Studies Towards the Synthesis of a Bicyclo[2.2.2]diazaoctane Ring System and Efforts Towards the Synthesis of SB-219383

Submitted by
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In partial fulfillment of the requirements
for the degree of
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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY **NICHOLAS C GEARHART** ENTITLED PROGRESS TOWARDS A BICYCLO[2.2.2]DIAZAOCTANE RING SYSTEM AND EFFORTS TO SB-219383 BE ACCEPTED AS FUFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE.

Committee on Graduate Work

Advisor

Department Head

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Chapter 1

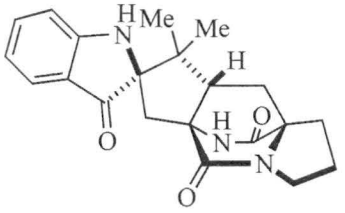
Introduction: Bicyclo[2.2.2]diazaoctane Ring System

1.1 Isolation and Structural Determination

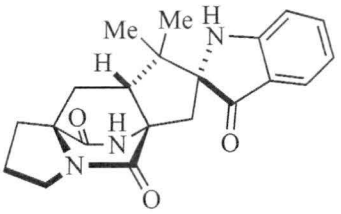
Marine organisms, particularly fungi, have become abundant sources of biologically active natural products possessing complex and diverse ring systems. Prenylated indole alkaloids such as the paraherquamides,[1] brevianamides,[2] stephacidins,[3] and notoamides[4] are fungal metabolites whose synthesis and biogenetic origin have been extensively investigated over the years.[5] To date, the number of natural products isolated in this family have surpassed fifty compounds. The majority of these natural products contain a unique bicyclo[2.2.2]diazaoctane ring system embedded in their core. The synthesis of this core would allow a general route to these natural products, which has not been achieved thus far. This would not only be beneficial to the total synthesis of these natural products, but also would provide easy access to a variety of analogs that would be beneficial for biological testing.

The first natural products observed to contain the bicyclo[2.2.2]diazaoctane ring system were isolated in 1969 when Birch and co-workers reported the isolation of brevianamides A and B (**1** and **2**, Figure 1) from *Penicillium brevicompactum*.[2] This marked the birth of this novel class of prenylated indole alkaloids, a family that in recent years has drawn much attention in part due to this unique characteristic ring system contained in their core. The brevianamides are made up of tryptophan, proline and one

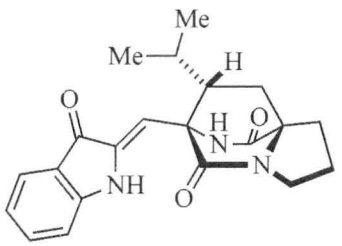
isoprene unit. Brevianamide B was shown to be a stereoisomer of A through interconversion (Scheme 1).[2] The relative and absolute stereochemistry of brevianamide A was determined through X-ray crystallography of 5-bromobrevianamide A.[6] Brevianamides C and D (**3** and **4**) were also isolated from the same culture, yet they were found to be artifacts of isolation due to light irridation.[2]



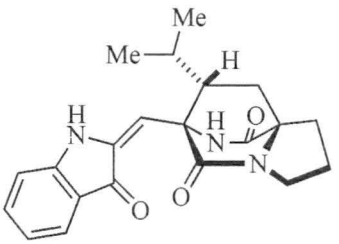
1, (+)-brevianamide A



2, (-)-brevianamide B

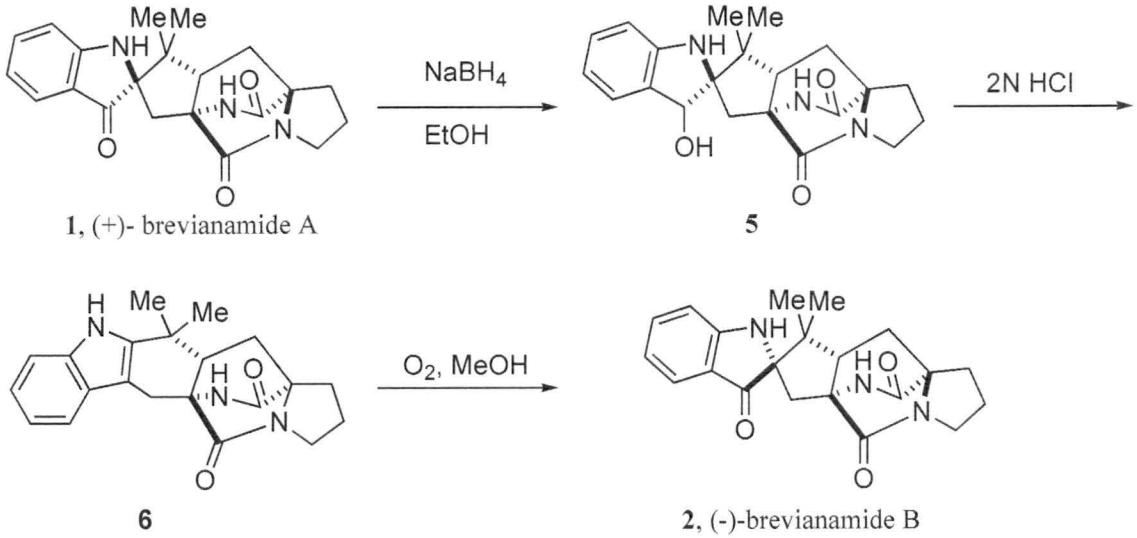


3, brevianamide C



4, brevianamide D

Figure 1. Structures of the brevianamides.[1]



1, (+)- brevianamide A

5

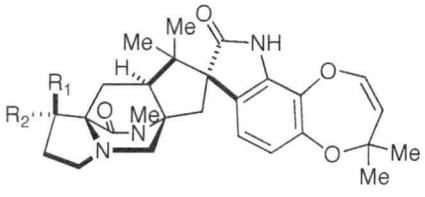
6

2, (-)-brevianamide B

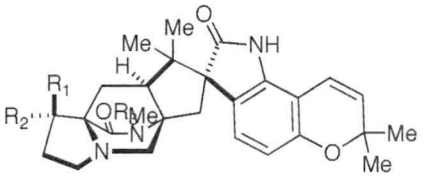
Scheme 1. Conversion of (+)-brevianamide A into (-)-brevianamide B.[1]

Over the next few decades this family has grown substantially to include the paraherquamides (A-G, **7-13**), the asperparalines (**15-18**), VM55599 (**19**), the marcofortines (**20-22**), and sclerotiamide (**23**) from *Penicillium* and *Aspergillus* species (Figure 2).[2,6-9] All of these compounds contain the characteristic bicyclo[2.2.2]diazaoctane core and are structurally more complex and diverse then their brevianamide counterparts, all sharing two isoprene units and a tryptophan (with the exception of the asperparalines, which contain a spiro-succinamide instead of the spiro-oxindole and only one isoprene unit). The various paraherquamides differ in substituted proline derivatives such as (**7-19**) compared to that of the marcfortines (**20-22**), which contain a pipecolic acid unit in place of the proline. One or two oxygens on the tryptophan moiety differentiate compounds (**7-11** *vs.* **12-14**, or **20-21** *vs.* **22-23**). All of these compounds with the exception of sclerotiamide (**23**) have their tertiary lactam reduced to the amine and VM55599 (**19**) remains as the 2,3-disubstituted indole, rather

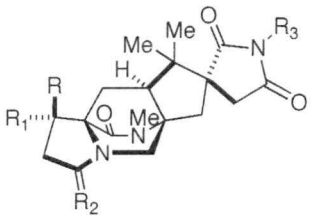
than being oxidized to the *spiro*-oxindole. It is also of great importance to note that all of these compounds in the paraherquamide family contain the *syn* orientation at the bicyclic core, compared to the *anti* configuration that is present in the brevianamides. The *syn* **(25)** and *anti* (**24**) relationship is determined by the position of the C-H proton at C19 of the bridgehead of the bicyclic core in relation to the bridging secondary lactam (Figure 3).[5]



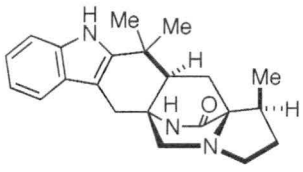
7, paraherquamide A, R_1 = OH, R_2 = Me
8, paraherquamide B, R_1 = H, R_2 = H
9, paraherquamide C, R_1 = R_2 = CH_2
10, paraherquamide D, R_1 = R_2 = OCH_2
11, paraherquamide E, R_1 = H, R_2 = Me



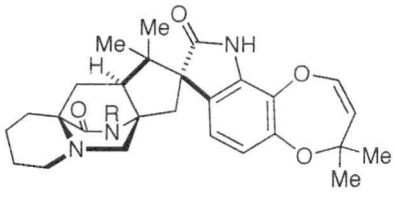
12, paraherquamide F, R_1=H, R_2=Me, R_3=Me
13, paraherquamide G, R_1=OH, R_2=Me, R_3=Me
14, VM55595, R_1=H, R_2=Me, R_3=H



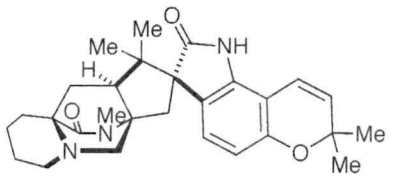
15, asperparaline A, R_1 = Me, R_2 = H_2, R_3 = Me
16, asperparaline B, R_1 = Me, R_2 = H_2, R_3 = H
17, asperparaline C, R_1 = H, R_2 = H_2, R_3 = Me
18, 16-keto-aspergillimide, R_1 = Me, R_2 = O, R_3 = Me



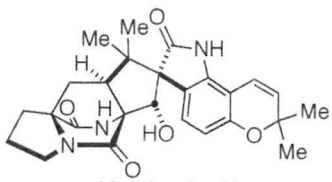
19, VM55599



20, marcfortine A, R = Me
21, marcfortine B, R = H

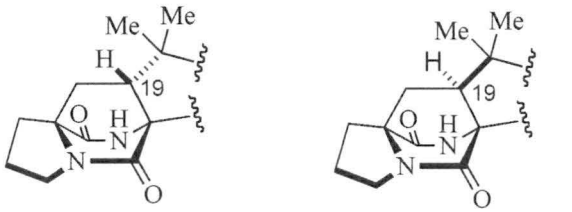


22, marcfortine C



23, sclerotiamide

Figure 2. Structures of the paraherquamides and related alkaloids.[5]



anti-diastereomer of the brevianamides **24**

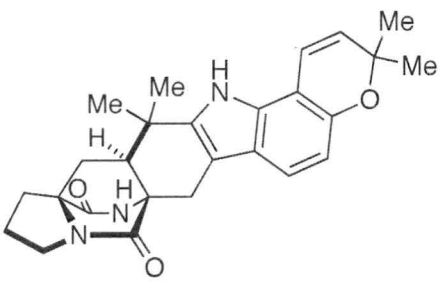
syn-diastereomer of the paraherquamides **25**

Figure 3. *Anti / syn* diastereomers at the bicyclo[2.2.2]diazaoctane core.

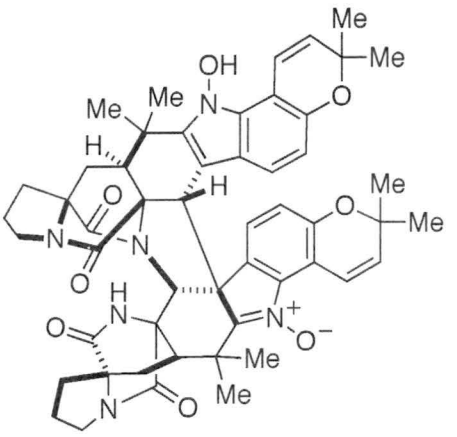
Another intriguing addition to the brevianamide and paraherquamide family occurred in 2002, when scientists from Bristol-Myers Squibb isolated stephacidin A and B **(26-27)** from the fungi *Aspergillus ochraceus* WC76466 (Figure 4).[10] A similar fungal alkaloid, avrainvillamide **(28)** (also named CJ-17665), was independently isolated from a different species of *Aspergillus*,[11] and later from the same species.[12] Aspergamides A and B **(29-30,** Figure 4**)** were isolated from *Aspergillus ochraceus* as well.[13] The stephacidins are similar to paraherquamide F by sharing a pyran ring on the tryptophan unit, yet are unique from most of the paraherquamides in that they contain a 2,3-disubstituted indole instead of the *spiro*-oxindole moiety, while the tertiary lactam remains intact. The very rare indole oxidation state of stephacidin B (**27**), avrainvillamide (**28**) and aspergamide A (**29**) is extremely intriguing. *N*-methoxyindoles are known to occur occasionally in nature, and there have been a few reported *N*-hydroxyindoles isolated.[14] However, stephacidin B, avrainvillamide and aspergamide A contain an indole nitrone moiety that has not been seen in any other natural products outside the *Aspergillus* species. Stephacidin B is believed to be the dimer of

avrainvillamide, which itself is proposed to be an oxidation product of stephacidin A.[10] Detailed 1D- and 2D-NMR spectral studies (DEPT, COSY, HETCOR, HMBC, HMQC, and NOESY) led to elucidation of the molecular structure of stephacidin A. The structure of stephacidin B was more challenging to decipher, especially due to the difficulty in finding a suitable NMR solvent to eliminate severe signal broadening and overlapping. A solvent mixture of DMSO-d_6 and acetonitrile-d_3 finally gave well-resolved NMR spectra. While extensive 2D-NMR studies (COSY, HMBC, and NOESY) led to the establishment of the structural fragments, the nature of the dimer linkage in stephacidin B remained unclear until a single-crystal X-ray structure was obtained. The dimer is formed from just 2 bonds, and the molecule has a butterfly-like structure. With 15 rings and 9 stereogenic centers, stephacidin B has been deemed to be one of the most structurally complex and novel alkaloids occurring in nature.[10]

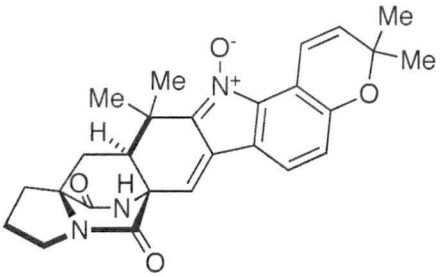
Early in 2006 the first chlorinated indole alkaloid belonging to this family was isolated. Malbrancheamide **(31,** figure 5**)** was isolated from the ascomycete *Malbranchea aurantiaca* and is the first in the family to be isolated outside of the *Penicillium* and *Aspergillus* genus.[15] Detailed 2D-NMR spectral analysis (COSY, HETCOR, HMBC, and NOESY) and X-ray analysis established that malbrancheamide had the same syn-diastereomeric relationship as the stephacidins and the paraherquamides. Also, similar to the paraherquamides, malbrancheamide's tertiary lactam is reduced.



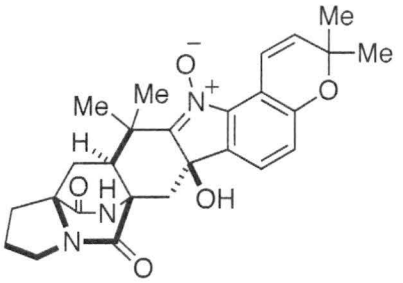
26, stephacidin A



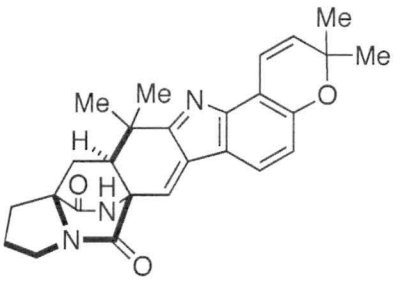
27, stephacidin B



28, avrainvillamide

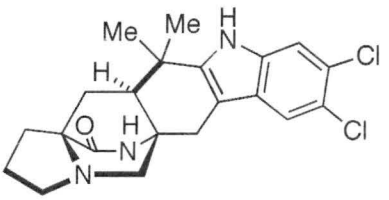


29, aspergamide A



30, aspergamide B

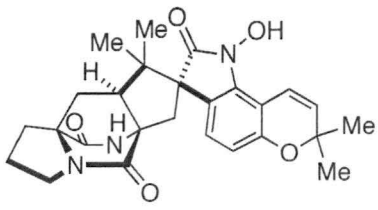
Figure 4. Structures of the stephacidins and related alkaloids. [10, 12]



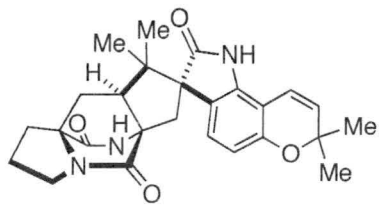
31, malbrancheamide

Figure 5. Structure of malbrancheamide.[15]

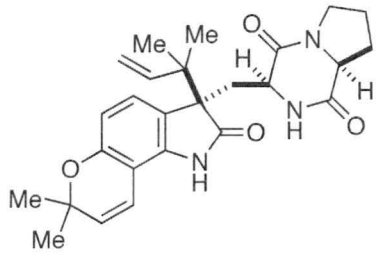
Recently, Tsukamoto and co-workers isolated four new prenylated indole alkaloids named the notoamides A-D **(32-35,** Figure 6).[16] The notoamides were isolated along with the known alkaloids sclerotiamide **(23)** and stephacidin A **(26)** from a marine strain of *Aspergillus sp.* cultivated from the common mussel, *Mytilus edulis* found off the Noto peninsula in the Sea of Japan. Similar to stephacidin A and B **(26-27)** the notoamides posses a sensitive indolopyran ring system and notoamides A and B **(32-33)** contain the bridged bicyclo[2.2.2]diazaoctane core characteristic of this growing family. Most recently, versicolamide B **(36,** figure 6**)** was isolated from *Aspergillus versicolor* NRRL 35600.[17] Other then the brevianamides, versicolamide B is the only member of the paraherquamide family to possess the *anti*-relative stereochemistry at the bridgehead.



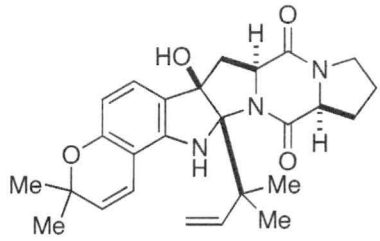
32, notoamide A



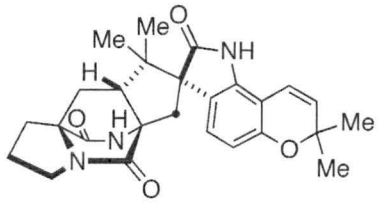
33, notoamide B



34, notoamide C



35, notoamide D

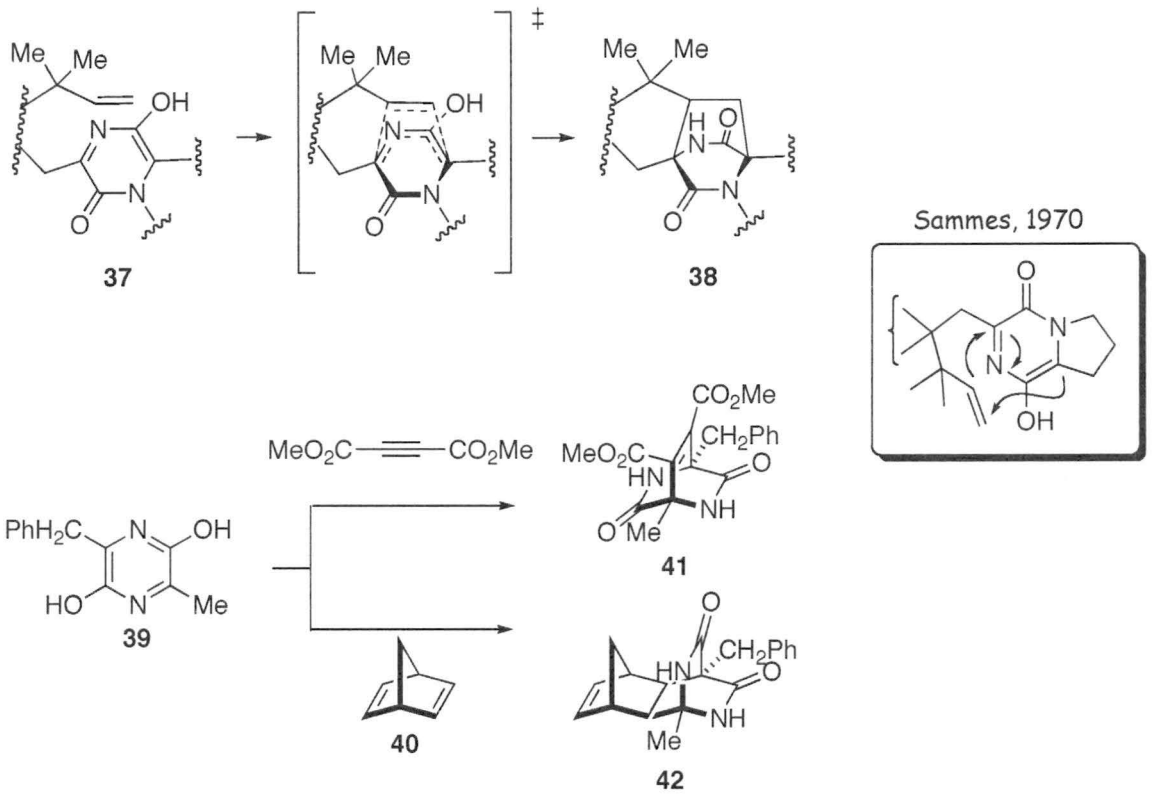


36, versicolamide B

Figure 6. Structure of the notoamides and versicolamide B.[16-17]

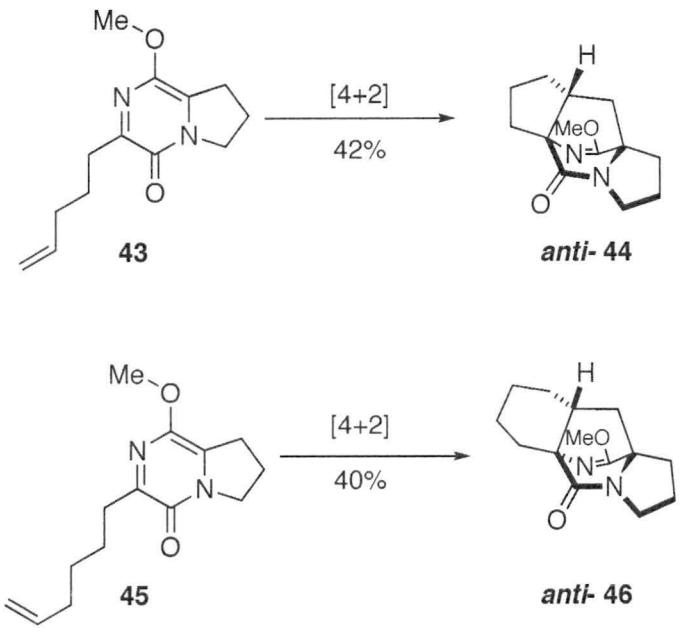
1.2 Biosynthetic Origin of the bicyclo[2,2,2]diazaoctane core.

Shortly after the brevianamides were isolated, Porter and Sammes proposed that the bicyclo[2.2.2]diazaoctane core could originate from a hetero Diels-Alder cycloaddition (**37-38**) (Scheme 2).[18] They showed this hypothesis to be possible by reacting the model dihydroxy pyrazine (**39**) with dimethyl acetylenedicarboxylate and with norbornadiene (**40**), to provide the Diels-Alder cycloadducts **41** and **42**.



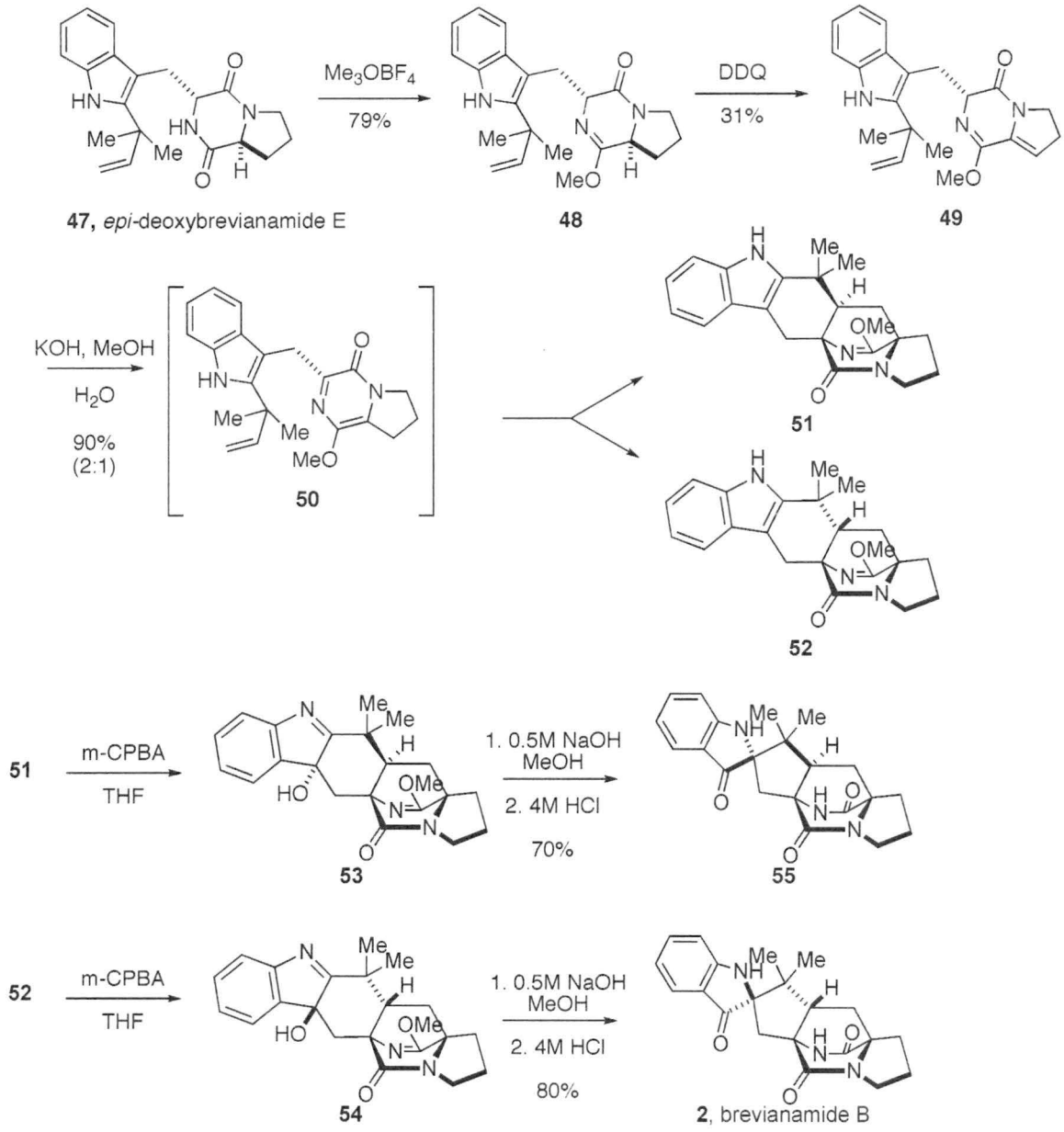
Scheme 2. Proposed biosynthesis of the brevianamides.[18]

Model systems using the diketopiperazine (**43, 45,** scheme 3) as the diene for the proposed [4+2] cycloaddition were prepared to test this biosynthetic hypothesis (Scheme 3).[19] It was found that the intramolecular Diels-Alder reaction with either **43** or **45** proceeded spontaneously at room temperature to give exclusively the *anti*-diastereomeric products **44** and **46**. Interestingly, an intermolecular reaction with an analogous azadiene and either cyclopentene or cyclohexene could only be effected by harsh Lewis acidic conditions. These results suggested that the proposed Diels-Alder biosynthesis of the brevianamide and paraherquamide family was indeed feasible. While a specific enzyme might not be needed to catalyze the spontaneous reaction, it was proposed that an enzyme would be needed to control the relative *anti* or *syn* configuration of the natural products.



Scheme 3. Model studies examining the Diels-Alder biosynthetic hypothesis.[19]

Williams and co-workers tested this enzymatic Diels-Alder biogenetic hypothesis in their synthesis of racemic brevianamide B (**2,** scheme 4). [20,21] They started by forming the lactim ether (**48**) from *epi*-deoxybrevianamide E (**47**) by treatment with trimethyloxonium tetrafluoroborate. Oxidation with DDQ gave the azadiene (**49**), which spontaneously cyclized upon tautomerization under basic conditions to give a 2:1 mixture of cycloadducts **51** and **52**, favoring the *syn*-diastereomer. Each cycloadduct was converted to the *spiro*-indoxyl moiety by diastereoselective oxidation, followed by a base-catalyzed pinacol-type rearrangement. Removal of the lactim ethers provided the desired racemic brevianamide B (**2**) and C19-*epi*-brevianamide A (**55**). These were the first results to show that the brevianamide natural products could be constructed through a biosynthetic Diels-Alder approach.



Scheme 4. Biomimetic synthesis of brevianamide B (**2**). [20,21]

1.3 Biological Activity

Brevianamides A and D have modest insecticidal activity, and recent studies have shown that some of the paraherquamides also display potent insecticidal activity.[10-15,22] The paraherquamides have been widely studied for their potential in veterinary medicine.

The paraherquamides have anthelmitic activity against several drug-resistant strains of nematodes.[7,8,23-26] The stephacidins have demonstrated in vitro cytotoxicity against numerous human tumor cell lines (Table 1).[10] Stephacidin B (**27**) is much more potent and shows more selective antitumor activity than stephacidin A (**26**). The best selectivity was seen with prostate-dependent LNCaP cells, where **27** had an IC_{50} value of 0.06 μM. Importantly, it was proposed that these cytotoxic compounds have a novel mechanism of action, which is not mediated by the p53, mdr, bc12, tubulin or topoisomerase II. Stephacidin A was re-isolated from another group in a culture broth of *Aspergillus ochraceus* and tested for biological activity as an inhibitor of the mammalian mitochondrial respiratory chain.[27] Stephacidin A had only slight inhibitory potency against NADH oxidase (IC_{50} value of 34.6 μM), suggesting that mitochondrial chain inhibition is not part of the mechanism of action for this cytotoxic compound.

Avrainvillamide (**28**) was tested against multi-drug resistant (MDR) strains of Gram-positive bacteria.[24] Vancomycin is typically used as the drug for the treatment of MDR, but vancomycin-resistant Enterococci (VRE) and vancomycin-intermediate resistant *Staphylococcus aureus* (VISA) have escalated the need for a novel antibacterial drug with activity against MDR, VRE and VISA.[25] Avrainvillamide was tested for activity against these strains, and compared to the antibiotics erythromycin, azithromycin and vancomycin (Table 2), avrainvillamide displayed good antibacterial activity against these MDR bacteria, yet had no antibacterial activity against *E. coli*. Avrainvillamide was also found to be cytotoxic to HeLa cells (cervical cancer cell lines), with an IC_{50} value of 1.1 μg/mL. [12]

cell line	histotype	characteristic	**26** (IC_{50})	**27** (IC_{50})
PC3	prostate	testosterone-independent	2.10	0.37
LNCaP	prostate	testosterone-sensitive	1.00	0.06
A2780	ovarian	parental	4.00	0.33
A2780/DDP	ovarian	mutp53/bcl2+	6.80	0.43
A2780/Tax	ovarian	taxol-resistant	3.60	0.26
HCT116	colon	parental	2.10	0.46
HCT116/mdr+	colon	overexpress mdr+	6.70	0.46
HCT116/topo	colon	resistant to etoposide	13.10	0.42
MCF-7	breast	estradiol-sensitive	4.20	0.27
SKBR3	breast	estradiol-independent	2.15	0.32
LX-1	lung	sensitive	4.22	0.38

Table 1. In vitro cytotoxicity of stephacidin A (**26**) and stephacidin B (**27**) (IC_{50} in μM).[10]

	MIC (μg/ml)			
Microorganism	CJ-17,665 (Avrainvillamide, **28**)	Erythromycin	Azithromycin	Vancomycin
Staphylococcus aureus 01A1105	12.5	>100	>100	1.56
Streptococcus pyogenes 02C1068	12.5	>100	>100	0.39
Enterococcus faecalis 03A1069	25	>100	>100	12.5
Esherichia coli 51A0266	>100	100	1.56	>100

Table 2. Antibacterial activities of avrainvillamide (CJ-17,665, **28**).[12]

Malbrancheamide (**31**) was shown to be a novel type of calmodulin (CaM)-inhibitor, where the compound competes with the formation of the CaM-PDE1 active complex. Malbrancheamide had an IC_{50} value of 3.65 μM, which is comparable to that of chlorpromazine (IC_{50} = 2.75 μM), a well-characterized CaM antagonist.[15]

The notoamides A-C (**32-34**) exhibit moderate cytotoxicity against a variety of cancer cell lines, however notoamide D (**35**) has not been shown to possess any similar activity.[16]

1.4 Past Synthetic Work Towards the bicyclo[2.2.2]diazaoctane Core

The hypothesis by Birch,[2] Sammes,[18] and Williams[5,19-21] that the bicyclo[2.2.2]diazaoctane core is derived biosynthetically from a Diels-Alder reaction has influenced several creative synthetic strategies for its construction (Figure 7). The groups of Williams[5] and Liebscher[30] have independently developed Diels-Alder strategies with success (**57,61,63**); the Williams group has also developed a novel stereoselective intramolecular S_N2' alkylation approach for the construction of the bicyclic core (**59**).[31] In Myers' recent total synthesis of stephacidin B, a very elegant acyl radical approach was utilized to access this core (**65**).[32] Baran and co-workers utilized a remarkable metal-mediated oxidative coupling of enolates which formed the bicyclic core (**86**) as a single diastereomer.[33] Williams' and Myers' approaches are the only examples that isolate the bicyclo[2.2.2]diazaoctane core as a key intermediate (**57, 65,** Figure 7) in the synthesis of their natural products without the indole nucleus intact. The Williams intermediate **57** shows the *anti*-relationship at C19 compared to the Myers intermediate **65,** which contains the *syn* relationship at C19. It is noteworthy that there is a far greater number of compounds in this family that contain the bicyclo[2.2.2]diazaoctane core constituted as the *syn*-diastereomer.

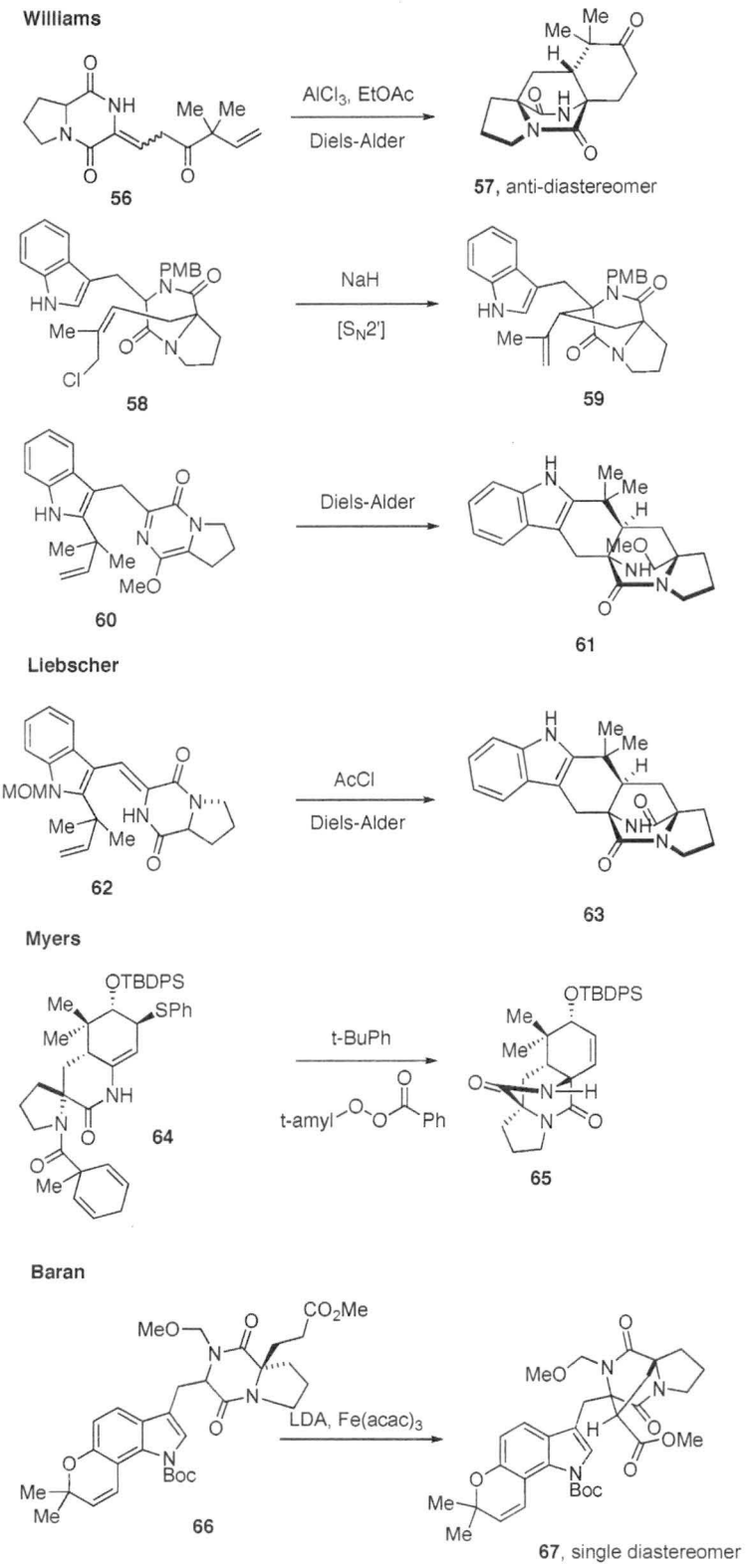


Figure 7. Williams,[5,19-21,31] Liebscher,[30] Myers'[32], and Baran's[33] approach to the bicyclo[2,2,2]diazaoctane core.

Williams and co-workers established an S_N2' cyclization for the construction of the bicyclic core in their synthesis of brevianamide B.[31] The known optically active allylated proline derivative **68** was prepared according to the literature. Conversion of **68** to the piperazinedione **70** was achieved by aminolysis with *p*-methoxybenzylamine followed by condensation with bromoacetyl bromide and ring closure. Ozonolysis of **70** afforded aldehyde **71,** which was homologated to the *E*-allylic alcohol **72** by Wittig reaction and $NaBH_4$ reduction. Silylation of **72** followed by carbomethoxylation afforded **73** as a 4:1 mixture, which was used in a Somei-Kametani condensation to afford **74** as a single diastereomer. Compound **74** was converted to **75** in four steps which then underwent the key intramolecular S_N2' cyclization with sodium hydride and 18-crown-6 to afford **76.** Completion of the synthesis involved removal of the BOC group with HCl in dioxane and cyclization to afford **77.** Oxidation with *m*-CPBA gave **78,** which was directly treated with NaOMe to furnish **79.** Treatment of **79** with excess *t*-butyllithium and O_2 removed the PMB group to provide brevianamide B (**2**) in 40% yield. Williams and co-workers have had much recent success in the synthesis of many of these natural products using either the S_N2' approach, or the Diels-Alder method.[5,19-21,31]

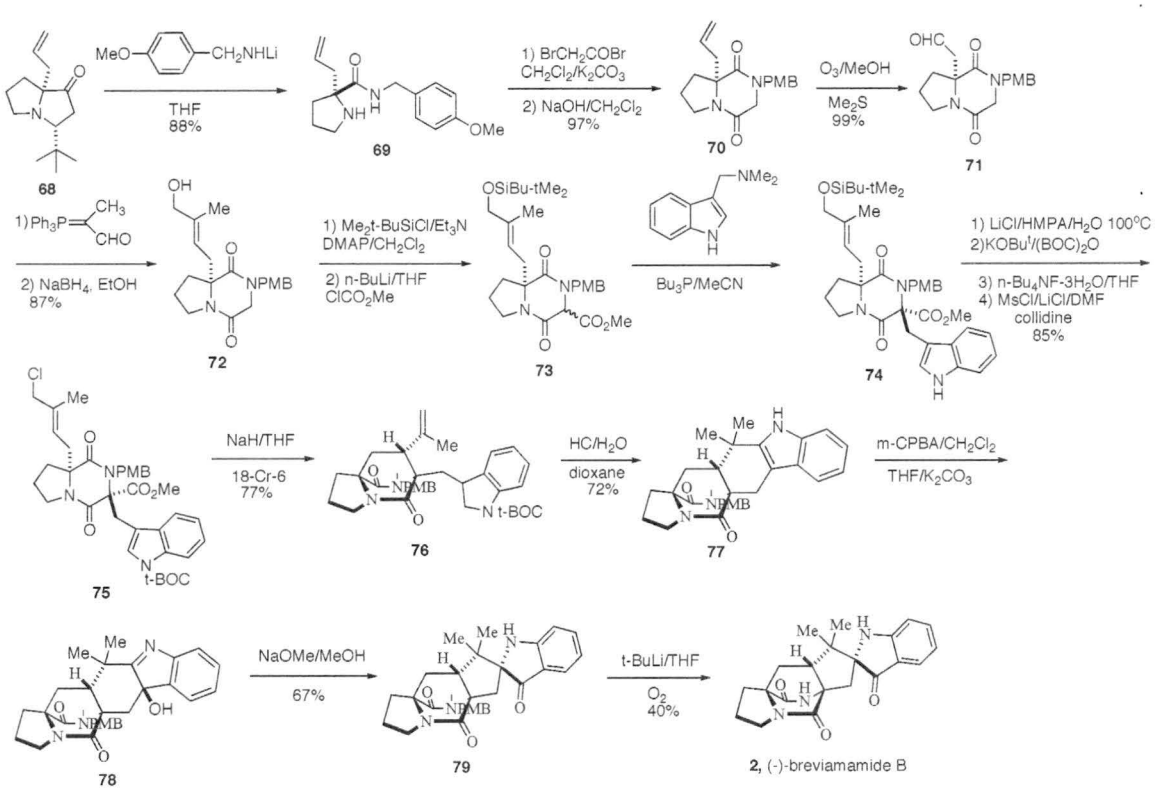
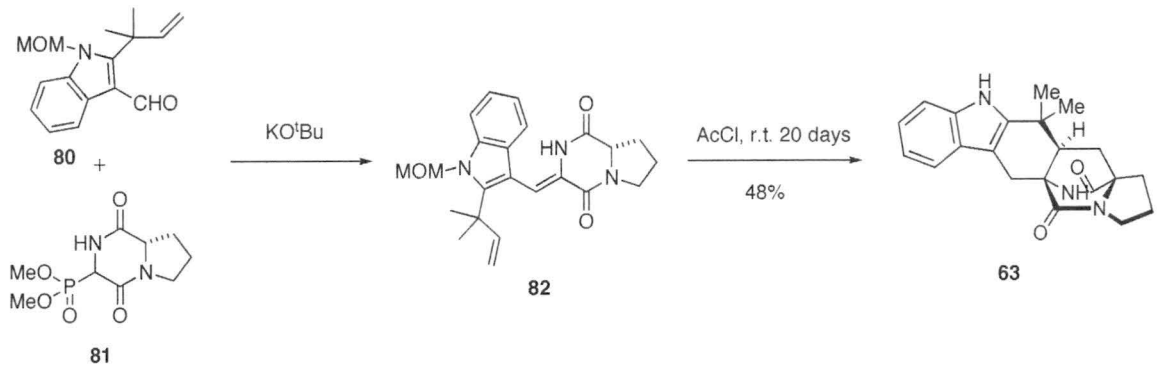


Figure 8. Williams Synthesis of brevianamide B, utilizing an S_N2 reaction.[31]

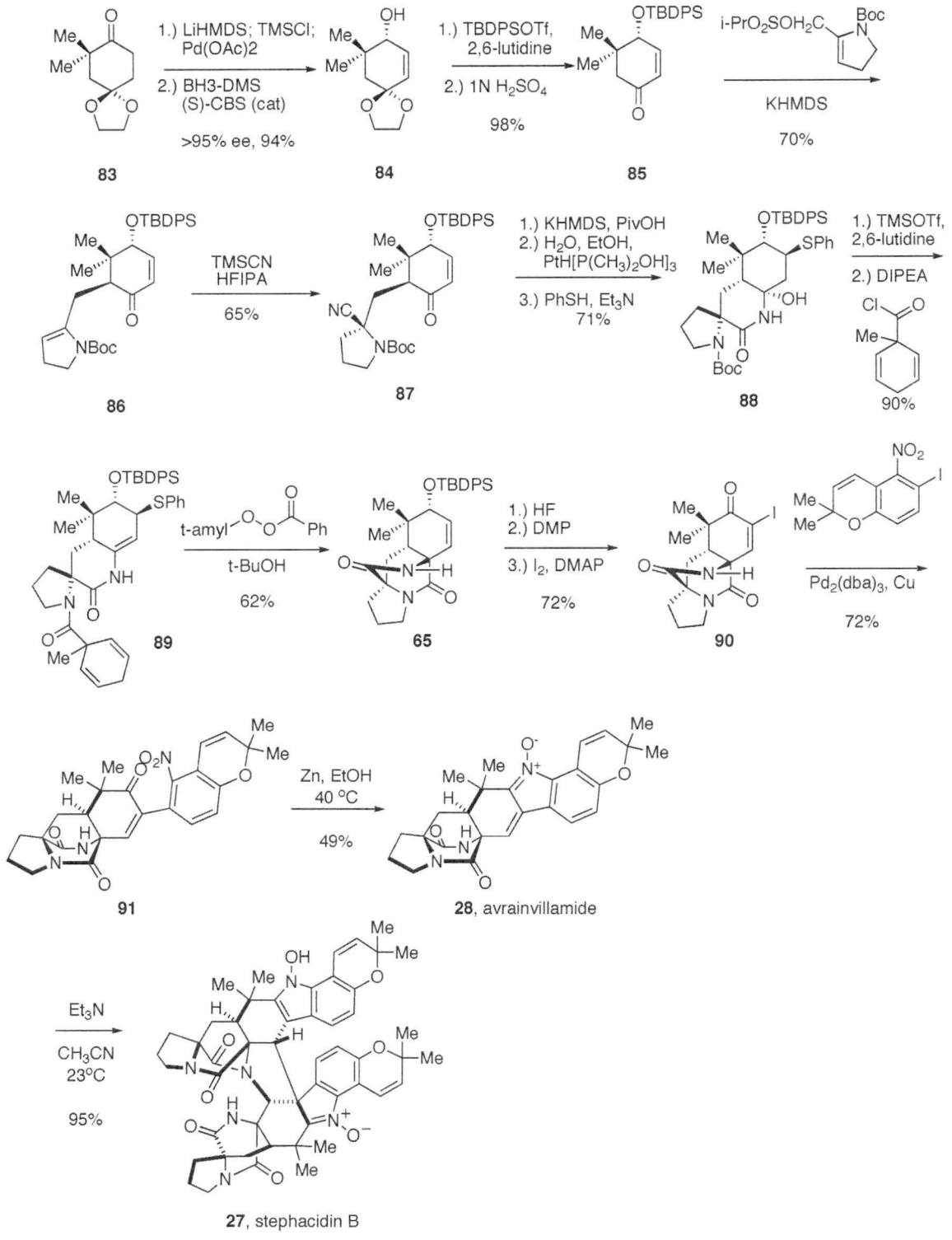
Liebscher and co-workers found that MOM-protected 3-indolylmethlidenepiperazine-2,5-dione **82** undergoes a straightforward intramolecular Diels-Alder reaction to lead to the bicyclic core (**63,** Scheme 5).[30] This reaction yields 43% of the product after letting the material stand in acetyl chloride at room temperature for 20 days. In an attempt to reduce the reaction time, refluxing formic acid with $BF_3 \bullet Et_2O$ in dichloromethane even under high pressure, or refluxing in DMF/DMAP or aqueous KOH in methanol all proved to be unsuccessful.



Scheme 5. Liebscher's approach to the bicyclo[2,2,2]diazaoctane core.[30]

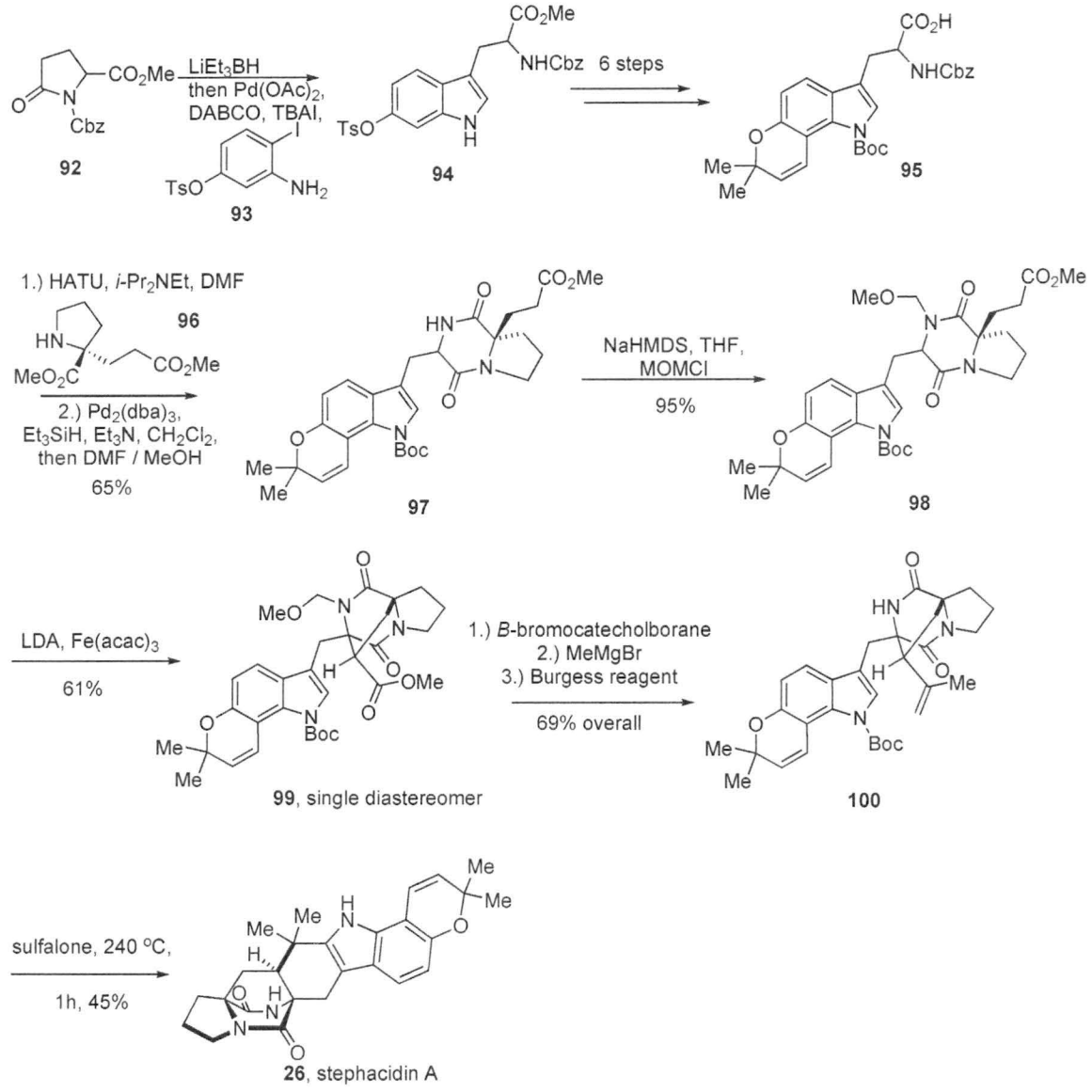
Herzon and Myers provided an elegant enantioselective synthesis of stephacidin B (**27**) through the synthesis of avrainvillamide (**28**, Scheme 6).[32] The stereochemistry that was set in **84** (>95% ee) from a Corey-Bakshi-Shibata (CBS) enantioselective reduction of the initial ketone **83** controlled the stereochemical outcome throughout the rest of the synthesis. At this point, the absolute stereochemistry of stephacidin A and B were not determined, so the authors randomly chose the (*S*)-CBS catalyst to illustrate their enantioselective route to stephacidin B. The silyl ether directed diastereoselective alkylation of the enolate of **85**, providing **86** as a single diastereomer. In a critical transformation, the alkylation product **86** was found to undergo Strecker-like addition of HCN in HFIPA that gave **87** as the major diastereomer in 65% yield. Epimerization followed by nitrile conversion of the amide allowed cyclic hemiaminal formation to give the tricyclic product **88**. Dehydration was accompanied with *N*-Boc deprotection, which after acylation led to the key acyl radical precursor **89**. The bicyclo[2.2.2]diazaoctane core **65** is believed to be formed by an aminoacyl radical intermediate followed by attack of the aminoacyl radical upon the more substituted position of the enamide C-C double bond and expulsion of phenylthiyl radical. The stage was then set for an Ullmann-like

coupling of the vinyl iodide **90** and the aryl iodide, to provide the nitroketone product **91**. Reductive cyclization then furnished avrainvillamide (**28**), which dimerized to stephacidin B (**27**) reversibly in the presence of excess triethylamine.

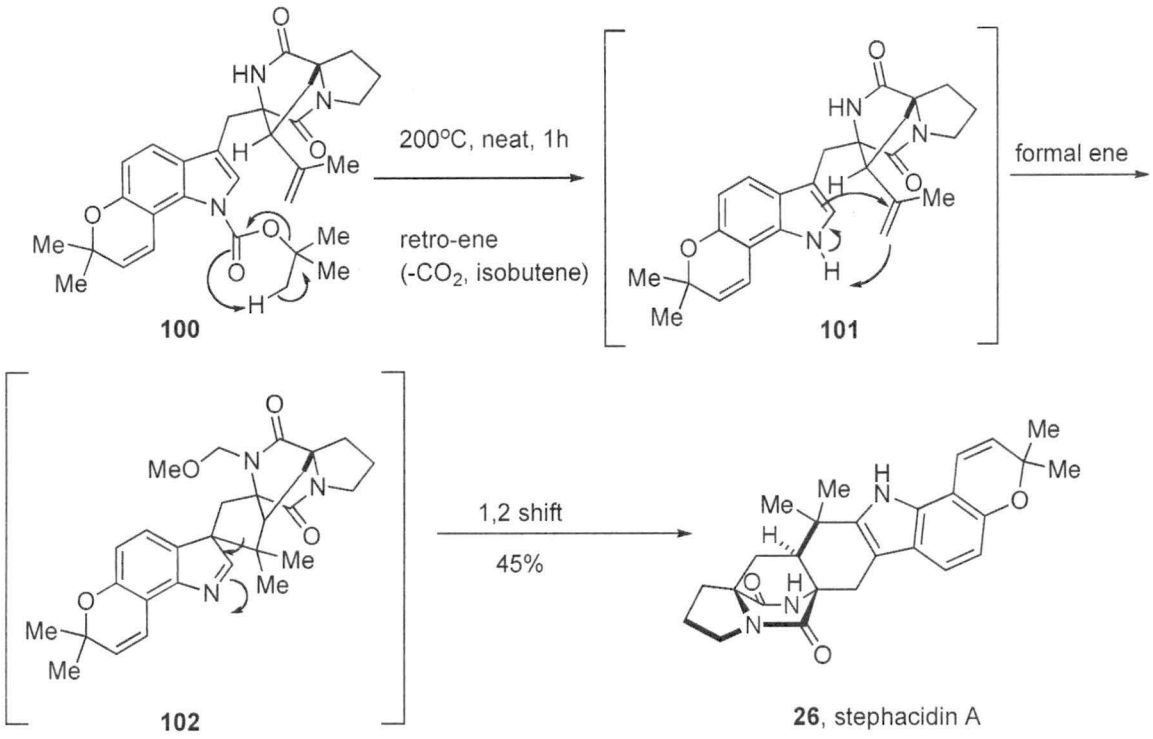


Scheme 6. Herzon and Myers synthesis of stephacidin B.[32]

In 2006 Baran and co-workers accomplished the total synthesis of stephacidin A (**26**) and then on to stephacidin B (**27**) utilizing an intramolecular oxidative heterocoupling to form the bicyclic core (Scheme 7).[33] Noting the difficulty in obtaining a practical and rapid synthesis of 6-hydroxytryptophan, the authors were finally able to obtain the precursor **94** in fantastic yield after extensive optimization. With the indole in hand, subsequent chromene installation by the approach developed by Williams provided **95**. Peptide coupling of **95** with the proline derivative **96**, followed by a chemoselective cleavage of the N-Cbz group, allowed the cyclization to the diketopiperazine **97**. After MOM-protection, the bicyclic core was formed in a rare metal-mediated oxidative enolate coupling. This remarkable reaction gave **99** as a single diastereomer. After removal of the MOM group with *B*-bromocatecholborane, reaction with MeMgBr provided a tertiary alcohol that was dehydrated with Burgess reagent to furnish **100**. The stage was now set for another remarkable reaction, where stephacidin A (**26**) was formed in one pot by simply heating **100** either neat or in sulfolane to 240°C (Scheme 8). The reaction is proposed to proceed by sequential thermolytic removal of the Boc group (by a retro-ene reaction) to give **101**, a formal ene reaction to give the *spiro*-cyclic intermediate **102**, which then finally undergoes a 1,2-shift to finish the cascade and provide stephacidin A (**26**).



Scheme 7. Baran's enantioselective synthesis of stephacidin A.[33]

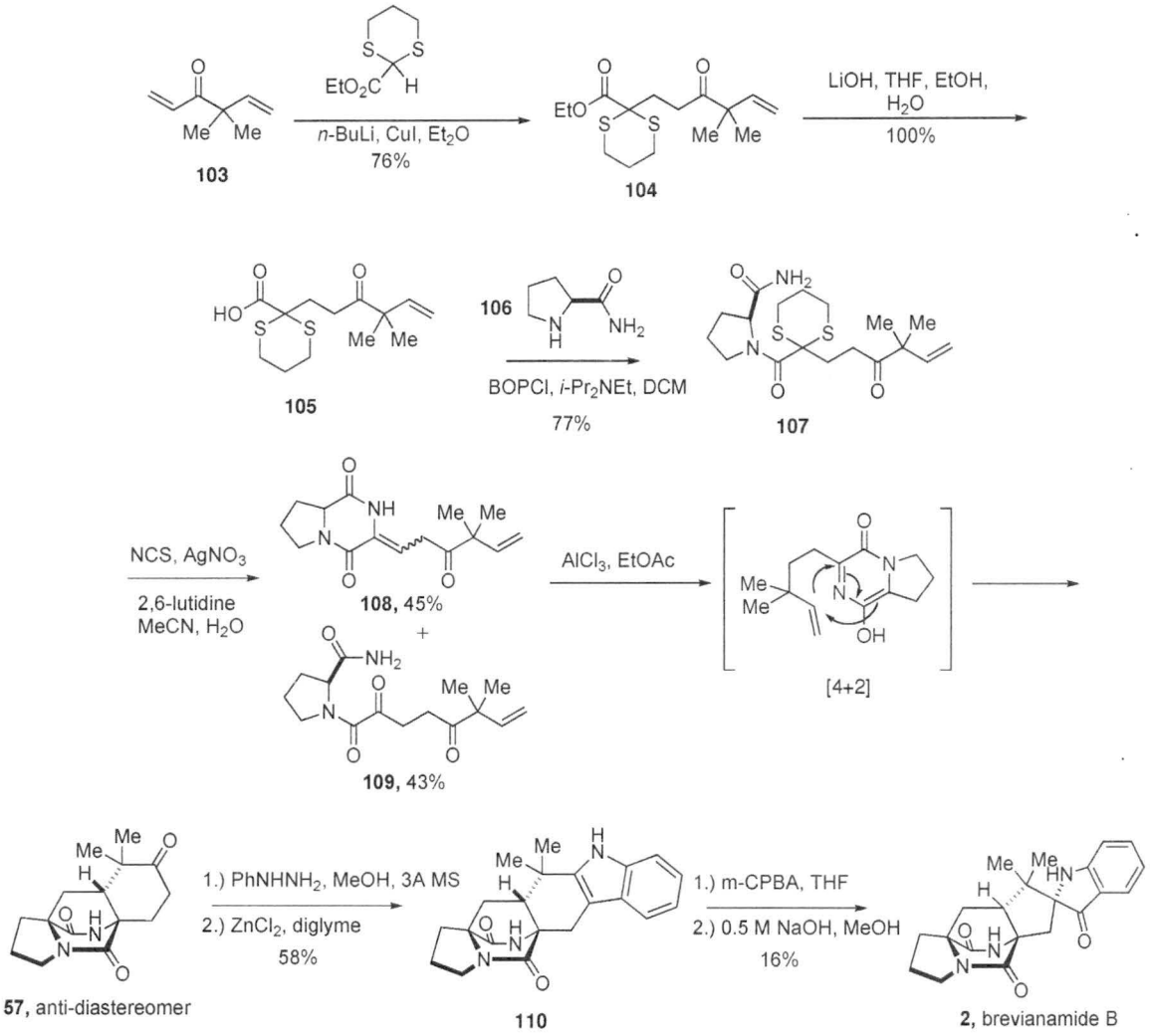


Scheme 8. Indole annulation cascade in the synthesis of stephacidin A.[33]

1.5 Potential Access To A Variety of Natural Products.

In 2006 Williams and co-workers reported a straightforward, concise conversion of the *anti*-diastereomer of the bicyclo[2.2.2]diazaoctane ring system to brevianamide B (**2,** Scheme 9).[34] Conjugate addition of ethyl 1,3-dithiane-2-carboxylate to known ketone **103** followed by basic hydrolysis of the ester to form the acid **105,** which was coupled with (L)-prolinamide (**106**) provided the protected peptide **107**. Oxidative deprotection of the dithiane gave a mixture of diketopiperazine **108** and the uncyclized amide **109**. Reacting this mixture with 3 equivalents of $AlCl_3$ in refluxing EtOAc for 24 hours gave the desired Diels-Alder product **57**. The relative stereochemistry of the cycloadduct was exclusively the *anti*-configuration. The bicyclic core **57** was converted to the phenyl hydrazone, which without purification, was rearranged to the indole **110** by the Fischer

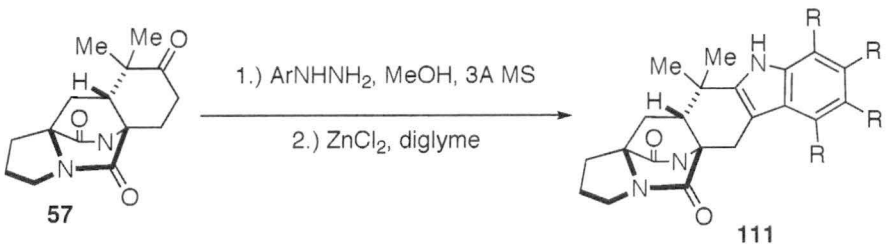
indole reaction. Indole **110** was stereoselectively oxidized to the 3-hydroxyindolenine, which undergoes a pinacol-type rearrangement under basic conditions to provide racemic brevianamide B (**2**).



Scheme 9. Williams synthesis of d,l-brevianamide B.[34]

It is believed that the Fischer indole reaction utilized to convert the bicyclo[2.2.2]diazaoctane core **57** into indole **110** could also be applied to the *syn*-diastereomer. If the *syn*-configuration of the bicyclic core is obtained, the general Fischer indole reaction can be utilized to construct a vast number of natural products or

analogs in this family that are constituted the *syn*-diastereomer of this ring system. It was found during this work that by varying the hydrazine substrate of the Fischer indole reaction a variety of substitution and functionality could be constructed off the bicyclic core (Table 3).[34,35] Using commercially available hydrazines (**112-121**), varying functionality such as methoxy (**113, 115, 116, 118**), methyl (**120**), and chloro (**122, 123**) groups could be installed using the Fischer indole reaction. The scope of this reaction might allow for fast and efficient construction of many natural products using a much more general method as compared to Myers' approach to stephacidin B (**27**). This might prove to be a valuble method in making analogs, which would allow the further exploration of the biological activity of these compounds.



57 111

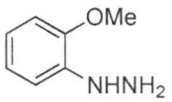
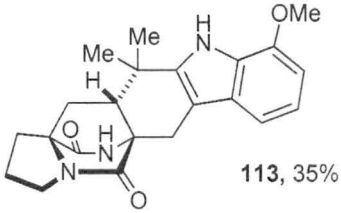
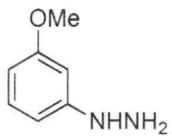
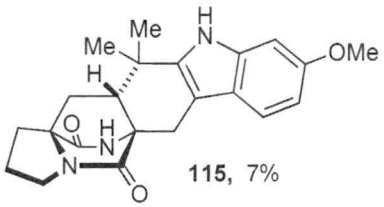
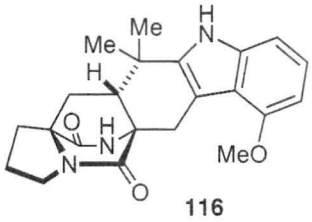
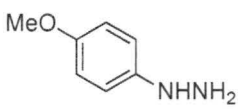
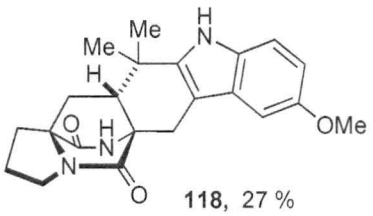
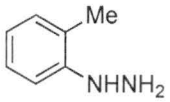
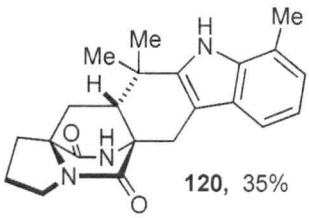
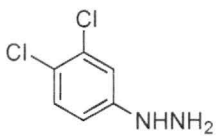
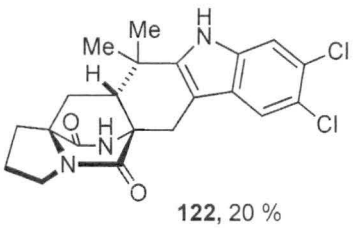
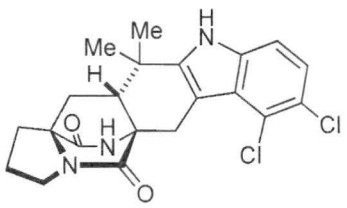
Hydrazine	Product
 112	 **113**, 35%
 114	 **115**, 7%  **116** Product ratio 2:1 for **115:116**
 117	 **118**, 27 %
 119	 **120**, 35%
 121	 **122**, 20 %  **123**, 10%

Table 3. Various Fischer indole reaction products. [35]

1.6 Research Objectives

The bicyclo[2.2.2]diazaoctane core, particularly the *syn*-diastereomer, of the ring system that this family of molecules possesses was the primary target. It is believed that easy access to this core will provide a very easy, general, and short route to a wide variety of natural products in this family that contain the characteristic bicyclic core. This route would allow the indole to be installed last, which is something that the previously discussed syntheses don't provide. A route of this type would not only be beneficial for the construction of known natural products, but also would be a very efficient route towards a number of analogs that could be used for biological testing of these very interesting structures.

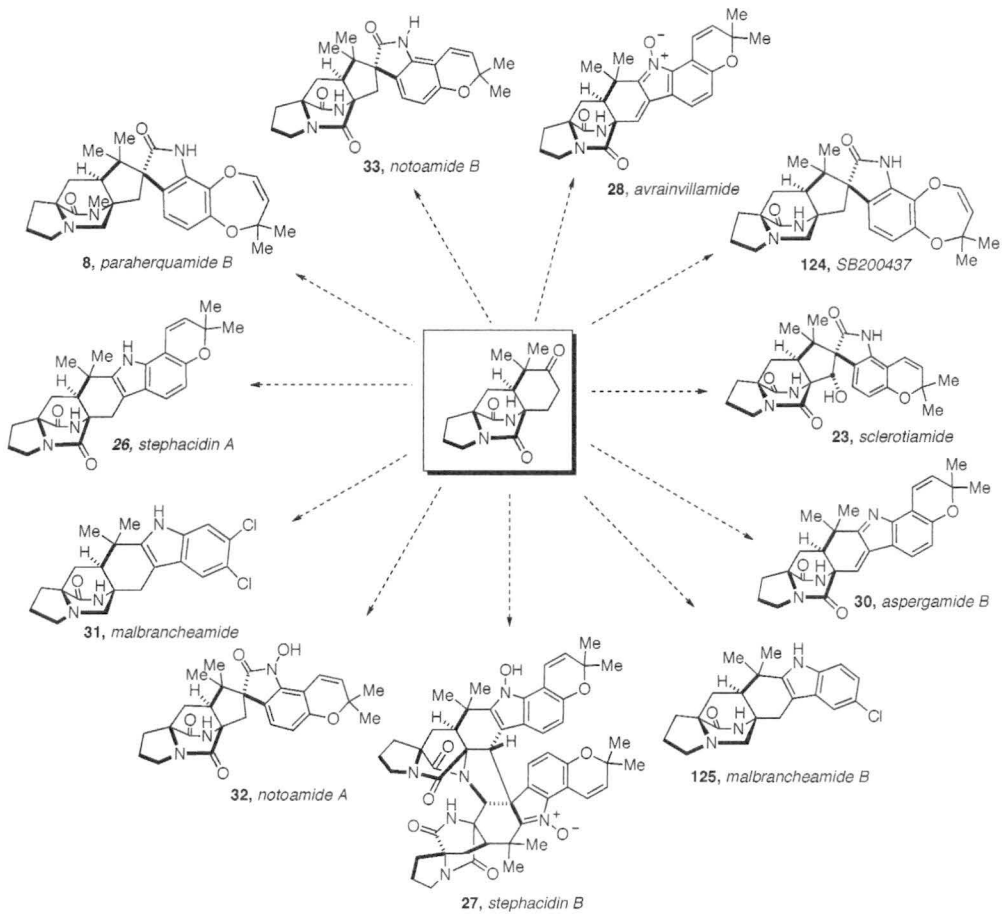


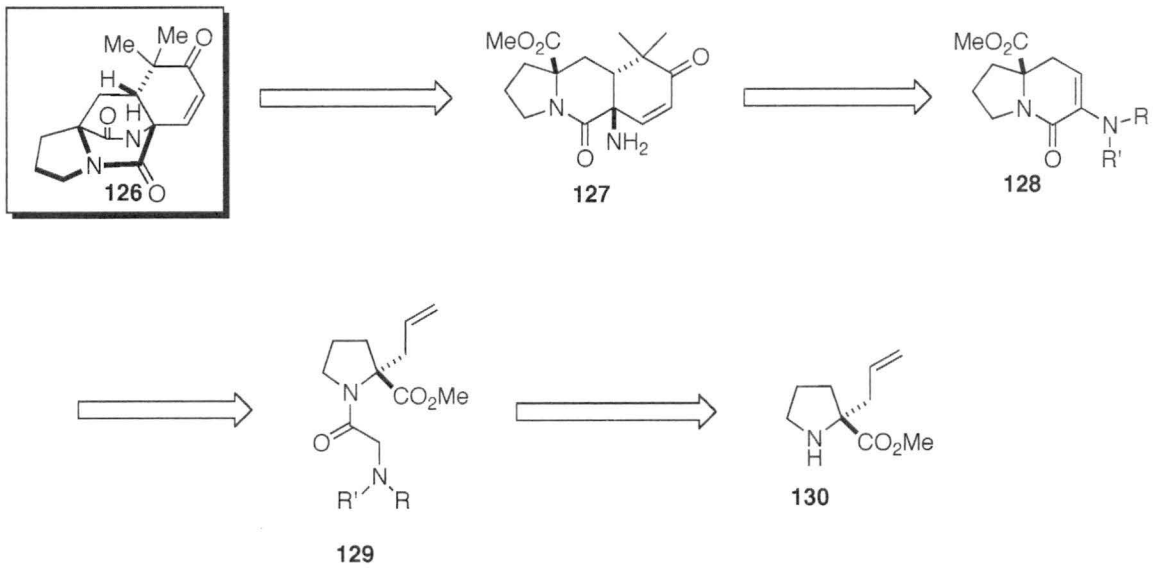
Figure 9. Various natural products that contain the bicyclo[2,2,2]diazaoctane core.

Chapter 2

Studies Toward The Total Synthesis Of The bicyclo[2,2,2]diazaoctane Ring System

2.1 Retrosynthetic Analysis

A short synthesis to the bicyclo[2.2.2]diazaoctane ring system will allow for future use of this method in the aforementioned construction of more complex natural products as well as the preparation of analogs to be used for biological testing. With this in mind the bicyclic core was envisioned to arise from amine **127**, which upon heating would cyclize to from the desired bicyclo[2.2.2]diazaoctane ring system (**126,** Scheme 10). Tetracycle **126** is a similar compound to the intermediate Myers constructed (**65**) in an elegant synthesis of stephacidin B (**27**). The two structures are identical aside from the TBDPS protecting group on the enone of Myers intermediate.[32] Amine **127** could be derived from a Diels-Alder reaction between the dieneophile **128** and Danishefsky's diene. The dieneophile **128** could be constructed from (*R*)-allyl proline methyl ester **130** and a glycine derivative, which will allow for the necessary nitrogen functionality. This route would provide relatively quick and efficient access to the bicyclic core. Dr Gerald Artman is credited with devising this strategy.

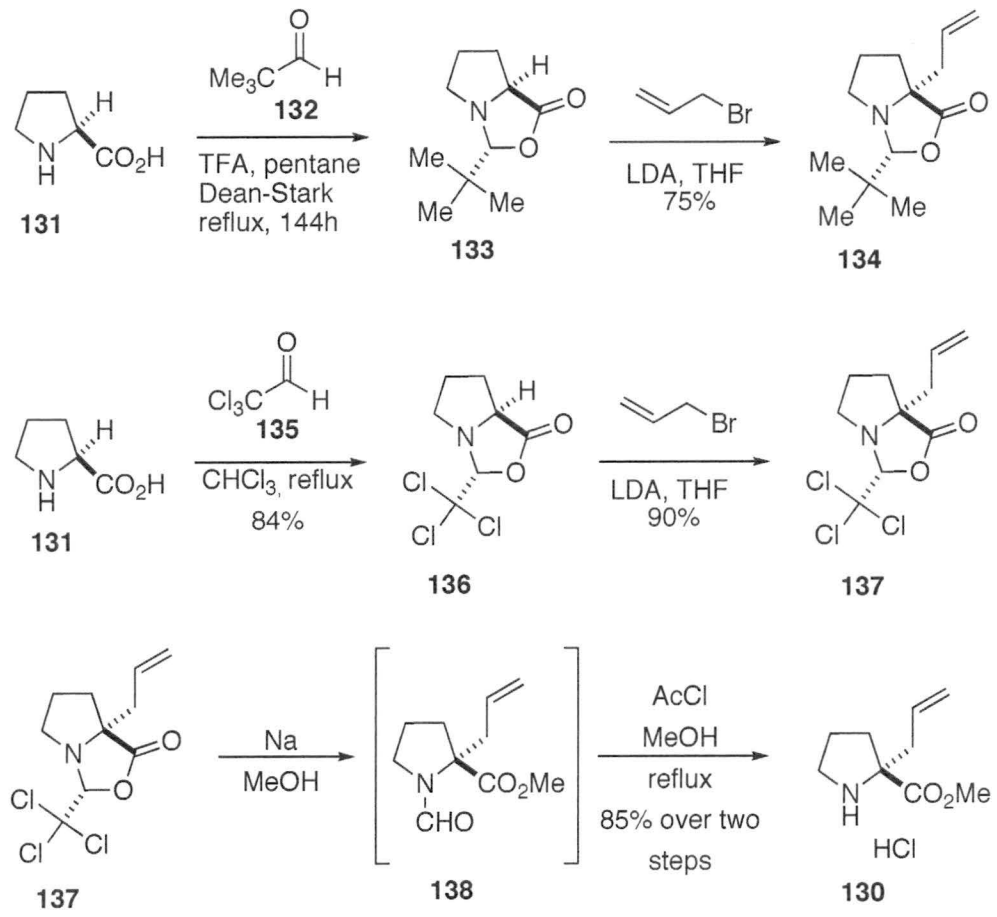


Scheme 10. 1st generation retrosynthesis to the bicyclic core.

2.2 Allyl Proline Synthesis

A practical method in preparing the (*R*)-allyl proline methyl ester (**130**) starting material is vital in the productivity of constructing the bicyclic ring. Seebach and co-workers developed a method for the construction of the desired proline, which commences with the condensation of commercially available (*S*)-proline **131** with pivaldehyde (**132**) in the presence of TFA under azeotropic conditions for 7-10 days to afford **133** (Scheme 11).[36] Following isolation of **133**, allylation can be achieved in a diastereoselective fashion to afford **134** and then subsequently the methyl ester **130** following removal of the auxiliary. However, the sensitive nature of **133** along with the high cost of pivaldehyde ($400/100 mL, Aldrich), which is required in 7-fold molar excess has made the synthesis of methyl ester **130** impracticle for the large amount of material needed.

Wang and Germanas have reported an alternative approach to **130** that can be prepared from inexpensive starting materials.[37] By combining trichloroacetaldehyde (500g/$90) and (*S*)-proline, trichloro oxazolinone **136** can be obtained as an air and moisture stable crystalline solid that can be stored at room temperature with no decomposition or loss of optical purity observed after several weeks. Furthermore, the preparation of the trichloro oxazolinone requires only a small excess of trichloroacetaldehyde. In a similar manner to the Seebach protocol, alkylation of the oxazolinine **136** in the presence of allyl bromide and LDA readily produces the allyl lactone **137** in good yield and as a single diastereomer. Cleavage of the chloral auxiliary from **137** to the amino ester salt **130** using the reported conditions of refluxing HCl/MeOH for 1 hour only provided <10% of the desired product. Modest yields of **130** could be obtained by refluxing for over 24 hours. Interestingly, Williams and co-workers, recognizing that the slow step for the cleavage of the oxazolinone **136** under acidic conditions must be the formation of the methyl ester, were able to devise a one-pot process to rapidly cleave the chloral auxiliary in high yield (Scheme 11).[31] Exposure of the allyl lactone **136** to sodium in methanol followed by the addition of AcCl and heating to reflux, rapidly removes the trichloroacetaldehyde auxiliary to produce the desired methyl ester hydrochloride salt **130** in 85% on a 20 gram scale.

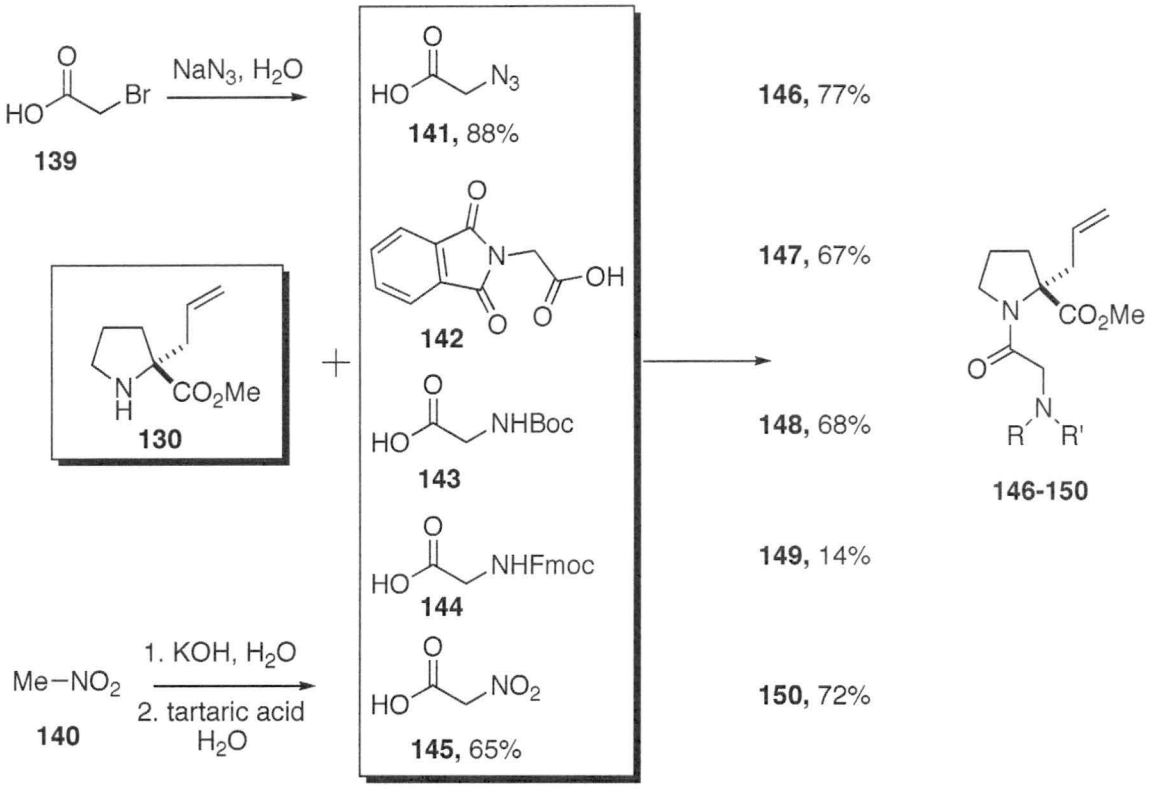


Scheme 11. Gram-Scale synthesis of (*R*)-allyl proline methyl ester.

2.3 Diels-Alder Reaction

With the (*R*)-allyl proline methyl ester in hand, it was then necessary to couple it to a variety of glycine derivatives, to make precursors of the dieneophiles necessary for the proposed Diels-Alder reaction (Scheme 12). The azido acetic acid (**141**) was prepared by reacting bromoacetic acid (**139**) with sodium azide and water. Azide **141** was coupled to the allyl proline methyl ester **130** with DCC in dichloromethane. Nitroacetic acid **145** was prepared by reacting nitromethane **140** with aqueous KOH and tartaric acid. It should be noted that this is an extremely exothermic reaction, for which the yield harshly depends on the addition rate of the nitromethane. Compound **145** was

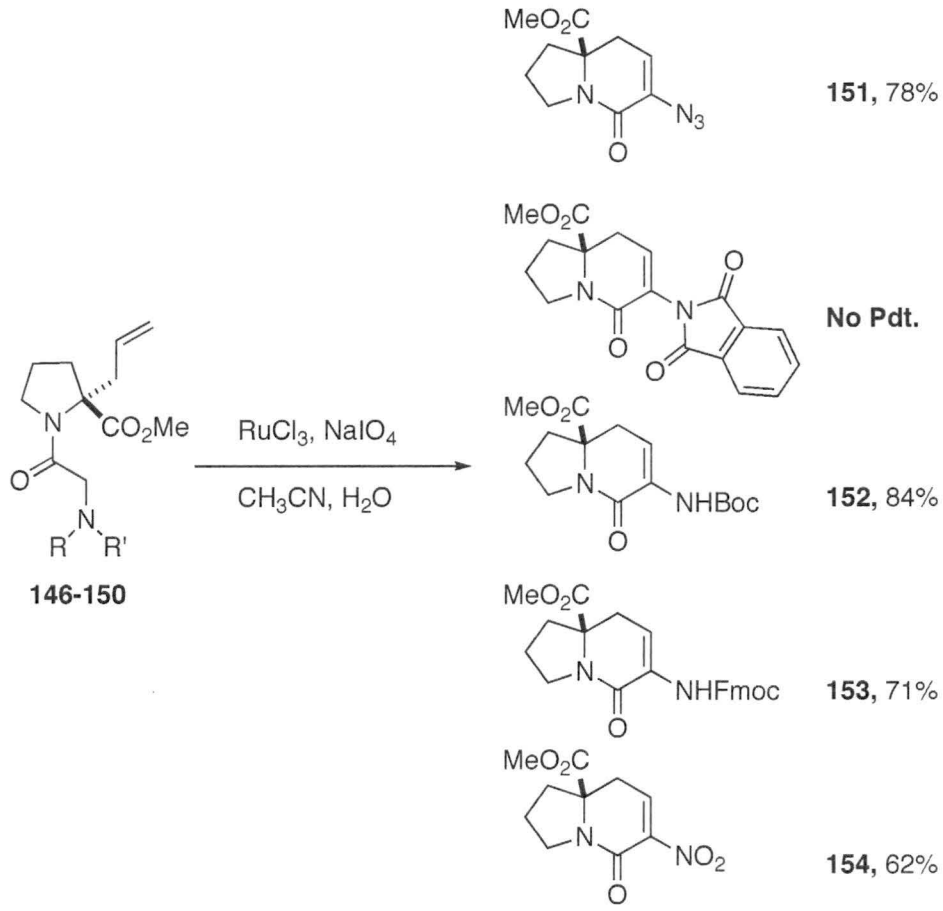
also coupled to the allyl proline using DCC with decent yields. When the commercially available phthalimide (**142**), *N*-Boc glycine (**143**), *N*-Fmoc glycine (**144**) were reacted with the allyl proline **130** under DCC coupling conditions poor yields were achieved. However, using BOP-Cl along with Hunig's Base in DCM provided the desired products **147, 148, 149** in modest yields in every case with the exception of Fmoc glycine.



Scheme 12. Coupling of the glycine derivatives to the (*R*)-aryl proline

The glycine derivatives were all subjected to cyclization conditions in an attempt to obtain the corresponding dieneophiles (**151-154,** Scheme 13). The olefin of the allyl proline was converted to the aldehyde using sodium periodate and a catalytic ruthenium chloride.[65] The aldehyde then undergoes condensation to provide the cyclized products (**151-154**) in good yields for all compounds with the exception of the phthalimide **147**,

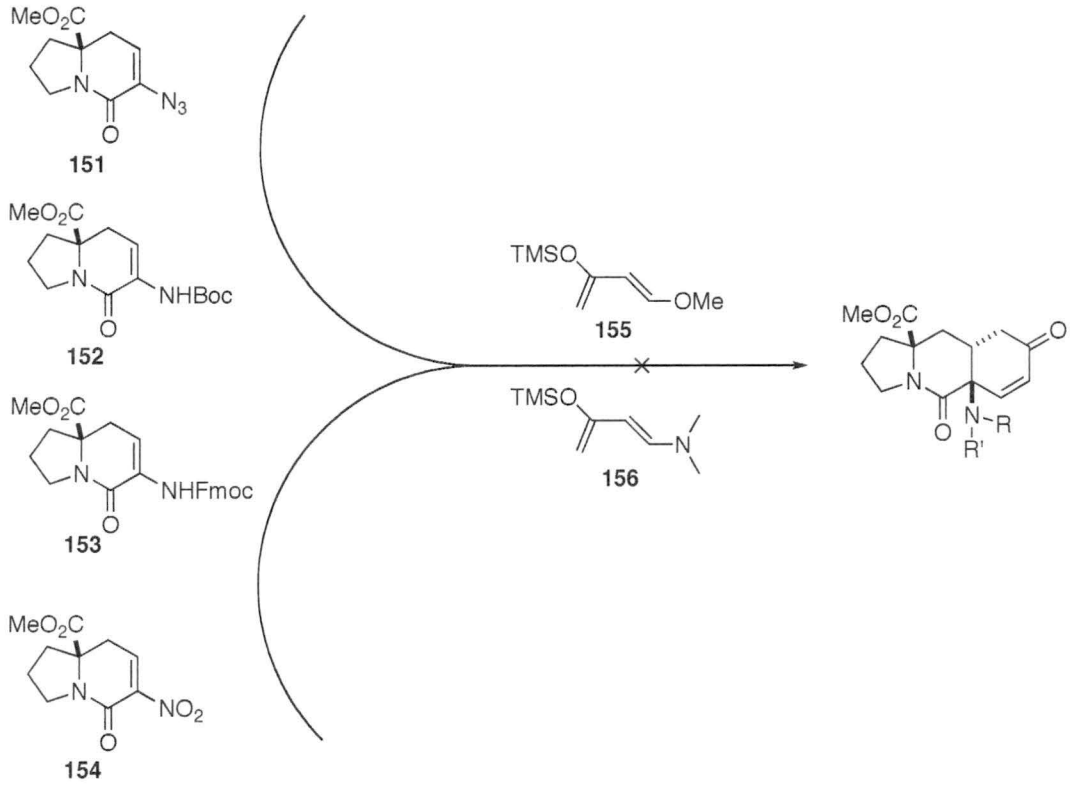
which provided no product. All attempts to cleave and cyclize **147** resulted in the recovery of starting material, and the desired product was not obtained. A drop in yield accompanied scale up to anything larger then 100 mg regardless of the conditions.



Scheme 13. Cyclization reactions with the various glycine derivatives.

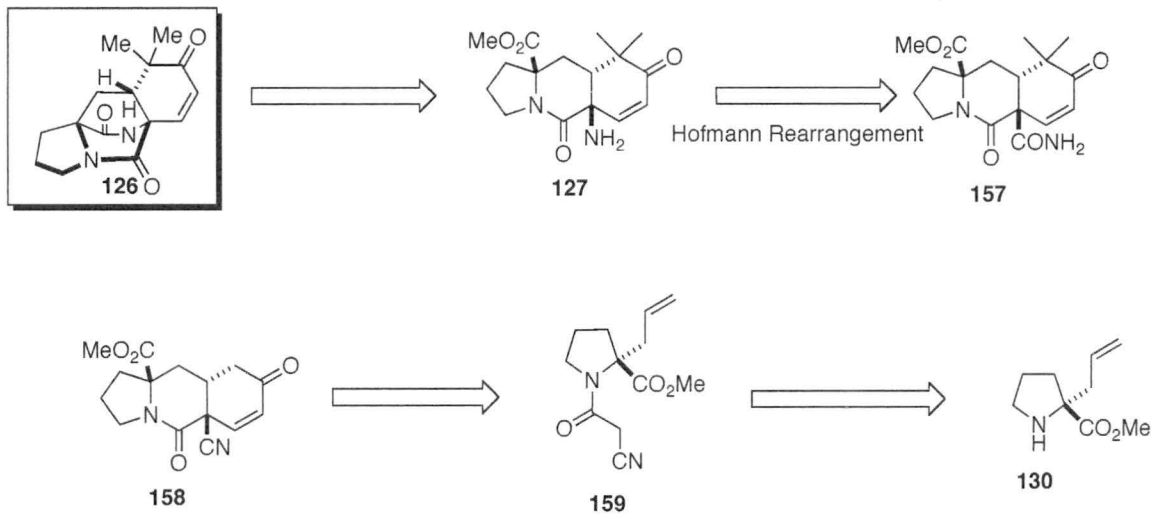
The corresponding dieneophiles (**151-154**) were all subjected to a variety of Diels-Alder conditions with both Danishesky's diene[38] **155** and Rawal diene[39] **156** (Scheme 14). Danishesky diene **155** was purified by distillation before every use to ensure the purity of the compound. It was found that no desired product was obtained using diene **155.** These reactions were carried out under a wide variety of temperatures (0 °C-120 °C), to no avail. Microwave conditions were also tried in toluene with no

desired product seen. It was found that with larger amine protecting groups, such as the Boc and Fmoc, no reaction took place and only starting material was isolated. These large groups might be hindering the cycloaddition. However, with azide **151** decomposition was seen in most cases at elevated temperatures and no reaction was observed at lower temperatures. The nitro compound **154** showed decomposition when subjected to all conditions, which indicates that the nitro dieneophile **154** might be decomposing before cyclization occurs. The Diels-Alder reaction was tried on **154** immediately after it was prepared, however decomposition was still seen. The literature suggests that the Rawal diene **156** might be more reactive than diene **155** and requires lower temperatures for cycloadditions than other similar dienes.[39] The Rawal diene was prepared following the literature precedent starting from *N,N*-dimethylthioformamide and bromoacetone.[39] Diene **156** is reported to be quite stable to normal handling and was not distilled before using. Diels-Alder conditions similar to those with diene **155** proved to give the same negative result on all accounts.



Scheme 14. Attempted Diels-Alder conditions on the various glycine derivatives.

The failure of these Diels-Alder reactions led to the reevaluation of the retosynthesis to the bicyclo[2.2.2]diazaoctane core. Knowing that amine **127** was still the primary objective in order to access the bicyclic core the approach was still intriguing. A new possibility was explored by changing the initial nitrogen functionality of the glycine derivatives that were used. Employing a relatively less hindered functional group, while continuing to be an efficient electron-withdrawing group, might better facilitate the cycloaddition. Employing a nitrile group could allow the use of a Hofmann rearrangement to access the amine **127** and potentially circumvent the previous Diels-Alder reaction problems (Scheme 15).



Scheme 15. 2nd generation retrosynthesis to the bicyclo[2.2.2]diazaoctane ring system

To that end, (*R*)-allyl proline **130** was coupled to the acid chloride of cyanoacetic acid using Hunig's Base in dichloromethane to give the cyano allyl proline **159** in high yield (Scheme 16). Attempted coupling of the allyl proline **130** directly to cyanoacetic acid proved to be very difficult and only trace amounts could be obtained with a variety of coupling agents. Because the acid chloride of cyanoacetic acid is readily coupled to the allyl proline and easily prepared it proved to be beneficial for this route. Compound **159** was subjected to the previous oxidative cleavage conditions to obtain the cyclized product **161.** The α,β-unsaturated cyano ketone **161** was then exposed to microwave conditions with Danishefsky diene **155** and upon acidic workup enone **158** was successfully obtained in excellent yield. We were also intrigued to find that cycloadduct **158** could also be obtained by refluxing **161** with the diene **155** in toluene for 4-5 days. It was observed that using the Rawal diene **156** resulted in very messy reactions that the products were nearly impossible to purify and isolate clean **158.** However, when using diene **155**, purification could be readily achieved with simple SiO_2 column

chromatography. Enone **148** was then bis-methylated with little difficulty to **162.** To convert the nitrile of **162** into the desired amide **157**, the use of a platinum phosphinito catalyst discovered by Parkins and co-workers was then explored.[40] Parkins' catalyst has proven to be a very powerful tool in recent years for the hydrolysis of nitriles to amides in very good yields, requiring minimal catalyst loading. Parkins catalyst is relatively inexpensive compared to other similar catalysts, and also hydrolizes nitriles to amides under neutral conditions. Nitrile **162** proved to be no exception; the amide **157** was achieved in very good yield with Parkins catalyst in an equal mixture of THF and water. A possible mechanism for this transformation is suggested by the authors (Figure 10).[40]

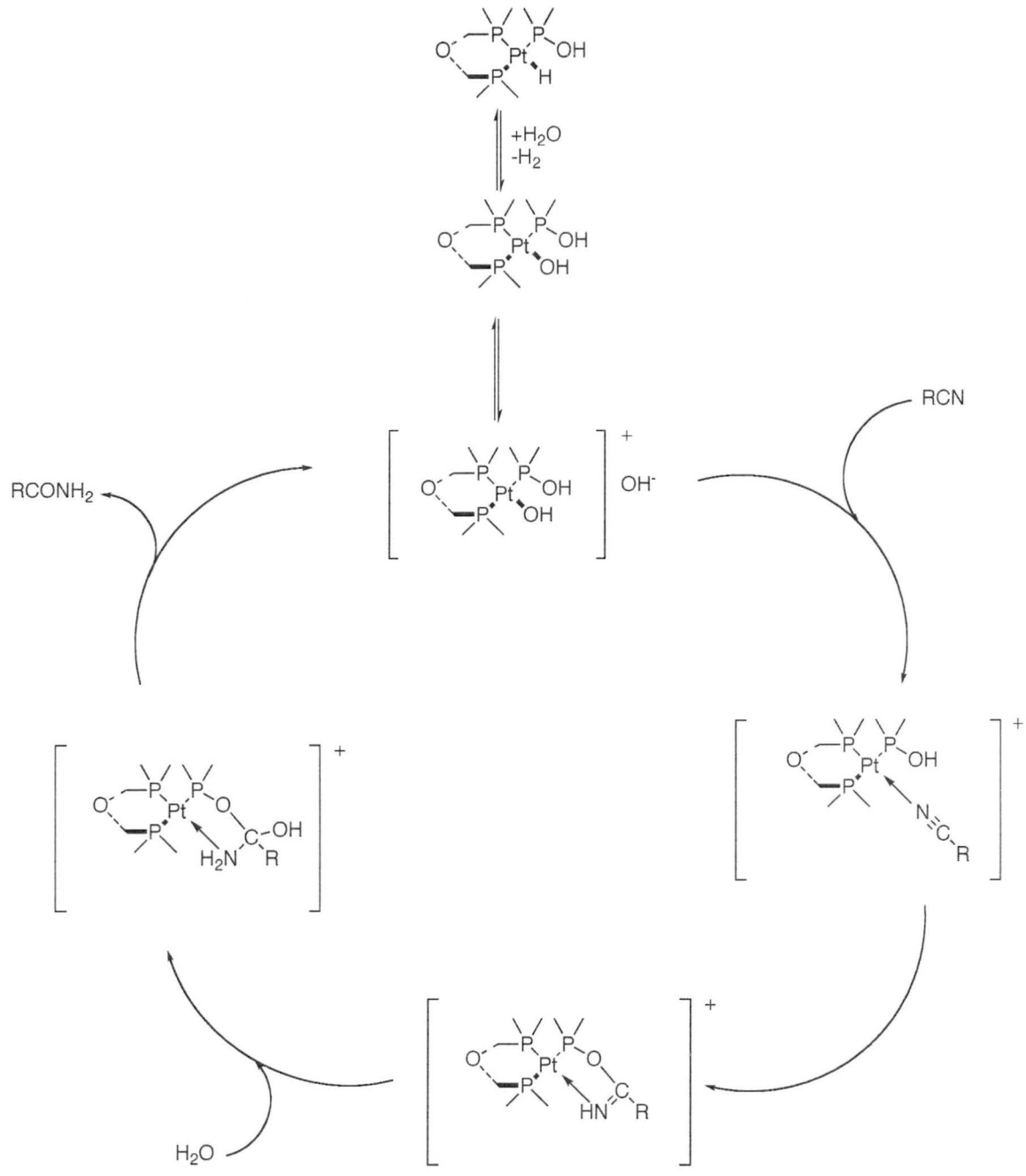
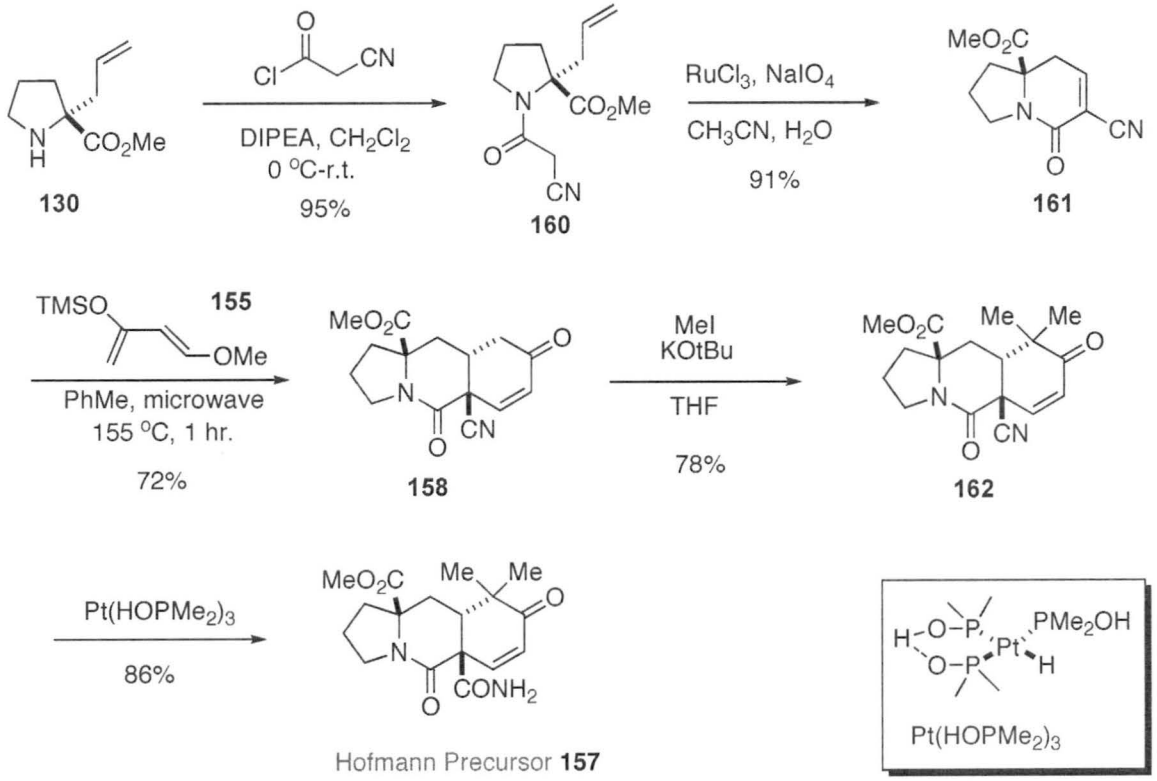
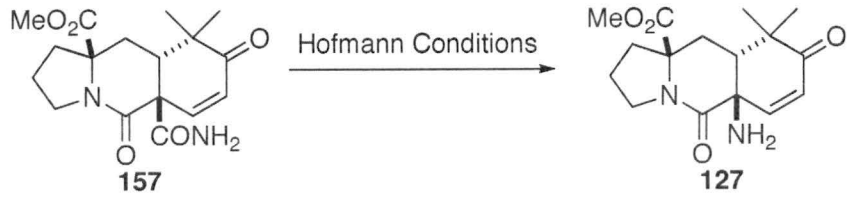


Figure 10. Suggested mechanism for the hydrolysis of nitriles by phosphinito complexes.[40]



Scheme 16. Synthesis to the Hofmann precursor **157**.

Amide **157** was subjected to several reaction conditions in an attempt to facilitate the Hofmann rearrangement (Table 4). Various types of iodide reagents[41], lead tetraacetate, bromine[43], and mercury acetate/NBS[42] reaction conditions were attempted. Unfortunately, the conditions that were tried failed in converting amide **157** into the desired amine **127.** In most cases no reaction took place and only starting material could be isolated. These reactions were monitored by TLC, staining with ninhydrin, however no new spots were seen. Crude ^{1}H NMR revealed that only starting material was present.



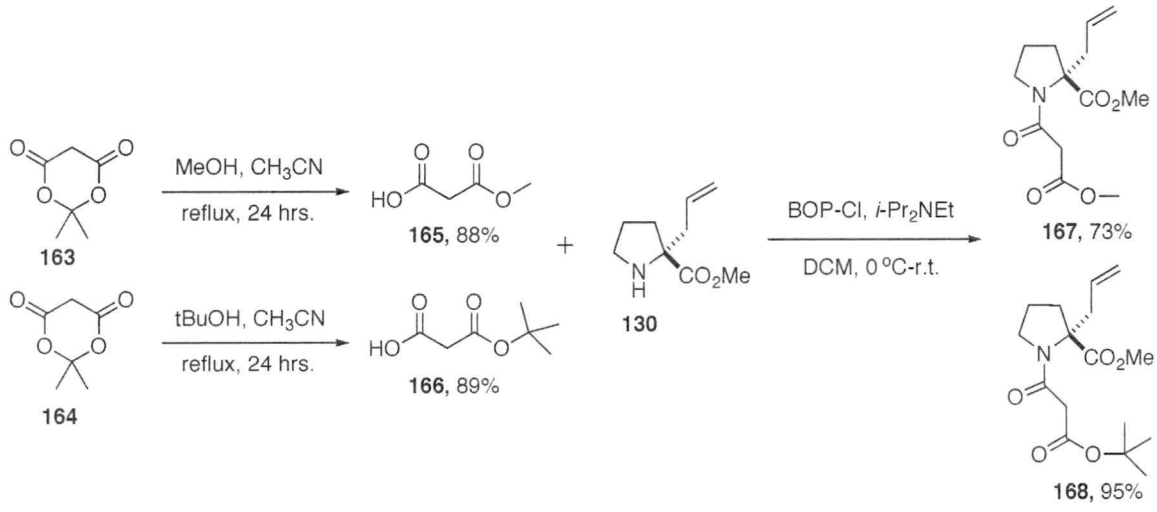
157 127

Reagents	Conditions	Result
$PhI(TFA)_2$, pyridine, DMF, H_2O	0 °C	NR
	0 °C-r.t.	NR
	50 °C	Decomposition
$Pb(OAc)_4$, DMF	5 mol %, r.t.	NR
	5 equiv. r.t.	NR
	1 equiv. r.t.	NR
	2 equiv. r.t.	NR
	2 equiv. 40 °C	NR
		Decomposition
Br_2, NaOH	-78 °C then reflux	NR
KOH	-78 °C then reflux	NR
NaOMe	-78 °C then reflux	NR
NBS, $Hg(OAc)_2$	DMF, 12 hrs	NR
	DMF, 24 hrs	NR
PhI(OTs)OH	0°-75 °C	NR
$PhI(OAc)_2$	0°-75 °C	NR

Table 4. Reaction conditions for the Hofmann reaction.[41,42,43]

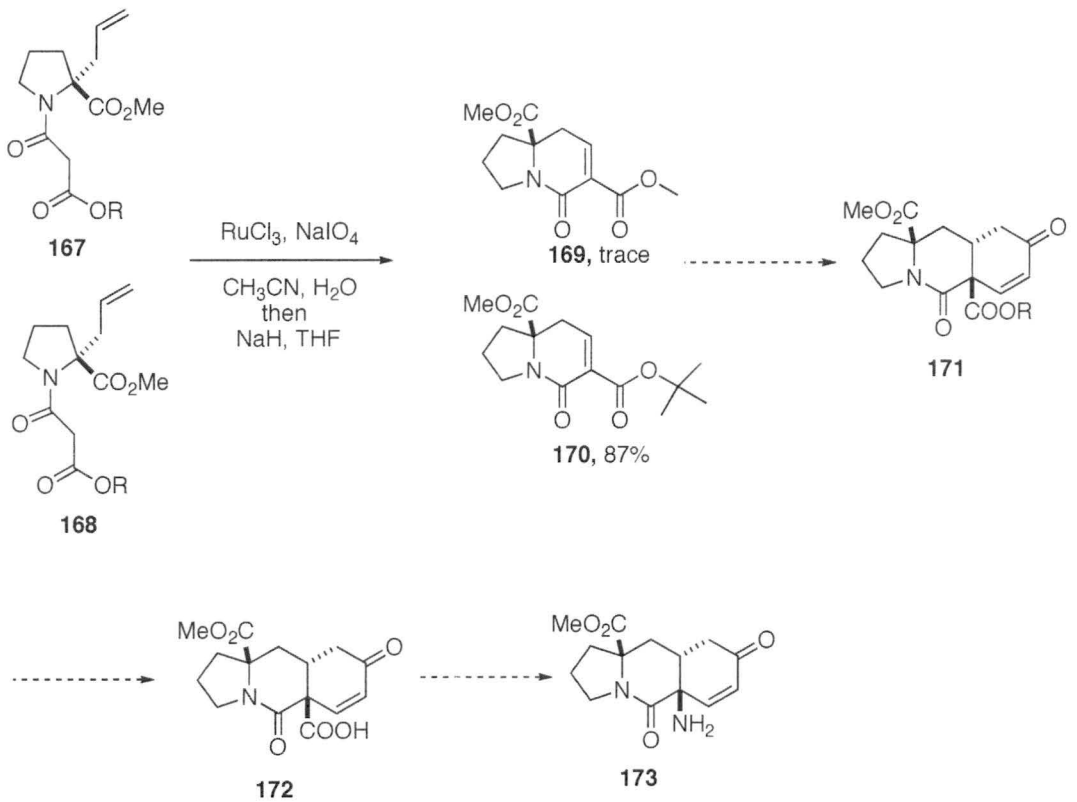
With this disappointing result, efforts were explored at replacing the amide with an acid, which could undergo a Curtius rearrangement to access amine **127** (Scheme 17-18). If the thus far problematic Diels-Alder reaction was completed with an ester (**169, 170**), that ester could be converted to the acid **172**, which could then undergo a Curtius rearrangement to afford the desired amine **173.** Starting from Meldrum's acid **163** the corresponding methyl ester **165** and *t*-Butyl-ester **166** were prepared following literature precedent by simply refluxing with methanol or *t*-Butyl-alcohol in acetonitrile for 24

hours.[45] The resulting esters were both coupled to the (*R*)-allyl proline methyl ester **130** using BOP-Cl and Hunig's Base to afford **167** and **168** in good yields.



Scheme 17. Synthesis of the proline esters **167, 168.**

The two esters **167** and **168** were then subjected to the previously described conditions of ruthenium (III) chloride and sodium periodate to afford the dieneophiles **169** and **170** (Scheme 18). It was found that these conditions readily converted the olefin to the aldehyde, however condensation and cyclization to the corresponding dieneophiles was not observed as previously seen under these conditions. The aldehydes were isolated and purified. Subsequent reactions with sodium hydride in THF effected the desired condensation to afford the cyclized products **169** and **170**. The *t*-Butyl-ester provided good yields whereas only trace amounts of the methyl ester were seen. Unfortunately, all cycloaddition attempts with the t-Butyl-ester **170** have been unsuccessful thus far. It should be noted that other esters such as an ethyl ester could also be readily prepared through the same synthetic method and subjected to the Diels-Alder conditions. An isonitrile was also tried, however the dieneophile was never obtained.



Scheme 18. Possible access to acid **172** for a Curtius rearrangement.

2.4 Conclusion

The problems associated with this synthesis have revolved around the required Diels-Alder reaction to afford the corresponding enone. The nitrile **161** is the only single compound thus far that has produced the desired enone **158**. Unfortunately the Hofmann rearrangement could not be achieved via this nitrile route. Other esters could possibly be looked into for attempts at the Curtius route. It appears that these substrates are very specific in relation to the Diels-Alder reaction. It is still believed that access to the bicyclo[2.2.2]diazaoctane core will provide easy access to a vast number of similar natural products in this family.

Chapter 3

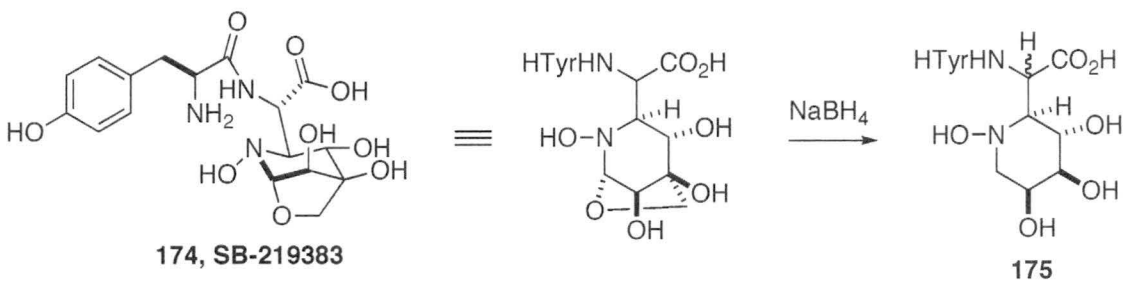
Introduction: Natural Product SB-219383

3.1 Isolation and Structural Determination

Aminoacyl-tRNA synthetases play an essential role in protein synthesis by producing charged tRNAs.[46] An amino acid is first condensed with an ATP molecule to form a stable aminoacyl-adenylate intermediate and is then transferred onto a cognate tRNA to form the desired product. Because the synthetases play such a critical role, compounds that inhibit bacterial aminoacyl-tRNA synthetases specifically could be useful as potent antibacterial drugs. Inhibition of bacterial isoleucyl-tRNA synthetase is the mode of action of know antibacterial agents such as mupirocin, which is marketed as Bactroban®.[47] Multi-drug-resistant bacteria are becoming an ever more persistent threat to public health: for instance, *Staphylococcus aureus* causes serious hospital-aquired infections that are difficult to treat. A new compound that shows competitive, inhibitory activity against *Staphylococcus aureus* YRS is the natural product SB-219383 (**174,** Figure 9)

SB-219383 **174** is a natural product isolated from *Micromonospora sp.* NCIMB 40684, which was found in the soil of South Africa.[48] SB-219383 is recovered from the cells by heat treatment (70 °C, 15 minutes) or methanol extraction followed by centrifugation to remove the cell debris. Extensive NMR studies and amino acid analysis showed SB-219383 **174** to be a dipeptide with an N-terminal L-tyrosine unit coupled to a novel highly functionalized bicyclic α-amino acid. This amino acid incorporates an

unprecedented N-hydroxylamino sugar C-glycosidically linked to the α-carbon of a glycine.[49] The relative stereochemistry between the amino acid α-stereogeniccenter and the ring system as well as the absolute configuration of the C-terminal amino acid remained elusive. To determine the correct stereochemistry, a stereoselective synthetic approach was utilized. Four stereoisomers (**176-179**) were synthesized from arabinose and tested against a reduced form of SB-219383 **175** (Figure 11).[50] The four stereiosomers were tested in a standard aminoacylation assay of YRS activity where **176** was the only structure to match the activity of **175.** The NMR spectra of SB-219383 and **176** are almost identical with exception of the methyleneoxy unit. From these results it was determined that the stereochemistry of SB-219383 **174** is as shown below.



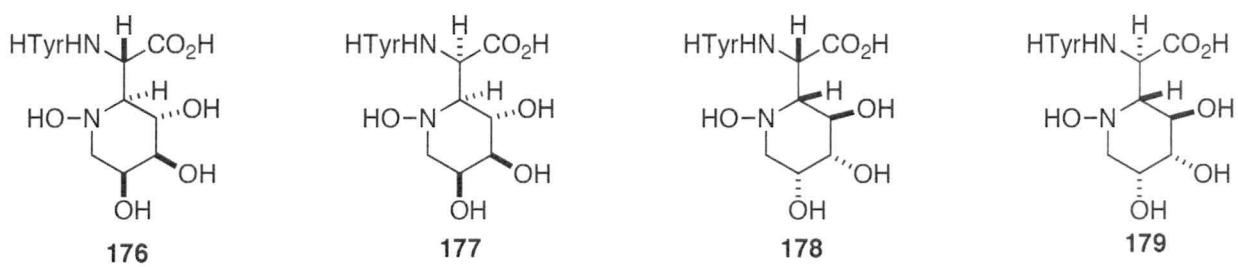


Figure 11. Structure of SB-219383 and analogues used to determine the correct stereochemistry.[50]

3.2 Biological Activity

SB-219383 **174** was found to be a highly potent time-dependent competitive inhibitor of bacterial tyrosyl tRNA synthetase (YRS) (*Staphylococcus aureus* YRS, IC_{50} 1.4 nM; mammalian YRS, IC_{50} 22 μM).[48] However, SB-219383 and other similar analogues show only weak antibacterial activity. Using a microtitre broth dilution method, the antibacterial activity of SB-219383 was determined against multiple organisms including *Escherichia coli, Haemphilus influenzae, Moraxella catarrhalis, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus pneumoniae,* and *Staphylococcus pyogenes.* No whole cell antibacterial activity was observed in any test. This absence of antibacterial activity is most likely due to the high polarity of the molecule, which would prevent penetration into the bacterial cell. Many different analogues have been made of SB-219383, however all show the same weak antibacterial activity.

3.3 Research Objectives

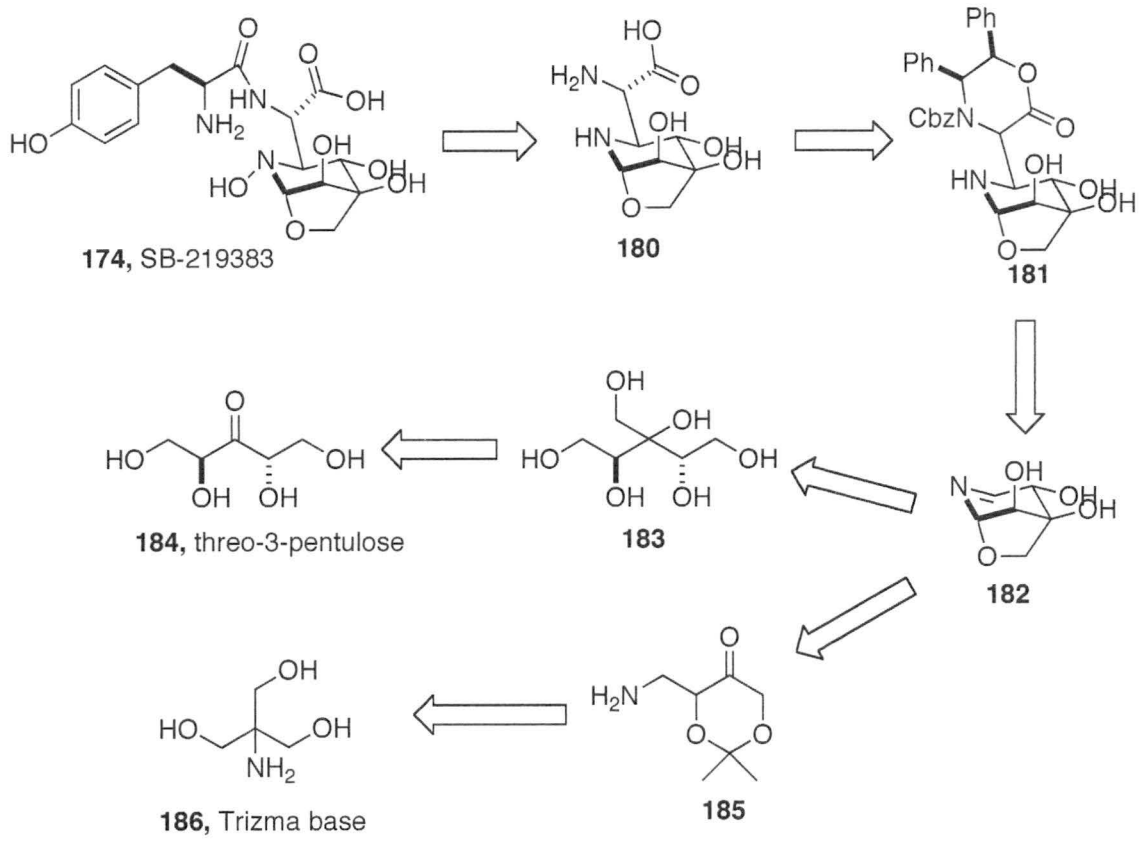
The main objective of the work on SB-219383 was to establish a route to the N-hydroxylamino sugar bicyclic core of the molecule. Once this core is established, the remaining functionality of the molecule is fairly straightforward. It should be noted that no synthesis has been reported on this molecule or any similar structure related to the bicyclic core of SB-219383.

Chapter 4

Studies Toward The Total Synthesis of SB-219383

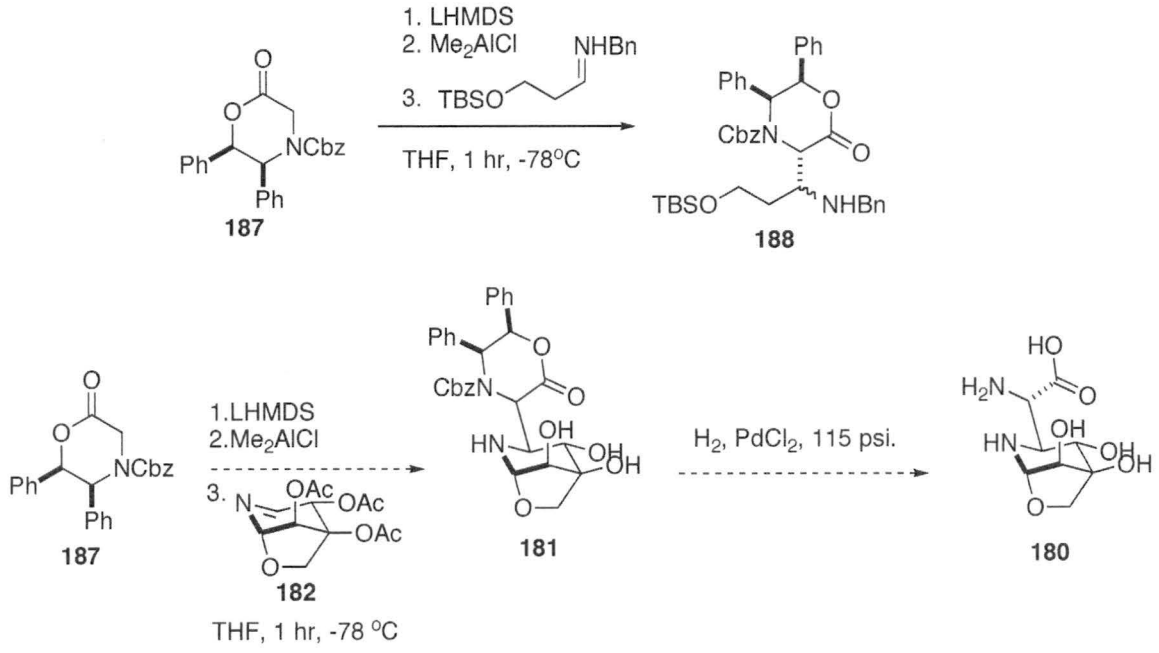
4.1 Retrosynthetic Analysis

Realizing the main synthetic challenge of this natural product will most definitely be the unique, unprecedented hydroxylamino sugar, the focus of my synthesis revolved around designing a route to this bicyclic ring (**182**). A late stage coupling of the tyrosine to the glycine is envisioned (**180-174**). The glycine could possibly be derived from cleaving the auxiliary of the lactone template, which could be coupled to the bicyclic core of **182** via an imine. Two paths were explored to the *N*-hydroxylamino sugar, one starting from thero-3-pentulose **184** which can be readily constructed from dihydroxy acetone and the other starting from Trizma base **186** which would utilize a sulfur ylide to form the six-membered piperidine ring.



Scheme 19. Retrosynthesis of SB-219383.

The Williams group has published precedent for the use of lactone template **187** to engage in coupling to the imine of the bicyclic core **182** (Scheme 20).[51] They found that they could readily couple the lactone template **187** to a benzyl imine. Preparation of the lithium enolate of the chiral auxiliary **187** with LHMDS, followed by transmetallation resulted in the formation of the corresponding aluminum enolate. Addition of the imine to the enolate resulted in the Mannich product **188**. This chiral auxiliary can be easily cleaved with H_2/palladium (II) chloride to produce the desired glycine derivative **180**.

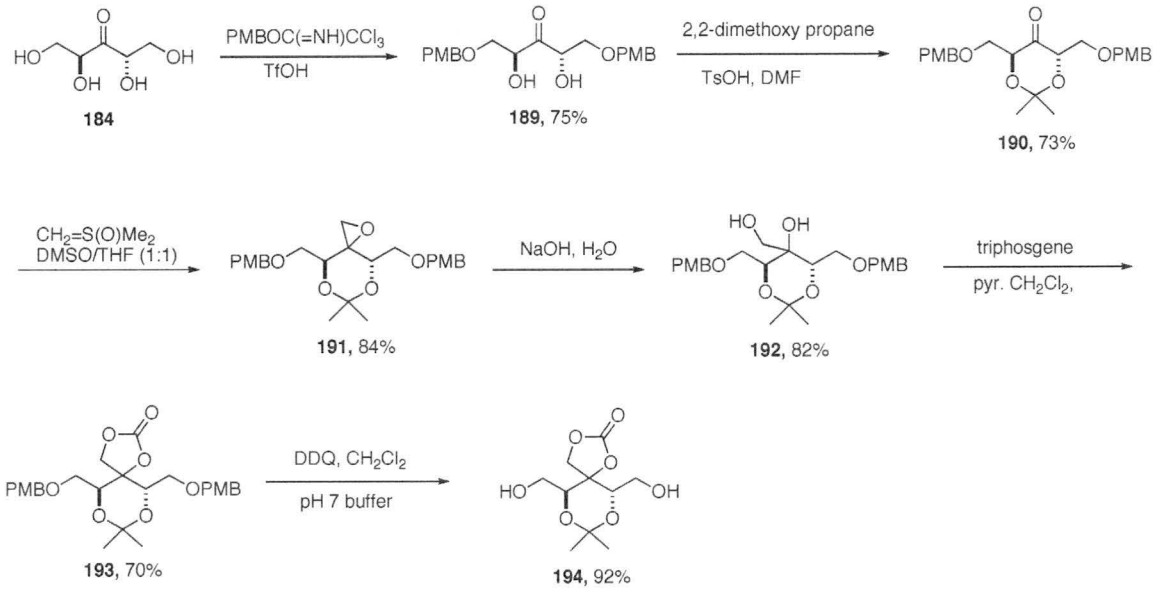


Scheme 20. Possible coupling of the lactone using Williams procedure.[51]

4.2 Progress Towards the *N*-hydroxylamino Sugar

The first route that was explored started from *threo*-3-pentulose **184**. This route utilized a variety of protecting group manipulations in order to maintain the highly functionalized structure and access the primary diols. Compound **184** could be prepared by a series of aldol reactions from dihydroxy acetone in the presence of formaldehyde.[52,53] *Threo*-3-pentulose was found to be the major product of the aldol reaction when the reaction was run in methanol in the presence of calcium chloride and potassium hydroxide. This was rationalized in part due to a much more soluble calcium cation in which a chelated enolate coordinates to the metal cation. Formaldehyde approaches as to reduce the steric congestion, which leads to *thero*-3-pentulose. Compound **184** proved to be difficult to handle and ultimately resulted in a brown syrup

that was accompanied by poor solubility in most organic solvents. The primary diols of **184** were selectively protected with a PMB group to afford diol **189**. At first, the secondary diols of **189** were protected with a TBS group. However once the material was taken forward it was found that the compound was much too hindered and no conditions could be carried out to modify the ketone functionality. Ultimately an acetonide protecting group for the secondary diol was utilized to provide the less hindered substance **190**. With the hydroxyl functionality protected, conditions were examined to convert the ketone into an olefin, which could then be converted to another diol. All attempts at olefination yielded very minimal or no product. Tebbe[54], Wittig[55], and Peterson[56] olefinations were all attempted. The Tebbe and Peterson conditions gave trace amounts of desired product, however there was never enough material that could be isolated to take forward and make this a worthwhile route. Ketone **190** seems to be fairly hindered so another route was explored to convert ketone **190** into an epoxide. Using Corey-Chaykovsky epoxidation conditions ketone **190** could be readily converted to epoxide **191**.[57] The epoxide was then opened to the diol **192**. Another protecting group manipulation was needed, and it was necessary to have a different protecting group than was already used in order to be able to remove the different protecting groups at different stages. Triphosgene was used to protect the diol of **192** as a cyclic carbonate to provide **193**. The PMB groups were then removed with DDQ in the presence of a pH 7 buffer to afford the primary diols of **194**.

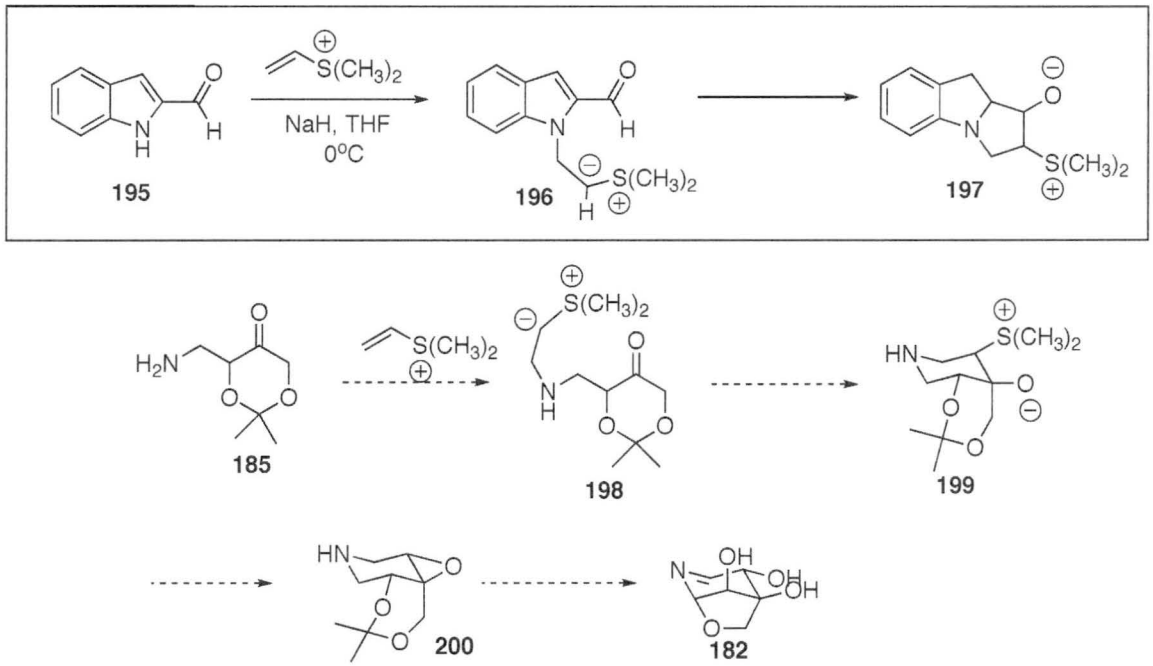


Scheme 21. Progress towards the bicyclic core of SB-219383.

The reactivity of the primary diols of **194** were explored in order to install nitrogen functionality that would be needed for the N-hydroxylamino sugar. Oxidation attempts were carried out under Swern[58], Dess-Martin[59], and Corey-Kim[60] conditions to afford an aldehyde, unfortunately no oxidation was seen and only starting material was isolated. Attempts at installing nitrogen functionality were carried out under Mitsunobu[61] conditions. Once again these conditions showed no reactivity and only starting material could be isolated. Structure **194** appears to be very unreactive possibly due to the protecting groups that are still present.

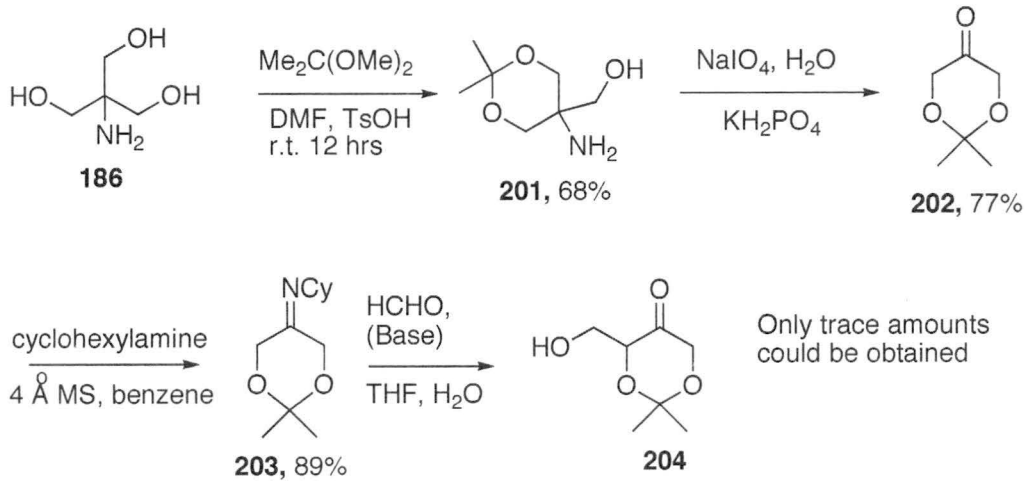
The second approach to the bicyclic core **182** was through a sulfur ylide that could close to form the nitrogen ring (Scheme 22). The Jimenez group from Rutgers has had success in forming nitrogen rings using this method.[62] Using aldehyde **195** and reacting it with dimethylvinylsulfonium iodide results in the sulfur ylide **196**. This sulfur ylide then adds into the aldehyde to form the 5-membered nitrogen ring **197**. This approach would start from amine **185**, and then utilize the Jimenez conditions to close

onto the ketone of **198** to form the 6-membered ring of **199**. This would also provide much of the hydroxyl functionality required to access the N-hydroxylamino sugar **182**.



Scheme 22. Jimenez's approach to nitrogen rings.[62]

The sulfur ylide route commenced with the cyclization of trizma base **186** into the acetonide **201** (Scheme 23).[63, 64] Acetonide **201** was then taken to the ketone **202** using sodium periodate. It was found that this ketone readily polymerizes and trying to perform reactions with this substrate was very difficult. In order to circumvent this problem, ketone **202** was converted to the cyclohexylimine **203** in the presence of cyclohexylamine, benzene, and molecular sieves. A variety of aldol reactions were attempted in the presence of formaldehyde and a number of bases, however only trace amounts of the desired product could be isolated and the resultant material was too little to carry on to the next step.



Scheme 23. Synthesis towards the bicyclic core.[63, 64]

4.3 Conclusion

Both of these routes could possibly still yield the desired N-hydroxylamino sugar **182**. Further investigation of the primary hydroxyl group reactivity of **194** might still prove to be useful, as well as the aldol reaction of **203** in order to explore the intriguing sulfur ylide ring closing of **198.** The natural product SB-219383 is a very interesting molecule from a synthetic standpoint. The unprecedented bicyclic core provides a very challenging synthetic target, and it would not be surprising to see more work on this structure in the future.

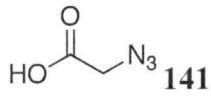
Chapter 5
Experimental

5.1 General Procedures and Conditions

Commercially available reagents were used as received without further purification. Reactions were run under an argon atmosphere unless noted otherwise. Thin layer chromatography was performed using 0.25 mm silica gel 60 (F254, Merck) plates visualizing at 254 nm, or developed with either vanillan, anisaldehyde, ninhydrin, or phosphomolybdic acid solutions by heating with a hot-air gun. Specified products were purified by flash column chromatography using silica gel 60 (230-400 mesh, Merck). IR absorptions on NaCl plates were run on a Perkin Elmer FT-IR 1600. ^{1}H NMR spectral data was obtained using Varian 300 or 400 MHz instruments. ^{13}C NMR spectral data was obtained using a Varian 100 or 125 MHz spectrometer. Mass spectra were obtained at Colorado State University's Central Instrument Facility. Chemical shifts are reported in ppm relative to $CHCl_3$ at δ 7.27 (^{1}H NMR) and δ 77.23 (^{13}C NMR). Chemical shifts are reported in ppm relative to CD_3OD at δ 3.31 (^{1}H NMR) and δ 49.15 (^{13}C NMR). For all NMR spectra, δ values are given in ppm and J values in Hz.

5.2 Chemical Synthesis Experiments for the Bicyclo[2,2,2]diazaoctane Ring.

Azide (141): Sodium azide (6.68g, .103mol, 2 equiv) was taken up in water (30mL) and cooled to 0 °C. Bromoacetic acid (7.08g, .051mol, 1 equiv) was added over 15 minutes and the reaction was stirred for 24 hours. The reaction was then acidified to a pH of 3 with acetic acid and separated with ether (3x 15 mL). The combined organic layers were dried (MgSO4) and concentrated to yield 17% (880 mg, 8.6 mmol) of **141** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.0 (2H, s); 11.55 (1H, s br)



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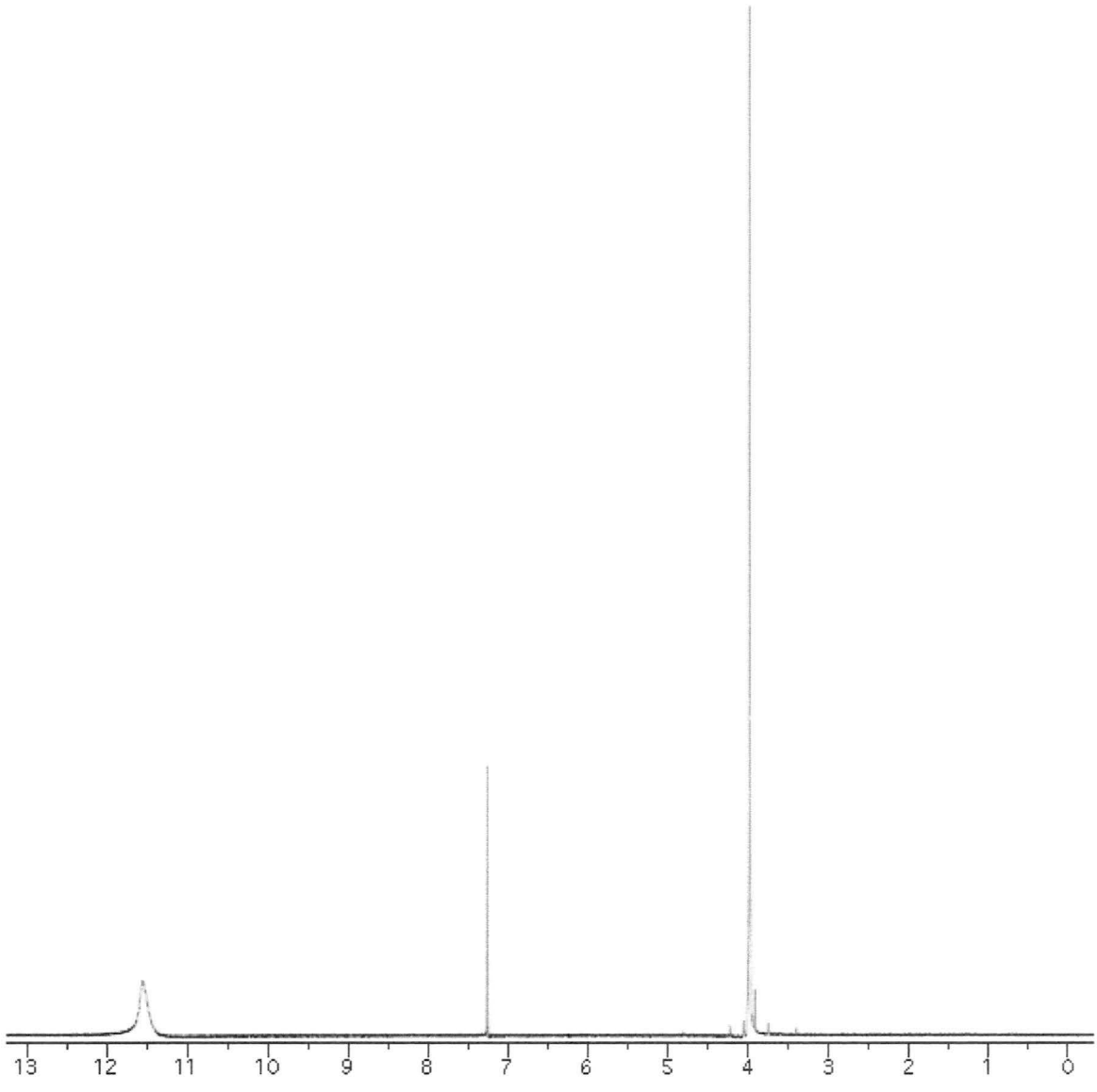
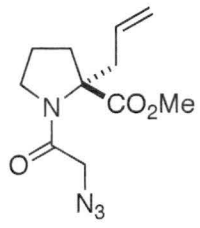


Azide Allyl Proline (146): Azidoacetic acid (36mg, ,36mmol, 1.5 equiv) was taken up in CH_2Cl_2 and then the allyl proline (50mg, .24mmol, 1 equiv) was added and cooled to 0 °C. DCC (74mg, .36mmol, 1.5 equiv) was added and the reaction stirred for 2 hours. Ether was added and the reaction was filtered and concentrated to yield a brown residue. This brown residue was purified by column chromatography on silica gel (ether) to yield 77% (53mg, .021 mmol) **146** as a light brown oil. ^1H NMR (300 MHz, $CDCl_3$): δ 1.18 (1H, m); 1.69 (1H, m); 2.07 (1H, s); 2.46 (1H d, J = 1.5 Hz); 2.66 (2H, t, J = 6.3); 3.72 (6H, s); 3.90 (3H, d, J = 14.1 Hz); 4.08 (3H, s) 5.10-5.38 (1H, m); 5.65 (1H, m). LRMS (FAB+): Calc. for $C_{11}H_{16}N_4O_3$: 252.12224, Found: 253.20015 (MH^+, 100%), 252.12345 (M^+, 15%).



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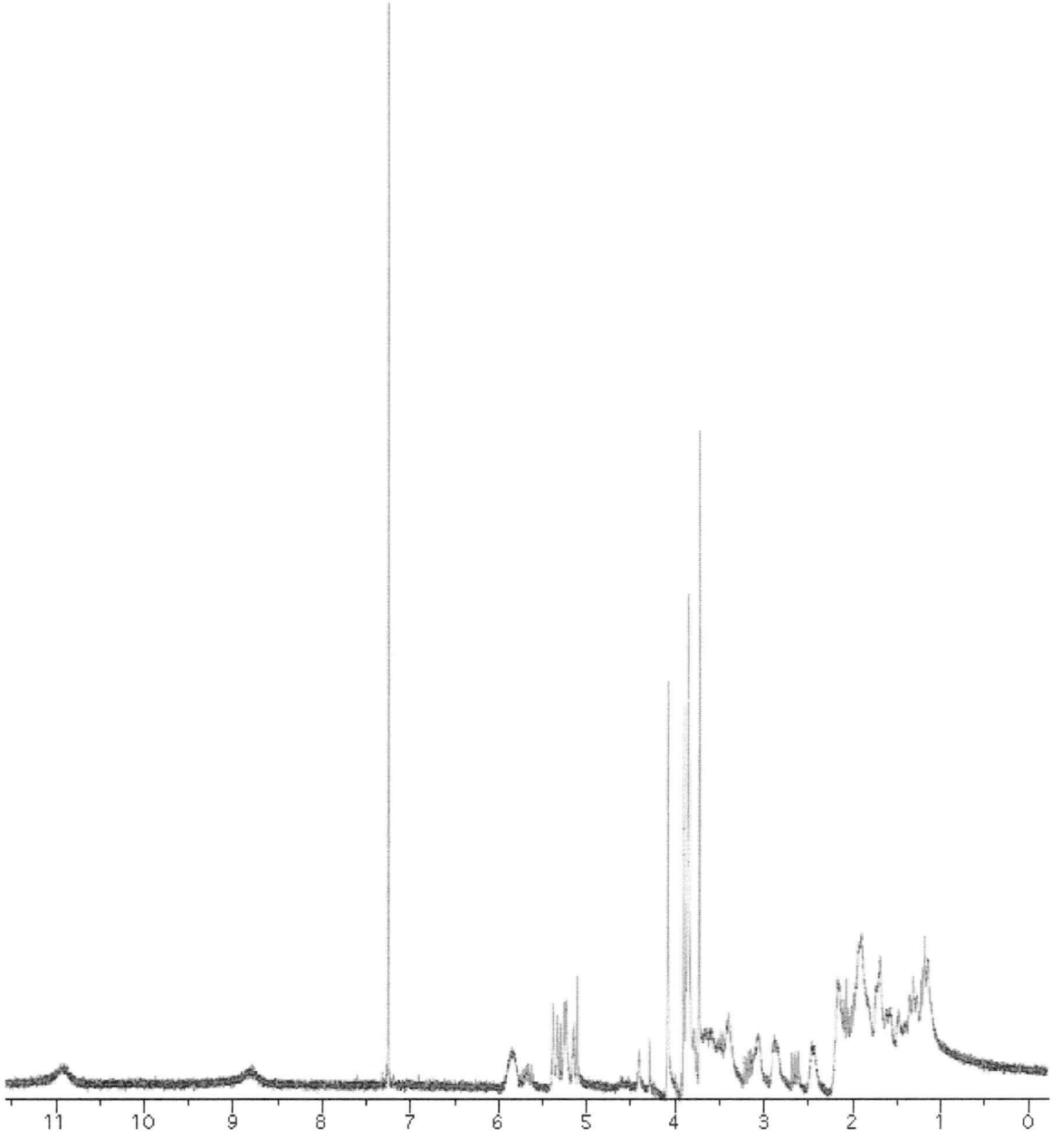
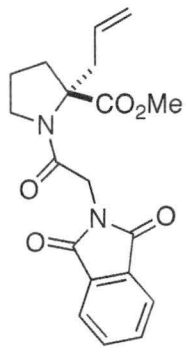
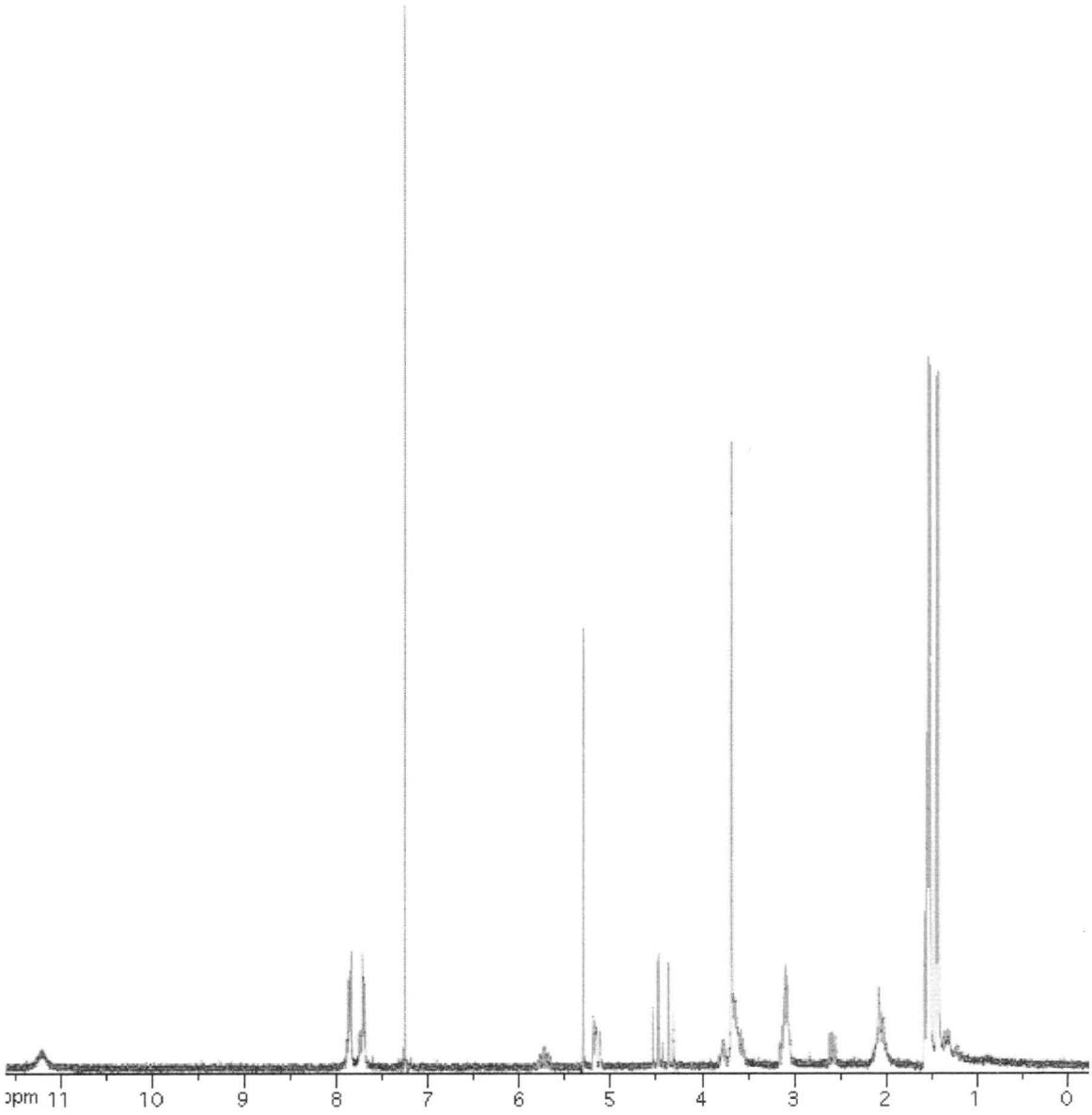


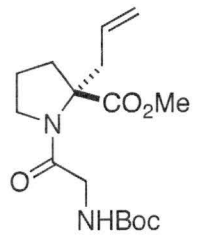
Thylimide Allyl proline (147): Allyl proline (50 mg, .24 mmol, 1 equiv.) was taken up in DCM (10 mL) and cooled to 0 °C. N-Phthaloglglycine (74 mg, .36 mmol, 1.5 equiv) was added followed by DCC (74 mg, .36 mmol, 1.5 equiv). This mixture was stirred for 4 hrs. The reaction was then diluted with ether filtered and conc. The crude product was purified by column chromatography on silica gel (3:1 Hexanes/EtOAc) to yield 67% (28 mg, .0792 mmol) of **147** as a yellow residue (R_f = 0.34) . ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (6H, d, J = 6.6, 6.6 Hz); 2.08 (3H, m); 2.59 (1H, m); 3.08 (2H, m); 3.6 (3H, s); 4.36 (1H, d, J = 16.2 Hz); 4.53 (2H, d, J = 18.9 Hz); 5.17 (1H, m); 5.74 (1H, q); 7.72-7.84 (2H, m). HRMS (FAB+): Calc. for $C_{19}H_{20}N_2O_5$: 356.13722, Found: 356.12997 ($M^{=}$).



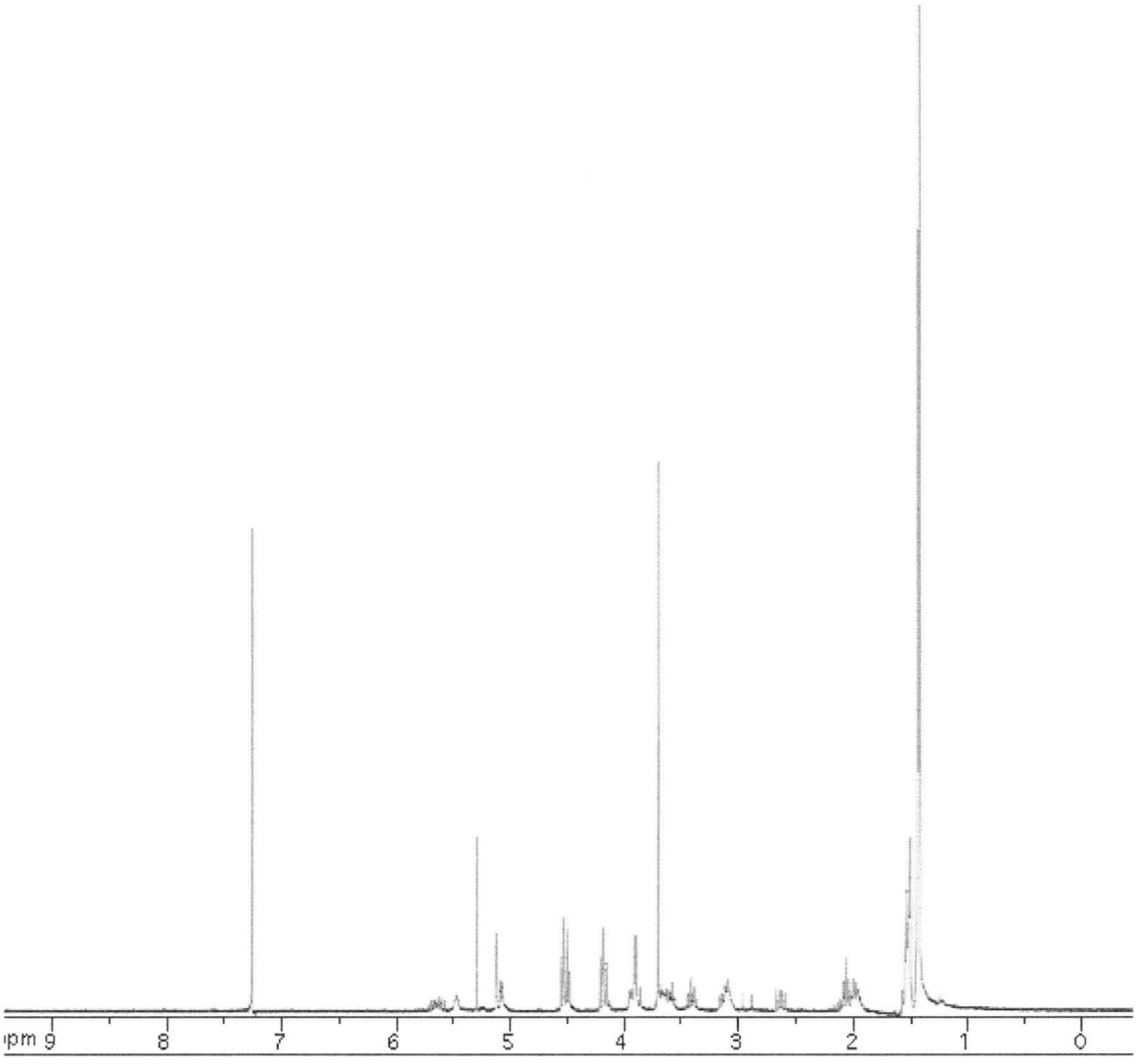
147



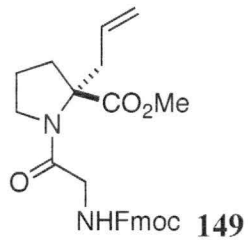
NHBoc Allyl Proline (148): Boc-Gly (851 mg, 4.86 mmol, 1 equiv.) was taken up in DCM (75 mL) and cooled to 0 °C. BOP-Cl (1.51 g, 5.832 mmol, 1.2 equiv.) and i-Pr_2NEt (1.06 mL, 5.832 mmol, 1.2 equiv.) were added and the mixture stirred for 5 minutes. Allyl Proline (1g, 4.86 mmol, 1 equiv.) is then added and the reaction is warmed to room temperature. The reaction is stirred at room temperature for 36 hrs. The reaction was then diluted with DCM and quenched with NH_4Cl. The layers were separated and the organic layers were extracted with DCM (3 x 50 mL) dried (Na_2SO_4) and conc. Only one spot was present by TLC (EtOAc) so the product was run through a short column on silica gel to yield 68% (1.03 g, 3.3 mmol) **148** as a brown syrup (R_f =0.78). ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (9H, s); 1.51 (4H, d, J = 3 Hz); 1.53 (4H, s); 1.97 (2H, m); 2.04 (2H, t, J = 6.3 Hz); 2.66 (1H, dd, J = 8.4, 17.41 Hz); 3.09 (2H, m); 3.38 (1H, m); 3.71 (3H, s); 3.91 (3H, d, J = 4.2 Hz); 4.18 (2H, t, J = 8.7, 8.4 Hz); 4.53 (3H, q, J = 7.8, 8.1, 6.9 Hz); 5.08 (2H, d, J = 3.9 Hz); 5.12 (2H, s); 5.29 (1H, s); 5.668 (1H, m).



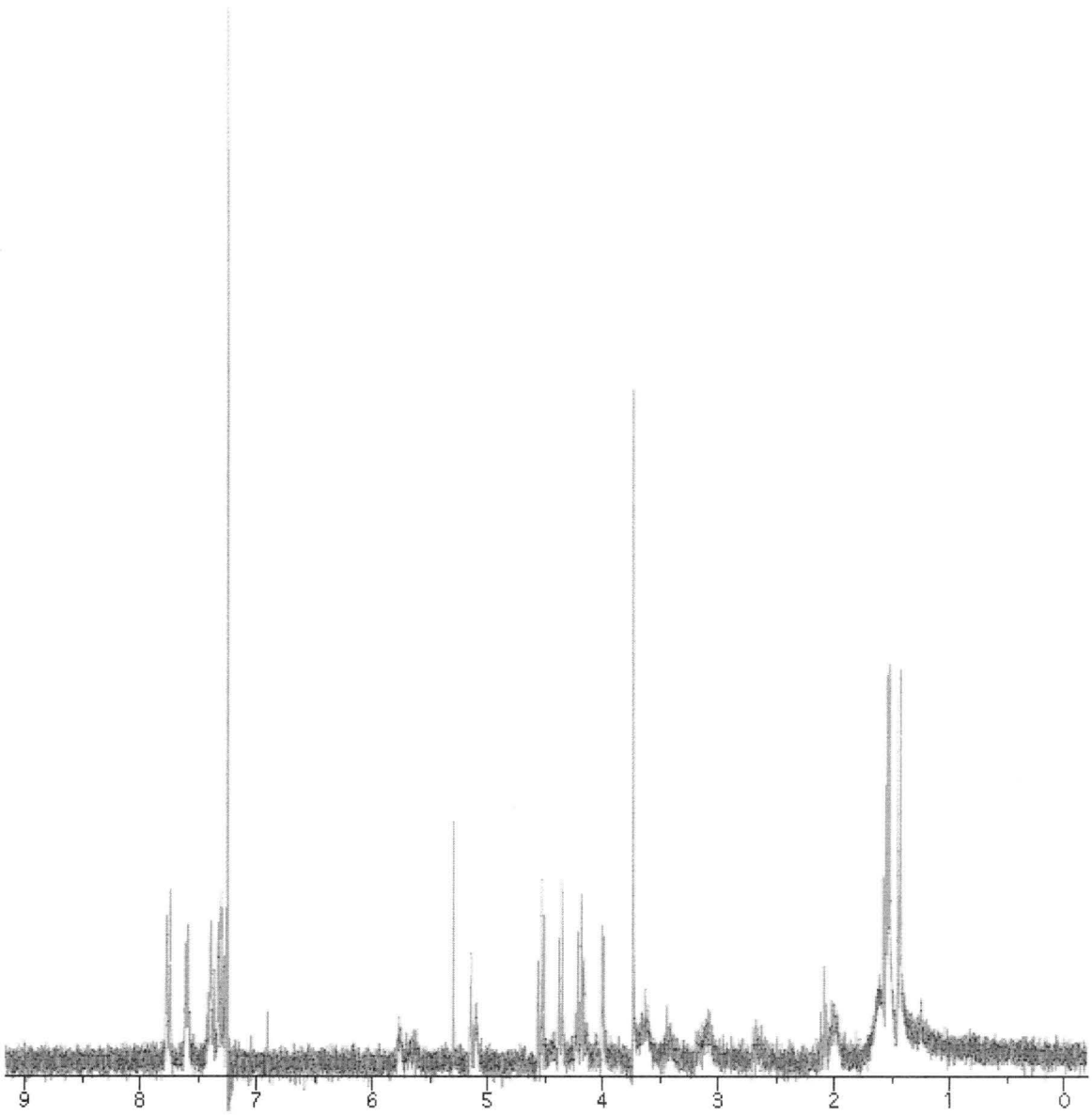
148



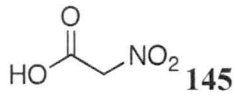
NHFmoc Allyl Proline (149): To a cold (0 °C) stirring solution of Fmoc-Gly (71 mg, .24 mmol, 1 equiv.) and DCM (15 mL) was added BOP-Cl (74 mg, .29 mmol, 1.2 equiv.) and i-Pr_2NEt (.05 mL, .29 mmol, 1.2 equiv.). This mixture was stirred for 10 minutes then allyl proline (50 mg, .24 mmol, 1 equiv.) was added. The reaction was warmed to room temperature and stirred for 48 hrs. The reaction was then quenched with NH_4Cl, the organic layer was extracted with DCM (3 x 15 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and conc. The crude product was purified by column chromatography on silica gel (1:1 Hexanes/EtOAc) to yield 14% (15 mg, .033 mmol) of **149** as a colorless oil (Rf = 0.23). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (2H, d, J = 6.6 Hz); 1.54 (2H, d, J = 6.9 Hz); 2.00 (2H, m); 2.08 (2H, m); 3.08 (1H, m); 3.63 (1H, m); 3.74 (3H, s); 4.01 (1H, d, J = 4.8 Hz); 4.18 (1H, m); 4.37 (1H, d, 6.9 Hz); 4.53 (1H, t, J = 9.3 Hz); 5.10 (1H, m); 5.3 (1H, s); 5.76 (1H, m); 7.33 (2H, m); 7.59 (2H, m); 7.77 (4H, m). HRMS (FAB+): Calc. for $C_{26}H_{28}N_2O_5$: 448.2066, Found: 448.10723 (M^+), 449.20709 (MH^+).



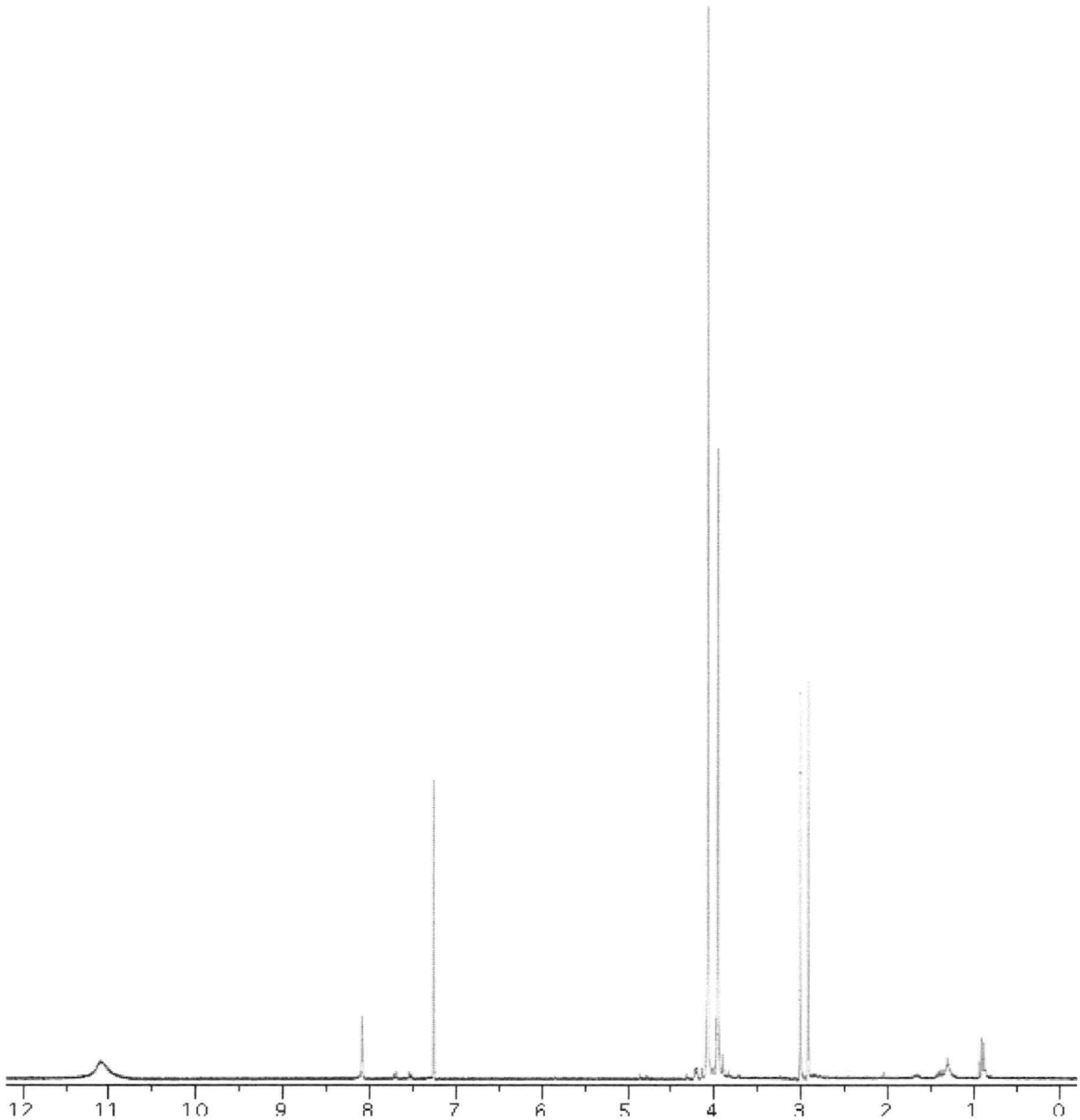
149



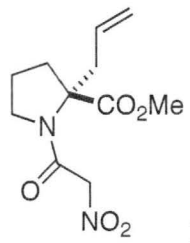
Nitro Acetic Acid (145): In a 3 necked flask equipped with a condenser and an addition funnel, KOH (224g, 3.99 mol, 4 equiv.) was dissolved in water (100ml). Nitromethane (61 g, 1 mol, 1 equiv.) was added dropwise from the addition funnel (1drop/sec) **making sure to vent the condenser**. The reaction is **extremely** exothermic. The reaction turns yellow then orange, followed by the formation of a yellow suspension. Once all of the nitromethane is added the reaction is refluxed for 1 hr then cooled to room temperature. The nitromethane needs to be added at a constant rate otherwise the yields suffer greatly. The reaction is then filtered and the solid was washed with cold MeOH and dried under vacuum. This Dipotassium nitroacetate was then dissolved in water (100ml) and cooled in an EtOH dry ice bath (-8 C) to which a cold solution of tartaric acid in water (100ml) was added over 20 minutes. The mixture was filtered and the filtrate was saturated with NaCl then extracted with ether (4 x 50 mL), dried (Na_2SO_4) and concentrated. Chloroform was added and evaporated several times. Upon cooling yields 13% of **145** as yellow needles (5.26 g, 0.05 mol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.98 (1H, d, J = 18.5); 3.987 (2H, s); 4.082 (2H, s); 11.1 (1H, s br).



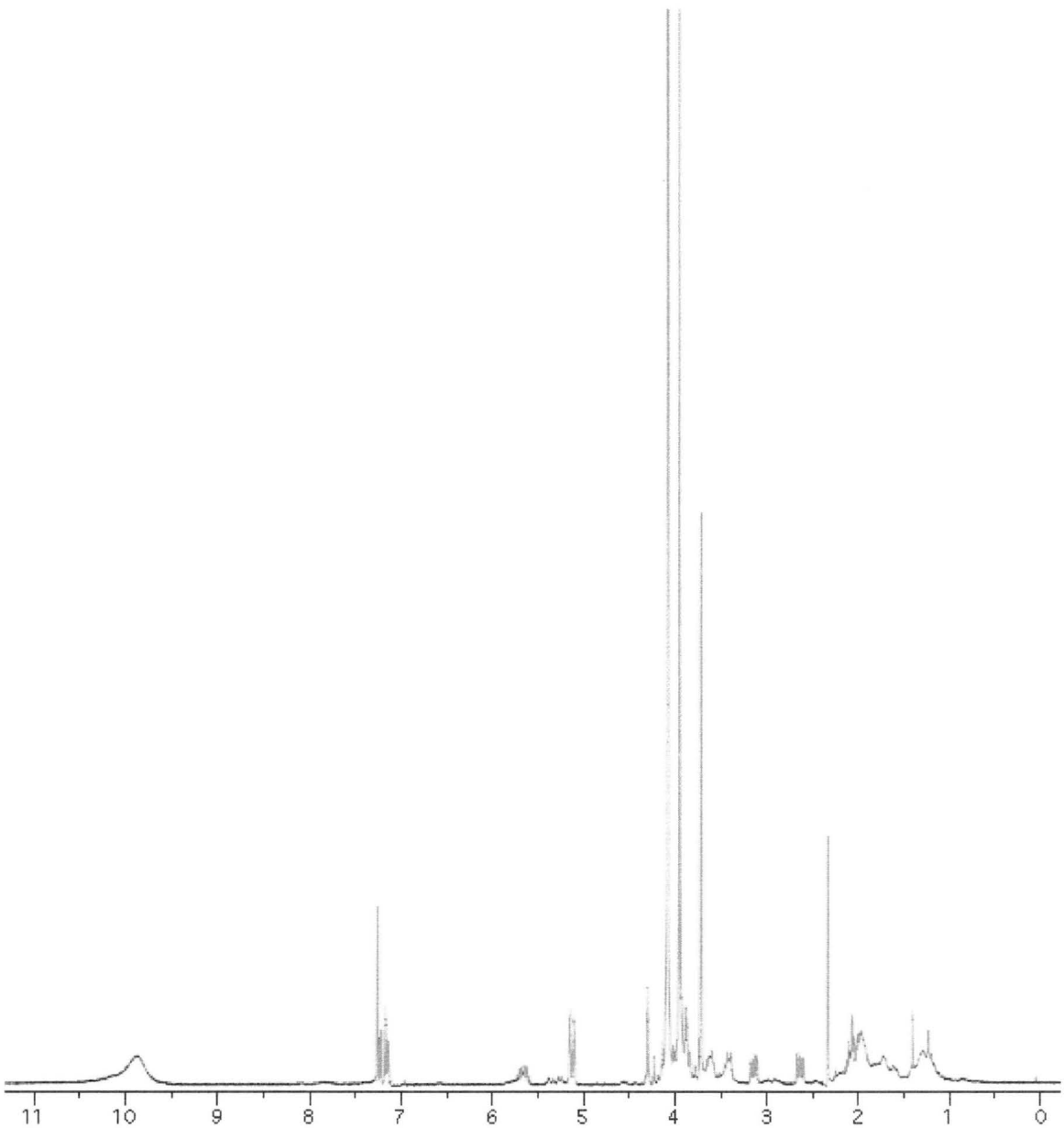
145



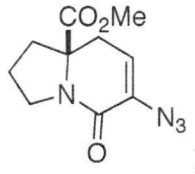
Nitro Allyl Proline (150): Ally proline (50 mg, .24 mmol, 1 equiv.) was dissolved in DCM (10 mL) and cooled to 0 °C. To this was added nitro acetic acid (37.8 mg, .36 mmol, 1.5 equiv.) followed by DCC (74.3 mg, .36 mmol, 1.5 equiv.) This mixture was stirred for 3.5 hrs. The mixture was then diluted with DCM and quenched with NH_4Cl. The layers were separated and the organic layer extracted with DCM (3 x 10 mL) washed with brine, dried ($MgSO_4$) and conc. The crude product was purified by column chromatography on silica gel (EtOac) to yield 72% (44.3 mg, .17 mmol) of the nitro allyl proline **150** as a colorless thick oil. ^1H NMR (300 MHz, $CDCl_3$): 1.2 (2H, m); 1.41 (1H, s); 2.07 (4H, m br); 2.67 (1H, dd, J = 15, 21.3 Hz); 3.15 (1H, dd, J = 6.9, 6.6 Hz); 4.402 (1H, m); 3.41 (2H, m); 3.71 (3H, s); 3.94 (6H, s); 4.07 (6H, s); 4.29 (1H, s); 5.14 (1H, d, J = 12.6 Hz); 5.64 (1H, q); 7.14-7.26 (2H, m). HRMS (FAB+): Calc. for $C_{11}H_{16}N_2O_5$: 256.10592, Found: 256.11035 ($M^=$).



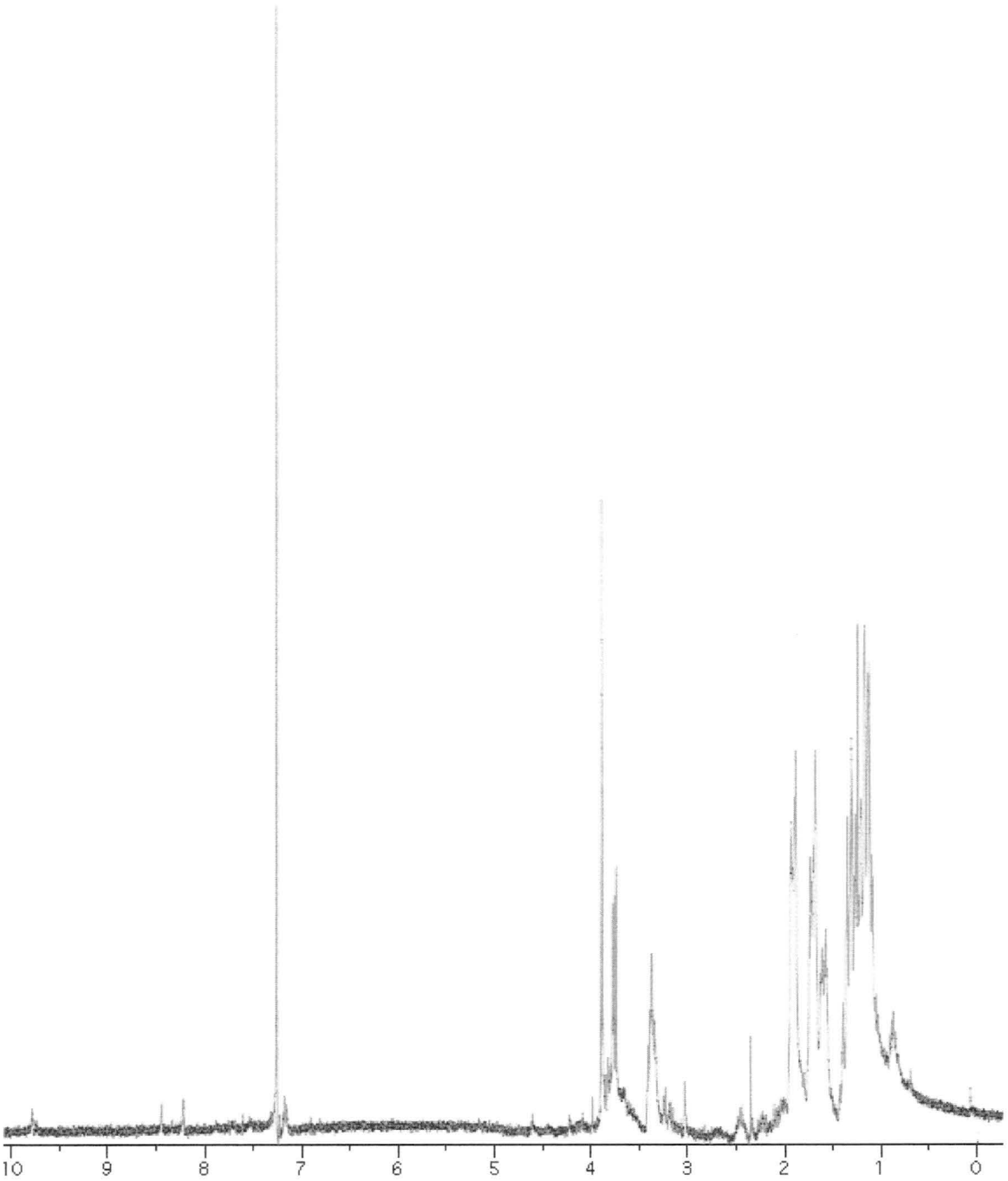
150



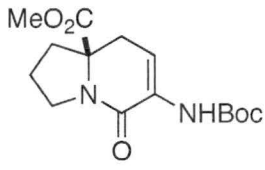
Azide Dienophile (151): Azide Allyl Proline (50mg, .2mmol, 1 equiv) was taken up in THF (25mL) and cooled to 0 °C. $NaIO_4$ (107mg, .5mmol, 2.5 equiv) is added followed by a solution of RuCl3 (5mg, .10 mmol, 5 mol%) in acetonitrile (5mL) dropwise via syringe. The solution is stirred for 4 hours then diluted with ether and filtered through celite. The layers are then separated and the combined organic layers were dried (Na_2SO_4) and concentrated to yield 78% (36 mg, .154 mmol) of **151** as a reddish residue. ^1H NMR (300 MHz, $CDCl_3$): δ 1.13 (1H, m); 1.21 (1H, m); 1.57 (1H, m); 1.62 (1H, m); 1.69 (2H, d, J = 3.3 Hz); 1.93 (2H, d, J = 9.3 Hz); 2.35 (1H, s); 2.46 (1H d, J = 7.21 Hz); 3.36 (4H, m); 3.75 (2H, t, J = 5.1 Hz); 3.87 (3H, s); 7.17 (1H, m). HRMS (FAB+): Calc. for $C_{10}H_{12}N_4O_3$: 236.09094, Found: 236.08133 (M^+), 237.09104 (MH^+).



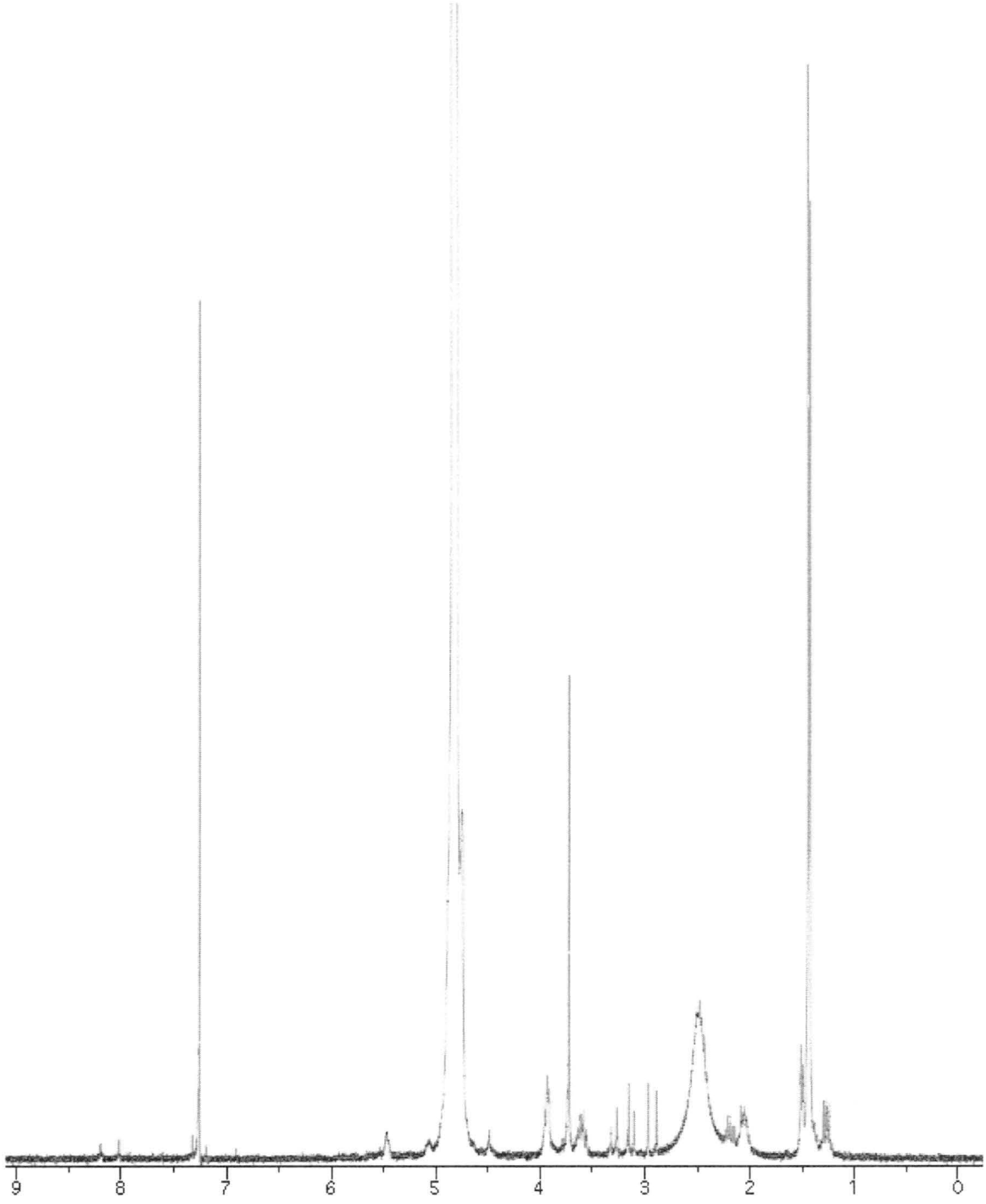
151



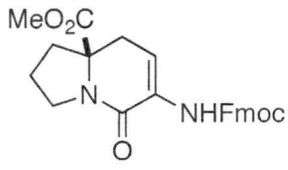
Boc Dieneophile (152): Boc allyl proline (70 mg, .214 mmol, 1 equiv.) is dissolved in acetonitrile (15 mL) and cooled to 0 °C. A solution of $NaIO_4$ (114 mg, .535 mmol, 2.5 equiv.) and $RuCl_3$ (2.2 mg, .0107 mmol, 5 mol%) in water (10 mL) is added to the allyl proline solution dropwise via cannula. The reaction is allowed to warm to room temperature and then quenched with $Na_2S_2O_3$. The organic layers were extracted with ether (3 x 15 mL), washed with brine, dried ($MgSO_4$) and conc. The crude product was purified by column chromatography on silica gel (10:1 DCM/MeOH) to yield 84% (40 mg, .129 mmol) of an orange oil **152** (R_f = 0.12). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24-1.27 (2H, m); 1.44 (9H, s); 1.49 (2H, d, J = 2.7 Hz); 1.52 (2H, d, J = 1.8 Hz); 2.05 (3H, m); 2.21 (2H, d, J = 6.6 Hz); 2.96 (1H, d, J = 23.7 Hz); 3.15 (2H, d, J = 15.9 Hz); 3.32 (2H, d, J = 15.9 Hz); 3.58 (2H, m); 3.61 (2H, m); 3.72 (3H, s); 3.92 (2H, m); 5.46 (1H, m).



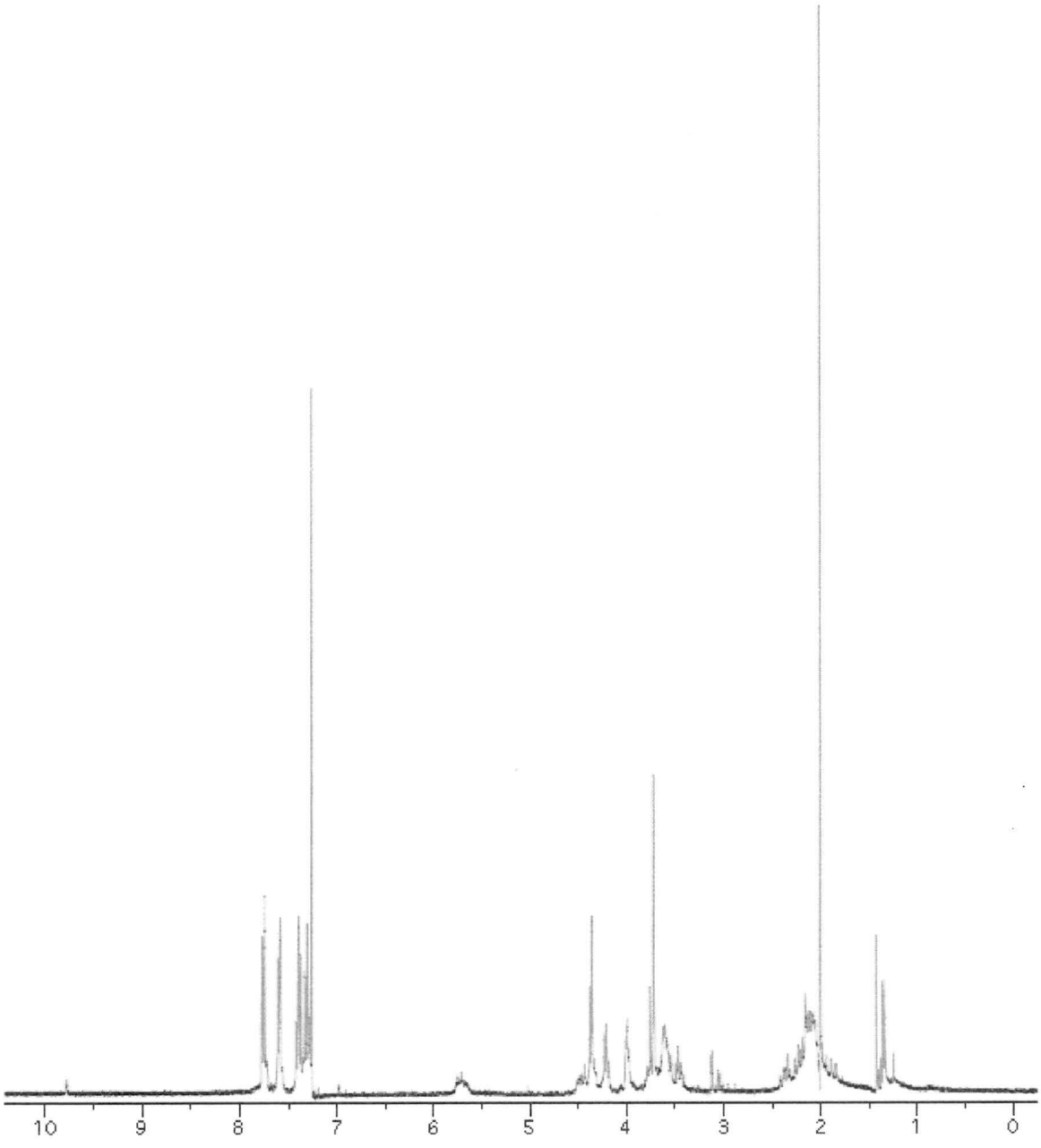
152



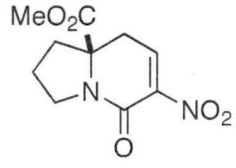
Fmoc Dieneophile (153): Fmoc alllyl proline (15 mg, .033 mmol, 1 equiv.) was taken up in acetonitrile (10 mL) under argon and cooled to 0 °C. A cooled solution of $NaIO_4$ (18 mg, .0825 mmol, 2.5 equiv.) and $RuCl_3$ (1 mg, 5 mol%) in water (5 mL) was added dropwise via cannula over approx. 10 minutes. The mixture was allowed to warm to room temperature over 2.5 hrs. A white precipitate that formed was filtered off and the remaining liquid was quenched with $Na_2S_2O_3$ and extracted with ether (3 x 10 mL), dried ($MgSO_4$) and conc. The crude product was purified by column chromatography on silica gel (10:1 DCM/MeOH) to yield 71% (10 mg, .023 mmol) clear yellow crystals of **153** (R_f = 0.39). ^1H NMR (300 MHz, $CDCl_3$): δ 1.36 (2H, d, J = 6.6 Hz); 1.42 (2H, s); 2.16 (1H, m); 2.36 (1H, m); 3.12 (1H d, J = 29.1 Hz); 3.46 (2H, m); 3.60 (2H, m); 3.72 (3H, s); 3.99 (1H, m); 3.99 (1H, m); 4.21 (1H, d, J = 7.5 Hz); 4.38 (2H, d, J = 7.2 Hz); 7.30-7.39 (4H, m); 7.6 (4H, d, J = 7.2 Hz) 7.7 (4H, d, 7.5 Hz).



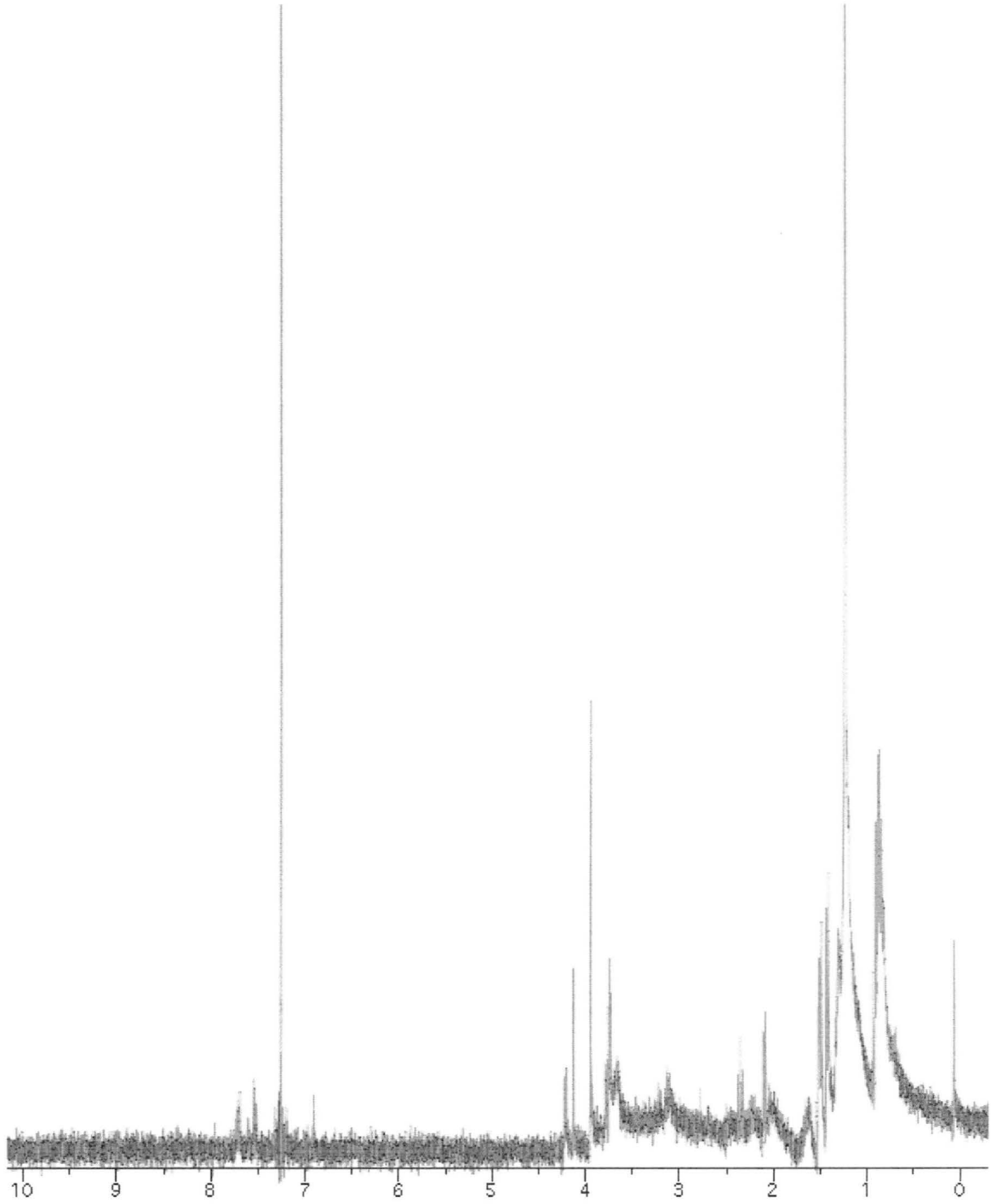
153



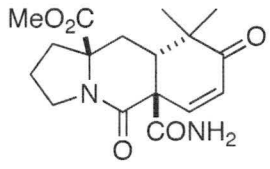
Nitro Dieneophile (154): Nitro Allyl Proline (10 mg, .039 mmol, 1 equiv.) was taken up in acetonitrile (10 mL) and cooled to 0 °C. $RuCl_3$ (.28 mg, .00136 mmol, 3.5 mol%) and water (2 mL) are added and stirred for 5 minutes. $NaIO_4$ (16.7 mg, .078 mmol, 2 equiv.) is added in small portions aver 5 minutes. The reaction was then stirred and warmed to room temperature over 3 hrs. The reaction was then quenched with $Na_2S_2O_3$. The layers were separated and the organic layer was extracted with EtOAc (3 x 15 mL) washed with water and brine respectfully, dried ($MgSO_4$) and conc. The crude product was purified by column chromatography on silica gel (5:1 DCM/MeOH) to yield 62% (5.6 mg, .024 mmol) of **154** as a brown oil (R_f = 0.81). ^1H NMR (300 MHz, $CDCl_3$): δ 0.87-0.89 (2H, m); 1.2 (2H, m); 1.44 (4H, dd, J = 6.9, 4.2 Hz); 2.35 (1H, t, J = 7.21 Hz); 3.12 (1H, m); 3.73 (2H, m); 3.94 (3H, s); 4.13 (1H, s); 4.21 (2H, dd, J = 3.9, 24.3 Hz); 6.9 (1H, m). HRMS (FAB+): Calc. for $C_{10}H_{12}N_2O_5$: 240.07162, Found: 240.08535 (M^+), 241.07452 (MH^+).



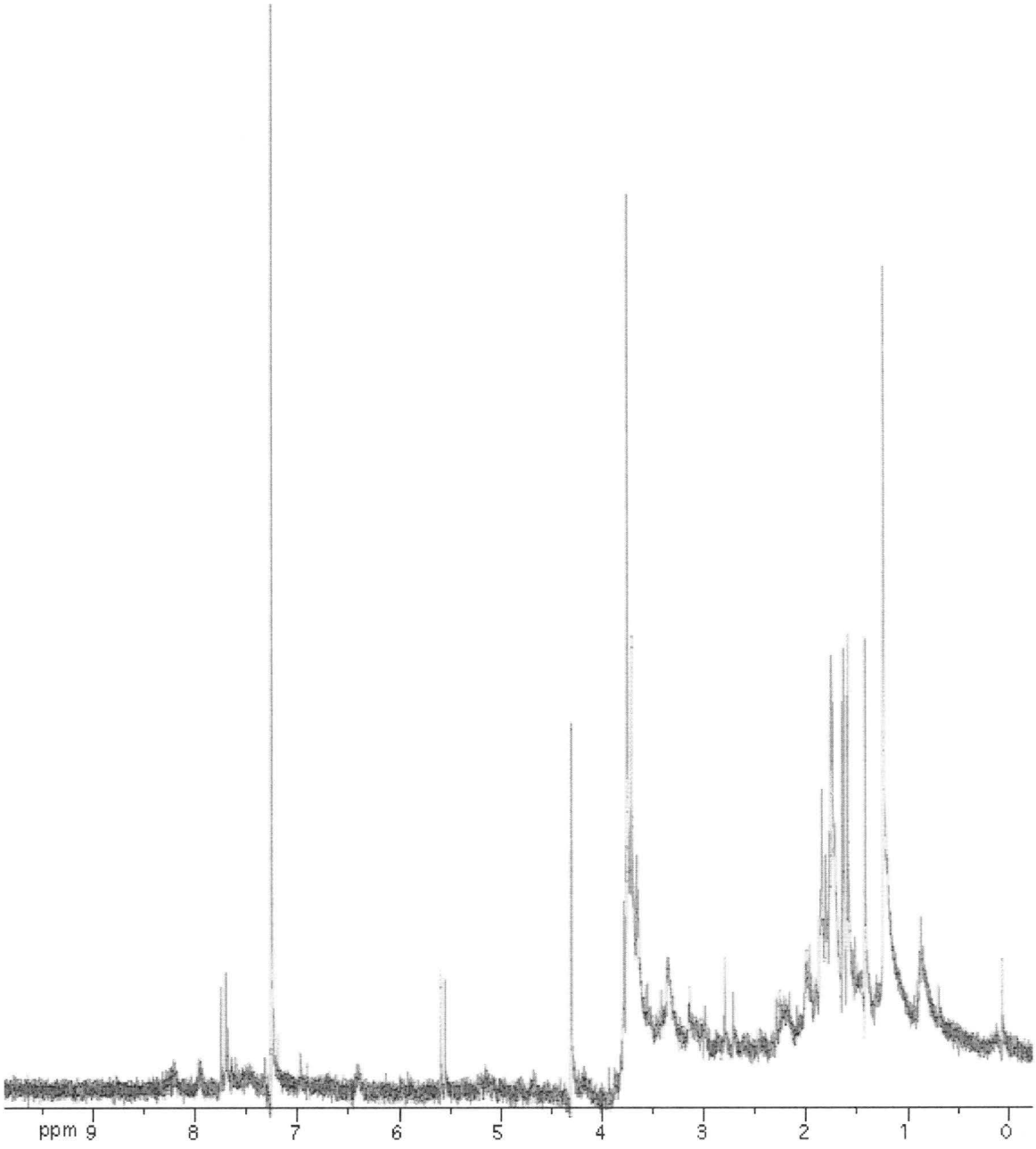
154



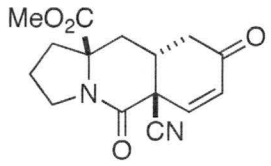
Amide (157): Methylated Enone (40mg, .126 mmol, 1equiv.) was taken up in a 1:1 mixture of THF/water at room temperature under argon. Parkins catalyst (3mg, .0063mmol, 5 mol%) was added and the reaction was fitted with a condenser and brought to reflux at 90 °C. The reaction was refluxed for 20 hrs and then cooled to room temperature. The remaining solvent was evaporated via rotovaporation to yield 85% (41 mg, .122 mmol) of a brown residue. This residue **157** was determined not to need any further purification.^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24 (6H, s); 1.27 (2H, s); 1.35 (1H, d, J = 3 Hz); 1.37 (2H, d, J = 6 Hz); 1.38 (1H, m); 1.64 (2H, s); 1.8 (2H, m); 2.21 (1H, s); 2.79 (2H, d, J = 22.5 Hz); 3.33 (2H, s); 3.7 (2H, m); 3.75 (3H, s); 4.3 (2H, s); 4.57 (1H, s br); 5.59 (1H, d, J = 12.9 Hz); 7.25 (1H, m); 7.74 (1H, d, J = 10.5 Hz).). ^{13}C NMR (100 MHz, $CDCl_3$): δ 5.66, 10.23, 13.99, 18.44, 20.44, 25.81, 36.33, 40.29, 42.90, 46.15, 49.40, 50.22, 73.50, 97.33, 102.59, 112.20, 128.35, 141.93, 175.03, 176.38, 199.22, 205.01. HRMS (FAB+): Calc. for $C_{17}H_{22}N_2O_5$: 334.15287, Found: 334.36676 (M^+), 335.15307 (MH^+).



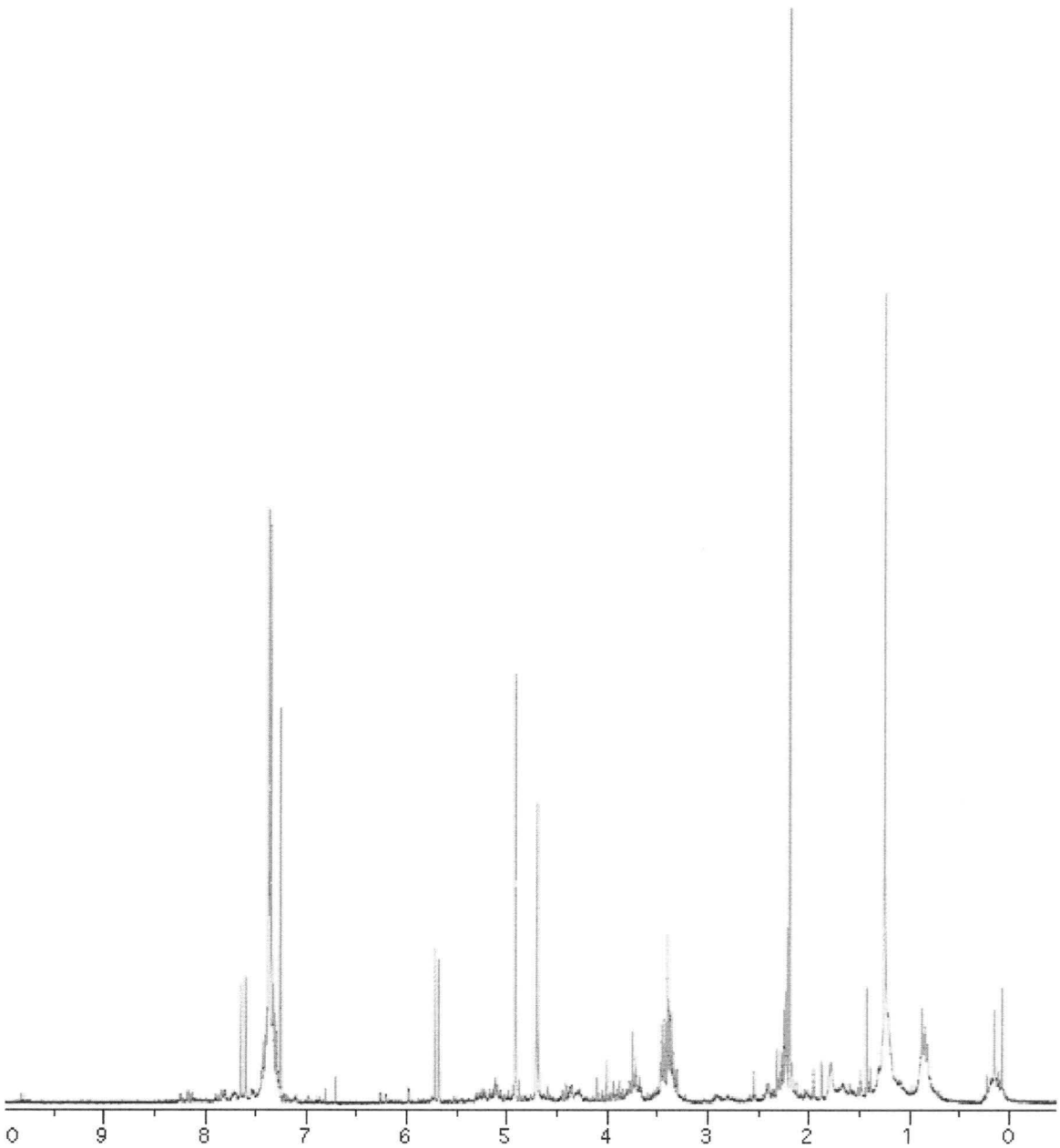
157



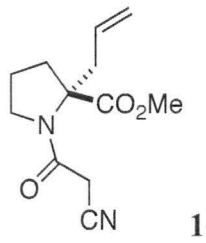
Cyano Enone (158): Cyanoalkene **7** (18mg, .082 mmol, 1 equiv.) was taken up in toluene (7ml) and Danishefsky's diene (70 mg, .41 mmol, 5 equiv.) was added and the reaction was put under microwave conditions (155 ^{o}C, 1 hr). The reaction was then taken out of the microwave and TFA (7-8 drops) was added and the reaction was stirred for 30 minutes. The reaction was then neutralized with Sat. Na_2CO_3 and the organic layers were extracted with ether (3 x 10 mL). The material was purified by column chromatography on silica gel (1:1 Hexanes/EtOAc) to yield 72% (17mg, .059 mmol) of **158** as a clear oil (R_f = 0.79). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82-0.88 (2H, m); 1.24 (2H, m); 1.42 (3H, s); 2.04 (2H, d, J = 7.4 Hz); 2.29 (2H, s); 2.54 (1H, d, J = 2.4 Hz); 3.40 (2H, m); 3.45 (2H, d, J = 6 Hz); 3.67 (1H, m); 3.74 (3H , s); 4.7 (2H, s); 4.92 (2H, s); 5.7 (1H, d, J = 12.9 Hz); 7.36 (1H, m); 7.64 (1H, d, J = 12.9 Hz). HRMS (FAB+): Calc. for $C_{15}H_{16}N_2O_4$, 288.11101, Found: 288.29854 (M^+), 289.20367 (MH^+).



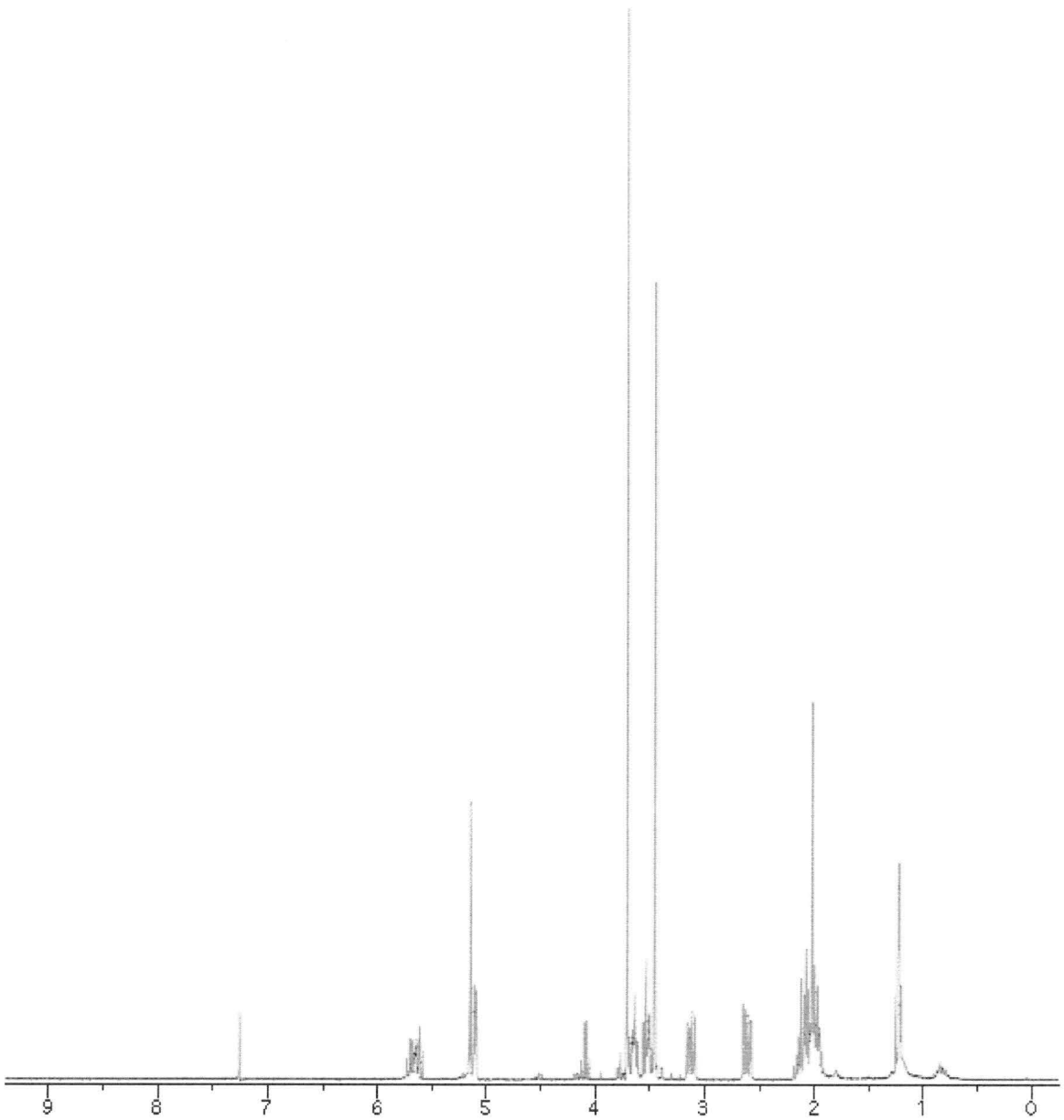
158



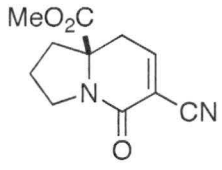
Cyano Allyl Proline (160): Cyano Acetic Acid (20 mg, .24 mmol, 1 equiv.) was taken up in DCM (20 mL) at 0 °C. BOPCl (73 mg, .288 mmol, 1.2 equiv.) followed by i-Pr_2Net (.05 mL, .288 mmol, 1.2 equiv.) were added and stirred for 10 minutes. Allyl proline (50 mg, .24 mmol, 1 equiv.) was then added and the reaction was warmed to room temperature after which the reaction was diluted with DCM and quenced with NH_4Cl. The organic layers were extracted with DCM (3 x 15 mL). The combined organic layers were washed with brine dried ($MgSO_4$) and conc. Purification was done with column chromatography on silica gel (3:1 EtOAc/Hexanes then EtOAc) to yield 88% (49.9 mg, .211 mmol) of a clear oil **160** (R_f = 0.42). ^1H NMR (300 MHz, $CDCl_3$): δ 1.21 (2H, d, J = 1.2 Hz); 1.23 (2H, s); 1.25 (2H, d, J = 3.4 Hz); 2.02 (2H, s); 2.07 (2H, m); 2.65 (1H, dd, J = 7.8 Hz); 3.16 (1H, dd, J = 6, 8.1 Hz); 3.45 (2H, s); 3.51 (1H, m); 3.66 (1H. m); 3.7 (3H, s); 5.11 (2H, d J = 4.2 Hz); 5.15 (2H, s); 5.67 (1H, m). HRMS (FAB+): Calc. for $C_{12}H_{16}N_2O_3$: 236.11609, Found: 236.26704 (M+), 237.16534 (MH+).



160

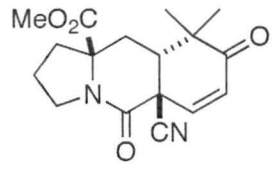


α, β-Unsaturated Cyano Ketone (161): Cyano Allyl Proline (25mg, .106 mmol, 1 equiv.) was taken up in acetonitrile (15ml) and cooled to 0 °C under argon. A solution of $NaIO_4$ (56mg, .265 mmol, 2.5 equiv.) and $RuCl_3$ (1.1mg, .0053 mmol, 5 mol%) in water (10ml) was added dropwise via cannula and the reaction was allowed to warm to room temperature overnight. The reaction was then quenched with Na_2SO_4 and extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with water and brine respectfully, dried with Na_2SO_4 and concentrated. The crude black residue was purified by preparative tlc (5:1 EtOAc/Hexanes) to yield 57% (13 mg, .057 mmol) **161** as a brown oil (R_f = 0.48). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.81-0.87 (2H, m); 1.25 (2H, m); 1.42 (3H, s); 2.04 (2H, d, J = 7.2 Hz); 2.2 (2H, s); 2.53 (1H, d, J = 2.4 Hz); 2.59 (1H, d, J = 2.4 Hz); 3.39 (2H, dd, J = 6.3, 6.9 Hz); 3.67 (1H, m); 3.75 (3H, s); 3.83 (1H. m); 7.22 (1H, t, J = 2.1 Hz). HRMS (FAB+): Calc. for $C_{11}H_{12}N_2O_3$: 221.08479, Found: 221.09301 (MH^+).

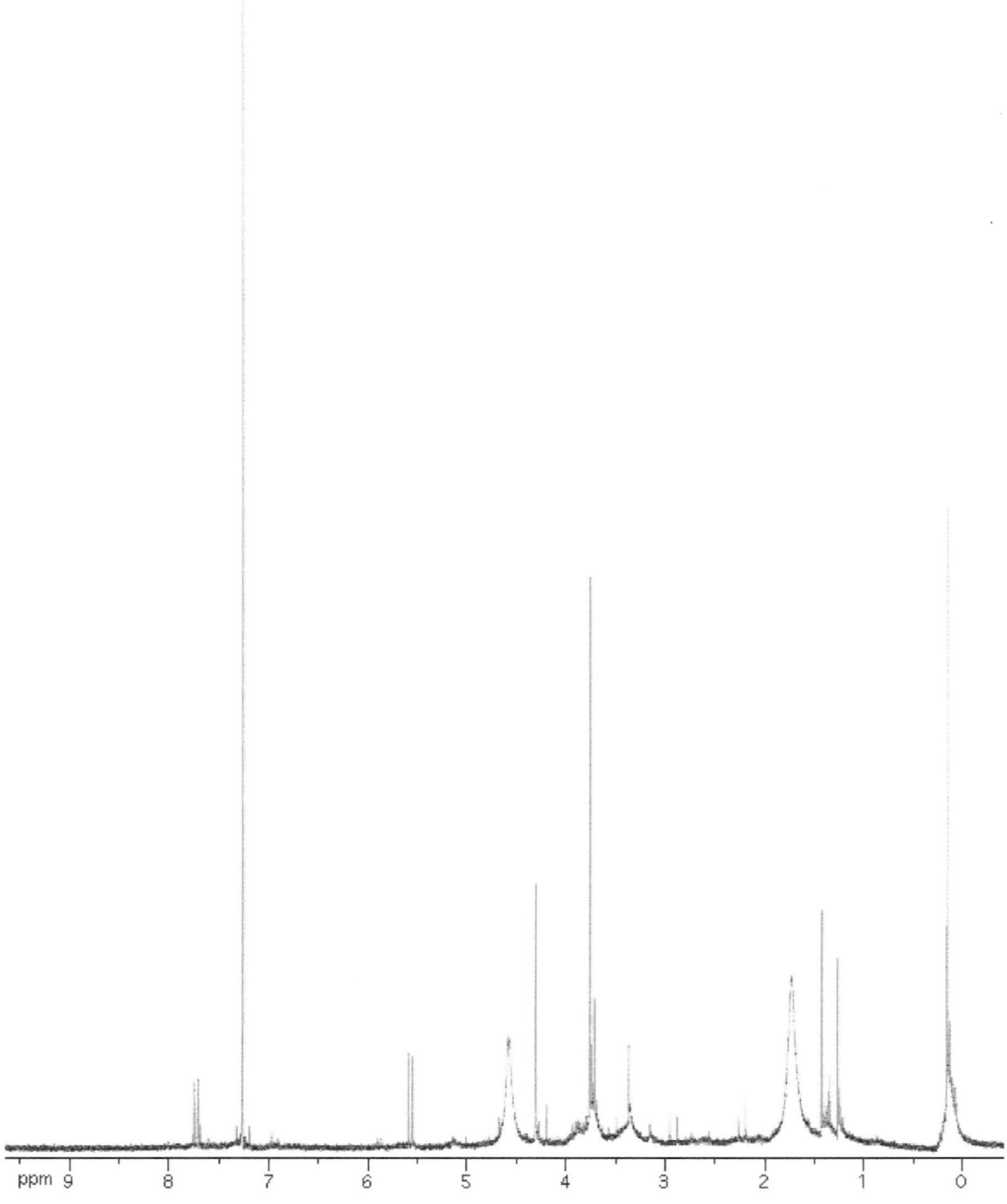


161

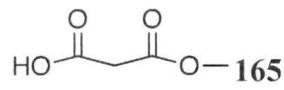
Methylated Enone (162): Enone **158** (75mg, .26 mmol, 1 equiv.) was taken up in THF (50ml) and KOtBu (58mg, ,52 mmol, 2 equiv.) was added and the reaction was stirred for 10 minutes. MeI (184mg, 1.3 mmol, 5 equiv.) was then added dropwise via syringe. The reaction was allowed to stir for 4 hrs. NH_4Cl was added and the organic layer was extracted with ether (3 x 15 mL). The crude product was purified by column chromatography on silica gel (10:1 DCM/MeOH) to yield 85% (70 mg, .22mmol) of an orange oil (R_f = 0.77). ^1H NMR (400 MHz, $CDCl_3$): δ 0.15 (6H, s); 1.27 (2H, s); 1.35 (1H, d, J = 3 Hz); 1.37 (2H, d, J = 6 Hz); 1.38 (1H, m); 1.40 (2H, s); 2.19 (1H, s); 2.95 (2H, d, J = 22.5 Hz); 3.36 (2H, s); 3.7 (2H, m); 3.75 (3H, s); 4.3 (2H, s); 4.57 (1H, s br); 5.59 (1H, d, J = 12.6 Hz); 7.25 (1H, m); 7.74 (1H, d, J = 12.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.32, 18.35, 20.3, 25.22, 36.11, 37.86, 40.21, 42.89, 46.00, 52.13, 55.64, 65.33, 69.21, 73.4, 114.25, 128.32, 145.32, 175.99, 176.48, 204.81. HRMS (FAB+): Calc. for $C_{17}H_{20}N_2O_4$: 316.14231, Found: 316.3517 (M^+).



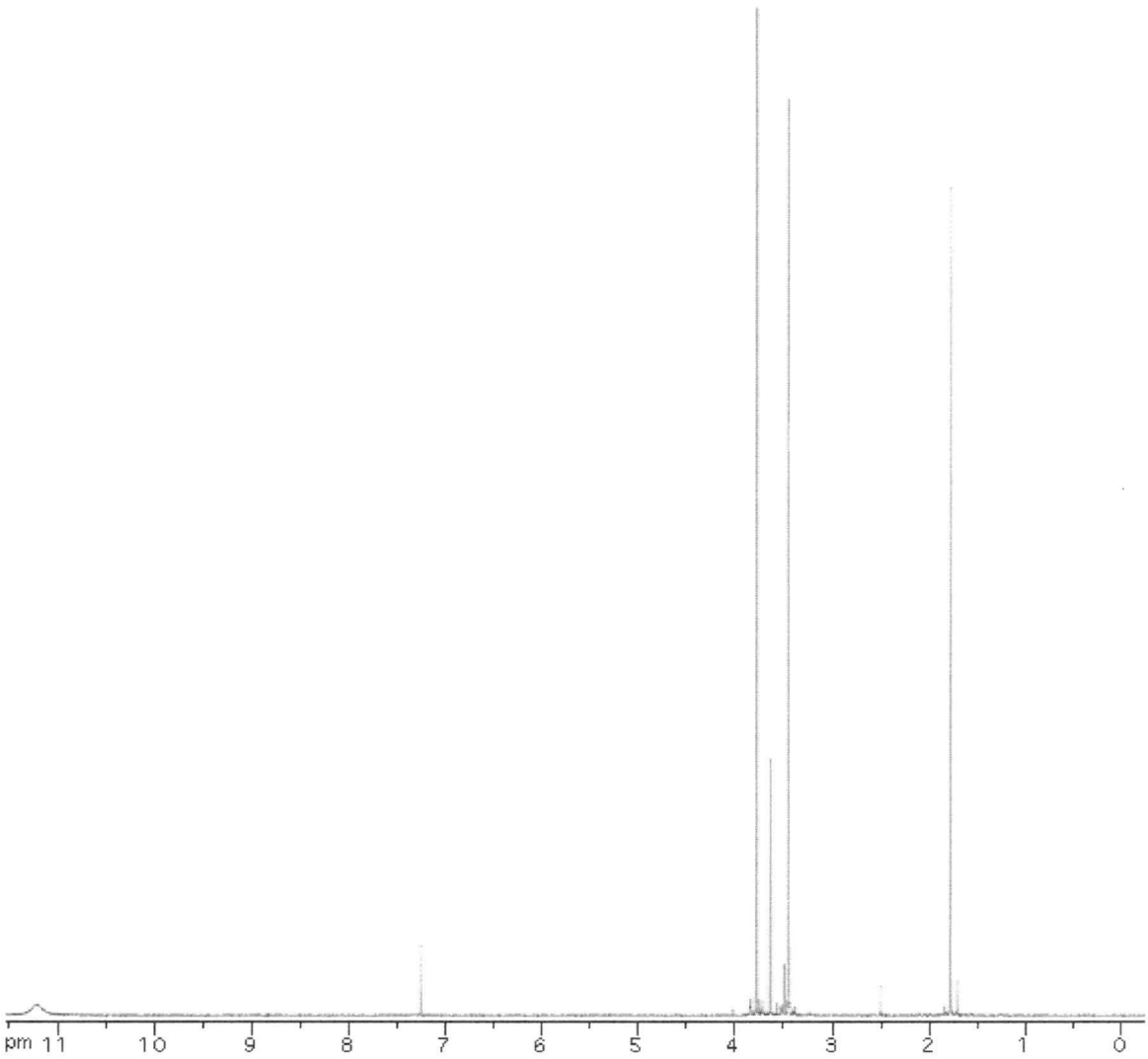
162



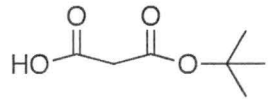
Acid Methyl Ester (165): In a 25 mL round bottom flask equipped with a condenser, Meldrum's acid (1.0 g, 6.94 mmol, 1 equiv.) and MeOH (.28 mL, 6.94 mmol, 1 equiv.) were heated to ruflux in acetonitrile (7 mL). The reaction was allowed to reflux for 24 hrs. The solvent was then concentrated via rotovap and the product was put through a short silica gel column (10:1 DCM/MeOH) to yield 88% (722 mg, 6.11 mmol) **165** as a clear, colorless liquid (R_f = 0.94). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.45 (2H, s); 3.634 (1H, s); 3.77 (3H, s); 11.2 (1H, s br).



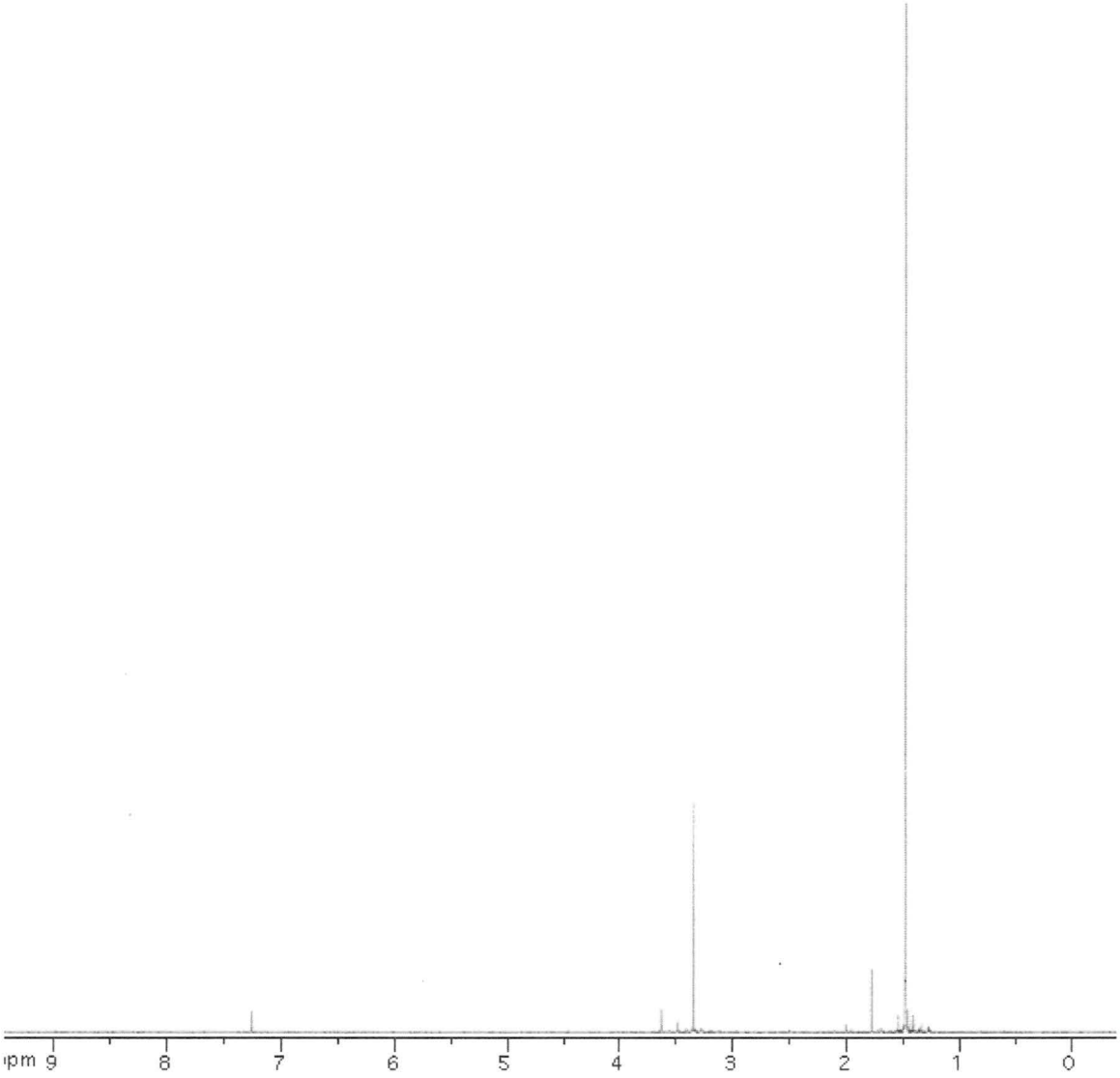
165



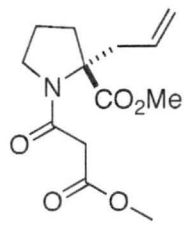
Acid tBu Ester (166): Meldrum's acid (1.0 g, 6.94 mmol, 1 equiv.) and tBuOH (518 mg, 6.94 mmol, 1 equiv.) were taken up in acetonitrile (10 mL) at room temperature. The reaction was allowed to stir for 10 minutes and then brought to reflux. The reaction was refluxed for 24 hrs. after which was cooled to room temperature. The remaining solvent was then conc. to yield 89% (988 mg, 6.17 mmol) of the pure tBu ester **166**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (9H, s); 3.34 (2H, s); 10.8 (1H, s br).



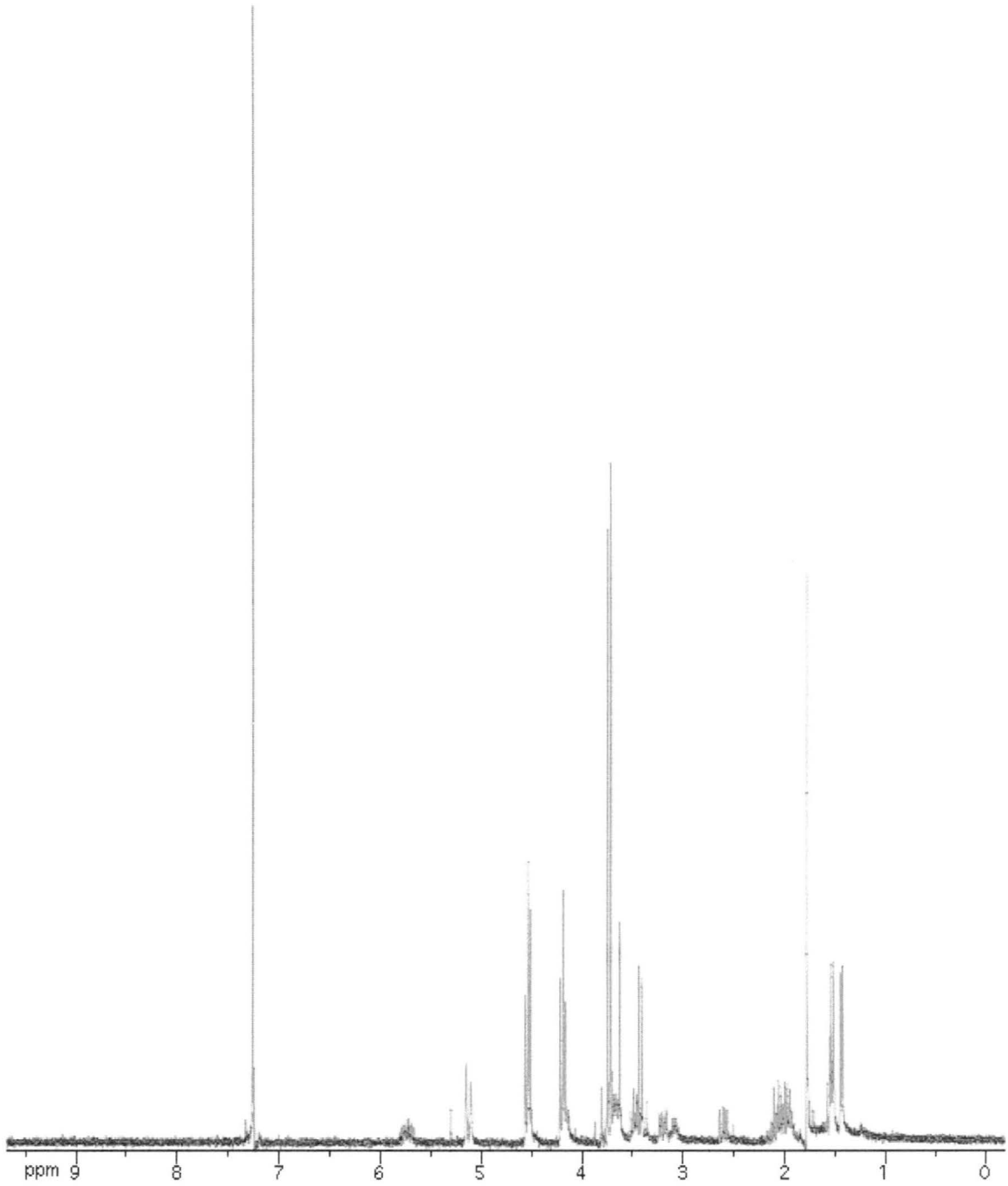
166



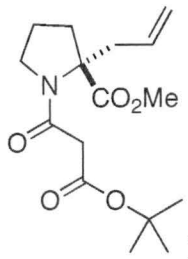
Methyl Ester Allyl Proline (167): The methyl ester (57 mg, .486 mmol, 1 equiv.) was dissolved in DCM (30 mL) at 0 °C. To this solution was added BOP-Cl (151 mg, .583 mmol, 1.2 equiv.) and i-Pr_2NEt (.11 mL, .583 mmol, 1.2 equiv.) in one portion. This mixture was stirred for 10 minutes then allyl proline (100 mg, .486 mmol, 1 equiv.) was added. The reaction was warmed to room temperature over night and then diluted with DCM. NH_4Cl was added and the layers were separated. The organic layers were extracted with DCM (3 x 15 mL) and the combined organic layers were dried ($MgSO_4$) and conc. The crude product was purified by column chromatography on silica gel (5:1 DCM/MeOH) to yield 73% (95 mg, ,35 mmol) of light brown oil (R_f = 0.77). ^1H NMR (300 MHz, $CDCl_3$): δ 1.45 (2H, d, J = 6.6 Hz); 1.54 (2H, d, J = 6.9 Hz); 1.95-2.10 (3H, m); 2.61 (1H, dd, J = 8.4, 22.8 Hz); 3.18 (1H, m); 3.43 (2H, d, J = 7.2 Hz); 3.62 (1H, s); 3.71 (3H, s); 3.74 (3H, s); 4.18 (3H, t, J = 8.4 Hz); 4.56 (4H, t, J = 7.8 Hz); 5.10 (1H, s); 5.73 (1H, m). HRMS (FAB+): Calc. for $C_{13}H_{19}NO_5$: 270.1328, Found: 270.13359 (M^+).



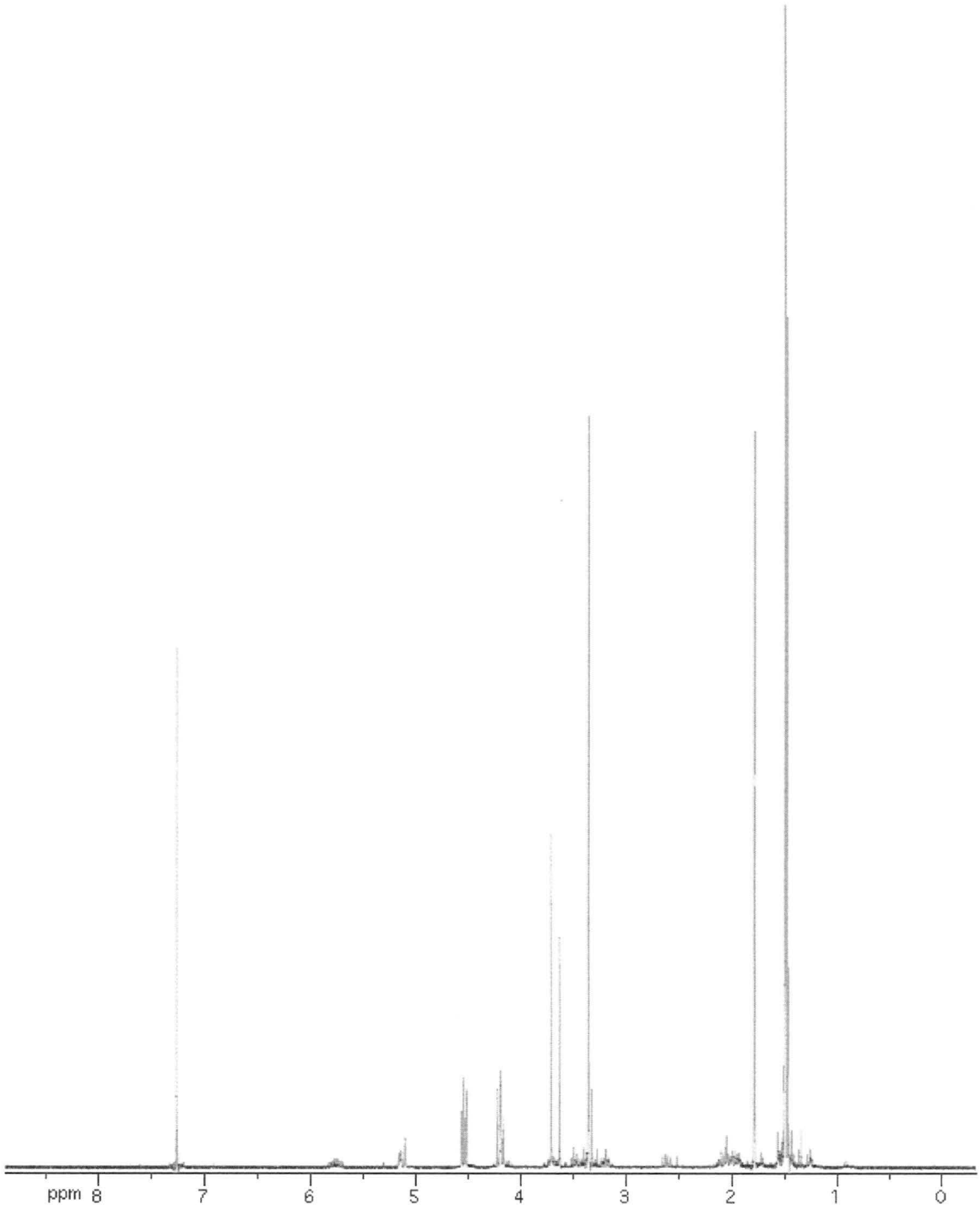
167



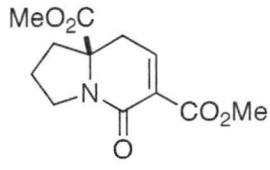
tBu Ester Allyl Proline (168): tBu ester (100 mg, .624 mmol, 1 equiv.) was taken up in DCM (20 mL) and cooled to 0 °C. To this stirring solution was added BOP-Cl (190 mg, .75 mmol, 1.2 equiv.) and i-Pr_2NEt (.14 mL, .75 mmol, 1.2 equiv.). This mixture was stirred for 1o minutes and then the allyl proline (128 mg, .624 mmol, 1 equiv.) was added. The reaction was warmed to room temperature and stirred an additional 18 hrs. The reaction was then diluted with DCM and quenched with NH_4Cl. The organic layers were extracted and combined, dried ($MgSO_4$) and conc. Only one spot was present by TLC (EtOAc) and the product was run through a short silica gel column to yield 95 % (184 mg, .591 mmol) of the tBu ester allyl proline **168** (R_f = 0.72). ^1H NMR (300 MHz, $CDCl_3$): δ 1.47 (9H, s); 1.78 (6H, s); 1.92 (4H, m); 1.96 (2H, m); 2.05 (4H, d, J = 1.5 Hz); 2.61 (1H, dd, J = 5.1, 15.7 Hz); 3.35 (3H, s); 3.48 (1H, m); 3.62 (2H, s); 3.71 (2H, s); 4.19 (4H, t, J = 6.6 Hz); 4.53 (4H, t, J = 8.1 Hz); 5.14 (1H, m); 5.76 (1H, m).



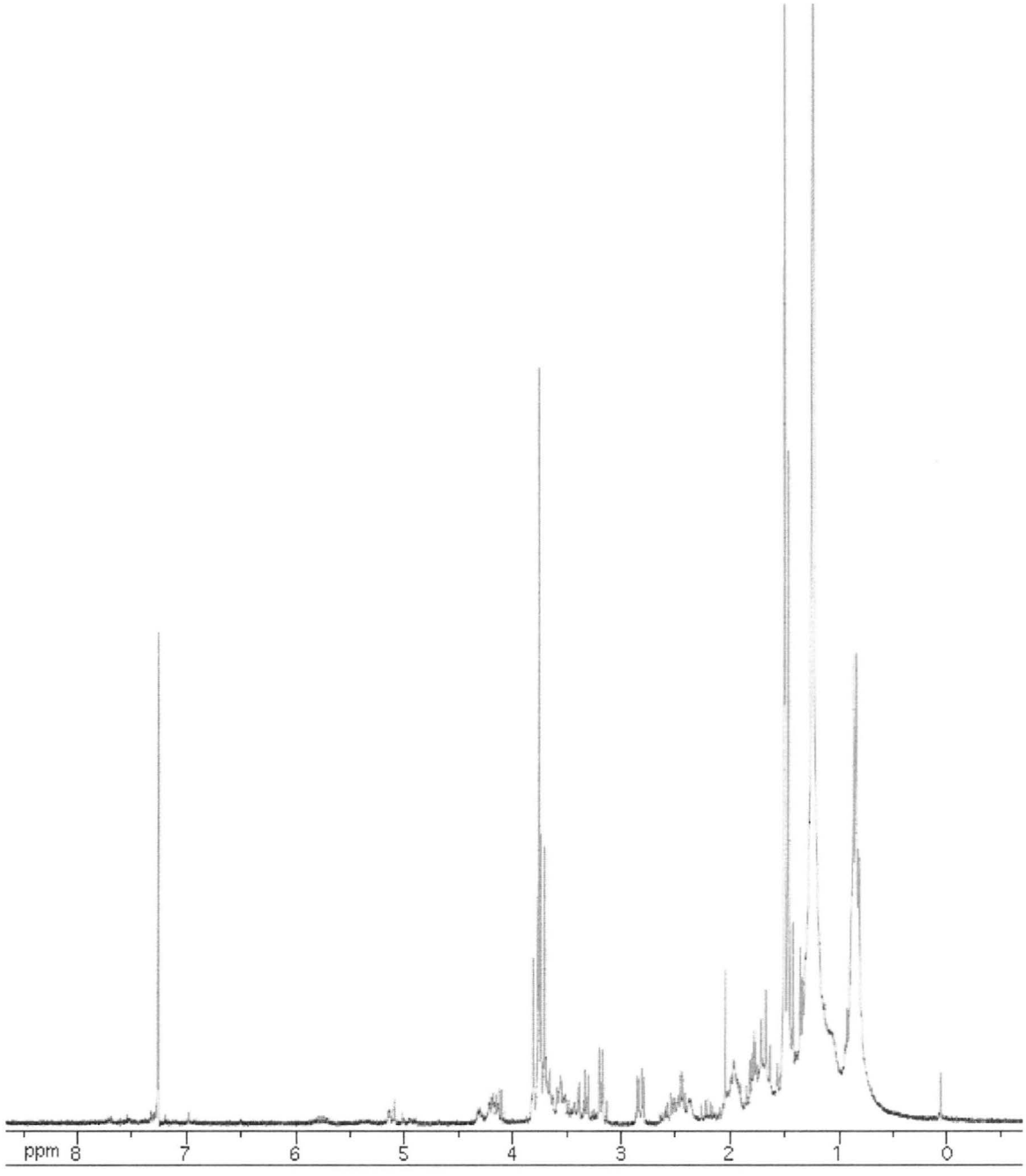
168



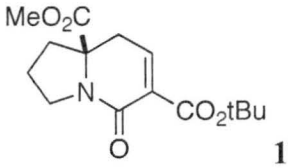
Methyl Ester Dieneophile (169): To a stirred solution of methyl ester allyl proline (30 mg, .11 mmol, 1 equiv.) and $RuCl_3$ (.80 mg, 3.5 mol%) in acetonitrile and water (6:1, 12 mL) was added $NaIO_4$ (47 mg, .22 mmol, 2 equiv.) in portions over 5 minutes. The reaction was allowed to stir for 2.5 hrs. The reaction was then quenched with $Na_2S_2O_3$ and the layers were separated. The organic layers were extracted with EtOAc (3 x 10 mL), washed with water then brine, dried ($MgSO_4$) and conc. It was determined from the crude ^1H NMR that only the aldehyde was forming and no cyclization was taking place. Taking the crude aldehyde (25 mg, .098 mmol, 1equiv.) up in THF (5 mL) at room temperature and adding NaH (2.3 mg, .098 mmol, 1equiv.), this mixture was stirred for 1 hr. The reaction was then diluted with EtOAc and washed with water dried ($MgSO_4$) and conc. Unfortunately upon purification only a very small amount of the desired product **169** could be obtained (<2 mg). ^1H NMR (300 MHz, $CDCl_3$): δ 0.87 (2H, d, J = 7.8 Hz); 1.245 (3H, s); 1.47 (2H, d, J = 6.6 Hz); 1.54 (2H, d, J = 6.9 Hz); 1.95-2.10 (3H, m); 2.61 (1H, dd, J = 8.4, 22.8 Hz); 3.18 (1H, m); 3.43 (2H, d, J = 7.2 Hz); 3.62 (1H, s); 3.71 (3H, s); 3.74 (3H, s); 4.18 (3H, t, J = 8.4 Hz).



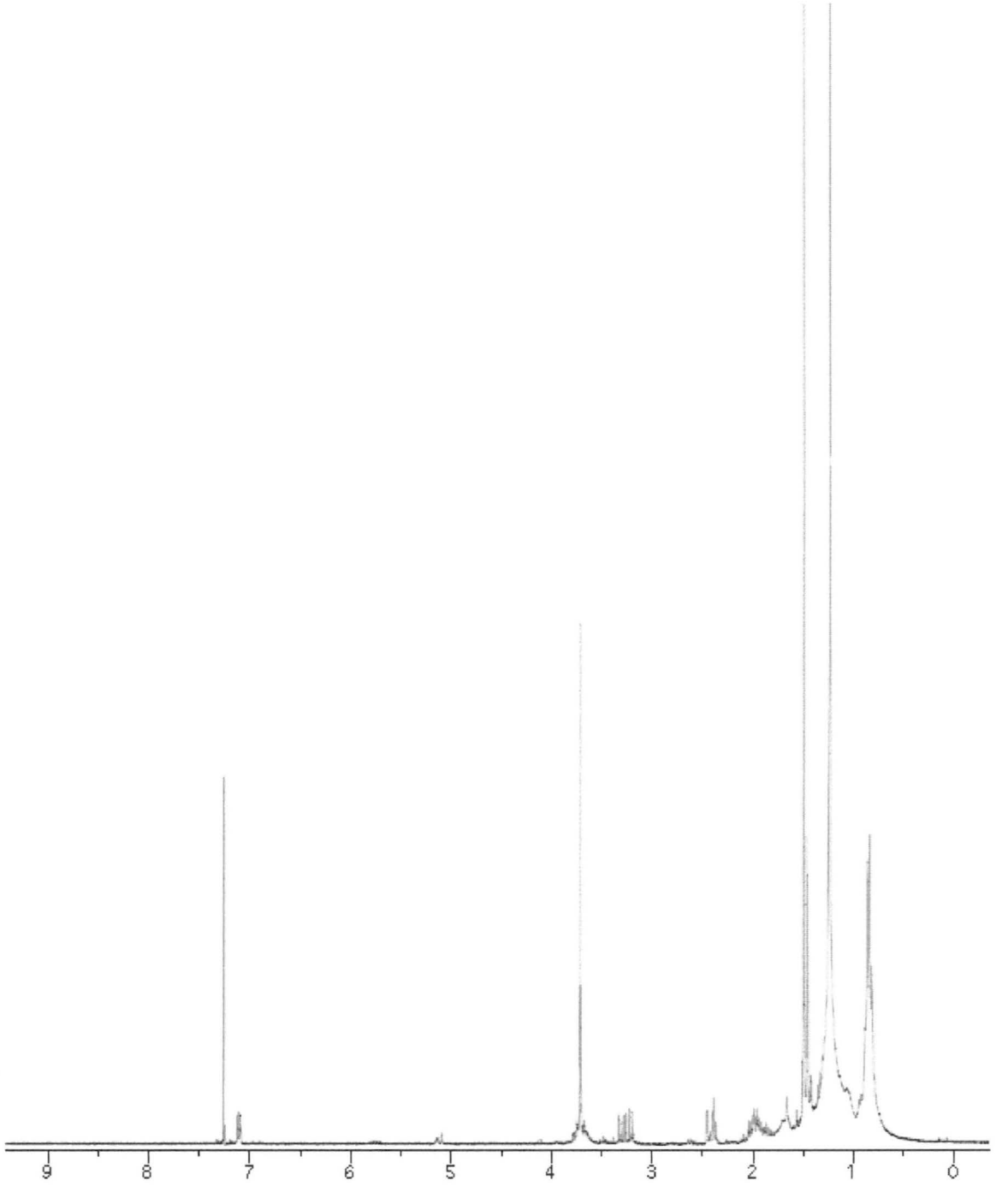
169



tBu Ester Dieneophile (170): To a stirred solution of tBu ester allyl proline (50 mg, .16 mmol, 1 equiv.) and $RuCl_3$ (1.1 mg, 3.5 mol%) in acetonitrile and water (6:1, 12 mL) was added $NaIO_4$ (68 mg, .32 mmol, 2 equiv.) in portions over 5 minutes. The reaction was allowed to stir for 2.5 hrs. The reaction was then quenched with $Na_2S_2O_3$ and the layers were separated. The organic layers were extracted with EtOAc (3 x 10 mL), washed with water then brine, dried ($MgSO_4$) and conc. It was determined from the crude ^1H NMR and TLC (5:1 DCM/MeOH) (R_f = 0.79) that only the aldehyde was forming. ^1H NMR (300 MHz, $CDCl_3$): δ 1.44 (9H, s); 1.71 (6H, s); 1.92 (4H, m); 1.96 (2H, m); 2.05 (4H, d, J = 1.5 Hz); 2.61 (1H, dd, J = 5.1, 15.7 Hz); 3.35 (3H, s); 3.48 (1H, m); 3.62 (2H, s); 3.71 (2H, s); 4.11 (4H, t, J = 6.6 Hz); 9.77 (1H, s). The aldehyde (25 mg, .08 mmol, 1equiv.) was immediately taken up in THF (5 mL) at room temperature to which NaH (2 mg, .08 mmol, 1 equiv.) was added. The reaction was stirred for 1 hr. then diluted with EtOAc and washed with water. The organic layer was separated and dried ($MgSO_4$) and conc. The crude product was purified by column chromatography on silica gel (EtOAc) to yield 87% (21 mg, .071 mmol) of **170** as a colorless oil (R_f = 0.26). ^1H NMR (300 MHz, $CDCl_3$): δ 0.87 (2H, m); 1.50 (9H, s); 1.71 (2H, s); 1.94 (4H, m); 1.96 (2H, m); 2.39 (2H, m); 2.45 (4H, d, J = 2.1 Hz); 3.304 (2H, m); 3.19 (1H, dd, J = 5.1, 15.7 Hz); 3.35 (3H, s); 3.71 (3H, s); 7.16 (1H, t, J = 2.1 Hz).

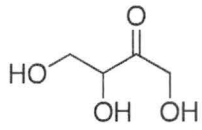


170

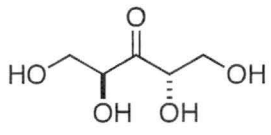


5.3 Chemical Synthesis Experiments for SB-219383 Research

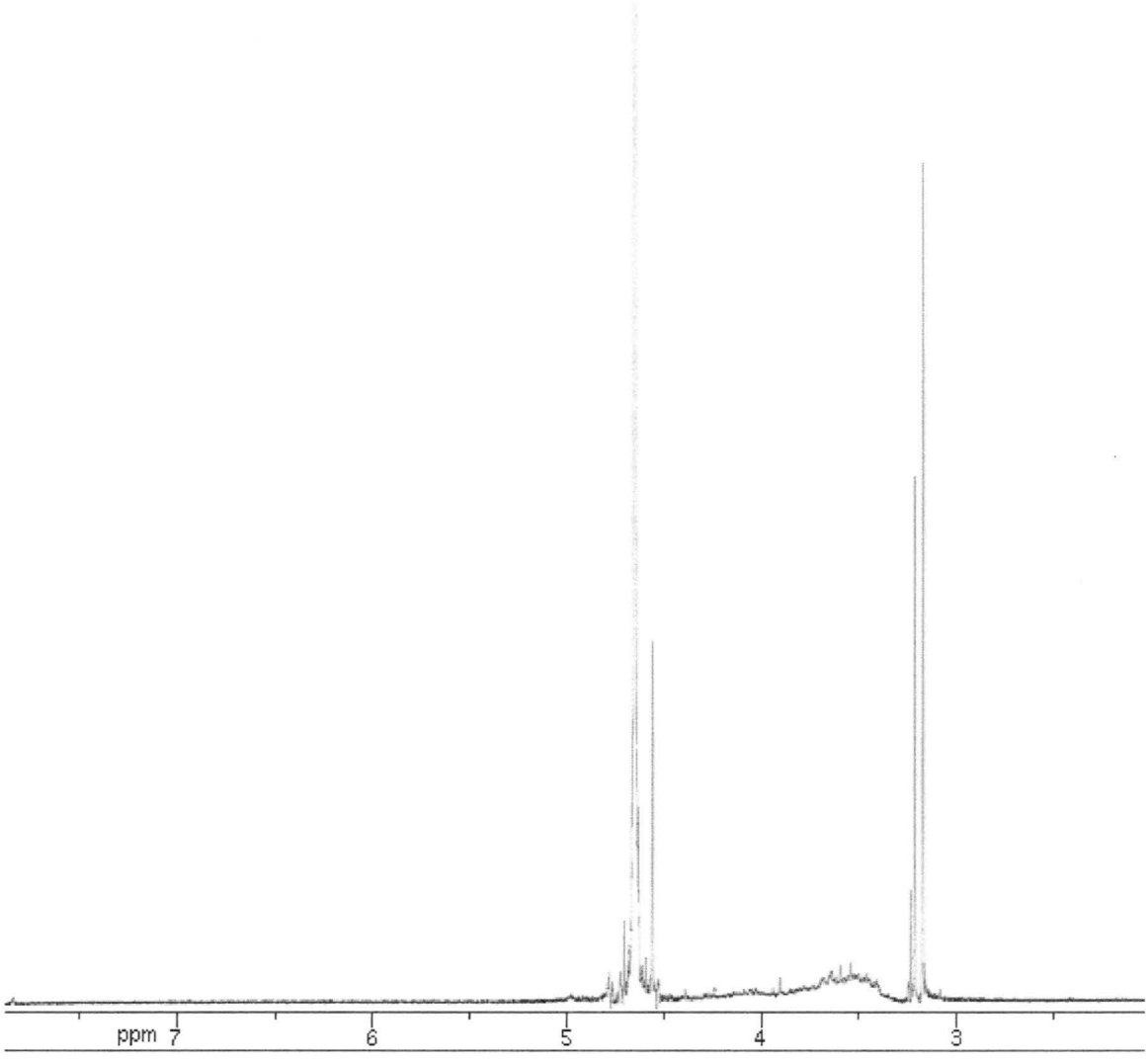
Glycero-tetrulose. Dihydroxyacetone (270 mg, 3.0 mmol, 1 equiv) was dissolved in water (50 ml) at 0°C under an argon atmosphere. A formaldehyde solution (37% wt. in water, .247 mL, 9.0mmol, 3 equiv.) was added to the reaction followed by sodium hydroxide (20 mg, 0.50 mmol). The mixture was stirred at 0°C for 2 hours. The reaction was then acidified with HCl and diluted with ether. The layers were separated and the aqueous layer was extracted with ether (3 x 25 mL). The combined aqueous layer was concentrated on a rotovap at 35 °C yielding 75% (270 mg, 13.4 mmol) of a sweet smelling gray flaky solid **9**. ^{1}H NMR (300 MHz, D2O): δ 3.8 (2H, s); 4.17 (2H, m); 4.69 (2H, s); 4.78 (1H, s); 5.56 (1H, s). IR (NaCl) 3000-3700, 1755 cm^{-1}.



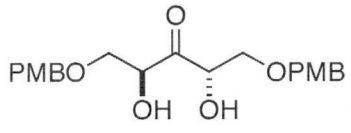
Threo-3-Pentulose (184). Glycero-tetrulose **(9)** (360 mg, 3.0 mmol, 1 equiv.) was dissolved in MeOH (10 mL) under argon at 0°C. A formaldehyde solution (37% wt. in water, 0.9 mL, 33 mmol, 11 equiv.) was added, and then a .4M solution of KOH in MeOH was added followed by $CaCl_2$ (166 mg, 1.5 mmol, .5 equiv). The reaction stirred at 0°C for 2 hours, after which the reaction was acidified with HCl then diluted with ether. Upon adding ether a precipitate formed, this was filtered off using gravity filtration. The remaining liquid was concentrated on a rotovap, EtOH was added to further wash. This yielded a sweet smelling brown syrup. It was determined that both threo-3 and erythro-3 pentulose were formed (threo:erythro,90:10), the desired threo-3 had an R_f 0.38 compared to 0.46 of the undesired erythro-3. The two were separated by preparative tlc using a gradient of 1-butanol/pyradine/water (6:4:3) to obtain 65% (300mg, 1.99 mmol) of pure threo-3-pentulose **184** as a light brown syrup. ^{1}H NMR (300 MHz, D2O): δ 3.8 (2H, dd, J =3.1, 11.6 Hz); 3.21 (2H, dd J = 4.7, 11.6 Hz); 4.58 (1H, t, J = 4.4 Hz); 4.8 (2H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 64.8, 77.9, 213. IR (NaCl) 3000-3700, 1720 cm^{-1}.



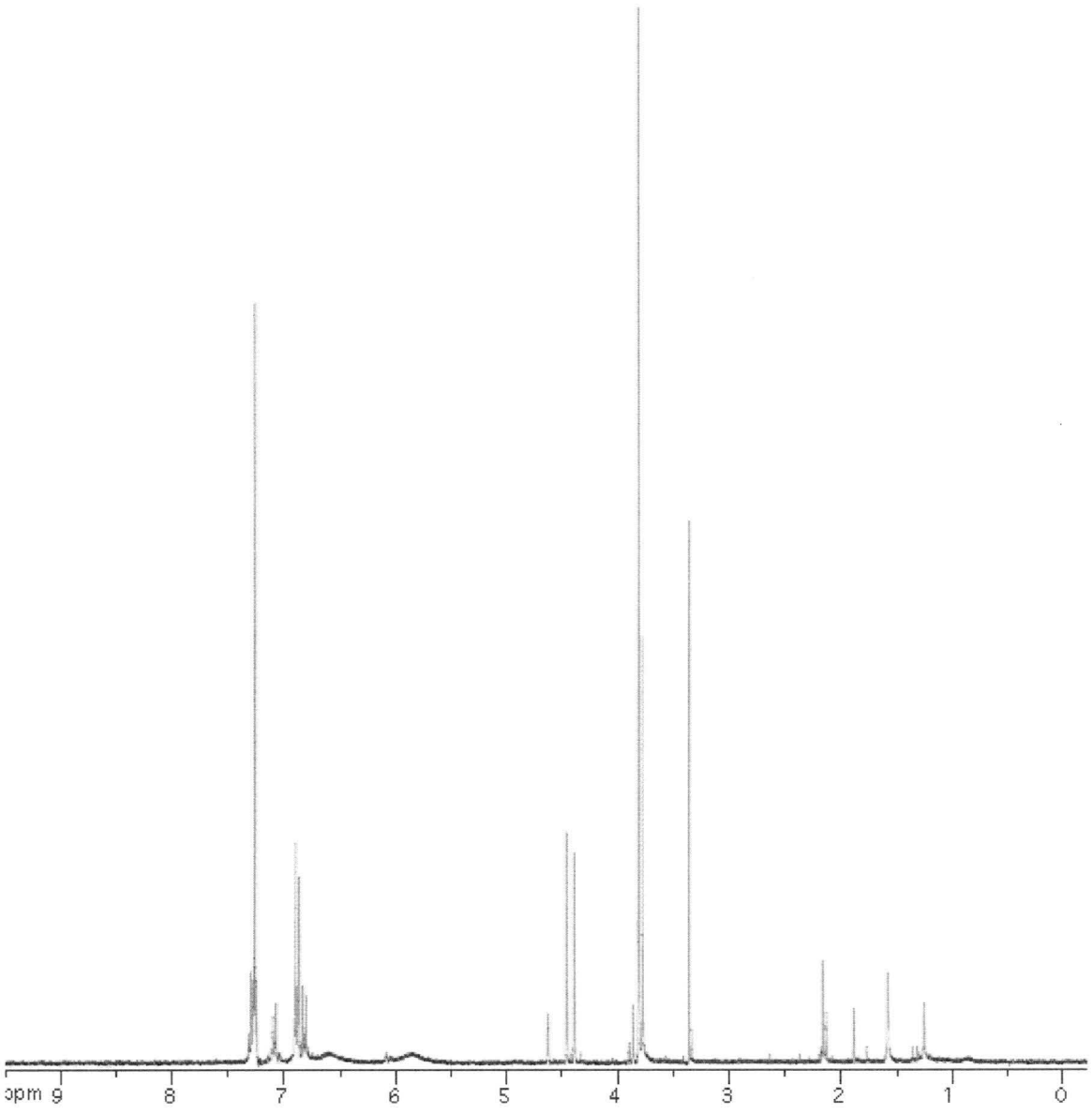
184



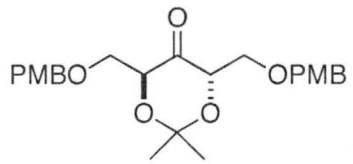
PMB Protected Ketone (184). Sodium hydride (58 mg, 2.4 mmol, 0.1 equiv.) was washed 3 times with hexanes in a 50 mL round bottom flask under argon. Ether (2 mL) was added via syringe, then a solution of 4-methoxybenzyl alcohol (3.324 g, 24 mmol, 1.1 equiv.) in ether (3 mL) was added to the NaH solution via cannula. The mixture was stirred for 30 min. and was then cooled to 0°C, trichloroacetonitrile (2.18 mL, 21.8 mmol, 1equiv.) was added dropwise. This mmixture was stirred at 0°C for 2.5 hours, after which the mixture was concentrated on the rotovap, the reaction was then diluted with pentane. A solution of MeOH (.1 mL) in pentane was added and stirred vigorously. This was filtered through celite then concentrated to afford a yellow liquid. This liquid is used immediately adding DCM (25 mL) and cyclohexane (25 mL). Threo-3-pentulose (2.76 g, 18.4 mmol, 1 equiv.) is added followed by triflic acid (.44 mL, 5.52 mmol, .3 equiv.) and the reaction is stirred for 22 hours. The reaction is then quenched with $NaHCO_3$ and diluted with DCM. The layers are separated and the organic layer is extracted with DCM (3 x 20 mL), the combined organic layers are dried with $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography on silica gel (3:1 Hexanes/EtOAc) to yield 92% (6.18 g, 16.9mmol) of **184** as a white paste (R_f = 2.9). ^1H NMR (300 MHz, $CDCl_3$): δ 1.56 (1H, s); 2.15 (2H, m); 3.45 (3H, s); 3.83 (6H, s); 3.94 (2H, d, J = 9.3 Hz); 4.48 (2H, d, J = 21.2 Hz); 4.56 (2H, s); 6.88-7.25 (4H, m). IR (NaCl) 3400-3350, 1691, 1612, 1513, 1381, 1247, 1109, 823, 753, 649 cm^{-1}.



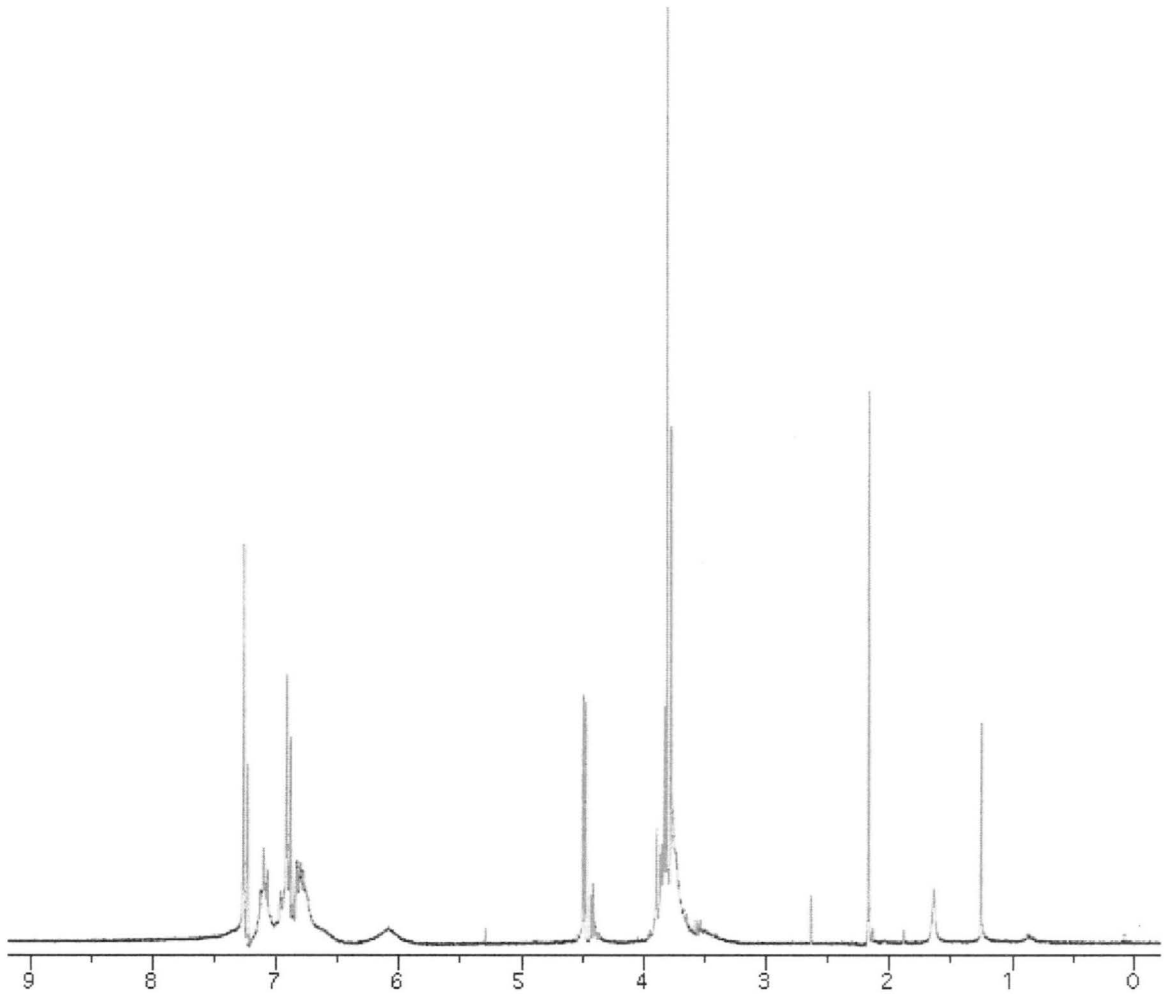
184



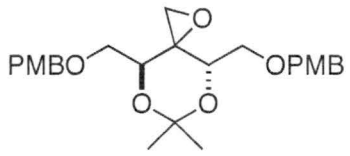
PMB and Acetonide Protected Ketone (190). **184** (100 mg, .27 mmol, 1 equiv.) is dissolved in acetone (50 mL) at room temperature under argon. P-toluenesulfonic acid (25 mg, .135 mmol, .5 equiv.) is added followed by 2,2-dimethyl propane (.40 mL, .297 mmol, 1.1 equiv.) and the reaction is allowed to stir for 3 days. The reaction is then quenched with $NaHCO_3$ and diluted with DCM. The layers were separated and the organic layer was extracted with DCM (3 x 50 mL). The combined organic layers were dried with $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel (3:1 Hex/EtOAc) to yield 75% (81 mg, .20 mmol) of the ketone **190** as an orange oil (R_f = 0.34). This reaction was tried without the addition of the 2,2-dimethyl propane to no success. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (1H, s); 2.17 (6H, s); 3.57 (6H, s); 4.38 (2H, dd, J = 12.7, 3.9 Hz); 4.5 (2H, dd, J = 10.2, 2.8 Hz); 6.15 (1H, s br); 6.89-7.25 (4H, m). IR (NaCl) 3463, 2984, 2908, 1732, 1447, 1373, 1245, 1044, 938, 847, 634, 607cm^{-1}. LRMS (FAB+) Calc. for $C_{24}H_{30}O_7$: 430. 19915, Found: 430.18984 (M^+, 100%), 431.1994 (MH^+, 20%).



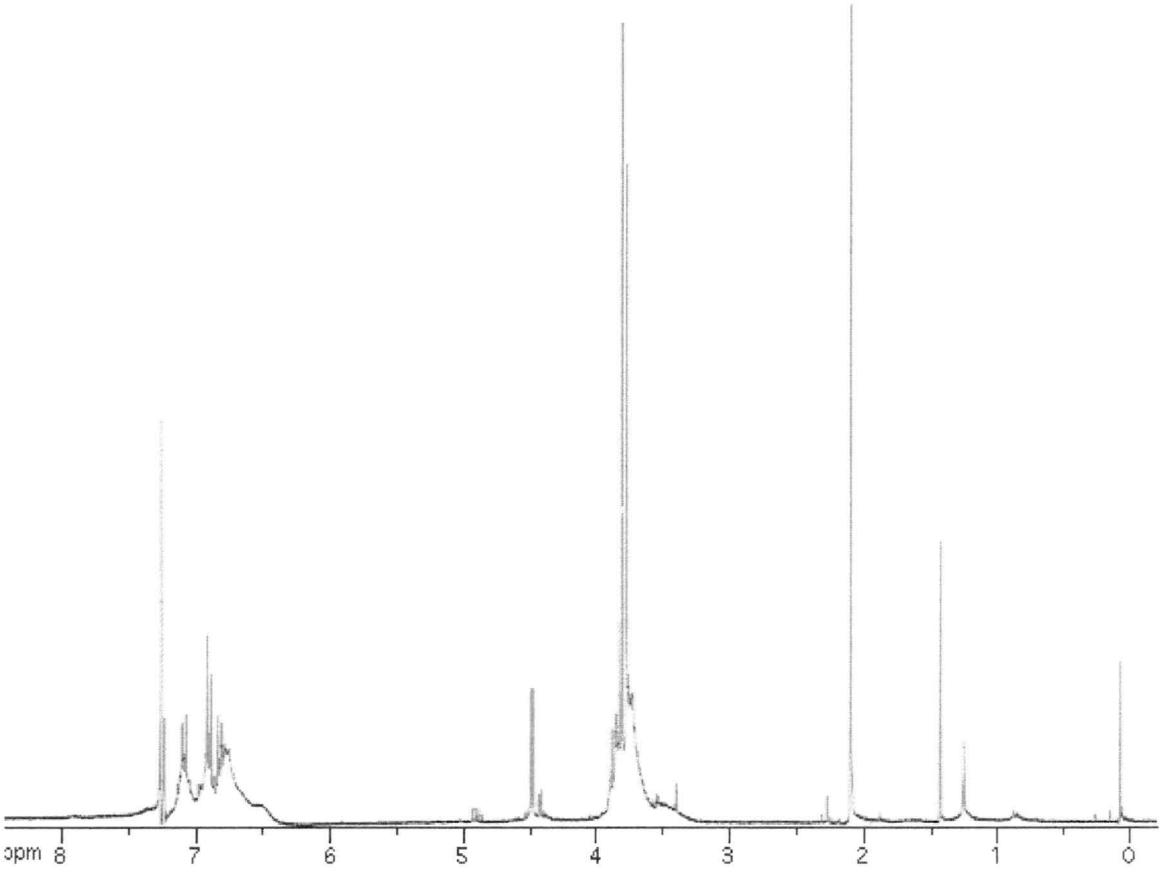
190



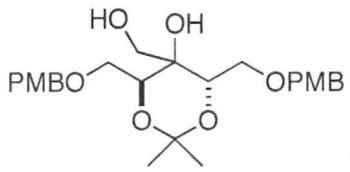
Epoxide (191). NaH (5 mg, .205 mmol, 1.5 eq.) and trimethyl sulfoxonium iodide (45 mg, .205 mmol, 1.5 eq.) were dissolved in DMSO (5 mL) at room temperature under argon. The mixture was stirred until gas evolution was stopped (roughly 7 minutes). This mixture was then added dropwise via syringe to a stirring solution of ketone **190** (50 mg, .137 mmol, 1 eq.) in a (1:1) mixture of THF/DMSO (10mL) at 0°C under argon. The reaction was stirred for 1.5 hours then warmed to room temperature. The reaction was stirred at room temperature for and additional 45 minutes. The reaction was quenched with Ammonium Chloride and extracted with ether (3 x 15 mL). The combined organic layers were washed with water then dried (MgSO4) and concentrated by rotovap. The product was purified by column chromatography on silica gel (4:1) hexanes/ethyl acetate to afford 85% (42.5 mg, .095 mmol) of the epoxide **191** as a clear oil (R_f =0.35). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (6H, s); 2.14 (1H, s); 3.83 (6H, s); 4.41 (2H, dd J = 5.1 Hz); 4.5 (2H, d, J = 14.5 Hz); 4.89 (1H, t, J = 5.23 Hz); 6.89-7.25 (8H, m). ^{13}C NMR (100MHz,$CDCl_3$):δ 26.82, 47.45, 55.89, 64.9, 69.6, 73.33, 77.43, 114.12, 116.34, 129.33, 159.22. HRMS (FAB+): Calc. for $C_{25}H_{32}O_7$: 454.2148, Found: 445.21671 (MH^+)



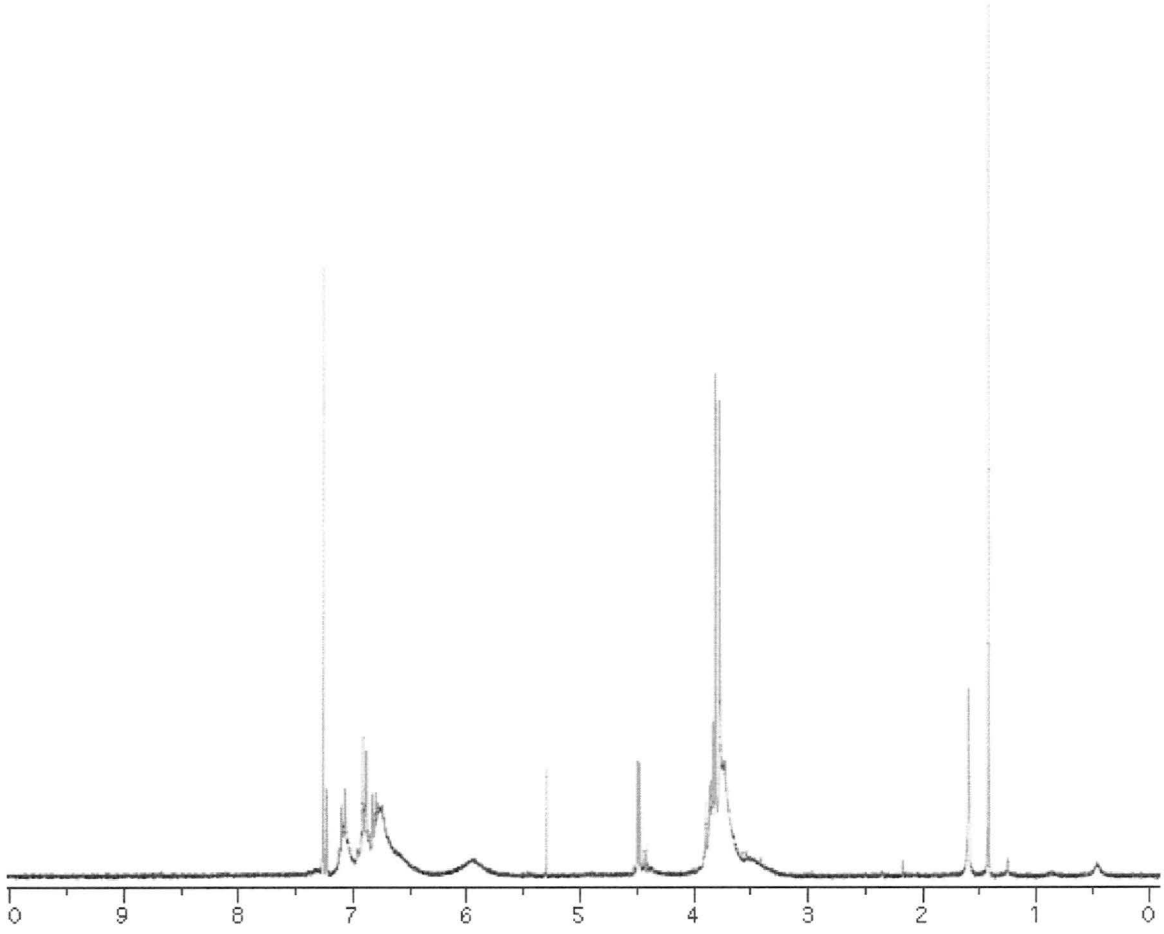
191



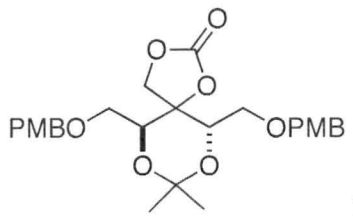
Diol (192). NaOH (4.3 mg, .106 mmol, 2 eq.) and water (5 mL) were added to epoxide **191** (25mg, 0.0543 mmol, 1 eq.) at room temperature under argon. The mixture was stirred for 30 minutes then quenched with dichloromethane, and the organic layer was extracted (3 x 10 mL), washed with water and brine respectfully, and dried with Na_2SO_4, then conc. to yield 72% (18 mg, .039 mmol) **192** as a cloudy oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (6H, s); 3.83 (6H, s); 4.41 (2H, dd, J = 12.3, 3.2 Hz); 4.49 (2H, d, J = 9.3 Hz); 5.3 (1H, s); 6.2 (1H, s br); 6.89-7.25 (8H, m). IR (NaCl) 3200, 1365, 1238, 1144, 803, 756, 669 cm^{-1}. HRMS (FAB+): Calc. for $C_{25}H_{34}O_8$: 462.22537, Found: 462.23010 (M^+), 463.22596 (MH^+).



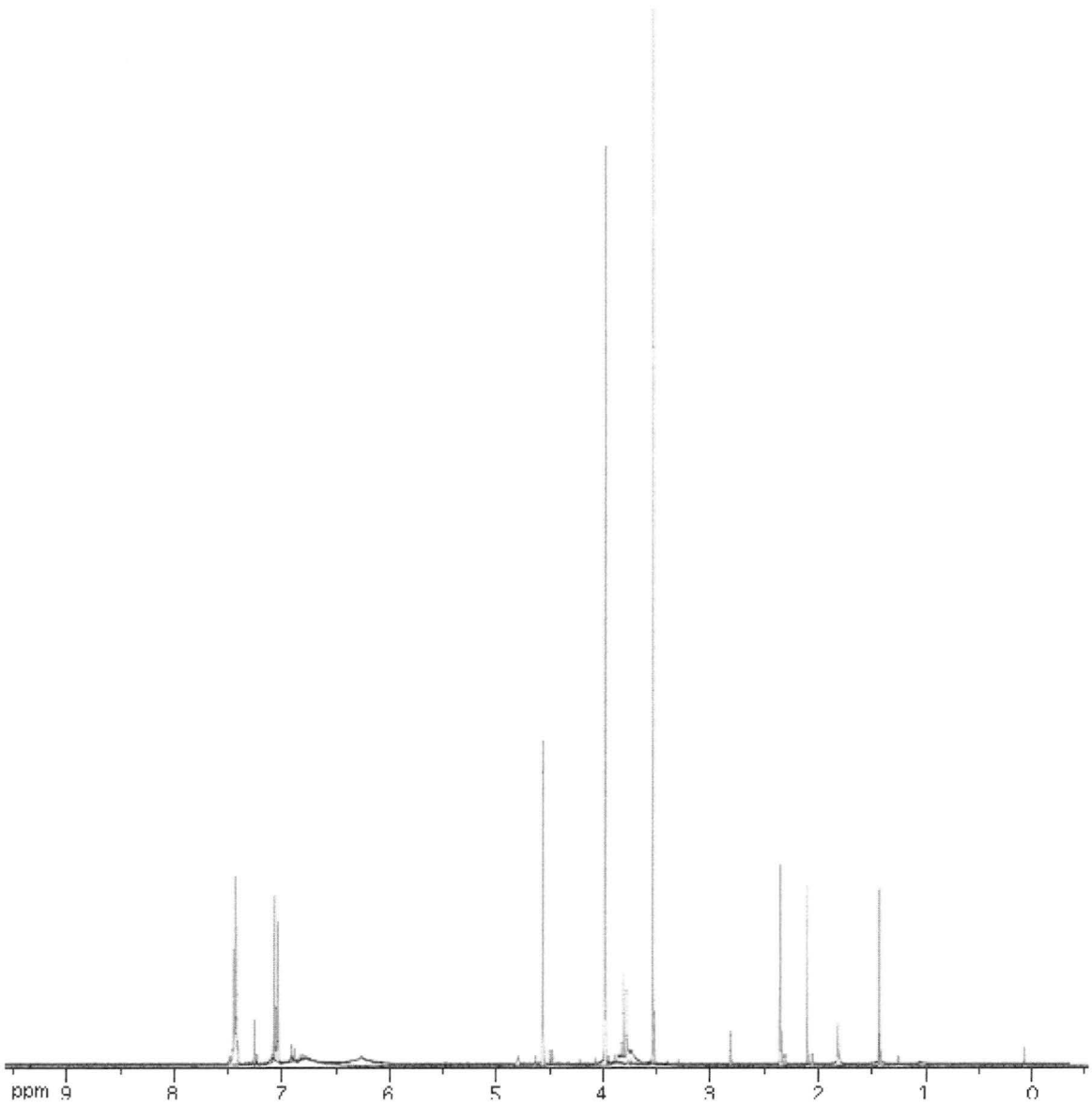
192



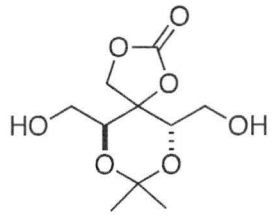
Cyclic Carbonate (193). A solution of triphosgene (5.5 mg, .02875 mmol, 0.5 eq.) in dichloromethane (2mL) was added to a cooled (-70°C) solution of **192** (25 mg, .0575 mmol, 1 eq.) and pyridine (.03mL, .345 mmol, 6 eq.) in dichloromethane (5mL) under argon. The reaction was allowed to warm to room temperature on its own accord. After warming the reaction was quenched with ammonium chloride. The organic layers were extracted with dichloromethane (3 x 25 mL), and the combined organic layers were washed with HCl, NaHCO3, then brine, dried (Na2SO4) then flitered and concentrated via rotovap. The product was purified by flash column chromatography on silica gel using (3:1) Hexanes/EtoAc as the gradient, to yield 60% (15 mg, .0325 mmol) of **193** as a yellow residue (R_f = 0.58). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (6H, s); 2.27 (6H, s); 3.51 (6H, s); 3.75 (2H, d, J = 12.5 Hz); 4.43 (2H, dd, J = 17.2, 9.4 Hz); 4.05 (1H, s); 4.6 (1H, s); 6.89-7.25 (8H, m). IR (NaCl) 3145, 1625, 1384, 1325, 1278, 1185, 1124, 813, 736, 669 cm^{-1}. HRMS (FAB+): Calc. for $C_{26}H_{32}O_9$: 489.20463, Found: 489.21331 (MH^+).



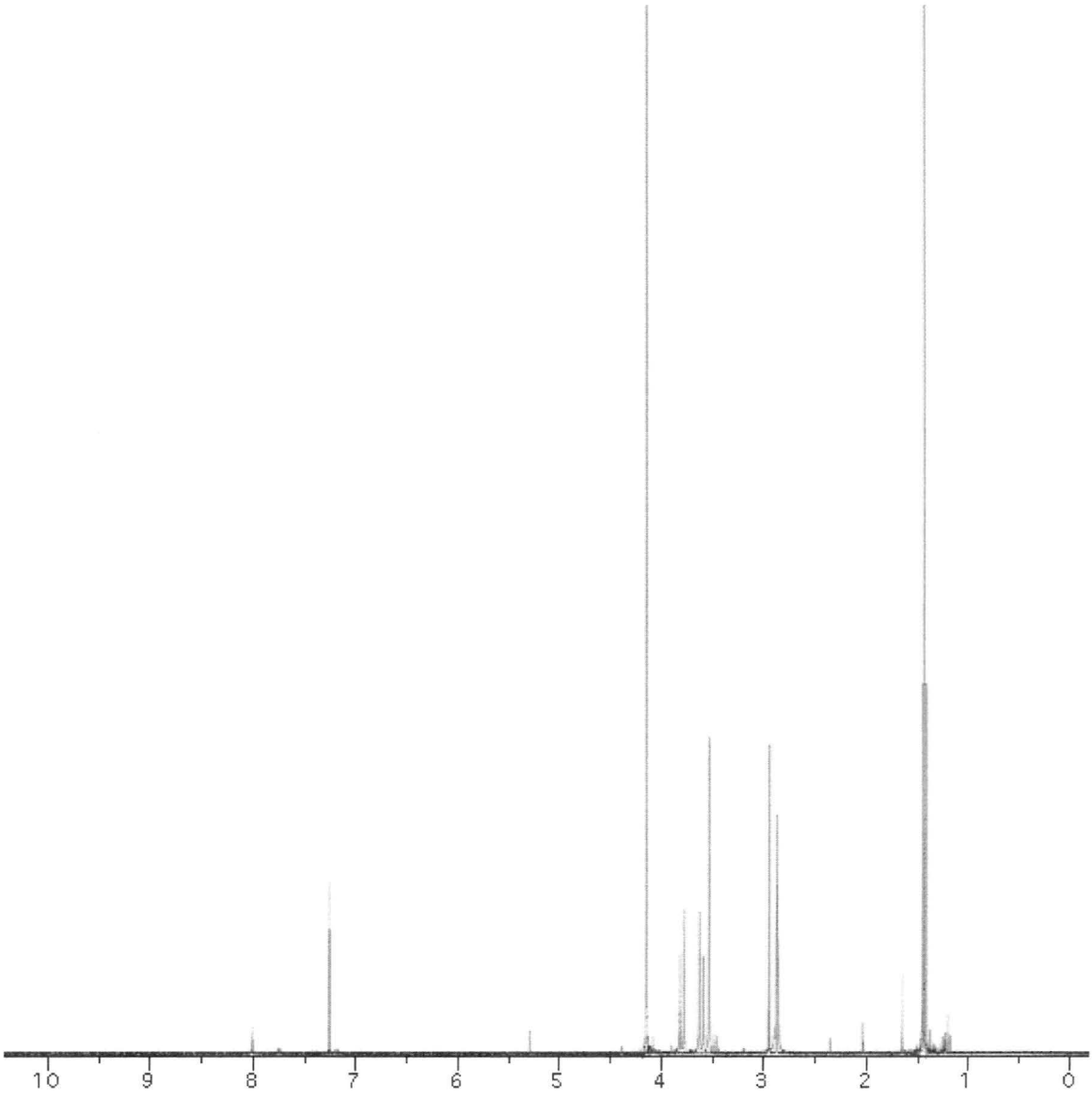
193



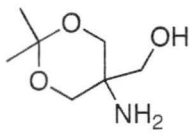
Primary Diol (194). 193 (10mg, .0217 mmol, 1 eq.) was taken up in a 10:1 solution of dichloromethane/pH 7 buffer (10 mL) under argon and cooled to 0°C. DDQ (24 mg, .108 mmol, 5 equiv.) was added and the reaction warmed to room temperature. It was found that the pH 7 buffer was the key reagent to remove the PMB group. The reaction was then quenched with NaHCO3 and the organic layers were extracted with dichloromethane (3 x 10 mL). The organic layers were dried (MgSO4) filtered and concentrated via rotovap. The crude product was purified by column chromatography on silica gel (3:1) Hexanes/EtOAc. This yielded 91% (6.1 mg, .0246 mmol) of a brown residue **194** (R_f = 0.53) ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48 (6H, s); 2.95 (2H, d, J = 4.25 Hz); 3.55 (2H, t, J = 6.23 Hz); 3.85 (2H, d, J = 4.88 Hz); 4.17 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.83, 57.22, 60.23, 82.66, 85.41, 116.32, 154.09. IR (NaCl) 3300-3055, 1627, 1378, 1245, 1059, 603. HRMS (FAB+): Calc. for $C_{10}H_{16}O_7$: 248.0896, Found: 248.0123 (M^+), 249.07539 (MH^+).



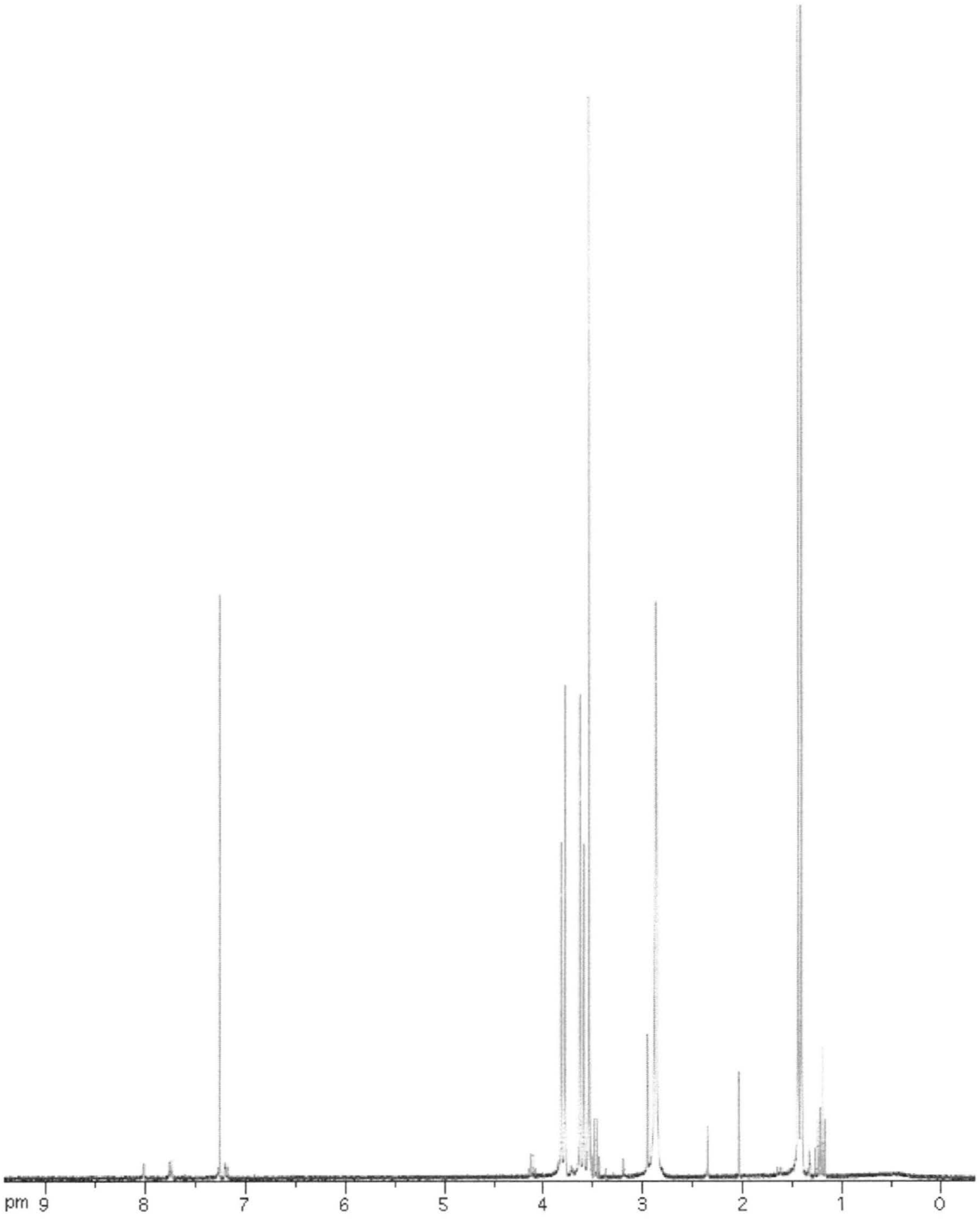
194



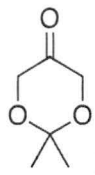
Amine (201). 2,2-dimethyl propane (18.64 mL, .152 mol, 1.2 equiv), pTsOH (1.21 g, 6.35 mmol, 0.05 equiv.) were added to a stirring solution of trizma base (20 g, .127 mol, 1 equiv.) in dry DMF (140 mL) at 40 °C. The mixture was allowed to stir for 3 days. The reaction was then quenched with NEt_3 (1 mL) and stirred for 10 minutes. The DMF was then concentrated via rotovap equipped with a high vac. NEt_3 (14 mL) and EtOAc (500 mL) were added, a white precipitate formed and was filtered off. The remaining liquid was evaporated off via rotovap, the flask was then placed under vacuum to insue all the DMF was gone. Eventually under vacuum a white solid was formed and was washed with ether several times. **201** proved to be clean by NMR and yielded 51% (10.35 g, 64.2 mmol). This material is stable to leave on the bench for an extended period of time. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.447 (6H, s); 2.87 (2H, s); 3.54 (2H, d, J = 12 Hz); 3.6 (2H, d, J = 11.5 Hz); 3.78 (2H, d, J = 12 Hz).



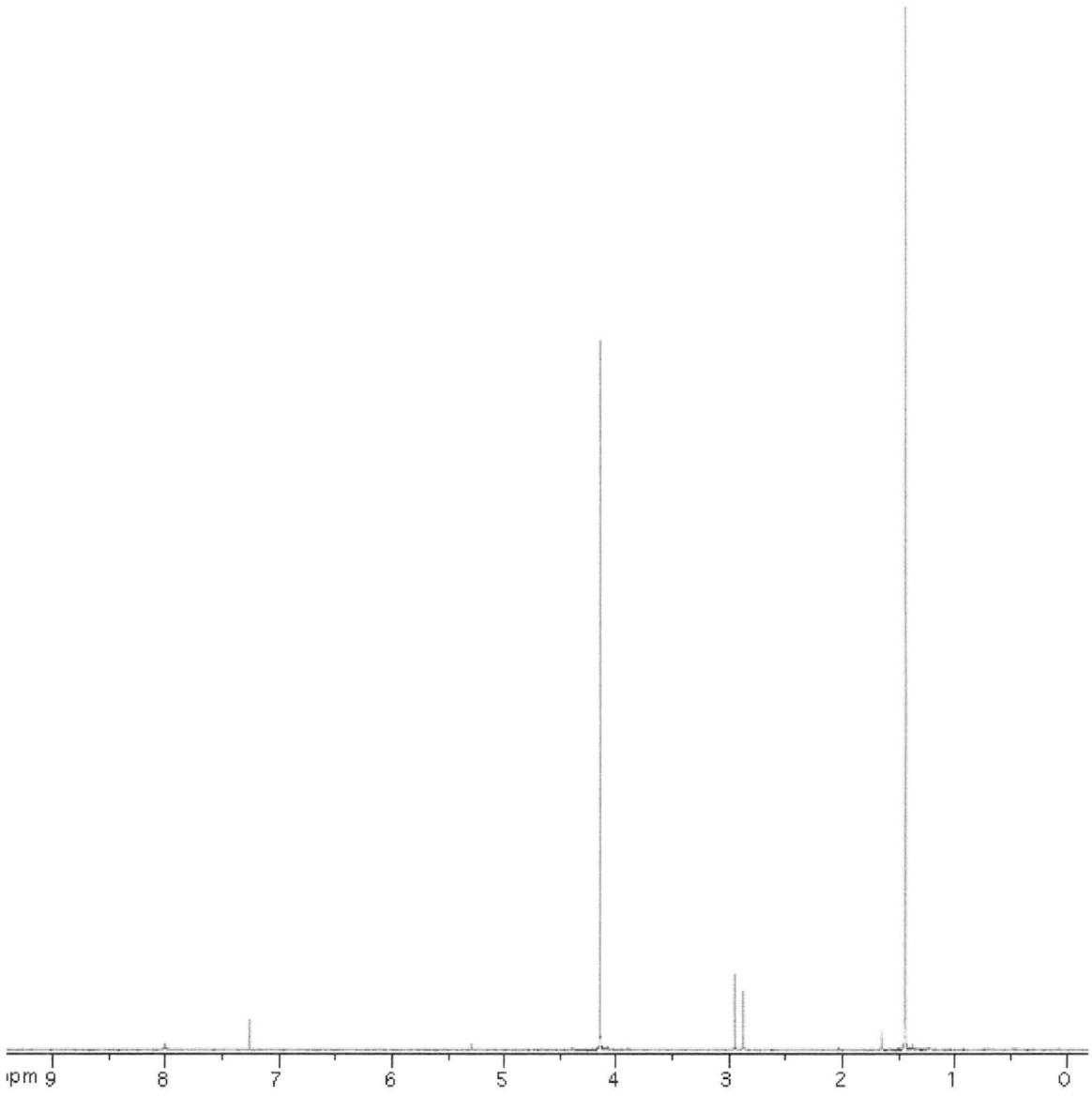
201



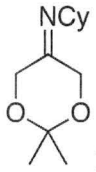
Ketone (202). NaIO4 (1.33g, 6.2 mmol, 1 equiv) in cold water (15mL) was added dropwise over 3 hours to a cooled solution (0 °C) of amine **201** (1g, 6.2mmol, 1equiv) and KH_2PO_4 (844mg, 6.2mmol, 1 equiv) in water (20mL) under Ar. Upon completion of the addition, the reaction was allowed to warm to room temperature overnight. The reaction was then separated with CH_2Cl_2 and the organic layer extracted with CH_2Cl_2 (4 x 15 mL). The combined organic layers were dried ($MgSO_4$) filtered and concentrated. The resulting yellow liquid was purified by distillation under reduced pressure using a short path distillation apparatus to yield 70% (4.34 mmol, 565 mg) of **202** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (6H, s), 2.95 (2H, d, J = 22.5 Hz); 4.13 (4H, s).



202

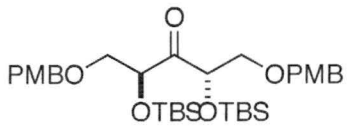


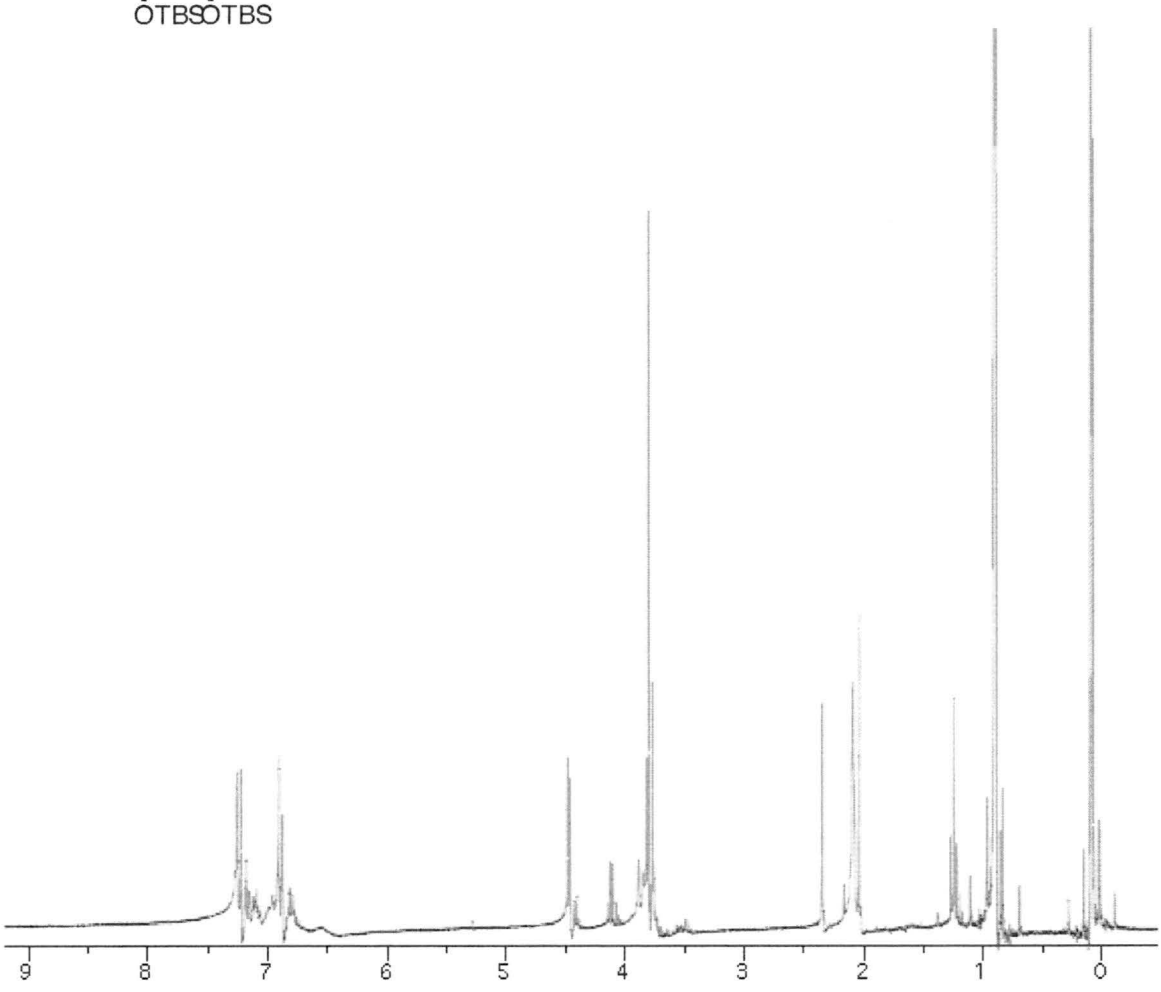
Cyclohexyl Imine (203): To a solution of ketone **202** (1g, 7.7mmol, 1 equiv) in benzene (40mL) at room temperature was added Mol. Sieves (1.3g, 4A) and cyclohexylamine (1.76mL, 15.4mmol, 2 equiv) via syringe. This mixture was allowed to stir overnight. The reaction was then filtered and concentrated to yield a brown slurry **203** (1.46g, 89.7%). No further purification was needed. It was necessary to convert to the imine due to the readily polymerization of the ketone. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (6H, s), 1.23-1.8 (2H, m), 3.14 (1H, m); 4.38 (4H, d, J = 51.9 Hz).



203

PMB and TBS protected ketone: Imidazole (150 mg, 2.2 mmol, 4 equiv.) was dissolved in DCM aunder argon and cooled to 0°C. **184** (200 mg, .55 mmol, 1 equiv.) was added and stirred for 5 min. TBSCl (330 mg 2.2 mmol, 4 equiv.) was added and the reaction was slowly warmed to room temperature. The reaction was concentrated and then the crude product was purified by column chromatography on silica gel (3:1 Hex/EtOAc) to yield 35% (112 mg, .189 mmol) of a yellow oil (R_f= 0.21). It should be noted that this compound decomposes if left in fridge for an extended period. ^{1}H NMR (300 MHz, $CDCl_3$): δ .21 (12H, s); .96 (18H, s); 2.13 (2H, d, J = 18.4 Hz); 3.83 (6H, s); 4.15 (2H, dd, J = 13.5, 4.1 Hz); 4.5 (2H, dd, J = 11.2, 4,3 Hz); 6.89-7.25 (4H, m). IR (NaCl) 1691, 1625, 1601, 1513, 1381, 1247, 1109, 823, 753, 649 cm^{-1}.


O
PMBO
OPMB
OTBS
OTBS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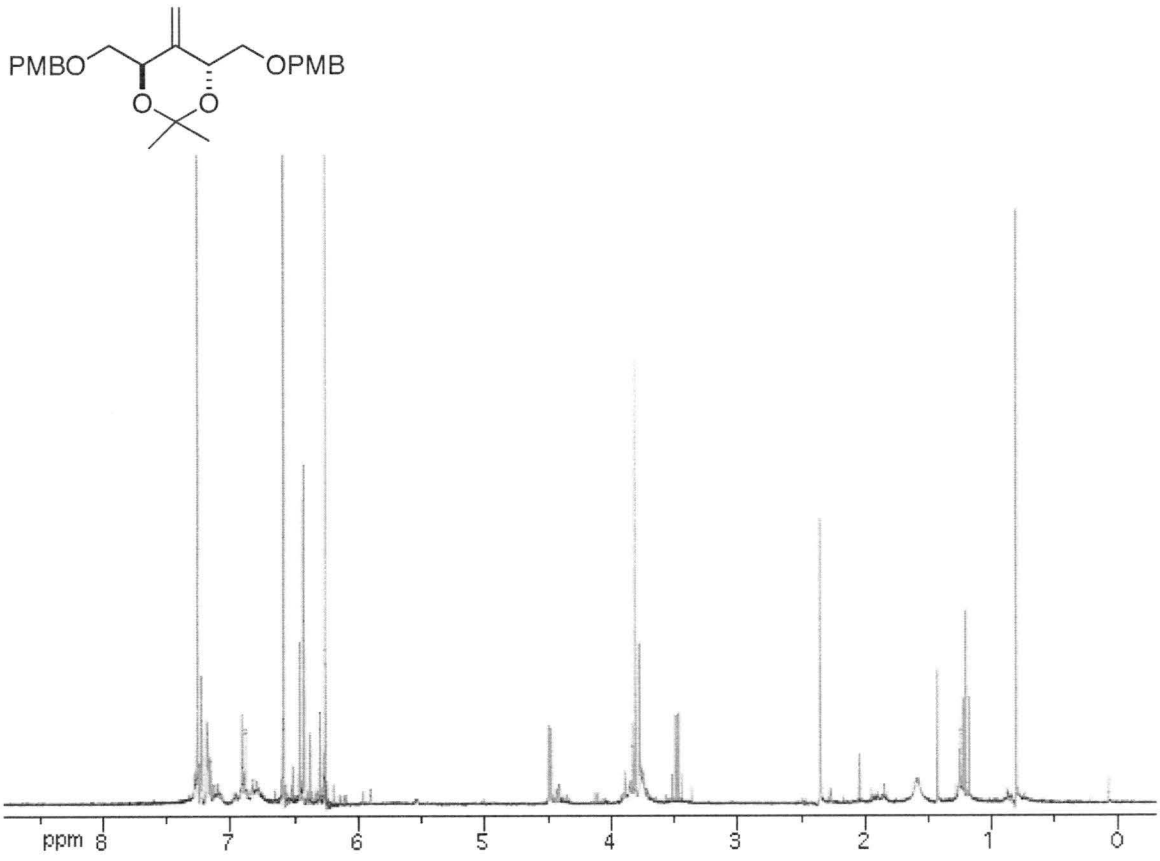
Olefin; Peterson conditions. (50 mg, .123 mmol, 1 equiv.) was dissolved in THF at room temperature under argon. Trimethylsilylmethyl magnesium chloride (.12 mL, .615 mmol, 5 equiv.) was added dropwise and then the reaction was heated to reflux. The reaction was refluxed for 18 hours, after which the reaction is cooled to room temperature and acetic acid (3:1 acid/water, 2 mL) is added and the reaction is stirred for 3 hours to afford elimination. The reaction was then quenched with NH_4Cl, and diluted with ether. The layers were separated and the organic layer was extracted with ether (3 x 15 mL). The combined organic layers were dried with Na_2SO_4 and concentrated to yield the crude product, which was purified by column chromatography on silica gel (3:1, Hexanes/EtOAc) to yield (<2 mg) of a brown oil (R_f = 0.43). ^1H NMR (300 MHz, $CDCl_3$): δ 0.76 (6H, s); 2.45 (2H, s); 3.49 (1H, t, J = 7.2 Hz) 3.83 (6H, s); 4.03 (2H, m); 4.43 (2H, d, J = 5.1 Hz); 4.49 (2H, d, J = 5.4 Hz); 5.58 (1H, s); 6.25 (1H, s); 6.55 (1H, s); 6.89-7.25 (8H, m). IR (NaCl) 1620, 1381, 1247, 1109, 823, 753, 649 cm^{-1}.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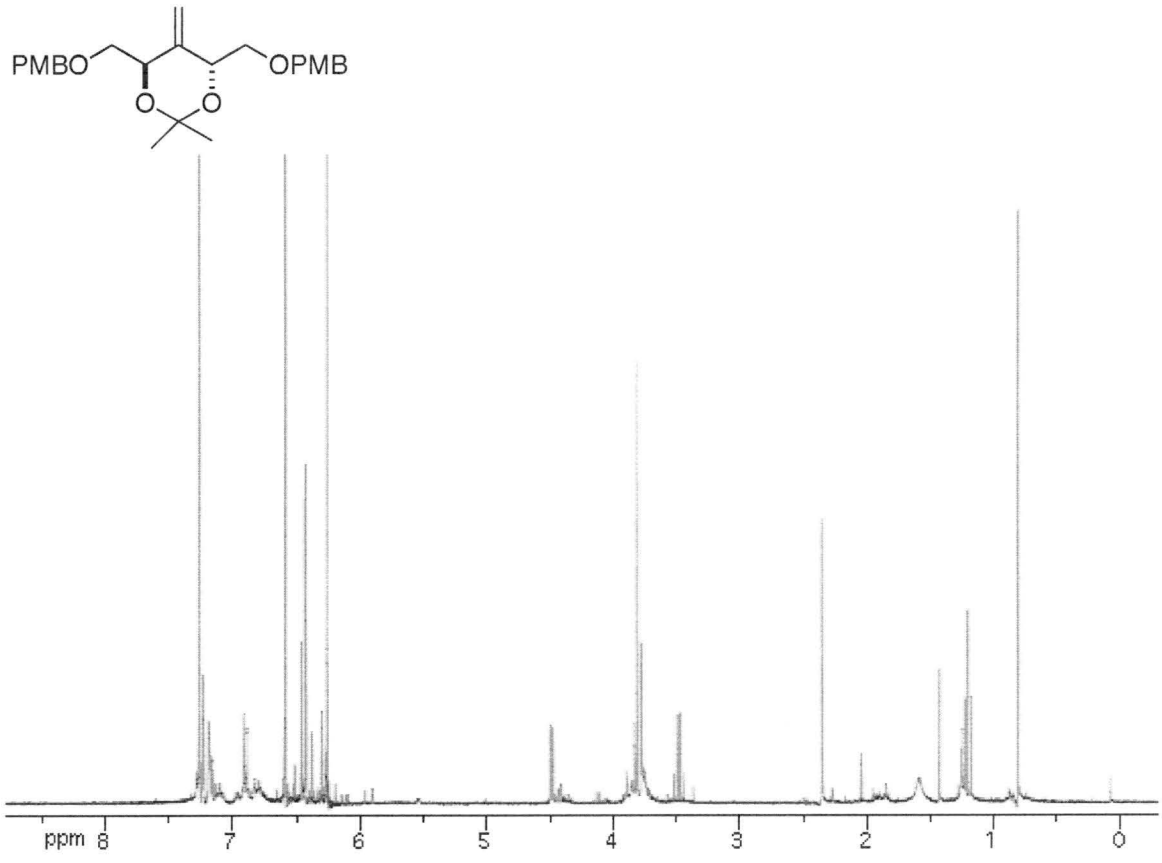
Olefin; Tebbe conditions. (50 mg, .123mmol, 1 equiv.) was dissolved in THF under argon and was cooled to -40°C. DMAP (75 mg, .615 mmol, 5 equiv.) was added followed by Tebbe reagent (.18 mL, .615 mmol, 5 equiv). The reaction was stirred for .5 hours then warmed to 0°C over 90 min. The reaction was quenched by the addition of aqueous NaOH, the reaction was then allowed to warm to room temperature. The mixture was diluted with ether dried with Na_2SO_4 and filtered through celite to remove excess Tebbe reagent then concentrated. The product was purified through a short column on silica gel (ether) to yield (<2 mg) of the olefin as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.76 (6H, s); 2.43 (2H, s); 3.83 (6H, s); 4.03 (2H, m); 4.414 (2H, d, J = 9.3 Hz); 4.49 (2H, d, J = 6.8 Hz); 5.39 (1H, s); 5.58 (1H, s); 6.25 (1H, s); 6.55 (1H, s) 6.89-7.25 (8H, m). IR (NaCl) 1620, 1391, 1242, 1115, 823, 764, 649 cm^{-1}.


PMBO
OPMB
O
O
ppm 8
7
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