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Communication

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

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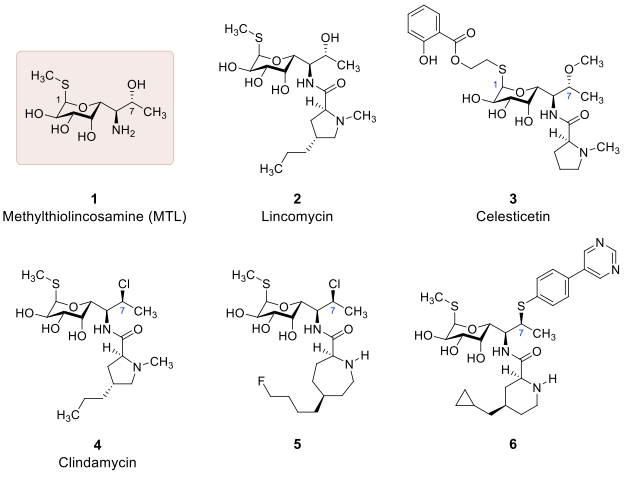
ABSTRACT: The development of a flexible, component-based synthetic route to the amino sugar fragment of the lincosamide antibiotics is described. This route hinges on the application and extension of nitroaldol chemistry to forge strategic bonds within complex amino sugar targets and employs a glycal epoxide as a versatile glycosyl donor for the installation of 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 safety concerns (specifically, a risk of *C. difficile* infection) have 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 lincosamides' cellular target. X-ray co-crystallography has 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 lincosamides, macrolides, and streptogramin B antibiotics, a multidrug resistance phenotype referred to as MLS_B.[2,3] Early semisynthetic modifications to the lincosamides support the idea that the 2,3,4-triol pharmacophore is indispensable, as deoxygenation, O-methylation, epimerization, and other modifications abolish activity.[4] On the other hand, 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 has been advanced since the approval of clindamycin (**4**) in 1970,[7] this is not for lack of effort. Vicuron scientists 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 aminoacyl portion of the molecule (**5**) greatly improved the spectrum and potency of antibacterial activity.[8] Meiji-Seika researchers have reported that semisynthetic modification of C7 with a biarylthio substituent (as well as aminoacyl modification, see **6**) imparted potency against multidrug-resistant strains.[10] These and other precedents encouraged us to attempt to develop a component-based, more streamlined route to MTL that would permit structural modification of positions 1 and 7. Here we report the successful realization of such a route.



Our original retrosynthetic analysis targeted 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 (Scheme 1),[11] following on earlier success with a related transformation.[12] 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**

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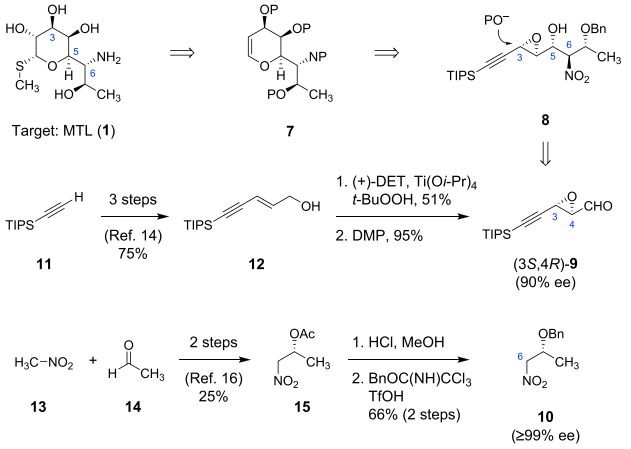
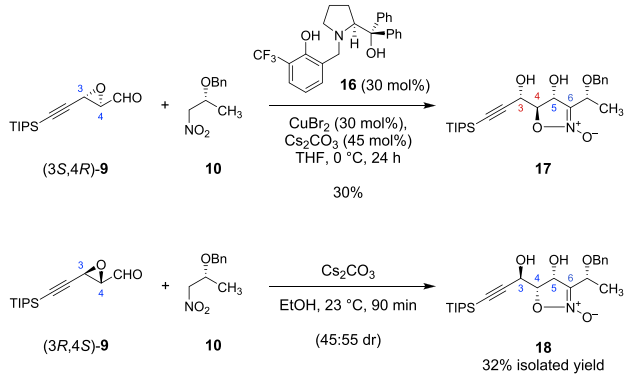
Scheme 1. Retrosynthetic Analysis and Construction of Building Blocks for a Proposed Nitroaldol Coupling



Scheme 2. Unanticipated Formation of a Cyclic Nitroaldol Adduct and Analogous Construction of the MTL 3,4,5-Stereotriad



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, particularly through inclusion of an easily varied element such as an alcohol, alkene, or masked aldehyde, would permit facile diversification of C7.

Building blocks **9** and **10** were each prepared in multigram amounts by known sequences of 4−5 steps from starting materials 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 epoxidation followed by Dess−Martin 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,[18] we observed the formation of a notably polar byproduct, which was isolated in 30% yield and proved to be isomeric with the desired Henry adduct. X-ray analysis of this crystalline material revealed it to be the isoxazoline *N*-oxide **17**, arising from the desired Henry adduct (**8**) by nitronate formation and consequent cyclization. Such cyclizations involving ethyl nitroacetate have been documented by Righi and Jørgensen.[19,20]

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 a 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 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.[21] 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 chromatography the desired cycloadduct **18** in 32% yield (minor) and, separately, the 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**[22] 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).[23] 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).[24] Thus, this optimized 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),[25] rhodium(I),[26] and ruthenium(II)[27] 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

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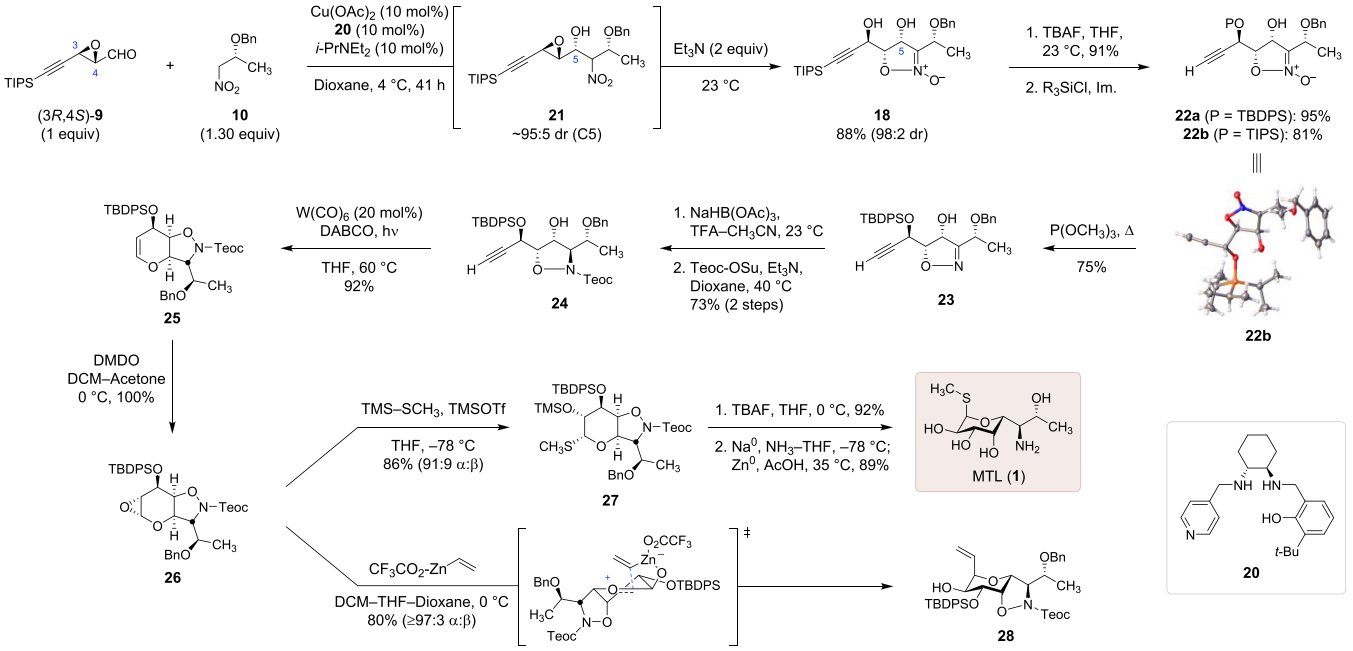
Scheme 3. Synthesis of MTL via Diastereoselective Nitroaldol Cyclization, Cycloisomerization, and *cis*-α-Thioglycosylation and Extension of This Route to the Assembly of a *C*-Glycosidic Variant

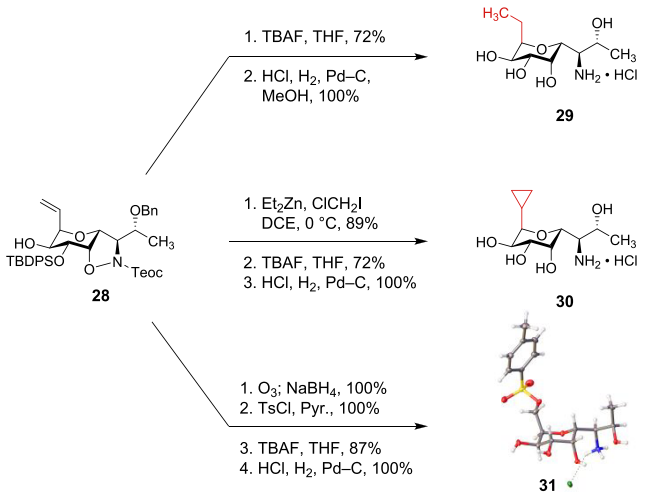


isoxazoline **23**, choosing conditions conducive toward internal hydroxyl-directed reduction. Thus, exposure of **23** to sodium triacetoxyborohydride in a mixed solvent system comprising trifluoroacetic acid[28] and acetonitrile led to smooth reduction of the C═N double bond to afford only a single diastereoisomer. 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 co-workers.[25a,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[29,30] proceeded with perfect selectivity for the convex face, providing the epoxide **26** in quantitative yield on scales up to 1.5 g. *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.[31] 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**.

Epoxide **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).[32] 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

Scheme 4. Elaboration of Vinyl Glycoside 28 to Diverse C1 Variants of MTL



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.

Amino sugar **29** was transformed into fully synthetic lincosamide analog **37** following established paths (Scheme 5).[8b] Subjecting **32**, the *N*-trifluoroacetamide of **29**, to 6 equiv of 1-(chloromethylene)piperidinium chloride (**33**) resulted in

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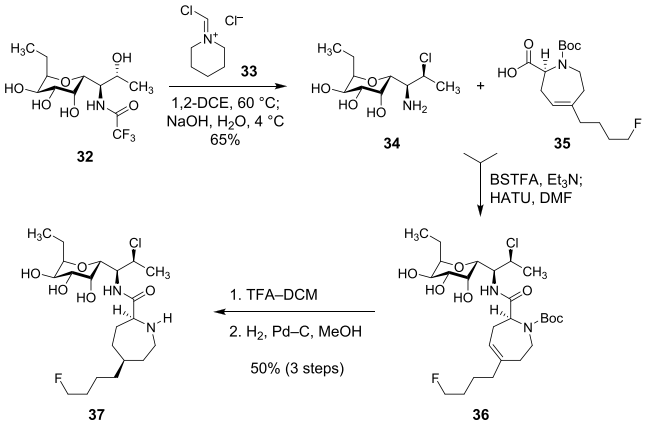
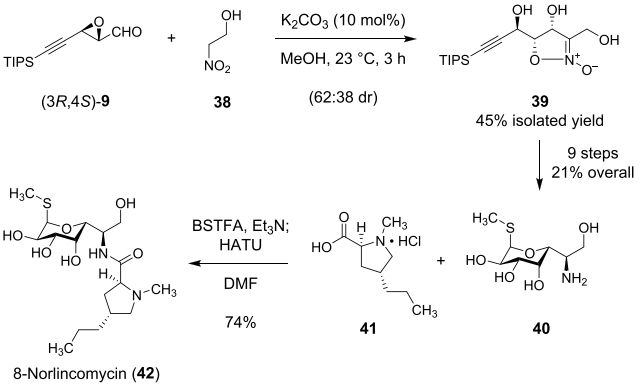
Scheme 5. Assembly of a Fully Synthetic Azepanamide



Scheme 6. Assembly of 8-Norlincomycin through Building-Block Exchange

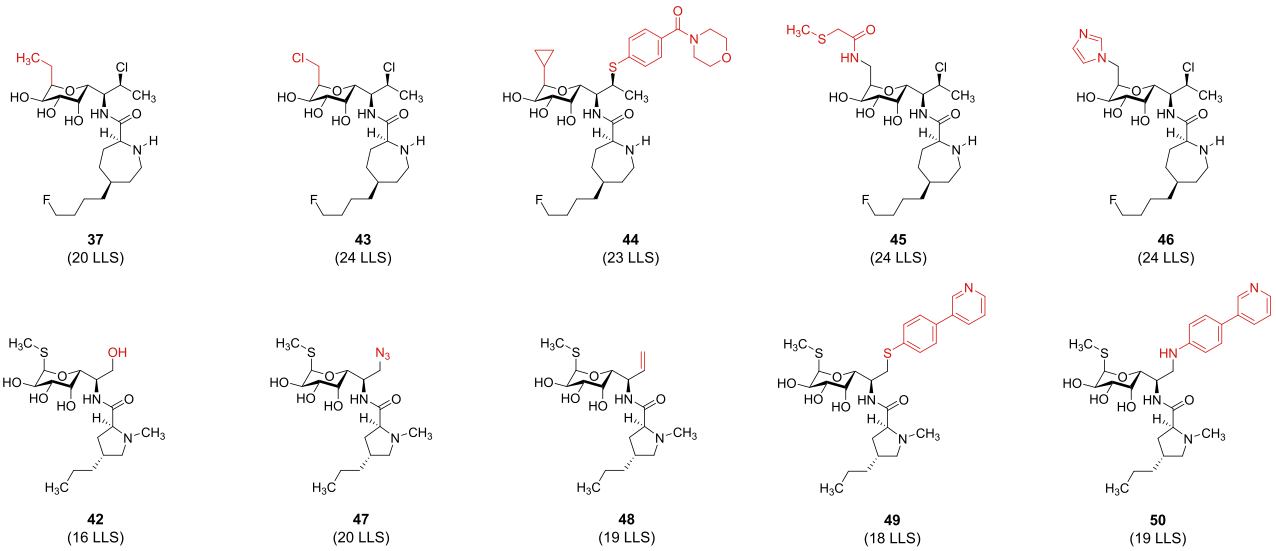


regioselective 7-deoxychlorination;[33] careful addition of sodium hydroxide then effected hydrolysis of pyranose formate esters and the nitrogen protecting group. Convergent coupling to the aminoacyl component discovered by Vicuron scientists was performed next, followed by *N*-Boc removal and diastereoselective azepane hydrogenation[34] to furnish azepanamide **37** in 50% yield from **34**.

In order to explore C7 substitution effects within the lincosamides, we substituted 2-nitroethanol (**38**) for the nitro ether **10** in the key nitroaldol coupling, obtaining 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**[35] 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.

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

Table 1. Structures and Minimum Inhibitory Concentrations (μg/mL) of Selected Lincosamides Prepared by the Route Described



37 (20 LLS) **43** (24 LLS) **44** (23 LLS) **45** (24 LLS) **46** (24 LLS)

42 (16 LLS) **47** (20 LLS) **48** (19 LLS) **49** (18 LLS) **50** (19 LLS)

	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; i-*ermA*	1	0.25	≤0.06	≤0.06	4	8	>64	>64	NT	NT	8	NT	NT
	S. pneumoniae	ATCC 49619	0.5	0.12	≤0.06	≤0.06	1	0.12	2	8	4	4	2	0.25	8
	S. pneumoniae	MMX 3028; c-*ermB*	>64	>64	8	64	>64	16	>64	>64	NT	>64	NT	>64	>64
	S. pneumoniae	MMX 3031; c-*mefA*	0.25	0.06	≤0.06	≤0.06	1	0.12	4	8	NT	0.12	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. pneumnoniae*	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

MIC Color Scale (μg/mL): ≤0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | >64

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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[36] 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.

Extending nitroaldol chemistry for amino sugar synthesis,[13] we have developed a platform to discover new lincosamides with variations at positions 1 and 7. These modifications would have been difficult or impossible to achieve using semisynthesis. The route features two-component assembly by a nitroaldol cyclization reaction with full diastereocontrol and a versatile glycal epoxide intermediate. With concurrent development of similarly flexible routes to novel southern-half residues,[37,38] this work provides the basis for broad discovery within this underexplored antibiotic class.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c03536.

Detailed experimental procedures and characterization data for all new compounds (PDF)

Accession Codes

CCDC 2072279−2072281 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare the following competing financial interest(s): A.G.M. and M.J.M. have filed an international patent application, WO/2019/032956, Lincosamide Antibiotics and Uses Thereof.

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