A practical synthesis of macrobicyclic thiolincosamines

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ABSTRACT: Scalable syntheses of the northern macrobicyclic thiolincosamine fragments of two structurally complex antibiotic candidates, BT-33 and cresomycin, are presented. A key transformation in each route is the highly diastereoselective addition of a putative allenylzinc nucleophile to a common Ellman sulfinimine intermediate using a zinc-promoted Barbier-type propargylation protocol that is detailed herein. These transformations proceed with dynamic kinetic resolution and use just 1.2 equivalents of each respective propargyl bromide precursor.

INTRODUCTION AND BACKGROUND

The lincosamide antibiotics inhibit bacterial protein synthesis by binding to the 50S subunit of the bacterial ribosome, near the peptidyl transferase center.[1] The last lincosamide antibiotic to receive FDA approval was clindamycin, in 1970, but after more than 50 years of use in hospitals and the community, bacterial resistance to clindamycin is now widespread.[2] For several years our laboratory has sought to explore and expand the lincosamides and other classes of antibiotics using convergent chemical synthesis. Recently, we reported the discovery of the potent, broad-spectrum antibiotics **BT-33**[3] and cresomycin (**CRM**)[4] (Figure 1).

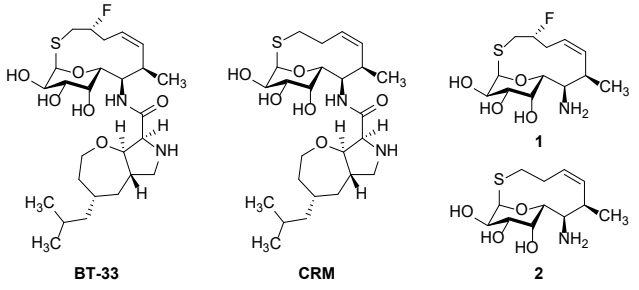
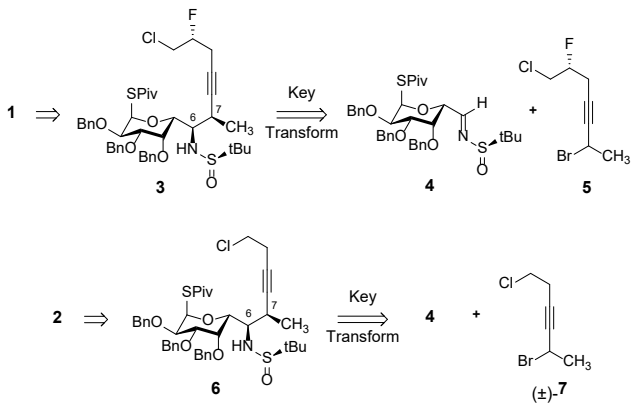


Figure 1. Chemical structures of **BT-33** and **CRM**, alongside their respective northern macrobicyclic fragments, **1** and **2**.

BT-33 and **CRM** comprise structurally distinct, conformationally restricted macrobicyclic northern halves, and share a common oxepanoprolinamide (OPP) southern scaffold, the latter emerging from the discovery of iboxamycin.[5] Like the macrobicyclic northern halves, the OPP southern fragment contains a new, fused ring that rigidifies the molecule with respect to natural or semi-synthetic lincosamides. In view of their promising in vitro and in vivo potencies, we hope to advance both **BT-33** and **CRM** in pre-clinical, investigational new drug (IND)–enabling studies, but these experiments will require multi-gram quantities of each of the structurally distinct northern fragments (11- and 10-membered macrobicyclic thiolincosamines **1** and **2**, respectively, Figure 1). Using the new, scalable synthetic routes reported herein, we believe that we can now meet these objectives. Both of the two routes described below employ a new carbon-carbon bond-forming reaction that creates adjoining stereocenters highly selectively, and both feature different strategies for transannular macrocyclization.

The discovery synthetic route to **CRM** employed a Grubbs ring-closing olefin metathesis[6] reaction for macrobicycle formation and was suitable for gram-scale synthesis[4]; however, **BT-33** was one of two co-products of a single deoxyfluorination reaction, and was isolated in low yields after a challenging chromatographic purification.[3] **BT-33** was the first 11-membered macrobicyclic antibiotic candidate we had synthesized and demonstrated extraordinary potencies against multidrug-resistant (MDR) Gram-positive and Gram-negative bacteria in in vitro MIC analyses, making it a particularly attractive molecule for advancement. Unfortunately, attempts to prepare **BT-33** and other 11-membered macrobicyclic analogs by using the Grubbs ring-closing metathesis reaction were not successful because (*E*)- not (*Z*)-alkene products were favored in these macrocyclization reactions (not depicted). For these reasons, we considered an entirely new strategy for macrocyclic construction, one centered on C–S bond formation rather than C=C bond formation for ring closure. The retrosynthetic analyses for the macrobicyclic thiolincosamines **1** and **2** are depicted in Scheme 1.

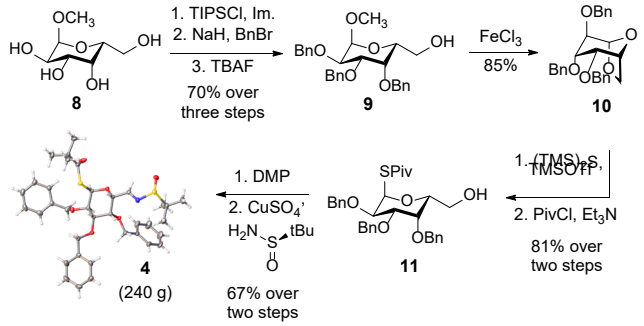
Scheme 1. Retrosynthetic Analyses of 1 and 2



RESULTS AND DISCUSSION

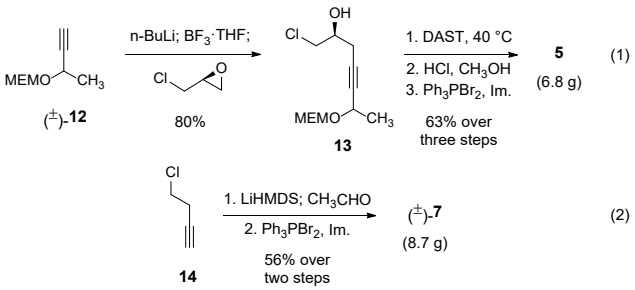
Retrosynthetic Analysis. We imagined forming the (*Z*)-alkenes of targets **1** and **2** by semihydrogenation of an alkyne precursor, either before or after macrocyclization by using an intramolecular C–S bond-forming reaction (see precursors **3** and **6**, respectively, Scheme 1). This in turn led us to consider establishing the C6-C7 *anti*-stereodiad within intermediates **3** and **6** by *anti*-addition of a (chiral) allenylzinc reagent to the Ellman sulfinimine substrate **4**. Allenylzinc reagents are readily obtained by γ-lithiation-transmetalation of an alkyne precursor,[7] or, as in this work, by direct insertion of zinc metal into a propargyl bromide precursor such as compound **5** or **7** (Scheme 1).[8] Foundational studies by others have shown that allenylzinc intermediates readily add to carbonyl compounds and imines (including Ellman *tert*-butylsulfinimines),[9–13] that in general the additions proceed with *anti*-selectivities,[9–13] and that at low temperatures the rate of racemization of chiral allenylzinc intermediates is typically slower than the rate of addition of the organometallic intermediates to the different electrophiles.[11,12] To study the key carbon-carbon bond-forming reactions proposed in Scheme 1, we developed scalable synthetic routes to the common Ellman *tert*-butylsulfinimine intermediate **4** (Scheme 2) and the two different propargyl bromides **5** and **7** (Scheme 3).

Scheme 2. Synthesis and Single-crystal X-ray Structure of the Ellman Sulfinimine 4



Synthesis of the Ellman Sulfinimine 4. Component **4** was prepared on 9-g scale in our laboratory, and subsequently on 240-g scale by a contract research organization, using the eight-step sequence depicted in Scheme 2, beginning with methyl α-D-galactopyranoside (**8**) as starting material. The latter is both inexpensive and commercially available in kilogram amounts. The primary hydroxyl group of tetraol **8** underwent selective protection in the presence of triisopropylsilyl chloride (TIPSCl) and imidazole; perbenzylation of the remaining three hydroxyl groups and removal of the silyl protecting group then afforded the primary alcohol **9** in 70% yield for the three-step sequence.[14] Intermediate **9** cyclized in the presence of ferric chloride, forming the tribenzylated derivative **10**,[15] a transformation we conducted routinely to prepare **10** in >10-g amounts. Alternatively, **10** can be prepared in one step by per-benzylation of 1,6-anhydro-β-D-galactopyranose,[16] which is also commercially available, but more expensive than **8**. Prepared by either sequence, intermediate **10** was next transformed in a stereospecific ring-opening reaction using bis(trimethylsilyl)sulfide as nucleophile and trimethylsilyl triflate as Lewis acid.[17] The resultant *S*-trimethylsilyl thioether hydrolyzed during aqueous workup; the free thiol was reprotected as the *S*-pivaloyl thioester **11** using pivaloyl chloride and triethylamine in dichloromethane. The two-step sequence afforded **11** exclusively as the α-anomer, in 81% yield. Oxidation of the primary hydroxyl group within **11** using the Dess-Martin periodinane (DMP) followed by condensation of the product aldehyde with (*R*)-*tert*-butylsulfinamide[18] completed the sequence to the Ellman sulfinimine **4** (67% yield over two steps). After purification by flash column chromatography, **4** was recrystallized from ethyl acetate–hexanes as white needle-like crystals. Analysis of these by single-crystal X-ray diffraction (thermal ellipsoids depicted in Scheme 2) unambiguously confirmed structure **4**, including all stereochemical assignments.

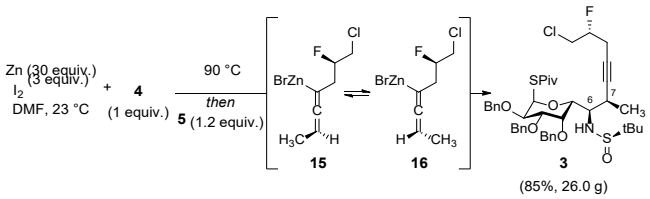
Scheme 3. Syntheses of Propargyl Bromides 5 and (±)-7



Syntheses of Propargyl Bromides 5 and (±)-7. The two different propargyl bromide allenylzinc precursors were prepared on multi-gram scale using the simple reaction sequences depicted in Scheme 3. The route to bromide(s) **5**, precursor to the thiolincosamine **1** and thereby **BT-33**, is depicted first (eq 1). Lithiation of the racemic terminal alkyne **12** and subsequent addition of boron trifluoride etherate and (*S*)-epichlorohydrin in sequence afforded the alcohol(s) **13** as a 1:1 mixture of diastereomers in 80% yield after purification by flash column chromatography. Stereoinvertive deoxyfluorination with diethylaminosulfur trifluoride (DAST) at 40 °C, MEM ether cleavage with HCl in methanol, and then bromination of the resulting diastereomeric mixture of secondary alcohols gave bromide(s) **5** in 63% yield for the three-step sequence (7-g scale). The bromide (±)-**7**, precursor to thiolincosamine **2** and thereby **CRM**, was prepared by coupling of lithiated 1-chloro-3-butyne (**14**) with acetaldehyde followed by bromination, as shown in eq 2 of Scheme 3 (9-g scale). Each of the depicted sequences to the two propargyl alcohol precursors of bromides **5** and (±)-**7** (Scheme 3) has been replicated on 50-g scale by a contract research organization.

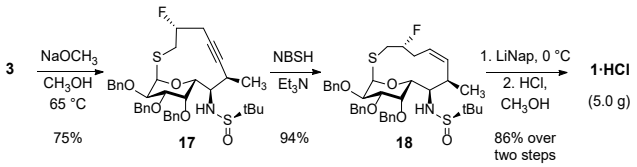
Synthesis of the 11-Membered Macrobicyclic Thiolincosamine 1, Precursor to BT-33. With readily scalable routes to the Ellman sulfinimine **4** and the two propargyl bromide coupling partners **5** and **7**, we next developed methodology that proved to be generally enabling for both coupling reactions on decagram scale. The chemistry is depicted for the specific instance of propargyl bromide **5** in Scheme 4, wherein we propose the occurrence of a dynamic kinetic resolution of allenylzinc diastereomers **15** and **16** at the reaction temperature of 90 °C in *N*,*N*-dimethylformamide (DMF) as solvent.

Scheme 4. Barbier-type Diastereoselective Propargylation of Sulfinimine 4 with Propargyl Bromide 5



As shown in Scheme 4, this Barbier-type protocol for in situ allenylzinc formation from just 1.2 equivalents of bromide **5** (relative to sulfinimine **4**) afforded the coupling product **3** as a single diastereomer (>49:1 dr at both C6 and C7 by ^{1}H NMR analysis) in 85% yield on 26-g scale.

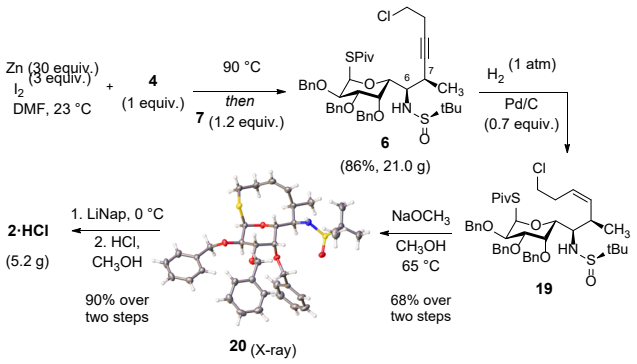
Scheme 5. Synthesis of 1·HCl



Heating thioester **3** with sodium methoxide (1.2 equiv.) in methanol at reflux effected concomitant thiopivaloate deprotection and intramolecular *S*-alkylation to provide the 11-membered macrobicyclic alkyne **17** in 75% yield (17-g scale, Scheme 5). Reduction of **17** with diimide, generated in situ by decomposition of *o*-nitrobenzenesulfonylhydrazide (NBSH, 2.0 equiv.)[19,20] at 23 °C, proceeded in 94% yield and afforded the (*Z*)-alkene product **18** stereospecifically. Benzyl deprotection of **18** with lithium naphthalenide (LiNap) at 0 °C, followed by sulfinamide cleavage with HCl in methanol at 23 °C, produced the macrobicyclic thiolincosamine **1** as its hydrochloride salt in 86% yield over two steps (5-g scale).

Synthesis of the 10-Membered Macrobicyclic Thiolincosamine 2, Precursor to CRM. The same Barbier-type protocol, when separately applied using the propargyl bromide (±)-**7** and the Ellman sulfinimine **4** as substrates (Scheme 6), afforded product **6** (86% yield on 21-g scale), also as a single diastereomer (>49:1 dr at both C6 and C7 by ^{1}H NMR analysis).

Scheme 6. Synthesis of 2·HCl



Attempts to transform the alkyne **6** into the corresponding 10-membered macrobicyclic alkyne (see the analogous transformation of **3** to **17**, Scheme 5) were unsuccessful; the 20-membered dimer was formed instead. Therefore, the alkyne **6** was exposed to hydrogen gas (1 atm) in the presence of palladium on carbon (0.7 equiv.) at 23 °C to furnish the (*Z*)-alkene **19** selectively, without overreduction or benzyl deprotection (Scheme 6). When the (*Z*)-alkene **19** was heated with sodium methoxide (1.2 equiv.) in methanol at reflux, macrobicycle **20** was obtained in 68% yield over two steps (12-g scale) as white block-like crystals suitable for X-ray diffraction (thermal ellipsoids shown in Scheme 6). The benzyl and sulfinamide groups were removed by the same two-step sequence employed for the synthesis of **1**, described above, to furnish macrobicyclic thiolincosamine **2** as its hydrochloride salt in 90% yield over two steps (5-g scale).

Discussion. Certain chiral allenylzinc reagents are known to be configurationally stable at temperatures up to –10 °C in tetrahydrofuran[11] and do not readily interconvert between enantiomeric forms even at room temperature in ethyl ether.[12] Highly diastereoselective propargylation reactions have been documented to occur using ≥2 equivalents of racemic allenylzinc nucleophiles and 1 equivalent of an enantiopure chiral imine as electrophile to form "matched pair" *anti*-products,[9,10,12,13] but in the present context such an approach was considered impractical, leading us to explore the dynamic kinetic resolutions we report, using just 1.2 equivalents of each of the two propargyl bromide substrates. We are unaware of previous examples of such an approach, and anticipate that the Barbier-type coupling process reported herein may prove to be of general value. It should be noted that attempts to transform bromide (±)-**7** into the corresponding allenylzinc reagent in DMF at 90 °C,[21] with subsequent addition of the Ellman sulfinimine coupling partner **4**, were not successful and provided complex product mixtures that were not easily characterized.

CONCLUSION

We envision that the synthetic routes reported herein will provide the foundations for a potential manufacturing route to both **BT-33** and **CRM**. There is great benefit to having sulfinimine **4** as a common, late-stage intermediate in both synthetic routes. First, sulfinimine **4** can be accessed on large scale (240 g) and is highly crystalline. Second, macrobicyclic thiolincosamines (such as **1** and **2**) can be synthesized in just five steps from sulfinimine **4** on multi-gram scale. Lastly, in addition to supporting the gram-scale syntheses of macrobicyclic thiolincosamines **1** and **2**, we expect that other Barbier-type propargylation reactions of sulfinimine **4** will be useful for the discovery of future novel northern macrobicyclic antibiotic precursors.

ASSOCIATED CONTENT

Detailed experimental procedures and NMR characterization data (PDF) are provided as supporting information. This material is available free of charge via the Internet at http://pubs.acs.org. Single-crystal X-ray crystallographic data for sulfinimine **4** and macrobicyclic thiolincosamine **20** are deposited at the Cambridge Crystallographic Data Centre (CCDC) under deposition numbers 2376059 and 2376060, respectively.

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Author Contributions

#These authors contributed equally.

Notes

K.J.Y.W., B.I.C.T., and A.G.M. have filed patent application WO/2023/205206, "Lincosamides and Uses Thereof." and US provisional patent application U.S. 63/676,017, "Synthesis of Macrobicyclic Thiolincosamines." B.I.C.T., K.J.Y.W., and A.G.M. are shareholders of Kinvard Bio, Inc. All other authors declare that they have no competing financial or non-financial interests.

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