DISSERTATION

ASPERPARALINE A: BIOSYNTHETIC STUDIES AND SYNTHETIC EFFORTS

Submitted by

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In partial fulfillment of the requirements

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WE HEREBY ROCOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY CHANDELE RAMSEY GRAY ENTITLED ASPERPARLINE A: BIOSYNTHETIC STUDIES AND SYNTHETIC EFFORTS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF SCIENCE.

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ABSTRACT OF DISSERTATION

ASPERPARALINE A: BIOSYNTHETIC STUDIES AND SYNTHETIC EFFORTS

Asperparaline A, a fungal metabolite isolated from *Aspergillus japonicus,* is of interest due to anthelmintic activity and structural similarities to the paraherquamides and brevianamides owing to the presence of bicyclo [2.2.2] diazaoctane core proposed to be derived from a biosynthetic [4+2] cycloaddition.

This communication details two aspects of research regarding asperparaline A. The first goal involves the elucidation of asperparaline A as being biosynthetically composed of dimethylallylpyrophosphate and the amino acids, tryptophan and L-isoleucine, analogous to the paraherquamides. The second goal addresses the desire to develop synthetic methodology amenable to the introduction of isotopic labels for further biosynthetic studies. The proposed retrosyntheses envision the spiro-succinimide ring of asperparaline A being introduced by the photooxidation of a suitably oxidized pyrrole ring.

Synthetic approaches toward asperparaline A presented include peptide coupling of β-methylproline with a prenylated pyrolylalanine, and Horner-Wadsworth-Emmons olefination of a diketopiperazine phosphonate with various aldehydes designed to allow for late stage pyrrole synthesis.

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This effort is dedicated to each of the individuals that participated with me in my journey through education. To my father, Dan Ramsey, thank you for answering my questions. You inspired in me a love of learning. To my mother, Paula DePerte, I treasure your guidance and friendship more with each passing day. To my husband, Randy Gray, your support and understanding have been unfailing and invaluable. To my brothers, Eric and Colin, and to the rest of my family, Kate, Steve, John, Mabel, Rich, Debbie, Adrienne, Nicole, Nik, Sunda, Sylvia and Joe, and anyone I may have forgotten, without whose support and encouragement none of this would have ever happened. To my advisor, Robert Williams whose involvement allowed me complete freedom to seek and learn completely on my own for the first time in my life. To the memory of the late Otto Phanstiel. In 1990, his chemistry class opened my eyes to a world I never imagined and ignited a fire that burns as intensely today as it did that first week of class. And finally to my students, whose presence in my life keeps my love of learning alive. Thank you all.

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List of Abbreviations

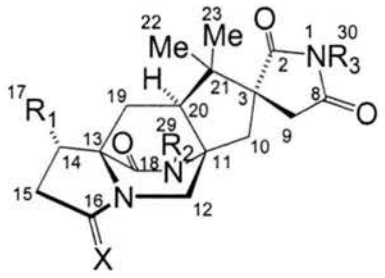
Abbreviation	Definition
[ox]	oxidation
1,3-DMB	1,3-dimethoxy benzene
2,2-DMP	2,2-dimethoxypropanone
9-BBN	9-Borabicyclo[3.3.1]nonane
BAIB	Iodobenzenediacetate
BOC	*tert*-butylcarbamate
Boc_2O	Di-*tert*-butyl dicarbonate
BOP	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
CH_3CN	acetonitrile
CSA	camphor sulfonic acid
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DDQ	dichlorodicyanoquinone
DIBAH	diisobutylaluminum hydride
DIC	diisopropylcarbodiimide
DIPEA	diisopropylethylamine
DKP	diketopiperazine
DMAP	dimethylaminopyridine
DMAPP	dimethylallylpyrophosphate
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
EDCI	*N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
HOMO	highest occupied molecular orbital
HRMS	high resolution mass spectrometry
HWE	Horner-Wadsworth-Emmons (olefination)
IMDA	intramolecular Diels-Alder (reaction)
IR	infrared (spectroscopy)
LAH	lithium aluminum hydride
LDA	lithium diisopropyl amide
LHMDS	lithium hexamethyldisilzane
LUMO	lowest unoccupied molecular orbital
MeOH	methanol
MOM	methoxymethyl (ether)
NMM	N-methyl morpholine
NMO	4-Methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
PG	protecting group
PhH	benzene
PMBOH	*para*-methoxybenzyl alcohol
PMP	pyridoxal monophosphate
PPTS	Pyridinium *p*-toluenesulfonate
PTLC	preparative thin layer chromatography
SAM	S-adenosyl methionine
TBAF	tetrabutyl ammonium fluoride
TBS	tri-tert-butyl silyl (ether)

TEMPO	2,2,6,6-Tetramethylpiperidine 1-oxyl
TES	triethylsilyl (ether)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TsOH	*p*-Toluenesulfonic acid

CHAPTER ONE

Introduction

1.1 Structure and Isolation



1, Asperparaline A (X=H_2, R_1=R_2=R_3=Me)
Aspergillamide (VM55598)

2, Asperparaline B (X=H_2, R_1=R_2=Me, R_3=H)

3, Asperparaline C (X=H_2, R_1=H, R_2=R_3=Me)

4, SB202327 (X=O, R_1=R_2=R_3=Me)

Asperparalines (A-C) are fungal metabolites isolated from the fermentation broth of *Aspergillus japonicus*[1] JV-23 by Hayashi and coworkers and have been shown to have potent paralytic activities against silkworm. Asperparaline A, named aspergillamide (VM55598) along with the 16-oxo-derivative were isolated along with several paraherquamide derivatives from *Aspergillus sp.* IMI 337664 by Everett and associates[2] and were reported to display anthelmintic activity.

Hayashi determined the structure of asperparaline A-C by NMR, HRMS, IR, and single crystal x-ray analysis[1]. Everett determined and confirmed the structure by NMR, HRMS, IR, and UV and the relative stereochemistry was unambiguously assigned using 2D NMR (HETCOR) and ^{1}H nOe experiments

and was in agreement with the published crystal structure of asperparaline A[2]. The 16-oxo-derivative was determined by HRMS to have a molecular weight 14 Daltons greater than asperparaline A and 2-D ^{1}H COSY-45 experiments were used to confirm the structure of SB202327.

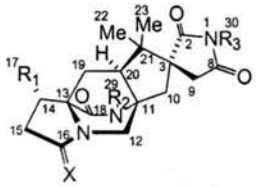
1.2 Structurally related metabolites

The asperparalines along with the brevianamides[3], paraherquamides[4], marcfortine A[5] and sclerotamide[6] (Figure 1) comprise an interesting class of structurally related indole alkaloids which have attracted much attention recently due to their anthelmintic, paralytic and insecticidal activities. These secondary metabolites are the result of mixed biogenetic origin and are comprised of the oxidative polycyclization of amino acids and isoprene units.

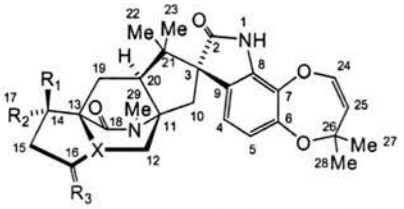
The brevianamides were isolated as early as 1969. Brevianamide A was isolated as the major fluorescent metabolite of *Penicillium brevicompactum*[7] and was shown to have modest insecticidal activity[8]. Brevianamides B-F were later isolated from the same fungus. The brevianamides are constructed from tryptophan in the form of a *spiro*-indoxyl, proline and a single isoprene unit.

The paraherquamides are the most structurally complex of this class of alkaloids. The most potent member of this class, Paraherquamide A, was isolated in 1980 from *Pennicillium paraherquei* by Yamazaki and coworkers[4a]. Since then, Paraherquamides B-G[4b-d], VM55595, VM55596, and VM55597[4e], SB203105 and SB200437[2] have been isolated from various *Penicillium* and *Aspergillus* species. The paraherquamides are constructed of tryptophan in the form of a *spiro*-oxindole, two isoprene units and variously substituted proline derivatives.

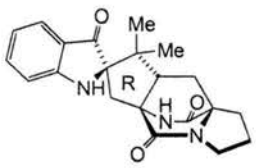
Other closely related compounds include the marcfortines[5] which have a pipicolic acid residue in place of proline, and VM55599[4e], aspergamides A and B[9] and CJ-17,665[10] which have a 2,3-disubstituted indole rather than the *spiro*-oxindole.



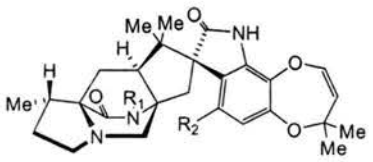
1, Asperparaline A (X=H_2, R_1=R_2=R_3=Me)
Aspergillamide (VM55598)
2, Asperparaline B (X=H_2, R_1=R_2=Me, R_3=H)
3, Asperparaline C (X=H_2, R_1=H, R_2=R_3=Me)
4, SB202327 (X=O, R_1=R_2=R_3=Me)



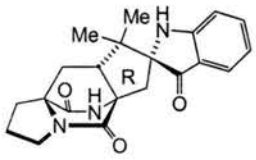
7, (–)-paraherquamide A, R_1 = OH, R_2 = Me, R_3 = H_2, X = N
8, (–)-paraherquamide B, R_1 = H, R_2 = H, R_3 = H_2, X = N
9, (–)-paraherquamide C, R_1 = R_2 = CH_2, R_3 = H_2, X = N
10, (–)-paraherquamide D, R_1 = O, R_2 = CH_2, R_3 = H_2, X = N
11, VM55596, R_1 = OH, R_2 = Me, R_3 = H_2, X = N^+-O^-
12, VM55597, R_1 = OH, R_2 = Me, R_3 = O, X = N



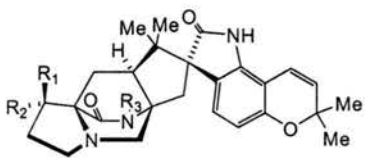
5, (+)-brevianamide A



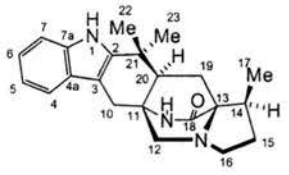
13,paraherquamide E, (VM54159), R_1 = Me, R_2 = H
14, SB203105, R_1 = Me, R_2 = OH
15, SB200437, R_1 = H, R_2 = H



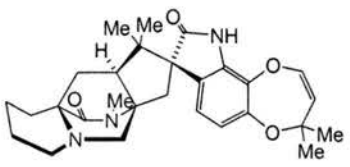
6, (+)-brevianamide B



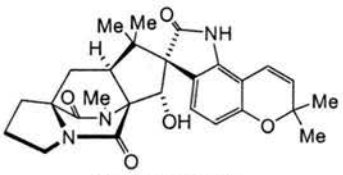
16, paraherquamide F (VM55594), R_1 = H, R_2 = Me, R_3 = Me
17, paraherquamide G (VM54158), R_1 = OH, R_2 = Me, R_3 = Me
18, VM55595, R_1 = H, R_2 = Me, R_3 = H



19, VM55599



20, marcfortine



21, sclerotamide

Figure 1: Structurally Related Secondary Metabolites.

Among its interesting structural features, asperparaline A contains an unusual 3-*spiro*-succinimide moiety, the orientation of which is consistent with the *spiro*-ring configuration of structures **5-21**. A search of the literature revealed that the *spiro*-succinimide ring system has not been reported as constituting part of any known natural products. Additionally, compounds 1-21 share the bicyclo [2.2.2] diazaoctane core.

1.3 Pharmacology

Nematode parasites infect both humans and animals resulting in disease with loss of productivity, debility and even death. Helminth infections of ruminants - namely sheep, pigs, and goats - result in decreased production of meat, milk and wool and diminishes the value of herds due to an increase in the mortality rate of young. According to the Food and Agriculture Organization (FAO) of the United Nations, in 2001 the global market for animal health products was $11,050M with 28.1% of sales going towards parasiticides[11].

Currently there are three classes of anthelmintic therapies available (Figure 2). The nicotinic acetylcholine receptor agonists represented by levamisole/morantel were discovered in the 1950's. The benzimidizoles (i.e. thiabendazole, mebendazole, etc.) which compete for nematode β-tubulin thereby preventing the formation of microtubules were discovered in the 1960's and the macrocyclic lactones (ivermectin and milbemycin) were discovered in the 1970's and increase chloride ion permeability by acting on the glutamate gated chloride channel.[12]

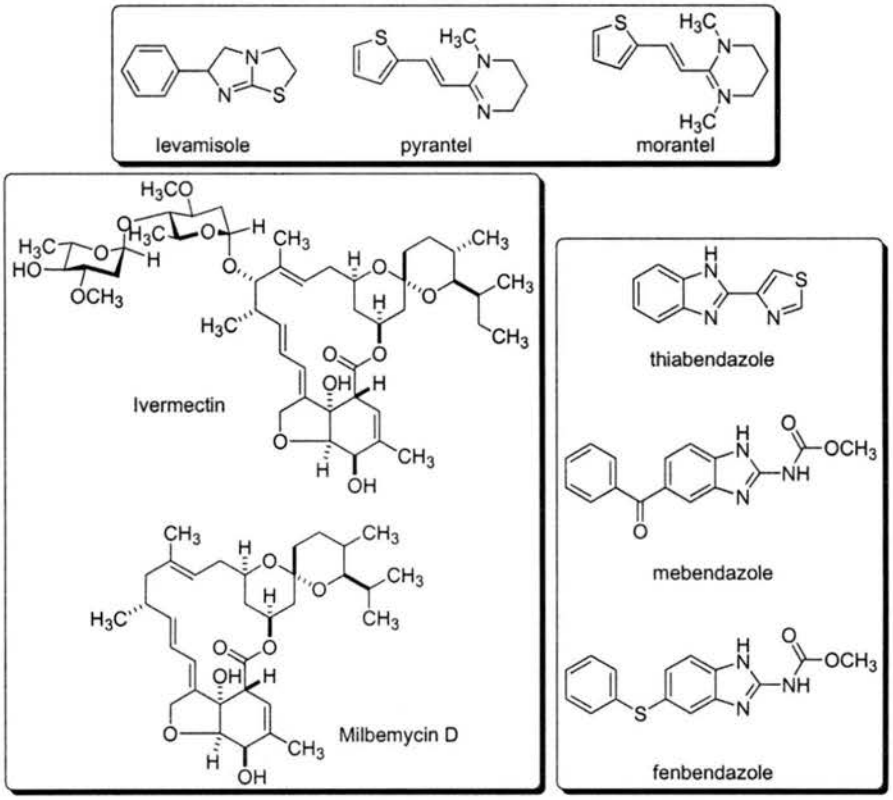


Figure 2. Commercially available therapeutics for anthelmintic infection in ruminants

Unfortunately widespread resistance to each of these classes of broad spectrum anthelmintics has been observed.[13]

The asperparalines, along with their more potent structural relatives the paraherquamides and marcfortines, exhibit activity against strains of parasites which are resistant to each of the known chemotherapeutic treatments available. Upon oral administration of asperparaline A at a dose of 10 μg/g of diet, the fourth instar larvae of silkworm exhibit paralysis within one hour and remain paralyzed for 7 to 10 hours.[1]

Table 1: Percent reduction of *T. colubriformis* in fecal egg count at stated dose.[2]

Compound	0.5 mg/kg	1 mg/kg	4 mg/kg	10 mg/kg	20 mg/kg
Asperparaline A	--	--	--	44	98
SB202327	--	0	--	--	--
Paraherquamide E	88	96	99	--	--
SB203105	--	--	73	67	--
SB200437	--	--	--	86[a]	--
Paraherquamide A	19	70	99	--	--

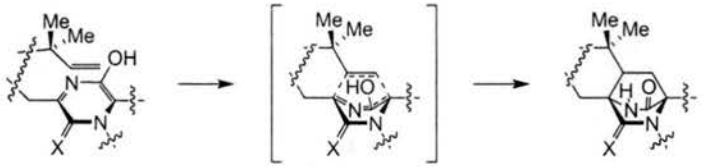
[a] Dosed orally at 7.7 mg/kg

The most potent of these structures is paraherquamide A. Geary and coworkers at Pharmacia Animal Health have studied the mode of action of paraherquamide[14]. Due to the close structural similarity between the asperparalines and paraherquamides it has been assumed that the mode of activity of the asperparalines is the same.

In vitro paraherquamide induces rapid flaccid paralysis of nematodes without affecting ATP levels, suggesting that these compounds act at a neuroreceptor in these organisms rather than as a metabolic poison. Geary and associates have observed that paraherquamide binds to nematode nicotinic acetylcholine receptors and act as an antagonist of acetylcholine at this site. In the classical model of a cholinergenic receptor there is an anionic site which binds to the quaternary ammonium of acetylcholine and another location which binds to the carbonyl oxygen of acetylcholine through hydrogen bonding. In acetylcholine these positions are separated by four atoms and Geary proposes that it is the structural similarity of the carbonyl of the *spiro*-ring and the nitrogen of the tertiary amide in paraherquamide which is responsible for its binding and observed activity. The asperparalines possess the same structural features so they are presumed to act accordingly.

1.4 Significance and Implications

An emerging body of evidence supports the notion that the bicyclo [2.2.2] diazaoctane core common to each of these metabolites is constructed by a biological intramolecular [4+2] cycloaddition of the isoprene derived olefin across the azadiene moiety of a pre-formed, oxidized piperazinedione (**A→B→C**).[15] While biological Diels-Alder reactions have been proposed for a variety of natural products there are very few proven examples of this cycloaddition occurring in biological systems and even fewer mediated by enzyme catalysis.[16] There is, as of yet, no report of an isolated Diels-Alderase from the fungi which produce these compounds, but if this family is indeed biosynthesized via a [4+2] cycloaddition, the producing organism may contain a rare but extremely important type of this enzyme.



A **B** **C**

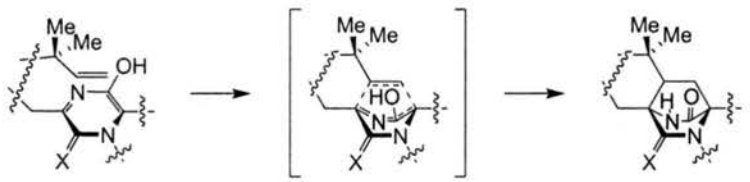
In these laboratories exists a program directed toward elucidating the mechanism for the biosynthetic formation of the bicyclo [2.2.2] diazaoctane core common to each of these compounds. Synthesis of, and feeding experiments with, isotopically labeled compounds proposed as biogenetic intermediates have proven to be useful methods for studying the biogenesis of these compounds. As a result, synthesis of asperparaline A and the elucidation of the building blocks responsible for the core framework of asperparaline A have been the primary research focus of this dissertation.

CHAPTER TWO

Biosynthetic Studies

2.0 Biosynthesis of Structurally Related Compounds

The biosynthesis of this family of alkaloids is of interest for two reasons. First, nature is unsurpassed in its elegance and efficiency in synthesizing a vast array of structurally interesting and pharmacologically useful compounds. There remains much to be learned with regards to the assembly and pre-organization of biosynthetic precursors. Understanding these processes is sure to fine tune the understanding necessary to efficiently synthesize these compounds in a laboratory setting. Second, it has been proposed that the bicyclo[2.2.2]diazaoctane core common to each of these compounds arises via a biosynthetic Diels-Alder reaction, an intramolecular [4+2] cycloaddition of the terminal olefin of the isoprene moiety across the azadiene derived from the preformed oxidized piperazinedione (**A→B→C**).



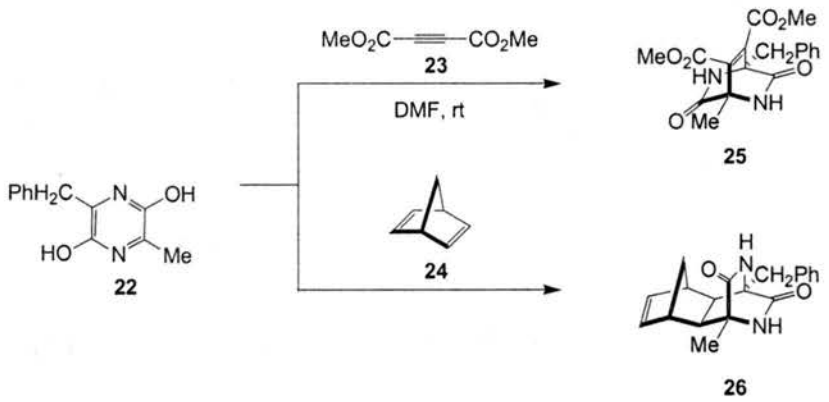
A **B** **C**

While the synthetic utility of the Diels-Alder reaction is well documented in the chemical literature there are few examples of confirmed Diels-Alder cycloadditions occurring in nature[17].

2.1 Biosynthetic Studies of the Brevianamides

In 1970, Porter and Sammes[18] proposed that the bicyclic core of brevianamide A originated from an intramolecular hetero-Diels-Alder cycloaddition, based on the results of a model study wherein dihydroxypyrazine **22** reacted with both dimethyl acetylenedicarboxylate (23) and norbornadiene (24) to give cycloadducts **25** and **26** respectively (Scheme 1).

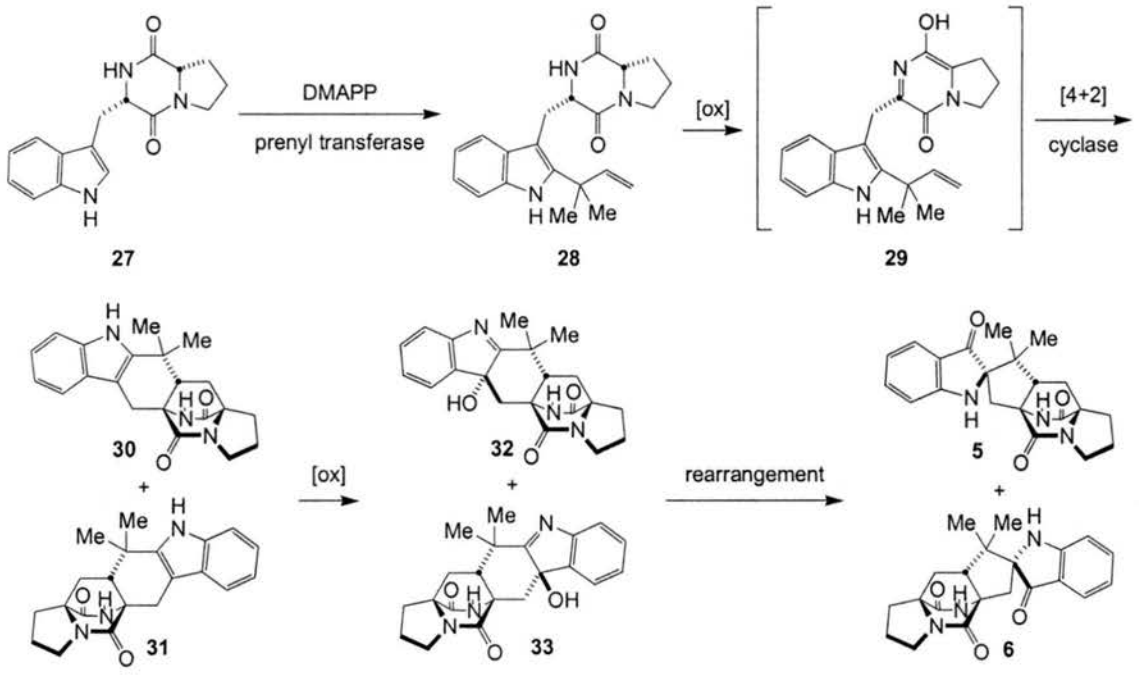
Scheme 1: Porter and Sammes model study for the proposed biosynthesis of brevianamide A.[2]



Radiolabeled feeding experiments by Birch and Wright[19] with *Penicillium brevicompactum* investigating the biosynthesis of brevianamide A confirmed that it is biogenetically derived from tryptophan, proline and dimethylallypyrophosphate (DMAPP). Later studies revealed brevianamide F (27) as a biosynthetic precursor and the reverse prenylated indole derivative, deoxybrevianamide E (28) which had been isolated from cultures of *Aspergillus ustus* was postulated as the next step in the biosynthetic pathway to brevianamide A[20] though it has not been observed as a metabolite of brevianamide A producing cultures. In 1993, Williams and coworkers performed feeding experiments with [8-$^3H_2$]-**28** and observed strong experimental evidence in favor of deoxybrevianamide E as a biosynthetic precursor to **5** and **6**[21]. Based on these results, Williams et al.[22] proposed a biosynthesis of brevianamide A and B wherein brevianamide F (27) undergoes reverse prenylation to deoxybrevianamide E (28) (Scheme 2). Two electron oxidation of **28** would result in the requisite azadiene which would then suffer an intramolecular hetero-Diels-Alder cycloaddition providing enantiomeric cycloadducts **30** and **31**. An R-selective

oxidation at the 3-position of the indole followed by a pinacol-type rearrangement to the indoxyl would thus provide **5** and **6**.

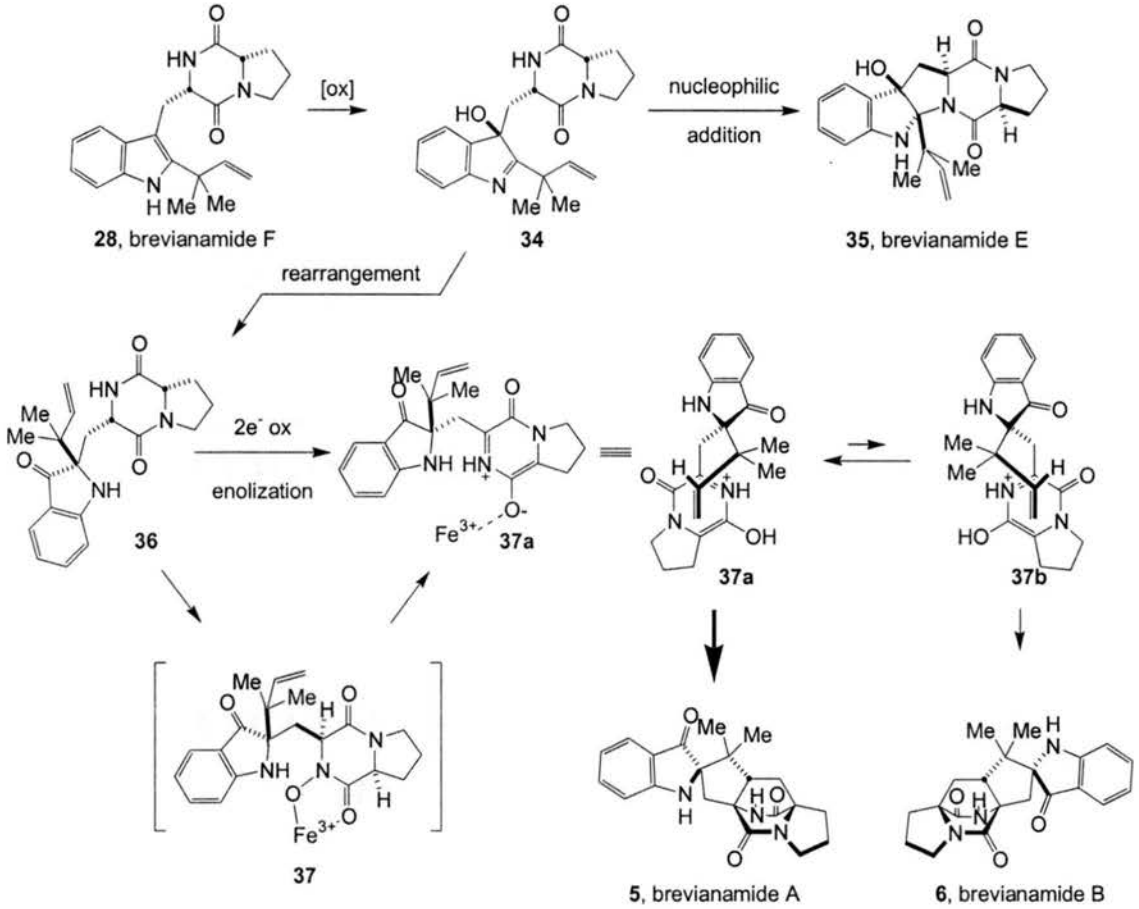
Scheme 2: Initial proposal by Williams et al. for the biosynthesis of the brevianamides.



To further investigate the biosynthetic construction of the brevianamides, ^{13}C-labeled cycloadducts **30** and **31** were synthesized and fed to cultures of *P. brevicompactum,* yet no detectable incorporation was observed[5]. While these results do not rigorously exclude the pathway in Scheme **2** as a viable biosynthetic route to the brevianamides, an alternate biosynthesis for the brevianamides was proposed.

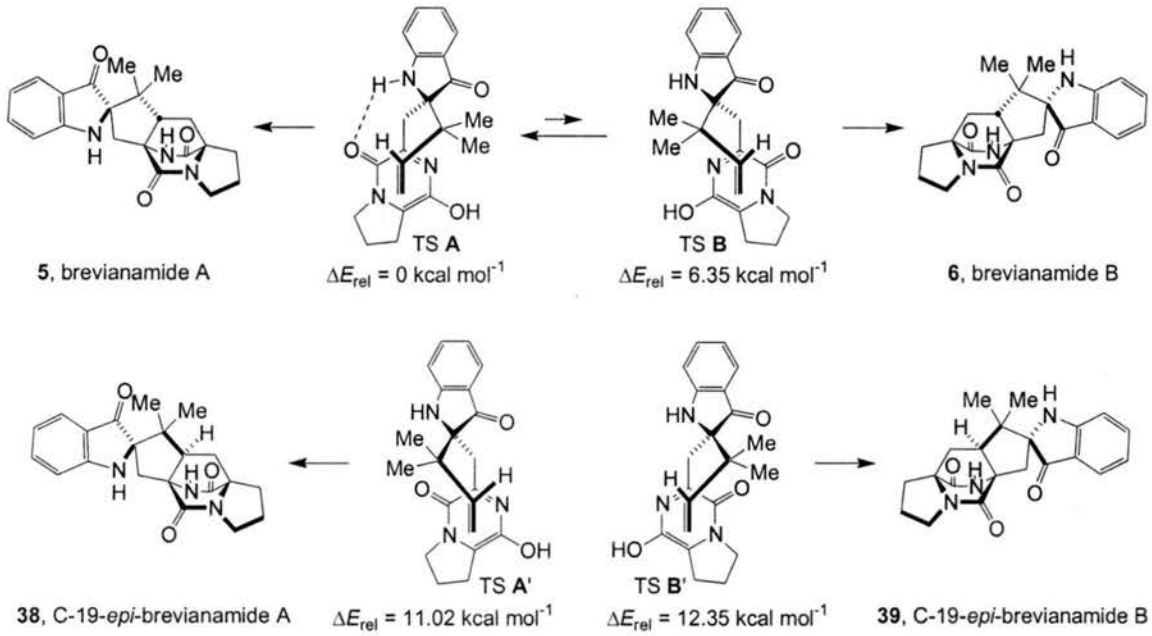
The alternate pathway, shown in Scheme **3** envisions deoxybrevianamide E undergoing R-selective oxidation at the 3-position of the indole. The resulting hydroxyindolenine **34** then suffers one of two possible fates. Ring closure by nucleophilic addition of the diketopiperazine amide nitrogen results in the formation of shunt metabolite, brevianamide E (35), while pinacol-type rearrangement yields *spiro*-indoxyl **36**. Two electron oxidation and enolization affords the requisite azadiene which would then suffer an intramolecular hetero-Diels-Alder cycloaddition revealing **5** and **6**.

Scheme 3: Revised biosynthetic proposal for the brevianamides.



This revised proposal was substantiated by *ab initio* calculations of the four possible diastereomeric transition states[23]. Calculations of the proposed transition state conformations (Scheme 4) estimated the potential energy barrier for transition state leading to brevianamide A (TS**A**) to be 38.68 kcal mol^{-1} and the potential energy barriers to TS**B**, TS**A'**, and TS**B'** were higher by 6.35, 11.02, and 12.73 kcal mol^{-1}, respectively. These values are in agreement with the observed product ratios of brevianamide A and B. The cycloadducts, **38** and **39** that would result from the highest energy transitions states, TS**A'** and TS**B'**, are unknown as natural products. In addition to the differential orientation of the alkene relative to the azadiene system, the preferential lowering of TS**A** relative to TS**B** is attributed to intramolecular hydrogen bonding between the indoxyl amine and the carbonyl of the azadiene ring.

Scheme 4: Calculated potential energy barriers of transitions states for possible brevianamide Diels-Alder cycoadditions.



2.2 Biosynthetic Studies of the Paraherquamides

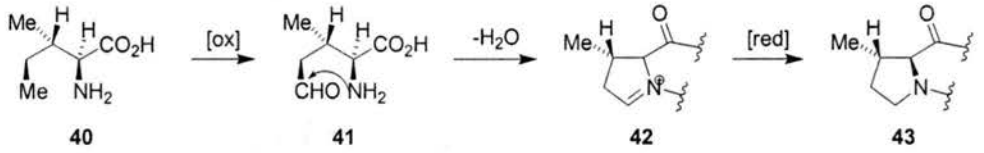
The paraherquamides are structurally complex indole alkaloids comprised of two isoprene units, tryptophan, and variously substituted proline derivatives. Many members of this family have attracted interest recently owing to their potent anthelmintic and antinematodal activity and have been under intense investigation for use in veterinary medicine for the treatment of intestinal parasites in ruminants. The parent and most potent member of this class of alkaloids is paraherquamide A (7). First isolated in 1980 from the fermentation cultures of *Penicillium paraherquei*[24], Paraherquamide A along with various paraherquamide analogs have since been isolated from *Penicillium charlesii (fellutanum)*[25], *Penicillium* sp (IMI332995)[26], and recently paraherquamide analogs, SB203105 and SB200437 were isolated from the asperparaline A producing fungus *Aspergillus* sp. (IMI337664)[27].

Feeding experiments have revealed L-methionine, L-tryptophan, and L-isoleucine to be the proteinogenic amino acids responsible for the core framework of paraherquamide A[28]. Feeding experiments with [$^{13}C_2$]-acetate and [$^{13}C_6$] glucose revealed the mevalonate origin of the isoprene units[29].

Feeding experiments also determined the unusual, non-proteinogenic amino acid L-β-methyl proline (43) to be biosynthetically derived from L-isoleucine rather than SAM mediated methylation of L-

proline as might otherwise be expected. Investigation of the mechanism[30] for the formation of β-methyl proline from L-isoleucine indicates that L-isoleucine suffers a four electron oxidation of the terminal methyl group such as via the putative intermediate **41** shown in Scheme 5.

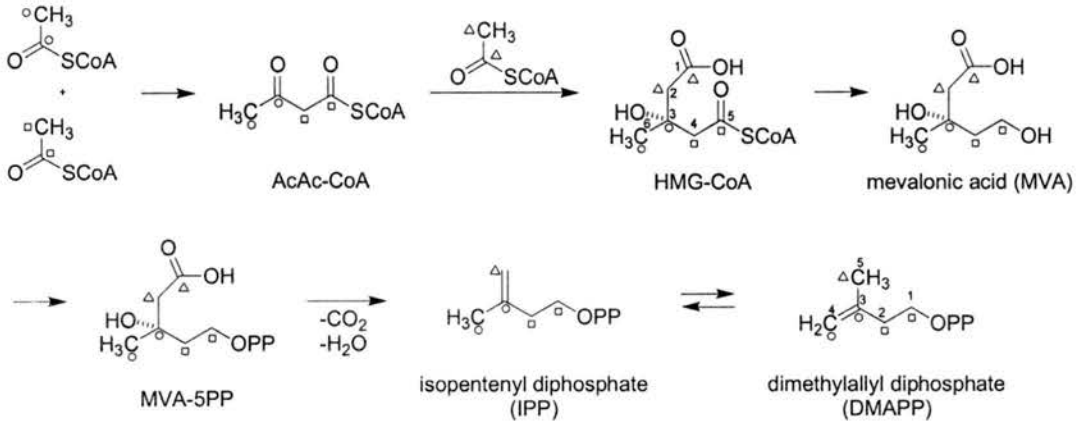
Scheme 5: Plausible mechanism for the formation of β-methyl proline from L-isoleucine.



Cyclodehydration and diastereoselective reduction from the same face as the methyl group of the proline ring results in the formation of β-methyl proline, which is converted to the β-hydroxy-β-methyl proline observed in paraherquamide A in a downstream transformation.

During the course of feeding experiments to determine the biogenesis of the isoprene units of paraherquamide A an interesting discovery was made. Close inspection of the ^{13}C NMR of the paraherquamide A isolated from feeding experiments on *P. fellutanum* with [$^{13}C_6$]-glucose and [$^{13}C_2$]-acetate indicated that while the stereochemical integrity of the *gem*-dimethyl groups of the isoprene unit of the dioxepin moiety was preserved, scrambling of the isotopic label for the methyl groups at C22 and C23 was evident. DMAPP obtained via the mevalonate pathway is constructed from three acetate units (Scheme 6) followed by loss of C1 in the decarboxylation of MVA-5PP to isopentenyl diphosphate (IPP).

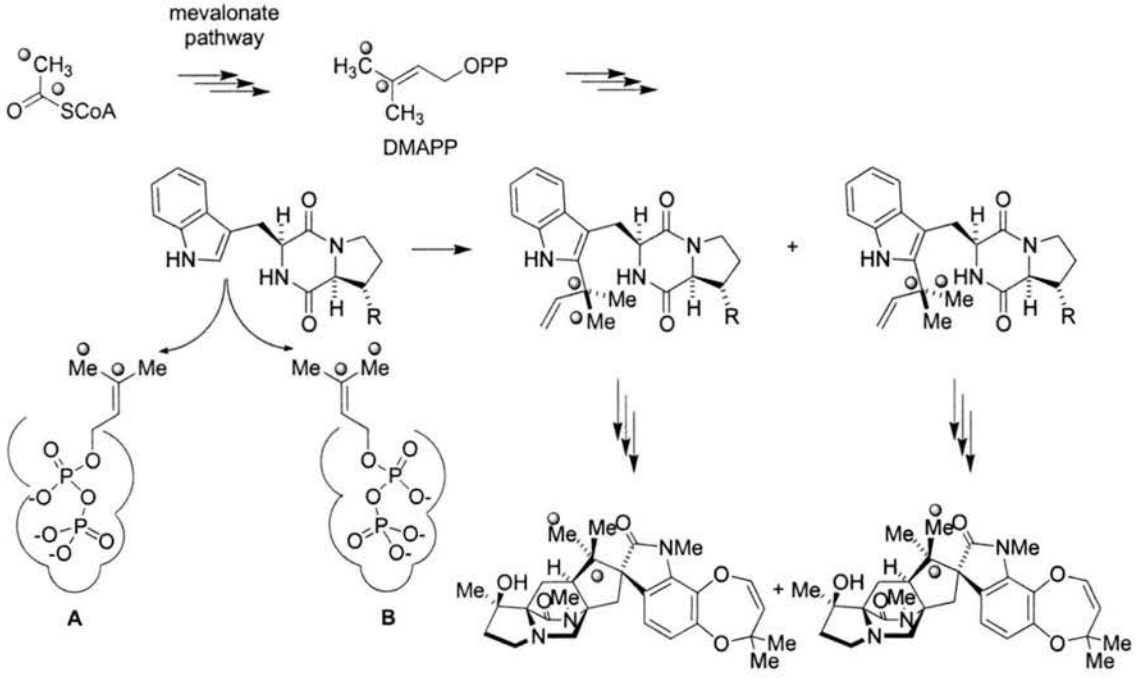
Scheme 6: The classical mevaonate pathway showing the labeling pattern via [$^{13}C_2$]-acetate.



Incorporation of [$^{13}C_2$]-acetate would be expected to exhibit coupling between C1 and C2 and between C3 and C4 of DMAPP, but no coupling between C3 and C5 should be observed.

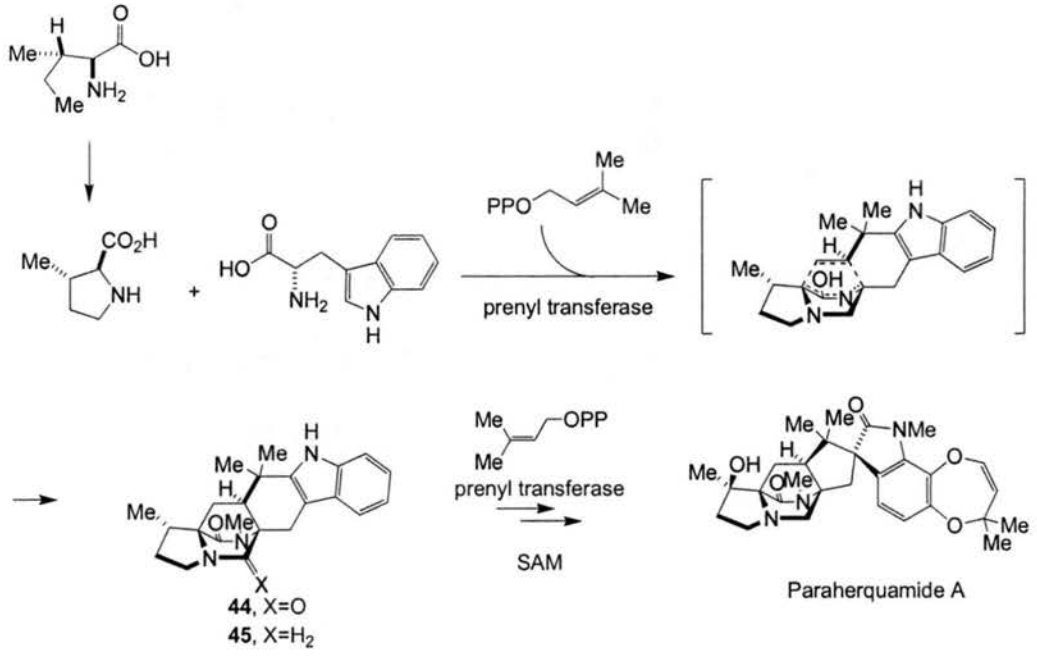
The ^{13}C NMR of paraherquamide A obtained from feeding experiments of [$^{13}C_2$]-acetate to *P.fellutanum* exhibits coupling between C24 and C25 and between C26 and C27, but no coupling is observed to C28, for the carbons of the isoprene unit in the dioxepin ring, as expected. In the carbons of the bridging isoprene unit (C19-C23), coupling is detected between C19 and C20, and C21 is coupled to both C22 and C23, but not simultaneously. Thus indicating that the stereochemical integrity of the isoprene unit of the dioxepin ring is retained, whereas, the stereochemical integrity of the isoprene unit, which comprises the bicyclo [2.2.2] core, is sacrificed during some stage of the biosynthesis. To explain these results, Williams et al.[13b] have proposed that the hydrophilic diphosphate portion of DMAPP is buried in the enzyme active site "upside-down" relative to "normal" prenyl transferases and presenting the olefinic portion of DMAPP in a facially indiscriminate manner such that both faces of the olefin are susceptible to, essentially an S_N2', attack by the 2-position of the indole (Scheme 7).

Scheme 7: A possible biosynthetic sequence to explain how the geminal dimethyl groups are rendered equivalent in the biosynthesis of paraherquamide A.



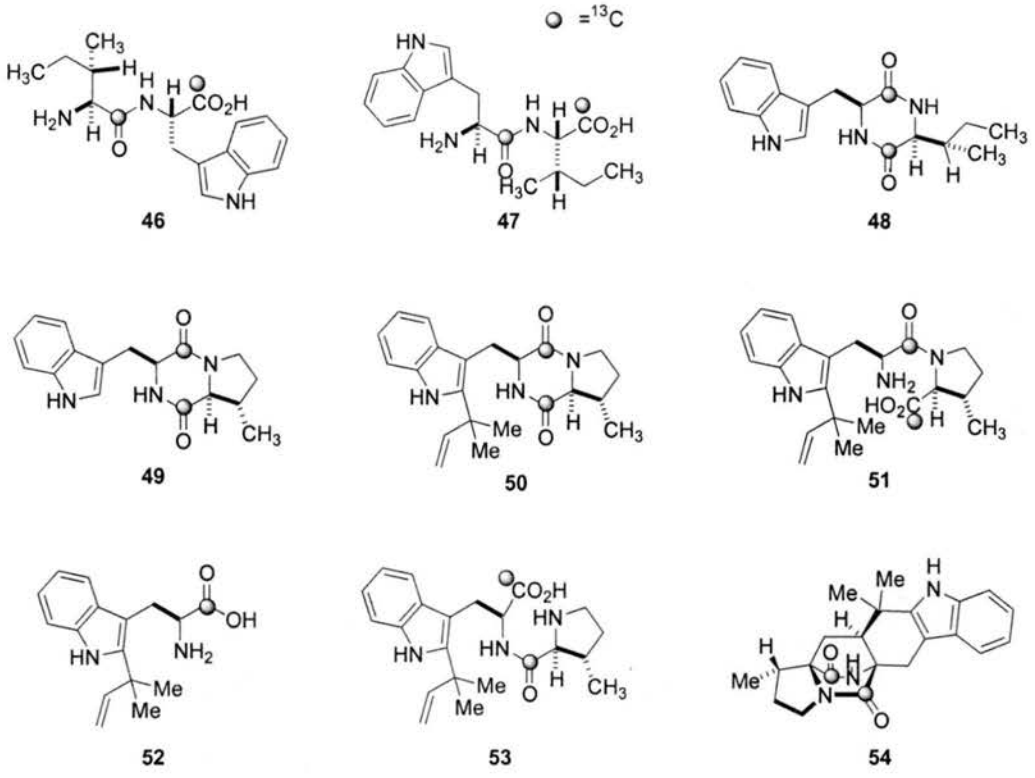
With respect to the timing of the biosynthetic sequence of events responsible for the construction of paraherquamide A there is much that remains uncertain. Confirmed biogenetic precursors include the amino acids listed earlier and DMAPP. Also confirmed is the 2,3-disubstituted indole **45** (Scheme 8)[31]. Synthesis and feeding of the [$^{13}C_2$]-cycloadducts **44** and **45** revealed that **45** is indeed a biosynthetic precursor to paraherquamide. Incorporation of its oxidized counterpart **44**, however, was not observed.

Scheme 8: Confirmed precursors in the biosynthesis of Paraherquamide A.



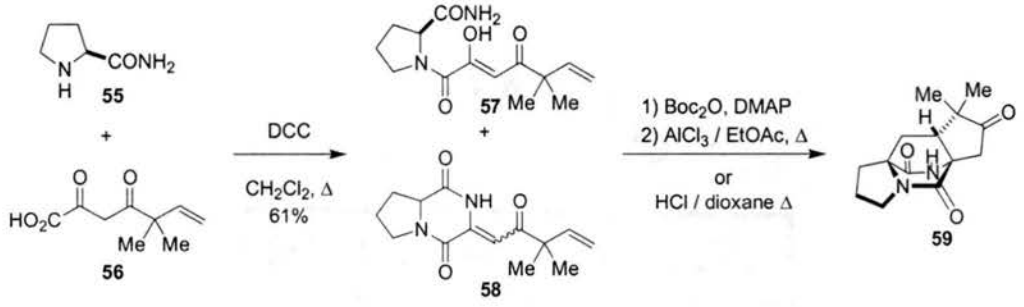
In addition, each of the ^{13}C-labeled compounds depicted in Figure 3 were independently prepared and fed to cultures of *P. fellutanum.* Spectral analysis of the paraherquamide A so produced did not provide compelling evidence for the site-specific incorporation for any of these proposed precursors. These results raise interesting questions regarding the timing of the reduction of the tryptophan derived carbonyl.

Figure 3: Proposed ^{13}C-labeled precursors fed to *P. fellutanum* which gave negative results.



As a result of the success in synthesizing the unsaturated diketopiperazine **58**[32] from the *alpha*-keto acid **56** and prolinamide **55** (Scheme 9) and the resulting formation of cycloadduct **59** a new biosynthetic proposal has been hypothesized.

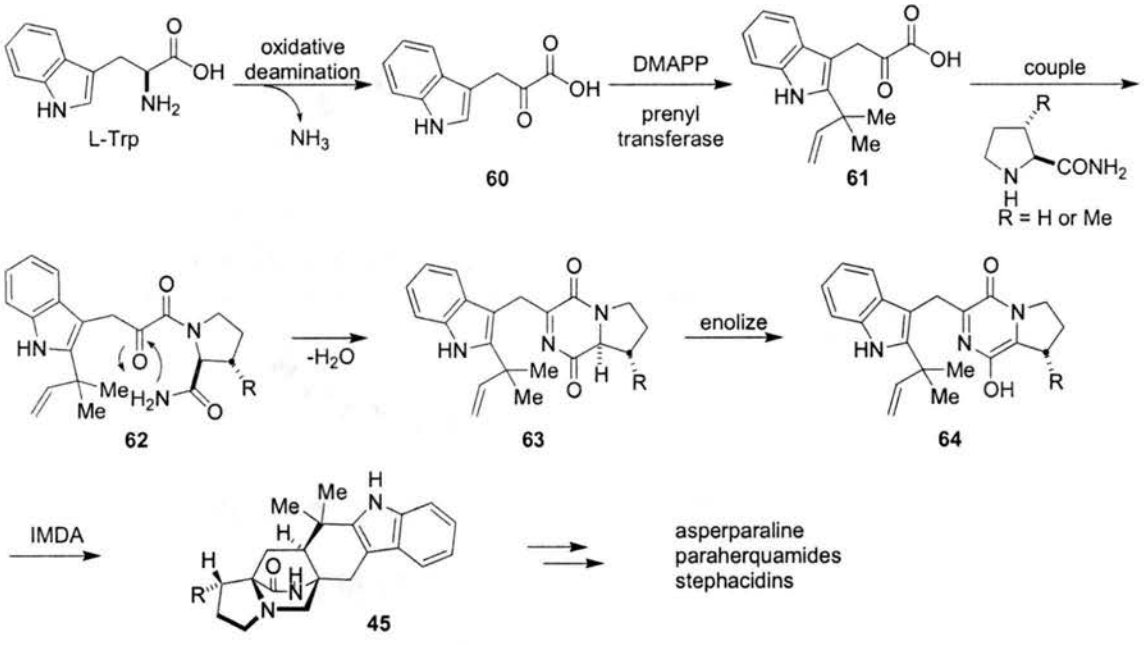
Scheme 9: Coupling of α-keto acid with prolinamide and formation of bicyclo [2.2.2] diazaoctane.



Oxidative deamination of tryptophan or a reverse prenylated tryptophan derivative would furnish the α-keto acid **60**, coupling with the appropriate prolinamide derivative would result in a potentially spontaneous

cascade of cyclodehydration, tautomerization and intramolecular cycloaddition to afford the key hexacyclic substance **45** (Scheme 10). Downstream oxidations, prenylations and further manipulations of necessary functional groups would provide the paraherquamides, and asperparalines as well as the stephacidins (via avrainvillamide).

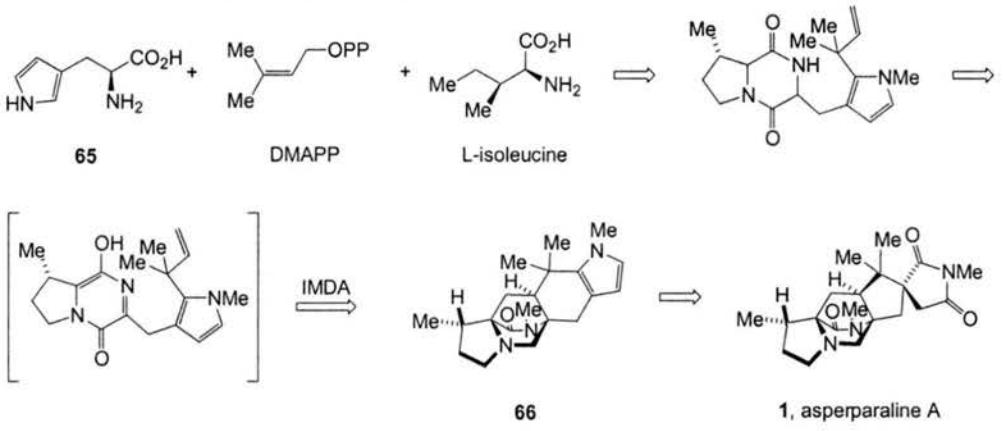
Scheme 10: A new biogenetic proposal.



2.3 Biosynthetic Studies of Asperparaline A

A recent addition to this family of alkaloids is asperparaline A. Structurally similar to the paraherquamides, it contains a β-methyl proline residue and has the same absolute stereochemistry at C20. In addition, the orientation of the *spiro*-succinimide ring is consistent with the relative configuration of the *spiro*-oxindole ring observed in the paraherquamides. It also exhibits modest anthelmintic activity. Given these similarities, the initial biosynthetic hypothesis proposed that it would be biogenetically derived from L-methionine, L-isoleucine, as the progenitor of the β-methyl proline ring, DMAPP and the novel amino acid pyrrolylalanine (Scheme 11). Prenylation of *cyclo*-L-pyrrolylalanine-L-β-methyl proline followed by oxidation of the diketopiperazine (65) and intramolecular [4+2] cycloaddition would give the putative hexacyclic intermediate **66** which would then undergo oxidation and spirocyclization to furnish asperparaline A.

Scheme 11: Initial biogentic proposal for asperparaline A.



2.3.1 Feeding Experiments – Introduction

A convenient method for conducting feeding experiments on filamentous fungi involves inoculation of a sterilized liquid growth media with spores of the metabolite producing fungus. The fungus is allowed to progress through the stage of primary metabolism and just prior to maturation and shifting into secondary metabolism (as evidenced by the growth of aerial hyphae and usually accompanied by a color change) the growth media is carefully decanted and replaced by sterile trace element solution containing the ^{13}C-labeled compound of interest. The trace element solution is comprised of only the

inorganic salts necessary for cellular function. As all other nutritive content has been removed, the fungus utilizes the ^{13}C-labeled compounds selectively introduced as a source of nutrition and as a result, the metabolites so produced exhibit ^{13}C enrichment which can be analyzed spectroscopically.

Aspergillus japonicus is a Japanese soil fungus and in all reports of its growth in a laboratory setting, a solid, agar based medium was used. Agar based media is unsuitable for performing feeding experiments as described above because it is not possible to separate the fungus from the growth medium once underway, thereby making it impossible to control the source of nutrition. As a result, it was of foremost importance to identify a liquid media upon which *A. japonicus* could be propagated. Healthy *A. japonicus* develops as white mycelia largely submerged which overtakes the surface onto which it has been inoculated. After approximately four days, abundant conidial structures colored a deep brownish-black cover the entire colony. The conidial heads are large and brownish-black in color. The resulting mature fungus appears as a lush, nearly black carpet completely obscuring the surface on which it is grown. Consultation of the available sourcebooks[33] indicated the use of corn steep liquor broth, Czapek's-dox broth and modified malt-extract broth as the liquid media upon which a variety of other *Aspergillus* sp. had been successfully grown. Each of the above listed media were prepared and inoculated with spores of *A. japonicus* and initial metabolism appeared to proceed as described above. However at no time did the cultures attain the lush brownish-black growth of conidial heads as observed in the cultures grown on solid media. Furthermore, these cultures failed to produce a detectable quantity of asperparaline A. Rather than exhaustively test each of the known liquid media formulations (which exceed 125) a method was devised to adapt the potato-dextrose agar to a liquid media.

The *A. japonicus* used to inoculate the culture flasks are grown on slants of potato-dextrose agar. Nutritionally, the agar supplies the fungus with a source of protein. It was reasoned that substituting the agar with an alternative source of protein would provide a means for growing *A. japonicus* that would eliminate the practical issues associated with the 'solidifying-effect' that the agar presented. To that end eight different experimental liquid media were prepared, sterilized and inoculated with spores of *A. japonicus* (Table 2). Four of the broths tested employed glucose as the sugar source and four employed maltose. The enzymatically digested proteins peptone, neopeptone, tryptone and tryptose were chosen as agar replacements.

Table 2: Ingredients of potato-sugar broths containing enzymatically digested proteins as an agar substitute.

Entry	Protein (0.75g)	Sugar (5g)	Potato* (g)	dH_2O (mL)
1	Peptone	Glucose	75	250
2	Peptone	Maltose	75	250
3	Neopeptone	Glucose	75	250
4	Neopeptone	Maltose	75	250
5	Tryptone	Glucose	75	250
6	Tryptone	Maltose	75	250
7	Tryptose	Glucose	75	250
8	Tryptose	Maltose	75	250

* unwashed russet baking potatoes

Each broth was autoclaved, allowed to cool and inoculated with spores of *A. japonicus* and incubated in the dark at ambient temperature. Within five days each flask contained cultures exhibiting all of the characteristics of the cultures grown on the solid media and each produced asperparaline A. The cultures grown on the broths prepared with dextrose appeared healthier than cultures grown in the presence of maltose and tryptose appeared to be the optimal choice of protein. Henceforth, all feeding experiments were performed by growing *A. japonicus* on potato-dextrose broth containing tryptose.

2.3.2 Feeding Experiments – Preliminary Results

[$^{13}C_2$]-acetate was fed to cultures of *A. japonicus* to establish the origin of the carbons presumably derived from DMAPP. As expected, incorporation of the ^{13}C-labeled compound was observed at carbons C19 to C23 indicative of a mevalonate pathway in accordance with the observations of previously reported for paraherquamide A. In addition, coupling is observed between C21 and C22 and between C21 and C23 albeit not simultaneously, indicating that the stereochemical integrity of the isoprene unit is sacrificed at some stage of the biosynthesis. These results suggest that, as in the case of the paraherquamide biosynthesis, a "reverse" prenyl transferase is presenting the olefinic portion of DMAPP in a facially indiscriminate manner.

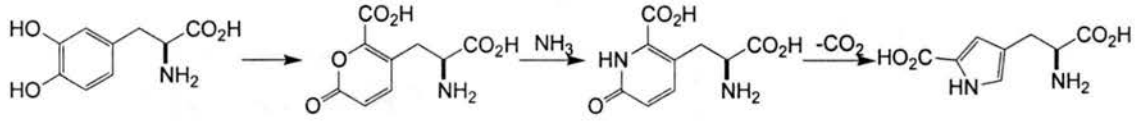
The results of feeding experiments with [^{13}C]-L-isoleucine showed enrichment (5.8%) at C18 as expected. Furthermore, feeding experiments with [*methyl*-^{13}C]-L-methionine only resulted in enrichment at positions C29 (25.2%), the N-methyl position of the piperazinone ring and at C30 (26.9%), the N-methyl position of the *spiro*-succinimide ring. No incorporation was detected at C17, corresponding to the methyl

substituent of the proline residue, thereby providing further evidence for isoleucine as the progenitor of the β-methyl proline ring.

2.3.3 Feeding Experiments – Probing the origin of the *spiro*-succinimide ring

The initial biogenetic proposal suggested the novel amino acid pyrrolylalanine as the progenitor of the *spiro*-succinimide ring in asperparaline A. In 1991, Shirahama[34] and coworkers reported the isolation of L-3-(2-carboxy-4-pyrrolyl)-alanine (69) from the mushroom *Clytocybe acromelalga*.

Scheme 12: Proposed biosynthesis of L-3-(2-carboxy-4-pyrrolyl)-alanine[34].



L-DOPA **67** **68** **69**

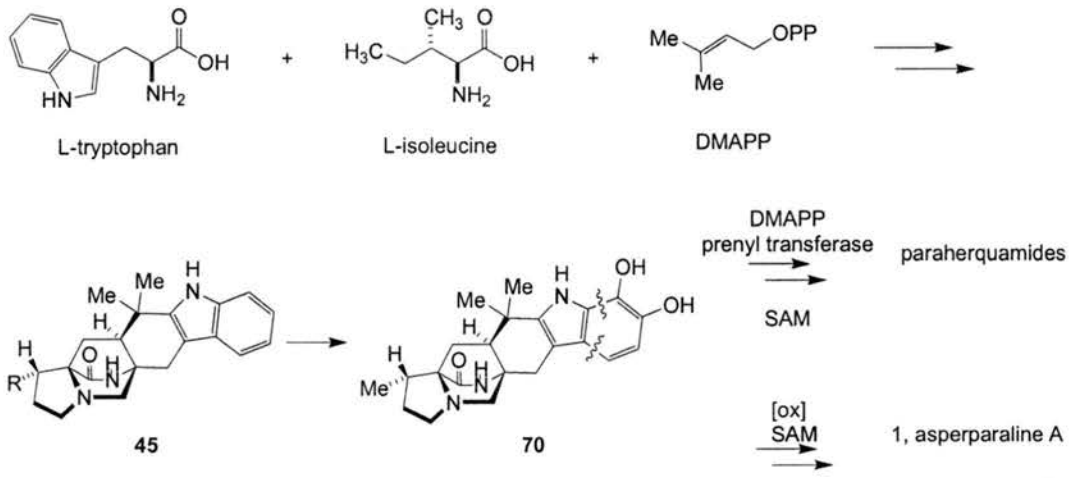
They proposed dihydroxy phenylalanine (L-DOPA) as the progenitor of this novel amino acid. To investigate this as a potential biosynthetic precursor to asperparaline A, [^{13}C]-L-tyrosine (the progenitor of L-DOPA[35]) was fed to cultures of *A. japonicus*. Spectral analysis of the asperparaline A so produced did not show evidence of incorporation at any location, thereby casting doubt on the probability of L-DOPA as the biosynthetic precursor of te *spiro*-succinimide ring via the pyrrole moiety proposed.

Pyrrolylalanine can also be envisioned as being biogenetically derived from one unit of acetate and L-glutamate or L-glutamine. Close inspection of ^{13}C NMR of the asperparaline A isolated from feeding experiments with $^{13}C_2$-acetate does not show any detectable enrichment at C8 or C9. Neither was the isotopic label from a-^{15}N-L-glutamate or a-^{15}N-L-glutamine observed to incorporate into asperparaline A.

Since the *spiro*-succinimide ring of asperparaline A is consistent with the relative orientation of the *spiro*-oxindole ring of paraherquamide A, and given the other biogenetic similarities shared between the two metabolites, it seemed plausible that the *spiro*-succinimide ring might be biosynthetically derived from an oxidative degradation of L-trytophan. To test this hypothesis, [1-^{13}C]-L-tryptophan was fed to cultures of *A. japonicus*. Isolation and spectral analysis of the asperparaline so produced indicated ^{13}C enrichment

(6.7-7.4%) at C12. Next, a feeding experiment with [*indole*-2-^{13}C]-L-tryptophan resulted in enrichment (12.6%) at C21, indicating that tryptophan is indeed the progenitor of the *spiro*-succinimide ring.

Scheme 13: A proposed unified biogenesis of asperparaline A and the paraherquamides.

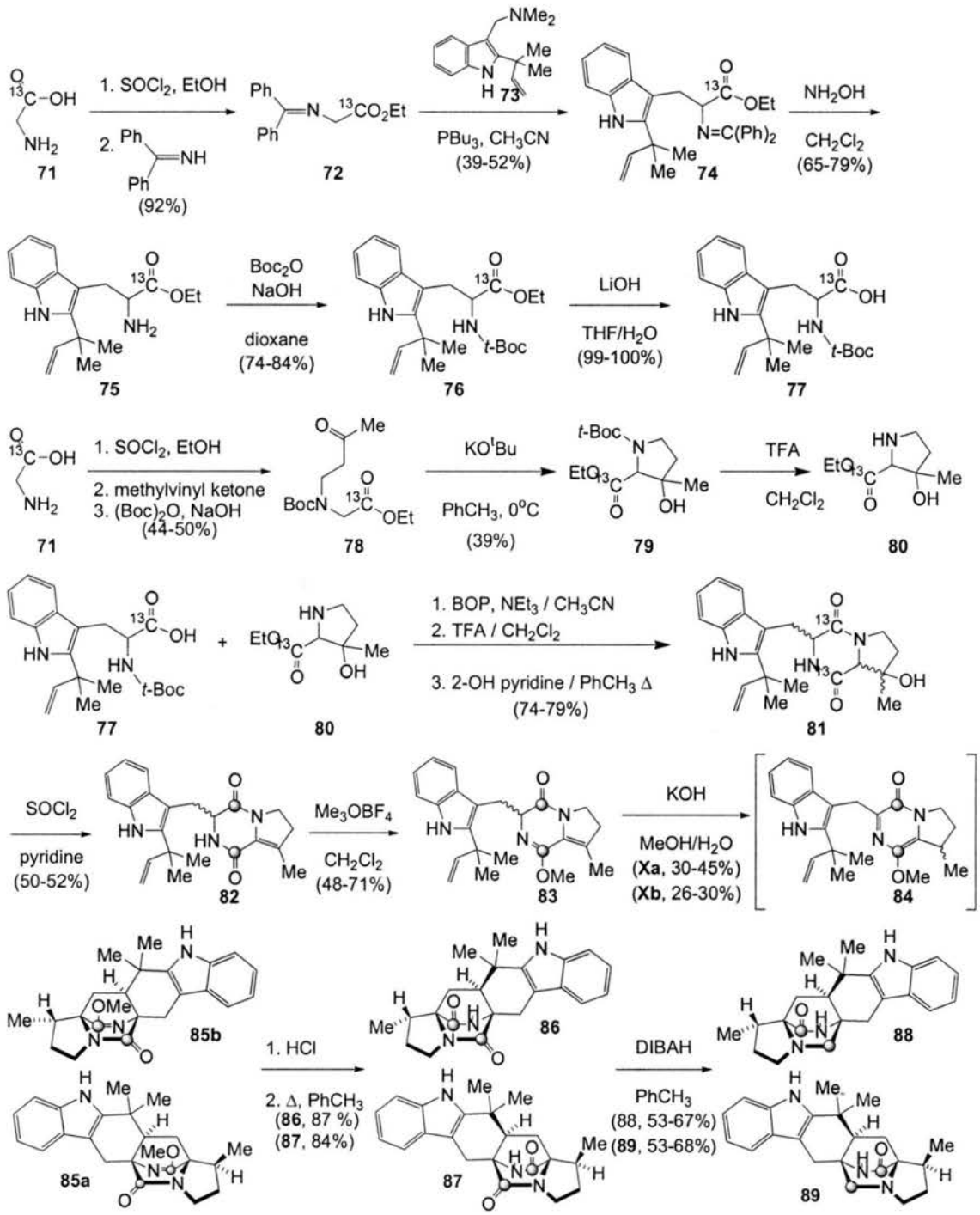


A unified biogenetic proposal for the aspeparalines and paraherquamides is shown in Scheme 13. Oxidative cyclization of isoleucine to provide β-methyl proline and coupling with tryptophan and DMAPP followed by a biosynthetic intramolecular [4+2] cycloaddition would provide the key hexacyclic substance **45.** Oxidation of the aromatic ring to the catechol derivative **70,** followed by prenylation, dioxepin formation and oxidation to the *spiro*-oxindole would yield paraherquamide. Interruption of the prenylation would provide a branch point wherein oxidative cleavage of four carbon atoms of the oxygenated aromatic ring could furnish the *spiro*-succinimde ring of asperparaline A.

To probe the intermediacy of cycloadduct **45** as a biosynthetic precursor, $^{13}C_2$-labeled cycloadduct was prepared according literature precedent[31] (Scheme 14). The synthesis begins with formation of the benzophenone imine of [1-^{13}C]-glycine ethyl ester (72). Somei-Kametani coupling of the glycinate with the gramine derivative **73** is followed by removal of the imine in the presence of ammonium hydroxide to reveal the free amine in 65-79% yields. The amine (75) is then protected as the *tert*-butyl carbamate (76) and saponified to yield the carboxylic acid (77) in quantitative yield. Synthesis of the tryptophan derivative (77) proceeded as reported. Removal of the imine however, was modified by subjecting the crude product of the Somei-Kametani coupling (74) to direct hydrolysis by stirring in an equal mixture of 1N HCl and

CH_2Cl_2. This procedure eliminated a difficult purification step and provided higher yields of the desired amine (75).

Scheme 14: Synthesis of [$^{13}C_2$]-*rac*-VM55599. ○ = ^{13}C



The β-methyl-β-hydroxy proline (80) is then prepared by Michael addition of [^{13}C]-glycine ethyl ester to methyl vinyl ketone followed by protection of the resulting amine as the *tert*-butyl carbamate using standard conditions yielding **78** in 44-50% over three steps. Dieckman cyclization with potassium *tert*-butoxide gives a 39% yield of the proline derivative (79) which is treated with trifluoroacetic acid to reveal the free amine (80). Unfortunately, I was unable to replicate this procedure. All attempts to remove the *tert*-butyl carbamate with TFA resulted in very poor mass recovery of the desired amine. Attempts to remove the carbamate under alternative conditions also proved unsuccessful. Ultimately, time constraints prevented the synthetic completion of $^{13}C_2$-labelled **88** and the corresponding feeding experiments. However, upon successful attainment of **80**, completion of $^{13}C_2$-labelled **88** would be expected to proceed as follows.

Peptide coupling of **77** and **80** with BOP reagent followed by removal of the carbamate as before and heating in the presence of 2-hydroxy pyridine results in the formation of the diketopiperazine (**81**). Dehydration is accomplished with thionyl chloride and treatment with trimethyloxonium tetrafluoroborate reveals 1-azadiene (**83**) which undergoes prototropic isomerization with potassium hydroxide in aqueous methanol to give the labile 2-azadiene (**84**) which suffers an intramolecular hetero-Diels-Alder cycloaddition. The resulting cycloadducts (**85a** and **85b**) can then be chromatographically separated and independently treated with aqueous acid to liberate the amides (**86** and **87**). Reduction of the tertiary amide with excess DIBAH would then provide racemic VM55599 (**88** and **89**) suitable for biosynthetic studies.

CHAPTER THREE

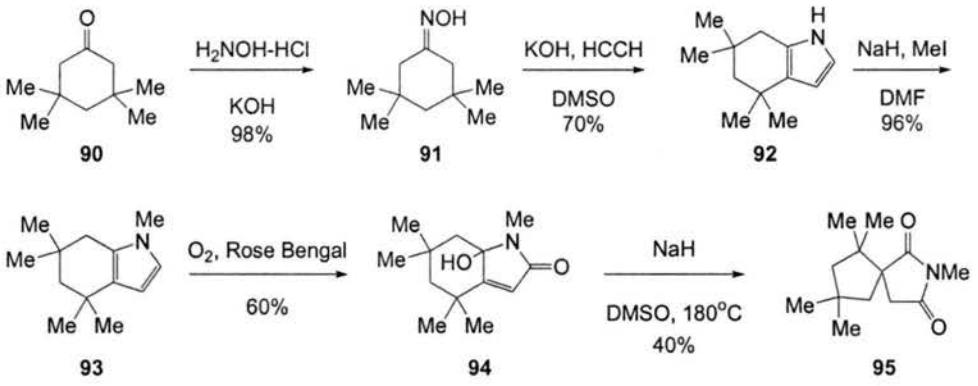
Synthetic Studies Toward the Asperparalines

3.0 Model Studies for the Formation of the *Spiro*-succinimide Ring

The *spiro*-succinimide ring common to the asperparalines has not been observed as constituting part of any other known natural products. Presently, two model studies have been reported for its construction.

The first, reported by Gonzalez and Williams[36] in 1999, utilizes a pinacol-type rearrangement of a hydroxypyrrolydinone (Scheme 15) to construct a *spiro*-succinimide ring.

Scheme 15: Williams' model of the *spiro*-succinimde formation.

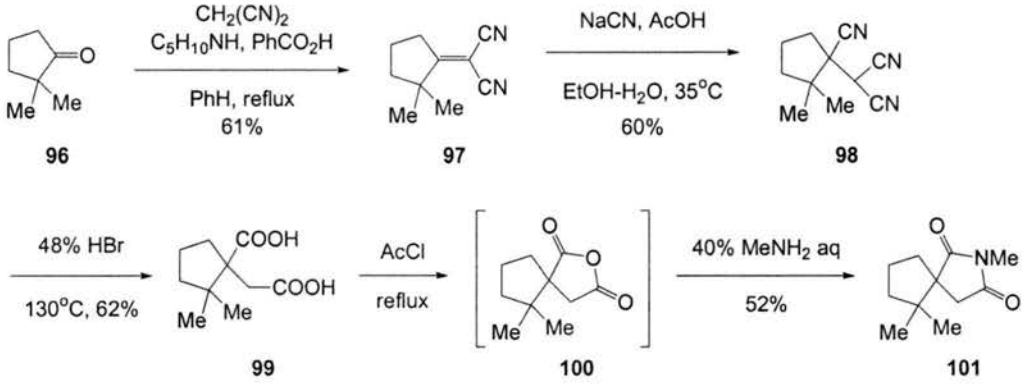


In this synthesis, the 2,3-disubstituted pyrrole compound **92** is formed by a Trofimov[37] reaction, wherein a keto-oxime is treated with acetylene under super-basic conditions. Following methylation of the pyrrole nitrogen, photooxidation, employing Rose Bengal as the photosensitizer, results in the formation of the hydroxypyrrolidinone (94). Upon treatment with NaH **94** suffers a pinacol-type rearrangement and provides the *spiro*-succinimde (95) in 40% yield.

The second model study for the formation of the *spiro*-succinimide ring was reported by Tanimori and co-workers[38] in 2000 and employs standard malonitrile chemistry (Scheme 16). Condensation of ketone **96** with malonitrile provides dinitrile **97**. This then undergoes Michael addition of cyanide anion to yield the trinitrile (98). Acid catalyzed hydrolysis accompanied by decarboxylation results in the formation

of acid **99**. *In situ* formation of the anhydride (100) is accomplished in refluxing acetyl chloride, which upon addition of aqueous $MeNH_2$ results in a 52% yield of the desired *spiro*-succinimide (101).

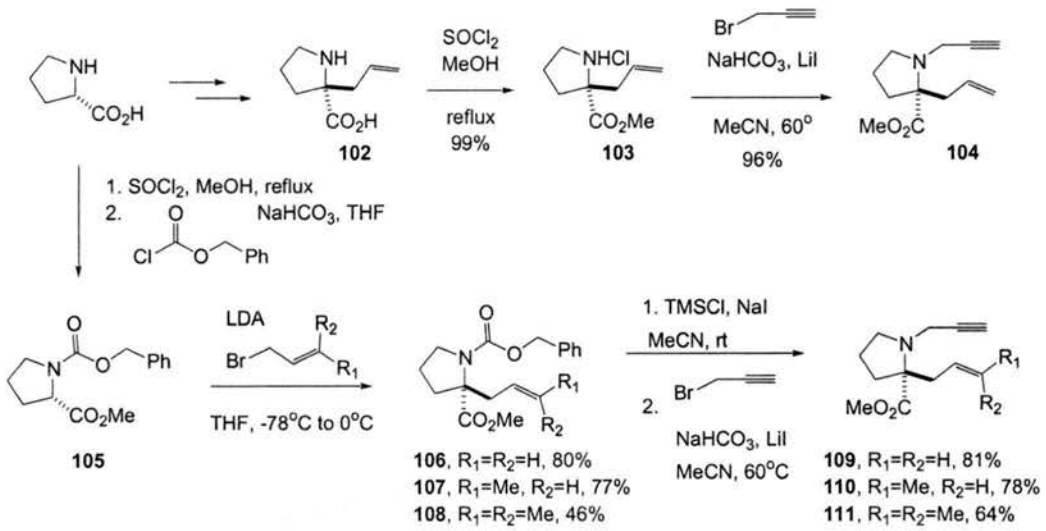
Scheme 16: Tanimori model for *spiro*-succinimide formation.



3.1 Tanimori synthesis of bicyclo[2.2.2]diazaoctane core

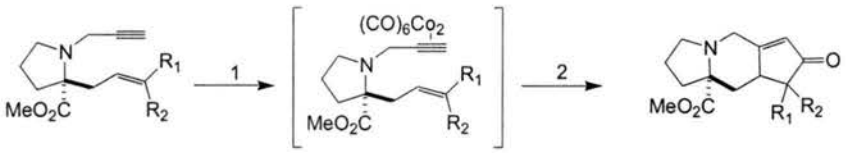
In 2001, Tanimori *et al.*[39] reported an approach for the formation of the bicyclo[2.2.2]diazaoctane core common to the asperparalines and paraherquamides which highlights the use of a [2+2+1] Pauson-Khand cycloaddition. The requisite Pauson-Khand enyne precursors (109-111) are prepared in a straightforward fashion by alkylation of proline (Scheme 17).

Scheme 17: Tanimori synthesis of Pauson-Khand precursors.



The [2+2+1] cycloaddition of simple enyne **109** proceeded in good yield using excess DMSO as a promoter (Scheme 18). Cycloaddition of enyne **110,** bearing a methyl-group at the terminus of the alkene, employing conditions suitable for the transformation of **109** resulted in poor yields of the desired cycloadduct. It was found, however, that replacing DMSO with NMO as the promoter improved the reaction yield nearly 2-fold resulting in a 4:1 mixture of diastereomers. Conditions could not be found which would affect the desired cycloaddition with dimethyl substituted enyne **111**. The authors propose that the steric bulk imposed by the *gem*-dimethyl substitution hinders the approach to the alkene by the alkyne-cobalt complex or destablilizes the transition state and results in decomposition of the intermediate.

Scheme 18: Tanimori Phauson-Khand cycloaddition.



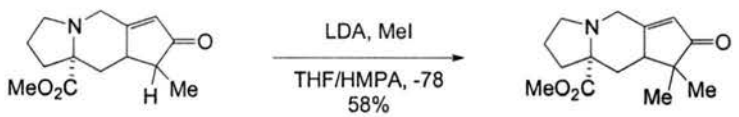
109, R_1=R_2=H
110, R_1=Me, R_2=H
111, R_1=R_2=Me

112, R_1=R_2=H, 94%[a]
113, R_1=Me, R_2=H, 61%[b]
114, R_1=R_2=Me, no reaction

a) conditions: 1) 1.05 eq $Co_2(CO)_6$, THF, Ar, rt, 2h, 2) 6 eq. DMSO, Ar, 50°C, 26h
b) conditions: 1) 1.0 eq $Co_2(CO)_6$, CH_2Cl_2Ar, rt, 2h, 2) 9 eq. NMO, Ar, rt, 22h

Since the requisite enone (114) for the synthesis of asperparaline was inaccessible via the Pauson-Khand reaction, it is synthesized from compound **113** by methylation with LDA and MeI to give a 58% yield of the dimethyl enone **114** (Scheme 19).

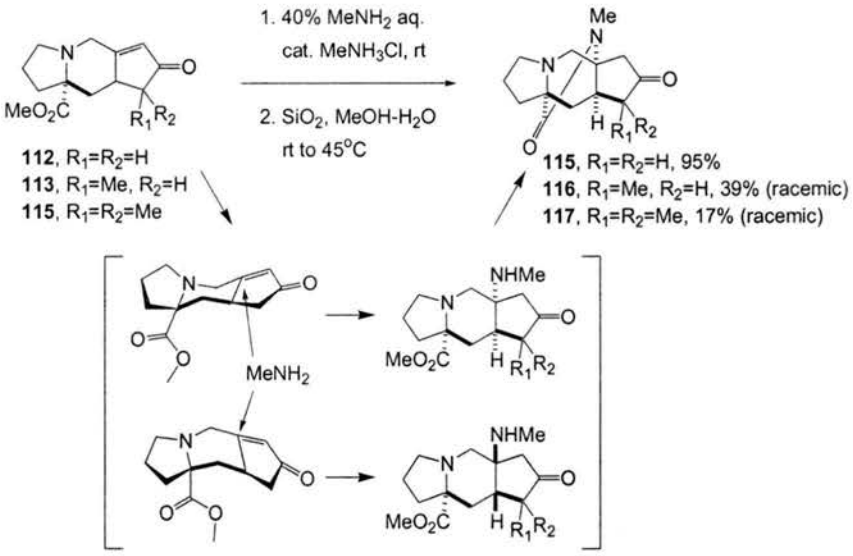
Scheme 19



113 **114**

Installation of the bridging lactam is achieved by Michael addition of $MeNH_2$ to the enone and treatment with silica-gel in aqueous methanol (Scheme 20).

Scheme 20: Tanimori formation of the bicyclo[2.2.2]diazaoctane core



In the absence of substitution *alpha* to the carbonyl, addition of methyl amine proceeds from the convex face resulting in a *cis*-relationship between the carbomethoxy group and methylamino substituent allowing for formation of the amide in 95% yield, since addition to the concave face would lead to an *anti*-relationship which would be unable to cyclize. Yields were observed to drop off considerably as substitution *alpha* to the carbonyl increased.

Presumably, synthesis of asperparaline C would be completed by applying the strategy of the model study shown in Scheme 16 to ketone 117 to install the *spiro*-succinimde ring, but no further reports have been issued to date.

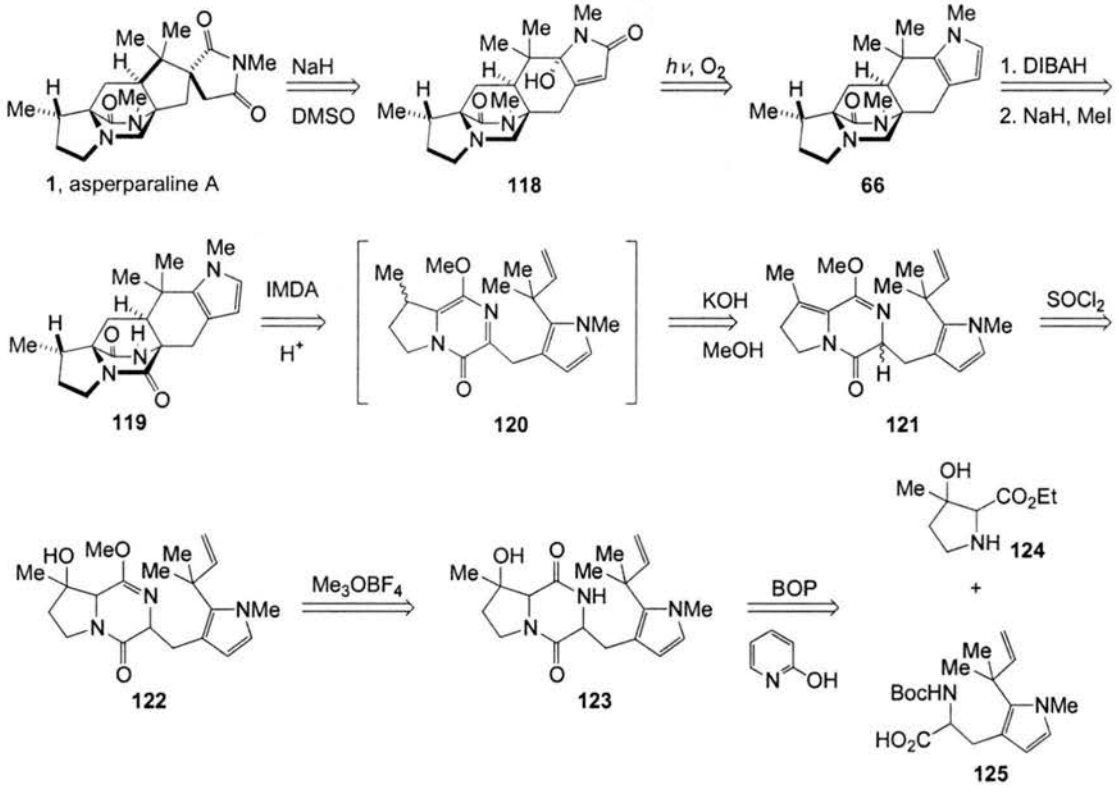
CHAPTER FOUR

First Generation Synthetic Approach

4.0 Retrosynthetic Analysis

The first generation retrosynthetic analysis was designed to mimic the biosynthetic proposal which envisioned the spirosuccinimide moiety being installed by an oxidative cyclization of a 2,3-disubstituted pyrrole (Scheme 21) as had been done in the model study by Williams and Gonzales[35].

Scheme 21: First generation retrosynthetic analysis.



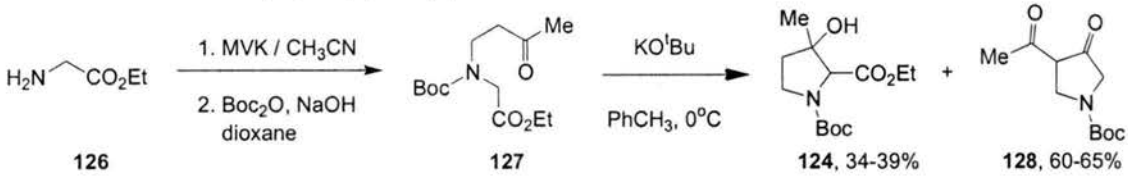
Under this plan, the *spiro*-succinimide ring of asperparaline A would be installed by a pinacol-type rearrangement of the hydroxypyrrolidinone (118) which would be prepared by a photooxidation of pyrrole **66**. An intramolecular hetero-Diels-Alder cycloaddition of the isoprene derived terminal olefin across the diketopiperazine (DKP) derived azadiene would yield cycloadduct **119**. The requisite 2-azadiene would be prepared by protropic isomerization of the 1-azadiene (121) prepared in two steps from the

diketopiperazine (123) by formation of the lactim ether followed by thionyl chloride mediated dehydration of the tertiary alcohol. Standard peptide coupling of β–hydroxy-β–methyl proline (124) with isoprenyl pyrrolylalanine (125) would provide DKP **123**.

4.1 Synthesis of β-hydroxy-β-methyl Proline

The β-hydroxy-β-methylproline (124) has been previously synthesized by Williams *et al.*[40] and involves a Diekmann cyclization of the N-Boc protected Michael addition product (127) of methyl vinyl ketone with glycine ethylester (Scheme 22).

Scheme 22: Formation of β-hydroxy-β-methyl proline.



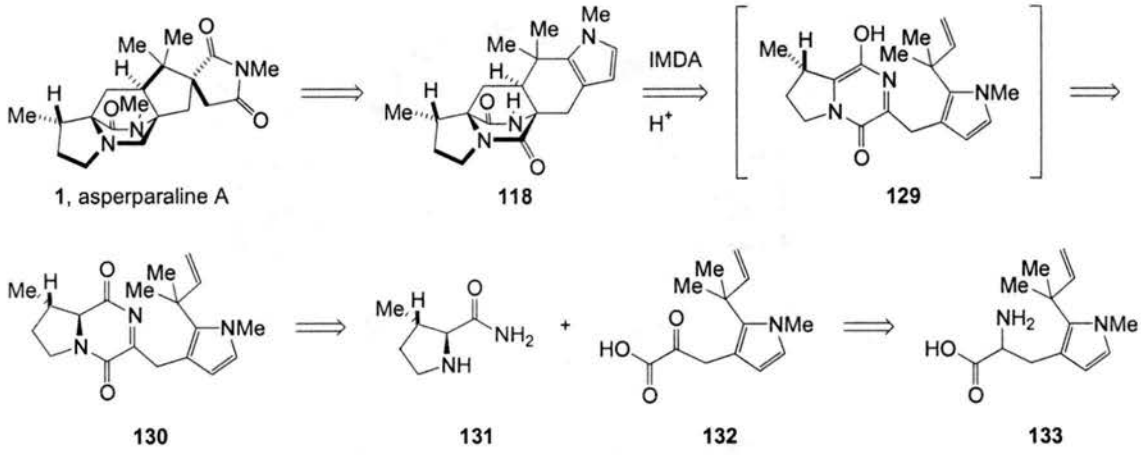
126 **127** **124**, 34-39% **128**, 60-65%

4.2 Model study for the synthesis of unsaturated diketopiperazines

One disadvantage of the synthetic plan outlined in the Scheme 21 is it necessarily results in a racemic synthesis of asperparaline A. The diketopiperazine must undergo a net two electron oxidation and enolization in order to construct the requisite 2-azadiene for the [4+2] cycloaddition. This transformation is affected by dehydration of the tertiary alcohol followed by formation of the lactim ether. As a consequence of the base mediated tautomerization the stereochemistry of the methyl substituent on the proline residue is racemized, thereby preventing synthesis of asperparaline A in an optically pure fashion. To circumvent this problem, it was reasoned that deliberate construction of an unsaturated diketopiperazine by coupling an α–keto acid with an amide would remove the need to perform the dehydration step that results in the undesired racemization (Scheme 23). This would allow for the use of optically pure β–methyl prolinamide, rather than β–hydroxy-β–methyl proline. Enolization and [4+2] cycloaddition would furnish the putative hexacyclic intermediate **118** and the remainder of the synthesis would proceed essentially unchanged. The

α–keto acid would be furnished by an oxidative deamination of amino acid **133** to allow for maximum flexibility in the overall synthetic plan and this transformation was investigated with a model system.

Scheme 23: Non-racemic retrosynthesis of asperparaline A.

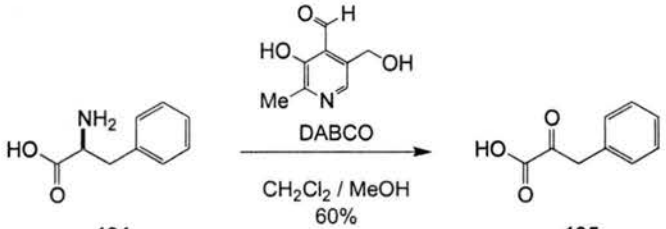


1, asperparaline A **118** **129**

130 **131** **132** **133**

Combining the procedures of Rapoport[41] and Martell[42], a method was devised for the oxidative deamination of pyrrolylalanine (133) using phenylalanine as a model substrate. In a one-pot, two-step process phenylalanine is biomimetically converted to the α–keto acid (135) by initial formation of the imine with pyridoxal, the synthetic equivalent of pyridoxal mono-phosphate (PMP), followed by treatment with [2.2.2]-diazabicyclooctane (DABCO) which effects the prototropic isomerization, upon workup the desired α–keto acid is obtained in 60% yield (Scheme 24).

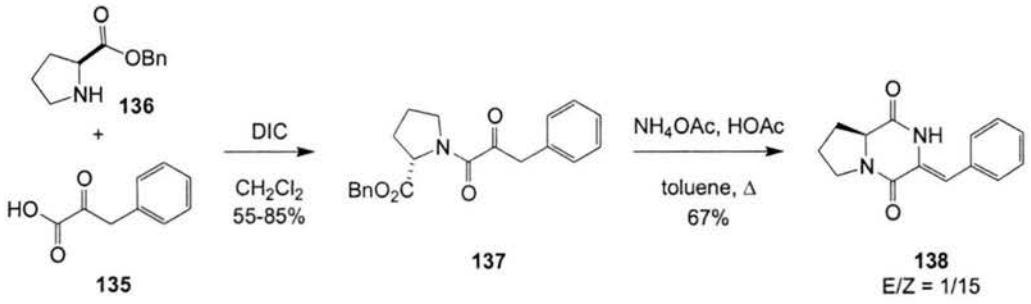
Scheme 24: Oxidative deamination model study.



134 **135**

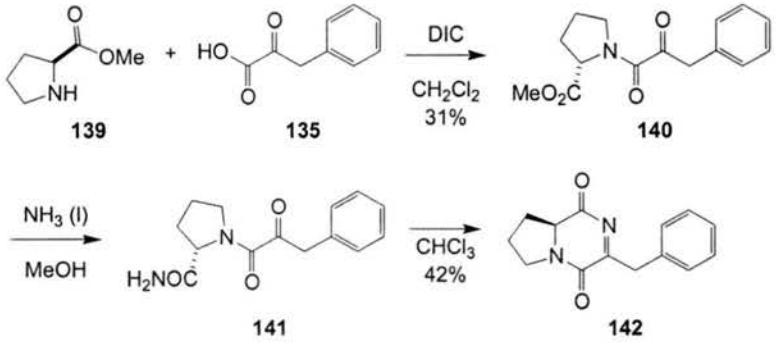
In 1998, Li and Peng[43] reported the synthesis of unsaturated diketopiperazine **138** by coupling proline benzyl ester (136) with phenylpyruvic acid (135) and treating the resulting α–keto amide **137** with ammonium acetate. This results in the *in-situ* formation of the amide from the ester which undergoes cyclodehydration to give unsaturated diketopiperazine **138** as a 15:1 mixture of Z to E isomers in 67% yield (Scheme 25).

Scheme 25: Li and Peng[42] synthesis of an unsaturated diketopiperazine.



Since the desired unsaturated DKP was envisioned as containing the double bond *within* the piperazine ring, conditions were sought which would allow for the cyclodehydration step to occur under milder conditions since **138** is believed to be the thermodynamic product resulting from tautomerization under the harsh conditions employed by Li and Peng. Coupling proline methyl ester with phenyl pyruvic acid furnished the α–keto amide in an un-optimized yield of 31% (Scheme 26). Treatment of ester **140** with methanolic ammonia afforded the amide (141) that underwent cyclodehydration to the desired unsaturated DKP upon stirring in an aprotic solvent. Additionally, compound **142** was observed to gradually tautomerize to **138** upon standing at room temperature.

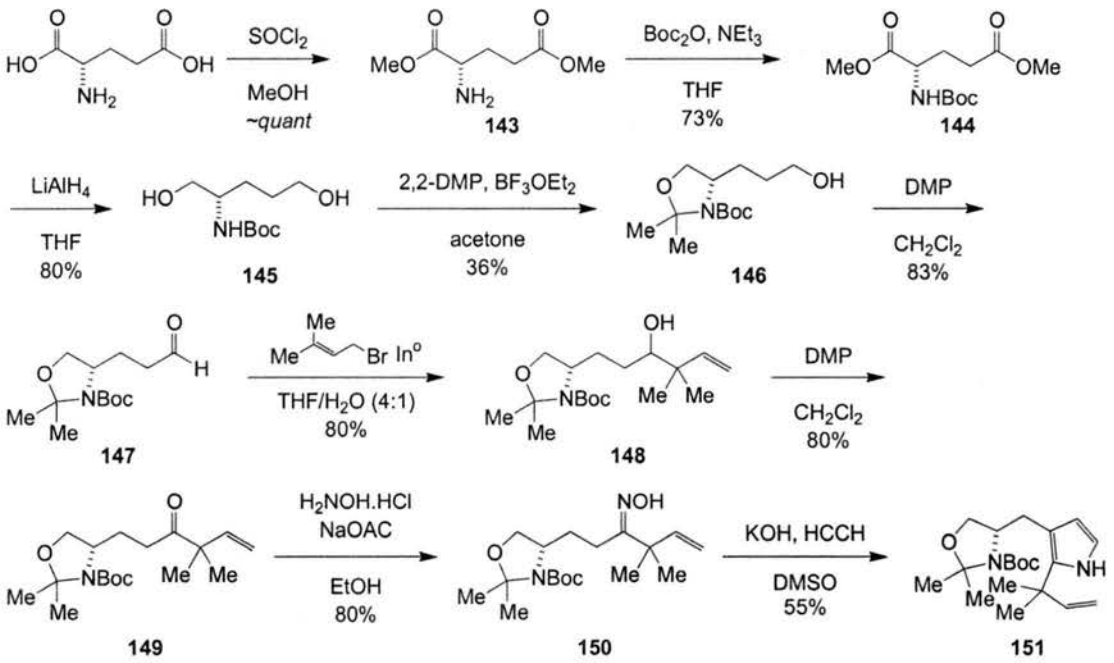
Scheme 26: Modified synthesis of a model unsaturated diketopiperazine.



4.3 Synthetic Efforts Toward Isoprenyl Pyrrolylalanine

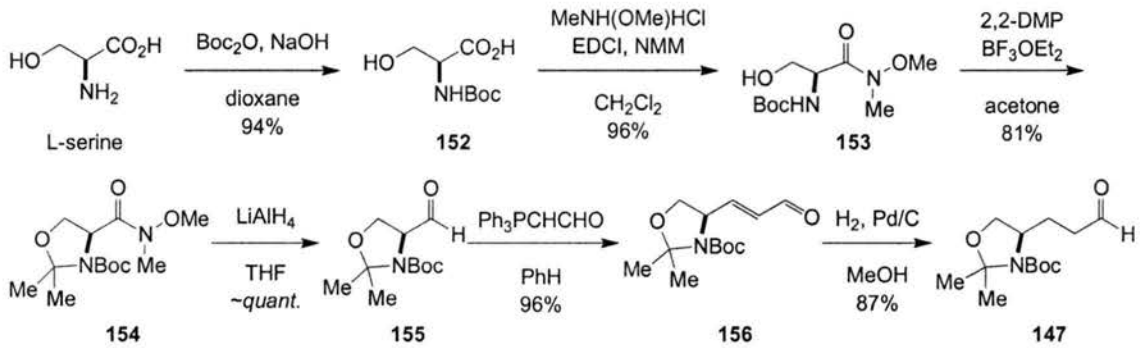
Initial efforts toward the synthesis of the isoprenyl pyrrolylalanine (125) began with the esterification of commercially available L-glutamic acid (Scheme 27), followed by protection of the amine with di-*tert*-butyl dicarbonate following a procedure outlined by Avenoza *et al.*[44] Reduction of the N-Boc-diester to aminodiol **145** proceeded cleanly with $LiAlH_4$. Formation of oxazolidine **146** with 2,2-dimethoxypropane and a catalytic amount of $BF_3{\bullet}OEt_2$ proved to be somewhat capricious, often yielding multiple products and inconsistent yields. Attempts to improve this reaction using a variety of techniques and conditions did not improve the yields nor minimize the formation of side products. Oxidation of the primary alcohol to aldehyde **147** proceeded neatly with Dess-Martin periodinane. The homoallylic ketone (149) was formed by the indium mediated allylation of aldehyde **147** to alcohol **148** using a modified procedure reported by Chan[45] with subsequent oxidation to the ketone. Formation of pyrrole **151** using conditions outlined by Trofimov[46] required that the ketone be converted to the oxime **150**. The Trofimov reaction produced the pyrrole in moderate yields.

Scheme 27: Glutamate route to isoprenyl pyrrolylalanine.



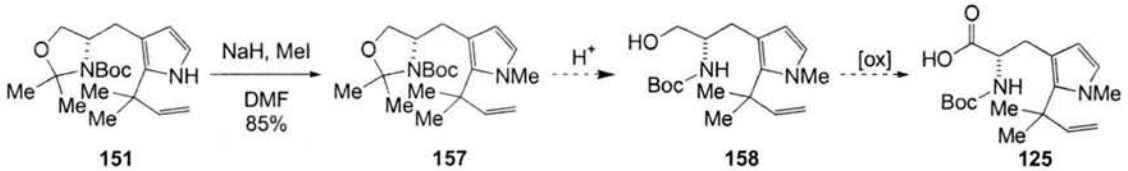
Due to the low yielding oxazolidine forming step and the difficulty purifying aminodiol **145**, another method was sought for the formation of aldehyde **147**. The method that was chosen involves use of the Garner aldehyde as prepared by Taylor *et al.*[47] (Scheme 28). In this synthesis, the N-Boc-L-serine was converted to the Weinreb amide (153) followed by formation of the oxazolidine with 2,2-DMP and BF_3OEt_2 and subsequent reduction with $LiAlH_4$ to give the Garner aldehyde (155) in 72% yield over 4 steps. The aldehyde was homologated using a Wittig reaction followed by hydrogenolysis to give aldehyde **147**. The two major advantages of this synthetic route are an increased yield of 60% over six steps (compared to ~20% over five steps) and minimal purification. Synthesis of the pyrrole from the aldehyde proceeded as previously described (Scheme 27).

Scheme 28: Improved Garner aldehyde route to key aldehyde **147.**



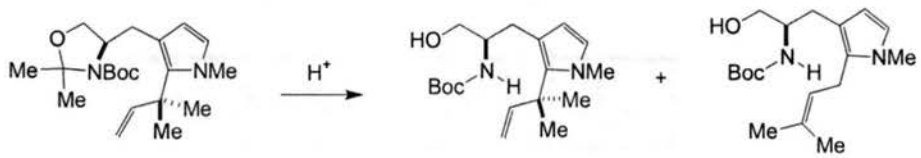
Completion of the amino acid synthon was anticipated to occur in the following fashion (Scheme 29). Methylation of the pyrrole nitrogen followed by opening of the oxizolidine to the amino alcohol (158) and oxidation to the carboxylic acid would provide the novel amino acid needed for a biomimetic synthesis of asperparaline A.

Scheme 29: Proposed completion of isoprenyl pyrrolylalanine.



Oxizolidines are ring opened to the corresponding amino alcohols using conditions similar to those needed for opening acetals and ketals. While methylation proceeded as expected, the acidic conditions required for revealing the amino alcohol produced unexpected results. During the ring opening of oxazolidine **157** two products were produced (Scheme 30).

Scheme 30: Acid catalysed ring opening of oxazolidine **157**.

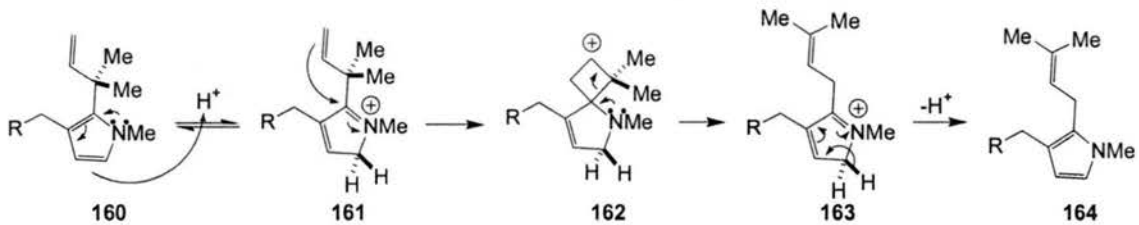


157 **158** **159**

Regardless of conditions the desired alcohol **158** was always obtained with varying quantities of alcohol **159** bearing the rearranged isoprene. This result is attributed to the basicity of pyrrole ring in the starting material. Electron rich pyrroles are known for their propensity to protonate under even very mild conditions. Rather than protonating at nitrogen as might be expected, they are observed to protonate at the 2-position[48].

Scheme **31** proposes a mechanism for the observed products. Protonation of the pyrrole gives iminium species **161**. Nucleophilic attack on the iminium by the terminal olefin results in a cyclobutyl cation (162) which then suffers retrocyclic ring opening to the more thermodynamically stable trisubstituted alkene (**164**).

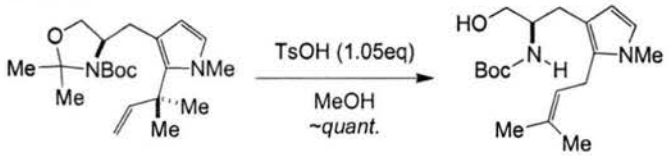
Scheme 31: Proposed mechanism for the observed isoprene rearrangement.



160 **161** **162** **163** **164**

This supposition is supported by the observation that treatment of oxizolidine **157** with stoichiometric TsOH results in the quantitative recovery of trisubstituted olefin **159** (Scheme 32).

Scheme 32



157 **159**

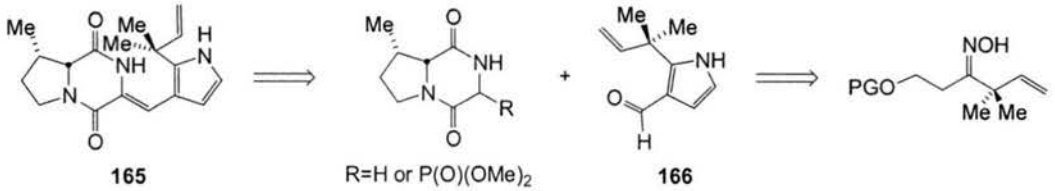
Attempts to improve the reaction conditions to minimize formation of **159** were casually explored while conditions were examined for oxidizing the primary alcohol of **158** to a carboxylic acid functionality.

Oxidations in the presence of pyrroles tend to be problematic as pyrroles are highly susceptible to oxidation. Most of the conditions attempted resulted in complex product mixtures with no detectable trace of desired product. Dess-Martin periodinane was a promising oxidant. Under typical conditions (0.1M concentration at room temperature) trace amounts of the desired aldehyde could be isolated, however, isomerization of the isoprene continued to be a problem. At low temperature and high dilution the reaction was appeared to proceed "spot to spot" by thin layer chromatography (TLC), but upon warming or concentration decomposition into complex mixtures was observed, preventing full characterization of the resulting product and conditions which would provide the desired amino acid (125) were not found.

4.4 Aldol approach to unsaturated diketopiperazines

An alternative for the direct formation of the putative intermediate **165** would be to do an aldol dehydration or olefination between pyrrolaldehyde **166** and a suitably substituted diketopiperazine (Scheme 33). This would obviate the difficult oxidation step but still allow for the synthesis of optically pure asperparaline A by way of the unsaturated diketopiperazine. The formypyrrole (166) would be furnished by a Trofimov reaction of a suitably protected ketoxime.

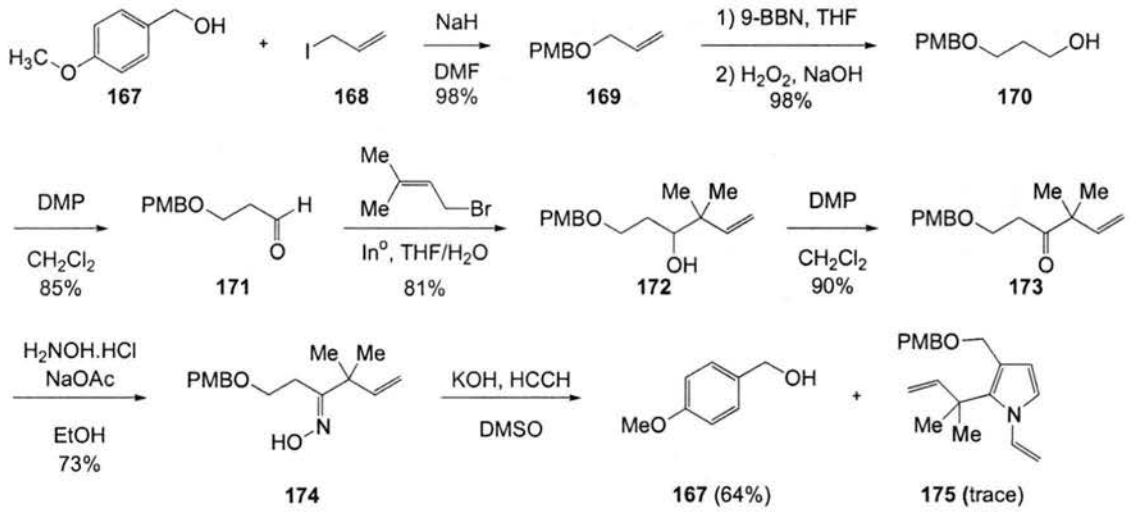
Scheme 33: Retrosynthesis of putative unsaturated diketopiperazine via pyrrolaldehyde.



165 R=H or $P(O)(OMe)_2$ **166**

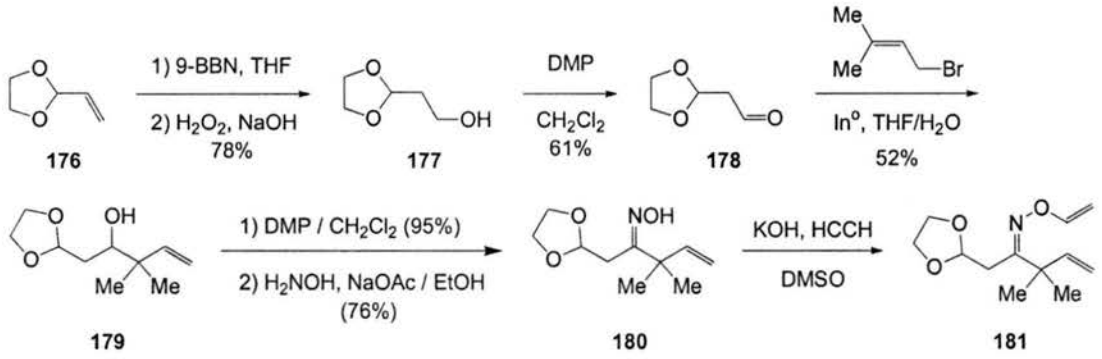
Ketoxime **174** was prepared from commercially available allyliodide (168) and *para*-methoxybenzyl alcohol (167) (Scheme 34). Hydroboration / oxidation provided primary alcohol **170** and Dess-Martin oxidation furnished the aldehyde (171) which underwent indium-mediated allylation to yield the homo-allylic alcohol (172) in 81% yield. Finally, oxidation and conversion to the oxime under standard conditions gave the requisite precurson for the Trofimov reaction. However, only a trace amount of a pyrrole product was isolated, the major product isolated was *para*-methoxybenzyl alcohol (167), suggesting that under the super basic reaction conditions, elimination was competing with pyrrole formation.

Scheme 34: Trofimov reaction of ketoxime bearing PMB ether.



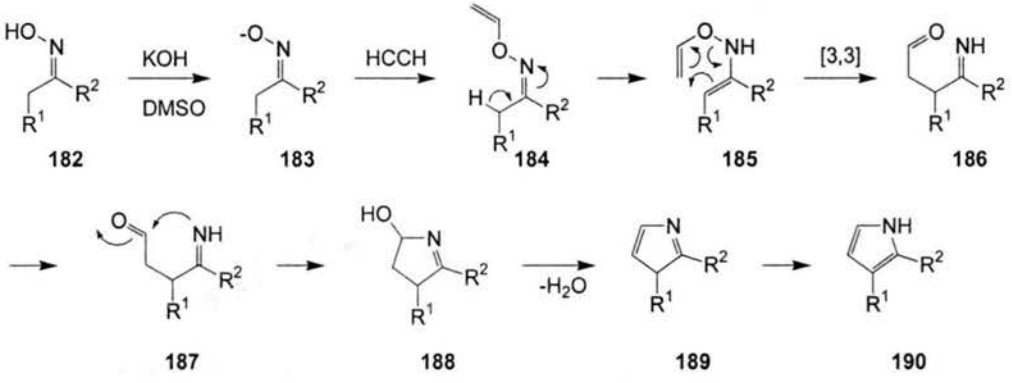
In a second generation approach to the formylpyrrole, the ketoxime was prepared with the aldehyde masked as an acetal (Scheme 35). Using the same chemistry as described above, ketoxime **180** was prepared from commercially available vinyl dioxolane (181). With this substrate, however, the Trofmov failed to produce any pyrrole product at all only the O-vinyl oxime (181) was isolated. Subjecting the isolated O-vinyl oxime to higher pressures and temperatures to effect pyrrole formation only resulted in the recovery of starting material.

Scheme 35: Trofimov reaction with ketoxime bearing acetal.



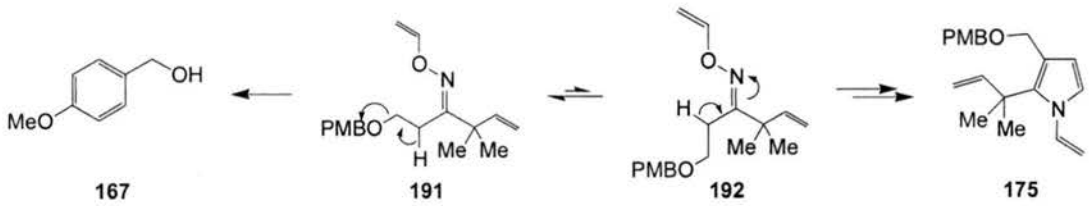
According to the proposed mechanism for the Trofimov reaction (Scheme 36)[49], an O-vinyl oxime would be an expected intermediate in the formation of the pyrrole. Deprotonation of the oxime followed by alkylation with acetylene would yield the O-vinyl oxime (184). Tautomerization to the E-enamine (185) is followed by a [3,3]-sigmatropic rearrangement which furnishes the imino-aldehyde (186). This compound suffers intramolecular addition, followed by dehydration and tautomerization to the 2,3-disubstituted pyrrole (190).

Scheme 36: Proposed mechanism of the Trofimov reaction[48].



Careful inspection of the mechanism with respect to both **174** and **180** aids in explaining the failings of each of these compounds as substrates for the Trofimov reaction. Isolation of *para*-methoxybenzyl alcohol suggests that the O-vinyl oxime **191** suffers from competitive elimination following the alkylation step but preceeding isomerization to the necessary enamine (192) (Scheme 37).

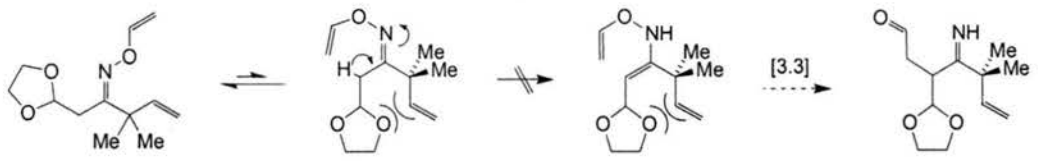
Scheme 37: Proposed mechanistic explanation for Trofimov reaction of ketoxime bearing PMB ether.



167 **191** **192** **175**

With respect to the ketoxime bearing the acetal, isolation of the O-vinyl oxime (181) and application of the proposed mechanism suggests that **181** is inhibited from adopting the necessary conformation to isomerize to the enamine by a steric interaction between the dimethylallyl substituent and the acetal moiety (Scheme 38). As a result of this steric impediment the reaction fails to undego the sigmatropic rearrangement to the imino aldehyde (194), thereby preventing formation of the pyrrole and resulting in the accumulation of O-vinyl oxime.

Scheme 38: Proposed mechanistic explanation for Trofimov reaction of ketoxime bearing acetal.



181 **193** **193** **194**

In order to prepare the formyl pyrrole, the protecting group used to mask either the alcohol or the aldehyde had to meet stringent requirements. It must tolerate the harsh alkaline conditions of the Trofimov reaction. It had to be small enough not to impose steric restrictions on the isomerzation of the imine to the enamine. It was also required that it could be removed under mild conditions that would not react with either the pyrrole ring or the isoprenyl side chain. Due to these stringent conditions this route to unsaturated diketopiperazines was not pursued further because finding a protecting group for the alcohol or aldehyde that would satisfy all of these requirements seemed unlikely. As a result, attention was refocused on a means of salvaging the synthetic route toward the isoprenyl pyrrolylalanine (125).

4.5 Salvage Attempts

Attainment of the isoprenyl pyrrolylalanine was tantalizingly close, but there were problems in need of being addressed. Overcoming the problem of performing the oxidation in the presence of the pyrrole was foremost, followed by the need to suppress the rearrangement of the isoprene side chain. The first and most obvious approach involved reducing the basicity of the pyrrole ring by introducing an electron withdrawing substituent onto the pyrrole nitrogen. This would be expected to reduce the propensity for the isoprene rearrangement and perhaps increase the range of possibilities for performing the oxidation. Also considered was the deliberate oxidation of the pyrrole to a less reactive, yet synthetically useful, functionality. Another option to be explored was protection of the terminal olefin, thereby preventing the isomerization and allowing for other options to be pursued for coupling the proline portion.

4.5.1 Attempts to protect pyrrole nitrogen with electron withdrawing substituent

Introduction of an electron withdrawing substituent at the pyrrole nitrogen was an obvious first choice as a means to overcome the difficulties of preparing the isoprenyl pyrrolylalanine. An electron withdrawing functionality would be expected to lower the basicity of the pyrrole ring thereby reducing its propensity to protonate and undergo isoprenyl rearrangement during the deprotection of the amino alcohol. Also, it was presumed that the reason for the instability of the aldehyde resulting from Dess-Martin oxidation of the alcohol was due to attack of the aldehyde by the pyrrole resulting in both inter- and intra-molecular addition products. Lowering the nucleophilicity of the pyrrole nucleus would perhaps allow for oxidation of the alcohol to the desired oxidation state without suffering inter- or intra-molecular addition or permit direct oxidation to the carboxylic acid which was expected to be less reactive. Unfortunately, conditions were not found which would allow for the installation of any desired functionality onto the pyrrole nitrogen (Table 3).

The steric influence exerted by the *gem*-dimethyl group of the isoprenyl side chain seems to be responsible for the observed results. In the case of alkylation with sodium hydride and methyl iodide, deprotonation was accompanied by a color change, which upon addition of the alkylating agent would disappear. In each of the attempts to introduce the desired protecting group, a similar change in solution color was observed indicating that deprotonation had likely occurred, however, only starting material was

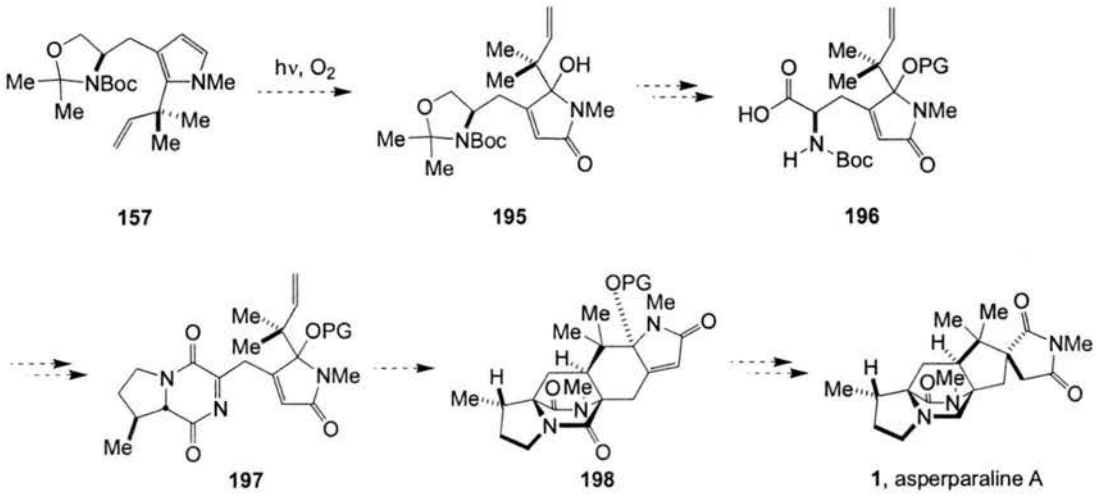
recovered from the reactions. It was possible to use the un-alkylated pyrrole in the following sequence but this was not an effective solution for suppressing the isoprene rearrangement or aldehyde decomposition.

Acylating Agent	Base	Solvent
TrocCl	NaH	DMF
TrocCl	NaOH	CH_2Cl_2
TFAA	NEt_3	CH_2Cl_2
$PhSO_2Cl$	KH, imidazole	DMF
$PhSO_2Cl$	NaH	DMF
EtO_2CCl	NEt_3	CH_2Cl_2
CH_3CO_2CHO	-	THF
MsCl	NaH	DMF
CH_3COCl	Pyridine, DMAP	THF
CH_3COCl	DIPEA, DMAP	THF
CH_3COCl	NaH	DMF
CH_3COCl	pyridine	DMF

4.5.2 Deliberate pyrrole oxidation

One option for dealing with the reactivity of the electron rich, 2,3-disubstituted pyrrole was deliberately oxidizing it to the hydroxypyrrolidinone. This functionality was expected to be less reactive than the analogous pyrrole and would be a synthetically useful alternative. Photooxidation of the pyrrole to the hydroxypyrrolidinone would precede the opening of the oxazolidine and the resulting functionality would be protected and carried through the synthesis as planned for the pyrrole moiety.

Scheme 39: Proposed hydroxypyrrolidinone route to asperparaline A.

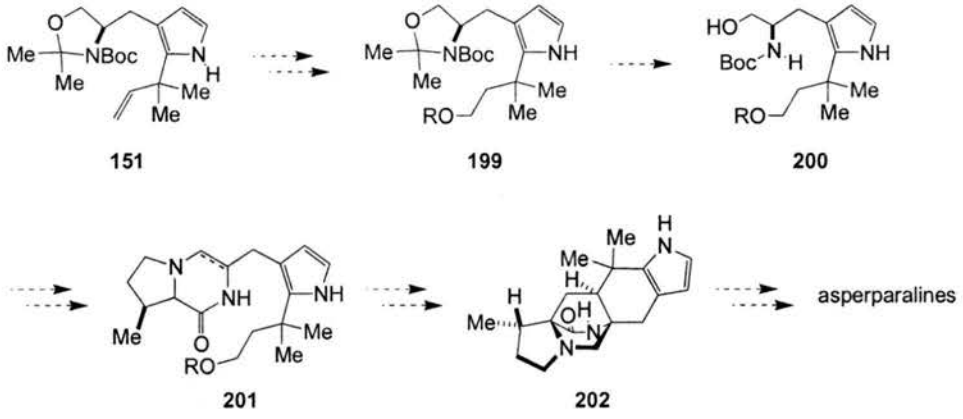


The photooxidation conditions employed in the model study (Scheme 15) were tested but the resulting product mixtures contained no evidence of the desired product. ^{1}H NMR of the oxidation products so produced indicated probable oxidation of the pyrrole accompanied by either oxidation of and/or reactions with the terminal olefin.

4.5.3 Protection of the terminal alkene

Protection of the terminal olefin provided another option for overcoming the difficulties associated with working with pyrrole **151**. By suppressing the isoprene rearrangement, larger quantities of the amino alcohol could be accessed and allowed for the alternative pursuit of the piperazine (Scheme 40). Since asperparaline bears a piperazinone ring rather than a piperazindione ring, it seemed possible to construct asperparaline without needing to fully oxidize the alcohol. Oxidation to the aldehyde followed by coupling with proline might allow for a similar intramolecular hetero-Diels-Alder cycloaddition. The resulting cycloadduct could then be converted to asperparaline using previously proposed chemistry.

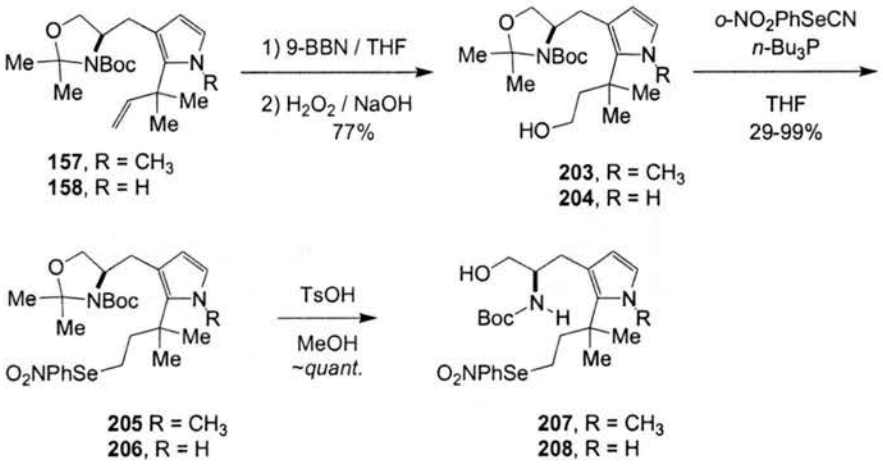
Scheme 40: Proposed alkene protection route to the asperparalines.



Hydroboration / oxidation of alkene **157** gave primary alcohol **203** (Scheme 41) which was then treated with *o*-nitrophenyl selenium cyanide and tri-*n*-butyl phosphine to produce the aryl selenide which could later be selectively oxidized to the alkene. Treatment of the oxazolidine (205) with catalytic TsOH resulted in a quantitative recovery of the primary alcohol (207). Pyrrole **151** was also subjected to the same conditions for protection of the terminal olefin with comparable results. Oxidation of the alcohol was

expected to suffer concomitant inter- and/or intra-molecular attack as observed previously and was not attempted.

Scheme 41: Protection of termainal alkene.



157, R = CH_3
158, R = H

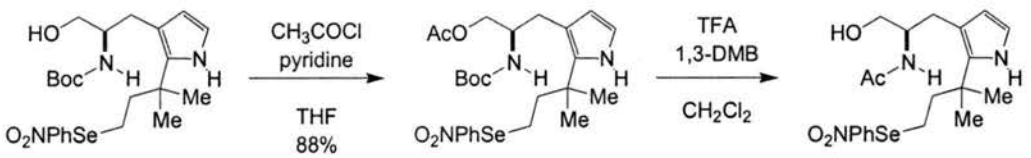
203, R = CH_3
204, R = H

205 R = CH_3
206, R = H

207, R = CH_3
208, R = H

Efforts to couple the proline portion of the molecule with the pyrrole portion first required protection of the primary alcohol followed by removal of the *tert*-butyl (Boc) carbamate. Introduction of the acetate was accomplished with acetyl chloride and pyridine to give an 88% yield of the ester (209), but upon removal of the Boc group acyl transfer was observed and resulted in irreversible formation of amide **210** (Scheme 42).

Scheme 42: Acylation and acyl transfer.



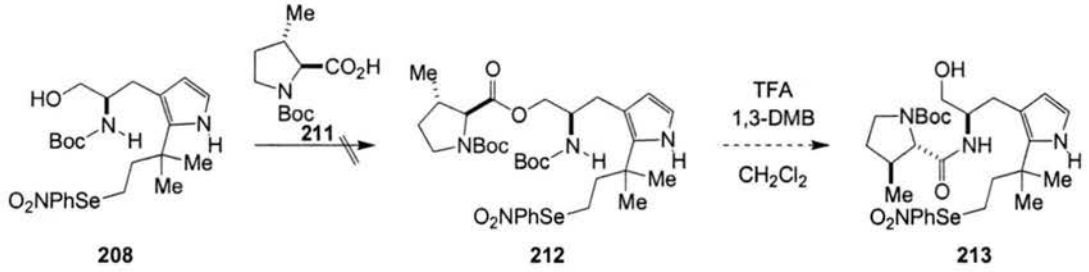
208 **209** **210**

Introduction of a protecting group that would not suffer acyl migration and be compatible with the conditions necessary for removal of the Boc group were not found. The *tert*-butyl carbamate evidently introduced steric congestion in the area of the primary alcohol and no reaction was observed when trying to install silyl ethers. Conditions for the formation of simple ethers (eg. -Me, -MOM) resulted in the

formation of multiple products or no reaction. While conditions for the conversion of the alcohol to a halide ($SOCl_2$/pyr; $SOCl_2$ / MeOH; PPh_3, CBr_4 / DMF; PPh_3, NBS), which could be directly coupled to the proline, resulted in halogenation of the pyrrole.

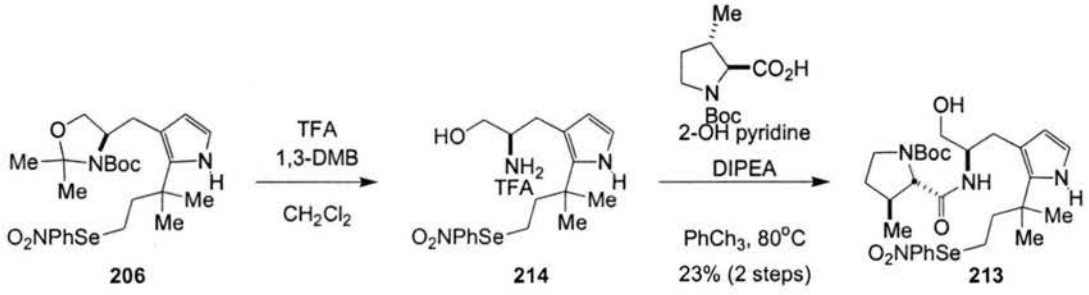
Since TFA affected acyl transfer of the acetate protecting group, a similar reactivity was probed with prolyl ester **212**. Again the *tert*-butyl carbamate exerted considerable steric influence and coupling

Scheme 43: Attempted esterification and acyl transfer with β-methyl proline



was not possible. However, this problem could be addressed by first treating the oxazolidine with TFA, resulting in removal of the Boc group accompanied by deprotection of the amino alcohol (Scheme 44). Direct coupling was then possible in modest yield by coupling alcohol **214** with β-methyl proline in the presence of 2-hydroxy pyridine and Hunig's base at elevated temperature. However, attempts to oxidize the resulting alcohol (213) to the aldehyde resulted in no reaction.

Scheme 44: Successful proline amide formation.



4.6 Conclusions

It is important to note that despite the usefulness of the Trofimov reaction for the synthesis of 2,3-disubstituted pyrroles, the reaction yields seemed to be largely affected by the scale of the reaction. Product yields in the 60-70% range were only observed when the reaction was done with less than 250mg of oxime **150**, increasing the scale of the reaction resulted in product yields in the range of 30-40%. The scale limitations of the Trofimov reaction coupled with the variable yields obtained in formation of the aryl selenide greatly hindered pursuing the olefin protection route at length.

Ultimately, introducing the pyrrole moiety so early in the synthesis proved to be a liability. Its sensitivity to oxidation limited the possibilities for final oxidation of the amino alcohol to the needed amino acid. The irreversible rearrangement of the isoprene side chain to the thermodynamically more stable tri-substituted alkene was an unexpected result of the basicity of di-alkyl substituted pyrrole. Inability to deactivate this reactivity by introduction of an electron-withdrawing substituent onto the pyrrole nitrogen by reason of the steric influence of the *gem*-dimethyl allyl chain *alpha* to the nitrogen further limited the usefulness of this route as a means of synthesizing asperparaline A. While it was possible to suppress the rearrangement of the isoprene moiety by conversion of the alkene to the aryl selenide, this process added four additional steps for the purpose of performing what would be, in the absence of the pyrrole ring, a rather elementary oxidation. Further, there is no guarantee that the oxidative conditions necessary for reinstalling the alkene would have been tolerated by the pyrrole, though given the limited success in coupling the proline residue it does perhaps merit further investigation. It is, however, doubtful that the conditions required for the dehydration ($SOCl_2$/pyridine) and formation of the lactim ether (Me_3OBF_4, Cs_2CO_3) would have given satisfactory results (Scheme 21).

Each of the synthetic problems encountered in the originally proposed, biomimetic route to asperparaline A was a result of the reactivity of the 2,3-di-substituted pyrrole and it was ultimately determine that a better course of action would be to introduce the pyrrole immediately prior to its conversion to the hydroxypyrrolidinone, rather than amend the synthesis at every step to its strict requirements. Furthermore, biosynthetic studies had, by this time, ruled out the probability of the *spiro*-succinimde ring as being biogenetically derived from the oxidative *spiro*-cyclization of a pyrrole. As such,

a synthesis of asperparaline A which involved the use of a pyrrole would not be biomimetic in any case and the stage at which it was to be introduced was no longer relevant.

CHAPTER FIVE

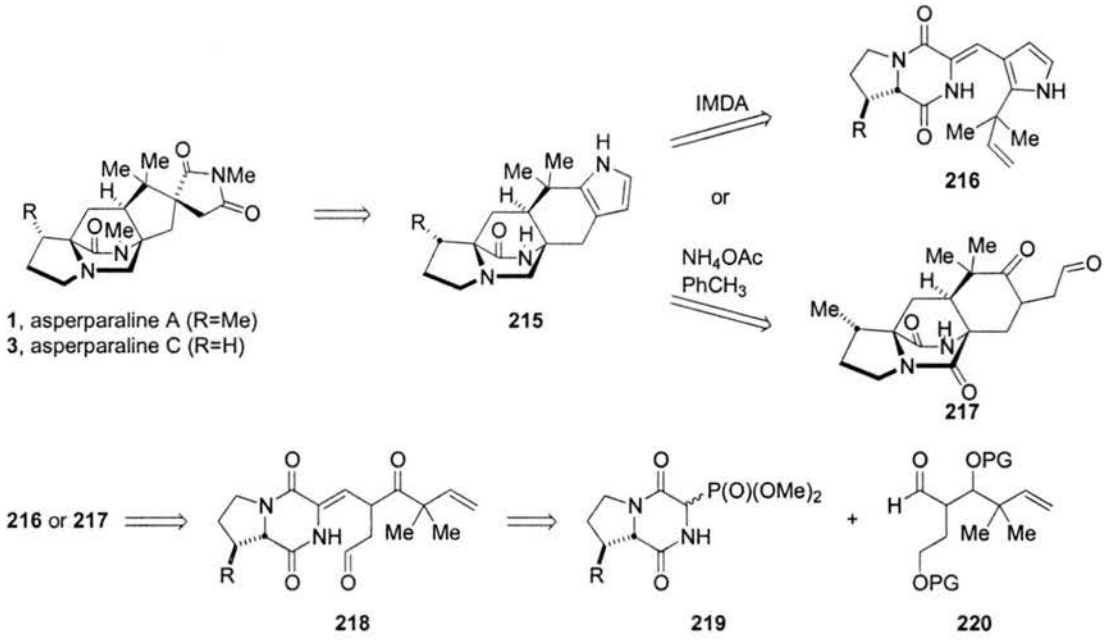
Second Generation Synthetic Approach

The revised approach to asperparaline A envisioned a late stage installation of the pyrrole functionality. Installing the pyrrole nucleus just prior to its oxidation to the hydroxypyrolidinone was expected to alleviate the difficulties in working with a pyrrole through multiple manipulations. The new approach is also consistent with the preexisting methodology for the construction of a *spiro*-succinimide ring from a 2,3-disubstituted pyrrole.

5.0 Retrosynthesis – First Generation Approach

The first approach for a synthesis of asperparaline A that would use a late stage installation of the pyrrole ring is given in Scheme 45. Again the *spiro*-succinimide ring would be derived from a pyrrole via photooxidation and a pinacol-type *spiro*-cyclization.

Scheme 45: Retrosynthetic Analysis with aldehyde containing all carbons present in core framework of asperparalines.

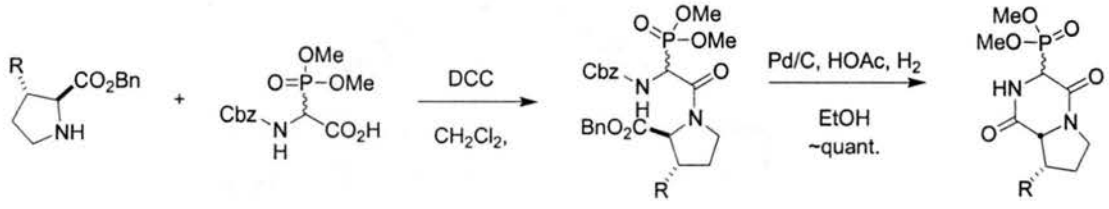


The putative hexacyclic intermediate **215** could be fashioned from either an intramolecular hetero-Diels-Alder cycloaddition of unsaturated DKP **216** or via ammonium acetate mediated pyrrole formation of cycloadduct **217**. Both **216** and **217** could be derived from di-carbonyl compound **218** which would be prepared by a Horner-Wadsworth-Emmons (HWE) olefination of the diketopiperazine phosphonate (219) and a suitably protected aldehyde (220).

5.1 Synthesis of Diketopiperazine Phosphonate

Diketopiperazine phosphonate **225** was prepared according to the procedure of Schmidt *et al.*[50] by coupling proline benzyl ester (136) with the glycine phosphinate **222** (Scheme 46). Hydrogenolysis of the resulting peptide in the presence of a catalytic quantity of acid resulted in removal of the benzyl carbamate and benzyl ester accompanied by ring closure to the DKP.

Scheme 46: Synthesis of DKP phosphonate.



136, R=H
221, R=Me

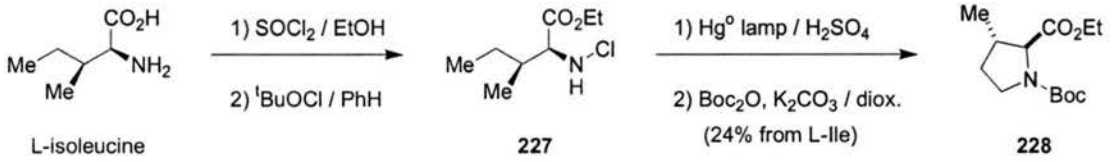
222

223, R=H
224, R=Me

225, R=H
226, R=Me

Initial studies of the HWE olefination were done with DKP phosphonate **225** because proline benzyl ester is readily available from commercially available proline. The β-methyl proline necessary to prepare DKP phosphonate **226**, however, is not commercially available and was prepared from L-isoleucine according to the procedure of Titouani[51] (Scheme 47). L-isoleucine is esterified and converted to the N-chloro compound **227**. Photolysis with a mercury lamp in 80% sulfuric acid triggers a Hoffman-Loeffler-Freitag reaction transferring the chloride from the nitrogen to the carbon of the terminal methyl group. Upon neutralization, nucleophilic attack by the amine furnishes β-methyl proline with complete retention of stereochemistry.

Scheme 47: Synthesis of β-methylproline[50].

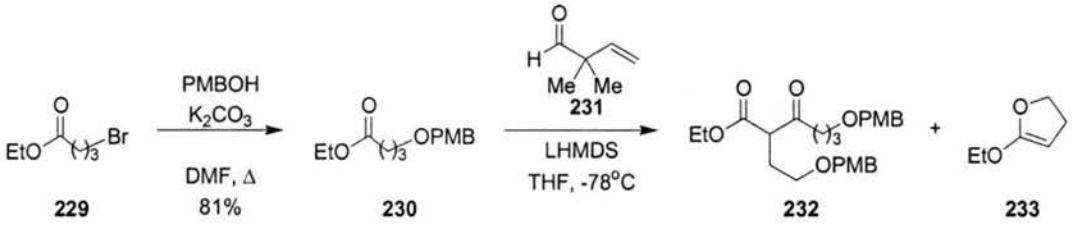


Transesterification and removal of the *tert*-butyl carbamate provides **221** in 87% over four steps. This sequence is necessary to prevent dimerization to the *cyclo*-L-proline anhydride.

5.2 Synthesis of Aldehyde 220 Candidates

The first of several approaches to aldehyde **220** is shown in Scheme 48. Ester **230** was prepared from ethyl bromobutyrate and *para*-methoxy benzyl alcohol.

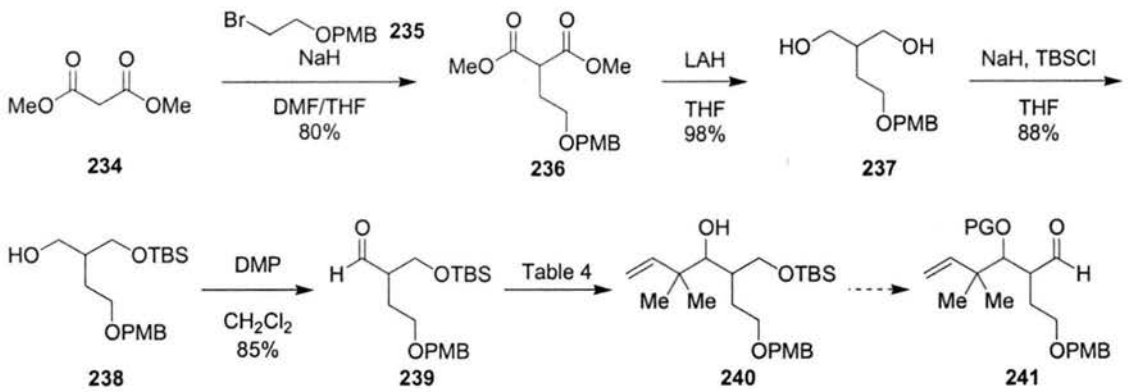
Scheme 48: Aldol coupling approach to aldehyde **220**



Efforts to couple this with aldehyde **231**[52], however, resulted only in the Claisen condensation of the ester and formation of dihydrofuran **233**.

The second approach to aldehyde **220** began with dimethyl malonate (234) (Scheme 49).

Scheme 49: Second approach to aldehyde **220**



Alkylation with bromide **235**[53] followed by reduction to the diol (237) and treatment with NaH and TBSCl[54] furnished the silyl ether (238). Dess-Martin oxidation provided the aldehyde (239) in 85% yield. The alkylation of aldehyde **239**, however, proved to be more difficult than expected (Table 4).

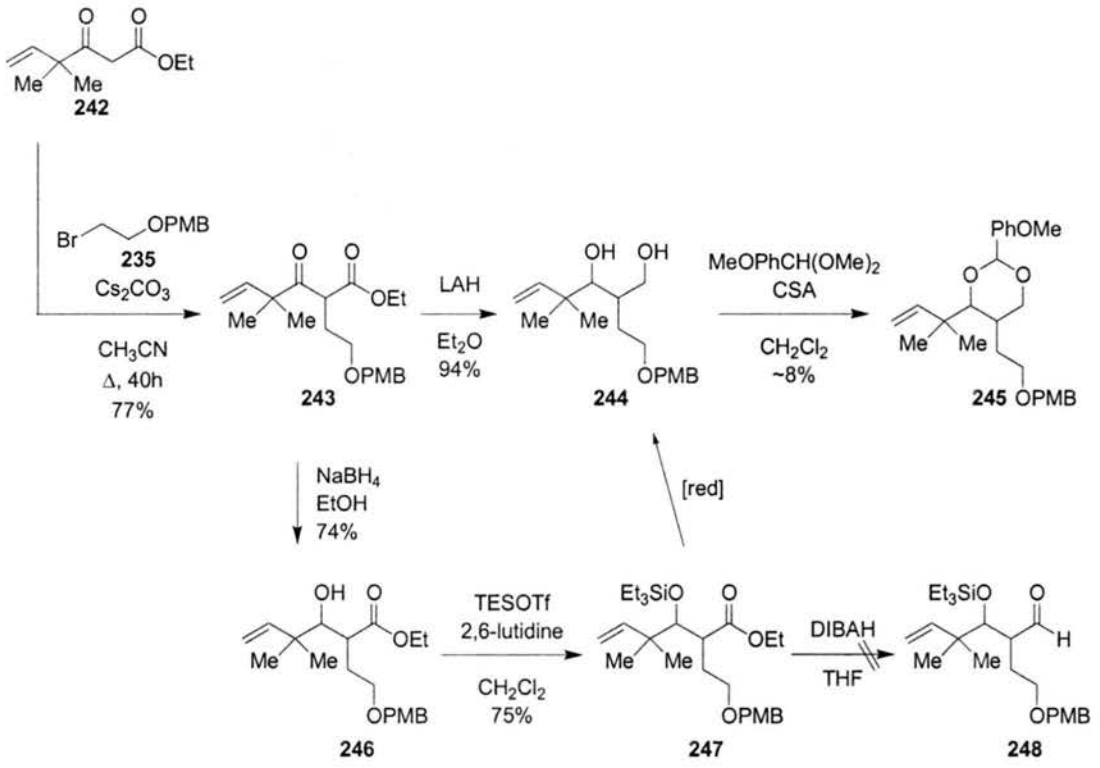
Table 4: Conditions of attempted aldyhyde alkylation.

Entry	Reagent/Conditions	Results	Ref.
1	prenyl bromide/In^0/DMF	Complex misture	
2	prenyl bromide / Zn^0 / Cp_2TiCl_2 / THF	Aldehyde decomposition	55
3	prenyl bromide/In^0/THF	Complex mixture	
4	prenyl bromide / In^0 / THF/H_2O	Complex mixture	
5	prenyl magnesium bromide / $MgBr_2$ / THF	No reaction	54
6	prenyl bromide / Mg^0 / $MgBr_2OEt_2$ / THF	No reaction	54

Indium mediated olefination resulted in a mixture of products, none of which resembled the desired product (Entries 1,3 and 4). Lewis acid mediated[55] addition of prenyl Grignard (Entries 5-6) gave no reaction. Ding and Zhao[56] reported successful zinc-promoted alkylation of carbonyl compounds catalyzed by Cp_2TiCl_2. Unfortunately, use of these reaction conditions resulted only in the slow decomposition of the aldehyde to the α,β-unsaturated aldehyde.

Another approach to aldehyde **220** began with the preparation of β-keto ester **242**, followed by alkylation with bromide **235** in refluxing CH_3CN with Cs_2CO_3 (Scheme 50). Reduction to the diol with LAH in THF proceeded satisfactorally, but formation of the *para*- methoxy benzylidene acetal **245**

Scheme 50: Synthesis of diol **244**



was low yielding, probably due to the steric bulk imposed by the dimethyl allyl substituent of the racemic diastereomers. Selective reduction of the ketone functionality of **243** with $NaBH_4$ followed by protection as the silyl ether, proceeded cleanly with TESOTf and lutidine in CH_2Cl_2. Unfortunately, treatment of ester **247** with DIBAH gave no reaction. A two-step reduction/oxidation sequence to prepare aldehyde **248** was also unsuccessful as treatment with LAH gave quantitative yields of the diol. Changing the hydroxyl protecting group to the more robust TBS ether was of no value as the same over reduction product (244) was likewise observed. Attempts to introduce either a MEM ether or SEM ether proved futile.

The next approach involved hydrolyzing ester **243** to the β-keto-acid (249), followed by formation of the Weinreb amide (**250**) and reduction to the β-hydroxy aldehyde (Scheme 51). But upon treatment of

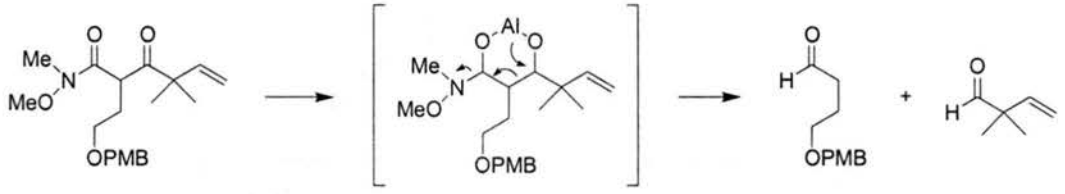
amide **250** with LAH, aldehyde **251** was the only product isolated. A proposed mechanism for this transformation is shown in Scheme 52.

Scheme 51: Weinreb amide approach to aldehyde **220**

243 **249** **250** **251**

Aldehyde **231** is known to be volatile from previous handling experience and was probably lost during the reaction workup since it was not observed in the ^{1}H NMR of the crude reaction mixture.

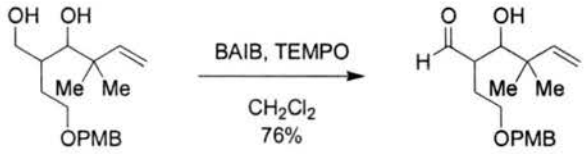
Scheme 52: Proposed mechanism for the observed results from reduction of amide **250**



250 **251** **231**

At last a successful candidate for aldehyde **220** was prepared by selective oxidation of the primary alcohol. Following the procedure of Piancatelli[57], alcohol **244** was treated with TEMPO and diacetoxyiodobenzoic acid (BAIB) to furnish the β-hydroxy aldehyde in 76% yield (Scheme 53).

Scheme 53: TEMPO oxidation of diol to β-hydroxy aldehyde.

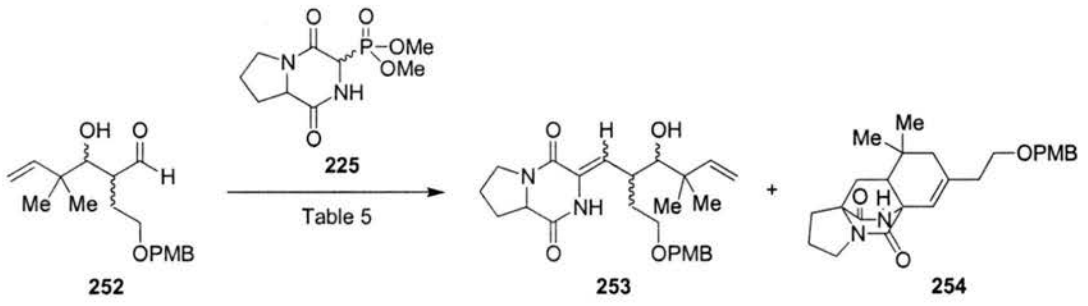


244 **252**

5.3 Horner-Wadsworth-Emmons (HWE) Olefination with Aldehyde 252

With a suitable candidate for the olefination reaction in hand, conditions were explored which would provide the desired alkene (Scheme 54). Use of sodium hydride resulted in no reaction.

Scheme 54: HWE olefination between DKP phosphonate **225** and aldehyde **252**



Both diazabicycloundecene (DBU) and potassium *tert*-butoxide resulted in the formation of the desired alkene (Table 5). In addition, an interesting result was observed when an excess amount of base was used. Bicycloadduct **254** would be formed in quantities that increased with increasing reaction times. In order to install the pyrrole ring, the six-membered ring required the presence of an oxygen, either in the form of

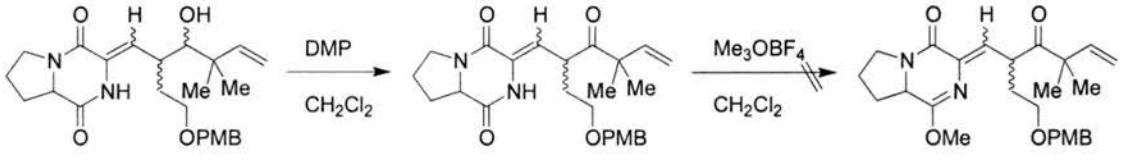
Table 5: Results of HWE olefination with different bases.

Entry	Base	Solvent	Time	253 (% yield)	254 (% yield)
1	NaH	THF		0	0
2	DBU	CH_2Cl_2		63	0
3	KO^{t}Bu	CH_2Cl_2		91	2

a hydroxyl substituent or a carbonyl. Given the location of the double bond, allylic oxidation was expected to occur on the exocyclic side chain rather than on the ring. At the time, it was viewed as a useless byproduct that detracted from the yield of the desired alkene. It did, however, lend optimism to success in the Diels-Alder cycloaddition soon to be attempted. Formation of **254** could be suppressed by carefully controlling the amount of base used to deprotonate the phosphonate (225).

Work toward asperparaline C* proceeded with the alkene obtained from the HWE reaction of aldehyde **252** and DKP phosphonate **225** (Scheme 55). Oxidation of the secondary alcohol (253) resulted

Scheme 55: Manipulation of secondary alcohol **253**



253 **255**

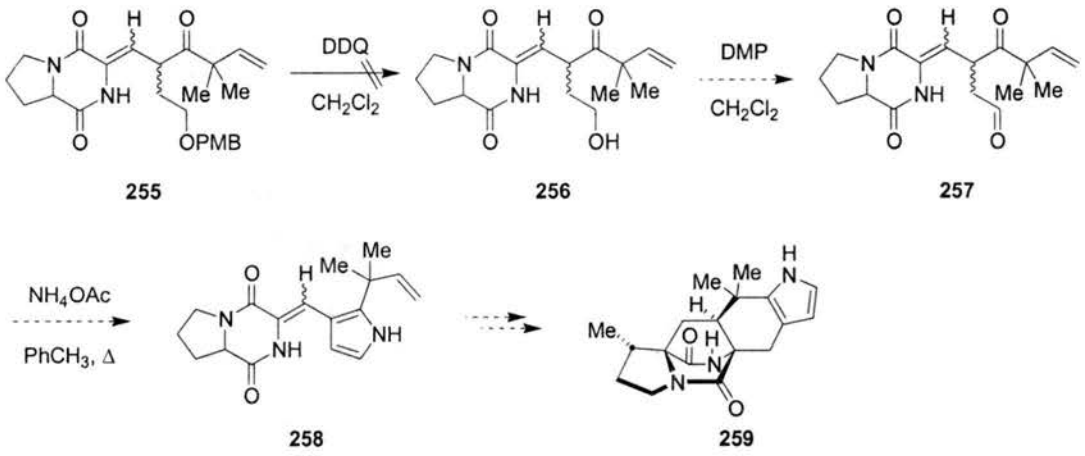
in the formation of ketone **255**. Efforts to convert this to the lactim ether with methyl Meerwein resulted in the formation of multiple products. Spectral analysis of the products isolated suggested that in addition to possible formation of the lactim ether, the compound had gained additional methyl groups and/or lost the PMB ether.

In 2001, Liebscher[58] reported a procedure for the formation of bicyclo[2.2.2]diazaoctane containing compounds from unsaturated diketopiperazines bearing pendant alkenes by stirring with acetyl chloride for 6-11 days. Both alcohol **253** and ketone **255** were subjected to these conditions but only decomposition was observed.

Oxidative removal of the PMB ether with DDQ was also attempted, with the idea that the resulting alcohol could be oxidized to the aldehyde and used to install the pyrrole prior to attempting the cycloaddition (Scheme 56), however, this only resulted in the formation of a complex mixture of undetermined products.

* While asperparaline A is the focus of this thesis, asperparaline C is a simpler target and also serves as an ideal model for studying asperparaline A. By lacking a methyl group on the proline ring, synthesis of asperparaline C allows for the use of commercially available proline and proline derivatives in synthetic studies rather than β-methyl proline - synthesis of which was a time consuming, laborious, and frequently unfruitful task.

Scheme 56: Attempted manipulation of ketone **255**



255 **256** **257**

258 **259**

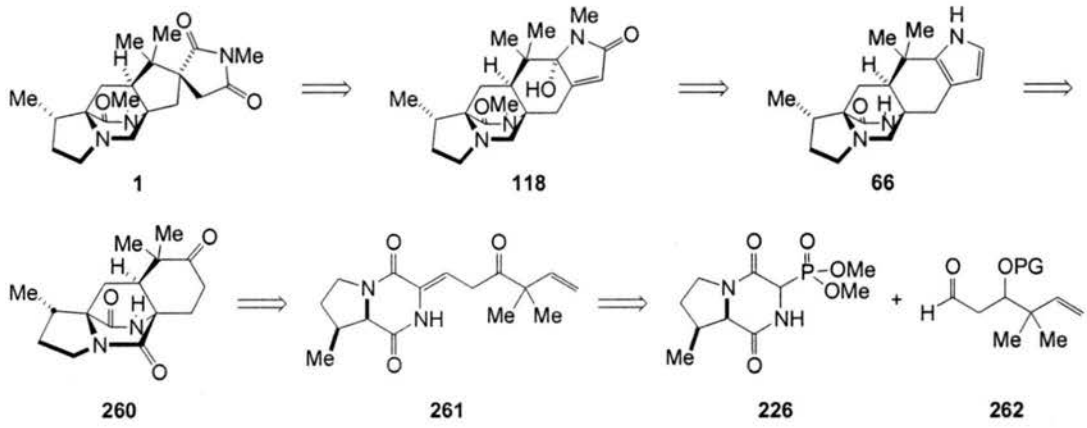
Alkylation of β-keto ester **242** resulted in **243** being formed as a racemate. Upon reduction, an inseparable mixture of racemic diastereomers was obtained. These were carried through the olefination step, which also produced a mixture of E and Z isomers. Attempts to separate this complex mixture by chromatography proved futile. Oxidation of the alcohol (253) to the ketone (255) effectively removed one of the stereocenters but the different isomers were still inseparable. This made it very difficult to determine exactly what was occurring in each subsequent reaction. Rather than pursue the synthesis of optically pure β-hydroxy aldehyde **252,** the synthetic plan was modified yet again to simplify the precursor of the [4+2] cycloaddition to a compound that could be easily accessed and subsequently modified.

5.4 Retrosynthesis of modified approach to [4+2] cycloaddition

Horner-Wadsworth-Emmons olefination had proven to be an effective means for synthesizing unsaturated diketopiperazines. It remained to be determined whether or not these compounds would be useful substrates for intramolecular hetero-Diels-Alder cycloadditions. Exploring this transformation with a substrate in which all of the carbons of the core framework of asperparaline A were present was complicated by inseparable mixtures of diastereomers. This was addressed by modifying the approach to asperparaline A in such a way that this inconvenience could be avoided without further complicating the synthesis. As shown in Scheme 57, the putative hexacyclic intermediate **66** would be derived by introduction of a pyrrole moiety onto a simplified cycloadduct (260). This would be prepared from an

intramolecular [4+2] cycloaddition of the terminal alkene across the requisite azadiene derived from unsaturated diketopiperazine **261**.

Scheme 57: Revised retrosynthesis.

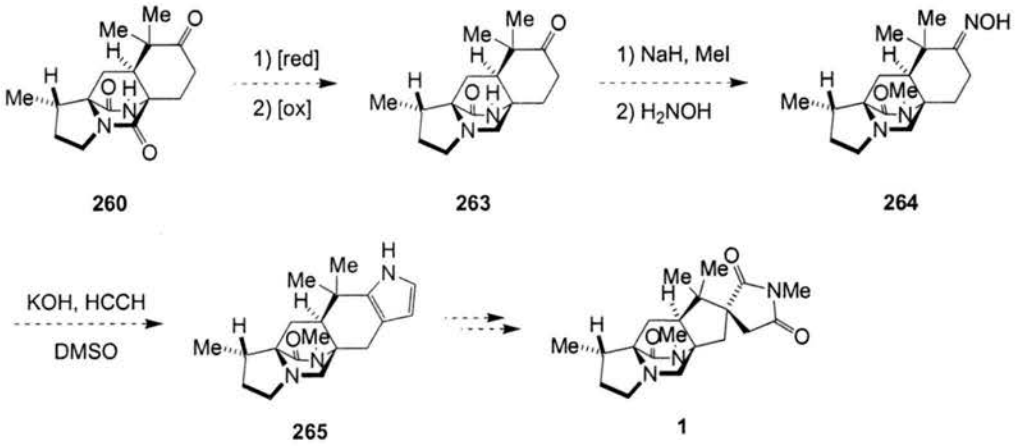


The unsaturated DKP could be easily accessed via an HWE olefination between the DKP phosphonate (226) and the appropriate aldehyde (262). This route would eliminate the hassle of complex diastereomeric mixtures and the introduction of the pyrrole ring onto cycloadduct **260** could be done by a variety of methods.

5.4.1 Possible methods for introducing a pyrrole ring onto cycloadduct 260

The Trofimov reaction has been previously demonstrated as a useful and direct means for preparing 2,3-disubstituted pyrroles. Reduction of the tertiary amide and re-oxidation of the reduced ketone would provide compound **263** (Scheme 58). Methylation of the amide and converstion of the ketone to the oxime would provide a substrate expected to fulfill all of the requirements for the Trofimov reaction. The initial reduction, oxidation methylation sequence would be necessary for two reasons. First, the selective reduction of the tertiary amide to the amine can only be accomplished if the secondary amide remains unalkylated. However, the secondary amide would need to be methylated prior to the Trofimov reaction to prevent unwanted side reactions in which alkylation of the amide nitrogen with acetylene would be expected.

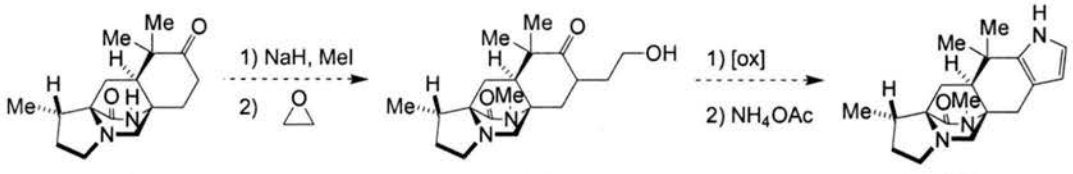
Scheme 58: Proposed completion of asperparaline A from cycloadduct **260** using Trofimov reaction.



260 **263** **264** **265** **1**

In the event that the Trofimov fails to produce expected results, the pyrrole can also be installed in a stepwise manner. Starting from the reduced/re-oxidized cycloadduct **263**, methylation of the amide nitrogen and alkylation *alpha* to the carbonyl to install the hydroxyethyl side chain would give pyrrole compound **265** after oxidation and treatment with ammonium acetate (Scheme 59).

Scheme 59: Proposed stepwise installation of pyrrole onto cycloadduct **263**



263 **266** **265**

5.5 Synthesis of Aldehyde 262 Candidates and Horner-Wadsworth-Emmons Olefination

In revising the precursor of the Diels-Alder cycloaddition reaction, the key consideration was a substrate easily amenable to manipulation to the pyrrole moiety upon acquisition of the cycloadduct. The Diels-Alder precursor would be prepared by a Horner-Wadsworth-Emmons olefination between diketopiperazine **226** and an aldehyde wherein the introduction of stereogenic centers would be limited in order to avoid complications in purification and structural elucidation of the products obtained from the cycloaddition. Two different aldehydes were synthesized and examined for this purpose. The first utilized

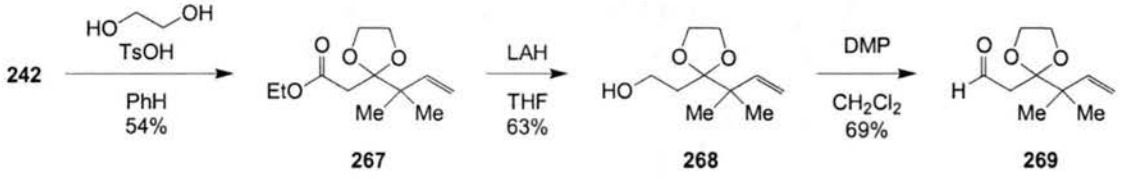
a ketal to protect the ketone functionality adjacent to the dimethyallyl chain, the second used a silyl ether to protect the hydroxyl group adjacent to the dimethallyl chain.

5.5.1 Aldehyde 262 featuring ketal protection of oxygen functionality.

The first choice of an aldehyde to couple with the piperazinedione phosphonate (226) utilized a ketal to protect the carbonyl adjacent to the dimethallyl chain. The ketal would be tolerant of the conditions of the olefination reaction and could removed under mild conditions following the cycloaddition. Introducing the oxygen as a symmetric ketal also assured that the products of the olefination reaction would be limited to the geometric isomers and purification would not be complicated by the presence of multiple diastereomers in addition to the E and Z alkene.

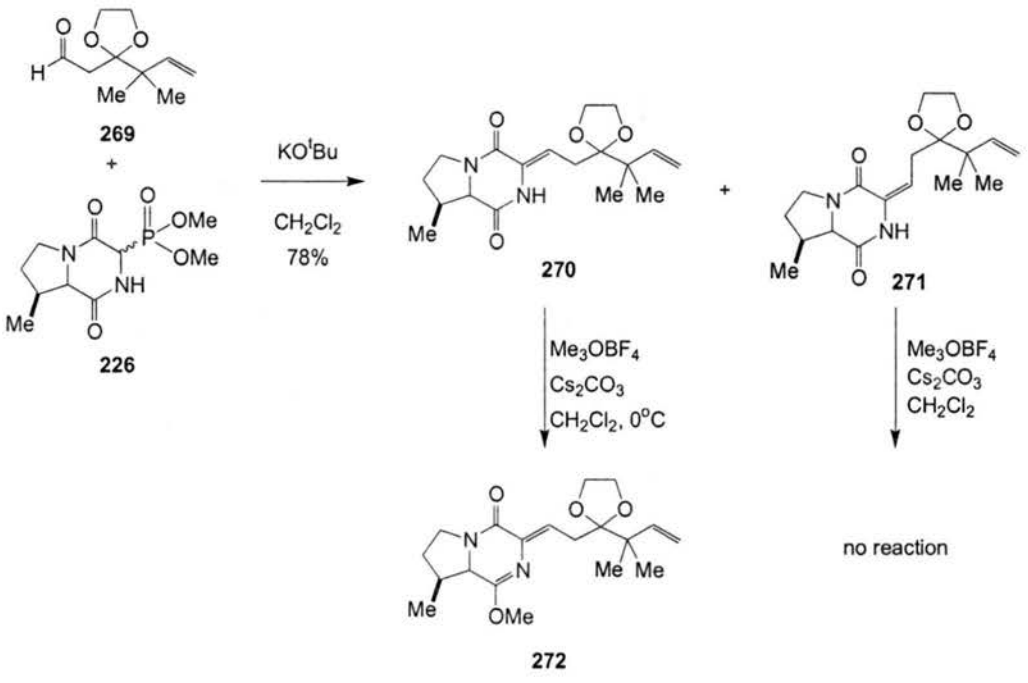
The ketal protected aldehyde **269** was prepared from β-keto ester **242** used earlier[9]. Ketalization with ethylene glycol under Dean-Stark conditions provided a 54% yield of the desired ketal. The yield of this reaction reflects the poor turnover of the β-keto ester into the ketal. However, it was not possible to separate the ketal from the β-keto ester chromatographically and recycle the β-keto ester. Rather, the mixture of ketal and ester were carried through the reduction step together, at which time the diol was chromatographically separable from the alcohol. Oxidation of the primary alcohol (268) to the aldehyde (269) was then performed with Dess-Martin periodinane (Scheme 60).

Scheme 60: Formation of ketal protected aldehyde **269**



This aldehyde was then successfully coupled with the DKP phosphonate (226) to give a 1:1 mixture of E and Z alkenes as expected (Scheme 61). After separation, each alkene was independently treated with trimethyloxonium tetrafluoroborate to form the lactim ether. However, only the Z-alkene provided the desired lactim ether (272) while the E-alkene was unreactive. The reasons for this remain unclear.

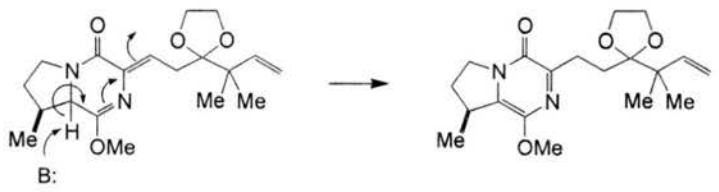
Scheme 61: Horner-Wadsworth olefination of DKP phosphonate **226** with aldehyde **269**



In addition, the Z-alkene was observed to isomerize to the unreactive E-alkene if the lactim ether was formed at room temperature, but this isomerization could be suppressed if the reaction was done at 0^0C over a longer period of time. It is also worth noting that replacing Cs_2CO_3 with K_2CO_3 for this reaction results in very poor product yields.

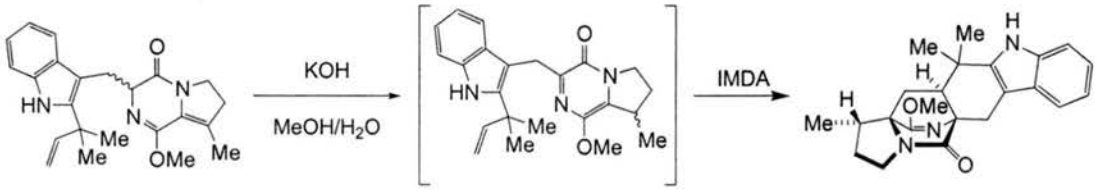
With the azadiene in hand, efforts turned to eliciting the prototropic rearrangement that would provide the azadiene needed for the cycloaddition (Scheme 62).

Scheme 62: Desired tautomerization and formation of requisite azadiene.



Deprotonation of the hydrogen on the carbon *alpha* to the lactim ether was expected to bring about the desired rearrangement. This was based on the precedence provided in the synthesis of VM55599 wherein the requisite azadiene for the intramolecular Diels-Alder cycloaddition was prepared from an α,β-unsaturated lactim ether upon treatment with aqueous KOH in methanol (Scheme 63).

Scheme 63: Azadiene tautomerization and concomitant IMDA cycloaddition in synthesis of VM55599.



273 **274** **275**

Atempted prototropic isomerization of azadiene **272** with these conditions resulted in no reaction. Other bases were attempted for this transformation (CsOH, NaH, DBU) without success. This was originally believed to be a result of the steric bulk imposed by the ketal, prohibiting the isoprene derived olefin from adopting the necessary geometry to allow for the cycloaddition. While exploring conditions to regenerate the ketone, an alternative aldehyde was prepared in which the oxygen functionality was protected as a silyl ether.

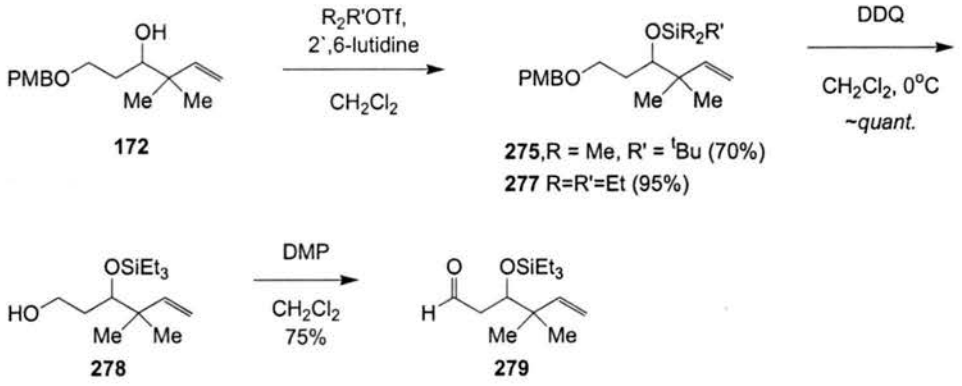
5.5.2 Aldehyde 262 featuring silyl ether protection of oxygen functionality.

The second choice of aldehyde partner for the Horner-Wadsworth-Emmons reaction introduced a silyl ether adjacent to the dimethallyl side chain for the introduction of the requisite oxygen functionality. Choice of the silyl ether as a protecting group was based in part on its stability to the conditions of the olefination reaction and also on its ease of removal under mild acidic conditions.

Starting with the homoallylic alcohol (172) used previously (Scheme 34), silyl-protected β-hydroxy aldehyde **279** was prepared (Scheme 64). Both the TBS and TES ether were considered, however, formation of the TES ether gave higher yields and the PMB ether protecting the primary alcohol could be

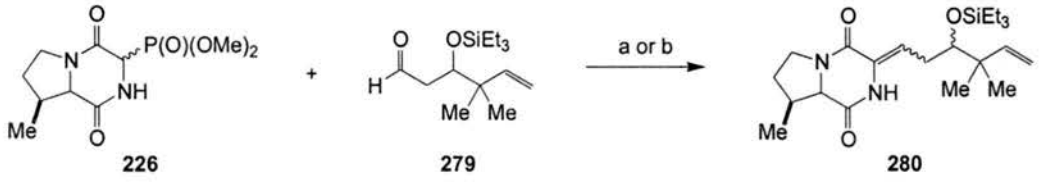
efficiently removed in its presence at low temperature with DDQ. Dess-Martin oxidation of the primary alcohol (278) provided the requisite aldehyde (279) in good yield.

Scheme 64: Synthesis of aldehyde **279**.



HWE olefination between aldehyde **279** and phosphonate **226** utilizing potassium *tert*-butoxide as the base resulted a 91% yield of the desired alkene as a sparingly separable mixture of all possible diastereomers. Using the conditions reported by Roush[59] improved the reaction yield to 94% in addition to showing a marked preference for the formation of the E-alkene, thereby simplifying purification (Scheme 65).

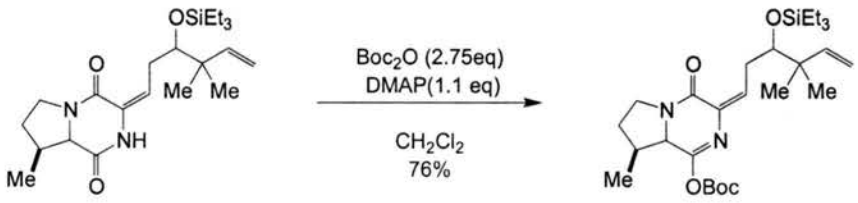
Scheme 65: HWE olefination between phosphonate **226** and aldehyde **279**.



Conditions: a) KO^{t}Bu, CH_2Cl_2 (91%); b) DBU, LiCl, CH_3CN (94-99%)

Due to the sensitivity of silyl ethers to acid, trimethyloxonium tetrafluoroborate is not suitable for the formation of the lactim ether. So the azadiene was prepared by treatment of the unsaturated DKP with Boc_2O and DMAP to provide the imino-carbonate (281) in excellent yield (Scheme 66).

Scheme 66: Formation of the imino-carbonate.



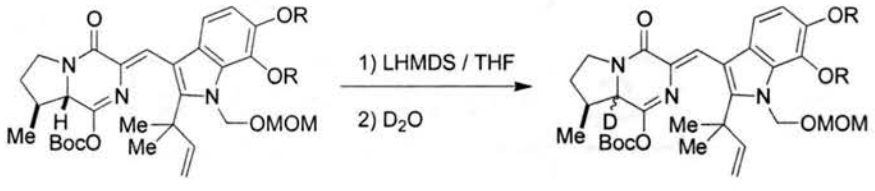
280 **281**

This azadiene was treated with a variety of bases (KOH, $NaOCH_3$, DBU, DABCO, Cs_2CO_3, NaH) in an attempt to elicit the desired isomerization to the requisite azadiene and concomitant cycloaddition again without success. The silyl ether was removed with TBAF to remove any possibility of steric encumbrance that it might be imposing but this did not aid in the formation of the desired cycloadduct.

5.6 Lewis acid Mediated Intramolecular Diels-Alder Cycloaddition

In a related effort, a study was done[60] to investigate the base-mediated isomerization of a similar azadiene. Treatment of azadiene **282** with LHMDS followed by protonation with D_2O only resulted in introduction of the deuterium label at the site of deprotonation, indicating that isomerization of the 1-azadiene to the requisite 2-azadiene was not occurring (Scheme 67).

Scheme 67: Deuterium labelling study of paraherquamide F precursor.

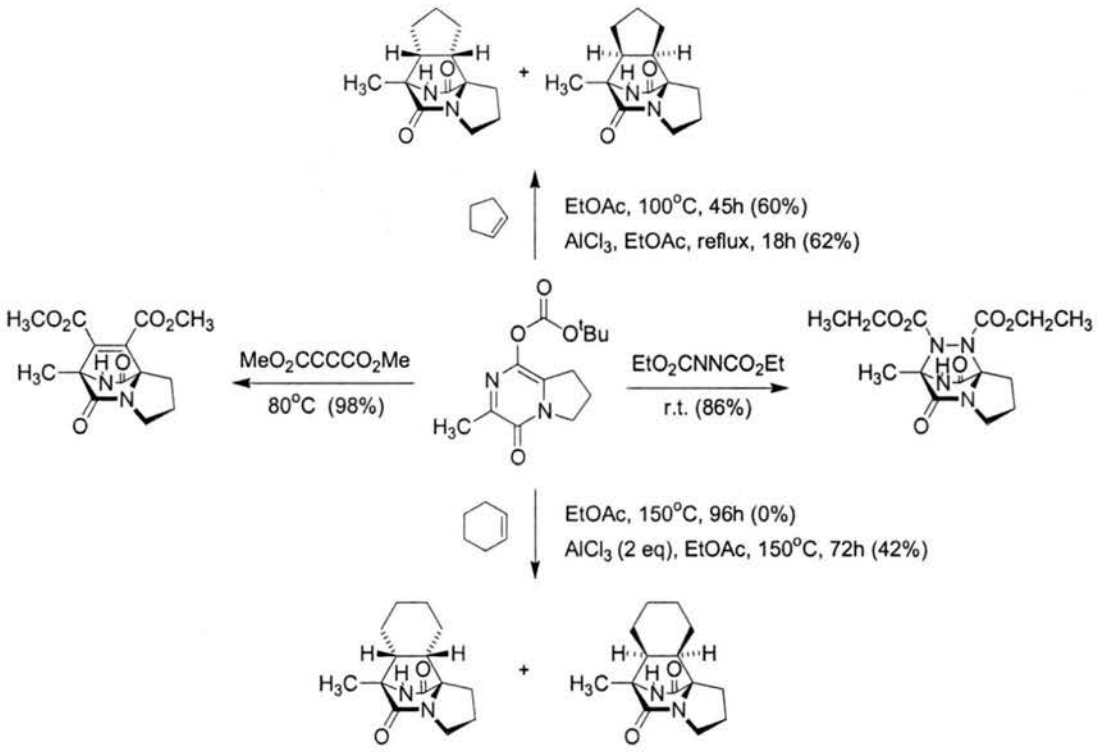


282 **283**

This result coupled with the observation that the 2-azadiene (281) slowly isomerized to the bis-enamine and consideration of the molecular orbitals involved in the desired rearrangement led to the belief that Lewis or Brønsted acid mediated prototropic rearrangement would provide the necessary azadiene for the intramolecular [4+2] cycloaddition. Furthermore, a model study done in 2000 by Williams *et al.*[61] reported

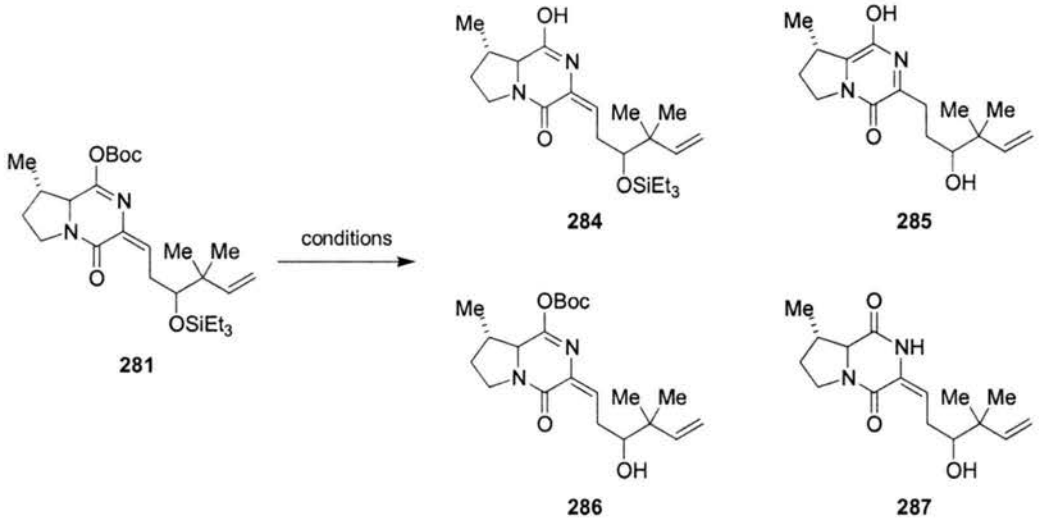
the intermolecular Diels-Alder cycloadditions between *tert*-butyl substituted azadienes and both activated and unactivated dienophiles (Scheme 68). Activated dienophiles were observed to give cycloaddition products at both ambient and elevated temperatures without the aid of Lewis acid activation. Unactivated dienophiles gave cycloaddition products at elevated temperatures when catalyzed by a Lewis acid.

Scheme 68: Williams et al. model study of Lewis acid mediated [4+2] cycloaddition.



Azadiene **281** was exposed to a variety of Lewis and Brønsted acids with varying success. Aluminum chloride ($AlCl_3$), and toluenesulfonic acid (TsOH) as well as hydrochloric acid (HCl) each induced the desired prototropic rearrangement. However, due to the sensitivity of both the *tert*-butyl carbonate and the silyl ether to acidic conditions, both were lost during the course of the reaction. Furthermore, azadiene **285** failed to spontaneously undergo the desired cycloaddition. This is perhaps the result of incompatible reactivity between the alkene and azadiene.

Figure 4: Results of treating azadiene **281** with Lewis and Bronsted acids.

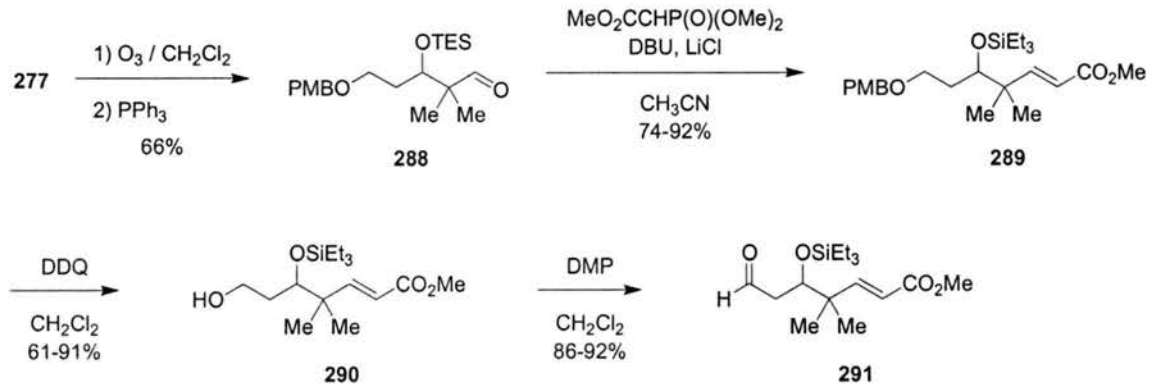


Lewis acid (eq.)	Solvent	Temp. (°C)	Pressure*	284 (%)	285 (%)	286 (%)	287 (%)
$AlCl_3$ (1.4)	EtOAc	71	0	0	60	0	0
$AlCl_3$ (3.0)	EtOAc	25	100	0	<5	0	0
$AlCl_3$ (2.8)	EtOAc	130	Sealed tube	0	0	0	38
HCl (1.01)	Dioxane	25	0	0	87	0	0
$ZnCl_2$ (3.7)	Benzene	25	80	78	0	0	0
$MgBr_2$ (3.6)	Benzene	115	Sealed tube	83	0	0	0
TsOH (1.1)	Benzene	25	100	0	58	0	0
TsOH (1.3)	Benzene	120	Sealed tube	0	52	0	0
PPTS (1.2)	Benzene	25	135	0	0	82	0
PPTS (1.2)	MeOH	25	135	0	0	77	0

* psi

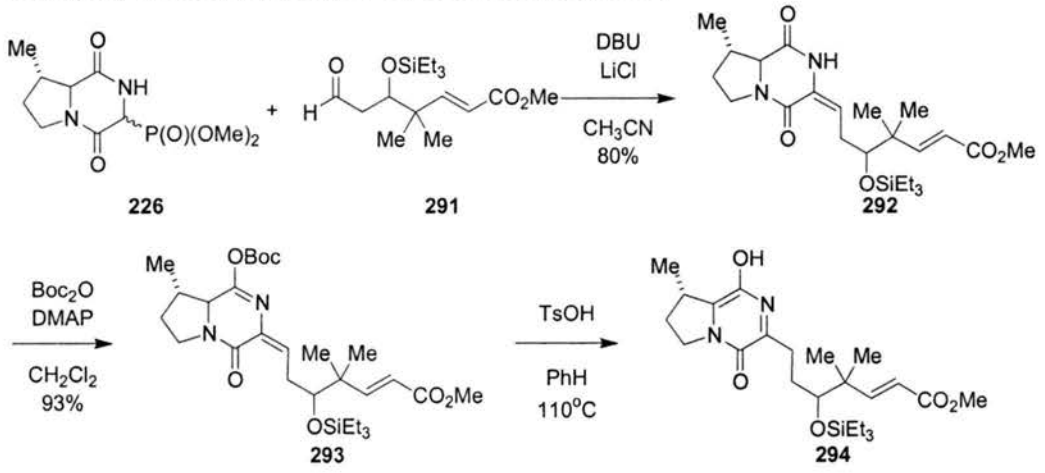
To improve the electrophilicity of the dienophile for the cycloaddition, aldehyde **291** was prepared from HWE olefination of the aldehyde prepared by ozonolysis of alkene **277** (Scheme 69). It should be noted that Me_2S was a poor reducing agent for this transformation, PPh_3 was a superior reducing agent despite making reaction workup more challenging.

Scheme 69: Synthesis of aldehyde featuring electron poor alkene.



Olefination between DKP phosphonate **226** and aldehyde **291** proceeded uneventfully in good yield (Scheme 70) and the resulting unsaturated DKP **292** was converted to the iminocarbonate (293). Upon treatment with Lewis and Bronsted acids, however, similar results were observed. The desired azadiene is apparently formed with concomitant loss of all protecting groups but fails to undergo the desired cycloaddition.

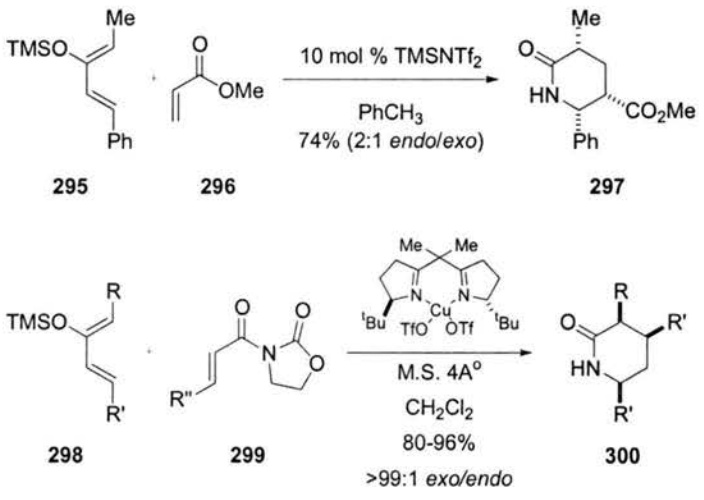
Scheme 70: HWE olefination of phosphonate **226** and aldehyde **291**.



Unlike the earlier syntheses of paraherquamide derivatives, this approach to asperparaline lacks the indole ring to impose steric control on the conformation of the Diels-Alder substrate. It also appears that substitution of the pyrazinone is critical for the cycloaddition to take place. Time constraints prevented exploration of this approach at greater depth, however, Ghosez[62] reported successful intermolecular Diels-Alder cycloadditions with similar 2-azadienes using $TMSNTf_2$ or the Evans' Lewis acid derived from copper (II) triflate and a C_2-symmetric bis(oxazoline) ligand (Scheme 71). Perhaps upon successful

substitution of the pyrazine alcohol, use of one of the following methodologies will facilitate the desired cycloaddition.

Scheme 71: Ghosez approach to intermolecular hetero-Diels-Alder cycloadditions.



5.7 Conclusions

In the second generation approach toward asperparaline A, a Horner-Wadsworth-Emmons olefination was used to construct an unsaturatede diketopiperazine. this methodology proved indispensable for the quick and efficient access of substrates for an intramolecular [4+2] cycloaddition. The stability of the 2-azadiene to tautomerization into the requisite azadiene for formation of the cycloadduct under basic conditions was overcome by the use of both Lewis and Brønsted-Lowry acids. These conditions, however, resulted in universal loss of protecting groups, thereby altering the reactivity of the precursor toward cycloaddition. A direct solution to this problem might be to use protecting groups stable to acidic conditions. In addition to the synthesis of asperparaline A, this route would give direct access to each of the asperparalines as well as to the paraherquamides, avrainvillamides and stephacidins and is worthy of further consideration.

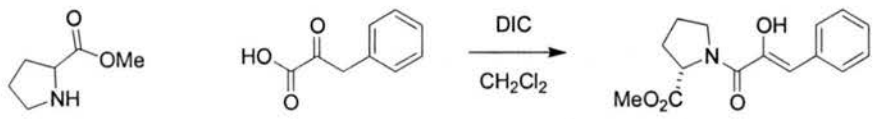
CHAPTER SIX

Experimental Data

6.1 General Procedures

Commercially available reagents were used as received without further purification except where stated. Thin layer chromatography was performed using 0.25 mm silica gel 60 (F254, Merck) plates visualizing at 254 nm, or developed with potassium permanganate solutions and heating with an hot-air gun. Specified products were purified by flash chromatography using silica gel 60 (230-400 mesh, Merck). Melting points were obtained using a MEL-TEMP melting point apparatus. IR absorptions on NaCl plates were run on a Perkin-Elmer FTIR 1600. ^{1}H NMR spectral data were obtained using a Varian 300 or 400 MHz instruments. ^{13}C NMR spectral data were obtained using a Varian 75.5 or 100 MHz spectrometer. Chemical shifts are reported in parts per million relative to $CHCl_3$ at δ 7.27 (^{1}H NMR) and δ 77.0 (^{13}C NMR). For all NMR spectra, δ values are given in parts per million and *J* values in Hertz. Mass spectra data were obtained at Colorado State University's Central Instrument Facility.

6.2 Preparation of Compounds, ^{1}H NMR and ^{13}C NMR Spectral Data



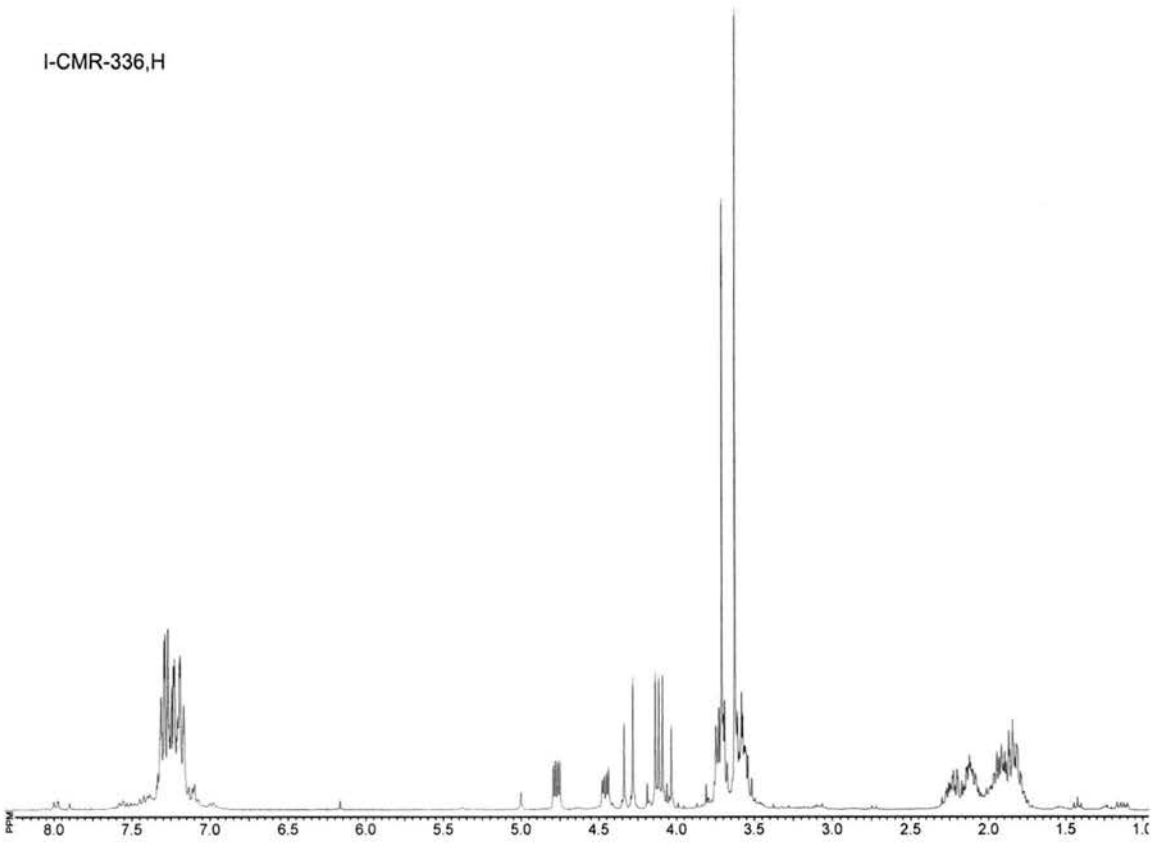
139 **133** **140**

2-hydroxy-1-phenyl-3-[(2-carbomethoxy)pyrrolidin-1-yl]prop-1-en-3-one (140): To a stirring solution of proline methyl ester (164 mg, 1.270 mmol) in CH_2Cl_2 (12.7 mL) at 0°C under argon was added phenylpyruvic acid (209 mg, 1.273 mmol). After all solids dissolved, diisopropylcarbodiimide (220μL, 1.405 mmol, 1.4 eq) was added. After stirring at 0°C for 1h, dilution with Et_2O (20 mL) and filtration through celite to remove urea, concentration by rotary evaporation give the crude product as a yellow oil. Purification by flash chromatography with 9:1 hexanes / ethyl acetate to 100 % ethyl acetate yields the product (a mixture of E/Z enols) as a clear yellow oil (109 mg, 0.3995 mmol, 31%).

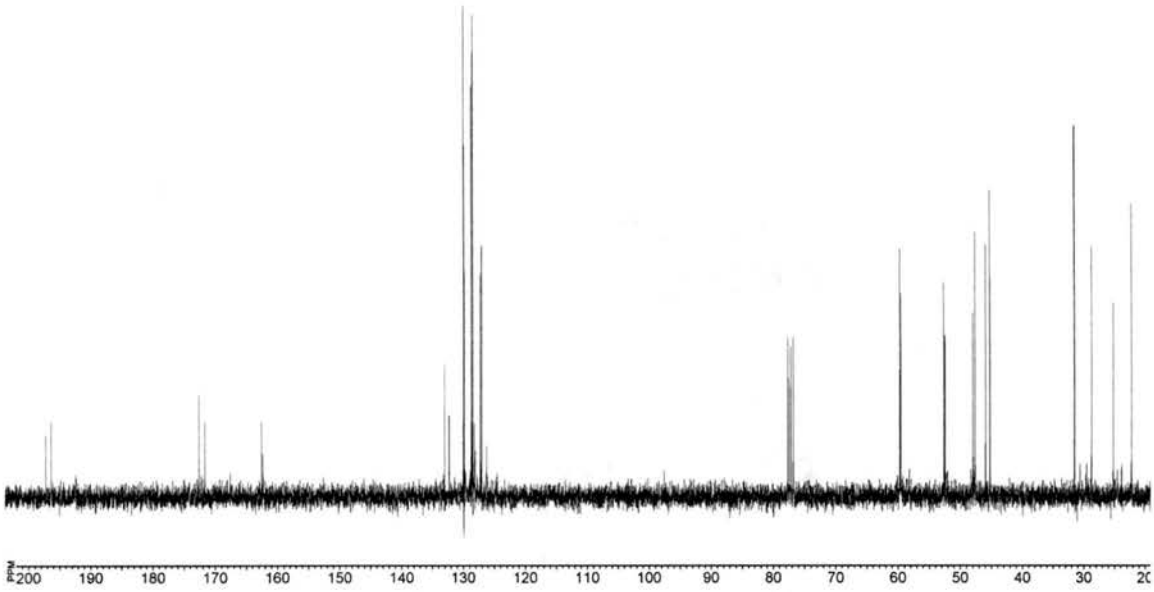
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.766-2.061 (mult. 2H), δ2.075-2.345 (mult, 2H), δ3.562-3.689 (mult., 2H), δ3.672 (d, J=0.5 Hz, 1H), δ3.755 (d, J=0.5 Hz, 1H), δ3.720-3.803 (mult, 1H), δ4.172 (dd, J=15.8, 22.4 Hz, 1H), δ4.232 (dd, J=17, 74.4 Hz, 1H). δ4.661 (ddd, J=4, 8.5, 94.3 Hz, 1H), δ7.183-7.338 (mult, 5H).

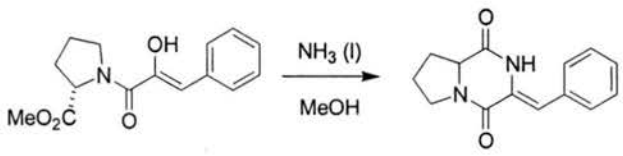
^{13}C NMR ($CDCl_3$, 75 MHz) δ 197.2, 196.4, 172.6, 171.7, 162.6, 132.0, 132.3, 130.0, 129.9, 128.7, 128.5, 127.3, 121.1, 59.7, 59.6, 52.7, 52.5, 48.0, 47.6, 46.0, 45.3, 31.5, 28.8, 25.2, 22.2.

I-CMR-336,H



I-CMR-336,C

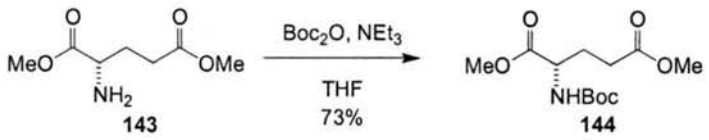




140 138

3-benzylidene-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (138): NH_3 bubbled through a solution of the amide (32 mg, 0.1162 mmol) in MeOH (11 mL) at –78°C for 1h. Reaction then allowed to warm to room temperature and NH_3 vented. Solvent removed by rotary evaporation. Residue suspended in $CHCl_3$ (10 mL) and stirred at room temperature overnight. White precipitate collected by filtration and recrystalized from methanol / ethyl acetate. Product isolated as a white crystalline solid. Mp 185-188°C (dec.) (11.7 mg, 0.04829 mmol, 42%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ1.514 – 1.678 (mult., 2H), δ1.829-2.022 (mult, 2H), δ2.301-2.359 (mult., 1H), δ2.963 (d, J=13 Hz, 1H), δ3.244 (s, 1H), δ3.455-3.588 (mult. 1H), δ7.165-7.197 (mult., 2H), δ 7.250-7.299 (mult., 3H).



***tert*-butyl (S)-1,3-di(methoxycarbonyl)propylcarbamate (144):** A stirring solution of the diester hydrochloride salt (21.6g, 102 mmol) in THF (300 mL) and NEt_3 (30.5 mL, 219 mmol) was cooled to 0°C and Boc_2O (21.2 g, 97 mmol) in THF (150 mL) was added dropwise. Upon complete addition, the mixture was allowed to warm to room temperature and stirred for an additional 6h, then heated to reflux for an additional 2h. Following removal of the solven by rotary evaporation the white solid was partitioned between ether (300 mL) and water (300 mL). The organic layer was separated and the aqueous layer was extracted with ether (200 mL x 2). The combined organic extracts were washed successively with aqueous 3% HCl (200 mL) sat. aq. $NaHCO_3$ (200 mL) and brine (200 mL) then dried (Na_2SO_4) filtered and concentrated by rotary evaporation. The crude product was isolated as a colorless oil and used without further purification (20.5g, 73%).

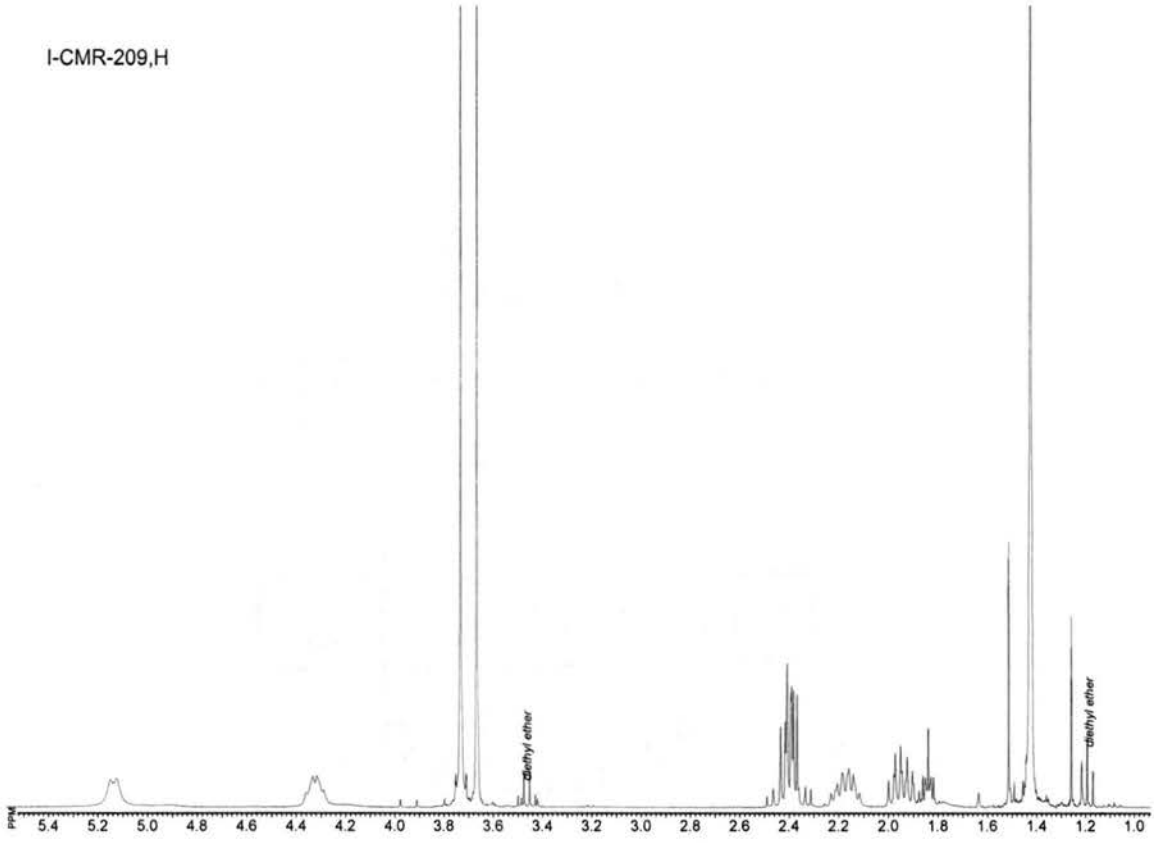
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.428 (s, 9H), δ 1.900-2.000 (mult., 1H), δ2.117-2.232 (mult., 1H), δ2.314-2.490 (mult., 2H), δ3.668 (s, 3H), δ3.735 (s, 3H), δ4.326 (d, J=7.8 Hz, 1H), δ5.138 (d, J=7.8 Hz, 1H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ173.0, 172.5, 155.2, 80.0, 67.9, 52.8, 52.4, 51.8, 30.1, 28.3.

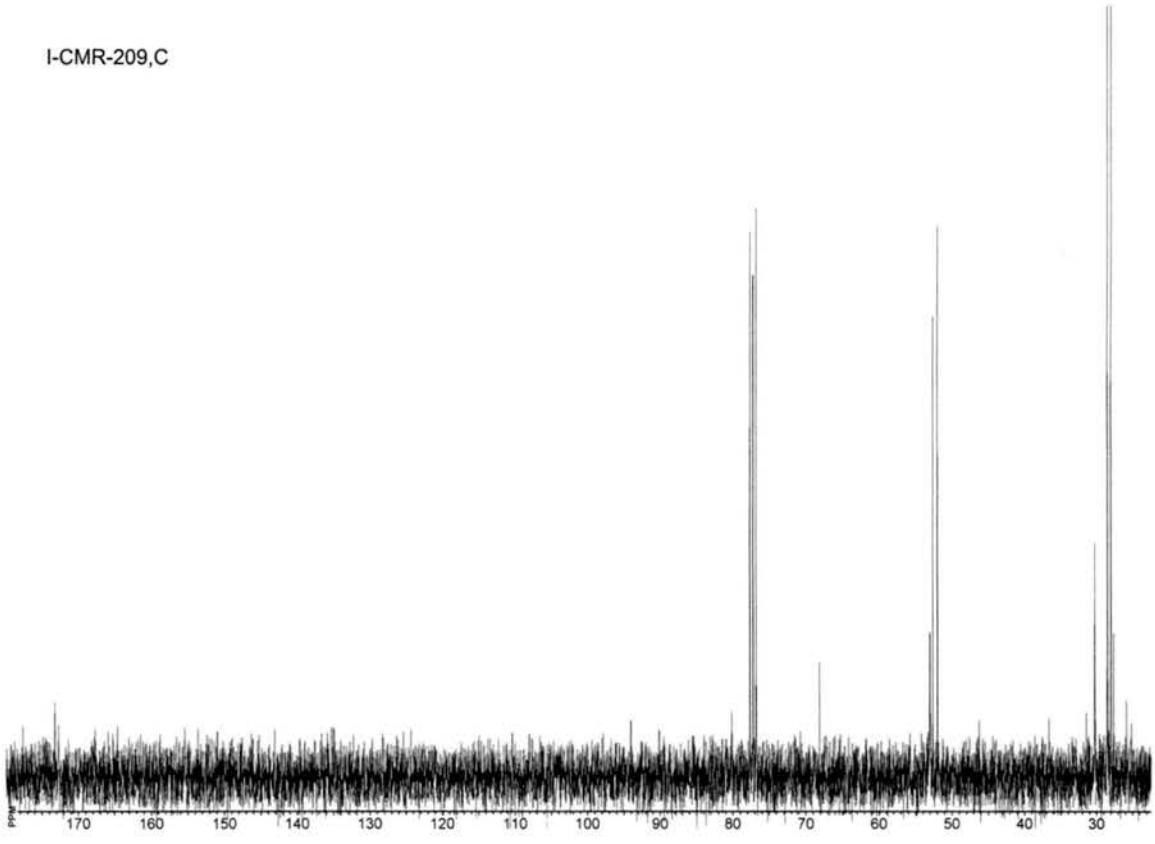
IR (NaCl, neat) 3369, 2978, 2955, 1741, 1715, 1517, 1438, 1367, 1252, 1212, 1168 cm^{-1}.

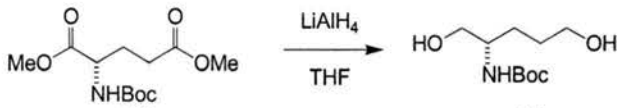
HRMS (FAB+) M+H calc'd. for $C_{12}H_{21}NO_6$ 276.1447, found 276.1445.

I-CMR-209,H



I-CMR-209,C





144 **145**

***tert*-butyl (S)-1,5-dihydroxypentan-2-ylcarbamate (145)**: To a stirring suspension of $LiAlH_4$ (18.47g, 487 mmol, 1.5 eq) in anhydrous THF (160 mL) at 0°C was slowly added di-ester (**144**, 90g, 326 mmol) in anhydrous THF (80 mL) via addition funnel. The mixture was allowed to warm to room temperature with stirring over 12h. Mixture cooled to 0°C and excess $LiAlH_4$ quenched by the careful addition of a minimum of sat. aq. Na_2SO_4. Addition of anhydrous Na_2SO_4 (~5g) followed by stirring for 2h resulted in the formation of a white precipitate which was removed by filtration. Concentration by rotary evaporation and purification on silica gel with ethyl acetate gave the product as a white solid (10.26g, 58%).

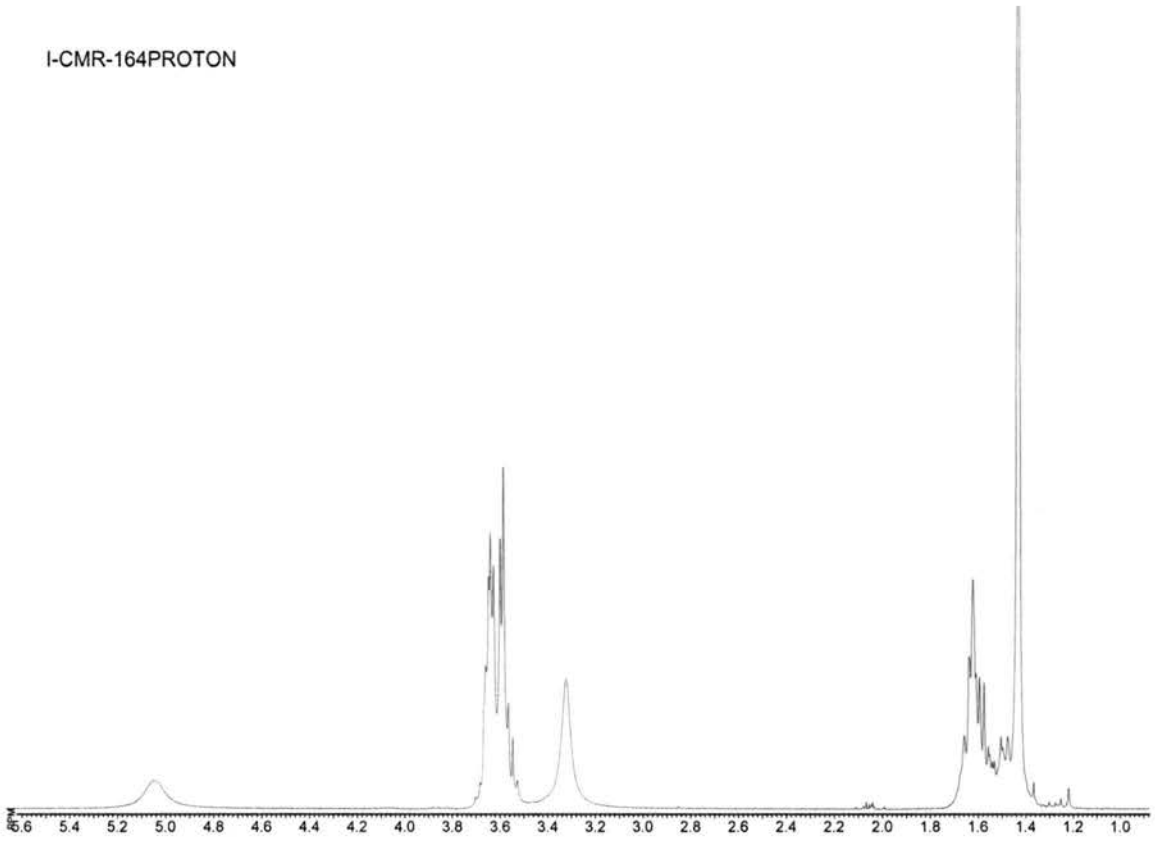
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.433 (s, 9H), δ1.533-1.660 (mult, 4H), δ3.324 (s, br, 2H), δ3.547-3.662 (mult., 5H), δ5.042 (s, br, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ156.4, δ79.6, 65.0, 62.2, 52.3, 28.7, 28.4, 28.0.

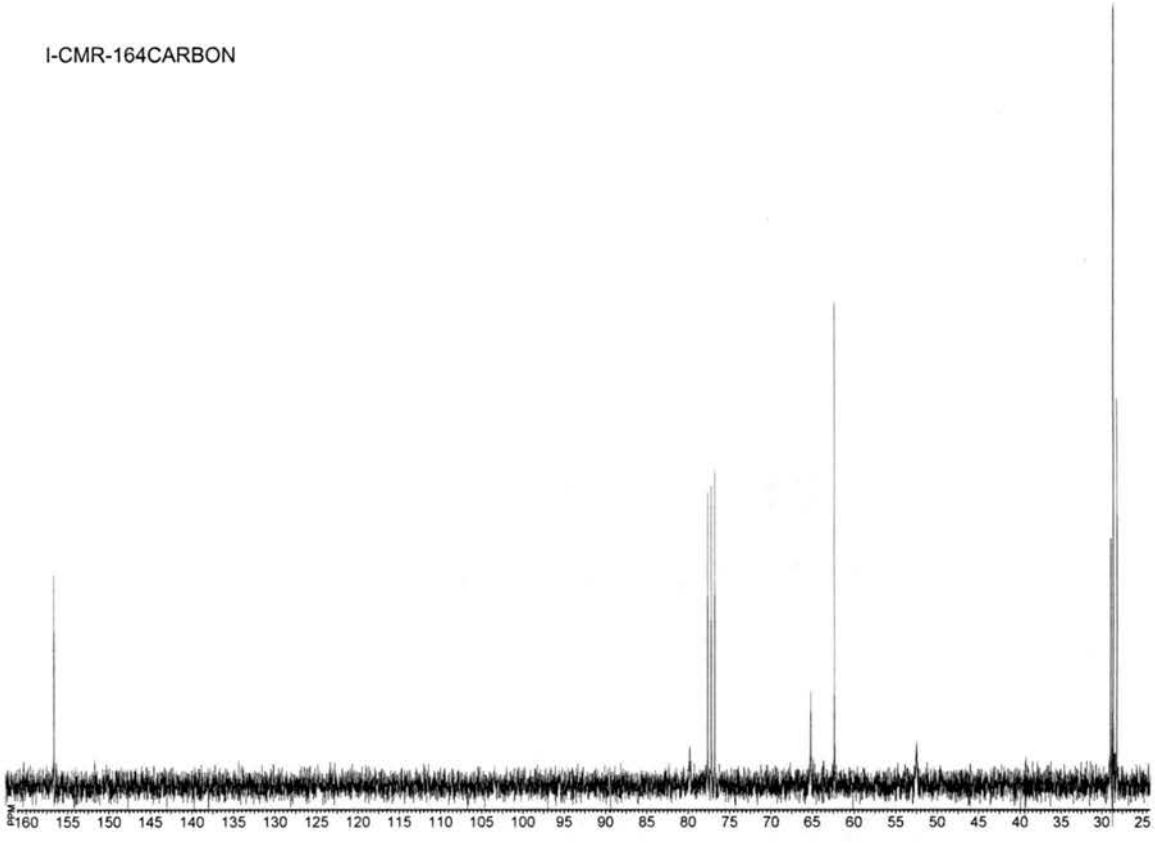
IR (NaCl, neat) 3340, 2936, 1686, 1528, 1366, 1250, 1170, 1049 cm^{-1}.

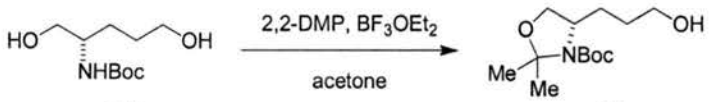
HRMS (FAB+) M+H calc'd. for $C_{10}H_{21}NO_4$ 220.1549, found 220.1542.

I-CMR-164PROTON



I-CMR-164CARBON





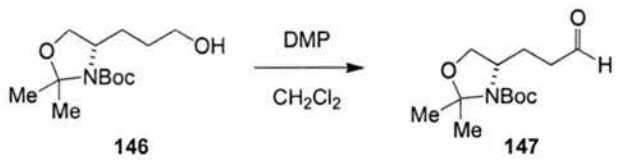
145 **146**

(S)-*tert*-butyl 4-(3-hydroxypropyl)-2,2-dimethyloxazolidine-3-carboxylate (146): To a stirring solution of the diol (**145**, 8.62g, 39 mmol) in acetone (195 mL) and 2,2-dimethoxypropanone (38 mL) at 0°C under argon was added BF_3OEt_2 (3.9 mL, 0.1 mmol) slowly dropwise via syringe. The reaction was warmed to room temperature and monitored for completion by TLC (EtOAc). The product was concentrated by rotary evaporation and crude residue suspended in CH_2Cl_2 (100 mL) and washed successively with sat. aq. $NaHCO_3$ (3 x 100 mL) and brine (100 mL), then dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification in silica gel with 7:3 hexanes/ethyl acetate to 100% ethyl acetate gave the product as a clear yellow oil (3.67g, 36%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ1.439 (s, 9H), δ1.509 (s, 3H), δ1.545 (s, 3H), δ1.794 (mult., 2H), δ2.915 (br s, 1H), δ3.623 (mult. 2H), δ3.701 (d, J=7.2 Hz, 1H), δ3.765 (br s, 1H), δ3.872-3.920 (mult., 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ152.2, 93.3, 79.8, 62.2, 60.3, 57.1, 53.8, 29.5, 28.4, 27.5.

IR (NaCl, neat) 3448, 2978, 2936, 2871, 1694, 1478, 1455, 1392, 1366, 1257, 1175, 1086, 845, 768 cm^{-1}.



146 147

(S)-*tert*-butyl 4-(2-formylethyl)-2,2-dimethyloxazolidine-3-carboxylate (147): To a stirring solution of the alcohol (**146**, 3.67g, 14.2 mmol) in CH_2Cl_2 (142 mL) was added Dess-Martin periodinane (9.03g, 21.3 mmol, 1.5 eq.). The resulting cloudy white solution was stirred at room temperature for 1.5h. The reaction was diluted with Et_2O (200 mL) and $Na_2S_2O_3$ (17.96g, 113.6 mmol) was added followed by sat. aq. $NaHCO_3$ (200 mL) and the biphasic mixture was stirred vigorously until it became clear. The aqueous layer was separated and the organic layer was washed with sat. aq. $NaHCO_3$ (100 ml x 3). Combined aqueous washes were extracted withEt_2O (100 mL x 3). Combined organic extracts were washed with bring (100 mL) then dried (Na_2SO_4) filtered and concentrated by rotary evaporation. Purification by flash chromatography on silica gel with 7:3 hexanes / ethyl acetate gave the product as a clear yellow oil (3.02g, 83%).

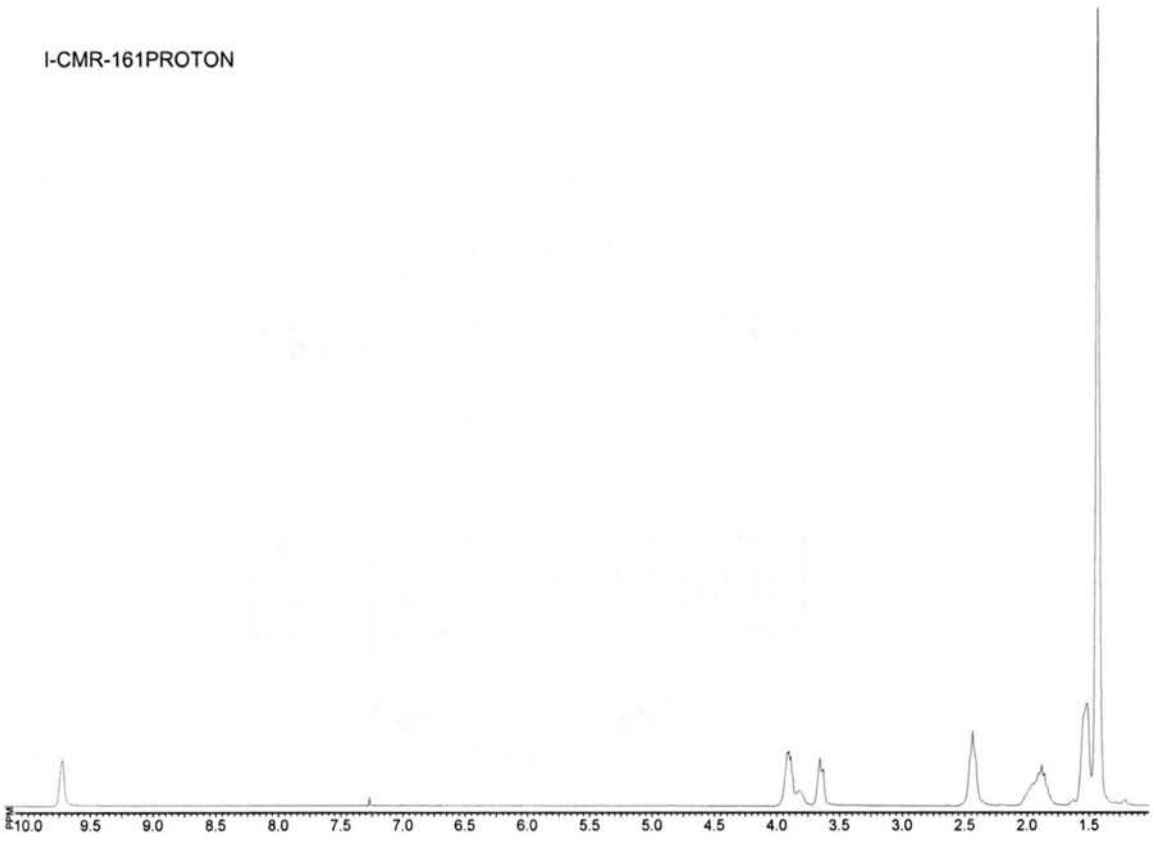
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.476 (s, 9H), δ1.561 (s, 3H), δ1.606 (s, 3H), δ1.870-2.042 (mult., 2H), δ2.463-2.518 (mult., 2H), δ3.696 (d, J=7.5, 1H), δ3.867 (br s, 1H), δ3.926-3.972 (mult., 1H), δ9.779 (s, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ201.3, 93.4, 80.1, 66.9, 56.5, 56.1, 40.4, 28.3, 25.8.

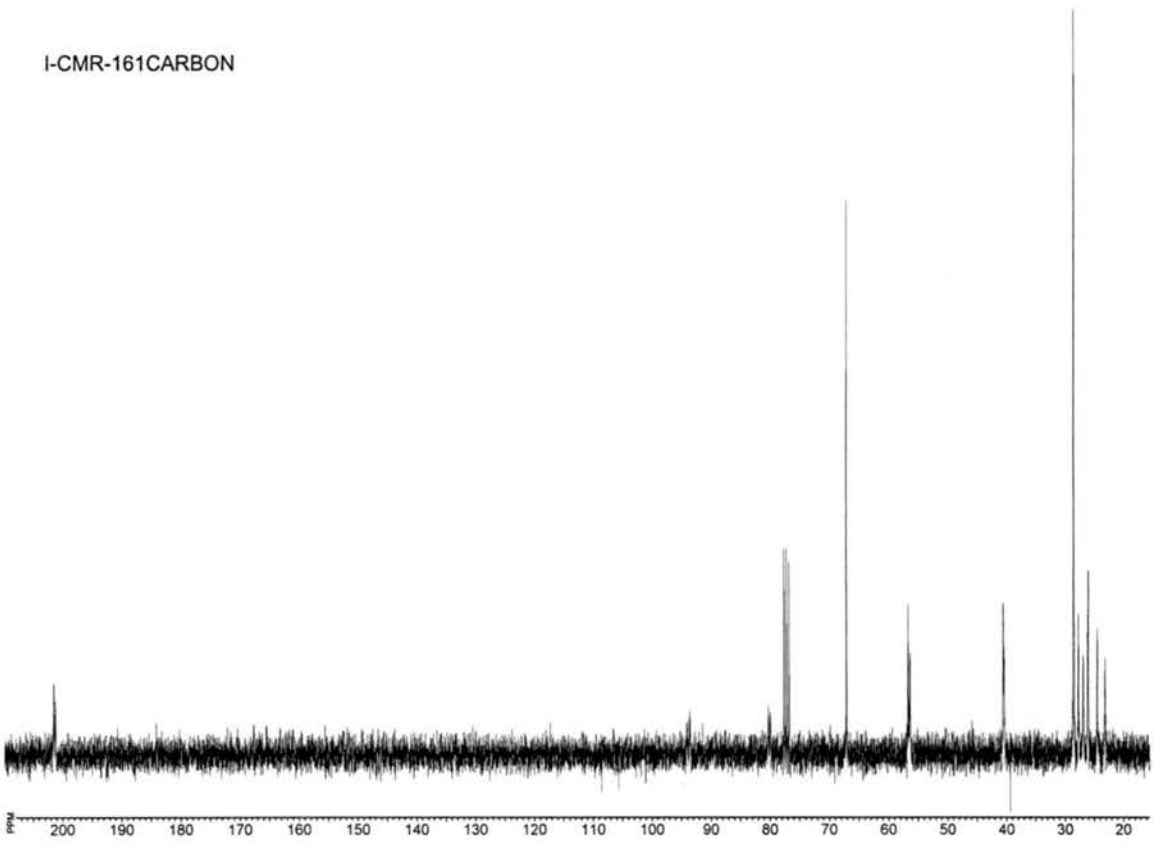
IR (NaCl, neat) 2979, 2936, 2876, 2720, 1725, 1693, 1478, 1455, 1390, 1366, 1257, 1175, 1150, 1083 cm^{-1}.

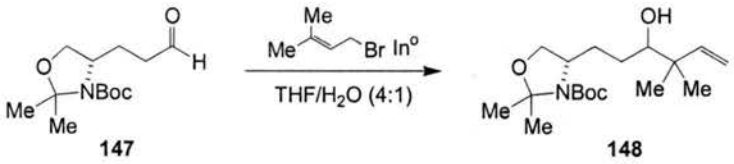
HRMS (FAB+) M+H calc'd. for $C_{13}H_{23}NO_4$ 258.1705, found 258.1710.

I-CMR-161PROTON



I-CMR-161CARBON





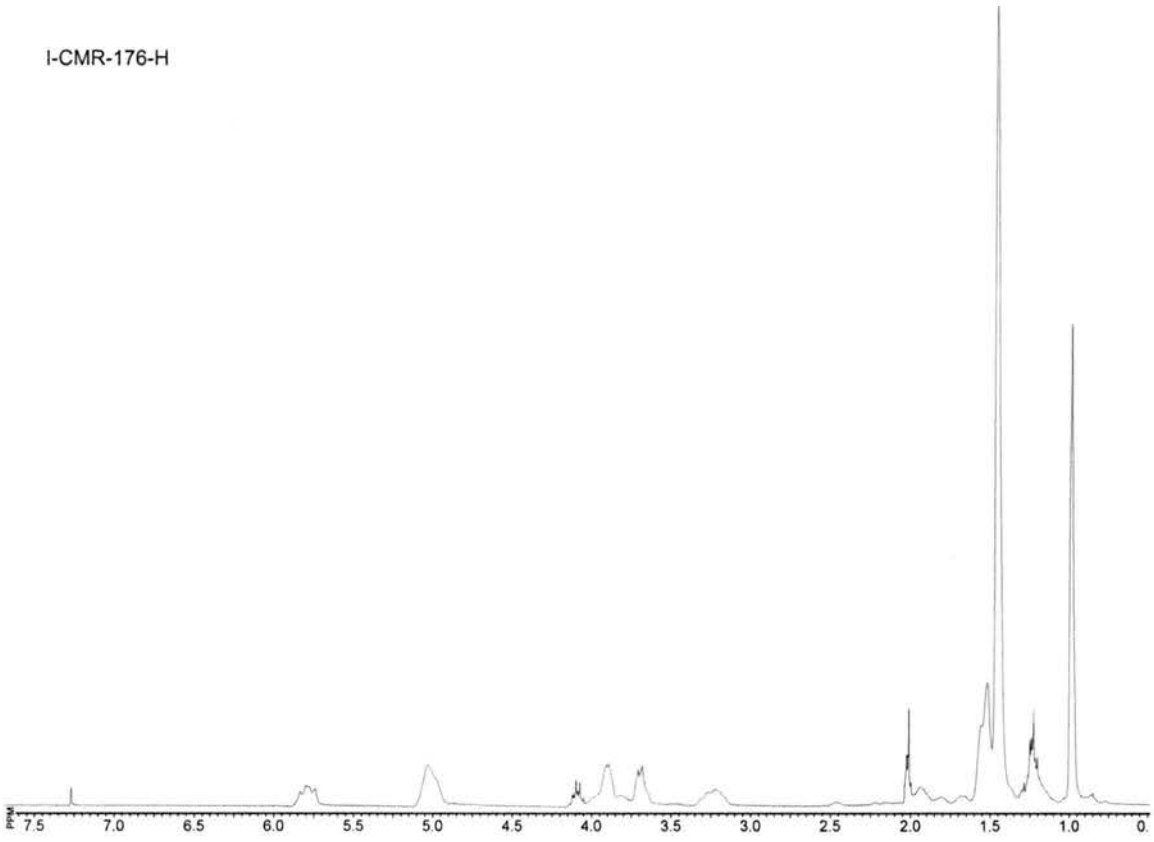
(S)-*tert*-butyl 4-(3-hydroxy-4,4-dimethylhex-5-enyl)-2,2-dimethyloxazolidine-3-carboxylate (148): To a stirring solution of the aldehyde (**147**, 3.491g, 13.6 mmol) in THF (54 mL) and H_20 (216 mL) was added prenyl bromide (2.34 mL, 20.3 mmol) and indium powder (1.714g, 14.9 mmol). The resulting mixture was stirred at room temperature for 1h then extracted with Et_2O (100 mL x 3). Combined organic extracts were washed with brine (100 mL) then dried (Na_2SO_4) filtered and concentrated by rotary evaporation. Purification by flash chromatography with 7:3 hexanes / ethyl acetate gave the product, a mixture of diastereomers, as a clear colorless oil (3.56g, 80%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ1.476 (s, 9H), δ1.561 (s, 3H), δ1.606 (s, 3H), δ1.870-2.042 (mult., 2H), δ2.463-2.518 (mult., 2H), δ3.696 (d, J=7.5, 1H), δ3.867 (br s, 1H), δ3.926-3.972 (mult., 1H), δ9.779 (s, 1H).

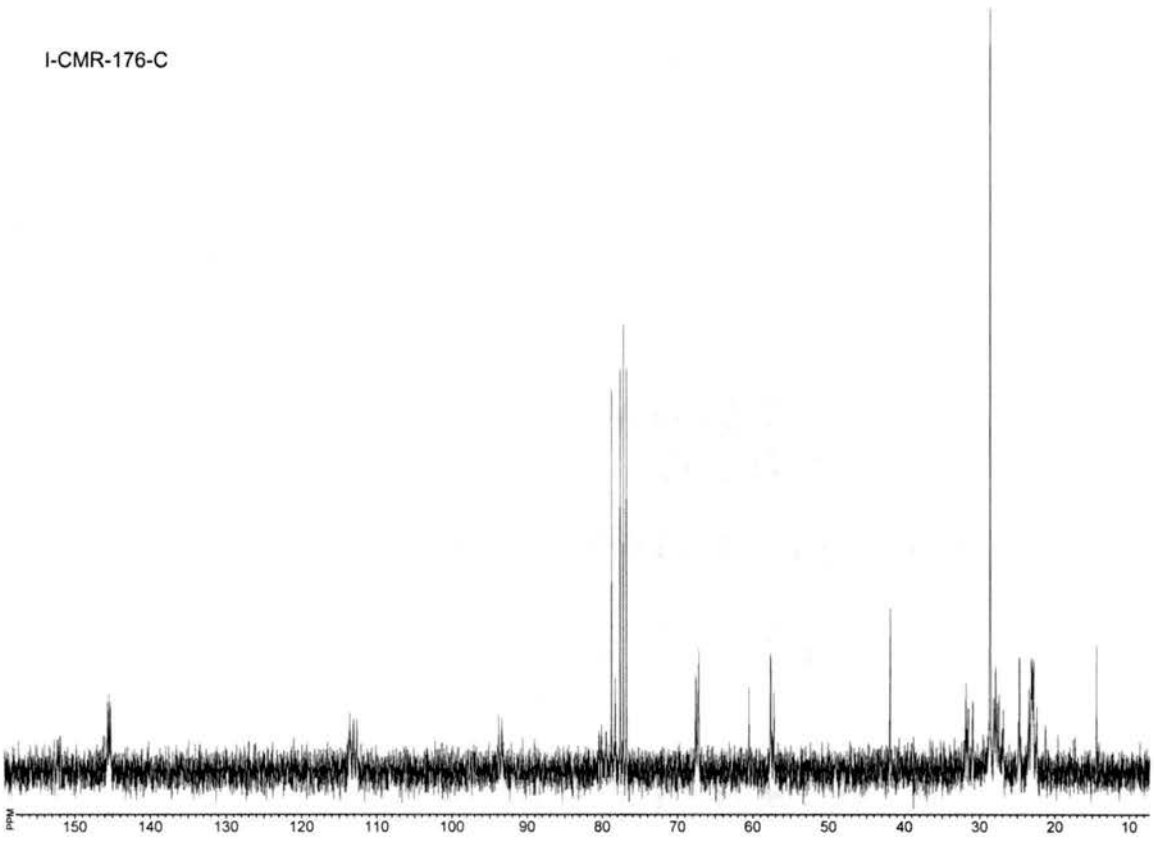
^{13}C NMR ($CDCl_3$, 75 MHz) δ145.5, 145.2, 145.1, 113.4, 112.9, 93.6, 93.2, 78.6, 78.1, 67.4, 67.0, 60.3, 57.5, 57.0, 41.7, 31.6, 31.3, 30.7, 28.4, 27.9, 27.7, 27.,5 27.2, 26.7, 24.5, 23.3, 22.9, 22.7, 22.2., 21.0, 14.2.

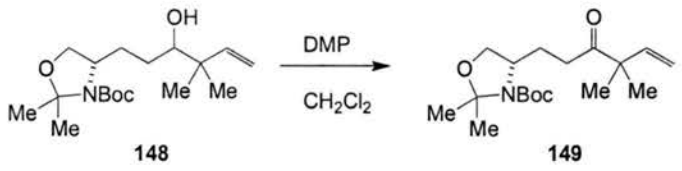
HRMS (FAB+) M+H calc'd. for $C_{18}H_{33}NO_4$ 328.2488, found 328.2484.

I-CMR-176-H



I-CMR-176-C





(S)-*tert*-butyl 2,2-dimethyl-4-(4,4-dimethyl-3-oxohex-5-enyl)oxazolidine-3-carboxylate (149): To a stirring solution of the alcohol (**148**, 1.00g, 3.05 mmol) in CH_2Cl_2 (30 mL) was added Dess-Martin periodinane (1.94g, 4.58 mmol, 1.5 eq.) at room temperature. The resulting cloudy white solution was stirred at room temperature for 2h. The reaction was then diluted with Et_2O (60 mL) and $Na_2S_2O_3$ (3.85g, 24.4 mmol) was added followed by sat. aq. $NaHCO_3$ (60 mL) and stirred vigorously until the biphasic solution became clear. The aqueous layer was separated and the organic layer was washed with sat. aq $NaHCO_3$ (50 ml x 3). The combined aqueous washings were then extracted with Et_2O (50 mL x 3). Combined organic extracts were washed with brine (50 mL) then dried (Na_2SO_4) filtered and concentrated by rotary evaporation. Purification by flash chromatography on silica gel with 7:3 hexanes / ethyl acetate gave the product as a clear colorless oil (794 mg, 80%).

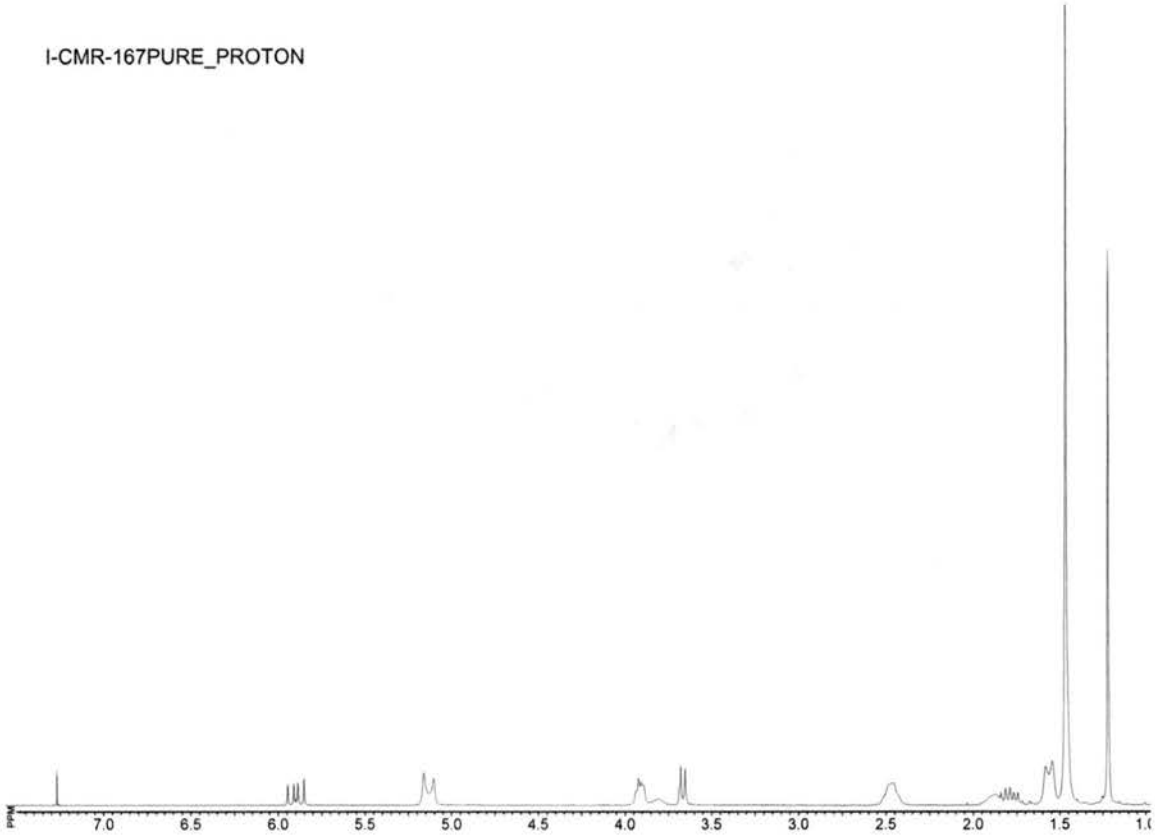
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.22 (s, 6H), δ1.47 (s, 12H), δ1.56 (d, J=11.35 Hz, 3H), δ1.70-1.98 (m, 2H), δ2.47 (d, J=5.49 Hz, 2H), δ3.67 (d, J=7.69 Hz, 1H), δ3.81 (bs. s, 1H), δ3.92(d, J=4.39Hz, 1H), δ5.13 (d, J=16.85 Hz, 2H), δ5.90 (dd, J=17.58, 10.62 Hz, 1H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ142.3, 114.4, 114.1, 80.0, 67.1, 56.8, 56.7, 34.2, 33.9, 28.5, 27.9, 27.7, 26.7, 24.5, 23.6, 23.2.

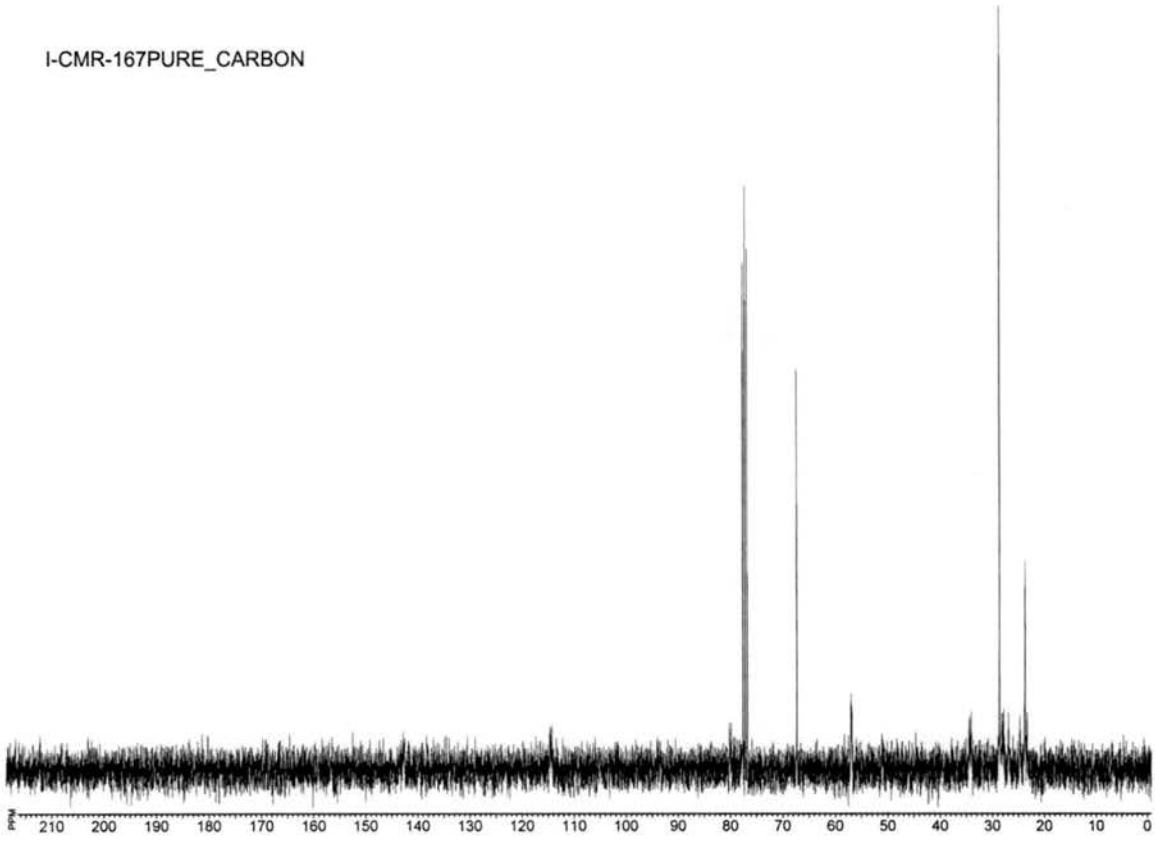
IR (NaCl, neat) 2977, 2935, 2873, 1697, 1388, 1365, 1257, 1175, 1087 cm^{-1}.

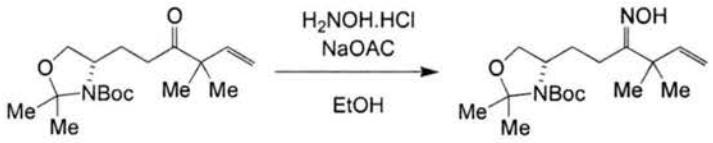
HRMS (FAB+) M+H calc'd. for $C_{18}H_{31}NO_4$ 326.2331, found 326.2332.

I-CMR-167PURE_PROTON



I-CMR-167PURE_CARBON





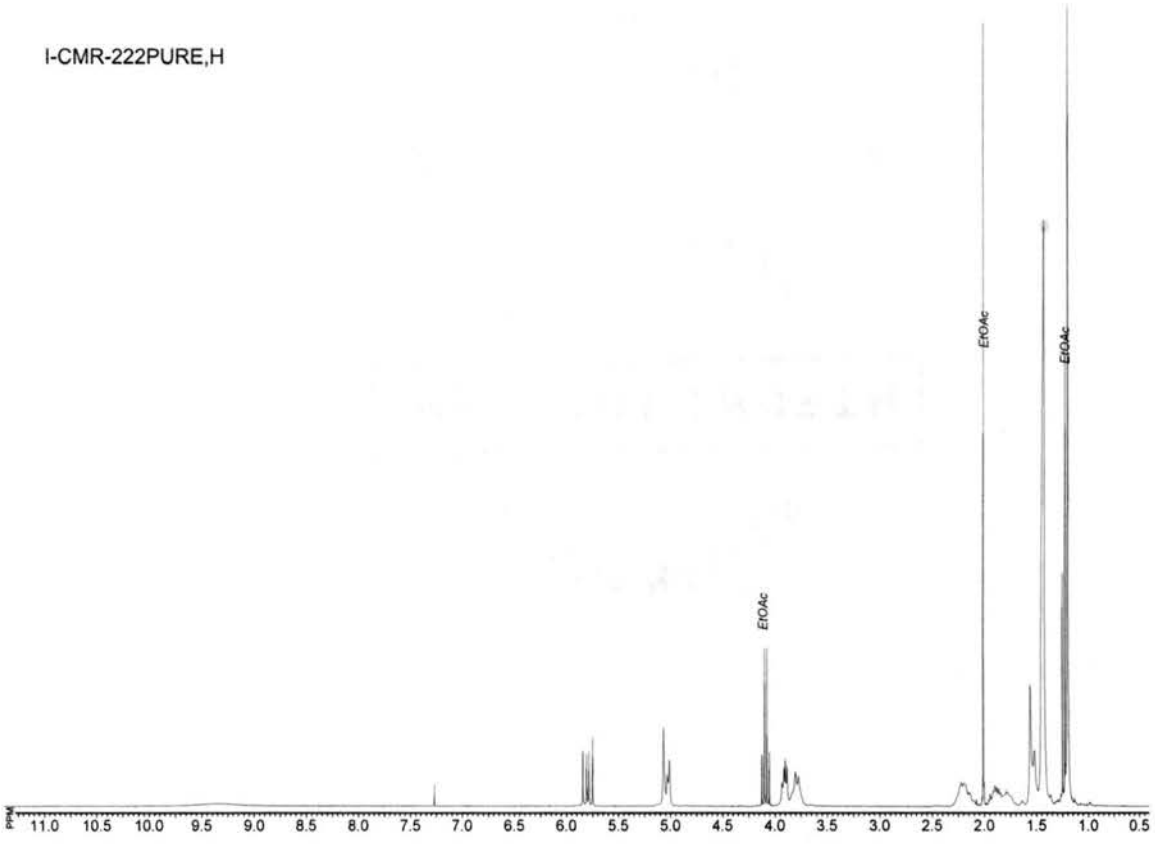
149 150

(S)-*tert*-butyl 2,2-dimethyl-4-(4,4-dimethyl-3-oxohex-5-enyl)oxazolidine-3-carboxylate oxime (150): NaOAc (0.195 g, 2.377 mmol, 3.0 eq) measured into a two-neck round bottom flask. Ketone **149** (0.258 g, 0.7924 mmol) dissolved in EtOH (4 x 2 ml) and transferred via pipet to the reaction flask and flushed with argon for 15min. $H_2NOH \cdot HCl$ (0.087 g, 1.252 mmol, 1.5 eq) was added and the reaction was heated to reflux for 26h. Solvent was removed by rotary evaporation and the solid residue was dissolved in 1M HCl (~3 ml) and diluted with $CHCl_3$ (~2 ml). Solution was made basic with with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ (3 x 25 ml); organic fractions were combined, washed with brine (50 ml), dried (Na_2SO_4), filtered and solvent removed by rotary evaporation. Crude oil was purified on silica gel with 4:1 diethyl ether/pentane to give the product as a clear thick oil (0.205 g, 0.6023 mmol, 76%).

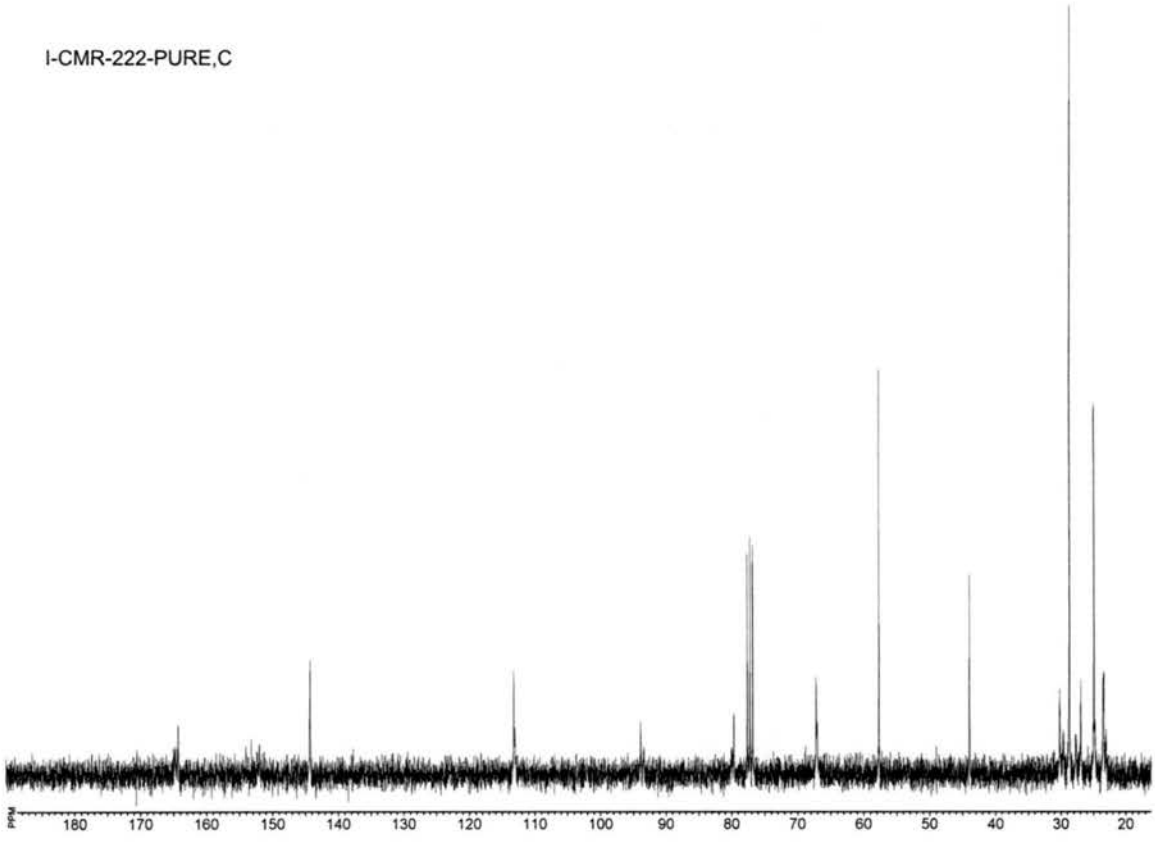
^1H NMR ($CDCl_3$, 300 MHz) δ1.22 (br. s., 6H), δ1.45 (br. s, 12H), δ1.56 (d, J=11 Hz, 6H), δ1.70-2.03 (m, 3H), δ2.21 (br. s, 2H), δ3.70-4.06 (m, 3H), δ5.06 (d, J=15.75 Hz, 2H), δ5.82 (dd, J=17.03, 10.8 Hz, 1H)

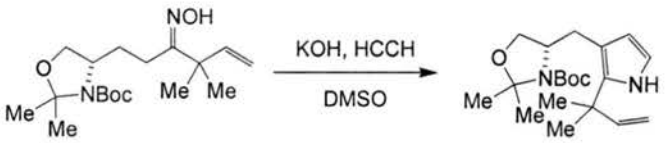
^{13}C NMR ($CDCl_3$, 75 MHz) δ16.6, 164.1, 152.2, 151.8, 144.1, 113.0, 93.7, 79.5, 67.0, 57.5, 43.7, 30.0, 28.5, 26.7, 24.7, 23.2.

I-CMR-222PURE,H



I-CMR-222-PURE,C





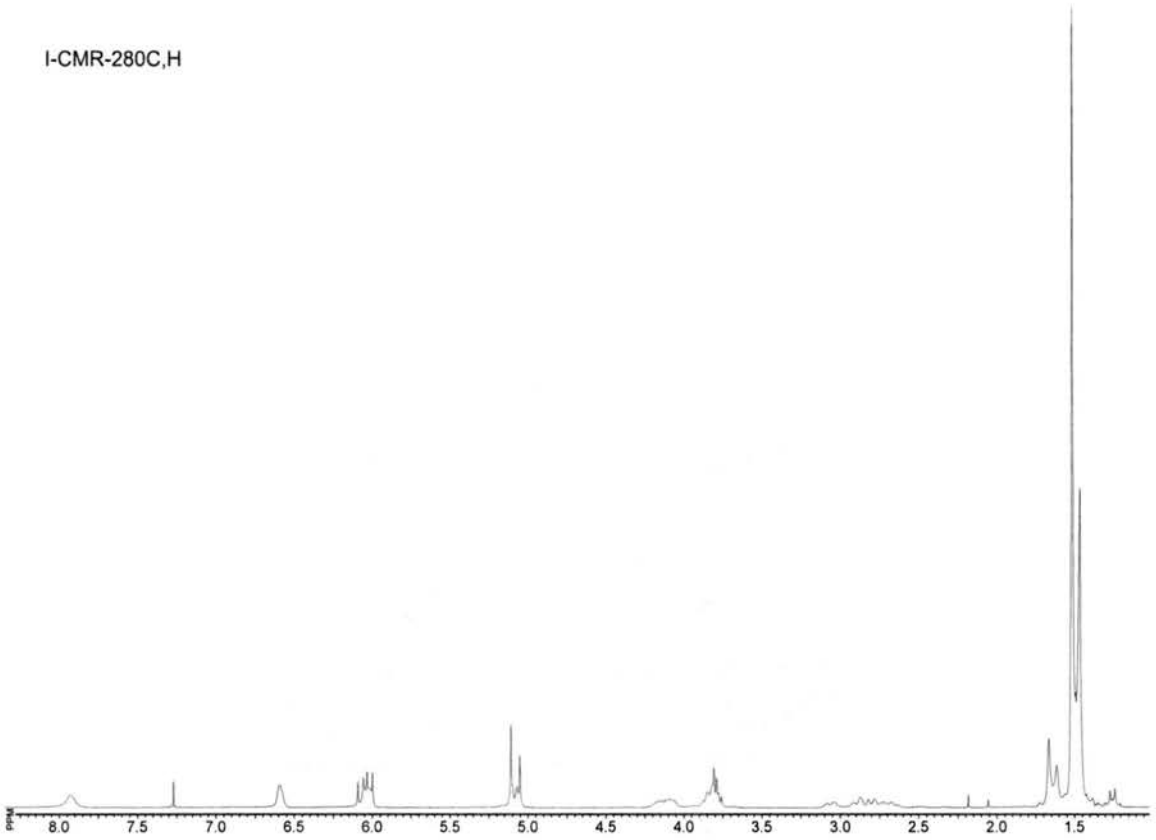
150 151

(S)-*tert*-butyl 2,2-dimethyl-4-((2-(2-methylbut-3-en-2-yl)-1H-pyrrol-3-yl)methyl)oxazolidine-3-carboxylate (151): To a stirring solution of pulverized KOH (19.3 mg, 0.343 mmol, 1.04 eq) in wet DMSO (7 mL) in a high pressure reaction vessel at 85°C was added the oxime (112 mg, 0.329 mmol). The reaction flask was fitted with a pressure head and the sealed flask was evacuated and filled with argon (repeated 3 times). The reaction flask was then evacuated and pressurized to 20 psi with acetylene (repeated 3 times). The acetylene pressure was then adjusted to 10 psi and the reaction was stirred at 85°C for 1h 15 min. After cooling to room temperature the reaction was diluted with H_2O (3 mL) and brine (9 mL) and extracted with 1:1 Et_2O / EtOAc (30 mL x 4). Combined organic etracts were dried ($MgSO_4$) filtered and concentrated by rotary evaporation. Purification by flash chromatography on silica gel with 6:1 hexanes / ethyl acetate gave the product as a pale yellow waxy solid (54 mg, 0.155 mmol, 47%).

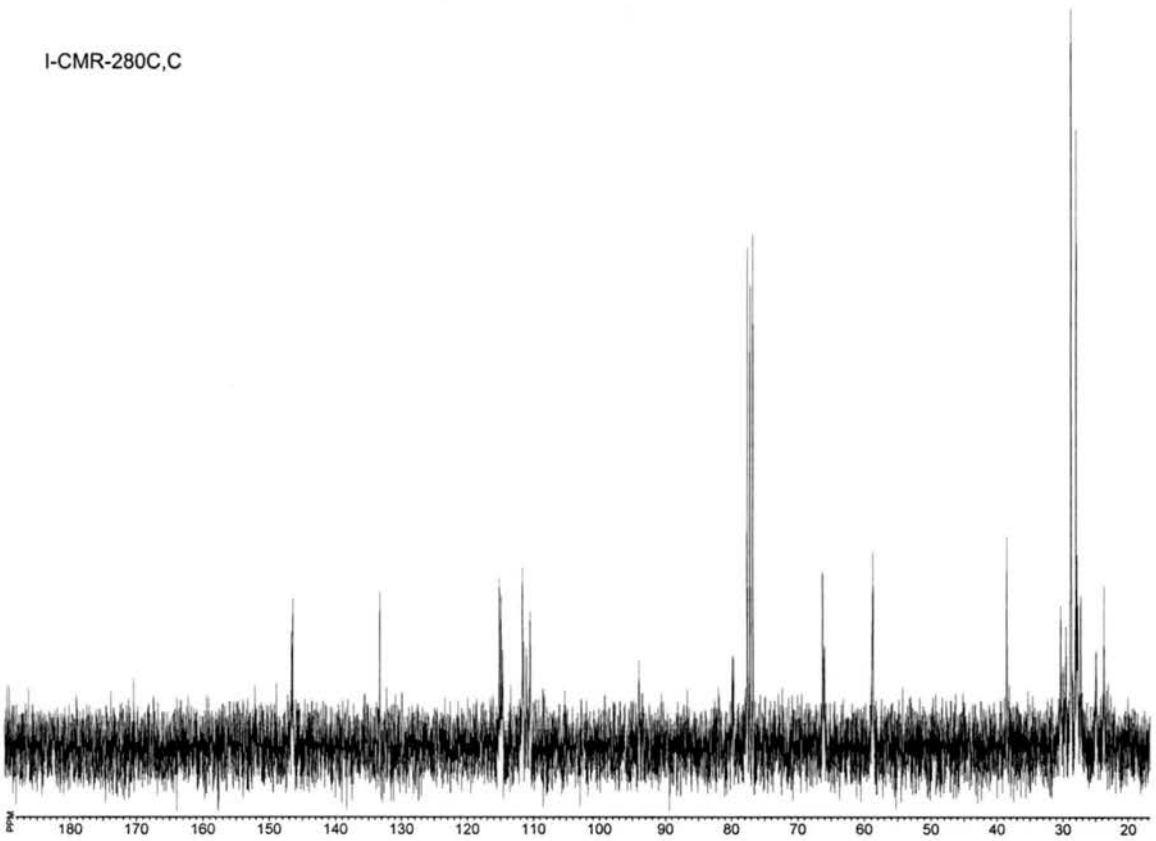
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.50 (d, J= 14 Hz, 6H), δ1.52 (s, 9H), δ1.62 (m, 6H), δ2.58-3.17 (m, 2H), δ3.70-3.92 (m, 2H), δ3.99-4.26 (m, 1H), 5.01-5.26,(m, 2H), δ5.95-6.15 (m, 2H), δ6.59 (br s, 1H), δ7.93 (br s, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ146.40, 133.04, 114.93, 111.44, 110.84, 110.29, 93.78, 79.72, 79.51, 66.08, 58.52, 38.34, 30.12, 28.59, 27.81, 27.06.

I-CMR-280C,H



I-CMR-280C,C



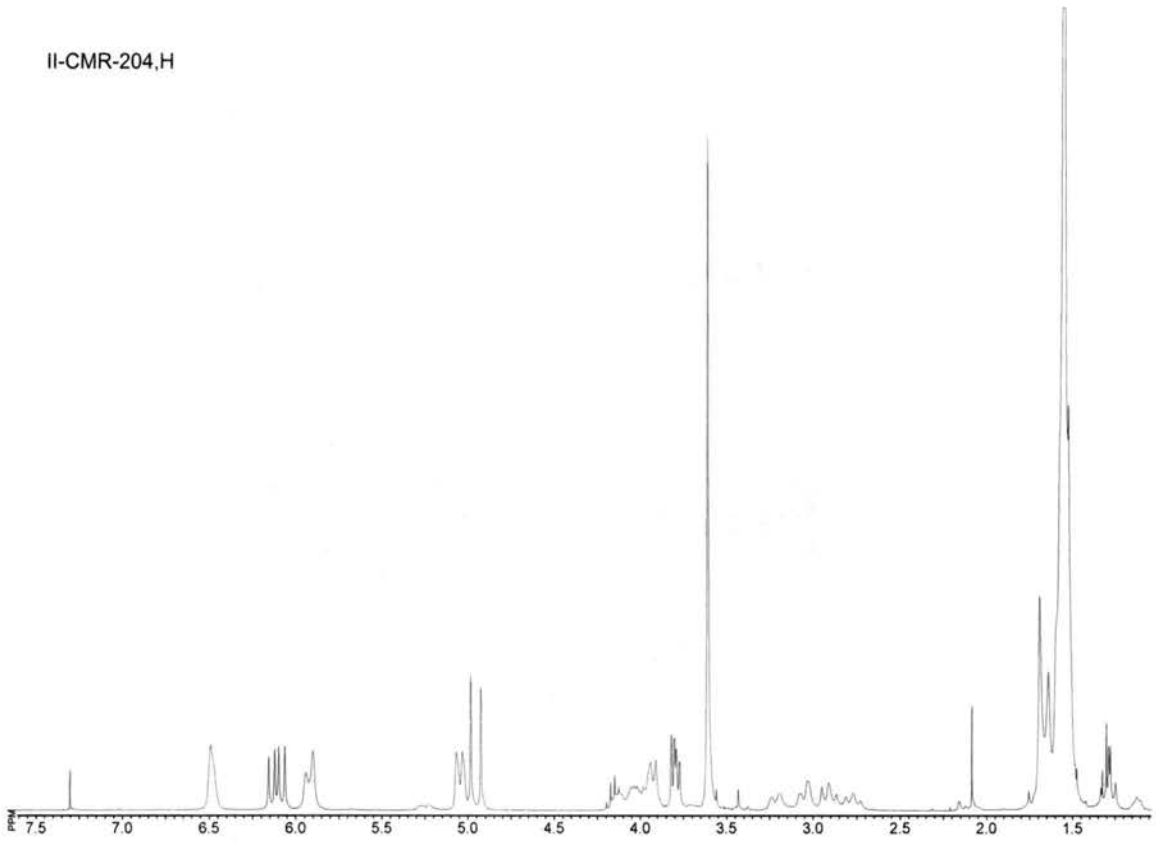
151 157

(S)-*tert*-butyl 2,2-dimethyl-4-((2-(2-methylbut-3-en-2-yl)-1-methyl--pyrrol-3-yl)methyl)oxazolidine-3-carboxylate (157): To a stirring solution of the pyrrole **151** (0.150 g, 0.4304 mmol) in dry DMF (4 mL) under argon was added NaH (0.039 g, 1.625 mmol, 3.8 eq) and stirred at room temperature for 45 minutes. Iodomethane (0.046 mL, 0.7389 mmol, 1.7 eq) was added and stirred under argon for an additional 25 hours. The mixture was cooled in an ice bath and the reaction was quenched by the drop-wise addition of distilled water and extracted with EtOAc (8 mL x 4). Combined organic extracts were dried (Na_2SO_4) filtered and concentrated by rotary evaporation. Purification by flash chromatography on silica gel with 6:1 hexanes / ethyl acetate gave the product as a waxy solid (0.153 g, 4.223 mmol, 98%)

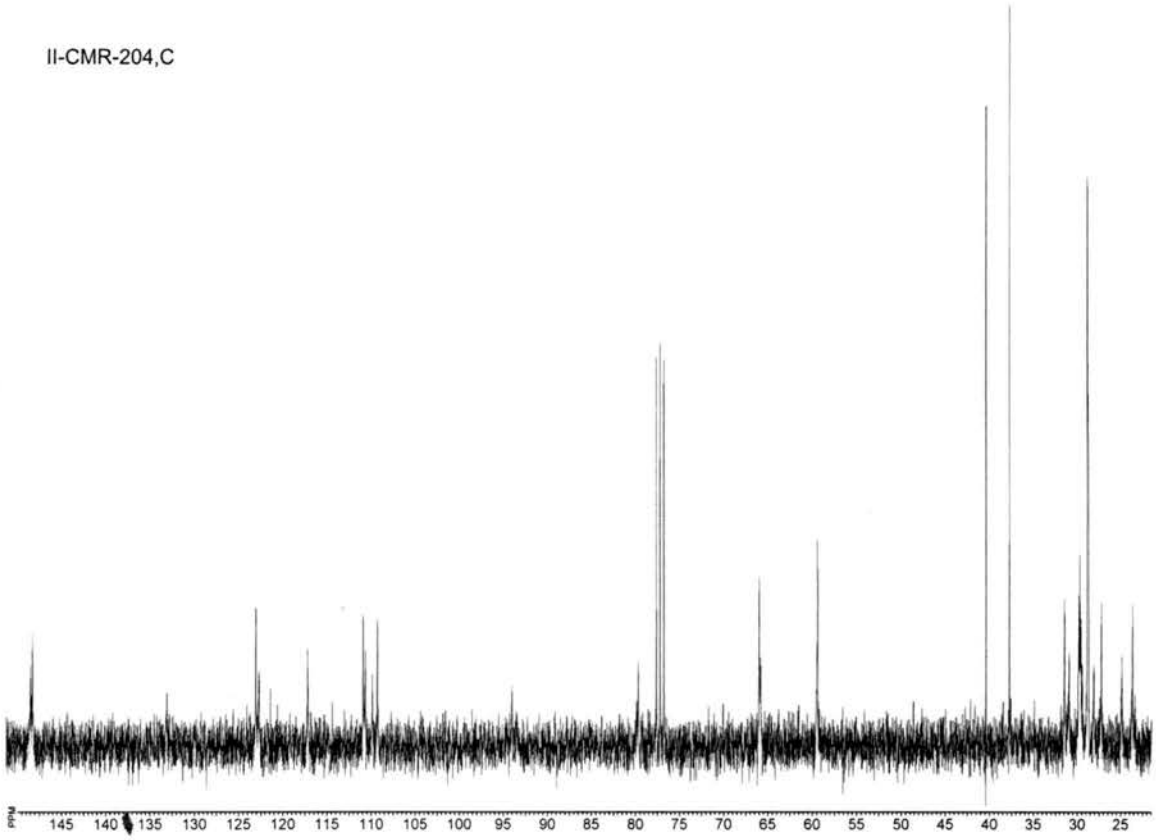
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.51 (s, 18H), δ1.63 (d, 6H, 14.65 Hz), δ2.66-2.80(m, 1H), δ 2.80-2.95 (m, 1H), δ2.96-3.09 (mult., 1H), δ3.18(d, J=13.18 Hz, 1H), δ3.58 (s, 3H), δ3.76 (dd, 1H, 8.42, 5.49 Hz), δ3.97-4.05 (mult., 1H), δ4.05-4.18 (m, 1H), δ4.92 (d, J=17.58 Hz, 1H), δ5.02 (d, 9.89 Hz, 1H), δ5.89 (d, 11.72 Hz, 1H), δ6.08 (dd, J=17.40, 10.44Hz, 2H) δ6.46 (br s., 1H).

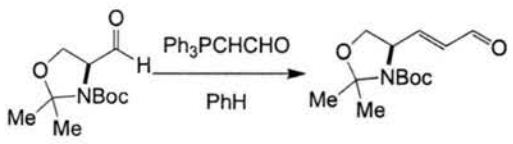
^{13}C NMR ($CDCl_3$, 75 MHz) δ148.03, 122.81, 122.24, 116.97, 110.68, 110.42, 109.06, 79.46, 65.77, 65.60, 59.18, 40.19, 37.53, 31.26, 29.52, 28.62, 27.90, 27.02, 24.68.

II-CMR-204,H



II-CMR-204,C





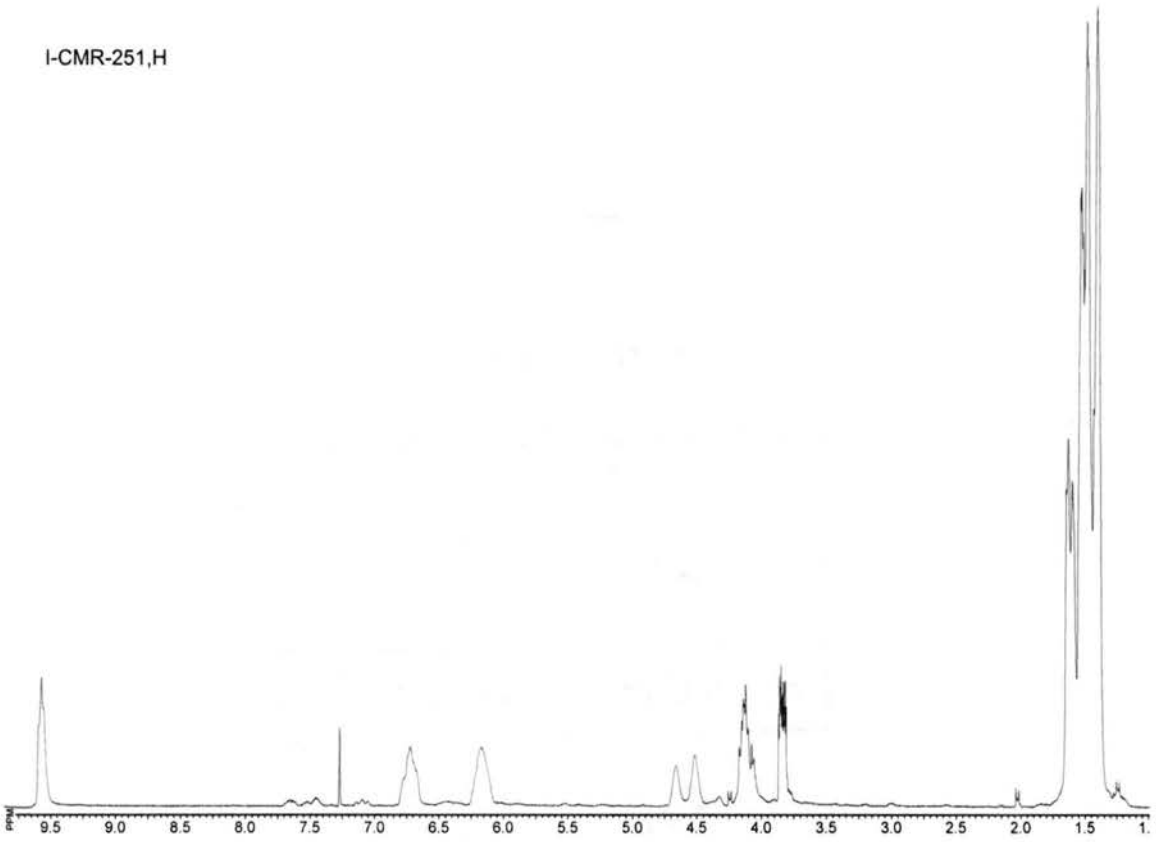
229 **255**

To a stirring solution of the aldehyde 229 (8.516 g, 37.14 mmol) in benzene (200 mL) was added (Triphenylphosphoranylidene)acetaldehyde (11.6 g, 38.12 mmol, 1.03 eq). The resulting solution was heated to reflux for 22h. After cooling the solvent was removed by rotary evaporation and the triphenylphosphine oxide was precipitated with EtOAc and removed by filtration. Solvent was again removed by rotary evaporation. Purification by flash chromatography on silica gel with 7:3 hexanes/EtOAc gave the product as a colorless oil. (7.445 g, 29.16 mmol, 78.5%)

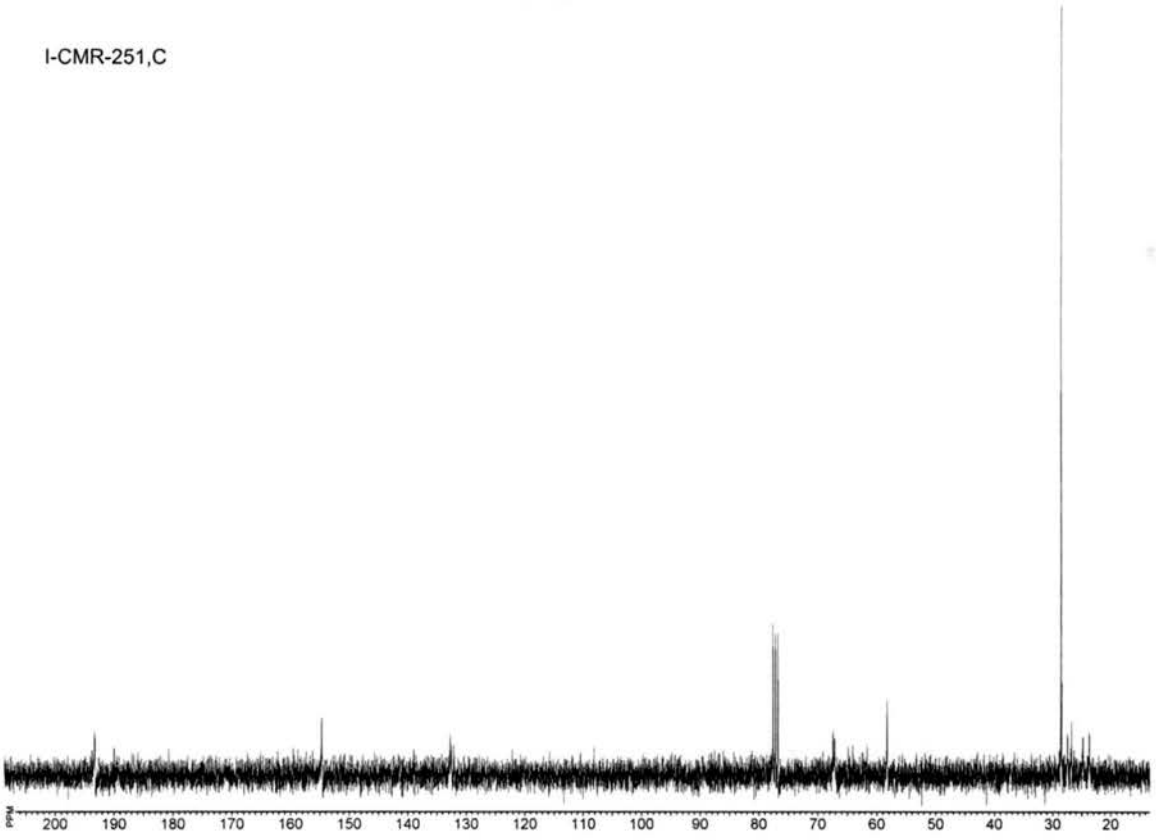
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.35-1.50 (m, 9H), δ1.52 (br. s, 3H), δ1.61 (d, J=9.52 Hz, 3H), δ3.84 (dt, J=5.95, 3.07, 3.07 Hz, 1H), δ4.00-4.26 (m, 1H), δ4.44-4.73 (m, 1H), δ6.17 (br. s, 1H), δ6.72 (br. s, 1H), 9.57 (br. s, 1H)

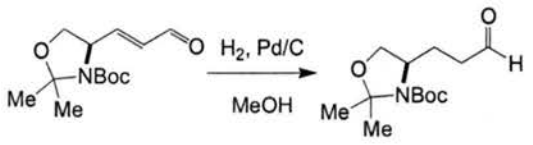
^{13}C NMR ($CDCl_3$, 75 MHz) δ15.4, 132.5, 132.3, 67.3, 67.0, 58.1, 28.4, 27.3, 26.6, 24.6, 23.5.

I-CMR-251,H



I-CMR-251,C





156 147

A stirring solution of the α,β-unsaturated aldehyde (156, 1.04 g, 4.073 mmol) in methanol (41mL) was degassed with argon for 30min. Under positive argon pressure was added Pd/C (0.020g, 0.005g/mmol). The resulting mixture was degassed under argon for 15 min, followed by hydrogen for 30 min then stirred under 1 atm hydrogen gas for 4h. After degassing with argon for 30 min the mixture was filtered through celite and contentrated by rotary evaporation. Purification by flash chromatography on silica gel with 7:3 hexanes/EtOAc gave the product as a colorless oil (0.75 g, 2.915 mmol, 72%)

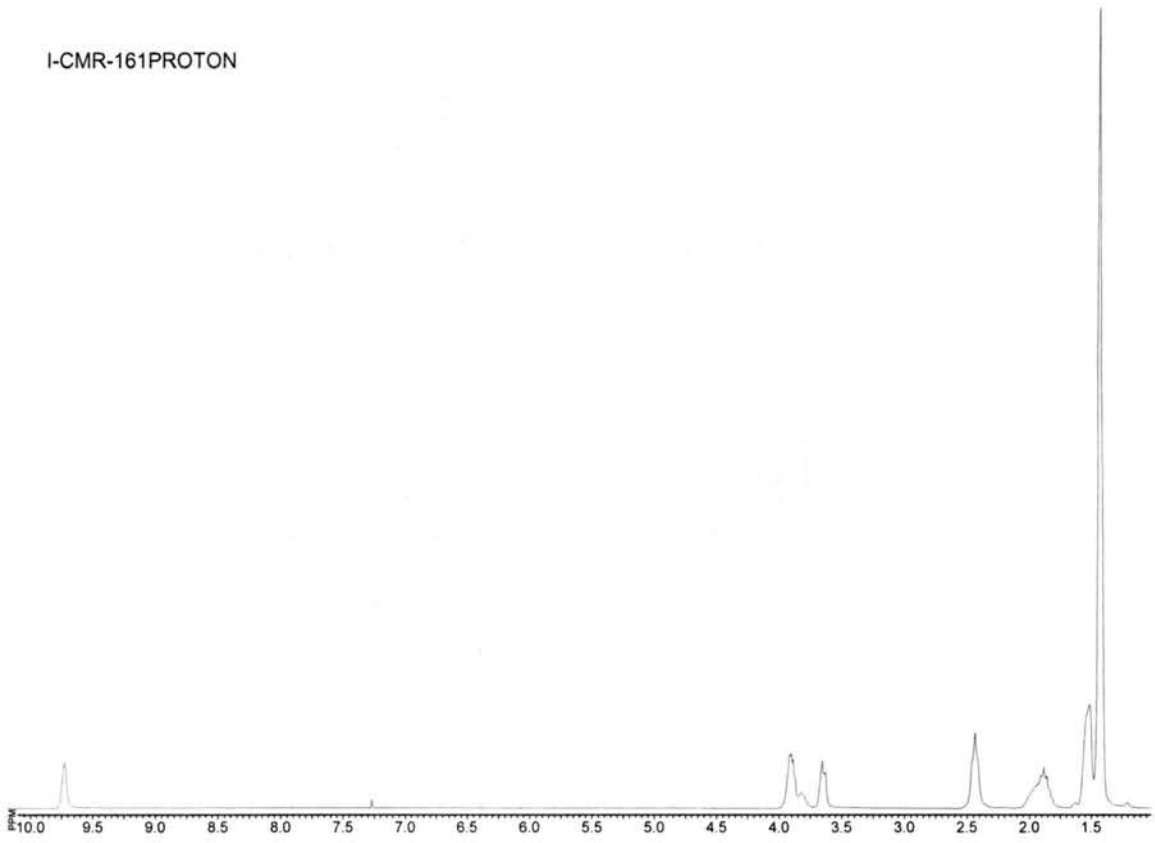
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.476 (s, 9H), δ1.561 (s, 3H), δ1.606 (s, 3H), δ1.870-2.042 (mult., 2H), δ2.463-2.518 (mult., 2H), δ3.696 (d, J=7.5, 1H), δ3.867 (br s, 1H), δ3.926-3.972 (mult., 1H), δ9.779 (s, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ201.3, 93.4, 80.1, 66.9, 56.5, 56.1, 40.4, 28.3, 25.8.

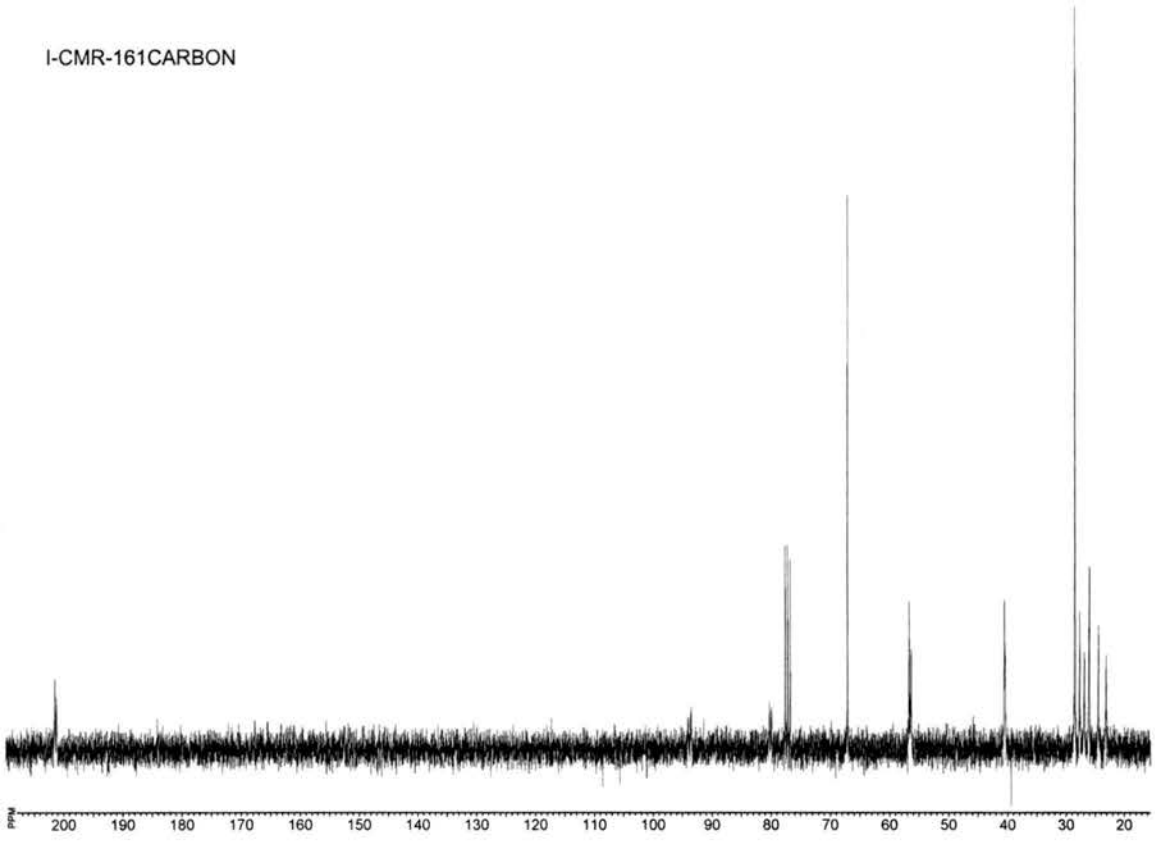
IR (NaCl, neat) 2979, 2936, 2876, 2720, 1725, 1693, 1478, 1455, 1390, 1366, 1257, 1175, 1150, 1083 cm^{-1}.

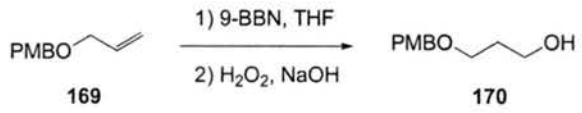
HRMS (FAB+) M+H calc'd. for $C_{13}H_{23}NO_4$ 258.1705, found 258.1710.

I-CMR-161PROTON



I-CMR-161CARBON





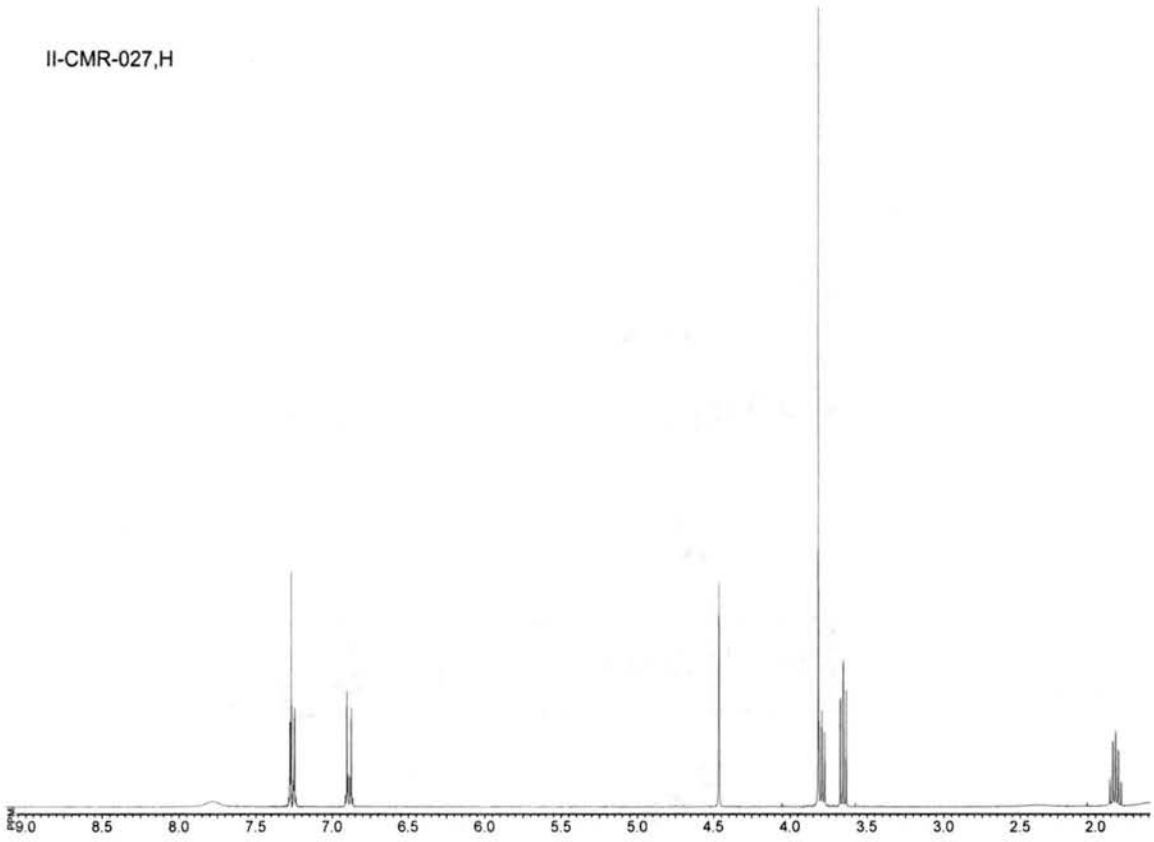
169 **170**

3-(4-methoxy-benzyloxy)-propan-1-ol (170): 9-BBN (35 ml, 0.5M in THF) added to a stirring solution of alkene **169** (1.964g, 11.96 mmol) in distilled THF (120 ml) under argon and heated to reflux for 5h. Reaction then allowed to cool to room temperature and stir under argon for 18h. 6M NaOH (12 ml) added followed by the slow addition of 30% aqueous H_2O_2 (24 ml). The resulting solution was heated to reflux under argon for 1.5h and then allowed to cool to room temperature and neutralized with saturated aqueous NH_4Cl. Solution was extracted with diethyl ether (3 x 120 ml); organic fractions combined, dried (Na_2SO_4), filtered and solvent removed by rotary evaporation. Crude oil was purified on silica gel with 1:1 hexanes/ethyl acetate to give the product as a clear oil (2.176g, 11.94 mmol, 99%).

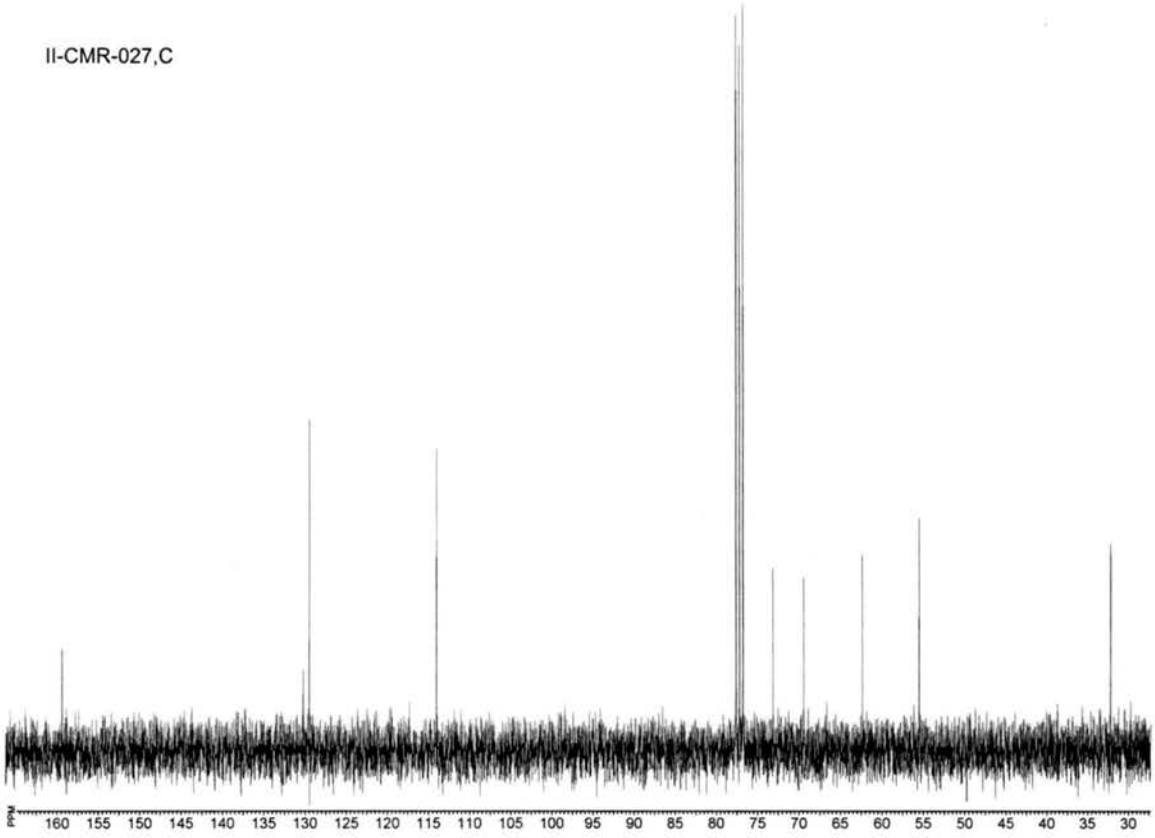
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.863 (., J = Hz, H), 3.648 (t, J = 5.86 Hz, 2H), 3.784(t, J = 5.86 Hz, H), 3.814(s, 3H), 4.462(s, 2H),8.388 (d, J = 8.79Hz, 2H), 7.261(d, J = 8.42 Hz, 2H),,.

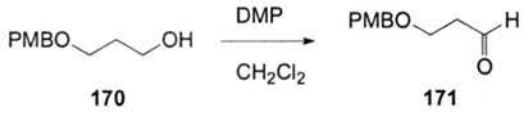
^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.1, 130.0, 129.3, 113.8, 73.0, 69.2, 62.2, 55.3, 32.0.

II-CMR-027,H



II-CMR-027,C





170 171

3-(4-methoxy-benzyloxy)-propionaldehyde (171):

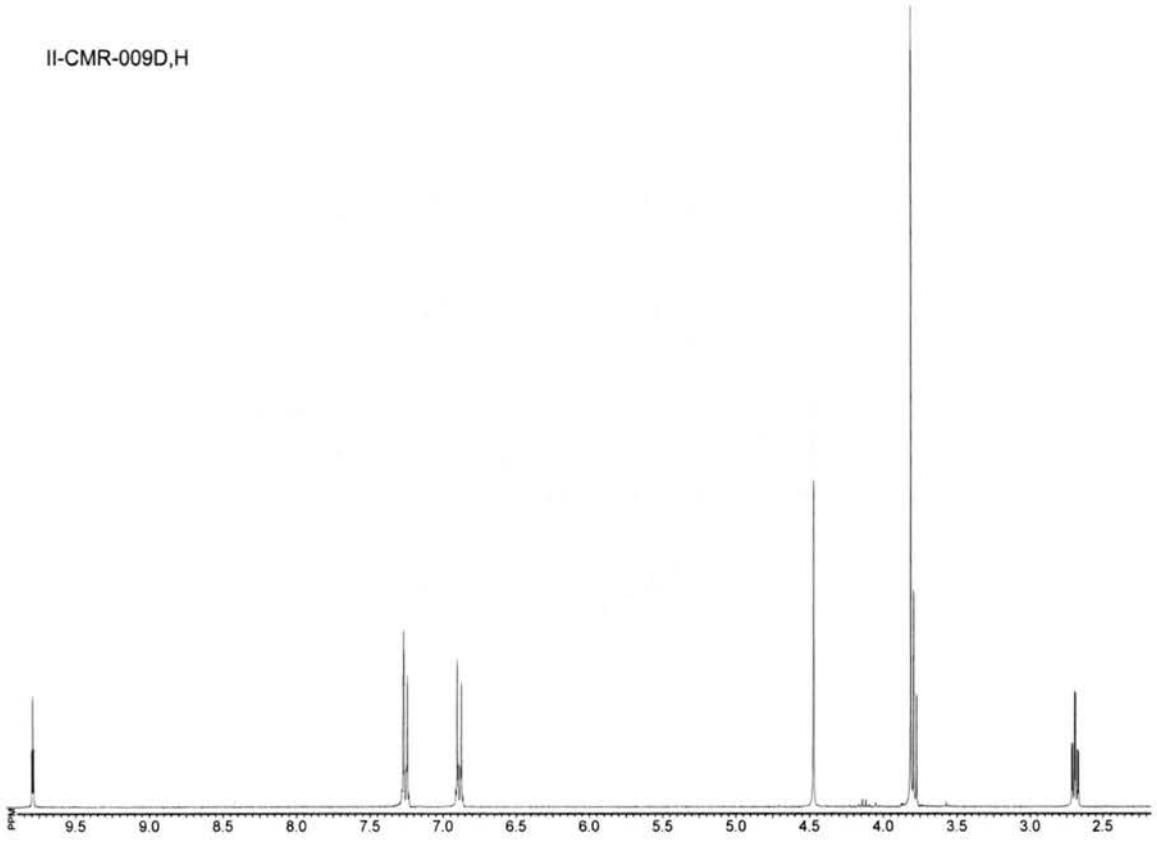
Dess-Martin reagent (8.69g, 20.49 mmol, 1.5 eq) added to a stirring solution of the alcohol **170** (2.60g, 13.25 mmol) in CH_2Cl_2 (110 ml) at room temperature for 1.5h. The cloudy white solution was diluted with diethyl ether (8 ml). $Na_2S_2O_3$ (19.19g, 121.4 mmol, 9 eq) was added followed by saturated aqueous $NaHCO_3$ (250 ml) and stirred until the biphasic layers were clear. The mixture was then poured into a separatory funnel; the aqueous layer was separated and the organic layer was washed with saturated aqueous $NaHCO_3$ (3 x 50 ml); the aqueous fractions were combined and extracted with diethyl ether (3 x 100 ml); organic fractions were combined, washed with brine (100 ml), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. The crude oil was purified on silica gel with 7:3 to 1:1 hexanes/ethyl acetate to give the product as a clear oil (1.35 g, 6.951 mmol, 70%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ2.697(dt, J = 5.86 Hz, 2H), 3.800(t ,J = 5.86 Hz, 2H), 3.821(s, 3 H), 4.477(s, 2H), 6.895(d, J = 8.43Hz, 2H), 7.263(d, J = 8.05Hz, 2H), 9.799(t, J = 1.47Hz, 1H).

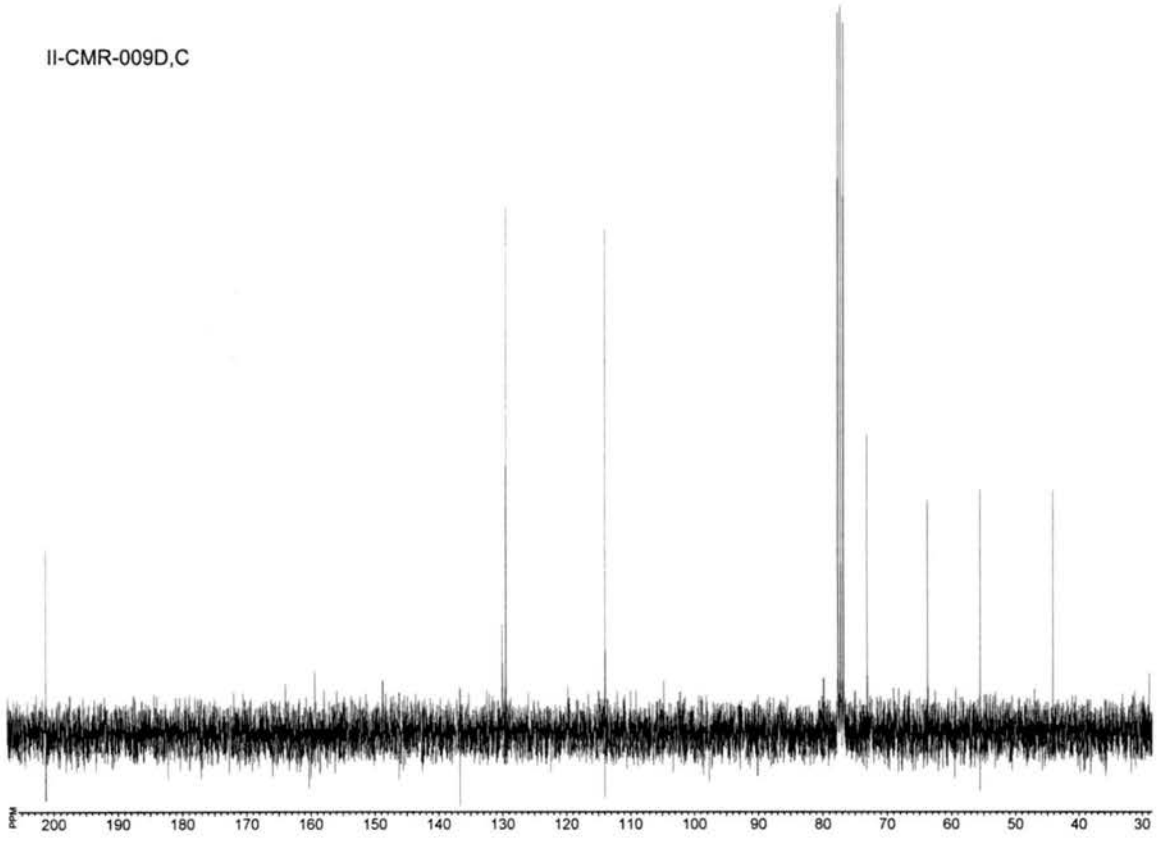
^{13}C NMR ($CDCl_3$, 75 MHz) δ201.1, 129.9, 129.3, 113.8, 72.9, 63.5, 55.3, 43.9. IR (NaCl, neat) 2837, 1723, 1612, 1513, 1247, 1091, 1032 cm^{-1}

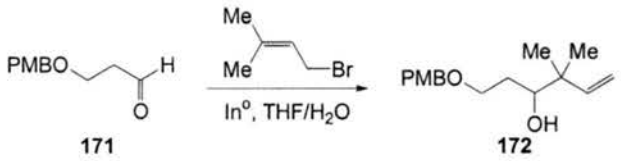
HRMS (FAB) M+H calcd. 193.086469, found 193.087042 .

II-CMR-009D,H



II-CMR-009D,C





171 172

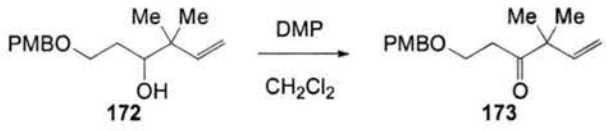
1-(4-methoxy-benzyloxy)-4,4-dimethyl-hex-5-en-3-ol (172): Prenyl bromide (0.245 ml, 2.126 mmol, 1.5 eq) and indium powder (0.179g, 1.559 mmol, 1.1 eq) added successively to a stirring solution of the aldehyde **171** in THF (2.8 ml) and H_2O (11.2 ml) at room temperature for 3h. The reaction was then poured into a separatory funnel and extracted with diethyl ether (3 x 15 ml); organic fractions combined and washed with brine (30 ml); dried (Na_2SO_4); filtered and solvent removed by rotary evaporation. The crude product was purified on silica gel with 3:1 hexanes/ethyl acetate to yield a clear oil (0.171g, 0.6468 mmol, 46%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.034(s, 6H), 1.696(mult., 2H), 2.871(bs, 1H), 3.513(dd,J = 10.25, 1.84Hz, 1H), 3.660(mult., 2H), 3.814(s, 3H), 4.461(s, 2H), 5.023(dd, J = 9.34, 1.46 Hz, 1H), 5.069(dd, J = 1.83Hz, 1H), 5.873(dd, J = 17.21, 10.99Hz, 1H), 6.886(d, J = 8.79Hz, 2H), 7.263(d, J = 8.79 Hz, 2H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.1, 145.4, 130.0, 129.3, 113.7, 112.5, 73.0, 69.7, 55.3, 41.3, 31.2, 22.8, 22.5.

IR (NaCl, neat) 3474, 2960, 1612, 1513, 1248, 1083cm^{-1}.

HRMS (FAB) M+H calcd. 265.180370, found 265.178870.



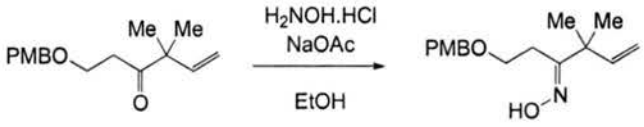
1-(4-methoxy-benzyloxy)-4,4-dimethyl-hex-5-en-3-one (173): Dess-Martin reagent (0.411g, 0.9690 mmol, 1.5 eq) added to a stirring solution of the alcohol **172** (0.171g, 0.6468 mmol) in CH_2Cl_2 (6.5 ml) at room temperature for 1.5h. The cloudy white solution was diluted with diethyl ether (8 ml). $Na_2S_2O_3$ (0.818g, 5.174 mmol, 8 eq) was added followed by saturated aqueous $NaHCO_3$ (9 ml) and stirred until the biphasic layers were clear. The mixture was then poured into a separatory funnel; the aqueous layer was saparated and the organic layer was washed with saturated aqueous $NaHCO_3$ (3 x 10 ml); the aqueous fractions were combined and extracted with diethyl ether (3 x 10 ml); organic fractions were combined, washed with brine (15 ml), dried (Na_2SO_4), filtered and solvent removed by rotary evaporation. The crude oil was purified on silica gel with 10:1 hexanes/ethyl acetate to give the product as a clear oil (0.152 g, 0.5794 mmol, 90%).

^1H NMR ($CDCl_3$, 300 MHz) δ 1.234(s, 6H), 2.780(t, J = 6.6Hz, 2H), 3.690(t, J = 6.6 Hz, 2H), 3.808(s, 3H), 4.432(s, 2H), 5.134(dd, J = 2.93, 1.1 Hz, 1H), 5.175(dd, J = 2.39, 0.73 Hz, 1H), 5.911(dd, J = 17.6, 10.62 Hz, 1H), 6.875(d, J = 8.79 Hz, 2H), 7.243(d, J = 8.43 Hz, 2H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 211.1, 159.1, 143.1, 130.3, 129.2, 114.5, 113.7, 99.9, 85.4, 72.9, 65.4, 55.3, 50.9, 37.8, 23.4.

IR (NaCl, neat) 2969, 1708, 1612, 1513, 1247, 1099 cm^{-1}.

HRMS (FAB) M+H calcd. 262.156895, found 262.155816.



173 **174**

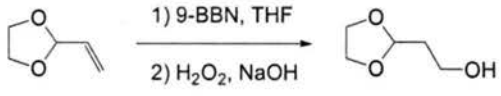
1-(4-methoxy-benzyloxy)-4,4-dimethyl-hex-5-en-3-one oxime (174): NaOAc (0.051 g, 0.6212 mmol, 3.3 eq) measured into a two-neck round bottom flask. Ketone **173** (0.049 g, 0.1868 mmol) dissolved in EtOH (5 x 0.5 ml) and transferred via syringe to the reaction flask and flushed with argon for 15min. $H_2NOH \cdot HCl$ (0.019 g, 0.2734 mmol, 1.5 eq) was added and the reaction was heated to reflux for 15.5h. Solvent was removed by rotary evapoartion and the solid residue was dissolved in 1M HCl (~0.5 ml) and diluted with $CHCl_3$ (~1 ml). Solution was made basic with with saturated aqueous $NaHCO_3$ (2.5 ml) and extracted with $CHCl_3$ (3 x 6 ml); organic fractions were combined, washed with brine(10 ml), dried (Na_2SO_4), filtered and solvent removed by rotary evaporation. Crude oil was purified on silica gel with 10:1 hexanes/ethyl acetate to give the product as a clear thick oil (0.036 g, 0.1298 mmol, 70%).

^1H NMR ($CDCl_3$, 300 MHz) δ 1.233(s, 6H), 2.657(dd, J = 7.7, 7.7 Hz, 2H), 3.674(dd, J = 7.88, 7.88 Hz, 2H), 4.464(s, 2H), 5.065(s, 1H), 5.110(d, J = 4.4 Hz, 1H), 5.825(dd, J = 17.58, 10.62 Hz, 1H), 6.883(d, J = 8.79 Hz, 2H), 7.275(d, J = 8.79 Hz, 2H), 9.170(bs, 1H),.

^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.3, 159.0, 143.8, 130.4, 129.1, 113.7, 113.1, 72.3, 66.0, 55.3, 43.6, 27.6, 24.6.

IR (NaCl, neat) 3302, 2971, 2933, 1612, 1513, 1249, 1089, 1036, 919 cm^{-1}.

HRMS (FAB) M+H calcd. 278.175619 , found 278.175113 .



176 177

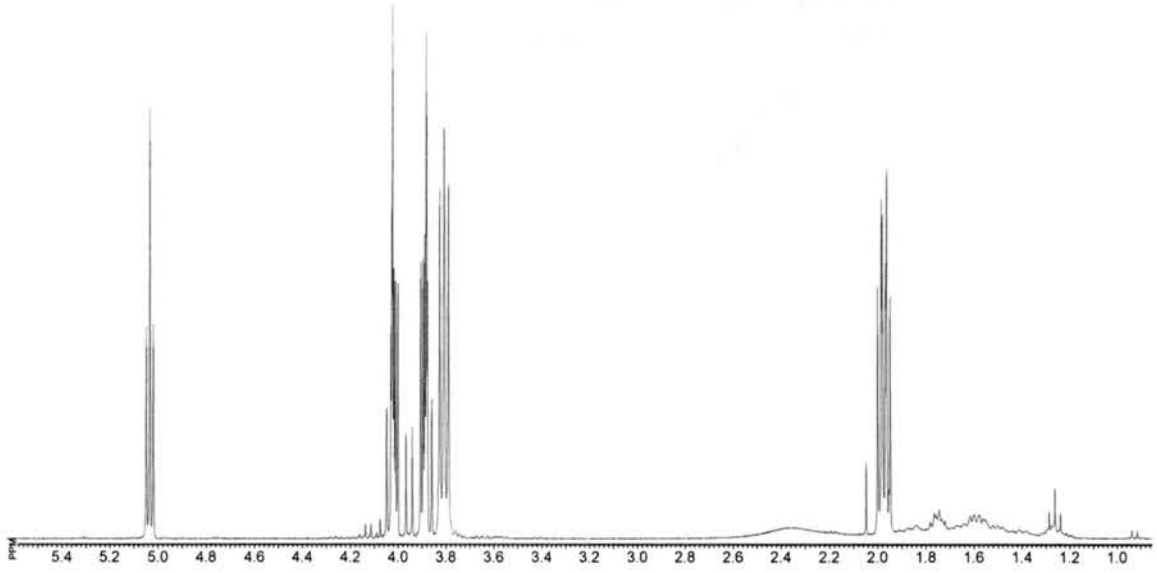
2-[1,3]Dioxolan-2-yl-ethanol (177): 9-BBN (24 ml, 0.5 M solution in THF, 1.2 eq) was added to a flask that had been flamed dried and cooled under argon then cooled further to 0°C. Vinyl dioxolane (1.0 ml, 9.998 mmol) was added via syringe and the reaction was allowed to warm to warm to room temperature with stirring under argon for 1.5h. Reaction cooled to 0°C and quenched with 1M NaOH (16 ml) followed by the slow addition of 30% aqueous H_2O_2 (12 ml) and allowed to warm to room temperature with stirring overnight. Reaction neutralized with saturated aqueous NH_4Cl and extracted with diethyl ether (4 x 50 ml). Organic fractions combined and dried (K_2CO_3). Crude oil purified on silica gel with 4:1 diethyl ether/pentane. CAUTION: alcohol is extremely volatile and should be handled with care to avoid loss of product. Alcohol is obtained as a clear oil (0.918g, 7.776 mmol, 78%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.976(ddd, J = 10.62, 5.13, 0.74 Hz, 2H), 2.370(bs, 1H), 3.809(dd, J = 5.5, 5.5 Hz, 2H), 3.900(m, 2H), 4.008(m, 2H), 5.034(t, J=4.4 Hz, 1H).

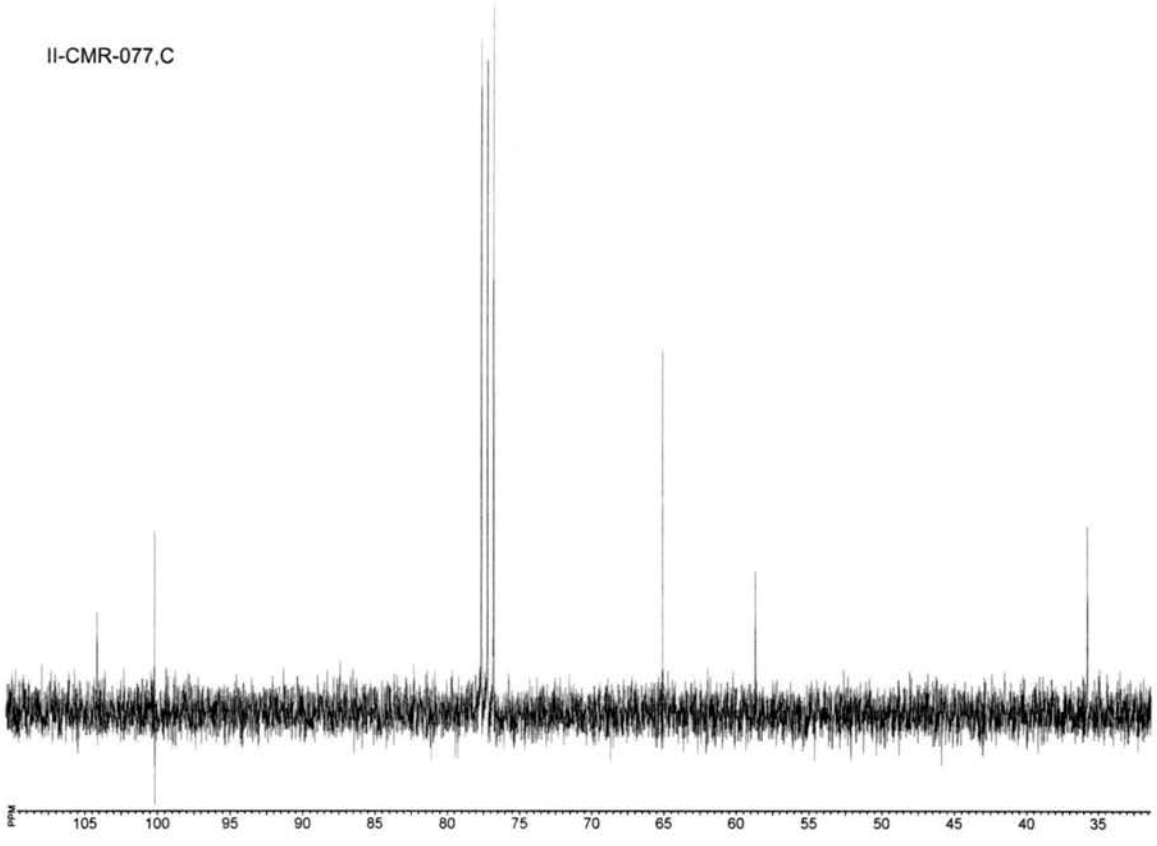
^{13}C NMR ($CDCl_3$, 75 MHz) δ103.9, 64.9, 58.5, 35.6.

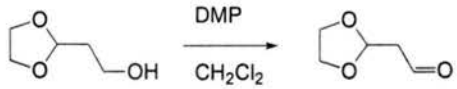
IR (NaCl, neat) 3394.5, 2889.2, 1651.0, 1415.7, 1139.9, 1024.1 cm^{-1}.

II-CMR-077,H



II-CMR-077,C



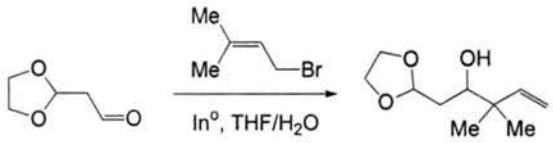


177 **178**

2-[1,3]Dioxolan-2-yl-acetaldehyde (178): PCC (1.290 g, 5.984 mmol, 2 eq) added to a stirring solution of alcohol **177** (0.350 g, 2.963 mmol) in distilled CH_2Cl_2 (15 ml) at room temperature and stirred for 3.5h. Resulting red solution was dituted with diethyl ether (75 ml) and a scoop of $MgSO_4$ was added to adsorb excess chromium then filtered through a short plug of $MgSO_4$. Solvent removed by rotary evapration and the crude yellow oil was purified on silica gel with 4:1 diethyl ether/pentane. CAUTION: Compound will begin to trimerize in the presence of a catalytic acid source in solution (unreacted chromic acid) and should be purified immediately. Compound isolated as a clear oil (0.209 g, 1.780 mmol, 61%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.685(dd, J = 4.4, 2.2 Hz, 2H), 3.828(m, 2H), 3.913(m, 2H), 5.169(t, J = 4.39 Hz, 1H), 9.696(t, J = 2.38 Hz, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 198.8, 99.9, 99.8, 65.6, 64.9.



178 179

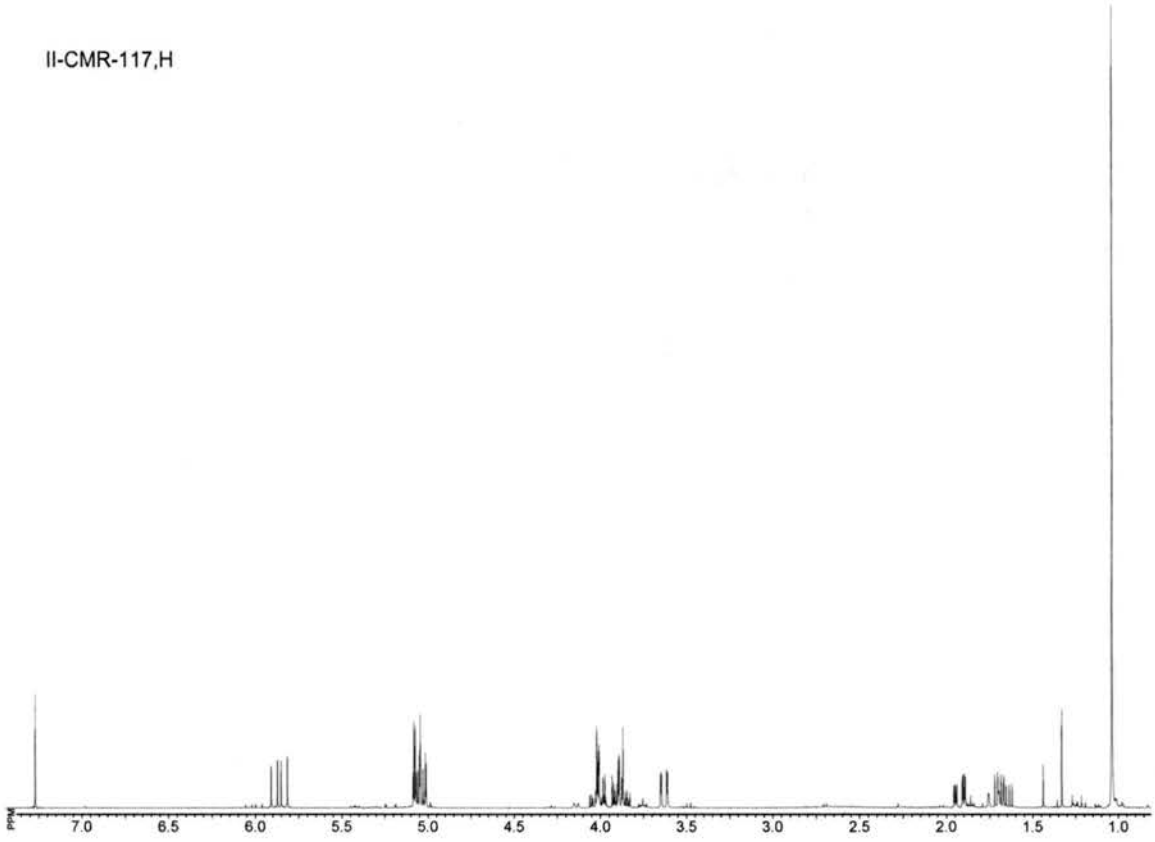
2-[1,3]Dioxolan-2-yl-3,3-dimethyl-pent-4-en-2-ol (179): Prenyl bromide (0.228 ml, 1.978 mmol, 1.1 eq) and indium powder (0..309g, 2.691 mmol, 1.5 eq) added successively to a stirring solution of the aldehyde **178** in THF (7 ml) and H_2O (28 ml) at room temperature for 50 min. The reaction was then poured into a separatory funnel and extracted with diethyl ether (3 x 25 ml); organic fractions combined and washed with brine (50 ml); dried (K_2CO_3); filtered through a plug of celite and solvent removed by rotary evaporation. Compound used without further purification (0.174g, 0.9342 mmol, 52%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.040(s, 6H), 1.672(ddd, J = 14.37, 10.53, 5.13 Hz, 1H), 1.926(ddd, J = 14.28, 4.03, 1.47 Hz, 1H), 3.632(dd, J = 10.62, 1.46 Hz, 1H), 5.050(m, 4H), 5.032(dd, J = 10.26, 1.46 Hz, 1H), 5.046(t, J = 4.58 Hz, 1H), 5.079(dd, J = 3.84, 1.46 Hz, 1H), 5.864(dd, J = 15.71, 10.99 Hz, 1H),.

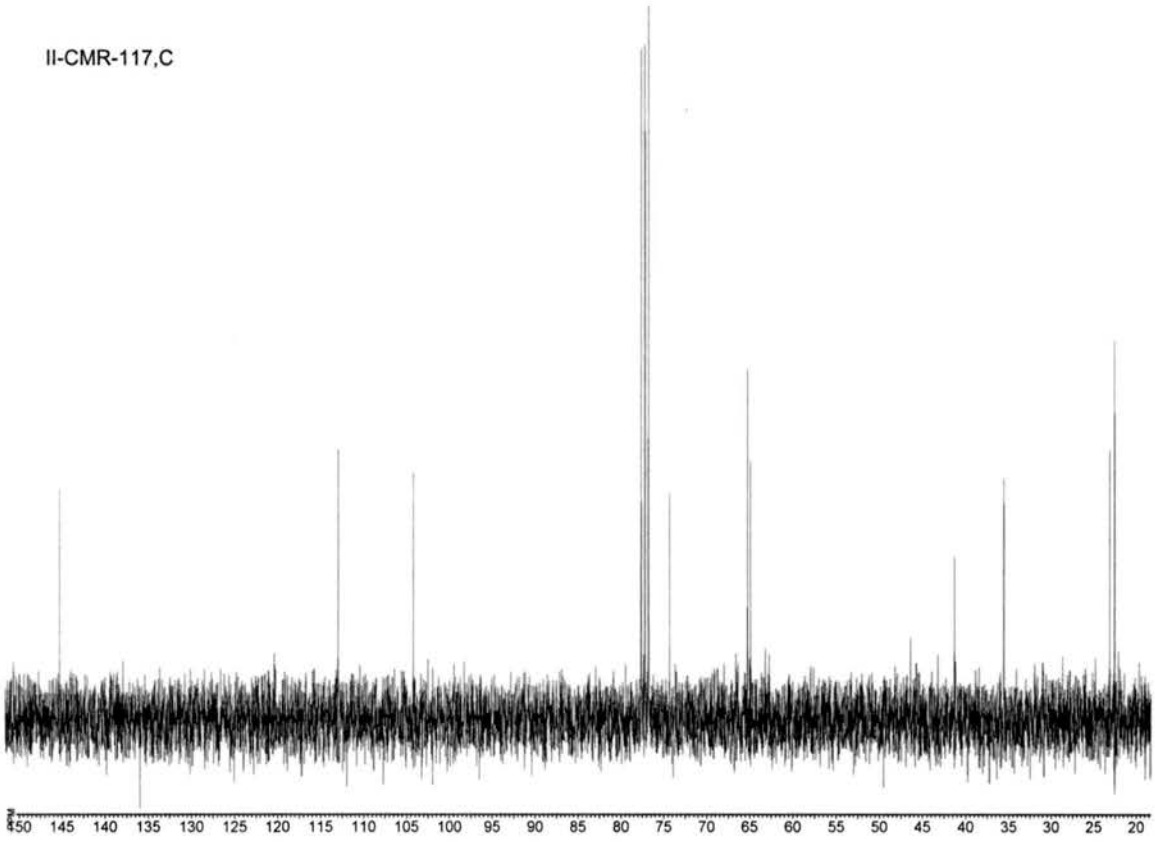
^{13}C NMR ($CDCl_3$, 75 MHz) δ 145.1, 112.8, 104.0, 74.1, 65.1, 64.7, 41.1, 35.3, 22.9, 22.4.

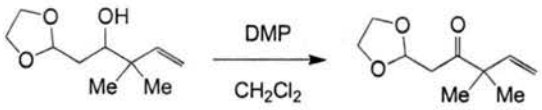
IR (NaCl, neat) 3506, 2966, 2883, 1416, 1136, 1080, 1028, 916 cm^{-1}.

II-CMR-117,H



II-CMR-117,C





179 **179a**

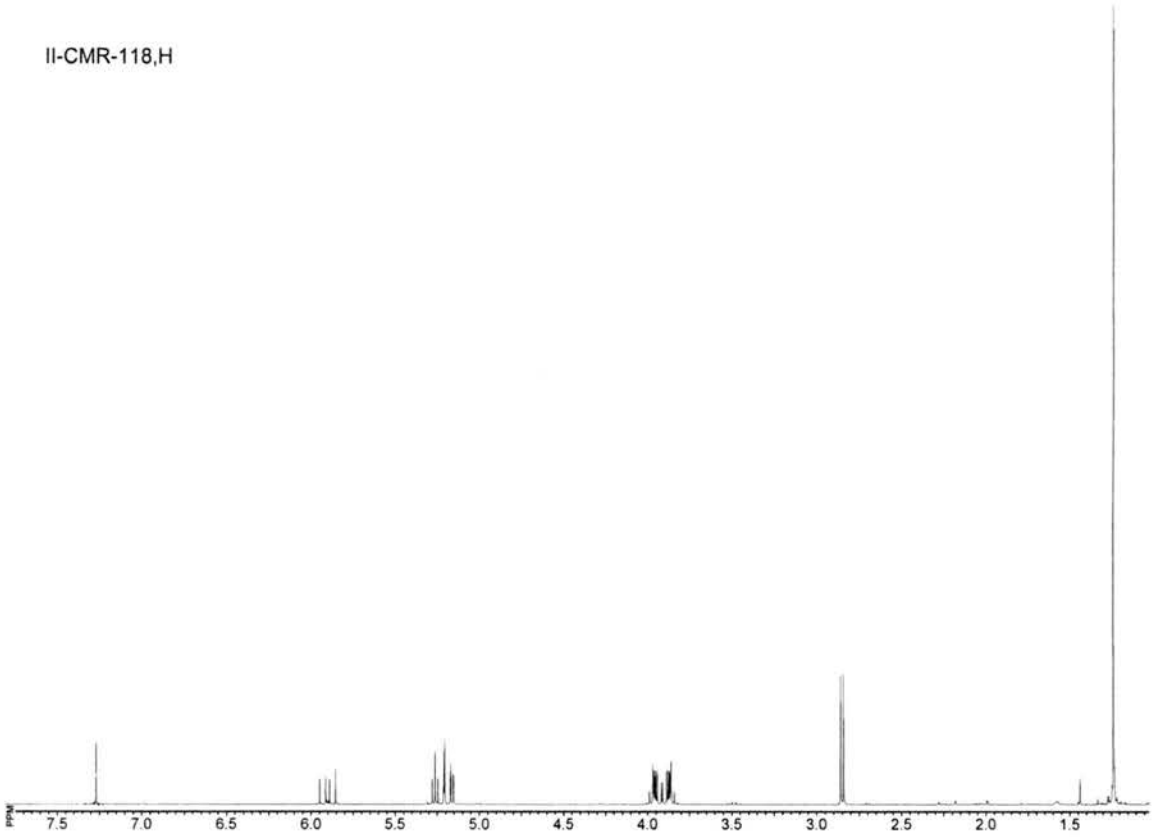
2-[1,3]Dioxolan-2-yl-3,3-dimethyl-pent-4-en-2-one (179a): Dess-Martin reagent (0.559g, 1.318 mmol, 1.5 eq) added to a stirring solution of the alcohol **179** (0..163g, 0.8757 mmol) in CH_2Cl_2 (9 ml) at room temperature for 1.5h. The cloudy white solution was diluted with diethyl ether (20 ml). $Na_2S_2O_3$ (1.051g, 6.647 mmol, 7.6 eq) was added followed by saturated aqueous $NaHCO_3$ (20 ml) and stirred until the biphasic layers were clear. The mixture was then poured into a separatory funnel; the aqueous layer was saparated and the organic layer was washed with saturated aqueous $NaHCO_3$ (3 x 20 ml); the aqueous fractions were combined and extracted with diethyl ether (3 x 25 ml); organic fractions were combined, washed with brine (50 ml), dried (Na_2SO_4), filtered and solvent removed by rotary evaporation. Compound used without further purification (0.154 g, 0.8359 mmol, 95%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.240(s, 6H), 2.854(d, J = 5.12 Hz, 2H), 3.878(m, 2H), 3.956(m, 2H), 5.162(d, J = 4.76 Hz, 1H), 5.211(d, J = 1.46 Hz, 1H), 5.264(t, J = 5.13 Hz, 1H), 5.904(dd, J = 17.57, 10.26 Hz, 1H).

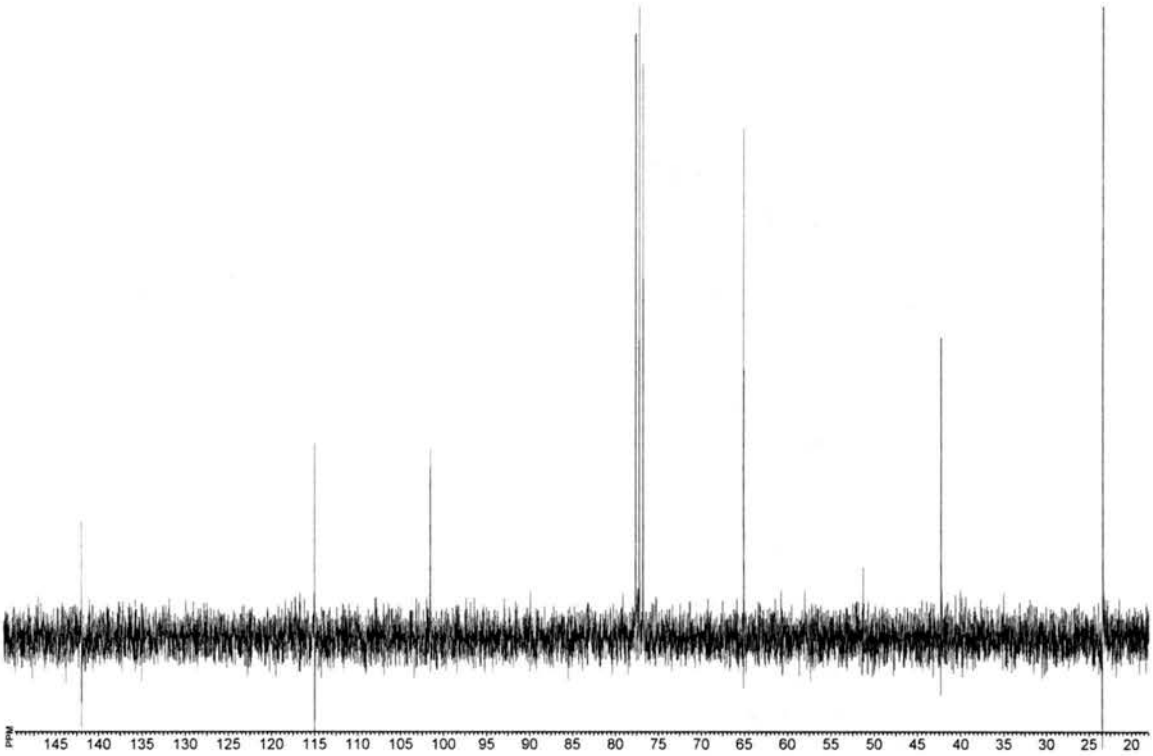
^{13}C NMR ($CDCl_3$, 75 MHz) δ 137.3, 110.4, 96.9, 95.5, 60.4, 46.6, 37.6, 18.8.

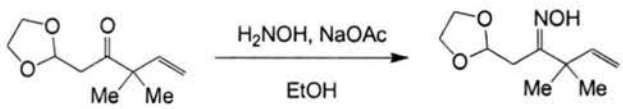
IR (NaCl, neat) 2974, 2887, 1713, 1134, 1018 cm^{-1}.

II-CMR-118,H



II-CMR-118,C





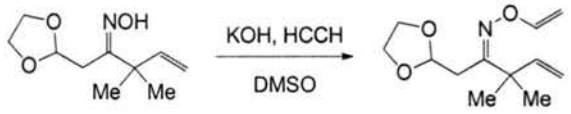
179a **180**

2-[1,3]Dioxolan-2-yl-3,3-dimethyl-pent-4-en-2-one oxime (180): NaOAc (0.195 g, 2.377 mmol, 3.0 eq) measured into a two-neck round bottom flask. Ketone **179a** (0.146 g, 0.7924 mmol) dissolved in EtOH (4 x 2 ml) and transferred via pipet to the reaction flask and flushed with argon for 15min. $H_2NOH \cdot HCl$ (0.087 g, 1.252 mmol, 1.5 eq) was added and the reaction was heated to reflux for 26h. Solvent was removed by rotary evaporation and the solid residue was dissolved in 1M HCl (~3 ml) and diluted with $CHCl_3$ (~2 ml). Solution was made basic with with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ (3 x 25 ml); organic fractions were combined, washed with brine(50 ml), dried (Na_2SO_4), filtered and solvent removed by rotary evaporation. Crude oil was purified on silica gel with 4:1 diethyl ether/pentane to give the product as a clear thick oil (0.120 g, 0.6023 mmol, 76%).

^1H NMR ($CDCl_3$, 300 MHz) δ 1.242(s, 6H), 2.653(d, J = 5.13 Hz, 2H), 3.836(m, 2H), 3.961(m, 2H), 5.038(dd, J = 3.6, 1.1 Hz, 1H), 5.085(dd, J = 3.3, 1.1 Hz, 1H), 5.462(t, J = 5.13 Hz, 1H), 5.864(dd, J = 17.58, 10.25 Hz, 1H), 9.283(s, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.6, 144.3, 112.6, 101.0, 65.8, 64.6, 32.5, 24.9.

HRMS (FAB+) calcd. for $C_{10}H_{18}NO_3$, found 200.128616 .

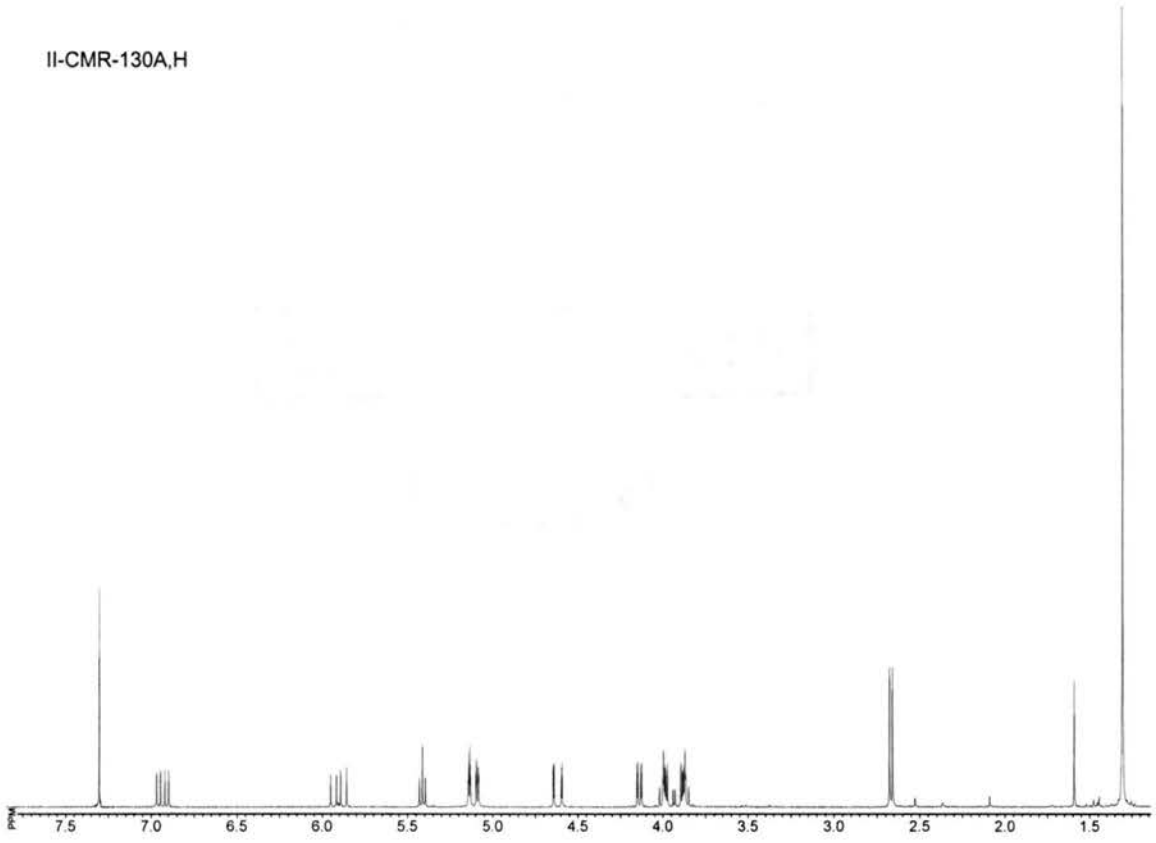


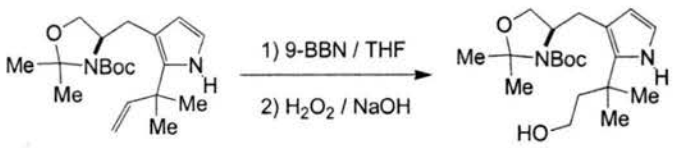
180 **181**

To a stirring solution of pulverized KOH (35 mg, 0.6238 mmol, 1.04 eq) and a trace amount of Al_2O_3 in DMSO (6 mL) in a high pressure reaction vessel at 85°C was added the oxime (120 mg, 0.6023 mmol) dissolved in DMSO (6mL) via pipet. The reaction flask was fitted with a pressure head and the sealed flask was evacuated and filled with argon (repeated 3 times). The reaction flask was then evacuated and pressurized to 20 psi with acetylene (repeated 3 times). The acetylene pressure was then adjusted to 10 psi and the reaction was stirred at 85°C for 30 min. After cooling to room temperature the reaction was diluted with 1:1 brine/H_2O (24 mL) and brine (9 mL) and extracted with EtOAc (25 mL x 4). Combined organic etracts were dried (Na_2SO_4) filtered and concentrated by rotary evaporation. Purification by flash chromatography on silica gel with 1:1 Et_2O / pentane gave the product as a colorless oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ1.27 (s, 9H), δ1.56 (s, 1H), δ2.63 (d, J=5.49, 2H), δ3.78-4.02 (m, 6H), 4.10 (dd, J=6.59, 1.46 Hz, 1H), δ4.48-4.66 (m, 1H), δ5.08 (dd, J=14.1, 2.75 Hz, 2H), δ5.38 (t, J=5.13 Hz, 1H), δ5.87 (dd, J=17.58, 10.25 Hz, 1 H), δ6.90 (dd, J=14.28, 6.96 Hz, 1H)

II-CMR-130A,H





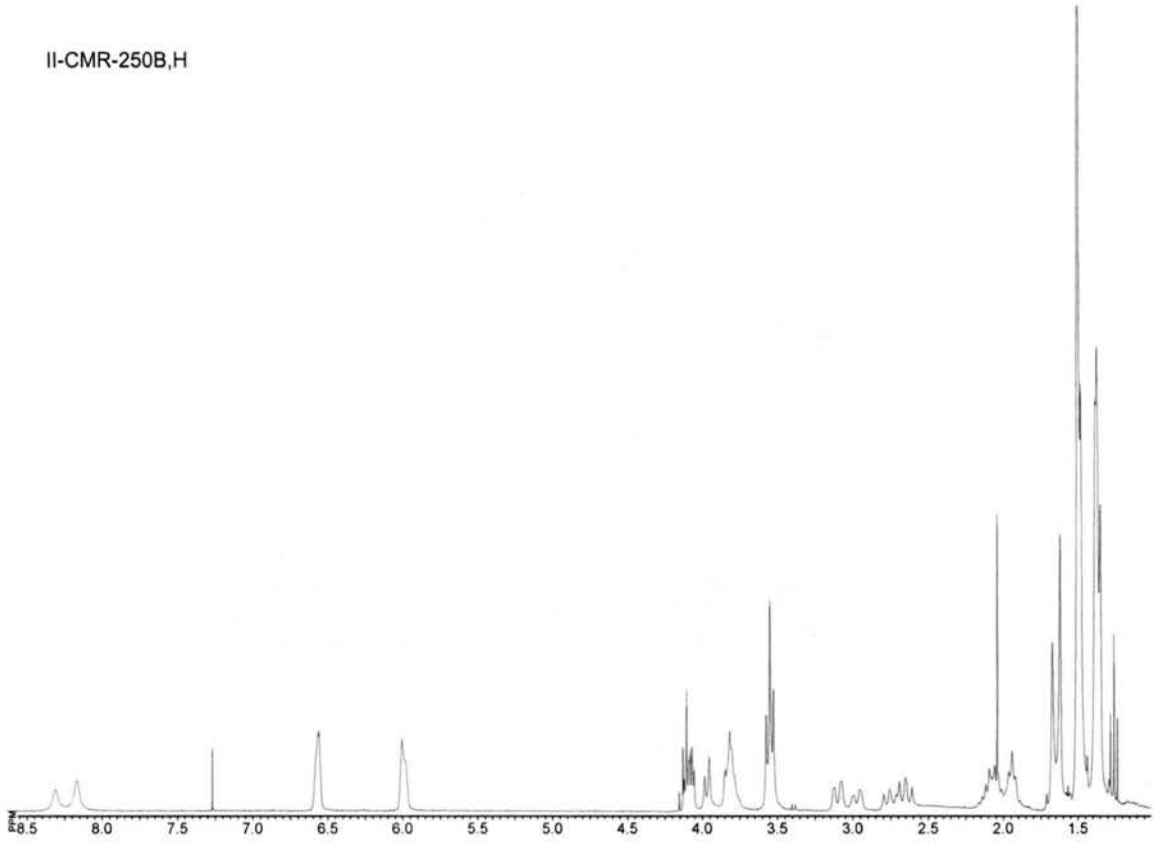
151 204

To a stirring solution of the alkene (151, 0.165g, 0.4735 mmol) in distilled THF (2.5mL) under argon was added a solution of 0.5 M 9-BBN in THF (1.9 mL, 0.9470, 2.0 eq). After stirring under argon for 7.5 h the resulting mixture was quenched with distilled water (1mL). The borane was converted to the alcohol by addition of 1M NaOH (2.0 mL) followed by 30% aq. H_2O_2 (4mL) and stirred at room temperature overnight. The reaction mixture was neutralized with saturated aqueous NH_4Cl (5mL) and extracted with Et_2O (20 mL x 3). Combined organic extracts werewashed with brine (35 mL) dried (Na_2SO_4) and concentrated by rotary evaporation. Purification by flash chromatography on silica gel with 7:3 to 1:1 hexanes/EtOAc gave the product as a colorless oil (0.122g, 0.3329 mmol, 70%)

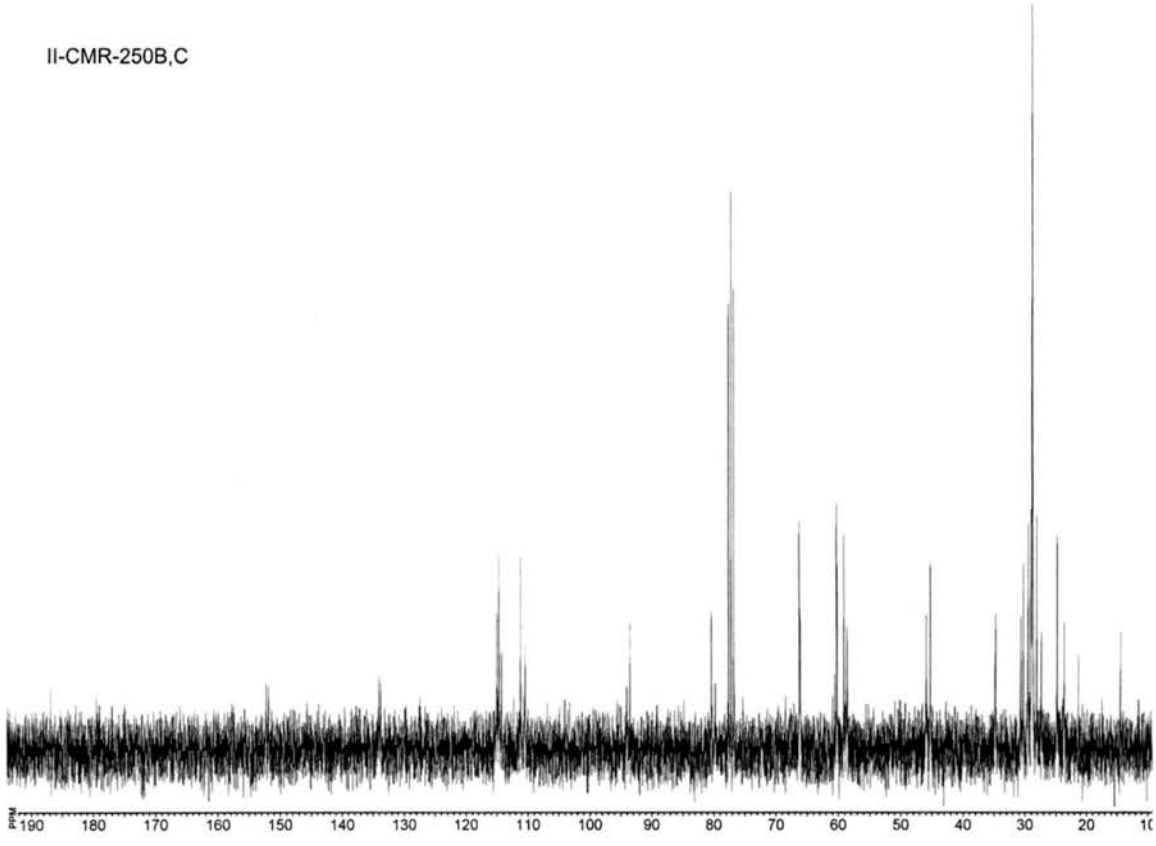
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.32-1.42 (m, 6H), δ1.50 (s, 9H), δ1.58-1.74 (m, 6H), δ1.87-2.19 (m, 4H), δ2.56-3.20 (m, 4H), 3.47-3.63 (m, 3H), δ3.71-3.91 (m, 3H), δ3.97 (d, J=8.79 Hz, 1H), δ4.03-4.19 (m, 2H), δ6.01 (br. s, 2H), 6.56 (br. s, 2H), δ8.17 (br. s, 1H), δ8.31 (br. s, 1H).

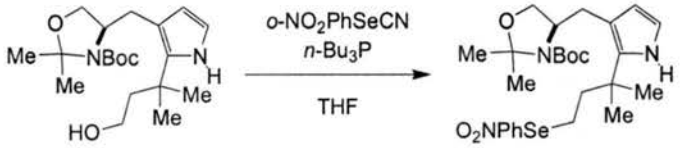
^{13}C NMR ($CDCl_3$, 75 MHz) δ133.7, 114.5, 111.0, 80.2, 66.1, 60.1, 58.9, 45.7, 45.1, 34.6, 30.5, 29.3, 28.5, 27.9, 27.1, 24.6, 23.4.

II-CMR-250B,H



II-CMR-250B,C





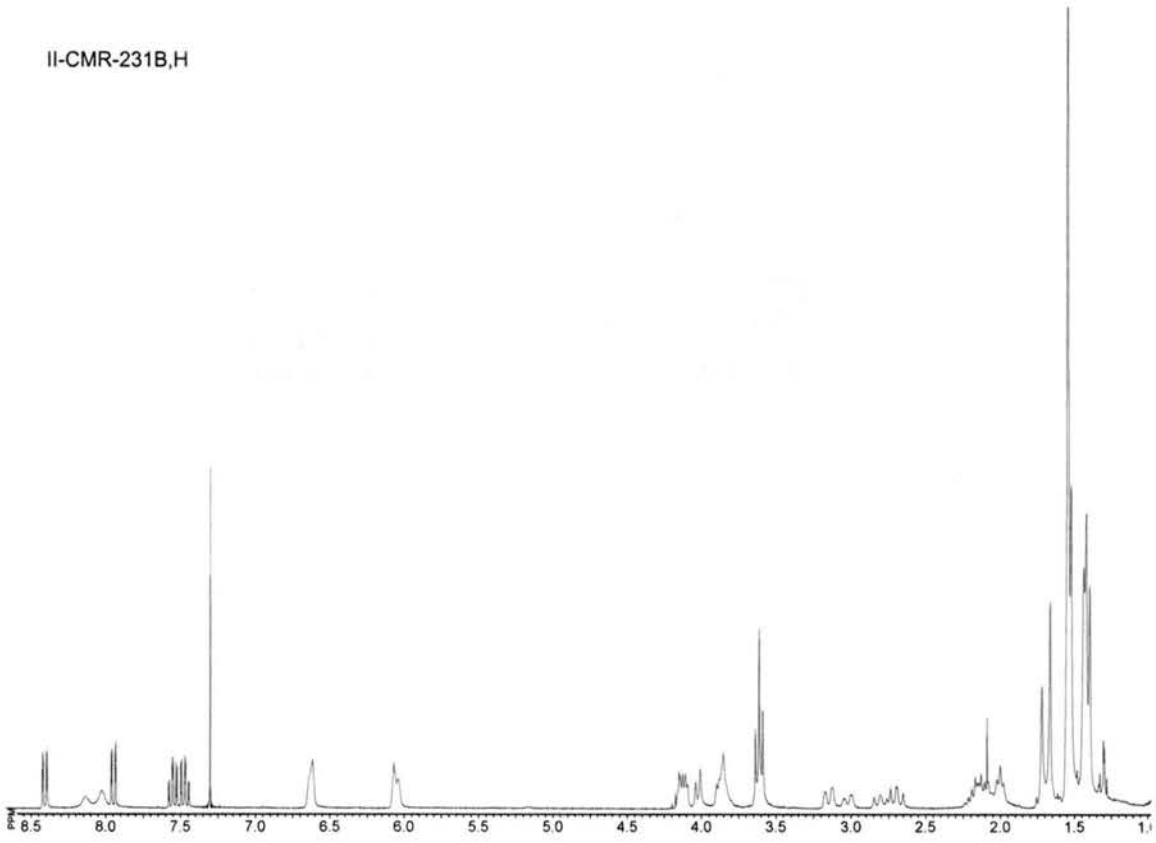
204 206

To a stirring solution of the alcohol (204, 0.122g, 0.3329 mmol) and *o*-NO_2PhSeCN (0.226g, 0.9952 mmol, 3.0 eq) in THF (3.5 mL) under argon was added PBu_3 (0.205 mL, 0.8228 mmol, 2.5 eq) via syringe. After 30 min, the reaction mixture was diluted with EtOAc (25 mL), washed with 1M qaqueous HCl (15 mL x 2) and brine (30 mL), dried (Na_2SO_4) and concentrated by rotary evaporation. Purification by flash chromatography on silica gel with 6:1 to 7:3 hexanes/EtOAc gave the product as a waxy yellow solid (0.180 g, 0.3269 mmol, 98%)

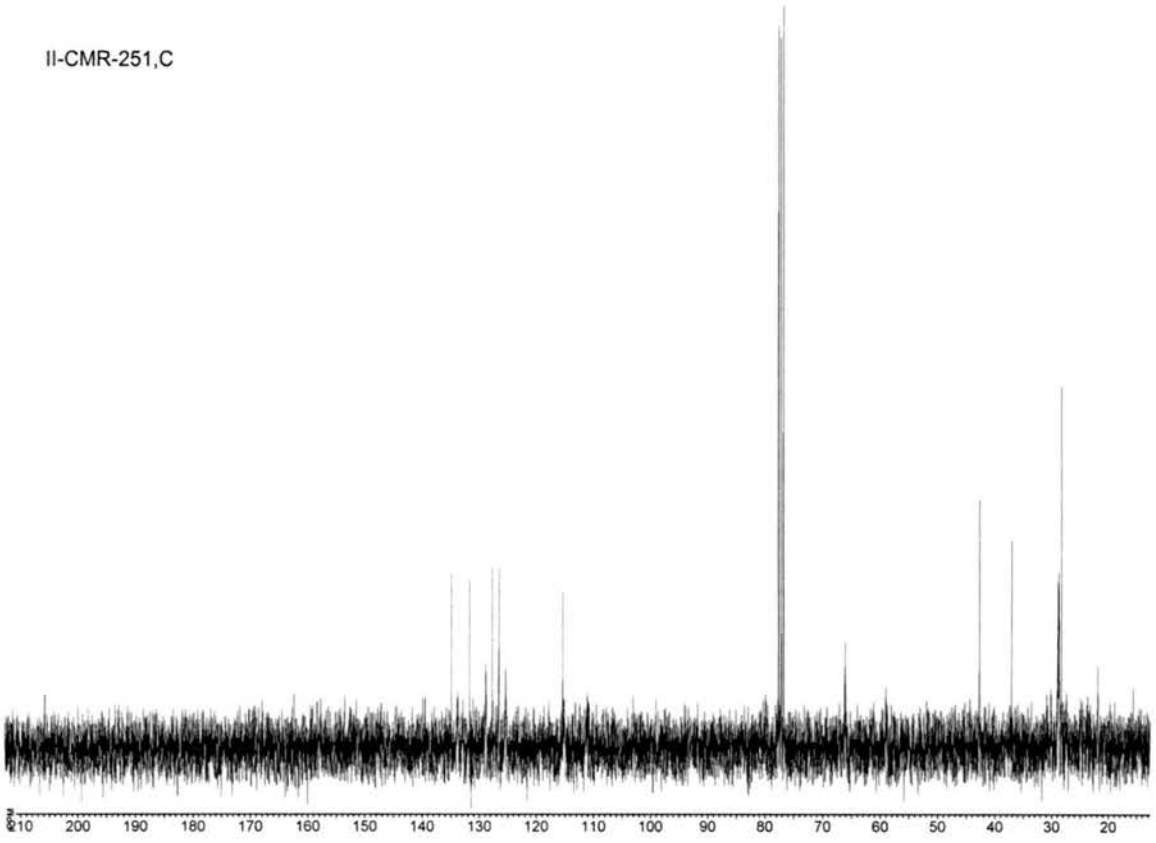
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.32-1.44 (m, 6H), δ1.45-1.56 (m, 9H), δ1.59-1.74 (m, 6H), δ2.58-2.86 (m, 2H), δ2.91-3.18 (m, 2H), δ3.58 (t, J=7.14 Hz, 2H), δ3.82 (br. s, 2H), δ3.99 (d, J=8.79, 1H), δ4.03-4.16 (m, 2H), δ5.93-6.11 (m, 2H), δ6.59 (br. s, 2H), δ7.38-7.48 (m, 1H), δ7.48-7.57 (m, 1H), δ7.92 (d, J=8.79 Hz, 1H), δ7.99 (br. s, 1H), δ8.11 (br. s, 1H), δ8.38 (dd, J=8.06, 1.47Hz, 1H).

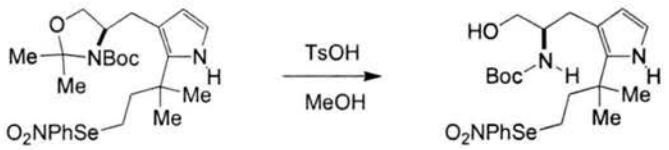
^{13}C NMR ($CDCl_3$, 75 MHz) δ168.3, 164.9, 145.0, 142.6, 134.7, 131.5, 127.5, 126.3, 124.3, 119.8, 118.5, 111.5, 95.2, 94.8, 67.8, 66.9, 66.2, 60.3, 59.0, 38.7, 29.4, 28.6, 25.6.

II-CMR-231B,H



II-CMR-251,C



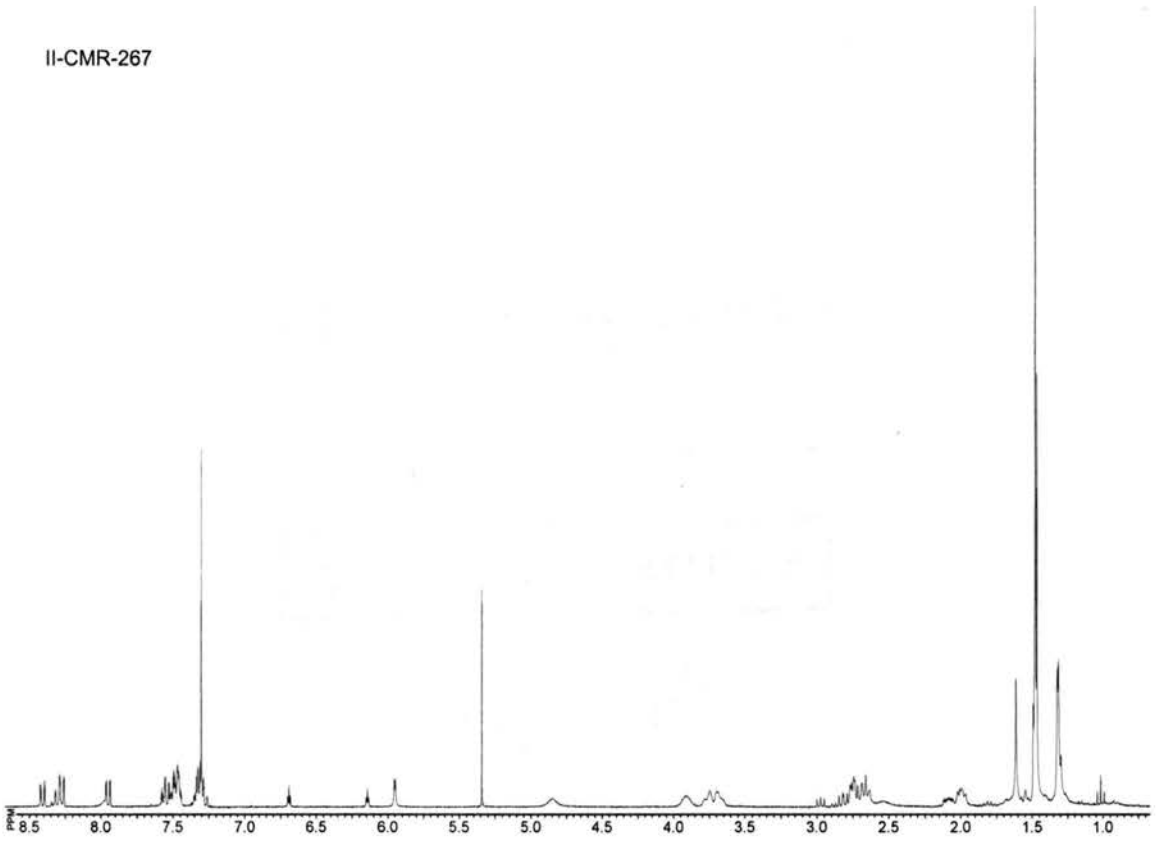


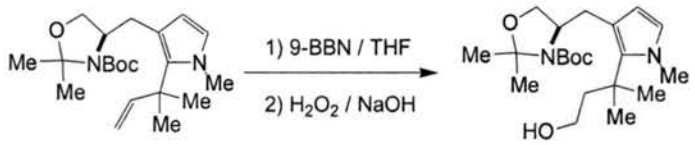
206 **208**

To a stirring solution of the oxazolidine (0.180g 0.3269 mmol) in MeOH (4.0mL) was added TsOH (0.068g, 0.3567 mmol, 1.1 eq) and the resulting mixture was stirred at room temperature. After 1h the reaction was quenched by addition of saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (10 mL x 3). Combined organic extracts were washed with brine (30 mL) dried (Na_2SO_4) filtered and concentrated by rotary evaporation. Purification by flash chromatography on silica gel with 1:1 hexanes/ EtOAc gave the product as an orange/yellow foam (0.106 g, 0.2076 mmol, 63.5%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ1.29 (s, 6H), δ1.44 (s, 9H), δ1.58 (s, 2H), δ1.89-2.14 (m, 2H), δ2.40-3.04 (m, 4H). δ3.69 (d, J=14.28, 4H), δ3.88 (br. s, 1H), 4.81 (br. s, 1H), δ5.31 (s, 1H), δ5.92 (d, J=2.56 Hz, 1H), δ6.07-6.70 (m, 1H), δ7.21-7.36 (m, 1H), δ7.36-7.58 (m, 1H), δ7.92 (d, J=7.69 Hz, 1H), δ8.17-8.33 (m, 2H), δ8.38 (d, J=8.06 Hz, 1H).

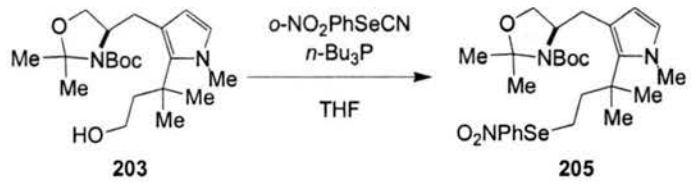
II-CMR-267





157 203

4-[2-(3-Hydroxy-1,1-dimethyl-propyl)-1-methyl-1H-pyrrol-3-ylmethyl] 2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (203): To a stirring solution of the N-methyl pyrrole (157, 0.033g, 0.09100 mmol, 1.0 eq) in THF (0.9 ml) under argon at room temperature was added BH_3 (0.185 ml, 1M in THF, 2.0 eq) and the resulting solution was stirred under argon at room temperature for 2 h 45 min. The reaction was quenched by the addition of 1M NaOH (4 ml) followed by the careful addition of 30% aq. H_2O_2 (4 ml) at 0°C and the resulting mixture stirred for 30 min. The reaction was neutralized with sat. aq NH_4Cl and extracted with diethyl ether (3 x 5 ml). The organic extracts were combined, washed with brine (10 ml), dried (K_2CO_3), filtered and the solvent was removed by rotary evaporation. Crude oil purified on silica gel with 1:1 hexanes / ethyl acetate to give the product as a clear oil (0.023g, 0.06044 mmol, 66%).

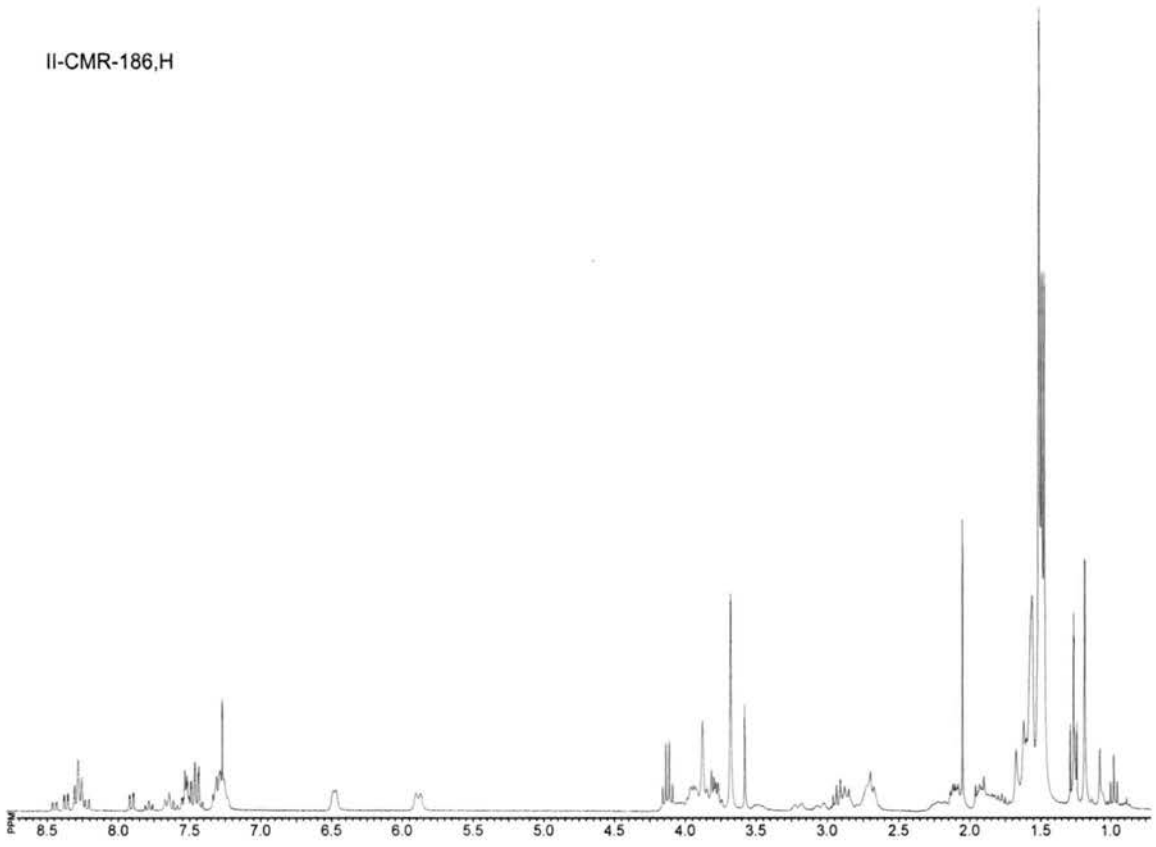


203 205

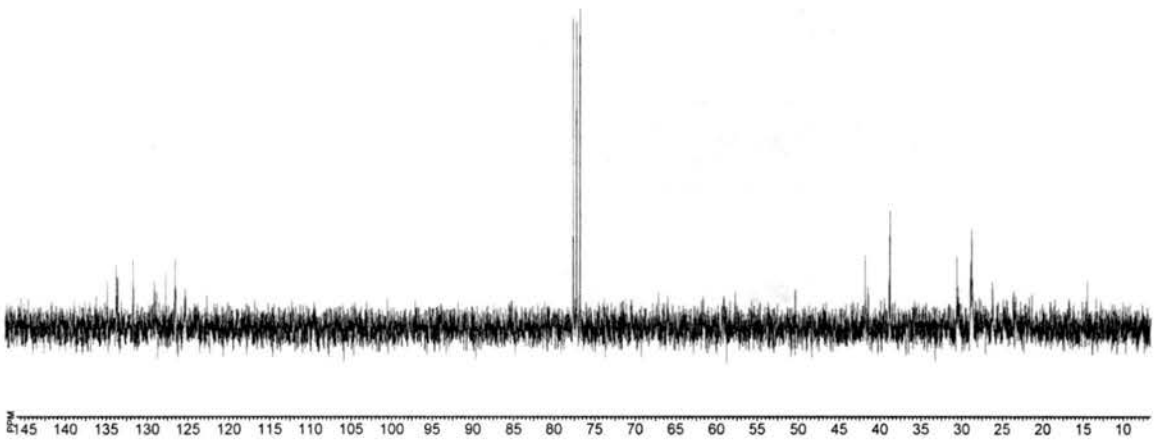
4-{2-[1,1-Dimethyl-3-(2-nitro-phenylselanyl)-propyl]-1-methyl-1H-pyrrol-3-ylmethyl}-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (205): To a stirring solution of **203** (0.034g, 0.08935 mmol, 1 eq.) in THF (1.0 ml) at room temperature was added *o*-NO_2PhSeCN (0.031g, 0.1365 mmol, 1.5 eq.) and PBu_3 (0.027 ml, 0.1084 mmol, 1.2 eq.) successively. The resulting orange/red solution was stirred at room temperature under argon for 30 min. Volatiles removed by rotary evaporation and the crude oil purified on silica gel with 7:3 hexanes / ethyl acetate to give the product as a clear yellow oil (0.035 g, 0.06022 mmol, 67%)

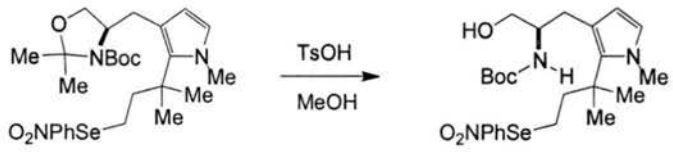
HRMS (FAB) M+H calc'd. 565.205492, found 565.205673.

II-CMR-186,H



II-CMR-186,C





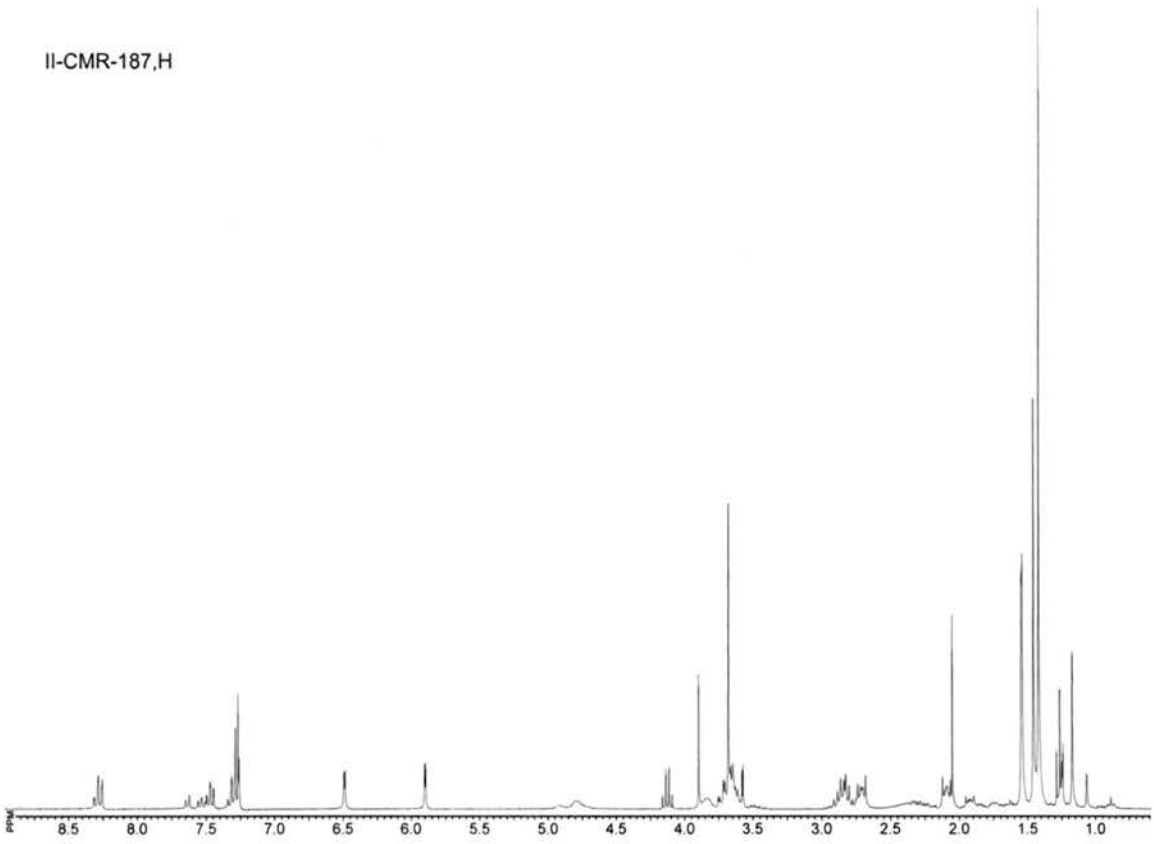
205 207

(1-{2-[1,1-Dimethyl-3-(2-nitro-phenylselanyl)-propyl]-1-methyl-1H-pyrrol-3-ylmethyl}-2-hydroxy-ethyl)-carbamic acid *tert*-butyl ester (207): To a stirring solution of **205** (0.034 g, 0.06022 mmol, 1.0 eq) in methanol (1.0 ml) was added pTsOH (0.0125g, 0.06557 mmol, 1.1 eq). The resulting yellow solution was stirred at room temperature for 1 h. The reaction was quenched with sat. aq $NaHCO_3$ (3 ml) and extracted with chloroform (3 x 5 ml). The organic extracts were combined, dried (K_2CO_3), filtered and the solvent was removed by rotary evaporation. The crude oil was purified on silica gel with 1:1 hexanes / ethyl acetate to give the product as a yellow oil (0.020 g, 0.03813 mmol, 63%).

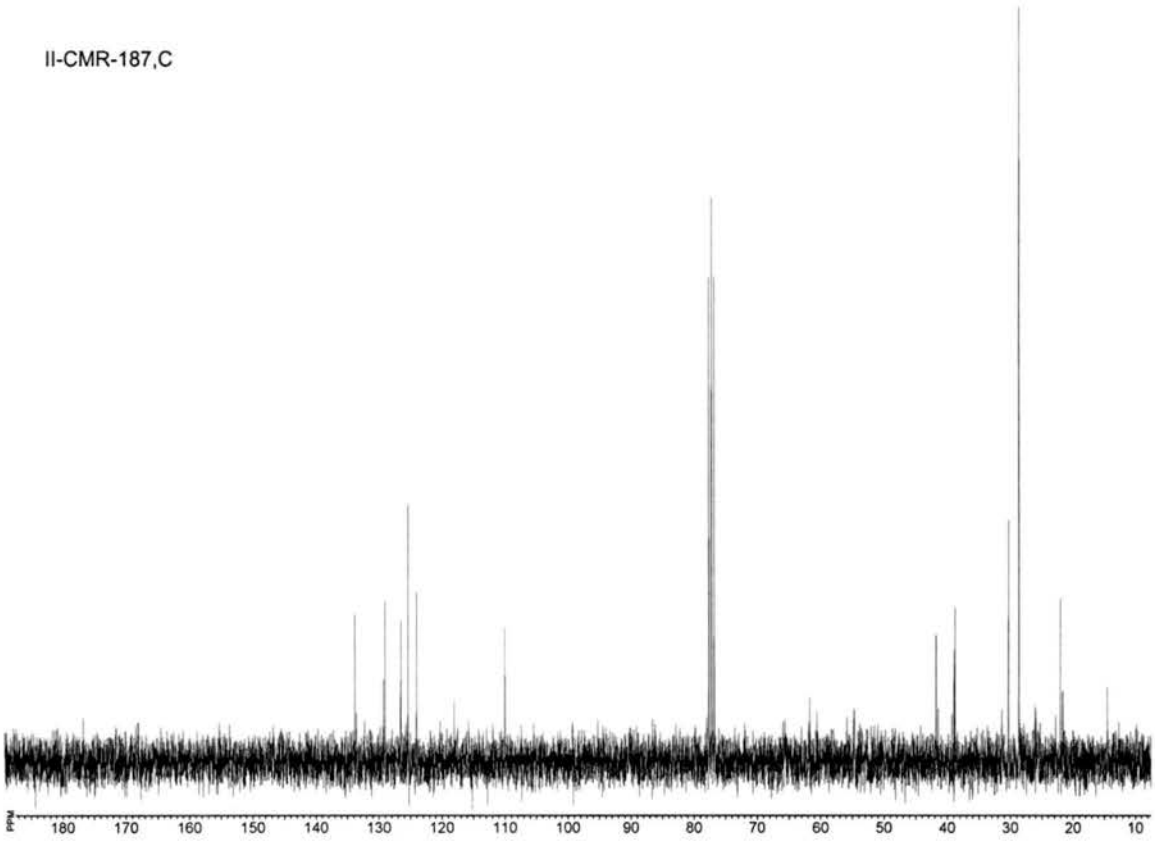
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.18 (s, 2H), δ1.22-1.31 (m, 2H), δ1.42 (s, 9H), δ1.46 (s, 3H), δ1.55 (s, 3H), δ2.63-2.98 (m, 4H), δ3.51-3.78 (m, 6H), δ3.90 (s, 1H), δ4.79 (br. s, 1H), δ5.90 (d, J=2.56 Hz, 1H), δ6.49 (d, J=2.56 Hz, 1H), δ7.22-7.37 (m, 1H), δ7.40-7.70 (m, 2H), δ8.21-8.38 (m, 1H).

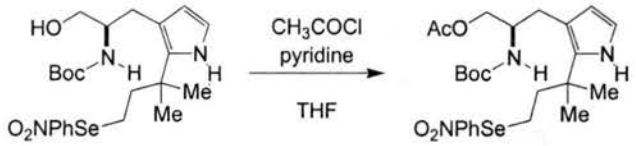
^{13}C NMR ($CDCl_3$, 75 MHz) δ133.6, 129.1, 128.8, 126.3, 125.2, 123.8, 117.9, 109.6, 105.3, 86.4, 61.5, 60.4, 41.6, 41.2, 39.1, 38.6, 31.1, 30.1, 28.4, 27.7, 25.8, 22.6, 21.8, 21.4, 14.2.

II-CMR-187,H



II-CMR-187,C





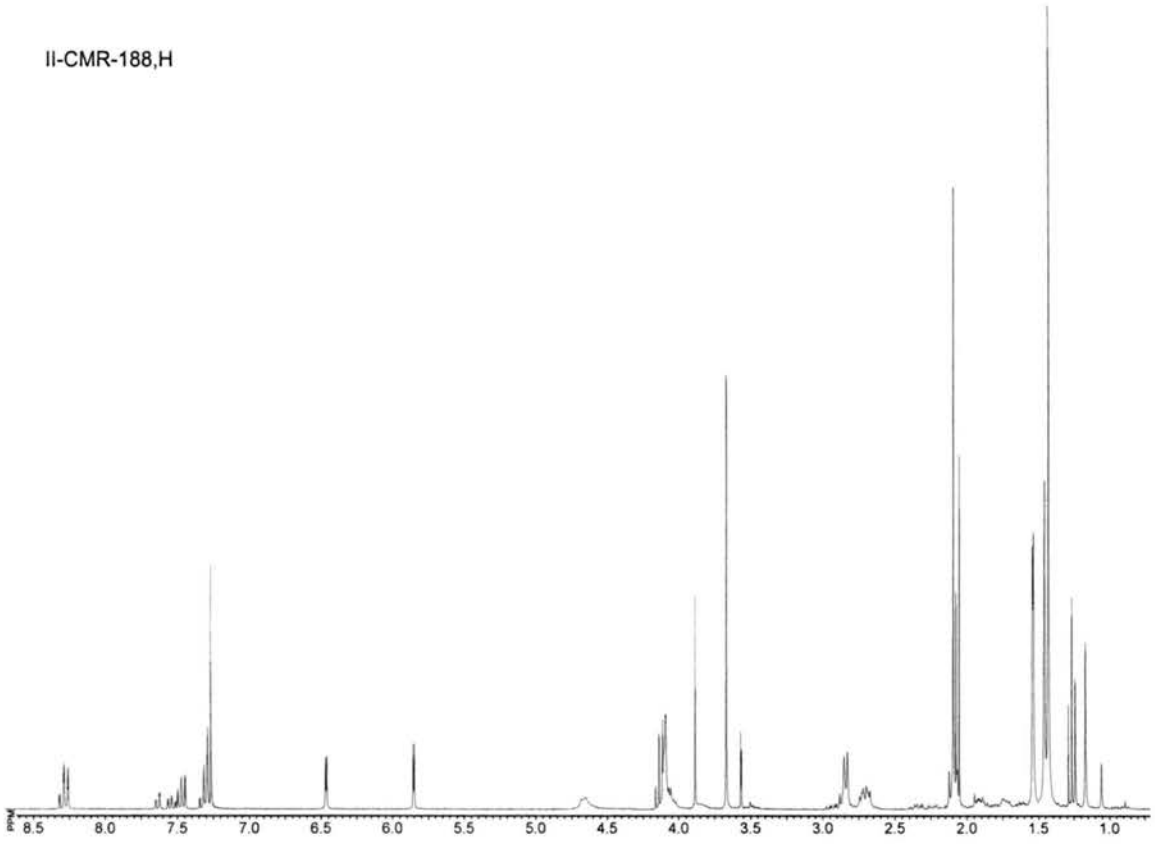
208 209

Acetic acid 2-*tert*-butoxycarbonylamino-3- (2-[1,1-dimethyl-3-(2-nitro-phenylselanyl)-propyl]-1-methyl-1H-pyrrol-3-yl}-propyl ester (209): To a stirring solution of **208** (0.020g 0.03813 mmol, 1.0 eq) in CH_2Cl_2 (0.5 ml) was added pyridine (0.0062 ml, 0.07666 mmol, 2.0 eq.) and acetyl chloride (0.0032 ml, 0.0450 mmol, 1.18 eq.) successively. The resulting yellow solution was stirred at room temperature for 1.5 h. Volatiles removed by rotary evaporation . The crude oil was purified on silica gel with 7:3 hexanes / ethyl acetate to give the product as a yellow oil (0.019g, 0.03354 mmol, 88%).

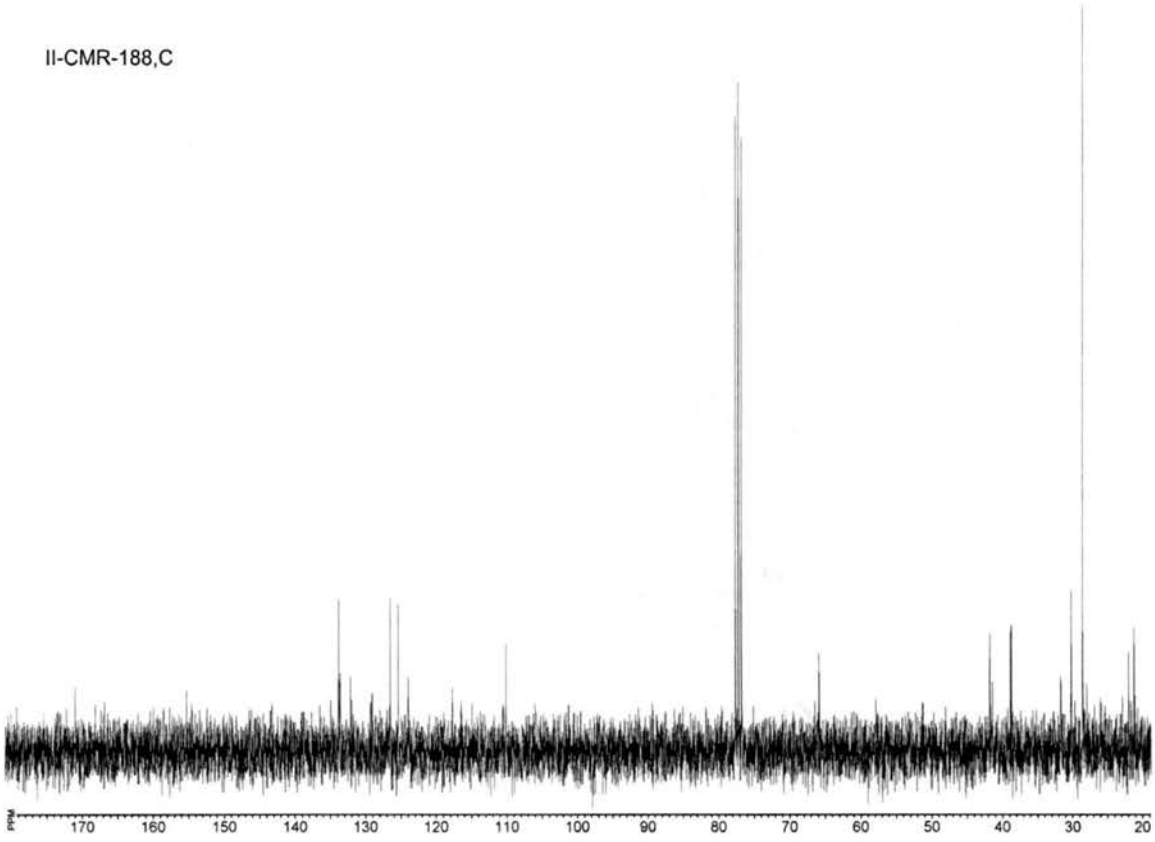
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.432 (s, 9H), δ 1.537 (s, 3H), δ 1.543 (s, 3H), δ 2.097 (s, 3H), δ 2.710 (m, 2H), δ 2.841 (m, 2H), δ 3.671 (s, 3H), δ 3.842 (bs, 1H), δ 4.095 (m, 2H), δ 4.652 (m, 1H), δ 5.854 (d, 1H, 2.93 Hz), δ 6.466(d, 1H, 2.93 Hz), δ 7.291(m, 2H), δ 7.474 (m, 1H).

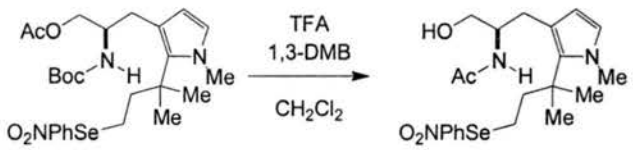
^{13}C NMR (75 MHz, $CDCl_3$) δ 155.1, 133.6, 133.4, 132.0, 128.9, 126.3, 125.2, 123.8, 65.7, 51.0, 41.6, 38.7, 38.6, 31.5, 30.1, 28.4, 27.8, 25.9, 22.7, 21.8, 21.0

II-CMR-188,H



II-CMR-188,C





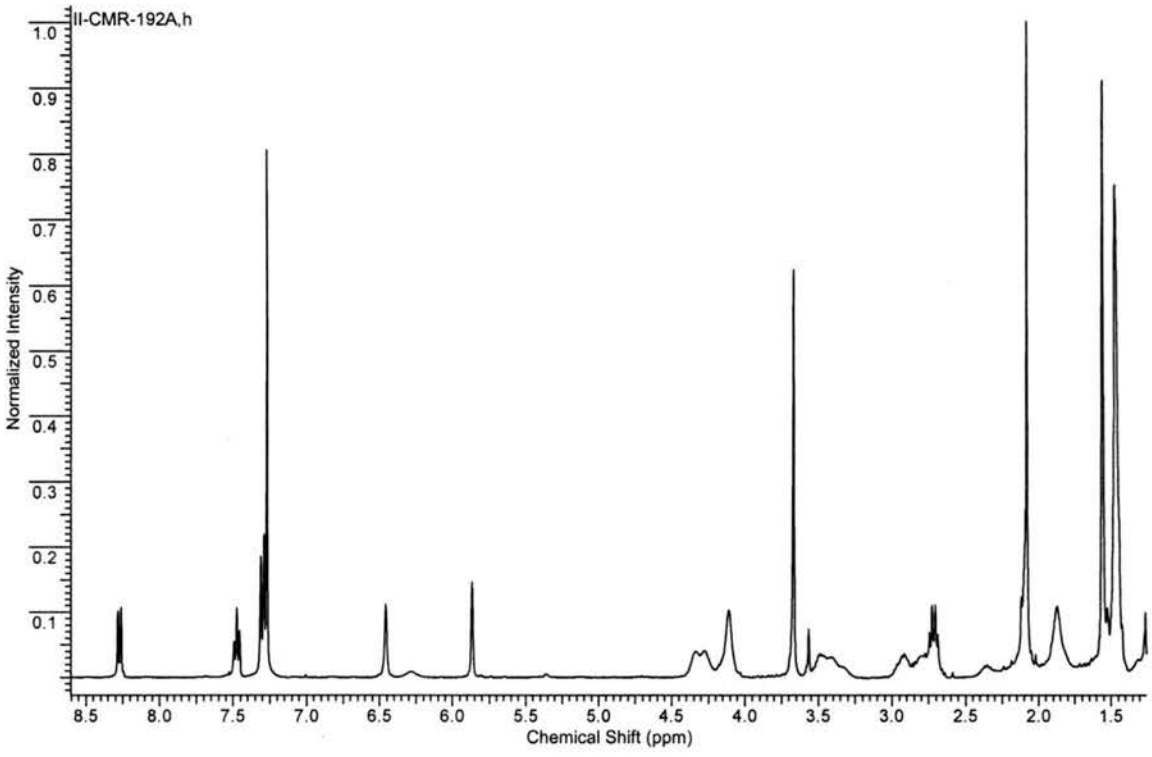
209 **210**

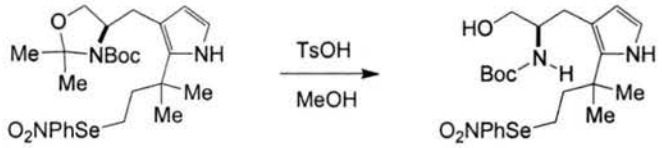
Acetic acid 2-amino-3- {2-[1,1-dimethyl-3-(2-nitro-phenylselanyl)-propyl]-1-methyl-1H-pyrrol-3-yl}-propyl ester (210): To a stirring solution of **209** (0.019g, 0.03354 mmol, 1 eq) in CH_2Cl_2 (0.3 ml) was added 1,3-dimethoxybenzene (0.01 ml, 0.07621 mmol, 2.3 eq.) and Trifluoroacetic acid (0.3 ml, 3.894 mmol, 116 eq.) successively. The resulting orange solution was stirred at room temperature for 30 min. The reaction was neutralized with sat. aq. $NaHCO_3$ over 1 h. The aqueous solution was extracted with dichloromethane (3 x 3 ml). The organic extracts were combined, washed with brine (10 ml) dried (Na_2SO_4), filtered and the solvent was removed by rotary evaporation. The crude oil was purified on silica gel 95:5 chloroform / methanol to give the product as a yellow oil (0.012g, 0.02573 mmol, 77%).

^1H NMR (300 MHz, $CDCl_3$) δ 1.535 (s, 6H), δ 2.013 (bs, 2H), δ 2.106 (s, 3H), δ 2.674 (m, 3H), δ 2.857 (m,1H), δ 3.306 (m, 1H), δ 3.691 (s, 3H), δ 3.966 (m, 2H), δ 4.177 (m, 2H), δ 5.921 (d, 1H, 2.56Hz), δ 6.497 (d, 1H, 2.58 Hz), δ 7.280 (m, 2H), δ 7.468 (dt, 1H, 7.51 Hz, 1.47 Hz), δ 8.278 (dd, 1H, 8.05 Hz, 1.47 Hz).

IR (NaCl, neat) 2925.8, 1735.8, 1589.2, 1565.3, 1511.6, 1331.1, 1303.8, 1242.2, 1037.4 cm^{-1}.

HRMS (FAB) M+H calc'd. 468.140152, found 468.140277.

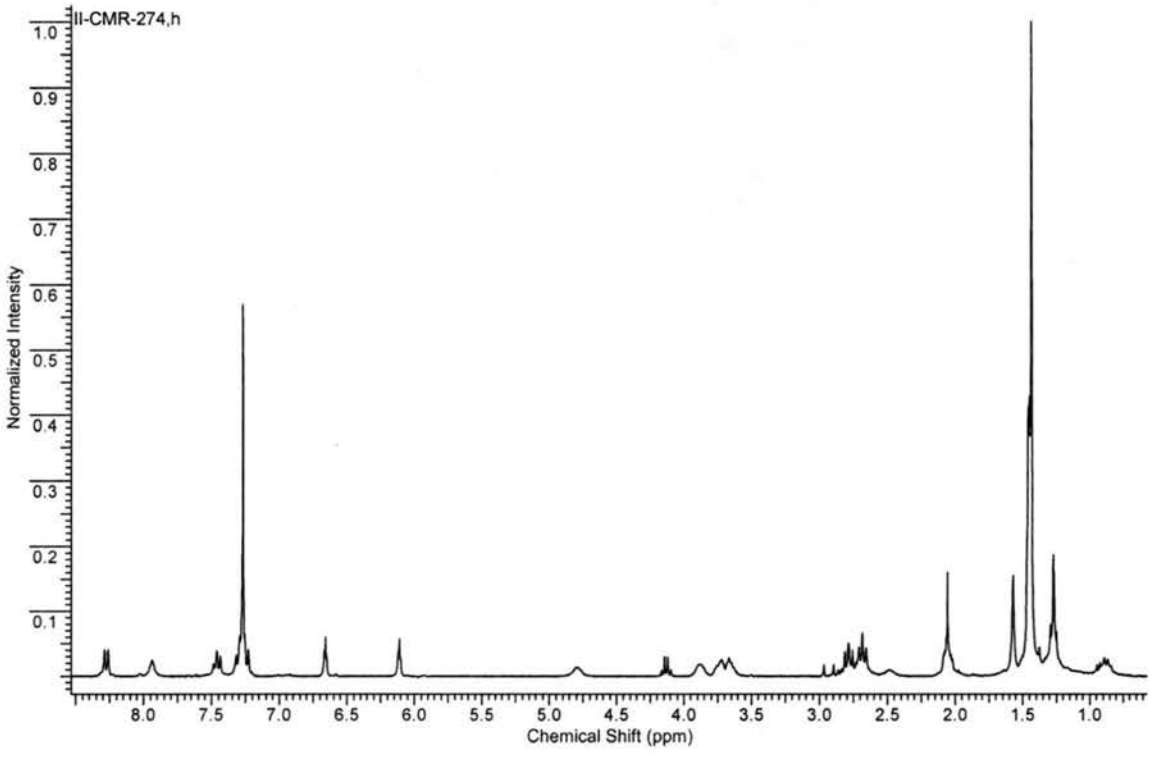

II-CMR-192A,h
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Chemical Shift (ppm)

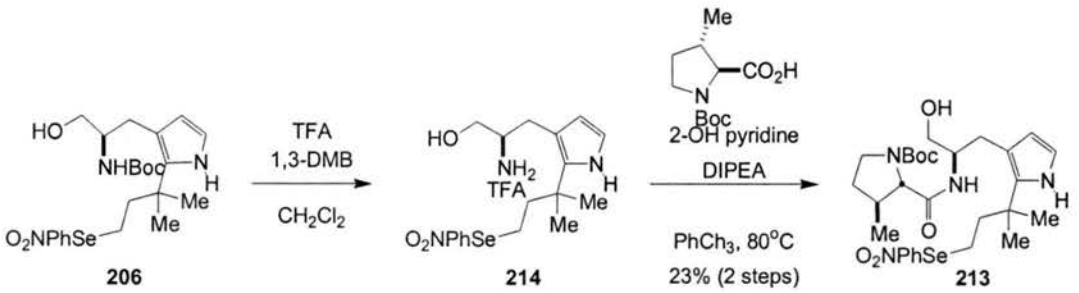


206 208

(1-{2-[1,1-Dimethyl-3-(2-nitro-phenylselanyl)-propyl]-1-methyl-1H-pyrrol-3-yl}-2-hydroxy-ethyl)-carbamic acid *tert*-butyl ester (208): To a stirring solution of the oxazolidine (0.036 g, 0.06539 mmol, 1.0 eq) in methanol (1.0 ml) was added pTsOH (0.014g, 0.07343 mmol, 1.1 eq). The resulting yellow solution was stirred at room temperature for 1 h. The reaction was quenched with sat. aq $NaHCO_3$ (3 ml) and extracted with chloroform (3 x 5 ml). The organic extracts were combined, dried (Na_2SO_4), filtered and the solvent was removed by rotary evaporation. The crude oil was purified on silica gel with 1:1 hexanes / ethyl acetate to give the product as a yellow oil (0.016 g, 0.03134 mmol, 48%).

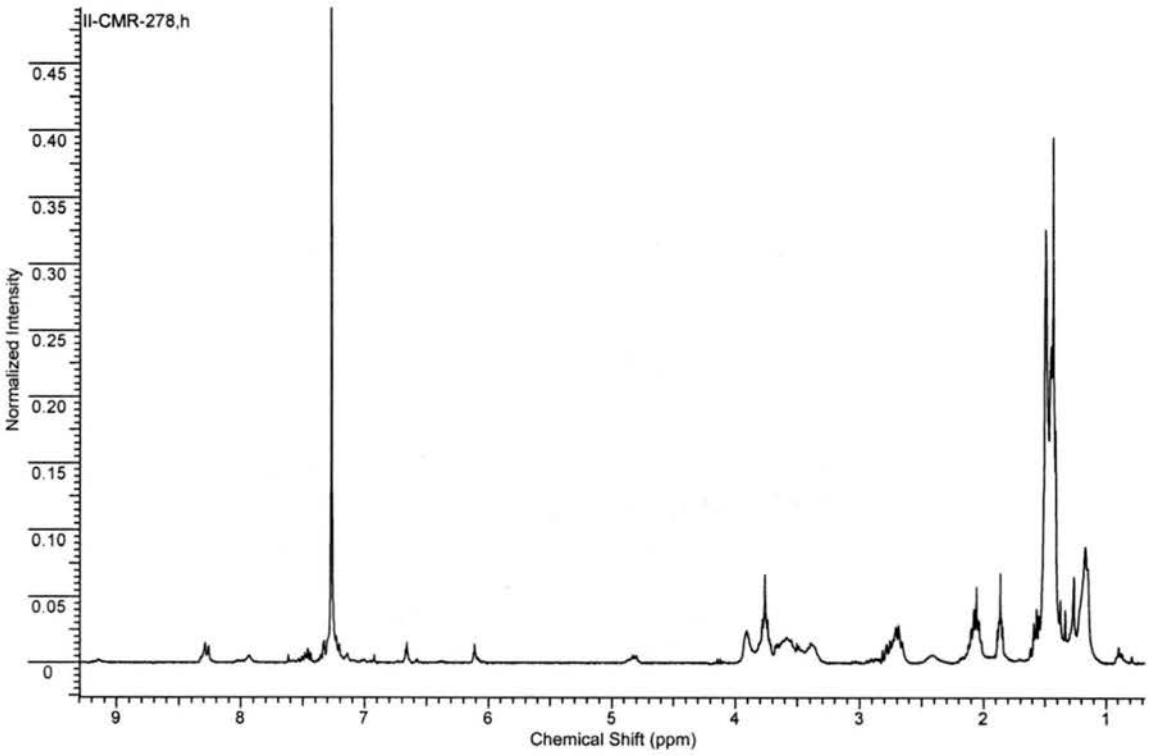
^{1}H NMR ($CDCl_3$, 300 MHz) δ1.29 (s, 6H), δ1.44 (s, 9H), δ1.58 (s, 2H), δ1.89-2.14 (m, 2H), δ2.40-3.04 (m, 4H). δ3.69 (d, J=14.28, 4H), δ3.88 (br. s, 1H), 4.81 (br. s, 1H), δ5.31 (s, 1H), δ5.92 (d, J=2.56 Hz, 1H), δ6.07-6.70 (m, 1H), δ7.21-7.36 (m, 1H), δ7.36-7.58 (m, 1H), δ7.92 (d, J=7.69 Hz, 1H), δ8.17-8.33 (m, 2H), δ8.38 (d, J=8.06 Hz, 1H).

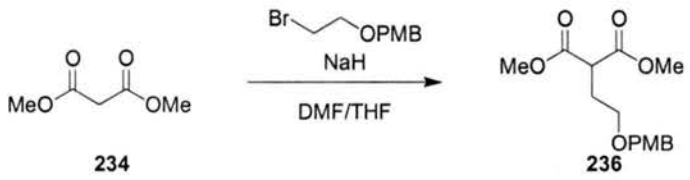

II-CMR-274,h
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)



To a stirring solution of the alcohol (206, 0.008g, 0.01567mmol) in distilled CH_2Cl_2 (1 mL) was successively added 1,3-dimethoxybenzene (0.005 mL, 0.0381 mmol, 2.4 eq) and trifluoroacetic acid (0.02 mL, .2596 mmol, 16.5 eq). After stirring at room temperature for 2.5 h solvent and volatiles were removed by rotary evaporation. Crude material used in next step without further purification.

To a flask containing the crude amino alcohol was added β-methyl proline (0.0054g, 0.02355 mmol, 1.5 eq) and 2-hydroxy pyridine (0.0072g, 0.07571 mmol, 4.8 eq). This mixture was diluted with toluene (o.5 mL) and stirred at room temperature for 25 min then heated under argon in a pre-warmed oil bath at 80°C for 22h. After cooling to room temperature the solvent was removed by rotary evaporation and puified by PTLC with 1:1 hexanes/EtOAc. Product isolated as a white film (0.022g, 0.003539 mmol, 23% over two steps)


II-CMR-278,h
Normalized Intensity
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
9
8
7
6
5
4
3
2
1
Chemical Shift (ppm)

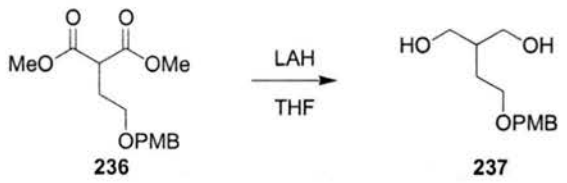


234 236

2-[2-(4-Methoxy-benzyloxy)-ethyl]-malonic acid dimethyl ester (236): Sodium hydride (0.706g, 29.42 mmol, 3.0 eq) added in one portion to a stirring solution of dimethyl malonate (5.5 mL, 48.12 mmol, 5.0 eq) and the bromide[63] (2.365g, 9.649 mmol) in dry DMF (10 mL) and dry THF (30 mL) at room temperature under argon. The resulting mixture heated to reflux for 4.5 h. Reaction mixture was then cooled to room temperature, diluted with d H_2O (60 mL) and extracted with 1:1 diethyl ether/hexanes (60 mL x 4). The combined organic extracts were washer with brine (60 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. The resulting crude oil was purified on silica gel with 6:1 hexanes/ethyl acetate. Products isolated as a clear colorless oil. Yield 1.83 g, 6.176 mmol (64%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.218 (dt, J=7.3, 6.0 Hz, 2H), 3.501(t, J=5.9 Hz, 2H), 3.627 (t, J=7.4Hz, 1H), 3.711 (s, 6H), 3.812 (s, 3H), 4.407 (s, 2H), 8.878 (d, 8.6Hz, 2H), 7.244 (d, J=8.8 Hz, 2H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ169.7, 159.0, 130.1, 129.2, 111.7, 72.6, 67.0, 55.3, 52.5, 48.8, 29.1.



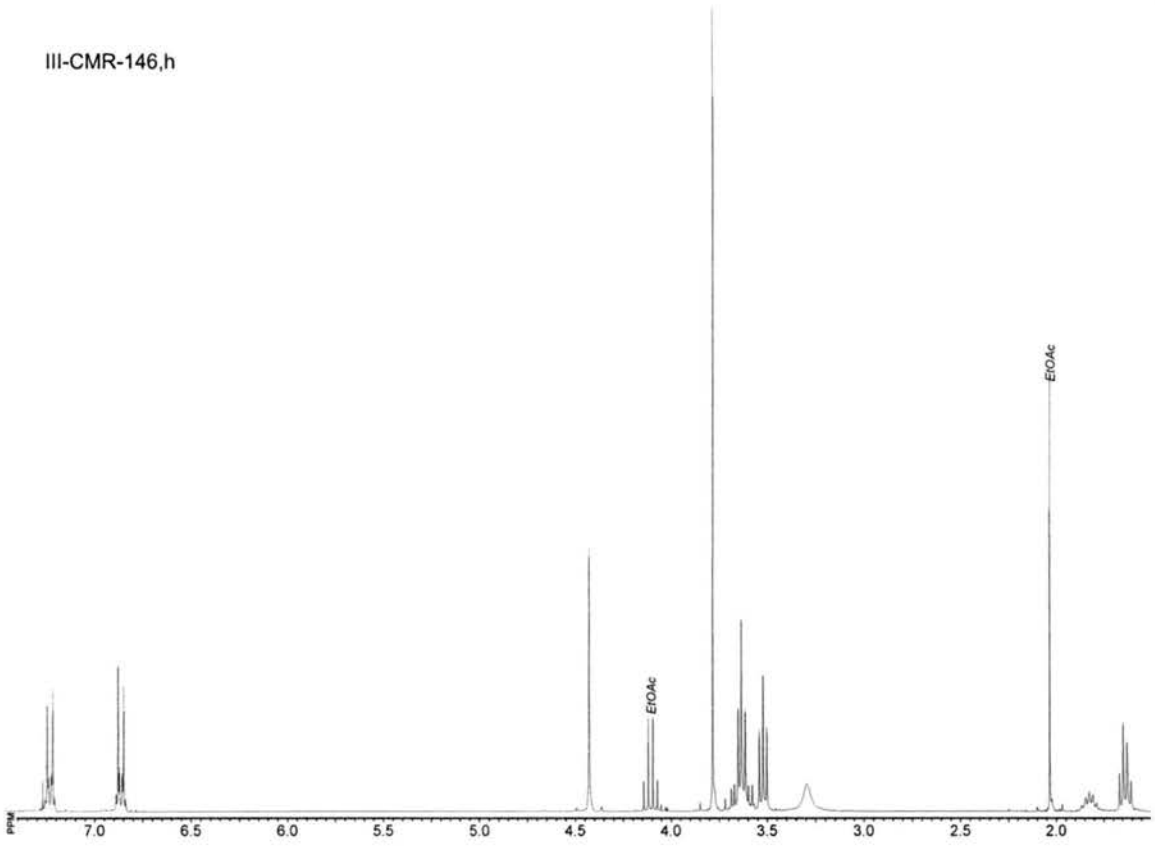
236 **237**

2-[2-(4-Methoxy-benzyloxy)-ethyl]-propane-1,3-diol (237): Diester **236** (1.83 g, 6.176 mmol) in dry THF (35 mL) added via addition funnel to a stirring suspension of LAH (1.406 g, 37.02 mmol, 6.0 eq) in dry THF (25 mL) at room temperature under argon. The resulting mixture stirred at room temperature for 14 h. The reaction was cooled to 0^0C and ice cold sat.aq. $KHSO_4$ (60 mL) was added slowly. After 30 min the resulting grayish slurry was extracted with diethyl ether (50 mL x 3). Combine organic extracts were washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Crude product isolated as a clear colorless oil and was sufficiently pure to be used without further purification. Yield 1.45 g, 6.034 mmol (98%).

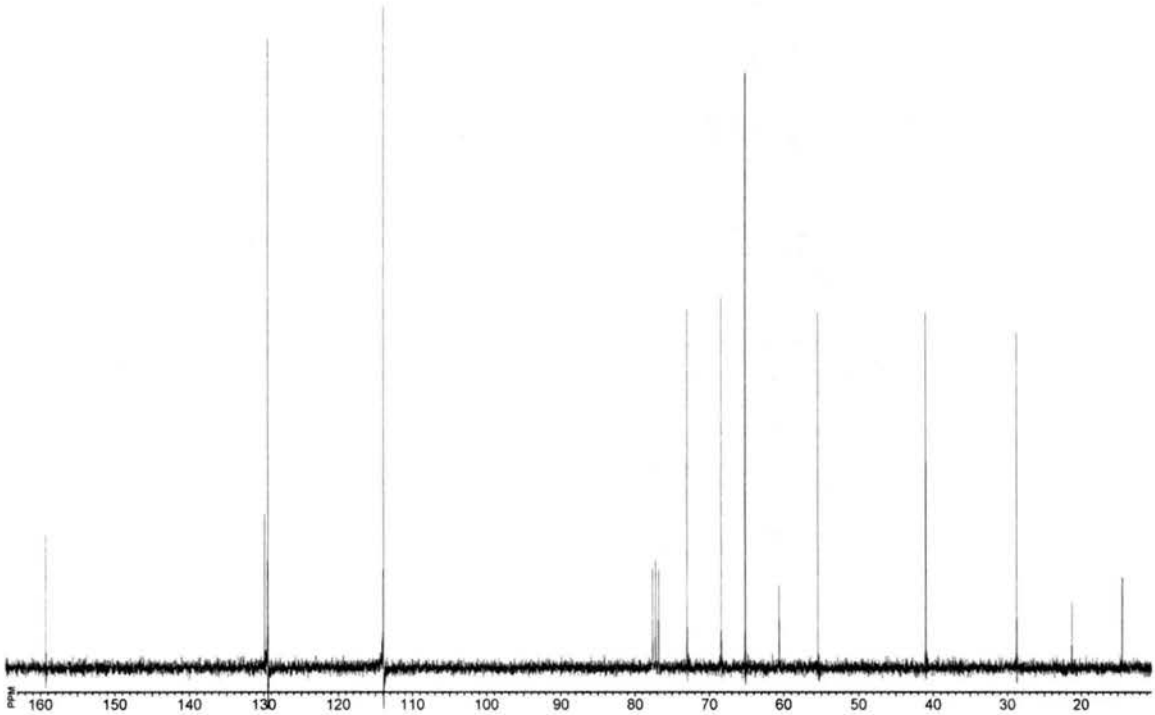
^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.711(q, J=5.8Hz, 2H), 1.868(sp, J=5.9 Hz, 1H), 2.150 (bs, 2H), 3.564 (t, J=5.8 Hz, 2H), 3.706 (ddd, J=15.7, 110., 5.5 Hz), 3.814 (s, 3H), 4.462 (s, 2H), 6.891 (d, J=8.7 Hz, 2H), 7.259 (d, J=9.1 Hz, 2H).

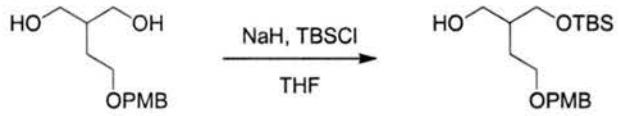
^{13}C NMR ($CDCl_3$, 75 MHz) δ175.1, 159.3, 129.5, 113.8, 72.9, 68.2, 65.4, 55.3, 41.1, 28.6.

III-CMR-146,h



III-CMR-146,c



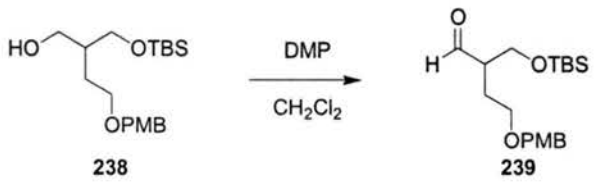


237 **238**

2-(tert-Butyl-dimethyl-silanyloxymethyl)-4-(4-methoxy-benzyloxy)-butan-1-ol (238): Sodium hydride (0.097 g, 4.042 mmol, 1.8 eq) added in one portion to a stirring solution of the diol **237** (0.537g, 2.235 mmol) in dry THF (4.5 mL) at room temperature under argon. After 1 h, TBSCl (0.338g, 2.242 mmol, 1.0 eq) was added to the resulting white slurry and stirred at room temperature for 1 h. The reaction mixture was then poured into a seraratory funnel containing diethyl ether (50 mL) and washed successively with 10% aq. Na_2CO_3 (25 mL) and brine (30 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Crude oil was purified on silica gel with 7:3 hexanes/ethyl acetate. The product wsa isolated as a clear colorless oil. Yield 0.785g, 2.214 mmol (99%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.058(d, J=1.3 Hz, 6H), 0.893 (s, 9H), 1.628 (q, J=6.2Hz, 2H), 1.88 (sp, J=5.6 Hz, 1H), 3.526 (m, 2H), 3,596 (dd, J=9.8, 6.8 Hz, 1H), 3.679 (d, J=2.5Hz, 1H), 3.666 (d, J=3.7 Hz, 1H), 3.733 (dd, J=9.8, 4.6 Hz, 1H), 3.814 (s, 3H), 4.449 (s, 2H), 6.886 (d, J=8.8 Hz, 2H), 7.259 (d, J=8.8 Hz, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz) δ 129.4, 113.8, 72.8, 68.2, 66.5, 65.7, 55.3, 40.3, 28.4, 25.9, 18.2, -5.6.

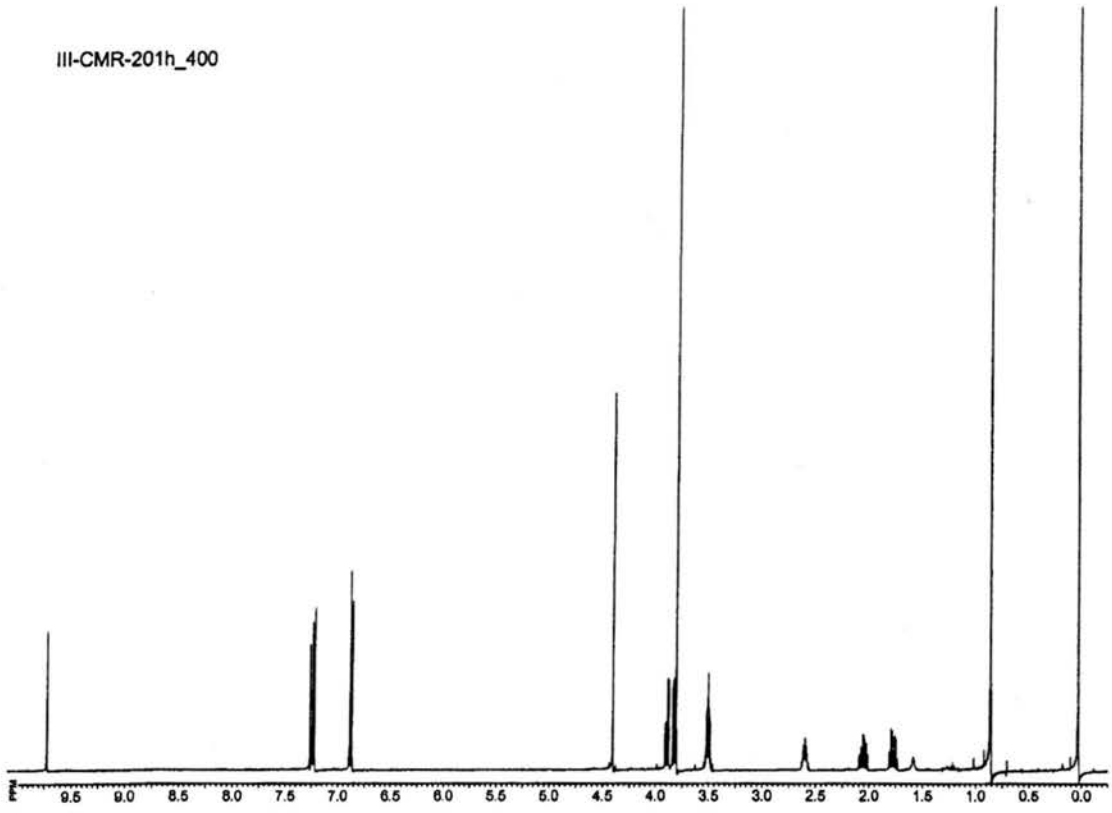


238 **239**

2-(tert-Butyl-dimethyl-silanyloxymethyl)-4-(4-methoxy-benzyloxy)-butyraldehyde (239): Dess-Martin periodinane (0.187 g, 0.4409 mmol, 1.5 eq) added to a stirring solution of the alcohol in CH_2Cl_2 (3.0 ml) at room temperature. The resulting mixture was stirred at room temperature for 1 h. Reaction was diluted with diethyl ether (4 mL), $Na_2S_2O_3$ (0.370g, 2.34 mmol, 7.9 eq) added followed by sat. aq $NaHCO_3$ (3 mL) and stirred vigorously until the biphasic mixture became clear (~15 min). The aqueous layer was separated and the organic layer was washed with sat. aq $NaHCO_3$ (3 mL). Combined aqueous washings were extracted with diethyl ether (5 mL x 3). The combined organic extracts were washed once more with sat. aq $NaHCO_3$ (5 mL), then brine (20 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Flash silica gel chromatography with 10:1 hexanes/ethyl acetate gave the pure compound as a clear colorless oil. Yield 0.089g, 0.2525 mmol (85%).

^1H NMR ($CDCl_3$, 400 MHz) δ 0.034 (s, 6H), 0.865 (s, 9H), 1.773 (dq, J=14.4, 5.9 Hz, 1H), 2.569-2.635 (m, 1H), 2.013-2.099 (m, 1H), 3.465-3.550 (m, 1H), 3.825 (dd, J=10.2, 6.0 Hz, 1H), 3.897 (dd, J=10.2, 4.9 Hz, 1H), 3.811 (s, 3H), 4.409 (s, 2H), 6.881 (d, J-8.5 Hz, 2H), 7.236 (d, J=8.5 Hz, 2H), 9.729 (d, J=2.2 Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.3, 159.2, 130.3, 129.3, 113.8, 72.6, 67.3, 61.8, 55.3, 51.6, 26.0, 25.8, 18.2, -5.6.


III-CMR-201h_400
PPM
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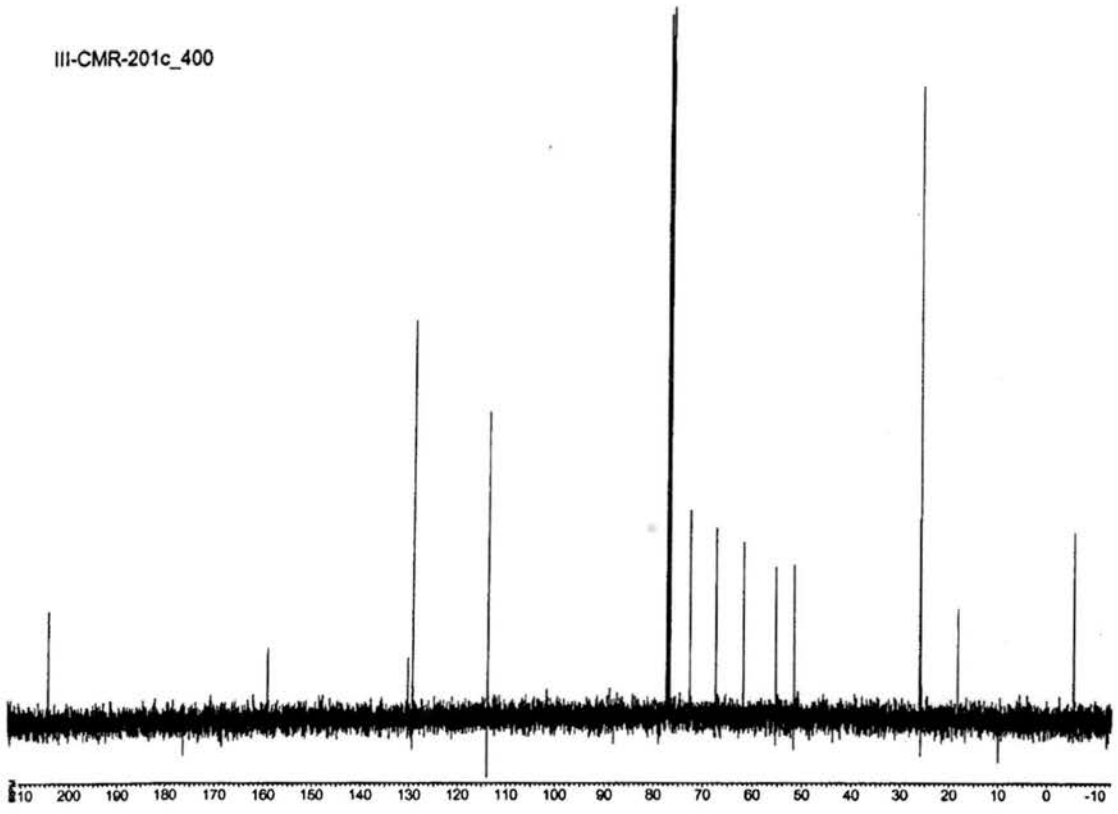
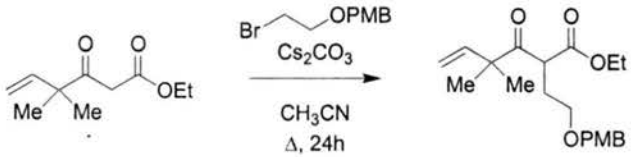

III-CMR-201c_400
PPM
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

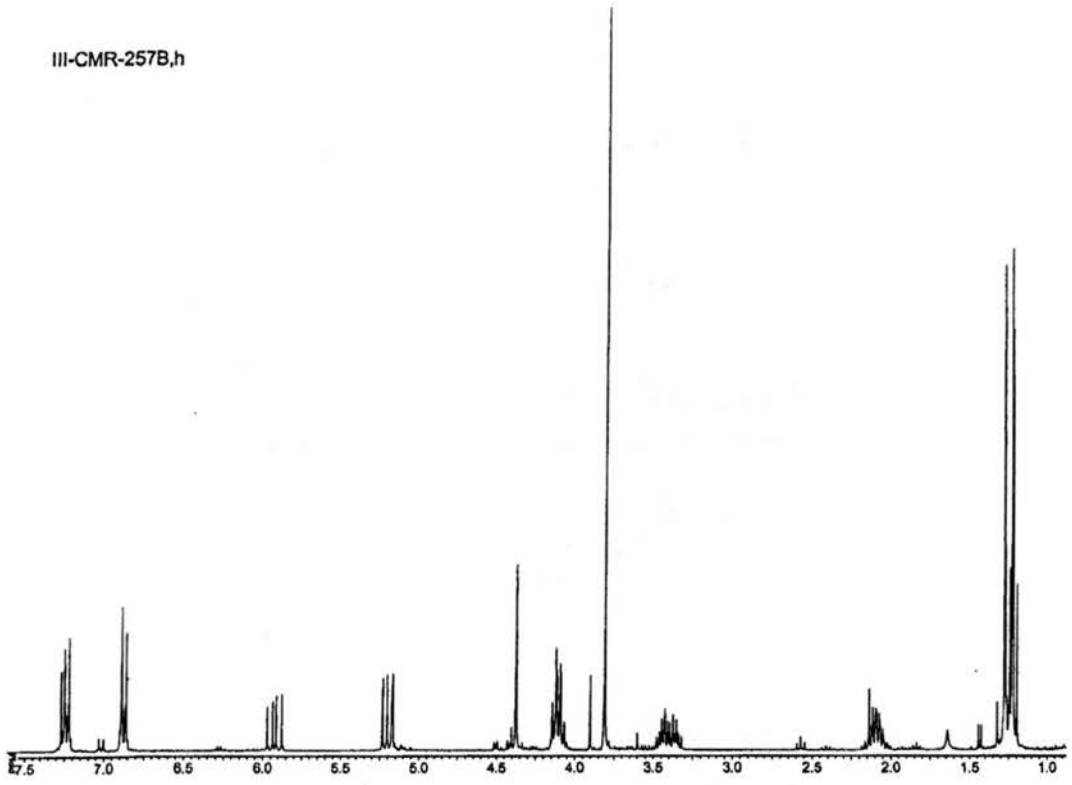


242 243

2-[2-(4-Methoxy-benzyloxy)-ethyl]-4,4-dimethyl-3-oxo-hex-5-enoic acid ethyl ester (243): Cesium carbonate (1.643g, 5.043 mmol, 3.0 eq) added to a stirring solution of the β-keto ester 30 (0.306g, 1.661 mmol) and the bromide (0.460g, 1.877 mmol, 1.1 eq) in anhydrous CH_3CN (17 mL). the resultion yellow solution was heated to reflux for 24h. The reaction was then acidified tp pH 2 with 1N HCl and extracted with EtOAc (25mL x 3). Combined organic extracts were then washed with brine (50 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Crude oil purified on silica gel with 10:1 hexanes/ethyl acetate. Product isolated as a clear, pale yellow oil. Yield 0.366g, 1.050 mmol (63%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.225 (t, J=7.1 Hz, 3H), 1.233 (s, 3H), 1.281 (s, 3H), 2.007-2.189 (m, 2H), 3.325-3.483 (m, 2H), 3.816 (s, 3H), 4.033-4.184 (m, 2H), 4.379 (s, 2H), 5.188 (dd, J= 0.9, 10.6 Hz, 1H), 5.208 (dd, J=0.9, 17.4 Hz, 1H), 5.929 (dd, J=10.6, 17.4 Hz, 1H), 6.879 (d, J=8.7 Hz, 2H), 7.237 (d, J=8.7 Hz, 2H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.2, 169.6, 141.2, 130.2, 129.2, 115.2, 113.7, 72.5, 67.2, 61.2, 55.3, 51.9, 49.8, 29.9, 23.4, 14.1.


III-CMR-257B,h
7.5
7.0
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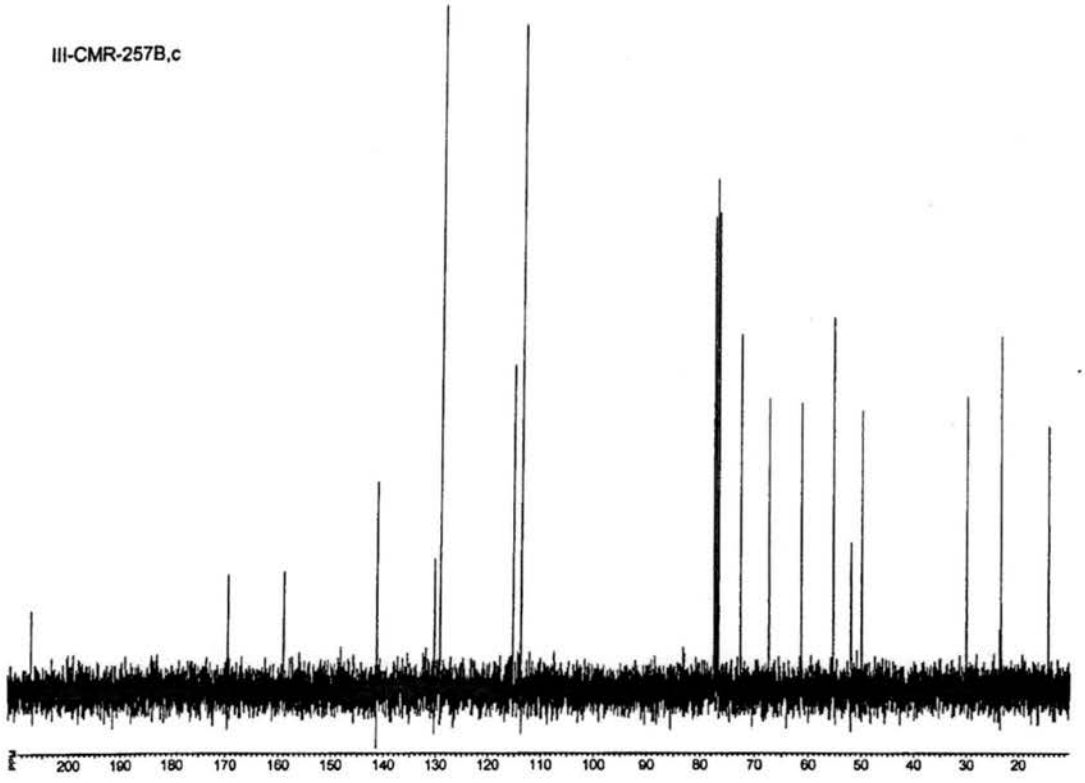
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


III-CMR-257B,c
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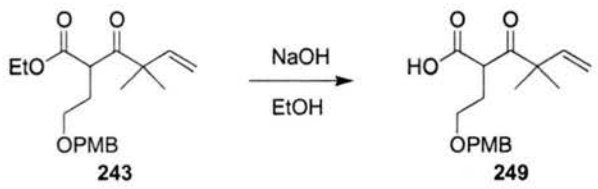
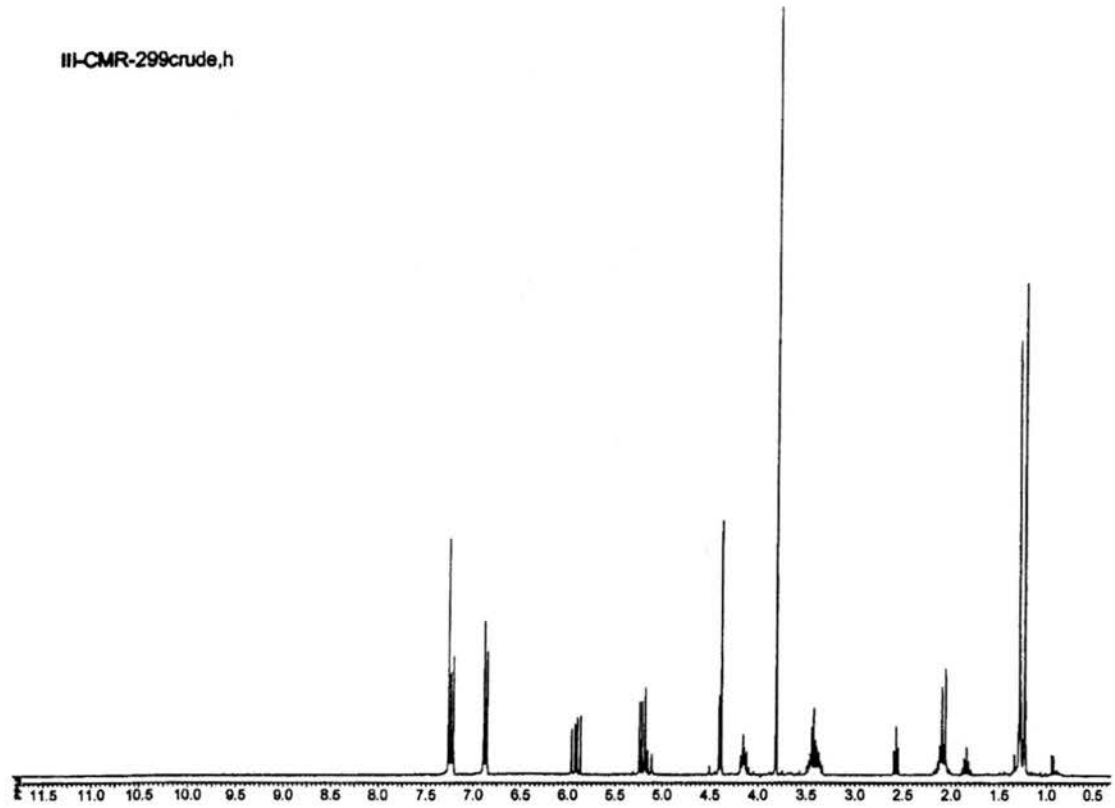
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

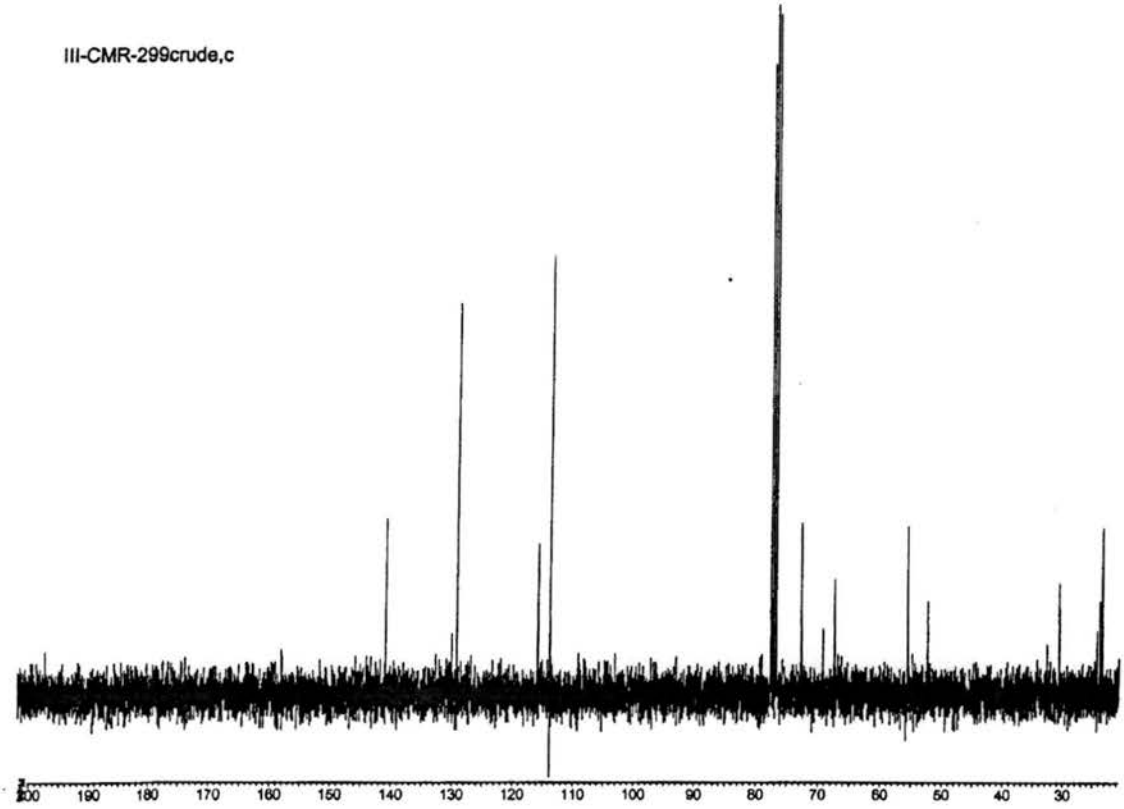


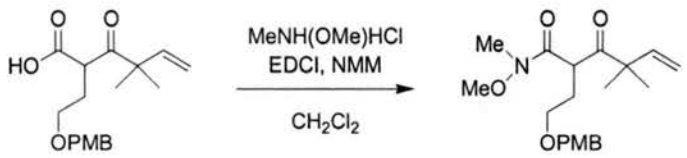
243 **249**

2-[2-(4-Methoxy-benzyloxy)-ethyl]-4,4-dimethyl-3-oxo-hex-5-enoic acid (249): 2M NaOH (0.050 mL, 0.1000 mmol, 3.2 eq) added to a stirring solution of the β-keto ester (0.011g, 0.03157 mmol) in abs. EtOH (0.5 mL) at room temperature and stirred for 18h. Solvent removed by rotary evaporation and the residue taken up in dH_2O (5mL) and extracted with Et_2O (5 mL). Organic extract discarded and aqueous layer acidified with 1N HCl and extracted with EtOAc (5 ml x 3). Combined organic extacts washed with brine (10 mL), dried (Na_2SO_4) an concentrated by rotary evaporation. Crude material isolated as a clear colorless oil was used without further purification. Yield 0.008g, 0.02497 mmol (79%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.226-1.231 (m, 3H), 1.284 (s, 3H), 2.016-2.190 (m, 2H), 3.340-3.487 (m, 2H), 3.812 (s, 3H), 4.169 (t, J=3.7 Hz, 1H), 4.385-4.410 (m, 2H), 5.114-5.248 (m, 2H), 5.917 (dd, J=9.6, 17.4, 1H), 6.876 (d, J-8.8 Hz, 2H), 7.231 (d, J= 8.8 Hz, 2H).


III-CMR-299crude,h
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5


III-CMR-299crude,c
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30

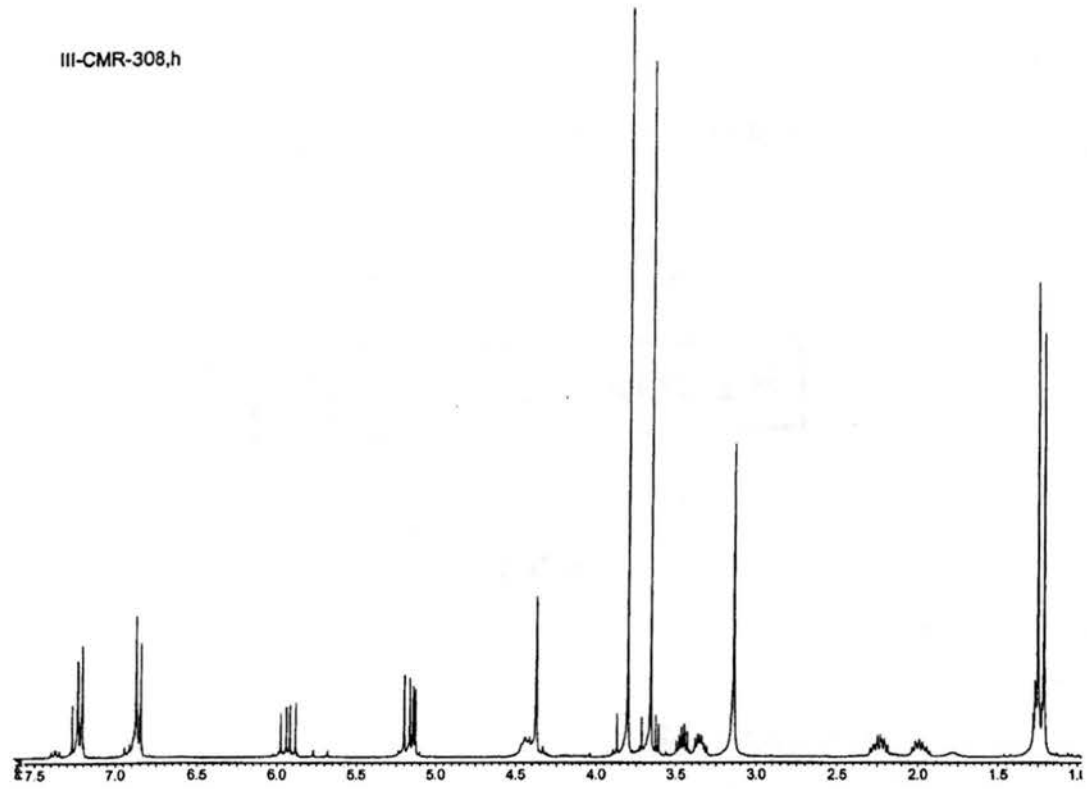


249 **250**

2-[2-(4-Methoxy-benzyloxy)-ethyl]-4,4-dimethyl-3-oxo-hex-5-enoic acid methoxy-methyl-amide (250): NMM (0.002 mL, 0.01819 mmol, 1 eq) and EDCI (0.0039 g, 0.02034 mmol, 1.1 eq) added successively to a stirring solution of the β-keto acid (0.006g, 0.01873 mmol) in anhydrous CH_2Cl_2 (0.5 mL) at 0°C and allowed to warm to room temperature oer 3h. Solvent was removed by rotary evaporation and the crude product was purified by PTLC with 8:2 hexanes/ethyl acetate. Product obtained as a clear, colorless oil. Yield 0.005g, 0.01376 (73%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.212 (s, 3H), 1.251 (s, 3H), 1.928-2.035 (m, 1H), 2.188-2.295 (m, 1H), 3.144 (s, 3H), 3.310-3.383 (m, 1H), 3.343-3.500 (m, 1H), 3.660 (s, 3H), 3.804 (s, 3H), 4.336-4.465 (m, 1H), 4.377 (s, 3H), 5.150 (dd, J=0.9, 10.6 Hz, 1H), 5.176 (dd, J=0.9, 17.6 Hz, 1H), 5.932 (dd, J=10.6, 17.6, 1H), 6.764 (d, J=8.6 Hz, 2H), 7.224 (d, J=8.6 Hz, 2H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.1, 158.9, 142.1, 130.4, 128.9, 114.4, 113.6, 72.3, 67.7, 61.3, 55.3, 51.1, 47.2, 33.0, 29.2, 24.1, 23.5.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6.0
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5.0
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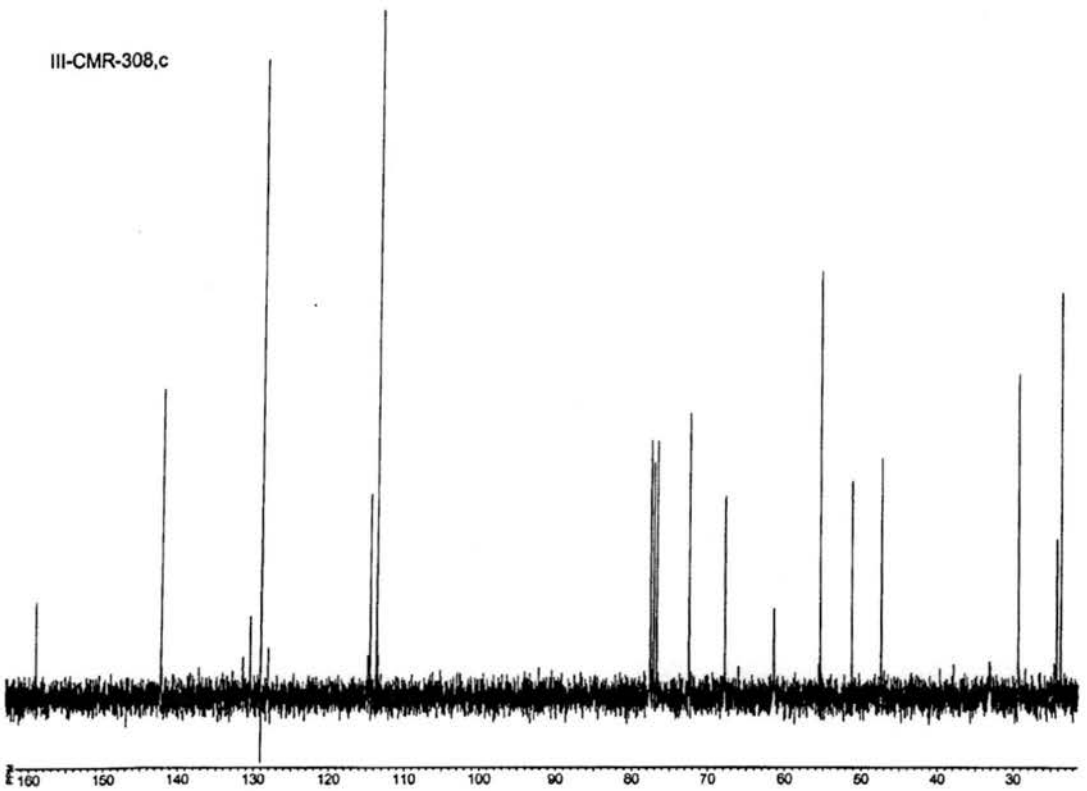
4.0
3.5
3.0
2.5
2.0
1.5


III-CMR-308,c
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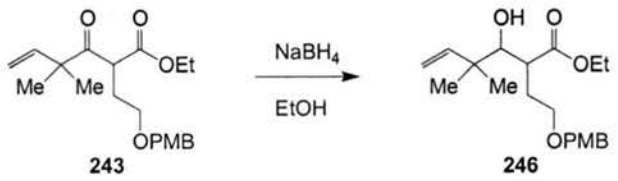
130
120
110
100
90
80
70
60
50
40
30

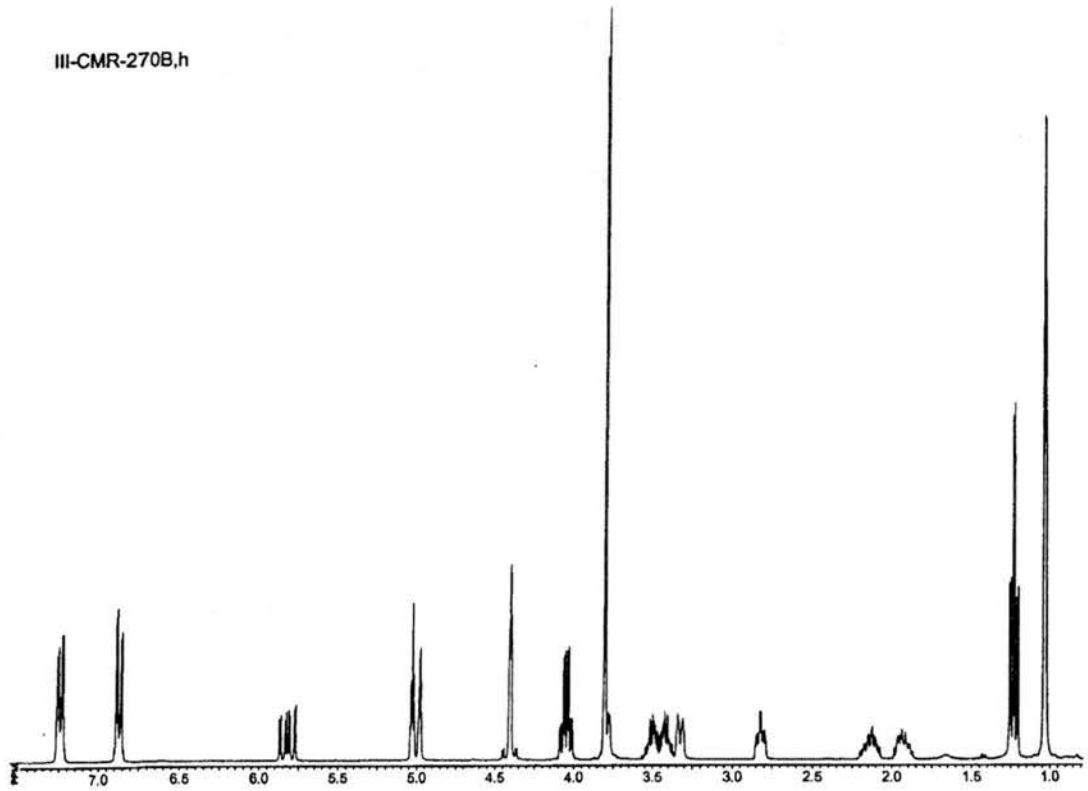


243 246

3-Hydroxy-2-[2-(4-methoxy-benzyloxy)-ethyl]-4,4-dimethyl-hex-5-enoic acid ethyl ester (246): $NaBH_4$ (0.010g, 0.2643 mmol, 1.3 eq) added to a stirring solution of the β-keto ester (0.069g, 0.1980 mmol) in abs. EtOH (2 mL) at room temperature. The resulting solution stirred at room temperature for 24h. Solvent removed by rotary evaporation. The white residue taken up in 1N HCl (5 mL) and washed with EtOAc (5mL x 3). Combined organic extracts washed with saturated $NaHCO_3$ (5 mL) and brine (15 mL), dried (Na_2SO_4), filtered and concentratd by rotary evaporation. Crude oil purified on silica gel with 10:1 hexanes/ethyl acetate. Product isolated as a clear colorless oil. Yield 0.043g, 0.1227 mmol (74%).

^1H NMR ($CDCl_3$, 300 MHz) δ 1.024-1.040 (m, 6H), 1.199-1.257 (m, 3H), 1.866-1.982 (m, 1H), 2.072-2.199 (m, 1H), 2.787-2.852 (m, 1H), 3.300-3.339 (m, 1H), 3.369-3.555 (m, 2H), 3.769-3.778 (m, 1H), 3.802 (d, J=2.9 Hz, 3H), 4.006-4.088 (m, 2H), 4.967-4.991 (m, 1H), 5.012-5.041 (m, 1H), 5.765-5.869 (m, 1H), 6.851-6.890 (m, 2H), 7.222-7.266 (m, 2H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.5, 159.0, 143.7, 130.2, 129.2, 113.6, 112.7, 80.2, 72.7, 67.3, 60.6, 55.3, 42.3, 41.6, 32.6, 24.0, 14.0.


III-CMR-270B,h
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4.5
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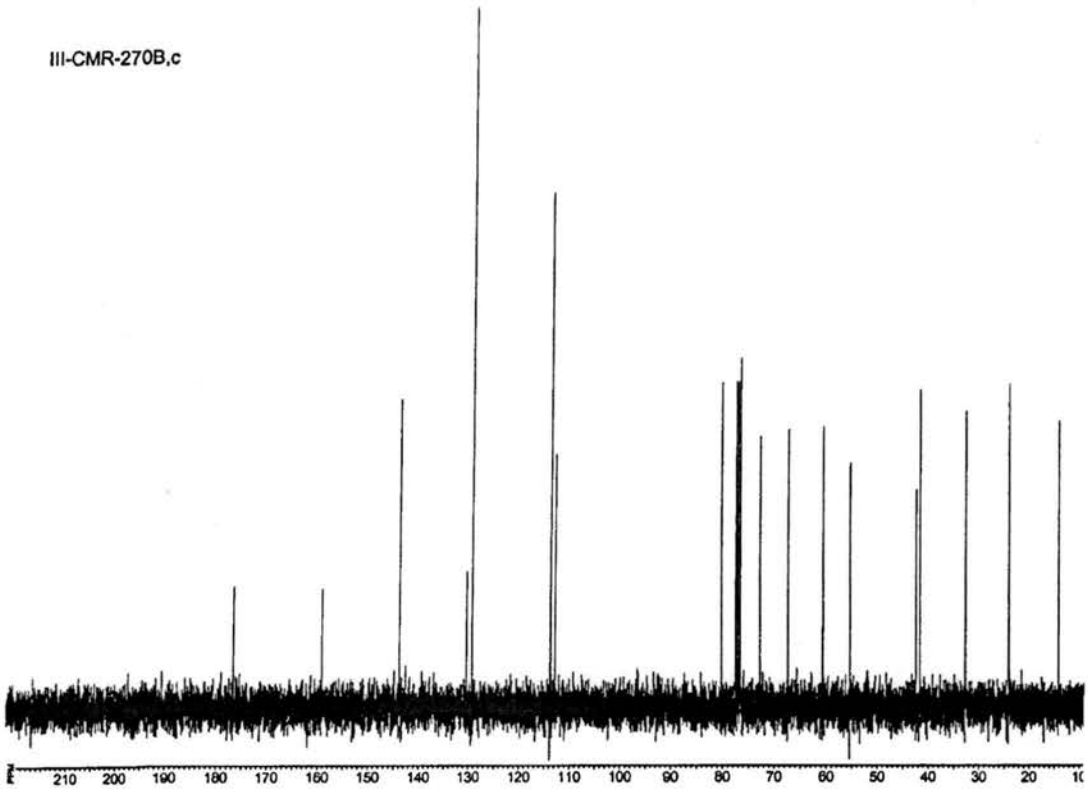
1.5
1.0


III-CMR-270B,c
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130
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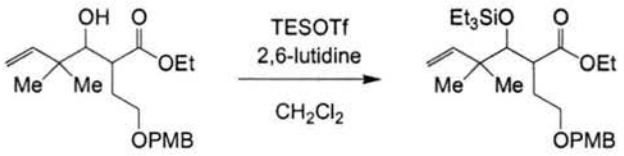
100
90
80
70
60
50
40
30
20

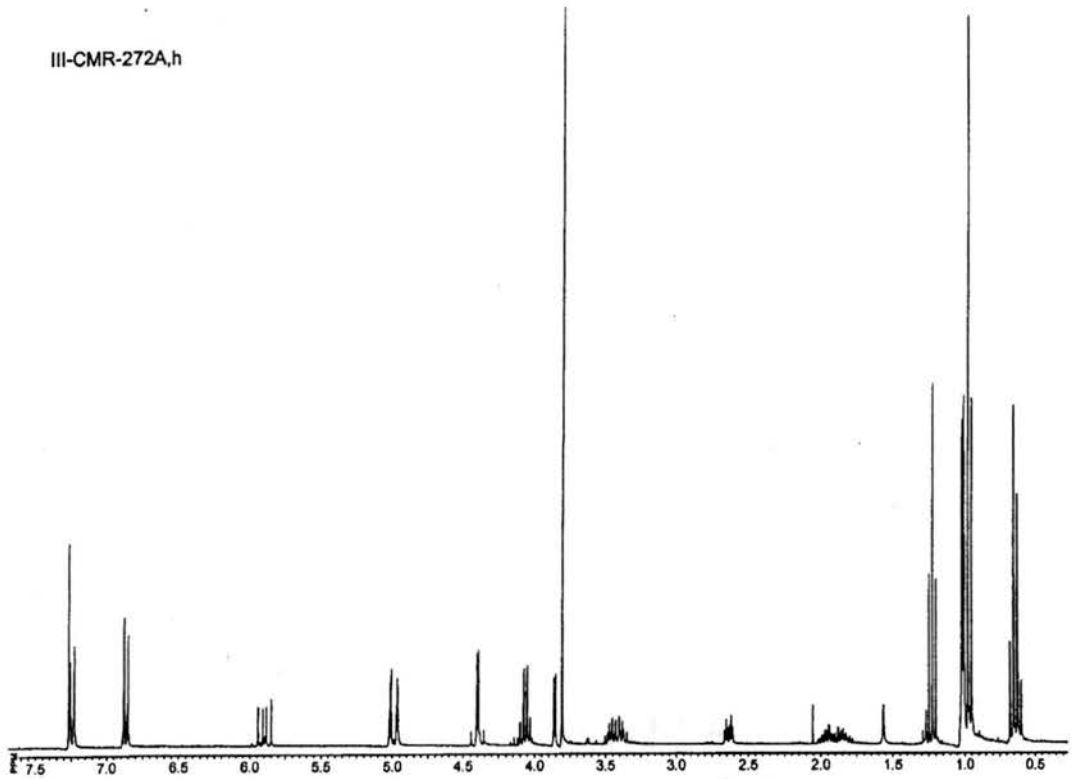


246 **247**

2-[2-(4-Methoxy-benzyloxy)-ethyl]-4,4-dimethyl-3-triethylsilanyloxy-hex-5-enoic acid ethyl ester (247): TESOTf (0.0063 mL, 0.03007 mmol, 1.3 eq) added to a stirring solution of the β-hydroxy ester (0.008g, 0.02283 mmol) and 2,6-lutidine (0.006 mL, 0.05151 mmol, 2.25 eq) in anhydrous CH_2Cl_2 (0.2 mL) at 0°C under argon. The resulting solution stirred at 0°C for 2.5h then quenched by the addition of abs. EtOH (2 mL). Reaction mixture was then concentrated by rotary evaporation and purified by PTLC with 8:2 hexanes/ethyl acetate. Product isolated as a clear colorless oil. Yield 0.008g, 0.01722 mmol (75%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ0.629 (q, J= 8 Hz, 6H), 0.956 (t, J=8 Hz, 9H), 1.003 (d, J=3.5 Hz, 6H), 1.213 (t, J=7.2 Hz, 3H), 1.849-2.007 (m, 2H), 2.596-2.654 (m, 2H), 3.338-3.494 (m, 2H), 3.797 (s, 3H), 3.848 (d, J=3.8 Hz, 1H), 4.017-4.092 (m, 2H), 4.389 (dd, J=11.7, 15.2 Hz, 2H), 4.938-4.974 (m, 1H), 4.979-5.016 (m, 1H), 5.885 (dd, J=10.6, 17.8, 1H), 6.857 (d, J=8.8 Hz, 2H), 7.233 (d, J=8.8 Hz, 2H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ175.5, 158.9, 145.0, 128.9, 113.6, 112.1, 79.4, 72.4, 68.8, 60.3, 55.3, 44.7, 43.4, 27.8, 24.6, 22.9, 14.7, 7.2, 5.4.


III-CMR-272A,h
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2.5
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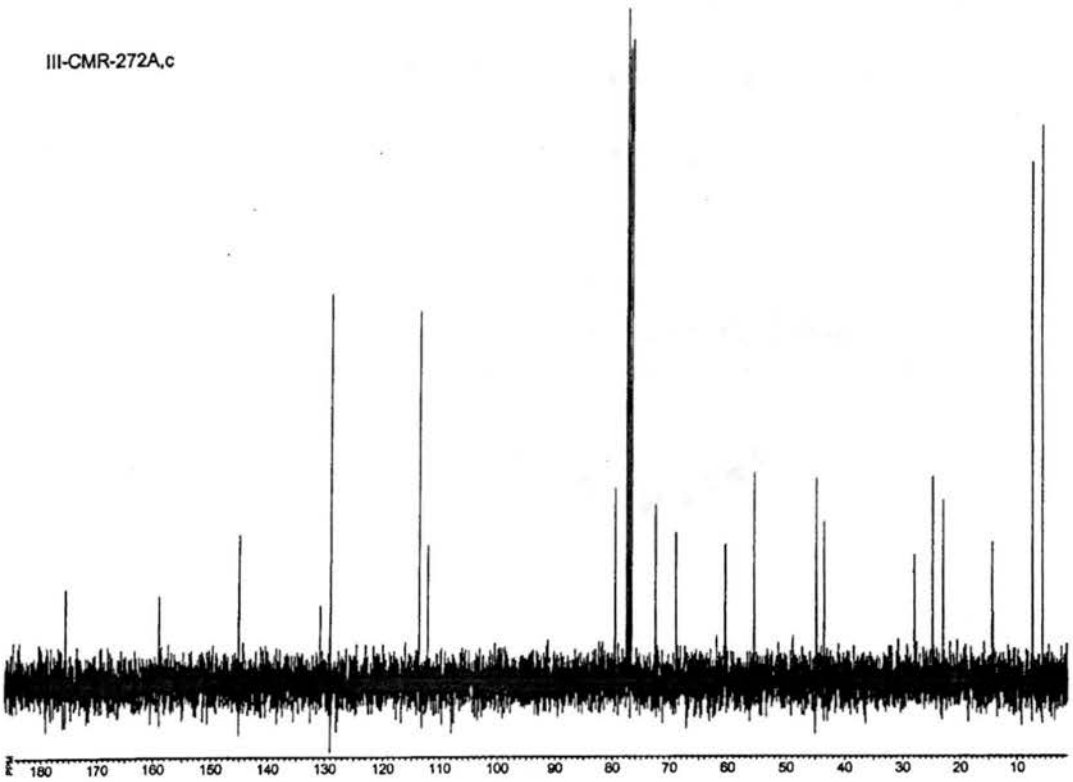
1.5
1.0
0.5


III-CMR-272A,c
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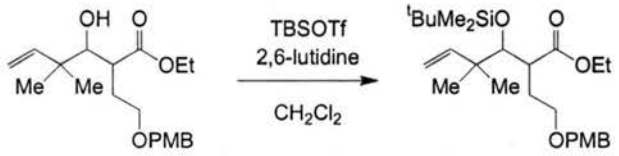
50
40
30
20
10



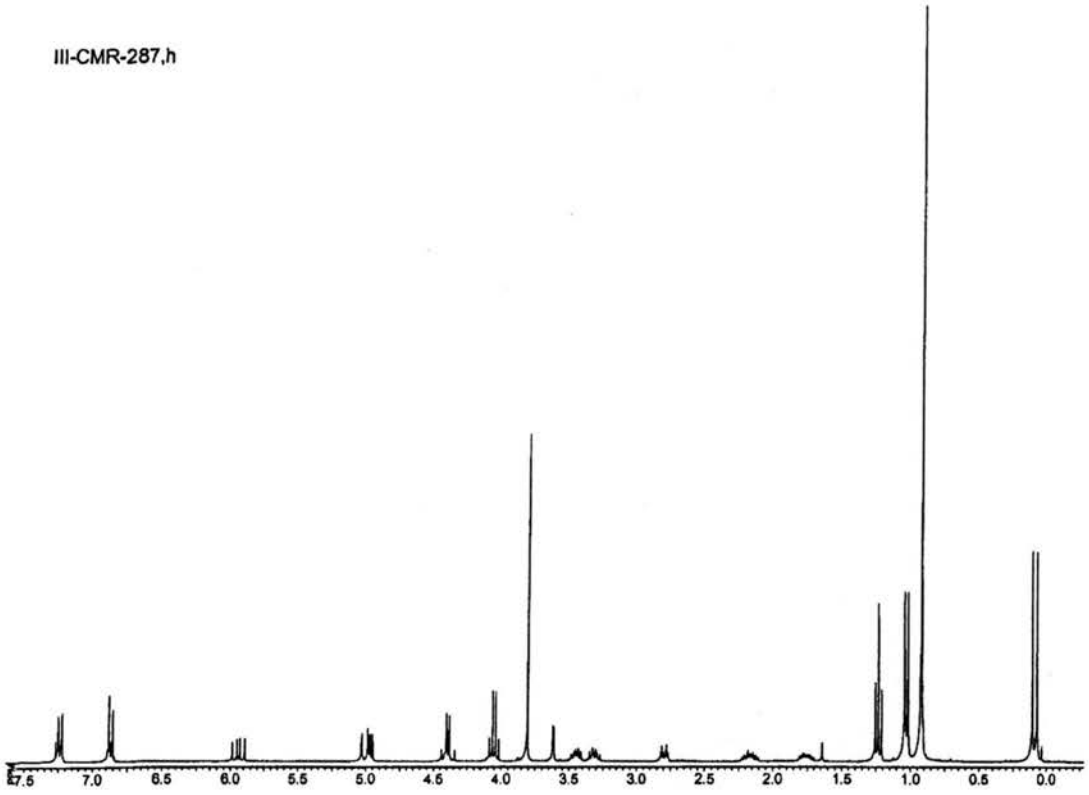
246

3-(tert-Butyl-dimethyl-silanyloxy)-2-[2-(4-methoxy-benzyloxy)-ethyl]-4,4-dimethyl-hex-5-enoic acid ethyl ester: TBSOTf (0.034 mL, 0.1480 mmol, 1.3 eq) added to a stirring solution of the b-hydroxy ester (0.039g, 0.1113 mmol) and 2,6-lutidine (0.028 mL, 0.2546 mmol, 2.3 eq) in anhydrous CH_2Cl_2 (1.5 mL) at 0°C under argon. The resulting solution stirred at 0°C for 3.5h then quenched by the addition of abs. EtOH (2 mL). Reaction mixture was then concentrated by rotary evaporation and purified on silica gel with 8:2 hexanes/ethyl acetate. Product isolated as a clear colorless oil. Yield 0.032g, 0.06892 mmol (62%).

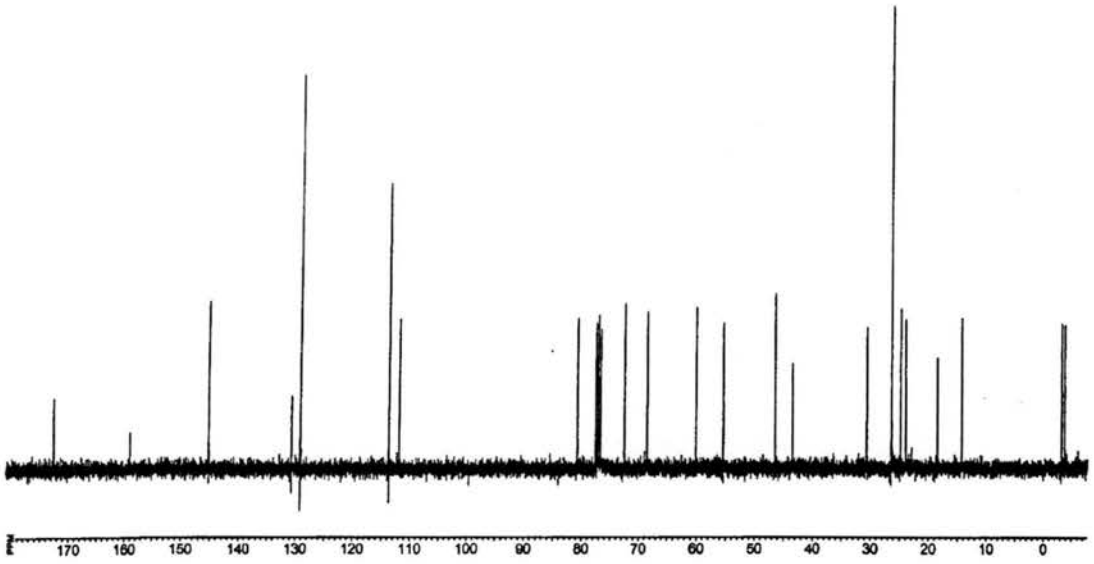
^{1}H NMR ($CDCl_3$, 300 MHz) δ0.071 (s, 3H), 0.106 (s, 3H), 0.918 (s, 9H), 1.018, (s, 3H), 1.043 (s, 3H), 1.232 (t, J=7.2 Hz, 3H), 1.704-1.810 (m, 1H), 2.107-2.226(m, 1H), 2.767-2.826 (m, 1H), 3.273-2.351 (m, 1H), 3.413-3.485 (m, 1H), 3.619 (d, J=3.1 Hz, 1H), 3.808 (s, 3H), 4.054 (q, J=7.2Hz, 2H), 4.393 (dd, J=11.5, 11.5 Hz, 2H), 4.971(dd, J=1.5, 10.8 Hz, 1H), 5.005 (dd, J=1.5, 17.6Hz, 1H), 5.942 (dd, J=10.8, 17.7 Hz, 1H), 6.868 (d, J=8.8 Hz, 2h), 7.239 (d, J=8.8 Hz, 2H).

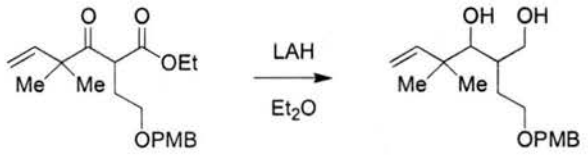
^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.5, 158.9, 145.1, 130.6, 129.0, 113.6, 111.8, 80.6, 72.4, 68.4, 60.0, 55.3, 46.3, 43.4, 30.4, 26.2, 24.7, 23.8, 18.3, 14.1, -3.4, -4.0.

III-CMR-287,h



III-CMR-287,c





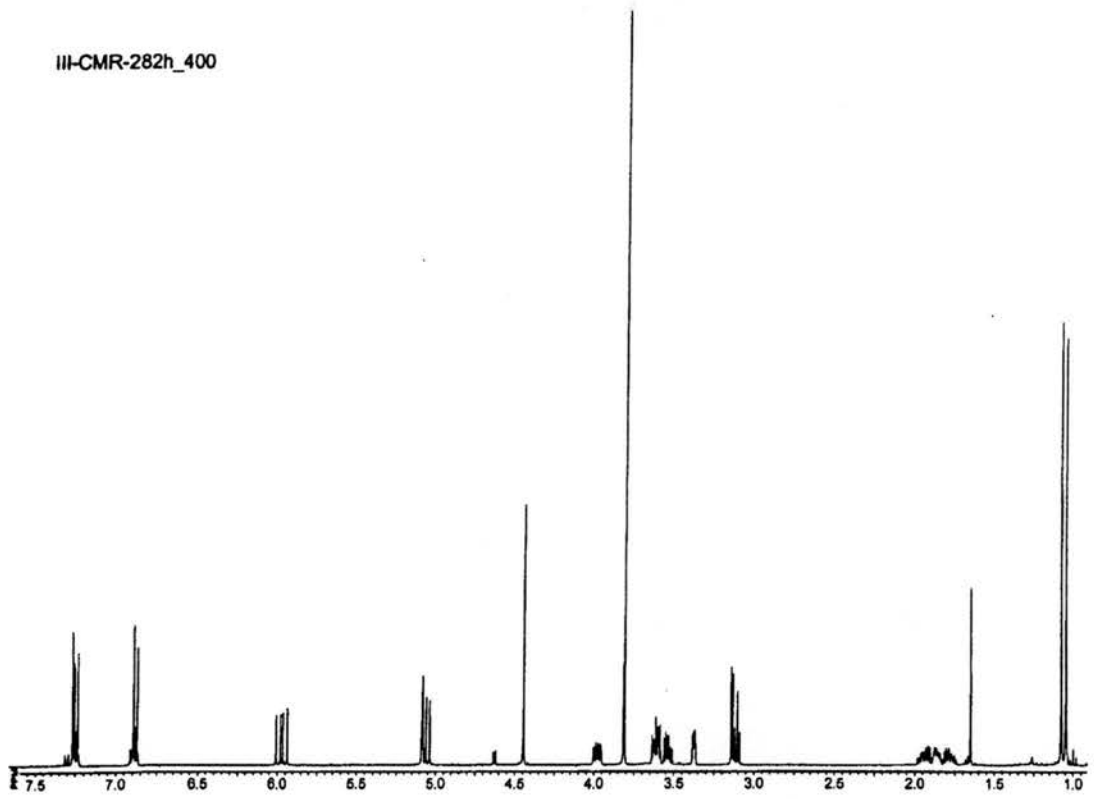
243 **244**

2-[2-(4-Methoxy-benzyloxy)-ethyl]-4,4-dimethyl-hex-5-ene-1,3-diol (244): $LiAlH_4$ (0.048g, 1.265 mmol, 5.8 eq) added to a stirring solution of the β-keto ester (0.075g, 0.2153 mmol) in anhydrous THF (3.0 mL) at room temperature under argon. The resulting reaction mixture was stirred for 2h then quenched by addition of excess $Na_2SO_4 \cdot 10\ H_2O$. After 30 min the crude suspension was filtered through celite with EtOAc and concentrated by rotary evaporation. The crude oil was purified on silica gel with 1:1 hexane/ethyl acetate. Product (a mixture of racemic diastereomers) was isolated as a clear colorless oil. Yield 0.064g, 0.2075 mmol (96%).

^1H NMR ($CDCl_3$, 400 MHz) δ 1.049 (s, 3H), 1.069 (s, 3H), 1.790-1.885 (m, 3H), 2.762-2.907 (m, 1H), 3.446-3.664 (m, 4H), 3.709-3.778 (m, 1H), 3.814 (s, 3H), 4.450 (s, 3H), 4.998-5.094 (m, 2H), 5.938-6.012 (m, 1H), 6.886 (d, J=8.8 Hz, 2H), 7.249 (d, J=8.8 Hz, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3, 145.5, 145.1, 129.9, 129.4, 113.8, 112.8, 112.3, 81.9, 80.9, 72.8, 81.0, 72.8, 68.7, 68.0, 67.1, 62.9, 61.9, 55.3, 41.9, 39.6, 37.7, 32.4, 25.4, 24.8, 24.4, 23.6, 22.7.

HRMS (FAB) M+H calc'd. 465.303628 found 465.302947.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7.5
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5.0
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4.0
3.5
3.0
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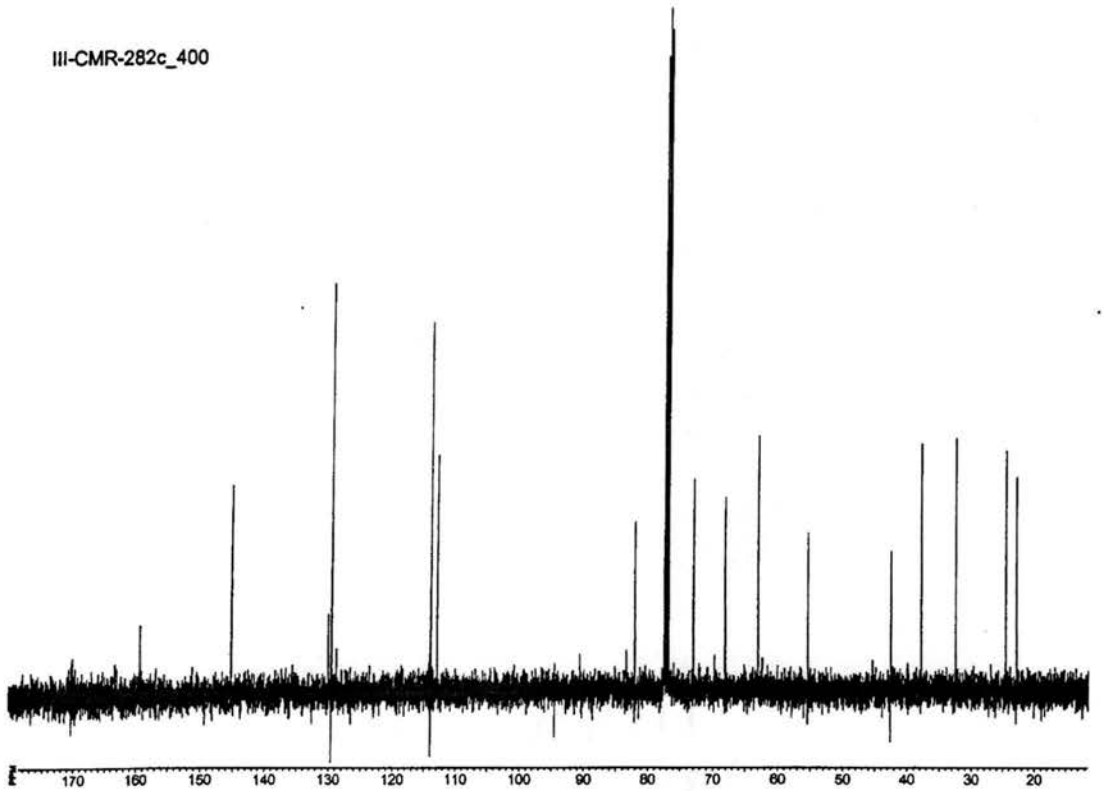
2.0
1.5
1.0


III-CMR-282c_400
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60
50
40
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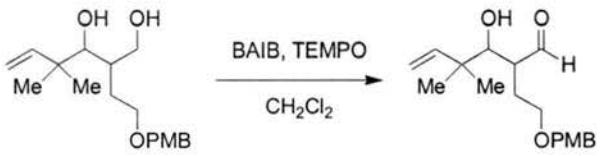
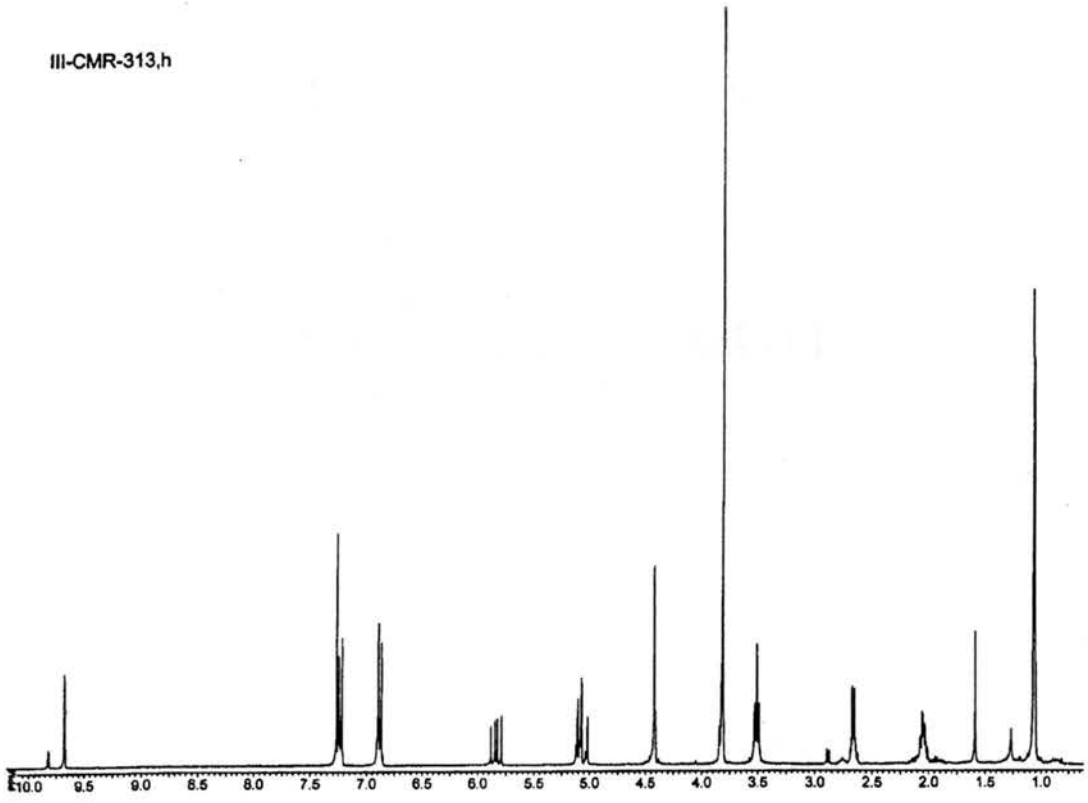


244 **252**

3-Hydroxy-2-[2-(4-methoxy-benzyloxy)-ethyl]-4,4-dimethyl-hex-5-enal (252): Iodobenzene diacetate (BAIB, 0.041g, 0.1273 mmol, 1.1 eq) and TEMPO (0.002g, 0.0128 mmol, 0.1 eq) added to a stirring solution of the diol (0.036 g, 0.1167 mmol) in anhydrous CH_2Cl_2 (1.2 mL) at room temperature. After stirring for 4h, $Na_2S_2O_3$ (0.070g) and sat. aq. $NaHCO_3$ (3mL) added with stirring for 30 min. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 mL x 3). Combined organic extracts were washed with brine (10 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Crude product purified on silica gel with 6:4 hexanes/ethyl acetate. Product isolated as a clear colorless oil. Yield 0.027g, 0.08818 mmol (76%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.051 (s, 3H), 1.060 (s, 3H), 2.011-2.083 (m, 2H), 2.669-2.689 (m, 2H), 3.491-3.533 (m, 3H), 3.809-3.846 (m, 1H), 3.816 (s, 3H), 4.424 (s, 2H), 5.016-5.126 (m 2H), 5.838 (dd, J=10.9, 17.4 Hz, 1H), 6.885 (d, J=8.6 Hz, 2H), 7.236 (d, J=8.6 Hz, 2H), 9.677 (d, J=2.3Hz, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.6, 144.7, 129.7, 129.2, 113.7, 113.6, 75.2, 72.8, 67.7, 55.3, 51.9, 42.2, 26.2, 24.0, 23.2.


III-CMR-313,h
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3.5
3.0
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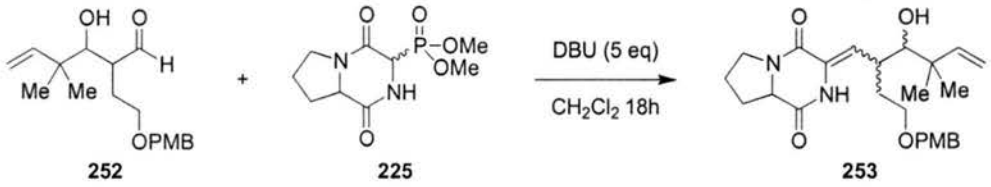
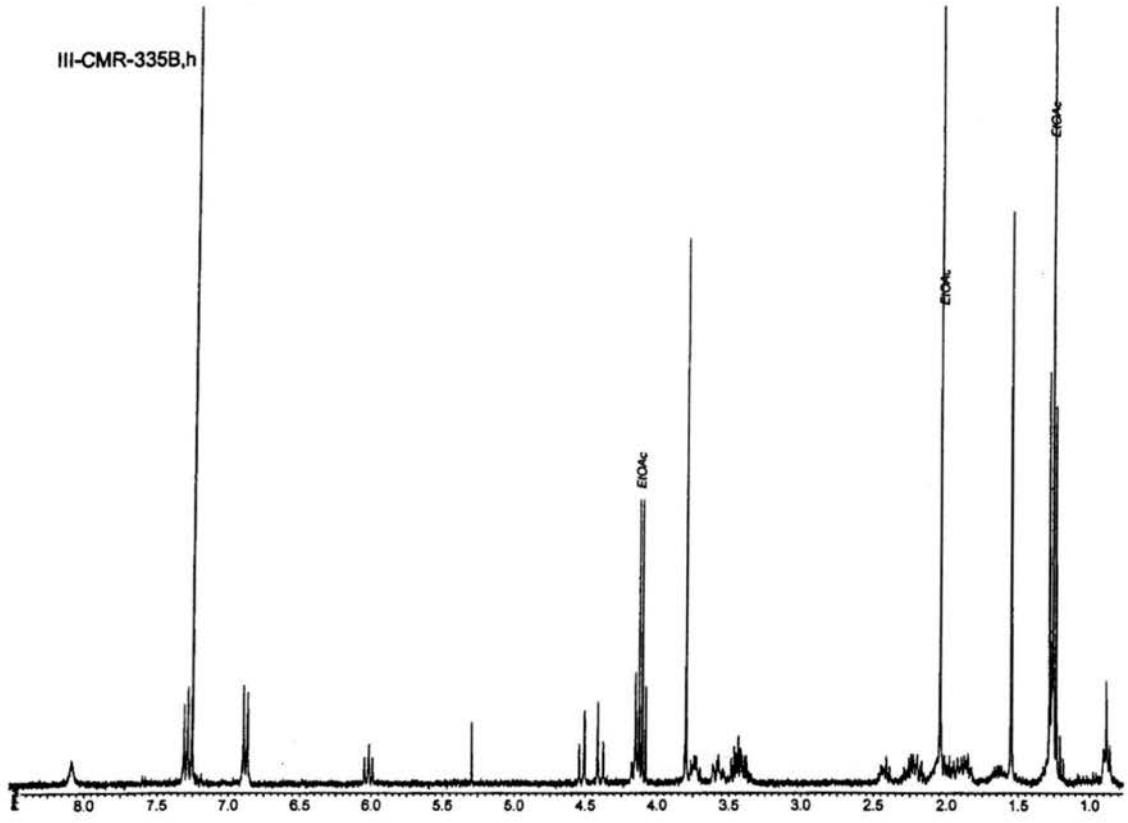
2.0
1.5
1.0



3-{3-Hydroxy-2-[2-(4-methoxy-benzyloxy)-ethyl]-4,4-dimethyl-hex-5-enylidene}-hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione (253): DBU (0.022 mL, 0.1471 mmol, 5.0 eq) added to a stirring solution of the phosphinate ester (0.008g, O.03051 mmol, 1.04 eq) in anhydrous CH_2Cl_2 (0.4 mL) at room temperature under an inert atmosphere. After 20 min of stirring the aldehyde (0.009g, 0.02937 mmol) in anhydrous CH_2Cl_2 was added and the resulting mixture was stirred at room temperature for 19h. Solvent and volatile reagents removed by rotary evaporation. The residue was then suspended in EtOAc (10 mL) and washed with 5% aq H_2SO_4 (4 mL x 2), dried (Na_2SO_4) filtered and concentrated by rotary evaporation. The crude product was purified by PTLC with 95:5 hexanes / ethyl acetate.

Alternate method: The DKP phosphinate (0.0207g, 0.07895 mmol, 1.2 eq) in anhydrous CH_2Cl_2 (0.35 x 2) was added via cannula to a stirring suspension of KO^{t}Bu (0.0093g, 0.08288 mmol, 1.27 eq) in anhydrous CH_2Cl_2 (0.5 mL) at -78°C under argon. After 15 min, the β-hydroxy aldehyde (0.020g, 0.06527 mmol) in anhydrous CH_2Cl_2 (0.4 mL x 2) was added via cannula. The resulting pale yellow solution was slowly warmed to room temperature and stirred for 16h. The crude product was purified on silica gel with 97:3 hexanes / ethyl acetate. Product isolated as a clear, colorless oil. Yield 0.022g, 0.04971 mmol (63%).


III-CMR-335B,h
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EtOAc
EtOAc
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3.5
3.0
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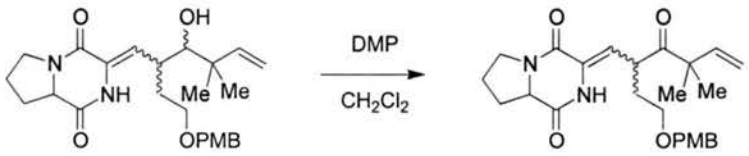
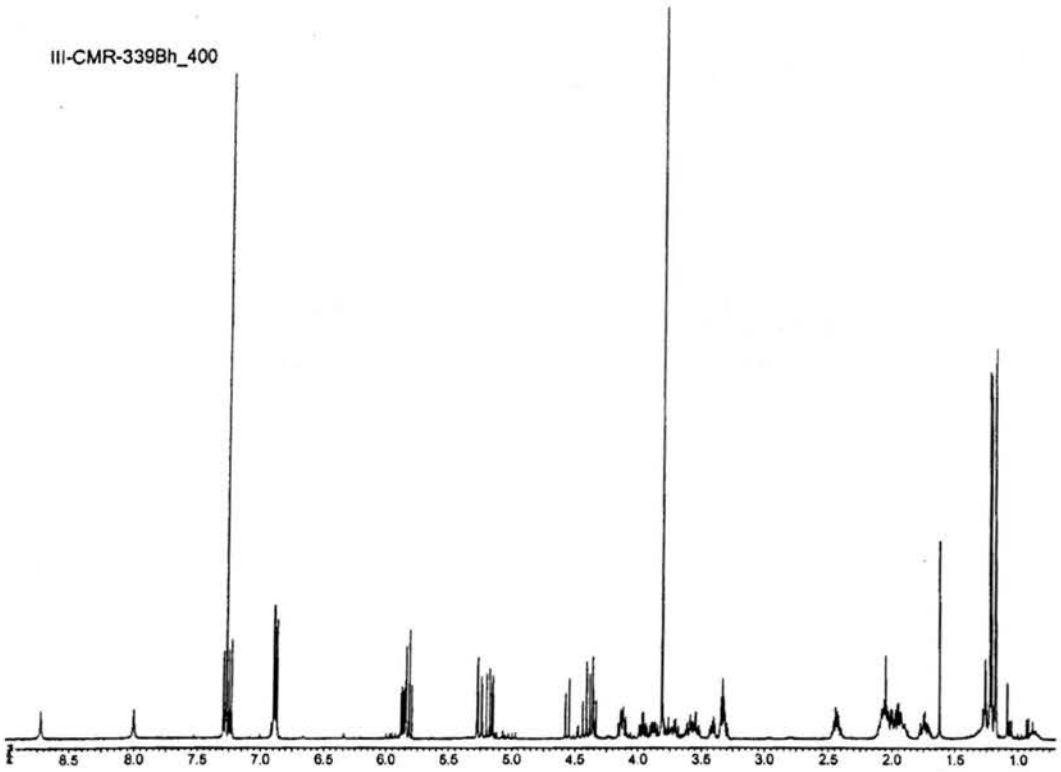
1.5
1.0



253 255

3-{2-[2-(4-Methoxy-benzyloxy)-ethyl]-4,4-dimethyl-3-oxo-hex-5-enylidene}-hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione (255): Dess-Martin periodinane (0.029g, 0.06837mmol, 1.4 eq) was added to a stirring solution of the alcohol (0.022g, 0.4971 mmol) in CH_2Cl_2 (1 mL) at room temperature and stirred for 1.5h. The resulting reaction was diluted with Et_2O (2 mL) and $Na_2S_2O_3$ (0.063g, 8 eq) and sat. aq. $NaHCO_3$ were added successively and stirred vigorously until the biphasic mixture became clear (15 min). The aqueous layer was separated and the organic layer was washed with sat. aq. $NaHCO_3$ (3 mL). Combined aqueous washings were extracted with CH_2Cl_2 (3 mL x 3). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification by PTLC with EtOAc (x2) provided the product as a clear, colorless oil. Yield 0.018g, 0.04086 mmol (82%).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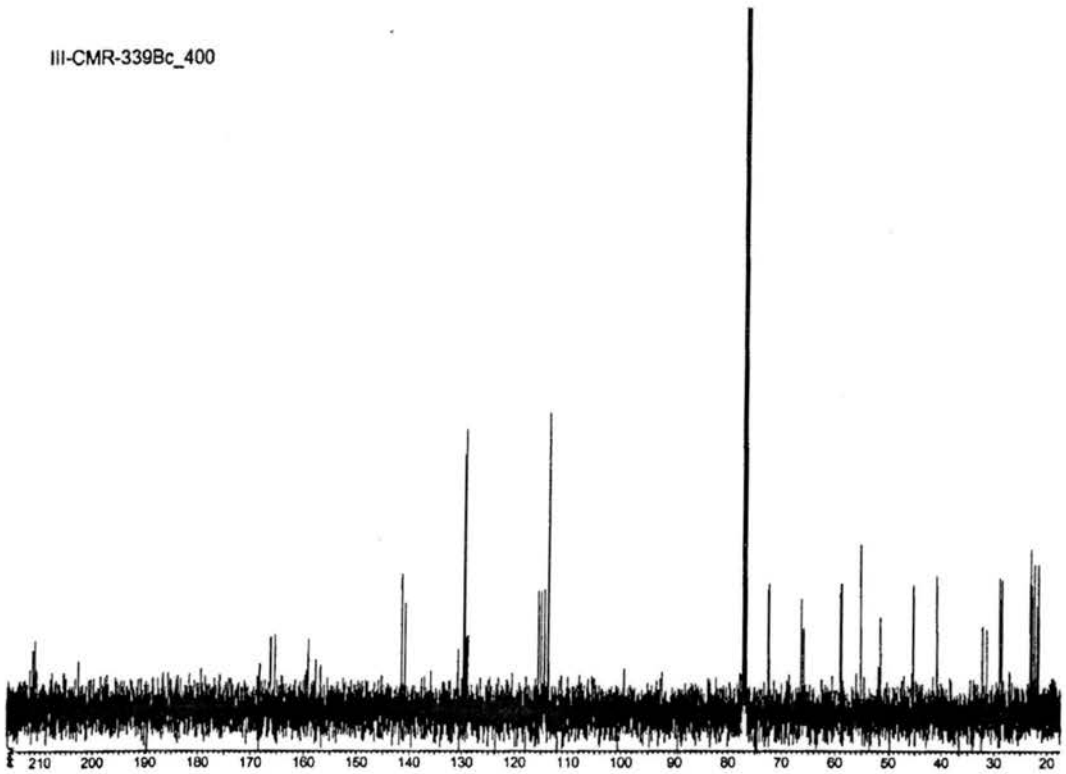
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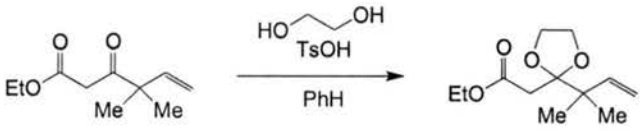
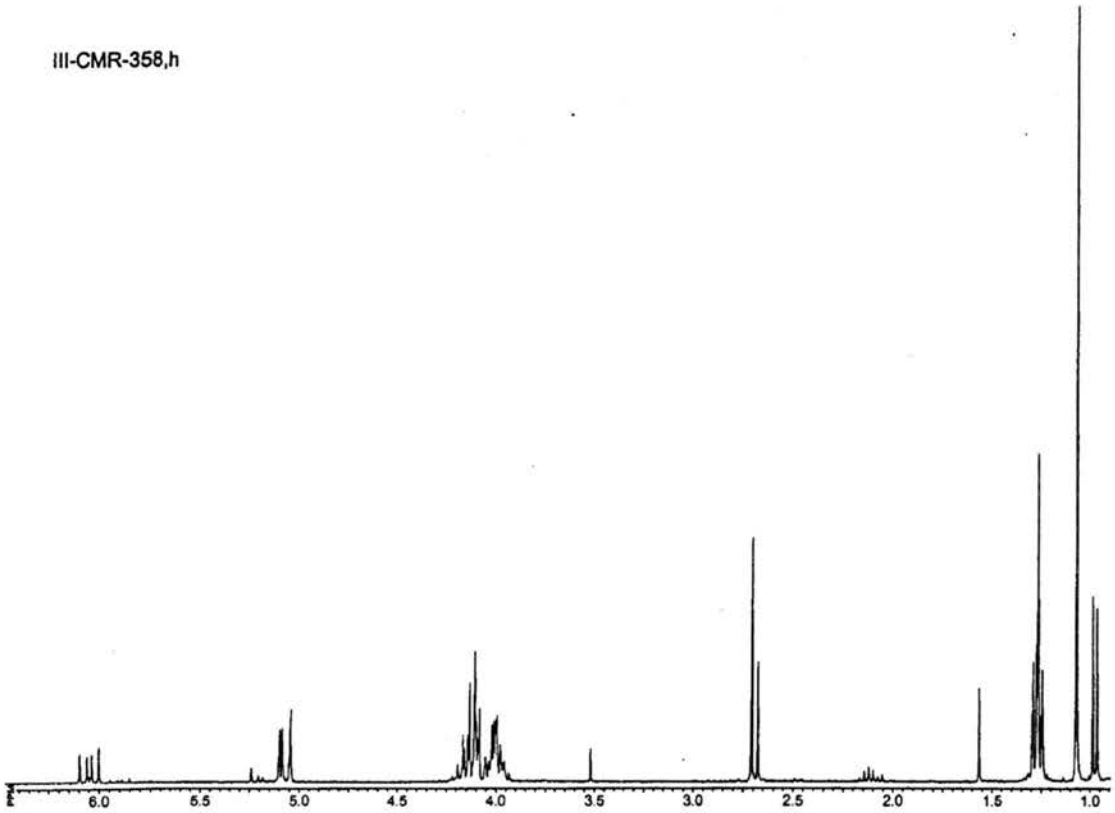
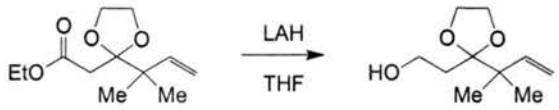


242 267

[2-(1,1-Dimethyl-allyl)-[1,3]dioxolan-2-yl]-acetic acid ethyl ester (267): 1,3-ethanediol (3.1 mL, 44.87 mmol, 2.5 eq) and TsOH (0.184g, 0.9673 mmol, 0.05 eq) added successively to a stirring solution of the β-keto ester (3.373g, 18.31 mmol) in freshly distilled benzene (60 mL) in a round bottom flask fitted with a Dean-Stark trap and a reflux condenser. The reaction heated to reflux for 24h. Reaction mixture concentrated by rotary evaporation. Purification on silica gel with 6:1 hexanes / ethyl acetate allows for partial separation of acetonide from unreacted β-keto ester. Yield 2.1g, 9.199 mmol (54%).

III-CMR-358,h

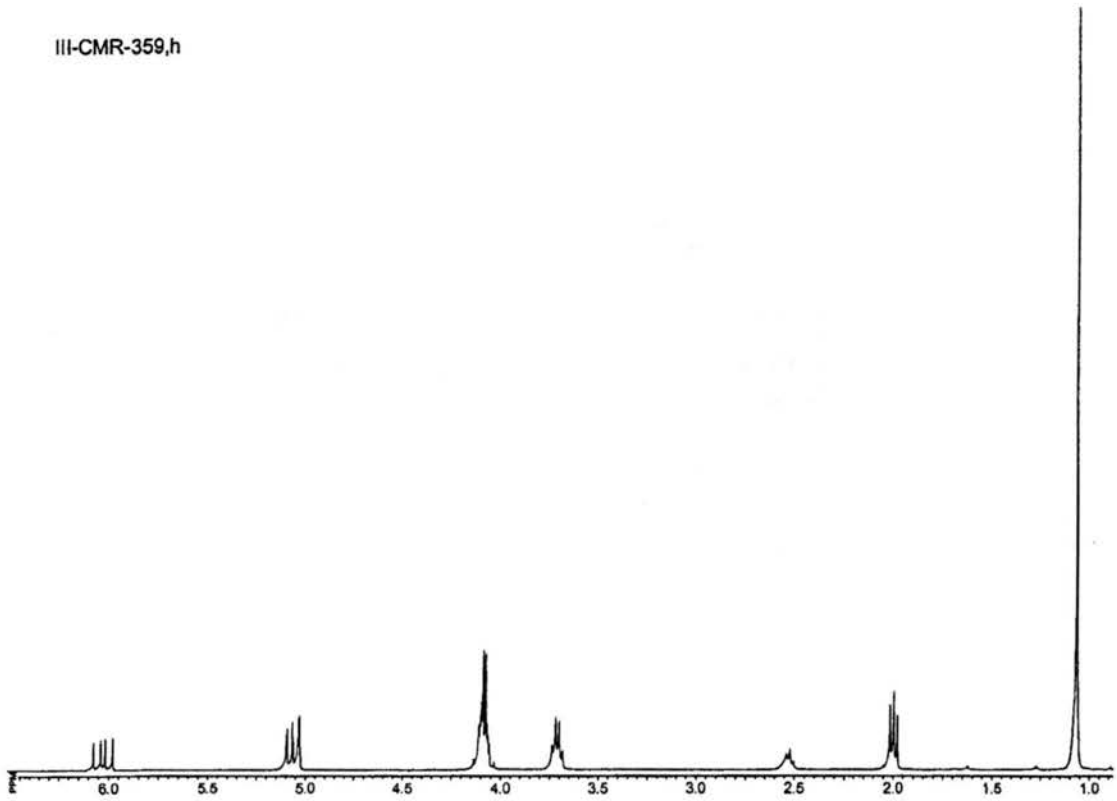




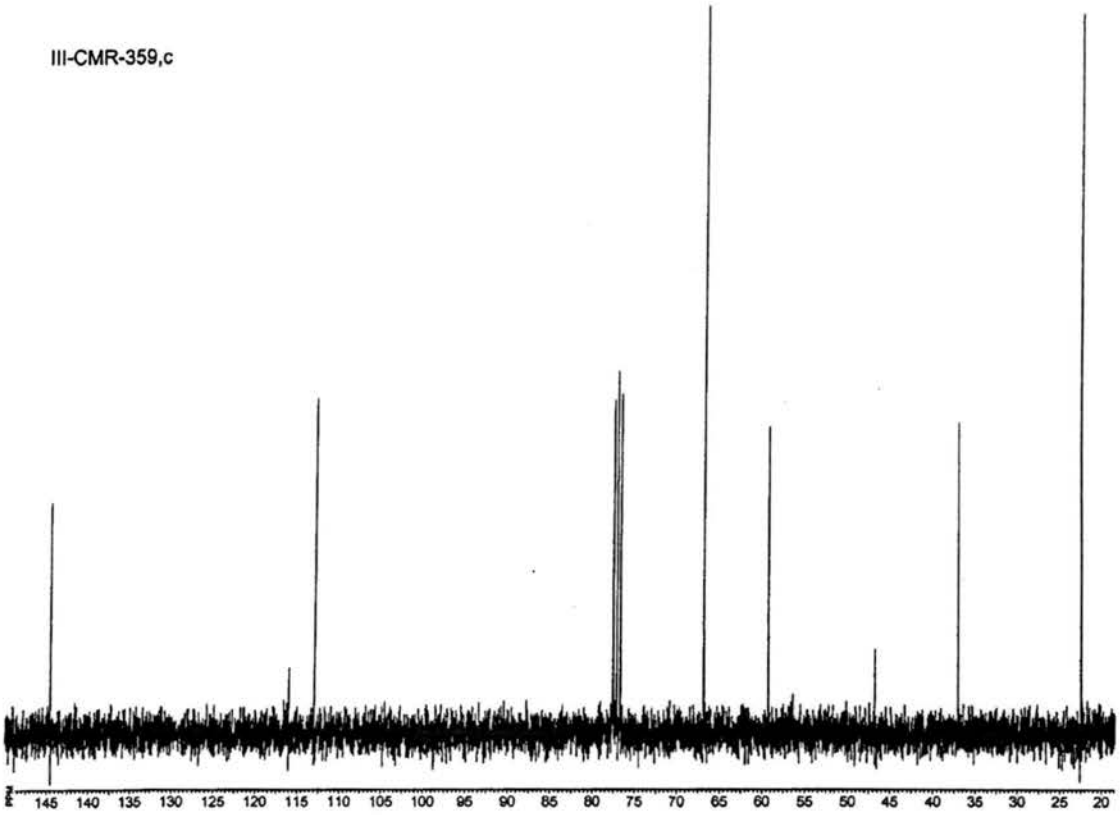
267 **268**

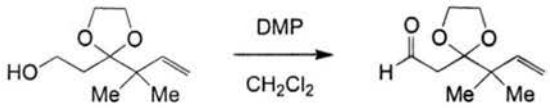
2-[2-(1,1-Dimethyl-allyl)-[1,3]dioxolan-2-yl]-ethanol (268): LAH (0.179g, 4.717 mmol, 4.8 eq) added to a stirring solution of the ester (0.225g, 0.9856 mmol) in anhydrous THF and stirred under argon for 12h. Reaction was quenched by addition of solid $Na_2SO_4 \cdot 10H_2O$ until bubbling ceased (0.520g). The resulting mixture was then filtered through celite with Et_2O. Contentration by rotary evaporation followed by purification on silica gel with 7:3 hexanes / ethyl acetate gave the alcohol as a clear colorless oil. Yield 0.115g, 0.6174 mmol (63%).

III-CMR-359,h



III-CMR-359,c

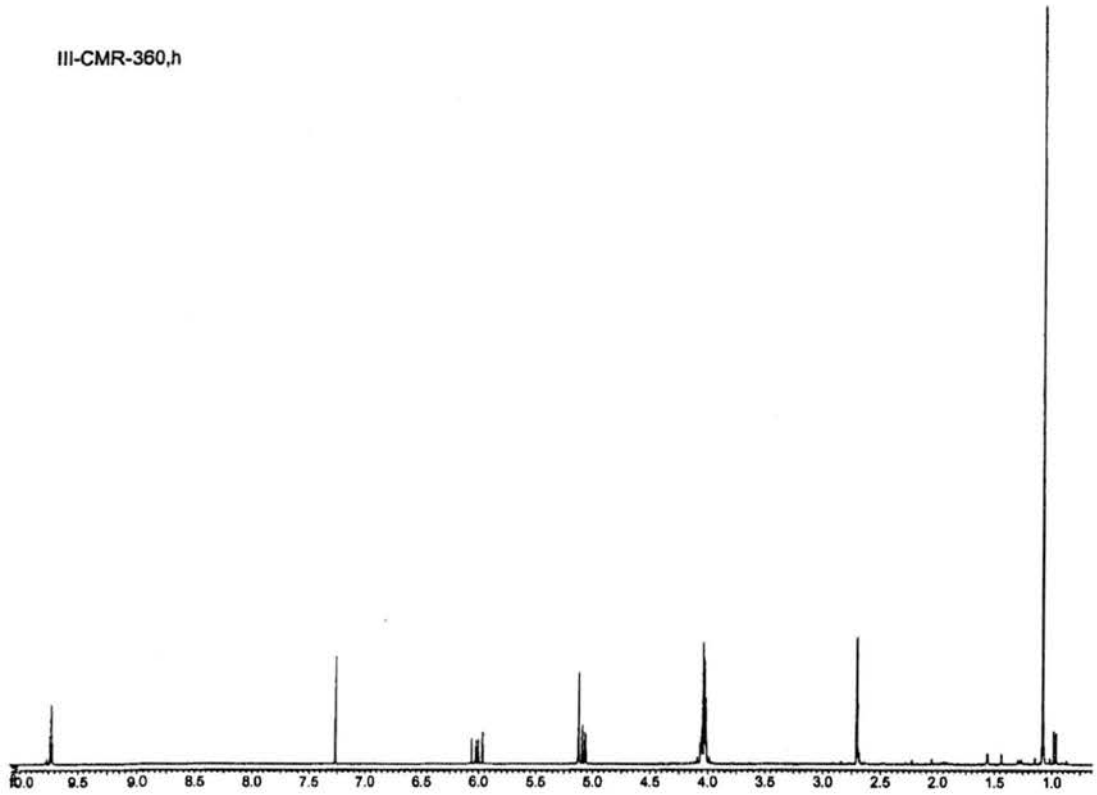




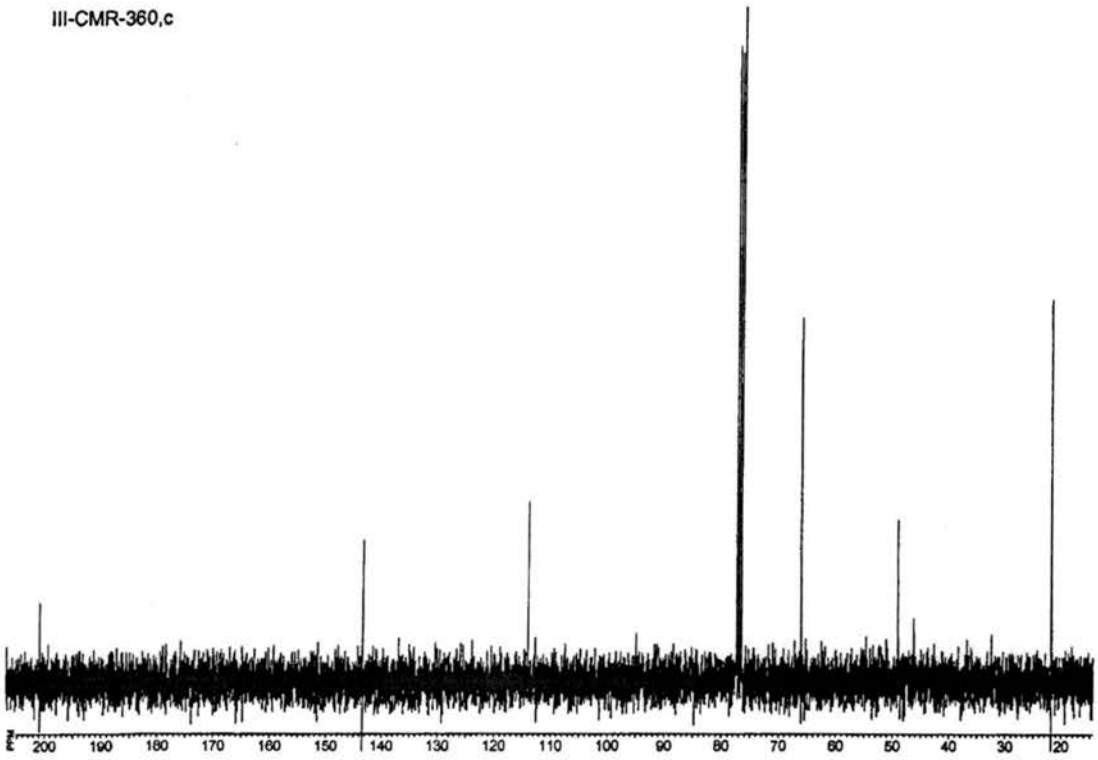
268 **269**

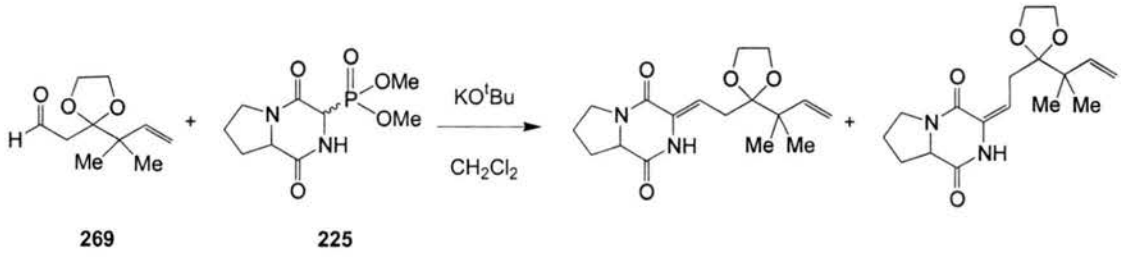
[2-(1,1-Dimethyl-allyl)-[1,3]dioxolan-2-yl]-acetaldehyde (269): Dess-Martin periodinane (5.029g, 11.86 mmol, 1.5 eq) was added to a stirring solution of the alcohol (1.5g, 8.054 mmol) in CH_2Cl_2 (80 mL) at room temperature and stirred for 1.5h. The resulting reaction was diluted with Et_2O (80 mL) and $Na_2S_2O_3$ (10.2g, 8 eq) and sat. aq. $NaHCO_3$ were added successively and stirred vigorously until the biphasic mixture became clear (15 min). The aqueous layer was separated and the organic layer was washed with sat. aq. $NaHCO_3$ (40 mL). Combined aqueous washings were extracted with CH_2Cl_2 (40 mL x 3). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification by PTLC with EtOAc (x2) provided the product as a clear, colorless oil. Yield 1.43g, 7.762 mmol (96%).

III-CMR-360,h



III-CMR-360,c

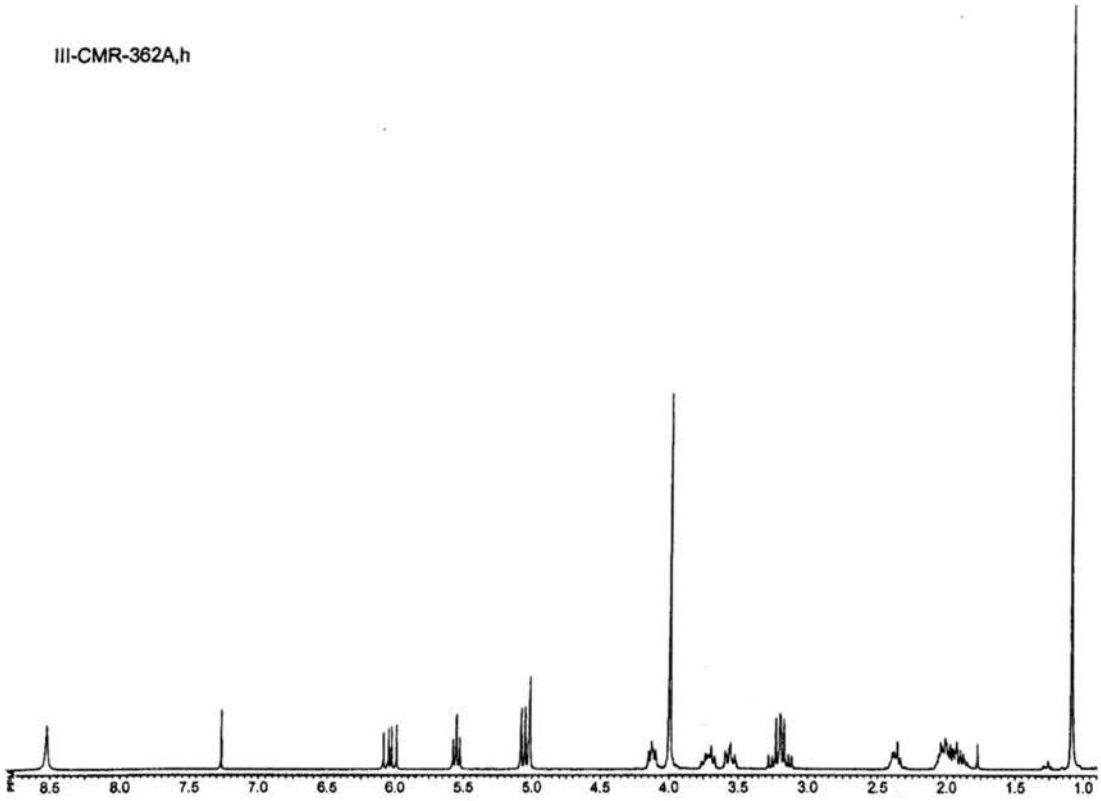




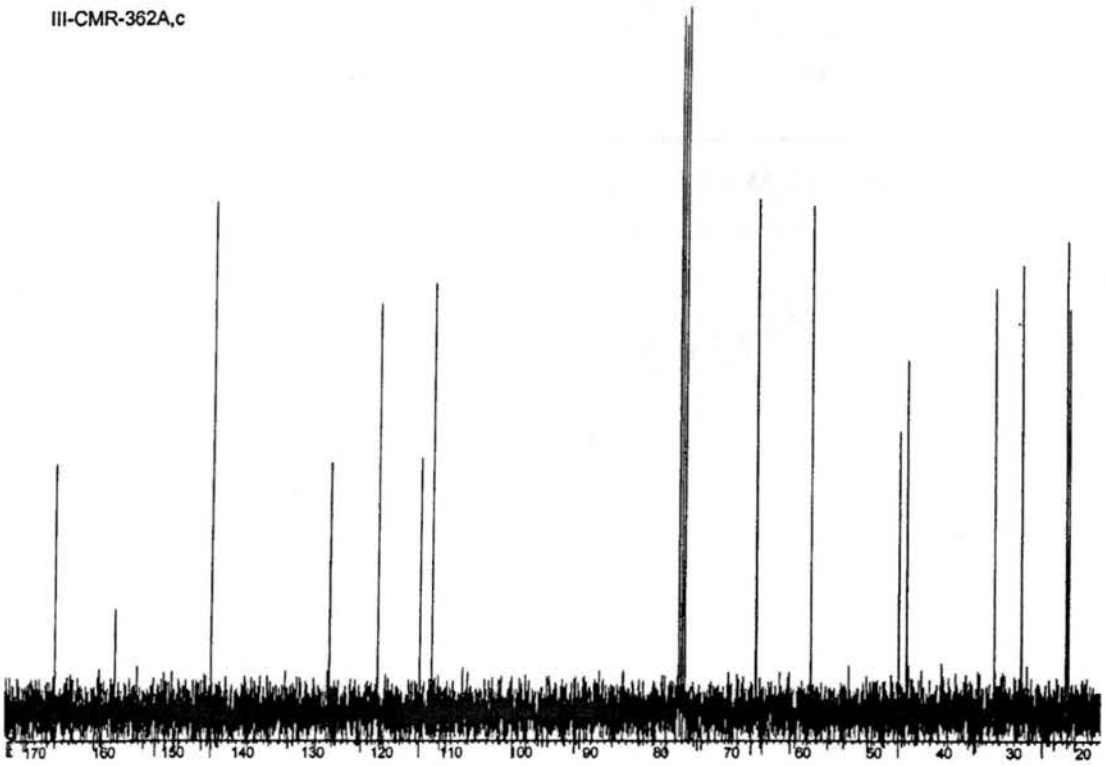
269 225

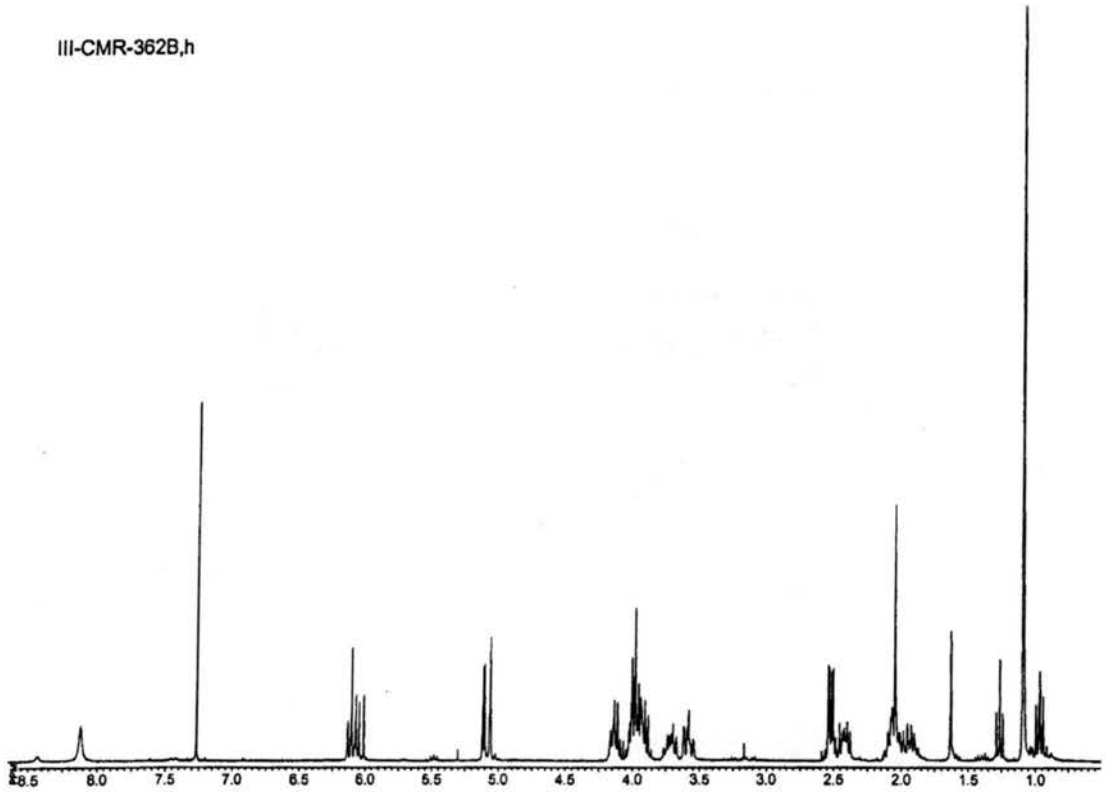
3-{2-[2-(1,1-Dimethyl-allyl)-[1,3]dioxolan-2-yl]-ethylidene}-hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione: KO^{t}Bu (0.340 g, 3.030 mmol, 1.3 eq) measured into a flame dried, conical 50 mL flask and carefully suspended in anhydrous CH_2Cl_2 (10 mL) and cooled to -78°C under argon. The DKP phosphonate (0.847 g, 3.230 mmol, 1.3 eq) in anhydrous CH_2Cl_2 (5 mL x 2) was added dropwise over 15 min. The resulting yellow suspension was stirred at -78°C for 15 min and the aldehyde (0.464 g, 2.519 mmol) in dry CH_2Cl_2 (3 mL + 5 mL) was added dropwise. The resulting orange solution was allowed to warm to room temperature and stirred under argon until rection complete by TLC. Silica gel was added directly to reaction mixture and solvent was removed by rotary evaporation. Purification by silica gel chromatography with EtOAc gave the product as a partially separable mixture of isomers. Yield 0.620g, 1.935 mmol (77%).

III-CMR-362A,h



III-CMR-362A,c




III-CMR-362B,h
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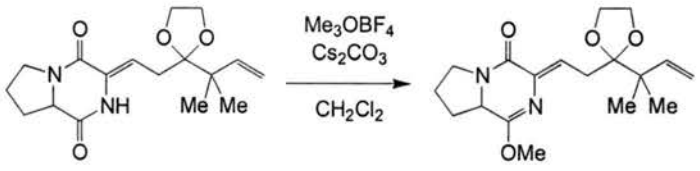
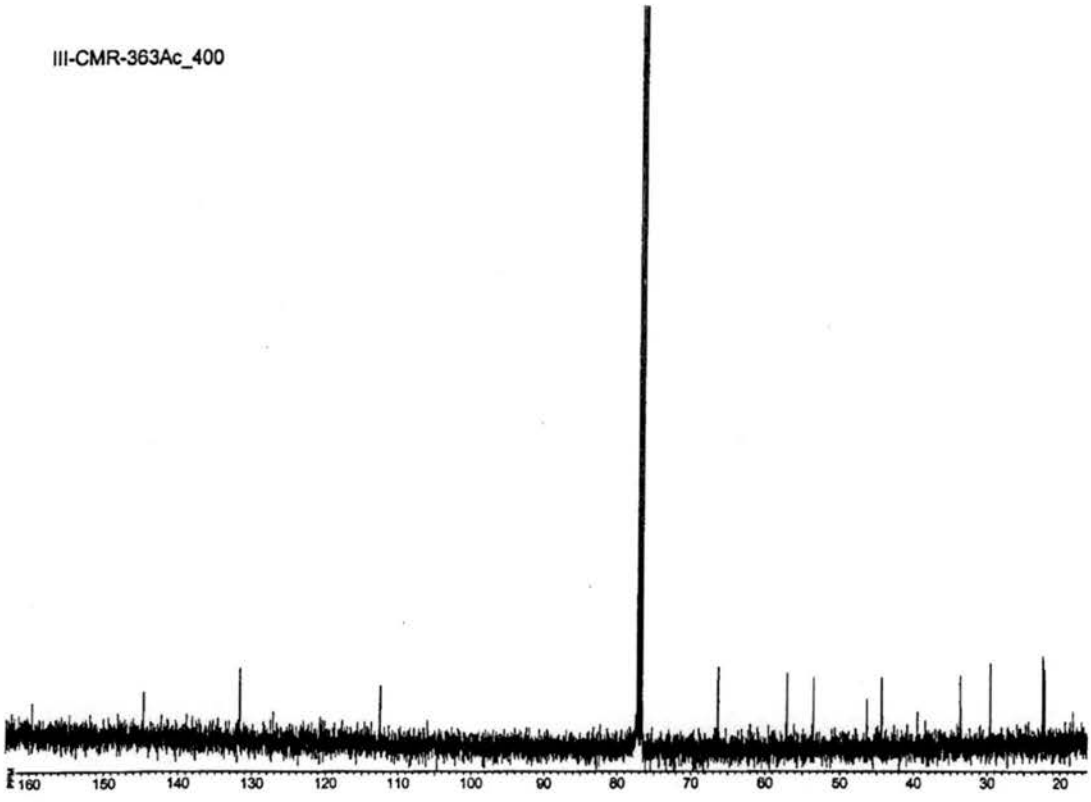
1.0



3-{2-[2-(1,1-Dimethyl-allyl)-[1,3]dioxolan-2-yl]-ethylidene}-1-methoxy-6,7,8,8a-tetrahydro-3H-pyrrolo[1,2-a]pyrazin-4-one: Me_3OBF_4 (0.153 g, 1.034 mmol, 3.0 eq) and Cs_2CO_3 (0.563 g, 1.74 mmol, 5.0 eq) added successively to a stirring solution of the DKP (0.110g, 0.3433 mmol) in anhydrous CH_2Cl_2 (4 mL) at 0°C under argon for 6h. The reaction mixture was poured into a sep funnel containing ice cold dH_2O (20 mL) and extracted with CH_2Cl_2 (10 mL x 3). Combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification by silica gel chromatography with 1:1 hexanes / ethyl acetate gave the lactim ether as a clear colorless oil. Yield 0.048 g, 0.1435 mmol (42%).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30
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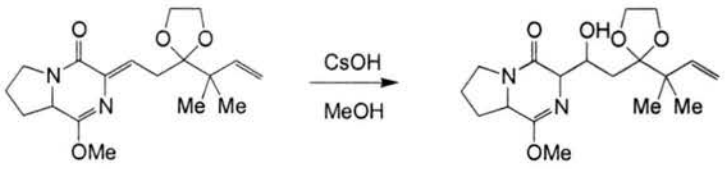
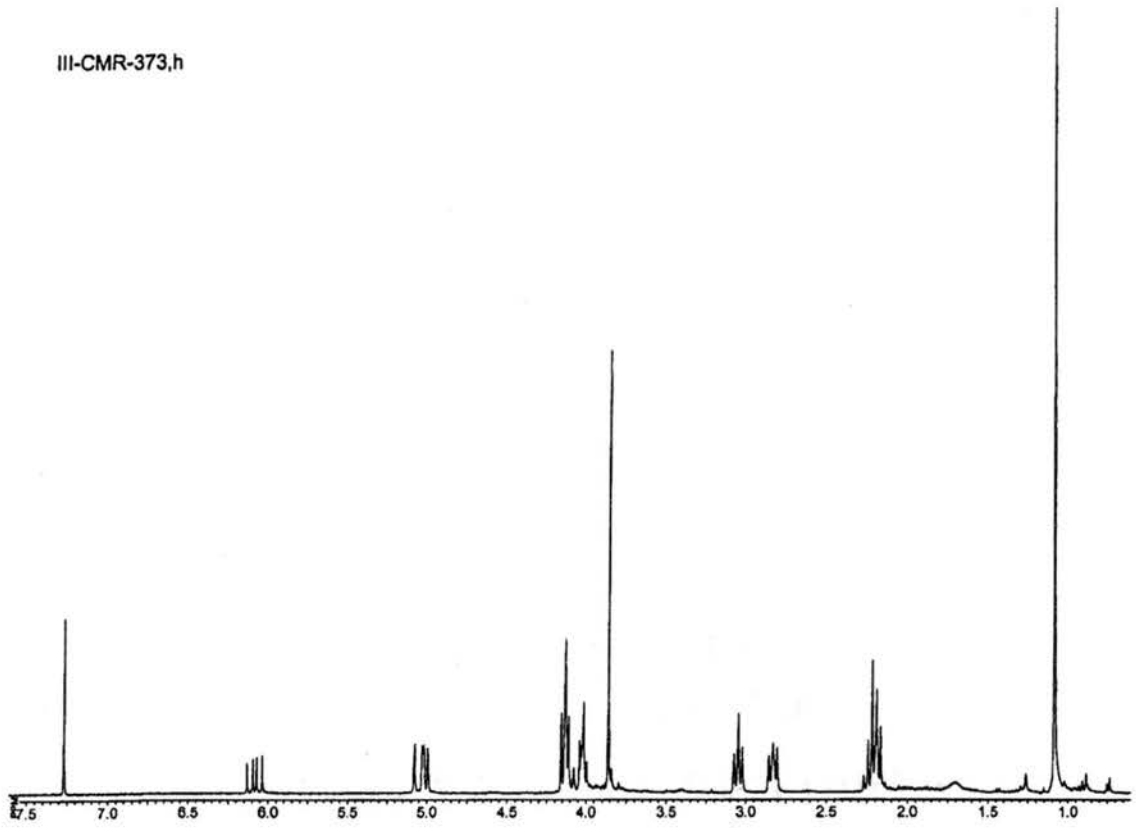


3-{2-[2-(1,1-Dimethyl-allyl)-[1,3]dioxolan-2-yl]-1-hydroxy-ethyl}-1-methoxy-6,7,8,8a-tetrahydro-3H-pyrrolo[1,2-a]pyrazin-4-one: $CsOH \cdot H_2O$ (0.015 g, 0.08932 mmol, 1.5 eq) added to a stirring solution of the lactim ether (0.020 g, 0.05981 mmol) in MeOH (0.6 mL) at room temperature for 5h. Solvent was removed by rotary evaporation and purification by PTLC with EtOAc gave the product as a clear colorless oil. Yield 0.005 g.


III-CMR-373,h
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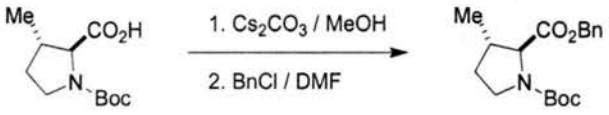
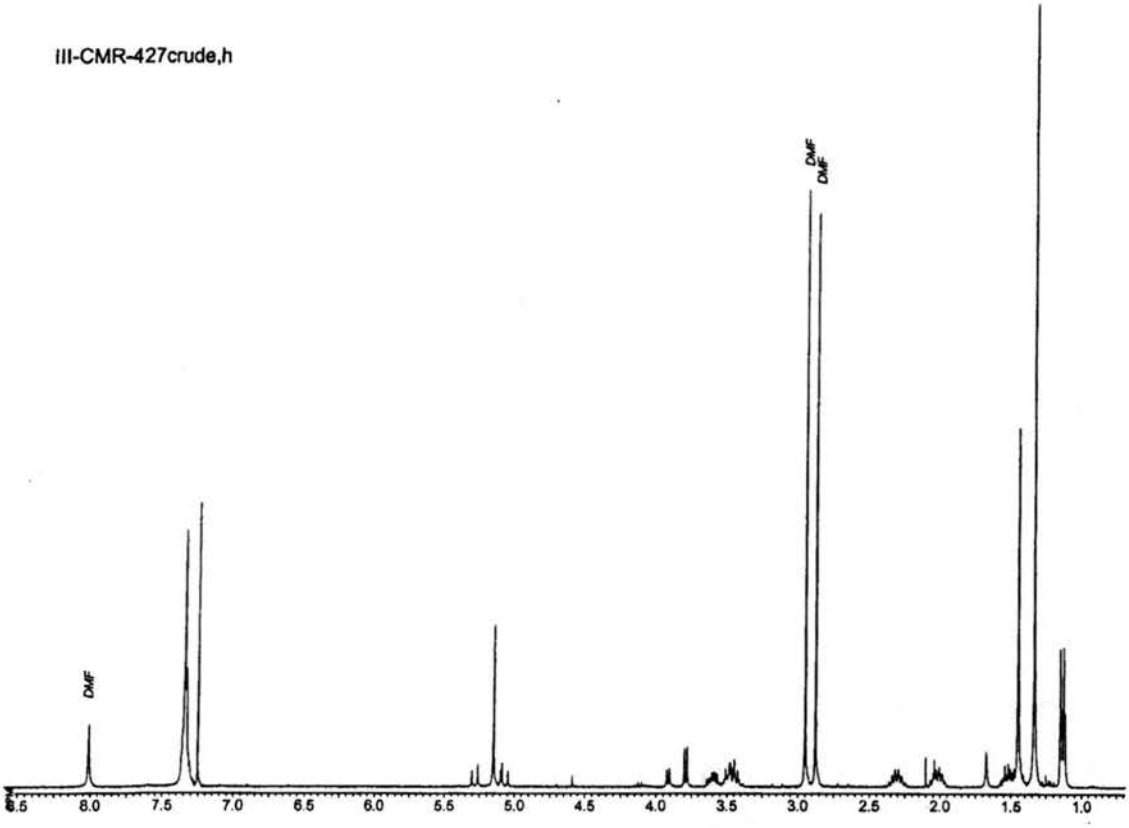
1.0



3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-benzyl ester 1-tert-butyl ester: 3-Methyl-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester (1.58g, 6.891 mmol) in MeOH (20 mL) was neutralized with 20% aq. Cs_2CO_3 (~11.5 mL). The aqueous residue was diluted with DMF (25mL) and concentrated by rotary evaporation. Azeotropic removal of water with DMF was repeated three times to give the cesium prolinate as a white solid. BnCl (0.795 mL, 6.908 mmol, 1 eq) was added to a stirring solution of the cesium prolinate dissolved in dry DMF (35 mL) and stirred at ambient temperature for 16h. The reaction mixture was then diluted with dH_2O (75 mL) and extracted with EtOAc (25 mL x 3). The combined organic extracts were washed with brine (75 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification on silica gel with 6:1 hexanes / ethyl acetate provided the product as a clear, colorless oil. Yield 2.07g, 6.481 mmol (94%).


III-CMR-427crude,h
DMF
DMF
DMF
8.5
8.0
7.5
7.0
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5.5
5.0
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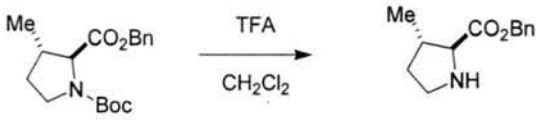
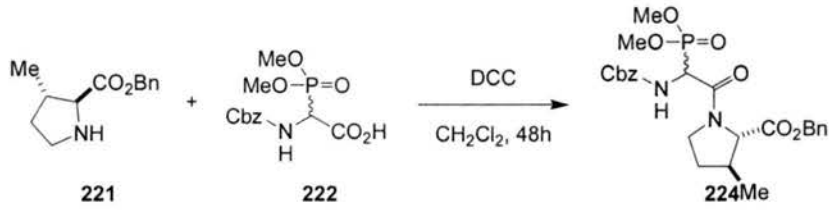
3.5
3.0
2.5
2.0
1.5
1.0

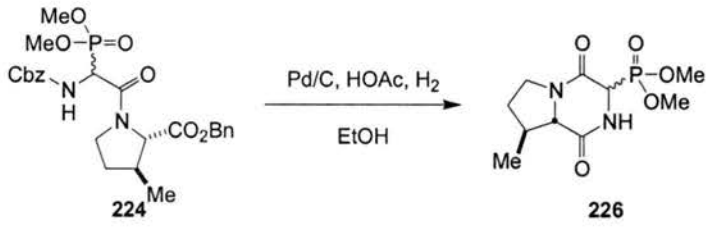


3-Methyl-pyrrolidine-2-carboxylic acid benzyl ester: TFA (6.6 mL, 85.67 mmol, 30 eq) was added to a stirring solution of ester (0.91g, 2.849 mmol) in CH_2Cl_2 (7 mL) at 0°C and stirred at 0°C for 30 min. Excess TFA was then carefully quenched at 0°C with sat. aq. $NaHCO_3$ until the aqueous layer maintained a pH<3. The aqueous layer was extracted with CH_2Cl_2 (20 mL x 3). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Material was suitably pure to be used in next step without further purification. Yield 0.625g, 2.85 mmol (~quant.)



221 222 224Me

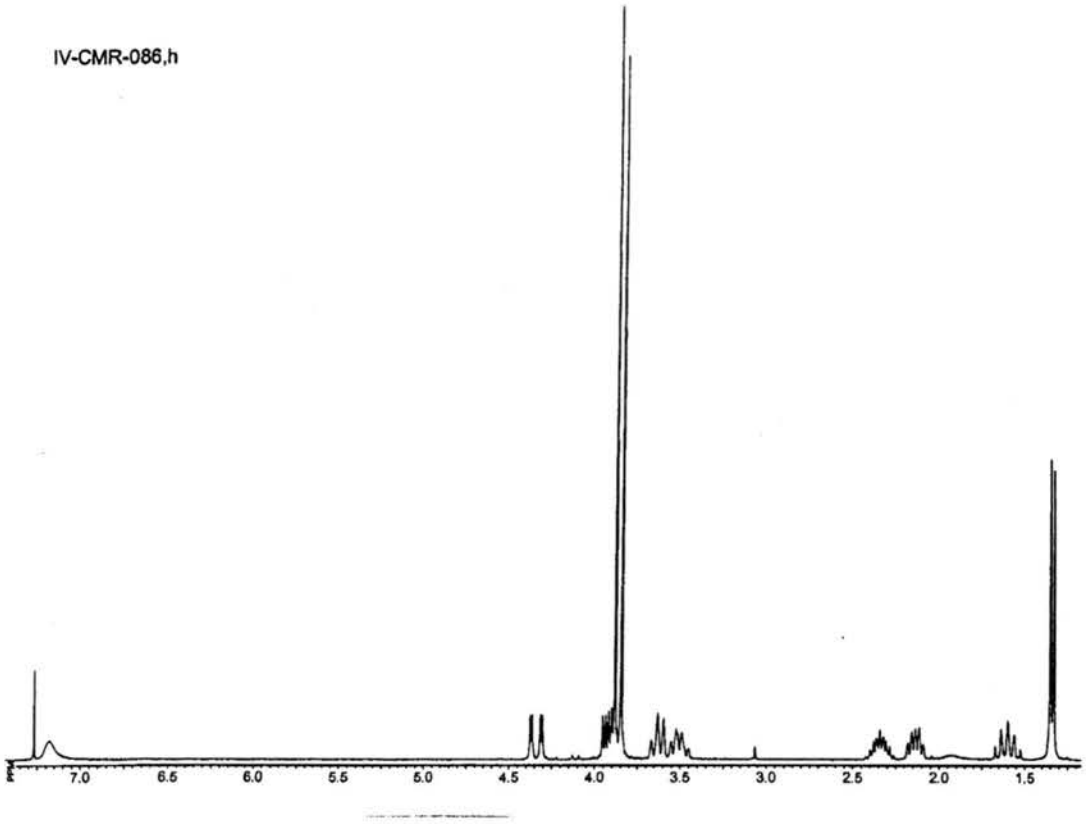
1-[2-Benzyloxycarbonylamino-2-(dimethoxy-phosphoryl)-acetyl]-3-methyl-pyrrolidine-2-carboxylic acid benzyl ester (224): DCC (0.902g, 4.372 mmol, 1.5 eq) was added to a stirring solution of 3-Methyl-pyrrolidine-2-carboxylic acid benzyl ester (**221**, 0.625g, 2.850 mmol) and Benzyloxycarbonylamino-(dimethoxy-phosphoryl)-acetic acid (0.905g, 2.853 mmol, 1 eq) in CH_2Cl_2 (15mL) and stirred at ambient temperature for 48h. After filtration on celite with EtOAc to remove excess urea the crude material was concentrated by rotary evaporation and purified on silica gel with EtOAc to give the product as a clear, colorless oil. Yield 1.29g, 2.488 mmol (87%).



(8-Methyl-1,4-dioxo-octahydro-pyrrolo[1,2-a]pyrazin-3-yl)-phosphonic acid dimethyl ester (226): 5% Pd/C (0.087g) was added to a stirring, argon degassed, solution of peptide **224** (1.5g, 2.893 mmol) and glacial AcOH (0.1 mL) in abs. EtOH (15mL) at ambient temperature and degassed with argon for 15 min. After H_2 was bubbled into the reaction for 30 min, the reaction was stirred at ambient temperature under H_2 (1 atm) for 14h. After degassing the reaction with argon for 30 min a small scoop of K_2CO_3 was added to neutralize the HOAc, the mixture was then filtered through celite with EtOAc to remove the Pd/C and then concentrated by rotary evaporation. Purification on silica gel with 9:1 ethyl acetate / methanol gives the product as a white solid. Yield 0.790g, 2.86 mmol (99%).

^{13}C NMR ($CDCl_3$, 75 MHz) δ169.8, 159.7, 63.2, 57.0, 55.1, 54.8, 54.7, 54.3, 54.2, 44.9, 37.8, 31.4, 18.3.

HRMS (FAB+) M+H calc'd. 277.095335, found 277.093994.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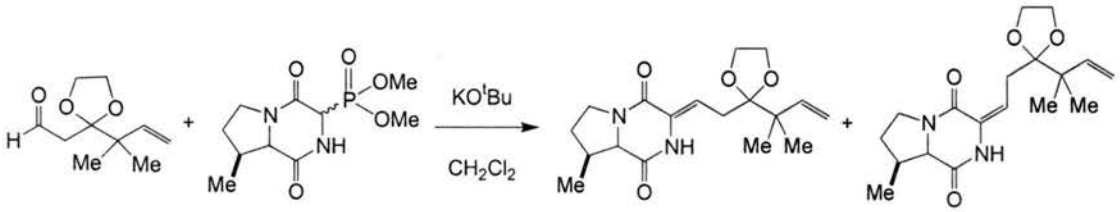
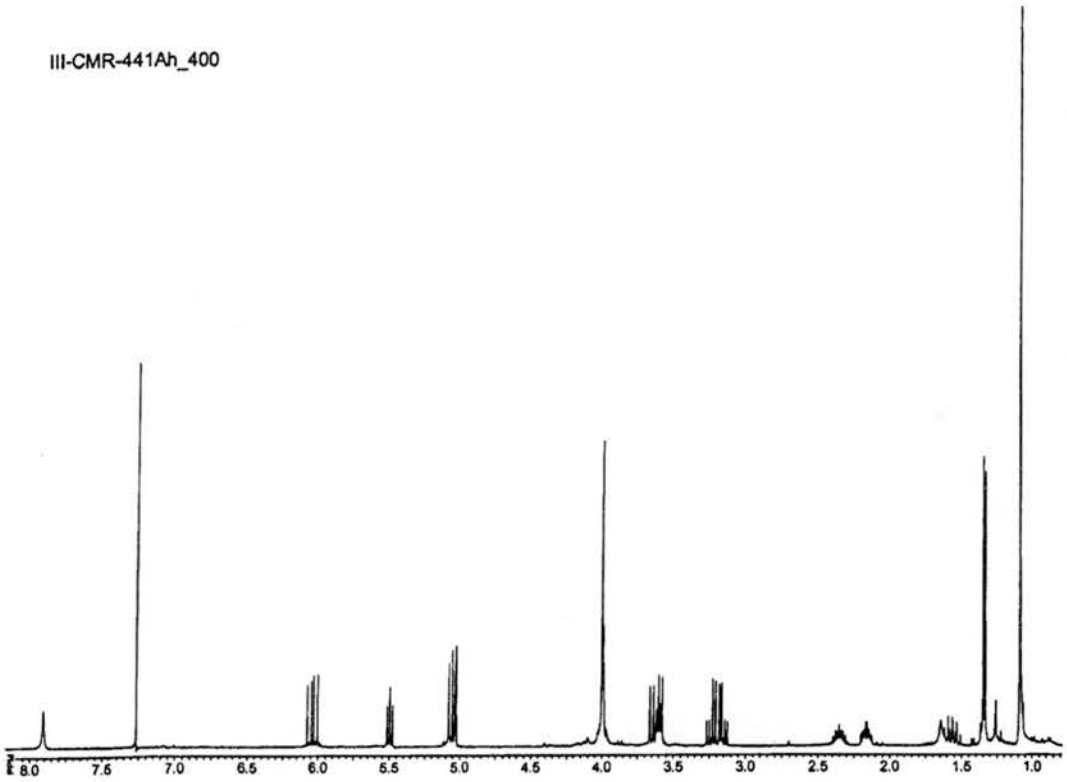
1.5



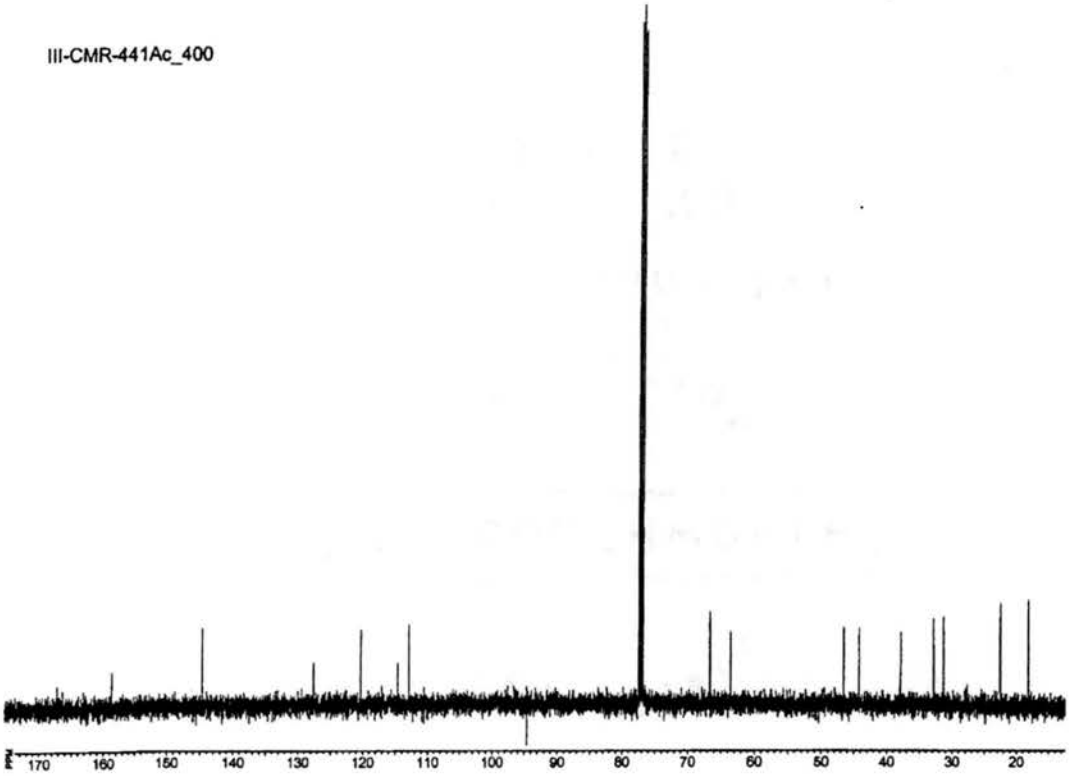
269 **226** **270** **271**

To a stirring suspension of KO^{t}Bu (0.186g, 1.658 mmol, 1.1 eq) in dry CH_2Cl_2 (5 mL) at -78°C under argon was added via cannula the DKP (0.409 g, 1.481 mmol) in dry CH_2Cl_2 (2mL + 2 mL + 1 mL). After stirring for 30 min, the aldehyde (0.300 g, 1.628 mmol, 1.1 eq) in dry CH_2Cl_2 (5 mL) via cannula and the mixture was allowed to warm to room temperature and stir for 46 h. After concentration by rotary evaporation and purification on silica gel (50 g/ 1g) with EtOAc the products were isolated as a partially separated mixture of geometric isomers (**270**: 0.187g, 0.5591 mmol; **271**: 0.095g, 0.2841 mmol; mix: 0.106 g, 0.317 mmol, 97% overall)

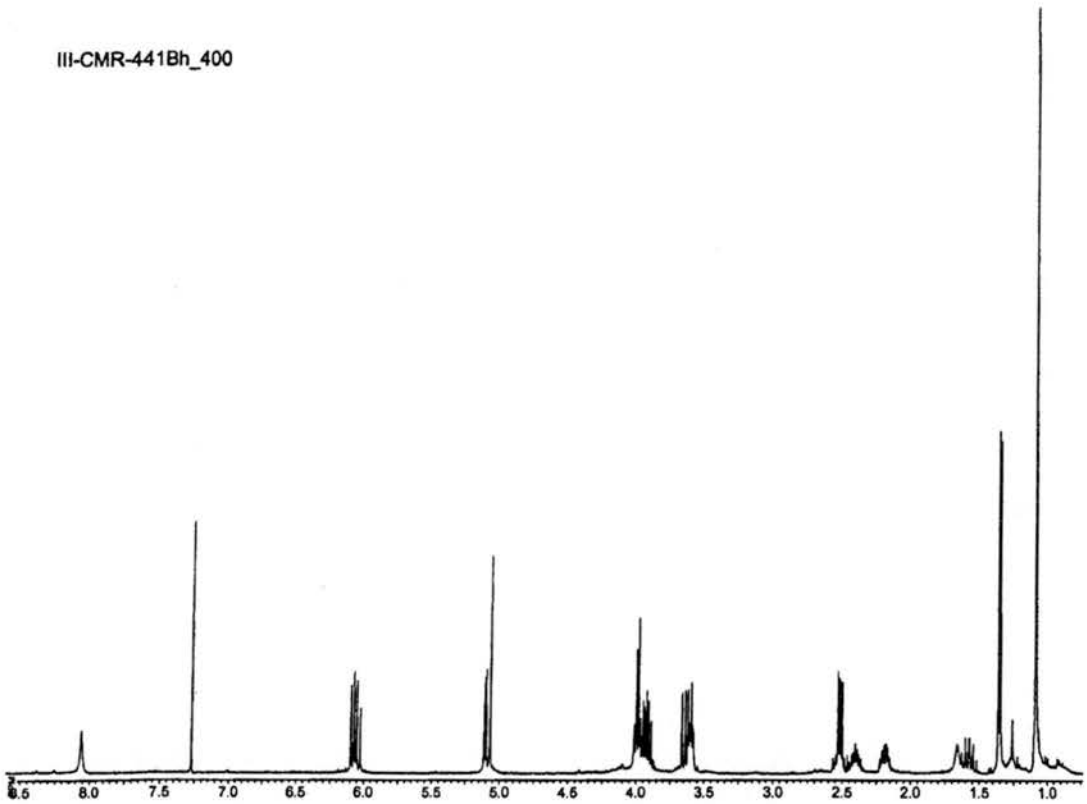
III-CMR-441Ah_400



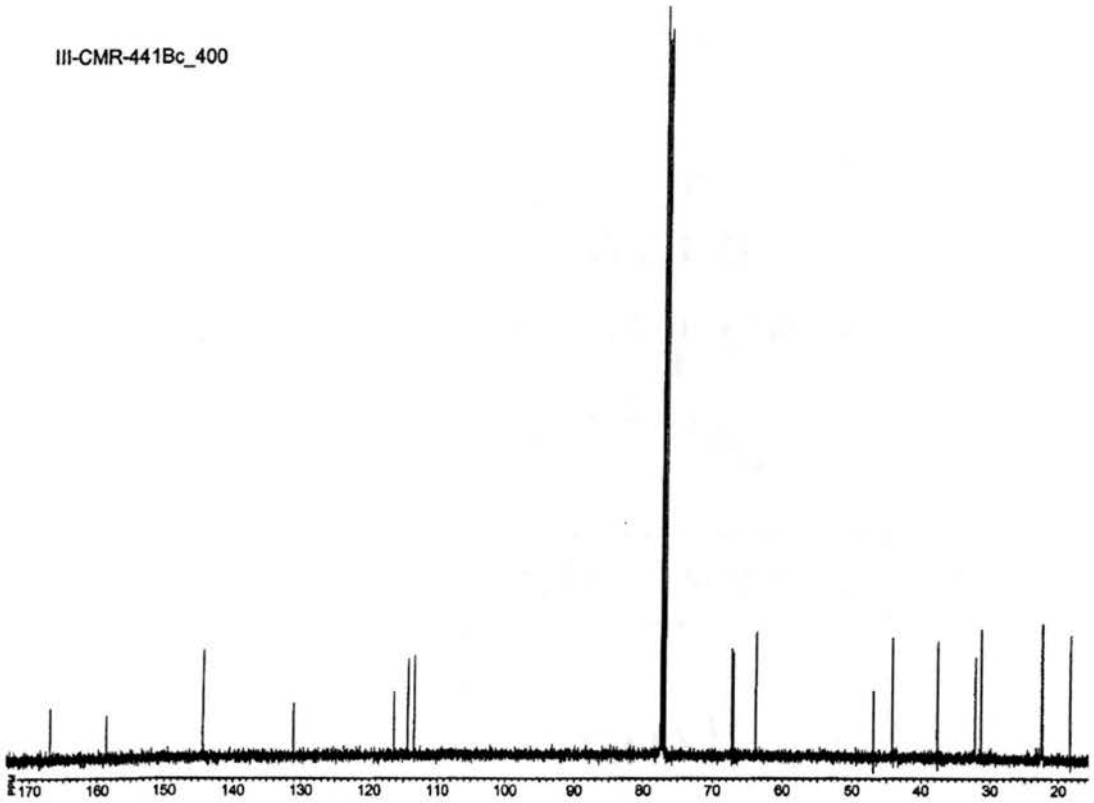
III-CMR-441Ac_400

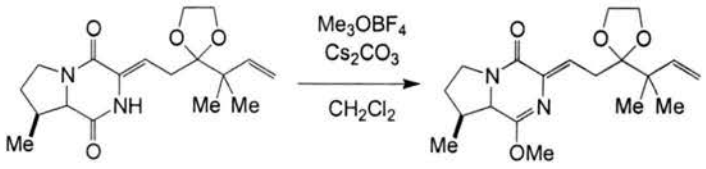


III-CMR-441Bh_400



III-CMR-441Bc_400

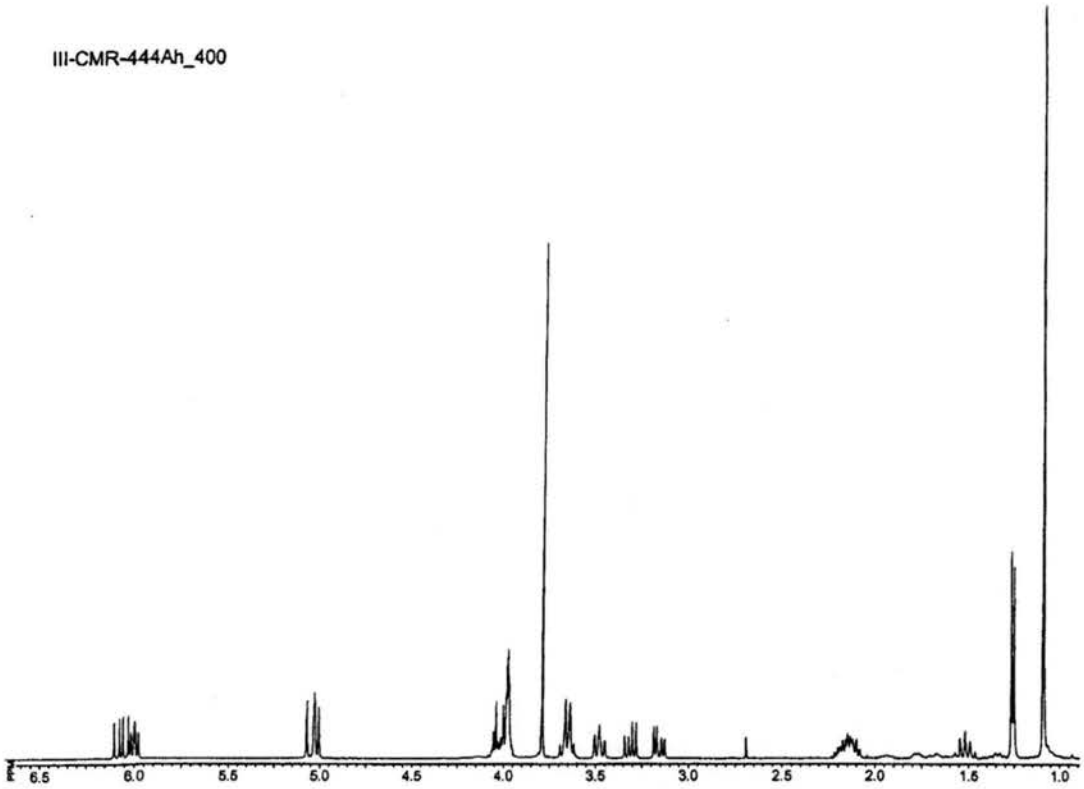




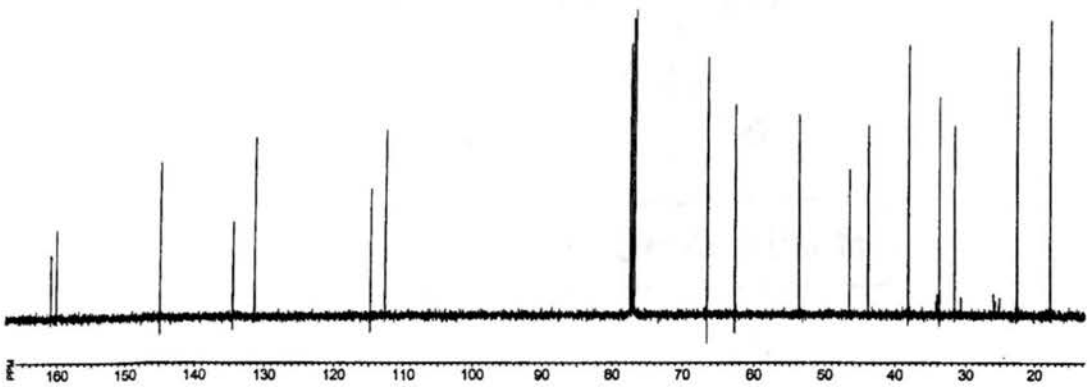
270 272

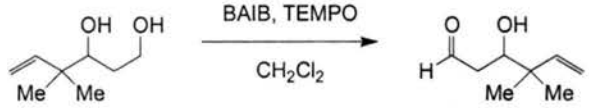
Me_3OBF_4 (0.251 g, 1.697 mmol, 3.0 eq) and Cs_2CO_3 (0.911 g, 2.796 mmol, 5.0 eq) added successively to a stirring solution of the DKP (0.187g, 0.5592 mmol) in anhydrous CH_2Cl_2 (6 mL) at 0°C under argon for 6h. The reaction mixture was poured into a sep funnel containing ice cold dH_2O (20 mL) and extracted with EtOAc (15 mL x 3). Combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification by silica gel chromatography with 1:1 hexanes / ethyl acetate gave the lactim ether as a clear colorless oil.

III-CMR-444Ah_400

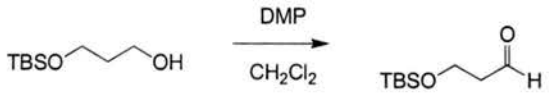


III-CMR-444Ac_400



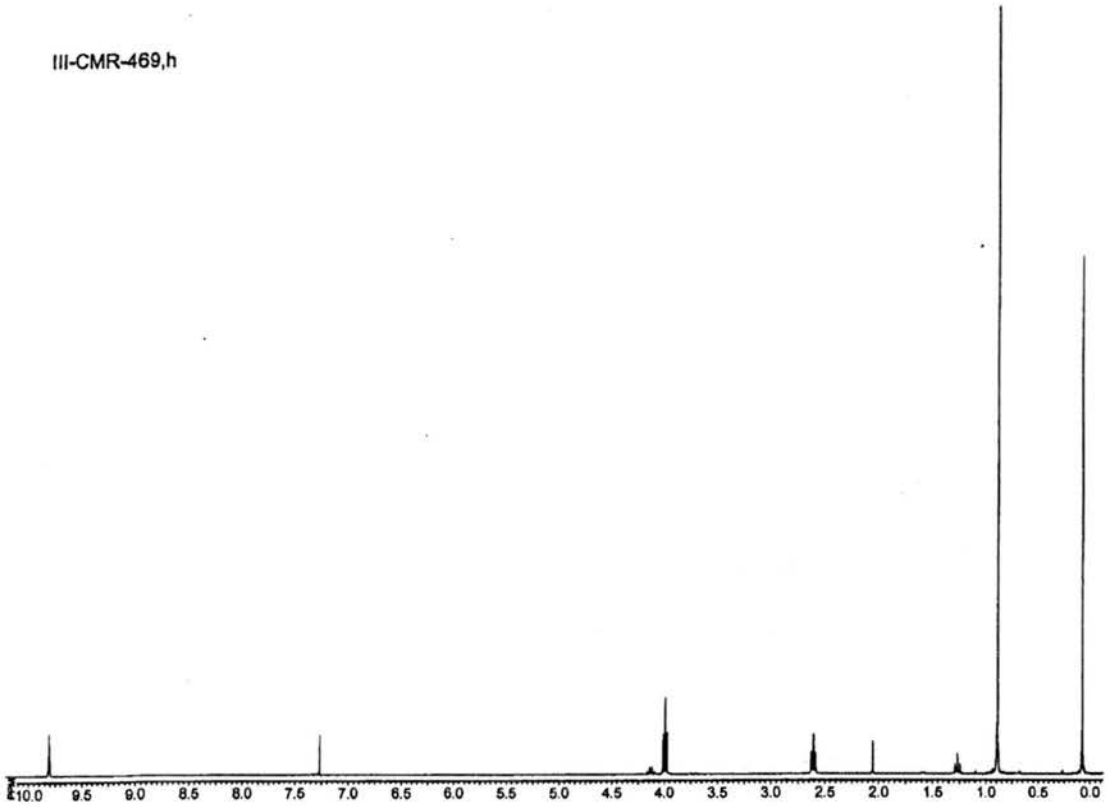


To a stirring solution of the diol (0.101 g, 0.9004 mmol) in CH_2Cl_2 (7 mL) at room temperature was successively added TEMPO (0.012 g, 0.0768 mmol, 0.1 eq) and BAIB (0.250 g, 0.7762 mmol, 1.1 eq). After 8 h $Na_2SO_3 \cdot 5H_2O$ (8eq) and saturated aqueous $NaHCO_3$(7 mL). Product isolated as a clear colorless oil (0.044g, 0.3094 mmol, 44%)

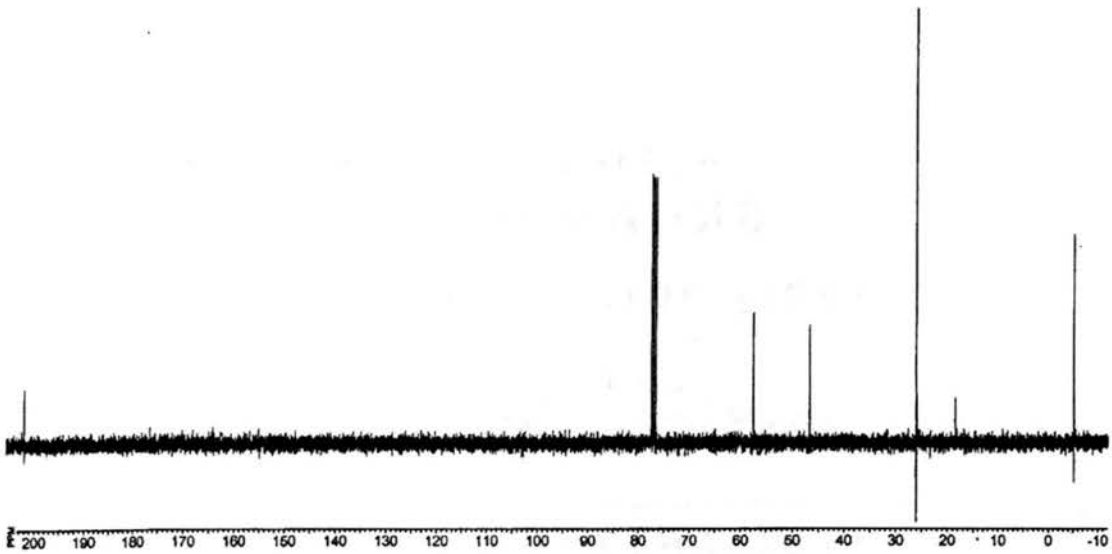


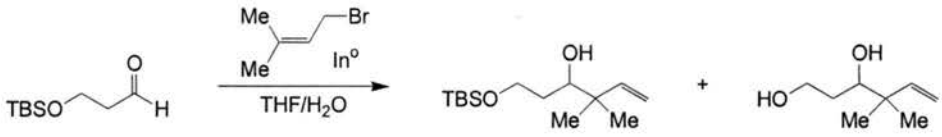
3-(tert-Butyl-dimethyl-silanyloxy)-propionaldehyde: Dess-Martin periodinane (3.16g, 7.450 mmol, 1.25 eq) was added to a stirring solution of the alcohol (0.760g, 4.035 mmol) in CH_2Cl_2 (50 mL) and stirred at ambient temperature for 1.5h. Reaction was diluted with Et_2O (100 mL) and sat. aq. $NaHCO_3$ (50 mL) and after addition of $Na_2S_2O_3$-H_2O (11.87g, 8 eq) was stirred vigorously until the biphasic mixture became clear. The aqueous layer was separated and the organic larer was washed with sat. aq. $NaHCO_3$ (50 mL x 2). The combined aqueous layers were then extracted with Et_2O (50 mL x 2). Combined organic layer were then washed with sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification on silica gel with 6:1 hexanes / ethyl acetate resulted in the product isolated as a clear, colorless oil. Yield 0.770g, 4.088 mmol (76%).

III-CMR-469,h



III-CMR-469,c

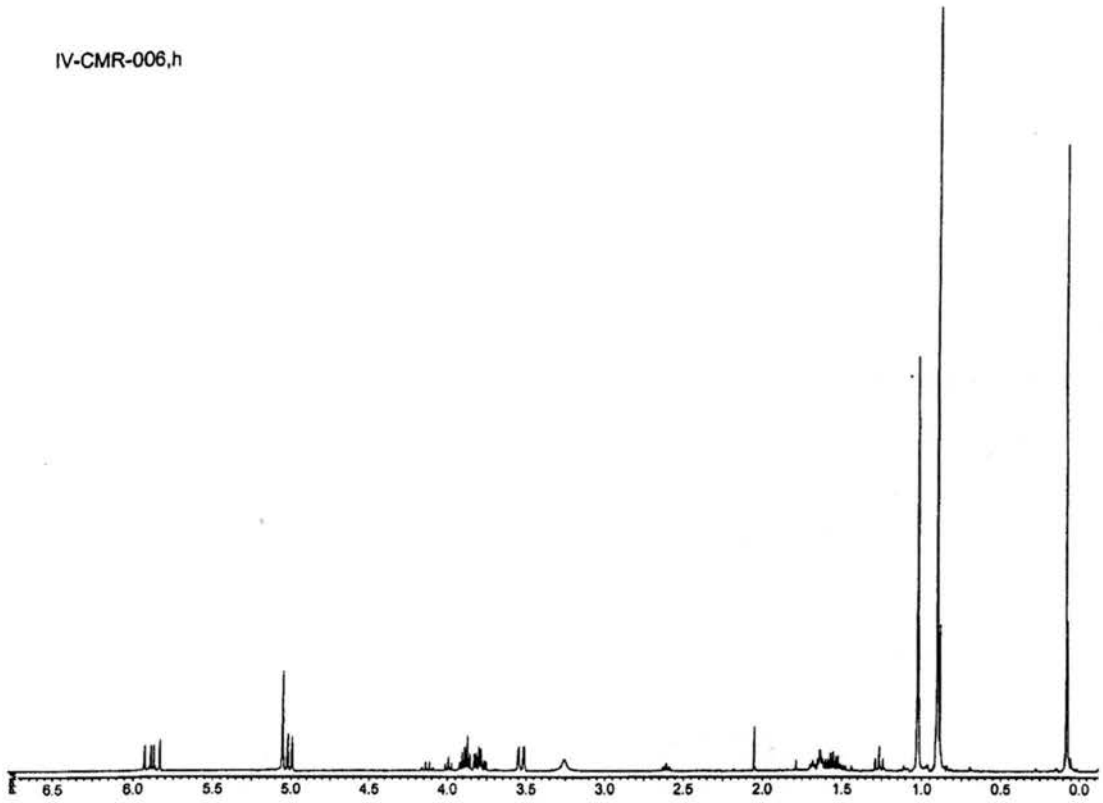




1-(tert-Butyl-dimethyl-silanyloxy)-4,4-dimethyl-hex-5-en-3-ol: Prenyl bromide (0.7 mL, 6.073 mmol, 1.5 eq) and indium powder (0.511g, 4.450 mmol, 1.1eq) were added successively to a stirring solution of aldehyde in THF (15 mL) and H_2O (65 mL) at ambient temperature and stirred for 1h. The aqueous solution was then extracted with Et_2O (25 mL x 3). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification on silica gel with 9:1 hexanes / ethyl acetate resulted in the 2° alcohol isolated as a clear colorless oil, yield 0.655g, 2.573 mmol (64%) along with the diol, yield 0.101g, 0.7004 mmol (17%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ0.081 (s, 6H), 0.905 (s, 9H), 1.028 (s, 6H), 1.476-1.702 (m, 2H), 3.260 (bs, 1H), 3.535 (dd, J = 10.0, 1.7 Hz, 1H), 3.756 - 3.832 (m, 1H), 3.860 - 3.924 (m, 1H), 4.990 - 5.059 (m, 2H), 5.881 (dd, J= 11.3, 17.1 Hz, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ145.6, 112.2, 78.6, 63.4, 41.2, 33.4, 26.0, 22.9, 22.5, 18.3, -5.4.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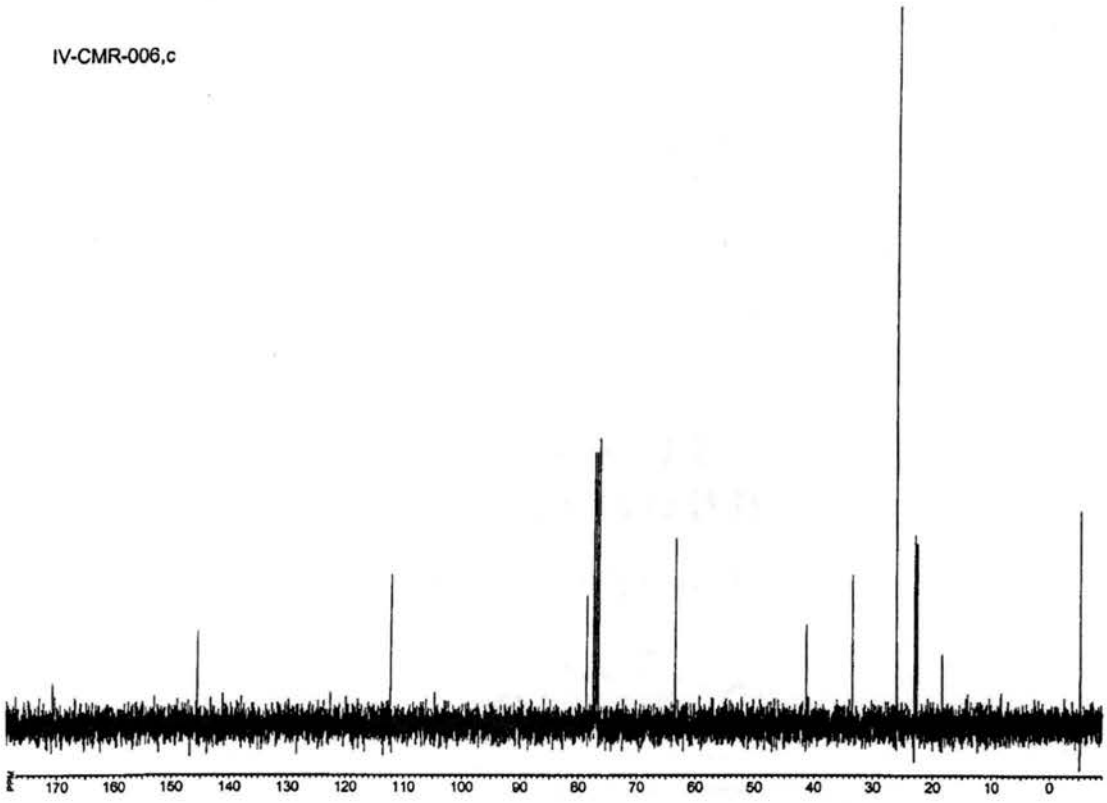
0.0


IV-CMR-006,c
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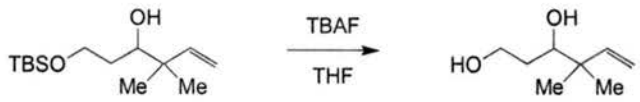
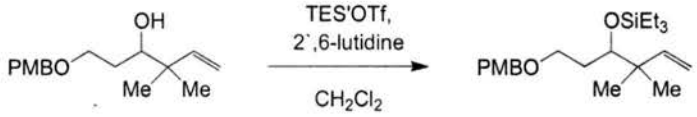
90
80
70
60
50
40
30
20
10
0



To a stirring solution of the ether (0.655 g, 2.534 mmol) in THF (3.0 mL) at 0°C was added a 1M solution of TBAF in THF (3.0 mL, 3.0 mmol, 1.2 eq). After stirring at room temperature for 1h, the solution was washed with saturated aqueous $NaHCO_3$ (15 mL), 1M HCl (15mL) and brine (15 mL), then dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification by column chromatography on silica gel with EtOAc dave the product as a colorless oil (0.212 g, 1.470 mmol, 58%)



172 **277**

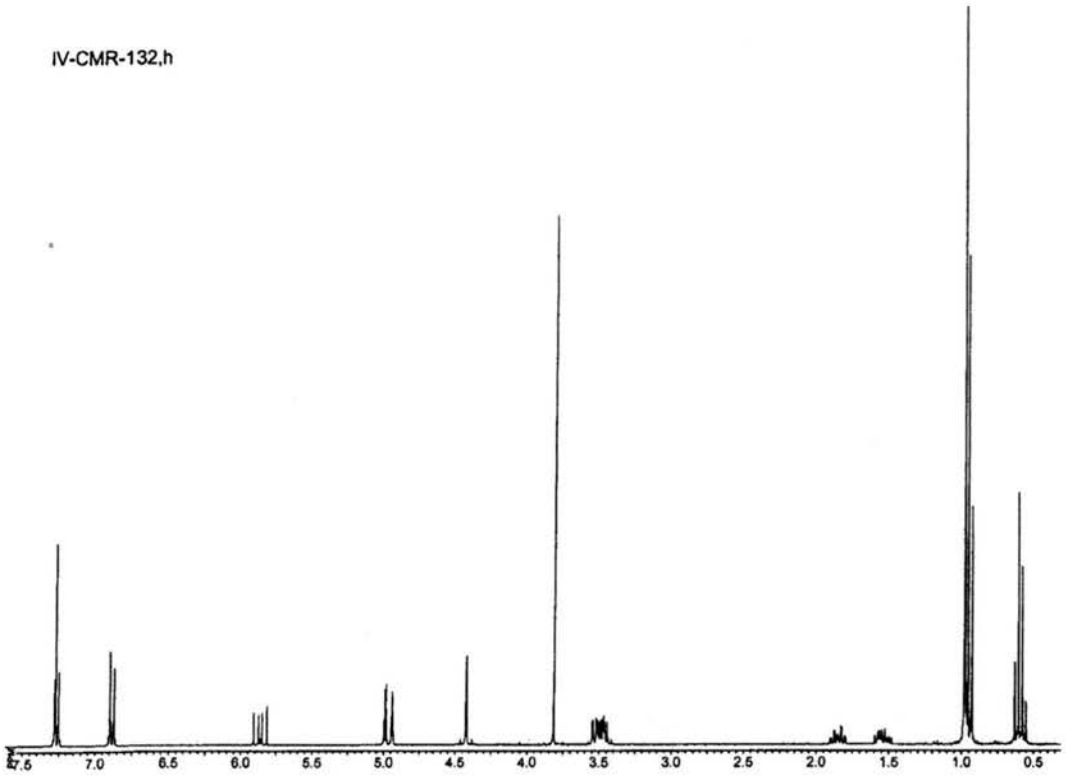
Triethyl-{1-[2-(4-methoxy-benzyloxy)-ethyl]-2,2-dimethyl-but-3-enyloxy}-silane (277): To a stirring solution of the alcohol (**172**, 1.17g, 4.426 mmol) in dry CH_2Cl_2 (45mL) at 0°C under argon was added 2,6-lutidine (1.1 mL, 10.00 mmol, 2.3 eq) and TESOTf (1.3 mL, 5.749 mmol, 1.3 eq) successively. The resulting solution was allowed to warm to room temperature and stirred under argon an additional 4h. Excess TESOTf was quenched by addition of MeOH (1 mL). Concentration by rotary evaporation followed by purification on silica gel with 15:1 hexanes / ethyl acetate gives the desired product as a clear colorless oil (1.38g, 3.645mmol, 82%).

^{1}H NMR ($CDCl_3$, 300 MHz) 0.594 (q, J=7.5 Hz, 6H), 0.955 (t, J=7.5Hz, 9H), 0.978 (s, 6H), 1.481-1.595 (mult, 1H), 1.797-1.915 (mult., 1H), 3.445-3.549 (mult., 3H), 3.817 (s, 3H), 4.423 (dd, J=11.5, 12.8 Hz, 2H), 4.943 (dd, J=1.7, 3.2 Hz, 1H), 4.990 (dd, J=1.7, 4.3 Hz, 1H), 5.865 (dd, J=10.4, 18 Hz, 1H), 6.885 (d, J=8.8 Hz, 2H), 7.27 (d, J=8.8 Hz, 2H).

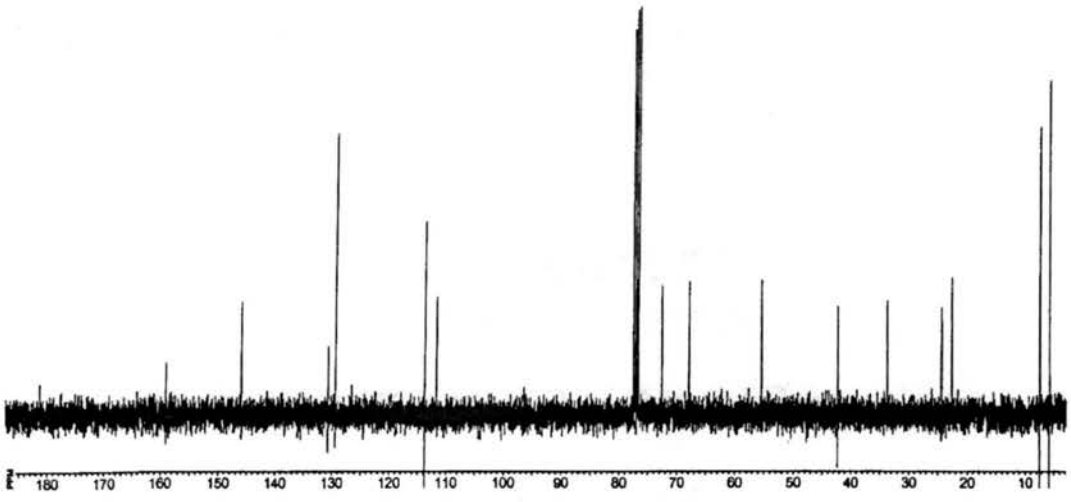
^{13}C NMR ($CDCl_3$, 75 MHz) δ159.2, 146.1, 129.5, 113.8, 111.8., 77.0, 72.7, 68.0, 55.5, 42.3, 33.8, 24.5, 22.7, 7.5, 5.8.

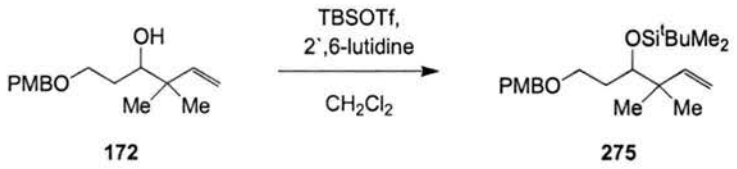
HRMS (FAB+) M+H calcd. 379.266849 Found 379.265053.

IV-CMR-132,h



IV-CMR-132,c



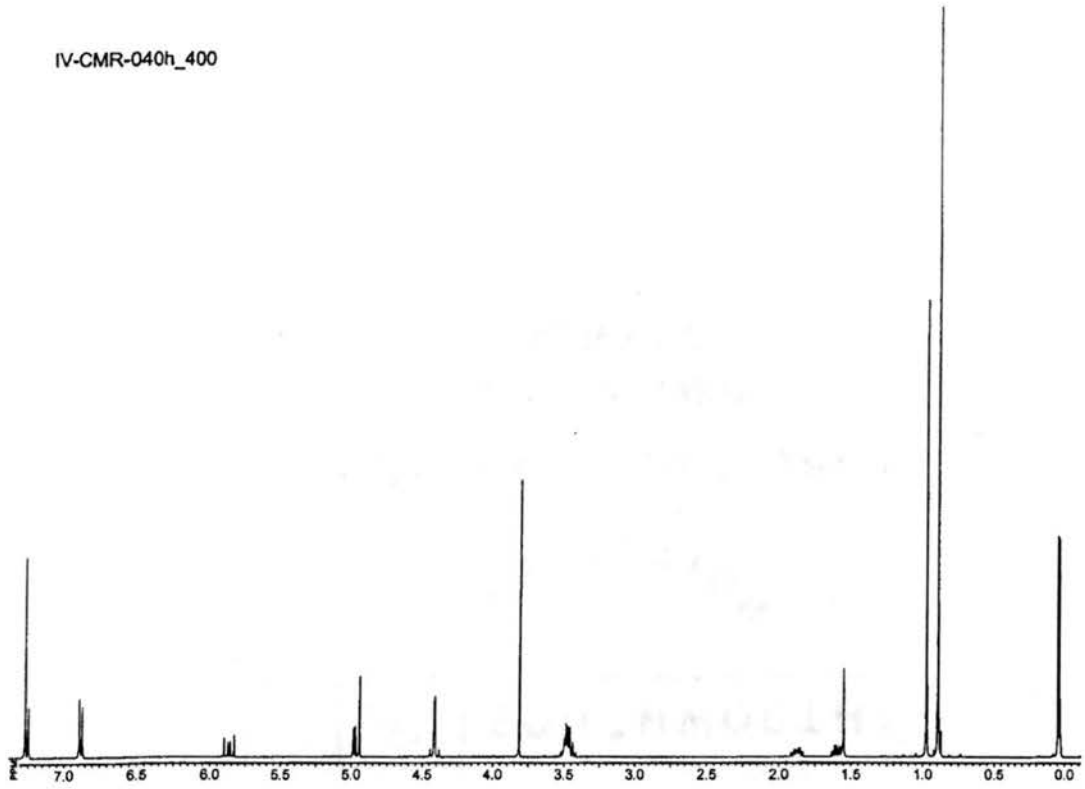


172 **275**

***tert*-Butyl-{1-[2-(4-methoxy-benzyloxy)-ethyl]-2,2-dimethyl-but-3-enyloxy}-dimethyl-silane (275):** To a stirring solution of the alcohol (**172**, 0.058Gg, 0.2194 mmol) in dry CH_2Cl_2 (2mL) at 0°C under argon was added 2,6-lutidine (0.055mL, 0.5002mmol, 2.3 eq) and TBDMSOTf (0.066 mL, 0.2874 mmol, 1.3 eq) successively. The resulting solution was allowed to warm to room temperature and stirred under argon an additional 4h. Excess TESOTf was quenched by addition of MeOH (1 mL). Concentration by rotary evaporation followed by purification on silica gel with 15:1 hexanes / ethyl acetate gives the desired product as a clear colorless oil (0.058g, 0.1532mmol, 70%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.033 (s, 3H), δ 0.046 (s, 3H), δ 0.896 (s, 9H), 0.976 (s, 6H), δ 1.550-1.634 (mult., 1H), δ 1.835-1.917 (mult., 1H), δ 3.421-3.522 (mult., 3H), δ 3.814, (s, 3H), δ 4.412 (m, 2H), δ 4.943-4.989 (mult., 2H), δ 5.872 (dd, J=11.3, 17 Hz, 1H), δ 6.882 (d, J=8.8 Hz, 2H), δ 7.260 (d, J=8.8 Hz, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz) δ146.3, 131.0, 129.5, 114.0, 111.8, 76.7, 72.7, 68.0, 55.5, 42.5, 34.1, 26.3, 24.7, 22.8, 18.6, -3.6.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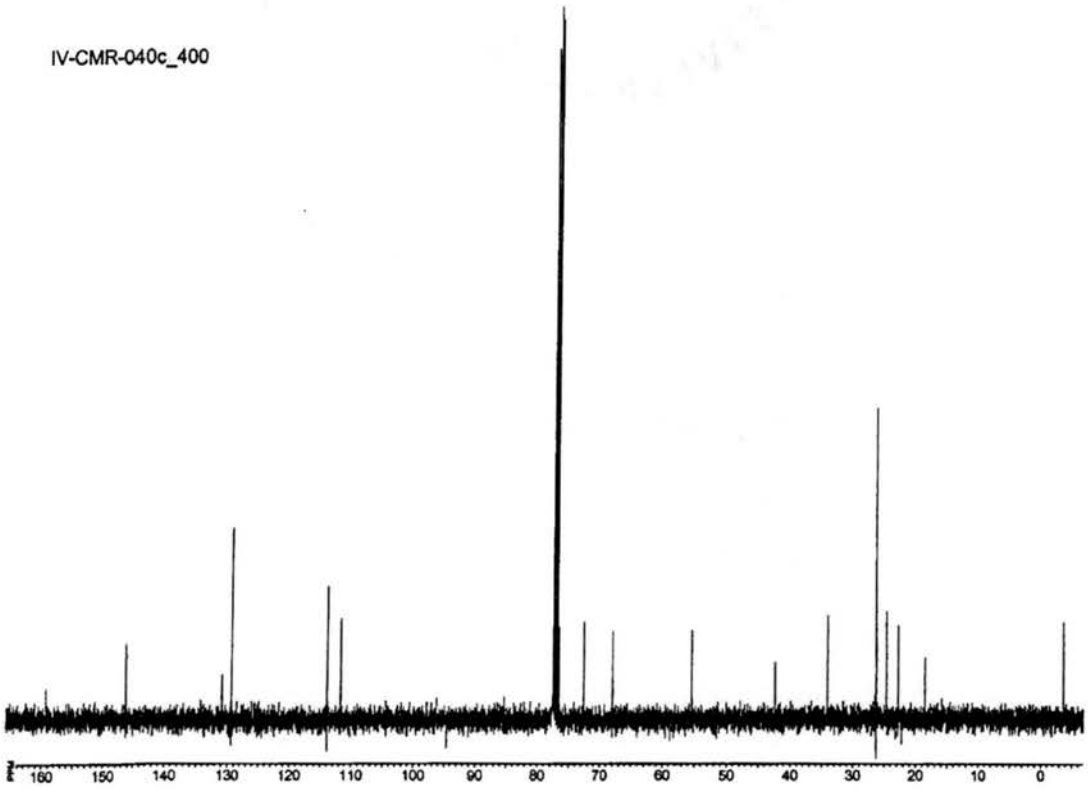
0.0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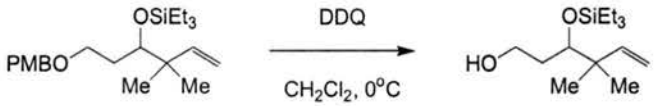


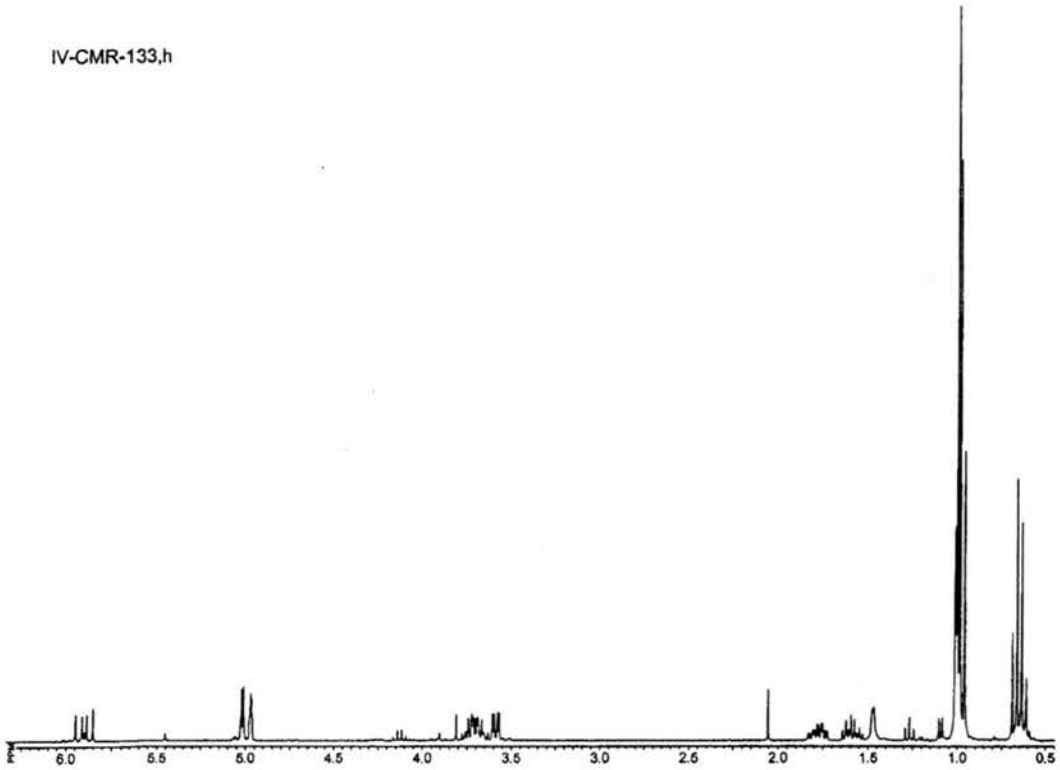
277 278

4,4-Dimethyl-3-triethylsilanyloxy-hex-5-en-1-ol (278): To A stirring solution of the PMB ether (**277**, 1.39g, 3.671 mmol) in wet CH_2Cl_2 (40 mL) at 0°C was added DDQ (1.003g, 4.418 mmol, 1.2 eq). After stirring at 0°C for 3h sat. aq. $NaHCO_3$ (40 mL) was added and stirred for 20 min. Aqueous layer was extracted with EtOAc (50 mL x 3). Combined organic extracts were washed with sat. aq. $NaHCO_3$ (50 mL x 2), and brine (50 mL), then dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Purification on silica gel with 9:1 hexanes / ethyl acetate gives the product as a clear colorless oil (0.846g, 3.273 mmol, 89%).

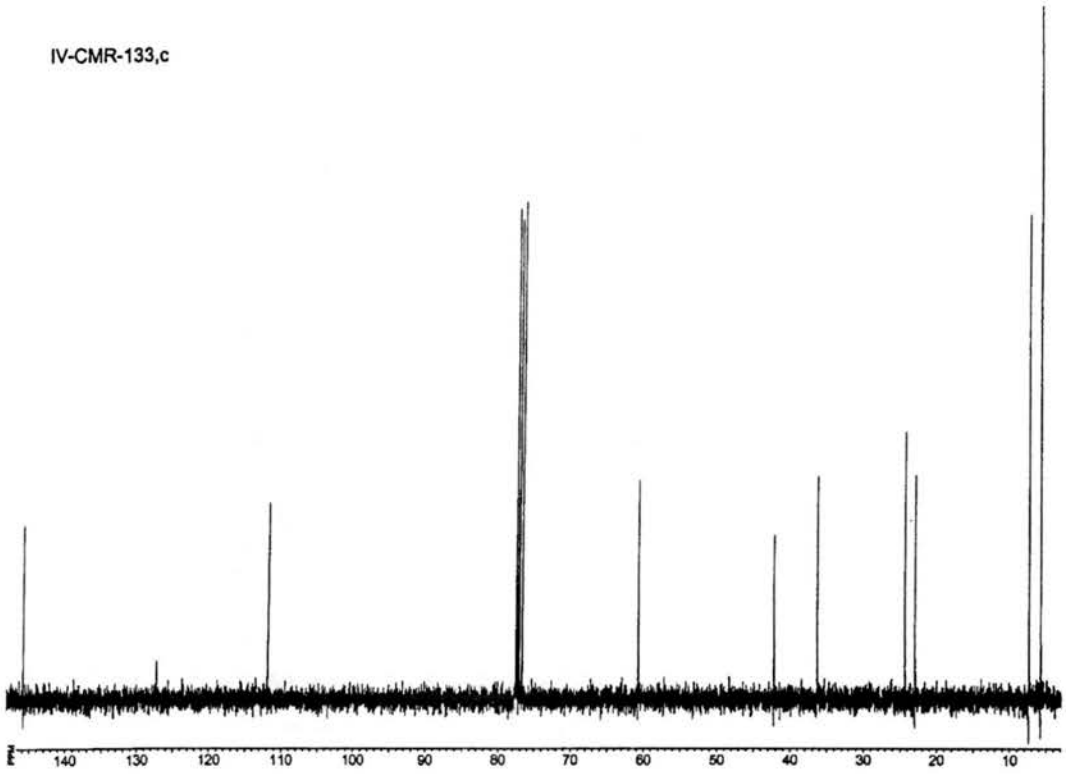
^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.647 (q, J=7.2 Hz, 6H), δ 0.981 (t, J=7.2 Hz, 9H), δ 1.000 (s, 6H), δ1.485 (bs, 1H), δ 1.522-1.638 (mult., 1H), δ 1.729-1.829 (mult., 1H), δ 3.586 (dd, 2.9, 8.3 Hz, 1H), δ 3.628-3.775 (mult., 2H), δ 4.99 (dd, J=2.6 14.1Hz, 2H), δ 5.898 (dd, J=10.4, 18Hz, 1H).

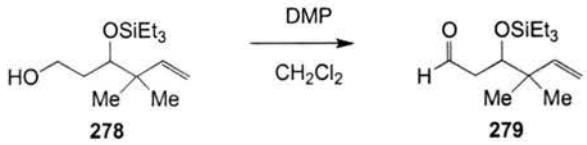
^{13}C NMR ($CDCl_3$, 75 MHz) δ146.0, 127.4, 112.0, 77.4, 60.9, 42.3, 36.4, 24.5, 23.1, 7.4, 5.8.

IV-CMR-133,h



IV-CMR-133,c





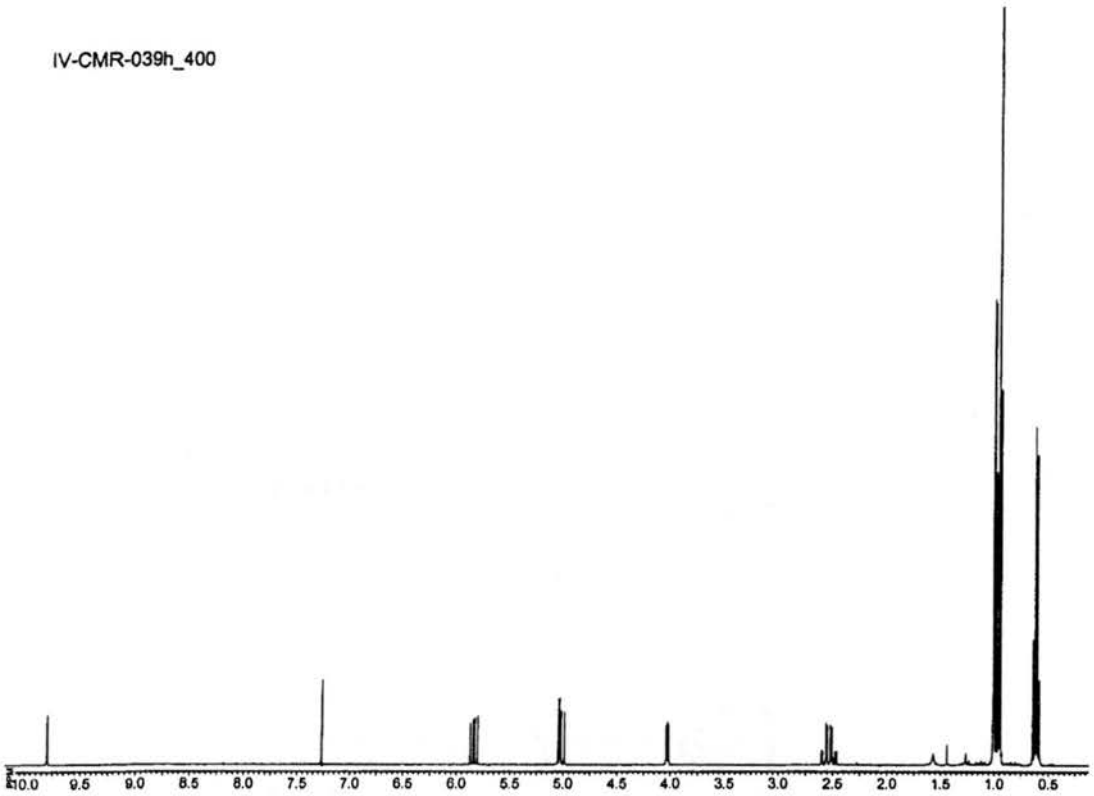
4,4-Dimethyl-3-triethylsilanyloxy-hex-5-enal (279): Dess-Martin reagent (8.69g, 20.49 mmol, 1.5 eq) added to a stirring solution of the alcohol **278** (0.846 g, 3.262 mmol) in CH_2Cl_2 (33 ml) at room temperature for 1.5h. The cloudy white solution was diluted with diethyl ether (35 ml). $Na_2S_2O_3.5H_2O$ (6.49g, 26.15mmol, 8 eq) was added followed by saturated aqueous $NaHCO_3$ (35 ml) and stirred until the biphasic layers were clear. The mixture was then poured into a separatory funnel; the aqueous layer was separated and the organic layer was washed with saturated aqueous $NaHCO_3$ (3 x 35 ml); the aqueous fractions were combined and extracted with diethyl ether (3 x 50 ml); organic fractions were combined, washed with brine (100 ml), dried (Na_2SO_4), filtered and concentrated by rotary evaporation. The crude oil was purified on silica gel with 9:1 hexanes/ethyl acetate to give the product as a clear oil (0.726g, 2.839 mmol, 87%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.616 (q, J=7.9 Hz, 6H), δ 0.962 (t, J=7.9 Hz, 9H), δ 1.001 (s, 3H), δ 1.011 (s, 3H), δ 2.530 (dq, J=2.5, 17Hz, 1H), δ 2.543 (dq, J=1.5, 17 Hz, 1H), δ 4.029 (dd, J=4.4, 6.3 Hz, 1H), δ 5.024 (ddd, J=1.5, 10.9, 18.6 Hz, 2H), δ 5.839 (dd, J= 10.8, 17.9 Hz, 1H), δ 9.797 (dd, J=1.6, 2.4 Hz, 1H).

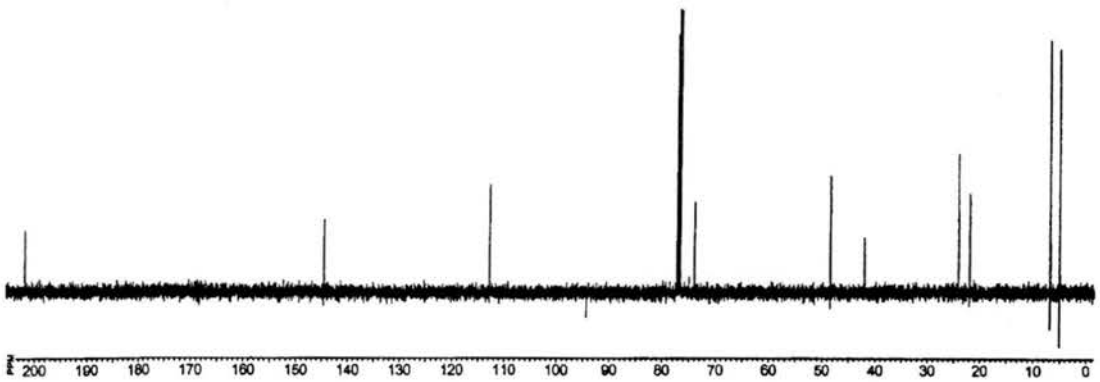
^{13}C NMR ($CDCl_3$, 75 MHz) δ

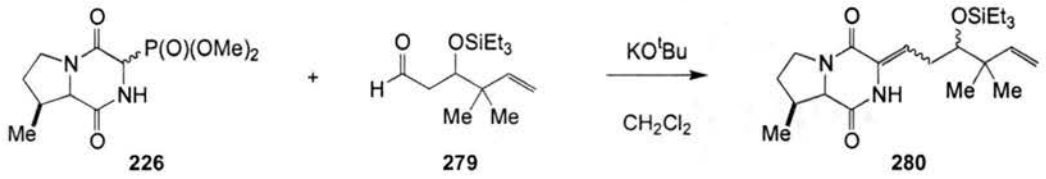
HRMS (FAB+) M+H calcd. 257.193684, found 257.193942.

IV-CMR-039h_400

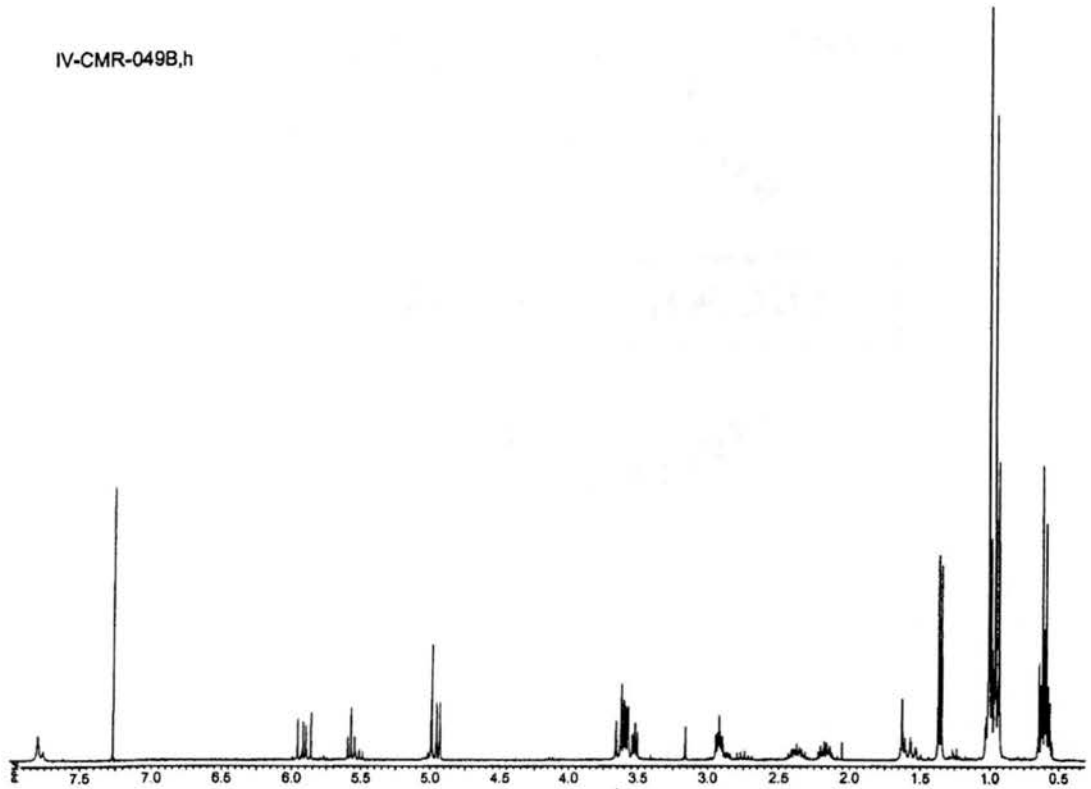


IV-CMR-039c_400





To a stirring suspension of KO^{t}Bu (0.035g, 0.3119 mmol, 1.7 eq) in dry CH_2Cl_2 (2 mL) at -78°C under argon was added the DKP (0.066g, 0.2389 mmol, 1.3 eq). After stirring for 1.5h, the aldehyde (0.048g, 0.1872 mmol) in dry CH_2Cl_2 (2 mL) was added and the mixture was allowed to warm to 0 °C for 4h. Concentration by rotary evaporation followed by purification on silica gel with 1:1 hexanes/EtOAc gave the product as a poorly separable mixture of isomers.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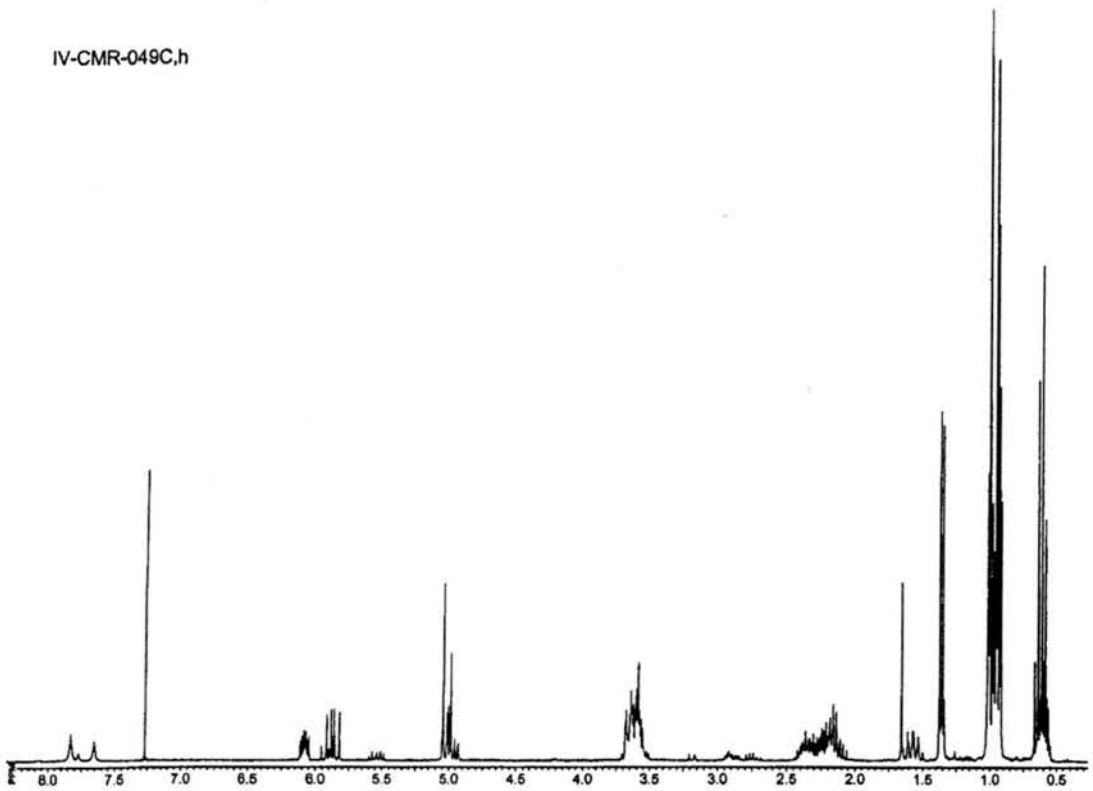
1.0
0.5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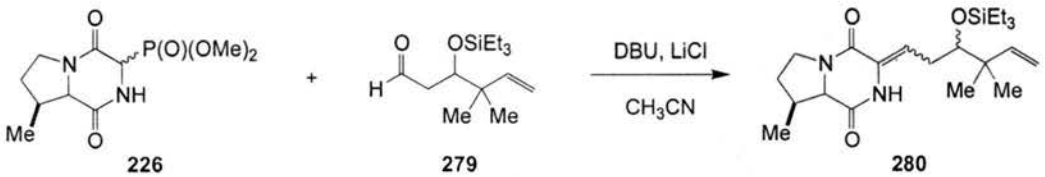
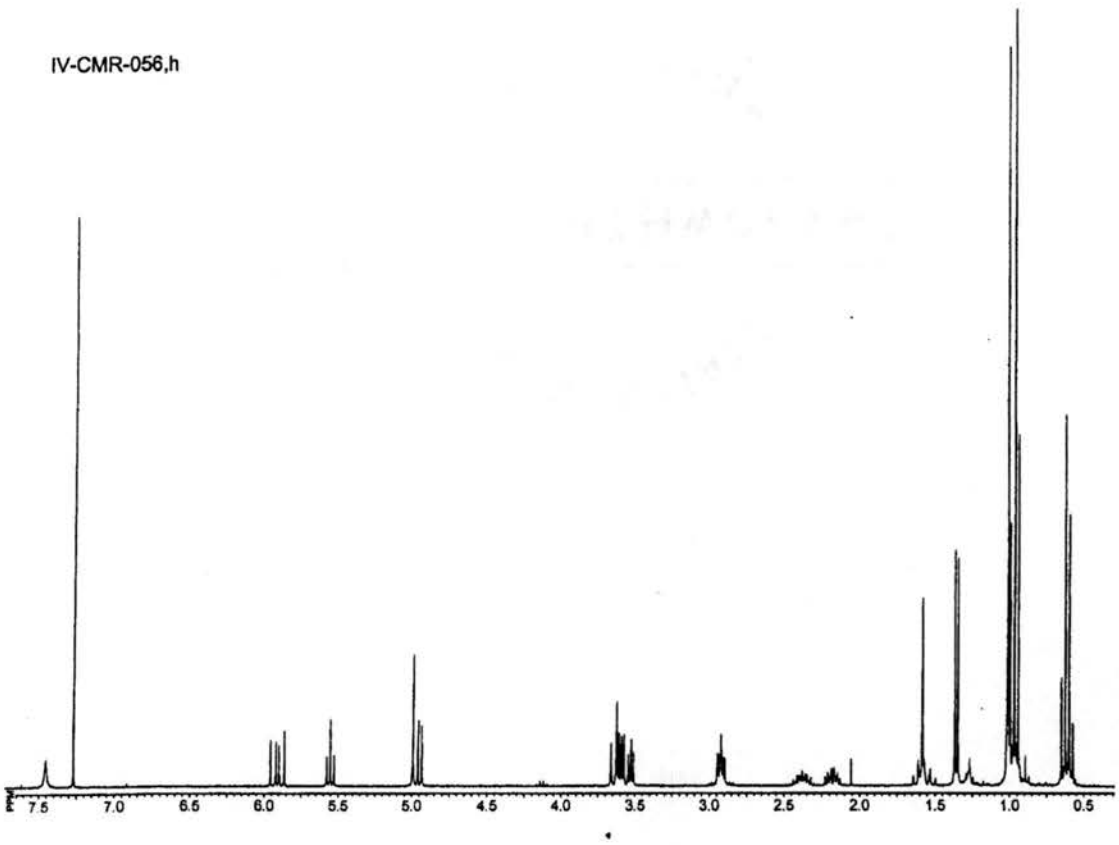
0.5



To a stirring suspension of the DKP (0.085g, 0.3077 mmol, 1.18 eq) and LiCl (0.014g, 0.3303 mmol, 1.26 eq) [NOTE: LiCl was prepared for this reaction by heating to 120 °C under high vacuum pressure overnight.] in dry CH_3CN under argon at room temperature was added DBU (0.040 mL, 0.2675 mmol, 1.02 eq). After 5 min the aldehyde (0.067g, 0.2612 mmol) in dry CH_3CN (1.1 mL) was added via cannula and stirred for 5.5 h. The reaction mixture was diluted with EtOAc (25 mL) and washed with saturated aqueous NH_4Cl (10 mL), brine (10 mL) and dried (Na_2SO_4) filtered and concentrated by rotary evaporation. Purification on silica gel (Whatman silica gel 60A°, 230-400 mesh ASTM, Cat # 4790-050) with 7:3 hexanes/EtOAc gave the product as a white solid (0.094g, 0.2312 mmol, 89%)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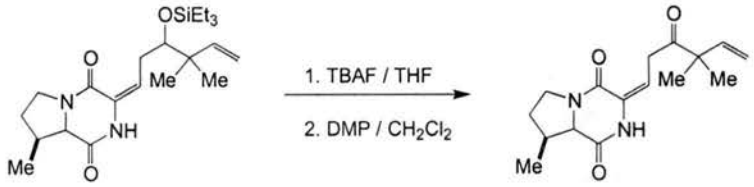
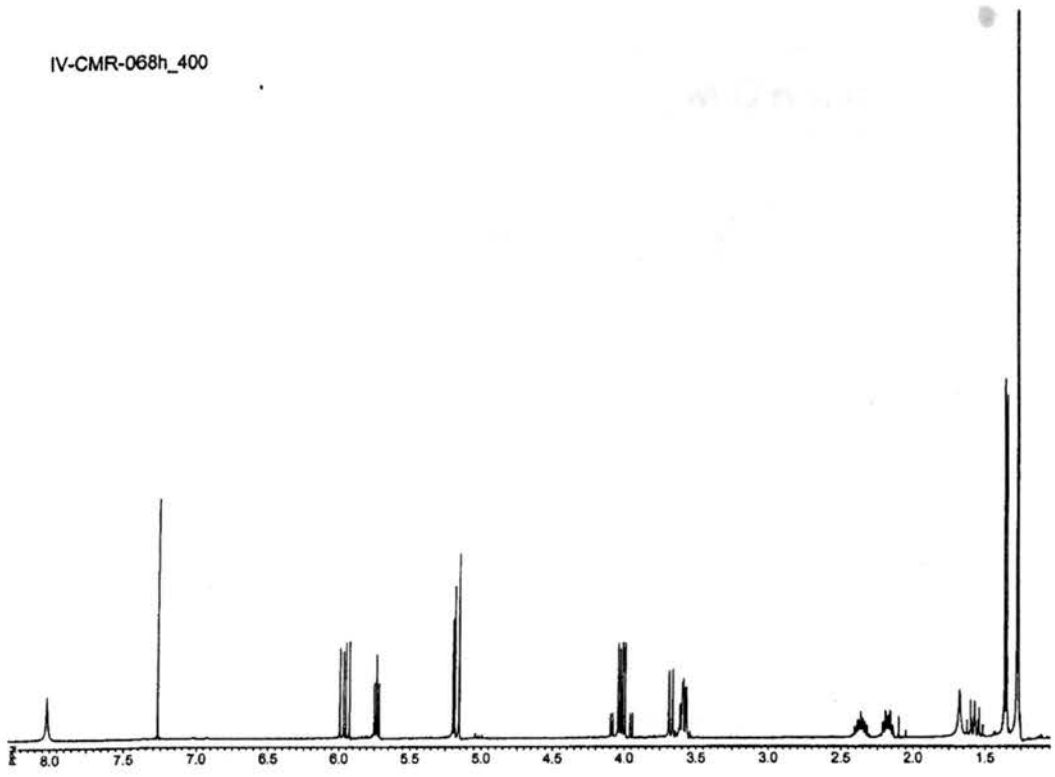


280a

3-(3-Hydroxy-4,4-dimethyl-hex-5-enylidene)-8-methyl-hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione:

To a stirring solution of silyl ether **280a** (0.128 g, 0.3147 mmol) at 0°C was added TBAF (1 M in THF, 0.650 mL, 2.06 eq). The resulting solution was allowed to warm to room temperature and stirred for an additional 18h. Concentration by rotary evaporation and purification on silica gel with EtOAc gives the product as a sticky oil (0.079g, 0.2702 mmol 86%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.068 (s, 3H), δ 1.073 (s, 3H), δ 1.356 (d, J=6.4 Hz, 3H), δ 1.509-1.656 (mult., 1H), δ 2.135-2.234 (mult., 1H), δ 2.285-2.453 (mult., 2H), δ 2.817-2.934 (mult., 1H), δ 3.303 (d, J=2.1, 11.3 Hz, 1H), δ 3.599 (dd, J=2.1, 9.85 Hz, 1H), δ 3.641 (dd, J=10, 16.1 Hz, 2H), δ 5.001-5.073 (mult., 2H), δ 5.605 (dd, J=7.9, 10.1 Hz, 1H), δ 5.914 (dd, J=11.2, 17.2 Hz, 1H), δ 8.260 (bs, 1H).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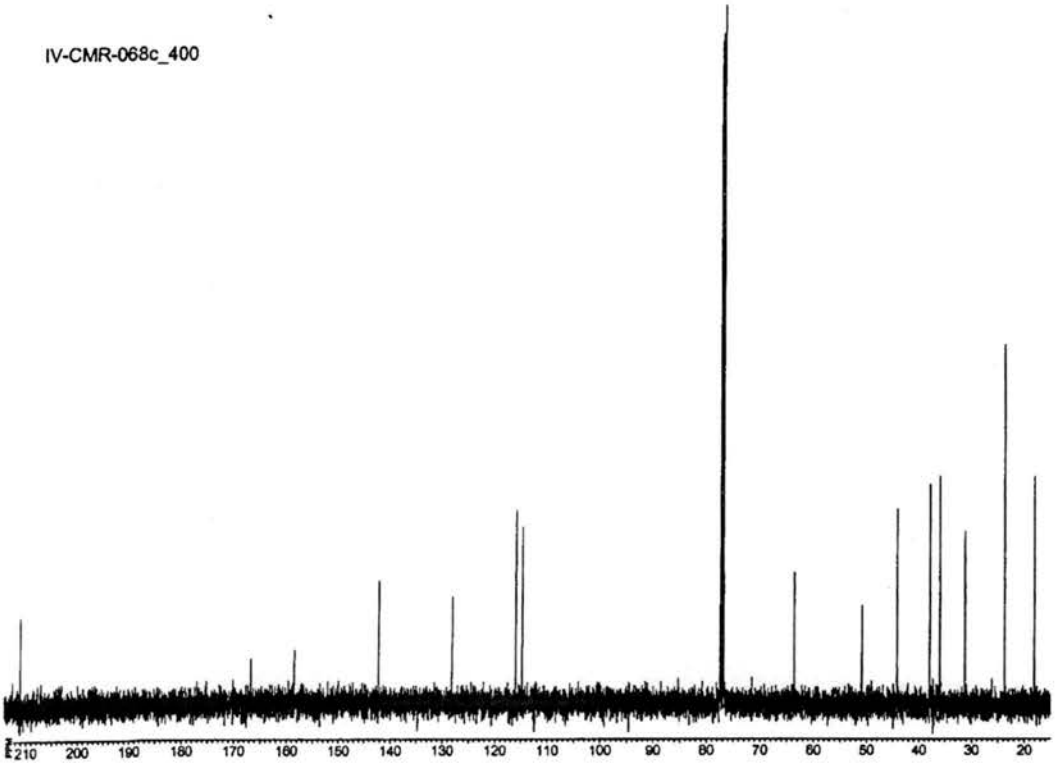
2.0
1.5


IV-CMR-068c_400
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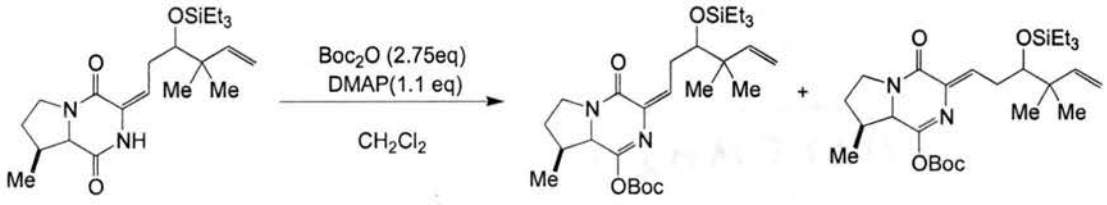
20



280a **281**

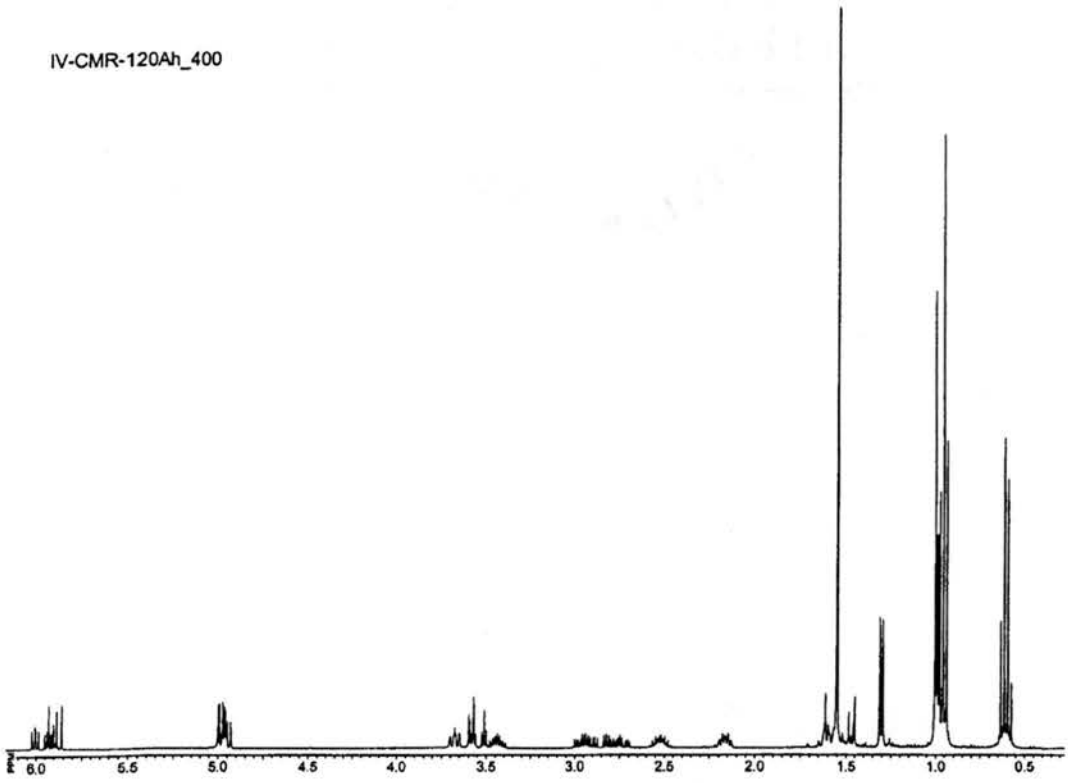
Carbonic acid tert-butyl ester 3-(4,4-dimethyl-3-triethylsilanyloxy-hex-5-enylidene)-8-methyl-4-oxo-3,4,6,7,8,8a-hexahydro-pyrrolo[1,2-a]pyrazin-1-yl ester (281): To a stirring solution of diketopiperazine **280a** (0.078g, 0.1918 mmol) in dry CH_2Cl_2 (2 mL) at 0°C was added DMAP (0.026 g, 0.2128 mmol, 1.1 eq) and Boc_2O (0.112g, 0.5132 mmol, 2.7 eq) and the resulting solution was stirred at 0°C for 30 min, then concentrated by rotary evaporation and purified on neutral silica gel with 8:2 hexanes / ethyl acetate. Product, a 1:1 mixture of inseparable diastereomers isolated as a clear colorless oil (0.098 g, 0.1914 mmol, 98%)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.606 (q, J=8.1 Hz, 6H), δ 0.960 (t, J=8.1 Hz, 9H), δ 0.994 (s, 3H), δ 1.005 (s, 3H), δ 1.304 (dd, J=1.6, 6.5, 3H), δ 1.552, (s, 9H), δ 2.130-2.197 (mult., 1H), δ 2.481-2.566 (mult., 1H), δ 2.696-2.841 (mult., 1H), δ 2.876-3.001 (mult., 1H), δ 3.393-3.482 (mult., 1H), δ 3.501-3.599 (mult., 2H), δ 3.649-3.702 (mult., 1H), δ 4.927-5.005 (mult, 2H), δ 5.861-6.026 (mult., 2H).

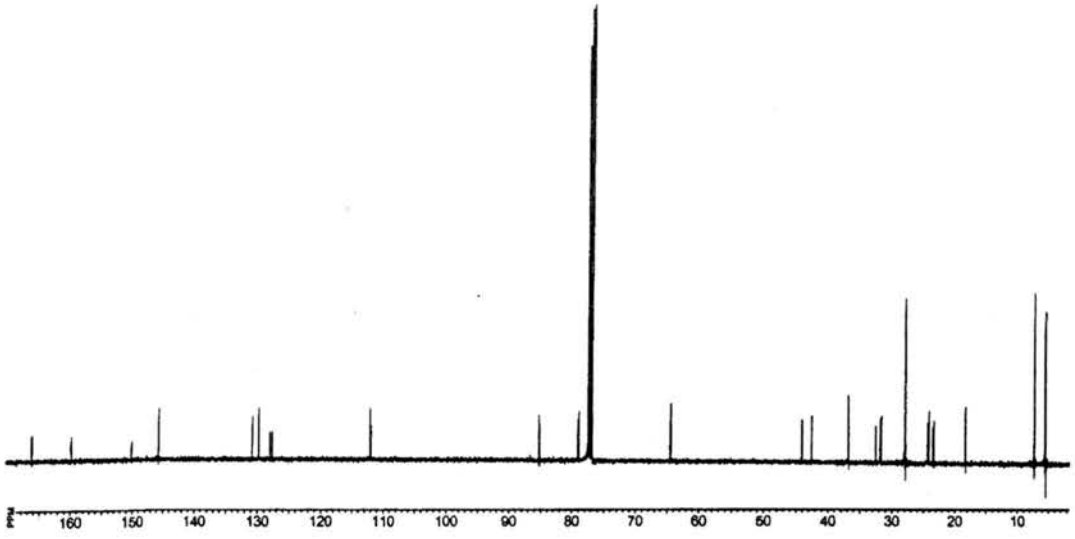
^{13}C NMR ($CDCl_3$, 75 MHz) δ166.4, 166.2, 160.0, 159.9, 150.3, 150.2, 145.8, 130.9, 129.9, 128.1, 127.8, 112.2, 112.1, 85.4, 85.2, 79.2, 79.0, 64.6, 64.4, 44.1, 44.0, 42.5, 36.9, 36.8, 32.5, 31.9, 31.8, 31.6, 28.1, 27.9, 24.2, 24.1, 23.5, 23.3, 18.3, 7.3, 5.6, 5.5.

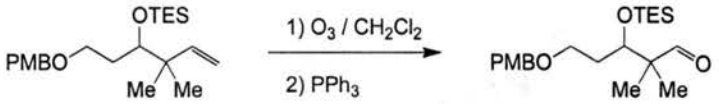
HRMS (FAB+) M+H calcd. 507.325426, found 507.325441.

IV-CMR-120Ah_400



IV-CMR-120Ac_400



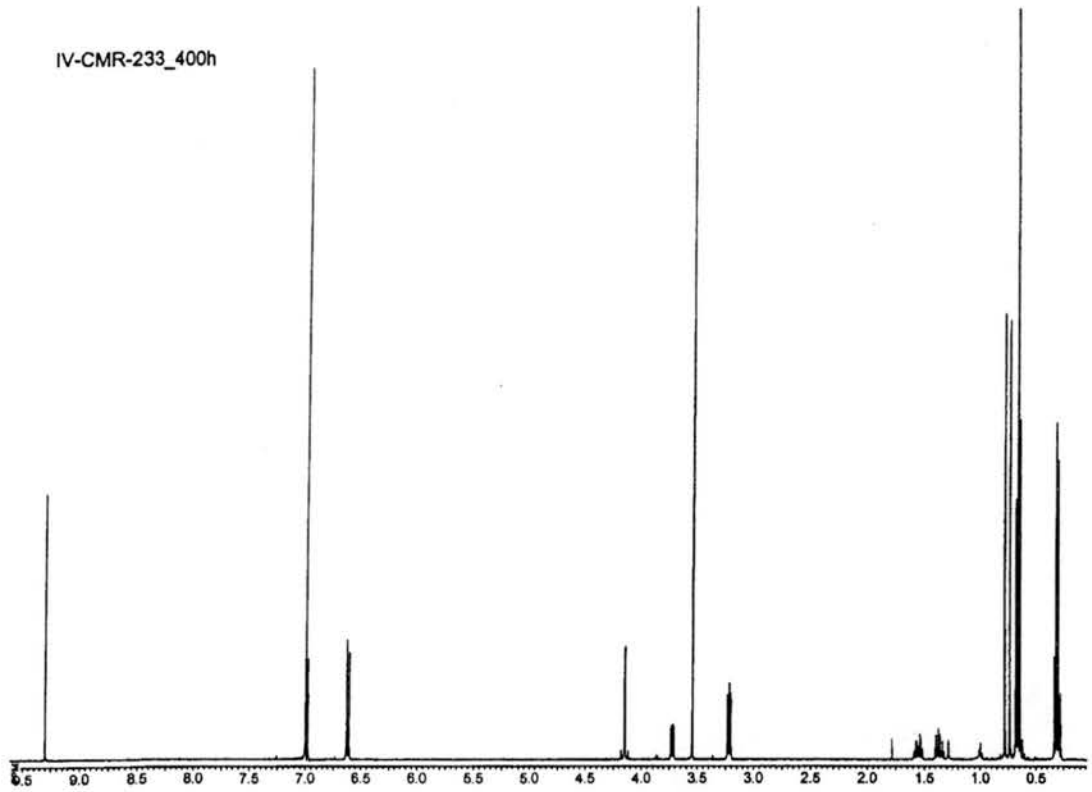


277 288

5-(4-Methoxy-benzyloxy)-2,2-dimethyl-3-triethylsilanyloxy-pentanal (288): Ozone bubbled through a stirring solution of alkene **277** (0.199g, 0.5256 mmol) in CH_2Cl_2 (6 mL) at -78°C until pale blue color persisted, then degassed with argon for 15 min. PPh_3 (0.152g, 0.5795 mmol, 1.1 eq.) added and reaction mixture warmed to room temperature over 2h. Following concentration by rotary evaporation, semi-solid residue suspended in 9:1 hexanes/ethyl acetate and filtered through celite to remove excess Ph_3PO. Crude material purified on silica gel with 9:1 hexanes/ethyl acetate. Product isolated as a clear colorless oil (0.133g, 0.3495 mmol, 66%)

^{1}H NMR (400 MHz, $CDCl_3$) δ0.543 (q, J=7.9 Hz, 6H), δ0.901 (t, J=8.0 Hz, 9H), δ0.974 (s, 3H), δ1.018 (s, 3H), δ1.559-1.642 (m, 1H), δ1.749-1.830 (m, 1H), 3.455 (dd, J=5.5, 7.7 Hz, 2H), δ3.783 (s, 3H), δ3.960 (dd, J=2.9, 8.6 Hz, 1H), δ4.388 (dd, J=11.5, 13.4, 2H), δ6.855 (d, J=8.8 Hz, 2H), δ7.228 (d, J=8.8 Hz, 2H), δ9.538 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ211.6, 206.6, 175.1, 159.4, 130.6, 129.6, 114.0, 73.7, 72.9, 66.9, 55.5, 51.4, 33.6, 19.2, 17.7, 7.2, 5.6.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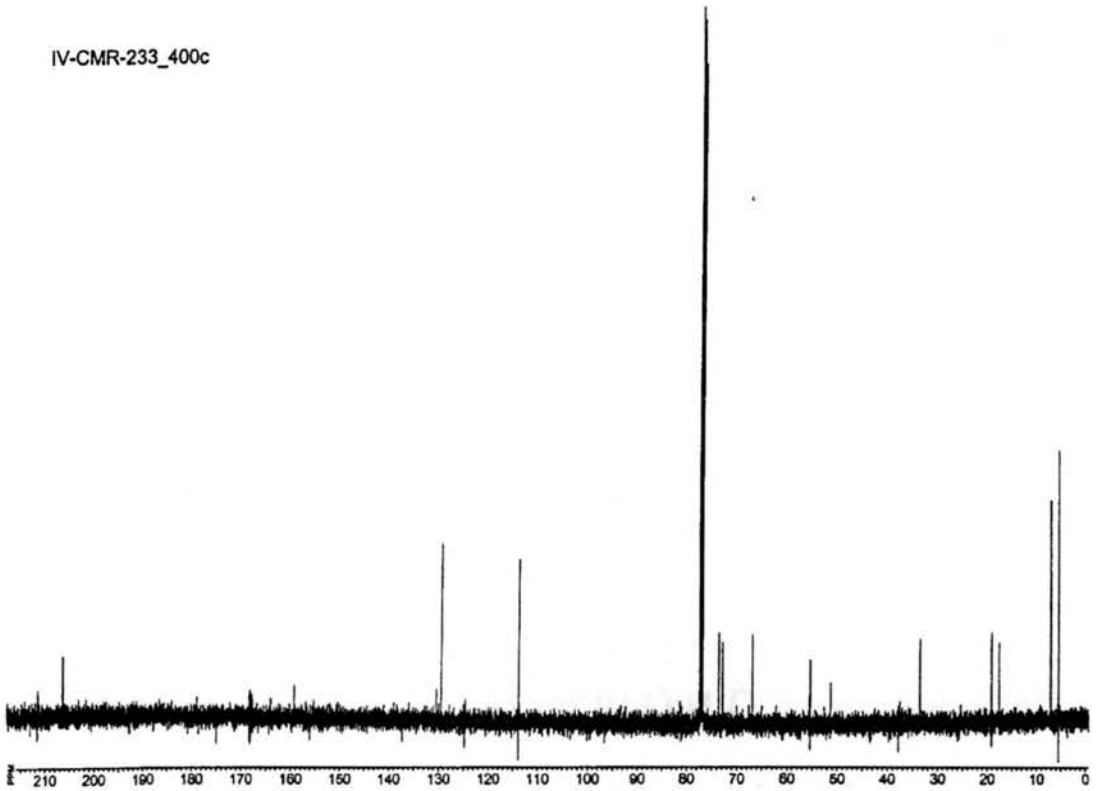
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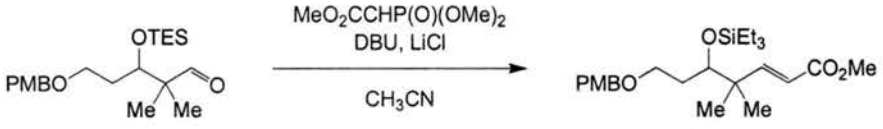


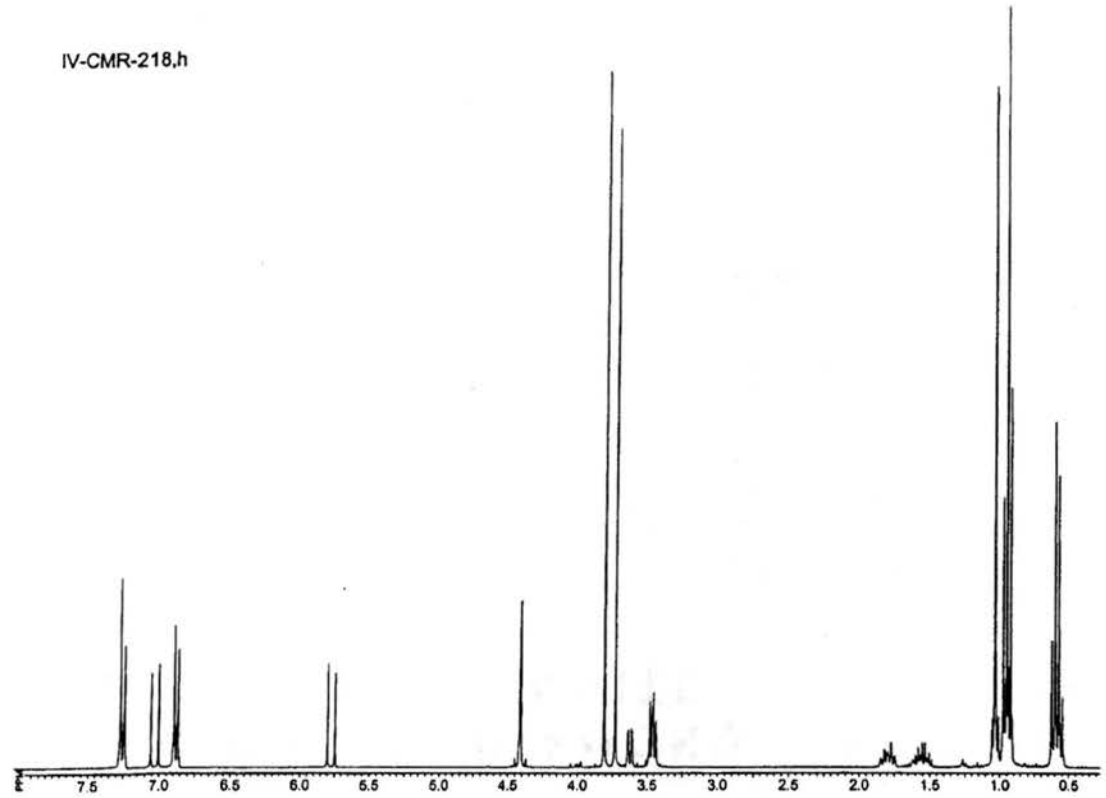
288 **289**

7-(4-Methoxy-benzyloxy)-4,4-dimethyl-5-triethylsilanyloxy-hept-2-enoic acid methyl ester (289): DBU (0.015 mL, 0.1003 mmol, 1.2 eq,) was added to a stirring solution of (Dimethoxy-phosphoryl)-acetic acid methyl ester (0.016 mL, 0.09884 mmol, 1.2 eq.) in dry CH_3CN (0.3 mL) in flame dried glassware under argon and stirred at room temperature for 15 min. Adehyde **288** in CH_3CN (0.4 mL x 3) added via cannula and the resulting mixture stirred at room temperature for 24 h. Reaction mixture diluted with EtOAc (6 mL) and washed with sat. aq. NH_4Cl (6 mL). Aqueous layer extracted with EtOAc (5 mL x 3). Combined organic extracts washed with brine (6 mL), then dried (Na_2SO_4) filtered and concentrated by rotary evaporation. Purified on silica gel with 9:1 hexanes/ethyl acetate. Product isolated as a clear colorless oil (0.027g, 0.06183 mmol, 74%).

^{1}H NMR (300 MHz, $CDCl_3$) δ0.590 (q, J=7.7 Hz, 6H), δ0.951 (t, J=7.9 Hz, 9H), δ1.036 (s, 6H), δ1.489-1.627 (m, 1H), δ1.744-1.852 (m, 1H), δ3.463 (dd, J=5.9, 7.8 Hz, 2H), δ3.628 (dd, J=2.6, 8.8 Hz, 1H), δ3.736 (s, 3H), δ3.815 (s, 3H), δ4.413 (s, 2H), 5.775 (d, J=16.1 Hz, 1H), δ6.884 (d, J=8.7 Hz, 2H), δ7.034 (d, J=16.2 Hz, 1H), δ7.256 (d, J=8.6 Hz, 2H).

^{13}C NMR (75 MHz, $CDCl_3$) δ167.4, 159.2, 156.5, 130.6, 129.5, 118.5, 113.9, 76.4, 72.8, 67.5, 55.5, 51.7, 42.7, 34.0, 23.7, 22.9, 7.4, 5.7.

HRMS (FAB+) M+H calcd. 437.272328, Found 437.271515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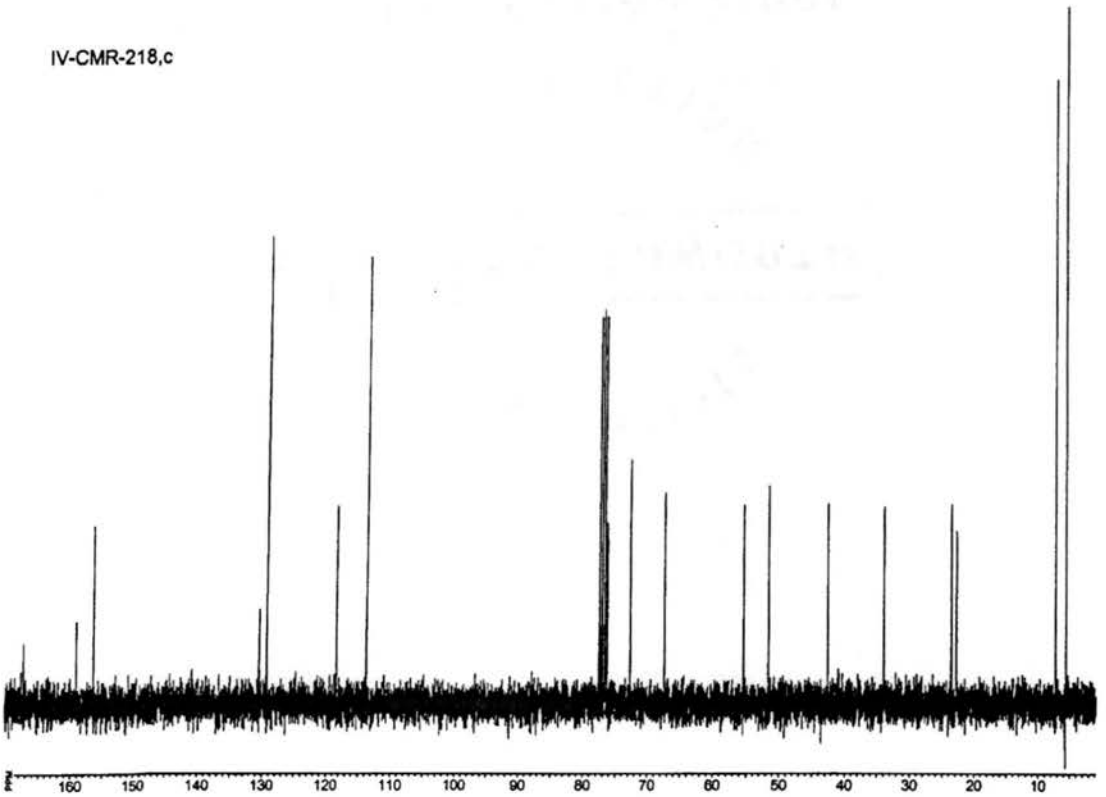
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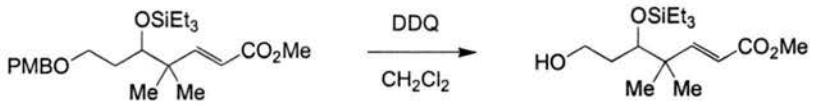
10



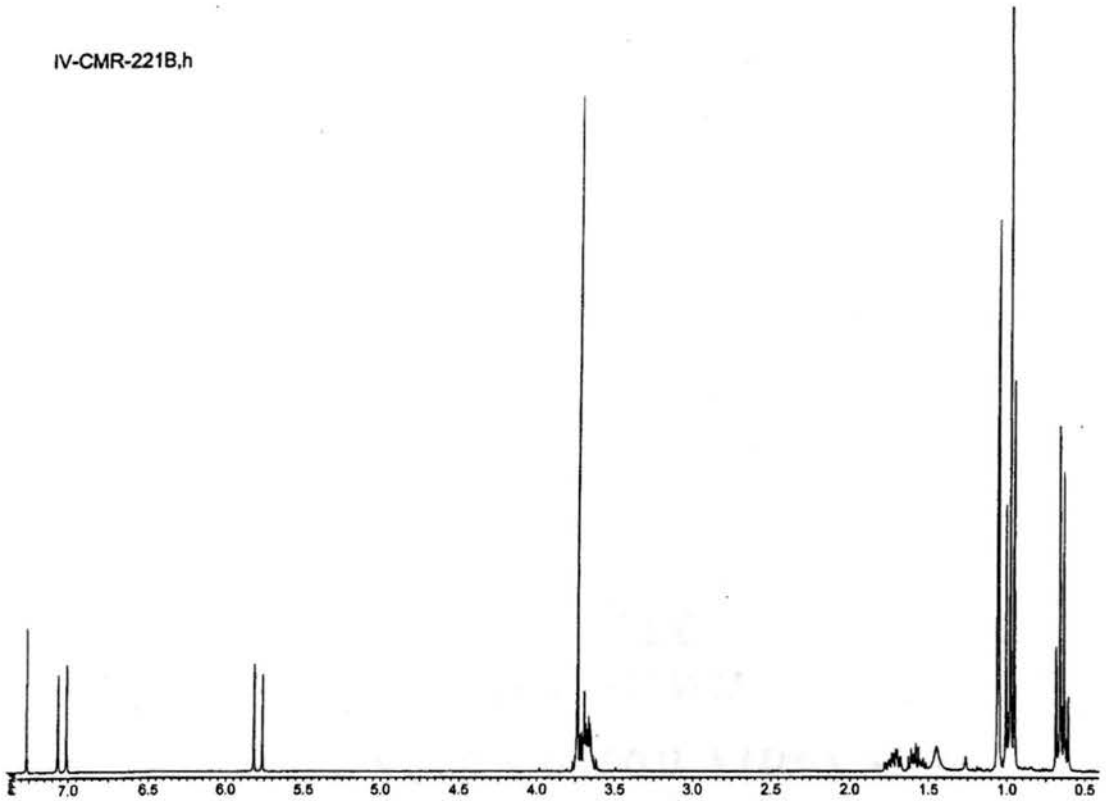
289 **290**

7-Hydroxy-4,4-dimethyl-5-triethylsilanyloxy-hept-2-enoic acid methyl ester (290): To a stirring solution of PMB ether 12 (0.027g, 0.06183 mmol) in wet CH_2Cl_2 (1 mL) was added DDQ (0.019g, 0.08370 mmol, 1.35 eq.). The resulting green solution was stirred at 0°C for 3h. The yellow/orange reaction mixture was diluted with EtOAc (5 mL) and washed with sat. aq. $NaHCO_3$ (5 mL). Aqueous layer was extracted with EtOAc (5 mL x 3). Combine organic extracts were washed with sat. aq. $NaHCO_3$ (5 mL x 3) and brine (10 mL) then dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Crude material purified on silica gel with 9:1 hexanes/ethyl acetate. Product isolated as a clear colorless oil (0.012g, 0.03791 mmol 61%). Material stored at 0°C until used.

^{1}H NMR (300 MHz, $CDCl_3$) δ0.649 (q, J=7.9 Hz, 6H), δ0.983 (t, J=7.9 Hz, 9H), δ1.057 (s, 3H), δ1.063 (s, 3H), δ1.451 (bs, 1H), δ1.519-1.631 (m, 1H), δ1.675-1.782 (m, 1H), δ3.620-3.773 (m, 3H). δ3.743 (s, 3H), δ5.800 (d, J=15.9 Hz, 1H), δ7.042 (d, J=16.1 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ156.4, 118.6, 76.6, 60.5, 51.7, 42.6, 36.6, 23.8, 22.9, 7.4, 5.7

HRMS (FAB+) M+H calcd. 317.214813, Found 317.214880.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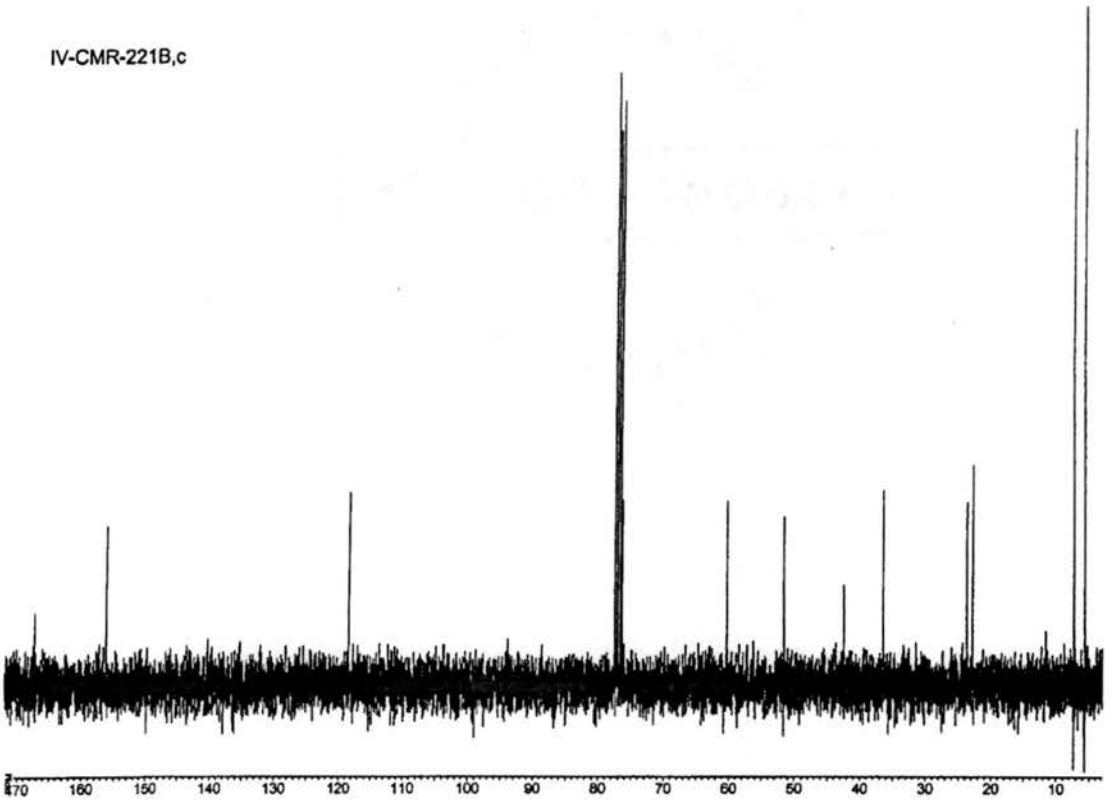
1.0
0.5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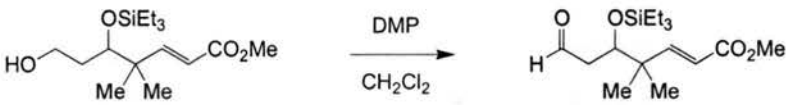
10

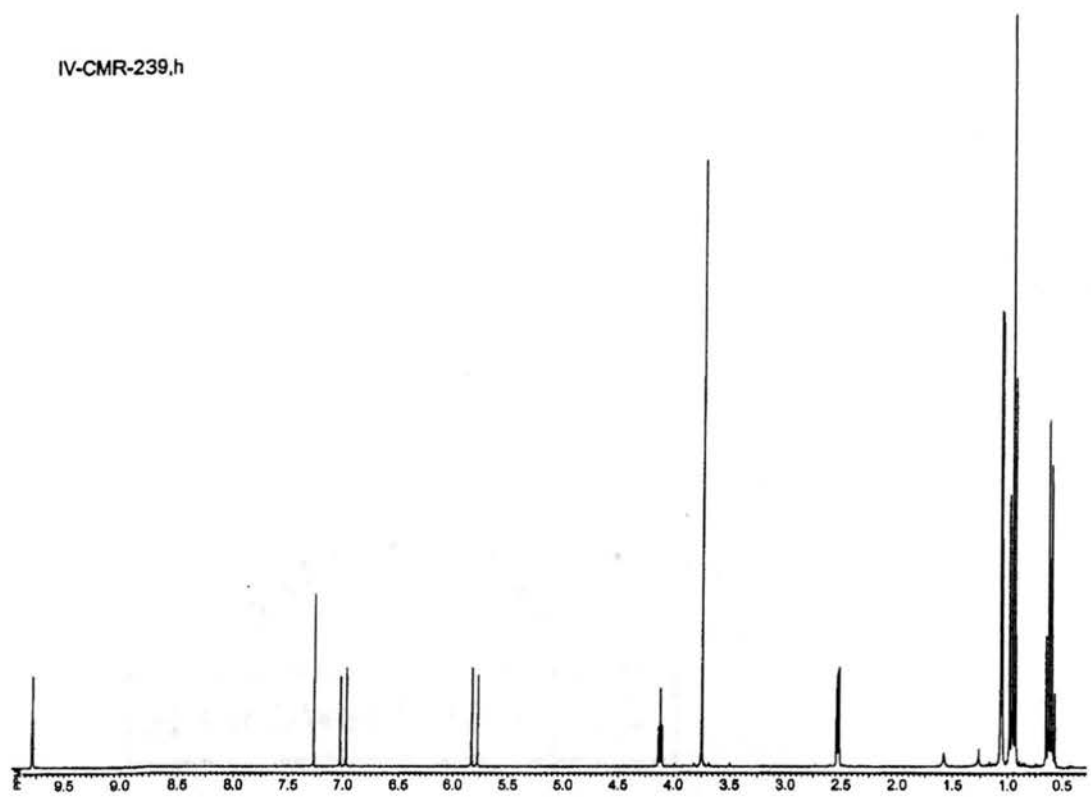


290 **291**

4,4-Dimethyl-7-oxo-5-triethylsilanyloxy-hept-2-enoic acid methyl ester (291): To a stirring solution of alcohol **290** (0.097g, 0.3065 mmol) in CH_2Cl_2 (3 mL) was added Dess-Martin periodinane (0.195g, 0.4598 mmol, 1.5 eq.). The resulting mixture was stirred at room temperature for 2h. Reaction mixture diluted with Et_2O (6 mL). $Na_2S_2O_3 \cdot 5H_2O$ (0.606g, 2.442 mmol, 8 eq) was added followed by sat. aq. $NaHCO_3$ (6 mL) and stirred until biphasic mixture became clear. Organic phase washed with sat. aq. $NaHCO_3$ (6 mL), then combined aqueous washings extracted with Et_2O (6 mL x 3). Combined organic extracts washed with sat. aq. $NaHCO_3$ (6 mL) and brine (6 mL), then dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Crude material purified on silica gel with 9:1 hexanes/ethyl acetate. Product isolated as a clear colorless oil (0.084g, 0.2671 mmol, 87%).

^{1}H NMR (300 MHz, $CDCl_3$) δ0.614 (q, J=7.9 Hz, 6H), δ0.958 (t, J=7.9 Hz, 9H), δ1.056 (s, 3H), δ1.069 (s, 3H), δ2.532 (dd, J=1.7, 5.2 Hz, 2H), δ3.749 (s, 3H), δ4.122 (t, J=5.3 Hz, 1H), δ5.805 (d, J=15.9 Hz, 1H), δ7.003 (d, J=16.1 Hz, 1H), δ9.789 (t, J=1.8 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ201.1, 154.9, 119.5, 73.6, 51.8, 48.7, 42.6, 23.6, 22.8, 7.2, 5.4. HRMS (FAB+) M+H calcd. 315.199163, Found 315.197851.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6.5
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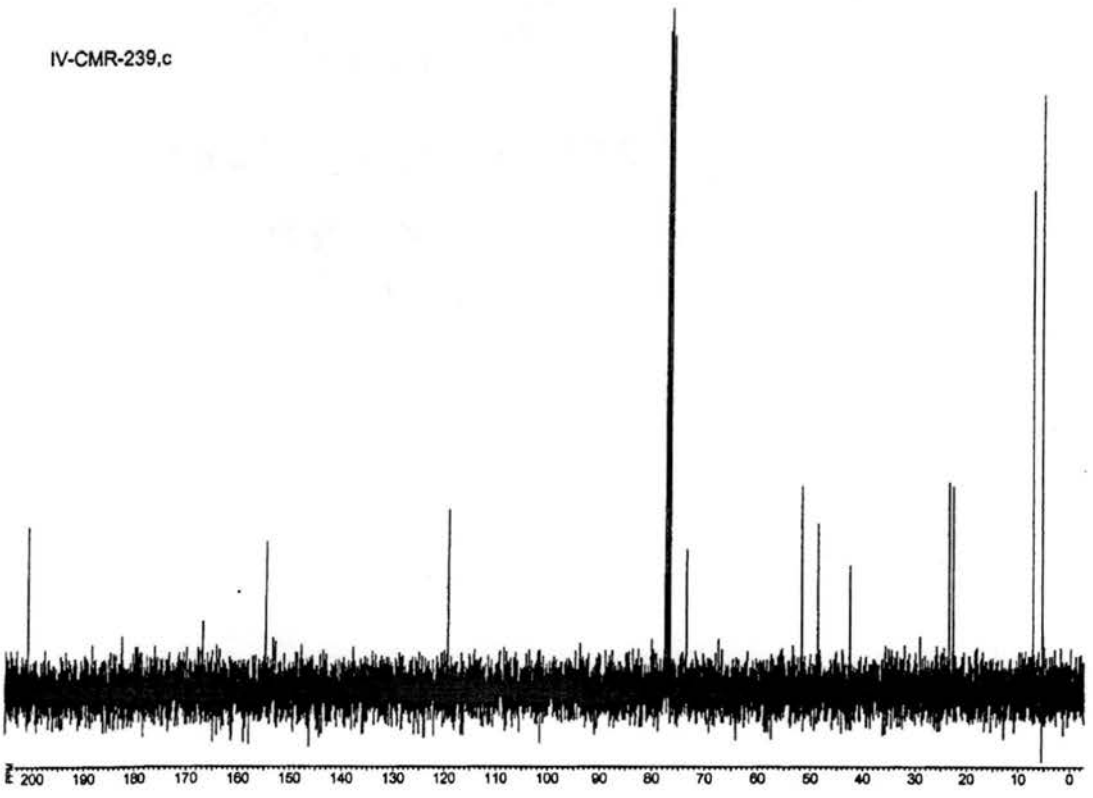
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


IV-CMR-239,c
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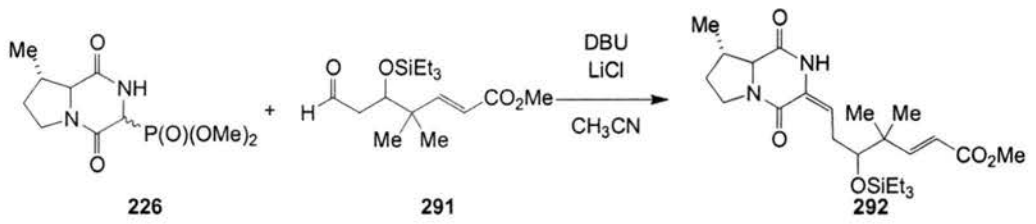
70
60
50
40
30
20
10
0



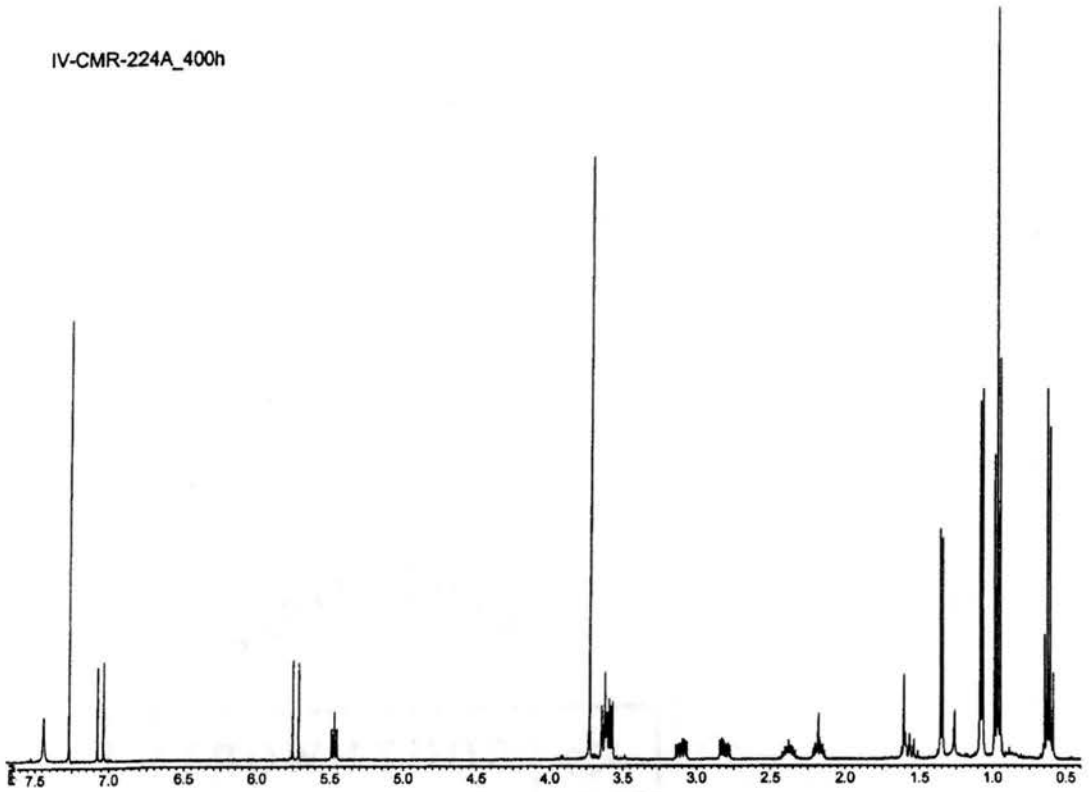
4,4-Dimethyl-7-(8-methyl-1,4-dioxo-hexahydro-pyrrolo[1,2-a]pyrazin-3-ylidene)-5-triethylsilanyloxy-hept-2-enoic acid methyl ester (292): DBU (0.008 mL, 0.05349 mmol, 1.7 eq.) was added to a stirring solution of (8-Methyl-1,4-dioxo-octahydro-pyrrolo[1,2-a]pyrazin-3-yl)-phosphonic acid dimethyl ester (0.012g, 0/04344 mmol, 1.37 eq.) and LiCl (0.016g, 0.03774 mmol, 1.2 eq.) in dry CH_3CN (0.1 mL) in flame dried glassware. The resulting solution was stirred at room temperature under argon for 15 min. The aldehyde (14, 0.010g, 0.03180 mmol) in dry CH_3CN (0.3 mL x 3) via cannula. After stirring at room temperature under argon for 3h the reaction was diluted with EtOAc (5 mL) and washed with sat. aq. NH_4Cl (5 mL). Aqueous layer was extracted with EtOAc (5 mL x 2). Combined organic extracts were washed with brine (5 mL) then dried (Na_2SO_4), filtered and concentrated by rotary evaporation. Crude material purified by PTLC with 6:4 hexanes/ethyl acetate. Product isolated as a clear colorless oil as a mixture of partially separable diastereomers (0.012g, 0.02582 mmol, 81%). Material used as a mixture of diastereomers, separated by PTLC for analytical purposes only.

^{1}H NMR (400 MHz, $CDCl_3$) δ0.617 (q, J=7.9 Hz, 6H), δ0.966 (t, J=7.9 Hz, 9H), 1.069 (s, 3H), 1.087 (s, 3H), δ1.352 (d, J=6.4 Hz, 3H), δ1.512-1.623 (m, 2H), δ2.140-2.214 (m, 1H), δ2.318-2.440 (m, 1H), δ2.776-2.846 (m, 1H), δ3.065-3.139 (m, 1H), δ3.573-3.649 (m, 4H), δ3.731 (s, 3H), δ5.455-5.492 (m, 1H), δ5.734 (d, J=16 Hz, 1H), δ7.057 (d, J=16 Hz, 1H), δ7.442 (bs, 1H).

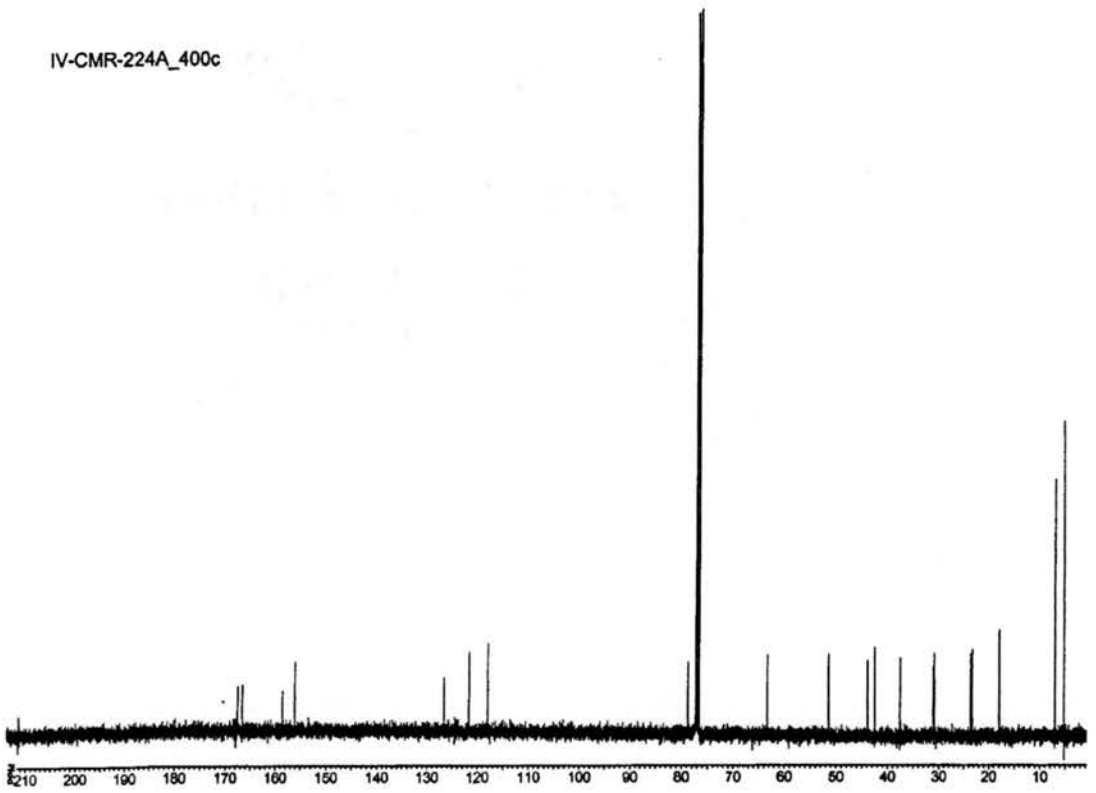
^{13}C NMR (100 MHz, $CDCl_3$) δ167.9, 166.9, 158.9, 156.4, 127.0, 122.0, 118.3, 79.1, 63.6, 51.8, 44.2. 42.8, 37.8, 31.4, 31.1, 37.8, 23.9, 23.5, 18.2, 7.2, 5.5.

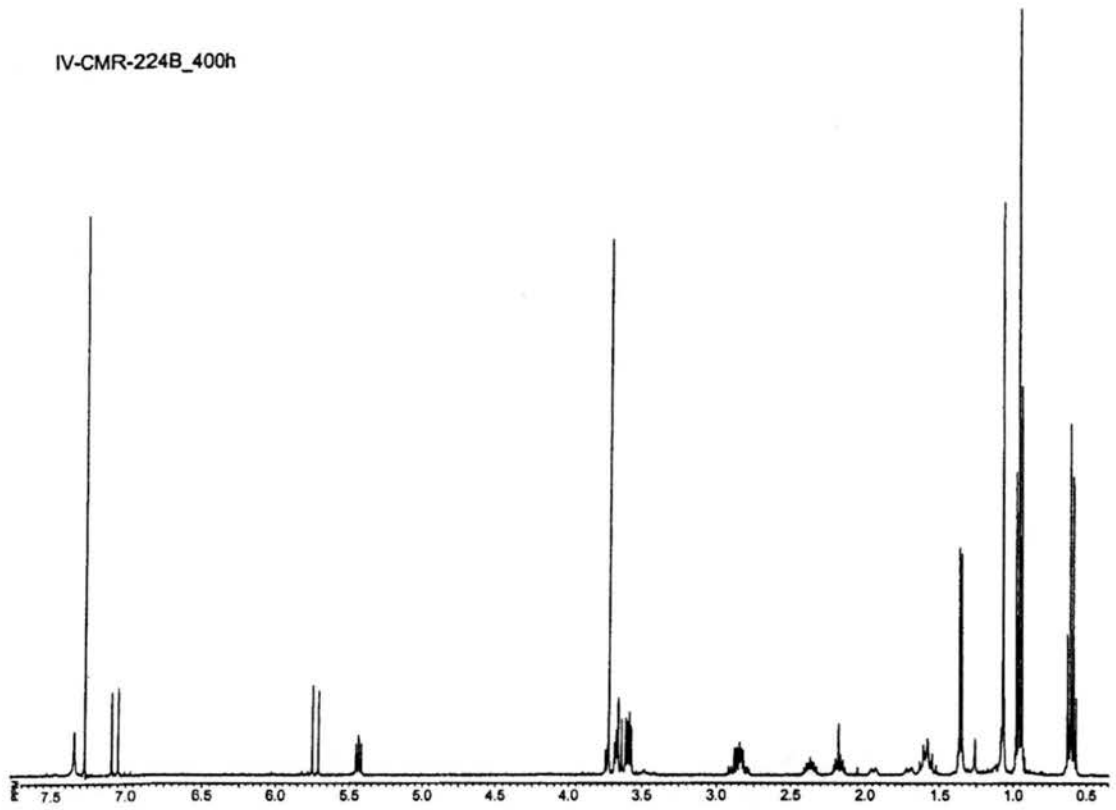
HRMS (FAB+) M+H calcd. 465.278476, Found 465.278601.

IV-CMR-224A_400h



IV-CMR-224A_400c

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4.0
3.5
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2.5
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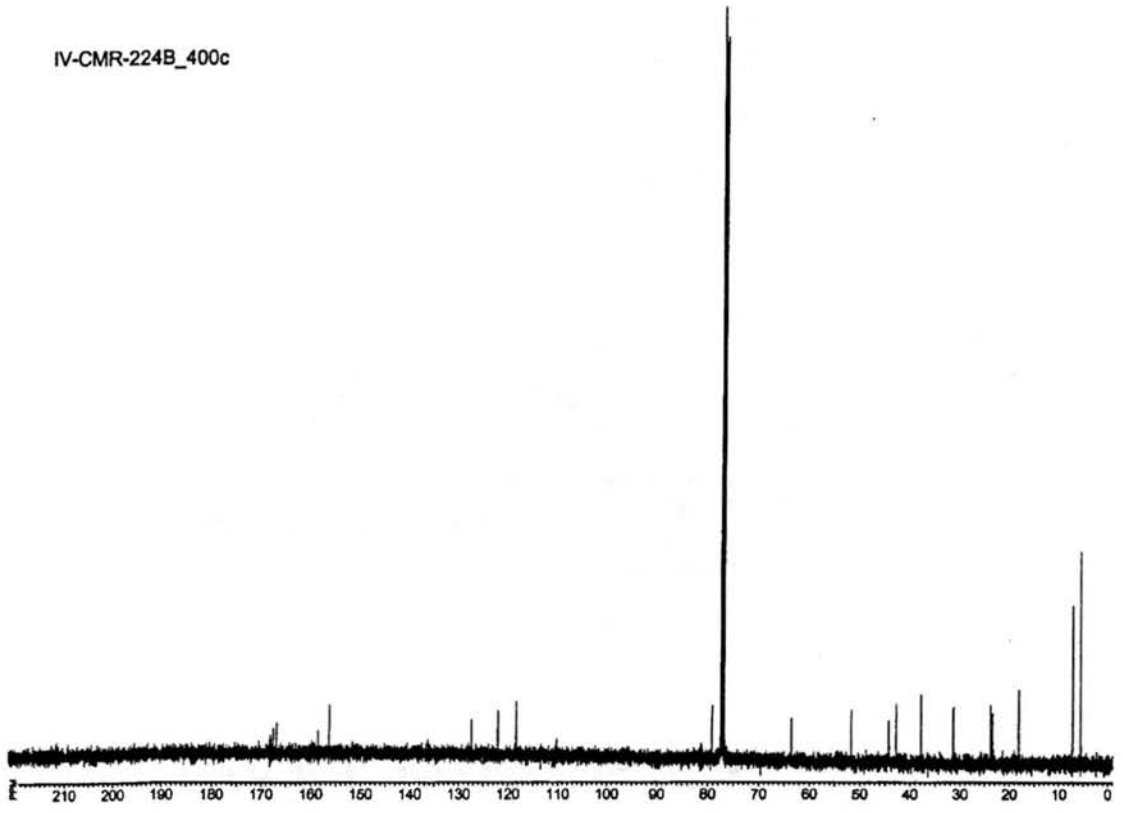
1.5
1.0
0.5


IV-CMR-224B_400c
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30
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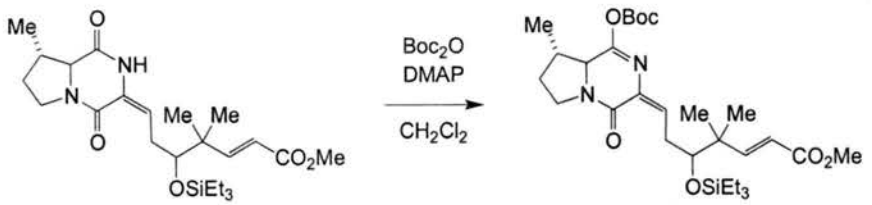
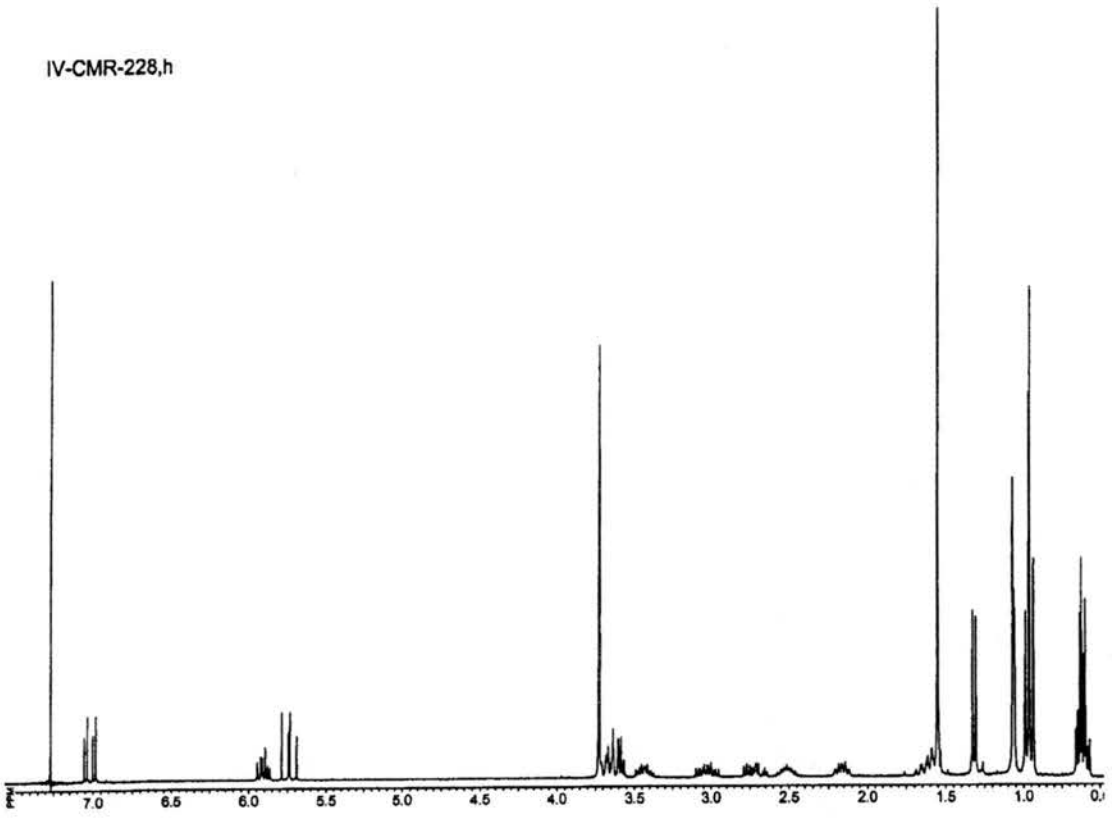
10
0



292 **293**

To a stirring solution of the DKP (0.078g, 0.1918mmol) in dry CH_2Cl_2 (2.0 mL) at 0°C was successively added DMAP (0.026 g, 0.2128 mmol, 1.1 eq) and Boc_2O (0.112g, 0.5132, 2.7 eq). After 30 min the reaction was concentrated by rotary evaporation. Purification on silica gel with 8:2 hexanes/EtOAc gave the product, a mixture of isomers, as a clear colorless oil (293, 0.074g, 0.1460 mmol, 76%; XX, 0.023g, 0.04539 mmol, 23%)


IV-CMR-228,h
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3.5
3.0
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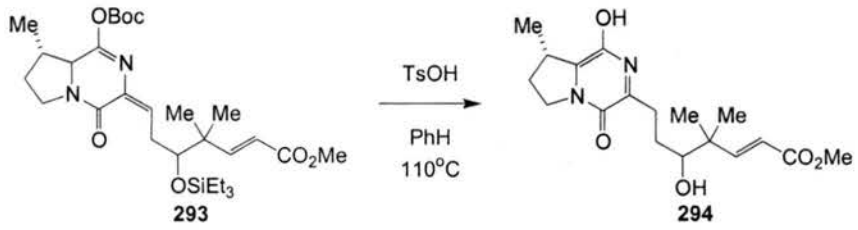
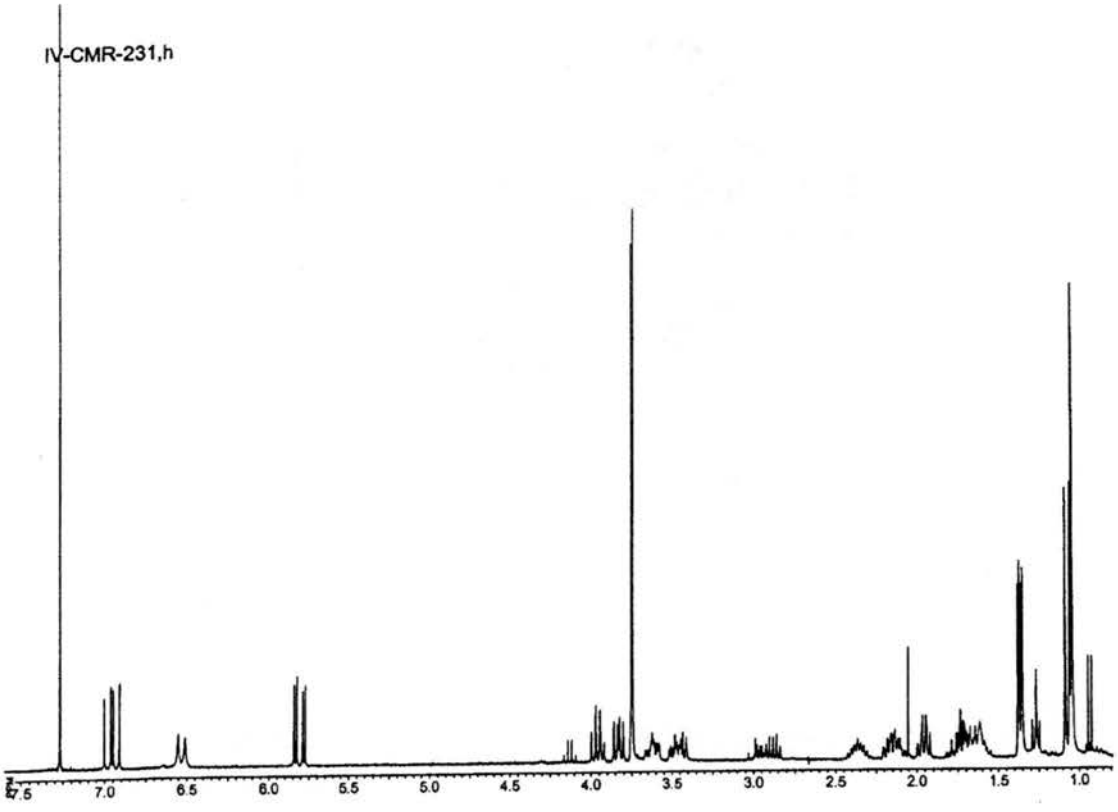
1.5
1.0



5-Hydroxy-7-(1-hydroxy-8-methyl-4-oxo-4,6,7,8-tetrahydro-pyrrolo[1,2-a]pyrazin-3-yl)-4,4-dimethyl-hept-2-enoic acid methyl ester (294): TsOH (0.004 g, 0.02130 mmol, 1 eq.) was added to a stirring solution of imino carbonate (293) in PhH (1 mL) at room temperature. The resulting solution was heated to 120°C in a sealed tube for 16h. Concentration by rotary evaporation followed by purification by PTLC with ethyl acetate gives the product as a clear colorless oil as a mixture of inseparable diastereomers (0.005g, 0.01427 mmol, 67%).

^{1}H NMR (300 MHz, CDCl3) δ1.044 (s, 6H), δ1.073 (d, J=8.1 Hz, 6H), δ1.355 (d, J=2.4 Hz, 3H), δ1.376 (d, J=2.3 Hz, 3H), δ1.550-1.819 (m, 7H), δ1.920-1.998 (m, 2H), δ2.077-2.208 (m, 3H), δ2.307-2.430 (m, 2H), δ2.839-3.033 (m, 2H), δ3.407-3.520 (m, 2H), δ3.581-3.670 (m, 2H), δ3.748 (s, 3H), δ3.757 (s, 3H), δ3.822 (d, J=10.1Hz, 1H), δ3.845 (d, J=10 Hz, 1H), δ3.919-3.999 (m, 2H), 5.901 (d, J=16.1 Hz, 1H), δ5.818 (d, J=16.1 Hz, 1H), δ6.513 (bs, 1H), δ6.554 (bs, 1H), δ6.938 (d, J=16.1 Hz, 1H), δ6.977 (d, J=16 Hz, 1H).

HRMS (FAB+) M+H calcd. 351.191997, Found 351.192243.


IV-CMR-231,h
7.5
7.0
6.5
6.0
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5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

Feeding Experiment with *A. japonicus* JV-23:

Each potato dextrose agar slant was streaked with *A. japonicus* JV-23 spores (50 μL of a 15% glycerol/water suspension). 500 mL of potato dextrose agar is prepared by boiling unwashed, cubed russet potatoes (150 g) in 500 mL dd water until cooked. Potatoes and broth filtered through cheesecloth and diluted to a volume of 500 mL as necessary. Broth is then combined with dextrose (10.0g) and agar (7.5g) and heated gently until all solids dissolve. The slants were then sterilized in an autoclave at 250°C for 45 minutes. After inoculation, the slants were incubated in the dark at 25 °C for 7-10 days.

The spores of two slants were shaken into a flask containing 400 ml sterile potato dextrose broth. One liter potato dextrose broth is prepared by boiling unwashed, cubed russet potatoes (300 g) in 1 L dd water until cooked. Potatoes and broth filtered through cheesecloth and diluted to a volume of 1L as necessary. Broth is then combined with dextrose (20.0g) and tryptose (3.0g) and heated gently until all solids dissolve. The broth was then sterilized in an autoclave at 250°C for 45 minutes. The inoculated flasks were incubated in the dark at 25°C for 4 days.

The potato dextrose broth was decanted from the flasks leaving a disk of *A. japonicus* JV-23. A sterile solution of the proposed biosynthetic precursor in trace element solution (see Table for the volume and molarity of each solution) was added by dripping it down the side of the flask and under the mycelia using a sterile syringe. Each labeled compound was added to six flasks. The flasks were left in the incubator another 14 days. The flasks were swirled daily to allow even distribution of the labeled compound.

The aqueous solution containing the precursor was decanted off. 1-2 mL methylene chloride was added to the solution which was then covered and stored at 4°C. The mycelia of each feeding experiment were combined with the mycelia of the duplicate experiment and pureed with 500 mL 1:1 MeOH/$CHCl_3$ in an Oster blender. The contents of the blender were poured into a 2L Erlenmeyer flask. The blender was rinsed three times with 1:1 MeOH/$CHCl_3$ and the suspension of mycelia cells was diluted to a volume of 1.2 L with 1:1 MeOH/$CHCl_3$. The mycelia cells were placed in a shaker at room temperature for 24 h.

Table:

Proposed Biosynthetic Precursor	Amount of Precursor (mmol)	Molarity of the Solution	Volume added to each flask (mL)	Amount of Asperparaline A produced (mmol)
[1,2-$^{13}C_2$]-acetate	14.3	2.38×10^{-2}	100	0.0139
[*methyl*-^{13}C]-L-methionine	0.999	1.66×10^{-3}	100	0.0147
[1-^{13}C]-L-isoleucine	1.01	1.68×10^{-3}	100	0.00556
[1-^{13}C]-L-tryptophan	0.731	1.22×10^{-3}	100	0.00862
[*indole*-2-^{13}C]-L-tryptophan	0.591	9.84×10^{-4}	100	0.00529
[3-^{13}C, $^{2}H_2$]-L-serine	1.11	1.85×10^{-3}	100	0.00390

Celite (30g) was added to the flask. The suspension was filtered through Whatman #2 paper. The filtrate was stored at 4°C. The residual celite and mycelia was suspended in 1.2 L 1:1 MeOH/$CHCl_3$ and shaken at room temperature for an additional 24 h.

The celite and mycelial suspension was filtered through Whatman #2 paper. The organic solvent from the combined filtrates was concentrated by rotary evaporation. The aqueous solution from the feeding experiment was combined with aqueous residue and the mixture was acidified to pH 3 with glacial acetic acid. The acidic solution was filtered through a pad of celite and extracted with ethyl acetate (400 ml x 3). The aqueous layer was made basic, pH 12, by the addition of 10% aqueous Na_2CO_3. The aqueous layer was extracted with ethyl acetate (450 mL x 4). The combined organic extracts from the basic extraction were washed with brine (800 mL), dried (Na_2CO_3) filtered and concentrated by rotary evaporation. The asperparaline A was purified by successive silica gel chromatography with 9:1 ethyl acetate/methanol and 95:5 chloroform/methanol. (See table for the yield of asperparaline isolated from each experiment.)

Calculation of the percent incorporation using ^{13}C NMR data:

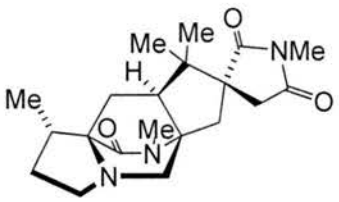
To interpret the integral results for a feeding experiment the integrals must be normalized to eliminate the effects of the nuclear Overhauser enhancements and different relaxation times that cause the difference in intensities of carbon signals. Since asperparaline has 20 carbon atoms, each signal should be ideally 1/20 = 5% of the total integral (except for the signal at 53.3 ppm which is the overlapping signal for both C16 and C20, and thus should be 10% of the total integral.) Dividing 5% by the real percentage of the total integral, calculates the correction factor. Multiplying the integral for the feeding experiment by the correction factor provides a corrected integral that now represents relative ^{13}C abundances that can be directly compared for different positions. To calculate the percentage of the total ^{13}C at each position, divide each corrected integral by the sum of the corrected integrals. The percentage of ^{13}C at each position

is determined by multiplying the percentage of the total ^{13}C in the molecule by the average ^{13}C percentage in the whole molecule (obtained from electrospray mass spectral data) by 1200 (as each of 100 molecules of asperparaline contains 20 carbon atoms) for each position. Enrichment is then calculated by subtracting the natural abundance of ^{13}C, which is 1.1%, from the percent of ^{13}C at each position.

Calculation of Percent Incorporation from Mass Spectra Data:

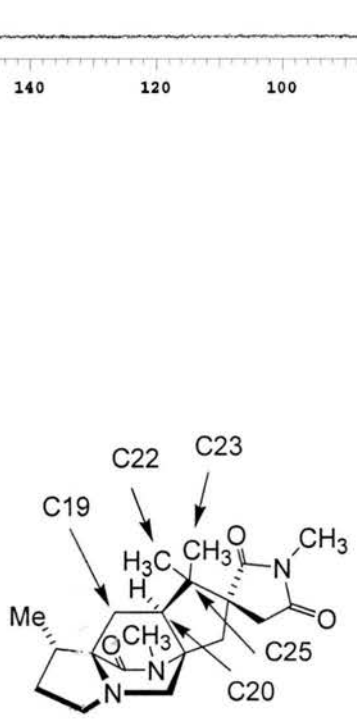
The percentage of ^{13}C-enrichment in the fungal metabolites from isotopically labeled biosynthetic precursors was calculated according to the method of Lambert et al.[64] These calculations are based on the comparison of the mass spectrum of the labeled material to the mass spectrum of the unlabeled material. For these experiments, electrospray mass spectroscopy was used, thus the base peak in the mass spectrum was the M+H peak.

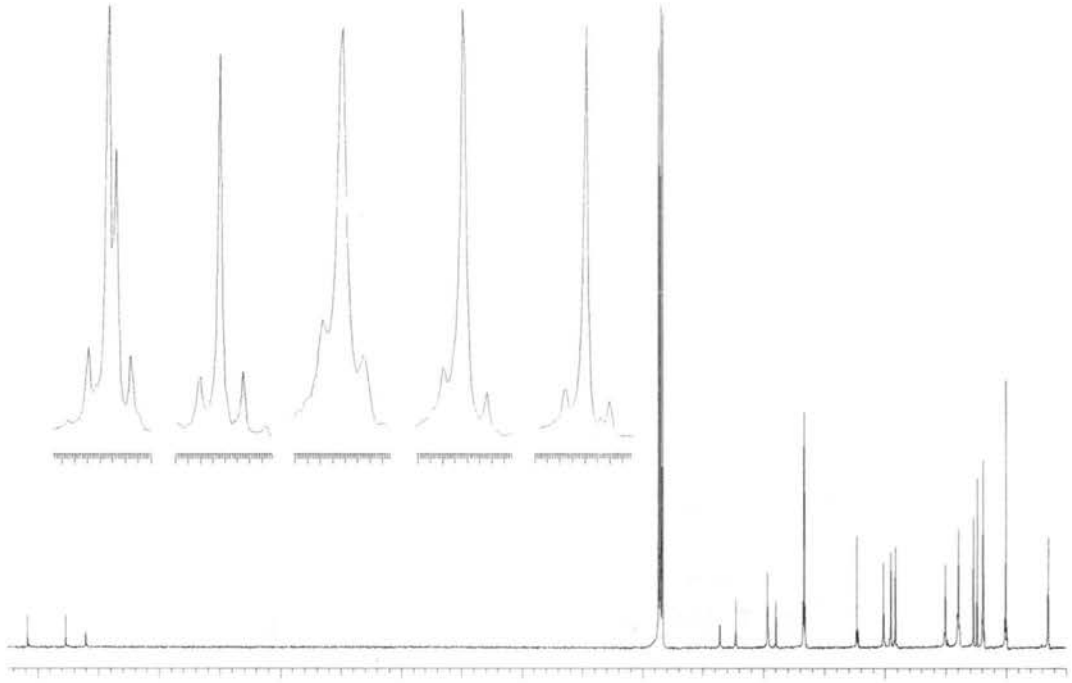
Authentic asperparaline A

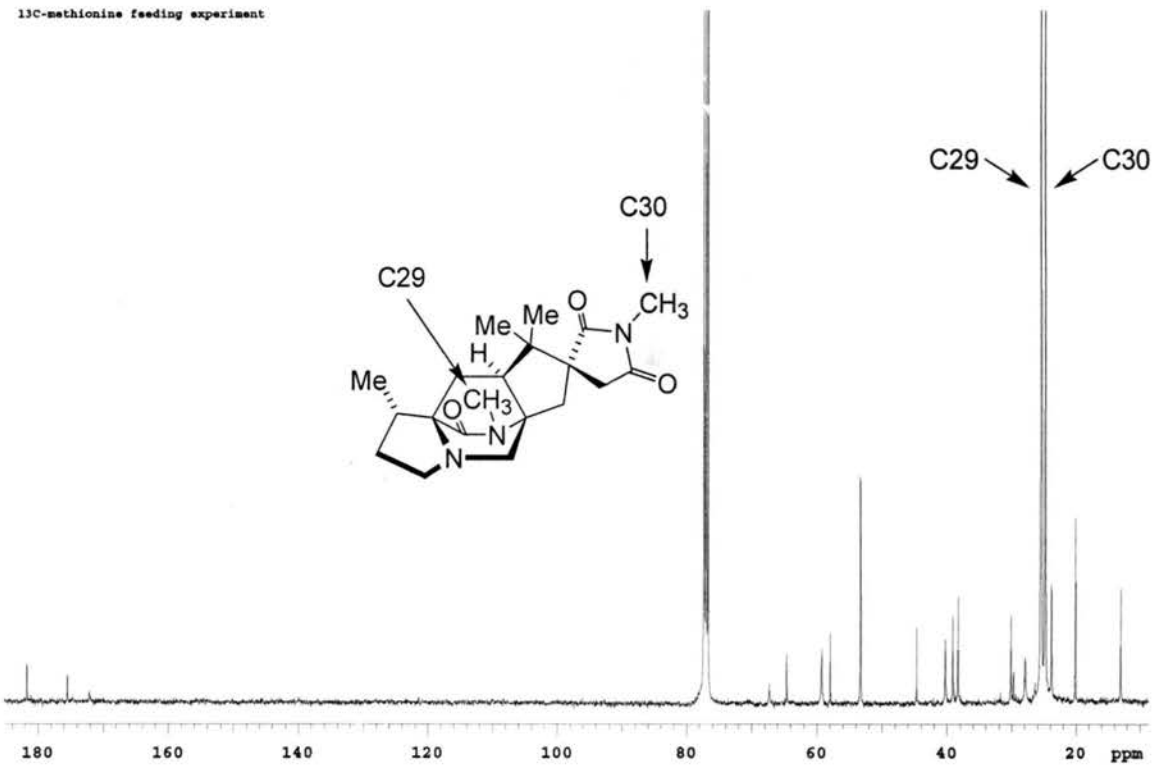


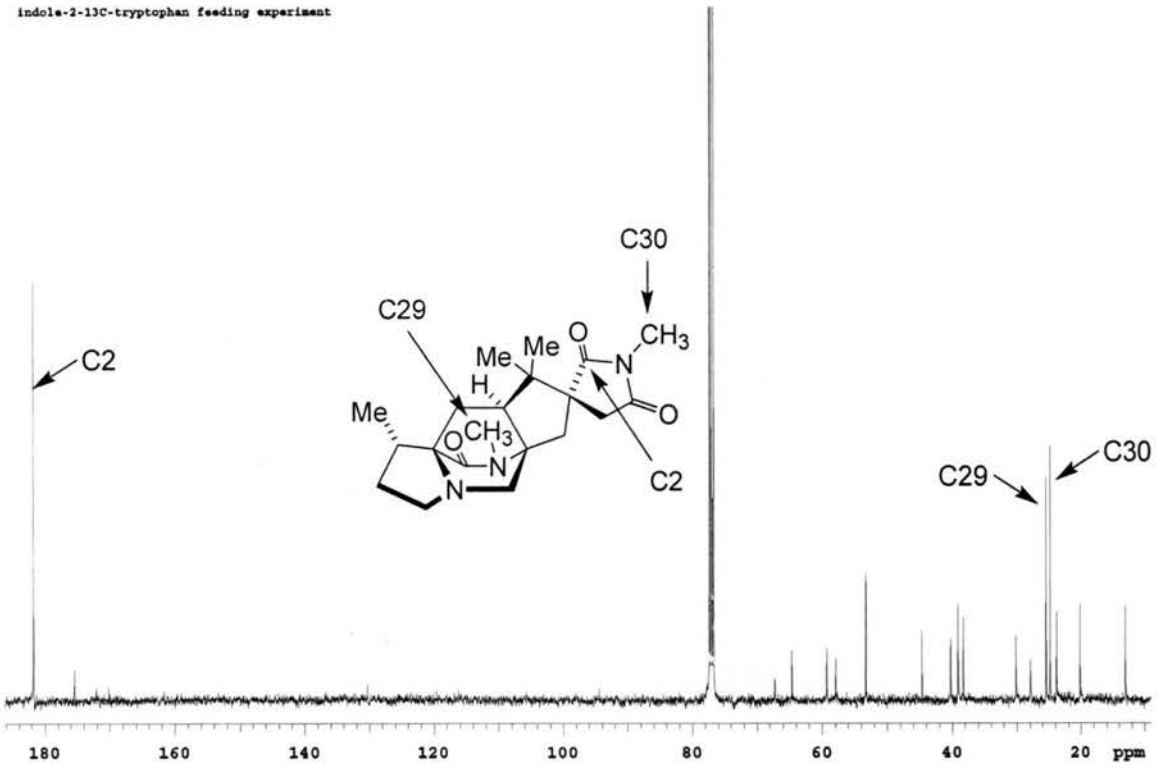
Unlabled Asperparaline A

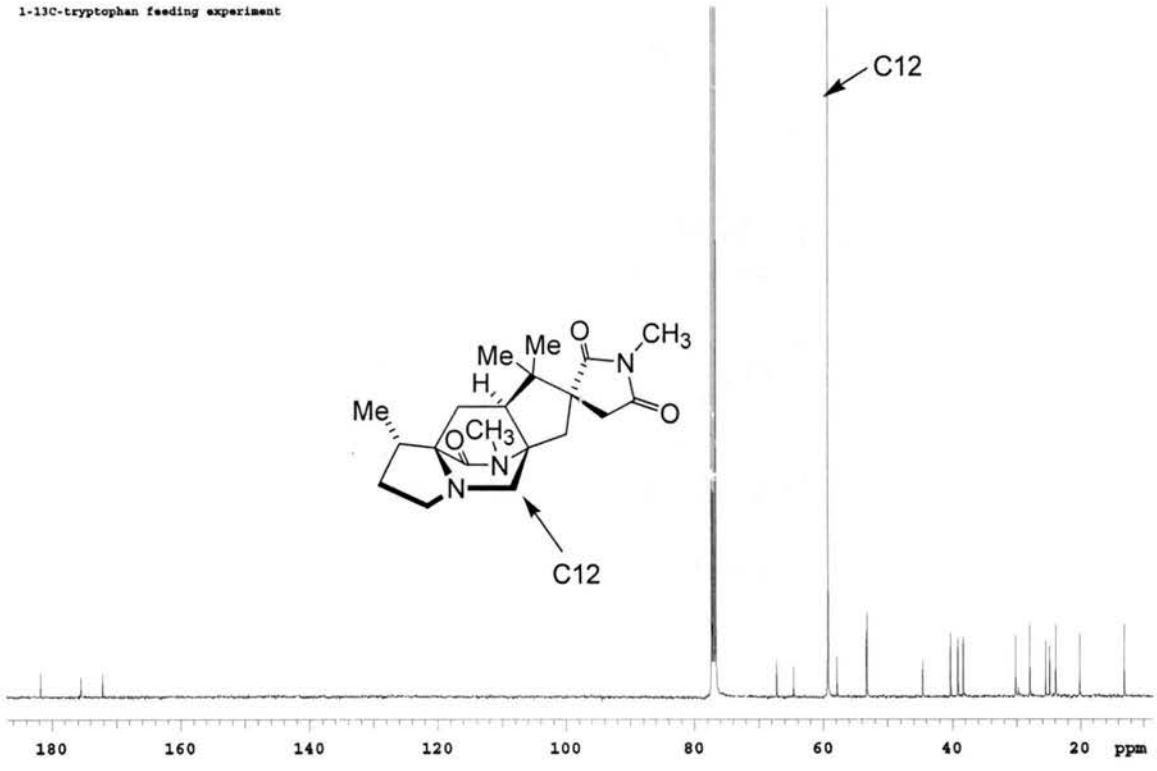
13C2-acetate feeding experiment

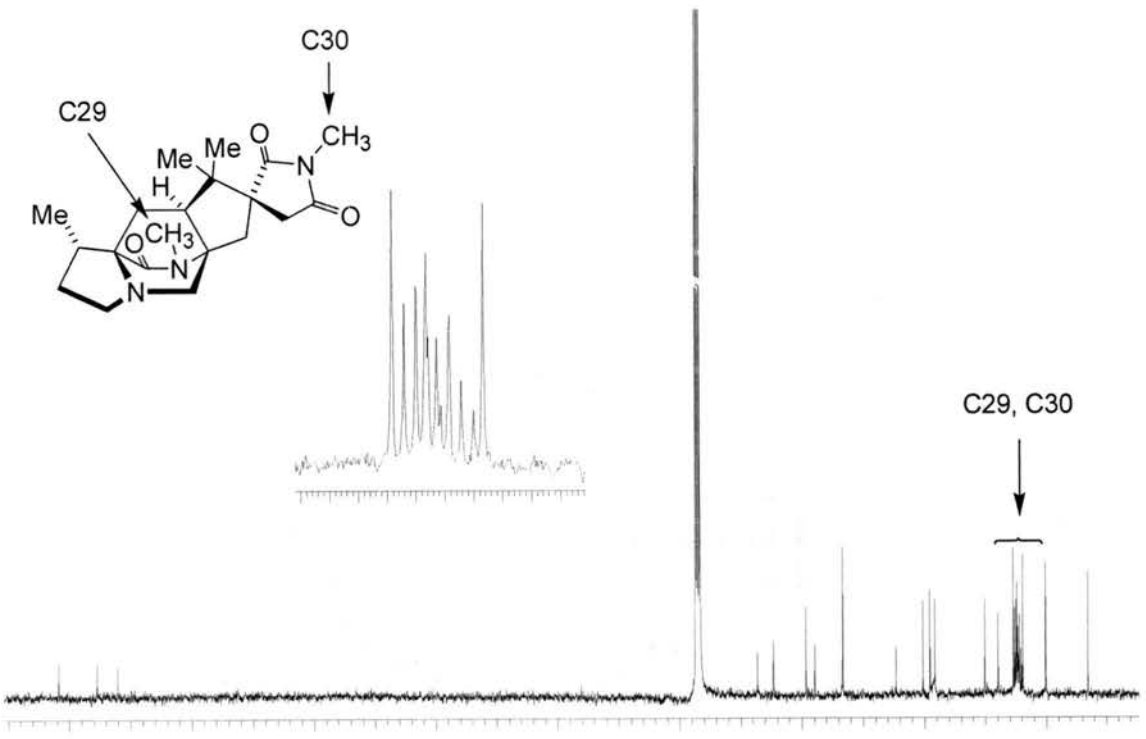





13C-methionine feeding experiment
C29
C30
C30
C29
Me
Me
H
Me
O
CH3
N
O
N
O
CH3
N
180
160
140
120
100
80
60
40
20
ppm


indole-2-13C-tryptophan feeding experiment
C30
C29
C2
Me
Me
H
Me
O
CH3
N
N
O
N
CH3
O
C2
C29
C30
180
160
140
120
100
80
60
40
20
ppm


1-13C-tryptophan feeding experiment
C12
Me
Me
H
Me
O
CH3
N
N
O
N
CH3
O
C12
180
160
140
120
100
80
60
40
20
ppm


C30
C29
Me
Me
H
Me
O
CH3
N
O
N
O
CH3
N
O
C29, C30

	^{13}C NMR Integral Values (uncorrected)																		
ppm	181.7	175.4	172.1	67.2	64.6	59.3	57.9	53.3	44.6	40.2	39.1	38.3	30.1	27.9	25.4	24.8	23.8	20.1	13.0
Corresponding carbon	2	8	18	13	11	12	3	20/16	25	14	10	9	15	19	29	30	22	23	17
Precursor																			
None (control)	1.06	1.06	1.00	1.88	2.15	5.06	1.81	9.68	2.03	4.67	4.98	5.13	4.76	4.67	3.61	3.04	4.93	4.76	4.37
[1,2-$^{13}C_2$]-acetate*	1.01	1.16	1.00	1.72	1.74	4.36	1.22	14.65	4.54	4.39	4.98	4.55	5.25	10.78	5.30	4.98	10.88	10.38	4.53
	0.96	1.27	1.00	1.07	1.76	3.33	1.33	11.17	4.15	3.80	4.07	3.71	4.09	8.68	4.31	4.22	8.64	7.98	3.49
[*methyl*-^{13}C]-L-methionine	1.65	1.34	1.00	1.26	2.19	5.36	1.95	13.18	2.53	5.60	6.63	6.43	6.42	6.81	140.57	125.73	10.01	7.16	6.61
[1-^{13}C]-L-isoleucine	0.11	0.30	1.00	0.36	0.42	0.71	0.18	1.33	0.36	0.56	0.53	0.64	0.49	0.55	0.40	0.46	0.64	0.47	0.78
	0.17	0.27	1.00	0.35	0.39	0.84	0.32	1.36	0.39	0.67	0.80	0.69	0.73	0.66	0.51	0.47	0.75	0.74	0.61
[1-^{13}C]-L-tryptophan	0.87	1.40	1.00	0.82	1.82	41.73	2.03	10.41	2.20	4.95	5.51	5.81	6.82	5.33	5.67	4.32	6.23	6.21	5.73
	1.15	1.43	1.00	1.87	1.78	40.84	2.02	8.54	2.05	4.21	4.48	3.97	4.75	4.44	3.69	3.31	4.33	4.21	4.13
[*indole*-2-^{13}C]-L-tryptophan	24.41	2.20	1.00	1.92	3.11	7.23	3.64	14.61	2.75	7.85	10.91	6.30	7.69	6.63	18.57	14.30	7.13	7.87	5.93
[3-^{13}C,$^{2}H_2$]-L-serine	1.62	1.51	1.00	3.19	2.93	3.98	1.36	12.07	2.37	5.04	5.75	8.25	5.14	5.85	18.36	22.41	5.71	7.89	5.07

Precursor	**Relative m/z intesity - from electrospray mass spectrometry**										**MS Incorporation (%)**				**Calculated Enrichment**
	360	**361**	**362**	**363**	**364**	**365**	**366**	**367**	**368**	**369**	**$^{13}C_1$**	**$^{13}C_2$**	**$^{13}C_3$**	**Avg.**	
None (control)	100	23.89	3.30	--	--	--	--	--	--	--					
[1,2-$^{13}C_2$]-acetate*	100	31.10	8.12	1.96	--	--	--	--	--	--	6.5	2.8	0.9	1.8	1.8%
	100	31.37	7.76	1.65	--	--	--	--	--	--	6.8	2.4	--	1.7	1.7%
[*methyl*-^{13}C]-L-methionine	100	55.60	36.29	6.06	--	--	--	--	--	--	20.4	16.3	--	3.5	25% (C29); 27% (C30)
[1-^{13}C]-L-isoleucine	100	26.48	4.98	--	--	--	--	--	--	--	2.5	1.0	--	1.3	5.8% (C18)
	100	26.36	4.56	1.10	0.29	--	--	--	--	--	2.4	0.6	0.8	1.4	5.7% (C18)
[1-^{13}C]-L-tryptophan	100	28.12	4.29	0.72	--	--	--	--	--	--	4.0	--	0.6	1.4	6.4% (C12)
	100	29.02	4.69	0.44	--	--	--	--	--	--	4.9	0.2	0.2	1.4	7.2% (C12)
[*indole*-2-^{13}C]-L-tryptophan	100	35.41	7.43	0.86	--	--	--	--	--	--	10.2	1.2	0.1	1.7	12.2% (C2); 1.9% (C29); 1.6% (C30)
[3-^{13}C,$^{2}H_2$]-L-serine	100	23.45	5.52	10.76	2.73	1.12	1.68	0.49	0.18	0.16	9.6	1.5	0.1	1.7	3.9%(C29); 6.1% (C30)

* NMR integral analysis for the incorporation of [1,2-$^{13}C_2$]-acetate was not performed because incorporation was low and the integrals were difficult to determine. The position of the incorporated label was determined by the presence of doublets surrounding the carbon signals, indicative of ^{13}C-^{13}C coupling interactions, corresponding to carbon signals in the unlabeled compound. The Enrichment calculation for [1,2-$^{13}C_2$]-acetate was determined solely from mass spectral data.

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