DISSERTATION

THE ASYMMETRIC SYNTHESIS OF (2*S*,3*R*)-CAPREOMYCIDINE AND THE TOTAL SYNTHESIS OF CAPREOMYCIN IB

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DUANE EUGENE DEMONG ENTITLED THE ASYMMETRIC SYNTHESIS OF (2*S*,3*R*)-CAPREOMYCIDINE AND THE TOTAL SYNTHESIS OF CAPREOMYCIN IB BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Adviser

Department Head

ABSTRACT OF DISSERTATION

THE ASYMMETRIC SYNTHESIS OF (2*S*,3*R*)-CAPREOMYCIDINE AND THE TOTAL SYNTHESIS OF CAPREOMYCIN IB

An efficient and asymmetric synthesis of the non-proteinogenic amino acid (2*S*,3*R*)-capreomycidine is presented. The synthesis features a novel aluminum enolate-aldimine reaction with a chiral glycinate, which sets both stereocenters.

A concise and high-yielding approach to the unnatural amino acid (2*S*,3*S*)-β-hydroxyornithine is also reported. The key step in this approach is a boron-mediated aldol reaction with a chiral glycinate.

Additionally, the first asymmetric syntheses of α-formylglycine dimethyl and diethyl acetals are described. This two-step approach employs a novel titanium enolate of a chiral glycinate, followed by addition of the requisite trialkyl orthoformate to provide a single diastereomer of the glycinate adduct. Hydrogenolysis provides the optically pure acetal of α-formylglycine.

Finally, the total synthesis of capreomycin IB is described. The synthesis features the incorporation of the previously prepared (2*S*,3*R*)-capreomycidine. Additionally, the number of protecting group manipulations required in the synthesis has been greatly reduced by the utilization of asparagine in the peptide preparations as a masked precursor

to diaminopropanoic acid. Conversion of the asparagine residue to the diaminopropanoic residue is accomplished by a Hofmann rearrangement.

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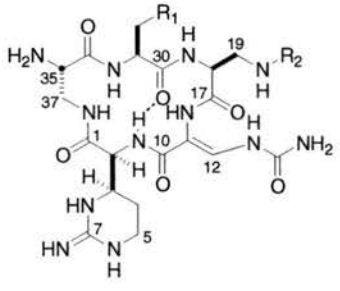
CHAPTER 1

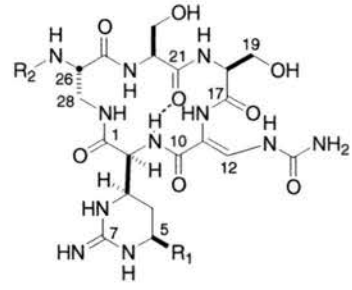
Background on the Capreomycins and Tuberactinomycins

1.1. Isolation of the Capreomycins and Tuberactinomycins.

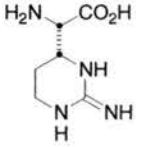
The capreomycins (**1a-d**) and the structurally similar tuberactinomycins (**2a-f**) are a group of cyclic peptide tuberculostatic compounds (Figure 1). Tuberactinomycin B (**2d**), also known as viomycin, was first isolated from several organisms including *Streptomyces puniceus* and *Streptomyces griseus* var. *purpureus* by Finlay and coworkers at Pfizer in 1951.[1] In 1970, Abe and coworkers isolated viomycin from another organism (*Streptomyces griseoverticulatus* var. *tuberacticus*) along with tuberactinomycins N, O, and A (**2a-c**).[2] The capreomycins (**1a-d**) were isolated from *Streptomyces capreolus* by Herr and coworkers at Eli Lilly in 1959.[3] Most recently, McGahren and co-workers at Lederle Laboratories reported the isolation of the structurally similar LL-BM547β (**2e**) from a proprietary *Nocardia* species.[4]



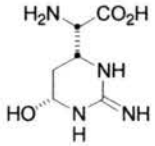
1a: capreomycin IA; R_1=OH, R_2=β-lysine
1b: capreomycin IB; R_1=H, R_2=β-lysine
1c: capreomycin IIA; R_1=OH, R_2=H
1d: capreomycin IIB; R_1=H, R_2=H



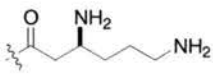
2a: tuberactinomycin N; R_1=H, R_2=γ-hydroxy-β-lysine(**5**)
2b: tuberactinomycin O; R_1=H, R_2=β-lysine(**6**)
2c: tuberactinomycin A; R_1=OH, R_2=γ-hydroxy-β-lysine(**5**)
2d: tuberactinomycin B (viomycin); R_1=OH, R_2=β-lysine(**6**)
2e: LL-BM547β; R_1=OH, R_2=N^{β}-methyl-β-arginine(**7**)
2f: tuberactinamine N; R_1=H, R_2=H



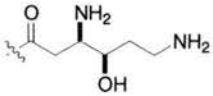
3: capreomycidine



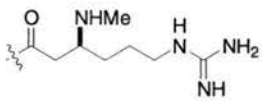
4: tuberactidine



5: β-lysine



6: γ-hydroxy-β-lysine



7: N^{β}-methyl-β-arginine

Figure 1. The capreomycins and tuberactinomycins.

1.2. Biological Activity of the Capreomycins and Tuberactinomycins.

The tuberactinomycins and capreomycins all have significant activity against *Mycobacterium tuberculosis*, with the capreomycins and viomycin being the most potent.[5,6] Capreomycin has long been known as a second-line tuberculostatic drug. This is, in part, due to its weaker activity against *Mycobacterium tuberculosis* compared to compounds such as isoniazid and ethambutol.[7] Another problem with capreomycin, making it less attractive, is its nephratoxicity. Regardless of the previously mentioned issues with capreomycin, the compound has seen renewed interest in the clinic due to emerging incidences of multi-drug resistant tuberculosis (MDR-TB).[7-9] The problem of MDR-TB and the need for drugs to combat it has been further exacerbated by the population increase of people with depressed immune systems (HIV, transplant recipients, etc.) due to their greater susceptibility to these infections when compared to people with healthy immune systems.[10] Capreomycin has shown some promise in inhibiting MDR-TB isolates *in vitro*. This is perhaps due to the belief that capreomycin possesses a different mode of action than that of other tuberculostatic drugs.[11]

The mode of action of capreomycin and the tuberactinomycins is thought to be similar to that of viomycin.[12] It has been proposed that viomycin inhibits polypeptide synthesis, perhaps *via* more than one mechanism.[11,13] Tanaka and Liou reported that, in a cell free *E. coli* system, viomycin prevented both the initiation step and elongation steps of protein synthesis. Viomycin was found to inhibit the peptide synthesis initiation complex between fMet-Trna and the 30s ribosomal subunit. Additionally, peptide chain elongation was halted by viomycin through the inhibition of peptidyl-Trna translocation from the acceptor to the donor site on the ribosome. Further support for the proposal that

viomycin inhibits ribosomal translocation was provided by Modolell and Vazquez.[14] They found that viomycin binds reversibly to AcPhe-Trna·ribosome·polyphenylalanine complexes, thus preventing translocation and subsequent peptide chain elongation. Yamada and co-workers also investigated the effect of viomycin on ribosomal function.[15] Their research found that when a combination of reconstituted 30s and 50s ribosomal subunits from viomycin-sensitive *Mycobacterium smegmatis* were treated with viomycin, a 70s couple was formed, suggesting that the drug aids in the formation and stabilization of couples of the 30s and 50s ribosomal subunits. Interestingly, when the 30s subunit from a viomycin-resistant strain of *M. smegmatis* was combined with the 50s subunit from a viomycin-sensitive strain, and viomycin itself, no 70s couple was observed. This would suggest that viomycin's biological activity is likely to be related to interaction with the 30s ribosomal subunit. A second report by Yamada focused on the composition of ribosomes in *M. smegmatis* strains that were found to be viomycin and capreomycin resistant.[16] Analysis of the ribosomes in these strains showed that mutations were present in both the 30s and 50s ribosomal subunits. This result suggests that the inhibition of ribosomal function by viomycin and capreomycin not only stems from an interaction of the compound with the 30s subunit as previously described, but also from an interaction of the compound with the 50s subunit.

More recently, Schroeder and co-workers looked at the effect of the capreomycins and tuberactinomycins on group I intron RNA splicing.[17] Previous work in this area had shown that a number of compounds including the aminoglycosides: gentamycin, kanamycin, and neomycin in addition to L-arginine were capable of inhibiting group I intron RNA splicing.[18-20] Viomycin and tuberactinomycin A, as well as the semisynthetic

di-β-lysyl capreomycin IIA were found to inhibit this splicing event. Guanosine-5'-phosphate is necessary for the first step of group I intron splicing to occur. It has been proposed that the capreomycins and tuberactinomycins act as a mimic for guanosine-5'-phosphate, thus reversibly occupying the catalytic domain and preventing splicing. Although these results are not likely to be related to the method by which the capreomycins and tuberactinomycins inhibit *Mycobacterium tuberculosis*, it is interesting to note that these compounds may possess the capability to inhibit other organisms.

1.3. Structure Determination.

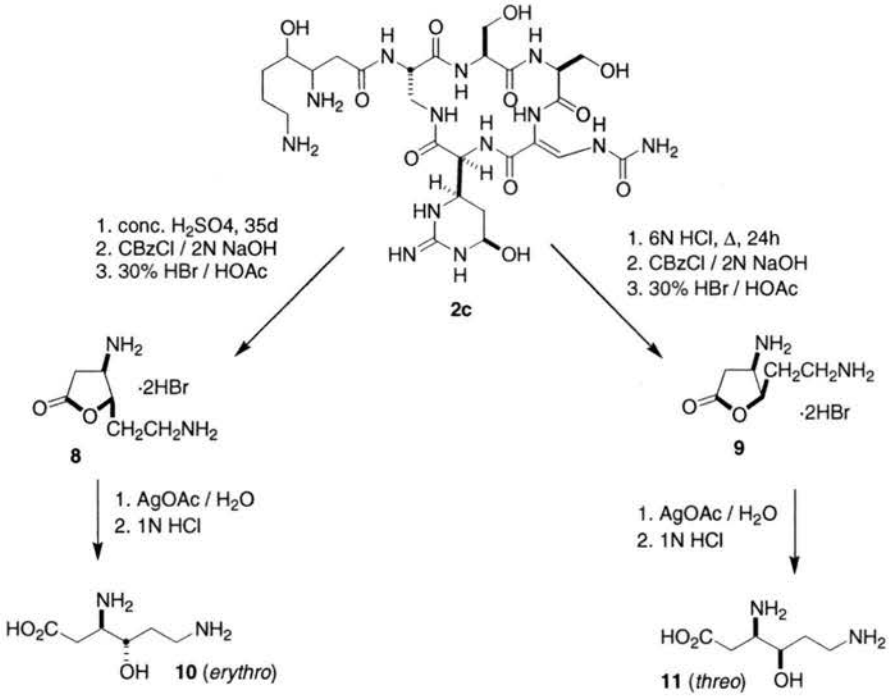
In 1972, both Shiba and Bycroft independently proposed the correct structure for viomycin (**2d,** Figure 1).[21,22] Shiba's proposal was based on comparisons between viomycin and tuberactinomycin O, a compound for which they had recently solved the crystal structure.[23] Bycroft was able to obtain a crystal structure of viomycin dihydrobromide monohydrochloride itself to confirm the structure. Viomycin, as with all of the tuberactinomycins and LL-BM547β, is composed of a 16-membered, macrocyclic pentapeptide core, with a diaminopropanoic acid, two serine residues, and the intriguing unsaturated amino acid β-ureidodehydroalanine. Compounds **2a-b** possess the unique cyclic guanidino amino acid L-capreomycidine (**3**), while the others contain the hydroxylated tuberactidine (**4**) in place of capreomycidine. The other structural difference of the tuberactinomycins is that tuberactinomycin O and viomycin contain a pendant β-lysine residue (**5**) on the α-amine of the diaminopropanoic acid residue, while tuberactinomycin N and A have a γ-hydroxy-β-lysine residue (**6**) at the same position. LL-BM547β stands alone in its possession an N^{β}-methyl-β-arginine (**7**) residue attached to the α-amine of the diaminopropanoic acid residue.[4] Tuberactinamine N (**2f**) was

subsequently prepared by acid hydrolysis of the γ-hydroxy-β-lysine moiety from tuberactinomycin N.[24] Both the crystal structure of tuberactinomycin O and the crystal structure of viomycin suggested an intramolecular hydrogen bond between the capreomycidine or tuberactidine amide proton and the serine carbonyl as depicted in Figure 1.1. Conformational analysis of the tuberactinomycins by ^{1}H-NMR also corroborated this proposal.[25]

An original proposal for the structure of the capreomycin was made by Bycroft and coworkers in 1971, but was revised to the correct structure (**1a-d**) by Shiba in 1977.[26,27] Structurally, the capreomycins differ in two ways. Capreomycins IA and IB have a pendant β-lysine (**5**) group in the N-20 position, while IIA and IIB are devoid of this substitiuent. Both "A" forms of the capreomycins have a serine residue present in the macrocycle, while the "B" forms have alanine in its place. All of the capreomycins possess capreomycidine (**3**). Common to all of the capreomycins are two diaminopropanoic acids and the unsaturated amino acid β-ureido-dehydroalanine.

As previously mentioned, the acid hydrolysis of tuberactinomycin A or N (**2c,a**) cleaved the then unknown γ-hydroxy-β-lysine (**6**) moiety to provide tuberactinamine N (**2f**).[24] This method was also used to isolate pure **6** so that its relative and absolute configuration could be determined. An interesting observation was made from the acid hydrolysis of **2c** (Scheme 1). Hydrolysis of **2c** with 6N HCl was followed by benzyloxycarbonylation, purification, and debenzyloxycarbonylation to provide lactone **9**. Coupling constants between the β and γ protons supported a *threo* configuration. Consequently, acidolysis of **2c** with concentrated sulfuric acid, benzyloxycarbonylation and subsequent debenzyloxycarbonylation provided lactone **8** which was of a different

(*erythro*) configuration than that of the aqueous HCl hydrolysis product. Based on the fact that other sulfuric acid-mediated *N,O*-acyl migrations of hydroxyamino acids in peptides have been shown to undergo an inversion of stereochemistry at the hydroxyl center, Shiba suggested that this could explain the formation of **8** from **2c** with sulfuric acid.[28] Shiba and coworkers also hypothesized based on Cotton effects of the lactones and comparison of synthetic derivatives that both compounds are of the L- configuration. Finally, lactones **8** and **9** were hydrolyzed with aqueous AgOAc followed by treatment with aqueous HCl to provide *erythro* (**10**) and *threo* (**11**) γ-hydroxy-β-lysine respectively.

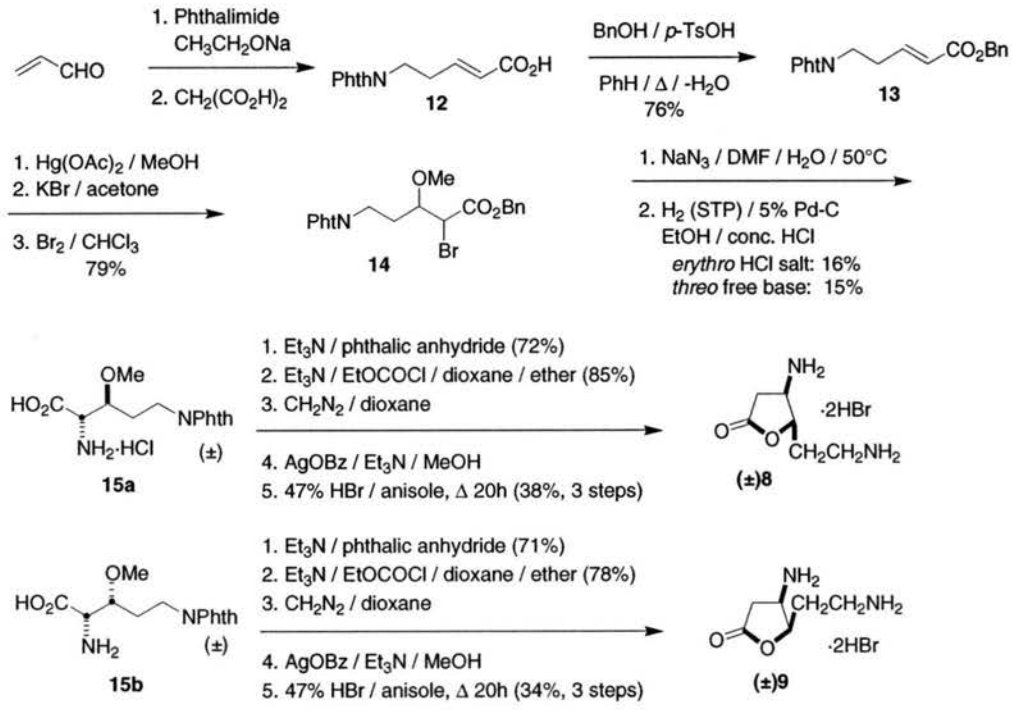


Scheme 1. Hydrolysis products of tuberactinomycin O.

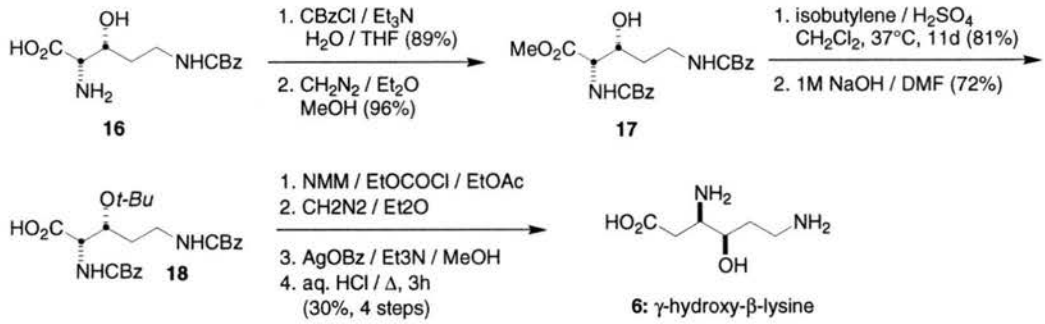
In order to further confirm the structures and relative configurations of compounds **8** through **11**, an independent, racemic synthesis was undertaken (Scheme 2).[29] Michael addition of phthalimide to acrolein followed by condensation with malonic acid provided the α,β-unsaturated acid **12**. Benzyl ester **13** was prepared in 76% yield.

Mercuration in the presence of methanol followed by bromination of the resulting alkyl mercuric salt provided **14** in 79% yield. Azide displacement of the bromide followed by hydrogenolysis to the amine resulted in a 16% recrystallized yield of racemic *erythro*-**15a** as its HCl salt and a 15% recrystallized yield of *threo*- **15b** as the free base. The configurations of **15a** and **15b** were confirmed by their deprotection followed by treatment with CBzCl to form the respective N^{δ}-CBz oxazolidinones, which are distinguishable by ^{1}H-NMR. Phthaloyl protection of the α-amines of **15a** and **15b** was followed by Arndt-Eistert preparation of the protected β-amino ester. Refluxing in 47% HBr provided racemic lactones (±) **8** and (±) **9** from **15a** and **15b** respectively. This confirmed the proposed structures of the compounds previously isolated from the aqueous HCl and concentrated sulfuric acid hydrolyses of **2c**. In turn, as previously described (Scheme 1), (±) **8** and (±) **9** can be converted to **(±) 10** and (±) **11** by treatment with silver acetate followed by aqueous HCl.

Finally, in order to confirm both the relative and absolute stereochemistry of **6**, a stereoselective synthesis was investigated by Shiba and co-workers (Scheme 3).[30] Starting from the optically pure N^{δ}-benzyloxycarbonyl-β-hydroxyornithine (**16**) that was available from Shiba's synthesis of (2*S*,3*R*)-capreomycidine (Chapter 3), benzyloxycarbonyl protection of the α-amine proceeded in 89% yield and subsequent methyl ester formation was achieved in 96% yield to provide **17**.[31] Formation of the t-butyl ether, followed by hydrolysis of the methyl ester afforded **18**. Subjection of **18** to Arndt-Eistert homologation conditions resulted in the formation, after deprotection with refluxing aqueous HCl, of optically pure **6**, which matched the natural amino acid in all respects.



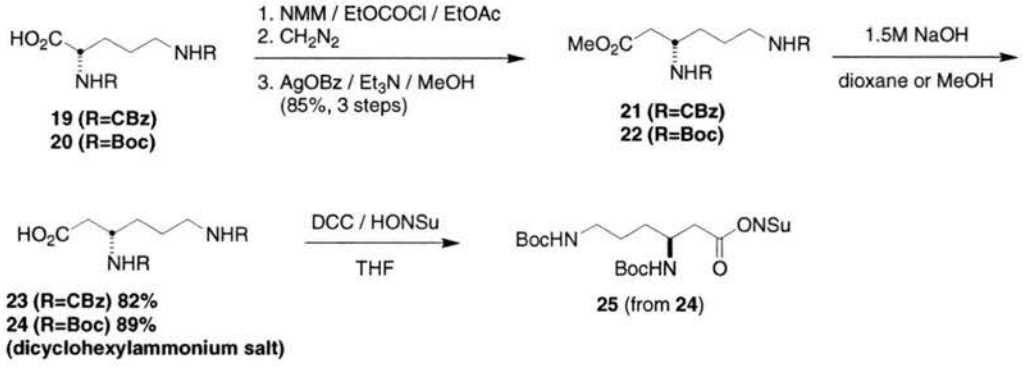
Scheme 2. Racemic syntheses of 4-hydroxy-β-lysine lactones.



Scheme 3. Optically pure preparation of 6-amino-4*R*-hydroxy-3*R*-aminohexanoic acid.

The Arndt-Eistert synthesis has also been used by Shiba for the preparation of synthetically useful *N*-acyl- derivatives of β-lysine (Scheme 4).[32] Starting from either the dibenzyloxycarbonyl protected ornithine **19**, or the di-*tert*-butoxycarbonyl protected ornithine **20**, formation of the mixed carbonic anhydride with ethyl chloroformate and *N*-methylmorpholine, was followed by exposure to diazomethane to afford the crude diazoketone. Subsequent Wolff rearrangement in the presence of AgOBz and Et_3N in methanol provided the protected β-lysine methyl esters **21** and **22** in 85% and 86% yields

respectively. Hydrolysis of **21** provided **23** as the free acid in 82% yield, while hydrolysis of **22** provided **24**, which was crystallized as its dicyclohexylammonium salt in 89% yield. In addition, the *N*-hydroxysuccinimidyl ester **25** can be prepared by carbodiimide-mediated coupling of **24** and HONSu.[33] No yield was reported for this transformation.



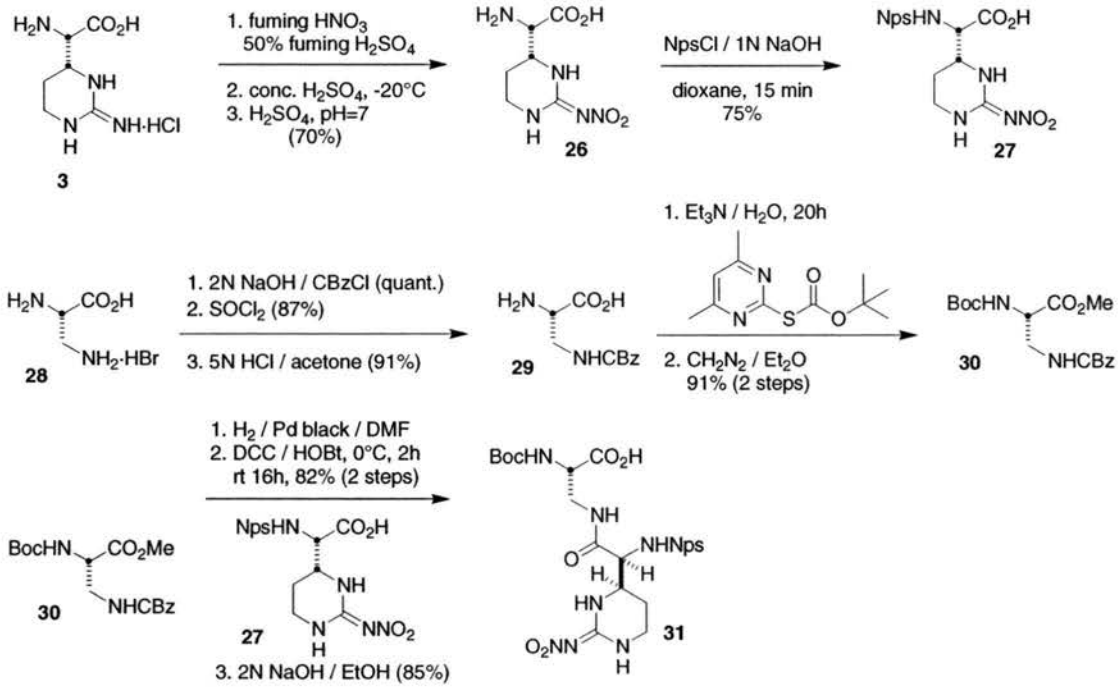
Scheme 4.

1.4. Previous Syntheses of Tuberactinomycin O and Capreomycin IA and IB.

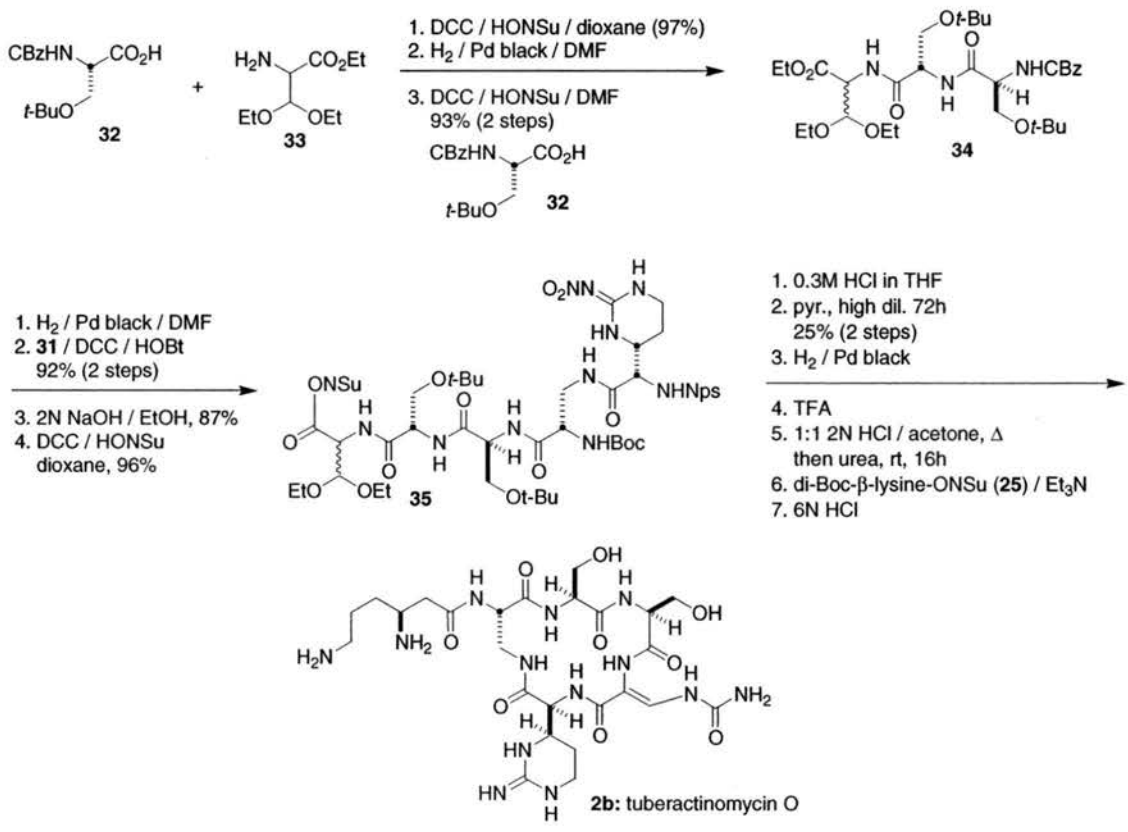
1.4.1. Shiba's Total Synthesis of Tuberactinomycin O.

In 1976, Shiba and coworkers published the first synthesis of tuberactinomycin O.[33] In order to do so, suitably protected forms of the non-proteinogenic amino acids (2*S*,3*R*)-capreomycidine (**3**) and diaminopropanoic acid had to be prepared (Scheme 5).[34,35] The capreomycidine used in the synthesis of tuberactinomycin O was, in fact, not prepared synthetically, but rather by isolation from the acid hydrolysate of tuberactinomycin N. Treatment of **3** with fuming HNO_3 and H_2SO_4, followed by concentrated H_2SO_4, resulted in nitration of the guanidine. Neutralization with aqueous $NaHCO_3$ provided **26** as a precipitate in 70% yield. Exposure of **26** to NpsCl provided the *o*-nitrophenyl sulfenamine **27** in 75% yield. For the preparation of the appropriately protected diaminopropanoic acid unit, **28** (prepared from L-aspartic acid *via* the Schmidt

reaction) was treated with CBzCl to provide the dibenzyloxycarbonyl protected diaminopropanoic acid in quantitative yield. Addition of this product to thionyl chloride resulted in the acid chloride, which reacted with the N^{α}-CBz group to provide the oxazolidinedione in 87% yield. Aqueous HCl in acetone cleaved the oxazolidinedione to provide the N^{β}-benzyloxycarbonyl-diaminopropanoic acid **29** in 91% yield. *Tert*-Butoxycarbonyl protection of the α-amine and subsequent methyl ester formation provided the orthogonally protected **30**. Formation of the dipeptide comprised of these two amino acids commenced with the removal of the CBz group from **30**. Coupling of the deprotected product with **27** via DCC and HOBt provided the dipeptide in 82% yield over the two steps. Hydrolysis of the methyl ester provided **31** in 85% yield.



Scheme 5. Preparation of the capreomycidine-diaminopropanoic acid fragment.



Scheme 6. The completion of Shiba's total synthesis of tuberactinomycin O.

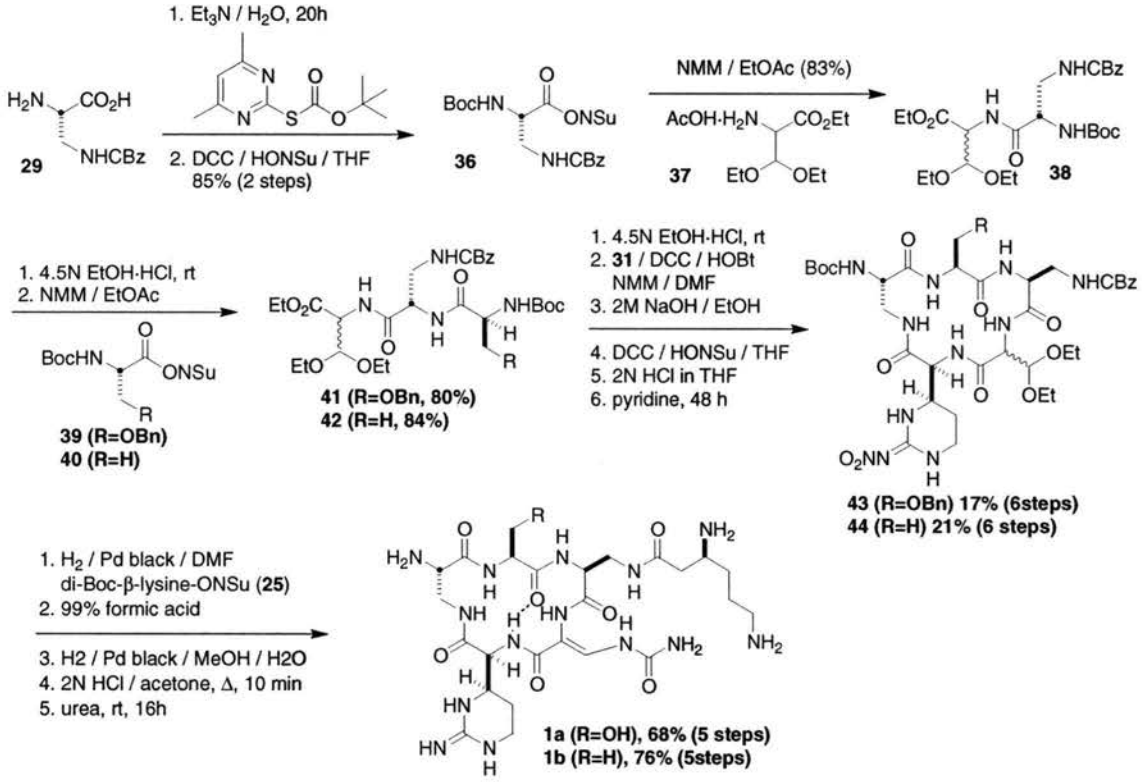
With the dipeptide in hand, preparation of the tripeptide fragment commenced (Scheme 6). Coupling of **32** with the previously described racemic ethyl ester of α-formylglycine diethylacetal **33** via DCC and HONSu proceeded in 97% yield. Hydrogenolysis of the CBz group from the dipeptide and coupling of another equivalent of **32** under the same conditions afforded the tripeptide **34** in 93% yield over the two steps. Deprotection of the CBz group was followed by coupling of the previously prepared dipeptide **31** with DCC/HOBt, resulting in the formation of the desired pentapeptide. Hydrolysis of the ethyl ester and treatment with DCC/HONSu provided **35**. Acidic cleavage of the Nps group from **35** and addition of the product to pyridine supplied the macrocyclic peptide in 25% yield. The nitroguanidine was cleaved with hydrogen and Pd black, while the Boc group and *t*-butyl ethers were cleaved with TFA.

Hydrolysis of the diethyl acetal was followed by the addition of urea to access the enamidourea after equilibration to a single geometric isomer. Coupling of the deprotected macrocycle with the previously prepared activated β-lysyl ester **25** and cleavage of the Boc groups with 6N HCl resulted in the completion of the synthesis of tuberactinomycin O (**2b**).

1.4.2. Shiba's Total Synthesis of Capreomycins IA and IB.

With the completion of the synthesis of **2b**, Shiba's group embarked on a synthesis of capreomycins IA and IB (**1a,b**) (Scheme 7).[36,37] While the synthetic approach to the capreomycins mirrored some of the work done in the synthesis of tuberactinomycin O additional protecting group considerations were necessary due to the presence of two diaminopropanoic acid residues in the macrocycle. Preparation of the fully protected activated ester **36** from the previously described **29** proceeded in 85% yield.[34] Coupling of **36** with acetate salt **37** afforded dipeptide **38** in 83%. This peptide is versatile in that it can be used for the preparation of both capreomycins IA and IB. Removal of the Boc group from **38** with EtOH·HCl and subsequent coupling with the *N*-hydroxysuccinimidyl esters of either *N*-Boc-*O*-benzyl serine (**39**) or *N*-Boc-alanine (**40**) provided similar yields of the tripeptides **41** and **42**. Cleavage of the Boc groups on **41** and **42** were followed by coupling with the previously prepared capreomycidine-containing dipeptide **31**. Hydrolysis of the ethyl ester and formation of the *N*-hydroxysuccinimidyl ester allowed for the ensuing deprotection of the Nps group and macrocyclization to provide **43** in 17% yield and **44** in 21% yield over the 6 steps. It was found to be necessary to remove the CBz groups of **43** and **44** by hydrogenolysis in the presence of activated β-lysyl ester **25** so that coupling would occur immediately.

Sequential hydrogenolysis and acylation led to significant side products due to *N,N*-acyl migration within the macrocycle. Another interesting observation in the final steps was the fact that attempts to cleave the nitroguanidine with the Boc groups intact on the molecule were unsuccessful. It was crucial to first remove the Boc groups with 99% formic acid, and then remove the nitro group by hydrogenolysis. Finally, hydrolysis of the diethyl acetal in refluxing 2N HCl in acetone, followed by the addition of urea afforded **1a** in 68% yield and **1b** in 76% yield.



Scheme 7. Shiba's total synthesis of capreomycin IA and IB.

1.5. Preparation of analogs of the tuberactinomycins and capreomycins.

1.5.1. Shiba's tuberactinomycin analogs.

In addition to the total syntheses of both tuberactinomycin O and the capreomycins, a significant amount of effort has been directed towards the preparation of fully synthetic as well as semisynthetic analogs of this class of antibiotics.

Shiba first used analogs to look at the structure/activity relationships (SAR) of the tuberactinomycins (Figure 2).[34,38] These investigations focused on the importance of the side chains as well as the two serine residues present in tuberactinomycins N and O.

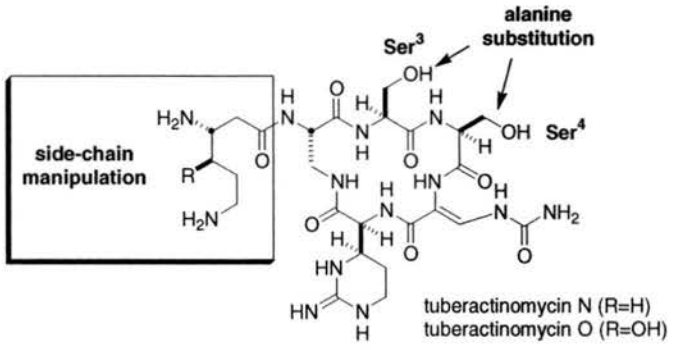
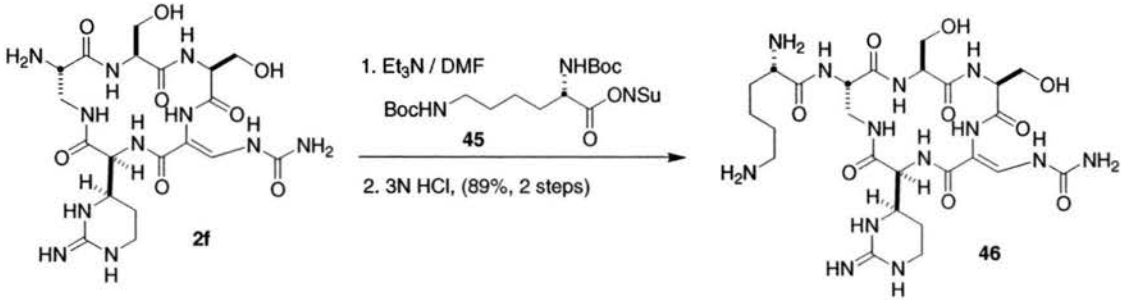


Figure 2. Shiba's tuberactinomycin analogs.

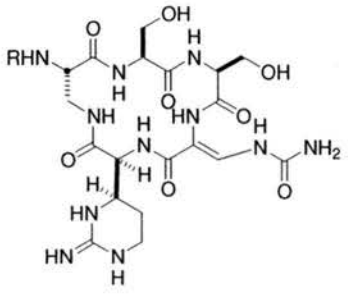
Initially, Shiba and coworkers investigated the biological importance of the amino acid side chain of the tuberactinomycins.[38] Tuberactinamine N (**2f**) was acylated with a wide range of protected α- and β-amino acids via their *N*-hydroxysuccinimidyl esters (Scheme 8). As an example, **2f** was treated with the *N*-hydroxysuccinimidyl ester of di-*tert*-butoxycarbonyl-L-lysine (**45**). Removal of the Boc groups with aqueous HCl provided *N*-lysyl tuberactinamine N (**46**) in 89% yield.



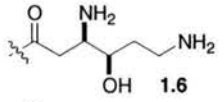
Scheme 8. Shiba's preparation of lysyl-tuberactinamine N.

Upon preparation of these *N*-acyl tuberactinamine N derivatives, their biological activities were tested against a variety of microorganisms and compared with that of tuberactinomycin N (Table 1). The first observation made from these studies was that

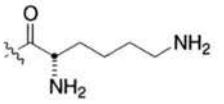
only the β-ornithine derivative (**46**) maintained the same broad-spectrum activity as tuberactinomycin N. Interestingly, *N*-acetyl-β-lysine derivatives **47** and **48** were devoid of any activtity, demonstrating the importance of the basic side chain. Hydrophobic side chains such as leucine (**49**) do not possess broad-spectrum acitivity, but do maintain activity similar to **2a** against *Mycobacterium* ATCC 607. Incorporation of an acidic side chain such as aspartic acid (**50**) results in loss of biological acitivity.



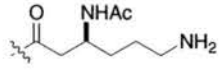
2a: R=



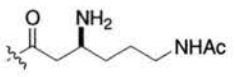
46: R=



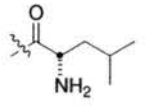
47: R=



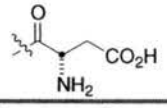
48: R=



49 R=



50 R=



Organism	2a	46	47	48	49	50
Corynebacterium diphtheriae	6.3	12.5	>100	100	25	>100
Bacillus subtilis ATCC 6633	25	25	>100	>100	>100	>100
Escherichia coli NIHJ	50	50	>100	>100	100	>100
Escherichia coli B	50	50	>100	>100	>100	>100
Salmonella typhosa H 901	50	50	>100	>100	>100	>100
Shigella sonnei E33	50	50	>100	>100	>100	>100
Klebsiella pneumonia	50	25	>100	>100	>100	>100
Mycobacterium ATCC 607	6.3	12.5	>100	100	6.3	>100

Table 1. MIC's in μg/mL of side-chain analogs against various microorganisms.

In the series of α,ω- and β,ω-diamino acid derivatives, another interesting trend was observed (Table 2, entries 1-4). When moving from longer chain length to shorter chain length, for example, from lysine to diaminopropanoic acid, the biological activity against *Mycobacterium* ATCC 607 drops 16-fold. This empirical observation was made, to a lesser extent, with the β-amino acids as well (Table 2, entries 5-7). Finally, it was also found that the analogs that maintained their antimicrobial activity possessed similar

CD spectra to that of tuberactinomycin N, suggesting that conformation of the macrocycle is also important to elicit a biological response.

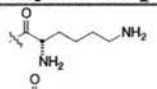
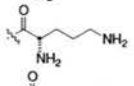
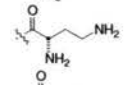
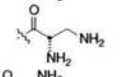
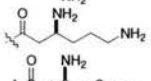
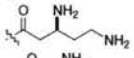
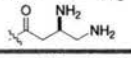
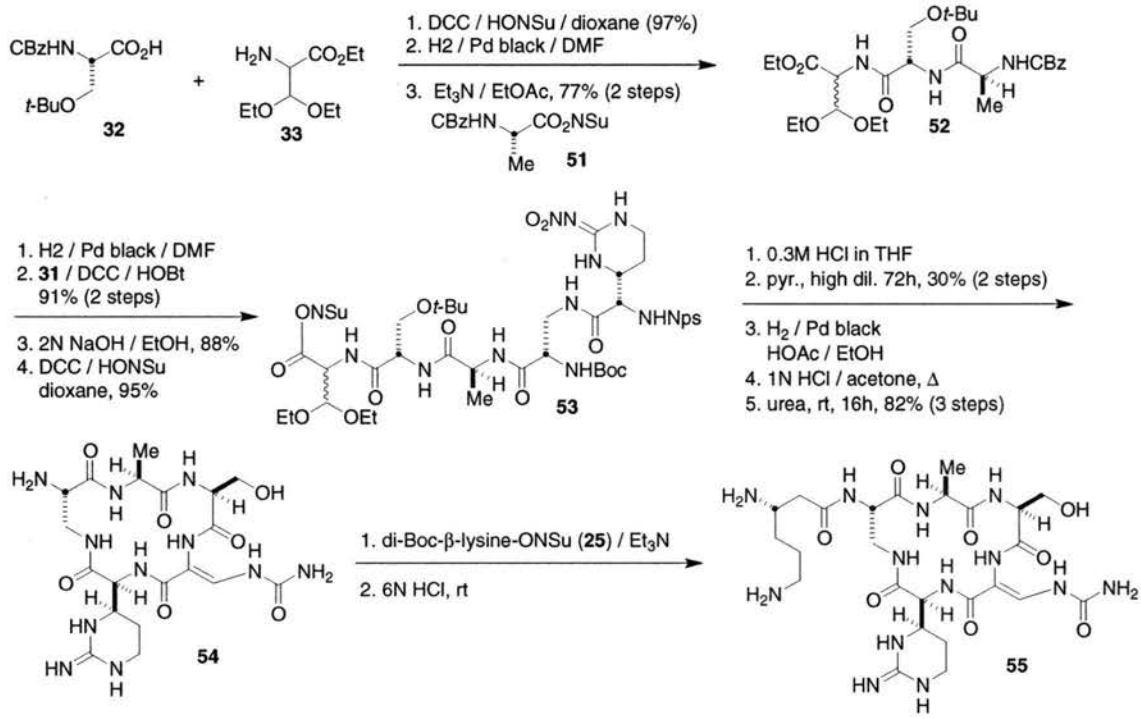
Entry	Acyl Group	MIC (μg/mL)
1		6.3
2		25
3		50
4		100
5		6.3
6		12.5
7		50

Table 2. Effect of diamino acid chain length on MIC against *Mycobacterium* ATCC 607.

Having investigated the importance of the side chains of the tuberactinomycins, Shiba and coworkers then set out to explore what effect replacing the serine residues present in the tuberactinomycins would have on biological activity.[34] In order to conduct this investigation, separate syntheses were required. Synthesis of the –[Ala^3]-[Ser^4]-analog **55** was begun using chemistry developed for the synthesis of tuberactinomycin O (Scheme 9). Coupling of **32** and **33** provided the necessary dipeptide, which was then deprotected at the N-terminus *via* hydrogen and palladium black. Addition of **51** provided the requisite tripeptide **52** in 77% yield. Removal of the CBz group from **52** was followed by coupling with the previously described capreomycidine-containing dipeptide **31**. Ethyl ester hydrolysis and subsequent *N*-hydroxysuccinimidyl ester formation afforded macrocyclization precursor **53**. Treatment of **53** with HCl in THF facilitated removal of the Nps group, while addition of the product to pyridine resulted in formation of the macrocycle in 30% yield. Deprotection, and enamidourea formation as previously seen in the tuberactinomycin O synthesis provided [Ala^3]- tuberactinamine N

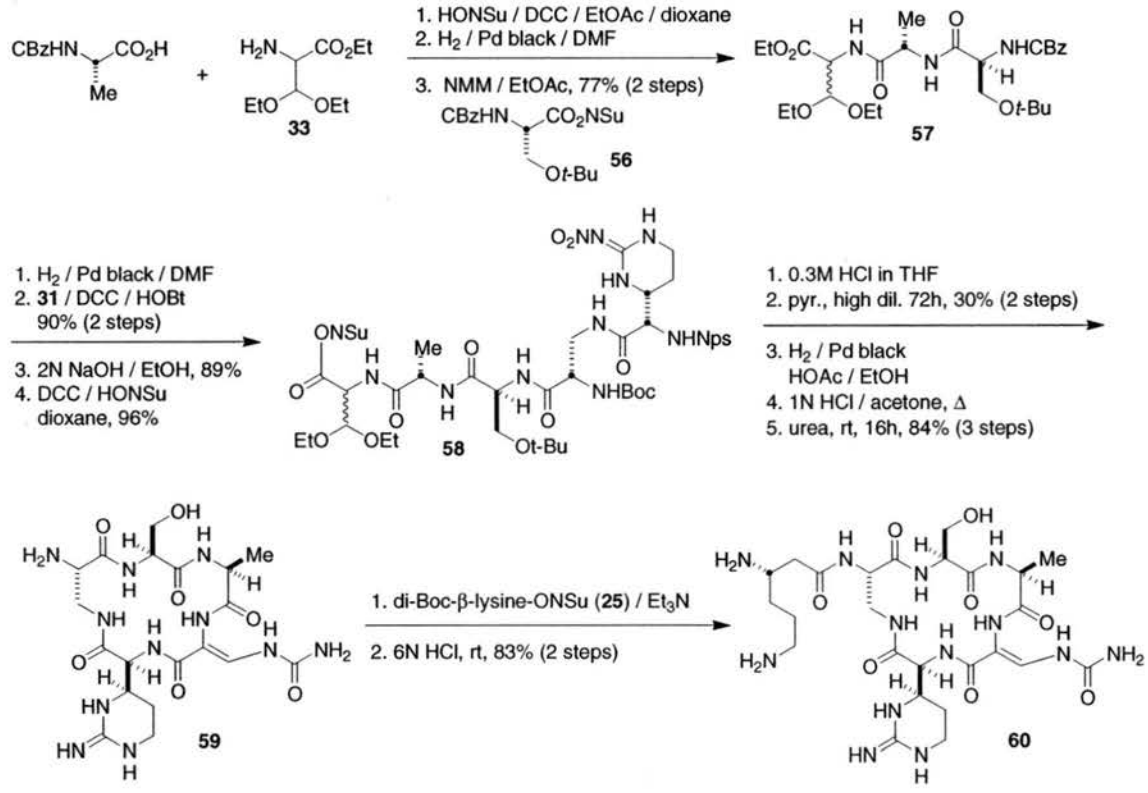
(**54**). Incorporation of the β-lysyl group was achieved by treatment of **54** with **25** and Et_3N, followed by Boc group deprotection to provide **55**.



Scheme 9. Shiba's preparation of [Ala^3]- tuberactinomycin O.

The preparation of -[Ser^3]-[Ala^4]- (**60**) was accomplished in a similar manner to that of **55** (Scheme 10). Coupling of *N*-CBz-alanine with **33** *via* HONSu and DCC was followed by hydrogenolysis of the CBz group and subsequent coupling with the activated ester of serine **56** to provide tripeptide **57**. Removal of the CBz group from **57** and coupling with **31** afforded the pentapeptide in 90% yield over the two steps. Ethyl ester hydrolysis and formation of the *N*-hydroxysuccinimidyl ester provided the macrocyclization precursor **58** in good yield. Removal of the Nps group followed by dilution with pyridine resulted in the isolation of the desired macrocycle in 30% yield. Removal of the protecting groups and enamidourea formation provided [Ala^4]

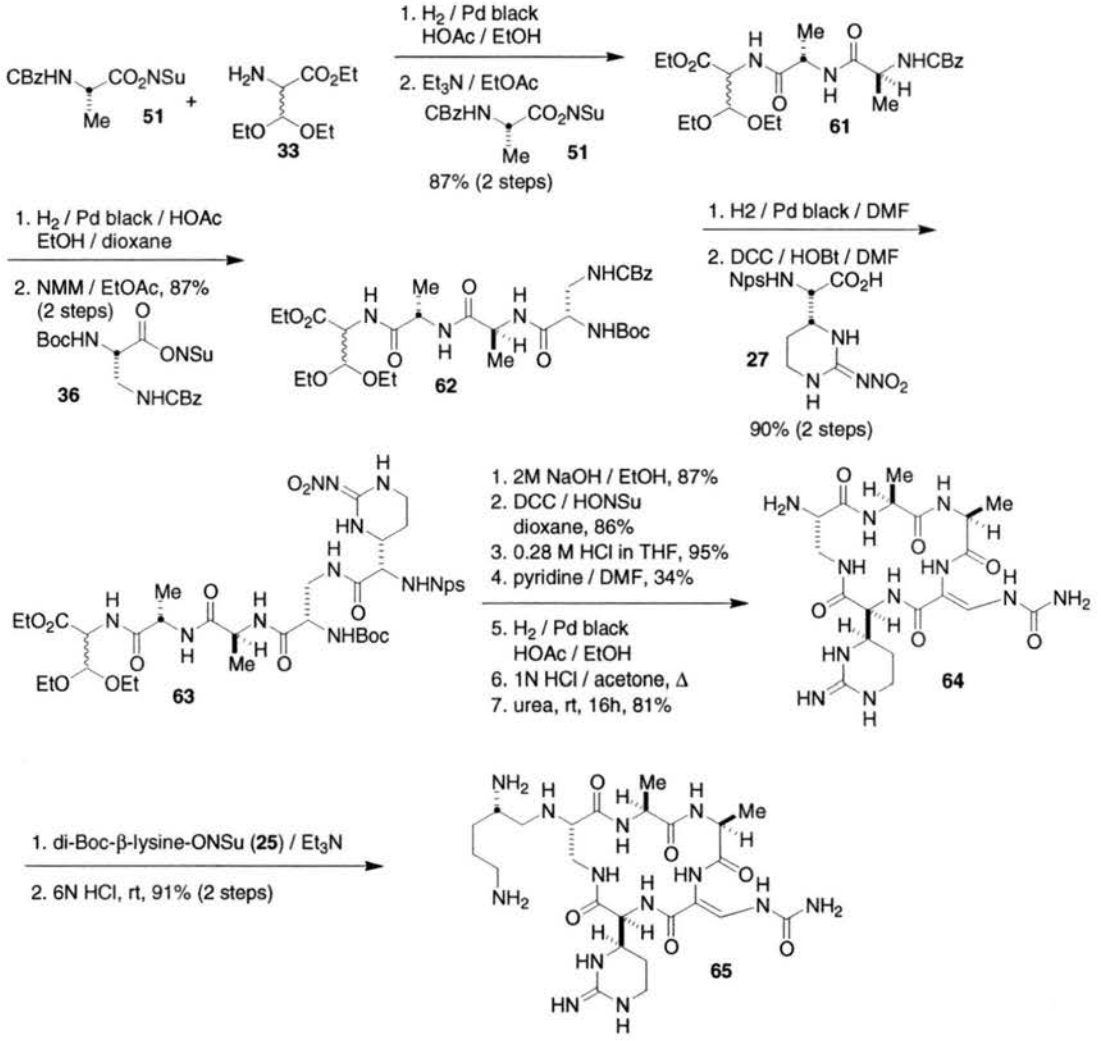
tuberactinamine N (**59**). Treatment of **59** with **25** and Et_3N followed by 6N HCl provided entry to *N*-β-lysyl analog **60**.



Scheme 10. Shiba's preparation of [Ala^4]- tuberactinomycin O.

A slightly more linear approach to -[Ala^3]- [Ala^4]- analog **65** compared to the other monosubstituted analogs was undertaken (Scheme 11). Coupling of **51** and **33** provided the dipeptide, which upon deprotection and coupling again with **51**, provided tripeptide **61**. Hydrogenolysis of the CBz group from **61** allowed for coupling with the orthogonally protected active ester of diaminopropanoic acid **36** resulting in the isolation of **62**. After removal of the CBz group from **62**, the previously prepared protected form of capreomycidine **27** was coupled to provide the linear pentapeptide **63** in 90% yield. Hydrolysis of the ethyl ester of **63** and formation of the *N*-hydroxysuccinimidyl ester proceeded in 75% yield over the two steps. Removal of the Nps group and subsequent

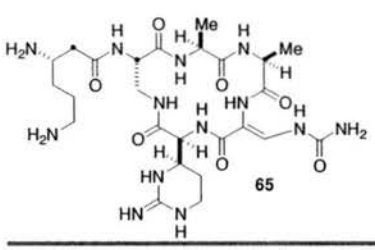
macrocyclization afforded the cyclic peptide in 32% yield. Deprotection and enamidourea formation gave the cyclic pentapeptide **64** in 81% yield. Treatment of **64** with **25** and subsequent deprotection provided **65** in 91% yield over the two steps.

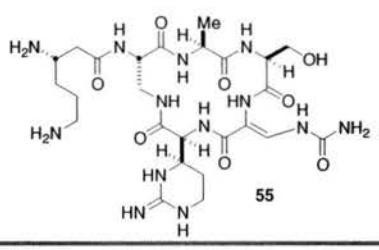


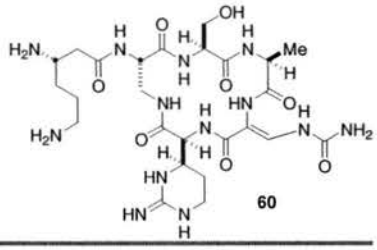
Scheme 11. Shiba's preparation of [Ala^3]-[Ala^4]-tuberactinomycin O.

The newly prepared analogs were then tested against a variety of microbes and the results compared with that of tuberactinomycin N (**2a**) (Table 3). It was interesting to note that substitution of one or the other serine residues with alanine (**55, 60**) did not have a large effect on the biological activity of the substrate when compared with **2a**. Substitution of both serine residues (**65**) had a more significant impact, but not to an

overwhelming extent. Shiba also proposed that similarity in the CD spectra of these analogs with that of tuberactinomycin N suggested that the conformation of the macrocyclic peptide is perhaps more important than the nature of the amino acid residues in the peptide.


65


55


60

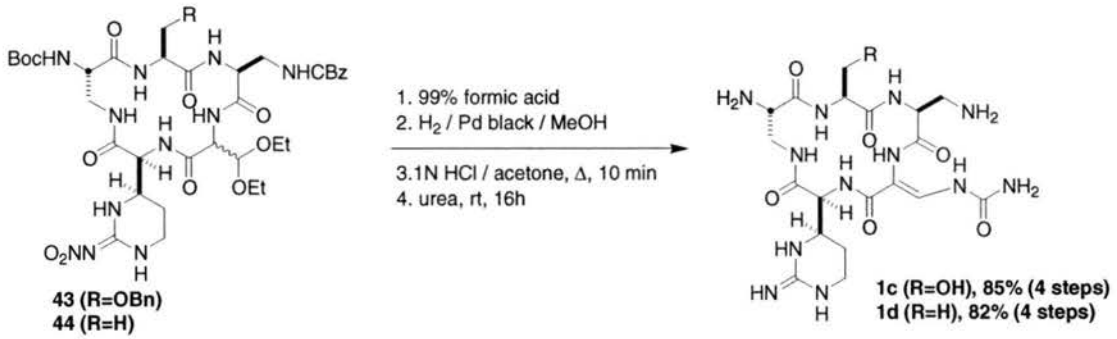
Organism	65	55	60	2a
Corynebacterium diphtheriae	12.5	6.3	6.3	6.3
Bacillus subtilis ATCC 6633	50	25	12.5	25
Escherichia coli NIHJ	100	50	12.5	12.5
Escherichia coli B	100	50	50	50
Salmonella typhosa H 901	100	25	25	25
Salmonella paratyphi PA 41-N-22	>100	25	>100	>100
Salmonella enteritidis Gaertner	>100	25	100	100
Shigella sonnei E33	>100	100	50	50
Klebsiella pneumonia	100	25	25	25
Proteus vulgaris OX 19	>100	50	50	50
Mycobacterium ATCC 607	12.5	6.3	6.3	6.3

Table 3. MIC's (μg/mL) of tuberactinomycin macrocycle analogs.

1.5.2. Shiba's capreomycin analogs.

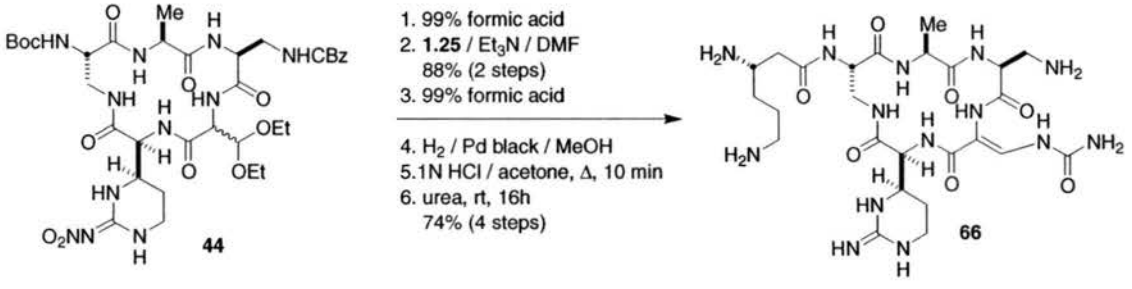
Having thus far looked at only derivatives of the tuberactinomycins, Shiba and co-workers investigated SAR of the capreomycins as well. As previously mentioned, the capreomycins contain two diaminopropanoic acid residues in the macrocycle whereas the tuberactinomycins contain only one. The following study looked at the importance of the capreomycin side chain, in addition to the arrangement of amino acid residues in the macrocycle.[39]

Capreomycins IIA and IIB were prepared synthetically from intermediates **43** and **44**, respectively. These intermediates were used in their total synthesis of capreomycin IA and IB (Scheme 12).



Scheme 12. Shiba's syntheses of capreomycin IIA and IIB.

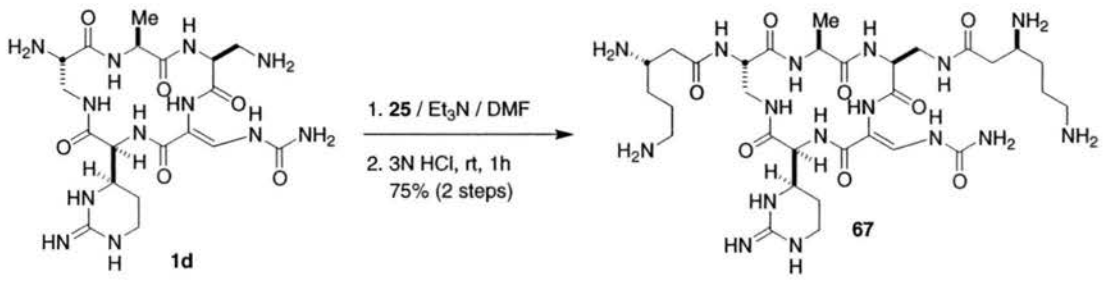
Preparation of the analog pseudocapreomycin IB (**66**) began again with macrocycle **44** (Scheme 13). Removal of the Boc group from **44** was achieved with 99% formic acid. Installation of the β-lysyl side chain to the α-amine of the diaminopropanoic acid residue in the N-36 position was accomplished by treatment with **25** and Et_3N, a method used in previous synthetic work with the capreomycins and tuberactinomycins. Removal of the protecting groups and enamidourea formation provided pseudocapreomycin IB (**66**) in six steps and 65% overall yield from **44**.



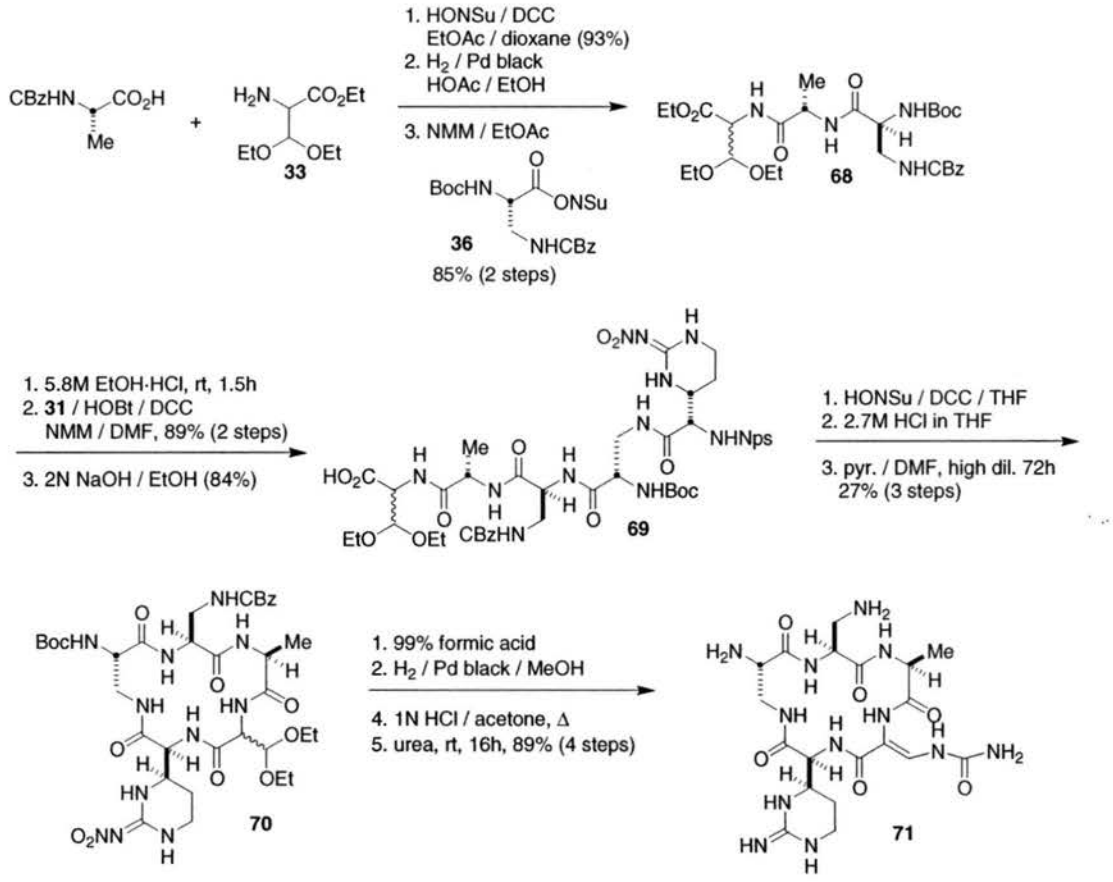
Scheme 13. Shiba's synthesis of pseudocapreomycin IB.

Di-β-lysyl-capreomycin IB (**67**) was prepared to probe whether or not an additional side chain would enhance biological activity (Scheme 14). Capreomcyin IIB

(**1d**) was treated with a slight excess of **25** in the presence of Et_3N. The crude product was then treated with aq. HCl, neutralized, and purified on Sephadex G10 to provide **67** in 75% yield.



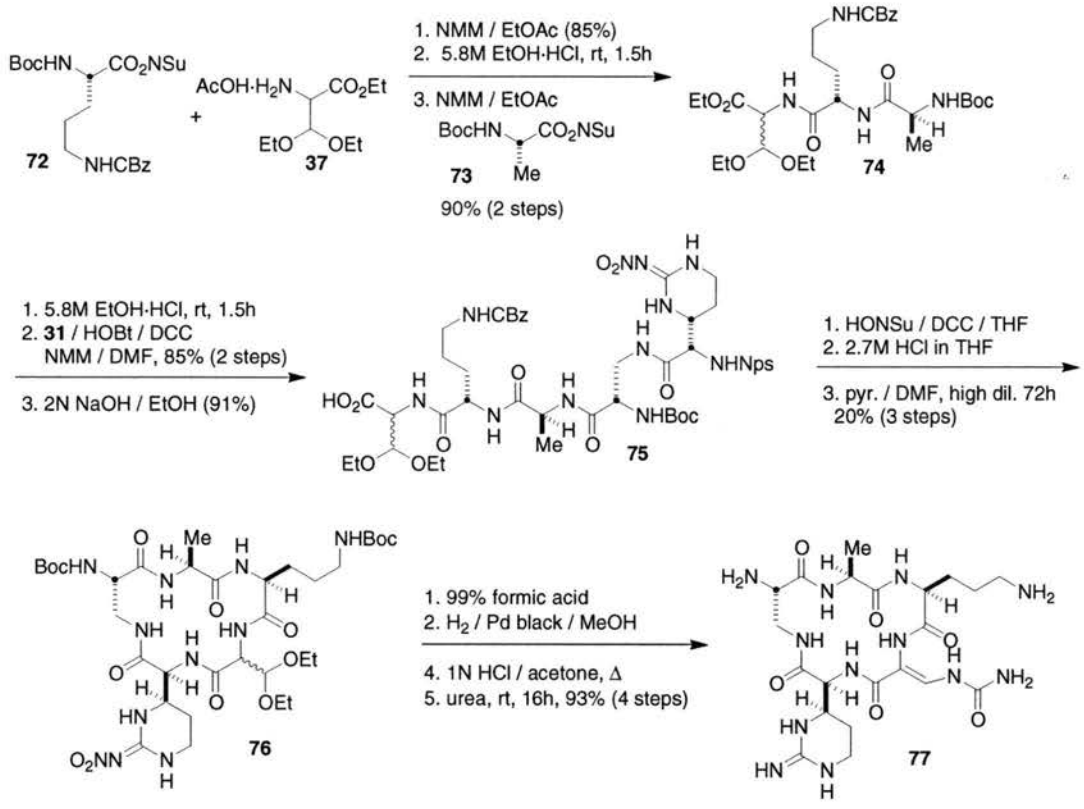
Scheme 14. Shiba's synthesis of di-β-lysyl-capreomycin IB.



Scheme 15. Shiba's preparation of reverse capreomycin IIB.

Unlike the afore mentioned capreomycin derivatives, reverse capreomycin IIB (**71**) could not be accessed *via* semisynthesis (Scheme 15). The dipeptide resulting from the

coupling of *N*-CBz-alanine and **33** was hydrogenolyzed and coupled with active ester **36** to prepare tripeptide **68** in 79% yield overall. Removal of the Boc group and elongation of the peptide chain by coupling with the capreomycidine-containing dipeptide **31** resulted in the formation of the linear pentapeptide in 89% yield. Free acid **69** was afforded in 84% yield by hydrolysis of the ethyl ester. Macrocyclization *via* the previously described HONSu method provided the cyclic peptide **70** in 27% yield. Deprotection and enamidourea formation resulted in the desired reverse capreomycin IIB (**71**) in 89% yield.



Scheme 16. Shiba's preparation of [Orn^4]-capreomycin IIB.

Similarly, the preparation of [Orn^4]-capreomycin IIB (**77**) was achieved by total synthesis (Scheme 16). Coupling of N^{α}-Boc-N^{δ}-CBz-ornithine-*N*-hydroxysuccinimidyl ester (**72**) with **37** afforded the desired dipeptide, which, upon removal of the Boc group,

was coupled with active ester **73** to give tripeptide **74**. Deprotection and coupling with **31**, followed by ester hydrolysis provided access to the pentapeptide **75** in good yield. Cyclic peptide **76** was realized in 20% yield via the HONSu method. Global deprotection and enamidourea formation resulted in the isolation of **77** in high yield.

Upon comparing the biological activities of the capreomycin analogs versus capreomycin IB against a variety of pathogens, two interesting observations were made (Table 4). First, the diaminopropanoic acid present in the 4-position (acylated or not) is crucial in order to elicit a biological response. This proposal was supported by the fact that a dramatic drop in the activity of reverse capreomycin IIB (**71**) and the structurally more subtle analog [Orn^4]-capreomycin IIB (**77**) was observed. Additionally, it was found that both the position and number of β-lysine side chains did not have a significant effect on biological activity as evidenced by the results for pseudocapreomycin IB (**66**) and di-β-lysylcapreomycin IIB (**67**).

Organism	66	67	71	77	1b
Staphylococcus epidermidis sp-a1-1	50	50	>100	>100	>100
Streptococcus pyogenes 1022	50	>100	100	100	>100
Streptococcus agalactiae 1020	>100	>100	>100	100	>100
Corynebacterium diphtheriae P.W.8	1.6	1.6	100	25	3.1
Bacillus subtilis ATCC 6633	6.3	3.1	100	50	25
Escherichia coli B	50	100	>100	>100	100
Klebsiella pneumonia ATCC 10031	25	50	>100	100	50
Salmonella typhosa H 901	25	50	>100	>100	50
Shigella sonnei E33	100	100	>100	>100	100
Proteus vulgaris OX 19	50	100	>100	>100	50
Mycobacterium ATCC 607	12.5	3.1	100	25	6.3

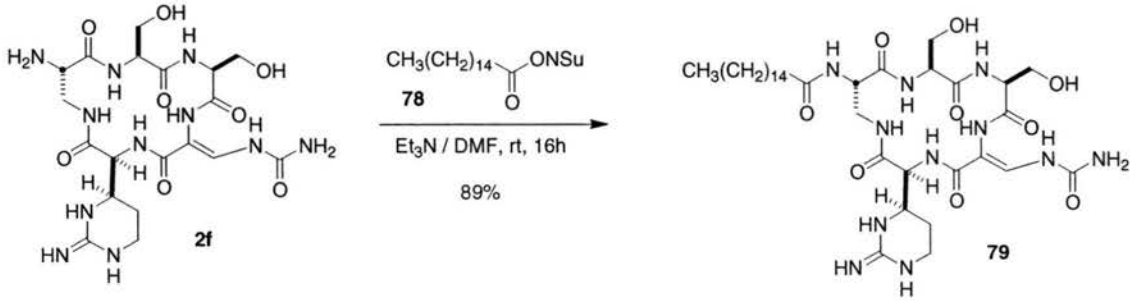
Table 4. Minimun inhibitory concentrations (μg/mL) of capreomycin analogs.

1.5.3. Other capreomycin and tuberactinomycin derivatives.

In the early 1980's the Shiba group published results from a second investigation into the effect of side chain variation on the biological activity of the tuberactinomycins

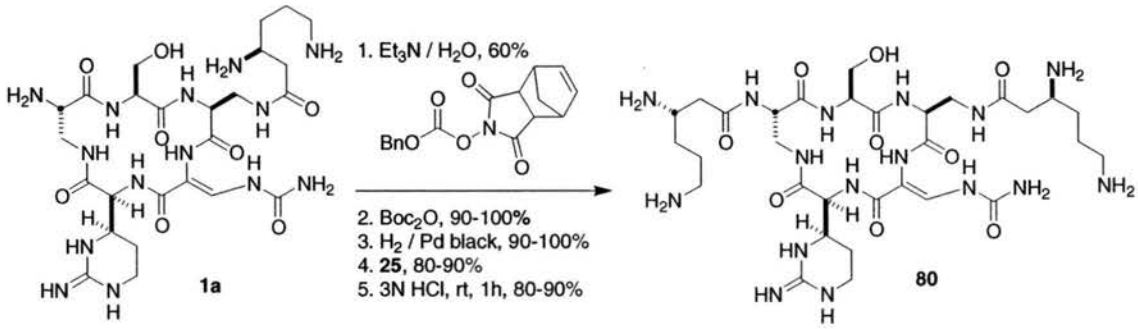
and capreomycins.[12,40] The main purpose of these studies was to prepare novel side-chain analogs of the capreomycins and tuberactinomycins, and test both their antimicrobial ability and their ability to halt polypeptide biosynthesis. Of the 61 derivatives prepared, the authors only discussed the results from two of the derivatives.

The first derivative, palmitoyl tuberactinamine N (**78**) was prepared semisynthetically from the previously described tuberactinamine N (**2f**) (Scheme 17). Tuberactinamine N was treated with *N*-hydroxysuccinimidyl palmitate (**78**) and triethylamine in DMF to provide **79** in 89% yield.

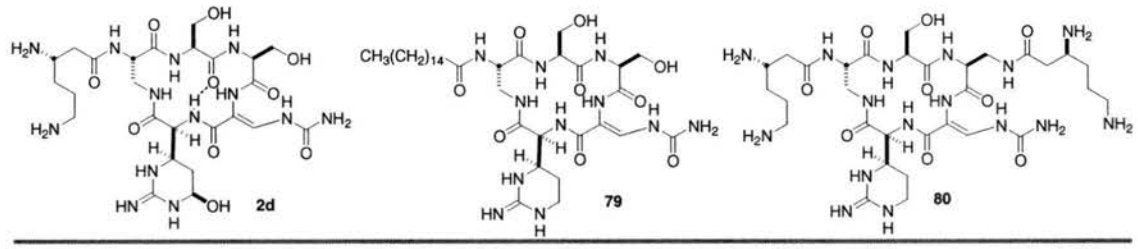


Scheme 17. Shiba's preparation of palmitoyltuberactinamine N.

Di-β-lysyl-capreomycin IIA (**80**) was the second analog described (Scheme 18). It was prepared semisynthetically from capreomycin IA (**1a**).[41] The α-amine of the diaminopropanoic acid residue in **1a** was selectively protected as a benzyl carbamate via CbzONb in 60% yield. Boc protection of the remaining two amines was achieved with Boc_2O in good yield. Hydrogenolysis of the CBz group was followed by coupling with the previously described active ester of *N,N'*-di-*tert*-butoxycarbonyl-β-lysine (**25**). Removal of the Boc groups with 3N HCl afforded di-β-lysylcapreomycin IIA (**80**) in useful yields.



Scheme 18. Shiba's semisynthetic preparation of di-β-lysylcapreomycin IIA.



COMPOUND	*M. smegmatis R15* % inhib. @ 1 μg/mL[a]	*M. smegmatis R31* MIC (μg/mL)[b]	*M. smegmatis R33* MIC (μg/mL)[b]
2d	95	900	80
79	2	20	20
80	96	100	20

Table 5. Percent inhibition and MIC's of analogs versus viomycin in *M. smegmatis R15*, *R31*, and *R33*. [a]Cell-free polypeptide synthesis assay. [b]Cell culture growth assay.

The newly prepared analogs **79** and **80** were then tested versus viomycin (**2d**) for their ability to inhibit polypeptide synthesis in viomycin sensitive *Mycobacterium smegmatis R15* and for their ability to inhibit culture growth of two viomycin resistant strains *Mycobacterium smegmatis R31* and *R33* (Table 5). *Mycobacterium smegmatis* is a non-pathogenic organism that is similar to *Mycobacterium tuberculosis*. As one can see from the data, both palmitoyl tuberactinamine N (**79**) and di-β-lysyl-capreomycin IIA (**80**) are significantly more active than viomycin against the viomycin resistant *R31* and *R33* strains of *M. smegmatis*. Perhaps even more interesting is the observation that, unlike viomycin and di-β-lysyl-capreomycin IIA, palmitoyl tuberactinamine N does not seem to inhibit *M. smegmatis* via polypeptide synthesis inhibition. This suggests that **79** does not

prevent ribosomal translocation, the proposed mode of action for the capreomycins and tuberactinomycins.[11,13-15]

Additionally, the tuberactinomyicins and **79** and **80** were analyzed for their capacity to prevent RNA synthesis. It was found that none of these compounds prevent RNA synthesis in cell-free systems.[40]

LABORATORY STRAINS	CLINICAL ISOLATES	Viomycin Resistant?	1.80	viomycin
M. tuberculosis $H_{37}Rv$		no	5	5
M. tuberculosis $H_{37}Rv$		yes	5	>40
	M. tuberculosis (1)	no	5	5
	M. tuberculosis (2)	yes	20	20
	M. tuberculosis (3)	yes	5	20
	M. tuberculosis (4)	yes	20	>40
	M. tuberculosis (5)	yes	20	>40
	M. tuberculosis (6)	yes	5	10

Table 6. MIC's (μg/mL) of capreomycin analog **80** vs. viomycin.

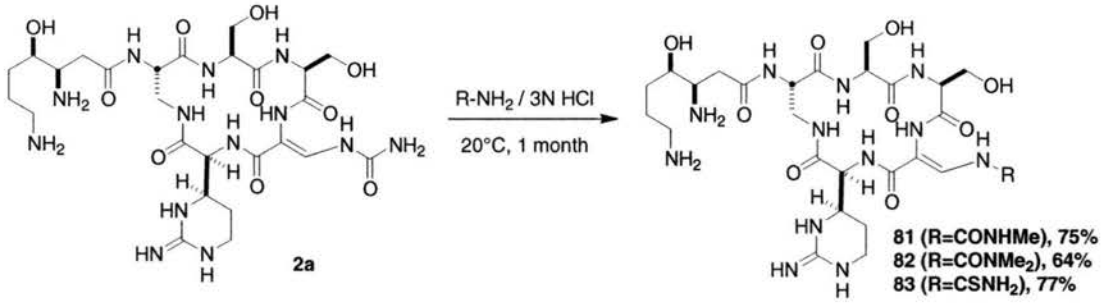
Di-β-lysyl-capreomycin IIA (**80**) was also tested against a variety of laboratory-derived viomycin resistant mutants and clinical isolates of *Mycobacterium tuberculosis* and the results compared with that of viomycin (Table 6). The trend shown in this experiment supports the greater potency of **80** against viomycin resistant strains of *M. tuberculosis.*

Finally, palmitoyl tuberactinamine N (**79**) was tested against a variety of microorganisms, and the results compared with that of viomycin (Table 7). Although **79** showed greater potency than viomycin against a variety of organisms, including *M. smegmatis R33*, it is remarkable to note that it is, in fact, less active than viomycin against *M. tuberculosis* and *M. bovis.*

MICROORGANISM	79	VIOMYCIN
Shigella dysenteriae	10	ND
Escherichia coli	7.5	>40
Klebsiella pneumoniae	20	7.5
Vibrio cholerae	10	20
Vibrio parahaemolyticus	20	>40
Cornybacterium diphtheriae	5	40
Bacillus subtilis	7.5	7.5
Staphylococcus aureus	7.5	20
Streptococcus pyogenes M-type	7.5	>80
Mycobacterium smegmatis R33	5	40
Mycobacterium bovis BCG	>20	5
Mycobacterium tuberculosis $H_{37}Rv$	>40	5

Table 7. MIC's (μg/mL) of **79** and viomycin against various microorganisms.

Curiosity as to the importance of the enamidourea in the biological activity of the capreomycins and tuberactinomycins led Shiba and coworkers to prepare three novel enamidoureas from tuberactinomycin N (**2a**) (Scheme 19).[42] The preparation of enamidourea derivatives **81, 82,** and **83** proved to be a tedious process, requiring one month for the urea exchange reaction to occur. Separate treatment of **2a** with *N*-methylurea, *N,N*-dimethylurea, and thiourea in 3N HCl provided urea exchange products **81** (R=CONHMe), **82** (R=$CONMe_2$), and **83** in 75, 64, and 77% yields respectively.



Scheme 19. Shiba's enamidourea substitutions on tuberactinomycin N.

Comparison of the newly formed enamidourea derivatives showed that a single methyl substitution on the urea nitrogen (**81**) or substitution with thiourea (**83**) had little effect on their biological activity when compared with tuberactinomycin N (Table 8). It

was observed, however, that substitution of the urea nitrogen with two methyl groups (**82**) did, in fact, decrease the biological activity when compared with tuberactinomycin N.

Organism	81	82	83	2a
Staphylococcus aureus MS353	>100	>100	100	>100
Staphylococcus aureus MS353 AO	>100	>100	100	>100
Streptococcus pyogenes 1022	>100	>100	100	>100
Streptococcus agalactiae 1020	>100	50	>100	>100
Corynebacterium diphtheriae P.W.8	6.3	12.5	3.1	3.1
Bacillus subtilis ATCC 6633	50	100	12.5	25
Escherichia coli NIHJ-JC2	50	>100	50	50
Escherichia coli B	50	>100	50	50
Klebsiella pneumonia ATCC 10031	25	>100	12.5	25
Salmonella typhosa H 901	50	>100	25	50
Salmonella enteritidis Gaertner	>100	>100	100	100
Shigella flexneri type 3a	>100	>100	100	100
Shigella sonnei E33	100	>100	100	100
Proteus vulgaris OX 19	50	>100	50	50
Mycobacterium ATCC 607	12.5	100	12.5	6.3
Mycobacterium 1088	25	>100	25	25

Table 8. MIC's (μg/mL) of enamidourea analogs against various microorganisms.

1.5.4. Dirlam's capreomycin and tuberactinomycin derivatives.

Recently, Dirlam and co-workers at Pfizer central research published a series of reports describing the semisynthetic preparation of a variety of capreomycin and tuberactinomycin derivatives which possessed antimicrobial activity against a broad spectrum of microorganisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and the vancomycin-resistant enterococci *Enterococcus faecalis* and *Enterococcus faecium* (Figure 3).[5,43-45] The analogs prepared from capreomycin primarily consisted of novel substitution at the enamidourea residue. A small number of manipulations of the β-lysine residue and free amine at C-16 were also reported. Derivatives of viomycin (R=OH) containing enamidourea substitution as well as tuberactidine C-19 substitution were also reported.

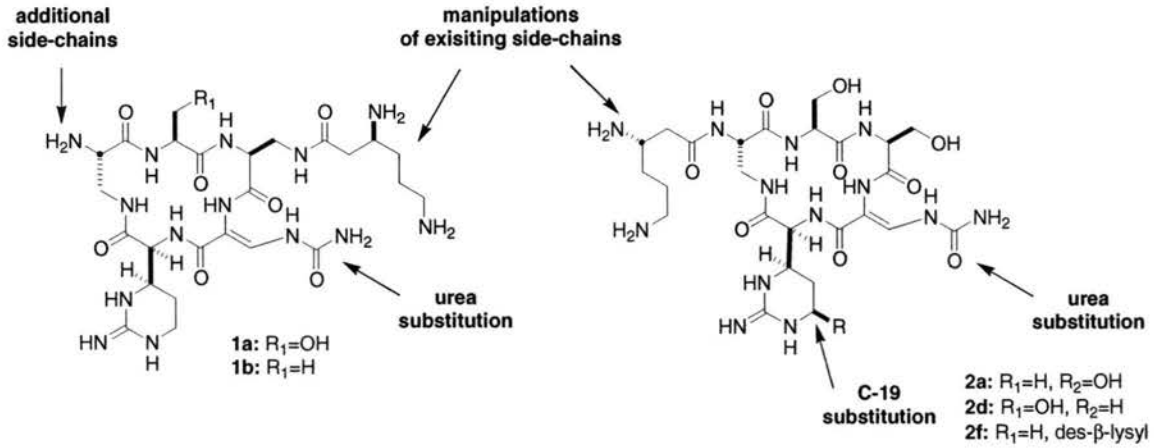
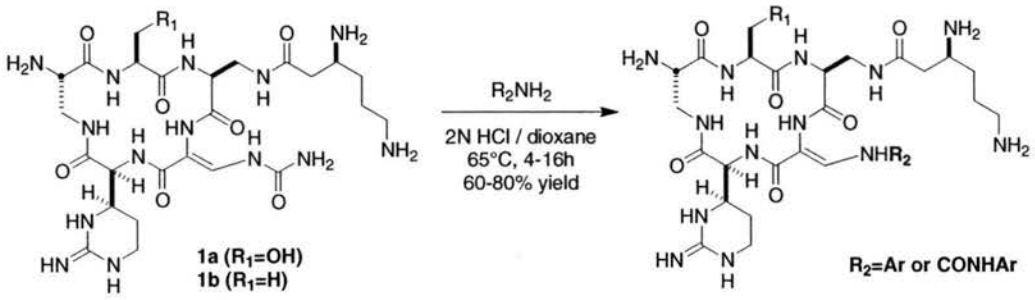


Figure 3. Overview or Dirlam's capreomycin and tuberactinomycin analogs.



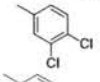
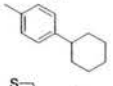
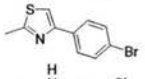
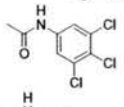
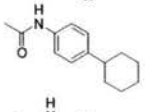
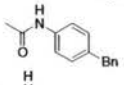
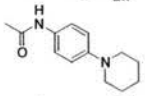
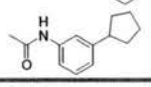
Cmpd.	R_1	R_2	*P. m.*[a]	*E. coli*	MRSA[b]	*E. f.*[c]	*E. fc.*[d]
1a,b	H, OH	$CONH_2$	50	200	100	>1000	>1000
84	H, OH		0.39	6.25	0.78	25	12.5
85	H, OH		0.2	6.25	1.56	12.5	6.25
86	H, OH		3.13	100	12.5	50	12.5
87	OH		0.2	12.5	0.78	3.12	1.56
88	OH		0.39	25	1.56	3.12	1.56
89	H, OH		0.78	12.58	1.56	6.25	3.12
90	OH		1.56	50	3.12	100	100
91	OH des-β-lysine		0.39	12.5	3.12	3.12	3.12

Table 9. MIC's (μg/mL) of enaniline and enamidourea analogs. [a] *Pasteurella multocida.* [b] Methicillin resistant *Staphylococcus aureus.* [c] *Enterococcus faecalis.* [d] *Enterococcus faecium.*

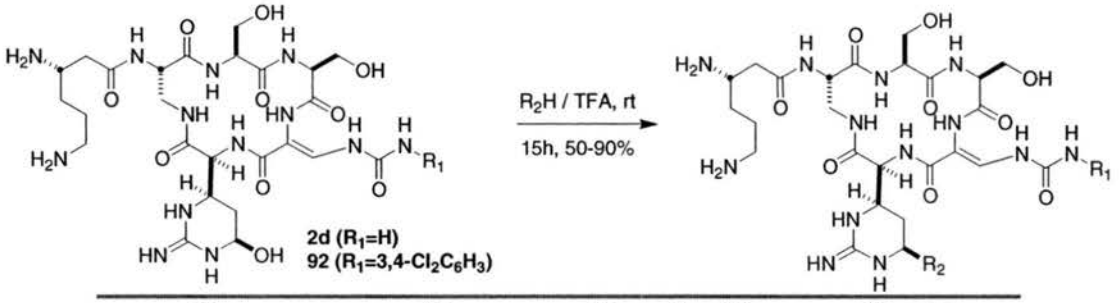
The first analogs prepared focused on the enamidourea portion of the capreomycins (Table 9). Pure capreomycin IA or mixtures of capreomycin IA and IB were treated with ~40 equivalents of various anilines or 2-10 equivalents of various phenylureas in 2N HCl and dioxane at 65°C for 4-16h. Organic extraction followed by purification with HP-21 resin facilitated removal of excess aniline or urea and capreomycin to provide the new enanilines or enamidoureas in 60-80% yield.

The resulting capreomycin analogs were compared with native capreomycin for their antibacterial acitivity against the gram-negative pathogen *Pasteurella multocida*, and the multidrug resistant gram-positive pathogens MRSA, *Enterococcus faecalis,* and *Enterococcus faecium.* Upon looking at the results of the experiment, a number of novel broad-spectrum antimicrobial compounds were prepared, possessing excellent activity against all of the organisms tested relative to capreomycin.

After investigating enamidourea derivatives of capreomycin, Dirlam and co-workers switched to the use of viomycin as an analog scaffold (Table 10). Treatment of viomycin and the 3,4-dichlorophenyl urea analog of viomycin (**92**) with TFA resulted in the formation of the guanidinium species at C-5. Addition of electron-rich aromatics, as well as various thiols and sulfonamides resulted in substitution at the C-5 position. Less electron-rich aromatics were found to be unreactive in this system.

The resulting compounds were then tested against *Pasteurella multocida* and *E. coli* to determine their antimicrobial activity (Table 10). As could perhaps be expected, all derivatives of **92** were significantly more potent than their viomycin-based counterparts. However, the C-5 aromatic derivatives were found to be less active than their complementary unsubstituted compounds. Sulfonamide did not improve activity

over viomycin either. Thiol substitution did not substantially increase or decrease biological activity compared with the parent compound.



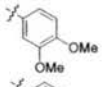
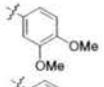
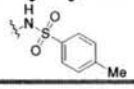
R_1	R_2	*P. multocida*	*E. coli*
H	OH	200	200
H		>200	>200
H		200	200
	OH	3.12	50
		12.5	12.5
		12.5	12.5
	$NH_2(CH_2)_2S$-	3.12	25
	L-cysteine	3.12	25
	L-methylcysteine	3.12	25
	*N*-acetylcysteine	6.25	50
H		200	200

Table 10. MIC's (μg/mL) of C-5 and C-12 substituted viomycin analogs.

A second series of C-5 substitutions was undertaken using benzylic and aryl-substituted ureas and carbamates (Table 11). C-5 substitution of viomycin with benzylic carbamates, *N*-benzyl ureas, and *N*-aryl ureas provided substrates with significantly better antimicrobial activity against *Pasteurella multocida*, *E. coli*, and MRSA as compared to the C-5 aryl compounds described in Table 10. The best activity was observed when the

aromatic ring contained halogen substitution. Hoping for a possible additive effect, **92** was substituted with both a benzylic carbamate as well as a phenyl urea. Unfortunately, these derivatives were no more active than the viomycin-based derivatives.

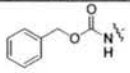
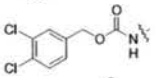
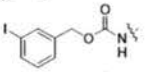
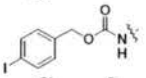
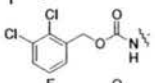
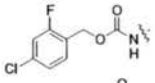
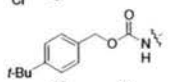
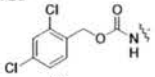
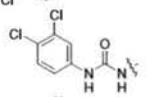
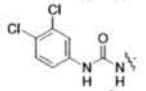
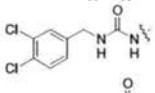
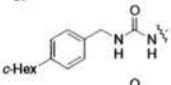
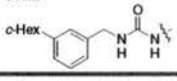
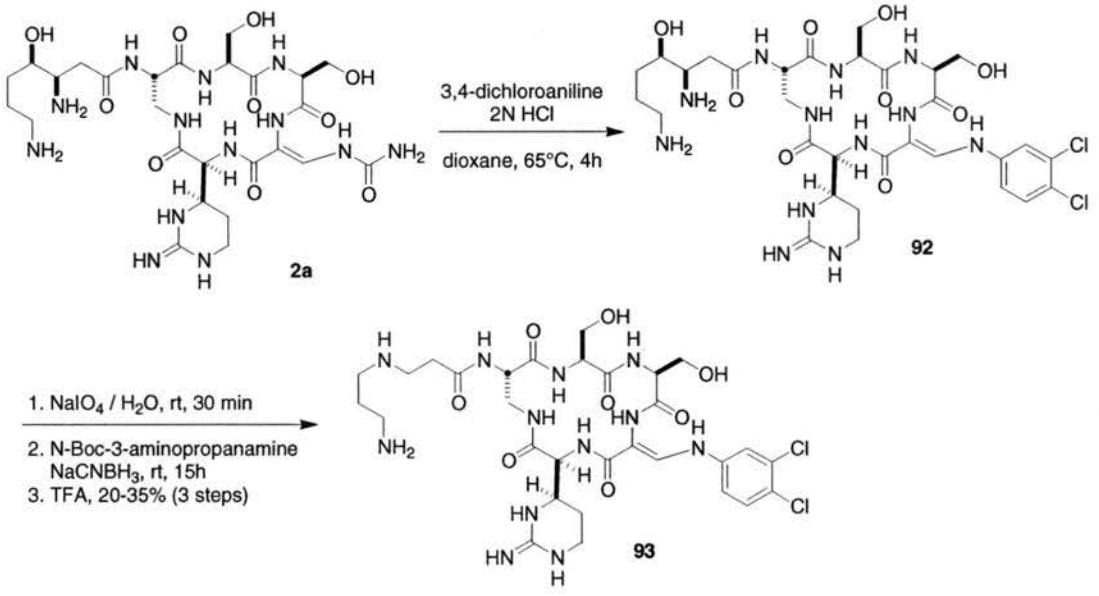
R_1	R_2	*P. multocida*	*E. coli*	MRSA
H		25	50	N.T.
H		6.25	25	3.12
H		6.25	6.25	12.5
H		3.13	12.5	12.5
H		3.13	25	12.5
H		3.13	12.5	N.T.
H		12.5	25	N.T.
		6.25	3.13	12.5
H		1.56	6.25	25
		6.25	6.25	25
H		3.13	12.5	N.T.
H		N.T.	N.T.	25
H		N.T.	N.T.	12.5

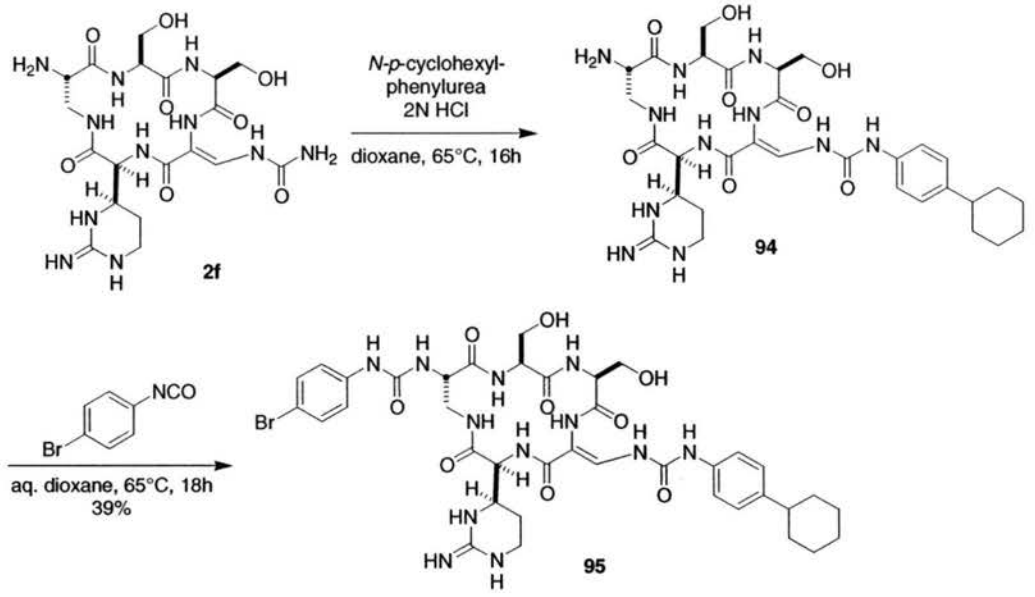
Table 11. MIC's (μg/mL) of C-5 carbamate and urea derivatives of **2d** and **92**.

Several additional enamidourea analogs, as well as some novel side chain analogs were prepared in a final study by Pfizer research. Treatment of tuberactinomycin N (**2a**) with 3,4-dichloroaniline in 2N HCl and dioxane provided the enaniline **92** (Scheme 20). Periodate cleavage of the γ-hydroxy-β-lysine group followed by reductive amination with *N*-Boc-3-aminopropanamine and TFA removal of the Boc group resulted in the isolation of side-chain derivative **93**.



Scheme 20. Enamidourea and side-chain manipulations of tuberactinomycin N.

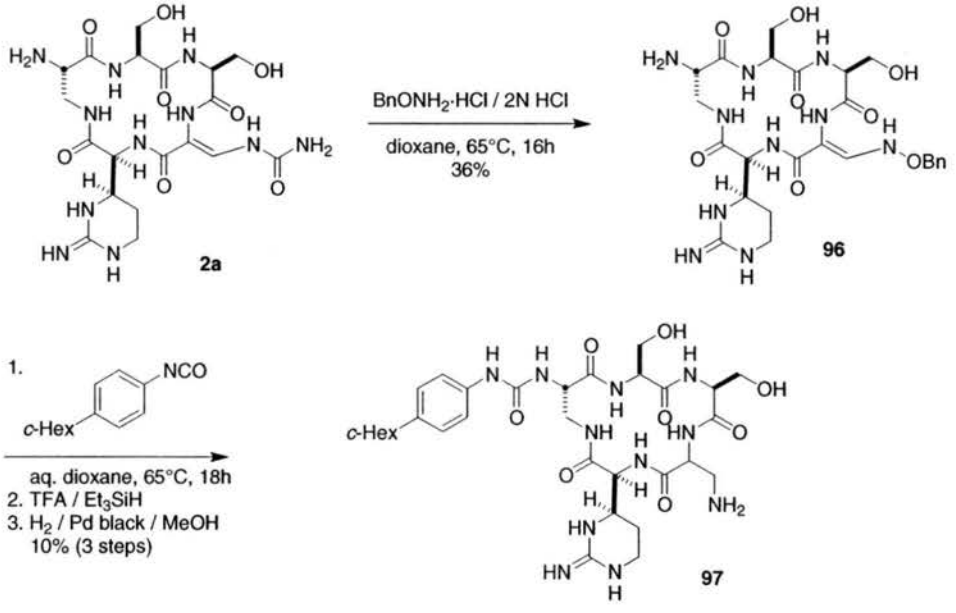
Exposure of tuberactinamine N (**2f**) to *N*-(*p*-cyclohexylphenyl)urea in 2N HCl in dioxane afforded enamidourea **94**, which was then treated with *p*-bromophenylisocyanate to access bromophenylurea **95** (Scheme 21).



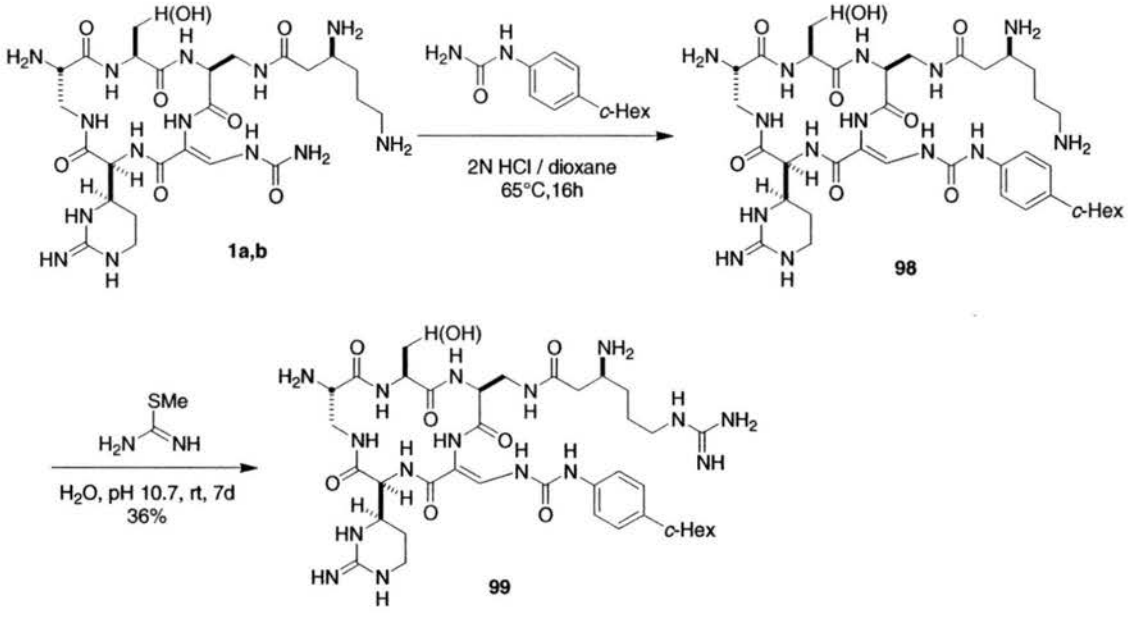
Scheme 21. Enamidourea and N-27 amine substitution of tuberactinamine N.

The enamidourea of tuberactinamine N was also exchanged with *O*-benzylhydroxylamine hydrochloride to afford oxime **96** in 36% yield (Scheme 22).

Reaction between *p*-cyclohexylphenylisocyanate and the amine at N-27 of **96** resulted in the formation of the *N*-(*p*-cyclohexylphenyl)urea, which was then subjected to a TFA and Et_3SiH reduction of the oxime, and subsequent palladium-mediated hydrogenolysis of the *O*-benzylhydroxylamine to provide **97** in 10% yield over three steps.



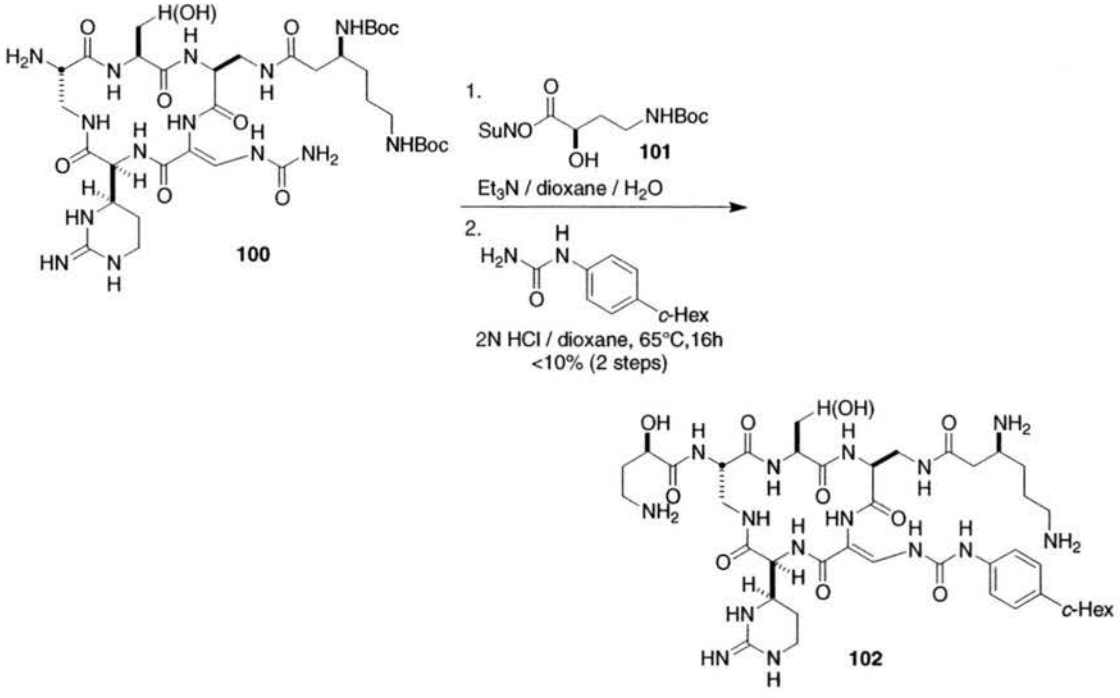
Scheme 22. Enamidourea and N-27 substitution of tuberactinamine N.



Scheme 23. Enamidourea and β-lysyl substitution of capreomycin IA and IB.

Capreomycin IA and IB as a mixture was subjected to *N*-(*p*-cyclohexylphenyl)urea under the previously described urea exchange conditions to provide **98** as a mixture of the two peptides (Scheme 23). Subsequent guanidinylation of the primary amine of the pendant β-lysine residue was accomplished with *S*-methylisothiourea in water in 36% yield to afford **99**.

Finally, the previously described di-*tert*-butoxycarbonyl capreomycin IA/B (**100**) was acylated with N-hydroxysuccinimidyl ester **101** (Scheme 24).[41] The acylated compound was then exposed to *N*-(p-cyclohexylphenyl)urea in 2N HCl and dioxane to exchange the urea and deprotect the Boc group to provide **102** in <10% yield.



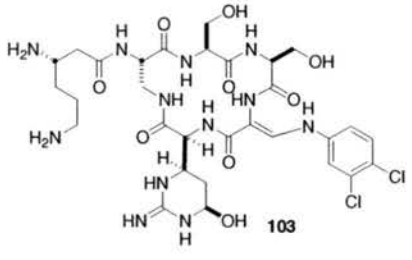
Scheme 24. Enamidourea and N-36 substitution of capreomycin IA and IB.

Comparison of the previously described derivatives to capreomycin (**1a,b**) and vancomycin against MRSA, and the vancomycin resistant *E. faecalis* and *E. faecium* are shown in Table 12. Compound **97**, which contains a C-12 *p*-cyclohexylphenyl urea and a diaminopropanoic acid residue in place of the enamidourea showed no improved activity

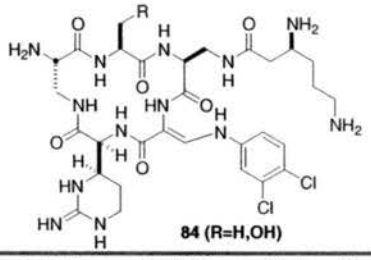
over capreomycin. The *p*-cyclohexylphenyl urea capreomycin analog (**98**) showed two to three orders of magnitude improvement in activity over that of capreomycin against all three organisms, and two orders of magnitude improvement in activity over vancomycin against the vancomycin resistant *Enteroccoci*. Interestingly, appending **98** with a more basic guanidine group (**99**) resulted in no change in activity from that of **98**.

COMPOUND	MRSA	*E. faecalis*	*E. faecium*
92	12.5	50	25
93	50	50	50
94	25	12.5	3.12
95	12.5	12.5	6.25
97	>100	>100	>100
98	1.56	3.12	3.12
99	1.56	3.12	3.12
1a,b	100	>1000	>1000
102	3.12	6.25	6.25
vancomycin	0.78	>100	>100

Table 12. MIC's (μg/mL) of enamidourea and side-chain derivatives.



103



84 (R=H,OH)

Bacteria	Strain #	1.2d	1.103	1.1a+b	1.84
A. pleuropneumoiniae	ATCC27088	>200	6.25	200	3.13
B. bronchiseptica	ATCC19395	>200	25	200	6.25
E. coli	ATCC25922	100	6.25	50	12.5
E. coli	W4680	25	3.13	25	3.13
E. coli	WZM120	25	3.13	25	3.13
H. somnus	ATCC43625	200	1.56	100	1.56
P. haemolytica	ATCC14003	200	12.5	200	6.25
P. multocida	ATCC15743	>200	12.5	>200	6.25
S. choleraesuis	ATCC19430	50	3.13	50	3.13
S. typhimurium	LT2 SGSC230	50	6.25	25	6.25
S. aureus	ATCC29213	200	6.25	100	1.56
S. uberis	ATCC27958	200	3.13	50	1.56

Table 13. Comparison of MIC's (μg/mL) of capreomycin and viomycin derivatives versus parent compounds against range of bacteria.

The antibacterial activity of viomycin derivative **103** and capreomycin derivative **84** were compared to their parent compounds against a wide variety of bacteria in order to determine the generality of their bioactivity (Table 13).[5] It was found that **103** and **84** were both significantly more active than viomycin (**2d**) and capreomycin (**1.1a,b**) against each bacteria tested. Additionally, **103** and **84** maintained antibacterial potency across a wide range of bacterial types. Generally, the culture medium used had little effect on the biological activity of the compounds tested, nor did the addition of EDTA. More alkaline conditions did result in an increase in potency in all of the compounds tested. These results demonstrate the promising possibilities that have thus far, and could still arise from further development of analogs of the capreomycins and tuberactinomycins.

1.6. Biosynthetic Studies on Viomycin and Capreomycin.

1.6.1. Carter's studies on viomycin biosynthesis.

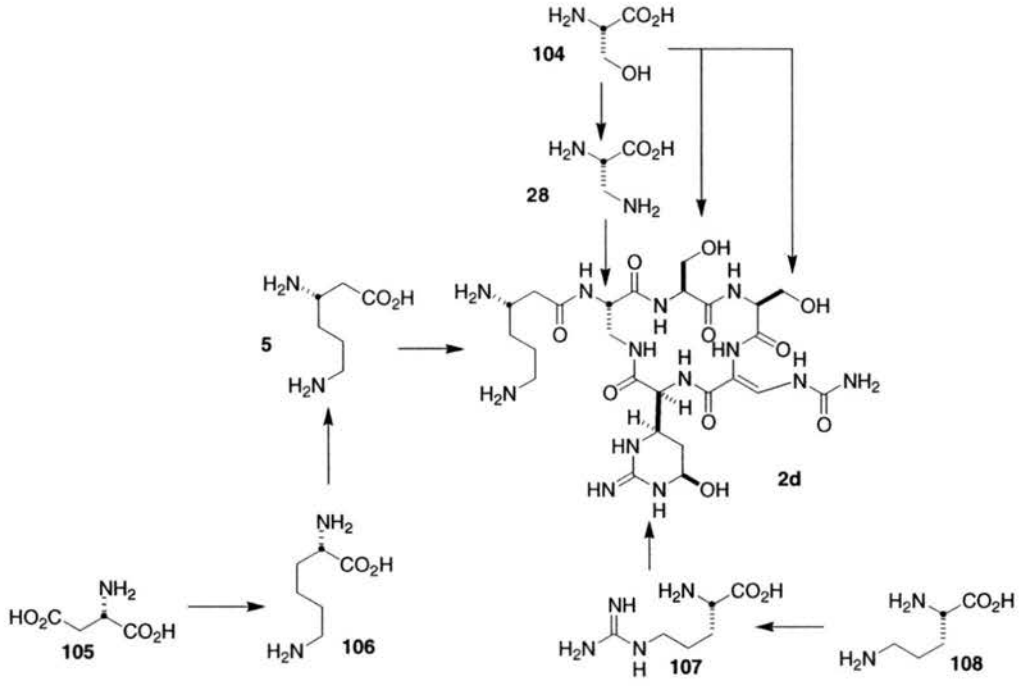


Figure 4. Carter's viomycin biosynthesis work.

Carter and co-workers in 1974 published their efforts towards the elucidation of the biosynthesis of viomycin (Figure 4).[46,47] By using ^{14}C labeled amino acids, they were able to gain insight into what the biosynthetic precursors to viomycin were, and at what stage they were incorporated. Cultures of an actinomycete responsible for the production of viomycin were fed separately DL-[1-^{14}C]serine and DL-[3-^{14}C]serine (**104**). Isolation of viomycin from these cultures showed significant incorporation of both labeled forms of serine into the serine residues as well as the diaminopropanoic residue present in the macrocyclic peptide. [U-^{14}C]diaminopropanoic acid (**28**) was found to be incorporated exclusively into the backbone of viomycin. This result suggests that diaminopropanoic acid is formed prior to incorporation into the peptide. The origin of the β-lysine portion of **2d** was probed *via* several experiments. Both DL-[1-^{14}C]lysine and DL-[2-^{14}C]lysine (**106**) were incorporated well into the β-lysine side chain. Although to a lesser extent, DL-[4-^{14}C]aspartic acid (**105**) was also incorporated exclusively into β-lysine, suggesting that it is an earlier intermediate along the biosynthetic pathway. Finally, L-[^{14}C]-β-lysine (**5**) was also incorporated into viomycin, indicative of pre-assembly of the amino acid, and subsequent coupling to the peptide. Finally, the tuberactinamine portion was found to be derived from arginine (**107**) as demonstrated by incorporation of DL-[5-^{14}C]arginine, DL-[1-^{14}C]arginine, and DL-[amidine-^{14}C]arginine. Additionally, it was found that ornithine (**108**), the biosynthetic precursor to arginine was also incorporated into viomycin.

1.6.2. Gould's studies on the biosynthesis of capreomycin.

In 1992, Gould and co-workers published the first of two reports on their investigations into the biosynthesis of the capreomycins (Figure 5).[48,49] Focusing on the

origin of the capreomycidine moiety, *Streptomyces capreolus* was fed with both [1-^{14}C]arginine and [2,3,3,5,5-$^{2}H_5$]arginine, and the resulting labeled capreomycins were isolated. As expected, the feeding experiment demonstrated that arginine (**107**) was, in fact, incorporated into capreomycidine. A more interesting observation, however, was made from the deuterated arginine experiment. The capreomycidine present in the natural product isolated from this experiment was found to be devoid of the deuterium label at C-2 that was present in the labeled arginine. This suggested that capreomycidine was most likely biosynthesized from a dehydroarginyl intermediate such as **109**, in which arginine was already incorporated into some manner of peptide.

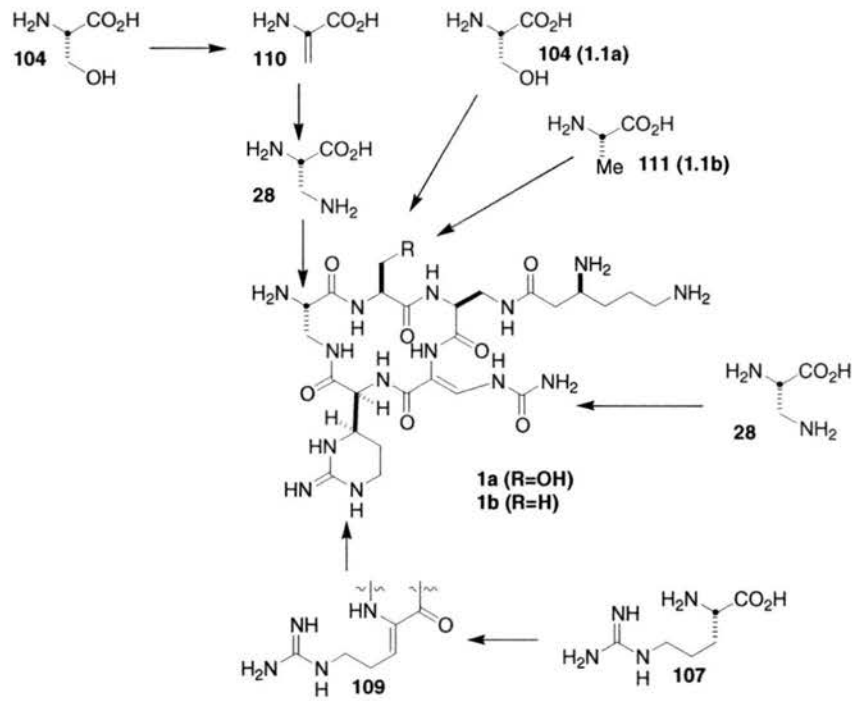


Figure 5. Gould's capreomycin biosynthesis work.

In the second report, Gould's main focus was on the origin of the serine and alanine units of **1a** and **1b** respectively, the origin of the diaminopropanoic acid residues, and the origin of the ureido-dehydroalanyl group. Initial feeding experiments with DL-[1-^{13}C]serine showed that the serine residue in **1a** came directly from incorporation of

serine into the peptide, rather than the potential post-peptide assembly oxidation of alanine from **1b**. Serine was also incorporated, to a lesser extent, into both diaminopropanoic acid residues and the ureido-dehydroalanine residue. More specifically, upon feeding of DL-[2,3,3-$^{2}H_{3}$]serine to *Streptomyces capreolus* it was found that the deuterium label was lost at C-35 and C-18, the α-protons of the two diaminopropanoic residues. This suggests that a dehydroalanyl intermediate (**110**) is responsible for the conversion of serine to diaminopropanoic acid. Labeled diaminopropanoic acid (**28**) was also incorporated into the ureido-dehydroalanyl residue in addition to the diaminopropanoic residues. Finally, alanine was directly incorporated into the macrocycle of **1b**.

As evidenced by the extensive work devoted to the study of the tuberactinomycins and capreomycins over the decades since their initial discovery, these compounds continue to intrigue the scientific community. Recently prepared analogs of these natural products may, in the future, find use in the treatment of human and animal health pathogens such as *Pasteurella*, MRSA, and vancomycin-resistant *Enterococci*.

CHAPTER 2

The Asymmetric Synthesis of (2*S*,3*S*)-, and (2*R*,3*R*)-β-Hydroxyornithine

INTRODUCTION

2.1. β-Hydroxyornithine Background.

The unnatural amino acid β-hydroxyornithine (**112a-d,** Figure 6) in its various stereoisomeric forms has been used as a biosynthetic probe for both the β-lactamase inhibitor clavulanic acid (**113**) and the antitumor antibiotic acivicin (**114**). In addition, (2S,3S)- β-hydroxyornithine **(112d)** has been proposed as a potential biosynthetic precursor to xanthobaccin A (**115**) and the related compounds discodermide (**116**), cylindramide (**117**), and alteramide A (**118**).

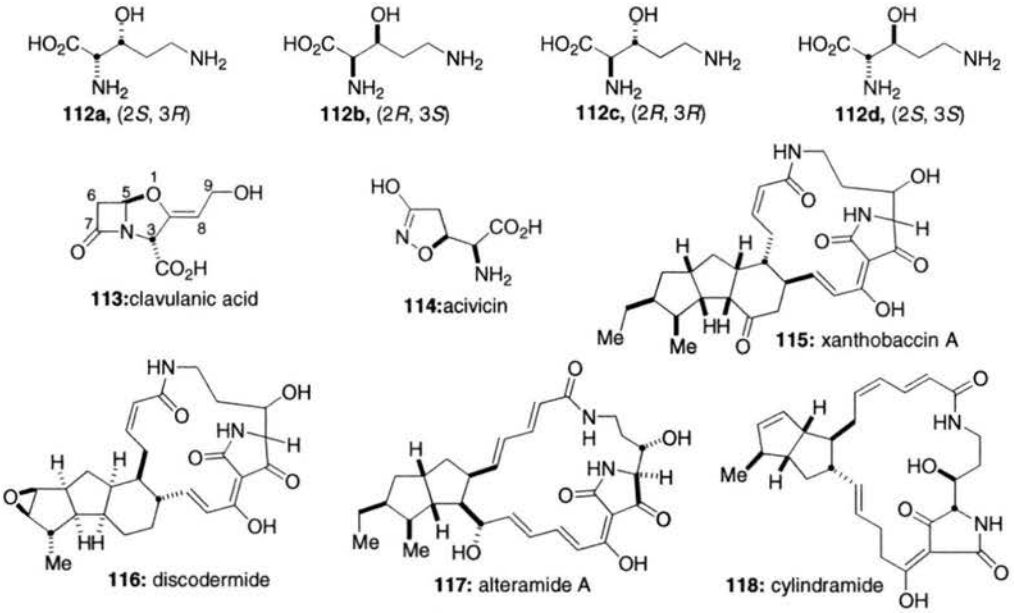
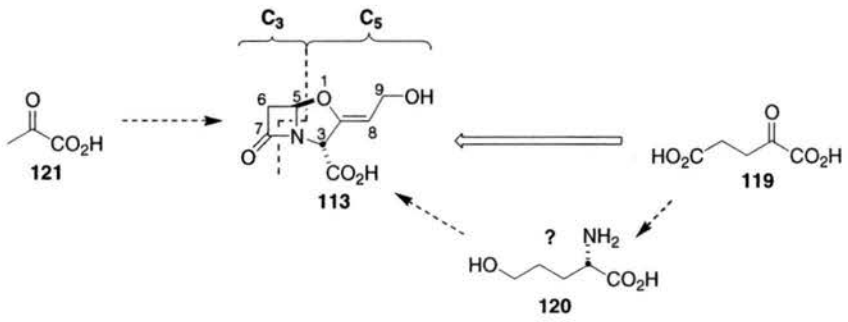


Figure 6. The stereoisomeric forms of β-hydroxyornithine, and related compounds.

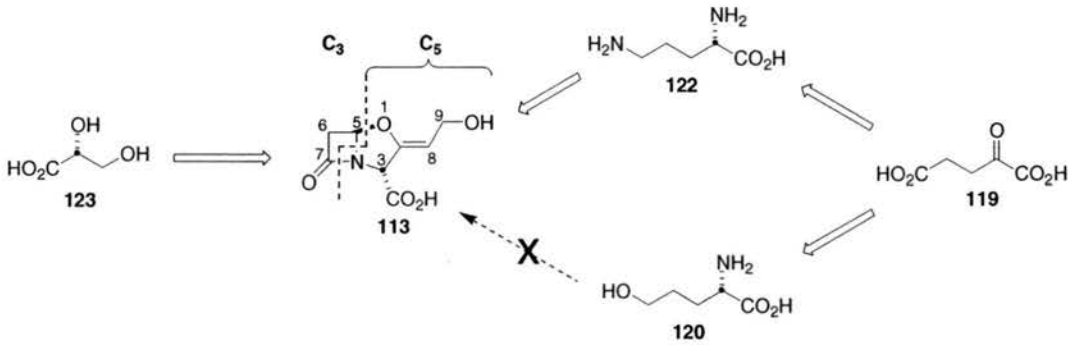
2.1.1. Clavulanic Acid Biosynthesis.

Clavulanic acid **(113)** is a potent β-lactamase inhibitor isolated from the fermentation of *Streptomyces clavulerigus.* Much effort has been put forth to understand the biosynthesis of this bicyclic β-lactam.



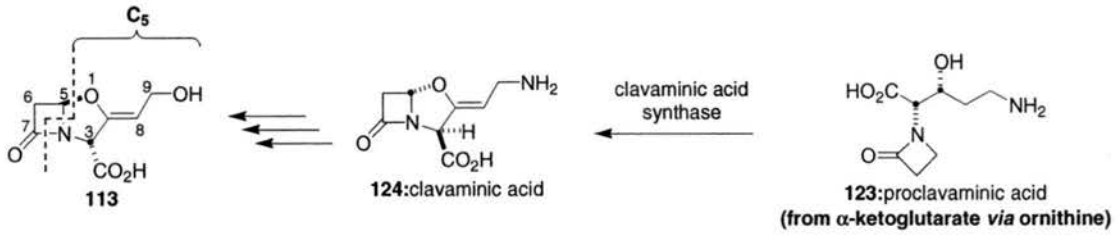
Scheme 25. Elson's initial biosynthetic proposal for clavulanic acid.

Original biosynthetic studies by Elson and coworkers proposed that the C_5 unit of clavulanic acid (Scheme 25) was derived from α-ketoglutarate (**119**) perhaps via δ-hydroxynorvaline (**120**), while the C_3 portion of the molecule was possibly accessed from a three-carbon glycolysis intermediate such as pyruvate (**121**).[50]



Scheme 26. Townsend's revised proposal for the biosynthesis of clavulanic acid.

Townsend and coworkers also engaged in an effort to elucidate the biosynthetic pathway of clavulanic acid.[51-53] When probing the origin of the C_5 portion of **113** (Scheme 26), it was determined that ornithine (**122**), which is biosynthetically derived from α-ketoglutarate (**119**), is a direct precursor to clavulanic acid, rather than the previously proposed δ-hydroxynorvaline (**120**) which is also biosynthesized from **119**. In addition, the Townsend group also discovered that the C_3 portion of **113** was derived from D-glycerate (**123**) rather than the previously postulated pyruvate.

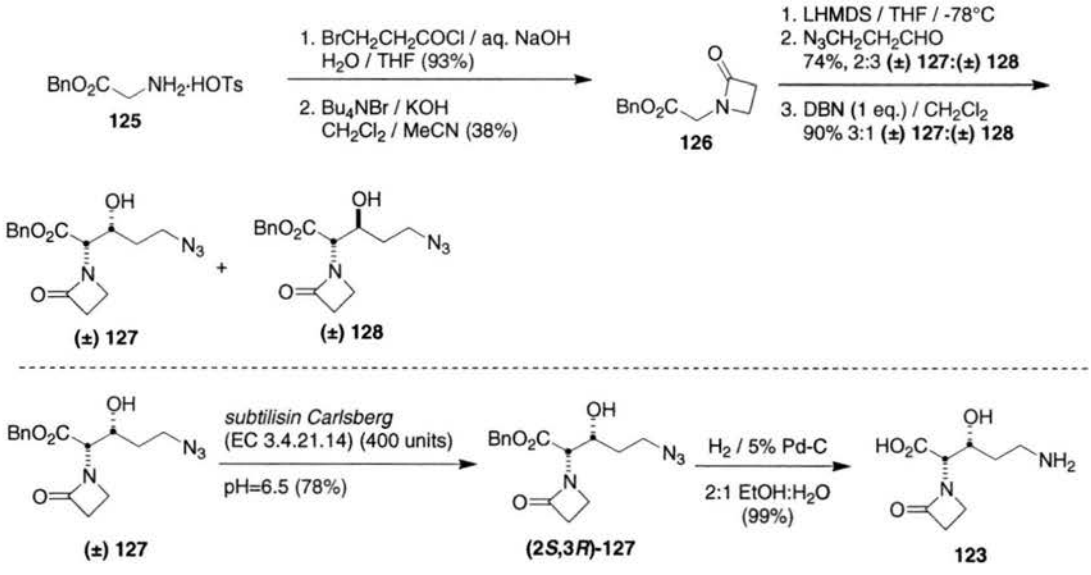


Scheme 27. Discovery of proclavaminic acid and clavaminic acid.

After Townsend's initial reports about the origins of the C_3 and C_5 portions of clavulanic acid, Elson and co-workers published a series of communications regarding later stage intermediates in the biosynthesis of the C_5 portion of **113**.[54-56] Having developed a cell-free method for the detection of late-stage intermediates in the biosynthetic pathway, they discovered two novel β-lactams, the monocyclic proclavaminic acid **(123)** and the bicyclic clavaminic acid **(124)** (Scheme 27). The enzyme responsible for the transformation of **123** to **124**, dubbed clavaminic acid synthase, requires Fe^{2+}, dioxygen, and α-ketoglutarate **(119)**. In addition, it was found that when both proclavaminic acid and clavaminic acid were incubated with *Streptomyces clavulerigus*, clavulanic acid **(113)** was produced. Baldwin and coworkers, in collaboration with Elson and co-workers at Beecham, also determined that the biotransformation of **123** to **124** occurs *via* a dihydroclavaminate species rather than a β-ketoproclavaminate intermediate.[57] It is interesting to note that the stereochemistry of **124** is opposite that of **113** which creates the necessity for inversion of both stereocenters to arrive at clavulanic acid.

Elson and Baggaley investigated several synthetic approaches to proclavaminic acid so that the absolute stereochemistry of **123** could be determined, and significant amounts of the substrate could be prepared for further biosynthetic studies.[58] The approach to **123** that was settled upon involved a racemic aldol reaction that was

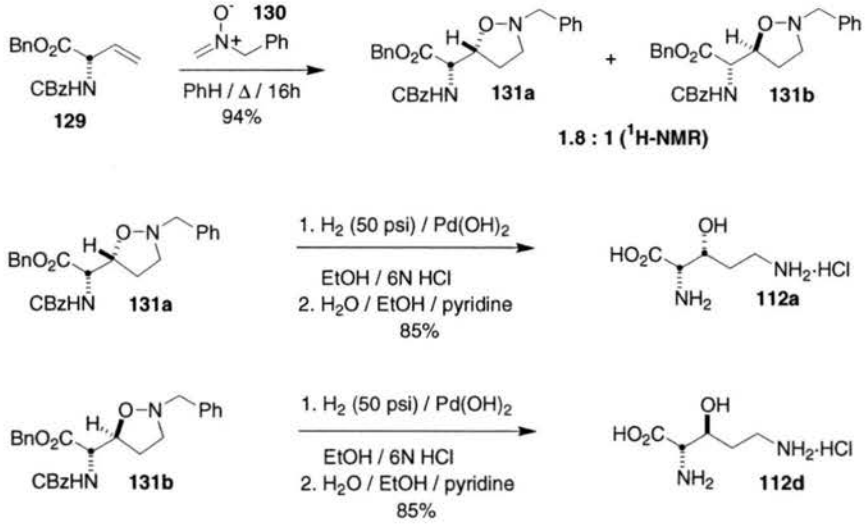
followed by an enzymatic resolution of the resulting aldol products (Scheme 28). Glycine benzyl ester tosylate salt (**125**) was treated with bromoacetyl chloride to form the α-bromoacetate and was then ring closed with tetrabutylammonium bromide and potassium hydroxide to provide the desired azetidinone **126** in 35% yield over the two steps. Lithium enolate formation followed by addition of 3-azidopropionaldehyde provided a 74% yield of racemic aldol products **127** and **128** in a 2:3 *threo* (**127**):*erythro* (**128**) ratio. Epimerization with DBN provided a 90% yield of the aldol products (±) **127** and (±) **128** in an improved ratio of 3:1 *threo*:*erythro*. Enzymatic resolution of the racemic mixture of the *threo* product **127** with *Subtilisin Carlsberg* afforded a 78% yield of the benzyl ester (2S,3R)-**127**. Hydrogenolysis of the benzyl ester and azide functionality provided optically pure proclavaminic acid (**123**) in quantitative yield.



Scheme 28. Elson's synthesis of proclavaminic acid.

Following Baggaley and Elson's synthesis of proclavaminic acid, the Townsend group published an article in which they described the asymmetric synthesis of (2*S*,3*S*)- and (2*S*,3*R*)-β-hydroxyornithine (Scheme 29).[59] Their hope was to find that either the

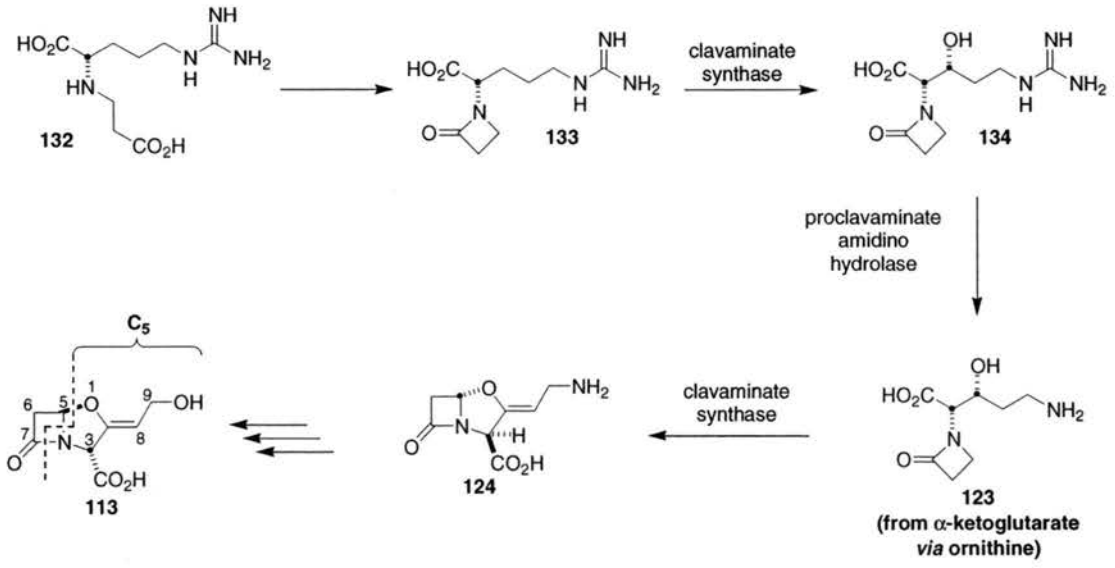
erythro- **(112d)** or *threo-* **(112a)** antipode of β-hydroxyornithine served as an intermediate between ornithine and proclavaminic acid in the biosynthesis of clavulanic acid. Treatment of protected vinylglycine **129** (prepared via Hanessian's method from glutamic acid) with nitrone **130** provided a modest 1.8:1 diastereomeric mixture of cycloadducts (**131a,b**). Separation of the products by column chromatography followed by hydrogenolysis provided *threo-* **(112a)** and *erythro*-β-hydroxyornithine **(112d)** in 85% yield in both cases. Although the approach to both diastereomers of β-hydroxyornithine was short, the selectivity in the cycloaddition was modest. In addition, the synthesis of the vinylglycine precursor was found to be fraught with varying degrees of epimerization. Unfortunately, upon feeding racemic *erythro-* and *threo-* [5-^{14}C]-β-hydroxyornithine to *Streptomyces clavulerigus*, no incorporation was observed.



Scheme 29. Townsend's synthesis of (2*S*,3*R*)- and (2*S*,3*S*)-β-hydroxyornithine.

A subsequent series of investigations by both the Elson and Townsend groups revealed an unexpected observation that proclavaminic acid (**123**) was biosynthesized via *N*-(3-propionyl)-L-arginine (**132**) rather than β-hydroxyornithine (Scheme 30).[60-63] Cyclization of **132** provided **133** which was then oxidized by clavaminate synthase to

afford **134**. Finally, hydrolysis of the guanidine present in **134** with proclavaminate amidino hydrolase resulted in the formation of proclavaminic acid (**123**) which was finally converted to clavulanic acid (**113**) by way of clavaminic acid (**124**).

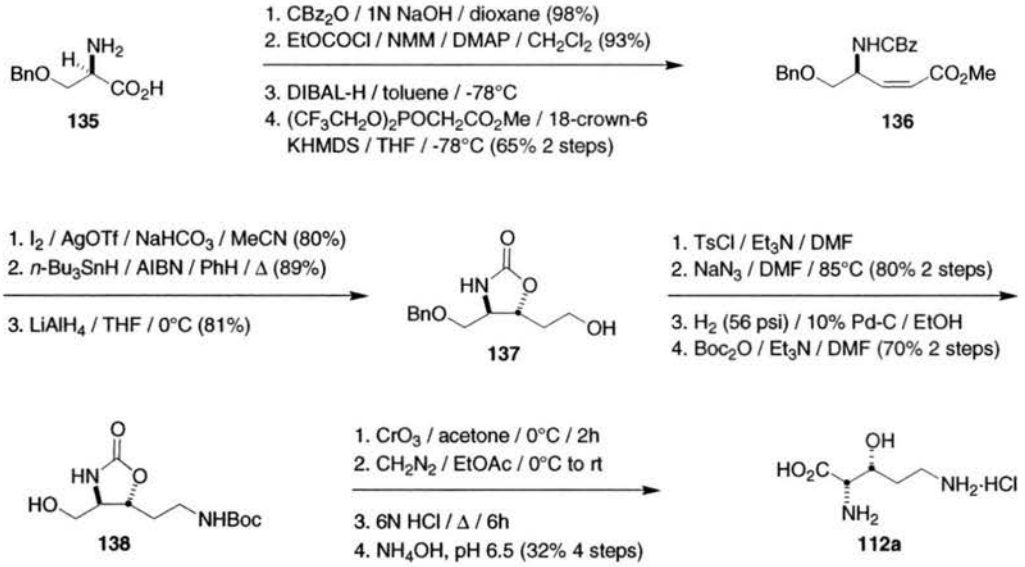


Scheme 30. Elucidation of the origin of proclavaminic acid.

Although it appears that β-hydroxyornithine is not along the biosynthetic pathway to clavulanic acid, the (2*S*,3*R*)- form **(112a)** has attracted considerable attention due to the fact that it can be transformed into the known biosynthetic precursor proclavaminic acid **(123)**.

Misiti and coworkers reported a stereoselective synthesis of **112a**, which derived its chirality from D-serine and utilized an iodocyclocarbamation as the key step (Scheme 31). Benzyloxycarbonyl protection of D-serine benzyl ether **(135)** was followed by ethyl ester formation, which proceeded in 91% yield over the two steps. Reduction of the ester with DIBAL provided the crude aldehyde, which was immediately subjected to a modified Still olefination to provide the desired α,β-unsaturated ester **136** in 65% yield. Iodocyclocarbamoylation with iodine, silver triflate, and sodium bicarbonate in

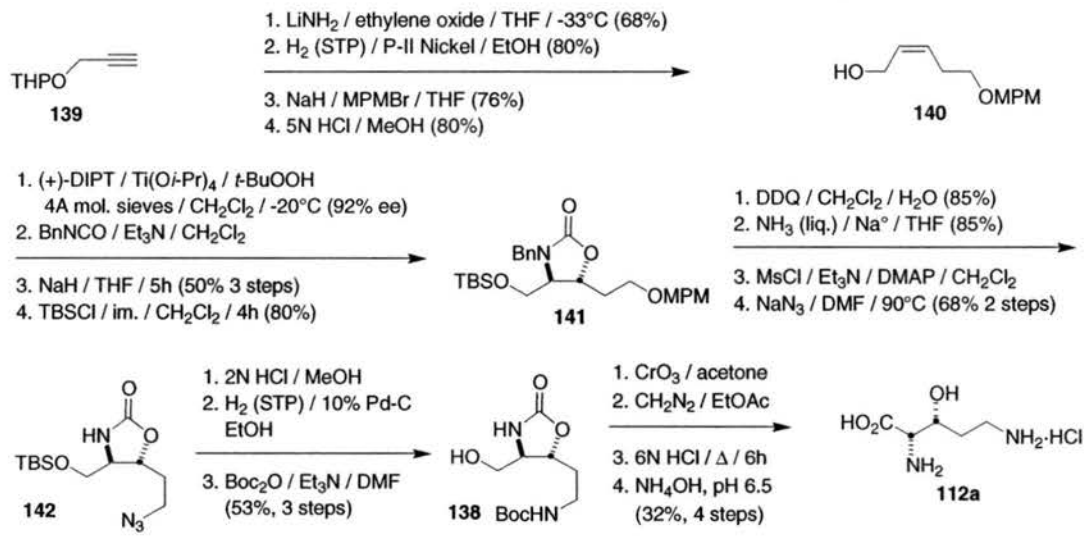
acetonitrile afforded a single diastereomer of the cyclic urethane, and an 11:1 mixture of epimers at the α-iodoester in 80% yield. This, however, was not a problem since the iodide was removed in the following step with tributyltin hydride and AIBN in benzene followed by reduction of the ester with $LiAlH_4$ to provide a 72% yield over two steps of the desired **137**. Tosylation of the newly formed primary alcohol was followed by displacement with sodium azide in DMF resulting in the primary azide in a yield of 80%. Hydrogenolysis of the azide and benzyl ether, followed by Boc protection of the resulting primary amine afforded **138**. Jones' oxidation of the primary hydroxyl to the acid, methyl ester formation and subsequent deprotection afforded **112a** in 32% yield (6% overall).



Scheme 31. Misiti's approach to (2*S*,3*R*)- β-hydroxyornithine.

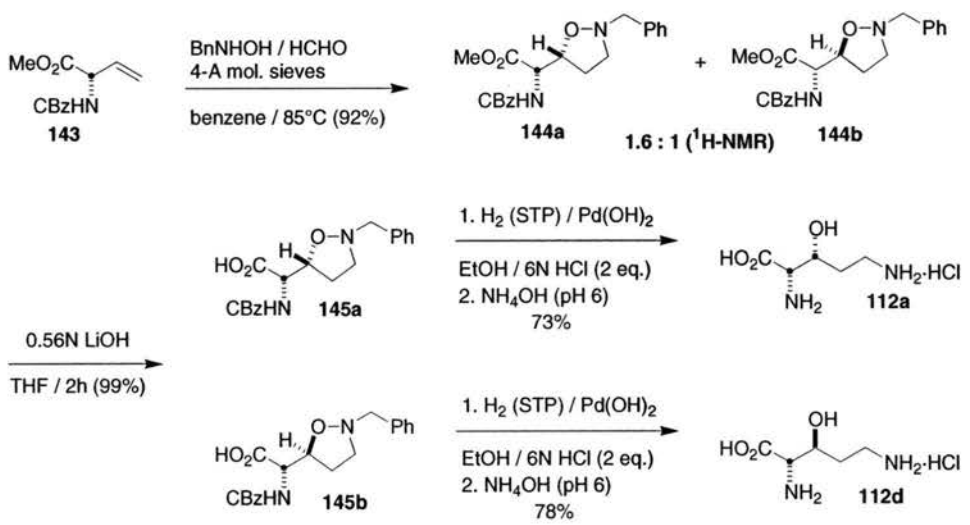
Gurjar and coworkers also developed an asymmetric synthesis of **112a** utilizing a Sharpless asymmetric epoxidation to provide the necessary stereochemistry (Scheme 32). The THP protected propargyllic alcohol **139** was treated with lithium amide and ethylene oxide in ammonia and THF to provide the desired homopropargyllic alcohol in 68% yield.

Partial reduction of the triple bond to the *cis*- double bond was achieved with hydrogen and Raney Nickel (80% yield). Protection of the homo-allylic alcohol as the MPM ether was followed by removal of the THP group to provide allylic alcohol **140** in 61% yield over the 2 steps. Sharpless asymmetric epoxidation provided the allylic epoxide in 92% ee. Treatment of the epoxide with benzyl isocyanate and Et_3N followed by NaH in THF provided the cyclic urethane in 50% yield from. Protection of the primary hydroxyl accessed the orthogonally protected **141**. Oxidative cleavage of the MPM group and benzyl group cleavage under Birch conditions provided the desired primary alcohol. Mesylation and azide displacement provided **142**. Removal of the TBS group, hydrogenolysis of the azide, and Boc protection of the newly formed primary amine resulted in Misiti's cyclic urethane intermediate **138** in 53% yield for the three steps. Oxidation of the primary hydroxyl to the acid, ester formation and deprotection provided **112a** in 19 steps and an overall yield of 1%.



Scheme 32. Gurjar's approach to (2*S*,3*R*)- β-hydroxyornithine.

2.1.2. The Biosynthesis of Acivicin.



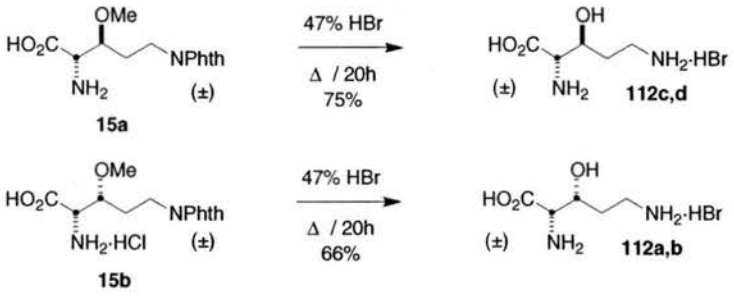
Scheme 33. Gould's synthesis of (2*S*,3*R*)- and (2*S*,3*S*)-β-hydroxyornithine.

Gould and coworkers undertook extensive investigations into the biosynthesis of the antitumor antibiotic acivicin (**114**).[64,65] Hypothesizing that either *erythro*- or *threo*-β-hydroxyornithine perhaps played a role in the biosynthesis of this compound, an efficient synthesis of **112a** and **112d** was required, one that would be amenable to isotope label incorporation (Scheme 33). The nitrone 1,3-dipolar cycloaddition approach to **112a** and **112d** that Townsend improved upon and used in his clavulanic acid studies, was originally developed by Gould in 1987. Using the methyl ester, *N*-benzyloxycarbonyl vinylglycine **143** rather than the benzyl ester used in Townsend's case, the nitrone 1,3-dipolar cycloaddition with formaldehyde and *N*-hydroxybenzylamine, a modest 1.6:1 mixture of diastereomeric cycloadducts **144a** and **144b** was obtained in 92% yield. Hydrolysis of the diastereomeric mixture of methyl esters provided acids **145a** and **145b**, which were then separated by flash chromatography. Hydrogenolysis and neutralization of **145a** provided (2*S*,3*R*)-β-hydroxyornithine **(112a)** in 73% yield, while hydrogenolysis and neutralization of **145b** provided (2*S*,3*S*)-β-hydroxyornithine **(112d)** in 78% yield.

The major disadvantage to this approach that was later addressed by Townsend's improvements was that a significant amount of racemization of **143** occurred, rendering a significant loss in enantiomeric purity of **144a** and **144b** and subsequent compounds. Townsend's vinylglycine **129** was a significant improvement due to the fact that it could be recrystallized to optical purity whereas **143** could not.

2.1.3. Additional syntheses of β-hydroxyornithine.

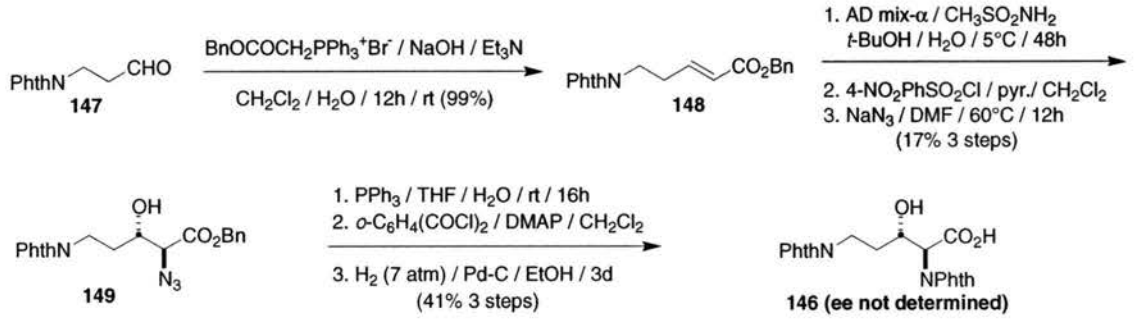
Shiba and coworkers in 1974 in their synthesis of γ-hydroxy-β-lysine (**6**), decided to access the substrate *via* an Arndt-Eistert synthesis from β-hydroxyornithine (Scheme 2).[29] In order to determine the relative stereochemistry of intermediates in the synthesis of **6**, **15a** and **15b** were deprotected with 47% HBr at reflux, providing *erythro*-β-hydroxyornithine **(112c,d)** and *threo*-β-hydroxyornithine **(112a,b)** in 75% and 66% yields respectively (Scheme 34).



Scheme 34. Shiba's racemic synthesis of *erythro*- and *threo* -β-hydroxyornithine.

More recently, Luzzio and coworkers developed an asymmetric synthesis of di-*N*-phthalimido-2*S*,3*S*-β-hydroxyornithine **(146)** to gain access to a variety of thalidomide analogs (Scheme 35).[66] Wittig olefination of phthalimido protected amino aldehyde **147** provided the desired unsaturated benzyl ester **148**. A Sharpless dihydroxylation was utilized as the key step to provide the necessary stereocenters. Nitrophenyl sulfonylation of the α-hydroxyl followed by azide displacement resulted in the azido ester **149** in a

17% yield over three steps. Staudinger reduction of the azide, phthalimide protection of the resulting amine, and reduction of the benzyl ester provided di-*N*-phthalimido-2*S*,3*S*-β-hydroxyornithine **(146)** in an overall yield of 7% over a total of 7 steps.



Scheme 35. Luzzio's preparation of di-*N*-phthalimido-2*S*,3*S*-β-hydroxyornithine.

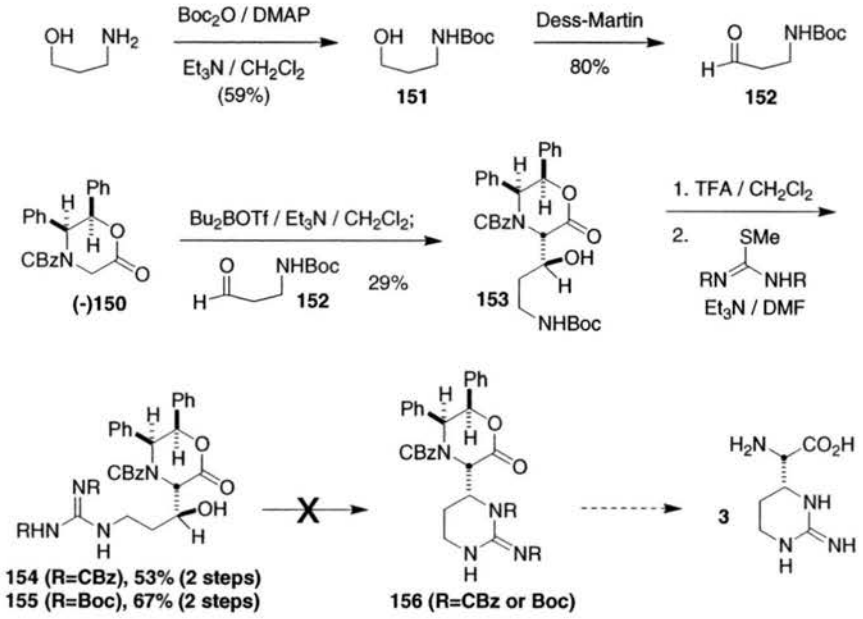
RESULTS

2.2. An Aldol-Based Approach to (2*S*,3*R*)-Capreomycidine and the Asymmetric Syntheses of (2*S*,3*S*)- and (2*R*,3*R*)-β-Hydroxyornithine.

One of the major goals of this research project was to develop a concise, and asymmetric synthesis of (2*S*,3*R*)-capreomycidine (**3**). Our original efforts towards the synthesis of capreomycidine proceeded in a two-tiered approach. Along with the enolate-aldimine investigations towards capreomycidine that will be discussed in Chapter 3, a synthetic plan using familiar aldol chemistry with Williams' chiral glycinate ((**-**) **150**) was probed (Scheme 36).[67,68]

Treatment of 3-aminopropanol with di-*tert*-butyl-dicarbonate in CH_2Cl_2 with Et_3N and DMAP provided a 59% yield of the desired protected amino alcohol **151**. Dess-Martin oxidation of **151** provided an 80% yield of protected amino aldehyde **152**. The boron enolate of **(-) 150** was formed and combined with **152** resulting in a 29% yield of aldol product **153**. Removal of the *tert*-butoxycarbonyl group and subsequent guanidinylation with both bis(*tert*-butoxycarbonyl)- and bis(benzyloxycarbonyl)-S-

methylisothiourea provided protected guanidines **154** and **155** in 53% and 67% yield respectively.[69,70] Unfortunately, attempts to displace the β-hydroxyl group of **154** or **155** with the guanidine by either a single step Mitsunobu inversion or a two-step activation / displacement approach were unsuccessful, resulting in either no reaction or trace elimination of the β-hydroxyl group in the case of more rigorous conditions. Since it seemed unlikely that any set of conditions would allow access to **156** from **154** or **155**, it was decided to abandon this approach to (2S,3R)-capreomycidine (**3**).



Scheme 36. Aldol-based approach to capreomycidine.

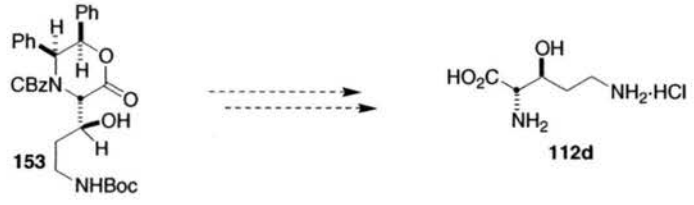
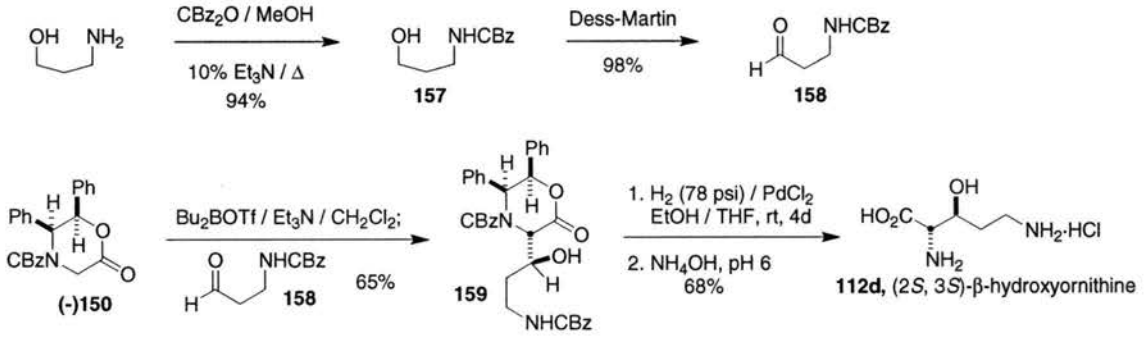


Figure 7. Fully protected precursor to (2*S*,3*S*)-β-hydroxyornithine.

Although we were unable to access capreomycidine by this approach, it was realized that aldol product **153** was a reasonable precursor to (2S,3S)-β-hydroxyornithine (**112d**) (Figure 7).



Scheme 37. Synthesis of (2*S*,3*S*)-β-hydroxyornithine from Williams' chiral glycinate.

Unlike the aldol-based approach to capreomycidine in which it was necessary to have an orthogonal protecting group on the δ-amine of aldol product **153** (Scheme 36), it was decided that it would be more advantageous for the synthesis of β-hydroxyornithine to prepare an aldol product that could be globally deprotected in a single hydrogenolysis step (Scheme 37). Protection of 3-hydroxypropylamine with dibenzyldicarbonate in refluxing 10% Et_3N in methanol provided a 99% yield of the desired carbamate **157**.[71] Dess-Martin periodinane oxidation of **157** provided the desired aldehyde **158** in 98% yield. The boron enolate-mediated aldol reaction between oxazinone **(-) 150** and **158** provided aldol product **159** in 65% yield and a diastereomeric ratio of 8:1 *erythro* : *threo* (^{1}H-NMR). Recrystalization from abs. EtOH facilitated removal of the minor diastereomer. Hydrogenolysis of **159** followed by neutralization and recrystalization provided (2*S*,3*S*)-β-hydroxyornithine **(112d)** in 41% overall yield from **(-) 150**.[72] In addition, the (2*R*,3*R*)- enantiomer (**112c**) was prepared in similar yield. Both were found to be prepared in >99.5:0.5 er by chiral HPLC.

This synthesis represents a short and high-yielding approach to both enantiomers of *erythro*-β-hydroxyornthine. For potential biosynthetic studies in molecules such xanthobaccin A, discodermide, alteramide A, and cylindramide, this approach has the added advantage of relatively simple isotope label incorporation via a suitably labeled form of oxazinone **2.2**.[73]

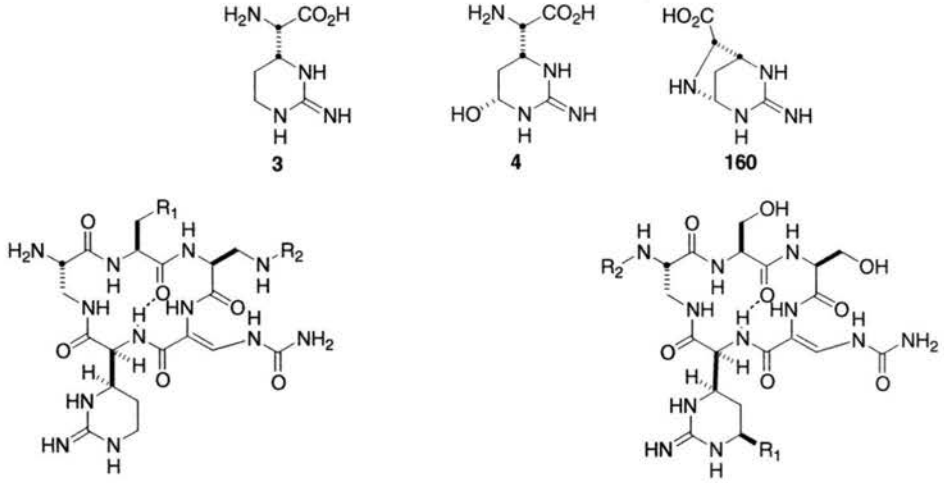
CHAPTER 3

The Asymmetric Synthesis of (2*S*,3*R*)-Capreomycidine

INTRODUCTION

3.1. The structure and prevalence of capreomycidine and related amino acids.

The cyclic guanidine-containing amino acid (2*S*,3*R*)-capreomycidine (**3**) is present in both the cyclic pentapeptide family of antibiotics the capreomycins (**1 a-d**) and the structurally similar tuberactinomycins N and O (**2a-b**) (Figure 9). Additionally, tuberactinomycins A and B (viomycin) (**2c-d**) contain an oxygenated form of capreomycidine known as tuberactidine (**4**). Upon hydrolysis of **2c** and **2d**, the tuberactidine moiety is isolated as the aminal viomycidine (**160**).



1a: capreomycin IA; R_1=OH, R_2=β-lysine
1b: capreomycin IB; R_1=H, R_2=β-lysine
1c: capreomycin IIA; R_1=OH, R_2=H
1d: capreomycin IIB; R_1=H, R_2=H

2a: tuberactinomycin N; R_1=H, R_2=γ-hydroxy-β-lysine(**5**)
2b: tuberactinomycin O; R_1=H, R_2=β-lysine(**6**)
2c: tuberactinomycin A; R_1=OH, R_2=γ-hydroxy-β-lysine(**5**)
2d: tuberactinomycin B (viomycin); R_1=OH, R_2=β-lysine(**6**)
2e: LL-BM547β; R_1=OH, R_2=N^{β}-methyl-β-arginine(**7**)
2f: tuberactinamine N; R_1=H, R_2=H

Figure 8. The capreomycins, tuberactinomycins, and component amino acids.

In an interesting example of biologically diverse organisms selecting for the biosynthesis of similar molecular structures, an entirely different group of organisms have incorporated (2*S*,3*S*)-epicapreomycidine (**161**) into unique natural products (Figure

9). Epicapreomycidine was first discovered by Umezawa and co-workers as a component of the chymotrypsin inhibitors, chymostatins A-C (**162a-c**).[74] Soon after, **161** was found to be a component of a similar pseudotetrapeptide elastinal (**163**).[75] Recently, **161** has also been found in the peptide nucleoside conjugate muraymycin A1 (**164**).[76]

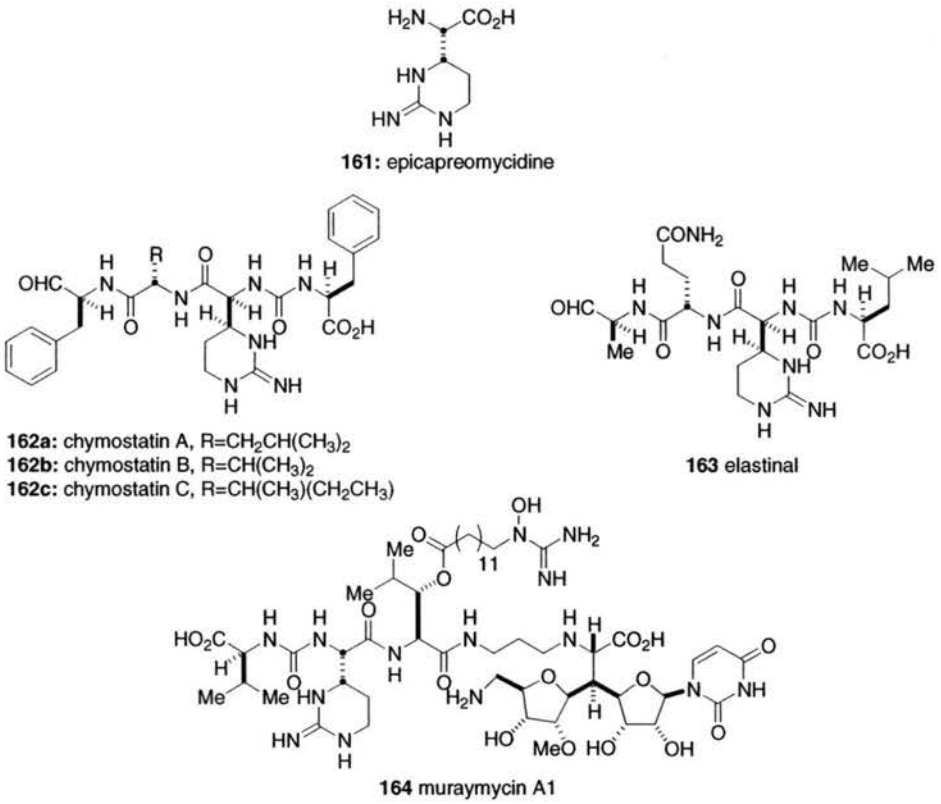


Figure 9. Natural products containing epicapreomycidine.

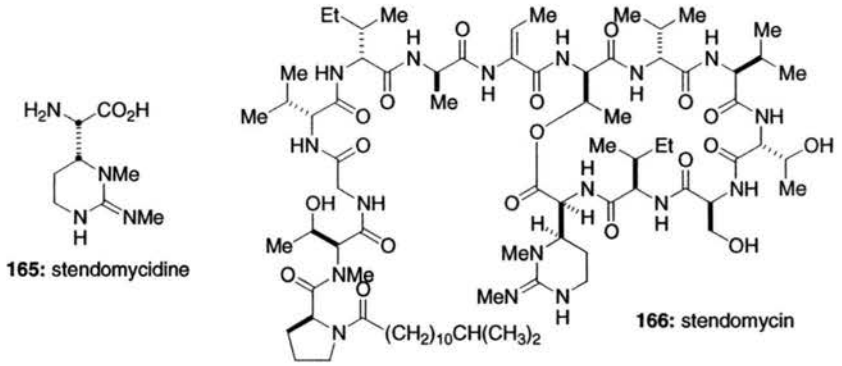
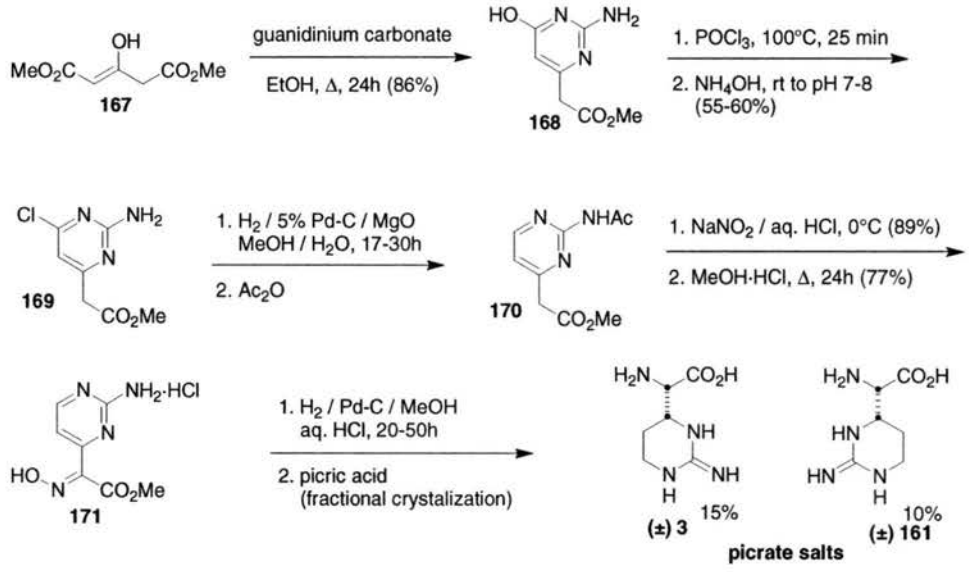


Figure 10. The structure of macrocyclic depsipeptide stendomycin.

In another example of cyclic guanidine-containing amino acids found as secondary metabolites in nature, stendomycidine (**165**) was found to be a constituent amino acid of the macrocyclic depsipeptide stendomycin (**166**) (Figure 10).[77,78] In addition to stendomycidine, stendomycin's unique structure contains a number of non-proteinogenic amino acids as well as an intriguing pendant fatty acid chain. The configuration of **165** was proposed to be (2*S*,3*R*)-, identical to that of capreomycidine. This was determined by ORD measurements of **165** and degradation products of **165**.

3.2. Bycroft's racemic synthesis of capreomycidine and epicapreomycidine.

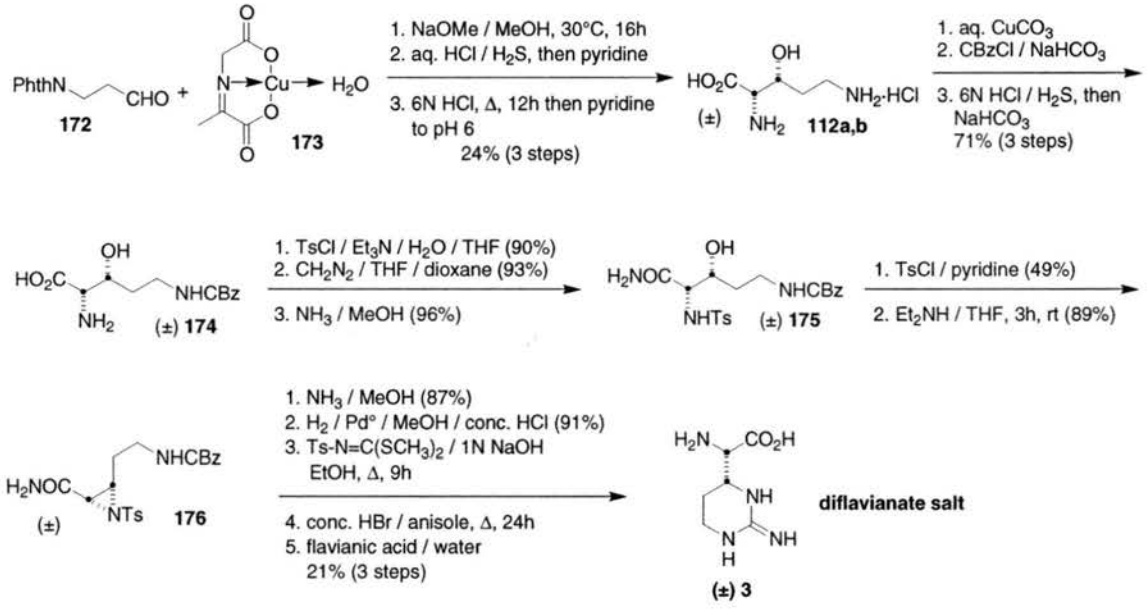


Scheme 38. Bycroft's racemic synthesis of capreomycidine and epicapreomycidine.

In an effort to confirm the structure of capreomycidine, Bycroft and co-workers developed a racemic synthesis of **3** and **161** starting from dimethyl acetonedicarboxylate (**167**) (Scheme 38).[79] Treatment of **167** with guanidinium carbonate at reflux in ethanol provided the substituted pyrimidyl acetate **168** in 86% yield. Exposure of **168** to phosphorus oxychloride afforded chloropyrimidine **169** in moderate yield. Palladium on carbon mediated hydrogenolysis of the chloride, and subsequent acetylation of the

exocyclic pyrimidine nitrogen provided **170**. Benzylic oxime formation with $NaNO_2$ and aq. HCl was followed by methanolysis of the acetate to provide **171** in 69% yield over the two steps. Finally, hydrogenation of **171** and treatment with picric acid, followed by fractional crystallization of the resulting picrate salts afforded (±) **3** in 15% yield and (±) **161** in 10% yield, thus confirming the proposed relative structures of **3** and **161**.

3.3. Shiba's capreomycidine work.



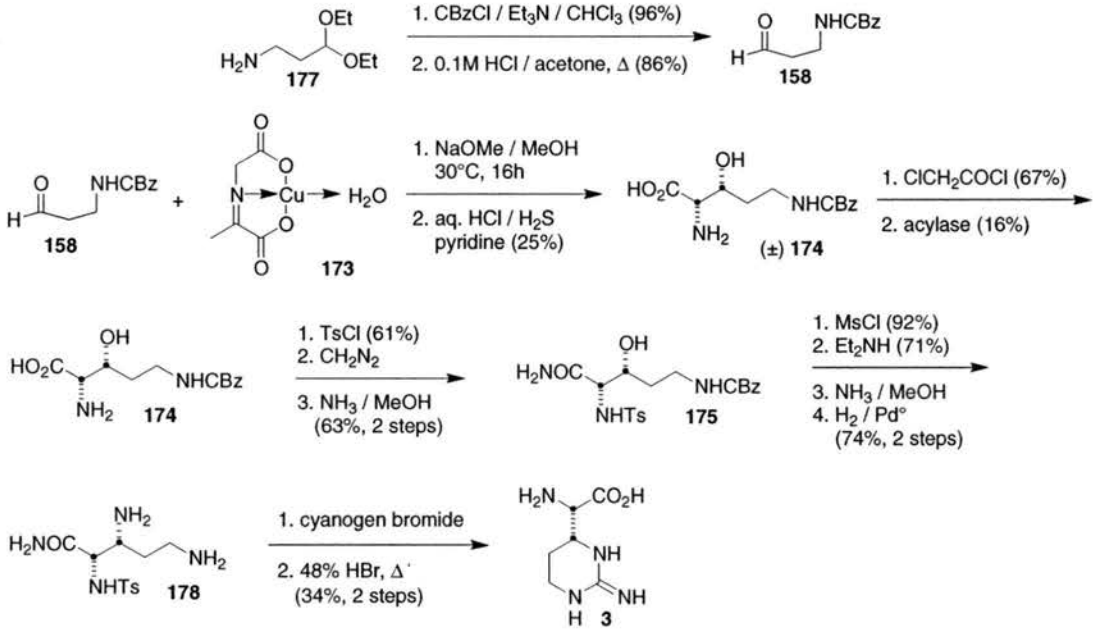
Scheme 39. Shiba's racemic synthesis of capreomycidine.

In line with their extensive synthetic work with the capreomycins and tuberactinomycins, Shiba and co-workers initially embarked on a racemic synthesis of **3** (Scheme 39).[80] Treatment of 3-phthalimido-propionaldehyde (**172**) with *N*-pyruvylideneglycinatoaquocopper (II) (**173**), followed by removal of the copper chelate and phthalimide group provided the racemic *threo*-β-hydroxyornithine (**112a,b**) in 24% yield over three steps. A three-step procedure was followed to selectively protect the δ-amine of **112a,b** as its benzyl carbamate ((±)**174**). Selective tosylation of **(±) 174** was followed by methyl ester formation and subsequent displacement of the ester with

ammonia, providing primary amide (±) **175** in 80% yield over the three steps. Upon tosylation of the hydroxyl group of (±) **175**, treatment with diethylamine provided the tosyl aziridine **176**. Regioselective opening of the aziridine from the β-position with ammonia provided access to the β-amino-ornithine derivative with net retention of stereochemistry from (±) **175** in 38% yield. Hydrogenolysis of the benzyloxycarbonyl group was accomplished in 91% yield. The resulting diamine was exposed to *N*-(*p*-toluenesulfonyl)bis(methylthio) methanamine to provide the tosyl guanidine which subsequently underwent global deprotection with concentrated HBr to provide (±) **3** which was isolated as its diflavianate salt in 21% yield over the last three steps. Shiba's preparation of (±) **3** was accomplished in 16 steps and an overall yield of 1% from **172**.

Having completed the racemic synthesis of capreomycidine, Shiba then pursued a stereoselective synthesis of (2*S*,3*R*)-capreromycidine (Scheme 40).[31] Although similar to the racemic synthesis of capreomycidine, the asymmetric synthesis incorporated some significant changes. Treament of **177** with CBzCl and Et_3N, followed by aq. HCl to remove the diethylacetal afforded the protected amino aldehyde **158** in 83% yield overall. Analogous to the racemic synthesis, **158** was treated with glycinatoaquocopper reagent **173**, resulting in a 25% yield of (±)**174**. Chloroacetylation (67%) was followed by acylase resolution of the two enantiomers to provide a 16% yield of optically pure **174**. Tosyl amide formation, methyl ester formation, and displacement of the ester with ammonia afforded amide **175** in 38% yield. Mesylation of the hydroxyl group and tosyl aziridine formation proceeded in 65% yield over the two steps. Regioselective opening of the aziridine with ammonia and hydrogenolysis of the CBz group resulted in the realization of diamine **178** in 74% yield. Exposure of **178** to cyanogen bromide followed

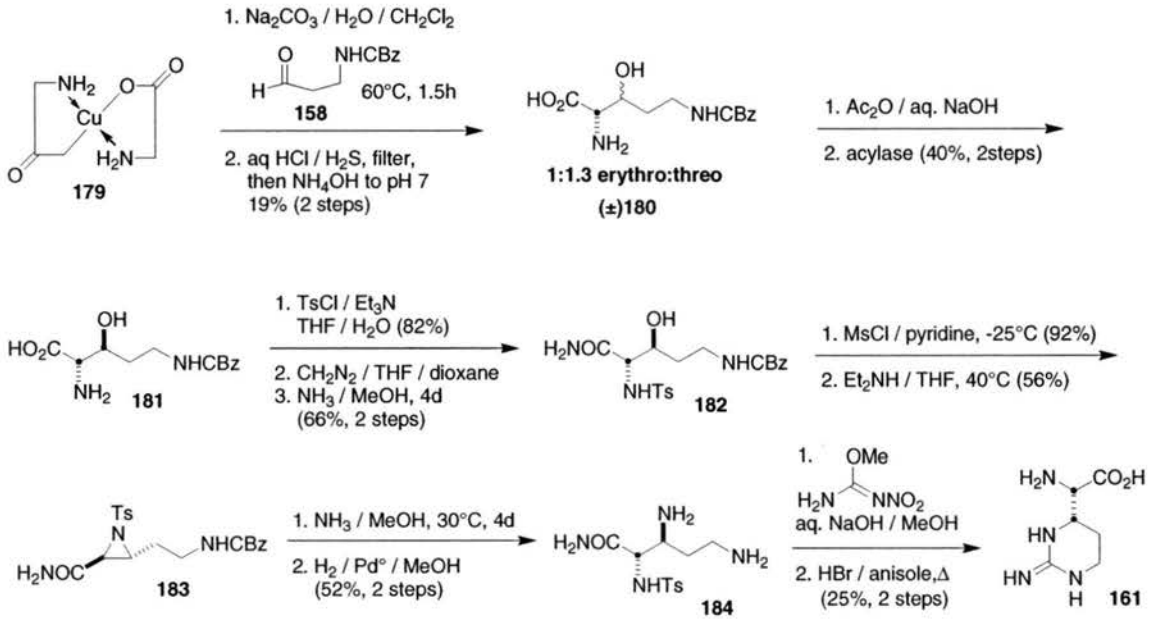
by deprotection with refluxing conc. HBr resulted in the isolation of (2*S*,3*R*)-capreomycidine (**3**) in 34% yield. This represented the first asymmetric synthesis of capreomycidine. However, due to the low overall yield of **3** (14 steps, 0.14%), this approach was not amenable to the preparation of significant quantities of the amino acid for a total synthesis of the capreomycins.



Scheme 40. Shiba's stereoselective synthesis of capreomycidine.

In an extension of the methodology developed for the asymmetric synthesis of **3**, Shiba also reported a synthesis of (2*S*,3*S*)-epicapreomycidine (**161**) (Scheme 41).[81] Unlike the synthesis of **3**, the *erythro* β-hydroxyornithine derivative would be needed instead of the *threo* isomer. Treatment of the previously described aldehyde **158** with the sodium enolate of bis(glycinato)copper (II) (**179**) provided a near equal mixture of the *erythro* and *threo* forms of **(±) 180**. In a useful observation, it was found that acetylation of the racemic *erythro/threo* mixture **(±) 180** followed by acylase resolution of the mixture of acetates provided **181** as a single enantiomer in 40% yield over the two steps.

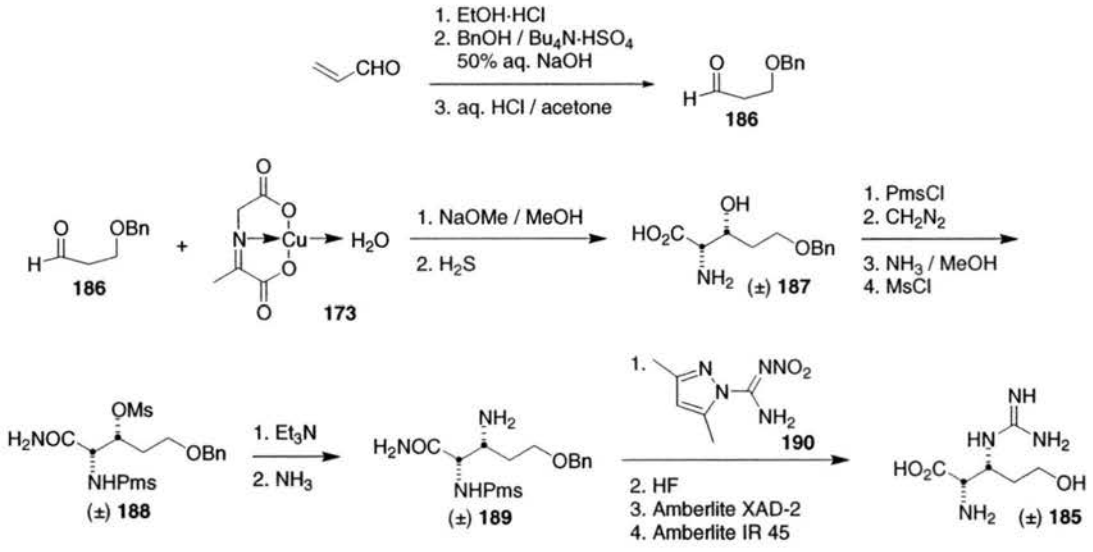
As in the previous synthesis of **3**, tosylation of the amine of **181** was followed by a two-step formation of the primary amide **182**. Mesylation of the free hydroxyl and treatment with diethylamine afforded the tosyl aziridine **183** in 52% yield (2 steps). Opening of the aziridine with ammonia and removal of the CBz group provided diamine **184**. Exposure of **184** to *O*-methyl-*N*-nitroisourea and subsequent deprotection with conc. HBr provided (2*S*,3*S*)-epicapreomycidine (**161**) in 25% yield.



Scheme 41. Shiba's asymmetric synthesis of epicapreomycidine.

In order to complete the synthesis of the series of cyclic guanidino amino acids isolated from both the capreomycins and tuberactinomycins, Shiba reported the racemic syntheses of dihydroviomycidine (**185**) and viomycidine (**160**) (Scheme 42 and 43).[82,83] Dihydroviomycidine (**185**) was originally prepared semisynthetically from **2d** by sodium borohydride reduction of the intact peptide followed by subsequent hydrolysis into its component amino acids. Shiba and coworkers developed a racemic synthesis of **185** in order to confirm its structure and relative stereochemistry (Scheme 42). Unfortunately, the communication in which this work was described provided no experimental details or

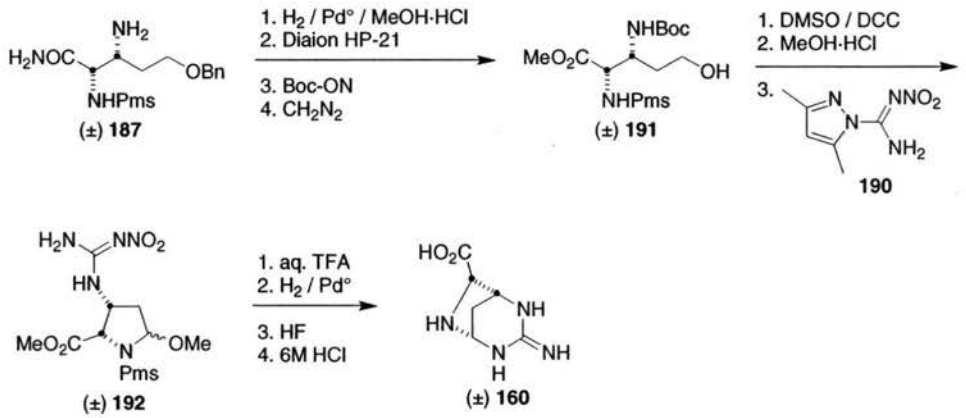
yields for their approaches to **185** and **160**. Preparation of 3-benzyloxypropanal (**186**) was achieved in three steps from acrolein. Aldol reaction of **186** with **173** provided the δ-benzyloxy-β-hydroxy amino acid (±) **187**. Protection of the amine as the *p*-tolylmethylsulfonamide (Pms) was followed by primary amide formation *via* the methyl ester. Mesylation of the secondary hydroxyl provided (±) **188**. Aziridine formation with Et_3N and subsequent regioselective ammonolysis afforded protected diamine (±) **189**. Guanidinylation with **190** and global deprotection resulted in the isolation of racemic dihydroviomycidine (±) **185**.



Scheme 42. Shiba's racemic synthesis of dihydroviomycidine.

In the same publication, Shiba also presented the racemic synthesis of viomycidine ((±) **160**) in order to confirm its structure and relative stereochemistry (Scheme 43). From the previously described **(±) 187**, hydrogenolysis of the benzyl ether resulted in closure of the primary hydroxyl onto the amide to form the lactone. Treament with Diaion HP-21 opened the lactone to the carboxylic acid. Boc protection of the free amine was followed by methyl ester formation to afford **(±)191**. Moffatt oxidation of the alcohol resulted in the carbinolamine, which was then treated with MeOH·HCl to remove

the Boc group and form the methoxy carbinolamine. Construction of nitroguanidine **(±)192** was then accomplished using the guanidinylating reagent **190**. A multi-step deprotection of **(±)192** resulted in the isolation of racemic viomycidine **(±)160**.



Scheme 43. Shiba's racemic synthesis of viomycidine.

3.4. Zabriskie's stereoselective synthesis of ^{13}C labeled capreomycidine.

Recently, Zabriskie and coworkers developed an asymmetric synthesis of ^{13}C labeled capreomycidine (**193**) for use in their studies on the biosynthesis of streptothricin F (**194**) (Figure 11).[84]

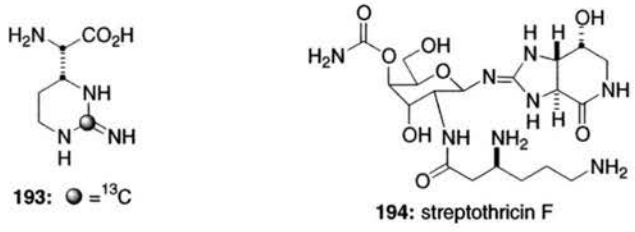
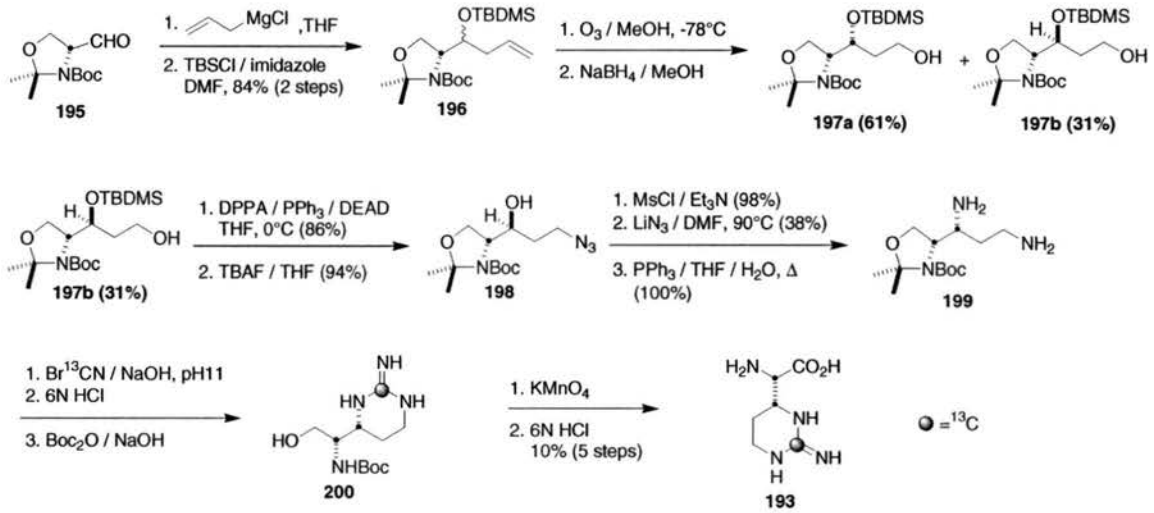


Figure 11. [*guanidino*-^{13}C]capreomycidine and streptothricin F.

Zabriskie's synthetic approach to capreomycidine was significantly different from previously published approaches (Scheme 44). Diastereoselective addition of allyl Grignard to the Garner aldehyde (**195**) provided a mixture of secondary alcohols, which were then protected as their TBS ethers **196**. Ozonolysis of the alkene followed by reductive workup with $NaBH_4$ provided a separable 2:1 mixture of silyl ethers (**197a,b**)

with the minor diastereomer (**197b**) being the desired product. Azidation of the hydroxymethyl group of **197b**, followed by removal of the silyl group afforded **198** in 81% yield over the two steps. Mesylation of the secondary hydroxyl and displacement with lithium azide provided the diazide, which was reduced under Staudinger conditions to provide the diamine **199** in 37% yield for the three steps. Tethering of the diamine with [^{13}C]cyanogen bromide accessed the desired guanidine. Hydrolysis of the acetonide and Boc group with aqueous HCl was followed by reprotection of the amine with Boc_2O to provide crude **200**. Oxidation of the primary alcohol to the acid was accomplished with potassium permanganate. Removal of the Boc group with aqueous HCl provided labeled capreomycidine (**193**) in 10% yield over the final five steps.

Attempts to incorporate **193** into streptothricin F were unsuccessful. This suggests that perhaps capreomycidine is not a precursor to streptothricin F. One explanation for this is the hypothesis that in capreomycin, capreomycidine is formed in a post-peptide assembly modification rather than as a single amino acid that is incorporated into capreomycin.[48,49]



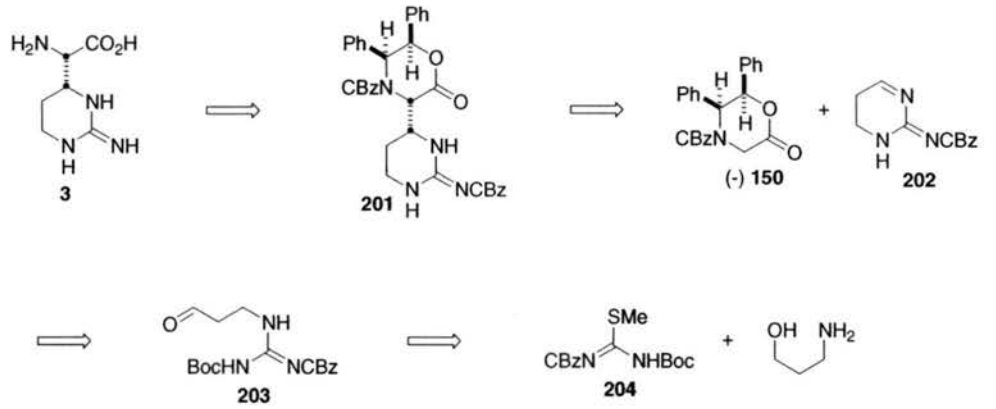
Scheme 44. Zabriskie's asymmetric synthesis of [*guanidino*-^{13}C]capreomycidine.

RESULTS

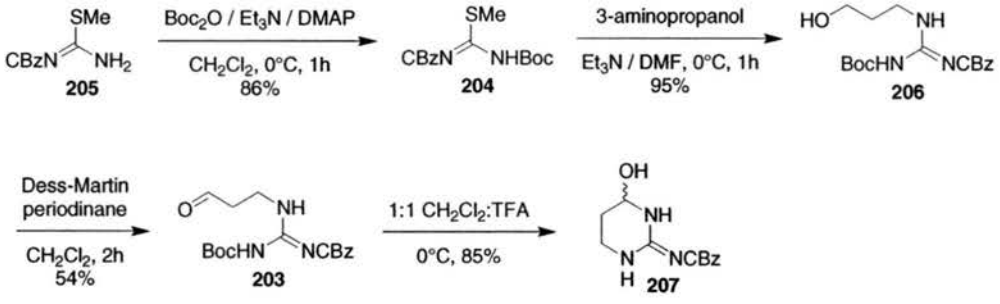
3.5. Synthesis of (2*S*,3*R*)-capreomycidine using Williams' chiral glycinate.

3.5.1. Enolate-cyclic imine approach.

Retrosynthetically, our initial approach to capreomycidine (**3**) revolved around the hydrogenolysis of lactone adduct **201** that would hopefully be arrived at by the addition of Williams' chiral glycinate ((**-**) **150**) to a requisite cyclic guanidine imine species **202** (Scheme 45). The cyclic guanidine portion could be prepared from guanidino aldehyde **203** which could be accessed from the guanidinylation of 3-aminopropanol with the differentially protected *S*-methylisothiourea **204**.



Scheme 45. Retrosynthesis of cyclic imine approach to (2*S*,3*R*)-capreomycidine.

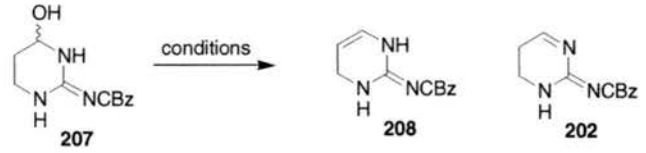


Scheme 46. Preparation of cyclic imine precursor.

Starting from the previously described *N*-CBz-*S*-methylisothiourea (**205**), treatment with Boc_2O in the presence of Et_3N and DMAP provided the orthogonally

protected **204** in 86% yield (Scheme 46).[70] Exposure of **204** to 3-aminopropanol afforded guanidino alcohol **206** in 95% yield. Dess-Martin oxidation of **206** provided a modest 54% yield of aldehyde **203**. Deprotection of the Boc group with TFA in CH_2Cl_2 resulted in carbinolamine **207**.

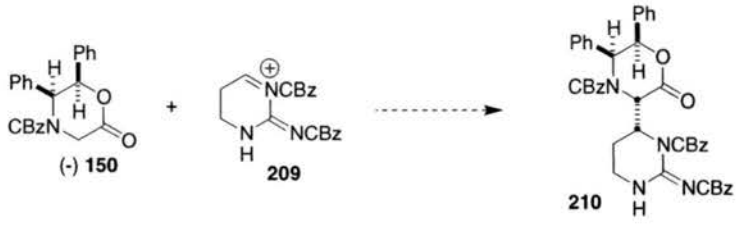
Attempts to dehydrate **207** to the desired imine **202** were met with either no reaction or dehydration followed by tautomerization to the undesired **208** under more forcing conditions (Table 14). In hindsight, this is not very surprising due to the fact that there is greater electron delocalization in **208** than in **202**.



CONDITIONS	RESULT
CH_2Cl_2 / $MgSO_4$, rt	No reaction
CH_2Cl_2 / $MgSO_4$, Δ	No reaction
PhH / mol. Sieves, Δ	**208**
PhH / TsOH, Δ	**208**

Table 14. Attempts to prepare cyclic imine.

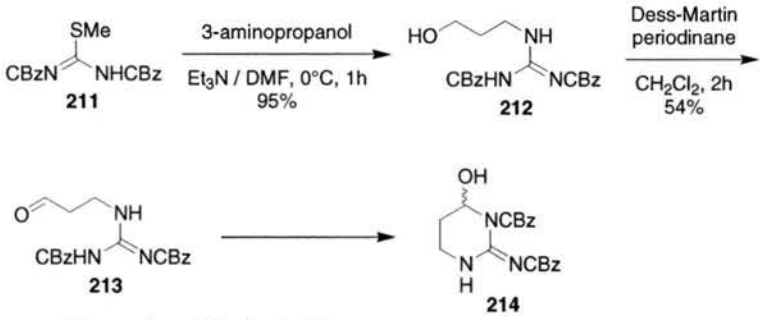
As a variation upon the initial cyclic imine approach, it was thought that perhaps a carbamoyl iminium ion such as **209** could be employed to access an oxazinone adduct such as **210** (Scheme 47).



Scheme 47. Potential cyclic iminium approach to (2*S*,3*R*)-capreomycidine.

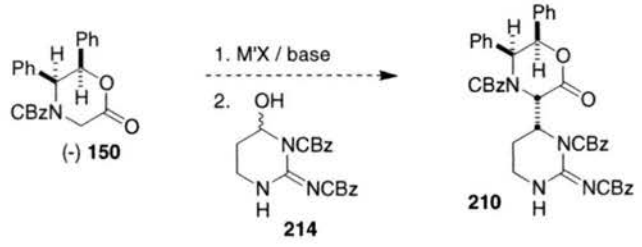
Guanidinylation of 3-aminopropanol with the commercially available *N,N'-bis*(benzyloxycarbonyl)-*S*-methylisothiourea (**211**) provided the desired guanidino

alcohol **212** in good yield (Scheme 48). Dess-Martin oxidation provided the guanidine aldehyde **213** in 50% yield. Over time **213** was found to cyclize to carbinolamine **214**.



Scheme 48. Preparation of cyclic iminium precursor.

It was envisioned that treatment of a Lewis-acid enolate of (-) **150** with carbinolamine **214** would promote the *in situ* formation of the cyclic iminium ion, allowing the formation of oxazinone adduct **210** (Table 15). Unfortunately, under no conditions was **210** formed. In addition, attempts to make the hydroxyl of **214** a better leaving group by mesylation or cyanide exchange were unsuccessful.



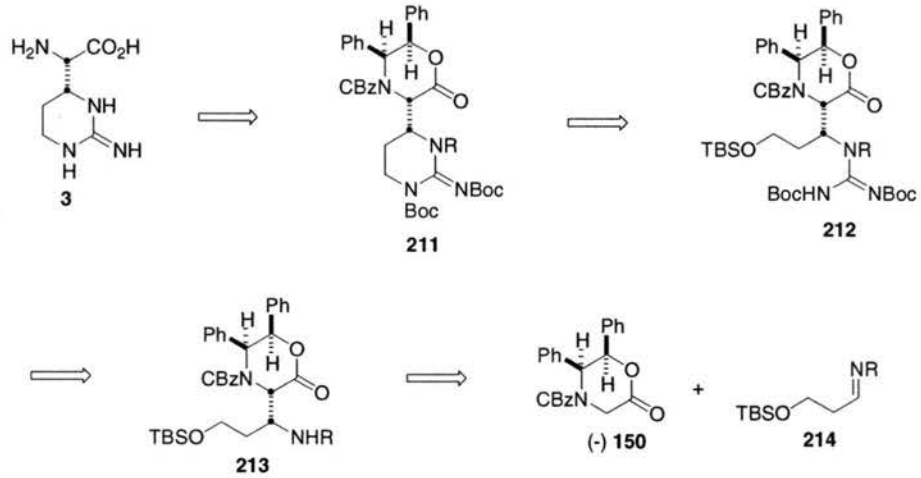
M'X / BASE	RESULT
Bu_2BOTf / Et_3N	No reaction
Me_2AlCl / LiHMDS	No reaction
$TiCl_4$ / Et_3N	No reaction

Table 15. Attempts to form and trap cyclic iminium ion with Williams' chiral glycinate.

3.5.2. Enolate-aldimine approach and the completion of (2*S*,3*R*)-capreomycidine.

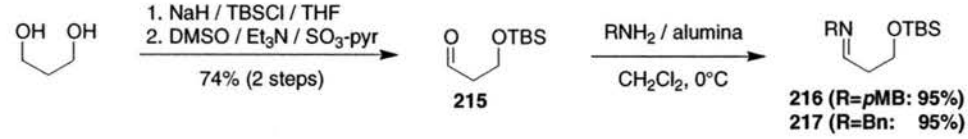
With the difficulties of the cyclic iminium approach to capreomycidine becoming apparent, investigations into a slightly more linear approach were embraced. It was felt that capreomycidine could be accessed from hydrogenolysis and deprotection of

oxazinone adduct **211** (Scheme 49). Capreomycidine precursor **211** may be prepared by desilylation and cyclization of **212**, which could, in turn, be accessed from **213**. Secondary amine **213** was to be provided from a Mannich-type reaction between chiral glycinate **(-) 150** and an aldimine of the general type **214**.



Scheme 49. Retrosynthetic analysis of enolate-aldimine approach to capreomycidine.

Monosilylation of 1,3-propanediol, followed by a modified Swern oxidation resulted in the desired aldehyde **215**, which matched spectroscopically with previously published data for the same aldehyde (Scheme 50).[85-87] Treatment of **215** with either *p*-methoxybenzylamine or benzylamine in the presence of alumina afforded both the *p*-methoxybenzyl imine **216** and the benzyl imine **217** in 95% yield.[88]



Scheme 50. Preparation of requisite aldimines.

Nuclear Overhauser enhancement (nOe) experiments with **216** showed the imine to be of the *E*-configuration (Figure 12). The assignment of an *E*-configuration to **216**

was supported by a strong nOe (3.2%) between the imine proton and benzylic proton of the *p*MB group.

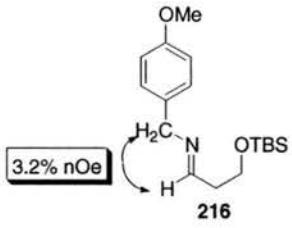
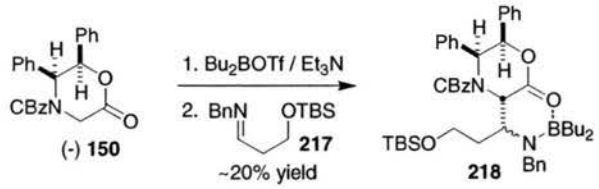
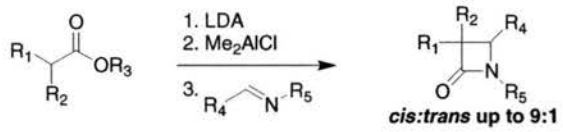


Figure 12. Confirmation of imine configuration in solution.



Scheme 51. Boron enolate addition to aldimine.

With the imines now in hand, investigation into the enolate-aldimine reaction was begun (Scheme 51). In an attempt to extend the aldol chemistry previously developed for glycinate (-) **150**, the di-butyl boron enolate was formed and treated with benzyl imine **217**.[89-91] Unfortunately, the only product isolated was an unbreakable boron chelate such as **218** in approximately 20% yield.

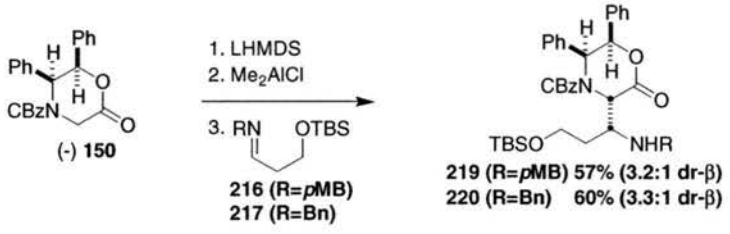


Scheme 52. Akiba's aluminum enolate investigations.

With the boron enolate not providing the desired results, our focus was turned to other enolate strategies. Akiba and Shibasaki both demonstrated the usefulness of aluminum enolates in the ester enolate-aldimine reaction manifold as a means of accessing β-lactams.[92,93] In Akiba's work, moderate to good diastereoselectivities were

observed with a variety of substrates when using dimethylaluminum ester enolates (Scheme 52).

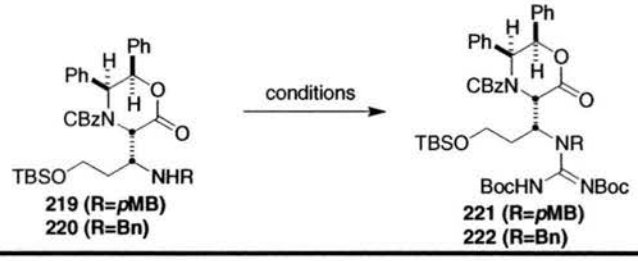
Taking note of the apparent generality of the reaction of aluminum based ester enolates with aldimines, it was decided to adapt this methodology to our system (Scheme 53). Formation of the lithium enolate of (-) **150** with LHMDS followed by transmetallation with Me_2AlCl provided the requisite aluminum enolate. Addition of the previously prepared imines **216** and **217** to the enolate of (-) **150** resulted in a 57% yield of the secondary *p*-methoxybenzylamine **219** and a 60% yield of the secondary benzylamine **220**, both as a mixture of diastereomers (~3:1) at the β-carbon (^{1}H-NMR).



Scheme 53. Aluminum enolate-based addition of glycinate to aldimines.

With a viable approach to the desired Mannich-type products **219** and **220** in hand, the subsequent guanidinylation was investigated (Table 16). Obtaining synthetically useful conditions for the guanidinylation of **219** and **220** proved to be non-trivial. Upon treatment of **220** under the standard *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea and triethylamine conditions (entry 3), no reaction was observed. Goodman's triflylguanidine reagent also proved ineffective (entry 1).[94] A 17% yield of guanidinylation product **221** was observed when treating **219** with *N,N'*-bis(*tert*-butoxycarbonyl)thiourea in the presence of $HgCl_2$ and Et_3N, a method developed by Kim and coworkers.[95,96] Further, *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea could be used with $HgCl_2$ to give **221** in a greatly improved 70% yield (entry 4). In addition,

exposure of **220** to the same conditions provided **222** in 67% yield. Subsequent to this realization, Cammidge and co-workers reported this method as a means of guanidinylating normally unreactive amines.[97] As an improvement to this approach, it was found that substituting AgOTf for $HgCl_2$ provided a higher yield (74% for **222**) and cleaner reaction, with the added benefit of eliminating the undesired mercury salts.



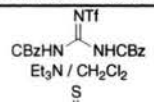
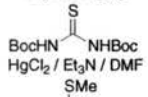
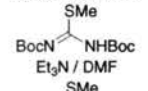
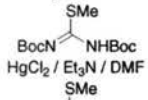
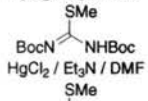
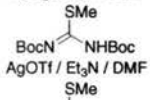
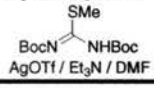
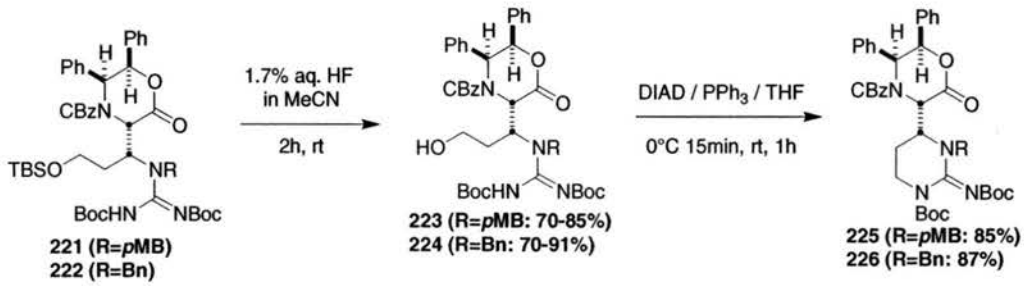
ENTRY	CONDITIONS	R	YIELD
1		*p*MB (**219**)	N.R.
2		*p*MB (**219**)	17%
3		Bn (**220**)	N.R.
4		*p*MB (**219**)	70%
5		Bn (**220**)	67%
6		*p*MB (**219**)	82%
7		Bn (**220**)	75%

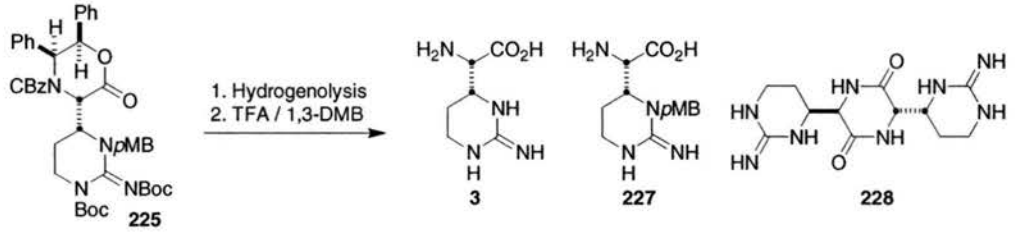
Table 16. Attempts to guanidinylate Mannich product.

Desilylation of **221** and **222** was met with some resistance. Attempts to remove the TBS group with either TBAF or acetic acid in THF / H_2O resulted primarily in decomposition. Use of HF-pyridine resulted in significant epimerization, presumably at the α-center. Finally, it was found that treatment of **221** with aqueous HF in MeCN, followed by rapid silica gel purification using Whatman® silica gel, afforded the desired primary alcohol **223** in 70-85% yield with minimal epimerization (Scheme 54). Similarly,

224 was obtained from **222** in 70-91% yield. Using conditions developed by Dodd and Kozikowski, the cyclic guanidine **225** was prepared from **223** under Mitsunobu conditions in 85% yield.[98] Likewise **226** was prepared from **224** in 87% yield.



Scheme 54. Desilylation and formation of cyclic guanidine.



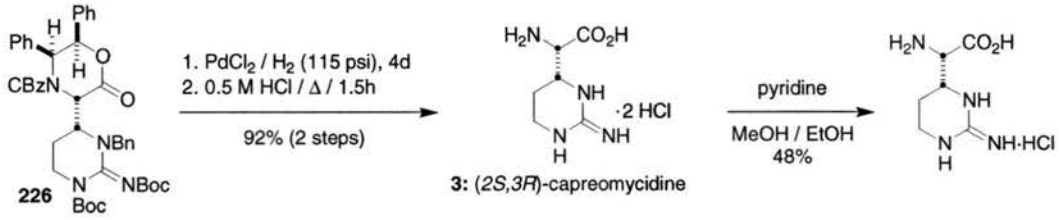
HYDROGENOLYSIS CONDITIONS	RESULT
$PdCl_2$, THF / EtOH, r.t. (60 psi H_2)	<10% **3**
$PdCl_2$, TFA / MeOH, r.t. (60 psi H_2)	mixture of reduction products
$Pd(OH)_2$, TFA / EtOH, r.t. (60 psi H_2)	mixture of reduction products
$PdCl_2$, THF / H_2O, 70°C, 2h (115 psi H_2)	**227**
$Pd(OH)_2$, THF / H_2O, 70°C, 16h (115 psi H_2)	**228**

Table 17. Attempts to hydrogenolyze **225**.

Attempts to access **3** by hydrogenolysis of **225** and subsequent Boc group removal proved unsuccesful (Table 17). Exposure of **225** to hydrogen (60 psi) in the presence of $PdCl_2$ in THF and EtOH (conditions commonly used for the hydrogenolysis of adducts of chiral glycinate **150**) gave less than 10% yield of (2*S*,3*R*)-capreomycidine (**3**). Attempts to increase the reaction rate by addition of trifluoroacetic acid to the hydrogenation with either $PdCl_2$ or $Pd(OH)_2$ were also unsuccessful. An interesting result was observed when hydrogenating **225** in the presence of $PdCl_2$ in THF / water at

115 psi H_2 and 70°C for 2h. The major product isolated was *p*-methoxybenzyl capreomycidine (**227**). Although not the desired product, **227** will be revisited in the synthesis of capreomycin IB (Chapter 5). Longer reaction times resulted in the removal of the *p*MB group and dimerization to diketopiperazine **228**. While unexpected, diketopiperazine formations such as this are known in the literature.[99]

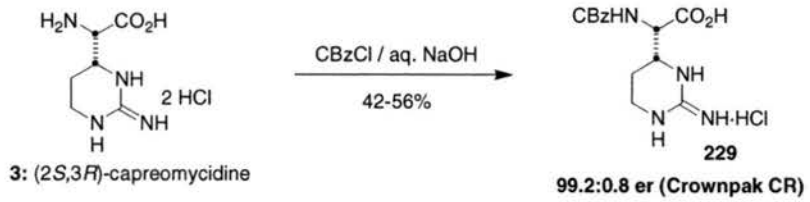
Thinking that perhaps the less electron-rich benzyl group would be easier to remove than the *p*MB group, attempts were made at the hydrogenolysis of **226**. It was found that treatment of **226** with hydrogen (115 psi) and $PdCl_2$ for four days provided a completely reduced product with one of the two Boc groups remaining (Scheme 55). Exposure of this product to refluxing aqueous HCl afforded crude **3** as its dihydrochloride salt in 95% yield over the two steps.[100] Preparation of the analytically pure monohydrochloride salt of **3** was achieved in 48% yield by treatment of the dihydrochloride salt with pyridine in methanol, followed by precipitation with ethanol. The synthetic **3** agreed with the natural product by spectroscopic methods and molar optical rotation (synthetic: $[M]_D^{20}$=+28.2, natural: $[M]_D^{20}$=+32.5). With the solution of the hydrogenolysis issue, the synthesis of (2*S*,3*R*)-capreomycidine was completed in six steps, and an overall yield of 28%.



Scheme 55. Completion of the synthesis of capreomycidine.

To further assure the enantiomeric purity of the synthetic capreomycidine, both enantiomers were prepared, and treated with benzyl chloroformate to afford N^{α}-

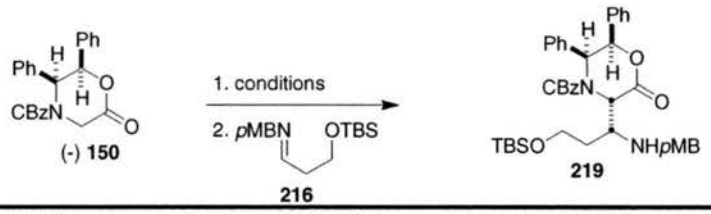
benzyloxycarbonyl-capreomycidine **229** (Scheme 56). Analysis of the protected amino acid on a Crownpak CR chiral HPLC column showed **3** to have been prepared in a 99.2:0.8 er.



Scheme 56. Preparation of N^{α}-CBz-capreomycidine and er determination.

3.6. Enolate-aldimine reaction selecitivity improvements.

With the completion of the synthesis of (2*S*,3*R*)-capreomycidine, efforts were focused on potential improvements in the selectivity of the enolate-aldimine reaction, as well as the broadening of its scope.



CONDITIONS	YIELD	dr[a]
1. LHMDS, 2. Me_2AlCl	57%	3.2:1
1. LHMDS, 2. Et_2AlCl	~50%	3.8:1
LDA	N.R.	N/A
1. $TiCl_4$, 2. Et_3N	<20%	nd[b]
1. LDA, 2. Cp_2ZrCl_3	N.R.	N/A

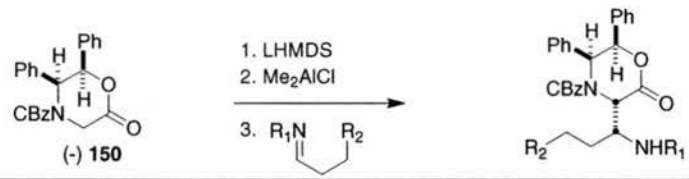
[a]determined by ^{1}H-NMR.[b]dr not determined

Table 18. Effect of enolate nature on diastereoselectivity.

When looking at different enolates of (-) **150**, a variety of results were observed (Table 18). As previously mentioned, the dimethylaluminum enolate provided a diastereomeric ratio at the β-position of 3.3:1. Upon switching to the diethylaluminum enolate, a slightly improved 3.8:1 selectivity was observed. The lithium enolate of (-) **150** provided no product at all, while the titanium enolate resulted in a less than 20%

yield of the desired product. In this limited investigation, the aluminum enolates were found to still be the best for the Mannich-type reaction.

The effect of the steric bulk of the imine on reaction selectivity was also investigated (Table 19). Using 3-(*p*-methoxybenzyloxy)-propanal, the benzyl (**230**), benzhydryl (**231**), and trityl (**232**) imines were formed. Addition of **230** to the aluminum enolate of (-) **150** provided a 32% yield of the desired Mannich-type product in a 3.3:1 diastereomeric ratio at the β-position (Similar to the 3-(*tert*-butyldimethylsiloxy)-propanal case). Addition of the aluminum enolate of (-) **150** to benzhydryl imine **231** provided a significantly improved dr of 6.5:1. Attempts to add (-) **150** to trityl imine **232** were unsuccessful, presumably due to the imine being too hindered to allow enolate addition.



IMINE	R_1	R_2	YIELD[a]	dr[b]
230	Bn	O*p*MB	32%	3.3:1
231	Ph_2CH	O*p*MB	22%	6.5:1
232	Ph_3C[c]	O*p*MB	0%	n/a
233	Bn	H	20%	1.8:1
234	Ph_2CH	H	25%	2.6:1

[a] Unoptimized yields. [b] dr determined by ^{1}H-NMR. [c] It was necessary to reflux imine forming reaction.

Table 19. Effect of imine substitution on diastereoselectivity.

After empirically observing the increase of diastereoselectivity in the enolate-aldimine reaction after increasing the steric bulk on the imine nitrogen from benzyl (**230**) to benzhydryl (**231**), it was decided to also look at the effect of C-substitution of the imine. Both the benzyl (**233**) and benzhydryl (**234**) imines of propanal were prepared and reacted with the enolate of (-) **150**. Although reaction with **234** provided a somewhat

greater dr than with **233** (2.6:1 vs. 1.8:1), both reactions were found to be considerably less selective than their 3-substituted counterparts.

The selectivity observed in the enolate-aldimine reaction of (-) **150** can be potentially explained by either a non-chelation or chelation mechanism. When looking at the various transition state models, it will be assumed that the imine is of the *E*-configuration based on the previously discussed nOe experiments on **216** (Figure 12).

Of the two possible non-chelation transition states, **235** will lead to the observed major Mannich product **236**, while **237** will lead to the minor Mannich product **238** (Figure 13). If a non-chelation model for the formation of **236** is to be considered valid, one would have to argue that the R_2 group plays a larger role than R_1 in the diastereoselectivity because although transition state model **235** eliminates an unfavorable interaction for R_2, it creates one for R_1. This may or may not be likely due to the fact that the results in Table 19 suggest that both R_1 and R_2 play a significant role in the diastereoselectivity of the reaction.

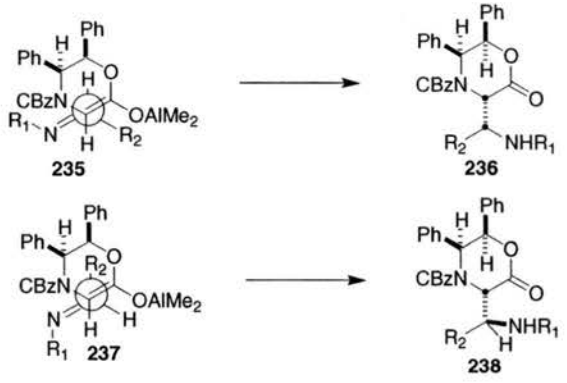


Figure 13. Non-chelation transition-state models for enolate-aldimine reaction.

When addressing a chelation-control model for the diastereoselectivity, the major product **241** can be accessed by way of a chair-like transition state model (**239,240**), while the minor product **244** is arrived at from a boat like transition state model (**242,243**)

(Figure 14). In this case, the desire for R_1 to avoid an undesired steric interaction such as the one in the boat configuration (**242,243**) would seem to explain the formation of **241** over **244**. However, the fact that Mannich reactions with imines formed from propionaldehyde result in significantly lower diastereoselectivity than reactions with the analogous imine from 3-silanoxy or 3-benzyloxypropionaldehydes requires additional explanation. It is possible that the oxygen present in the 3-substituted propionaldehyde engages in an additional 6-membered ring chelation with the aluminum, further stabilizing the chair transition state over that of the boat, thus enhancing the overall diastereoselectivity from that of the propionaldehyde system.

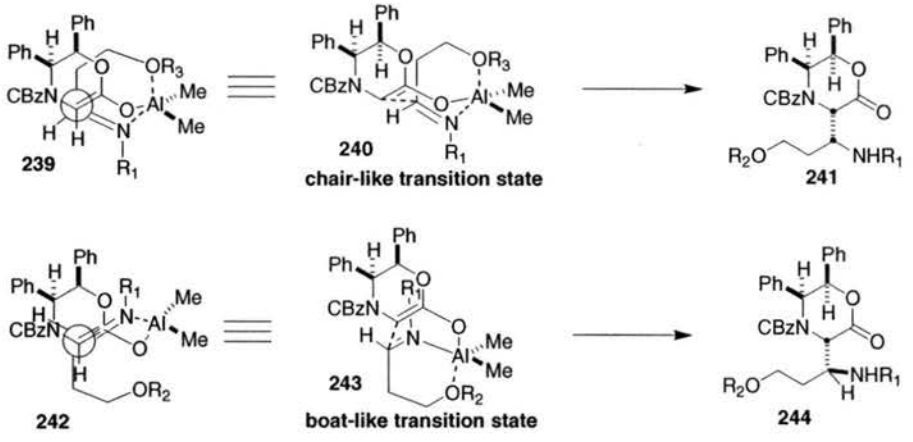


Figure 14. Chelation-controlled transition-state model for enolate-aldimine reaction.

As is apparent with these results, there seems to be a delicate interplay between the nature of the N-substituent and C-substituent of the imines used in Mannich reactions with (-) **150** that have a substantial effect in the diastereomeric outcome of the reaction. In order to gain a clearer picture of the diastereoselectivity issue, additional studies with this system will be required.

In conclusion, a concise asymmetric synthesis of (2*S*,3*R*)-capreomycidine and its enantiomer has been developed. This has been achieved *via* a novel enolate-aldimine

reaction with Williams' chiral glycinate ((-) **150**). The success of the key step has revolved around the deployment of a previously unexplored aluminum enolate of (-) **150**. Further exploration of this transformation is warranted in hopes of developing a more selective means of accessing other α,β-diamino acids.

CHAPTER 4

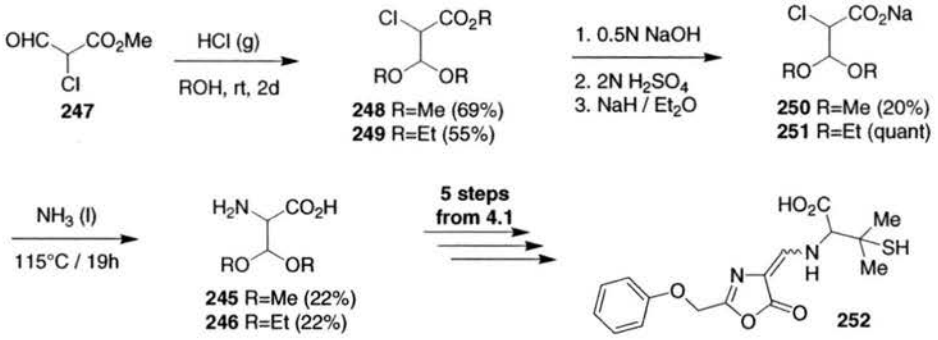
The First Asymmetric Synthesis of Acetals of α-Formylglycine

INTRODUCTION

4.1. Previous Syntheses and Work with Acetals of α-Formylglycine.

The dimethyl (**245**) and diethyl (**246**) acetals of α-formylglycine (Figure 15) have found use as versatile building blocks in a wide variety of synthetic endeavors. Several different approaches to acetals of α-formylglycine have been previously described in the literature.

Figure 15. Dimethyl- and diethylacetals of α-formylglycine.

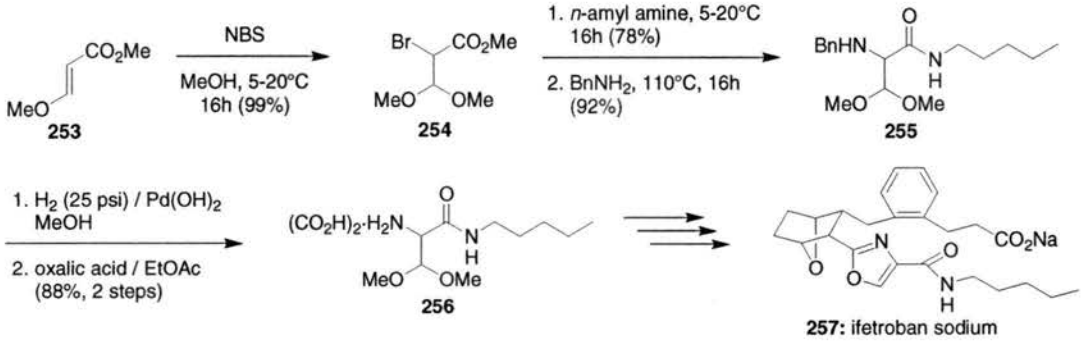


Scheme 57. Dudley's synthesis of 2-phenoxymethylpenicillenic acid.

Dudley *et al.* described racemic syntheses of both the dimethyl (**245**) and diethyl (**246**) acetals of α-formylglycine in their synthesis of DL-2-phenoxymethylpenicillenic acid (**252**) (Scheme 57).[101] Methyl chloromalonaldehydate **247** was separately treated with both methanolic HCl and ethanolic HCl to provide acetals **248** and **249** in 69% and 55% yields respectively. Ester hydrolysis of **248** followed by sodium carboxylate formation provided **250** in 20% yield. Conversely, the transformation of diethyl acetal

249 to carboxylate **251** proceeded in quantitative yield. Treatment of chlorides **249** and **251** with ammonia at 115°C provided a 22% yield of both α-formylglycine dimethylacetal **245** and diethyl acetal **246** respectively. Acetal **245** was then converted to DL-2-phenoxymethylpenicillenic acid **(252)** over 5 steps. Although lengthy, this was an effective method of synthesizing the free amino acid of both α-formylglycine dimethylacetal **(245)** and diethylacetal **(246)**.

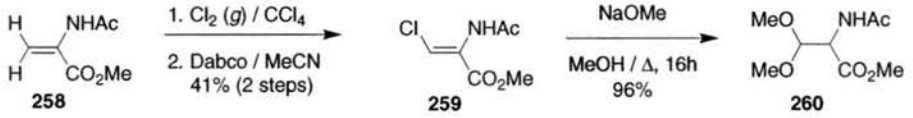
Swaminathan and co-workers at Bristol-Meyers Squibb developed a synthesis of the *n*-amylamide of α-formylglycine dimethylacetal **256** as an intermediate in their synthesis of ifetroban sodium **(257)** (Scheme 58).[102] Utilizing a method similar to Dudley *et al.* in their synthesis of **245**, methoxy methacrylate **253** was treated with *N*-bromosuccinimide in methanol to provide the α-bromo ester **254** in near-quantitative yield. Exposure of **254** to *n*-amylamine, followed by treatment with benzylamine at elevated temperature resulted in isolation of **255** in 72% yield over the two steps. Hydrogenolysis over Pearlman's catalyst and subsequent oxalate salt formation afforded the *n*-amyl amide of α-formylglycine dimethylacetal **256** in 88% yield.



Scheme 58. Swaminathan's preparation of ifetroban sodium.

Vederas and co-workers in their synthesis of deuterium labeled serine, developed a preparation of *N*-acetyl-2-amino-3,3-dimethoxypropanoic acid, methyl ester **(260)**

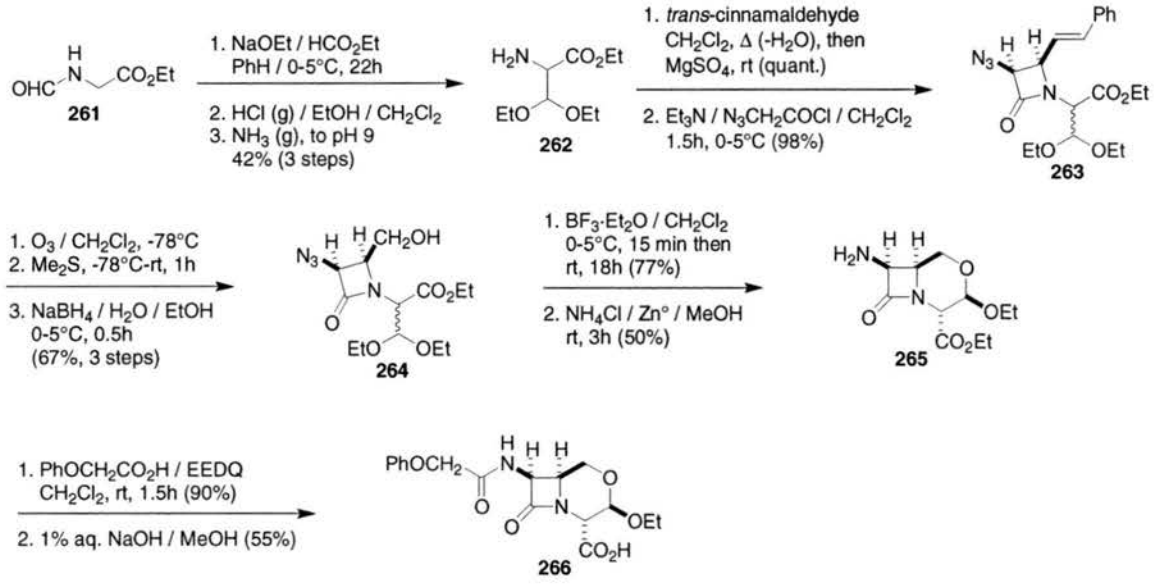
starting from *N*-acetyl-dehydroalanine, methyl ester (**258**) (Scheme 59)[103]. Exposure of **258** to chlorine gas, followed by DABCO-mediated elimination of HCl resulted in the formation of chloro-dehydroalanine **259** in 41% yield over the two steps. Treatment of **259** with NaOMe in refluxing methanol provided *N*-acetyl-3,3-dimethoxypropanoic acid, methyl ester (**260**) in 96% yield.



Scheme 59. Vederas' preparation of α-formylglycine dimethylacetal derivatives.

The original preparation of 2-amino-3,3-diethoxy-propanoic acid, ethyl ester (**262**) was published by Brown and co-workers in 1949[104], however the overall yield of **262** was quite low. Taking this into account, Belleau and coworkers published an improvement to Brown's synthesis of **262** for use in their preparation of *O*-2-isocepham **266** (Scheme 60).[105] Although a racemic synthesis, the α-center of the α-formylglycine moiety is kept intact, leaving open the possibility for a stereoselective synthesis if an optically pure form of **262** could be prepared. Claisen condensation of **261** with ethyl formate was followed by treatment with EtOH·HCl to provide the ethyl ester of α-formylglycine diethylacetal **262** in 42% yield. Exposure of **262** to *trans*-cinnamaldehyde and $MgSO_4$ provided the cinnamaldimine, which then underwent a Staudinger-type reaction with azidoacetylchloride and triethylamine to afford the desired β-lactam **263** in 98% yield. Ozonolysis of **263** followed by Me_2S workup accessed the aldehyde, which was subsequently reduced to alcohol **264** with $NaBH_4$ (67%, 3 steps). Lewis acid catalyzed cyclization of the alcohol onto the diethyl acetal with $BF_3{\cdot}Et_2O$ and reduction of the azide with zinc and NH_4Cl in methanol afforded *O*-isocepham **265** in 39% yield over the two

steps. Coupling of phenoxyacetic acid to the amine and ester hydrolysis provided a 50% yield of the desired 7β-(aminophenoxyacetoyl)-3β-ethoxy-*O*-2-isocepham-4α-carboxylic acid (**266**).



Scheme 60. Belleau's *O*-2-isocepham investigations.

Belleau again used **262** in his synthesis of the diketopiperazine **267** (Figure 16).[106] In addition, Belleau's approach to **262** has also been used in Dodd's synthesis of ethyl β-carboline-3-carboxylate (**268**) and Franchetti's preparation of the imidazole portion of imidazofurin (**269**).[107,108]

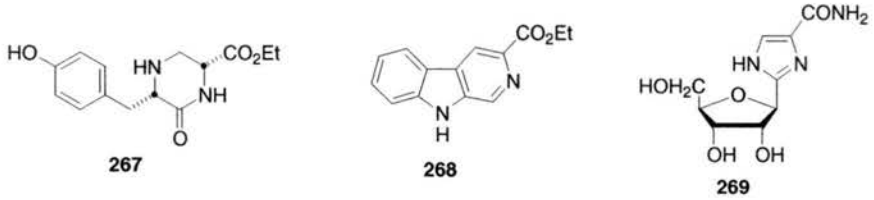
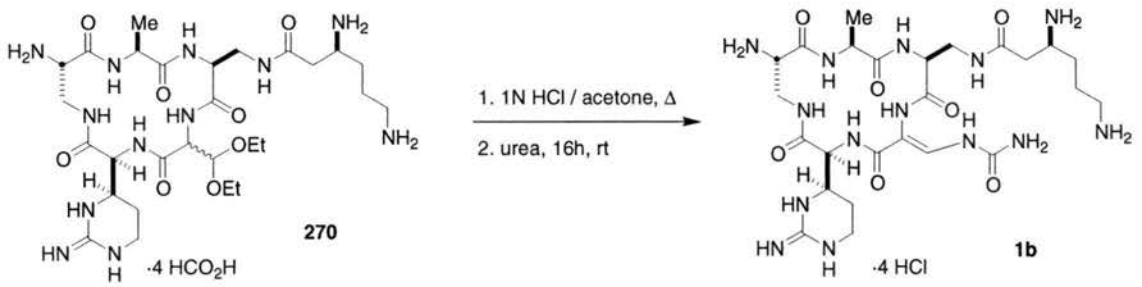


Figure 16. Additional compounds prepared from α-formylglycine **262**.

More important to the research in these laboratories, it had been shown by Shiba and co-workers that α-formylglycine diethylacetal can be used as a suitable precursor to the enamidourea functionality present in the capreomycins and tuberactinomycins

(Scheme 61).[33,34,36,39] Shiba showed that treatment of cyclic pentapeptide **270**, which contains the α-formylglycine diethylacetal moiety, with aqueous HCl in the presence of urea provides the desired enamidourea of capreomycin 1B (**1b**) as a single geometric isomer.

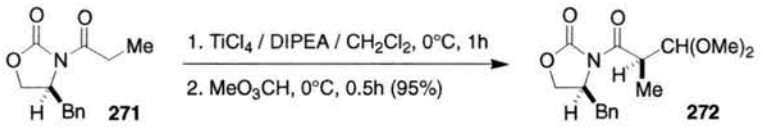


Scheme 61. Shiba's enamidourea formation in their synthesis of capreomycin IB.

RESULTS

4.2. The first asymmetric synthesis of α-formylglycine dimethyl acetal.

In our approach to the total synthesis of capreomycin 1B, it was desired to investigate a possible asymmetric synthesis of the dimethylacetal of α-formylglycine (**245**). The reasoning for this was twofold: First, upon incorporating an enantiopure α-formylglycine dimethylacetal into our capreomycin synthesis, it would eliminate the necessity of carrying two diastereomers through the final stages of the synthesis. Second, by preparing both diastereomeric hexapeptides, it would be possible to see if there was a preference for one diastereomer to cyclize over the other.

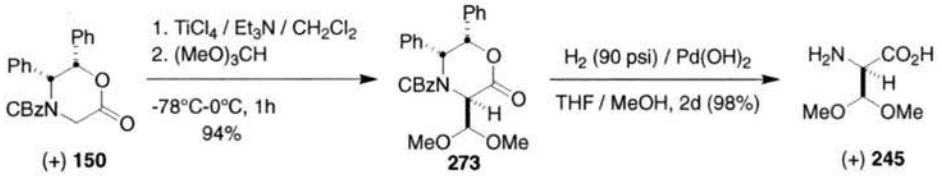


Scheme 62. Evans' titanium enolate chemistry.

For our approach to the asymmetric synthesis of **245**, our attention was focused on previous work with titanium enolates published by Evans and co-workers (Scheme

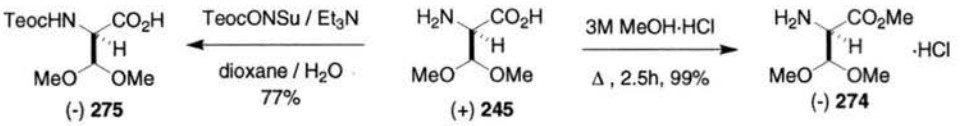
62).[109] In this report it was shown that treatment of the propionate derivative of Evans' oxazolidinone (**271**) with $TiCl_4$ followed by DIPEA provides the titanium enolate that undergoes an aldol-type reaction with trimethyl orthoformate to provide the desired dimethylacetal adduct **272** in 95% yield (Scheme 64).

To begin the asymmetric synthesis of **245**, a solution of optically pure Williams' chiral glycinate ((+) **150**) in methylene chloride at –78°C was first treated with $TiCl_4$, followed by Et_3N to form the requisite titanium enolate (Scheme 63). Addition of an excess of trimethyl orthoformate and slow warming of the reaction to 0°C over 1h resulted in the formation of the desired dimethyl acetal **273** in 94% yield. Hydrogenolysis of **273** over Pearlman's catalyst afforded enantiopure *R*-α-formylglycine dimethylacetal ((+) **245**) in 98% yield. This achievement represents the first asymmetric synthesis of α-formylglycine dimethylacetal reported in the literature.[110]



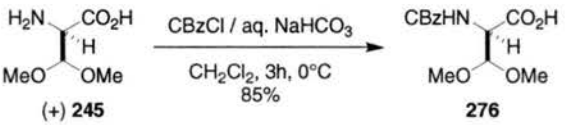
Scheme 63. The first asymmetric synthesis of α-formylglycine dimethylacetal.

In addition to the synthesis of (+) **245**, it has also been shown that both the amine and carboxyl functionalities can be manipulated for incorporation into peptides (Scheme 64). Treatment of (+) **245** with refluxing MeOH·HCl provided the desired methyl ester hydrochloride salt (-) **274** in 99% yield as a single enantiomer by Mosher's amide analysis. In addition, exposure of (+) **245** to TeocONSu and Et_3N in dioxane provided a 77% yield of the trimethylsilylethyl carbamate (-) **275**.[111]



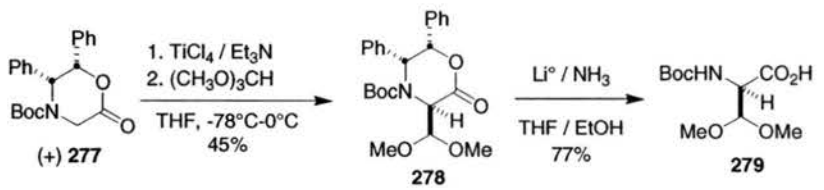
Scheme 64. Carbamate protection and methyl ester formation from **(+) 245**.

Access to both the CBz- and Boc- protected forms of (+) **245** was also desired. Treatment of (+) **245** with benzyl chloroformate and saturated aqueous $NaHCO_3$ under Schotten-Baumann conditions afforded **276** in 85% yield (Scheme 65).



Scheme 65. CBz protection of (+) **245**.

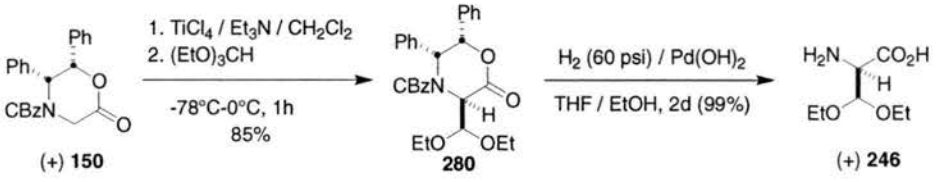
For the preparation of enantiopure *N-tert*-butoxycarbonyl-α-formylglycine dimethylacetal (**279**), a more direct approach was envisioned that would eliminate the need to hydrogenolyze the previously prepared glycinate adduct **273** and subsequently protect the free amino acid (Scheme 66). Using the optically pure, Boc-protected form of Williams chiral glycinate ((+) **277**), the titanium enolate was formed in THF and then exposed to trimethyl orthoformate to afford the desired dimethyl acetal glycinate adduct **278** in a modest 45% yield. (*R*)-*N-tert*-butoxycarbonyl-α-formylglycine dimethyl acetal (**279**) was accessed *via* Birch reduction of **278** in 77% yield.



Scheme 66. Preparation of *N*-Boc-α-formylglycine dimethylacetal.

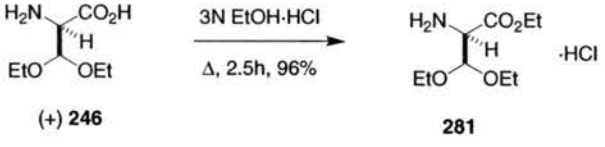
4.3. The asymmetric synthesis of α-formylglycine diethylacetal.

Finally, for reasons that will be discussed further in Chapter 5, it was desired to obtain optically pure forms of α-formylglycine diethylacetal (**246**) in addition to the previously prepared dimethyl acetal (Scheme 67). Formation of the titanium enolate of (+) **150** was now followed by addition of triethylorthoformate, and the reaction warmed to 0°C. The desired diethylacetal **280** was obtained in 85% yield. Hydrogenolysis of **280** afforded (+) **246** in 99% yield. This result proved that both the dimethyl and diethyl acetals of α-formylglycine could be prepared asymmetrically in an efficiently in high yield.



Scheme 67. Asymmetric synthesis of *R*-α-formylglycine diethylacetal.

Furthermore, treatment of (+) **246** with 3N EtOH·HCl provided the ethyl ester hydrochloride salt **281** in 96% crude yield (Scheme 68). This crude product can be subsequently used in peptide coupling reactions.



Scheme 68. Preparation of α-formylglycine diethylacetal ethyl ester hydrochloride salt.

The first asymmetric syntheses of α-formylglycine dimethyl- and diethylacetals have been described herein. A novel titanium enolate reaction with Williams' chiral glycinate ((+) **150)** has been used to access both of these unnatural amino acids. The

resulting acetals of α-formylglycine can be protected at either the amine or carboxyl termini, and incorporated into peptide synthesis.

CHAPTER 5

The total synthesis of capreomycin IB

5.1. Our initial approaches to capreomycin IB.

With the completion of asymmetric syntheses of (2*S*,3*R*)-capreomycidine and α-formylglycine dimethylacetal, it was now possible to undertake the total synthesis of capreomycin IB. When embarking on the total synthesis of capreomycin IB (**1.1b**) one would be remised to not consider the previous synthesis reported by Shiba.[36,37] Two drawbacks in Shiba's synthesis of capreomycin IB were apparent upon its completion: multiple protecting group manipulations were required for the preparation of the individual amino acid fragments used in the assembly of the peptide, thus significantly lengthening the number of overall synthetic steps, and (2*S*,3*R*)-capreomycidine needed to be supplied from the acid hydrolyzate of natural capreomycin rather than by synthetic preparation due to the poor yields encountered in their asymmetric synthesis of (2*S*,3*R*)-capreomycidine. This being the case, it was imperative, if one was to begin a novel total synthesis of capreomycin IB, to develop a synthetic approach that would avoid the previously mentioned pitfalls if at all possible. Our novel preparation of (2*S*,3*R*)-capreomycidine, as described in Chapter 3, has made it possible to prepare significant amounts of the amino acid for undertaking a total synthesis of capreomycin IB.

In planning the total synthesis of **1.1b**, it was decided to make the synthesis as convergent as possible, not only for the purpose of keeping the number of overall steps in the synthesis to a minimum, but also to allow for easy access to analogs if so desired. That being the case, it was decided to disconnect the molecule into three dipeptide fragments (Figure 17). In an effort to eliminate some of the protection/deprotection

issues associated with the incorporation of diaminopropanoic acid residues into peptides, it was decided to use an asparagine residue in dipeptide **282** as a masked form of diaminopropanoic acid. It was hoped that the primary amide in **282** could then undergo Hoffman rearrangement to provide the diaminopropanoic acid-alanine fragment **283** with the β-amine of the diaminopropanoic acid residue ready for coupling.[112-114] Dipeptide **284** could be arrived at from the coupling of N^{α}-CBz-diaminopropanoic acid methyl ester (**285**) and the previously described *N*-hydroxysuccinimidyl ester of *N,N'*-bis(*tert*-butoxycarbonyl)-β-lysine (**25**). Finally, it was proposed that **286** would arise from the coupling of *N*-trimethylsilylethylcarbamoyl-α-formylglycine dimethylacetal ((-) **275**) with *N-tert*-butoxycarbonyl-capreomycidine (**287**) a dipeptide in which the α-formylglycine dimethylacetal residue would serve as a precursor to the enamidourea functionality, a transformation analogous to Shiba's approach to the capreomycins and tuberactinomycins.

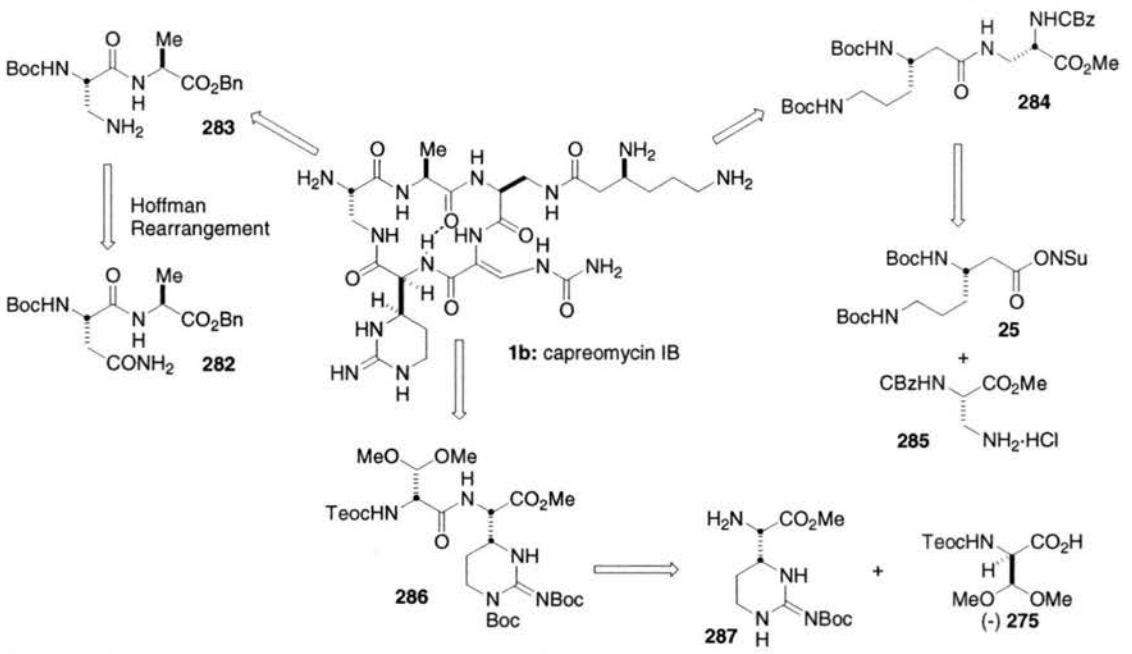
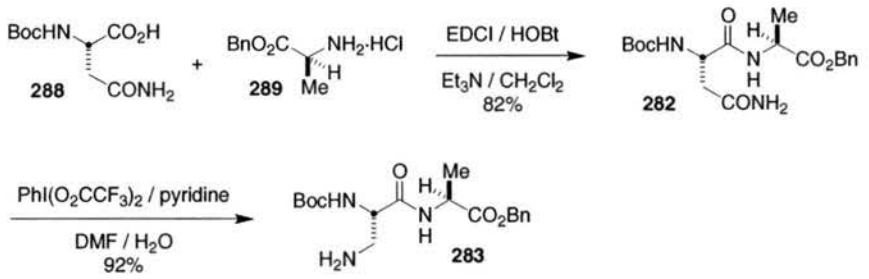
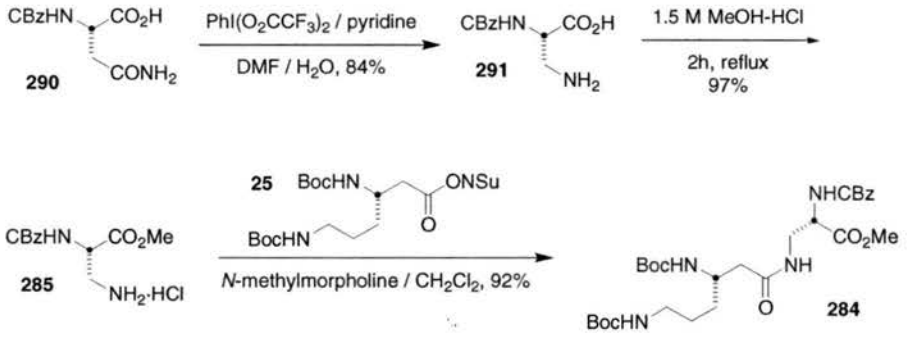


Figure 17. Initial retrosynthetic analysis of capreomycin IB.

To prepare the desired diaminopropanoic acid-alanine fragment **283**, *N*-Boc-asparagine (**288**) was first coupled with the hydrochloride salt of alanine benzyl ester (**289**) using EDCI, HOBt and Et_3N to provide an 82% yield of dipeptide **282** (Scheme 69). Much to our satisfaction, Hofmann rearrangement of **282** with bis(trifluoroacetoxy) iodobenzene and pyridine provided the desired diaminopropanoic acid-containing dipeptide **283** in an excellent 92% yield.



Scheme 69. Preparation of the diaminopropanoic acid-alanine fragment.

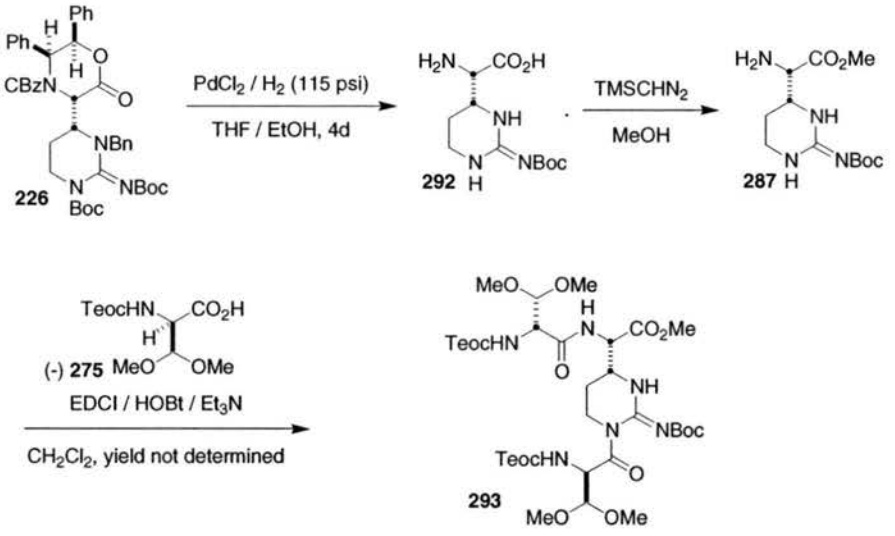


Scheme 70. Preparation of the diaminopropanoic acid-β-lysine fragment.

Preparation of dipeptide fragment **284** also proved to be rather straightforward (Scheme 70). Waki and co-workers previously described the preparation of N^{α}-CBz-diaminopropanoic acid (**291**) *via* Hofmann rearrangement of *N*-CBz-asparagine (**290**).[112] This is a robust transformation that provides good yields of **291** even upon scale-up. Refluxing **291** in MeOH·HCl provides the desired amino ester hydrochloride salt **285** as a crystalline solid in near-quantitative yield. Coupling of **285** with the previously

described β-lysine derivative **25** afforded **284** in 92% yield. This highly efficient approach to dipeptide **284** allowed for the straightforward preparation of significant amounts of this material in a short period of time.

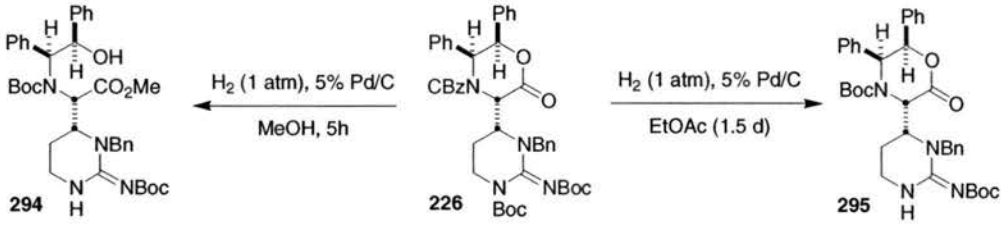
With the completion of **284**, attempts to prepare the capreomycidine and α-formylglycine dimethylacetal fragment (**286**) were begun (Scheme 71). In our previously described synthesis of (2*S*,3*R*)capreomycidine (**3**), hydrogenolysis of lactone adduct **226** provided a crude mixture of Boc-protected products (**292**). After treatment with $TMSCHN_2$, **287** was isolated as the major product. Unfortunately, attempts to couple (-) **275** to **287** resulted in a mixture of products in which the major product appeared to be a diacylated product such as **293**.



Scheme 71. Initial attempt to form α-formylglycine-capreomycidine dipeptide.

In an attempt to avoid undesired di-acylation in the preparation of a capreomycidine-α-formylglycine dimethylacetal dipeptide, it was decided to attempt a selective removal of the CBz group from **226** followed by acylation of the resulting secondary amine with (-) **275** (Scheme 72). A new problem, however, was encountered with this approach. Hydrogenolysis of **226** in methanol resulted in the desired cleavage

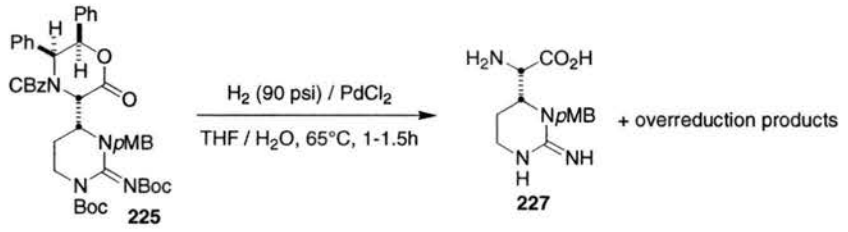
of the CBz group, followed by rapid opening of the lactone with methanol and Boc group migration to provide **294**. The same reaction in ethyl acetate resulted in the same Boc group migration to provide **295**, but at a much slower rate, presumably due to the fact that the lactone remained intact. Attempts to trap the secondary amine prior to Boc group migration by coupling with (-) **275** were unsuccessful.



Scheme 72. CBz group removal and subsequent Boc group migration.

At this point it was becoming apparent that coupling of **(-) 275** with carbamoyl-protected forms of capreomycidine was not going to become a reality, so alternative approaches were investigated.

It can be recalled from Chapter 3 that the hydrogenolysis of lactone adduct **225** with $PdCl_2$ in THF and water at elevated temperatures provided *p*-methoxybenzyl-capreomycidine (**227**) as the major product along with some over-reduction products (Scheme 73). Although it was not possible to separate **227** from these impurities, the hydrogenation conditions were such that it was possible to keep these undesired products to a minimum (~20% or less).



Scheme 73. Preparation of *p*-methoxybenzyl-capreomycidine from **225**.

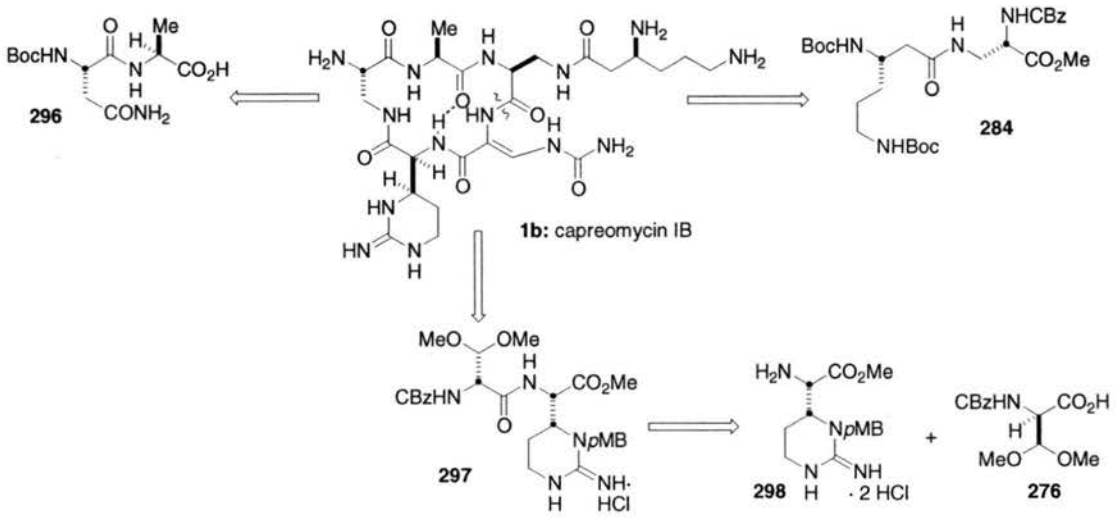
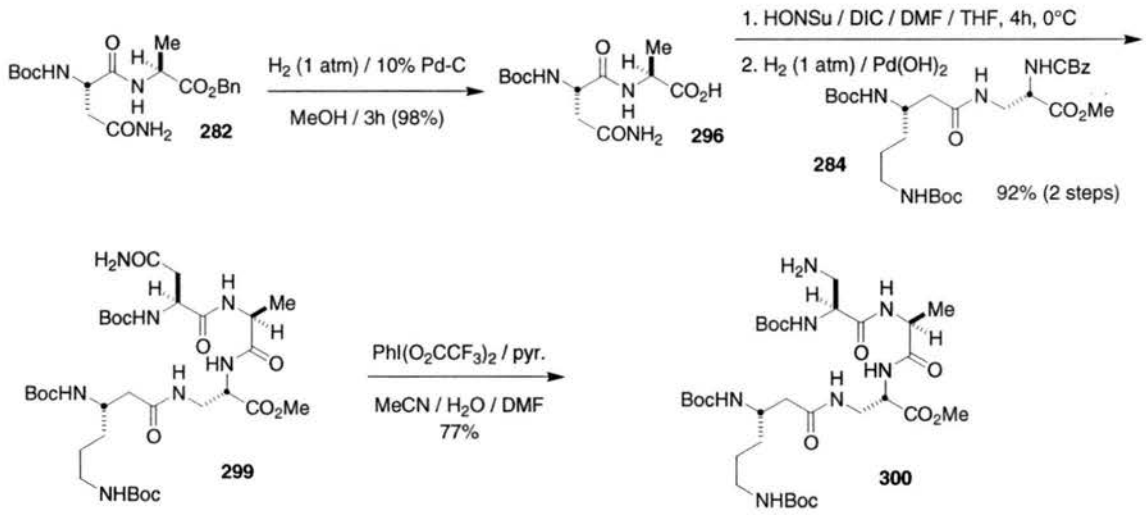


Figure 18. Revised approach to capreomycin IB.

It has been known in the literature that peptide couplings with arginine can be performed without a protecting group on the guanidine.[115-117] A hydrochloride salt of the guanidine of arginine can serve as a suitable "protecting group" due to the difference in pKa's between the α-amine and the guanidine of arginine. This being the case, it was decided to attempt to use **227** as an intermediate in the preparation of capreomycin (Figure 18). For this approach to become a reality, it would be necessary to alter the coupling strategy for the assembly of the dipeptide fragments. Peptide **296** (prepared from the previously described **282**) and peptide **284**, upon cleavage of the CBz group, would be coupled with one another and subjected to a Hofmann rearrangement to provide the requisite tetrapeptide. This tetrapeptide would then be coupled with dipeptide **297** which would result from the coupling of **298** and **276**. Deprotection of both ends of the linear hexapeptide would be followed by macrocyclization between the amine of the α-formylglycine dimethylacetal residue and the carboxyl of the diaminopropanoic acid residue. Hydrolysis of the dimethyl acetal and treatment with urea should provide the ureidodehydroalanine residue in a manner similar to that of Shiba's.

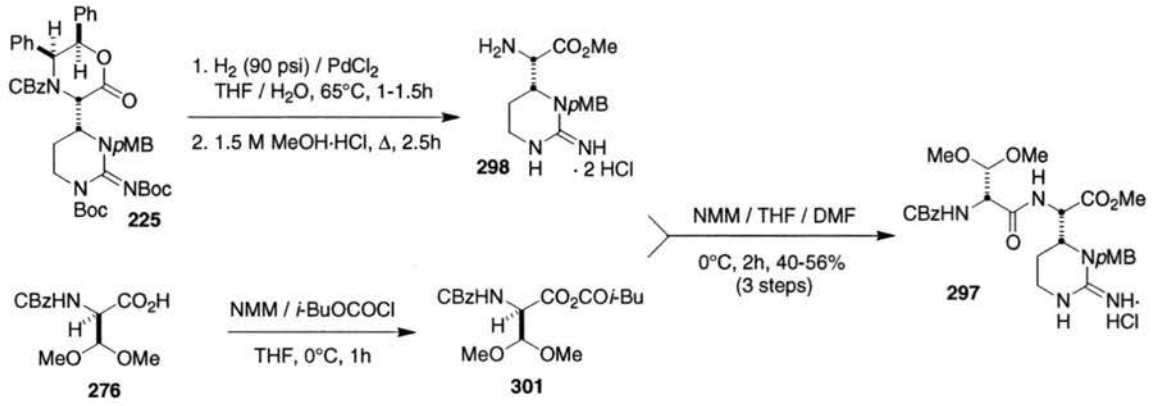
To assemble the tetrapeptide, the benzyl ester of **282** was hydrogenolyzed to provide a near-quantitative yield of the desired carboxylic acid **296** (Scheme 74). Tetrapeptide **299** was prepared in a two-step, one-pot sequence from **296**. Treatment of **296** with DIC and HONSu provided the intermediate *N*-hydroxysuccinimidyl ester. Addition of the previously described **284** and $Pd(OH)_2$ to the reaction mixture and saturation of the solution with hydrogen resulted in hydrogenolysis of the CBz group from **284** and simultaneous coupling with the awaiting *N*-hydroxysuccinimidyl ester of **296** to afford tetrapeptide **299** in good yield. Hofmann rearrangement of **299** provided a satisfactory yield of the desired primary amine **300**.



Scheme 74. Assembly of the tetrapeptide fragment.

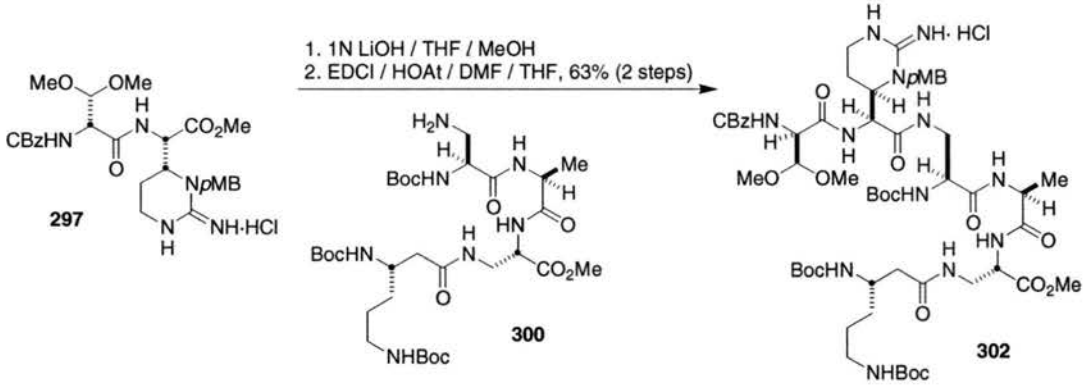
With the desired tetrapeptide in hand, the preparation of the third dipeptide (**297**) was begun (Scheme 75). Hydrogenolysis of lactone adduct **225** was followed by refluxing of the crude mixture in MeOH·HCl to provide the methyl ester dihydrochloride salt **298** which was taken on crude to the next step. Treatment of **276** with *N*-methylmorpholine and isobutylchloroformate afforded the mixed carbonic anhydride **301**,

which was added to **298**, resulting in the formation of dipeptide **297** as a single diastereomer in 60% yield over the three steps.



Scheme 75. Preparation of capreomycidine-α-formylglycine dimethylacetal dipeptide.

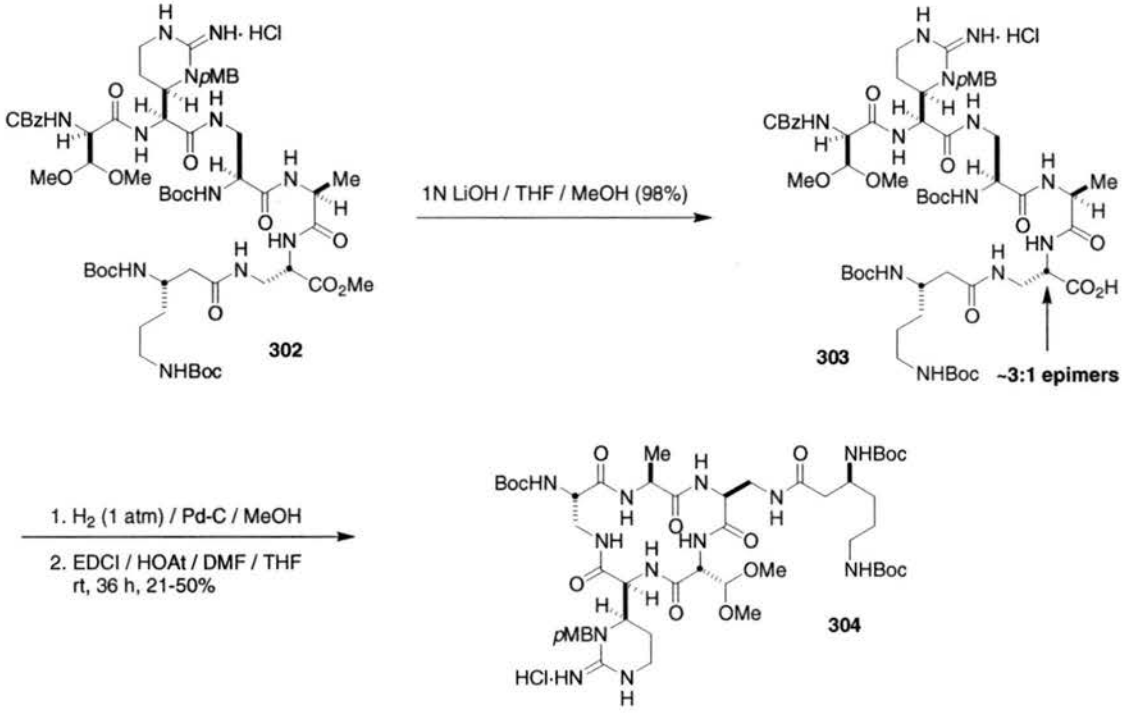
Cleavage of methyl ester **297** with 1N lithium hydroxide, followed by neutralization, provided the crude carboxylic acid which was coupled to **300** with EDCI and HOAt to provide a 70% yield of the desired hexapeptide **302** over the two steps (Scheme 76).[118] This completed the synthesis of the linear macrocyclization precursor.



Scheme 76. Preparation of linear hexapeptide.

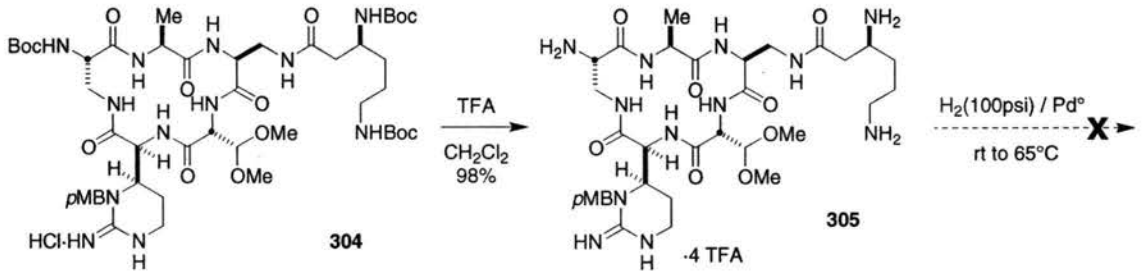
Hydrolysis of the methyl ester (**302**) with lithium hydroxide afforded carboxylic acid **303** as an ~3:1 mixture of epimers, presumably at the diaminopropanoic acid residue (Scheme 77). Cleavage of the CBz group was followed by macrocyclization of the

resulting amino acid with EDCI and HOAt in DMF / THF under high dilution conditions (~0.9 Mm) to provide **304** in anywhere from 21-50% yield.



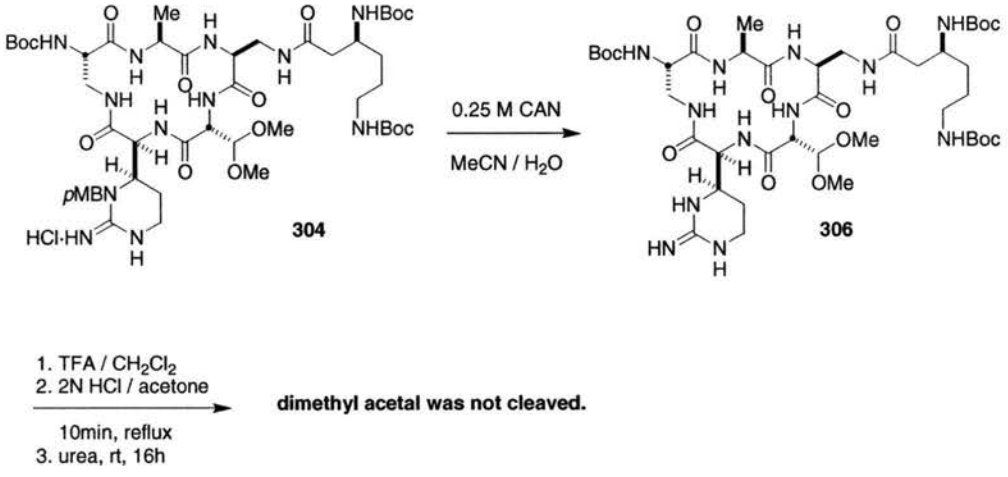
Scheme 77. Deprotection and macrocyclization.

With macrocycle **304** in hand, it was felt that conversion to capreomycin IB would be a straightforward task. However, this proved to be anything but the case. Knowing that in Shiba's total synthesis, removal of the Boc groups from the macrocycle was required prior to cleavage of the nitroguanidine protecting group, it was decided to use the same tactic in our synthesis (Scheme 78).[36,37] Removal of the Boc groups from **304** was accomplished readily with trifluoroacetic acid in methylene chloride to afford the triamine **305**. Unfortunately, under a variety of hydrogenolysis conditions, cleavage of the *p*-methoxybenzyl group was not realized.



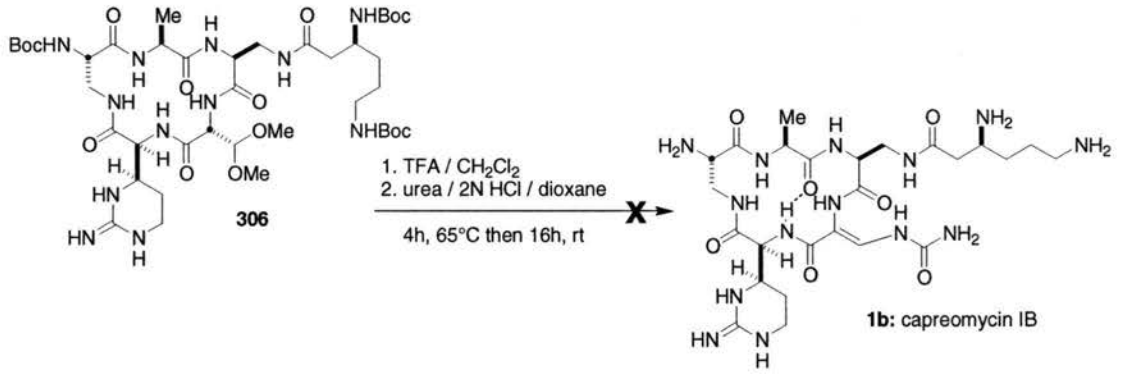
Scheme 78. Attempted hydrogenolysis of *p*-methoxybenzyl group.

It was at this point that oxidative cleavage of the *p*-methoxybenzyl group was investigated. Previous reports have described the use of ceric ammonium nitrate (CAN) for the cleavage of *p*-methoxybenzyl groups from amides.[99,119] This being the case, it was decided to extrapolate this method to our *p*-methoxybenzylguanidine (Scheme 79). Since CAN is known to oxidize amines, it was necessary to attempt the cleavage reaction with the Boc groups on **304** left intact. Exposure of **304** to 0.25 M CAN in acetonitrile and water provided a product lacking a *p*MB group with the desired mass for the deprotected product (**306**). Unfortunately, much to our surprise, the dimethylacetal was not cleaved under the conditions that Shiba used for the cleavage of the diethylacetal in their capreomycin IB synthesis.



Scheme 79. Attempted oxidative cleavage of *p*MB group.

Even upon heating **306** in 2N HCl and dioxane for several hours in the presence of urea, a method similar to that of Dirlam's in his urea exchange reactions with the capreomycins, a small amount of dimethyl acetal still remained and no desired product was observed (Scheme 80).[5,43-45]



Scheme 80. Second attempt at dimethylacetal hydrolysis.

5.2. The completion of capreomycin IB.

After many unsuccessful attempts at converting macrocycle **304** to the natural product, it was decided to abandon this route and investigate one final approach (Figure 19). Keeping in mind our previous failures, it was decided to incorporate α-formylglycine diethylacetal into the synthesis *via* tripeptide **307**. In addition, it was decided to attempt to incorporate capreomycidine into the synthesis at a late stage as the previously prepared free guanidine species **229**.

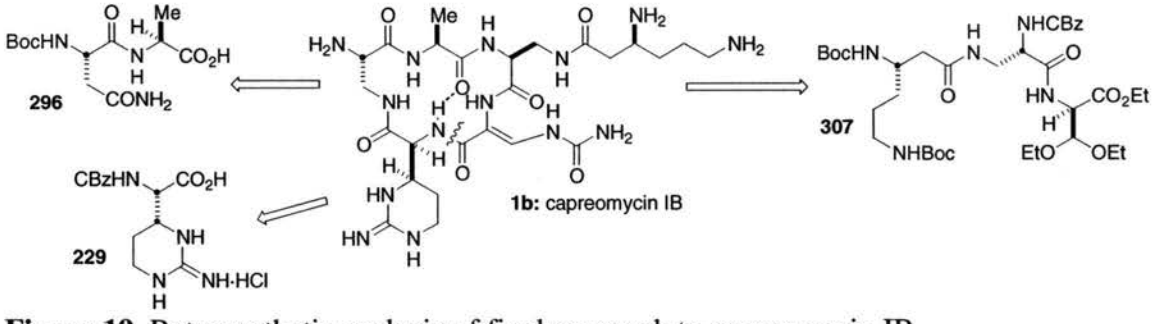
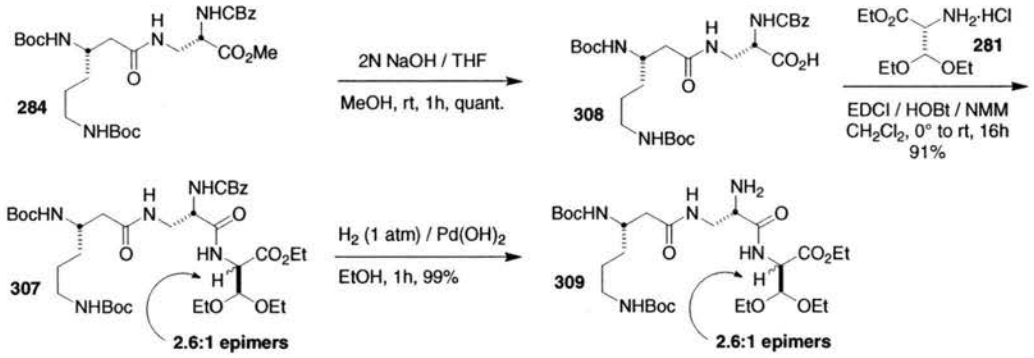


Figure 19. Retrosynthetic analysis of final approach to capreomycin IB.

Preparation of tripeptide **309** began with methyl ester cleavage of **284** to provide **308** in quantitative yield (Scheme 81). Coupling of **308** with the previously described ethyl ester hydrochloride salt of α-formylglycine diethylacetal (**281**) provided the desired tripeptide **307** in 90% yield. Unfortunately, an approximately 2.6:1 mixture of inseparable epimers was observed in the coupling product by ^{1}H NMR. These epimers were carried through together until separation was possible at the macrocyclization stage. Hydrogenolysis of the CBz group in the presence of palladium hydroxide provided the free amine **309** in 99% yield. It was necessary to immediately use **309** in the subsequent coupling reaction in order to avoid undesired *N,N'*-acyl transfer.

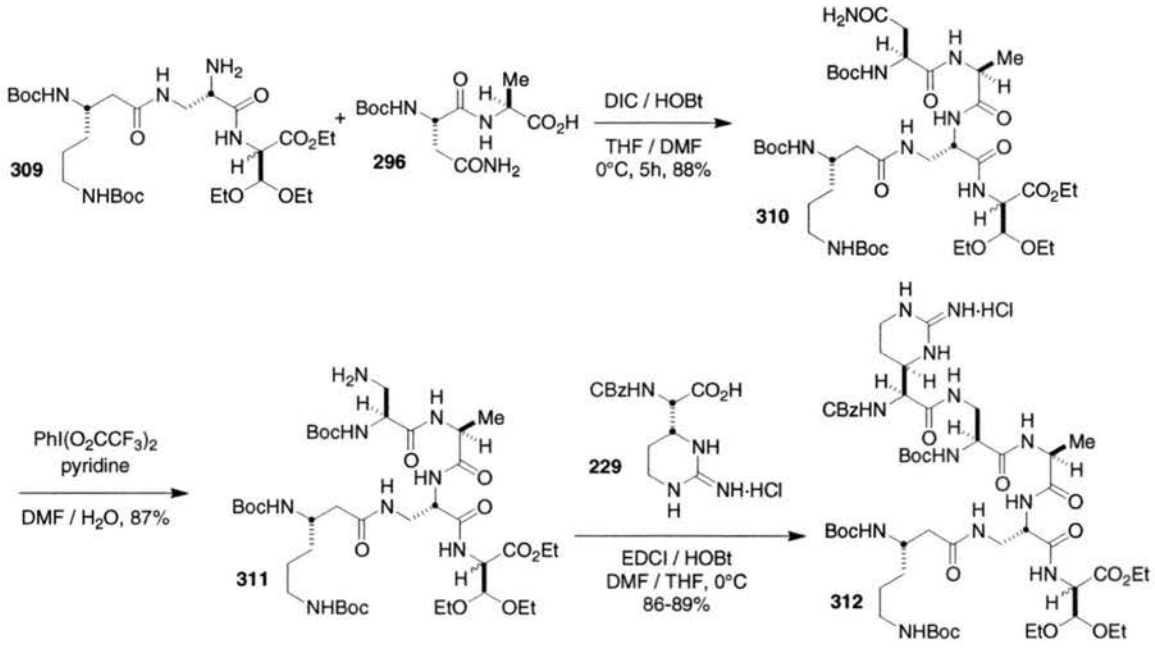


Scheme 81. Preparation of the tripeptide fragment.

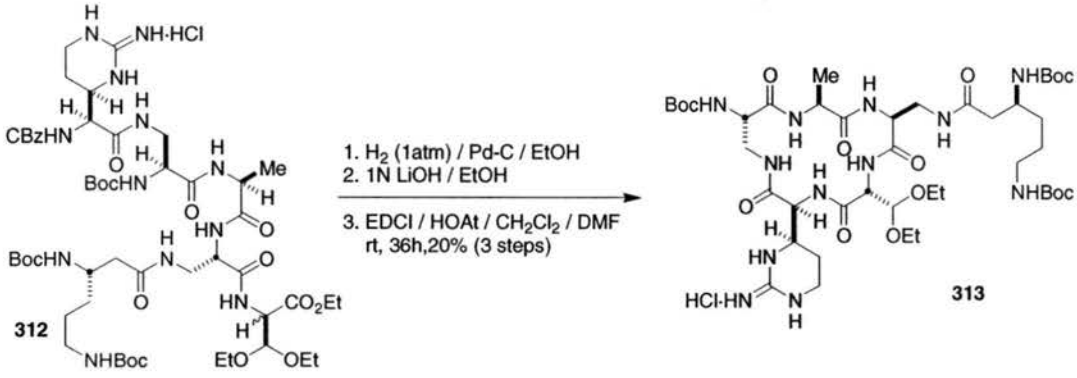
Coupling of **309** with the previously prepared dipeptide **296** resulted in an 88% yield of the pentapeptide **310** (Scheme 82). Hofmann rearrangement of **310** proceeded in 87% yield to provide primary amine **311**. Coupling of **311** with N^{α}-CBz-capreomycidine (**229**) afforded the desired hexapeptide **312** in 86-89% yield.

Initial attempts to hydrolyze the ethyl ester of **312** with sodium hydroxide in methanol resulted in a complex mixture of products arising from the hydrolysis of both the ethyl ester and CBz groups. This problem was avoided by first removing the CBz group of **312** by hydrogenolysis (Scheme 83). The crude product was then subjected to

1N lithium hydroxide in methanol, which hydrolyzed the ethyl ester. Upon neutralization of the hydrolysis reaction and desalting with a Waters C18 Sep-Pak cartridge, the crude product was treated with EDCI and HOAt in CH_2Cl_2 / DMF for 36h to provide the desired macrocyclization product **313** in 20% yield over the three steps.



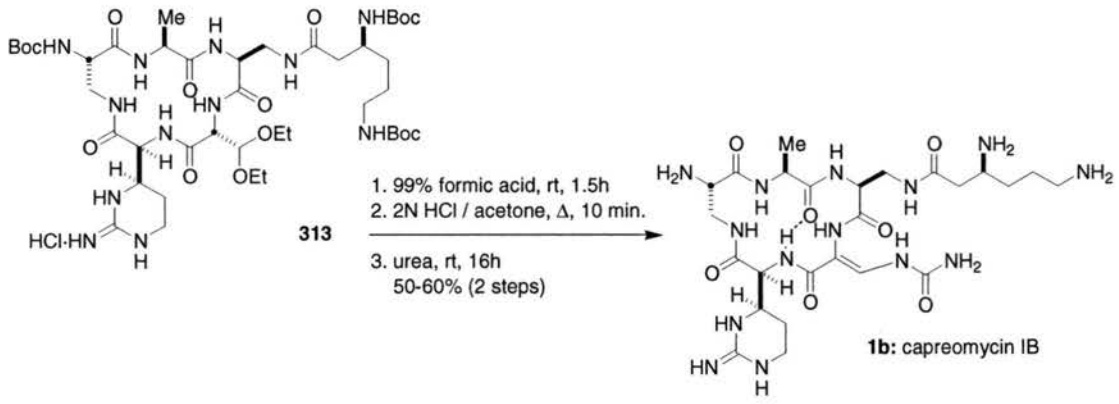
Scheme 82. Synthesis of linear hexapeptide **312**.



Scheme 83. Deprotection and macrocyclization.

Using conditions developed by Shiba in his synthesis of capreomycin IB, **313** was treated with 99% formic acid to remove the Boc groups (Scheme 84). The resulting triamine was exposed to refluxing 2N HCl and acetone for 10 min, at which time the

reaction was cooled to room temperature, urea was added, and the solution stirred overnight at room temperature. Evaporation of the reaction and addition of ethanol resulted in the precipitation of pure capreomycin IB (**1b**) as its tetra-hydrochloride salt in 50-60% yield for the two steps. The synthetic material matched both by ^{1}H NMR and optical rotation to the natural product $[\alpha]_D^{25}$, synthetic: -48 (c=0.05, dd H_2O), natural: -44.6 (c=0.5, dd H_2O).



Scheme 84. Completion of the total synthesis of capreomycin IB.

A concise total synthesis of capreomycin IB has been completed (27 steps, 2% overall yield). Highlights of the synthesis included the efficient asymmetric synthesis of (2*S*,3*R*)-capreomycidine, the asymmetric synthesis of α-formylglycine diethylacetal, and the use of a Hofmann rearrangement on asparagine-containing peptides as a means of accessing the diaminopropanoic acid residues, a strategy that dramatically decreased the number of protection and deprotection steps required for the synthesis.

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ABBREVIATION LIST

AgOAc	silver benzoate
CBzCl	benzylchloroformate
CBz	benzyloxycarbonyl
Et_3N	triethylamine
PhH	benzene
$Hg(Oac)_2$	mercury (II) acetate
DMF	dimethylformamide
HOAc	acetic acid
BnOH	benzyl alcohol
p-TsOH	*p*-toluenesulfonic acid
EtOCOCl	ethylchloroformate
AgOBz	silver benzoate
NMM	*N*-methylmorpholine
HONSu	*N*-hydroxysuccinimide
Boc	*tert*-butoxycarbonyl
NpsCl	*o*-nitrophenylsulfenyl chloride
DCC	dicyclohexylcarbodiimide
HOBt	1-hydroxybenzotriazole
THF	tetrahydrofuran
Nps	*o*-nitrophenylsulfenyl
TFA	trifluoroacetic acid
EtOAc	ethyl acetate

Boc_2O	di-*tert*-butyldicarbonate
CBz_2O	dibenzyldicarbonate
DMAP	4-(dimethylamino)-pyridine
DIBAL-H	diisobutylaluminum hydride
AIBN	2,2'-azobisisobutyronitrile
KHMDS	potassium bis(trimethylsilyl)amide
MeCN	acetonitrile
TsCl	*p*-toluenesulfonyl chloride
AgOTf	silver trifluoromethanesulfonate
DIPT	diisopropyltartrate
TBSCl	*tert*-butyldimethylsilyl chloride
DDQ	dichloro-dicyanoquinone
Bu_2BOTf	dibutylboron trifluoromethanesulfonate
t-BuOOH	*tert*-butyl-hydroperoxide
Ac_2O	acetic anhydride
Pms	*p*-tolylmethylsulfonyl
Boc-ON	2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile
MsCl	methanesulfonyl chloride
DPPA	diphenylphosphoryl azide
DEAD	diethyl azodicarboxylate
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilyl)amide
DIAD	diisopropyl azodicarboxylate

NBS	*N*-bromosuccinimide
DABCO	1,4-diazabicyclo[2.2.2]octane
DIPEA	diisopropylethylamine
TeocONSu	trimethylsilylethyl-*N*-hydroxysuccinimidyl carbonate
EDCI	ethyl-dimethylaminopropylcarbodiimide hydrochloride
$TMSCHN_2$	trimethylsilyldiazomethane
DIC	diisopropylcarbodiimide
HOAt	1-hydroxyazabenzotriazole
Pd-C	palladium on carbon

EXPERIMENTAL SECTION

6.1. General Procedures

Unless otherwise noted, materials were obtained from commercially available sources and used without purification. Toluene was freshly distilled from CaH_2. Diethyl ether and THF were either freshly distilled from sodium benzophenone ketyl, or degassed with argon and passed through a solvent purification system (J.C. Meyer of Glass Contour) containing alumina or molecular sieves. Dimethylformamide was degassed with argon and passed through a solvent purification system (J.C. Meyer of Glass Contour) containing alumina or molecular sieves. 3Å molecular sieves were activated by heating for three minutes at the highest setting in a microwave followed by cooling under argon.

All reactions requiring anhydrous conditions were performed under a positive pressure of argon using oven-dried glassware (120°C) that was cooled in a dessicator, unless stated otherwise.

Column chromatography was performed on Merck silica gel Kiesel 60 (230-400 mesh).

Mass spectra were obtained on Fisons VG Autospec.

HPLC data were obtained on a Waters 600 HPLC.

^{1}H NMR, ^{13}C NMR, HSQC and nOe experiments were recorded on a Varian 300 or 400 MHz spectrometer. Chemical shifts (δ) were given in ppm and were recorded relative to the residual solvent peak unless otherwise noted. Proton ^{1}H NMR were tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant in hertz, and number of protons. When a signal was

deemed "broad" it was noted as such. Additionally, the term "partially buried" is used to refer to resonances that are obscured by another resonance.

IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer.

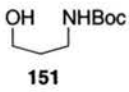
Optical rotations were determined with a Rudolph Research Autopol III automatic polarimeter referenced to the D-line of sodium.

6.2. Experimental Procedures.

151

3-(*N*-Boc-amino)-propanol: To a solution of 3-aminopropanol (1.00 g, 13.3 mmol, 1 eq.) in dichloromethane (10 mL) at 0°C, was added Boc_2O (2.9 g, 13.3 mmol, 1 eq.), and 4-dimethylaminopyridine (81 mg, 0.67 mmol, 0.05 eq.). This mixture was stirred until the solution became clear. Triethylamine (4.04 g, 39.9 mmol, 3 eq.) was added, and the reaction was stirred for 16 h, allowing to warm to rt. The solvent was evaporated (in vacuo) resulting in a white solid, which chromatographed on silica gel (75:20:5 CH_2Cl_2:EtOAc:MeOH) to provide 1.37 g (59%) of **151** as a pale oil.

^{1}H NMR (300 MHz) ($CDCl_3$) δ $CHCl_3$: 1.45 (9H, s); 1.67 (2H, quintet, J=5.9 Hz); 3.04 (1H, broad s); 3.29 (2H, q, J=6.2 Hz); 3.66 (2H, t, J=5.5 Hz); 4.79 (1H, broad s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ $CDCl_3$: 28.5, 32.8, 37.1, 59.4, 79.5, 157.2. IR (NaCl, deposited from CH_2Cl_2): 3347, 2976, 2935, 2877, 1688, 1526, 1453, 1392, 1366, 1278, 1253, 1172, 1046 cm^{-1}.



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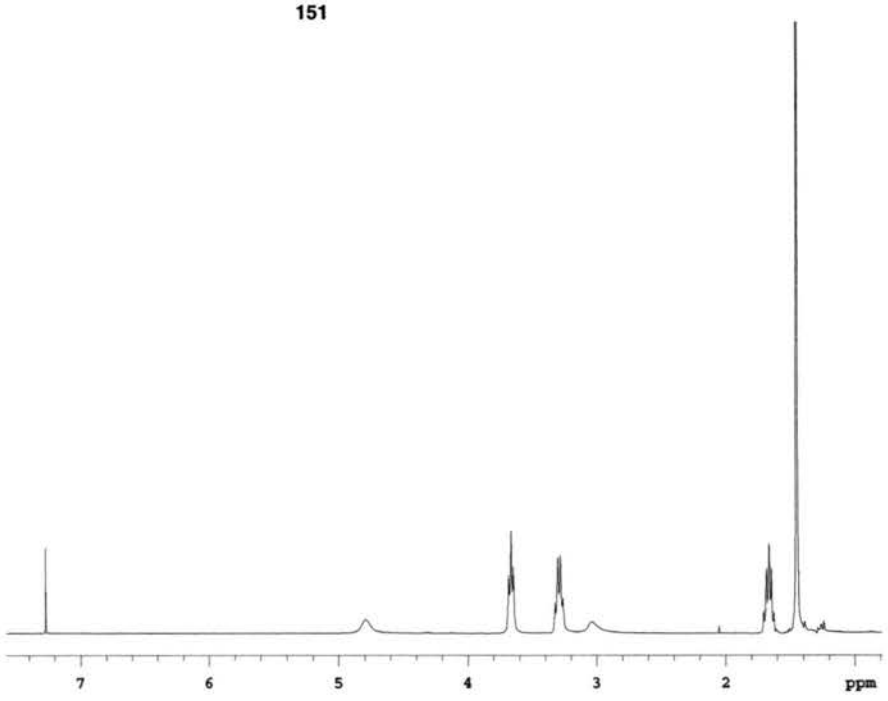

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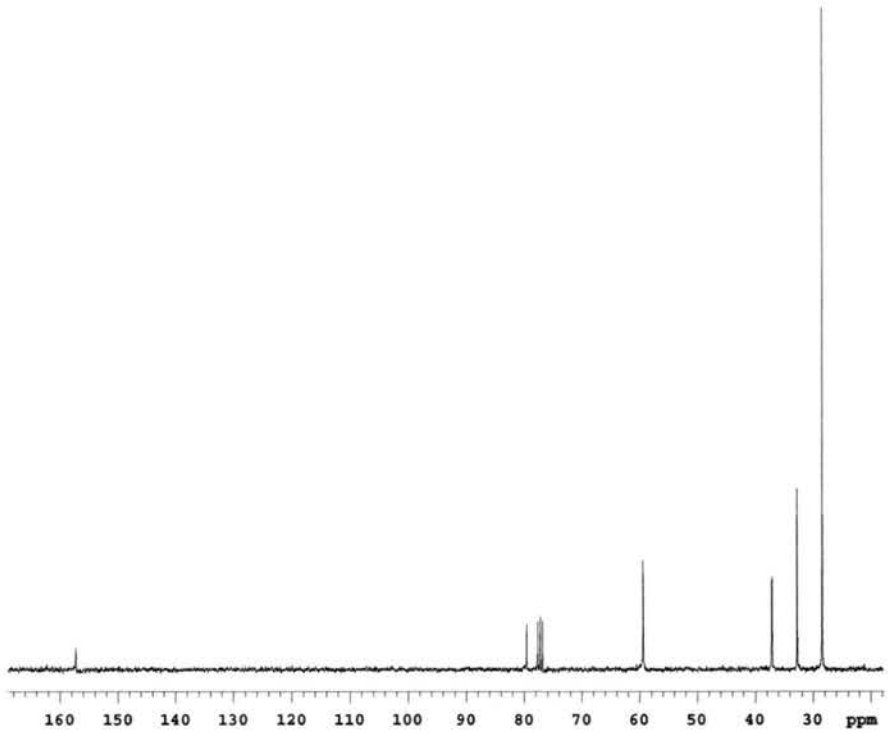

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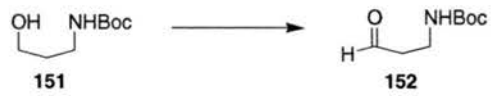
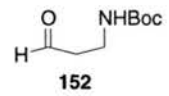


151 152

3-(*N*-Boc-amino)-propanal (152). Compound **151** (1.37g, 7.82 mmol, 1 eq.) was taken up in dichloromethane (50 mL). Dess-Martin Periodinane (5.64 g, 13.29 mmol, 1.7 eq.) was added, and the reaction stirred 6 h at room temp. The reaction was then poured into a 1:1 mixture of diethyl ether and sat. aq. $NaHCO_3$ (400 mL). Sodium thiosulfate (23.1 g, 93.06 mmol, 11.9 eq.) was added, and the solution stirred for 20 minutes. The organic layer was removed, and the aqueous layer was extracted twice with ether. The organic layers were then combined and dried over anhydrous Na_2SO_4. The organic layer was removed from the Na_2SO_4 by filtration evaporated (in vacuo) to provide a crude pale oil. Silica gel chromatography of the crude product (1.5:1 hexanes:EtOAc) provided 1.08 g (80%) of **152** as a clear oil.

^1H NMR (300 MHz) ($CDCl_3$) δ $CHCl_3$: 1.43 (9H, s); 2.72 (2H, t, J=5.9 Hz); 3.43 (2H, t, J=5.9 Hz); 4.91 (1H, broad s); 9.81 (1H, s). ^{13}C NMR (75 MHz) ($CDCl_3$): δ $CDCl_3$: 28.5, 34.2, 44.5, 79.6, 156.0, 201.6. IR (NaCl, deposited from CH_2Cl_2): 3362, 2978, 2931, 2734, 1717, 1696, 1681, 1522, 1458, 1392, 1366, 1276, 1252, 1171 cm^{-1}.



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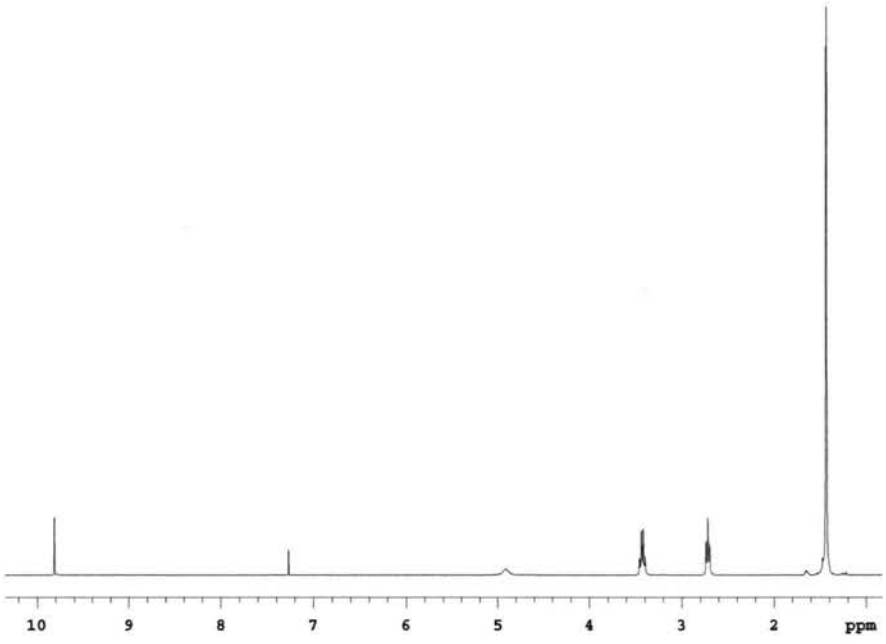

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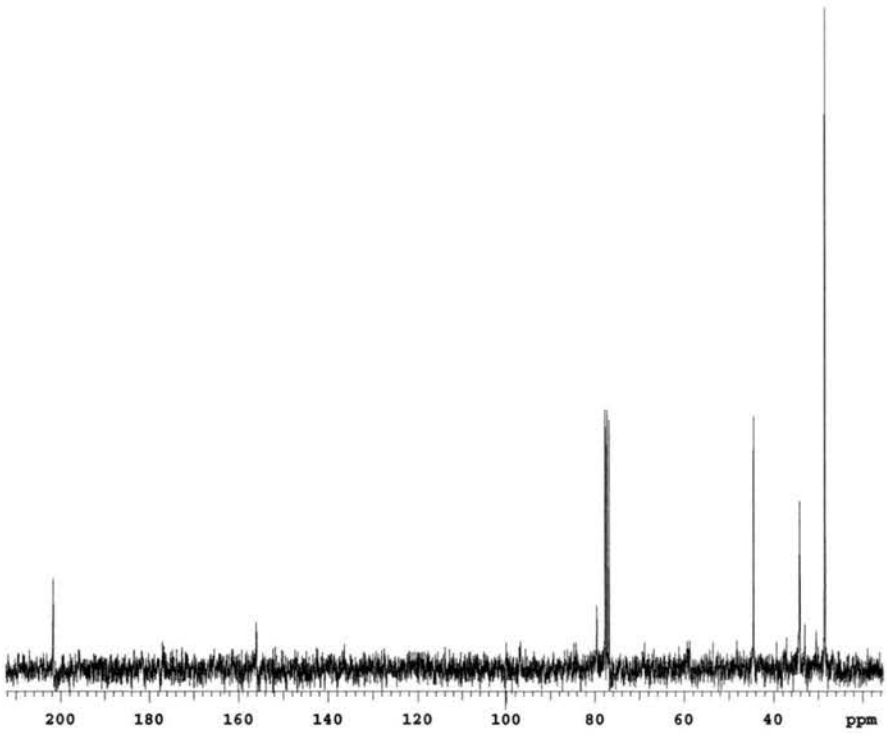

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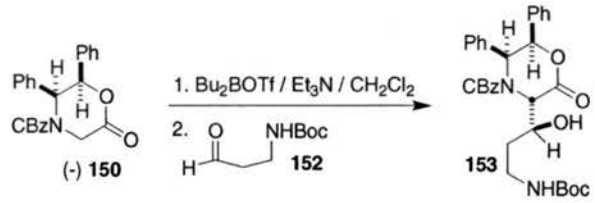
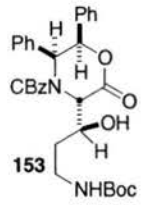
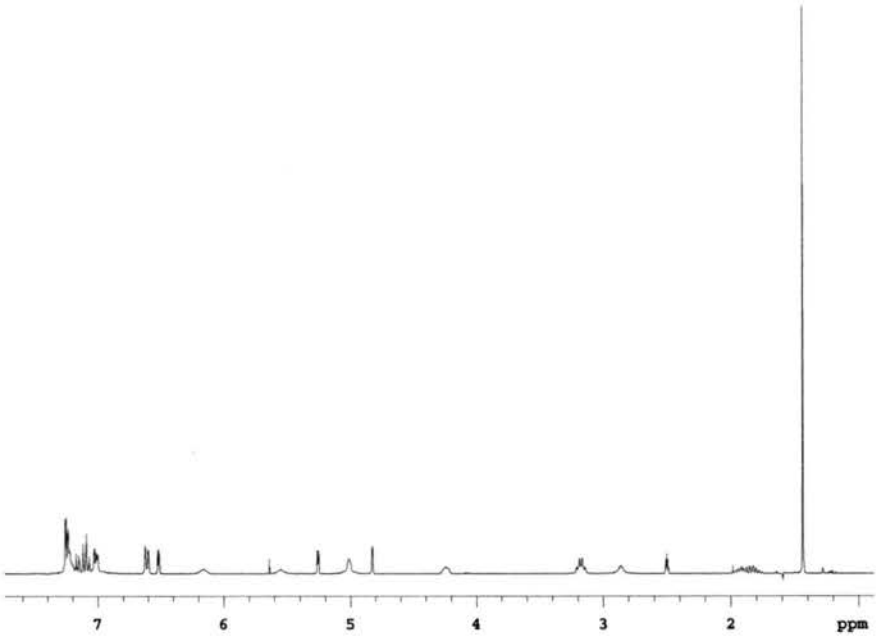


Aldol product (153). To a solution of oxazinone (-) **150** (737 mg, 1.90 mmol, 1 eq.) in dichloromethane (19 mL) under an argon atmosphere at -5°C, was added Bu_2BOTf (1M solution in CH_2Cl_2, 3.8 mL, 3.80 mmol, 2 eq.) followed by Et_3N (795 µL, 5.70 mmol, 3 eq.). The resulting solution was stirred 15 minutes at -5°C then 15 minutes at -78°C. A solution of aldehyde **152** (494 mg, 2.85 mmol, 1.5 eq.) in dichloromethane (10 mL) under argon atmosphere was added by canula into the flask containing the enolate. The resulting mixture was stirred at -78°C for 1 h, at which point the reaction was warmed to room temperature and quenched with 0.025 M pH 7 potassium phosphate buffer. Pouring of the reaction mixture into water was followed by removal of the organic layer. Upon extraction of the aqueous layer twice with dichloromethane, the combined organic layers were dried over Na_2SO_4. Removal of the Na_2SO_4 by filtration and evaporation of the filtrate (in vacuo) provided a crude orange oil that was pre-adsorbed onto silica gel and subjected to silica gel chromatography (30% EtOAc in hexanes) to separate a mixture of **152** and **153** from (-) **150**. A second silica column (3:1:1 CH_2Cl_2:EtOAc:hexanes) was used to separate 309 mg (29%) of pure **153** from **152** as a white solid.

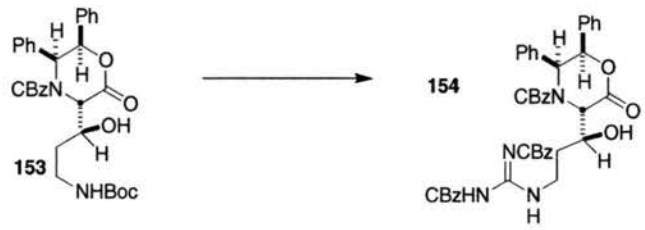
^{1}H-NMR (300 MHz) (DMSO-d_6, 393K) δ DMSO: 1.44 (9H, s); 1.76-1.99 (2H, m); 3.18 (2H, q, J=6.6 Hz); 4.24 (1H, broad m); 4.83(1H, d, J=2.2 Hz); 4.99 (1H, ½ ABq, J=14.7 Hz); 5.03(1H, ½ ABq, J=14.7 Hz); 5.26 (1H, d, J=3.3 Hz); 5.55 (1H, broad s); 6.16 (1H,

broad s); 6.52(1H, d, J=2.9 Hz); 6.61 (2H, d, J=7.3 Hz); 7.00-7.26 (13H, m). IR-(NaCl, deposited from CH_2Cl_2): 3375, 3033, 2976, 1751, 1704, 1686, 1515, 1454, 1402, 1355, 1282, 1168, 1118, 1062, 1003, 912 cm^{-1}. LRMS M+1: 561.6 and a major peak at 461.5 (loss of Boc group).



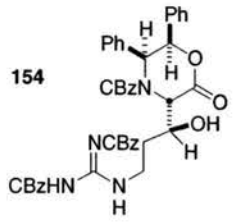


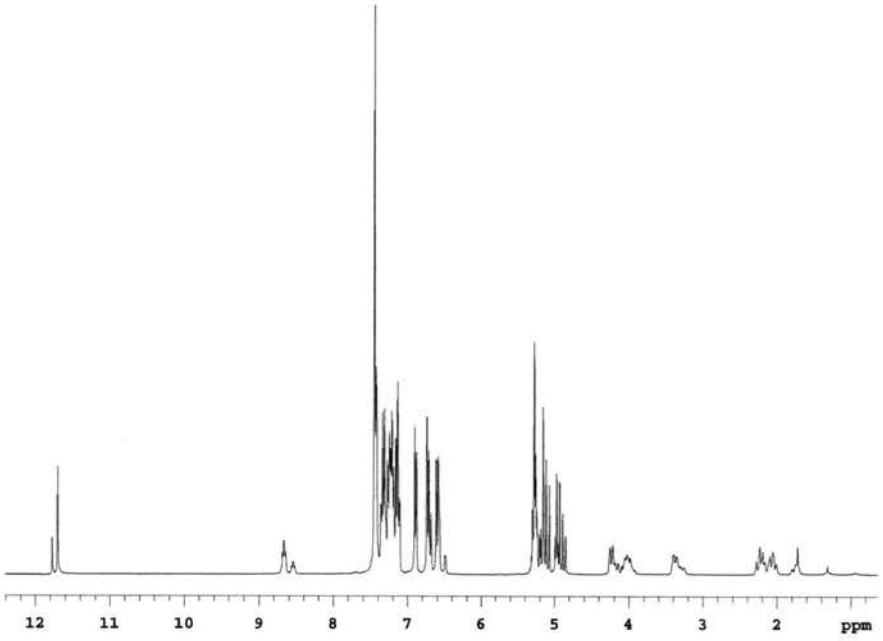
dd212



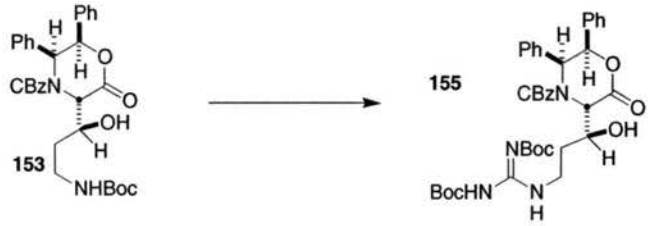
Guanidine 154. Compound **153** (185 mg, 0.33 mmol, 1 eq) was added to a 1:1 mixture of TFA and dichloromethane (10 mL) at 0°C. The reaction was stirred for 3h followed by removal of solvents *in vacuo*. Dichloromethane was added to resulting yellow oil and evaporated (in vacuo) to remove residual TFA. This was repeated 3 times. The crude product was then dissolved in DMF (3 mL) with *N,N'*-di-benzyloxycarbonyl-S-methylisothiourea (118 mg, 0.33 mmol, 1 eq), to which Et_3N (100 mg, 0.99 mmol, 3 eq.) was added dropwise at 0°C. The reaction mixture was subsequently stirred 16 h, at which point it was poured into dichloromethane and washed with 1N HCl, sat. aq. $NaHCO_3$, and brine (4x). After drying over anhydrous Na_2SO_4, the organic layer was filtered off and evaporated (in vacuo). Purification of the crude product by silica gel chromatography (30% EtOAc in hexanes) provided 135 mg (53%) of **154** as a white solid. ^1H NMR (300 MHz) ($CDCl_3$) (~2:1 mixture of major and minor rotamers) δ $CHCl_3$: 2.01-2.10 (1H, m); 2.17-2.28 (1H, m); 3.26-3.31 (minor) and 3.31-3.41 (major) (1H, m); 3.92-4.09 (1H, m); 4.16 (minor, 1H, d, J=12.1) and 4.24 (major, 1H, d, J=11.7); 4.85-4.99 (2H, m); 5.07-5.30 (6H, m); 6.49-6.74 (5H, m); 6.89 (2H, d, J=7.0 Hz); 7.11-7.45 (19H, m); 8.54 (minor, 1H, t, J=5.9 Hz) and 8.67 (major, 1H, t, J=7.0 Hz); 11.70 (major, 1H, s) and 11.77 (minor, 1H, s). IR (NaCl, deposited from CH_2Cl_2): 3328, 3064, 3032, 2924, 1750, 1702, 1652, 1624, 1576, 1498, 1454, 1405, 1382, 1322, 1283, 1209, 1143, 1116, 1056, 1029, 1002, 972 cm^{-1}. LRMS M+1: 771.5

154



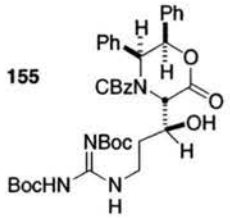


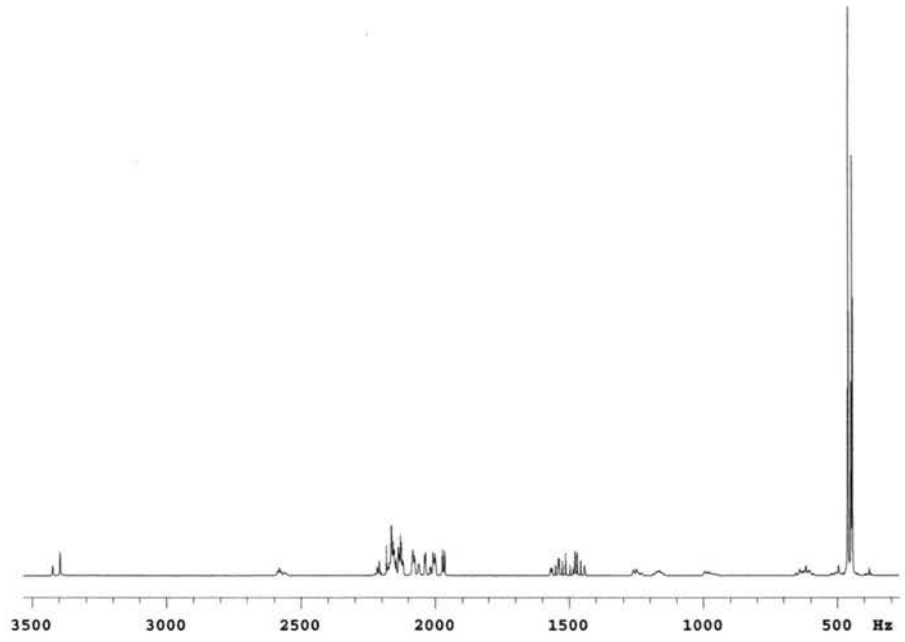
dd194



Guanidine 155. Compound **153** (67 mg, 0.12 mmol, 1 eq) was added to a 1:1 mixture of TFA and dichloromethane (5 mL) at 0°C. The reaction was stirred for 3h followed by removal of solvents *in vacuo*. Dichloromethane was added to resulting yellow oil and evaporated (in vacuo) to remove residual TFA. This was repeated 3 times. The crude product was then dissolved in DMF (3 mL) with *N,N'*-bis(*tert*-butoxycarbonyl)-S-methylisothiourea (35 mg, 0.12 mmol, 1 eq), to which Et_3N (36 mg, 0.36 mmol, 3 eq.) was added dropwise at 0°C. The reaction mixture was subsequently stirred 3 h, at which point an additional 24 mg Et_3N was added. After stirring for 16 h, the reaction mixture was poured into dichloromethane and washed with 1N HCl, sat. aq. $NaHCO_3$, and brine (4x). After drying over anhydrous Na_2SO_4, the organic layer was filtered off and evaporated (in vacuo). Purification of the crude product by silica gel chromatography (gradient elution 9:1 hexanes:EtOAc to 30% EtOAc in hexanes) provided 565 mg (67%) of **155** as a white solid.

^{1}H NMR (300 MHz) ($CDCl_3$) (~2:1 mixture of major and minor rotamers) δ $CHCl_3$: 1.48 (major, 9H, s) and 1.50 (minor, 9H, s); 1.53 (9H, s); 1.95-2.19 (2H, m); 3.81-3.96 (1H, m); 4.10-4.20 (1H, m); 4.81 (minor, 1H, s) and 4.92 (major, 1H, s); 4.88 (major, ½ ABq, J=12.5 Hz) and 4.97 (minor, ½ ABq, J=12.1 Hz); 5.06 (major, ½ ABq, J=12.5 Hz) and 4.18 (minor, ½ ABq, J=12.1 Hz); 5.13 (major, 1H, d, J=3.3 Hz) and 5.22 (minor, 1H, d, J=3.3 Hz); 6.55-7.39 (16H, m); 8.53 (minor, 1H, t, J=7.0 Hz) and 8.60 (major, 1H, t, J=6.2 Hz); 11.32 (major, 1H, s) and 11.41 (minor, 1H, s).


155
Ph
H
Ph
O
H
CBzN
O
OH
NBoc
H
BocHN
N
H

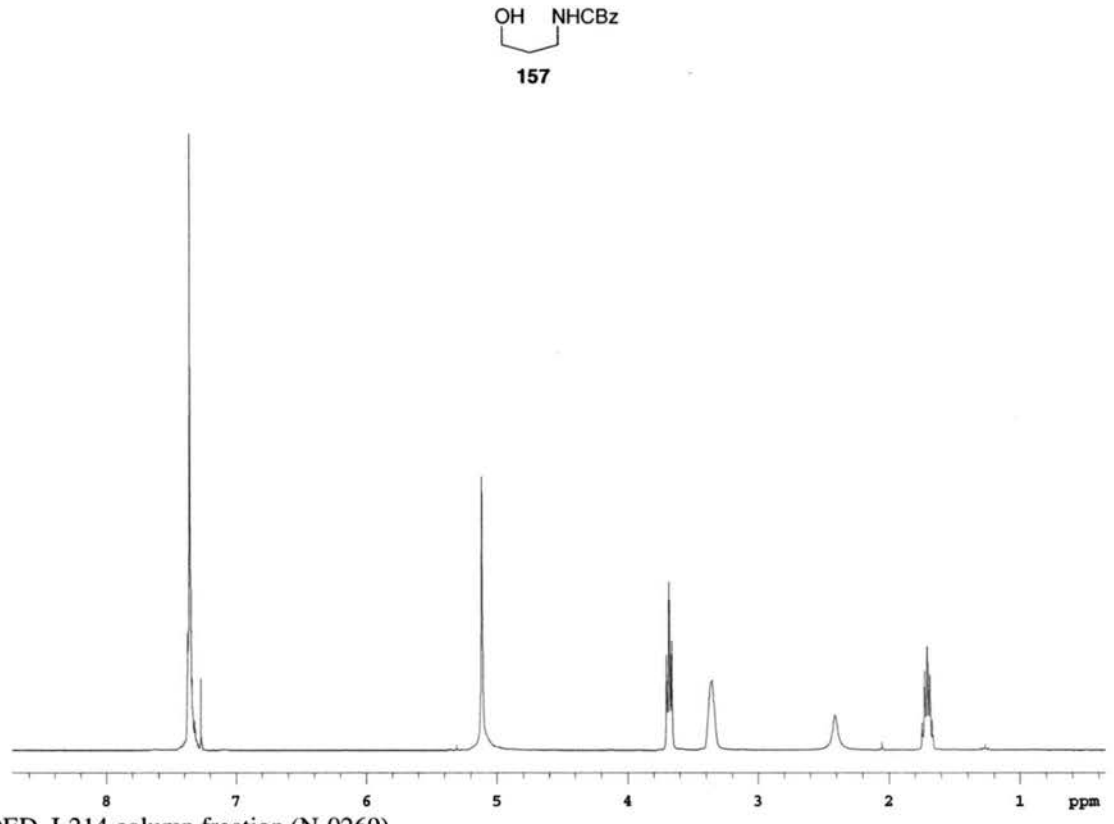

3500
3000
2500
2000
1500
1000
500
Hz

dd182

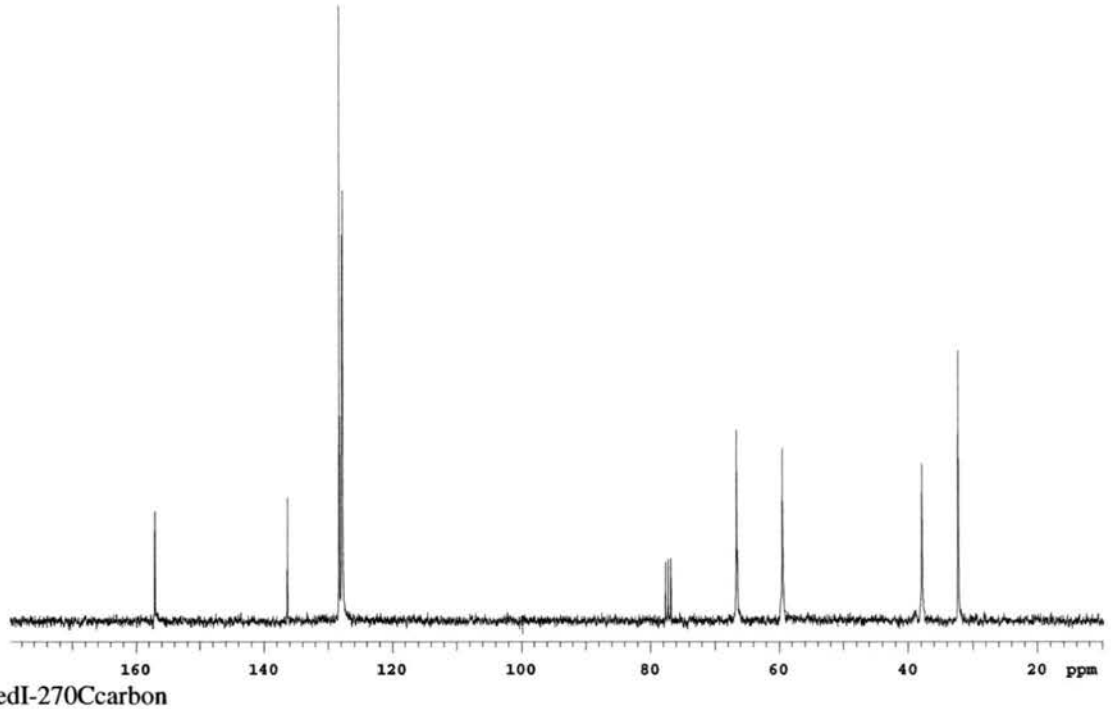
157

3-(*N*-CBz-amino)-propanol (157). To a 10% solution of triethylamine in methanol (2 mL), was added 3-aminopropanol (150 mg, 2.00 mmol., 1 eq.), and dibenzyl dicarbonate (802 mg, 2.80 mmol., 1.4 eq.). The resulting solution was refluxed under argon for 1 h, at which time the solvent was removed *in vacuo* resulting in a clear oil. Silica gel chromatography of the crude oil (eluted with 75:20:5 CH_2Cl_2:EtOAc:MeOH) provided 427 mg (99%) of **157** as a white solid. M.p. 51-52 °C (recryst. CH_2Cl_2:EtOAc).

^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.71 (2H, m); 2.47 (1H, bs); 3.36 (2H, bm); 3.69 (2H, t); 5.09 (1H, bs); 5.12 (2H, s); 7.32-7.38 (5H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ 32.6, 38.0, 59.7, 66.9, 128.1, 128.2, 128.6, 136.5, 157.4. IR (NaCl, Neat) 3326, 3030, 2955, 2931, 2873, 1684, 1651, 1586, 1535, 1499, 1489, 1454, 1374, 1327, 1298, 1266, 1216, 1144, 1116, 1086, 1066, 1023, 984, 966 cm^{-1}. HRMS (FAB) calc. for $C_{11}H_{15}NO_3$ (MH^+) 210.1130; found 210.1125.



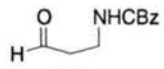
DED, I-214 column fraction (N-0269)



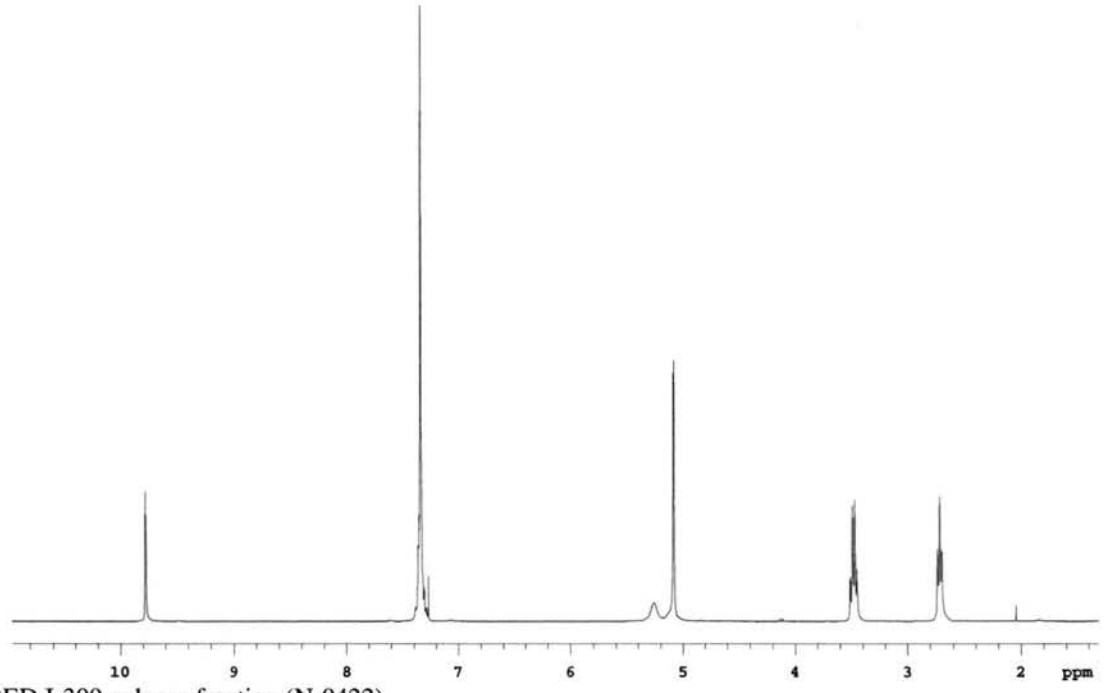
dedI-270Ccarbon

3-(N-CBz-amino)-propanal (158). To a solution of **157**(427 mg, 2.04 mmol., 1 eq.) in non-distilled methylene chloride (17 mL), was added the Dess-Martin Periodinane (1.47 g, 3.47 mmol., 1.7 eq.). The resulting heterogeneous mixture was stirred overnight at room temp. Upon taking up the reaction mixture in diethyl ether (70 mL) and sat. aq. $NaHCO_3$ (70 mL), sodium thiosulfate (6.03 g, 24.3 mmol., 11.9 eq.) was added, and the biphasic solution was stirred for 30 min. After removing the organic layer *via* separatory funnel, the aqueous layer was extracted twice more with ether. The organic layers were combined and dried over anhydrous $MgSO_4$. Filtration followed by removal of solvent under reduced pressure provided a crude white solid that was purified by silica gel chromatography (eluted with 1:1 EtOAc:hexanes) to provide 416 mg (98%) of **158** as a white solid. M.p. 53-54°C (recryst. EtOAc/hexanes).

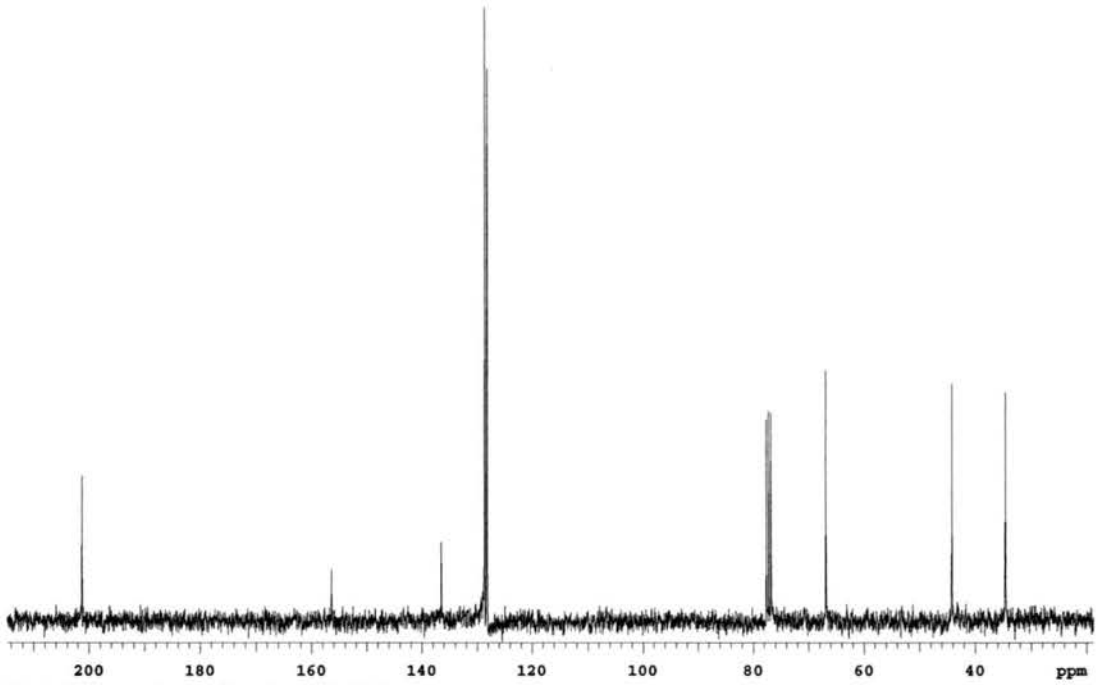
^{1}H NMR (300 MHz) ($CDCl_3$) δ 2.75 (2H, t); 3.50 (2H, q); 5.09 (2H, s); 5.18 (1H, bs); 7.32-7.37 (5H, m); 9.81 (1H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ 34.7, 44.3, 66.9, 128.2, 128.3, 128.6, 136.5, 156.4, 201.2. IR (NaCl, Neat) 3449, 3054, 2986, 2305, 2254, 1720, 1512, 1422, 1265, 1144, 1094, 1027, 909 cm^{-1}. HRMS (FAB) calc. for $C_{11}H_{13}NO_3$ (MH^+) 208.0974; found 208.0975.



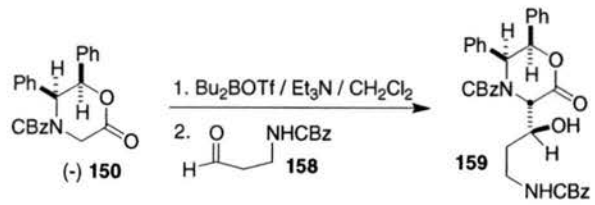
158



DED,I-309 column fraction (N-0422)

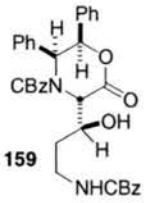


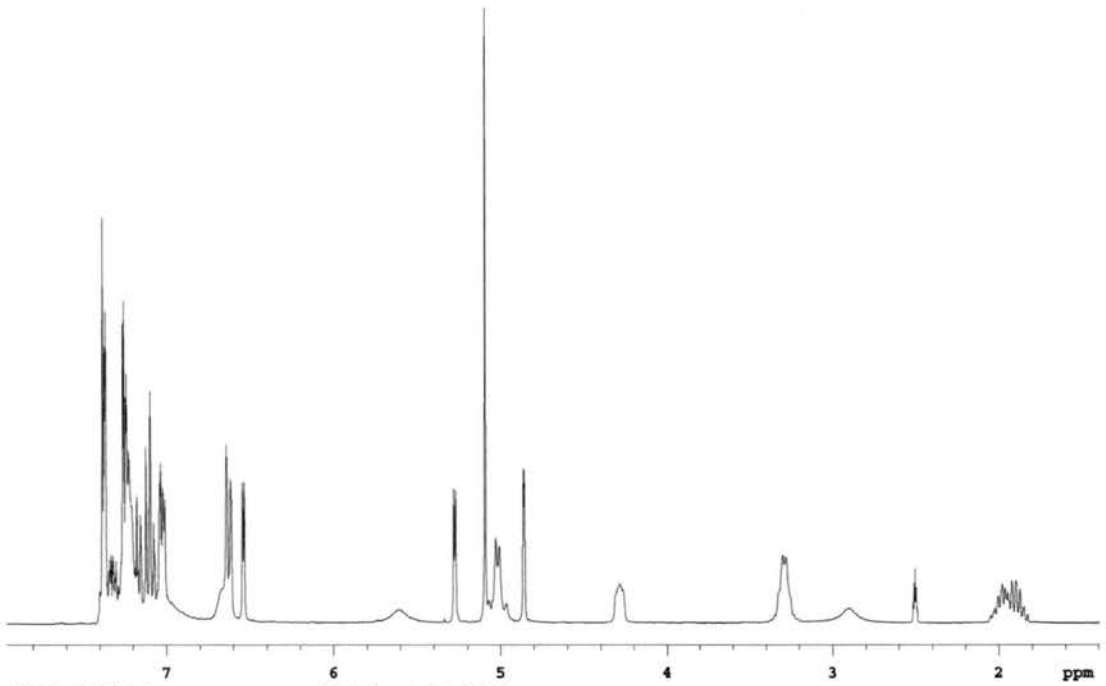
DED, I-309A column fraction (C-0042)



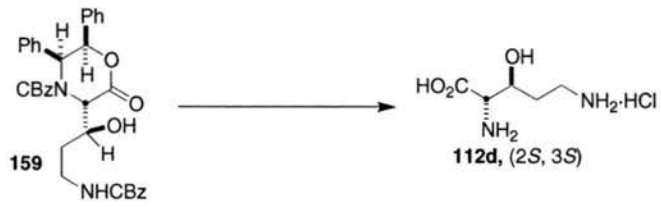
Aldol product 159. Under argon atmosphere, compound (-) **150** (624 mg, 1.61 mmol., 1 eq.) was dissolved in dry methylene chloride (19 mL). The resulting solution was then cooled to –5°C (ice/acetone bath). Di-*n*-butylboron triflate (1M in CH_2Cl_2) (3.22 mL, 3.22 mmol., 2 eq.) was added *via* syringe followed by addition of triethylamine (673 µL, 4.83 mmol., 3 eq.). The mixture was stirred 15 min. at –5 °C then cooled to -78 °C. In a separate flask, compound **158** (400 mg, 1.93 mmol., 1.2 eq.) was dissolved in methylene chloride (3.5 mL) and the resulting aldehyde solution was added *via* canula to the boron enolate, and the reaction stirred 1h at -78 °C. The reaction was quenched by the addition of 0.025 M pH 7 potassium phosphate buffer at -78 °C, and the mixture was allowed to warm to room temperature. The organic layer was separated and the aqueous layer was extracted twice more with methylene chloride. The organic layers were combined and dried over anhydrous sodium sulfate. Filtration and removal of the solvent under reduced pressure produced an orange oil that was purified by silica gel chromatography (eluted with 30% EtOAc in hexanes). This initial separation resulted in co-elution of both the aldehyde and aldol product. A second silica gel chromatographic separation (eluted with 3:1:1 CH_2Cl_2:EtOAc:hexanes) resulted in the isolation of 657 mg (69%) of **159** as an 8:1 mixture of diastereomers (^{1}H-NMR). The minor diastereomer was removed by recrystallization from EtOAc/hexanes. M.p. 175°C (recryst. EtOAc/hexanes).

^{1}H-NMR (300 MHz) (DMSO-d_6, 393K) δ DMSO: 1.84-2.06(2H, m); 3.30(2H, m); 4.29(1H, m); 4.87(1H, d, *J*=1.8Hz); 5.02(1H, 1/2 ABq, *J*=12.9Hz); 5.07(1H, 1/2 ABq, *J*=12.9Hz); 5.12(2H, s); 5.30(1H, d, *J*=3.3Hz); 5.62(1H, bs); 6.55(1H, d, *J*=3.3Hz); 6.64-7.41(21H, m). IR (NaCl, Neat) 3365, 3032, 2951, 1750, 1734, 1700, 1684, 1653, 1540, 1404, 1274, 1120, 967 cm^{-1}. Anal. Calc. for $C_{35}H_{34}N_2O_7$: C, 70.69; H, 5.76; N, 4.71. Found C, 70.50; H, 6.02; N, 4.89. **159.** $[\alpha]_D^{25}$ = -5.7 (c=1.5, CH_2Cl_2). ***Ent*-159.** $[\alpha]_D^{25}$ = +5.7 (c=1.5, CH_2Cl_2).

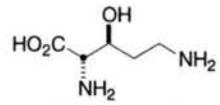




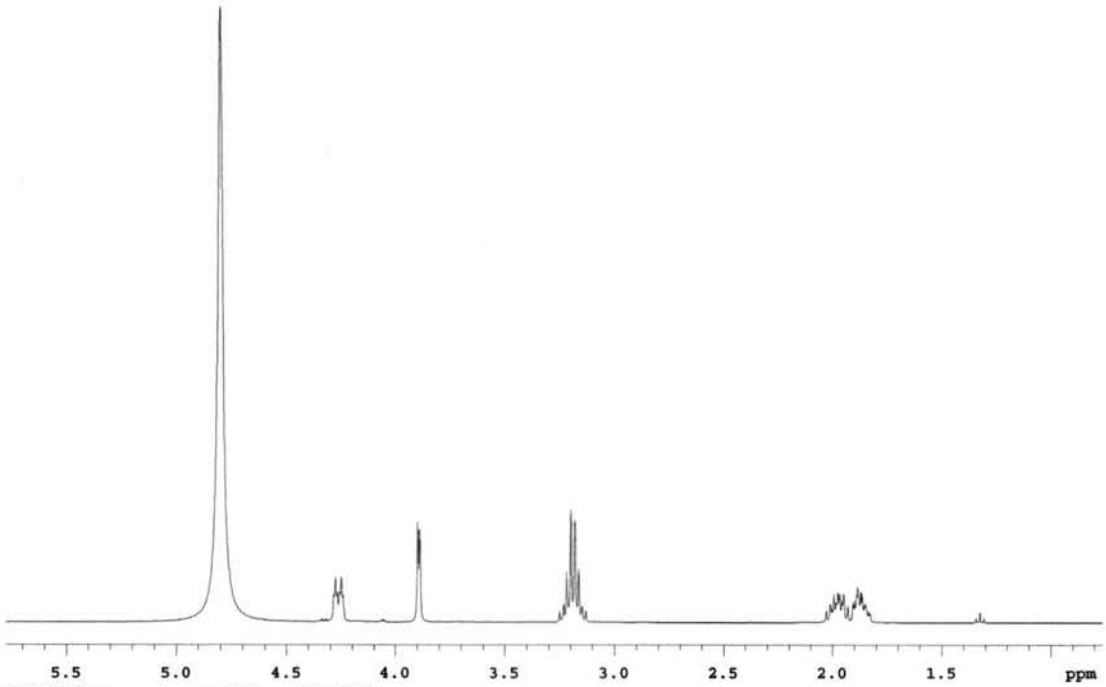
dedIII-515B1-postrecryst-120deg-DMSO



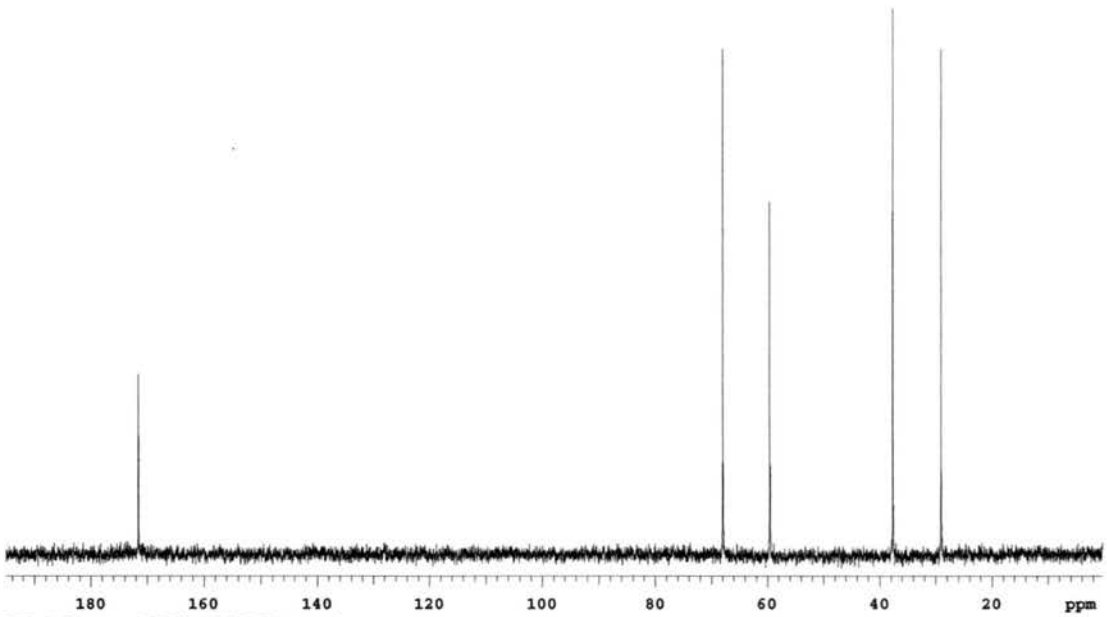
(2*S*,3*S*)-β-hydroxyornithine. A pressure tube containing **159** (100 mg, 0.17 mmol., 1 eq.) in dry THF (4 mL) and absolute ethanol (2 mL) was purged with argon for 15 minutes. $PdCl_2$ (60 mg, 0.34 mmol., 2 eq.) was added to this solution and the tube pressurized to 78 psi with hydrogen gas. The reaction was stirred at room temperature for 4 days. The catalyst was removed by filtration through Celite. The Celite pad was washed several times with a 2:1 THF:EtOH solution. Into a separate flask, the Celite pad was washed with five volumes of deionized water. The water volume was reduced by lyophilization and brought to pH 6.5 with NH_4OH. Addition of EtOH resulted in a white precipitate that was collected by filtration and dried to yield 21 mg (68%) of **112d** as the mono-HCl salt. ^{1}H NMR (300 MHz) (D_2O) δ 1.82-2.04 (2H, m); 3.18 (2H, sym m); 3.89 (1H, d, *J*=3.7Hz); 4.26 (1H, ddd, *J*=3.3, 3.3, 10.6Hz).^{13}C NMR (100 MHz) (D_2O) δ 29.0, 37.7, 59.5, 68.0, 171.6. IR (NaCl, 1% KBr) 3074, 1612, 1576, 1529, 1508, 1431, 1358, 1325, 1180, 1140, 1065, 1032 cm^{-1}. m.p. 232°C dec (lit.[5] mp 232°C dec). **112d.** $[\alpha]_D^{25}$ = +24.1 (c = 0.56, 6N HCl) [lit.[5] $[\alpha]_D$ = +18.0 (c = 2.2, 6N HCl)]. **112c.** $[\alpha]_D^{25}$ = -20.2 (c=0.47, 6N HCl). The enantiomeric purities of **112d** and **112c** were determined to be >99.5:0.5 er by chiral HPLC analysis (Daicel Chiral Pak WH, column temperature 50°C, 0.25 mM $CuSO_4$ mobile phase, Waters 600 HPLC, dual wavelength UV detection at 210 and 254 nm).



112d, (2*S*, 3*S*)



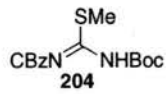
dedII-519-aq-postneut-filter-400MHz



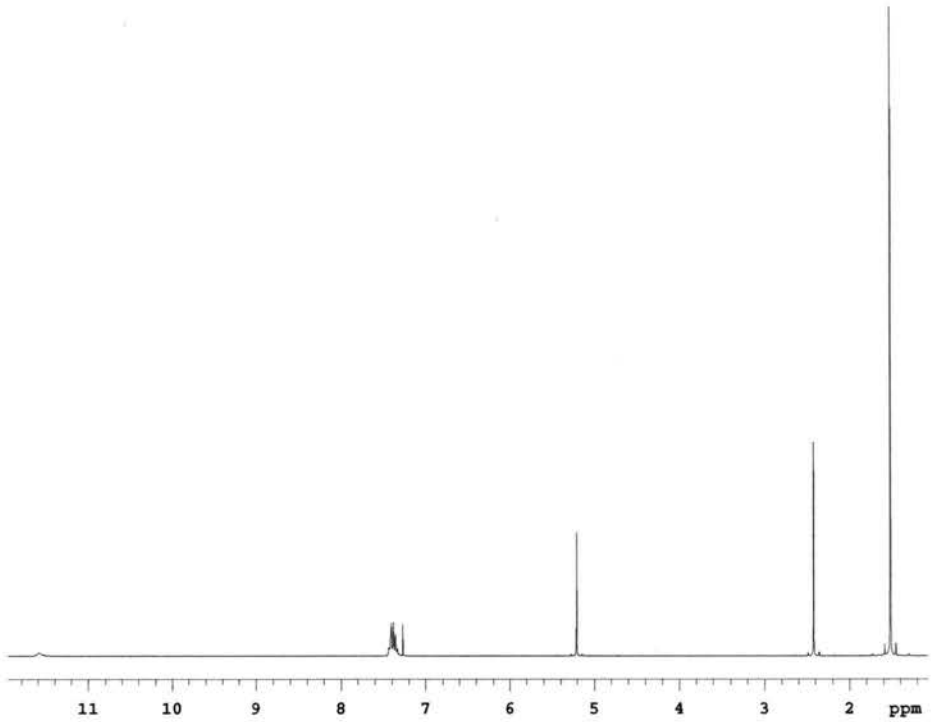
dedII-564monoHCl-100MHz-carbon

***N*-Boc-*N'*-CBz-*S*-methylisotiourea (204).** To a solution of dichloromethane (90 mL) and *N*-Cbz-*S*-methylisothiourea (**205**) (3.60 g, 16.1 mmol, 1 eq.) at 0°C, was added Boc_2O (3.50 g, 16.1 mmol, 1 eq.), followed by DMAP (2.16 g, 17.7 mmol, 1.1 eq.). This mixture was stirred until clear solution resulted. Et_3N (2.44 g, 24.2 mmol, 1.5 eq.) was then added, and the reaction stirred for one hour at 0°C. After 1 hour, the solvent was removed *in vacuo* to yield a white solid. This solid was taken up in 9:1 CH_2Cl_2:EtOAc and run through a silica gel plug under these solvent conditions to remove DMAP and other salts. The resulting pale oil was subjected to a silica gel column using 30% EtOAc in hexanes to yield compound **204** as a white solid in 86% yield (m.p. 63°C, recryst. hexanes).

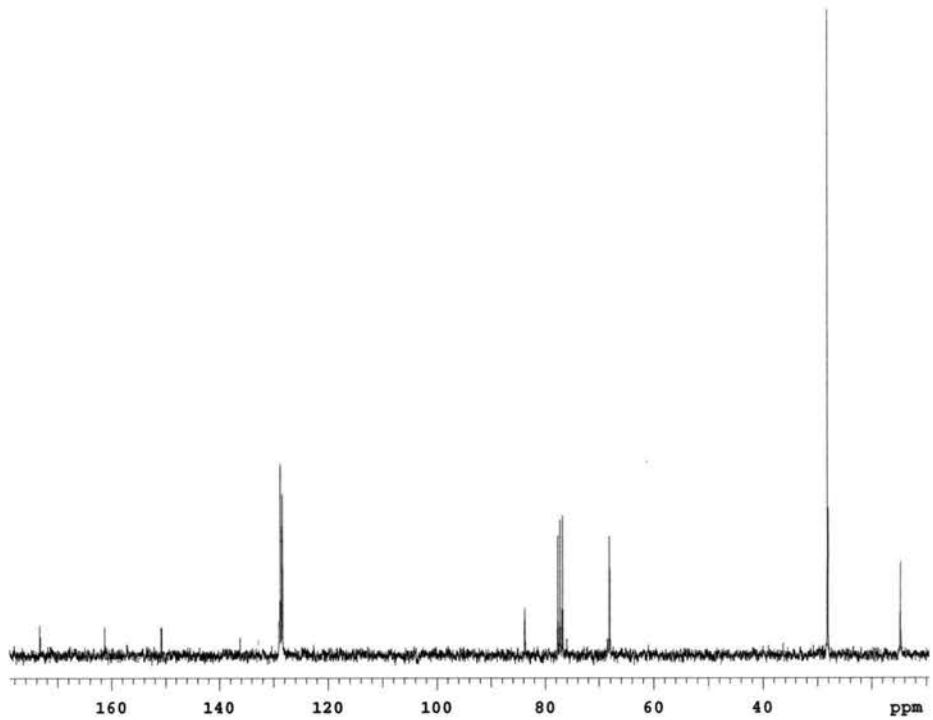
^{1}H-NMR (300 MHz) ($CDCl_3$): δ $CHCl_3$ 1.52 (9H, s); 2.42 (3H, s); 5.21 (2H, s); 7.38 (5H, m); 11.59 (1H, broad s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ $CHCl_3$: 14.7, 28.2, 68.1, 83.7, 128.4, 128.4, 128.7, 136.2, 150.7, 161.3, 173.3. IR (NaCl, depos. from CH_2Cl_2): 3168, 3034, 2980, 2931, 1750, 1651, 1568, 1498, 1455, 1417, 1370, 1315, 1238, 1146, 1065, 1025 cm^{-1}.



204



dd094



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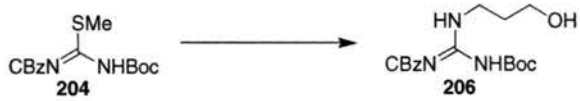
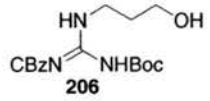
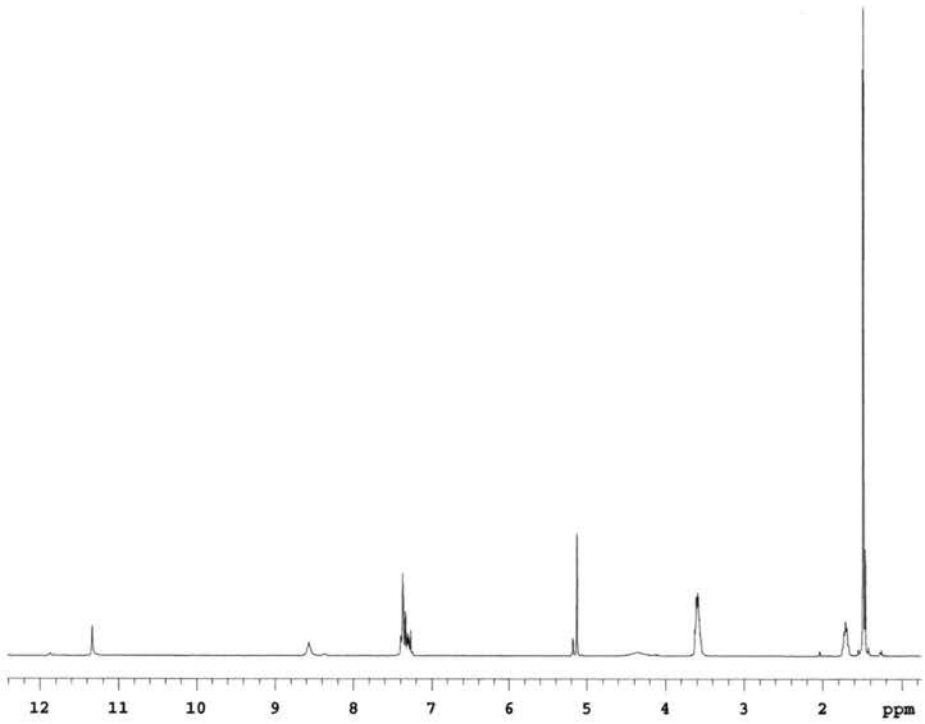


3-(*N*-Boc-*N'*-CBz-guanidino)propanol (206). To a solution of **204** (973 mg, 3 mmol, 1 eq.) in DMF (25 mL) at 0°C, 3-amino-1-propanol (338 mg, 4.5 mmol, 1.5 eq.) was added with stirring. Triethylamine (911 mg, 9 mmol, 3 eq.) was added dropwise, and the reaction was stirred 1 hour. The reaction was then poured into dichloromethane and washed with 1M HCl, sat. aq. $NaHCO_3$, and brine (4 times). The organic layer was then dried over sodium sulfate, filtered, and concentrated (in vacuo) to yield a pale oil. The crude product was separated on silica gel (4:1 CH_2Cl_2:EtOAc) to yield **206** as an oil in 95% yield.

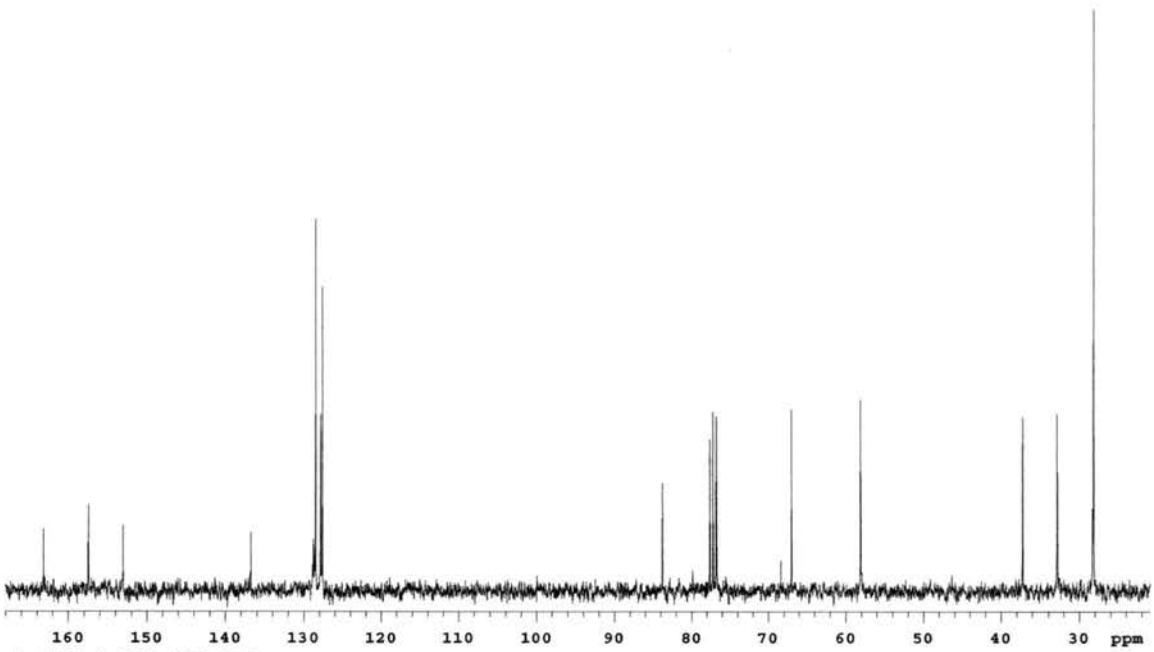
^{1}H-NMR (300 MHz) ($CDCl_3$): δ $CHCl_3$ 11.34 (1H s), 8.57 (1H broad t), 7.35 (5H m), 5.14 (2H s), 3.61 (4H m), 1.73 (2H m), 1.50 (9H s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ $CDCl_3$: 28.2, 32.8, 37.2, 58.1, 67.0, 83.8, 127.6, 127.9, 128.5, 136.8, 153.0, 157.4, 163.1. IR (NaCl film, cm^{-1}): 3325.0, 2978.4, 2938.2, 2877.8, 1724.7, 1644.2, 1619.0, 1573.8, 1498.3, 1453.0, 1417.8, 1382.6, 1272.0, 1151.3, 1136.2, 1103.0, 1050.0, 965.1.



206



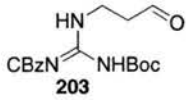
dd101



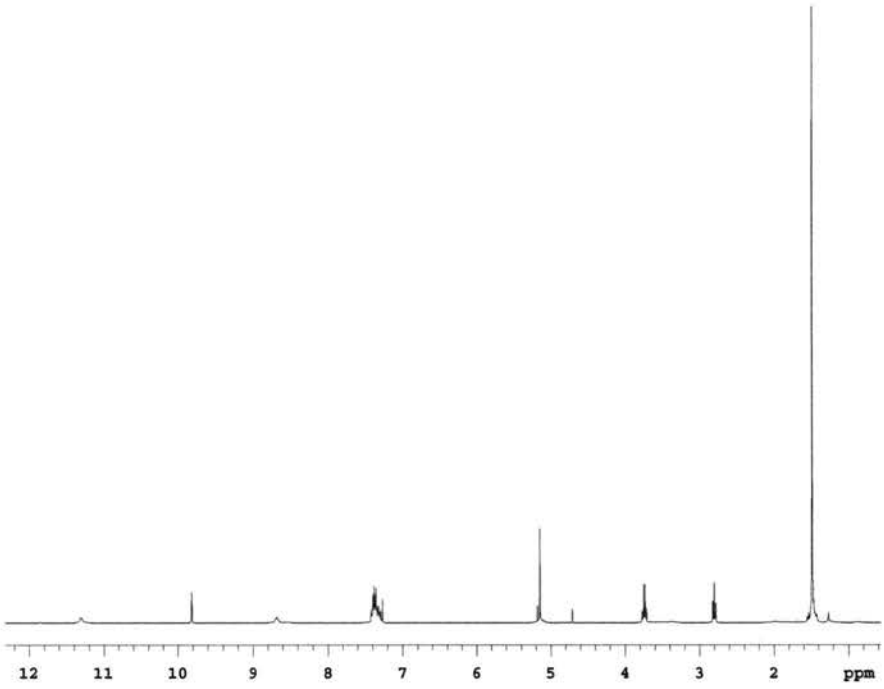
dedIII-1484-75MHz

Guanidino aldehyde 203. To a solution of **206** (317 mg, 0.90 mmol, 1 eq.) in dichloromethane (12 mL), was added Dess-Martin periodinane (650 mg, 1.53 mmol, 1.7 eq.). The resulting heterogeneous mixture was stirred for 2 hours. Reaction was then poured into a 1:1 mixture of diethyl ether and sat. aq. $NaHCO_3$. Sodium Thiosulfate (2.66 g, 10.7 mmol, 12.0 eq.) was added, and the solution stirred 30 minutes. The organic layer was removed, and the aqueous layer was extracted once with ether. The organic layers were then combined, and washed three times with sat. aq. $NaHCO_3$, and three times with water. The organic layer was evaporated *in vacuo* and a pale oil resulted. Crude product was purified on silica gel (1:1 EtOAc:Hexanes w/ 1% Et_3N), yielding 154 mg (50%) of **203** as a clear oil.

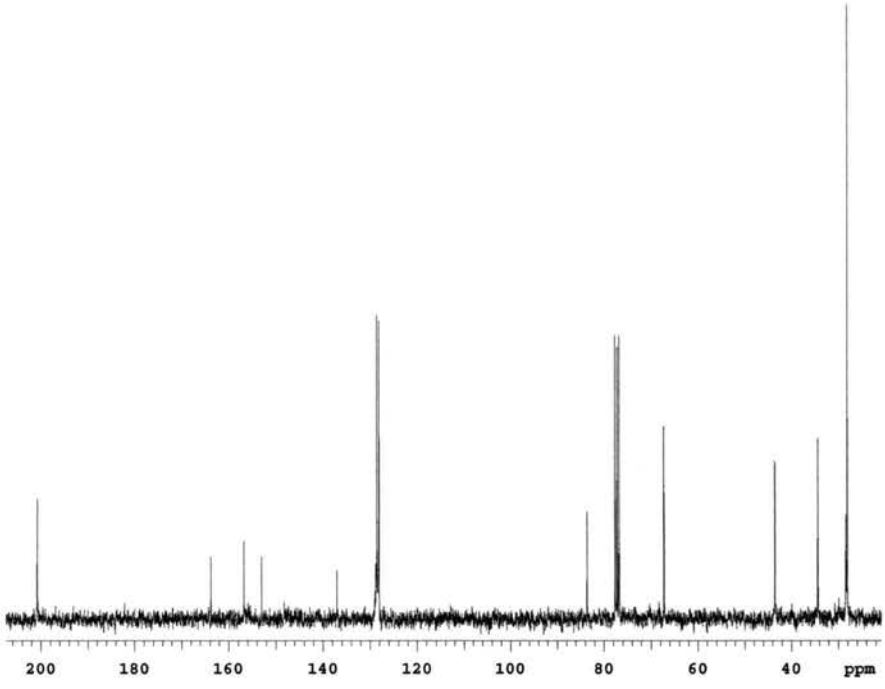
^{1}H-NMR (300 MHz, $CDCl_3$ vs $CHCl_3$): δ 11.31 (1H broad s), 9.82 (1H s), 8.68 (1H broad t), 7.35 (5H m), 5.15 (2H s), 3.74 (2H q), 2.80 (2H t), 1.49 (9H s). ^{13}C-NMR (75 MHz, $CDCl_3$ vs $CDCl_3$): δ 200.73, 163.69, 156.73, 153.03, 137.07, 128.83, 128.59, 128.12, 128.07, 83.65, 67.21, 43.57, 34.38, 28.17 ppm. IR (NaCl film, cm^{-1}): 3333.9, 3146.5, 2979.6, 2727.7, 1724.8, 1638.8, 1570.8, 1497.6, 1420.1, 1369.2, 1322.1, 1279.5, 1250.4, 1207.0, 1140.2, 1110.0, 1052.1, 1028.6.



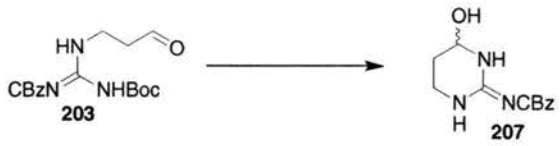
203



dd110

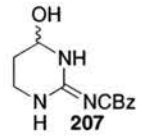


ddc013



Carbinolamine 207. Aldehyde **203** (38.9 mg, 0.11 mmol, 1 eq.) was added to a 1:1 mixture of trifluoroacetic acid and dichloromethane (10 mL) at 0°C. The solution was stirred for 2 hours. The solvent was removed *in vacuo*, leaving a yellow oil. Dichloromethane was added to the oil, and was evaporated again to remove residual TFA. This was repeated 3 more times. The resulting crude oil was subjected to a silica gel column (10% MeOH in CH_2Cl_2) yielding **207** as an off-white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ $CHCl_3$: 1.72 (1H, dddd, J=3.3, 5.1, 13.2, 13.2 Hz); 1.88 (1H, dddd, J=2.6, 2.6, 3.7, 13.2 Hz); 2.18 (1H, s); 3.28 (1H, ddd, J=2.2, 5.1, 12.8 Hz); 3.51 (1H, ddd, J=4.0, 12.5, 12.5 Hz); 5.04 (1H, t, J=2.6); 5.11 (2H, s); 7.30-7.38 (5H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ $CDCl_3$: 26.5, 34.2, 67.3, 70.9, 128.4, 128.5, 128.8, 136.3, 155.9, 160.0.



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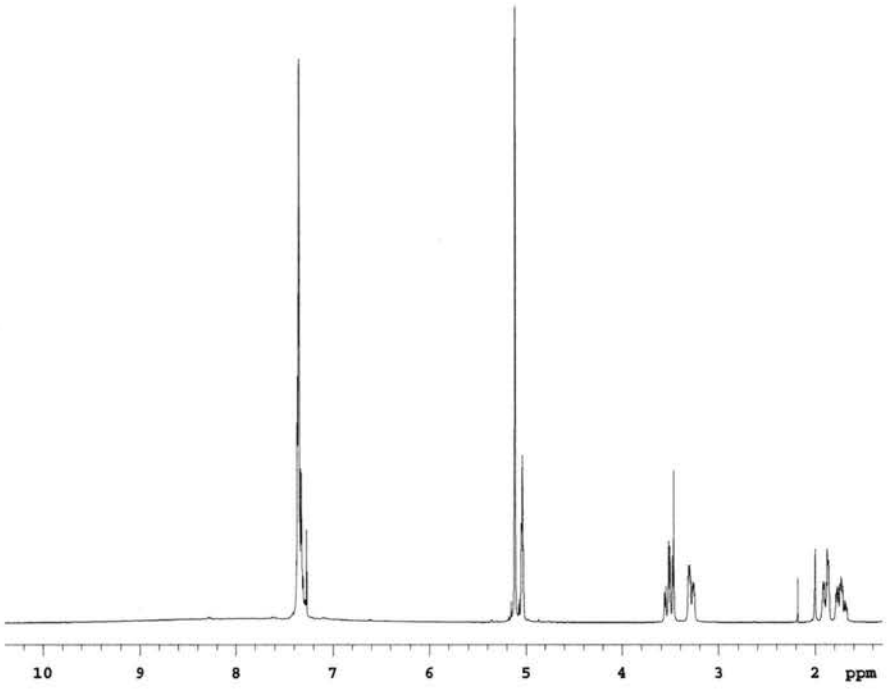
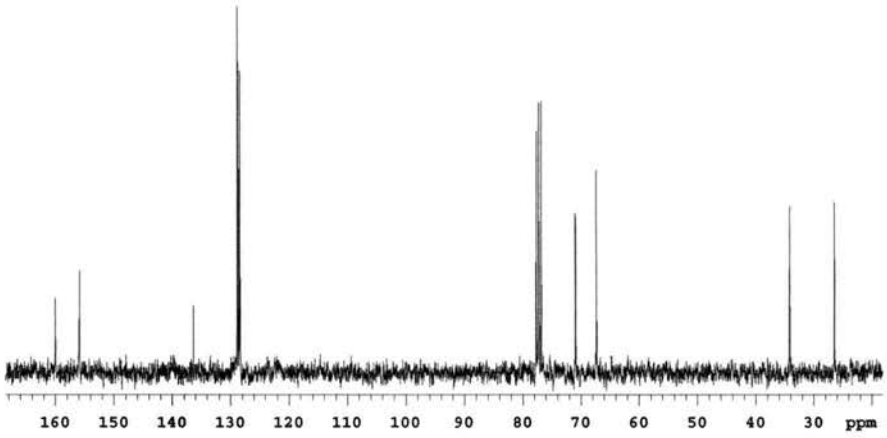


dd111



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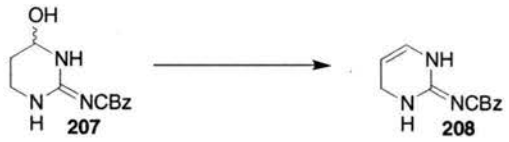
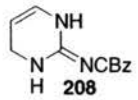
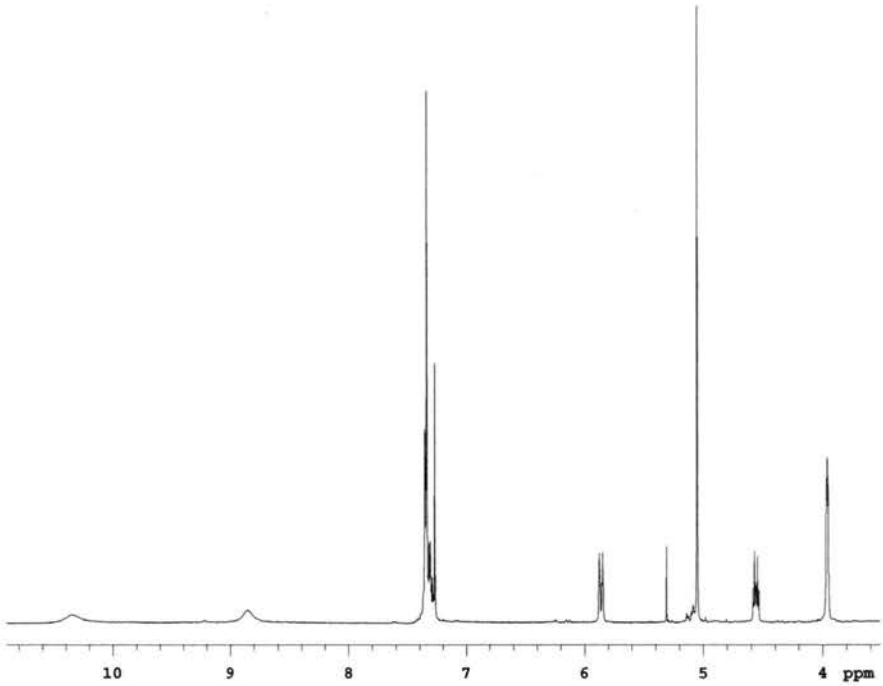


Elimination product 208. Carbinolamine **207** was taken up in benzene with molecular sieves and refluxed overnight. Reaction was cooled and filtered through Celite. The solvent was evaporated, and the resulting residue purified via PTLC (10% MeOH in CH_2Cl_2) to provide **208** as a pure white solid.

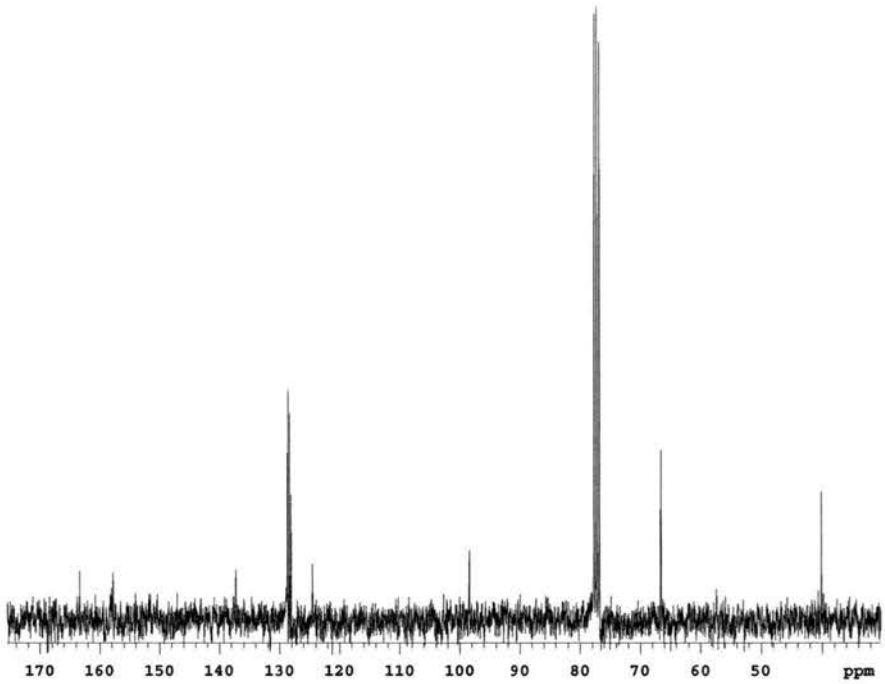
^{1}H-NMR (300 MHz, $CDCl_3$): δ $CHCl_3$: 3.96 (2H, dd, J=1.8, 3.3 Hz); 4.55 (1H, ddd, J=3.3, 3.3, 8.1 Hz); 5.05 (2H, s); 5.86 (1H, ddd, J=1.8, 1.8, 8.1 Hz); 7.27-7.37 (5H, m); 8.85 (1H, broad s); 10.34 (1H, broad s). ^{13}C-NMR (75 MHz, $CDCl_3$): δ $CDCl_3$: 40.1, 66.6, 98.4, 124.5, 128.1, 128.3, 128.7, 137.3, 157.8, 163.3.



208



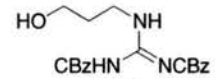
dd115



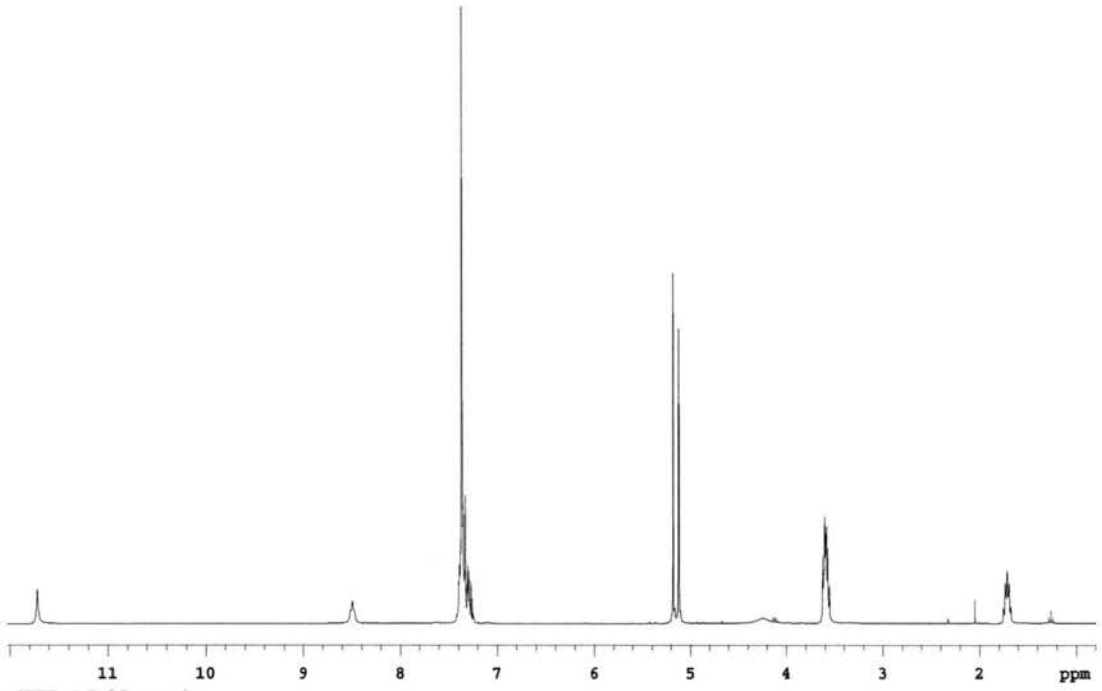
ddc016

Guanidino alcohol 212. A solution of **211** (799 mg, 2.23 mmol, 1 eq) and 3-amino-1-propanol (246 mg, 3.35 mmol, 1.5 eq) in DMF (18 mL) was prepared with stirring under argon. Triethylamine (663 mg, 6.69 mmol, 3 eq) was then added dropwise to the reaction mixture and stirred overnight. The mixture was diluted with methylene chloride and washed with 1M HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated to provide 705 mg (82%) of **212** as an analytically pure oil. If further purification was necessary, flash chromatography (silica gel, 3:1 hexanes:EtOAc) was used.

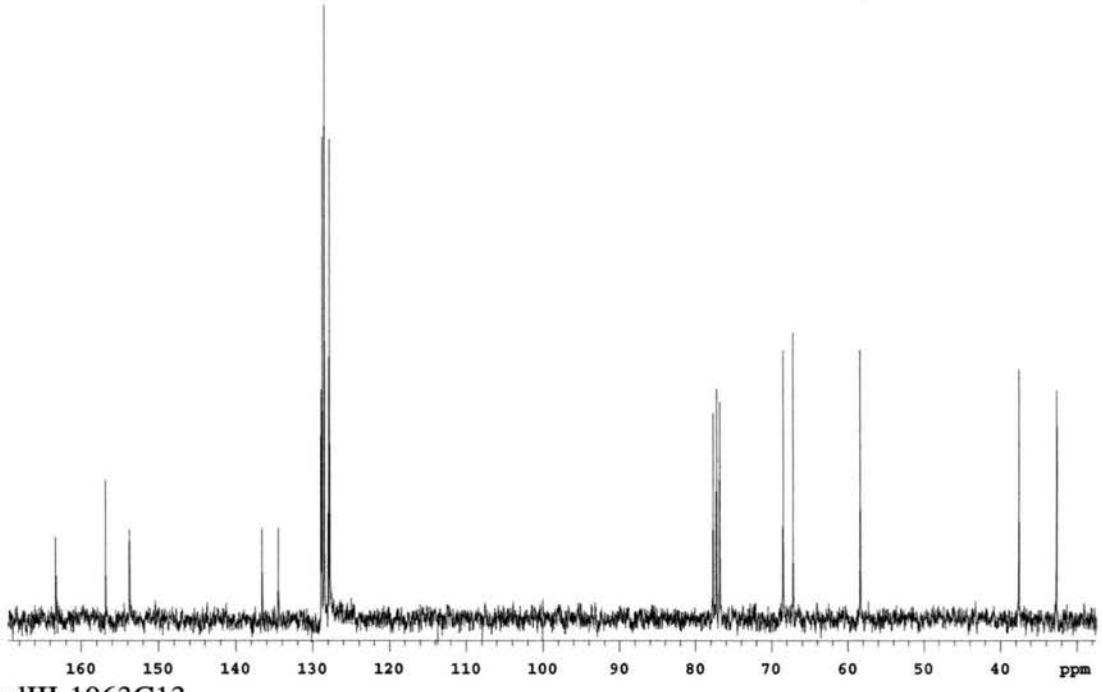
^{1}H NMR (300 MHz) ($CDCl_3$) δ $CHCl_3$: 1.71 (2H, quintet, J=5.9 Hz); 3.55-3.62 (4H, m); 5.12 (2H, s); 5.18 (2H, s); 7.24-7.42 (5H, m); 8.49 (1H, broad t, J=5.9); 11.72 (1H, s). ^{13}C NMR (75 MHz) ($CDCl_3$): δ $CDCl_3$ 32.6, 37.6, 58.3, 67.1, 68.5, 127.8, 127.9, 128.5, 128.5, 128.8, 128.9, 134.5, 136.6, 153.7, 156.9, 163.2.



212



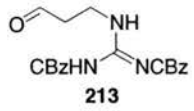
dedIII-1063crude



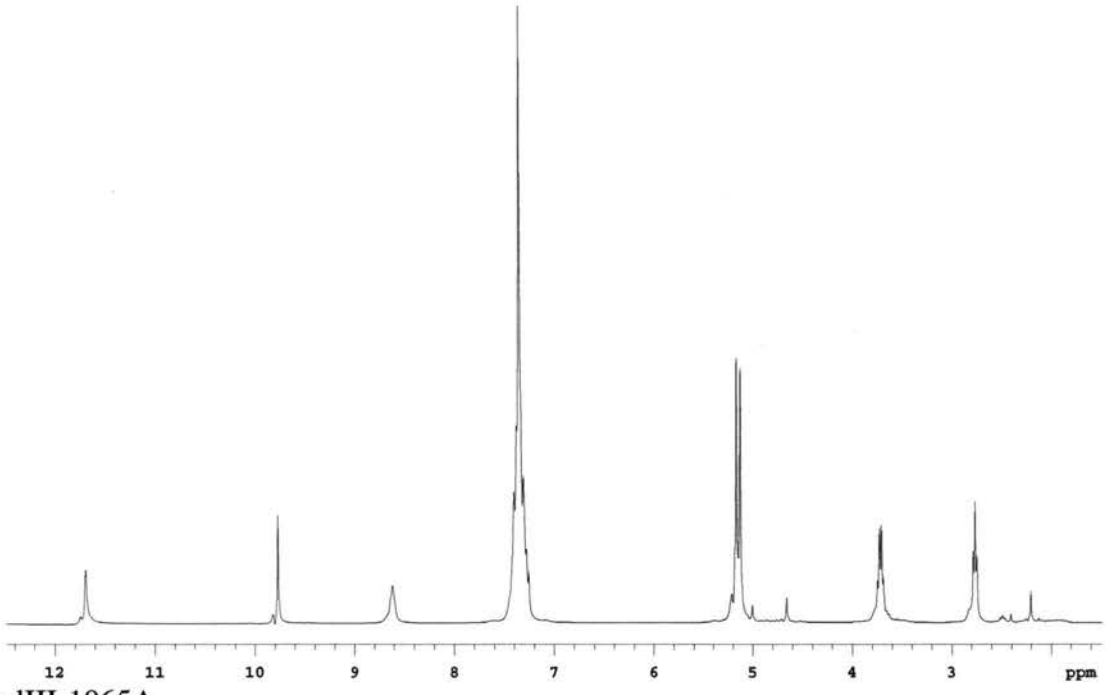
dedIII-1063C13

Guanidino aldehyde 213. Guanidino alcohol **212** (1.04 g, 2.70 mmol, 1 eq.) was added to dichloromethane (40 mL), and the solution stirred until starting material was dissolved. Dess-Martin Periodinane (1.95 g, 4.59 mmol, 1.7 eq.) was added, and the resulting heterogeneous solution stirred for 3 hours. The reaction was then poured into a 1:1 mixture of diethyl ether and sat. aq. $NaHCO_3$. Sodium thiosulfate (7.97 g, 32.1 mmol, 11.9 eq.) was added, and the solution stirred for 20 minutes. The organic layer was removed, and the aqueous layer was extracted once with ether. The organic layers were then combined, and washed three times with sat. aq. $NaHCO_3$, and three times with water. The organic layer was evaporated *in vacuo* and a pale oil resulted. The crude product was purified on silica gel (1:1 EtOAc:Hexanes w/ 1% triethylamine), yielding 560 mg of **213** as a clear oil in 54% yield.

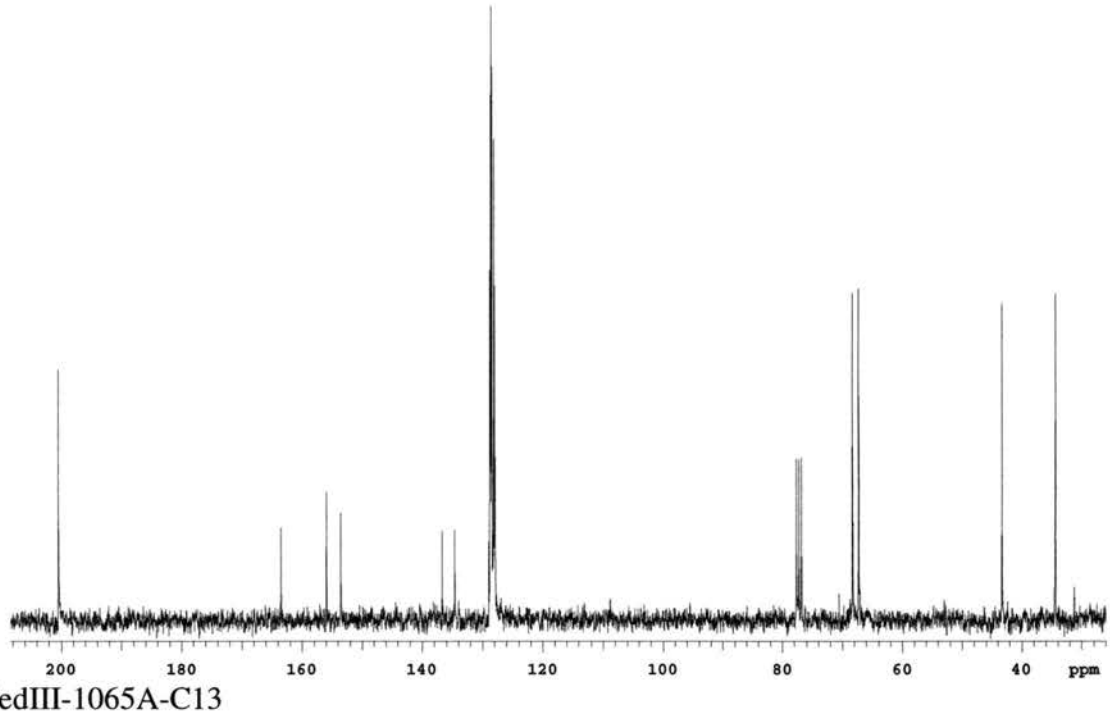
^1H NMR (300 MHz) ($CDCl_3$) δ $CHCl_3$: 2.76 (2H, t, J=5.9Hz); 3.72 (2H, q, J=6.2Hz); 5.13 (2H, s); 5.16 (2H, s); 7.26-7.50 (5H, m); 8.62 (1H, broad t); 9.77 (1H, s); 11.69 (1H, s). ^{13}C NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 34.4, 43.3, 67.3, 68.3, 127.9, 128.1, 128.4, 128.5, 128.6, 128.8, 128.9, 134.6, 136.7, 153.6, 155.9, 163.5, 200.4.



213



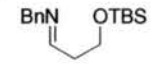
dedIII-1065A



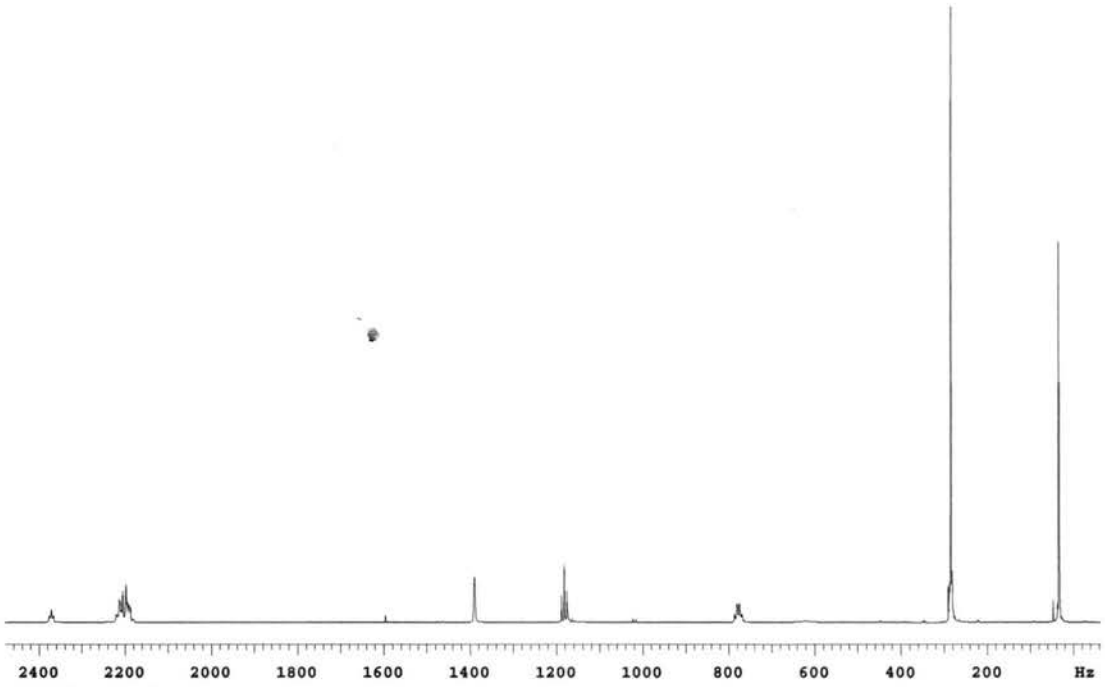
dedIII-1065A-C13

3-(*tert*-Butyldimethylsiloxy)-*N*-benzyl-propanaldimine (217). To benzylamine (209 mg, 1.95 mmol, 1 eq.) and alumina (1.3 g), was added a solution of aldehyde **215** (405 mg, 2.15 mmol, 1.1 eq.) in CH_2Cl_2 (8.1 mL) at 0°C. The heterogeneous mixture was stirred for 25 min. at 0°C then filtered and evaporated *in vacuo*. The product (**217**), isolated as a pale oil (540 mg, 99%), was used crude for the next step.

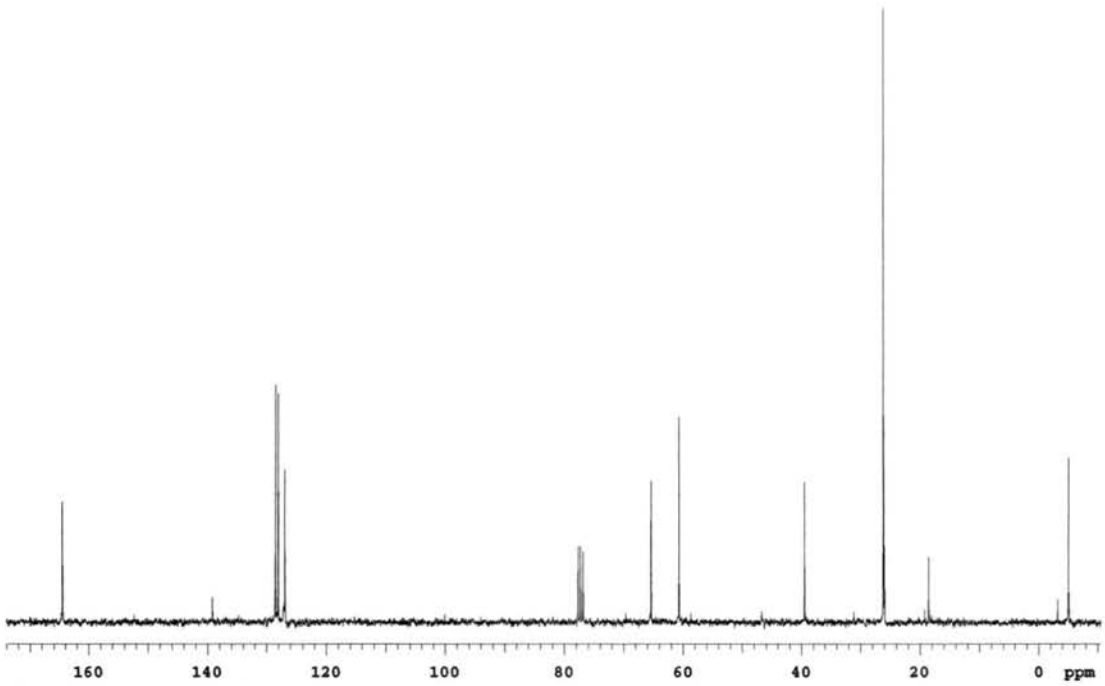
^{1}H NMR (300 MHz) ($CDCl_3$) δ $CHCl_3$: 0.11 (6H, s); 0.95 (9H, s); 2.59 (2H, q, J=6.2 Hz); 3.94 (2H, t, J=6.2Hz); 4.63 (2H, s); 7.26-7.40 (5H, m); 7.90 (1H, t, J=4.8Hz). ^{13}C NMR (75 MHz) ($CDCl_3$) δ $CDCl_3$: -5.0, 18.5, 26.1, 39.4, 60.7, 65.4, 127.0, 128.0, 128.5, 139.2, 164.5. IR (NaCl, neat) 3087, 3064, 3029, 2955, 2928, 2885, 2856, 1669, 1495, 1472, 1463, 1387, 1255, 1100, 837 cm^{-1}.



217

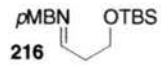


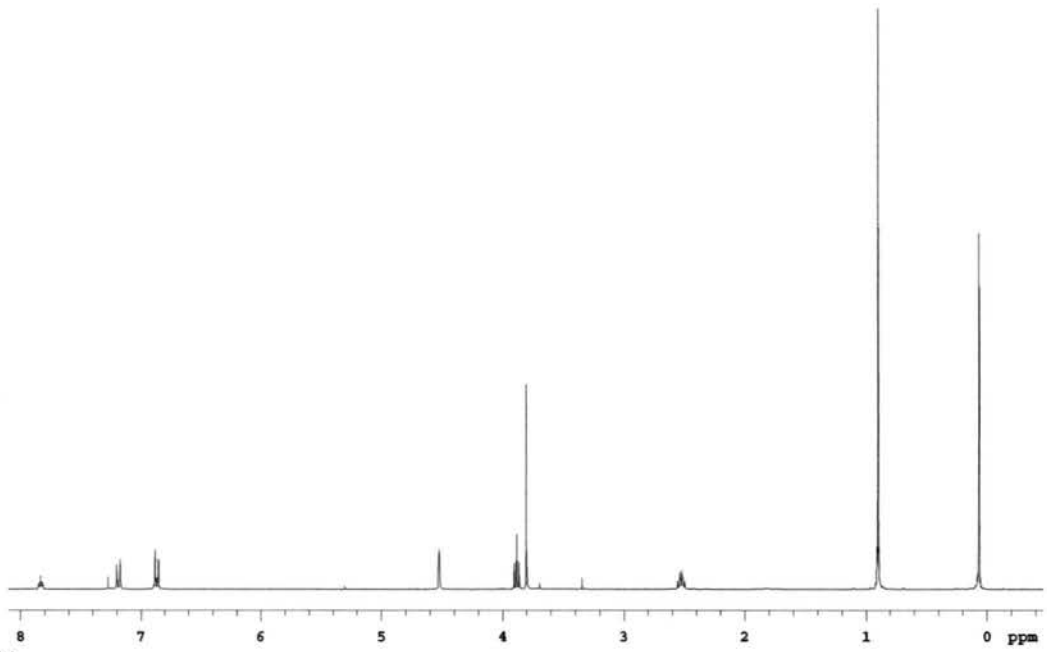
dedII-659crude



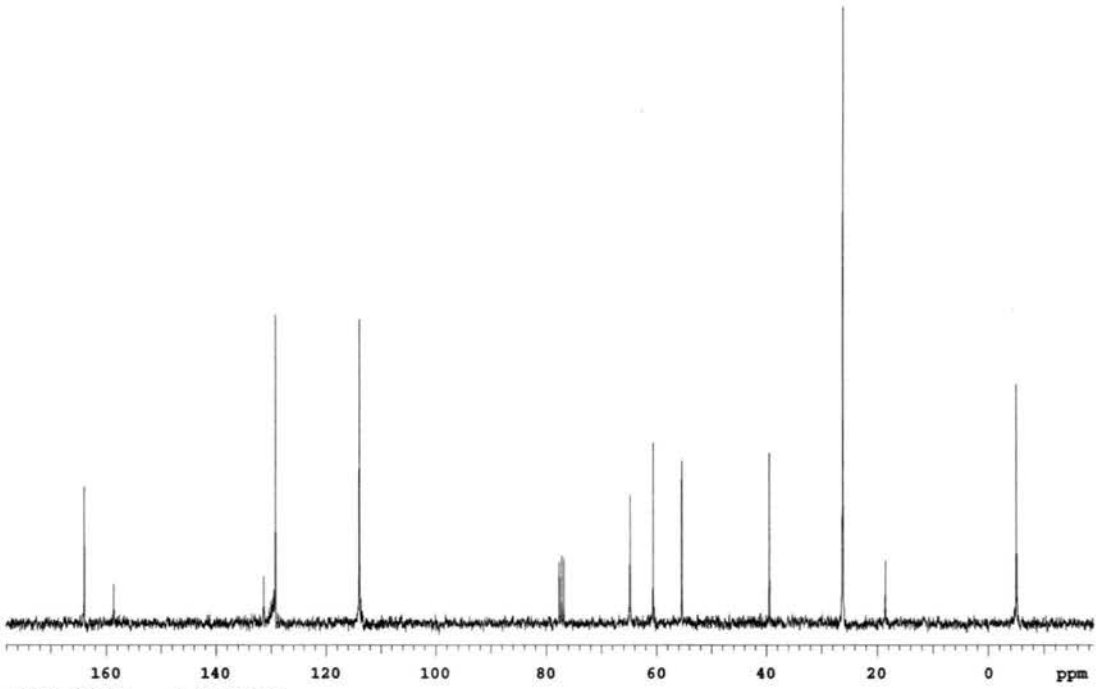
dedII-659crude-75Mhzcarbon

To *p*-methoxybenzylamine (427 mg, 3.11 mmol, 1 eq.) and alumina (2.1 g), was added a solution of aldehyde **215** (645 mg, 3.42 mmol, 1.1 eq.) in CH_2Cl_2 (13 mL) at 0°C. The heterogeneous mixture was stirred for 25 min. at 0°C then filtered and evaporated *in vacuo* to provide 867 mg (91%) of **216** as a pale oil which was used crude in the next step. ^{1}H NMR (300 MHz) ($CDCl_3$) δ $CHCl_3$: 0.69 (6H, s); 0.90 (9H, s); 2.53 (2H, q, J=6.2 Hz); 3.80 (3H, s); 3.88 (2H, t, J=6.6 Hz); 4.53 (2H, s); 6.87 (2H, d, J=8.8 Hz); 7.18 (2H, d, J=8.8Hz); 7.83 (1H, ddd, J=1.1, 1.1, 4.8, 4.8Hz) ^{13}C NMR (75 MHz) ($CDCl_3$) δ $CDCl_3$: -5.0, 18.5, 26.1, 39.4, 55.4, 60.7, 64.8, 113.9, 129.2, 131.3, 158.6, 163.9. IR (NaCl film, cm^{-1}): 2954, 2928, 2856, 1667, 1612, 1585, 1512, 1463, 1441, 1387, 1361, 1301, 1247, 1174, 1098, 1037, 1006. nOe data: irradiation of H1 enhanced H2,2' (3.29%)

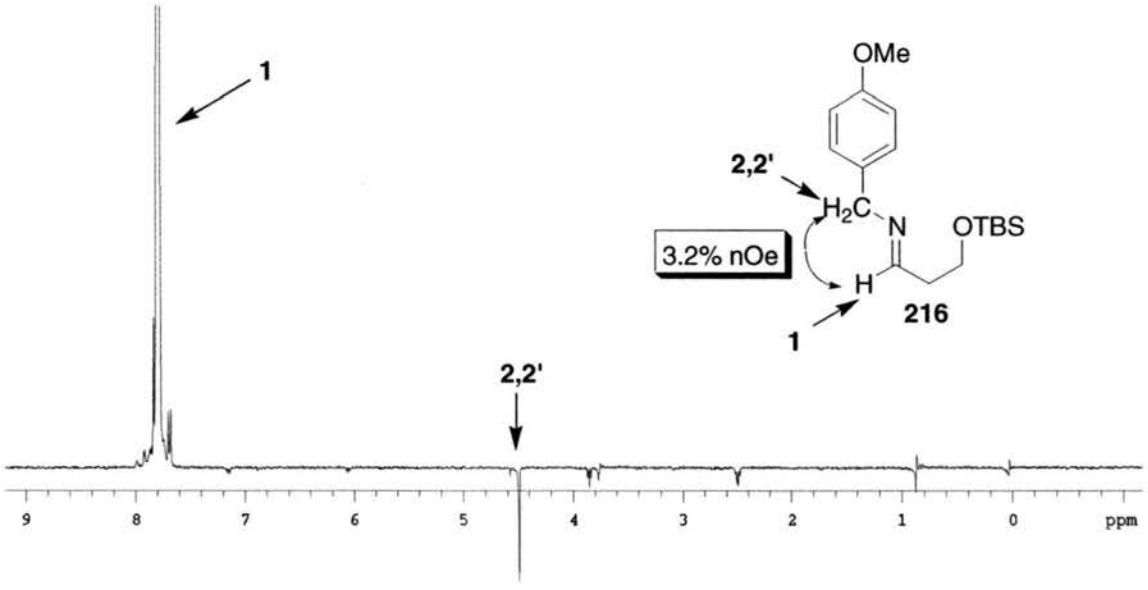




dd421



dedIII-1511crude75MHz



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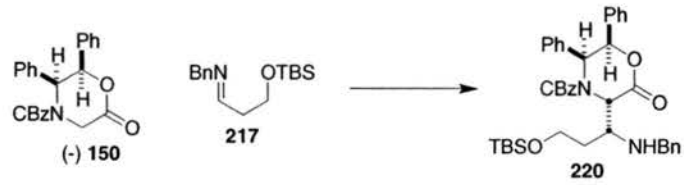
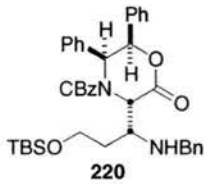


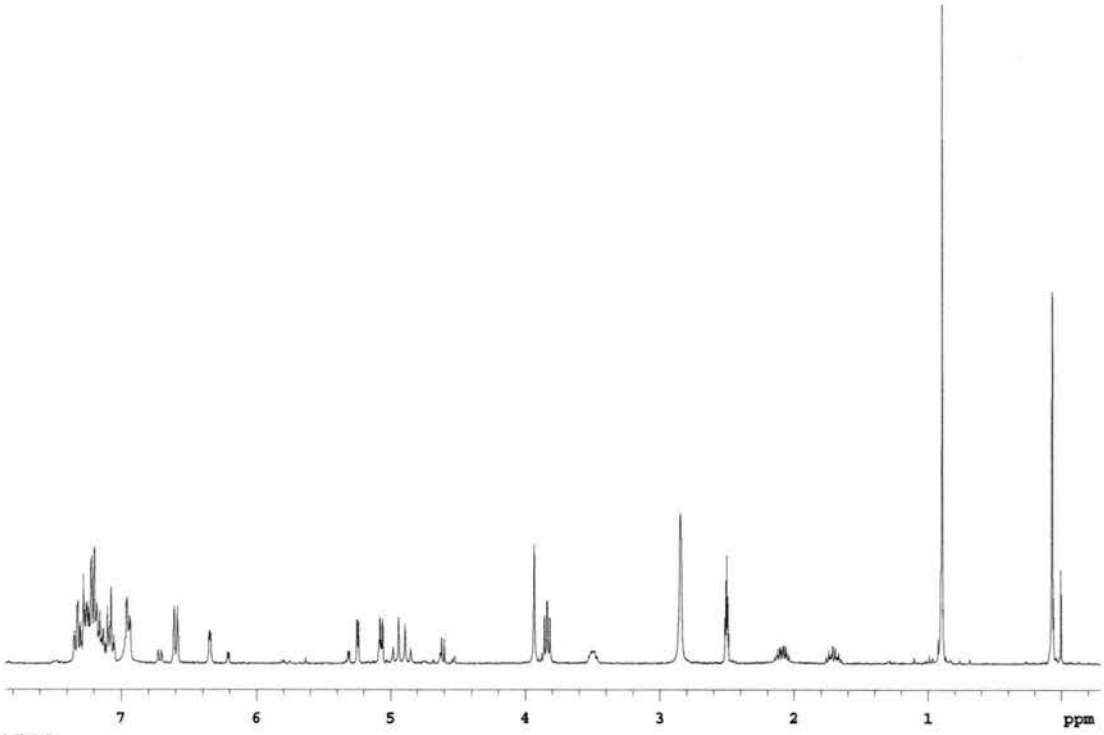
Mannich product (220). Under argon atmosphere, compound (-) **150** (755 mg, 1.95 mmol, 1 eq.) was dissolved in dry tetrahydrofuran (42 mL). The resulting solution was cooled to -78°C. LHMDS (3.3 mL, 2.0 mmol, 1.03 eq., 0.61 M in THF) was added, and the reaction stirred 15 min. Dimethylaluminum chloride (2.0 mL, 2.0 mmol, 1.03 eq., 1M in hexanes) was added dropwise, and the mixture stirred 15 min. In a separate flask, compound **217** was dissolved in 4 mL dry THF under argon atmosphere, and added *via* canula to the awaiting aluminum enolate. The reaction was stirred 1 hour at -78°C. Saturated aqueous sodium bicarbonate was added and the quenched reaction was warmed to room temp. After filtering the resulting suspension through Celite, the mother liquor was extracted 3x with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated *in vacuo*. This resulted in a pale orange oil that was purified by silica gel chromatography (eluted with 9:1 hexanes:ethyl acetate) to yield 659 mg (51%) of **220** as an inseparable 3.3:1 mixture of diastereomers (^{1}H-NMR). Both diastereomers were taken on to the next step.

^{1}H NMR (300 MHz) (DMSO-d_6, 393K) (major diastereomer) δ DMSO: 0.07 (6H, s); 0.90 (9H, s); 1.70 (1H, dddd, J=14.3, 9.2, 5.9, 5.9 Hz); 2.09 (1H, dddd, J=11.0, 6.6, 4.4, 4.4 Hz); 3.50(1H, sym. M); 3.83 (1H, ½ ABq, J=5.5 Hz); 3.85 (1H, ½ ABq, J=5.5 Hz); 3.93 (2H, s); 4.88 (1H, ½ ABq, J=12.5 Hz); 4.96 (1H, ½ ABq, J=12.5 Hz); 5.07 (1H, d, J=5.9 Hz); 5.25 (1H, d, J=3.3 Hz); 6.35 (1H, d, J=3.3 Hz); 6.60 (2H, d, J=7.0 Hz); 6.93-

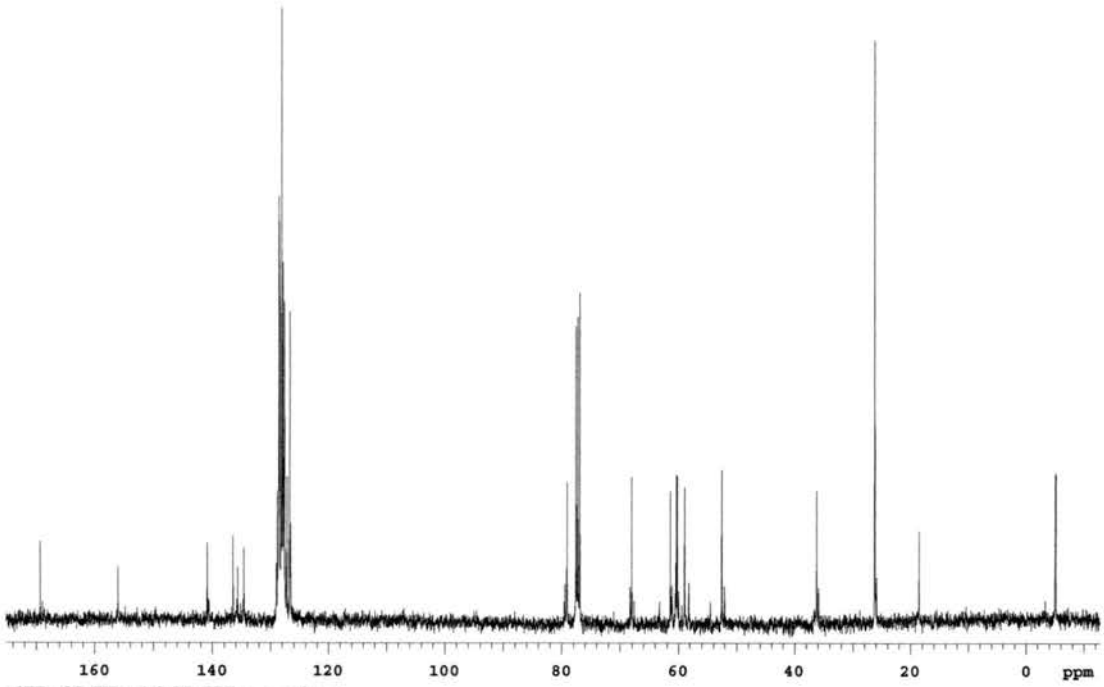
7.35 (18H, m). ^{13}C NMR (100 MHz) ($CDCl_3$) (major diastereomer) δ -5.1, 18.5, 26.2, 36.2, 52.5, 58.9, 60.2, 60.3, 61.4, 68.0, 79.1, 126.6, 126.7, 127.2, 127.5, 127.6, 127.8, 127.9, 127.9, 128.1, 128.1, 128.4, 128.6, 128.6, 128.8, 129.0, 134.6, 135.6, 136.5, 140.9, 156.0, 169.3. IR (NaCl, deposited from CH_2Cl_2) 3032, 2928, 2855, 1752, 1708, 1497, 1454, 1398, 1250, 1102, 836, 775 cm^{-1}. HRMS (FAB+) calc. for $C_{40}H_{49}N_2O_5Si$ (MH^+) 665.3411; found 665.3417.



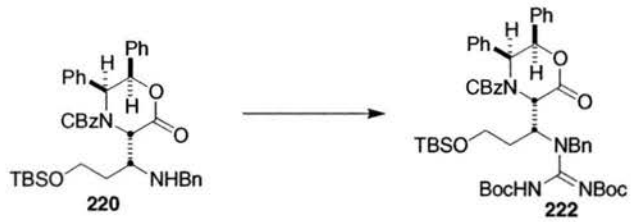
220



dd518



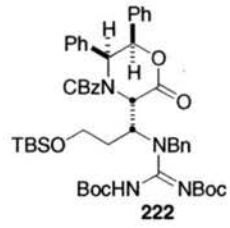
dedII-625B-100MHz-carbon



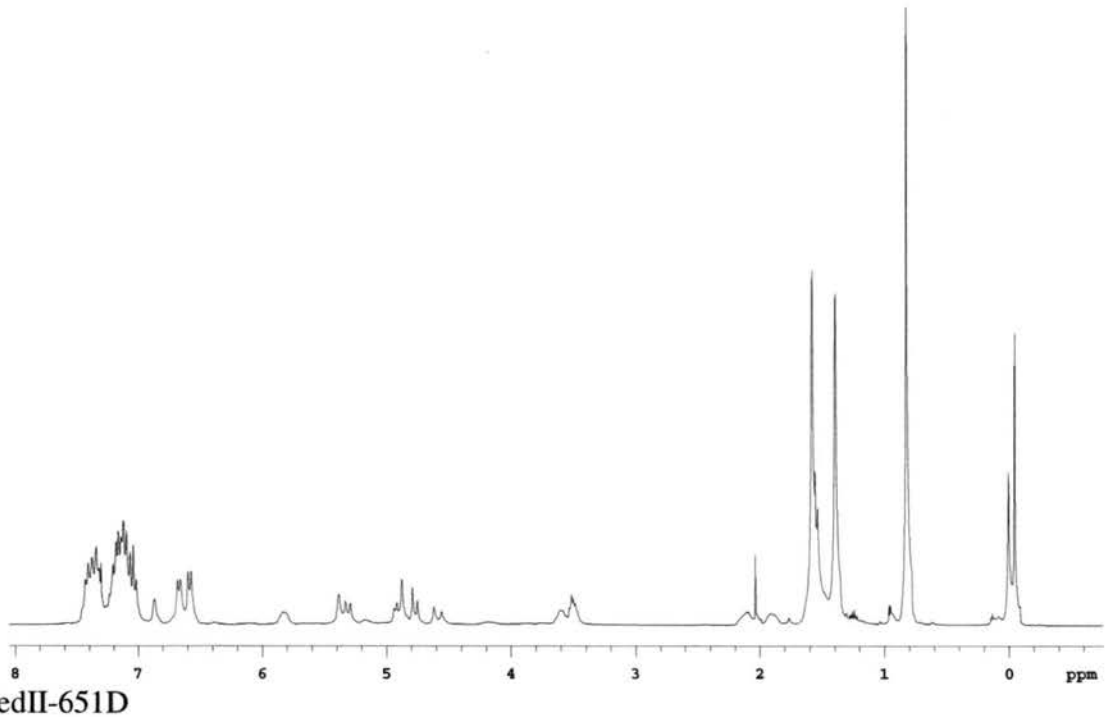
Guanidine 222. To a solution of **220** (510 mg, 0.77 mmol., 1 eq.) and *N,N'*-bis-*tert*-butoxycarbonyl-*S*-methylisothiourea (290 mg, 1.00 mmol., 1.3 eq.) under an argon atmosphere in DMF (3.9 mL), was added triethylamine (322 mL, 2.31 mmol., 3.0 eq.). After dissolution, silver triflate (277 mg, 1.08 mmol., 1.4 eq.) was added and the heterogeneous reaction stirred 3h. Dilution of the reaction with ethyl acetate was followed by filtration through celite to remove any solids. The resulting organic layer was washed twice with brine and dried over anhydrous sodium sulfate. Filtration, followed by removal of the solvent under reduced pressure resulted in an orange oil that was subjected to silica gel chromatography (eluted with 7:1 hexanes:ethyl acetate), providing 518 mg (74%) of **222** as a white foam.

^{1}H NMR (300 MHz) ($CDCl_3$) (unresolvable rotamers) spectrum appears as follows: δ $CHCl_3$: -0.04 and 0.01 (6H, s); 0.83 (9H, s); 1.40 (9H, s); 1.58 (9H, s); 1.81-1.98 (1H, broad m); 2.04-2.22 (1H, broad m); 3.41-3.55 (1H, m); 3.55-3.68 (1H, m); 4.59 (1H, broad d, J=18.7 Hz); 4.77 (1H, broad d, J=12.1 Hz); 4.83-4.99 (2H, m); 5.31 (1H, broad d, J=11.0 Hz); 5.38 (1H, broad s); 5.74-5.91 (1H, broad m); 6.58 (2H, broad d, J=7.3 Hz); 6.67 (2H, broad d, J=7.0 Hz); 6.87 (1H, broad s); 7.02-7.48 (16H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ -5.2, 18.3, 26.1, 28.3, 28.6, 32.1, 49.0, 54.8, 59.0, 59.7, 62.3, 68.0, 78.7, 79.2, 81.7, 125.9, 127.1, 127.2, 127.5, 127.7, 128.0, 128.1, 129.2, 134.6, 135.8, 137.3, 149.8,

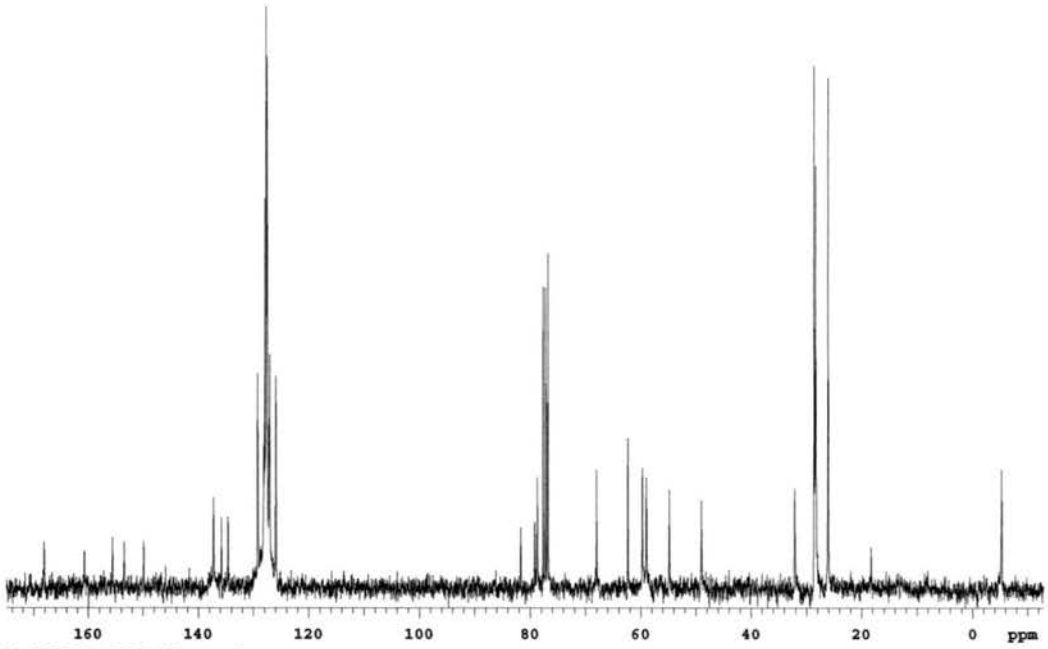
153.4, 155.5, 160.7, 168.1. IR (NaCl, neat) 3403, 3064, 3033, 2955, 2930, 1760, 1701, 1600, 1489, 1455, 1393, 1367, 1297, 1252, 1146, 1123, 837 cm^{-1}. HRMS (FAB) calc. for $C_{51}H_{67}N_4O_9Si$ (MH^+) 907.4677; found 907.4671. $[\alpha]_D^{25}$=+15.6 (*c*=0.55, CH_2Cl_2).



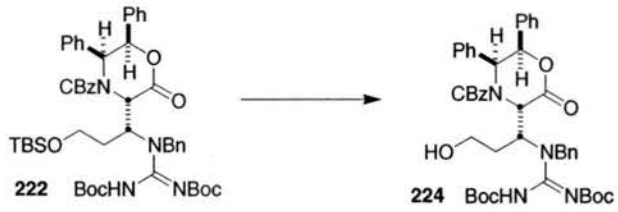
222



dedII-651D

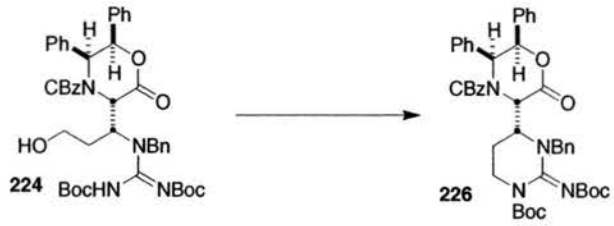


dedII-651D-75Mhzcarbon



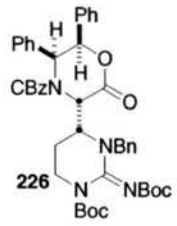
Alcohol 224. Compound **222** (198 mg, 0.22 mmol., 1 eq.) was dissolved in acetonitrile (14.5 mL). Upon addition of 5% aqueous HF in acetonitrile (7.3 mL) the mixture was stirred until the starting material was consumed (2.5 h.). At that point, solid sodium bicarbonate was added and the mixture stirred until the bubbling ceased (30 min.). After filtration to remove the remaining bicarbonate and evaporation of the solvent under reduced pressure, the crude product was subjected to silica gel chromatography (Whatman brand silica gel (230-400 mesh), eluted with 4:1 dichloromethane:ethyl acetate). This resulted in the isolation of 141 mg (81%) of **224** as a pale foam. It was necessary to use **224** immediately for the next step as it is unstable.

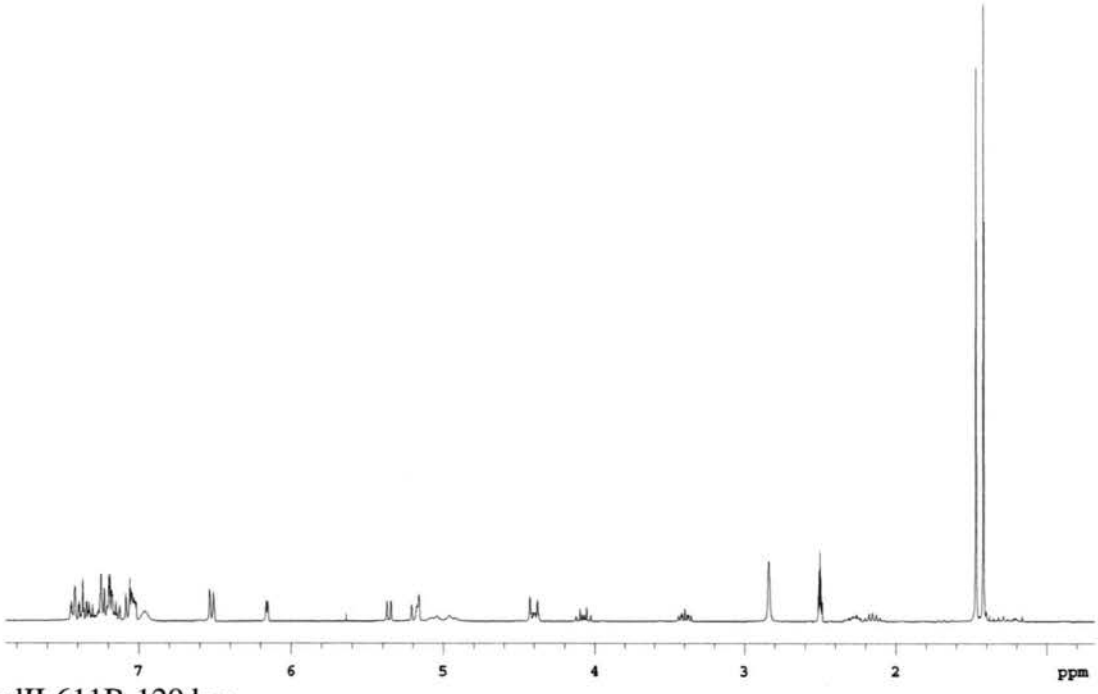
^{1}H NMR (unresolvable rotamers). ^{13}C NMR (unresolvable rotamers). IR (NaCl, neat) 3393, 3064, 3032, 2978, 1757, 1700, 1653, 1635, 1601, 1497, 1394, 1287, 1147 cm^{-1}. HRMS (FAB) calc. for $C_{45}H_{53}N_4O_9$ (MH^+) 793.3812; found 793.3807.



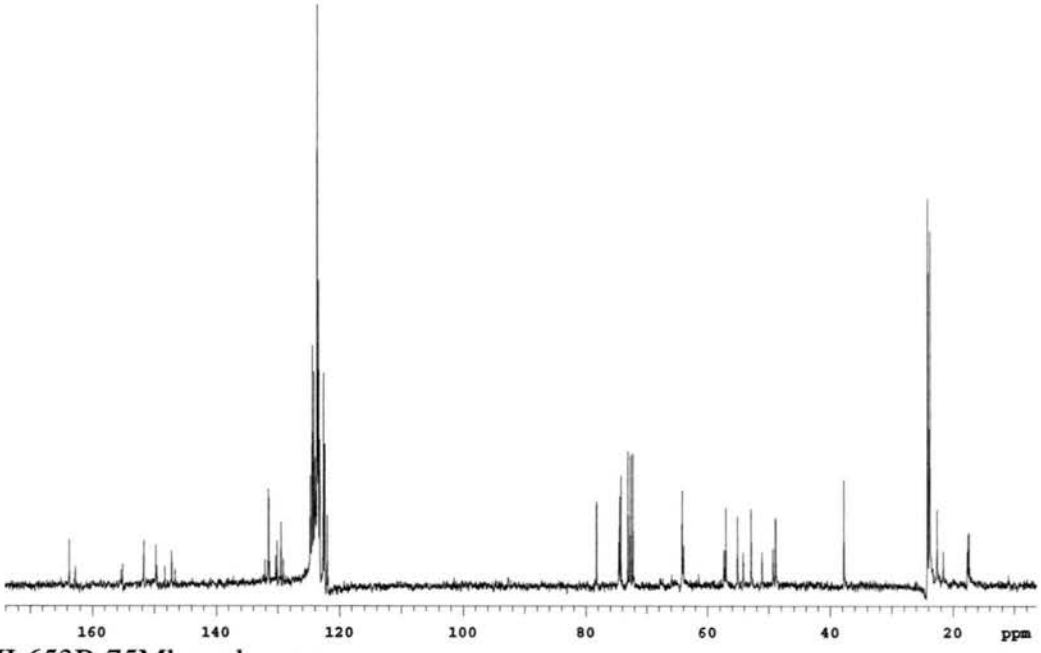
Cyclic guanidine 226. Diisopropylazodicarboxylate (45 mL, 0.23 mmol., 1.5 eq.) was added *via* syringe to a solution of **224** (122 mg, 0.15 mmol., 1 eq.) and triphenylphosphine (60 mg, 0.23 mmol., 1.5 eq.) in 5.3 mL THF at 0°C under argon atmosphere. After stirring 10 minutes at 0°C, the reaction was allowed to warm to room temperature and stirred 30 min. The THF was removed *in vacuo*, and the crude oil subjected to silica gel chromatography (eluted with 88:12 dichloromethane:ether). This purification provided 102 mg (88%) of **226** as a white amorphous solid. M.p. 199°C (recryst. *i*-PrOH/water).

^{1}H NMR (300 MHz) (DMSO-d_6, 393K) δ DMSO: 1.42 (9H, s); 1.47 (9H, s); 2.08-2.34 (2H, m); 3.40 (1H, ddd, *J*=12.8, 7.7, 5.1 Hz); 4.08 (1H, ddd, *J*=12.8, 8.4, 8.4 Hz); 4.40 (1H, m); 4.41 (1H, ½ ABq, *J*=14.7 Hz); 4.92-5.09 (2H, sym. M); 5.17 (1H, d, *J*=3.3 Hz); 5.18 (1H, ½ ABq, *J*=14.7 Hz); 5.36 (1H, d, *J*=8.1 Hz); 6.17 (1H, d, *J*=3.3 Hz); 6.52 (2H, d, *J*=7.3 Hz); 6.96-7.45 (18H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) (unresolvable rotamers, resonances are reported as observed at 298 K, * denotes minor rotamer) δ $CDCl_3$:21.9, 22.2, 26.2, 27.2, 28.4, 28.8, 42.4, 53.5, *53.9, *55.7, 57.4, *58.7, 59.7, 61.6, *61.9, 68.7, 78.8, 79.0, 82.8, 126.5, 127.2, 127.8, 128.2, 128.7, 129.0, 129.3, *133.7, 134.1, 134.7, 136.0, *136.6, *151.2, 151.8, *152.8, 154.3, 156.3, 159.6, *159.9, *167.3, 168.3. IR (NaCl, neat): 2976, 1751, 1734, 1717, 1701, 1684, 1676, 1616, 1576, 1456, 1394, 1314, 1247, 1139 cm^{-1}. HRMS (FAB) calc. for $C_{45}H_{51}N_4O_8$ (MH^+) 775.3707; found 775.3707. $[\alpha]_D^{25}$=+16.7 (*c*=0.55, CH_2Cl_2).

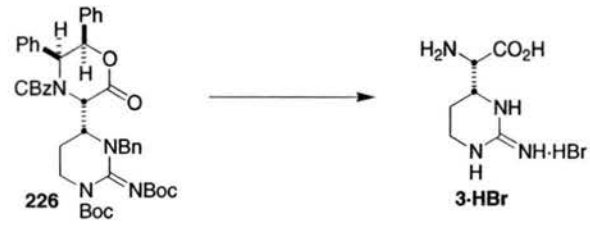




dedII-611B-120deg



dedII-653B-75Mhzcarbon-ne



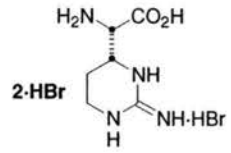
Capreomycidine·HCl (3·HBr). A solution of **226** (215 mg, 0.28 mmol., 1 eq) in 3:1 THF:EtOH (32.4 mL) was purged with argon for 5 min. Palladium chloride (146 mg, 0.83 mmol., 3 eq.) was added to the solution in a pressure tube. The tube was then pressurized and evacuated five times with hydrogen gas. Pressurization of the tube to 100 psi with hydrogen gas was followed by stirring of the reaction for 4 days at room temperature. Release of the hydrogen pressure was followed by purging with argon. The solution was filtered through Celite and evaporated *in vacuo*. The off-white residue was triturated with ether and dried under vacuum. This residue was then taken up in 2.5 mL 0.5 M HCl (prepared with dd H_2O) and refluxed for 1.5 h. Lyophilization of this reaction mixture provided crude (2*S*,3*R*)-capreomycidine **3** as its dihydrochloride salt. Dissolution of the crude product in MeOH was followed by addition of pyridine to ~pH 5. Addition of absolute EtOH resulted in the precipitation of 28 mg (48%) capreomycidine monohydrochloride as a white amorphous solid. The synthetic **3** agreed with the natural product by spectroscopic methods and molar optical rotation (synthetic: $[M]_D^{20}$=+28.2, natural: $[M]_D^{20}$=+32.5). Molar optical rotation is defined as $[M]_D^{20}=[\alpha]_D^{20}$ x MW / 100.

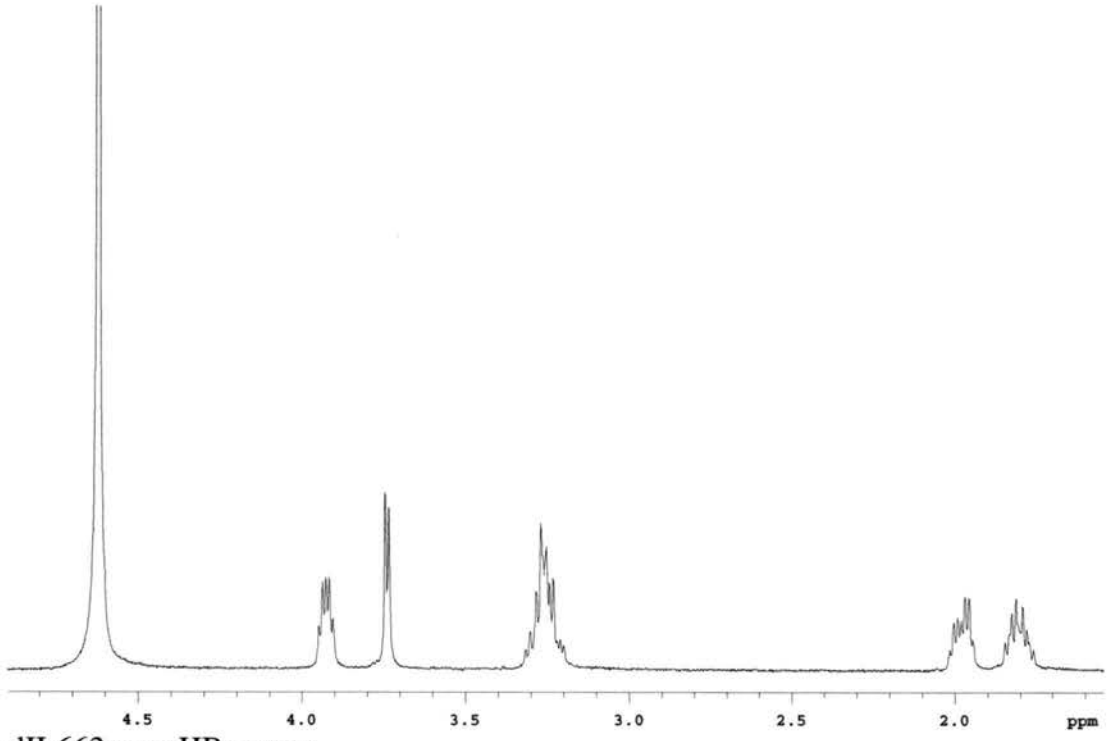
For analytical purposes, the monohydrobromide salt was formed in the following manner: The dihydrochloride salt was taken up in deionized water, treated with 2 drops of 28% aqueous HBr, and lyophilized overnight. The resulting off-white residue was dissolved in a minimal amount of methanol, and the solution neutralized with pyridine.

Absolute ethanol was added until capreomycidine mono-HBr (**3·HBr**) began to precipitate as a white solid which was recovered by filtration and dried.

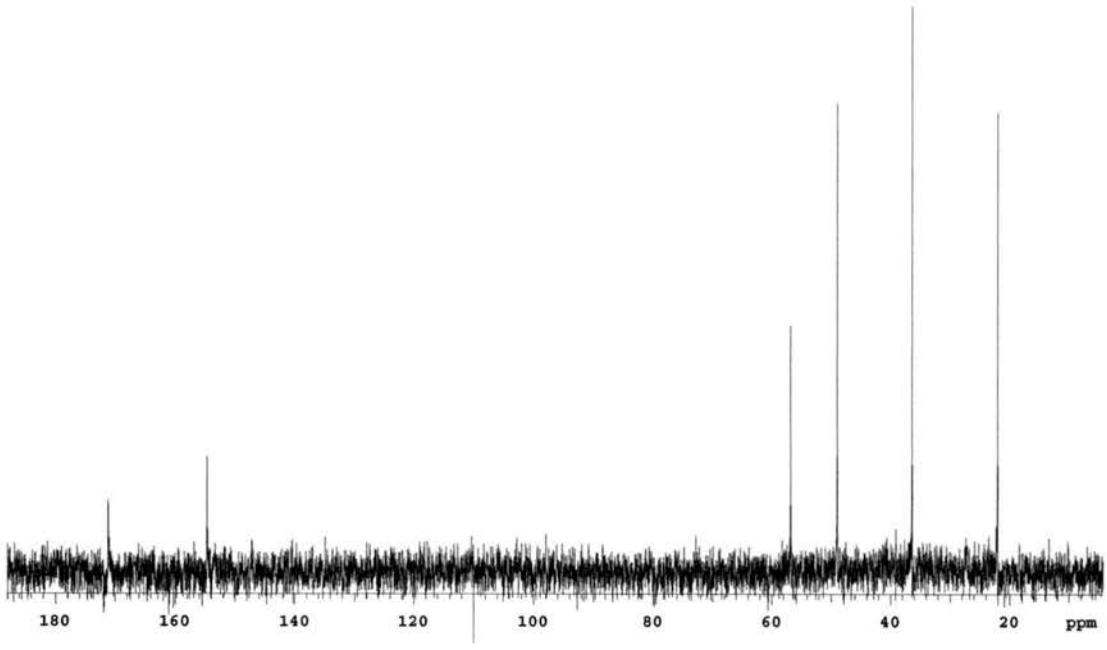
To compare to natural **3**, synthetic **3** was passed down a column of Dowex 50WX2-100 (H^+ form), washed with dd H_2O and eluted with 1.5% NH_4OH. The eluent was evaporated, dissolved in a minimal amount of dd H_2O containing several equivalents of NH_4Oac, and lyophilized overnight. The resulting residue (capreomycidine·2HOAc) was found to be identical to natural **3** by ^1H and ^{13}C-NMR.

^1H NMR (monohydrobromide) (400 MHz) (D_2O) δ 1.94-2.02 (1H, m); 2.12-2.20 (1H, m); 3.38-3.49 (2H, m); 3.91 (1H, d, J=4.7 Hz); 4.11 (1H, ddd, J=8.6, 4.3, 4.3 Hz). ^{13}C NMR (100 MHz) (D_2O): δ 22.0, 36.4, 48.9, 56.8, 154.7, 171.1. IR (1% KBr):. 3566, 3397, 3066, 2927, 1669, 1663, 1646, 1617, 1576, 1569, 1533, 1448, 1418, 1374, 1339, 1113.





dedII-662monoHBr-recry



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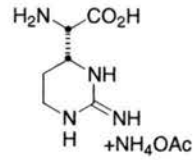
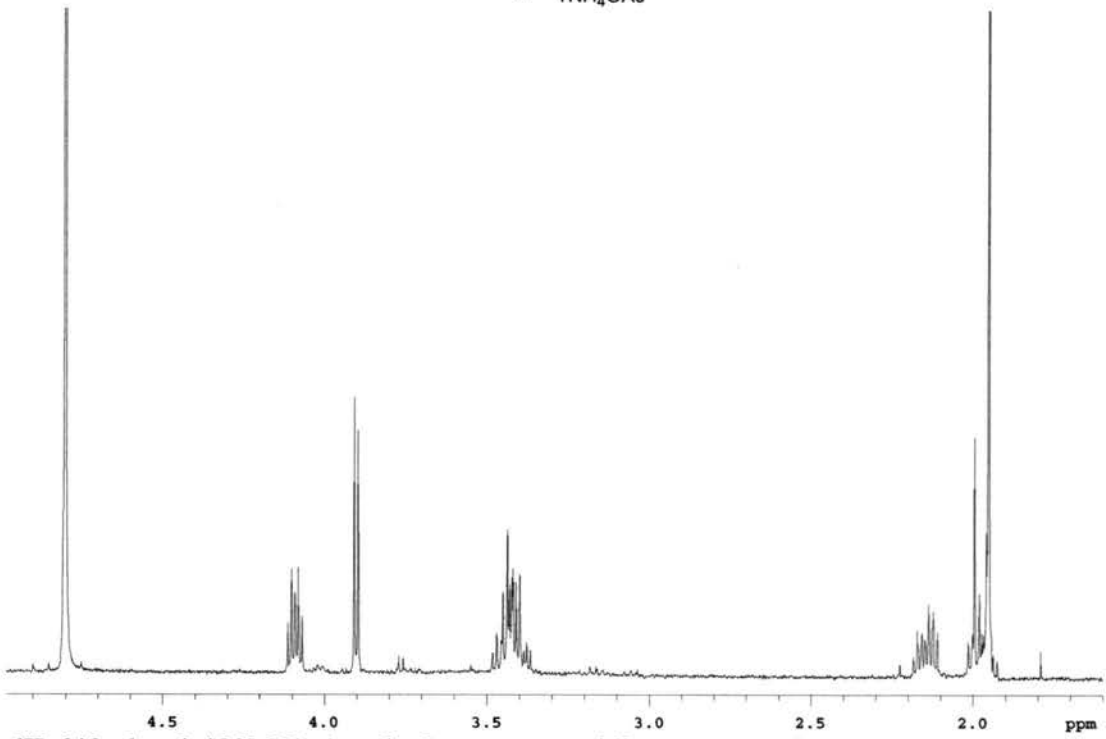
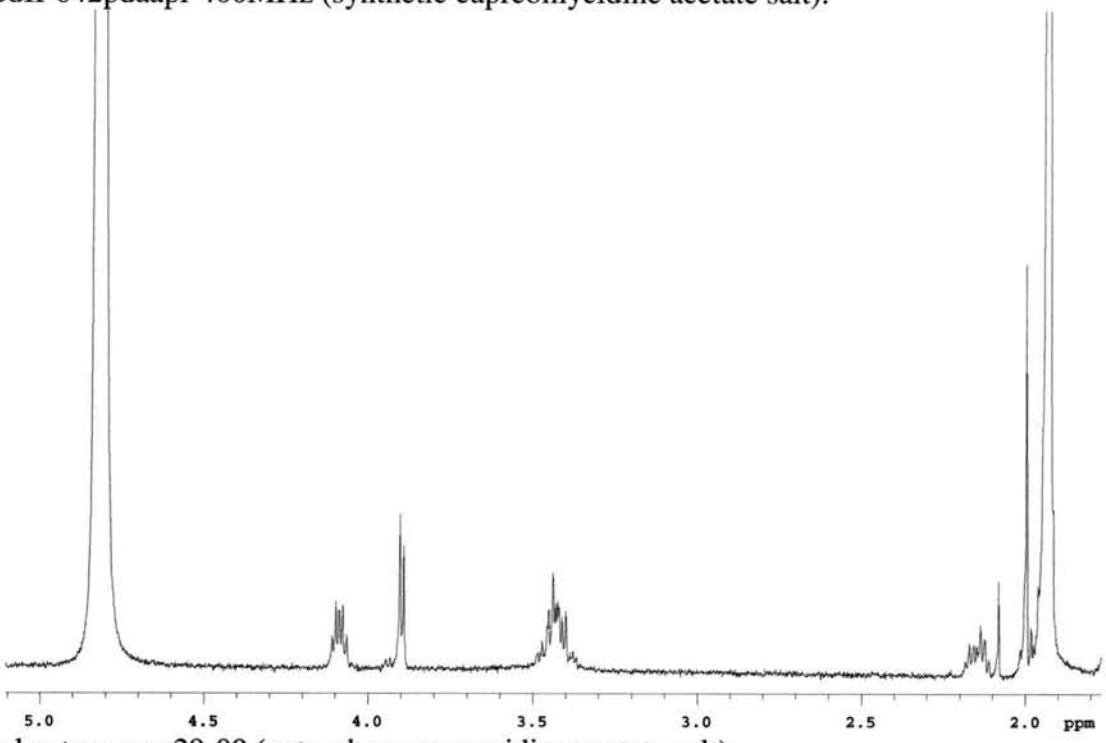




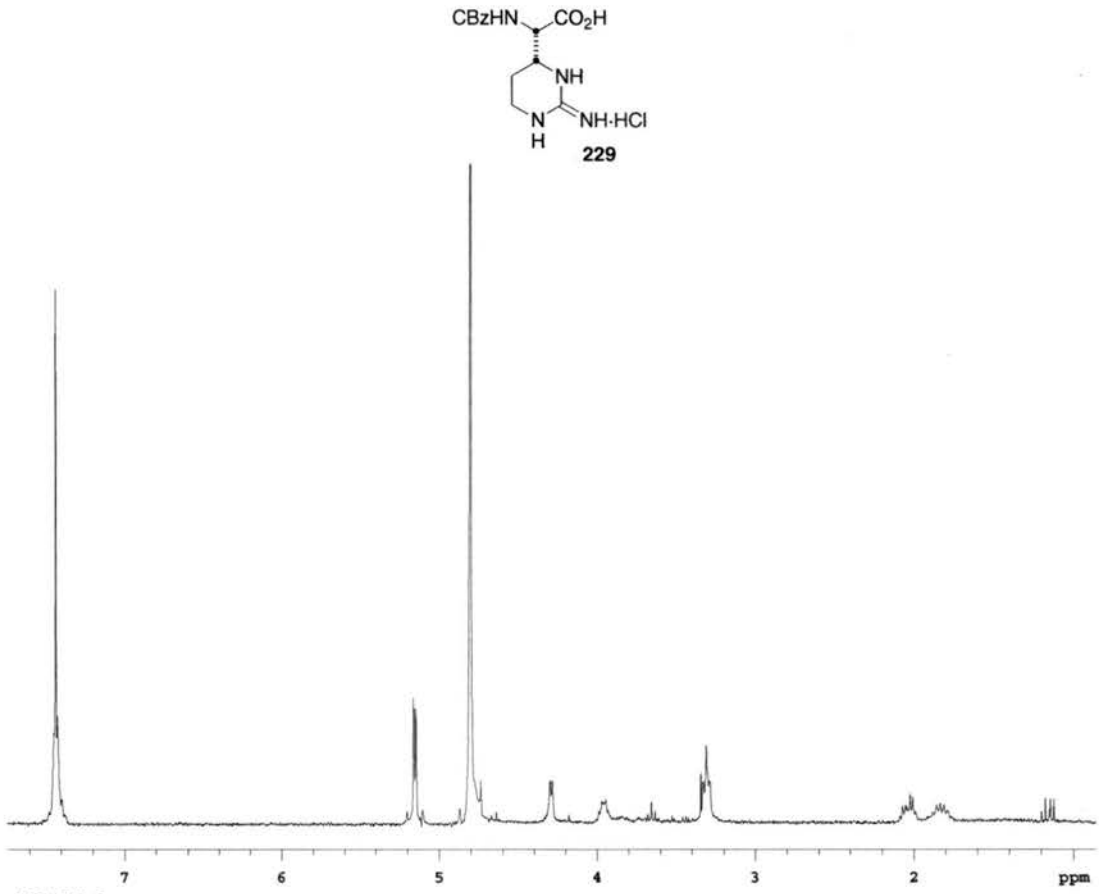
dedII-642pdaapl-400MHz (synthetic capreomycidine acetate salt).



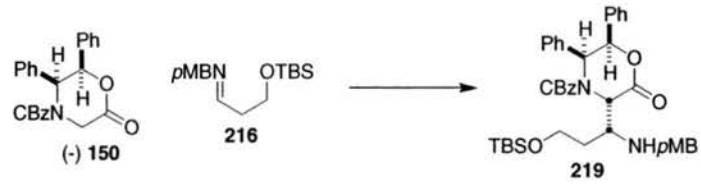
ded natcap-nov29-00 (natural capreomycidine acetate salt)

***N^{α}*-CBz-capreomycidine (229).** A solution of **3** (19 mg, 0.08 mmol, 1 eq) in dd H_2O (1.5 mL) and 2N NaOH (50 µL, prepared from dd H_2O) was treated with benzyl chloroformate (23 µL, 0.16 mmol, 2 eq) and stirred at room temp for 1h. At that point, and additional 100 µL of 2N NaOH was added and the reaction stirred an additional 1h. The mixture was diluted with dd H_2O and extracted 2x with CH_2Cl_2. Adjustment of the cloudy aqueous layer to pH 3 with aq. HCl (prepared from dd H_2O), was followed by evaporation to dryness. The residue was then dissolved with dd H_2O and loaded onto a Waters C18 Sep-Pak cartridge (prepared by washing with 3 x 5 mL MeCN followed by 3 x 5 mL dd H_2O). Any water insoluble material was kept in the flask. After washing the loaded Sep-Pak cartridge with dd H_2O (~7 mL) the product was eluted back into the flask containing the water insoluble material with 3 x 5 mL MeCN and 3 x 5 mL MeOH. The solvent was removed *in vacuo* to provide 15 mg (56%) of crude **229** as a white solid. This material was used crude in the subsequent coupling reaction.

^{1}H NMR (300 MHz) (D_2O) δ 1.83 (1H, m); 2.03 (1H, m); 3.31 (2H, t, J=6.2 Hz); 3.96 (1H, m); 4.29 (1H, d, J=4.4 Hz); 5.13 (1H, ½ ABq, J=12.5 Hz); 5.17 (1H, ½ ABq, J=12.5 Hz); 7.37-7.48 (5H, m).



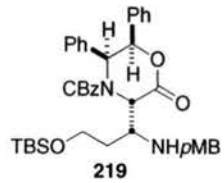
dedII-736.



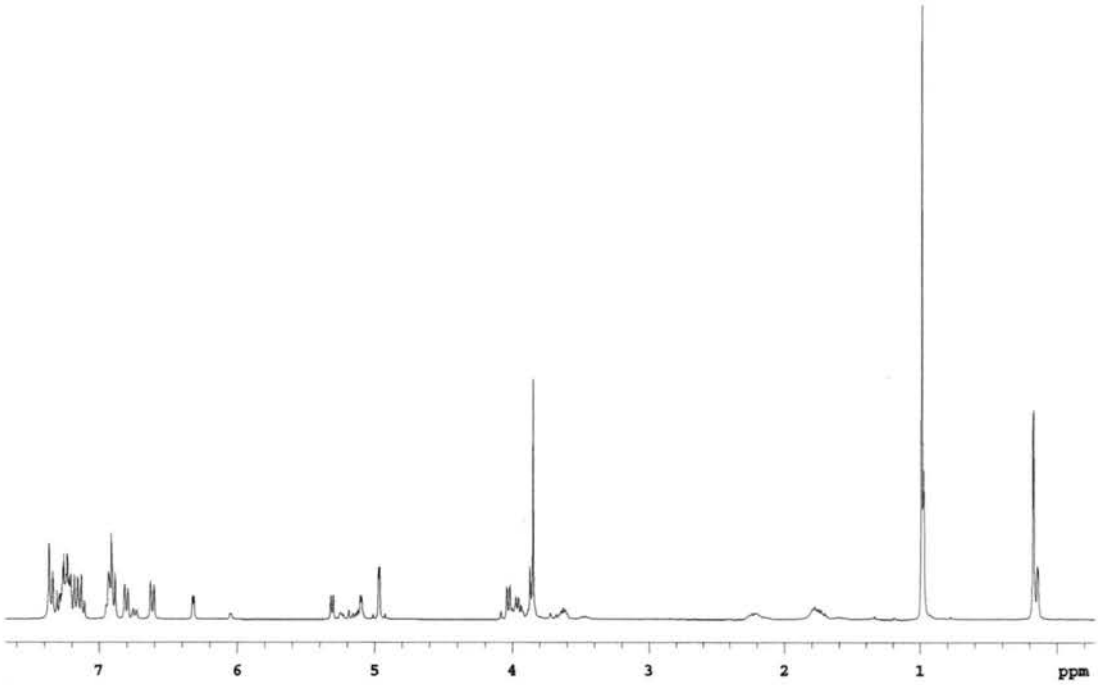
Mannich product (219). Under argon, racemic 2-oxo-4-benzyloxycarbonyl-5,6-diphenylmorpholine (240 mg, 0.62 mmol, 1 eq.) was dissolved in 13 mL dry tetrahydrofuran. After cooling to -78°C, LHMDS (0.62 mL, 0.62 mmol, 1 eq., 1M in THF) was added, and stirred 15 minutes. Dimethylaluminum chloride (0.62 mL, 0.62 mmol, 1 eq., 1M in hexanes) was added dropwise and the reaction stirred 15 minutes. A solution of **216** was dissolved in 3 mL dry THF, and added *via* canula into the enolate. After stirring for 1h at -78°C, aqueous sodium bicarbonate was added and the miture

warmed to room temp. The solids were removed by filtration through Celite and the filtrate partitioned between brine and ethyl acetate. The organic layer was removed and the aqueous layer extracted 3x more with ethyl acetate. The organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated *in vacuo*. The resulting orange residue was purified *via* silica gel chromatography with 9:1 hexanes:ethyl actetate to yield 205 mg of **219** as an amorphous solid (48% yield).

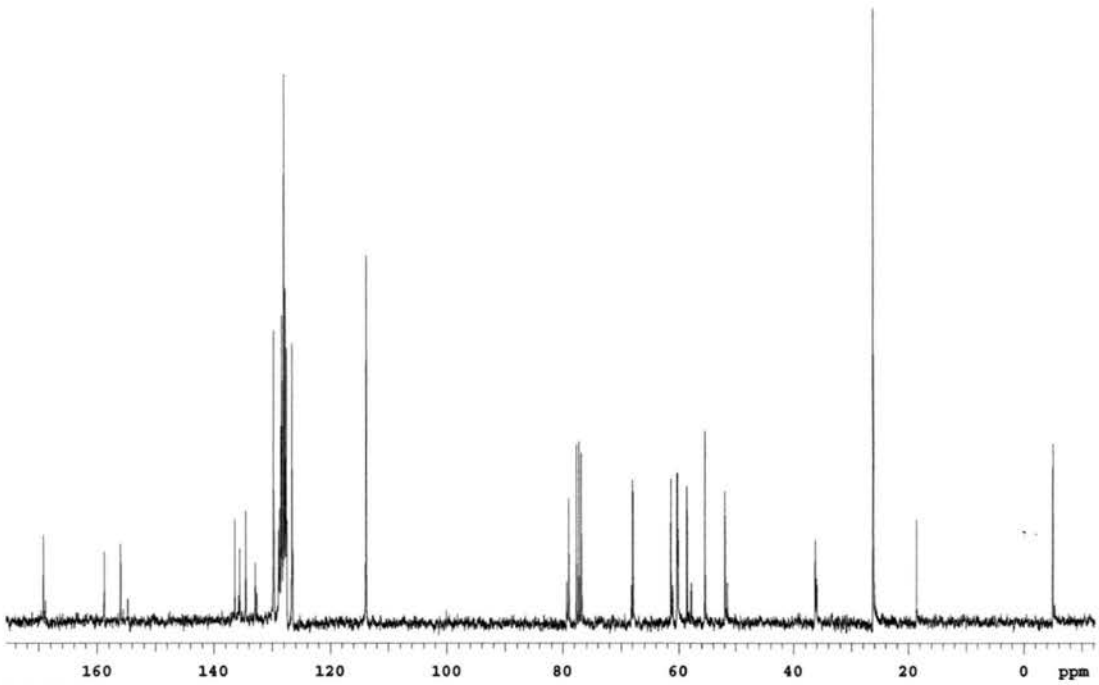
^{1}H-NMR (300 MHz) ($CDCl_3$) (3.3:1 mixture of diastereomers, * denotes minor diastereomer): δ $CHCl_3$: *0.13 (3H, s); *0.14 (3H, s); 0.17 (3H, s); 0.17 (3H, s); *0.97 (9H, s); 0.99 (9H, s); *1.60 (1H, m); 1.73 (1H, m); 1.79 (1H, broad s); *2.14 (1H, m); *3.47 (1H, m); 3.63 (1H, ddd, J=4.4, 4.4, 9.2 Hz); *3.70 (1H, ½ ABq, J=12.8 Hz); 3.85 (3H, s); *3.87 (3H, s); 3.96 (2H, m); 4.01 (1H, ½ ABq, J=12.8 Hz); 4.05 (1H, ½ ABq, J=12.8 Hz); 4.95 (1H, ½ ABq, J=12.5 Hz); 4.98 (1H, ½ ABq, J=12.5 Hz); 5.10 (1H, d, J=2.9 Hz); *5.11 (1H, ½ ABq, J=11.7 Hz); *5.15 (1H, d, J=4.8 Hz); *5.21 (1H, ½ ABq, J=11.7 Hz); *5.24 (1H, d, J=3.3 Hz); 5.31 (1H, d, J=3.3 Hz); *6.05 (1H, d, J=2.9 Hz); 6.32 (1H, d, J=2.9 Hz); 6.62 (2H, d, J=7.3 Hz); *6.74 (2H, d, J=7.3 Hz); 6.80 (2H, d, J=7.0); 6.88-6.95 (4H, m); 7.11-7.36 (13H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) (3.3:1 mixture of diastereomers, * denotes minor diastereomer): δ $CDCl_3$: -5.0, -5.0, 18.5, 26.2, *36.0, 36.2, *51.5, 51.9, 55.4, *57.8, 58.6, 60.1, 60.2, 60.3, *61.0, 61.4, 67.9, *68.2, 79.0, *79.3, 113.8, 126.5, 126.6, 127.5, 127.6, 127.7, 127.8, 128.0, 128.0, 128.3, 128.5, 128.7, 128.9, 129.6, 129.8, *132.6, 132.9, 134.5, 135.6, *135.8, 136.4, *154.7, 155.9, 158.7, *168.9, 169.2. IR (NaCl, deposited from CH_2Cl_2): 3347.4, 3063.7, 3032.5, 2952.9, 2928.9, 2855.3, 1753.5, 1708.3, 1611.0, 1585.7, 1512.4, 1498.0, 1454.7, 1397.8, 1338.0, 1247.7, 1179.4, 1104.4, 1061.9, 1032.6 cm^{-1}.



219



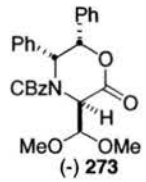
dd424



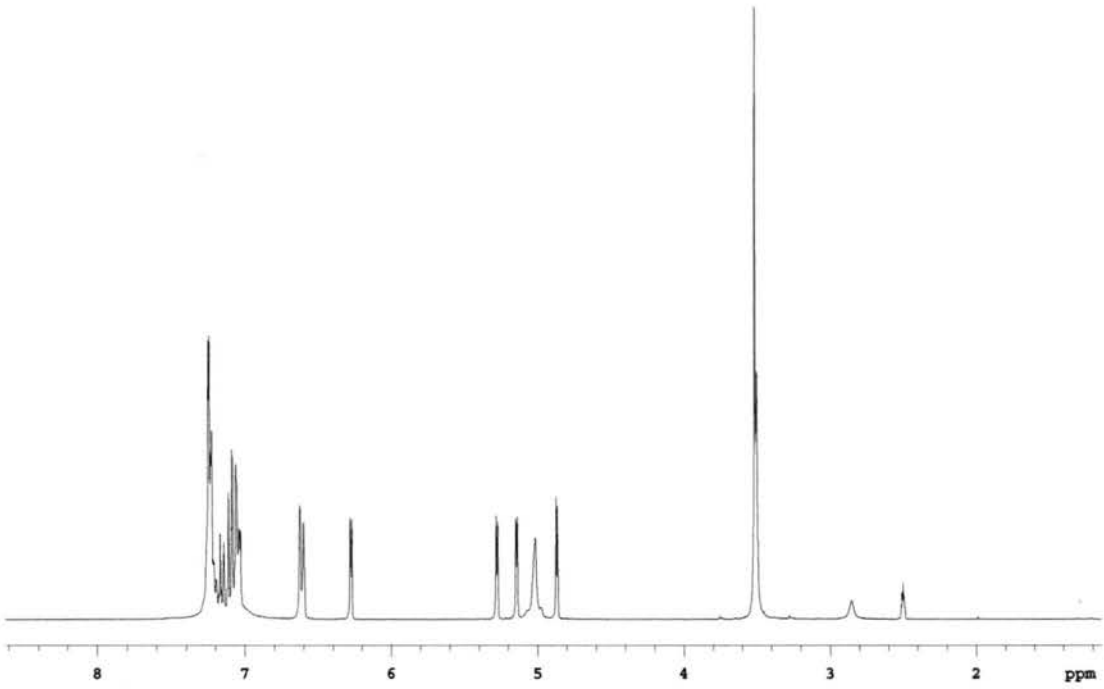
ddc043

Dimethylacetal (-) 273: A solution of (+) **150** (1.24 g, 3.2 mmol., 1 eq.) was dissolved in CH_2Cl_2 (50 mL) and cooled to -78°C. While stirring, $TiCl_4$ (700 µL, 6.4 mmol., 2 eq.) was added, followed by triethylamine (900 µL, 6.4 mmol., 2 eq.) to provide a dark blue enolate solution. After stirring for 15 min., trimethyl orthoformate (2.1 mL, 19.2 mmol., 6 eq.) was added, and the solution warmed slowly to 0°C. After stirring 45 min. at 0°C, 0.025 M pH 7 phosphate buffer was added and stirred 30 min. The quenched reaction was filtered through Celite, diluted with CH_2Cl_2, and washed twice with brine. Upon drying the organic layer over anhydrous sodium sulfate, the solution was filtered and evaporated to provide an off white solid. Silica gel chromatography (eluted with 6:3:1 CH_2Cl_2:hexanes:EtOAc) provided 1.37g (93%) of pure (-) **273** as a white solid.

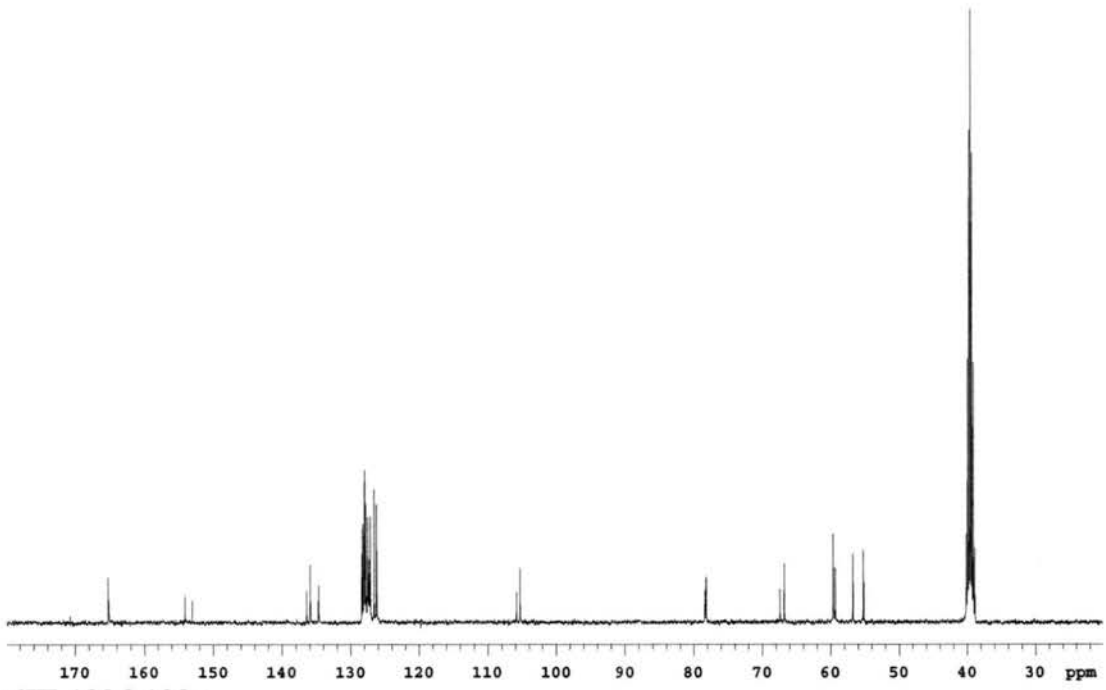
^{1}H NMR (300MHz) (DMSO-d_6, 393K) δ DMSO: 3.50 (3H, bs); 3.51 (3H, s); 4.87 (1H, d, J=2.9 Hz); 5.02 (2H, broad m); 5.14 (1H, d, *J*=2.9 Hz); 5.28 (1H, d, J=3.3 Hz); 6.28 (1H, d, J=3.3 Hz); 6.61 (2H, d, J=7.0 Hz); 7.01-7.29 (13H, m). ^{13}C NMR (100 MHz) (DMSO-d_6, 298K, mixture of rotamers, * indicates minor rotamer) δ DMSO: 55.2, 56.8, 59.3, 59.6, 66.7, 67.4, 78.2, 78.3, 105.3, *105.8, 126.3, 126.6, 127.1, 127.3, 127.4, 127.5, 127.5, 127.8, 127.9, 128.0, 128.2, 128.2, 128.4, 134.7, *134.7, 135.9, *136.0, 136.5, *153.1, 154.1, *165.1, 165.2. IR (NaCl, deposited from CH_2Cl_2): 3031, 2940, 2837, 1753, 1706, 1454, 1401, 1348, 1288, 1267, 1250, 1207, 1189, 1109, 1081 cm^{-1}. HRMS (FAB^+) calc. for $C_{27}H_{28}NO_6$ (MH^+) 462.1917; found 462.1917. (-) **273.** $[\alpha]_D^{25}$ = -1.8 (*c*=1.0, CH_2Cl_2). (+) **273.** $[\alpha]_D^{25}$ = +1.7 (*c*=1.0, CH_2Cl_2).



(-) **273**



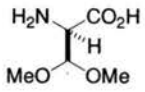
dedIII-1296-120deg



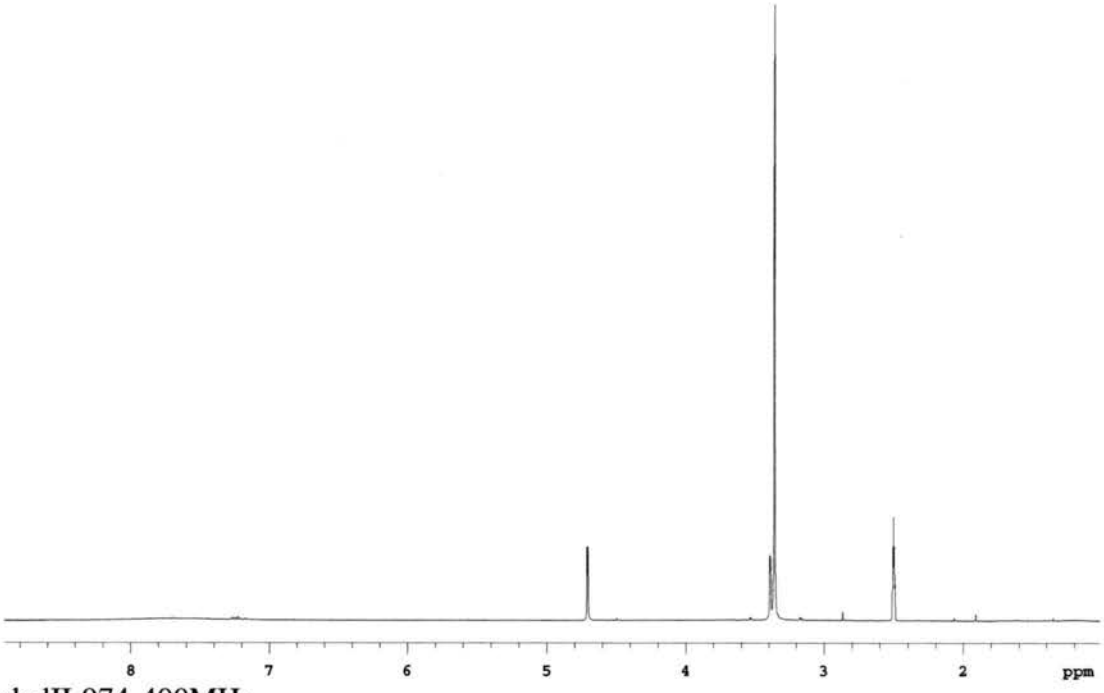
dedIII-1296-100rt

***R*-α-formylglycine dimethyl acetal ((+) 245).** A solution of (-) **273** (472 mg, 1.02 mmol., 1 eq.) in 3:1 THF:MeOH (32 mL) was purged with argon for 10 min. To this solution in a pressure tube, 20% $Pd(OH)_2$ on activated carbon (360 mg, 0.51 mmol., 0.5 eq.) was added, and the tube filled with hydrogen gas to 95 psi. The pressure was released, and the tube refilled. This was repeated 4 times more. The pressurized tube was then stirred for 2 days at room temperature. After the 2 days, the pressure was released, the solution purged with argon, and the 20% $Pd(OH)_2$ on activated carbon removed by filtration through Celite. Evaporation of the filtrate and trituration of the residue with ether provided 152 mg (99%) of (+) **245** as an oily solid.

^{1}H NMR (400MHz) (DMSO-d_6) δ DMSO: 3.35 (3H, s); 3.36 (3H, s); 3.39 (1H, d, J=2.1 Hz); 4.71 (1H, d, J=2.1 Hz); 6.80-8.40 (3H, bs). ^{13}C NMR (100 MHz) (DMSO-d_6) δ DMSO: 55.0, 55.6, 56.8, 103.7, 166.3. IR (NaCl, Neat): 2939, 1641, 1506, 1406, 1342, 1272, 1218, 1194, 1067 cm^{-1}. HRMS (FAB^+) calc. for $C_5H_{12}NO_4$ (MH^+) 150.0766; found 150.0768. (+) **245.** $[\alpha]_D^{25}$ = +7.6 (*c*=0.67, MeOH). (-) **245.** $[\alpha]_D^{25}$ = -7.4 (*c*=0.67, MeOH).



(+) **245**



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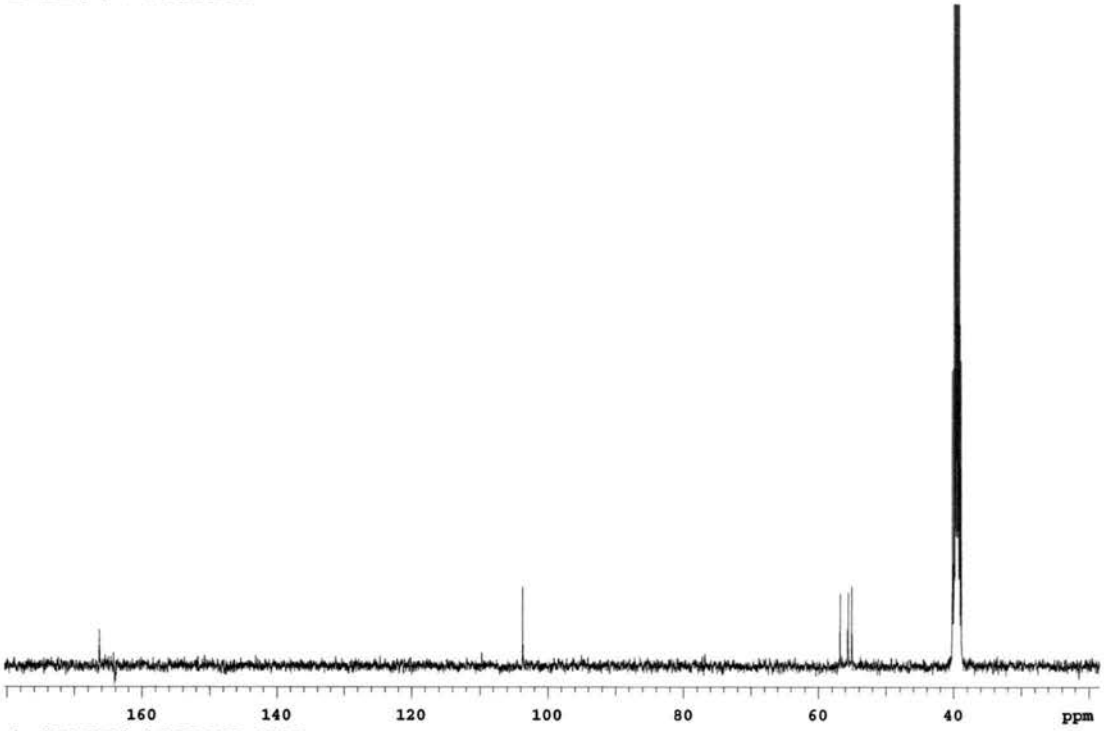

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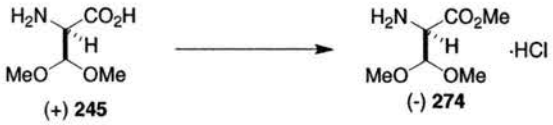
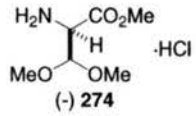
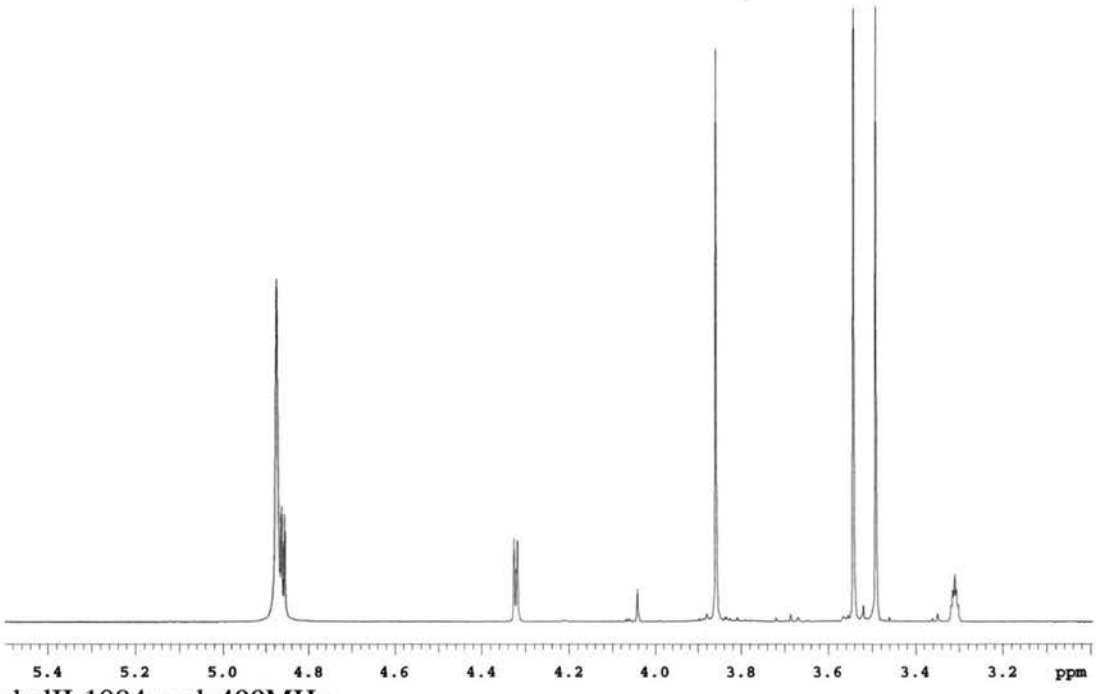


***R*-α-Formylglycine dimethylacetal, methyl ester hydrochloride salt ((-) 274).** A stirred solution of MeOH (10 mL) at 0°C was treated with acetyl chloride (2 mL, 30 mmol). This mixture was warmed to room temperature and stirred for 20 min. The resulting methanolic HCl solution was added to a round bottomed flask containing (+) **245** (95 mg, 0.64 mmol, 1 eq.). After stirring the reaction at reflux for 2.5 h, the solvent was removed *in vacuo* to provide 126 mg (99%) of (+) **274** as a clear oil.

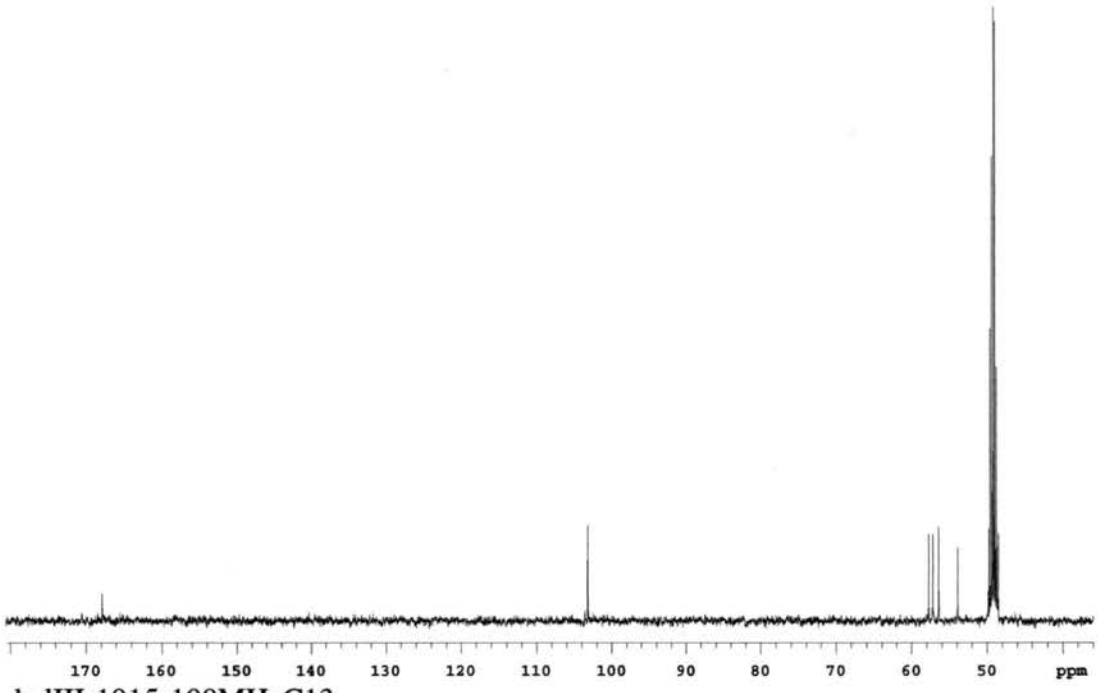
^{1}H NMR (400MHz) (CD_3OD) δ: 3.49 (3H, s); 3.54 (3H, s); 3.86 (3H, s); 4.33 (1H, d, *J*=2.8 Hz); 4.86 (1H, d, =2.8 Hz). ^{13}C NMR (100 MHz) (CD_3OD) δ: 53.9; 56.4; 57.2; 57.7; 103.2; 167.8. IR (NaCl, Neat): 3583, 3408, 2956, 2843, 1749, 1643, 1591, 1503, 1443, 1378, 1306, 1241, 1195, 1111, 1070 cm^{-1}. HRMS (FAB^+) calc. for $C_6H_{14}NO_4$ (MH^+) 164.0923; found 164.0922. **(+)274.** $[\alpha]_D^{25}$ = +2.7 (*c*=0.66, MeOH). **(-)274.** $[\alpha]_D^{25}$ = -2.7 (*c*=0.66, MeOH). The enantiomeric purity of **(+)274** was found to be >95% ee by formation of the Mosher's amide *via* both the optically pure and racemic Mosher's acid chlorides and comparison of the resulting diastereomers by ^{1}H-NMR. None of the minor diastereomer was observed in the optically pure Mosher's acid chloride case.



(-) **274**



dedII-1004crude400MHz

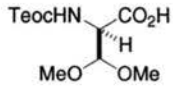


dedIII-1015-100MHzC13

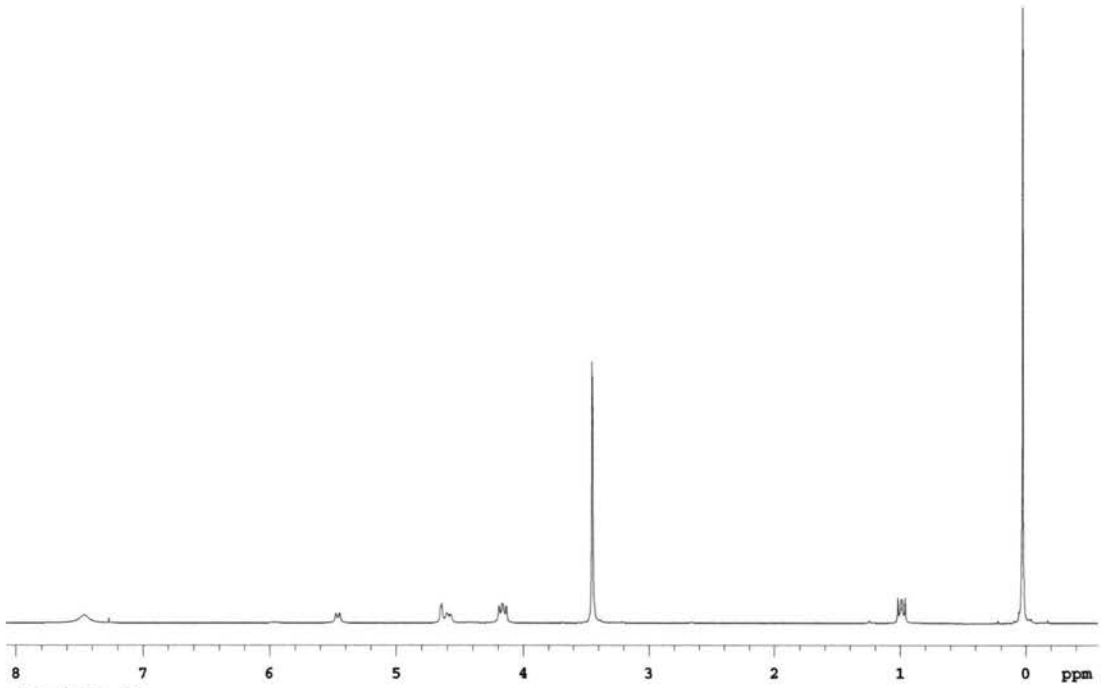
(+) **245** (-) **275**

2*R*-(*N*-Trimethylsilylethylcarbonyl)-α-formylglycine. To a solution of (+) **245** (59 mg, 0.40 mmol., 1 eq.) in 1:1 dioxane:water (3 mL) was added triethylamine (166 μL, 1.19 mmol., 3 eq.) and the mixture stirred under argon. TeocONSu[6] (109 mg, 0.42 mmol., 1.05 eq.) was added to the solution, and the reaction stirred overnight at room temperature. The reaction was diluted, acidified to pH 4 with 0.5 M citric acid, and extracted twice with ether. The combined ether layers were washed twice with water, then dried over $MgSO_4$. Removal of the drying agent by filtration and evaporation provided 90 mg (77%) of (-) **275** as a pale oil.

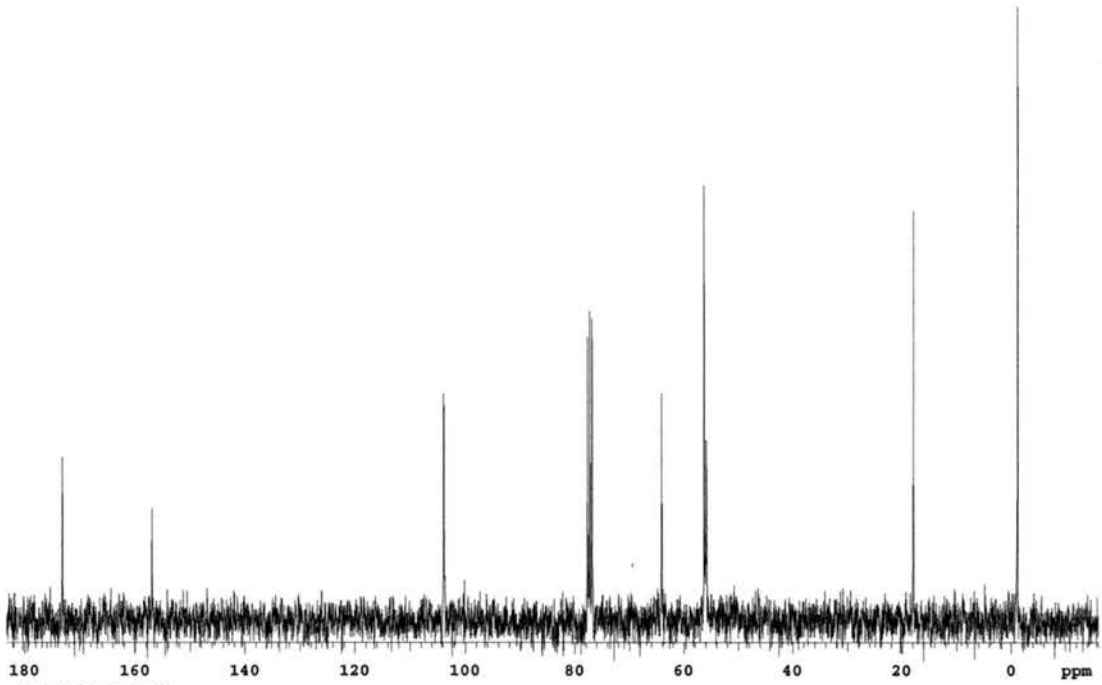
^{1}H NMR (300MHz) ($CDCl_3$) δ $CDCl_3$: 0.04 (9H, s); 1.00 (2H, ddd, J=0, 8.4, 9.5 Hz); 3.47 (6H, s); 4.19 (2H, ddd, J=0, 6.6, 9.5 Hz); 4.61 (1H, d, J=8.4 Hz); 4.66 (1H, s); 5.42 (1H, d, J=7.7 Hz); 9.29 (1H, bs). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: -1.2, 17.9, 55.9, 56.3, 64.1, 103.9, 156.9, 173.1. IR (NaCl, Neat): 3452, 3319, 3107, 2954, 2838, 1725, 1525, 1448, 1415, 1317, 1251, 1214, 1186, 1118, 1068 cm^{-1}. HRMS (FAB$^+$) calc. for $C_{11}H_{24}NO_6Si$ (MH^+) 294.1373; found 294.1379. (-) **275.** $[\alpha]_D^{25}$ = -22.2 (*c*=2.0, CH_2Cl_2).



(-) **275**



dedII-852old

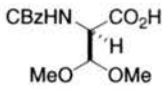


dedII-1010C13

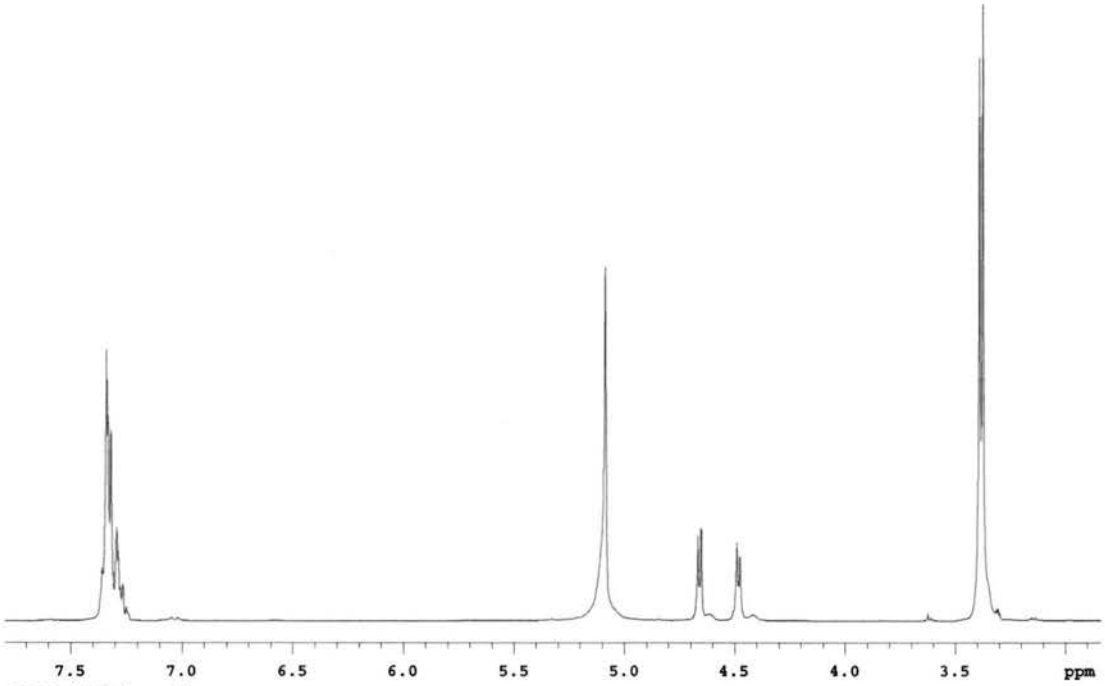
(+) **245** **276**

***N*-CBz-α-formylglycine dimethylacetal (276).** A solution of 2*R*-amino-3,3-dimethoxypropanoic acid ((+) **245**) (424 mg, 2.84 mmol, 1 eq) was dissolved in saturated aqueous $NaHCO_3$ (26 mL) and dioxane (5 mL). After cooling the mixture to 0°C, benzyl chloroformate (446 μL, 3.12 mmol, 1.1 eq) was added dropwise with stirring. The reaction was stirred for 3h then diluted with water and extracted with ether. While stirring the aqueous layer with ethyl acetate in an ice bath, aqueous HCl was added until the solution reached pH 3. The organic layer was removed, and the aqueous layer was extracted twice more with ethyl acetate. The organic layers were combined, washed twice with brine, and dried over anhydrous sodium sulfate. Filtration to remove the sodium sulfate was followed by evaporation of the solvent to provide **276** (687 mg, 85%) as a viscous oil.

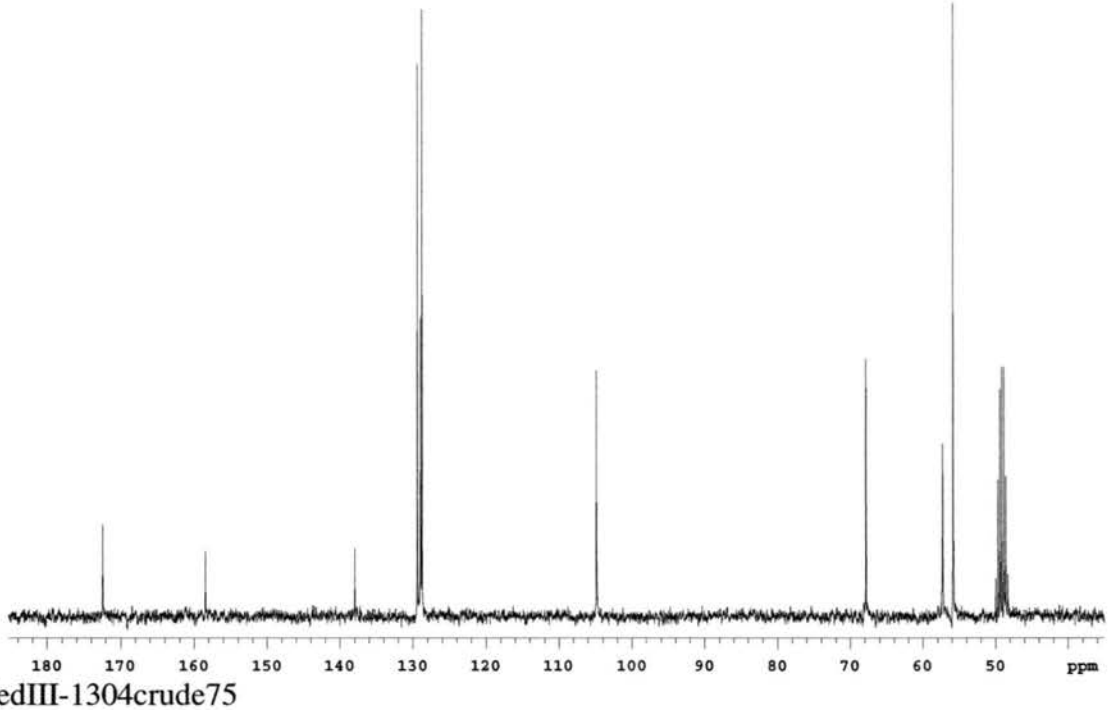
^{1}H NMR (300MHz) (CD_3OD) δ CD_2HOD: 3.38 (3H, s); 3.39 (3H, s); 4.48 (1H, d, J=4.4 Hz); 4.66 (1H, d, J=4.4 Hz); 7.23-7.38 (5H, m). ^{13}C NMR (75MHz) (CD_3OD) δ: 55.9, 55.9, 57.3, 67.8, 104.9, 128.8, 128.9, 129.4, 138.0, 158.4, 172.4.



276



dedIII-1304crude

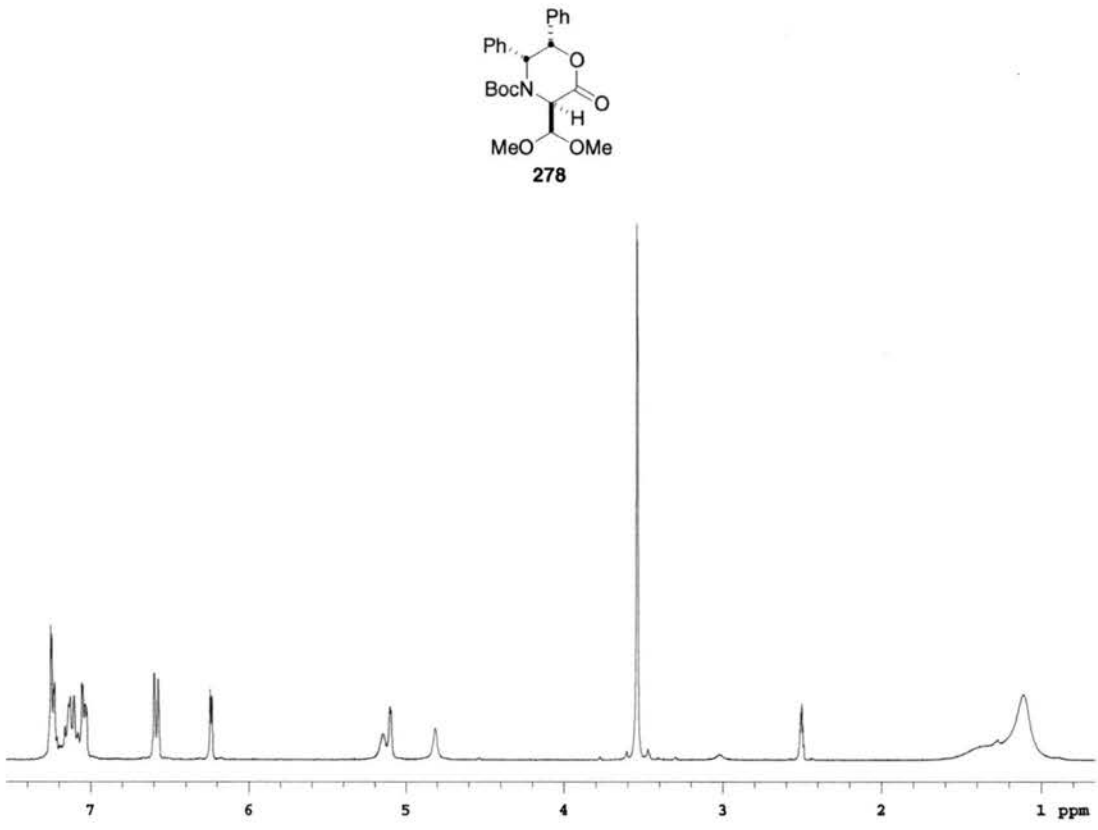


dedIII-1304crude75

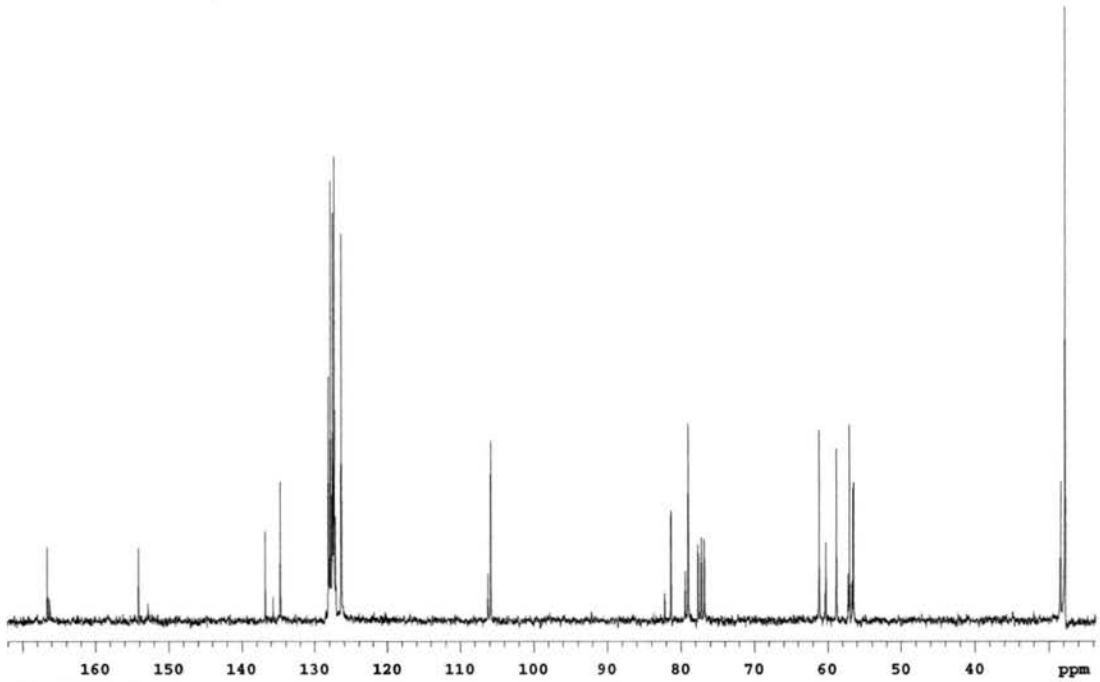
Dimethyl acetal 278. A solution of (+) **277** (912 mg, 2.58 mmol., 1 eq.) was dissolved in THF (40 mL) and cooled to -78°C. While stirring, $TiCl_4$ (566 µL, 5.16 mmol., 2 eq.) was added, followed by triethylamine (719 µL, 5.16 mmol., 2 eq.) to provide a dark blue enolate solution. After stirring for 15 min., trimethyl orthoformate (1.7 mL, 15.5 mmol., 6 eq.) was added, and the solution warmed slowly to 0°C. After stirring 45 min. at 0°C, 0.025 M pH 7 phosphate buffer was added and stirred 30 min. The quenched reaction was filtered through celite, diluted with CH_2Cl_2, and washed twice with brine. Upon drying the organic layer over anhydrous sodium sulfate, the solution was filtered and evaporated to provide an off white solid. Silica gel chromatography (eluted with 6:5:1 CH_2Cl_2:hexanes:EtOAc) provided 496 mg (45%) of pure **278** as a white solid. Recrystalization from CH_2Cl_2/hexanes provided clear prisms (m.p. 200°C).

^{1}H NMR (300MHz) (DMSO-d_6, 373K) δ DMSO: 1.00-1.60 (9H, broad m); 3.54 (6H, s); 4.81 (1H, broad s); 5.10 (1H, d, J=2.2 Hz); 5.15 (1H, broad s); 6.24 (1H, d, J=3.2 Hz); 6.59 (2H, d, J=6.8 Hz); 7.02-7.25 (8H, m). ^{13}C NMR (75 MHz, 298K) ($CDCl_3$) (unresolvable rotamers, * denotes minor rotamer) δ $CDCl_3$: 27.8, *28.4, 56.5, *56.7, 57.0, *57.2, 58.8, 60.2, 61.2, 79.0, *79.4, 81.4, *82.2, 105.8, *106.2, 126.4, 127.2, 127.4, 127.5, 127.7, 127.9, 128.2, 134.7, 135.7, 136.8, *152.8, 154.1, *166.3, 166.6. IR (NaCl,

deposited from CH_2Cl_2): 3065, 3031, 3001, 2979, 2911, 2835, 1754, 1688, 1456, 1390, 1376, 1367, 1349, 1328, 1275, 1256, 1199, 1189, 1165, 1108, 1080, 1030 cm^{-1}.



dedII-1013r100deg



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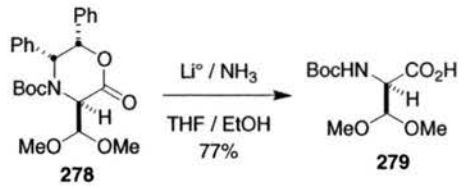
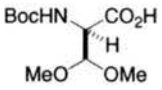
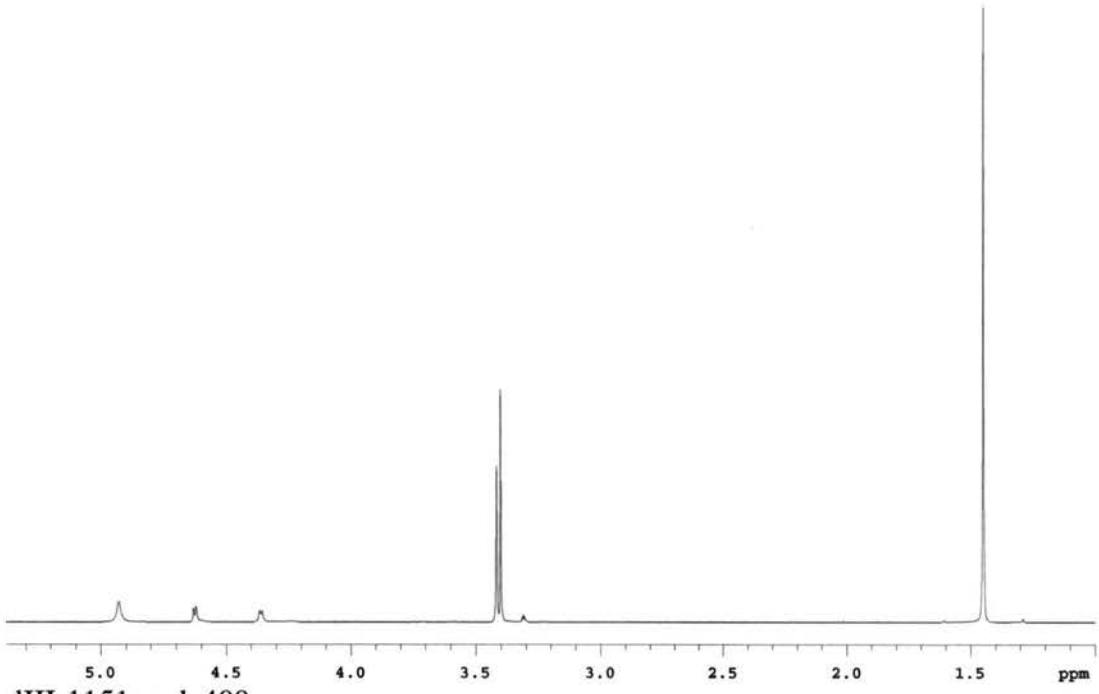


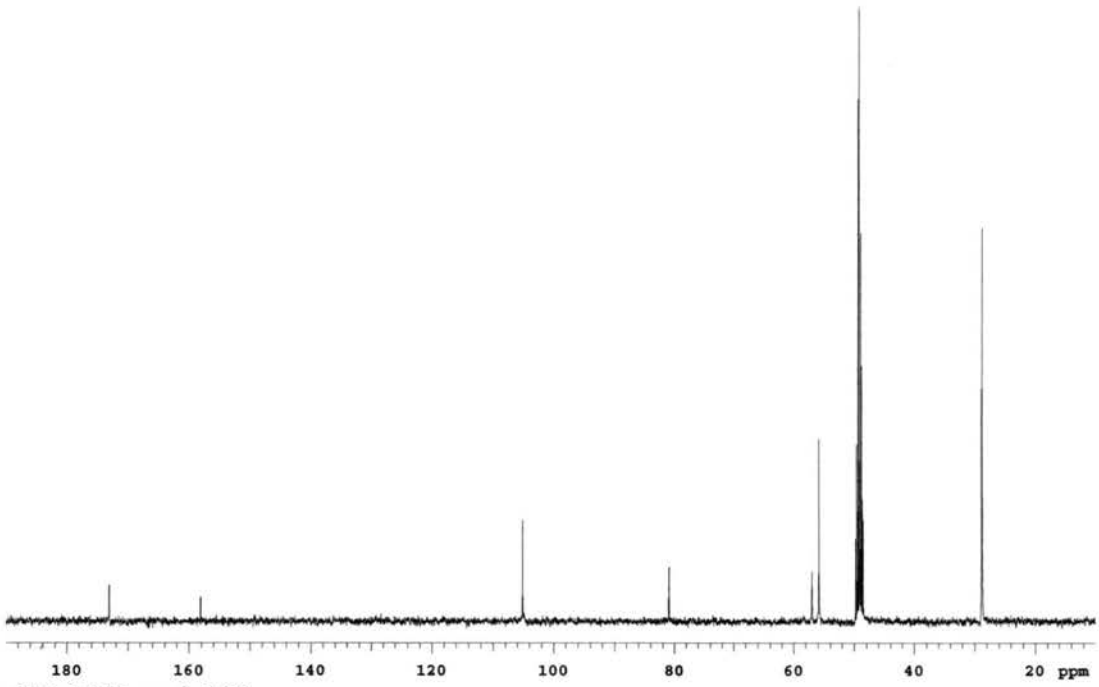
2*R*-*tert*-Butoxycarbonylamino-3,3-dimethoxypropanoic acid. Into a 100 mL round bottomed flask in a Dry Ice/acetone bath was condensed ammonia (40 mL). Lithium wire (21 mg, 3.04 mmol, 13 eq) was added to the liquid ammonia and a dark blue solution formed. A solution of **278** (100 mg, 0.23 mmol, 1 eq) and absolute ethanol (136 μL, 2.34 mmol, 10 eq) in tetrahydrofuran (5 mL) was added to the lithium and ammonia solution, and the solution allowed to warm to reflux (-33°C). At that point, the blue color of the reaction began to disappear and additional lithium wire (21 mg, 3.04 mmol, 13 eq) was added, and the solution refluxed for 1h. Upon cooling the reaction again to -78°C, the reaction was quenched with solid NH_4Cl (quenching is complete when blue color disappears). The reaction was then warmed slowly to rt to allow the excess ammonia to evaporate. After dissolving the resulting white solid in water, the aqueous solution was extracted with Et_2O twice, then acidified to pHh 2 and extracted three times with EtOAc. The combined EtOAc extracts were washed twice with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to provide **279** (44 mg, 77% yield) as a pale yellow oil. ^1H NMR (400MHz) (CD_3OD) δ MeOH: 1.45 (9H, s); 3.40 (3H, s); 3.42 (3H, s); 4.36 (1H, d, J=4.4Hz); 4.63 (1H, d, J=4.4Hz); 4.93 (2H, broad s). ^{13}C NMR (100 MHz) (CD_3OD) δ CD_3OD: 28.8, 55.8, 55.8, 57.0, 80.9, 105.1, 158.0, 173.1. IR (NaCl, deposited from $CHCl_3$): 3323, 2978, 2937, 2839, 1720, 1513, 1503, 1453, 1393, 1368, 1322, 1250, 1212, 1165, 1117, 1081.



279



dedIII-1151crude400

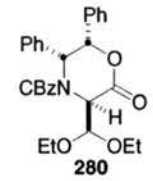


dedIII-1151crude100

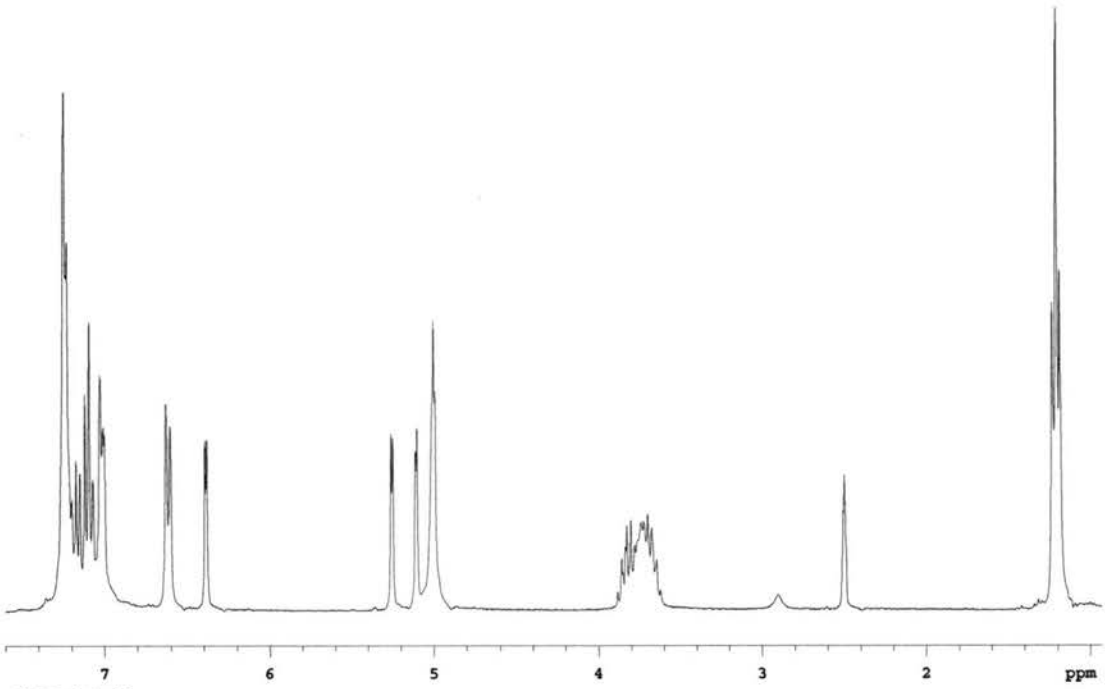
Diethylacetal 280. A solution of **(+) 150** (500 mg, 1.29 mmol., 1 eq.) was dissolved in CH_2Cl_2 (20 mL) and cooled to -78°C. While stirring, $TiCl_4$ (280 µL, 2.58 mmol., 2 eq.) was added, followed by triethylamine (360 µL, 2.58 mmol., 2 eq.) to provide a dark blue enolate solution. After stirring for 15 min., triethyl orthoformate (1.3 mL, 7.74 mmol., 6 eq.) was added, and the solution warmed slowly to 0°C. After stirring 45 min. at 0°C, 0.025 M Ph 7 phosphate buffer was added, and the mixture stirred 15 min. The quenched reaction was partitioned between sat. aq. $NaHCO_3$ and CH_2Cl_2. The organic layer was removed and washed twice with water. Upon drying the organic layer over anhydrous sodium sulfate, the solution was filtered and evaporated to provide an off white solid. Silica gel chromatography (eluted with 6:4:1 CH_2Cl_2:hexanes:EtOAc) provided 539 mg (85%) of pure **280** as a white solid. Recrstalization from CH_2Cl_2 / hexanes provided heavy white needles (m.p. 151°C).

^{1}H NMR (300MHz) (DMSO-d_6, 373K) δ DMSO: 1.21 (3H, t, J=7.0 Hz); 3.70 (2H, m); 3.81 (2H, m); 5.00 (2H, m); 5.11 (1H, d, J=2.8 Hz); 5.26 (1H, d, J=3.1 Hz); 6.39 (1H, d, J=3.1 Hz); 6.61 (2H, d, J=7.5 Hz); 7.00 (1H, d, J=2.6 Hz); 7.01-7.27 (13H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) (unresolvable rotamers, resonances reported as observed at 298K, *denotes minor rotamer) δ $CDCl_3$: 15.3, 60.0, 60.7, 60.9, *64.5 , 64.6, 65.1, 65.2, 67.8, *68.4, 79.0, *79.3, 102.8, *103.5, 126.4, 126.5, 127.4, 127.4, 127.5, 127.6, 127.8, 128.0, 128.2, 128.3, 128.6, 128.7, 134.7, 135.4, 135.5, 136.1, *153.7, 155.0, *166.0, 166.3. IR (NaCl, deposited from CH_2Cl_2): 3031, 2975, 2894, 1754, 1705, 1497, 1454, 1402, 1372,

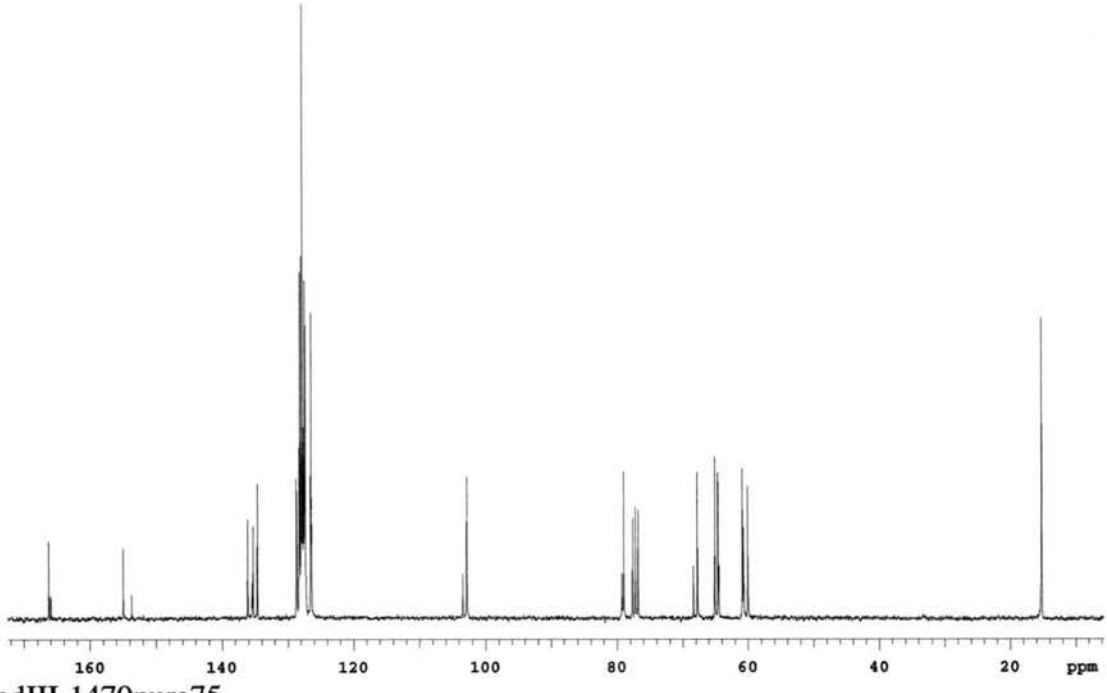
1344, 1319, 1302, 1268, 1245, 1107, 1070, 1031, 980. HRMS (FAB^+) calc. for $C_{29}H_{32}NO_6$ (MH^+) 490.2229; found 490.2213. $[\alpha]_D^{25} = -20.6$ (*c*=0.5, CH_2Cl_2).



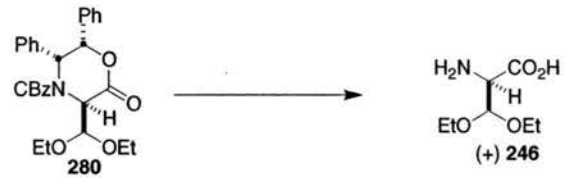
280



dedIII-1470pure

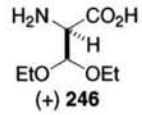


dedIII-1470pure75

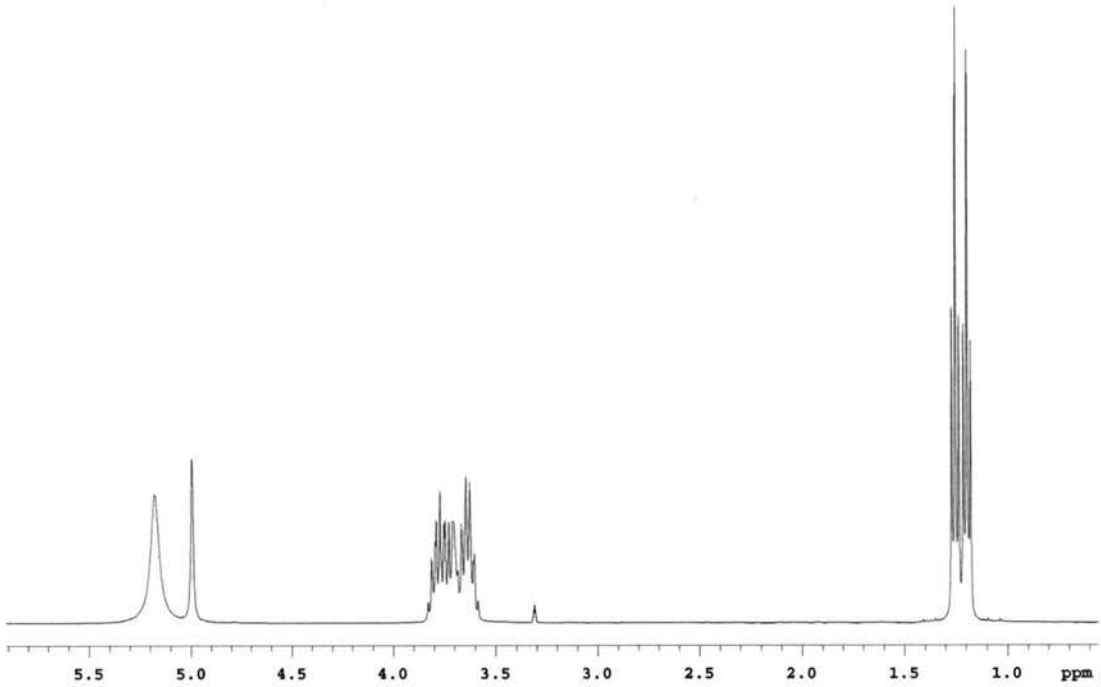


***R*-α-Formylglycine diethylacetal.** A solution of **280** (370 mg, 0.76 mmol., 1 eq.) and 20% $Pd(OH)_2$ on activated carbon (106 mg, 0.15 mmol., 0.2 eq.) in 3:1 THF:EtOH (23.5 mL) was purged with argon for 10 min. The tube was then filled with hydrogen gas to 85 psi. The pressure was released, and the tube refilled. This was repeated 3 times more. The pressurized tube was then stirred for 2 days at room temperature. After the 2 days, the pressure was released, the solution purged with argon, and the solution diluted with 15 mL MeOH. The 20% $Pd(OH)_2$ on activated carbon was removed by filtration through Celite. Evaporation of the filtrate and trituration of the residue with ether provided 134 mg (99%) of (+) **246** as a white solid (m.p. m.p. 160-165°C (decomp), recryst. $MeOH/CH_2Cl_2$).

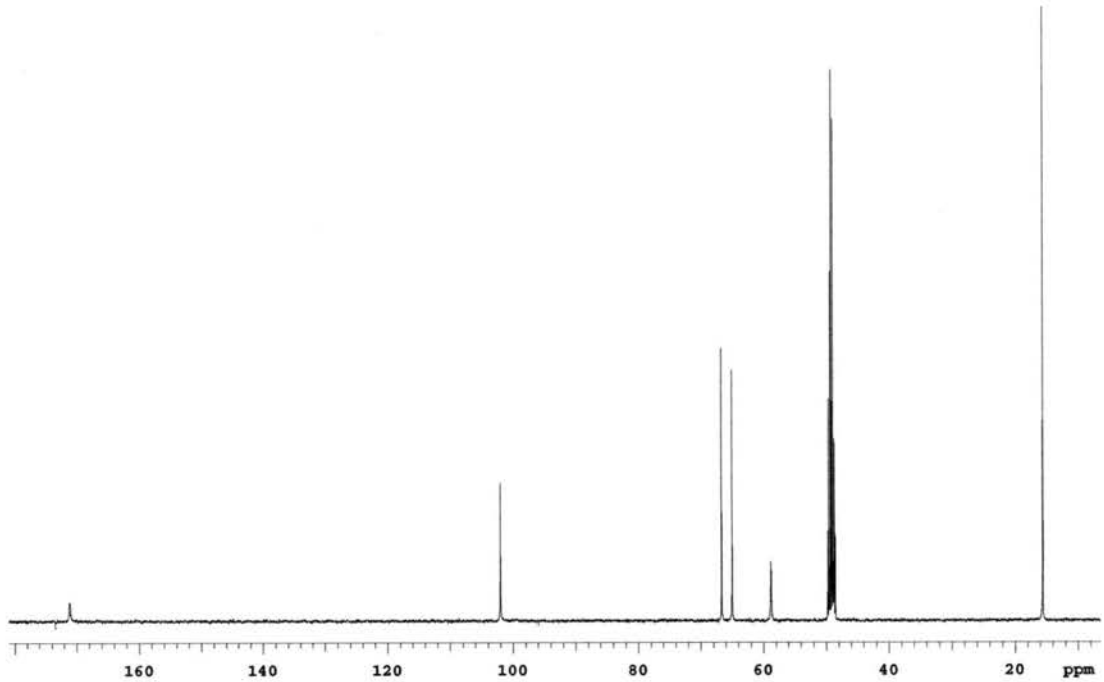
^1H NMR (400MHz) (CD_3OD) δ CD_2HOD: 1.20 (3H, t, J=7.0 Hz); 1.25 (3H, t, J=7.0 Hz); 3.59-3.83 (5H, m); 5.00 (1H, broad s). ^{13}C NMR (100 MHz) (CD_3OD) δ CD_3OD: 15.6, 15.6, 58.8, 65.0, 66.7, 102.0, 171.1. IR (NaCl, Neat): cm^{-1}. HRMS (FAB^+) calc. for $C_7H_{16}NO_4$ (MH^+) 178.1079; found 178.1082. $[\alpha]_D^{25}$ = +14.4 (*c*=0.55, MeOH).



(+) **246**

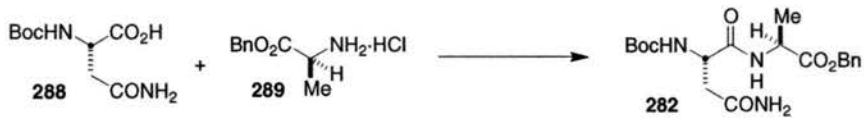


dedIII-1472-400



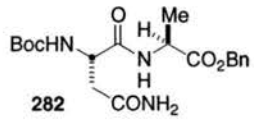
dedIII-1472-100

***R*-α-Formylglycine diethylacetal, ethyl ester hydrochloride salt**. A stirred solution of EtOH (10 mL) at 0°C was treated with acetyl chloride (2 mL, 30 mmol.). This mixture was warmed to room temperature and stirred for 30 min. The resulting ethanolic HCl solution was added to a round bottomed flask containing (+) **246** (109 mg, 0.62 mmol., 1 eq.). After stirring the reaction at reflux for 2.5 h, the solvent was removed *in vacuo* to provide 144 mg (96%) of **281** as a yellow solid which was used crude in the next step.

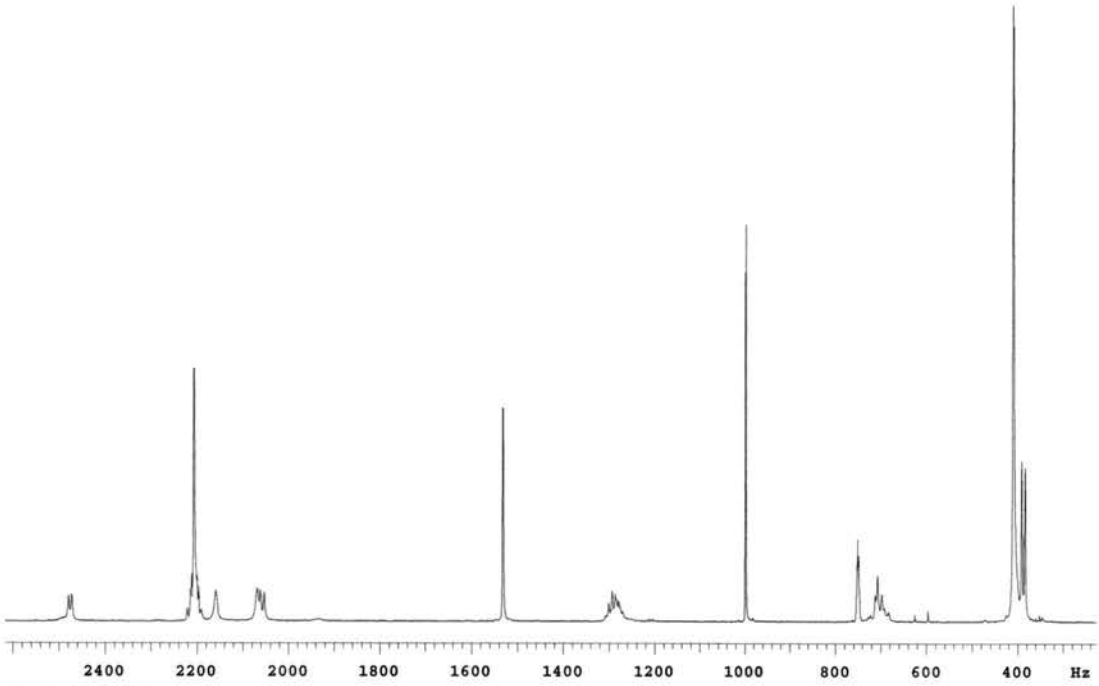


Boc-Asn-Ala-OBn (282). A round bottomed flask was charged with **288** (464 mg, 2 mmol., 1 eq.) and **289** (431 mg, 2 mmol., 1 eq.) under argon. Methylene chloride (40 mL) was added and the resulting suspension was cooled to 0°C. Triethylamine (558 μL, 4 mmol., 2 eq.) was added and the suspension became clear. EDCI (403 mg, 2.1 mmol., 1.05 eq.) and HOBt (405 mg, 3 mmol., 1.5 eq.) were added at 0°C, and the reaction was allowed to warm to room temperature with stirring overnight. The reaction mixture was diluted with ethyl acetate, washed with sat. aq. sodium bicarbonate, dilute aqueous HCl, and brine three times. The organic layer was dried over sodium sulfate, filtered, and evaporated to provide 646 mg (82 %) of **282** as a white solid (m.p. 144°C, recryst. EtOAc).

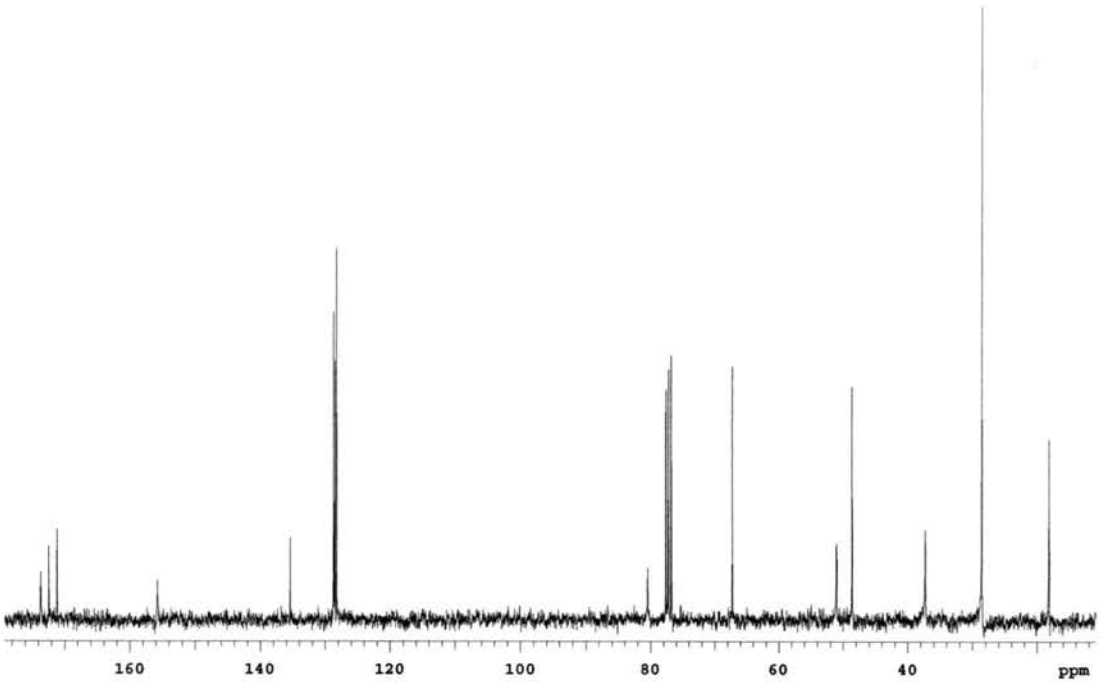
^{1}H NMR (300MHz) (DMSO-d_6) δ DMSO: 1.29 (3H, d, J=7.3 Hz); 1.36 (9H, s); 2.32 (1H, dd, J=15.0, 8.8 Hz); 2.38 (1H, dd, J=15.0, 4.8 Hz); 4.23-4.36 (2H, m); 5.1 (2H, s); 6.86 (1H, d, J=8.1Hz); 6.89 (1H, s); 7.19 (1H, s); 7.30-7.40 (5H, m); 8.25 (1H, d, J=7.0 Hz). ^{13}C NMR (75MHz) ($CDCl_3$) δ $CDCl_3$: 18.2, 28.6, 37.3, 48.6, 51.1, 67.3, 80.5, 128.3, 128.5, 128.7, 135.4, 155.8, 171.1, 172.4, 173.6. IR (NaCl, depos. from CH_2Cl_2): 3400, 3329, 3206, 2982, 1740, 1692, 1654, 1526, 1456, 1410, 1391, 1368, 1325, 1254, 1169, 1053 cm-1. HRMS (FAB^+) calc. for $C_{19}H_{28}N_3O_6$ (MH^+) 394.1978; found 394.1967. $[\alpha]_D^{25}$: +5.8 (*c*=0.55, CH_2Cl_2).



282



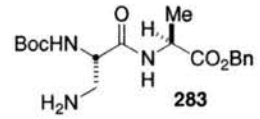
dedIII-1131old



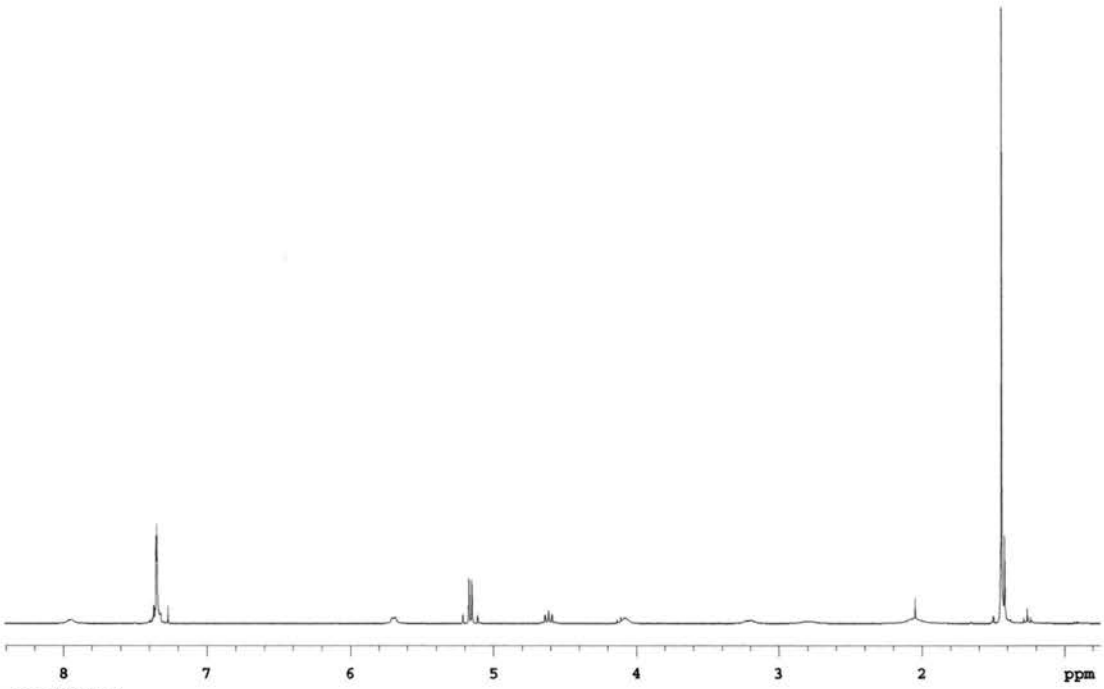
dedII-688pr75MHzC13

***N*$^{\alpha}$-Boc-DAPA-Ala-OBn (283).** A solution of bis(trifluoroacetoxy)iodobenzene (162 mg, 0.38 mmol., 1.5 eq.) in 1:1 MeCN:water (3 mL) was prepared at room temperature. To this solution was added **282** (99 mg, 0.25 mmol., 1 eq.), and stirring was continued for 15 min. Pyridine (40 µL, 0.5 mmol., 2 eq.) was then added, and the mixture stirred for 3 h. The solvent was removed by rotary evaporation to provide **283** as a yellow crude oil. The residue was dissolved in EtOAc and washed three times with brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. Silica gel chromatography (10% MeOH in CH_2Cl_2) provided 57 mg (62%) of **283** as a glass.

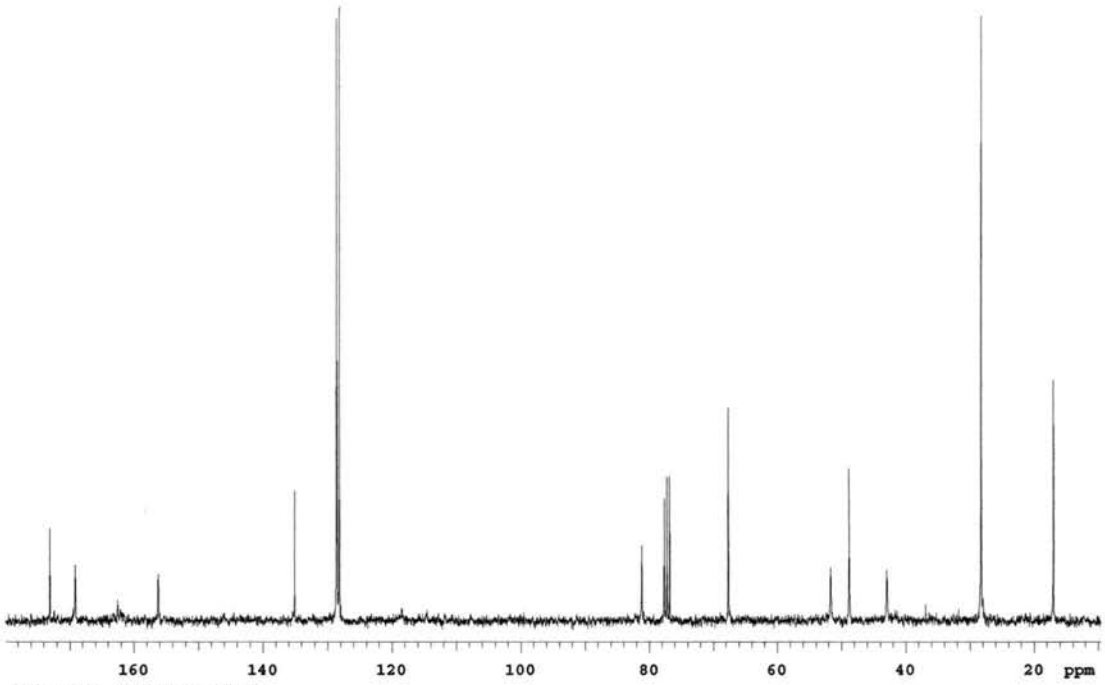
^{1}H NMR (300MHz) ($CDCl_3$) δ $CHCl_3$: 1.43 (12H, s); 3.39 (2H, broad s); 4.56-4.65 (2H, m); 5.13 (1H, ½ ABq, *J*=12.5 Hz); 5.20 (1H, ½ ABq, *J*=12.5 Hz); 6.37 (1H, broad d, *J*=5.5 Hz); 7.30-7.41 (5H, m); 8.30 (1H, broad d, *J*=6.2 Hz); 8.47 (2H, broad s). ^{13}C NMR (75MHz) ($CDCl_3$) δ $CDCl_3$: 17.0, 28.3, 42.9, 48.8, 51.7, 67.7, 81.2, 128.2, 128.5, 128.6, 135.1, 156.2, 169.1, 173.1.



283



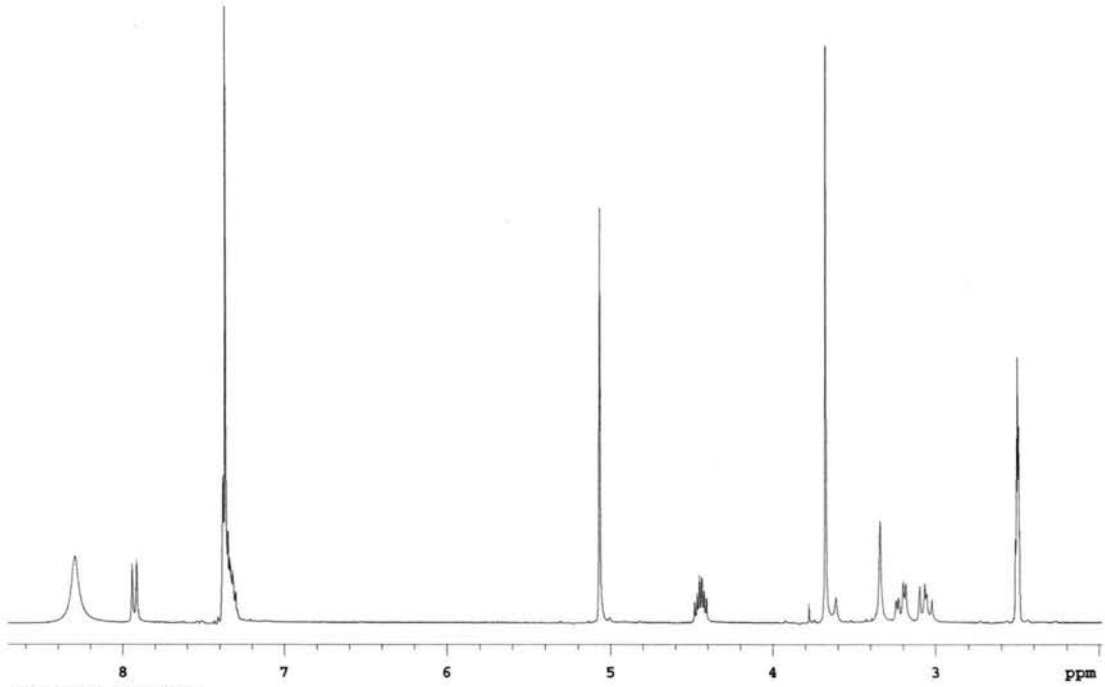
dedII-797C



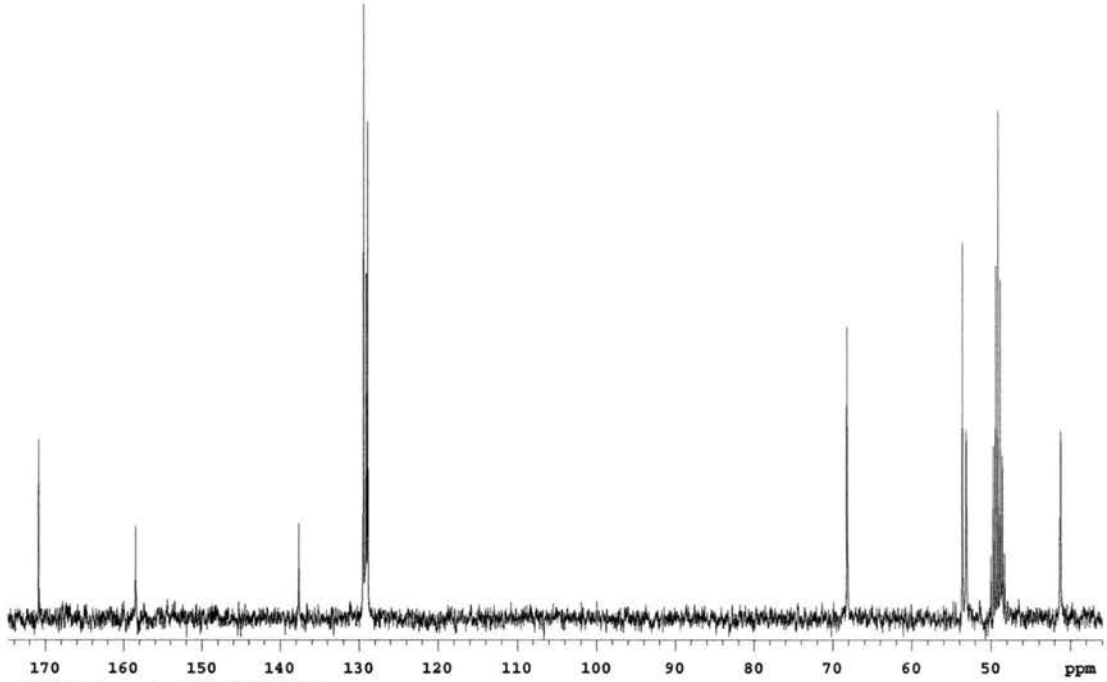
dedII-690-75MHzC13

N^{α}-CBz-DAPA-OMe·HCl (285). Cool 10 mL dry methanol to 0°C under argon. Acetyl chloride (1.07 mL, 15 mmol) was added dropwise with stirring. After stirring for 15 min. at room temperature, **290** (516 mg, 2.17 mmol, 1 eq) was added and the mixture refluxed for 2 hours. The reaction was then cooled and the solvent removed *in vacuo* to provide 608 mg (97%) of methyl ester hydrochloride **285** as a white solid (m.p.=169°C, recryst. MeOH / ether).

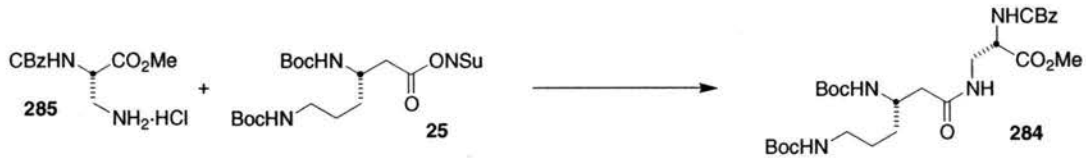
^{1}H NMR (300MHz) (DMSO-d_6) δ DMSO: 3.06 (1H, dd, J=9.2, 12.8 Hz); 3.22 (1H, dd, J=4.8, 13.2); 3.67 (3H, s); 4.45 (1H, ddd, J=9.2, 9.2, 4.8 Hz); 5.07 (2H, s); 7.30-7.38 (5H, m); 7.93 (1H, d, J=8.4); 8.30 (3H, bs). ^{13}C NMR (75MHz) (CD_3OD) δ CD_3OD: 41.3, 53.1, 53.6, 68.2, 128.9, 129.1, 129.4, 137.6, 158.5, 170.8. IR (NaCl, depos. from MeOH): 3221, 3032, 2954, 1720, 1587, 1526, 1455, 1438, 1305, 1262, 1227, 1180, 1055, 1026, 1008 cm-1. HRMS (FAB^+) calc. for $C_{12}H_{17}N_2O_4$ (MH^+) 253.1188; found 253.1191. $[\alpha]_D^{23}$= -42.6 (*c*=0.5, dd H_2O).



dedII-716-DMSO

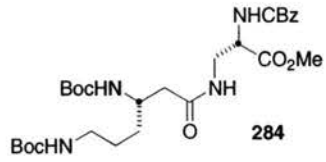


dedII-716crude75MHzC13

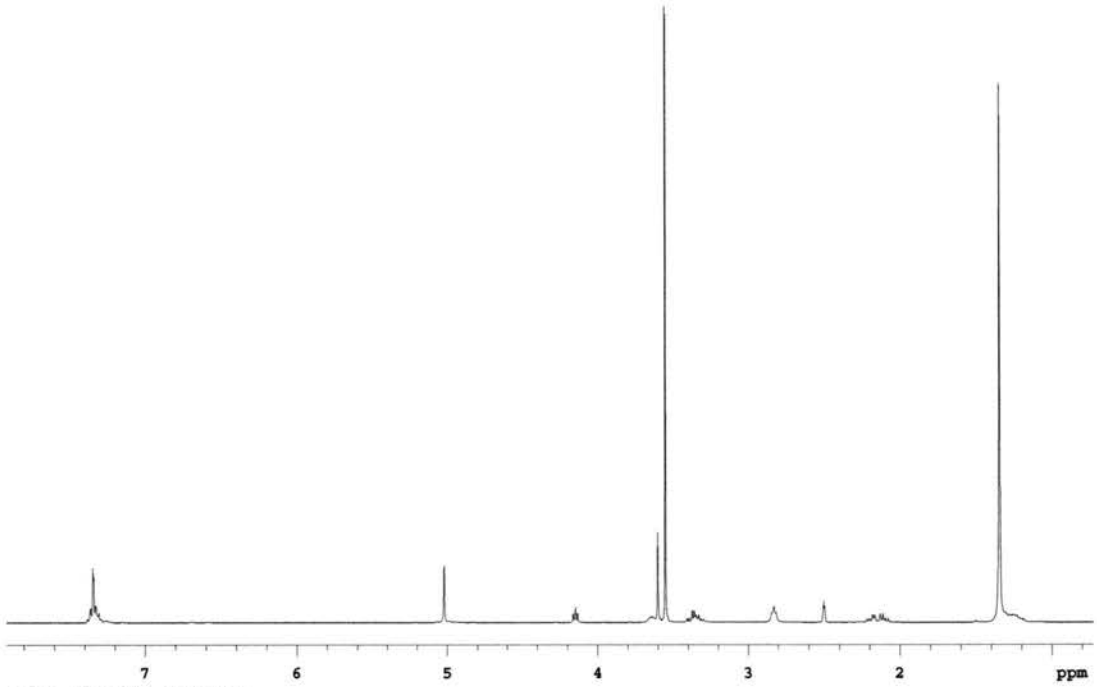


N^{α}-CBz-DAPA-N^{β}-(*N,N'*-di-Boc-β-Lys)-OMe (284): To a suspension of **285** (130 mg, 0.45 mmol., 1 eq.) and **25** (200 mg, 0.45 mmol., 1 eq.) in methylene chloride (8 mL) was added *N*-methylmorpholine (99 μL, 0.90 mmol, 2 eq.) at 0°C under argon. This mixture was stirred 1h. at 0°C and overnight at room temperature. The reaction mixture was then evaporated and taken up in EtOAc with a small amount of methylene chloride and methanol. The organic solution was washed with 0.5 M citric acid, saturated aqueous sodium bicarbonate, and twice with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to provide 240 mg (92%) of **284** as a white amorphous solid.

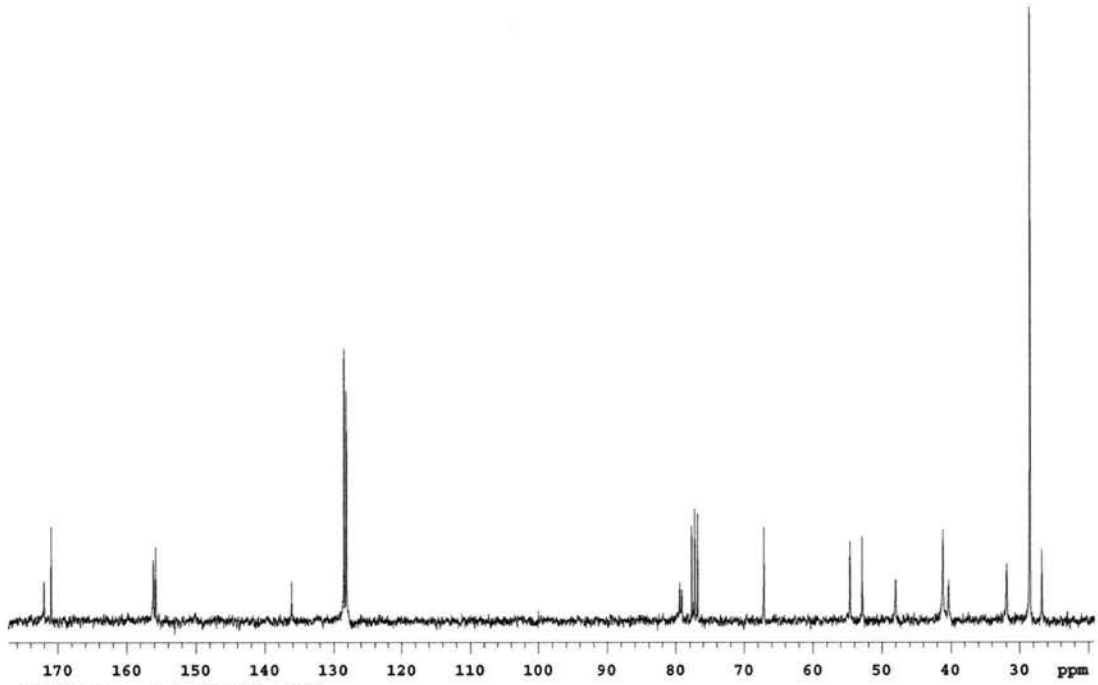
^{1}H NMR (400MHz) (DMSO-d_6, D exchange) δ DMSO: 1.16-1.40 (4H, m); 1.34 (18H, s); 2.10 (1H, dd, J=7.5, 14.5 Hz); 2.19 (1H, dd, J=6.0, 14.5 Hz); 2.83 (2H, broad t.); 3.32 (1H, dd, J=7.0, 13.5 Hz); 3.38 (1H, dd, J=5.5, 13.5); 3.60 (3H, s); 3.65 (1H, m); 4.15 (1H, dd, J=6.0, 6.0 Hz); 5.02 (2H, s); 7.22-7.40 (5H, m). ^{13}C NMR (75MHz) ($CDCl_3$) δ $CDCl_3$: 26.8, 28.5, 28.6, 31.9, 40.3, 41.1, 48.0, 52.8, 54.6, 67.1, 79.1, 79.4, 128.2, 128.5, 136.1, 155.8, 156.2, 171.0, 172.0. IR (NaCl, depos. from $CHCl_3$) 3345, 3064, 3035, 2981, 2939, 1748, 1685, 1651, 1530, 1449, 1391, 1366, 1329, 1276, 1169, 1109, 1062, 1028, 1014, 970. HRMS (FAB^+) calc. for $C_{28}H_{45}N_4O_9$ (MH^+) 581.3187, found 581.3179. $[\alpha]_D^{25}$=+14.5 (*c*=0.55, $CHCl_3$).



284



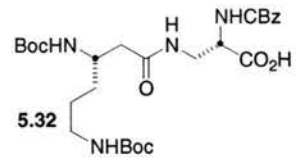
dedIII-1275Dx2d400.

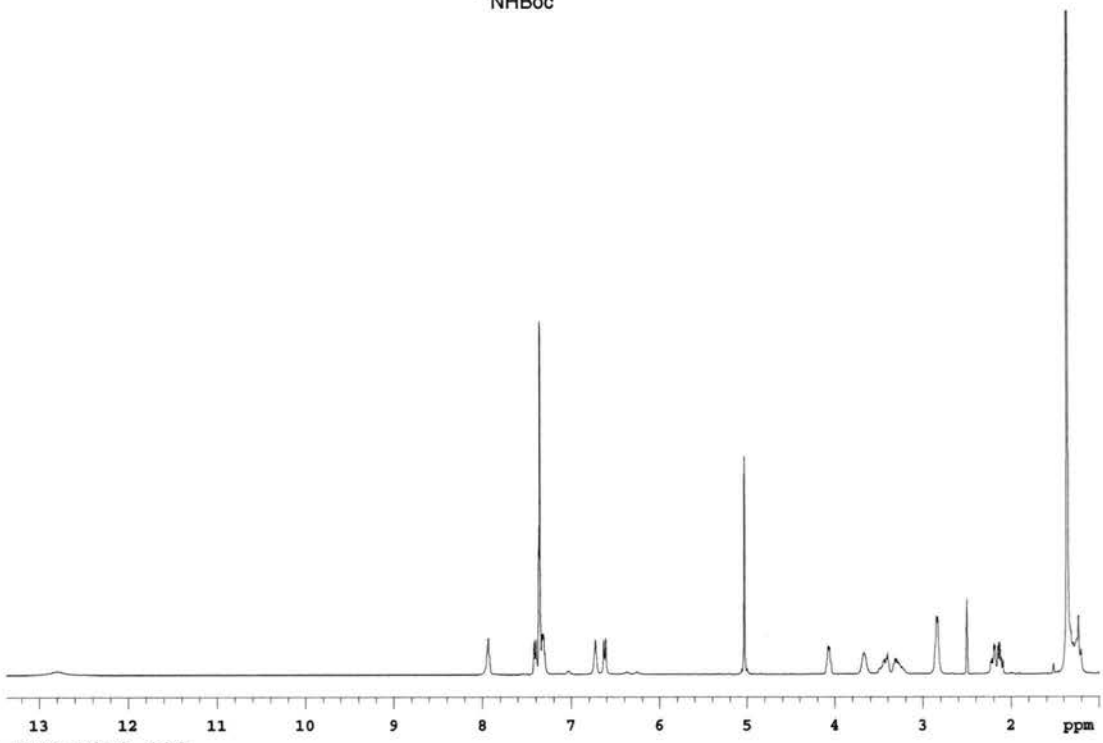


dedII-728crude75MHzC13.

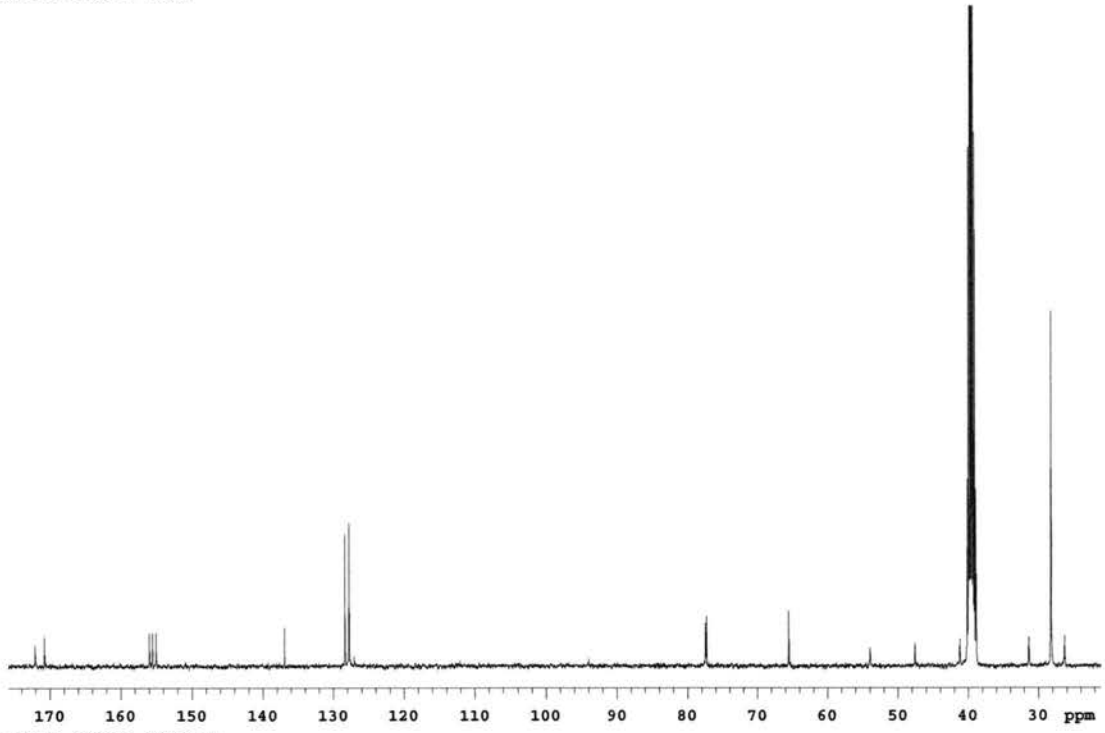
N^{α}-CBz-DAPA-N^{β}-(*N,N'*-di-Boc-β-Lys)-OH (307): To a solution of **284** (99 mg, 0.17 mmol, 1 eq) in THF (1.8 mL) and MeOH (1.8 mL) was added 2N NaOH (217 mL, 0.43 mmol, 2.6 eq) dropwise. After the starting material was consumed by TLC (1h), the reaction was diluted with water and acidified to pH 3 with aq. HCl. The solution was then extracted three times with EtOAc, and then the combined extracts were washed twice with brine. Drying of the organic layer over anhydrous Na_2SO_4 was followed by filtration and evaporation to provide 96 mg (quant) of **308** as a clear oil.

^{1}H NMR (400MHz) (DMSO-d_6) δ DMSO: 1.20-1.40 (4H, m); 1.36 (18H, s); 2.12 (1H, dd, J=7.9, 14.3 Hz); 2.21 (1H, dd, J=5.5, 13.9 Hz); 2.84 (2H, broad m); 3.27 (1H, m); 3.40-3.50 (1H, m); 3.67 (1H, broad s); 4.07 (1H, m); 5.02 (1H, ½ ABq, J=13.2 Hz); 5.04 (1H, ½ Abq, J=13.2 Hz); 6.62 (1H, d, J=8.7 Hz); 6.73 (1H, broad t); 7.30-7.36 (5H, m); 7.41 (1H, d, J=7.9 Hz); 7.93 (1H, broad t). ^{13}C NMR (100MHz) (DMSO-d_6) δ DMSO: 26.3, 28.3, 28.3, 31.3, 41.2, 47.5, 54.0, 65.5, 77.3, 77.4, 127.8, 127.8, 128.3, 136.9, 155.0, 155.5, 155.9, 170.7, 172.0. IR (NaCl, depos. from $CHCl_3$): 3334, 3066, 3035, 2977, 2934, 1693, 1526, 1454, 1411, 1393, 1367, 1342, 1291, 1251, 1170, 1064, 1028. HRMS (FAB^+) calc. for $C_{27}H_{43}N_4O_9$ (MH^+) 567.3030, found 567.3033. $[\alpha]_D^{25}$=-3.3 (*c*=1.0, $CHCl_3$).





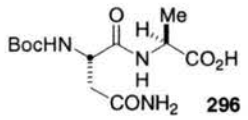
dedIII-1533-400



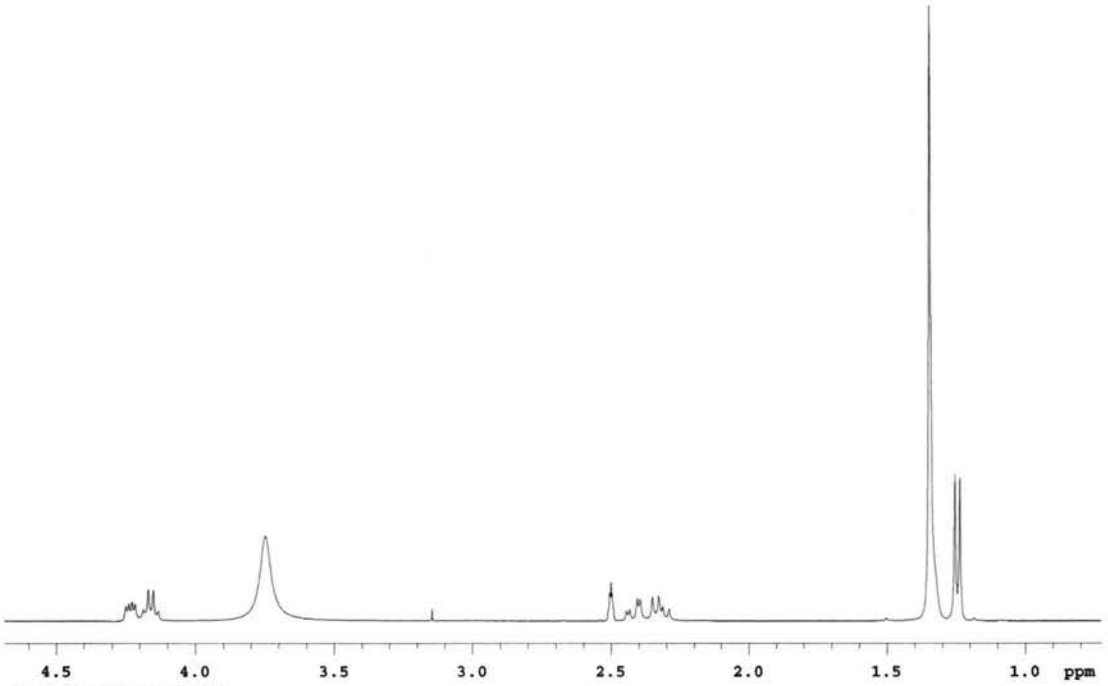
dedIII-1533-100-2

Boc-Asn-Ala-OH (296). A solution of **282** (386 mg, 0.98 mmol, 1 eq) in MeOH (18 mL) was purged with argon. To this solution was added 10% palladium on carbon (103 mg, 0.097 mmol, 0.1 eq). Hydrogen gas was bubbled through the mixture and a balloon of hydrogen attached to the flask. After stirring for 3h, the starting material was shown to be consumed by TLC (75:20:5 CH_2Cl_2:EtOAc:*i*-PrOH). Argon was bubbled through the reaction and the palladium on carbon removed by filtration through celite. Evaporation of the solvent provided **5** (291 mg, 98% yield) as a white crystalline solid (m.p.=195-197°C, recryst. MeOH / Et_2O).

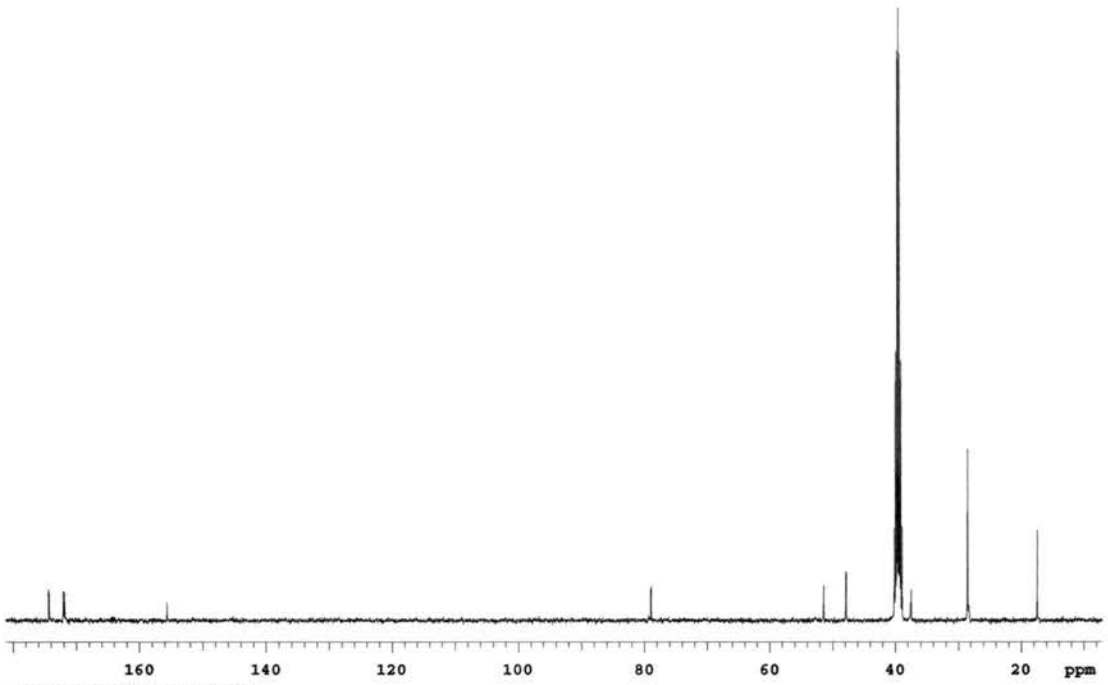
^{1}H NMR (400 MHz) (DMSO-d_6, D_2O exchange) δ: 1.24 (3H, d, J=7.5 Hz); 1.35 (9H, s); 2.32 (1H, dd, J=9.0, 15.0 Hz); 2.42 (1H, dd, J=4.5, 15.0 Hz); 4.16 (1H, q, J=7.5 Hz); 4.23 (1H, dd, J=4.5, 9.0 Hz). ^{13}C NMR (100 MHz) (DMSO-d_6, D_2O exchange) δ: 17.5, 28.5, 37.5, 47.9, 51.4, 78.9, 155.6, 171.9, 172.1, 174.3. IR (NaCl, depos. from MeOH): 3584, 3320, 3210, 2980, 2936, 1717, 1662, 1615, 1558, 1539, 1507, 1456, 1393, 1367, 1318, 1296, 1238, 1162, 1054, 1022. HRMS (FAB$^+$) calc. for $C_{12}H_{22}N_3O_6$ (MH^+) 304.1509; found 304.1502. $[\alpha]_D^{25}$=-14.7 (*c*=0.6, MeOH).



296



dedIII-1138rt2d400



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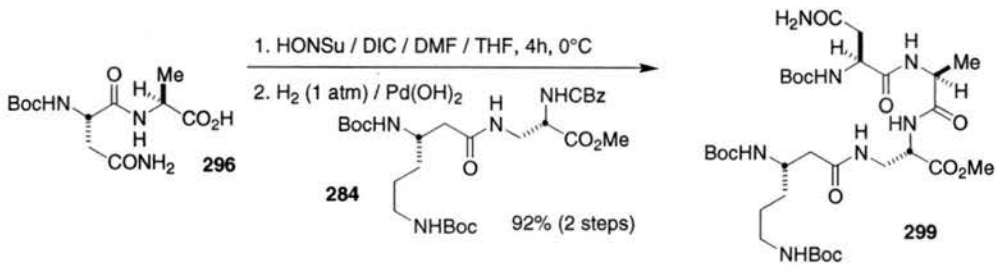
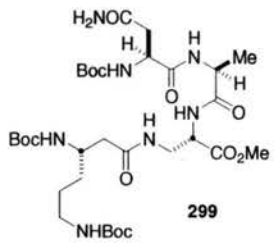
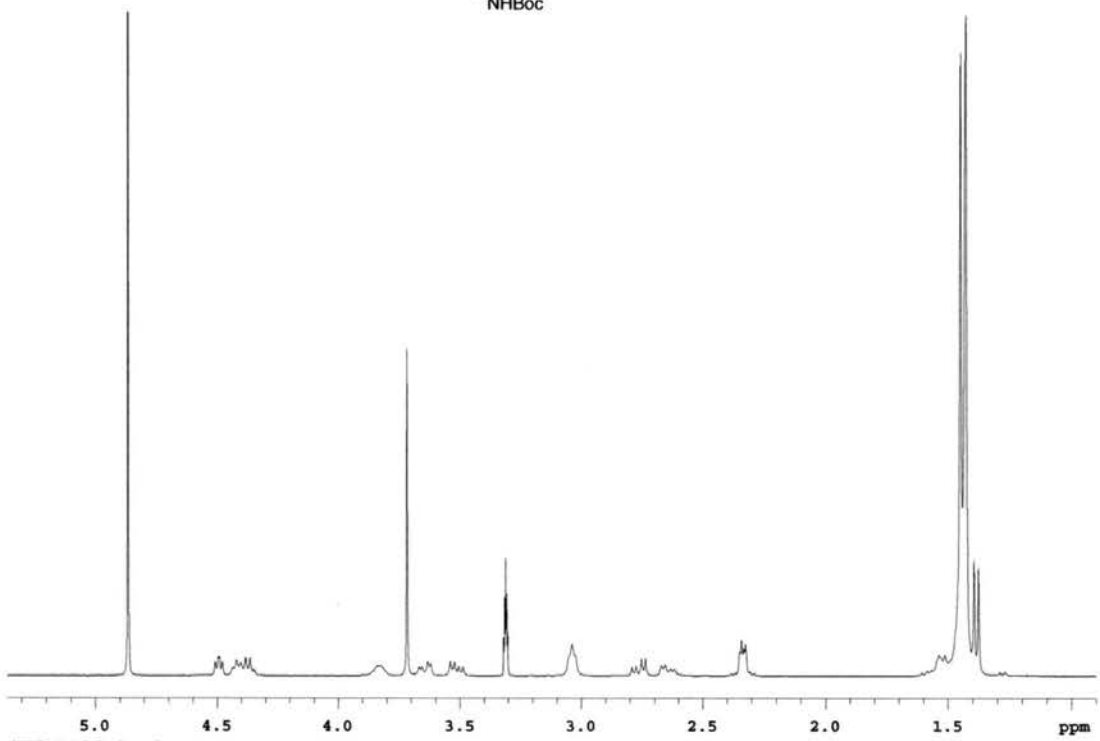


3-(3*S*,6-Bis-*tert*-butoxycarbonylamino-hexanoylamino)-2*S*-[2*S*-(2*S*-*tert*-butoxycarbonylamino-3-carbamoyl-propionylamino)-propionylamino]-propanoic acid methyl ester (299). A solution of **296** (50 mg, 0.16 mmol, 1 eq) and HONSu (66 mg, 0.64 mmol, 4 eq) in THF (3 ml) and DMF (700 μL) was cooled to 0°C and treated with diisopropylcarbodiimide (25 μL, 0.19 mmol, 1.2 eq). After stirring for 4h, allowing the *N*-hydroxysuccinimidyl ester to form, **284** (93 mg, 0.16 mmol, 1 eq) and $Pd(OH)_2$ (22 mg, 0.030 mmol, 0.2 eq) were added to the reaction mixture, and the solution purged with argon. After cooling to 0°C, hydrogen was bubbled through the solution and a hydrogen balloon subsequently attached to the flask. The hydrogenation was stirred 16h, allowing to warm to room temperature. After purging the reaction with argon, the mixture was diluted with methanol and filtered through Celite. Additional dilution with CH_2Cl_2 was followed by washing with dilute aqueous HCl, saturated sodium bicarbonate, and brine twice. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to provide a crude solid. Silica gel chromatography (gradient elution from 5% to 20% MeOH in CH_2Cl_2) provided 108 mg (92%) of **299** as an amorphous solid. (m.p.: 185°C, precip. from MeOH / Et_2O).

^{1}H NMR (400MHz) (CD_3OD) δ MeOH: 1.39 (3H, d, J=7.3 Hz); 1.43 (18H, s); 1.45 (9H, s); 1.45-1.62 (4H, m); 2.32 (1H, dd, J=6.8, 14.3 Hz); 2.36 (1H, dd, J=7.0, 14.3 Hz); 2.64 (1H, dd, J=6.2, 15.4 Hz); 2.76 (1H, dd, J=6.6, 15.4 Hz); 3.04 (2H, broad t); 3.51 (1H, dd, J=7.0, 13.9 Hz); 3.64 (1H, dd, J=4.9, 13.9 Hz); 3.72 (3H, s); 3.83 (1H, m); 4.38 (1H, q, J=7.3 Hz); 4.42 (1H, m); 4.49 (1H, dd, J=4.9, 7.0). ^{13}C NMR (100 MHz) (CD_3OD) δ MeOH: 17.7, 27.7, 28.8, 29.0, 29.0, 33.0, 38.4, 41.2, 41.3, 42.9, 50.6, 52.9, 53.2, 54.0, 79.9, 80.1, 81.0, 157.6, 158.0, 158.6, 171.9, 173.8, 174.2, 175.0, 175.3. IR (NaCl, deposited from: MeOH / CH_2Cl_2): 3283, 3083, 2978, 2933, 1736, 1689, 1645, 1555, 1524, 1447, 1392, 1367, 1251, 1172, 1056, 1014. HRMS (FAB^+) calc. for $C_{32}H_{58}N_7O_{12}$ (MH^+) 732.4143; found 732.4157.



299



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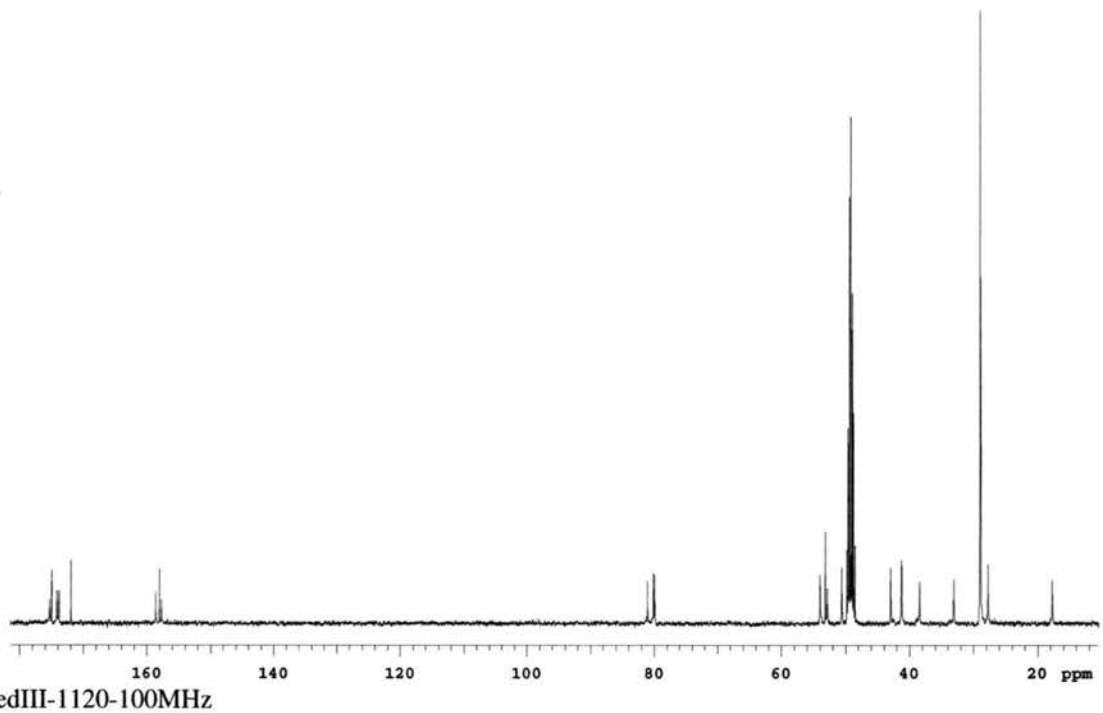

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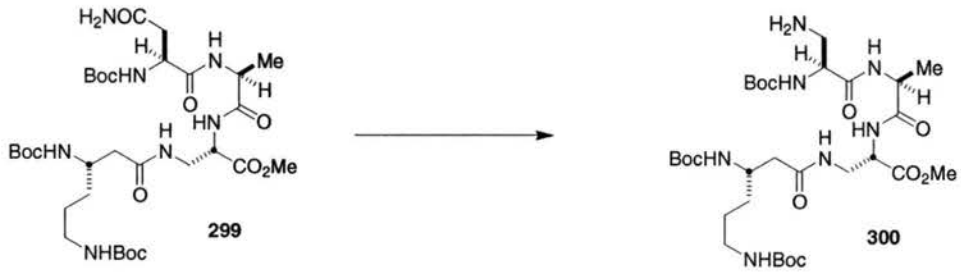
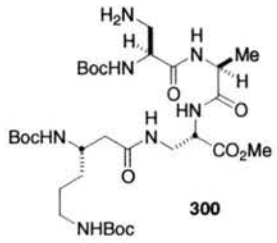


Amine-methyl ester 300. To a solution of **299** (273 mg, 0.37 mmol, 1 eq) in MeCN (7 ml) and water (7 ml) bis(trifluoroacetoxy)iodobenzene (242 mg, 0.56 mmol, 1.5 eq) was added with stirring. DMF (7 ml) was then added and the stirring continued for 15 minutes. Pyridine (74 μL, 1.11 mmol, 3 eq) was added, and the reaction stirred for 6 h. The reaction was then evaporated *in vacuo* to provide a yellow syrup. The residue was then dissolved in EtOAc and poured into aq. $NaHCO_3$. After mixing the layers, the organic layer was removed and the aqueous layer extracted again with EtOAc. The organic layers were combined and washed twice with brine. After drying the organic layer over Na_2SO_4, it was filtered and evaporated *in vacuo* to provide a clear oil. The oil was then purified via silica gel chromatography (gradient elution 10% MeOH in CH_2Cl_2 to 20% MeOH in CH_2Cl_2) resulting in isolation of 201 mg (77%) of **300** as a glass after evaporation of the compound containing fractions.

^{1}H NMR (300MHz) (DMSO-d_6, 2 drops of D_2O, 353K) δ: 1.26 (3H, d, J=7.0 Hz); 1.37 (9H, s); 1.38 (9H, s); 1.39 (9H, s); 1.31-1.46 (4H, m); 2.19 (1H, dd, J=7.0, 14.0 Hz); 2.26 (1H, dd, J=6.0, 14.0 Hz); 2.77 (1H, dd, J=6.0, 13.5 Hz); 2.83 (1H, dd, J=6.5, 13.5 Hz); 2.90 (2H, broad t.); 3.34 (1H, dd, J=7.0, 13.5 Hz); 3.43 (1H, dd, J=6.0, 13.5 Hz); 3.62 (3H, s); 3.64-3.73 (1H, m); 3.96 (1H, dd, J=6.0, 6.0 Hz); 4.30 (1H, q, J=7.0 Hz); 4.35 (1H,

dd, J=6.5, 6.5 Hz). ^{13}C NMR (100 MHz) (CD_3OD) δ: 17.8, 27.7, 28.9, 29.0, 29.0, 33.1, 41.3, 41.4, 42.9, 44.6, 50.7, 53.2, 54.1, 57.5, 80.0, 80.2, 81.0, 158.0, 158.0, 158.6, 172.0, 173.5, 174.4, 175.1. IR (NaCl, deposited from: MeOH / CH_2Cl_2): 3304, 3056, 2978, 2934, 1685, 1526, 1454, 1392, 1367, 1251, 1170 cm^{-1}. HRMS (FAB^+) calc. for $C_{31}H_{58}N_7O_{11}$ (MH^+) 704.4194; found 704.4183.



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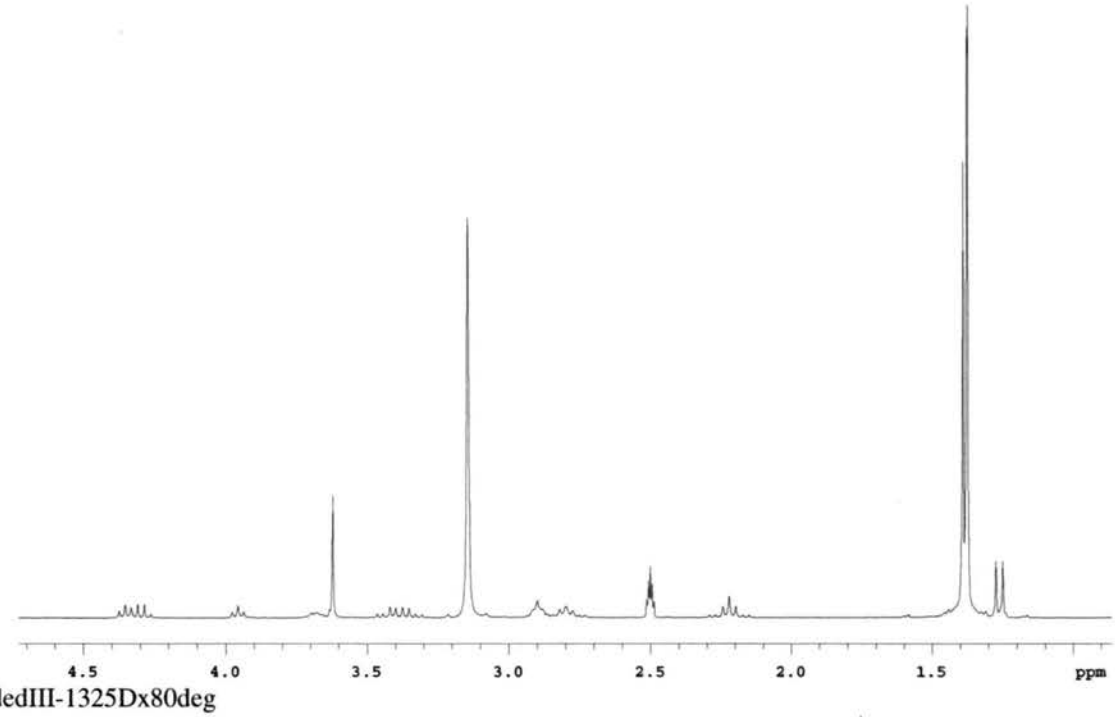

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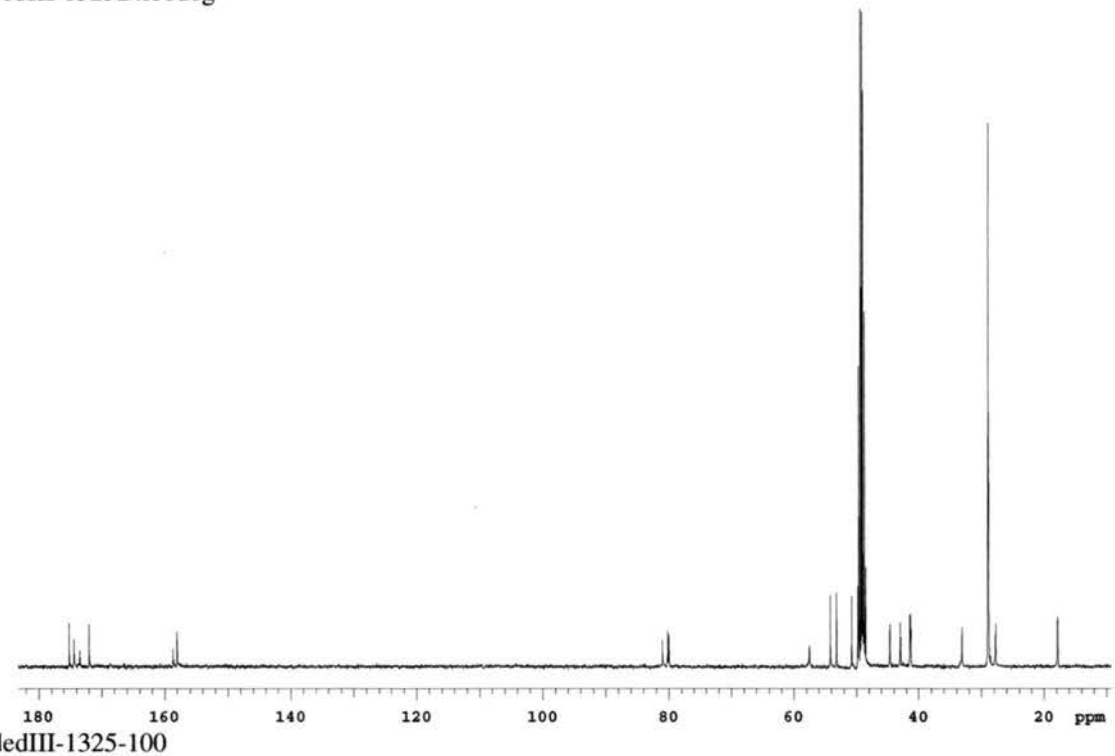
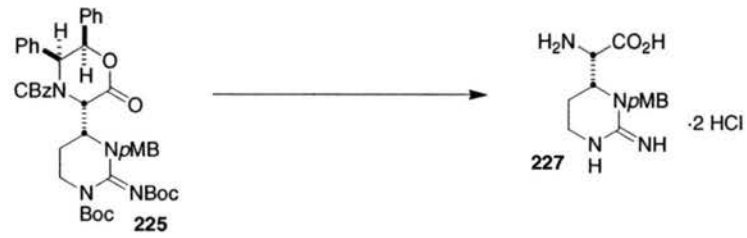
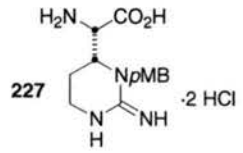


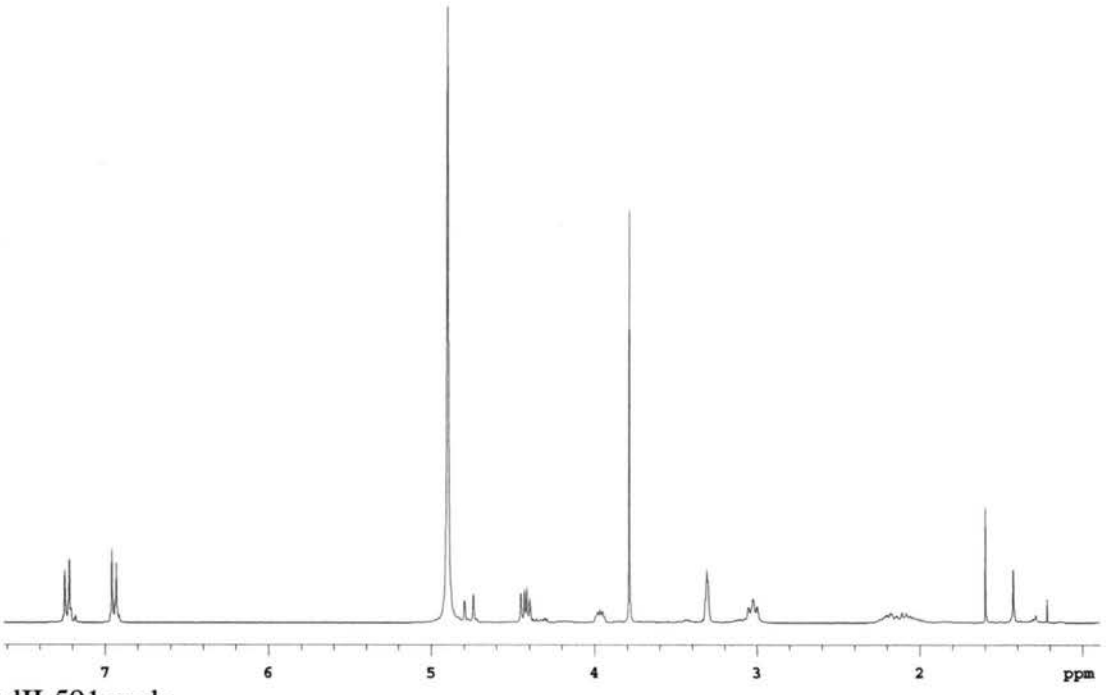
dedIII-1325-100



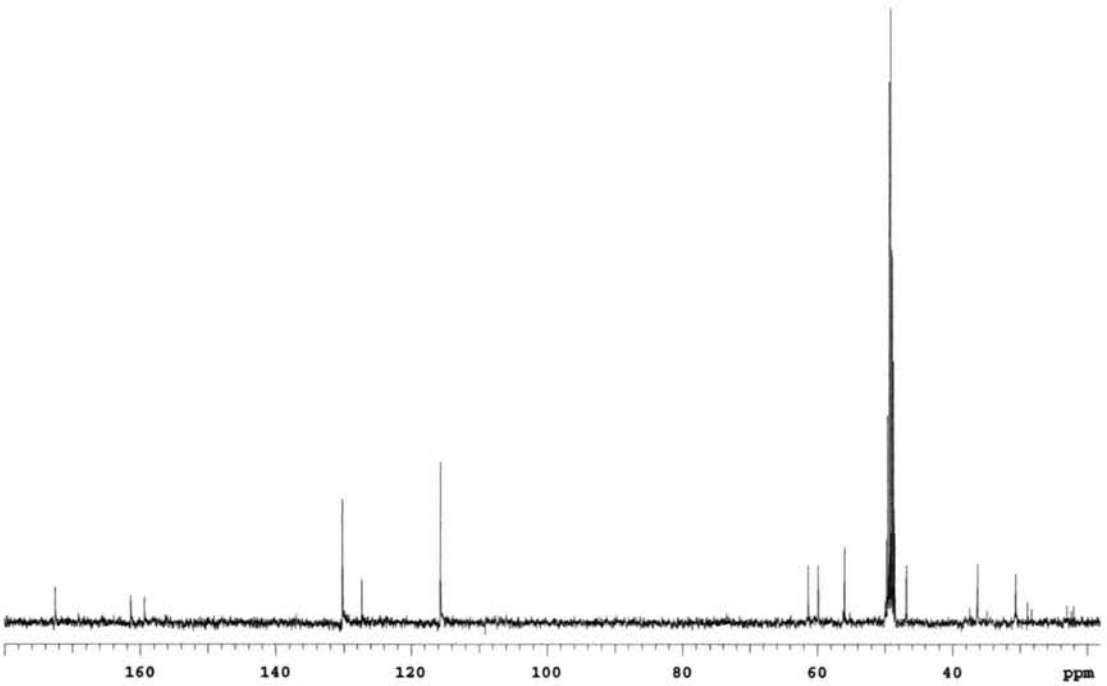
***N^{β}-p*-Methoxybenzyl-capreomycidine:** A solution of **225** (122 mg, 0.15 mmol., 1eq.) in 1.5:1 THF:H_2O (19.5 ml) was added to a pressure tube and purged with argon. To this solution was added $PdCl_2$ (88mg, 0.45 mmol., 3 eq.) and the tube pressurized with hydrogen gas (90 psi). The pressure was released, and the tube filled again to the same pressure. This was repeated 3 times more. The pressurized tube was then placed in a 68 °C oil bath and the reaction stirred for 1h. After removing the pressure vessel from the oil bath, and release of the hydrogen gas, the solution was purged with argon. Filtration through a glass wool plug (not Celite) to remove the palladium, and evaporation provided 52 mg (95%) of crude **227** as an off-white solid. This material is used crude in the next step. It should be noted that over the many times that this reaction has been performed, varying amounts of over-reduction (5-20%) have been observed.

^{1}H NMR (300MHz) (CD_3OD) δ MeOH: 1.90-2.30 (2H, m); 3.02 (2H, t); 3.79 (3H, s); 3.97 (1H, ddd, J=8.1, 3.3, 3.3 Hz); 4.43 (1H, d, J=4.4 Hz); 4.43 (1H, ½ ABq, J=16.3 Hz); 4.77 (1H, ½ ABq, J=16.3 Hz); 6.95 (2H, d, J=8.8 Hz); 7.24 (2H, d, J=8.8 Hz). ^{13}C NMR (100 MHz) (CD_3OD) δ MeOH: 30.6, 36.3, 46.8, 56.0, 59.8, 61.3, 115.7, 127.3, 130.2, 159.4, 161.4, 172.6.



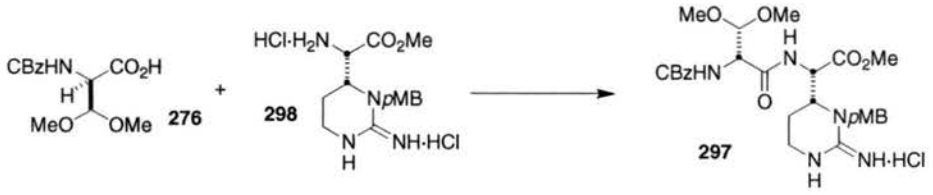


dedII-591crude



dedIII-1025-100MHzC13

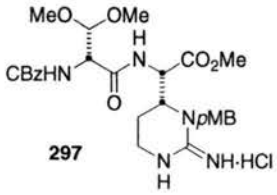
Dipeptide 297. Acetyl chloride (1 mL) was added to methanol (10 mL) and the resulting solution stirred 15 min. This freshly prepared solution of 1.5 M MeOH·HCl was then added to a flask containing crude **227** (37 mg, 0.10 mmol, 1 eq), and the mixture was heated at reflux for 2.5h. Removal of the solvent from the reaction *in vacuo* was followed by trituration with ether to provide the crude methyl ester hydrochloride salt (**298**), which was used immediately for the next step.



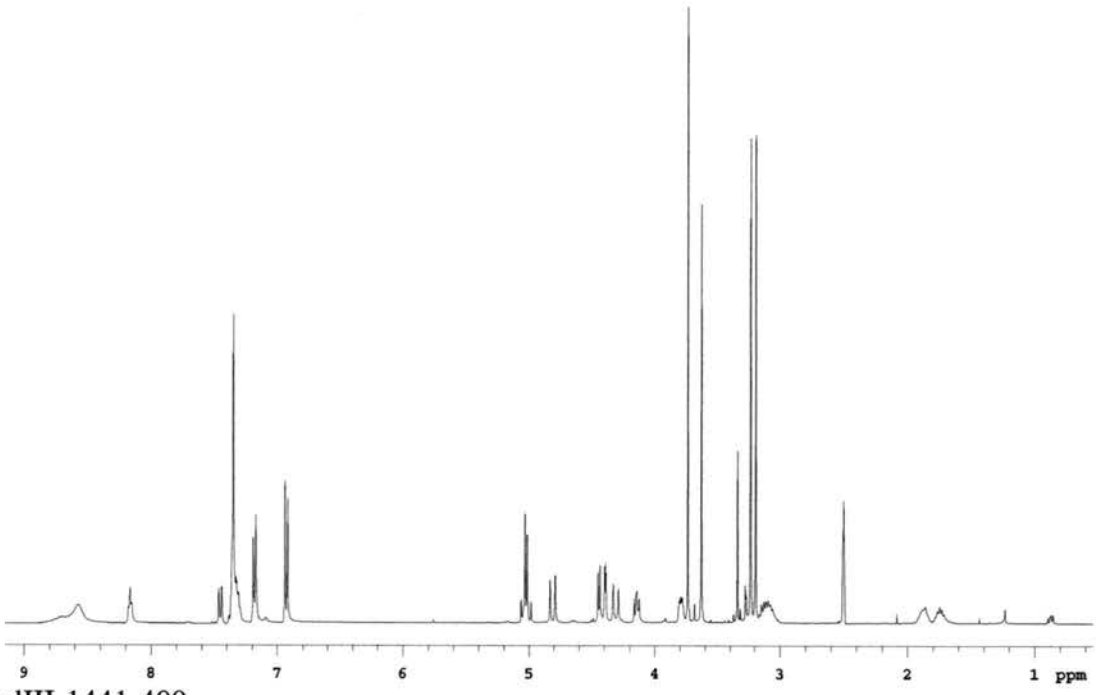
A solution of **276** (28 mg, 0.10 mmol, 1 eq) in THF (2 mL) was cooled to 0°C with stirring. *N*-methylmorpholine (11 μL, 0.10 mmol, 1eq) was added dropwise to the solution followed by the dropwise addition of isobutylchloroformate (13 μL, 0.10 mmol, 1 eq). After stirring for 30 min, the resulting mixed carbonic anhydride was added to a solution of the previously prepared **298** in DMF (2 mL) and THF (2 mL) at 0°C. *N*-methylmorpholine (11 μL, 0.10 mmol, 1eq) in THF (100 μL) was added dropwise followed by DMF (2.5 mL) and THF (2 mL). The reaction was stirred at 0°C for 3h at which time methanol (5 mL) was added and the solvent removed *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed twice with brine. After drying of the organic layer over Na_2SO_4, and filtration, the solvent was removed in vacuo to provide a clear oil which was chromatographed on silica gel (gradient elution from 15% to 25% MeOH in

CH_2Cl_2) to provide 34 mg (56%) **297** as a glass. Typically, the yield for this reaction ranged from 40-55%.

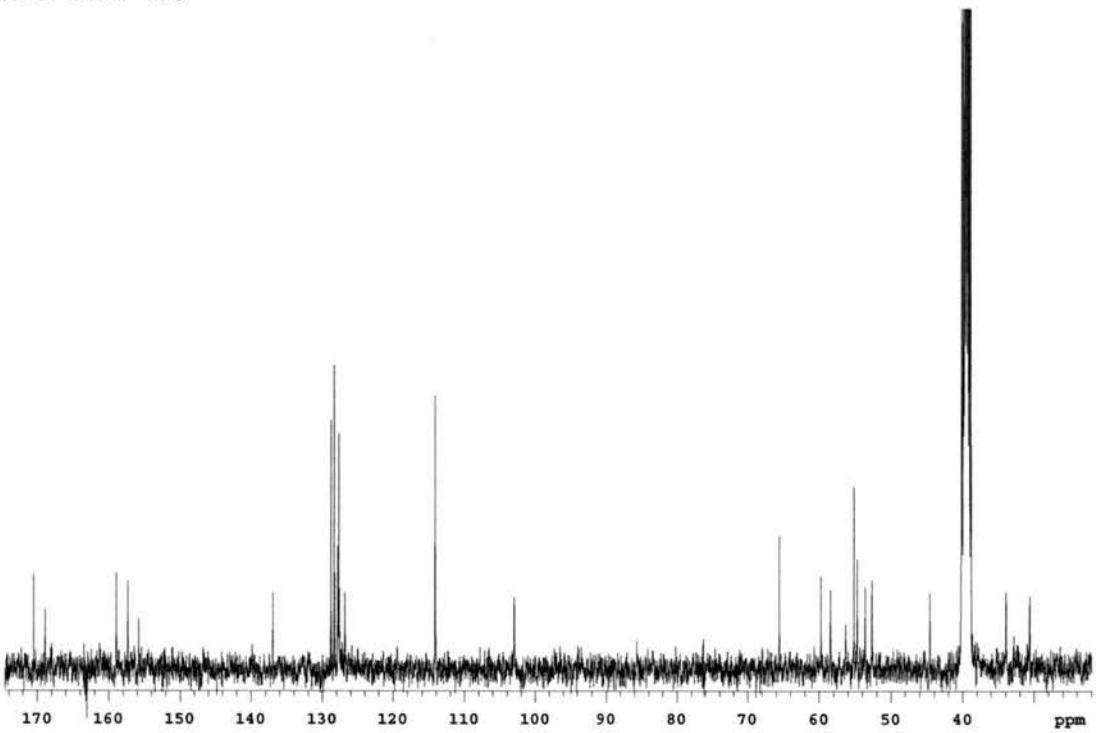
^{1}H NMR (400MHz) (DMSO-d_6) δ DMSO: 1.67-1.80 (1H, m); 1.80-1.94 (1H, m); 3.01-3.17 (2H, m); 3.19 (3H, s); 3.23 (3H, s); 3.63 (3H, s); 3.74 (3H, s); 3.79 (1H, ddd, J=3.6, 3.6, 7.9 Hz); 4.14 (1H, dd, J=6.8, 8.7 Hz); 4.31 (1H, ½ ABq, J=16.4 Hz); 4.39 (1H, d, J=4.3 Hz); 4.44 (1H, d, J=6.8 Hz); 4.81 (1H, ½ ABq, J=16.2 Hz); 5.00 (1H, ½ ABq, J=12.8 Hz); 5.04 (1H, ½ ABq, J=12.8 Hz); 6.93 (2H, d, J=8.7 Hz); 7.18 (2H, d, J=8.5 Hz); 7.28-7.39 (5H, m); 7.45 (1H, d, J=9.0 Hz); 8.17 (1H, t, J=5.1 Hz); 8.42-8.85 (3H, broad m). ^{13}C NMR (100 MHz) DMSO-d_6) δ DMSO 30.6, 34.0, 44.6, 52.7, 53.6, 54.7, 55.1, 56.3, 58.4, 59.7, 65.5, 103.0, 114.1, 126.8, 127.7, 127.8, 128.3, 128.8, 136.9, 155.8, 157.3, 158.9, 168.9, 170.4.



297

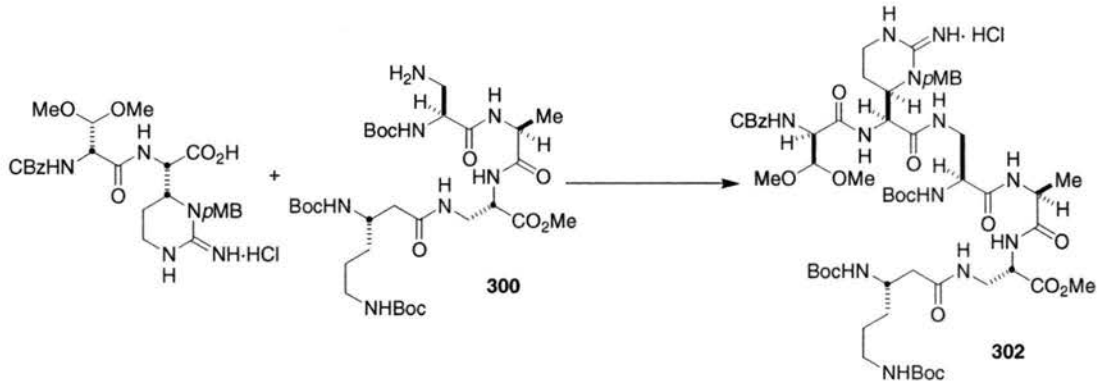


dedIII-1441-400



dedIII-1441-100

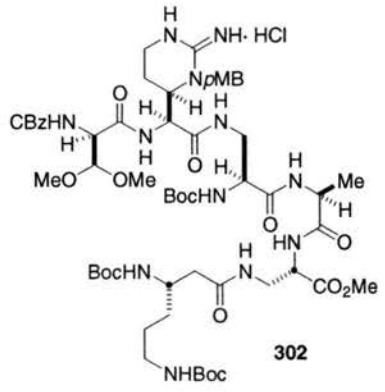
Hexapeptide 302. Compound **297** (32 mg, 0.053 mmol, 1 eq) was dissolved in methanol (3 mL) and THF (0.4 mL). Dropwise addition of 1N LiOH (0.13 mL) was followed by stirring for 1h. An additional amount of 1N LiOH was added (0.04 mL) and the reaction stirred for 1h more. The reaction was then diluted with dd H_2O and adjusted to pH 6 with dilute aq. HCl. The solvent was evaporated *in vacuo*, and the resulting carboxylic acid was used crude in the next step.



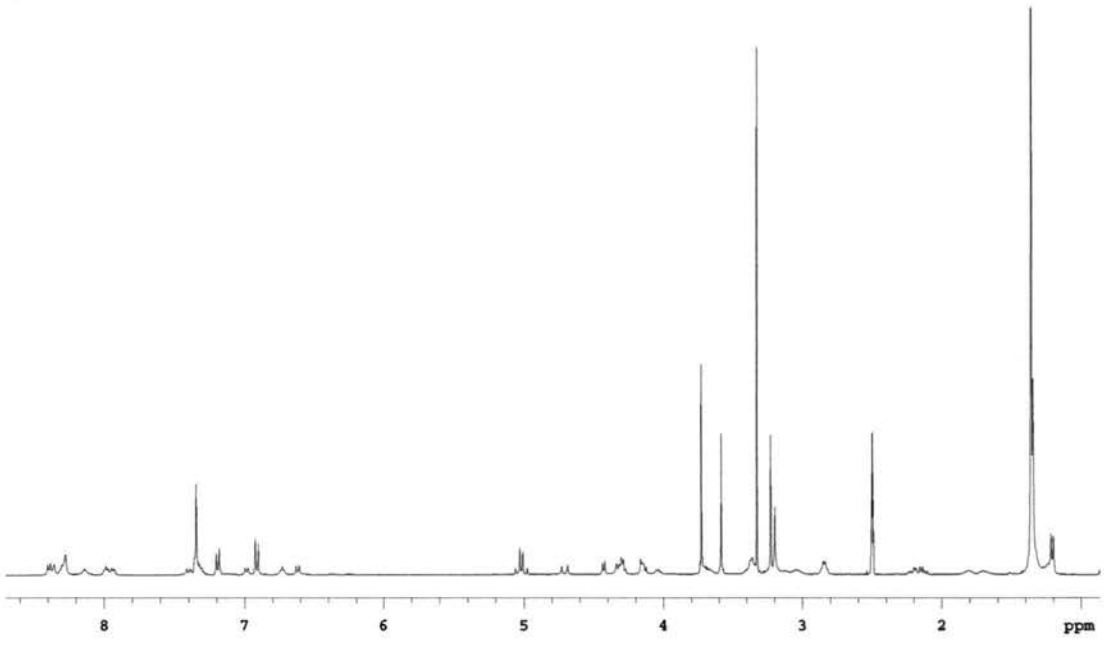
The crude carboxylic acid (0.031 mmol, 1 eq, based on starting **297**) was then dissolved with **300** (22 mg, 0.031 mmol, 1 eq) into THF (3 mL) and DMF (2 mL) and cooled to 0°C. To this solution was added HOAt (11 mg, 0.081 mmol, 2.6 eq) and EDCI (16 mg, 0.083 mmol, 2.7 eq) and the reaction was stirred 16h, allowing to warm to room temperature. The reaction mixture was then partitioned between CH_2Cl_2 and brine. The organic layer was removed and the aqueous layer was again extracted with CH_2Cl_2. The combined organics were washed once with brine, dried over Na_2SO_4, filtered, and

evaporated to provide an off-white crude solid. This solid can be further purified by silica gel chromatography (gradient elution from 15% to 25% MeOH in CH_2Cl_2) to provide 25 mg (63%) of hexapeptide **302** as a glass. Yields for this reaction ranged from 50-90%.

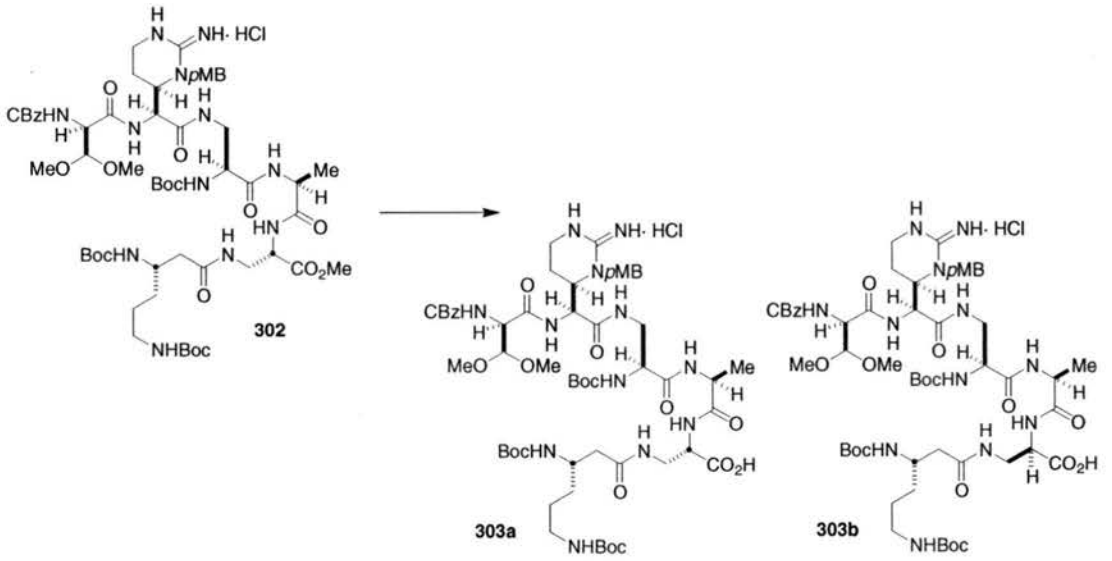
^{1}H NMR (400MHz) (DMSO-d_6) δ DMSO: 1.18-1.38 (4H, m); 1.21 (3H, d, J=6.8 Hz); 1.35 (9H, s); 1.36 (18H, s); 1.63-1.76 (1H, m); 1.79-1.88 (1H, m); 2.13 (1H, dd, J=7.7, 14.1 Hz); 2.21 (1H, dd, J=6.0, 14.5 Hz); 2.84 (2H, symm. m); 2.98-3.09 (1H, m); 3.09-3.19 (1H, m); 3.20 (3H, s); 3.23 (3H, s); 3.31-3.43 (4H, m); 3.58 (3H, s); 3.63-3.74 (2H, m); 3.73 (3H, s); 4.04 (1H, q, J=7.5 Hz); 4.13-4.16 (2H, m); 4.27-4.34 (3H, m); 4.43 (1H, d, J=6.6 Hz); 4.71 (1H, ½ Abq, J=16.4 Hz); 5.00 (1H, ½ Abq, J=12.8 Hz); 5.04 (1H, ½ Abq, J=12.8 Hz); 6.62 (1H, d, J=8.7 Hz); 6.73 (1H, t, J=5.3 Hz); 6.91 (2H, d, J=8.7 Hz); 6.99 (1H, d, J=8.3 Hz); 7.19 (2H, d, J=8.5 Hz); 7.28-7.36 (5H, m); 7.40 (1H, d, J=9.0 Hz); 7.94 (1H, d, J=7.5 Hz); 7.99 (1H, t, J=5.5 Hz); 8.14 (1H, m); 8.25-8.33 (3H, m); 8.33-8.43 (2H, m).



302

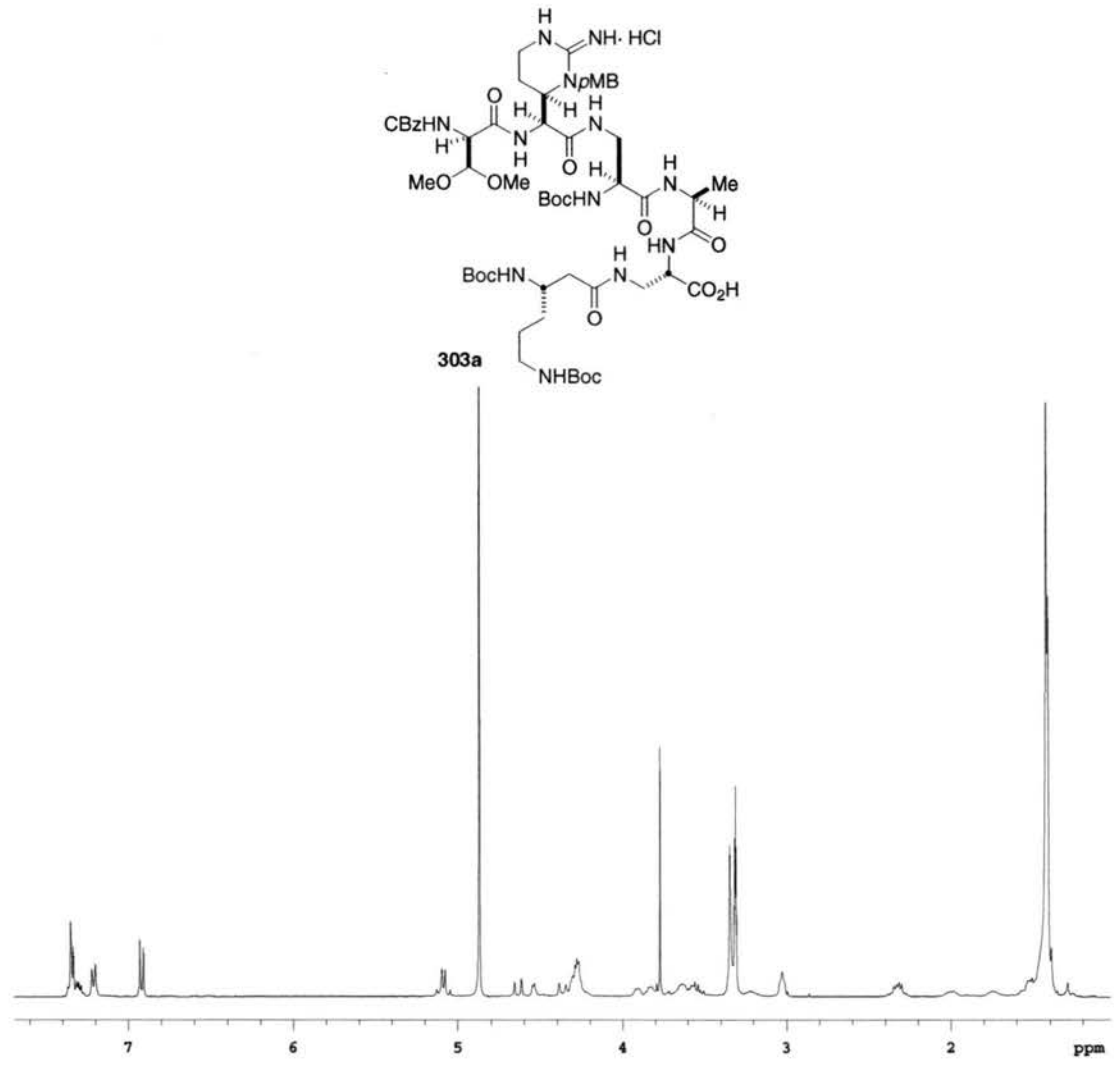


dedIII-1458-400

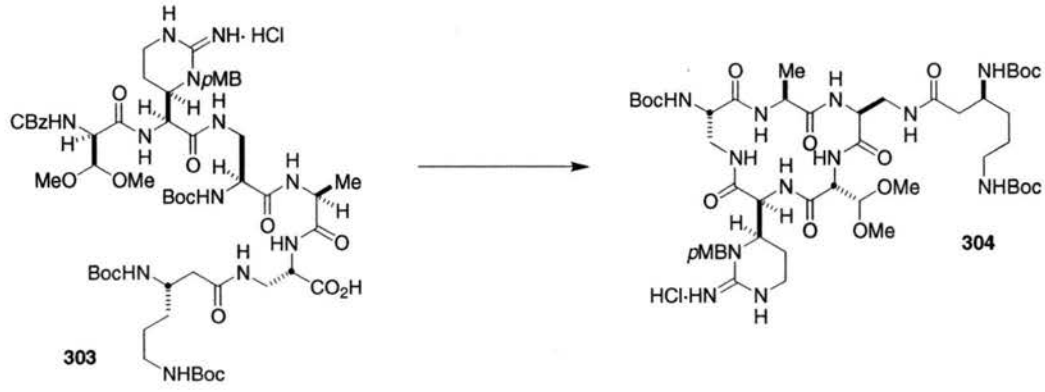


Carboxylic acids 303a and 303b. To a solution of **302** (31 mg, 0.024 mmol, 1 eq) in methanol (1.4 mL) and THF (0.19 mL), 1N LiOH (0.09 mL, 0.090 mmol, 3.8 eq) was added, and the reaction stirred for 1h at room temp. Dilution of the mixture with dd H_2O was followed by adjustment of the solution to pH 6 with dilute aqueous HCl. Evaporation of the solvent *in vacuo* provided a residue that was triturated with dd H_2O to remove the salts and provide crude epimers **303a** and **303b** (~3:1 by mass). PTLC of the crude product (15% MeOH in CH_2Cl_2, eluted with 2.5:1 CH_2Cl_2:MeOH) provided **303a** (20 mg) and **303b** (7 mg).

^{1}H NMR (400MHz) (CD_3OD) δ CD_2HOD: 1.38-1.60 (34H, m); 1.68-1.81 (1H, m); 1.93-2.06 (1H, m); 2.29 (1H, dd, J=6.8, 14.1 Hz); 2.36 (1H, dd, J=7.3, 14.7 Hz); 3.03 (2H, broad s); 3.13-3.28 (2H, m); 3.35 (6H, s); 3.53 (1H, dd, J=7.5, 13.6 Hz); 3.56-3.73 (3H, m); 3.77 (3H, s); 3.80-3.87 (1H, m); 3.87-3.95 (1H, m); 4.19-4.33 (5H, m); 4.37 (1H, ½ ABq, J=16.2 Hz); 4.54 (1H, d, J=4.5 Hz); 4.64 (1H, ½ ABq, J=16.2 Hz); 5.07 (1H, ½ ABq, J=12.6 Hz); 5.11 (1H, ½ ABq, J=12.8 Hz); 6.92 (2H, d, J=8.7 Hz); 7.21 (2H, d, J=8.3 Hz); 7.26-7.38 (5H, m).

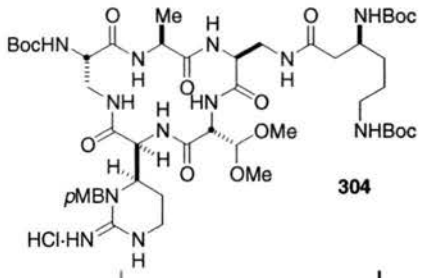


dedIII-1468B-400

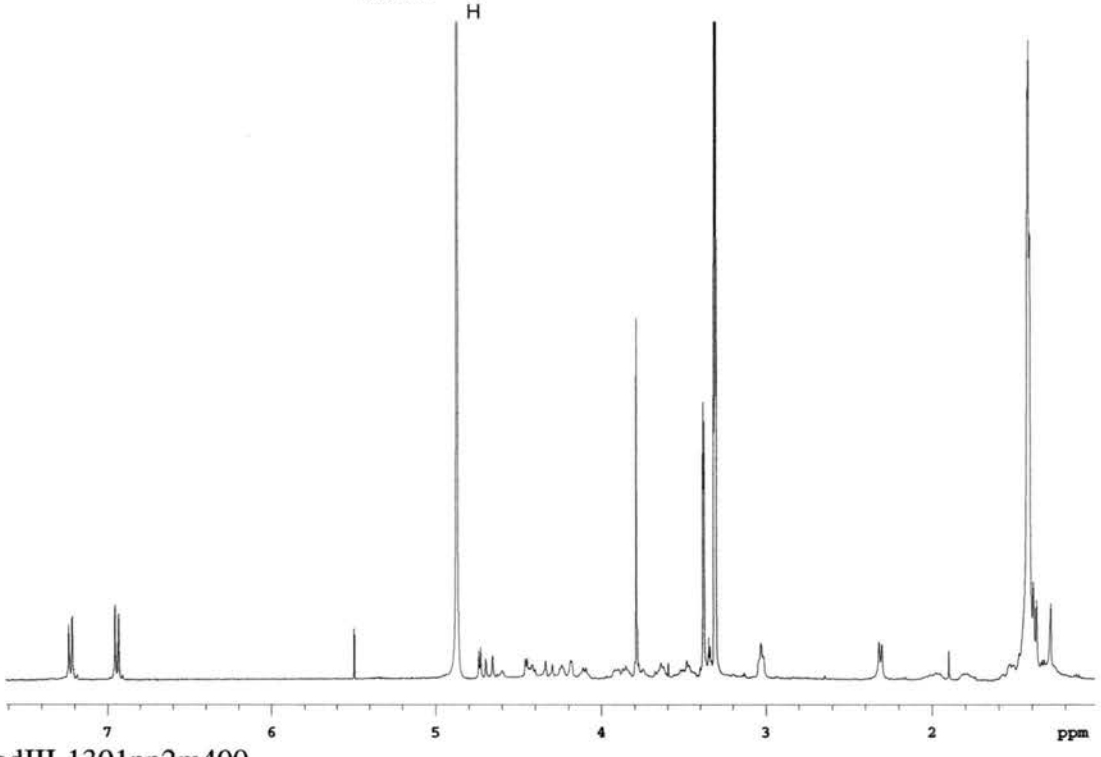


Macrocycle 304. Crude product **303** (0.027 mmol (based on **302**), 1eq) was dissolved in methanol (3.5 mL) and purged with argon. Careful addition of 10% Pd/C (15 mg, 0.014 mmol, 0.5 eq) was followed by the bubbling of hydrogen through the solution. A balloon of hydrogen was fixed to the top of the flask and the reaction was stirred 1h. The completed reaction was then purged with argon and filtered through Celite to remove the Pd/C. Evaporation of the solvent *in vacuo* provided the crude amino acid as a glass. The crude amino acid was then dissolved in DMF (4 mL) and THF (25 mL) under argon. At room temperature, EDCI (42 mg, 0.22 mmol, 8 eq) and HOAt (30 mg, 0.22 mmol, 8 eq) were added and the reaction stirred for 48h. The solvents were subsequently evaporated *in vacuo* and the resulting residue partitioned between CH_2Cl_2 and brine. Removal of the organic layer was followed by extraction of the aqueous layer again with CH_2Cl_2. The combined organic extracts were washed once with brine and dried over anhydrous Na_2SO_4 . Removal of the Na_2SO_4 by filtration and evaporation of the solvent provided a crude solid which was chromatographed on silica gel (15% to 20% MeOH in CH_2Cl_2) to provide 14 mg (48%) of macrocycle **304**. Material of additional purity can be obtained by PTLC (15% MeOH in CH_2Cl_2, eluted with 2.5:1 CH_2Cl_2:MeOH). The yields for this reaction ranged from 21-50%.

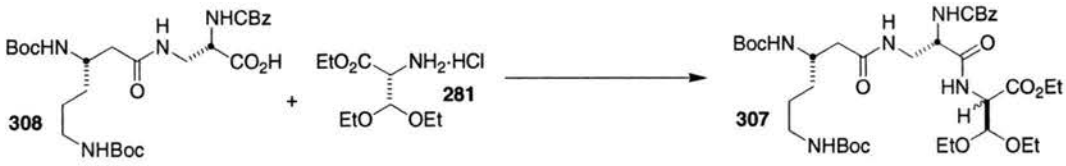
^{1}H NMR (400MHz) (CD_3OD) δ CD_2HOD: 1.32-1.59 (4H, m); 1.38 (3H, d, J=7.2 Hz); 1.42 (9H, s); 1.43 (9H, s); 1.43 (9H, s); 1.73-1.86 (1H, m); 1.91-2.07 (1H, m); 2.31 (2H, d, J=6.8 Hz); 3.03 (2H, t, J=6.3 Hz); 3.33-3.40 (2H, m); 3.38 (3H, s); 3.38 (3H, s); 3.42-3.56 (2H, m); 3.59-3.69 (2H, m); 3.79 (3H, s); 3.81-3.88 (1H, m); 3.88-3.94 (1H, m); 4.10 (1H, q, J=7.7 Hz); 4.18 (1H, d, J=3.2 Hz); 4.24 (1H, m); 4.32 (1H, ½ ABq, J=16.6 Hz); 4.41 (1H, dd, J=6.2, 7.9 Hz); 4.45 (1H, d, J=4.5 Hz); 4.68 (1H, ½ ABq, J=16.6 Hz); 4.74 (1H, d, J=4.5 Hz); 6.94 (2H, d, J=8.7 Hz); 7.22 (2H, d, J=8.7 Hz). HRMS (FAB^+) calc. for $C_{49}H_{81}N_{12}O_{15}$ (MH^+) 1077.5944; found 1077.5973.



304



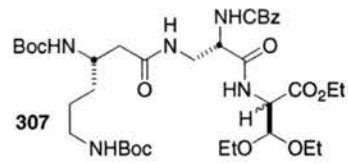
dedIII-1301pp2m400

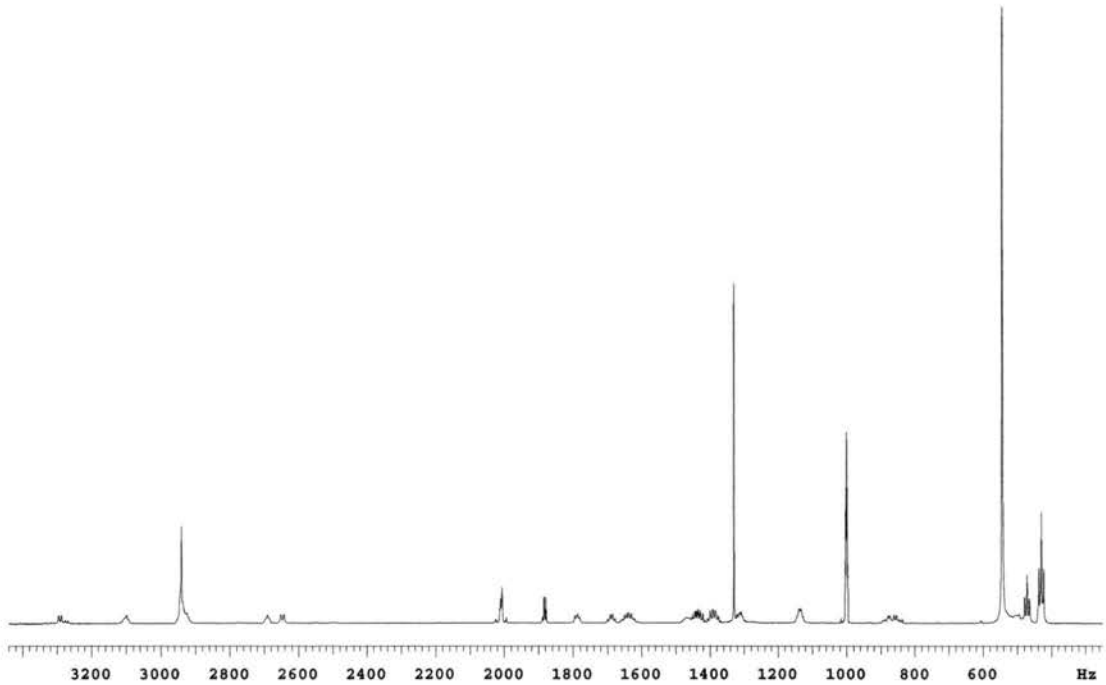


N^{α}-CBz-DAPA-N^{β}-(*N,N'*-di-Boc-β-Lys)-DEA-OEt (307): A solution of **308** (50 mg, 0.088 mmol, 1 eq) and **281** (21 mg, 0.088 mmol, 1 eq) in CH_2Cl_2 (1.8 mL) was cooled to 0°C. Dropwise addition of *N*-methylmorpholine (20 μL, 0.18 mmol, 2.05 eq) to the solution was followed by the addition of HOBt (18 mg, 0.13 mmol, 1.5 eq) and EDCI (18 mg, 0.092 mmol, 1.05 eq). The reaction was stirred for 1h at 0°C, diluted with EtOAc and washed with sat. aq. $NaHCO_3$, dilute aq. HCl, and brine twice. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to provide a crude yellow solid which was purified by silica gel chromatography (gradient elution from 25:1 MeOH:CH_2Cl_2 to 20:1 MeOH:CH_2Cl_2) to provide 60 mg (91%) of **307** as an amorphous white solid that is an ~2.6:1 mixture of epimers (^{1}H NMR).

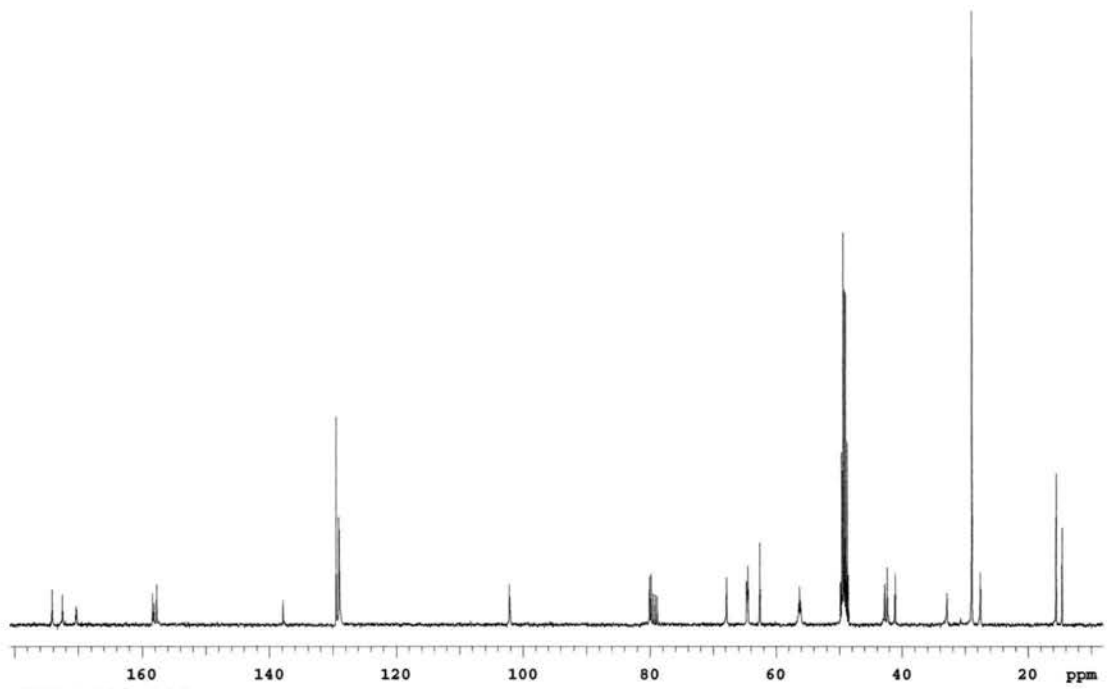
^{1}H NMR (400MHz) (DMSO-d_6) (mixture of epimers, * denotes minor epimer) δ DMSO: 1.07 (3H, t, J=7.0 Hz); 1.07 (3H, t, J=7.0 Hz); 1.18 (3H, t, J=7.0 Hz); *1.17 (3H, t, J=7.0 Hz); 2.12 (1H, dd, J=7.5, 13.9 Hz); 2.21 (1H, dd, J=5.8, 14.1 Hz); 2.84 (2H, symm. M); 3.15-3.40 (2H, m); 3.42-3.52 (2H, m); 3.53-3.73 (3H, m); 4.03-4.16 (2H, symm. m); 4.22 (1H, q, J=7.9 Hz); 4.44-4.49 (1H, m); 4.70 (1H, d, J=5.8 Hz); *4.71 (1H, d, J=4.9 Hz); 5.00 (1H, ½ ABq, J=12.6 Hz); *5.01 (1H, ½ ABq, J=12.6 Hz); 5.04 (1H, ½ ABq, J=12.6 Hz); *5.04 (1H, ½ ABq, J=12.6 Hz); 6.61 (1H, d, J=8.7 Hz); 6.72 (1H, t, 5.7 Hz); 7.28-7.39 (6H, m); 7.74 (1H, m); *8.18 (1H, d, J=8.1 Hz); 8.23 (1H, d, J=8.3 Hz). ^{13}C NMR (100 MHz) (5:1 CD_3OD:$CDCl_3$) δ CD_3OD 14.6, 15.6, 15.6, 27.6, 29.0,29.0, 32.9, 41.1, 42.3, 42.8, 56.1, 56.3, 56.4, 62.6, 64.4, 64.6, 64.7, 67.9, 79.8, 80.1, *102.1, 102.2, 129.0,

129.1, 129.5, 137.9, 157.7, 158.2, 158.4, 170.3, 172.5, 174.1. IR (NaCl, depos. from CH_2Cl_2): 3304, 2977, 2933, 2481, 2422, 1743, 1680, 1662, 1534, 1421, 1393, 1367, 1327, 1299, 1253, 1176, 1116, 1071, 1029, 1002 cm-1. HRMS (FAB^+) calc. for $C_{36}H_{60}N_5O_{12}$ (MH^+) 754.4238; found 754.4251.





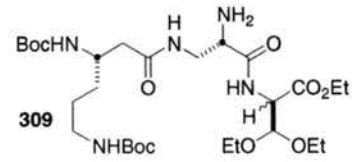
dedIII-1482-400

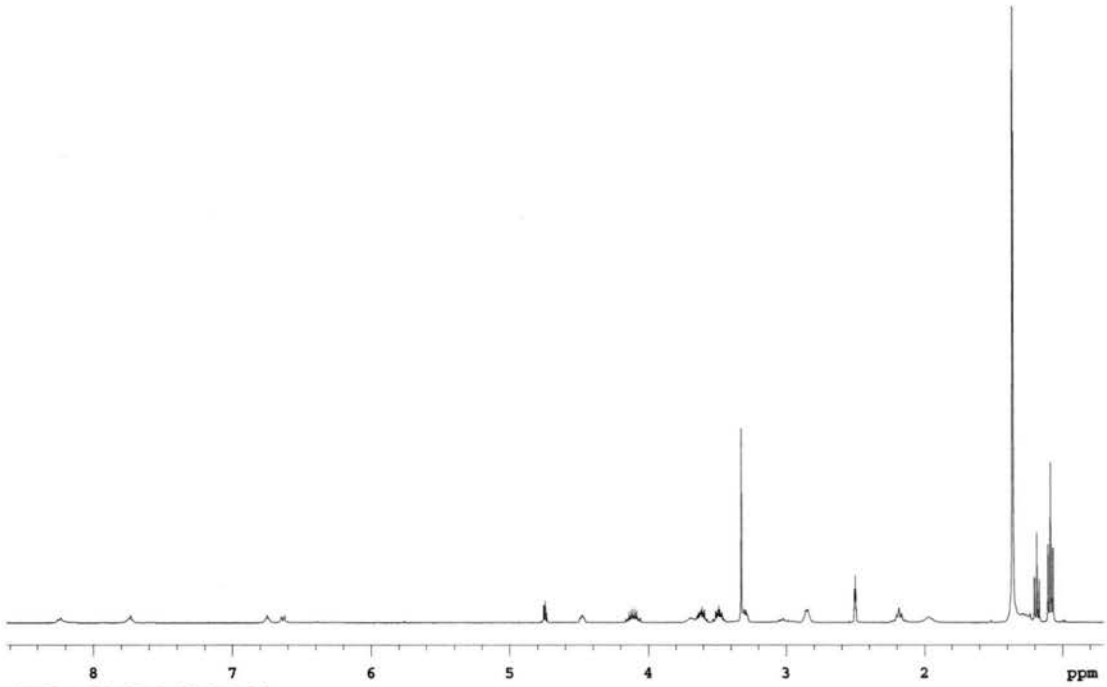


dedIII-1486-100

N^{β}-(*N,N'*-di-Boc-β-Lys)-DAPA-DEA-OEt (309): A solution of **307** (50 mg, 0.066 mmol, 1 eq) and $Pd(OH)_2$ (23 mg, 0.033 mmol, 0.5 eq) in absolute ethanol (2 mL) was purged with argon for 10 min. Hydrogen was then bubbled through the solution and a balloon of hydrogen fixed to the top of the flask. After stirring for 1h, the starting material was shown to be consumed by TLC. The reaction was then purged with argon, filtered through celite and evaporated to provide 41 mg (quant) of **309** as a clear oil and an ~2:1 mixture of epimers.

^{1}H NMR (400 MHz) (DMSO-d_6, * denotes minor diastereomer) δ: 1.09 (3H, t, J=7.0 Hz); 1.09 (3H, t, J=7.0 Hz); 1.19 (3H, t, J=7.0 Hz); 1.21-1.38 (4H, m); 1.36 (18H, broad s); 1.97 (2H, broad s); 2.16 (1H, dd, J=7.2, 14.1 Hz); 2.21 (1H, dd, J=6.8, 14.3 Hz); 2.85 (2H, m); 3.02 (2H, m); 3.30 (1H, m,); 3.49 (2H, m); 3.62 (2H, m); 3.69 (1H, broad s); 4.11 (2H, m); 4.48 (1H, m); *4.74 (1H, d, J=4.3 Hz); 4.75 (1H, d, J=3.0 Hz); 6.63 (1H, d, J=9.0 Hz); 6.75 (1H, t, J=5.1 Hz); 7.74 (1H, m); 8.23 (1H, m). ^{13}C NMR (75 MHz) (CD_3OD) δ CD_3OD: 14.7, 15.7, 27.8, 29.0, 29.0, 33.5, 41.2, 43.3, 44.8, 55.8, 56.3, 62.7, 64.9, 79.9, 80.1, 102.5, 157.8, 158.4, 170.4, 174.0, 175.7. IR (NaCl, deposited from CH_2Cl_2): 3330, 2977, 2933, 1734, 1690, 1520, 1451, 1415, 1392, 1366, 1344, 1271, 1251, 1171, 1112, 1068, 1025 cm^{-1}. HRMS (FAB$^+$) calc. for $C_{28}H_{54}N_5O_{10}$ (MH^+) 620.3871; found 620.3853.





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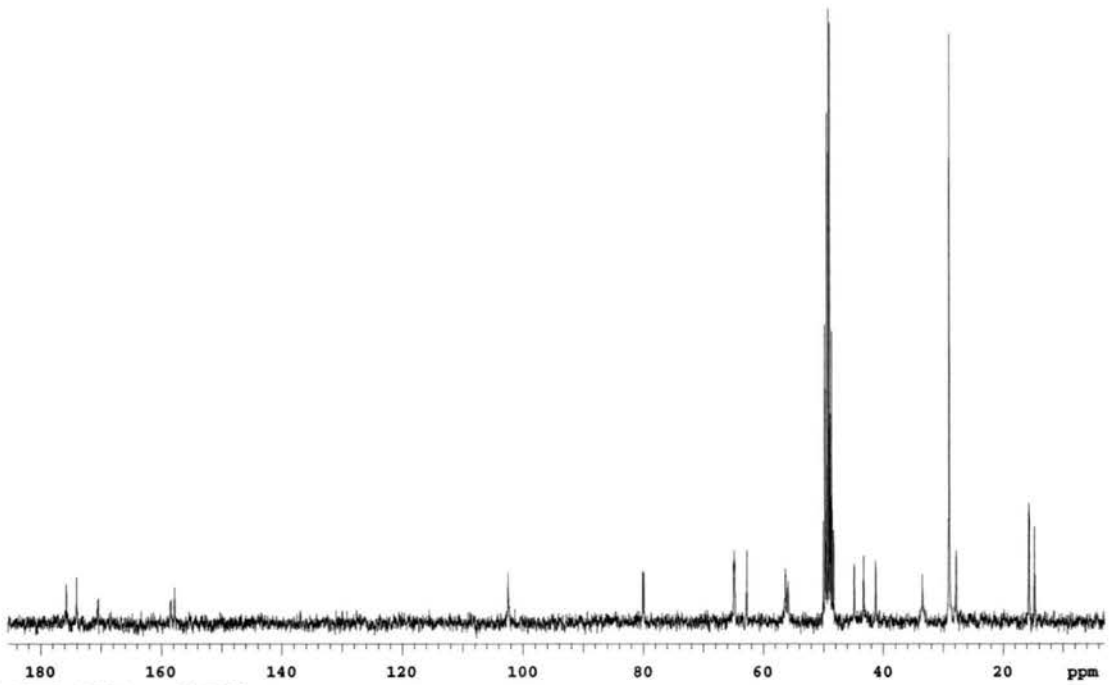
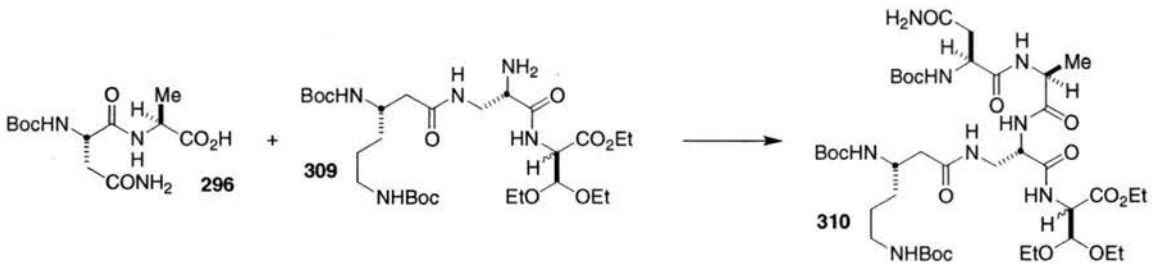


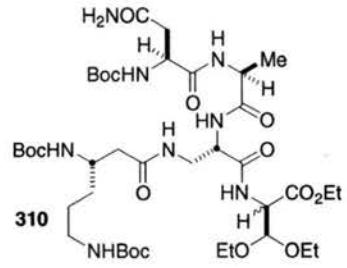
dedIII-1521crude75late

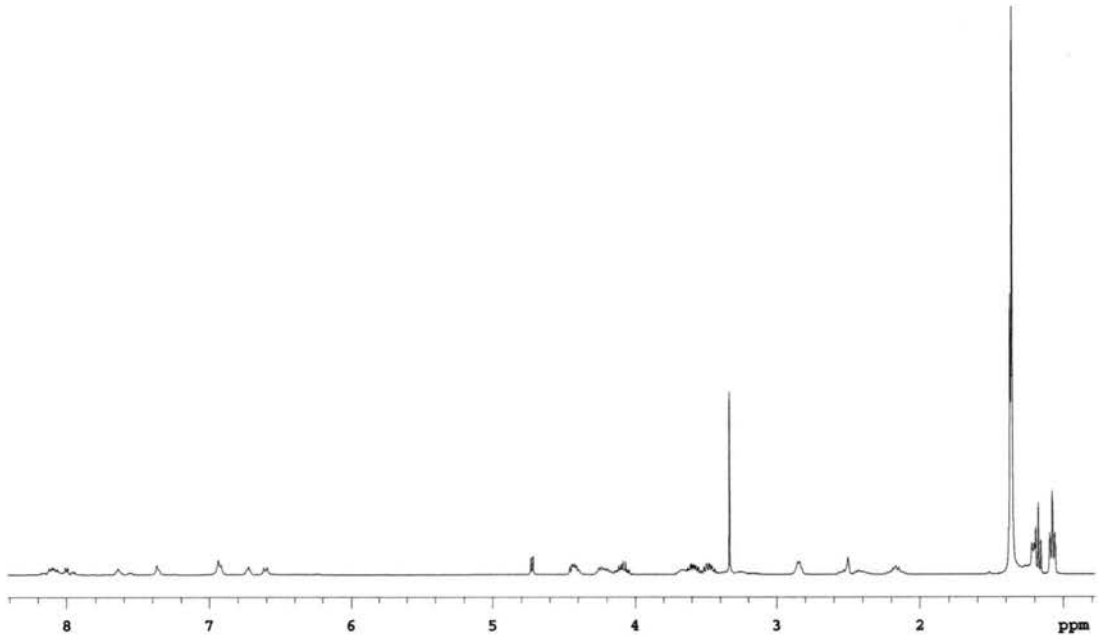


N^{α}-(Boc-Asn-Ala-)-DAPA-N^{β}-(*N,N'*-di-Boc-β-Lys)-DEA-OEt (310): A solution of **296** (22 mg, 0.072 mmol, 1 eq), **309** (45 mg, 0.072 mmol, 1 eq), and HOBt (14 mg, 0.11 mmol, 1.5 eq) in THF (2.3 mL) and DMF (1.3 mL) was cooled to 0°C. Diisopropylcarbodiimide (17 μL, 0.11 mmol, 1.5 eq) was added dropwise and the reaction stirred 5h, allowing to warm to room temp. The reaction mixture was then treated with two drops of water, and the solvent removed *in vacuo*. The crude residue was purified by silica gel chromatography (gradient elution from 5% to 15% MeOH in CH_2Cl_2) to provide the desired dipeptide with some HOBt contamination. A second silica gel purification using the same solvent conditions provided 57 mg (88%) of **310** (2.5:1 mixture of inseparable epimers) as a white amorphous solid.

^{1}H NMR (400 MHz) (DMSO-d_6, *denotes minor epimer) δ: 1.07 (3H, t, J=6.8 Hz); 1.08 (3H, t, J=7.0 Hz); 1.17 (3H, t, J=7.0 Hz); 1.21 (3H, d, J=7.0 Hz); 1.20-1.49 (4H, m, partially buried); 1.36 (18H, s); 1.37 (9H, s); 2.17 (2H, m); 2.41 (1H, m); 2.54 (1H, dd, J=5.8, 14.9 Hz, partially buried); 2.84 (2H, m); *3.15 (1H, m); 3.25 (1H, m); 3.37 (1H, m, partially buried); 3.48 (2H, m); 3.59 (2H, m); 3.67 (1H, broad m, partially buried); 4.09 (2H, m); 4.20 (1H, m, partially buried); 4.24 (1H, q, J=7.3 Hz, partially buried); 4.41 (1H, m, partially buried); 4.44 (1H, dd, J=6.0, 8.1 Hz partially buried); 4.72 (1H, d, J=6.0 Hz); 6.61 (1H, d, J=8.7 Hz); 6.73 (1H, t, J=5.3 Hz); 6.93 (2H, m); *7.36 (1H, broad s); 7.37

(1H, broad s); *7.55 (1H, t, J=5.9 Hz); 7.64 (1H, t, J=5.6 Hz); *7.95 (1H, d, J=6.8 Hz); 8.00 (1H, d, J=6.6 Hz); *8.04 (1H, d, J=8.9 Hz); 8.08 (1H, d, J=8.3 Hz); 8.11 (1H, d, J=8.3 Hz); *8.16 (1H, d, J=8.1 Hz). ^{13}C NMR (100 MHz) (DMSO-d_6, *denotes minor epimer) δ: 14.0, 15.0, 15.0, 17.9, *18.2, 26.4, 28.2, 28.3, 28.3, 31.6, 37.2, 40.3, 41.2, 47.5, 48.7, 51.1, 52.4, 54.8, 60.6, 62.3, 62.5, 62.7, 77.3, 77.4, 78.3, 100.3, 155.1, 155.1, 155.5, 168.9, 169.8, 170.5, 171.4, 171.9, 172.1. IR (NaCl, depos. from 10% MeOH in CH_2Cl_2): 3461, 3285, 3077, 2978, 2932, 1744, 1691, 1665, 1641, 1547, 1529, 1449, 1392, 1367, 1326, 1272, 1253, 1174, 1121, 1068, 1028. HRMS (FAB^+) calc. for $C_{40}H_{73}N_8O_{15}$ (MH^+) 905.5195; found 905.5199.





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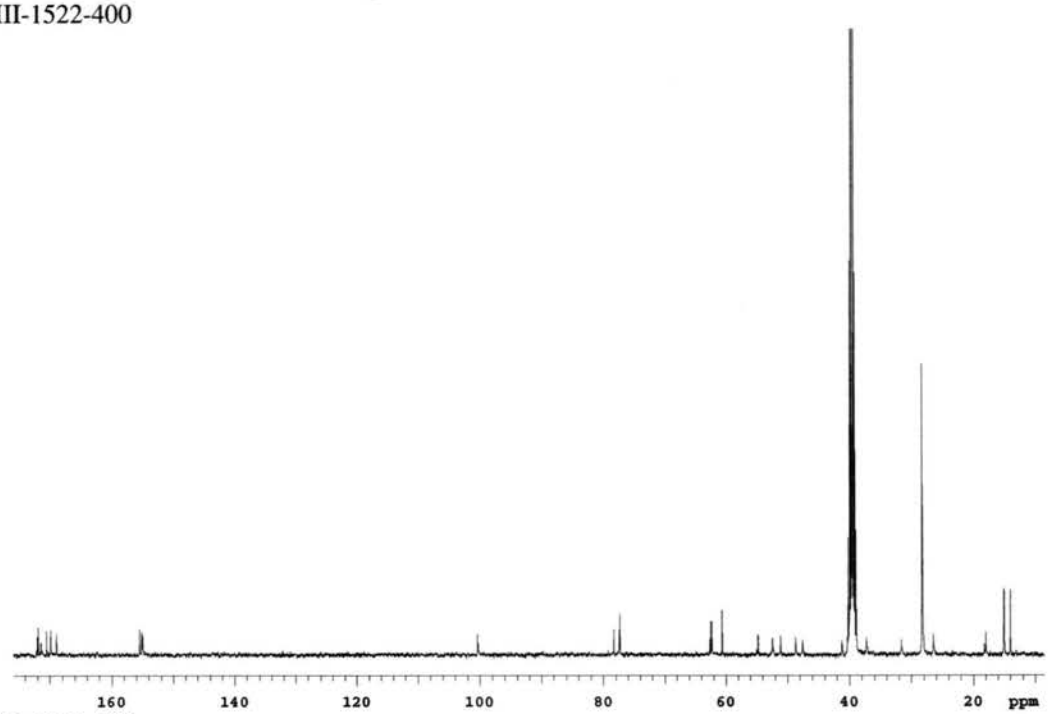

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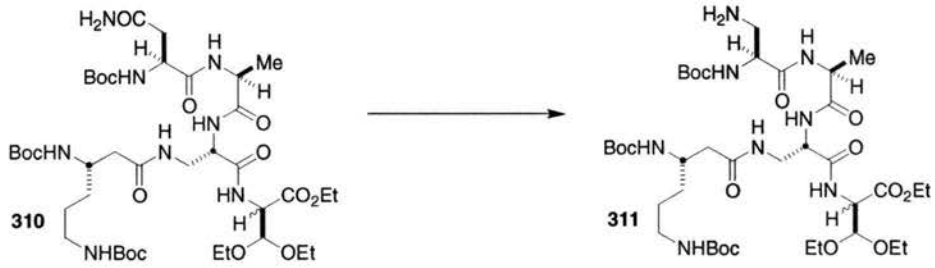
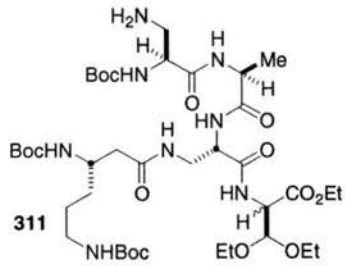
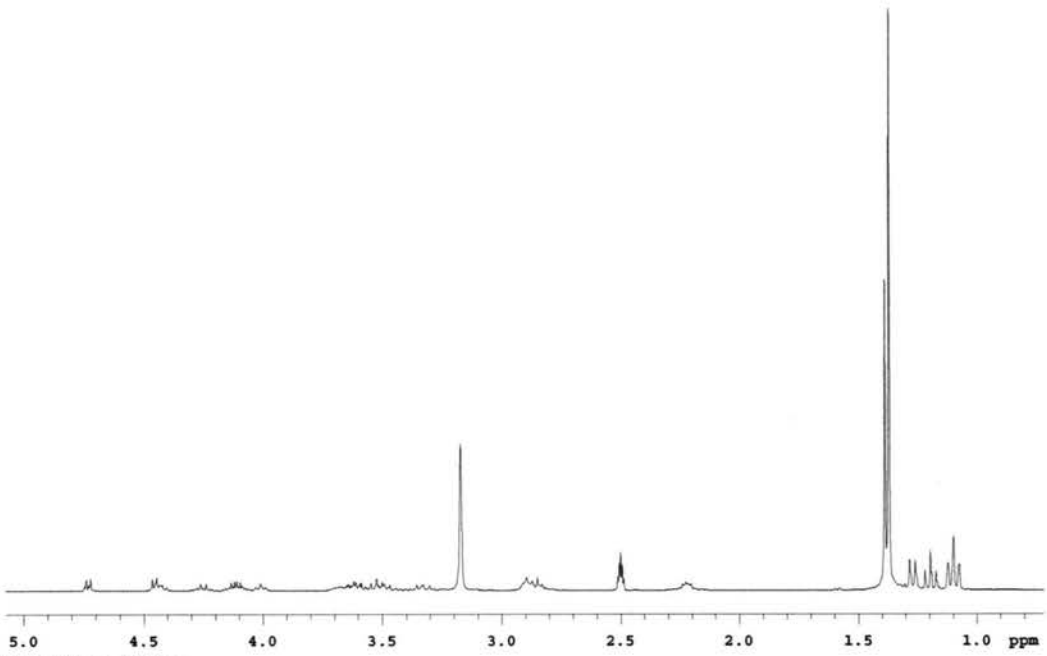


N^{α}-(N^{α}-Boc-DAPA-Ala-)-DAPA-N^{β}-(*N,N'*-di-Boc-β-Lys)-DEA-OEt (311): To a solution of **310** (57 mg, 0.063 mmol, 1 eq) in acetonitrile (1.15 mL) and water (1.15 mL), bis(trifluoroacetoxy)iodosobenzene (41 mg, 0.094 mmol, 1.5 eq) was added followed by □imethylformamide (1.15 mL). After stirring the reaction for 15 min, pyridine (15 mL, 0.19 mmol, 3 eq) was added, and the resulting solution stirred for an additional 2.5 h. The reaction was then reduced to ca. ½ its volume by rotary evaporation, diluted with EtOAc, and partitioned with brine. Removal of the organic layer was followed by extraction of the aqueous layer with EtOAc. Upon drying of the combined organic extracts over anhydrous Na_2SO4, the solids were removed by filtration and the filtrate concentrated to dryness. Silica gel chromatography (gradient elution from 5% to 15% MeOH in CH_2Cl_2) provided 48 mg (87%) of **311** as a clear glass that was an ~2.6:1 mixture of epimers. The product was used immediately for the subsequent coupling reaction in order to avoid *N,N'*-acyl migration.

^{1}H NMR (300 MHz) (DMSO-d_6, 353 K, D_2O exchange, * denotes minor diastereomer) δ DMSO: 1.10 (3H, t, J=7.0 Hz); 1.10 (3H, t, J=7.0 Hz); *1.19 (3H, t, J=7.1 Hz); 1.20 (3H, t, J=7.1 Hz); *1.27 (3H, d, J=7.2 Hz); 1.27 (3H, d, J=7.1 Hz); 1.29-1.46 (4H, m, partially buried); 1.37 (18H, s); 1.39 (9H, s); 2.22 (2H, m); 2.86 (2H, m, partially buried); 2.89 (2H, m, partially buried); 3.29 (1H, dd, J=7.9, 13.6); 3.37 (1H, dd, J=5.7, 13.6); 3.52 (2H, m, partially buried); 3.61 (2H, m, partially buried); 3.69 (1H, broad m, partially buried);

4.01 (1H, t, J=6.1 Hz); 4.11 (2H, m); 4.25 (1H, q, J=7.1 Hz); *4.26 (1H, q, J=7.1 Hz); 4.43 (1H, m, partially buried); 4.45 (1H, d, J=5.3 Hz); 4.73 (1H, d, J=5.3 Hz); *4.74 (1H, d, J=5.3 Hz). ^{13}C NMR (100 MHz) (CD_3OD) δ CD_3OD: 14.7, 15.6, 15.6, 15.6, 17.7, 27.8, 28.8, 29.0, 29.0, 33.1, 41.3, 42.1, 43.1, 44.5, 51.3, 51.4, 54.9, 56.6, 62.7, 64.6, 64.8, 80.0, 80.2, 81.0, 102.4, 158.0, 158.1, 158.7, 170.8, 172.0, 173.8, 174.5, 175.3. IR (NaCl, depos. from $CHCl_3$): 3306, 3061, 2978, 2934, 1683, 1668, 1523, 1454, 1392, 1367, 1345, 1250, 1202, 1170, 1113, 1065, 1024. HRMS (FAB^+) calc. for $C_{39}H_{73}N_8O_{14}$ (MH^+) 877.5246, found 877.5236.





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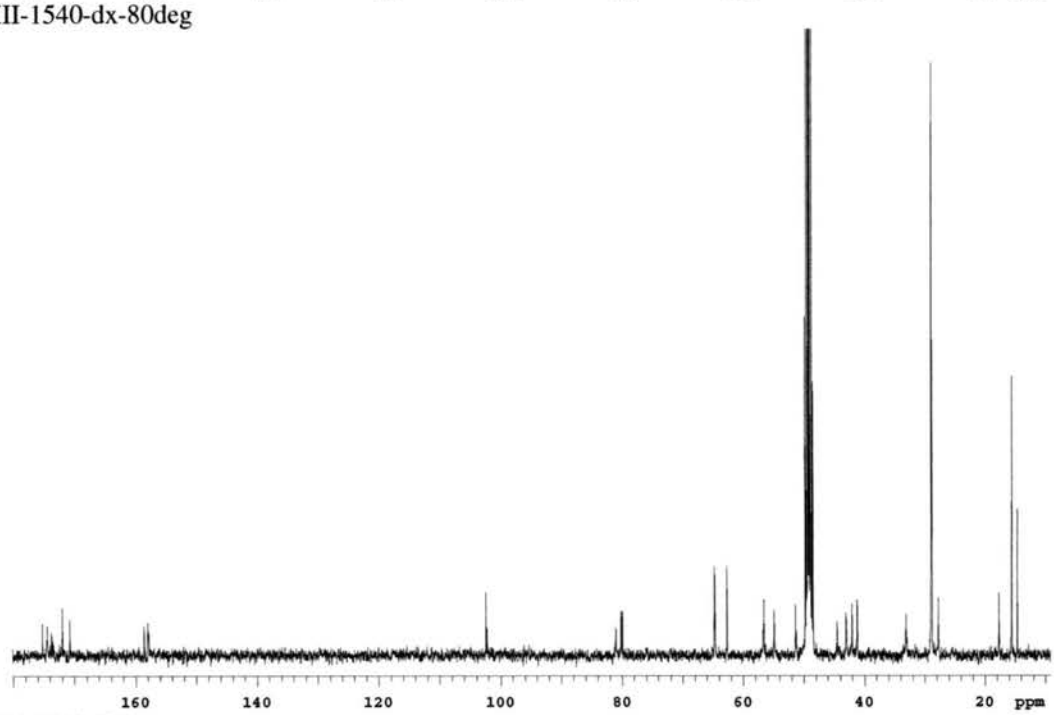

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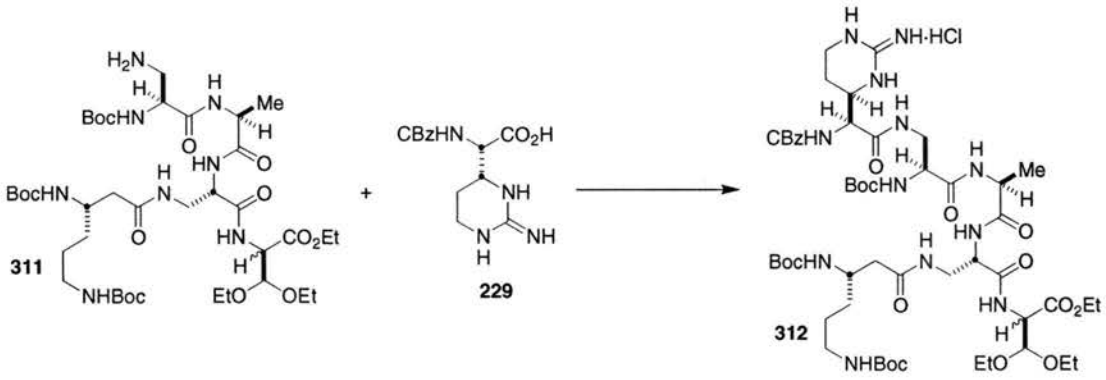
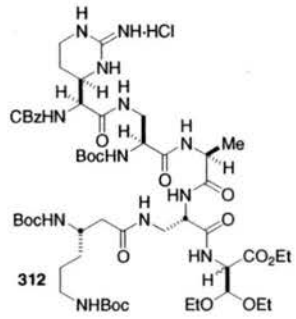
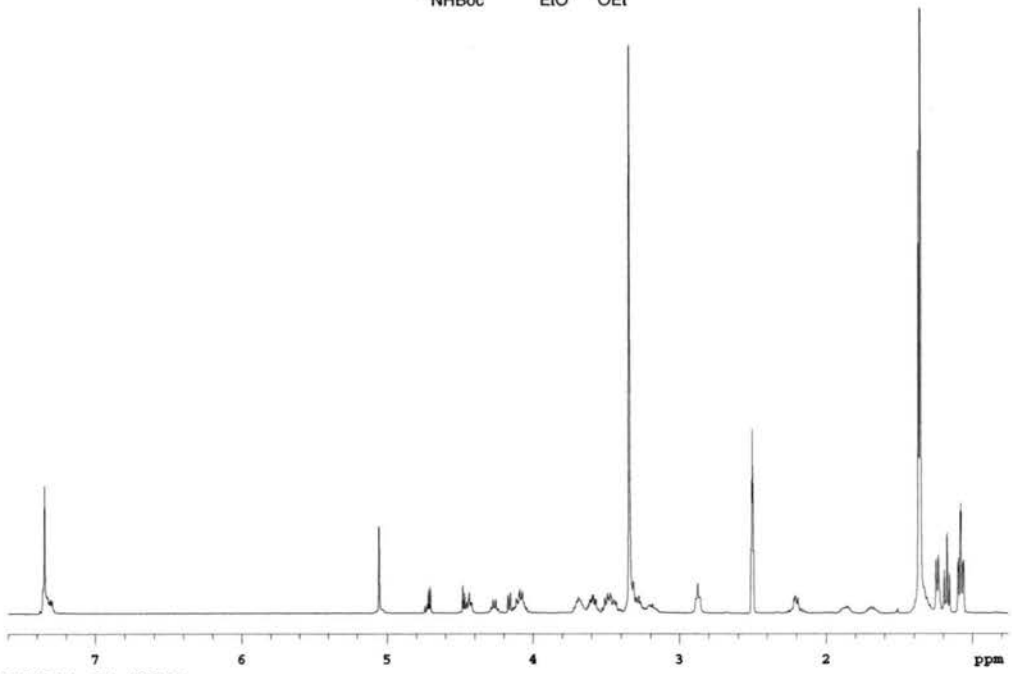


Hexapeptide 312. A solution of **311** (27 mg, 0.031 mmol, 1 eq), **229** (11 mg, 0.031 mmol, 1 eq), and HOBt (6 mg, 0.047 mmol, 1.5 eq) in DMF (1 mL) and THF (0.6 mL) was cooled to 0°C and stirred. EDCI (12 mg, 0.062 mmol, 2 eq) was then added, and the reaction stirred for 5h, at which point the starting material appeared to be consumed by TLC. The solvent was then removed *in vacuo* and the resulting residue was subjected to silica gel chromatography (gradient elution from 15% to 25% MeOH in CH_2Cl_2) to provide a partially pure product which was chromatographed again under the same conditions to provide 33 mg (89%) of **312** as a glass. The product was isolated as an inseparable 2.6:1 mixture of epimers.

^{1}H NMR (400 MHz) (DMSO-d_6, 338 K, D_2O exchange, * denotes minor diastereomer) δ DMSO: 1.08 (3H, t, J=6.8 Hz); 1.08 (3H, t, J=7.0 Hz); 1.17 (3H, t, J=7.0 Hz); *1.24 (3H, d, J=7.3 Hz); 1.24 (3H, d, J=7.0 Hz); 1.25-1.42 (4H, m, partially buried); 1.35 (18H, s); 1.38 (9H, s); 1.68 (1H, m); 1.87 (1H, m); 2.20 (2H, m); 2.87 (2H, t, J=5.8 Hz); 3.20 (1H, m); 3.23-3.35 (2H, m, partially buried); 3.40-3.53 (3H, m); 3.59 (2H, m); 3.69 (2H, m); 4.09 (3H, m); 4.16 (1H, d, J=6.8 Hz); 4.26 (1H, q, J=6.8 Hz); 4.44 (1H, m); 4.47 (1H, d, J=5.3 Hz); 4.71 (1H, d, J=5.3 Hz); *4.73 (1H, d, J=5.5 Hz); 5.05 (2H, s); 7.30-7.36 (5H, m). ^{13}C NMR (100 MHz) (CD_3OD, * denotes minor diastereomer) δ CD_3OD: 14.7, 15.6, 15.7, 17.8, 23.9, 27.8, 28.9, 29.0, 29.0, 33.3, 37.9, 41.3, 42.2, 42.6, 43.0, 43.3, 51.3, 51.4,

54.3, 54.9, 55.2, 56.5, 59.4, 62.8, 64.7, 64.8, 64.9, 68.4, 80.0, 80.3, 81.1, *102.3, 102.5, 129.4, 129.5, 129.7, 138.0, 156.0, 157.6, *158.1, 158.7, 158.8, 170.5, 172.3, 172.9, 173.4, 174.3, 175.4. IR (NaCl, deposited from CH_2Cl_2/MeOH): 3302, 3064, 2977, 2934, 1669, 1522, 1455, 1392, 1367, 1249, 1169, 1064. HRMS (FAB^+) calc. for $C_{53}H_{89}N_{12}O_{17}$ (MH^+) 1165.6469; found 1165.6459.





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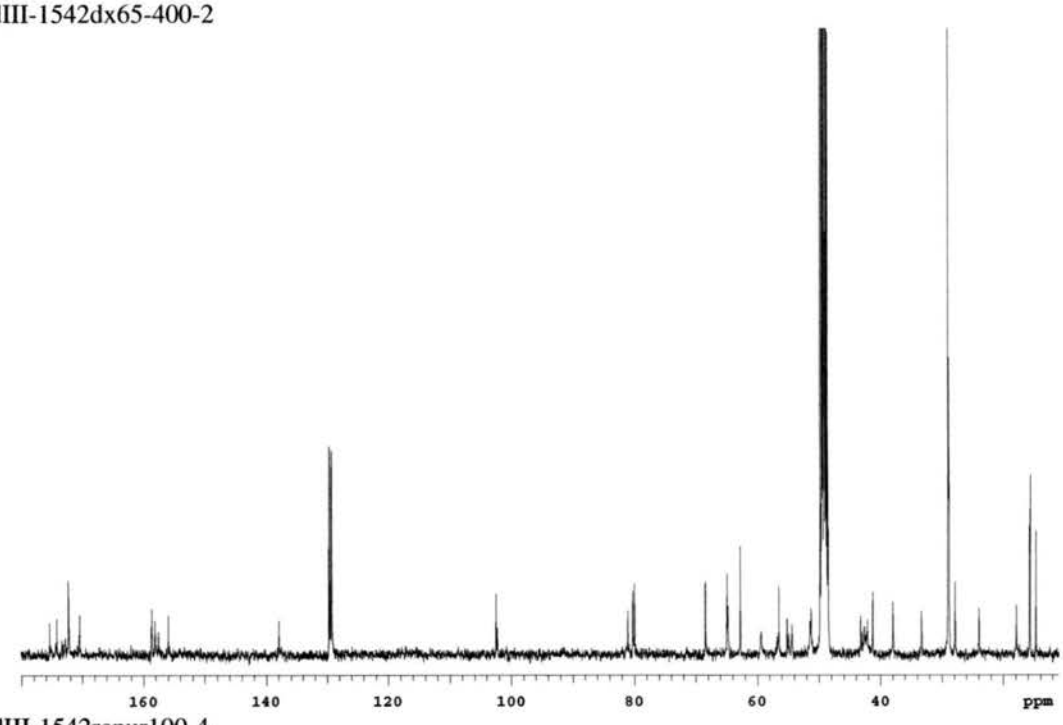

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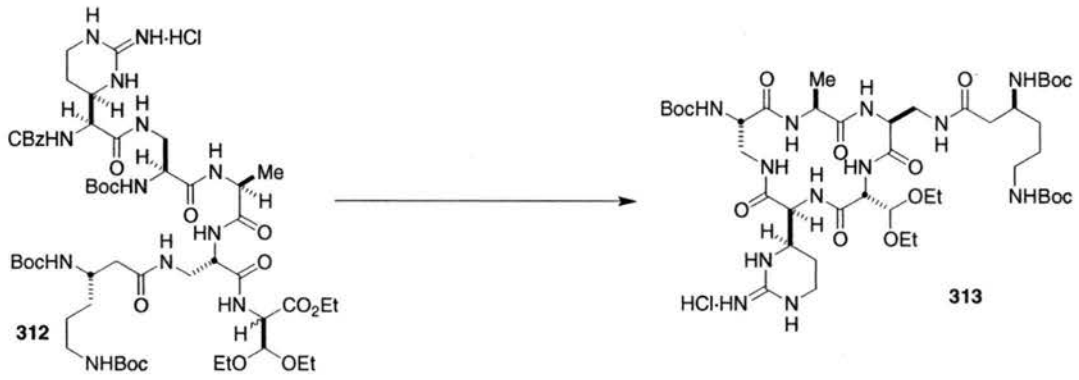
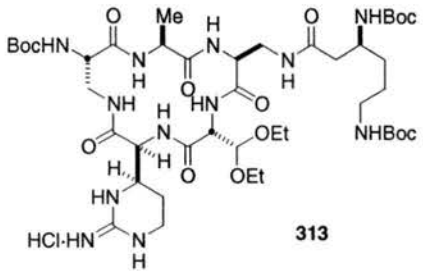


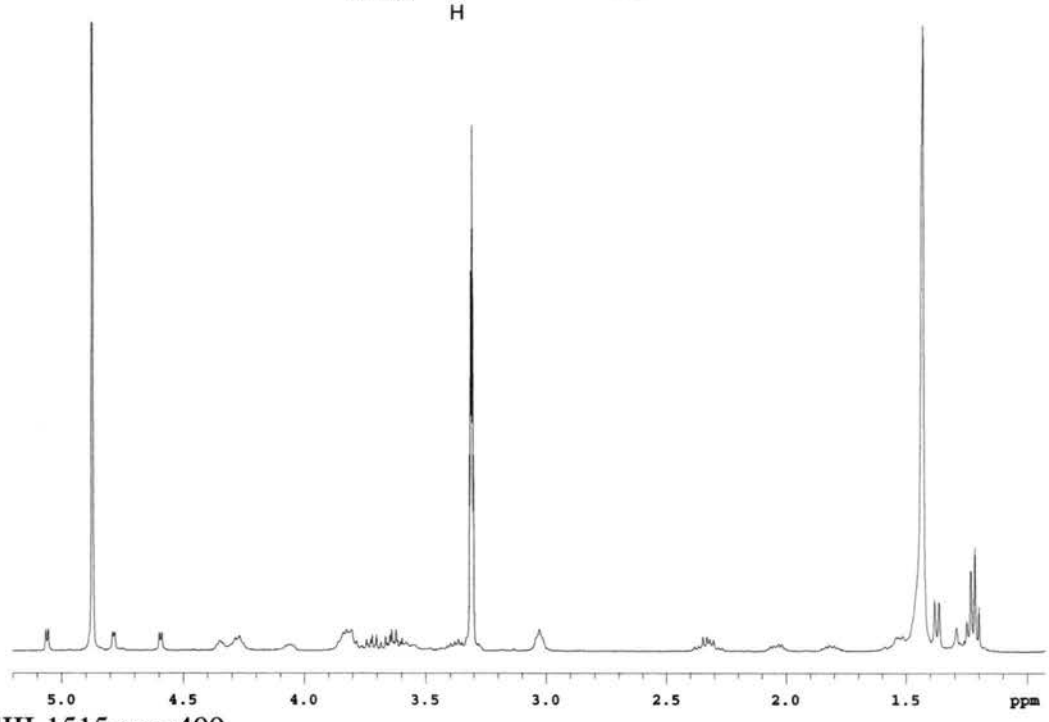
Macrocycle 313: A solution of **312** (24 mg, 0.020 mmol, 1 eq)) in ethanol (3 mL) was purged with argon for 10 min. Addition of 10% Pd/C (15 mg, 0.014 mmol, 0.7 eq) was followed by careful bubbling of hydrogen gas through the solution. After fixing a hydrogen balloon to the flask, the reaction was stirred for 2h then purged with argon. The 10% Pd/C was removed by filtration through celite and the filter cake was washed with absolute ethanol. The filtrate was concentrated to dryness. Dissolution of the crude hydrogenation product in absolute ethanol (1.3 mL) was followed by addition of 1N LiOH (80μL, 0.080 mmol, 4 eq). At 1h, and additional amount of 1N LiOH (40 mL, 0.040 mmol, 2 eq) was added. After 1.5h total reaction time, the reaction mixture was diluted with dd H_2O and adjusted to ~pH 6 with aqueous HCl (prepared from dd H_2O) and evaporated to provide an off-white residue. Dissolution of the residue in dd H_2O was followed by loading onto a Waters C18 sep-pak cartridge (prepared by washing with 3 x 5 mL acetonitrile followed by 3 x 5 mL dd H_2O). The cartridge was washed with 2 x 2 mL dd H_2O which was subsequently discarded. Isolation of the amino acid was accomplished by elution from the Sep-Pak cartridge with 3 x 5 mL acetonitrile and 3 x 5 mL methanol followed by evaporation of the solvent. The crude amino acid was then dissolved in DMF (4 mL) and CH_2Cl_2 (20 mL) followed by the addition of EDCI (30 mg,

0.16 mmol, 7.8 eq) and HOAt (20 mg, 0.15 mmol, 7.3 eq). After stirring the reaction at room temperature for 36h, the solvent was removed *in vacuo* to provide a yellow oily residue. The residue was chromatographed on silica gel (gradient elution from 15% to 30% MeOH in CH_2Cl_2) to provide **313** with some HOAt contaminant present. After dissolution of the residue in 10% MeOH in CH_2Cl_2 and partitioning with brine, the organic layer was removed, dried over Na_2SO_4, filtered, and evaporated. The resulting residue was triturated with 10% MeOH in CH_2Cl_2 and the solvent evaporated to provide 4 mg (20%, opaque glass) of pure **313** as a single diastereomer.

^{1}H NMR (400 MHz) (CD_3OD) δ: 1.22 (3H, t, J=7.0 Hz); 1.23 (3H, t, J=6.4 Hz); 1.37 (3H, d, J=7.3 Hz); 1.40-1.61 (4H, m); 1.43 (27H, broad s); 1.81 (1H, m); 2.04 (1H, m); 2.29 (1H, dd, J=6.4, 14.3 Hz); 2.36 (1H, dd, J=7.0, 14.3 Hz); 3.03 (2H, broad t); 3.27-3.41 (2H, m, partially obscured); 3.54-3.86 (9H, m); 4.06 (1H, dd, J=4.9, 9.2 Hz); 4.28 (2H, m); 4.35 (1H, m); 4.59 (1H, d, J=3.6 Hz); 4.79 (1H, d, J=3.2 Hz); 5.06 (1H, d, J=3.8 Hz). ^{13}C NMR (125 MHz) (CD_3OD) δ CD_3OD: 15.7, 15.7, 18.0, 24.2, 27.9, 28.8, 29.0, 29.0, 33.3, 37.7, 40.1, 41.3, 42.3, 43.2, 50.4, 51.2, 54.2, 55.0, 56.4, 58.0, 65.1, 65.3, 65.3, 80.1, 80.3, 81.1, 102.0, 156.2, 157.4, 158.1, 158.7, 171.1, 171.9, 172.2, 172.5, 174.4, 177.1. IR (NaCl, depos. from CH_2Cl_2): 3583, 3287, 3070, 2976, 2931, 1667, 1518, 1454, 1392, 1366, 1303, 1249, 1169, 1107, 1065. HRMS (FAB^+) calc. for $C_{43}H_{77}N_{12}O_{14}$ (MH^+) 985.5682; found 985.5678. $[\alpha]_D^{25}$=-47 (*c*=0.1, MeOH).



313



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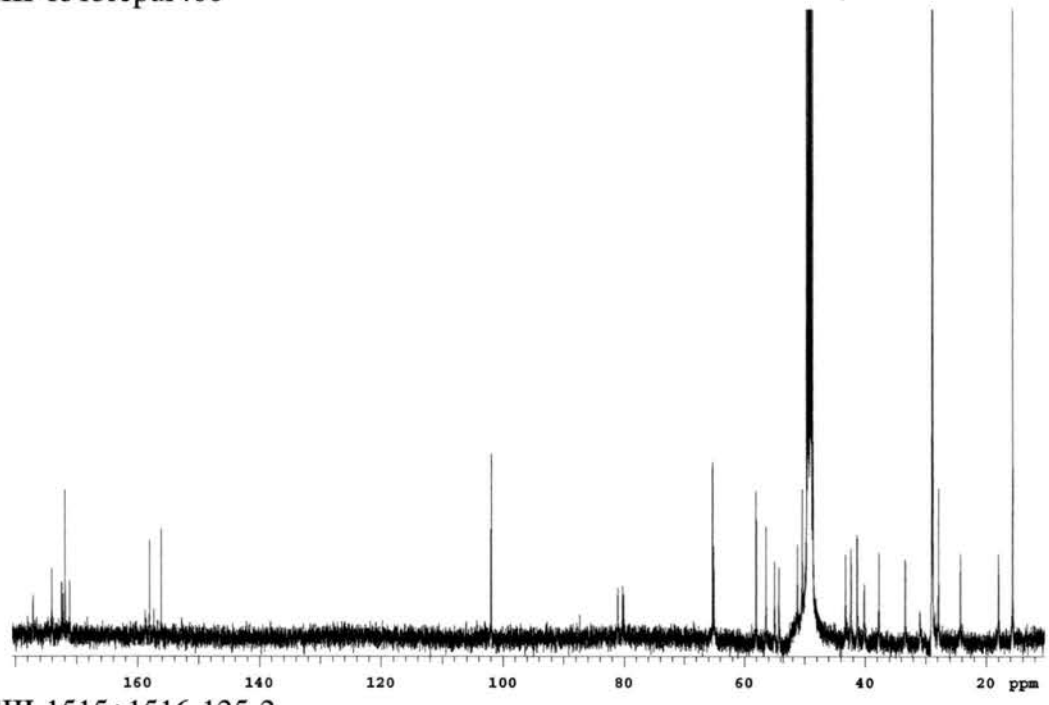

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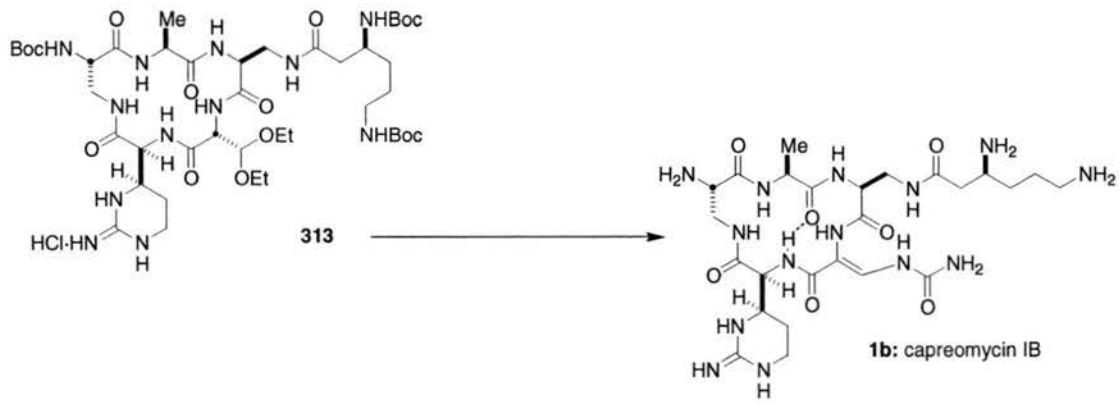
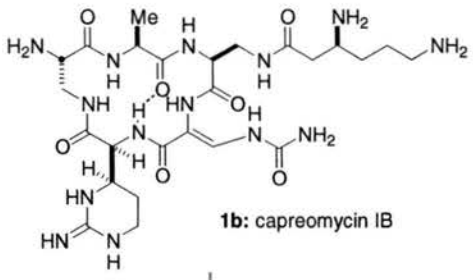


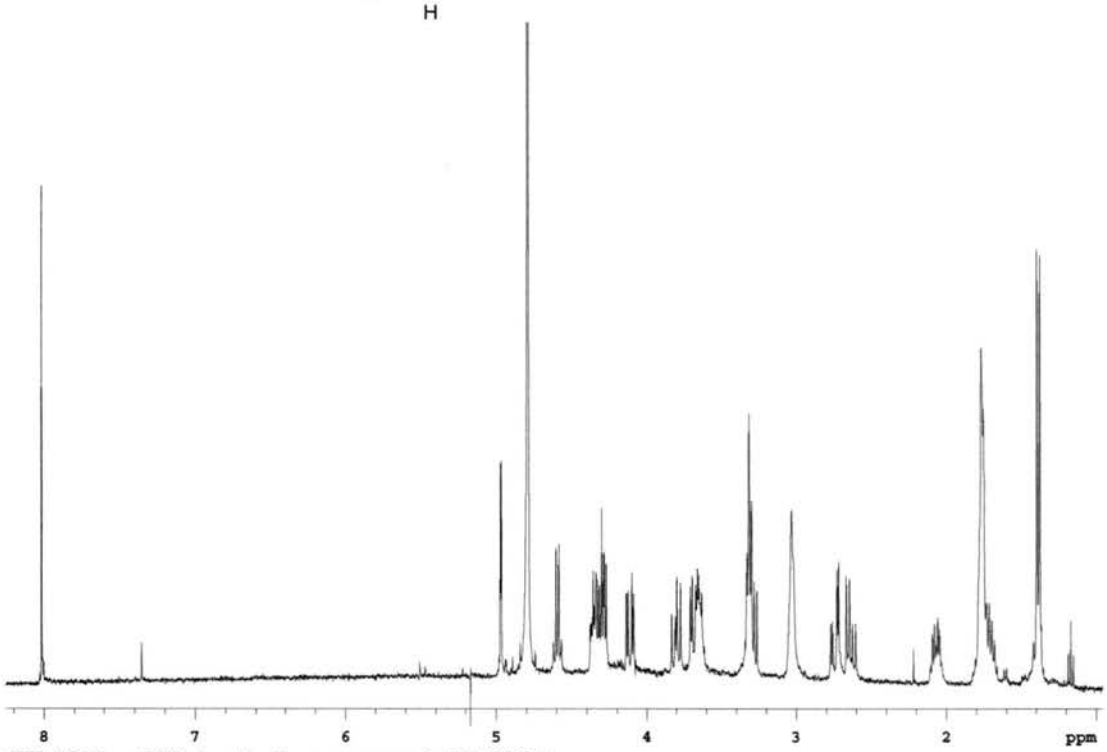
Capreomycin IB·4HCl (1b). Macrocycle **313** (5 mg, 0.0049 mmol, 1 eq) was dissolved in 99% formic acid (500 μL, distilled from phthallic anhydride) and stirred at room temperature for 1.25h. The formic acid was removed *in vacuo*, and the resulting residue dissolved in acetone (400 μL) and 2N HCl (400 μL). After refluxing the resulting solution under argon for 10 min, the solution was cooled to room temperature and urea (35 mg, 0.58 mmol, 119 eq) was added. After stirring for 14h, the solvent was removed *in vacuo*, and the resulting residue was triturated with absolute ethanol to provide 2 mg (50%) of capreomycin IB·4HCl (**1b**) as a white precipitate. The synthetic product matched the natural product by 1H NMR, optical rotation, and TLC (30:10:1 phenol : H_2O : 28% aqueous ammonia, R_f=0.29).

Synthetic **1b** was then combined with a mixture of natural capreomycin IA and IB (**1a,b**) in water, 2 drops of concentrated sulfuric acid were added and the solution evaporated. Absolute ethanol was added to the residue, and the precipitate collected. Spectral analysis of the mixture of synthetic and natural material (^{1}H NMR) showed that all peaks corresponding to **1b** increased in intensity compared to **1a**.

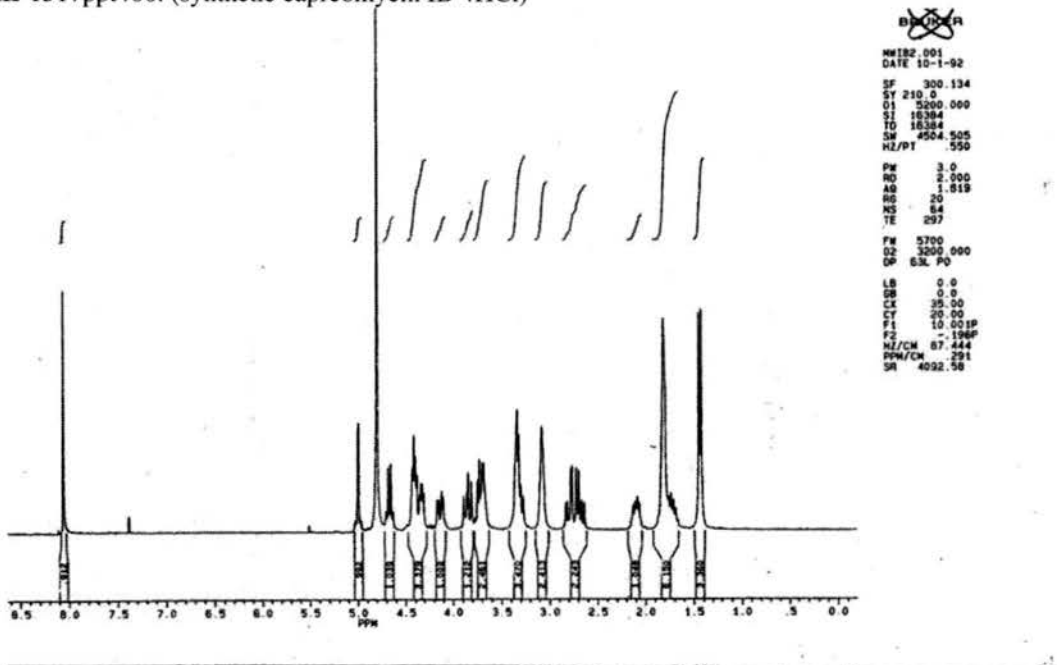
^{1}H NMR (400MHz) (D_2O) δ EtOH: 1.38 (3H, d, J=7.3 Hz); 1.72 (1H, m, partially buried); 1.75 (2H, broad s); 1.77 (2H, broad s); 2.07 (1H, dddd, J=5.1, 5.1, 5.1, 13.6 Hz); 2.64 (1H, dd, J=8.3, 16.4 Hz); 2.74 (1H, dd, J=4.7, 16.2 Hz); 3.03 (2H, broad s); 3.29 (1H, dd, J=7.7, 14.5 Hz, partially buried); 3.31 (2H, t, J=5.8 Hz); 3.65 (1H, m, partially buried); 3.68 (1H, dd, J=5.3, 14.1 Hz, partially buried); 3.80 (1H, dd, J=9.6, 14.1 Hz); 3.68 (1H, dd, J=4.9, 14.1 Hz); 4.27-4.34 (2H, m, partially buried); 4.36 (1H, ddd, J=2.8, 5.3, 8.1 Hz, partially buried); 4.59 (1H, q, J=7.0 Hz); 4.97 (1H, d, J=2.8 Hz); 8.02 (1H, s). $[\alpha]_D^{25}$, synthetic: -48 (*c*=0.05, dd H_2O), natural: -44.6 (*c*=0.5, dd H_2O). Electrospray MS (ES^+) ($M+H^+$): calc.: 653.35, found 653.40.



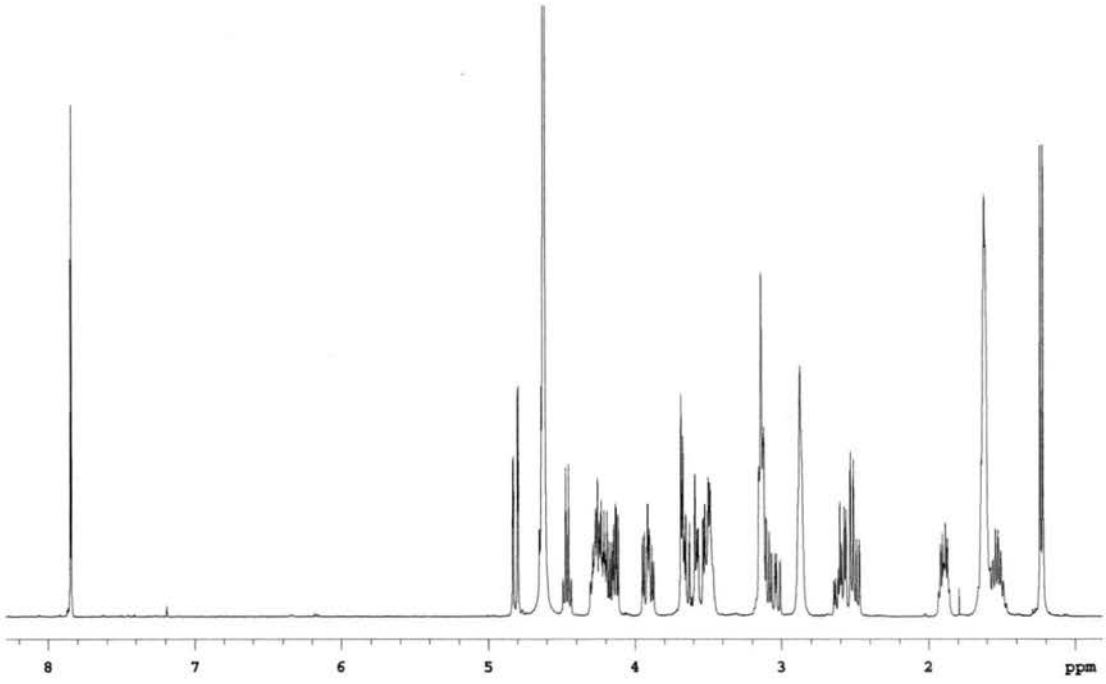
1b: capreomycin IB



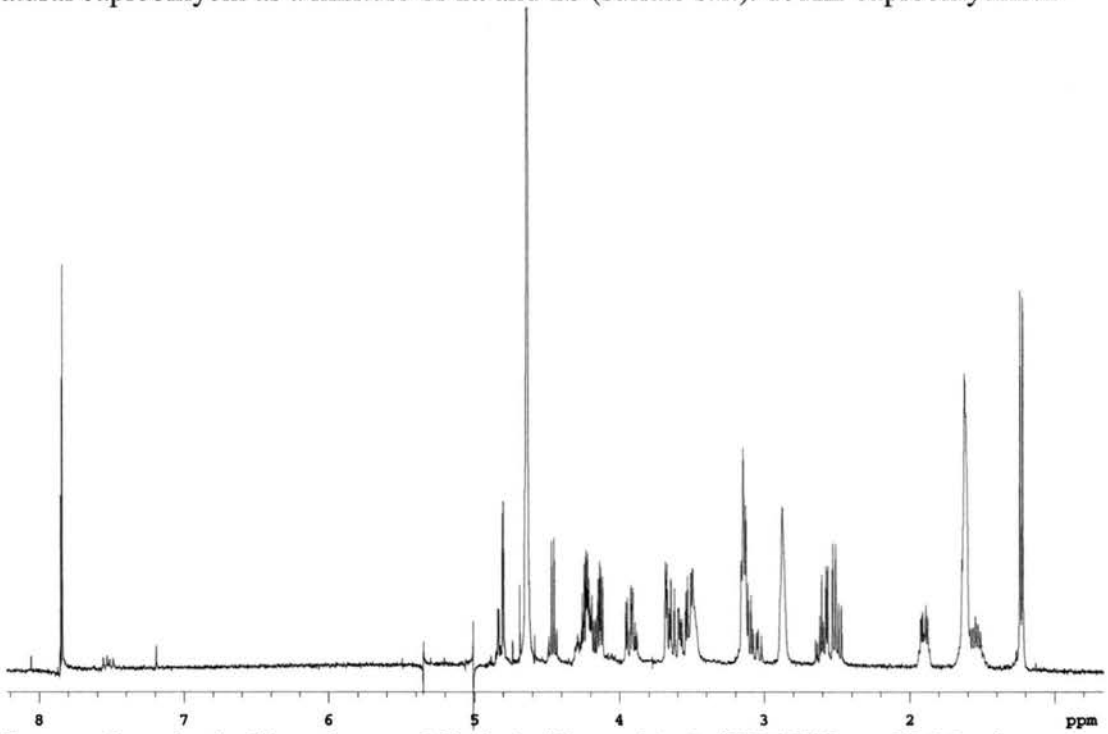
dedIII-1517ppt400. (synthetic capreomycin IB·4HCl)



300 MHz spectrum of natural capreomycin IB.



Natural capreomycin as a mixture of **1a** and **1b** (sulfate salt). dedIII-capreomycinAB



Mixture of synthetic **1b** and natural **1a,b** (sulfate salt). dedIII-1517+natH_2SO_4-4.

APPENDIX 1

PUBLICATIONS

Pergamon

Tetrahedron Letters 42 (2001) 183–185

TETRAHEDRON LETTERS

An efficient asymmetric synthesis of (2*S*,3*S*)- and (2*R*,3*R*)-β-hydroxyornithine

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Abstract—Asymmetric syntheses of (2*S*,3*S*)- and (2*R*,3*R*)-β-hydroxyornithine have been achieved in four steps and 46% overall yield. The key step in this synthesis involved an aldol reaction between a chiral glycine boron enolate and (3-oxo-propyl)-carbamic acid benzyl ester.

β-Hydroxyornithine in its various stereoisomeric forms **1a–d** (Fig. 1) has been previously investigated as a potential biosynthetic precursor to both the β-lactamase inhibitor clavulanic acid (**2**) and the antibiotic and anticancer agent acivicin (**3**). Clavulanic acid has been shown to be derived from (2*S*,3*R*)-proclavaminic acid **4**, a molecule that is structurally similar to β-hydroxyornithine.[1] In addition, both (2*S*,3*R*)- and (2*S*,3*S*)-β-hydroxyornithine have been shown by Gould et al. not to be incorporated into acivicin.[2] Recently, it has been suggested that a new antibiotic xanthobaccin A could also be derived from this unusual amino acid.[3]

Along with these biosynthetic investigations, efforts have been made to develop efficient syntheses of the *erythro*- and *threo*-forms of β-hydroxyornithine. Gould et al.[4] and Townsend et al.[5] have accessed both diastereomers of 2*S*-β-hydroxyornithine via the dipolar cycloaddition of vinyl glycine derivatives with a suitable nitrone. Misiti and co-workers developed a synthesis of (2*S*,3*R*)-β-hydroxyornithine, starting from D-serine.[6] More recently, Gurjar and co-workers synthesized the same enantiomer as that reported by Misiti by opening an enantiomerically pure epoxy alcohol with benzyl isocyanate.[7] Baldwin, Baggaley, and Wakamiya have used aldol chemistry to synthesize protected forms or derivatives of β-hydroxyornithine.[1,8]

Given the important roles that various isotopomers of β-hydroxyornithine have played in biosynthetic studies, an efficient asymmetric synthesis of both (2*S*,3*S*)- and (2*R*,3*R*)-β-hydroxyornithine that is compatible with various simple strategies to incorporate both stable- and radioisotopes was deemed justified. Herein, we

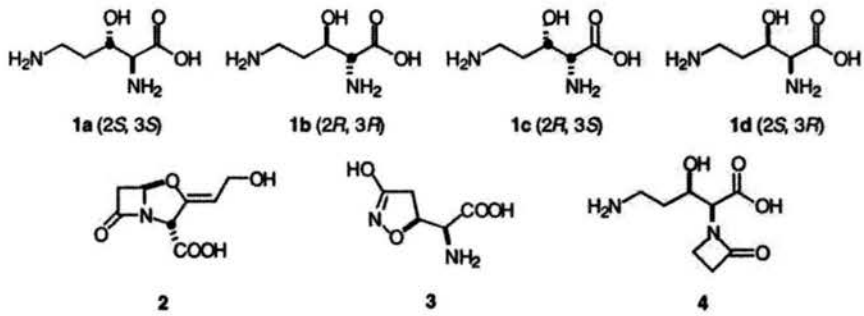

Figure 1.

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report an efficient and stereoselective synthesis of (2*S*,3*S*)- and (2*R*,3*R*)-β-hydroxyornithine. In order to arrive at the desired hydroxy amino acid, an aldol reaction between the benzyloxycarbonyl protected 3-amino-propanal **6** and the commercially available chiral oxazinone **7a** was investigated (Scheme 1).[9] Upon treatment of 3-amino-propanol with dibenzyl dicarbonate in refluxing 10% triethylamine in methanol, a quantitative yield of the desired benzyloxycarbamate **5** was achieved. Treatment of **5** under Dess–Martin periodinane conditions resulted in a 98% yield of the requisite (3-oxo-propyl)-carbamic acid benzyl ester **6**. Lactone **7a** was then treated with di-*n*-butyl boron triflate to yield the resulting boron enolate. Addition of aldehyde **6** to this enolate provided the expected aldol product **8a** in 69% yield with a diastereoselectivity of 8:1 (^{1}H NMR). The undesired diastereomer was easily removed by recrystallization of **8a** from ethyl acetate and hexanes. Hydrogenolysis of the benzyloxycarbonyl groups as well as the chiral auxiliary was achieved by treatment of **8a** with palladium chloride and hydrogen. The dihydrochloride salt of the amino acid, obtained from this protocol, was neutralized with ammonium hydroxide (pH 6.5); recrystallization from water/ethanol, afforded (2*S*,3*S*)-β-hydroxyornithine **1a** in 68% yield and >99.5:0.5 er as determined by chiral HPLC analysis.

Using the commercially available antipode of the oxazinone (**7b**)[10] the enantiomeric (2*R*,3*R*)-β-hydroxyornithine **1b** was prepared in a similar manner. Full experimental details are provided.

It has been demonstrated that both (2*S*,3*S*)- and (2*R*,3*R*)-β-hydroxyornithine can be prepared from commercially available starting materials in an efficient and stereocontrolled manner. The synthetic approach described herein, maintaining a 46% overall yield, compares favorably with other published syntheses that contain longer sequences, lower overall yields, and lower stereoselectivity.

Experimental

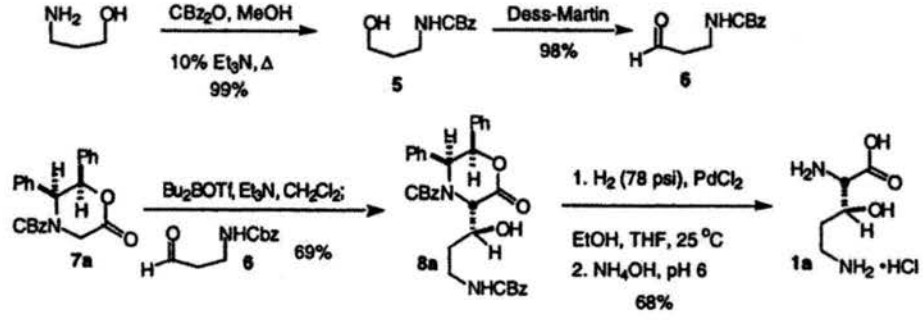
Compound **5**: To a 10% solution of triethylamine in methanol (2 mL) was added 3-aminopropanol (150 mg, 2.00 mmol, 1 equiv.) and dibenzyl dicarbonate (802 mg, 2.80 mmol, 1.4 equiv.). The resulting solution was refluxed under argon for 1 h, at which time the solvent was removed in vacuo resulting in a clear oil. Silica gel chromatography of the crude oil (eluted with 75:20:5 CH_2Cl_2:EtOAc:MeOH) provided 427 mg (99%) of **5** as a white solid. Mp 51–52°C (recryst. CH_2Cl_2:EtOAc).

^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.71 (2H, m); 2.47 (1H, bs); 3.36 (2H, bm); 3.69 (2H, t); 5.09 (1H, bs); 5.12 (2H, s); 7.32–7.38 (5H, m). ^{13}C NMR (75 MHz) ($CDCl_3$) δ 32.6, 38.0, 59.7, 66.9, 128.1, 128.2, 128.6, 136.5, 157.4. IR (NaCl, Neat) 3326, 3030, 2955, 2931, 2873, 1684, 1651, 1586, 1535, 1499, 1489, 1454, 1374, 1327, 1298, 1266, 1216, 1144, 1116, 1086, 1066, 1023, 984, 966 cm^{-1}. HRMS (FAB) calcd for $C_{11}H_{15}NO_3$ (MH^+) 210.1130; found 210.1125.

Compound **6**: To a solution of **5** (427 mg, 2.04 mmol, 1 equiv.) in non-distilled methylene chloride (17 mL) was added the Dess–Martin periodinane (1.47 g, 3.47 mmol, 1.7 equiv.). The resulting heterogeneous mixture was stirred overnight at room temp. Upon taking up the reaction mixture in diethyl ether (70 mL) and satd. aq. $NaHCO_3$ (70 mL), sodium thiosulfate (6.03 g, 24.3 mmol, 11.9 equiv.) was added, and the biphasic solution was stirred for 30 min. After removing the organic layer via separatory funnel, the aqueous layer was extracted twice more with ether. The organic layers were combined and dried over anhydrous $MgSO_4$. Filtration followed by removal of solvent under reduced pressure provided a crude white solid that was purified by silica gel chromatography (eluted with 1:1 EtOAc:hexanes) to provide 416 mg (98%) of **6** as a white solid. Mp 53–54°C (recryst. EtOAc/hexanes).

^{1}H NMR (300 MHz) ($CDCl_3$) δ 2.75 (2H, t); 3.50 (2H, q); 5.09 (2H, s); 5.18 (1H, bs); 7.32–7.37 (5H, m); 9.81 (1H, s). ^{13}C NMR (75 MHz) ($CDCl_3$) δ 34.7, 44.3, 66.9, 128.2, 128.3, 128.6, 136.5, 156.4, 201.2. IR (NaCl, Neat) 3449, 3054, 2986, 2305, 2254, 1720, 1512, 1422, 1265, 1144, 1094, 1027, 909 cm^{-1}. HRMS (FAB) calcd for $C_{11}H_{13}NO_3$ (MH^+) 208.0974; found 208.0975.

Compound **8a**: Under argon atmosphere, compound **7a** (624 mg, 1.61 mmol, 1 equiv.) was dissolved in dry methylene chloride (19 mL). The resulting solution is then cooled to −5°C (ice/acetone bath). Di-*n*-butyl-



Scheme 1.

boron triflate (1 M in CH_2Cl_2) (3.22 mL, 3.22 mmol, 2 equiv.) was added via syringe followed by addition of triethylamine (673 μL, 4.83 mmol, 3 equiv.). The mixture was stirred 15 min at −5°C then cooled to −78°C. In a separate flask, compound **6** (400 mg, 1.93 mmol, 1.2 equiv.) was dissolved in methylene chloride (3.5 mL) and the resulting aldehyde solution was added via canula to the boron enolate, and the reaction was stirred one hour at −78°C. The reaction was quenched by the addition of 0.025 M pH 7 potassium phosphate buffer at −78°C, and the mixture was allowed to warm to room temperature. The organic layer was separated and the aqueous layer was extracted twice more with methylene chloride. The organic layers were combined and dried over anhydrous sodium sulfate. Filtration and removal of the solvent under reduced pressure produced an orange oil that was purified by silica gel chromatography (eluted with 30% EtOAc in hexanes). This initial separation resulted in co-elution of both the aldehyde and aldol product. A second silica gel chromatographic separation (eluted with 3:1:1 CH_2Cl_2:EtOAc:hexanes) resulted in the isolation of 657 mg (69%) of **8a** as an 8:1 mixture of diastereomers (^{1}H NMR). The minor diastereomer was removed by recrystallization from EtOAc/hexanes. Mp 175°C (recryst. EtOAc/hexanes).

^{1}H NMR (300 MHz) (DMSO-d_6, 393 K) δ DMSO: 1.84–2.06 (2H, m); 3.30 (2H, m); 4.29 (1H, m); 4.87 (1H, d, $J = 1.8$ Hz); 5.02 (1H, 1/2 ABq, $J = 12.9$ Hz); 5.07 (1H, 1/2 ABq, $J = 12.9$ Hz); 5.12 (2H, s); 5.30 (1H, d, $J = 3.3$ Hz); 5.62 (1H, bs); 6.55 (1H, d, $J = 3.3$ Hz); 6.64–7.41 (21H, m). IR (NaCl, Neat) 3365, 3032, 2951, 1750, 1734, 1700, 1684, 1653, 1540, 1404, 1274, 1120, 967 cm^{-1}. Anal. calcd for $C_{35}H_{34}N_2O_7$: C, 70.69; H, 5.76; N, 4.71. Found C, 70.50; H, 6.02; N, 4.89. Compound **8a**. $[\alpha]_D^{25} = -5.7$ ($c = 1.5$, CH_2Cl_2). Compound **8b**. $[\alpha]_D^{25} = +5.7$ ($c = 1.5$, CH_2Cl_2).

Compound 1a (mono-HCl salt): A pressure tube containing **8a** (100 mg, 0.17 mmol, 1 equiv.) in dry THF (4 mL) and absolute ethanol (2 mL) was purged with argon for 15 min. $PdCl_2$ (60 mg, 0.34 mmol, 2 equiv.) was added to this solution and the tube pressurized to 78 psi with hydrogen gas. The reaction was stirred at room temperature for 4 days. The catalyst was removed by filtration through Celite. The Celite pad was washed several times with a 2:1 THF:EtOH solution. Into a separate flask, the Celite pad was washed with five volumes of deionized water. The water volume was reduced by lyophilization and brought to pH 6.5 with NH_4OH. Addition of EtOH resulted in a white precipitate that was collected by filtration and dried to yield 21 mg (68%) of **1a** as the mono-HCl salt.

^{1}H NMR (300 MHz) (D_2O) δ 1.82–2.04 (2H, m); 3.18 (2H, sym m); 3.89 (1H, d, $J = 3.7$ Hz); 4.26 (1H, ddd, $J = 3.3$, 3.3, 10.6 Hz).^{13}C NMR (100 MHz) (D_2O) δ 29.0, 37.7, 59.5, 68.0, 171.6. IR (NaCl, 1% KBr) 3074, 1612, 1576, 1529, 1508, 1431, 1358, 1325, 1180, 1140, 1065, 1032 cm^{-1}. Mp 232°C dec (lit.[5] mp 232°C dec). Compound **1a**. $[\alpha]_D^{25} = +24.1$ ($c = 0.56$, 6N HCl) [lit.[5] $[\alpha]_D = +18.0$ ($c = 2.2$, 6N HCl)]. Compound **1b**. $[\alpha]_D^{25} = -20.2$ ($c = 0.47$, 6N HCl). The enantiomeric purities of **1a** and **1b** were determined to be >99.5:0.5 er by chiral HPLC analysis (Daicel Chiral Pak WH, column temperature 50°C, 0.25 mM $CuSO_4$ mobile phase, Waters 600 HPLC, dual wavelength UV detection at 210 and 254 nm).

Acknowledgements

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10. Lactones **7** are commercially available from Aldrich Chemical Co.; **7a**: catalog # 33-185-6; **7b** catalog # 33,187-2.

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The asymmetric synthesis of (2*S*,3*R*)-capreomycidine

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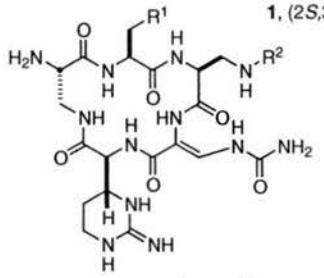
Abstract—An improved asymmetric synthesis of the guanidine-containing amino acid (2*S*,3*R*)-capreomycidine has been achieved in seven steps and 28% overall yield. The key synthetic step involved a Mannich-type reaction between a chiral glycine aluminum enolate and the benzyl-imine of 3-*tert*-butyldimethylsiloxy-propionaldehyde.

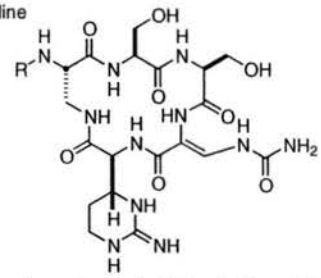
Capreomycidine **1** is a non-proteinogenic amino acid that is a constituent of the capreomycins **2a–d** and the tuberactinomycins **3a–b** (Fig. 1).[1,2] These cyclic pentapeptides are known for their unique tuberculostatic properties. First discovered by Herr et al.[3] in 1960, the capreomycins have recently attracted attention due to their demonstrated effectiveness against resistant strains of *Mycobacterium tuberculosis*.[4]

Various derivatives of both the capreomycins and tuberactinomycins have been made by synthetic modifications to the amino acid side chains and peptide backbone of the intact natural product.[5] This was done in order to determine the sectors of the molecule that are important for the expression of biological activity and also to perhaps discover more potent synthetic variants. Some of these derivatives have been shown to

1, (2*S*,3*R*)-capreomycidine



2a: capreomycin IA; R^1=OH, R^2=β-lysine
2b: capreomycin IB; R^1=H, R^2=β-lysine
2c: capreomycin IIA; R^1=OH, R^2=H
2d: capreomycin IIB; R^1=H, R^2=H



3a: tuberactinomycin N; R=γ-hydroxy-β-lysine
3b: tuberactinomycin O; R=β-lysine

Figure 1.

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be effective against other pathogens as well. The capreomycidne moiety contained in the macrocyclic portion of these substances has been shown to be essential for the expression of biological activity.[6]

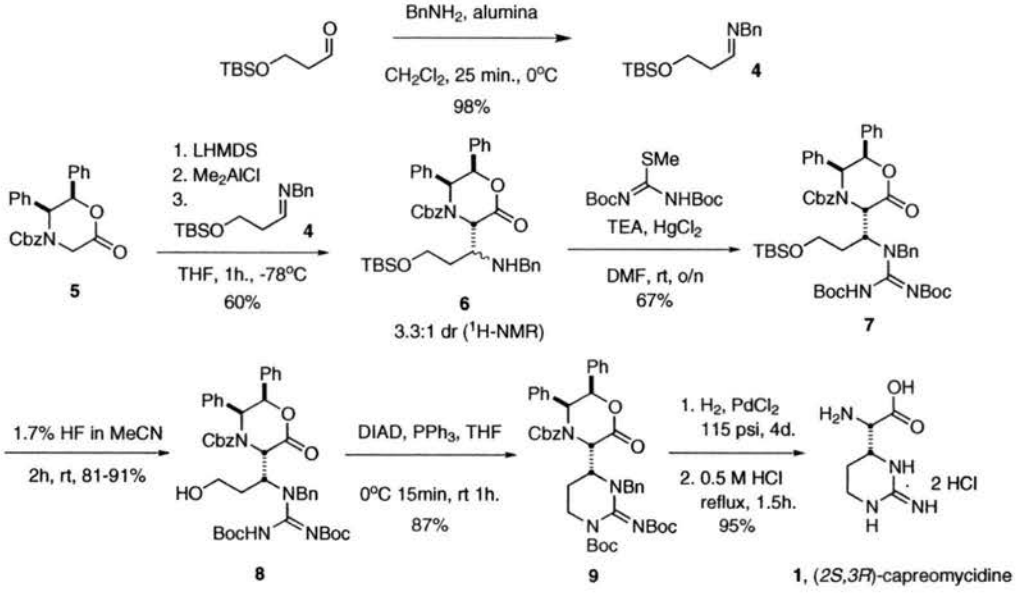
Total syntheses of the capreomycins as well as tuberactinomycin O have been reported by Shiba et al.[1,7] However, the capreomycidine moiety used in these syntheses was obtained not synthetically, but rather semi-synthetically by acid hydrolysis of the natural product.

Syntheses of capreomycidine and its epimer in both racemic and optically active form have previously been reported. Cameron and Bycroft were the first to report a racemic synthesis of both capreomycidine and *epi*-capreomycidine.[8] The desired product was obtained by elaboration of 2-aminopyrimid-4-ylacetate. Fractional recrystallization of picrate salts of the two diastereomers resulted in a tedious isolation of racemic capreomycidine and racemic *epi*-capreomycidine. Shiba and co-workers prepared racemic capreomycidine via a diastereoselective aldol reaction which produced a protected version of β-hydroxyornithine that was further elaborated to the final product.[9] Using a chloroacetylated intermediate in this same synthesis, Shiba enzymatically resolved two enantiomers with an acylase. This allowed for the synthesis of optically pure (2*S*,3*R*)-capreomycidine.[10] In a similar manner, the synthesis of *epi*-capreomycidine was also accomplished.[11]

In order to investigate a broader array of capreomycin derivatives for potential antituberculosis activity, it is desirable to have an effective means of obtaining significant quantities of a suitably protected form of (2*S*,3*R*)-capreomycidine via synthesis rather than by degradation of the natural product. Shiba's synthetic approach to (2*S*,3*R*)-capreomycidine was lengthy and proceeded in a low overall yield (~0.2%). With this as a backdrop, we sought to develop a more efficient synthesis of (2*S*,3*R*)-capreomycidine.

As shown in Scheme 1, treatment of 3-(*tert*-butyldimethylsiloxy)-propionaldehyde with benzylamine on alumina, gave the desired imine **4** in 98% yield.[12,13] Preparation of the lithium enolate of the chiral oxazinone **5** with lithium bis(trimethylsilyl)amide, followed by transmetallation with dimethylaluminum chloride, resulted in the formation of the corresponding aluminum enolate.[14,15] Addition of **4** to the enolate resulted in a 60% yield of the Mannich product **6** as an inseparable mixture of two diastereomers (3.3:1 *dr* by ^{1}H NMR) both arising from the approach of the imine to the face opposite that of the phenyls.

The guanidinylation of Mannich product **6** proved to be a far more challenging step than expected. Treatment of **6** with *N*,*N*′-di-*tert*-butoxycarbonyl-*S*-methylisothiourea and triethylamine in DMF as well as treatment of **6** with Goodman's benzyloxycarbonyl protected triflylguanidine reagent resulted in no reaction.[16,17] Subjecting **6** to a combination of *N*,*N*′-di-*tert*-butoxycarbonylthiourea, mercuric chloride and triethylamine in DMF according to the protocol described by Kim et al., the desired product **7** was obtained in a modest yield of 50% as a single diastereomer (determined by ^{13}C NMR).[18] It was then decided to determine what effect switching from *N*,*N*′-di-*tert*-butoxycarbonylthiourea to the corresponding *N*,*N*′-di-*tert*-butoxycarbonyl-*S*-methylisothiourea might have on the guanidinylation yield. This approach, also recently reported by Cammidge and co-workers, resulted in an improved 67% yield of the desired



Scheme 1.

guanidine **7**.[19] Judging from the analysis of recovered starting material, the guanidinylation only occurs with the major diastereomer of Mannich product **6**, therefore explaining the moderate yield and single product from this reaction.

Removal of the *tert*-butyldimethylsilyl protecting group from **7** with 1.7% aqueous HF in acetonitrile provided the primary alcohol **8** in 81–91% yield. Unfortunately, this cyclization precursor proved unstable to both acid and base. Rapid silica gel purification using Whatman brand silica gel gave satisfactory results. Attempted removal of the TBS group by other means proved unsuccessful.

Using the method described by Dodd and Kozikowski, the cyclic guanidine **9** was formed by treatment of **8** under Mitsunobu conditions in 87% yield.[20] The bicyclic compound **9** was then subjected to hydrogenolysis in 3:1 THF:EtOH using $PdCl_2$ and 115 psi hydrogen gas for 4 days at room temperature. Upon removal of the catalyst by filtration through Celite, and evaporation, the residue was triturated twice with ether. The hydrogenation product was refluxed in 0.5 M aqueous HCl to remove the remaining Boc group and lyophilized to provide the di-hydrochloride salt of capreomycidine **1** in 95% yield from **9**. Neutralization with ammonium hydroxide, followed by desalting via Dowex 50WX2-100 cationic exchange resin (product eluted with 1.5% NH_4OH) provided the free amino acid. Spiking of this free amino acid with ammonium acetate provided our synthetic capreomycidine in a form that was identical to the form of the natural capreomycidine obtained from Oregon State University and resulted in proton and carbon spectra that matched the natural sample. Upon synthesis of the enantiomer of natural capreomycidine in similar yield from the antipode of **5**, the α-amines of both (2*S*,3*R*)- and (2*R*,3*S*)-capreomycidine were protected as the corresponding benzyl carbamates. Chiral HPLC analysis of these enantiomeric carbamates showed that our synthetic capreomycidine possessed an *er* of 99.2:0.8 (>99% ee).[21] The optical rotations of the synthetic and natural mono-HCl salts of capreomycidine were also agreeable (synthetic: $[M]_D^{20} = +28.2$ ($c = 0.75$, H_2O); natural: $[M]_D^{20} = +32.5$ ($c \sim 0.75$, H_2O)).[22,23]

Since the desired (2*S*,3*R*)-capreomycidine was obtained from the major diastereomer of **6**, one can infer that the major diastereomer of **6** was of the (2*S*,3*R*)-configuration. This can be explained by a Zimmerman–Traxler 'chair' transition state between the *E*-enolate and the *E*-imine.

In summary, we have employed a novel and moderately diastereoselective Mannich-type reaction with the chiral glycine template **5** as a key step in the asymmetric synthesis of (2*S*,3*R*)-capreomycidine. The synthesis recorded here proceeds in six steps with an overall yield of 28%. Application of this methodology to the total synthesis of capreomycin and derivatives is currently under study in these laboratories.

Acknowledgements

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21. Enantiomeric ratios were determined using a Daicel Crownpak CR chiral HPLC column (pH 2 aqueous perchloric acid, 1.6 mL/min, UV detection at 205 nm). Retention times: (2*S*,3*R*)-capreomycidine: 21.22 min; (2*R*,3*S*)-capreomycidine: 31.95 min.
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Asymmetric Synthesis of (+)-Hypusine

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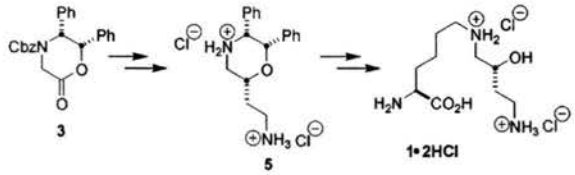
ABSTRACT



Wittig reaction of (triphenylphosphoranylidene)acetonitrile with the lactone carbonyl of (5*R*,6*S*)-4-(benzyloxycarbonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (3) and subsequent reduction generates morpholinylethylamine dihydrochloride (5) in quantitative yield and with excellent diastereoselectivity. Compound 5 was readily converted into hypusine dihydrochloride (1·2HCl) in overall 53% yield.

(+)-Hypusine (**1**) (Hpu, (2*S*,9*R*)-2,11-diamino-9-hydroxy-7-azaundecanoic acid) is an unusual naturally occurring amino acid that was first isolated from extracts of bovine brain in 1971 by Shiba et al.,[1] who also established its absolute configuration in 1982.[2] (+)-Hypusine (**1**) is formally a conjugate of hydroxyputrescine (**2**, Figure 1) and lysine.[1]

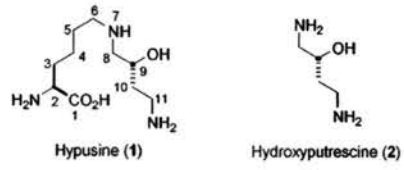


Figure 1. Structures of hypusine (**1**) and hydroxyputrescine (**2**).

In 1983, Folk and co-workers[3] found that a precursor protein of eukaryotic initiation factor 5A (eIF-5A, formerly known as eIF-4D), found in all animal cells, undergoes posttranslational modification in growing cells to form hypusine. In 1986, Park et al.[4] isolated the eIF-5A protein from human red blood cells and determined the amino acid sequence around the single hypusine residue (Hpu) as Thr-Gly-Hpu-His-Gly-His-Ala-Lys. Recently, eIF-5A has been shown to play a key role in the replication of human immunodeficiency virus-1 (HIV-1).[5]

Synthesis of a reagent that enables the incorporation of hypusine into peptide sequences has also recently been reported.[6]

A number of approaches have been reported in the literature for the synthesis of hypusine, including (1) *N*-alkylation of a L-lysine fragment with a 4-amino-1-bromo-2-butanol derivative;[2] (2) reductive amination of a hydroxyputrescine fragment with a 2-amino-6-oxo-hexanoic acid derivative;[7] (3) reductive amination of a L-lysine fragment with a 4-amino-2-hydroxy-butyraldehyde derivative;[8] and (4) *N*-alkylation of a L-lysine fragment with epichlorohydrin, subsequent displacement of chloride with cyanide, and reduction.[9] In most of these approaches, L-lysine was utilized as a key substrate.

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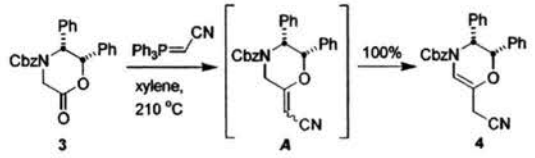
Scheme 1

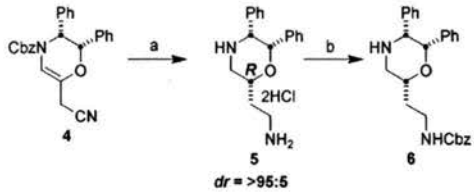


Herein, we report a concise method for the synthesis of (+)-hypusine by employing (5*R*,6*S*)-4-(benzyloxycarbonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**3**)[10] as a starting material. This approach involves a de novo synthesis of both the L-lysine fragment and the hydroxyputrescine moiety and is amenable to the site-specific incorporation of stable and/or radioisotopes.

As shown in Scheme 1, Wittig reaction of (triphenylphosphoranylidene)acetonitrile[11] with the lactone carbonyl group of **3** (xylene, 210 °C, 2.5 h) generated the adduct **4** in quantitative yield. This species is reasonably presumed to arise via tautomerization of the initial olefination product ***A*** to the thermodynamically more stable trisubstituted olefin. Although a few isolated cases of stabilized Wittig olefinations of lactones and esters have appeared in the literature, this condensation is an underutilized reaction in synthetic organic chemistry.[12-18] Wittig reactions of stabilized ylides with the carbonyl groups of lactones,[12] esters,[13] thioesters,[14] anhydrides,[15] thioanhydrides,[16] amides[17] and imides[18] have been reported in the literature, but many of these systems were intramolecular ring-closure reactions. Attempts to conduct the reaction at lower temperatures (in toluene or xylene at reflux) required longer reaction times and incomplete transformations with poor yields of **4**.

Hydrogenation of **4** with $PdCl_2$ (30 mol %, 120 psi of H_2, MeOH, 4 equiv concentrated HCl, rt, 72 h) resulted in the formation of desired all *syn*-substituted oxazine **5** in essentially quantitative yield and with >95:5 diastereomeric ratio (by ^{1}H NMR, Scheme 2).

Scheme 2[a]



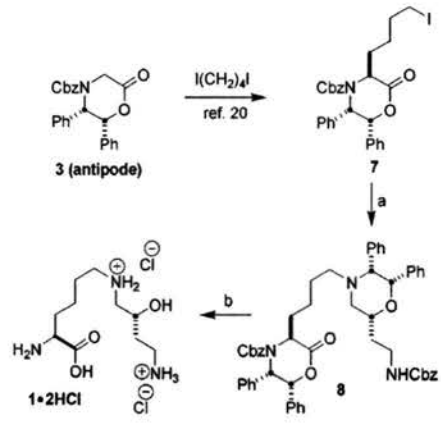
[a] Reagents and conditions: (a) 120 psi H_2, $PdCl_2$, MeOH, concentrated HCl, rt, 100%; (b) THF, 2 M NaOH, reflux; then Cbz_2O, rt, 70%.

The relative and absolute stereochemistry of the newly created stereogenic center in the major diastereomer of **5** was determined as *R* by ^{1}H NMR NOE measurements that revealed a *syn*-relationship of the protons at C2, C5, and C6 of the oxazine ring. The high degree of asymmetric induction in the reduction step can be explained by adsorption on the catalyst surface and subsequent hydrogenation of the double bond from the sterically less hindered face of the molecule.

Selective protection of primary amine in **5** was achieved by treatment of **5** with Cbz_2O (1 equiv, THF/2 M NaOH) yielding the desired monoprotected morpholine **6** in 70% yield (Scheme 2).

Compound **6** is a protected version of hydroxyputrescine **2** and may be suitable for related applications in the regioselective coupling of this fragment to other amino acids. The conversion of **6** into (+)-hypusine (**1**) required *N*-alkylation of the morpholine nitrogen in **6** with an electrophilic L-lysine fragment. The desired L-lysine fragment **7** required for this coupling reaction was easily synthesized by the glycine enolate alkylation method[19] by using the commercially available antipode of **3**[10] and 1,4-diiodobutane, as reported previously by our group[20] (Scheme 3).

Scheme 3[a]



[a] Reagents and conditions: (a) **6**, *N,N*-diisopropylethylamine, xylene, reflux, 78%; (b) 80 psi H_2, $PdCl_2$, THF/H_2O, 80-85 °C, 98%.

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Treatment of **6** with **7** in the presence of *N,N*-diisopropylethylamine in xylene at reflux (2.5 h) generated the desired coupling product **8** in 78% yield. Complete hydrogenolysis of **8** with $PdCl_2$ (6 equiv, THF/H_2O, 80 psi of H_2, 80−85 °C, 6 h) resulted in the formation of (+)-hypusine (**1**) in essentially quantitative yield as its dihydrochloride salt ($[\alpha]^{25}_D = +7.3$ (*c* 0.52, 6M HCl); lit.[9] $[\alpha]^{23}_D = +7.6$ (*c* 0.5, 6 M HCl)). The spectral data for this substance matched that reported in the literature for hypusine.[2,7−9]

In summary, we have demonstrated a concise, asymmetric, and stereocontrolled method for the synthesis of (+)-hypusine. Since both antipodes of oxazinone **3** are commercially available,[10] the present method can provide access to all four stereoisomers of hypusine. Current efforts are focused on extending the stabilized Wittig homologation on the oxazinones to other amino acid-derived natural products and alkaloids.

Acknowledgment. This work was supported by National Science Foundation (grant CHE 9731947).

Supporting Information Available: Experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL016959O

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TETRAHEDRON LETTERS

The first asymmetric synthesis of (2*S*)- and (2*R*)-amino-3,3-dimethoxypropanoic acid

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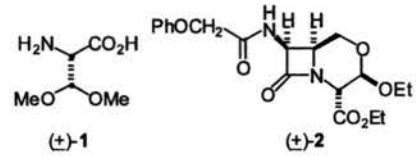
Abstract—The first asymmetric synthesis of (2*S*)-, and (2*R*)-amino-3,3-dimethoxypropanoic acid (α-formylglycine dimethylacetal) has been achieved in two steps and 91% overall yield. The key step involved the quenching of a chiral glycine titanium enolate with trimethyl orthoformate.

The unnatural amino acid 2-amino-3,3-dimethoxypropanoic acid or α-formylglycine dimethylacetal (±)-**1** has been utilized in a wide variety of synthetic transformations (Fig. 1).[1] This compound and its diethylacetal counterpart, until now, have only been synthesized in racemic form via a variety of tedious approaches. In many of the literature references using this compound, the stereogenic center present in α-formylglycine dimethylacetal is destroyed such as, in cases where a dehydroamino acid moiety is constructed. Doyle and co-workers demonstrated the usefulness of α-formylglycine diethylacetal in their synthesis of isocepham (±)-**2** (Fig. 1).[2] Although, in this case, the stereogenic center from the α-formylglycine diethylacetal portion was retained in the final product, compound **2** was synthesized in racemic form.

Our desire to prepare both enantiomers of α-formylglycine dimethylacetal is related to our investigations on the total synthesis of capreomycin IB (**3**) (Fig. 2). Shiba, in his total syntheses of the capreomycins and the structurally similar tuberactinomycins, used racemic α-formylglycine diethylacetal as a precursor to the enamidourea functionality present in both of these cyclic pentapeptides.[3] Our goal was to synthesize both diastereomers of our macrocyclization precursor arising from the incorporation of both enantiomers of α-formylglycine dimethylacetal. This was desired so that we could investigate whether or not one diastereomeric hexapeptide undergoes more facile macrocyclization than the other.



(±)-**1** (±)-**2**

Figure 1.

Adapting chemistry developed by Evans to our strategy, chiral glycinate (−)-**4** was treated with $TiCl_4$ at −78°C, followed by the addition of triethylamine (Scheme 1).[4] After allowing the enolate to form, trimethylorthoformate was added, and the mixture was allowed to warm to 0°C over an hour. The result was a single diastereomer of the desired dimethylacetal (+)-**5** in 93% yield. Hydrogenolysis of the lactone adduct, followed by trituration with ether to remove the dibenzyl byproduct provided (2*R*)-amino-3,3-dimethoxypropanoic acid (−)-**1** in 98% yield. Treatment of (−)-**1** with refluxing methanolic HCl resulted in the formation of (+)-**6** in 99% yield. The ee of (+)-**6** was found to be >95% by Mosher's amide analysis (^{1}H NMR). In addition, both enantiomers of α-formylglycine dimethylacetal (**1**) can be prepared from the commercially available antipodes of oxazinone **4**.[5]

The amino functionality of compound **1** can also be protected for use in peptide synthesis strategies as illustrated with the example in Scheme 2. Treatment of (+)-**1** with TeocONSu[6] and triethylamine in dioxane:water provided the trimethylsilylethyl carbamate (−)-**7** in 77% yield.[7]

In summary, a titanium enolate/trimethyl orthoformate condensation with chiral glycine template **4** has been

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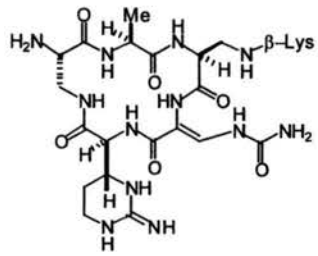
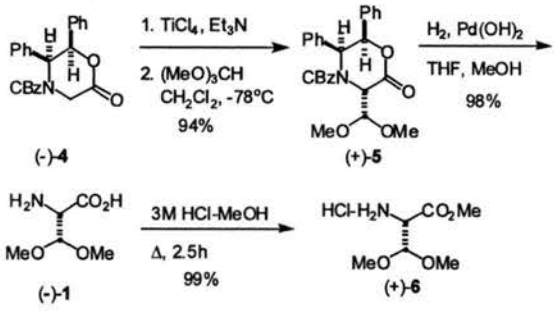
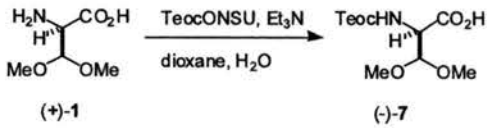


Figure 2.



Scheme 1.



Scheme 2.

accomplished in excellent yield. This has served as the key step in the first asymmetric synthesis of (2*S*)- and (2*R*)-amino-3,3-dimethoxypropanoic acid. This work constitutes the first example of the successful deployment of a titanium enolate generated from **4** that may find other useful applications in amino acid synthesis. In addition, the condensation product **5** is a potentially useful chiral building block, and studies are currently underway to explore other uses for this substance.

Experimental

Compound (+)-**5**: A solution of (−)-**4** (1.24 g, 3.2 mmol, 1 equiv.) is dissolved in CH_2Cl_2 (50 mL) and cooled to −78°C. While stirring, $TiCl_4$ (700 μL, 6.4 mmol, 2 equiv.) was added, followed by triethylamine (900 μL, 6.4 mmol, 2 equiv.) to provide a dark blue enolate solution. After stirring for 15 min, trimethyl orthoformate (2.1 mL, 19.2 mmol, 6 equiv.) was added, and the solution warmed slowly to 0°C. After stirring 45 min at 0°C, 0.025 M pH 7 phosphate buffer was added and stirred 30 min. The quenched reaction was filtered through Celite, diluted with CH_2Cl_2, and washed twice with brine. Upon drying the organic layer over anhydrous sodium sulfate, the solution was filtered and evaporated to provide an off white solid. Silica gel chromatography (eluted with 6:3:1 CH_2Cl_2:hexanes: EtOAc) provided 1.37 g (93%) of pure (+)-**5** as a white solid.

^{1}H NMR (300 MHz) (DMSO-d_6, 393K) δ DMSO: 3.50 (3H, bs); 3.51 (3H, s); 4.87 (1H, d, J=2.9 Hz); 5.02 (2H, broad m); 5.14 (1H, d, J=2.9 Hz); 5.28 (1H, d, J=3.3 Hz); 6.28 (1H, d, J=3.3 Hz); 6.61 (2H, d, J=7.0 Hz); 7.01–7.29 (13H, m). IR (NaCl, neat) 3031, 2940, 2837, 1753, 1706, 1454, 1401, 1348, 1288, 1267, 1250, 1207, 1189, 1109, 1081 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{27}H_{28}NO_6$ (MH$^+$) 462.1917; found 462.1917. (+)-**5**. $[\alpha]_D^{25}$=+1.7 (c=1.0, CH_2Cl_2). (−)-**5**. $[\alpha]_D^{25}$=−1.8 (c=1.0, CH_2Cl_2).

Compound (−)-**1**: A solution of (+)-**5** (472 mg, 1.02 mmol, 1 equiv.) in 3:1 THF:MeOH (32 mL) was purged with argon for 10 min. To this solution in a pressure tube, 20% $Pd(OH)_2$ on activated carbon (360 mg, 0.51 mmol, 0.5 equiv.) was added, and the tube filled with hydrogen gas to 95 psi. The pressure was released, and the tube refilled. This was repeated 4 times more. The pressurized tube was then stirred for 2 days at room temperature. After the 2 days, the pressure was released, the solution purged with argon, and the 20% $Pd(OH)_2$ on activated carbon removed by filtration through Celite. Evaporation of the filtrate and trituration of the residue with ether provided 152 mg (99%) of (−)-**1** as an oily solid.

^{1}H NMR (400 MHz) (DMSO-d_6) δ DMSO: 3.35 (3H, s); 3.36 (3H, s); 3.39 (1H, d, J=2.1 Hz); 4.71 (1H, d, J=2.1 Hz); 6.80–8.40 (3H, bs). ^{13}C NMR (100 MHz) (DMSO-d_6) δ DMSO: 55.0, 55.6, 56.8, 103.7, 166.3. IR (NaCl, neat): 2939, 1641, 1506, 1406, 1342, 1272, 1218, 1194, 1067 cm^{-1}. HRMS (FAB$^+$) calcd for $C_5H_{12}NO_4$ (MH$^+$) 150.0766; found 150.0768. (−)-**1**. $[\alpha]_D^{25}$=−7.4 (c=0.50, MeOH). (+)-**1**. $[\alpha]_D^{25}$=+7.6 (c=0.67, MeOH).

Compound (+)-**6**: A stirred solution of MeOH (10 mL) at 0°C was treated with acetyl chloride (2 mL, 30 mmol). This mixture was warmed to room temperature and stirred for 20 min. The resulting methanolic HCl solution was added to a round bottomed flask containing (−)-**1** (95 mg, 0.64 mmol, 1 equiv.). After stirring the reaction at reflux for 2.5 h, the solvent was removed in vacuo to provide 126 mg (99%) of (+)-**6** as a clear oil.

^{1}H NMR (400 MHz) (CD_3OD) δ CD_3OD: 3.49 (3H, s); 3.54 (3H, s); 3.86 (3H, s); 4.33 (1H, d, J=2.8 Hz); 4.86 (1H, d, J=2.8 Hz). ^{13}C NMR (100 MHz) (CD_3OD) δ: 53.9; 56.4; 57.2; 57.7; 103.2; 167.8. IR (NaCl, neat): 3583, 3408, 2956, 2843, 1749, 1643, 1591, 1503, 1443, 1378, 1306, 1241, 1195, 1111, 1070 cm^{-1}. HRMS (FAB$^+$) calcd for $C_6H_{14}NO_4$ (MH$^+$) 164.0923; found 164.0922. (+)-**6**. $[\alpha]_D^{25}$=+2.7 (c=0.66, MeOH). (−)-**6**. $[\alpha]_D^{25}$=−2.7 (c=0.66, MeOH). The enantiomeric purity of (+)-**6** was found to be >95% ee by formation of the Mosher's amide via both the optically pure and racemic Mosher's acid chlorides and comparison of the resulting diastereomers by ^{1}H NMR. None of the minor

diastereomer was observed in the optically pure Mosher's acid chloride case.

Compound (−)-**7**: To a solution of (+)-**1** (59 mg, 0.40 mmol, 1 equiv.) in 1:1 dioxane:water (3 ml) was added triethylamine (166 μL, 1.19 mmol, 3 equiv.) and the mixture stirred under argon. TeocONSu[6] (109 mg, 0.42 mmol, 1.05 equiv.) was added to the solution, and the reaction stirred overnight at room temperature. The reaction was diluted, acidified to pH 4 with 0.5 M citric acid, and extracted twice with ether. The combined ether layers were washed twice with water, then dried over $MgSO_4$. Removal of the drying agent by filtration and evaporation provided 90 mg (77%) of (−)-**7** as a pale oil.

^{1}H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 0.04 (9H, s); 1.00 (2H, ddd, J=0, 8.4, 9.5 Hz); 3.47 (6H, s); 4.19 (2H, ddd, J=0, 6.6, 9.5 Hz); 4.61 (1H, d, J=8.4 Hz); 4.66 (1H, s); 5.42 (1H, d, J=7.7 Hz); 9.29 (1H, bs). ^{13}C NMR (75 MHz) ($CDCl_3$) δ: −1.2, 17.9, 55.9, 56.3, 64.1, 103.9, 156.9, 173.1. IR (NaCl, neat): 3452, 3319, 3107, 2954, 2838, 1725, 1525, 1448, 1415, 1317, 1251, 1214, 1186, 1118, 1068 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{11}H_{24}NO_6Si$ (MH^+) 294.1373; found 294.1379. (−)-**7**. $[\alpha]_D^{25}$ = −22.2 (c=2.0, CH_2Cl_2).

Acknowledgements

This work was supported by the National Science Foundation (Grant CHE 9731947).

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7. Shute, R. E.; Rich, D. H. *Synthesis* **1987**, 346–349.

APPENDIX 2

INDEPENDENT RESEARCH PROPOSAL

The Total Synthesis of Nomofungin and the Synthesis of Structural Analogs

Introduction:

Nomofungin (**1**) is an indole alkaloid recently isolated by Hemscheidt and coworkers from the fermentation broth of an unknown endophytic fungus (Figure 1).[1] The fungus in question was found on the bark of *Ficus microcarpa* L., a native plant of Hawaii. Ethyl acetate extracts of the fermentation broth were bioassayed for microfilament disruption in A-10 rat smooth muscle cells and were found to be highly active. Normal phase silica gel chromatography of the ethyl acetate extracts was followed by bioassay of the resulting fractions. Additional chromatography and fraction bioassay resulted in the isolation of **1** as a colorless solid. This optically pure compound was determined to be 0.9% of the beginning extraction mass. Structure determination was performed using mass spectrometry and high-field NMR experiments. The absolute stereochemistry was proposed based on an exciton chirality method. Unfortunately, attempts to repeat the initial fermentation and isolate additional amounts of **1** by fungal subculture have been unsuccessful.

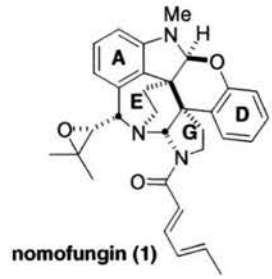


Figure 1. Structure of nomofungin.

In addition to its microfilament disruption activity, nomofungin was also found to be cytotoxic against both LoVo (human colon adenocarcinoma) cells with an MIC of

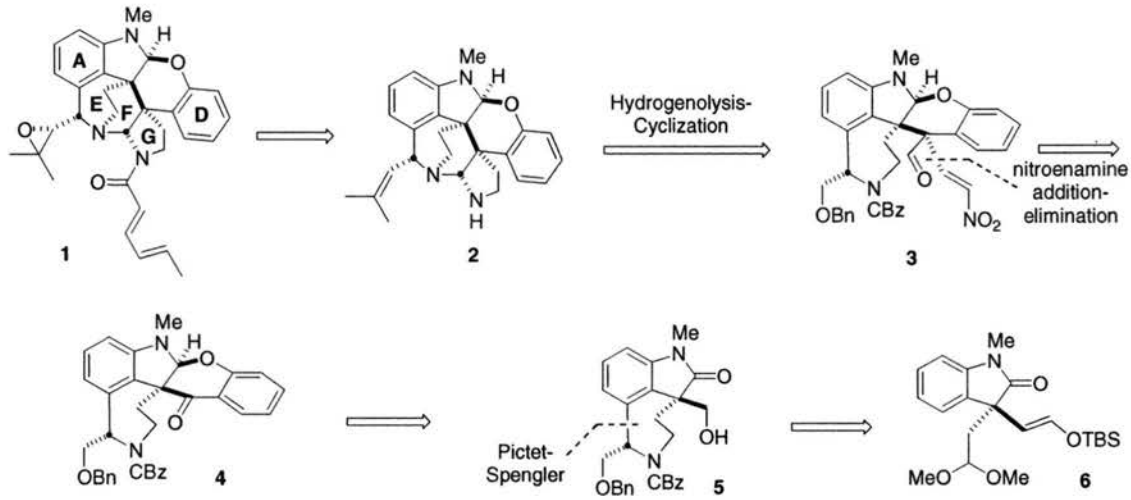
2 μg/mL and KB (human nasopharyngeal carcinoma) cells with an MIC of 4.5 μg/mL. This cytotoxic effect is presumably due to microfilament disruption as in the A-10 cell line case. Nomofungin possesses activities that are one to two orders of magnitude less potent that other microfilament disrupters such as phalloidin, lyngbyabellin A, and the cytochalasins.[2,3] The afore mentioned compounds, however, have not seen therapeutic use due to their significant *in vivo* toxicities. The *in vivo* toxicity of nomofungin has yet to be determined, and may never be, due to the lack of additional material.

There are several reasons for wanting to embark upon a total synthesis of nomofungin (**1**). From the standpoint of the synthetic organic chemist, the complex and unique structure of nomofungin presents some interesting challenges. This unique structure possesses seven fused rings with vicinal quaternary spirocyclic centers. Pendant to the fused ring system are a sorbate amide and an oxygenated prenyl group. Completion of a synthesis of nomofungin will serve to confirm both the structure and absolute stereochemistry of the molecule. The mode of action of **1** can also be probed by designing the synthesis in a manner that allows for the preparation of analogs not readily accessible from the natural product. Since the responsible endophyte has thus far stopped producing nomofungin, the only way in which to carry out further toxicity studies would be to access **1** by synthetic means. This is perhaps the most pressing reason for engaging in a total synthesis.

Experimental Design:

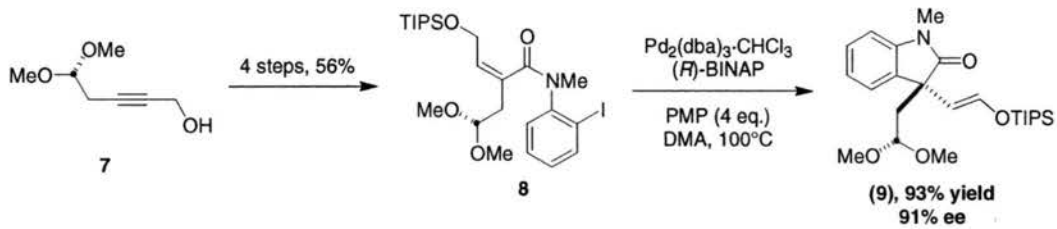
The proposed synthetic approach to nomofungin will allow for the total synthesis to be completed in a concise and enantioselective manner. In addition, the synthetic

design allows for analogs with a wide range of structural diversity to be prepared. In a late stage of the synthesis of nomofungin (**1**), both the epoxide and sorbate groups will be installed into the heptacyclic core **2** (Scheme 1). Rings **F** and **G** will be assembled from **3** by simultaneously reducing the nitroalkene, benzyloxycarbonyl, and benzyl ether followed by condensation with the aldehyde. The **G**-ring quaternary center in **3** will be prepared from **4** *via* transformation of the benzylic ketone to a spiroepoxide, rearrangement to the formyl enolate and subsequent Michael addition-elimination with a nitroenamine. Installation of the **C** and **D** rings in **4** will accessed by oxidation of the hydroxymethyl group present in **5** to an aldehyde followed by addition of a phenolate species and reductive cyclization upon the oxindole to form the fused carbinolamine. Finally, azepine **5** can be accessed from the previously reported 3,3-disubstituted oxindole **6**. This will be accomplished by transformation of **6** to a suitable carbamoyl-alcohol precursor for a Pictet-Spengler type reaction with 2-benzyloxyacetaldehyde to access the desired substituted benzazepine.



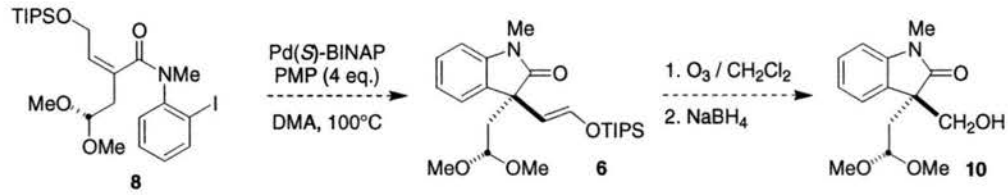
Scheme 1. Retrosynthetic analysis.

In 1998, Overman and coworkers published two initial reports describing some very elegant work on catalytic asymmetric intramolecular Heck reactions.[4,5] In particular, using palladium/BINAP systems, they were able to prepare a variety of α,α-disubstituted oxindoles in an enantioselective fashion. Important to the synthesis of nomofungin is their synthesis of oxindole **9** (Scheme 2). Butenanilide **8** was synthesized in 56% yield over four steps from propargyl alcohol **7**. Heck reaction of **8** provided oxindole **9** in an impressive 93% yield and 91% ee.



Scheme 2. Overman's asymmetric Heck reaction.

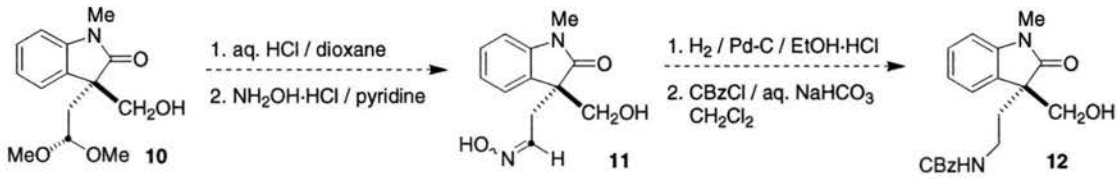
For the synthesis of nomofungin, it will be necessary to prepare the antipode of **9** (compound **6**) using (*S*)-BINAP (Scheme 3). After preparation of **6** the first step in the nomofungin synthesis will be installation of the **E** ring. Ozonolysis of enol silane **6** followed by reductive workup will provide hydroxymethyl oxindole **10** (Scheme 3).[6,7]



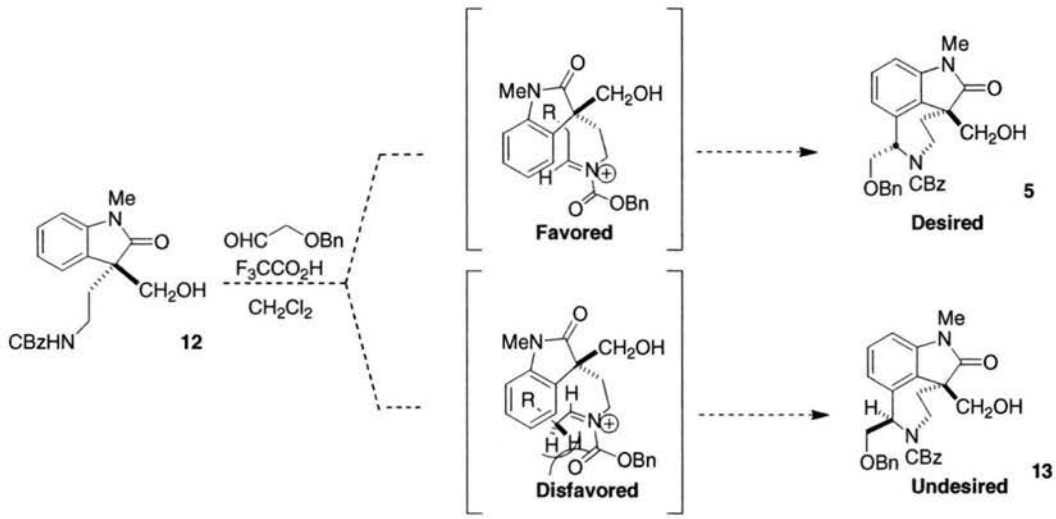
Scheme 3. Synthesis of 3,3-disubstituted oxindole.

In order to prepare a suitable precursor to the Pictet-Spengler reaction, acetal **10** will be hydrolyzed with aqueous HCl and treated with hydroxylamine hydrochloride and pyridine to provide oxime **11** (Scheme 4).[8] Palladium-catalyzed hydrogenation of oxime

11 followed by CBz protection of the resulting amine will afford the carbamate protected aminoethyl group **12**.



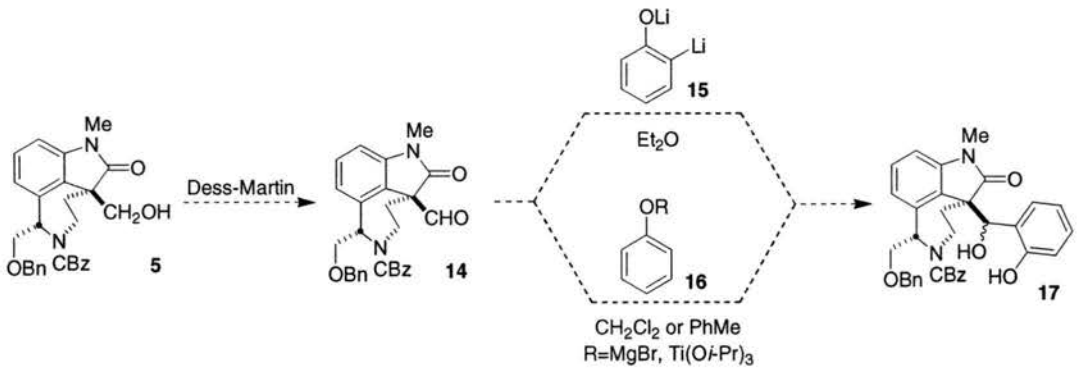
Scheme 4. Synthesis of Pictet-Spengler precursor.



Scheme 5. Benzazepine formation.

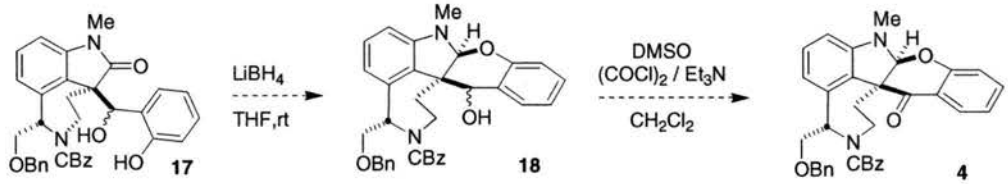
To install the benzazepine **E** ring portion of nomofungin, it was decided to use a Pictet-Spengler type reaction *via* an *N*-acyliminium intermediate (Scheme 5).[9] Treatment of **12** with 2-benzyloxyacetaldehyde[10] and trifluoroacetic acid will generate the necessary *N*-acyliminium ion. Trapping of the iminium ion by the oxindole aromatic ring, followed by rearomatization will provide the desired benzyloxymethyl benzazepine **5**. The 4-position of the oxindole should be the center to react with the iminium ion due to the fact that it is the only sterically accessible site on the aromatic ring. When looking at the two possible *N*-acyliminium conformations one can expect preference for the desired product **5**, due to the fact that the benzyloxymethyl group is situated away from the carbamate

carbonyl. The *N*-acyliminium geometry that would provide Pictet-Spengler product **13** places the benzyloxymethyl group on the same side as the carbamate, which creates an undesired steric interaction that is not present in the other transition state.



Scheme 6. D ring installation.

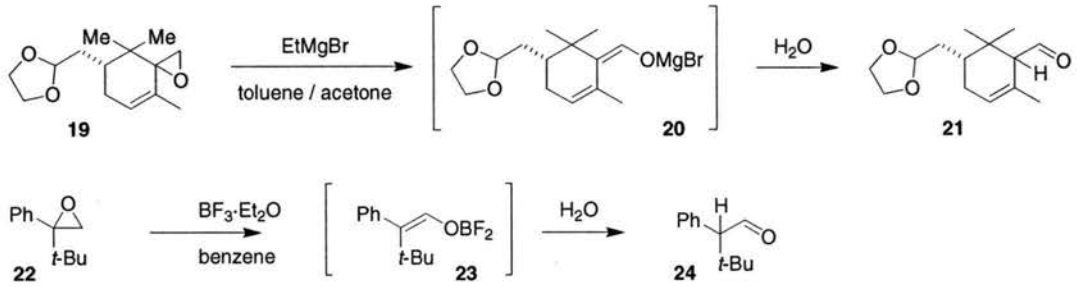
The hydroxymethyl group present in benzazepine **5** will next be oxidized to aldehyde **14** with the Dess-Martin periodinane to facilitate the incorporation of the **D** ring (Scheme 6).[11] Two possible means of adding phenol to aldehyde **14** have been outlined. Exposure of **14** to lithium *o*-lithiophenoxide **15** will afford phenol adduct **17**.[12] Addition of a magnesium or titanium phenolate (**16**) to **14** is an alternative possibility to provide access to **17**.[13-15] The stereochemistry of the addition product is unimportant due to the fact that it will be oxidized to a ketone in a later stage.



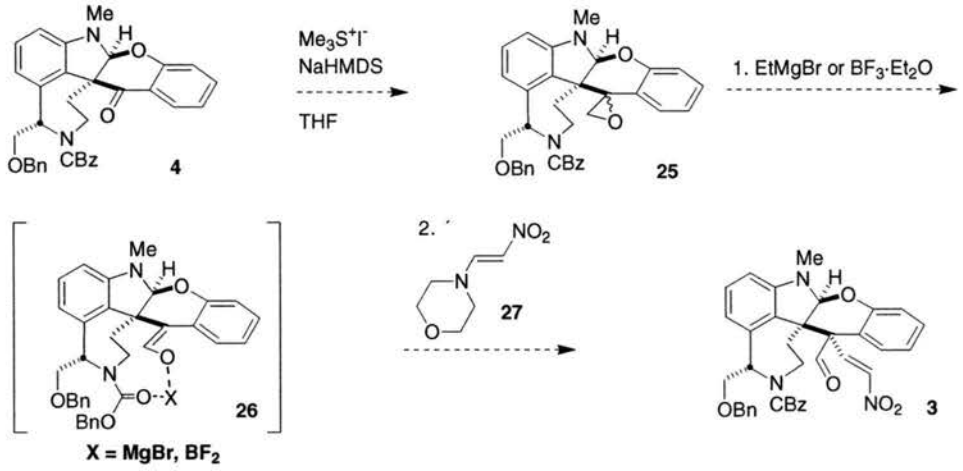
Scheme 7. Reductive cyclization.

Treatment of **17** with lithium borohydride will exact reduction of the oxindole carbonyl to the carbinolamine and facilitate closure of the phenol to the *N,O*-acetal **18** (Scheme 7).[16,17] The reductive cyclization should supply the more stable *cis*- 6-

membered ring.[18] Subsequent Swern oxidation of the benzylic alcohol will complete the preparation of the desired pentacyclic ketone **4**.[19,20]



Scheme 8. Previous spiroepoxide rearrangements.



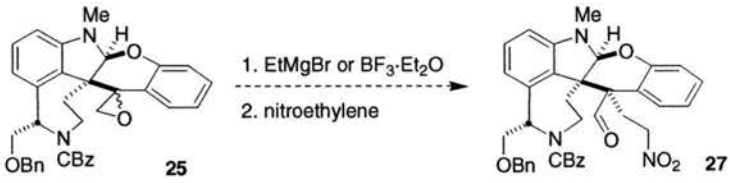
Scheme 9. Tandem spiroepoxide rearrangement-nitroenamine addition-elimination.

In order to install the **F** and **G** rings of nomofungin, the benzylic ketone in compound **4** needs to be transformed into a quaternary center. Two previous reports describe the single-step transformation of spiroepoxides to aldehydes (Scheme 8).[21,22] Pfander and coworkers treated epoxide **19** with EtMgBr to open the epoxide to magnesium enolate **20** which, upon workup, resulted in aldehyde **21**. Likewise, Ibarra described the opening of epoxide **22** to the boron enolate **23** which provided aldehyde **24** after quenching. In addition, Heathcock and coworkers prepared **24** from **22** by

hydrogenolysis of the epoxide followed by Swern oxidation to the aldehyde (not shown).[23]

Adapting the afore mentioned epoxide rearrangement methods to this synthesis, exposure of **4** to trimethyl sulfonium iodide and NaHMDS will access the desired spiroepoxide **25** (Scheme 9).[23,24] The stereochemical outcome of the spiroepoxide formation is again insignificant due to the fact that the following step will eliminate the newly created spirocenter. Treatment of **25** with EtMgBr or $BF_3 \cdot Et_2O$ will form the intermediate enolate **26**, which can undergo Michael addition with nitroenamine **27** to afford nitroalkene **3.**[25] Although, it is ultimately unclear whether or not there will be a significant preference for the desired stereochemistry shown in **3**, it is reasonable to propose that the enolate will coordinate with the sufficiently close CBz group carbonyl and direct the nitroenamine to the bottom (desired) face of enolate **26**. If preferred selectivity for the desired face of enolate **26** is not achieved, it is also possible to explore the use of chiral nitroenamines for this transformation.[26]

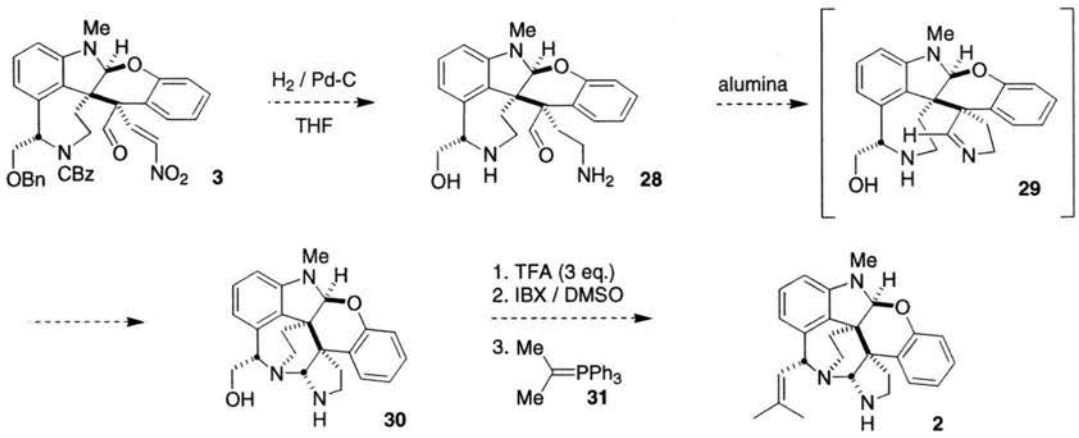
Alternatively, epoxide **25** could again be rearranged to the formyl enolate and treated with nitroethylene to provide the nitroethyl Michael adduct **27** (Scheme 10).[27,28]



Scheme 10. Alternate approach to vicinal quaternary center.

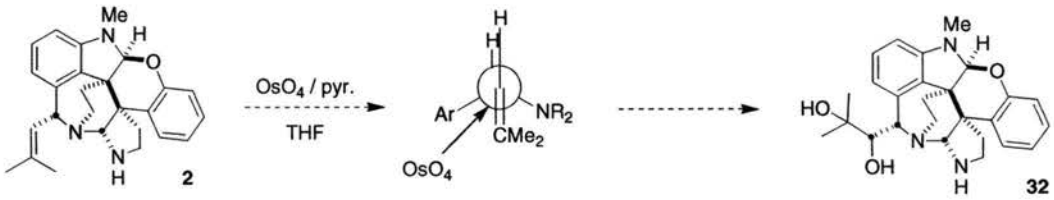
Hydrogenation of **3** (or **27**) in the presence of Pd/C will remove both the CBz group and benzyl ether, and reduce the nitroalkene to the desired aminoethyl group present in compound **28** (Scheme 11).[29] Treatment of **28** with alumina will promote the dehydrative cyclization and provide the heptacyclic core molecule **30** *via* the intermediate

imine **29**. Due to the spatial orientation of the intermediate imine **29** the only accessible geminal diamine is that of **30**. Protonation of the free amines will be followed by IBX oxidation of the alcohol to the aldehyde.[30] Wittig homologation of the aldehyde with phosphorus ylide **31** provide the desired prenyl azepine **2**.[31]



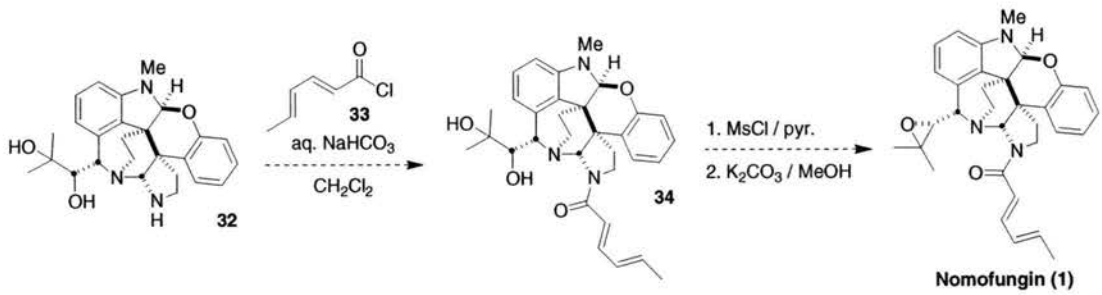
Scheme 11. Completion of the heptacyclic core.

Direct epoxidation of prenyl azepine **2** will not be possible due to the oxidizable amines that are present. In order to circumvent this problem, and to access the desired epoxide stereochemistry, a three-step epoxide forming sequence is appropriate (Scheme 12). Several reports have established that OsO_4-mediated oxidations of allylic heteroatom systems undergo dihydroxylation from the alkene face opposite that of the heteroatom.[32,33] Assuming that the alkene will be pointing away from the ring system, dihydroxylation of **2** should provide diol **32** with the desired stereochemistry.



Scheme 12. Prenyl group dihydroxylation.

Coupling of sorbic acid chloride **33** with pyrrolidine **32** will supply sorbate amide **34** (Scheme 13).[34] Mesylation of the secondary hydroxyl present in compound **34** followed by base mediated displacement of the mesylate by the adjacent tertiary hydroxyl will form the epoxide in the requisite configuration[35-37], thus completing the synthesis of nomofungin (**1**). In addition, if the mesylation approach is unsuccessful, Mitsunobu chemistry may be alternatively employed to form the epoxide.[38,39]



Scheme 13. Completion of nomofungin.

Since both enantiomers of the starting α,α-disubstituted oxindole are accessible, it is also desired to synthesize ***ent*-1** and investigate its cytotoxicity (Figure 2). In addition, it would also be interesting to investigate the activity of some deprotected synthetic intermediates such as **35**, **36**, and **37** in order to determine the importance of the side chains as well as the fused ring systems.

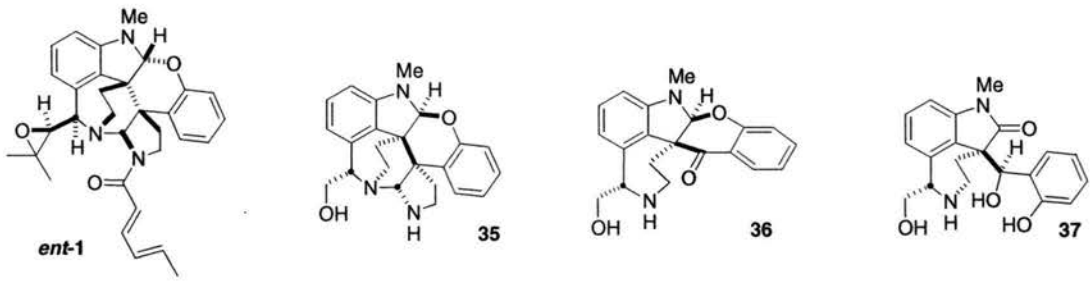
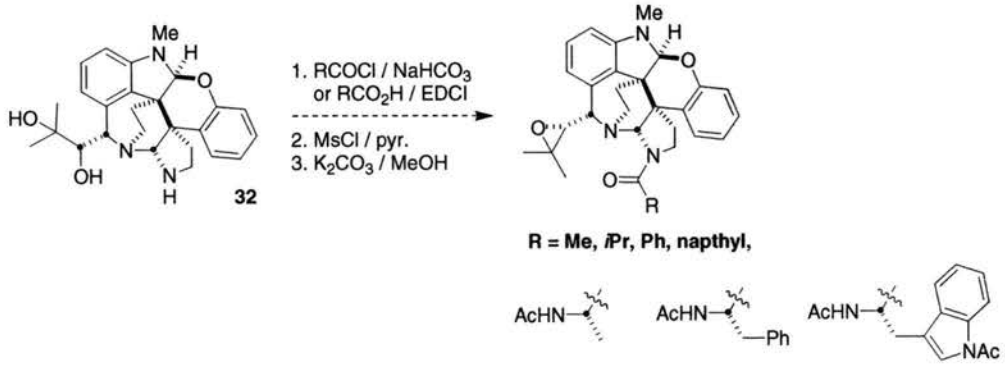


Figure 2. Intermediate analogs.

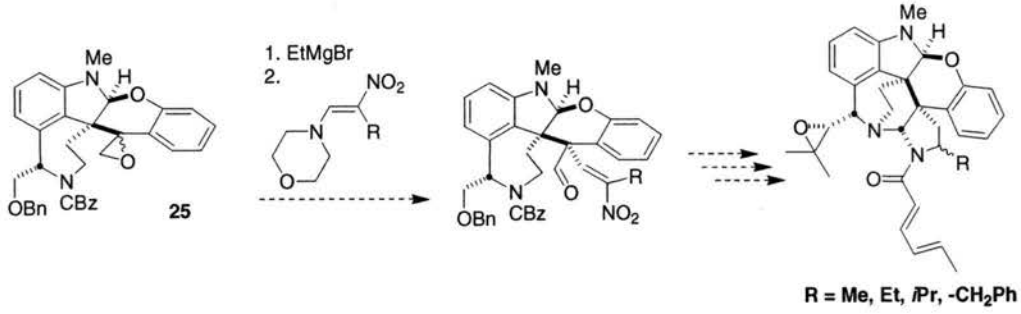
With the completion of the synthesis of nomofungin, it would also be of interest to synthesize a group of analogs that could be used to determine the mode of action of **1**.

In the first series of analogs, the pyrrolidine amide substituents will be varied (Scheme 14).

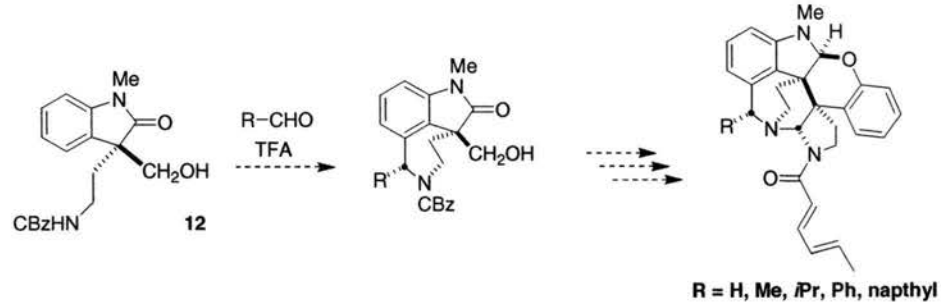


Scheme 14. Acyl group modifications.

In addition to acyl derivatives, pyrrolidine substitution could also be investigated *via* a variety of substituted nitroenamines (Scheme 15).



Scheme 15. Fused spiropyrrolidine analogs.



Scheme 16. Benzazepine analogs.

Finally, the nomofungin synthesis allows for access to a variety of azepine substitutions as well (Scheme 16). This will allow for investigation of the biological importance of the azepine side chain in nomofungin.

In conclusion, an asymmetric synthesis of the indole alkaloid nomofungin has been proposed. An asymmetric Heck reaction will set the stereochemistry from which other diastereoselective transformations will build upon. Formation of the fused spiropyrrolidine ring will be attempted using a tandem spiroepoxide rearrangement / nitroenamine addition / elimination reaction. Additionally, the synthesis has been designed in a manner that allows for the preparation of a variety of analogs that could be used to determine the biologically important structural motifs of nomofungin.

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