INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

RICE UNIVERSITY

SYNTHETIC STUDIES OF LUZOPEPTINS

by

NING XI

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE

M. A. Ciufolini
Professor of Chemistry, Chair

P. S. Engel
Professor of Chemistry

K. M. Beckingham
Professor of Biochemistry and Cell Biology

Houston, Texas

April, 1997

UMI Number: 9727630

UMI Microform 9727630
Copyright 1997, by UMI Company. All rights reserved.

This microform edition is protected against unauthorized copying under Title 17, United States Code.

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

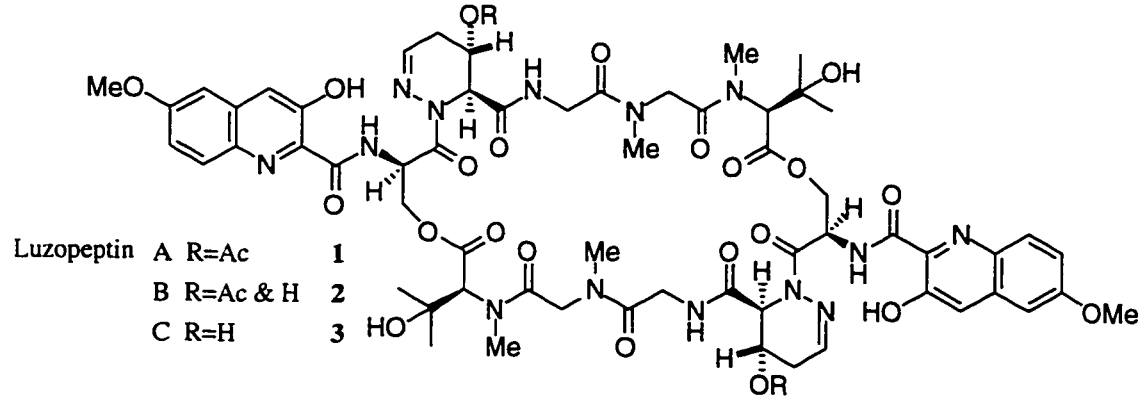
ABSTRACT

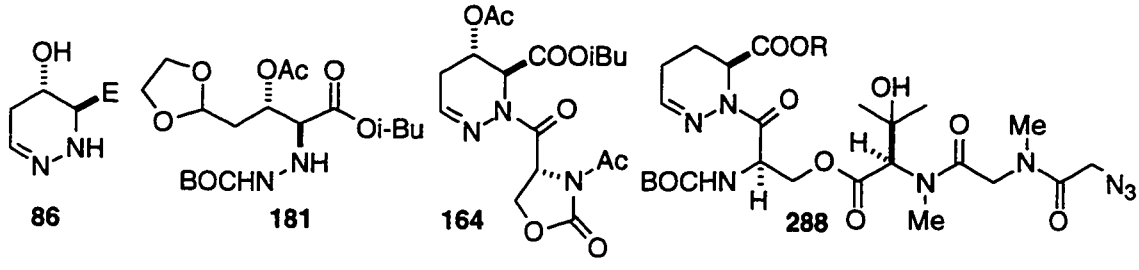
Synthetic Studies of Luzopeptins

by

Ning Xi

Luzopeptins are a series of cyclic depsipeptides which possesses potent antitumor and antiviral activities. There is no total synthesis of Luzopeptins has been achieved. In our own effort to synthesize the luzopeptins, we developed a concise and practical route to PCA, **86**, and an efficient protocol to synthesize the mono-BOC, **181**. A novel serinyl chloride was devised in conjunction with the formation of PCA-serine dipeptide derivatives, **164**. All these methodologies were successfully applied to the synthesis of monomeric derivative of luzopeptin E_2, **288**.





86 181 164 288

ACKNOWLEDGEMENTS

To my advisor, Dr. Marco Ciufolini, I would like to express my sincere gratitude. Your dedication, enthusiasm and love for chemistry are always inspirations to me.

To members of the Ciufolini group, both present and past, thanks for their friendship and creating an exciting and rewarding research environment. I am also grateful to Dr. Terry Marriot for teaching me how to use Mass Spectroscopy.

I would like to express my special thanks to my family for their support and sacrifice. Most of of all to my wife, Yijia Jiang, for her love, understanding and support.

Finally, I would like to express my appreciation to the Department of Chemistry, Rice University, the National Science Foundation, the National Institutes of Health, the Robert A. Welch Foundation and Lodieska Stockbridge Vaughan Fellowship for financial support.

TABLE OF CONTENTS

Chapter 1	Introduction	1
Chapter 2	Background	7
Chapter 3	Synthetic Studies on Luzopeptin C	19
3.1	Synthetic Strategy	19
3.2.	Synthesis and Chemistry of (±)-PCA	22
3.3.	Novel Serinyl Chlorides as Powerful Acylating Agents	34
3.4.	Synthesis of Protected PCA-Serine Dipeptides	46
3.5.	Deprotection of the Blocked PCA-Serine Dipeptide	55
Chapter 4	Synthesis of Tripeptides and Monomeric Derivatives of Luzopeptin E_2	62
4.1	Synthesis of dPCA-serine Dipeptide	62
4.2	Preparation of a Key Tripeptide Segment of Luzopeptins	66
4.3	Monomeric Precursors to the Framework of Luzopeptin E_2	79
4.4	Conclusions	82
Chapter 5	Experimental	83

Table of Abbreviations

Ac	acetate
Alloc	allyloxycarbonyl
B	base
Bn	benzyl
BOC	*tert* -butyloxycarbonyl
BOP-Cl	*N, N'* – bis (2 – keto 3 – oxazolidinyl) phosphinic chloride
DBP	2,4' – dibromophenacyl
Bz	benzoyl
Cbz	benzyloxycarbonyl
18-C-6	18-crown-6
DCC	1,3 – dicyclohexylcarbodiimide
DIBAL	diisobutylaluminum hydride
DMPU	*N, N'* – dimethylpropyleneurea
de	diastereomeric excess
DBAD	di – tert – butyl azodicarboxylate
DMAP	4 – dimethylaminopyridine
DMF	N,N – dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPA	diphenyl azodiphosphonate
ee	enantiomeric excess
Fmoc	9 – fluorenylmethyloxycarbonyl

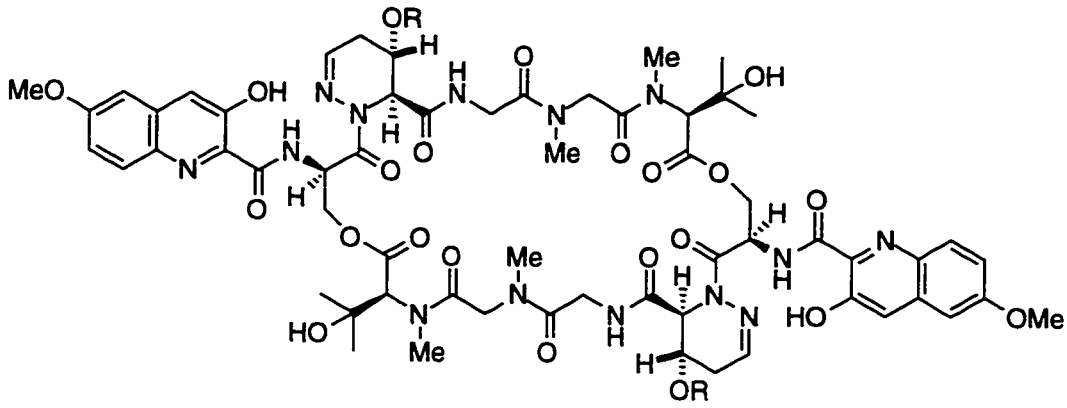
HBTU	*O* – benzotriazolyl – tetramethyl-isouronium hexafluorophosphate
HMPA	hexamethylphosphoramide
HOBt	1 – hydroxybenzotriazole
LDA	lithium diisopropylamide
Mukaiyama reagent	2 – chloro – 1 – methylpyridium iodide
NBS	N – bromosuccinimide
NMM	N – methylmorpholine
NMO	N – methylmorpholine – N – oxide
NMR	nuclear magnetic resonance
Nu	nucleophile
Ph	phenyl
Pht	Phthaloyl
PPTS	pyridinium 4 –toluenesulfonate
RNA	ribonucleic acid
SES	b - trimethylsilylethanesulfonyl
TBAF	tetrabutylammonium fluoride
TBDMS	tert – butyldimethylsilyl
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TPAP	Tetrapropylammonium perruthenate
TROC	2, 2, 2 – trichloroethoxycarbonyl
Ts	4 – toluenesulfonyl

To my parents and Yijia

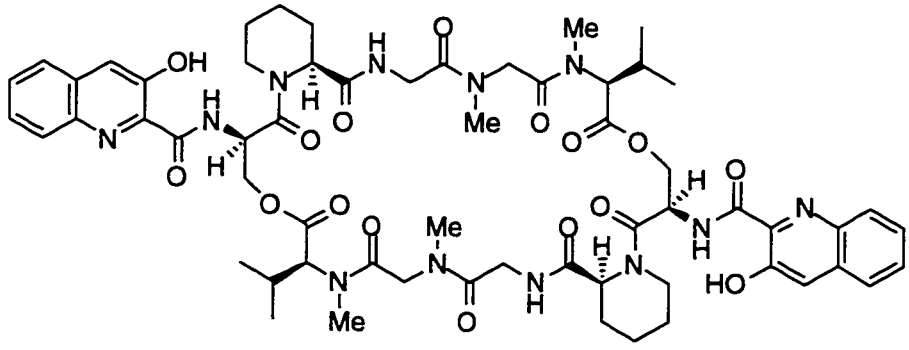
Chapter 1
Introduction

Luzopeptins, originally named BBM928, are a series of cyclic depsipeptides which possesses potent antitumor and antiviral activities. They are produced by an actinomycete strain, *Actinomadura luzonensis*, and were discovered in the mid 1970's at Bristol-Myers Japan.[1] Luzopeptins belong to a growing class of cyclic decadepsipeptides that include quinoxapeptins[2] and sandramycin[3], which we refer to as the peptin family. All members of this group display a dimeric cyclodepsipeptide scaffolding that possesses a two-fold axis of symmetry and that carries two heteroaromatic chromophoric units such as quinolines or quinoxalines (Figure 1-1).

The luzopeptin complex contains six components: three major (luzopeptins A, B and C, **1-3**) and three minor ones (luzopeptins D, E and F). The structures of luzopeptins, A, B, and C, were first characterized by extensive chemical degradation and spectroscopic studies,[4] and later were unequivocally confirmed by crystallographic analysis of luzopeptin A.[5] Two unusual amino acid components, L-N-**m**ethyl (3)-**h**ydroxy**v**aline, hereinafter referred to as **mhv**, **5**, and the unique *trans*-(3S,4S)-4-hydroxy-2,3,4,5-tetrahydro**p**yridazine-3-**c**arboxylic **a**cid ("**PCA**," **6**) were previously unknown in nature (Figure 1-2). The chromophore of luzopeptins is the novel heterocyclic compound, 3-**h**ydroxy-6-**m**ethoxy**q**uinaldic acid, henceforth referred to as **hmq**, **7**. The antibiotics differ only in the degree of acetylation of their PCA component.

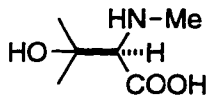


Luzopeptin A R=Ac **1**
B R=Ac & H **2**
C R=H **3**

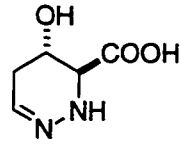


Sandramycin **4**

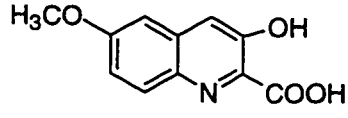
Figure 1-1



(L)-N-Methyl-3-hydroxyvaline **5**



PCA **6**

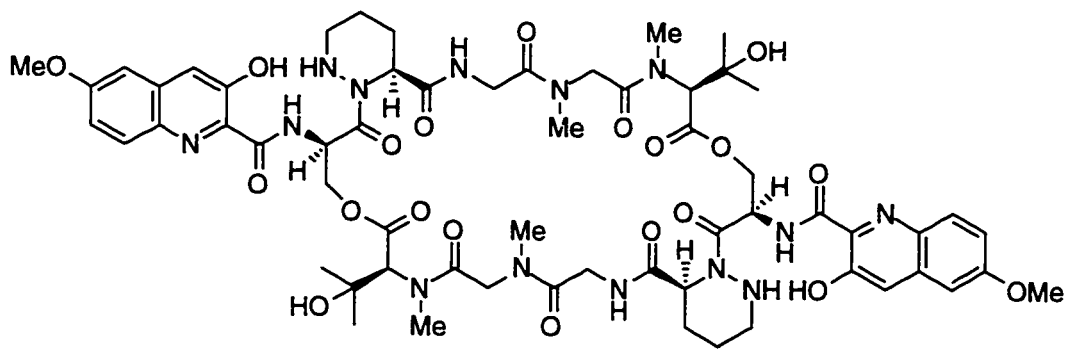


Quinoline acid **7**

Figure 1-2

The structures of luzopeptins D and F are not yet known, or at least they are

not in the public domain. The E series of these antibiotics is actually a mixture of compounds, among which luzopeptin E_2, **8**, is the only component with a secure structure[6] (Figure 1 - 3). The main difference between the E and the A, B, C series of natural products is the presence of (3S)-piperazic acid in lieu of PCA. It will be later seen that this minor difference imparts considerably greater stability to luzopeptin E relative to its PCA-containing congeners.



Luzopeptin E_2 **8**

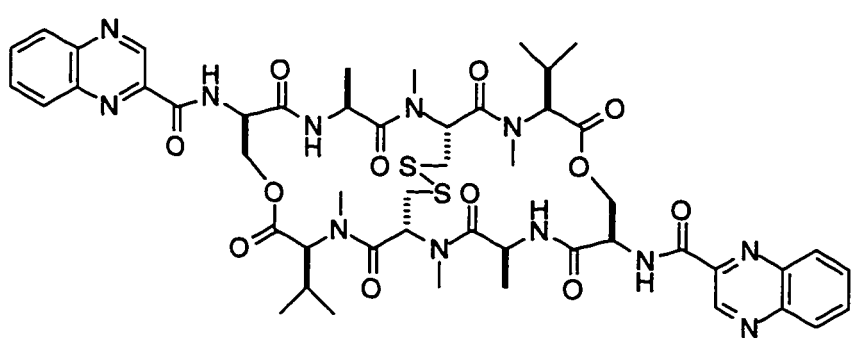
Figure 1-3

The extreme potency of luzopeptins against various tumors soon stimulated a great deal of interest, both from the biomedical and the chemical communities. Early assays revealed that the activity of luzopeptin A against P388 leukemia was approximately 3-fold greater than echinomycin and over 100-fold higher than mytomycin C, a clinically used chemotherapeutic resource. Moreover, luzopeptin A was approximately 3-times as potent as luzopeptin B, while luzopeptin C was considerably less active.[7]

Binding studies of luzopeptin A to nucleic acids were also carried out.[8] The compound was shown to intercalate bifunctionally into DNA with one binding site for every 5 or 6 base-pairs. Footprinting experiments suggest that luzopeptin binds best to regions containing alternating A and T residues. The

sequence selectivity and characteristics of luzopeptin binding are quite different from those of echinomycin, also a bifunctional intercalator that binds to GC-rich regions in DNA.

Interest in luzopeptins increased even more with the discovery by Y. Inouye and collaborators in 1987[9] that the antibiotics are powerful inhibitors of reverse transcriptase (RT), a crucial enzyme for retrovirus proliferation. More significantly, activity was especially pronounced in the weakly cytotoxic C series at doses that did not elicit adverse effects on the cell lines used for the assay. Thus, while luzopeptin A and B showed marked cytotoxicity against L5178Y cells at effective concentrations, luzopeptin C completely suppressed replication of HIV-1 in infected MT-4 cells at concentrations around 2.5 - 5.0 μg/mL without compromising cell viability. This immediately led to the speculation that luzopeptin C and related substances may be useful as experimental anti-HIV agents. Inouye's findings are particularly significant in light of the fact that luzopeptins, together with actinomycin D, are the only non-nucleotide inhibitors of RT ever discovered, raising several important questions



Triostin A **9**

Figure 1-4

about their mechanism of action. More interestingly, quinoxaline antibiotics such as triostin A, **9**, (Figure 1-4) and echinomycin[10] were inactive against RT, despite their structural similarity to luzopeptins.

In early 1996, a Merck group described the isolation of quinoxapeptin A, **10**, and B, **11**, from a culture of nocardioform actinomycete.[11] The structure of the new antibiotics is strikingly similar to that of luzopeptins, and indeed, quinoxapeptins are exceedingly potent anti-HIV agents. The two antibiotic families share the same peptide backbone. Differences are evident only at the level of the pendant chromophores and of the acyl substituent on the the PCA residues (Figure 1-5). The absolute stereochemistry of the *trans*-2-methyl-cyclopropanecaboxylic acid unit on the PCA component remains unknown to this day.

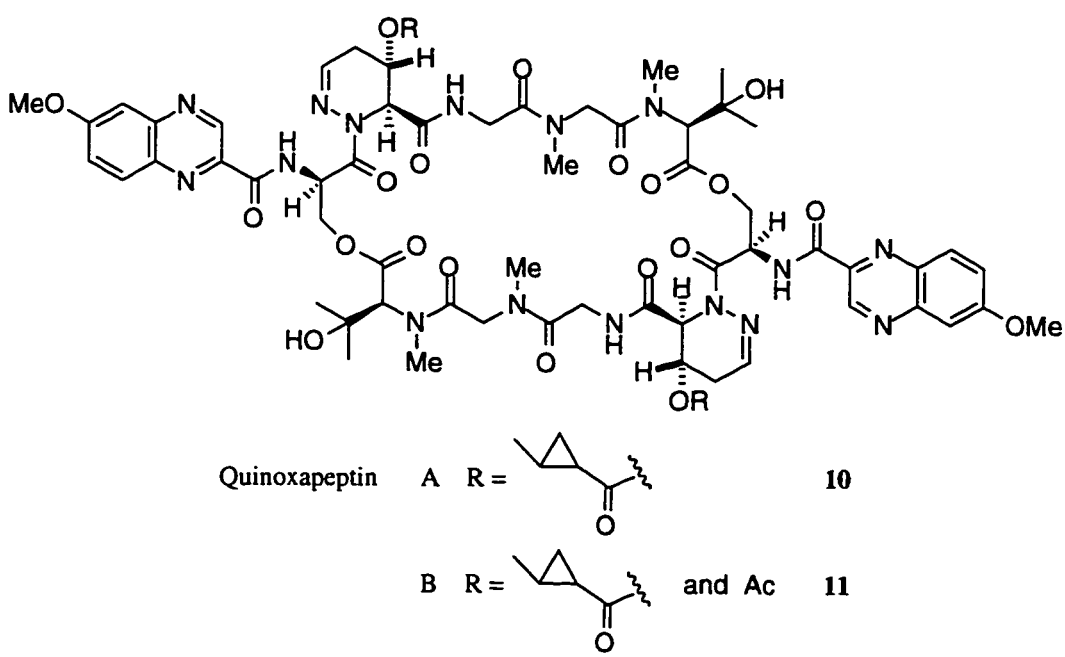


Figure 1-5

Quinoxapeptins were evaluated for inhibition of HIV-1 and HIV-2 RT and assessed for their selectivity towards RT. Compound **10** inhibited HIV-1 and HIV-2 RT with IC_{50} values (the concentration of compound that elicits 50% inhibition) of 4 and 40nM, respectively, while **11** was less potent and inhibited HIV-1 and HIV-2 RT with IC_{50} values of 10 and 100nM, respectively. These activity data are comparable to those reported for luzopeptins. Quinoxapeptin A was also very active against three mutant forms of HIV-1 RT. Tests against several mammalian DNA polymerases showed that quinoxapeptin A was an extremely poor inhibitor of these enzymes, with IC_{50} values of 2563, 615, 1798 and 494 nM against DNA polymerase a, b, g and d, respectively. This confirmed its elevated specificity for HIV-1 and HIV-2 RT, and its potential as a candidate anti-AIDS agent.

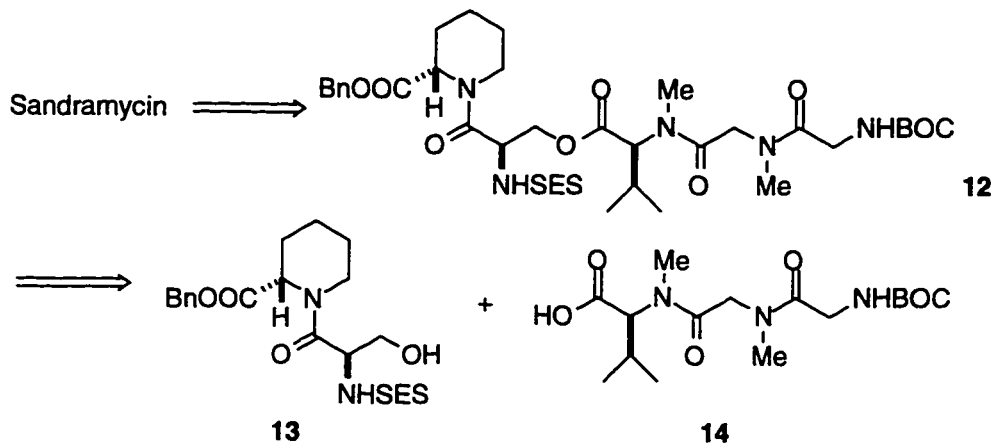
Despite the excitement elicited by the above findings, the mechanism of anti-HIV action by members of the peptin family still remains a mystery, because the extreme scarcity of luzopeptins and quinoxapeptins has hampered any further medicinal chemistry and molecular biology investigations. The success of these studies depends on the availability of synthetic peptins, a demand that may be satisfied only through the development of reliable chemical methodology. It is conceivable that only portions of these complex antibiotic molecules may be responsible for anti-HIV action. Once research has defined such "minimal active substructures," it should be possible to prepare simplified analogues with amplified antiretroviral potency.

Chapter 2
Background

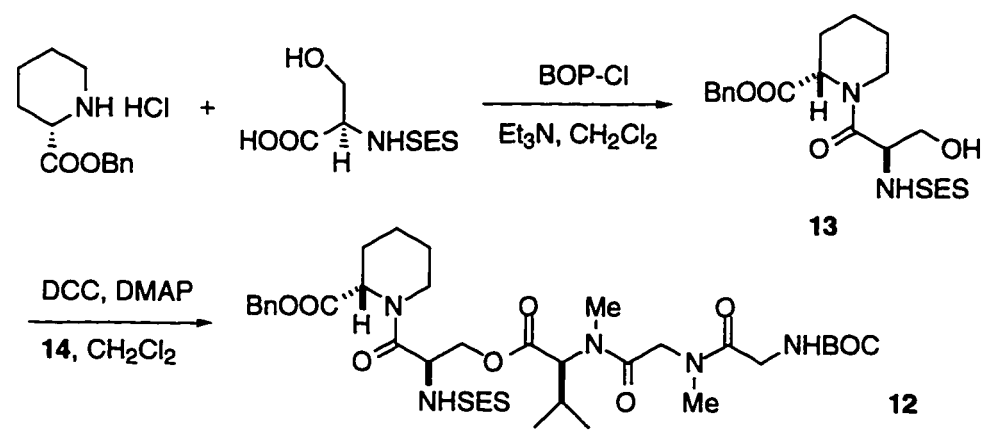
The only natural product in the peptin family, sandramycin, **4**, was synthesized in late 1993 by Boger.[12] However, sandramycin does not contain the hallmark PCA or piperazic acid components of more complex peptins. Furthermore, all aminoacids present in **4** are relatively common. Regardless, the route to this important synthetic objective represents a valuable guide for anyone who might wish to venture into the peptin area, especially with regard to the difficulties which may be encountered in the synthesis of more complex members of the family and to possible strategies for an obligatory final macrocyclization.

Boger's strategy for the total synthesis of sandramycin envisioned preparation and subsequent dimerization of monomer **12**, available through the merger of peptides **13** and **14** (Scheme 2-1 and 2-2). A wide range of coupling procedures afforded only modest yields of dipeptide **13**, formed from the benzyl ester of pipecolinic acid and N-SES-(D)-serine. The best yield was eventually obtained with BOP-Cl as coupling reagent. A subsequent esterification of the serine terminus of **13** with N-methyl valine tripeptide **14** was anticipated to be problematic, since the α-N-methyl amide present in the carboxylic acid coupling partner is known to retard or preclude esterifications and to increase the propensity for racemization. In the end, it was found that the rate of this crucial esterification was not seriously affected by the sterically demanding valine

segment; however, racemization was a serious problem. Fortunately, the use of 1 full equivalent of DMAP in the Steglich coupling (DCC-DMAP), instead of the customary 5-10 mol%, reduced the extent of epimerization to only 2-8%.

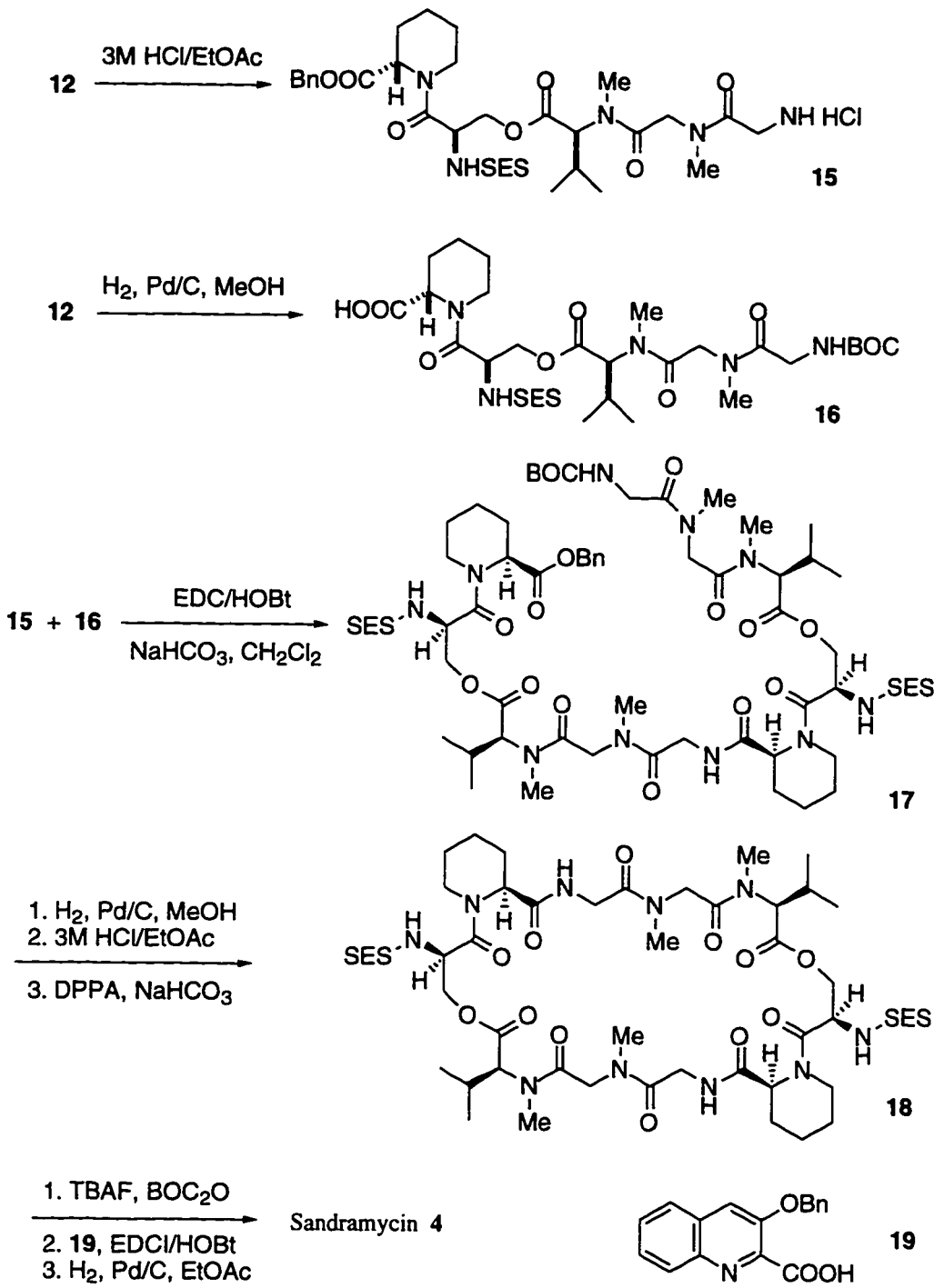


Scheme 2-1



Scheme 2-2

Formation of the *seco* decadepsipeptide was accomplished by deprotection of the amine (3 M HCl-EtOAc) and carboxylic acid (H_2, 10% Pd/C, CH_3OH) termini of **12** to provide **15** and **16**, respectively, which were coupled with formation of the secondary amide (EDCI/HOBT) to furnish **17** (Scheme 2-3). Cyclization of **17** to provide the 32-membered cyclic decadepsipeptide **18**

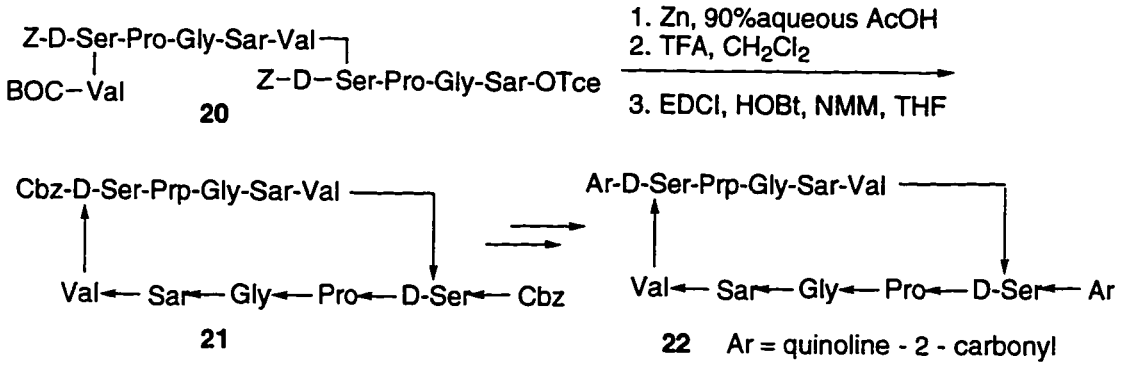


Scheme 2-3

was accomplished by sequential benzyl ester and BOC deprotection followed

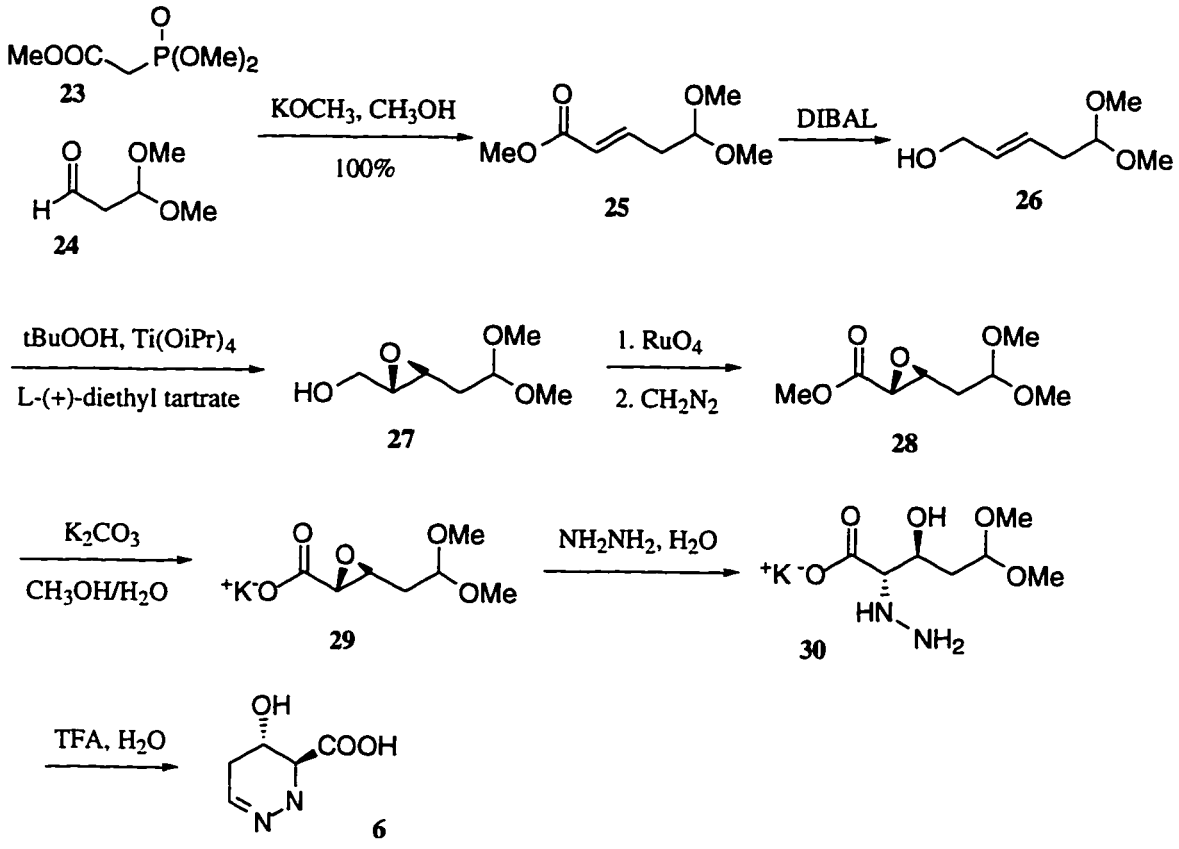
by treatment of **18** with DPPA. It is noteworthy that ring closure was conducted at the sole secondary amide site in the molecule. The total synthesis of sandramycin was completed by removal of the serine N-protecting group, coupling of chromophore **19** to the macrocyclic ring and final deprotection, as shown in Scheme 2-3.

Synthetic studies toward luzopeptin itself have not been reported, although the preparation of related systems has been investigated. Olsen and collaborators[13] synthesized analogue **22** of luzopeptin C in order to define suitable conditions for macrocycle formation (Scheme 2-4). This model system incorporated L-proline and L-valine in lieu of PCA and mhv, respectively. A simpler quinaldic acid unit also replaced the more complex chromophore of luzopeptins. The synthesis of peptide **22** was straightforward and will not be discussed in detail. Suffice it to state that the terminal carboxylic acid was protected as either a p-chlorophenacyl or a 2,2,2-trichloroethyl ester, both of which may be cleaved under mild reductive conditions without causing disturbance to the serine ester subunit. Macrocyclization of **21** by peptide bond formation between sarcosine (COOH terminus) and valine proceeded in a respectable 66 % yield.



Scheme 2-4

Methods for the preparation of key components of luzopeptins have also been described. In particular, the first synthesis of PCA was reported in 1989 by Hughes and Clardy.[14] The molecule was obtained in scalemic form through Sharpless epoxidation of an early key intermediate (Scheme 2-5).

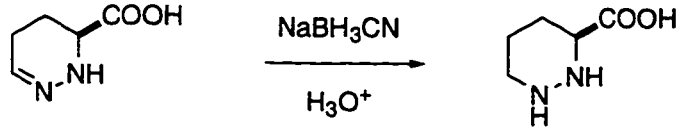


Scheme 2-5

Propargyl alcohol was advanced in three steps to malonaldehyde dimethyl acetal, **24**, which was condensed with Wadsworth-Emmons reagent **23** to form ester **25**. DIBAL reduction furnished allyl alcohol **26** in 87% yield. Sharpless epoxidation (>97% ee) followed by oxidation of intermediate **27** to an acid and diazomethane esterification produced glycidic ester **28**. This substance was converted to the final product as follows. Sponification of the ester **28**,

concentration of the reaction mixture, and treatment of the resulting potassium salt of the glycidic acid with hydrazine hydrate resulted in clean regioselective opening of the epoxide at the α-position and afforded hydrazino acid **30**. Subsequent treatment with TFA/water yielded unstable (+)-PCA, **6**, as glassy solid.

A close relative of PCA, (3S)-2,3,4,5-tetrahydropyridazine-3-carboxylic acid, **31**, is shown in Scheme 2-6. This substance, which we refer to as dPCA (deoxy PCA), is a constituent of cirratiomycins[15] and antrimycins,[16] peptide antibiotics with antituberculostatic activity. We,[17] as well as other researchers, have demonstrated that dPCA derivatives are easily reduced by sodium cyanoborohydride[18] to piperazic acid, **32**, which is a component of luzopeptin E_2, **8**. It will be seen that the best route to advanced intermediates for **8** proceeds through dPCA. Therefore, it seems appropriate to review known syntheses of dPCA and of piperazic acid.

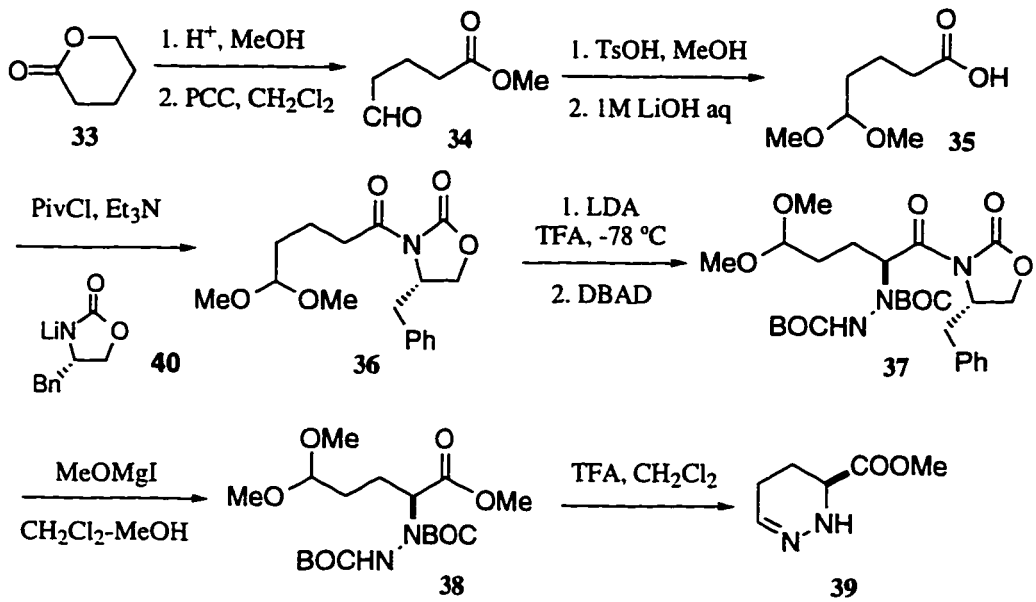


dPCA **31** Piperazic acid **32**

Scheme 2-6

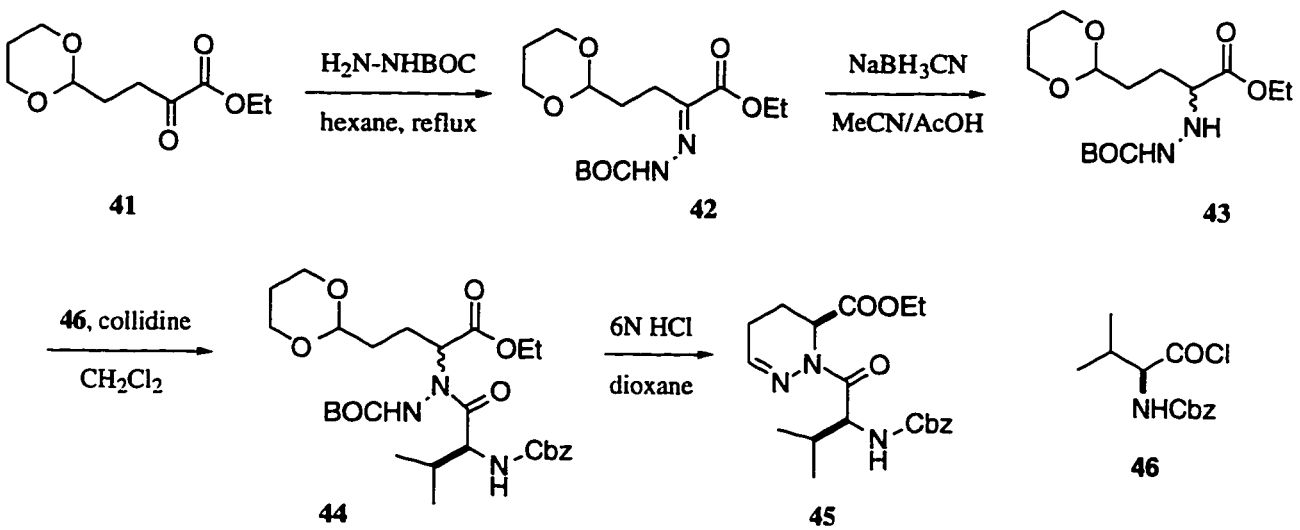
The first asymmetric avenue to **31** was reported by Shin[19] in connection with synthetic work on antrimycins. As illustrated in Scheme 2-7, acid **35** was derived from lactone **33** and converted to Evans imide **36**. The enolate of **36** was condensed with di-*tert*-butyl azodicarboxylate (DBAD) to give adduct **37**. Cleavage of the chiral auxiliary with MeOMgBr and subsequent exposure of **38**

to acid induced cyclization to dPCA ester **39**.



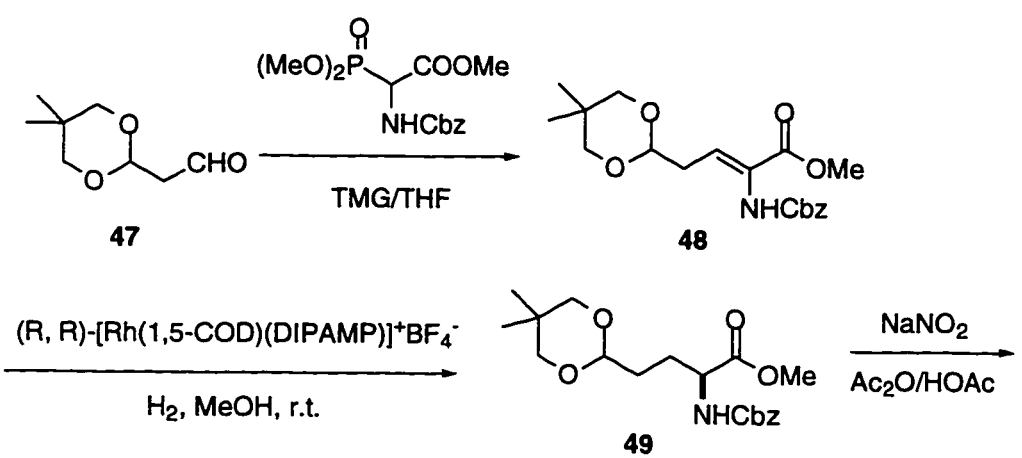
Scheme 2-7

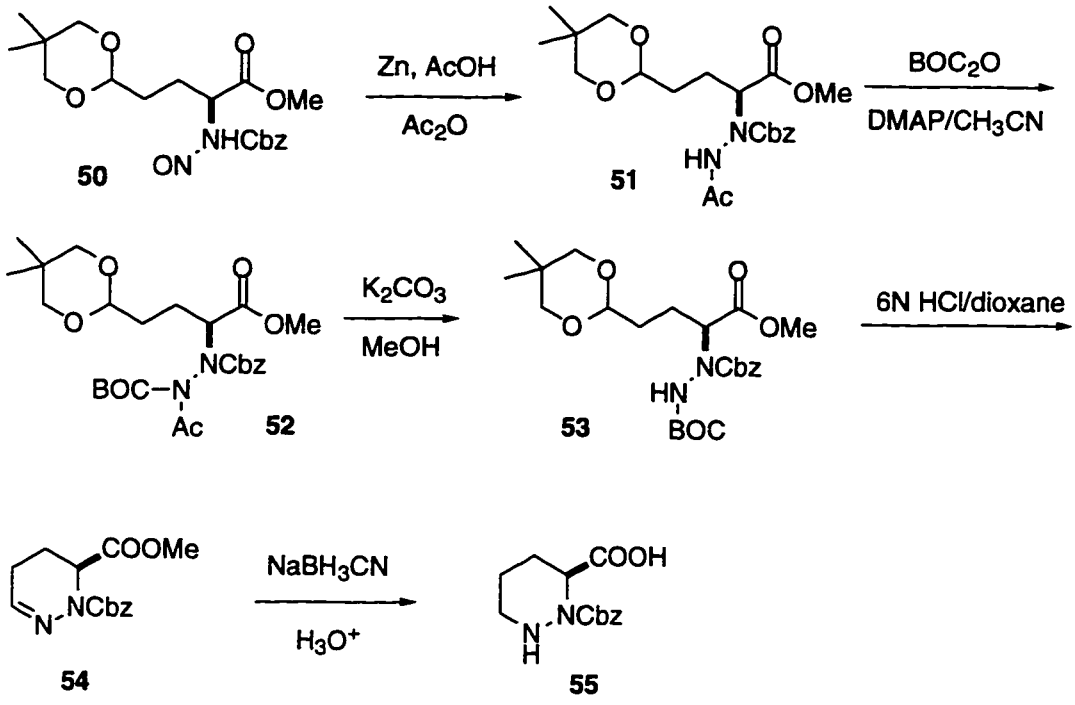
In the course of the total synthesis of antrimycin Dv by Schmidt,[20] it was found that dPCA could not be coupled with an acid at the N-2 position under standard peptide-forming conditions (Scheme 2-8). The problem was circumvented by coupling a racemic PCA precursor **43** with N-Cbz-valine acid chloride **46** to give compound **44**. Ring closure was effected by treatment with 6N HCl in dioxane. Subsequent chromatographic separation of the diastereomers afforded optically active product **45**. It must be stressed that coupling could be effected only with the acid chloride of protected valine, while more common protocols for peptide formation failed. This is a reflection of the unusually poor nucleophilicity of the free NH group in **43**, which we also observed in structurally related entities,[21] and that later induced us to devise a special procedure for the formation of PCA-serine dipeptides (*vide infra*).



Scheme 2-8

An interesting enantioselective synthesis of dPCA and piperazic acid was subsequently developed as shown in Scheme 2-9.[22] Asymmetry was created by Noyori-type hydrogenation of dehydroamino acid **48**. The chiral aminoacid **49** was advanced to **51** via nitrosation and reduction. Simple manipulations afforded dPCA **54**, reduction of which led to scalemic piperazic acid.

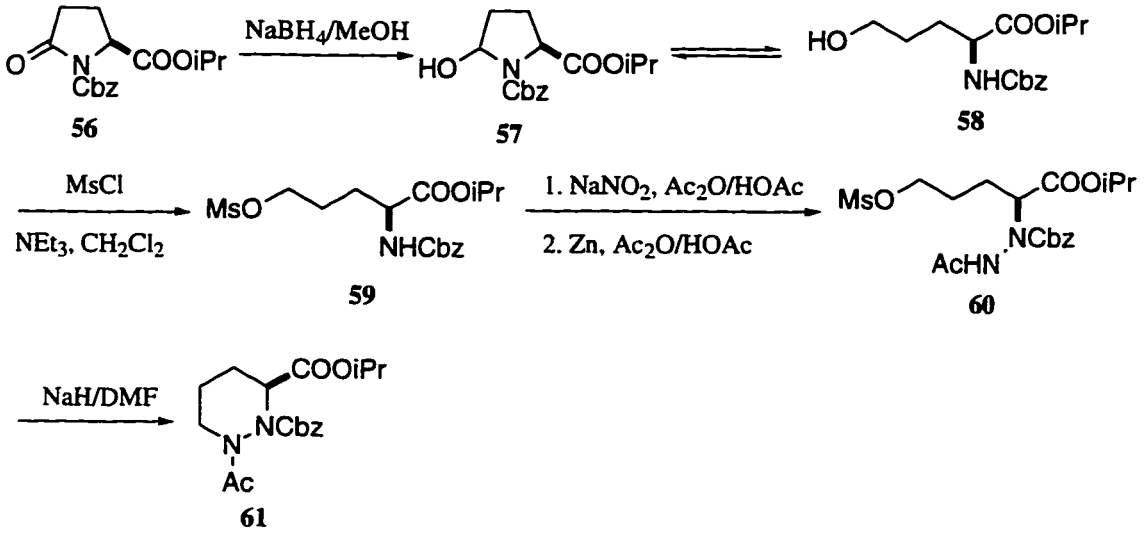




Scheme 2-9

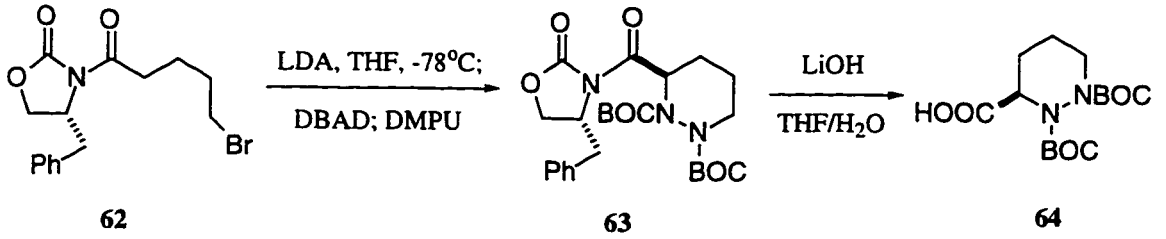
Piperazic acid **32** is found not only in luzopeptin E_2, but also in certain fungal metabolites of current interest such as the azinothricin family of antitumor antibiotics,[23] the matlystatins,[24] inhibitors of type IV collagenases, as well as the antibiotic L-156,602.[25] The preparation of **32** was studied extensively by Hassall[26] in the late 1960's in connection with work on the monamycin antibiotics. These earlier routes to **32** relied on resolution of enantiomers and are inadequate for the preparation of unsaturated analogues such as dPCA. Practical asymmetric syntheses have been developed only in recent times.

Schmidt reached **61** starting with L-pyroglutamic acid. His strategy parallels the logic of the synthesis of dPCA (Scheme 2-9), as is evident from Scheme 2-10.



Scheme 2-10

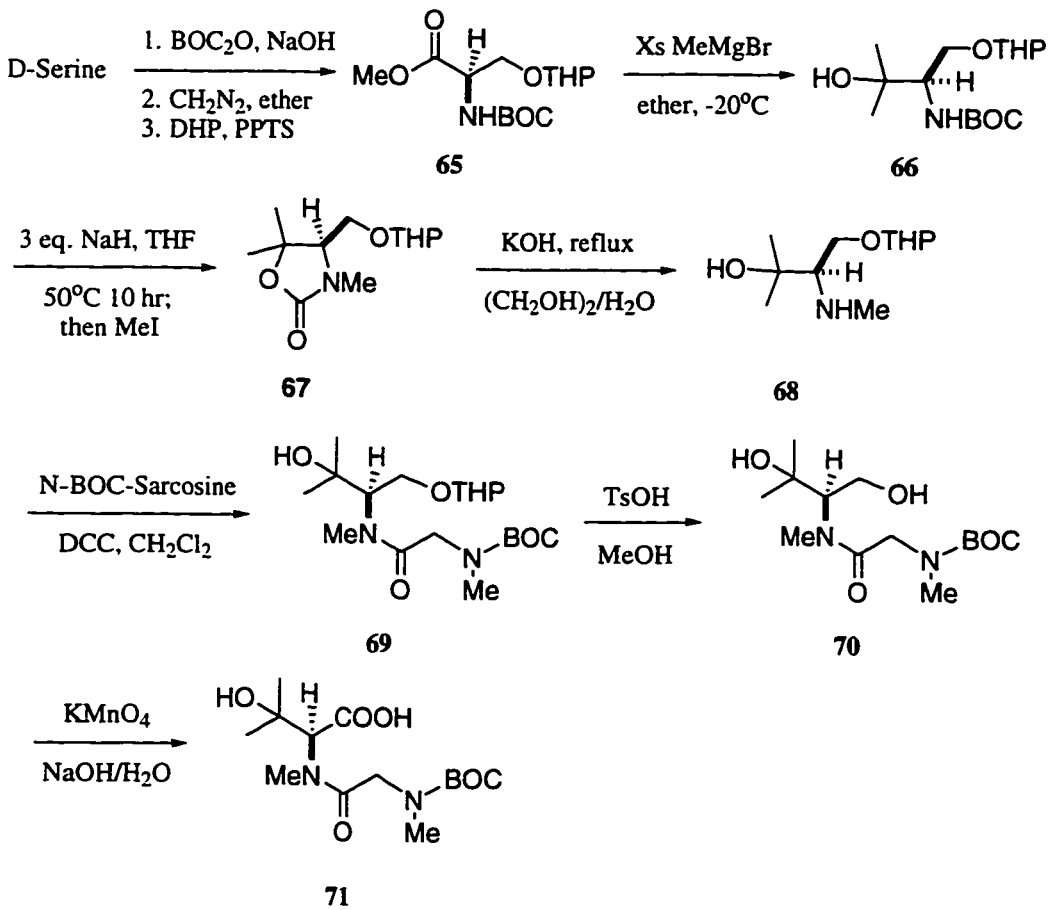
An elegant asymmetric synthesis of both (3R)- and (3S)-piperazic acids based on electrophilic hydrazination have been developed by Hale[27] in connection with the synthesis of azinothricin. As illustrated in Scheme 2-11, the ring system of piperazic acid was completed in one step through condensation of the enolate of Evans imide **62** with DBAD and *in situ* displacement of halide from the intermediate hydrazine anion.



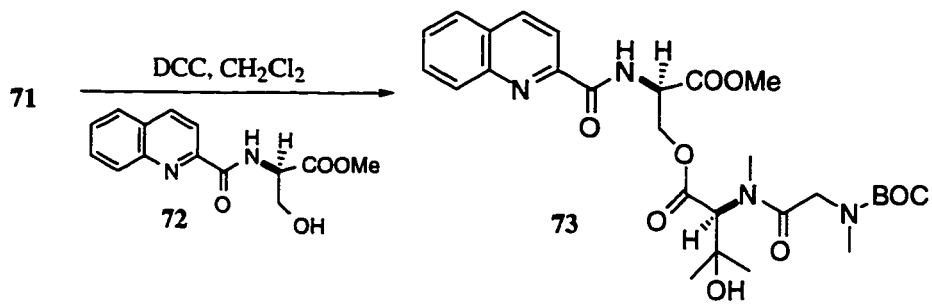
Scheme 2-11

Earlier work from this laboratory established methodology for the synthesis of optically active N-methyl-3-hydroxyvaline, **5**,[28] as shown in Scheme 2-12. The key feature of this synthesis was the Rapport-like inversion of hydroxy and

carboxy group of serine.[29] Fully protected D-serine, **65**, reacted with MeMgBr to form tertiary alcohol **66**. Without purification, this intermediate was cyclized to oxazolone **67** (NaH, THF, heat) and N-methylated *in situ* with MeI. Vigorous base hydrolysis opened the oxazolone **67** to form aminoalcohol **68**, which was directly coupled with N-BOC sarcosine. After removal of the THP group, alcohol **70** was subjected to $KMnO_4$ oxidation in basic solution under Garner conditions[30] to form carboxylic acid **71** in 65 % yield (75% based on recovered starting material). The incorporation of this dipeptide into a model depsipeptide, **73**, corresponding to the lower sector of luzopeptin was also reported (Scheme 2-13).



Scheme 2-12



Scheme 2-13

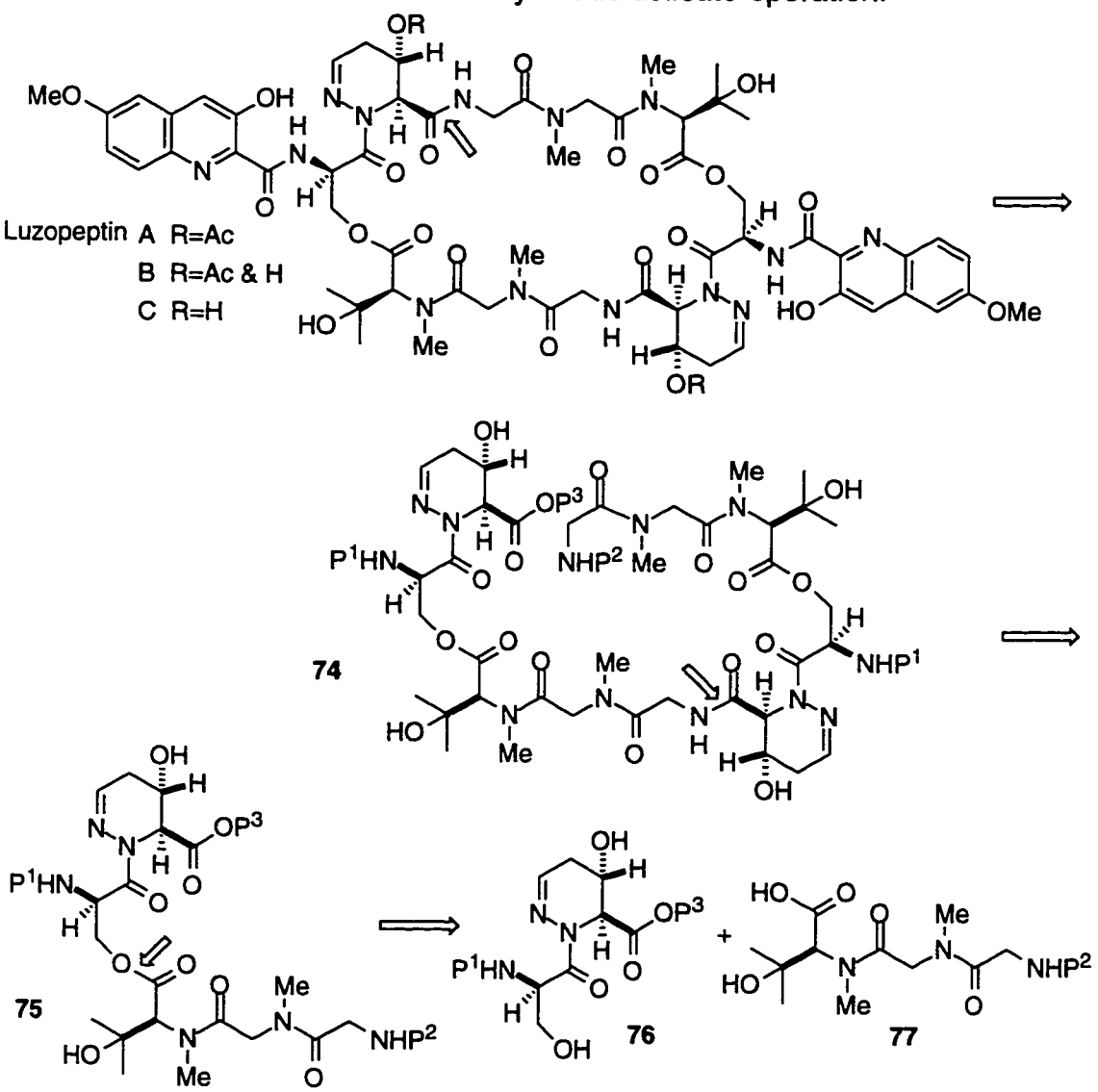
In summary, none of the synthetic studies addressed the issue of the coupling of PCA and serine segment to form the key dipeptide **76**. Our primary goal, therefore, was to develope a concise, practical synthesis of dipeptide **76**. The total synthesis of luzopeptins could be realized only after all the subunits of luzopeptins were available.

Chapter 3
Synthetic Studies of Luzopeptin C

3.1 Synthetic Strategy

Our retrosynthetic analysis of luzopeptins and quinoxapeptins was influenced by several principles that have been developed in connection with the synthesis of complex cyclodepsipeptides.[31] Peptide bond formation is generally preferred over lactone formation as a means to accomplish macrocyclization, because the difficulties associated with macrolactonization are generally greater than those encountered in macrolactamization. Furthermore, the likelihood of a successful, efficient macrocyclization increases with decreasing steric hindrance around the cyclization termini, primary amines being especially desirable as nucleophiles unless branching is present in their surroundings (such as, e.g., in valine and leucine). Greatest efficiency is usually realized through a convergent synthesis involving the merger of two peptide fragments of similar size. Finally, any pendant groups on the peptide scaffolding are best connected after ring closure. Accordingly, and on the basis of Boger's synthesis of sandramycin, it seemed that a viable attack on luzopeptins may be based on macrocyclization of *seco* intermediate **74** through amide bond formation between the PCA's COOH and the glycine's NH_2 termini. This initial retrosynthetic dissection leads to monomeric intermediate **75**, wherein the serine amino group later destined to accept the heteroaromatic chromophore of the antibiotics is now blocked using a suitable protecting group

(Scheme 3-1). One needs to be mindful, however, that the efficiency of macrocyclization is sequence-dependent: an adaptation of reaction conditions (coupling agent, concentration, temperature, etc.) is often required for each kind of specific compound; furthermore, seemingly minor structural changes may have a marked effect on the feasibility of this delicate operation.[32]



Scheme 3-1

The monomeric pentapeptide can be made from PCA-serine dipeptide **76**

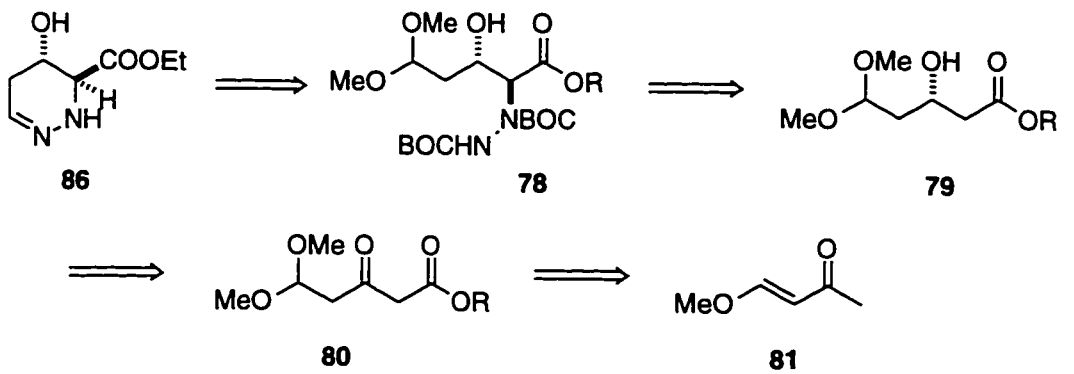
and tripeptide **77** by ester bond formation. Three of the five required aminoacids are commercially available: glycine, sarcosine, and D-serine. The others, mhv, **5**, PCA, **6**, as a well as the quinaldic acid **7**, must be obtained by total synthesis. Theoretically, **7** is available by chemical degradation of the native antibiotic, but because of the extreme rarity of luzopeptins, a synthesis of this fragment is clearly necessary. By contrast, mhv and PCA do not survive degradation. Needless to say, a synthesis of these components must be concise, efficient, and highly enantioselective.

Additional issues that must be taken into careful account in charting a route to **1-3** relate to lability of the β-hydroxyester and β-hydroxyamide functionalities found in the natural products and to selection of protecting groups. Three amino acid subunits of luzopeptins, serine, PCA and mhv, display a β-hydroxycarbonyl motif that may easily undergo β-elimination, especially in basic media.[33] These subunits are more tolerant of acidic conditions, although long exposure to strong acids is detrimental. The sensitivity of such β-hydroxycarbonyl structures must be factored into the choice of protecting groups for the remainder of the molecules. This selection is even further narrowed by the presence of delicate ester bonds in these depsipeptides. Problems relating to the proper choice of blocking groups are especially acute when considering a PCA synthesis, because this small molecule contains an elevated density of highly sensitive functionality. It was not clear how those issues would be best addressed at the onset of our own efforts toward luzopeptins, but it was already apparent that the number of protecting groups had to be limited to an absolute minimum.[34] The following paragraphs detail problems and solutions pertaining to the general approach of Scheme 3-1.

3.2. Synthesis and Chemistry of (±)-PCA

Our first goal was to develop a concise, practical synthesis of PCA and to study its chemistry. We were aware of the instability of this molecule, as reported by Hughes and Clardy;[35] however, detailed knowledge of its chemical properties was regarded as crucial for the formulation of a rational synthetic plan for luzopeptins. The issue of asymmetry was therefore regarded as secondary at this stage.

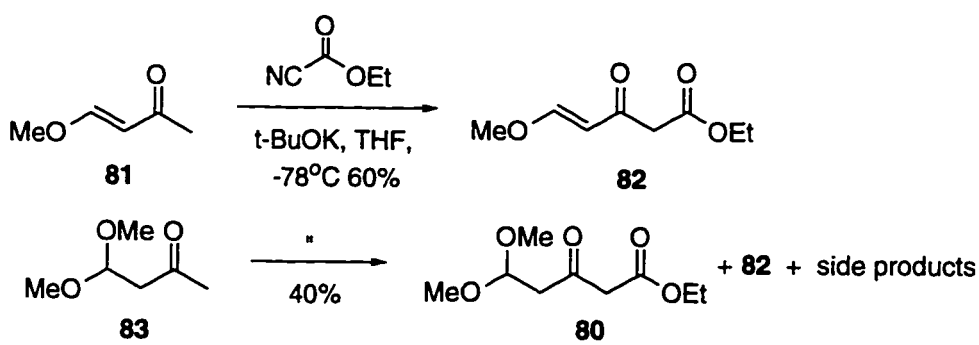
Our strategy for PCA synthesis is shown retrosynthetically in Scheme 3-2. We envisioned that the Gennari-Evans-Vederas hydrazination[36] of the dianion of β-hydroxy ester **79** would lead to intermediate **78**, which possesses the correct relative stereochemistry for PCA. The hydroxyester may be obtained by chemoselective reduction of a corresponding β-ketoester **80**, a transformation that may be readily carried out in an enantiocontrolled fashion in number of ways. A logical precursor to **80** was identified in commercial enone **81**.[37]



Scheme 3-2

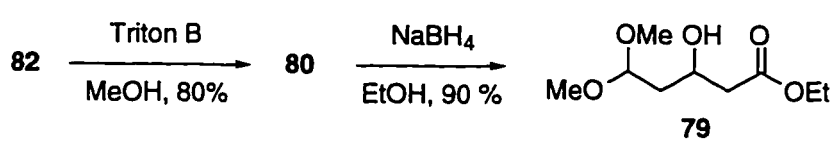
The enolate of **81** was generated using tBuOK[38] as the base and was condensed with the Mander reagent[39] to give β-ketoester **82** in 60 % yield. This material appeared to exist substantially only as the keto tautomer (^{1}H

NMR). The enolate of **81** has been prepared also by deprotonation with LDA.[40] In the present case, however, we observed superior results with tBuOK. A similar sequence evolving from acetal **83**, also commercially available, gave poor results. Elimination of MeOH during the condensation reaction furnished significant amounts of compound **82** besides the expected **80**. While this was not a serious problem, the excess tBuOK required for complete consumption of **83**, and the KOMe liberated in the reaction medium, seemed to have an adverse effect and promoted formation of several side products that were not thoroughly characterized (Scheme 3-3).



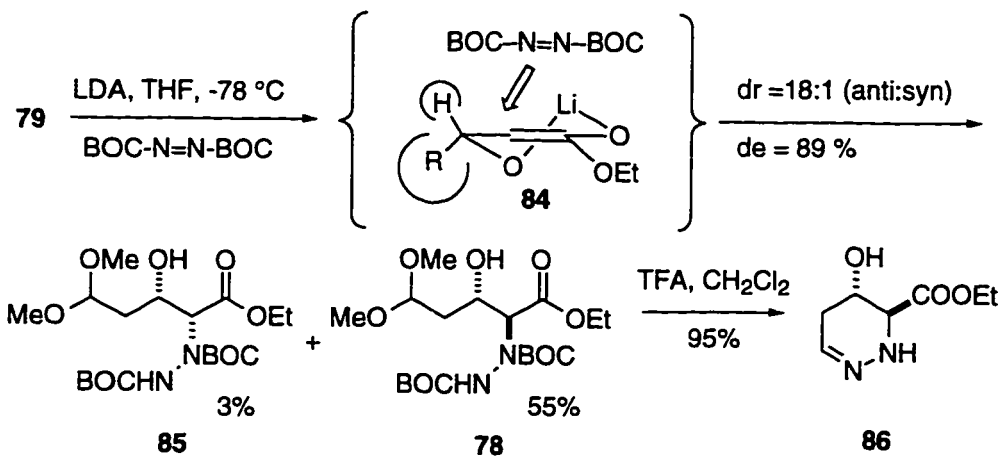
Scheme 3-3

Ketoester **80** emerged upon conjugate addition of MeOH to **82**, a reaction that was best effected with methanolic Triton B.[41] Selective reduction of the ketone with $NaBH_4$ in ethanol furnished (±)-β-hydroxyester **79** in 90% yield, as illustrated in Scheme 3-4.



Scheme 3-4

The dianion of this hydroxyester **79** was condensed with DBAD to furnish an 18:1 mixture (500MHz) of *anti* (major) and *syn* (minor) diastereomers of the adduct, which were separated by column chromatography and recrystallization. This stereochemical outcome is in agreement with earlier observations reported by Guanti,[42] and it is attributed to a steric effect. Briefly, the dianion is believed to exist as chelate **84**. Incoming electrophiles tend to approach the reactive site of **84** from the least hindered face, thereby accounting for the observed diastereoselectivity. Exposure of compound **78** to 50% TFA in CH_2Cl_2 induced rapid (15 min) and quantitative conversion to PCA ethyl ester **86**, a delicate substance that was difficult to purify, but that, fortunately, emerged in high purity (^{13}C, ^1H NMR) when analytically pure, crystalline **78**, mp 90-91 °C, was used in this step (Scheme 3-5).



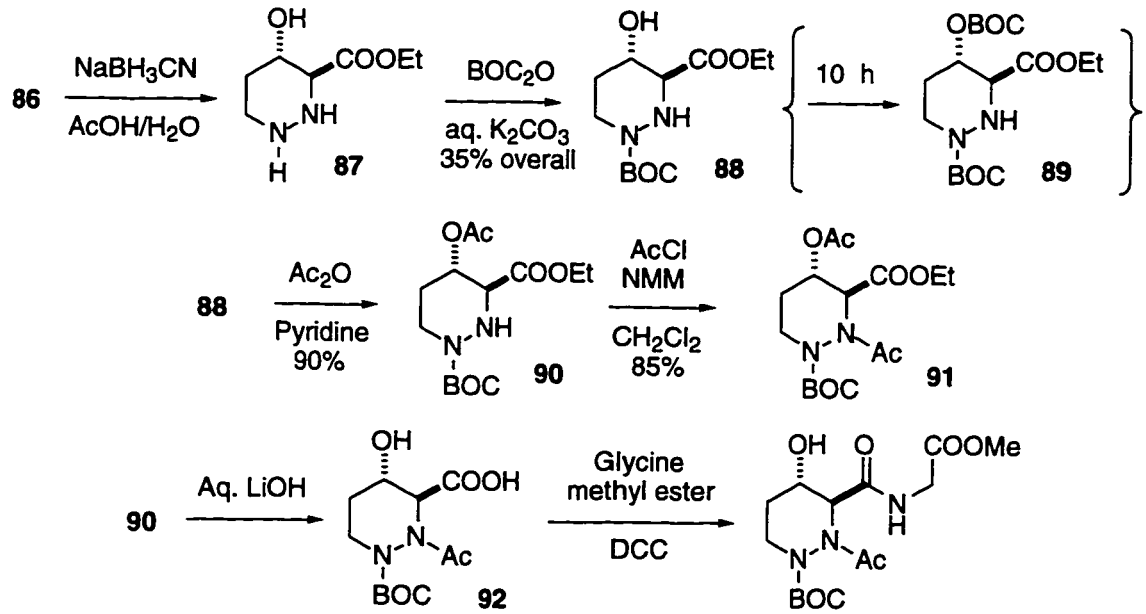
Scheme 3-5

As alluded-to earlier, no literature record existed regarding the chemical properties of free PCA, despite the obvious relevance of such knowledge to a

possible synthesis of the luzopeptins. Such studies were now possible, and the first reaction we tested was acylation. To our surprise, subjecting of **86** to various acylating conditions resulted in rapid destruction of the substrate and formation of complex mixtures. Acylating agents examined including active esters, symmetrical and mixed anhydrides, imidazolides, and acid chlorides. To illustrate, a commonly used, mild acylation protocol in peptide synthesis involves the use of reactive 4-nitrophenyl esters in combination with catalytic HOBt and DMAP. PCA decomposed in a matter of few minutes upon attempted reaction with N-BOC 4-nitrophenyl serinate. Intermediates obtained through addition of carboxylic acids to DCC may also be regarded as active esters. Complete decomposition again resulted upon attempted coupling of **86** with N-BOC glycine under the influence of DCC. Attempted acylation with acetic anhydride and pyridine, acetyl chloride and triethylamine, N-acetylimidazole and catalytic trifluoroacetic acid, as well as octanoyl chloride and N-methylmorpholine fared no better. Even mild hydrolytic conditions (LiOH, THF) rapidly obliterated the substrate, as did exposure to strong acids (TFA, CH_2Cl_2) for periods longer than 2 hours. Other researchers encountered analogous problems in similar systems.[43]

The disconcerting fragility of PCA raised serious doubts about the possibility of incorporating it directly into a peptide chain. A modestly successful protocol to circumvent the instability of **86** was developed as shown in Scheme 3-6. Reduction of PCA ester with $NaBH_3CN$ in aqueous acetic acid furnished a presumed hydrazine **87**, which was intercepted *in situ* with BOC_2O to yield monocarbamate **88** in 40% yield after brief contact time (2 hours). Compound **89** was observed as a byproduct if the reaction was allowed to proceed overnight. The elevated regioselectivity observed in this reaction is a

consequence of the exceptionally poor nucleophilicity of the N-2 nitrogen atom (pyridazine numbering). Indeed, the OH group of **88** reacts selectively over the NH group with acylating agents. For instance, treatment with acetic anhydride and pyridine cleanly furnished **90** in 90% yield plus only minor amounts of diacetyl derivative **91**. Reaction of **90** with acid chlorides, e.g., acetyl chloride, in the presence of N-methylmorpholine (NMM) proceeded normally to give **91** in 70% yield. Thus, the three nucleophilic sites of hydrazine **87** can be readily differentiated. Furthermore, the ester group in **90** underwent hydrolysis (aq. LiOH) without incident, and the resulting free acid **92** condensed normally with



Scheme 3-6

methyl glycinate under the influence of DCC. Reoxidation of N-2 monoacyl derivatives of piperazic acids back to hydrazones may be carried out with *t*BuOCl[44] or by Swern reaction,[45] so that a total synthesis of luzopeptins using the present approach was regarded as feasible (Figure 3-1).

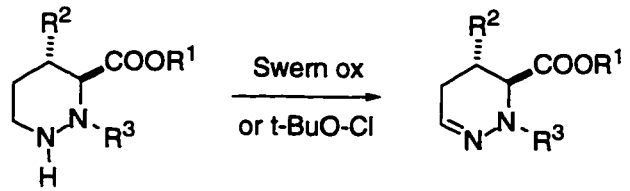
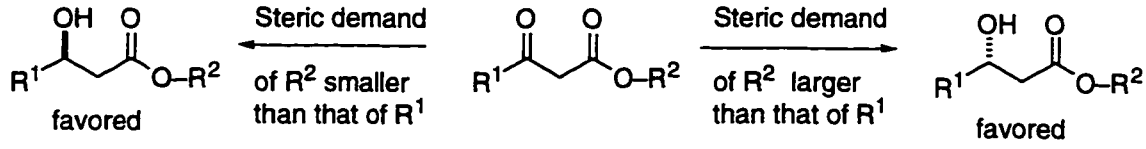


Figure 3-1

The success of our racemic synthesis of PCA induced us to consider the possibility of an enantioselective reduction of the keto functionality in **80** as a means to generate scalemic material. There are several well established protocols for the enantioselective reduction of β-ketoesters, among which the Noyori asymmetric hydrogenation[46] is especially popular thanks to its wide scope. An alternative was the biocatalytic reduction with baker's yeast,[47] a very well studied transformation that was even more attractive to us, given its ease of execution and great economy (Noyori hydrogenation require expensive metal complexes and chiral ligands; furthermore, the catalysts are extremely air sensitive), and given its environmental friendliness.[48]

The sense of enantioselectivity in baker's yeast reduction may be predicted using the Prelog's rule,[49] a simple, but effective, model that takes into account the relative steric demand of the substituents occupying the two sides of a ketone carbonyl. As applied to β-ketoesters, the Prelog model may be simplified as shown in Scheme 3-7.[50] It should also be stressed that this model is rather qualitative: precise ee's tend to vary a great deal upon seemingly minor modifications of the substrate, so some tailoring of reaction conditions is often necessary.



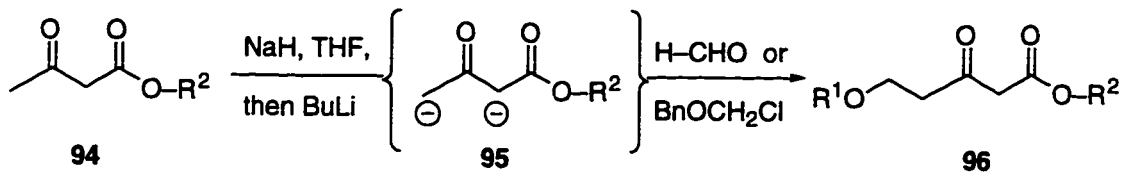
Scheme 3-7

A series of acetal-containing ketoesters was synthesized in preparation for baker's yeast reduction. In all cases, the compounds were obtained by condensation of **81** with an appropriate cyanoformate as discussed earlier in Scheme 3-3. In turn, the cyanoformates were prepared by treatment of the corresponding chloroformates, available simply by reaction of the alcohols with phosgene, with sodium cyanide under the conditions described by Weber.[51]

Unfortunately, yeast reduction gave rather disappointing results. The best enantioselectivity, 40 % ee, was obtained with the pentyl ester, as determined by scrutiny of the ^{1}H NMR spectrum of the Mosher ester[52] of the product. All other ketoesters gave either poor ee's and chemical yields, or were not substrates at all (no reduction) (Table 1).

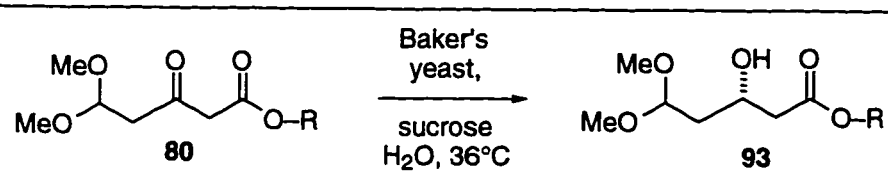
Substrates of the type **96** have been reported to undergo highly successful yeast reduction.[53] This led us to conclude that the mediocre results observed in our own experiments may be attributable to the steric similarity between the acetal and the ester units of **80**, and that removal of one of the oxygen functionalities now part of the acetal group may alleviate or correct the problem. This proved to be the case. Compounds **96** were prepared in good yield by reaction of the dianion of acetoacetate esters **94**[54] with formaldehyde or with benzyloxymethyl chloride ("BOM-Cl").[55] Ethyl and isobutyl acetoacetates are articles of commerce, whereas pentyl acetoacetate is not and

was made by reaction of pentanol with diketene (98%).[56]



Scheme 3-8

Table 1: Enantioselective Reduction of Acetal Ketoesters with Baker's Yeast

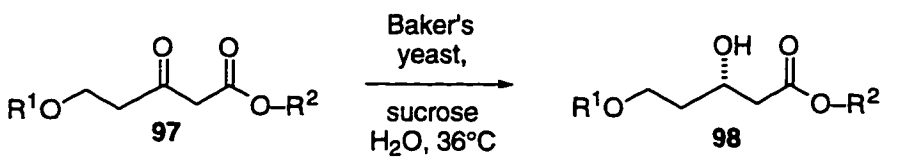


R	Entry	Unoptimized chemical yield	Enantiomeric excess (ee)
iso-butyl	**a**	50 %	20 %
n-pentyl	**b**	30 %	40 %
n-hexyl	**c**	not a substrate	–
n-octyl	**d**	not a substrate	–

The reduction of **97c** (**99**) was readily confirmed to proceeds cleanly and efficiently. We thus isolated hydroxyester **98c** (**100**) in 70% chemical yield and 89% ee (Table 2). However, puzzling results were obtained in experiments with ketoesters **97a** and **97b**, reportedly good, though not outstanding, substrates for yeast reduction.[57] Briefly, these compounds were not substrates for our yeast, which was store-bought *Saccharomyces cerevisiae* from the Fleischman

Co. We attribute this discrepancy to the fact that a cell-free yeast extract was apparently utilized in previous studies; furthermore, a different yeast cell line might have been used in those experiments. With hydroxyester **100** in hand, an asymmetric synthesis of PCA could be achieved according to the protocol described in Scheme 3-5.

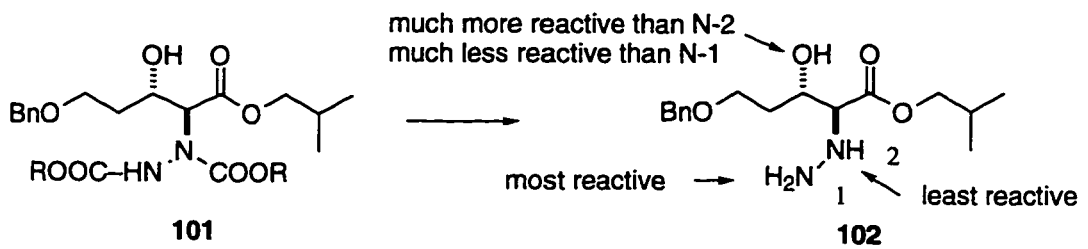
Table 2: Enantioselective Reduction of Oxy Ketoesters with Baker's Yeast



R^1	R^2	Entry	Optimized chemical yield	Enantiomeric excess (ee)
H	ethyl	**a**	not a substrate	–
H	n-pentyl	**b**	not a substrate	–
benzyl	iso-butyl	**c**	70 %	89 %

Our research on PCA ethyl ester had revealed that acylation of N-2 is possible only after reduction of the C=N bond. We rapidly realized that an approach relying on this manoeuvre was unattractive, because it would needlessly lengthen the synthesis and introduce additional protection on sensitive intermediates. It became apparent that it may be best to incorporate an appropriate PCA *precursor*, not PCA *itself*, into a growing peptide chain. A suitable strategy materialized as we learned more about the chemistry of PCA

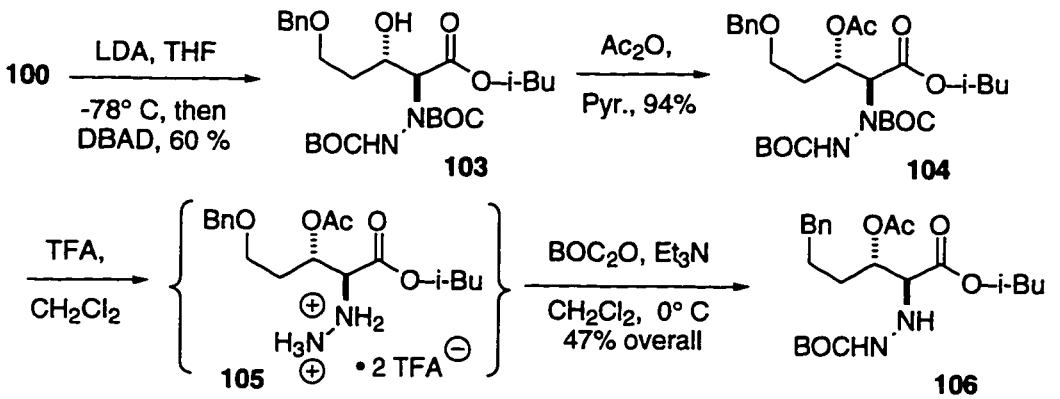
and of its precursors and derivatives, and it relied on the difference in reactivity among the three nucleophilic sites of **102**: N-1, the most reactive one, OH, a site of intermediate reactivity, and N-2, by far the least nucleophilic center.[58] If an intermediate of the type **101** were to be converted to a free hydrazine such as **102**, it would then be possible to block N-1, e.g. as a BOC derivative, and protect the OH, e.g. as an acetate, leaving a free N-2 ready to interact with an opportune acylating agent.



Scheme 3-9

This general idea was readily translated into practice. Reaction of the dianion of **100** with DBAD provided the expected hydrazine **103**. Purification of this intermediate was necessitated by the presence of considerable quantities of di-*tert*-butyl hydrazodicarboxylate (DBAD-H_2), a troublesome side product of Gennari-Evans-Vederas reactions that forms through reduction of excess DBAD. Unfortunately, compound **103** displayed a chromatographic mobility almost identical to that of DBAD-H_2, rendering its purification extremely laborious and painstaking. The quantity of DBAD-H_2 byproduct could not be diminished by decreasing the amount of the parent DBAD introduced into the hydrazination reaction, because it is well known, and we confirmed, that the use of less than 2 - 2.5 equivalents of this reagent results in severely reduced yields. These complications precluded removal of BOC from **103** at this stage. If this

step were to be carried out with material contaminated by DBAD-H_2, large quantities of hydrazine trifluoroacetate would form together with **102**. Congeners of **102** are known to be exceedingly unstable and are best intercepted *in situ* with suitable acylating agents, without purification. In our case, reaction of the mixture of **102** and hydrazine trifluoroacetate with BOC_2O would result in consumption of large amounts of this expensive reagent, as the hydrazine is converted back to DBAD-H_2. Furthermore, experience indicated that separation of DBAD-H_2 from an N-1 mono-BOC derivative of **102** containing a free OH would be even more troublesome (Scheme 3-10).



Scheme 3-10

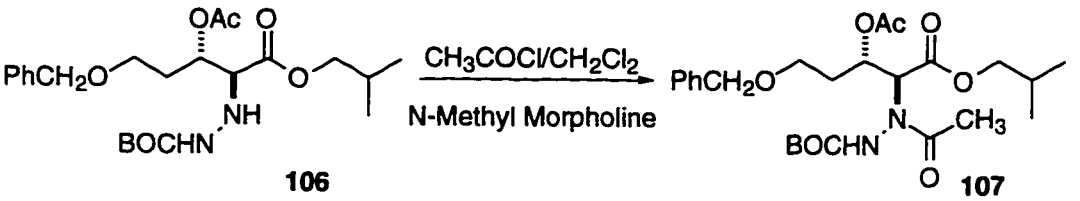
These observed and / or anticipated difficulties were partially circumvented as follows. Careful chromatography of **103** removed substantial quantities of DBAD-H_2. The partially purified material thus obtained was acetylated to furnish **104**. This compound was still difficult to purify, but less so than **103**, and it could be obtained substantially free from DBAD-H_2 after two cycles of column chromatography. Acid treatment led to the TFA salt of sensitive

hydrazine **105**, which was not isolated, but was rather intercepted *in situ* with BOC_2O in the presence of triethylamine and after removal of most of the TFA. Weaker base such as pyridine and lutidine were not efficient for this reaction due to the acidic sensitivity of BOC group. As expected, monoprotected hydrazinoester **106** emerged as the sole identifiable product in a moderate 45-48% yield. It was highly advisable not to expose **105** to the air for any significant length of time, nor to attempt complete removal of TFA from it, otherwise the transformation **104**→**106** would proceed in considerably lower yield. The protocol thus developed was deemed to be satisfactory for a subsequent exploration of the acylation chemistry of **106**. It was clear, however, that a much more efficient route would later have to be developed for a realistic chance of success of a total synthesis of a molecule as complex as luzopeptin.

Extensive experimentation was undertaken at this juncture in order to define good conditions for the acylation of N-2 in **106** with a serine residue. Our own research, as well as Olson's work,[79] showed that a wide range of methods for carboxyl activation, such as DCC, DCC-HOBt, BOP-Cl, mixed anhydrides, active esters, etc., failed completely to deliver a serinyl derivative of **106**. These failures may be attributed not only to the innate lack of nucleophilicity of the N-2-atom, which in the present case is exacerbated by the presence of an electron-withdrawing acyl group on N-1, or to steric congestion in its surroundings, but also to the ease of β-elimination of the OH functionality (or a protected variant thereof) in serine derivatives wherein strong activation has been provided to the COOH terminus. Additionally, long contact with acylating mixtures containing triethylamine, collidine, or similar bases (as well as their salts) may also have an adverse effect on the stability of PCA precursor

106, itself a β-acyloxy ester that could readily eliminate.

Schmidt demonstrated that only N-Cbz-valinyl chloride is sufficiently reactive to acylate compound **43** (Background, p. 14). Our own studies showed that acetyl chloride, but not acetic anhydride, acetylated **106** in CH_2Cl_2 in the presence of N-methylmorpholine (NMM) as base (Scheme 3-11). It became apparent that delivery of a serinyl unit to **106** would be possible only through the use of highly reactive agents such as serinyl chlorides,[59] fluorides,[60] or, possibly, cyclic N-carboxy anhydrides (NCA's).[61]

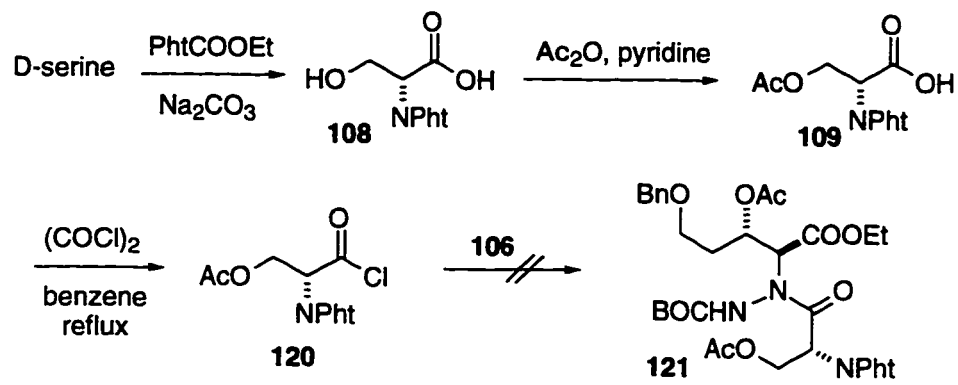


Scheme 3-11

3.3. Novel Serinyl Chlorides as Powerful Acylating Agents

Acid chlorides are much more readily available that the other acylating agents mentioned above. Indeed, various serinyl chlorides are known in the literature; therefore these well-studied species were selected for an initial foray into the acylation chemistry of **106**.

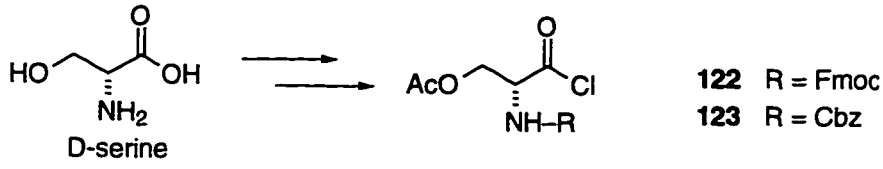
Literature procedures readily afforded serinyl chloride **120**, in which the OH group is protected as an acetate (Scheme 12).[62] Acylation of mono-BOC **106** with this acid chloride furnished none of the desired product, despite numerous attempts involving a variety of techniques and conditions. Acylations with N-



Scheme 3-12

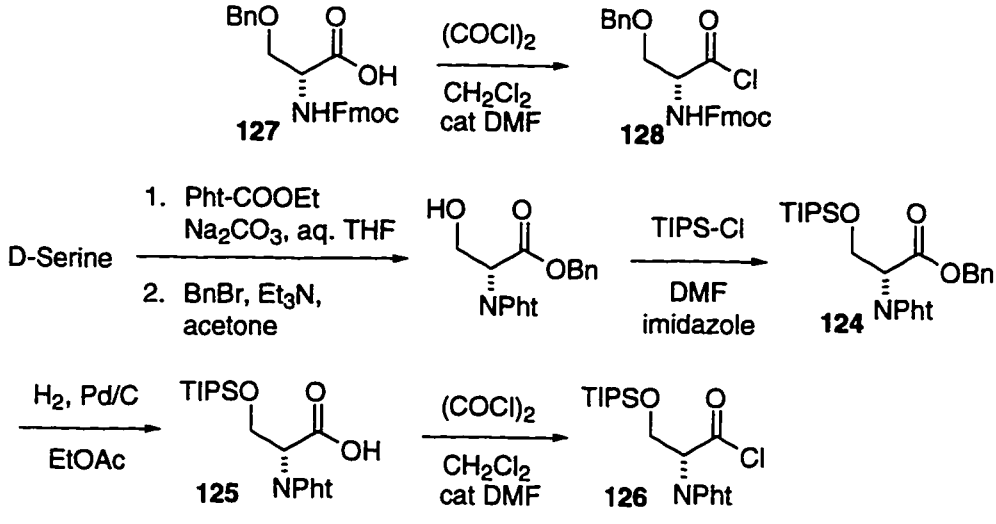
protected amino acid chlorides are generally best carried out in biphasic aqueous-organic media (Schotten-Baumann conditions).[59] In our case, the use of such biphasic systems (CH_2Cl_2 or THF with aq. $NaHCO_3$ or MgO as the bases) caused the acid chloride to react faster with water than with the poorly nucleophilic receptor. It is also recognized that acylation with acid chlorides in homogeneous organic solutions may be problematic. In our case, β-elimination of AcOH from **120** and polymerization of the resulting dehydroalanine, even under the influence of the mildly basic agents customarily employed in such steps, competed effectively with the coupling reaction regardless of solvent (CH_2Cl_2, THF, $CHCl_3$, EtOAc), base (Et_3N, NMM, 2,6-lutidine and 2,4,6-collidine), or temperature (–40°C to room temperature) employed. In all cases, immediate darkening of the solution was observed, and although most of the starting **106** could be recovered, no substance related to serine could be readily discerned in the NMR spectra of crude reaction mixtures. Identical results were obtained with chlorides **122** and **123**, which seemingly have not been recorded in the literature, but that we prepared by the same procedure

used for **120** (Scheme 3-13). Compound **122** appeared to be a particularly promising candidate for our reaction, on the basis of Carpino's important observation that N-Fmoc protected acid chlorides are especially well-behaved reagents.[59]



Scheme 3-13

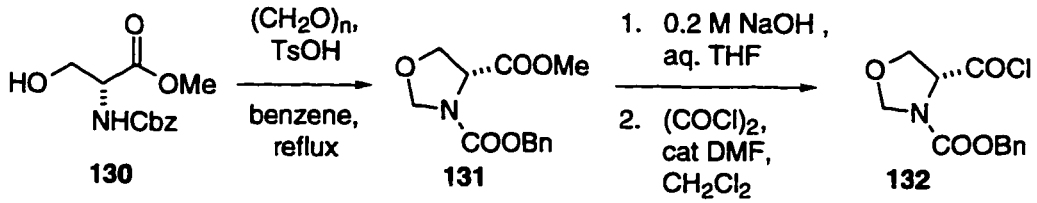
We reasoned that replacement of acetate, a good leaving group in E1cb reactions, with a protecting group that would not significantly activate the serine OH for departure, e.g., an alkyl or silyl ether, might alleviate the troublesome elimination problem. Two derivatives of serine containing acid-stable O-triisopropyl silyl (TIPS) and O-benzyl ethers were converted to the chlorides as shown in Scheme 3-14. Compound **125** was prepared by silylation of N-Pht



Scheme 3-14

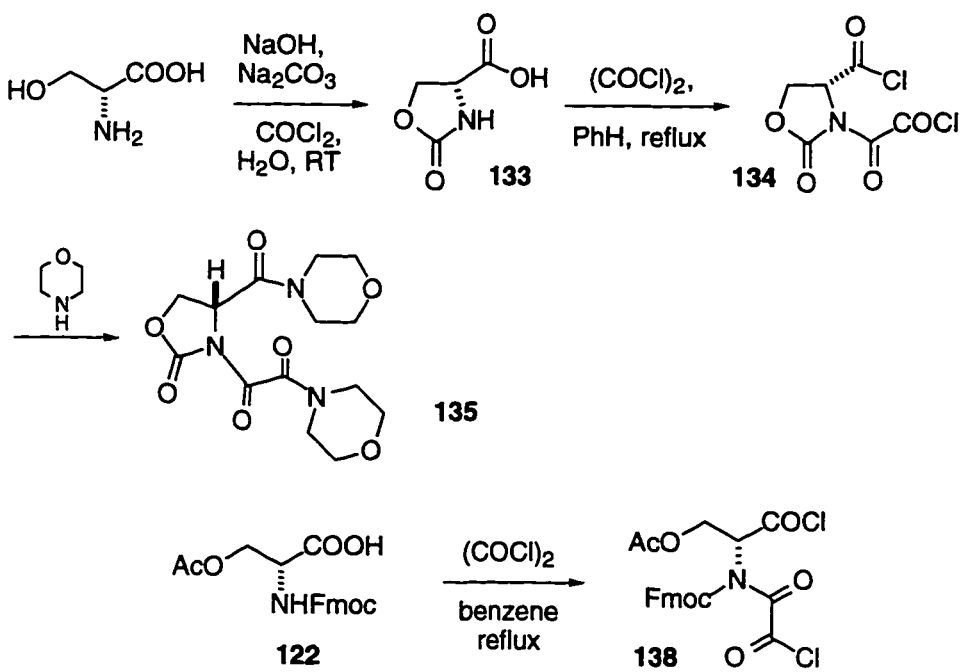
benzyl serinate followed by hydrogenolysis, while **127** was obtained commercially.[63] The choice of benzyl and TIPS protecting groups was suggested by the mildness of the conditions required for their removal (hydrogenolysis and treatment with F^-, respectively). To our disappointment, these acid chlorides also failed to acylate **106**. Even the powerful promoter, AgCN,[64] in refluxing benzene without added organic bases, was of no use: not only did the acid chlorides still decompose, but even the substrate was severely damaged. It is not clear whether injury to **106** was the result of some oxidative process [Ag(I) is an oxidant] or of interaction with protonic acid formed *in situ*.

Our results suggested that a more powerful inhibition mechanism must be introduced into the serinyl chloride to prevent β-elimination. We entertained the possibility of retarding such a reaction through a stereoelectronic effect described in detail by Baldwin.[65] Briefly, if the N and O atoms of the serine were made part of a 5-membered cyclic structure, β-elimination would become a disallowed, reverse "5-endo-trig" process. Two plausible solutions were identified: formation of an oxazolidine or of a cyclic carbamate such as an oxazolidinone ("oxazolone"). Acetonide derivatives of N-acyl serine are well known; however, the acetonide is rather acid-sensitive and we had serious doubts that a manageable acid chloride could be obtained from such intermediates. Accordingly, we elected to prepare formaldehyde-derived oxazolidine **132**, which could be predicted to be more acid-stable (Scheme 3-15). Unfortunately, **132** also failed to acylate **106**. Although the cyclic structure of **132** may retard the β-elimination process, acid-catalyzed opening of the five-membered ring during the reaction could undermine the stability of **132**.



Scheme 3-15

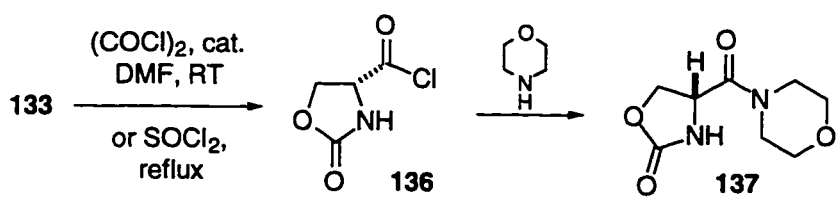
We then turned our attention to the sturdier oxazolone derivative **133**. A literature procedure for its synthesis[66] was improved by bubbling phosgene into an aqueous solution of serine containing NaOH and Na_2CO_3. Compound **133** *per se* was not suitable for conversion to an acid chloride: Reaction with $(COCl)_2$ in refluxing benzene for 1 hour and evaporation of the volatiles provided a substance that furnished a reasonable proton NMR spectrum, but



Scheme 3-16

that exhibited two extra ^{13}C NMR peaks in the carbonyl region at 160.7 and 165.6 ppm. Clearly, a molecule of oxalyl chloride had condensed with the substrate. Reaction with morpholine delivered a product that was unambiguously identified as **135**, confirming that the NH group on the oxazolone ring had reacted with $(COCl)_2$. A closer examination of this matter revealed that reaction of serine-derived acid **122** with $(COCl)_2$ in refluxing benzene, instead of at room temperature in the presence of a catalytic amount of DMF (*vide supra*), furnished a similar product (Scheme 3-16).

The desired acid chloride **136** was finally made from **133** through the DMF catalyzed reaction or, more simply, by refluxing in $SOCl_2$. This material was thoroughly characterized; furthermore, its reaction with morpholine proceeded as a expected to furnish compound **137** (Scheme 3-17).

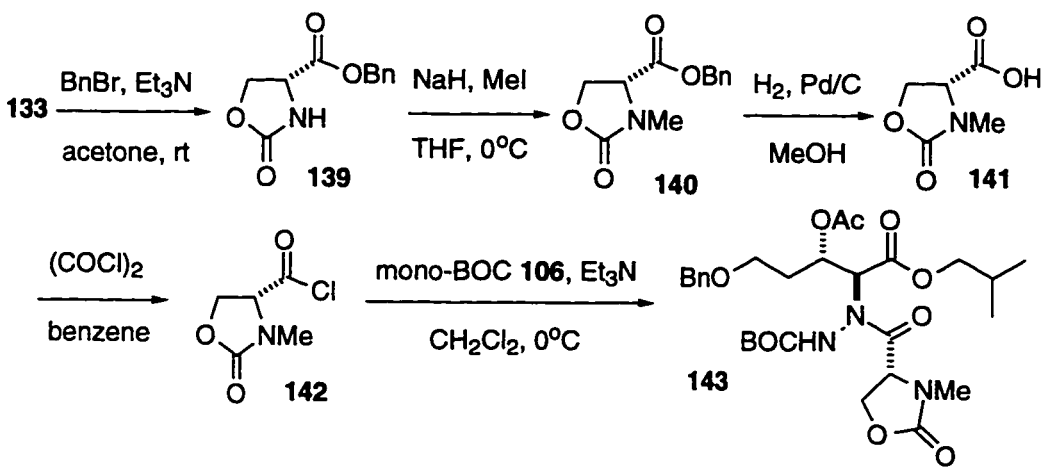


Scheme 3-17

To our surprise, the reaction of **136** with **106** still failed to deliver any coupling product. This time, however, we detected intact oxazolone acid **133** from the aqueous phase generated during partitioning of the reaction mixture. This confirmed that the failure of the acylation step was not caused by opening of the oxazolone ring or β-elimination.

These observations indicated that the NH group in the oxazolone interfered

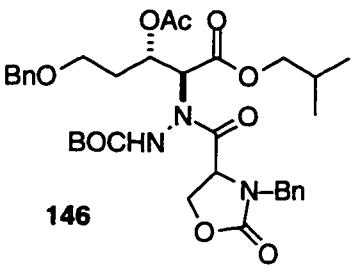
with the coupling reaction and had to be blocked. The behavior of **142** nicely verified this hypothesis. Oxazolone **133** is very polar and does not dissolve well in organic solvents, so it was first transformed into a benzyl ester **139**. Installation of an N-Me group, a simple, sturdy masking unit for the NH, was achieved by using MeI as methylating reagent and NaH as base. The benzyl ester was then hydrogenolyzed to afford free acid **141**, which was converted to the acid chloride by refluxing with $(COCl)_2$ in benzene. We were delighted to find that the resulting **142** coupled efficiently with **106** in CH_2Cl_2 using NMM as base, and the long-sought **143** was isolated in 40-50% yield after purification by column chromatography (Scheme 3-18).



Scheme 3-18

Of course, an N-methyl blocking group is not suitable in a synthetic sequence. A protecting group that is easy to remove under mild basic conditions, but that is stable to acid, is necessary as a temporary form of protection for the oxazolone NH. A cyanomethyl units seemed to be a reasonable choice, since it is stable to acid and base and may be removed

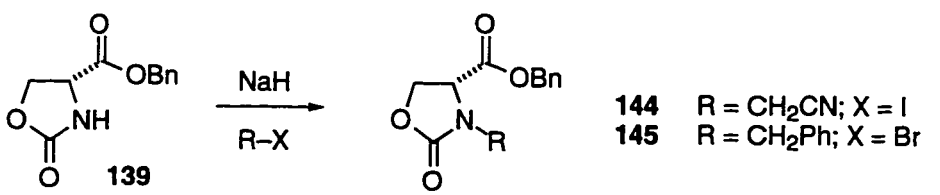
under very mild conditions with ethanolic $AgNO_3$.[67] An N-benzyl group might also have been adequate. However, hydrogenolytic cleavage of N-benzyl requires vigorous conditions, engendering some apprehension about the survival of the N-N bond also present in the final product. Differentiation of the the two benzyl groups in the desired **146** was not a concern, given the considerably greater ease of O-debenzylation vs. N-debenzylation (Figure 3-2).



146

Figure 3-2

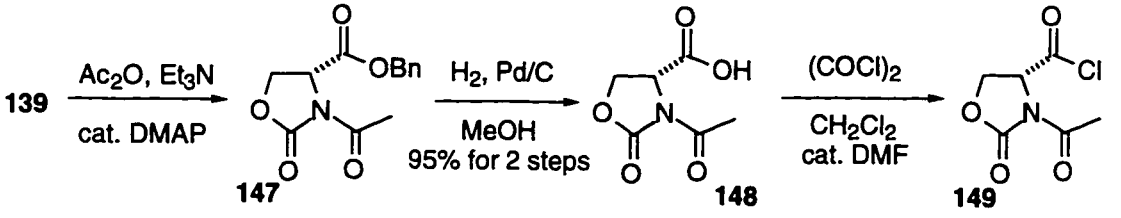
These blocking groups were quickly discarded when we found that it was not possible to introduce them by the use of mild bases such as Et_3N, etc., presumably due to the poor nucleophilic character of the N atom, while the use of stronger condensing agents such as NaH promoted formation of complex mixtures (Scheme 3-19).



144 R = CH_2CN; X = I
145 R = CH_2Ph; X = Br

Scheme 3-19

It is well-known that the NH group of the oxazolone ring may be readily acylated, e.g., with BOC_2O.[68] Thus it seemed reasonable that an N- acyl group that might later be readily removed could be used to block the molecule. Oxazolone imides prepared from simple carboxylic anhydrides are stable in acidic media, but they may be cleaved under mild nucleophilic condition. This logic induced us to prepare imide **148** as shown in Scheme 3-20. Reaction of **139** with Ac_2O in the presence of Et_3N and catalytic DMAP furnished acetimide **147** in 97% yield. The benzyl group was hydrogenolytically removed and acid **148** was converted to the chloride by refluxing in benzene with $(COCl)_2$ for 1 hour, or, more conveniently, by reaction with $(COCl)_2$ at room temperature in the presence of a catalytic amount of DMF.[59]



Scheme 3-20

Chloride **149** is a highly reactive substance that was obtained as a thick dark oil and that could not be further purified. However, it appeared to be free from contaminants by ^1H and ^{13}C NMR spectroscopy and it produced a fully satisfactory mass spectrum. As the first acid chloride of its kind, its chemistry was thoroughly studied. In particular, its ability to acylate derivatives of amino acids containing a secondary amino group, which react with greater difficulty relative to their primary amine congeners, was investigated in detail. Results of experiments with L-proline and L-pipecolinic esters are summarized in Table 3

(cf p. 45). Good to excellent yields of acylated products were obtained in all cases. The ease of reaction in the pipecolinic system is noteworthy, given its notoriously poor reactivity. It should also be pointed out that while this problem is often ascribed to steric hindrance around the N atom, it is more likely to result primarily from stereoelectronic phenomena.[69] Briefly, the N atom in a piperidine ring is quite reluctant to rehybridize from an sp^3 state to substantially sp^2, as required for amide formation, because ring flattening induces severe A-1,3-like interactions between the N-acyl group and the adjacent equatorial H atoms and it forces the ester group to the axial position, to avoid even greater nonbonding interactions between it and the N-acyl unit (Figure 3-3).

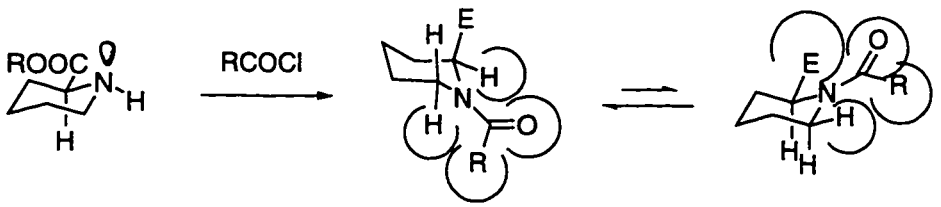
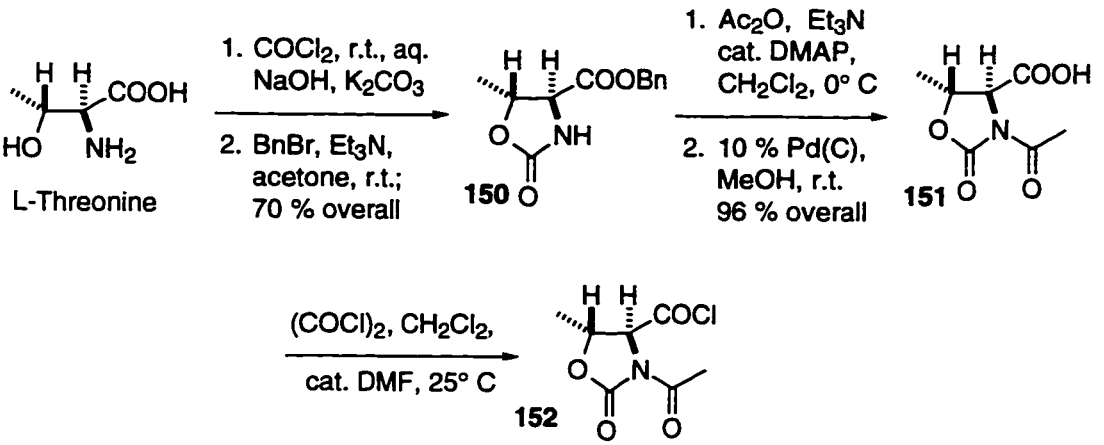


Figure 3-3

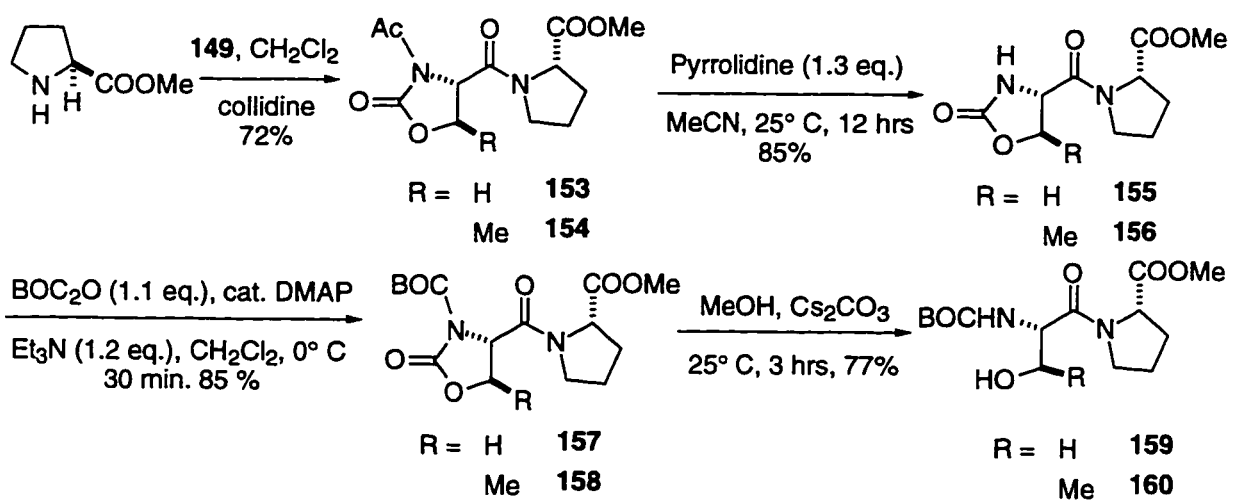
A similar acid chloride **152** derived from L-threonine was also prepared and studied. This material displayed reactivity identical to that of **149** (Scheme 3-21 and Table 3).[70]

A typical coupling procedure involved slow addition of a solution of crude acid chloride (1.3 eq.) in CH_2Cl_2 to a CH_2Cl_2 solution of receptor (1 eq.) and collidine (2.3 eq.) at 0° C. After stirring at 0° C for 2 hrs, the cooling bath was removed and the mixture was stirred at room temperature overnight. Extractive workup (partition between CH_2Cl_2 and dilute aq. HCl) and chromatography



Scheme 3-21

afforded pure products, all of which were obtained as thick, hygroscopic syrups that did not crystallize. Threonine derivatives were obtained in slightly better yields than the serine analogues. This is attributable to greater solubility in organic solvents, a property that facilitated extraction and chromatography.

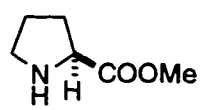
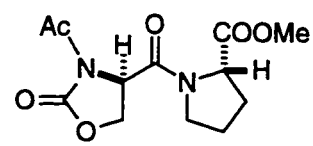
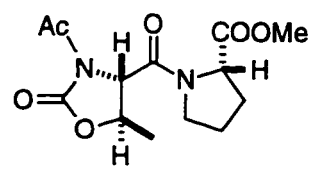
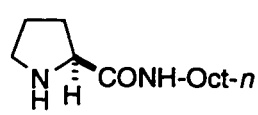
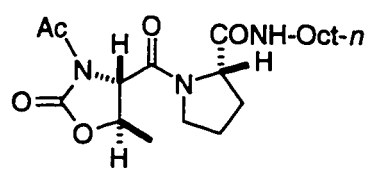
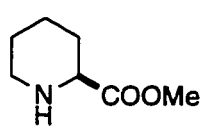
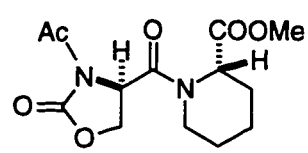
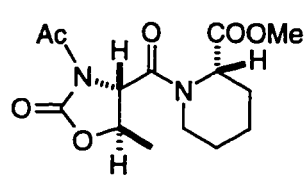


Scheme 3-22

Full deblocking of dipeptides **153** and **154** commenced with N-

deacetylation using pyrolidine in acetonitrile. Free oxazolones **155** and **156** were extremely polar and were not purified or extensively characterized. N-Derivatization with BOC_2O/cat. DMAP provided **158**, m.p. 177.0-177.5 (effervescence; recr. EtOH), and **157**, thick oil, $[\alpha]_D^{25}$ = –62.7°(c=0.063, CH_2Cl_2), in 85 % and 72 % yield, respectively. Because of extremely unfavorable solubility properties, the rotation of **158** could not be measured.

Table 3: Representative coupling reactions of acid chlorides **149** and **152**

chloride	receptor	product[a]	entry	$[\alpha]_D^{25}$ [b]	% yield[c]
149			**153**	–102.3° [d]	70
152	"		**154**	–36.6° [e]	72
152			**214**	–46.3° [f]	90
149			**215**	–51.1° [g]	91
152	"		**216**	–50.7° [h]	95

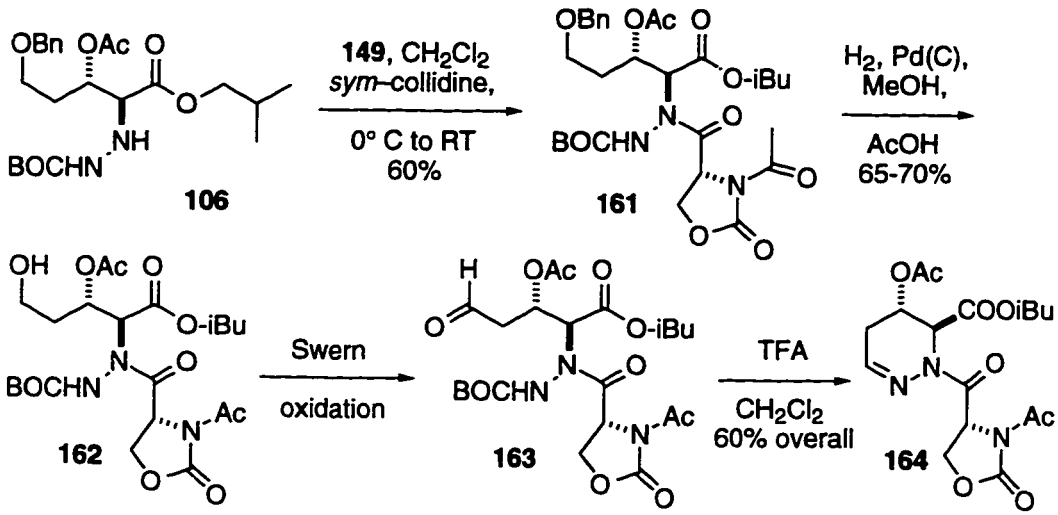
a. see text. b. All rotations were measured in CH_2Cl_2 solutions. c. Chromatographed (silica gel; ethyl acetate/hexanes mixtures) d c=0.110; e c=0.046; f c=0.130; g c=0.041; h c=0.019; m.p. 142.5-143.0° C.

Oxazolone cleavage was effected under Kunieda conditions[71] with Cs_2CO_3 in MeOH, resulting in N-BOC dipeptides **159**, oil, 77%, $[\alpha]_D^{25}$ = –35.1° (c=0.078, CH_2Cl_2), and **160**, oil, 79 %, $[\alpha]_D^{25}$ = –57.5° (c=0.057, CH_2Cl_2). Compound **158** is poorly soluble in MeOH. Fortunately, a methanolic suspension of this material was fully satisfactory for the Kunieda reaction, completion of which was visually signaled by the mixture becoming homogeneous. Proton and ^{13}C NMR spectra of **159** and **160** showed no evidence of erosion of stereochemical integrity of the aminoacid units; moreover, they revealed that **159** exists as a 4:1 mixture of rotamers, while in **160** the rotamer ratio is ca. 15:1.

3.4. Synthesis of Protected PCA-Serine Dipeptides

Armed with acid chloride **149**, we began to investigate the acylation of **106** and the formation of the PCA ring. We were greatly relieved to discover that the long-sought coupling product **161** emerged in about 50 % yield after chromatography when **106** and **149** were allowed to react in CH_2Cl_2 at 0° C in the presence of NMM. Further research indicated that the use of 2,4,6-collidine as the base in this reaction afforded better yields (55 - 60 % yield). Hydrogenation in methanol using Pd(C) as catalyst proceed very slowly: after 48 hours only about 20 to 25% of starting material had reacted. A proton source, AcOH, was then added to accelerate the reaction; however, acid also promoted formation of side products, the nature of which was not ascertained. Chromatography ultimately provided pure alcohol **162** in 65-70% yield. Swern oxidation of this alcohol smoothly yielded sensitive aldehyde **163**, which cyclized in 60 % overall chromatographed yield to protected PCA-serine

dipeptide **164** upon exposure to TFA in CH_2Cl_2 for 0.5 hours.

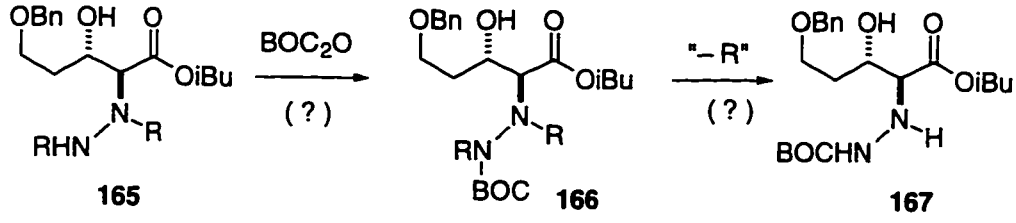


Scheme 3-23

If one considers the difficulties of the coupling reaction, this approach was quite straightforward: only 7 steps are needed after yeast reduction to reach dipeptide **164**. There are also few protection / deprotection sequences in the synthesis. However, several steps in this protocol needed to be improved to secure sizable quantities of **164** and to minimize the number of chromatographic separations, some of which were quite tedious. The most significant problem remained the conversion of bis-BOC **104** to mono-BOC **106**. This transformation, which proceed in a moderate 45-48% yield, could not be scaled up to more than 2 grams. The yield of the debenzylation step also needed to be improved: it is well recognized that this reaction can proceed in high yield. Finally, it seemed prudent to minimize the number of manipulations of sensitive intermediates containing the α-hydrazinoester unit. In that regard, we felt that adjustment of the oxidation state of C-5 of **100** would be best

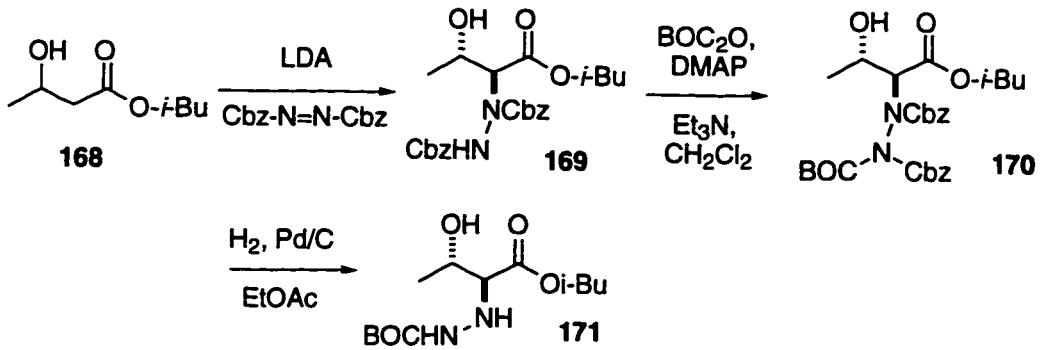
conducted prior to Gennari-Evans-Vederas ("GEV") reaction. At the same time, we deemed it desirable to retain formation of the PCA ring under acidic conditions, as demonstrated in the conversion of **163** to **164** (Scheme 3-23).

An excellent solution to the first problem was formulated as follows. A generic GEV compound, **165**, may be selectively acylated at the terminal hydrazine atom. If acyl groups R in **166** were selectively removable over a BOC moiety, then it might be possible to reach intermediate **167** by selective carbamate cleavage. However, R must give rise to an azodicarboxylate reagent, R-N=N-R, that functions well in GEV reactions.



Scheme 3-24

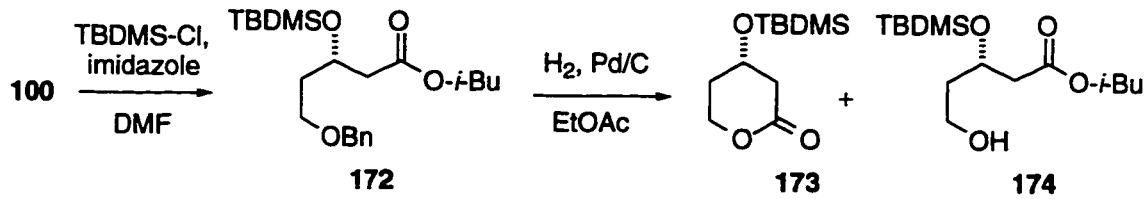
These requirements were nicely satisfied for R = benzyloxycarbonyl ("Cbz"), a commercial reagent that displays normal GEV reactivity. The Cbz groups in **165** would later be cleaved by hydrogenolysis. It was not known whether the NH-NHBOC group in product **167** would survive hydrogenation conditions, thus a model study was carried out with simple (±)-β-hydroxyester **168**. The dianion of this molecule condensed well with dibenzyl azocarboxylate, and treatment of the resulting **169** with BOC_2O in CH_2Cl_2 in the presence of Et_3N and DMAP as catalyst gave the desired **170**. It was pleasing to observe that the N-N bond in mono-protected hydrazine **171** survived the hydrogenation reaction unscathed: mono-BOC **171** compound was thus isolated in 94% yield (Scheme 3-25).



Scheme 3-25

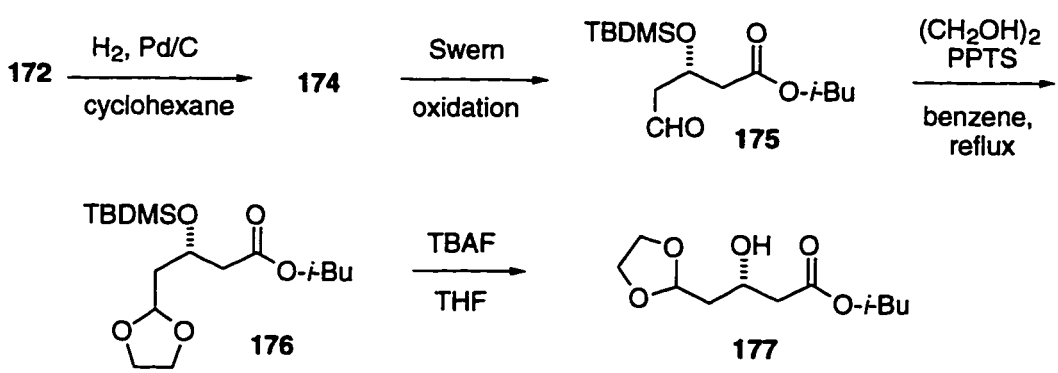
The advent of this new mild procedure for the preparation of mono-BOC hydrazine **167** allowed us to refocus our efforts on adjustment of the oxidation state of **100**. Introduction of an aldehyde equivalent before the GEV reaction was anticipated to be advantageous for several reasons. We would have more latitude in selecting conditions that might improve the debenzylation reaction and the following oxidation, since these steps would be carried out on simple, less sensitive compounds; furthermore, overall efficiency was expected to increase because fewer steps would be necessary to reach the PCA dipeptide after coupling with the serine reagent.

Among several protocols studied, the following solution proved to be especially effective. The secondary alcohol was protected as a tert-butyl dimethylsilyl (TBDMS) ether and the benzyl ether was hydrogenolyzed. Hydrogenation was most efficiently conducted in cyclohexane (95% yield), because polar solvents such as methanol or ethyl acetate promoted cyclization of alcohol **174** to lactone **173** as shown in Scheme 3-26.



Scheme 3-26

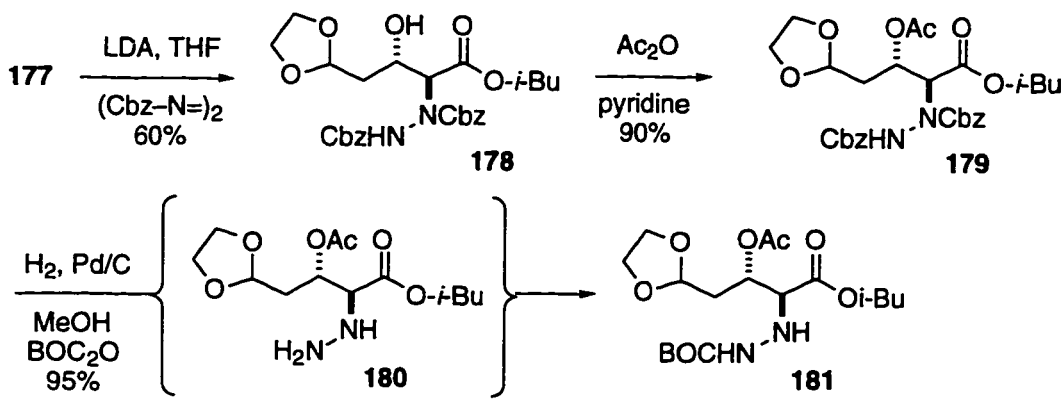
Swern oxidation of the primary alcohol **174** afford aldehyde **175** in 92% yield. Without further purification, the aldehyde was protected as the ethylene ketal using pyridinium para-toluenesulfonate (PPTS) as the catalyst. About 90% of the TBDMS group survived this transformation. Use of TsOH instead of PPTS as the acid catalyst resulted in much diminished yields of **176** (60-70%), probably because of to the instability of the β-oxyester function to the reaction conditions. Complete desylation of **176** was accomplished with tetrabutyl-ammonium fluoride (TBAF) and furnished alcohol **177** in 85% yield. Some β-elimination product (<5 %) was also detected in the reaction mixture (Scheme 3-27).



Scheme 3-27

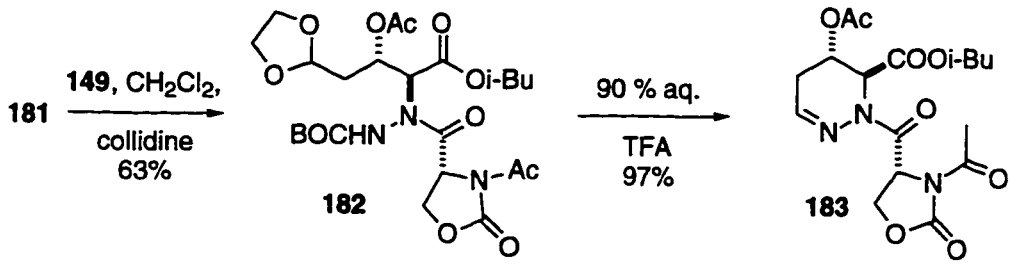
Compound **177** was deprotonated with excess LDA to form a dianion,

which reacted with Cbz-N=N-Cbz to afford hydrazine **178** in 60% yield. As before, the secondary alcohol was acetylated with Ac_2O in pyridine. Whereas the two-step sequence demonstrated in Scheme 3-25 worked quite nicely for the conversion of **179** to **181**, an even more direct and convenient route involved simply hydrogenolysis of **179** in methanol, in the presence of 1.5 mol equivalents of BOC_2O. Evidently, the sensitive hydrazine intermediate **180** was acylated by the BOC reagent faster that it could be hydrogenolytically cleaved. Compound **181** thus emerged in an outstanding 95 % yield (Scheme 3-28).



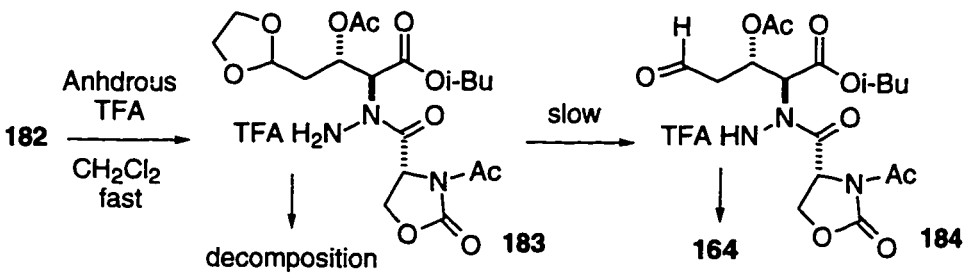
Scheme 3-28

The coupling of serinyl chloride **149** with **181** was performed in CH_2Cl_2 with *sym*-collidine as the base (63 %). Cyclization of **182** to PCA-serine dipeptide **164** occurred in 97% yield upon exposure to 90 % aqueous TFA (Scheme 3-29).



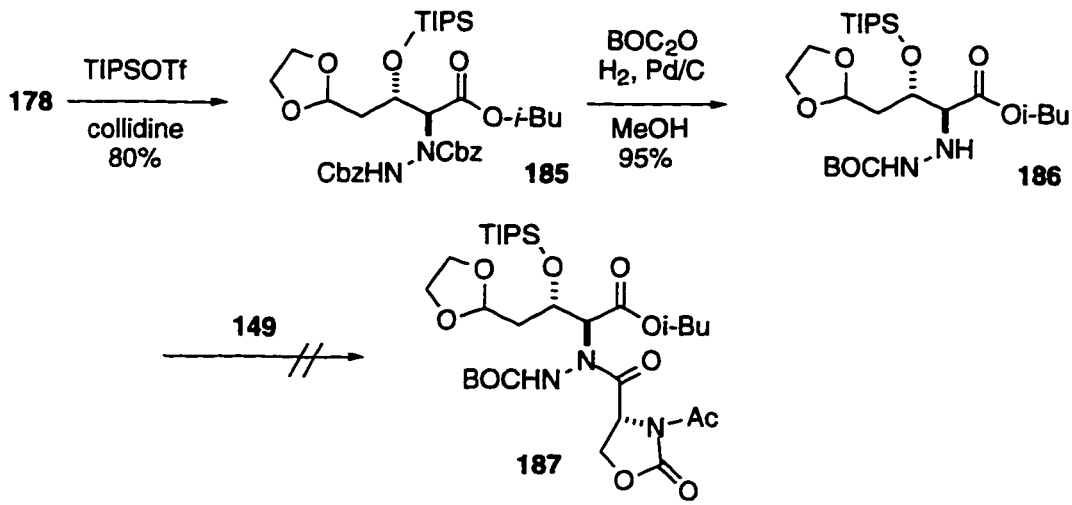
Scheme 3-29

Several aspects of this chemistry deserve additional comment. In sharp contrast to aqueous TFA, anhydrous 50% TFA in CH_2Cl_2 was entirely unsatisfactory for cyclization of **182** to **164** (only 20-30% yield). We suspect that the reason for this is the slow rate of dioxalane ring cleavage in the absence of water. Thus, the initially formed **183** can decompose faster than cyclization might occur.



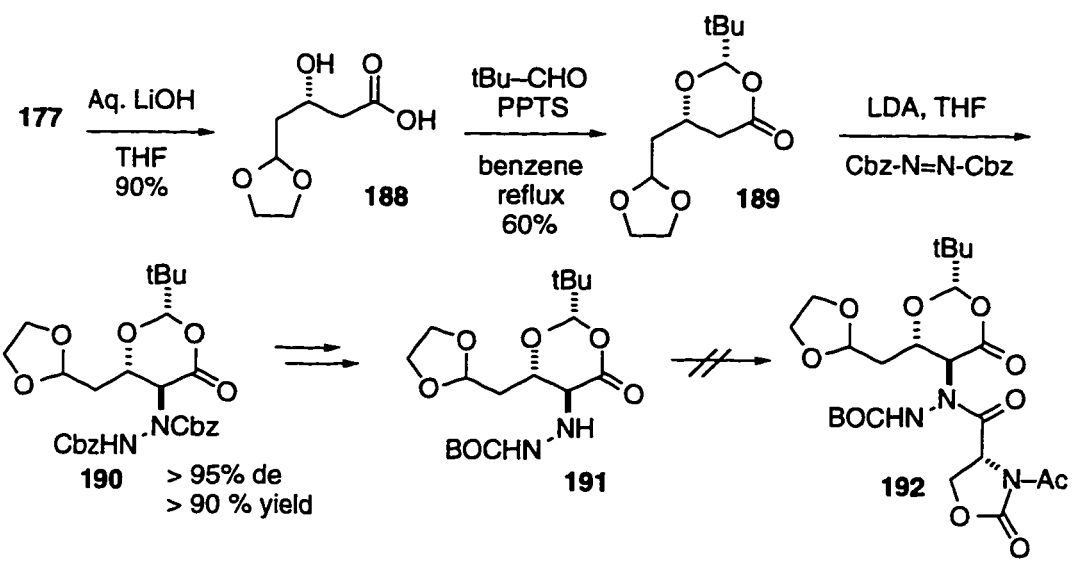
Scheme 3-30

Serinylation of mono-BOC PCA precursors of the type **178** succeeded only when the secondary alcohol was protected as an acetate. Thus, reaction of compound **178** with triisopropylsilyl (TIPS) triflate produced silyl ether **185** (80 %). Hydrogenolysis of **185** in the presence of BOC_2O afforded mono-BOC **186**, which decomposed upon attempted condensation with **149**.



Scheme 3-31

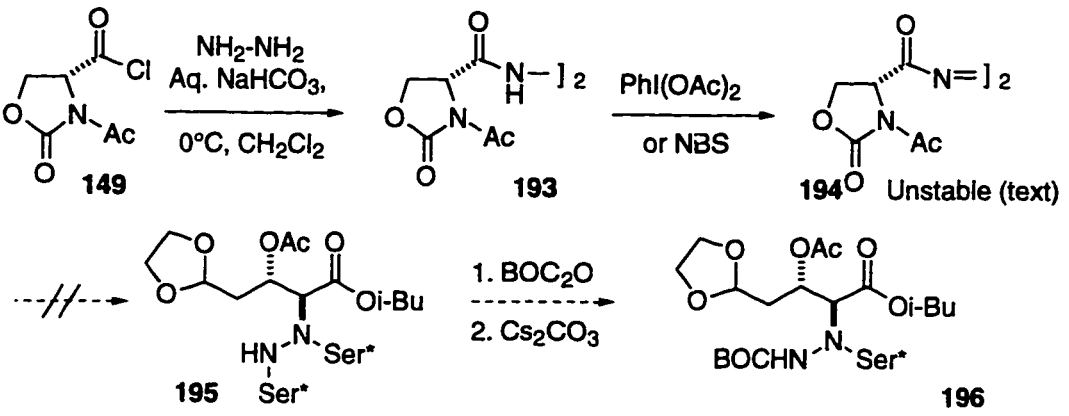
Similarly, 1,3-dioxanes such as **189**, the anions of which are known to react highly efficiently and diastereoselectively with azodicarboxylates, led to intermediates that failed in the acylation reaction.[72] Compound **191** was



Scheme 3-32

manufactured beginning with hydrolysis of **177** to β-hydroxyacid **188**. This material reacted with pivaldehyde in the presence of PPTS to give dioxane **189** in 55% yield.[73] Excellent chemical yield (90%) and diastereoselectivity (95% de) were achieved in the condensation of **189** with Cbz-N=N-Cbz and in the subsequent conversion of **190** to **191** (94%) (Scheme 3-32). However, the dioxane structure incurred major damage during attempted coupling with our serinyl chlorides.

An interesting idea that unfortunately led only to a synthetic dead end developed as follows. If an azodicarboxylate **195** could be produced from our serinyl chloride, it might be possible to cause it to condense with the dianion of **177**. Subsequent BOC derivatization of the terminal N atom might permit selective cleavage as shown in Scheme 3-33. Hydrazine **194** was obtained in high yield from **149**; however, treatment with oxidants normally employed for azodicarboxylate formation resulted in extremely fast decomposition of the intermediate **194**. This substance was spectroscopically detectable when oxidation was attempted in an NMR tube with constant ^1H monitoring, but it self-destructed in no longer than 2-3 minutes with copious evolution of gas, presumably N_2. A brief examination of the oxidation of various symmetrical and unsymmetrical diacyl hydrazines revealed that only bis-carbamoyl structures $R^1OOC\text{-}NH\text{-}NH\text{-}COOR^2$ (R^1, R^2 = allyl, tBu, Bn, 2,2,2-trichloroethyl) produced stable azodicarboxylates. Mixed carbamoyl-acyl and carbamoyl-sulfonyl species, $R^1OOC\text{-}NH\text{-}NH\text{-}COR^2$ (R^1 = Bn or tBu; R^2 = Me or n-octyl) or $R^1OOC\text{-}NH\text{-}NH\text{-}SO_2R^2$ (R^1 = Bn or tBu; R^2 = p-tolyl) underwent oxidation to presumed azodicarboxylates that decomposed instantly (gas evolution).[74] Decomposition was particularly vigorous, even violent, in the sulfonyl series.



Scheme 3-33

3.5. Deprotection of the Blocked PCA-Serine Dipeptide

The successful production of dipeptide **164** presented us with the task to develop a suitable deblocking procedure to reach **197** (Figure 3-4). It will be recalled that our general approach was to apply the Kunieda procedure. This requires N-deacetylation of **164** and introduction of an N-BOC group (cf. Scheme 3-22).

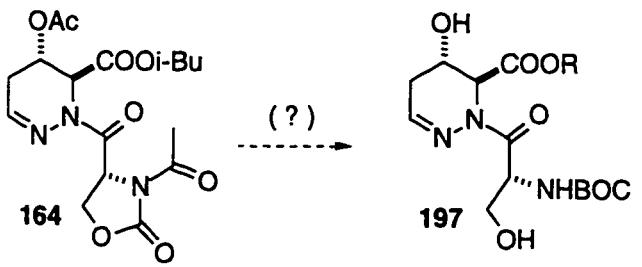
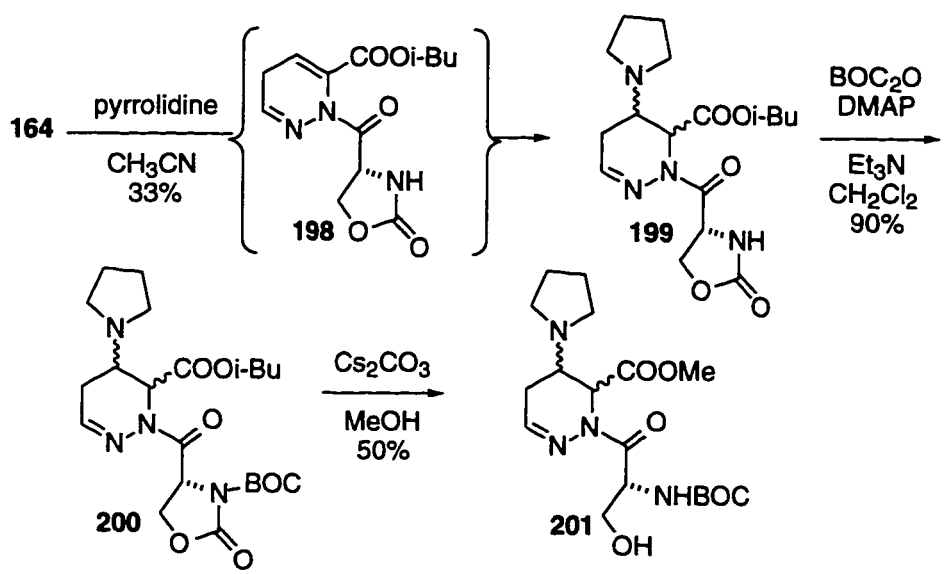


Figure 3-4

Unlike the dipeptides of Table 3-3, compound **164** did not undergo clean deacetylation. Pyrrolidine in acetonitrile, so successful with **153-154**, reacted with **164** to yield 30-35 % of compound **199**. Evidently, the reagent was sufficiently basic to promote β-elimination of acetate prior to undergoing Michael-type addition into intermediate **198**. We presume that a significant fraction of this material was lost to polymerization, thus accounting for the low yield. Substance **199**, formed as a mixture of diastereomers, was nevertheless



Scheme 3-34

useful to ascertain whether the serine unit would survive the Kunieda reaction. To that end, the oxazolone was derivatized with BOC_2O and the resulting imide **200** was subjected to the action of methanolic Cs_2CO_3. Oxazolone cleavage occurred normally (ca. 50 % yield) to afford **201**, the only side-reaction being the transesterification of the PCA portion as shown in Scheme 3-34. More

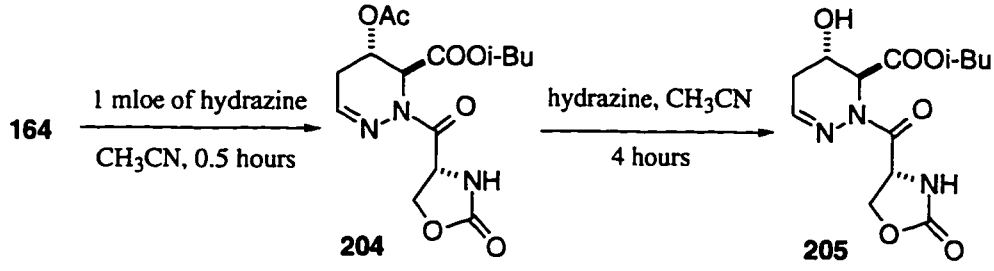
importantly, no obvious damage to the serine sector was apparent. Of course, the conditions of this step were not optimized. Other aliphatic amines, such as ethylamine, diethylamine, propylamine, piperidine and morpholine, also caused slow decomposition of **164** with no clean deprotection.

Deacetylation under hydrolytic or other nucleophilic conditions also failed, despite numerous attempts. In particular, the **164** was quite unstable in the presence of aqueous / organic bases such as LiOH, $KHCO_3$ and KCN. Available evidence suggests that, again, rapid β-elimination of acetate and polymerization of the resultant **198** were responsible for the almost immediate destruction of the substrate. The acyclic precursor **182** was even more base-sensitive, a fact that we account for by invoking the following stereoelectronic effect. The acyclic nature of **182** permits access to conformer **202**, which could suffer β-elimination through an E1cb mechanism displaying significant E2 character. In this manner, the extent of charge accumulation on the C atom α to the COOR (enolate formation) at or near the transition state would not be as great as in a *bona fide* E1cb process. This would lower the activation energy for elimination and facilitate its occurrence. No such conformational freedom is available to **164**. This molecule is likely to eliminate by a more energetic, true E1cb mechanism, because the *cis* relative stereochemistry of the acetoxy group and the proton α to the COOR bars them from attaining the *anti* relationship. In addition, the molecule greatly favors conformation **203**, in which both ester and acetoxy groups occupy axial positions, and in which the dihedral angle between the acetoxy group and the proton a to the COOR is essentially locked to about 60° (Figure 3-5).

202 203

Figure 3-5

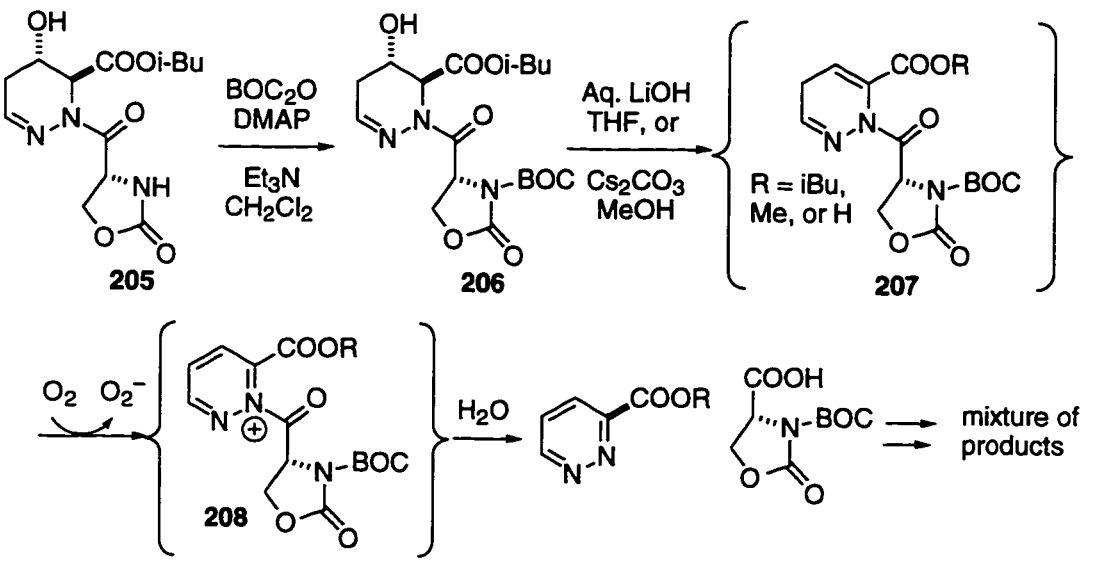
Success was finally attained by the use of hydrazine hydrate as the deacetylation agent. One stoichiometric equivalent of this highly nucleophilic, but feebly basic, reagent cleanly N-deacetylated the oxazolone in **164** in just 30 minutes. A small amount of alcohol **205** (2-3 %) was also formed during this step. Complete O-deacetylation was readily accomplished by exposing **164** to 2 equivalents of reagent for 4 hours (Scheme 3-35). We were now ready to pursue oxazolone cleavage.



Scheme 3-35

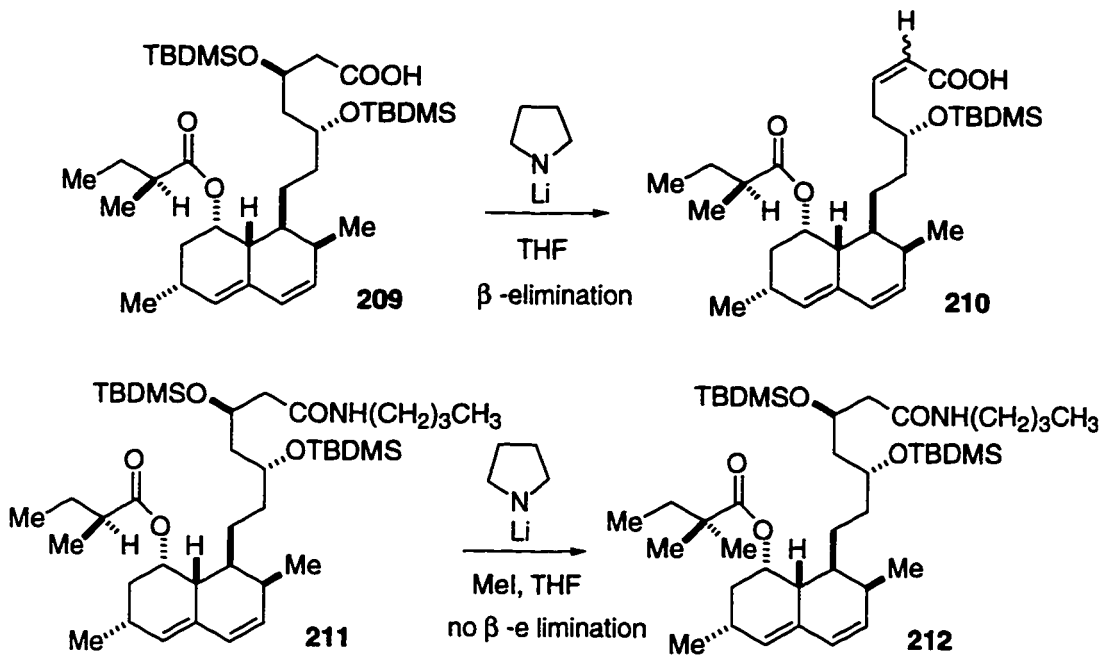
Treatment of **205** with BOC_2O afforded imide **206** in 95% yield. However, a complex mixture was obtained when **206** was exposed either to methanolic Cs_2CO_3 at room temperature, or to aqueous LiOH at temperatures ranging from 0° to 25°C. A close examination of the reaction mixture revealed that the amide bond between PCA and serine had been cleaved. Our hydrolytic conditions

were too mild to possibly cleave an amide, as evident also from the successful deblocking of the oxazolone in compound **200**. We suspect that release of serine from PCA involved initial, rapid β-elimination to intermediate **207**, which subsequently underwent air-oxidation to **208**. Rapid hydrolysis of this species would be expected even in the presence of plain water (Scheme 3-36).



Scheme 3-36

These experiments clearly established that a technique to suppress β-elimination had to be devised in order to take **206** on to more advanced intermediates. We based our subsequent moves on the hypothesis that elimination was proceeding though the E1cb mechanism. If so, drastic reduction of the Brønsted acidity of the proton α to the ester in **206** might permit survival of the PCA segment under basic conditions. A very good literature analogy was found in a report by a Merck group (Scheme 3-37),[75] wherein it was shown that conversion of free acid **209** to a secondary amide allowed

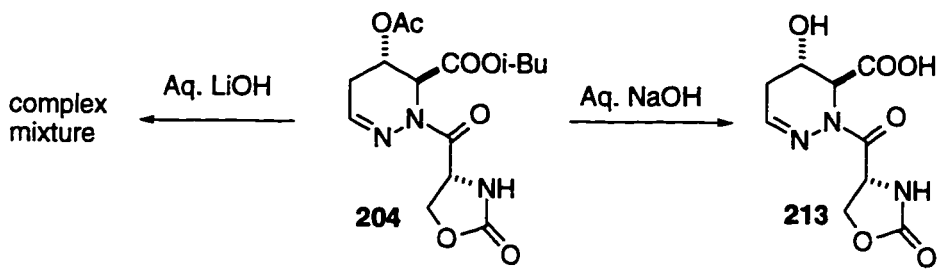


Scheme 3-37

based-mediated reactions elsewhere in the molecule without harm to the otherwise exceedingly sensitive β-hydroxycarbonyl unit (it has been reported that the effective pK_a of lithium carboxylates in THF is only a few units higher than that of the corresponding esters[76]). We concluded that it was necessary to convert the ester in **206** into a secondary amide,[77] and in that regard, it seemed especially reasonable to connect a glycine residue to **206**, as dictated by the structure of the target product.

Our new objective required prior hydrolysis of **205** to an acid, in preparation for coupling with a suitable glycine derivative. Compound **204** was easily soluble in water, removing the need for an organic co-solvent during base hydrolysis. Reaction of acetate **204** with aqueous LiOH again produced complex mixtures; however, aqueous NaOH proved to be satisfactory.

Compound **213** was obtained in essentially quantitative yield after only 5 minutes of contact time. We attribute the poor results observed with LiOH to the fact that the acetate carbonyl in **204** might have strongly coordinated the Lewis acidic Li^+ ion, thereby becoming activated for departure. (Scheme 3-38) It should be mentioned that contact of **213** with aquoues NaOH for longer times (12 hours) also resulted in decomposition.



Scheme 3-38

Protocols for the conversion of **213** to a suitable glycine amide derivative have not yet been developed. We preferred to refocus all of our attention to parallel studies directed toward the synthesis of luzopeptin E_2. It will be recalled that d-PCA, the PCA-like component of luzopeptin E_2, lacks the vulnerable β-hydroxycarbonyl function; therefore it is largely insensitive to the action of basic reagents that would rapidly destroy **206**. Our efforts to incorporate d-PCA into a pentapeptide monomer for luzopeptin E_2 were considerably more successful and are described in the following section.

Chapter 4
Synthesis of Tripeptides and Monomeric Derivatives of Luzopeptin E_2

4.1 Synthesis of dPCA-serine Dipeptide

The results described in the previous chapter allowed us to chart a very smooth route to the key fragment of luzopeptin E_2: the piperazic acid-serine dipeptide **217**. Our intent was to create intermediate dPCA-serine **218** by applying the same strategy detailed earlier for **164**, followed by reduction of the imino linkage at an appropriate time. Furthermore, it was anticipated that work on luzopeptin E would provide valuable insight into the question of macrocycle formation, an obligatory step in the synthesis of any member of the family.

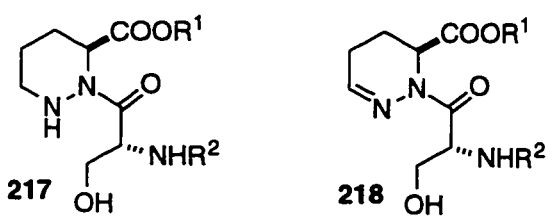
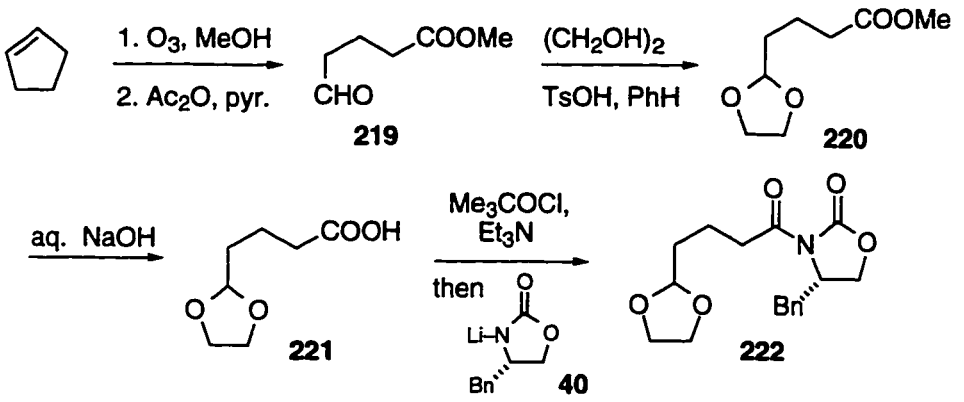


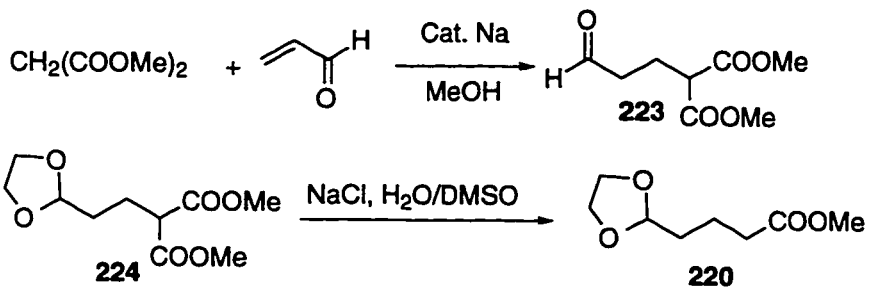
Figure 4-1

The nature of **218** dictated the use of Evans methodology for the creation of chirality. Accordingly, compound **219** was prepared by Schreiber ozonolysis[78] of cyclopentene followed by ketalization of the aldehyde, and the corresponding acid **221** was coupled with Evans auxiliary **40** (Scheme 4-1) after conversion

into the mixed pivalic anhydride. This procedure was decidedly superior to the literature method for the synthesis of **220** *via* Michael addition of malonic ester to acrolein, ketalization, and decarboxylation (Scheme 4-2).[79]



Scheme 4-1

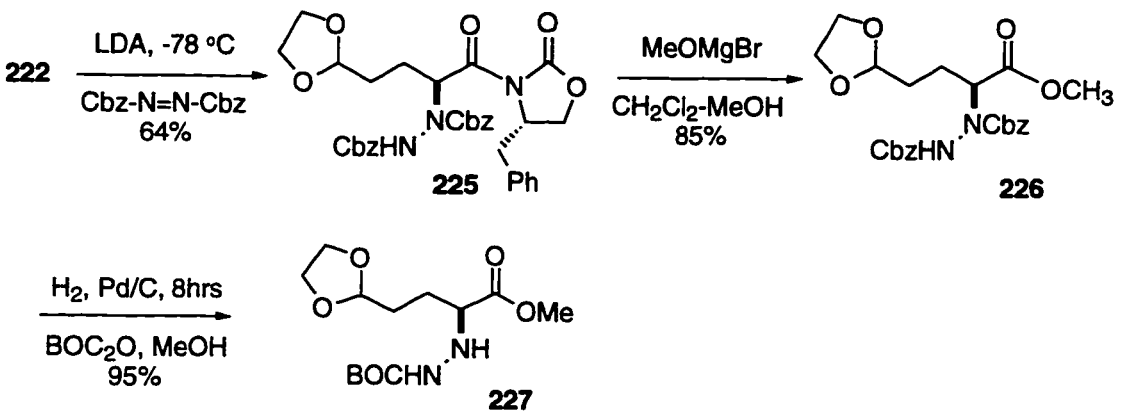


Scheme 4-2

Condensation of acetal **222** with Cbz-N=N-Cbz give imide **225** in 64% yield and in at least 97 % de (250 MHz ^{1}H NMR revealed only one diastereomer). At this juncture, the chiral auxiliary had completed its function and was excised by transesterification with MgOMgBr (Scheme 4-3).[80]

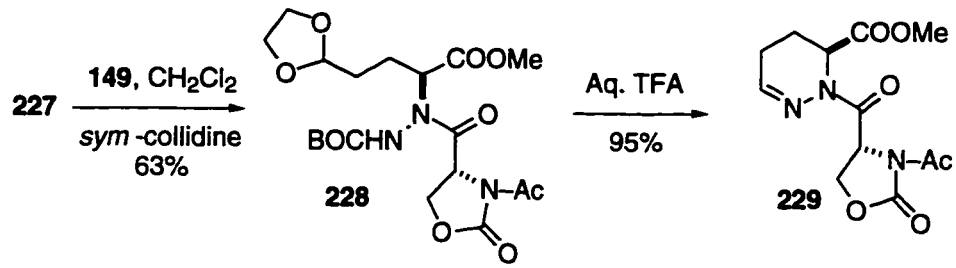
Hydrogenolysis of **226** in methanol with Pd/C as catalyst in the presence of BOC_2O completed in 8 hours and delivered **227** in 95 % yield. As discussed

earlier (p. 51), hydrogenolysis was sluggish when ethyl acetate was utilized as the solvent, regardless of whether a BOC group was present at the N(1) atom: even after 36 hours, 20-25 % of starting material still remained.



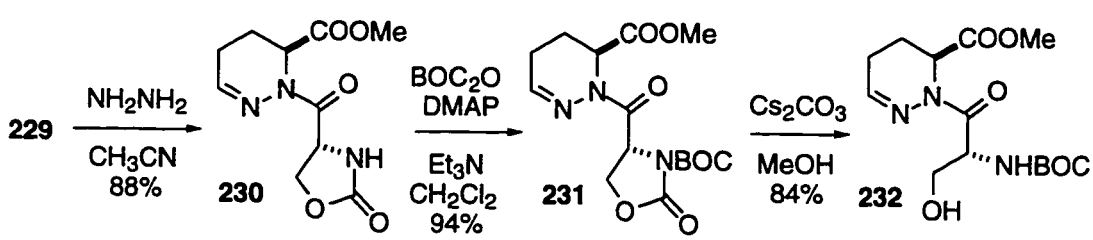
Scheme 4-3

The merger of **227** with serinyl chloride **149** proceeded well, so long as the contact time was limited to about 30 minutes, whereas longer exposure in an attempt to force completion resulted in diminished yields. A non-aqueous workup was also beneficial for maximum yield. Thus, compound **228** was reached in 63% yield after reaction of **227** with **149** at 0°C in the presence of collidine, followed by direct application of the reaction mixture to a silica gel column and elution with a gradient 50% EtOAc / hexanes → 100% EtOAc. Finally, ring closure with aqueous TFA at room temperature produced **229** as colorless crystals, m.p. 152-153°C, in 95% yield (Scheme 4-4).



Scheme 4-4

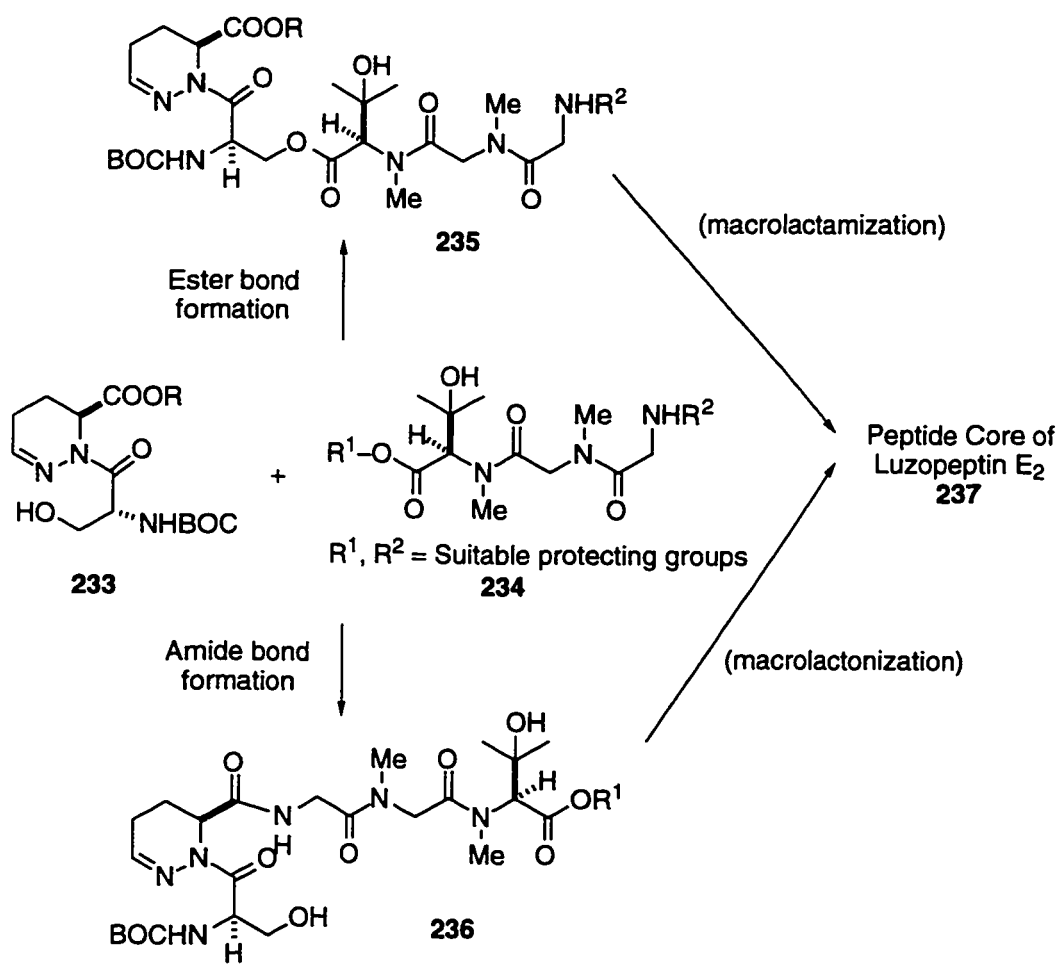
N-Deacetylation of the oxazolone ring with NH_2NH_2 in CH_3CN proceeded cleanly, and the resultant **230**, unlike its PCA-serine congener **205**, was quite stable to basic reagents. For instance, the compound was recovered untouched after 6 hours in methanolic Cs_2CO_3, a useful test of stability in light of the forthcoming Kunieda reaction. In that connection, an N-BOC group was introduced into the oxazolone under standard conditions, followed by methanolysis using Cs_2CO_3 as the catalyst. Alcohol **232** resulted in 84% yield (Scheme 4-5). It was particularly reassuring to observe that no harm resulted to the β-hydroxyamide unit of the serine sector. This reinforced our expectation that almost certainly a PCA amide (p. 60) would be a perfectly good substrate for this step.



Scheme 4-5

4.2 Preparation of a Key Tripeptide Segment of Luzopeptins

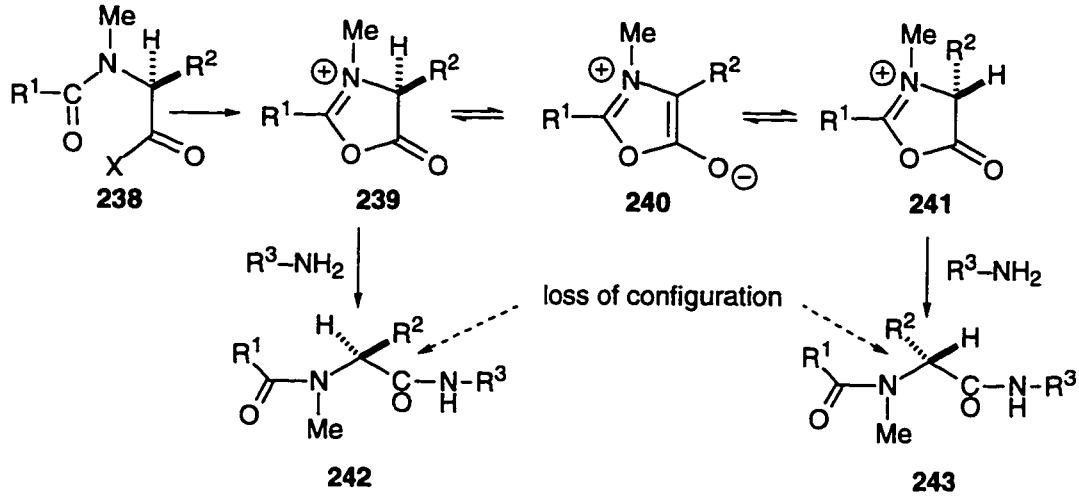
The successful preparation of dPCA-serine dipeptide **232** induced us to consider possible modes of incorporation into a monomeric precursor to the peptide framework of luzopeptin E_2. The precise nature of this monomer is a function of the strategy for macrocycle formation (p. 20): macrolactonization requires compound **236**, while macrolactamization demands peptide **235**. In either case, dipeptide **233** must be merged with tripeptide **234**, wherein the



Scheme 4-6

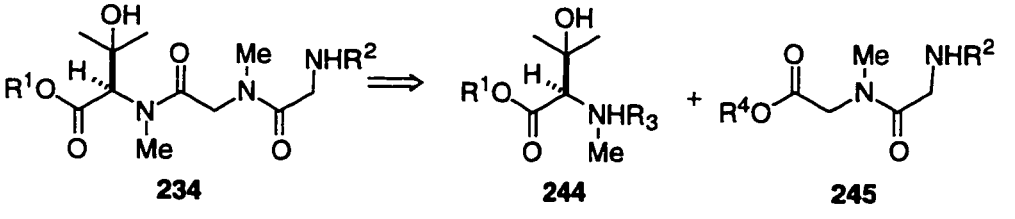
level of protection provided to the COOH and NH_2 termini must be different from that present in **234** (Scheme 4-6). The development of a good route to the tripeptide became our top priority at this time.

Our target peptide **234** contains two simple, commercially available amino acids, glycine and sarcosine, and an unusual one, L-N-methyl-3-hydroxylvaline, mhv, **5**. Two out of three residues in **234** are N-methyl amino acids, which present special difficulties in peptide chemistry. Steric hindrance is sometimes a problem during peptide bond formation with an N-methylamino group. Esters of N-methyl aminoacids tend to recemize by direct enolization, e.g., under saponification conditions, considerably faster than ordinary aminoacid esters. Finally, N-methyl amino acids are more prone to recemization during peptide synthesis than their unsubstituted counterparts, seemingly because of an increased tendency of the rather nucleophilic tertiary amide or carbamate carbonyl to cyclize to mesoionic species **240** after COOH activation (Scheme 4-7).



Scheme 4-7

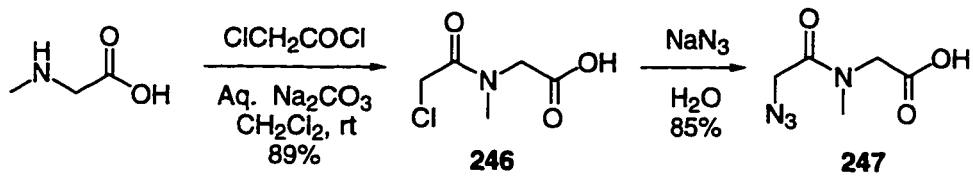
The most efficient way to synthesize **234** appeared to be the coupling of a preformed glycine-sarcosine dipeptide to mhv. In this manner, the most complex aminoacid unit would be incorporated into the peptide chain at a late stage, minimizing the number of manipulations of intermediates containing a residue available only by total synthesis (Scheme 4-8).



Scheme 4-8

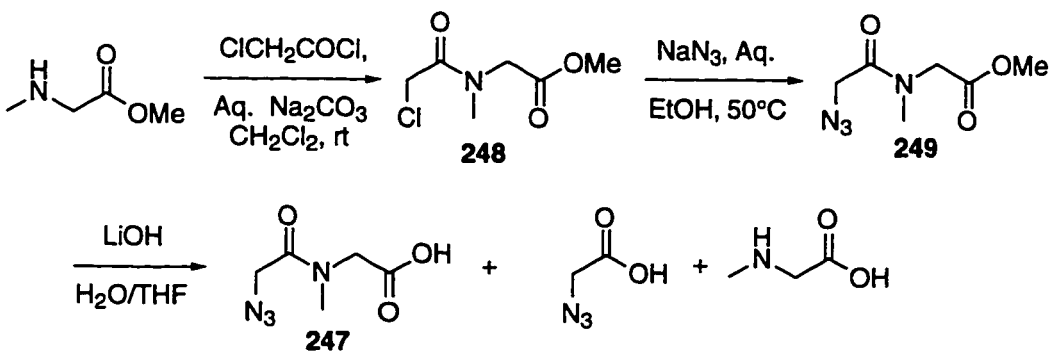
Most derivatives of glycine and sarcosine are only slightly soluble in common organic solvent, a fact that complicated the preparation of **245**. Poor yields where obtained when coupling of N-BOC or N-Cbz glycine with methyl sarcosinate was attempted with condensing agents such as DCC, EDCI, BOP-Cl and Mukaiyama reagent, even when the reactions were carried out in DMF as the solvent. A combination of poor solubility and steric effects conspired against the desired outcome.[81] It was surmised that the use of a readily handled glycine equivalent would greatly simplify the problem, and an especially good solution finally emerged as follows. Reaction of chloroacetyl chloride with free sarcosine in a biphasic medium composed of aqueous Na_2CO_3 and CH_2Cl_2 afforded acid **246** in 89 % yield (Scheme 4-9). Interestingly, this material proved to be readily amenable to silica gel chromatographic purification (100% EtOAc). Further reaction of freely water-

soluble **246** with sodium azide and purification of the crude product by chromatography afforded pure azido dipeptide **247** in 85% yield. The future NH_2 unit of glycine is present in this molecule as a readily handled, nonpolar azido group, which requires no protection.



Scheme 4-9

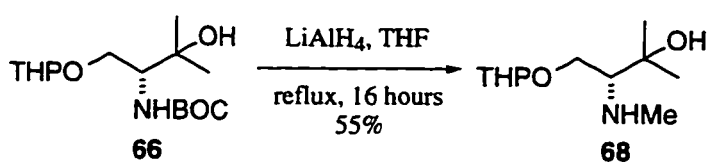
The same chemistry did not perform adequately with the methyl ester of sarcosine. Whereas the expected **249** was obtained in 76% overall yield, attempted basic hydrolysis of the methyl ester produced a mixture of desired product **247**, azidoacetic acid and free sarcosine. Such a facile amide cleavage was unexpected: perhaps the electron withdrawing azide group activated the acetyl group toward nucleophilic attack (Scheme 4-10).



Scheme 4-10

Parallel investigations were directed toward improving our original route to

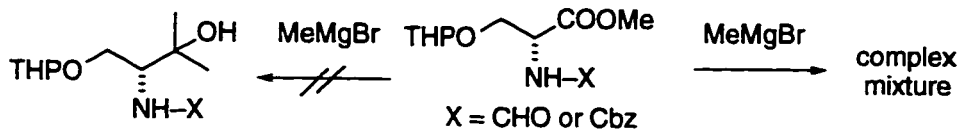
mhv, **5**. Two areas that required additional attention were the introduction of an N-methyl group on **66** and the oxidation of the primary OH to a carboxylic acid. A three-step sequence had been necessary to reach the first objective (Scheme 2-12, p. 17, ch. 2). It was realized that hydride reduction of BOC, or of an equivalent carbamate, would accomplish the same transformation in fewer steps. Direct reduction of BOC to a methyl group can be difficult; and indeed, when compound **66** was treated with 1.2 eq $LiAlH_4$ in THF at room temperature for 16 hours, less than 10% conversion to amine **68** occurred. However, conducting the same reaction in refluxing THF for 16 hours resulted in transformation of 50-60% of starting material to the product. For reasons that are not fully understood, lower yields were obtained when the reaction was refluxed for longer times. Fortunately, unreacted **66** could be easily separated from **68** (chromatography) and recycled (Scheme 4-11).



66 **68**

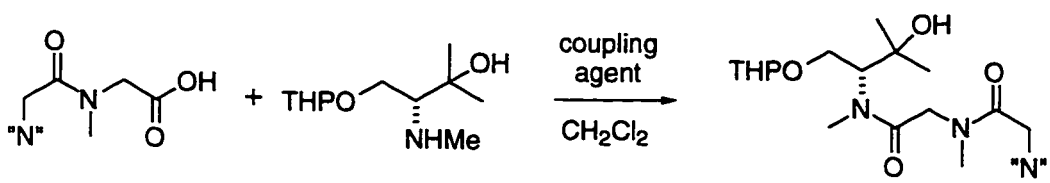
Scheme 4-11

Various attempts to improve this step by changing the BOC in **66** to a more readily reducible N-blocking group were not particularly successful, because the resulting intermediates, e.g., N-formyl or N-Cbz serine methyl esters, were poor substrates for the obligatory Gignard addition (mixtures of products) (Scheme 4-12).



Scheme 4-12

In principle, amine **68** could be coupled either with dipeptide **247**, or protected as a carbamate, in preparation for oxidation of the primary alcohol to a carboxylic acid. The most direct route to the desired **234** was clearly the first one, because it would minimize protection / deprotection steps. The seemingly trivial merger of **68** with **234** in fact required extensive experimentation. The efficiency of the reaction was a function of solvent, condensing agent, and nature of the protection present on **247** (Scheme 4-13). Briefly, best results were obtained using azidoacid **247** itself in CH_2Cl_2 with BOP-Cl as the coupling reagent. A summary of several experiments is presented in Table 4. Acids **250-252** were made from **247** by reduction of the azide with W-2 Raney Nickel and Schotten-Bauman N-acylation with appropriate chloroformate reagents or with BOC_2O (for **251**).



Scheme 4-13

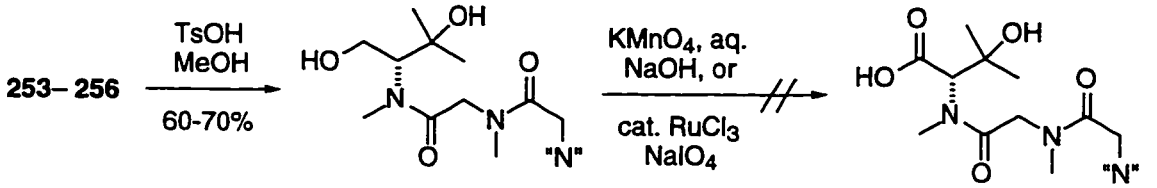
Table 4

Formation of the Tripeptides

Entry	"N"	Coupling Reagent	Chrom. yield %	Coupling product
250	Cbz-NH	DCC	30	**253**
250	Cbz-NH	BOP-Cl	50	**253**
251	BOC-NH	DCC	33	**254**
251	BOC-NH	BOP-Cl	45	**254**
252	Troc-NH	BOP-Cl	50	**255**
247	N_3	DCC	35	**256**
247	N_3	Mukaiyama	30	**256**
247	N_3	BOP-Cl	62	**256**

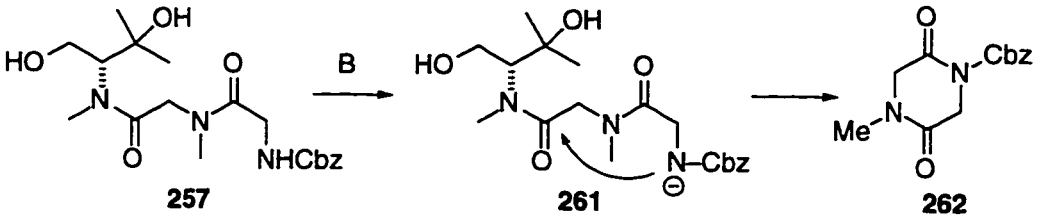
Removal of the THP group in **253-256** was effected by transacetalization using TsOH as catalyst in methanol. Compound **257-260**, extremely polar compounds, were thus obtained in 60-70% yield after brief filtration-type chromatography. The elevated polarity of **257-260** and some difficulties encountered in its visualization on TLC plates probably contributed to lower the yield of this step. An even more troublesome problem was observed during the subsequent oxidation of the alcohol to the acid. Unlike our earlier system **70** (Scheme 2-12, p. 17, ch. 2), compound **257-260** reacted poorly with either $KMnO_4$ / NaOH (Garner conditions)[30] or with $RuCl_3$ / $NaIO_4$ (Sharpless conditions)[82] (Scheme 4-14) The precise nature of the problem remains unclear. The basic conditions of the Garner oxidation may have promoted the now familiar cleavage of azidoacetyl group in **260**, while substrates **257-258**, wherein the glycine terminus is present as a secondary carbamate, may have experienced reversible formation of anion **261**, which might well have been

oxidized by KMnO4. The same anion might also have undergone cyclization to diketopiperazine **262** (Scheme 4-15).[34] It is noteworthy that the successful substrate **70** displays only tertiary amides / carbamates, which cannot engage in similar reactions. The reasons behind the failure of the Sharpless oxidation are even less obvious.



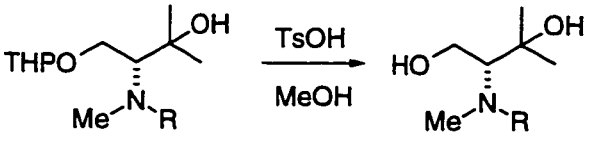
"N" = **257** Cbz-NH
258 BOC-NH
259 Troc-NH
260 N_3

Scheme 4-14



257 **261** **262**

Scheme 4-15

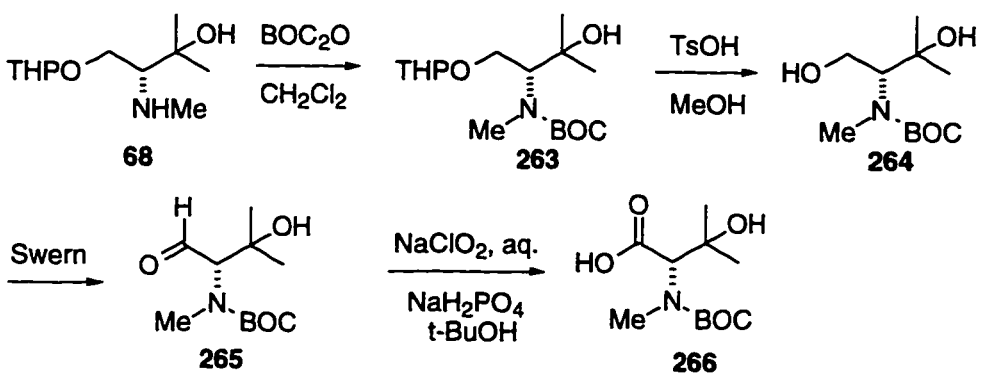


R = BOC, Cbz, Troc or Fmoc

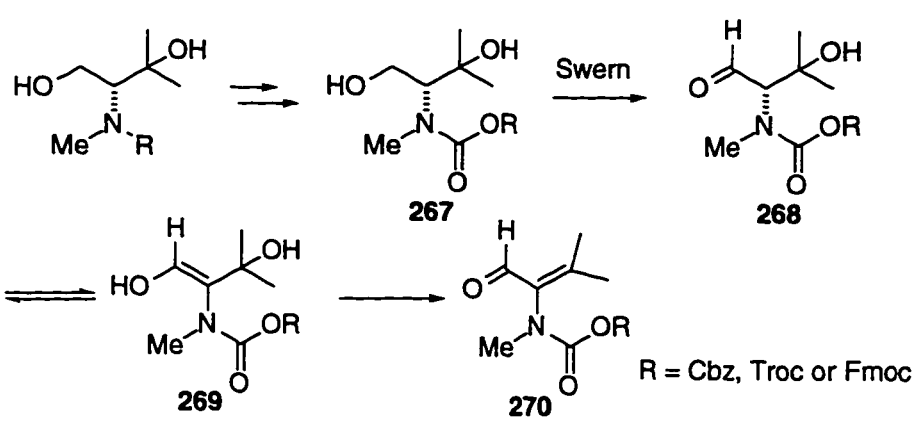
Figure 4-2

This turn of events led us to explore the second option. Amine **68** was

protected as several carbamate derivatives prior to THP removal (Figure 4-2). The resulting alcohols, which were subjected to Garner or Sharpless oxidation, gave mixed results. Fortunately, the following two-step protocol resolved most of these difficulties. Swern oxidation of **264** to the highly sensitive aldehyde **265**[83] was effected in THF (–78°C), since the starting **264** is poorly soluble in CH_2Cl_2, the traditional solvent for this reaction.[84] Further oxidation of **265** was carried out with $NaClO_2$ in buffered aqueous solution,[85] whereupon acid **266**



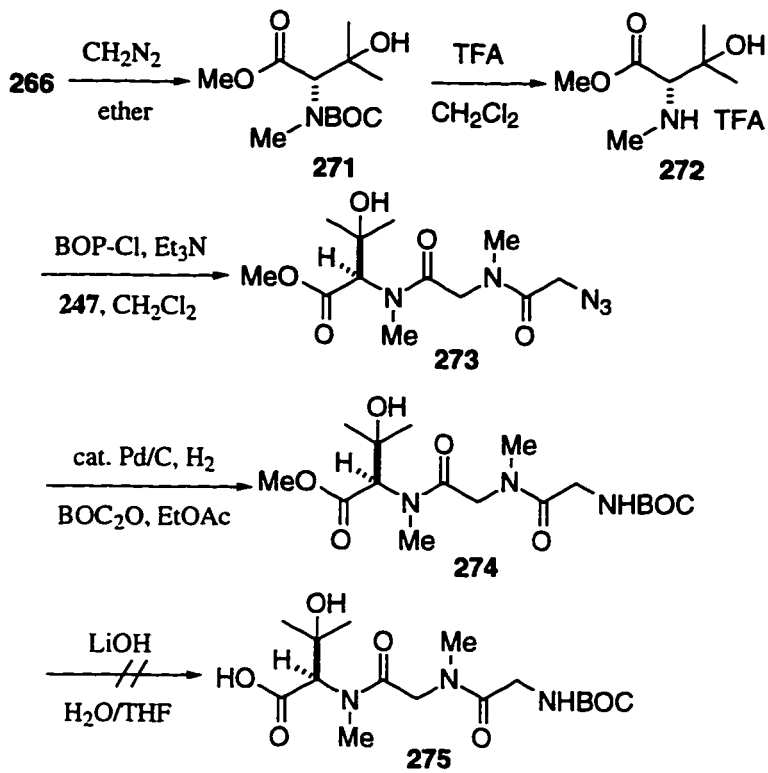
Scheme 4-16



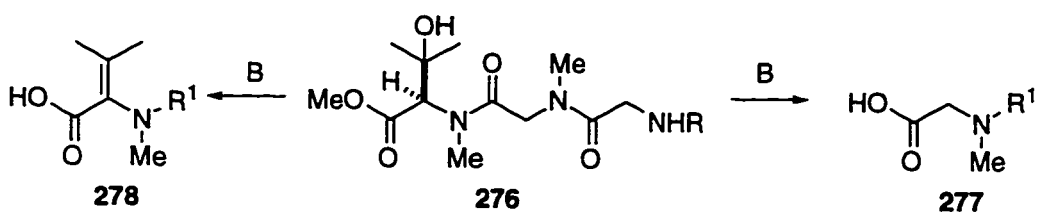
Scheme 4-17

was obtained in 85 % overall yield. We were surprised to find that only BOC derivatives gave high yields in this sequence (Scheme 4-16). Swern or TPAP/NMO oxidation of Cbz, Fmoc and Troc analogues of **264** produced substantial amounts of dehydroamino acids **270** (Scheme 4-17). Seemingly, a BOC group strongly disfavors enolization of the aldehyde and consequent β-elimination of the OH group.

In preparation for coupling with **247**, acid **266** was converted to the methyl ester with CH_2N_2. It was our hope that we could later cleave the methyl ester under mild basic conditions. Treatment of **271** with TFA in CH_2Cl_2 produced amine salt **272**, which, without purification, was coupled with dipeptide **247** using BOP-Cl. Tripeptide **273** was thus formed in 80% chromatographed yield. Unfortunately the azide in **273** was not compatible with the basic conditions required for ester hydrolysis. Two corrective measures were studied, the first one being reduction of the azide to an amine and subsequent N-protection as a carbamate. This transformation was accomplished in one pot by hydrogenolysis with Pd/C in the presence of BOC_2O, resulting in formation of compound **274**. However, hydrolysis of the methyl ester with LiOH in aqueous THF/MeOH again failed to deliver the desired acid **275** (Scheme 4-18). Although considerable care was used in the execution of the reaction, even at low temperature and in the absence of excess alkali, decomposition still accompanied saponification. Possible side reactions again included cyclization to a diketopiperazine (Scheme 4-15), as well as β-elimination of water from mhv and retroaldol loss of acetone (Scheme 4-19).



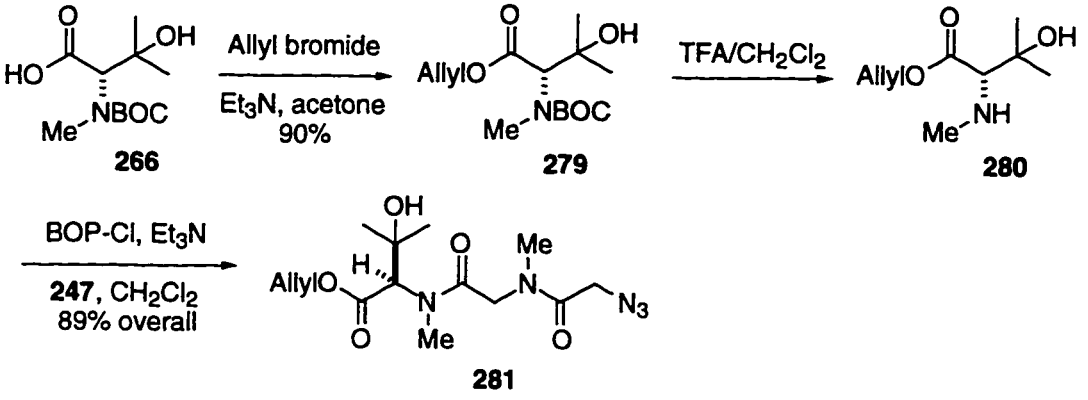
Scheme 4-18



Scheme 4-19

The apparent instability of **274** to basic conditions placed upon us the requirement for COOH and amino blocking groups for **234** that could be removed under neutral conditions. The azido unit fits this requirement very nicely, as it may be converted to a free NH_2 by catalytic as well as chemical reduction, e.g., under Staudinger conditions.[86] An ideal COOH protecting unit was identified in the allyl ester, which may be readily cleaved under the catalytic

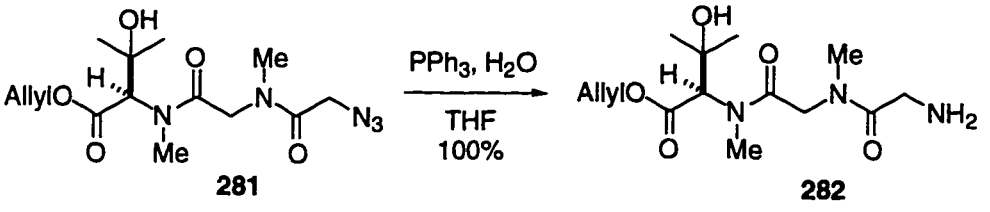
influence of Pd(0). Allyl bromide reacted with acid **266** to give allyl ester **279** in 90% yield without noticeable side reactions. BOC cleavage with TFA in CH_2Cl_2 afforded amine salt **280**, which again coupled with **247** in very good yield (88 %) under the influence of BOP-Cl (Scheme 4-20).



266 **279** **280** **281**

Scheme 4-20

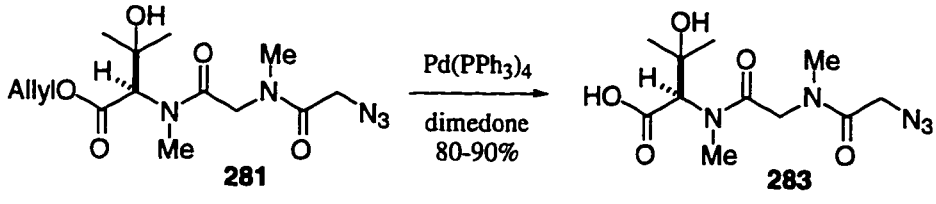
With tripeptide **281** now securely in hand, selective release of COOH and amino termini was investigated in detail. Reduction of the azide was readily achieved under Staudinger conditions[87] with PPh_3 in the presence of water.[88] Although the by-product of this reaction, $O{=}PPh_3$, was difficult to separate from **282**, the crude mixture could be directly used in the next step, the coupling of **282** with dPCA-serine dipeptide **233** (R = H, **284**). It was not likely that $O{=}PPh_3$ would interfere the coupling reaction, and indeed, it might assist it.[89]



281 **282**

Scheme 4-21

Selective deprotection of the allyl ester was best accomplished using catalytic $Pd(PPh_3)_4$ and dimedone as the acceptor. The product, free acid **283**, was purified by chromatography (Scheme 4-22).



Scheme 4-22

Complex mixtures were obtained when dimedone was replaced by amines, probably because of the base sensitivity of **283**. Even weakly basic morpholine, which is commonly used in similar operations, was entirely unsatisfactory in the present case.

Our experience with the synthesis of deceptively simple tripeptide **281**, as well as with the PCA-serine dipeptides **197** and **232**, led us to a most thorough appreciation of the following statement, found in the introduction to M. Bodanszky's landmark book, "*Peptide Chemistry*"[90]

> *The synthesis of a peptide with a well defined sequence of amino acid residues is a fairly complex process which appears simple only for those who have never been involved in it. This complexity does not follow from the construction of the peptide bond: numerous methods are available for that task, most of them quite good, and the only problem in*

this respect is to select the most suited for the actual bond under consideration. The more demanding part of the discipline of peptide synthesis is the need to block those functional groups which should not participate in the peptide bond forming reaction.....

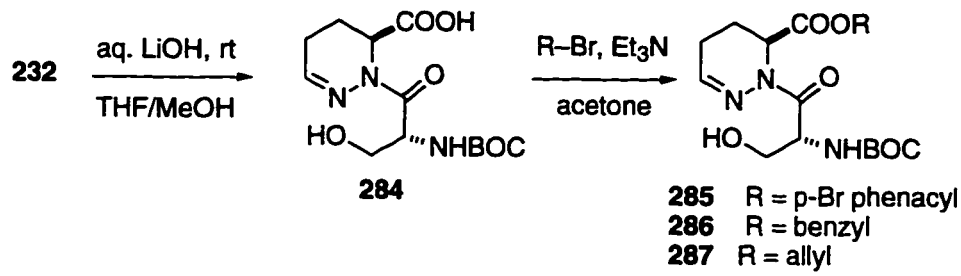
The events detailed in the following paragraphs further reinforce the veracity of the above comments.

4.3 Monomeric Precursors to the Framework of Luzopeptin E_2

The successful conclusion of our efforts toward dipeptide **232** and tripeptide **281** prepared the grounds for an investigation of their merger into pentapetides **235** or **236**. It will be recalled that these substances are the monomers of the dimeric peptide scaffolding of luzopeptin E_2, and that their dimerization would involve macrolactamization and macrolactonization, respectively.

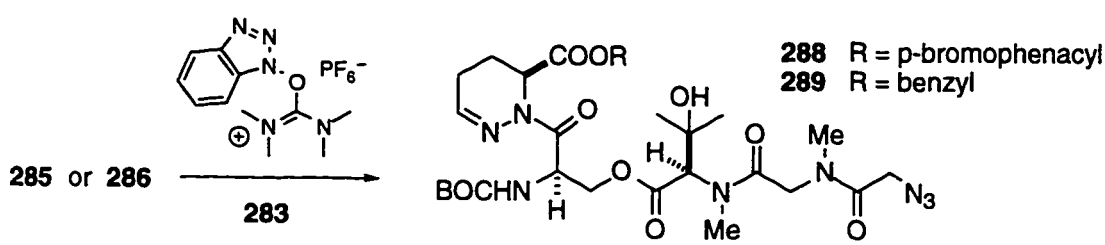
Pentadepsipeptide **235** seemed available by esterification of **283** with the free serine OH. However, the base-sensitivity of the tripeptide was likely to be carried over into **235**. Therefore, it seemed prudent to replace the COOMe in **232** with a different ester that might be cleavable under essentially neutral conditions. Hydrolysis of **232** with aqueous LiOH proceeded without incident to furnish acid **284**. Three different esters, **285**, **286**, and **287**, were subsequently prepared through reaction of **284** with p-bromophenacyl, benzyl, and allyl bromide, respectively (Scheme 4-23). Methods for the release of esters **286** an **287** have been discussed in the preceding sections. Para-bromophenacyl (BPH) ester **285** may be cleaved by treatment with soft

nucleophiles such as thiophenoxide ion or under mild reductive conditions with Zn and acid.[91] The latter protocol, however, would also reduce the azide.



Scheme 4-23

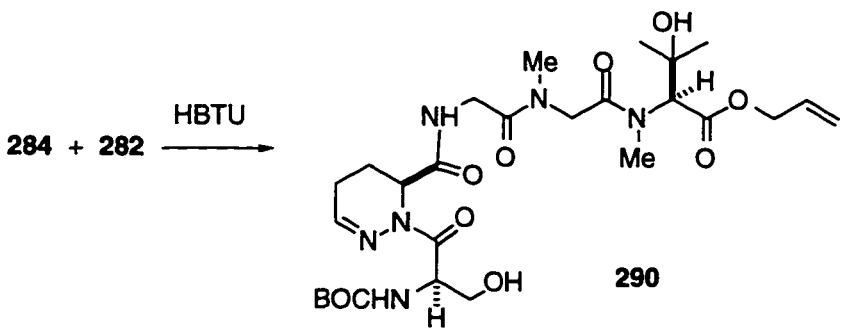
The crucial coupling of tripeptide **283** with esters **285-287** was quite sensitive to the nature of the condensing agent. Benzyl and BPH esters behaved similarly in coupling reactions. The Steglich procedure (DCC/DMAP) did afford depsipeptide monomers **288** and **289**, but only in 15 % yield after 24 hours. Much unreacted starting material remained. When 3 equivalents of acid **283** was used to drive the reaction to completion, the yield of **288** or **289** increased to 40% after 24 hours. When HBTU was used as coupling reagent, the reaction finished in 10 hours to give monomers **288** or **289** in 45-50% yield, but more than 3 equivalents of acid was still needed to force the reaction to proceed (Scheme 4-24).



Scheme 4-24

By contrast, the allyl ester **287** proved to be a poor substrate for this step. While the reasons for this remain obscure, a slate of condensing agents (DCC/DMAP; BOP-Cl/NMM; HBTU; Mukaiyama's reagent/NMM) produced virtually none of the desired depsipeptide. Neutral coupling conditions permitted recovery of unchanged starting materials, while basic agents, that is, those requiring NMM, promoted destruction of the substrates.

The synthesis of pentapeptide **290** was more successful, undoubtedly because it is generally easier to prepare an amide than an ester, and because the PCA acid and glycine amine components are less sterically encumbered. Thus the free amine **282** was condensed with acid **284** under the influence of DCC, or, better, of HBTU. The latter reagent furnished compound **290** in 55% chromatographed yield. Again, this coupling reaction was carried out without any base present (Scheme 4-25).



Scheme 4-25

4.4 Conclusions

In an effort to synthesize the luzopeptins, a concise and practical route to PCA was developed. A novel serinyl chloride was devised in conjunction with the formation of PCA-serine dipeptide derivatives. An efficient protocol to the mono-BOC **181** is now available for the further synthetic endeavor in the luzopeptin area. All these methodologies were successfully applied to the problems of synthesis of luzopeptin E_2 and two monomeric derivatives have been realized. Those achievements have paved the road to the ultimate goal of total synthesis of luzopeptins.

Chapter 5
Experimental

TECHNICAL NOTES

Melting points (mp.), determined on a Fisher-Johns apparatus, are uncorrected.

Infrared (IR) spectra were recorded on a Nicolet 205 FT-IR Spectrometer and are reported in wavenumbers (cm^{-1}).

^{1}H NMR (250 MHz) and ^{13}C NMR (62.5 MHz) spectra were determined on a Bruker AC-250 instrument. Chemical shifts are reported in parts per million (ppm). The following abbreviations are used for spin multiplicity: br.=broad, s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, m=complex multiplet.

Mass spectra (MS) were obtained on a Finnigan MAT95. Base peak was indicated as "(100)"

Analytical thin layer chromatography (TLC) was performed on Merck precoated analytical plates, 0.25 mm thick, silica gel 60 F_{254}.

Column chromatography was performed on grade 62 silica gel, 60-200 mesh, 150 A.

Reagents and solvents were commercial grade and were used as supplied with the following exceptions:

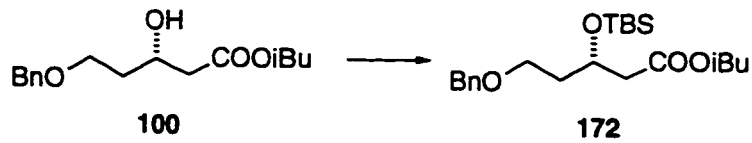
Dichloromethane: distilled over calcium hydride.

Tetrahydrofuran: distilled from sodium benzophenone ketyl

Ethyl acetate: distilled

Methanol: distilled form CaH_2

All moisture or oxygen sensitive reaction were conducted under an argon atmosphere.



100 **172**

To a solution of compound **100** (10g, 0.0357mol) in 6mL of DMF was added 2.91g imidazole (0.0428mol, 1.2eq) and 5.92g TBS-Cl (0.0393mol, 1.1eq). The reaction was stirred at room temperature for 16 hours. The mixture was then diluted with 100mL of EtOAc and was washed with 20mL of water, then with 20mL of brine. The organic phase was dried over Na_2SO_4, concentrated *in vacuo* and passed through a short silica gel column to afford 12.7g of **172** as a yellow oil (0.0321mol, 90%).

$[\alpha]_D^{25}$ = +3.58° (0.081g/mL CH_2Cl_2)

^{1}H NMR ($CDCl_3$): 0.042(3H, s), 0.055 (3H, s), 0.86 (9H, s), 0.92 (6H, d, J = 6.6Hz), 1.8 - 2.0 (3H, m, overlapping), 2.49 (2H, d, J = 6.1Hz), 3.55 (2H, d, J = 6.6Hz), 3.84 (2H, dd, J_1 = 3.4Hz, J_2 = 6.6Hz), 4.48 (2H, d, J = 2.4Hz), 7.2 - 7.4 (5H, m)

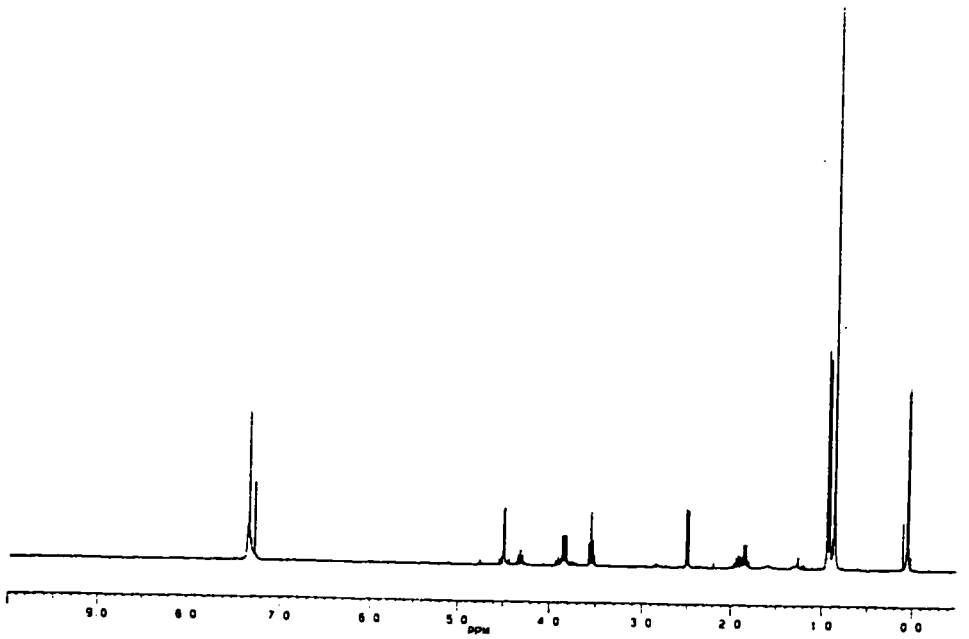
^{13}C NMR ($CDCl_3$): 17.9, 19.0, 19.1, 25.7, 25.9, 27.6, 37.3, 42.9, 43.1, 66.5, 66.9, 70.5, 72.8, 127.4, 127.5, 128.3, 138.4, 171.6

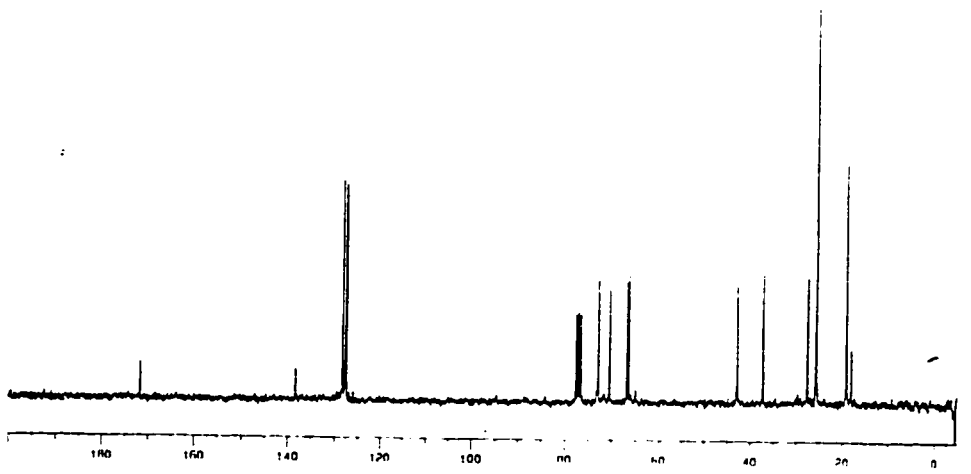
IR (film): 698, 737, 778, 838, 939, 1007, 1100, 1171, 1256, 1379, 1472, 1737, 2855, 2926, 2957

MS (EI): 57, 75, 91 (100), 105, 131, 145, 173, 189, 203, 245, 287, 321, 337, 351, 395 (M^++1)

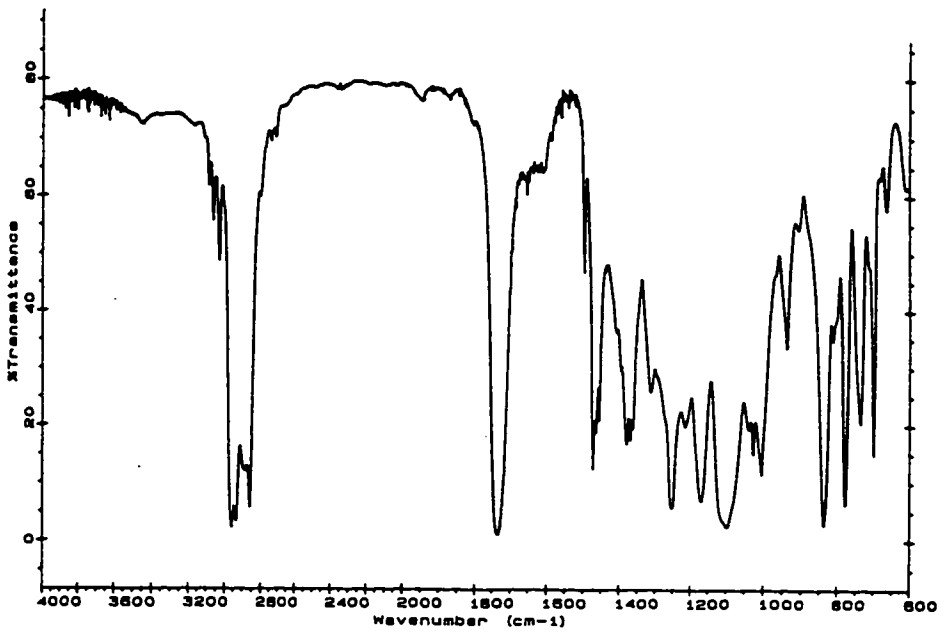
HRMS: expected ($C_{22}H_{38}O_4Si$, M^+): 394.2540; observed: 394.2539

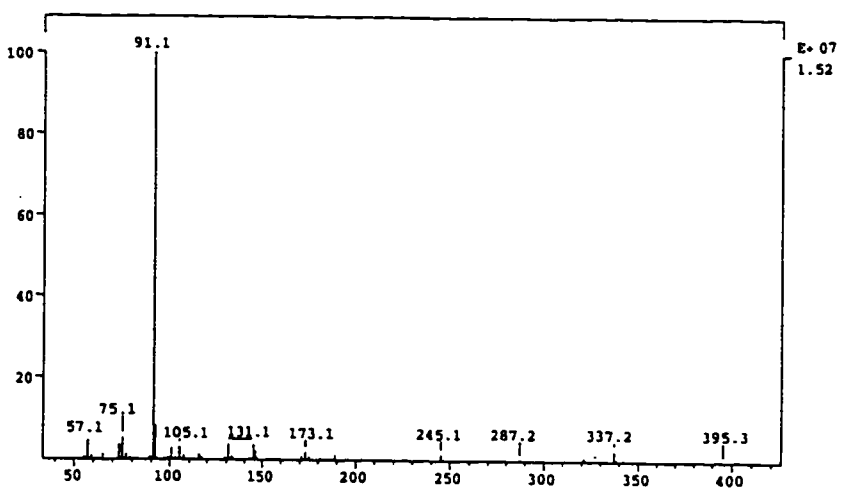
Compound **172**

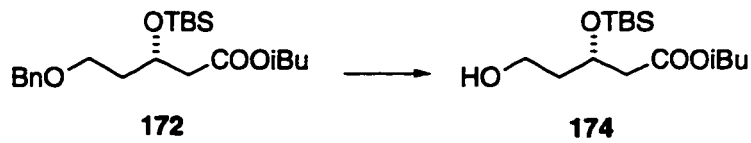




Compound **172** continued





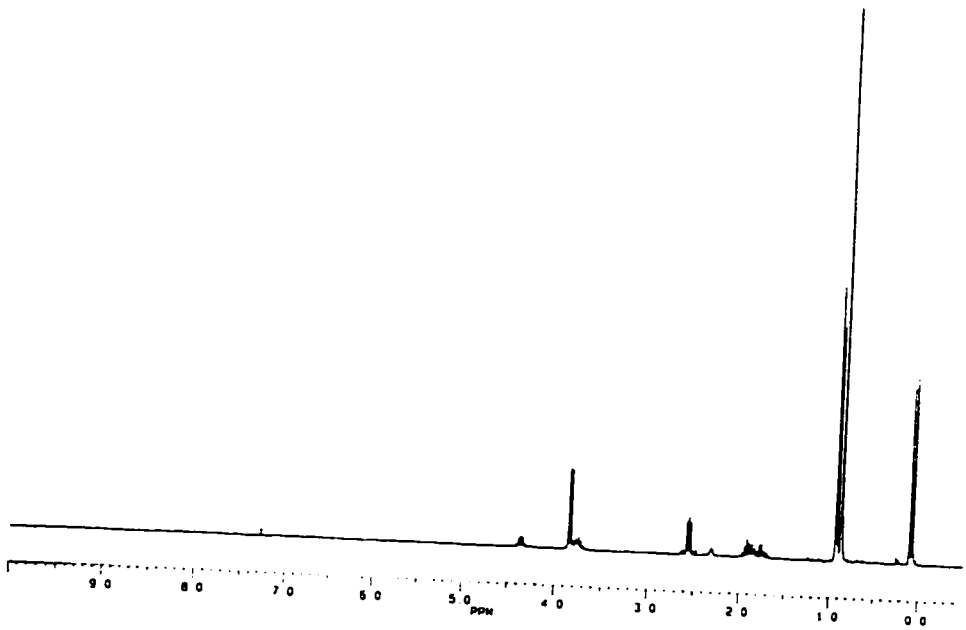


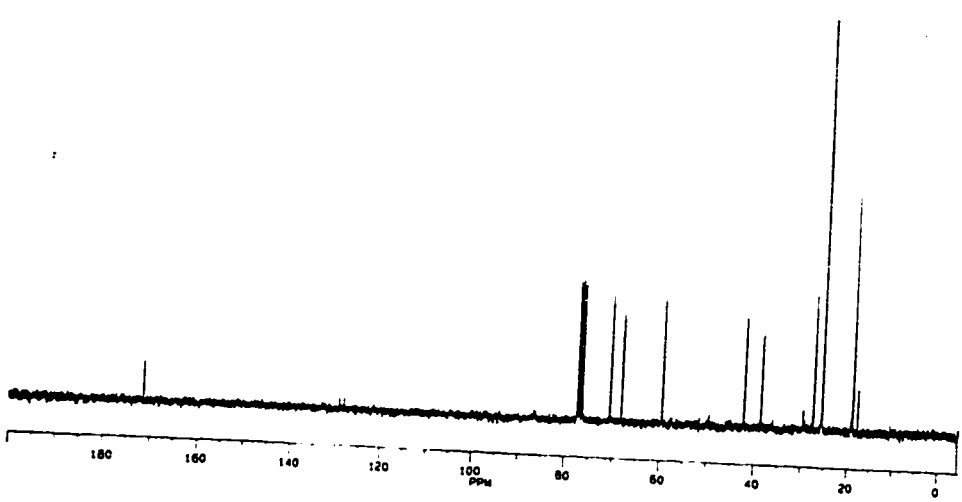
172 **174**

To a solution of compound **172** (8g, 0.0203mol) in 500mL cyclohexane was added Pd/C catalyst (0.4g, 5% weight of **172**.). The reaction was stirred at room temperature for 2 hours. The catalyst was then removed by filtration through a celite pad and the solution was concentrated *in vacuo* at no more than 35°C to afforded 5.87g of **174** as a yellow oil (0.0193mol, 95%).

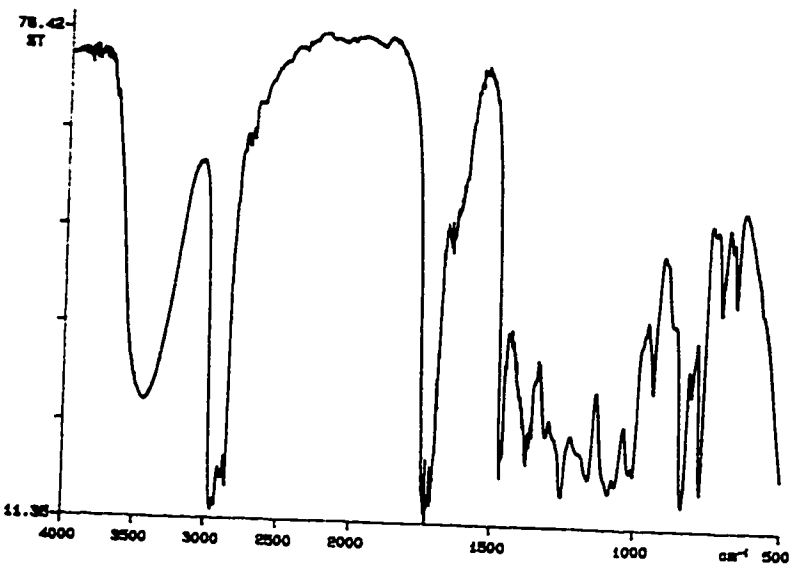
$[\alpha]_D^{25}$ =	+2.36° (0.125g/mL in CH_2Cl_2)
^1H NMR ($CDCl_3$):	0.07 (3H, s), 0.10 (3H, s), 0.87 (9H, s), 0.92 (6H, d, J = 6.70Hz), 1.66-2.04 (3H, m), 2.56 (2H, m), 3.67-3.90 (2H, m), 3.84 (2H, d, J = 6.6Hz), 4.36 (1H, m)
^{13}C NMR ($CDCl_3$):	17.7, 19.1, 25.7, 27.6, 29.6, 38.7, 42.3, 59.6, 68.2, 70.7, 171.5
IR (film):	663, 712, 777, 837, 939, 1005, 1092, 1162, 1253, 1363, 1381, 1463, 1465, 1470, 1698, 1716, 1733, 2857, 2868, 2957, 3443
MS (EI):	57, 75, 89, 101, 105, 155, 131 (100), 145, 173, 191, 203, 232, 247, 259, 287, 305 (M^++1)
HRMS:	expected ($C_{15}H_{33}O_4Si$, M^++1): 305.2148; observed: 305.2148

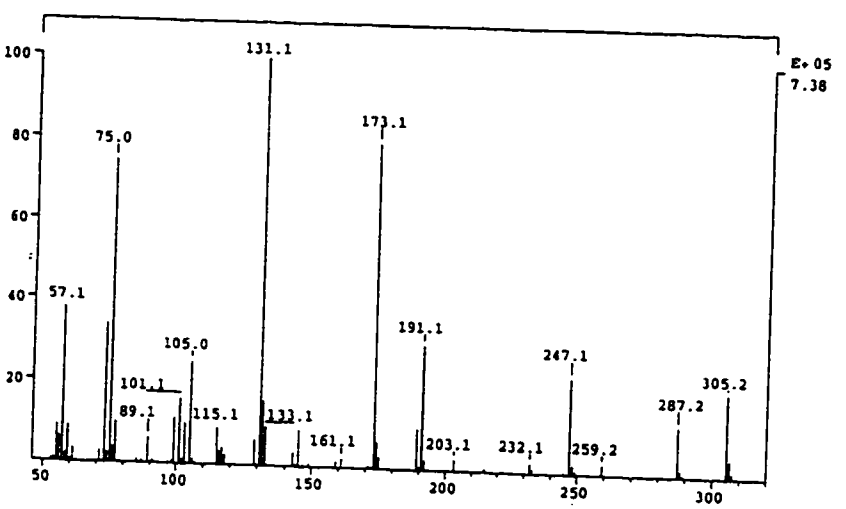
Compound **174**:

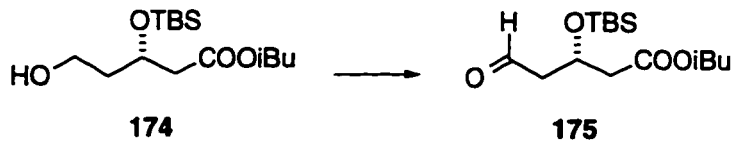




Compound **174** continued:







174 **175**

0.81mL of dimethyl sulfoxide (10.53mmol, 3.2eq) was added dropwise to a stirred solution of oxalyl chloride (0.43mL, 4.94mmol, 1.5eq) in 10 mL of methylene chloride at –78°C. The mixture was continued to stir 30 minutes at –78°C, and a solution of compound **174** (1.0g, 3.29mmol) in 10 mL methylene chloride was added dropwise via a syringe. The residue was rinsed with 5 mL of mthylene chloride and added to the reaction. After 30 minutes of additional stirring at –78°C, 4.56 mL (32.9mmol, 10eq) of triethylamine was added. The resulting solution was stirred for an additional 15 minutes at –78°C and then allowed to warm to room temperature. After one hour, the reaction was poured into 20 mL of saturated aqueous sodium bicarbonate solution. The two layers were shaken and separated, and the aqueous layer was extracted with 60mL of methylene chloride. The combined organic layers were washed with 20 mL brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was pruified by a short silica gel column (10% EtOAc/hexane) to afford 0.92g of **175** as a orange color oil (3.03mmol, 92%)

$[\alpha]_D^{25}$ = +5.76° (0.042g/mL in CH_2Cl_2)

^{1}H NMR ($CDCl_3$): 0.034 (3H, s), 0.037 (3H, s), 0.84 (9H, s), 0.89 (6H, d, J = 6.70Hz), 1.88 (1H, 6 peaks, J=6.70Hz), 2.52 (2H, dd, J_1 = 2.77Hz, J_2 = 6.23Hz), 2.62 (2H, ddd, J_1 = 1.84Hz, J_2 = 2.30Hz, J_3 = 6.23Hz), 3.80 (2H, dd, J_1 = 3.59Hz, J_2 = 6.71Hz), 4.59 (1H, quintet, J = 6.23Hz), 9.76 (1H, t, J = 2.30Hz)

^{13}C NMR ($CDCl_3$): 17.8, 19.0, 25.5, 25.6, 27.5, 42.4, 50.7, 64.9, 70.7, 170.7,

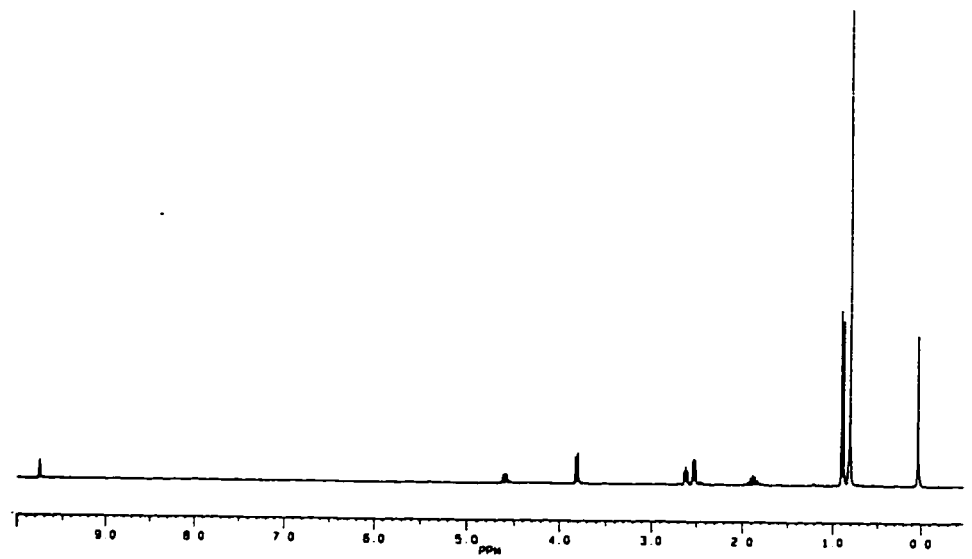
200.8

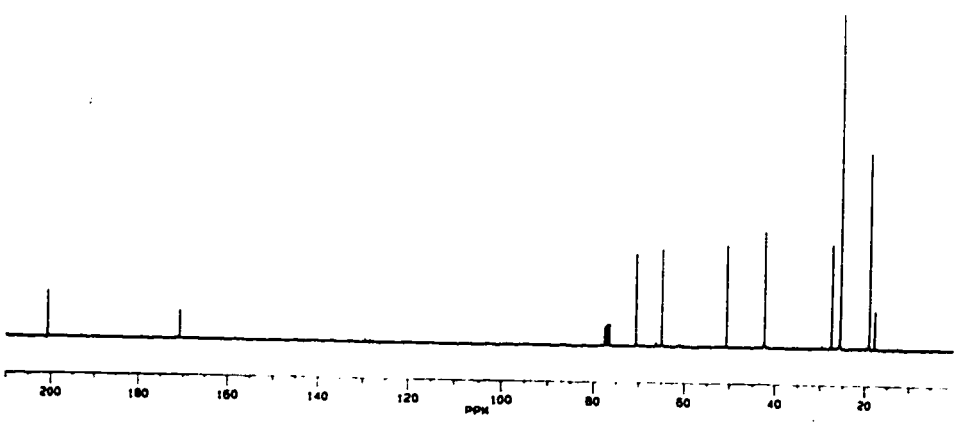
IR (film): 778, 837, 1005, 1098, 1172, 1257, 1381, 1472, 1732, 1738, 2857, 2911, 2958

MS (EI): 89, 99, 115, 145, 169, 173, 184, 189, 203, 231, 259, 287 (100), 301(M^+)

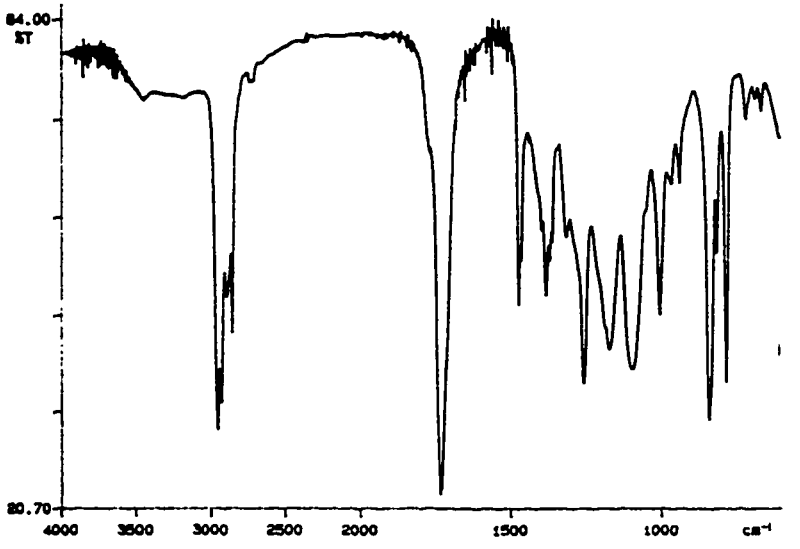
HRMS (EI): expected ($C_{15}H_{31}O_4Si$, M^+): 302.1913; observed: 302.1906

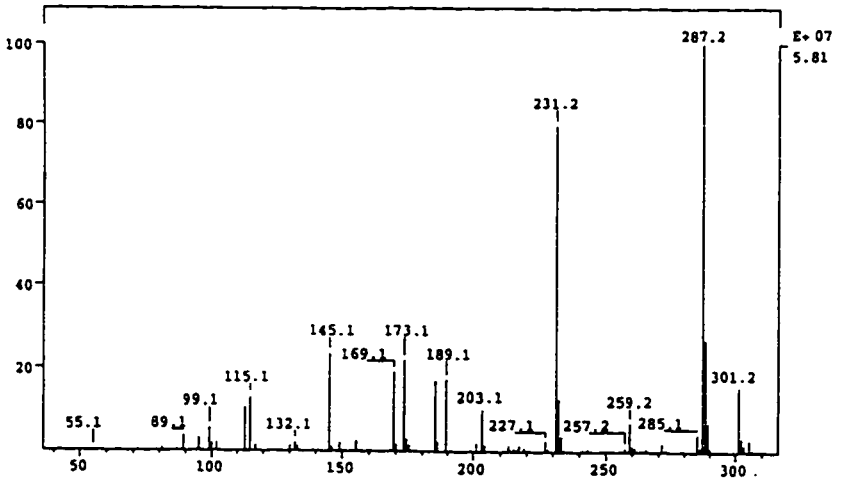
Compound **175**:





Compound **175** continued:





175 **176**

To a solution of compound **175** (5g, 0.0165mol) and ethylene glycol (1.84 mL, 0.033mol, 2eq) in 110 mL of benzene was added catalytic amount of PPTS solid (0.25g, 5% weight of **175**). The solution was heated at reflux for 3 hours. After cooling the solution to room temperature, 60 mL of saturated $NaHCO_3$ was added. The two layers were separated and the aqueous phase was extracted with 80 mL of EtOAc. The combined organic phase was washed with 50 mL of brine, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The product was chromatographed (20% EtOAc/hexane) to yield 5.02g of **176** (0.0145mol, 88%) as yellow oil.

$[\alpha]_D^{25}$ = +4.5° (0.064g/mL in CH_2Cl_2)

^1H NMR ($CDCl_3$): 0.05 (3H, s), 0.08 (3H, s), 0.85 (9H, s), 0.92 (6H, d, J = 6.73Hz), 1.8 - 2.0 (3H, m, overlapping), 2.53 (2H, dd, J_1 = 3.33Hz, J_2 = 6.24Hz), 3.8 - 4.0 (4H, m, overlapping), 3.9 - 4.0 (2H, m), 4.34 (1H, 5peaks, J = 6.24Hz), 4.94 (1H, t, J = 5.42Hz)

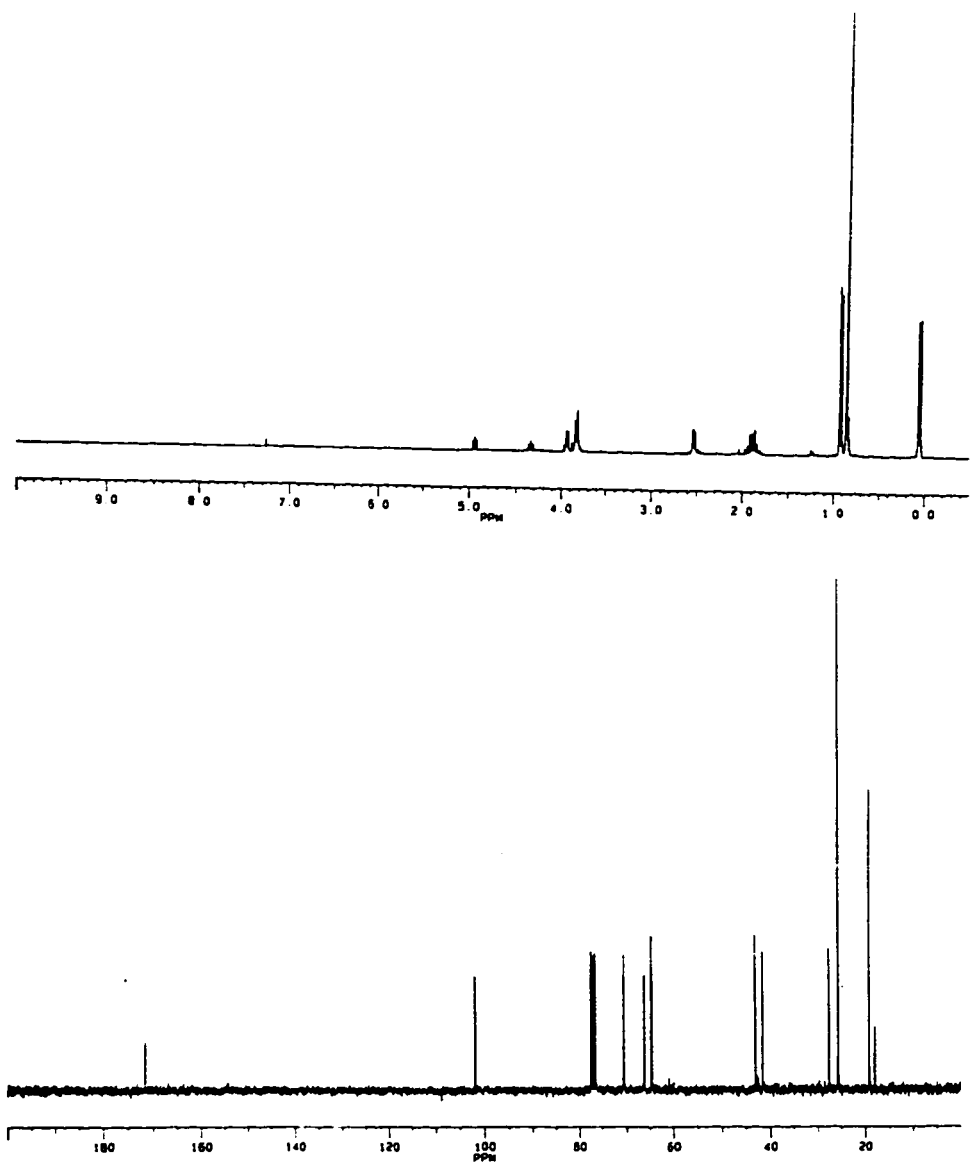
^{13}C NMR ($CDCl_3$): 17.9, 19.1, 25.6, 25.7, 27.6, 41.5, 43.1, 64.6, 64.7, 66.2, 70.5, 101.9, 171.5

IR (film): 662, 712, 778, 812, 837, 940, 1006, 1038, 1102, 1144, 1171, 1257, 1315, 1382, 1472, 1738, 2858, 2931, 2888, 2959

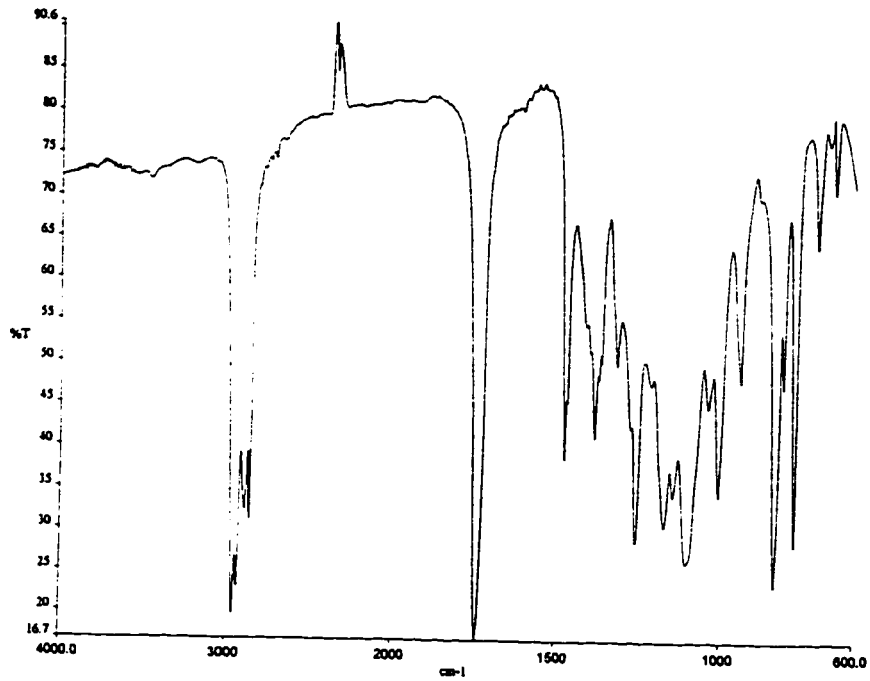
MS (EI): 73, 101, 119, 145 (100), 161, 171, 189, 233, 273, 289, 345 (M^+-H)

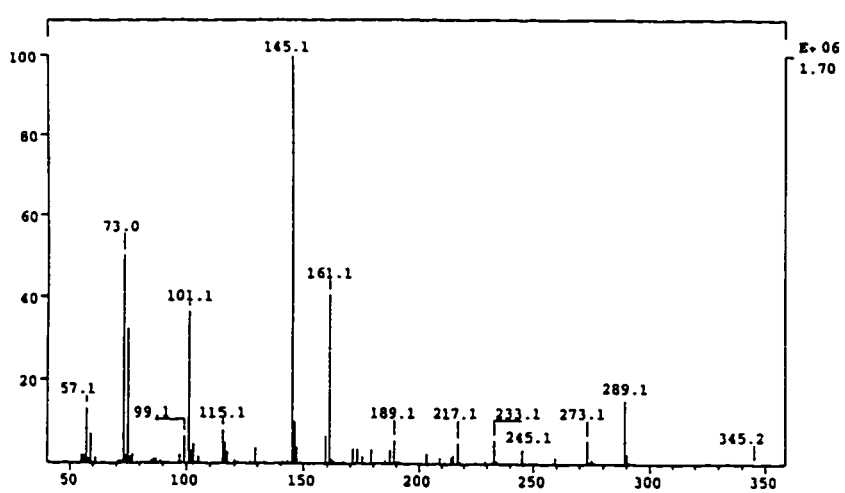
HRMS (EI) expected ($C_{17}H_{34}O_5Si$, M^+): 346.2176; observed: 346.2177

Compound **176**:



Compound **176** continued:



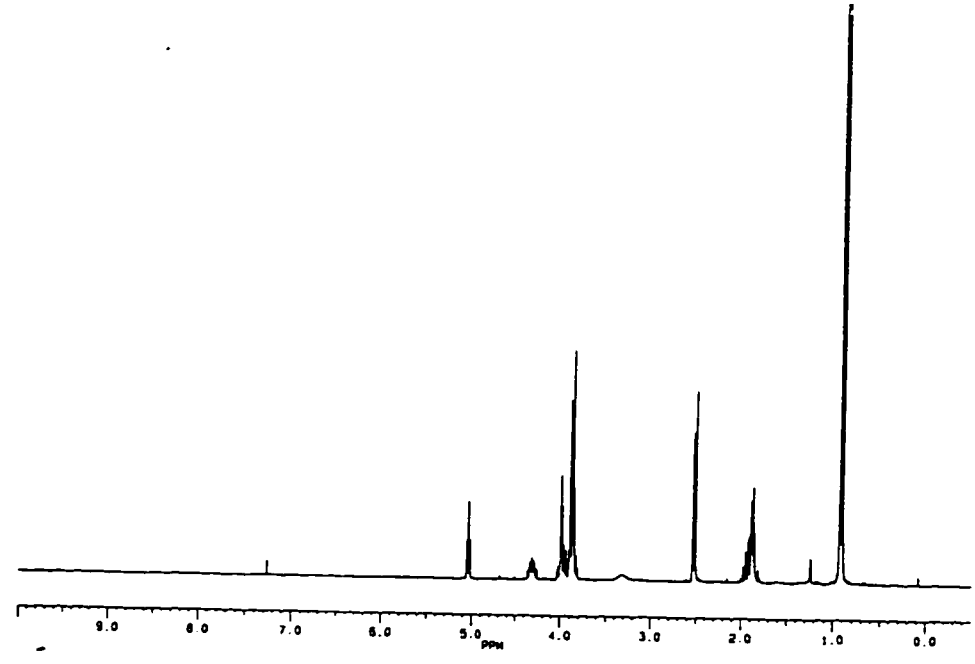


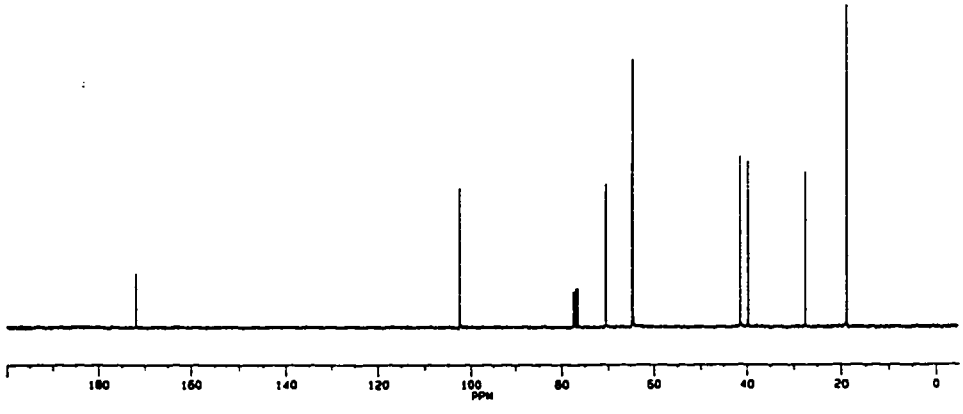
176 **177**

Compound **176** (5.02g, 0.0145mol) was treated with 21.7 mL of TBAF (1.0 M in THF) at room temperature for 4 hours. The reaction mixture was then diluted with 60 mL EtOAc and washed with 20 mL of water followed by 20 mL of brine. The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and chromatographed (40% EtOAc/hexane) to give 2.85g of **177** as a yellow oil (0.0123mol, 85%).

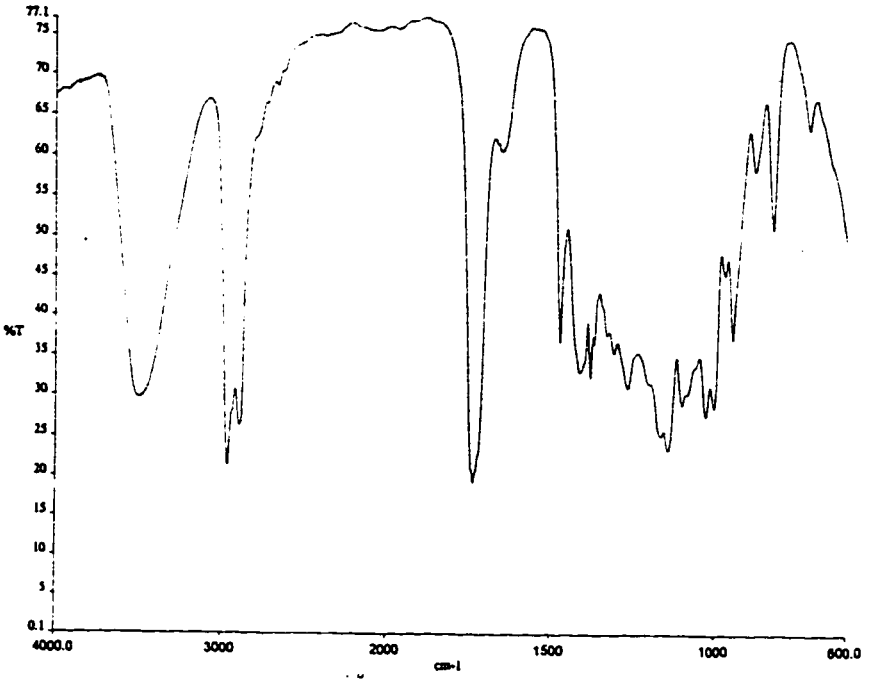
$[\alpha]_D^{25}$ =	+6.53° (0.0406g/mL in CH_2Cl_2)
^{1}H NMR ($CDCl_3$):	0.92 (6H, d, J = 6.71Hz), 1.86-1.92 (3H, m), 2.52 (2H, d, J = 6.31Hz), 3.37 (1H, d, br, J = 2.93Hz), 3.85-3.99 (4H, m), 3.83 (2H, d, overlapping, J = 6.59Hz), 4.26 (1H, m), 5.04 (1H, t, J=4.64Hz)
^{13}C NMR ($CDCl_3$):	18.9, 27.5, 39.7, 41.4, 64.5, 64.7, 70.5, 102.5, 172.0
IR (film):	714, 823, 878, 945, 969, 1001, 1028, 1098, 1143, 1269, 1381, 1415, 1472, 1734, 2891, 2963, 2500
MS (EI)	57, 73 (100), 86, 99, 115, 130, 142, 159, 187, 215, 233 (M^+)
HRMS (EI):	expected ($C_{11}H_{21}O_5$, M^+): 233.1389; observed: 233.1384

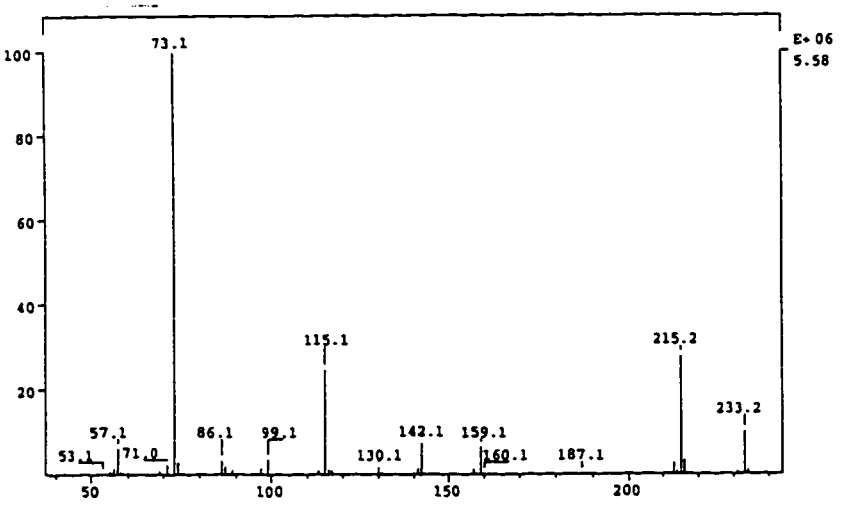
Compound **177**:

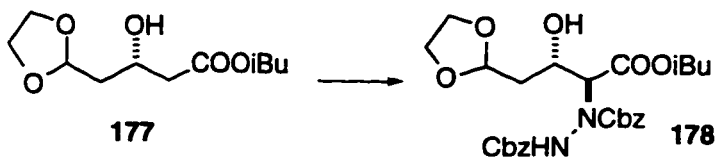




Compound **177** continued:







A crystal of phenanthroline was added to a solution of i-Pr_2NH (3.64mL, 0.026mol, 3eq) in 20mL of freshly distilled THF at –78°C. A small amount of BuLi in hexane (2.5M) was added to the solution via syringe until the solution turned red color, then the required amount of BuLi (8.8mL, 0.022mol, 2.5eq) was added. The solution was allowed to warm to 0°C and then cooled to –78°C again. Compound **177** (2g, 8.62mmol) in 10mL of THF was added to the reaction mixture slowly via syringe. The resulting solution was allowed to warm to –20°C over 30 minutes. This dianion solution was cooled to –78°C and Cbz-N=N-Cbz (6.56g, 0.022mol, 2.5eq) in 20 mL of THF was added. The residue was rinsed with 5 mL of THF. After 2 minutes the reaction was quenched with 3 mL of acetic acid, stirred for 10 minutes at –78°C, and then allowed to warm up to room temperature. The reaction mixture was diluted with 40 mL of EtOAc and 20 mL of water and the two layer were separated. The organic phase was washed with 20 mL of saturated $NaHCO_3$ aqueous solution followed by 10 mL of brine, dried over Na_2SO_4, concentrated *in vacuo* and chromatographed (15% EtOAc in hexane) to afford 2.79g of **178** (5.26mmol, 61%) as a viscous orange oil.

$[\alpha]_D^{25}$ = +4.55° (0.052g/mL in CH_2Cl_2)

^1H NMR(C_6D_6, 70° C): 0.80 (6H, d, J = 6.73Hz), 1.78 (1H, 7peaks, J = 6.73Hz), 2.2 - 2.4 (2H, m), 3.4 - 3.5 (3H, m, overlapping), 3.5 - 3.7 (2H,

m), 3.8 - 4.0 (2H, m), 4.70 (1H, br), 4.9 - 5.2 (5H, m, overlapping), 7.0 - 7.3 (10H, m)

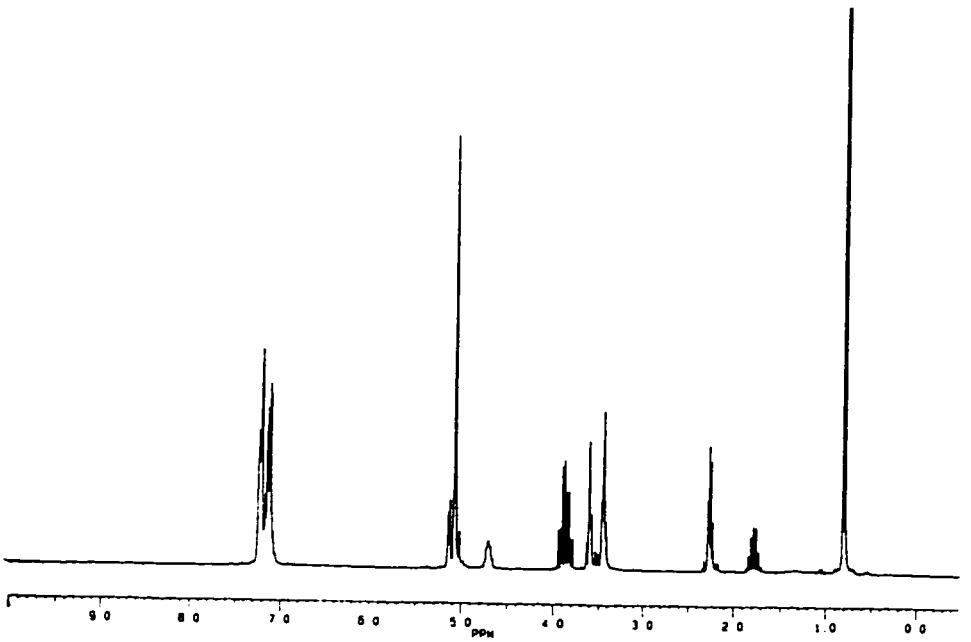
^{13}C NMR (C_6D_6, 70° C): 18.4, 27.3, 38.4, 64.2, 64.3, 65.7, 67.3 (two peaks overlapping), 68.2, 71.1, 102.7, 127.0, 127.4, 127.5, 127.6, 127.7, 127.8, 128.1, 135.9, 136.1, 155.9, 156.2, 169.3

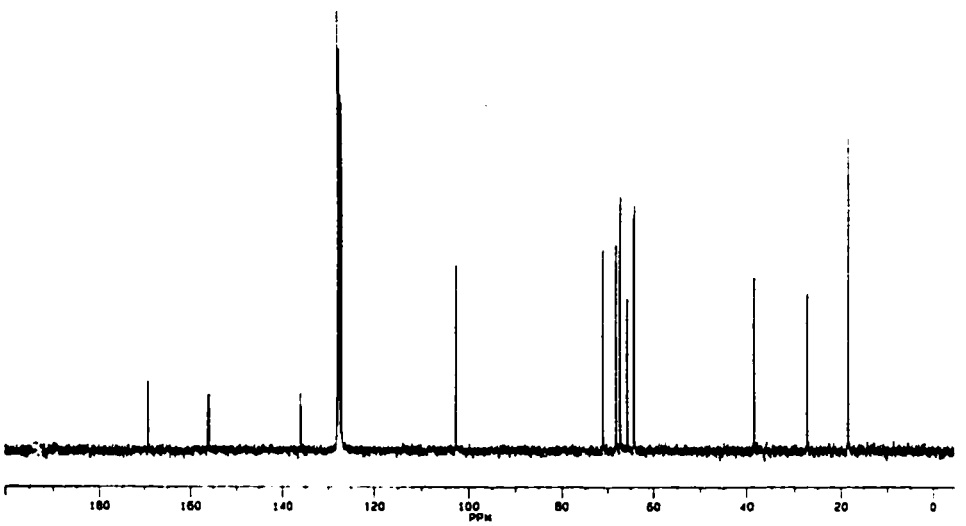
IR (film): 698, 743, 825, 946, 1056, 1094, 1139, 1217, 1406, 1456, 1499, 1587, 1728, 2892, 2963, 3034, 3065, 3299, 3502

MS (EI): 73, 91 (100), 108, 148, 161, 220, 235, 265, 279, 295, 414, 512, 530(M^+)

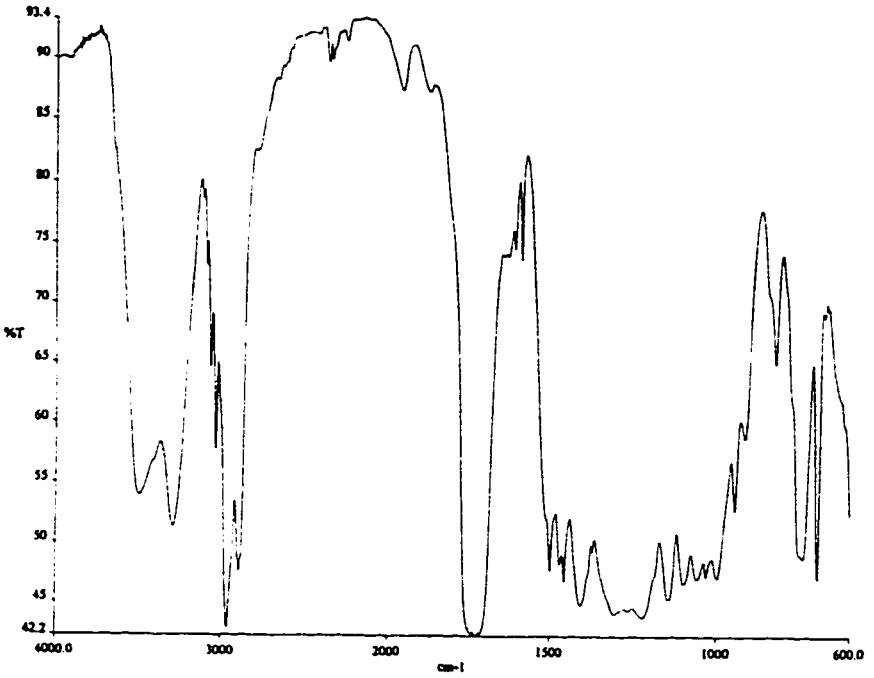
HRMS (EI): expected ($C_{27}H_{34}N_2O_9$, M^+): 530.2264; observed: 530.2263

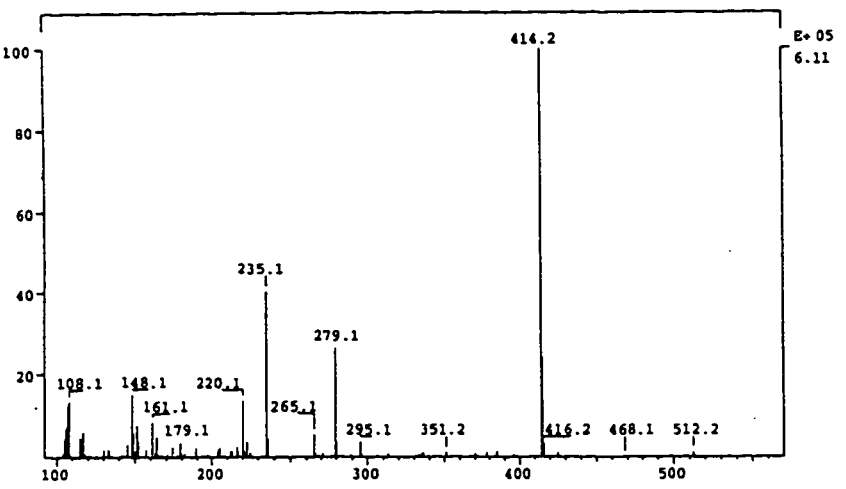
Compound **178**:

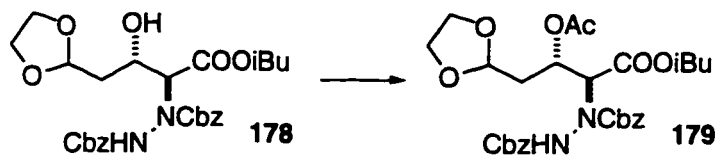




Compound **178** continued:







A solution of alcohol **178** (8.37g. 0.0158mol) and 4.47 mL of Ac_2O (0.0474mol, 3eq) in pyridine (30mL) was stirred at room temperature for 16 hours. Pyridine was removed *in vacuo* and the residue was dissolved in 80 mL of EtOAc, washed with 20 mL of dilute HCl aqueous solution (0.01N) followed by 20 mL of saturated $NaHCO_3$ aqueous solution, dried over Na_2SO_4, concentrated *in vacuo* and chromatographed (20% EtOAc in hexane) to afford 8.58g of **179** as a yellow oil (0.015mol, 95%).

$[\alpha]_D^{25}$ = $-3.37°$ (0.127g/mL in CH_2Cl_2)

^1H NMR (C_6D_6, 70° C): 0.80 (6H, d, J = 6.72Hz), 1.79 (3H, s), 1.7 - 1.8 (1H, m, overlapping), 2.3 - 2.4 (1H, m), 2.4 - 2.5 (1H, m), 3.35 - 3.45 (2H, m), 3.55 - 3.65 (2H, m), 3.75 - 3.95 (2H, m), 5.0 - 5.2 (4H, m, overlapping), 5.0 - 5.1 (1H, m, overlapping), 5.47 (1H, d, J =5.44Hz), 5.93 (1H, q, J = 5.44Hz), 7.0 - 7.3 (10H, m)

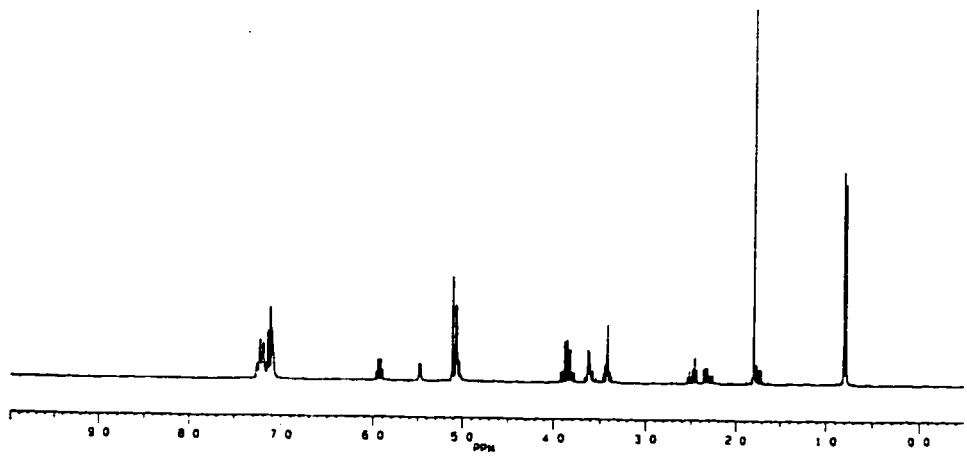
^{13}C NMR (C_6D_6, 70° C): 18.4, 19.9, 27.3, 36.6, 63.0, 64.3, 64.4, 67.3, 68.2, 68.5, 71.2, 102.0, 127.0, 127.4, 127.5, 127.6, 127.8, 128.0, 135.9, 136.2, 155.6, 155.8, 168.2, 168.5

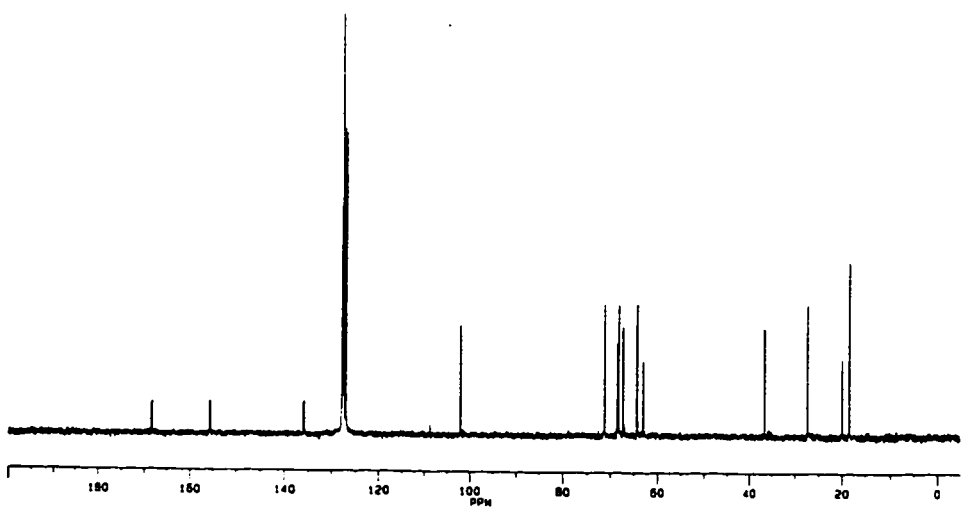
IR (film): 698, 742, 946, 1028, 1053, 1140, 1231, 1372, 1405, 1456, 1498, 1744, 2892, 2963, 3034, 3302

MS (EI): 73, 91(100), 106, 157, 216, 271, 333, 378, 427, 468, 512, 572(M^+)

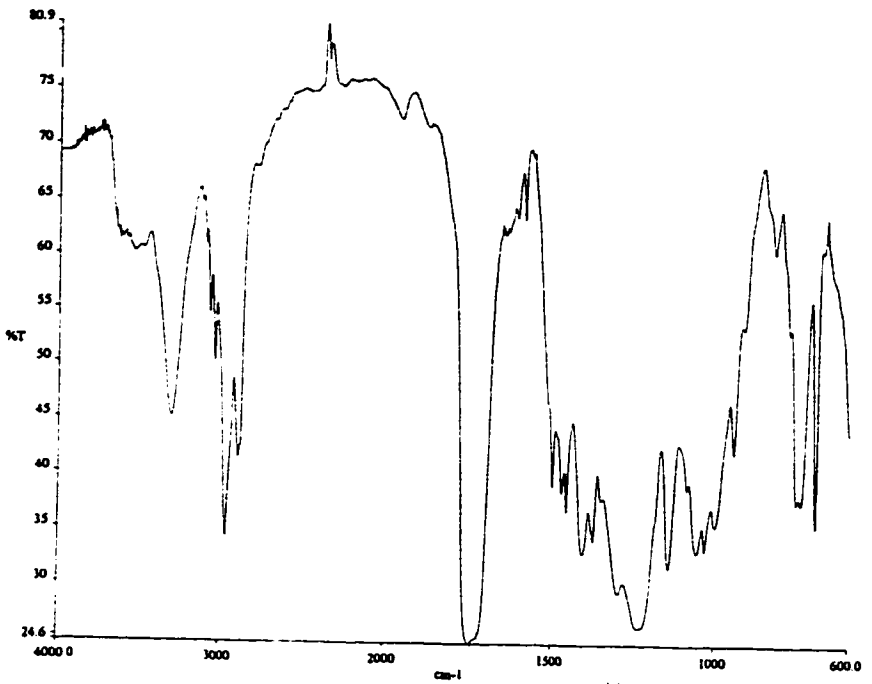
HRMS (EI): expected ($C_{29}H_{36}N_2O_{10}$, M^+): 572.2730; observed: 572.2730

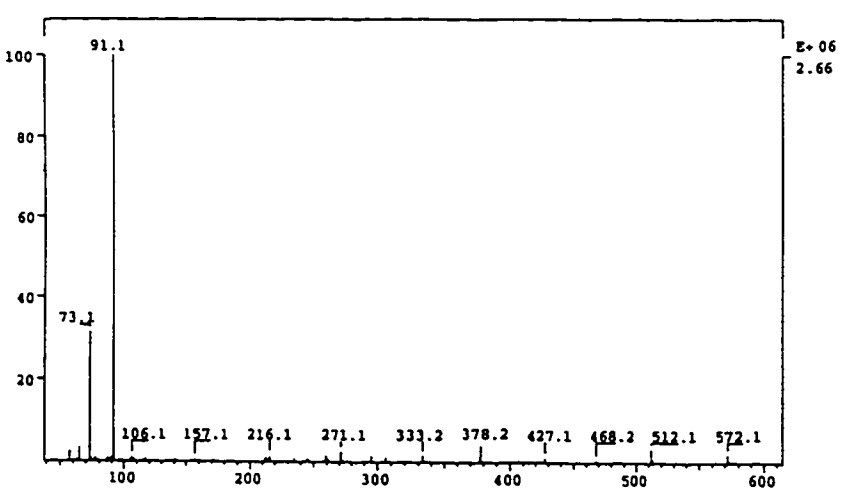
Compound **179**:

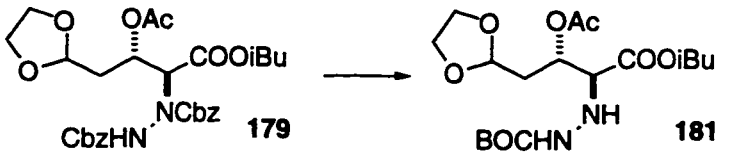




Compound **179** continued:



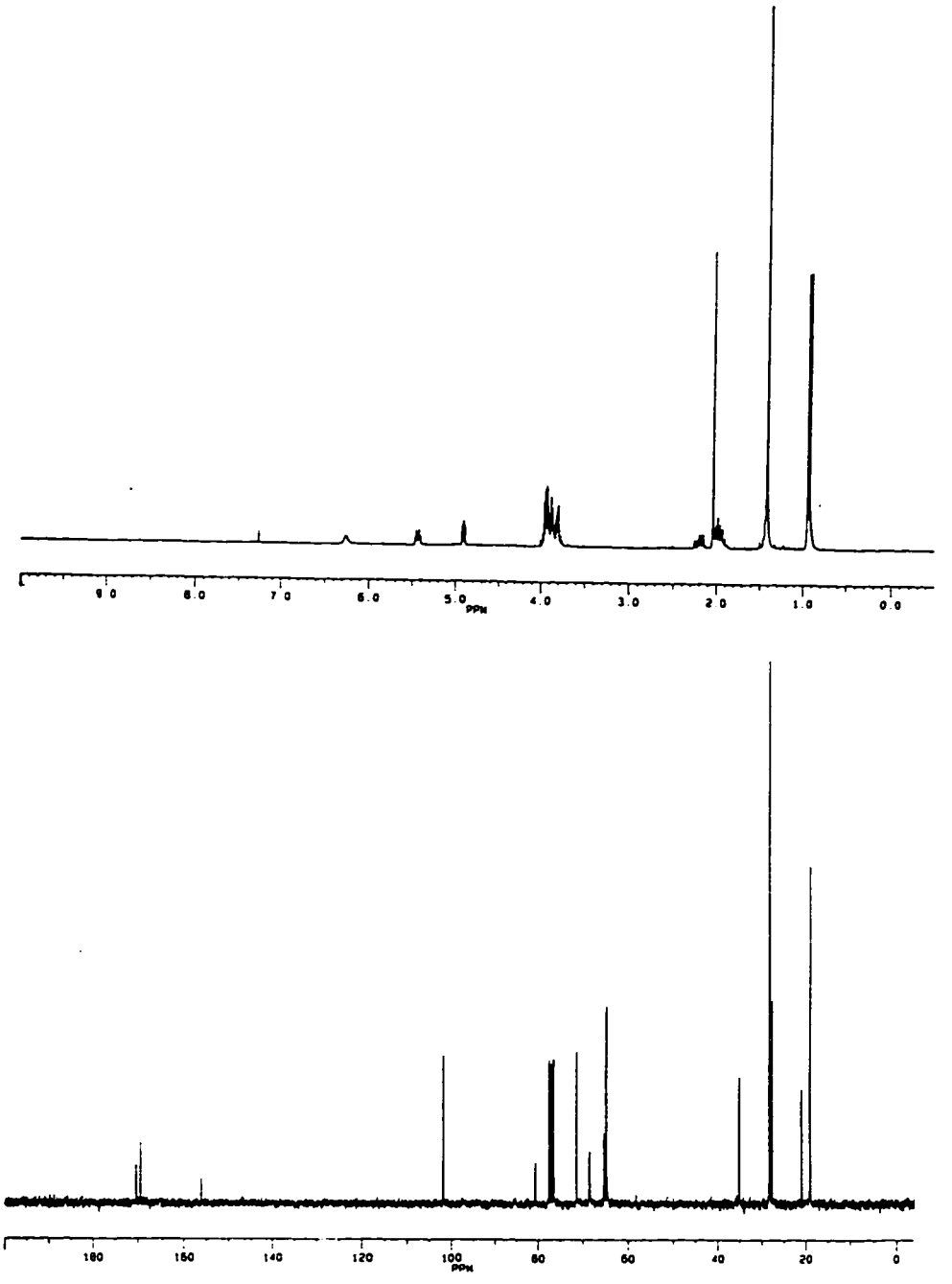




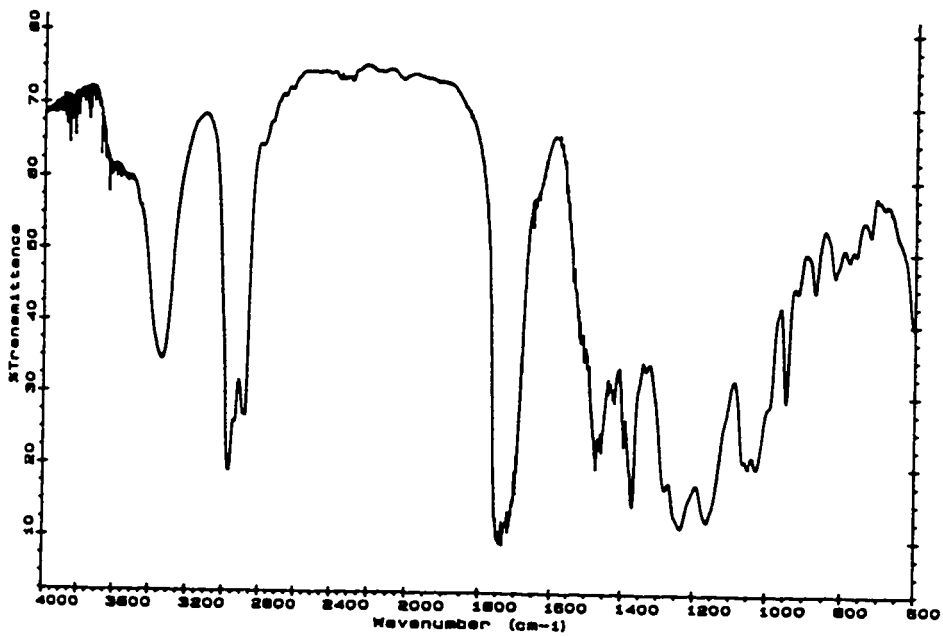
To a solution of acetate **179** (8.58g, 0.015mol) in 200mL of MeOH was added 0.43g of Pd/C (5% weight of **179** catalyst). The solution was stirred at room temperature under H_2 balloon for 8 hours. The reaction solution was filtered through a celite pad to remove the catalyst, concentrated *in vacuo*, Chromatographed (40% EtOAc/hexane) to afford 5.87g g of **181** (0.0145mol, 97%) as a yellow oil.

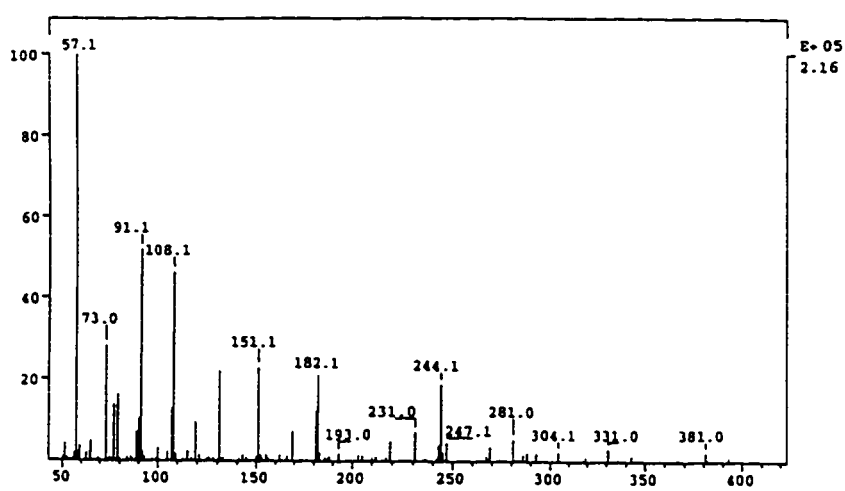
$[\alpha]_D^{25}$ =	+0.48° (0.033g/mL in CH_2Cl_2)
^{1}H NMR ($CDCl_3$):	0.94 (6H, d, J = 6.83Hz), 1.41 (9H, s), 1.9 - 2.0 (2H, m), 2.04 (3H, s), 2.1 - 2.3 (1H, m), 3.7 - 4.0 (6H, m, overlapping), 4.91 (1H, dd, J_1 = 3.85Hz, J_2 = 5.35Hz), 5.44 (1H, dt, J_1 = 3.60Hz, J_2 = 8.90Hz), 6.26 (1H, br)
^{13}C NMR ($CDCl_3$):	19.0, 21.0, 27.6, 28.2, 35.0, 64.8, 64.9, 65.3, 68.5, 71.4, 80.6, 101.8, 156.2, 169.4, 170.4
IR(film):	964, 1049, 1162, 1236, 1331, 1407, 1426, 1436, 1464, 1501, 1684, 1700, 1739, 2892, 2972, 3356.
MS (EI):	57 (100), 73, 108, 131, 151, 182, 231, 244, 247, 281, 304, 331, 381, 404 (M^+)
HRMS:	expected ($C_{18}H_{32}N_2O_8$, M^+): 404.2158; observed.:404.2163

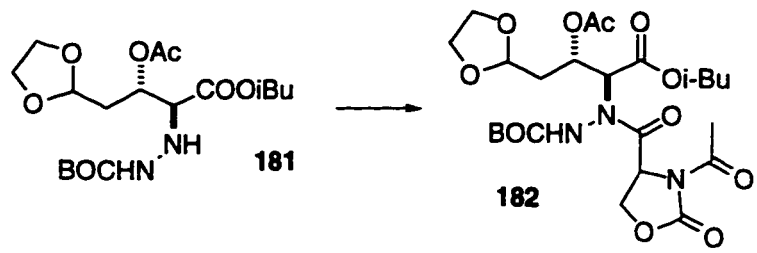
Compound **181**:



Compound **181** continued:





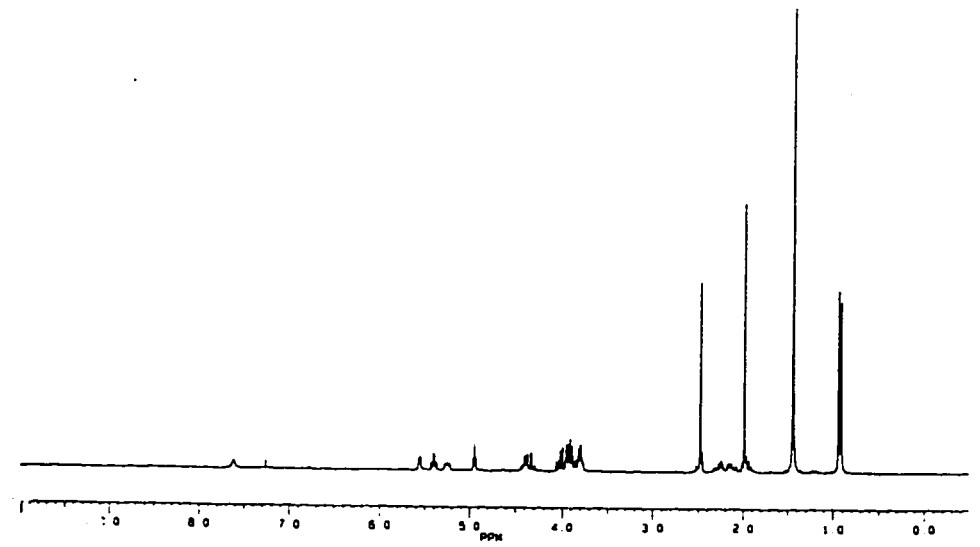


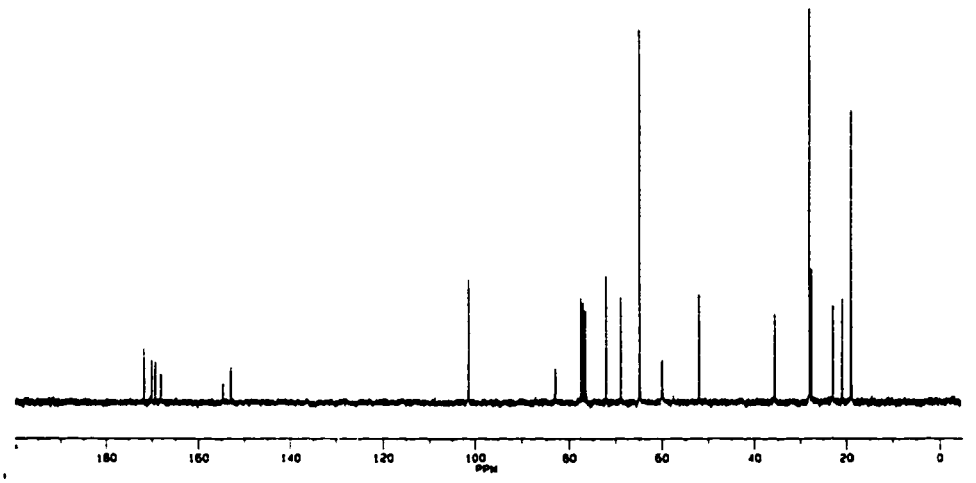
A solution of mono-BOC **181** (3g, 7.42mmol) and 2.95 mL of colindine (0.0223mol, 3eq) in 15 mL of anhydrous CH_2Cl_2 was cooled to 0°C. 2.12g of acid chloride (11.1mmol, 1.5eq) in 10 mL CH_2Cl_2 was slowly added to the solution via syringe. The reaction was allowed to stirred at 0°C for 0.5 hours and the mixture was directly passed through a short silica gel column to remove most of the byproduct. Another silica gel column was used to purify the product (40% → 80% EtOAc/hexane) to afford 2.49g of **182** as a white foam (4.45mol, 60%).

$[\alpha]_D^{25}$ =	+34.45° (0.085g/mL in CH_2Cl_2)
^{1}H NMR ($CDCl_3$):	0.93 (6H, d, J = 6.72Hz), 1.9 - 2.1 (1H, m, overlapping), 1.99 (3H, s), 2.0 - 2.4 (2H, m, overlapping), 2.45 (3H, s), 3.7 - 4.1 (6H, m, overlapping), 4.3 - 4.5 (2H, m), 4.95 (1H, t, J = 4.42Hz), 5.2 - 5.3 (1H, m), 4.35 - 4.45 (1H, m), 5.0 - 5.1 (1H, m), 7.6 (1H, br)
^{13}C NMR ($CDCl_3$):	18.9, 20.9, 22.8, 27.5, 27.9, 35.6, 52.0, 60.0, 64.8, 68.9, 72.1, 83.0, 101.5, 152.9, 154.6, 168.0, 169.2, 170.0, 171.7
IR (film):	732, 757, 917, 945, 1052, 1154, 1233, 1322, 1373, 1473, 1700, 1747, 1792, 2973, 3299
MS (EI):	57, 73 (100), 91, 128, 156, 182, 212, 236, 270, 309, 383, 414, 459, 486, 559 (M^+)

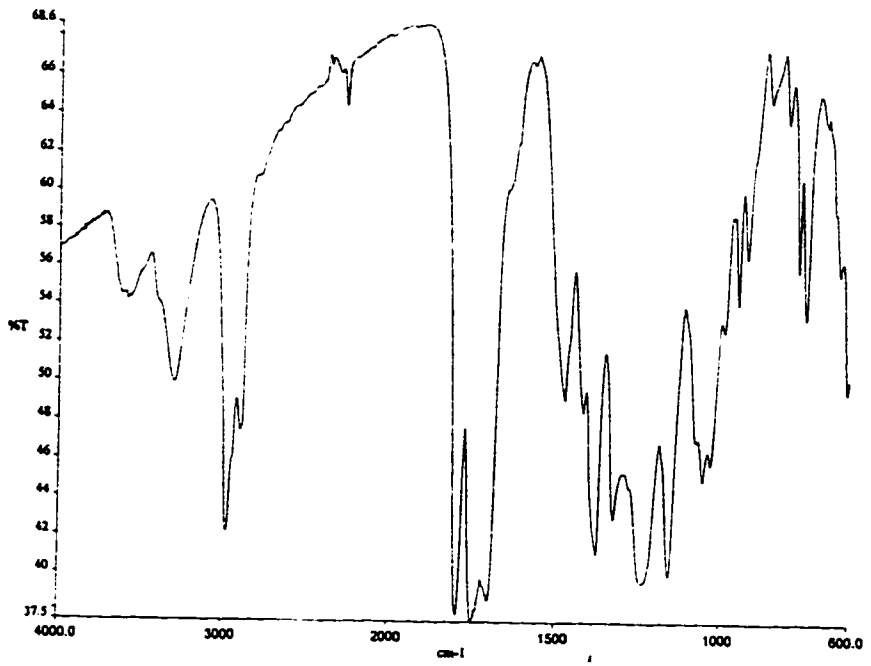
HRMS (EI): expected ($C_{24}H_{37}N_3O_{12}$, M^+): 559.2377; observed: 559.2368

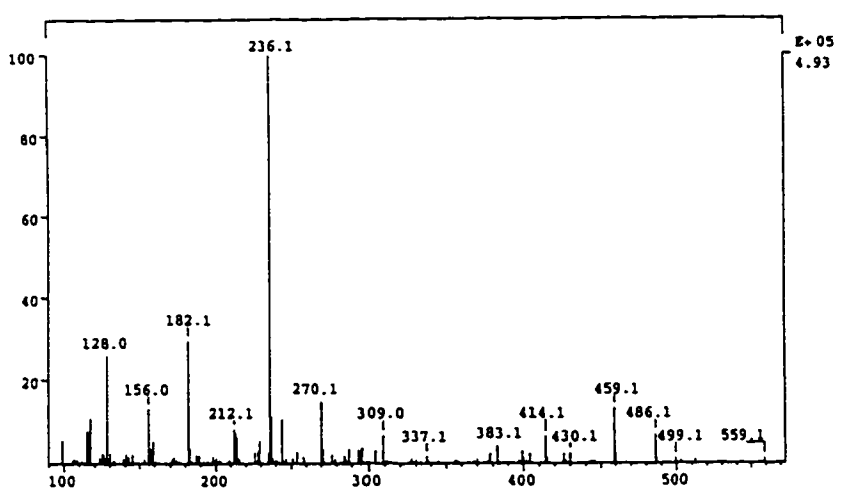
Compound **182**:

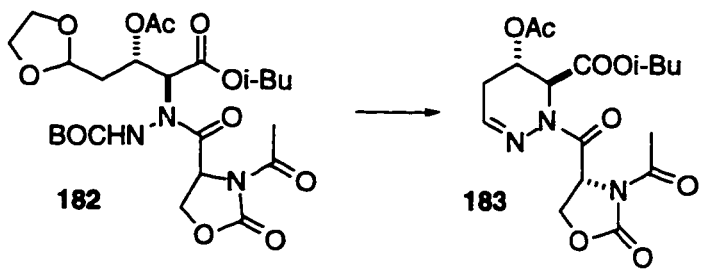




Compound **182** continued:



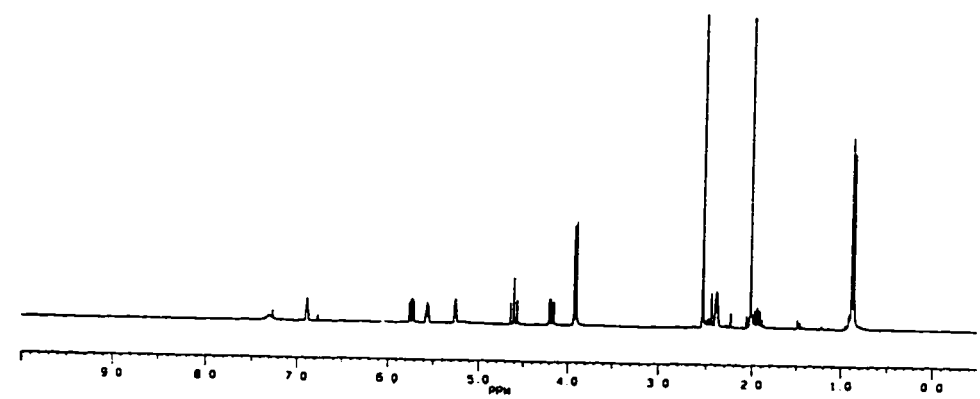


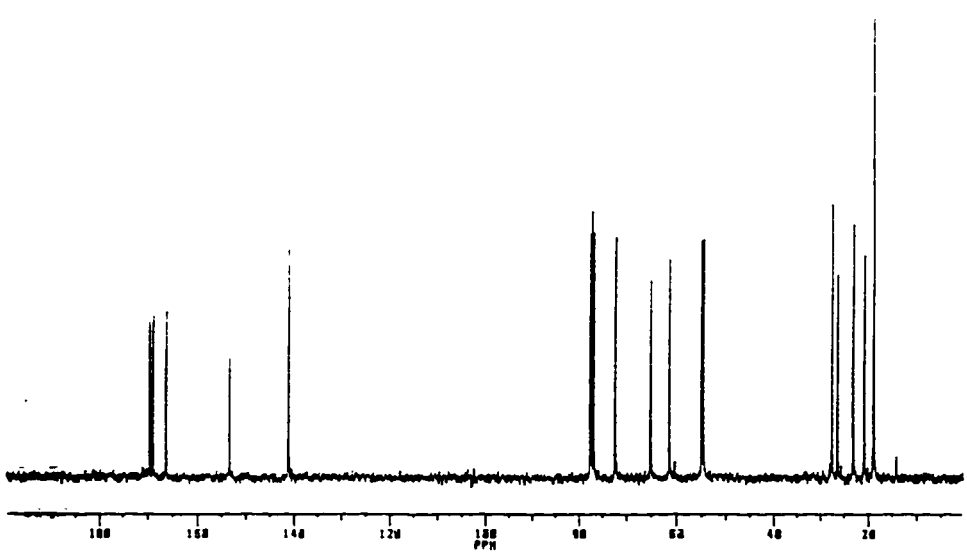


A solution of compound **182** (2.49g, 4.45mmol) in 10 mL of 90% TFA in H_2O was stirred at room temperature for 30 minutes. The solvent was then concentrated *in vacuo* and the residue was dissolved in 30 mL of CH_2Cl_2. The organic solution was washed with saturated $NaHCO_3$ aqueous solution, dried over Na_2SO_4, and concentrated *in vacuo* to afford 1.78g of **183** as a red oil (4.32mmol, 97%).

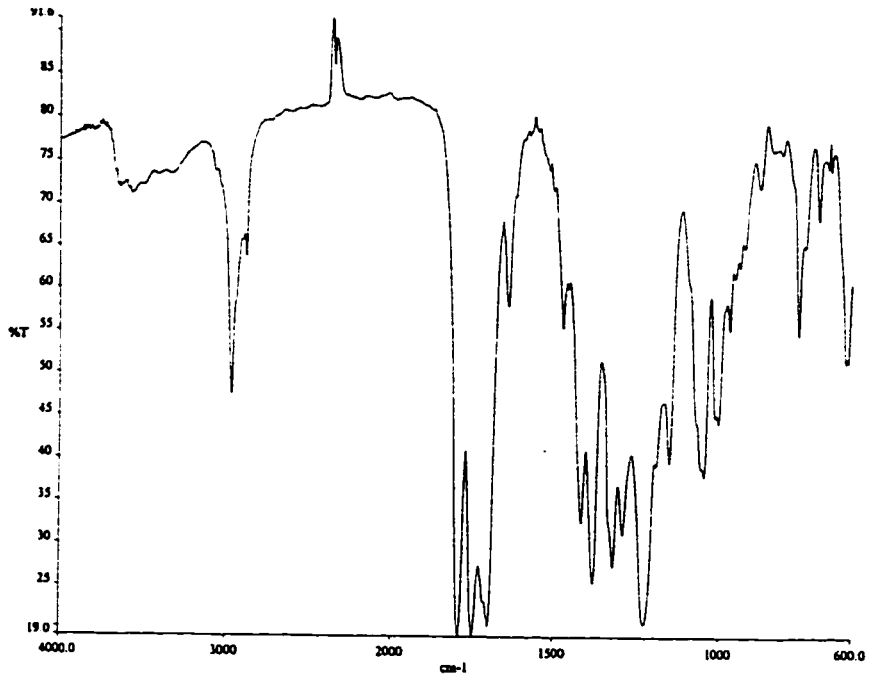
$[\alpha]_D^{25}$ =	+51.05° (0.0304g/mL in CH_2Cl_2)
^1H NMR ($CDCl_3$):	0.88 (6H, distorted d, J = 6.67Hz), 1.85 - 2.05 (1H, sept. J= 6.67Hz, overlapping), 2.00 (3H, s), 2.38 (2H, m), 2.54 (3H, s), 3.91 (2H, d, J = 6.67Hz), 4.18 (1H, dd, J_1 = 3.93Hz, J_2 = 9.57Hz), 4.61 (1H, t, J = 9.57Hz), 5.26 (1H, m), 5.57 (1H, m), 5.73 (1H, dd, J_1 = 3.93Hz, J_2 = 9.57Hz), 6.89 (1H, m)
^{13}C NMR ($CDCl_3$):	18.7, 20.7, 23.1, 26.2, 27.4, 54.6, 55.0, 61.3, 65.0, 72.4, 121.3, 140.7, 153.2, 166.2, 168.7, 169.3, 169.6
IR (film):	618, 700, 760, 968, 1002, 1045, 1151, 1227, 1290, 1319, 1378, 1415, 1637, 1699, 1748, 1790, 2965
MS (EI):	57, 81 (100), 99, 128, 150, 182, 236, 251, 277, 355, 397 (M^+).
HRMS:	expected ($C_{17}H_{23}N_3O_8$, M^+): 397.1485; observed: 397.1485.

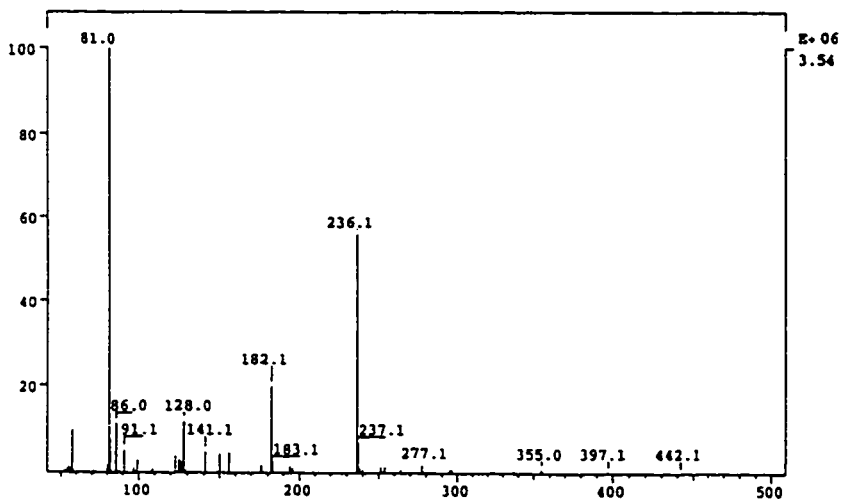
Compound **183**:

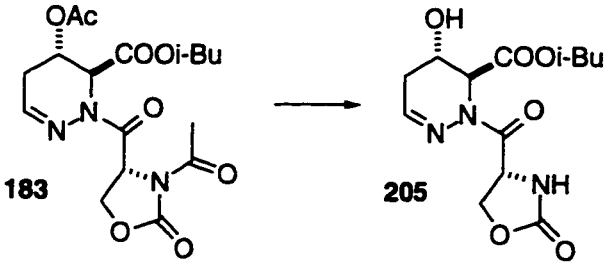




Compound **183** continued:



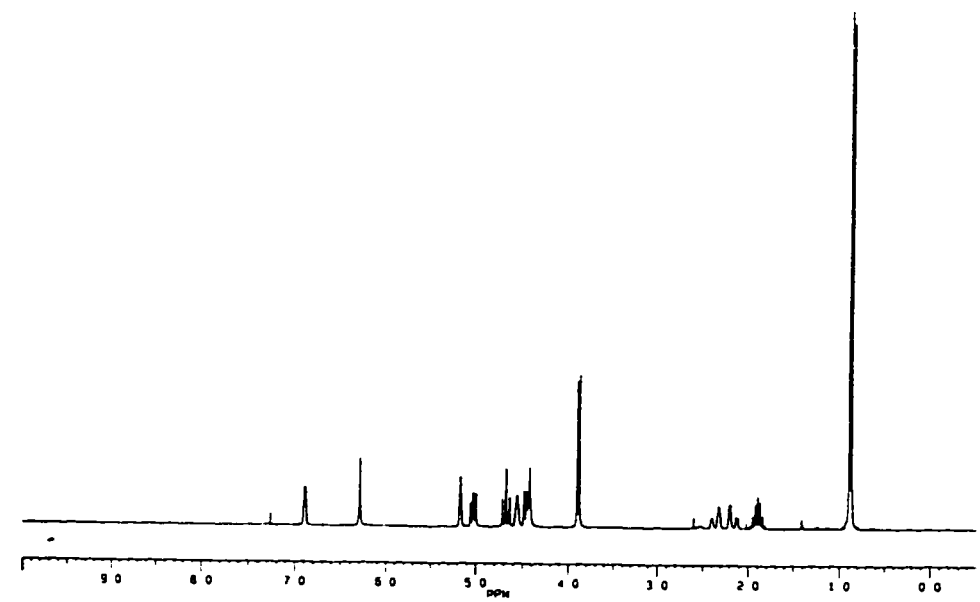


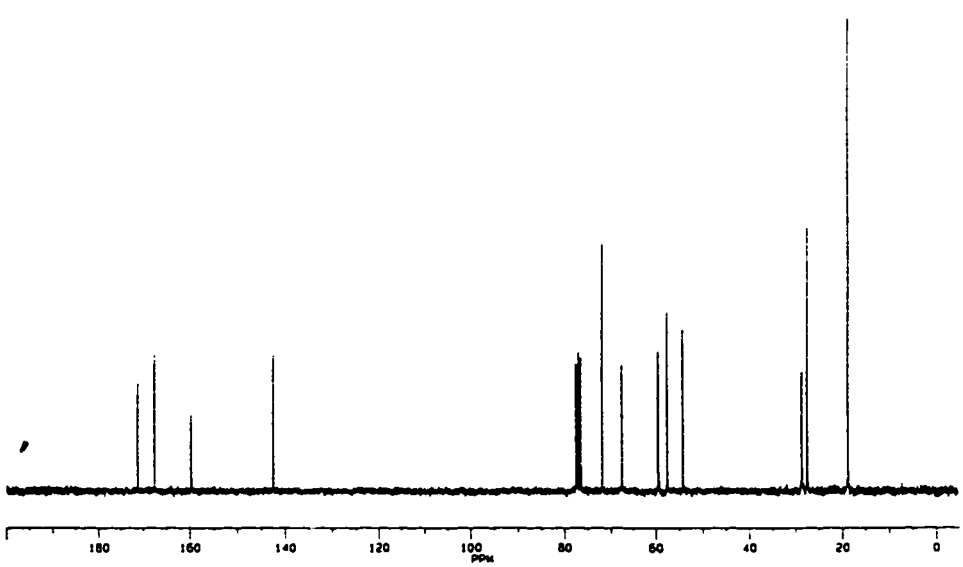


A solution of compound **183** (1.78g, 4.32mmol) and 0.42 mL of H_2N-NH_2 (8.64mmol, 2eq) in CH_3CN was stirred at room temperature for 4 hours. The solution was pass through a short silica gel column and concentrated *in vacuo* to afford 1.08g of **205** as yellow oil (3.46mmol, 80%).

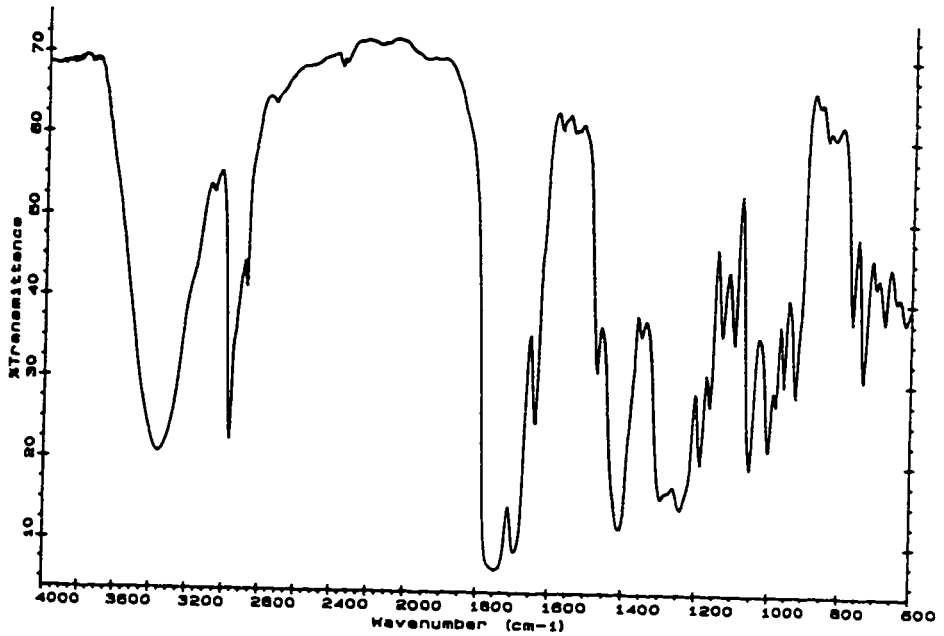
$[\alpha]_D^{25}$ =	$-4.16°$ (0.036g/mL in CH_2Cl_2)
^1H NMR ($CDCl_3$):	0.88 (6H, d, J = 6.6Hz), 1.89 (1H, sept. J = 6.6Hz), 2.16 (1H, distorted dd, J_1 = 18.2Hz, J_2 = 3.3Hz), 2.35 (1H, distorted d, J_1 = 18.2, J_2 = 1.8Hz), 3.88 (2H, J = 6.6Hz), 4.42 (1H, br), 4.43 (1H, dd, J_1 = 5.8Hz, J_2 = 9.4Hz), 4.56 (1H, br), 4.67 (1H, t, J = 9.4Hz), 5.02 (1H, dd, J_1 = 5.8Hz, J_2 = 9.4Hz), 5.17 (1H, distorted d, J = 2.2Hz), 6.29 (1H, br), 6.89 (1H, distorted t, J = 1.8Hz)
^{13}C NMR ($CDCl_3$):	18.8, 27.5, 28.7, 54.2, 57.5, 59.5, 67.6, 71.9, 142.4, 159.7, 167.5, 171.1
IR (film):	737, 928, 1002, 1054, 1243, 1409, 1691, 1759, 2965, 3367
MS (CI):	91, 106, 122, 131, 165, 193, 209, 252 (100), 270, 298, 314 (M^++1).
HRMS:	expected ($C_{13}H_{20}N_3O_6$, M^++H): 314.1352; observed: 314.1346.

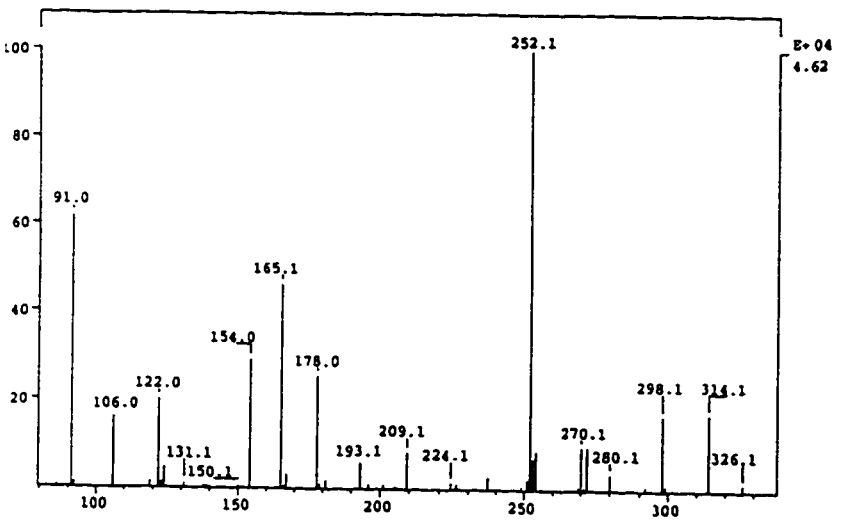
Compound **205**:

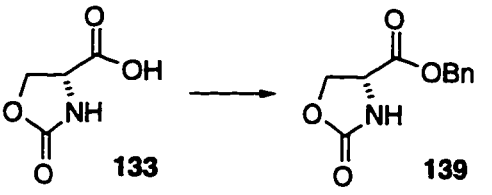




Compound **205** continued:



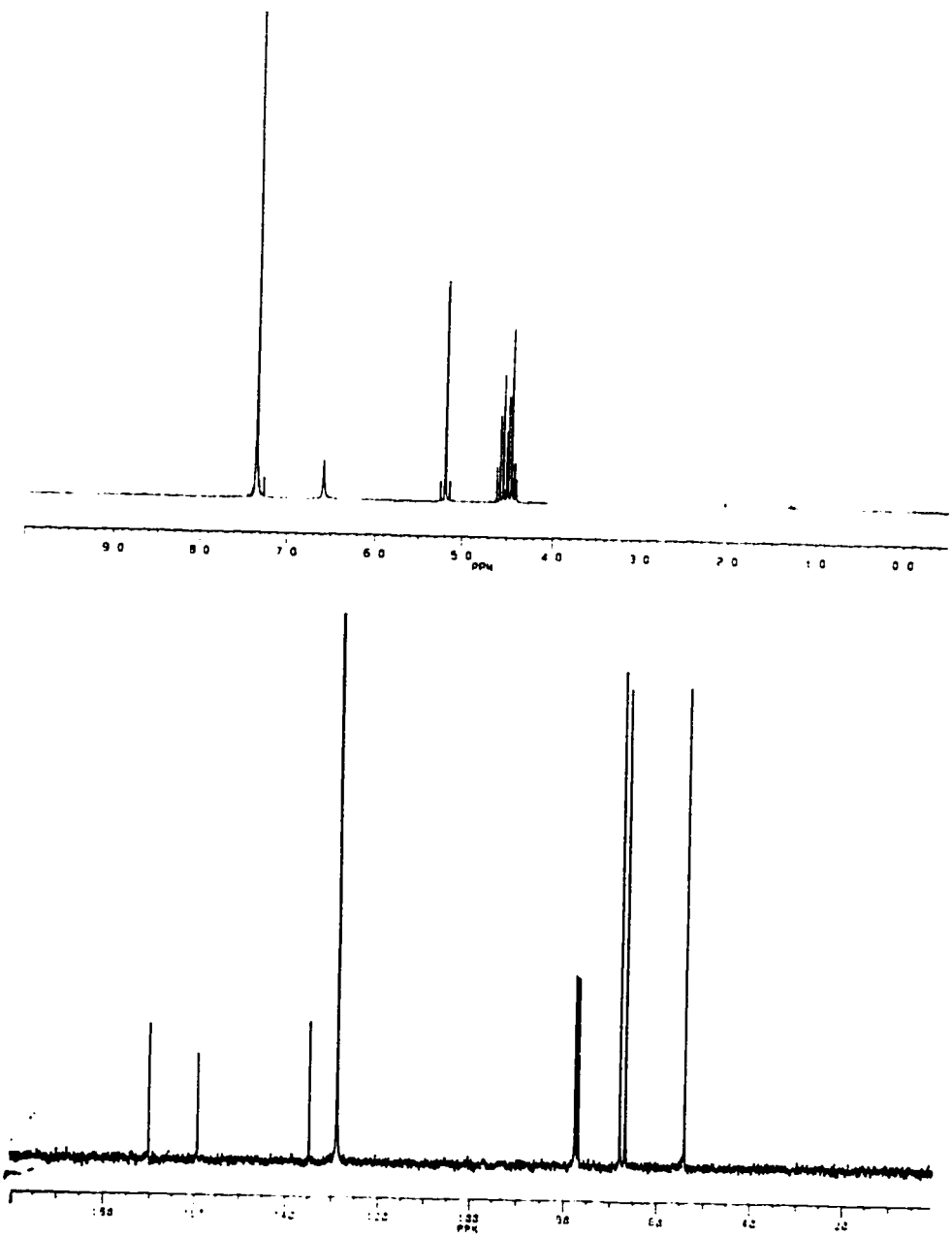




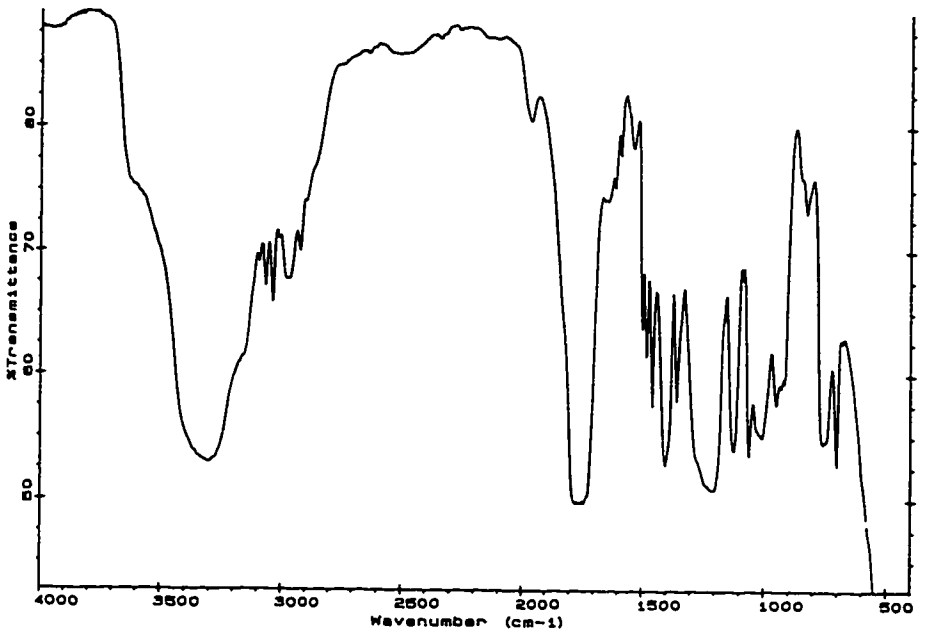
To a suspended solution of oxazolone acid **133** (5g, 0.0382mol) and Et_3N (1.60 mL 0.115mol, 3eq) in 80 mL of acetone was added BnBr (13.7 mL, 0.115mol, 3eq). After 10 minutes the reaction became warm and cloudy and more white solid was formed after 8 hours. The solid was removed by filtration and the organic solution was concentrated *in vacuo*. The residue was dissolved in 50 mL of EtOAc and the undissolved white solid was removed through filtration. The organic solution was concentrated *in vacuo* and the residue was chromatographed (silica gel, 40 → 70% EtOAc/hexane) to afford 7.18g of **139** as colorless oil, which on standing form colorless crystal (0.0325mol, 85%)

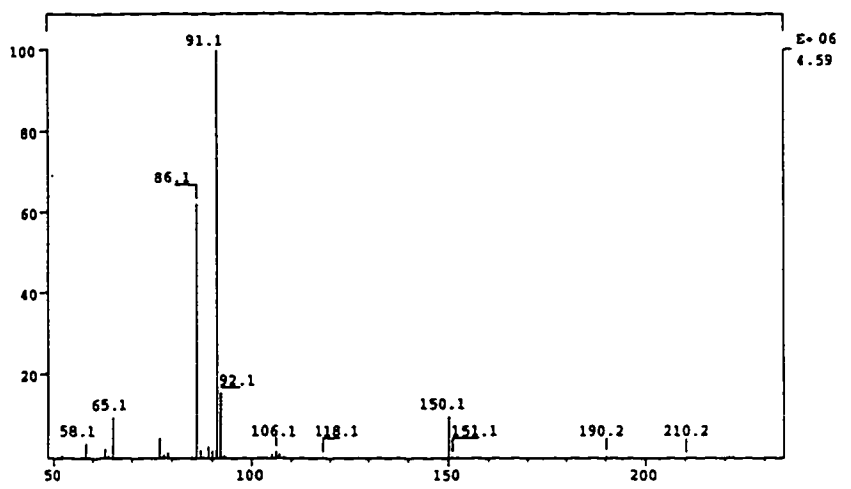
$[\alpha]_D^{25}$ =	+16.9° (0.115g/mL in CH_2Cl_2)
mp =	80–80.5° C
^1H NMR ($CDCl_3$):	4.4-4.6 (3H, m), 6.58 (1H, br), 7.35 (5H, s)
^{13}C NMR ($CDCl_3$):	53.8, 66.6, 67.7, 129.4, 129.8, 134.6, 159.1, 170.0
IR (film):	699, 757, 913, 927, 948, 1003, 1028, 1058, 1122, 1202, 1272, 1354, 1400, 1456, 1499, 1725, 1758, 1786, 2970, 3037, 3276
MS (EI)	58, 65, 77, 86, 91 (100), 106, 118, 150, 180, 190, 221 (M^+)
HRMS (EI):	expected ($C_{11}H_{11}NO_4$, M^+): 221.0688; observed: 221.0688

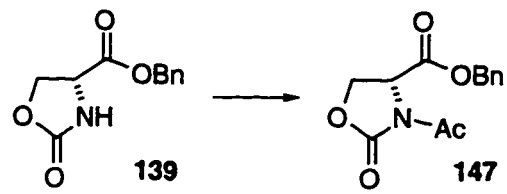
Compound **139**:



Compound **139** continued:



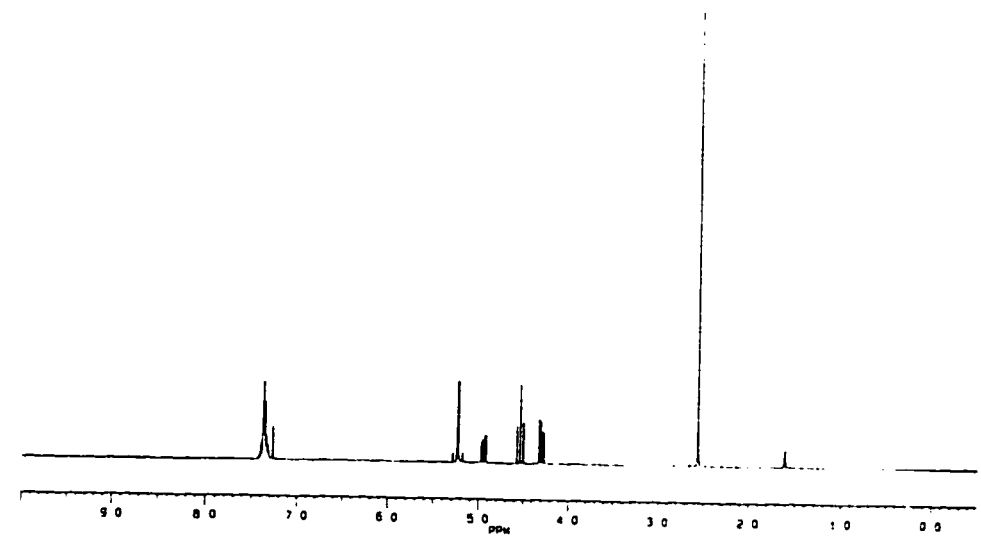


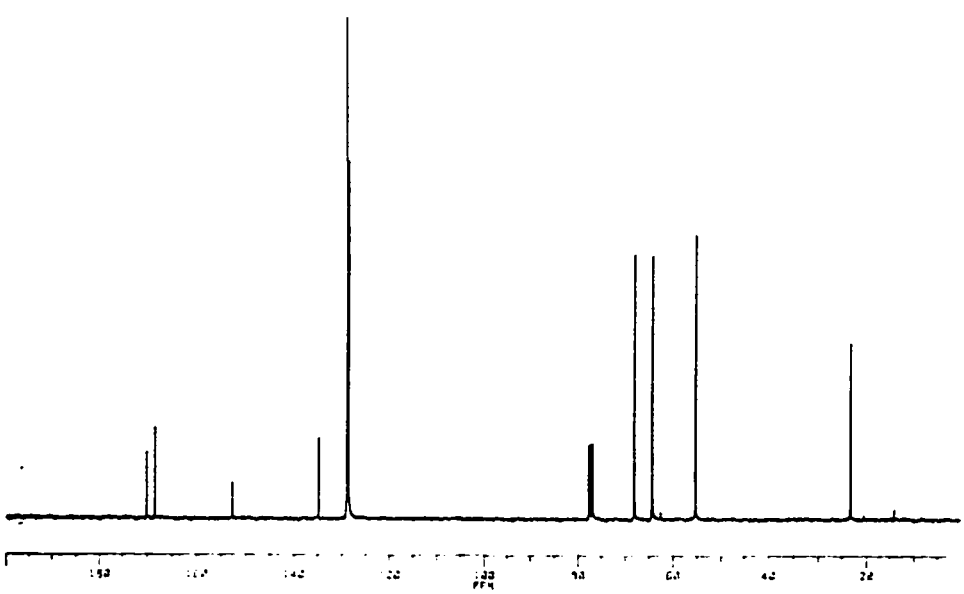


To a stirred solution of oxazolone **139** (7.18g, 0.0325mol, 1eq) in 60 mL of CH_2Cl_2 was added 4.98 mL of Et_3N (0.0358mol, 1.1eq), followed by 3.38 mL of Ac_2O (0.0358mol, 1.1eq) and catalyst DMAP (0.36g, 5%wt of **139**) at 0°C. The reaction was stirred for 2 hours at 0°C and then quenched with 15 mL of pH 7 phosphate buffer (0.5M). The product was partitioned between CH_2Cl_2 and phosphate solution. The two layers were separated and the aqueous layer was extracted with two 50 mL-portions of CH_2Cl_2. The combined organic solution was washed with 40 mL of brine, dried over Na_2SO_4, concentrated *in vacuo* and chromatographed (40% → 60% EtOAc/hexane) to yield 8.36g of **147** as light yellow oil (0.0318mol, 98%).

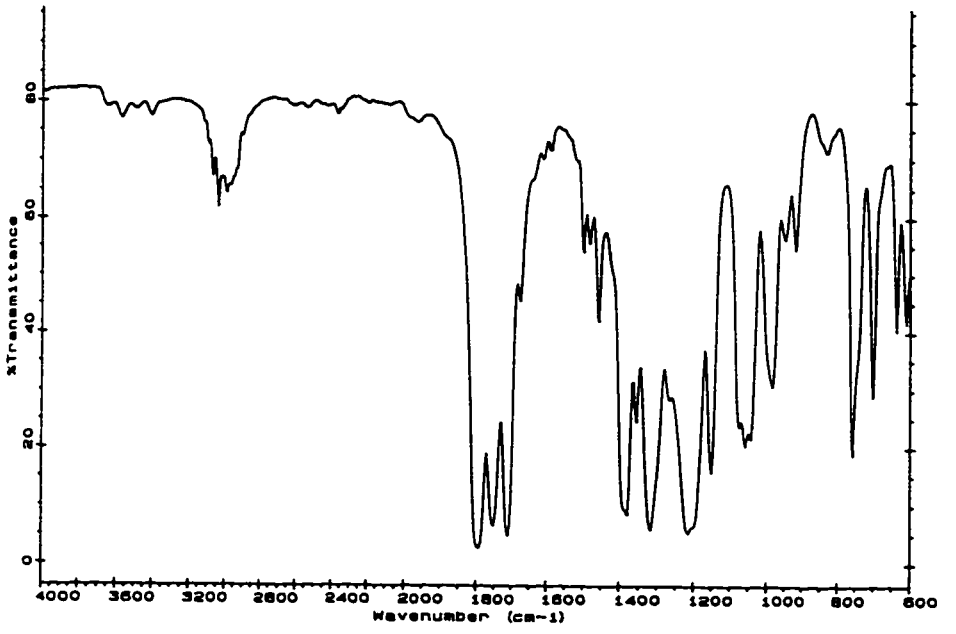
$[\alpha]_D^{25}$ =	+63° (0.14g/mL in CH_2Cl_2)
^{1}H NMR ($CDCl_3$):	2.56 (3H, s), 4.29 (1H, dd, J_1 = 9.36Hz, J_2 = 3.90Hz), 4.53 (1H, t, J = 9.36Hz), 4.94 (1H, dd, J_1 = 9.36Hz, J_2 = 3.90Hz)
^{13}C NMR ($CDCl_3$):	22.3, 55.1, 64.1, 67.9, 128.2, 128.6, 134.4, 152.5, 168.1, 169.9
IR (film):	638, 698, 758, 1062, 1152, 1218, 1319, 1377, 1459, 1713, 1751, 1798, 2984, 3039
MS (EI):	69, 86, 91 (100), 119, 169, 181, 221, 263 (M^+)
HRMS (EI):	expected ($C_{13}H_{13}NO_5$, M^+): 263.0794; observed: 263.0796

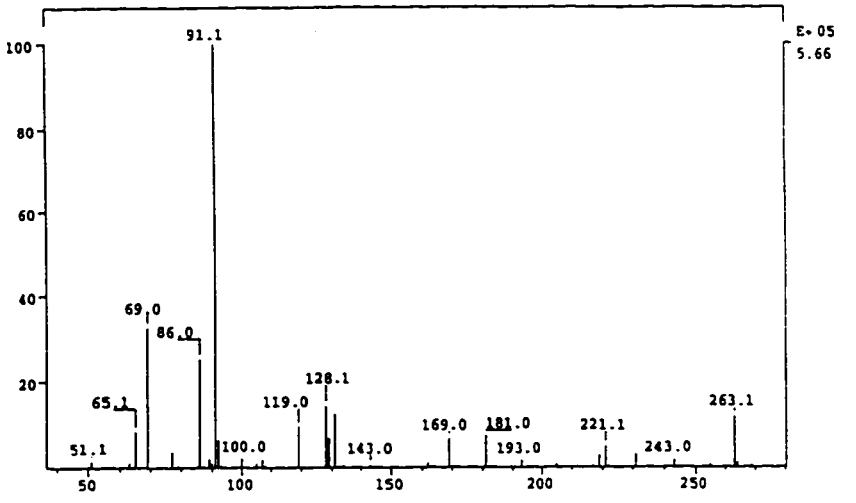
Compound **147**:

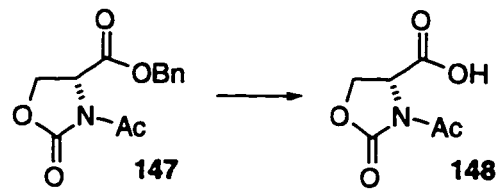




Compound **147** continued:



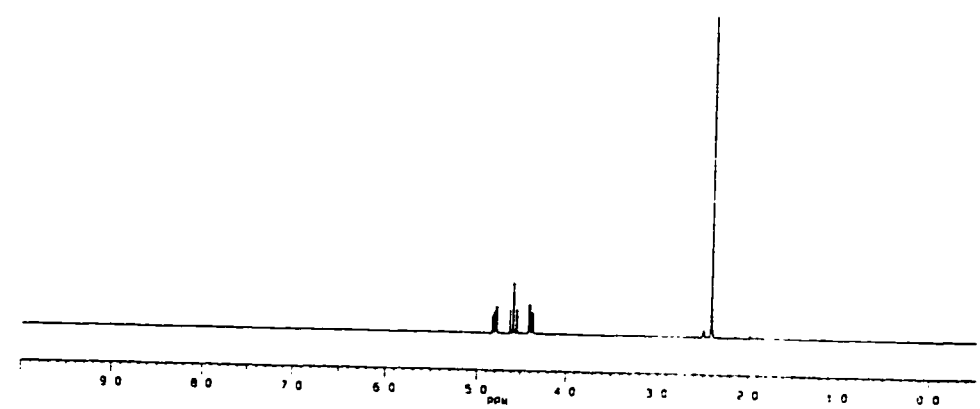


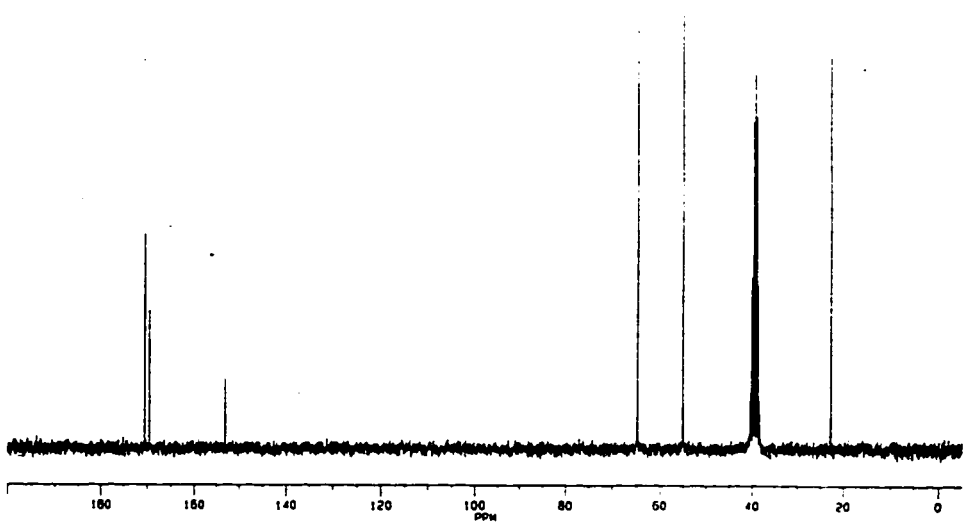


A solution of compound **147** (3.3g, 0.015mol) and Pd/C catalyst (0.16g, 5% wt of **147**) in 200 mL of EtOAc was stirred at room temperature under H_2 atmosphere for 4 hours. The reaction mixture was filtered through a celite pad to remove the catalyst. The filtrate was concentrated *in vacuo* to afford **148** as colorless oil, which upon standing formed colorless crystal (2.54g, 0.0147mol, 98%)

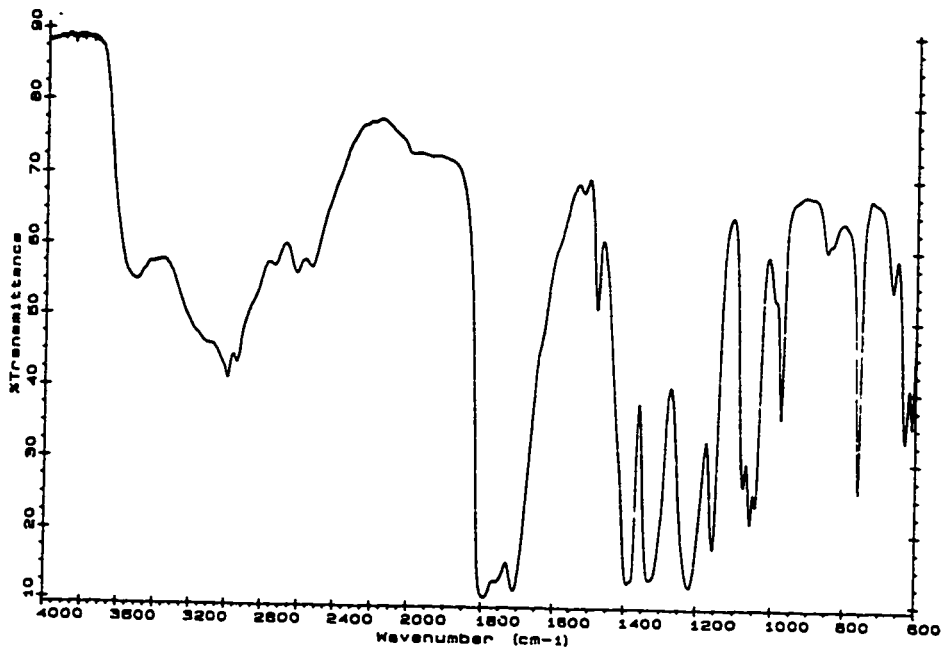
$[a]_D^{25}$ =	+74.66 (0.216g/mL CH_2Cl_2)
mp =	81 - 82° C
^1H NMR (DMSO-d_6):	2.41 (3H, s), 4.39 (1H, dd, J_1 = 9.16Hz, J_2 = 3.49Hz), 4.58 (1H, t, J = 9.16Hz), 4.80 (1H, dd, J_1 = 9.16Hz, J_2 = 3.49Hz)
^{13}C NMR (DMSO-d_6):	22.8, 55.1, 64.8, 153.1, 169.5, 170.4
IR (film):	610, 629, 758, 975, 1043, 1058, 1077, 1155, 1219, 1330, 1390, 1710, 1756, 1797, 2994, 3529
MS (EI):	58, 69, 86, 128 (100), 132, 145, 156, 174 (M^+ +1)
HRMS (EI):	expected ($C_6H_7NO_5$, M^+): 173.0324; observed: 173.0323

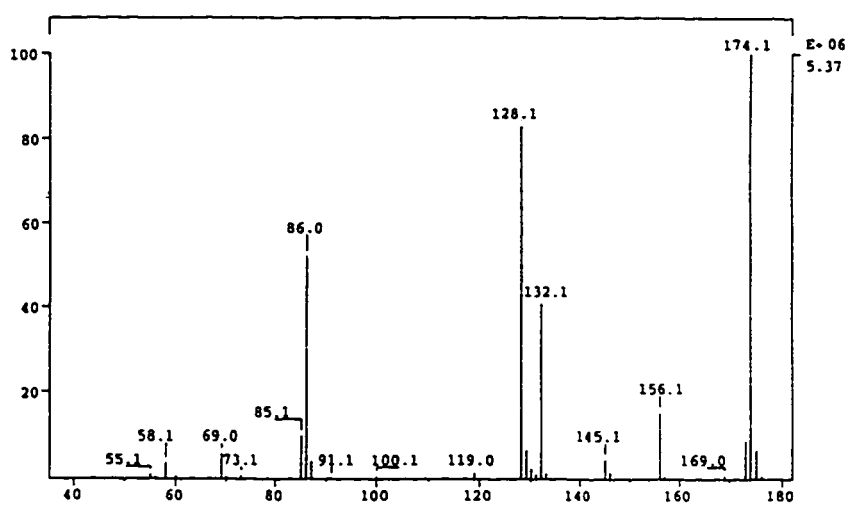
Compound **148**:

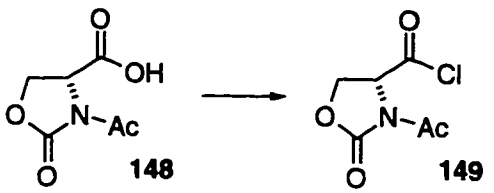




Compound **148** continued:





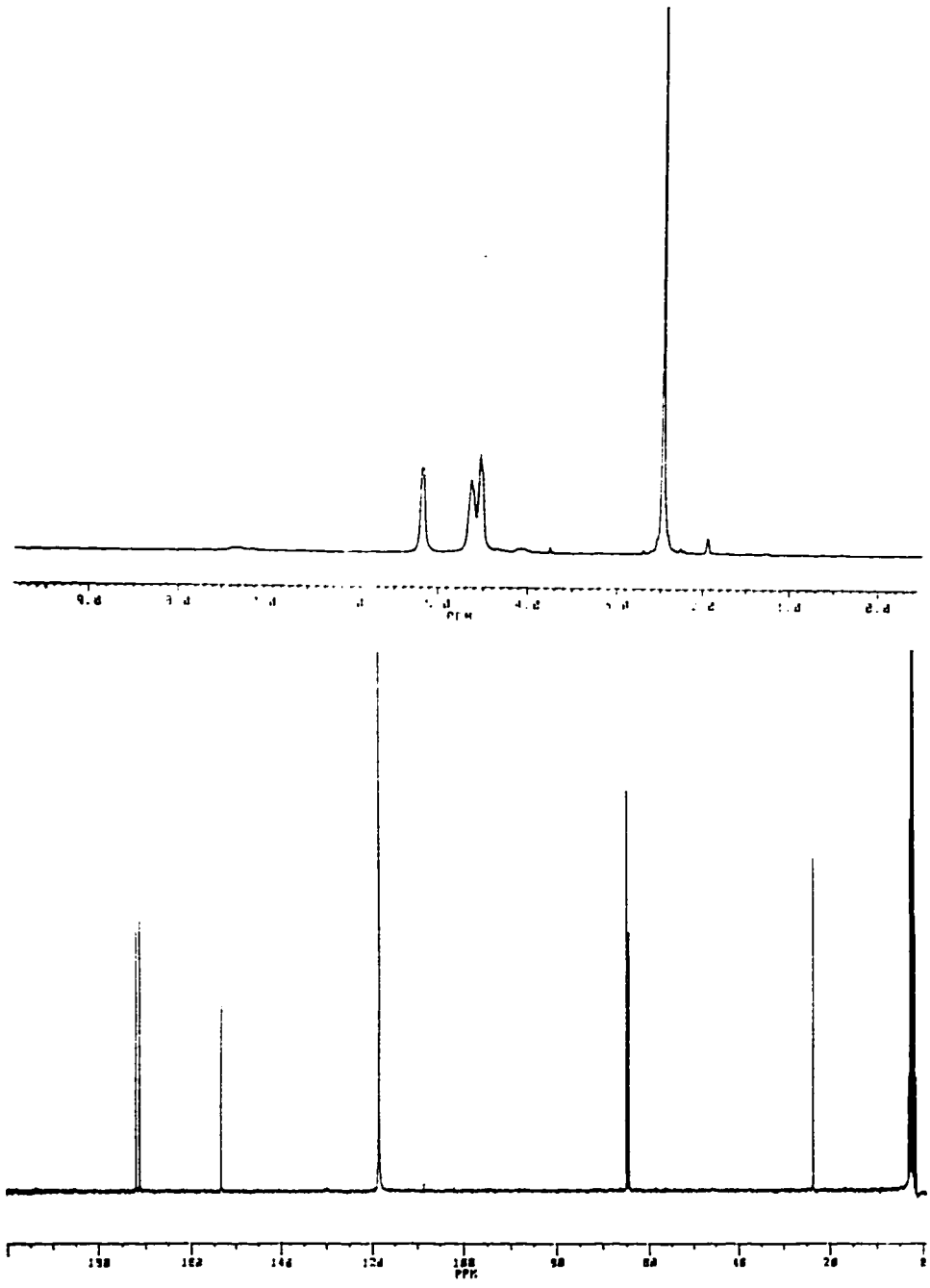


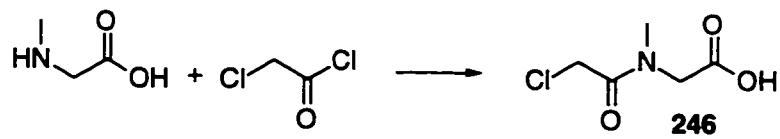
To a solution of the acid **148** (1.5g, 8.7mmol) in 8 mL of anhydrous CH_2Cl_2 was added 1.14 mL of $(COCl)_2$ (0.013mol, 1.5eq) at room temperature. After 10 minutes, two drops of DMF was added to the solution, and the reaction was stirred at room temperature for an additional hour. The solution was concentrated *in vacuo* to afford dark brown solid (1.7g), which was used directly without further purification.

^{1}H NMR (CD_3CN): 2.49 (3H, s), 4.56 (1H, br, overlapping), 4.62 (1H, br, overlapping), 5.20 (1H, br)

^{13}C NMR (CD_3CN): 23.4, 64.2, 64.7, 153.2, 171.0, 171.8

Compound **149**:



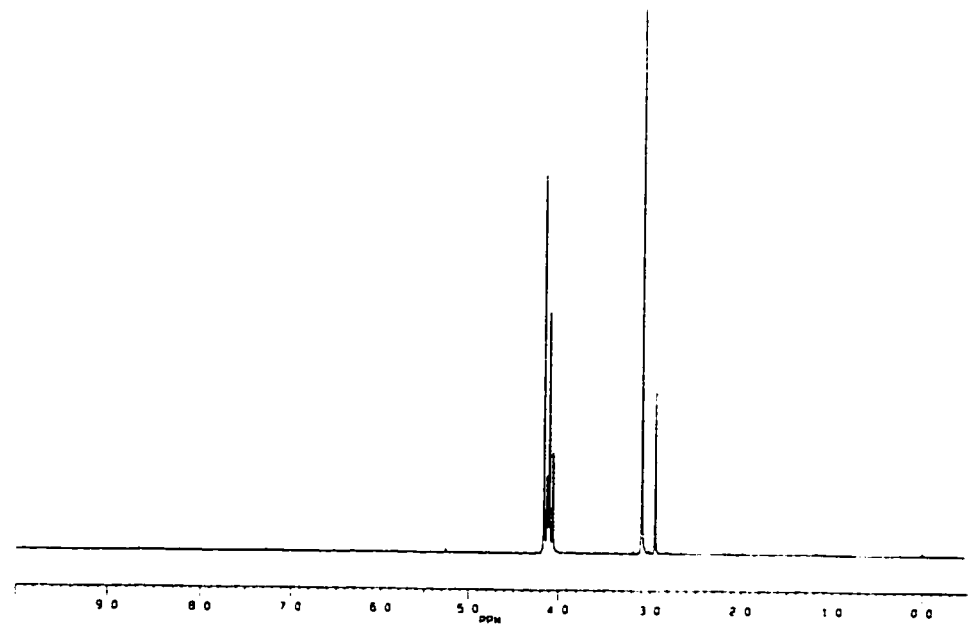


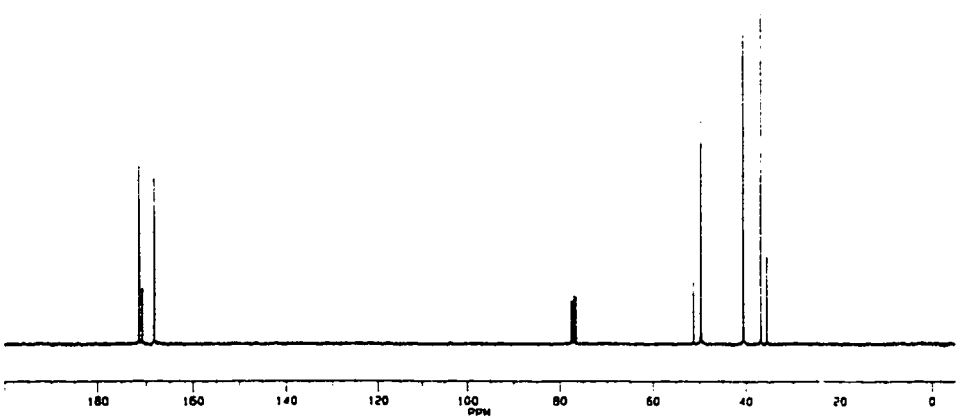
246

8.9g of sarcosine (0.1mol) and 10.6g of $NaHCO_3$ (0.1mol, 1eq) were dissolved in 50 mL of aqueous NaOH solution (10%wt, 0.12mol, 1.2eq). Additional 30mL of water and 40 mL of CH_2Cl_2 were added and the mixture was cooled to 0°C. 15.9 mL of chloroacetyl chloride (0.2mol, 2eq) was added slowly through an additional funnel. The reaction was warmed up to room temperature and was stirred for an additional 30 minutes. The pH of the mixture was then adjusted to 1-2 with concentrated HCl and the product was extracted with three 100 mL-portions of EtOAc. The organic solutions were combined, concentrated *in vacuo* and chromatographed (silica gel, 80% EtOAc/hexane to EtOAc as eluent) to afford **246** as colorless oil (13.1g, 0.079mol, 79%)

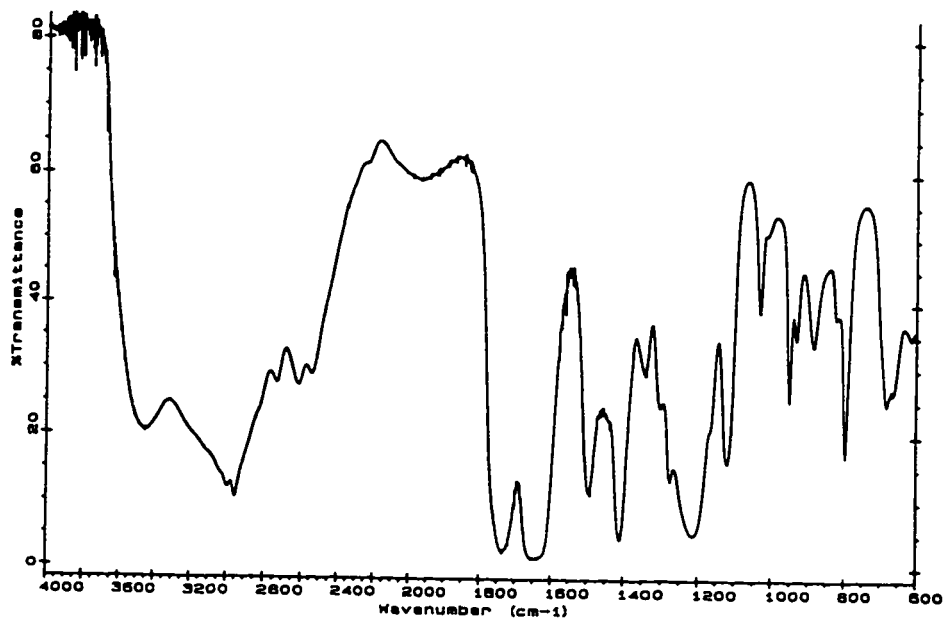
^{1}H NMR ($CDCl_3$):	Two rotomers exist with the ratio of about 3:10 at 25°C Major: 3.09 (3H, s), 4.09 (2H, s), 4.15 (2H, s), 11.04 (1H, br, overlapping with minor rotomer) Moinor: 2.94 (3H, s), 4.05 (2H, s), 4.12 (2H, s), 11.04 (1H, br, overlapping with major rotomer)
^{13}H NMR ($CDCl_3$):	Two rotomers exist at 25°C Major: 36.7, 40.6, 49.7, 168.2, 171.5; Minor: 35.5, 40.5, 51.2, 168.1, 170.8
IR (Film):	682, 797, 953, 1032, 1120, 1215, 1409, 1494, 1644, 1735, 2617, 2730, 2949, 3449
MS (EI):	57, 77, 88, 102, 120 (100), 130, 165 (M^+)
HRMS (EI):	expected ($C_5H_8NO_3Cl$): 165.0193; observed: 165.0191

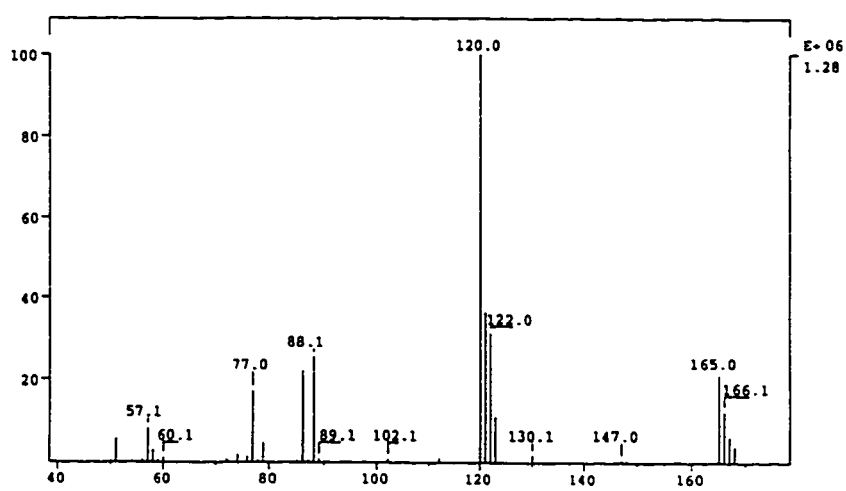
Compound **246**:

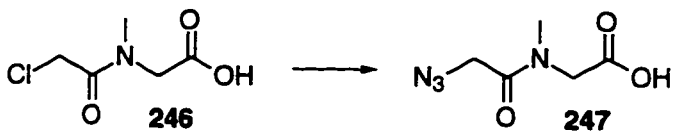




Compound **246** continued:



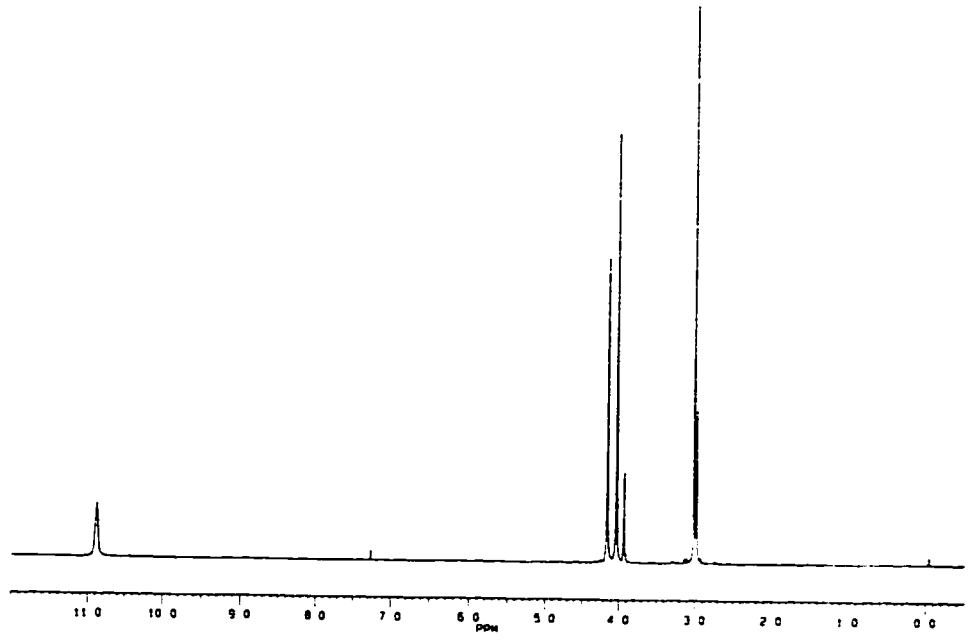


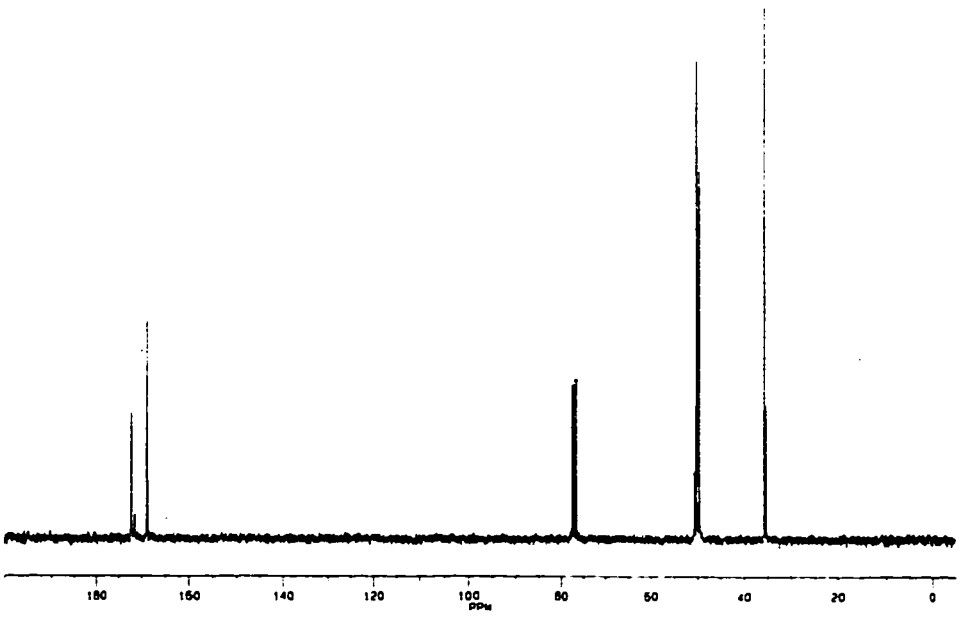


25g of **246** (0.15mol, 1eq) and 19.5 g of NaN_3 (0.30mol, 2eq) was dissolved in 80 mL of water and the resulting solution was stirred at room temperature for 36 hours. Concentrated HCl was added until the orange color of the reaction mixture disappeared. The product was extracted with three 100mL-portions of EtOAc, and the combined organic solutions were dried over Na_2SO_4, and concentrated *in vacuo* to afford **247** as colorless oil (22.2g, 0.13mol, 86%).

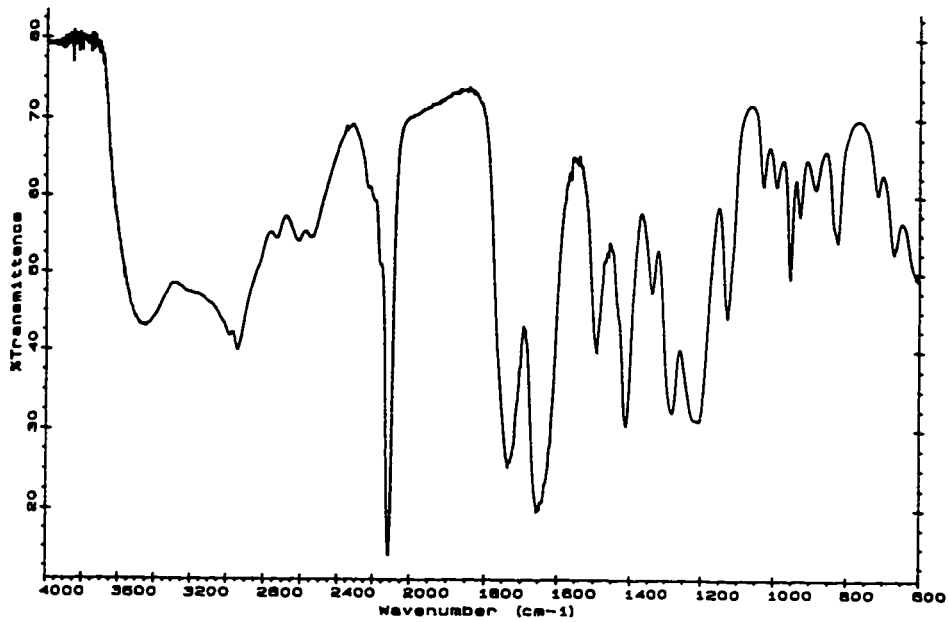
^{1}H NMR ($CDCl_3$):	Two rotomers exist in a ratio of about 3:10 Major: 3.02 (3H, s), 4.03 (2H, s, overlaping with minor rotomer), 4.15 (2H, s), 10.88 (1H, br, overlapping with minor rotomer) Minor: 2.98 (2H, s), 3.93 (2H, s), 4.03 (2H, s, overlaping with minor rotomer), 4.15 (2H, s), 10.88 (1H, br, overlapping with major rotomer)
^{13}C NMR ($CDCl_3$):	Two rotomers: Major: 35.8, 49.6, 50.2, 168.9, 172.3; Minor: 35.4, 50.3, 50.7, 168.8, 171.5
IR (film):	671, 824, 956, 1125, 1213, 1281, 1415, 1491, 1650, 1735, 2113, 2941, 3465
MS (EI):	57, 71, 88 (100), 99, 102, 116, 145, 155, 173 (M^+)
HRMS (EI):	expaected ($C_5H_9N_4O_3$, M^+ +H): 173.0674; observed: 173.0674

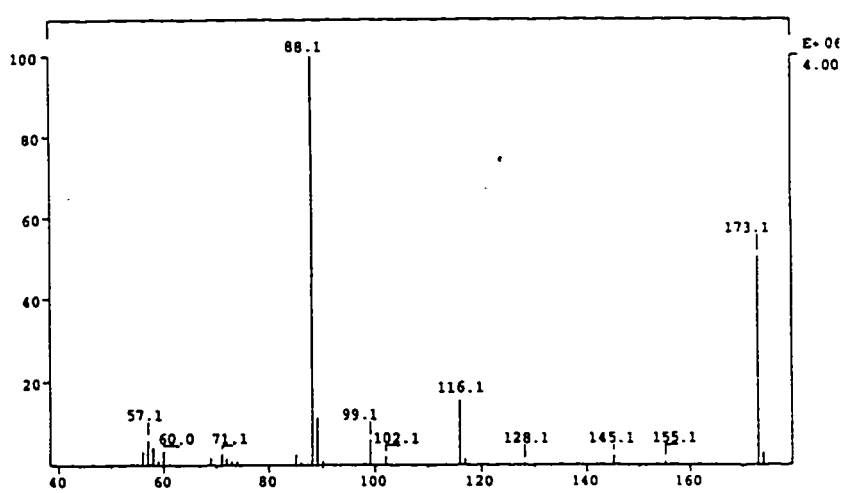
Compound **247**:





Compound **247** continued:



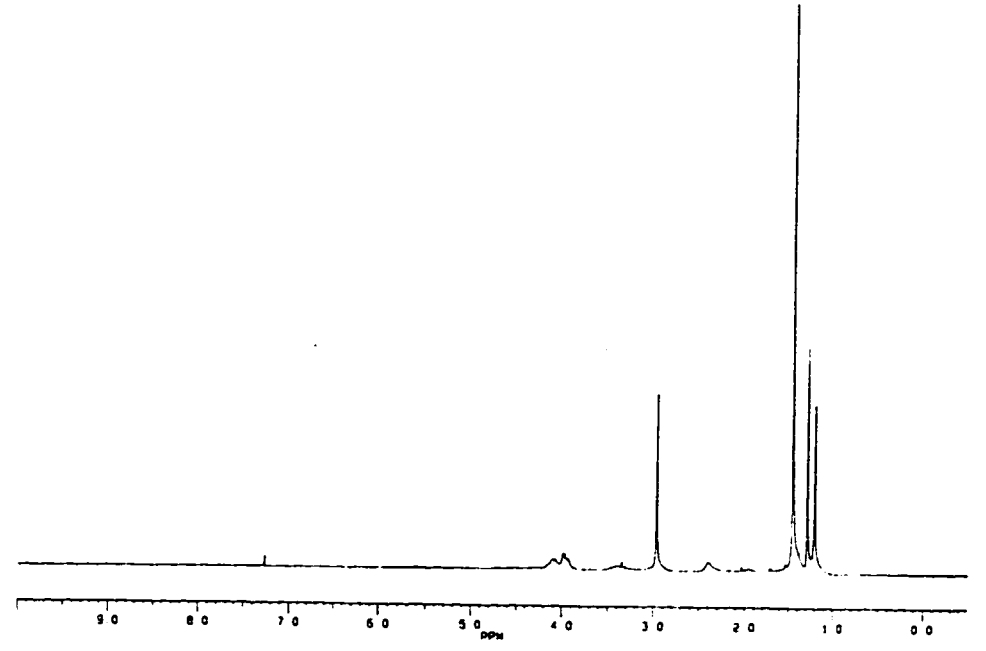


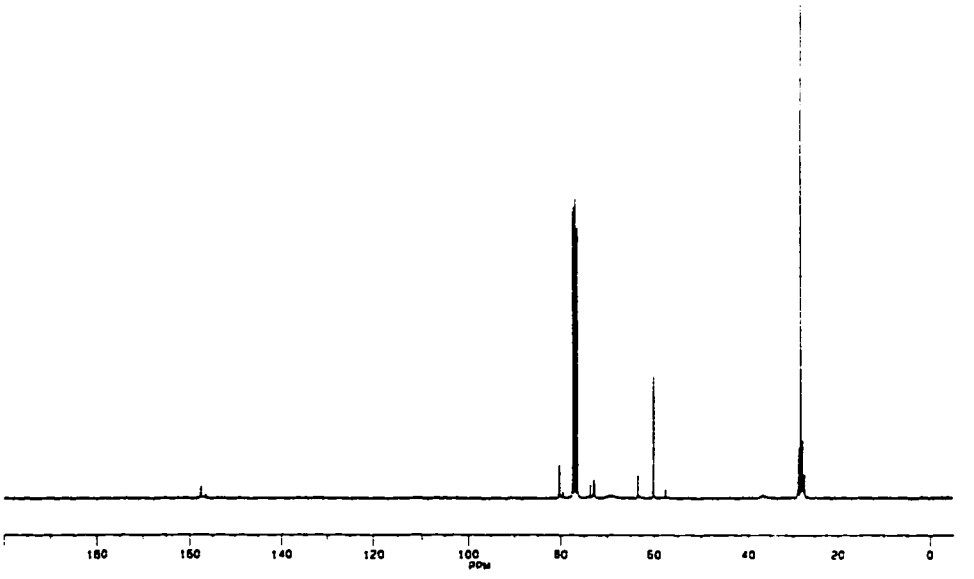
To a solution of compound **263** (5.7g, 18.0 mmol) in 60 mL of MeOH was added 0.57g of $TsOH \cdot H_2O$ (10% wt of **263**) and the reaction was stirred at room temperature for 2 hours. Upon the disappearance of the starting material (TLC), solid $KHCO_3$ was added to the mixture to neutralize acid. MeOH was then evaporated *in vacuo* and the residue was partitioned between 100 mL of EtOAc and 30 mL of water. The solution was washed with 40mL of brine, dried over Na_2SO_4, concentrated *in vacuo* to give the crude product (14.44mmol, 97%) as white solid. The product was recrystallized in 50% EtOAc/hexane to afford 3.32g of **264** as colorless crystal (14.20mmol, 79%).

$[\alpha]_D^{25}$ =	+34.81 (0.049g/mL MeOH)
m.p.=	134–135°C
^{1}H NMR ($CDCl_3$):	1.20 (3H, s), 1.29 (3H, s), 1.46 (9H, s), 2.40 (IH, br), 2.97 (3H, s), 3.3 (1H, br), 3.8-4.0 (2H, multiple complx), 4.0-4.2 (1H, multiple complx)
^{13}C NMR ($CDCl_3$):	Two rotomers: major: 27.9, 28.4, 28.8, 60.2, 63.4, 72.8, 80.3, 157.5 minor: 27.5, 27.3, 28.8, 57.6, 63.4, 73.6,79.5, 156.4
IR (KBr):	693, 763, 774, 813, 828, 873, 929, 954, 974, 1040, 1060, 1077, 1165, 1236, 1369, 1408, 1442, 1475, 1661, 2973, 3387
MS (EI):	58, 74, 102 (100), 118, 128, 144, 157, 174, 204, 217, 234 (M^++1)

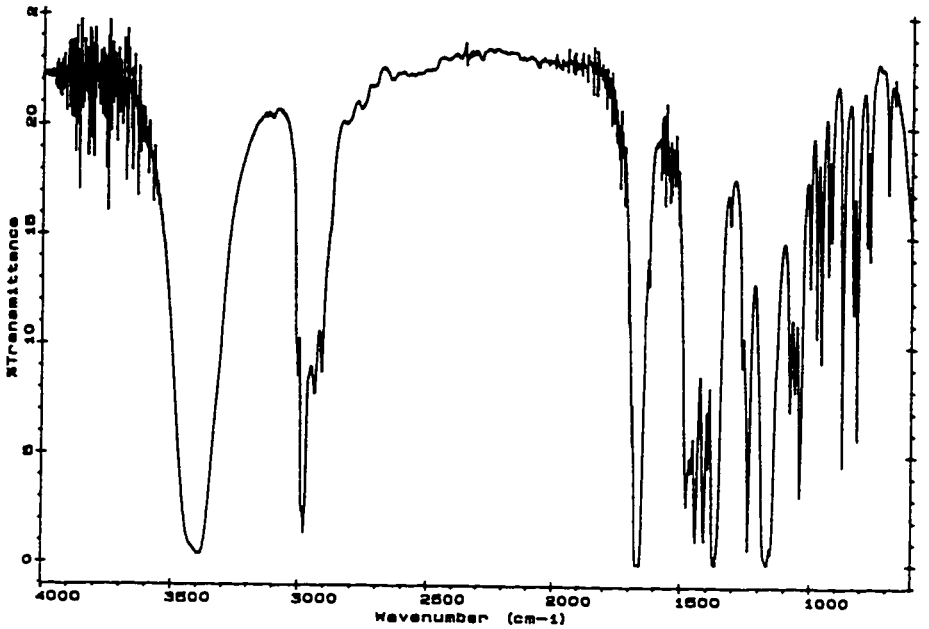
HRMS (EI): expected ($C_{11}H_{24}NO_4$, M^+ +H): 234.1705; observed: 234.1706

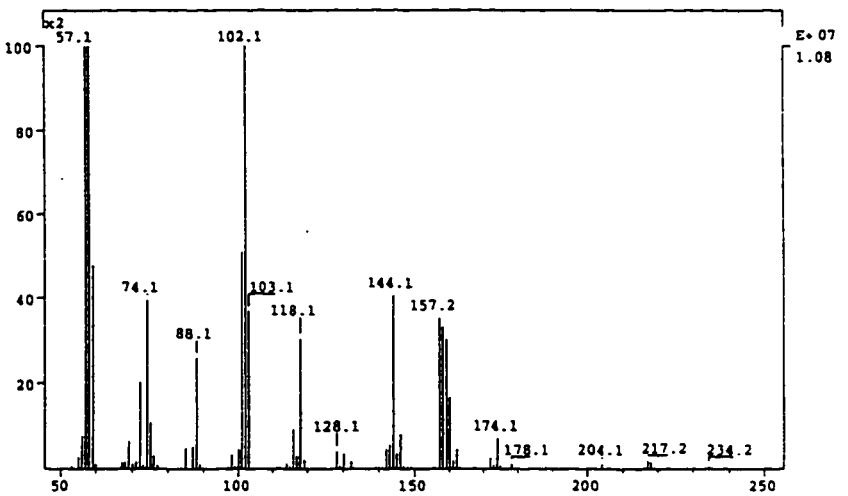
Compound **264**:

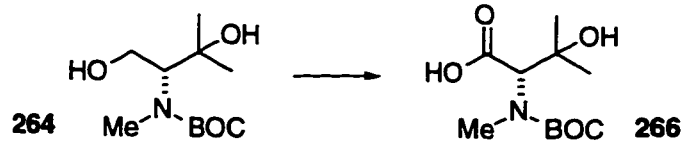




Compound **264** continued:







264 **266**

DMSO (2.3 mL, 0.032mol) was added dropwise to a stirred solution of oxalyl chloride (2.06 mL, 0.024mol) in 50 mL THF at –78°C under Ar, and the solution was stirred for additional 20 minutes. A solution of alcohol (5g, 0.022mol) in 40 mL of THF was added to the reaction solution slowly via syringe. After 30 minutes additional stirring at –78°C, triethylamine (15 mL, 0.20 mol) was added, and the reaction was allowed to warm up to room temperature. After one hour, the reaction was poured into 50 mL of saturated aqueous $NaHCO_3$ solution. Ethyl ether (100 mL) was added to the mixture and the two layers were shaken and separated. The aqueous layer was extracted with 100 mL of ethyl ether. The combined organic layers were washed with 50 mL of brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford 5.1g of crude aldehyde **265** as orange oil.

Without any purification, the aldehyde was dissolved in a 50 mL of 3:2 t-BuOH/2-methyl-2-butene and the mixture was cooled to 0°C. A solution of $NaClO_2$ (2.92g, 0.032mol) in water (20 mL) was added dropwise followed by NaH_2PO_4 solution (4.45g in 25 mL of H_2O). The reaction was stirred at 0°C for 0.5 hours and then at room temperature for 2 hours. 100 mL of ethyl acetate was added to the mixture and the two layers were separated. The aqueous layer was extracted with additional 100 mL of ethyl acetate and the combined organic layers were washed with 50 mL of brine, dried over Na_2SO_4, concentrated *in vacuo* to yield light yellow oil (5.3g crude, 0.021mol 100%).

$[\alpha]_D^{25}$ = –31.34 (0.054g/mL CH_2Cl_2)

^1H NMR ($CDCl_3$, 50°C): 1.26 (3H, s), 1.38 (3H, s), 1.46 (9H, s), 2.94 (3H, s), 4.55 (1H, s), 6.94 (1H, br)

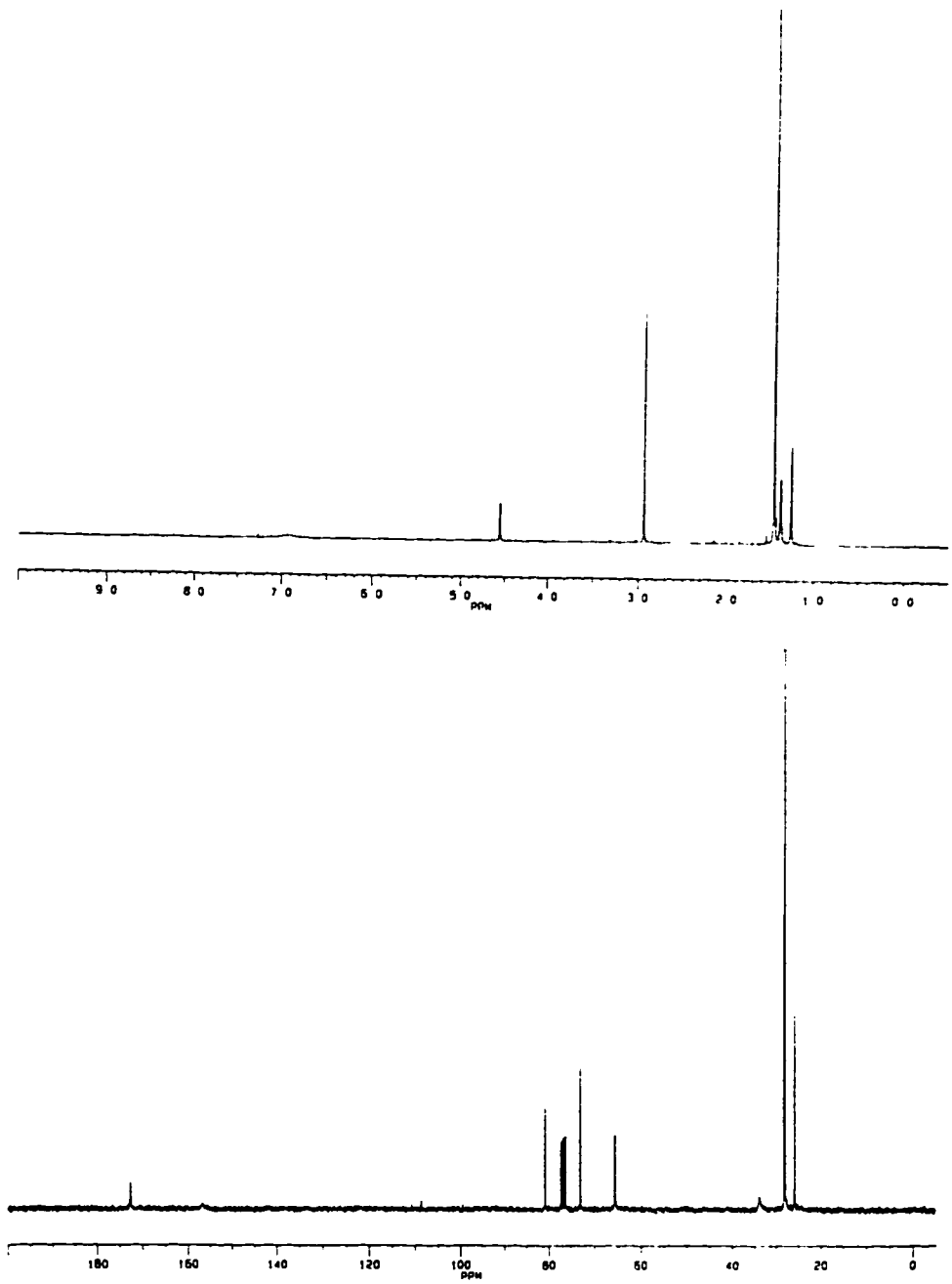
^{13}C NMR ($CDCl_3$, 50°C): 26.2, 28.3, 28.6, 34.2, 65.9, 73.3, 81.1, 157.0, 173.0

IR (Film): 726.2, 772.7, 859.0, 918.8, 1064.8, 1157.8, 1244.1, 1330.5, 1403.5, 1456.6, 1489.9, 1689.1, 2598.8, 2930.8, 2984.0, 3455.5

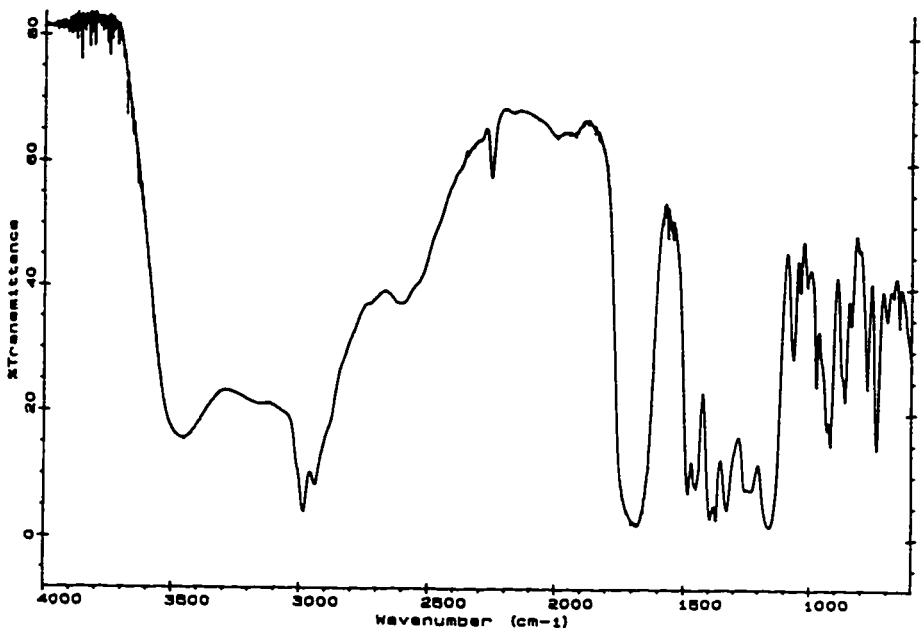
MS (EI): 57, 69, 84, 102, 115 (100), 133, 148, 156, 174, 192, 205, 248 (M^+ +1)

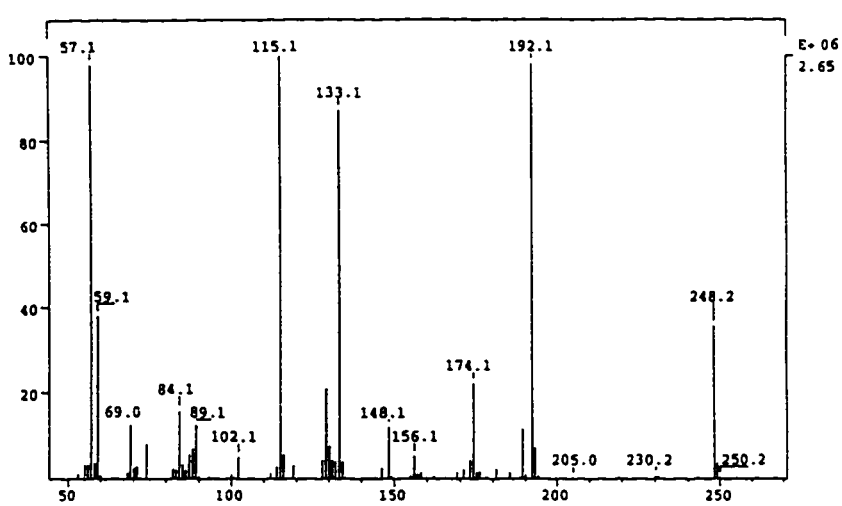
HRMS (EI) expected ($C_{11}H_{21}NO_5$, M^+ +H): 248.1498; observed: 248.1503

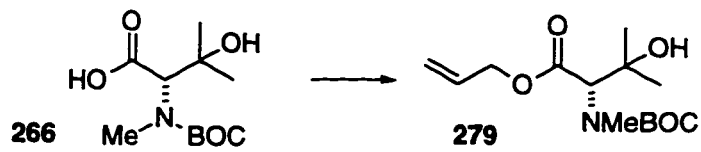
Compound **266**:



Compound **266** continued:







266 279

To a solution of acid **266** (5.3g crude, 0.021mol) in 100 mL of acetone was added 8.93 mL of Et_3N (0.064mol, 3eq), followed by 5.56 mL of allyl bromide (0.0642mol, 3eq) under Ar. The solution became cloudy in 5 minutes and more white solid was formed after 3 hours. The solid was removed from the mixture by filtration. The organic solution was concentrated *in vacuo* and the residue partitioned between 100 mL of EtOAc and 40 mL of pH 7 phosphate solution. The aqueous layer was extracted with 50 mL of EtOAc. The combined organic layers were washed with 40 mL of brine, dried over Na_2SO_4, concentrated *in vacuo* and chromatographed (silica gel, 5%-10% EtOAc/hexane) to yield 4.68g of **279** as light yellow oil (0.016mol, 78%).

$[\alpha]_D^{25}$ = – 58.66 (0.1431g/mL in CH_2Cl_2)

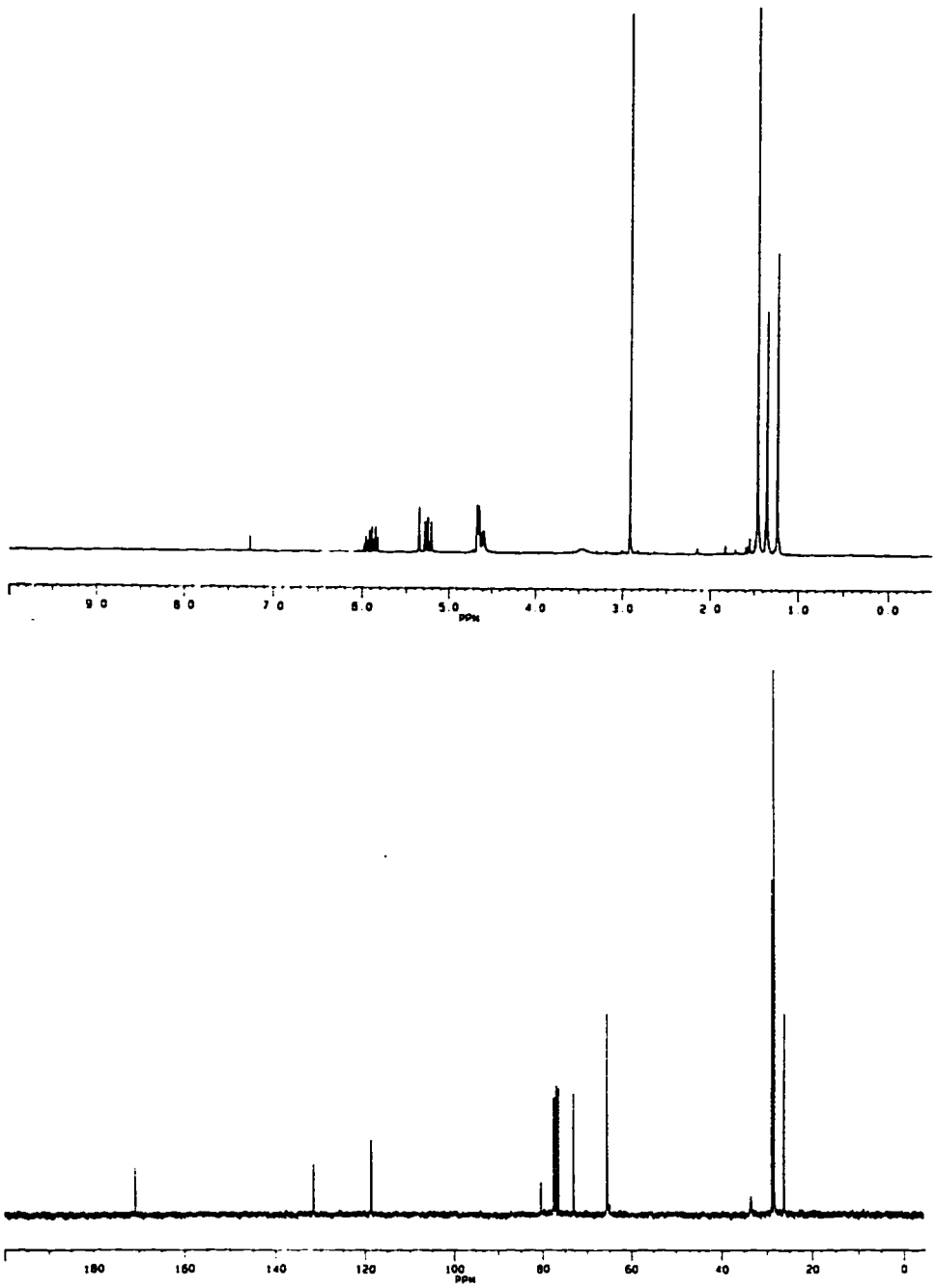
^{1}H NMR ($CDCl_3$, 50°C): 1.24 (3H, s), 1.36 (3H, s), 1.46 (9H, s), 2.92 (3H, s), 4.59 (1H, br), 4.65 (2H, dt, J_1 = 5.62Hz, J_2 = 1.46Hz), 5.24 (1H, dq, J_1=10.50Hz, J_2 = 1.46Hz), 5.32 (1H, dq, J_1 = 17.33Hz, J_2 = 1.46Hz), 5.91 (1H, ddt, J_1 = 17.33Hz, J_2 = 10.50Hz, J_3 = 5.62Hz)

^{13}C NMR ($CDCl_3$): There are two major rotomer exist at room temperature:
Major: 25.9, 28.2, 28.8, 33.7, 64.8, 65.5, 72.8, 80.3, 118.5, 131.4, 156.5, 170.8
Minor: 25.8, 28.2, 28.6, 33.1, 65.2, 65.6, 72.8, 80.6, 118.8, 131.1, 155.3, 170.8

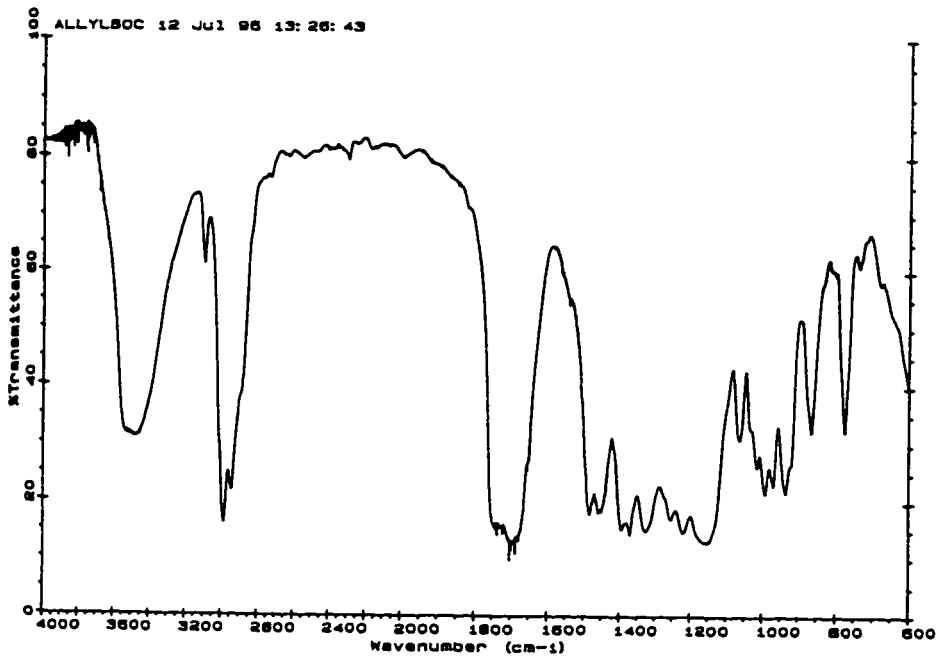
At 50°C: 26.2, 28.3, 28.9, 33.2, 65.4, 65.5, 73.0, 80.4, 118.6, 131.6, 170.8

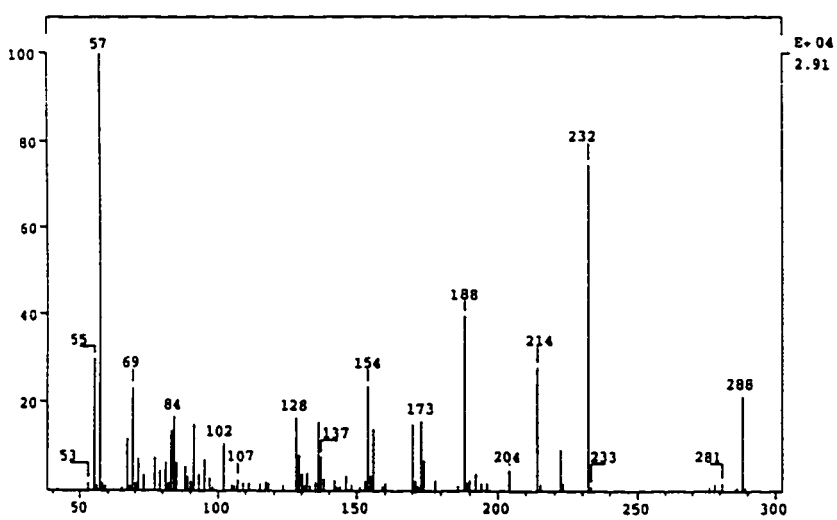
IR (film):	773, 859, 931, 991, 1062, 1152, 1325, 1366, 1450, 1483, 1691, 1743, 2934, 2981, 3488
MS (FAB):	57 (100), 69, 84, 102, 128, 137, 154, 173, 188, 204, 214, 232, 281, 288 (M^++1)
HRMS	expected ($C_{14}H_{26}NO_5$, M^+ +H): 288.1811; observed: 288.1806

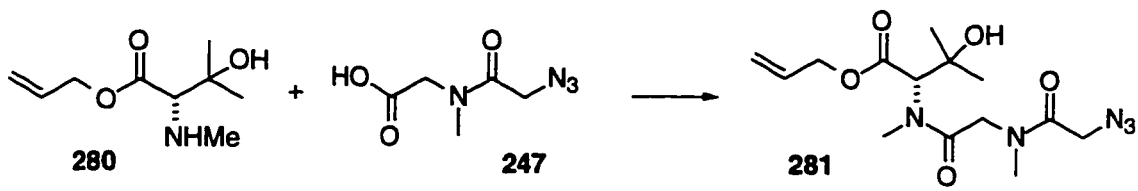
Compound **279**:



Compound **279** continued:







280 **247** **281**

A solution of 1.07g of allyl ester **279** (3.74mmol, 1eq) and 10 mL of 50% TFA/CH_2Cl_2 was stirred at room temperature for 30 minutes. The solution was then concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2 and concentrated again. This process was repeated twice to remove most of the TFA residue.

A solution of the amine·TFA salt **280** and the acid **247** (0.77g, 4.49mmol, 1.2eq) in 10 mL of CH_2Cl_2 was cooled to 0°C under Ar. 1.56 mL of Et_3N (11.22mmol, 3eq) was added to the reaction followed by slow addition of BOP-Cl solid (1.14g, 4.49mmol, 1.2eq). If needed, more Et_3N was added to keep the reaction under basic condition. The reaction was stirred at 0°C for one hours (monitored by TLC) and then quenched with 10 mL of pH 7 phosphate solution. The reaction mixture was partitioned between 40 mL of CH_2Cl_2 and 20 mL of pH 7 phosphate and the aqueous layer was extracted with 40 mL of CH_2Cl_2. The combined organic layers were washed with 20 mL of brine, dried over Na_2SO_4, concentrated *in vacuo*, and chromatographed (silica gel, 40%→60% EtOAc/hexane) to afford 1.19g of **281** as light yellow oil (3.49mmol, 93%).

$[\alpha]_D^{25}$ = – 64.67 (0.077g/mL, CH_2Cl_2)

^{1}H NMR ($CDCl_3$, 50°C): 1.16 (3H,s), 1.37 (3H, s), 3.00 (3H, s), 3.08 (3H, s), 3.95 (2H, s), 4.22 (2H, s), 3.55 (1H, br), 4.63 (2H, dd, J_1 = 5.86Hz, J_2 = 1.46Hz), 5.02 (1H, br), 5.23 (1H, d,

overlapping, J = 10.50Hz), 5.30 (1H, dq, J_1 = 17.33Hz, J_2 = 1.46Hz), 5.88 (1H, ddt, J_1 = 17.33HZ, J_2 = 10.50HZ, J_3 = 5.86Hz)

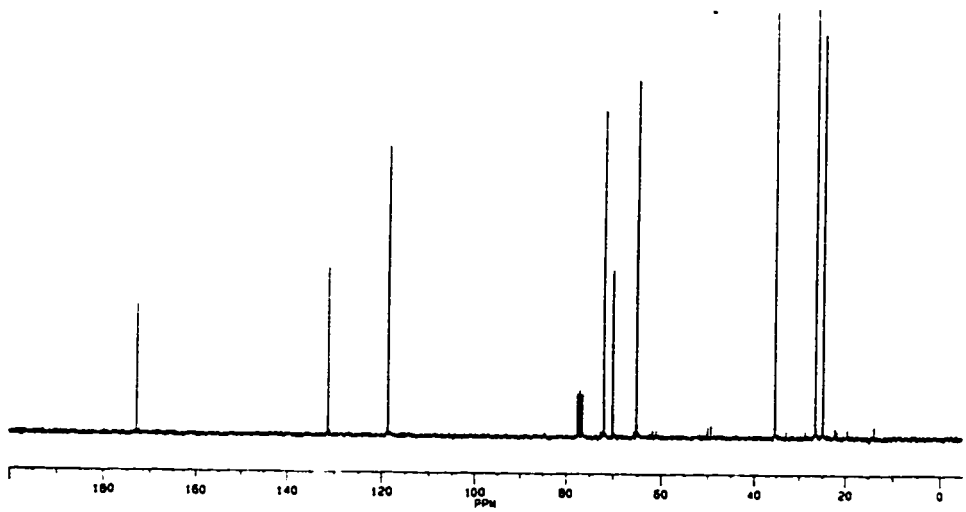
^{13}C NMR ($CDCl_3$, 50°C): 26.4, 28.8, 33.4, 35.7, 49.4, 50.3, 63.7, 65.8, 72.7, 119.0, 131.4, 167.8, 168.7, 169.8

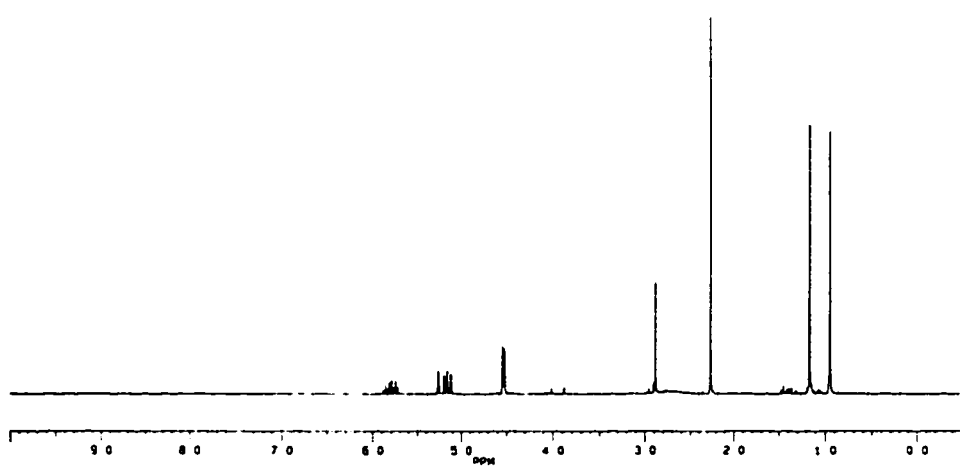
IR (film): 734, 827, 920, 950, 1029, 1120, 1215, 1286, 1409, 1485, 1650, 1732, 2109, 2938, 2981, 3414

MS (CI): 41, 88, 99, 116, 127, 155(100), 170, 198, 228, 253, 271, 284, 324, 342 (M^++1)

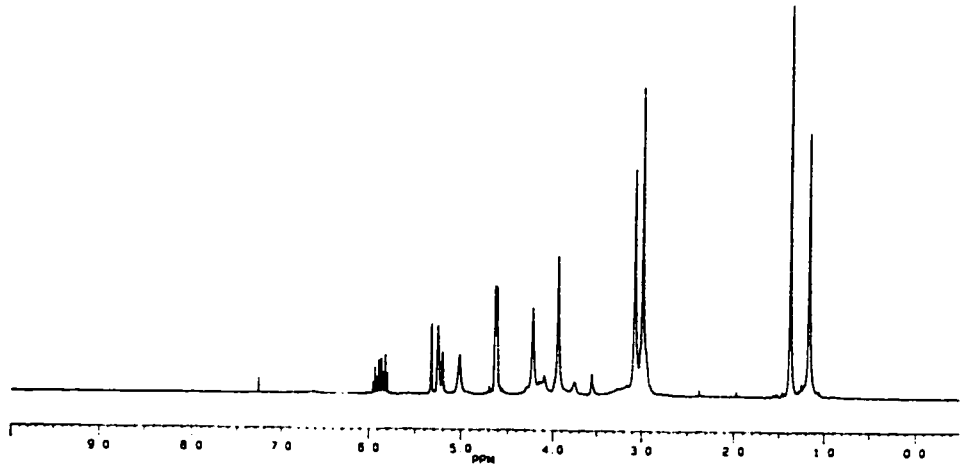
HRMS (EI): expected ($C_{14}H_{24}N_5O_5$, M^+ +H): 342.1777; observed: 342.1778

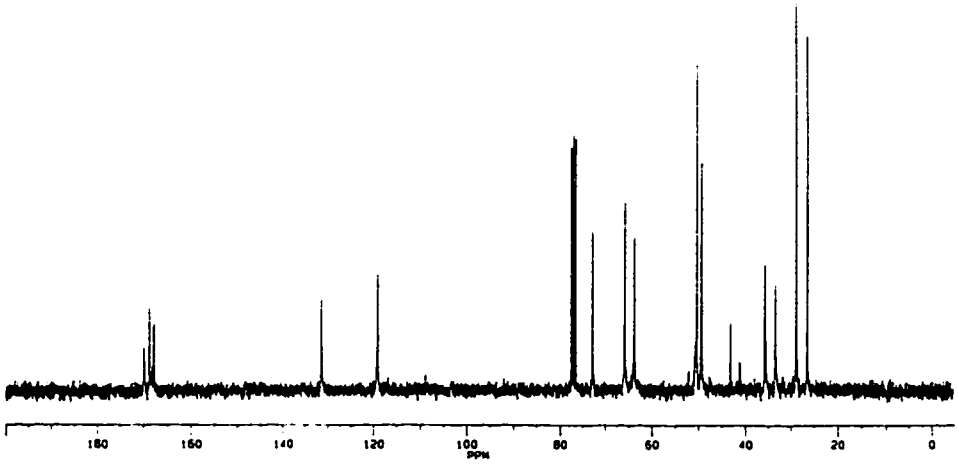
Compound **280**:



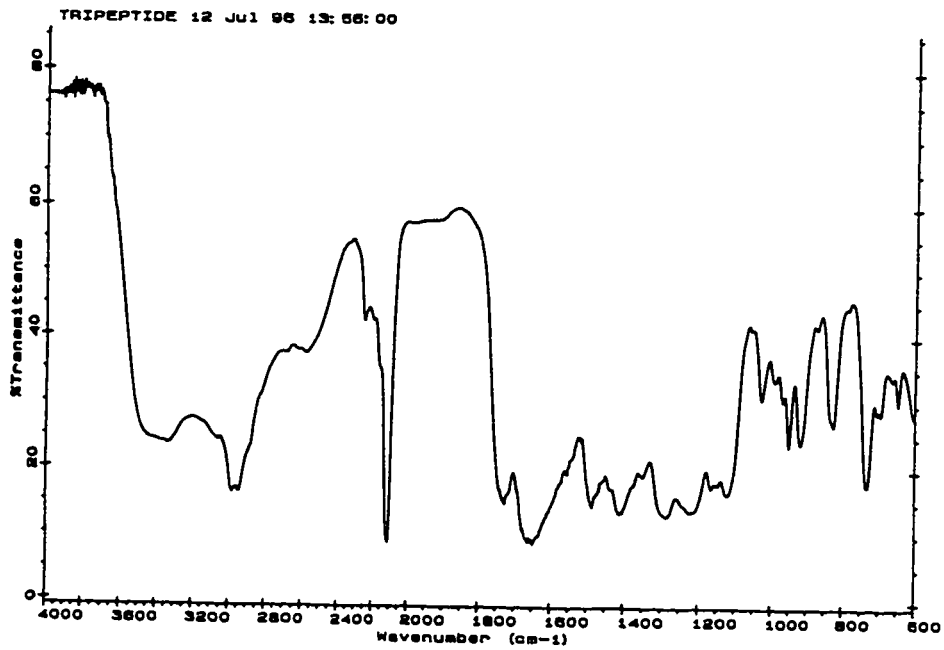


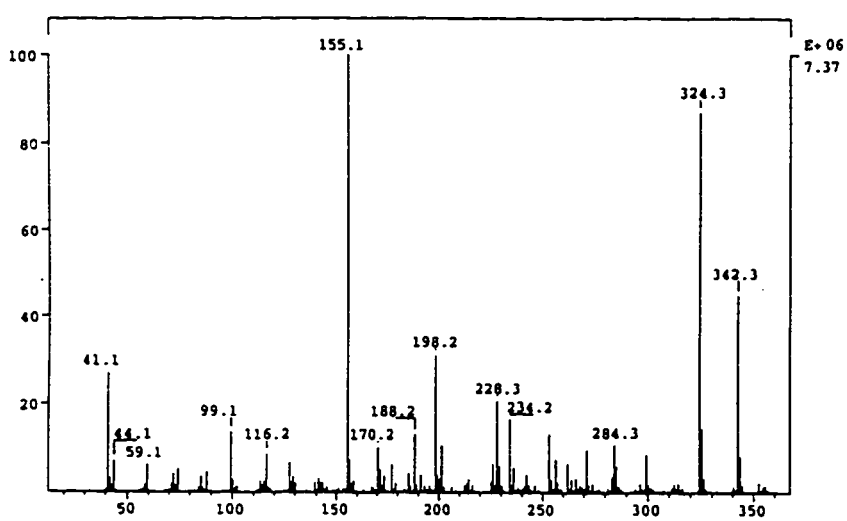
Compound **281**:

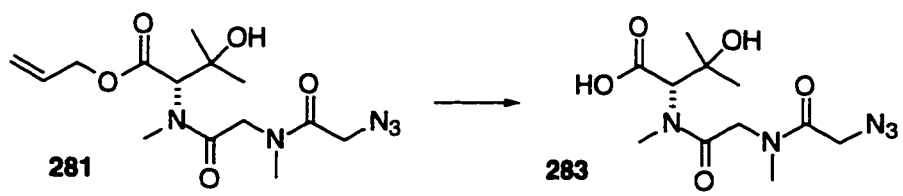




Compound **281** continued:





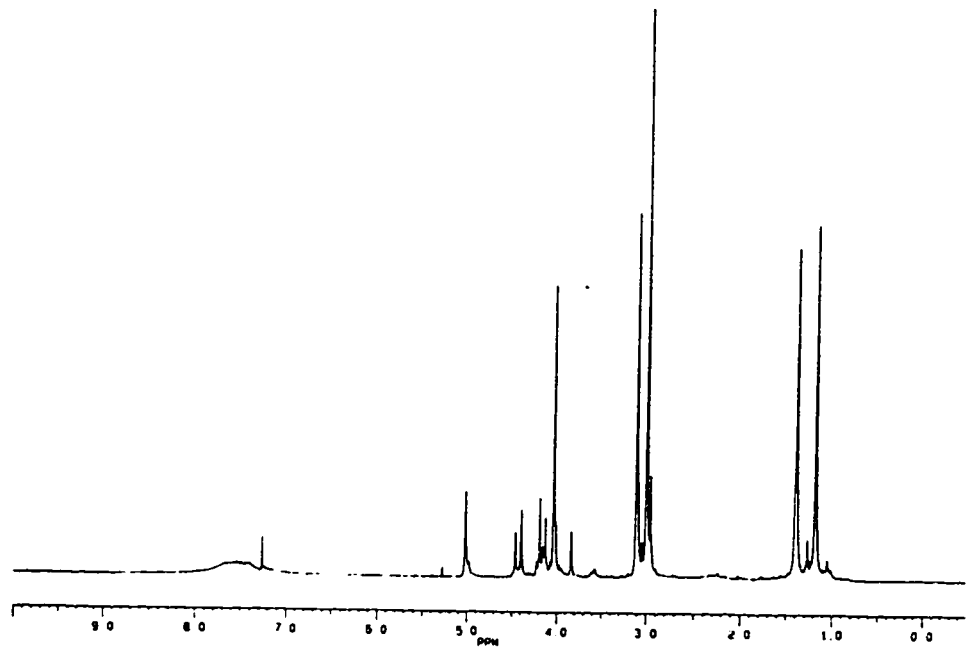


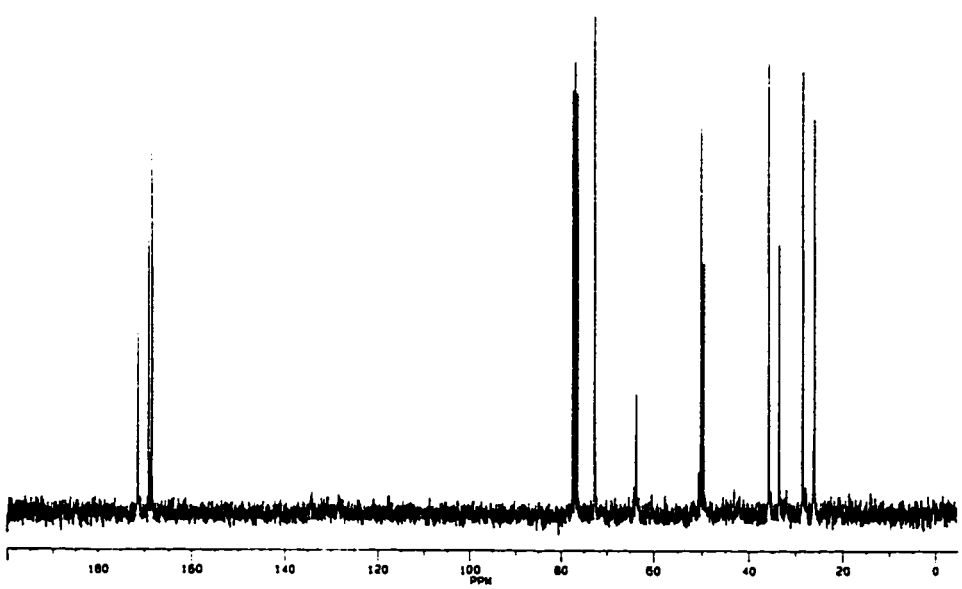
281 283

To a solution of tripeptide **281** (0.72g, 2.1mmol) and dimedone (0.29g. 2.1mmol, 1eq) in 10mL of freshly distilled THF was added 48.5mg of $Pd(PPh_3)_4$ catalyst under Ar (2% mol). The reaction was stirred at room temperature and the progress of the reaction was monitored by NMR. Upon the disappearance of the starting material (4 to 6hrs), the mixture was applied to a silica gel column and 80% EtOAc/hexane was used to remove dimedone and its derivatives. The product was recovered from the column with 10% $MeOH/CH_2Cl_2$. Evaporating the solvent *in vacuo* afforded 0.57g of **283** as yellow foam (1.90mmol, 91%).

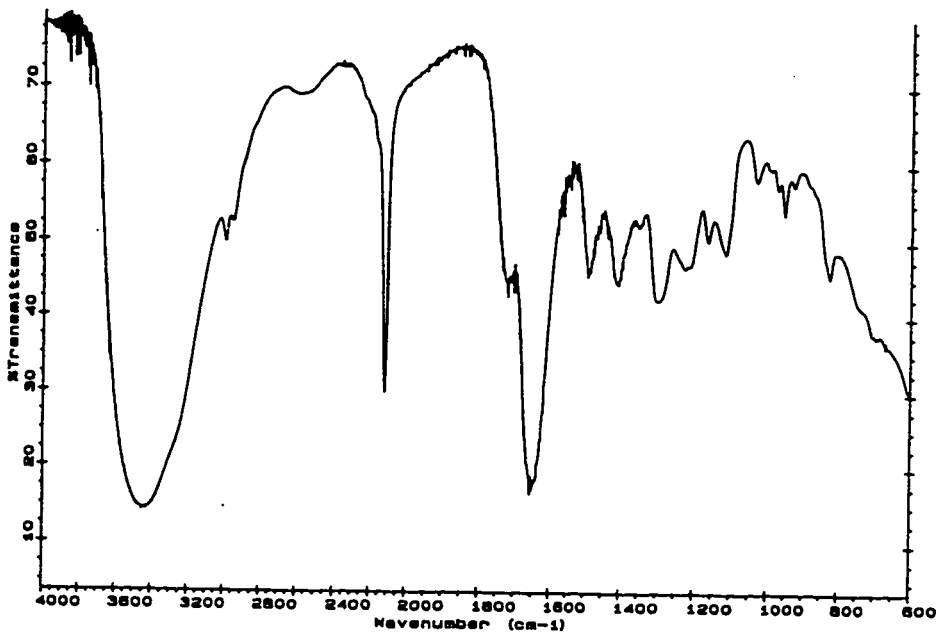
$[\alpha]_D^{25}$ =	-20° (0.023g/mL, CH_2Cl_2)
^1H NMR ($CDCl_3$):	1.18 (3H, s), 1.38 (3H, s), 2.99 (3H, s), 3.10 (3H, s), 4.03 (2H, s), 4.16 (1H, d, J = 16.84Hz), 4.43 (1H, d, J = 16.84Hz), 5.02 (1H, s), 7.58 (1H, br)
^{13}C NMR ($CDCl_3$):	26.1, 28.6, 33.6, 35.9, 49.7, 50.2, 63.6, 72.8, 168.5, 169.3, 171.5
IR (Film):	824.6, 952.9, 1114.9, 1217.6, 1297.3, 1403.5, 1496.5, 1649.2, 2107.4, 2977.3, 3442.2
MS (FAB):	57, 69, 81, 107, 136, 154 (100), 176, 284, 289, 302 (M^++1), 324 (M+ Na^+)
HRMS(FAB):	expected ($C_{11}H_{20}N_5O_5$, M^++H): 302.1464; observed: 302.1445

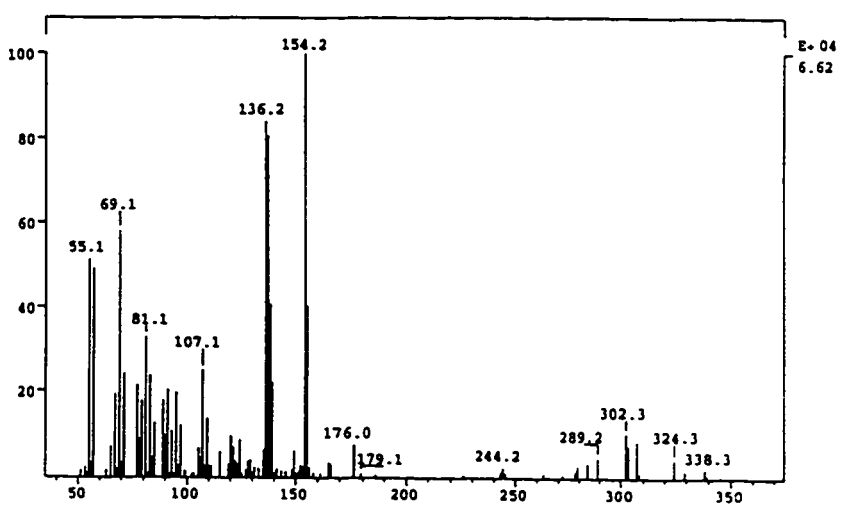
Compound **283**:

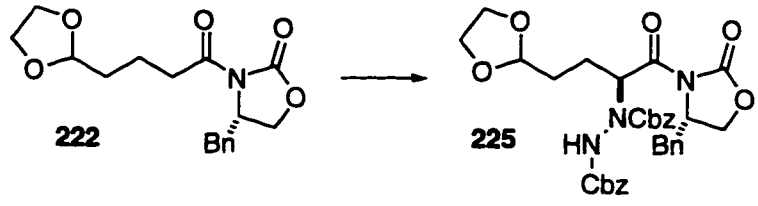




Compound **283** continued:







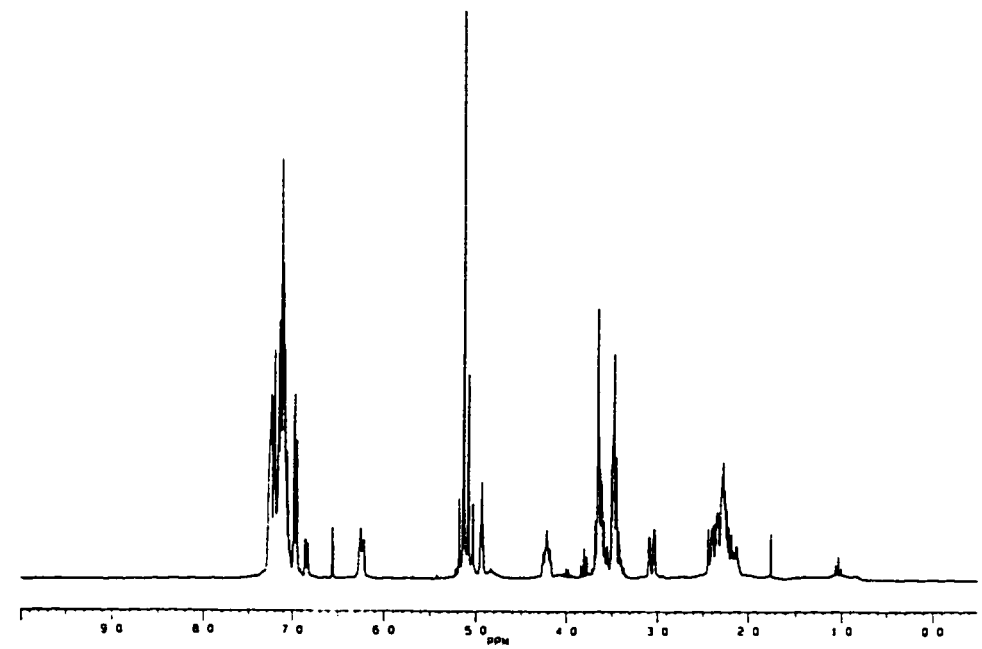
Two crystals of 1,10-phenanthroline were added to 50 mL of freshly distilled THF in a flame dried flask under Ar. The solution was cooled to –78°C and 3.95 mL of Di-isopropylamine (28.2mmol, 1.5eq) was added to the flask followed by small amount of BuLi (in hexane) until indicator turned to orange color. Full amount of BuLi (9.0 mL 2.5M, 22.6mmol, 1.2eq) was then added, and the solution was warmed up to 0°C and cooled to –78°C again. **222** (6g, 18.8 mmol, 1eq) in 30 mL of THF was added slowly to the cooled solution via syringe. Residual **222** was rinsed in with two 5 mL portions of THF and stirring continued at –78°C for 30 minutes. A solution of Cbz-N=N-Cbz (11.2g. 37.6mol, 2.0eq) in 40 mL of THF was added via syringe to the above enolate solution and after an additional 2 minutes the reaction was quenched with 5 mL of glacial acetic acid. The mixture was partitioned between 150 mL of CH_2Cl_2 and 50 mL of pH 7 phosphate buffer. The aqueous phase was extracted with three 70 mL portions of CH_2Cl_2. The combined organic phases were washed with 50 mL of saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, concentrated *in vacuo*, and chromatographed (40% EtOAc/hexane) to yield 7.5g of **225** as white waxy solid (12.2mmol, 65%).

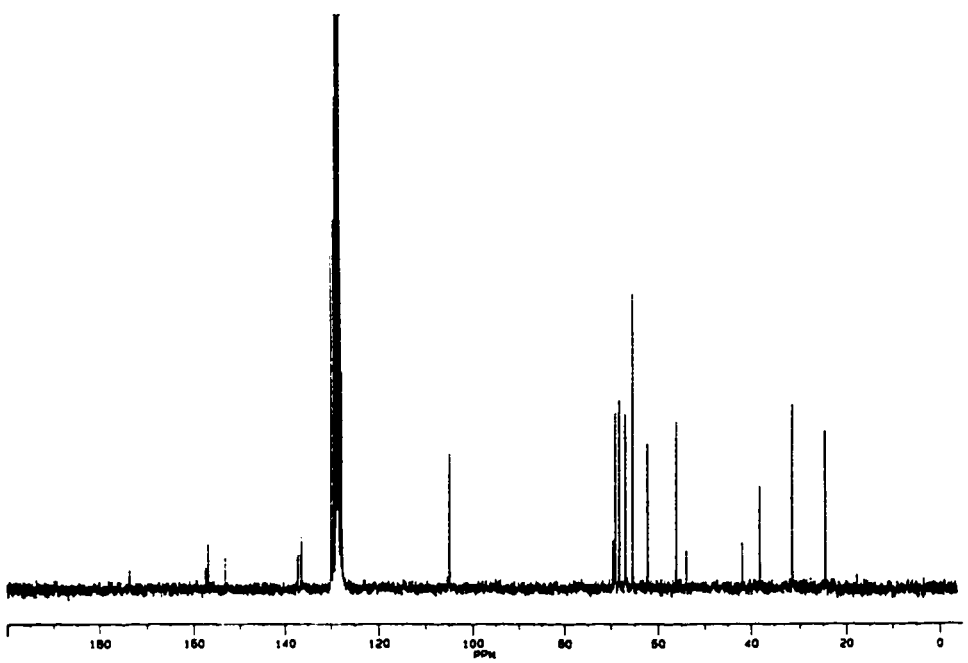
$[\alpha]_D^{25}$ = +35.1° (0.073g/mL, CH_2Cl_2)

^1H NMR (Benzene, 70°C): 2.08 - 2.46 (6H, m), 3.06 (1H, dd, J_1 = 2.9Hz, J_2 =

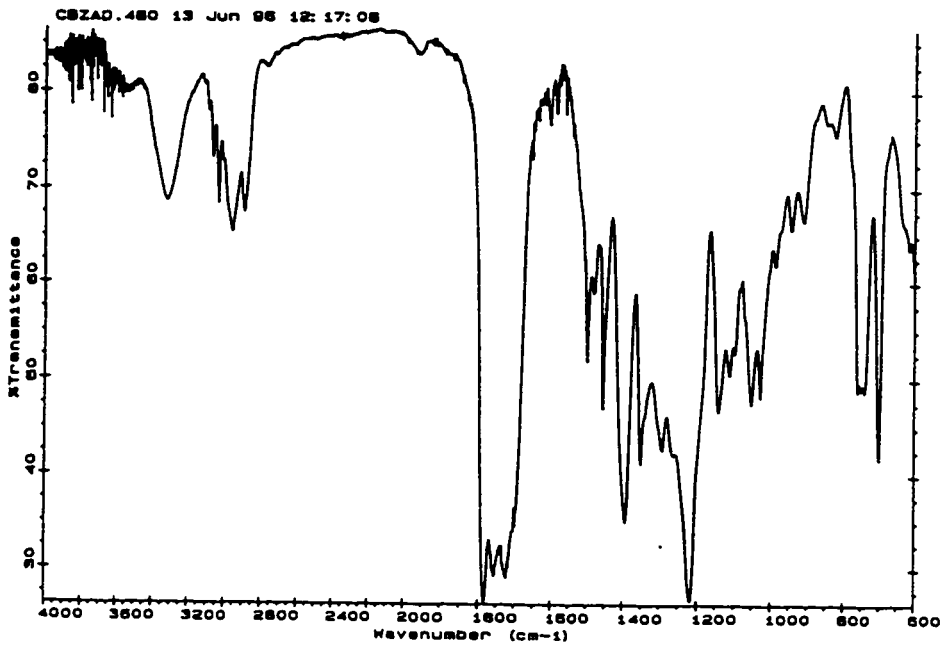
	13.67Hz), 3.3 - 3.7 (4H, m), 4.21 (1H, m), 4.9 - 5.0 (1H, m), 5.06 (1H, d, J = 12.45Hz), 5.16 (1H, d, J = 12.45Hz, overlapping), 5.13 (2H, s, overlapping), 6.2 - 6.3 (1H, m), 6.9 - 7.3 (15H, m)
^{13}C NMR (Benzene, 70°C):	24.4, 31.4, 38.0, 41.8, 56.1, 62.2, 65.3, 66.9, 68.1, 69.0, 69.5, 104.9, 127.5, 127.7, 128.0, 128.4, 128.5, 128.8, 129.0, 129.4, 129.5, 130.0, 136.4, 137.1, 137.3, 153.3, 156.8, 157.4, 173.7
IR (film):	698, 756, 1032, 1057, 1139, 1221, 1352, 1396, 1456, 1500, 1721, 1759, 1787, 2887, 2957, 3316
MS (FAB):	57, 91 (100), 95, 136, 154, 178, 207, 307, 369, 422, 512, 618 (M^++1)
HRMS (EI):	expected ($C_{33}H_{35}N_3O_9$): 617.2373; observed: 617.2373

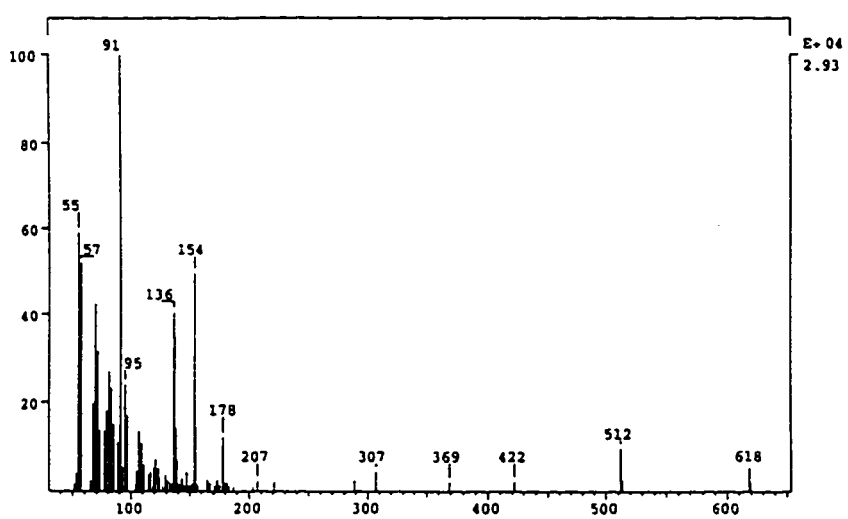
Compound **225**:

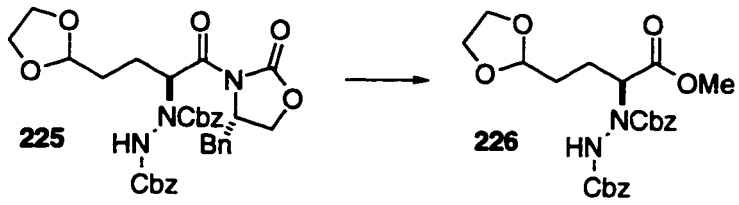




Compound **225** continued:







To a 50 mL of dry MeOH, cooled to 0°C under Ar, was added dropwise 8.9 mL of MeMgBr (26.8mmol of 3M solution in Et_2O, 1.5eq). After stirring the above solution at 0°C for 5 minutes, a solution of 11.0g amide **225** (17.8mmol, 1.0eq) in 40 mL of dry CH_2Cl_2 was added slowly via syringe, residual was rinsed in with two 5 mL-portions of CH_2Cl_2. The reaction was stirred at 0°C for 0.5 hours and then quenched with 30 mL of saturated aqueous NH_4Cl solution. The two layers were separated and the aqueous layer was extracted with three 30 mL-portions of CH_2Cl_2. The organic layers were combined, washed with 40 mL of brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product, which contained both acetal and chiral auxiliary, were separated by chromatography (silica gel, 40 → 70% EtOAc/hexane) to yield 6.7g of **226** as white waxy solid (14.3mmol, 80%).

$[\alpha]_D^{25}$ = −7.42° (0.084g/mL, CH_2Cl_2)

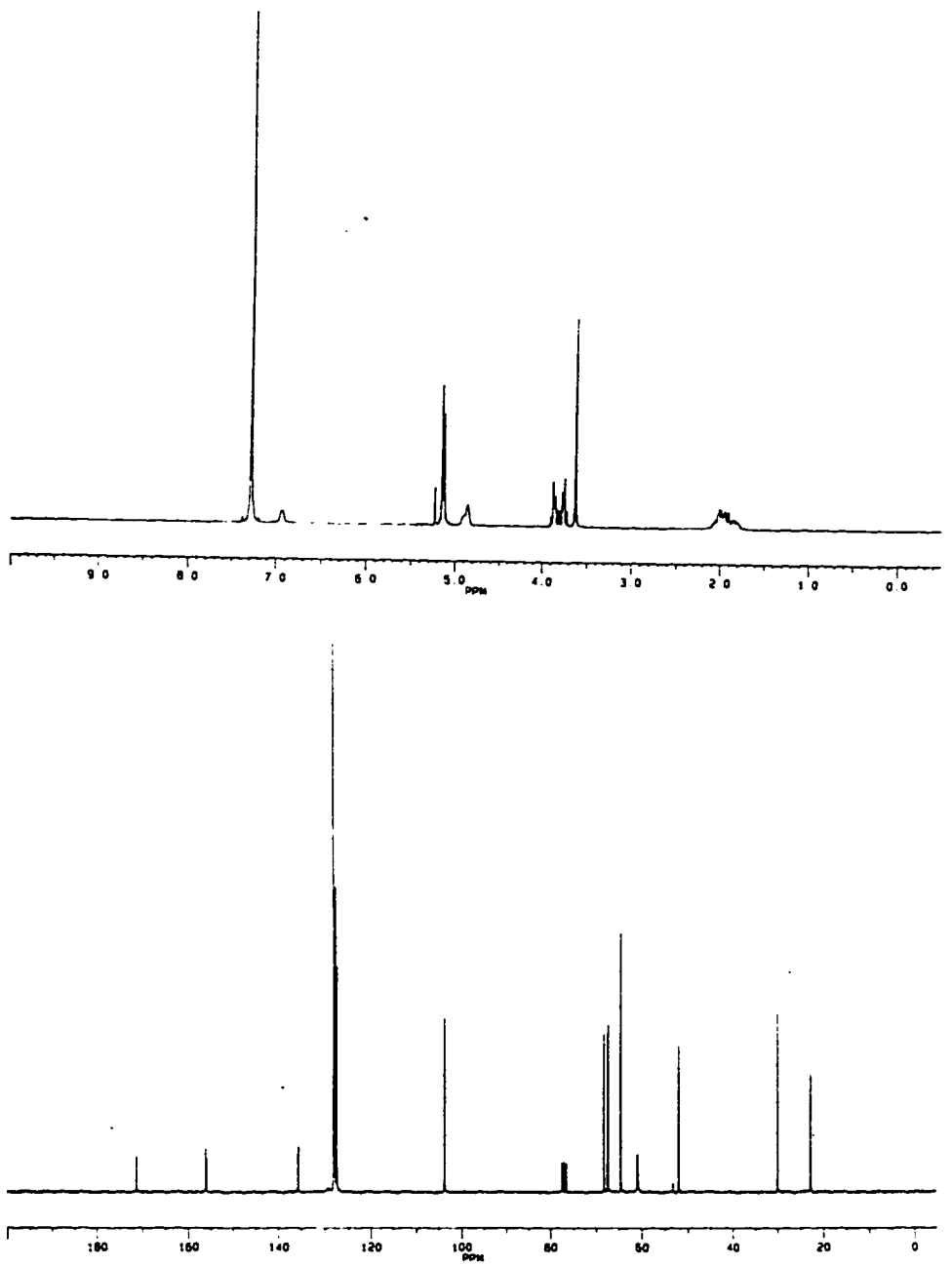
^{1}H NMR (CH_2Cl_2, 50°C): 1.70-2.10 (4H, m), 3.62 (3H, s), 3.70-3.90 (4H, m), 4.80-4.96 (2H, m, overlapping), 5.12 (2H, s), 5.14 (2H, s), 6.93 (1H, br), 7.28 (5H, br)

^{13}H NMR (CH_2Cl_2, 50°C): 22.8, 30.0, 51.9, 60.8, 65.6, 67.4, 68.2, 103.8, 127.5, 127.8, 127.9, 128.0, 128.2, 135.6, 135.7, 155.9, 156.2, 171.4

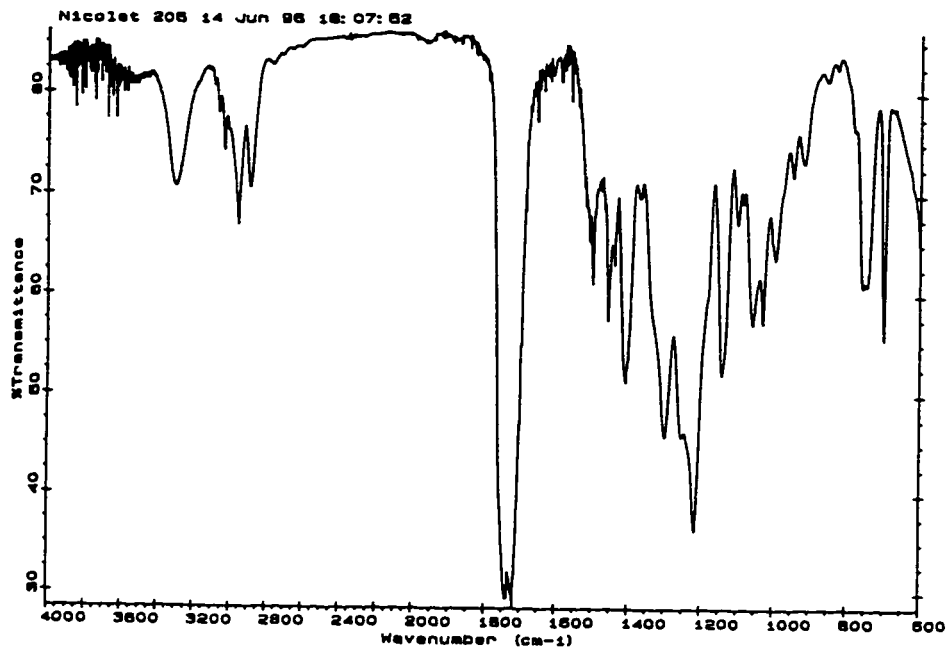
IR (film): 698, 750, 1029, 1054, 1144, 1213, 1295, 1407, 1453,

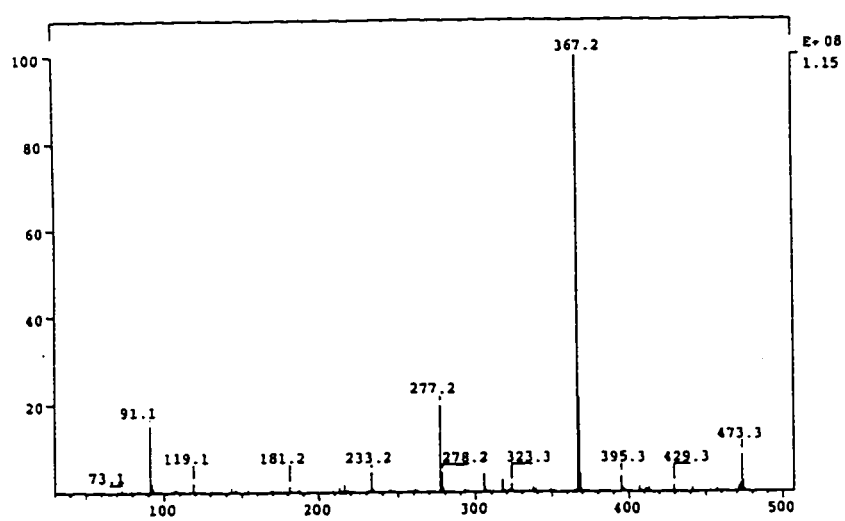
	1500, 1716, 1740, 2887, 2953, 3301
MS (CI):	91, 119, 181, 233, 277, 323, 367 (100), 395, 429, 473 (M^++1)
HRMS (EI):	expected ($C_{24}H_{28}N_2O_8$): 472.1845; observed: 472.1849

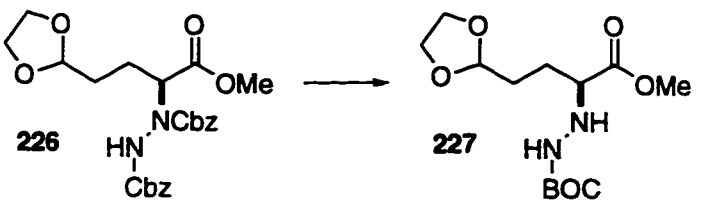
Compound **226**:



Compound **226** continued:



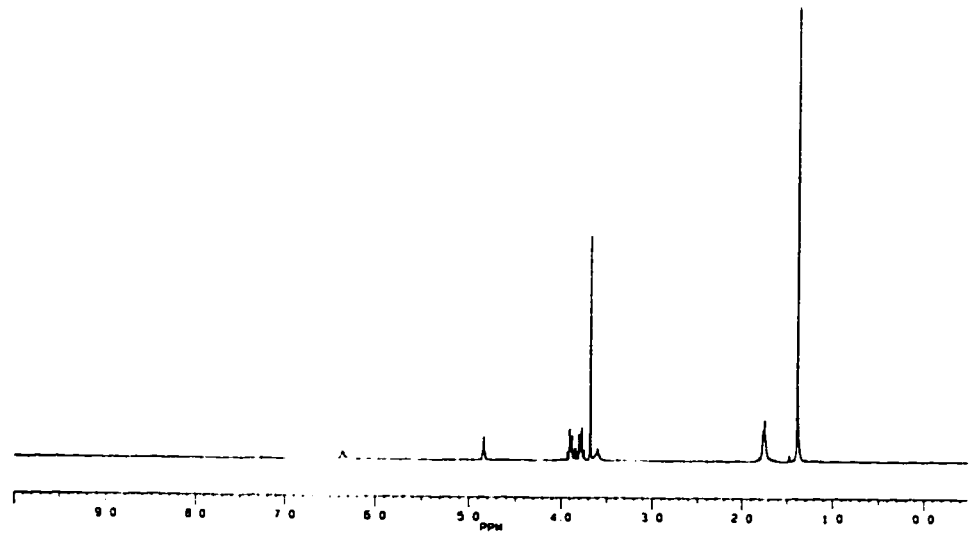


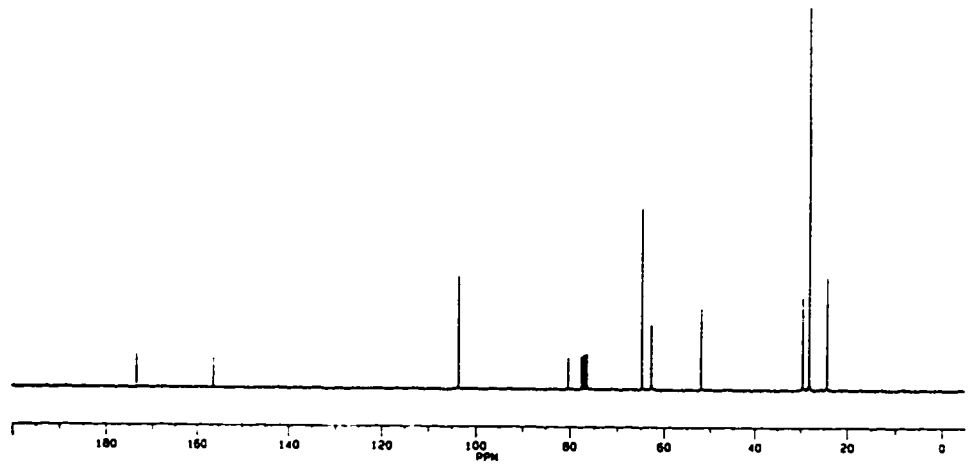


To a solution of di-Cbz **226** (6.0g, 12.7mmol, 1.0eq) and 3.3g BOC_2O(15.3mmol, 1.2eq) in 200 mL of MeOH was added 0.6g of 10% Pd/C catalyst (10% wt of **226**). The reaction was stirred at room temperature for 12 hours under H_2 atmosphere (balloon). Upon the disappearance of the starting material (TLC), the mixture was filtered through a pad of celite to remove the catalyst. The solution was concentrated *in vacuo* and chromatographed (40% EtOAc/hexane) to yield 3.6g of **227** as light yellow oil (11.8mmol, 93%).

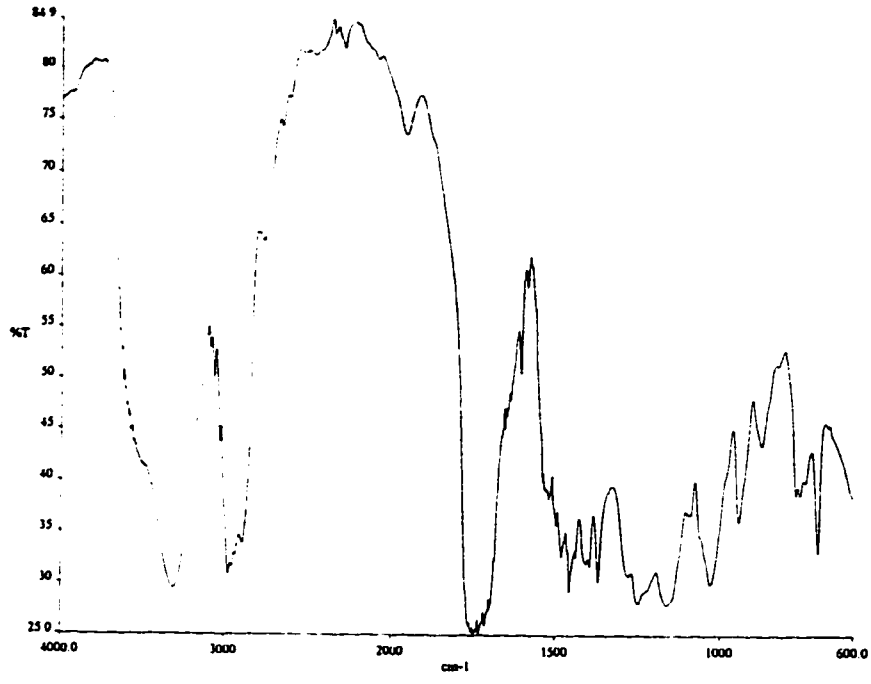
$[\alpha]_D^{25}$ =	−15° (0.306g/mL, CH_2Cl_2)
^1H NMR ($CDCl_3$):	1.40 (9H, s), 1.69-1.84 (4H, m), 3.62 (1H, br), 3,70 (3H, s), 3.76-3.95 (4H, m), 4.86 (1H, distorted t, J = 4.0Hz), 6,32 (1H, br)
^{13}C NMR ($CDCl_3$):	24.3, 28.1, 29.6, 51.9, 62.7, 64.8, 80.4, 103.7, 156.2, 173.5
IR (film):	704, 770, 874, 941, 1028, 1162, 1248, 1368, 1394, 1455, 1480, 1732, 2977, 3316
MS (EI):	57 (100), 73, 101, 127, 142, 145, 169, 186, 189, 204, 232, 248, 304 (M^++1)
HRMS (EI):	expected ($C_{13}H_{24}N_2O_6$, M^+ +H): 304.1634; observed: 304.1628

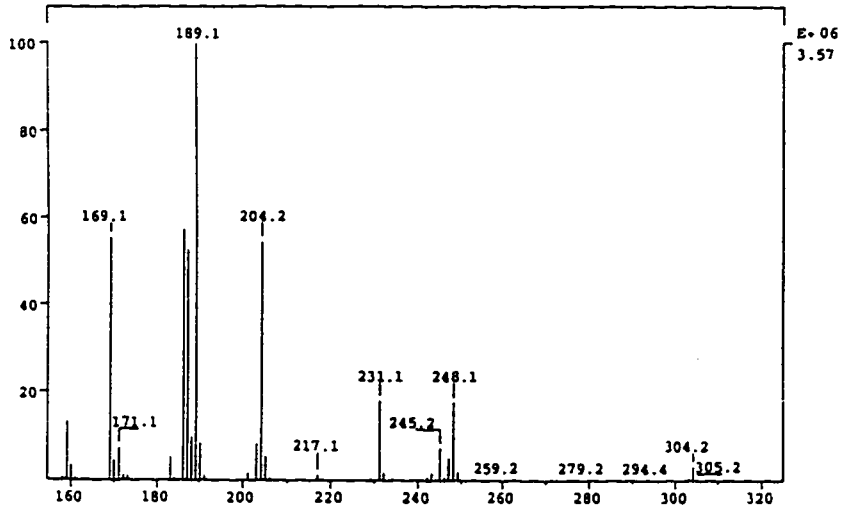
Compound **227**:

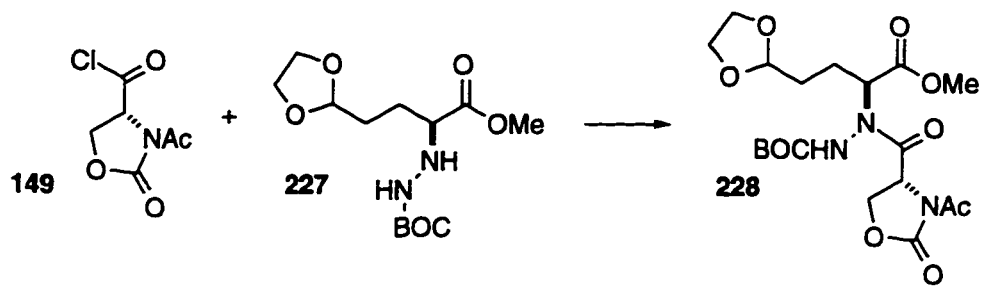




Compound **227** continued:





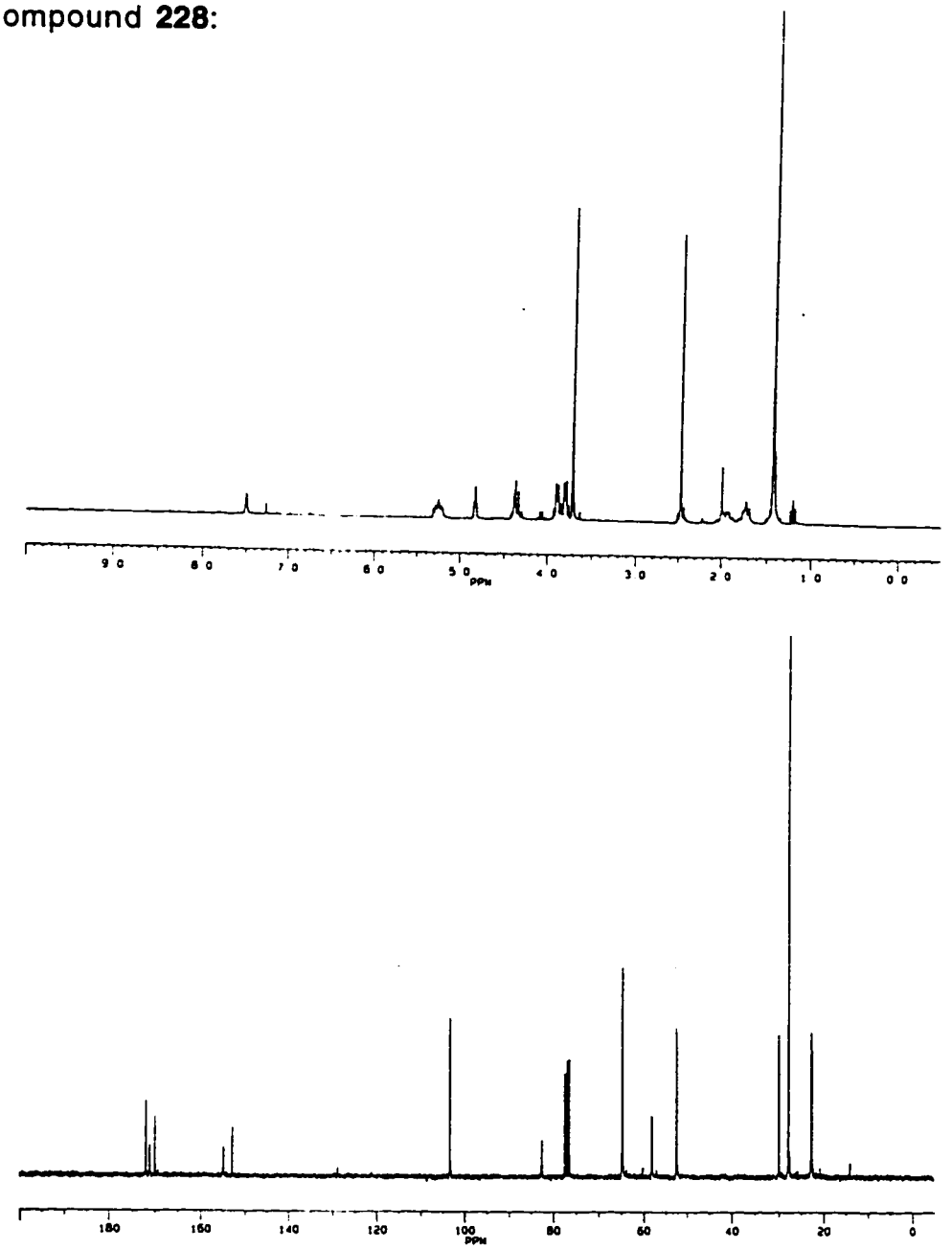


To a solution of monoBOC **227** (2.1g, 6.9mmol, 1.0eq) in 10 mL of dry CH_2Cl_2 was added 2.65 mL of collidine (20.7mmol, 3eq) under Ar. The mixture was cooled to 0°C and a solution of acid chloride **149** (2.4g, 13.8mmol, 2.0eq) in 6 mL of CH_2Cl_2 was added slowly via syringe. The residue was rinsed in with two 2 mL portions of CH_2Cl_2. The reaction was continued to stir at 0°C for 20 minutes and then directly applied to a short silica gel column. EtOAc was used to elute the product along with collidine. The eluant was concentrated *in vacuo* and chromatographed (20-50% EtOAc/hexane) again to yield 2.0g of **228** (4.4mmol, 63%) as yellow oil, which upon standing, turned to waxy solid.

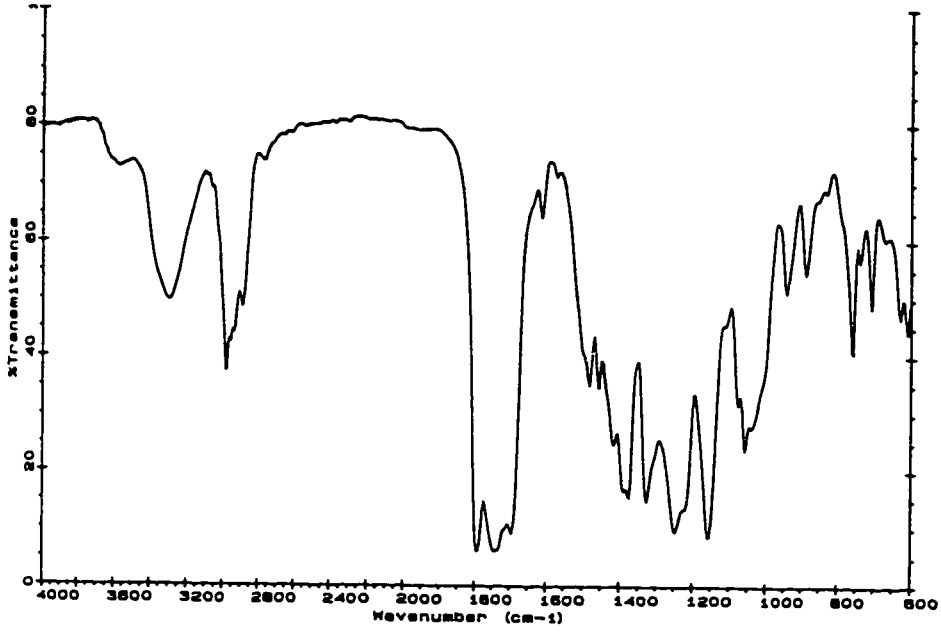
$[\alpha]_D^{25}$ =	+ 17° (0.055g/mL, CH_2Cl_2)
^{1}H NMR ($CDCl_3$):	1.46 (9H, s), 1.6 - 1.8 (2H, m), 1.8 - 2.0 (2H, m), 2.47 (3H, m), 3.71 (3H, s), 3.8 - 4.0 (4H, m), 4.3 - 4.4 (2H, m), 4.83 (1H, distorted t, J = 3.8Hz), 5.2 - 5.3 (2H, m), 7.50 (1H, br)
^{13}C NMR ($CDCl_3$):	22.6, 22.8, 27.5, 27.8, 30.0, 52.4, 52.5, 58.2, 64.8, 94.9, 82.6, 103.4, 152.9, 154.9, 170.1, 171.2, 172.1
IR (film):	704, 756, 890, 942, 1054, 1155, 1245, 1325, 1371, 1456, 1699, 1740, 1792, 2890, 2981, 3297
MS (EI):	57 (100), 73, 83, 128, 142, 171, 211, 237, 256, 297, 343, 359, 386, 403, 458, 459 (M^+)

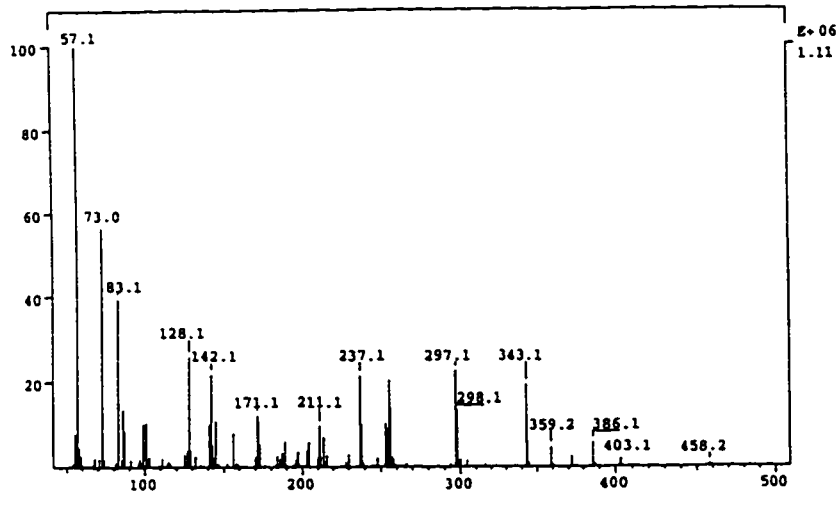
HRMS:(EI): expected ($C_{19}H_{29}N_3O_{10}$, M^+): 459.1853; observed: 459.1858

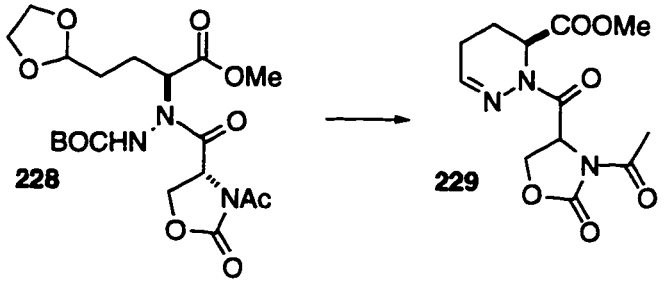
Compound **228**:



Compound **228** continued:







A solution of the PCA precursor **228** (2.4g, 5.2mmol) in 25 mL of 90% TFA in H_2O was stirred at room temperature for 30 minutes. The solvent was then evaporated *in vacuo* and the residue was and partitioned between 50 mL of CH_2Cl_2 and 20 mL of saturated aqueous $NaHCO_3$ solution (solid $NaHCO_3$ may added to neutralize the excess TFA residue). The aqueous layer was extracted with two 30 mL-portions of CH_2Cl_2. The combined organic solutions were washed with 20 mL of brine, dried over Na_2SO_4 and concentrated *in vacuo* to afford 1.54g of **229** as white solid (5.2mmol, 100%). White crystal (1.5g,4.9mmol, 95%) was obtained after recrystallization in 30% EtOAc/hexane.

$[\alpha]_D^{25}$ = + 37.1° (0.062g/mL in CH_2Cl_2)

m.p. = 152–153°C

^{1}H NMR ($CDCl_3$): 1.78-2.45 (4H, m), 2.52 (3H, s), 3.71 (3H, s), 4.21 (1H, dd, J_1 = 3.91, J_2 = 9.52), 4.56 (1H, t, J = 9.52), 5.13 (1H, m), 5.68 (1H, dd, J_1 = 3.91, J_2 = 9.52), 6.92 (1H, m)

^{13}C NMR ($CDCl_3$): 18.1, 20.4, 23.1, 51.2, 52.8, 54.8, 65.0, 144.0, 153.3, 168.3, 169.3, 169.6

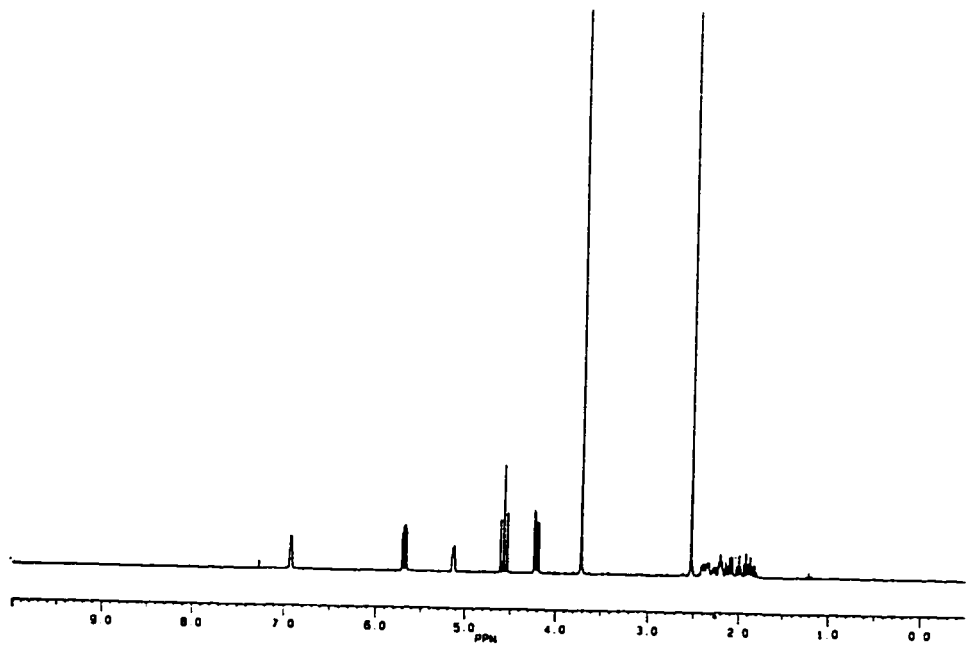
IR (KBr): 616, 653, 684, 748, 758, 770, 790, 876, 894, 929, 964, 988, 1048, 1060, 1080, 1152, 1208, 1224, 1270, 1300, 1324, 1333, 1347, 1380, 1393, 1417, 1458, 1641, 1689, 1708, 1748, 1782,

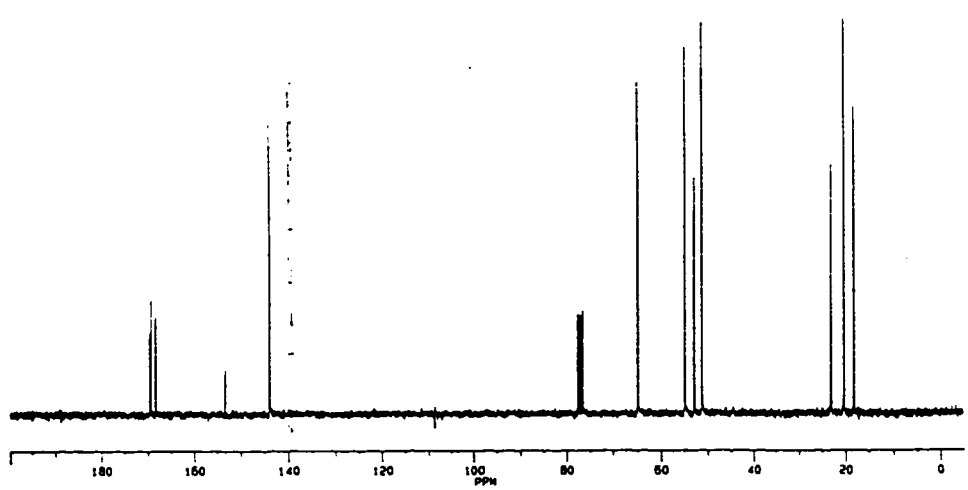
2966, 3021

MS (EI): 69, 83 (100), 125, 128, 142, 211, 231, 237, 255, 297 (M^+)

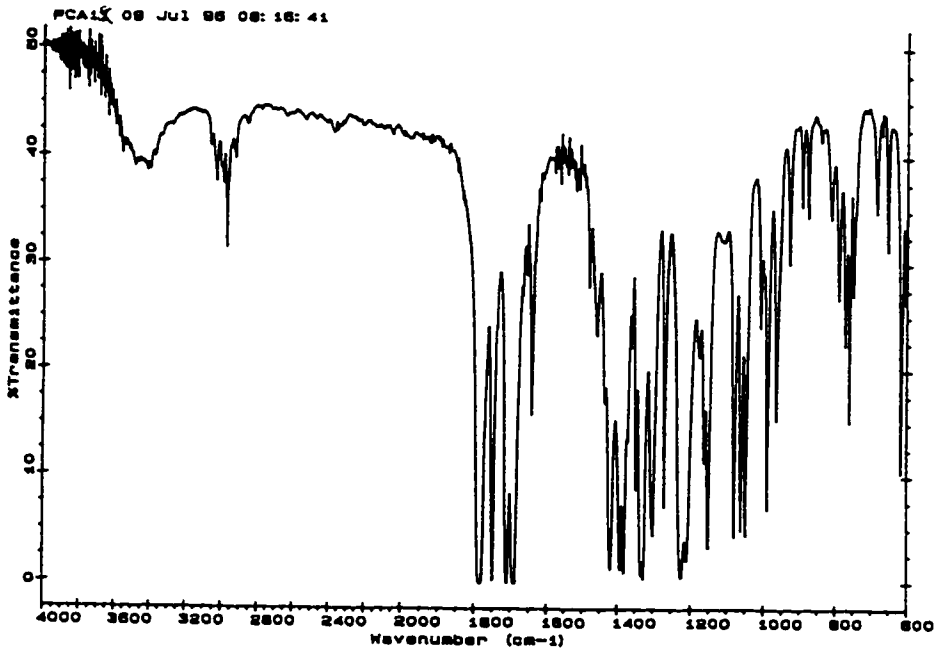
HRMS (EI): expected ($C_{12}H_{15}N_3O_6$): 297.0961; observed: 297.0962

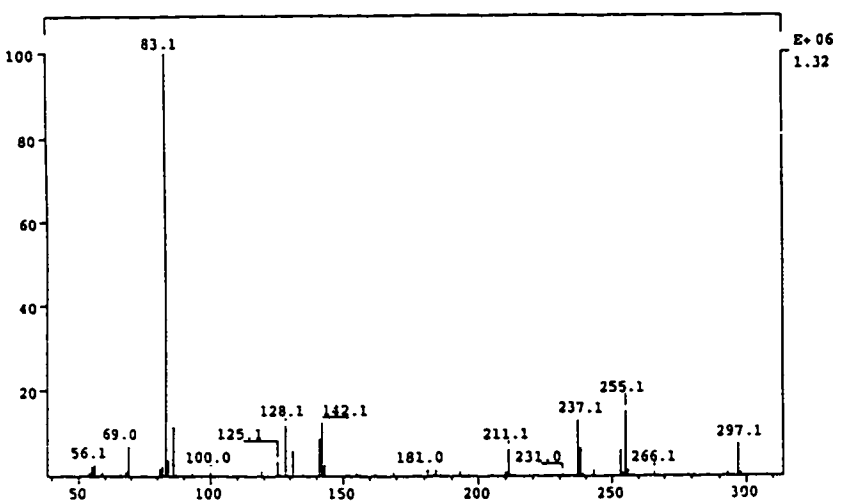
Compound **229**:

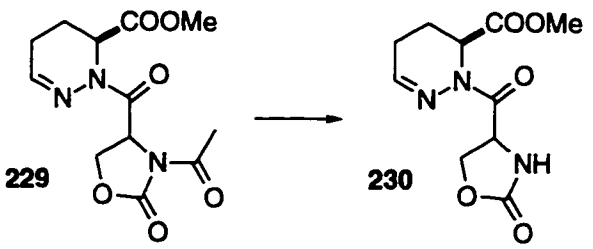




Compound **229** continued:



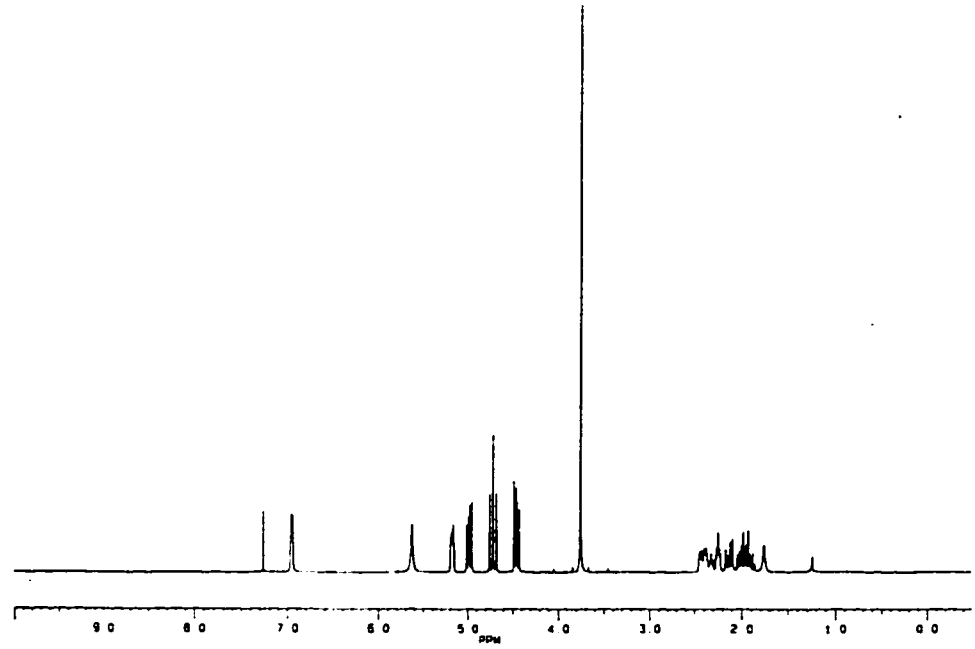


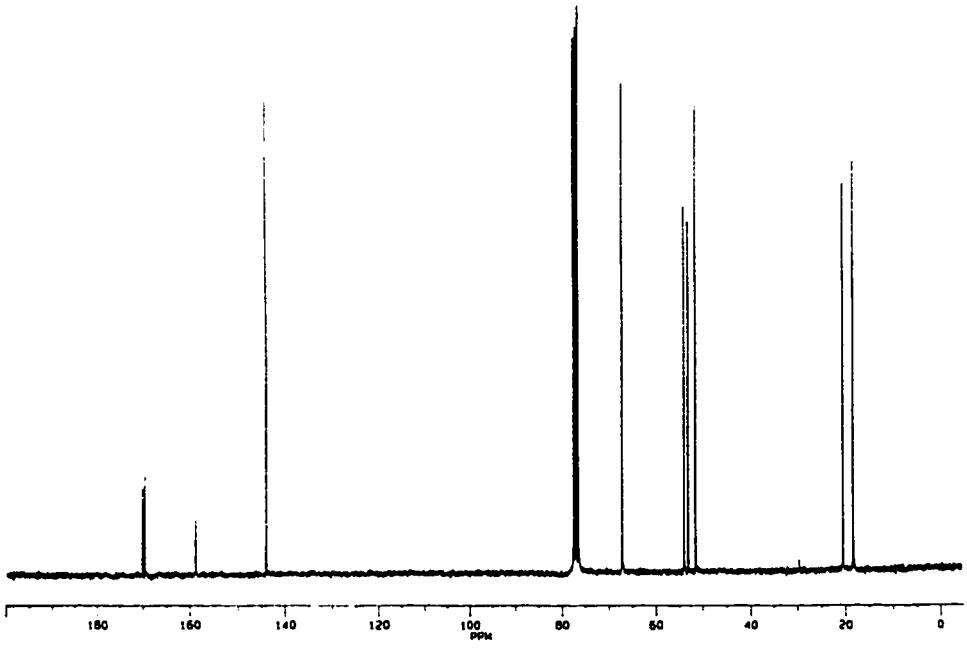


To a solution of oxazolone **229** (1.48g, 5.0mmol, 1eq) in 15 mL of CH_3CN was added 0.29 mL of $NH_2NH_2 \cdot H_2O$ (6.0mmol, 1.2eq) at room temperature. The progress of the reaction was monitored with TLC. Upon the disappearance of the starting material (about 30 minutes), the reaction mixture was concentrated in vacuo and the residue was applied to a short silica gel column and eluted with 50% to 100% EtOAc/hexane. The solution containing product was collected and concentrated *in vacuo* to afford **230** as colorless oil (1.15g, 4.5mmol, 90%).

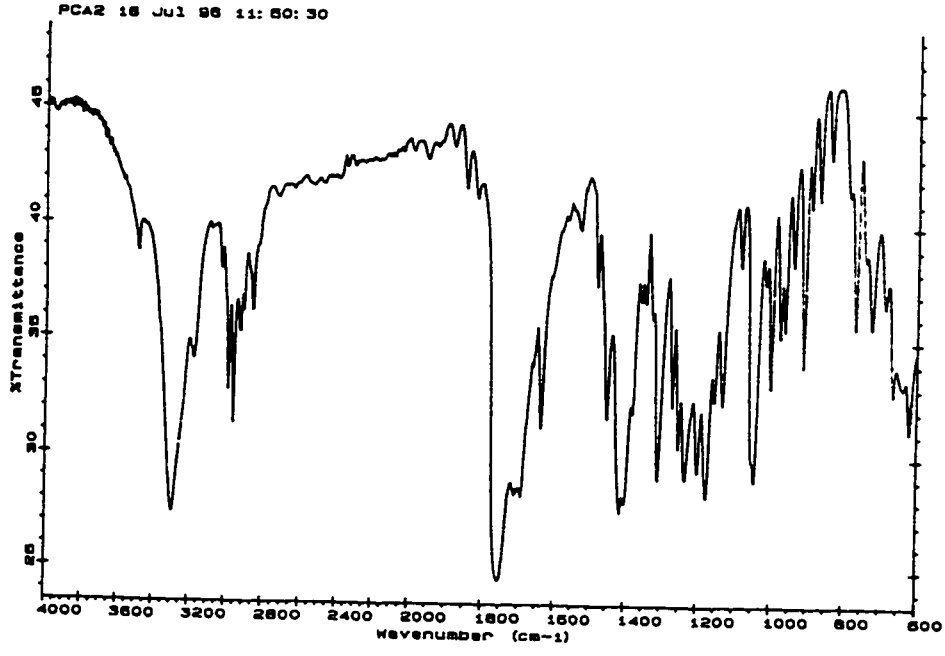
$[\alpha]_D^{25}$ =	$-9.54°$ (0.022g/mL in CH_2Cl_2)
^1H NMR ($CDCl_3$):	1.87-2.45 (4H, m), 3.75 (3H, s), 4.46 (1H, dd, J_1 = 9.26Hz, J_2 = 6.06Hz), 4.72 (1H, t, J = 9.26Hz), 4.99 (1H, dd, J_1 = 9.26Hz, J_2 = 6.06Hz), 5.17 (1H, m), 5.60 (1H, br), 6.95 (1H, t, J = 2.07)
^{13}C NMR ($CDCl_3$):	18.2, 20.4, 51.2, 52.8, 53.8, 67.0, 143.9, 158.6, 169.4, 170.0
IR (film):	720, 769, 909, 1001, 1043, 1130, 1174, 1234, 1308, 1401, 1412, 1450, 1639, 1702, 1754, 2957, 2984, 3289
MS (EI):	56, 83 (100), 126, 142, 170, 196, 211, 255 (M^+)
HRMS (EI):	expected ($C_{10}H_{13}N_3O_5$): 255.0855; observed: 255.0855

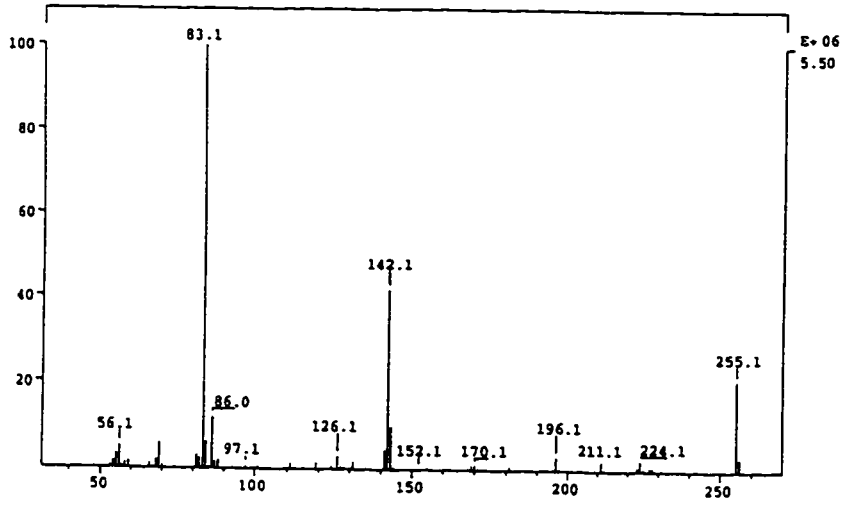
Compound **230**:

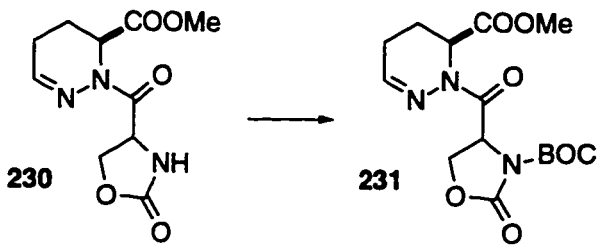




Compound **230** continued:







To a stirred solution of oxazolone **230** (1.23g, 4.8mmol, 1eq), 0.67 mL of Et_3N (4.8mmol, 1.0eq), and 1.57g of BOC_2O (7.2mmol,1.5eq) in 30 mL of CH_2Cl_2 was added catalyst DMAP (62mg, 5%wt) at 0°C. The reaction was stirred for 30 minutes at 0°C and then warmed to room temperature. The progress of the reaction was monitored by TLC. Upon the disappearance of the oxazolone, 15 mL of pH 7 phosphate buffer (0.5M) was added to the reaction. The two layers were separated and the aqueous layer was extracted with two 30 mL-portions of CH_2Cl_2. The combined organic solutions were washed with 20 mL of brine, dried over Na_2SO_4, concentrated *in vacuo*. The crude product was recrystallized in 40% EtOAc/hexane to yield 1.70g of **231** (4.8mmol, 100%) as white solid.

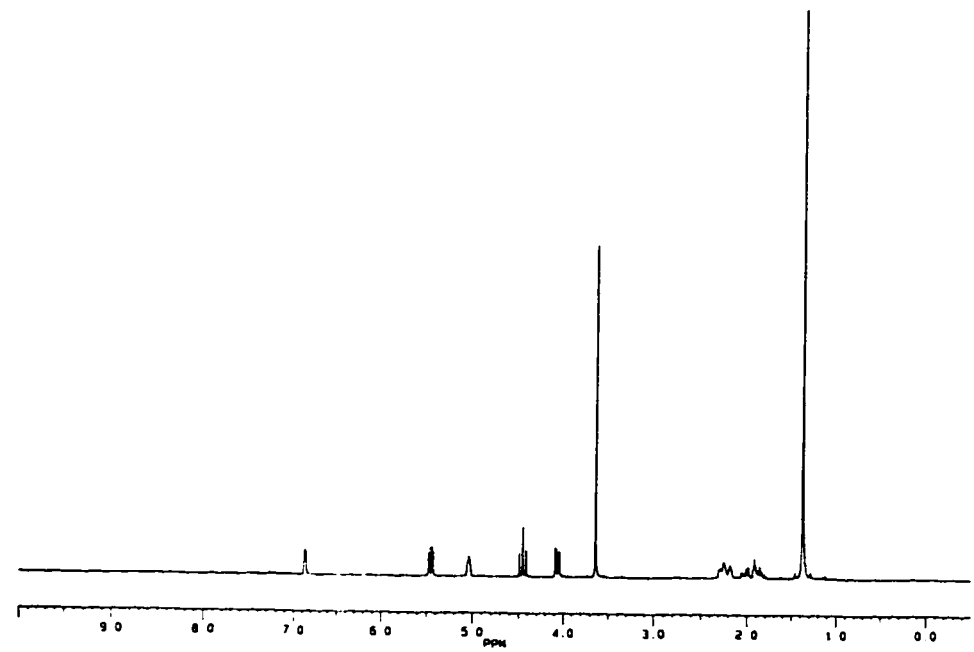
$[\alpha]_D^{25}$ =	+ 32.28 (0.048g/mL CH_2Cl_2)
m.p.=	166–167°C
^1H NMR ($CDCl_3$):	1.37 (9H, s), 1.77-2.33 (4H, m), 3.64 (3H, s), 4.06 (1H, dd, J_1 = 9.52, J_2 = 3.91), 4.45 (1H, t, J = 9.52), 5.04 (1H, m), 5.45 (1H, dd, J_1 = 9.52, J_2 = 3.91), 6.87 (1H, distorted d, J=4.15).
^{13}C NMR ($CDCl_3$):	18.0, 20.1, 27.2, 51.1, 52.4, 55.8, 64.3, 83.3, 144.1, 148.2, 152.0, 168.5, 169.3
IR (KBr):	625, 748, 778, 830, 893, 966, 996, 1054, 1073, 1160, 1193, 1218, 1264, 1305, 1338, 1374, 1387, 1420, 1634, 1691, 1724,

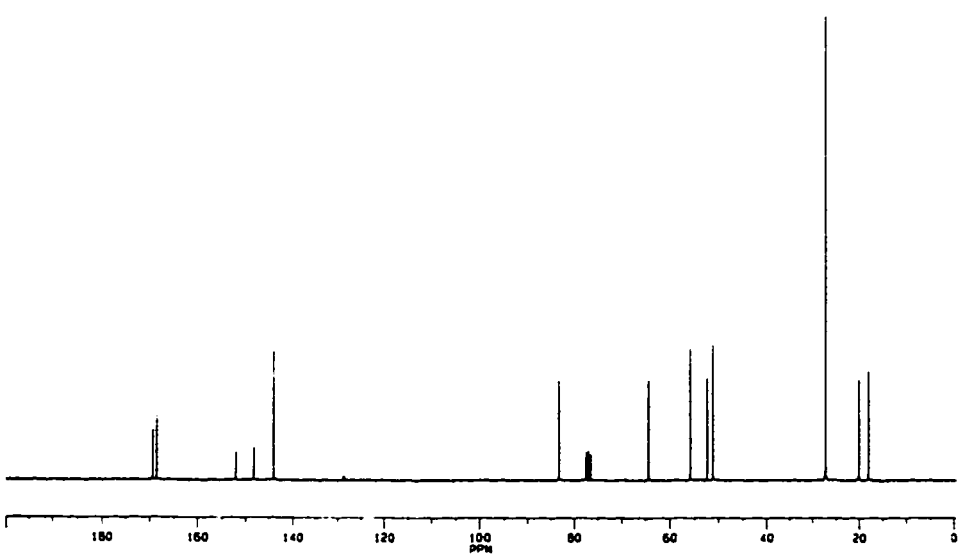
1746, 1819, 2941, 2959, 2992, 3039

MS (EI): 57 (100), 83, 118, 128, 142, 196, 211, 238, 255, 282, 311, 340, 356 (M^++1)

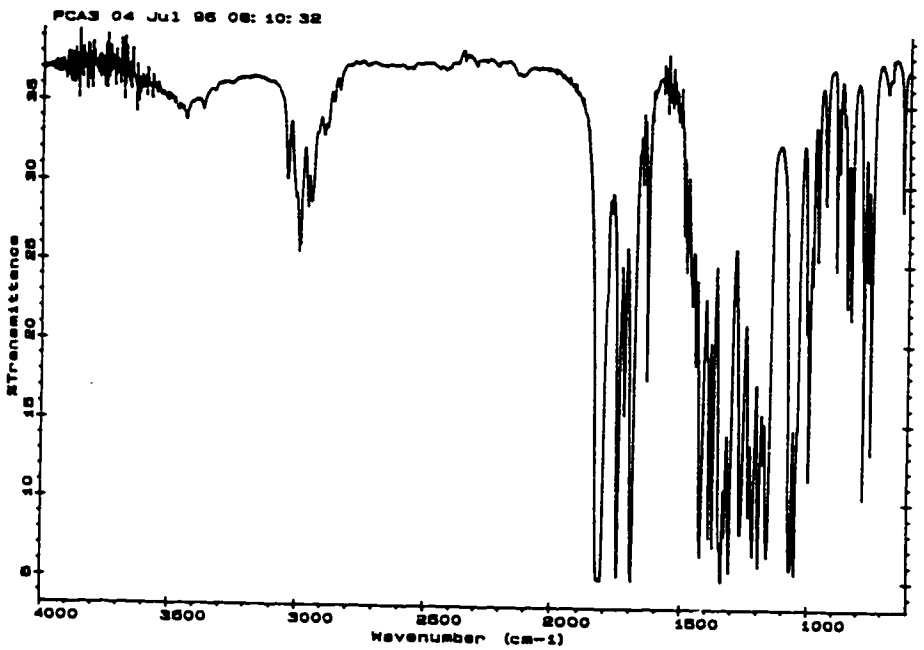
HRMS (EI): expected ($C_{15}H_{21}N_3O_7$, M^+): 355.1379; observed: 355.1378

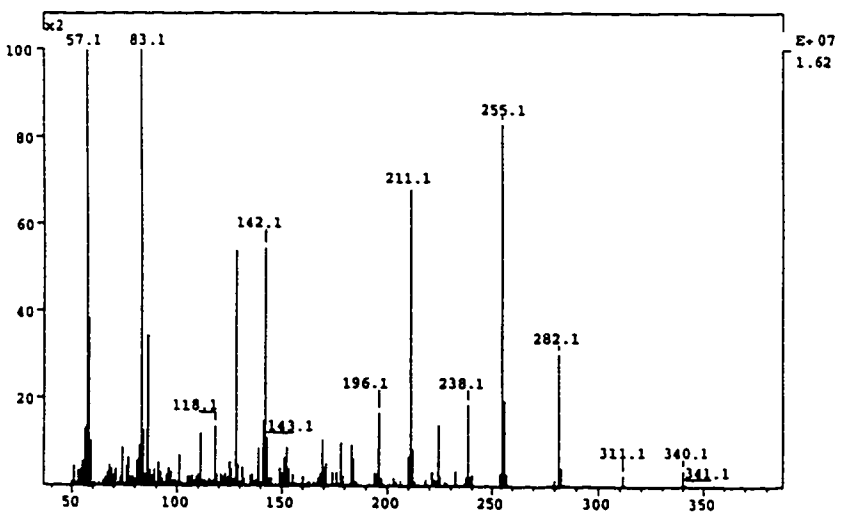
Compound **231**:

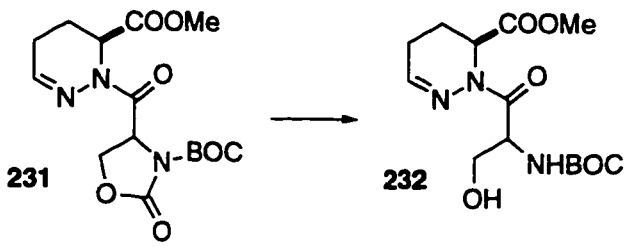




Compound **231** continued:



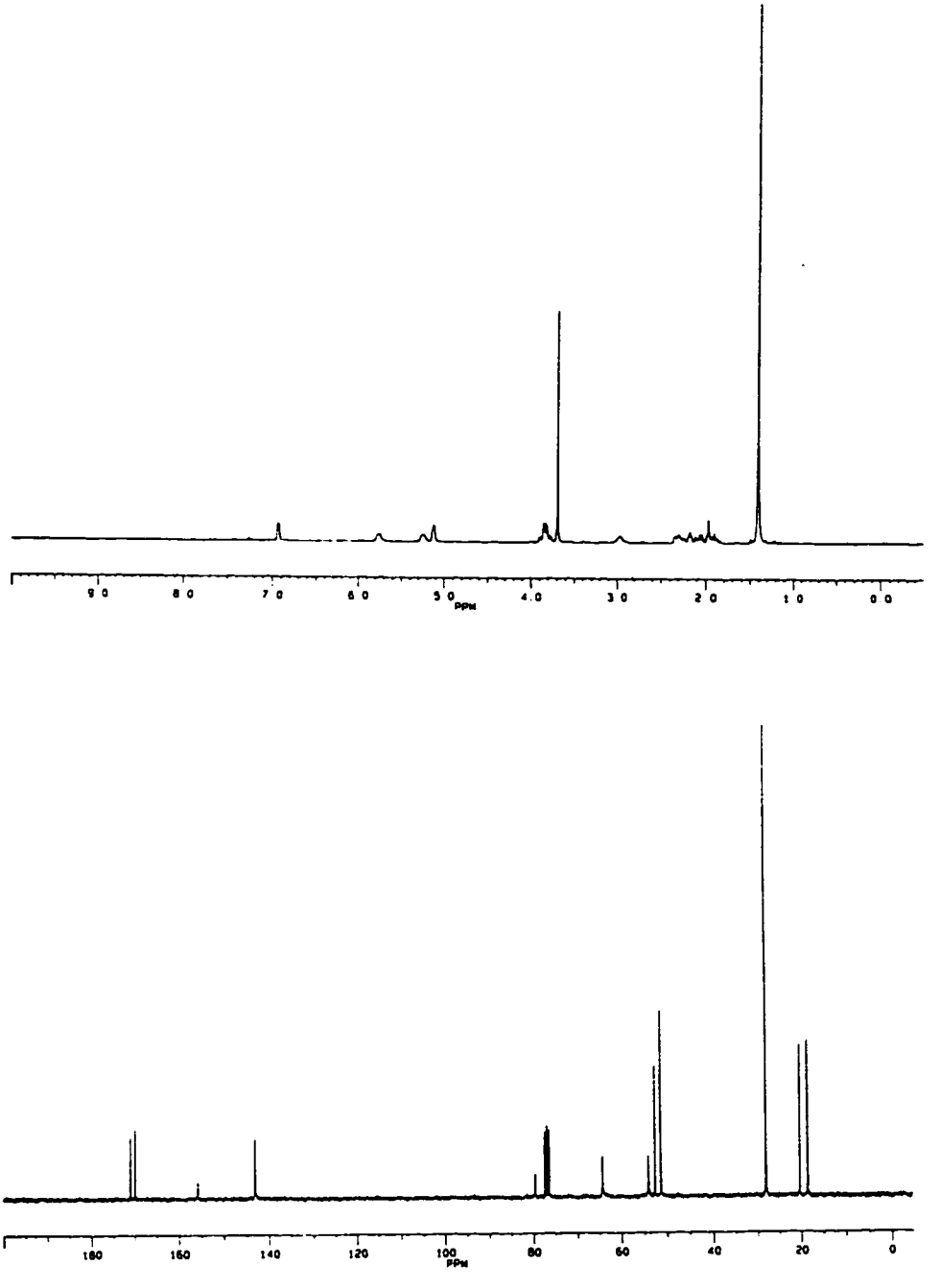




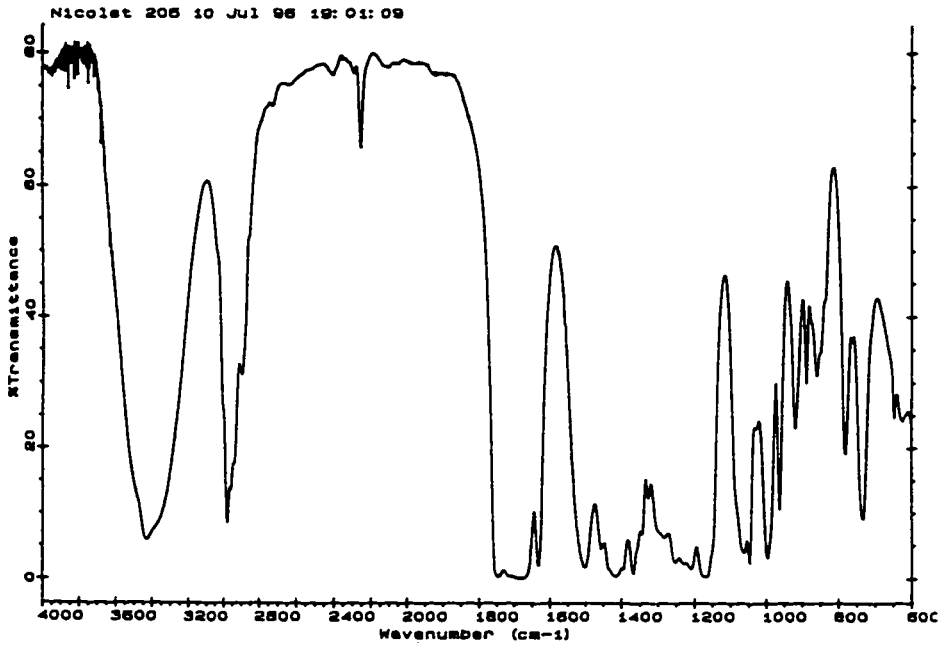
To a solution of BOC **231** (1.70g, 4.8mmol) in 10 mL of MeOH was added catalytic amount Cs_2CO_3 (170mg, 10%wt) at room temperature. After 1 hours, NMR of the crude reaction aliquot showed no starting material left. The reaction mixture was applied to a short silica gel column and eluted with 50% to 80% of EtOAc/Hexane. The product containing eluant was collected, concentrated *in vacuo* to afford **232** as colorless oil (1.39g, 4.2mmol, 88%).

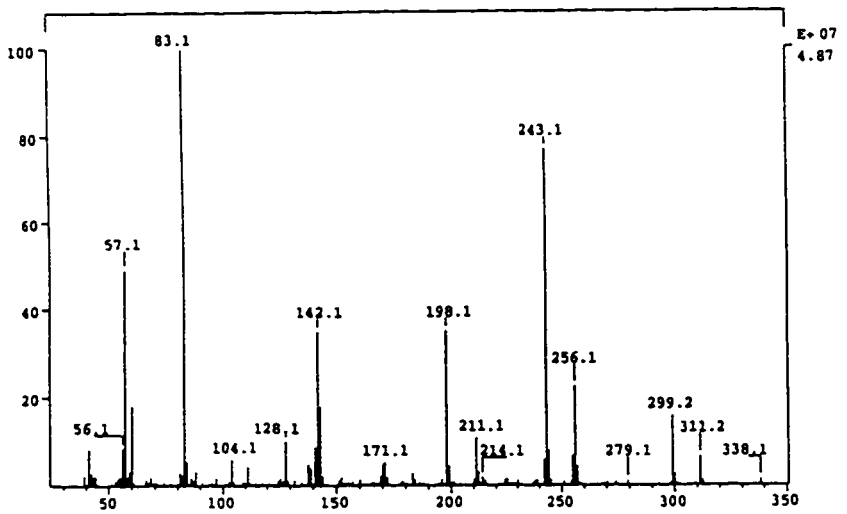
$[\alpha]_D^{25}$ =	$-45°$ (0.11g/mL, CH_2Cl_2)
^1H NMR ($CDCl_3$):	1.40 (9H, s), 1.81-2.38 (4H, m), 2.97 (1H, br), 3.69 (3H, s), 3.84(2H, oct, AB protion of ABX system), 5.12 (1H, m), 5.24 (1H, X portion of ABX system, m), 5.76 (1H, d, J = 6.83Hz), 6.93 (1H, d, J = 3.91Hz)
^{13}C NMR ($CDCl_3$):	18.6, 20.3, 28.2, 51.3, 52.6, 54.1, 64.4, 79.7, 143.1, 169.9, 171.1
IR (film):	734, 780, 860, 920, 961, 996, 1046, 1065, 1174, 1234, 1368, 1418, 1502, 1631, 1691, 1713, 1748, 2980, 3434
MS (EI):	57, 83 (100), 104, 128, 142, 171, 198, 121, 243, 256, 279, 299, 311, 330 (M^++1)
HRMS (EI):	expected ($C_{14}H_{24}N_3O_6$, M^++H): 330.1665; observed: 330.1668

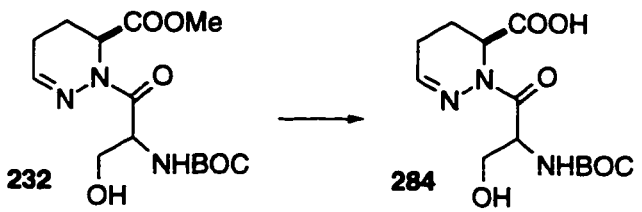
Compound **232**:



Compound **232** continued:







To a mixture of 4mL of MeOH/water (1:1) was added 0.2g of $LiOH \cdot H_2O$ (4.8mmol, 1.1eq), followed by a solution of methyl ester **232** (1.43g, 4.35mmol) in 5mL of THF. The reaction was stirred at room temperature for 2 hours and then acidified to pH 2 with 1N HCl. 20mL of EtOAc and 5mL of water was added and the two layers were separated. The aqueous layer was extracted with 20mL of EtOAc. The combined organic layers were were washed with 10mL of brine, dried over Na_2SO_4, concentarted *in vacuo* to afford 1.23g light yellow oil (3.9mmol, 90%).

$[\alpha]_D^{25}$ = -2.5° (0.07g/mL in MeOH)

^1H NMR (CD_3CN): 1.40 (9H, s), 1.8 - 2.3 (4H, cm), 3.27 (H signal from OH, br), 3.70 (2H, d, J = 4.4 Hz), 4.99 (1H, m), 5.06 (1H, m), 7.00 (1H, m)

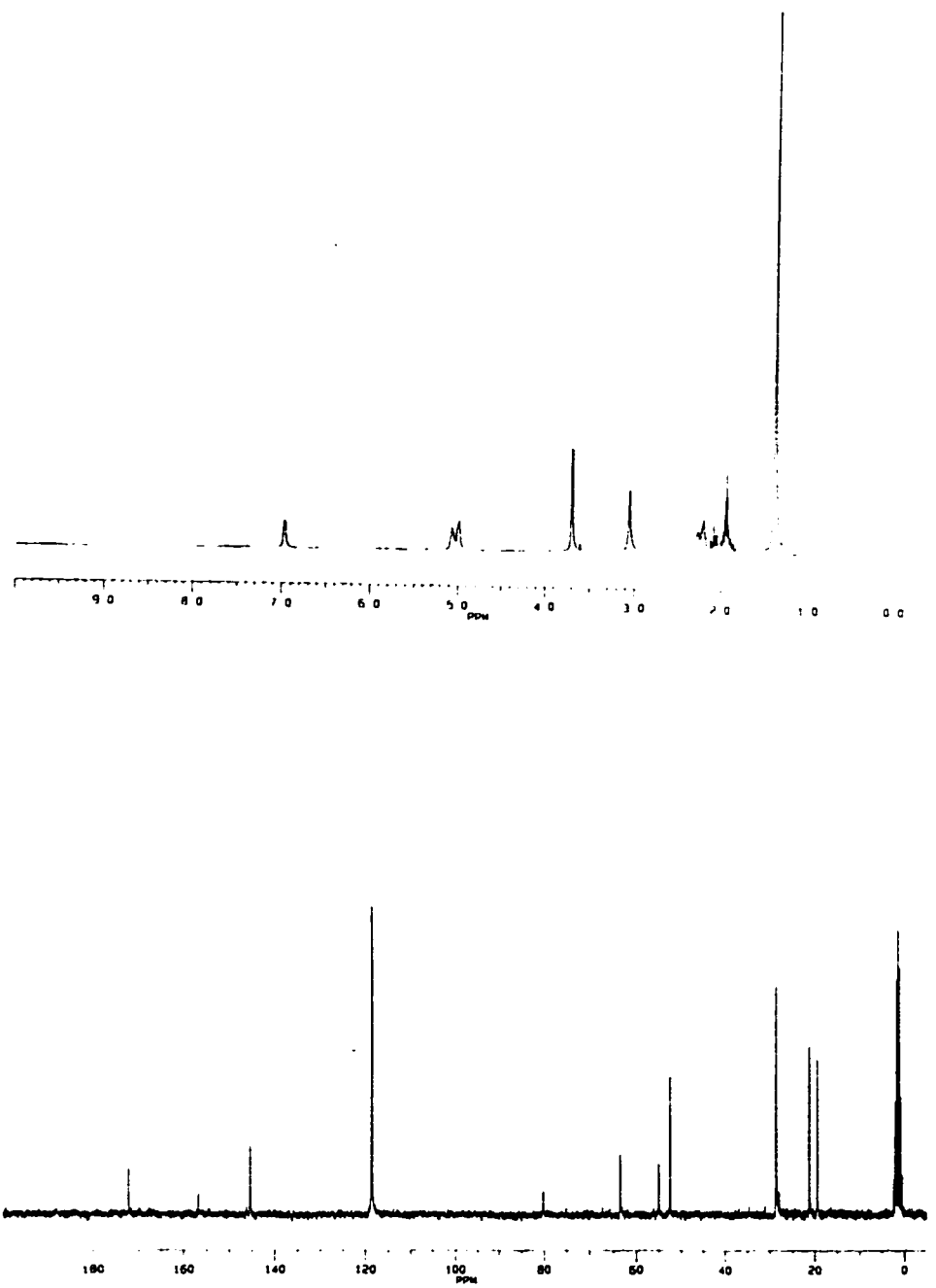
^{13}C NMR (CD_3CN): 19.4, 21.2, 28.6, 52.5, 55.0, 63.5, 80.2, 145.4, 156.8, 172.3

IR (film): 882, 964, 994, 1068, 1169, 1256, 1368, 1420, 1508, 1631, 1680, 2934, 2981, 3352

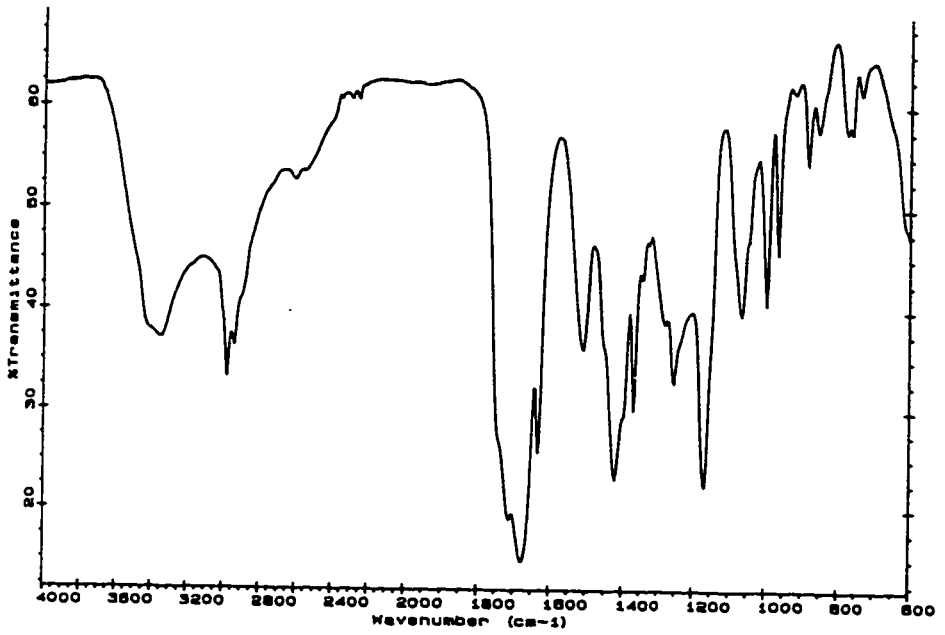
MS (FAB): 57, 83, 107, 136, 154 (100), 216, 260, 295, 316 (M + H^+), 338 (M + Na^+)

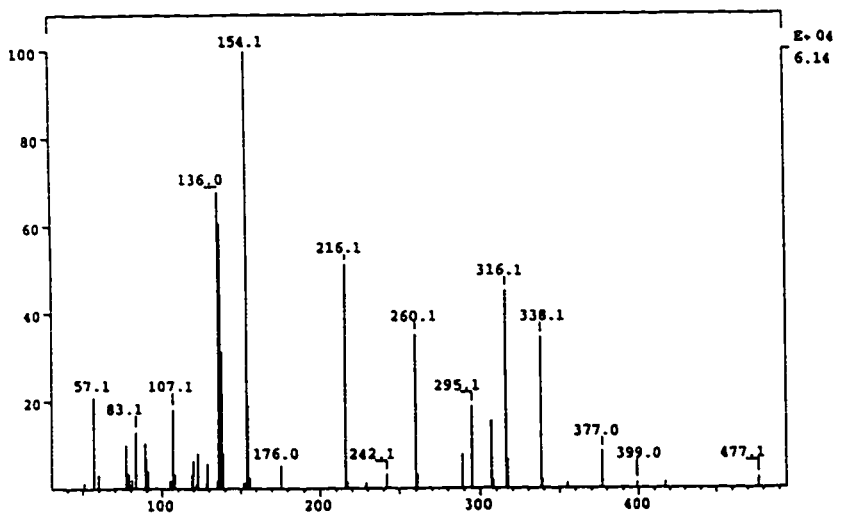
HRMS (FAB): expected ($C_{13}H_{22}N_3O_6$, M^++H): 316.1509; observed: 316.1507

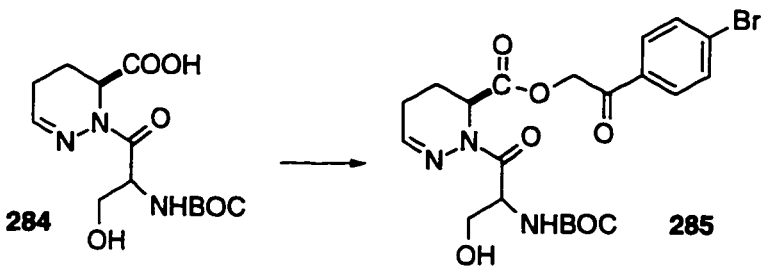
Compound **284**:



Compound **284** continued:







To a solution of acid **284** (1.2g, 3.9mmol, 1eq) in 20mL of acetone was added 4.9mL of Et_3N (11.82mmol, 3eq), followed by 3.3g of phenacyl bromide (11.8mmol, 3eq). The solution became cloudy in 5 minutes and more white solid was formed after 3 hours. The solid was removed from the solution by filtration. The solution was concentrated *in vacuo* and the residue partitioned between 50 mL of CH_2Cl_2 and 20 mL of pH 7 phosphate solution. The aqueous layer was extracted with 50 mL of CH_2Cl_2. The combined organic layers were washed with 30 mL of brine, dried over Na_2SO_4, and concentrated *in vacuo* to yield 2.0g of crude product (3.94mmol, 100%) as white solid. The crude product was recrystallized in 50% EtOAc/hexane to afford 1.94g of **285** (3.79mmol, 96%) as white power.

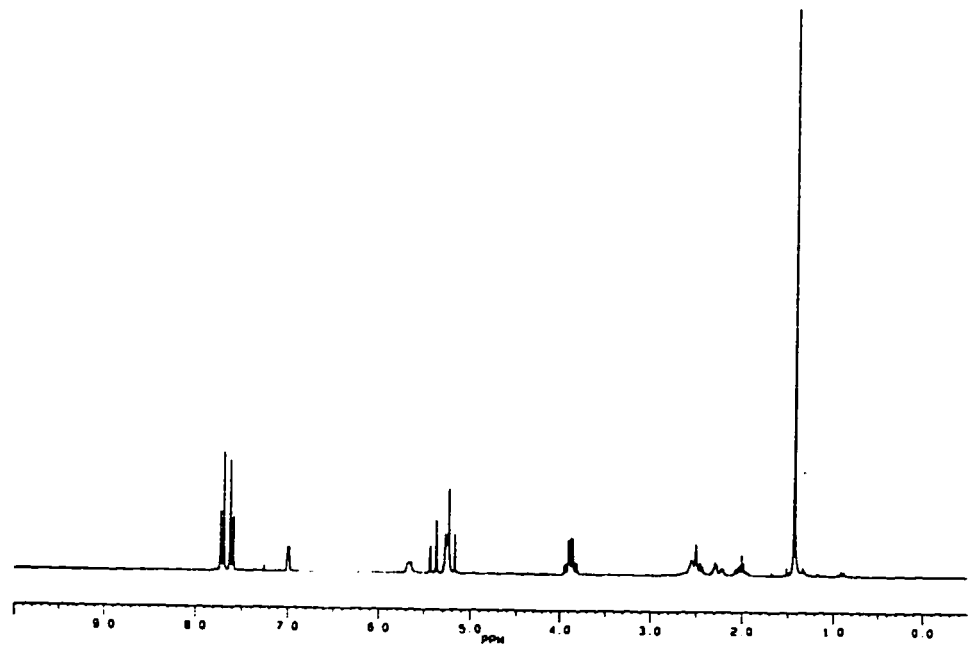
$[\alpha]_D^{25}$ = -29.60 (0.021g/mL CH_2Cl_2)

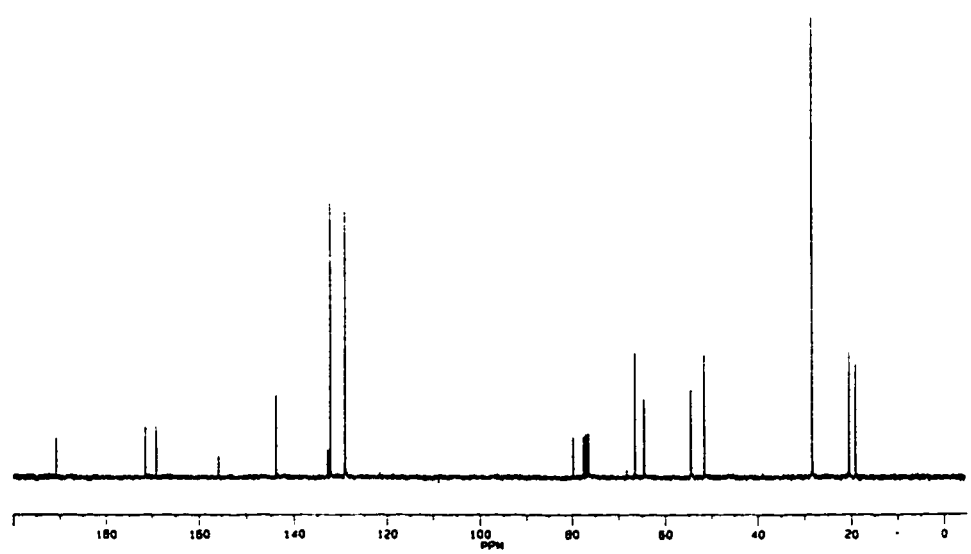
m.p.= 189–190°C

^{1}H NMR ($CDCl_3$): 1.43 (9H, s), 1.91-2.08 (1H, m), 2.18-2.34 (1H, m), 2.40-2.62 (2H, m), 3.88 (2H, oct, AB protions of ABX system), 5.21-5.30 (2H, overlaping, m), 5.21 (1H, d, J = 16.3Hz, A portion of AB system), 5.39 (1H, d, J = 16.3Hz, B portion of AB system), 5.65 (1H, d, J = 7.32Hz), 7.00 (1H, distorted d, J = 4.64Hz), 7.61 (2H, dt, J_1 = 8.79Hz, J_2 = 2.1Hz, A portion of AB system), 7.71 (2H, dt, J_1 = 8.79Hz, J_2 = 2.1Hz, A portion of AB system)

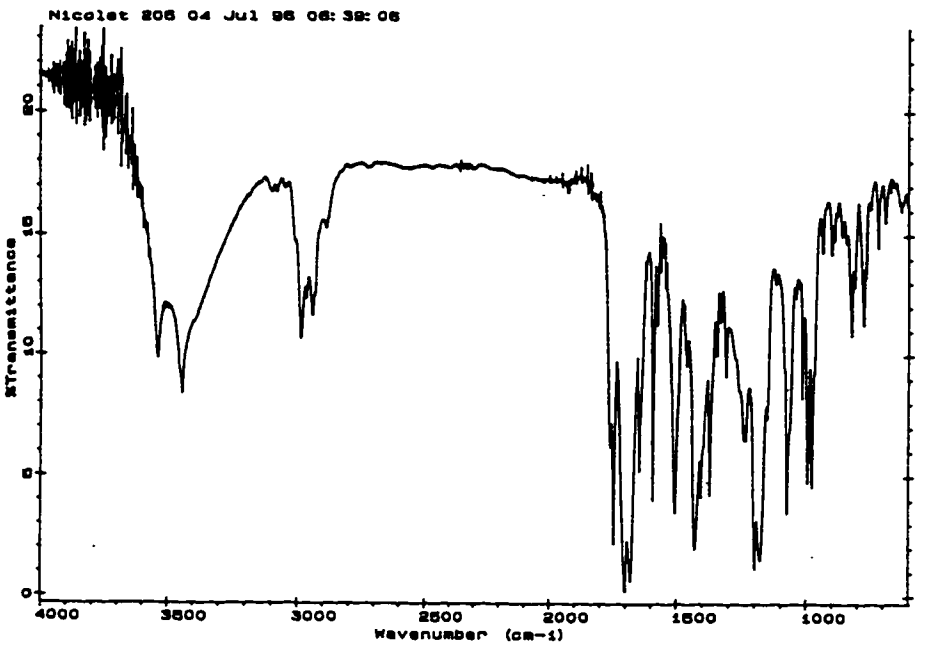
^{13}C NMR ($CDCl_3$):	19.0, 20.4, 28.3, 51.6, 54.5, 64.4, 66.4, 79.8, 129.1, 129.2, 132.3, 132.9, 143.8, 155.8, 169.0, 171.4
IR (KBr):	771, 823, 975, 989, 1073, 1172, 1195, 1369, 1427, 1500, 1587, 1638, 1676, 1699, 1741, 2934,
MS (EI):	57, 83 (100), 131, 157, 183, 229, 310, 324, 380, 393, 425, 428, 438, 481, 493 (M^+)
HRMS (EI):	expected ($C_{21}H_{26}N_3O_7Br$ - H_2O): 493.0848; observed: 493.0855

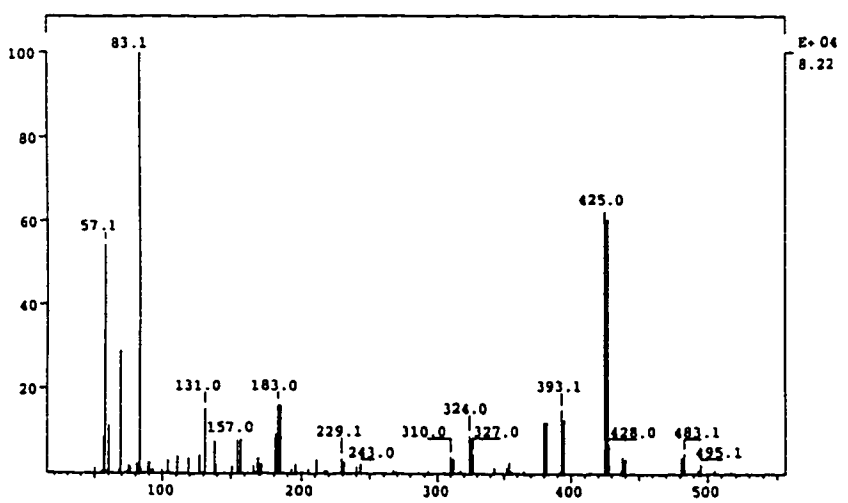
Compound **285**:

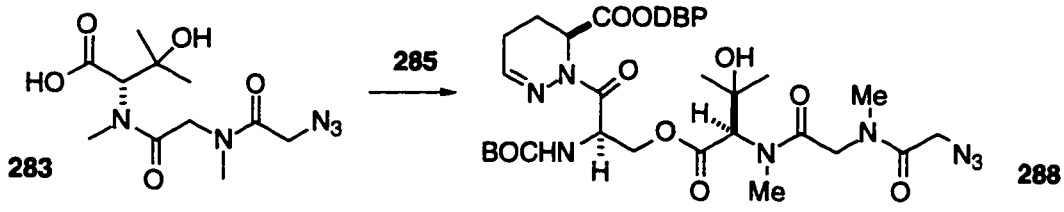




Compound **285** continued:







Alcohol **285** (0.22g, 0.43mmol) and acid **283** (0.39g, 1.3mmol, 3eq) was dissolved in 2 mL of CH_2Cl_2. 0.33g of HBTU (0.86mmol, 2eq) was added to the solution at 0°C, followed by catalyst DMAP (53mg, 0.43mmol, 1eq). The reaction was stirred at room temperature for 8 hours and then quenched with 10mL of pH 7 phosphate aqueous solution. The mixture was partitioned between CH_2Cl_2 and aqueous phosphate solution. The aqueous layer was extracted with 15 mL of CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and the white solid (HBTU by-product) was removed by filtration. The solution, which still contained some HBTU impurities, was concentrated *in vacuo* and chromatographed (50% EtOAc/hexane) to give 0.16g of colorless oil (0.20mmol, 46%)

^{1}H NMR ($CDCl_3$): 1.20 (3H, s), 1.45 (9H, s), 1.48 (3H, s), 2.0 - 2.2 (1H, m), 2.2 - 2.4 (1H, m), 2.4 - 2.6 (2H, m), 3.07 (3H, s), 3.13 (3H, s), 3.98 (2H, s), 4.28 (1H, m), 4.59 (2H, distorted q, J = 10.7Hz), 5.01 (1H, s), 5.23 (1H, d, J = 16.1Hz, overlapping), 5.24 (1H, m, overlapping), 5.39 (1H, d, J = 16.1Hz, overlapping), 5.40 (1H, m, overlapping), 5.57 (1H, m), 7.04 (1H, m), 7.64 (2H, distorted d, J = 8.6Hz), 7.72 (2H, distorted d, J = 8.6Hz)

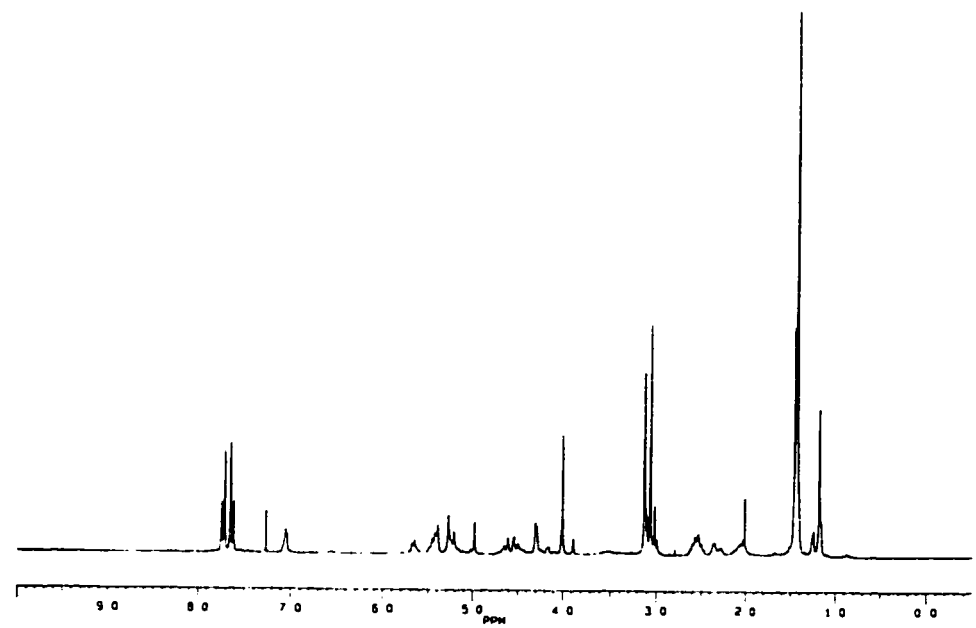
^{13}C NMR ($CDCl_3$): 18.9, 20.4, 26.5, 28.1, 28.3, 29.1, 33.7, 35.6, 35.9, 49.5, 50.3, 51.6, 64.0, 65.3, 66.4, 72.6, 79.9, 129.1, 129.4, 132.3, 132.4, 144.8, 145.0, 154.8, 168.0, 169.0, 169.9, 190.5

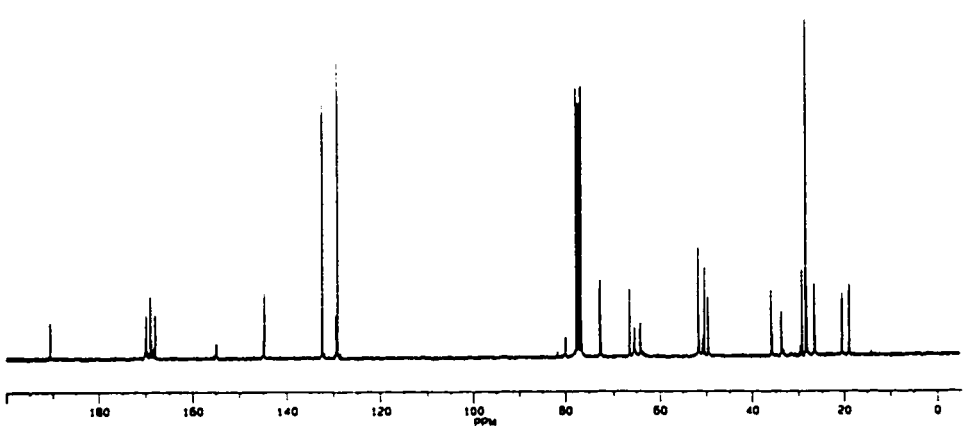
IR (film): 746, 782, 845, 973, 996, 1070, 1170, 1284, 1412, 1508, 1587, 1664, 1702, 1752, 2108, 2938, 3432

MS (FAB): 57, 69, 136, 154 (100), 225, 307, 695, 795 (M^++1)

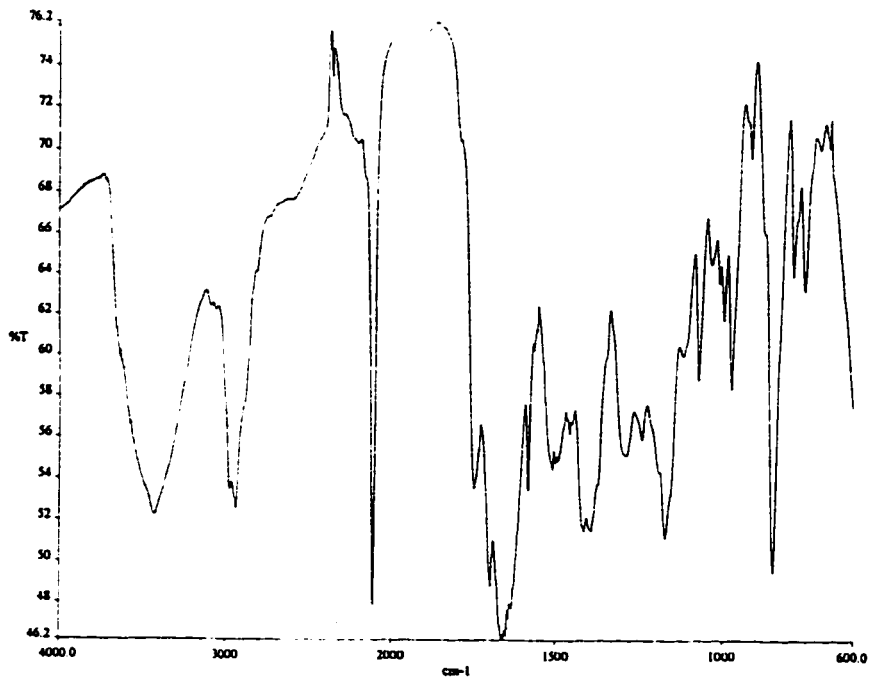
HRMS (FAB): Expected ($C_{32}H_{43}N_8O_{11}BrNa$, M + Na^+): 817.2133; Observed: 817.2128

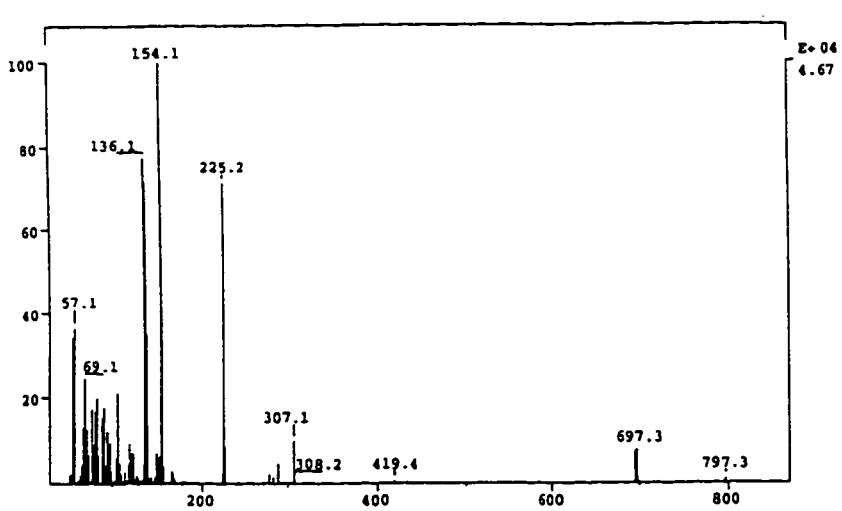
Compound **288**:





Compound **288** continued:





References

1. (a) Ohkuma, H.; Sakai, F.; Nishiyama, Y.; Ohbayashi, M.; Imanishi, H.; Konishi, M.; Miyaki, T.; Koshiyama, H.; Kawaguchi, H. *J. Antibiot.* **1980**, *33*, 1087; (b) Tomita, K.; Hoshino, Y.; Sasahira, T.; Kawaguchi. *J. Antibiot.* **1980**, *33*, 1098

2. Lingham, R. B.; Hsu, A. H. M.; O'Brien, J. A.; Sigmund, J. M.; Sanchez, M.; Gagliardi, M. M.; Heimbuch, B. K.; Genilloud, O.; Martin, I.; Diez, M. T.; Firsch, C. F.; Zink, D. L.; Liesch, J. M.; Koch, G. E.; Gartner, S. E.; Garrity, G. M.; Tsou, N. N.; Salituro, G. M. *J. Antibiot.* **1996**, *49*, 253

3. Matson, J. A.; Bush, J. A. *J. Antibiot.* **1989**, *42*, 1763. Matson, J. A.; Colson, K. L.; Belofsky, G. N.; Bleiberg, B. B. *J. Antibiot.* **1993**, *46*, 162

4. (a) Konishi, M.; Ohkuma, H.; Sakai, F.; Tsuno, T.; Koshiyama, H.; Naito, T.; Kawaguchi, H. *J. Antibiot.* **1981**, *34*, 148; (b) Konishi, M.; Ohkuma, H.; Sakai, F.; Tsuno, T.; Koshiyama, H.; Naito, T.; Kawaguchi, H. *J. Am. Chem. Soc.* **1981**, *103*, 1241

5. Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 1243

6. *Netherlands Pat.*, **1984**, 84 00 237 (*Chem Abs.* **1985**, *102*, 22795q)

7. Ohkuma, H.; Sakai, F.; Nishiyama, Y.; Ohbayashi, M.; Imanishi, H.; Konishi, M.; Miyaki, T.; Koshiyama, H.; Kawaguchi, H. *J. Antibiot.* **1980**, *33*, 1087

8. (a) Fox, K. R.; Davies, H.; Adams, G. R.; Portugal, J.; Waring, M. J. *Nucleic Acids Res.* **1988**, *16*, 2489; (b) Fox, K. R.; Wooley, C. *Biochem. Pharmacol.* **1990**, *39*, 941; (c) Hung, C.-H.; Mong, S.; Crook, S. T. *Biochemistry*, **1980**, *19*, 5537; (d) Hung, C.-H.; Prestayko, A. W.; Crook, S. T. *Biochemistry*, **1982**, *21*, 3704; (e) Hung, C.-H.; Crook, S. T. *Cancer Res.*, **1985**, *45*, 3768; (f) Hung, C.-H.; Mong, S.; Crook, S. T. *Cancer*

Res., **1983**, *43*, 2718; (g) Leroy, J. L.; Gao, X.; Misra, V.; Gueron, M.; Patel, D. J. *Biochemistry*, **1992**, *31*, 1407; (h) Zhang, X.; Patel, D. J. *Biochemistry*, **1991**, *30*, 4026

9. (a) Inouye, Y.; Take, Y.; Nakamura, S. *J. Antibiot.* **1987**, *40*, 100; (b) Take, Y.; Inouye, Y.; Nakamura, S.; Allaudeen, H. S.; Kubo, A. *J. Antibiot.* **1989**, *44*, 107

10. (a) Wang, A. H.-J.; Ughetto, G.; Quigley, G. J.; Hakoshima, T.; van der Marel, G. A.; van Boom, J. H.; Rich, A. *Science*, **1984**, *225*, 1115; (b) van Dyke, M. M.; Dervan, P. B. *Science*, **1984**, *225*, 1122

11. Lingham, R. B.; Hsu, A. H. M.; O'Brien, J. A.; Sigmund, J. M.; Sanchez, M.; Gagliardi, M. M.; Heimbuch, B. K.; Genilloud, O.; Martin, I.; Diez, M. T.; Firsch, C. F.; Zink, D. L.; Liesch, J. M.; Koch, G. E.; Gartner, S. E.; Garrity, G. M.; Tsou, N. N.; Salituro, G. M. *J. Antibiot.* **1996**, *49*, 253

12. Boger, D. L.; Chen, J.-H. *J. Am. Chem. Soc.* **1993**, *115*, 11624

13. Olsen, R. K.; Apparao, S.; Bhat, K. L. *J. Org. Chem.* **1986**, *51*, 3079

14. Hughes, P.; Clardy. J. *J. Org. Chem.* **1989**, *54*, 3260.

15. Shiroza, T.; Ebisawa, N.; Kojima, A.; Furihata, K.; Shimazu, A.; Endo, T.; Seto, H.; Otake, N. *Agric. Biol. Chem.* **1982**, *46*, 1891

16. Shimada, N.; Morimoto, H.; Naganawa, H.; Takita, T.; Hamada, M.; Maeda, K.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 1613

17. Ciufolini, M. A.; Xi, N. *J. Chem. Soc., Chem. Commun.* **1994**, 1867.

18. Manescalchi, M.; Nardi, A. R.; Savoia, D. *Tetrahedron Lett.* **1994**, *35*, 2775

19. Nakamura, K.; Shin, C. *Chem. Lett.* **1991**, 1953

20. Schmidt, U.; Riedl, B. *Synthesis* **1993**, 809

21. Full detail will be discussed in Chapter 3; See also ref. 17

22. Schmidt, U.; Braun, C.; Sutoris, H. *Synthesis* **1996**, 223

23. Maehr, H.; Liu, C.-M.; Palleroni, N. J.; Smallheer, J.; Todaro, L.; Williams, T. H.; Blount, J. H.; *J. Antibiot.* **1986**, *39*, 1723

24. Ogita, T.; Sato, A.; Enokita, R.; Suzuki, K.; Ishii, M.; Negishi, T.; Okazaki, T.; Tamaki, K.; Tanzawa, K. *J. Antibiot.* **1992**, *45*, 17

25. Hurley, T. R.; Bunge, R. H.; Willmer, N. E.; Hokanson, G. C.; French, J. C.; *J. Antibiot.* **1986**, *39*, 1651

26. (a) Hassall, C. H.; Ogihara, R. B.; Thomas, W. A. *Chem. Comm.* **1969**, 1079; (b) Hassall, C. H.; Ramachandran, K. L. *Hetercycles* **1977**, *7*, 119

27. Hale, K.; Delisser, V. M.; Manaviazar, S. *Tetrahedron Lett.* **1992**, *33*, 7613

28. Ciufolini, M. A.; Swaminathan, S. *Tetrahedron Lett.* **1989**, *30*, 3027

29. Maurer, P. J.; Takahata, H.; Rapoport, H. *J. Am. Chem. Soc.* **1984**, *106*, 1094

30. Garner, P. *Tetrahedron Lett.* **1984**, *25*, 5855

31. Dory, Y. L.; Mellor, J. M.; McAleer, J. F. *Tetrahedron* **1996**, *52*, 1343; (b) Calvelier, F.; Jacquier, R.; Mercadier, J.-L.; Verducci, J. *Tetrahedron* **1996**, *52*, 6173; (c) Schmidt, U. *Pure. Appl. Chem.* **1986**, *58*, 295

32. Meng, Q.; Hesse, M. in: "*Tropics in Current Chemistry: Macrocycles*"; Vol. 161, Weber, E.; Vogtle, F., Eds.; Springer-Verlag: Berlin, Germany, 1992; p. 107

33. (a) Sjolin, P.; Elofsson, M.; Kihlberg, J. *J. Org. Chem.* **1996**, *61*, 560; (b) Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294

34. Bondanszky, M. "*Principles of Peptide Synthesis*", Spronger Laboratory: New York, 2nd Edition, 1993

35. See ref. 14 & 20

36. (a) Gennari, C.; Colombo, L.; Bertolini, G. *J. Am. Chem. Soc.* **1986**, *108*, 6394; (b) Evans, D. A.; Britton, T. C.; Dorow, R. L.; Dellaria, J. F. *J. Am. Chem. Soc.* **1986**, *108*, 6395; (c) Trimble, L. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 6397

37. Benetti, S.; Romagnoli, R.; Risi, C. D.; Spalluto, G.; Zanirato, V. *Chem. Rev.* **1995**, *95*, 1065

38. Ganem, B.; Morgan, T. A. *Tetrahedron Lett.* **1980**, *21*, 2773

39. Mander, L. N.; Sethi, S. P. *Tetrahedron Lett.* **1983**, *24*, 5425

40. Koreeda, M.; Akagi, H. *Tetrahedron Lett.* **1980**, *21*, 1197

41. Dasaradhi, L.; Fadnavis, N. W.; Bhalerao, U. T. *J. Chem. Soc., Chem. Commun.* **1990**, 729

42. Guanti, G.; Banfi, L.; Narisano, E. *Tetrahedron* **1988**, *44*, 5553

43. See ref. 20 &79

44. Pettibone, D. J.; Clineschmidt, B. V.; Anderson, P. S.; Freidinger, R. M.; Lundell, G. F.; Koupal, L. R.; Schwartz, C. D.; Williamson, J. M.; Goetz, M. A.; Hensens, O. D.; Liesch, J. M.; Springer, J. P. *Endocrinology*, **1989**, *125*, 217

45. Gentilucci, L.; Grijzen, Y.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett.* **1995**, *36*, 4665

46. (a) Noyori, R.; Ohkuma, T.; Kitamura, M.; Takaya, H.; Sayo, N.; Kumobayashi, H.; Akutagawa, S. *J. Am. Chem. Soc.* **1987**, *109*, 5856; (b) Kitamura, M.; Tokunaga, M.; Ohkuma, T.; Noyori, R. *Org. Synth.*, **1992**, *71*, 1

47. Seebach, D.; Sutter, M. A.; Weber, R. H.; Zuger, M. F. *Org. Synth., Coll. Vol. VII* **1990**, 215.

48. (a) Csuk, R.; Glanzer, B. I. *Chem. Rev.*, **1991**, *91*, 49; (b) Servi, S. *Synthesis*, **1990**, 1

49. Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119

50. Sih, C. J.; Chen, C.-S. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 570

51. Childs, M. E.; Weber, W. P. *J. Org. Chem.* **1976**, *41*, 3486

52. Dale, J. A.; Dull, D. L.; Mosher, S. H. *J. Org. Chem.* **1969**, *34*, 2543

53. (a) Hirama, M.; Nakamine, T.; Ito, S. *Chem. Lett.* **1986**, 1381; (b) Brooks, D.; Kellogg, R. P.; Cooper, C. S. *J. Org. Chem.* **1987**, *52*, 192

54. Huckin, S. N.; Weiler, L. *Can. J. Chem.* **1974**, *52*, 2157

55. Connor, D. S.; Klein, G. W.; Taylor, G. N.; Bockman, R. K.; Medwid, J. B. *Org. Synth., Coll. Vol. VI*, **1988**, 101

56. Mauz, O. *Justus Liebigs Ann. Chem.* **1974**, 345

57. Hirama, M.; Nakamine, T.; Ito, S. *Chem. Lett.* **1986**, 1381

58. Relative reactivities were deduced on the basis of the behavior of compounds related to **87** toward BOC_2O and Ac_2O

59. (a) Carpino,L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalaee, S. Y.; Tien, J.-H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732; (b) Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.*, **1996**, *29*, 268

60. Kim, H.-O.; Gardner, B.; Kahn, M. *Tetrahedron Lett.* **1995**, *36*, 6013

61. Saragovi, H. U.; Fitzpatrick, D.; Raktabutr, A.; Nakanishi, H.; Kahn, M.; Greene, M. I. *Science*, **1991**, *253*, 792

62. (a) Newman, H. *J. Am. Chem. Soc.* **1973**, *95*, 4098; (b) Saitoh, Y.; Moriyama, H.; Hirota, H.; Takahashi, T.; Khuong-Huu, Q. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 488

63. This material was purchased from Advanced ChemTech Inc.

64. (a) Durette, P. L.; Baker, F.; Barker, P. L.; Boger, J.; Bondy, S. S.; Hammond, M. L.; Lanza, T. J.; Pessolano, A. A.; Caldwell, C. G. *Tetrahedron Lett.* **1990**, *31*, 1237; (b) Hale, K. J.; Delisser, V. M. Yeh, L.-K. Peak,S. A.; Manaviazar, S.; Bhatia, G. S. *Tetrahedron Lett.* **1994**, *35*, 7685

65. Baldwin, J. *J. Chem. Soc. Chem. Commun.* **1976**, 734

66. Kaneko, T.; Takeuchi, I.; Inui, T. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 974

67. Aoyagi, S.; Wang, T.-C.; Kibayashi, C.; *J. Am. Chem. Soc.* **1993**, *115*, 11393

68. Shizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185

69. (a) Ciufolini, M. A.; Hermann, C. W.; Whitmire, K. H.; Byrne, N. E. *J. Am. Chem. Soc.*

1989, *111*, 3473; (b) Ciufolini, M. A.; Rivera-Fortin, M. A..; Zuzukin, V.; Whitmire, K. H. *J. Am. Chem. Soc.* **1994**, *116*, 1272

70. Xi, N.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36*, 6595

71. Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185

72. Genet, J. P.; Juge, S.; Mallart, S. *Tetrahedron Lett.* **1988**, *29*, 6765

73. Condensation of **188** with benzaldehyde proceeded in better yields (65 %), but it formed a mixture of diastereomers of the resulting phenyl analogue of **189**.

74. Some of these investigations were carried out by Mr. Kirill Poltevskii, formerly of these laboratories.

75. Askin, D.; Verhoeven, T. R.; Liu, T. M.-H.; Shinkai, I. *J. Org. Chem.* **1991**, *56*, 4929

76. Gronert, S.; Streitweiser, A. *J. Am. Chem. Soc.* **1988**, *110*, 4418

77. Jones, J. *"The Chemical Synthesis of Peptides"*; Oxford Science Pubications: Oxford, UK, 1991

78. Claus, R. E.; Schreiber, S. L. *Org. Synth. Coll. Vol. VII* **1990**, 168

79. Rebert, N. W. *Dissertation*, Utah State University, Logan, Utah, 1987

80. Evans, D. A.; Britton, T. C.; Dorow, R. L.; Dellaria, F. F. *Tetrahedron*, **1988**, *44*, 5525

81. Mixed anhydrides has been successfully applied to this coupling reaction to form gly-sar dipeptide; see Seebach, D.; Bossler, H.; Grundler, H.; Shoda, S.; Wenger, R. *Helv. Chim, Acta* **1991**, *74*, 197

82. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936

83. The aldehyde could also be obtained by TPAP oxidation, but the Swern protocol was superior.

84. Tidwell, T. T. *Org. Reac.* **1990**, 297.

85. (a) Hillis, L. R.; Ronald, R. C. *J. Org. Chem.* **1985**, *50*, 470; (b) Bal, B. S.; Childers, W. E.; Pinnick, H. W. *Tetrahedron Lett.*, **1981**, *37*, 2091

86. Scriven, E. F.; Turnbull, K. *Chem. Rev.* **1988**, *88*, 297

87. Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron*, **1992**, *48*, 1353

88. The azide **281** can be reduced with Zn dust in AcOH. However, AcOH residue could not be removed from the reaction mixture: base (aqueous $NaHCO_3$ or NaOH solution) treatment caused amine **282** instant decomposition. Substituting AcOH with either dilute HCl or pivalic acid or NH_4Cl in Zn dust reduction resulted a complex mixture.

89. Efimov, V. A.; Chakhmakhcheva, O. G.; Ovchinnikov, Yu. A. *Nucl. Acids Res.* **1985**, *13*, 3651.

90. Bodanzsky, M. "*Peptide Chemistry*", Springer-Verlag, Berlin, Germany, 1993

91. (a) Bamberg, P.; Eckstrom, B.; Sjoberg, B. *Acta Chem. Scand.* **1967**, *21*, 2210; (b) Hendrickson, J. B.; Kandall, C. *Tetrahedron Lett.*, **1970**, 343