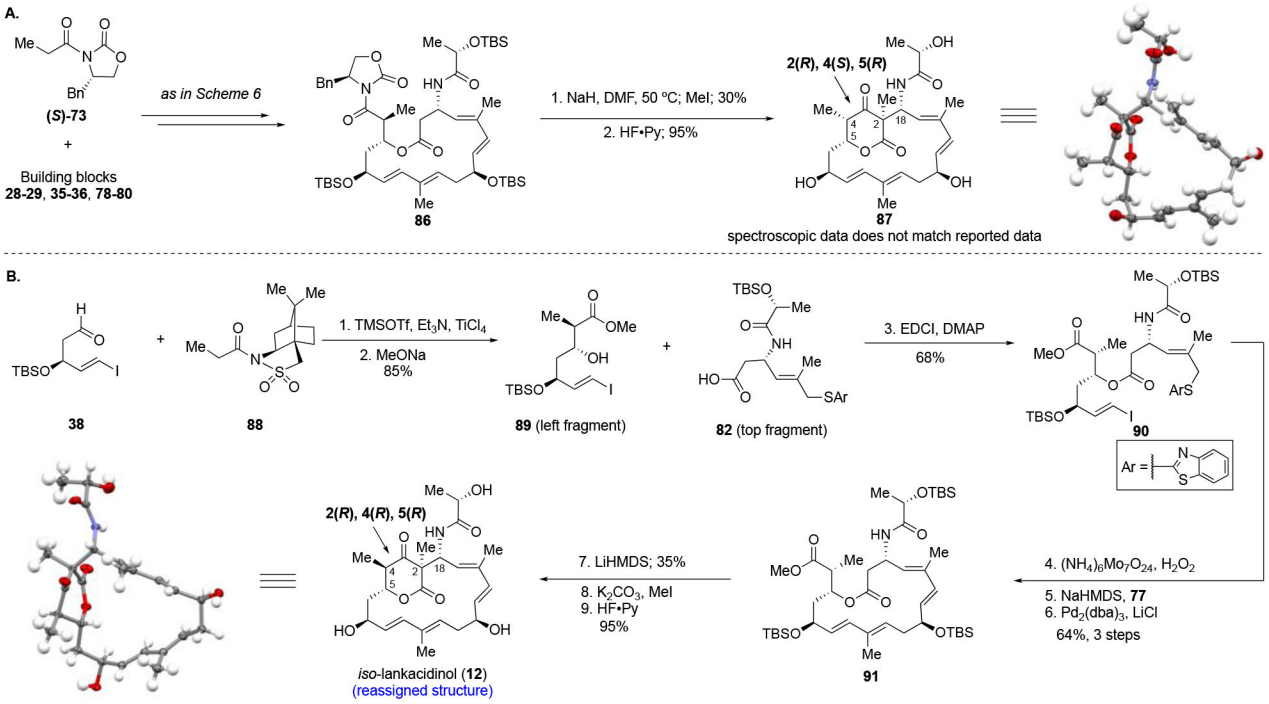


Scheme 6.

Proposed structures *iso*-lankacidinol based on degradation studies from lankacidinol (**3**).



Scheme 7.
Synthesis of the reported structure of *iso*-lankacidinol.



Scheme 8.

Reassignment of *iso*-lankacidinol by means of modular chemical synthesis.

Table 1.

Antimicrobial activity of *seco*-lankacidinols.[a]

	Minimal Inhibitory Concentration (MIC) Values (μg/mL									
	Gram-negative					Gram-positive				
Compound	*A. baumannii* ATCC 19606	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 43816	*P. aeruginosa* ATCC 27853	*H. influenzae* ATCC 49247	*E. faecalis* ATCC 29212	*E. faecium* ATCC 35667	*S. aureus* ATCC 29213	*S. aureus (MRSA)* ATCC 33591	*S. pneumoniae* ATCC 49619
9	>64	>64	>64	>64	32	>64	>64	>64	>64	>64
10	>64	>64	>64	>64	32	>64	>64	>64	>64	>64

[a]MIC values were obtained by the microdilution method following CLSI standards and are given in μg/mL.