A Practical, Component-Based Synthetic Route to Methylthiolincosamine Permitting Facile Northern-Half Diversification of Lincosamide Antibiotics

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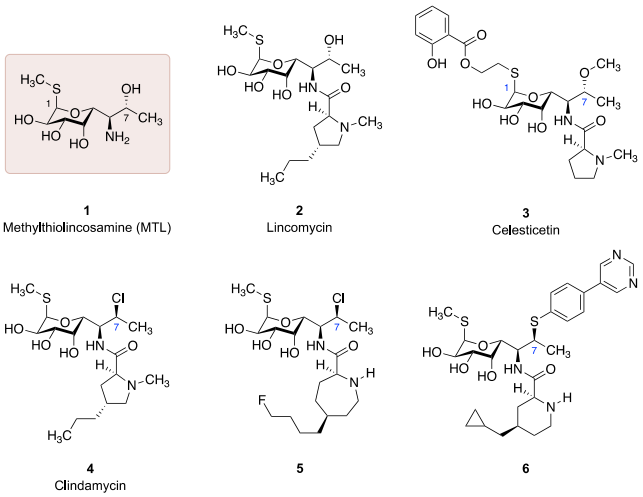
ABSTRACT: The development of a flexible, component-based synthetic route to the aminosugar fragment of the lincosamide antibiotics is described. This synthetic route hinges on the application and extension of nitroaldol chemistry to forge strategic bonds within complex aminosugar targets, and employs a glycal epoxide as a versatile glycosyl donor for the installation of various anomeric groups. Through building-block exchange and late-stage functionalization, this route affords access to a host of rationally designed lincosamides otherwise inaccessible by semisynthesis, and underpins a platform for the discovery of new lincosamide antibiotics.

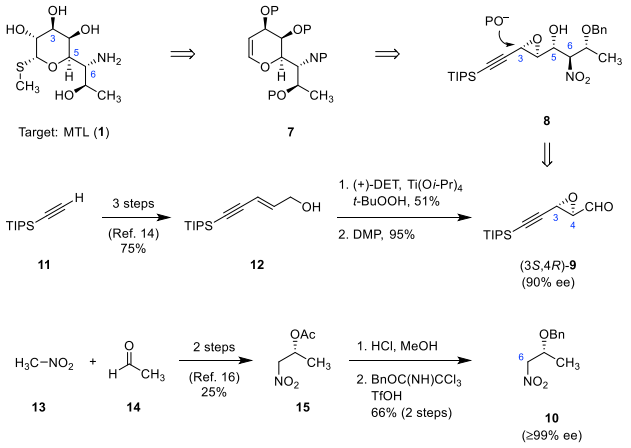
The lincosamide antibiotics have been used to treat staphylococcal and streptococcal infections in humans for more than 50 years, but widespread bacterial resistance and growing safety concerns (specifically, the promotion of opportunistic *C. difficile* infections) have greatly diminished their use in patients. Methylthiolincosamine (MTL, **1**) is the northern-half component of the prototypical lincosamide, lincomycin (**2**),[1] for which the thiogalactopyranose plays an essential role in binding to the bacterial ribosome, the cellular target of the class. X-ray co-crystallographic experiments have shown that the pyranose hydroxyl groups of MTL form an extensive hydrogen-bond network with the neck of the peptide-exit tunnel, including with adenosine 2058, a key residue whose mutation or post-transcriptional modification confers resistance to canonical lincosamides, macrolides and streptogramin B antibiotics – a multidrug resistance phenotype referred to as MLS_B.[2,3] Results from early semisynthetic modifications of the MTL region of lincosamides support the idea that the 2,3,4-triol pharmacophore is indispensable for successful inhibition of translation,[4] whereas modifications of positions C1 and C7 are tolerated,[5] a finding perhaps presaged by the naturally occurring lincosamide antibiotic celesticetin (**3**, reported in 1955).[6]

Although no new lincosamide antibiotic has been advanced since the approval of clindamycin (**4**) in 1970,[7] this is not for lack of effort. Scientists at the former antibiotics enterprise Vicuron discovered beneficial modifications of C7 using both semisynthesis and de novo construction from a carbohydrate precursor employing an extant method.[8,9] In addition, they discovered that azepane replacement of the lower portion of the molecule (**5**) greatly improved the spectrum and potency of antibacterial activity.[8] Scientists at Meiji-Seika have reported that semisynthetic modification of C7 with a biarylthio substituent (as well as lower-half modification, see **6**) imparted exceptional potency against multidrug-resistant Staphylococcus and Streptococcus isolates.[10] These and other precedents encouraged us to attempt to develop a component-based, more streamlined route to MTL that would permit facile structural modifications of positions 1 and 7. Here we report the successful realization of such a route.



In our initial retrosynthetic analysis we envisioned the protected glycal **7** as a key subgoal, imagining in the forward direction selective epoxidation of that intermediate from the bottom face (as drawn) followed by stereoselective addition of various nucleophiles to C1, also along the bottom face (Scheme 1).[11] Based on an earlier success with a related transformation[12] we were optimistic that we could develop methodology for the required stereoretentive nucleophilic addition, and thereby satisfy the goal of late-stage introduction of diverse C1 substituents. In turn, we reasoned that glycal **7** might be assembled by transition metal–catalyzed cycloisomerization of a linear alkynol precursor, such as one formed by regioselective opening of propargylic epoxide **8** with a suitably protected oxygen nucleophile. The alkynyl epoxide **8** by design also comprises a β-hydroxy nitro function,[13] permitting its convergent assembly by a proposed diastereoselective Henry reaction of components **9**, an epoxy aldehyde, and the nitro ether **10**. Varying the latter component would permit facile diversification of C7.

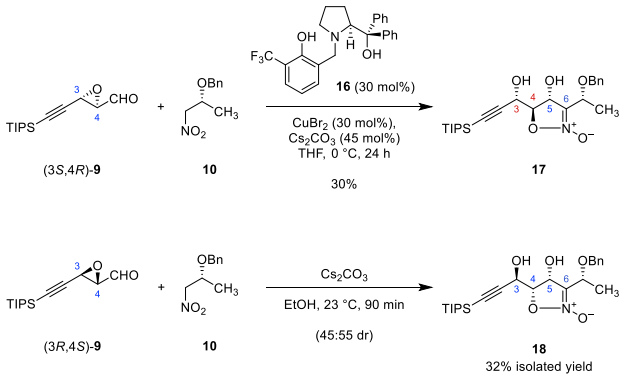
Scheme 1. Retrosynthetic analysis and construction of building blocks for a proposed nitroaldol coupling.



Building blocks **9** and **10** were each readily prepared in multi-gram amounts by known sequences of 4–5 steps from simple starting materials that are available in bulk. The allylic alcohol precursor **12** was prepared by formylation of tri-*iso*-propylsilylacetylene (**11**), Horner–Wadsworth–Emmons olefination, then ester reduction.[14] Sharpless asymmetric epoxidation followed by Dess–Martin periodinane oxidation then furnished epoxy aldehyde (3*S*,4*R*)-**9** (90% ee, determined by Mosher analysis of the epoxy alcohol precursor).[15] The nitro ether **10** was prepared from enantiopure nitro acetate **15**[16] by acetate hydrolysis and *O*-benzylation under acidic conditions, then recrystallization from ethyl acetate–hexane (17.6 g, 66% yield from enantiopure **15**). Spectroscopic data and melting-point determination of the resulting white solid matched literature reports for **10**;[17] the product was found to be optically pure (≥99% ee) by chiral HPLC analysis.

With building blocks **9** and **10** in hand, we investigated their proposed coupling to form nitroaldol adduct **8** (Scheme 2). Under a variety of conditions commonly employed for such couplings (e.g., potassium *tert*-butoxide–tetrahydrofuran, potassium carbonate–methanol, potassium fluoride–isopropanol, silica gel), we observed complex mixtures of diastereomeric nitroaldol addition products, the separation and characterization of which were complicated by their apparent instability toward retro-Henry fragmentation on silica gel. However, when we attempted coupling of **9** and **10** using the chiral ligand **16** and copper(II) bromide, a catalyst system described by Xu et al,[18] we observed the formation of a notably polar by-product, which proved to be an isomer of the desired Henry adduct **8**. Two-dimensional thin-layer chromatography experiments suggested that this product was stable toward silica-gel, permitting its successful chromatographic isolation as a white solid (30% yield). X-ray analysis of this crystalline material revealed it to be the isoxazoline *N*-oxide **17**, evidently arising from the desired Henry adduct (**8**) by nitronate formation and consequent cyclization. Such cyclizations have been previously documented by Righi and co-workers.[19]

Scheme 2. Unanticipated formation of a cyclic nitroaldol adduct, and analogous construction of the MTL 3,4,5-stereotriad.



We recognized that, while unanticipated, isoxazoline *N*-oxide **17** presented several beneficial features in the context of our synthesis goals. These included the stability of the product toward silica gel (in contrast to **8** itself), the potential use of cyclic stereocontrol to bias the subsequent reduction of C6, and the concise internal protection of the functionality at positions C4 and C6 in the form of an *N*–*O* linkage. We wondered whether the 3,4,5-stereotriad of MTL might be established by a similar chemical transformation – similar but not identical, because our original retrosynthetic analysis anticipated inversion at C3 rather than C4 (Scheme 1), as occurs in the formation of **17**. In theory, this could be rectified simply by using the enantiomer of epoxy aldehyde **9**, which we prepared by a two-step route analogous to the one used to provide the (3*S*,4*R*) isomer, with some changes to address scalability.[20] When we prepared (3*R*,4*S*)-**9** and attempted coupling with **10** in the presence of cesium carbonate (EtOH, 23 °C), we obtained after purification by flash-column chromatography the desired cycloadduct **18** in 32% yield (minor) and separately, the corresponding C5 epimeric cycloadduct in 50% yield (major, not depicted, dr 45:55).

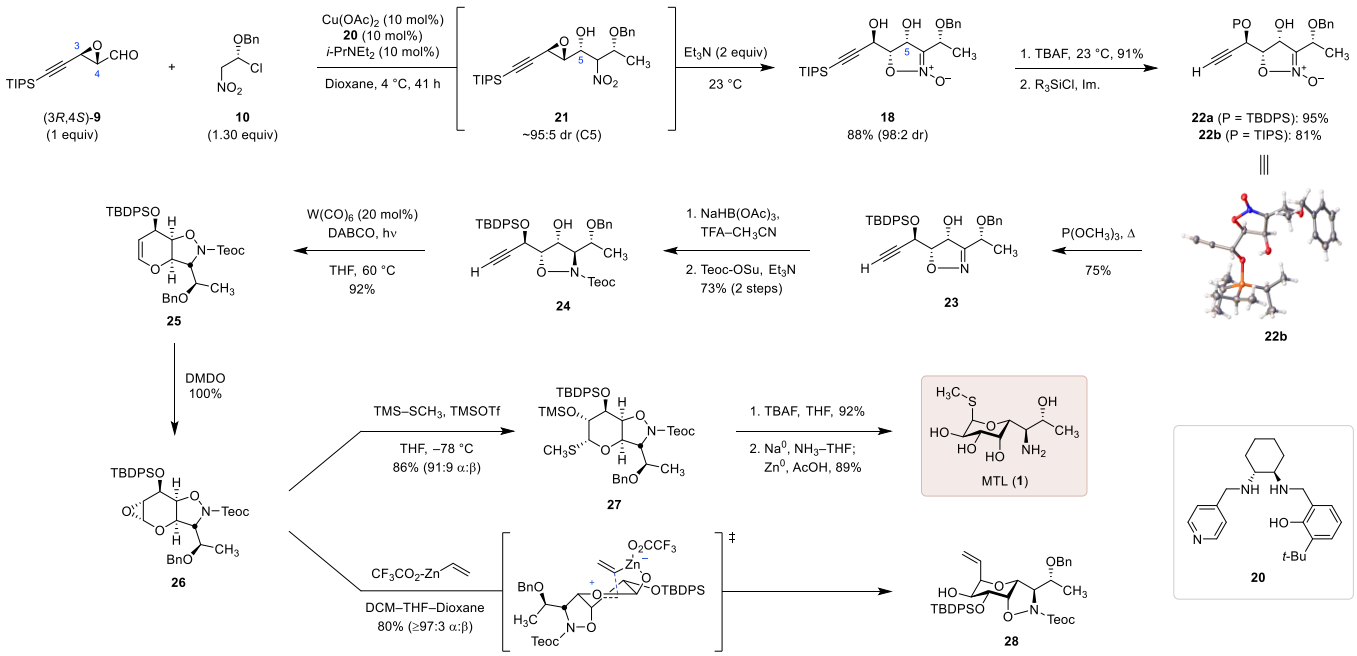
From a screen examining the ability of various chiral catalysts to steer the diastereoselectivity of the coupling of (3*R*,4*S*)-**9** and **10** toward the desired diastereoisomer **18**, we found that a copper(II) system employing cyclohexanediamine ligand **20**[21] at 4 °C afforded the initial nitroaldol product **21** with a C5 dr of ~95:5 (^{1}H NMR analysis; though inconsequential, the distribution of indeterminate C6 epimers was estimated to be ~85:15).[22] Following disappearance of the limiting epoxyaldehyde component, triethylamine was introduced, and the mixture was warmed to promote smooth cyclization of **21** to isoxazoline *N*-oxide **18** (Scheme 3). The overall diastereoselectivity of this transformation was determined by ^{1}H NMR analysis of the reaction mixture at completion, revealing by integration of epimeric C5 methine resonances a dr of 98:2. Thus optimized, this coupling was scaled to produce 16.4 g of **18** in 88% isolated yield in one operation. *C*-Desilylation of this product with tetra-*n*-butylammonium fluoride, followed by selective *O*-protection of the sterically less encumbered propargylic alcohol provided silyl ethers **22** (**a** = TBDPS, **b** = TIPS) in good yield; the crystallinity of **22b** permitted unambiguous assignment of all stereochemistry by single-crystal X-ray diffractometry.

With suitably protected alkynols **22** in hand, we then sought to identify conditions for transition metal-catalyzed cycloisomerization to form the corresponding glycal. We observed

that both the isoxazoline *N*-oxide **22a** and its reduced counterpart **23** (formed in 70% yield upon warming **22a** with trimethylphosphite) were unreactive toward tungsten(0),[23] rhodium(I),[24] and ruthenium(II)[25] catalysts for glycal formation, leading us to speculate that the polar isoxazoline *N*-oxide and isoxazoline functional groups might serve as catalyst poisons. We elected instead to reduce the isoxazoline **23**, choosing conditions conducive toward internal hydroxyl-directed reduction. Thus, exposure of **23** to sodium triacetoxyborohydride in a mixed solvent system comprising trifluoroacetic acid[26] and acetonitrile led to smooth reduction of the C=N double bond to afford only a single diasteroisomer. Protection of the resulting isoxazolidine as its 2-(trimethylsilyl)ethoxycarbonyl (Teoc) derivative then furnished **24**, which proved to be an excellent substrate for tungsten(0)-catalyzed glycal formation using conditions reported by McDonald and coworkers.[23a,b] We found that this sequence of transformations was readily scaled, providing up to 3.5 g of glycal **25** in a single run.

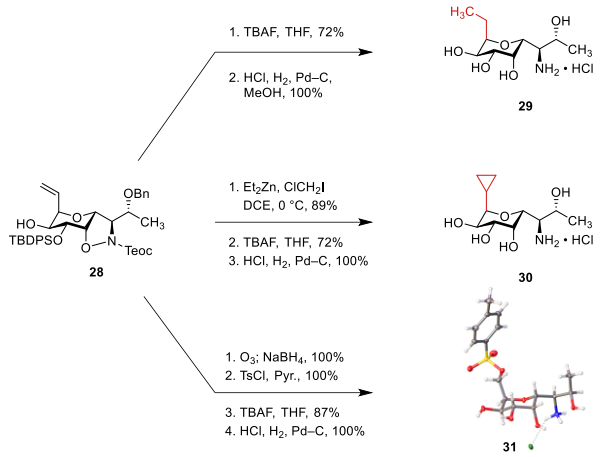
The remaining steps of our original retrosynthetic plan proceeded as envisioned, permitting the synthesis of MTL by a straightforward sequence of epoxidation, thioglycosylation, and deprotection. Epoxidation of glycal **25** with dimethyldioxirane[27,28] proceeded with perfect selectivity for the convex face, providing the epoxide **26** in quantitative yield on scales up to 1.5 g, following evaporation of volatile reaction components, including residual organic oxidant. Cis-α-thioglycosylation was then achieved using trimethyl(methylthio)silane as glycosyl acceptor and trimethylsilyl trifluoromethanesulfonate as a Lewis-acid promoter, producing **27** in 86% yield and 91:9 dr when tetrahydrofuran was used as solvent. Notably, the diastereoselectivity of this transformation was highly dependent on the solvent, and is consistent with a double-displacement model involving ethereal solvent participation.[29] Finally, *O*-desilylation, dissolving-metal debenzylation, and *N*–*O* bond cleavage with zinc in acetic acid (the latter two steps may be performed in the same flask) furnished fully synthetic MTL (**1**) in 82% overall yield from **27**.

Scheme 3. Synthesis of MTL via diastereoselective nitroaldol–cyclization, cycloisomerization, and cis-α-thioglycosylation, and extension of this route to the assembly of a *C*-glycosidic variant.



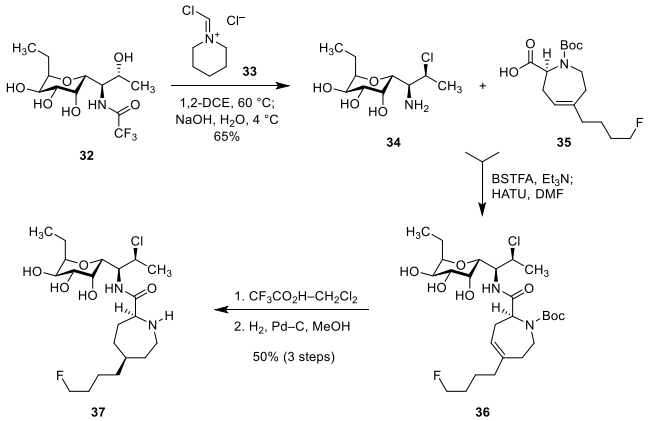
We also discovered that the epoxy glycal intermediate **26** provided a means by which to install alkyl groups at the C1 position with high α-selectivity. Treatment of **26** with vinylzinc trifluoroacetate, an amphiphilic reagent that putatively serves to activate the glycosyl donor while directing nucleophilic attack to the same face of the nascent oxocarbenium ion (pictured), provided the desired *cis*-α-*C*-glycoside in 80% yield as a single diastereomer (Scheme 3).[30] The resulting α-vinylated product **28** was readily transformed into a number of diverse MTL analogs (Scheme 4). For example, isosteric replacement of the methylthio group within MTL was possible through desilylation and hydrogenation of **28**, providing the α-ethyl MTL analog **29**, while cyclopropanation provided access to the α-cyclopropyl analog **30**. Sequential exposure of a methanolic solution of **28** to ozone gas and sodium borohydride produced the corresponding α-hydroxymethyl analog, which could be selectively activated with *p*-toluenesulfonyl chloride. Desilylation and hydrogenation as before then furnished 1-(tosyloxy)methyl lincosamine analog **31**, whose structure was established unambiguously through single-crystal X-ray diffractometry. The latter compound served as a valuable precursor to diverse lincosamides bearing non-natural substitution at the C1 position.

Scheme 4. Elaboration of vinyl glycoside 28 to diverse C1 variants of MTL



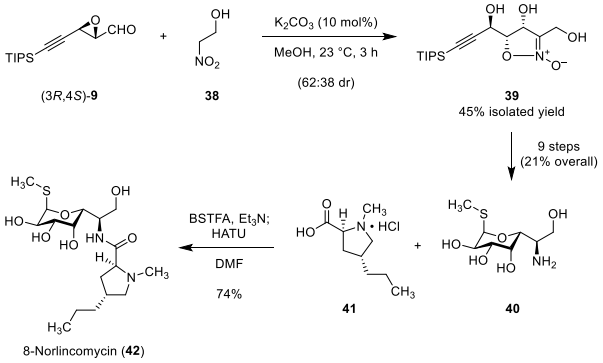
Aminosugar analogs such as **29** were transformed into fully synthetic lincosamide analogs along established paths. First, an abbreviated sequence to protect **29** as its corresponding *N*-trifluoroacetyl derivative was developed: methyl trifluoroacetate and triethylamine were added to the methanolic, heterogeneous hydrogenation reaction mixture used to prepare **29** (Scheme 4) affording analytically pure trifluoroacetamide **32** in 63% yield after aqueous workup. Exposure of the resulting tetraol to 6 equivalents of 1-(chloromethylene)piperidinium chloride (**33**) in hot 1,2-dichloroethane resulted in regioselective, invertive 7-deoxychlorination (Scheme 5).[8b,31] Careful addition of aqueous sodium hydroxide to this reaction mixture then effected hydrolysis of any formate esters that had formed as well as of the trifluoroacetyl group, permitting the isolation of **34** in 65% yield. Convergent coupling to the *N*-Boc–protected southern-half azepane component discovered by Vicuron scientists[8b] was achieved by first protecting the 2,3,4-triol motif within **34** by exposure to 1.5 equivalents of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, followed by the addition of **35**and HATU as the coupling reagent. When amide bond formation was complete, desilylation was performed by the addition of acetic acid and methanol. Removal of the *N*-Boc group of **36** and diastereoselective azepene hydrogenation with palladium on carbon[32] furnished azepanamide **37** in 50% yield from **34**.

Scheme 5. Assembly of a fully synthetic azepanamide.



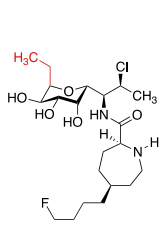
To demonstrate the efficacy of our modular pathway to diversify position C7 of lincosamides, we substituted 2-nitroethanol (**38**) for the nitro ether **10** in the key nitroaldol coupling, obtaining after purification by flash-column chromatography the pure isoxazoline **39** in 45% yield on multigram scale (Scheme 6). The diastereoselectivity of the latter addition was modest (62:38 dr in favor of **39**) but serviceable; efforts to screen chiral catalysts as with **10** afforded no practical advantage in this case. Elaboration of **39** to aminotetraol **40** was achieved as in the synthesis of MTL and proceeded in 21% yield over the 9 steps. Finally, in this illustration we chose to couple with *trans*-4-*n*-propylhygric acid **41**[33] by an otherwise identical sequential silylation–acylation–desilylation protocol to produce 8-norlincomycin (**42**) in 74% yield. Selective functionalization of the primary alcohol group within **42** was possible, permitting the synthesis of 15 additional propylhygramides bearing non-natural substitution to C7.

Scheme 6. Assembly of 8-norlincomycin through building-block exchange.



In this fashion, and through a combination of building-block exchange and late-stage derivatization, a library of 41 lincosamides bearing diverse substitutions at positions 1 and 7 was prepared for evaluation against a panel of pathogenic bacteria (Table 1). Of these analogs, 18 displayed minimum inhibitory concentrations (MICs) of ≤ 4 μg/mL against the standard *Streptococcus pneumoniae* strain ATCC 49619, a Gram-positive isolate susceptible toward canonical lincosamides such as lincomycin and clindamycin (MICs = 0.5 and 0.125 μg/mL respectively). Analysis of structure–activity relationships of a selection of representative lincosamides illuminates a subtle reliance on the C1 thiomethyl substituent of **5** on activity, for instance, as nearly isosteric replacement with ethyl (**37**) or chloromethyl (**43**) groups produced analogs with diminished activities, particularly against Gram–negative and MLS_B Gram–positive strains. Similarly, C7 methylation (as in lincomycin [**2**]) proved critically beneficial to antimicrobial activity, as 8-norlincomycin (**42**), 7-azidolincomycin **47**, and biarylsulfide **49** each displayed significantly greater MICs in all tested organisms relative to their methylated counterparts (for a complete listing of lincosamides synthesized by the routes described here, with corresponding MIC data, see Ref. 5). Together these results suggest particular electronic requirements at the C1 position, and conformationally rigidifying requirements at C7, which may be incorporated into the design and evaluation of new lincosamides targeting challenging drug-resistant pathogens.

Table 1. Structures and minimum inhibitory concentrations (μg/mL) of selected lincosamides prepared by the route described.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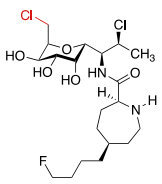
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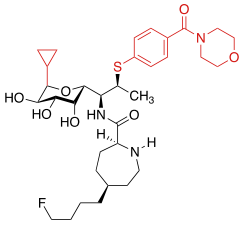
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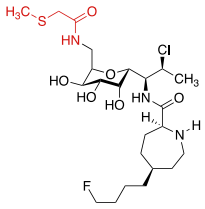
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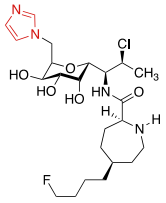
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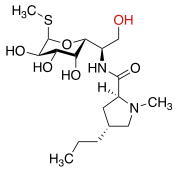
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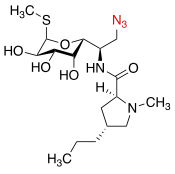
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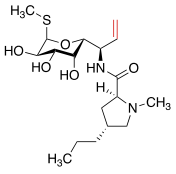
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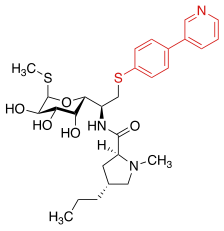
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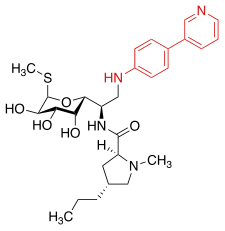
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	Species	Description	Linco	Clinda	5	37	43	44	45	46	42	47	48	49	50
Gram +	*S. aureus*	ATCC 29213	1	0.25	≤0.06	≤0.06	16	8	>64	>64	32	8	8	0.5	32
	S. aureus	BAA 977; iErmA	1	0.25	≤0.06	≤0.06	4	8	>64	>64	NT	NT	8	NT	NT
	S. pneumoniae	ATCC 49619	0.5	0.125	≤0.06	≤0.06	1	0.12	2	8	4	4	2	0.25	8
	S. pneumoniae	MMX 3028; cErmB	>64	>64	8	64	>64	16	>64	>64	NT	>64	NT	>64	>64
	S. pneumoniae	MMX 3031; cMefA	0.25	0.06	≤0.06	≤0.06	1	0.12	4	8	NT	0.125	NT	0.25	32
	S. pyogenes	ATCC 19615	≤0.06	0.06	≤0.06	≤0.06	4	≤0.06	2	4	8	2	2	≤0.06	8
	S. pyogenes	MMX 946; MLS_B	>64	>64	4	64	>64	4	>64	>64	NT	>64	NT	>64	>64
	E. faecalis	ATCC 29212	32	16	≤0.06	>64	>64	>64	>64	>64	64	>64	NT	>64	>64
Gram –	*K. pneumoniae*	ATCC 10031	NT	8	0.5	1	NT	NT	NT	NT	NT	NT	>64	NT	NT
	E. coli	ATCC 25922	>64	>64	4	32	>64	>64	>64	>64	>64	>64	>64	>64	>64
	P. aeruginosa	ATCC 27853	>64	>64	>64	>64	>64	>64	>64	>64	NT	NT	NT	NT	NT
	H. influenzae	ATCC 49247	32	16	0.25	1	>64	>64	>64	>64	NT	NT	NT	NT	NT

By the application and extension of nitroaldol chemistry for complex aminosugar synthesis,[13] we have developed a fully synthetic route to discover new lincosamides bearing modifications at key positions of the methylthiolincosamine moiety. These modifications, which would be difficult or impossible to perform by the semisynthetic approaches historically employed, illuminate the subtle, beneficial effects of the natural C1-thiomethyl and C7-methyl substitutions upon antimicrobial activity of lincosamides.[8a] The route described hinges both upon nitroaldol-based two-component assembly of the aminosugar backbone of MTL with full diastereocontrol, and upon diversification of a glycal epoxide intermediate, the combined flexibility of which permits multiplicative expansion of molecular diversity at positions 1 and 7 of methylthiolincosamine. Together with the concurrent development and application of similarly flexible, convergent routes to novel southern-half residues,[34,35] this work forms the basis of a platform for antibiotic discovery of this underexplored class.

ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and characterization data for all new compounds. Single-crystal X-ray crystallographic data have been deposited at the Cambridge Crystallographic Data Centre under deposition numbers 2072279 (**17**), 2072280 (**22b**), and 2072281 (**31**).

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Notes

A.G.M. and M.J.M. have filed an international patent application WO/2019/032956 'Lincosamide Antibiotics and Uses Thereof.'

ACKNOWLEDGMENT

This work was generously supported by the Blavatnik Biomedical Accelerator Program at Harvard University, and by the LEO Foundation (grant LF18006). M.J.M. was supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE1144152. J.W.S. thanks the Harvard College Research Program for summer undergraduate research funding. The authors thank Dr. Shao-Liang Zheng for conducting X-ray crystallographic analyses, Dr. William Weiss (University of North Texas Health Science Center) and Micromyx L.L.C. for performing antimicrobial screening, and Drs. Ziyang Zhang, Fan Liu, Thomas Dougherty, and Su Chiang for their valuable insights contributed during the execution of this work.

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