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Asymmetric Synthesis of (2*S*,3*R*)-Capreomycidine and the Total Synthesis of Capreomycin IB

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Abstract: A 27 step total synthesis of the tuberculostatic macrocyclic peptide antibiotic capreomycin IB has been accomplished. The synthesis features the use of an enolate−aldimine condensation between a chiral glycine aluminum enolate and the benzyl imine of 3-*tert*-butyldimethylsiloxy-propanal as a means of preparing the cyclic guanidine amino acid (2*S*,3*R*)-capreomycidine. Additionally, a Hofmann rearrangement was exacted on a late-stage pentapeptide in order to transform an asparagine residue into a diaminopropanoic acid residue.

Introduction

The tuberculostatic cyclic peptide antibiotics capreomycins IA, IB, IIA, and IIB (**1a**−**d**) were isolated from *Streptomyces capreolus* by Herr and co-workers at Eli Lilly in 1959 (Figure 1).[1] An original structural proposal for capreomycin IB made by Bycroft et al.[2] was later corrected by Shiba and co-workers who completed the first total syntheses of capreomycins IA and IB.[3] The capreomycins all contain two diaminopropanoic acid residues, the α,*β*-unsaturated amino acid ureido-dehydroalanine and the intriguing cyclic guanidino amino acid (2*S*,3*R*)-capreomycidine (**2**). Capreomycins IA and IIA both contain serine in the cyclic peptide structure (R_1 = OH), while capreomycins IB and IIB contain alanine (R_1 = H). Additionally, the IA and IB forms contain a pendant *β*-lysine (**3**) group, while the IIA and IIB forms are devoid of this substituent.

The capreomycins, which have enjoyed clinical utility for many years, have been the subject of renewed interest recently due to their effectiveness against multidrug-resistant strains of *Mycobacterium tuberculosis*.[4] This is of particular importance to individuals with compromised immune systems who are especially susceptible to these infections.[5] The capreomycins are thought to have the same mode of action as the structurally similar peptide antibiotic viomycin (*aka* tuberactinomycin B).[6] It has been proposed that capreomycin inhibits protein biosynthesis in prokaryotes in two ways: (1) inhibition of the translocation of peptidyl tRNA and (2) inhibition of the dissociation of the ribosomal subunits. Various structural analogues of the capreomycins have been prepared semisynthetically, and some of these agents have been shown to have broad-spectrum antimicrobial activity unlike that of capreomycin itself.[7]

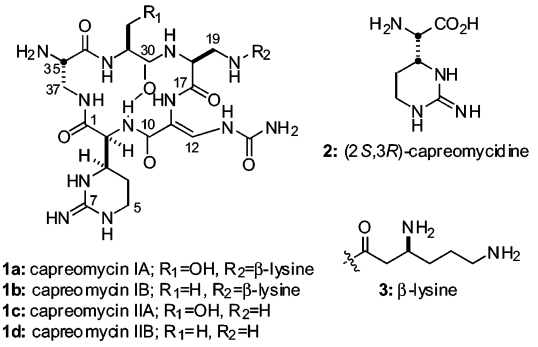

Figure 1. Structures of the capreomycins.

One of the key constituents of the capreomycins and the related tuberactinomycins is the cyclic guanidine-containing

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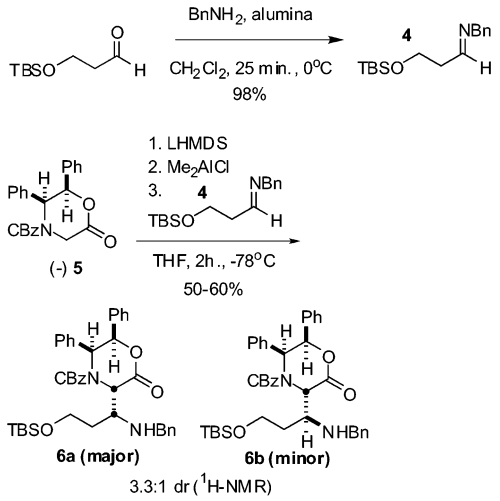
Scheme 1. Enolate−Aldimine Reaction



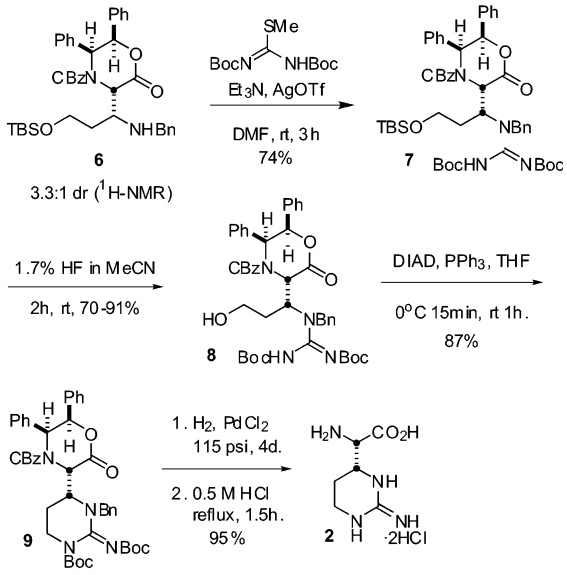
amino acid capreomycidine. Cameron and Bycroft reported the first synthesis of racemic capreomycidine (**2**) in 1971.[8] Subsequently, Shiba et al. reported both a racemic and an enantioselective synthesis of **2**.[8] Shiba's approach to enantiomerically pure (2*S*,3*R*)-capreomycidine was accomplished in 14 steps and an overall yield of 0.14%. Recently, Zabriskie and co-workers completed a ^{13}C-labeled synthesis of **2** that was developed for biosynthetic studies.[9] Our objectives were to develop a more efficient synthesis of this distinctive amino acid which could be seamlessly incorporated into an efficient total synthesis of capreomycin IB and congeners. In addition, the unique structural motifs present in the capreomycins and their interesting biological activity render this family of antimicrobial agents attractive targets for total synthesis. Herein, we report a concise total synthesis of capreomycin IB that is suitable for the preparation of analogues not readily accessible semisynthetically from natural material.

Results and Discussion

The synthesis of capreomycin IB began with our investigations into a concise synthesis of the cyclic guanidino amino acid (2*S*,3*R*)-capreomycidine (**2**).[10] We envisioned that **2** could be accessed via a novel enolate−aldimine reaction between chiral glycinate (−)-**5** and benzyl imine **4** (Scheme 1).

Imine **4** was prepared in 98% yield by treatment of 3-*tert*-butyldimethylsiloxy-propanal with benzylamine on alumina.[11] Initial attempts to exact a condensation of imine **4** with the boron enolate of (−)-**5** resulted in a less than 20% yield of a stable boron-chelate product that proved recalcitrant to transformation into **6**. As previously described, formation of the lithium enolate of (−)-**5** with lithium hexamethyldisilazide at −78 °C was followed by transmetalation with dimethylaluminum chloride to provide the requisite aluminum enolate. Addition of imine **4** resulted in a 50−60% yield of Mannich products **6a** and **6b** as an inseparable 3.3:1 mixture of diastereomers epimeric at the

Scheme 2. Completion of (2*S*,3*R*)-Capreomycidine



β-carbon (^{1}H NMR). Attempts to improve the selectivity of this transformation by employing diverse enolate motifs with the analogous *p*-methoxybenzyl imine resulted in either no reaction or significantly lower yield and/or selectivity when compared with the aluminum enolate (see Supporting Information). By changing the imine nitrogen substituent in a similar system, it was found that the use of a benzhydryl imine rather than a benzyl imine resulted in a significant improvement in the diastereoselectivity (6.5:1 vs 3.3:1) but a lower yield (22%).[12] Attempts to guanidinylate this Mannich product were unsuccessful, presumably due to the added steric hindrance of the benzhydryl group. Although not further pursued in the synthesis of capreomycidine, the result leaves open the possibility for additional optimization of this methodology.

In our initial report, guanidinylation of Mannich product **6** was accomplished with triethylamine, mercuric chloride, and *N*,*N*′-di-*tert*-butoxycarbonyl-*S*-methyl isothiourea.[10] After our communication of these results, we found that silver triflate could be substituted for mercuric chloride, providing an improved 75% yield of **7** (Scheme 2). The use of silver triflate provided a significantly cleaner reaction profile and had the added benefit of eliminating the need for difficult to remove mercury salts. The completion of the synthesis of capreomycidine was accomplished by the method previously described.

With the completion of the synthesis of (2*S*,3*R*)-capreomycidine, our attention was turned to the total synthesis of cap-

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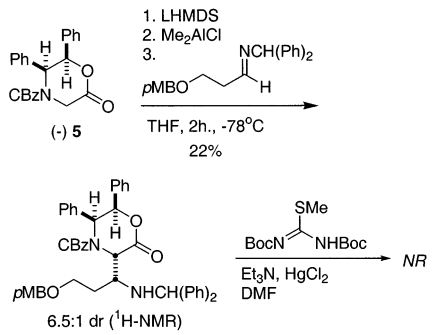
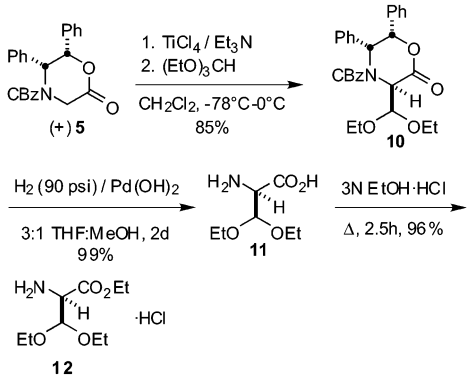
(11) Texier-Boullet, F. *Synthesis* **1985**, 679−681.

(12) The results mentioned were observed with the 3-(*p*-methoxy)benzyloxy-propanaldimine:



Scheme 3. Preparation of the Diethylacetal of *R*-α-Formylglycine



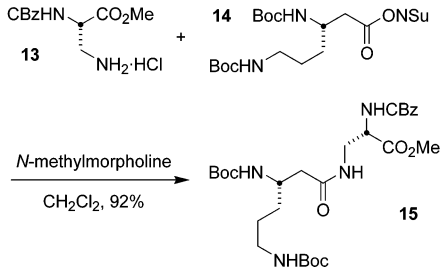
reomycin IB. In his synthetic work with both the capreomycins and structurally similar tuberactinomycins, Shiba showed that the enamidourea functionality could be accessed from the diethylacetal of α-formylglycine.[13] Previous preparations of acetals of α-formylglycine have all been carried out in racemic form.[14] It was our goal to prepare an enantiomerically pure form of α-formylglycine dimethylacetal such that both diastereomeric linear peptides (constituted with each antipode of the α-formylglycine) could be prepared and the macrocyclization propensities of the two species independently assessed.

In this work previously described from our laboratory, the preparation of *R*-α-formylglycine dimethylacetal was accomplished in two steps from (+)-**5**.[15] The resulting amino acid can be either amine- or carboxyl-protected and incorporated into peptides. While epimerization appeared to be minimal when coupling *N*-protected forms of *R*-α-formylglycine dimethylacetal, attempts to incorporate the methyl ester hydrochloride salt of *R*-α-formylglycine dimethylacetal into peptide coupling sequences resulted in a significant amount of epimerization. Furthermore, when attempting to hydrolyze the dimethylacetal after incorporation into a cyclic peptide, it was found that even after several hours at reflux in 2 N HCl in acetone (conditions used to cleave Shiba's diethylacetal after only 10 min), some of the dimethylacetal still remained. The unexpected difference in the acid lability of the methyl- and ethylacetals mandated the employment of the more labile diethylacetal.

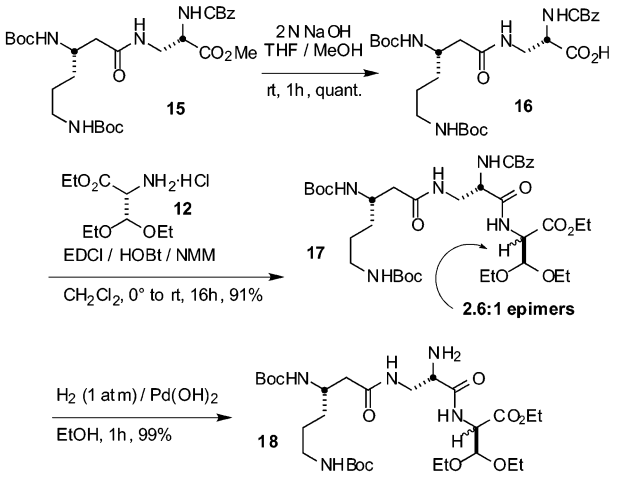
We thus adapted the titanium enolate chemistry to the synthesis of enantiomerically pure α-formylglycine diethylacetal **11** (Scheme 3). Formation of the titanium enolate of (+)-**5** was followed by addition of triethylorthoformate to provide an 85% yield of diethylacetal **10**. Hydrogenolysis of **10** provided clean conversion to *R*-α-formylglycine diethylacetal (**11**). Additionally, exposure of **11** to refluxing EtOH·HCl afforded the ethyl ester hydrochloride salt **12**, which was used crude in subsequent peptide couplings.

The synthesis of capreomycin IB began with the preparation of the diaminopropanoic acid-β-lysine dipeptide (Scheme 4).

Scheme 4. Preparation of DAPA-β-lysine Dipeptide



Scheme 5. Tripeptide Formation



The suitably protected diaminopropanoic acid **13** was prepared from an oxidative Hofmann rearrangement of *N*-CBz-asparagine using previously published methodology.[16,17] Coupling of **13** with the previously reported *N*-hydroxysuccinimidyl ester of bis(*tert*-butoxycarbonyl)-β-lysine[18] **14** in the presence of *N*-methylmorpholine provided dipeptide **15** in 92% yield.

Hydrolysis of the methyl ester present in **15** provided a quanititative yield of carboxylic acid **16** (Scheme 5). Coupling of **16** with the previously described *R*-α-formylglycine diethylacetal ethyl ester hydrochloride salt **12** with EDCI, HOBt, and NMM provided the desired tripeptide **17** in 91% yield.

Unfortunately, the coupling reaction resulted in an inseparable 2.6:1 mixture of epimers at the α-formylglycine diethylacetal center. This was an unexpected result due to the fact that formation of the Mosher's amide of the *R*-α-formylglycine diethylacetal methyl ester hydrochloride salt resulted in no epimerization. Since pyridine was used in the Mosher's amide for-

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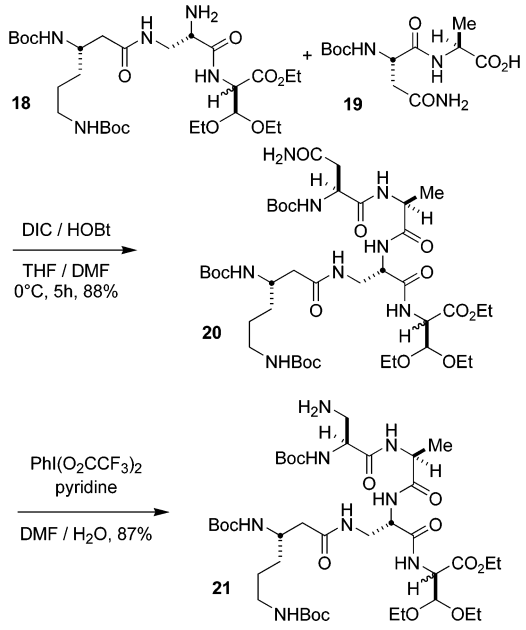
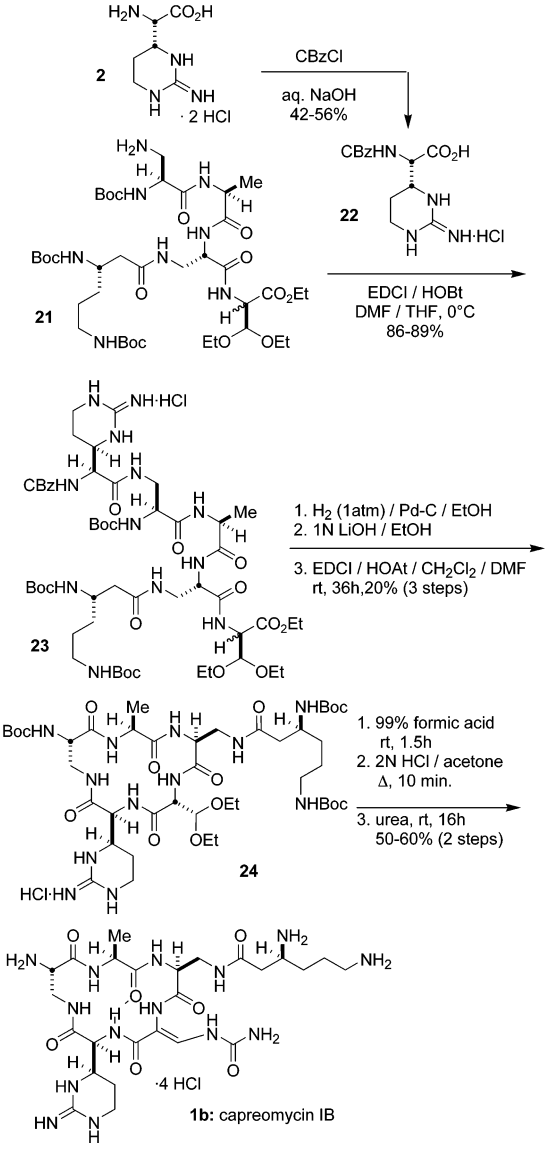
Scheme 6. Pentapeptide Formation



Scheme 7. Completion of Capreomycin IB



mation rather than NMM, it is likely that the increased basicity of NMM is responsible for the epimerization. Although this was an undesired result, it proved practical to complete the capreomycin IB synthesis with this material. Removal of the benzyloxycarbonyl group was accomplished with hydrogen in the presence of Pearlman's catalyst to provide a 99% yield of amine **18** which was used immediately in the subsequent coupling reaction in order to avoid a possible *N*,*N*-acyl migration.

In an attempt to make our approach to capreomycin IB as convergent as possible, the previously reported dipeptide fragment of asparagine and alanine (**19**) was prepared.[19] Coupling of tripeptide **18** and **19** with diisopropylcarbodiimide and HOBt resulted in an 88% yield of pentapeptide **20** (Scheme 6). We decided to incorporate the asparagine residue into pentapeptide **20** as a "masked" form of the second diaminopropanoic acid residue. Our hope was that the primary amide could, via a chemoselective Hofmann rearrangement, be transformed into a primary amine, which would be ready to couple with a suitably protected capreomycidine species.[20] The major reason for this endeavor was to attempt to avoid an undesired series of protection and deprotection steps that would be required if we were to incorporate the diaminopropanoic acid residue directly. To that end, Hofmann rearrangement of **20** with bis(trifluoroacetoxy)iodosobenzene and pyridine provided an 88% yield of the primary amine **21**.

It was at this point that it became necessary to prepare a suitably protected form of capreomycidine for incorporation into the peptide. After extensive investigation of protecting group strategies for this amino acid, it was found that the protection of the α-amine of **2** with a benzyl carbamate was the most effective and straightforward approach (Scheme 7). Treatment of the crude dihydrochloride salt of **2** with benzylchloroformate and aqueous sodium hydroxide provided N^{α}-CBz-capreomycidine (**22**), which was used crude in the subsequent coupling reaction. Coupling of **22** with pentapeptide **21** resulted in an 89% yield of the desired linear hexapeptide **23**. The CBz group of **23** was removed by hydrogenolysis in the presence of 10% palladium on carbon. Cleavage of the ethyl ester was accomplished with 1 N LiOH in ethanol. The crude amino acid was then desalted with a Waters C18 Sep-Pak cartridge.

Treatment of the desalted amino acid with EDCI and HOAt resulted in a 20% yield (3 steps) of the macrocycle **24** as a single diastereomer. The 20% combined yield observed in the deprotections and subsequent macrocyclization is owed, in part, to the multiple silica gel purifications required to obtain pure macrocycle. Additionally, the isolation of a single diastereomer from the macrocyclization suggests that one of the diastereomers cyclizes more efficiently than the other, leading also to a decrease in reaction yield. That being the case, however, the result does compare similarly to the yield observed by Shiba in their capreomycin IB macrocylization (23%, four steps). Using conditions previously developed by Shiba, removal of the Boc groups was achieved by exposure to 99% formic acid. After

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removal of the formic acid, the residue was dissolved in 1:1 acetone:2 N HCl and refluxed for 10 min to cleave the diethylacetal. Upon cooling of the reaction mixture to room temp, an excess of urea was added and the resulting solution was stirred overnight. Evaporation of the solvent and addition of absolute ethanol resulted in precipitation of capreomycin IB (**1b**) (50−60%) as a white solid, which matched by ^{1}H NMR, optical rotation, and TLC with the natural product. Additionally, mixing of the synthetic capreomycin IB with an approximately 1:1 mixture of natural capreomycins IA and IB resulted in an increase in intensity of all ^{1}H NMR resonances associated with capreomycin IB.

Conclusion

In summary, a concise total synthesis of capreomycin IB has been accomplished. The synthesis was completed in 27 total steps (14 steps in the longest linear sequence) and an overall yield of 2% from (−)-**5**. It is significant to compare this to Shiba's total synthesis that required 45 total steps (19 steps in the longest linear sequence) and proceeded in 0.008% overall yield. Our approach featured the asymmetric synthesis of (2*S*,3*R*)-capreomycidine, in which a novel aluminum enolate−aldimine reaction with a chiral glycinate was utilized. Finally, the use of an asparagine residue as a "masked" form of diaminopropanoic acid allowed for a significant decrease in the number of protection and deprotection steps required to complete the synthesis. Efforts to utilize this synthetic approach to prepare novel congeners of the capreomycins as potential agents for treatment of MDR-tuberculosis is under study in these laboratories.

Acknowledgment. This work was supported by the National Science Foundation (Grant CHE0202827). We thank Dr. Mark Zabriskie of Oregon State University for kindly providing an authentic specimen of (2*S*,3*R*)-capreomycidine and ^{1}H and ^{13}C spectra for capreomycin IB.

Supporting Information Available: Full experimental details and characterization (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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