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Development of novel synthetic methodologies and their use in approaches for the preparation of lactacystin, lepadiformine and a Giardia lamblia fructose-1,6-bisphosphate aldolase inhibitor

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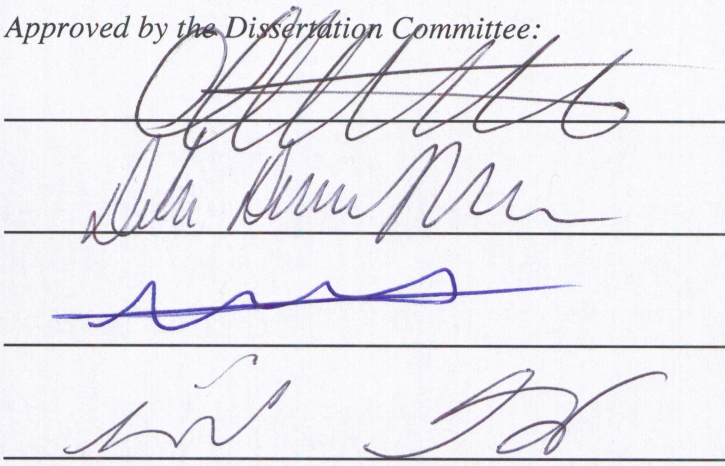
This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:



, Chairperson

Development of Novel Synthetic Methodologies and Their Use in Approaches for the Preparation of Lactacystin, Lepadiformine and a *Giardia Lamblia* Fructose-1,6-bisphosphate Aldolase Inhibitor

BY

Jiwen Zou

B.S., Chemistry, Beijing University, 1989
M.S., Organic Chemistry, Beijing University, 1992

DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy

Chemistry

The University of New Mexico
Albuquerque, New Mexico

December, 2009

DEDICATION

This dissertation is dedicated to my parent.

Yifang Zou and Guinan Fu

ACKNOWLEDGMENTS

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ABSTRACT OF DISSERTATION

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ABSTRACT

The investigations carried pot in my doctoral studies focus on (1) applications of pyridinium salt photochemical and tandem ene-yne-ene metathesis reactions to the synthesis of the natural products (+)-lactacystin and lepadiformine C, and (2) the preparation of a novel inhibitor for the class II *Giardia lamblia* fructose-1,6-bisphosphate aldolase.

New developments in the area of pyridinium salt photochemistry were explored in the context of approaches to the natural product (+)-lactacystin. The results of this effort show that irradiation of a substituted 1,2-cyclopenta-fused pyridinium salt in aqueous solution followed by treatment with sodium

bicarbonate leads to selective production of an unusual, structural and functional complex, and stereodefined tetracyclic carbamate. This substance contains structural features and a functionality array that would make it applicable as a late stage intermediate in a synthesis of the proteosome inhibitor, lactacystin.

A novel strategy was developed for the synthesis Lepadiformine C, a structurally interesting, biologically active marine alkaloid derived from the marine organism *Claveline Moluccensis*. that has a tricyclic structure. The approach, which begins with L-proline, relies on implementation of a ruthenium-alkylidene catalyzed, tandem ene-yne-ene metathesis process to construct the tricyclic structure.

Class I and class II fructose-1,6-bisphosphate aldolases (FBPA), enzymes that exhibit no amino acid sequence homology and utilize different catalytic mechanisms, promote the retro-aldol conversion of fructose-1,6-bisphosphate (FBP) to dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate as part of energy producing glycolysis pathways in bacteria, protests and humans. The mammalian class I FBPA employs a Schiff base mechanism, involving an active site lysine amine group, whereas the parasitic protozoan *Giardia lamblia* relies on a class II FBPA that utilizes an active

site Zn^{+2} to stabilize the forming enolate of dihdroxyacetone phosphate. One subgoal of my studies was to develop a novel strategy for the design of inhibitors of the class II *Giardia lamblia* FBP (*gl*FBPA). As part of an overall effort in this area, potential inhibitors that possess Zn^{+2} binding 3-hydroxy-2-pyridone moieties were designed and prepared. The inhibitory properties of these substances against *gl*FBPA were determined. The results show that the structure-based inhibitor design is effective in identifying new 3-hydroxy-2-pyridone based *gl*FBPA inhibitors that have modestly tight (low micromolar) binding affinities.

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LIST OF ABBREVIATION

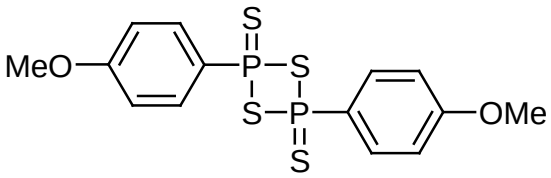
Organic Chemistry Acronyms:

Ac	Acetyl
AIBN	Azobis(isobutyronitrile)
Ar	Aryl
Bn	Benzyl
Boc	t-Butyloxycarbonyl
Bu	Butyl
Bz	Benzoyl
Cbz	Carbonbenzyloxy
CSA	Camphorsulfonic acid
Cy	Cyclohexyl
DBU	1,8-Diazabicycloundec-7-ene
DCC	Dicyclohexyl carbodiimide
DEAD	Diethyl azodicarboxylate
DMAP	N,N-Dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	Dimethyl Sulfoxide
ee	Enantiomeric excess
Et	Ethyl

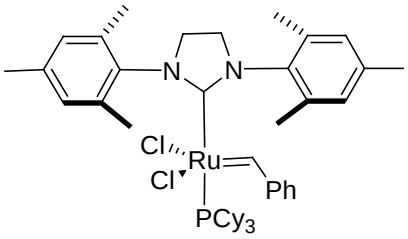
HMPA	Hexamethylphosphoramide
LDEA	Lithium Diethylamide
Me	Methyl
MCPBA	Meta-Chloroperoxybenoic acid
MOM	Methoxymethyl
Ms	Methanesulfonyl
NMO	N-Methylmorphorine-N-oxide
NMP	N-Methylpyrrolidone
PCC	Pyridinium Chlorochromate
Ph	Phenyl
Pr	Propyl
PTSA	p-Toluenesulfonic acid
Py	Pyridine
TBAF	Tetra-n-Butylammonium Fluoride
TBS	t-Butyldimethylsilyl
TEA	Triethylamine
Tf	Triflate
TFA	Trifluoroacetic acid
THF	Tetrahydronfuran
TIPS	Triisopropylsilyl
Ts	Tosyl

Named Reagents:

Lawesson's Reagent



Grubbs II catalyst



Wittig Reagent $Ph_3P{=}CR_2$

Spectroscopy Acronyms:

MS	Mass Spectrum
NMR	Nuclear Magnetic Resonance
J	Coupling Constant(NMR)
DEPT	Distortionless enhancement by polarization transfer(NMR)
COSY	Correlation Spectroscopy(NMR)

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CHAPTER ONE

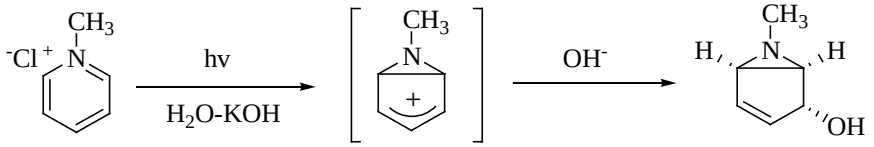
A Photochemical Approach to the Synthesis of (+)-Lactacystin

(Adapted from Jiwen Zou, Maozhen Gong, Patrick S. Mariano and Ung Chan Yoon. *Bull.Korean Chem.Soc.* **2008**, 29(1), 89-93.)

1.1 Background. Pyridinium Salt Photochemistry.

In the early 1970's, Wilzbach and his coworkers[1] first reported that irradiation of N-methylpyridinium chloride in an aqueous basic solution results in stereoselective formation of the bicyclic aziridine **1** (Scheme 3).

Scheme 3

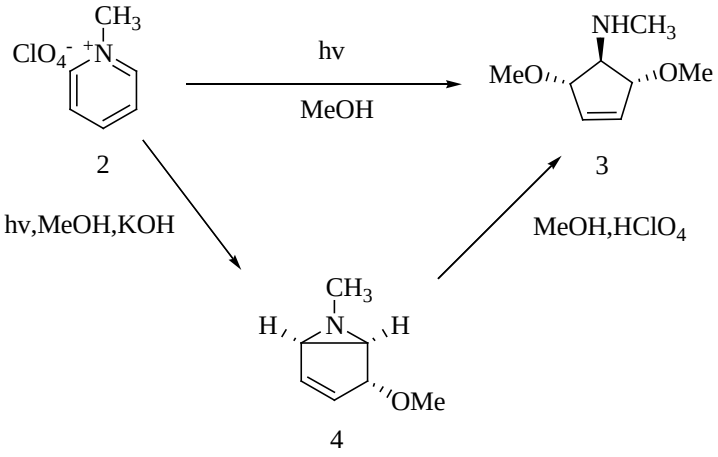


1

This remarkable transformation had gone relatively unnoticed since the time of its discovery. The only other early observations of this type of reactivity of pyridinium salts were made by Mariano and his coworkers[2] in the 1980's. In the course of a broad investigation of N-heteroarmatic salt single electron transfer photochemistry, Mariano and his coworkers found that irradiation of methanol solutions of N-methyl- or N-allyl- pyridinium perchlorate **2** results in efficient production of the trans,trans-aminocyclopentenes **3**. In addition, the bicyclic aziridine photoproducts **4**, formed by irradiation of these salts in basic methanol solutions, were found to

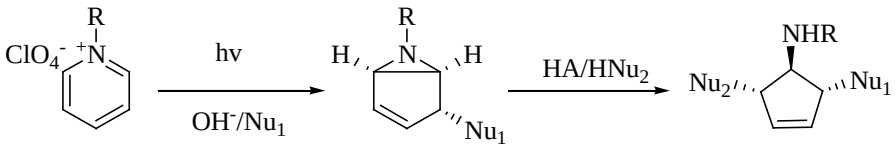
react with methanol under acid catalyzed conditions to produce the corresponding trans,trans-aminocyclopentenes **3** (Scheme 4).

Scheme 4



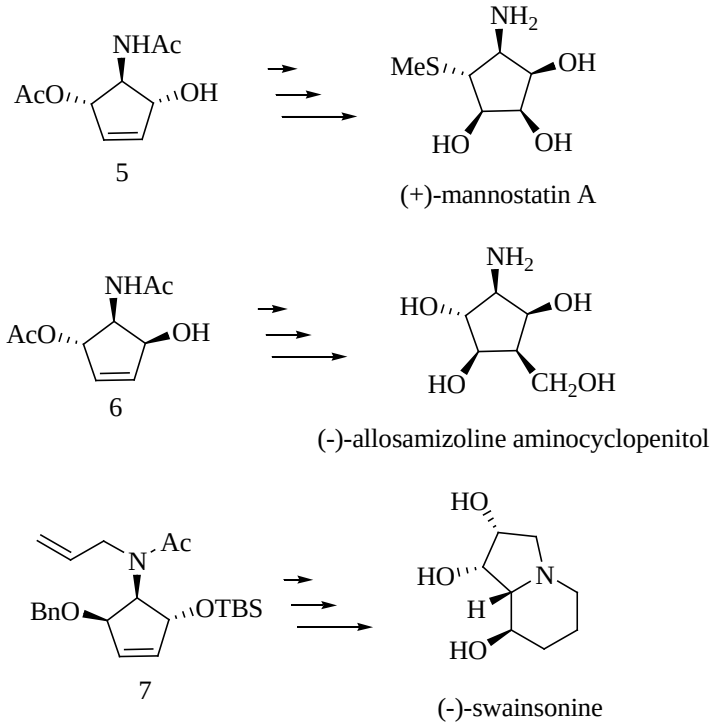
In the 1990s, Mariano and his coworkers[3] investigated the potential synthetic power of tandem sequences, involving (1) photoinduced cyclization of pyridinium cations coupled with stereocontrolled nucleophilic addition to produce bicyclic aziridines, and (2) stereocontrolled nucleophilic cleavage of bicyclic aziridine to produce highly functionalized aminocyclopentenes. These efforts demonstrated that N-alkylpyidinium perchlorates undergo photocyclization reactions in a number of polar nucleophilic solvents, such as water and methanol, to efficiently produce bicyclic aziridines. The bicyclic aziridine photoproducts react with a number of different nucleophiles (e.g. H_2O, MeOH , AcOH, AcSH) under acid catalyzed conditions to produce aminocyclopentenes (Scheme 5). The aziridine ring opening processes are both regioselective and stereoselective, yielding trans, trans-3,4,5-trisubstituted cyclopentenes exclusively as a consequence of the operation of a SN_2 mechanism.

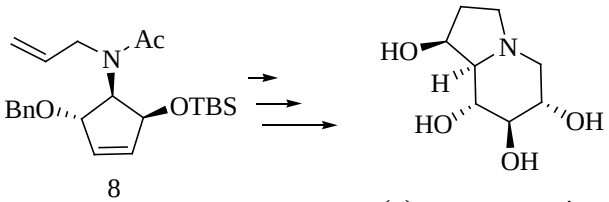
Scheme 5



In explorations of synthetic applications of pyridinium salt photochemistry, Mariano and his coworkers showed that this new methodology can be used for the synthesis of (+)-mannostatin A[4], (-)-allosamizoline aminocyclopenitol[5], (-)-swainsonine[6] and (+)-castanospermine[6] (Scheme 6).

Scheme 6

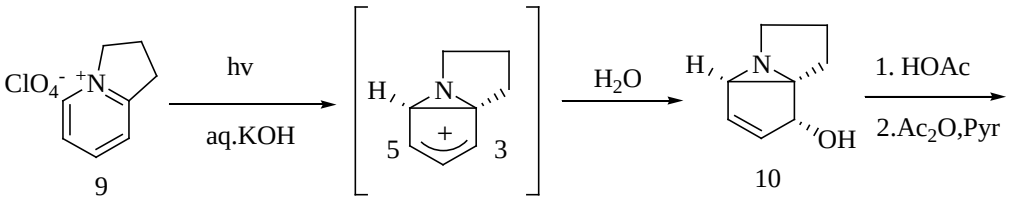




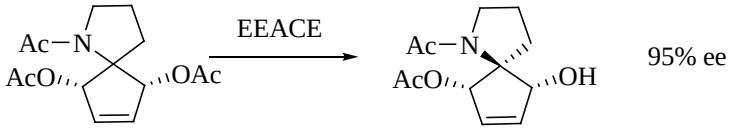
8 (+)-castanospermine

In more recent studies, Mariano and his coworkers[7] investigated the potential synthetic power of 1,2-cyclopenta- and 1,2-cyclohexa-fused pyridinium salt photochemistry. They discovered that irradiation of 1,2-cyclopenta-fused pyridinium perchlorate **9** leads to selective production of the tricyclic allylic alcohol **10** involving unusual nucleophilic addition at the C-3 position rather than the C-5 position of the intermediate allylic cation. Acetic acid induced ring opening of the tricyclic allylic alcohol **10**, followed by peracetylation results in clean formation of spirocyclic amido-diester **11** (Scheme 7). This two-step procedure serves as a highly effective method to construct the spirocyclic compound **11**, a meso-diester that is readily desymmetrized by enantioselective enzymatic hydrolysis with electric eel acetyl cholinesterase to yield the enantio-monoalcohol **12** (>95% ee following crystallization).

Scheme 7



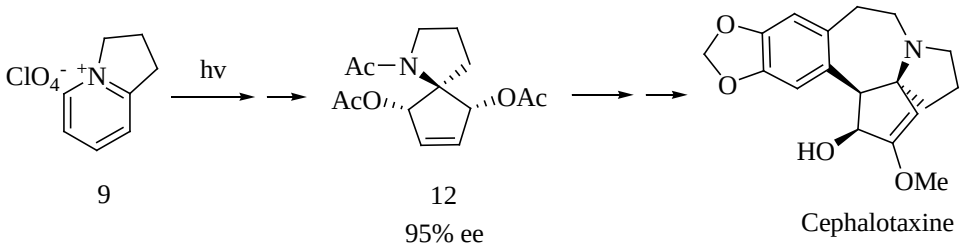
9 10



11 12

In investigations aimed at showcasing the synthetic potential of this chemistry, Mariano and his coworkers[8] completed a formal total synthesis of (+)-cephalotaxine (Scheme 8).

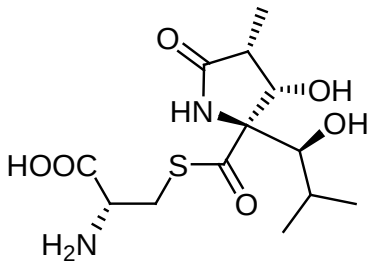
Scheme 8



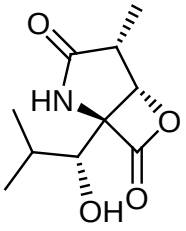
9 12 95% ee Cephalotaxine

1.1 Background. (+)-Lactacystin.

(+)-Lactacystin is a potent and selective proteasome inhibitor that was isolated from *Streptomyces sp.* by Mura.[9] The cell-permeable, biologically active form derived from lactacystin is (-)-*clasto*-lactacystin[10], which is generated by lactonization with elimination of cystein. The highly strained β-lactone of (-)-*clasto*-lactacystin can acylate the N-terminal threonine of the proteasome, a crucial amino acid for protease activity.[11] Thus, lactacystin is a proteasome inhibitor that operates by forming a covalent adduct in the active site of the enzyme.[12]



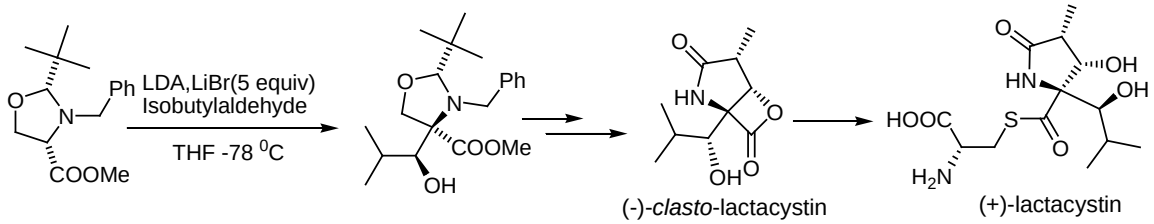
(+)-lactacystin



(-)-*clasto*-lactacystin

The first total synthesis of lactacystin was achieved by Corey[13] in 1992 (Scheme 9). Subsequently, two different synthetic routes for the preparation of lactacystin were reported by the Corey group.[14]

Scheme 9

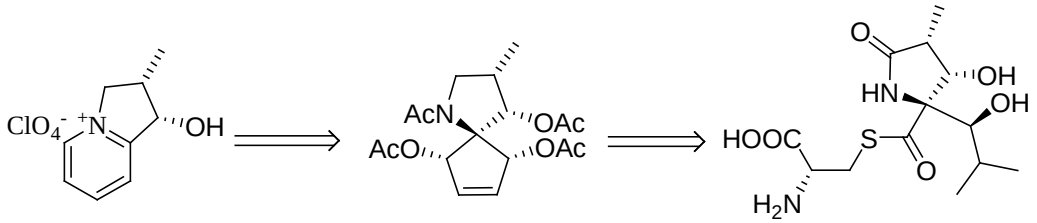


Other approaches have been employed to prepare (+)-lactacystin that rely on a broad array of modern synthetic strategies and methodologies. Syntheses of this target and related compounds have been thoroughly reviewed recently by Shibasaki.[15]

1.2 Model Reactions Probing a Novel Route for the Total Synthesis of (+)-Lactacystin.

As mentioned above, irradiation of 1,2-cyclopenta-fused pyridinium perchlorate **9** leads to selective production of the tricyclic allylic alcohol **10**. In the synthetic plan developed for preparation of (+)-lactacystin shown in Scheme 18, we anticipated that a substituted 1,2-cyclopenta-fused pyridinium perchlorate **28** could serve as the starting material for photochemical-aziridine ring opening sequence leading to formation of the spirocyclic aminocyclopentene **31**. We envisaged that **31** would serve as a key intermediate in a (+)-lactacystin preparative route (Scheme 10).

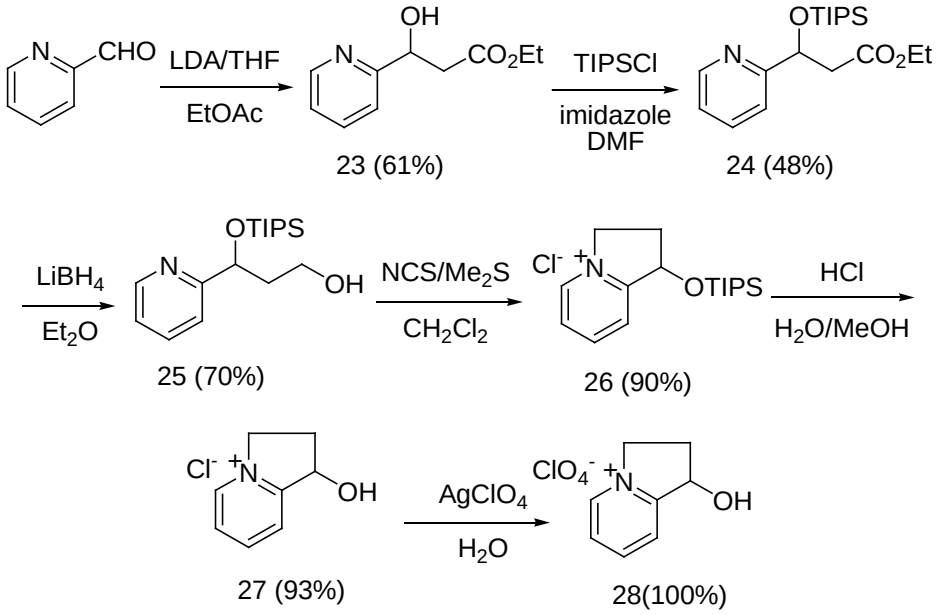
Scheme 10



28 31 (+)-lactacystin

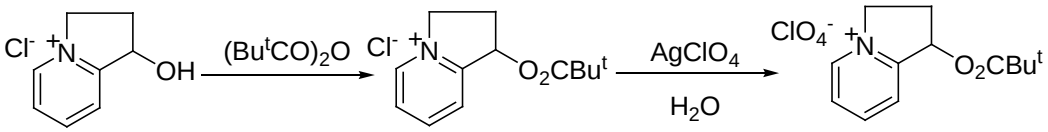
In order to test this strategy, the model substituted 1,2-cyclopenta-fused pyridinium perchlorate **28** was prepared starting with 2-pyridinecarboxaldehyde. Aldol reaction of the ethyl acetate enolate with 2-pyridinecarboxaldehyde, followed by TIPS protection of the alcohol **23** and reduction of ester group in **24** by lithium borohydride gave alcohol **25**. Cyclization of **25** was accomplished by using a Corey-Kim type reaction in good yield. After ion exchange of **27** with silver perchlorate, the desired pyridinium salt **28** was produced (Scheme 11).

Scheme 11



The trimethylacetyl protected pyridinium salt **30** was prepared for photochemical studies by using the sequence shown in Scheme 12.

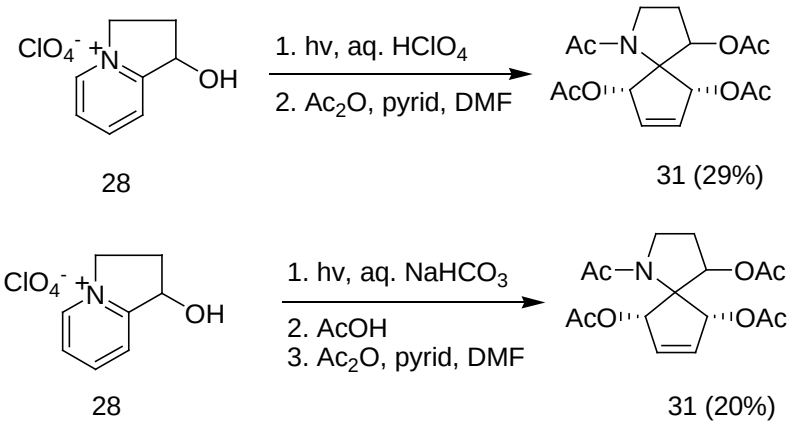
Scheme 12



27 29 (80%) 30 (100%)

A number of acidic and basic conditions for conducting the photochemical reaction of **28** were screened. Irradiation of an aqueous perchloric acid solution of **28**, followed by acetylation led to formation of spirocyclic product **31** in a 29% yield (Scheme 21). Similarly, irradiation of an aqueous sodium bicarbonate solution of **28**, followed by acetic acid promoted aziridine ring opening and peracetylation, led to isolation of **31** in a 20% yield (Scheme 13).

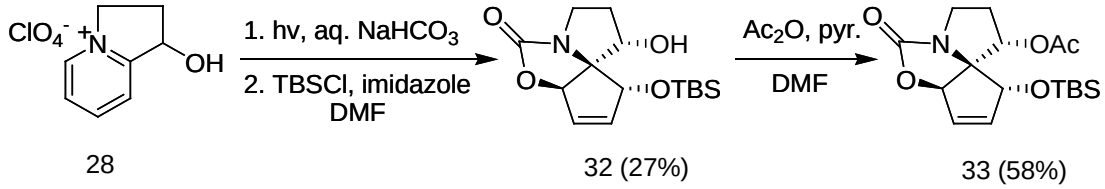
Scheme 13



However, a surprising result came from studies of the photoreaction of **28**, promoted by irradiation of in an aqueous sodium bicarbonate solution, followed by treatment of the crude photolysate with t-butyldimethylsilyl

chloride in order to form less polar and more chromatographically amenable alcohol protected products. In this case, the unusual ring opened, tricyclic cyclic carbamate product **32** was produced in a 27% yield (Scheme 14). X-crystallographic analysis (Figure 1) showed that **32** is generated with a remarkably high level of stereochemical control.

Scheme 14



28 32 (27%) 33 (58%)

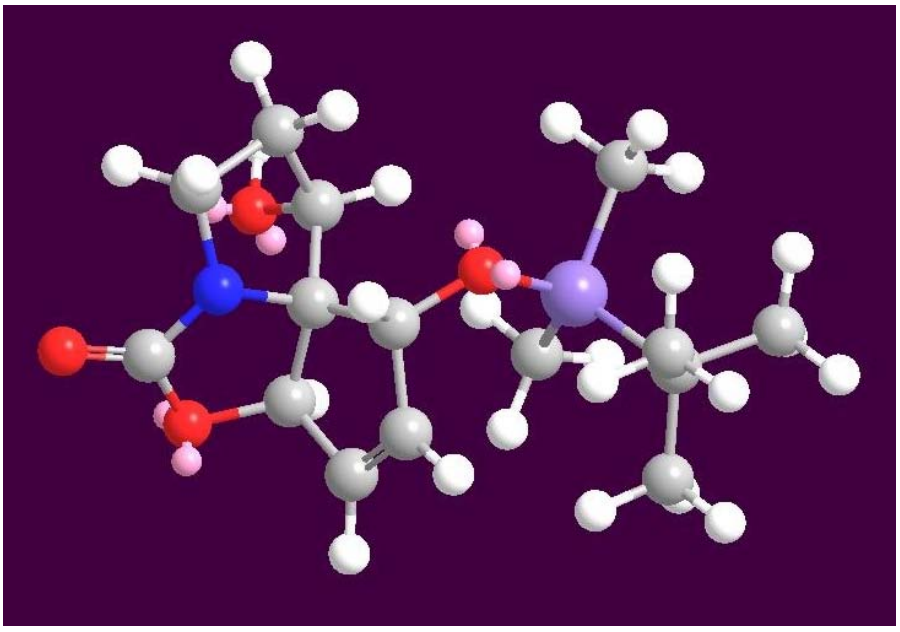
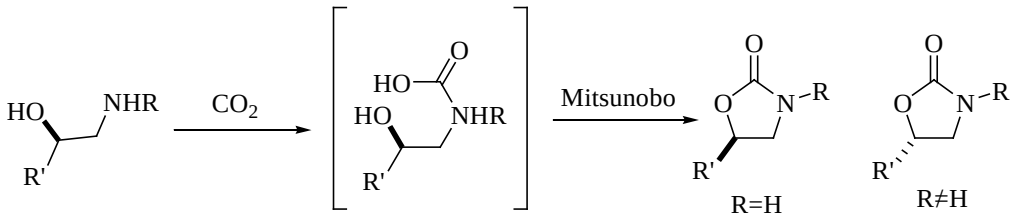


Figure 1. Chem-3D plot of the X-ray structure of **32**

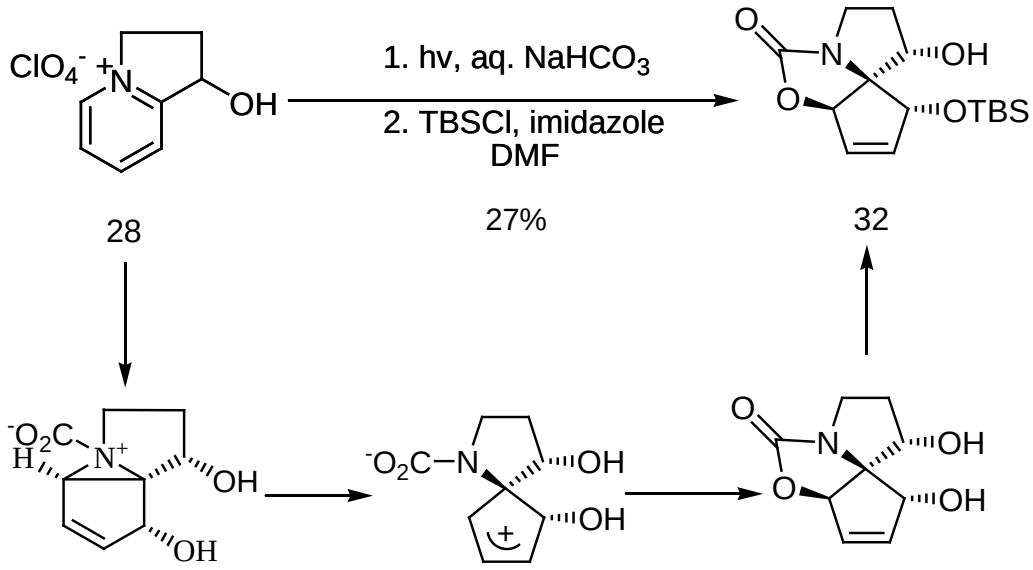
The formation of **32** can be rationalized on the basis of a carbon dioxide promoted carboxylation reaction of amines, described earlier by a Merck group (Scheme 15).[16] In our case, it is possible that CO_2, liberated during the photochemical process by decomposition of sodium bicarbonate with acid, reacts with the initially formed photoproduct to form an ammomium

carboxylate, which then undergoes ring opening and carbamate formation to generate **32** (Scheme 16).

Scheme 15



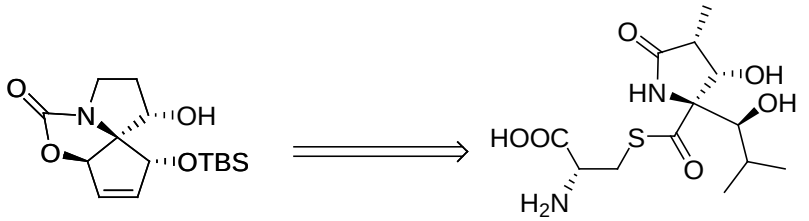
Scheme 16



It is interesting that the photochemical process to form **32** would be compatible with the approach designed for synthesis of (+)-lactacystin (Scheme 17). Specificaaly, although the yield of the process is low, **32** contains structural features and functionality that would enable stereocontrolled installation of the pyrrolidone ring and side chains of the target. However, we decided to abandon this project since the less than

acceptable yield of the photoreactions would make execution of a multistep synthetic sequence difficult.

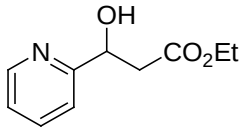
Scheme 17



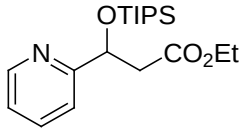
32 (+)-lactacystin

1.3 Experimental

General. Unless otherwise noted, all reagents were obtained from commercial sources and used without further purification. All compounds were isolated and shown to be >90% pure by ^{1}H and/or ^{13}C NMR, unless otherwise noted. Dynamic Adsorbents' 60A silica gel was used for chromatography, and Analtech silica gel plates with fluorescence F254 were used for thin-layer chromatography (TLC) analysis. ^{1}H and ^{13}C NMR spectra were recorded on Broker Advance 500 and tetramethylsilane (TMS) was used as a reference. Data for ^{1}H are reported as follows: chemical shift (ppm), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for ^{13}C NMR are reported as ppm. ^{13}C NMR resonance assignments were aided by the use of the DEPT technique to determine numbers of attached hydrogens.

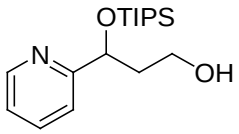


Compound 23 To a solution of LDA, made from diisopropylamine (28.0 g, 0.28 mol) in THF (150 mL) and n-butyllithium (112 mL, 0.28 mol, 2.5 M in hexane), at -78 ^{0}C was added a solution of ethyl acetate (24.0 g, 0.27 mol) in THF (50 mL). After stirring at -78 ^{0}C for 1 h, a solution of 2-pyridinecarboxaldehyde (20.0 g, 0.19 mol) in THF (50 mL) was added. The resulting mixture was stirred overnight at room temperature, diluted with satd. aq. ammonium chloride and separated. The organic layer was dried and concentrated in vacuo to give the residue which was subjected to chromatography on silica gel (1:4 to 1:1 ethyl acetate-hexane) to provide 22.0 g (61%) of **23**. ^{1}H NMR 1.12 (m,3H),2.64 (q, J = 8.5 Hz,1H), 2.80 (q, J = 4 Hz,1H),4.04 (q, J = 7 Hz, 2H), 5.09 (q, J = 4.5 Hz,1H),7.09 (q, J = 6 Hz,1H), 7.34 (d, J = 8 Hz,1H), 7.59 (m,1H),8.41 (t, J = 4 Hz, 1H); ^{13}C NMR, 13.9, 42.3, 60.4, 69.9, 120.1, 122.3, 136.7, 148.3, 160.9, 171.7; HRMS (ES) m/z 196.0974, calcd for $C_{10}H_{14}NO_3$ 196.0977.

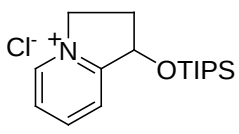


Compound 24 A solution of **23** (30.0 g, 0.15 mol), imidazole (16.0 g, 0.24 mol) and triisopropylsilyl chloride (40 mL, 0.19 mol) in DMF (50 mL) was stirred overnight at room temperature, diluted with satd. aq. sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with aq. sodium chloride, dried and concentrated in vacuo to give a residue which was subjected to column chromatography on silica gel (hexane to 1:4

ethyl acetate-hexane) to afford 26.0 g (48%) of **24**. ^{1}H NMR 0.96 (m, 21H), 1.15 (m, 3H),2.77 (m, 2H), 4.04 (m, 2H), 5.34 (d, J = 5.5 Hz, 1H), 7.11 (d, J = 5 Hz, 1H), 7.51 (d, J = 8 Hz, 1H), 7.65 (d, J = 6 Hz, 1H), 8.46 (s,1H); ^{13}C NMR 12.3, 14.0, 17.9, 44.6, 60.3, 73.3, 120.5, 122.2, 136.4, 148.4, 163.3, 170.6; HRMS (ES) m/z 352.2308, calcd for $C_{19}H_{34}NO_3$ Si 352.2303.

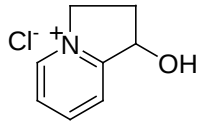


Compound 25 To a solution of **24** (5.0 g, 14 mmol) in ethyl ether (150 mL), was added a solution of lithium borohydride (15 mL, 30 mmol, 2M in THF) at 0 ^{0}C. The resulting solution was stirred for 24 h at room temperature, diluted with satd. aq. sodium bicarbonate, and the organic layer was separated, dried over magnesium sulfate, and concentrated in vacuo to give a residue which was subjected to column chromatography on silica gel(1:4 to 1:1 ethyl acetate-hexane) to afford 3.1 g (70%) of **25**. ^{1}H NMR 1.01 (d, J = 22.5 Hz,18H), 1.13 (m, 3H), 2.03 (q, J = 6.9 Hz,1H), 2.20 (q, J = 5.5 Hz, 1H), 3.54 (t, J = 5.5 Hz, 1H), 3.68 (t, J = 5.5 Hz, 1H), 3.81 (s, 1H), 5.19 (d, J = 5.1 Hz, 1H), 7.17 (t, J = 5.7 Hz, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.73 (t, J = 7.4 Hz, 1H),8.48 (s, 1H); ^{13}C NMR 12.1, 17.9, 40.9, 59.1, 74.8, 120.4, 122.1, 136.8, 148.0, 163.9; HRMS (ES) m/z 310.2202, calcd for $C_{17}H_{32}NO_2Si$ 310.2202.

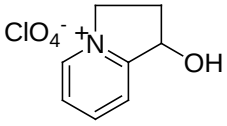


Compound 26 To a solution of N-chlorosuccinimide (4.7 g, 35 mmol) in methylene chloride (150 mL) was added dimethylsulfide (2.8 mL, 38 mmol)

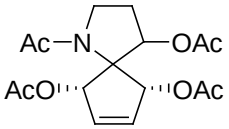
at 0 ^{0}C. To the resulting mixture at -30 ^{0}C was added a solution of **25** (9.0 g, 29 mmol) in methylene chloride (20 mL). The resulting solution was stirred for 4 h at 0 ^{0}C, poured into satd. aq. Sodium chloride at 0 ^{0}C, and extracted with methylene chloride. The organic layers were dried and concentrated in vacuo to give a residue which triturated with acetonitrile and ethyl acetate to afford 8.6 g (90%) of **26**. ^{1}H NMR (D_2O) 0.97 (d, J = 4 Hz, 18H), 1.12 (m, 3H), 2.28 (m, 1H), 2.86 (m, 1H),4.57 (m, 1H), 4.78 (t, J = 9.5 Hz, 1H), 5.73 (t, J = 7.5 Hz, 1H), 7.82 (t, J = 7 Hz, 1H), 7.98 (d, J = 8 Hz, 1H), 8.36 (t, J = 7.5 Hz, 1H), 8.64 (d, J = 6 Hz,1H); ^{13}C NMR (D_2O) 13.3, 35.0, 58.0, 76.1, 126.0, 128.9, 142.8, 147.9; HRMS (ES) m/z 292.2097, calcd for $C_{17}H_{30}NOSi$ 292.2102.



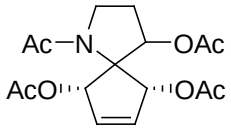
Compound 27 A solution of **26** (10.4 g, 32 mmol) in a mixture of distilled water (50 mL), conc. HCl (36%, 50mL) and methanol (100 mL) was stirred overnight at room temperature, concentrated in vacuo to give a residue which triturated with acetonitrile and ethyl acetate to afford 5.0 g (93%) of the **27**. ^{1}H NMR (D_2O) 2.21 (m, 1H), 2.74 (m, 1H), 4.58 (m, 1H), 4.77 (m, 1H), 5.50 (t, J = 7.5 Hz, 1H), 7.80 (d, J = 6.5 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 8.36 (t, J = 8.0 Hz, 1H), 8.63 (d, J = 6.0 Hz, 1H); ^{13}C NMR (D_2O) 33.0, 58.1, 74.1, 125.9, 128.8, 142.6, 147.8,1 59.5; HRMS (ES) m/z 136.0689, calcd for $C_8H_{10}NO$ 136.0762.



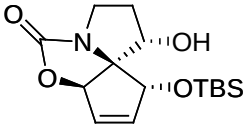
Compound 28 A solution of **27** (1.0 g, 6 mmol) and silver perchlorate hydrate (1.1 g, 5.3 mmol) in in distilled water (220 mL) was stirred for 4 h at room temperature and filtered. The filtrate was concentrated in vacuo to afford 0.65 g of **28**. ^{1}H NMR (D_2O) 2.19 (m, 1H), 2.74 (m, 1H), 4.58 (m, 1H), 4.77 (m, 1H), 5.49 (t, J = 7.5 Hz, 1H), 7.79 (m, 1H), 7.93 (d, J = 8 Hz, 1H), 8.36 (t, J = 8 Hz, 1H), 8.62 (d, J = 6 Hz, 1H); ^{13}C NMR (D_2O) 32.8, 57.9, 74.0, 125.8, 128.7, 142.5, 147.6, 159.4; HRMS (ES) m/z 136.0689, calcd for $C_8H_{10}NO$ 136.0762.



Reaction A Compound 31 A solution of **28** (0.65 g, 2.8 mmol) and sodium bicarbonate (0.6 g, 7.1 mmol) in water (600 mL) was irradiated for 11 h at room temperature (70% conversion), concentrated in vacuo to afford a residue, which was dissolved in anhydrous DMF (20 mL) and glacial acetic acid (2 mL). The resulting solution was stirred overnight at room temperature and then mixed with pyridine (2 mL) and acetic anhydride (2.5 mL), stirred overnight at room temperature, diluted with satd. aq. sodium bicarbonate and extracted with ethyl acetate (2x100 mL). The extracts were dried and concentrated in vacuo to give a residue which was subjected to column chromatography on silica gel (1:4 acetone-hexane) to afford 0.13 g (yield 20%) of **31**. ^{1}H NMR 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 3.43 (m, 1H), 3.58 (m, 1H), 5.44 (q, J = 5.5 Hz, 1H), 5.88 (m, 1H), 6.10 (m, 1H), 6.12 (s, 1H), 6.35 (s, 1H); ^{13}C NMR 20.9, 21.0, 21.1, 23.5, 28.3, 45.4, 73.2, 75.9, 78.8, 79.0, 130.4, 134.6, 169.7, 169.9, 170.2; HRMS (ES) m/z 340.1395 (M+1), calcd for $C_{16}H_{22}NO_7$ 340.1396.

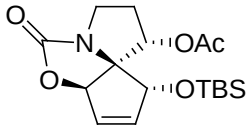


Reaction B Compound 31 A solution of **28** (0.6 g, 2.6 mmol) in water (600 mL) containing perchloric acid (2.5 mL, 70%) was irradiated for 14 h at room temperature (40% conversion), concnetrated in vacuo to afford a residue, which was mixed with DMF (20 mL), pyridine (2 mL) and acetic anhydride (2.5 mL). The resulting mixture was stirred overnight at room temperature, diluted with satd. aq. sodium bicarbonate (20 mL), and extracted with ethyl acetate. The extracts were dried and concentrated in vacuo to give a residue, which was subjected to column chromatography on silica gel (1:4 acetone:hexane) to afford 0.10 g (29%) of **31**.

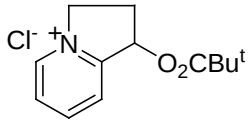


Compound 32 A solution of **28** (0.6 g, 2.6 mmol) and sodium bicarbonate (0.6 g, 7.1 mmol) in water (600 mL) was irradiated for 10 h at room temperature (70% conversion) and concentrated in vacuo giving a residue, which was mixed with DMF (20 mL), imidazole (1.0 g, 15 mmol) and tert-butyldimethylsilyl chloride (1.0 g, 6.6 mmol). The resulting mixture was stirred overnight at room temperature, diluted with satd. aq. sodium bicarbonate and extracted with ethyl acetate. The extracts were dried and concentrated in vacuo to give a residue16 which was subjected to column chromatography on silica gel (1:4 acetone-hexane) to afford 0.13 g (yield 27%) of **32** 16as a crystalline solid, mp 152.1-152.9 ^{o}C (20:1 EtOAc-hexane)16 ^{1}H NMR 0.05 (s, 3H), 0.06 (s, 3H), 0.85 (s, 9H), 2.15 (m, 2H),

3.22 (m, 1H), 3.73 (m, 1H), 4.42 (s, 1H), 4.56 (s, 1H), 5.55 (s, 1H), 5.97 (q, J = 1.5 Hz, 1H), 6.06 (q, J = 1.5 Hz, 1H); ^{13}C NMR -5.1, -4.0, 17.9, 25.6, 34.6, 43.3, 70.1, 79.6, 81.4, 83.1, 132.1, 136.9, 161.1; HRMS (ES) m/z 312.1638, calcd for $C_{14}H_{26}NO_4Si$ 312.1631.

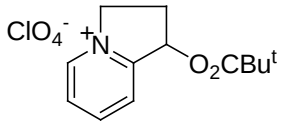


Compound 33 A mixture of **32** (30 mg, 0.11 mmol) and acetic anhydride (0.5 mL) in anhydrous DMF (2 mL) was mixed with pyridine (1.0 mL) and mixture was stirred overnight at room temperature, diluted with satd. aq. sodium bicarbonate and extracted with ethyl acetate. The extracts were washed with aq. sodium chloride, dried and concentrated in vacuo to give a residue which was subjected to column chromatography on silica gel (1:4 ethyl acetate-hexane) to afford 20 mg (58%) of **33**. ^{1}H NMR 0.07 (s, 3H), 0.09 (s, 3H), 0.88 (s, 9H), 2.05 (s, 3H), 2.25 (m, 2H), 3.29 (m,1H), 3.72 (m,1H), 4.46 (s, 1H), 5.36 (s, 1H), 5.63 (s, 1H), 6.01 (m, 1H), 6.06 (t, J = 4 Hz, 1H); ^{13}C NMR -5.0, -3.9, 17.9, 21.1, 25.6, 32.4, 43.4, 73.6, 79.7, 80.0, 83.0, 132.1, 136.7, 160.2, 170.2; HRMS (ES) m/z 354.1735, calcd for $C_{17}H_{28}NO_5Si$ 354.1737.



Compound 29 A mixture of **27** (2.0 g, 12 mmol) and pivalic anhydride (30 mL) was stirred at 170 ^{0}C for 1 h, cooled to room temperature and triturated with ethyl acetate to afford 3.0 g (80%) of **29**. ^{1}H NMR (D2O) 1.18 (s, 9H), 1.21 (s, 9H), 2.58 (m, 1H), 3.01 (m, 1H), 4.90 (m, 1H), 5.06 (m, 1H), 6.53 (t,

J = 7.5 Hz, 1H), 8.05 (t, J = 7 Hz, 1H), 8.13 (d, J = 8 Hz, 1H), 8.55 (t, J = 8 Hz, 1H), 8.90 (d, J = 6 Hz,1H); ^{13}C NMR 26.7, 26.9, 29.0, 38.7, 39.1, 57.7, 74.9, 125.9, 128.6, 142.1, 147.0, 154.5, 180.5, 184.4;



Compound 30 A solution of **29** (3.0 g, 9.3 mmol) and silver perchlorate hydrate (2.0 g, 9.7 mmol) in water (220 mL) was stirred for 4 h at room temperature and filtered. The filtrate was concentrated in vacuo giving a residue which was crystallized **30** ^{1}HNMR(D_2O) 1.24(s, 9H),2.59(m, 1H),3.04(m, 1H),4.93(m, 1H),5.09 (m, 1H), 6.56(t,18J=7.5Hz), 8.08(t, J=6.5Hz,181H), 8.16(d, J=8Hz, 1H), 8.58(t, J=8Hz, 1H), 8.92(d, J=6Hz),^{13}C, 26.5, 28.9, 39.1, 57.6, 74.9, 125.9, 128.4, 142.0, 146.8, 154.5, 180.6.

1.4 References

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CHAPTER TWO

Total Synthesis of Lepadiformine C

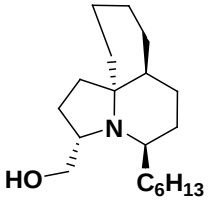
2.1 Background

2.1.1 Isolation and Biological Activity of Members of the Lepadiformine Family

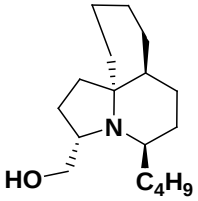
In 1994, Biard and his coworkers described the isolation of a new marine alkaloid, lepadiformine A, obtained by HCl extraction of the methyl chloride soluble portion of the marine tunicate *Claveline Lepadiformis* (Figure 2), collected in the Mediterranean Sea off the coast of Tunisia[1] and later isolated from *Claveline Moluccensis* (Figure 2) obtained near Djibouti.[2] Very recently,[3] two additional alkaloids closely related to lepadiformine A were isolated from *Claveline Moluccensis.* These compounds include lepadiformine B, which bears a butyl group rather than hexyl at C(2), and lepadiformine C, which does not contain the hydroxylmethyl group at C(13).

Figure 2.

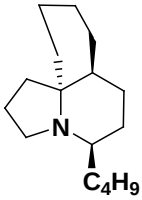
Claveline Lepadiformis *Claveline Moluccensis*



Lepadiformine A



Lepadiformine B



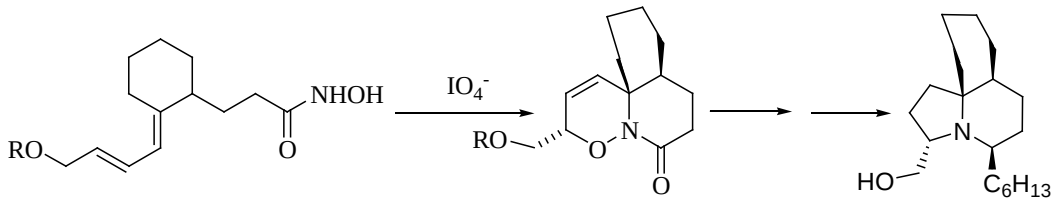
Lepadiformine C

Lepadiformine A has been reported to have moderate in vitro cytotoxicity against nasopharynx carcinoma (KB) and non-small-cell lung carcinoma (NSCLC-N6).[1] In addition, the cardiovascular effects of this alkaloid have been investigated *in vivo*. The alkaloid caused a variety of effects when tested in rats, including induction of bradycardia and prolongation of ECG parameters, and it led to a transient reduction of blood pressure.[2] It was also suggested that lepadiformine A may have antiarrythmic properties. Recently members of the lepadiformine family were found to block the cardiac muscle K_{ir} channel.[3]

2.1.2 Synthetic Approaches to Lepadiformine A and Related Compounds

In 2000, Kibayashi and his coworkers[4] achieved the first total synthesis of racemic lepadiformine A using an intramolecular acylnitroso Diels-Alder strategy (Scheme 1).

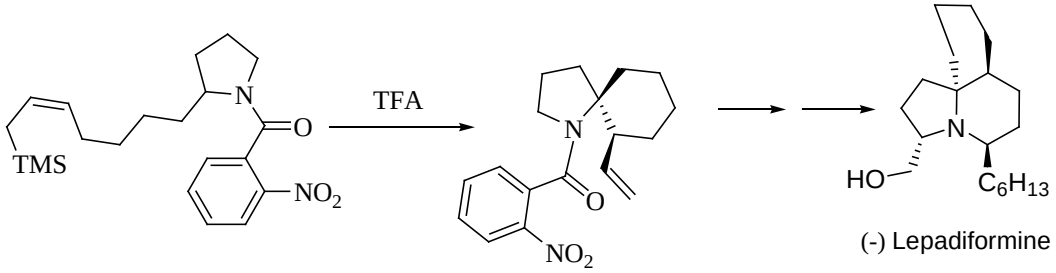
Scheme 1



Racemic Lepadiformine

In 2001, Weinreb and his coworkers[5] were the first to assign the absolute stereochemistry of lepadiformine by using a total synthesis of the natural enantiomer based on an intramolecular spirocyclization of a cyclic N-acyliminium ion with an allylsilane as a pivotal step (Scheme 2).

Scheme 2

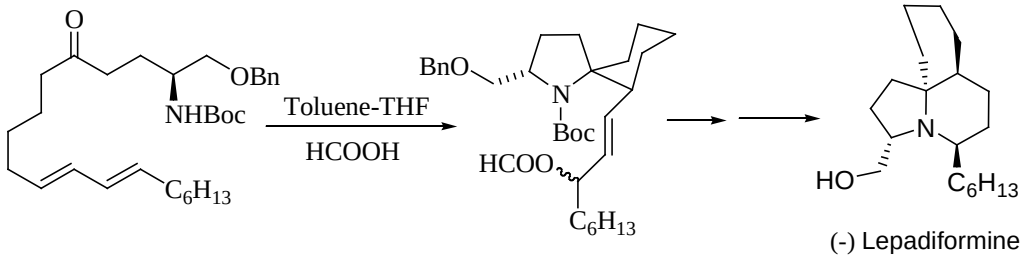


(-) Lepadiformine

In 2005, Kibayashi and his coworkers[6] accomplished the enantioselective total synthesis of (-)lepadiformine A by employing a conjugate

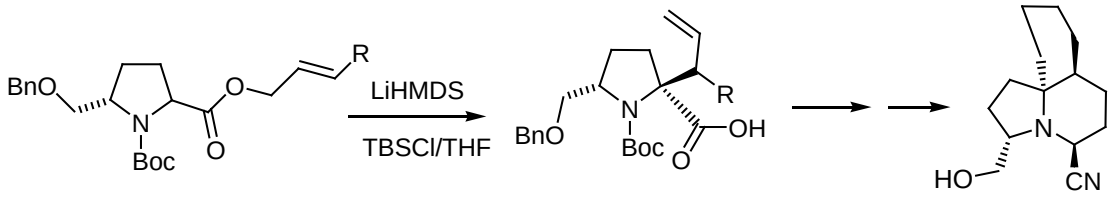
azaspirocyclization methodology in seven steps from the common spirocyclic formate (Scheme 3).

Scheme 3



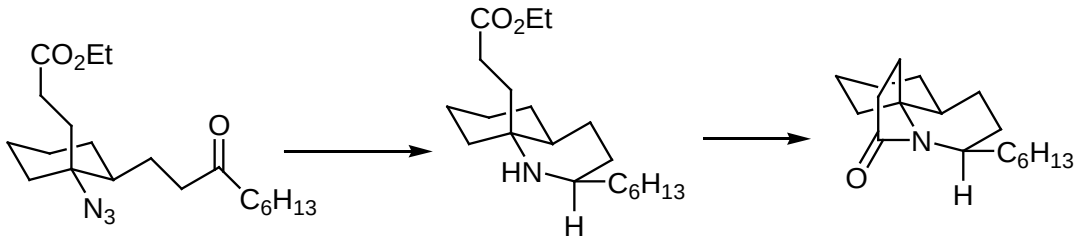
In 2006, Kim et al.[7] reported a new approach to (-)-lepadiformine A which uses an amino acid ester-enolate Claisen rearrangement and a ring closing metathesis as key step. (Scheme 4).

Scheme 4



Later, Schar and Renaud [8] described a new approach to racemic lapediformine A, which makes use of an interesting free radical carboazidation strategy developed by this group (Scheme 5).

Scheme 5

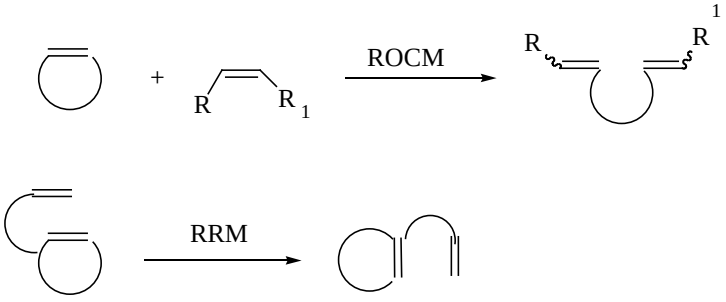


Other varied approaches, relying on a broad array of modern synthetic methodologies, that have been employed to prepare lepadiformine A and the closely related cylindricines. Syntheses of these targets have been thoroughly reviewed recently by Weinreb.[9]

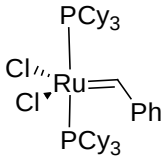
2.1.3 Ru(0) Catalyzed RRM Metathesis Reactions

Olefin metathesis is an important method for the sequential cleavage and formation of carbon-carbon double bonds. This transformation plays a key role in a variety of methodologies including ring opening metathesis (ROM), ring closing metathesis (RCM), cross metathesis (CM), ring opening cross metathesis (ROCM) and ring rearrangement metathesis (RRM) (Scheme 6).

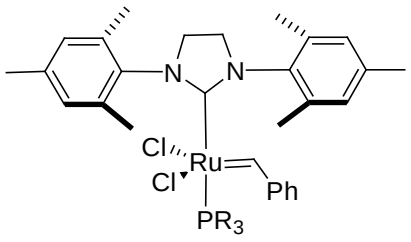
Scheme 6



The now famous Grubbs's metathesis catalysts[10] (1st generation catalyst Ru1 and 2nd generation catalyst Ru2) have found extensive use as catalysts for these processes due to their high activity, stability and compatibility with most functional groups.



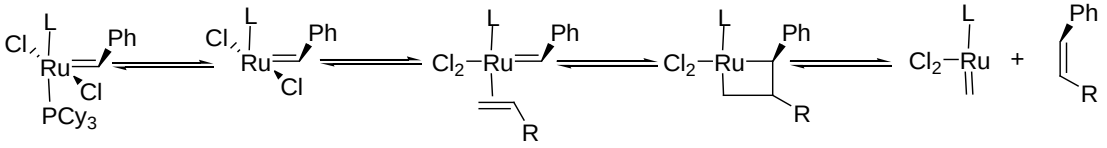
Ru1



Ru2

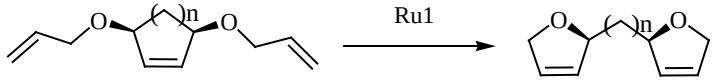
Studies of the mechanism of metathesis promoted by the Grubb's catalysts indicate that the initial substitution of phosphine by the olefinic substrate in Ru1 and Ru2 proceeds in a dissociative fashion involving a 14-electron intermediate.[11] The high activity of the N-heterocyclic carbene coordinated catalyst Ru2 is due to its improved selectivity for binding π-nucleophilic olefinic substrates in the presence of σ-donating free phosphine. The Ru-olefin intermediate forms a four member ring ruthenocyclobutane from which bond rearrangement takes place to generate a new double bond and a catalytically active ruthenium alkylidene (Scheme 7).

Scheme 7



Several thorough reviews[12] on Ru(0)-catalyzed olefin metathesis have been published. Here we focus on the Ru(0)-catalyzed ring rearrangement metathesis (RRM) reaction, which serves as a key step in our approach to (-)-lepadiformine C. Ring rearrangement metathesis (RRM) is an intramolecular metathesis reaction taking place between a strained endocyclic olefin and an exocyclic olefin in which the ring is opened (ROM) and concomitantly a new ring is formed (RCM). Tandem Ru(0) catalyzed RRM was first reported by Grubbs and his coworkers.[13] An example of the use of this process is found in the reaction of cyclic olefins **1**, which contain two terminal olefin groups. These substances undergo Ru1 promoted sequential RRM and RCM to produce bicyclic ethers **2**. The reaction is entropically driven by the production of ethylene. The reactivities of substances are related to the size of the cycloalkene ring in the substrate. The cyclobutene derivative reacts most rapidly due to its high ring strain, followed by the five and eight-membered ring analogs. Substrates with cycloheptene and cyclohexene ring systems react the most slowly (Scheme 8).

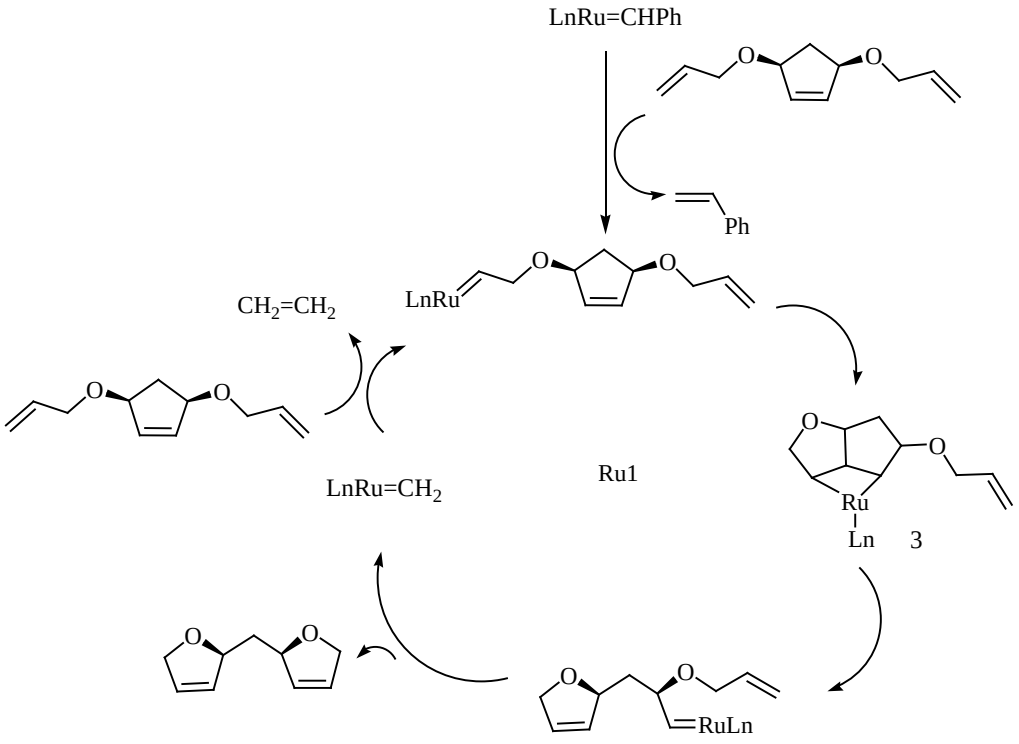
Scheme 8



1 2

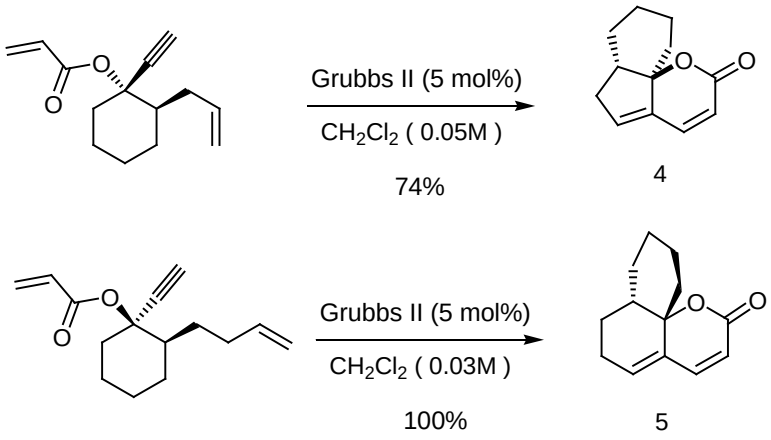
The results of mechanistic investigations suggest that the RRM reactions proceeds by initial cross metathesis at the terminal olefin unit, followed by metathesis at the cyclic olefin to form the first ring. The formation of four-membered ruthenocyclobutane intermediate **3** is the key step, whose energy determines whether or not the reaction proceeds (Scheme 9).

Scheme 9



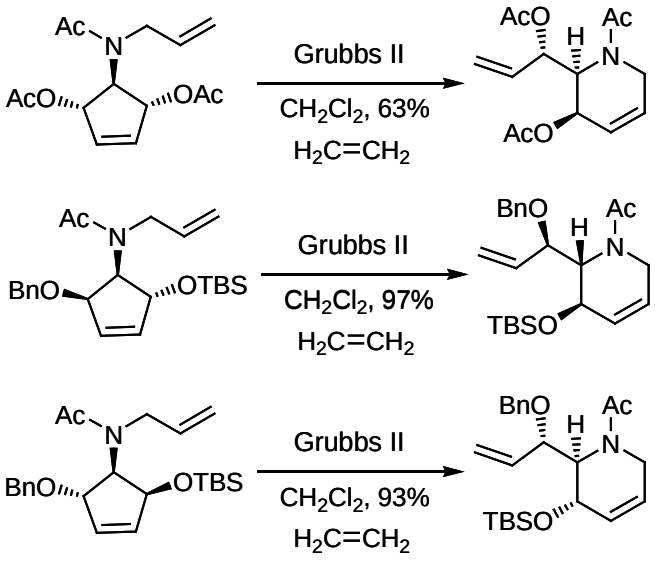
Grubbs and his coworkers[14] have utilized ene-yne-ene RRM metathesis reactions to produce the functionalized fused tricyclic compound **4** and **5**. In this process, the triple bond positioned between the two olefins acts as an olefin metathesis relay (Scheme 10).

Scheme 10



In their total synthesis of (-)-swainsonine[15] and (+)-castanospermine,[15] Mariano and his coworkers employed an asymmetric RRM reaction of N-allylacetamidocyclopentenes to produce a 6-allyl-1,2,5,6-tetrahydropyridines (Scheme 11). Relative and absolute stereochemistry at three chiral centers of the 6-allyl-1,2,5,6-tetrahydropyridine products were controlled by the choice of the amidocyclopentene used as starting materials for the RRM reactions.

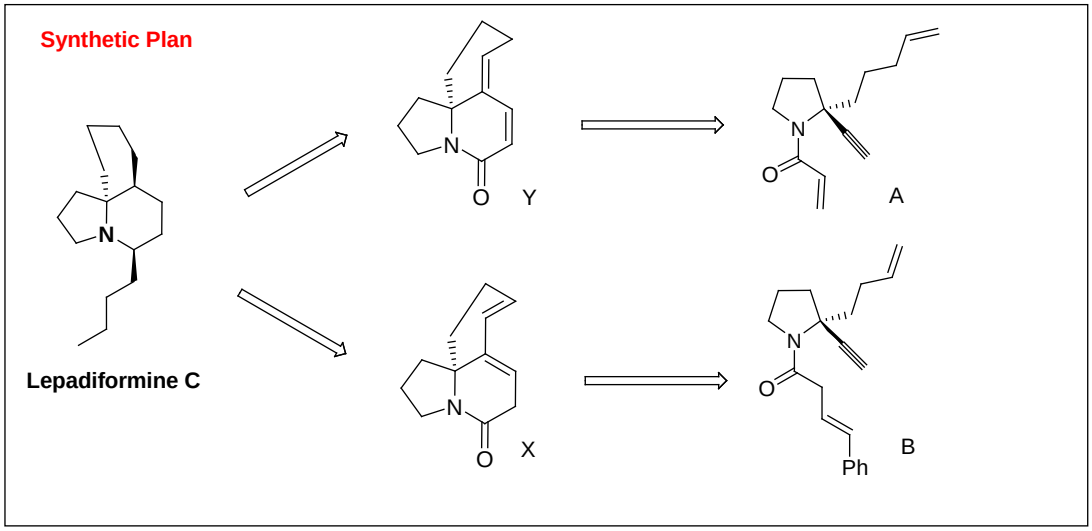
Scheme 11

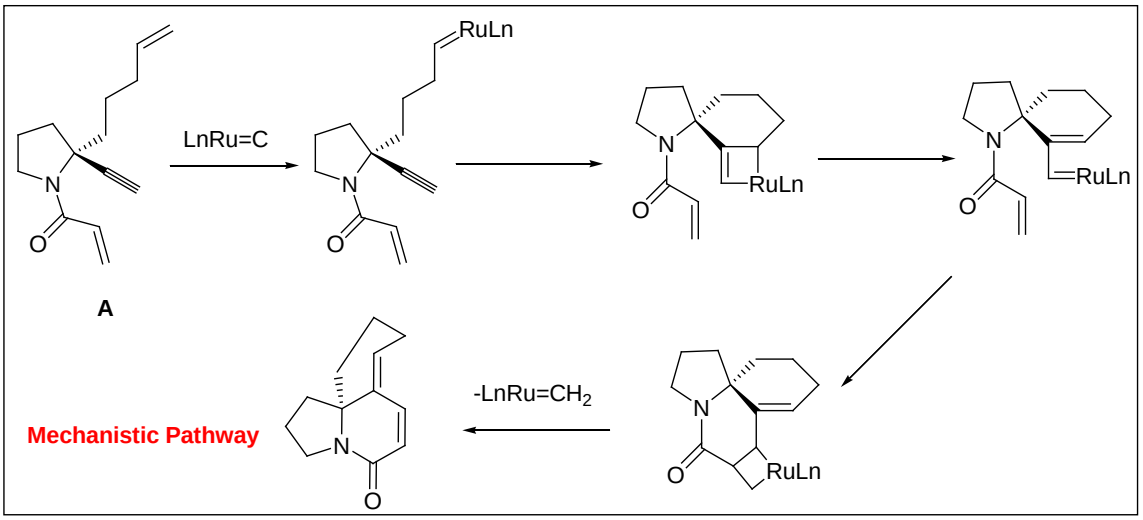


2.2 Research Design

One phase of my doctoral research program was focused on the development of new synthetic strategies for the synthesis of the natural product lepadifomine C that make use of tandem ene-yne-ene metathesis reaction (Scheme 12).

Scheme 12





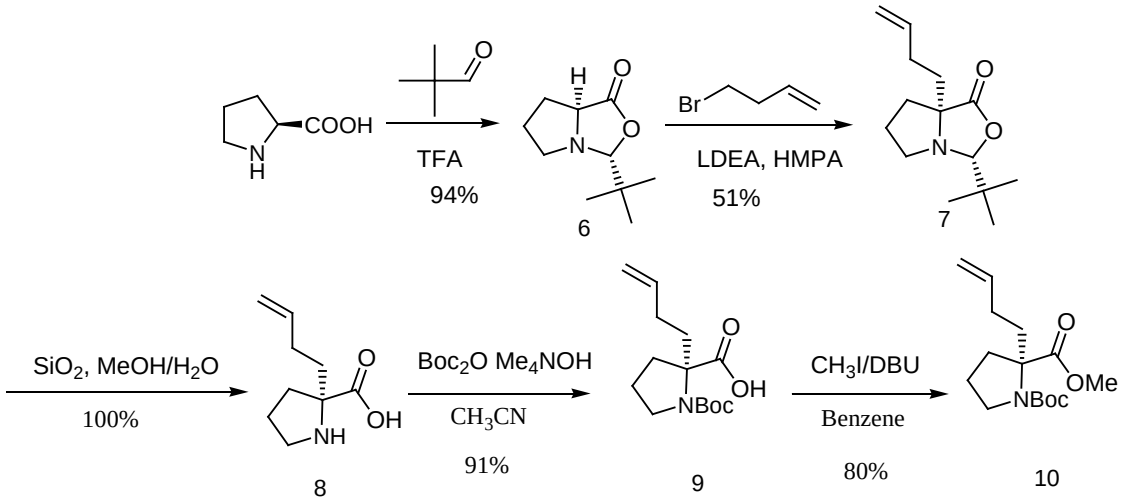
In the synthesis planning stage, I elected to focus my efforts on the tandem ene-yne-ene metathesis reaction for the formation of unsaturated tricyclic amide (X or **Y** in Scheme 12). As shown in Scheme 12, two possible approaches to the formation of unsaturated tricyclic amide exist. Based on

analysis of the mechanistic pathway, one approach relies on the formation of tricyclic diene amide **X** and other approach involves formation of tricyclic conjugate diene amide **Y**. Methods for promoting these types of tandem ene-yne-ene metathesis reactions have been reported by in the literature.[14]

2.3 Results and Discussion

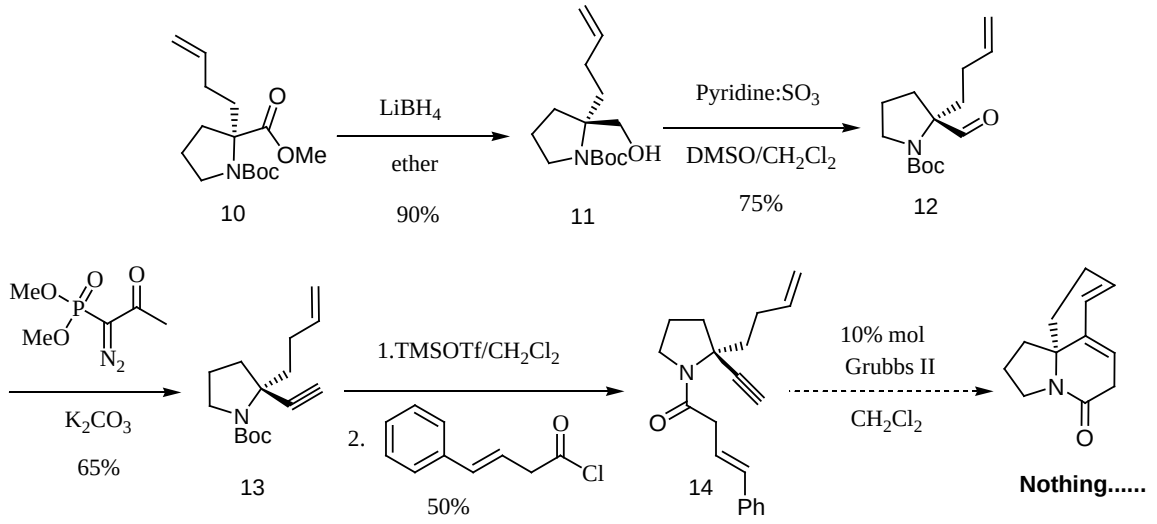
The first approach began with implementation of a modification of the methodology described by Seebach.[16] [17] Accordingly, alkylation of oxazolidinone **6**, derived from L-proline, with the relatively unactivated electrophile 4-bromo-1-butene was accomplished in the presence of HMPA to give **7**. This alkylated oxazolidinone was deprotected to yield **8** by using the procedure reported by Genin et al.[18] This was followed by protection of amino group in **8** under the conditions of suggested by Khalil et al.[19] According to the procedure reported by Ono et al,[20] the carboxylic acid functionality of **9** was protected as the corresponding methyl ester. (Scheme13)

Scheme 13



The methyl ester group in **10** was selectively reduced by using lithium borohydride in ether. Subsequent Swern oxidation afforded the aldehyde **12**. By using methodology developed by Bestmann,[21] aldehyde **12** was converted to the corresponding Boc-protected 2-butenyl-2-ethynyl pyrrolidine **13**. Finally, removal of the Boc group with TMSOTf and followed by acrylamide formation gave the key metathesis substrate yne-diene **14** (Scheme 14).

Scheme 14

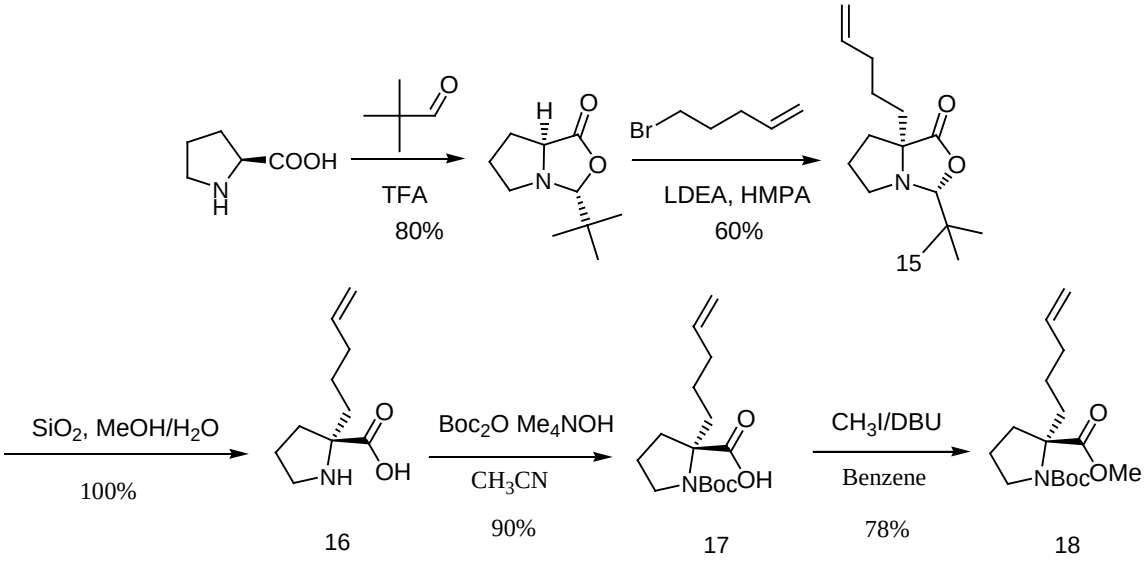


The tandem ene-yne-ene metathesis reaction **14** was carried out under mild conditions involving refluxing methylene chloride and 10 mol % of the Grubbs II generation catalyst. Surprisingly, a complex product mixture was formed and the starting material was completely consumed. Importantly, the desired product was not obtained in this process.

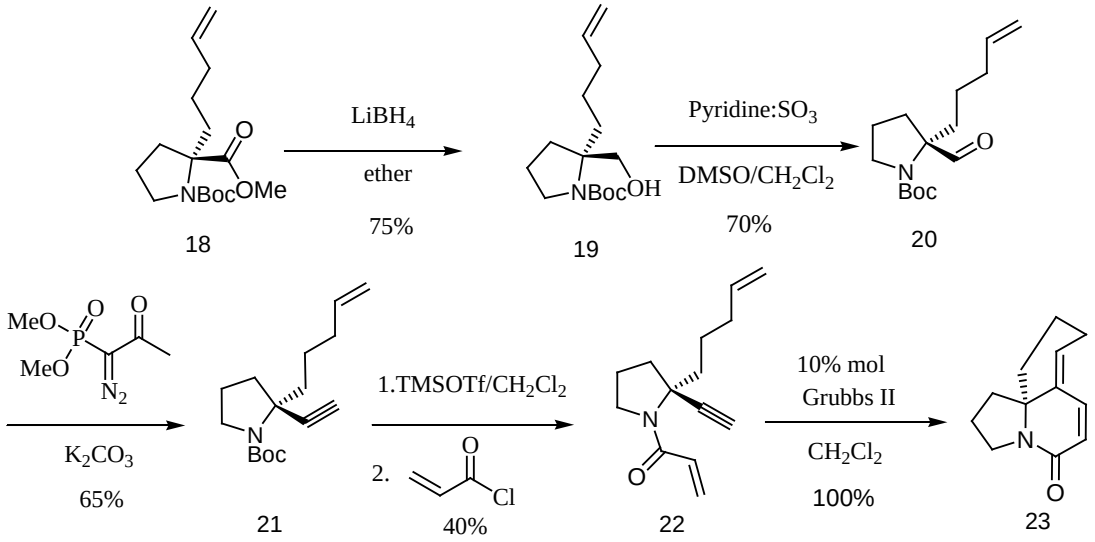
In the second approach, I again followed a modification of the methodology prescribed by Seebach.[16] [17] In this case, L-proline was transformed to the corresponding oxazolidinone, which underwent alkylation with 5-bromo-1-pentene in the presence of HMPA to give **15**. The alkylated oxazolidinone was deprotected to form **16** again by using the procedure of Genin et al.[18] This was followed by protection of amino group employing the

conditions suggested by Khalil et al.[19] Following the procedure of Ono et al,[20] the carboxylic acid functionality in **17** was protected as the methyl ester **18** (Scheme15), which was selectively reduced with lithium borohydride in ether to afford alcohol **19**. Swern oxidation of **19** affords aldehyde **20**. By using the methodology developed by Bestman,[21] this aldehyde was converted to the corresponding Boc-protected 2-pentenyl-2-ethynyl pyrrolidine **21**. Boc group removal with TMSOTf, followed by acrylamide formation, gave the metathesis substrate yne-diene **22** (Scheme16).

Scheme 15



Scheme 16

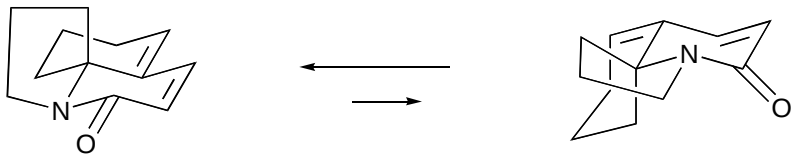


Tandem ene-yne-ene metathesis reaction of **22** was carried out under mild condition involving refluxing methylene chloride and 10 mol % of the Grubbs II generation catalyst. This led to formation of the desired tricyclic amide **23** in a quantitative yield.

The final stage of the lepadiformine C synthesis began with what we believed would be stereoselective hydrogenation of conjugate unsaturated tricyclic amide **23**. Although the results of AM1 calculations were not conclusive, we anticipated that hydrogenation of **23** would take place on a lower energy conformer **23a** from the sterically less hindered face to give the tricyclic amide **24** selectively (Scheme 17). Specifically, inspection of

conformation **23a** shows that the presence of axial pyrrolidine methylene group would block hydrogen addition from the β-face of the dienamide grouping. In fact, this catalytic process (Pd/C, H_2) gave a saturated tricyclic amide **24**, whose stereochemistry was assigned based on the above reasoning.

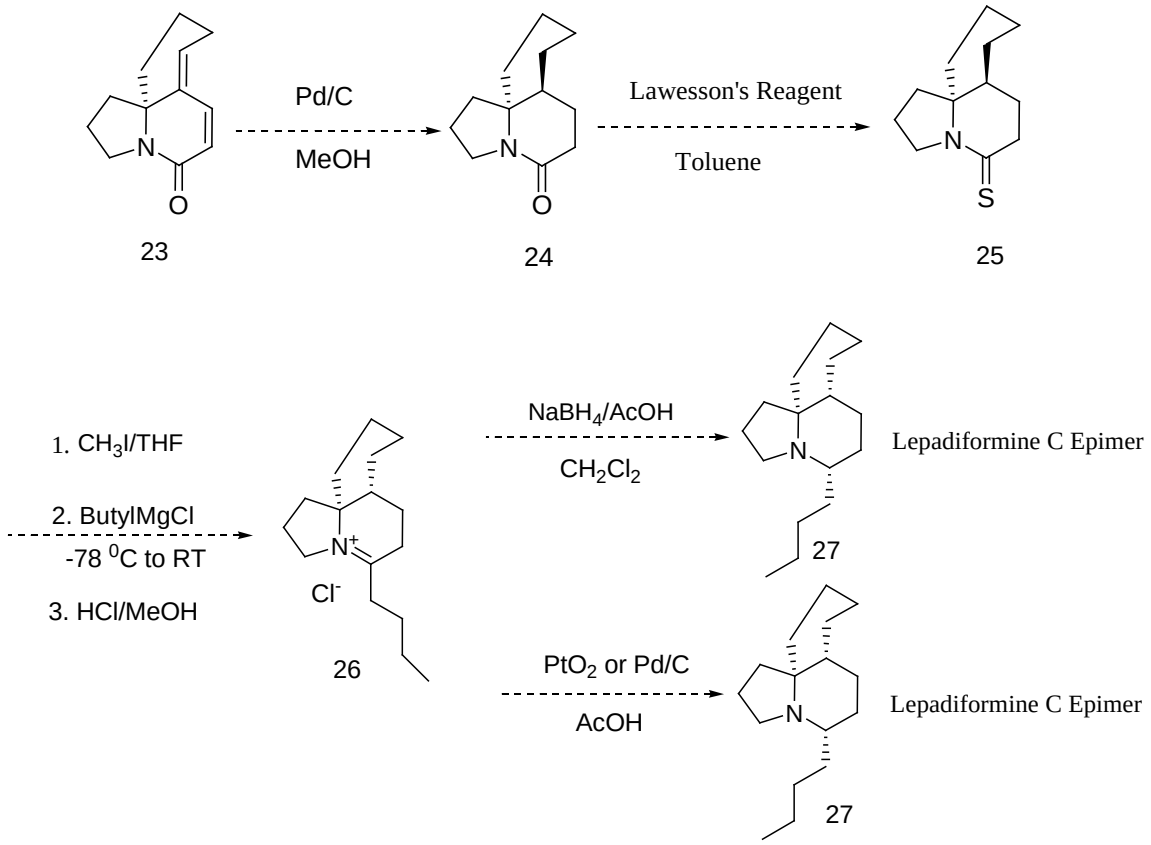
Scheme 17



23a 23b

Treatment of **24** with the Lawesson's reagent in refluxing toluene affords the saturated tricyclic thioamide **25**. By using the methodology developed by Speckamp et al.,[22] the thioamide **25** was treated with methyl iodide and followed by addition of the butyl magnesium chloride, followed acidification gave the intermediate iminium salt **26**, which was purified by silica gel chromatography. Reduction of **26** by using either metal hydride ($NaBH_4$, AcOH) or catalytic hydrogenation (PtO_2/H_2 or Pd/H_2) was found to afford a tricyclic product, that, unfortunately did not have NMR properties that are identical to those reported for Lepadiformine C (Scheme 18).

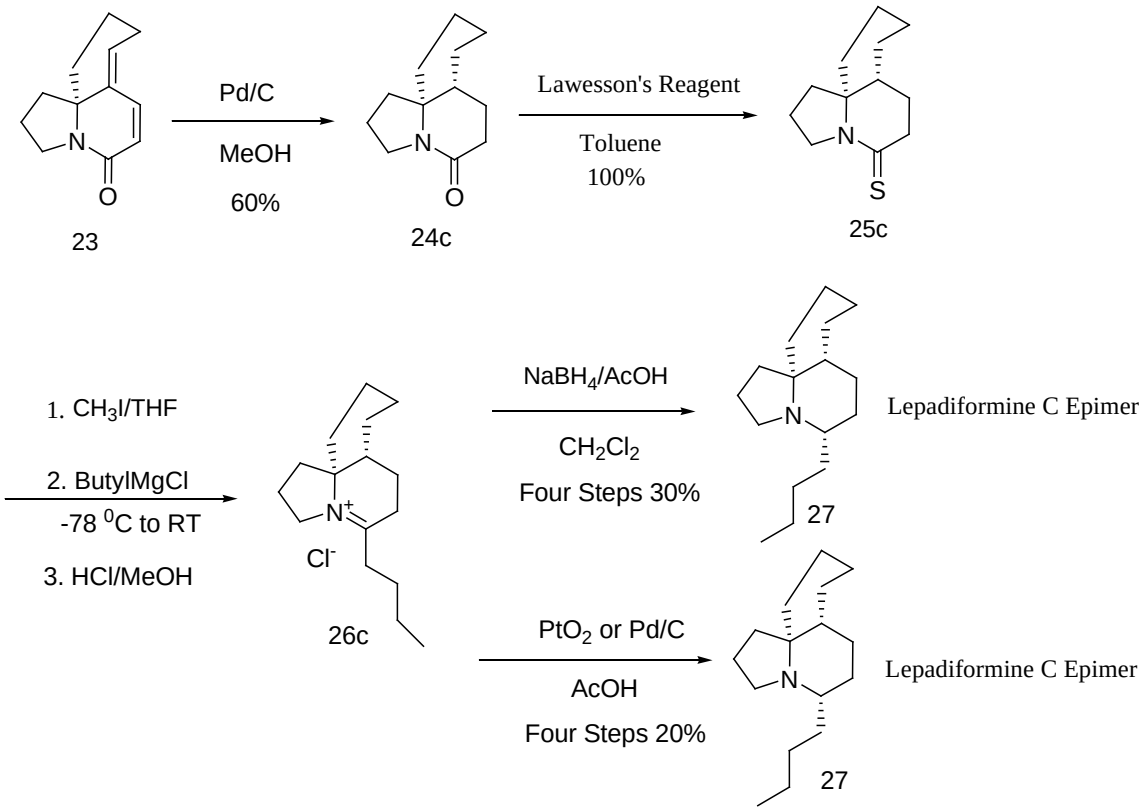
Scheme 18



As a result of this finding, we assumed that either the structure/sterochemistry of the natural product was incorrectly assigned or that the stereochemistry expected for reduction of the dienamide **23** is incorrect. Fortunately, identification of the source of the problem was facilitated by the finding that the thioamide **25** could be crystallized and that the crystals were acceptable for x-ray crystallographic analysis. Surprisingly

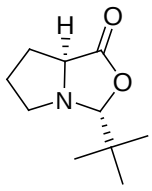
(see above), the stereochemistry of this amide (**25c**, Scheme 19) is not that predicted. As a result, it appears that catalytic hydrogenation of 23 takes place from the least hindered face of the opposite conformation **23b** (Scheme 17) to give the tricyclic amide **24c**. Accordingly, Grignard addition to the S-methyl iminium salt formed from the thioamide **25c** yields the iminium **26c**, which is then converted to the lepadiformine C epimer 27 (Scheme 19).

Scheme 19



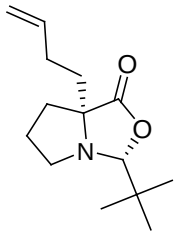
2.4 Experimental

General. Unless otherwise noted, all reagents were obtained from commercial sources and used without further purification. All compounds were isolated and shown to be >90% pure by ^{1}H and/or ^{13}C NMR analysis, unless otherwise noted. Dynamic Adsorbents' 60A silica gel was used for chromatography, and Analtech silica gel plates with fluorescence F254 were used for thin-layer chromatography (TLC) analysis. ^{1}H and ^{13}C NMR spectra were recorded on Broker Advance 500 and tetramethylsilane (TMS) was used as a reference. Data for ^{1}H are reported as follows: chemical shift (ppm), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for ^{13}C NMR are reported as ppm. ^{13}C NMR resonance assignments were aided by the use of the DEPT technique to determine numbers of attached hydrogens.



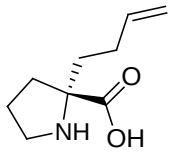
Compound 6. To a suspension of L-proline (40.0 g, 0.35 mol) in pentane (1500 mL) was added 200 mL (1.87 mol) of pivaladehyde and 3 mL of trifluoroacetic acid. The mixture was stirred at reflux for 6 days with

azeotropic removal of the water. The resulting solution was concentrated in vacuo to afford a residue, which was subjected to short path distillation(bp 80-90 ^{0}C, 0.2 mm Hg)to afford **6** (58 g, 92%). ^{1}H NMR 0.85 (s,9H), 1.10-1.20 (m,1H), 1.60-1.70 (m, 1H), 1.70-1.80 (m, 1H), 1.90-2.00 (m,1H), 2.00-2.10 (m,1H), 2.70-2.85 (m,1H), 3.10-3.25 (m,1H), 3.80-3.95 (m,1H), 4.47 (s,1H); ^{13}C NMR, 23.88, 24.84, 29.42, 37.07, 58.16, 62.52, 107.53, 178.50. The data are consistent those reported for this substance earlier. [17]

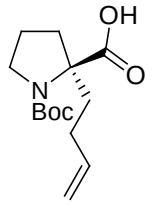


Compound 7. To a solution of LEDA (made from diethylamine (16 mL, 0.154 mol) in THF (700 mL) and n-butyllithium (60 mL, 0.15 mol, 2.5 M in hexane)) at -78 ˚C was added a solution of oxazolidinone **6** (23 g, 0.13 mol) in dry THF (50 mL). The mixture was stirred at -78 ˚C for 20 min and then HMPA (26 mL, 0.17 mol) was added. After stirring for an additional 30 min, a solution of 5-bromo-1-pentene (23 g, 0.16 mol) in THF (50 mL) was added. The resulting mixture was stirred overnight at room temperature, concentrated in vacuo to afford a residue, which was dissolved in dichloromethane (400 mL) and washed with water. The organic layer was dried and concentrated in

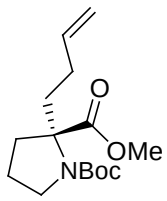
vacuo to give a residue which was purified by short path distillation (bp 80-100 ^{0}C, 0.2 mm Hg) to provide **7** as a clear oil (15.2 g, 51%). ^{1}H NMR 0.92 (s,9H),1.60-1.90 (m,5H), 2.05-2.20 (m, 2H), 2.20-2.30 (m, 1H), 2.80-2.90 (m,1H), 2.90-3.05 (m,1H), 4.90-5.10 (m,2H), 5.70-5.90 (m,1H); ^{13}C NMR, 24.20, 24.72, 28.06, 35.59, 36.77, 57.35, 71.57, 104.95, 114.59, 137.93, 178.17; The data are consistent those reported for this substance earlier. [23]



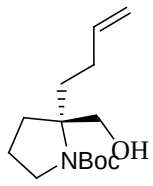
Compound 8. Oxazolidinone **7** (7.6g, 0.03 mol) was dissolved in 70 mL of MeOH/H_2O(6:1). Silica gel(7.6 g,200-400mesh) was added to the solution and the resulting suspension was stirred at room temperature for 48 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo to give a white solid which was triturated with ethyl ether to give **8** as a white solid (5.3 g, 95%); mp 280-281 ^{0}C(dec). ^{1}H NMR (D_2O) 1.90-2.20 (m,6H),2.20-2.30 (m,1H), 2.45-2.55 (m,1H), 3.35-3.50 (m,2H), 5.10-5.20 (m,2H); 5.80-5.97 (m,1H); ^{13}C NMR, 15.78, 21.79, 27.53, 28.61, 38.89, 67.46, 108.73, 129.89, 168.86; The data are consistent those reported for this substance earlier.[23]



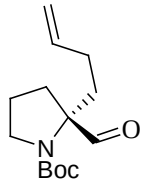
Compound 9. Substituted L-proline **8** (2.0 g, 0.011mol) and Me_4NOH (2.2 g, 0.012mol) were dissolved in 50 mL of dry acetonitrile. The mixture was stirred at room temperature until a clear solution was formed. $(Boc)_2O$ (3.9 g, 0.018 mol) was then added and stirring was continued for 2 days. On the third day, another 0.82 equiv of $(Boc)_2O$ (1.8 g, 0.009 mol) was added, and the mixture was then stirred for an additional 2 days. The acetonitrile was removed in vacuo, and the residue was partitioned between ethyl acetate and water. The aqueous layer was acidified with 1 N HCl aqueous solution to pH 2-3. The aqueous solution was extracted with ethyl acetate and dried with $MgSO_4$. The ethyl acetate extracts were concentrated in vacuo to give **9** as white crystals (2.9g, 94%), mp 89-90 ^{0}C. ^{1}H NMR 1.50 (s,9H), 1.70-1.90 (m,3H), 1.90-2.10 (m,4H), 2.30-2.40 (m,1H), 2.70-2.90 (m,1H), 3.25-3.85 (m,2H), 4.95-5.30 (m,2H), 5.70-5.90 (m,1H); ^{13}C NMR, 22.72, 28.36, 33.52, 35.07, 37.49, 49.30, 70.25, 82.11, 114.68, 115.19, 137.19, 138.02, 153.73, 157.08, 174.81, 180.55. The data are consistent those reported for this substance earlier. [23]



Compound 10. By following the procedure described by Ono et al,[20] **9** (3.0g, 11 mmol) was dissolved in benzene. DBU(1.7 g, 11 mmol) was added followed by a solution of methyl iodide(2 ml, 32 mmol) in benzene. A white precipitate appeared after ca. 10 min. The mixture was stirred at reflux overnight and concentrated in vacuo to give a residue, which was washed with water and extracted with ethyl acetate. The organic layers were dried and concentrated in vacuo to give a residue which was subjected to column chromatography (10% acetone/hexane) on silica gel to afford 10 as a clear liquid (2.5 g, 80%) ^{1}H NMR 1.30-1.50 (d, 9H), 1.70-2.10 (m, 6H), 2.15-2.40 (m, 1H), 3.30-3.45 (m, 1H), 3.69 (s, 3H), 4.80-5.10 (m, 2H), 5.70-5.90 (m, 1H); ^{13}C NMR, 22.65, 28.28, 33.30, 37.39, 48.48, 52.02, 67.26, 79.92, 114.54, 138.15, 153.70, 175.32; HRMS (FAB) m/z calcd for $C_{15}H_{25}NO_4$ (M+Na) 306.1681, found 306.1678.

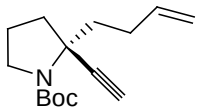


Compound 11. To a solution of **10** (10 g, 0.035 mol) in dry ethyl ether (400 mL) was added a solution of lithium borohydride (35 mL, 0.07 mol, 2M in THF) at 0˚C. The mixture was stirred for 8 hours at room tempera ture and quenched with saturated aq. sodium bicarbonate and separated. The organic layer was dried and concentrated in vacuo to afford a residue, which was subjected to column chromatography (50% acetone/hexane) on silica gel to provide **11** (8.1 g, 90%) as an oil. ^{1}H NMR 1.40 (s, 9H),1.60-2.00 (m, 6H), 2.10-2.20 (m, 1H), 3.25-3.40 (m, 1H), 3.50-3.60 (m, 1H), 4.80-5.00 (m, 2H), 5.20-5.35 (m, 1H), 5.70-5.85 (m, 1H); ^{13}C NMR, 21.89, 28.30, 31.67, 33.94, 48.61, 67.25, 68.94, 79.78, 114.17, 138.39, 155.88; HRMS (FAB) m/z calcd for $C_{14}H_{26}NO_3$ (M+1) 256.1913, found 256.1901.



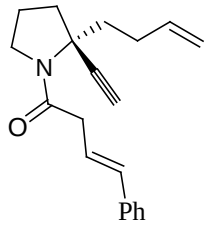
Compound 12. To a solution of **11** (6.0, 0.047 mol) in dry methylene chloride (100 mL) at 0 ˚C under nitrogen was added triethylamine (7 mL) and 40 mL of the sulfur trioxide pyridine complex(12 g) in dimethyl sulfoxide. The mixture was stirred overnight at room temperature, quenched with saturated aq. sodium chloride, washed with water, dried and concentrated in

vacuo to give a residue, which was subjected to column chromatography (20% acetone/hexane) on silica gel to provide **12** (8 g, 100%) as an oil. ^{1}H NMR 1.30-1.50(d, 9H), 1.70-2.20 (m, 8H), 3.40-3.70 (m, 2H), 4.90-5.10 (m, 2H), 5.70-5.85 (m, 1H), 9.30-9.50 (d, 1H); ^{13}C NMR, 22.76, 28.11, 31.87, 33.75, 48.09, 70.60, 80.85, 114.70, 137.83, 153.34, 199.51; HRMS (FAB) m/z calcd for $C_{14}H_{23}NO_3$ (M+Na) 276.1576, found 276.1579.



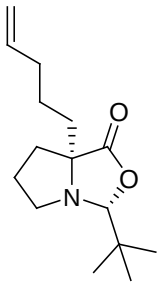
Compound 13. To a solution of $CH_3COCN_2PO(OEt)_2$ (6.0, 47 mmol) in methanol (50 mL) at room temperature under nitrogen was added potassium carbonate(8.0 g,76 mmol) resulting in a pale yellow solution. After stirring for 10 min, a solution of **12**(6.0 g, 24 mmol) in methanol (5 mL) was added to the mixture, which was then stirred overnight at room temperature. The mixture was filtered and the filtrate was concentrated in vacuo to give a residue, which was subjected to column chromatography (20% acetone/hexane) on silica gel to provide **13** (5.9 g, 100%) as an oil. ^{1}H NMR 1.47 (s, 9H),1.70-2.10 (m, 5H), 2.10-2.40 (m, 3H), 3.20-3.40 (m, 1H), 1.90-2.00 (m, 1H), 2.00-2.10 (m, 1H), 2.70-2.85 (m, 1H), 3.10-3.25 (m, 1H), 3.50-3.80 (m, 1H), 4.80-5.10 (m, 2H), 5.70-5.90(m, 1H); ^{13}C NMR, 22.54, 29.06, 38.26, 40.64,

47.86, 60.00, 69.56, 114.56, 138.03, 154.60; HRMS (FAB) m/z calcd for $C_{15}H_{23}NO_2$ (M+Na) 272.1626, found 272.1618.



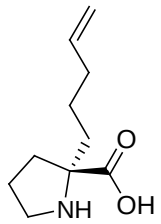
Compound 14. To a solution of compound **13** (6.0, 0.047 mol) in dry dichloromethane (10 mL) at room temperature under nitrogen was added TMSOTf (5.0 g). After stirring for 30 min, the mixture was concentrated in vacuo to afford a pale yellow residue, to which dichloromethane was added and cooled in ice-water bath. Dry triethylamine, trans-styrylacetyl chloride (made from trans-styrylacetic acid, 1.6 g, 10 mmol) and thionyl chloride (20 mL) were added. The resulting mixture was stirred overnight at room temperature, washed with 1 N HCl aqueous solution followed by water, dried and concentrated in vacuo to give a residue, which was subjected to column chromatography (20% acetone/hexane) on silica gel to provide **14** as a liquid (5.0 g, 71 %).^{1}H NMR 1.80-2.20 (m, 5H), 2.20-2.40 (m, 2H), 2.50-2.70 (m, 1H), 3.20-3.25 (m, 2H), 3.40-3.55 (m, 1H), 3.65-3.75(m, 1H), 4.85-5.15 (m, 2H), 5.70-5.90 (m, 1H), 6.35-6.55 (m, 2H), 7.20-7.55 (m, 5H); ^{13}C NMR, 23.55, 29.43, 36.67, 39.64, 40.42, 48.46, 60.84, 70.47, 85.46, 114.68,

123.01, 126.22, 127.31, 128.45, 132.61, 137.97, 168.99; HRMS (FAB) m/z calcd for $C_{20}H_{23}NO$ (M+1) 294.1858, found 294.1864.

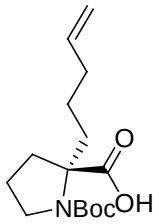


Compound 15. To a solution of LEDA (made from diethylamine (16 mL, 0.154 mol) in THF (700 mL) and n-butyllithium (60 mL, 0.15 mol, 2.5 M in hexane)) at -78 ˚C was added a solution of oxazolidinone **6** (23 g, 0.126 mol) in dry THF (50 mL). The mixture was stirred at -78 ˚C for 20 min and then HMPA (26 mL, 0.173 mol) was added. After an additional 30 min, a solution of 5-bromo-1-pentene (23 g, 0.16 mol) in THF (50 mL) was added. The resulting mixture was stirred overnight at room temperature, concentrated in vacuo to afford a residue, which was dissolved in dichloromethane (400 mL), washed with water. The organic layer was dried and concentrated in vacuo to give a residue which was purified by short path distillation (bp 90-120 ^{0}C,0.2 mmHg) to provide **15** as a clear oil (15.6 g, 50%). $[\alpha]_D^{23}$ -13.20 (*c* 2.1, MeOH); ^{1}H NMR 0.87 (s, 9H), 1.40-1.90 (m, 6H), 2.00-2.15 (m, 3H), 2.80-2.90 (m, 1H), 2.90-3.05 (m, 1H), 4.10-4.20 (s, 1H), 4.90-5.00 (m, 2H),

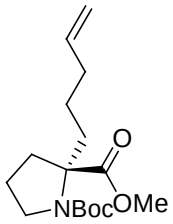
5.70-5.85 (m, 1H); ^{13}C NMR, 23.02, 24.20, 24.72, 33.70, 35.63, 36.30, 57.37, 71.74, 104.93, 114.72, 138.29, 178.45. HRMS (FAB) m/z calcd for $C_{15}H_{25}NO_2$ (M+1) 252.1964, found 252.1960.



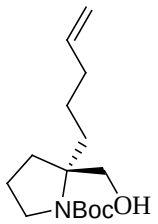
Compound 16. Oxazolidinone **15** (7.6 g, 0.03 mol) was dissolved in 100 mL of MeOH/H_2O (6:1). Silica gel (7.6 g, 200-400 mesh) was added to the solution and the resulting suspension was stirred at room temperature for 48 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo to give a white solid which was triturated with ethyl ether to give **16** as a white solid (5.3 g, 95%), mp 300 ^{0}C(dec). $[\alpha]_D^{23}$ -6.40 (*c* 1.1, MeOH); ^{1}H NMR (D_2O) 1.30-1.50 (m, 2H), 1.75-1.85 (m, 1H), 1.90-2.20 (m, 7H), 2.30-2.50 (m, 1H), 3.35-3.50 (m,2H), 5.05-5.20 (m, 2H); 5.80-5.97 (m, 1H); ^{13}C NMR, 15.89, 16.58, 25.59, 27.84, 28.36, 38.68, 67.59, 107.98, 131.34, 169.21. HRMS (FAB) m/z calcd for $C_{10}H_{17}NO_2$ (M+1) 184.1338, found 184.1337.



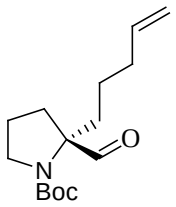
Compound 17. Substituted L-proline **16** (2.0 g, 11 mmol) and Me_4NOH (2.2 g, 0.012mol) were dissolved in 50 mL of dry acetonitrile. The mixture was stirred at room temperature until a clear solution formed. $(Boc)_2O$ (3.9 g, 0.018 mol) was then added and stirring was continued for 2 d. On the third day, another 0.82 equiv of $(Boc)_2O$ (1.8 g, 0.009 mol) was added, and the mixture was stirred for 2 d. The acetonitrile was removed in vacuo, and the residue was partitioned between ethyl acetate and water. The aqueous layer was acidified with 1 N HCl aqueous solution to pH 2-3 and extracted with ethyl acetate. The extracts were dried and concentrated in vacuo to give 17 as white crystals (2.9 g, 94%), mp 92-93 ^{0}C. $[\alpha]_D^{23}$ -50.40 (*c* 30.0, MeOH); ^{1}H NMR 1.20-1.40 (m, 2H), 1.40-1.70 (d, 9H), 1.70-2.00 (m, 4H), 2.00-2.40 (m, 4H), 2.60-2.90 (s, 1H), 3.30-3.90 (m, 2H), 4.90-5.20 (m, 2H), 5.70-6.00 (m, 1H); ^{13}C NMR, 22.70, 23.36, 28.35, 33.49, 33.76, 35.08, 49.32, 70.45, 82.06, 115.05, 137.99, 157.13, 174.91, 180.55. HRMS (FAB) m/z calcd for $C_{15}H_{25}NO_4$ (M+Na) 306.1681, found 306.1677.



Compound 18. Following the procedure described by Ono et al,[20] **17** (6.0 g, 21 mmol) was dissolved in benzene. DBU(4.0 g, 26 mmol) was added followed by a solution of methyl iodide(4.0 mL,64 mmol) in benzene. A white precipitate appeared after ca. 10 min. The mixture was stirred at reflux overnight and concentrated in vacuo to give a residue, which was washed with water and extracted with ethyl acetate. The organic layer was dried and concentrated to give a residue which was subjected to column chromatography (10% acetone/hexane) on silica gel to afford 18 as a clear liquid (5.4 g, 80%) 98% ee; $[\alpha]_D^{23}$ +13.80 (*c* 2.2, MeOH); ^{1}H NMR 1.30-1.50 (d, 11H), 1.75-2.20 (m, 9H), 3.30-3.45 (m, 1H), 3.70 (s, 3H), 4.90-5.10 (m, 2H), 5.70-5.90 (m, 1H); ^{13}C NMR, 22.75, 28.31, 33.87, 34.58, 37.45, 48.51, 52.04, 67.39, 79.87, 114.90, 138.47, 153.77, 175.56. HRMS (FAB) m/z calcd for $C_{16}H_{27}NO_4$ (M+1) 320.1838, found 320.1831.

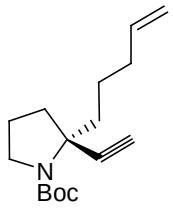


Compound 19. To a solution of **18** (10 g, 0.035 mol) in dry ethyl ether (200 mL) was added a solution of lithium borohydride (35 mL, 0.07 mol, 2M in THF) at 0°C. the mixture was stirred overnight at room temperature and quenched by the addition of satd aq. sodium bicarbonate. The organic layer was separated, dried and concentrated in vacuo to afford a residue, which was subjected to column chromatography (50% acetone/hexane) on silica gel to provide **19** (8.1 g, 90%) as an oil. $[\alpha]_D^{23}$ +6.80 (*c* 0.90, MeOH); ^{1}H NMR 1.40-1.70 (m, 13H), 1.70-2.20 (m, 8H), 3.30-3.40 (m, 2H), 3.63 (s, 2H), 4.90-5.05 (m, 2H), 5.20-5.50 (s, 1H), 5.70-5.90 (m, 1H); ^{13}C NMR, 22.07, 23.84, 28.48, 32.03, 34.11, 48.78, 67.62, 69.36, 80.00, 114.54, 138.76, 156.17. HRMS (FAB) m/z calcd for $C_{15}H_{27}NO_3$(M+Na) 292.1889, found 292.1886.



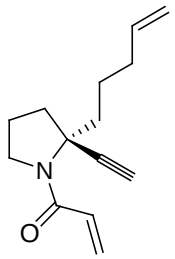
Compound 20. To a solution of **19** (6.0 g, 0.047 mol) in dry methylene chloride (100 mL) at 0 °C under nitrogen was added triethylamine (7 mL) and 40 mL of the sulfur trioxide pyridine complex(12 g) in dimethyl sulfoxide. The mixture was stirred overnight at room temperature, quenched by the

addition of satd aq. sodium chloride. The organic layer was separated, washed with water, dried and concentrated in vacuo to give a residue, which was subjected to column chromatography (20% acetone/hexane) on silica gel to provide **20** (8 g, 100%) as an oil. $[\alpha]_D^{23}$ +2.60 (*c* 0.6, MeOH); ^{1}H NMR 1.20-1.50 (d, 9H), 1.70-2.20 (m, 8H), 3.20-3.70 (m, 2H), 4.90-5.30 (m, 5H), 5.70-5.90 (m, 1H), 9.30-9.50 (d, 1H); ^{13}C NMR, 22.85, 28.19, 32.14, 33.97, 70.86, 80.88, 115.05 138.18, 153.49, 199.88. HRMS (FAB) m/z calcd for $C_{15}H_{25}NO_3$ (M+Na) 290.1732, found 290.1739.



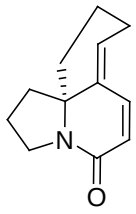
Compound 21. To a solution of $CH_3COCN_2PO(OEt)_2$ (6.0, 47 mmol) in methanol (30 mL) at room temperature under nitrogen was added potassium carbonate(8.0 g,76 mmol). After stirring for 10 min, a solution of **20** (6.0 g, 24 mmol) in methanol (5 mL) was added to the mixture, which was then stirred overnight at room temperature. Filtration gave a filtrate which was concentrated in vacuo to give a residue, which was subjected to column chromatography (20% acetone/hexane) on silica gel to provide **21** (6.4 g, 100%) as an oil. $[\alpha]_D^{23}$ -9.20 (*c* 0.8, MeOH); ^{1}H NMR 1.47 (s, 9H), 1.50-

1.60 (m, 1H), 1.60-1.90 (m, 2H), 1.90-2.40 (m, 7H), 3.20-3.35 (m, 1H), 3.50-3.80 (m, 1H); 4.80-5.10 (m, 2H), 5.70-5.90 (m, 1H); ^{13}C NMR, 22.51, 28.49, 33.69, 38.60, 40.70, 47.88, 59.50, 69.29, 79.84, 86.83, 114.74, 138.45, 154.23. HRMS (FAB) m/z calcd for $C_{16}H_{25}NO_2$ (M+1) 286.1783, found 286.1792.

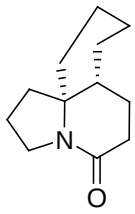


Compound 22. To a solution of **21** (6.0 g, 21 mol) in dry dichloromethane (10 mL) at room temperature under nitrogen was added TMSOTf (10.0 g,), After stirring for 30 min, the mixture was concentrated in vacuo to afford a pale yellow residue to which dichloromethane was added. After cooling the solution to 0 ^{o}C, dry triethylamine(7 mL) and then acryloyl chloride(5 ml,62 mmol) were added. The resulting mixture was stirred overnight at room temperature, washed with 1 N HCl followed by water, dried, and concentrated in vacuo to give a residue, which was subjected to column chromatography (20% acetone/hexane) on silica gel to provide **22** as a liquid (2.3 g, 50%).$[\alpha]_D^{23}$ -88.20 (*c* 24.0, MeOH); ^{1}H NMR 1.30-1.40 (m, 1H),

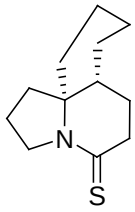
1.60-1.90 (m, 3H), 1.90-2.20 (m, 6H), 2.30-2.40 (m, 1H), 2.40-2.45 (m, 1H), 3.55-3.65 (m, 1H), 3.75-3.95 (m, 1H), 4.90-5.10 (m, 2H), 5.65-5.70 (m, 1H), 5.70-5.90 (m, 1H), 6.30-6.40 (m, 1.6H), 6.90-7.05 (m, 0.4H); ^{13}C NMR, 21.95, 23.55, 24.18, 33.64, 36.98, 39.58, 40.41, 41.70, 48.00, 60.98, 70.19, 72.85, 85.58, 114.69, 126.74, 127.86, 129.54, 138.52, 163.58. HRMS (FAB) m/z calcd for $C_{13}H_{17}NO$ (M+1) 218.1545, found 218.1561.



Compound 23. A solution of **22** (0.5 g, 2.3 mmol) in dry methylene chloride (500 mL) containing 10% mol Grubbs II generation catalyst was stirred at reflux for 5 h, cooled and concentrated in vacuo to give a residue, which was subjected to column chromatography (10% acetone/hexane) on silica gel to afford **23** (0.31 g, 70%) as an oil. $[\alpha]_D^{23}$ -5.60 (*c* 1.1, MeOH); ^{1}H NMR 1.40-1.60 (m, 2H), 1.70-1.90 (m, 4H), 1.90-2.10 (m, 2H), 2.20-2.40 (m, 2H), 3.00-3.20 (m, 1H), 4.30-3.45 (m, 1H), 5.65-5.75 (m, 1H), 5.80-5.90 (m, 1H), 5.55-5.65 (m, 1H); ^{13}C NMR, 17.78, 19.98, 23.80, 30.11, 32.99, 42.04, 63.71, 119.71, 131.74, 135.10, 138.34, 165.61. HRMS (FAB) m/z calcd for $C_{12}H_{15}NO$ (M+1) 190.1232, found 190.1237.

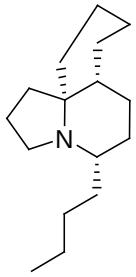


Compound 24. A solution of **23** (0.30 g, 1.6 mmol) and Pd/C (10%, 30 mg) in MeOH (100 mL) under a hydrogen atmosphere was stirred for 5 h. The filtrate obtained by filtration was concentrated in vacuo giving a residue which was subjected to column chromatography (50% acetone/hexane) on silica gel to afford **24** (0.25 g, 81%) as an oil. $[\alpha]_D^{23}$ -4.60 (*c* 0.58, MeOH); ^{1}H NMR 1.30-2.00 (m, 12H), 2.10-2.20 (m, 1H), 2.30-2.40 (m, 2H), 2.45-2.55 (m, 1H), 3.40-3.50 (m, 1H), 3.75-3.85 (m, 1H); ^{13}C NMR, 19.60, 20.07, 22.71, 23.39, 28.78, 29.89, 30.84, 35.87, 40.06, 43.76, 64.01, 168.67. HRMS (FAB) m/z calcd for $C_{12}H_{19}NO$ (M+1) 194.1545, found 194.1550.



Compound 25. A solution of Lawesson's reagent (2.1 g, 5.2 mmol) and **24** (0.5 g, 2.6 mmol) in toluene (50 mL) was stirred at reflux for 4-24 h, cooled and concentrated in vacuo to give a residue, which was subjected to column chromatography (dichloromethane to 20% acetone/hexane) on silica gel to

afford **25** as a white solid (0.54 g, 100%), mp 86-88 ^{0}C. $[\alpha]_D^{23}$ -4.20 (*c* 0.65, MeOH); ^{1}H NMR 1.30-1.80 (m, 12H), 1.90-2.20 (m, 3H), 2.45-2.55 (m, 1H), 2.90-3.05 (m, 1H), 3.15-3.25 (m, 1H), 3.75-3.85(m, 1H), 4.15-4.25 (m, 1H); ^{13}C NMR, 19.36, 22.28, 23.24, 28.82, 29.06, 35.39, 39.28, 39.97, 52.06, 67.70, 195.44. HRMS (FAB) m/z calcd for $C_{12}H_{19}NS$ (M+1) 210.1316, found 210.1316.



Lepadiformine C Epimer.

1. A solution of **25** (48 mg, 0.23 mmol) in anhydrous ethyl ether (2 mL) containing 0.2 mL of methyl iodide was stirred overnight at room temperature, the solvent was decanted. The remaining crystalline solid was washed with Et_2O and dried to give the S-methyl-thioiminium salt of **25**. To a solution of 10 mL of the S-methyl-thioiminium salt of **25** in dry dichloromethane at -78 ^{o}C, 0.15 mL of a solution of butylmagnesium chloride (2.0 M in ethyl ether) was added. The reaction mixture was allowed to warm to room over 4 hours period. After lowering the temperature to -20 ^{0}C,

sodium borohydride (15 mg, 0.41 mmol) was added, and the resulting mixture was stirred for 10 min and quenched by the addition of acetic acid (0.5 mL). After stirring for 1 h while the temperature was raised from 0 ^{0}C to 25 ^{0}C, the solution poured into water, made alkaline by addition of solid sodium carbonate, and extracted with ether. The combined organic extracts were dried, filtered, and concentrated to give lepadiformine C Epimer **as** a oil. HPLC purification gave pure material (16 mg, 30%).

2. A solution of **25** (48 mg, 0.23 mmol) in anhydrous ethyl ether (2 mL) containing 0.2 mL of methyl iodide was stirred overnight at room temperature, the solvent was decanted. The remaining crystalline solid was washed with Et_2O and dried to give the S-methyl-thioiminium salt of **25**. To a solution of 10 mL of the S-methyl-thioiminium salt of **25** in dry dichloromethane at -78 ^{o}C, 0.15 mL of a solution of butylmagnesium chloride (2.0 M in ethyl ether) was added. The reaction mixture was allowed to warm to room over 4 hours period. Methanol(2 mL) was added, and the resulting solution was concentrated under reduced pressure. HCl(4 M) in methanol(5 mL) was added and stirred for 4-8 hours at room temperature. The resulting mixture was concentrated under reduced pressure to afford a residue, which was subjected to chromatography on silica gel(methanol:dichloromethane:4M

HCl/methanol 3:7:0.5) to give the iminium salt, which was hydrogenated with PtO_2 or Pd/C in acetic acid(5 mL) to give lepadiformine C Epimer **as** a oil. HPLC purification gave pure material (11 mg, 20%). ^{1}H NMR 0.89 (t, 3H), 1.20-1.50 (m, 12H), 1.60-2.20 (m, 11H), 2.60-2.90 (m, 2H), 3.00-3.20 (m, 1H); ^{13}C NMR, 14.09, 20.38, 22.79, 24.31, 24.84, 26.00, 28.79, 29.70, 34.86, 37.01, 44.00, 49.17, 53.40. HRMS (FAB) m/z calcd for $C_{16}H_{29}N$ (M+1) 236.2378, found 236.2371.

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CHAPTER THREE

Design and Synthesis of

A Novel Inhibitor of the *Giardia lamblia* Class II Frutose-1,6-Bisphosphate Aldolase

3.1 Introduction

Background. *Giardia lamblia* is a flagellated protozoan that causes severe diarrhea in humans and it is the most common cause of waterborne outbreaks of diarrhea in the United States.[1] *Giardia* infections, termed giardiasis, are prevalent in developing countries and chronic giardiasis at a young age results in growth retardation. Water contaminated with *Giardia lamblia* cysts is a common cause of travel-related giardiasis in tourists. In a 1998 World Health Organization press release[2] it was reported that there were close to 1,000 million cases of giardiasis at any one time in the world that contributed to 2.5 million deaths annually.

Giardia lamblia has been classified as a category B organism by the Center for Disease Control as part of its plan to respond to bioterrorism threats. *Giardia Lamblia* compromised water and food supplies could affect

US populations as well as the military since the cysts are highly stable in the environment and only a few are sufficient to infect mammalian hosts. Transfection and transformation protocols for this protist are well developed and, as a result, it is easy to manipulate the organism in the laboratory. Because giardiasis is considered primarily a third-world problem, little in the way of profit motive exists to drive drug development by the pharmaceutical industry. In addition, given the ease of induction of resistance,[3,4] there is a need to identify Giardia drug targets that will provide alternative treatments when resistance to the traditional drugs becomes widely spread.

A major strategy to identify antimicrobial drugs which act at the enzymatic level involves a biological rational and a genomic approach. Specifically, candidate enzyme targets are selected based on a consideration of those that are essential for the survival of the pathogen and, ideally, that are either absent in the human genome or sufficiently diverged from the human counterpart.[5,6] Discrimination between human and pathogen enzymes is an important factor to consider in order to prevent or minimize deleterious consequences of the therapy.

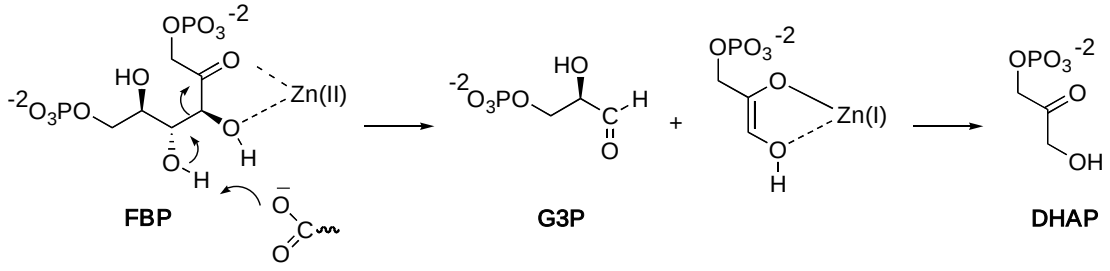
Class II Fructose-1,6-bisphosphate Aldoloase. Enzymes that effect viability (inactivation causes death) or growth (inactivation causes stasis)

should be essential for Giardia survival. Both types of enzymes can serve as potential drug targets. Among many essential enzymes in Giardia of interest in the collaborative group headed by Herzberg, Dunaway-Mariano and Mariano, the class II fructose-1,6-biphosphate aldolase (glFBPA), which catalyzes the reversible cleavage of D-fructose-1,6-biphosphate (FBP) to dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (G3P) has been targeted for study. glFBPA is involved in a key step in the energy (ATP) producing Embden-Meyerhof-Parnas glycolytic pathway. Because *Giardia lamblia* lacks mitochondria as well as the components of oxidative phosphorylation, glucose degradation via glycolysis serves as its major source of ATP.[7-9] As a result, selective inhibition of the *gl*FBPA might disrupt the functioning of the glycolytic pathway and thereby hinder survival of this organism. Support for this proposal comes from the observation that inhibition of FBPA gene transcription brings about the death of Giardia trophozoites (unpublished results of experiments performed in a collaborative effort with Nash at NIH). This result suggests that FBPA is necessary for the survival of the organism under optimal laboratory growth conditions.

Unlike the mammalian FBPA, which is a class I aldolase, the *gl*FBPA belongs to the class II family.[10] The former FBPAs use an active site lysine

residue to activate the substrate for C(3)–C(4) bond cleavage via protonated Schiff base formation. In contrast, class II FBPAs use a Zn(II) cofactor to coordinate both the C(2) carbonyl and C(3) hydroxyl oxygens of the substrate, consequently stabilizing the enediolate intermediate formed by C(3)–C(4) retro-aldol promoted bond cleavage (Scheme 1).[11,12] Importantly, a genomic sequence analysis shows that FBPA is the only Giardia class II aldolase and that the Giardia genome does not encode a class I aldolase. The divergence in class I and II aldolase structures and their catalytic mechanisms forms the basis of a potentially novel strategy, based on selective inhibition of FBPA, for the design of drugs for treatment of infections caused by *Giardia lamblia*.

Scheme 1. The catalytic mechanism of class II FBPAs



Structural Information for *gl*FBPA. Recently, crystal structures of both apo-glFBPA and of the enzyme complexed with the dihroxyacetone-phosphate analog, phosphoglycolohydroxamate (PGH) were solved (Figure

3).[13] The crystal structure of *gl*FBPA in complex with PGH shows that the enzyme is homodimeric and that its active site contains a Zn(II) ion. The structure, together with the kinetic properties of site-directed mutants (Table 1) measured in parallel unpublished studies provide significant insight into active site residues that participate in substrate binding and catalysis by this enzyme.[14-16] The *gl*FBPA active site is a highly polar cavity located at the C-terminal end of the β–strands of the barrel, a feature shared with other α/β barrels.[17] For discussion purposes, the active site is subdivided into the Zn^{2+}, dihydroxyacetone phosphate (DHAP) and glyceraldehydes-3-phosphate (G3P) binding sites. The DHAP site is occupied by the inhibitor PGH, whereas the G3P site is unoccupied, and its properties will be inferred based on a model of the *gl*FBPA containing the bound intact substrate. The catalytic Zn^{2+} is coordinated to the imidazole rings of His-84, His-178, and His-210 located at this site and with the C(2)=O and C(3) hydroxyl oxygen atoms of the substrate, fructose-1,6-bisphosphate (Figure 4). The tight binding inhibitor phosphoglycollohydroxamate[18] (PGH) mimics the enediolate intermediate formed in the retro-aldol process which, in the ensuing step of catalysis, undergoes protonation to form DHAP (Scheme 1). The inhibitor binds in a deep polar cavity with the phosphate moiety stationed in a site enriched with

the positive dipoles of four backbone amide groups (G-179, S-213, D-255, and S-256) (Figure 4). In addition, the phosphate group interacts with the ammonium group of K-182 on the 174-194 flexible loop and with the hydroxyl groups of S-213 and S-256.

Figure 3. Crystal structure of glFBPA II with PGH inhibitor

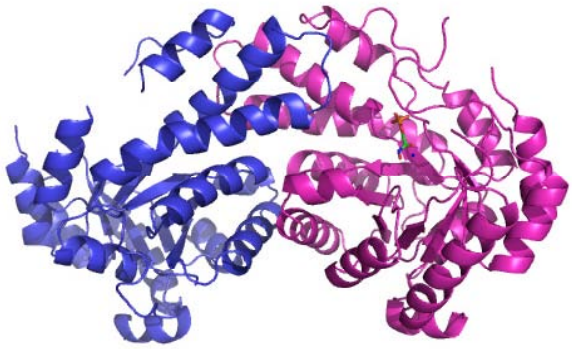


Table 1. Steady State Kinetic Constants of wild type and mutant glFBPAs.[a.b]

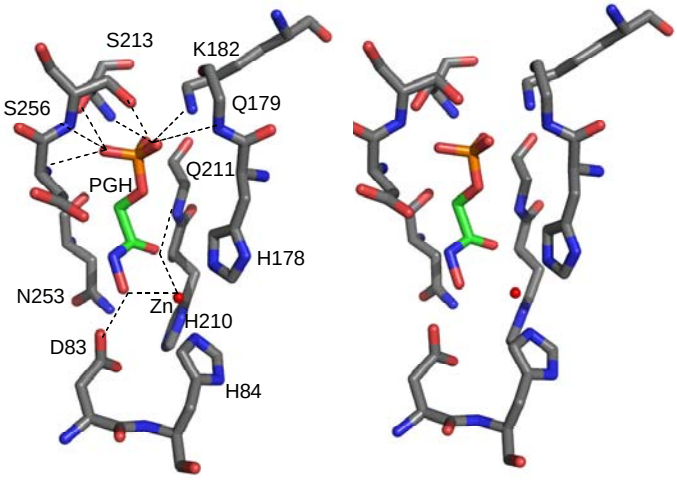
*gl*FBPA	k_{cat} (s^{-1})	K_m (µM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
WT	3.55 ± 0.05	1.7 ± 0.1	2.1 x 10^6
N253D	0.025 ± 0.001	240 ± 31	100
D83A	1.47 x 10^{-5}	ND	ND
N253A	0.37 ± 0.02	15 ± 3	2.5 x 10^4
E135A	0.005 ± 0.0005	34 ± 5	150
E143A	0.045 ± 0.0005	1.7 ± 0.1	2.87 x 10^4
G137/140/141A	0.005 ± 0.0001	9.0 ± 0.6	600

N24A	0.014 ± 0.0001	8.4 ± 0.4	1.7×10^3
S50A	0.0006 ± 0.00002	31 ± 4	18

(a) Reactions were performed using FBP as substrate at pH 7.5

(b) Data from unpublished work of Ling Li and Catherine Wright

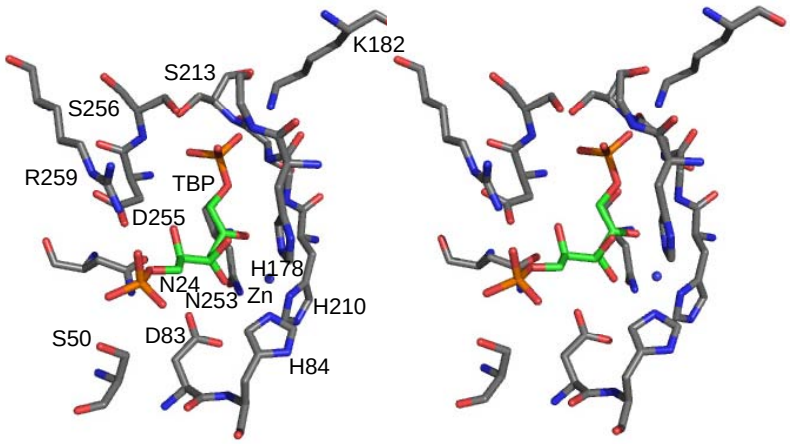
Figure 4. Binding site of the glFBPA complex with PGH.



The interaction of groups in *gl*FBPA with the G3P moiety is assessed by first using a model of the enzyme-FBP complex (not shown) and then by inspection of the structure of *gl*FBPA complexed to the tight binding inhibitor D-tagatose-1,6-bisphosphate (TBP) (Figure 5), a C(4) hydroxyl epimer of FBP, that binds tight to the enzyme but it can not be converted to products.[19] In constructing the model of the enzyme-FBP complex, the PGH-binding mode was used to define the orientation of the DHAP-forming end of FBP

and interaction with the G3P-forming end was optimized. Inspection of the model leads to the prediction that D-83 binds the FBP through electrostatic interactions formed between the side chain carboxylate group and the substrate C(3) and C(4) hydroxyl groups. In addition, the model assumes that D-83 functions as the general base to deprotonate the C(4) hydroxyl as part of the retro-aldol cleavage pathway. The *gl*FBPA TBP structure confirms many features of the model.

Figure 5. Binding site of the glFBPA complex with D-tagatose-1,6-bisphosphate (TBP).



Strategy for *gl*FBPA Inhibitor Design. The strategy we have devised for the design of class II *gl*FBPA inhibitors involves the incorporation of three moieties that individually bind to the catalytic Zn(II) ion (zinc-binding site), and interact through hydrogen bonding or charge attraction with residues in

the active site that bind phosphate dianion groups at C-1 (DHAP site) and C-6 (G3P site) of the natural substrate FBP.[20] Properly positioned phosphate, or better yet phosphonate, groups will be employed to take advantage of the latter interactions. 3-Hydroxy-4-pyrone, 3-hydroxy-2-pyridone and 3-hydroxy-4-pyridone moieties (Scheme 2) have been selected to play the role of Zn(II) binding groups owing to the fact that the substances possessing these groups are known to have (1) good zinc binding properties,[21,22] (2) ready synthetic accessibility,[23] and (3) potentially high biocompatibility.[24] To determine if potential inhibitors based on this design would be accommodated in the active site of *gl*FBPA and if interactions between their phosphate/phosphonate and zinc binding groups would mimic those with the natural substrate FBP, the 3-hydroxy-4-pyranones and 3-hydroxy-2-pyridones were modeled into the active site of the enzyme. As can be seen by viewing the structures of the model complex between *gl*FBA and the (R)- and (S)-enantiomer of 3-hydroxy-2-pyridone **10** (Figure 6), the phosphonate, phosphate and hydroxy-carbonyl moieties of pyridone can be properly located in regions responsible for binding the C-1 and C-6 phosphate and hydroxyketone groups of the natural substrate FBA.

Scheme 2.

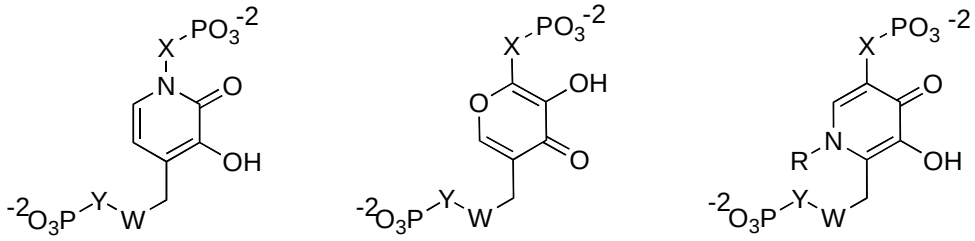
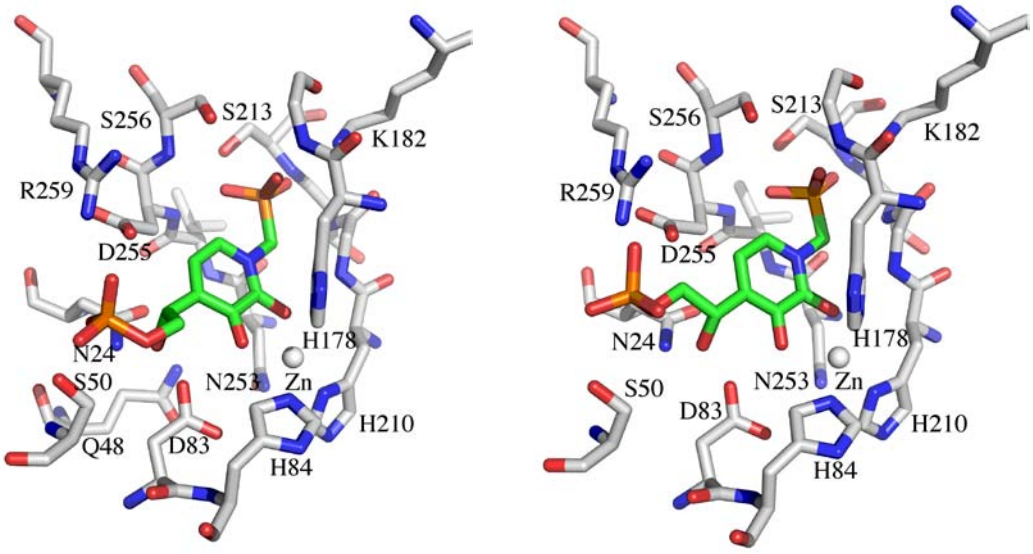


Figure 6. Docking derived structure of (R)- and (S)-enantiomer of pyridone **10** with the *gl*FBPA.

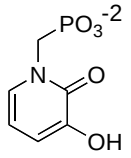


Research Plan. In initial studies testing the validity of the design strategy, we have prepared representative examples from the 3-hydroxy-2-pyridone and 3-hydroxy-4-pyrone families (Scheme 3) that contain two or three of the key components we identified as being required for binding to the DHAP, G3P and Zn(II) sites of *gl*FBPA. For example, N-phosponomethyl-3-hydroxy-2-pyridone **1** possesses an ideal Zn(III) binding moiety along with a

phosphonate positioned to interact with amino acid side chains of *gl*FBPA (*eg.*, S213, K182) that aid in binding the C-1 phosphate dianion of the substrate FBP. In contrast, the C-4 substituted-3-hydroxypyridones **2-5** and hydroxy-pyranone **6** contain side chains that should mimic the C-6 phosphate dianion of FBP which interacts with amino acid side chains of R258 and S50 in *gl*FBPA. Finally, the C-4 substituted N-phosphonomethyl-3-hydroxy-pyridones **7-10** should take abvantage of all three key binding interactions associate with the DHAP, G3P and Zn(II) sites in the aldolase.

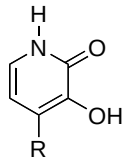
Scheme 3.

DHAP + Zn(II) Sites

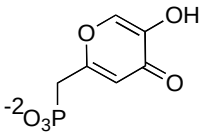


1

G3P + Zn(II) Sites

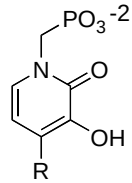


2 (R = $CH_2CH_2PO_3^{-2}$)
3 (R = $CH_2OCH_2PO_3^{-2}$)
4 (R = $CH_2CH_2OPO_3^{-2}$)
5 (R = (R)-$CHOHCH_2OPO_3^{-2}$)



6

DHAP + G3P + Zn(II) Sites

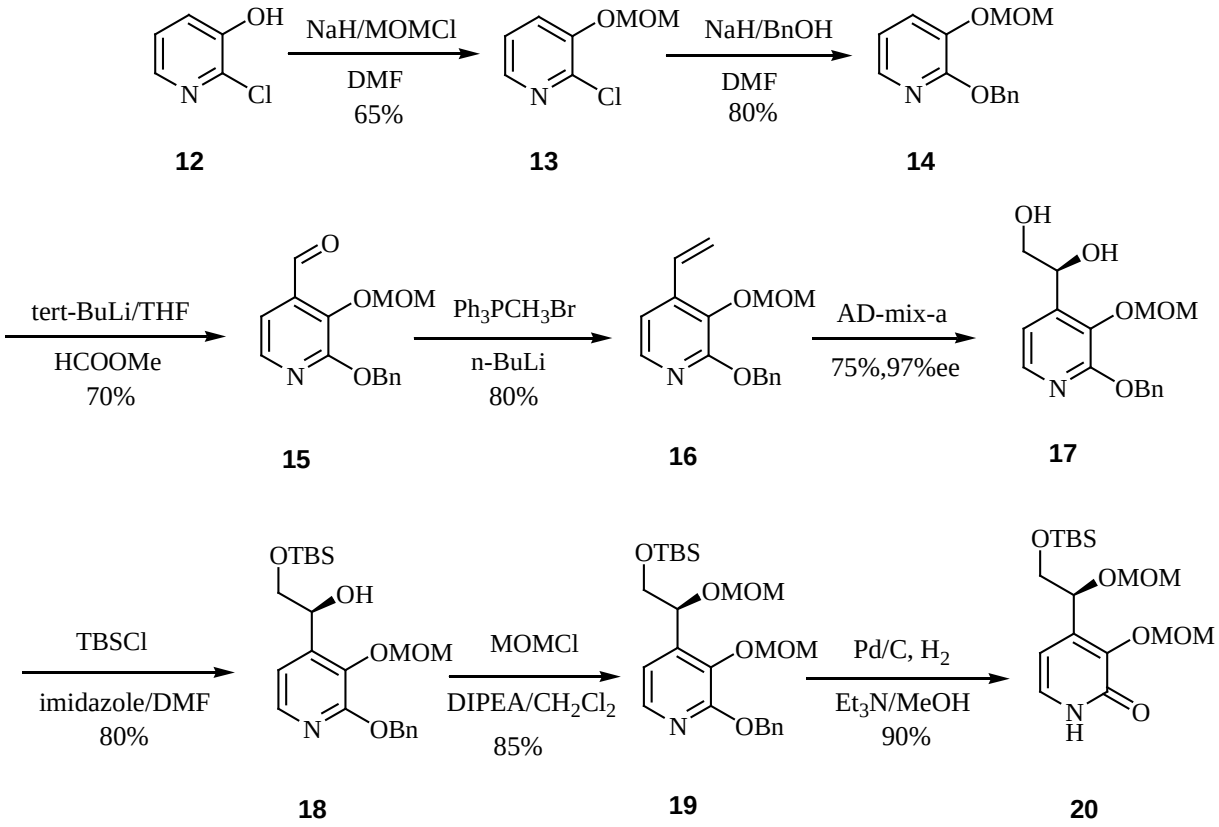


7 (R = $CH_2CH_2PO_3^{-2}$)
8 (R = $CH_2OCH_2PO_3^{-2}$)
9 (R = $CH_2CH_2OPO_3^{-2}$)
10 (R = (R)-$CHOHCH_2OPO_3^{-2}$)
10 (R = (S)-$CHOHCH_2OPO_3^{-2}$)

3.2 Total Synthesis of Novel Inhibitor of the Class II *Giardia lamblia* Fructose-bisphosphate Aldolase

In the beginning of the route targeted at the synthesis of the (S)-enantiomer of the alcohol side chain containing N-phosphonomethyl-3-hydroxy-pyridone **10** (Scheme 4), MOM-protected 2-chloro-3-hydroxypyridine **13**[25] was generated and converted to the benzyloxy-analog **14** (Scheme 4) Sequential C-4 formylation of **14** followed by Wittig olefination of the derived aldehyde **15** produces alkene **16**.

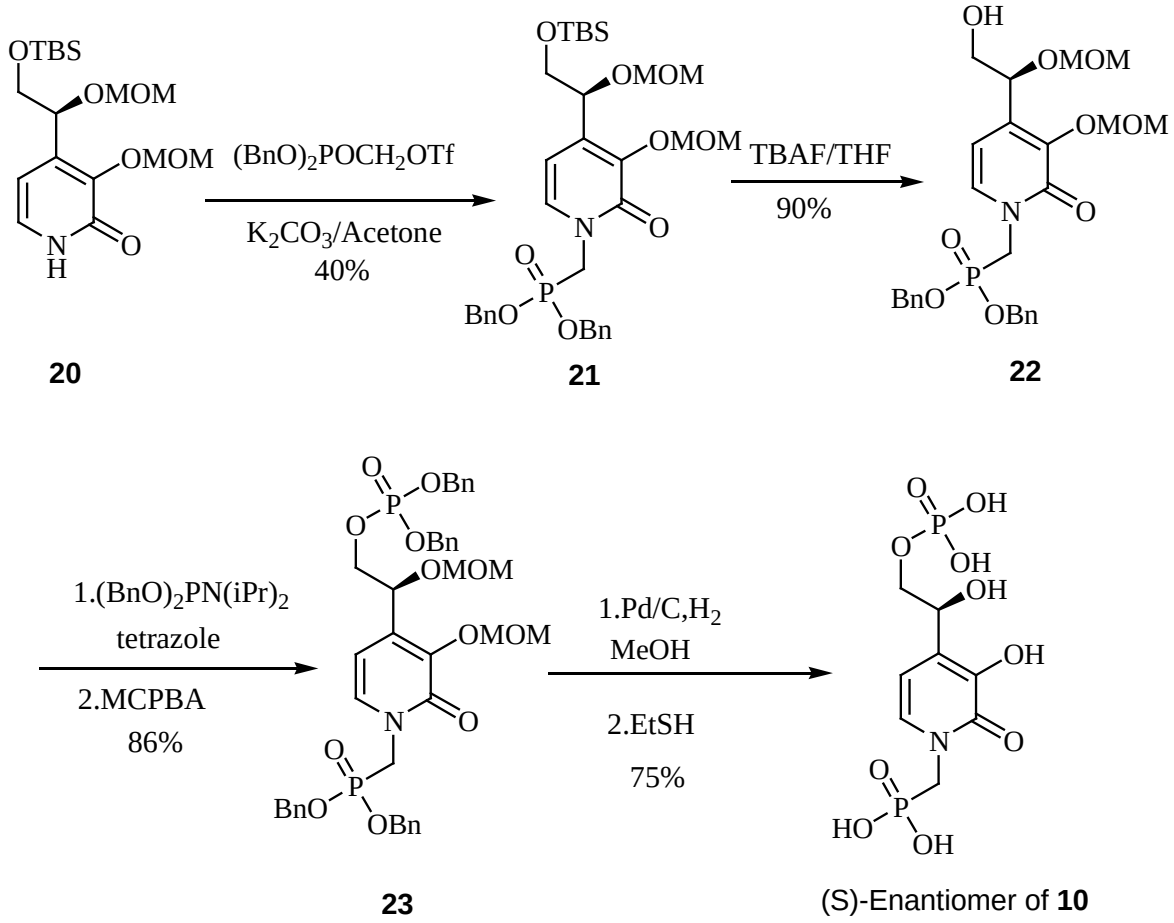
Scheme 4.



Sharpless dihydroxylation[26] with AD-mix-α was used to enatioselectively generate diol **17** with a 97% ee. Differential protection of the hydroxyl groups in **17** followed by hydrogenolysis leads to generation of the pyridone **20**.

Pyridone **20** is then N-alkylated by using the known[27] dibenzyl phosphonomethyl triflate to form **21** (Scheme 5). Selective liberation of the terminal hydroxyl group provides alcohol **22** which is then converted to the phosphate **23** by using a known procedure.[28] Finally the (S)-enantiomer of **10** is obtained by using a phosphorylation and MOM deprotection sequence (Scheme 5).

Scheme 5.



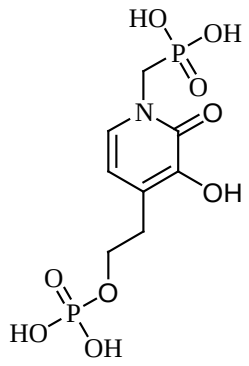
3.3 Inhibition Constants of Novel Inhibitors of the Class II *Giardia lamblia* Fructose-bisphosphate Aldolase

The inhibition constants for inhibitor **10** prepared in the effort described above, as well the related hydroxy-2-pyridones **9** and **(R)-10** synthesized by my collaborator Dr. Zhengang Liu, were determined by my collaborator

Zhiming Li. For this purpose, a spectrophotometric based coupled assay system was employed to monitor the initial velocity of *gl*FBPA catalyzed reaction of FBP to produce DHAP and G3P. The assay involves measurement of absorbance at 340 nm, associated with glycero-3-phosphate dehydrogenase promoted, NADH dependent conversion of DHAP to glycerol-3-phosphate. Initial velocities were measured for reactions initiated at 25 °C by the addition of FBP (0.5 K_m-10K_m) to 1 mL (final volume) solutions containing *gl*FBPA (0.02 μM) in 50 mM HEPES buffer at pH 7.5, 200 μM NADH, 5 units (1 μg/mL) of triosephosphate isomerase, and 2 units (11.76 μg/mL) of glycerol-3-phosphate dehydrogenase. The kinetic parameters of V_{max} and K_m were obtained from the analysis of initial velocity *vs.* FBP concentration data by using eq. 1 for competitive inhibition or eq 2 for mixed-type inhibition and the computer program KinetAsystI. In equations 1, [S] = substrate concentration, [I] = inhibitor concentration V_0 = initial velocity, V_{max} = maximum velocity and K_m = Michaelis constant, K_I is the inhibition constant, and K_{is} and K_{ii} are the respective slope and intercept inhibition constants.

$$V = V\text{max}[S] / [K\text{m}(1+[I] / K\text{i})+[S]] \quad (1)$$

$$V = V\text{max}[S] / [K\text{m}(1+[I] / K\text{is})+[S](1+[I] / K\text{ii})] \quad (2)$$



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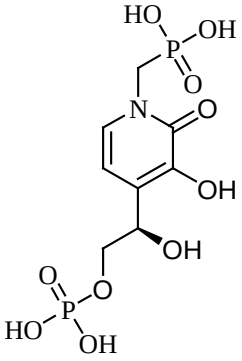
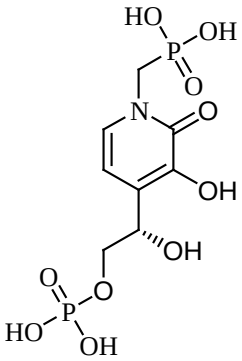


(R)-Enantiomer of **10**



(S)-Enantiomer of **10**

K_i =14 ± 4 µM

K_i =90 ± 6 µM

K_i =10 ± 2 µM

These results have demonstrated that the inhibitory constants (K_I) of inhibitors **9**, **(R)-10** and **(S)-10** against the Giardia *lamblia* fructose-bis-1,6-phosphate aldolase are in the low µM region. Interestingly, the (R)-enantiomer of **10** is a more weakly binding inhibitor of this enzyme than is the non-hydroxyl counterpart **9**. The (S)-enantiomer of **10** shows much better inhibition than the (R)-enantiomer and slightly better inhibition than **9**. Through comparison of the docking derived structure in the active site of *gl*FBPA (Figure 4), it appears that the proper orientation of phosphate groups and zinc binding groups accounts for the better inhibition by **(S)-10**.

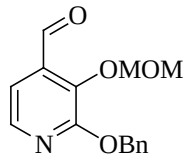
3.4 Conclusion

A novel inhibitor of the Giardia *lamblia* fructose-bis-1,6-phosphate aldolase that possess Zn^{+2} binding 3-hydroxy-2-pyridone moiety was prepared. The inhibitory properties of this substance against Giardia *lamblia* fructose-bis-1,6-phosphate aldolase was determined to be in the low μM region. The results show that a structure-based inhibitor design that derives from knowledge of enzyme mechanism and structure is an effective for the identification of new 3-hydroxy-2-pyridone based *gl*FBPA inhibitors that have modestly tight binding affinities to Giardia *lamblia* fructose-bis-1,6-phosphate aldolase .

3.5 Experimental

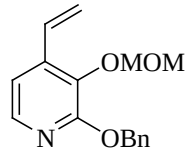
General. Unless otherwise noted, all reagents were obtained from commercial sources and used without further purification. All compounds were isolated and shown to be >90% pure by ^{1}H and/or ^{13}C NMR, unless otherwise noted. Dynamic Adsorbents' 60A silica gel was used for chromatography, and Analtech silica gel plates with fluorescence F254 were used for thin-layer chromatography (TLC) analysis. ^{1}H and ^{13}C NMR spectra were recorded on Broker Advance 500 and tetramethylsilane (TMS) was

used as a reference. Data for ^{1}H are reported as follows: chemical shift (ppm), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for ^{13}C NMR are reported as ppm. ^{13}C NMR resonance assignments were aided by the use of the DEPT technique to determine numbers of attached hydrogens.



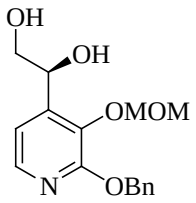
Compound 15. A solution of *tert*-BuLi (35.3 mL, 1.7 N in pentane) was added dropwise to a solution of the MOM-protected benzyloxy anlog 14 (12.3 g, 50 mmol) in Et_2O at -95^{o}C under N_2. A precipitate formed and the resulting suspension was stirred for 20 min before methyl formate (12 g, 200 mmol) was added dropwise at -95^{o}C. The resulting mixture was stirred for 3 h at -95^{o}C, diluted by addition of 20 mL of methanol and 100 mL of water, and extracted with Et_2O. The ether extracts were dried and concentrated in *vacuo* to give a residue which was subjected to flash column chromatography (silica gel, 10-20% acetone-hexanes) to yield 9.56 g (70%) of the 4-pyridinecarboxaldehyde 15 as a pale yellow oil. ^{1}H NMR ($CDCl_3$) 3.46 (s, 3H), 5.25 (s, 2H), 5.44 (s, 2H), 7.21 (d, *J* = 5.0 Hz, 1H), 7.29-7.44 (m, 5H), 7.97 (d, *J* = 5.0 Hz, 1H), 10.49 (s, 1H); ^{13}C NMR ($CDCl_3$) 57.60, 68.31,

98.56, 112.82, 127.80, 127.87, 128.32, 135.18, 136.41, 141.00, 142.76, 157.48, 189.11; HRMS (FAB) m/z calcd for $C_{15}H_{16}NO_4$ (M+1) 274.1079, found 274. 1085.



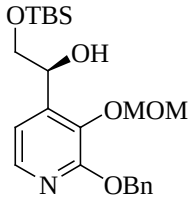
Compound 16. To a stirred suspension of methyltriphenylphosphonium bromide (6.30 g, 17.6 mmol) in THF (40 mL) at 0 ºC was added dropwise *n*-BuLi (11.0 mL, 17.6 mmol, 1.6 N solution in hexanes). After stirring for 30 min, the resulting solution was added dropwise a solution of 4-pyridinecarboxadehyde 15 (3.82 g, 14.0 mmol) in THF (20 mL). The resulting solution was stirred overnight at room temperature, diluted with water, and extracted with EtOAc. The extracts were dried and concentrated in vacuo to give a residue which was subjected to flash column chromatography (silica gel, 10-30% acetone-hexanes) to afford 3.04 g (80%) of vinylpyridine 16 as a colorless oil. ^{1}H NMR ($CDCl_3$) 3.47 (s, 3H), 5.13 (s, 2H), 5.42 (s, 2H), 5.45 (d, *J* = 11.5 Hz, 1H), 5.86 (d, *J* = 18.0 Hz, 1H), 6.99 (d, *J* = 5.5 Hz, 1H), 7.08 (dd, *J* = 11.5 Hz, 18.0Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 2H),7.44 (d, *J* = 7.5 Hz, 2H), 7.83 (d, *J* = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) 57.51, 67.80, 98.35, 113.53, 118.44, 127.68,

127.79, 128.31, 129.69, 137.16, 137.60, 138.89, 140.58, 157.23; HRMS (FAB) m/z calcd for $C_{16}H_{18}NO_3$ (M+1) 272.1287, found 272.1290.



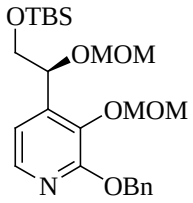
Compound 17. To a stirred suspension of AD-mix-α (16.8 g, 12.0 mmol) in *t*-BuOH/H_2O (1:1, 116.0 mL) at 0 ºC was added vinylpyridine 16 (3.12 g, 11.5 mmol). The mixture was stirred at 0 ºC for 6 h and quenched with sodium sulfite (18.0 g, 12.0 mmol). The resulting mixture was stirred for 0.5 h and extracted with EtOAc. The extracts were washed with H_2O, dried over and concentrated in vacuo giving aresidue which was subjected to flash column chromatography (silica gel, 20-50% acetone-hexanes) to afford 2.46 g (70%) of the (R or S)-enantiomer of 4-dihydroxyethylpyridine 17 as a colorless oil with a 97%ee (chiral HPLC with chiralcell OD-H column (10% Isopropanol/hexane, 0.6 mL/min), retention times, 17.35 min (one enantiomer), 21.21 min (another enantiomer)). $[\alpha]_D^{23}$ -7.0 (*c* 10.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 3.40 (s, 3H), 3.59 (dd, *J* = 8.0 Hz, 11.0Hz, 1H), 3.80 (dd, *J* = 3.0 Hz, 11.5Hz, 1H), 3.97 (b, 1H), 4.49 (b, 1H), 5.07 (dd, *J* = 6.0 Hz, 23.0Hz, 2H), 5.16 (dd, *J* = 3.0 Hz, 8.0 Hz, 1H), 5.36 (d, *J* = 12.0 Hz, 2H),

7.01 (d, *J* = 5.5 Hz, 1H), 7.26-7.41 (m, 5H), 7.85 (d, *J* = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) 57.37, 65.98, 67.90, 68.81, 98.30, 115.13, 127.75, 128.28, 136.79, 137.39, 141.05, 143.24, 155.99; HRMS (FAB) m/z calcd for $C_{16}H_{20}NO_5$ (M+1) 306.1341, found 306.1352.



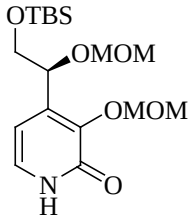
Compound 18. To a stirred solution of diol 17 (3.78 g, 12.4 mmol) in DMF (30 mL) at 0 ºC were added sequentially TBSCl (2.25 g, 14.8 mmol) and imidazole (1.26 g, 18.6 mmol). The resulting solution was stirred at 0 ºC for 5 h diluted with H_2O and extracted with EtOAc. The extracts were washed with water, dried and concentrated in vacuo giving a residue which was subjected to flash column chromatography (10-20% acetone-hexanes) to afford 4.16 g (80%) of mono-TBS-protected 4-dihydroxyethylpyridine 18 as a colorless oil. $[\alpha]_D^{23}$ +10.0 (*c* 12.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 0.06 (s, 3H), 0.07 (s, 3H), 0.91 (s, 9H), 3.49 (s, 3H), 3.55 (m, 1H), 3.96 (dd, *J* = 3.5Hz, 10Hz, 1H), 5.12 (d, *J* = 6.0 Hz, 1H), 5.17 (m, 2H), 5.43 (d, *J* = 10.0 Hz, 2H), 7.11 (d, *J* = 5.0 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.45 (d, *J* = 7.5 Hz, 2H), 7.92 (d, *J* = 5.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) -5.52,

18.18, 25.76, 57.45, 66.70, 67.82, 68.71, 98.43, 115.38, 127.72, 127.81, 128.32, 137.07, 137.59, 140.87, 143.01, 155.97; HRMS (FAB) m/z calcd for $C_{22}H_{33}NO_5NaSi$ (M+23) 442.2026, found 442.2028.



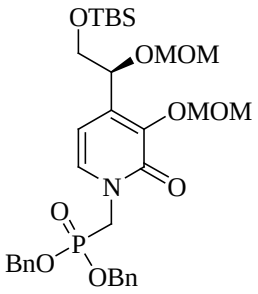
Compound 19. To a stirred solution of mono-TBS protected 4-dihydroxyethylpyridine 18 (4.11 g, 9.8 mmol) and diisopropylethylamine (6.0 mL, 34 mmol) in dry CH_2Cl_2 (50 mL) at room temperature was added dropwise MOMCl (1.47 mL, 19 mmol). The solution was stirred overnight, diluted with satd aq $NaHCO_3$, and extracted with ethyl acetate. The extracts were dried and concentrated *in vacuo* to afford a residue which was subjected to flash column chromatography (10-30% acetone-hexanes) to give 3.86 g (85%) of 19 as a colorless oil. $[\alpha]_D^{23}$ +30.2 (*c* 18.0, methanol); ^{1}H NMR ($CDCl_3$) 0.05 (s, 6H), 0.89 (s, 9H), 3.37 (s, 3H), 3.50 (s, 3H), 3.72 (dd, *J* = 7.5 Hz, 10.5 Hz, 1H), 3.82 (dd, *J* = 3.0 Hz, 10.5 Hz, 1H), 4.60 (d, *J* = 7.0 Hz, 1H), 4.70 (d, *J* = 7.0 Hz, 1H), 5.10 (d, *J* = 6.0 Hz, 1H), 5.23 (m, 2H), 5.41 (d, *J* = 5.0 Hz, 2H), 7.02 (d, *J* = 5.0 Hz, 1H), 7.25 (t, *J* = 7.5 Hz, 1H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 2H), 7.88 (d, *J* = 5.0 Hz, 1H);

^{13}C NMR ($CDCl_3$) -5.76, 18.04, 25.61, 55.05, 57.24, 66.27, 67.61, 72.77, 94.90, 98.07, 115.62, 127.50, 127.64, 128.12, 136.97, 137.85, 140.51, 141.68, 155.99; HRMS (FAB) m/z calcd for $C_{24}H_{37}NO_6NaSi$ (M+23) 486.2288, found 486.2283.



Compound 20. A solution of mono-TBS mono-MOM protected 4-dihydroxyethylpyridine 19 (5.00 g, 10.8 mmol) in 25 mL of MeOH conatining a catalytic amount of 5% Pd/C was added 3mL of triethylamine and stirred under an hydrogen atmosphere for 5 h. Concentration of the filtrate obtained by filtration gave 3.04 g (90%) of mono-TBS mono-MOM protected 4-dihydroxyethyl-2-pyridone 20 as a colorless. $[\alpha]_D^{23}$ +34.0 (*c* 3.4, $CHCl_3$); 3.63 ^{1}H NMR ($CDCl_3$) 0.05 (s, 6H), 0.89 (s, 9H), 3.37 (s, 3H), 3.58 (s, 3H), 3.74 (m, 1H), 3.81 (m, 1H), 4.63 (d, *J* = 6.0 Hz, 1H), 4.72 (d, *J* = 6.0 Hz, 1H), 5.17 (m, 1H), 5.23 (d, *J* = 5.5 Hz, 1H), 5.51 (d, *J* = 5.5 Hz, 1H), 6.45 (d, *J* = 6.5 Hz, 1H), 7.21 (d, *J* = 6.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) -5.73, 18.02, 25.57, 55.14, 57.32, 65.69, 72.63, 94.95, 96.83, 105.49, 128.53,

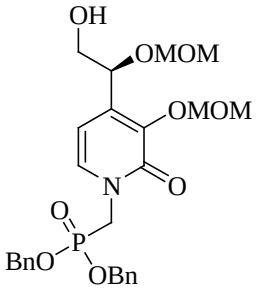
141.66, 142.61, 160.64; HRMS (FAB) m/z calcd for $C_{17}H_{32}NO_6Si$ (M+1) 374.1999, found 374.2005.



Compound 21. To a stirred solution of pyridine 20 (3.06 g, 8.2 mmol) and K_2CO_3 (2.27 g, 16.4 mmol) in acetone (10 mL) at room temperature was added the phosphonomethyl triflate 35[1] (4.17 g, 9.84 mmol). The solution was stirred overnight, diluted with H_2O, and extracted with EtOAc. The extracts were washed with H_2O, dried and concentrated under vacuum giving a residue which was subjected to flash column chromatograph (silica gel, 20-40% acetone-hexanes) to afford 2.12 g (40%) of 1-phosphonomethyl-2-pyridone 21 as an colorless oil. $[\alpha]_D^{23}$ +10.02 (*c* 28.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 0.03 (s, 6H), 0.87 (s, 9H), 3.32 (s, 3H), 3.48 (s, 3H), 3.68 (m, 1H), 3.75 (m, 1H), 4.44 (m, 2H), 4.56 (d, *J* = 6.5 Hz, 1H), 4.65 (d, *J* = 6.5 Hz, 1H), 5.00 (m, 5H), 5.14 (d, *J* = 6.0 Hz, 1H), 5.41 (d, *J* = 6.0 Hz, 1H), 6.26 (d, *J* = 7.0 Hz, 1H), 7.11 (d, *J* = 7.0 Hz, 1H), 7.25 (s, 10H); ^{13}C NMR

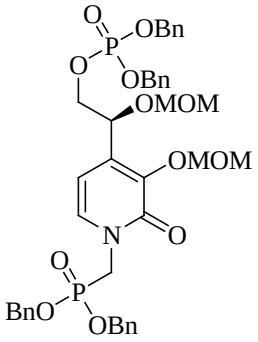
[1] Xu, Yibo.; Flavin, Michael T.; Xu, Ze-Qi. J.Org.Chem. 1996, 61, 7697-7701.

($CDCl_3$) 5.40, 17.78, 25.36, 42.48 (d, *J* = 611.0 Hz), 54.88, 56.96, 65.41, 67.68, 72.22, 94.66, 96.42, 103.91, 127.56, 128.02, 131.28, 135.30, 138.80, 142.33, 157.55; ^{31}P NMR ($CDCl_3$) 20.65; HRMS (FAB) m/z calcd for $C_{32}H_{46}NO_9NaSiP$ (M+23) 670.2577, found 670.2565.



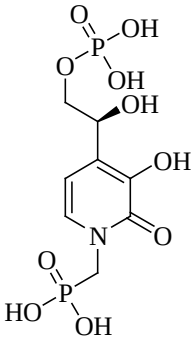
Compound 22. To a solution of 1-phosphonomethyl-2-pyridone 21 (2.59 g, 4.00 mmol) in THF (5 mL) was added TBAF (6 mmol, 6.0 mL, 1 N in THF). The resulting solution was stirred for 4 h, diluted with H_2O, and extracted with EtOAc. The extracts were washed with H_2O, dried and concentrated in vacuo giving a residue which was subjected to flash column chromatography (silica gel, 20-50% acetone-hexanes) to afford 1.92 g (90%) of 1-phosphonomethyl-4-hydroxyethyl-2-pyridone 22 as a colorless oil. $[\alpha]_D^{23}$+65.0 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) 3.35 (s, 3H), 3.50 (s, 3H), 3.66 (m, 1H), 3.72 (m, 1H), 4.43 (m, 2H), 4.60 (d, *J* = 6.5 Hz, 1H), 4.65 (d, *J* = 6.5 Hz, 1H), 5.06 (m, 5H), 5.21 (d, *J* = 6.0 Hz, 1H), 5.34 (d, *J* = 6.0 Hz, 1H), 6.26 (d, *J* = 7.0 Hz, 1H), 7.12 (d, *J* = 7.0 Hz, 1H), 7.29 (s, 10H); ^{13}C

NMR ($CDCl_3$) 42.66 (d, J = 613.0 Hz), 55.39, 57.18, 64.63, 67.95, 73.34, 95.23, 96.69, 103.91, 127.72, 128.23, 131.65, 135.28, 135.32, 138.85, 142.54, 157.73; ^{31}P NMR ($CDCl_3$) 20.67; HRMS (FAB) m/z calcd for $C_{26}H_{32}NO_9NaP$ (M+23) 556.1712, found 556.1710.



Compound 23. A mixture of 1-phosphonomethyl-4-hydroxyethyl-2-pyridone 22 (3.77, 10.0 mmol) and dibenzyl N,N-diisopropylphosphoramidite (5.18 g, 15.0 mmol) in a tetrazole solution (44.4 mL, 20 mmol, 0.45 N) in acetonitrile was stirred at room temperature for 24 h and diluted with chloroform. To the solution at -30^{o}C was added a solution of MCPBA (2.68 g, 12.0 mmol, 77%) in chloroform. The resulting mixture was stirred at room temperature until the starting material was consumed, diluted with aq. sodium bisulfite and extracted with chloroform. The chloroform extracts were dried and concentrated in *vacuo* to give a residue which was subjected to flash column chromatography (silica gel, 20-30 % acetone-hexanes) to afford 5,41 g (85%) of 1-phosphonomethyl-4-phosphatoethyl-2-pyridone **23** as a colorless oil

$[\alpha]_D^{23}$ +26.20 (*c* 14.4, $CHCl_3$); ^1H NMR ($CDCl_3$) 3.29 (s, 3H), 3.44 (s, 3H), 4.10 (m, 1H), 4.15 (m, 1H), 4.43 (m, 2H), 4.55 (d, *J* = 6.5 Hz, 1H), 4.60 (d, *J* = 6.5 Hz, 1H), 5.03 (m, 8H), 5.14 (d, *J* = 5.5 Hz, 1H), 5.23 (m, 1H), 5.37 (d, *J* = 5.5 Hz, 1H), 6.21 (d, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 5.5 Hz, 1H), 7.29 (s, 10H), 7.32 (s, 10H); ^{13}C NMR ($CDCl_3$) 42.78 (d, *J* = 613.5 Hz), 55, 40, 57.23, 68.01, 68.48, 68.93, 70.11, 94.90, 96.71, 103.72, 127.54, 127.80, 128.23, 128.28, 131.74, 135.36, 135.50, 137.11, 142.83, 157.61; ^{31}P NMR ($CDCl_3$) -0.26, 20.76; HRMS (FAB) m/z calcd for $C_{40}H_{45}NO_{12}NaP_2$ (M+23) 816.2315, found 816.2312.



Compound (S)-10. A solution of phosphonomethyl-4-phosphatoethyl-2-pyridone 23 (0.88 g, 1.1 mmol) and Pd/C (10%, 30 mg) in MeOH (5 mL) under a hydrogen atmosphere was stirred for 5 h. The filtrate obtained by filtration was concentrated in vacuo giving a residue which was diluted with methanol (2 mL) and ethanethiol (5 mL). After stirring overnight, the mixture was concentrated in vacuo to give a resdiue which was subjected to C18

reverse-phase column chromatography (water) to yield 0.29 g (76%) of 1-phosphonomethyl-3-hydroxy-4-phosphohydroxyethyl-2-pyridone as a colorless oil with a 97% ee (chiral HPLC with chiralcell OD-H column (0.46 × 25 cm, 90% hexane-iPrOH, 0.6 mL/min), retention times, 17.32 min (major enantiomer), 20.26 min (minor enantiomer)). $[\alpha]_D^{23}$ +54.2 (*c* 0.9, methanol); ^{1}H NMR (D_2O) 3.52 (m, 1H), 3.61 (m, 1H), 3.88 (d, *J* = 12.5 Hz, 2H), 4.63 (m, 1H), 5.98 (d, *J* = 7.0 Hz, 1H), 6.66 (d, *J* = 7.0 Hz, 1H); ^{13}C NMR (D_2O) 45.80 (d, *J* = 586 Hz), 67.06, 68.48, 106.95, 128.90, 129.86, 142.37, 157.80; ^{31}P NMR (D_2O) 2.47, 12.75; HRMS (FAB) m/z calcd for $C_8H_{12}NO_{10}P_2$ (M-1) 343.9936, found 343.9931.

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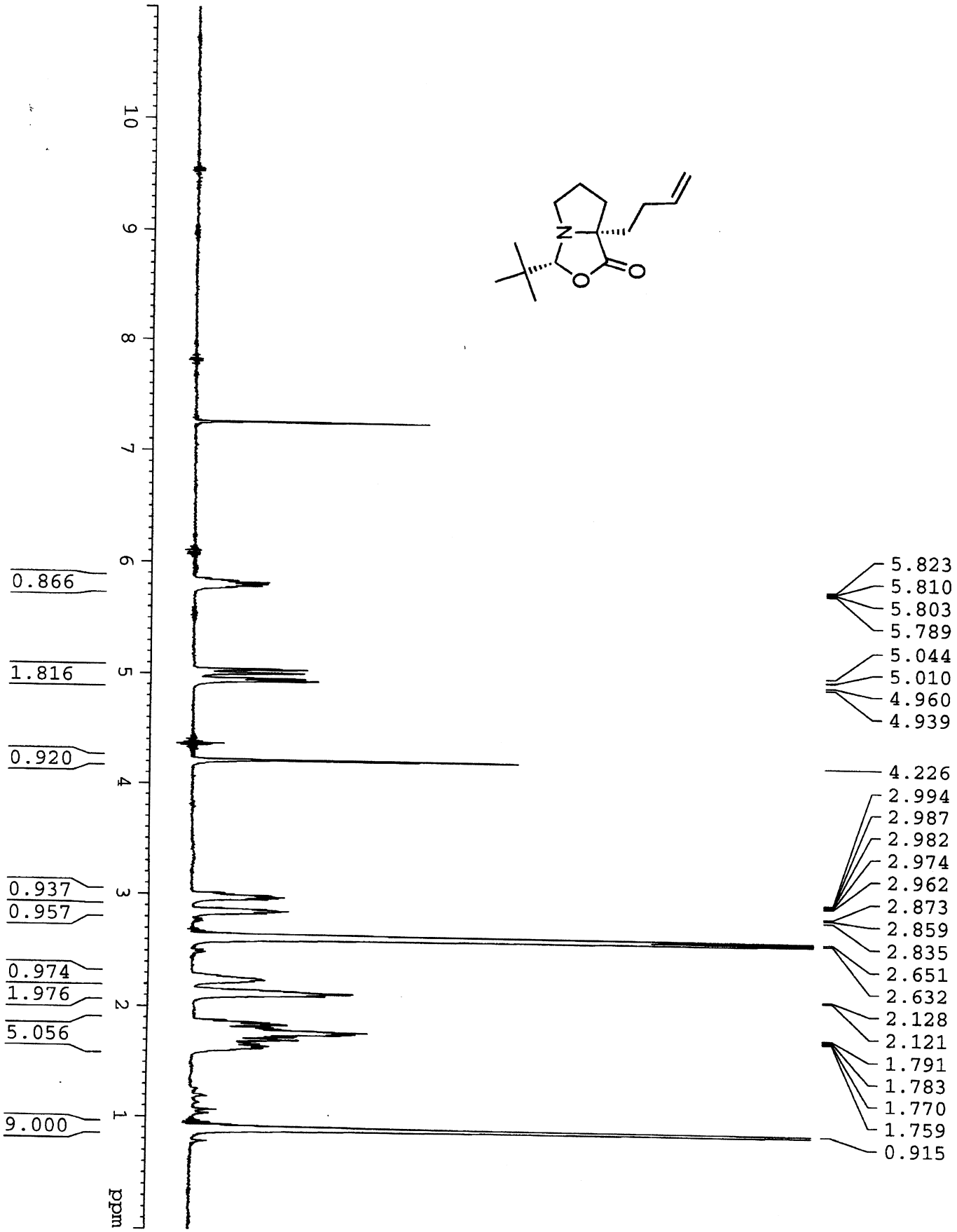
APPENDIX

NMR Spectra and MS Spectra

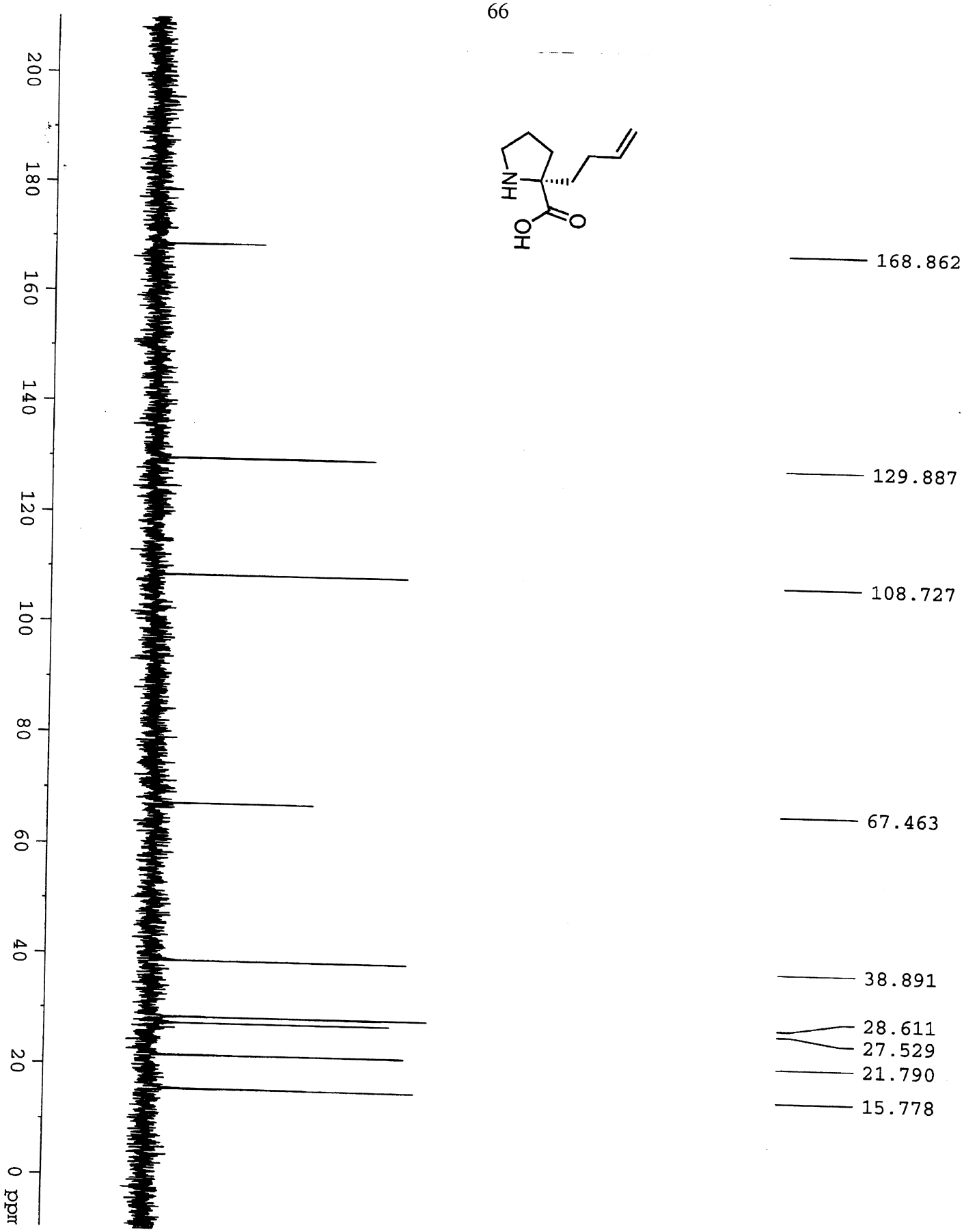
0.857
1.127
1.619
1.631
1.644
1.741
1.754
1.766
1.960
1.969
2.072
2.089
2.769
2.778
2.790
3.168
3.176
3.181
3.189
3.801
3.810
3.819
4.472
ppm
1
2
3
4
5
6
7
8
9
10
9.918
1.230
1.024
1.023
1.054
1.067
1.010
1.016
1.011
1.000
O
O
H
N

23.877
24.840
29.420
37.072
58.163
62.524
76.746
77.002
77.257
107.530
0 ppm
20
40
60
80
100
120
140
160
180
200
O
O
H
N


0.866
1.816
0.920
0.937
0.957
0.974
1.976
5.056
9.000
5.823
5.810
5.803
5.789
5.044
5.010
4.960
4.939
4.226
2.994
2.987
2.982
2.974
2.962
2.873
2.859
2.835
2.651
2.632
2.128
2.121
1.791
1.783
1.770
1.759
0.915
ppm

178.171
137.925
114.585
104.948
71.572
57.352
36.772
35.594
28.056
24.715
24.195
200
180
160
140
120
100
80
60
40
20
0 ppm

5.901
5.880
5.867
5.846
5.161
5.127
5.090
5.070
3.455
3.445
3.433
2.511
2.500
2.483
2.477
2.288
2.278
2.272
2.259
2.142
2.088
2.081
1.984
ppm
1
2
3
4
5
6
7
8
9
10
O
OH
NH


NH
O
OH
168.862
129.887
108.727
67.463
38.891
28.611
27.529
21.790
15.778
200
180
160
140
120
100
80
60
40
20
0 ppm

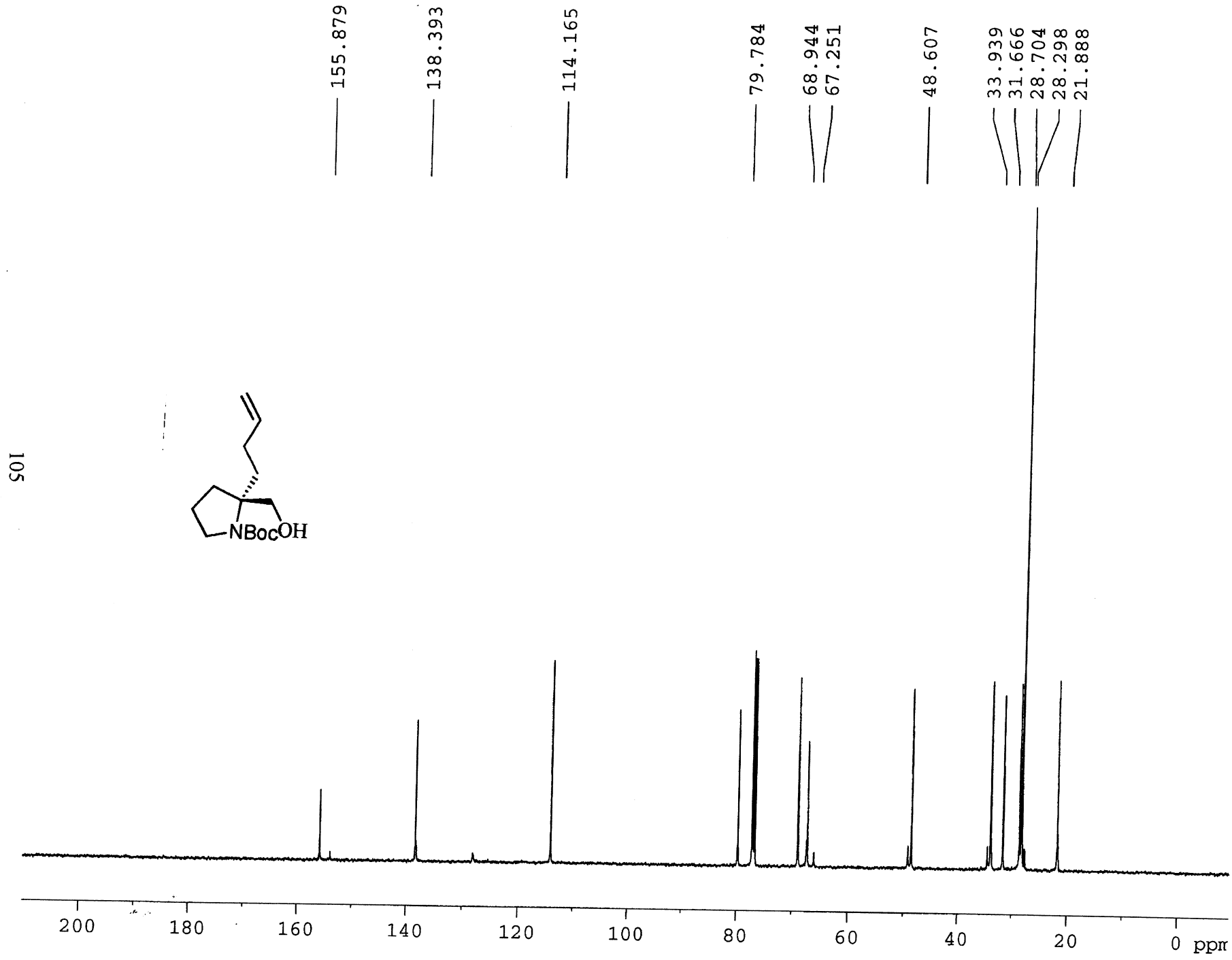
1.496
1.782
1.933
1.957
2.003
2.015
2.337
2.787
3.309
3.503
3.522
4.964
4.984
5.052
5.771
5.784
1.000
2.136
2.237
0.930
1.012
4.058
3.042
9.302
ppm
1
2
3
4
5
6
7
8
9
10
OH
O
NBoc

180.546
174.810
157.077
153.728
138.017
137.189
115.193
114.676
82.105
70.249
49.297
37.493
35.071
33.516
28.361
22.719
O
OH
NBoc
200
180
160
140
120
100
80
60
40
20
0 ppm

1.394
1.957
2.070
2.084
2.207
2.232
3.391
3.406
3.688
3.691
4.940
4.962
5.010
5.044
5.787
5.807
5.820
9.000
6.275
0.982
0.929
3.611
1.519
0.720
ppm
1
2
3
4
5
6
7
8
9
10
O
OMe
NBoc

175.321
153.704
138.151
114.544
79.919
67.264
52.017
48.483
37.394
36.130
34.362
33.302
28.277
23.179
22.651
NBoc
OMe
O
0 ppm
20
40
60
80
100
120
140
160
180
200

1.470
1.495
1.692
1.706
1.817
1.830
1.919
1.945
1.952
2.131
2.143
3.316
3.332
3.345
3.566
3.577
4.853
4.873
4.942
4.976
5.281
5.752
5.766
0.844
0.711
0.874
0.915
1.562
1.755
0.780
5.996
9.000
ppm
NBocOH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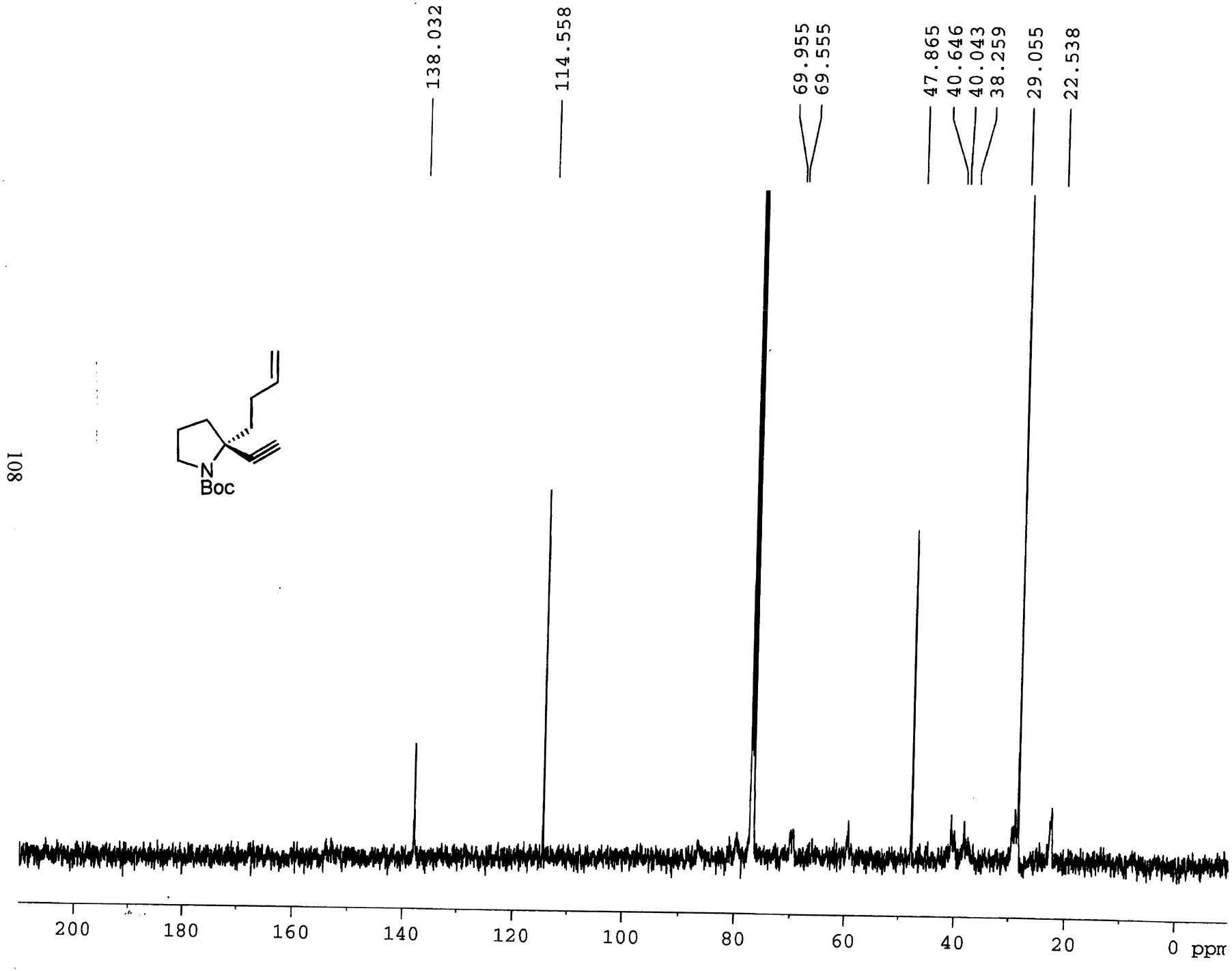
138.393
114.165
79.784
68.944
67.251
48.607
33.939
31.666
28.704
28.298
21.888
NBocOH
200
180
160
140
120
100
80
60
40
20
0 ppm

9.475
9.378
5.790
5.777
5.034
5.000
4.958
4.938
3.635
3.498
3.489
2.062
1.887
1.429
1.388
ppm
1
2
3
4
5
6
7
8
9
10
9.000
8.066
1.947
1.796
0.869
0.817
O
N
Boc

22.759
23.453
27.935
28.117
28.193
28.282
31.206
31.872
32.532
33.754
48.088
48.212
70.598
70.882
79.962
80.848
114.463
114.703
137.829
138.124
153.338
154.034
199.512
200.156
O
N
Boc
0 ppm
20
40
60
80
100
120
140
160
180
200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69.955
69.555
47.865
40.646
40.043
38.259
29.055
22.538
N
Boc
200
180
160
140
120
100
80
60
40
20
0 ppm

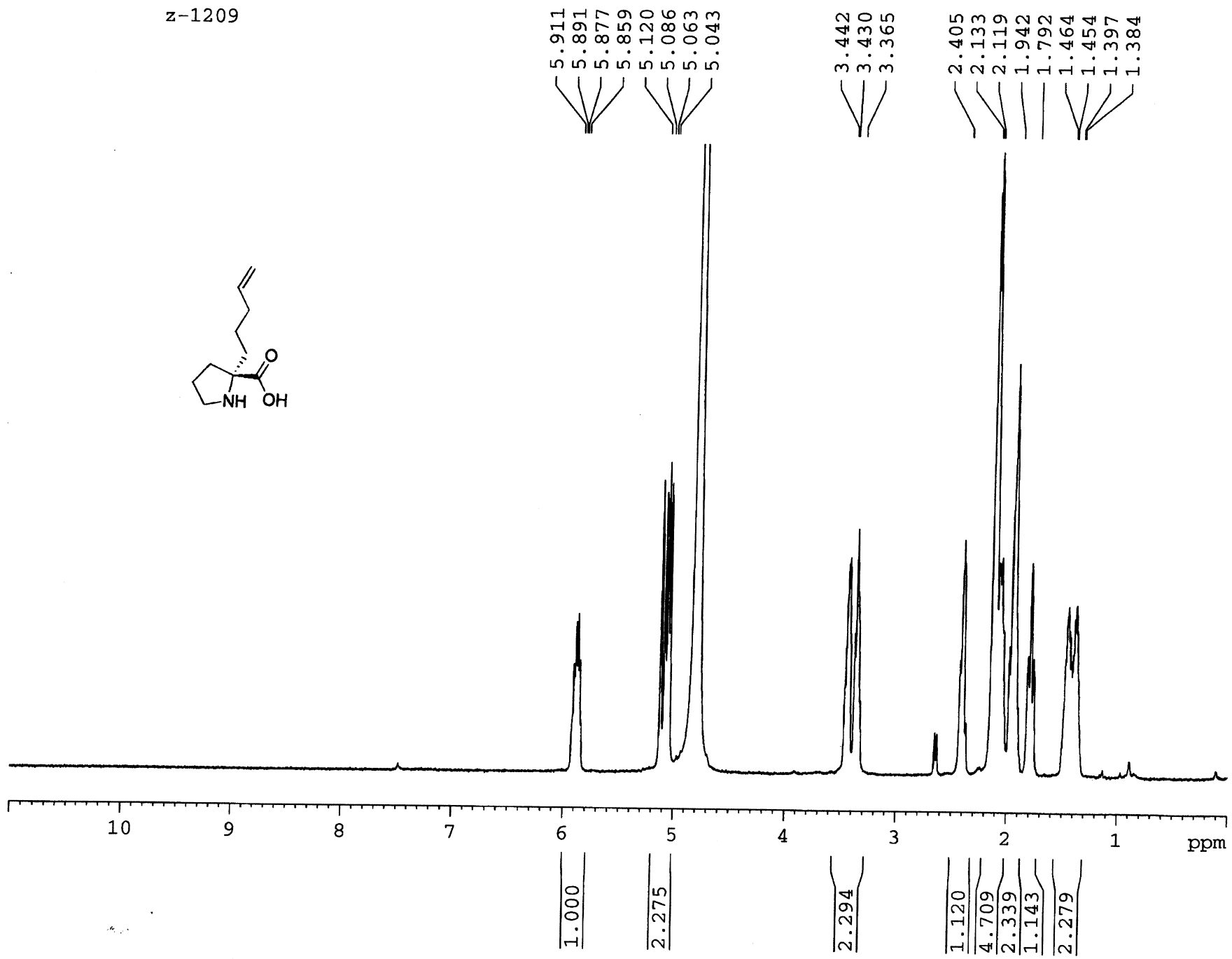
1.472
1.779
1.791
1.802
1.923
1.939
2.022
2.047
2.180
2.192
2.204
2.293
3.282
3.302
3.626
4.938
4.957
5.009
5.043
5.793
5.814
5.827
ppm
1
2
3
4
5
6
7
8
9
10
9.000
5.098
3.449
0.938
0.934
1.825
0.862
N
Boc

7.374
7.359
7.305
7.290
7.275
7.259
7.208
6.456
6.414
5.823
5.810
5.789
5.044
5.012
4.949
4.929
3.678
3.485
3.469
3.245
3.234
3.221
2.596
2.588
2.551
2.243
2.051
2.043
1.924
1.829
2.203
2.181
1.119
2.029
1.000
2.040
1.036
1.324
1.664
1.003
2.308
5.408
ppm
1
2
3
4
5
6
7
8
9
10
Ph
N
O

168.986
137.910
132.606
128.454
127.313
126.219
123.010
114.676
85.459
70.473
60.839
48.456
40.420
39.642
36.668
29.434
23.553
Ph
N
O
200
180
160
140
120
100
80
60
40
20
0 ppm

Z-1220
0.873
1.432
1.571
1.770
1.796
2.012
2.025
2.814
2.828
2.913
2.924
2.933
2.945
4.179
4.899
4.920
4.944
4.978
5.740
5.753
ppm
9.000
6.536
2.779
0.914
0.872
0.817
1.673
0.758
O
O
N

178.449
138.290
114.721
104.931
71.740
57.372
37.123
36.778
36.755
36.299
35.630
33.700
24.723
24.201
23.018
200
180
160
140
120
100
80
60
40
20
0 ppm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5.120
5.086
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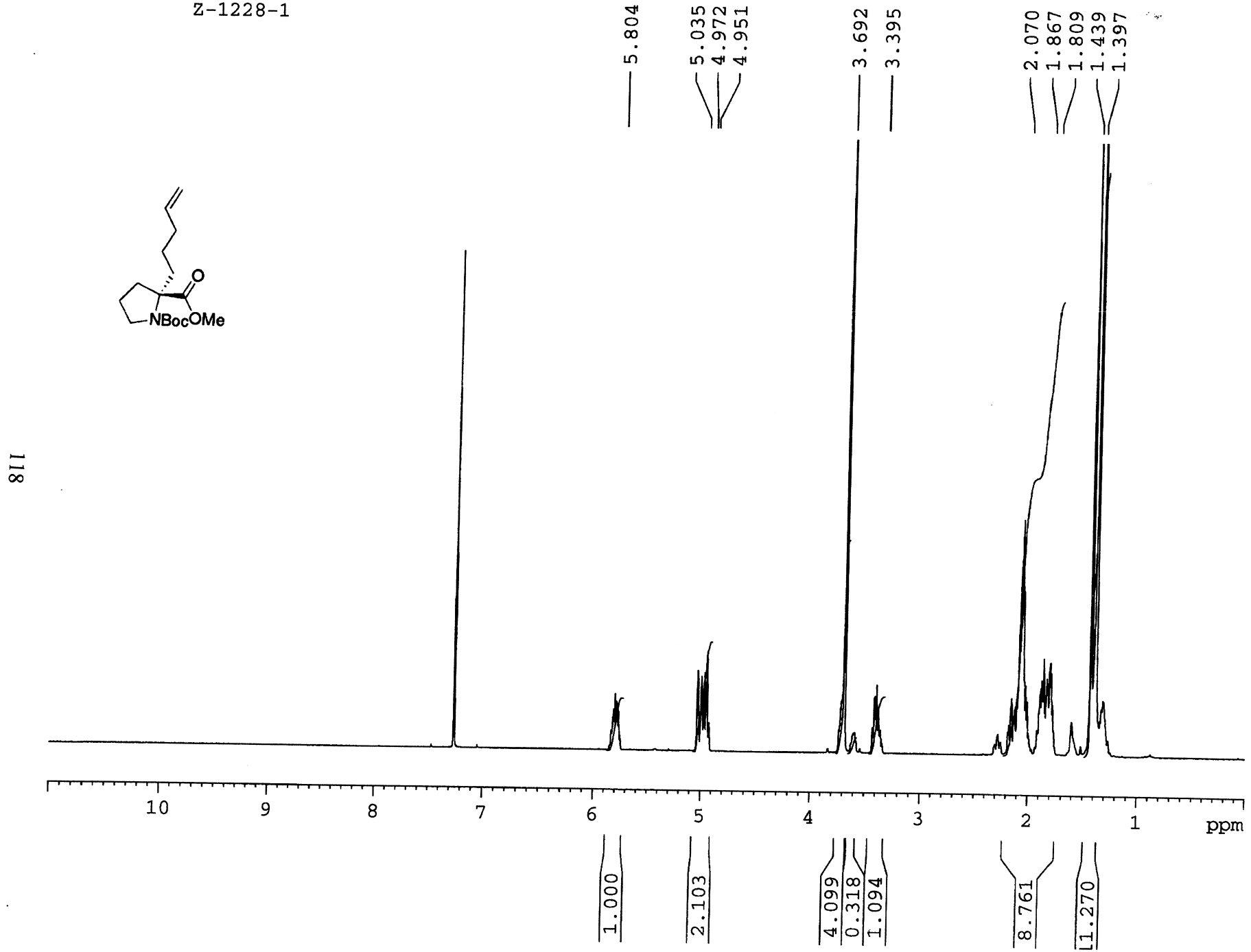
5.043
3.442
3.430
3.365
2.405
2.133
2.119
1.942
1.792
1.464
1.454
1.397
1.384
NH
OH
O
10
9
8
7
6
5
4
3
2
1
ppm
1.000
2.275
2.294
1.120
4.709
2.339
1.143
2.279

169.209
131.339
107.983
67.586
38.685
28.359
27.841
25.587
16.583
15.899
HO
O
NH
200
180
160
140
120
100
80
60
40
20
0 ppm

z-090320
NBocOH
O
ppm
1
2
3
4
5
6
7
8
9
10
1.530
9.200
4.270
4.254
0.584
1.975
2.000
0.747
1.477
1.772
1.782
2.067
2.663
3.310
3.496
3.506
4.964
5.753
5.766

174.909
157.126
137.988
115.054
82.062
80.314
70.450
67.302
49.327
48.552
35.083
33.767
33.494
28.354
23.355
22.701
NBoc
OH
O
200
180
160
140
120
100
80
60
40
20
0 ppm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5.035
4.972
4.951
3.692
3.395
2.070
1.867
1.809
1.439
1.397
NBocOMe
O
10
9
8
7
6
5
4
3
2
1
ppm
1.000
2.103
4.099
0.318
1.094
8.761
11.270

175.555
153.772
138.468
114.900
79.867
67.388
52.044
48.511
37.448
34.581
33.873
28.307
22.861
22.752
NBocOMe
O
0 ppm
20
40
60
80
100
120
140
160
180
200

University of New Mexico

Department of Chemistry & Chemical Biology

Method Name: C:\EZStart\Projects\Default\Method\zls1869.met
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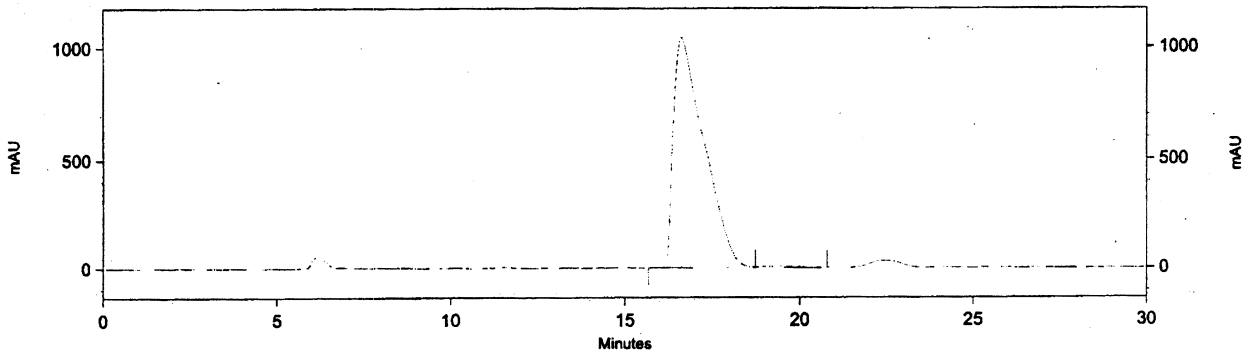
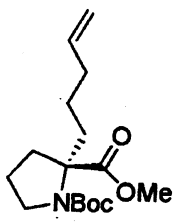


SPD-20A
Ch2-210nm Results

Pk #	Retention Time	Area	Area Percent
1	16.617	63080989	99.299
2	19.500	445155	0.701
Totals		63526144	100.000



University of New Mexico

Department of Chemistry & Chemical Biology

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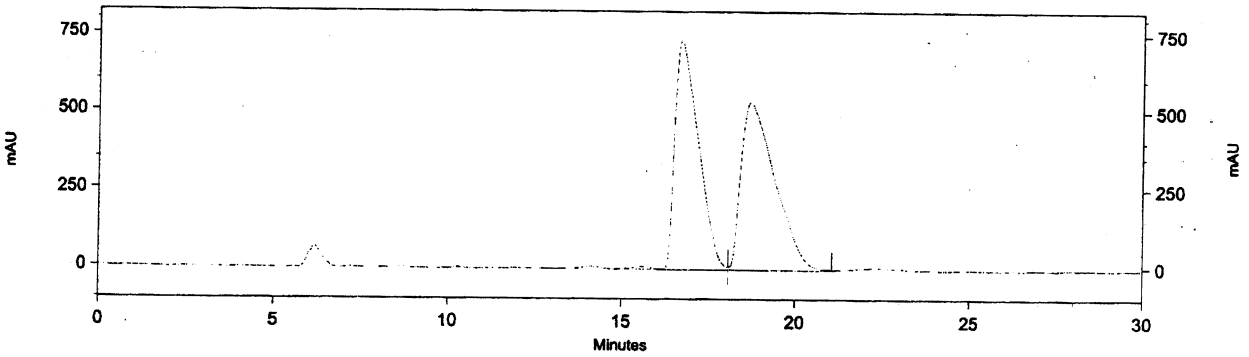
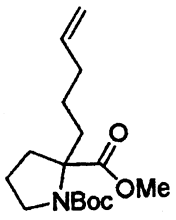


SPD-20A
Ch2-210nm Results

Pk #	Retention Time	Area	Area Percent
1	16.717	35334068	49.494
2	18.708	36056469	50.506
Totals		71390537	100.000



Z-1221-2
NBoc
OH
ppm
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0.675
2.130
1.948
2.416
8.543
12.869
5.819
5.805
5.785
5.024
4.990
4.934
3.629
3.412
3.371
3.357
2.067
2.052
1.860
1.848
1.742
1.449
1.212

156.172
138.758
114.544
79.997
69.363
67.621
48.780
34.113
32.033
28.480
23.842
22.066
NBocOH
200
180
160
140
120
100
80
60
40
20
0 ppm

ppm
1
2
3
4
5
6
7
8
9
10
9.000
7.604
1.939
5.406
0.666
0.653
1.403
1.445
1.728
1.856
1.871
1.883
1.962
1.971
2.060
2.073
2.085
3.183
3.503
3.517
3.645
3.653
3.666
4.954
4.976
5.030
5.072
5.775
5.788
9.502
9.396
Boc
N
O

199.881
153.492
138.177
115.045
80.884
70.862
33.974
33.831
32.143
28.177
23.009
22.856
O
N
Boc
200
180
160
140
120
100
80
60
40
20
0 ppm

Z-1219
N
Boc
5.800
5.786
5.021
4.987
4.956
4.937
3.625
3.289
3.268
2.262
2.184
2.173
2.161
2.067
2.056
1.944
1.931
1.921
1.906
1.782
1.771
1.759
1.750
1.464
ppm
1
2
3
4
5
6
7
8
9
10
9.000
0.935
2.070
6.721
0.934
0.917
1.794
0.854

22.511
23.888
28.494
33.690
38.604
40.702
47.883
59.500
69.291
79.847
86.826
114.740
138.455
154.230
N
Boc
200
180
160
140
120
100
80
60
40
20
0 ppm

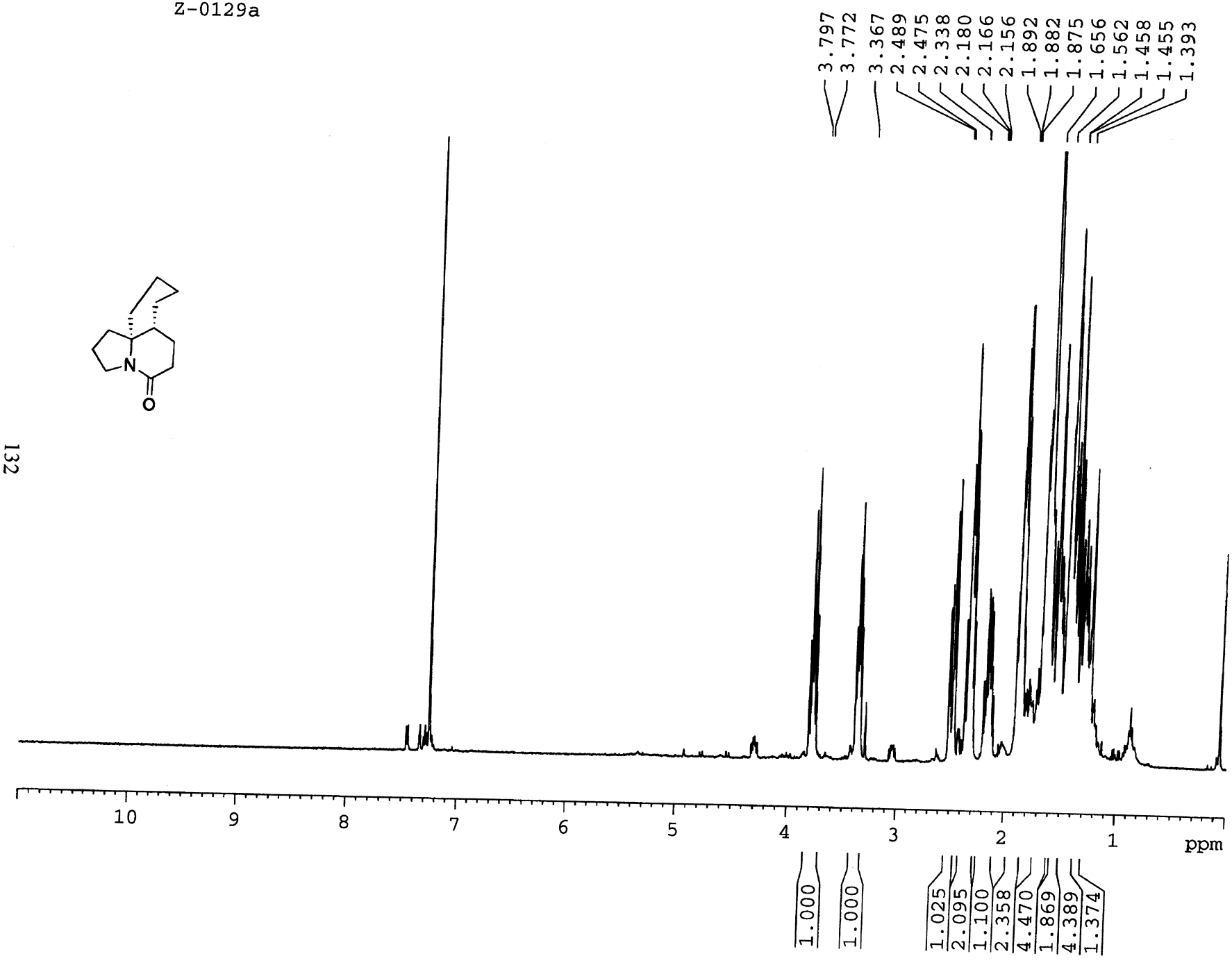
z-0112b
6.988
6.975
6.954
6.406
6.393
5.787
5.766
5.656
5.645
4.980
3.812
3.731
3.717
3.705
3.698
3.528
3.514
2.489
2.361
2.065
2.052
1.776
1.768
1.635
1.623
1.353
1.340
0.381
1.639
0.978
1.045
2.000
1.042
1.050
6.050
1.013
1.032
2.756
1.254
ppm

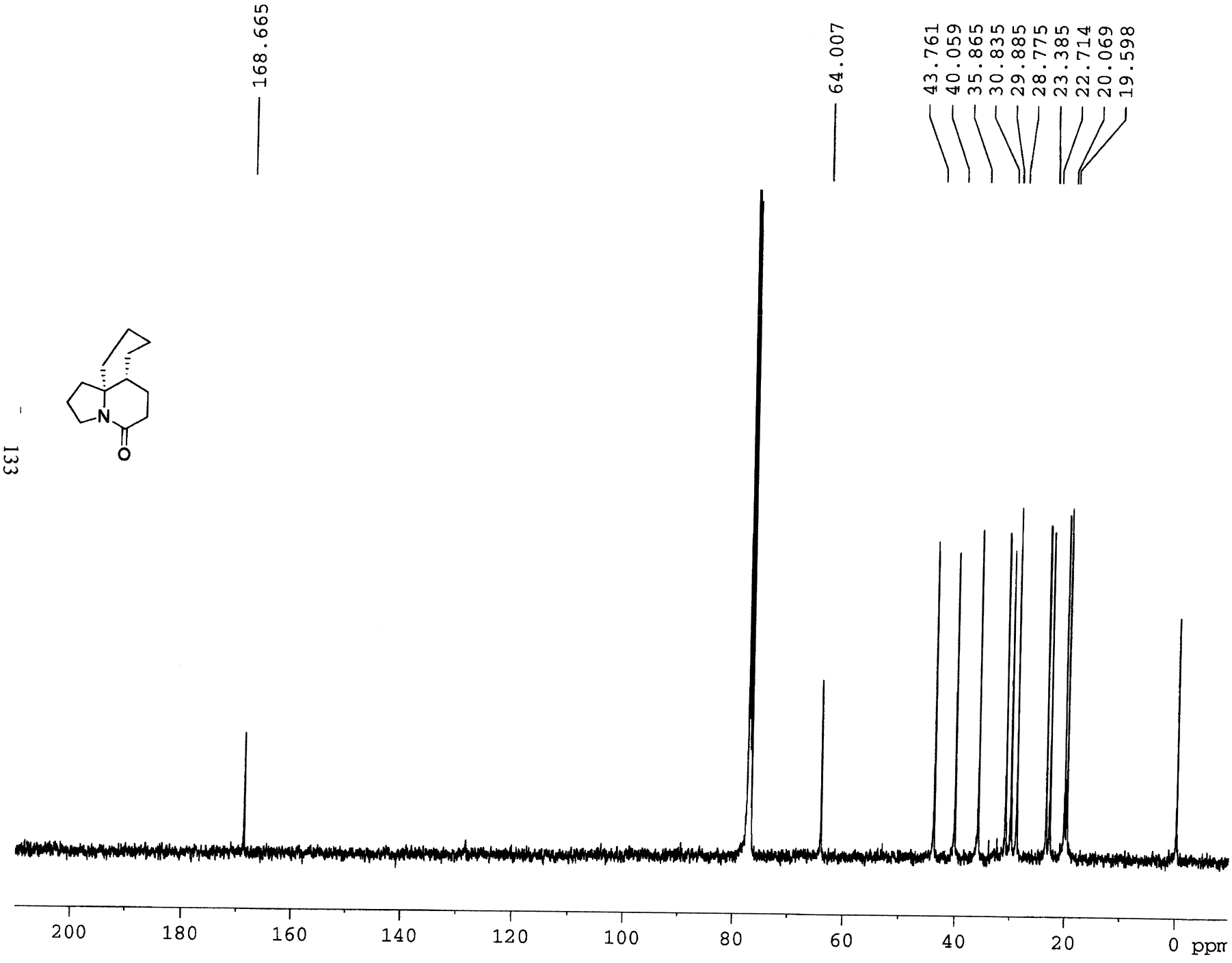
21.948
23.535
24.179
24.359
33.635
36.977
39.584
40.406
41.700
59.180
60.974
70.191
72.834
85.575
114.686
126.743
127.856
129.533
129.617
137.847
138.523
163.977
165.230
0 ppm
20
40
60
80
100
120
140
160
180
200
N
O

Z-0124
1.505
1.526
1.718
1.728
1.746
1.799
1.900
1.906
1.909
1.925
1.931
1.935
1.947
1.956
2.274
2.283
3.110
3.120
3.135
4.365
4.389
5.679
5.698
5.888
6.572
6.591
0.910
0.932
0.887
0.957
0.972
2.000
2.196
4.382
2.167
ppm
1
2
3
4
5
6
7
8
9
10
O
N

165.612
138.337
135.104
131.742
119.705
63.709
42.043
32.987
30.107
23.804
19.975
17.778
O
N
200
180
160
140
120
100
80
60
40
20
0 ppm

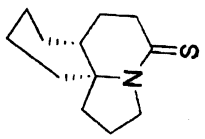
Z-0129a

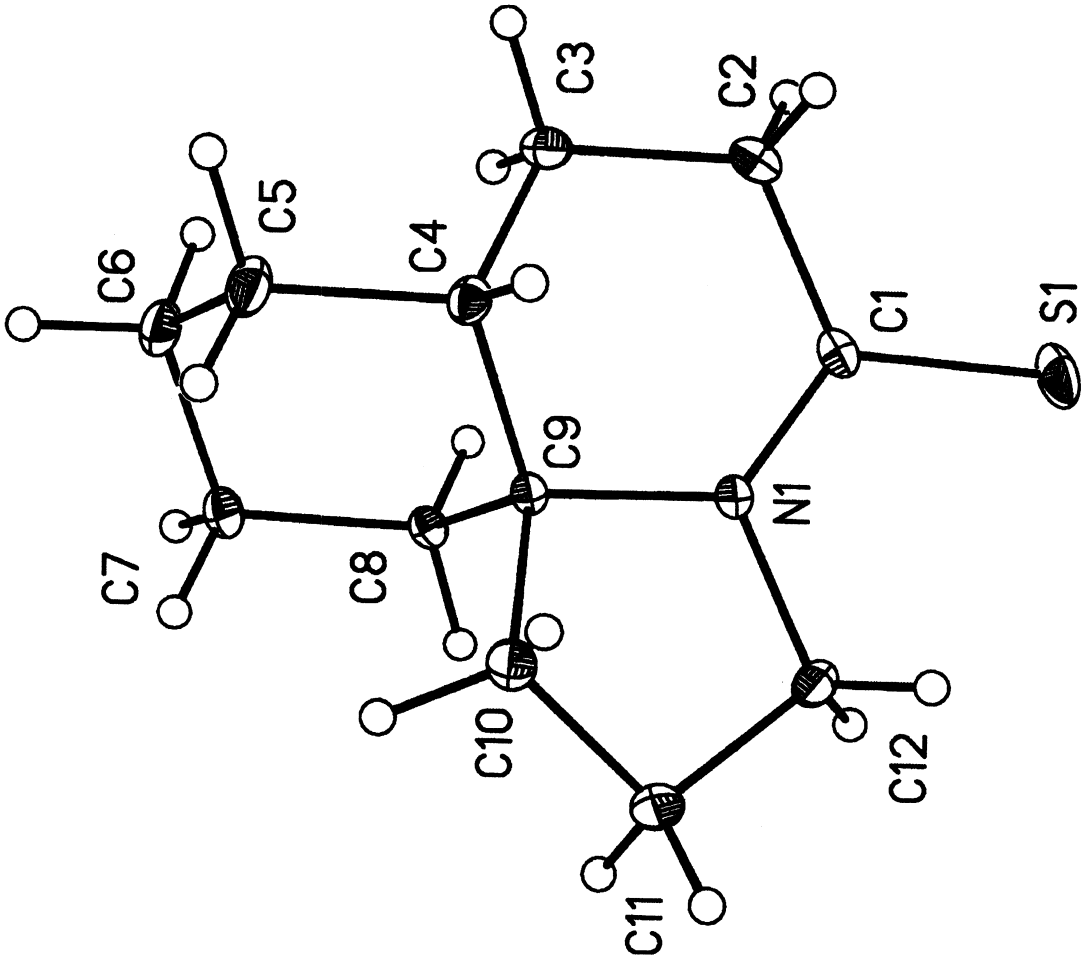

3.797
3.772
3.367
2.489
2.475
2.338
2.180
2.166
2.156
1.892
1.882
1.875
1.656
1.562
1.458
1.455
1.393
N
O
10
9
8
7
6
5
4
3
2
1
ppm
1.000
1.000
1.025
2.095
1.100
2.358
4.470
1.869
4.389
1.374


168.665
64.007
43.761
40.059
35.865
30.835
29.885
28.775
23.385
22.714
20.069
19.598
N
O
200
180
160
140
120
100
80
60
40
20
0 ppm

Z-080925b
ppm
1
2
3
4
5
6
7
8
9
10
0.934
0.975
0.935
0.931
0.977
0.969
2.000
5.010
6.182
1.211
4.208
3.773
3.175
3.161
2.956
2.484
2.468
2.457
2.110
2.095
1.556
1.490
1.481
S
N

195.437
67.704
52.058
39.970
39.276
35.390
29.057
28.821
23.241
22.280
19.356
S
N
200
180
160
140
120
100
80
60
40
20
0 ppm


S
N


C3
C2
C5
C4
C6
C1
S1
C9
N1
C7
C8
C10
C12
C11

0.889
1.096
1.253
1.321
1.353
1.477
1.508
1.625
1.684
1.725
1.743
1.822
1.841
2.675
2.778
3.005
3.022
7.258
2.299
12.257
12.097
2.429
1.000
ppm

53.403
49.167
44.344
44.005
37.634
37.017
35.602
35.280
34.860
29.700
29.563
28.786
27.974
26.005
25.610
24.845
24.612
24.310
23.032
22.793
20.569
20.378
0 ppm
20
40
60
80
100
120
140
160
180
200
N

Elemental Composition Report

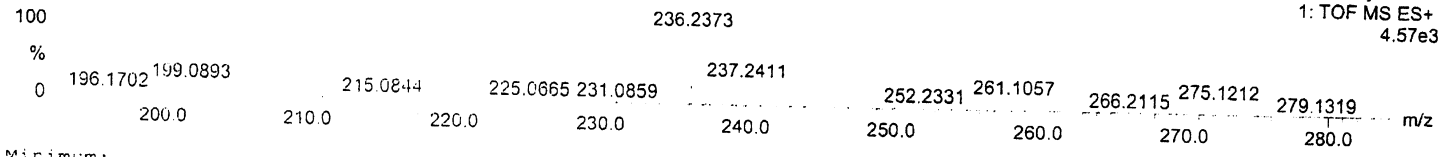
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
40 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

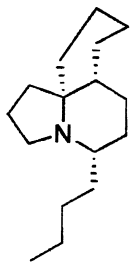
Jiwen Zhou / P.Mariano C16H29N
090602_210 61 (1.247) Cm (60:96)

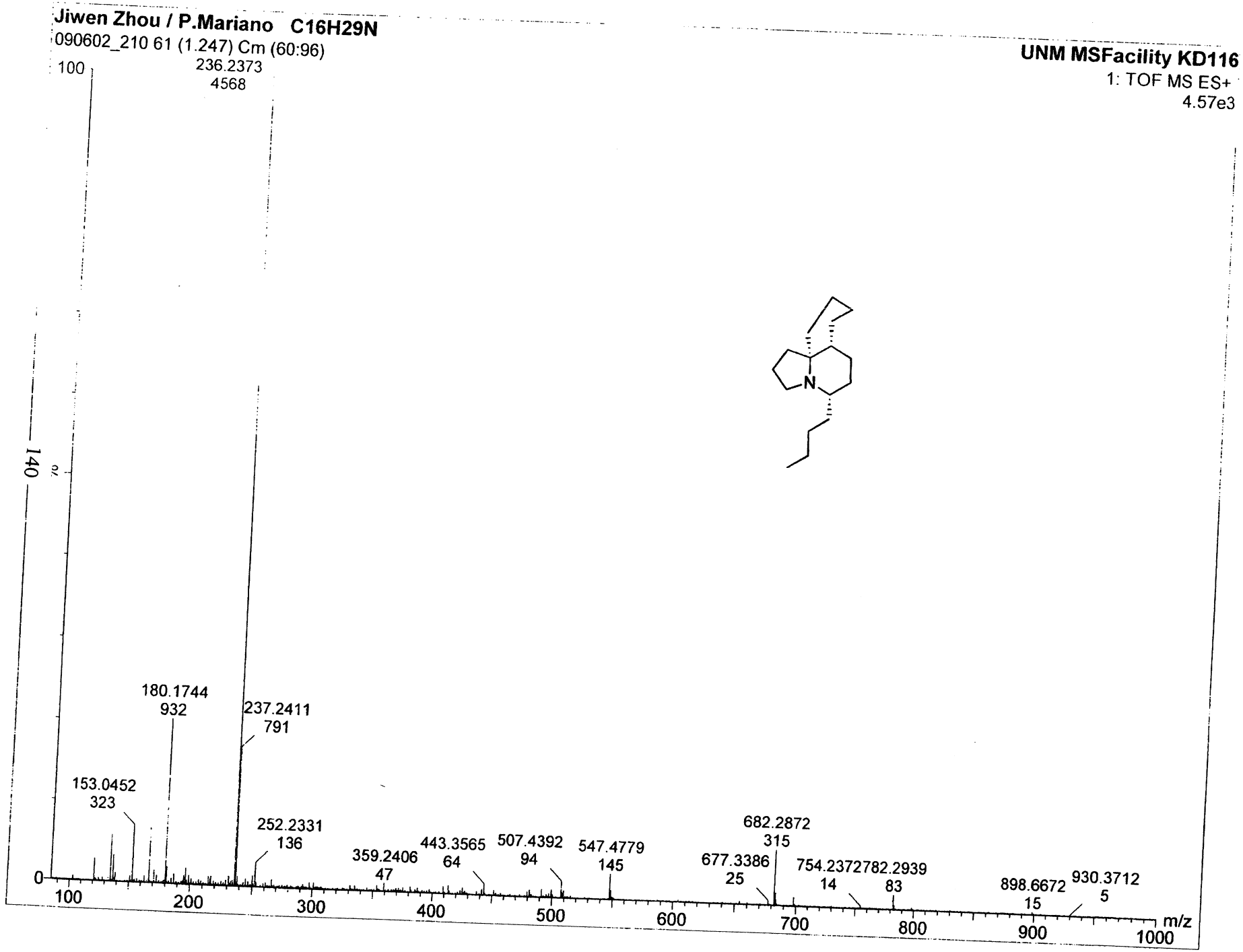
UNM MSFacility KD116
1: TOF MS ES+
4.57e3



Minimum:				-1.5		
Maximum:		200.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
236.2373	236.2378	-0.5	-2.2	2.5	1	C16 H30 N

[M+H]




Jiwen Zhou / P.Mariano C16H29N
090602_210 61 (1.247) Cm (60:96)
UNM MSFacility KD116
1: TOF MS ES+
4.57e3
100
236.2373
4568
%
140
180.1744
932
237.2411
791
153.0452
323
252.2331
136
359.2406
47
443.3565
64
507.4392
94
547.4779
145
682.2872
315
677.3386
25
754.2372
14
782.2939
83
898.6672
15
930.3712
5
0
100
200
300
400
500
600
700
800
900
1000
m/z
N

0.000
3.469
5.130
5.421
5.437
5.460
5.844
5.880
6.987
6.998
7.056
7.078
7.092
7.114
7.262
7.277
7.291
7.322
7.337
7.352
7.430
7.445
7.829
7.840
OMOM
OBn
N
ppm
0
1
2
3
4
5
6
7
8
9
10
11
3.173
2.134
1.998
1.189
1.049
1.036
1.036
1.059
2.030
2.008
1.000

-0.100
57.510
67.802
76.750
77.001
77.258
98.354
113.534
118.435
127.680
127.789
128.307
129.686
137.155
137.598
138.890
140.579
157.230
OMOM
OBn
N
ppm
0
20
40
60
80
100
120
140
160
180
200

Z-080910
7.923
7.913
7.449
7.434
7.369
7.024
7.014
5.426
5.413
5.157
3.882
3.860
3.723
3.708
3.506
0.782
4.928
0.914
2.000
2.859
1.089
1.149
3.010
ppm
OMOM
OBn
OH
HO

155.988
143.236
141.052
137.388
136.792
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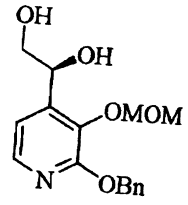
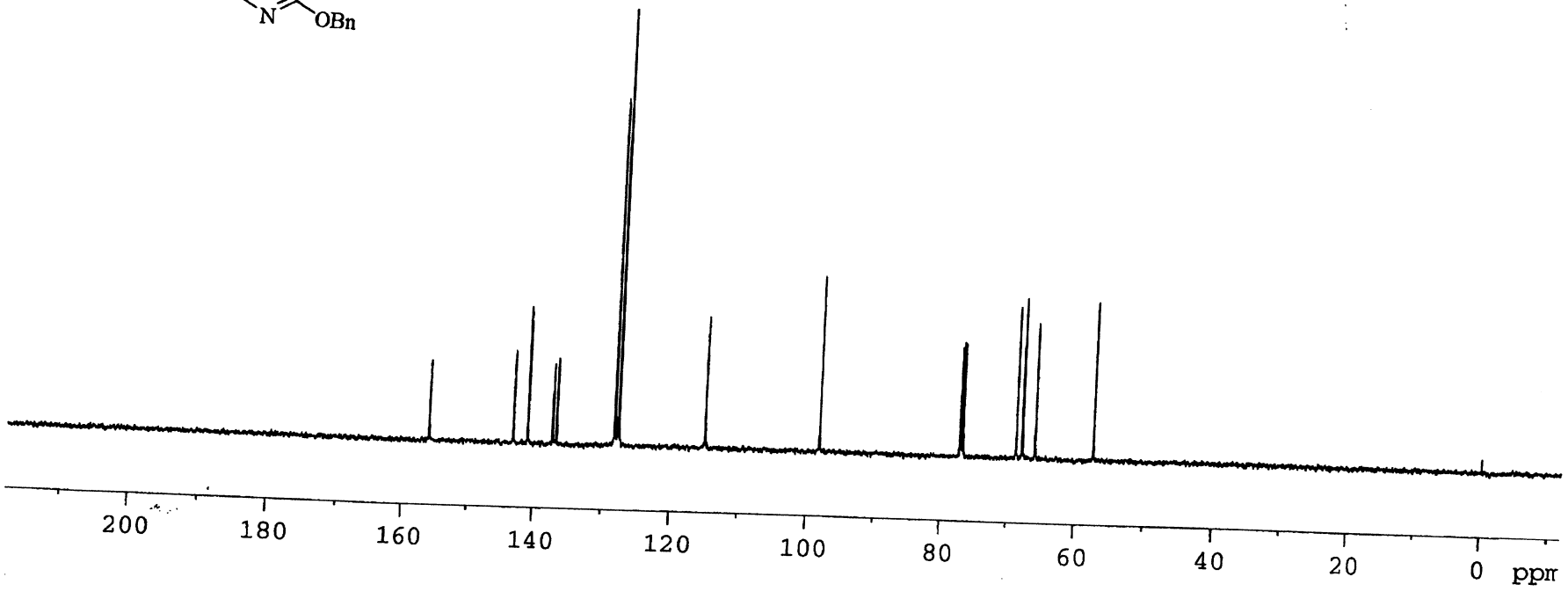
127.754
115.132
98.303
77.261
77.007
76.752
68.809
67.901
65.984
57.368

144





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Department of Chemistry

Method Name: C:\EZStart\Projects\WeiWang\xhx12.met
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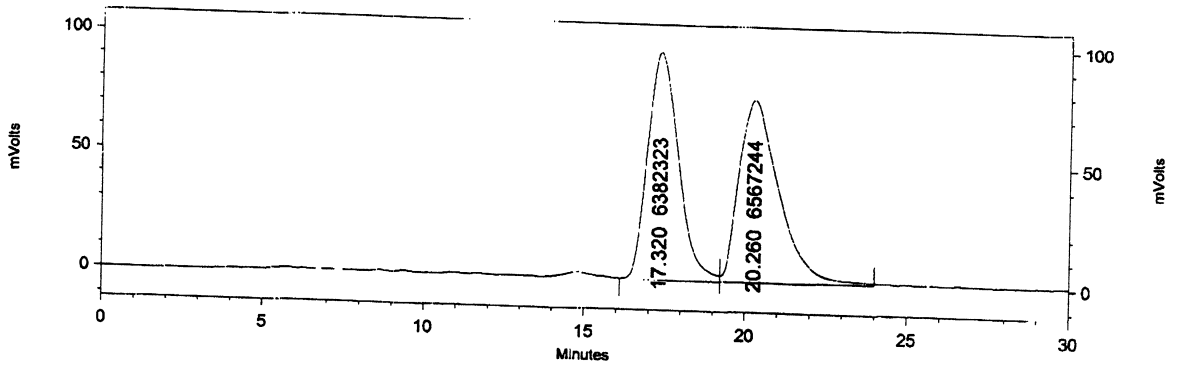
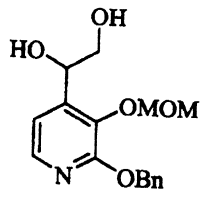
Sample ID: zgliu-1



SPD-10Avp
Ch1-254nm Results

Pk #	RT	Area	Area %
1	17.320	6382323	49.286
2	20.260	6567244	50.714
Totals		12949567	100.000



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Method Name: C:\EZStart\Projects\WeiWang\ddm.met
Data File: C:\EZStart\Projects\WeiWang\ddm1-1.dat
Date Acquired: 9/12/2008 2:26:46 PM Date Printed: 09/12/2008 03:04:31 PM
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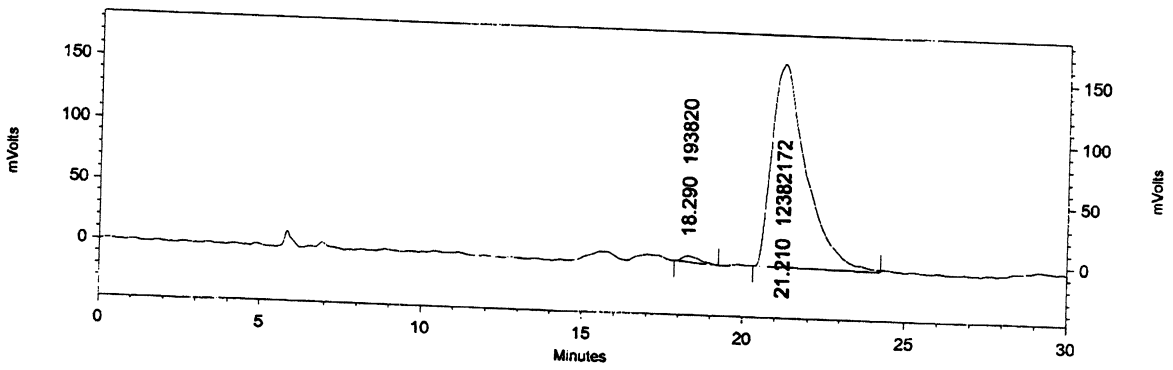
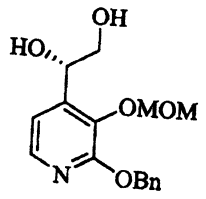


SPD-10Avp
Ch1-254nm Results

Pk #	RT	Area	Area %
1	18.290	193820	1.541
2	21.210	12382172	98.459
Totals		12575992	100.000



University Of New Mexico
Department of Chemistry

Method Name: C:\EZStart\Projects\WeiWang\xhx12.met
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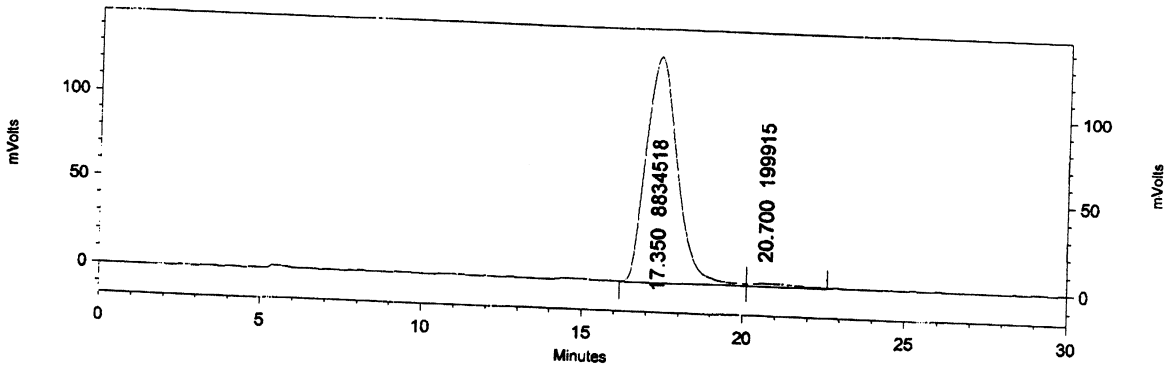

SPD-10Avp
Ch1-254nm Results

Pk #	RT	Area	Area %
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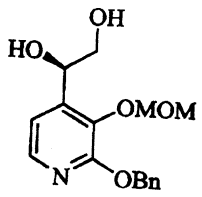
2	20.700	199915	2.213
Totals		9034433	100.000



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7.455
7.440
7.367
7.090
5.435
5.413
5.180
5.125
3.955
3.948
3.936
3.493
3.087
0.898
0.060
0.042
OTBS
OMOM
OBn
HO
N
ppm
0
1
2
3
4
5
6
7
8
9
6.120
10.057
4.043
0.912
2.864
2.000
0.857
5.193
0.933

-5.516
18.184
25.759
57.452
66.695
67.824
68.712
76.751
77.005
77.260
98.427
115.384
127.719
127.805
128.321
137.067
137.588
140.871
143.012
155.974
OTBS
OH
OMOM
OBn
N
ppm
0
20
40
60
80
100
120
140
160
180
200

Z-04306
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7.886
7.444
7.368
7.313
7.010
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5.237
5.226
5.176
5.117
5.106
4.706
4.613
4.601
3.785
3.699
3.523
3.356
0.875
0.020
OTBS
OMOM
OBn
MOMO
ppm
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2.118
1.209
0.842
2.107
1.967
1.115
0.883
1.260
0.856
1.162
3.410
2.794
10.714
5.929

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-5.622
18.039
25.610
55.053
57.243
66.270
67.608
72.767
76.749
77.004
77.259
94.897
98.067
115.617
127.502
127.640
128.119
136.971
137.847
140.508
141.676
155.987
OTBS
OMOM
OMOM
OBn
N
ppm
0
20
40
60
80
100
120
140
160
180
200

Z-080910b
7.142
6.442
5.502
5.490
5.223
5.211
5.155
5.149
5.141
4.696
4.619
3.787
3.728
3.714
3.565
3.361
0.875
0.034
1.000
1.014
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2.166
3.333
3.175
2.171
10.179
6.653
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OTBS
OMOM
MOMO
O
N
H

-5.792
-5.676
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55.136
57.324
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72.631
76.747
77.002
77.257
94.951
96.832
105.489
128.527
141.655
142.610
160.636
OTBS
OMOM
OMOM
O
N
H
ppm
0
20
40
60
80
100
120
140
160
180
200

Z-080910e
7.314
7.113
7.098
6.285
6.270
5.407
5.395
5.152
5.141
5.090
5.083
5.075
5.027
4.688
4.680
4.667
4.590
4.576
4.478
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4.429
4.404
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3.345
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0.031
ppm
0
1
2
3
4
5
6
7
8
9
6.000
9.200
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2.887
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1.000
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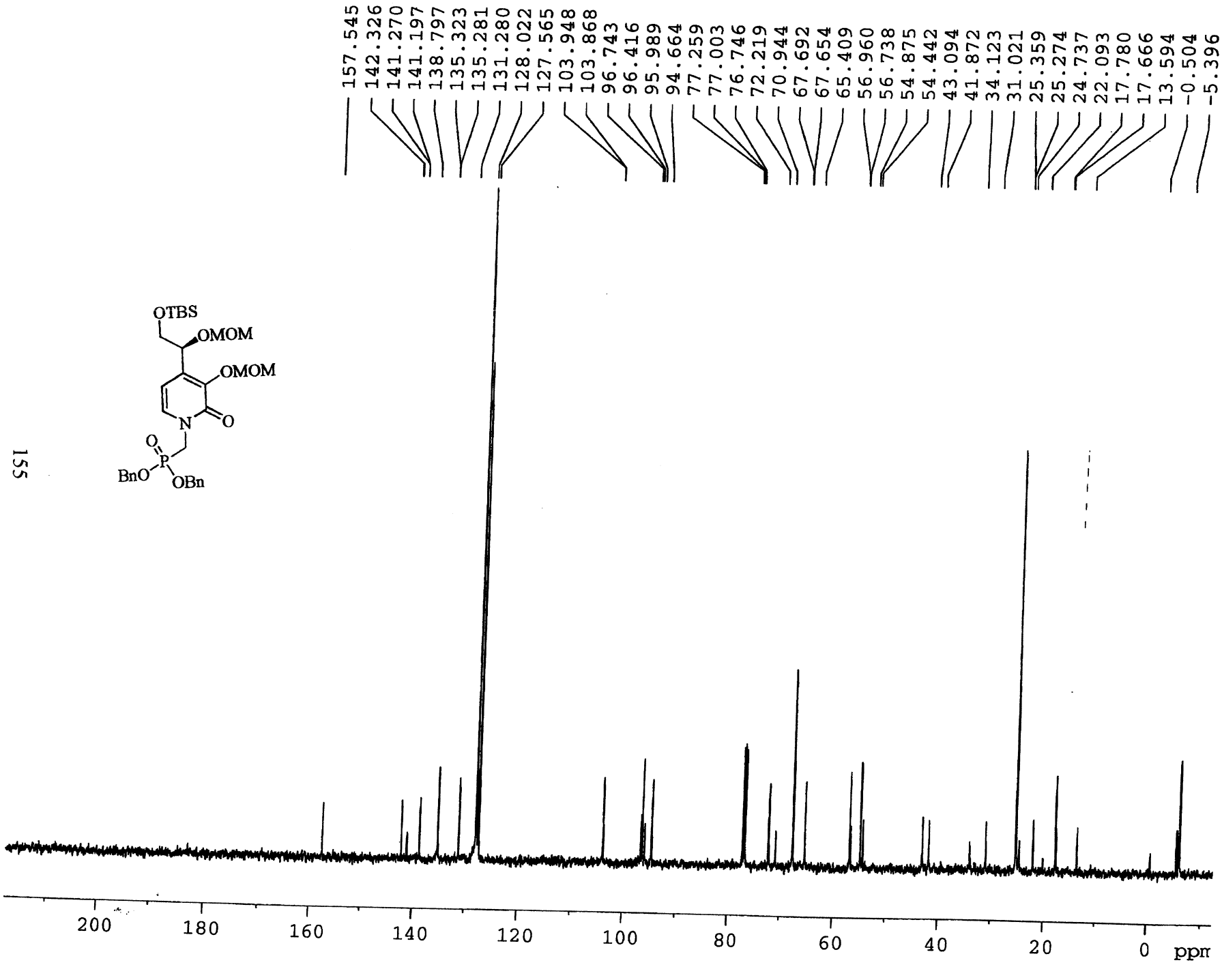
OTBS
OMOM
MOMO
OBn
BnO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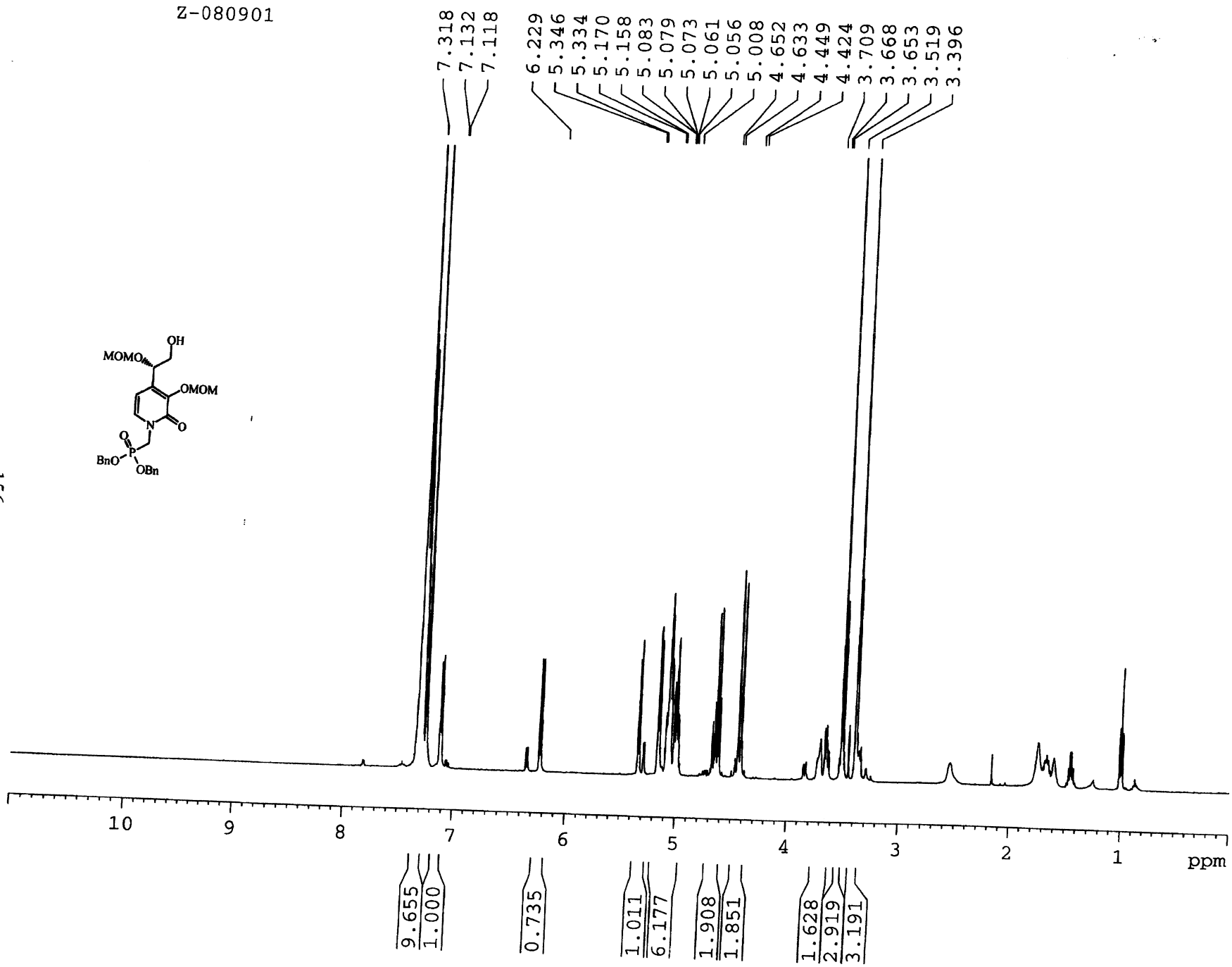
142.326
141.270
141.197
138.797
135.323
135.281
131.280
128.022
127.565
103.948
103.868
96.743
96.416
95.989
94.664
77.259
77.003
76.746
72.219
70.944
67.692
67.654
65.409
56.960
56.738
54.875
54.442
43.094
41.872
34.123
31.021
25.359
25.274
24.737
22.093
17.780
17.666
13.594
-0.504
-5.396
OTBS
OMOM
OMOM
N
O
O
P
BnO
OBn
200
180
160
140
120
100
80
60
40
20
0
ppm

Z-080901


7.318
7.132
7.118
6.229
5.346
5.334
5.170
5.158
5.083
5.079
5.073
5.061
5.056
5.008
4.652
4.633
4.449
4.424
3.709
3.668
3.653
3.519
3.396
OH
MOMO
OMOM
O
N
O
P
BnO
OBn
10
9
8
7
6
5
4
3
2
1
ppm
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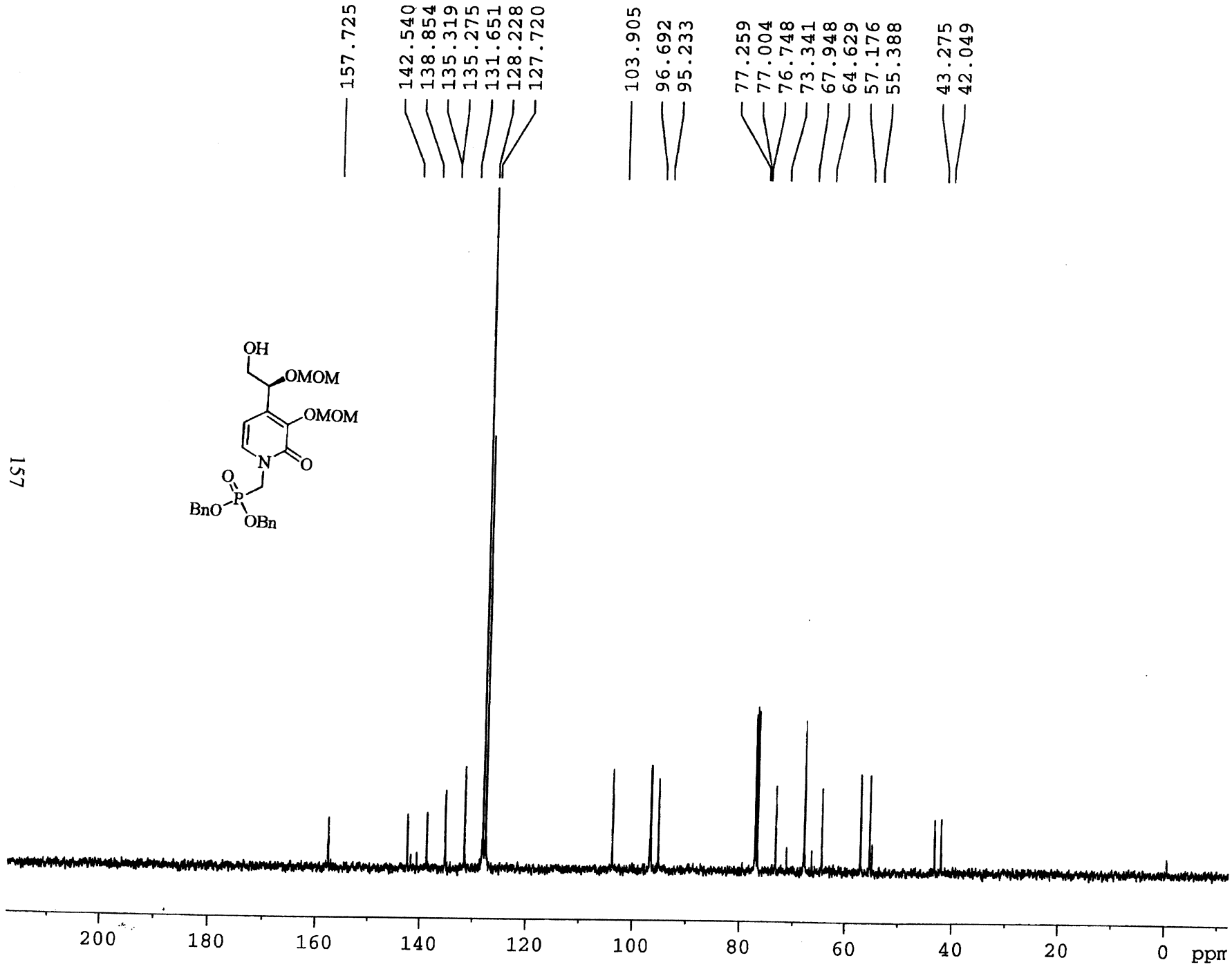
1.851
1.628
2.919
3.191

156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127.720
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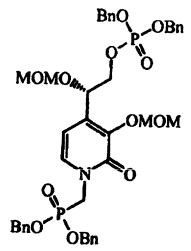
96.692
95.233
77.259
77.004
76.748
73.341
67.948
64.629
57.176
55.388
43.275
42.049
OH
OMOM
OMOM
N
O
O
P
BnO
OBn
200
180
160
140
120
100
80
60
40
20
0
ppm

157

Z-080908



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7.075
6.218
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5.367
5.355
5.044
4.600
4.557
4.459
4.433
4.389
4.363
4.146
4.139
4.134
4.074
4.053
3.448
3.302

10 9 8 7 6 5 4 3 2 1 ppm

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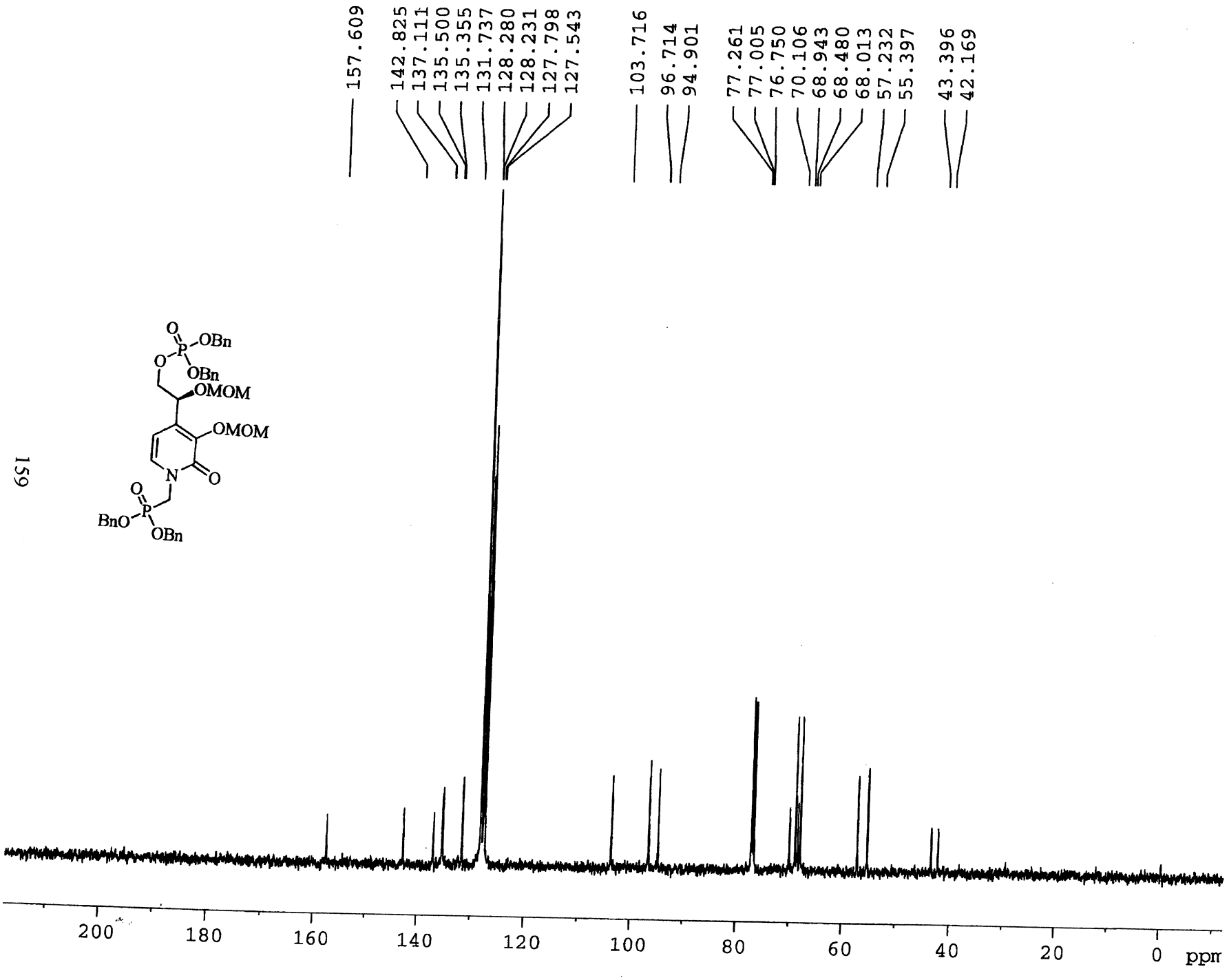
1.804
1.769
3.188
2.638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128.231
127.798
127.543
103.716
96.714
94.901
77.261
77.005
76.750
70.106
68.943
68.480
68.013
57.232
55.397
43.396
42.169
OBn
OBn
OMOM
OMOM
N
O
BnO
OBn
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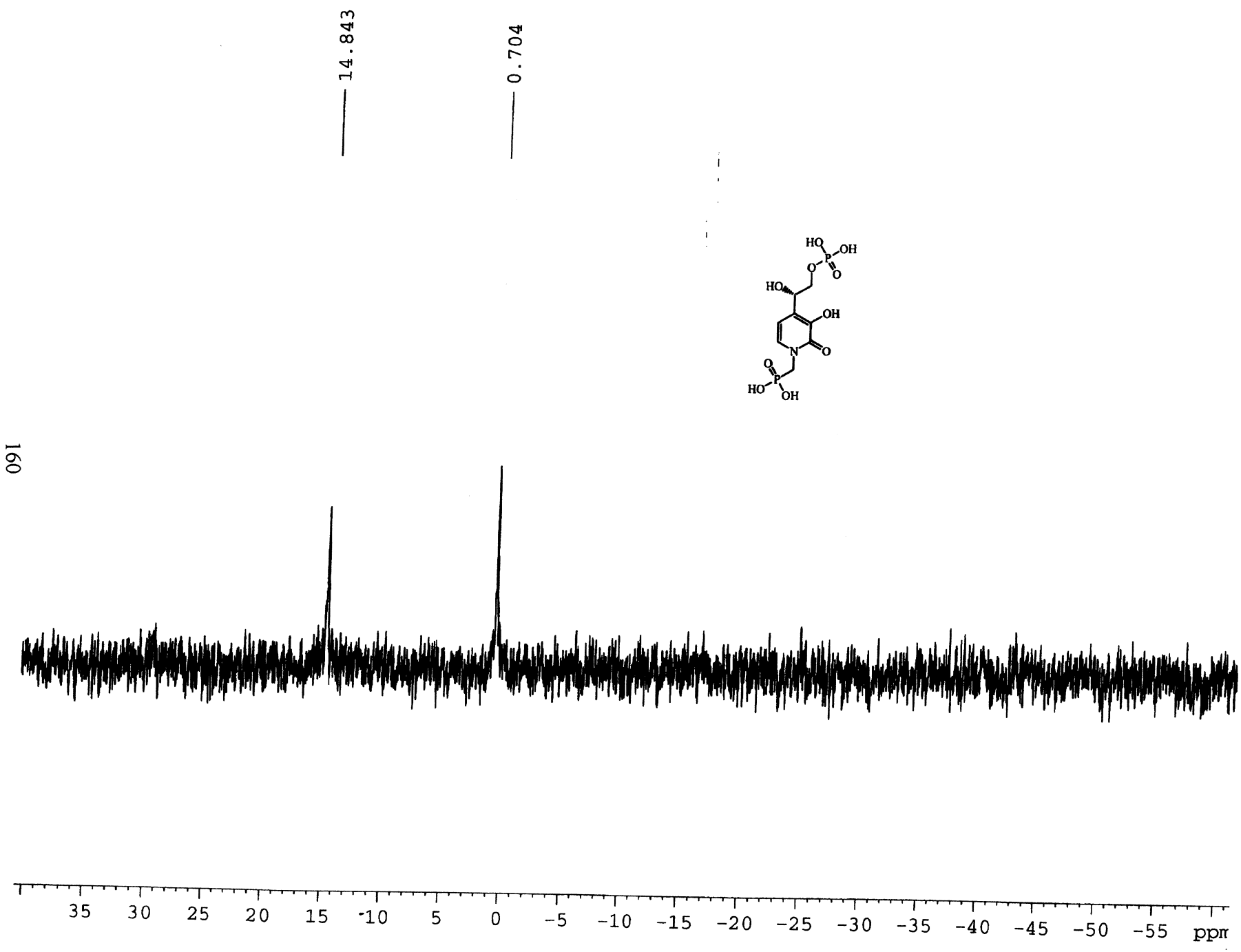
160
140
120
100
80
60
40
20
0
ppm

159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160
35 30 25 20 15 10 5 0 -5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 ppm

Z-081008
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7.280
6.590
6.576
5.238
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4.383
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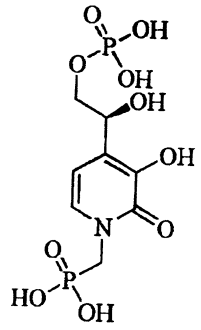
1.764
ppm



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67.056
49.372
46.582
45.409
200
180
160
140
120
100
80
60
40
20
0
ppm