THESIS

SYNTHESIS OF D-ALANYL-D-ALANINE DIPEPTIDE ISOSTERES AND CEPHALOSPORIN PRODRUGS

Submitted by

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In partial fulfillment of the requirements

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY PAUL B. GANSLE, JR. ENTITLED SYNTHESIS OF D-ALANYL-D-ALANINE DIPEPTIDE ISOSTERES AND CEPHALOSPORIN PRODRUGS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT OF THESIS

SYNTHESIS OF D-ALANYL-D-ALANINE

DIPEPTIDE ISOSTERES AND CEPHALOSPORIN PRODRUGS

Resistance to vancomycin by enterococci is of great clinical importance. Vancomycin inhibits bacterial growth by binding to terminal D-alanyl-D-alanine linkages in the growing peptidoglycan cell wall. Synthesis of dipeptide isosteres which may be incorporated into the growing cell wall is described. Previously published syntheses were employed as well as a Wittig reaction using a chiral Wittig reagent, a reaction not used in the synthesis of isosteres before. If the isosteres are directly incorporated, cell wall synthesis will be inhibited. Additionally, it is believed that the isosteres will act as mechanism-based inhibitors of the enzymes which are responsible for vancomycin resistance.

Delivery of dipeptides into bacteria is difficult due to selective transport mechanisms. Several reports have been made which use a cephalosporin prodrug with a dual mode of action. Antibiotics are coupled to cephalosporin intermediates and produce a compound which is capable of acting as an antibiotic on its own as well as delivering the coupled antibiotic into the periplasm by enzymatic hydrolysis of the prodrug. A novel phase-transfer reaction was used to couple dipeptide isosteres to a cephalosporin intermediate.

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DEDICATION

I would like to dedicate this thesis to my wife, Kristina. Without her patience, love, and support, I would not be where I am today. Thank you and I love you!

Table of Contents

Abbreviations

Boc	*tert*-butoxycarbonyl
$(BOC)_2O$	di-*tert*-butyl dicarbonate
DIBAH	diisobutylaluminum hydride
DIEA	diisopropylethylamine
DMAP	4-dimethylamino pyridine
DMF	N,N-dimethylformamide
EDCI	1-(3-dimthylaminopropyl)-3-ethylcarbodiimide hydrochloride
HMPA	hexamethylphosphoramide
HOAc	acetic acid
HRMS	high resolution mass spectrum
KHMDS	potassium hexamethyldisilazide
LAH	lithium aluminum hydride
NMM	4-methylmorpholine
PCC	pyidinium chlorochromate
PDC	pyridinium dichromate
TBSCl	*tert*-butyldimethylsilyl chloride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

Chapter 1

A. Introduction

Vancomycin (**1**), a glycopeptide antibiotic, is synthesized by a soil microorganism, *Amylocalatopsis orientalis*, collected in a Borneo jungle in 1956.[1] By 1958, it was introduced as an antibiotic, acting against gram-positive bacteria which were resistant to β-lactam antibiotics. Vancomycin has been used to treat endocarditis, meningitis, staphylococcus infections, and enterocolitis. Gram-negative organisms are intrinsically resistant to the antibiotic due to the drug's impermeability of the outer cell wall membrane which is absent in gram-positive organisms. The primary use of vancomycin is in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA), organisms which are resistant to most known β-lactams. Since its introduction over 30 years ago, vancomycin has been used as a 'last line of defense'. Recently, bacterial strains have emerged which are resistant to vancomycin, as well as the β-lactam antibiotics. Current research has focused on developing new antibacterial agents which will fight these infections or reverse the organism's susceptibility to vancomycin.

1. Vancomycin Resistance in Enterococci

The overuse of β-lactam antibiotics has lead to the increase in the number of bacteria resistant to these antibiotics. With this spread of resistance has also come the greater need to use vancomycin. As such, bacteria have developed a resistance to vancomycin as well. During the late 1980's, a report was made showing the emergence of strains of Enterococcus bacteria that were resistant to vancomycin.[2] The *Enterococcus*

(Streptococcus) faecium species was notable with its inducible, high-level resistance. In 1988, Patrice Courvalin and coworkers[3] isolated a plasmid from *Enterococcus faecium* BM4147 which contained the genes necessary for expression of high-level resistance to vancomycin. The mechanism of this resistance has been elucidated over the past decade.[4,5]

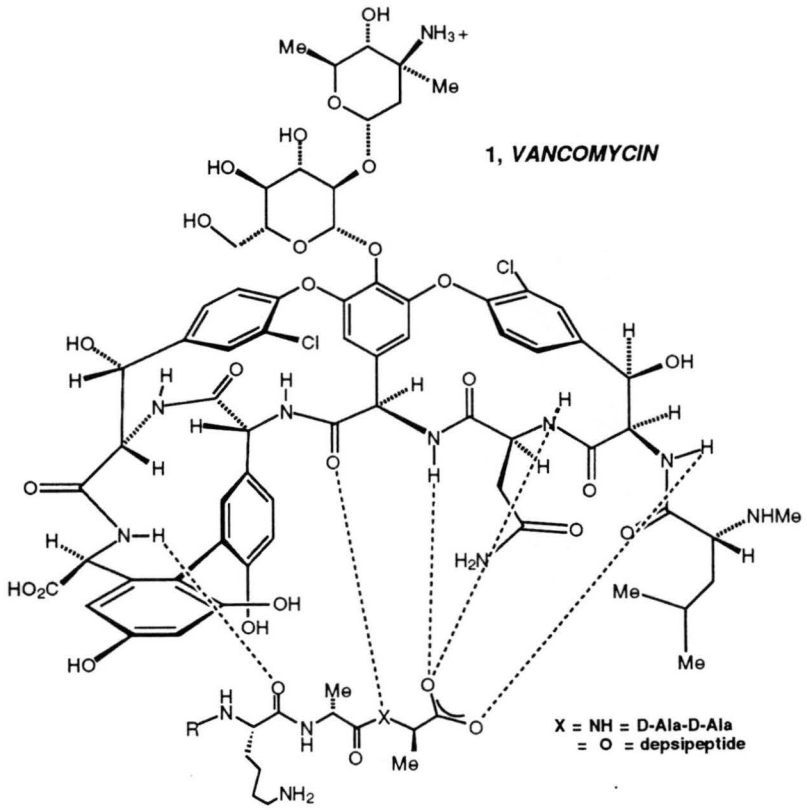


Figure 1

Vancomycin exhibits its antibiotic action by complexing with peptidoglycan precursors of the Gram-positive bacterial cell wall.[1] The cell wall contains a disaccharyl pentapeptide unit which terminates in D-alanyl-D-alanine (D-Ala-D-Ala), the site of vancomycin complexation. Vancomycin has a binding pocket which forms five hydrogen bonds to the dipeptide and effectively blocks transglycosylation and transpeptidation (cross-linking) of the cell wall precursors (Figure **1**, R=NH, see also

Figure 2). The D-Ala-D-Ala terminus is the site where vancomycin resistance is conferred. Five genes are necessary for expression of this resistance.[6]

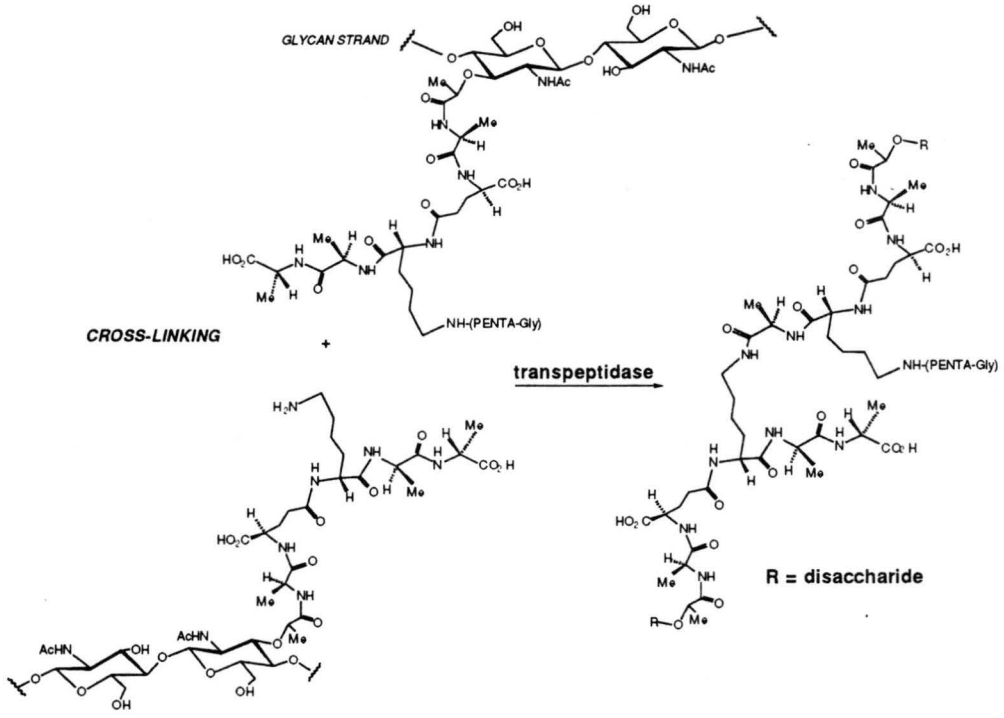
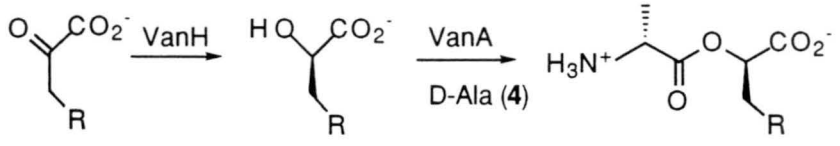


Figure 2

Courvalin and coworkers first isolated plasmid pIP816 in 1988 from *Enterococcus faecium* BM4147. At this time, the mechanism of resistance was completely unknown. However, they reported that the resistance plasmids were self-transferable through conjugation to other strains of bacteria.[3,7] Transfer of vancomycin resistance to other bacteria is of great concern. Unraveling of the molecular logic of the resistance followed quickly after this report.

The first gene to be decoded was the *vanA* gene. It was determined that the protein produced, VanA, was related to the D-Ala-D-Ala ligase involved in cell wall biosynthesis.[8] Shortly thereafter, VanA was found to be a D-Ala-D-Ala ligase with altered substrate specificity.[9] VanA will preferentially ligate more hydrophobic amino acids such as D-methionine and D-phenylalanine to form D-Ala-D-X dipeptides. The VanH protein was determined next and found to be related to 2-hydroxycarboxylic acid

dehydrogenases.[10] This led Courvalin and coworkers to the conclusion that VanA and VanH lead to the production of a depsipeptide peptidoglycan precursor.[11] VanH reduction of 2-ketobutyrate (**2**) leads to D-2-hydroxybutyrate (**3**) which can be condensed with D-alanine (**4**) to give depsipeptide **5**. Kinetic data showed that 2-hydroxybutyrate (K_m=0.6 mM vs. 38 mM for D-Ala) was the best substrate for VanA. However, further studies showed that 2-hydroxybutyrate was not the substrate, but that D-lactate was incorporated instead.[12] D-lactate (**7**), from pyruvate (**6**) was shown to be incorporated into peptidoglycan precursors both *in vitro* and *in vivo*. Therefore, the combined expression of *vanA* and *vanH* causes the dipeptide D-alanyl-D-lactate (**8**), a depsipeptide, to be synthesized and incorporated into the cell wall precursors. This effectively replaces the peptide bond with an ester linkage (Figure **1**, R=O), thus eliminating a crucial hydrogen bond to vancomycin, thereby precluding binding of the drug. Binding studies showed that the replacement of the amide nitrogen by oxygen caused a 1000-fold lower binding of vancomycin to the depsipeptide.



R=Me : 2
R=H : 6

R=Me : 3
R=H : 7

R=Me : 5
R=H : 8

Scheme 1

Continued work on the mechanism of resistance showed that two other genes, *vanR* and *vanS*, encoded for two proteins that formed a two-component regulatory system for expression of the remaining three genes, *vanA*, *H*, and *X*.[13] It was also determined that the five genes, *vanR*, *S*, *H*, *A*, and *X*, were necessary and sufficient to confer resistance to vancomycin. VanR is a response regulator while VanS is a transmembrane histidine protein kinase. The transmembrane protein, VanS, responds to some extra cellular signal causing it to autophosphorylate and transfer the phosphate group to VanR.

The phosphorylation of VanR cause the transcription of the remaining genes. It is known that the presence of vancomycin induces the production of the altered peptidoglycan precursors.[1] However, two groups recently showed that the signal to induce transcriptional activation of the *van* gene cluster may not be a structural feature of vancomycin itself.[14,15] It is currently unknown exactly what signals the regulatory system responds to in the presence of vancomycin. Any inhibition of cell wall biosynthesis may lead to the induction signal.

Continued investigation of the plasmid, pIP816, showed another gene sequence, *vanY*, which encoded for a D,D-carboxypeptidase.[16] The enzyme, VanY, is not necessary for vancomycin resistance to be expressed, but increases the minimum inhibitory concentration (MIC) four-fold.[17] The VanY protein is responsible for cleaving the terminal D-Ala or D-Lac of the pentapeptide or pentadepsipeptide, respectively. At this time, it was also found that VanX (described below) was responsible for hydrolyzing D-Ala-D-Ala. It was shown that the expression of Van R, S, H, A, and X resulted in peptidoglycan precursors that consisited of 76% pentadepsipeptide, less than 1.5% tetrapeptide, and 24% pentapeptide. The pentapeptide can compete with the depsipeptide for incorporation into the cell wall. Pentapeptide incorporation increases the sensitivity to vancomycin. However, if the gene cluster includes VanY, the cell wall precursors consist of 74% pentadepsipeptide, 26% tetrapeptide, and less than 1.5% pentapeptide. This lowers the chance of incorporation of D-Ala-D-Ala into the growing peptidoglycan chain where it can be complexed by vancomycin.

Finally, in 1994 and 1995, VanX was determined to be a metal-activated D,D-dipeptidase which hydrolyzes D-Ala-D-Ala.[18,19] The expression of VanH and A results in the synthesis of D-Ala-D-Lac. Any D-Ala-D-Ala synthesized will compete for incorporation into the growing peptidoglycan precursors. Hydrolysis of D-Ala-D-Ala allows incorporation of D-Ala-D-Lac and the efficient production of cytoplasmic

precursors consisting of 98% depsipeptide. Incorporation of the depsipeptide into the cell wall matrix renders vancomycin ineffective as an antibiotic.

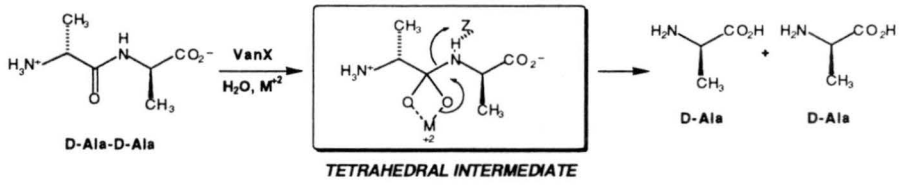


Figure 3

The entire transposon on plasmid pIP816, conferring vancomycin resistance to E. faecium, was decoded.[20] In addition to the *van* genes already mentioned, an unknown *vanZ* gene was discovered. It was later determined that *vanZ* encodes for an unknown protein that is responsible for teicoplanin resistance.[21] Both VanY and VanZ are accessory proteins and are not required for the expression of a high level of vancomycin resistance.

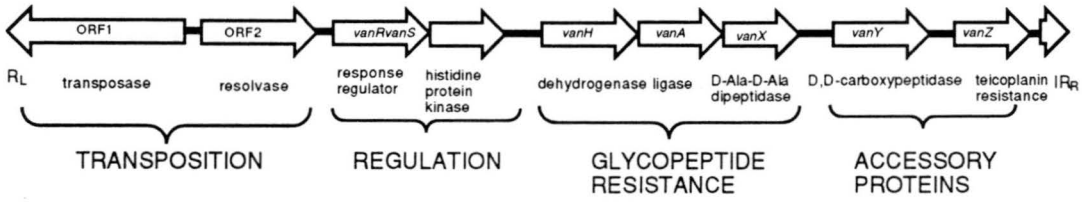


Figure 4

2. Dipeptide Isosteres

Isosteric replacements, represented as Ψ, consist of an exchange of atoms that alter the nature of a compound, yet leave its conformation essentially untouched. The mechanism for vancomycin resistance is an isosteric replacement of the amide bond. The exchange of an oxygen atom for the amide nitrogen causes a 1000-fold decrease in binding of vancomycin to the bacterial cell wall. It is believed that dipeptide isosteres

that mimic D-Ala-D-Ala could either be incorporated into the growing peptidoglycan precursors or act as inhibitors of the enzymes responsible for vancomycin resistance.

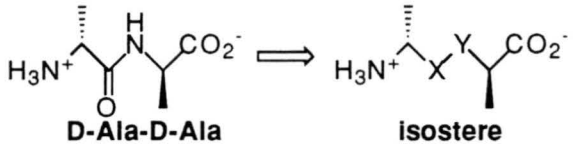


Figure 5

The enzyme responsible for incorporating D-Ala-D-Ala into the peptidoglycan muramyl tripeptide is known as the D-Ala-D-Ala-adding enzyme.[22] It has been demonstrated that the adding enzyme will process D-alanine analogs.[23] However, D,D-dipeptides are generally not antibacterial due to the difficulty in crossing the cytoplasmic membrane. The membrane permeases recognize the L,L-centers and transport these instead. Once a D,D-dipeptide analog is brought into the cytoplasm, it is conceivable that the adding enzyme will process it. If the analog were to contain an isosteric replacement of the amide bond, which is nonhydrolyzable, cross-linking of the pentapeptide will not occur. Without substantial cross-linking, the cell wall is weakened and osmotic lysis can occur.

In addition to direct incorporation of a dipeptide isostere into the growing cell wall, it is possible that a D-Ala-D-Ala mimic would bind to the enzymes responsible for vancomycin resistance. Inhibition of these enzymes would render the bacteria susceptible to vancomycin once again. The two enzymes proposed to be targeted by D-Ala-D-Ala mimics are the VanA and VanX proteins. The dipeptide isosteres are thought to act as transition-state analogs that mimic the tetrahedral intermediate as shown in figure **3**. Several groups have reported a series of phosphonate and phosphinate inhibitors of D-Ala-D-Ala ligases, VanA, and VanX.[19,24-27]

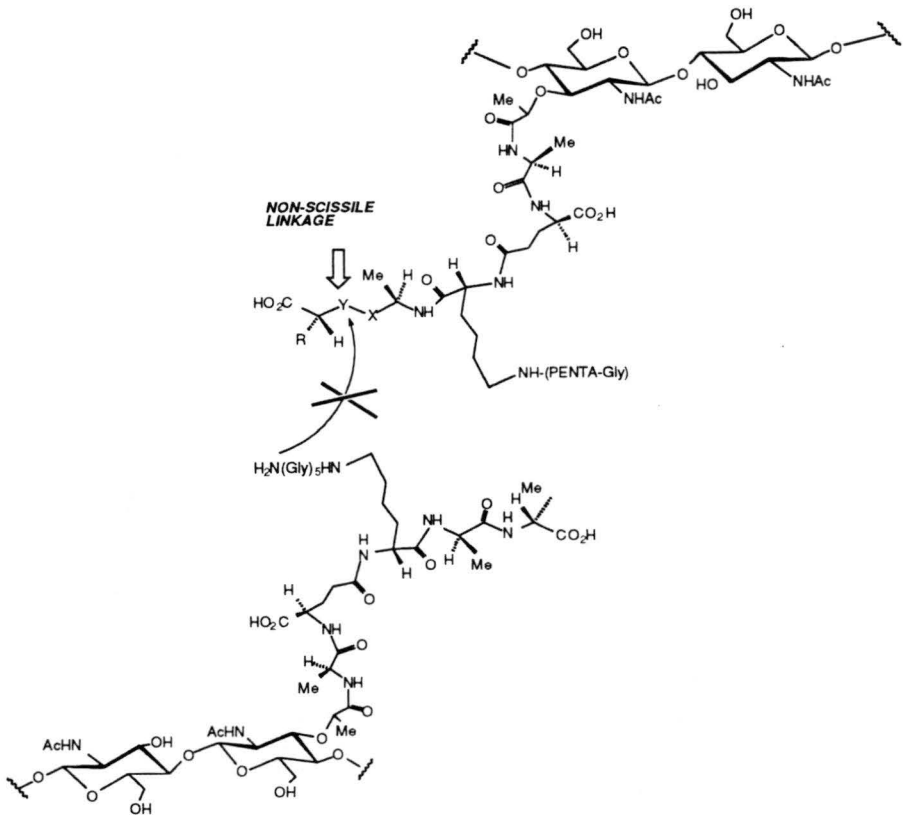
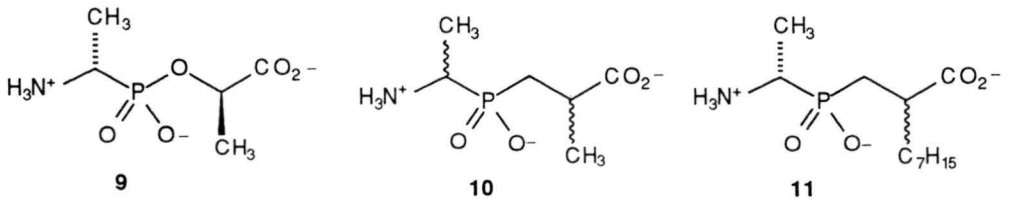


Figure 6

Duncan and Walsh[24] evaluated **11** as an inhibitor of *Salmonella typhimurium* D-Ala-D-Ala ligase. Phosphinate **11** was found to be an ATP-dependent, slow binding, competitive inhibitor of the ligase. Kinetic analysis gave a K_i of 1.2 μM where the K_m for D-Ala is 1.4 mM. The phosphinate showed a 1000-fold greater affinity for the enzyme than the substrate.



9 **10** **11**

Figure 7

Parsons and coworkers[25] evaluated a series of phosphinic acid inhibitors including **10** (D,D-configuration) against the D-Ala-D-Ala ligase of *Streptococcus faecalis*. Compound **10** was only modest in its antibacterial activity, but was quite potent

when combined with the alanine racemase inhibitor, fluoro-D-alanine. It was suggested that the lower levels of inhibition may be due to the poor transport of the analogs into the bacteria.

Walsh and coworkers[19,26] evaluated compounds **9**, **10**, and **11** for inhibition against VanX. Compound **9** was the weakest inhibitor with a K_i of 0.3 mM. They hypothesized that the lower inhibition of the phosphonate was due to a decreased nucleophilicity of the P-O group as a result of the bridging oxygen. Also, the bridging oxygen could cause subtle conformational changes that would affect the binding affinity to the enzyme. Phosphinates **10** and **11** were found to be mixed-type, noncompetitive inhibitors with apparent K_{is}'s of 0.32 and 0.40 μM, respectively. Both of these were analyzed as a mixture of diastereomers. Kinetic analysis of phosphonate **10** as a pure D,D-isomer established a K_{is} of 0.46 μM. In addition to heptyl derivative **11**, they examined ethyl and propyl derivatives as well. They gave K_i^*'s of .35 and 1.7 μM, respectively. All compounds showed slow-binding inhibition of VanX.

Bartlett and coworkers[27] evaluated several inhibitors of both *E. coli* D-Ala-D-Ala ligases (DdlA and DdlB) and VanA. They found that phosphinates **10**, **13**, and **14** inhibited DdlA and DdlB potently (K_i = 2-55 nM) and VanA weakly (K_i = 750-4100 nM). Phosphonates **9** and **12** were potent inhibitors of DdlB (K_i = 12 and 13 nM) while DdlA and VanA were weakly inhibited (K_i = 1600-8800 nM).

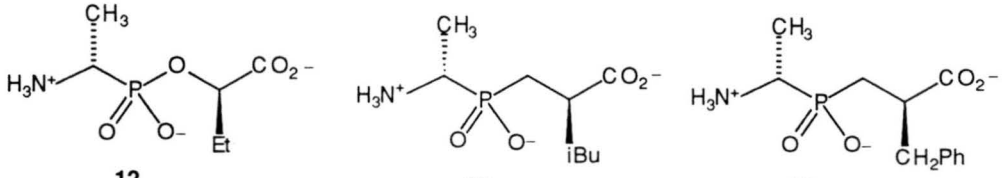


Figure 8

These examples clearly show that inhibitors of VanA and VanX can be developed. With these phosphorous-based dipeptide mimics, one of the major problems discussed by several of the groups was the inability to cross the inner membrane barrier.

Several of the studies show that hydrophobic analogs are better inhibitors than polar analogs. Phosphorous analogs are not the only dipeptide isosteres reported in the literature. It is believed that more hydrophobic inhibitors could be developed using isosteres of the amide bond linkage in D-Ala-D-Ala. If problems with inner membrane crossing still exist, analogs synthesized could be coupled to cephalosporin prodrugs that are capable of crossing the membrane.

Recent reviews of peptide mimics[28,29] detail the many isosteres that have been developed for the amide bond over the last few decades. The discovery of pepstatin, a peptide containing a hydroxymethylene isostere, by Umezawa[30] in 1970 paved the way for numerous syntheses of peptide isosteres. Table **1** lists some of the many isosteres that have been used in synthesizing peptidomimetic antibiotics.

Table 1. Peptide Isosteres

Isostere	Formula
hydroxymethylene	Ψ[CHOH]
ketomethylene	Ψ[$COCH_2$]
hydroxyethylene	Ψ[$CHOHCH_2$]
methyleneamino	Ψ[CH_2NH]
methyleneoxy	Ψ[CH_2O]
ketodifluoromethylene	Ψ[$COCF_2$]
ethylene	Ψ[CH_2CH_2]
alkene	Ψ[CH=CH]
ester	Ψ[CO_2]

Of particular interest in the Williams group are the following D-Ala-D-Ala isosteres: hydroxyethylene, ψ[$CH(OH)CH_2$] (**15**); ketomethylene, ψ[$COCH_2$] (**16**); methyleneamino, ψ[CH_2NH] (**17**); ketodifluoromethylene, ψ[$COCF_2$] (**18**); E-alkene, ψ[CH=CH] (**19**); and ethylene, ψ[CH_2CH_2] (**20**).

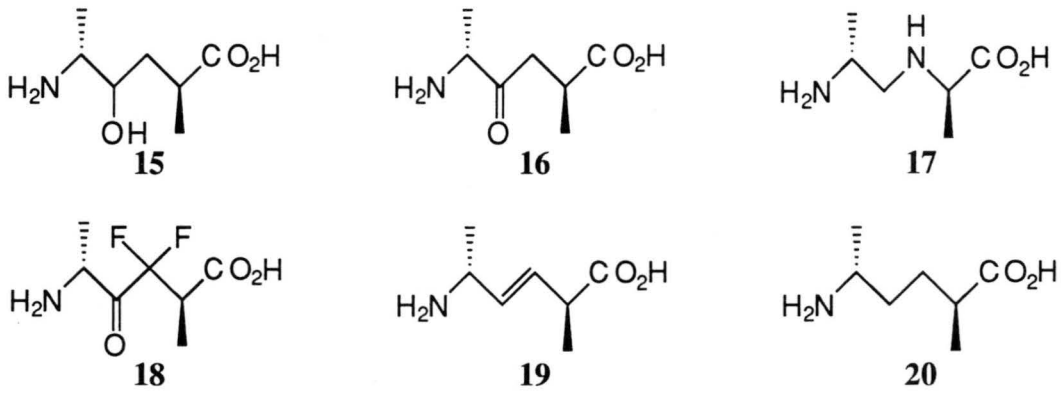


Figure 9

The above phosphonate, phosphinate, and amide isosteres should all act as transition-state analog inhibitors. In the case of VanX, D-Ala-D-Ala is hydrolyzed into two D-alanine molecules. Amide bond hydrolysis occurs through a tetrahedral intermediate, which the peptide isosteres are designed to mimic. It is hoped that tight-binding of the inhibitor will occur in the active-site of the enzyme. The research detailed below is an attempt to synthesize D-Ala-D-Ala mimics which will either form non-scissile precursors for bacterial wall synthesis or mechanism-based inhibitors of the enzymes VanA and VanX, two of the enzymes responsible for vancomycin resistance in *E. faecium* bacteria.

3. Biological Evaluation of V^R ---> V^S Bacteria

The Williams group routinely carries out bioassays of synthetic compounds using a panel of about a dozen microorganisms. The dipeptides mentioned above are proposed to be inhibitors of VanA and VanX. Inhibition of either of these enzymes would result in a conversion of a vancomycin-resistant (V^R) bacteria to a vancomycin-sensitive (V^S) one. A double-disc synergy test[31] must be employed that evaluates the combination of the peptide isostere and vancomycin. Patrice Courvalin of the Pasteur Institute, France, has

graciously donated samples of *Enterococcus faecium* BM4147 (V^R) and BM4147-1 (V^S) for use in our laboratories.

The assay consists of preparing an agar plate which has been inoculated with the vancomycin-resistant strain, BM4147. Two discs, a commercially available disc with 30 μg vancomycin and a second disc with the inhibitor, are placed 10 mm apart and incubated overnight at 37°C. If the V^R ---> V^S conversion occurs, a zone of inhibition will occur between the two discs where no bacterial growth occurs. Additionally, if the inhibitor acts as an antibiotic in its own right, there will be the standard ring of no growth surrounding the entire inhibitor disc. All isosteres will be evaluated at concentrations of 0.1, 1.0, and 10 mg/mL. Control experiments with the vancomycin-sensitive strain, BM4147-1, will be conducted in parallel.

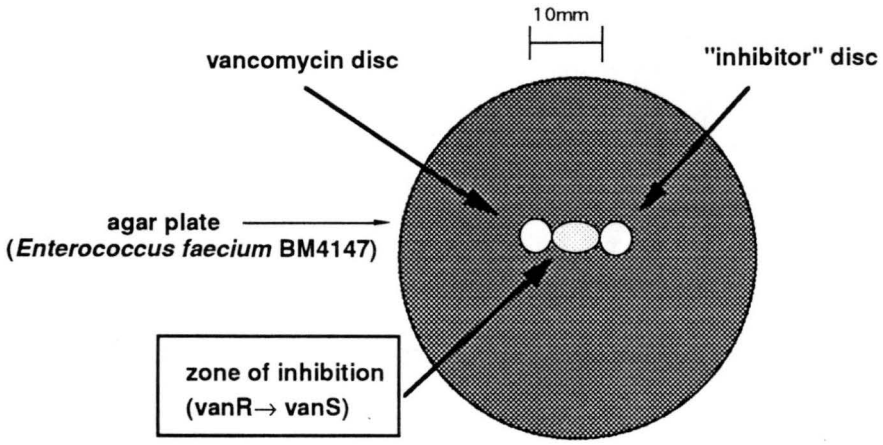


Figure 10

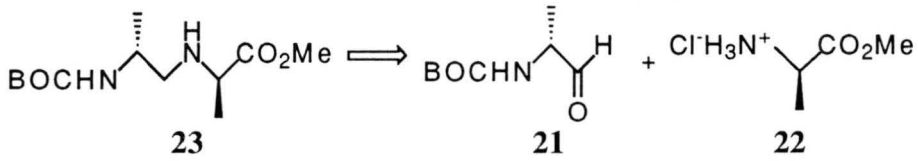
B. Results and Discussion

Dipeptide isosteres are proposed to act as cell wall precursors or mechanism-based inhibitors of VanA and/or VanX. Several of the isosteres were synthesized following previous literature methods. A novel synthesis of the alkene isostere using a

chiral Wittig reagent was also accomplished. Isosteres were assayed using the double-disc synergy test to see if they would act in conjunction with vancomycin to inhibit bacterial growth. Each of the compounds prepared were isosteres of the amide bond and mimics of D-alanyl-D-alanine.

1. Synthesis of Ψ[CH_2NH]

The protected methyleneamino isostere, or 'reduced amide', was envisioned to be derived from the aldehyde of D-alanine and the amine hydrochloride of D-alanine *via* a reductive amination. It was found, after completion of the synthesis, that another group had prepared this same isostere, but had not carried any further studies pertaining to this research.[32] Therefore, the experimental details used in this synthesis are slightly different from the published results.

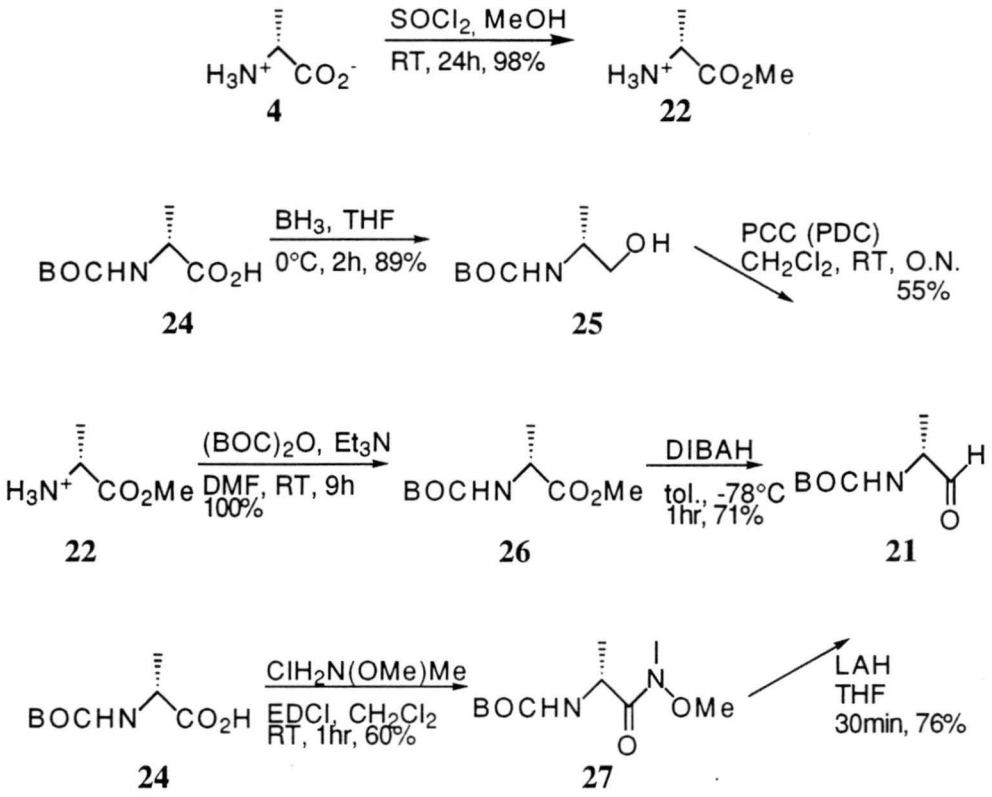


23 **21** **22**

Scheme 2

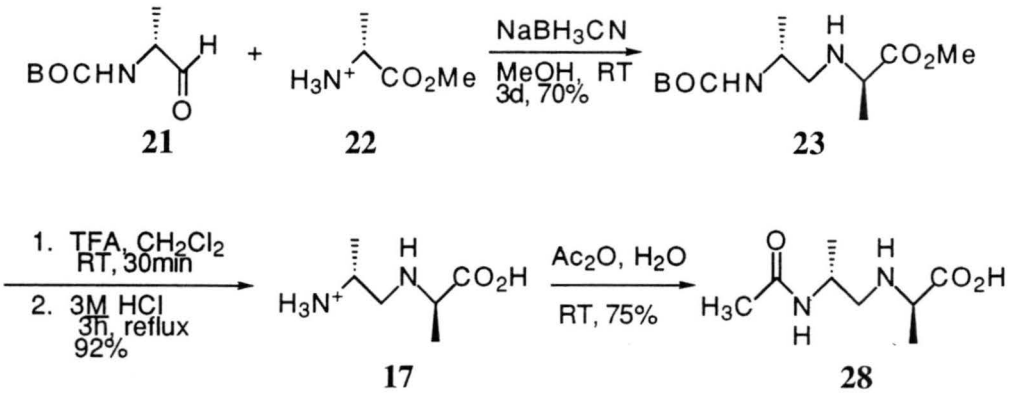
Protected methyl ester **22** was synthesized by *in situ* acid formation in methanol starting from D-alanine (**4**).[33] The synthesis of aminoaldehyde **21** was pivotal in that it was used as the starting material for almost all of the peptide isosteres. It was arrived at *via* three pathways. Boc-protected D-alanine (**24**) was reduced to primary alcohol **25** with BH_3·THF, then oxidized to aldehyde **21** using PCC or PDC.[34] A second attempt was made by a reductive route. The amine group of methyl ester **22** was Boc- protected to give **26**, followed by reduction of the ester with DIBAH.[35] This gave some overreduction to alcohol **25** and resulted in the need to purify by flash chromatography. The final and most successful attempt was the route introduced by Fehrentz and Castro[36]

using a Weinreb amidation.[37] Starting from **24**, dimethylhydroxamate **27** was formed *via* EDCI coupling. Reduction with lithium aluminum hydride afforded aminoaldehyde **21** in 65% yield, without the need for further purification. Since aminoaldehyde **21** is fairly unstable, hydroxamate **27** was synthesized and stored in gram quantities. As the aldehyde was needed, the reduction was accomplished and the product used immediately.



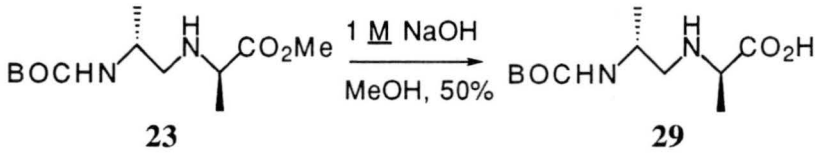
Scheme 3

Reductive amination of aminoaldehyde **21** with methyl ester **22** proceeded without incident and yielded the protected methyleneamino isostere **23**. Deprotection afforded isostere **17** as its hydrochloride salt in 70% yield. Acetylation of **17** with acetic anhydride afforded analog **28**. Biological testing of the isosteres **23**, **17**, and **28** have shown no activity by the double-disc diffusion assay.



Scheme 4

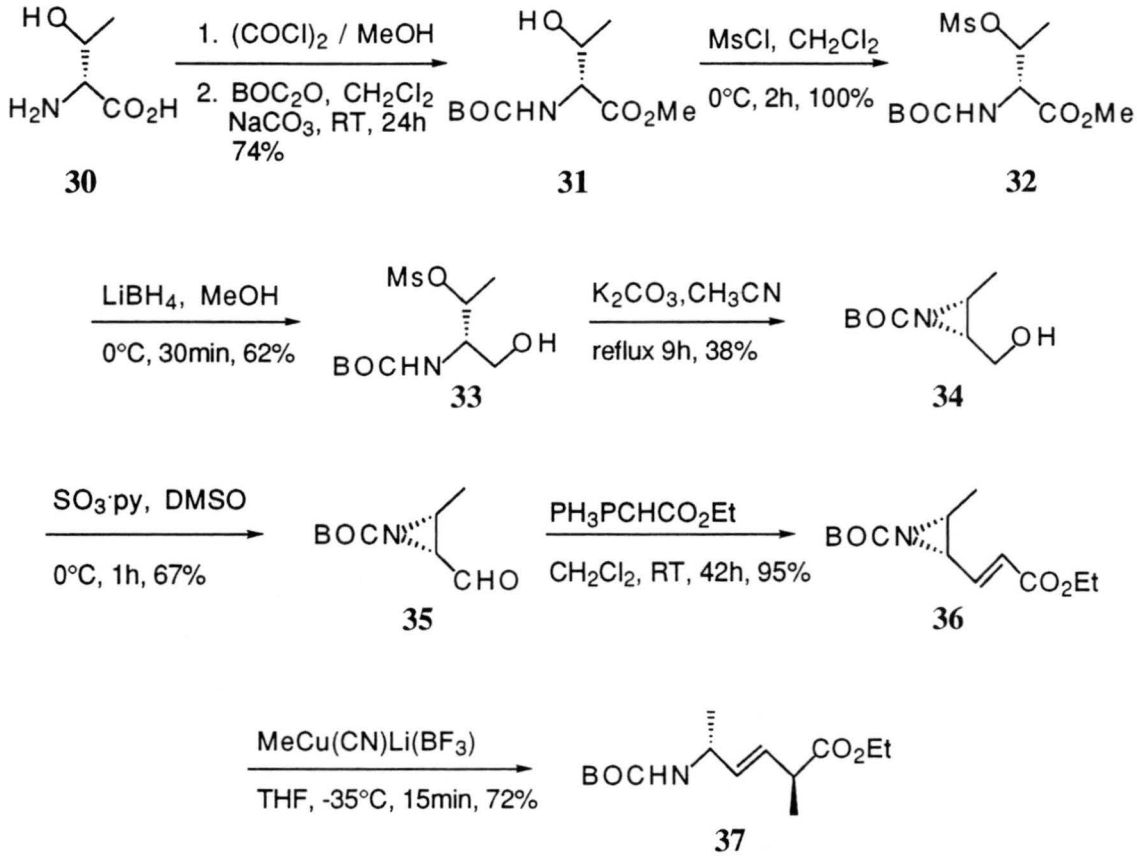
In order to facilitate coupling of the peptide isosteres for incorporation as prodrugs using synthetic cephalosporins, it was necessary to have the peptides in a form that was easily accessible. Saponification of protected isostere **23** was accomplished using NaOH in MeOH affording the Boc-protected amino acid **29** in a 50% yield.



Scheme 5

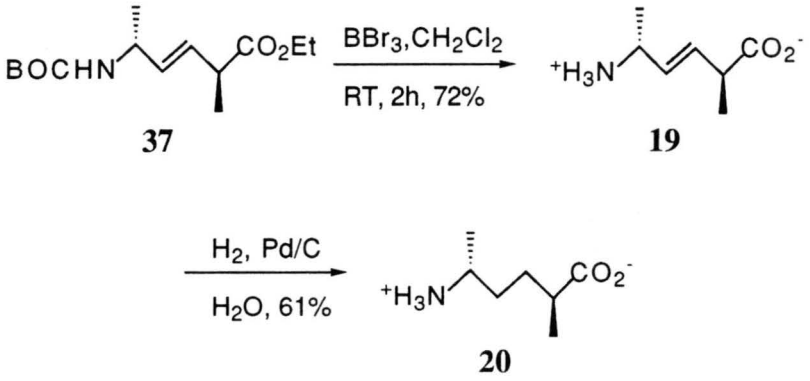
2. Synthesis of Ψ[CH=CH]

A recent report by Wipf and coworkers[38] detailed the synthesis of the protected L,L-alkene dipeptide isostere of D-Ala-D-Ala. The two chiral centers in L-threonine were used to set the stereochemistry of the final product. Use of D-threonine gave the desired D,D-isomer. The key step in this sequence is an anti-S_N2' displacement of an aziridine by an organocuprate.



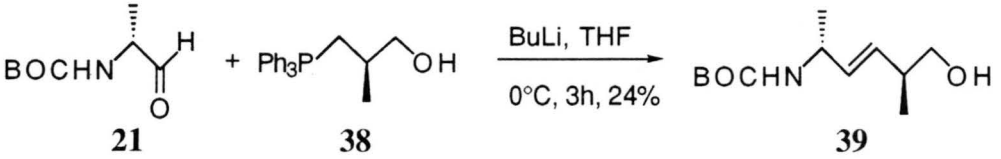
Scheme 6

D-Threonine (**30**) was protected as the Boc methyl ester **31**, followed by mesylation to afford **32**. Reduction of the ester to the alcohol was necessary due to competing elimination of the mesylate under basic conditions. Therefore, reduction with $LiBH_4$ afforded **33** and cyclization using K_2CO_3 yielded aziridine **34** in 38% yield. Parikh-Doering[39] oxidation using SO_3·pyridine afforded aldehyde **35** in 67% yield. Wittig chain extension afforded **36** with greater than 75% E-selectivity. Cuprate addition in an anti-S_N2' fashion afforded the protected isostere **37** in 72% yield as a 7:1 mixture of **37**:γ-alkylation product. In order to complete the synthesis, deprotection of the Boc group and methyl ester proceeded in a single step using BBr_3 to afford dipeptide isostere **19**.[40] Hydrogenation of the alkene moiety afforded ethylene isostere **20** in 61% yield. Biological testing was performed on both isosteres and, as before, none were found to be active.



Scheme 7

Though the synthesis of the E-alkene isostere by Wipf's methodology had already been accomplished, a shorter and more concise route was investigated. Wipf's route is long and cumbersome and a novel one-step route was devised using a Wittig reaction. Boc-D-alaninal (**21**) was coupled with chiral Wittig reagent **38** to afford the reduced E-alkene isostere **39**.[41] For our purposes, this is a much superior route as it reaches the dipeptide stage in a single step at a 24% yield. The Wipf route entails eight steps with an overall yield of less than 10%.



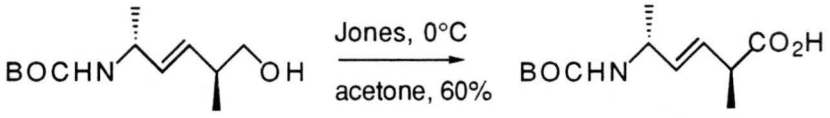
Scheme 8

The yield in this reaction was extremely low and some optimization was necessary. The reaction temperature was varied as well as the number of equivalents of n-butyllithium. The optimum reaction conditions achieved were the use of two equivalents of n-butyllithium at 0°C.

Table 2. Conditions for Wittig reaction

Temperature	Equivalents BuLi	yield (%)
-78C	2	7
0°C	2	24
RT	2	12
RT	3	0

After Wittig coupling, oxidation of isostere **39** gave the Boc-protected E-alkene dipeptide isostere **40**. The first attempt, using PDC in DMF as the oxidizing agent, was unsuccessful. The use of Jones reagent[42] produced **40** in a 60% yield. Compound **40** will be used in the coupling reactions with cephalosporin prodrugs as is the methyleneamino isostere **29**.



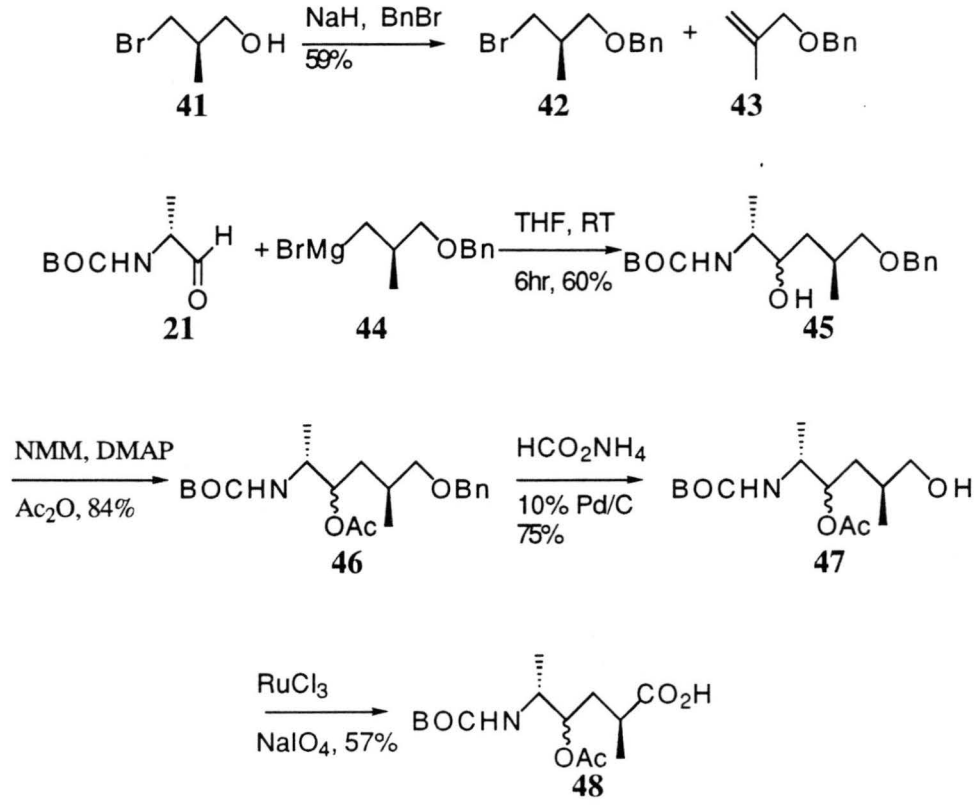
39 **40**

Scheme 9

3. Synthesis of Ψ[CHOHCH$_2$]

The hydroxyethylene isosteres were synthesized by the method of Rich and coworkers.[43] Starting with chiral bromoalcohol **41**, benzyl ether protection gave **42**, the precursor to Grignard reagent **44**.[44] The formation of **42** also yielded the elimination product **43**. It was difficult to completely remove this by-product, but it did not adversely affect the Grignard reaction. The Grignard coupling reaction of Boc-D-alaninal (**21**) with Grignard reagent **44** to form the protected hydroxyethylene isostere **45** proceeded in a 60% yield. Protection of the alcohol with acetic anhydride, followed by phase-transfer hydrogenation of the benzyl ether, and oxidation of the resulting alcohol gave **46**, **47**, and

48, respectively. Acid **48** was found to be a 60:40 mixture of two diastereomers using the integration of the acetyl methyl singlets in the ^1H NMR.



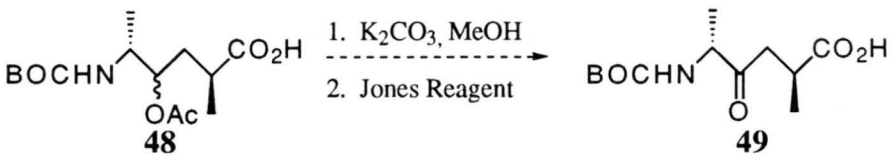
Scheme 10

To complete the synthesis of the hydoxyethylene isostere, compound **48** must be deprotected and then the two diastereomers separated. However, this has not been attempted yet as acid **48** is the needed precursor for coupling to a cephalosporin prodrug. Additionally, Rich and coworkers have reported that the isolation of a free hydroxyethylene isostere is extremely difficult due to facile cyclization to the lactone.[43]

4. Synthesis of Ψ[COCH$_2$] and Ψ[COCF$_2$]

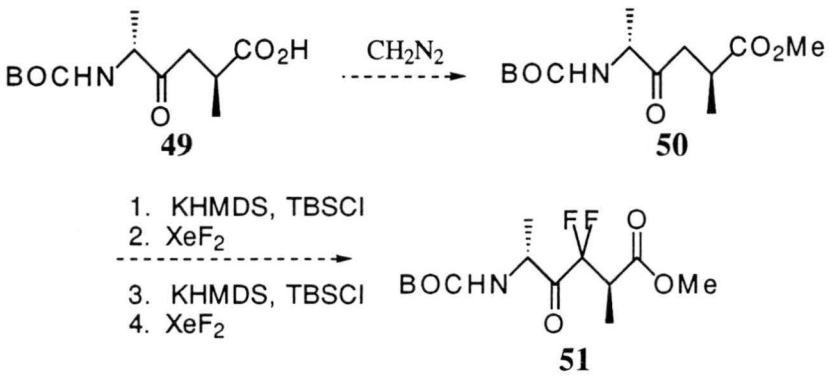
Synthesis of the ketomethylene and ketodifluoromethylene isosteres have not been accomplished, but both could follow directly from protected hydroxyethylene

isostere **48**. A two step deprotection-oxidation sequence would yield the protected ketomethylene isostere **49**.



Scheme 11

The ketodifluoromethylene isostere can be arrived at through the ketomethylene isostere **49**. Methyl ester formation using diazomethane followed by two consecutive electrophilic fluorinations with XeF_2 would yield compounds **50** and **51**, respectively.[45]



Scheme 12

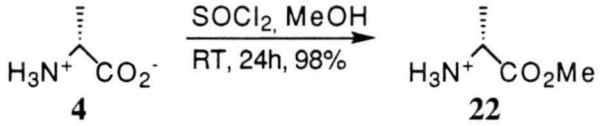
5. Conclusion

All peptide isosteres, with the exception of the ketomethylene isosteres have been synthesized. Unfortunately, none have resulted in a biologically active compound. Since it is thought that the problem lies in an inability to cross the cell membrane, the isosteres will be coupled to cephalosporin prodrugs in an effort to 'smuggle' the dipeptide surrogates into the cytoplasmic space. The next chapter details the efforts in this area.

C. Experimental

General Procedures

^{1}H NMR spectra were obtained on a Bruker AC 300 MHz spectrometer and chemical shifts were reported in parts per million relative to TMS (0.00), deuterated chloroform (7.24), deuterated methanol (4.87), or deuterium oxide (4.80). The numbers in parentheses were specified by Cambridge Isotope Labs, Andover MA. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR as KBr pellets or thin films from methylene chloride on NaCl plates. Optical rotations were determined on a Rudolph research Autopol III automatic polarimeter at a wavelength of 589 nm (sodium D line) with a 1.0 dm cell and a volume of 1 mL. Specific rotations, $[\alpha]_D$, are reported in degrees per decimeter at a specified temperature and a concentration of grams per 100 mL. Melting points were obtained using a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories in Phoenix, AZ and have an error of ±0.4%. Mass spectra were obtained on a 1992 Fisions VG Autospec at the Chemistry Department of Colorado State University. Column chromatography was performed with Merck silica gel grade 60, 230-400 mesh, 60Å. Analytical and preparatory thin layer chromatography was performed with Merck Kieselgel 60 F_{254} plates. Reagents and solvents were all commercial grade and used without further purification with the exception of THF (distilled over sodium, benzophenone), methylene chloride (distilled over CaH_2), ether (distilled over sodium, benzophenone), and DMF and HMPA (dried over 4Å molecular sieves). All air-sensitive reactions were run under an atmosphere of nitrogen. All glassware was oven or flame-dried prior to use.

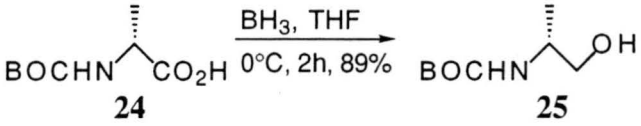


4 **22**

D-Alanine Methyl Ester Hydrochloride (22).

To a stirred mixture of **4** (1.044 g, 11.7 mmol, 1.0 eq) in MeOH (15 mL) at 0°C was added $SOCl_2$ (2.5 mL, 34.3 mmol, 2.9 eq) by addition funnel. The solution was warmed to room temperature, stirred for 18 hours, and concentrated to afford **22** (1.542 g, 94%) as a white solid.

R_f = 0.08 (90:8:2 CH_2Cl_2:MeOH:HOAc); mp 107-110°C (lit. 108-110°C); ^1H NMR (200 MHz) (D_2O) δ D_2O : 1.59 (3H, d, J = 7.4 Hz); 3.87 (3H, s); 4.23 (1H, q, J = 7.4 Hz); $[\alpha]_D^{27}$ = -7.6° (*c* 0.8, MeOH) (lit. $[\alpha]_D^{20}$ = -8.0° (*c* 1.6, MeOH).



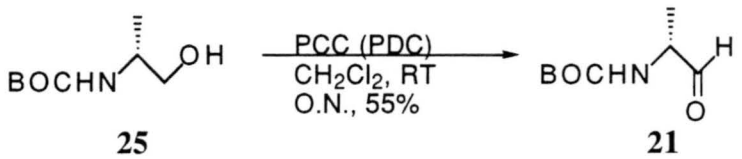
24 **25**

N-(t-Butoxycarbonyl)-D-alaninol (25).

To a stirred solution of BH_3·THF (5 ml, 5.0 mmol, 4.5 eq) at 0°C was added a solution of **24** (210 mg, 1.1 mmol, 1.0 eq) in THF (1 mL). The solution was stirred for 5 hours at 0°C, then quenched with a solution of 10% HOAc in MeOH (1.5 mL). After stirring for 1 hour, the solvent was evaporated, the residue taken up in EtOAc (10 mL) and washed with 1M HCl (10 mL), H_2O (10 mL), and 1M $NaHCO_3$ (10 mL), dried over anhydrous sodium sulfate, filtered, and evaporated to afford **7** (99 mg, 52%) as a white solid.

R_f = 0.51 (90:8:2 CH_2Cl_2:MeOH:HOAc); mp 46-49°C (lit. 52-53°C); ^1H NMR (200 MHz) ($CDCl_3$) δ $CDCl_3$: 1.12 (3H, d, J = 6.6 Hz); 1.42 (9H, s); 2.5 (1H, bs);

3.47 (1H, dd, J_{AB} = 11 Hz, J_{XC} = 6.2 Hz); 3.61 (1H, dd, J_{AB} = 11 Hz, J_{XC} = 3.9Hz); 3.66-3.82 (1H, m); 4.62 (1H, b). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 17.3, 28.4, 48.7, 67.4, 79.7, 156.4.

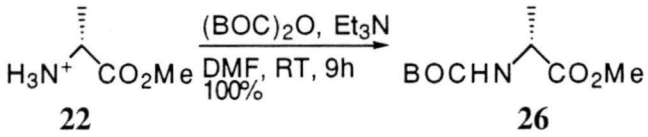


25 **21**

N-(t-Butoxycarbonyl)-D-alaninal (21).

To a stirred solution of **25** (89 mg, 0.5 mmol, 1.0 eq) in CH_2Cl_2 (2 mL) at room temperature was added PCC (or PDC) (166 mg, 0.77 mmol, 1.5 eq). The mixture was stirred for 17 hours, filtered, dissolved in EtOAc (10 mL), refiltered, and the solvent evaporated. The residue was dissolved in 1 mL of EtOAc and eluted rapidle with hexanes through a 2x12 silica gel column to afford **21** (48 mg, 55%) as a colorless oil which crystallized on standing to a semi-solid.

R_f = 0.50 (1:1 hexanes:EtOAc); ^1H NMR (200 MHz) ($CDCl_3$) δ $CDCl_3$: 1.31 (3H, d, J = 7.4 Hz); 1.42 (9H, s); 4.6 (1H, m); 9.53 (1H, s).



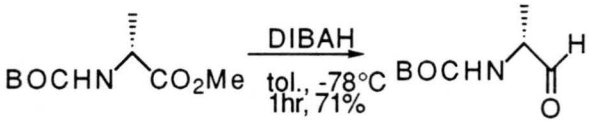
22 **26**

N-(t-Butoxycarbonyl)-D-alanine Methyl Ester (26).

To a stirred solution of **22** (3.708 g, 26.6 mmol, 1.0 eq) in DMF (60 mL) at 0°C was added Et_3N (7.42 mL, 53.2 mmol, 2.0 eq). To this solution was added a solution of $(Boc)_2O$ (6.410 g, 29.4 mmol, 1.1 eq) in DMF (30 mL). The mixture was warmed to room temperature, stirred for 12 hours, filtered, and concentrated. The residue was

dissolved in CH_2Cl_2 (100 mL) and washed with 1M HCl (100 mL), 1M $NaHCO_3$ (50 mL), and H_2O (50 mL). The organic layer was dried over anhydous sodium sulfate, filtered, evaporated, and separated on silica gel (1:1 hexanes/EtOAc) to afford **26** as a light yellow oil.

R_f = 0.55 (1:1 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.35 (3H, d, J = 7.2 Hz); 1.42 (9H, s); 3.72 (3H, s); 4.3 (1H, m); 5.05 (1H, s).

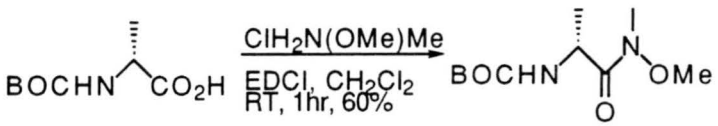


26 **21**

N-(t-Butoxycarbonyl)-D-alaninal (21).

To a stirred solution of **26** (2.749 g, 13.5 mmol, 1.0 eq) in toluene (45 mL) at -78°C was added DIBAH (22 mL, 33 mmol, 2.4 eq). The solution was stirred at -78°C for two hours, quenched with methanol (5 mL) and warmed to room temperature. The reaction mixture was poured into a solution of 1M potassium sodium tartrate (300 ml) and stirred for 2.5 hours. The mixture was extracted with Et_2O (3x200 mL), dried over anhydrous sodium sulfate, filtered, evaporated, and separated on silica gel (1:1 hexanes:EtOAc) to afford **21** (1.575 g, 67%) as a white solid.

R_f = 0.51 (1:1 hexanes:EtOAc); mp 82-84°C (lit. 88-89°C); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.31 (3H, d, J = 7.4 Hz); 1.43 (9H, s); 4.2 (1H, m); 5.1 (1H, s); 9.54 (1H, s). $[\alpha]_D^{25}$ = -24° (*c* 1.0, CH_2Cl_2).

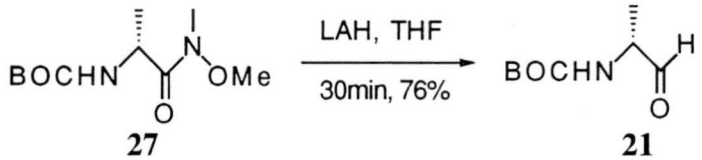


24 **27**

N^{α}-(t-butoxycarbonylamino)-D-alanine N-Methoxy-N-methylamide (27).

To a stirred solution of **24** (195 mg, 1.03 mmol, 1.0 eq) in CH_2Cl_2 at room temperature was added Et_3N (145 µL, 1.04 mmol, 1.0 eq), EDCI (198 mg, 1.03 mmol, 1.0 eq) and followed after a few minutes by HCl·HN(OMe)Me (111 mg, 1.14 mmol, 1.1 eq) and Et_3N (160 µL, 1.15 mmol, 1.1 eq). After 1 hour, the solution was diluted with CH_2Cl_2 (25 mL), washed consecutively (3x10 mL) with 1M HCl, 1M $NaHCO_3$, and brine, dried over anhydrous sodium sulfate, filtered, and evaporated to afford **27** (101 mg, 42%) as a white solid.

R_f = 0.40 (1:1 hexanes:EtOAc); mp 146-150°C (lit. 150°C); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.29 (3H, d, J = 6.9 Hz); 1.42 (9H, s); 3.19 (3H, s), 3.75 (3H, s); 4.65 (1H, m); 5.2 (1H, s).

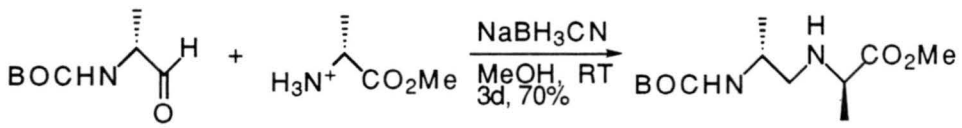


27 **21**

N-(t-Butoxycarbonyl)-D-alaninal (21).

To a stirred solution of **27** (476 mg, 2.0 mmol, 1.0 eq) in THF (10 mL) at room temperature was added LAH (120 mg, 3.16 mmol, 6.2 eq). After 30 minutes, the reaction was quenched with $NaHSO_4 \cdot H_2O$ (805 mg in 5mL). The aqueous layer was diluted with Et_2O (25 mL) and extracted with Et_2O (3x25 mL). The combined extracts were washed with 1M HCl (3x10 mL), 1M $NaHCO_3$ (3x10 mL), and brine (3x10 mL), dried over magnesium sulfate, filtered, and evaporated to afford **21** (267 mg, 76%) as a white solid.

mp 82-84°C (lit. 88-89°C); ^{1}H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.31 (3H, d, J = 7.4 Hz); 1.43 (9H, s); 4.2 (1H, m); 5.1 (1H, s); 9.54 (1H, s). $[\alpha]_D^{25}$ = -28.6° (*c* 0.5, CH_2Cl_2). [(lit. for L-isomer $[\alpha]_D^{20}$ = +36.7° (*c* 1.0, CH_2Cl_2).

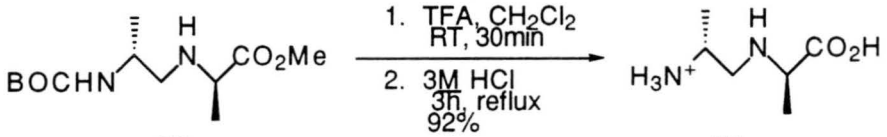


21 **22** **23**

N-(t-Butoxycarbonyl)-D-Ala-Ψ[CH_2NH]-D-Ala Methyl Ester (23).

To a stirred solution of **21** (200 mg, 1.16 mmol, 1.0 eq) and **22** (809 mg, 5.8 mmol, 5.0 eq) in MeOH (5 mL) at room temperature was added $NaBH_3CN$ (80 mg, 1.21 mmol, 1.0 eq). After stirring three days, the solvent was evaporated, the residue dissolved in 1M HCl (10 mL), followed by extraction with Et_2O (2x10 mL). The aqueous layer was made basic to pH = 10 with 6M NaOH, saturated with NaCl, and extracted with Et_2O (3x20 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to afford **23** (212 mg, 70%) as a colorless oil.

R_f = 0.14 (1:1 hexanes:EtOAc); ^{1}H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.12 (3H, d, J = 6.6 Hz); 1.29 (3H, d, J = 7.0 Hz); 1.42 (9H, s); 2.4 (1H, s); 2.48 (1H, dd, J = 6.8, 12 Hz); 2.65 (1H, dd, J = 5.1, 12 Hz); 3.37 (1H, q, J = 7.0 Hz); 3.66 (1H, m); 3.70 (3H, s); 4.7 (1H, s). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 18.9, 19.3, 28.7, 52.2, 53.0, 56.9, 156.1, 175.9. IR (NaCl, film) 3352, 2976, 1714, 1529, 1455, 1171, 1058. $[\alpha]_D^{26}$ = +24.0° (*c* 1.0, CH_2Cl_2). HRMS (FAB+) m/e 261.1819 ($C_{12}H_{25}N_2O_4$ requires 261.1814). Anal. Calcd. for $C_{12}H_{24}N_2O_4$: C, 55.36; H, 9.29; N, 10.76. Found: C, 55.16; H, 9.19; N, 10.65.

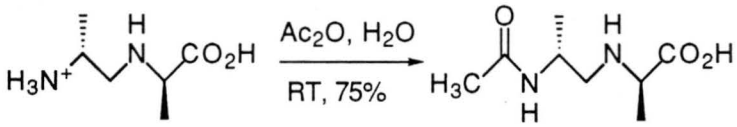


23 **17**

D-Ala-Ψ[CH_2NH]-D-Ala Hydrochloride (17).

To a stirred solution of **23** (182 mg, 0.70 mmol, 1.0 eq) in CH_2Cl_2 (5 mL) at 0°C was added TFA (1 mL, 13 mmol, 19eq). The flask was warmed to room temperature, stirred for 30 minutes, and concentrated. The residue was dissolved in 3M HCl (10 mL) and refluxed for 3 hours, followed by concentration. Recrystallization from MeOH and Et_2O afforded **17** (86.3 mg, 64%) as a white solid.

mp 44°C (dec.); ^1H NMR (300 MHz) (D_2O) δ D_2O : 1.47 (3H, d, J = 6.7 Hz); 1.60 (3H, d, J = 7.3 Hz); 3.37 (1H, dd, J = 6.6, 12 Hz); 3.46 (1H, dd, J = 6.1, 13 Hz); 3.83 (sextet, J = 6.6 Hz); 4.09 (1H, q, J = 7.3 Hz). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 15.5, 17.2, 46.2, 50.1, 57.8, 172. IR (NaCl, Nujol) 3583, 2923, 2360, 2341, 1744, 1199, 802. $[\alpha]_D^{25}$ = +5.0° (*c* 1.0, H_2O). Mass spectrum (ES+) m/e 147.1 (M-Cl).



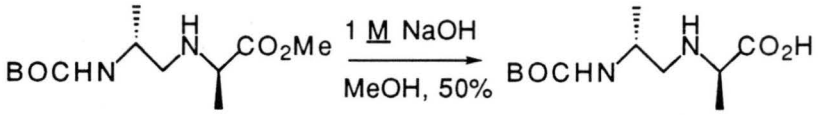
17 **28**

N-Acetyl-D-Ala-Ψ[CH_2NH]-D-Ala (28).

To a stirred solution of **17** (49.5 mg, 0.271 mmol, 1.0 eq) in H_2O (0.5 mL) at room temperature was added 1M NaOH (271 μL, 0.271 mmol, 1.0 eq). This was followed by twelve consecutive additions, one every five minutes, of 1M NaOH (27 μL, 0.0271 mmol, 0.1 eq) and acetic anhydride (3 μL, 0.032 mmol, 0.1 eq). Then 1M HCl (271 μL, 0.271 mmol, 1.0 eq) was added to quench the reaction and the solvent was

stripped. Flash chromatography, twice, using CH_2Cl_2:MeOH:HOAc (10:10:1) afforded 38.0mg (75%) of **28** as a white solid.

R_f = 0.21 (10:10:1 CH_2Cl_2:MeOH:HOAc); mp 202°C (dec.); ^1H NMR (300 MHz) (D_2O) δ D_2O : 1.25 (3H, d, 6.79 Hz); 1.51 (3H, d, J = 7.11 Hz); 2.00 (3H, s); 3.03 (1H, dd, J = 12.6, 8.7 Hz); 3.12 (1H, dd, J = 12.6, 8.7 Hz); 3.65 (1H, q, J = 7.1 Hz); 4.15-4.3 (1H, m). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 15.4, 18.5, 22.9, 44.8, 52.7, 58.5, 173.5, 174.5. IR (NaCl, Nujol) 3339, 2729, 1653, 1574, 1530, 1305, 1159, 1080. $[\alpha]_D^{25}$ = +7.6° (*c* 0.79, MeOH). Mass spectrum (ES-) m/e 187.3 (M-H).

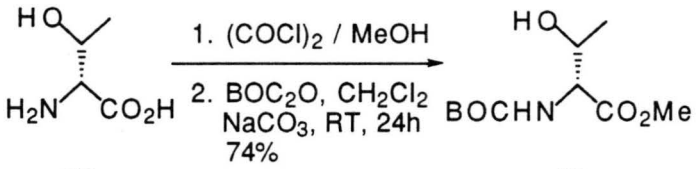


23 **29**

N-(t-Butoxycarbonyl)-D-Ala-Ψ[CH_2NH]-D-Ala (29).

To a stirred solution of **23** (50.7 mg, 0.195 mmol, 1 eq) in MeOH (500 μL) at room temperature was added 1M NaOH (500 μL, 0.5 mmol, 2.6 eq). After stirring for 4 hours at room temperature, the reaction was quenched with 1M HCl (500 μL). The solvent was removed under reduced pressure. Flash chromatography using CH_2Cl_2 : MeOH (1:1) afforded **29**, 23.8 mg (50%) as a white solid.

R_f = 0.36 (1:1 CH_2Cl_2:MeOH); mp 240°C (dec.); ^1H NMR (300 MHz) (D_2O) δ D_2O : 1.23 (3H, d, J = 6.6 Hz); 1.47 (9H, s); 1.52 (3H, d, J = 6.9 Hz); 2.85-3.27 (1H, m); 3.63-3.71 (1H, m); 3.90-4.00 (1H, m). IR (NaCl, Nujol) 3375, 2721, 1690, 1574, 1523, 1305, 1159, 1065. $[\alpha]_D^{25}$ = +129° (*c* 0.35, MeOH). Mass spectrum (ES+) m/e (rel. intensity) 191.2 (M-isobutylene, 100), 247.2 (M+H, 18).

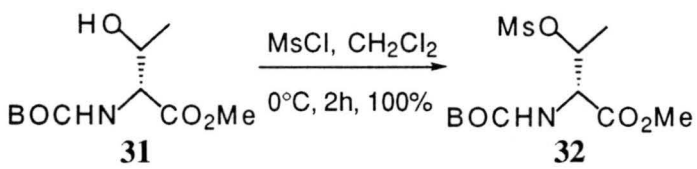


30 **31**

Boc-D-threonine Methyl Ester (31).

To a stirred suspension of **30** (503.4 mg, 4.23 mmol, 1.0 eq) in MeOH (5 mL) at 0°C was added oxalyl chloride (1.8 mL, 20.6 mmol, 4.88 eq). The reaction was warmed to room temperature then refluxed for two hours. The solvent was stripped to afford a white solid and colorless oil. This was taken up in CH_2Cl_2 (10 mL) and saturated $NaHCO_3$ (10 mL). To $(Boc)_2O$ (2.73 g, 12.5 mmol, 2.96 eq) in a flask was added the above mixture. This was stirred for 20 hours at room temperature, partitioned, and separated. The aqueous layer was extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and stripped. Flash chromatography using hexanes:EtOAc (1:1) afforded 728.9 mg (74%) of **31** as a colorless oil.

$R_f = 0.30$ (1:1 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.22 (3H, d, J = 6.36 Hz); 1.42 (9H, s); 2.30 (1H, d, J = 4.82 Hz); 3.74 (3H, s); 4.18-4.32 (2H, m); 5.33 (1H, d, J = 8.2 Hz). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 20.1, 28.5, 52.7, 58.9, 68.3, 80.3, 156.3, 172.2.



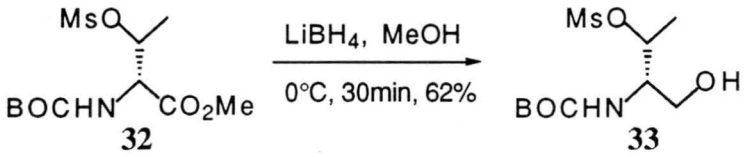
31 **32**

Mesylate 32.

To a stirred solution of **31** (716 mg, 3.07 mmol, 1.0 eq) in CH_2Cl_2 (7 mL) at 0°C was added DIEA (535 µL, 3.07 mmol, 1.0 eq) followed by a solution of MsCl (475 µL,

6.14 mmol, 2.0 eq) in CH_2Cl_2 (5 mL). The solution was stirred at 0°C for 1 hour, warmed to room temperature, and stirred for an additional hour. The reaction was quenched with 10 mL of ice and stirred for 15 minutes. The organic layer was partitioned and separated. The aqueous layer was extracted with CH_2Cl_2 (1x15 mL). The combined organic layers were washed with brine (3x30 mL), dried over Na_2SO_4, filtered, and stripped to afford 1.02 g (>100%) of **32** as a yellow oil. This was used without further purification.

^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.45 (9H, s); 1.48 (3H, d, J = 6.45 Hz); 2.97 (3H, s); 3.78 (3H, s); 4.49 (1H, dd, J = 9.6, 2.1 Hz); 5.2-5.3 (2H, m). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 18.4, 28.3, 38.6, 53.0, 57.5, 78.5, 80.6, 155.8, 169.9.

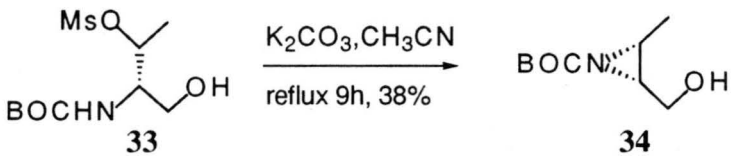


32 **33**

N-[(1S,2S)-1-(hydroxymethyl)-2-(methanesulfonyloxy)-ethyl]-1-(1,1-dimethylethoxy) methanamide (33).

To a stirred solution of **32** (956 mg, 3.07 mmol, 1.0 eq) in Et_2O (20 mL) at room temperature was added MeOH (190 μL, 4.70 mmol, 1.57 eq) followed by $LiBH_4$ (109 mg, 5.00 mmol, 1.67 eq), portionwise. The mixture was stirred for 30 minutes, cooled to 0°C, and quenched with 1M HCl (3 mL). After gas evolution ceased, the mixture was diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and stripped to afford 749.0 mg of a colorless oil. Flash chromatography using hexanes:EtOAc (40:60) afforded 479.8 mg (55%) of **33** as a colorless oil.

$R_f = 0.17$ (40:60 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.42 (9H, s); 1.45 (3H, d, J = 6.48 Hz); 2.65 (1H, bs); 3.04 (3H, s); 3.5-3.63 (1H, m); 3.64-3.83 (2H, m); 4.82 (1H, d, J = 8.8 Hz); 5.07 (1H, dq, J = 6.4, 2.6 Hz). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 18.6, 28.6, 38.6, 55.7, 61.9, 78.3, 80.5, 156.3. IR (NaCl, film) 3389, 2978, 2933, 1711, 1689, 1517, 1344, 1172, 911. $[\alpha]_D^{27} = -5.0°$ (*c* 1.0, CH_2Cl_2). Anal. Calcd. for $C_{10}H_{21}NO_6S$: C, 42.39; H, 7.47; N, 4.94. Found: C, 42.16; H, 7.64; N, 5.16.

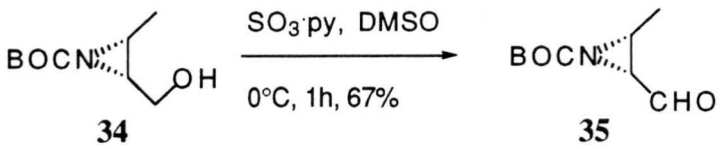


33 **34**

(2R,3R)-N-Boc-2-(hydroxymethyl)-3-methylaziridine (34).

To a stirred solution of **33** (1.26 g, 4.44 mmol, 1.0 eq) in CH_3CN (30 mL) at room temperature was added K_2CO_3 (1.23g, 8.88mmol, 2.0eq). The reaction mixture was refluxed for 8 hours then cooled to room temperature. The mixture was diluted with CH_2Cl_2 (20 mL), filtered, and stripped. Flash chromatography using hexanes:EtOAc (1:2) afforded 314.9 mg (38%) of **34**.

$R_f = 0.43$ (1:2 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.26 (3H, d, J = 5.64 Hz); 1.43 (9H, s); 1.92 (1H, dd, J = 7.4, 4.8 Hz); 2.53-2.65 (2H, m); 3.65 (1H, ddd, J = 12, 7.4, 4.9 Hz). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 13.2, 28.1, 37.5, 42.7, 60.5, 81.6, 162.6. IR (NaCl, film) 3422, 2971, 2933, 2355, 2333, 1811, 1711, 1689, 1300, 1233, 1156, 1039. $[\alpha]_D^{26} = +8.7°$ (*c* 1.0, CH_2Cl_2). Anal. Calcd. for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.59; H, 8.93; N, 7.26.

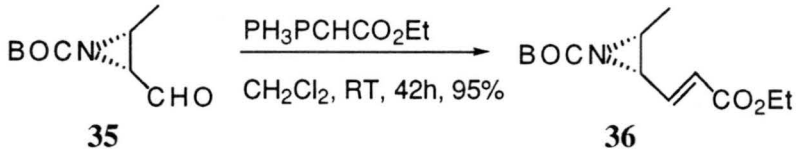


34 35

Aldehyde 35.

To a stirred solution of **34** (314.9 mg, 1.68 mmol, 1.0 eq) in CH_2Cl_2 (11 mL) at room temperature was added Et_3N (730 μL, 6.72 mmol, 4.0 eq). The flask was cooled to 0°C and a solution of SO_3·py (801 mg, 5.04 mmol, 3.0 eq) in DMSO (7 mL) was added. The reaction was stirred for 1 hour at 0°C then partitioned between hexanes:Et_2O (2:1, 150 mL) and saturated $NaHCO_3$ (70 mL). The aqueous layer was extracted with hexanes:Et_2O (2:1, 40 mL). The combined organic layers were washed with saturated NaH_2PO_4 (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and stripped. Flash chromatography using hexanes:EtOAc (1:1) afforded 209.8 mg (67%) of **35** as a light yellow oil.

R_f = 0.66 (1:1 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.36 (3H, d, J = 5.80 Hz); 1.42 (9H, s); 2.78 (1H, dq, J = 6.72, 5.81 Hz); 2.92 (1H, dd, J = 6.87, 5.18 Hz); 9.32 (1H, d, J = 5.18 Hz).



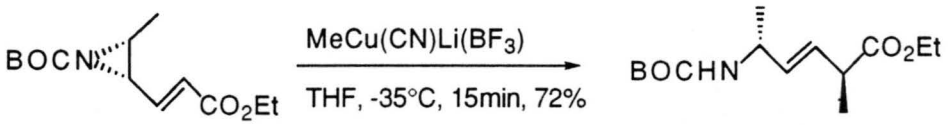
35 36

(2S,3R)-N-Boc-2-[(E)-2-(ethoxycarbonyl)ethenyl]-3-methylaziridine (36).

To a stirred solution of **35** (209.8 mg, 1.13 mmol, 1.0 eq) in CH_2Cl_2 (7 mL) at room temperature was added a solution of phosphorane (987 mg, 2.83 mmol, 2.5 eq) in CH_2Cl_2 (7 mL). The reaction was stirred for 42.5 hours then diluted with CH_2Cl_2 (150 mL). The organic layer was washed with 40 mL each of H_2O, brine, and saturated

$CuSO_4$. The organic layer was dried over $MgSO_4$, filtered, and stripped. Flash chromatography using hexanes:EtOAc (4:1) afforded 274.9 mg (95%) of **36** as a colorless oil.

R_f = 0.38 (4:1 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.20 (3H, d, J = 5.62 Hz); 1.27 (3H, t, J = 5.71 Hz); 1.43 (9H, s); 2.65-2.75 (1H, m); 3.01 (1H, ddd, J = 6.6, 6.6, 0.8 Hz); 4.13-4.23 (2H, m); 6.11 (1H, dd, J = 15.6, 0.8 Hz); 6.72 (1H, dd, J = 15.6, 6.7 Hz). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 13.6, 14.4, 28.1, 40.2, 41.5, 60.7, 81.7, 125.4, 142.1, 162.1, 166.0. IR (NaCl, film) 3361, 2976, 2932, 1704, 1515, 1276, 1167, 1036, 978, 847.

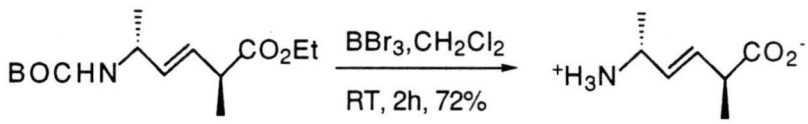


36 **37**

Ethyl (3E,2S,3R)-2-Methyl-5-[N-(tert-butoxycarbonyl)-amino]-3-hexenoate (37).

To a slurry of CuCN (55.6 mg, 0.621 mmol, 3.0 eq) in THF (2 mL) at -30°C was added MeLi (0.45 mL, 0.63 mmol, 3.0 eq). The flask was warmed to 0°C over 10 minutes then cooled to -70°C, after which, $BF_3 \cdot Et_2O$ (77 μL, 0.626 mmol, 3.0 eq) was added and the flask warmed to -35°C. To this colorless solution was added a solution of **36** (52.6 mg, 0.206 mmol, 1.0 eq) in THF (1 mL). The solution turned yellow. After stirring 15 minutes at -35°C, the reaction was quenched with saturated NH_4Cl (2 mL). The reaction was extracted with Et_2O (3x10 mL) and the combined organic extracts dried over $MgSO_4$, filtered, and stripped. The products were separated with preparatory TLC (3:1 hexanes:EtOAc) to afford 21.5 mg (38%) of **37** as a colorless oil.

$R_f = 0.41$ (3:1 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.15-1.3 (9H, m); 1.41 (9H, s); 3.0-3.13 (1H, m); 4.06-4.23 (1H, m); 4.10 (2H, q, J = 7.1 Hz); 4.40 (1H, b); 5.49 (1H, dd, J = 15.7, 4.8 Hz); 5.64 (1H, ddd, J = 15.6, 7.5, 1.3 Hz). IR (NaCl, film) 3361, 2976, 2925, 1712, 1515, 1370, 1247, 1167, 1043.

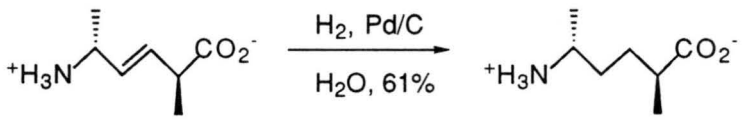


37 **19**

D-Ala-Ψ[CH=CH]-D-Ala (19).

To a stirred mixture of **37** (21.5 mg, 0.079 mmol, 1.0 eq) in CH_2Cl_2 (2 mL) at -8°C was added BBr_3 (80 μL, 0.08 mmol, 1.0 eq). After 1 hour the flask was warmed to room temperature and stirred a second hour. The reaction was quenched by dropwise addition of H_2O (2 mL) then separated. The organic phase was extracted with H_2O (3x10 mL) and the aqueous phases were combined. The H_2O was removed under reduced pressure and the resulting oil was eluted through a Dowex-50X2 ion exchange column using 2% HN_4OH to afford 8.1 mg (72%) of **19**, as a white semi-solid.

^1H NMR (300 MHz) (D_2O) δ D_2O : 1.18 (3H, d, J=7.1 Hz); 1.37 (3H, d, J = 6.7 Hz); 2.96-3.10 (1H, m); 3.87-4.00 (1H, m); 5.57 (1H, ddd, J = 15.3, 7.3, 1.2 Hz); 5.88-5.99 (1H, m).

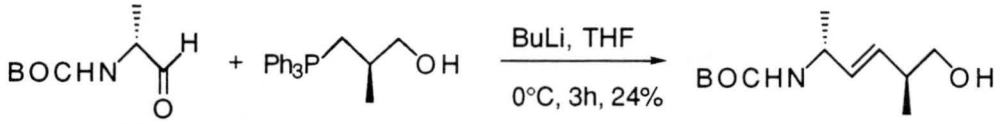


19 **20**

D-Ala-Ψ[CH_2CH_2]-D-Ala (20).

To a stirred solution of **19** (8.1 mg, 0.057 mmol, 1.0 eq) in H_2O (1 mL) at room temperature was added 10% Pd/C (7.0 mg). The flask was flushed with H_2 gas and stirred for 19 hours. The solvent was filtered through Celite then removed under reduced pressure to afford 5.0 mg (61%, crude) of **20**, as a colorless oily solid.

^1H NMR (300 MHz) (D_2O) δ D_2O : 1.09 (3H, d, J = 6.9 Hz); 1.29 (3H, d, J = 6.6 Hz); 1.0-1.8 (m); 2.2-2.4 (1H, m); 3.3-3.5 (1H, m).



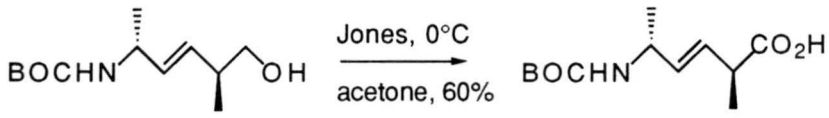
21 **38** **39**

(3E,2S,3R)-2-Methyl-5-[N-tert-butoxycarbonyl)-amino]-3-hexenol (39).

To a slurry of **38** (1.007 g, 2.425 mmol, 2.5 eq) in THF (15 mL) at -78°C was added 1.4M BuLi (3.5 mL, 4.9 mmol, 4.9 eq). The flask was warmed to room temperature and stirred for 3 hours then cooled again to -78°C. A solution of **21** (159 mg, 0.92 mmol, 1.0 eq) in THF (10 mL) was added dropwise. The flask was warmed to 0°C and stirred for 3 hours. The reaction mixture was quenched with saturated NH_4Cl (15 mL) at 0°C and stirred for 15 minutes, then extracted with EtOAc (3x30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Flash chromatography using hexanes:EtOAc (1:1) afforded 50.9 mg (24%) of **29** as a colorless oil.

^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 0.97 (3H, d, J = 6.7 Hz); 1.18 (3H, d, J = 6.9 Hz); 1.41 (9H, s); 1.81 (1H, bs); 2.27-2.38 (1H, m); 3.35 (1H, dd, J = 10.5, 9.4 Hz); 3.48 (1H, dd, J = 10.5, 5.6 Hz); 4.11 (1H, bs); 4.49 (1H, bs); 5.41 (1H, dd, J = 15.6, 6.4 Hz); 5.48 (1H, dd, J = 15.6, 4.6 Hz). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 16.4, 21.3, 28.6, 39.3, 67.3, 79.3, 132.3, 133.4, 155.4. IR (NaCl, film) 3339,

2976, 2932, 2874, 1697, 1515, 1457, 1363, 1247, 1167, 1050, 971. $[\alpha]_D^{29}$ = +9.3° (*c* 1.5, CH_2Cl_2) (lit. for enantiomer $[\alpha]_D^{22}$ = -7.4° (*c* 1.5, CH_2Cl_2).

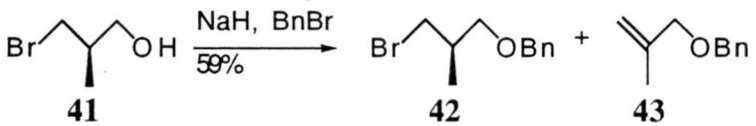


39 **40**

(3E,2S,3R)-2-Methyl-5-[N-tert-butoxycarbonyl)-amino]-3-hexenoic acid (40).

To a stirred solution of **39** (113 mg, 0.49 mmol, 1 eq) in acetone (3 mL) at 0°C was added Jones reagent (200 μL). The reaction was stirred for 30 minutes at 0°C an quenched with 1M $NaHSO_3$ (10 mL). The mixture was extracted with Et_2O (3x20 mL), dried over $MgSO_4$, filtered, evaporated, and separated on silica gel (1:1 hexanes:EtOAc) to afford **40** as a colorless oil (72 mg, 60%).

R_f = 0.12 (1:1 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.19 (3H, d, J = 6.6 Hz); 1.26 (3H, d, J = 6.9 Hz); 1.47 (9H, s); 3.08-3.18 (1H, m); 3.46 (1H, dq, J = 7.2, 0.9 Hz); 4.19 (1H, bs); 4.45 (1H, bs); 5.50-5.60 (1H, m); 5.62-5.72 (1H, m).



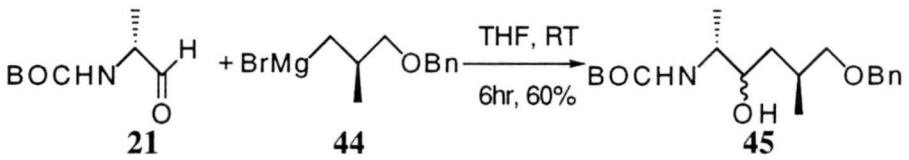
41 **42** **43**

R-1-Benzyloxy-3-bromo-2-methylpropane (42).

To a stirred solution of **41** (250 μL, 2.4 mmol, 1.0 eq) in THF (4 mL) at -78°C was added NaH (110 mg, 2.75 mmol, 1.1 eq). After 20 minutes, an additional 4 mL of THF was added followed by $INBu_4$ (94 mg, 0.25 mmol, 0.1 eq) and BnBr (320 μL, 2.69 mmol, 1.1 eq). The solution was warmed to room temperature, stirred for 1 hour, and

then Florisil (0.5 g) was added to the reaction mixture. The solvent was stripped and the resulting powder washed with hexanes through a plug of Florisil to give a colorless oil (418 mg). Chromatography on silica gel (1:1 hexanes:EtOAc) afforded **5** and **6** (347 mg, 59%) as a yellow oil. These were inseparable, but NMR gave a ratio of 39:1 for the two products.

R_f = 0.41 (1:1 hexanes:EtOAc); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.03 (3H, d); 2.14 (1H, m); 3.4-3.5 (4H, m); 4.52 (2H, s); 7.25-7.34 (5H, m). ^{13}C NMR (75.47 MHz) ($CDCl_3$) δ $CDCl_3$: 15.9, 35.7, 38.2, 72.8, 73.2, 127.6, 128.4, 128.7, 138.3. IR (NaCl, film) 2965, 2931, 2858, 1453, 1361, 1098. $[\alpha]_D^{27}$ = -12.7° (*c* 1.0, CH_2Cl_2) (lit. $[\alpha]_D^{24}$ = +13° (*c* 1.0, EtOH). Anal. Calcd. for $C_{11}H_{15}BrO$: C, 54.34; H, 6.22. Found: C, 54.60; H, 6.26.



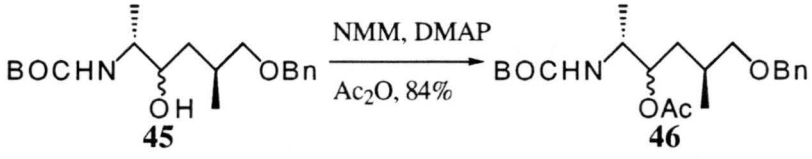
21 **44** **45**

(2S,4RS,5R)-1-(Benzyloxy)-5-[(t-butyloxycarbonyl)-amino]-4-hydroxy-2-methylhexane (45).

To a mixture of Mg (102.3 mg, 4.21 mmol, 2.7 eq) in THF (5 mL) at room temperature was added a crystal of iodine. The flask was warmed while the halide of **44** (849 mg, 3.49 mmol, 2.2 eq) in THF (5 mL) was added dropwise. The mixture was refluxed for 1 hour then cooled to room temperature. To the grignard solution was added a solution of **21** (267.3 mg, 1.55 mmol, 1.0 eq) in THF (5 mL), dropwise, over 30 minutes. After stirring at room temperature for 6 hours, the reaction was quenched with saturated NH_4Cl (10 mL) and stirred for 2 hours. The mixture was poured into H_2O (30 mL) and extracted with Et_2O (3x40 mL). The combined organic layers were dried over

$MgSO_4$, filtered, and the solvent removed under reduced pressure. Flash chromatography using hexanes:EtOAc (1:1) afforded 313.6 mg (60%) of a diastereomeric mixture of **45** as a colorless oil.

^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 0.90 (3H, d, J = 6.87 Hz); 0.93 (3H, d, J = 7.0 Hz); 1.05 (3H, d, J = 6.6 Hz)); 1.15 (3H, d, J = 6.6 Hz); 1.40-1.43 (18H, m); 1.9-2.1 (2H, m); 3.2-3.45 (6H, m); 3.47-3.71 (4H, m); 4.48 (2H, s); 4.51 (2H, s); 4.82 (1H, d, J = 9 Hz); 4.95 (1H, d, J = 9 Hz); 7.2-7.4 (10H, m). IR (NaCl, film) 3346, 2976, 2932, 2874, 1697, 1508, 1457, 1392, 1370, 1247, 1167, 1094, 1058, 1029, 992. HRMS (FAB+) m/e 338.2352 ($C_{19}H_{32}NO_4$ requires 338.2331).

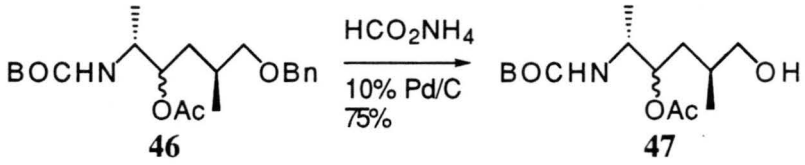


Acetate 46.

To a stirred solution of **45** (87.5 mg, 0.26 mmol, 1.0 eq) in EtOAc (1 mL) at room temperature was added, sequentially, NMM (5 μL, 0.027 mmol, 0.1 eq), DMAP (3 mg, 0.025 mmol, 0.1 eq), and Ac_2O (74 μL, 0.78 mmol, 3.0 eq). The reaction was stirred for 22 hours then quenched with saturated $NaHCO_3$ (1 mL) and stirred for 30 minutes. The reaction mixture was diluted with EtOAc (10 mL) then washed with saturated $NaHCO_3$ (3x5 mL) and ice-cold 0.5M HCl (2x5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and stripped to afford 82.6 mg (84%) of **46** as a colorless oil that was used without further purification.

^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 0.92 (3H, d, J = 6.7 Hz); 0.95 (3H, d, J = 6.7 Hz); 1.06 (3H, d, J = 6.6 Hz); 1.07 (3H, d, J = 6.9 Hz); 1.42 (18H, s); 1.55-1.75 (6H, m); 2.030 (3H, s); 2.033 (3H, s); 3.2-3.4 (4H, m); 4.54 (2H, s); 4.48 (2H, s);

4.51-4.68 (2H, m); 4.9-5.1 (2H, m); 7.25-7.35 (10H, m). IR (NaCl, film) 3361, 2968, 2932, 2881, 1712, 1515, 1457, 1370, 1312, 1239, 1159, 1029, 977.

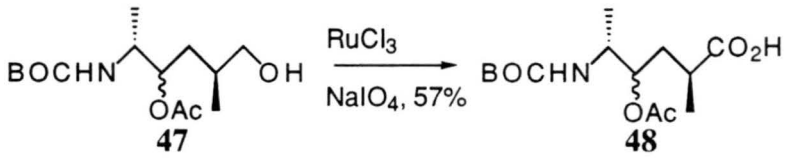


46 **47**

Alcohol 47.

A solution of **46** (81.2 mg, 0.214 mmol, 1.0 eq) in 1:1 iPrOH/HOAc (2 mL) at room temperature was flushed with N_2 gas for 10 minutes followed by the addition of HCO_2NH_4 (135 mg, 2.14 mmol, 10 eq) and 10% Pd/C (53 mg). The mixture was stirred for 6 hours then filtered through Celite using EtOAc (10 mL). The filtrate was washed with H_2O (3x5 mL) and saturated $NaHCO_3$ (3x5 mL), dried over $MgSO_4$, filtered, and stripped to afford 46.4 mg (75%) of **47** as a colorless oil. This was used without further purification.

^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 0.90 (3H, d, J = 6.6 Hz); 0.92 (3H, d, J = 6.7 Hz); 1.07 (3H, d, J = 6.9 Hz); 1.08 (3H, d, J = 6.6 Hz); 1.41 (9H, s); 1.41 (9H, s); 1.58-1.8 (4H, m); 2.04 (3H, s); 2.04 (3H, s); 3.30-3.54 (4H, m); 3.8-3.95 (2H, m); 4.61 (2H, d, J = 9 Hz); 4.87-5.0 (2H, m). IR (NaCl, film) 3360, 2976, 2932, 2881, 1704, 1515, 1450, 1370, 1312, 1239, 1167, 1036, 985.



(2S,4RS,5R)-4-Acetoxy-5-[(t-butyloxycarbonyl)-amino]-2-methylhexanoic Acid (48).

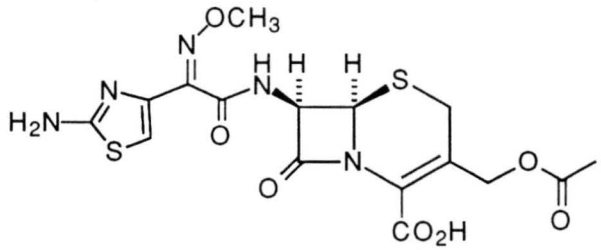
To a mixture of **47** (357 mg, 1.24 mmol, 1.0eq) in 1:1:1 $CCl_4/CH_3CN/H_2O$ (4.5 mL) at room temperature was added $NaIO_4$ (797 mg, 3.73 mmol, 3.0 eq) and $RuCl_3$ (4.7 mg, 0.02 mmol, 0.02eq). The reaction was stirred for 3 hours then quenched with ice-cold 0.5M HCl (100 mL) and stirred for 5 minutes. The reaction mixture was extracted with Et_2O (3x40 mL) and the combined organic layers dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography using hexanes:EtOAc (1:1) afforded 192 mg (57%) of an oil as an inseparable mixture of diastereomers (60:40).

^1H NMR (300 MHz) ($CDCl_3$) δ $CDCl_3$: 1.09 (6H, d, 6.9 Hz); 1.20 (6H, d, 7.2 Hz); 1.42 (18H, s); 2.03 (3H, s); 2.04 (3H, s); 2.4-2.6 (4H, m); 3.8-3.9 (2H, m); 4.55-4.65 (2H, m); 4.85-5.0 (2H, m). IR (NaCl, film) 3339, 2976, 2932, 2874, 1733, 1312, 1515, 1457, 1370, 1239, 1167, 1087, 1029.

Chapter 2

A. Introduction

Antibiotic resistance to β-lactams is due to the efficient production of β-lactamases which hydrolyze the lactam ring, resulting in inactivation of the drug. Around 1960, the first cephalosporin came into clinical use. As research progressed, cephalosporins were produced that had a much greater stability to the bacterial β-lactamases. These became known as the second and third-generation cephalosporins. The prototypical third-generation cephalosporin was cefotaxime. It possessed a broad range of activity against both gram-positive and gram-negative microorganisms.[46]

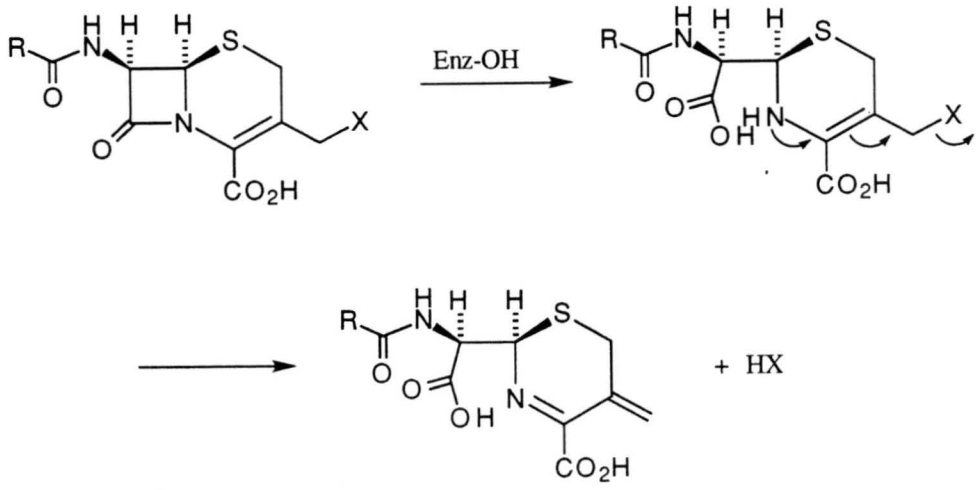


Cefotaxime

In 1965, Sabath and coworkers determined that the attack of a β-lactamase on a cephalosporin resulted in the expulsion of a substituent at the 3' (C_{10})-position.[47] They determined that enzymatic hydrolysis of cephalothin (Scheme **13**, X=OAc) resulted in the elimination of an acetoxy group while hydrolysis of cephaloridine (X=pyridine) resulted in the expulsion of the pyridine moiety.

If the 3'-substituent were an antibiotic itself, expulsion of the drug within the cytoplasm could result in increased antibacterial activity. A bacterium with no β-lactamase activity would still be susceptible to the cephalosporin prodrug as it is an

antibiotic itself. We set out to design such a system to deliver a prodrug with the potential to act synergistically with vancomycin.

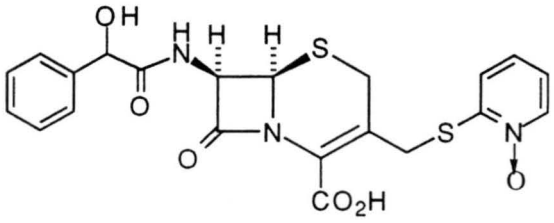


Scheme 13

1. Trojan Horse Delivery System

An antibiotic that is coupled to a β-lactamase susceptible cephalosporin should be released within the bacteria upon attack of the β-lactamase. This 'Trojan horse' delivery system was named after the Greek attack on Troy where soldiers were smuggled into the city by hiding in the hollowed-out belly of a wooden horse. The coupled cephalosporin may then act with a dual mode of action. First, the cephalosporin itself may show antibacterial action as it is a β-lactam drug. Second, expulsion of the 3'-substituent, an antibiotic itself, may also act against the bacteria. O'Callaghan and coworkers synthesized the first antibiotic with a dual mode of action.[48] They reported that an antibiotic, MCO, which had 2-mercaptopyridine-N-oxide as the 3'-substituent, exhibited antibacterial activity. MCO displayed a broad range of activity, inhibiting both gram-positive and gram-negative organisms as well as yeasts and fungi. They suggested that

the drug's activity was due to the prodrug itself as well as the activity of 2-mercaptopyridine-N-oxide, released as a result of beta-lactamase activity.



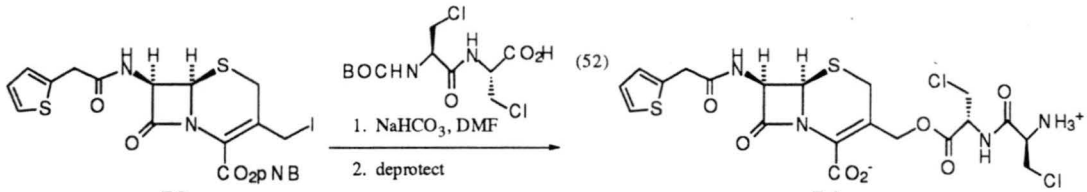
MCO

Table 3. Mean MIC's (μg/mL) of MCO

ORGANISM	Number of strains tested	Mean MIC of MCO
Staphylococcus aureus	25	2.7
Escherichia coli	24	1.8
Citrobacter freundii	8	11
Enterobacter cloacae	4	3.4
Enterobacter hafniae	10	3.7
Klebsiella aerogenes	13	16
Proteus rettgeri	2	2
Proteus morganii	1	4

Michael Johnston and coworkers applied the principle of a dual mode of action to deliver peptides which were linked as a 3'-ester to the cephalosporin nucleus.[49-51] They synthesized alanine analogs to act as suicide inhibitors of alanine racemase, capable of covalently binding to the enzyme. One of the most potent antibiotics was the dipeptide β-Cl-L-Ala-β-Cl-L-Ala (**52**)[52], which was active against 12 of 16 gram-positive and

negative organisms. An antibacterial agent that could deliver the drug directly to the site of action should show a much greater activity both *in vitro* and *in vivo*. In turn, they synthesized a dual action cephalosporin, **54**, which showed activity against six of the eight organisms tested.



53 **54**

Scheme 14

Table 4. MIC's (μg/mL) of Compound 54

ORGANISM	MIC of 54
Enterobacter aerogenes	100
Enterobacter cloacae	14.1
Escherichia coli JSR-O	14.1
Escherichia coli JSR-O[a]	>200
Escherichia coli JSR-O[b]	7.05
Escherichia coli[c]	7.05
Staphylococcus aureus[d]	0.85
Corynebacterium K	1.70

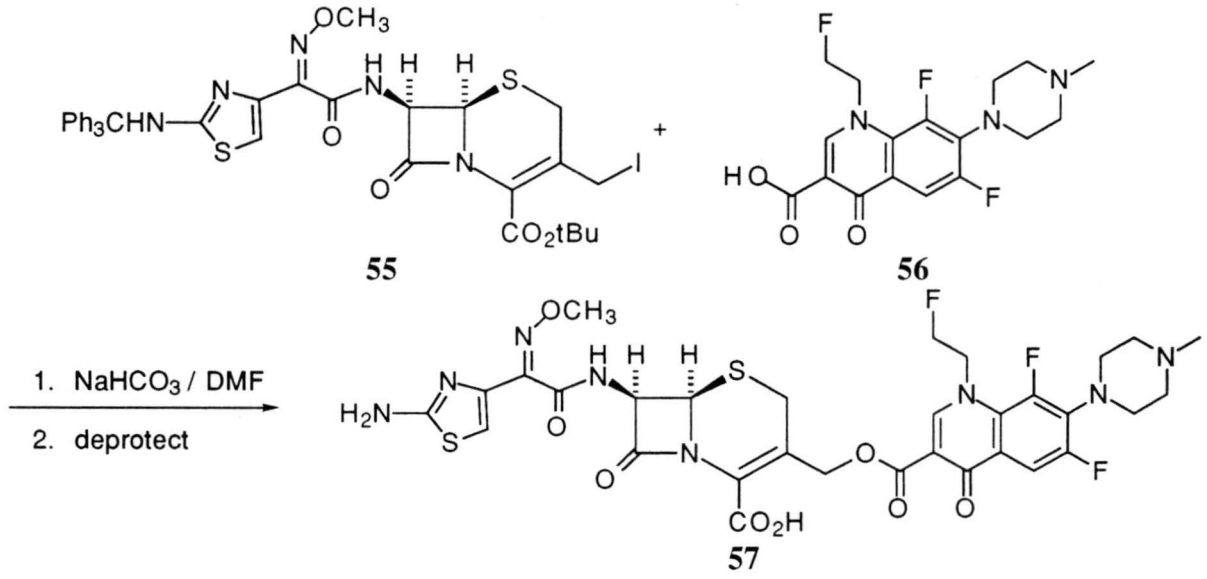
a. This is an E. coli strain selected for resistance to the dipeptide β-Cl-L-Ala-β-Cl-L- Ala (MIC>100 μg/mL).
b. Contains the plasmid gene encoding for the TEM β-lactamase.
c. Resistant to cephalothin (MIC>200 μg/mL).
d. Resistant to penicillin G (MIC>200μg/mL).

A general concern for the activity of the dual-action antibiotics is the need for transport through the periplasmic space to the cytoplasm. This is not a problem in gram-

negative organisms as their beta-lactamases are located in the periplasm. However, it is known that gram-positive bacteria excrete their beta-lactamases into the area surrounding the cell.[53] If enzymatic inactivation of the cephalosporin occurs outside the cell with release of the 3'-substituent, the dipeptide will not be able to enter the cell as before and the dual mode of action will not be in effect.

Researchers at Hoffmann-La Roche Inc. have synthesized a variety of dual-action cephalosporins exhibiting a broad spectrum of antibacterial activity.[54] It appears that concern for a lack of activity against gram-positive organisms is unwarranted. In the search for a potent antibiotic, the researchers thought that the combination of a cephalosporin and a quinoline antibiotic would give the necessary activity for clinical trials. As such, cephalosporin **57** was synthesized by the method of Johnston, coupling iodocephalosporin **55**, Ro 18-8511, with fleroxacin (**56**).

Cephalosporin **57**, Ro 23-9424, was chosen for clinical candidacy as it possessed a broad spectrum of antibiotic activity. The question researchers wanted to answer was whether or not the antibiotic acted with a dual mode of action. A variety of evidence has been reported which suggests that a dual mode of action is in effect and antibacterial activity is the result of both the cephalosporin and quinoline moieties.[55]



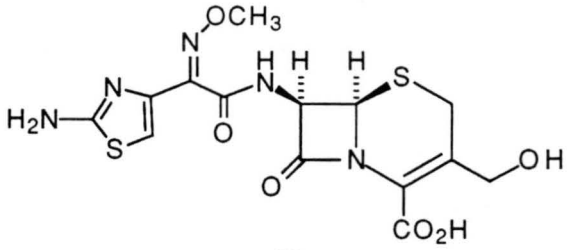
Scheme 15

Table 5. MIC's (μg/mL) of Compound 57 and Cefotaxime

ORGANISM	Compound 57	Cefotaxime
E. coli ATCC 25922	0.125	0.063
E. coli TEM-1	0.125	0.031
C. freundii BS-16	2	128
E. cloacae P99	0.125	32
S. marcesens 1071	0.5	8
P. vulgaris 1028 BC	0.25	16
P. mirabilis 90	0.25	0.016
P. aeruginosa ATCC 27853	8	64
P. aeruginosa 18S/H	4	128
S. aureus ATCC 29213	1	2
S. aureus 1059B	1	4
S. aureus 95	2	32
S. pneumoniae 6301	≤0.008	0.016
S. pyogenes 4	0.031	≤0.008
E. faecalis ATCC 29212	8	>128

Nonenzymatic hydrolysis of the 3'-ester can complicate matters when determining the mode of action for this antibiotic. In order to determine the effect of hydrolysis, the researchers compared the susceptibility of **57**, fleroxacin (**56**) and desacetylcefotaxime (**58**), the products of hydrolysis, and a 1:1 combination of **56** and **58**. It was found that cephalosporin **57** was significantly more active than any of the others and clearly showed that **57** can act as an antibiotic in its own right. It is known that both quinolines and

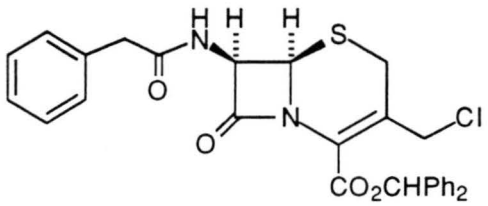
cephalosporins cause filamentation in growing *E. coli*. Inhibition of septum formation allows cells to proliferate, but not divide. This causes chains of the bacteria to form. Additionally, only quinolines disrupt nucleoid segregation in the bacteria.[56] This is seen as a grouping of the nucleoids at the center of the filaments instead of a regular separation throughout the filament. In an assay of activity, only filamentation was seen during the first hour of incubation and nucleoid segregation was not seen until after two hours. This suggests that initially the cephalosporin is acting alone and after release of the quinoline antibiotic, both filamentation and nucleoid segregation occurs.



58

B. Results and Discussion

Since all the peptide isosteres synthesized were biologically inactive, it was necessary to find a method to deliver them within the bacteria. Antibiotics with a dual mode of action seemed to be the best method as it provides a means of delivery as well as the possibility of acting as an antibiotic itself. Two prodrug intermediates were graciously given to the Williams group by Dr. John Roberts of Hoffmann-La Roche Inc. and Otsuka Chemical Company. Iodocephalosporin **55** was provided by Hoffmann-La Roche; chlorocephalosporin **59** was provided by Otsuka Chemical Company. With these intermediates in hand, direct coupling with the synthesized peptide isosteres was possible.



59

1. Coupling Using $NaHCO_3$ and DMF

Employing the method of Johnson, $NaHCO_3$ in DMF was used to couple cephalosporins **55** and **59** with peptide isosteres.[49] As **59** had a simpler ^1H NMR spectrum, it was chosen first for coupling. Initial reaction conditions included the addition of molecular sieves to trap any water present. All attempts at coupling failed. There seems to be a common product in almost all of the reactions which as yet, has not been identified. The unknown product has two characteristic doublets centered about 4.1 ppm in it's ^1H NMR spectrum. In order save the synthesized isosteres, NaOAc was used in the coupling reactions to determine the optimum reaction conditions. Table **3** details the attempted reaction conditions.

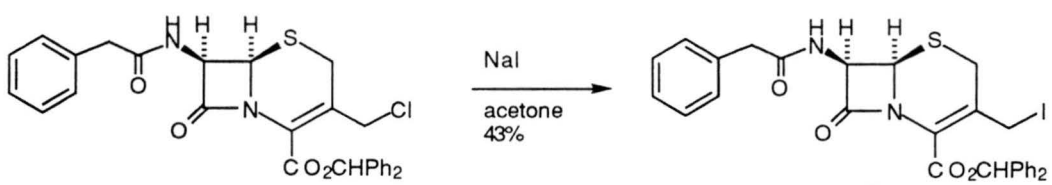
A major problem thought to have contributed to the unsuccessful reactions was the presence of water. Originally, the reactions were run in the presence of 4Å molecular sieves.[54] It was found that they are unnecessary and possibly detrimental to the reaction. All solid starting materials were now placed under a vacuum overnight in the presence of P_2O_5. The vacuum was removed under nitrogen pressure and the starting materials were placed into a single vial and evacuated for an additional hour. The second evacuation was done with a stir-bar, serum stopper, and syringe needle in place. The vacuum was

Table 6. Unsuccessful Couplings with 59

reactant	conditions
29	$NaHCO_3$, DMF, RT
29	Et_3N, DMF, RT
29	NaOH, DMF, RT
29	AgOTf, CH_3CN, RT
NaOAc	DMF, RT
NaOAc	CH_3CN, 60°C
NaOAc	1:1 DMF:dioxane, RT
28	NaOH, DMF, RT
28	NaOMe, DMF, RT
28	DMF, RT
Boc-L-Ala	$NaHCO_3$, DMF, RT

removed again under nitrogen pressure and the vial placed directly onto a nitrogen line to begin the reaction.

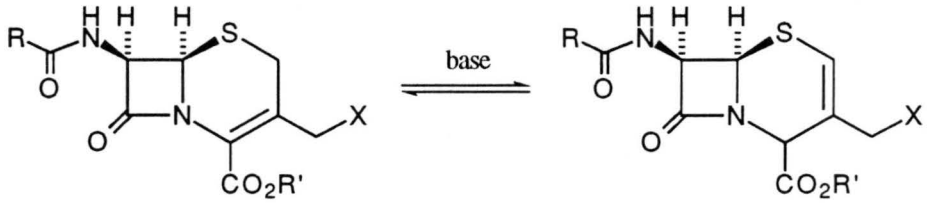
An additional problem may be the fact that the C-10 chloro group is too unreactive for coupling to occur. It was thought that if this could be converted to the iodo derivative, the reaction would proceed. Reaction of **59** with NaI in acetone yielded **60** in a 43% yield after chromatography.[57] Compound **60** was used as well as an *in situ* formation of the iodide by adding NaI to the reaction mixture containing **59**.



59 **60**

Scheme 16

The method of Johnston has a drawback in that isomerization of the C-3 double bond of the cephalosporin can occur under the basic conditions used.[58] The isomerization to the C-2 position yields an inactive antibiotic. Addtionally, expulsion of the 3'-substituent cannot occur.[59] The Δ^2-isomer was almost always present to some degree.

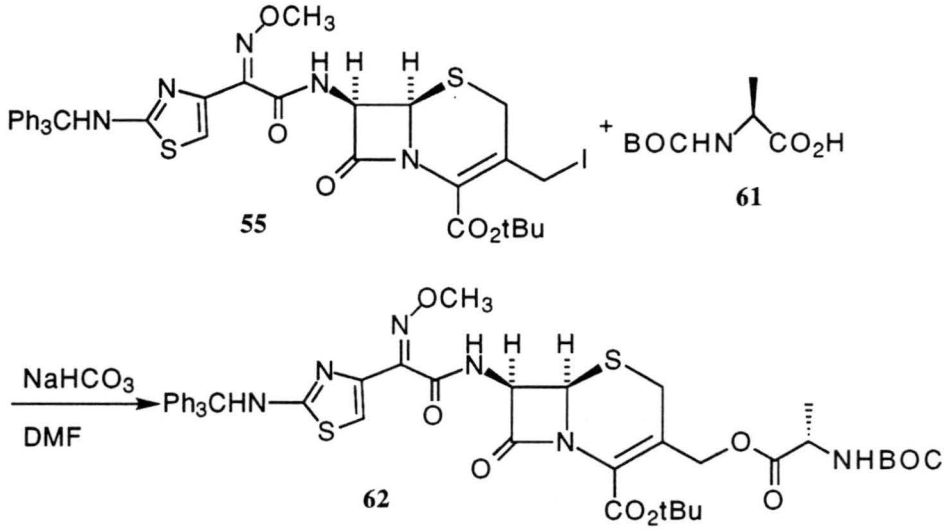


Δ^3-isomer **Δ^2-isomer**

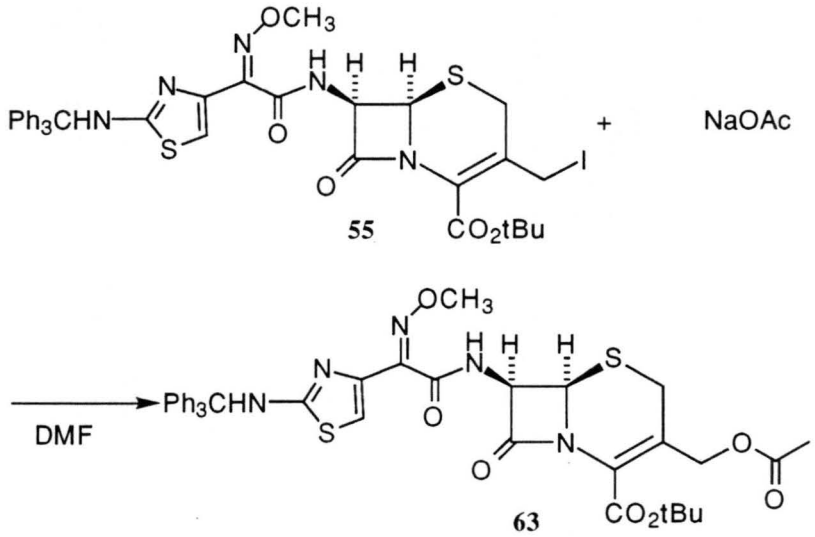
Scheme 17

Attempts at coupling using **60** directly and *in situ* formation of **60** were successful. However, there was a high degree of isomerization. It appears that the attack on the cephalosporin by the base occurs more rapidly than attack of the carboxylate species being coupled. Reaction of **59** with Boc-D-Ala, NaI, and $NaHCO_3$ in DMF gave a mixture of Δ^3 / Δ^2-isomers in 40:60 ratio. Reaction of **60** with NaOAc in DMF gave a 45:55 mixture while reaction in a 5:3 DMF / dioxane solvent system gave a 36:64 mixture of products.[60] Since isomerization of this degree is unacceptable, all work with the Otsuka cephalosporin was abandoned.

Work was turned to the Hoffmann-La Roche cephalosporin. The first coupling reaction to be successful was the coupling of **55** with Boc-L-alanine (**61**) yielding cephalosporin-conjugate **62** in a 38% yield.[54] Additionally, reaction of **55** with NaOAc gave compound **63** in a 45% yield. Both of these reaction followed the above procedure attempting to exclude all traces of water.



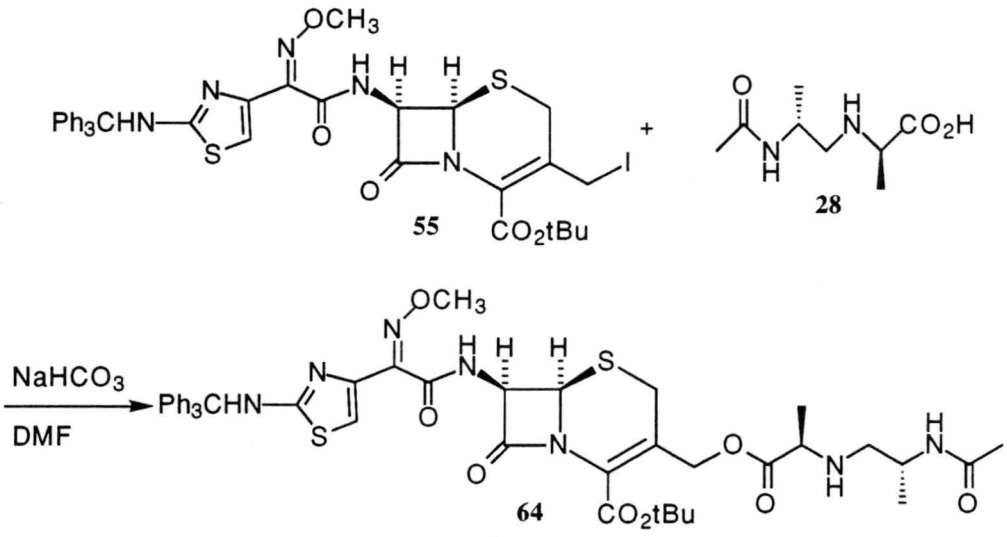
Scheme 18



Scheme 19

Next, coupling of methyleneamino isostere **28** and **55**, yielded compound **64** in a 38% yield. The one new aspect of this reaction was the addition of HMPA to help dissolve the starting isostere in DMF. It is not known whether this was actually necessary due to the difficulty in reading the TLC plates as the reaction was monitored. There is a side-product which co-spots with the starting material and therefore it is difficult to see when the reaction has been completed. Although coupling of a peptide isostere had now been acheived, attempts at coupling alkene isostere **40** failed. The

Johnston route proved difficult for coupling of the peptide isosteres and was abandoned for a more efficient route.

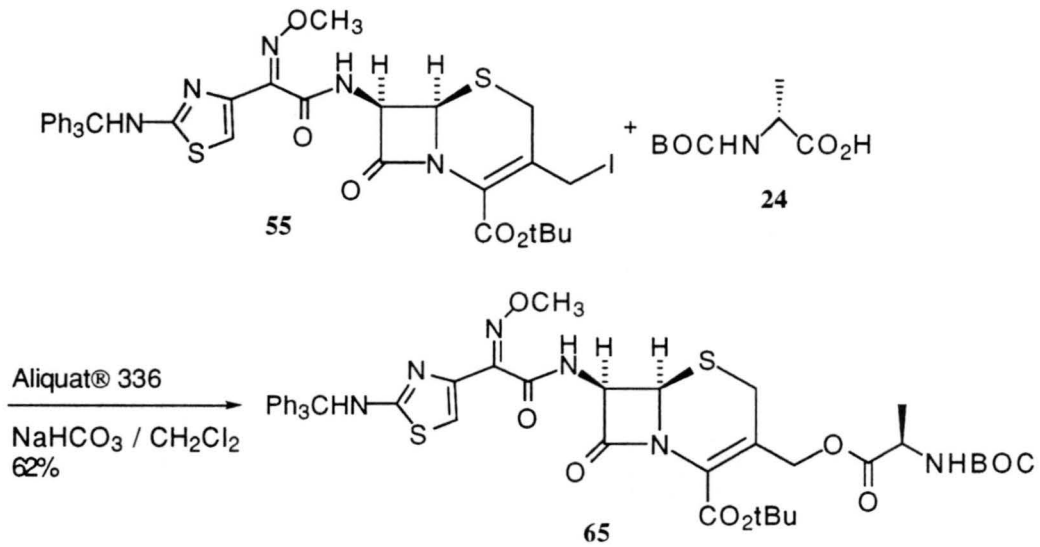


Scheme 20

2. Coupling Using Phase Transfer Catalysis

A novel method for coupling 3'-halocephalosporins with carboxylic acids to form 3'-esters has been developed. The difficulties of the Johnston method have been noted above. Rigorous exclusion of water proved to be the most cumbersome aspect of the coupling procedure. A 1979 communication detailed the esterification of protected amino acids and dipeptides using phase transfer catalysis (PTC).[61] A mixture of trioctylmethylammonium chloride (Adogen-464) and alkyl halide in methylene chloride was added to a solution of amino acid in saturated aqueous sodium bicarbonate. Reaction was complete at room temperature in less than 24 hours. This appeared to give an ideal situation, exclusion of water was unnecessary and attack of the bicarbonate ion would not occur as it does not have an appreciable solubility in the organic phase.[62] Double bond isomeration could only occur if the amino acid carboxylate attacked as a base before nucleophilic substitution occured. Reaction of **55** with Boc-D-Ala (**24**) using Aliquat®

336 (same as Adogen-464) gave **65** in a 62% yield. Additionally, reaction in EtOAc under the same conditions gave **65** in a 60% yield. Reaction of Otsuka cephalosporin **59** failed under identical conditions as well as with the addition of NaI. Purification was extremely difficult with these reactions as Aliquat® 336 eluted with the product and all atempts to completely purify **65** failed. A variety of phase transfer catalysts exist, so a search began for a catalyst which would be easy to separate from the product as well as give the highest yield.



Scheme 21

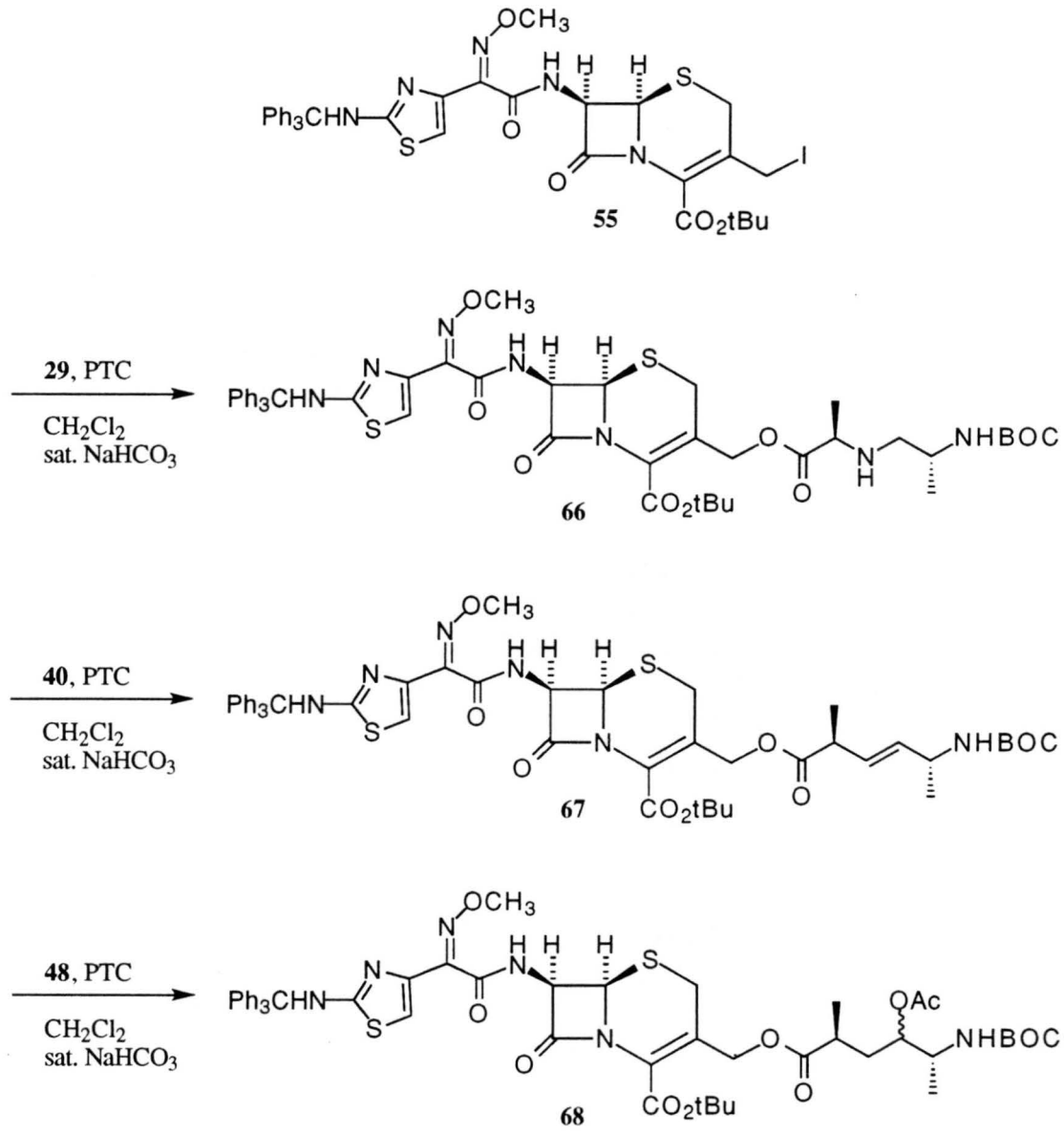
Using Boc-D-alanine as a model compound, various phase trasfer catalysts were employed to form the 3'-ester linkage to cephalosporin **55**. Research has shown that when an alkyl iodide is used in conjunction with a PTC, the iodide poisons the catalyst by forming a tight ion pair.[63] This keeps the PTC in the organic phase and stops the catalytic process. Therefore, at least one equivalent of catalyst must be used or extremely low yields result. Additionally, in the prescence of base, the catalysts have a half-life of about 17 hours[62] and some reactions were run up to 24 hours. The optimum reaction conditions were found using two equivalents of methyltributylammonium chloride as the PTC.

Table 7. Phase-Transfer Conditions

Phase Transfer Catalyst	eq PTC	yield
benzyltriethylammonium chloride	1	incomplete
tetramethylammonium chloride	1	incomplete
tetrabutylammonium hydrogen sulfate	1	31%
tetrabutylammonium hydrogen sulfate	10	62% (Δ^3/Δ^2)
tetrabutylammonium hydrogen sulfate	2	76%, crude
methyltributylammonium chloride	2	71%, crude

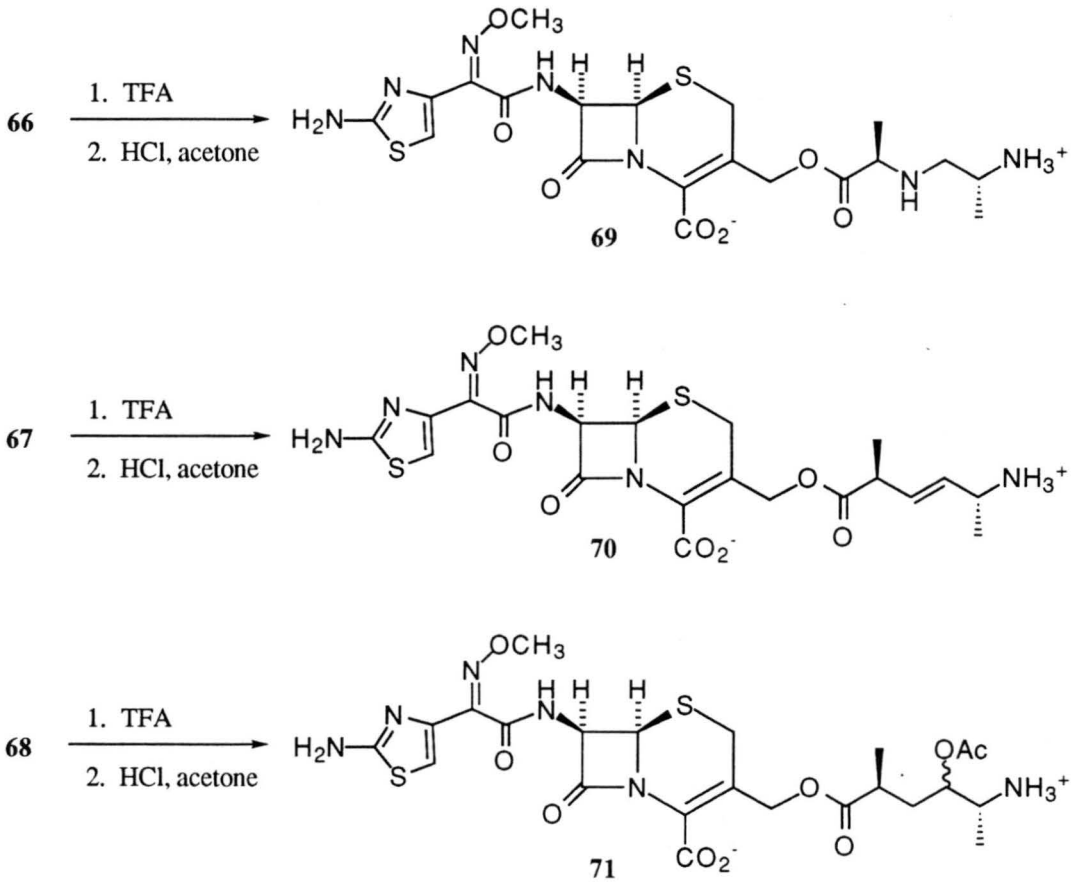
Reactions with the tetrabutyl and methyltributyl catalysts were very similar. In the case of Boc-D-Ala, no evidence for the formation of the Δ^2-isomer was found by ^{1}H NMR. However, reaction with alkene isostere **40**, resulted in a 3:1 mixture with the desired Δ^3-isomer in the highest yield. Since the undesired isomer probably results from the attack of the carboxylate ion as a base, a decrease in the accessability of the ion would increase the chance for a nucleophilic attack.[62] The replacement of one of the butyl groups for a methyl group allows for a tighter ion pair that results in a decrease in accessibilty as it approaches another molecule. There was one drawback to the use of the methyltributyl catalyst in that it is obtained as a water solution. The tetrabutyl catalyst is a crystalline solid.

Reaction of iodocephalosporin **55** with dipeptide isosteres **29**, **40**, and **48** under phases transfer conditions using two equivalents of methyltributylammonium chloride proceeded smoothly to afford **66**, **67**, and **68** with yields of 42%, 53%, and 53%, respectively. In all cases, a small amount of the Δ^2-isomer was evidenced by the ^{1}H NMR. This was seen as singlet at about 6.4 ppm. In order to complete the synthesis of the cephalosporin prodrugs, deprotection of the three protecting groups was necessary. Partial deprotection of any of the three groups as opposed to full deprotection will lower the yield of product.



Scheme 22

In an attempt to circumvent this problem, the reactions were run in TFA overnight at room temperature. If any starting material was present in the morning, additional TFA was added and the reaction stirred for another few hours. For the purposes of biological testing, it was desirable to have the free amino acid or hydrochloride salt present as opposed to a TFA salt. Using a procedure by the Hoffmann-La Roche group, the protected prodrugs were first deprotected with TFA.[64] The TFA salt was obtained by



Scheme 23

evaporation of the solvent and excess TFA. The residue was dissolved in a 1:1 solution of acetone and 1M HCl and stirred at 0°C for 45 minutes. The solvent was removed and the salt purified by preparatory TLC. Cephalosporins **69**, **70**, and **71** were obtained in 70% (crude), 51%, and 38% yields respectively by this method. No Δ^2-isomers were evident by ^{1}H NMR.

Biological testing on compounds **69**, **70**, and **71** by the double-disc synergy method against *E. faecium* BM4147 were all negative. This organism is completely resistant against the cephalosporins and against the cephalosporins in conjunction with sulbactam, a β-lactamase inhibitor. Since it was possible that the organism would secrete a β-lactamase outside the cell wall, it was thought that an inhibitor would allow the compounds to get through the cell wall barrier and into the cytoplasm where they could react.

Although the above compounds were not active against *E. faecium*, they may be active against other bacteria. Three strains were chosen as representatives to test against both gram-positive and gram-negative organisms. The gram-positive organism was *Staphylococcus aureus* ATCC 25923. The two gram-negative organisms were *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 10031. Compounds **69** and **70** proved to be active against all three strains and **71** was active against *K. pneumonia* (the only organism it was tested against). The following table gives the inhibitory concentrations (IC's) for the organisms tested. The minimum inhibitory concentrations and further testing of compound 71 were not completed for lack of a significant amount of compound. However, further testing of the above compounds is warranted as it was found that *S. aureus* and *K. pneumonia* were the only organisms with the β-lactamase enzyme. This was found using the DrySlide™ Beta-Lactamase kit from Difco Laboratories. In this test, a pH indicator is used to test for the presence of penicilloic acid, the product of β-lactamase attack of the penicillin nucleus. Since the enzyme is neccesary for the dual-mode of action to occur, more organisms must be tested in order to determine the mechanism of action.

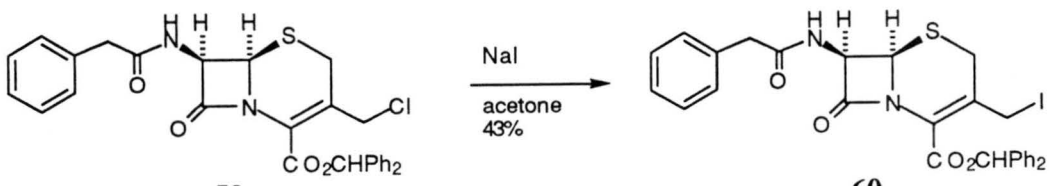
Table 8. IC's (μg/mL) of Synthetic Compounds

ORGANISM	69	70	71	Cefotaxime
S. aureus	1000 (15mm)	100 (13mm)	---	100 (7mm)
E. coli	1000 (16mm)	100 (19mm)	---	100 (11mm)
K. pneumonia	100 (15mm)	100 (25mm)	100 (19mm)	100 (16mm)
E. faecium	R	R	R	R

3. Conclusion

The completion of the synthesis of three cephalosporin prodrugs has been achieved by a novel method using phase-transfer catalysis. Additionally, they were all active against several strains of bacteria. Unfortunately the true goal of this research project has not been realized. All testing against the vancomycin-resistant strain *E. faecium* BM4147 have been unsuccessful. It would be very beneficial to obtain the purified enzymes responsible for vancomycin resistance. Like the previous research described, several groups obtained active compounds against the purified enzyme, but all *in vitro* tests failed.

C. Experimental

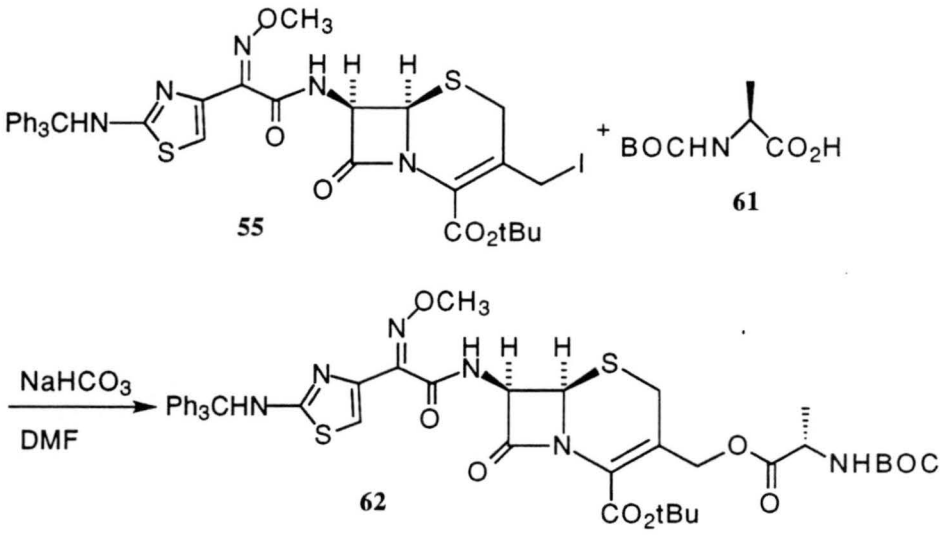


59 **60**

Iodocephalosporin 60.

To a stirred solution of **59** (20.2 mg, 0.0379 mmol, 1.0 eq) in acetone (500 μL) at room temperature was added NaI (29 mg, 0.193 mmol, 5.0 eq). The mixture was stirred for 2 hours then concentrated. The residue was taken up in EtOAc (10 mL) and washed with water (5 mL) and ice-cold brine (5 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated to afford 22.4 mg (95%) of **60** as an yellow solid.

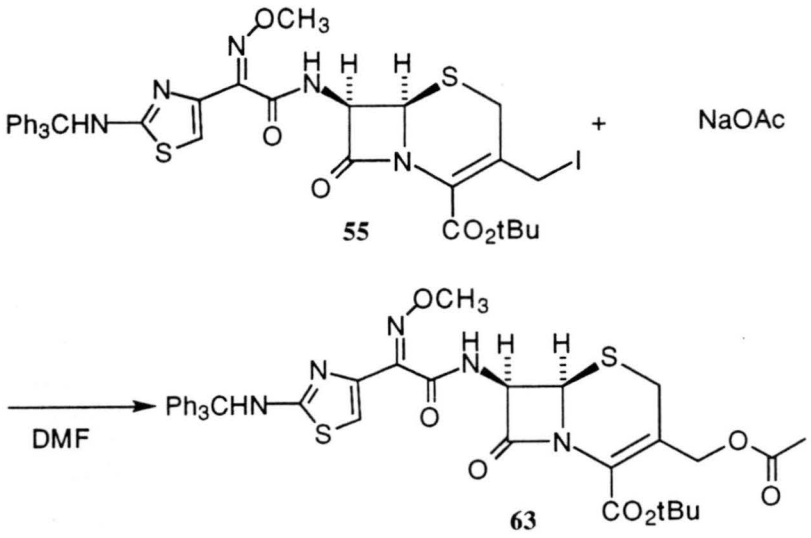
R_f = 0.67 (1:1 hexanes:EtOAc); mp 66°C (dec.) (lit. amorphous solid); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 3.14 (1H, d, J = 18 Hz); 3.59 (1H, d, J = 16 Hz); 3.66 (1H, d, J = 16 Hz); 3.67 (1H, d, J = 18 Hz); 4.22-4.31 (2H, m); 4.93 (1H, d, J = 4.8 Hz); 5.78 (1H, dd, J = 5 Hz, 9 Hz); 6.18 (1H, d, J = 9 Hz); 6.95 (1H, s); 7.1-7.5 (15H, m). IR (NaCl, film) 3302, 3063, 3027, 2925, 2852, 1777, 1726, 1653, 1529, 1459, 1377, 1181, 1080, 1029.



Cephalosporin 62.

To a vial at room temperature was added dried **55** (19.9 mg, 0.0242 mmol, 1.0 eq), **61** (4.6 mg, 0.0243 mmol, 1.0 eq), and $NaHCO_3$ (2.0 mg, 0.0238 mmol, 1.0 eq) followed by DMF (500 μL). The mixture was stirred for 20 hours then concentrated. Water (5 mL) and a 1:1 mixture of CH_2Cl_2 and EtOAc (10 mL) were added to the residue. The organic phase was dried over $MgSO_4$, filtered, concentrated, and separated on preparatory TLC (1:1 hexanes:EtOAc) to afford 8.2 mg (38%) of **62** as an orange solid.

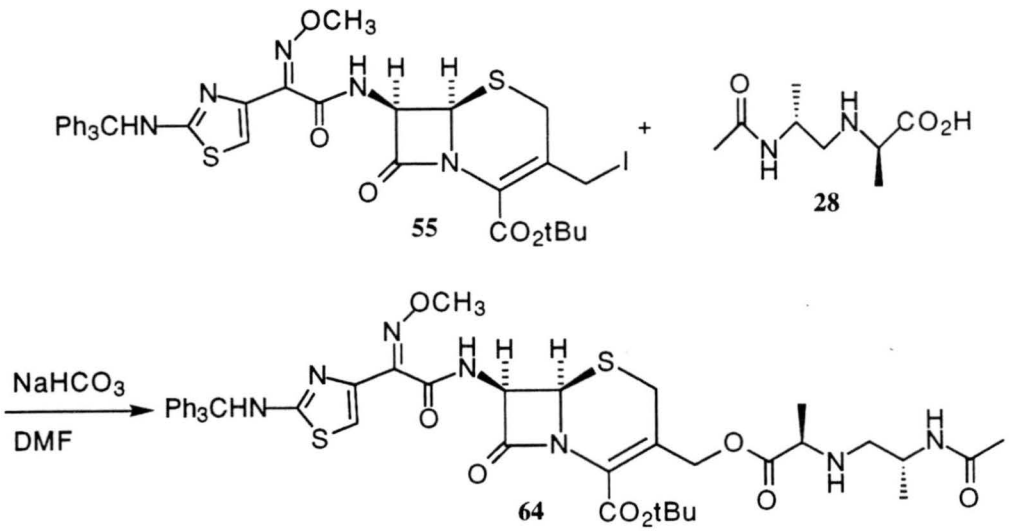
R_f = 0.37 (1:1 hexanes:EtOAc); mp 102°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.37 (3H, d, J = 7.2 Hz); 1.42 (9H, s); 1.52 (9H, s); 3.33 (1H, d, J = 18.6 Hz); 3.54 (1H, d, J = 18.6 Hz); 4.06 (3H, s); 4.23-4.35 (1H, m); 4.90 (1H, d, J = 13.5 Hz); 4.95 (1H, bs); 5.02 (1H, d, J = 4.8 Hz); 5.19 (1H, d, J = 13.5 Hz); 5.91 (1H, m); 6.71 (1H, bs); 7.00 (1H, bs); 7.30 (15H, bs).



Cephalosporin 63.

To a vial at room temperature was added dried **55** (20.2 mg, 0.0246 mmol, 1.0 eq) and NaOAc (2.1 mg, 0.0256 mmol, 1.1 eq) followed by DMF (500 μL). The mixture was stirred for 10.5 hours then concentrated. Water (5 mL) and a 1:1 mixture of CH_2Cl_2 and EtOAc (10 mL) were added to the residue. The organic phase was dried over $MgSO_4$, filtered, concentrated, and separated on preparatory TLC silica gel (1:1 hexanes:EtOAc) to afford 8.4 mg (45%) of **63** as a yellow solid.

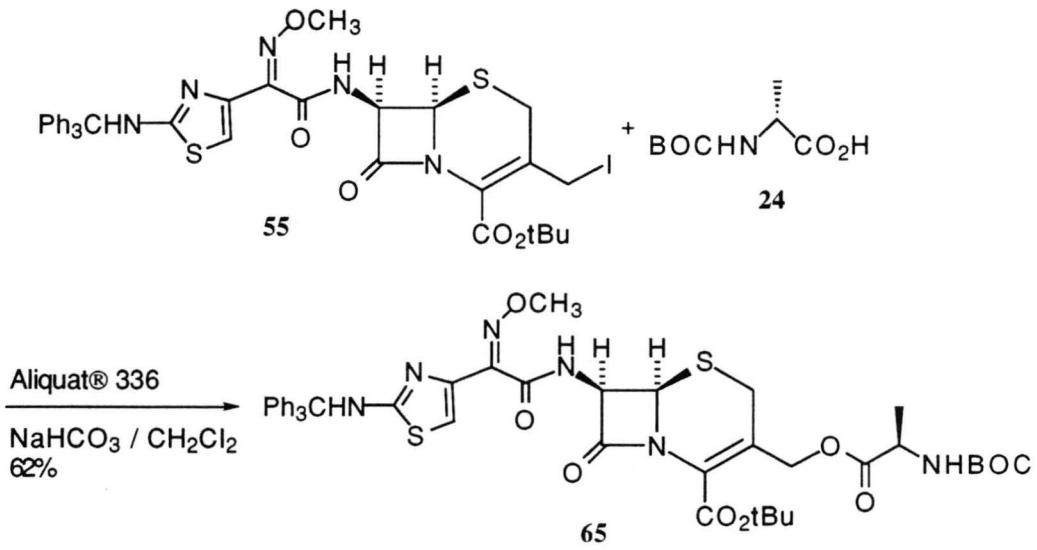
R_f = 0.34 (1:1 hexanes:EtOAc); mp 116°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.52 (9H, s); 2.08 (3H, s); 3.35 (1H, d, J = 18 Hz); 3.55 (1H, d, J = 18 Hz); 4.06 (3H, s); 5.00-5.02 (2H, m); 5.85-6.0 (1H, m); 6.73 (1H, bs); 7.00 (1H, bs); 7.33 (15H, bs). IR (NaCl, film) 3289, 3056, 2974, 2933, 1778, 1723, 1682, 1532, 1459, 1368, 1231, 1156, 1039.



Cephalosporin 64.

To a vial at room temperature was added dried **55** (43.5 mg, 0.0529 mmol, 1.0 eq), **28** (10.0 mg, 0.0532 mmol, 1.0 eq), and $NaHCO_3$ (4.5 mg, 0.0536 mmol, 1.0 eq) followed by DMF (500 μL). The mixture was stirred for 27 hours then HMPA (100 μL) was added and stirring continued for another 3 hours. The reaction was concentrated and water (5 mL) and a 1:1 mixture of CH_2Cl_2 and EtOAc (10 mL) were added to the residue. The organic phase was washed with water (5x10 mL), dried over $MgSO_4$, filtered, concentrated, and separated on preparatory TLC (1:1 hexanes:EtOAc) to afford 17.5 mg (38%) of **64** as an orange solid.

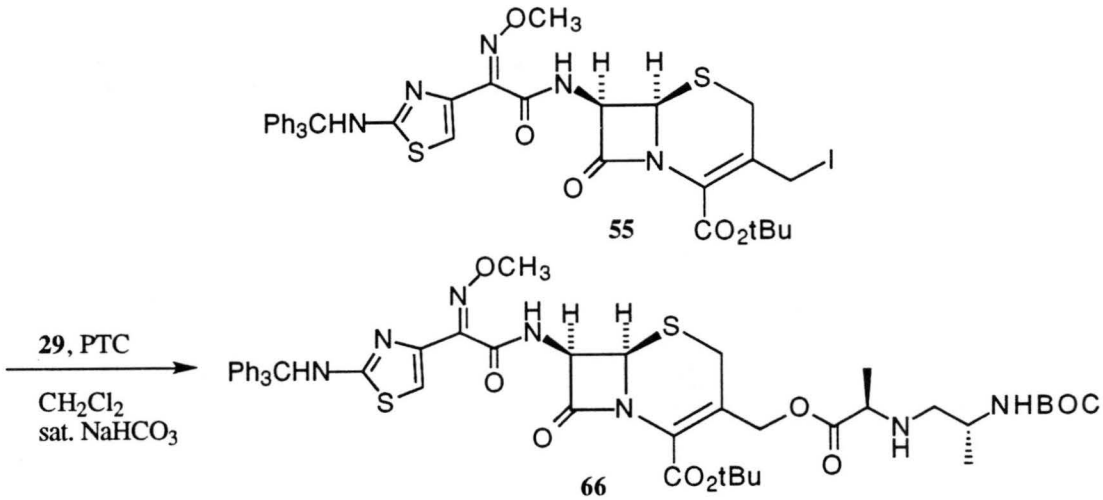
R_f = 0.05 (1:1 hexanes:EtOAc); mp 149°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.09 (3H, d, J = 6.6 Hz); 1.52 (9H, s); 1.93 (3H, s); 2.45-2.6 (2H, m); 3.3-3.7 (3H, m); 4.09 (3H, bs); 4.93 (1H, d, J = 13.5 Hz); 5.00-5.08 (2H, m); 5.7-6.0 (1H, m); 6.71 (1H, bs); 7.05 (1H, bs); 7.3 (15H, s). IR (NaCl, film) 3324, 3055, 2976, 2932, 1777, 1726, 1668, 1530, 1450, 1305, 1261, 1159, 1101, 1043.



Cephalosporin 65.

To a stirred solution of **55** (21.4 mg, 0.0620 mmol, 1.0 eq) in CH_2Cl_2 (1 mL) was added a solution of **24** (4.92 mg, 0.0260 mmol, 1.0 eq) in saturated $NaHCO_3$ (500 μL) followed by Aliquat® 336 (11.9 μL, 0.0260 mmol, 1.0 eq). The mixture was stirred vigorously for 17 hours followed by addition of CH_2Cl_2 (20 mL) and water (10 mL). The organic phase was separated, dried over $MgSO_4$, filtered, and purified on preparatory TLC (1:1 hexanes:EtOAc) to afford 14.2 mg (62%) of **65** as a light yellow solid.

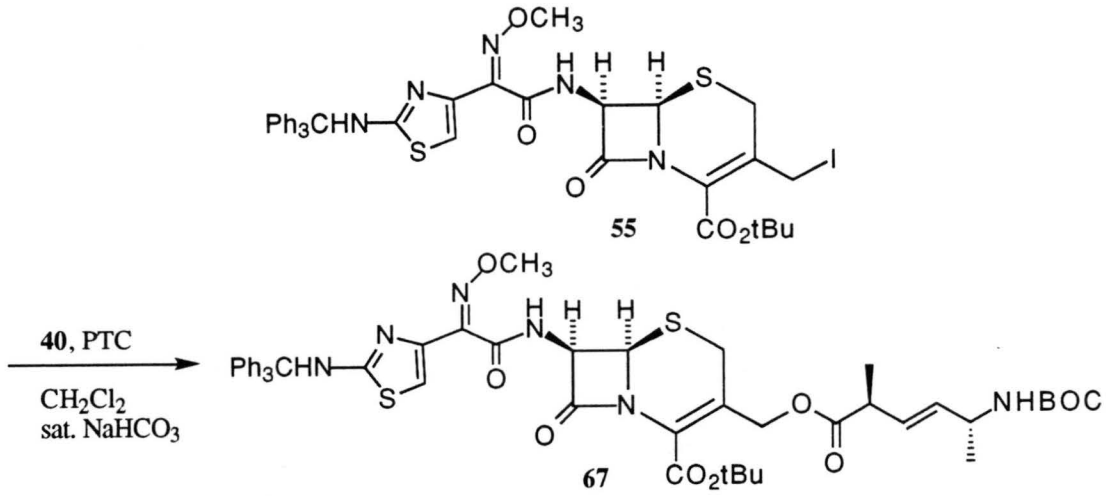
R_f = 0.32 (1:1 hexanes:EtOAc); mp 109°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.40 (3H, d, J = 7.2 Hz); 1.44 (9H, s); 1.53 (9H, s); 3.36 (1H, d, J = 18.6 Hz); 3.56 (1H, d, J = 18.6 Hz); 4.07 (3H, s); 4.24-4.35 (1H, m); 4.90 (1H, d, J = 13.2 Hz); 4.97 (1H, d, J = 6.6 Hz); 5.03 (1H, d, J = 4.5 Hz); 5.18 (1H, d, J = 13.5 Hz); 5.95 (1H, dd, J = 4.8, 9.0 Hz); 6.7-6.8 (2H, m); 7.04 (1H, s); 7.25 (15H, s). IR (NaCl, film) 3303, 3056, 2976, 2932, 1777, 1712, 1523, 1459, 1370, 1305, 1254, 1109, 1050, 702.



Cephalosporin 66.

To a stirred solution of **55** (54.9 mg, 0.0668 mmol, 1.0 eq) in CH_2Cl_2 (500 μL) was added a solution of **29** (16.4 mg, 0.0667 mmol, 1.0 eq) in saturated $NaHCO_3$ (500 μL) followed by methyltributylammonium chloride (45 μL, 0.143 mmol, 2.1 eq). The mixture was stirred vigorously for 24 hours followed by addition of CH_2Cl_2 (20 mL) and water (10 mL). The organic phase was separated, dried over $MgSO_4$, filtered, and purified on preparatory TLC (1:1 hexanes:EtOAc) to afford 26.2 mg (42%) of **66** as a light yellow solid.

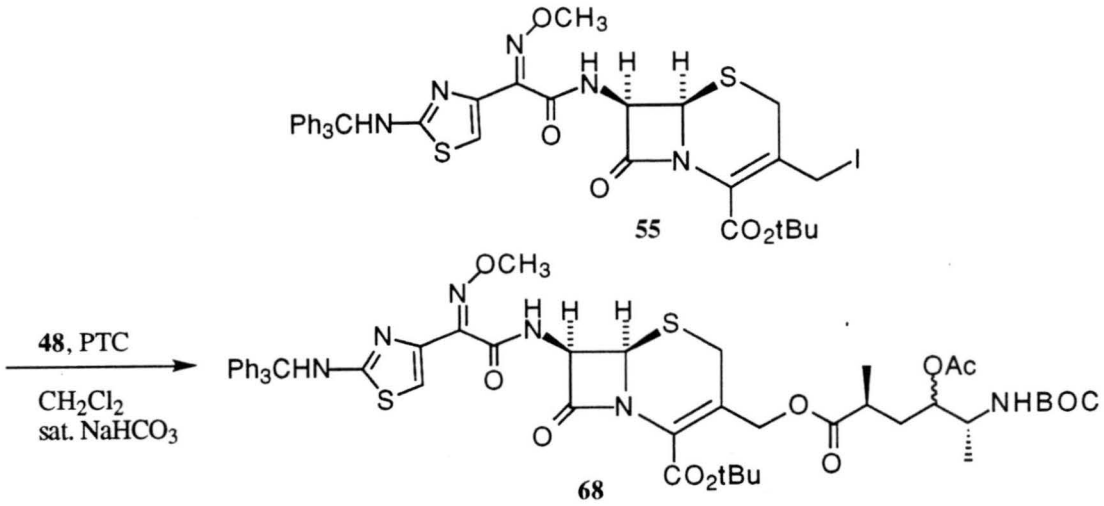
R_f = 0.13 (1:1 hexanes:EtOAc); mp 156°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.09 (3H, d, J = 6.0 Hz); 1.41 (9H, s); 1.52 (9H, s); 2.45-2.7 (2H, m); 3.3-3.8 (4H, m); 4.06 (3H, s); 4.72 (1H, d, J = 6.6 Hz); 4.92 (1H, d, J = 13.5 Hz); 5.0-5.1 (2H, m); 5.85-5.98 (1H, m); 6.72 (2H, bs); 7.04 (1H, m); 7.29 (15H, s). IR (NaCl, film) 3288, 3056, 2976, 2932, 1777, 1683, 1523, 1450, 1370, 1312, 1247, 1159, 1101, 1050. $[\alpha]_D^{26}$ = +27° (*c* 0.23, CH_2Cl_2). HRMS (FAB+) m/e 940.3781 ($C_{48}H_{58}N_7O_9S_2$ requires 940.3737).



Cephalosporin 67.

To a stirred solution of **55** (37.5 mg, 0.0456 mmol, 1.0 eq) in CH_2Cl_2 (500 μL) was added a solution of **40** (11.1 mg, 0.0457 mmol, 1.0 eq) in saturated $NaHCO_3$ (500 μL) followed by methyltributylammonium chloride (29 μL, 0.922 mmol, 2.0 eq). The mixture was stirred vigorously for 14 hours followed by addition of CH_2Cl_2 (20 mL) and water (10 mL). The organic phase was separated, dried over $MgSO_4$, filtered, and purified on preparatory TLC (1:1 hexanes:EtOAc) to afford 22.5 mg (53%) of **67** as a light yellow solid.

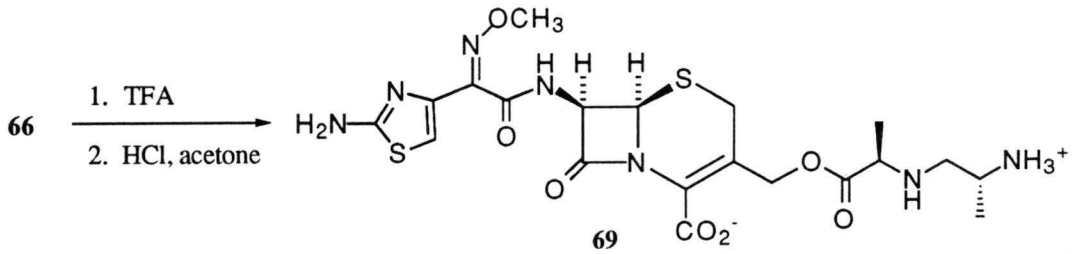
R_f = 0.34 (1:1 hexanes:EtOAc); mp 103°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.19 (3H, d, J = 6.9 Hz); 1.26 (3H, d, J = 6.9 Hz); 1.43 (9H, s); 1.53 (9H, s); 3.16 (1H, m); 3.33 (1H, d, J = 18.3 Hz); 3.53 (1H, d, J = 18.3 Hz); 4.07 (3H, s); 4.2 (1H, m); 4.42 (1H, d, J = 9.0 Hz); 4.84 (1H, d, J=13.5 Hz); 5.05 (1H, d, J = 4.8 Hz); 5.11 (1H, d, J = 13.2 Hz); 5.5-5.7 (2H, m); 5.93 (1H, dd, J = 5.1, 9.0 Hz); 6.73 (1H, s); 6.7-6.9 (1H, m); 7.02 (1H, s); 7.35 (15H, s). IR (NaCl, film) 3394, 3293, 2978, 2357, 1790, 1770, 1682, 1557, 1316. $[\alpha]_D^{26}$ = +27° (*c* 0.59, CH_2Cl_2). HRMS (FAB+) m/e 937.3657 ($C_{49}H_{57}N_6O_9S_2$ requires 937.3628).



Cephalosporin 68.

To a stirred solution of **55** (52.0 mg, 0.0633 mmol, 1.0 eq) in CH_2Cl_2 (500 μL) was added a solution of **48** (19.2 mg, 0.0634 mmol, 1.0 eq) in saturated $NaHCO_3$ (500 μL) followed by methyltributylammonium chloride (40 μL, 0.127 mmol, 2.0 eq). The mixture was stirred vigorously for 4 hours followed by addition of CH_2Cl_2 (20 mL) and water (10 mL). The organic phase was separated, dried over $MgSO_4$, filtered, and purified on preparatory TLC (1:1 hexanes:EtOAc) to afford 33.3 mg (53%) of **68** as a light yellow solid.

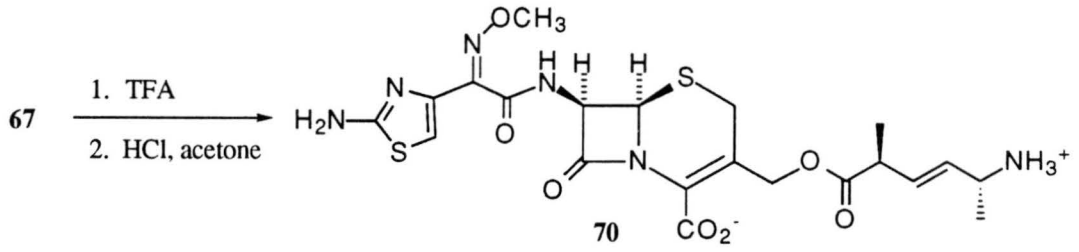
R_f = 0.29 (1:1 hexanes:EtOAc); mp 73°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.07-1.12 (3H, m); 1.17-1.22 (3H, m); 1.44 (9H, s); 1.54 (9H, s); 2.05 (3H, s); 2.45-2.54 (2H, m); 3.34-3.62 (3H, m); 3.78-3.90 (1H, m); 4.08 (3H, s); 4.8-5.2 (5H, m); 5.94 (1H, m); 6.74 (2H, m); 7.01 (1H, s); 7.31 (15H, s). IR (NaCl, film) 3310, 3057, 2976, 2939, 1784, 1733, 1523, 1450, 1370, 1305, 1239, 1167, 1109, 1043. $[\alpha]_D^{26}$ = +45° (*c* 0.27, CH_2Cl_2). HRMS (FAB+) m/e 997.3855 ($C_{51}H_{61}N_6O_{11}S_2$ requires 997.3840).



Cephalosporin 69.

To a solution of **66** (14.3 mg, 0.0152 mmol, 1.0 eq) in CH_2Cl_2 (1 mL) was added anisole (35 μL, 0.321 mmol, 21 eq) followed by TFA (140 μL, 1.82 mmol, 120 eq). The reaction was stirred overnight after which additional TFA (100 μL) was added. After reacting a total of 18 hours, the solvent was removed under reduced pressure. The residue was taken up in acetone (1 mL), cooled to 0°C, and 1 M HCl (1 mL) was added. The reaction was stirred for 45 minutes and evaporated. The residue was taken up in Et_2O and stirred for 1 hour. The ether was decanted affording 7.5 mg (70%, crude) of **69** as a light yellow solid.

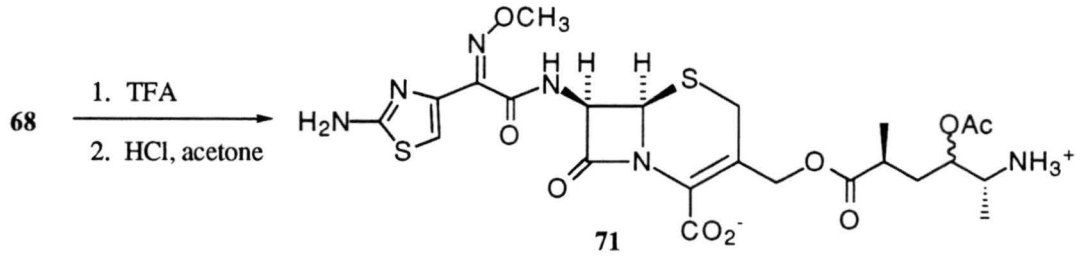
R_f = 0.21 (9:1 acetone:water); mp 200°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.31 (3H, d, J = 6.3 Hz); 1.69 (3H, d, J = 6.9 Hz); 3.3-4.1 (6H, m); 4.07 (3H, s); 5.1-5.4 (3H, m); 5.83 (1H, d, J = 4.8 Hz); 7.15 (1H, s). IR (KBr) 3430, 1778, 1714, 1682, 1652, 1634, 1557, 1557, 1040. Mass spectrum (FAB+) m/e 542.0 (M+H).



Cephalosporin 70.

To a solution of **67** (21.4 mg, 0.0228 mmol, 1.0 eq) in CH_2Cl_2 (1 mL) was added anisole (50 μL, 0.459 mmol, 20 eq) followed by TFA (210 μL, 2.73 mmol, 120 eq). The reaction was stirred overnight after which additional TFA (100 μL) was added. After reacting a total of 15 hours, the solvent was removed under reduced pressure. The residue was taken up in acetone (1 mL), cooled to 0°C, and 1 M HCl (1 mL) was added. The reaction was stirred for 45 minutes and evaporated. The residue was taken up in Et_2O and stirred for 1 hour. The ether was decanted and the residue purifed on preparatory TLC (9:1 acetone H2O) affording 6.7 mg (51%) of **70** as a light yellow solid.

R_f = 0.23 (9:1 acetone:water); mp 176°C (dec.); ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.18 (3H, d, J = 7.2 Hz); 1.37 (3H, d, J = 6.9 Hz); 3.35 (1H, m); 3.41 (1H, d, J = 18.0 Hz); 3.72 (1H, d, J = 18.0 Hz); 3.95-4.05 (1H, m); 4.01 (3H, s); 5.01 (1H, d, J = 12.3 Hz); 5.23 (1H, d, J = 4.5 Hz); 5.7 (1H, dd, J = 6.9, 15.9 Hz); 5.84 (1H, d, 4.3 Hz); 5.97 (1H, dd, J = 7.7, 15.6 Hz); 7.04 (1H, s). HRMS (FAB+) m/e 539.1390 ($C_{21}H_{27}N_6O_7S_2$ requires 539.1383).



Cephalosporin 71.

To a solution of **68** (17.0 mg, 0.0170 mmol, 1.0 eq) in CH_2Cl_2 (1 mL) was added anisole (40 μL, 0.367 mmol, 22 eq) followed by TFA (140 μL, 1.81 mmol, 107 eq). The reaction was stirred overnight after which additional TFA (100 μL) was added. After reacting a total of 18 hours, the solvent was removed under reduced pressure. The residue was taken up in acetone (1 mL), cooled to 0°C, and 1 M HCl (1 mL) was added. The reaction was stirred for 45 minutes and evaporated. The residue was taken up in Et_2O and stirred for 1 hour. The ether was decanted and the residue purifed on preparatory TLC (9:1 acetone H2O) affording 3.9 mg (38%) of **71** as a light yellow solid.

R_f = 0.28 (9:1 acetone:water); mp 123°C (dec.); ^1H NMR (300 MHz) ($CDCl_3$) δ TMS : 1.16-1.23 (6H, m); 1.29-1.34 (6H, m); 2.14 (3h, s); 2.11 (3H, s); 2.47-2.53 (2H, m); 2.55-2.72 (1H, m); 3.49 (1H, d, 18.0 Hz); 3.52-3.65 (1H, m); 3.78 (1H, d, 18.0 Hz); 4.07 (3H, s); 5.05 (1H, d, J = 12.6 Hz); 5.09 (1H, d, J = 12.6 Hz); 5.25 (1H, d, J = 4.2 Hz); 5.27 (1H, d, J = 4.2 Hz); 5.83 (1H, d, J = 4.8 Hz); 7.15 (1H, s). HRMS (FAB+) m/e 599.1603 ($C_{23}H_{31}N_6O_9S_2$ requires 599.1594).

References

1. *D-Alanyl-D-alanine Ligases and the Molecular Mechanism of Vancomycin Resistance,* Wright, G.D.; Walsh, C.T., *Acc. Chem. Res.* (1992) **25**, 468.
2. *Vancomycin-Resistant Enterococci,* Uttley, A.H.; Collins, C.H.; Naidoo, J.; George, R.C., *The Lancet* (1988) **1**, 57.
3. *Plasmid-Mediated Resistance to Vancomycin and Teicoplanin in Enterococcus faecium,* Leclercq, R.; Derlot, E.; Duval, J.; Courvalin, P., *New Engl. J. Med.* (1988) **319**, 157.
4. *Bacterial Resistance to vancomycin: five genes and one missing hydrogen bond tell the story,* Walsh, C.T.; Fisher, S.L.; Park, I-S; Prahalad, M.; Wu, Z., *Chem. and Biol.* (1996) **3**, 21.
5. *Glycopeptide resistance in enterococci,* Arthur, M.; Reynolds, P.; Courvalin, P., *Trends in Microbiology* (1996) **4**, 401.
6. *Genetics and Mechanisms of Glycopeptide Resistance in Enterococci,* Arthur, M.; Courvalin, P., *Antimicrob. Agents Chemother.* (1993) **37**, 1563.
7. *Transferable Vancomycin and Teicoplanin Resistance in Enterococcus faecium,* Leclercq, R.; Derlot, E.; Weber, M.; Duval, J.; Courvalin, P., *Antimicrob. Agents Chemother.* (1989) **33**, 10.
8. *The VANA glycopeptide resistance protein is related to D-alanyl-D-alanine ligase cell wall biosynthesis enzymes,* Dutka-Malen, S.; Molinas, C.; Arthur, M.; Courvalin, P., *Mol. Gen. Genet.* (1990) **224**, 364.

9. *Identification of Vancomycin Resistance Protein VanA as a D-Alanine:D-Alanine Ligase of Altered Substrate Specificity,* Bugg, T.; Dutka-Malen, S.; Arthur, M.; Courvalin, P.; Walsh, C.T., *Biochemistry* (1991) **30**, 2017.

10. *Structural relationship between the vancomycin resistance protein VanH and 2-hydroxycarboxylic acid dehydrogenases,* Arthur, M.; Molinas, C.; Dutka-Malen, S.; Courvalin, P., *Gene* (1991) **103**, 133.

11. *Molecular Basis for Vancomycin Resistance in Enterococcus faecium BM4147: Biosynthesis of a Depsipeptide Peptidoglycan Precursor by Vancomycin Resistance Proteins VanH and VanA,* Bugg, T.; Wright, G.D.; Dutka-Malen, S.; Arthur, M.; Courvalin, P.; Walsh, C.T., *Biochemistry* (1991) **30**, 10408.

12. *Evidence for In Vivo Incorporation of D-Lactate into Peptidoglycan Precursors of Vancomycin-Resistant Enterococci,* Arthur, M.; Molinas, C.; Bugg, T.; Wright, G.D.; Walsh, C.T.; Courvalin, P., *Antimicrob. Agents Chemother.* (1992) **36**, 867.

13. *The VanS-VanR Two-Component Regulatory System Controls Synthesis of Depsipeptide Precursors in Enterococcus faecium BM4147,* Arthur, M.; Molinas, C.; Courvalin, P., *J. Bacteriol.* (1992) **174**, 2582.

14. *Induction of vancomycin resistance in Enterococcus faecium by non-glycopeptide antibiotics,* Allen, N.E.; Hobbs Jr., J.N., *FEMS Microbiol. Lett.* (1995) **132**, 107.

15. *Induction Signals for Vancomycin Resistance Encoded by the vanA Gene Cluster in Enterococcus faecium,* Lai, M.H.; Kirsch, D.R., *Antimicrob. Agents Chemother.* (1996) **40**, 1645.

16. *Characterization of VanY, a DD-Carboxypeptidase from Vancomycin-Resistant Enterococcus faecium BM4147,* Wright, G.D.; Molinas, C.; Arthur, M.; Courvalin, P.; Walsh, C.T., *Antimicrob. Agents Chemother.* (1992) **36**, 1514.

17. *Contribution of VanY D,D-Carboxypeptidase to Glycopeptide Resistance in Enterocccus faecalis by Hydrolysis of Peptidoglycan Precursors,* Arthur, M.; Depardieu, F.; Snaith, H.A.; Reynolds, P.E.; Courvalin, P., *Antimicrob. Agents Chemother.* (1994) **38**, 1899.

18. *Glycopeptide resistance mediated by enterococcal transposon Tn1546 requires production of VanX for hydrolysis of D-alanyl-D-alanine,* Reynolds, P.E.; Depardieu, F.; Dutka-Malen, S.; Arthur, M.; Courvalin, P., *Mol. Microbiol.* (1994) **13,** 1065.

19. *Overexpression, Purification, and Characterization of VanX, a D-,D-Dipeptidase which Is Essential for Vancomycin Resistance in Enterococcus faecium BM4147,* Wu, Z.; Wright, G.D.; Walsh, C.T., *Biochemistry* (1995) **34**, 2455.

20. *Characterization of Tn1546, a Tn3-Related Transposon Conferring Glycopeptide Resistance by Synthesis of Depsipeptide Precursors in Enterococcus faecium BM4147,* Arthur, M.; Molinas, C.; Depardieu, F.; Courvalin, P., *J. Bacteriol.* (1993) **175**, 117.

21. *The vanZ gene of Tn1546 from Enterococcus faecium BM4147 confers resistance to teicoplanin,* Arthur, M.; Depardieu, F.; Molinas, C.; Reynolds, P.; Courvalin, P., *Gene* (1995) **154**, 87.

22. *Enzymes in the D-Alanine Branch of Bacterial Cell Wall Peptidoglycan Assembly,* Walsh, C.T., *J. Biol. Chem.* (1989) **264**, 2393.

23. *Inhibition of Cell Wall Biosynthesis by Analogs of Alanine,* Neuhaus, F.C.; Hammes, W.P., *Pharmacol. Ther.* (1981) **14**, 265.

24. *ATP-Dependent Activation and Slow Binding Inhibition of Salmonella typhimurium D-Alanine:D-Alanine Ligase (ADP) by (Aminoalkyl)phosphinate and Aminophosphonate Analogues of D-Alanine,* Duncan, K.; Walsh, C.T., *Biochemistry* (1988) **27**, 3709.

25. *Phosphinic Acid Inhibitors of D-Alanyl-D-alanine Ligase,* Parsons, W.H.; *et al.*, *J. Med. Chem.* (1988) **31**, 1772.

26. *Phosphinate analogs of D-,D-dipeptides: Slow-binding inhibition and proteolysis protection of VanX, a D-,D-dipeptidase required for vancomycin resistance in Enterococcus faecium,* Wu, Z.; Walsh, C.T., *Proc. Natl. Acad. Sci.* (1995) **92**, 11603.

27. *Synthesis and evaluation of inhibitors of bacterial D-alanaine:D-alanine ligases,* Ellsworth, B.A.; Tom, N.J.; Bartlett, P.A., *Chem. and Biol.* (1996) **3**, 37.

28. *Bioisosterism: A Rational Approach to Drug Design,* Patani, G.A.; LaVoie, E.J., *Chem. Rev.* (1996) **96**, 3147.

29. *Design and Synthesis of Biologically Active Peptide Mimics,* Williams, R.M. in *Biologically Active Peptides: Design, Synthesis, and Utilization* Williams, W.V.; Weiner, D.B., eds., Technomic Publishing Co., Inc.: Lancaster, 1993; pp 187-215; and references cited therein.

30. *Pepstatin, a New Pepsin Inhibitor Produced by Actinomycetes,* Umezawa, H.; Aoyagi, T.; Morishima, H.; Matsuzaki, M.; Hamada, M.; Takeuchi, T., *J. Antibiotics* (1970) **23**, 259.

31. *Effects of Combinations of β-Lactams, Daptomycin, Gentamycin, and Glycopeptides against Glycopeptide-Resistant Enterococci,* Leclercq, R.; Bingen, E.; Su, Q.H.; Lambert-Zechovski, N.; Courvalin, P.; Duval, J., *Antimicrob. Agents Chemother.* (1991) **35**, 92.

32. *Determination of the Chiral Purity of Dipeptide Isosteres Containing a Reduced Peptide Bond by Gas Chromatographic Analysis,* De Bondt, M.; Couder, J.; Van Der Auwera, L.; Van Marsenille, M.; Elseviers, M.; Delaet, N.; Laus, G.; Tourwe, D.; Van Binst, G., *J. Chromatography* (1988) **442**, 165.

33. *Herstellung von α-Aminosäureestern durch Alkoholyse der Methylester,* Brenner, M.; Huber, W., *Helv. Chim. Acta* (1953) **36**, 1109.

34. *Preparation of Protected Amino Aldehydes,* Stanfield, C.F.; Parker, J.E.; Kanellis, P., *J. Org. Chem* (1981) **46**, 4797.

35. *On the Double Bond Isostere of the Peptide Bond: Preparation of Modified Di- and Tri-peptides incorporating Proline and Alanine Analogues,* Miles, N.J.; Sammes, P.G.; Kennewell, P.D.; Westwood, R., *J. Chem. Soc. Perkin Trans. I* (1985) 2299.

36. *An Efficient Synthesis of Optically Active α-(t-Butoxycarbonylamino)-aldehydes from α-Amino Acids,* Fehrentz, J-A; Castro, B., *Synthesis* (1983) 676.

37. *N-Methoxy-N-methylamides as Effective Acylating Agents,* Nahm, S.; Weinreb, S.M., *Tet. Lett.* (1981) **22**, 3815.

38. *S_N2'-Reactions of Peptide Aziridines. A Cuprate-Based Approach to (E)-Alkene Isosteres,* Wipf, P.; Fritch, P.C., *J. Org. Chem.* (1994) **59**, 4875.

39. *Sulfur Trioxide in the Oxidation of Alcohols by Dimethyl Sulfoxide,* Parikh, J.R.; von E. Doering, W.J., *J. Am. Chem. Soc.* (1967) **89**, 5505.

40. *Cleavage of Protecting Groups with Boron Tribromide,* Felix, A.M., *J. Org. Chem.* (1974) **39**, 1427.

41. *The Intramolecular Nitrile Oxide Cycloaddition (INOC) Route to the Ergot Alkaloids: Use of the Isooxazoline to γ-Amino Alcohol Conversion in the Total Synthesis of (+)-Paliclavine,* Kozikowski, A.P.; Chen, Y-Y; Wang, B.C., Xy, Z-B, *Tetrahedron* (1984) **40**, 2345.

42. *Synthesis of E- and Z-alkene Dipeptide Isosteres,* Bohnstedt, A.C.; Vara Prasad, J.V.N.; Rich, D.H., *Tet. Lett.* (1993) **34**, 5217.

43. *Inhibition of Aminopeptidases by Peptides Containing Ketomethylene and Hydroxyethylene Amide Bond Replacements,* Harbeson, S.L.; Rich, D.H., *J. Med. Chem.* (1989) **32**, 1378.

44. *Nouvelle Methode De Benzylation D'Hydroxyles Glucidiques Encombres,* Czernecki, S.; Georgoulis, C.; Provelenghiou, C., *Tet. Lett.* (1976) **17**, 3535.

45. *Synthesis of the Monofluoro Ketone Peptide Isostere,* Garrett, G.S.; Emge, T.J.; Lee, S.C.; Fischer, E.M.; Dyehouse, K.; McIver, J.M., *J. Org. Chem.* (1991) **56**, 4823.

46. *β-Lactams Antibiotics in Clinical Medicine*, Kammer, R.B. in *Chemistry and Biology of β-Lactams Antibiotics Vol. 3*, Morin, R.B. and Gorman, M., ed., Academic Press, Inc.: New York, 1982; pp 291-296.

47. *Cephalosporinase and Penicillinase Activities of a β-Lactamase from Pseudomonas pyocyanea,* Sabath, L.D.; Jago, M.; Abraham, E.P., *Biochem. J.* (1965) **96**, 739.

48. *A New Cephalosporin with a Dual Mode of Action,* O'Callaghan, C.H.; Sykes, R.B.; Staniforth, S.E., *Antimicrob. Agents Chemother.* (1976) **10**, 245.

49. *Conscripting β-Lactamase for Use in Drug Delivery. Synthesis and Biological Activity of a Cephalosporin C-10-Ester of an Antibiotic Dipeptide,* Mobashery, S.; Lerner, S.A.; Johnston, M., *J. Am. Chem. Soc.* (1986) **108**, 1685.

50. *Reactions of Escherichia coli TEM β-Lactamase with Cephalothin and with C-10-Dipeptidyl Cephalosporin Esters,* Mobashery, S.; Johnston, M., *J. Biol. Chem.* (1986) **261**, 7879.

51. *Inactivation of Alanine Racemase by β-Chloro-L-alanine Released Enzymatically from Amino Acid and Peptide C-10-Esters of Deacetylcephalothin,* Mobashery, S.; Johnston, M., *Biochemistry* (1987) **26**, 5878.

52. *Chloroalanyl and Propargylglycyl Dipeptides. Suicide Substrate Containing Antibacterials,* Cheung, K-S; Wasserman, S.A.; Dudek, E.; Lerner, S.A.; Johnston, M., *J. Med. Chem.* (1983) **26**, 1733.

53. *Carbapenems: Special Properties Contributing to Their Activity,* Neu, H.C., *Am. J. Med.* (1985) **78** (suppl 6A), 33.

54. *Cephalosporin 3'-Quinolone Esters with a Dual Mode of Action,* Albrecht, H.A., *et al., J. Med. Chem.* (1990) **33**, 77.

55. *Mechanism-based Dual-action Cephalosporins,* Keith, D.D., *et al.* in *Recent Advances in the Chemistry of Anti-infective Agents,* Bentley, P.H.; Ponsford, R., eds., The Royal Society of Chmeistry: Cambridge, 1993; pp 79-92.

56. *Effects of Quinolones on Nucleoid Segregation in Escherichia coli,* Georgpapadakou, N.H.; Bertasso, A., *Antimicrob. Agents Chemother.* (1991) **35** 2645.

57. *Dual-Action Cephalosporins Incorporating a 3'-Tertiary-Amine-Linked Quinoline,* Albrecht, H.A.; *et al., J. Med Chem.* (1994) **37**, 400.

58. *A Facile and General Synthesis of 3-(Acyloxy)cephalosporins,* Wei, C.C; Barktkovitz; West, K.F., *J. Org. Chem.* (1992) **57**, 4027.

59. *The Mechanisms of Reactions of β-Lactam Antibiotics,* Page, M.I., *Acc. Chem. Res.* (1984) **17**, 144.

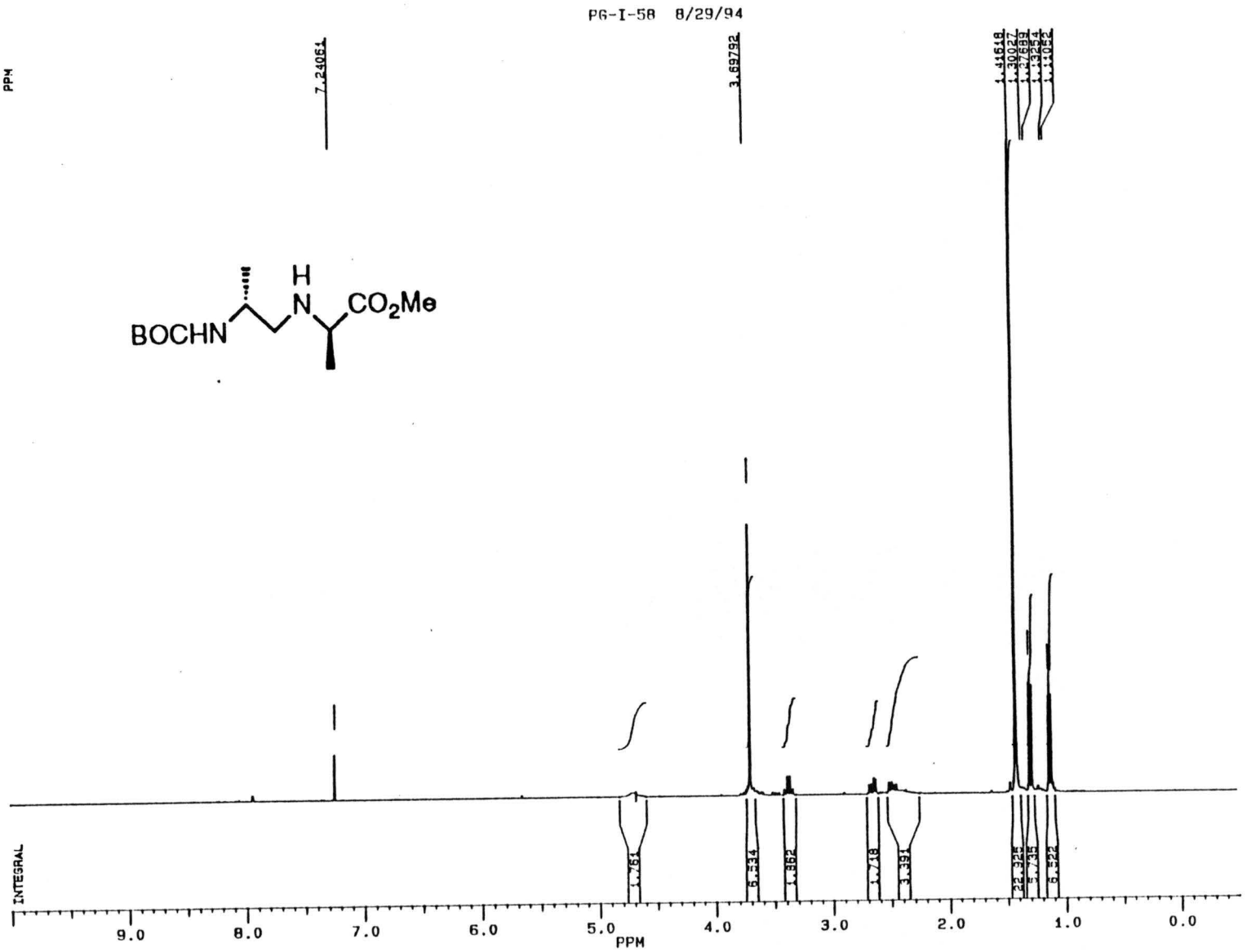
60. *Preparation of Ceph-3-em Esters Unaccompanied by Δ^3 ---> Δ^2 Isomerization of the Cephalosporin,* Mobashery, S.; Johnston, M., *J. Org. Chem.* (1986) **51**, 4723.

61. *Esterification of Amino Acids and Dipeptides under Mild Conditions; Part I: via Phase Transfer Catalysis,* Bocchi, V.; Casnati, G.; Dossena, A.; Marchelli, R., *Synthesis* (1979) 957.

62. Starks, C.M.; Liotta, C.L.; and Halpern, M., *Phase-Transfer Catalysis: Fundamentals, Applications, and Industrial Perspectives*; Chapman & Hall: New York, 1994; pp 27, 270, 289.

63. *A Convenient, High-yielding Method for the Methylenation of Catechols,* Bashall, A.P.; Collins, J.F., *Tet. Lett.* (1975) **16**, 3489.

64. *Synthesis of a Dual-Action Cephalosporin: A Novel Approach to 3-Acyloxymethyl-3-cephems,* Okabe, M.; Sun, R-C, *Synthesis* (1992) 1160.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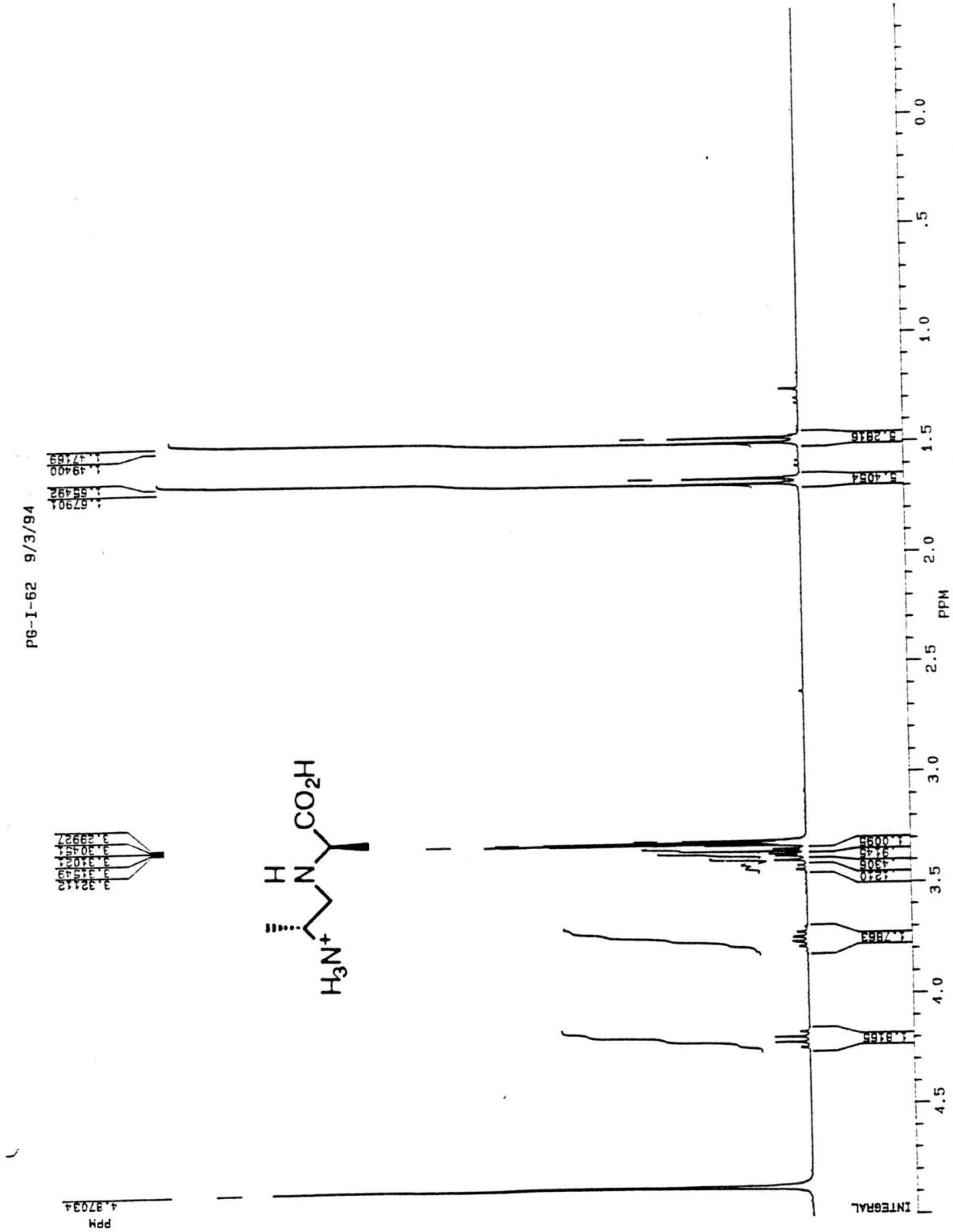
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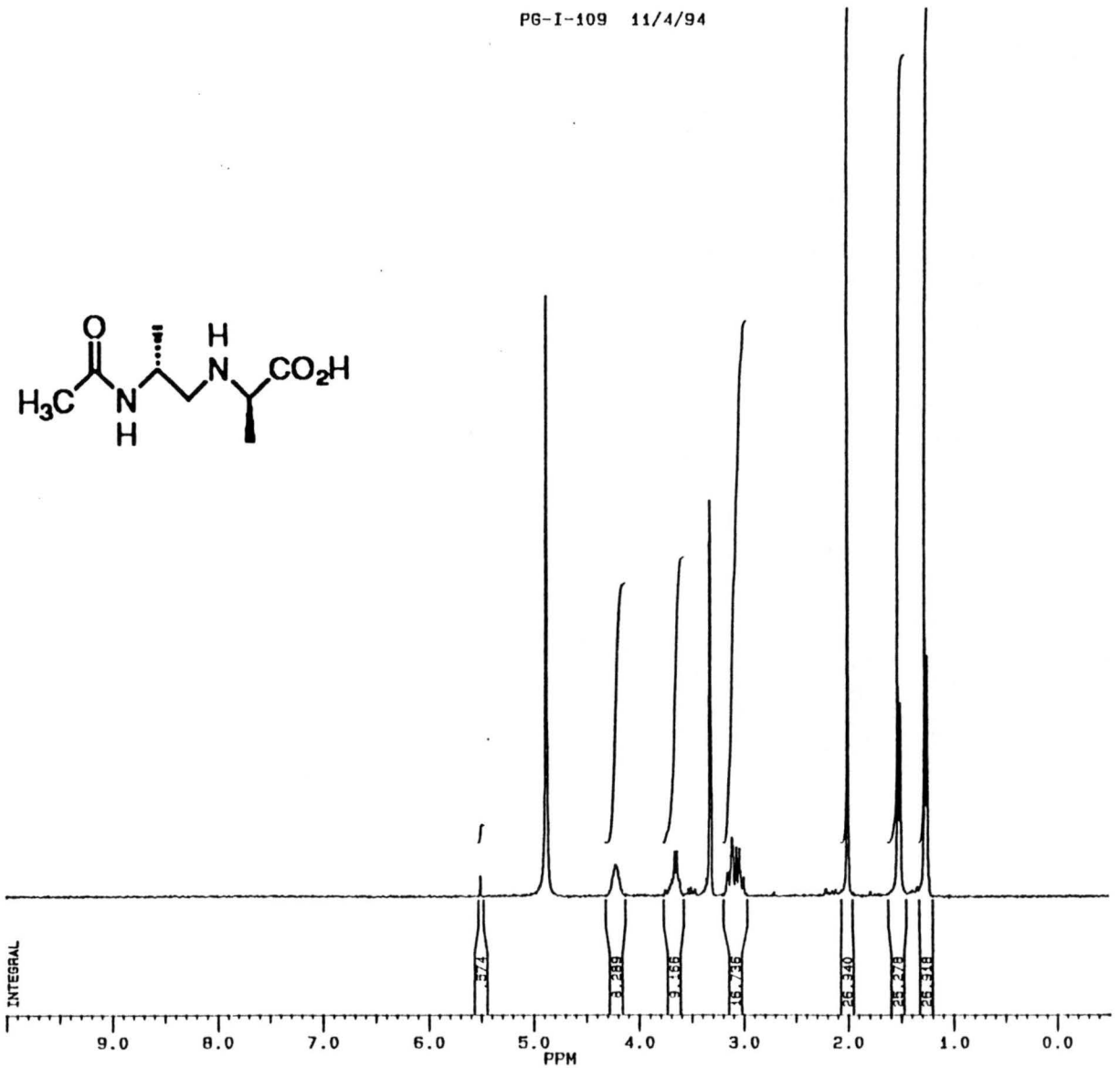

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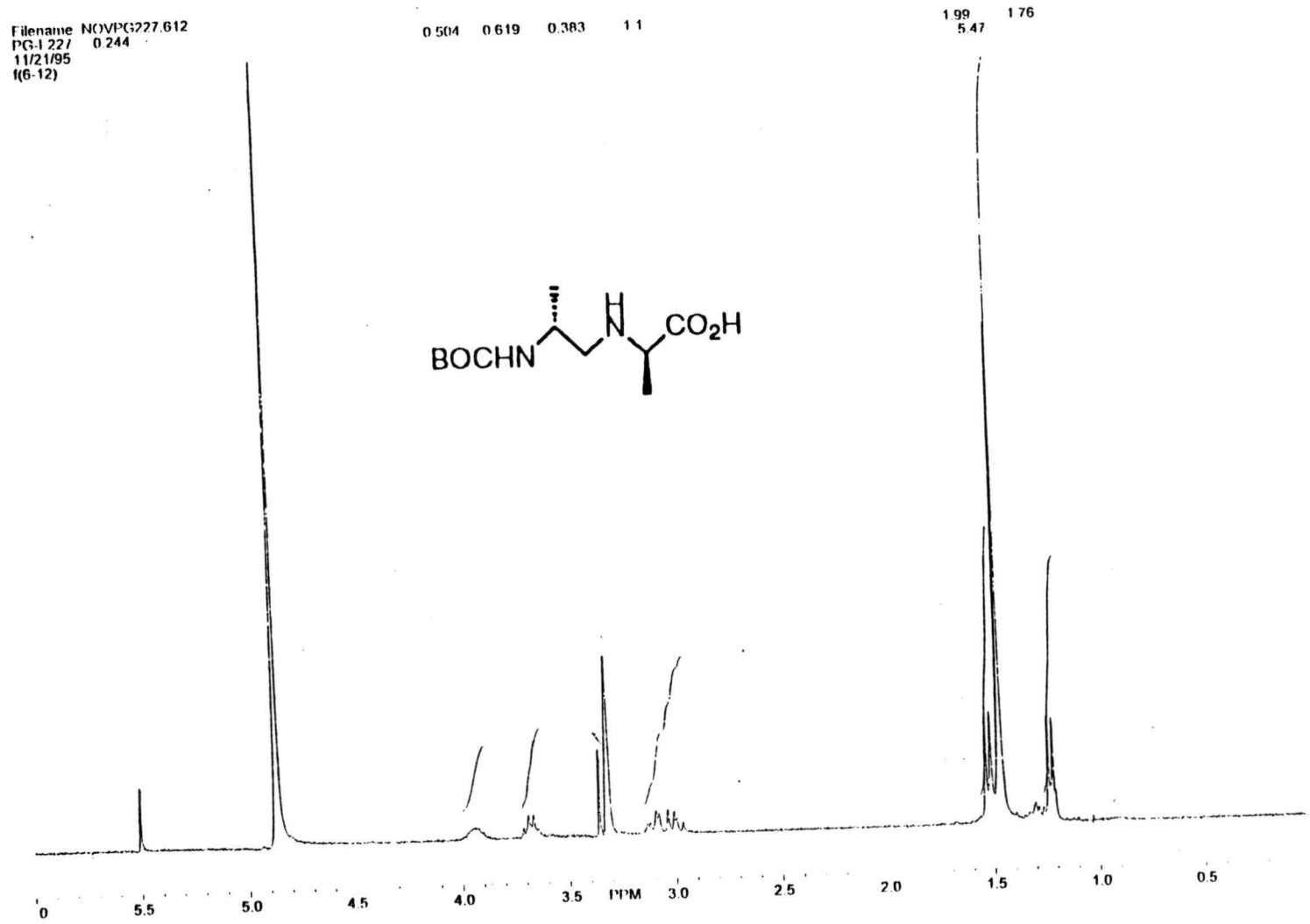
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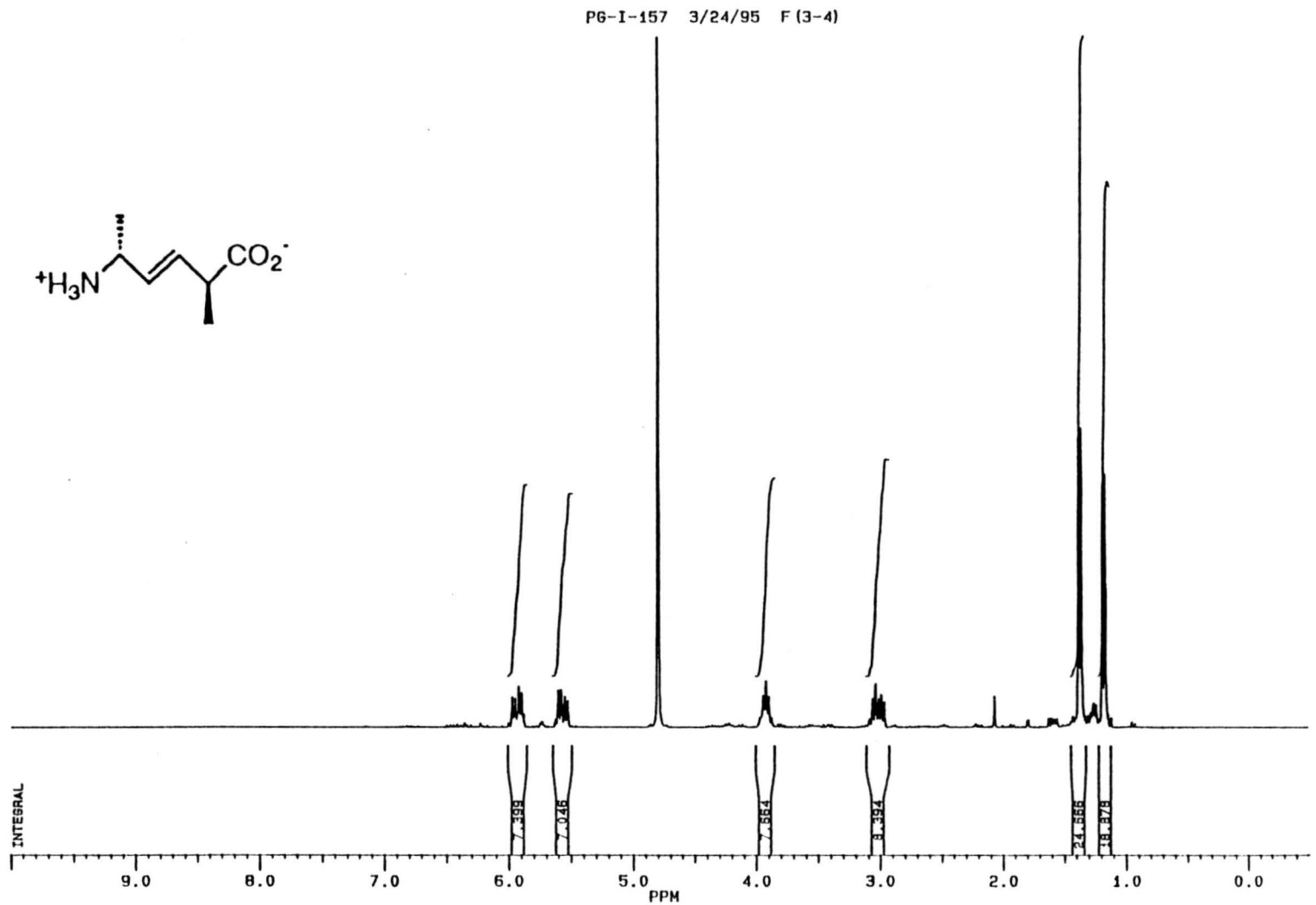
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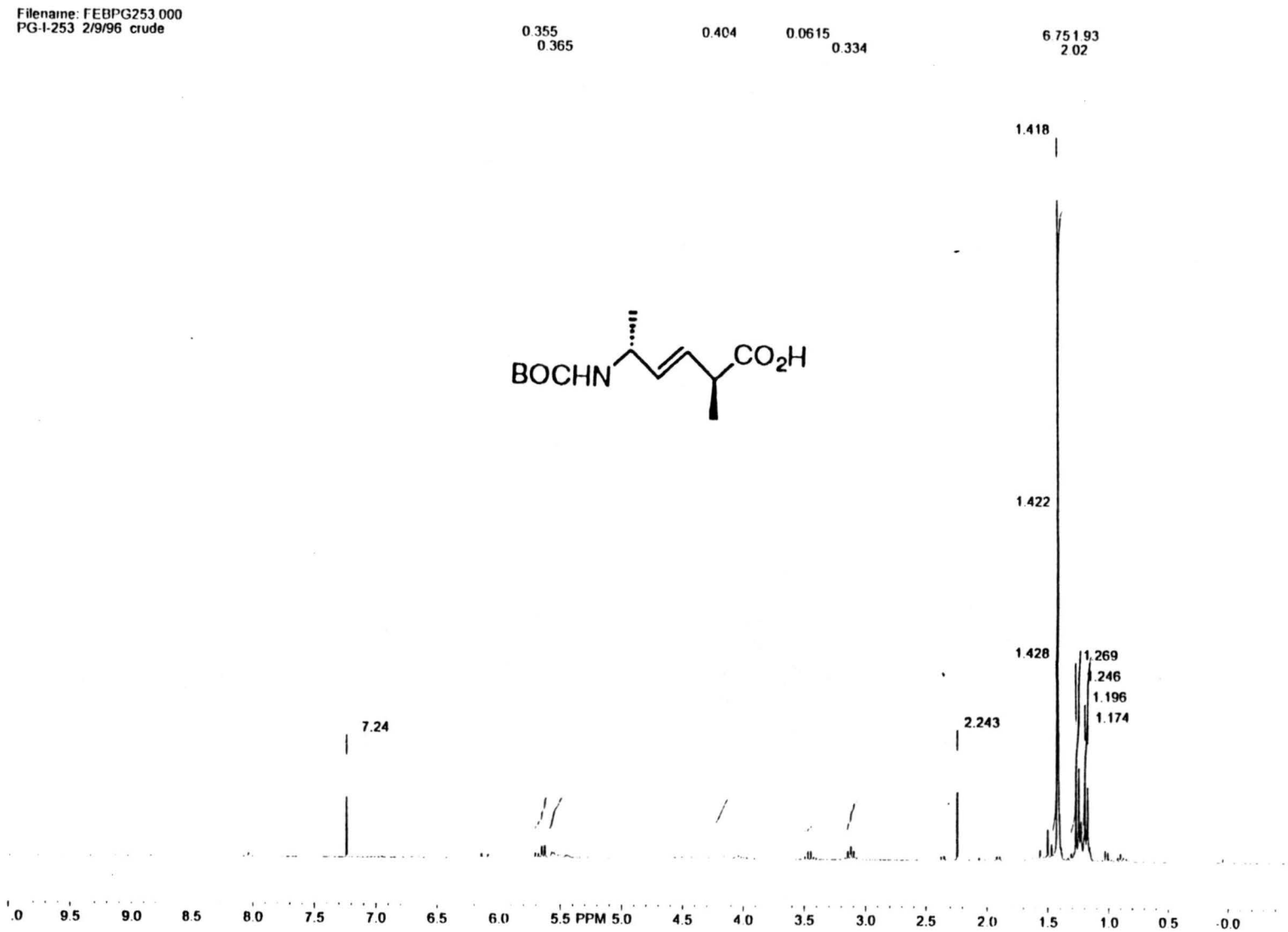
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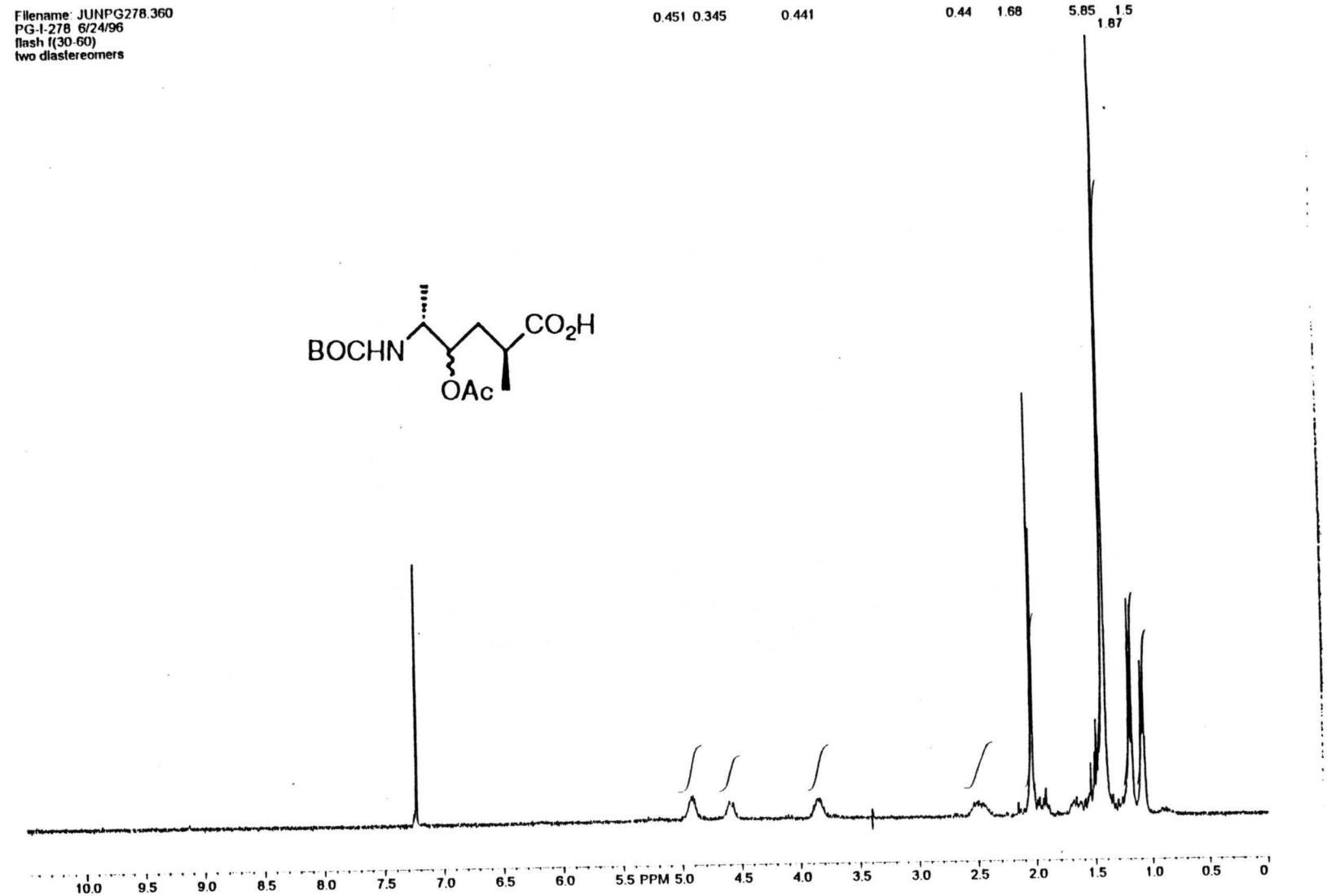
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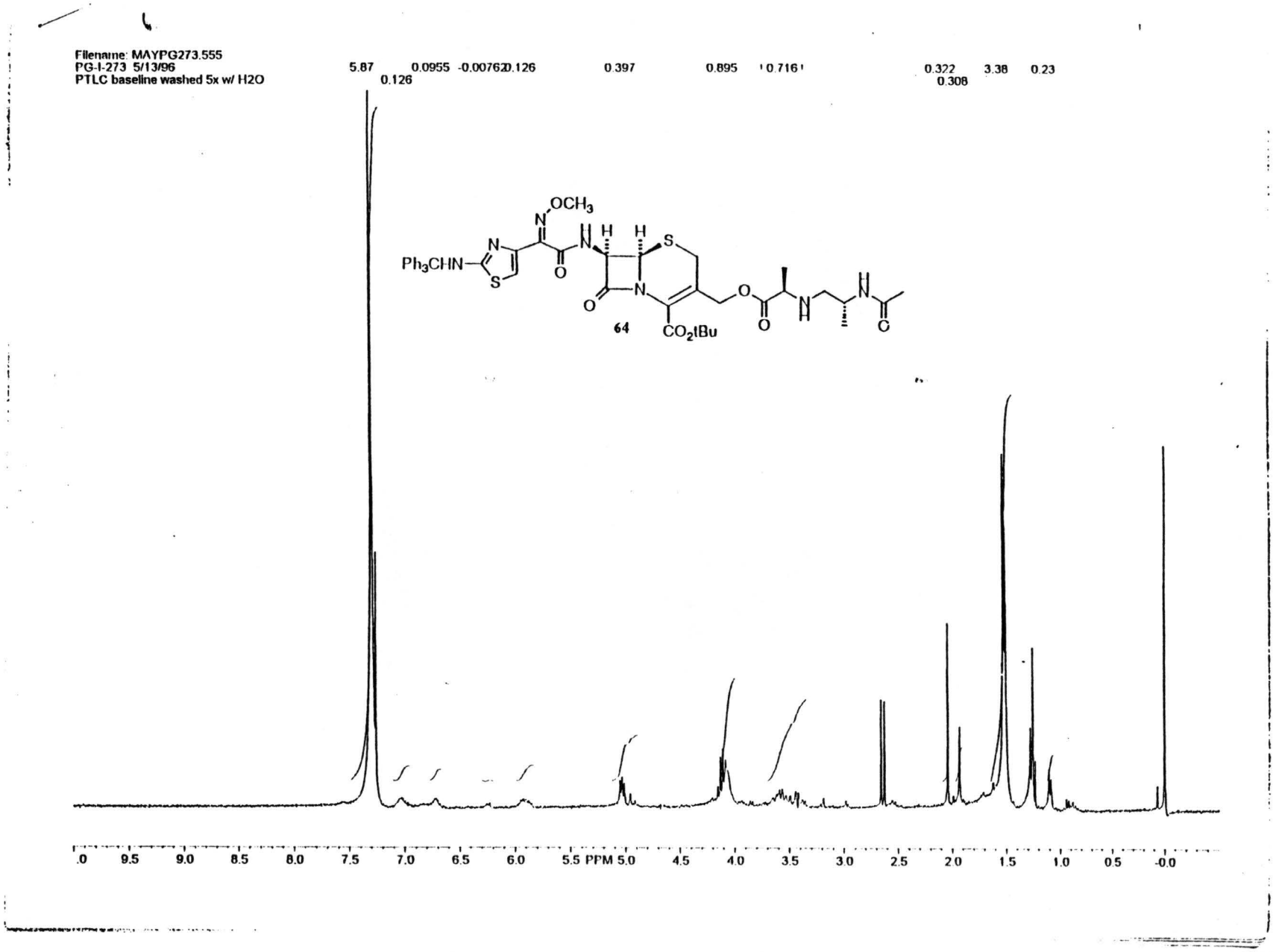
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