Communication

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Biomimetic Synthesis of Lankacidin Antibiotics

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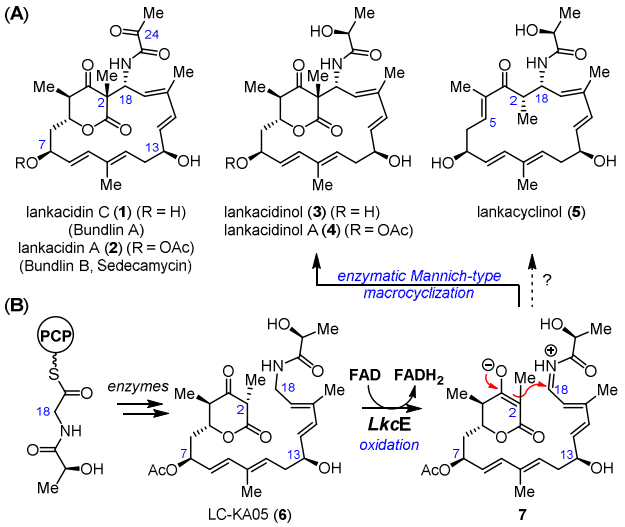
Supporting Information Placeholder

ABSTRACT: We devised short syntheses of lankacidinol and lankacyclinol that feature biomimetic Mannich macrocyclizations. The modular construction of the carbon framework of these compounds is amenable to rapid structural diversification for the development of antibiotic and antitumor agents.

The growing emergence of bacterial resistance, especially for multidrug resistance phenotypes, has become a major concern to public health.[1] In the past seven decades, the activity-guided identification of antibiotics from microbial sources has disclosed nearly 28,000 compounds, but only less than 1% these compounds have found direct use as clinical drugs.[2] It is no doubt that the majority of structurally "old" scaffolds remain to be scrutinized. For those structures with condensed functional groups, the opportunity to reveal novel mechanisms of action is particularly intriguing for the development of next-generation durable antibiotics with additional synergistic effect.[3] Previously demonstrated as oral antibiotics for infected livestock, lankacidins (Scheme 1) have the potential to be part of a new generation of antibiotics to combat conventional macrolide-resistant pathogenic strains.[4] However, the labile and densely functionalized moieties within this family of complex polyketides pose a formidable challenge for chemical total synthesis and derivatization, thus impeding further medicinal development for clinical use.

Lankacidins consist of a class of hybrid polyketide-peptide metabolites that feature a transannulated heptadecane (17-membered) framework. The first congener of this category is lankacidin C (**1**), which was first described by Gäumann and coworkers in 1960,[5] and the stereochemistry was firmly established by X-ray analysis of its hydrazone derivative.[6] Lankacidins typically possess dense functional groups that feature two skipped *E*,*E*-dienylic alcohols, a fully substituted β-oxo-δ-lactone and a rare amide residue adjacent to the bridgehead all-carbon quaternary stereocenter (**1**-**4**, Scheme 1A). The amide side chain is varied by lactoyl or pyruvoyl groups. Decarboxylated derivatives (losing C1, such as **5**) were also isolated from either *Streptomyces rochei* or other natural sources.[7] In contrast to a variety of glycosidic macrolides,[8] the biological evaluation of these aglycone compounds revealed strong antimicrobial activities against Gram-positive pathogens[4] and *in vivo* antitumor activity against certain cancer cell lines.[9] Its impressive antimicrobial activity guided the launch of lankacidin A (**2**) (drug name: Sedecamycin) into the market as an oral veterinary antibiotic (e.g., treatment of swine dysentery).[10] Although the mechanism of action remains unclear, crystallographic analysis revealed that lankacidin C (**1**) and lankamycin synergistically target the eubacterial large ribosomal subunit at the peptidyl transferase center, reigniting their promise for medicinal applications.[11] More interestingly, recent studies have revealed that lankacidin enhances tubulin assembly and displaces taxoids from their binding site.[12]

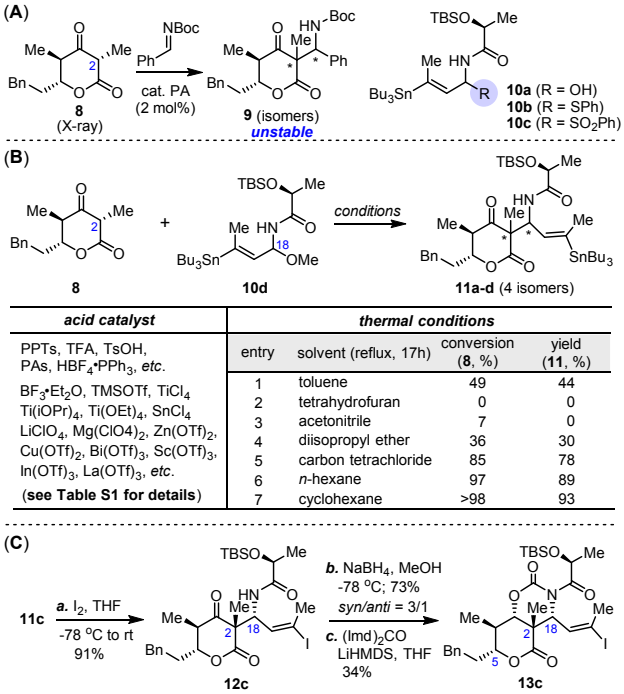


Scheme 1. Lankacidins: structures and biosynthesis. (**A**) Selected structures of lankacidins (the carbon numbering is adapted to the biosynthesis proposal, see ref 19); other trivial names are shown in parentheses. (**B**) Biogenesis proposal. FAD, flavin adenine dinucleotide; PCP, peptidyl carrier protein.

Although a certain number of lankacidin derivatives have been prepared through enzymatic esterification or sophisticated chemical transformations, these approaches are rather limited, as the modification of the parent structure was found to be difficult due to facile cleavage of the C2-C18 bond upon exposure to even mild acidic or basic conditions.[9,10,13] A *de novo* synthesis arguably provides far greater opportunities and flexibility for discovering new bioactive entities.[14] Over the past three decades, the fragile and congested structure has enticed significant interest from several groups to forge the C2-C18 bond with elaborate functional groups at an early stage in their synthetic blueprint.[15,16] Only the Kende group accomplished the landmark synthesis of lankacidin C (**1**) with an enormous 34 LLS (longest linear steps) (46 total steps) from two chiral building blocks.[17] Alternatively, a flavin-dependent amine oxidoreductase (*Lkc*E) was preliminary identified to biosynthetically participate in the unprecedented intramo-

lecular Mannich-type cyclization (Scheme 1B).[18,19] Kinashi and others also uncovered that polyketide synthases from *Streptomyces rochei* constructed the entire carbon chain first (such as LC-KA05, **6**) and that subsequent oxidation at C18 revealed a reactive alkenyl *N*-acyl imine.[19-21] Although the detailed enzymatic underpinnings for this extremely rare ring-forming reaction remain to be clarified (such as involving an enolate-iminium pair **7**), from a synthetic standpoint, a biomimetic approach would drastically decrease the number of steps if the proper precursor and reaction conditions could be defined.

To strive to imitate nature's ingenuity, we envisaged that non-productive refunctionalizations (such as redox and protective manipulation at C3),[22] which have plagued all the previous approaches, could be confined to a minimum if all the native oxidation states are preinstalled within a single linear precursor. However, such a fundamentally disparate tactic presents several challenges. First, the presumptive *N*-acyl-1-azahexatrienes are notoriously unstable to handle, and few protocols for their generation are available.[23] Additionally, β-oxo-δ-lactones have been categorized as the least reactive β-ketoester derivatives for nucleophilic addition.[24] Furthermore, the Mannich adducts are energetically unfavorable due to their strained structures and would undergo facile retro-Mannich reactions under a variety of conditions. A similar model substrate was declared by Thomas and coworkers to be "rather unstable, decomposing to give complex mixtures of products when stored at room temperature".[15f] Finally, the essential macrocyclization with critical stereocontrol remains elusive due to the insufficient data from conformational studies toward the synthesis of lankacidins.



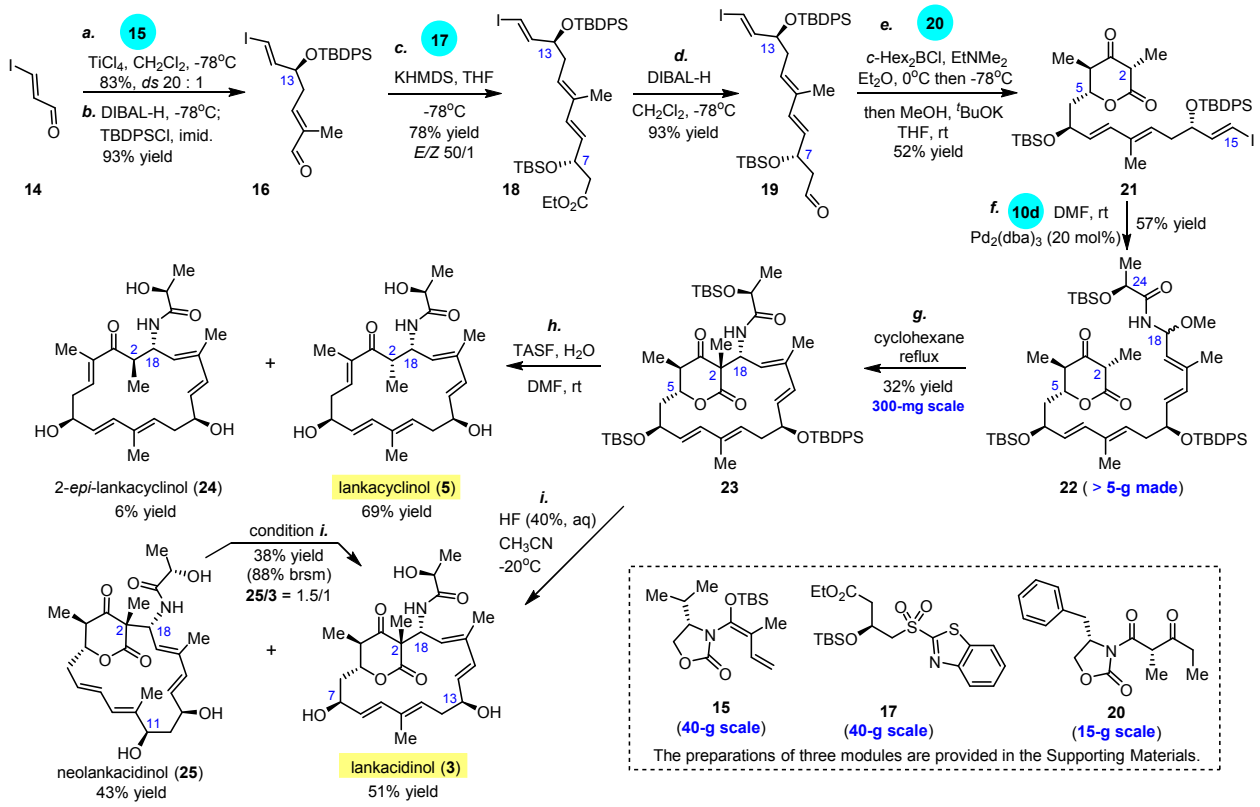
acid catalyst	*thermal conditions*			
PPTs, TFA, TsOH, PAs, HBF_4•PPh_3, *etc.*	entry	solvent (reflux, 17h)	conversion (**8**, %)	yield (**11**, %)
BF_3•Et_2O, TMSOTf, $TiCl_4$ $Ti(iOPr)_4$, $Ti(OEt)_4$, $SnCl_4$ $LiClO_4$, $Mg(ClO4)_2$, $Zn(OTf)_2$, $Cu(OTf)_2$, $Bi(OTf)_3$, $Sc(OTf)_3$, $In(OTf)_3$, $La(OTf)_3$, *etc.*	1	toluene	49	44
	2	tetrahydrofuran	0	0
	3	acetonitrile	7	0
	4	diisopropyl ether	36	30
	5	carbon tetrachloride	85	78
	6	*n*-hexane	97	89
(**see Table S1 for details**)	7	cyclohexane	>98	93

Scheme 2. Reaction design. (**A**) Initial attempts at the Mannich reaction. (**B**) Mannich reaction with model substrates. (**C**) Stereochemical determination of the adducts (**13c** as an example, see the Supporting Information for details). PA = *rac*-binaphthol-derived phosphoric acid.

With the above perspectives in mind, an intermolecular model study was performed using truncated β-oxo-δ-lactone **8** as the principal nucleophile (Scheme 2). The Mannich reaction of **8** with premade *N*-Boc benzaldimine proceeded smoothly; however, the corresponding Mannich adduct **9** readily underwent the retro-Mannich reaction upon exposure to silica gel and mild acidic conditions (Figure S7).[25] Strategically, a stannylated vinylogous imine precursor **10** was envisioned since chain elongation can be achieved by Stille coupling.[26] Initial forays verifying the R substituent as imine precursors **10a/b** resulted in decomposition without formation of any addition products (Scheme 2A). Although the Mannich reaction of **10c** with lactone **8** resulted in the requisite adducts, the vulnerability of the sulphonyl group under Stille coupling conditions hampered further modification. However, when the more stable *N*-acyl *N,O*-acetal **10d** was synthesized, after an exhaustive evaluation of conventional methods, including using Brønsted acids and Lewis acids,[27] the Mannich reaction between **8** and **10d** remained unfruitful (Scheme 2B). In most cases, the in situ generated imine was geometrically unstable, extremely susceptible to tautomerization and possessed only marginal reactivity toward nucleophilic addition. Based on the alcohol exchange phenomenon reported by Ben-Ishai and coworkers in the 1960s during the pyrolysis of *N,O*-acetals,[28] we anticipated that thermal activation of **10d** via methanol depletion would presumably release a transient imine under strictly neutral conditions that may be compatible with our delicate substrates. After a survey of different solvents (Table S2),[25] less polar solvents impressively facilitated the conversion of δ-lactone **8** to the Mannich adducts in good yields (Scheme 2B). Of particular interest to us is that *N,O*-acetal **10d** alone was stable in refluxing cyclohexane or toluene and that a drastic conversion occurred after the addition of δ-lactone **8**. The optimized conditions were readily applied on a 200-mg scale to generate the requisite Mannich adduct (**11**) as a mixture of four stereoisomers in a combined yield of 93%.

To clarify the stereochemical outcome, the coupling products were individually subjected to tin-iodine exchange.[29] The structures of vinyl iodides **12a** and **12b** were unambiguously determined by X-ray crystal structure analysis of their corresponding desilylation derivatives (see SI for details).[25] For vinyl iodide **12c**, reduction of the C3-ketone delivered diastereomeric alcohols (*ds* 3:1), from which the major isomer was readily identified as carbamate **13c** (Scheme 2C). From extensive NMR studies (Figure S8),[25] the newly generated vicinal stereocenters were unambiguously assigned as (2*S*,18*R*), which is congruent with those in natural lankacidins **1**-**4** (Scheme 1). With the stereogenic centers in both components identified (**8** and **10d**), the preference of the C2-Me/C5-H *cis*-adduct will be anticipated through the introduction of a long C5-tether in the forthcoming intramolecular event. Although the stereochemical outcome at C18 is difficult to discern, the *Si*-face addition of the in situ generated imine to yield an *R*-configuration would be a fascinating reward via a biomimetic macrocyclization.

Given the establishment of Mannich reaction in hand, a strategic disconnection of the requisite long carbon chain was realized in a polyketide synthase (PKS)-inspired pipeline.[14] 3-Iodoacrolein (**14**) was applied to the vinylogous Mukaiyama aldol reaction of a known silyl enolate **15**[30] to afford the allylic alcohol in excellent yield and diastereoselectivity (85% yield, *ds* 20/1) (Scheme 3). The removal of the oxazolidinone group with diisobutylaluminum hydride and immediate protection of the hydroxyl group with the *tert*-butyldiphenylsilyl group provided enal **16** in excellent yield (93%). After the Julia-Kocienski olefination[31] with sulfone module **17**, reduction of the corresponding ester delivered advanced aldehyde **19** in 73% yield over two steps. The final dipropionate motif was introduced by an Evans-aldol reaction of **19** with chiral β-keto imide **20**.[32] Although the diastereoselectivity (two *anti*-isomers, *ds* 3/1) was moderate, the subsequent KO^{t}Bu-promoted lactonization smoothly removed the chiral auxiliary,



Scheme 3. Syntheses of lankacidinol and lankacyclinol. (brsm = based on the recovered starting material)

and the major congener of δ-lactone **21** bearing a *trans*-configuration of C2-Me and C4-Me in the keto form was isolated in 51% yield. The two transformations were realized in one flask without any interrupted extraction. With the successful model study of the Mannich reaction of the *N,O*-acetal, we were poised to preinstall a higher oxidation state at C18 through Stille cross-coupling with the *N,O*-acetal. Surprisingly, it was found to be difficult due to the facile displacement of the methoxy group in the *N,O*-acetal when phosphine ligands were employed (Table S3).[26] After examination of a plethora of conditions, tris(dibenzylideneacetone)dipalladium(0) was identified as a suitable catalyst to form the critical C15-C16 bond, and the acid-labile precursor **22** was isolated in 57% yield. The key biomimetic Mannich reaction was then executed under the optimal conditions (c = 0.0005 M). After complete consumption of the starting material, the major cyclized product **23** was isolated in 32% yield (48% NMR yield).[33] At this juncture, the stereochemistry of **23** was not determined from the severe overlap of the proton signals in the upfield range. For example, although the cross 2D-NOE correlation of C2-Me/C5-H supported the *cis* relative configuration between C2-Me and C4-Me, the chirality at C18 was not conclusive due to the unclear conformation of the macrocyclic ring. The fragile nature of the δ-lactone motif bearing a bridgehead quaternary center at C2[10,19] was thus adopted to furnish lankacyclinol (**5**), whose structure was unambiguously confirmed by the synthetic work of the Williams group (25 LLS, 39 TS).[34] After surveying desilylation conditions, a combination of TASF[35] and 40 equivalents of water effectively triggered decarboxylation and desilylation in one pot. Lankacyclinol (**5**) was isolated in 69% yield as the major product, and the spectral data were consistent with those reported in the literature.[34] Therefore, the C18 stereochemistry of major isomer **23** was inferred to be the (*R*)-configuration based on the successful elaboration of this material to the natural product. In addition, the minor C2-epimer **24** was isolated in 6% yield. The favorable facial selectivity (*ds* 12/1) in the protonation reaction may be attributed to the rigid conformation of the polyene macrocyclic ring system and a possible hydrogen bond of the amide N-H group to the C3-enolate.[36]

The next objective was to identify the optimal reaction conditions to retain the fragile δ-lactone moiety. Gratifyingly, an aqueous solution of HF (40 wt%) at -20 °C was found suitable for global desilylation (Table S4).[25] Lankacidinol (**3**), which is the most complex congener in this family, was isolated in 51% yield from **23** as a white powder. The spectroscopic characteristics of the synthetic sample were identical to the previously reported data.[37] In this reaction mixture, the new congener **25** (coined as neolankacidinol) of the lankacidins was isolated in 43% yield as a single diastereomer, and extensive NMR studies revealed a formal hydroxyl shift occurred along the diene chain in a stereoselective manner. We assume that this facile rearrangement proceeded through a cationic intermediate and that the preferred stereochemical outcome was most likely controlled by the rigid conformation of the macrocyclic ring. Additional experiments established that neolankacidinol (**25**) was interconvertable with lankacidinol (**3**) under the identical conditions, which can maximize material throughput to access either target (**3** or **25**). Although compound **25** has not been previously disclosed, the facile propensity of hydroxyl relocation indicates a similar process may also occur under biologically relevant conditions.

In summary, we developed short total syntheses of lankacidinol and lankacyclinol. The salient features of the work include a modular approach for the rapid installation of all essential stereocenters in the linear carbon chain, a unique biomimetic Mannich-type macrocyclization via thermal activation of an imine, and judicious desilylation to access the congeners of the lankacidins. Our results also revealed the possible biogenesis of lankacyclinol through decarboxylation and stereoselective protonation. An unexpected stereoselective formal [1,5]-hydroxy shift through the

conjugated system likely resulted from the rigid conformation of the macrocyclic ring system. Together with the newly synthesized 2-*epi*-lankacyclinol, this study may implicate that more unknown natural products are yet to be identified, as the optimal conditions approximated a physiological environment. In comparison to previous syntheses of lankacidin antibiotics, the current diverted synthesis was concise and highly efficient (both in 8 LLS, 3.0~4.0% overall yield) from readily available materials (such as **14** and **15**) and provided a platform for structural modification to previously inaccessible derivatives. Studies along this line as well as collaborative investigation on biosynthesis are currently underway in this laboratory and will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.
Detailed experimental procedure, and ^{1}H NMR and ^{13}C NMR spectra, as well as X-ray data information (PDF)
Crystallographic data (CIF)

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Notes

The authors declare no competing financial interest.

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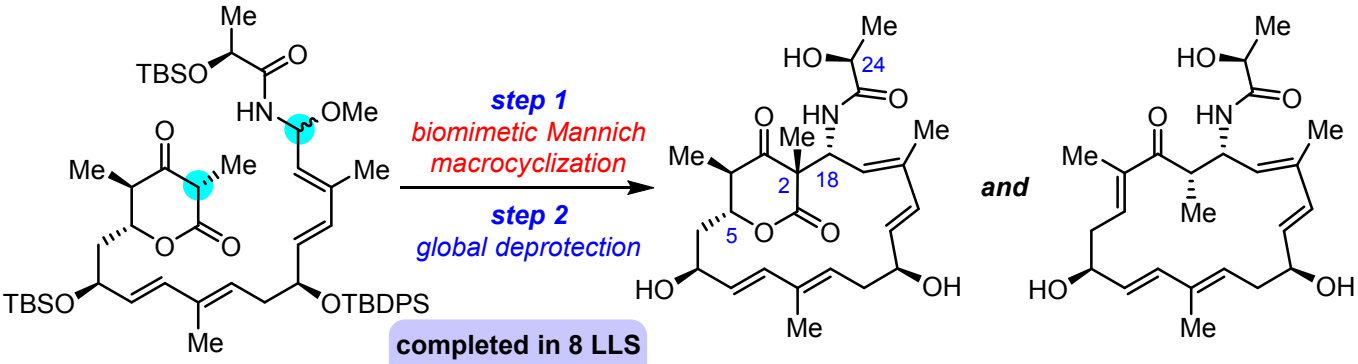
TOC artwork



modular synthesis;
6 steps; gram scale

lankacidinol
previous [C24-oxo]: 34 LLS, 46 TS

lankacyclinol
previous: 25 LLS, 39 TS