# SYNTHESIS OF HAPTENS FOR THE MARINE TOXIN, GYMNODIMINE; SYNTHESIS OF BETA-LACTONE FUSED CARBOCYCLES AND NITROGEN HETEROCYCLES; EFFORTS TOWARD THE SYNTHESIS OF THE PROPOSED STRUCTURE OF THIOLYNGBYAN

A Dissertation

by

CHANG SUK LEE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2010

Major Subject: Chemistry

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Approved by:

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May 2010

Major Subject: Chemistry

# ABSTRACT

Synthesis of Haptens for the Marine Toxin, Gymnodimine; Synthesis of Beta-Lactone Fused Carbocycles and Nitrogen Heterocycles; Efforts toward the Synthesis of the Proposed Structure of Thiolyngbyan.

(May 2010)

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Chair of Advisory Committee: Dr. Daniel Romo

Contamination of seafood by marine toxins has been a consistent public health problem. Gymnodimine (GYM) is a member of a family of spirocyclic imine containing marine natural products which was shown to be highly toxic ($LD_{50}$ 96 μg/kg, intraperitoneal injection); thus ensuring public safety requires stringent monitoring of gymnodimine. Current detection methods for GYM and spirolides include the mouse bioassay and LC-MS-based detection techniques which, however, have significant limitations. Therefore, more efficient and convenient detection methods are required. Building on our recently completed total synthesis of (-)-gymnodimine, the synthesis of two haptens were targeted for eventual production of monoclonal antibodies (mAb) to be used in an eventual Enzyme-Linked Immunosorbent Assay (ELISA) for gymnodimine.

As an extension of the intramolecular nucleophilic catalyzed aldol lactonization (NCAL) process from aldehyde acid to keto acid substrates, carbocyclic and nitrogen heterocyclic β-lactones were synthesized. Demonstration of the utility of the NCAL process for keto acids was applied to the synthesis of dihydroplakevulin A and the core of tussilagine. In addition, although initial attempts to develop guanidine catalysts for the asymmetric NCAL process were unsuccessful, homobenzotetramisole (HBTM) was found to be a suitable asymmetric catalyst for keto acid substrates.

Finally, synthetic studies toward the proposed structure of thiolyngbyan are described. Thiolyngbyan was isolated from a blue-green algae and it exhibited antifungal activity.

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# CHAPTER I

# INTRODUCTION: ANTIBODIES FROM SYNTHETIC HAPTENS FOR MARINE TOXINS DETECTION

## 1.1 Methods for Detection of Marin Toxins

Contamination of seafood by marine toxins has been a consistent public health problem. Significant shellfish poisoning cases occur worldwide and a great deal of these incidents is associated with microalgae-produced natural marine biotoxins. Most algal toxins cause human illness by disrupting electrical conductance, uncoupling communication between nerve and muscle, and impeding critical physiological processes. Thus, ensuring public safety requires stringent monitoring of shellfish. Various methods have been used for toxins detection. Most of these methods are modifications of the intraperitoneal mouse bioassay procedure but all have suffered from inaccuracy of the test methods, length of time required to prepare the samples and animal ethics associated with the 24 h duration of the animal testing.[1] Analytical methods have also been developed using high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled with selective detection systems. However, they suffer from being laborious and expensive and unsuitable for monitoring a large number of samples. Antibody-based assays have been developed due to a better detection limit, fast analysis of numerous samples and lower cost.  They would be further classified by detection methods. Surface plasmon resonance (SPR) measured the difference of reflected intensity on the surface, which is caused by the complex between antibodies and analytes.[2]  Radioimmunoassay (RIA) is measured the concentration of labeled iodine, which was developed by Rosalyn Yalow and Solomon Aaron Bersin in the 1950s.[3] To perform a radioimmunoassay, a known quantity of an antigen is labeling with gamma-radioactive isotopes of iodine attached to tyrosine.

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This dissertation follows the style of *The Journal of Organic Chemistry*.

This radiolabeled antigen is mixed with a known amount of antibody for that antigen. To this radiolabeled antigen-antibody complex is added an unknown amount of sample. If a sample of serum competes with radiolabeled antigen, the concentration of unbound radiolabeled antigen would be increased. Therefore radioactivity of the radiolabeled antigen is measured using known standard binding curves, which allows the amount of antigen in the sample of serum to be quantitated. Rosalyn Yalow developed the RIA for insulin, which allowed the precise quantification of minute amounts of such a hormone.[2] RIA overcomes limitations of other methods in crude sample matrices by not requiring animals, a tissue culture facility or extensive sample preparation. While the RIA technique is extremely sensitive and specific, it necessitates specialized equipment and is costly. It commonly requires a licensed laboratory facility, and presents waste disposal problems. Therefore, today it has been largely supplanted by the Enzyme-Linked Immunosorbent Assay (ELISA) method, where the antigen-antibody reaction is measured using colorimetric signals instead of a radioactive signal.[4] In order to replace the radioactive signal to colorimetric signal, the first antibody should be fixed to the surface of the container and secondary antibody need to link to an enzyme, which elicits a chromogenic signal. In 1966, Wide and coworkers developed the linking technique.[3] ELISA is a biochemical technique mainly used in immunology to detect the presence of an antibody in a sample. For its sensitivity, accuracy, portability and cost-affectivity, ELISA has found applications in the food industry and in the toxicology for drugs. In a typical direct ELISA, an antigen is added to a solid surface, to which antibodies specific for the antigen and labeled with an enzyme such as a peroxidase or a lactase are added (Figure 1.1).[5] The enzyme would then catalyze a reaction that emits light by adding chromogenic substrate such as tetramethylbenzidine (TMB) or 2,2'-azino-bis(3-ethylbenzethiazoline-6-sulphonic acid) (ATBS), which serves as an in situ readout of the concentration of the antigen both qualitatively and quantitatively.

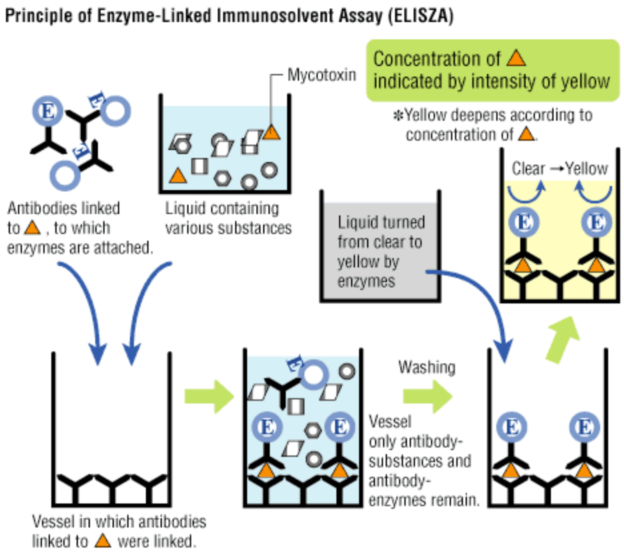

Principle of Enzyme-Linked Immunosolvent Assay (ELISZA)
Mycotoxin
Concentration of ▲ indicated by intensity of yellow
*Yellow deepens according to concentration of ▲.
Clear →Yellow
E
Antibodies linked to ▲, to which enzymes are attached.
Liquid containing various substances
Liquid turned from clear to yellow by enzymes
Washing
Vessel only antibody-substances and antibody-enzymes remain.
Vessel in which antibodies linked to ▲ were linked.

**Figure 1.1** Working mechanism of ELISA.[5]

Stewart and coworkers reported a rapid analytical optical biosensor-based immunoassay for the detection of okadaic acid (OA) and its structurally related toxins.[6] Although several antibody-based kits are commercially available for the detection of OA including ERFA biotech, DSP-Check (Sceti, Japan), Rougier Bio-Tech ELISA, issues exist regarding the cross-reactivity to congeners. Since ELISA detection is structural recognition and not toxicity, present ELISAs did not detect other members of the OA group of toxins. Therefore this technique could be useful in parallel in order to provide a full individual toxin determination and quantification.

Botana and coworkers reported fluorescence polarization technique for the detection of gymnodimine (GYM) and 13-desmethyl C spirolide.[7] Current detection methods for GYM and spirolides include mouse bioassay and LC-MS-based detection techniques. Although these toxins are not still regulated, efficient detection methods need to be developed. Recent studies have provided direct evidence that GYM and spirolides target muscular and nicotinic acetylcholine receptors (nAChR) subtypes with high affinity. Therefore these toxins inhibited the interaction of fluorescent-labled α-bungarotoxin with nAChR. This analytical method allowed detection of these toxins up to 1μg/kg of shellfish meat and could be useful for detection of toxins, which showed the affinity to nAChR.

## 1.2 Derivatization of Natural Products to Produce Antibodies

One of the first attempts to study antibodies from the viewpoint of chemistry was taken up by Arrhenius.[8] Considerably more important work was done by Landsteiner who investigated many of the fundamental principles. One important observation that came out of Landsteiner's investigations was that small molecules, by themselves, are not capable of inducing an immune response. Therefore small molecules, called "haptens", could be covalently attached to a carrier protein to generate an immune response. In fact, a number of ELISAs were already developed and used to monitor the environment. Most haptens were derived from natural products. For example, saxitoxin,[9] brevetoxin,[10] domoic acid,[11] and yessotoxin[12] are marine toxins and their antibodies were produced from their protein conjugates. The isolated antibodies showed high affinity to the natural product.

The conjugation of saxitoxin to keyhole limpets hemocyanin (KLH) or bovine serum albumin (BSA) involve an one-step formaldehyde method (Figure 1.2).[13] Polyclonal antibodies against saxitoxin antigen were collected from rabbits. The detection limit of saxitoxin, defined as the concentration of toxin standard equivalent to three standard deviation at $A_0$ (no competition), was 10 pg mL$^{-1}$ for the indirect assay and 3 pg mL$^{-1}$ for the direct format.

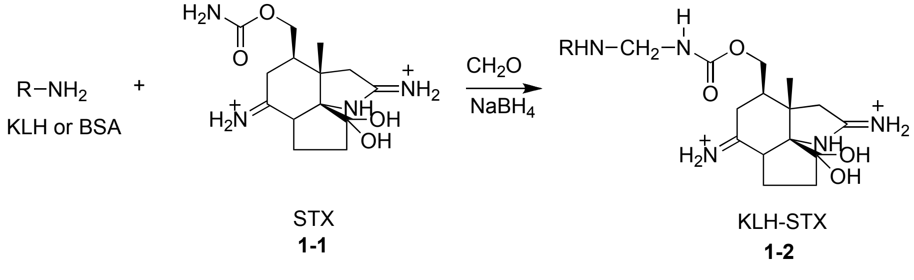


**Figure 1.2** The conjugation reaction between STX and KLH or BSA.

The conjugation of brevetoxin to BSA involved a three-step sequence.[9] Reduction of brevetoxin with sodium borohydride followed by succinylating with succinic anhydride in pyridine at 85 °C. The free carboxyl group on the succinate derivative **1-5** was covalently coupled to BSA using standard carbodiimide condensation (Figure 1.3).

Baden and coworkers developed efficient ELISA-based tools to monitor brevetoxins and demonstrated that ELISA have quantitative resolving capabilities (0.04-0.4 pM) which are higher than RIA (1.6-43 nM) due to secondary antibody enzyme conjugates which act as reporter molecules.

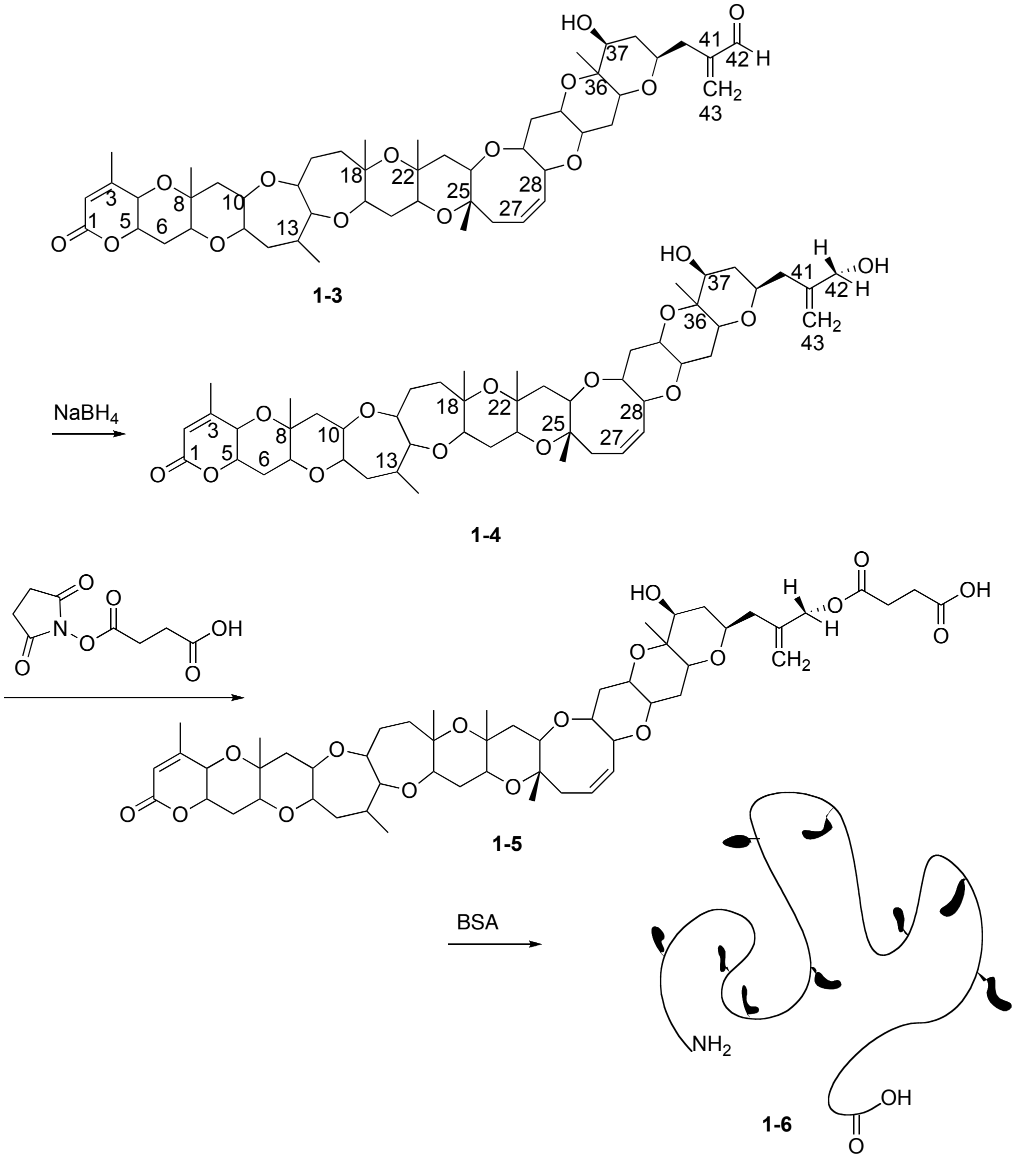
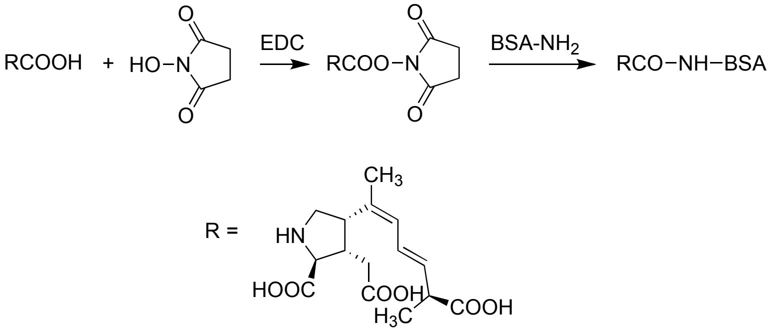


**Figure 1**.3 The conjugation reaction between brevetoxin and BSA.

The conjugation of domoic acid to BSA was done using a modified carbodiimide method. Briefly, domoic acid and carbodiimide and *N*-hydroxysucinimide were dissolved in DMSO at room temperature. After stirring for 1.5 h, BSA solution in phosphate buffered saline (PBS) was added to the mixture (Figure 1.4). The detection limit was 0.41 ng well$^{-1}$ in PBS.



**1-7**

**Figure 1.4** The conjugation reaction between domoic acid and BSA.

The conjugation of yessotoxin to BSA was achieved using the olefinic moiety. Briefly, yessotoxin was readily oxidized by ozone and the resulting ozonized yessotoxin **1-9** was conjugated with BSA via reductive amination by adding a solution of sodium cyanoborohydride in 1 mL PBS buffer. Yessotoxin was also readily oxidized with bromine, and examined by $^{1}$H NMR. The resulting bromo derivative **1-10** was conjugated via nucleophilic amination with ovalbumin (OVA) in carbonate buffer (Figure 1.5). Although the structure of both **1-11** and **1-12** were tentatively assigned, both of them elicited antibodies that showed high affinity to the yessotoxin.

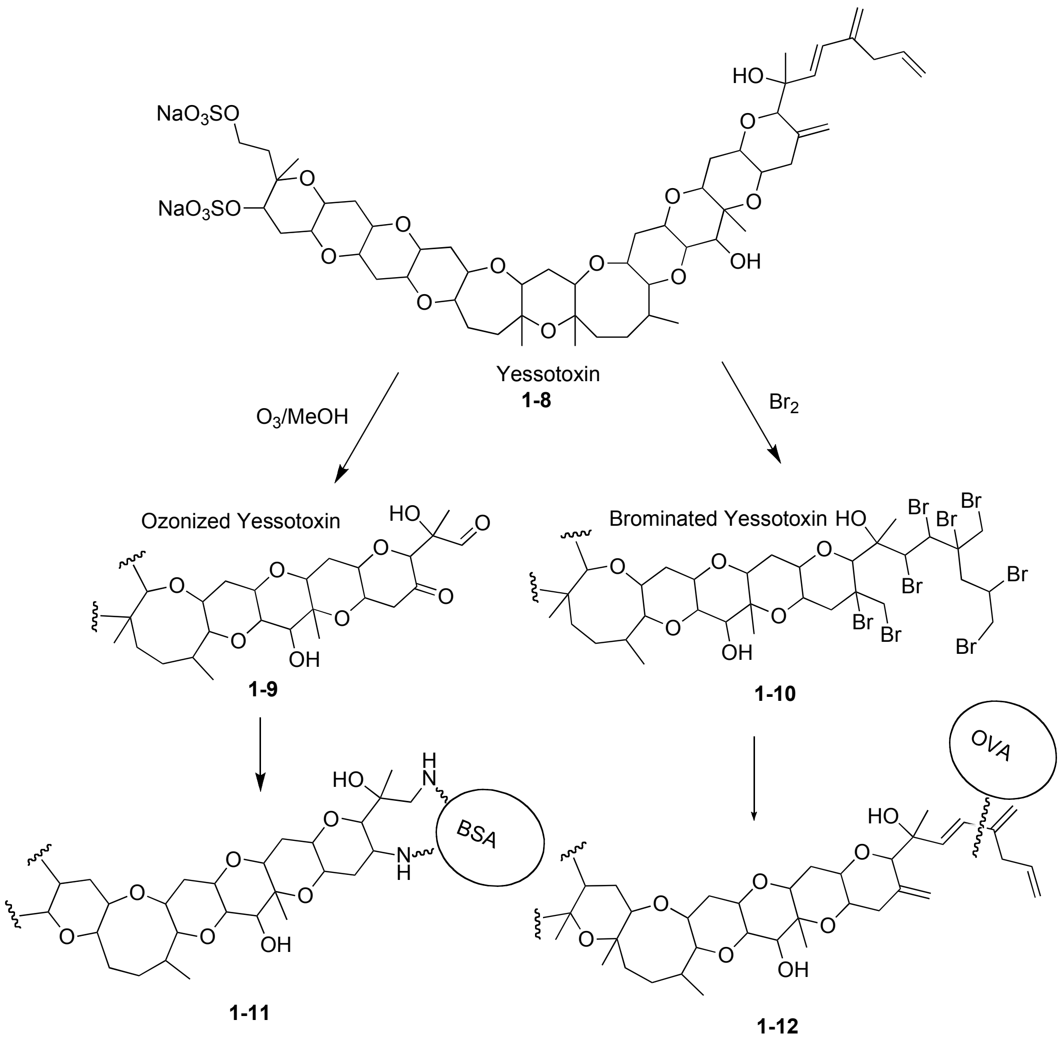

NaO$_3$SO
NaO$_3$SO
HO
OH
Yessotoxin
1-8
O$_3$/MeOH
Br$_2$
Ozonized Yessotoxin
1-9
Brominated Yessotoxin
1-10
BSA
1-11
OVA
1-12

**Figure 1.5** Preparation of immunizing and coating conjugates by derivatization of yessotoxin by ozonolysis and by bromination.

However, one important drawback of the use of natural product itself as a hapten is the loss of toxicity because the primary amine or carboxylic acid functional groups used for protein conjugation could be essential for its bioactivity. Indeed, Serum from rabbits immunized with a conjugate, in which palytoxin's amino group was covalently bound to a carboxyl function of BSA in the presence of carbodiimide, neutralized the biological activities of palytoxin (Figure 1.6).[14]

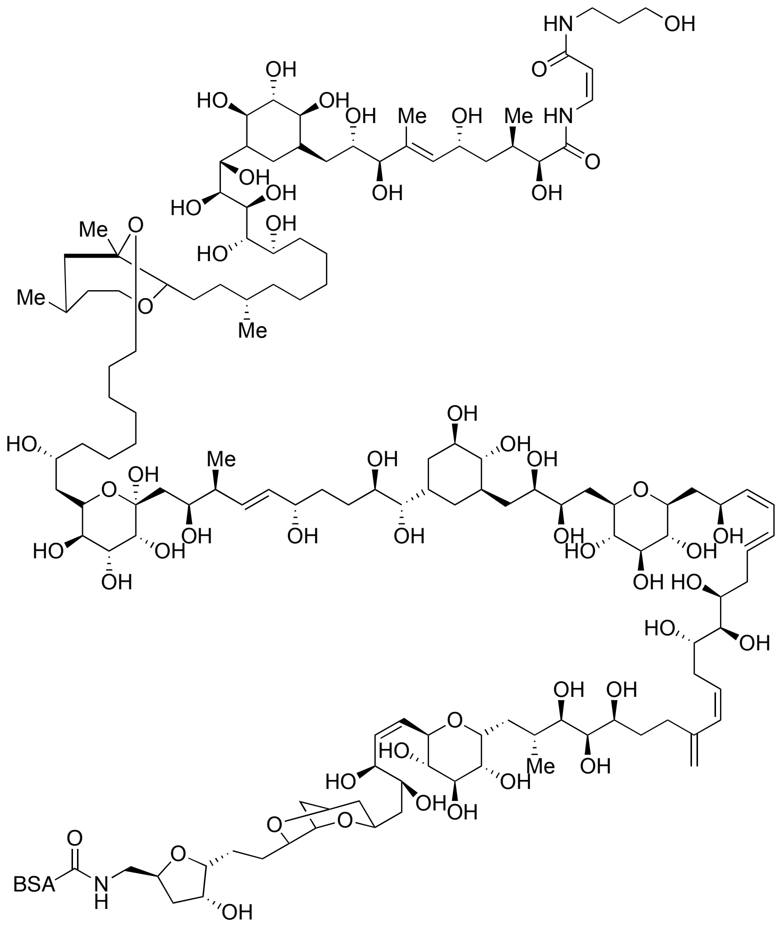


**Figure 1.6** Structure of palytoxin-BSA conjugation.

## 1.3 Synthetic Haptens to Produce Antibodies

### 1.3.1 Direct Sandwich Immunoassay for Ciguatoxin 1B and 3C

Coupling of a nonnative functionality that can be chemically reacted with the carrier protein can be difficult to fulfill in the case of complex natural products. Therefore synthetic haptens are more readily accessible to antigens without losing its bioactivity. Although many marine toxins have been synthesized, only ciguatoxin 3C (CTX3C)[15] and azaspiracid (AZA)[16] were successfully demonstrated to have the advantage as synthetic haptens.

Ciguatera fish poisoning is caused by the ingestion of a variety of reef fish, which have bioaccumulated ciguatoxins in their tissues. Ciguatoxins are produced by the marine dinoflagellate *gambieriscus toxicus*. Since over twenty thousand people in the tropics suffer from ciguatera poisoning, several methods have been developed to detect ciguatoxins. Although Hokama and coworkers prepared a monoclonal antibody (mAb) using Ciguatoxin 1B (CTX1B) **14**, this antibody showed a high cross reactivity to another marine toxin, okadaic acid (Figure 1.7).[17]

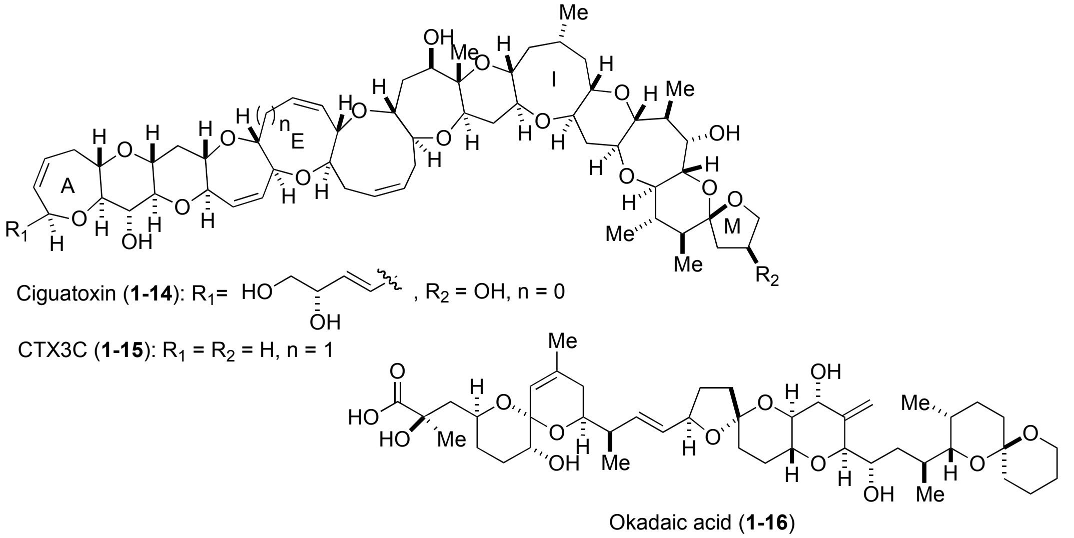
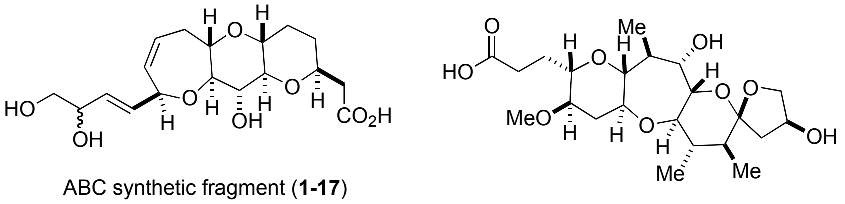

Ciguatoxin (**1-14**): R1= HO OH , R2 = OH, n = 0
CTX3C (**1-15**): R1 = R2 = H, n = 1
Okadaic acid (**1-16**)
A
E
I
M
R1
R2
n
Me
OH
H
O

**Figure 1**.7 Structure of ciguatoxins and okadaic acid.

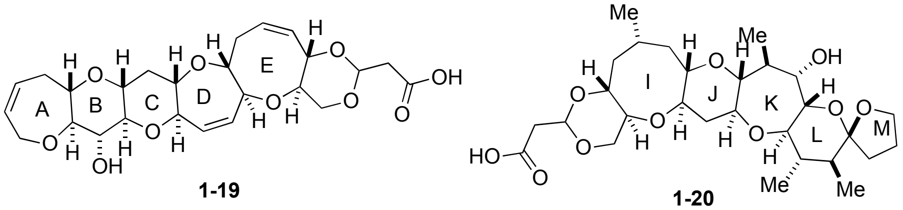
The extremely low content of ciguatoxin 1B (CTX1B) **14** in fish has hampered the further development of ciguatoxin antibodies. As a solution to this problem, two groups have developed the synthetic haptens of CTX1B independently. Hirama and coworkers synthesized the ABC ring fragment as a haptenic group with a carboxylic acid linker. Although the antibodies elicited by the antigen bound strongly to the ABC ring fragment, they did not recognize CTX1B itself. They speculated that the size of the ABC ring hapten would be too small to get enough information of CTX1B (Figure 1.8). Therefore they synthesized bigger hapten to elucidate their questions. Tachibana and coworkers synthesized a hapten, a carboxylic derivative of the right hand tetracyclic terminus portion of CTX1B, which was conjugated to carrier proteins.[18] Although the antibodies elicited by the JKLM ring antigen bound to CTX1B itself, they showed the cross reactivity to other natural products.


ABC synthetic fragment (**1-17**)

JKLM synthetic fragment (**1-18**)

**Figure 1.8** Synthetic haptens of ciguatoxin 1B.

A direct sandwich ELISA method developed by Hirma, Fugii and coworkers solved the aforementioned cross-reactivity problem.[19] This assay requires two distinct antibodies for detection. Since they synthesized the ABCDE ring fragment **1-19** and IJKLM ring fragment **1-20** to complete the total synthesis of CTX3C, both were used as haptens (Figure 1.9). Interestingly, antibodies (mAb 10C9) from an antigen (ABCDE ring hapten conjugated with protein) larger than ABC ring hapten conjugated with protein tightly bound to CTX3C itself. More importantly mAb 10C9 showed no cross reactivity with the IJKLM ring hapten. This result strongly suggested that mAb 10C9 recognized the pentacyclic ABCDE ring fragment of CTX3C. Similarly the other antibodies (mAb 3D11) showed strongly bound to CTX3C itself and did not cross-react with ABCDE ring hapten. For this assay they used mAb 10C9 to capture CTX3C and mAb 3D11 as a detector. This protocol could detect CTX3C at ppb (part per billion) level. Importantly none of the other marine toxins gave any detectable signals at 20 μM concentration.

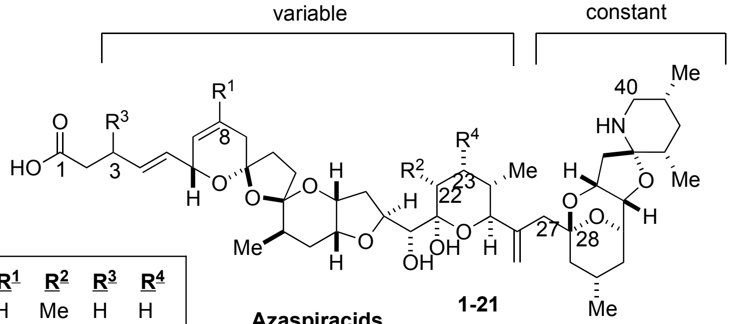

**1-19**

**1-20**

**Figure 1.9** Synthetic haptens of ciguatoxin 3C.

## 1.3.2 Congener Independent Immunoassay for Azaspiracids

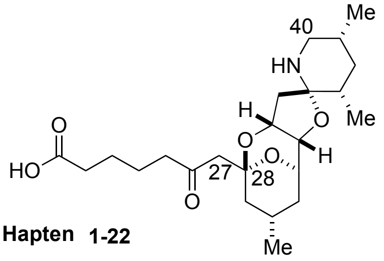
The azaspiracids are natural marine toxin witch have been proposed to originate from the dinoflagellate *protoperidium crassipes*.[20] The Yasumoto and Satake group[21] proposed a structure for azaspiracid-1 and Nicolaou and coworkers later revised the structure through degradative and synthetic efforts (Figure 1.10).[22]



**Azaspiracids**

**1-21**

| **Name** | **R$^1$** | **R$^2$** | **R$^3$** | **R$^4$** |
|---|---|---|---|---|
| **Aza1** | H | Me | H | H |
| **Aza2** | Me | Me | H | H |
| **Aza3** | H | H | H | H |
| **Aza4** | H | H | OH | H |
| **Aza5** | H | H | H | OH |
| **Aza6** | Me | H | H | H |
| **Aza7** | H | Me | OH | H |
| **Aza8** | H | Me | H | OH |
| **Aza9** | Me | H | OH | H |
| **Aza10** | Me | H | H | OH |
| **Aza11** | Me | Me | OH | H |



**General Hapten 1-22**

**Figure 1.10** Structures of azaspiracids and synthetic general hapten.

In general, antibodies that are raised against specific congeners tend to be sensitive to a relatively narrow range of natural product analogues. If detection and quantitation of numerous congeners including structurally similar other toxins are needed, congener dependent assays are not ideal for screening because of the possibility of false negatives in the presence of toxic congeners to which the assay is insensitive. Since all eleven azaspiracids shared the C28-C40 domain, Forsyth, Miles, and coworkers designed hapten **1-22**, which would eventually provide congener independent ELISA.[13] The conjugation of carboxylic acid **1-22** to Bovine Serum Albumin (BSA) was prepared using a modified carbodiimide method. Briefly, carboxylic acid **1-22** and carbodiimide and *N*-hydroxysucinimide dissolved in DMF at room temperature. After stirring for 0.5 h, BSA solution in PBS was added to the mixture. Indeed, antibodies raised against this hapten **1-22** cross-reacted with AZA 1, AZA 2, AZA 3 and AZA 6 with similar affinity.

Nicolaou and coworkers have published studies toward the development of a sandwich immunoassay for azaspiracids.[17] As mention before, these assays require two distinct antibodies for detection. Although azaspiracids was of sufficient size to have one antibody bind to some portion of the C1-C26 domain (ABCDE ring), antibodies raised against antigen (ABCDE ring conjugated with protein) showed weak or no binding to azaspiracids.

## 1.4 Design of Synthetic Haptens of Gymnodimine

The advantages and success of synthetic haptens-based ELISA encouraged us to delvelop an antibody probe for the gymnodimine congeners detection. Our goal was to isolate antibody proteins that recognize gymnodimine congeners without cross-reactivity with other marine toxins. An ideal hapten should be a near perfect mimic of the target structure in size, shape (geometry) and electronic properties. The hapten handle (required for conjugating the target mimic to a carrier protein) should in itself not elicit antibody recognition. Thus, an innocuous alkyl handle often is most appropriate, the length of which should be evaluated using one or more homologs. An appropriate functional group for covalent attachment of the hapten handle to the protein must be compatible with the chemistry of the functional groups on the protein. Building on our recently completed total synthesis of (-)-gymondimine, two haptens were proposed for eventual production of monoclonal antibodies (mAb). Toward a congener-independent immunoassay, an invariable sector of the toxin between all known congeners of the toxin of interest including degradation fragments must be identified. Careful examination of gymnodimines isolated to date and also of proposed and of potential degradation products lead to the analysis shown in Figure 1-11. It should be noted that hydrolysis of the butenolide ring under basic and neutral conditions ($t_{1/2}$ = 33 h at pH 7.6, 37°C) is known to occur readily, and thus a congener-independent hapten should exclude this portion of the molecule (Figure 1.11, eastern green area).[23] Also, the recent isolation of toxic gymnodimine congeners B and C (see the figure on page 17) that only differs in bearing an additional allylic alcohol suggests avoiding this sector. It should be noted that hydrolysis of the cyclic imine is expected to provide a mechanism of detoxification based on related studies of the spirolides and thus a congener-independent assay should include this region.

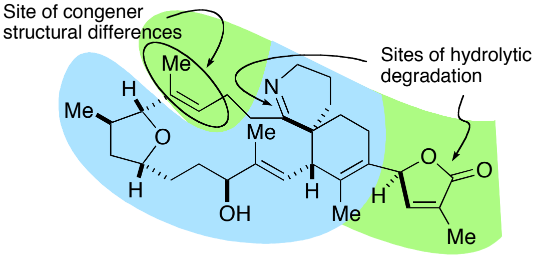

Site of congener structural differences
Sites of hydrolytic degradation
Me
N
H
Me
O
Me
H
OH
H
H
Me
O
O
Me

**Figure 1.11** Proposed regions of gymnodimine suitable for congener specific (green) and congener-independent (blue) hapten synthesis.

Our target macrocyclic gymnodimine hapten **M** will be prepared starting from known ketone **M-1**, an intermediate in our total synthesis (Figure 1.12). This hapten **M** retains most structural and streochemical information of the natural product. Therefore this hapten will ideally provide an antibody that recognizes gymnodimine. However, it would require more synthetic effort to prepare hapten **M** and antibodies raised from it might recognize only gymnodimine without recognition of its congeners. One example of a congener-independent hapten to be prepared is the spirocycle hapten **S**, and the synthesis makes use of an unfortunate (but useful for this purpose) by-product available during Nozaki-Hiyama-Kishi (NHK) couplings in the total synthesis, namely the deiodinated alkene **S-1**. Hapten **S** contains the imine moiety, which is the pharmacophore of gymnodimine. However, its size might not be sufficient to allow the preparation of specific antibody for gymnodimine. Although hapten **T** derived from the tetrahydrofuran fragment does not seem ideal, it is structurally complementary to the hapten **S**. If the antibodies elicited from antigen of hapten **T** binds to gymnodimine, it could have potentially valuable applications in development of a sandwich ELISA. Since antigen of hapten **S** has higher potential to elicite antibodies which recognize gymnodimine, we will have two sets of complimentary antibodies for a sandwich ELISA. Furthermore, these results could provide some insight into the structure-activity relationship of the toxicity of gymnodimine.

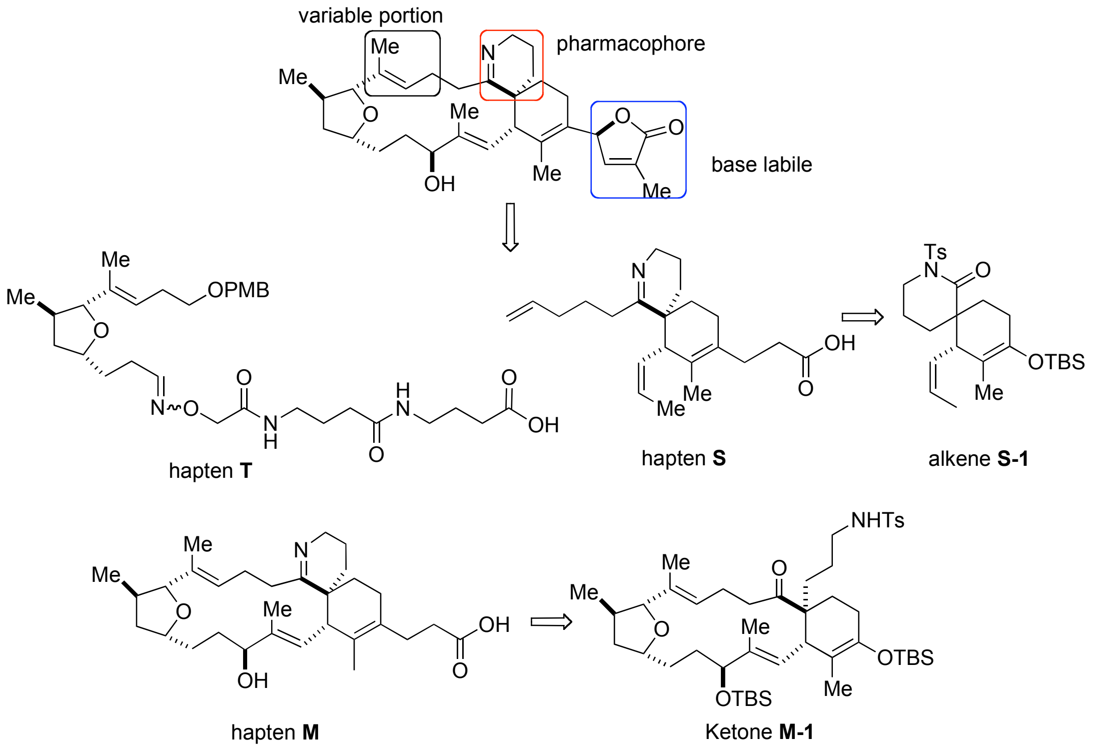
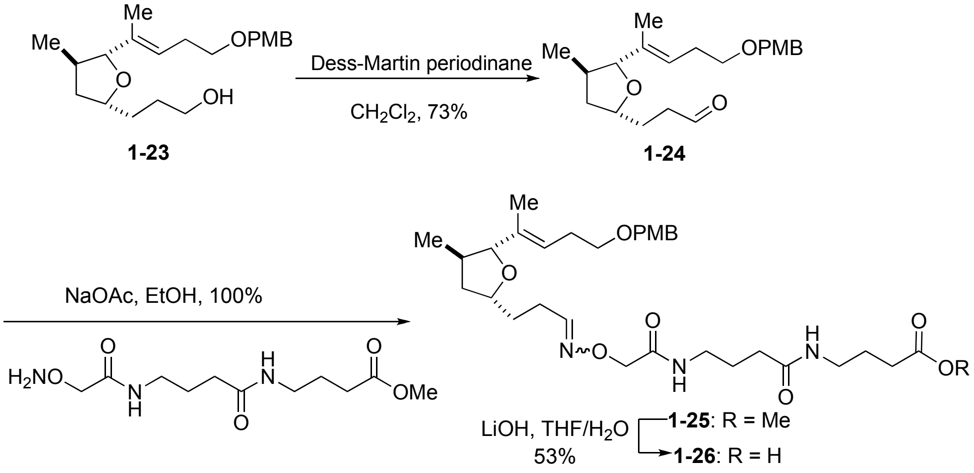

variable portion
pharmacophore
base labile
Me
N
O
OH
OPMB
hapten T
hapten S
alkene S-1
Ts
OTBS
NHTs
hapten M
Ketone M-1

**Figure 1.12** Designed haptens based on structures of gymnodimines.

## 1.5 Previously Synthesized Hapten of Gymnodimine

The synthesis of the hapten **T** was achieved from the tetrahydrofuran intermediate in our total synthesis. Our collaborators Prof. Chris Elliott from university of Belfast, Ireland conjugated it to a carrier protein. Immunization with this antigen led to the production of antibodies (mAbs). Although the antibodies raised by antigen of hapten **T** binds strongly to the hapten **T**, it did not recognize gymnodimine itself. We anticipate that haptens containing the spiroimine phamacophore and larger in size than the hapten **T** are required to induce mAbs that will recognize gymnodimine.

**Scheme 1**


Me
Me
OPMB
O
OH
1-23
Dess-Martin periodinane
CH2Cl2, 73%
Me
Me
OPMB
O
O
1-24
NaOAc, EtOH, 100%
H2NO
N
H
O
H
N
O
O
OMe
Me
Me
OPMB
O
N
O
O
N
H
O
H
N
O
OR
LiOH, THF/H2O
53%
1-25: R = Me
1-26: R = H

## 1.6 Summary and Outlook

Food and water intoxications posing serious threats to public health and the food industry and ELISAs are one of the best methods that efficiently monitor massive samples. A lot of ELISAs for marine toxins were already developed and showed high sensitivity. The main component of ELISA, which is antibodies, was elicited by natural product antigen. However, it is difficult to control the affinity of antibodies toward target toxins if antibodies have a problem such as cross reactivity to another marine toxins. Therefore, synthetic haptens are ideal and efficient to develop ELISAs. In order to obtain successful synthetic haptens, synthetic methodologies to acess marine toxins should be achieved beforehand. Since we completed the first total synthesis of gymnodimine, taking advantage of this, we have developed synthetic haptens. Although first example hapten **T** did not give promising results, hapten **S** and hapten **M** will give us valuable information to achieve our goal.

# CHAPTER II

# SYNTHESIS OF GYMNODIMINE IMMUNOGENS*

## 2.1 Biological Studies of Gymnodimine

Numerous cases of shellfish poisoning occur worldwide each year and a significant number of these incidents are associated with natural marine biotoxins produced by microalgae. Seafood intoxications posing serious threats to public health and the shellfish industry. Shellfish can concentrate phycotoxins in their edible tissue and act as vectors for transferring toxic chemical compounds to crabs, fish, birds marine mammals and unltimately to humans. Three main species of dinoflagellates that produce the toxins are: *Alexadrium* sp., *Gymnodinium* and *Pyrodinium*.[24] The marine biotoxin gymnodimine was first isolated in 1995 from oysters (*Tiostrea chilensis*) collected at Foveaux Strait in the South Island of New Zealand by Yasumoto and coworkers.[25] Gymnodimine was later shown to be widely distributed around the New Zealand coastline and has recently been identified in shellfish from both Tunisia and Canada. The gross structure was assigned by detailed spectroscopic studies. In 1997, Munro and coworkers elucidated the relative and absolute stereochemistry through X-ray crystallographic analysis of a *N*-acylated derivative.[26] The structural features of gymnodimine include a trisubstituted tetrahydrofuran within a 16-membered carbocycle, a chiral butenolide, and an azaspiro[5.5]undecadiene moiety. Two additional analogs were isolated and named gymnodimine B and C, respectively, that appear to be allylic oxidation products of the C17-C18 olefin of gymnodimine (Figure 2.1).[27] Gymnodimine toxicity was associated with a bloom of a gymnodinoid dinoflagellate within the *Karenia selliforms*.[28]

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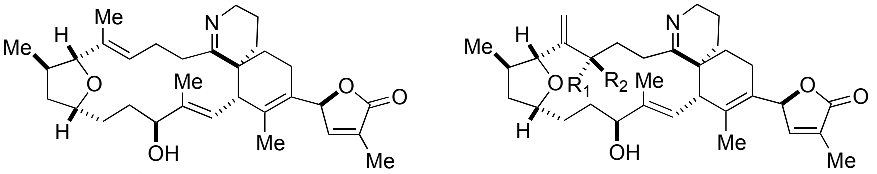
*Reprinted with permission from “Enantioselective Total Synthesis of the Marine Toxin (-)-Gymnodimine Employing a Barbier-Type Macrocyclization” by Kong, K.; Romo, D.; Lee, C. *Angew. Chem., Int. Ed.* **2009**, *48*, 7402-7405. Copyright 2009 Wiely-VCH.



Gymnodimine (**2-1**)

Gymnodimine B (**2-2**): $R_1$ = OH, $R_2$ = H
Gymnodimine C (**2-3**): $R_1$= H, $R_2$ = OH

**Figure 2.1** Structures of gymnodimines.

Gymnodimine was first suggested to act on voltage-gated $Na^+$ ion channels, but direct proof of this hypothesis was not provided. Gymnodimine **2-1** was highly toxic by intraperitioneal injection, $LD_{50}$ 96 μg/kg. However, much toxicity was lost $LD_{50}$ 755 μg/kg after oral administration by gavage.[29] No signs of toxicity were seen in mice voluntarily ingesting food containing gymnodimine at sufficient level as to give a dose of 7500 μg/kg. One study showed that gymnodine and its analogues had weak effects on Neuro2a cells alone, yet indeed tended to reduce Neuro2a cell number.[30] In contrast, gymnodimine and its analogues produced a strong and consistant sensitization to the toxic effects of another toxin, okadaic acid. Algal blooms possibly involve production of both okadaic acid-type molecules and gymnodimine thus enhencing toxicity and posing a greater public health problem. However, the direct combined effects presently remain ambigous. Interestingly, symtoms observed after intraperitoneal injection of gymnodimine were identical to those recorded with tubocurarine. Since the latter substance is a competitive non-depolarizing neuromuscular blocking agent which binds reversibly with postjunctional nicotinic receptors, thereby blocking the acetylcholine transmitter. If gymnodimine acted reversibly at the nicotinic receptors, it would be expected that neostigmine and physostigmine would similarly protect against gymnodimine toxicity in the same manner. This was found to be true as pre-treatment with physostigmine or neostigmine protected against injected gymnodimine, suggesting that gymnodimine exerts its toxic effects via blockade of nicotinic receptors at the neuromuscular junction. More recently, this hypothesis was investigated to test whether gymnodimine induced nicotinic acetylcholine receptor (nAChR) blockade (Figure 2.2).[31] Current-voltage relationships were performed with ionophoretic acetylcholine receptor (ACh) because it is well known that the binding of ACh to nAChRs caused inward nicotinic currents. The ACh-evoked current peaks and the membrane potential from -140 to -60 mV range under control conditions and after 10 nM gymnodimine treatment that blocked about 50-60 % of the nicotinic peak current amplitudes were block which indicated that gymnodimine shows high affinity with nAChRs.

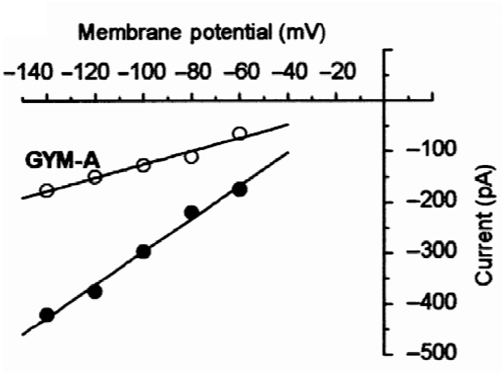
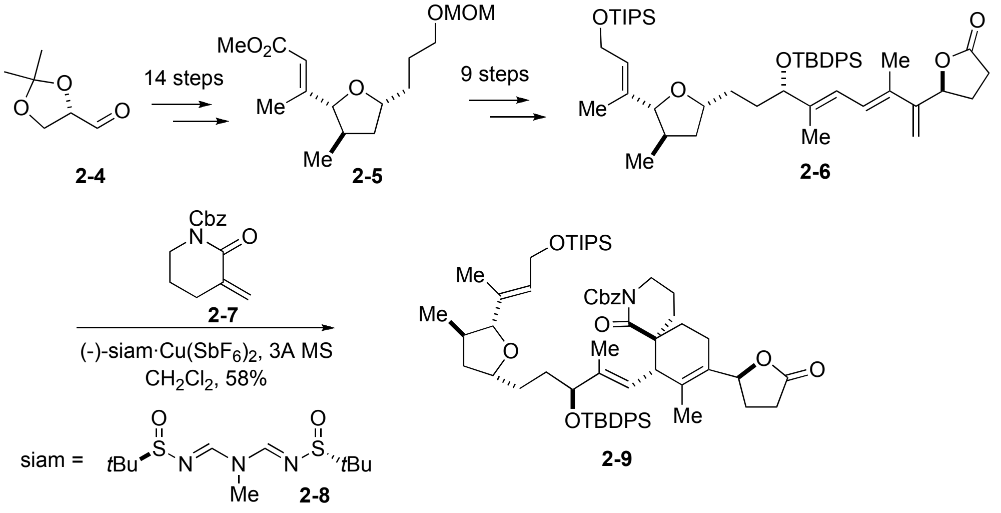


**Figure 2.2** Representative current-voltage relationship obtained with AChRs under control conditions and after addition of 10 nM GYM.[28]

### 2.2 Previous Synthetic Approaches to Gymnodimine

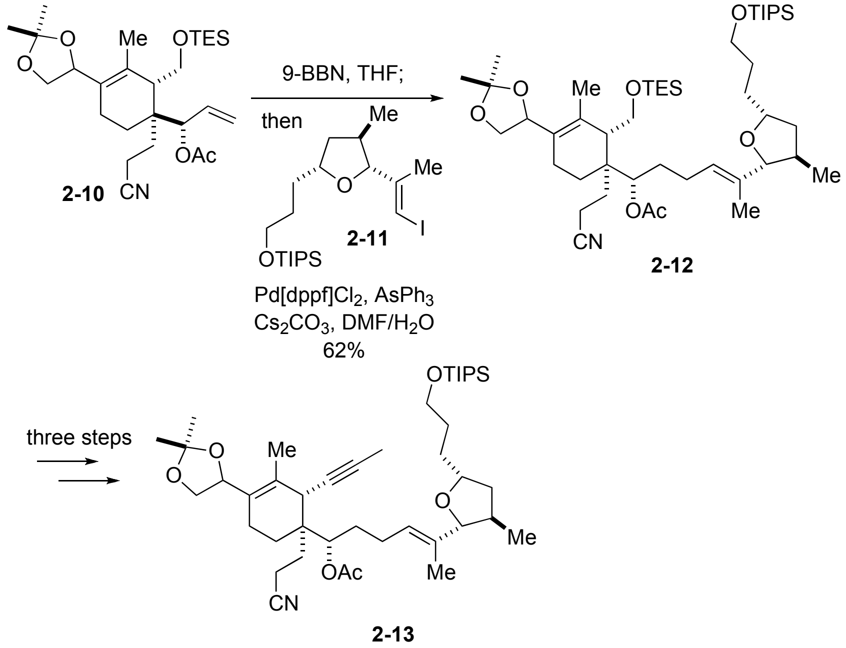
Several groups have disclosed their synthetic studies toward gymnodimine. Murai and coworkers synthesized the tetrahydrofuran segment **2-5** of gymnodimine in a highly stereoselective fashion (Scheme 2).[32] The key intermolecular Diels-Alder reaction of the triene **2-6** with α-methylene Cbz-lactam **2-7** was conducted in the presence of a full equivalent of Ellman's copper bis(sulfinyl)-imidoamidine (siam) complex **2-8**.[33] A double diastereoselective Diels-Alder reaction to construct the spirocyclic lactam provided the spirolactam **2-9** in good yield and remarkably high diastereoselectivity (dr > 20:1).

## Scheme 2


2-4
14 steps
MeO2C
OMOM
Me
O
Me
2-5
9 steps
OTIPS
OTBDPS
Me
O
Me
2-6
Cbz
N
O
2-7
(-)-siam·Cu(SbF6)2, 3A MS
CH2Cl2, 58%
siam =
tBu
S
N
N
Me
N
S
tBu
2-8
OTIPS
Me
CbzN
O
Me
OTBDPS
2-9

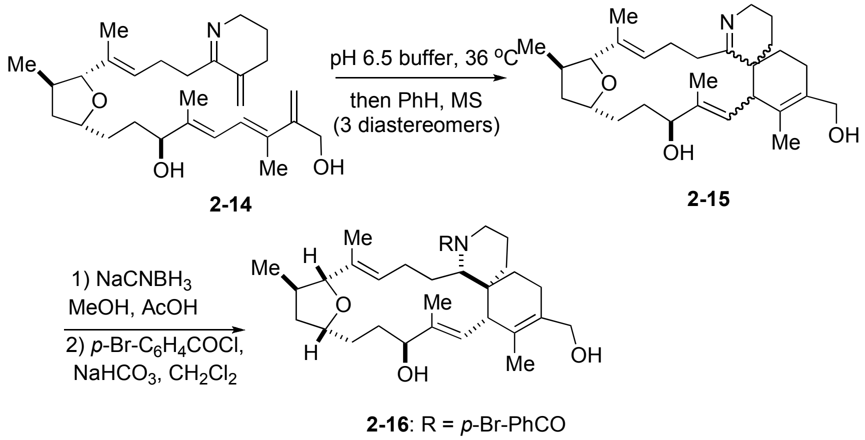
White and coworkers employed a convergent coupling of the optically active spirolactam and tetrahydrofuran moiety through a B-alkyl Suzuki-Miyaura coupling (Scheme 3).[34] Subsequent attempts to move alkyne **2-13** toward a substrate suitable for closing the macrocylic core met difficulties due to the relatively hindered enviroment of the alkyne **2-13**. A variety of conditions such as silylcupration-iodination, hydrozirconation-iodination, and palladium-catalyzed hydrostannylation-iodination of alkyne **2-13** all returned starting material.

**Scheme 3**


2-10
9-BBN, THF;
then
2-11
Pd[dppf]Cl2, AsPh3
Cs2CO3, DMF/H2O
62%
2-12
three steps
2-13
Me
OTES
OAc
CN
OTIPS
O
Me

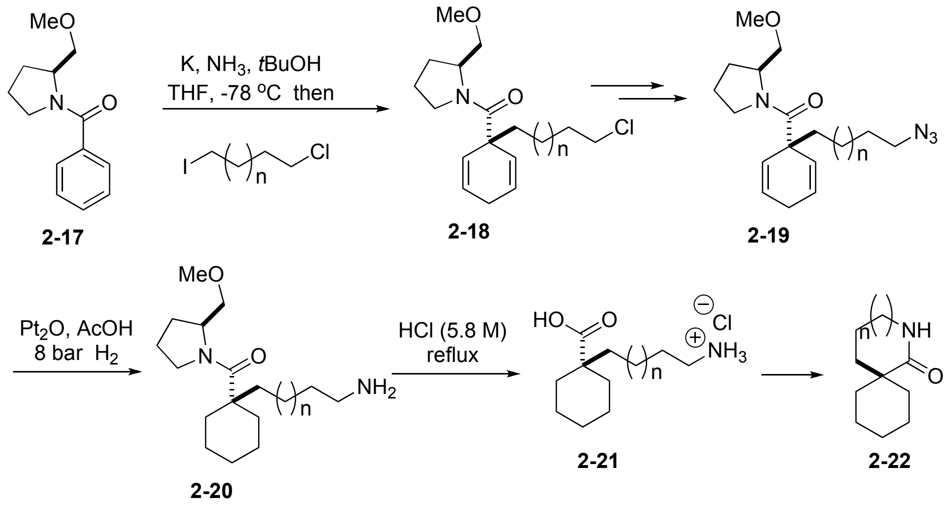
In a manner related to his pinnatoxin studies, Kishi and coworkers utilized a Diels-Alder/ macrocyclization process for an approach to gymnodimine (Scheme 4).[35] Diels-Alder macrocyclization of this imine **2-14** occurred in water at pH 6.5 under dilute conditions to give the desired product **2-15** as a mixture of three diastereomers. The desired natural product derivative **2-16** was isolated after reduction of a mixture of three imines **2-15** followed by acylation.

**Scheme 4**


2-14
pH 6.5 buffer, 36 °C
then PhH, MS
(3 diastereomers)
2-15
1) NaCNBH3
MeOH, AcOH
2) p-Br-C6H4COCl,
NaHCO3, CH2Cl2
2-16: R = p-Br-PhCO
Me
N
RN
OH
O
H

Brimble and Trzoss developed an alternative approach other than Diels-Alder reaction for synthesis of the spirolactam (Scheme 5).[36] Their method involved a diastereoselective Birch reductive alkylation as the key step. The resulting cyclohexadienes **2-18** was reduced under hydrogenation conditions followed by acidolysis to remove chiral auxiliary delivered amino acid **2-21**. The final inramolecular stylization of the amino acids provided enatiopure spirolactams **2-22**. However, the potential utility of this methodology for gymnodimine synthesis has not been demonstrated.

**Scheme 5**



## 2.3 Large Scale Synthesis of Gymnodimine Precursors

### 2.3.1 Retrosynthetic Strategy toward Gymnodimine

Our synthetic plan called for a convergent coupling of tetrahydrofuran **2-23** the optically active spirolactam **2-24** (Figure 2.3). Nozaki–Hiyama–Kishi (NHK) macrocyclization,[37] which is well-established in both inter- and intramolecular settings, was initially envisioned for the proposed merging of C9 and C10. The formation of the C20–C21 bond through nucleophilic opening of a δ-lactam by an sp$^3$carbanion is rare, especially in a complex setting.[38] The fragile butenolide would be annulated at a late stage by a vinylogous Mukaiyama aldol reaction of a hypothetical furanone anion **2-25** to a ketone at C5 after unmasking of the silylenol ether in spirolactam **2-24**.

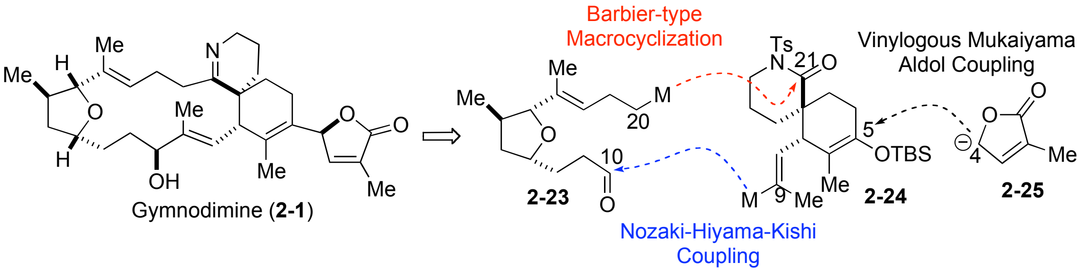
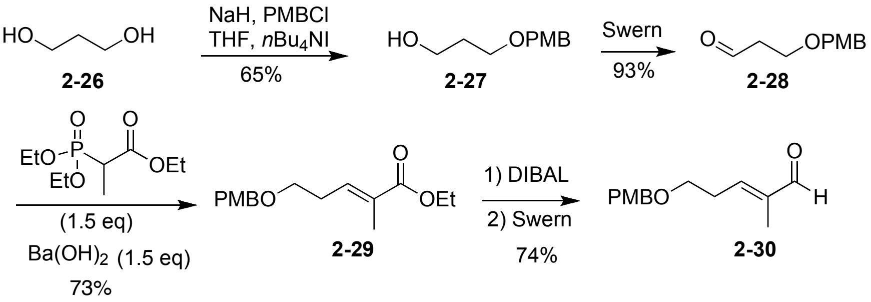

Me
H
Me
N
O
Me
H
OH
Me
O
O
Me
Gymnodimine (2-1)
Barbier-type
Macrocyclization
Me
Me
M
20
O
10
O
2-23
Ts
N
21
O
Vinylogous Mukaiyama
Aldol Coupling
5
OTBS
M
9
Me
Me
2-24
O
O
4
Me
2-25
Nozaki-Hiyama-Kishi
Coupling

**Figure 2**.3 Retrosynthetic strategy toward gymnodimine.

### 2.3.2 Synthesis of the Tetrahydrofuran Fragment

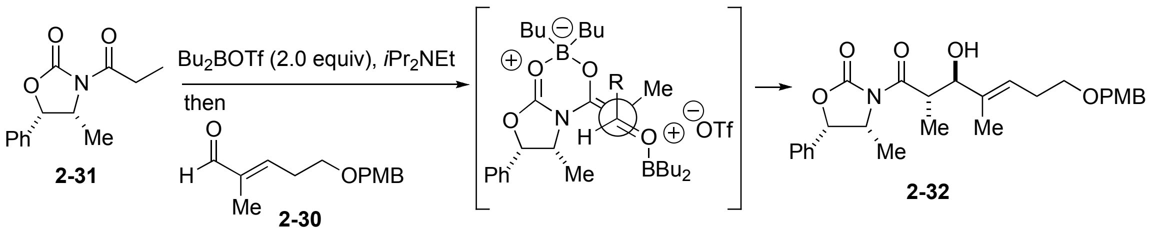
The conditions were already optimized in our group.[39] However, problemes were encountered in reproducing some of the reactions in terms of yields and diastereoselectivity. Optimized conditions were followed through the synthesis unless otherwise I mentioned. The synthesis of the tetrahydrofuran fragment began with monoprotection of 1,3-propanediol **2-26** followed by PCC oxidation to deliver the known aldehyde **2-28** (Scheme 6). Horner-Wadsworth-Emmons (HWE) reaction of aldehyde **2-28** gave the known trans α,β-unsaturated ester **2-29**. Reduction followed by Swern oxidation afforded the known α,β-unsaturated aldehyde **2-30**.[40]

**Scheme 6**


HO
OH
2-26
NaH, PMBCl
THF, nBu4NI
65%
HO
OPMB
2-27
Swern
93%
O
OPMB
2-28
EtO
EtO
P
O
O
OEt
(1.5 eq)
Ba(OH)2 (1.5 eq)
73%
PMBO
O
OEt
2-29
1) DIBAL
2) Swern
74%
PMBO
O
H
2-30

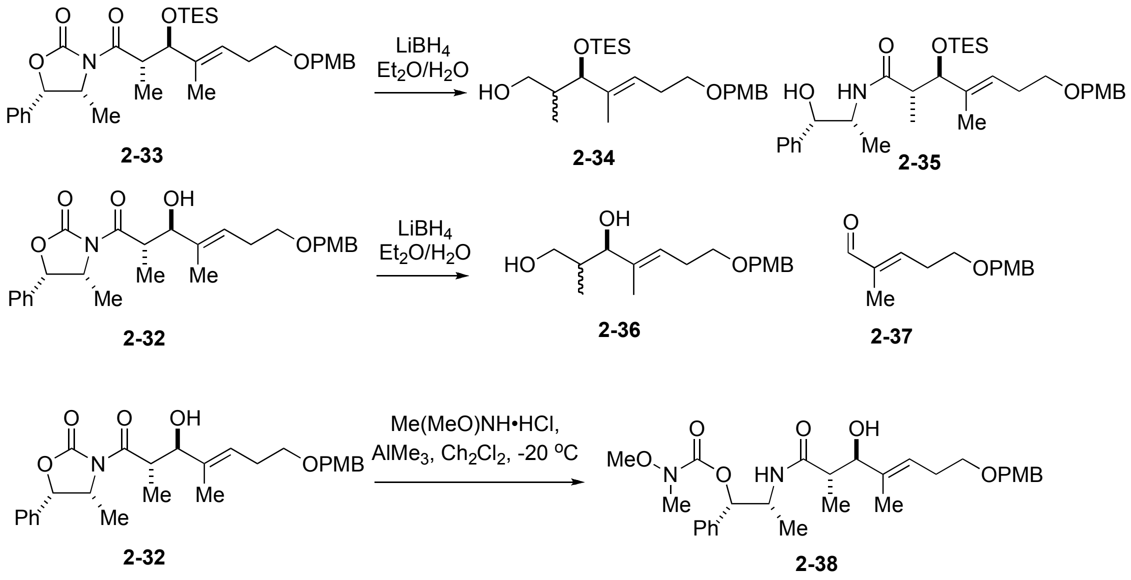
The chiral auxilary, (*S*,*S*)-5-phenyl-3-propionyl-2-oxzolidinone **2-31**, was synthesized according to the proceure of Evans.[41] In order to obtain the anti-aldol adduct **2-32** between C15 and C16 (gynodimine numbering) our synthesis commenced with the Heathcock's typical procedure employing $BBu_2OTf$ followed by aldehyde **2-30** addition (Scheme 7).

**Scheme 7**



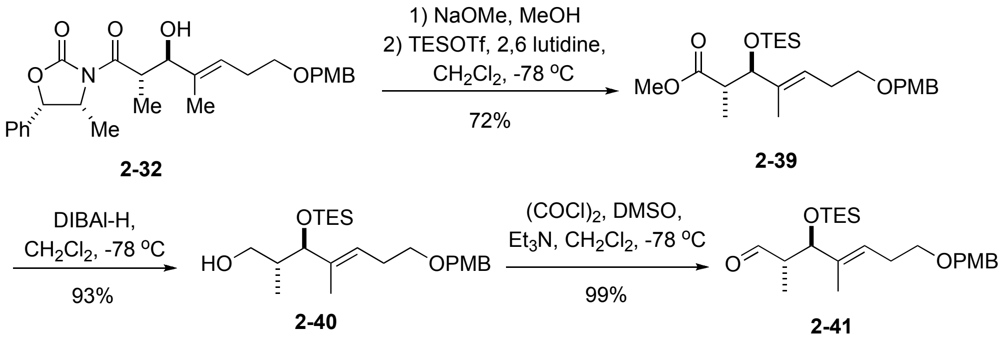
Previous studies by Yang, in the our group, indicated that reductive cleavage of the TES ether **2-33** from the chiral auxiliary with $LiBH_4$ led to extensive epimerization **2-34** and *endo*-carbonyl reduction product **2-35** (Scheme 8). Whereas a reduction of unprotected alcohol **2-32** resulted in a retro-aldol product **2-37**. However, the cleavage of the chiral auxiliary **2-32** with the Weinreb amide resulted in cleavage at the endo carbonyl group with either allylic alcohol protection present or absent due to the extremely hindered nature of the *exo*-carbonyl blocked by the substituents.

## Scheme 8


LiBH4
Et2O/H2O
2-33
2-34
2-35
LiBH4
Et2O/H2O
2-32
2-36
2-37
Me(MeO)NH•HCl,
AlMe3, CH2Cl2, -20 °C
2-32
2-38

After extensive studies, it was determined that methanolysis provided the best conditions for cleavage of the chiral auxiliary from the aldol adduct **2-32** (Scheme 9). Alcohol protection, DIBAL-H reduction, followed by Swern oxidation gave aldehye **2-38**. However, Swern oxidation conditions on large scale led to deprotection of triethyl silyl (TES) group. Therefore, the TES group was switched to the more robust triisopropyl silyl (TIPS) group.

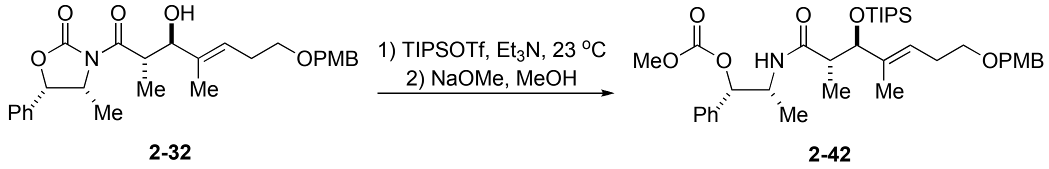
## Scheme 9


1) NaOMe, MeOH
2) TESOTf, 2,6 lutidine,
CH2Cl2, -78 °C
72%
2-32
2-39
DIBAl-H,
CH2Cl2, -78 °C
93%
2-40
(COCl)2, DMSO,
Et3N, CH2Cl2, -78 °C
99%
2-41

When alcohol protection have done prior to methanolysis low regioselectity and slow reactivity were observed due to the extremly hindered nature of the *exo*-

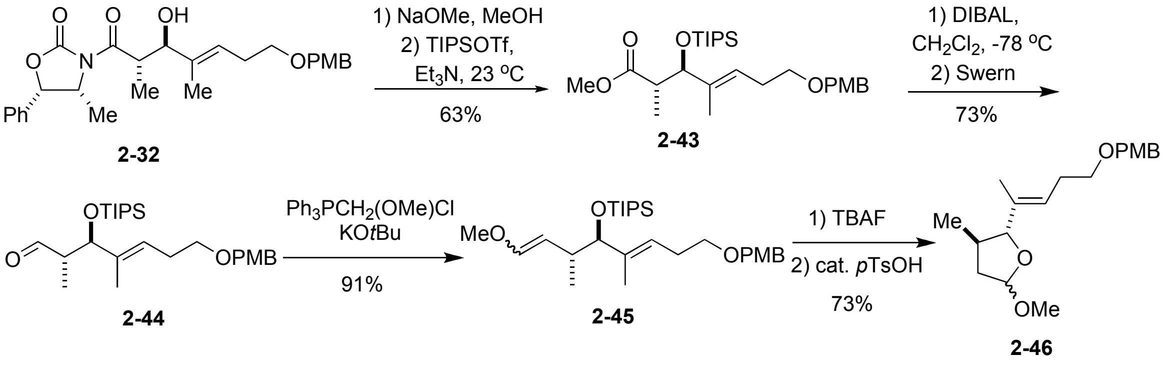
carbonyl group (Scheme 10).

**Scheme 10**


1) TIPSOTf, Et3N, 23 °C
2) NaOMe, MeOH
2-32
2-42

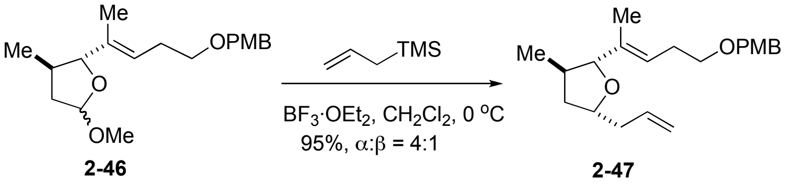
Therefore, removal of the chiral auxiliary **2-32** by methanolysis then allylic alcohol protection with TIPSOTf followed by a reduction-oxidation sequence provided aldehyde **2-44** (Scheme 11). After Wittig reaction, the TIPS group on **2-44** in this sequence did not cleavege to cyclize onto the enol ether to provide furanose **2-46**, so one extra step requiring deprotection was necessary followed by subsequent acid catalyzed cyclization to provide epimeric methoxyfuranose **2-46**.

**Scheme 11**


1) NaOMe, MeOH
2) TIPSOTf, Et3N, 23 °C
63%
1) DIBAL, CH2Cl2, -78 °C
2) Swern
73%
Ph3PCH2(OMe)Cl
KOtBu
91%
1) TBAF
2) cat. pTsOH
73%
2-32
2-43
2-44
2-45
2-46

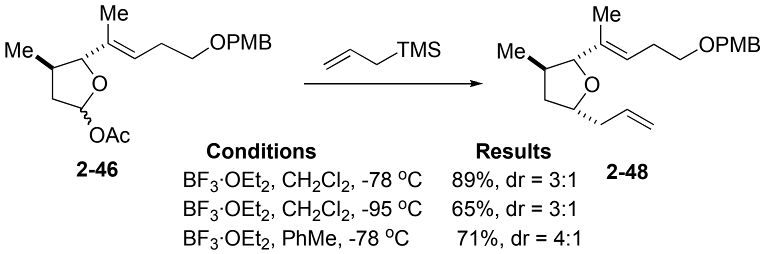
Previous studies by Yang in our group indicated that the allylation of furanose **2-46** with allyl trimethylsilane in the presence of exogenous Lewis acid $BF_3 \bullet OEt_2$ led to moderate diastereoselectivity and excellent yield (Scheme 12).

**Scheme 12**


Me
Me
OPMB
O
OMe
2-46
TMS
$BF_3 \cdot OEt_2$, $CH_2Cl_2$, 0 °C
95%, α:β = 4:1
Me
Me
OPMB
O
2-47

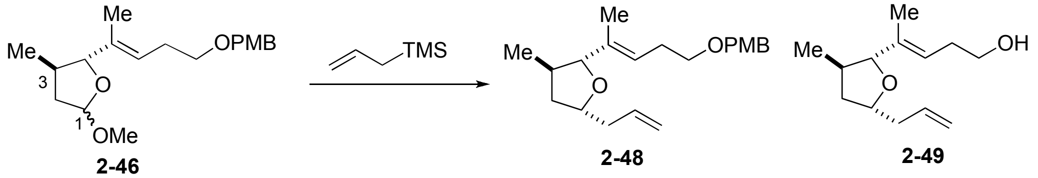
Base on these results, Dr. Kong in our group explored these conditions to enhance the selectivity (Scheme 13). Since the allylation to methoxyfuranose **2-46** did not proceed at lower temperature, Dr. Kong synthesized the more reactive acetoxyfuranose, however reaction temperature and Lewis acids had no effect on the diastereoselectivity. Interestingly, toluene provided comparable diastereoselectivity with good reproducibility.

**Scheme 13**


Me
Me
OPMB
O
OAc
2-46
TMS
Me
Me
OPMB
O
2-48
Conditions
Results
$BF_3 \cdot OEt_2$, $CH_2Cl_2$, -78 °C
89%, dr = 3:1
$BF_3 \cdot OEt_2$, $CH_2Cl_2$, -95 °C
65%, dr = 3:1
$BF_3 \cdot OEt_2$, PhMe, -78 °C
71%, dr = 4:1

These optimized conditions were difficult to reproduce on large scale. Interestingly, some of the primary alcohol **2-49** was observed resulting from cleavage of the PMB group under Lewis acid in Methanol (Scheme 14). Since the next step was PMB deprotection, this result was not a problem. However, low selectivity became a serious problem. Since Dr. Kong mentioned that solvent affected the allylation reaction, dichloromethane was switched to toluene providing similar diastereoselectivity.

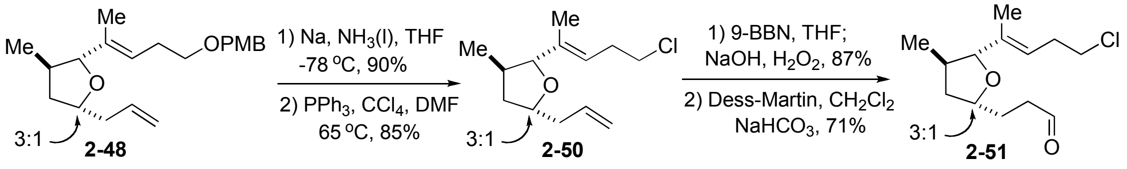
**Scheme 14**


Me
OPMB
Me
3
O
1
OMe
2-46
TMS
Me
OPMB
Me
O
2-48
Me
OH
Me
O
2-49

| Conditions | Results | |
|---|---|---|
| $BF_3$·$OEt_2$, $CH_2Cl_2$, 0 °C | 61%, 111a α:β = 2:1 | 25%, 111b α:β = 2:1 |
| $BF_3$·$OEt_2$, PhMe, 0 °C | 55%, 111a α:β = 3:1 | 19%, 111b α:β = 3:1 |

Reductive cleavage of the PMB ether **2-48** under dissolving metal conditions afforded the alcohol **2-49**. The primary alcohol **2-49** was transformed to the alkyl chloride **2-50** which was then subjected to hydroboration and oxidation to the aldehyde **2-51** (Scheme 15).

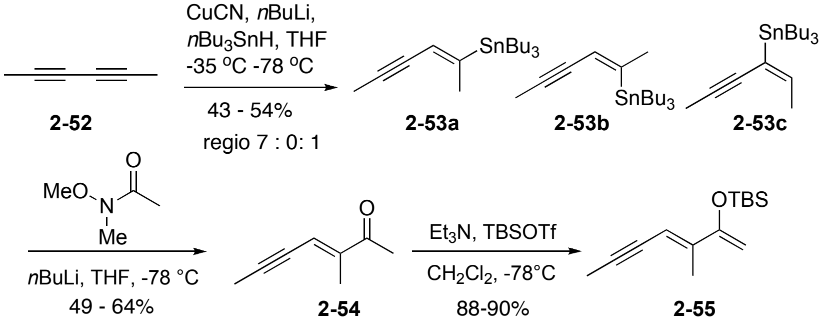
**Scheme 15**


Me
Me
OPMB
O
3:1
2-48
1) Na, NH3(l), THF
-78 °C, 90%
2) PPh3, CCl4, DMF
65 °C, 85%
Me
Me
Cl
O
3:1
2-50
1) 9-BBN, THF;
NaOH, H2O2, 87%
2) Dess-Martin, CH2Cl2
NaHCO3, 71%
Me
Me
Cl
O
3:1
2-51
O

### 2.3.3 Asymmetric Synthesis of the Spirolactam

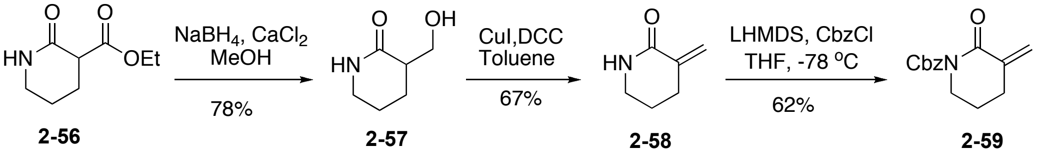
Previous studies from the our group have resulted in concise synthesis of the chiral spirolactam moiety. In the racemic spirolactam synthesis, Yang found that geometry of diene did not effect reaction outcome since both *E*/*Z* olefin lead to the same diastereomer with high diastereoselectivity with a strong Lewis acid such as $Et_2AlCl$. However, in the chiral spirolactam synthesis, Dr. Kong found that only the *E* olefin isomer promoted the Diels-Alder reaction with Evans' copper-bis(oxazoline)hexafluoroantimony complex.[42] Synthesis of diene commenced with stannylcupration of the commercially available 2,4-hexadiyne **2-52** to provide high regioselectivity to give *E* vinyl stannane **2-53a**. In order to obtain similar regioselctivity, slow addition of an external proton source over 30 min as well as slow warming of the reaction to room temperature over 1 hour was important. Fast addition of methanol to quench the reaction provided mixture of **2-53a**, **2-53b**, and **2-53c** (Scheme 16). The optimal conditions used required four equivalents of tributyltinhydride and a large exess of reagents which made it difficult to separate the desired product **2-53a** because tin reagent has similar $R_f$ value with desired compound **2-53a** ($R_f$ = 0.65; hexanes). Although the stannylcupration yield was diminished by using less reagent quivalents, it is beneficial due to the toxicity of tributyltinhydride as well as its price. Tin-lithium exchange of stannane **2-53a** followed by reaction with *N*-methoxy-*N*-methyl acetamide gave the corresponding ketone **2-54**. Subsequent formation of silyenol ether led to the diene **2-55**.

**Scheme 16**


CuCN, *n*BuLi,
*n*Bu$_3$SnH, THF
-35 °C -78 °C
43 - 54%
regio 7 : 0: 1
2-52
SnBu$_3$
2-53a
SnBu$_3$
2-53b
SnBu$_3$
2-53c
MeO
N
Me
O
*n*BuLi, THF, -78 °C
49 - 64%
O
2-54
Et$_3$N, TBSOTf
CH$_2$Cl$_2$, -78°C
88-90%
OTBS
2-55

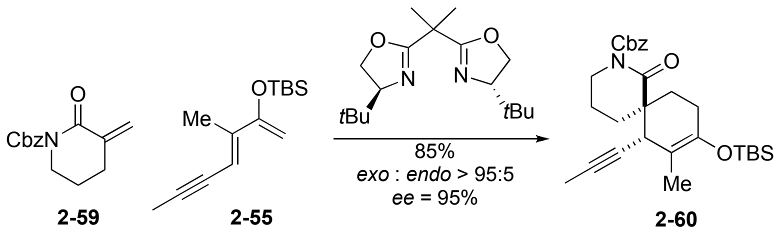
The synthesis of the α-methylene lactam dienophile **2-59** began with reduction of commercially available 3-carbethoxy-2-piperidone **2-56** to alcohol **2-57** and elimination with dicyclohexylcarbodiimide (DCC) produced the α-methylene lactam dienophile **2-58** (Scheme 17). Subsequent protection of lactam **2-58** led to the dienophile **2-59**.

**Scheme 17**


O
O
HN
OEt
2-56
NaBH$_4$, CaCl$_2$
MeOH
78%
O
OH
HN
2-57
CuI,DCC
Toluene
67%
O
HN
2-58
LHMDS, CbzCl
THF, -78 °C
62%
O
CbzN
2-59

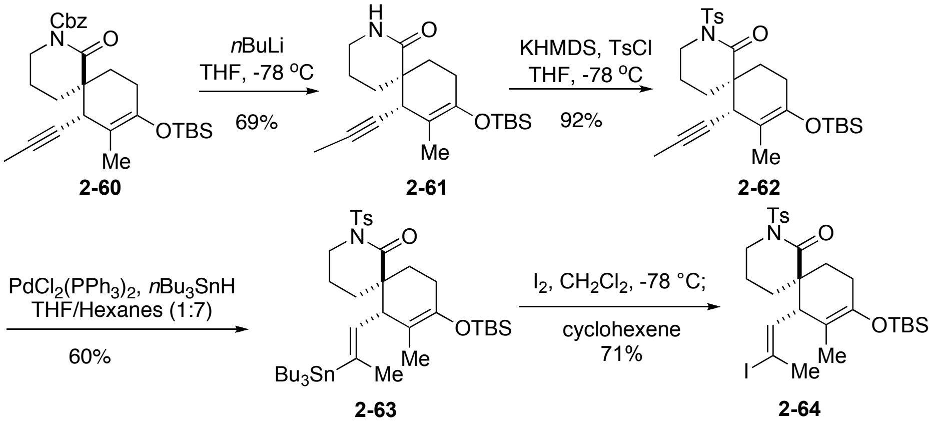
The asymmetric Diels-Alder reaction leading to spirolactam **2-60** proceeded with high enantioselectivity (Scheme 18). Due to the instability of the diene **2-55**, short basic $Al_2O_3$ was used for purification. The asymmetric Diels-Alder was also met with a reproducibility problem on large scale. When the Diels-Alder reaction was carried out on smaller scale it provide good yields and diastereoselectivity. However, when the diene **2-55** was used on a one gram or larger scale the reaction provided very low yields and enantioselectivity. It was fixed simply by purifing the diene **2-55** 2-3 time with the short chromatographies with basic $Al_2O_3$. Since 10 mol % of Evans' copper catalyst was used to promote the asymmetric Diels-Alder reaction, trace amounts of triethyl amine possibly deactivited the copper catalyst.

**Scheme 18**


O
CbzN
2-59
OTBS
Me
2-55
O
N
tBu
O
N
tBu
85%
exo : endo > 95:5
ee = 95%
Cbz
N
O
OTBS
Me
2-60

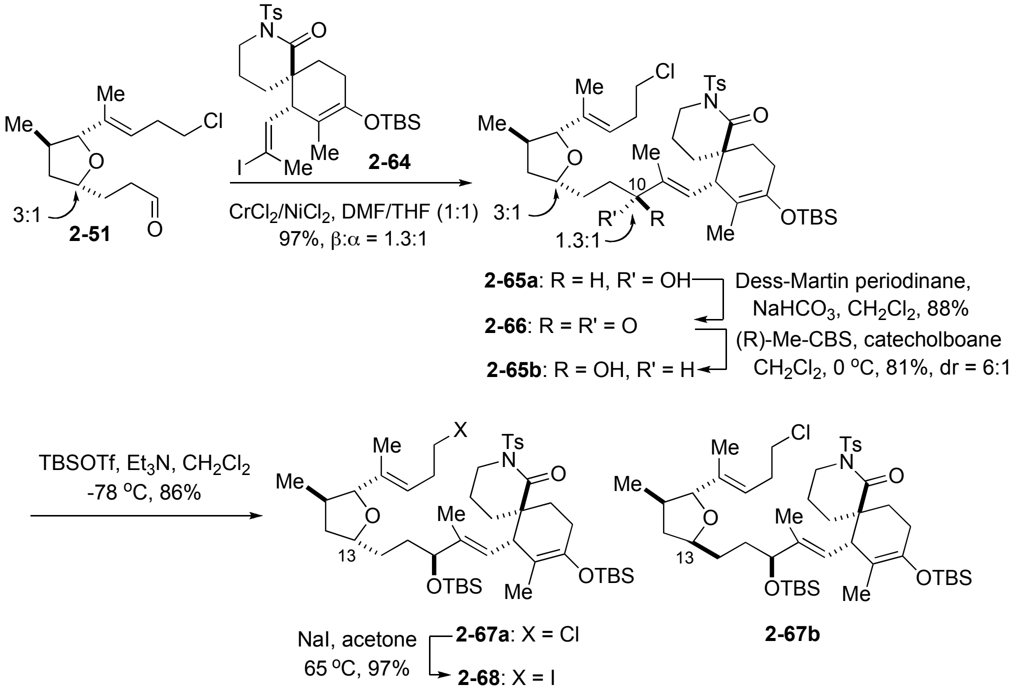
A chemoselective deprotection and conversion to the N-tosyl lactam **2-62** followed by hydrostannylation led to the vinyl stannane **2-63** (Scheme 19).[43] The subsquent conversion of the stannane **2-63** to the corresponding iodide **2-64** required for the Nozaki-Hiyama-Kishi coupling proceeded smoothly.

**Scheme 19**


Cbz
N
O
OTBS
Me
2-60
nBuLi
THF, -78 °C
69%
H
N
O
OTBS
Me
2-61
KHMDS, TsCl
THF, -78 °C
92%
Ts
N
O
OTBS
Me
2-62
PdCl2(PPh3)2, nBu3SnH
THF/Hexanes (1:7)
60%
Ts
N
O
OTBS
Bu3Sn
Me
Me
2-63
I2, CH2Cl2, -78 °C;
cyclohexene
71%
Ts
N
O
OTBS
I
Me
Me
2-64

The smooth intermolecular coupling between vinyl iodide **2-64** and the aldehyde **2-51** provided the allylic alcohols in excellent yield as a 1:1.3 mixture of epimers **2-65a** and **2-65b** (Scheme 20). The undesired $\alpha$-epimer **2-65a** could be converted to desired diastereomer **2-65b** via an oxidation-reduction sequence using the Itsuno-Corey reduction protocol (dr, 6:1) enabling greater material throughput.[44] Allylic alcohol **2-65b** was protected as silyl ether and the C-13 epimer (derived from minor diastereomer from the allylation of the furanose **2-46**, Scheme 14) could be separated at this stage thus allowing a clean pure spectrum to be obtained after seven steps from the allylation of the furanose **2-46**, Scheme 14. Subsequent Finkelstein reaction furnished the requisite alkyl iodide **2-68**.

**Scheme 20**

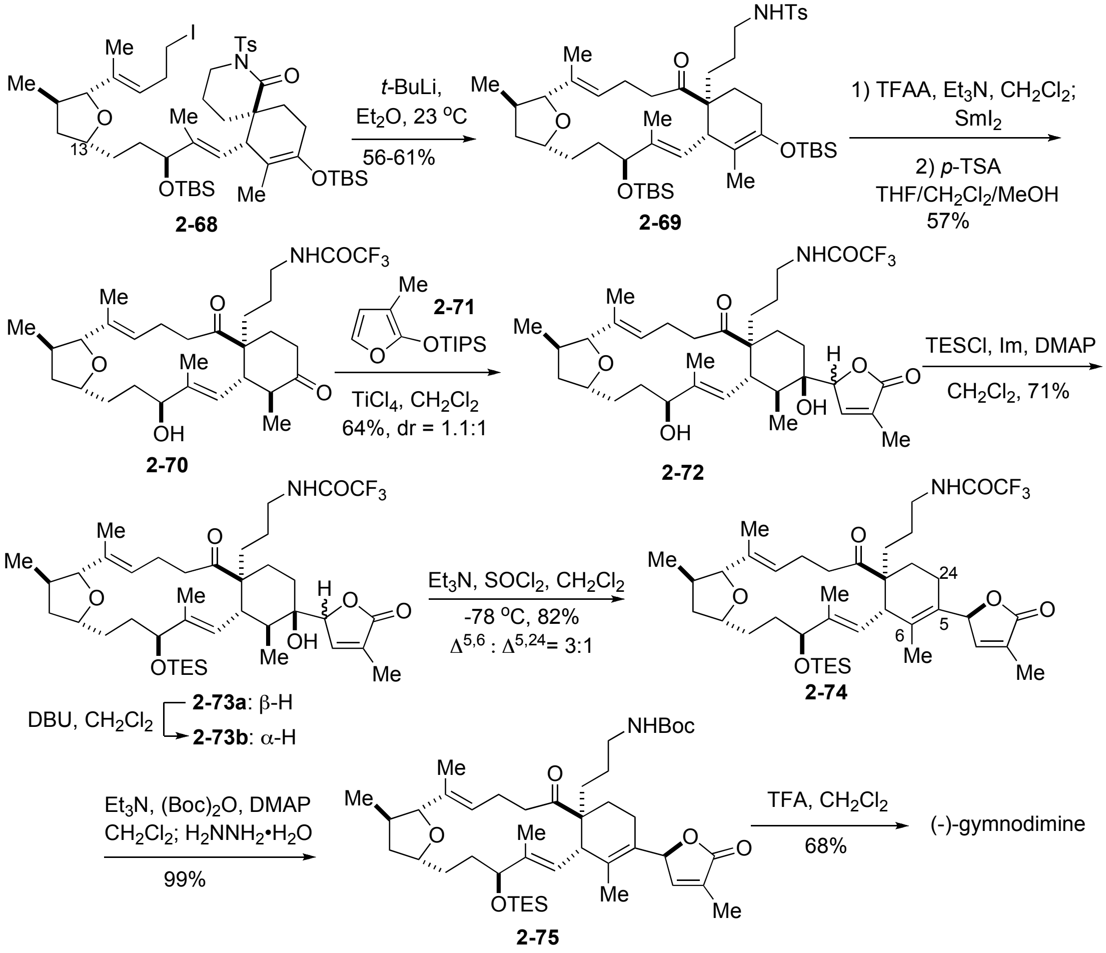


2-51
3:1
2-64
CrCl2/NiCl2, DMF/THF (1:1)
97%, β:α = 1.3:1
3:1
1.3:1
10
2-65a: R = H, R' = OH
2-66: R = R' = O
2-65b: R = OH, R' = H
Dess-Martin periodinane, NaHCO3, CH2Cl2, 88%
(R)-Me-CBS, catecholboane CH2Cl2, 0 °C, 81%, dr = 6:1
TBSOTf, Et3N, CH2Cl2 -78 °C, 86%
13
NaI, acetone 65 °C, 97%
2-67a: X = Cl
2-68: X = I
2-67b

### 2.3.4 Completion of the Synthesis of Gymnodimine

After some experimentation by Dr. Kong in our group, optimal conditions were found for an intramolecular Barbier-type coupling involving halogen-lithium exchange in the presence of the N-tosyl lactam electrophile which provided adduct **2-69** in 56-61 % yields (Scheme 21). The robust tosyl group was switched to a more labile trifluoroacetamide by sequential treatment with base, trifluoroacetic anhydride and samarium diiodide.[45] Both silyl groups in **2-69** were then removed under acidic conditions furnishing the hydroxyl ketone **2-70**. The ketone **2-70** and silyloxyfuran **2-71** was exposed to $TiCl_4$ and provided the $\gamma$-hydroxy-$\alpha$,$\beta$-unsaturated lactone as a mixture diastereomers **2-72**.[46] The two epimeric tertiary alcohols **2-72** were readily seperated after silyl protection. Dehydration of the tertiary alcohol **2-73b** under $SOCl_2/NEt_3$ conditions delivered the tetrasubstituted olefin ($\Delta^{4,5}$) regioisomer **2-74** as the major product.[47] However, tertiary alcohol **2-73a** provided both regioisomers without preference. A variety of basic conditions such as; $K_2CO_3$/MeOH, $NH_3{\cdot}H_2O$/MeOH, LiOH/THF/$H_2O$, $NaHCO_3$/MeOH were screened and all led to decomposition of this sensitive substrate, in agreement with Miles' finding that gymnodimine is unstable under neutral and alkaline pH values.[48] Attempted acid hydrolysis also proved unsuitable. Therefore, the trifluoroacetamide group was switched to a more acid labile group, *t*-butoxycarbonyl group. Hence, treatment of the trifluoroacetamide **2-74** with $(Boc)_2O$ followed by brief exposure to hydrazine provided the *N*-Boc amine **2-75**.[49] Careful treatment of the Boc amine **2-75** with trifluoroacetic acid led to both *t*-butylcarbamate and silylether cleavage. Finally, cyclization to the cyclic imine under vaccum led to (-)-gymnodimine.[44]

## Scheme 21

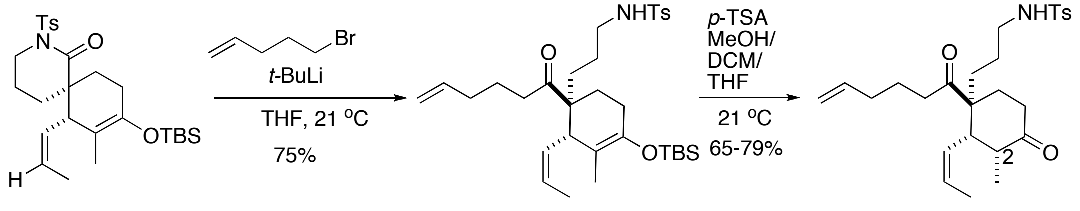

2-68
t-BuLi, Et2O, 23 °C
56-61%
2-69
1) TFAA, Et3N, CH2Cl2; SmI2
2) p-TSA THF/CH2Cl2/MeOH
57%
2-70
2-71
TiCl4, CH2Cl2
64%, dr = 1.1:1
2-72
TESCl, Im, DMAP
CH2Cl2, 71%
2-73a: β-H
DBU, CH2Cl2
2-73b: α-H
Et3N, SOCl2, CH2Cl2
-78 °C, 82%
Δ5,6 : Δ5,24 = 3:1
2-74
Et3N, (Boc)2O, DMAP
CH2Cl2; H2NNH2•H2O
99%
2-75
TFA, CH2Cl2
68%
(-)-gymnodimine

## 2.4 Synthesis of Gymnodimine Haptens

### 2.4.1 Synthesis toward Hapten S

The principal objectives of this research were to develop ELISA, which selectively quantify one chemical compound in the presence of others in its class and to meet the demands of regulatory agencies for inexpensive, accurate and sensitive, routine and portable use. Since selectivity among classes depends on the immunogen, structural design of the hapten becomes increasingly important. Our goal was to develop antibodies recognized gymnodimine congeners without cross-reactivity with other marine toxins. An efficient hapten should be a near perfect mimic of the target structure in size, shape (geometry) and electronic properties. The hapten handle (required for connecting the target mimic to a carrier protein) should not itself elicit antibody recognition. Building on our recently completed total synthesis of (-)-gymnodimine,[50] two haptens are proposed for eventual production of monoclonal antibodies (mAb). One example of a congener-independent hapten to be prepared is the spirocycle hapten **S** (Figure 1.12). Synthesis of hapten **S** began with spirolactam **2-76**, which is the by-product of the NHK reaction (Scheme 22). Treatment of lactam **2-76** with the alkyl lithium reagent afforded the desired amino ketone **2-77** in good yield. Silyl deprotection gave the corresponding ketone **2-78** as 5:1 mixture of two diastereomers. The major diastereomer was α-methyl group of cyclohexanone occupied equatorial and *cis* configuration with β-alkene group. Since stereocenter at C2 will be lost during dehyratation reaction, no need to improve diastereoselectivity of this reaction.

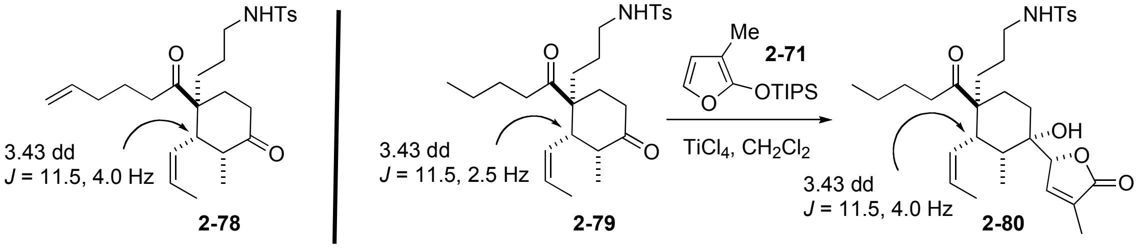
**Scheme 22**



**2-76** **2-77** **2-78**

The relative stereochemistry of ketone **2-78** was determined *via* γ-hydroxy-α,β-unsaturated lactone **2-80** which was derived from addition of siloxyfuran **2-71** to ketone **2-79** (Scheme 23).

**Scheme 23**


NHTs
O
3.43 dd
$J$ = 11.5, 4.0 Hz
O
2-78
NHTs
O
3.43 dd
$J$ = 11.5, 2.5 Hz
O
2-79
Me
2-71
OTIPS
O
TiCl$_4$, CH$_2$Cl$_2$
NHTs
O
OH
O
O
3.43 dd
$J$ = 11.5, 4.0 Hz
2-80

X-ray crystal structure of lactone **2-80** showed substituents on cyclohexane are *syn* to each other (Figure 2.4).

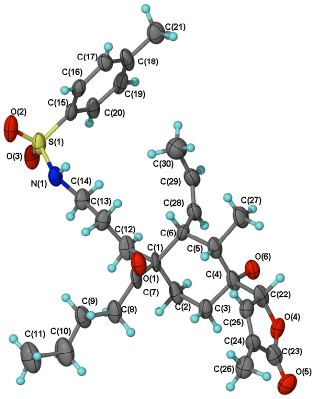
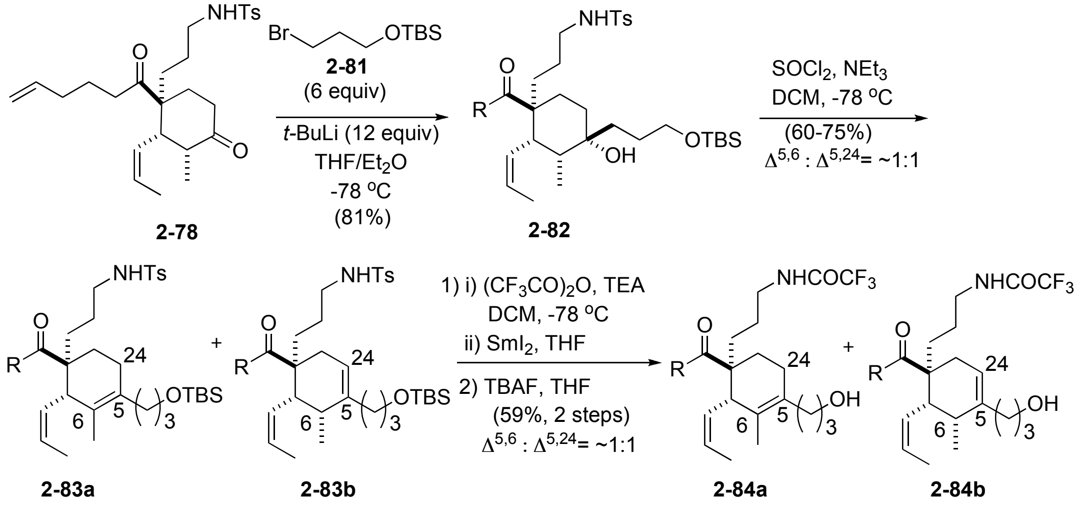

C(21)
C(17)
C(18)
C(16)
C(19)
C(15)
C(20)
O(2)
S(1)
O(3)
N(1)
C(14)
C(13)
C(30)
C(29)
C(28)
C(27)
C(12)
C(6)
C(5)
O(6)
C(1)
O(1)
C(4)
C(7)
C(22)
C(9)
C(8)
C(2)
C(3)
O(4)
C(25)
C(11)
C(10)
C(24)
C(23)
C(26)
O(5)

**Figure 2.4** ORTEP representation of X-ray crystal structure of **2-80**.

Treatment of ketone **2-78** with alkyl bromide **2-81** and *t*-butyl lithium provided corresponding tertiary alcohol **2-82** (Scheme 24). Unexpectedly dehydration of tertiary alcohol **2-82** under thionyl chloride/triethylamine conditions, used successfully in the total synthesis of gymnodimine, led to cyclohexene **2-83a** and **2-83b** as inseparable regioisomers (~1:1). It would be interesting to know weather olefin geometry of haptens is important to generate ideal antibodies which can recognize gymnodimine. In order to seperate two regioisomers, two olefin isomers **2-**

**83a** and **2-83b** were further functionalized. However, they were not seperable at any stage.

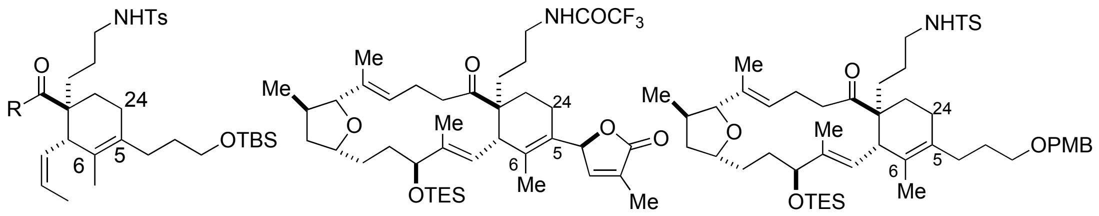
**Scheme 24**


NHTs
Br
OTBS
2-81
(6 equiv)
t-BuLi (12 equiv)
THF/Et2O
-78 °C
(81%)
2-78
R
OH
OTBS
2-82
SOCl2, NEt3
DCM, -78 °C
(60-75%)
Δ5,6 : Δ5,24= ~1:1
24
6
5
3
2-83a
2-83b
1) i) (CF3CO)2O, TEA
DCM, -78 °C
ii) SmI2, THF
2) TBAF, THF
(59%, 2 steps)
Δ5,6 : Δ5,24= ~1:1
NHCOCF3
OH
2-84a
2-84b

Therefore, other dehydration conditions were reconsidered. In order to obtain the desired cyclohexene **2-84**, higher reaction temperatures were tested. Extensive screening of dehydration protocols such as pyridine and $SOCl_2$, $I_2$ and $PPh_3$ and CSA, under refluxing conditions each gave unsatisfactory results.

### 2.4.2. Synthesis of Hapten M

Although dehydration conditions toward hapten **S** provided inseperable mixture of two regioisomers **2-83a** and **2-83b**, the macrocycle intermediate **2-85** (Figure 2.5) toward synthesis of hapten **M** (Figure 1.12) would more likely yield separable isomers based on results toward gymnodimine synthesis. Since geometry of the macrocycle intermediate **2-85** could make conformation change more than acyclic olefin **2-83a** and **2-83b**, physical property of two regioisomers would be more different.


NHTs
O
R
24
6
5
OTBS
NHCOCF3
Me
O
Me
Me
O
24
O
5
O
6
Me
OTES
Me
NHTS
Me
O
Me
O
Me
24
5
OPMB
6
Me
OTES

**2-83a**
inseperable
$\Delta^{5,6} : \Delta^{5,24} = \sim 1{:}1$

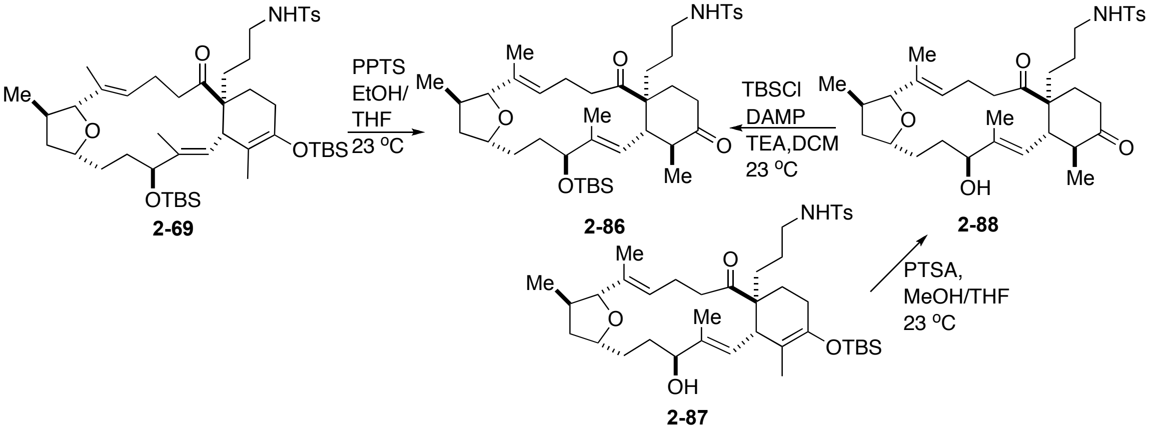
**2-74**
seperable
$\Delta^{5,6} : \Delta^{5,24} = 3{:}1$

**2-85**
seperable ?
$\Delta^{5,6} : \Delta^{5,24} = ?$

**Figure 2.5**. Dehydration results and desired intermediate of hapten M.

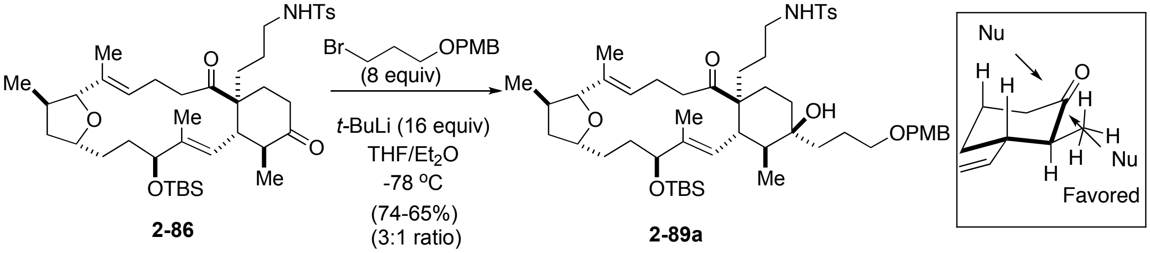
The macrocyclic hapten **M** started from known ketone **2-69** (Scheme 21). With macrocycle **2-69** in hand, the site selective deprotection of the silyl group was studied. The acidic conditions toward synthesis of gymnodimine did not differentiate two silyl group under various temperature. Therefore relatively strong acid conditions was switched to buffered PPTS conditions (Scheme 25). After stirring for 48h, 53 % of desired product **2-86**, ~4% of allylic alcohol **2-87**, and ~11 % of ketone **2-88** was obtained. Although desired product **2-85** was obtained in moderate yields, the rest of material could be recycled. Allylic alcohol **2-87** could be converted to ketone **2-88** and allylic alcohol **2-87** could be protected with TBSCl, to provide the desired ketone **2-86**. After recycled undesired products, overall yield was good. Therefore, further optimization would not be necessary.

**Scheme 25**


NHTs
O
Me
O
OTBS
OTBS
2-69
PPTS
EtOH/
THF
23 °C
NHTs
Me
O
Me
O
Me
O
Me
OTBS
2-86
TBSCl
DAMP
TEA,DCM
23 °C
NHTs
Me
O
Me
O
Me
O
Me
OH
2-88
NHTs
Me
O
Me
O
Me
OTBS
OH
2-87
PTSA,
MeOH/THF
23 °C

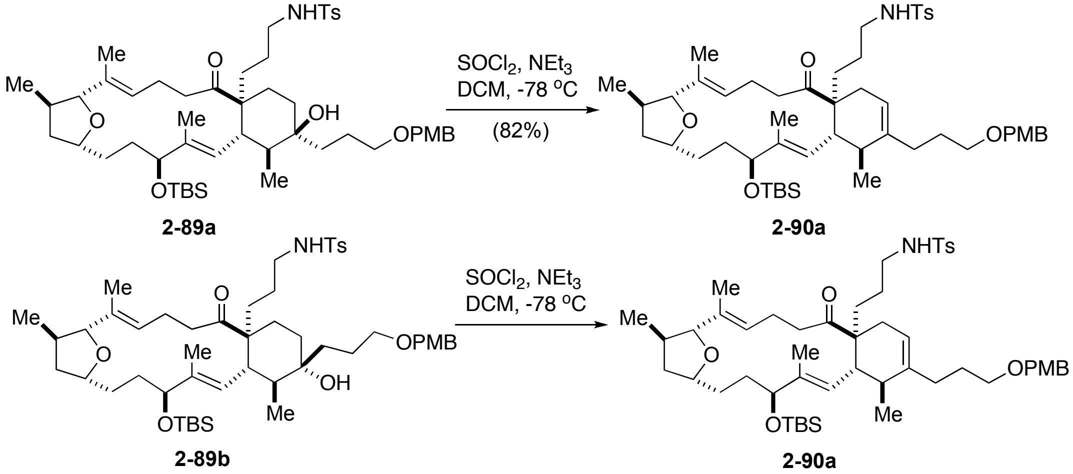
As mentioned before, the butenolide moiety will be replaced with a linker, which will connect with a carrier protein. It was known that addition of organoalkali reagents to the α-methyl group of cyclohexanone is attacked from equatorial position (Scheme 26). Since the α-methyl group introduces a pseudo-axial hydrogen into the molecule which increase hinderance of attack from the axial position.[51] Unexpectely these conditions provided two diastereomers **2-89a** and relative stereochemistry was tentativly assigned.

**Scheme 26**


NHTs
Me
O
Me
O
Me
O
OTBS
Me
2-86
Br
OPMB
(8 equiv)
t-BuLi (16 equiv)
THF/Et2O
-78 °C
(74-65%)
(3:1 ratio)
OH
OPMB
2-89a
Nu
H
H
O
H
H
H
Nu
H
Favored

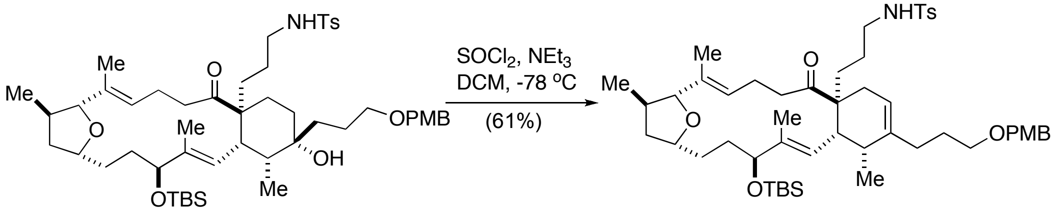
Newly generated stereogenic center will be lost during dehydration. Therefore diastereomerically enriched tertiary alcohol **2-89a** was subjected to dehydration conditions (Scheme 27). However, undesired trisubstituted olefin isomer **2-90a** was found to be the sole product under thionyl chloride/triethylamine conditions. Diastereomer tertiary alcohol **2-89b** also gave trisubstituted olefin isomer **2-90a** but unexpectedly $^1$H NMR spectrum of **2-90a** from **2-89a** and **2-89b** did not match.

**Scheme 27**


NHTs
Me
O
Me
O
Me
OH
OPMB
OTBS
Me
2-89a
SOCl2, NEt3
DCM, -78 °C
(82%)
2-90a
OH
2-89b
SOCl2, NEt3
DCM, -78 °C
2-90a

If the assignment of tertiary alcohol **2-89a** and **2-89b** was correct, both diastereomers **2-89a** and **2-89b** would give same product **2-90a**. Therefore structure of tertiary alcool **2-89b** would be more likely **2-89c** (Scheme 28).

**Scheme 28**

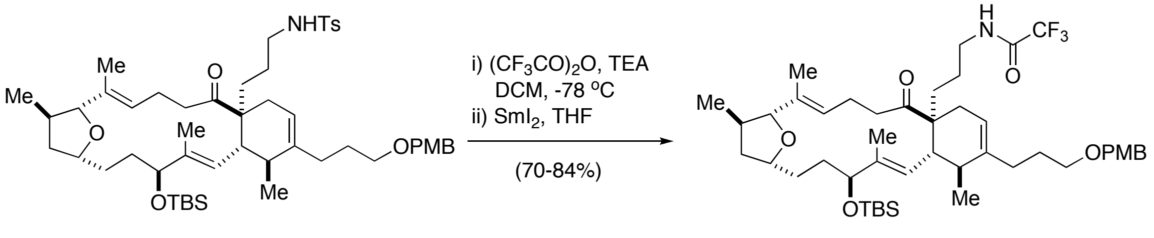

NHTs
Me
O
Me
O
Me
OTBS
Me
OPMB
OH
SOCl2, NEt3
DCM, -78 °C
(61%)
NHTs
Me
O
Me
O
Me
OTBS
Me
OPMB

**2-89c**

**2-90b**

In order to obtain the desired cyclohexene, higher reaction temperatures were tested. As I expected, extensive screening of dehydration protocols such as Pyridine and $SOCl_2$, $I_2$ and $PPh_3$ and CSA, under refluxing conditions each did not change the regioselectivity. Since the main goal of this project is to produce an antibody, which could recognize gymnodimine congeners, the geometry of the cyclohexene component might not be important in this respect. Therefore, macrocycle haxene **2-90a** was carried on in future steps (Scheme 29). The robust tosyl group was switched to a more labile trifluoroacetamide by sequential treatment with base, trifluoroacetic anhydride ad samarium diiodie and desired product **2-91** was obtained.

**Scheme 29**

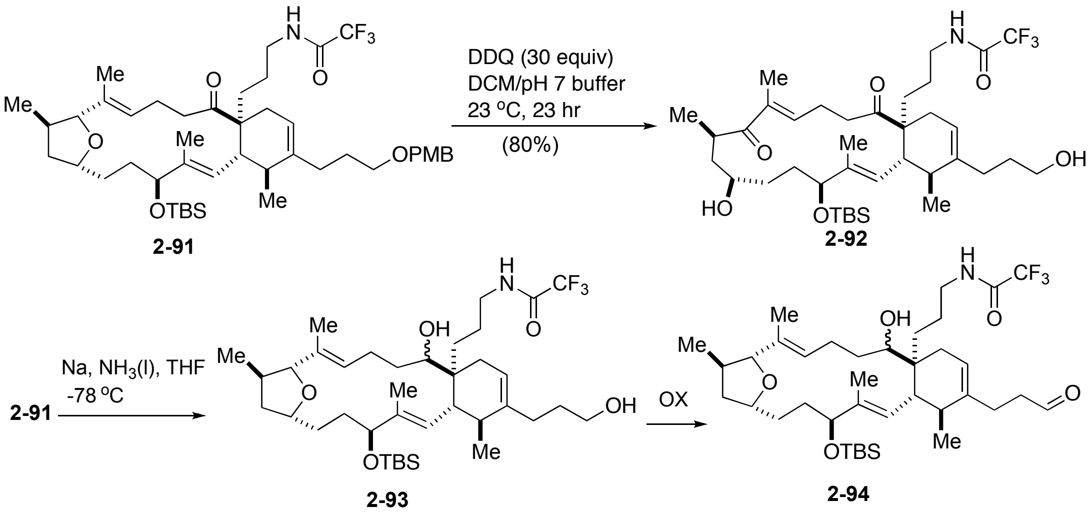

NHTs
Me
O
Me
O
Me
OTBS
Me
OPMB
i) (CF3CO)2O, TEA
DCM, -78 °C
ii) SmI2, THF
(70-84%)
H
N
CF3
O
Me
O
Me
O
Me
OTBS
Me
OPMB

**2-90a**

**2-91**

Deprotection of the PMB group was performed under DDQ and pH 7 buffer conditions.[52] Although PMB deprotection conditions with DDQ and CAN required relatively higher concentration (0.1-0.05 M), the conditions used here were quite dilute. In order to overcome lower substrate concentration, large excess amount of DDQ was employed. Although the PMB group was removed under these conditions, suprisingly furan ring also oxidized to the compound **2-92** (Scheme 30). During the synthesis of THF fragment toward gymnodimine, Birch conditions provided better yields than oxidative cleavage of PMB ether **2-49** (Scheme 15) using DDQ/H2O conditions. Since dissolving metal conditions reduced carbonyl group as well, oxidation of resulting alcohol **2-93** would be required. However, various oxidation conditions such as Dess-Martin periodinane (DMP), Swern and collins oxidation gave multiple products. Presumably oxidation was only occurred at primary alcohol to deliver the aldehyde **2-94**. Since we have limited amount macrocycle intermediate **2-69** (Scheme 25), this route was abandoned and a more productive strategy was pursued.

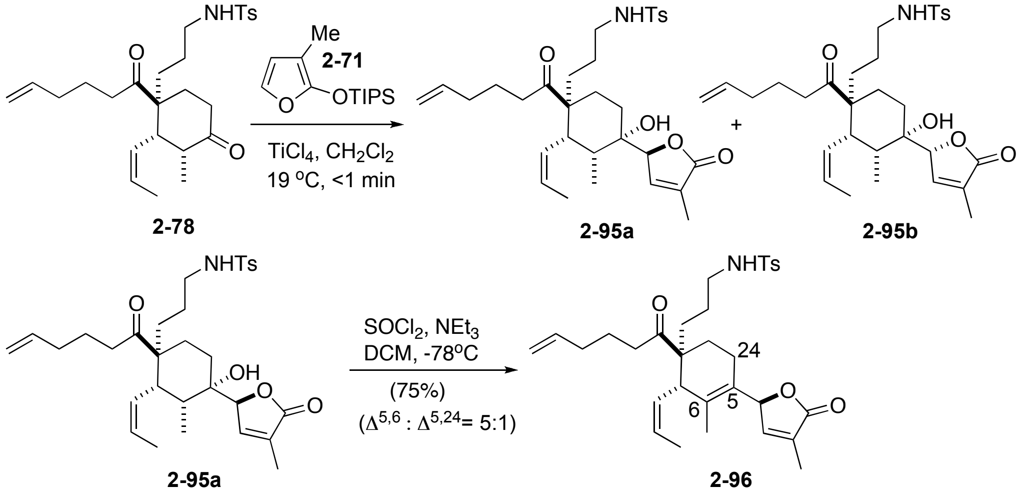
**Scheme 30**



### 2.4.3 Second Generation Synthesis toward Hapten S

In order to obtain the desired olefin isomer, we reexamined the original approach with butenolide addition followed by dehydration. We envisioned a late-stage opening of the butenolide with amino acid derivatives. This approach would also provide an acid tether suitable for further manipulation. The mukaiyama aldol addition to ketone **2-95** in moderate yields with mixture of diastereomers **2-95a** and **2-95b** (Scheme 31). Pure tertiary alcohols **2-95a** was subjected to dehydration conditions and as expected, desired tetrasubstituted olefin **2-96** was obtained with good regioselctivity.

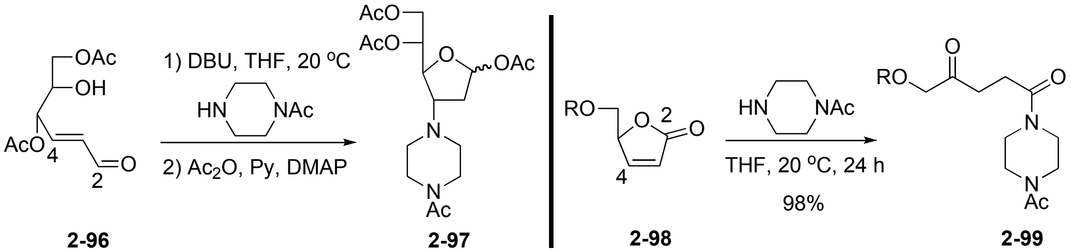
**Scheme 31**



Previously, Dr. Ziad, a former postdoc in our group found that the tosyl group was difficult to cleave under a variety of conditions. Specially, the unstable betenolide group caused many problems during the protecting group swich. Therefore the trifluoroacetyl group was installed at early stage due to the presumed instability of butenolide. However, for the hapten synthesis, butenolide **2-96** will be opened so the trifluoroacetyl group could be installed after amide bond formation. In addition, the opening of the butenolide **2-96** would be the biggest challenging step for hapten synthesis which was desired to carry out at the early state.

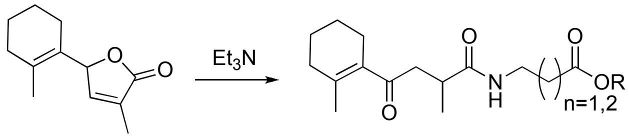
Wengel and coworkers reported interesting observations which piperazine added at C2 position of furanone **2-98** instead of C4 position (Scheme 32).[53] The formation of **2-98** can be explained by a nucleophic attack at C2 and concomitant ring opening assisted by the acidity of the C5 proton.

**Scheme 32**

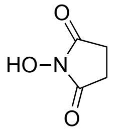
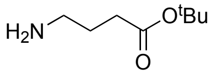
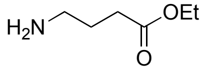
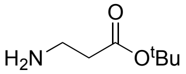
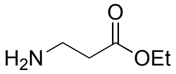
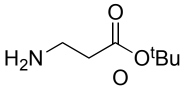
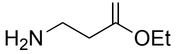


Inspired by these conditions, several nucleophiles were chosen instead of piperidine and explored at room temperature and no desired product was obtained (entry 1-6, Table 2.1). The reaction might require Lewis acid catalyst or higher temperature. Lewis acid catalyst showed no desired products and some of starting material was recovered (entry 5, 6). The butenolide **2-100** was mixed with excess aminoacid ester in triethylamine as a solvent at 40 °C (entry 7, 8). Interestingly, β-alanine ethylester gave desired product **2-101** but *t*-butyl aminobutyrate did not gave desired product. Initially, I speculated that different solubility of amino esters in triethylamine caused different results, but using co-solvent with triethylamine did not improve the yields.

**Table 2.1**. Optimization of the opening of butenolide moiety

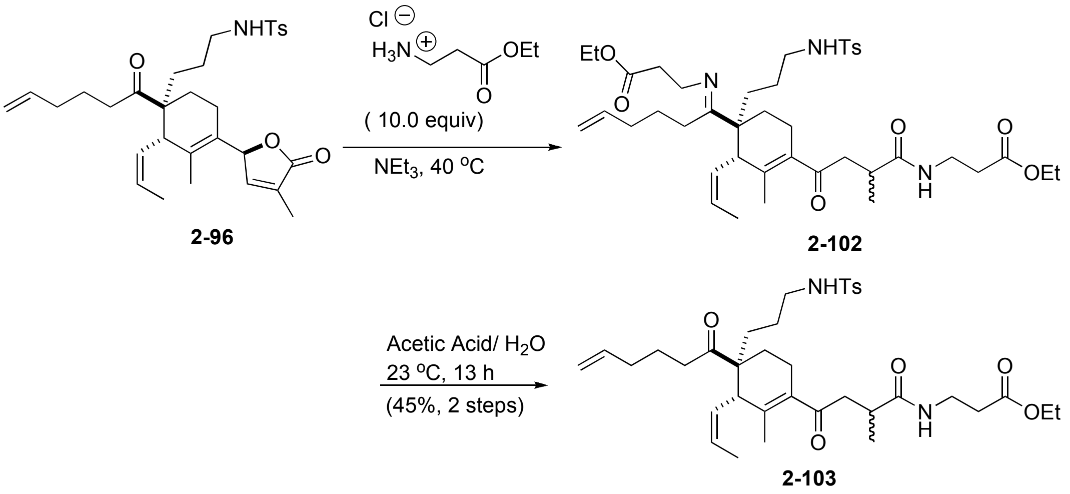


**2-100** **2-101**

| Entry | Nucleophile | catalyst | solvent | Temperature | product |
|---|---|---|---|---|---|
| 1 | LiOH | | MeOH | 23 °C | No desired Product |
| 2 |  | DMAP | | 23 °C | No desired Product |
| 3 |  | | THF | 23 °C | SM |
| 4 |  | | THF | 23 °C | SM |
| 5 |  | Me$_2$AlCl | CH$_2$Cl$_2$ | 23 °C | No desired Product |
| 6 |  | Me$_2$AlCl | CH$_2$Cl$_2$ | 23 °C | No desired Product |
| 7 |  | | Et$_3$N | 40 °C | No desired Product |
| 8 |  | | Et$_3$N | 40 °C | 46 % |

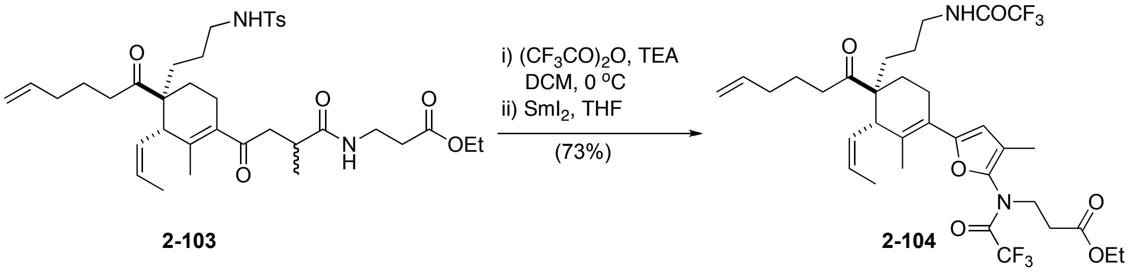
After developing a protocol for opening butenolide with aminoacids, this conditions applied to the butenolide **2-96** (Scheme 33). Interestingly, $^1$H NMR and MS supported intermolecular imine **2-102** as a major compound which was hydrolyzed under acid conditions to give mixture of ketone **2-103**.

**Scheme 33**


NHTs
O
Cl ⊖
H3N ⊕
OEt
O
( 10.0 equiv)
NEt3, 40 °C
O
O
2-96
EtO
O
N
NHTs
O
O
N
H
OEt
O
2-102
Acetic Acid/ H2O
23 °C, 13 h
(45%, 2 steps)
NHTs
O
O
O
N
H
OEt
O
2-103

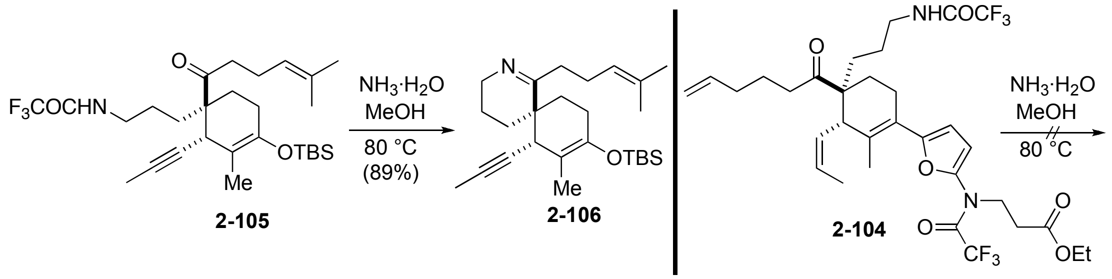
The robust tosyl group was switched to a more labile trifluoroacetamide by sequential treatment with base, trifluoroacetic anhydride and samarium diiodide (Scheme 34). Interestingly mixture of two diastereomers **2-103** became a single product due to formation of furan **2-104**.

**Scheme 34**


NHTs
O
O
O
N
H
OEt
O
2-103
i) (CF3CO)2O, TEA
DCM, 0 °C
ii) SmI2, THF
(73%)
NHCOCF3
O
O
N
O
CF3
O
OEt
2-104

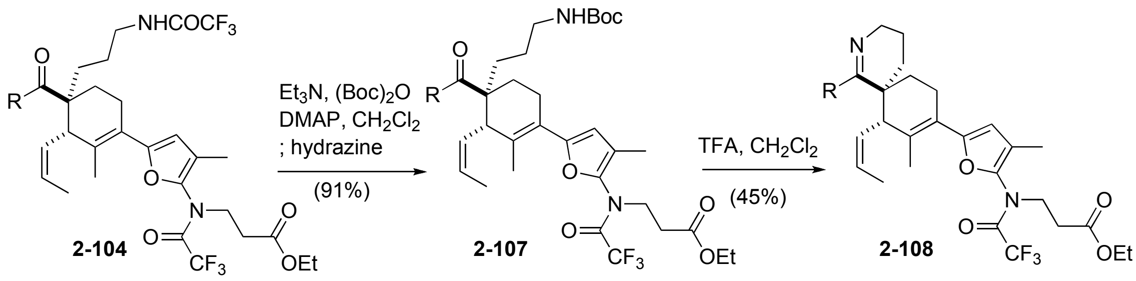
Priviously cleavage of the trifluoroacetamide **2-105** with warm ammonium hydroxide led to concomitant cyclization to provide imine **2-106**[39] but this condition led to many products (Scheme 35).

**Scheme 35**


F3COCHN
O
OTBS
Me
2-105
NH3·H2O
MeOH
80 °C
(89%)
N
OTBS
Me
2-106
NHCOCF3
O
NH3·H2O
MeOH
80 °C
O
N
O
O
CF3
OEt
2-104

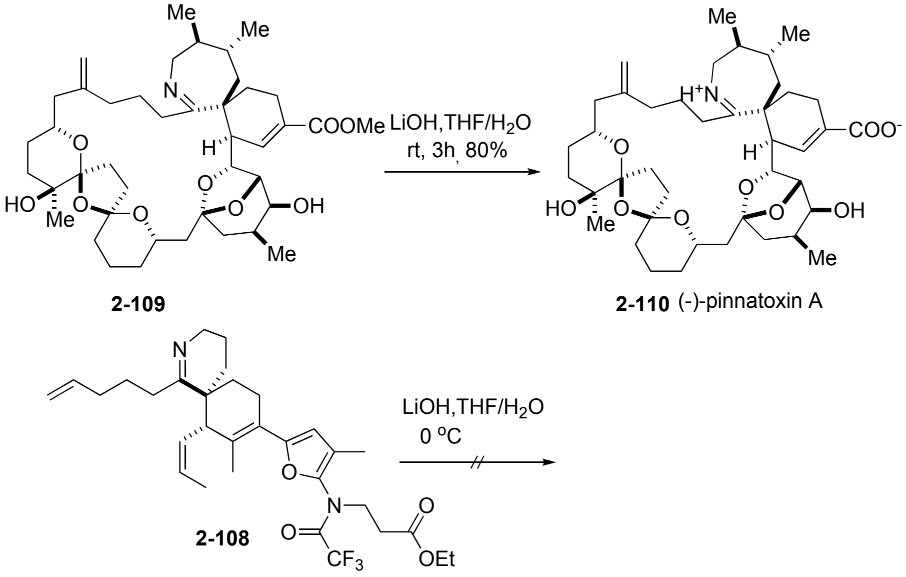
Therefore mild protocol for imine formation of gymnodimine was employed. Hence, treatment of the trifluoroacetamide **2-104** with $(Boc)_2O$ followed by brief exposure to hydrazine provided the *N*-Boc amine **2-107**. Careful treatment of the Boc amine **2-107** with trifluoroacetic acid led to both *t*-butylcarbamate and silylether cleavage (Scheme 36). Finally, cyclization to the cyclic imine under vaccum led to desired imine **2-108**.

**Scheme 36**


NHCOCF3
O
R
O
N
O
O
CF3
OEt
2-104
Et3N, (Boc)2O
DMAP, CH2Cl2
; hydrazine
(91%)
NHBoc
O
R
O
N
O
O
CF3
OEt
2-107
TFA, CH2Cl2
(45%)
N
R
O
N
O
O
CF3
OEt
2-108

In order to conjugate with carrier protein, hyderolysis of ethyl ester **108** was exposure to lithium hydroxide in aqueous tetrahydrofuran (Scheme 37). These conditions were utilized to synthesis of pinnatoxin A by Zakarian and coworkers.[54] However, imine **2-108** provided multiple products under these conditions.

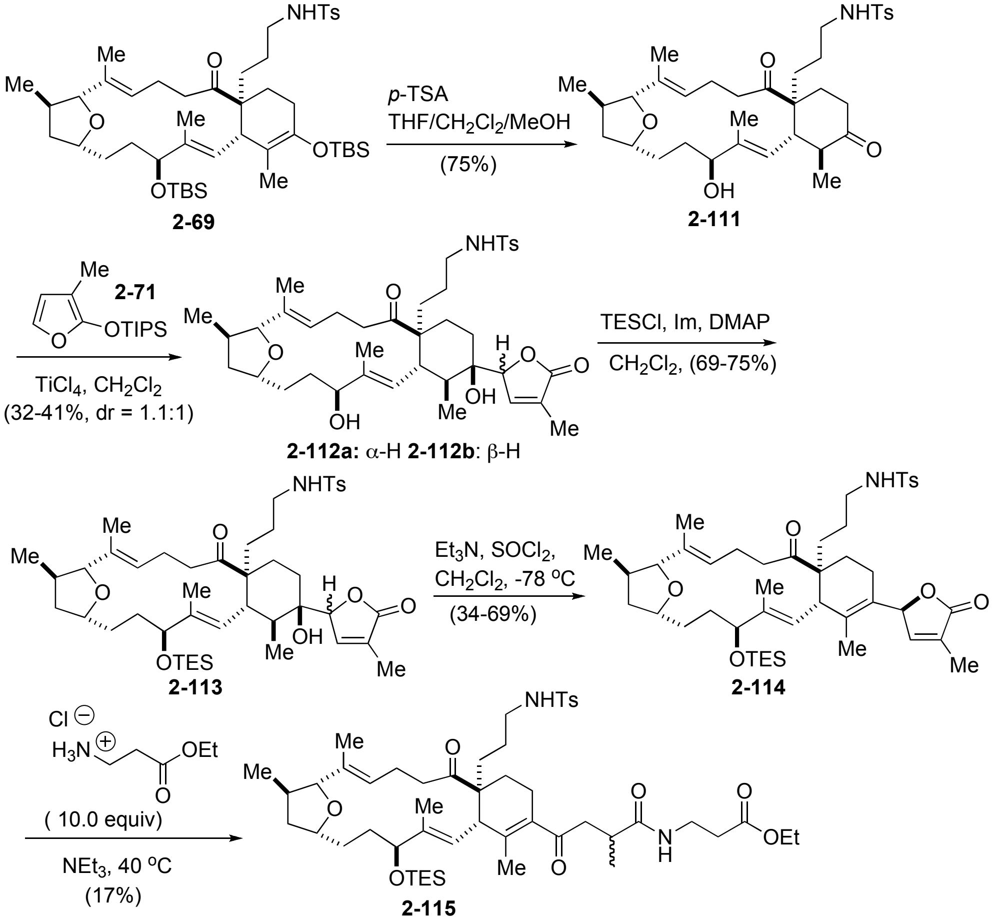
**Scheme 37**



### 2.4.4. Second Generation Synthesis toward Hapten M

In order to obtain the desired olefin isomer, we reexamined the original approach with butenolide addition followed by dehydration. We envisioned a late-stage opening of the butenolide with aminoacid derivatives. The mukaiyama aldol addition to ketone **2-69** in moderate yields with mixture of diastereomers **2-112a** and **2-112b** (Scheme 38). The two epimeric tertiary alcohols **2-112** were readily seperated after silyl protection. Dehydration of the tertiary alcohol **2-112b** under $SOCl_2/NEt_3$ conditions delivered the tetrasubstituted olefin ($\Delta^{4,5}$) regioisomer **2-113** as the major product. The butenolide **2-114** was mixed with excess aminoacid ester in triethylamine as a solvent at 40 °C. Although these conditions provided desired product **2-115**, complex molecule **2-114** provided more compounds than **2-96** such as dehyderation of allylic alcohol and other substrate related compounds. Using same methods of gymnodimine synthesis required a lot of material input and the key step to generate linker on gymnodimine moiety needed to study more to find better conditions.

**Scheme 38**


2-69
p-TSA
THF/CH2Cl2/MeOH
(75%)
2-111
2-71
TiCl4, CH2Cl2
(32-41%, dr = 1.1:1)
2-112a: α-H 2-112b: β-H
TESCl, Im, DMAP
CH2Cl2, (69-75%)
2-113
Et3N, SOCl2,
CH2Cl2, -78 °C
(34-69%)
2-114
( 10.0 equiv)
NEt3, 40 °C
(17%)
2-115

.

## 2.5 Conclusions

Consumption of seafood contaminated by marine toxins has been a long-standing public health problem. Gymnodimine is a member of the spirocyclic imine family of marine toxins initially isolated from oysters collected off the coast of New Zealand. Current detection methods for gymnodimines include mouse bioassay and LC-MS and recently Botana and coworkers developed fluoresence polarization mathods which used the high binding affinity of gymnodimine to nAChRs.[6] This method is an inhibition assay so it will recognized any toxins which can interact with nAChRs. Since antibody immunoassay is structural recognition, ELISA will be complementary analytical techniques. Therefore, building on our recently completed total synthesis of (-)-gymondimine, haptens were designed and studied for eventual production of monoclonal antibodies (Mab). We have completed the synthesis of the cores of hapten S and hapten M. This will enable subsequent conjugation to carrier proteins.

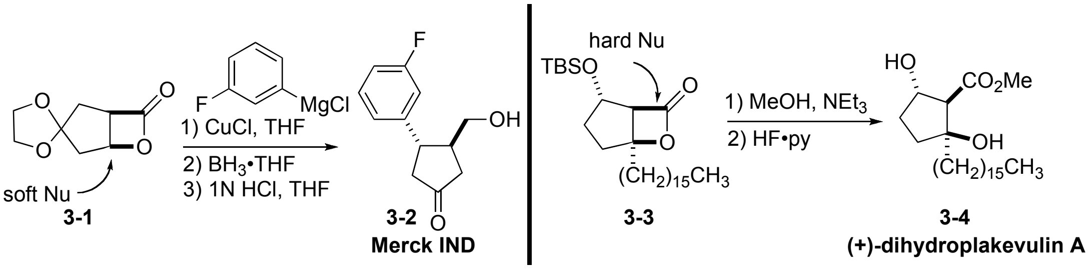
# CHAPTER III

# INTRAMOLECULAR NUCLEOPHILE CATALYZED, ALDOL-LACTONIZATIONS (NCAL) OF KETO ACIDS*

## 3.1 Previous Nucleophile Catalyzed Aldol-Lactonizations

Although the first β -lactone synthesis was demonstrated by Einhorn in 1883, it was not until almost 100 years later that β-lactones have attracted much attention, primarily due to their versatility as synthetic intermediates.[55] β-lactones have unique bielectrophilic character as a result of the strain present in their compact structures. As a result, soft nucleophiles such as Norman reagent ($R_2CuMgX$) lead to alkyl C-O cleavage, whereas hard nucleophiles such as alkoxy or amino group lead to acylation.[56] Our group synthesized two representative natural products such as Merck IND [57] and dihydroplakevulin A[58] utilizing this dual electrophilic character of β-lactones (Scheme 39).

Scheme 39



soft Nu
3-1
F
MgCl
1) CuCl, THF
2) BH3•THF
3) 1N HCl, THF
F
OH
3-2
Merck IND
hard Nu
TBSO
(CH2)15CH3
3-3
1) MeOH, NEt3
2) HF•py
HO
CO2Me
OH
(CH2)15CH3
3-4
(+)-dihydroplakevulin A

In addition, many β-lactone-containing natural products have been isolated and

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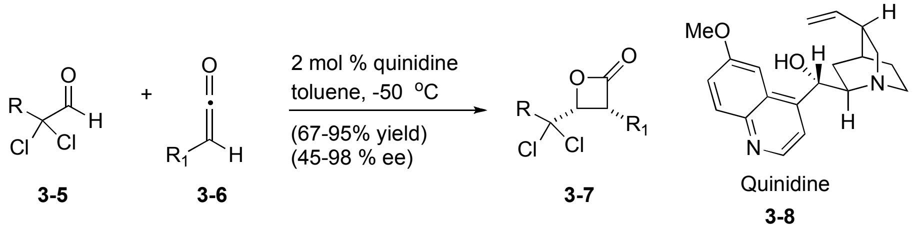
*Reprinted with permission from "Bicyclic and Tricyclic-β-lactones via Organonucleophile Promoted Bis-Cyclization of Keto Acids: Enantioselective Synthesis of (+)-Dihydroplakevulin" by Henry-Riyad, H.; Lee, C.; Purohit, V. C.; Romo, D. *Org. Lett.* **2006**, *8*, 4363-4366. Copyright 2006 American Chemical Society.

synthesized including panclicin D, orlistat, valilactone, salinosporamide A, and oxazolomycin. Besides drug or drug-like biological properties of these natural products, we have elucidated important information regarding structure-activity relationships and enzyme inhibition. Classical routes to β-lactones have generally involved the cyclization of β-halocarboxylate salts and the related "deaminative cyclization" which occurs upon diazotization of β-amino acids.[59] β-Hydroxy acids acids have been reported to undergo a similar cyclization under Mitsunobu[60] conditions, and the halolactonization of α, β-unsaturated acids[61] is a related process of some interest. Ketene participation in Lewis acid catalyzed [2+2] cycloadditions involving carbonyl compounds,[62] and addition reactions involving alkynolate[63] have been other utilized routes for generating β-lactone intermediates. Although these classical methods have been successfully employed for the preparation of a variety of β-lactones, it should be noted that their utility is often limited by undesired side reactions including β-elimination (to form α,β-unsaturated acids) and decarboxylative elimination (to generate alkenes). Few methods have been reported to permit the direct conversion of ketones and aldehdyes to β-lactones in a single step. The most popular method for this transformation of ketones and aldehydes to β-lactones employs a two-stage protocol: condensation of the carbonyl compound with a carboxylic acid dianion,[64] followed by benzenesulfonyl chloride in pyridine induced cyclization.[65] In 1982, Wynberg and Staring,[56a] building on earlier work by Borrmann and Wegler,[66] reported an asymmetric route for construction of β-lactones where the stereochemical setting step was dictated by a chiral Lewis base (Scheme 40).

**Scheme 40**



The mechanism proposed by Wynberg involves initial attack of ketene **3-6** by the chiral amine **3-8** to form the neutral ammonium enolate intermediate **A** (Figure 3.1). The enolate **A** then undergoes an aldol reaction with aldehyde **3-5** to form aldolate **B**, followed by cyclization to give oxetane **C**. Subsequent elimination delivers the chiral β-lactone **3-8**, thus regenerating the catalyst.

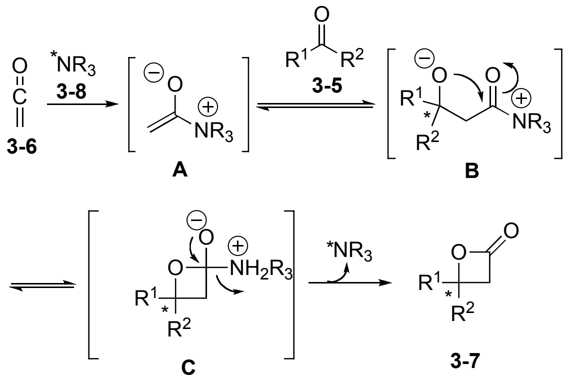


**Figure 3.1** Wynberg's proposed catalytic cycle with quinidine catalyst.

While the Wynberg process is an important method for generating β-lactones, this method requires use of activated aldehyde and a ketene generator. Therefore, several other research groups were prompted to expand upon and improve this promising advancement. In 1999, Nelson and coworkers reported this transformation by using chiral Al(III)[triamine] **3-9** catalyzed cyclo-condensations of acyl halides and non-activated aldehydes (Figure 3.2).[67] Fu and co-workers have been exploring the use of planar-chiral, 4-pyrrolidinopyridine derivatives such as **3-10** (PPY), now shown to provide disubstituted β-lactones from disubstituted ketenes.[68] Most recently, the groups of Bode, Glorious, Nair, and Scheidt groups employed *N*-heterocyclic carbenes (NHC) **3-11** and **3-12** as nucleophile to promote aldol-lactonizations leading to β- and γ-lactones and cyclopentens.[69]

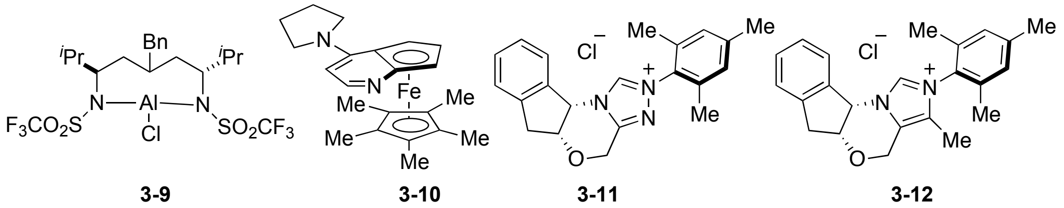
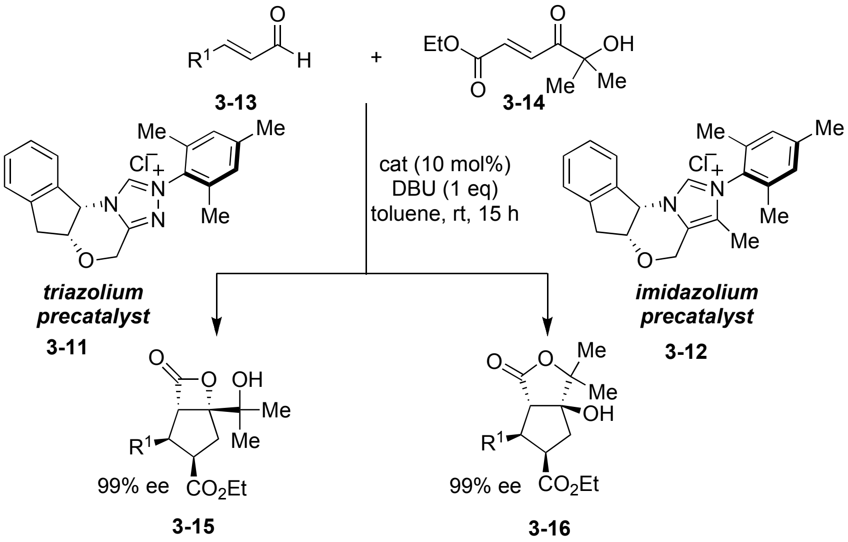


**Figure 3.2** Chiral catalysts for aldol lactonization.

In most cases, these carbene catalysts resulted in the formation of cyclopentens by spontaneous decarboxylation of a β-lactone intermediate. In order to trap the products at the lactone stage, Bode and coworkers used α-hydroxy enones (Scheme 41). To capture the stereochemical and functional complexity of these transient intermediates.[70] Furthermore, They disclosed that structurally identical triazolium and imidazolium carbene catalysts **3-11** and **3-12** provide routes to β- and γ-lactones respectively.

**Scheme 41**



R¹
O
H
3-13
+
EtO
O
O
OH
Me
Me
3-14
cat (10 mol%)
DBU (1 eq)
toluene, rt, 15 h
Me
Me
Me
Cl⁻
+
N
N
N
O
triazolium precatalyst
3-11
imidazolium precatalyst
3-12
OH
Me
99% ee
CO₂Et
3-15
3-16

They proposed two intermediates, **D** and **E,** based on leaving group ability of catalysts **3-11** and **3-12** (Figure 3.3). Mechanistic studies provide evidence for invoking a reversible benzoin condensation to result in anionic species **A**, which undergoes an Oxy-Cope rearrangement to produce **C** and further intramolecular aldol cyclization generating **D**. In the case of the triazolium catalyst **3-11**, the catalyst seems s a good leaving group in the tetrahedral intermediate, forming the β-lactone products. In contrast, imidazolium catalyst **3-12** preferred to expel the alkoxide leading to acyl imidazolium **E**, which undergoes a retro-aldol/aldol sequence inverting the stereochemistry and leading to the formation of the more stable γ-lactone.

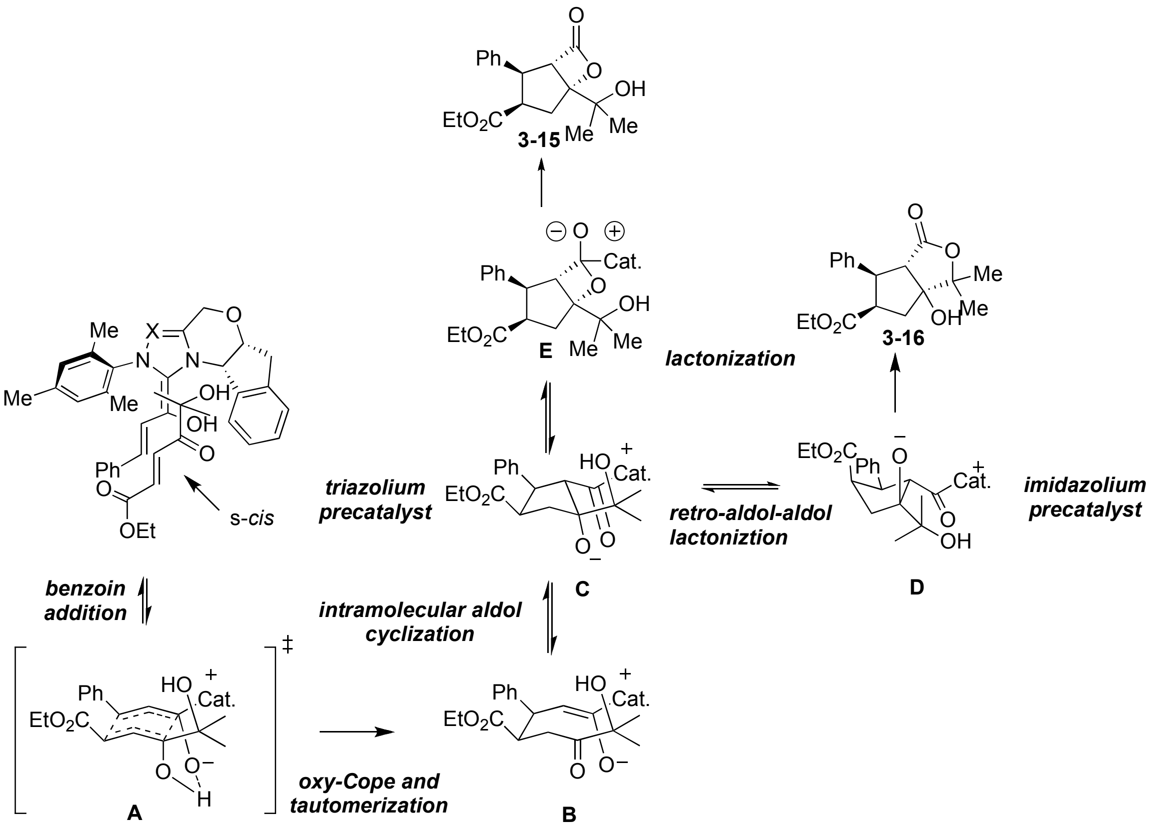
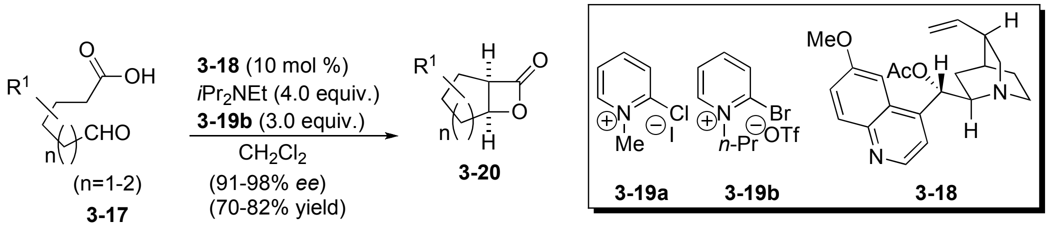

3-15
E
lactonization
3-16
s-cis
triazolium precatalyst
C
retro-aldol-aldol lactoniztion
D
imidazolium precatalyst
benzoin addition
intramolecular aldol cyclization
A
oxy-Cope and tautomerization
B

**Figure 3.3** Proposed mechanism via benzoin-oxy-cope reaction through boatlike transition state.

In 1992, our group also developed an intramolecular version of this nucleophilic catalyzed aldol-lactonization (NCAL) process building on work of Wynberg, which provided the first strategy to achieve the formation of β-lactones with non-activated aldehydes.[71] After several unsuccessful attempts with various activating agents and conditions, we were pleased to find that addition of an aldehyde acid to a mixture of Mukaiyama's reagent **3-19a**, Hunig's base and *O*-acetyl quinidine **3-18** in dichloromethane gave the bicyclic β-lactone **3-20** in high enantioselectivity in moderate yields (Scheme 42). Previous work in our group has shown that modified Mukaiyama reagent **3-19b** led to greatly improved conversion and efficiency (70-82% yield) with shorter reaction times and no diminution of enantioselectivity (91-98 % *ee*).[72]

**Scheme 42**

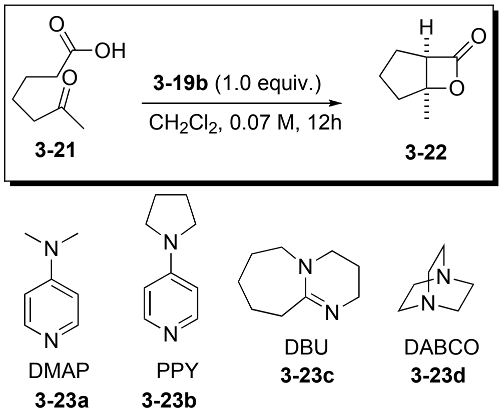


3-18 (10 mol %)
*i*Pr2NEt (4.0 equiv.)
3-19b (3.0 equiv.)
CH2Cl2
(91-98% *ee*)
(70-82% yield)
(n=1-2)
3-17
3-20
3-19a
3-19b
3-18

## 3.2 Optimization of the NCAL with Keto Acids

In our earlier studies, we reported that use of 6-oxoheptanoic acid (**3-21**) as substrate led to only to a 3% isolated yield of the corresponding bicyclic-β-lactone while employing $Et_3N$ as both a base and a nucleophilic promoter.[65b] More recently, we have focused on applying this process to ketoacid substrates. Initial studies with *p*-dimethylamino pyridine (DMAP) using previously described conditions led to more than a 5-fold increase in yield of β-lactone (19%). Further conversion was observed with the more nucleophilic 4-pyrrolidinopyridine (PPY) (Table 3.1). However, under the same conditions, it was determined that other *N*-heterocyclic nucleophiles including pyridine, diazabicyclooctane (DABCO), diazabicycloundecane (DBU), and Phosphorous nucleophiles such as triphenyl phosphine and tributylphosphine showed similar results to those of *N*-heterocyclic nucleophilic promoters. Use of only Hünig's base led to formation of no β-lactone, which is suggestive of a nucleophile-catalyzed process.

**Table 3.1**. Screen of nucleophile catalysts for NCAL process



3-19b (1.0 equiv.)
CH2Cl2, 0.07 M, 12h
3-21
3-22
DMAP
3-23a
PPY
3-23b
DBU
3-23c
DABCO
3-23d

| entry | pyridinium salt | i-$Pr_2NEt$ | Nu | % yield |
|---|---|---|---|---|
| 1 | **3-19b**(3 equiv) | 0 | $Et_3N$ (4 equiv) | 3 |
| 2 | **3-19b** (3 equiv) | 4 | Py (1 equiv) | 0 |
| 3 | **3-19b** (3 equiv) | 4 | DMAP (1 equiv) | 19 |
| 4 | **3-19b** (3 equiv) | 4 | PPY (1 equiv) | 48 |
| 5 | **3-19b** (3 equiv) | 4 | none | 0 |

$PPh_3$, $PBu_3$, DBU, DABCO gave no *β*-lactone.

Based on these results, we have concluded that more nucleophilic catalysts are essential for generation of a more nucleophilic ammonium enolate intermediate **B** and **B’** (Figure 3.4), which serves to favor formation of the aldolate intermediate **C,** increasing the rate of the presumed rate-determining oxetanone (**D)** formation step. Ketene intermediate **E**, derived from elimination of activated acid **A**, can undergo nucleophilic attack by the catalyst to generate ammonium enolates **B** and **B’**. The same pair of ammonium enolates **B** and **B’** can be generated by direct acylation of the catalyst leading to the acyl ammonium species, which can then undergo deprotonation. Regardless of which intermediates **C** and **C’** were formed, only the *cis* aldolate **C** would cyclize to an oxetane species **D** due to ring strain considerations.

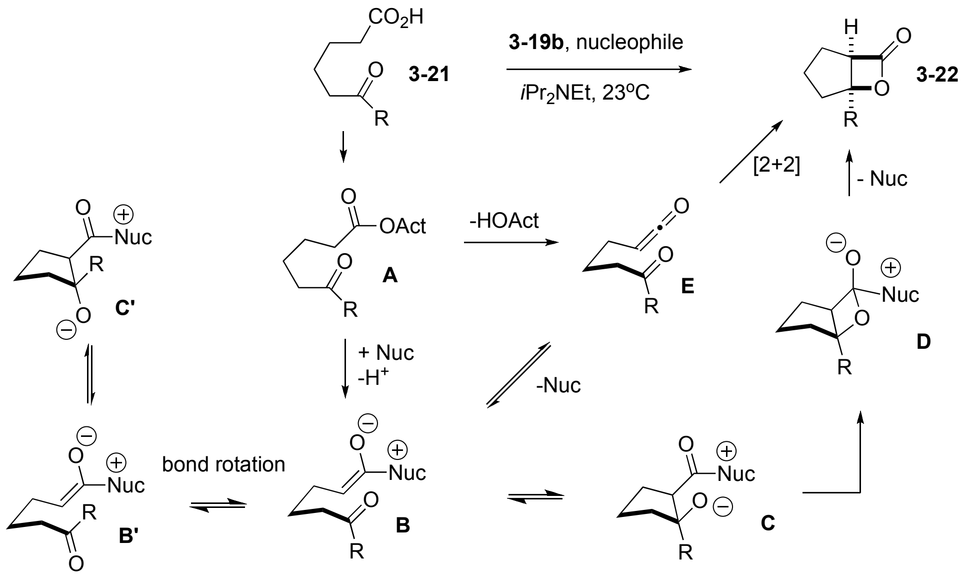
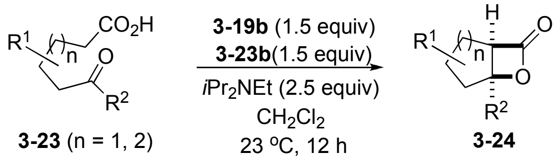
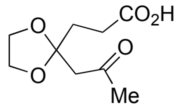
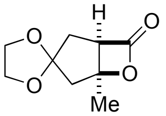
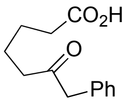
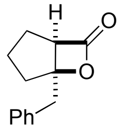
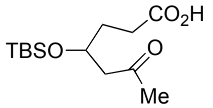
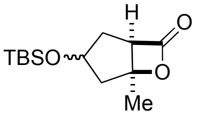
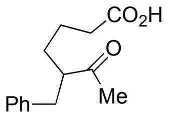
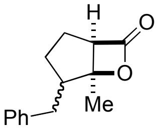
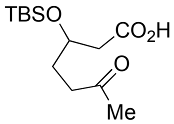
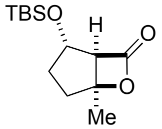


**Figure 3.4** Possible mechanistic pathways for intramolecular nucleophile catalyzed bis-cyclization process of aldehyde acids and keto acids.

The scope of this cyclization process was explored using various keto acids. As expected, all examples provided only *cis*-cyclopentyl-fused β-lactones due to ring strain considerations (Table 3.2). Keto-acids bearing β but not γ or δ-stereocenters relative to the carboxylic acid provided excellent diastereoselectivities (*cf.* β-lactones **3-23f** vs. **3-23d**, **e**).

**Table 3.2**. Substrate scope of the NCAL process



| entry | keto acid | cmpd. no. | b-lactone[a] | cmpd. no. | % yield[b] | dr[d] |
|---|---|---|---|---|---|---|
| 1 |  | **3-23b** |  | **3-24b** | 78[c] | - |
| 2 |  | **3-23c** |  | **3-24c** | 58 | - |
| 3 |  | **3-23d** |  | **3-24d** | 51 | 2:1 |
| 4 |  | **3-23e** |  | **3-24e** | 75[c] | ~1:1 |
| 5 |  | **3-23f** |  | **3-24f** | 67 | >19:1 |

[a]Relative stereochemistry is based on strain arguments (**3-24a-b**), and eventual conversion of **3-24f** to dihydroplakevulin (**3-34**). [b]Yields refer to isolated (silica gel), purified product. [c]0.7 equiv PPY, 1.0 equiv **2-19b**, and 2.0 equiv Hünig's base were employed and both β-lactones **3-24a** and **3-24e** were achieved by Dr Huda in our group. [d]Ratios were determined by 500 MHz $^1$H NMR on crude reaction mixtures and a dr of >19:1 indicates that minor diastereomers could not be detected.

Regarding the mechanism of this bis-cyclization process, we favor an aldol-lactonization route over a [2+2] cycloaddition pathway. The high diastereoselectivity (>19:1, dr) observed with β-substituted acid substrates, strongly supports a NCAL process. Namely, only keto-acids bearing a β-constituent to the carboxylic acid give **anti 3-24f** with high diastereoselectivities raised from intermediate **A**. This is consistent with A$^{1,3}$ strain for an ammonium enolate intermediate **B** leading to β-lactone **syn 3-24f**

(Figure 3.5). Other possible envelope intermediates **C** and **D** would be disfavored due to developing 1,3-diaxial interactions. In the case of a [2+2] cycloaddition pathway, such strain would be absent and thus intermediates **E** and **F** would have no difference of enthalpy resulting in low to no diastereoselectivity. More recent studies in our group have identified conditions for a catalytic asymmetric synthesis of β-lactones using keto-acid substrates. The asymmetric intramolecular NCAL process will be discussed in the subsequent chapter.

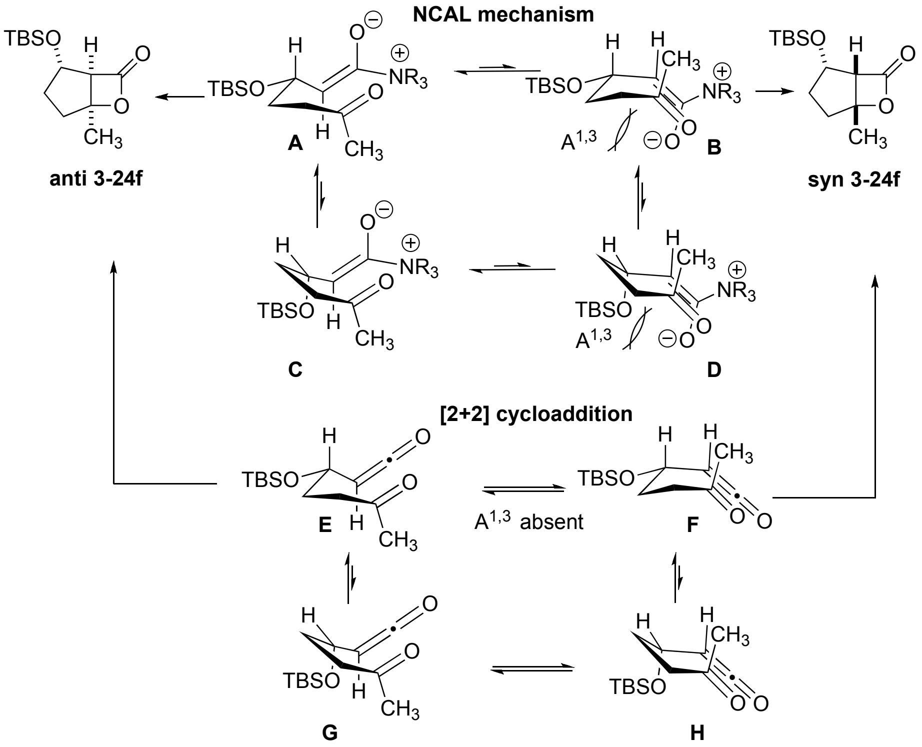


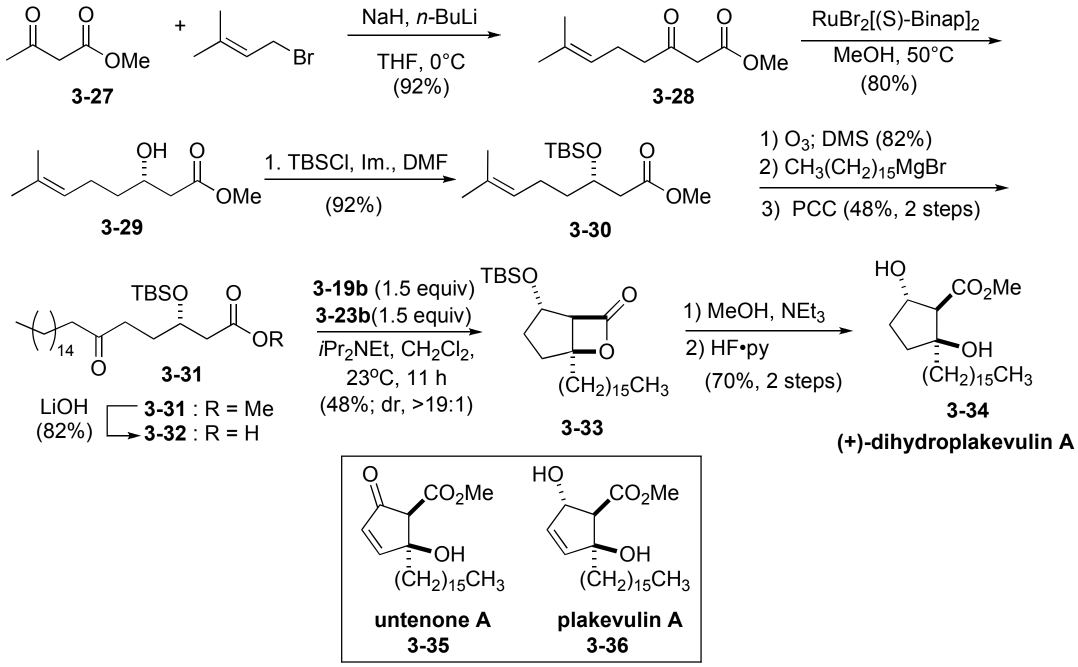
**Figure 3.5** Rationale for high diastereoselectivity with β-substituted acids.

## 3.3 Application to the Synthesis of (−)-Dihydroplakevulin

Having developed this NCAL route to β-lactones, we interested in applying this route in natural product synthesis. Untenone A, natural product structurally related to plakevulin A, was isolated from marine sponges of the genus plakortis. In 2003, Kobayashi and co-workers reported it role as an inhibitor of mammalian DNA polymerase α and β, and human terminal deoxynucleotidyl transferase.[73] An approach by

Kobayashi and co-workers approach toward synthesis of plakevulin A began with untenone A (Scheme 43). In order to determine the relative stereochemistry for plakevulin A, three derivatives of plakevulin A were prepared. The latter derivative dihydroplakevulin A became a synthetic target for the bis-cyclization of keto acids because the core of dihydroplakevulin A was almost identical with **3-23f**. The proposed route to the ketoacid **3-32** began with alkylation of the acetoacetate **3-27** with ally bromide, providing ketoester **3-28** in 92% yield.[74] The keto group of **3-28** was subjected to Noyori hydrogenation conditions, in the presence of $RuBr_2[(S)\text{-(Binap)}]$catalyst generated *in situ* from $Ru(Cod)(2\text{-methylallyl})_2$.[75] Known hydroxy ester **3-29** was obtained in > 80% yield and high enantioselectivity (97% *ee*).[68] TBS protection of alcohol **3-30**, followed by ozonolysis and reduction of the ozonide with triphenylphosphine provided the corresponding aldehyde. Reaction with alkylmagnesium bromide followed by oxidation afforded the ketoester **3-31**. Subsequent hydrolysis of the ester provided the corresponding carboxylic acid **3-32**, which was then exposed to our NCAL conditions. The desired bicyclic β-lactone **3-33** was obtained in moderate yield with high diastereoselectivity. Subsequent methanolysis and deprotection provided dihydroplakevulin A **3-34**.

**Scheme 43**


3-27
3-28
NaH, n-BuLi
THF, 0°C
(92%)
RuBr2[(S)-Binap]2
MeOH, 50°C
(80%)
3-29
1. TBSCl, Im., DMF
(92%)
3-30
1) O3; DMS (82%)
2) CH3(CH2)15MgBr
3) PCC (48%, 2 steps)
3-31
LiOH
(82%)
3-31 : R = Me
3-32 : R = H
3-19b (1.5 equiv)
3-23b(1.5 equiv)
iPr2NEt, CH2Cl2,
23°C, 11 h
(48%; dr, >19:1)
3-33
1) MeOH, NEt3
2) HF•py
(70%, 2 steps)
3-34
(+)-dihydroplakevulin A
untenone A
3-35
plakevulin A
3-36

### 3.4 Design and Attempted Synthesis of a Cyclic Guanidine Catalyst

Although extension of the NCAL process to ketoacid substrates was shown to be successful, stoichiometric amounts of nucleophile were required for obtaining synthetically useful yields. Use of stoichiometric nucleophile is undesirable in the asymmetric NCAL process due to the availability and expense of chiral catalysts. Therefore, our focus was shifted to synthesis and testing of several pyridine nucleophiles and other related nucleophiles, which possess greater nucleophilicity compared to 4-PPY **3-23b**. Highly planar nucleophiles minimize developing torsional strain with the alkyl group (R) of ketone substrates, and efficient syntheses of both achiral and chiral versions of the nucleophilic catalyst were pursued (Figure 3.6). If the catalyst was able to enhance the stability of the reactive acylammonium intermediate, the rate of NCAL process could be increased by perturbing the equilibrium in favor of the aldolate. Zipse and coworkers reported energy calculations of pyridine derivatives, which were ranked according to their relative acylation enthalpy.[76] Based on their calculations, the synthesis of two guanidine derivatives, which would have greater $-\Delta H_{acylation}$ than 4-PPY were developed. Commercially available tetramisole **3-40** and structurally related benzotetramisole **3-41**[77] and homobenzotetramisole (HBTM) catalysts **3-42**[78] also showed promise (Figure 3.6). Tetramisole-derived catalysts have recently been explored as asymmetric acylation catalysts by Birman and can be easily synthesized from the corresponding 2-amino alcohols.[79]

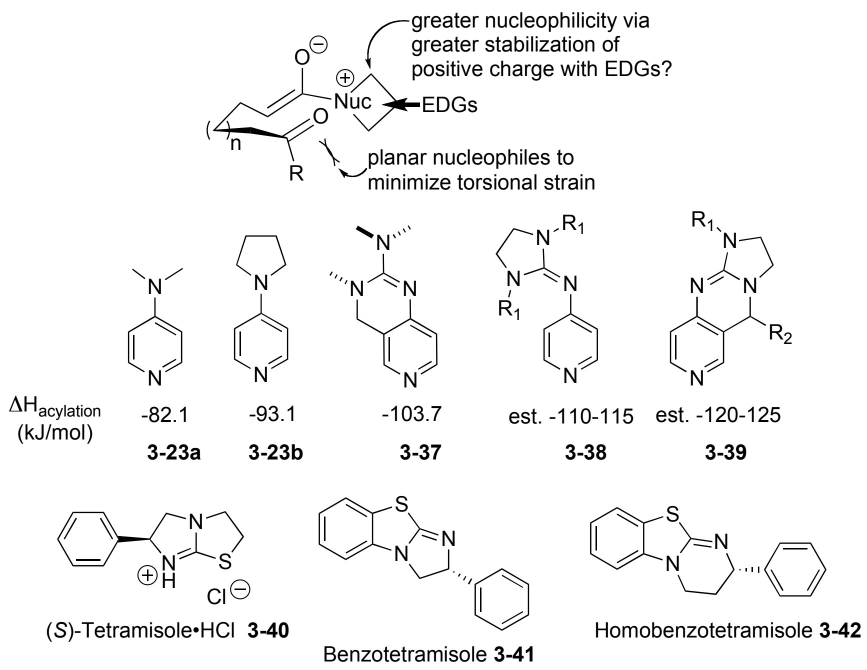
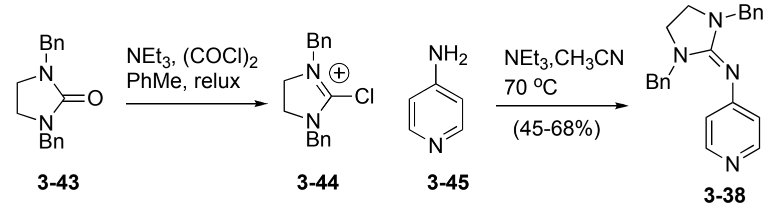

greater nucleophilicity via greater stabilization of positive charge with EDGs?
EDGs
planar nucleophiles to minimize torsional strain
Nuc
n
R
ΔH acylation (kJ/mol)
-82.1
-93.1
-103.7
est. -110-115
est. -120-125
3-23a
3-23b
3-37
3-38
3-39
(S)-Tetramisole•HCl 3-40
Benzotetramisole 3-41
Homobenzotetramisole 3-42

**Figure 3.6** Possible nucleophile catalyst candidates and criteria for catalyst selection.

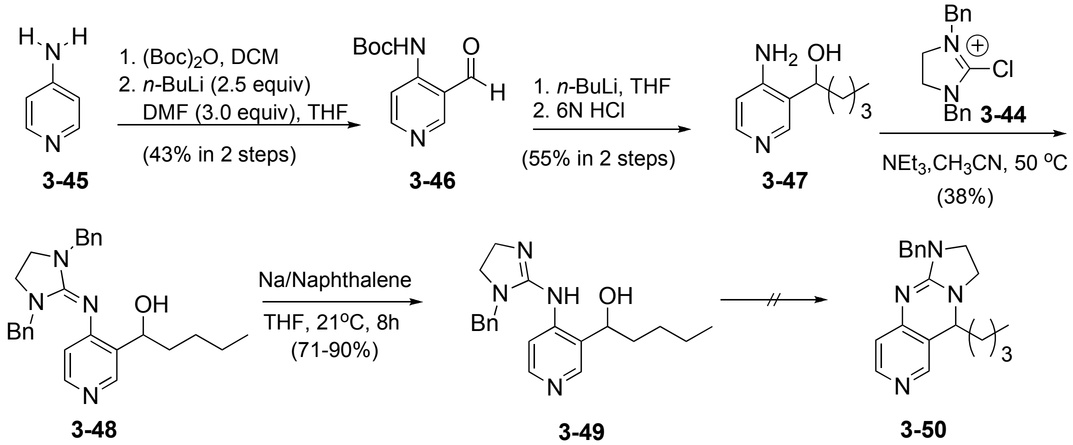
First, in order to determine the usefulness of guanidine nucleophiles in the process, synthesis of racemic **3-38** was first undertaken. The proposed achiral guanidine nucleophile **3-38** was generated from the guanidinylation of chloroimidazolinium chloride **3-44** with 4-amino pyridine **3-45** (Scheme 44). This highly reactive Vilsmeier salt **3-44** was prepared according to a known procedure.[80]

**Scheme 44**


NEt3, (COCl)2
PhMe, relux
NEt3,CH3CN
70 °C
(45-68%)
3-43
3-44
3-45
3-38

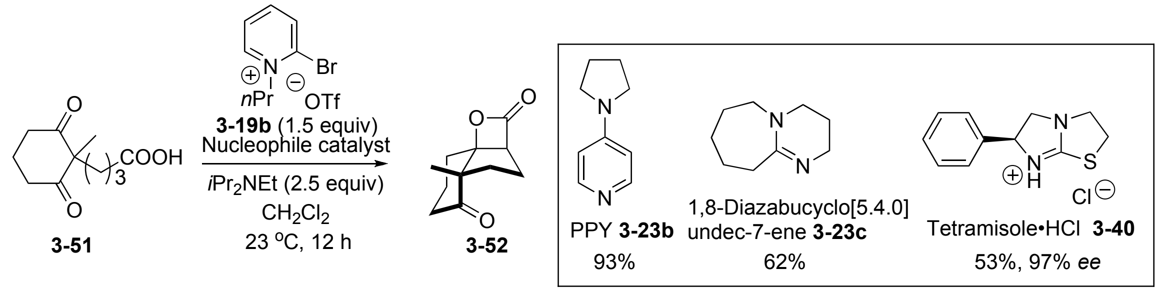
The second achiral guanidine nucleophile **3-39** began with *N*-Boc protection of aminopyridine **3-45** followed by formylation to give aminopyridine aldehyde **3-46** (Scheme 45).[81] Alkyllithium addition to aldehyde **3-46** followed by cleavage of the carbamate delivered secondary alcohol **3-47**. Guanidinylation of amino alcohol **3-47** afforded the corresponding guanidine **3-48**. Surprisingly only one benzyl group was cleavaged under dissolving metal conditions. In order to promote the crucial cyclization, the alcohol was activated and exposed to various temperatures and solvents, yet the desired tricyclic guanidine **3-50** was not generated, likely due to the large energy barrier present when the guanidine and the activated alcohol came in close proximity to each other.

**Scheme 45**


1. (Boc)2O, DCM
2. n-BuLi (2.5 equiv)
DMF (3.0 equiv), THF
(43% in 2 steps)
3-45
BocHN
3-46
1. n-BuLi, THF
2. 6N HCl
(55% in 2 steps)
NH2 OH
3-47
Bn 3-44
NEt3,CH3CN, 50 °C
(38%)
3-48
Na/Naphthalene
THF, 21°C, 8h
(71-90%)
3-49
BnN
3-50

Since the development of a new catalyst required extensive effort and promising results with commercially available tetramisole **3-40** were being observed, this project was halted. Related work in our group showed that a 5,6 carbocycle-fused β-lactone **3-52** was obtained in greater than 90% yield under identical NCAL conditions with 1,3-cycloalkanedione acid **3-51** (Scheme 46).[82] Furthermore, the asymmetric syntheses of β-lactones from 1,3-cycloalkanedione acid substrates were achieved with the chiral tetramisole catalyst **3-40** in 53% (97% ee). Even though the NCAL process is expected to remain the same with these substrates, the inherent reactivity of 1,3-cycloalkanedione acid substrates differs from non-dione keto acid substrates. For example, DBU **3-23c** promoted the bis-cyclization process in 62% yield with 1,3-cycloalkanedione acid. However, no β-lactone product was observed with non-dione keto acid substrates. These results provided an important starting point for the development of a catalytic asymmetric process with keto acid substrates.

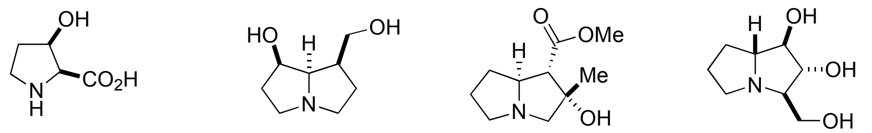
**Scheme 46**


O
O
COOH
3
3-51
Br
N
nPr
OTf
3-19b (1.5 equiv)
Nucleophile catalyst
iPr2NEt (2.5 equiv)
CH2Cl2
23 °C, 12 h
O
O
O
3-52
N
N
PPY 3-23b
93%
N
N
1,8-Diazabucyclo[5.4.0]
undec-7-ene 3-23c
62%
N
N
H
S
Cl
Tetramisole•HCl 3-40
53%, 97% ee

Very recently, this dione and non-dione NCAL process has been explored with chiral catalysts such as benzotetramisole **3-41** and homobenzotetramisole (HBTM) **3-42**, which are structurally related to tetramisole **3-40**. Results show that HBTM **3-42** catalyst promotes formation of asymmetric β-lactones with non-dione keto acid substrates. More importantly 20 mol% HBTM catalyst **3-42** delivered β-lactones in moderate yields with high enantioselectivity.

## 3.5 Synthesis of β-lactone Fused Nitrogen Heterocycles

Polyhydroxylated pyrrolidine and pyrrolizidine moieties are found in a wide variety of natural and synthetic compounds of biomedical significance.[83] Alkaloids such as (2*S*, 3*R*)-3-hydroxyproline (**3-53**),[84] tussilagine (**3-54**),[85] hyacinthacine $A_2$ (**3-55**),[86] and platynecine (**3-56**)[87] have received much attention by both synthetic chemists and biochemists due to their challenging structures and diverse biological activities (Figure 3.7). Although many racemic and enantioselective routes toward these alkaloids have been published, the NCAL process would be a practical new route to access these alkaloids.

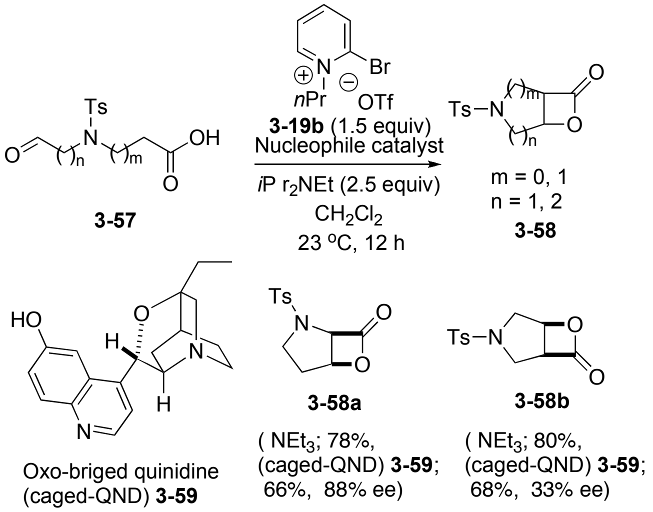

OH
CO2H
N
H
HO
H
OH
N
O
OMe
H
Me
N
OH
H
OH
OH
N
OH

(*2S, 3R*)-3-Hydroxyproline (**3-53**) Platynecine (**3-54**) Tussilagine (**3-55**) (+)-Hyacinthacine $A_2$ (**3-56**)

**Figure 3**.7 Examples of pyrrolidine and pyrrolizidine alkaloids.

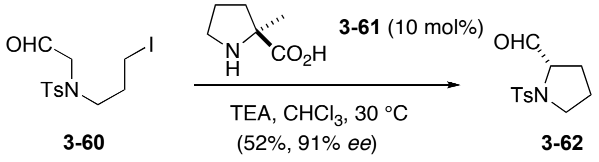
Previously we have synthesized nitrogen containing bicyclic β-lactones **3-58** from the corresponding aldehyde acid substrates **3-57** by an intramolecular NCAL process (Scheme 47).[88] Pyrrolidine fused β-lactones **3-58a** and **3-58b** were obtained in yields up to 80% in the racemic series and up to 60% yields in (88% *ee*) for the asymmetric series.

**Scheme 47**


Ts
N
OH
O
O
n
m
3-57
N
Br
nPr
OTf
3-19b (1.5 equiv)
Nucleophile catalyst
iP r2NEt (2.5 equiv)
CH2Cl2
23 °C, 12 h
Ts–N
m
n
O
O
m = 0, 1
n = 1, 2
3-58
HO
H
O
N
N
H
Oxo-briged quinidine
(caged-QND) 3-59
Ts
N
O
O
3-58a
( NEt3; 78%,
(caged-QND) 3-59;
66%, 88% ee)
Ts–N
O
O
3-58b
( NEt3; 80%,
(caged-QND) 3-59;
68%, 33% ee)

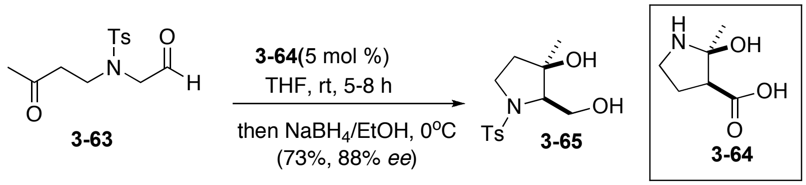
Construction of the pyrrolidine framework has also been widely reported using alternate methods. List and co-workers discovered the first catalytic asymmetric α-alkylation of aldehyde **3-60** by an intramolecular pathway (Scheme 48).[89] Their process furnished chiral substituted pyrrolidine **3-62** in moderate yield and enantioselectivity.

**Scheme 48**


OHC
I
TsN
3-60
N
H
CO2H
3-61 (10 mol%)
TEA, CHCl3, 30 °C
(52%, 91% ee)
OHC
TsN
3-62

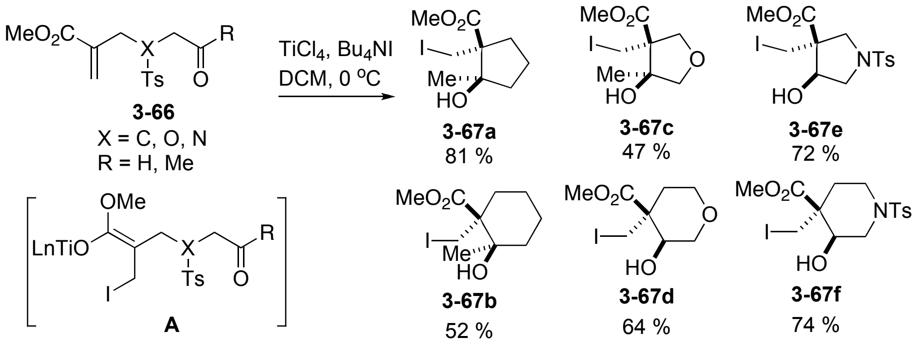
Hamada and coworkers reported the asymmetric synthesis of (2*S*,3*R*)-3-hydroxy-3-methylproline (OHMePro) **3-64** via an intramolecular asymmetric aldol reaction (Scheme 49).[90] Interestingly, final target molecule, OHMePro **3-64** is a better catalyst over (*S*)-proline toward the synthesis of (2*S*,3*R*)-3-hydroxy-3-methylproline (OHMePro) **3-65**.

Scheme 49


3-63
3-64(5 mol %)
THF, rt, 5-8 h
then NaBH4/EtOH, 0°C
(73%, 88% ee)
3-65
3-64

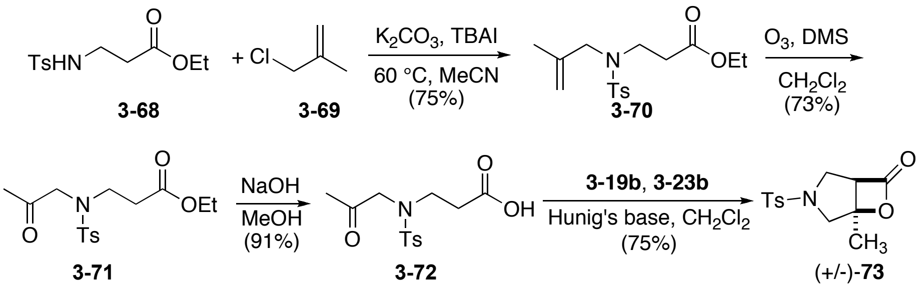
Pyrrolidines have also been obtained via the intramolecular iodo-aldol cyclization, which is part of the Morita-Baylis-Hillman (MBH) family of tandem aldol reactions. A current limitation of the iodo-aldol process is the inability to apply this method to the construction of quaternary centers. Greaney and coworkers have explored the scope of the iodo-aldol process (Scheme 50),[91] and have shown that the reaction of enolate-aldehyde or ketones **3-66** produces hindered γ-iodoalcohols **3-67** with excellent *trans* selectivity in moderate to good yields.

Scheme 50


3-66
X = C, O, N
R = H, Me
A
TiCl4, Bu4NI
DCM, 0 °C
3-67a
81 %
3-67c
47 %
3-67e
72 %
3-67b
52 %
3-67d
64 %
3-67f
74 %

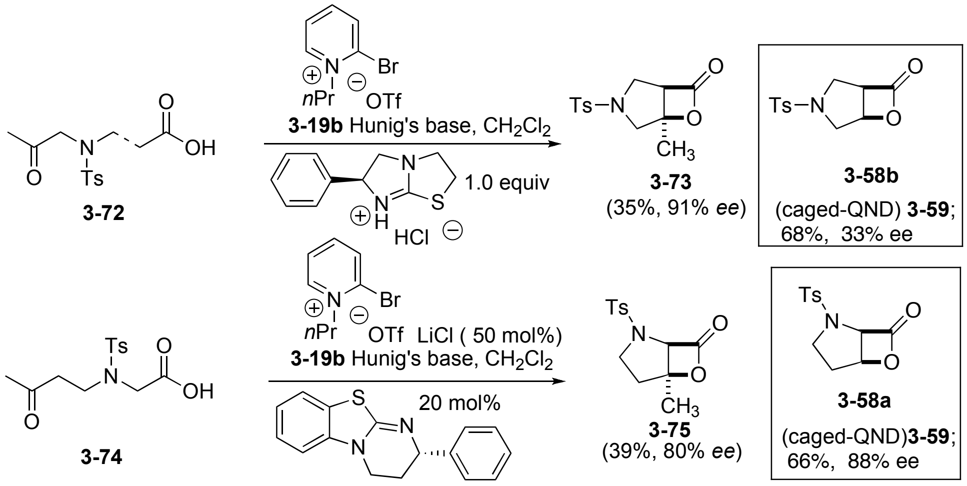
As mentioned previously, our group discovered that 4-pyrrolidinopyridine (PPY) **3-23b**, tetramisole **3-40**, and homobenzotetramisole (HBTM) **3-42** catalysts promoted intramolecular NCAL reactions with keto acids for generating bicyclic β-lactones. The scope of substrates was extended to include nitrogen-containing keto acid substrates. Keto acid **3-72** was readily synthesized from tosyl protected β-alanine ester **3-68** (Scheme 51). Alkylation of β-alanine **3-68**, followed by ozonolysis and ester hydrolysis provided ketoacid **3-72**. The NCAL reaction was conducted using PPY as a promoter, and the desired nitrogen containing bicyclic β-lactone **3-73** was isolated in 75% yield.[88]

**Scheme 51**


TsHN
OEt
O
3-68
+ Cl
3-69
$K_2CO_3$, TBAI
60 °C, MeCN
(75%)
N
Ts
OEt
O
3-70
$O_3$, DMS
$CH_2Cl_2$
(73%)
O
N
Ts
OEt
O
3-71
NaOH
MeOH
(91%)
O
N
Ts
OH
O
3-72
3-19b, 3-23b
Hunig's base, $CH_2Cl_2$
(75%)
Ts–N
O
O
$CH_3$
(+/-)-73

In order to determine the use of this substrate in an asymmetric NCAL process, ketoacid **3-72** was subjected to previously developed NCAL conditions using tetramisole **3-40**.[82] The desired bicyclic β-lactone **3-73** was obtained in 35% yield (unoptimized) and 91% *ee* (Scheme 52). The absolute configuration has not yet been determined. Bicyclic β-lactone **3-58b** was previously obtained with lower enantiomeric excess than bicyclic β-lactone **3-58a**,[88] but structurally similar β-lactone **3-73** was obtained with excellent enantiomeric excess. Therefore NCAL of ketoacid **3-74** was expected to give as similar results as ketoacid **3-72**. However, tetramisole catalyst provided very low conversion (> 5%) with excellent enantiomeric excess (93% *ee*) with ketoacid **3-74**, while the homobenzotetramisole (HBTM) catalyst **3-42** provided better yield.

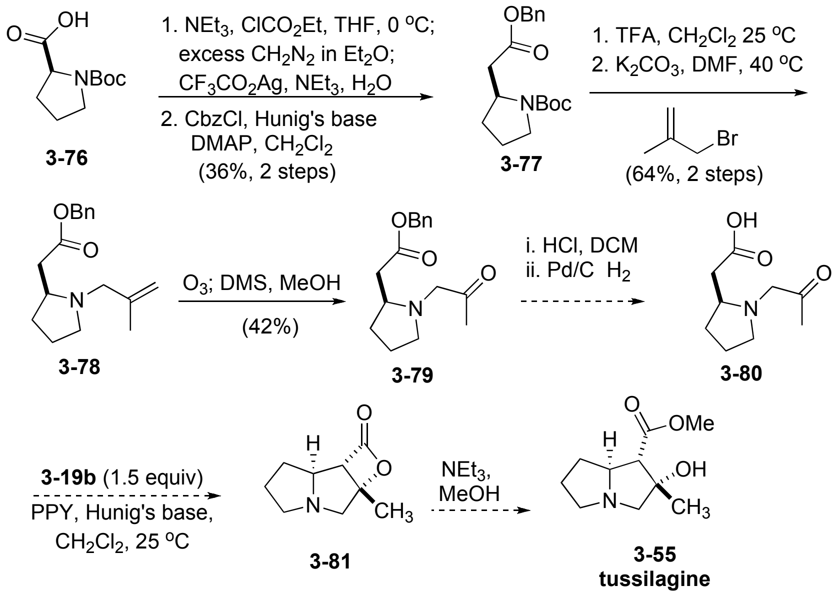
**Scheme 52**


O
N
Ts
OH
O
3-72
N
Br
nPr
OTf
3-19b Hunig's base, $CH_2Cl_2$
N
N
H
S
HCl
1.0 equiv
Ts–N
O
O
$CH_3$
3-73
(35%, 91% ee)
Ts–N
O
O
3-58b
(caged-QND) 3-59;
68%, 33% ee
Ts
N
O
OH
O
3-74
N
Br
nPr
OTf
LiCl ( 50 mol%)
3-19b Hunig's base, $CH_2Cl_2$
S
N
N
20 mol%
Ts
N
O
O
$CH_3$
3-75
(39%, 80% ee)
Ts
N
O
O
3-58a
(caged-QND)3-59;
66%, 88% ee

After determining the utility of the NCAL with nitrogen-containing keto-acid substrates, we are interested in applying this method to more complex nitrogen containing natural products such as tussilagine. Among hundreds of isolated pyrrolizidines, tussilagine is anomalous and non-toxic. It has been found to exist in *Tussilago farara*, *Echinacea purpurea*, and *Arnica. angustifolia.*[92] In 1998, Ma and co-workers reported an enantioselective protocol from 1,4-butanediol in an overall 8% yield in 9 steps.[93]

Our synthesis of tussilagine commences with homoproline **3-77** and a key step is the NCAL reaction with ketoacid **3-80**. Although homoproline is commercially available, it is rather expensive and instead is easily accessed from (s)-proline **3-76** via Arndt-Eistert homologation in two steps (Scheme 53).[94] The resulting carboxylic acid was then benzyl protected and the Boc group subsequently deprotected under acidic conditions. *N*-alkylation followed by ozonolysis provided ketoester **3-79**. In the future, after deprotection of the benzyl group, the corresponding ketoacid **3-80** is expected to deliver tricyclic β-lactone **3-81** via the NCAL process and further ring opening of β-lactone **3-81** should generate tussilagine **3-55**.

**Scheme 53**


3-76
1. NEt3, ClCO2Et, THF, 0 °C; excess CH2N2 in Et2O; CF3CO2Ag, NEt3, H2O
2. CbzCl, Hunig's base DMAP, CH2Cl2
(36%, 2 steps)
3-77
1. TFA, CH2Cl2 25 °C
2. K2CO3, DMF, 40 °C
(64%, 2 steps)
3-78
O3; DMS, MeOH
(42%)
3-79
i. HCl, DCM
ii. Pd/C H2
3-80
3-19b (1.5 equiv)
PPY, Hunig's base, CH2Cl2, 25 °C
3-81
NEt3, MeOH
3-55
tussilagine

## 3.6 Conclusions

In an effort to expand utility of the nucleophile promoted aldol lactonization process, we explored keto-acid substrates using established protocols previously proven highly useful with aldehyde-acids substrates. However, *N*-heterocyclic nucleophiles cinchona alkaloids, pyridine, diazabicyclooctane (DABCO), diazabicycloundecane (DBU), and phosphorous nucleophiles did not successfully promote the NCAL process. We were pleased to find that more nucleophilic catalysts such as 4-pyrrolidinopyridine (PPY) **3-23b**, tetramisole **3-40**, and homobenzotetramisole (HBTM) catalyst **3-42** promoted intramolecular NCAL reactions with keto-acid substrates for bicyclic β-lactones. This reaction produced β-lactones possessing up to three stereocenters, which include a masked tertiary carbinol center and a reactive β-lactone moiety. This protocol for keto-acids was applied toward asymmetric synthesis of polyhydroxylated pyrrolidine and pyrrolizidine alkaloids. In addition, the application of the NCAL process to the total synthesis of dihydroplakevulin A was achieved and synthesis of tussilagine is under study. Finally, we have developed a catalytic asymmetric NCAL reaction using homobenzotetramisole (HBTM) as a nucleophile and additional studies are underway in our laboratory.

# CHAPTER IV

# SYNTHETIC STUDIES TOWARD THIOLYGBYAN

## 4.1 Isolation and Background

The role of thiols and disulfides in biological systems is well known such as the thiol-disulfide interchange reaction and the related formation and cleavage under physiological conditions. The most well known disulfide natural products is the lipoic acid **4-1**, which plays a dual role in living organisms as acyl group transfer agent to coenzyme A in the metabolic process and as antioxidant against the free radical (Figure 4.1). Other natural products such as *epi*-zeylaoxide B **4-2**,[95] somocystinamide A **4-3**,[96] and rostratin C **4-4**[97] possessing disulfide moiety have been isolated and showed interesting bioactivity. *Epi*-zeylaoxide B **4-2** completely inhibited root growth of rice seedlings at 3 mM and somocystinamide A **4-3** exhibited cytotoxicity against mouse neuro-2a neuroblastoma cells with $IC_{50}$ values of 1.4 μg/mL, rostratins C **4-4** showed *in vitro* cytotoxicity against human colon carcinoma (HCT-116) with $IC_{50}$ values of 0.76 μg/mL.

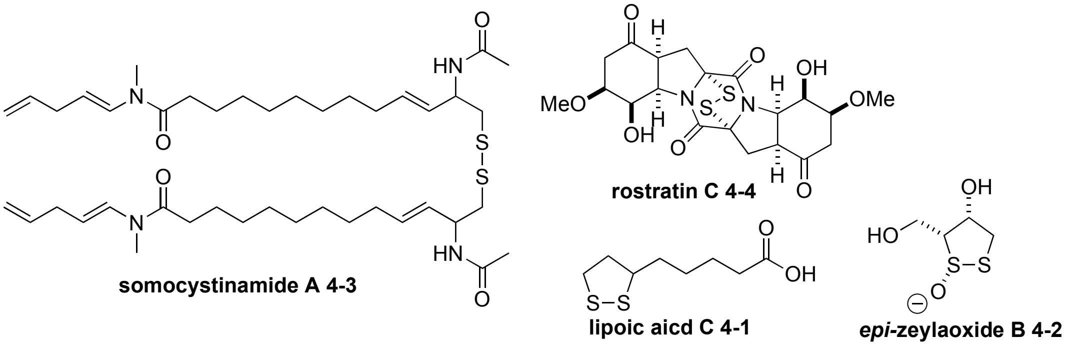
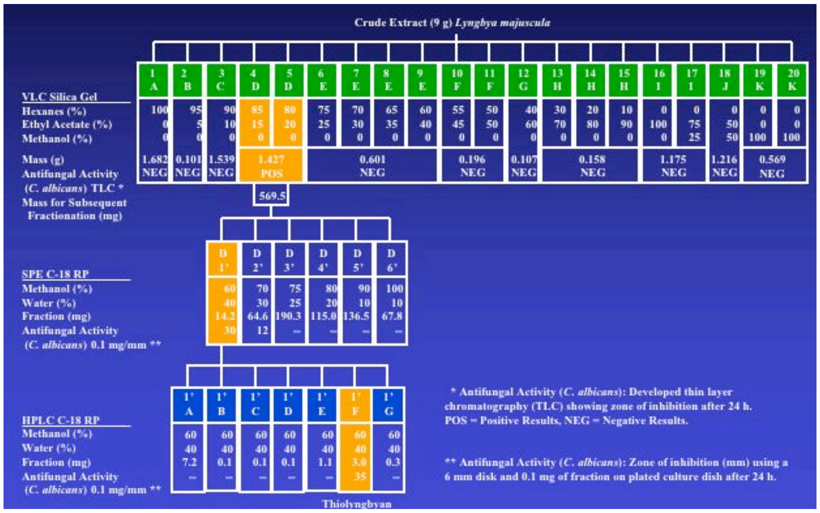


**Figure 4.1**. Examples of disulfide moiety containing natural product.

Marine organisms are known producers of compounds with novel chemical structures and interesting biological activities. The marine cyanobacteria (blue-green algae), *Lyngbya majuscula* are a rich source of novel bioactive natural products. Many structurally diverse metabolites have been isolated from this microoganism. Its compounds have been classified into various families such as the *lyngbyabelliums*, the *majuscamides*, and the *curacins* based on structural and functional similarities.[98] One such metabolite, thiolyngbyan, was first isolated and characterized by Dr. William Gerwick's research group at the University of California San Diego in 2003.[99] After HPLC purification of the crude extraction of *Lyngbya majuscula* (strain 19L), a blue-green algae collected from the coastal waters of Curacão, an island located near Venezuela, the isolated 0.1 mg sample of thiolyngbyan exhibited a 35 mm zone of inhibition with the yeast *Candida albicans* in a 6 mm disk after 24 hours (Figure 4.2). Because of the biological activity, thiolyngbyan may function as a useful antifungal agent.



Crude Extract (9 g) *Lyngbya majuscula*

| VLC Silica Gel | 1 A | 2 B | 3 C | 4 D | 5 D | 6 E | 7 E | 8 E | 9 E | 10 F | 11 F | 12 G | 13 H | 14 H | 15 H | 16 I | 17 I | 18 J | 19 K | 20 K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hexanes (%) | 100 | 95 | 90 | 85 | 80 | 75 | 70 | 65 | 60 | 55 | 50 | 40 | 30 | 20 | 10 | 0 | 0 | 0 | 0 | 0 |
| Ethyl Acetate (%) | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 60 | 70 | 80 | 90 | 100 | 75 | 50 | 0 | 0 |
| Methanol (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 50 | 100 | 100 |
| Mass (g) | 1.682 | 0.101 | 1.539 | 1.427 | | 0.601 | | | | 0.196 | | 0.107 | 0.158 | | | 1.175 | | 1.216 | 0.569 | |
| Antifungal Activity (*C. albicans*) TLC * | NEG | NEG | NEG | POS | | NEG | | | | NEG | | NEG | NEG | | | NEG | | NEG | NEG | |
| Mass for Subsequent Fractionation (mg) | | | | 569.5 | | | | | | | | | | | | | | | | |

| SPE C-18 RP | D 1' | D 2' | D 3' | D 4' | D 5' | D 6' |
|---|---|---|---|---|---|---|
| Methanol (%) | 60 | 70 | 75 | 80 | 90 | 100 |
| Water (%) | 40 | 30 | 25 | 20 | 10 | 10 |
| Fraction (mg) | 14.2 | 64.6 | 190.3 | 115.0 | 136.5 | 67.8 |
| Antifungal Activity (*C. albicans*) 0.1 mg/mm ** | 30 | 12 | – | – | – | – |

| HPLC C-18 RP | 1' A | 1' B | 1' C | 1' D | 1' E | 1' F | 1' G |
|---|---|---|---|---|---|---|---|
| Methanol (%) | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Water (%) | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| Fraction (mg) | 7.2 | 0.1 | 0.1 | 0.1 | 1.1 | 3.0 | 0.3 |
| Antifungal Activity (*C. albicans*) 0.1 mg/mm ** | – | – | – | – | – | 35 | – |

* Antifungal Activity (*C. albicans*): Developed thin layer chromatography (TLC) showing zone of inhibition after 24 h. POS = Positive Results, NEG = Negative Results.

** Antifungal Activity (*C. albicans*): Zone of inhibition (mm) using a 6 mm disk and 0.1 mg of fraction on plated culture dish after 24 h.

**Figure 4.2** Fraction of organic extract from *L*. majuscula.[88]

The gross structure of thiolyngbyan was tentatively assigned by spectroscopic studies including, accurate mass measurements and 1D and 2D NMR correlation methods (Figure 4.3). The proposed structural features of thiolyngbyan include an exo vinyl bromide, one stereogenic center, and a cyclic disulfide moiety. Unit peak patterns of thiolyngbyan mass spectrum indicated the presence of bromine because a unit peak and a base peak were approximately equal in intensity. The mass fragment peak of 208.8 indicated the present of an alcohol. The $^1$H NMR spectra, exhibited a signal at δ 6.37 ppm consistent with the presence of vinyl bromine. Furthermore, the carbon signal at δ 128 ppm was 15 ppm more down field than a typical external olefin signal. This likely demonstrates that vinyl halogen is present. The proton signals at δ 3.00 ppm to δ 4.00 ppm suggested the present of sulfur atoms. In addition, the signals at around δ 35 ppm indicated carbon atoms bearing sulfur. The $^{13}$C signal at δ 123 ppm is indicative of a quaternary olefin especially due to relatively small intensity.

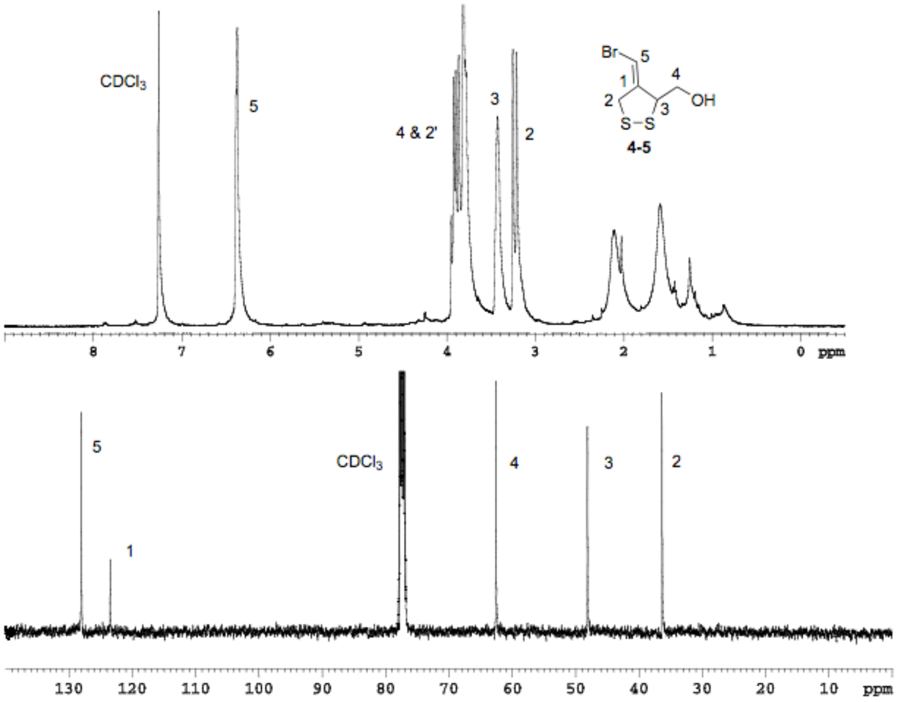


**Figure 4.3** 1D spectrum data of proposed thiolyngbyan.

2D NMR studies were ambiguous so the geometry of the vinyl bromide and the stereochemical assignment still need to be verified. As its structure has not been confirmed, structural verification of thiolyngbyan is the main goal of this project. In addition, the simple structure of the molecule should facilitate pinpointing the functionality responsible for thiolyngbyan's antifungal activity.

## 4.2 Synthetic Studies toward the Proposed Structure of Thiolyngbyan

We envisioned formation of the disulfide unit in thiolyngbyan **4-5** *via* oxidation of a 1,3-dithiol derivative, which in turn can arise by Mistunobu esterification of 1,3-diol **4-6** (Figure 4.4). The ketone **4-7** can be formed from known diol **4-8**,[100] *via* selective hydroxyl group protection followed by Swern oxidation. The diol **4-8** can be formed from D-glucose **4-9**, *via* selective hydroxyl protection followed by oxidative cleavage.

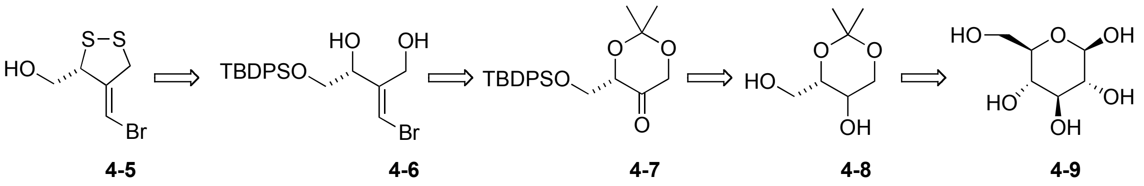
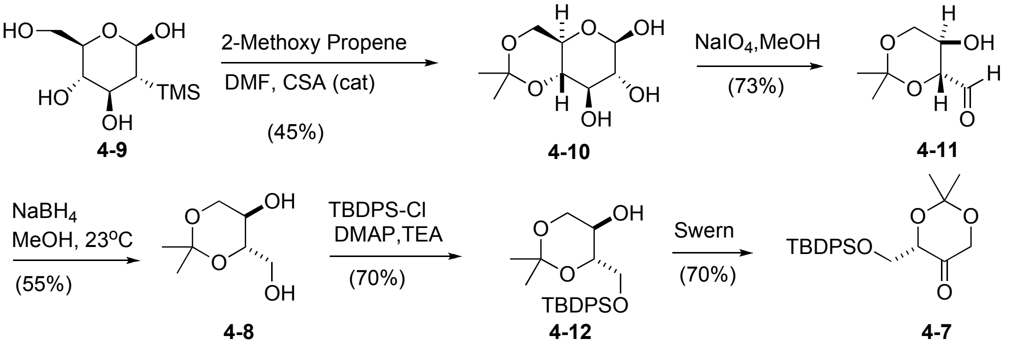

S–S
HO
Br
TBDPSO
HO
OH
Br
TBDPSO
O
O
O
HO
O
O
OH
HO
O
OH
HO
OH
OH
4-5
4-6
4-7
4-8
4-9

**Figure 4.4** Retrosynthetic analysis of thiolyngbyan.

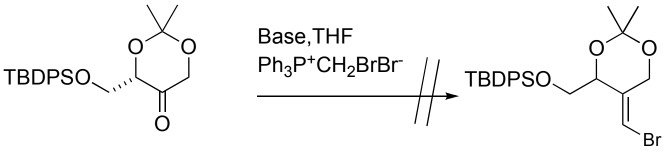
The selective protection of hydroxyl groups from D-glucose **4-9** was carried out according to the protocol developed by Barili *et al* (Scheme 54).[101] The initial reaction was performed on seven grams scale that provided 4 g of desired product in 45% yield. The oxidative cleavage of triol **4-10** followed by reduction of the resulting aldehyde **4-11** gave the 2,4-*O*-isopropylidene-D-erythritol **4-8**. Problems were encountered on scale up of the oxidative cleavage and above two gram-scale, the yield of this reaction diminished. Since the desired product was very soluble in water, it was difficult to extract it back to the organic layer. Therefore, the solvent was switched to methanol and the reproducibility problem was resolved. Resulting intermediate aldehyde **4-11** was not isolated due to its instability and direct reduction of the crude material provided primary alcohol **4-8**, which was selectively protected with TBDPSCl. Swern oxidation of alcohol **4-12** then provided ketone **4-7**.

**Scheme 54**


HO
O
OH
HO
TMS
OH
4-9
2-Methoxy Propene
DMF, CSA (cat)
(45%)
H
O
O
OH
O
H
OH
OH
4-10
NaIO4,MeOH
(73%)
H
O
OH
O
H
H
O
4-11
NaBH4
MeOH, 23°C
(55%)
O
OH
O
OH
4-8
TBDPS-Cl
DMAP,TEA
(70%)
O
OH
O
TBDPSO
4-12
Swern
(70%)
TBDPSO
O
O
O
4-7

To install the vinyl bromide, Wittig conditions were initially employed, but most of the starting material (> 90 %) was recovered under various conditions (Table 4.1).[102] We reasoned that the Wittig procedure might be unsuitable to convert ketone **4-7** to vinyl bromide **4-13** because of the steric hindrance.

**Table 4.1**. Optimization of the Wittig conditions



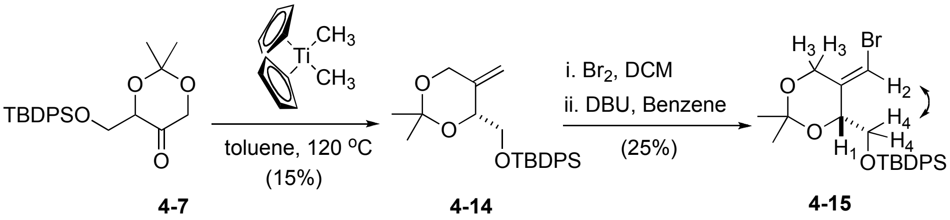
**4-7** **4-13**

| Entry | Base | Base equiv. | Phosponium bromide | Temp. | Major |
|---|---|---|---|---|---|
| 1 | KHMDS | 1.1 | 1.2 equiv. | 25 °C | SM |
| 2 | LiHMDS | 4.0 | 4.0 equiv. | 110 °C | SM |
| 3 | LDA | 4.0 | 4.0 equiv. | 110 °C | SM |
| 4 | NaH | 4.0 | 4.0 equiv. | 110 °C | SM |

SM = starting material

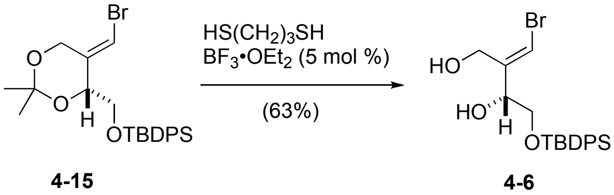
However, when the bulky silyl group was removed to minimize the steric hindrance of ketone **4-7**, results did not improve. Among alternative olefination methods, only Petasis conditions provided the desired olefin **4-14** (Scheme 55),[103] however the product was only obtained in low yield. As these conditions did not directly give vinyl bromide **4-13**, bromination of the olefin **4-14** followed by elimination provided a vinyl bromide **4-15**. nOe experiments was used to determine the stereochemistry of vinyl bromide **4-15**. We assumed that we have the desired diastereomer, because nOe analysis of vinyl bromide **4-15** indicated only existing relationship between H2 and H4.

**Scheme 55**


TBDPSO
4-7
CH3
Ti
CH3
toluene, 120 °C
(15%)
OTBDPS
4-14
i. Br2, DCM
ii. DBU, Benzene
(25%)
H3 H3
Br
H2
H4
H4
H1
OTBDPS
4-15

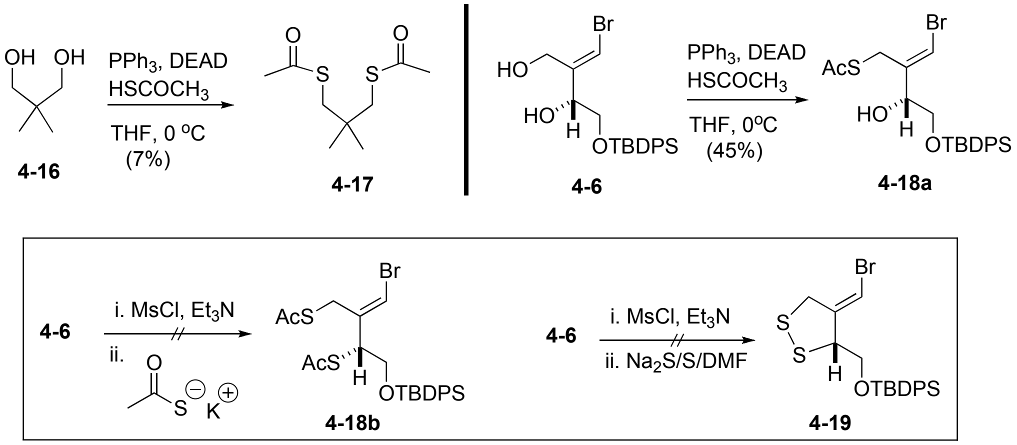
Cleavage of the acetonide group generated Mistunobu substrate diol **4-6** (Scheme 56).

**Scheme 56**


Br
H
OTBDPS
4-15
HS(CH2)3SH
BF3•OEt2 (5 mol %)
(63%)
HO
HO
Br
H
OTBDPS
4-6

Previously, an undergraduate student Jennifer Foulke in our group explored Mistunobu condition[104] with simplified diol **4-16** (Scheme 57) and obtained desired product **4-17**. However, under similar conditions, the mono-substituted thioacetate derivative **4-18a** was only observed due to the steric hindrance of the carbon atom bearing secondary hydroxyl group. Therefore, a relatively small activating reagent methanesulfonyl chloride or trifluoroacetic anhydride was employed. Subsequent replacement with a small nucleophile such as sodium sulfide or potassium thioacetate at various temperatures,[105] gave only decomposition, as suggested by the disappearance of the vinyl proton signal by analysis of the crude reaction mixture by NMR.

**Scheme 57**



4-16
PPh3, DEAD
HSCOCH3
THF, 0 °C
(7%)
4-17
4-6
PPh3, DEAD
HSCOCH3
THF, 0°C
(45%)
4-18a
4-6
i. MsCl, Et3N
ii.
4-18b
4-6
i. MsCl, Et3N
ii. Na2S/S/DMF
4-19

## 4.3 Conclusions

Thiolyngbyan, was first isolated from *Lyngbya majuscula*, a blue-green algae collected from the coastal waters of Curacão, an island located near Venezuela and characterized by Dr. William Gerwick's research group. The proposed structural features of thiolyngbyan include an exo vinyl bromide, one stereogenic center, and a cyclic disulfide moiety and although its structure has not been verified, thiolyngbyan may function as a useful antifungal agent. In our approach to the proposed structure of thiolyngbyan from chiral pool D-glucose **4-9**, the hydroxy groups of 1,3 diol **4-6** could not be replaced with several thiol reagents due to the inherent steric hindrance of the secondary hydroxyl group. Although several potential ways to solve this problem remain unexplored, this project was halted.

# CHAPTER V

# CONCLUSIONS

In order to develop efficient and convenient detection methods for the marine toxin gymnodimine and to study its mode of action, synthetic haptens of gymnodimine were proposed. Building on our recently completed total synthesis of (-)-gymnodimine, core intermediates were prepared and linkers were installed which will be used to conjugate the haptens with the carrier protein. Treatment of ketone **2-78** with alkyl bromide **2-81** and *t*-butyl lithium provided corresponding tertiary alcohol **2-82**. Unexpectedly dehydration of tertiary alcohol **2-82** under thionyl chloride/triethylamine conditions, used successfully in the total synthesis of gymnodimine, led to cyclohexene **2-83a** and **2-83b** as inseparable regioisomers (~1:1) and dehydration of the tertiary alcohol **2-89a** led to exclusively undesired trisubstituted olefin isomer **2-90a** under various conditions. Therefore more advanced intermediate, which bear the butenolide moiety was studied. Although developing a protocol to open the butenolide of hapten **S** with amino acids provided the desired product in moderate yield, much lower yield was observed with hapten **M**. Thus further optimization is currently under study.

β-lactones continue to attract interest as versatile intermediates in synthesis, and these heterocycles are also integral to many biologically active natural products. Early work in the Romo group led to the discovery of a nucleophile-catalyzed aldol lactonization (NCAL) process, resulting in a one-pot formation of bicyclic β-lactones from linear aldehyde-acids in high yields and enantioselectivites. We greatly expanded the scope and utility of this process to keto acid substrates leading to highly versatile carbocycles or heterocycles possessing up to three stereocenters including a masked tertiary carbinol center. We have been able to apply this method to the synthesis of dihydroplakevulin A and the core of tussilagine. Although initial attempts to develop guanidine catalysts for the asymmetric NCAL process were unsuccessful, homobenzotetramisole (HBTM) was found to be a suitable asymmetric catalyst for keto acid substrates. Studies are currently underway in our laboratory to further explore asymmetric NCAL process, which will lead to highly versatile carbocyclic or heterocyclic bicyclic β-lactones.

Thiolyngbyan was first isolated from *Lyngbya majuscula*, a blue-green algae and showed potential as a useful antifungal agent. The synthesis of the proposed structure of thiolyngbyan was starting from D-glucose **4-9**, however, the hydroxy groups of 1,3 diol **4-6** could not be replaced with several thiol reagents due to the inherent steric hindrance of the secondary hydroxyl group. In contrast, when reactions were carried in the present of sulfides, installation of the vinyl bromide was problematic. Although this project was halted, the potential antifungal activity of thiolyngbyan warrants further investigation.

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# APPENDIX A

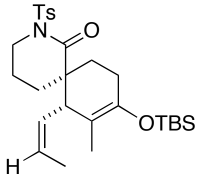
# EXPERIMENTAL AND SELECTED SPECTRAL DATA

# EXPERIMENTAL

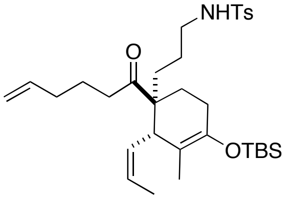
## General

All reactions were carried out under nitrogen atmosphere in oven-dried (120 °C) glassware unless noted otherwise. All non aqueous reactions were carried out under nitrogen atmosphere in oven-dried (120 ºC) glassware. Acetonitrile, dichloromethane, tetrahydrofuran, diethyl ether, *N*,*N*-dimethylformamide, toluene were dried by an Mbraun solvent drying system. Methanol was distilled from magnesium prior to use. Triethyl amine was distilled from calcium hydride prior to use. The molarities indicated for organolithium reagents were established by titration with 2,6-di-tert-butyl-4-methylphenol and 1,10-phenanthroline as indicator. All other commercially obtained reagents were used as received. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. All optical rotation measurements were made at 16-23 °C in a 10 millimeter cell (length); concentration *c* is reported in g/100mL. Infrared spectra were recorded with a Nicolet Impact 410 FTIR spectromer. Enantiomeric excess (ee) was determined by HPLC (Rainin SD-200) analysis using a chiralcell OD column. Flash column chromatography was performed using 60Å Silica Gel (Baker, 230-400 mesh) as a stationary phase. Sometimes basic $Al_2O_3$ (Brochmann, 150 mesh) was used for purification of acid labile compounds. Mass spectra were obtained on a VG analytical 70S high resolution, double focusing, sectored (EB) mass spectrometer at the center for Chemical Characterization and Analysis. Thin layer chromatography (TLC) was performed using glass-backed silica gel 60F254 (Merck, 250 μm thickness). $^1$H NMR and $^{13}$C NMR spectra were recorded on a Varian Unity-500, VXR-300 spectrometer. $^1$H NMR chemical shifts are reported as δ values in ppm relative to tetramethylsilane (TMS, 0.00 ppm). $^1$H NMR coupling constants (J) are reported in Hertz (Hz) and multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), sep (septet), m (multiplet), bs (broad singlet), dd (doublet of doublet), dt (doublet of triplet), dq (doublet of quartets), tt (triplet of triplet), ddd (doublet of doublet of doublet). Unless indicated otherwise, deuterochloroform ($CDCl_3$) served as an internal standard (77.0 ppm) for all $^{13}$C spectra.

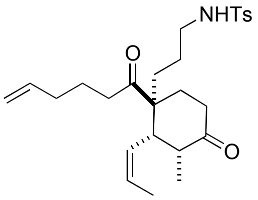
**Procedure**



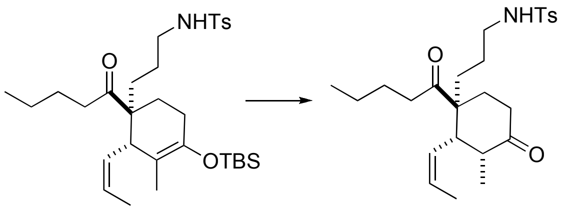
**Olefin 2-76**: By-product obtained during Nozaki-Hiyama-Kishi (NHK) coupling. m.p.122-126 ºC; $R_f$ = 0.57 (20% EtOAc/hexanes); $[\alpha]_D^{21}$ +95.5 (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 8.5 Hz, 2H), 5.41 (dq, *J* = 11.0, 7.0 Hz, 1H), 5.07 (ddq, *J* = 11.0, 11.0, 1.5 Hz, 1H), 3.83 (ddd, *J* = 12.0, 6.0, 6.0 Hz, 1H), 3.60 (ddd, *J* = 12.0, 7.5, 5.0 Hz, 1H), 3.60 (d, *J* = 11.0 Hz, 1H), , 2.44 (s, 3H), 2.06-2.00 (m, 2H), 1.94-1.87 (m, 3H), 1.87-1.80 (m, 1H), 1.69 (ddd, *J* = 13.0, 5.0, 5.0 Hz, 1H), 1.67-1.61 (m, 1H), 1.41 (s, 3H), 1.28 (dd, *J* = 7.0, 1.5 Hz, 3H), 0.94 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 175.3, 144.2, 142.1, 140.1, 136.5, 129.1, 128.9, 128.5, 127.0, 111.9, 47.3, 46.9, 42.8, 31.4, 26.8, 26.7, 25.8, 21.6, 20.7, 18.1, 14.1, 12.7, -3.9, -4.0; IR (thin film): 1686 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{27}H_{42}NO_4SSi$ [M+H]: 504.2598. Found: 504.2626.



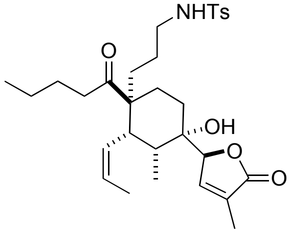
**Amino ketone 2-77**: To a solution of 5-bromo-1-pentene (355 mg, 2.382 mmol) in $Et_2O$ (10 mL) at -78 ºC was added *t*-BuLi (1.5 M in pentane, 3.17 ml, 4.764 mmol) dropwise. After stirring for 30 min, a solution of olefin **2-76** (200 mg, 0.397 mmol) in THF (3 mL) was added. The reaction mixture was stirred at 0 ºC for 30 min, warmed to room temperature and the reaction was quenched by pH 7 buffer. The aqueous layer was extracted with $Et_2O$, and the combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash chromatography (15% → 20% EtOAc/Hexanes) to afford 172 mg (75%) of the desired product **2-77** as a colorless oil. $R_f$ = 0.28 (20% EtOAc/hexanes); $[\alpha]^{17}_D$ + 188.9 (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 5,72-5.80 (m, 1H), 5.69 (dq, *J* = 11.0, 6.5 Hz, 1H). 4.96-5.03 (m, 2H), 4.50 (dd, $J$ = 6.0, 6.0 Hz, 1H), 3.43 (d, $J$ = 10.5 Hz, 1H), 2.79 (ddd, $J$ = 6.5, 6.5, 6.5 Hz, 2H), 2.43 (s, 3H), 2.41 (dd, $J$ = 7.5, 7.5 Hz, 2H), 1.91-2.06 (m, 4H), 1.70 (dd, $J$ = 7.0, 2.0 Hz, 3H), 1.53-1.62 (m, 3H), 1.51 (s, 3H), 1.31-1.35 (m, 3H), 1.12-1.15 (m, 2H), 0.90 (s, 3H), 0.80-0.95 (m, 2H), 0.034 (s, 3H), 0.026 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 212.9, 143.6, 143.4, 138.1, 136.8, 129.7, 127.0, 125.3, 115.1, 113.7, 53.0, 43.4, 41.0, 35.9, 33.7, 33.1, 27.6, 26.2, 25.8, 24.0, 22.6, 21.5, 18.1, 15.0, 13.5, -3.8, -4.1; IR (thin film): 3280, 1691 cm$^{-1}$; HRMS (ESI) calcd for $C_{32}H_{52}NO_4SSi$ [M+H]:574.33863. Found: 574.3336.



**Ketone 2-78**: To a solution of the silyl ether **2-77** (349 mg, 0.608 mmol) in THF/CH$_2$Cl$_2$/MeOH (1:1:1, 15 mL) at 21 °C was added *p*-toluenesulfonic acid monohydrate (306 mg, 1.216 mmol). The reaction mixture was stirred at room temperature for 7 h and quenched by saturated NaHCO$_3$ solution. The aqueous layer was extracted with Et$_2$O and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (20%→30% EtOAc/hexanes) to afford 239 mg (85%) of the desired product as a white foam. R$_f$ = 0.26 (30% EtOAc/hexanes); $[\alpha]^{21}_D$ –54.7 (*c* 2.47, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.71 (d, $J$ = 8.5 Hz, 2H), 7.31 (d, $J$ = 8.5 Hz, 2H), 5,75-5.83 (m, 2H), 5.02-5.09 (m, 2H), 4.90 (dd, $J$ = 11.5, 11.5 Hz, 1H), 4.25 (dd, $J$ = 6.0, 6.0 Hz, 1H), 3.43 (dd, $J$ = 11.5, 4.5 Hz, 1H), 2.89 (dd, $J$ = 13.5, 7.0 Hz, 2H), 2.52-2.67 (m, 2H), 2.44 (s, 3H), 2.27-2.44 (m, 4H), 2.12 (dd, $J$ = 14.5, 8.0 Hz, 2H), 1.74 (dt, $J$ = 7.0, 1.5 Hz, 2H), 1.65 (dd, $J$ = 7.0, 1.5 Hz, 3H), 1.40-1.53 (m, 3H), 1.03-1.21 (m, 2H), 0.87 (d, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 213.3, 212.0, 143.4, 137.8, 136.7, 130.0, 130.0, 126.9, 124.5, 115.3, 55.2, 45.4, 45.1, 43.2, 38.6, 35.2, 34.1, 32.9, 29.6, 24.1, 22.7, 21.4, 13.5, 12.0; IR (thin film): 3284, 1703, 1638 cm$^{-1}$; HRMS (ESI) calcd for $C_{26}H_{38}NO_4S$ [M+H]:460.25215. Found: 460.2681.

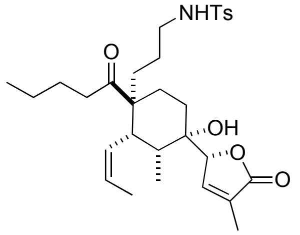


**Ketone 2-79**: To a solution of the silyl ether (81 mg, 0.144 mmol) in THF/$CH_2Cl_2$/MeOH (1:1:1, 6.0 mL) at room temperature was added *p*-toluenesulfonic acid monohydrate (109 mg, 0.58 mmol). The reaction mixture was stirred at 21 °C for 2.5 h and quenched by saturated $NaHCO_3$ solution. The aqueous layer was extracted with $Et_2O$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography 20%→30% EtOAc/hexanes) to afford 63 mg (98%) of the desired product as a white foam. $R_f$ = 0.24 (30% EtOAc/hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.5 Hz, 2H), 7.32 (d, *J* = 7.5 Hz, 2H), 5,77 (dq, *J* = 11.5, 7.5 Hz, 1H), 4.90 (ddq, *J* = 11.5, 11.5, 2.0 Hz, 1H), 4.18 (dd, *J* = 6.5, 6.5 Hz, 1H), 3.43 (dd, *J* = 11.5, 2.5 Hz, 1H), 2.80-2.88 (m, 2H), 2.50-2.64 (m, 2H), 2.44 (s, 3H), 2.27-2.42 (m, 4H), 1.60 (dd, *J* = 7.0, 1.5 Hz, 3H), 1.45-1.64 (m, 5H), 1.35-1.39 (m, 2H), 1.08-1.22 (m, 2H), 0.95, (t, *J* = 7.5 Hz, 3H), 0.87 (d, *J* = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 213.5, 212.0, 143.5, 136.7, 130.1, 129.7, 127.0, 124.6, 55.3, 45.5, 45.1, 43.3, 38.7, 36.0, 34.1, 29.3, 26.0, 24.2, 22.5, 21.5, 14.0, 13.6, 12.0; IR (thin film): 3287, 1706 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{25}H_{38}NO_4S$ [M+H]: 448.25215. Found: 448.2437.

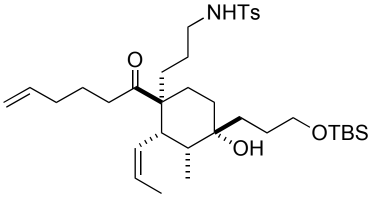


Butenolide **2-80**: A mixture of the racemic ketone **2-79** (14.5 mg, 0.0334 mmol) and silyloxyfuran **2-71** (85 mg, 0.334 mmol) was azeotropically dried with PhMe and dissolved in $CH_2Cl_2$ (1.5 mL). To this vigorously stirred solution was added $TiCl_4$ (1.0 M in $CH_2Cl_2$, 130 μL) dropwise at 21 °C. The cloudy yellow reaction mixture was stirred at 21 °C for 30 s and quenched by saturated $NH_4Cl$ solution. The mixture was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated in vacuo. The residue was purified by flash chromatography (10%→20%→30%→40% EtOAc/hexanes) to

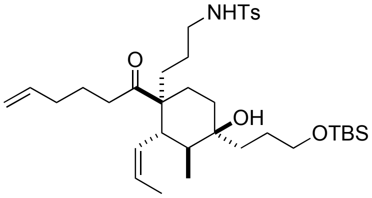
afford two diastereomers **2-80a** (less polar, 6.2 mg, 34% yield) and **2-80b** (more polar, 5.9 mg, 32% yield), respectively. **2-80a**: R$_f$ = 0.24 (40% EtOAc/hexanes); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.69 (d, *J* = 7.5 Hz, 2H), 7.29 (d, *J* = 7.5 Hz, 2H), 6.88 (s, 1H), 5,58-5.59 (m, 2H), 4.93 (s, 1H), 4.23 (t, *J* = 6.5 Hz, 1H), 3.17 (dd, *J* = 11.0, 4.5 Hz, 1H), 2.78 (dd, *J* = 13.5, 6.5 Hz, 2H), 2.42 (s, 3H), 2.32-2.42 (m, 2H), 1.80-1.91 (m, 1H), 1.74 (dt, *J* = 12.0, 7.0 Hz, 1H), 1.62 (d, *J* = 5.5, 3H) 1.60 (s, 3H), 1.32-1.56 (m, 4H), 1.23-1.31 (m, 4H), 1.06-1.16 (m, 2H), 0.97 (d, *J* = 4.5 Hz, 3H), 0.88-0.96 (m, 2H), 0.92 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 214.5, 173.7, 144.6, 143.5, 131.5, 129.7, 128.1, 127.0, 126.5, 86.3, 75.2, 54.6, 43.2, 39.2, 37.0, 35.9, 34.6, 29.7, 29.3, 26.0, 25.4, 23.3, 22.5, 21.5, 14.0, 13.5, 13.4, 10.8; IR (thin film): 3500, 3281, 1756, 1694 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{30}H_{44}NO_6S$ [M+H]: 546.28893. Found: 546.3023. Recrystallization from $CH_2Cl_2$ provided colorless crystals suitable for X-ray analysis.



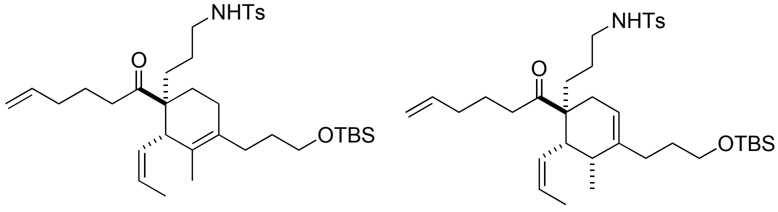
**2-80b**: R$_f$ = 0.17 (40% EtOAc/hexanes); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.68 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.09 (s, 1H), 5,69 (dq, *J* = 11.5, 7.0 Hz, 1H), 5.52 (dd, *J* = 11.0, 11.5 Hz, 1H), 4.73 (s, 1H), 4.21 (t, *J* = 6.0 Hz, 1H), 3.19 (dd, *J* = 11.5, 4.0 Hz, 1H), 2.75 (dd, *J* = 11.5, 6.0 Hz, 2H), 2.42 (s, 3H), 2.28-2.46 (m, 2H), 1.71-1.91 (m, 2H), 1.64 (d, *J* = 5.5 Hz, 3H), 1.60 (s, 3H), 1.41-1.58 (m, 2H), 1.20-1.41 (m, 6H), 1.06-1.16 (m, 2H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.81-0.96 (m, 2H), 0.90 (t, *J* = 7.5 Hz, 3H); ); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 213.1, 173.9, 147.2, 136.7, 130.8, 129.7,128.1, 127.5, 127.0, 83.1, 75.0, 54.3, 43.3, 39.3, 36.4, 34.7, 28.7, 25.8, 25.0, 25.4, 23.2, 22.4, 21.5, 14.0, 13.7, 12.7, 10.7; IR (thin film): 3496, 3284, 1762, 1699 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{30}H_{44}NO_6S$ [M+H]: 546.28893. Found: 546.3026.



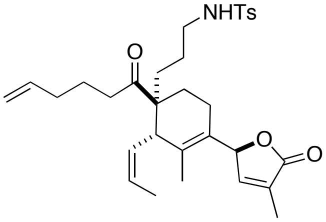
**Tertiary alcohol 2-82**: To a solution of alkyl bromide (531 mg, 0.0571 mmol) in $Et_2O$ (30 mL) at -78 ºC was added *t*-BuLi (1.5 M in pentane, 2.8 mL, 4.2 mmol) dropwise. After stirring for 10 min, a solution of olefin **2-82** (173 mg, 0.376 mmol) in THF (9 mL) was added. The reaction mixture was stirred at -78 ºC for 1 h, warmed to room temperature. The reaction was quenched by pH 7 buffer. The aqueous layer was extracted with $Et_2O$, and the combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash chromatography (10% → 30% EtOAc/Hexanes) to afford two diastereomers **2-82a** (less polar, ~118 mg, 50% yield) and **2-82b** (more polar, 75 mg, 31% yield), respectively. **2-82a**: $R_f$ = 0.50 (30% EtOAc/hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 5,70-5.80 (m, 1H), 5.54-5.65 (m, 2H), 4.89-5.09 (m, 2H), 4.46 (t, *J* = 6.0 Hz, 1H), 3.51-3.64 (m, 2H), 3.11 (dd, *J* = 11.0, 4.5 Hz, 1H), 2.75 (dd, *J* = 13.0, 6.5 Hz, 2H), 2.35-2.49 (m, 2H), 2.42 (s, 3H), 1.91-2.16 (m, 2H), 1.71-1.86 (m, 3H), 1.60 (d, *J* = 6.5 Hz, 3H), 1.50-1.66 (m, 2H), 1.39-1.49 (m, 4H), 1.26-1.39 (m, 4H), 1.09-1.25 (m, 3H), 0.87 (s, 9H), 0.81 (d, *J* = 7.0 Hz, 3H), 0.068 (s, 3H), 0.025 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 214.3, 143.4, 138.1, 136.7, 129.6, 128.9, 127.1, 125.8, 115.1, 72.8, 63.6, 54.7, 43.3, 39.2, 38.6, 38.4, 35.4, 34.9, 33.9, 33.1, 26.8, 26.1, 25.9, 23.2, 22.8, 21.5, 18.2, 13.4, 12.6, -5.4; IR (thin film): 3532, 3284, 1697 cm$^{-1}$; HRMS (ESI) calcd for $C_{35}H_{60}NO_5SSi$ [M+H]:634.39615 Found: 634.3571.



**2-82b**: $R_f$ = 0.47 (30% EtOAc/hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 5,71-5.79 (m, 1H), 5.46 (dq, *J* = 13.5, 6.0 Hz, 2H), 4.96-5.02 (m, 3H), 4.88 (t, *J* = 7.5 Hz, 1H), 3.66-3.76 (m, 2H), 2.93 (dd, *J* = 13.5, 6.5 Hz, 2H), 2.91 (dd, *J* = 4.0, 4.0 Hz, 2H), 2.43 (s, 3H), 2.31-2.42 (m, 2H), 2.01 (dd, *J* = 14.0, 7.0 Hz, 2H), 1.78-1.93 (m, 2H), 1.55-1.78 (m, 2H), 1.56-1.65 (m, 4H), 1.42-1.53 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H), 0.92 (s, 9H), 0.77 (d, *J* = 6.5 Hz, 3H), 0.092 (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 213.7, 143.2, 138.1, 136.8, 129.6, 128.8, 127.1, 125.6, 115.0, 72.9, 63.8, 55.0, 44.5, 43.6, 42.4, 37.0, 33.0, 31.4, 26.3, 25.9, 25.6, 24.4, 23.0, 21.4, 18.2, 13.3, 12.2, -5.393, -5.419; LRMS (ESI) calcd for $C_{35}H_{60}NO_5SSi$ [M+H]:634.39615 Found: 634.3789.

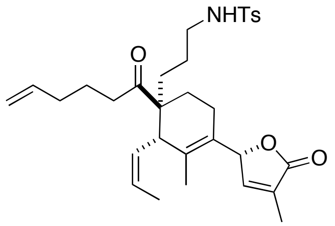


**Olefin 2-83**: To a solution of the alcohol **2-82** (63 mg, 0.00265 mmol, azeotropically dried with PhMe) in $CH_2Cl_2$ (10 mL) at -78 ºC was added 140 μL of $Et_3N$ and 140 μL of a solution of $SOCl_2$ (36 μL) in $CH_2Cl_2$ (1 mL). The reaction mixture was stirred at -78 ºC for 30 min and quenched by pH 7 buffer. The aqueous layer was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (10% EtOAc/hexanes) to afford 46 mg (75%) of the inseparable mixture of olefin **2-83a** and its regioisomer **2-83b** ($\Delta^{5,6}$ : $\Delta^{5,24}$ = ~1:1). $R_f$ = 0.61 (10% EtOAc/hexanes); IR (thin film): 3289, 1700 cm$^{-1}$; HRMS (ESI) calcd for $C_{35}H_{58}NO_4SSi$ [M+H]:616.38558 Found: 616.3706.

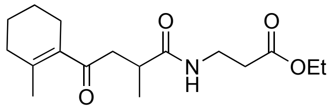


**Olefin 2-96**: To a solution of the alcohol **2-95a** (19 mg, 0.034 mmol, azeotropically dried with PhMe) in $CH_2Cl_2$ (2 mL) at -78 ºC was added 140 μL of a solution of $SOCl_2$ followed by 24 μL of $Et_3N$. The reaction mixture was stirred at -78 ºC for 10 min and quenched by pH 7 buffer. The aqueous layer was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (20% EtOAc/hexanes) to afford 11 mg (60%) of the desired product **2-96** as a colorless oil. $R_f$ = 0.23 (30% EtOAc/hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 6.72 (s, 1H), 5,70-5.78 (m, 1H), 5.72

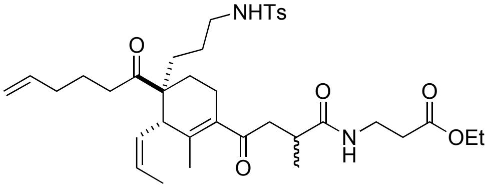
(s, 1H), 5.63 (dq, *J* = 11.0, 6.5 Hz, 1H), 5.06 (dt, *J* = 11.0, 1.5 Hz, 1H), 5.01-4.96 (m, 2H), 4.81 (t, *J* = 6.0 Hz, 1H), 3.39 (d, *J* = 12.0 Hz, 1H), 2.77 (dd, *J* = 13.5, 6.5 Hz, 2H), 2.41 (s, 3H), 2.30-2.42 (m, 2H), 2.00 (dd, *J* = 11.5, 6.5 Hz, 2H), 1.88 (s, 3H), 1.86 (dt, *J* = 19.5, 6.0 Hz, 2H), 1.75 (s, 3H), 1.73 (dd, *J* = 7.0, 1.5 Hz, 3H), 1.54-1.60 (m, 3H), 1.33-1.46 (m, 2H), 1.15-1.22 (m, 1H), 1.01-1.14 (m, 1H), 0.81-0.91 (m, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 212.8, 174.6, 147.6, 143.5, 137.9, 136.7, 136.4, 129.7, 128.3, 127.0, 126.8, 123.1, 115.2, 79.6, 53.2, 43.3, 41.4, 35.1, 33.8, 33.0, 26.5, 23.8, 22.7, 21.5, 20.6, 17.7, 13.8, 10.6; HRMS (ESI) Calcd. for $C_{31}H_{42}NO_5S$ [M+H]: 540.27837. Found: 540.2450.



**Olefin 2-96b**: To a solution of the alcohol **2-95b** (38 mg, 0.068 mmol, azeotropically dried with PhMe) in $CH_2Cl_2$ (6 mL) at -78 ºC was added 140 μL of a solution of $SOCl_2$ followed by 48 μL of $Et_3N$. The reaction mixture was stirred at -78 ºC for 30 min and quenched by pH 7 buffer. The aqueous layer was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (20% EtOAc/hexanes) to afford 20 mg (68%) of the desired product **2-96b** as a colorless oil. $R_f$ = 0.21 (30% EtOAc/hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 6.79 (s, 1H), 5,72-5.80 (m, 1H), 5.72 (s, 1H), 5.63 (dq, *J* = 11.0, 7.0 Hz, 1H), 4.97-5.06 (m, 3H), 4.31 (t, *J* = 6.0 Hz, 1H), 3.46 (d, *J* = 11.0 Hz, 1H), 2.79 (dd, *J* = 13.0, 6.0 Hz, 2H), 2.43 (s, 3H), 2.38 (t, *J* = 6.0 Hz, 2H), 2.03 (dd, *J* = 14.0, 7.0 Hz, 2H), 1.92 (s, 3H), 1.88-1.96 (m, 1H), 1.74 (s, 3H), 1.50-1.66 (m, 5H), 1.34-1.44 (m, 2H), 1.16-1.24 (m, 1H), 0.61-1.06 (m, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 212.4, 174.7, 147.3, 143.5, 138.1, 136.8, 135.0, 129.9, 129.7, 127.0, 126.5, 123.3, 115.2, 79.9, 53.1, 43.3, 41.3, 35.4, 33.9, 33.0, 26.6, 23.7, 22.6, 21.7, 17.7, 13.8, 10.6; HRMS (ESI) Calcd. for $C_{31}H_{42}NO_5S$ [M+H]: 540.27837. Found: 540.2133.

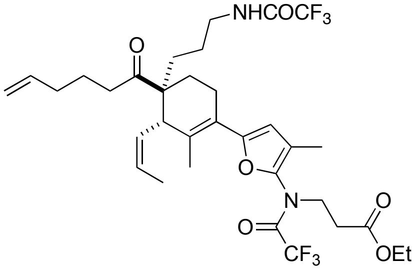


**Amide 2-101**: A mixture of the Butenolide **2-100** (15.0 mg, 0.078 mmol) and β-alanine ethylester (120 mg, 0.78 mmol) was dissolved in $Et_3N$ (0.3 mL). The reaction mixture was stirring for 12h at 40 °C. The volatiles were evaporated and the residue was purified by flash chromatography (20%→30%→40% EtOAc/hexanes) to afford 11 mg (46%) of the desired product as a colorless oil. $R_f$ = 0.45 (40% EtOAc/hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 6.29 (s, 1H), 4.16 (q, *J* = 8.0 Hz, 2H), 3.49 (dd, *J* = 12.0, 6.0 Hz, 2H), 3.00 (dd, *J* = 18.0, 8.5 Hz, 1H), 2.74-2.82 (m, 1H), 2.48 (ddd, *J* = 8.0 6.0, 4.0 Hz, 2H), 2.44 (d, *J* = 4.5 Hz, 1H), 2.14-2.28 (m, 2H), 2.07 (br s, 2H), 1.81 (s, 3H), 1.56-1.62 (m, 4H), 1.46 (s, 3H), 1.27 (s, 3H), 1.47 (t, *J* = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 205.0, 175.9, 172.5, 141.0, 132.6, 45.5, 35.9, 34.8, 34.1, 33.0, 28.0, 26.4, 21.6, 17.8, 14.2, 9.7; HRMS (ESI) Calcd. for $C_{17}H_{28}NO_4$ [M+H]: 310.20183. Found: 310.1960.

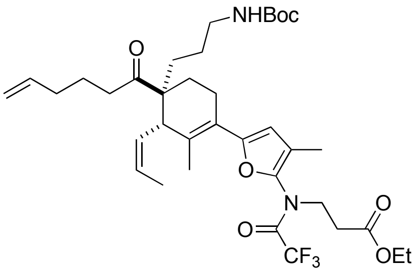


**Amide 2-103**: A mixture of the Butenolide **2-100** (11.0 mg, 0.0204 mmol) and β-alanine ethylester (31 mg, 0.202 mmol) was dissolved in $Et_3N$ (0.3 mL). The reaction mixture was stirring for 12h at 40 °C. The volatiles were evaporated and the residue was passed through a short pad of $SiO_2$ flushing with EtOAc. The crude was dissolved in acetic acid/$H_2O$ (1:1, 0.6 mL) at room temperature and the reaction mixture was stirred at room temperature for 18 h and the aqueous layer was extracted with EtOAc and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (40%→70%→100% EtOAc/hexanes) to afford the inseparable mixture of amide **2-103** 6 mg (45%) as a colorless oil. $R_f$ = 0.28 (40% EtOAc/hexanes); Key $^1$H NMR signals; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, *J* = 14.0 Hz, 2H), 7.29 (d, *J* = 13.5 Hz, 2H), 6.25 (t, *J* = 6.0 Hz, 1H), 5,73-5.79 (m, 1H), 5.60-5.70 (m, 1H), 4.98-5.08 (m, 3H), 4.26 (t, *J* = 6.5 Hz, 1H), 4.15 (q, *J* = 7.5 Hz, 1H), 3.49 (dd, *J* = 12.0, 6.0 Hz, 1H), 3.44 (d, *J* = 10.5 Hz, 1H), 2.96 (dd, *J* = 18.0, 9.0 Hz, 1H), 2.81 (dd, *J* = 13.0, 6.5 Hz, 2H), 2.66-2.71 (m, 1H), 2.50 (t, *J* = 6.5 Hz, 2H), 2.34-2.48 (m, 4H), 2.45 (s, 3H), 2.18-2.28

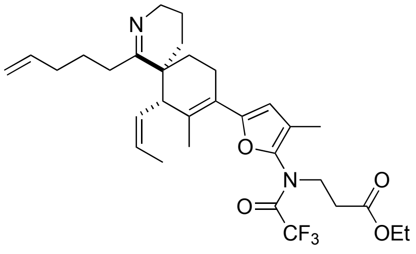
(m, 1H), 1.96-2.16 (m, 3H), 1.77 (s, 3H), 1.74 (d, *J* = 7.0 Hz, 3H), 1.59-1.64 (m, 4H), 1.36-1.48 (m, 3H), 1.27 (t, *J* = 7.5 Hz, 3H), 1.11 (d, *J* = 7.0 Hz, 3H); Key $^{13}$C NMR signals; $^{13}$C NMR (125 MHz, CDCl$_3$) δ 212.6, 205.2, 175.7, 172.5, 143.4, 141.3, 138.0, 136.8, 131.6, 129.7, 128.1, 127.2, 127.0, 115.2, 60.6, 53.0, 45.8, 43.3, 35.9, 35.4, 34.8, 34.0, 33.6, 33.0, 26.3, 24.4, 23,7, 22.7, 21.5, 20.2, 17.9, 14.1, 13.8; HaRMS (ESI) Calcd. for $C_{36}H_{53}N_2O_7S$ [M+H]: 657.35735. Found: 657.3439.



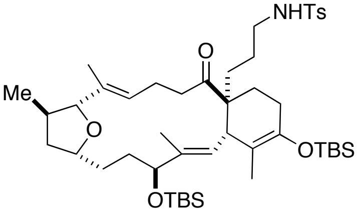
**Furan 2-104**: To a solution of Tosylamine **2-103** (28 mg, 0.0426 mmol) in THF (4 mL) was added triethylamine (0.12 mL, 0.852 mmol) and trifluoroacetic anhydride (0.072 mL, 0.512 mmol) at 0 °C. After stirring for 15 min, samarium iodide (0.1 M in THF) was added until the mixture stayed blue (totally about 5 mL, 0.5 mmol). The mixture was stirred for 5 min, diluted with $CH_2Cl_2$, and quenched with 10% sodium thiosulfate solution. The aqueous layer was extracted with $Et_2O$, and the combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash chromatography (10% → 30% EtOAc/Hexanes) to afford 21 mg (73%) of the desired product as a slightly yellow oil. R$_f$ = 0.53 (30% EtOAc/hexanes); [α]$^{19}_{D}$ + 196.4 (*c* 0.6, CHCl$_3$); $^{1}$H NMR (500 MHz, CDCl$_3$) δ 6.25 (s, 1H), 6.03 (s, 1H), 5.71-5.80 (m,1H), 5.70 (dq, *J* = 11.0, 7.0 Hz 1H), 5.17 (ddq, *J* = 11.0, 11.0, 2.0 Hz, 1H), 4.96-5.02 (m, 2H), 4.10 (q, *J* = 7.0 Hz, 2H), 3.96 (t, *J* = 7.0 Hz, 2H), 3.58 (d, *J* = 10.5 Hz, 1H), 3.21-3.32 (m, 2H), 2.61 (t, *J* = 7.5 Hz, 2H), 2.43-2.54 (m, 3H), 2.31-2.41 (m, 1H), 2.00-2.19 (m, 3H), 2.19-2.01 (m, 3H), 1.90 (s, 3H), 1.88 (s, 3H), 1.72-1.81 (m, 1H), 1.82 (dd, *J* = 7.0, 1.5 Hz, 3H) 1.52-1.68 (m, 1H), 1.48- 1.52 (m, 2H), 1.16,-1.41 (m, 1H), 1.24 (t, *J* = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 212.9, 170.5, 158.2 (q, *J* = 36.0 Hz), 157.2 (q, *J* = 36.6 Hz), 152.5, 138.0, 137.6, 135.2, 128.6, 126.7, 120.0, 117.5, 115.8 (q, *J* = 286.1 Hz), 115.2, 115.7 (q, *J* = 286.5 Hz), 111.0, 60.9, 53.1, 45.9, 42.2, 40.0, 35.6, 34.0, 33.0, 32.3, 26.5, 24.8, 23.3, 22.6, 20.4, 14.1, 13.9, 9.5; HRMS calcd for $C_{33}H_{42}F_6N_2NaO_6$ [M+Na]: 699.28448. Found: 699.2344.



**Boc amine 2-107**: To a solution of the trifluoroacetamide **2-104** (18 mg, 0.0228 mmol) was added $Et_3N$ (110 μL), DMAP (10 mg) and a solution of $(Boc)_2O$ (50 mg, 0.228 mmol) in $CH_2Cl_2$ (1.2 mL) at room temperature. After 25 min, TLC indicated the starting material was completely converted to a new intermediate ($R_f$ = 0.63, 30% EtOAc/hexanes). Hydrazine hydrate (50 μL) was added and the cloudy reaction mixture was stirred at room temperature for 5 min (TLC indicated the complete hydrolysis of this intermediate). The mixture was then quenched with saturated $NH_4Cl$ solution. The aqueous layer was extracted with EtOAc and the combined organic layers were concentrated *in vacuo* and purified by flash chromatography (10%→20%→30% EtOAc/hexanes) to provide 13 mg (84%) of the desired Boc amine **2-107** as a colorless oil. $R_f$ = 0.53 (30% EtOAc/hexanes); $[\alpha]^{19}_D$ + 161.5 (*c* 1.2, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) δ 6.02 (s, 1H), 5.70-5.81 (m,1H), 5.67 (dq, *J* = 11.0, 7.0 Hz 1H), 5.15 (t, *J* = 11.0 Hz, 1H), 4.96-5.02 (m, 2H), 4.10 (q, *J* = 7.5 Hz, 2H), 3.96 (t, *J* = 7.5 Hz, 2H), 3.70 (d, *J* = 10.5 Hz, 1H), 2.84-3.15 (m, 2H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.40-2.54 (m, 3H), 2.28-2.38 (m, 1H), 2.00-2.18 (m, 4H), 1.90 (s, 3H), 1.72-1.84 (m, 3H), 1.88 (s, 3H), 1.82 (dd, *J* = 7.0, 1.5 Hz, 3H), 1.42- 1.52 (m, 2H), 1.44 (s, 9H), 1.16,-1.31 (m, 1H), 1.23 (t, *J* = 7.5 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 213.0, 170.4, 158.2 (q, *J* = 36.1 Hz), 155..8, 152.7, 138.1, 137.5, 135.5, 128.6, 126.6, 119.8, 117.4, 116.8 (q, *J* = 287.0 Hz), 110.9, 79.1, 60.9, 53.3, 45.9, 42.4, 40.7, 35.5, 34.3, 33.1, 32.3, 29.7, 28.4, 28.3, 28.1, 26.5, 24.9, 24.4, 22.7, 20.4, 14.1, 13.9, 9.5; HRMS (MALDI) calcd for $C_{36}H_{51}F_3N_2NaO_7$ [M+Na]: 703.35461. Found: 703.3273.

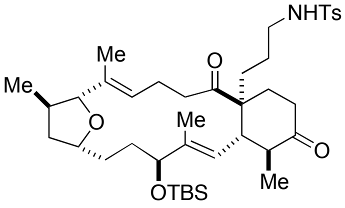


**Spirocyclic imine 2-108**: To a solution of the Boc amine **26** (13.0 mg) in $CH_2Cl_2$ (0.8 mL) was added trifluoroacetic acid (200 μL). After 30 min, the reaction was quenched by careful addition of solid $NaHCO_3$ (320 mg). After stirring for 5 min, saturated $NaHCO_3$ (1 mL) was added. The mixture was extracted with EtOAc/$CH_2Cl_2$ (5:1, 1.5 mL) for 6 times. The combined organic layers were concentrated *in vacuo* and purified by flash chromatography (basic $Al_2O_3$, $CH_2Cl_2 \rightarrow 10\%$ MeOH/$CH_2Cl_2$) to provide a primary amine ($R_f = 0.13$, 7% MeOH/$CH_2Cl_2$). Upon standing under high vacuum for 10 h, this amine cyclized to provide 7.1 mg (66%) of the free base of gymnodimine as a colorless oil. $R_f = 0.32$ (7% MeOH/$CH_2Cl_2$); $[\alpha]^{19}_D + 59.0$ (*c* 0.71, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) δ 6.10 (s, 1H), 5.76-5.82 (m,1H), 5.69 (dq, *J* = 11.0, 7.0 Hz 1H), 5.30 (ddq, *J* = 11.0, 11.0, 1.5 Hz, 1H), 5.03 (dd, *J* = 12.0, 1.5 Hz 1H), 4.96 (dd, *J* = 11.5, 2.0 Hz 1H), 4.12 (q, *J* = 7.5 Hz 1H), 3.99 (t, *J* = 7.5 Hz, 2H), 3.51-3.61 (m, 2H), 3.54 (d, *J* = 11.0 Hz, 1H), 2.64 (t, *J* = 7.5 Hz, 2H), 2.36-2.49 (m, 1H), 2.26-2.36 (m, 3H), 2.18-2.24 (m, 1H), 1.99-2.11 (m, 2H),1.84-1.98 (m, 2H), 1.95 (s, 3H), 1.72-1.84 (m, 2H), 1.80 (s, 3H), 1.69 (dd, *J* = 7.0, 1.5 Hz, 3H), 1.51-1.71 (m, 2H), 1.21-1.39 (m, 1H), 1.26 (t, *J* = 7.5 Hz, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 174.7, 170.5, 158.2 (q, *J* = 36.3 Hz), 152.7, 138.8, 137.7, 134.5, 129.2, 127.0, 121.1, 117.5, 115.7 (q, *J* = 287.1 Hz), 114.6, 111.0, 60.9, 49.4, 45.9, 45.0, 41.3, 33.9, 33.4, 32.3, 31.2, 29.7, 26.8, 26.5, 24.0, 19.9, 19.8, 14.1, 13.6; IR (thin film): 1738, 1720, 1643 $cm^{-1}$; HRMS (ESI) Calcd. for $C_{31}H_{42}F_3N_2NaO_4$ [M+H]: 563.30967. Found: 563.3093.

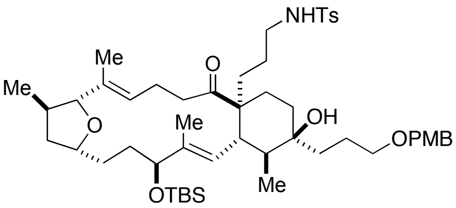


***N*-Tosyl amine 2-69**: Known macro cyclic amine **2-69** was prepared via Barbier-type coupling protocol, which was developed in our laboratory as colorless foam. $R_f = 0.30$

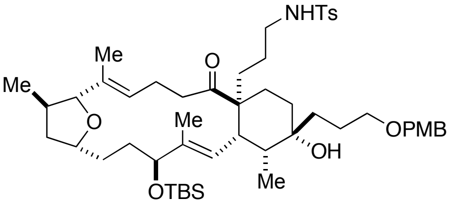
(20% EtOAc/hexanes); $[\alpha]^{20}_{D}$ + 20.2 (*c* 1.0, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 5.01 (br t, *J* = 5.0 Hz, 1H), 4.94 (dd, *J* = 11.5 Hz, 1.5 Hz, 1H), 4.86 (t, *J* = 5.5 Hz, 1H, NH), 4.09 (m, 1H), 3.99 (br s, 1H), 3.84 (dd, *J* = 3.5 Hz, 11.5 Hz, 1H), 3.68 (d, *J* = 11.5 Hz, 1H), 2.94 (m, 2H), 2.74 (ddd, *J* = 3.0 Hz, 12.5 Hz, 19.0 Hz, 1H), 2.61 (ddd, *J* = 3.0 Hz, 3.0 Hz, 19.0 Hz, 1H), 2.44 (s, 3H), 2.21 (m, 2H), 2.10-1.96 (m, 5H), 1.82 (m, 1H), 1.73 (m, 2H), 1.69 (d, *J* = 1.5 Hz, 3H), 1.66-1.56 (m, 2H), 1.53 (s, 3H), 1.50 (m, 3H), 1.46 (s, 3H), 1.41 (m, 2H), 1.11 (d, *J* = 7.0 Hz, 3H), 0.96 (s, 9H), 0.86 (s, 9H), 0.142 (s, 3H), 0.135 (s, 3H), 0.05 (s, 3H), 0.01 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 213.1, 143.1, 143.0, 140.5, 136.8, 135.5, 129.5, 127.2, 123.8, 122.6, 111.9, 89.1, 79.4, 78.1, 53.3, 45.4, 43.7, 37.6, 36.9, 34.6, 32.9, 32.1, 28.8, 27.3, 25.8, 25.6, 24.6, 23.4, 21.5, 20.6, 20.0, 18.1, 18.0, 14.2, 13.8, 10.7, -3.6, -3.8, -4.76, -4.82; IR (thin film): 3281, 1701, 1683 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{46}H_{77}NO_6SSi_2Li$ [M+Li]: 834.5170. Found: 834.5163.



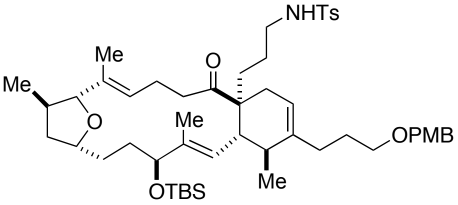
**Macrocyclic ketone 2-86**: To a solution of macrocycle **2-69** (37 mg, 0.0447 mmol) in THF/EtOH (1:1, 4 mL) at 23 °C was added pyridinium *p*-toluenesulfonate (23 mg, 0.0893 mg). The reaction mixture was stirred at 23 °C for 48 h and quenched by saturated $NaHCO_3$ solution. The aqueous layer was extracted with $Et_2O$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (40%→70%→100% EtOAc/hexanes) to afford 17 mg (53%) of the ketone **2-86** as a colorless oil, 14 mg (~34%) of the silyl enolether **2-87** and the recovered starting material of **2-86**. $R_f$ = 0.25 (30% EtOAc/hexanes); $[\alpha]^{21}_{D}$ –47.4 (*c* 0.75, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 8.5 Hz, 2H), 4.97-4.99 (m, 2H), 4.86 (br t, *J* = 5.5 Hz, 1H), 4.06-4.11 (m, 1H), 3.97 (br s, 1H), 3.81 (dd, *J* = 11.5 Hz, 3.0 Hz, 1H), 3.02 (dd, *J* = 13.0 Hz, 1.5 Hz, 1H), 3.02 (dd, *J* = 11.0 Hz, 11.0 Hz, 1H), 2.56-2.68 (m, 2H), 2.44 (s, 3H), 2.41 (dt, *J* = 14.5 Hz, 3.5 Hz, 1H), 2.17-2.37 (m, 2H), 2.15-2.19 (m, 3H), 1.92-2.15 (m, 6H), 1.65-1.72 (m, 3H), 1.63 (s, 3H), 1.51 (s, 3H), 1.28-1.56 (m, 3H), 1.11 (d, *J* = 7.0 Hz, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.92-1.06 (m, 1H), 0.85 (s, 9H), 0.04 (s, 3H), -0.01 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 211.4, 210.1, 143.4, 141.2, 136.9, 129.7, 127.1, 123.0, 122.2, 89.1, 79.5, 78.0, 54.4, 50.4, 46.1, 43.6, 37.6, 37.3, 37.0, 34.7, 32.8, 32.4, 31.3, 25.6, 24.5, 23.2, 21.5, 20.6, 20.0, 18.0, 14.2, 11.7, 11.1, -4.7, -4.8; IR (thin film): 3278, 1738, 1706 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{40}H_{63}LiNO_6$ [M+Li]: 720.43054. Found: 720.4333.



**Tertiary alcohol 2-89a**: To a solution of alkyl bromide (102 mg, 0.392 mmol) in $Et_2O$ (2.5 mL) at -78 ºC was added *t*-BuLi (1.68 M in pentane, 0.45 mL, 0.784 mmol) dropwise. After stirring for 10 min, a solution of olefin **2-86** (35 mg, 0.376 mmol) in THF (3 x 0.8 mL) was added. The reaction mixture was stirred at -78 ºC for 1 h, warmed to room temperature. The reaction was quenched by pH 7 buffer. The aqueous layer was extracted with $Et_2O$, and the combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash chromatography (20% → 30% EtOAc/Hexanes) to afford two diastereomers **2-89a** (less polar, ~22 mg, 50% yield) and **2-89c** (more polar, ~9 mg, 15% yield), respectively. **2-89a**: $R_f$ = 0.22 (40% EtOAc/hexanes); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.75 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 6.88 (d, *J* = 8.0 Hz, 2H), 5.0 (br s, 1H), 4.87 (t, *J* = 5.5 Hz, 1H), 4.86 (d, *J* = 11.5 Hz, 1H), 4.49 (dd, *J* = 11.5, 6.5 Hz, 2H), 4.06-4.11 (m, 1H), 3.97 (br s, 1H), 3.80 (dd, *J* = 12.5 Hz, 3.0 Hz, 1H), 3.80 (s, 3H), 3.50-3.58 (m, 2H), 2.88-3.00 (m, 2H), 2.71 (dd, *J* = 11.0, 11.0 Hz, 1H), 2.62 (dd, *J* = 5.0 Hz, 5.0 Hz, 1H), 2.43 (s, 3H), 2.16-2.26 (m, 2H), 1.96-2.05 (m, 2H), 1.78-1.94 (m, 3H), 1.44-1.78 (m, 9H), 1.65 (s, 3H), 1.52 (s, 3H), 1.17-1.48 (m, 8H), 1.10 (d, *J* = 7.5 Hz, 3H), 0.85 (s, 9H), 0.83 (d, *J* = 6.5 Hz, 3H), 0.043 (s, 3H), -0.004 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 213.1, 159.2, 143.2, 139.6, 136.9, 135.5, 129.6, 129.3, 127.2, 124.0, 122.6, 113.8, 113.8, 89.1, 79.8, 78.1, 72.8, 71.6, 70.4, 59.3, 54.6, 43.6, 43.6, 43.4, 39.1, 38.6, 37.6, 37.1, 34.5, 32.8, 32.5, 31.7, 26.0, 25.6, 24.2, 24.1, 22.5, 21.5, 20.7, 20.0, 18.0, 14.2, 11.2, 11.0, -4.65, -4.80; IR (thin film): 3520, 3275, 1700, 1614 cm$^{-1}$; HRMS (MALDI) Calcd. for $C_{51}H_{79}NNaO_8SSi$ [M+Na]: 916.51934. Found: 916.6692.

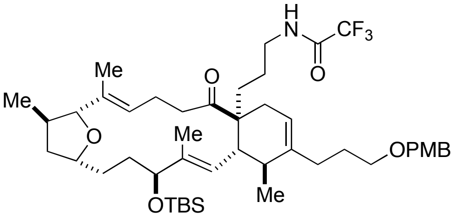


**2-89c**: $R_f$ = 0.20 (40% EtOAc/hexanes); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.75 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.01 (br s, 1H), 4.88 (t, *J* = 5.5 Hz, 1H), 4.83 (d, *J* = 12.0 Hz, 1H), 4.46 (br s, 2H), 4.06-4.12 (m, 1H), 3.98 (br s, 1H), 3.77-3.84 (m, 1H), 3.82 (s, 3H), 3.41-3.51 (m, 2H), 2.87-2.98 (m, 2H), 2.74 (dd, *J* = 9.0, 3.0 Hz, 1H), 2.58-2.78 (m, 1H), 2.43 (s, 3H), 2.14-2.26 (m, 3H), 1.94-2.06 (m, 2H), 1.66-1.88 (m, 2H), 1.65 (s, 3H), 1.60 (s, 3H), 1.49-1.65 (m, 3H), 1.52 (s 3H), 1.36-1.48 (m, 4H), 1.28-1.36 (m, 2H), 1.16-1.23 (m, 1H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.85 (s, 9H), 0.84 (d, *J* = 7.0 Hz, 3H), 0.042 (s, 3H), -0.005 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 213.9, 159.2, 143.1, 139.9, 136.9, 135.3, 130.2, 129.6, 129.3, 127.2, 124.8, 122.9, 113.8, 89.1, 79.9, 78.1, 73.1, 72.8, 71.6, 70.4, 59.3, 54.6, 45.8, 43.6, 43.2, 37.5, 37.1, 34.4, 32.9, 32.4, 31.7, 28.3, 28.2, 25.7, 24.2, 23.4, 23.0, 21.5, 20.6, 20.0, 18.0, 14.2, 11.5, 11.3, -4.7, -4.8.

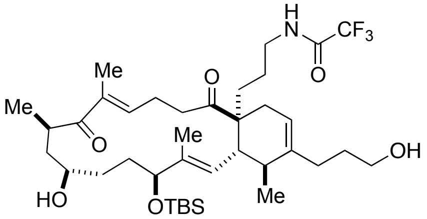


**Olefin 2-90a**: To a solution of Tertiary alcohol **2-89a** (9.8 mg, 0.0011 mmol, azeotropically dried with PhMe) in CH$_2$Cl$_2$ (1 mL) at -78 ºC was added 15 μL of Et$_3$N followed by 25 μL of a solution of SOCl$_2$ (16 μL) in CH$_2$Cl$_2$ (0.1 mL). The reaction mixture was stirred at -78 ºC for 1 h and quenched by pH 7 buffer. The aqueous layer was extracted with CH$_2$Cl$_2$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (20% EtOAc/hexanes) to afford 7.9 mg (82%) of olefin **2-90a** as a colorless oil; $R_f$ = 0.58 (40% EtOAc/hexanes); $[\alpha]^{21}_D$ –77.4 (*c* 0.59, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.75 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 2H), 6.88 (d, *J* = 8.0 Hz, 2H), 5.21 (t, *J* = 7.0 Hz, 1H), 4.99 (br s, 1H), 4.94 (d, *J* = 11.0 Hz, 1H), 4.85 (t, *J* = 5.5 Hz, 1H), 4.47 (s, 2H), 4.06-4.12 (m, 1H), 3.97 (br s, 1H), 3.77-3.85 (m, 1H), 3.81 (s, 3H), 3.42-3.50 (m, 2H), 2.93-3.00 (m,

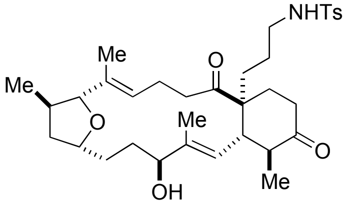
2H), 2.55-2.64 (m, 2H), 2.51 (t, *J* = 11.0 Hz, 1H), 2.43 (s, 3H), 2.30-2.42 (m, 2H), 2.15-2.23 (m, 2H), 1.90-2.03 (m, 2H), 1.56-1.75 (m, 4H), 1.62 (s, 3H), 1.60 (s, 3H), 1.42-1.55 (m, 3H), 1.51 (s, 3H), 1.34-1.42 (m, 2H),1.16-1.28 (m, 1H), 1.10 (d, *J* = 7.0 Hz, 3H), 0.97 (s, 3H), 0.86 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 213.1, 159.1, 143.2, 142.5, 139.9, 136.9, 135.4, 130.6, 130.6, 129.6, 129.6, 129.2, 127.2, 125.0, 122.8, 116.7, 113.7, 89.1, 79.9, 78.1, 72.6, 69.9, 55.3, 55.2, 50.4, 43.7, 37.9, 37.6, 37.1, 34.4, 32.9, 32.7, 32.5, 28.2, 25.7, 24.6, 24.3, 23.4, 21.5, 20.6, 20.0, 18.0, 15.0, 14.2, 11.2, -4.65, -4.80; IR (thin film): 3520, 3275, 1700, 1614 cm$^{-1}$; LRMS (MALDI) Calcd. for $C_{51}H_{77}NNaO_7SSi$ [M+Na]: 898.50877. Found: 898.7725.



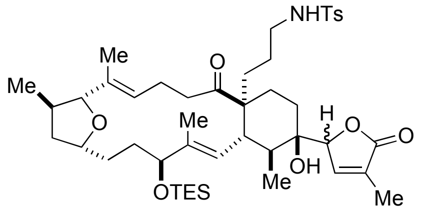
**Trifluoroacetamide 2-91**: To a solution of the tosylamine **2-90a** (10.2 mg, 0.0116 mmol) in $CH_2Cl_2$ (2 mL) at 0 ºC was added triethylamine (33 μL, 0.233 mmol) and trifluoroacetic anhydride (20 μL, 0.139 mml). After stirring at 0 ºC for 15 min, $SmI_2$ (0.1 M in THF) was added until the intermediate disappeared as monitored by TLC (*ca*. 1.2 ml of $SmI_2$ solution was added). The reaction mixture was stirred for additional 5 min and quenched by half saturated $Na_2S_2O_3$ solution (0.3 mL). The aqueous layer was extracted with $Et_2O$ and the organic layers were concentrated *in vacuo* and purified by flash chromatography (5%→10%→15% EtOAc/hexanes) to afford 7 mg (74%) of the desired product as a colorless oil. $R_f$ = 0.35 (20% EtOAc/hexanes); $[\alpha]^{21}_{D}$ –95.4 (*c* 0.79, $CHCl_3$); $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.43 (br s, 1H), 7.28 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.24 (t, *J* = 7.0 Hz, 1H), 4.98 (d, *J* = 11.0 Hz, 1H), 3.81 (br s, 1H), 3.45-3.50 (m, 2H), 3.34-3.40 (m, 2H), 2.62-2.69 (m, 2H), 2.56 (t, *J* = 11.0 Hz, 1H), 2.33-2.46 (m, 2H), 2.12-2.24 (m, 3H), 1.94-2.06 (m, 4H), 1.74-1.82 (m, 3H), 1.62-1.73 (m, 4H), 1.64 (s, 3H), 1.51 (s, 3H), 1.38-1.49 (m, 3H), 1.25-1.36 (m, 4H), 1.26 (s, 3H), 1.10 (d, *J* = 7.0 Hz, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.86 (s, 9H), 0.04 (s, 3H), 0.004 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 214.3, 162.4 ($COCF_3$, q, *J* = 37 Hz), 159.2, 142.4, 140.2, 135.6, 130.6,129.7, 12.8, 122.7, 120.8 ($CF_3$, q, *J* = 287 Hz), 116.9, 113.7, 89.1, 79.8, 78.1, 72.6, 69.9, 55.7, 50.4, 40.5, 38.0, 37.5, 36.7, 34.5, 32.8, 32.7, 32.2, 29.7, 28.2, 25.7, 24.7, 23.3, 23.1, 20.6, 19.9, 18.0, 15.0, 14.2, 11.2, -4.68, -4.84; IR (thin film): 3313, 1726, 1614 cm$^{-1}$; HRMS (MALDI) Calcd. for $C_{46}H_{70}F_3NNaO_7SSi$ [M+Na]: 840.48222. Found: 840.6652.



**Enone 2-92**: To a mixture of the PMB ether **2-91** (8.1 mg, 0.010 mmol) in $CH_2Cl_2/H_2O$ (3:1 0.4 mL) at 0 ºC was added dichloro-dicyanbenzoquione (DDQ) (36 mg, 0.16 mmol) in four portions. The mixture was stirred room temperature for 10 h and diluted with saturated $NaHCO_3$. The aqueous layer was extracted with $CH_2Cl_2$ and the organic layers were concentrated *in vacuo* and purified by flash chromatography (10%→30% EtOAc/hexanes) to afford 5.5 mg (80%) of the desired product as a colorless oil. $R_f$ = 0.11 (25% EtOAc/hexanes); δ 7.30 (s, 1H), 7.09 (t, *J* = 7.5 Hz, 2H), 5.25 (t, *J* = 7.5 Hz, 1H), 4.99 (d, *J* = 11.0 Hz, 1H), 3.84 (dd, *J* = 9.0, 5.5 Hz, 1H), 3.64-3.71 (m, 2H), 3.36-3.48 (m, 2H), 3.03 (dt, *J* = 19.0, 4.0 Hz, 1H), 2.71 (d, *J* = 11.0 Hz, 1H), 2.45-2.52 (m, 1H), 2.28-2.42 (m, 6H), 2.12-2.18 (m, 1H), 1.88-2.11 (m, 3H), 1.72-1.84 (m, 3H), 1.75 (s, 3H), 1.54-1.72 (m, 2H), 1.36-1.48 (m, 3H), 1.39 (s, 3H), 1.21-1.35 (m, 3H), 1.06 (d, *J* = 6.5 Hz, 1H), 0.96-1.04 (m, 1H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.84 (s, 9H), 0.025 (s, 3H), -0.019 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 214.9, 207.4, missed ($COCF_3$), 143.3, 141.7, 140.6, 137.7, 124.9, 116.8, missed ($CF_3$), 80.0, 69.6, 62.6, 55.6, 49.5, 41.8, 40.5, 38.3, 35.4, 35.0, 32.7,31.4, 31.0, 30.8, 25.7, 24.5, 23.8, 23.4, 22.8, 19.0, 18.0, 15.3, 11.4, 11.2, -4.56, -4.84; IR (thin film): 3313, 1726, 1614 cm$^{-1}$; HRMS (MALDI) Calcd. for $C_{38}H_{62}F_3NNaO_6Si$ [M+Na]: 736.41962. Found: 736.6912.



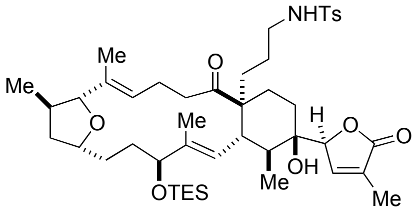
**Macrocyclic ketone 2-111**: To a solution of macrocycle **2-69** (82 mg, 0.0995 mmol) in THF/$CH_2Cl_2$/MeOH (1:1:1, 9.0 mL) at 23 $^{o}$C was added *p*-toluenesulfonic acid monohydrate (38 mg, 0.199 mL). The reaction mixture was stirred at 23 $^{o}$C for 2.5 h and quenched by saturated $NaHCO_3$ solution. The aqueous layer was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (30%→40%→50% EtOAc/hexanes) to afford 44 mg (74%) of the ketone **2-111** as a white foam. $R_f$ = 0.42 (70% EtOAc/hexanes); $[\alpha]^{20}_D$ –42.9 (*c* 1.4, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 5.12 (d, *J* = 11.0 Hz, 1H), 4.96 (t, *J* = 5.5 Hz, 1H), 4.94 (t, *J* = 5.5 Hz, 1H), 4.06-4.11 (m, 1H), 3.97 (br s, 1H), 2.96-3.08 (m, 2H), 2.93 (dd, *J* = 11.5 Hz, 11.5 Hz, 1H), 2.58-2.65 (m, 2H), 2.43 (s, 3H), 2.38-2.42 (m, 2H), 2.35-2.37 (m, 1H), 2.26-2.34 (m, 1H), 2.14, 2.24 (m, 2H), 2.09-2.14 (m, 2H), 2.02-2.08 (m, 1H), 1.94-2.00 (m, 3H), 1.66 (s, 3H), 1.51 (s, 3H), 1.35-1.43 (m, 1H), 1.29-1.39 (m, 1H), 1.10 (d, *J* = 7.0 Hz, 3H), 0.94-1.10 (m, 1H), 0.99 (d, *J* = 6.5 Hz, 1H); IR (thin film): 3414, 1732, 1643 $cm^{-1}$; HRMS (MALDI) Calcd. for $C_{34}H_{49}NNaO_6S$ [M+Na]: 622.31783. Found: 622.1585.



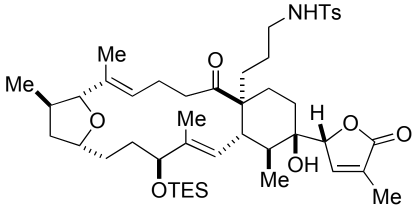
**TES ether butenolides 2-113**: A mixture of the ketone **2-111** (44 mg, 0.073 mmol) and silyloxyfuran **2-71** (187 mg, 0.73 mmol) was azeotropically dried with PhMe and dissolved in $CH_2Cl_2$ (4 mL). To this vigorously stirred solution was added $TiCl_4$ (1.0 M in $CH_2Cl_2$, 183 μL) at 23 °C dropwise over 60 s. The cloudy yellow reaction mixture was stirred for an additional 30 s and quickly quenched by saturated $NH_4Cl$ solution. The mixture was extracted with $Et_2O$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (40→70→100% EtOAc/hexanes) to afford 24 mg (47%) of the hydroxy butenolides **2-112a/b** as a white foam and as a mixture of two inseparable diastereomers (dr = 1:1, epimeric at C4). $R_f$ = 0.16 (70% EtOAc/hexanes).

Butenolides **2-112a** and **2-112b** (24 mg, 0.0344 mmol) as a mixture of diastereomers, imidazole (47 mg, 0.688 mmol) and DMAP (10 mg, 0.082 mmol) were azeotropically dried with PhMe. The mixture was dissolved in $CH_2Cl_2$ (3 mL) and at 23 °C was treated with TESCl (58 μL, 0.344 mmol). A white precipitate formed immediately. The reaction mixture was stirred at 23 °C for 5 min and then quenched by saturated $NaHCO_3$ solution.

The aqueous layer was extracted with $Et_2O$ and the combined organic layers were concentrated and purified by flash chromatography (10→20→30% EtOAc/hexanes) to afford 11 mg (39%) of TES ether butenolide **2-113a** and 10 mg (36%) of the C-4 epimer **2-113b**, respectively.

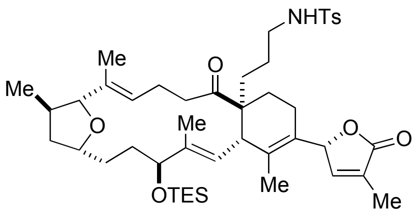


**butenolide 2-113a**: $R_f$ = 0.15 (40% EtOAc/hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, $J$ = 8.0 Hz, 2H), 7.32 (d, $J$ = 8.0 Hz, 2H), 7.21 (s, 1H), 5.01 (t, $J$ = 5.0, 1H), 4.93 (s, 1H), 4.83 (d, $J$ = 11.0 Hz, 1H), 4.07-4.12 (m, 1H), 3.98 (br s, 1H), 3.82 (dd, $J$ = 11.5, 3.0 Hz, 1H), 2.91-2.98 (m, 2H), 2.90 (dd, $J$ = 11.0, 11.0 Hz, 1H), 2.82-2.90 (m, 1H), 2.63-2.75 (m, 2H), 2.45 (s, 3H), 2.17-2.26 (m, 2H), 2.07-2.15 (m, 2H), 1.89-2.04 (m, 3H), 1.97 (s, 3H), 1.69-1.79 (m, 2H), 1.67 (s, 3H), 1.54-1.65 (m, 3H), 1.52 (s, 3H), 1.42-1.50 (m, 3H), 1.22-1.37 (m, 4H), 1.27 (d, $J$ = 7.5 Hz, 3H), 1.10 (d, $J$ = 7.5 Hz, 3H), 0.96 (t, $J$ = 8.0 Hz, 9H), 0.52-0.62 (m, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 212.8, 173.9, 147.0, 143.2, 141.0, 136.7, 135.5, 131.1, 129.6, 127.2, 124.2, 122.6, 89.2, 83.2, 79.8, 78.1, 73.8, 54.5, 43.3, 37.6, 37.1, 36.6, 34.6, 32.0, 32.6, 31.6, 26.5, 24.1, 22.6, 22.3, 20.7, 20.0, 14.2, 11.3, 11.0, 10.8, 6.9, 5.0.

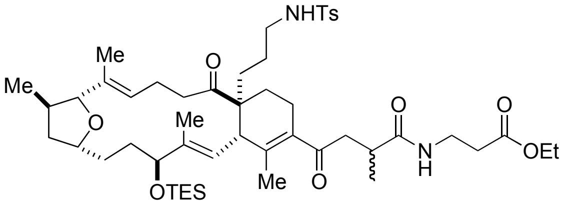


**Diastereomeric butenolide 2-113b**: $R_f$ = 0.3 (40% EtOAc/hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, $J$ = 8.0 Hz, 2H), 7.32 (d, $J$ = 8.0 Hz, 2H), 7.08 (s, 1H), 5.15 (s, 1H), 5.01 (s, 1H), 4.86 (d, $J$ = 11.0 Hz, 1H), 4.76 (t, $J$ = 5.5 Hz, 1H), 4.04-4.12 (m, 1H), 3.97 (s, 1H), 3.82 (dd, $J$ = 11.5, 3.0 Hz, 1H), 3.09 (dd, $J$ = 11.0, 11.0 Hz, 1H), 2.91-2.98 (m, 2H), 2.90 (dd, $J$ = 11.0, 11.0 Hz, 1H), 2.87-2.98 (m, 2H), 2.67-2.79 (m, 2H), 2.44 (s, 3H), 2.17-2.24 (m, 3H), 2.17-2.24 (m, 3H), 1.96-2.04 (m, 2H), 2.00 (s, 3H), 1.86-1.92 (m, 2H), 1.69 (s, 3H), 1.58- 1.74 (m, 4H), 1.69 (s, 3H), 1.60-1.67 (m, 3H), 1.51 (s, 3H), 1.45-1.54 (m, 1H), 1.36-1.44 (m, 3H), 1.19-1.32 (m, 3H), 1.03 (d, $J$ = 7.0 Hz, 3H), 0.94

(t, *J* = 7.5 Hz, 9H), 0.52-0.65 (m, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 212.8, 173.9, 147.0, 143.2, 141.0, 136.7, 135.5, 131.1, 129.6, 127.2, 124.2, 122.6, 89.2, 83.2, 79.8, 78.1, 73.8, 54.5, 43.3, 37.6, 37.1, 36.6, 34.6, 32.0, 32.6, 31.6, 26.5, 24.1, 22.6, 22.3, 20.7, 20.0, 14.2, 11.3, 11.0, 10.8, 6.9, 5.0.

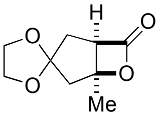


**Olefin 2-114**: To a solution of alcohol **2-113b** (10 mg, 0.00265 mmol, azeotropically dried with PhMe) in $CH_2Cl_2$ (1 mL) at -78 ºC was added 25 μL of a solution of $SOCl_2$ (18 μL) in $CH_2Cl_2$ (0.1 mL) followed by 9 μl of $Et_3N$. The reaction mixture was stirred at -78 ºC for 5 min and quenched by pH 7 buffer. The aqueous layer was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated *in vacuo*. The residue was purified by flash chromatography (10→20% EtOAc/hexanes) to afford 7.8 mg (80%) of the desired product **2-114** as a colorless oil. $R_f$ = 0.58 (40% EtOAc/hexanes); $[\alpha]^{19}_D$ +36.2 (*c* 0.75, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.01 (s, 1H), 5.85 (s, 1H), 4.98 (d, *J* = 8.0 Hz, 2H), 4.64 (t, *J* = 6.0 Hz, 1H), 4.06-4.11 (m, 1H), 3.98 (s, 1H), 3.87 (dd, *J* = 12.5, 2.5 Hz, 1H), 3.65 (d, *J* = 10.5 Hz, 1H ), 2.88-2.95 (m, 2H), 2.58-2.74 (m, 2H), 2.44 (s, 3H), 2.16-2.28 (m, 2H), 1.97-2.08 (m, 2H), 1.99 (s, 3H), 1.82-1.97 (m, 4H), 1.66- 1.74 (m, 4H), 1.74 (s, 3H), 1.66-1.78 (m, 4H), 1.59 (s, 3H), 1.52 (s, 3H), 1.46-1.48 (m, 2H), 1.26-1.38 (m, 3H), 1.10 (d, *J* = 7.0 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 9H), 0.52-0.62 (m, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 212.6, 174.6, 147.7, 143.4, 142.3, 136.8, 135.6, 135.5, 131.0, 129.7, 127.1, 124.9, 122.8, 122.6, 89.1, 79.6, 79.4, 78.1, 52.8, 47.0, 43.7, 37.6, 37.0, 34.6, 33.1, 32.3, 28.2, 24.4, 23.6, 22.5, 20.6, 20.3, 20.0, 16.9, 14.2, 11.0, 10.8, 6.9, 5.0. IR (thin film): 3271, 1759, 1700 cm$^{-1}$; HRMS (MALDI) Calcd. for $C_{45}H_{67}KNO_7SSi$ [M+K]: 832.40446. Found: 832.4055.



**Amide 2-115**: A mixture of the Butenolide **2-114a** (7.0 mg, 0.0088 mmol) and β-alanine ethylester (14 mg, 0.088 mmol) was dissolved in $Et_3N$ (0.3 ml). The reaction mixture was stirring for 12h at 40 °C. The volatiles were evaporated and the residue was purified by flash chromatography (20%→30%→40% EtOAc/hexanes) to afford the inseparable mixture of amide **2-115** 1.4 mg (17%) as a colorless oil. $R_f$ = 0.20 (40% EtOAc/hexanes); Key $^1$H NMR signals; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, $J$ = 8.0 Hz, 2H), 7.31 (d, $J$ = 8.0 Hz, 2H), 6.31 (m, 1H), 5,44-5.24 (m, 2H), 5.00 (t, $J$ = 3.5 Hz, 1H), 4.96 (d, $J$ = 11.0 Hz, 1H), 4.16-4.16 (m, 2H), 4.06-4.14 (m, 1H), 3.98 (s, 1H), 3.87 (dd, $J$ = 11.5, 2.5 Hz, 1H), 3.05-3.14 (m, 1H), 2.90-3.01 (m, 2H).

**Representative Procedure (Method A) as Described for 5-methyl-6-oxaspiro[bicyclo[3.2.0]heptane-3,2'-[1,3]dioxolan]-7-one (±)-3-24b:**



Pyridinium salt **19b** (250 mg, 0.5 mmol, 1.0 equiv) was dissolved in 3 mL of $CH_2Cl_2$ and 4-pyrrolidinopyridine (30 mg, 0.4 mmol, 0.7 equiv) was dissolved in 2 mL of $CH_2Cl_2$. These solutions were separately transferred to a flame-dried 25 mL round-bottomed flask resulting in a white slurry. Hünig's base (0.17 mL, 1.0 mmol, 2.0 equiv) was added dropwise to the reaction mixture by microliter syringe causing the reaction mixture to become a yellowish color. The reaction mixture was left stirring for 10 minutes to ensure a uniform milky slurry and then a solution of keto-acid **23b**[1] (100 mg, 0.5 mmol, 1.0

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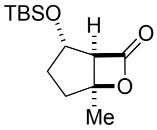
1) Keto acids **3-23b, 3-23c**, **3-23d**, **3-23e** were prepared by (a) alkylation of cyclohexanone (Michel, P.; Ivan, J.; Gilbert, R. *J. Chem. Soc. Perkin Trans. I.* **1993**, 1935-1936.) (b) formation of the corresponding thermodynamic silyl enol ether (Krafft, M. E.; Holton, R. A. *J. Org. Chem.* **1984**, *49*, 3669-3670.; Behenna, D. C.; Stoltz, B. M. *J. Am. Chem. Soc.* **2004**, *126*, 15044-15045.) and (c) ozonolysis by our previously described procedure.

equiv) dissolved in 5 mL of $CH_2Cl_2$ was added via syringe pump over 1 h. After 1-2 h, the reaction mixture became a homogeneous red solution. After stirring for a total of 12 h at 23 °C, the volatiles were removed under reduced pressure to give a dark red residue. The crude reaction mixture was then partitioned between aq. $NH_4Cl$ and diethyl ether (10 mL each). The biphasic mixture was transferred to a 125 mL separatory funnel and the organic phase was washed with 10% HCl (1 x 10 mL), aq. NaCl (1 x 10 mL), dried over $MgSO_4$, filtered, and concentrated to afford a light red residue that was purified by flash chromatography on $SiO_2$ (1:1, hexanes:EtOAc) to provide *cis-β*-lactone (±)-**24b** (71 mg, 78 %) as a pale yellow oil: $R_f$ 0.68 (1:1, hexanes:EtOAc); IR (thin film) 1815 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.01-4.08 (m, 2H), 3.89-3.99 (m, 2H), 3.56 (app d, $J$ = 8.7 Hz, 1H), 2.03-2.45 (m, 4H), 1.73 (s, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 170.5, 115.8, 83.7, 65.2, 65.1, 57.2, 44.9, 37.8, 22.8; LRMS (APCI) Calcd for $C_9H_{13}O_4$ [M+H]: 185. Found: 185.

**Representative Procedure (Method B) as Described for 2-(*tert*-butyldimethylsilyloxy)-5-methyl-6-oxabicyclo[3.2.0]heptan-7-one ((±)-3-24f):**



The pyridinium salt **3-19b** (191 mg, 0.55 mmol, 1.5 equiv) and 4-pyrrolidinopyridine (81 mg, 0.55 mmol, 1.5 equiv) are weighed out and transferred to a flame-dried 25 mL round-bottomed flask and dissolved in 6 mL of $CH_2Cl_2$. Hünig's base (0.16 mL, 0.91 mmol, 2.5 equiv) was transferred via syringe and then keto-acid **3-23f**[2] (100 mg, 0.36 mmol, 1.0 equiv) in 2 mL $CH_2Cl_2$ was added via syringe pump over 1 h. The resulting dark solution was stirred for another 12 h, at which point the volatiles were removed under reduced pressure to give a dark red residue. The crude reaction mixture was diluted with $CH_2Cl_2$ (10 mL) and washed with aqueous $NH_4Cl$ solution (10 mL each). After

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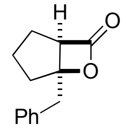
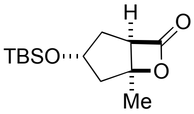
2) Keto acids **3-23f** were prepared by alkylation of methyl acetoacetate, reduction of the resulting β-ketoester with $NaBH_4$, protection of the resulting alcohol as the TBS silyl ether, ozonolysis by the previously described procedure and hydrolysis of the methyl ester.

separating phases, the aqueous phase was back extracted with $CH_2Cl_2$ (2 x 10 mL). The combined organics were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated to afford a light red residue. Alternatively, the crude residue could also be loaded directly on a silica gel column with minimal loss in yield. Analysis of the crude reaction mixture indicated a diastereomeric ratio of >19:1 (based on unidentified minor impurities in the spectrum). Purification by flash chromatography on $SiO_2$ (9:1, hexanes:EtOAc) to give *β*-lactone (±)-**3-24f** (63 mg, 68%) as a colorless oil and as a single diastereomer ($^1$H NMR): $R_f$ 0.22 (9:1, hexanes:EtOAc); IR (thin film) 1820 cm$^{-1}$, $^1$H NMR (500 MHz, $CDCl_3$) δ 4.47 (d, *J* = 3.0 Hz, 1H), 3.33 (d, *J* = 2.0 Hz, 1H), 2.13 (dd, *J* = 5.5, 12.0 Hz, 1H), 2.00-2.07 (m, 2H), 1.90 (ddd, *J* = 1.5, 5.5, 12.0 Hz, 1H), 1.73 (s, 3H), 0.86 (s, 9H), 0.074 (s, 3H), 0.070 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 168.5, 87.7, 73.1, 67.7, 33.8, 33.5, 25.6 (3), 21.9, 17.8, –4.9, –5.0; LRMS (ESI) Calcd for $C_{13}H_{24}LiO_3Si$ [M+Li]: 263. Found: 263.



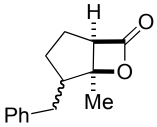
**5-benzyl-6-oxabicyclo[3.2.0]heptan-7-one ((±)-3-24c):** Prepared according to the representative procedure (Method B) using pyridinium salt **3-19b** (240 mg, 0.68 mmol, 1.5 equiv), 4-pyrrolidinopyridine (100 mg, 0.68 mmol, 1.5 equiv), and Hünig's base (0.16 mL, 0.91 mmol, 2.0 equiv) in 6 mL of $CH_2Cl_2$. Keto-acid **3-23c** (100 mg, 0.5 mmol, 0.45 equiv) was dissolved in 2 mL of $CH_2Cl_2$. Purification by flash chromatography on $SiO_2$ (9:1, hexanes:EtOAc) to give *cis*-bicyclic β-lactone (±)-**3-24c** (53 mg, 58 %) as a colorless oil and as a single diastereomer: $R_f$ 0.12 (9:1, hexanes:EtOAc); IR ($CDCl_3$) 1824 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.23-7.34 (m, 5H), 3.51 (app d, *J* = 8.0 Hz, 1H), 3.24 (app t, *J* = 15.0 Hz, 2H), 2.08 (app dt, *J* = 6.0, 12.0, 2H), 1.78-1.94 (m, 2H), 1.54-1.65 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 171.3, 136.0, 123.0 (2), 128.8 (2), 127.4, 89.9, 57.8, 41.5, 34.3, 27.2, 23.8. LRMS (ESI) Calcd for $C_{13}H_{14}NaO_2$ [M+Na]: 225. Found: 225.



***Trans*** **and** ***cis*****-3-(*****tert*****-butyldimethylsilyloxy)-5-methyl-6-oxabicyclo[3.2.0]heptan-7-one ((±)3-24d):** Prepared according to the representative procedure (Method B) using pyridinium salt **3-19b** (155 mg, 0.44 mmol, 1.5 equiv), 4-pyrrolidinopyridine (65 mg, 0.44 mmol, 1.5 equiv), Hünig's base (0.13 mL, 0.74 mmol, 2.5 equiv), and keto-acid **3-23d** (80 mg, 0.29 mmol, 1.0 equiv) in 7 mL of $CH_2Cl_2$. Purification by flash chromatography on $SiO_2$ (9:1, hexanes:EtOAc) gave a 2:1 ratio of *trans* and *cis*-bicyclic *β*-lactone (±)-**3-24d** (42 mg, ~56%) as a colorless oil contaminated with *N*-propyl-2-pyridone. A second purification allowed separation of *trans*- and *cis*-*β*-lactone (±)-**3-24d.**

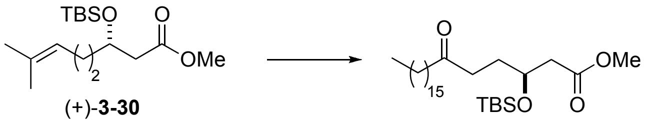
*Trans*-*β*-lactone (±)-**3-24d** (28 mg, 37 %)**:** $R_f$ 0.30 (9:1, hexanes:EtOAc); IR (thin film) 1820 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 4.43-4.49 (m, 1H), 3.46 (d, *J* = 8.5 Hz, 1H), 2.48 (dd, *J* = 6.0, 14.5 Hz, 1H), 2.34 (dd, *J* = 6.0, 13.0 Hz, 1H), 1.75 (ddd, *J* = 8.5, 13.0, 17.5 Hz, 1H), 1.67 (s, 3H), 1.60 (dd, *J* = 9.5, 17.5 Hz, 1H), 0.88 (s, 9H), 0.64 (s, 6H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 170.8, 85.2, 71.5, 56.5, 44.6, 35.5, 25.7(3), 22.3, 18.0, −4.89, −4.92; HRMS (ESI) Calcd for $C_{13}H_{24}LiO_3Si$ [M+Li]: 263. Found: 263.

*Cis*-*β*-lactone (±)-**3-24d** (10 mg, 13 %): $R_f$ 0.20 (9:1, hexanes:EtOAc) ; IR (thin film) 1824 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 4.50 (app t, *J* = 4.5 Hz, 1H), 3.46 (d, *J* = 8.0 Hz, 1H), 2.27 (dd, *J* = 2.0, 15.5 Hz, 1H), 2.21 (dd, *J* = 2.0, 14.0 Hz, 1H), 1.94 (ddd, *J* = 4.0, 8.0, 14.0 Hz, 1H), 1.74 (dd, *J* = 5.0, 15.5 Hz, 1H), 1.66 (s, 3H), 1.60 (dd, *J* = 9.5, 17.5 Hz, 1H), 0.87 (s, 9H), 0.050 (s, 3H), 0.043 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 171.3, 86.4, 74.2, 58.3, 44.7, 37.9, 25.5(3), 22.2, 17.7, −4.99, −5.06; LRMS (ESI) Calcd for $C_{13}H_{24}LiO_3Si$ [M+Li]: 263. Found: 263.



**4-benzyl-5-methyl-6-oxabicyclo[3.2.0]heptan-7-one ((±)-3-24e):** Prepared according to the representative procedure (Method A) using pyridinium salt **3-19b** (145 mg, 0.4 mmol, 1 equiv), 4-pyrrolidinopyridine (40 mg, 0.3 mmol, 0.7 equiv), Hünig's base (0.14 mL, 0.8 mmol, 2 equiv), and keto acid keto-acid **3-23e** (100 mg, 0.4 mmol, 1 equiv) in 10 mL of

$CH_2Cl_2$. The crude residue was purified by flash chromatography on $SiO_2$ ($CH_2Cl_2$) to give β-lactone (±)-**3-24e** (54 mg, 60 %) as yellow oil of inseparable mixture of diastereomers (~1:1) based on $^1$H NMR integration: $R_f$ 0.75 ($CH_2Cl_2$); IR (thin film) 1807 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.14-7.33 (m, 10H), 3.50 (app t, *J* = 7.0 Hz, 2H), 2.97 (dd, *J* = 5.0, 13.5 Hz, 1H), 2.82 (dd, *J* = 4.5, 13.0, 1H), 2.64 (dd, *J* = 10.0, 13.5, 1H), 2.59 (dd, *J* = 5.0, 11.5 Hz, 1H), 2.16 (t, *J* = 13.5, 1H), 1.82-2.05 (m, 7H), 1.61-1.68 (m, 2H), 1.59 (s, 3H), 1.56 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 171.4, 171.3, 140.4, 139.4, 128.83 (2), 128.75 (2) 128.61 (2), 128.48 (2), 126.4, 126.2, 89.1, 87.5, 60.2, 59.2, 49.9, 46.2, 34.5, 34.3, 30.0, 26.7, 24.9, 24.6, 20.4, 19.1; LRMS (ESI) Calcd for $C_{14}H_{16}LiO_2Si$ [M+Li]: 223. Found: 223.



(+)-**3-30**

**(*S*)-methyl 3-(*tert*-butyldimethylsilyloxy)-6-oxodocosanoate 3-31:** (+)-Ester **3-30**[3] (1.23 g, 4.1 mmol) was dissolved in 30 mL of dichloromethane, cooled to –78 °C, and a stream of ozone was bubbled through the solution for 15 min. Ozone addition was stopped, and excess ozone was removed by bubbling nitrogen through the solution for 30 min. The ozonide was quenched by addition of triphenylphosphine (1.2 g, 4.7 mmol). The reaction mixture was allowed to warm to 25 °C, and the solvent was removed under reduced pressure. The resulting residue was purified by flash chromatography on $SiO_2$ (9:1, hexanes:EtOAc) to afford the intermediate aldehyde (900 mg, 82 %) as a colorless oil: $R_f$ 0.16 (9:1, hexanes:EtOAc); $^1$H NMR (500 MHz, $CDCl_3$) δ 9.72 (t, *J*= 1.5 Hz, 1H), 4.15 (dq, *J*= 5.0, 6.0 Hz, 1H), 3.60 (s, 3H), 2.46 (dt, *J*= 1.5, 7.5 Hz, 1H), 2.43 (dd, *J*= 6.5, 14.5 Hz, 1H), 2.34 (dd, *J*= 6.0, 15.0 Hz, 1H),1.81-1.88 (m, 1H), 1.70-1.77 (m, 1H), 0.80 (s, 9H), 0.002 (s, 3H), -0.018 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 201.7, 171.4, 68.0, 51.4, 42.0, 39.1, 29.2, 25.6 (3), 17.8, –4.82, –5.01.

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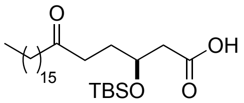
[3] Optically active silylether ester (+)-**10** was obtained by silyl protection of hydroxy ester **10'** (not shown) by standard conditions. Hydroxy ester **10'** has been reported previously in the literarture (Hirama, M.; Shimizu, M.; Iwashita, M. *J. Chem. Soc., Chem. Commun.* **1983***,* 599). Lit. $[\alpha]^{25}_D$ -15.9 (*c* 1.0, $CHCl_3$), >99% ee. This study: $[\alpha]^{25}_D$ +15.78 (*c* 1.0, $CHCl_3$), >98% ee.

To a stirred solution of aldehyde (600 mg, 2.0 mmol) in 20 mL of THF at –78 °C was added 1 M solution of hexadecyl magnesium bromide[4] (3 mL, 3.0 mmol) in THF. After stirring at –78 °C for 4 h, the reaction mixture was poured into 10% HCl solution and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated to afford a light yellow residue that was used passed quickly through a $SiO_2$ plug to remove polar and non-polar impurities. This material was then used directly in the oxidation step. To a solution of crude alcohol in 10 mL of $CH_2Cl_2$ was added PCC (260 mg, 1.2 mmol) at 0 °C. After 20 min, the reaction mixture was warmed to 25 °C and then stirred for 8 h. The solution was passed through a pad of celite and concentrated to afford a light yellow residue. The resulting residue was purified by flash chromatography on $SiO_2$ (9:1, hexanes:EtOAc) to afford the intermediate keto ester **3-31** (330 mg, 0.661 mmol, 33%, 2 steps) as a pale yellow oil: $R_f$ 0.35 (9:1, hexanes:EtOAc); $[\alpha]^{25}_{D}$ +7.5° (*c* 1.0, $CHCl_3$); IR (thin film) 1745, 1717 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.18 (app quintet, $J$ = 6 Hz, 1H), 3.66 (s, 3H), 2.35-2.50 (m, 6H), 1.67-1.87 (m, 2H), 1.55 (t, $J$ = 7.2 Hz, 2H), 1.25 (br s, 26H), 0.87 (br t, $J$ = 11 Hz, 2H), 0.86 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 210.8, 171.8, 68.3, 51.5, 42.9, 42.3, 37.3, 31.9, 30.8, 29.7 (3), 29.6, 29.64 (3), 29.59, 29.5, 29.4, 29.3, 29.2, 25.7 (3), 23.9, 17.9, 14.1, –4.6, –4.9; LRMS (ESI) Calcd for $C_{29}H_{59}O_4Si$ [M+H]: 499. Found: 499.



**(*S*)-methyl 3-(*tert*-butyldimethylsilyloxy)-6-oxodocosanoic acid (3-32):** A solution of keto ester **3-31** (330 mg, 0.662 mmol) in 10 mL of THF was cooled to 0 °C and treated with 1N LiOH (1.0 mL, 10.0 mmol). After 20 min, the reaction mixture was warmed to 25 °C and then stirred 12 h. The volatiles were removed under reduced pressure to give a residue that was dissolved in 10 mL of $H_2O$ and subsequently washed with 10 mL of hexanes and the aqueous layer was acidified with 1% HCl solution. Extraction with

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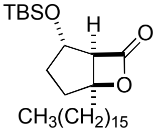
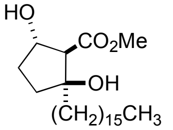
4) This Grignard reagent was prepared by standard conditions from hexadecyl bromide and activated $Mg^o$ turnings.

EtOAc (3 x 10 mL) was followed by combination of the organic layers, drying over $MgSO_4$, filtration, and concentration to afford a white solid that was purified by flash chromatography on $SiO_2$ (8:2, hexanes:EtOAc) to afford acid **3-22** (200 mg, 67%) as a colorless oil: $R_f$ 0.23 (8:2, hexanes:EtOAc); $[\alpha]^{25}_{D}$ -2.3° (*c* 1.0, $CHCl_3$); IR (thin film) 2928, 1713 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 4.19 (app quintet, *J*= 5.5 Hz, 1H), 2.47-2.50 (m, 4H), 2.40 (t, *J*= 7.5 Hz, 2H), 1.76-1.88 (m, 2H), 1.56 (t, *J*= 7.0 Hz, 2H), 1.26 (br s, 27H), 0.89 (br s, 12H), 0.09 (s, 3H), 0.08 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 210.6, 175.8, 68.2, 42.8, 41.7, 37.7, 31.9, 30.6, 29.68 (3), 29.67, 29.64 (3), 29.60, 29.5, 29.4, 29.35, 29.2, 25.7 (3), 23.9, 22.7, 17.9, 14.1, −4.7, −4.9.



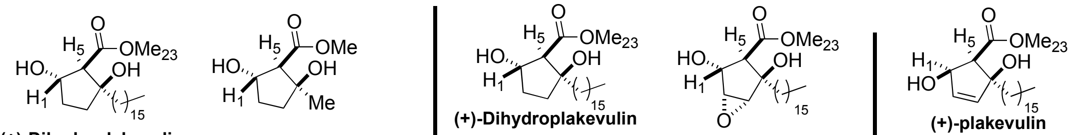
**(1*R*,2*S*,5*S*)-2-(*tert*-butyldimethylsilyloxy)-5-hexyadecyl-6-oxabicyclo[3,2,0]heptan-7-one (3-33):** Prepared according to the general procedure using pyridinium salt **3-19b** (152 mg, 0.43 mmol, 1.5 equiv), 4-pyrrolidinopyridine (64 mg, 0.43 mmol, 1.5 equiv), Hünig's base (0.12 mL, 0.72 mmol, 2.5 equiv), and keto-acid **3-32** (140 mg, 0.29 mmol, 1.0 equiv) in 8 mL of $CH_2Cl_2$. The crude residue was purified by flash chromatography on $SiO_2$ (95:5, hexanes:EtOAc) to give *β*-lactone (-)-**3-33** (65 mg, 48%) as a white waxy solid: $R_f$ 0.42 (95:5, hexanes:EtOAc); $[\alpha]^{25}_{D}$ –9.02° (*c* 1.0, $CHCl_3$); IR (thin film) 2921, 1824$cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 4.46 (d, *J* = 2.0 Hz, 1H), 3.34 (d, *J* = 2.0 Hz, 1H), 1.96-2.06 (m, 4H), 1.87-1.93 (m, 2H), 1.40-1.45 (m, 2H), 1.33-1.35 (m, 2H), 1.26 (br s, 24H), 0.87 (t, *J* = 8.0, Hz, 3H), 0.865 (s, 9H), 0.067 (s, 3H), 0.062 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 168.9, 90.8, 72.7, 66.3, 35.2, 33.5, 31.9, 31.1, 29.69 29.68 (3), 29.67, 29.65 (2), 29.62, 29.5, 29.44, 29.35 25.6 (3), 24.8, 22.7, 17.8, 14.1, −4.88, −4.94; LRMS (ESI) Calcd for $C_{13}H_{24}LiO_3Si$ $[M+Li]^+$: 473. Found: 473.



**Synthesis of Dihydroplakevulin A 3-34:** To a stirred solution of *β*-lactone **3-33** (15.0 mg, 0.032mmol) in 1.5 mL of MeOH at 25 °C was added triethylamine (18 μL, 0.128

mmol). After stirring at 50 °C for 8 h, the volatilies were removed under reduced pressure to give a white foam that was used directly in the following step. To a solution of the crude hydroxy ester in 4 mL of $CH_3CN$ was added a 1M solution of HF/Pyridine (0.52 mL, 0.52 mmol) at 0 °C. After 1 h, the reaction mixture was washed with brine (5 mL), dried over $MgSO_4$, filtered, and concentrated to afford a white solid. The resulting residue was purified by flash chromatography on $SiO_2$ (7:3, hexanes:EtOAc) to afford dihydroplakevulin A **3-34** (4 mg, 0.01 mmol, 32% over two steps) as a white solid: $R_f$ 0.30 (4:6, hexanes:EtOAc); $[\alpha]^{25}_{D}$ +7.13 (*c* 1.0, $CHCl_3$); IR (thin film) 3422, 1729 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 4.65 (q, *J* = 8.0 Hz, 1H), 3.79 (s, 1H), 2.99 (br s, 1H), 2.56 (d, *J* = 8.0 Hz, 1H), 2.27-2.34 (m, 1H), 2.07 (s, 1H), 1.89 (ddd, *J*= 7.0, 10.0, 17.5 Hz, 1H), 1.78 (dd, *J*= 5.0, 10.5 Hz, 1H), 1.75 (dd, *J*= 5.0, 10.5 Hz, 1H), 1.54-1.64 (m, 4H), 1.26 (br s, 26H), 0.89 (t, *J* = 7.0 Hz, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 174.6, 82.0, 75.8, 61.1, 52.3, 41.2, 35.9, 32.2, 31.7, 30.3, 29.94 (4), 29.92, 29.90 (2), 29.83, 29.82, 29.6, 24.4, 22.9, 14.4; LRMS (ESI) Calcd for $C_{23}H_{44}LiO_4$[M+Li]: 391.3 Found $C_{29}H_{58}O_4Si$: 391.3

**Comparison of Selected $^1H$ NMR Chemical Shifts and Couplings for Dihydroplakevullin and Derivatives.**



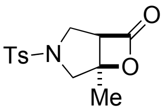
**(+)-Dihydroplakevulin**
This study (500 MHz $^1H$ NMR).

**(+)-Dihydroplakevulin**
Kobayashi, J. *et. al. Tetrahedron* **2003**, *59*, 1137
(600 MHz $^1H$ NMR)

**(+)-plakevulin**
Mizutani, H. *et. al.*
*Synlett* **2005**, 793.

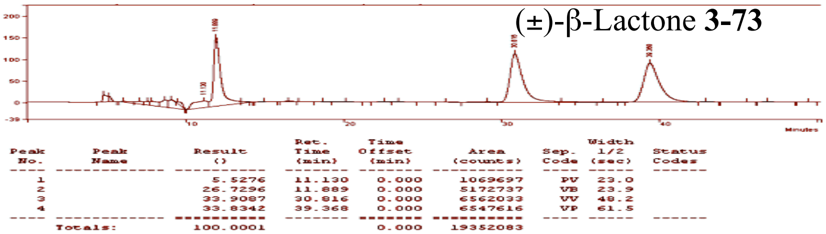
| | | | | | |
|---|---|---|---|---|---|
| $H_1$ | 4.65 (app q, *J* =8.0 Hz) | 4.68 (app dt, *J* = 13.5, 6.0 Hz) | 4.65 (ddd ,*J* = 7.9, 3.7, 2.0 Hz) | 4.47 (br d, *J* = 8.3 Hz) | 5.84 (dd, *J* = 5.7, 1.5 Hz) |
| $H_5$ | 2.56 (d, *J* =8.0 Hz) | 2.57 (d, *J* =13.5 Hz) | 2.55 (m) | 2.49 (d, *J* = 8.3 Hz) | 2.83 (d, *J* = 5.3 Hz) |
| $Me_{23}$ | 3.79 s | 3.83 s | 3.33* | 3.26* | 3.79 (3.78†) |

†Data from Kobayashi, J. *et. al. Tetrahedron* **2003**, *59*, 1137.
*Chemical shifts for methyl ester in red appear to be anomalous based on expected chemical shifts for methyl ester protons, data from Mizutani, and may be due to typographical errors in Kobayashi, et. al. We had no response to several inquiries regarding this spurious data.



**(±)-β-Lactone 3-73**: To a solution of pyridinium salt **3-19b** (117 mg, 0.334 mmol), 4-PPY **3-23b** (50 mg, 0.33 mmol) and Hunig's base (118 μL, 0.676 mmol) in 8 mL of $CH_2Cl_2$ at ambient temperature was added via syringe pump a solution of keto acid **3-72** (100 mg, 0.334 mmol) in 4 mL of $CH_2Cl_2$ over 1 h. After the addition was complete, the reaction was stirred for an additional 11 h. The solvent was then removed *in vacuo* and the orange residue was partitioned between ethyl acetate (10 mL) and water (8 mL). The phases were separated, and the aqueous layer was extracted with ethyl acetate (2 x 5 mL). The combined organic phases were washed with saturated $NH_4Cl$ solution (6 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by flash chromatography on $SiO_2$ (1:1, hexanes:ethyl acetate) gave **3-73** (70 mg, 75%) as a white amorphous solid: $R_f$ 0.35 (1:1, hexanes:ethyl aceatae). IR ($CHCl_3$) 1845 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.0, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 3.95 (d, *J* = 11.0 Hz, 1H), 3.90 (d, *J* = 12.0 Hz, 1H), 3.60 (d, *J* = 6.5 Hz, 1H), 2.99 (dd, *J* = 10.5, 6.5 Hz, 1H), 2.78 (d, *J* = 12.0 Hz, 1H), 2.46 (s, 3H), 1.67 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 166.7, 144.2, 132.4, 130.0 (2), 128.0 (2), 81.9, 59.2, 54.6, 48.0, 21.9, 19.7; HRMS (ESI) Calcd for $C_{13}H_{16}NO_4S$ [M+H]: 282.0800. Found: 282.0791.

**(−)-β-Lactone 3-73**: To a 10 mL round-bottomed flask containing pyridinium salt **3-19b** (126 mg, 1.5 equiv, 0.241 mmol) and levamisole•HCl **3-40** (58 mg, 1.0 equiv, 0.241 mmol) was added 2.5 mL $CH_2Cl_2$ and the reaction was cooled to 0°C. Diisopropylethylamine (307 μL, 5.0 equiv, 1.77 mmol) was added by syringe, followed by a solution of ketoacid **3-72** (72 mg, 1.0 equiv, 0.241 mmol) in 1.0 mL $CH_2Cl_2$ via syringe pump over 1 h. After 20 h, the reaction was concentrated *in vacuo*. Purification of the residue by flash chromatography on $SiO_2$ (1:1, hexanes:ethyl acetate) gave (−)-β-lactone **3-73** (24 mg, 35%) as a white amorphous solide. $[\alpha]^{16}_D$ −9.13 (*c* 1.0, $CHCl_3$); Enantiomeric excess was determined to be 91% ee by chiral HPLC (Chiral OD, 250 x 4.5 mm (L x I.D.), solvent (isocratic) 85:15 hexanes/2-propanol), flow rate 1.0 mL/min,wavelength λ = 230 nm). Retention times: 31.13 min and 39.08 min.


(±)-β-Lactone **3-73**

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                                 Ret.     Time                       Width
Peak     Peak        Result      Time    Offset      Area      Sep.   1/2    Status
 No.     Name         ()        (min)    (min)     (counts)    Code  (sec)   Codes
---- ------------ ----------  -------- -------- -----------  ---- -----   ------
   1                  5.5276    11.130    0.000     1069697    PV   23.0
   2                 26.7296    11.889    0.000     5172737    VB   23.9
   3                 33.9087    30.816    0.000     6562033    VV   48.2
   4                 33.8342    39.368    0.000     6547616    VP   61.5
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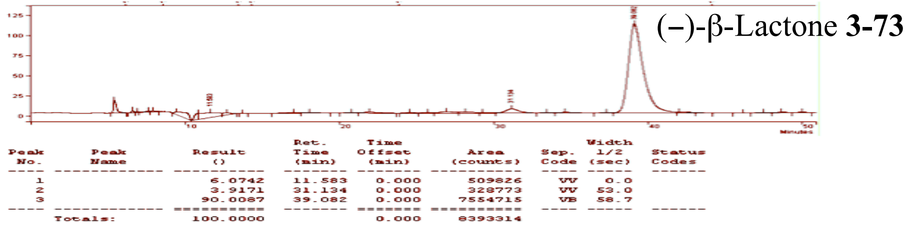
(−)-β-Lactone **3-73**

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                                 Ret.     Time                       Width
Peak     Peak        Result      Time    Offset      Area      Sep.   1/2    Status
 No.     Name         ()        (min)    (min)     (counts)    Code  (sec)   Codes
---- ------------ ----------  -------- -------- -----------  ---- -----   ------
   1                  6.0742    11.583    0.000      509826    VV    0.0
   2                  3.9171    31.134    0.000      328773    VV   53.0
   3                 90.0087    39.082    0.000     7554715    VB   58.7
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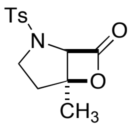
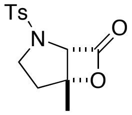
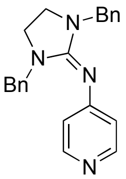


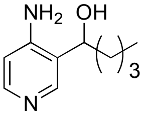
**(±)-β-Lactone 3-75**: To a solution of pyridinium salt **3-19b** (84 mg, 0.24 mmol), 4-PPY **3-23b** (36 mg, 0.24 mmol) and Hunig's base (84 μL, 0.48 mmol) in 3 mL of $CH_2Cl_2$ at ambient temperature was added via syringe pump a solution of keto acid **3-74** (72 mg, 0.24 mmol) in 1 mL of $CH_2Cl_2$ over 1 h. After the addition was complete, the reaction was stirred for an additional 24 h. The volatiles were evaporated and the residue was purified by flash chromatography (20%→30% EtOAc/hexanes) to afford the **3-75** (49 mg, 72%) as a white solid: R*f* 0.37 (30% EtOAc/hexanes). IR ($CHCl_3$) 1833 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.0, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 5.08 (s, 1H), 3.99 (dd, *J* = 11.0, 9.0 Hz, 1H), 3.15 (dt, *J* = 11.0, 6.0 Hz, 1H), 2.44 (s, 3H), 2.22 (dd, *J* = 14.5, 6.0 Hz, 1H), 1.81 (ddd, *J* = 14.5, 11.5, 8.0 Hz, 1H), f1.67 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 164.5, 144.5, 134.8, 129.8 (2), 127.8 (2), 87.3, 73.4, 46.7, 35.1, 21.6, 20.6; HRMS (ESI) Calcd for $C_{13}H_{16}NO_4S$ [M+H]: 282.0800. Found: 282.0872.



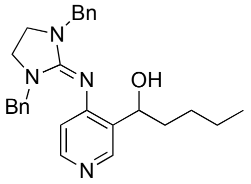
**(−)-β-Lactone 3-75**: To a 10 mL round-bottomed flask containing pyridinium salt **3-19b** (101 mg, 1.2 equiv, 0.289 mmol), LiCl (5.0 mg, 0.5 equiv, 0.12 mmol) and HBTM (12.8 mg, 0.2 equiv, 0.048 mmol) was added 1.5 mL $CH_2Cl_2$ at 19 °C. Diisopropylethylamine (210 μL, 5.0 equiv, 1.20 mmol) was added by syringe, followed by a solution of ketoacid **3-74** (72 mg, 1.0 equiv, 0.24 mmol) in 2.5 mL $CH_2Cl_2$ via syringe pump over 1 h. After the addition was complete, the reaction was stirred for an additional 36 h. The volatiles were evaporated and the residue was purified by flash chromatography (20%→30% EtOAc/hexanes) to afford the (−)-β-lactone **3-75** (26 mg, 39%) as a white solid: $[\alpha]^{17}_D$ −0.65 (*c* 1.08, $CHCl_3$); Enantiomeric excess was determined to be 80% ee by chiral HPLC (Chiral IA, 250 x 4.5 mm (L x I.D.), solvent (isocratic) 70:30 hexanes/2-propanol), flow rate 0.5 mL/min,wavelength λ = 254 nm). Retention times: 17.61 min and 21.08 min.



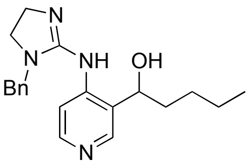
**Guanidine 3-38**: To a mixture of the 2-chloro-imidazolinium **3-44** (912 mg, 3.19 mmol) in $CH_3CN$ (25 mL) and $Et_3N$ (0.9 mL) was added amino pyridine **3-45** (300 mg, 3.19 mmol) at 22 °C then warmed up 70 °C and stirred for another 5 h. The reaction mixture was quenched by saturated $NaHCO_3$ solution. The mixture was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated in vacuo. The residue was purified by flash chromatography ($CH_2Cl_2 \rightarrow 10\%$ MeOH/$CH_2Cl_2$) to afford guanidine **3-38** (749 mg, 69%) as a colorless oil: $R_f = 0.21$ (10% MeOH/$CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, $J = 5.0$ Hz, 2H), 7.23-7.36 (m, 10H), 6.81 (d, $J = 6.0$ Hz, 2H), 4.34 (s, 4H), 3.27 (s, 4H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 157.5, 155.8, 149.8 (2), 136.5(2), 128.6(4), 128.0(4), 17.6(2), 117.1(2), 50.8(2), 44.9(2); IR (thin film): 3029, 1632 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{22}H_{23}N_4$ [M+H]: 343.19227. Found: 343.1995.



**Aminopyridine alcohol 3-47**: To a solution of pyridine carboxaldehyde **3-46** (825 mg, 3.7 mmol) in THF (30 mL) at 0 °C was added *n*-BuLi (2.2 M in hexane, 5 mL, 11.1 mmol) dropwise. After stirring for 30 min, the reaction was quenched 1N HCl (ca. 12 mL). The aqueous layer was made basic (pH = 8-9 pH paper) with saturated $NaHCO_3$ then extracted with $CH_2Cl_2$. The organic layers were dried ($MgSO_4$), concentrated *in vacuo*. The residue was purified by flash chromatography (50% → 70% EtOAc/Hexanes) to afford *tert*-butyl-3-(1-hydroxypentyl)pyridin-4-ylcarbamate (749 mg, 94%) as colorless oil. The crude alcohol (178 mg, 0.635 mmol) was dissolved in MeOH (4 mL) and 3N HCl (2 mL) was added at 23 °C then warmed up to 40 °C for 18 h. The reaction mixture was quenched by saturated $NaHCO_3$ solution. The mixture was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated in vacuo. The residue was purified by flash chromatography chromatography ($CH_2Cl_2 \rightarrow 10\%$ MeOH/$CH_2Cl_2$) to afford aminopyridine alcohol **3-47**(82 mg, 72%) as a colorless oil: $R_f = 0.21$ (10% MeOH/$CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, $J = 5.5$ Hz, 1H), 7.63 (s, 1H), 6.40 (d, $J$ = 5.5 Hz, 1H), 5.09 (s, 2H), missed (OH), 4.52 (t, $J$ = 7.0 Hz, 1H), 1.83-1.90 (m, 1H), 1.72-1.79 (m, 1H), 1.30-1.40 (m, 1H), 1.30 (q, $J$ = 7.0 Hz, 2H), 1.20-1.23 (m, 1H), 0.89 (t, $J$ = 2.0 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 154.0, 148.4, 140.6, 123.5, 111.1, 73.3, 35.1, 29.0, 23.1, 14.6; IR (thin film): 3349, 1623 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{10}H_{17}N_2O$ [M+H]: 181.1341. Found: 181.1314.

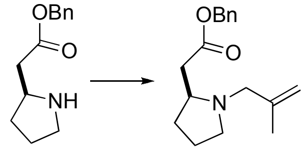


**Guanidine 3-48**: To a solution of the 2-chloro-imidazolinium **3-44** (1.1 g, 3.84 mmol) in $CH_3CN$ (20 mL) and $Et_3N$ (0.7 mL) was added amino pyridine **3-47** (231 mg, 1.28 mmol) at 22 °C then warmed up 50 °C and stirred for 36 h. The reaction mixture was quenched by saturated $NaHCO_3$ solution. The mixture was extracted with $CH_2Cl_2$ and the combined organic layers were concentrated in vacuo. The residue was purified by flash chromatography chromatography ($CH_2Cl_2$→10% MeOH/$CH_2Cl_2$) to afford **3-48** (209 mg, 38%) as a colorless oil: R$_f$ = 0.21 (10% MeOH/$CH_2Cl_2$); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.12 (s, 1H), 7.99 (d, $J$ = 4.0 Hz, 1H), 7.30-7.37 (m, 6H), 7.18 (d, $J$ = 8.5 Hz, 4H), 6.62 (d, $J$ = 6.0 Hz, 1H), 4.76 (t, $J$ = 7.0 Hz, 1H), 4.35 (s, 4H), 3.52 (s, 4H), 1.75-1.81 (m, 2H), 1.39-1.47 (m, 1H), 1.26-1.34 (m, 3H), 0.85 (t, $J$ = 6.5 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 157.5, 155.0, 146.3, 146.2, 135.7(4), 130.0, 128.8(4), 127.8(2), 127.7(2), 115.2, 72.5, 50.7(2), 44.9(2), 36.3, 28.3, 22.6, 14.0; HRMS (ESI) Calcd. for $C_{27}H_{33}N_4O$ [M+H]: 429.26544. Found: 429.2627.



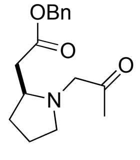
**Guanidine 3-49**: To a solution of the guanidine **3-48** (87 mg, 0.20 mmol) in THF (10 mL) was added dropwise a solution of Na°/naphthalenide (0.28 M, 1.4 mL) at 22 °C and stirred for 6 h. The reaction mixture was quenched by saturated $NH_4Cl$ solution and extracted with $CH_2Cl_2$. The combined organic layers were concentrated in vacuo and the residue was purified by flash chromatography ($CH_2Cl_2$→10% MeOH/$CH_2Cl_2$) to afford **3-48** (48 mg, 71%) as a yellow oil: R$_f$ = 0.13 (10% MeOH/$CH_2Cl_2$); $^1$H NMR (500 MHz,

$CDCl_3$) δ 8.34 (s, 1H), 8.22, (s, 1H), 7.28-7.37 (m, 5H), 6.89 (d, *J* = 6.0 Hz, 1H), 5.97 (dd, *J* = 7.5, 5.5 Hz, 1H), 4.61 (d, *J* = 15.0 Hz, 1H), 4.50 (d, *J* = 15.0 Hz, 1H), 3.35-3.48 (m, 4H), 2.55 (br s, 2H), 1.78-1.83 (m, 2H), 1.27-1.37 (m, 4H), 0.84 (t, *J* = 6.5 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 170.81, 155.5, 146.5, 144.6, 137.1, 128.6(2), 128.1(2), 127.5, 71.3, 48.6, 45.7, 40.5, 34.8, 29.7, 27.7, 22.4, 21.3, 14.0; IR (thin film): 3239, 1735 cm$^{-1}$.



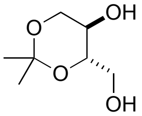
**3-77b**

**Methylallylpyrrolidine 3-78**: To a solution of potassium carbonate (290 mg, 2.1 mmol), and 3-bromo-2-methyl-1-propene (0.1 mL, 1.05 mmol) in 8 mL of DMF was added a solution of pyrrolidine **3-77b** (231 mg, 1.05 mmol) in DMF and heated to 40 °C for 24 h. After cooling to 21 °C, the mixture was filtrated through a celite pad and concentrated *in vacuo*. Purification of the residue by flash chromatography on $SiO_2$ (10%→20% EtOAc/hexanes) afforded **3-78** (135 mg, 47 %) as a colorless oil: $R_f$ 0.20 (20%, EtOAc/hexanes); $[\alpha]^{21}_D$ –52.2 (*c* 0.75, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) δ 7.37-7.40 (m, 5H), 5.18 (d, *J* = 12.0 Hz, 1H), 5.12 (d, *J* = 12.0 Hz, 1H), 4.91 (br s, 1H), 4.81 (br s, 1H), 3.29 (d, *J* = 13.0 Hz, 1H), 2.96-3.03 (ddd, *J* = 9.9, 6.9, 3.6 Hz, 1H), 2.79-2.87 (m, 1H), 2.74 (d, *J* = 13.0 Hz, 1H), 2.69 (dd, *J* = 15.0, 4.5 Hz, 1H ), 2.37 (dd, *J* = 15.0, 9.0 Hz, 1H ), 2.14 (d, *J* = 17.0, 9.0 Hz, 1H), 1.99-2.08 (m, 1H), 1.70-1.80 (m, 2H), 1.57-1.68 (m, 1H), 1.75 (s,3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 172.2, 144.1, 136.0, 128.5(2), 128.11(2), 128.08, 118.8, 66.2, 61.4, 60.9, 53.8, 39.8, 30.9, 22.3, 20.7; IR (thin film): 3079, 1735 cm$^{-1}$; HRMS (ESI) Calcd. for $C_{17}H_{24}NO_2$ [M+H]: 274.1807. Found: 274.1719.

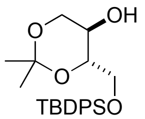


**ketone 3-79** : To a solution of alkene **3-78** ( 82 mg, 0.3 mmol) in diethyl ether (3 mL) was added 160 μL of a solution of 4N HCl (1 mL) in dioxane (1 mL) and stirred 30 min at 23 °C. After the reaction mixture was concentrated *in vacuo*, it was taken to the

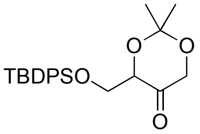
ozonolysis step. An ozone-oxygen mixture was passed through a mixture of alkene salt in $CH_2Cl_2$/MeOH (9:1, 10 mL) at –78 until a pale-blue color appeared in the solution. The reaction mixture was purged with $N_2$ and dimethyl sulfide (0.5 mL, 6.9 mmol) was added. The reaction mixture was warmed to 21 °C and concentrated *in vacuo*. Purification of the residue by flash chromatography on $SiO_2$ (50%→70% EtOAc/hexanes) afforded **3-79** (35 mg, 42 %) as a colorless oil: $R_f$ = 0.37 (EtOAc); $[\alpha]^{19}_D$ –52.9 (*c* 1.0, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.31-7.39 (m, 5H), 5.13 (d, *J* = 12.5 Hz, 1H), 5.10 (d, *J* = 12.5 Hz, 1H), 3.57 (d, *J* = 17.5 Hz, 1H), 3.35 (d, *J* = 17.5 Hz, 1H), 3.15 (dt, *J* = 6.0, 1.5 Hz, 1H), 2.95-3.00 (m, 1H), 2.64 (dd, *J* = 15.0, 5.0 Hz, 1H), 2.42 (dd, *J* = 15.0, 6.5 Hz, 1H ), 2.36 (dd, *J* = 17.0, 8.5 Hz, 1H ), 2.09 (s, 3H), 2.02-2.06 (m, 1H), 1.72-1.87 (m, 2H), 1.57-1.64 (m, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 210.8, 166.4, 130.2, 122.9, 122.7, 122.69, 60.69, 58.7, 55.1, 48.8, 34.2, 25.3, 21.9, 17.2.



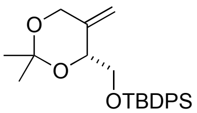
**Diol 4-8**: To a solution of triol **4-10** (2.0 g, 9.08 mmol) in dry MeOH (30 mL) was added $NaIO_4$ (3.0 g 14. 04 mmol), and the mixture was stirred at 23 °C for 24 h. The mixture was filtered, and the filtrated was concentrated and the resulting residue was passed through a short silicagel column eluted with MeOH. The fraction containing the desired product was evaporated and dissolved in dry MeOH (20 mL) and the solution was stirred at 0 °C while $NaBH_4$ (0.4 g) was slowly added. After 30 min, acetone was added to quench the reaction. The reaction mixture was evaporated and the residue was passed through a silica gel column eluted with (20% hexanes/EtOAc) to give diol **4-8** (0.81g, 5.0 mmol) as a yellow viscous oil. $R_f$ 0.28 (20% hexanes/EtOAc). IR (thin film) 3723-3032, 1665 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 3.90 (dd, *J* = 6.6, 3.3 Hz, 1H), 3.78 (d, *J* = 2.4 Hz, 2H), 3.75 (dd, *J* = 5.4, 3.3 Hz, 1H), 3.71-3.62 (m, 2H), 1.48 (s, 3H), 1.39 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 99.0, 74.1, 64.5, 63.9, 63.1, 28.7, 19.6.



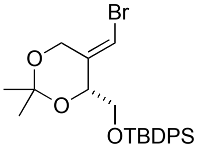
**Alcohol 4-12**: To a solution of diol **4-8** (0.6 g, 3.7 mmol) in $CH_2Cl_2$ (40 mL) was added TBDPSCl (1.2 g, 4.4 mmol) followed by and $Et_3N$ (1.5 mL, 11 mmol) and a crystal of DAMP at 22 °C. This solution was stirred for 15 h at which time it was washed with sat. aq. $NH_4Cl$ (2 x 30 mL), and water (30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (20% hexanes/EtOAc) to provide silyl ether **4-12** (1.0 g, 2.6 mmol, 70%) as a yellowish oil. $R_f$ 0.35 (20% hexanes/EtOAc); IR (thin film) 3673-3200, 3076, 1476, $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.72-7.69 (m, 4H), 7.50-7.38 (m, 6H), 3.96-3.77 (m, 5H), 3.70-3.63 (m, 1H), 1.46 (s, 3H), 1.36 (s, 3H), 1.09 (s, 9H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 135.6, 132.5, 132.5, 130.0, 127.8, 98.5, 72.4, 66.9, 66.9, 63.8, 28.4, 26.8, 19.2, 19.1.



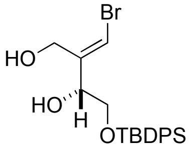
**Cyclic Ketone 4-7**. To stirred solution of oxalyl chloride (0.68 mL, 7.8 mmol) in $CH_2Cl_2$ (20 mL) was added DMSO dropwise (0.73 mL, 10 mmol) at –78°C. The reaction mixture was stirred for 10 min, after which time alcohol **4-12** (1.0 g, 2.6 mmol) was added in $CH_2Cl_2$ (10 mL) over 30 min. Triethylamine (2.3 mL, 13 mmol) was added and the reaction was stirred for 20 min then allowed to warm to 23 °C then stirred for 20 min. Water (30 mL) was added and the aqueous layer was extracted with diethyl ether (2 x 30 mL). The combined organic layers were dried over ($MgSO_4$), and solvent removed *in vacuo* then the residue was put on silica gel column (hexanes/EtOAc 96:4) to provide the ketone **4-7** (720 mg, 70%) as yellow oil. $R_f$ 0.3 (hexanes/EtOAc 9:1); IR (thin film) 3068, 1747 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.72-7.69 (m, 4H), 7.50-7.38 (m, 6H), 4.36 (ddd, *J* = 5.4, 3.0, 1.5 Hz, 1H), 4.29 (dd, *J* = 16.5, 1.5 Hz, 1H), 4.40 (d, *J* = 3.0 Hz, 1H), 4.15 (d, *J* = 5.4 Hz, 1H), 4.02 (d, *J* = 16.5 Hz, 1H), 1.49 (s, 3H), 1.49 (s, 3H), 1.09 (s, 9H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 207.3, 135.5, 133.2, 133.1, 129.6, 127.5, 127.5, 100.3, 76.5, 66.8, 62.4, 26.6, 24.2, 23.3, 19.1.



**Ethylene 4-14**. Bis-(cyclopentadienyl)dimethyltitanium (330 mg, 1.6 mmol) was prepared by the known procedure. To a solution of Petasis reagent in toluene (5 mL) was added ketone **4-7** (250 mg, 0.63 mmol) in toluene (5 mL). The reaction mixture was stirred under reflux conditions. After 12 h, the reaction was cooled to 23 °C. The precipitates were filtered through Celite and the filtrate was concentrated under reduced pressure. Purification by flash column chromatography (2% hexanes/EtOAc) to provide the ethylene **4-14** (37 mg, 0.09 mmol, 15%) as a yellow oil. $R_f$ 0.30 (hexanes/EtOAc 96:4); IR (thin film) 3073, 1475 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) δ 7.72-7.69 (m, 4H), 7.50-7.38 (m, 6H), 4.91 (dd, *J* = 13.2, 1.2 Hz, 2H), 4.45 (m, 1H), 3.93 (dd, *J* = 10.8, 5.1 Hz, 1H), 3.83 (dd, *J* = 10.8, 6.0 Hz, 1H), 1.44 (s, 3H), 1.38 (s, 3H), 1.07 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 143.5, 135.7, 135.6, 133.7, 129.6, 127.6, 127.6, 107.5, 99.5, 71.8, 65.5, 64.2, 27.2, 26.8, 22.3, 19.3.; HRMS (ESI) calcd for $C_{24}H_{32}O_3SiLi$ [M+Li]: 403.2281. Found: 403.2283



**Vinyl bromide 4-15**: A solution of ethylene **4-14** (37 mg, 0.09 mmol) in $CH_2Cl_2$ (4 mL) was treat with a solution of $Br_2$ (28 mg, 0.18 mmol) in $CH_2Cl_2$ (2 mL) at –78 °C. After 20 min, the solution was allowed to warm to 23 °C and concentrated under reduced pressure to give crude dibromide as a yellow oil. To the solution of the crude dibromide in benzene (8 mL) was added DBU **3-23** (55 mg, 0.36 mmol). The mixture was refluxed for 7 h then allowed to cool to 23 °C. The precipitates were filtered through Celite and the filtrate was concentrated under reduced pressure. Purification by flash column chromatography (2% hexanes/EtOAc) to provide the vinyl bromide **4-15** (11 mg, 0.02 mmol, 25%) as a yellow oil. $R_f$ 0.30 (hexanes/EtOAc 96:4); IR (thin film) 3069, 2938, 2851, 1469, 1425, 1382, 1222, 1105, 822, 785, 705 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) δ 7.72-7.69 (m, 4H), 7.50-7.38 (m, 6H), 4.91 (dd, *J* = 13.2, 1.2 Hz, 2H), 4.45 (m, 1H), 3.93 (dd, *J* = 10.8, 5.1 Hz, 1H), 3.83 (dd, *J* = 10.8, 6.0 Hz, 1H), 1.44 (s, 3H), 1.38 (s, 3H), 1.07 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 140.9, 135.9, 135.9, 133.8, 130.0, 128.0, 128.0, 100.5, 100.2, 72.2, 65.2, 62.5, 27.0, 26.1, 23.7, 19.4; HRMS (ESI) calcd for $C_{24}H_{32}O_3SiLi$ [M+Li]: 481.1386. Found: 481.1340



**Butane-1,3-diol 4-6**: $BF_3{\cdot}OEt_2$ (0.82 mg, 0.0058 mmol) was added to a mixture of vinyl bromide **4-15** (28 mg, 0.058 mmol) and 1,3-propanedithiol (15.6 mg, 0.143 mmol) in $CH_2Cl_2$ (3 mL) at 0 $^{o}$C. The mixture was stirred at 0 $^{o}$C for 1 h and quenched with saturated $NaHCO_3$ (3 drops). The aqueous layer was extracted with $CH_2Cl_2$ (2 x 10 mL) and the combined organic layers were dried over $MgSO_4$ and concentrated. Purification by flash column chromatography (20% hexanes/EtOAc) to provide the diol **4-6** (16 mg, 0.037 mmol, 63%) as a yellow oil. $R_f$ 0.27 (hexanes/EtOAc 7:3); IR (thin film) 3680-3127, 3069, 2924, 2851, 1469, 1425, 1113, 822, 698, 705 cm$^{-1}$; $^{1}$H NMR (300 MHz, $CDCl_3$) δ 7.67-7.63 (m, 4H), 7.46-7.37 (m, 6H), 6.33 (d, $J$ = 0.8, Hz, 1H), 4.40 (m, 1H), 4.28 (s, 2H), 3.79 (dd, $J$ = 10.5, 4.5 Hz, 1H), 3.72 (dd, $J$ = 10.5, 6.6 Hz, 1H), 1.07 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 142.6, 135.8, 132.9, 130.3, 128.1, 108.0, 75.0, 66.9, 61.6, 27.1, 19.4.

## Crystal and Molecular Structure Determination

X-ray Diffraction Laboratory
Department of Chemistry
Texas A&M University
Report: April 14, 2008
Structure: DR67 (**3-80b**)

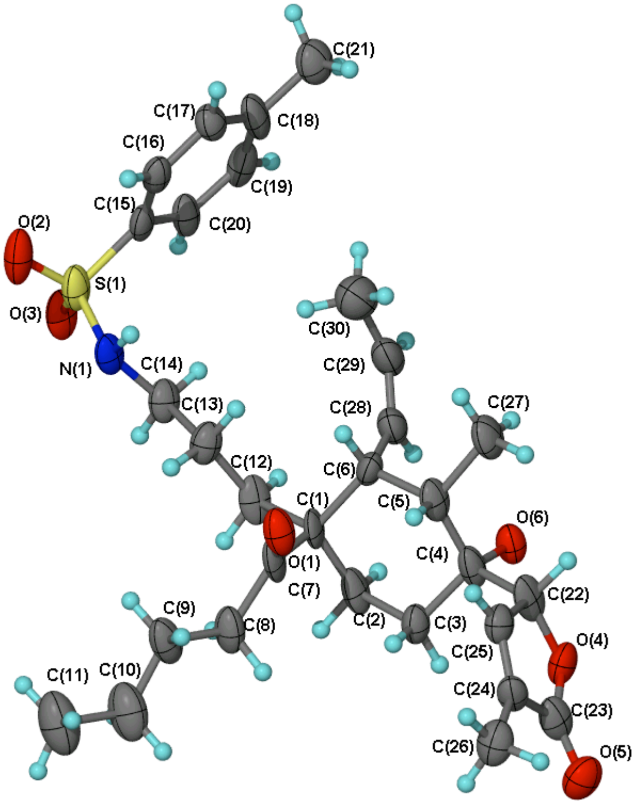

C(21)
C(17)
C(18)
C(16)
C(19)
C(15)
C(20)
O(2)
S(1)
O(3)
C(30)
N(1)
C(14)
C(29)
C(13)
C(28)
C(27)
C(12)
C(6)
C(1)
C(5)
O(6)
O(1)
C(4)
C(7)
C(22)
C(9)
C(8)
C(2)
C(3)
C(25)
O(4)
C(11)
C(10)
C(24)
C(23)
C(26)
O(5)

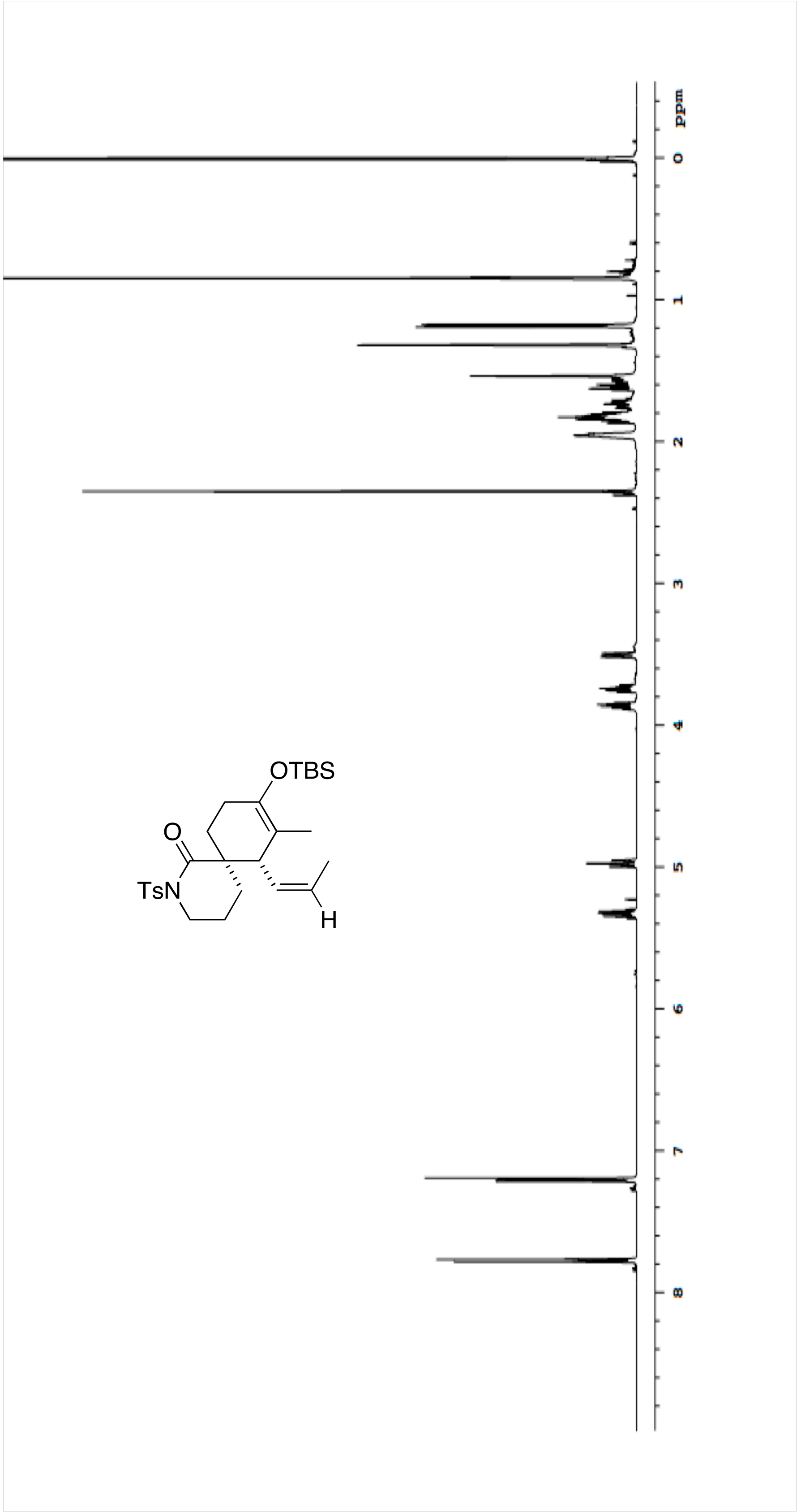
Table 1. Crystal data and structure refinement for dr67.

| | | |
|---|---|---|
| Identification code | dr67 | |
| Empirical formula | C30 H42 N O6 S | |
| Formula weight | 544.71 | |
| Temperature | 110(2) K | |
| Wavelength | 1.54178 Å | |
| Crystal system | Triclinic | |
| Space group | P-1 | |
| Unit cell dimensions | a = 9.016(3) Å | $\alpha$= 96.61(2)°. |
| | b = 12.970(5) Å | $\beta$= 94.392(19)°. |
| | c = 13.568(6) Å | $\gamma$ = 107.975(18)°. |
| Volume | 1488.5(10) Å$^3$ | |
| Z | 2 | |
| Density (calculated) | 1.215 Mg/m$^3$ | |
| Absorption coefficient | 1.302 mm$^{-1}$ | |
| F(000) | 586 | |
| Crystal size | 0.20 x 0.20 x 0.02 mm$^3$ | |
| Theta range for data collection | 5.19 to 59.90°. | |
| Index ranges | -9<=h<=10, -14<=k<=14, -15<=l<=15 | |
| Reflections collected | 11310 | |
| Independent reflections | 4019 [R(int) = 0.2140] | |
| Completeness to theta = 59.90° | 91.3 % | |
| Absorption correction | Semi-empirical from equivalents | |
| Max. and min. transmission | 0.9744 and 0.7807 | |
| Refinement method | Full-matrix least-squares on F$^2$ | |
| Data / restraints / parameters | 4019 / 0 / 343 | |
| Goodness-of-fit on F$^2$ | 1.006 | |
| Final R indices [I>2sigma(I)] | R1 = 0.0735, wR2 = 0.1128 | |
| R indices (all data) | R1 = 0.2317, wR2 = 0.1473 | |
| Largest diff. peak and hole | 0.251 and -0.368 e.Å$^{-3}$ | |

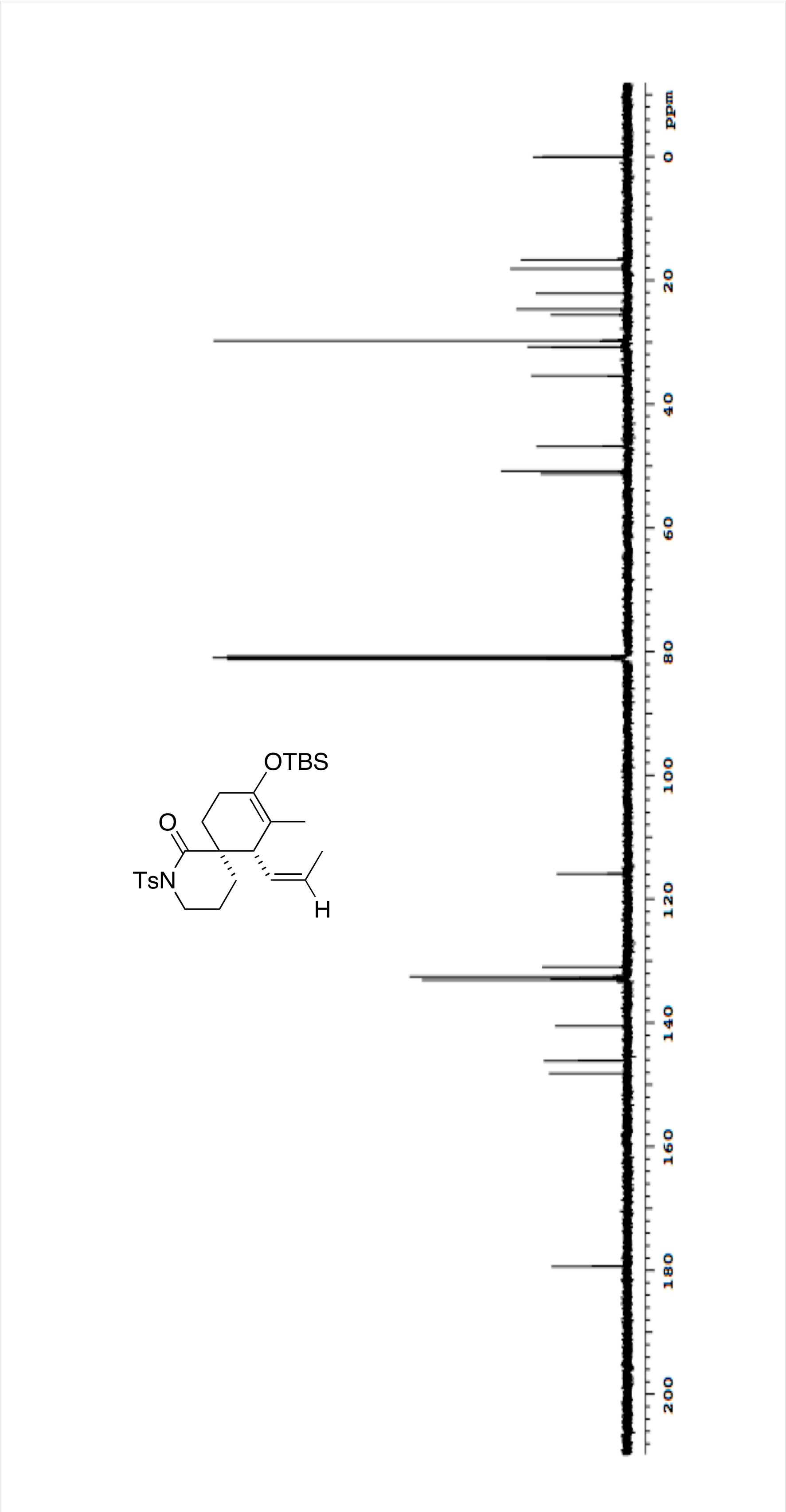
Table 2. Atomic coordinates ( x $10^4$) and equivalent isotropic displacement parameters (Å$^2$x $10^3$) for dr67. U(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| S(1) | 261(2) | 748(2) | 3404(2) | 55(1) |
| O(1) | -5654(6) | 674(4) | 6512(4) | 55(2) |
| O(2) | -166(5) | -290(4) | 2755(5) | 68(2) |
| O(3) | 1843(5) | 1247(4) | 3925(4) | 60(2) |
| O(4) | -6434(5) | 4436(4) | 9044(4) | 49(2) |
| O(5) | -7362(6) | 4131(4) | 10505(5) | 62(2) |
| O(6) | -4264(5) | 4862(3) | 7580(4) | 47(2) |
| N(1) | -965(6) | 579(4) | 4233(5) | 45(2) |
| C(1) | -3388(7) | 2299(5) | 6879(6) | 42(2) |
| C(2) | -2937(7) | 3068(6) | 7902(6) | 53(3) |
| C(3) | -4394(7) | 3233(5) | 8333(6) | 46(2) |
| C(4) | -5234(8) | 3769(6) | 7632(7) | 49(2) |
| C(5) | -5653(8) | 3084(6) | 6567(6) | 48(3) |
| C(6) | -4235(8) | 2819(5) | 6140(6) | 42(2) |
| C(7) | -4421(9) | 1153(6) | 7044(7) | 52(3) |
| C(8) | -3852(8) | 656(5) | 7905(6) | 56(3) |
| C(9) | -4308(8) | -553(6) | 7788(7) | 62(3) |
| C(10) | -3809(9) | -985(6) | 8686(8) | 81(3) |
| C(11) | -4212(9) | -2239(6) | 8568(8) | 95(4) |
| C(12) | -1873(7) | 2162(5) | 6511(6) | 52(3) |
| C(13) | -2221(7) | 1328(5) | 5556(6) | 49(2) |
| C(14) | -673(7) | 1490(5) | 5067(6) | 49(2) |
| C(15) | -99(9) | 1668(6) | 2693(7) | 45(2) |
| C(16) | -1400(9) | 1337(6) | 1929(6) | 46(2) |
| C(17) | -1772(8) | 2053(6) | 1349(6) | 47(2) |
| C(18) | -899(10) | 3165(7) | 1522(8) | 61(3) |
| C(19) | 384(10) | 3507(6) | 2281(7) | 57(3) |
| C(20) | 754(7) | 2762(6) | 2838(6) | 45(2) |
| C(21) | -1253(8) | 3966(6) | 925(6) | 64(3) |
| C(22) | -6804(8) | 3835(6) | 8017(7) | 57(3) |
| C(23) | -7379(9) | 3800(7) | 9626(8) | 54(3) |

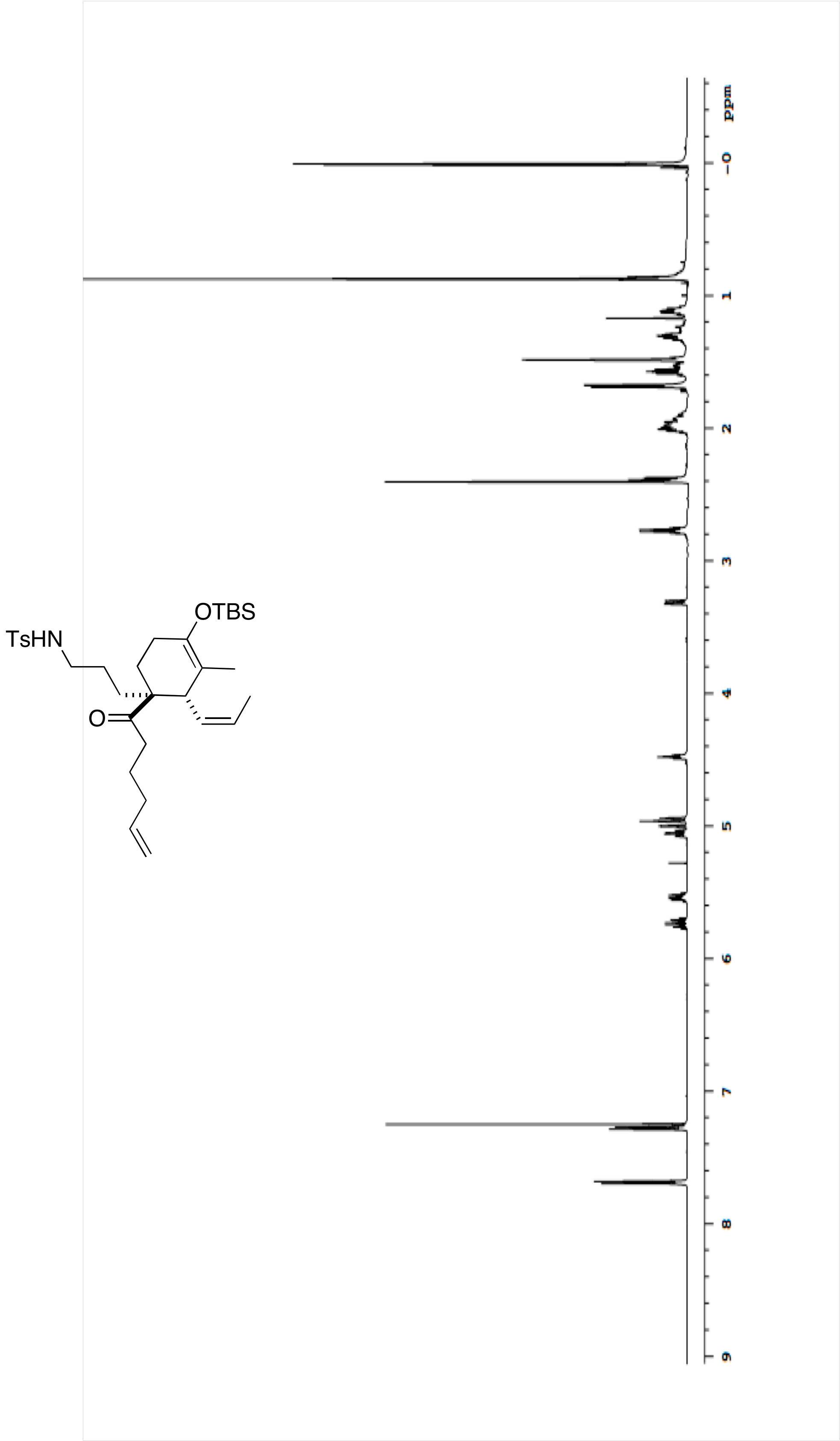
| | | | | |
|---|---|---|---|---|
| C(24) | -8357(8) | 2704(6) | 9029(7) | 47(2) |
| C(25) | -7978(7) | 2781(5) | 8112(6) | 45(2) |
| C(26) | -9408(8) | 1865(5) | 9489(6) | 60(3) |
| C(27) | -6428(8) | 3605(6) | 5795(6) | 61(3) |
| C(28) | -3091(8) | 3775(6) | 5791(7) | 52(2) |
| C(29) | -2782(9) | 3949(7) | 4895(8) | 62(3) |
| C(30) | -3547(10) | 3184(7) | 3908(7) | 80(3) |



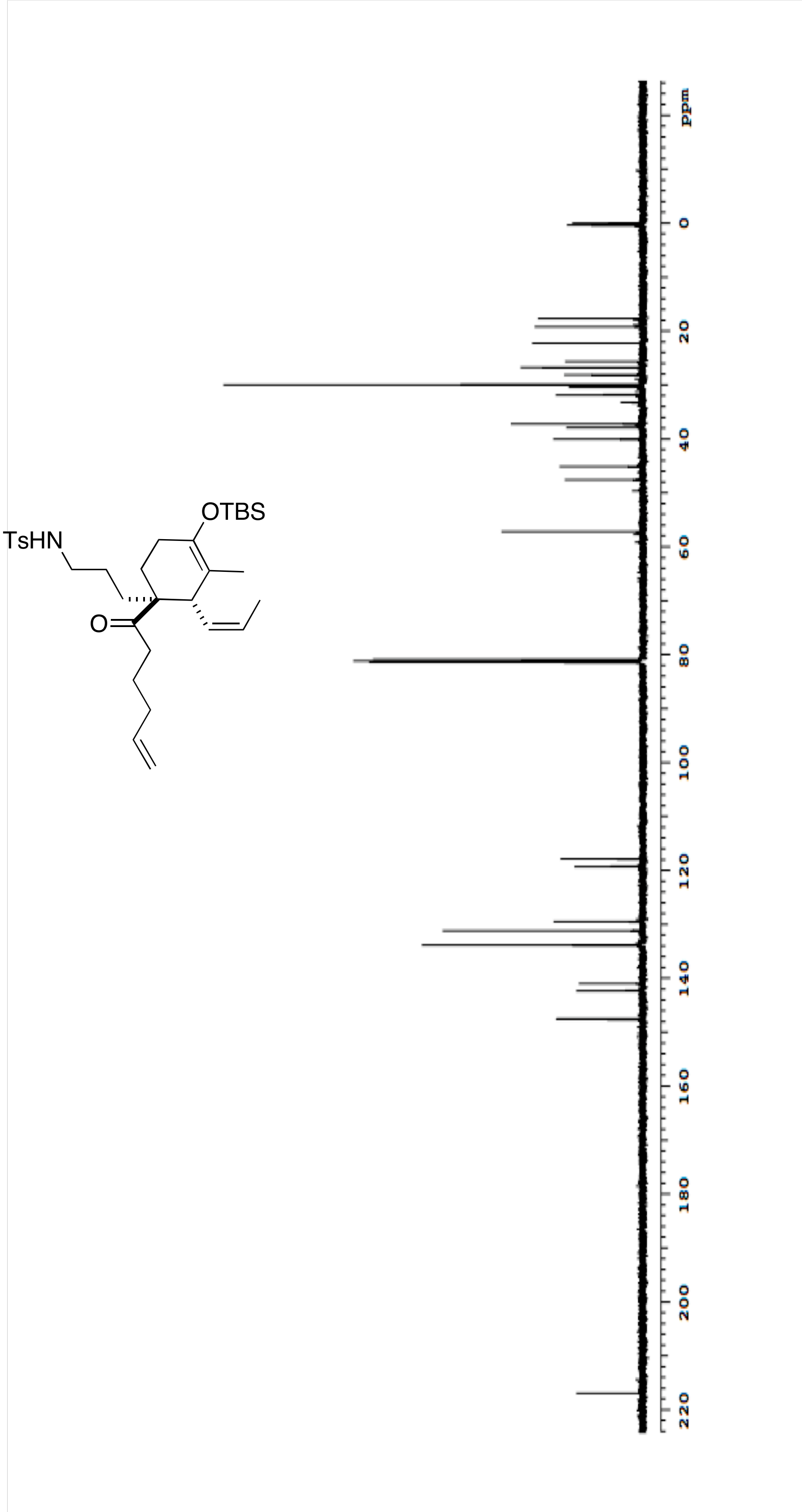
$^1$H-NMR (500 MHz) of **2-76** in $CDCl_3$



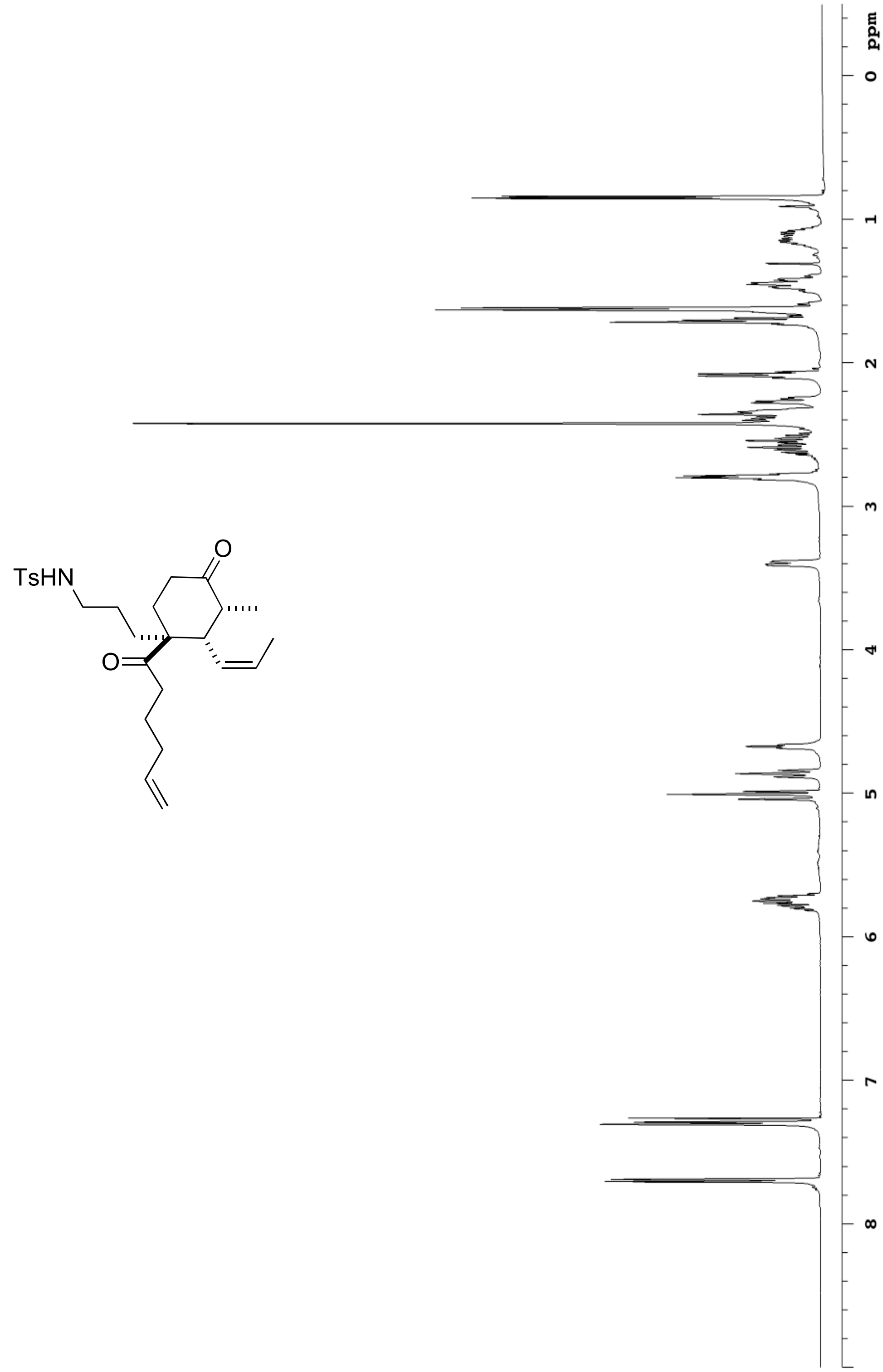
$^{13}$C-NMR (125 MHz) of **2-76** in $CDCl_3$



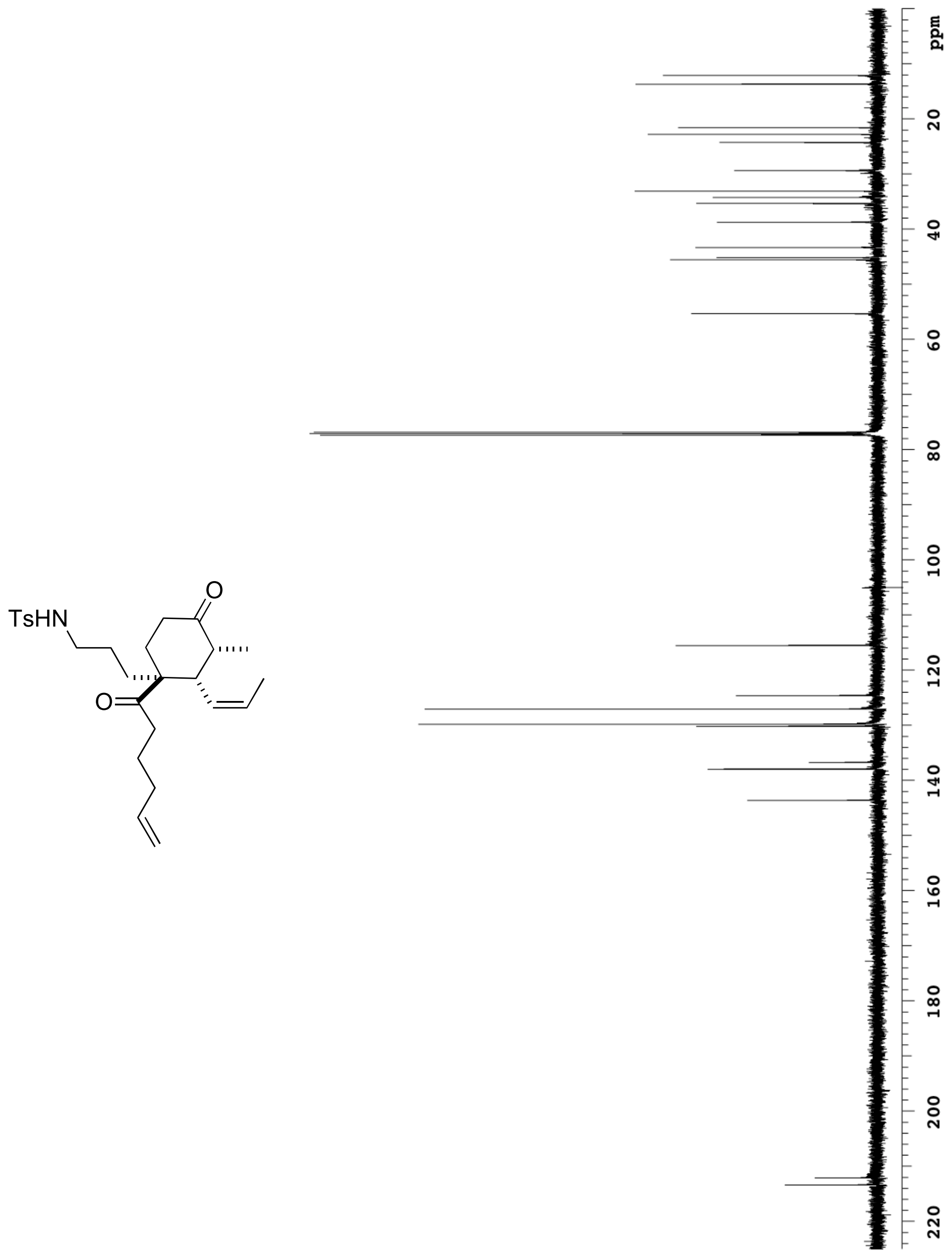
$^1$H-NMR (500 MHz) of **2-77** in $CDCl_3$



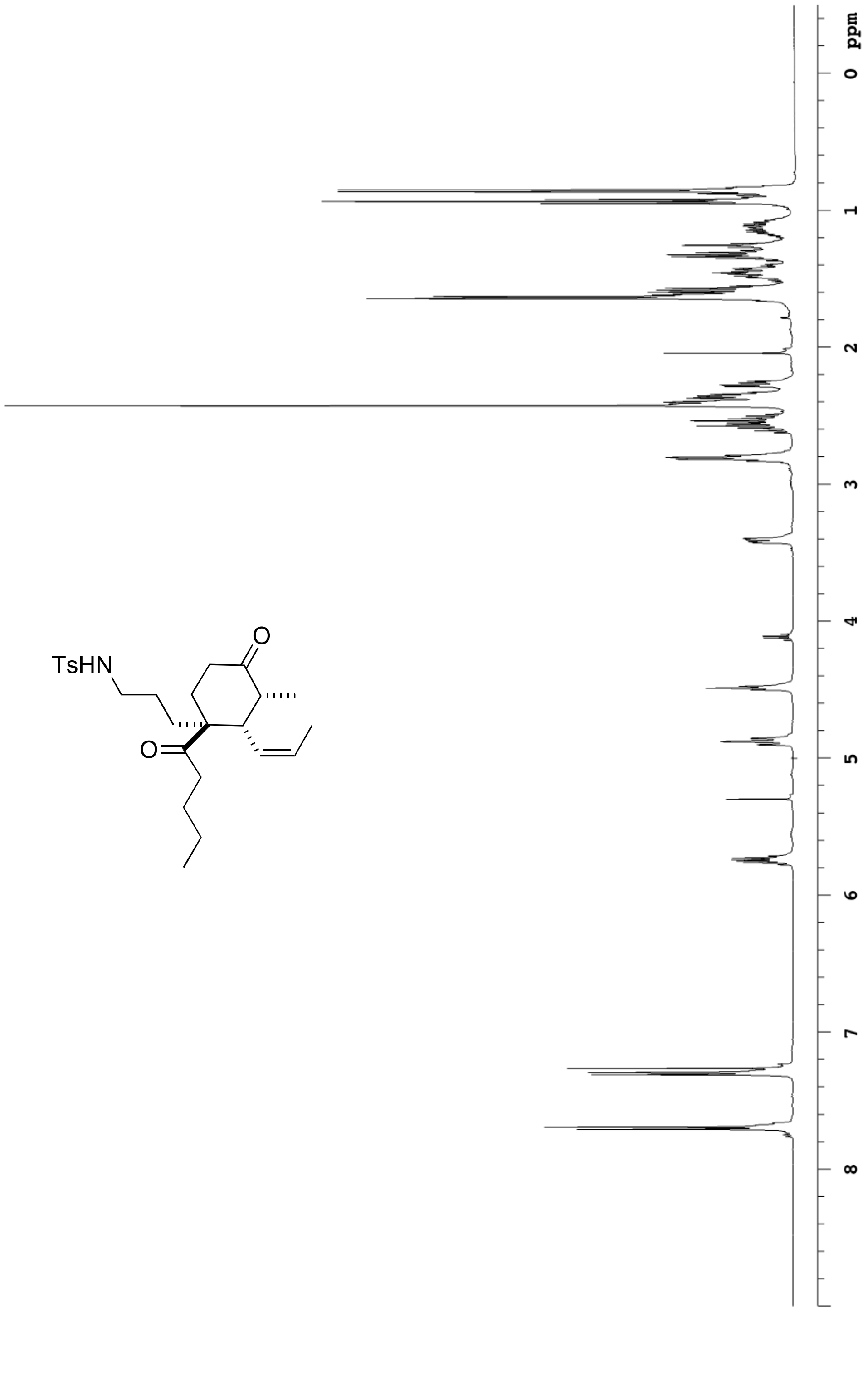
$^{13}$C-NMR (125 MHz) of **2-77** in $CDCl_3$



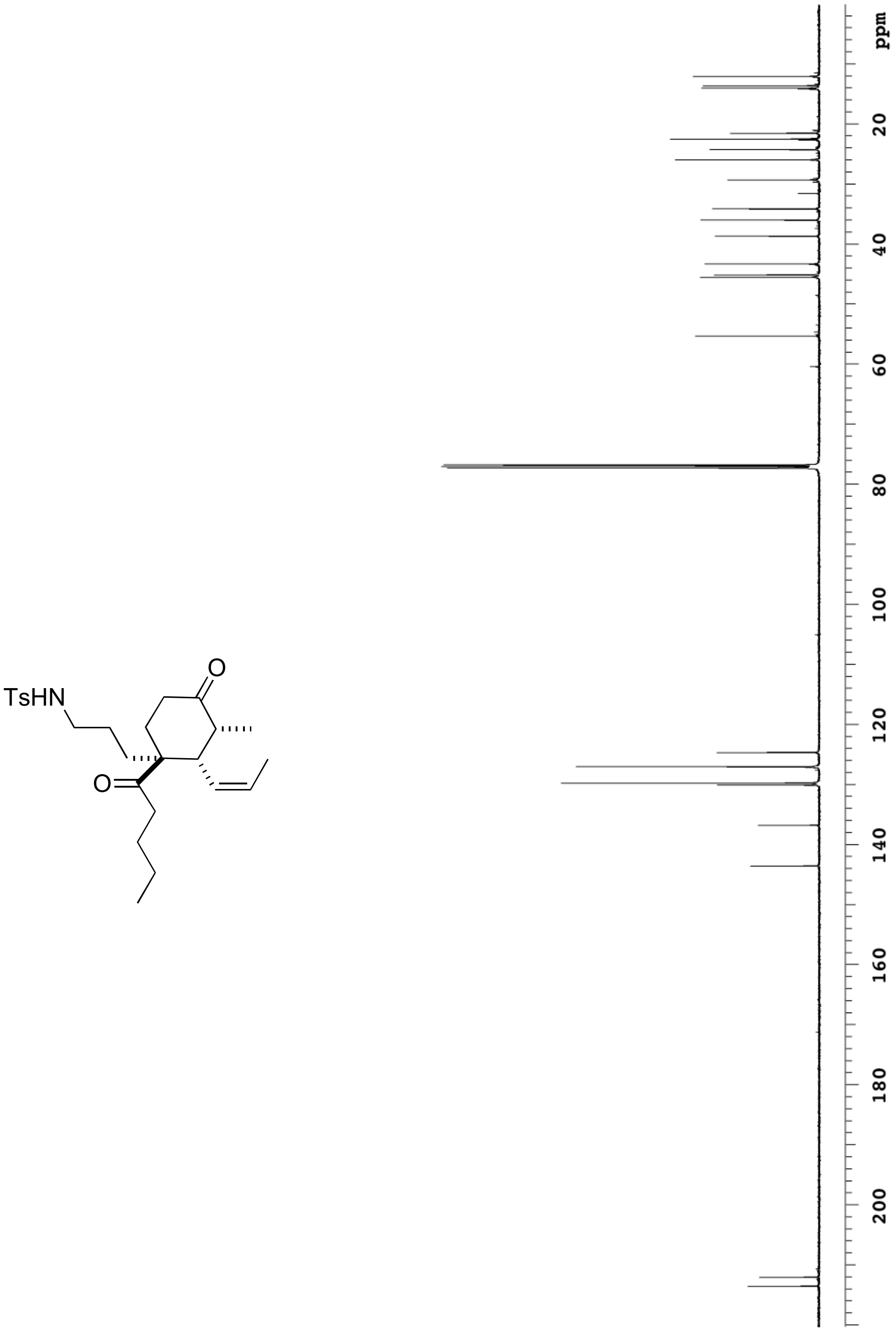
$^{1}$H-NMR (500 MHz) of **2-78** in $CDCl_3$



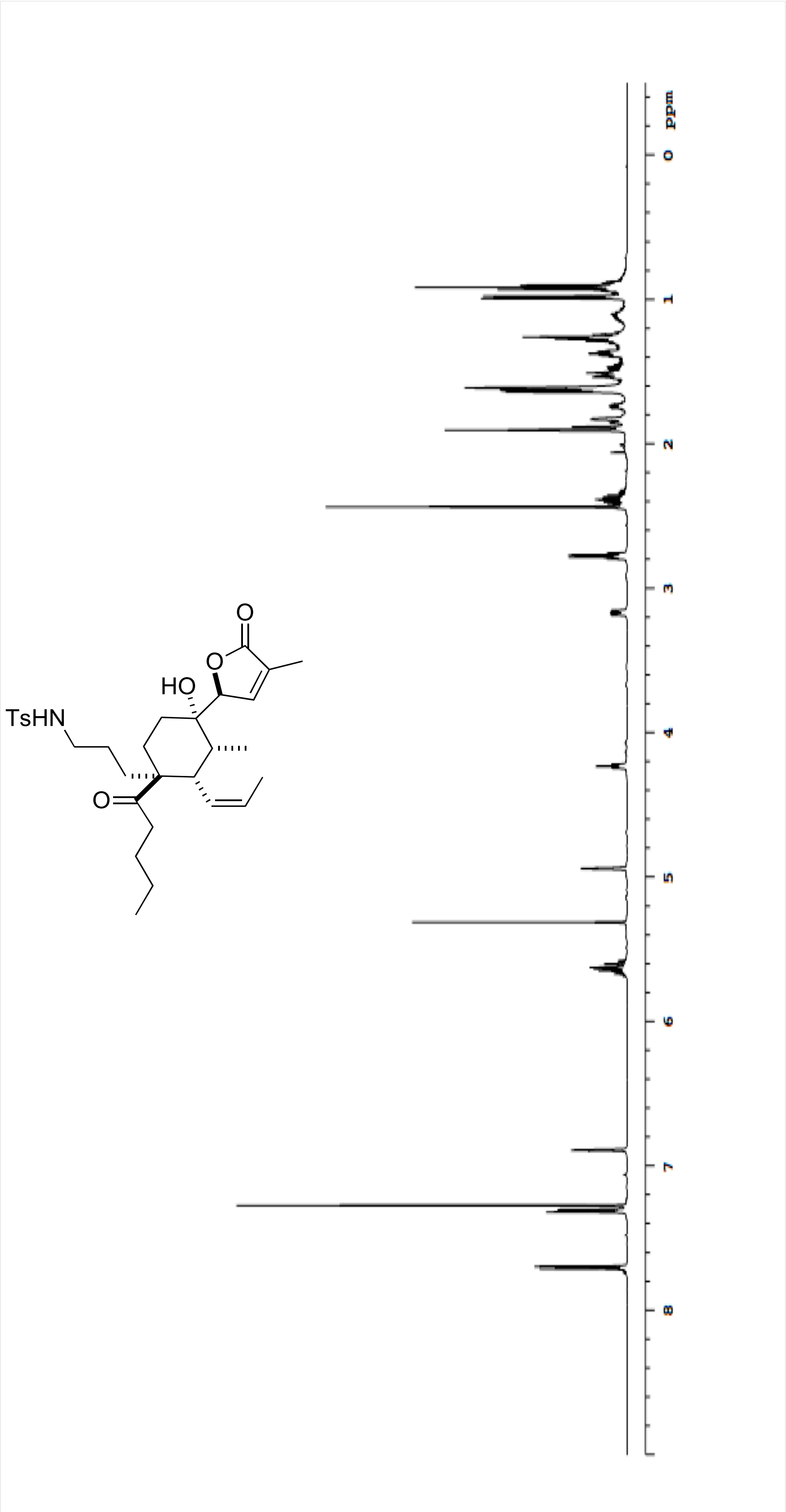
$^{13}$C-NMR (125 MHz) of **2-78** in $CDCl_3$



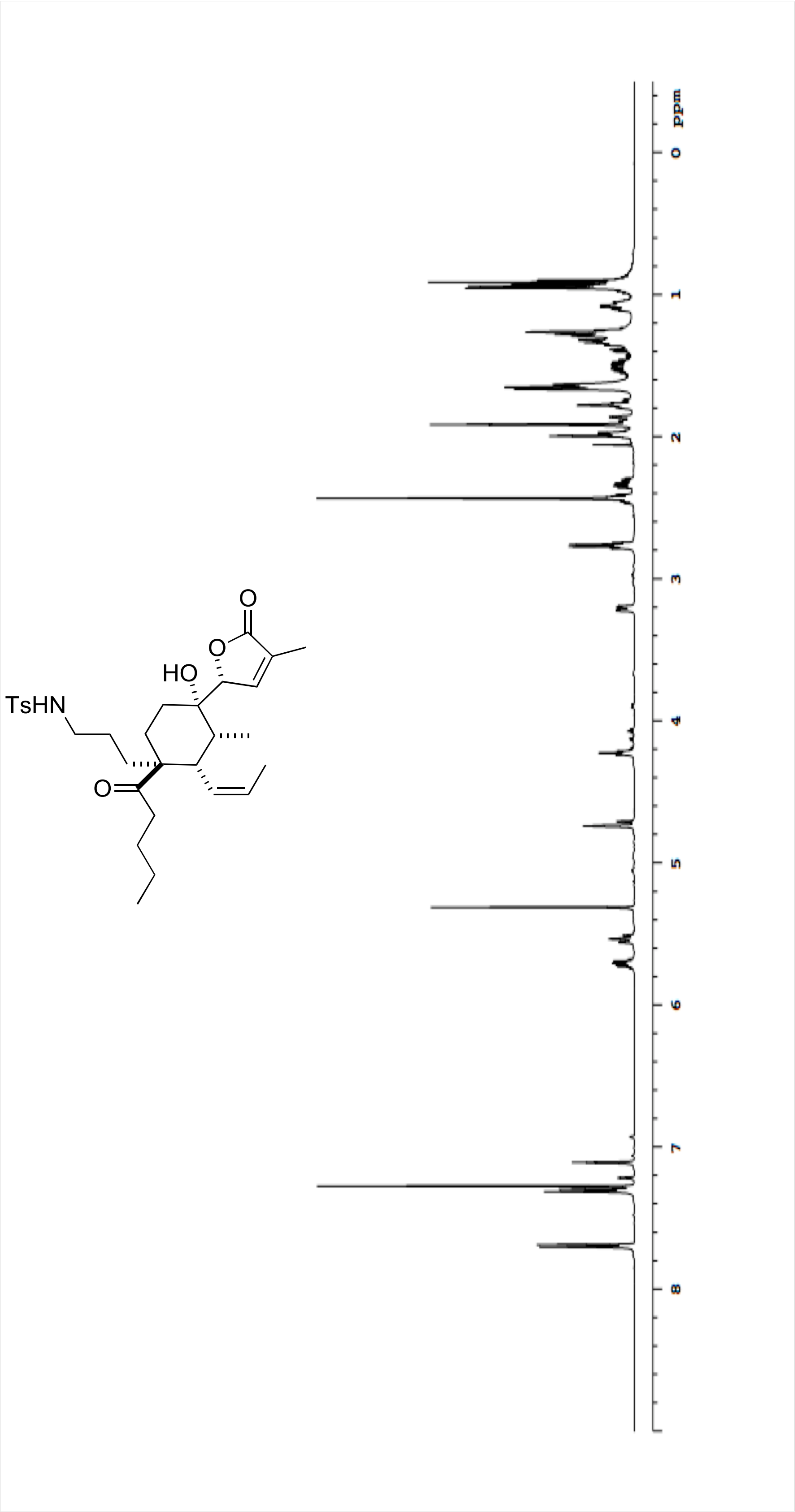
$^1$H-NMR (500 MHz) of **2-79** in $CDCl_3$



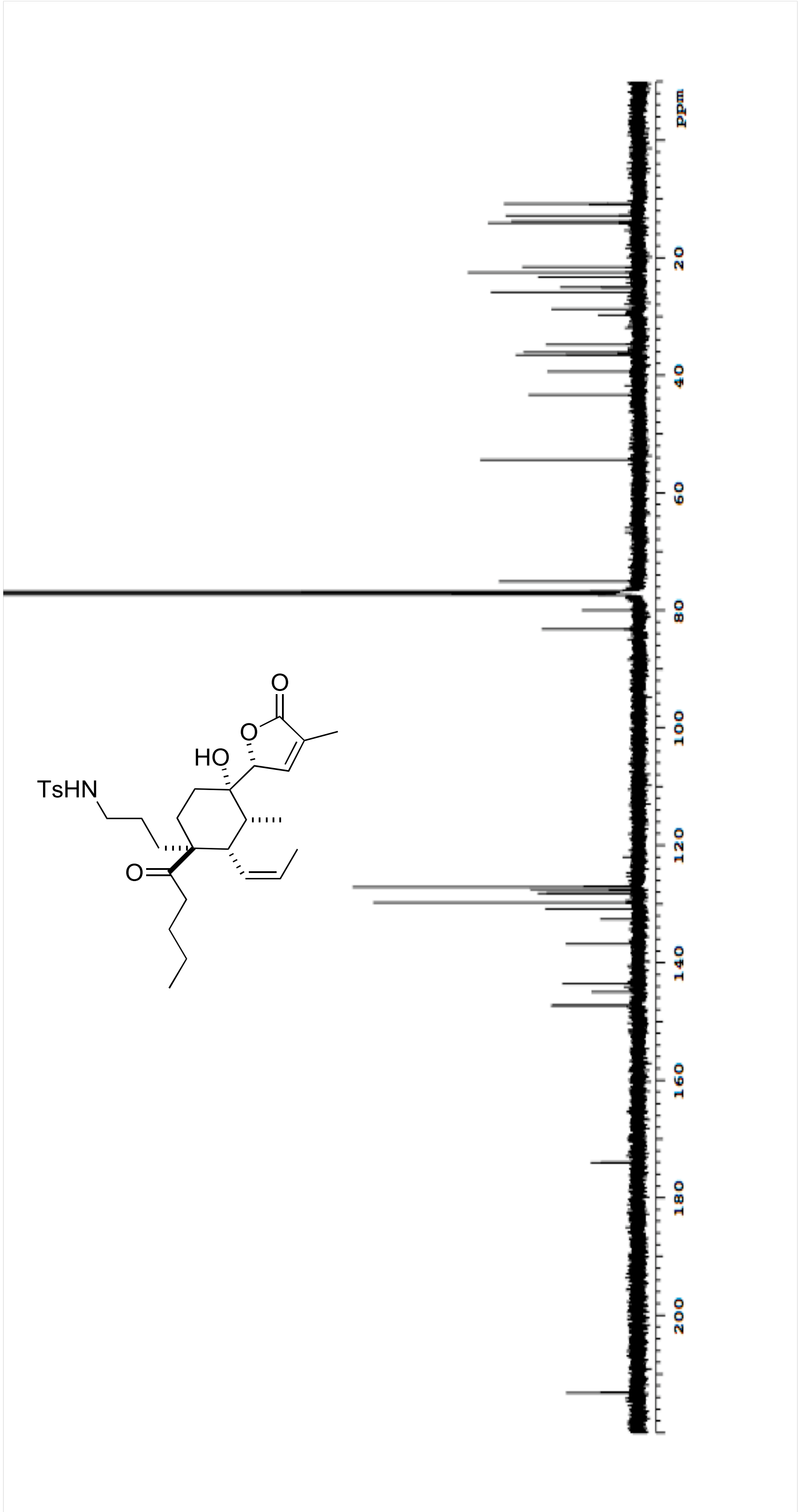
$^{13}$C-NMR (125 MHz) of **2-79** in $CDCl_3$



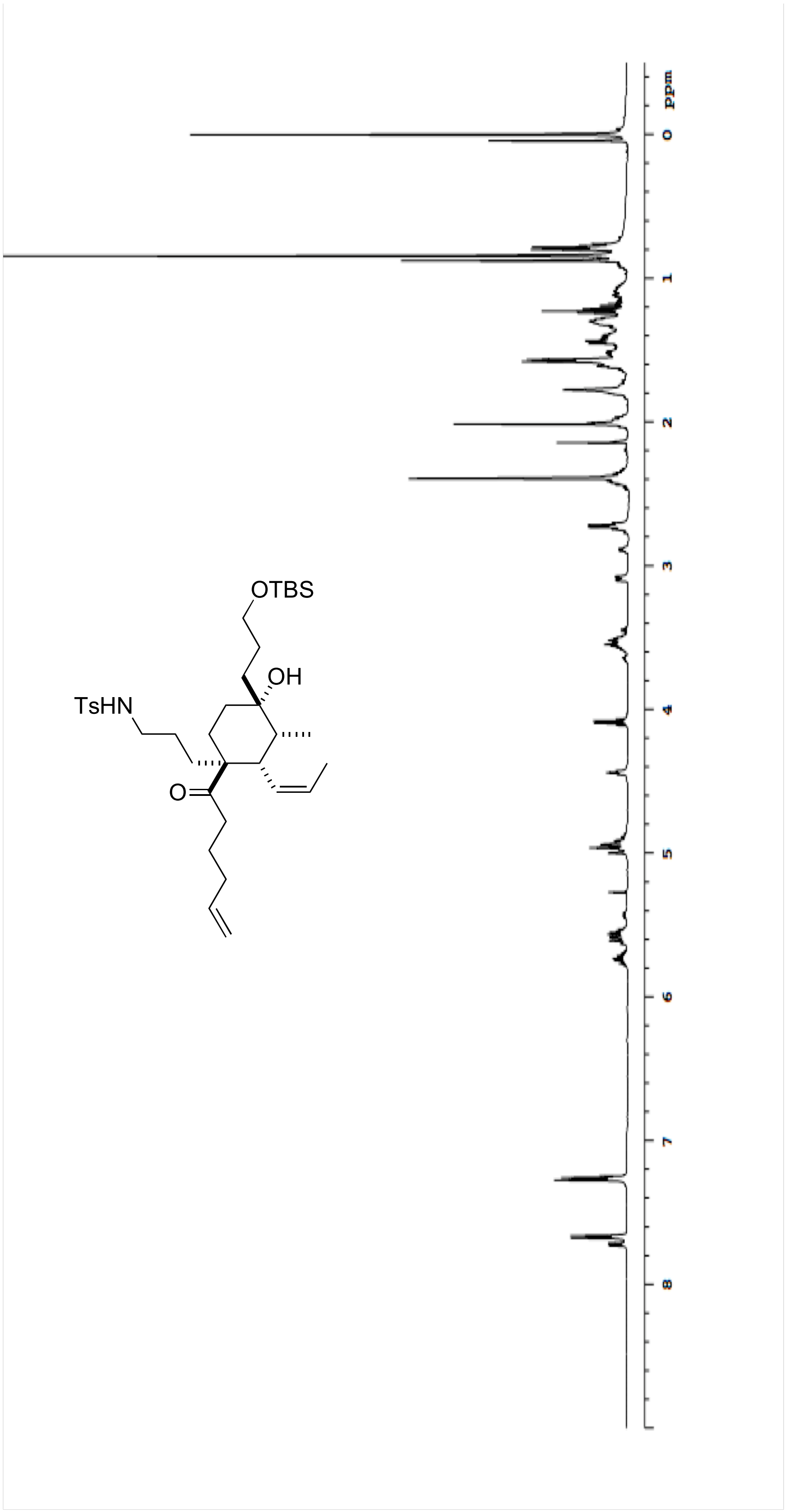
$^1$H-NMR (500 MHz) of **2-80a** in $CDCl_3$



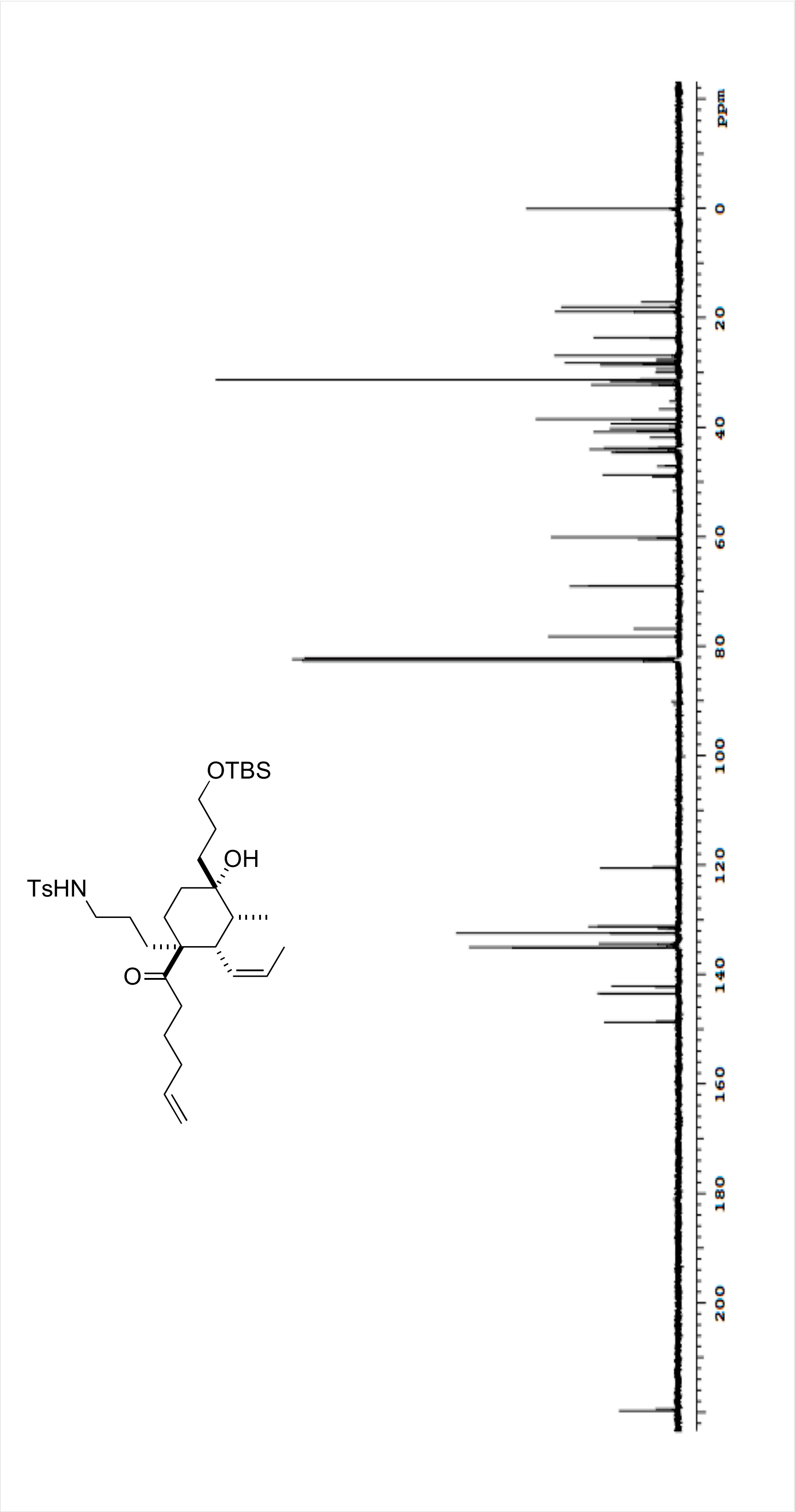
$^1$H-NMR (500 MHz) of **2-80b** in $CDCl_3$



$^{13}$C-NMR (125 MHz) of **2-80b** in CDCl$_3$



$^1$H-NMR (500 MHz) of **2-82a** in $CDCl_3$



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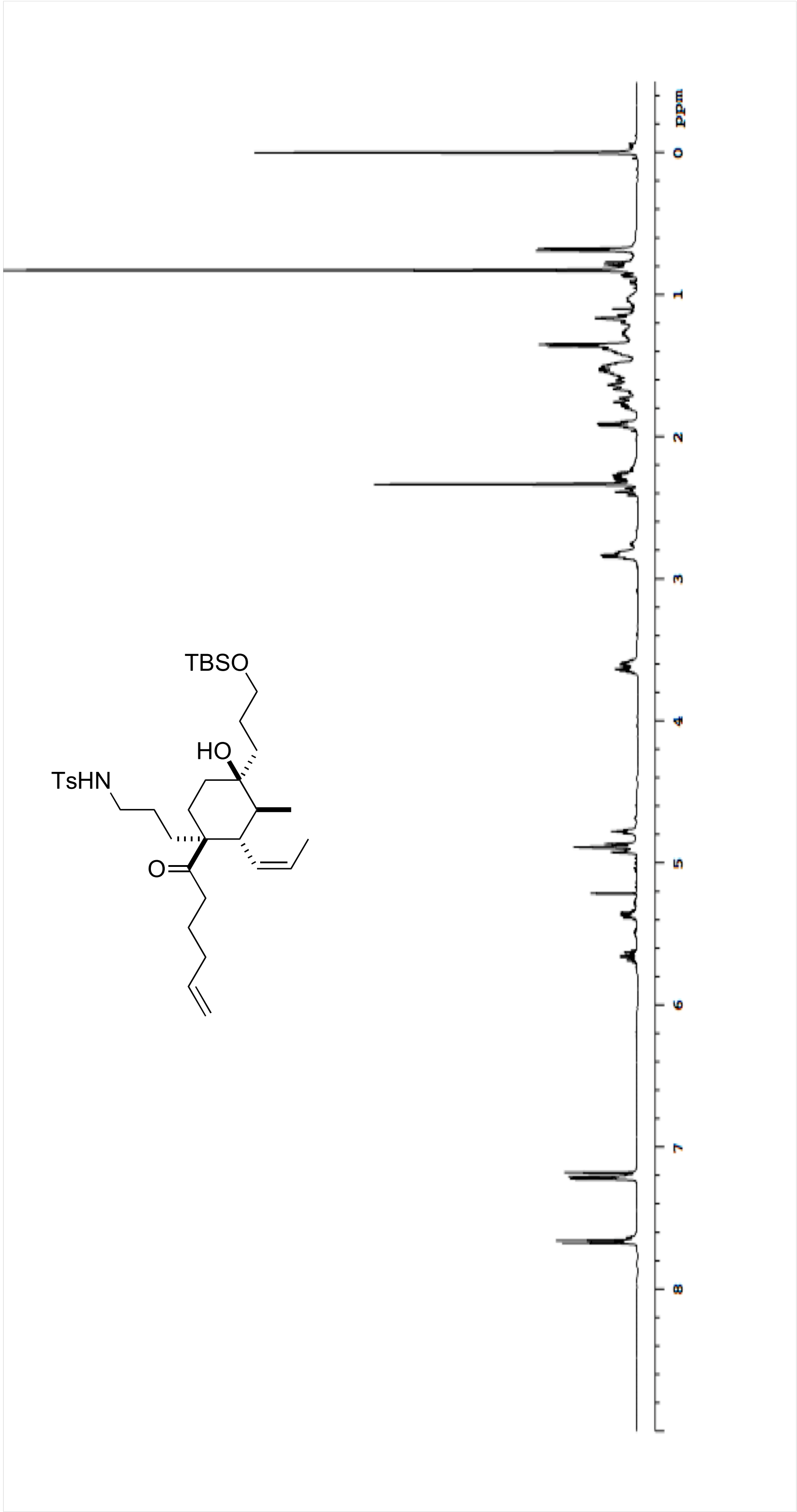
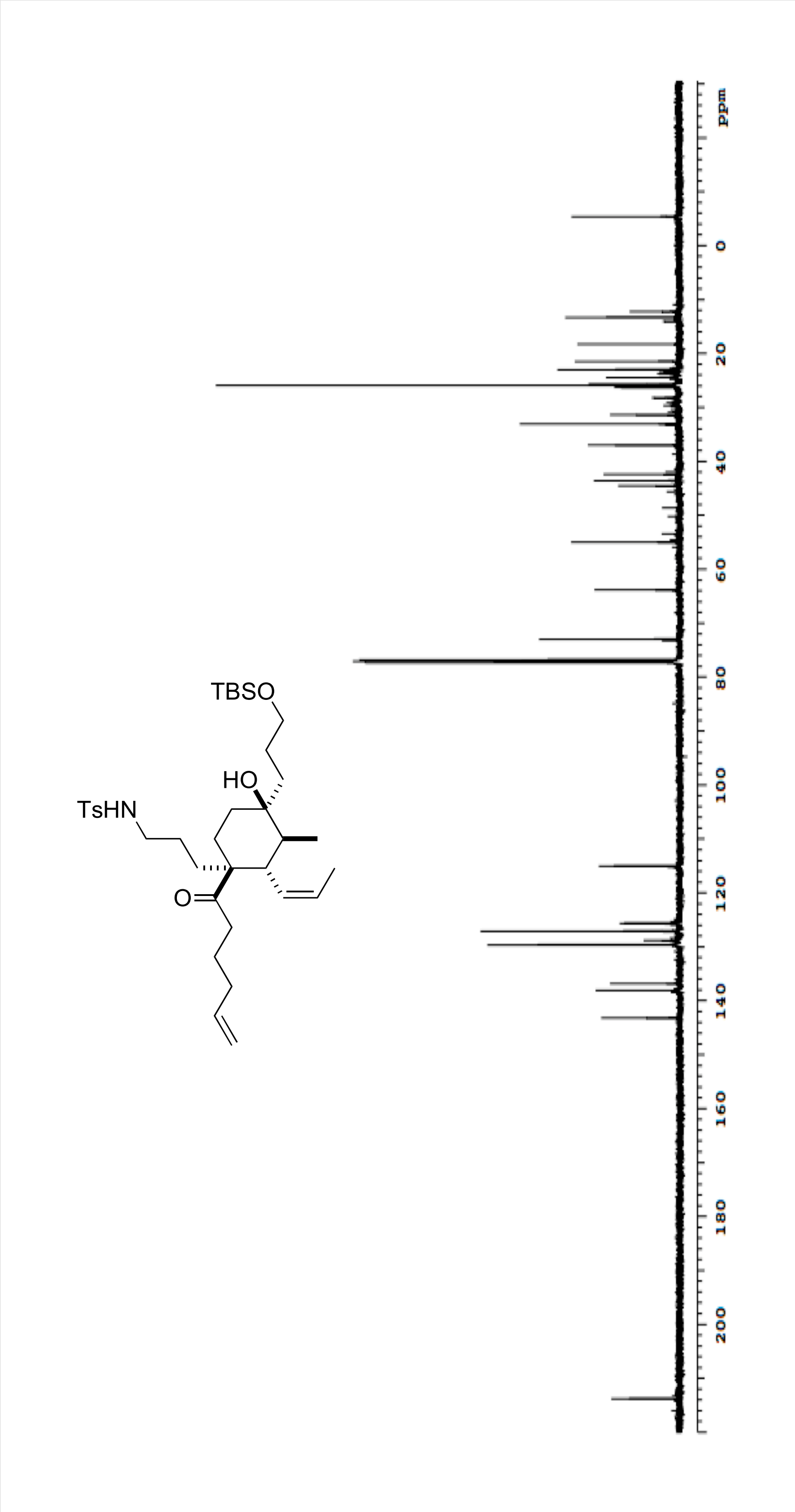
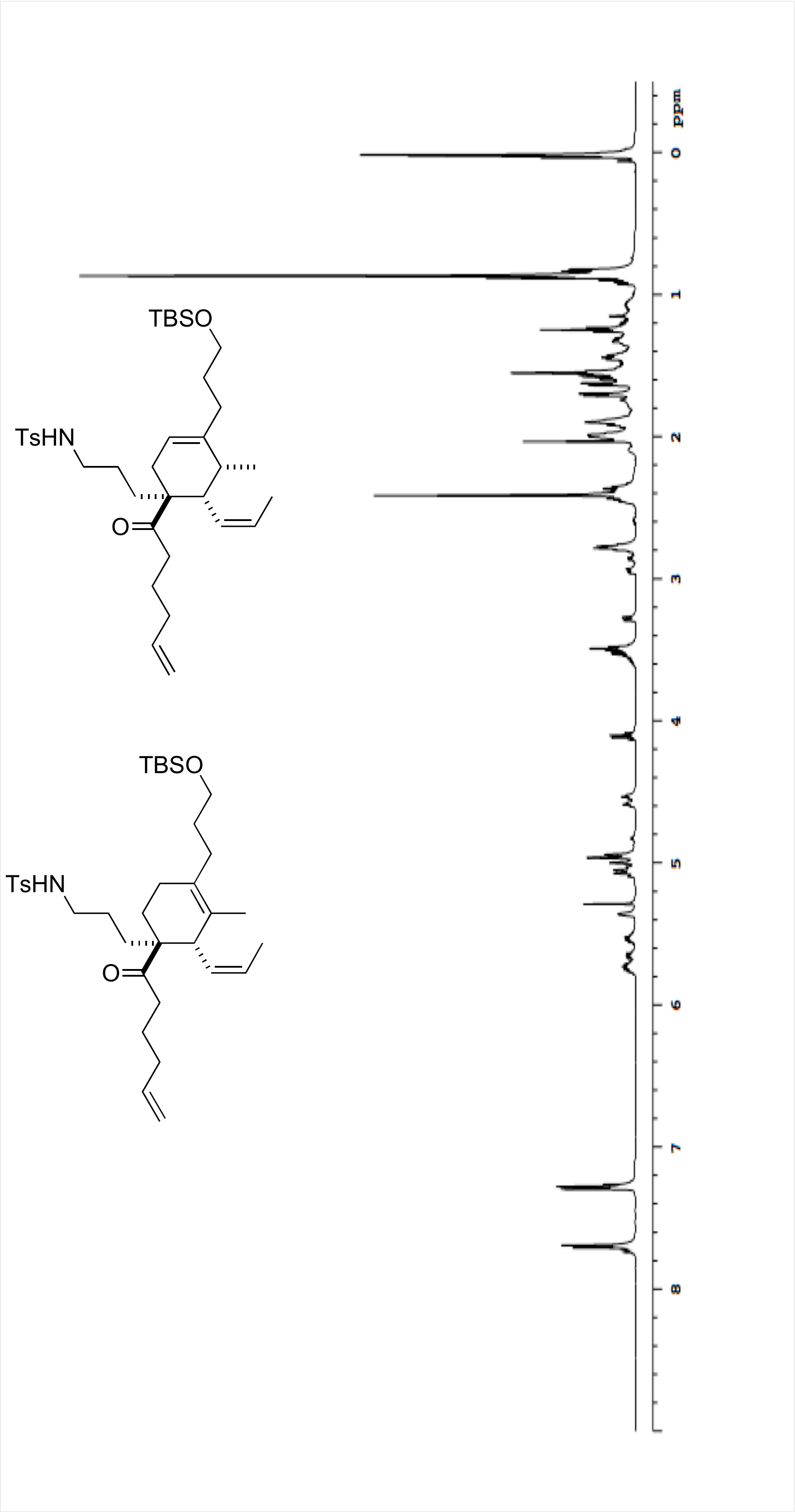


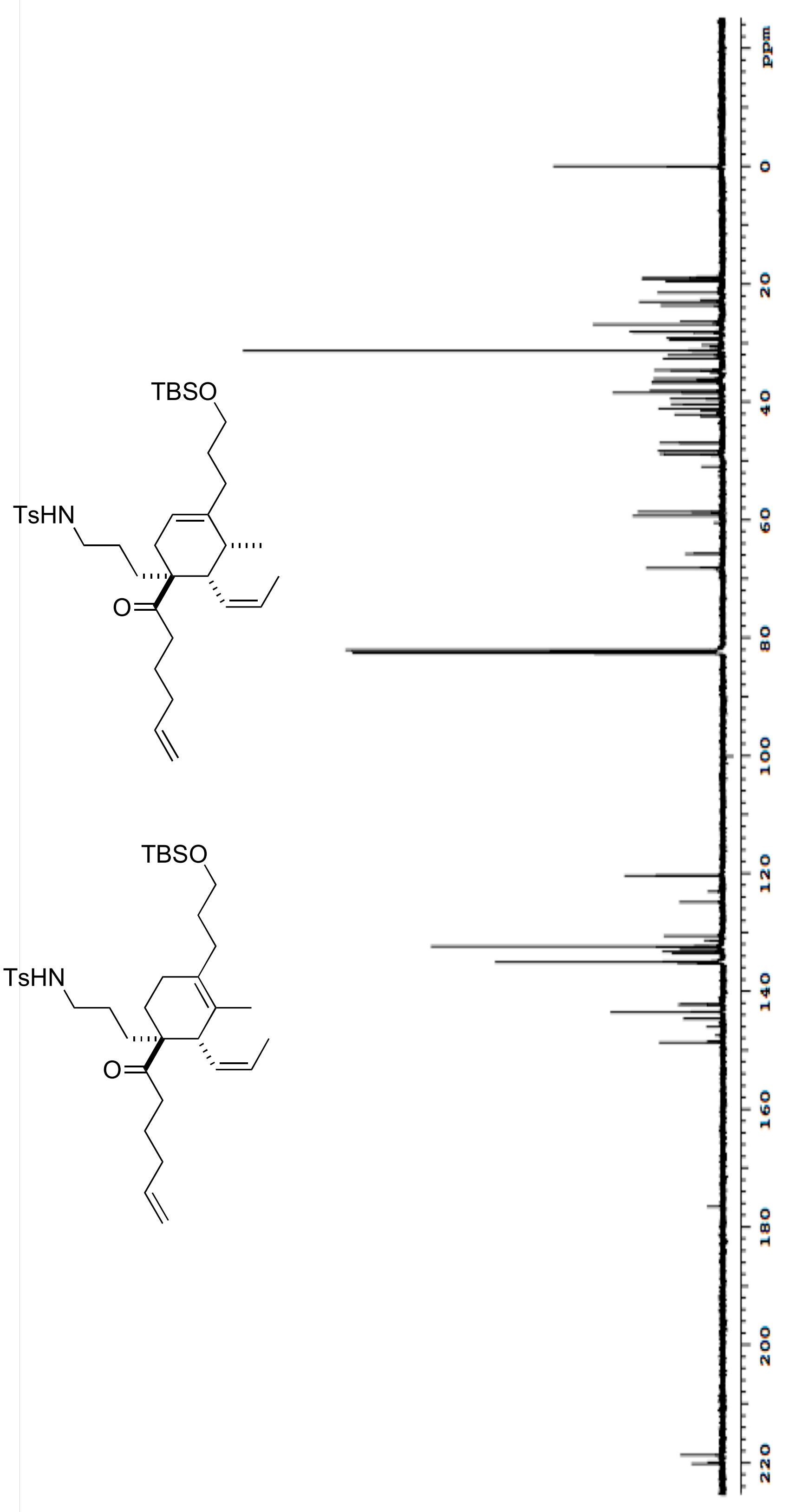
$^1$H-NMR (500 MHz) of **2-82b** in $CDCl_3$



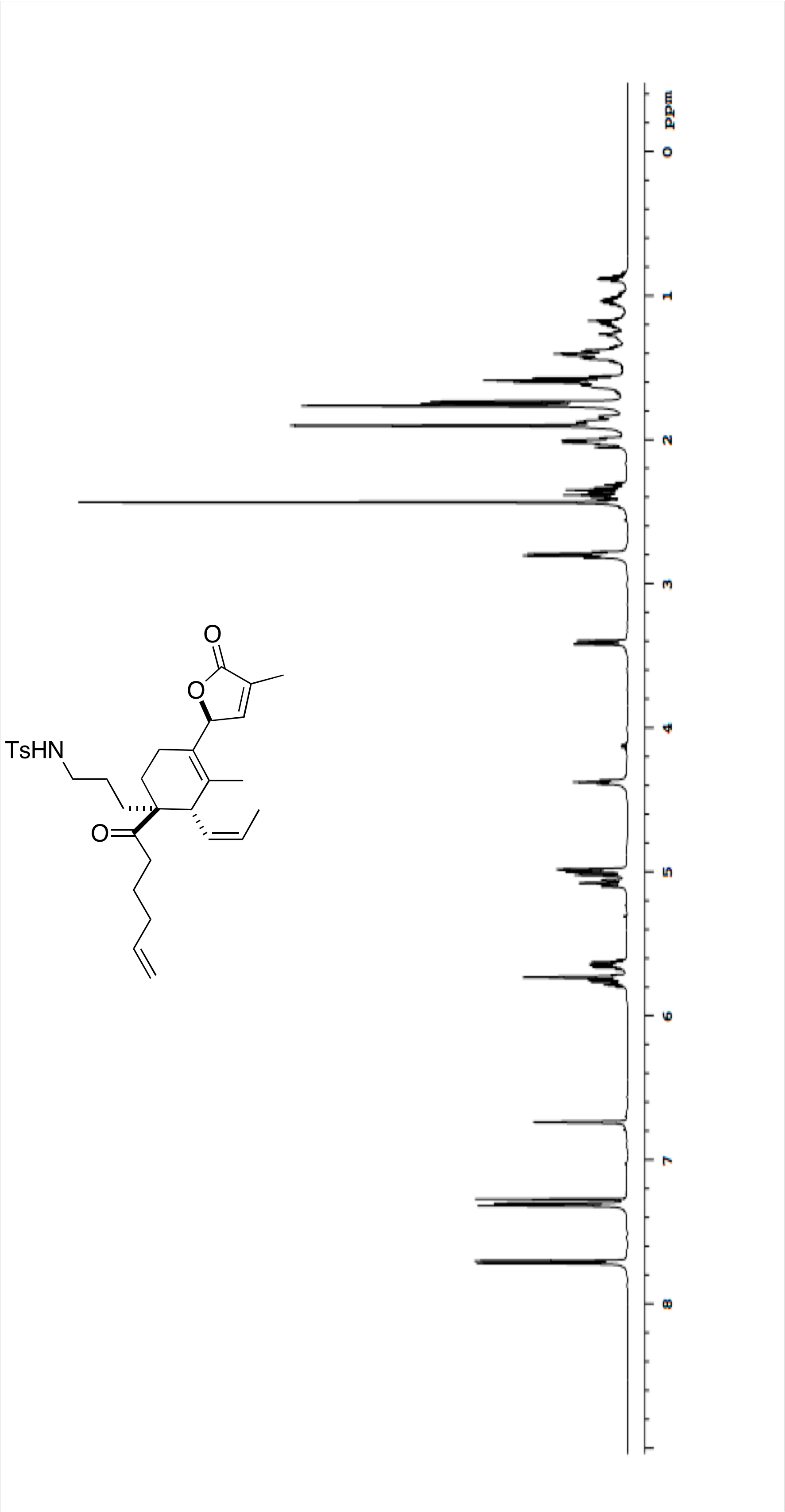
$^{13}$C-NMR (125 MHz) of **2-82b** in $CDCl_3$



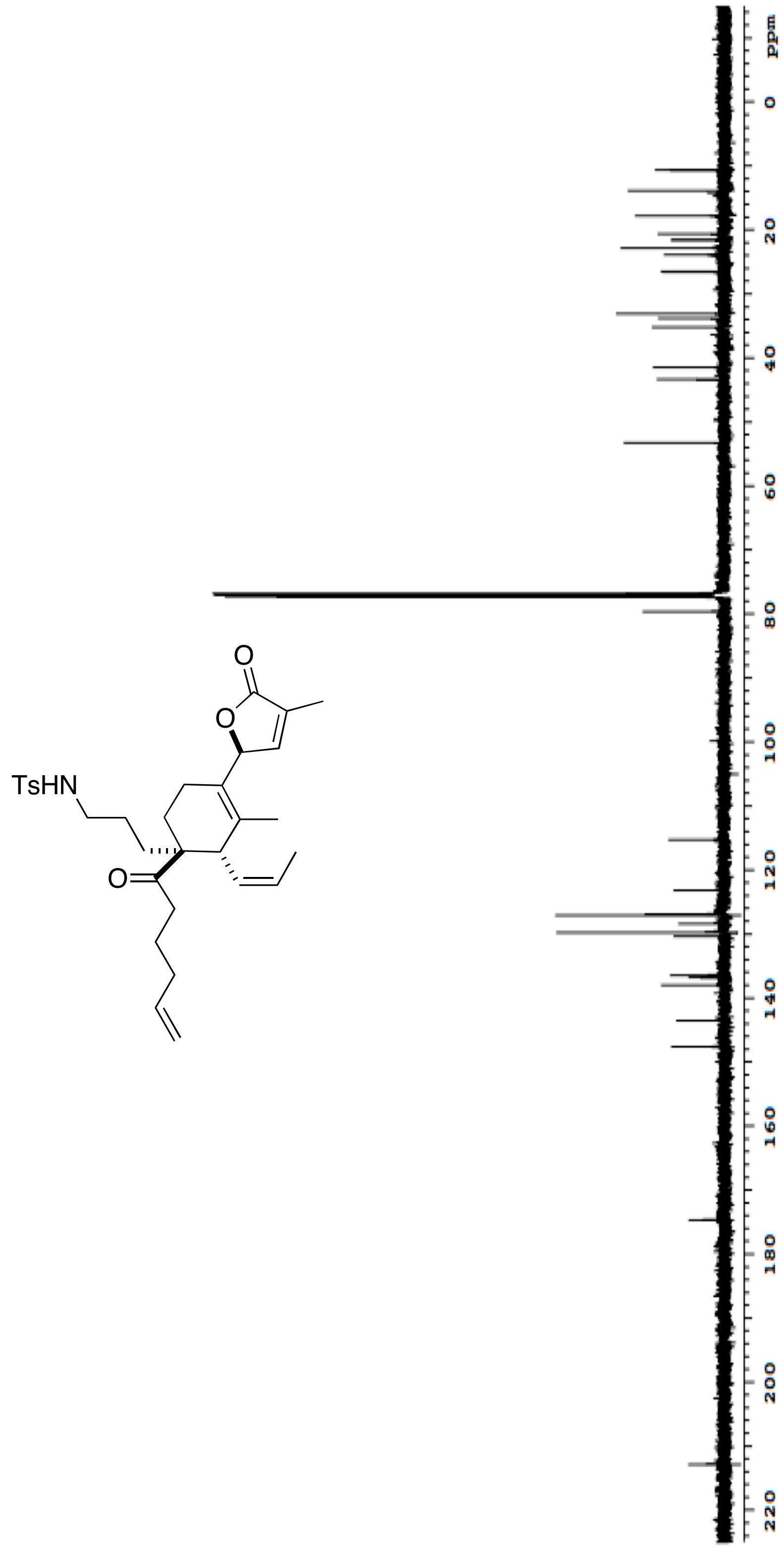
$^1$H-NMR (500 MHz) of **2-83** in $CDCl_3$



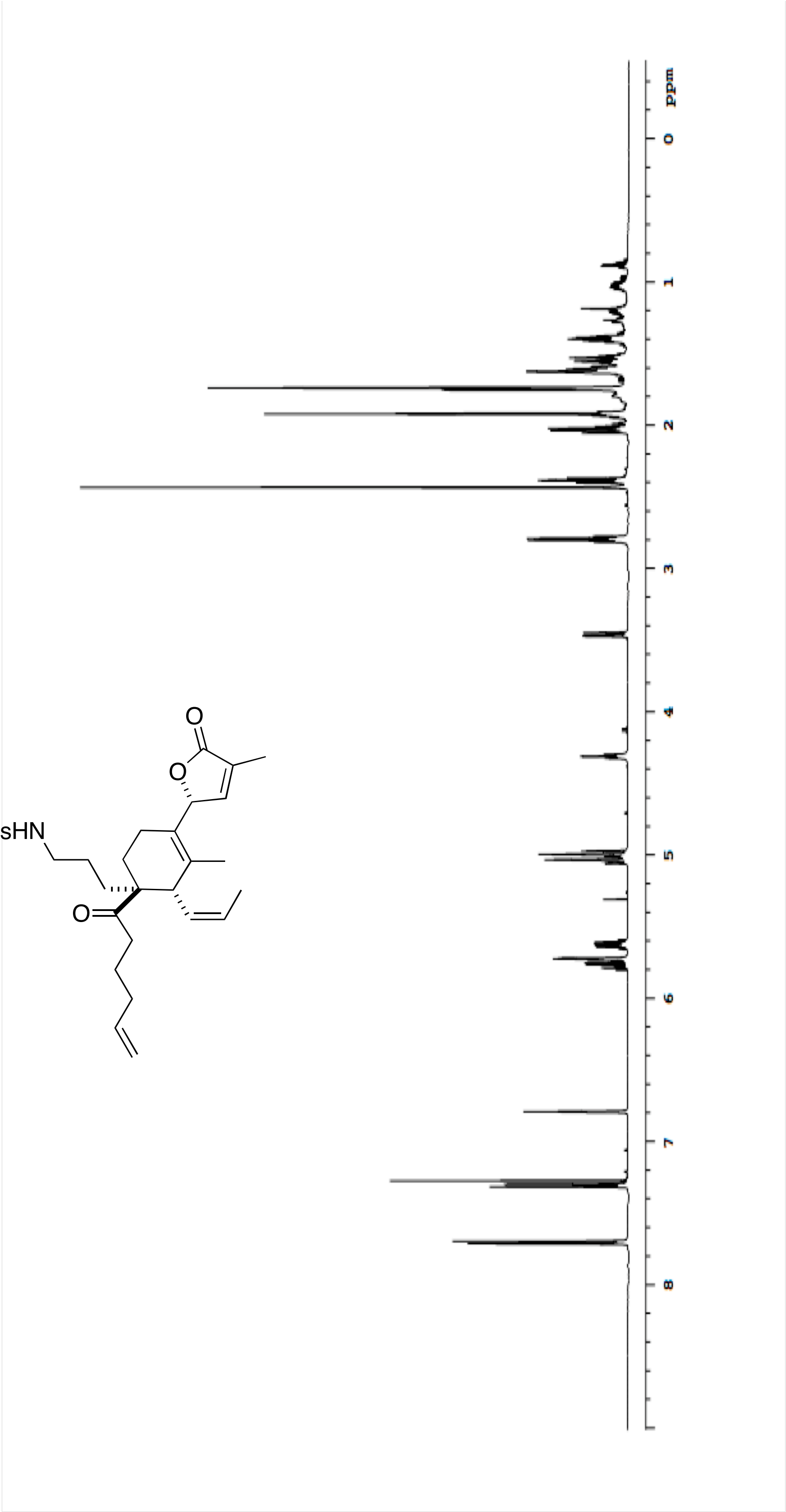
$^{13}$C-NMR (125 MHz) of **2-83** in $CDCl_3$



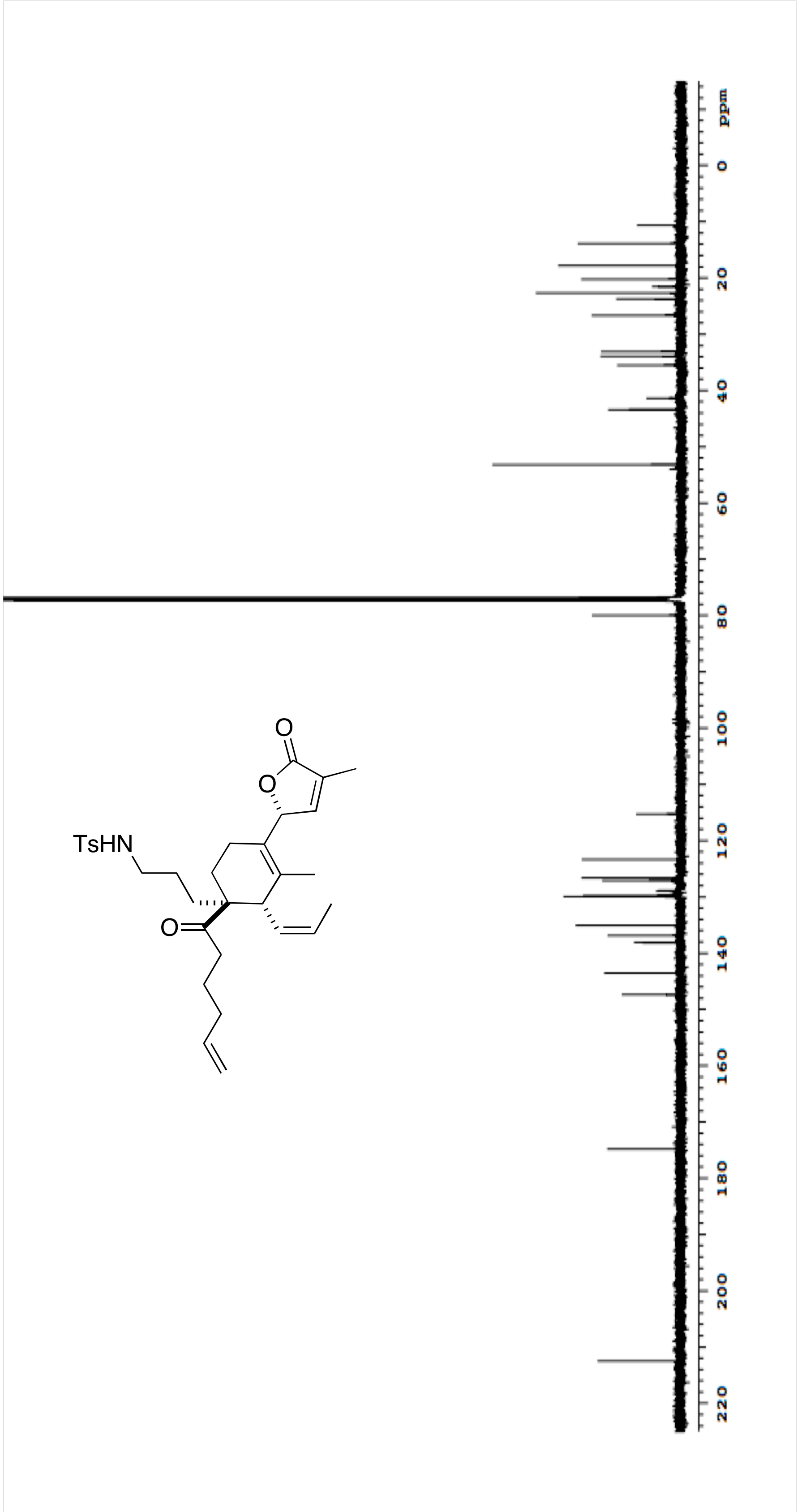
$^1$H-NMR (500 MHz) of **2-96a** in $CDCl_3$



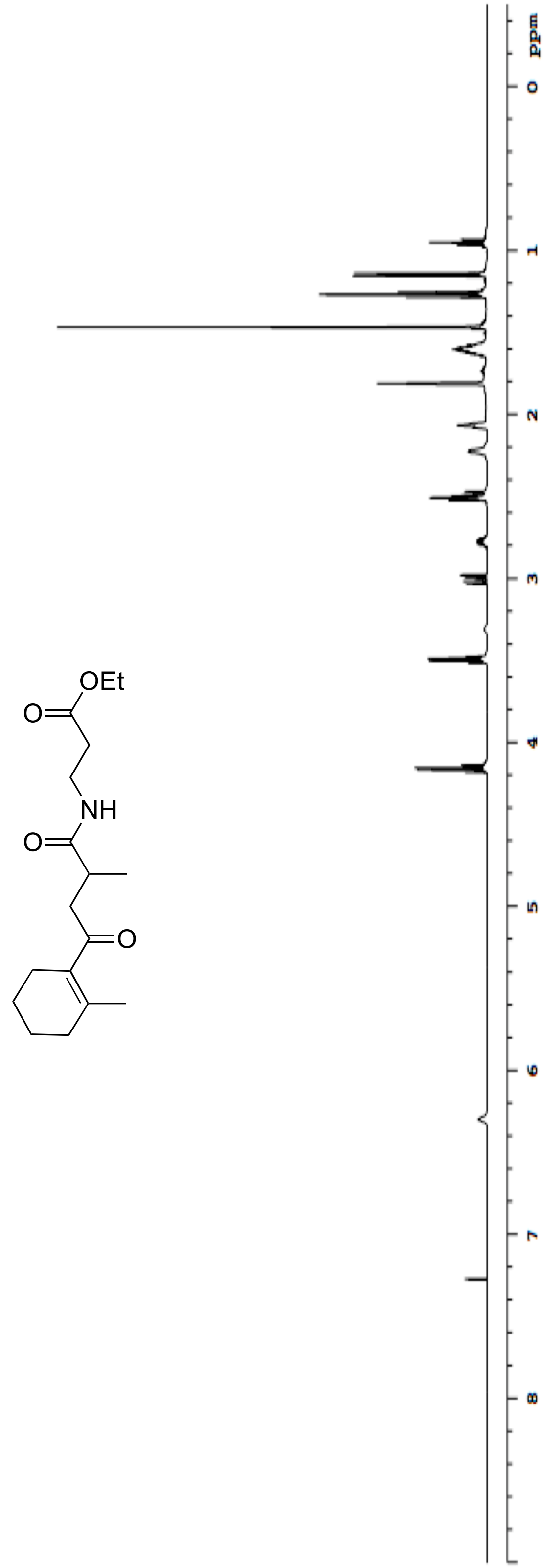
$^{13}$C-NMR (125 MHz) of **2-96a** in CDCl$_3$



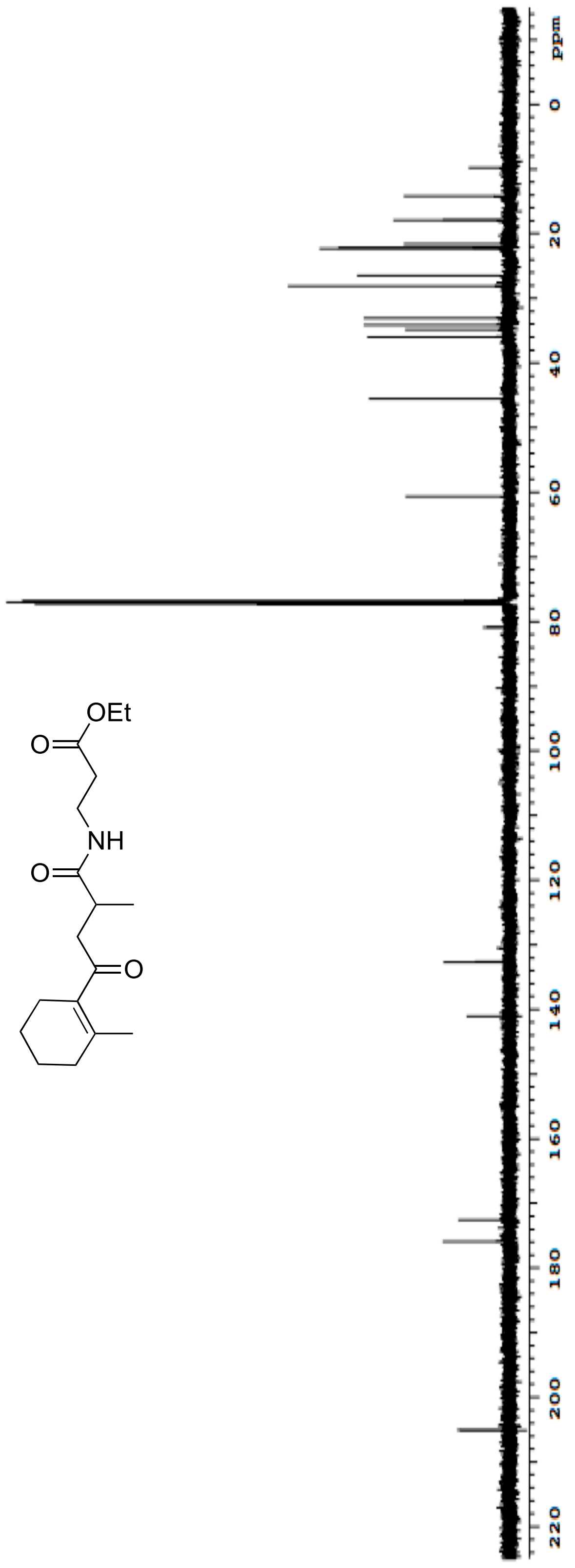
$^1$H-NMR (500 MHz) of **2-96b** in $CDCl_3$



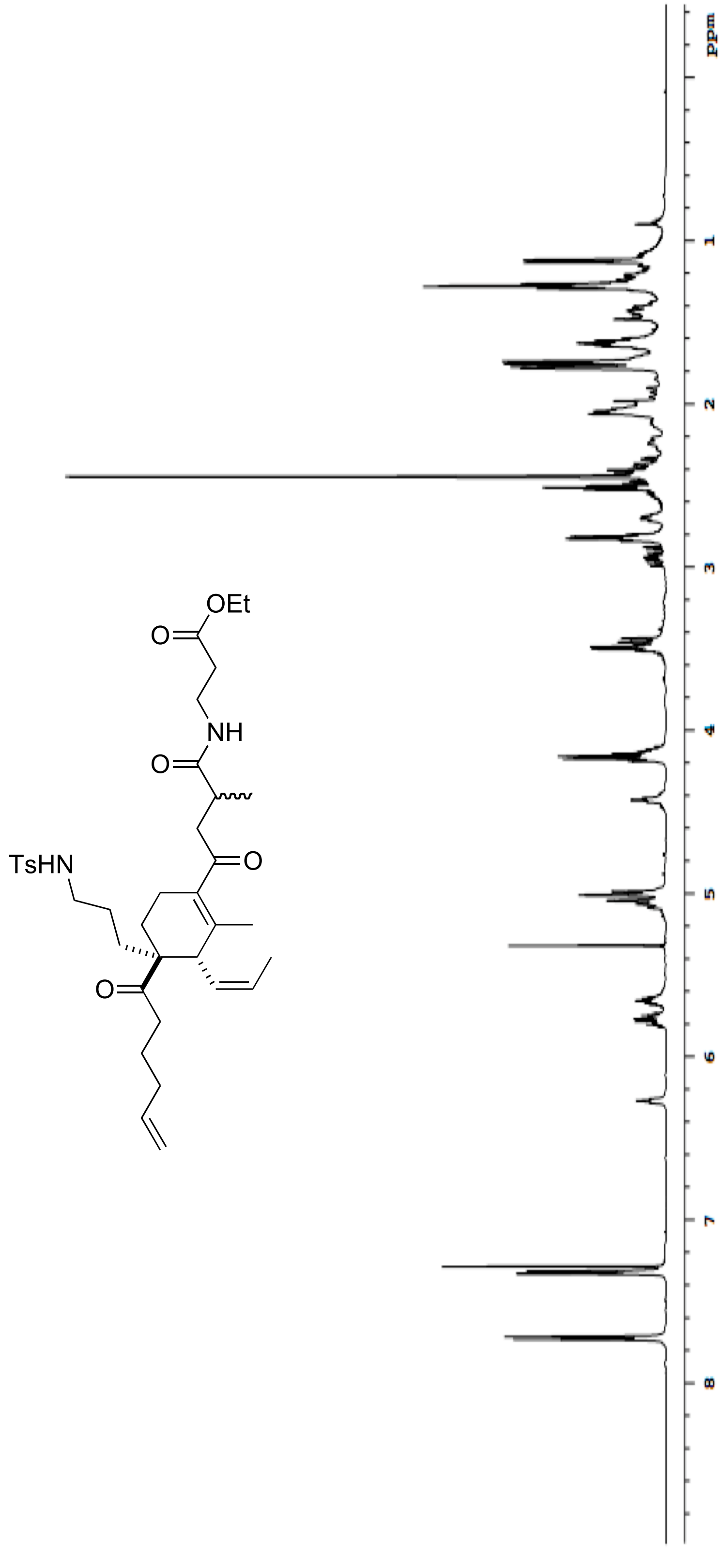
$^{13}$C-NMR (125 MHz) of **2-96**b in $CDCl_3$



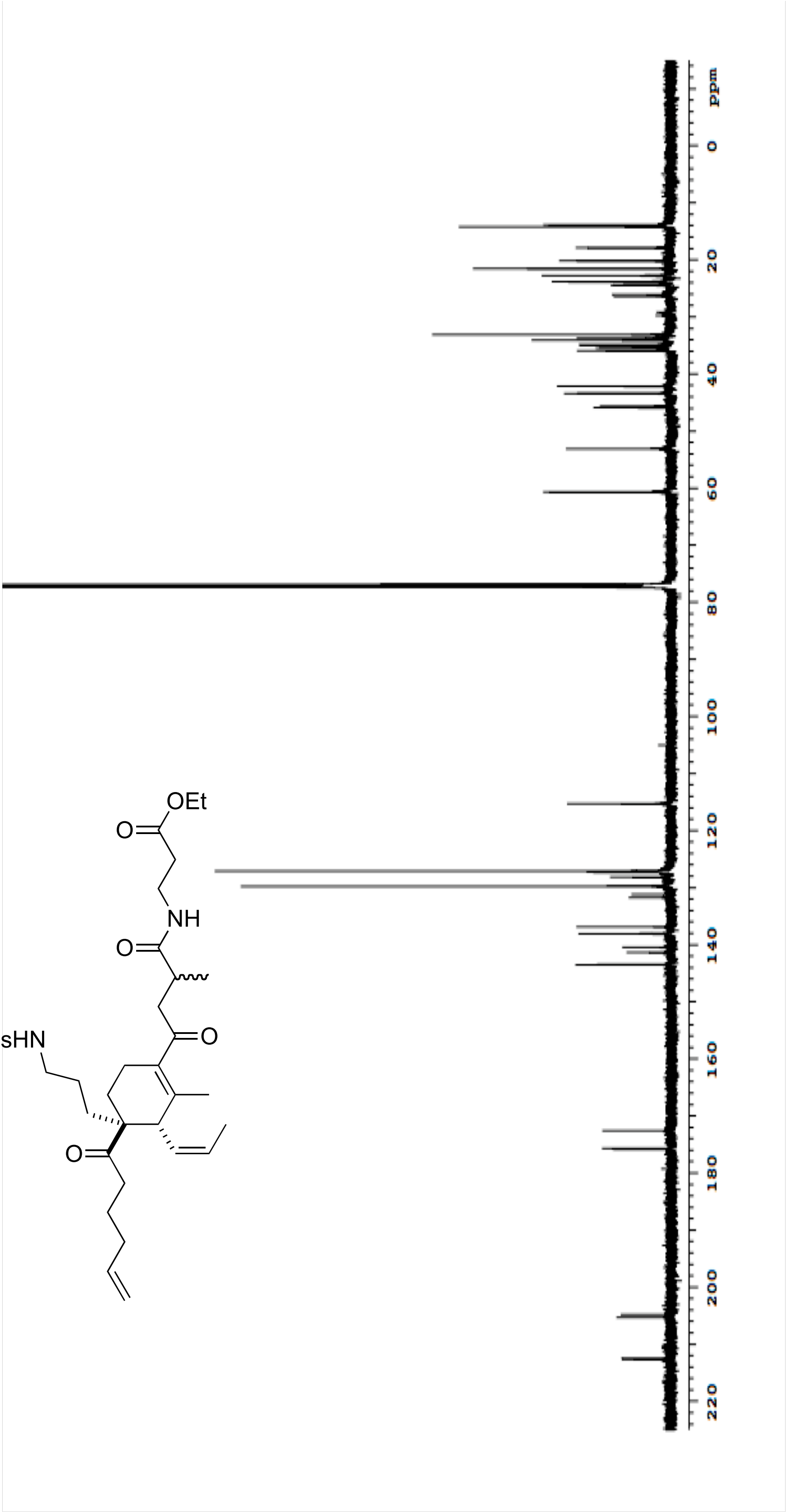
$^1$H-NMR (500 MHz) of **2-101** in CDCl$_3$



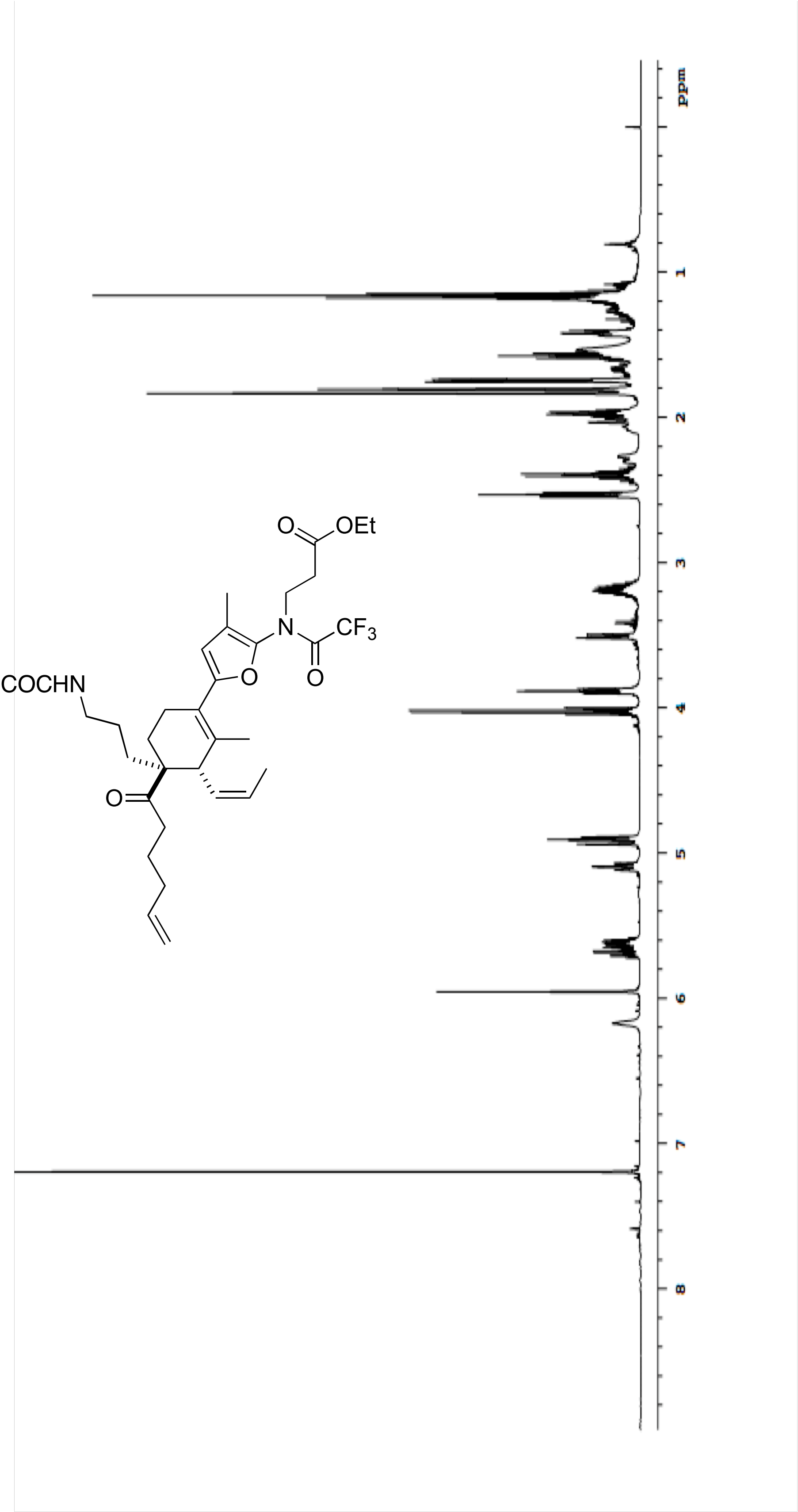
$^{13}$C-NMR (125 MHz) of **2-101** in $CDCl_3$



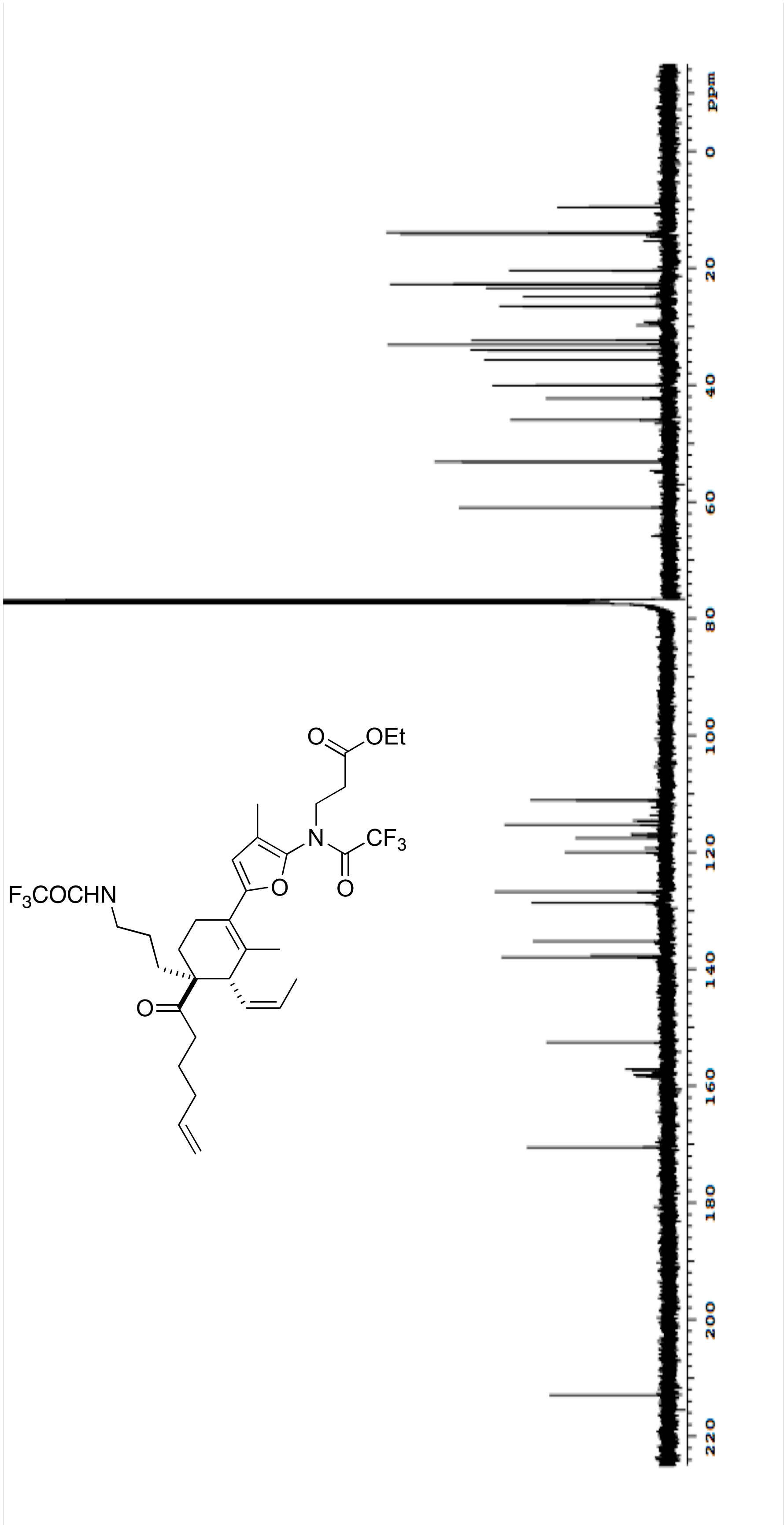
$^1$H-NMR (500 MHz) of **2-103** in $CDCl_3$



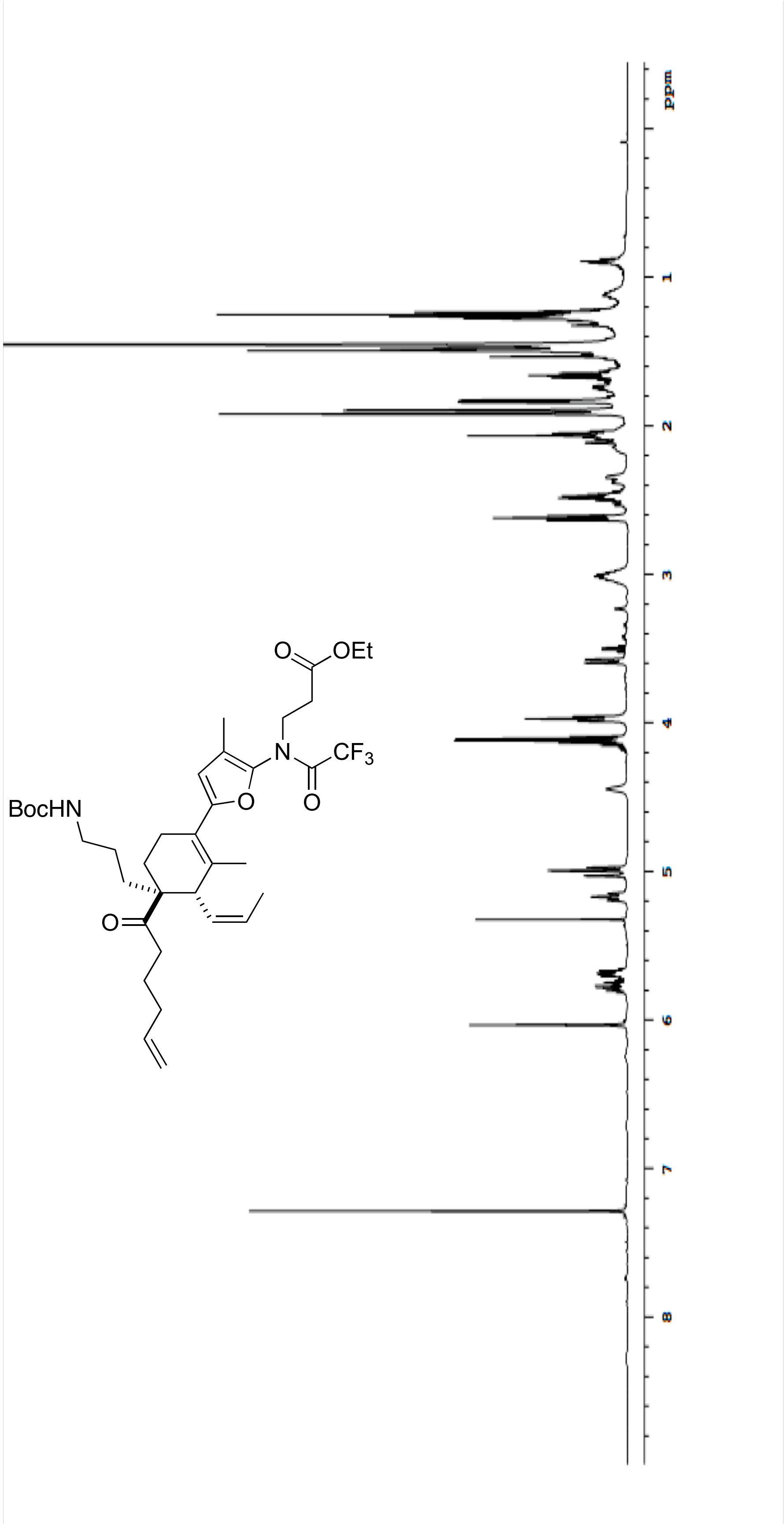
$^{13}$C-NMR (125 MHz) of **2-103** in $CDCl_3$



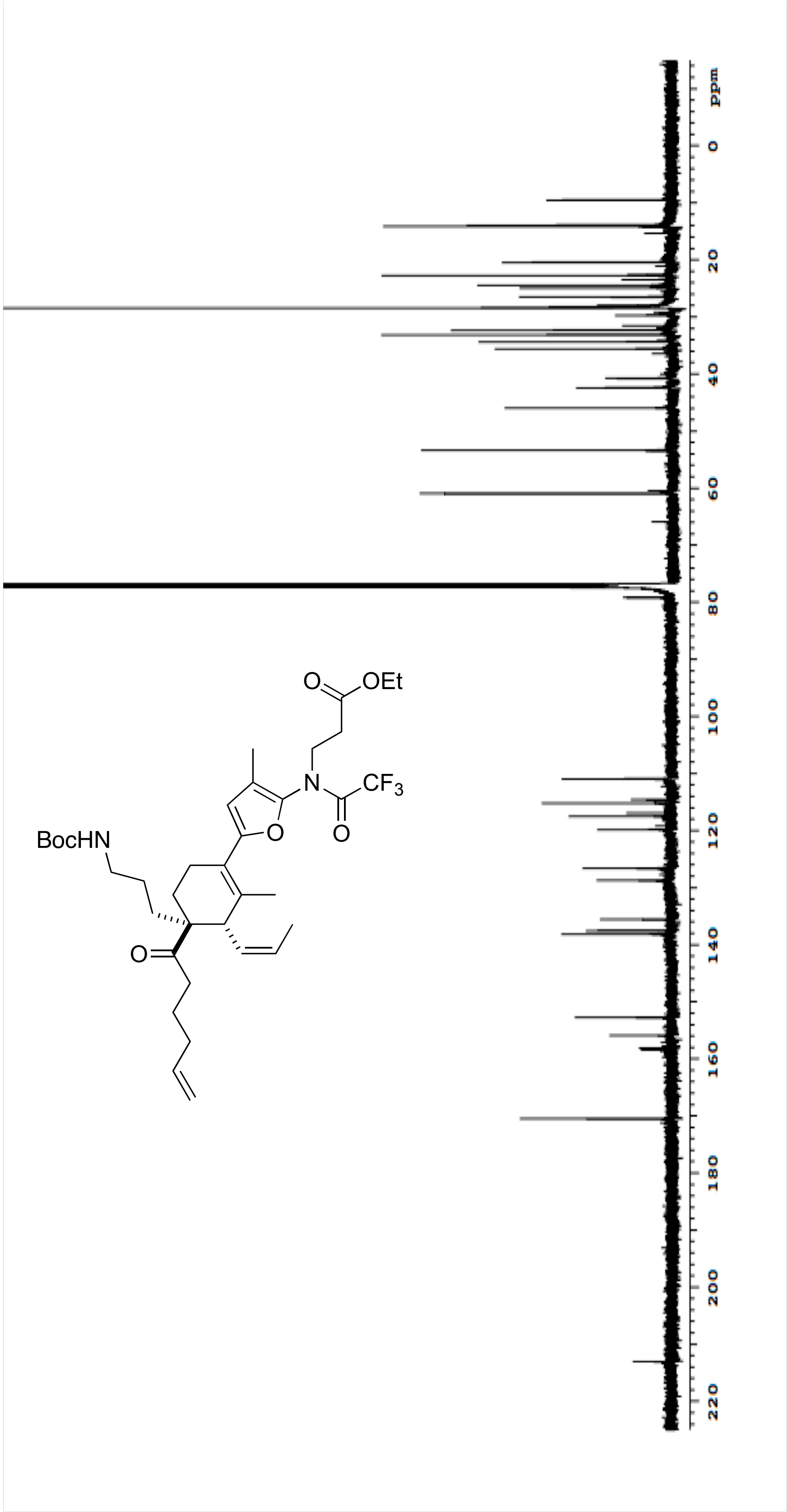
$^1$H-NMR (500 MHz) of **2-104** in $CDCl_3$



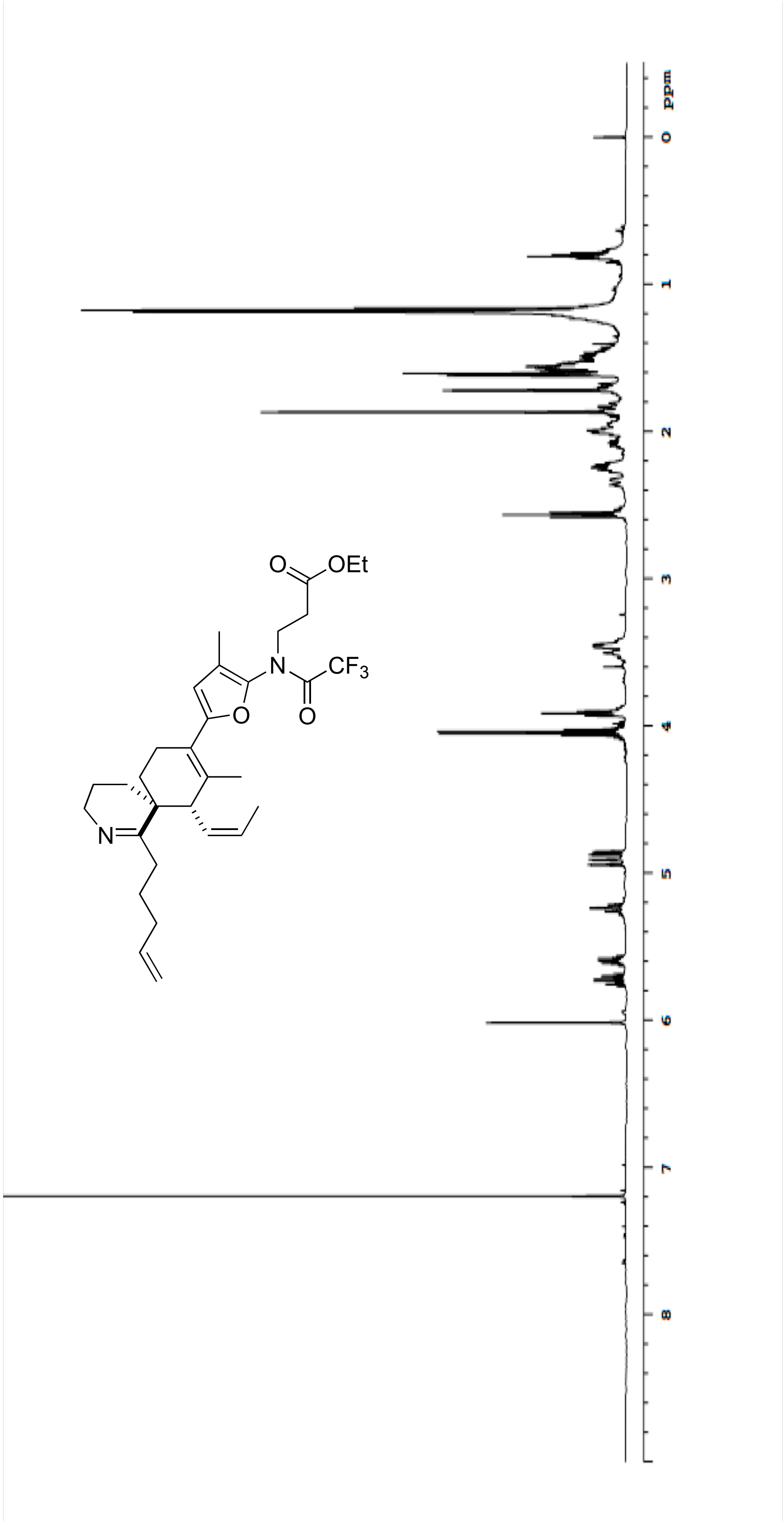
$^{13}$C-NMR (125 MHz) of **2-104** in CDCl$_3$



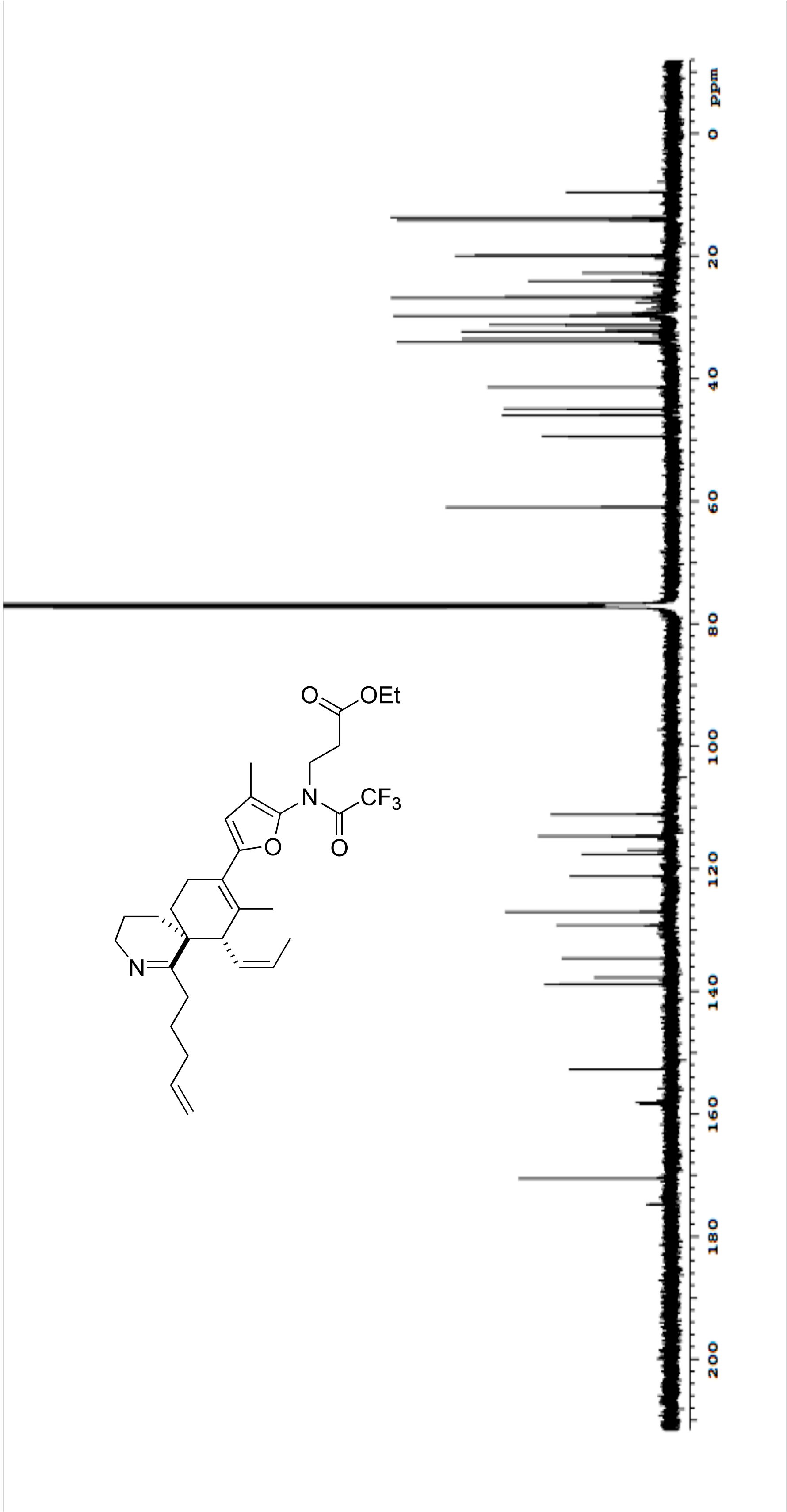
$^1$H-NMR (500 MHz) of **2-107** in $CDCl_3$



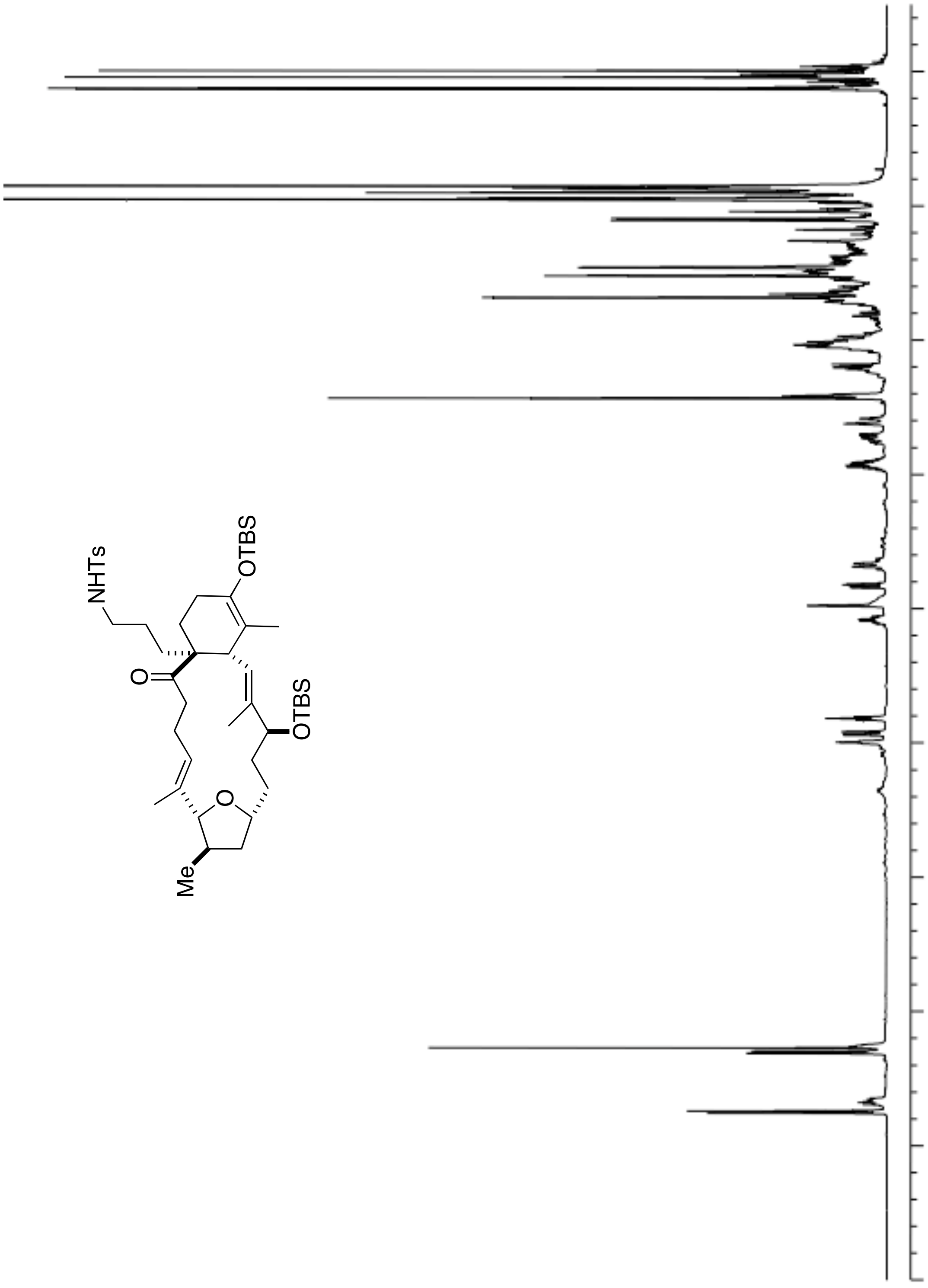
$^{13}$C-NMR (125 MHz) of **2-107** in $CDCl_3$



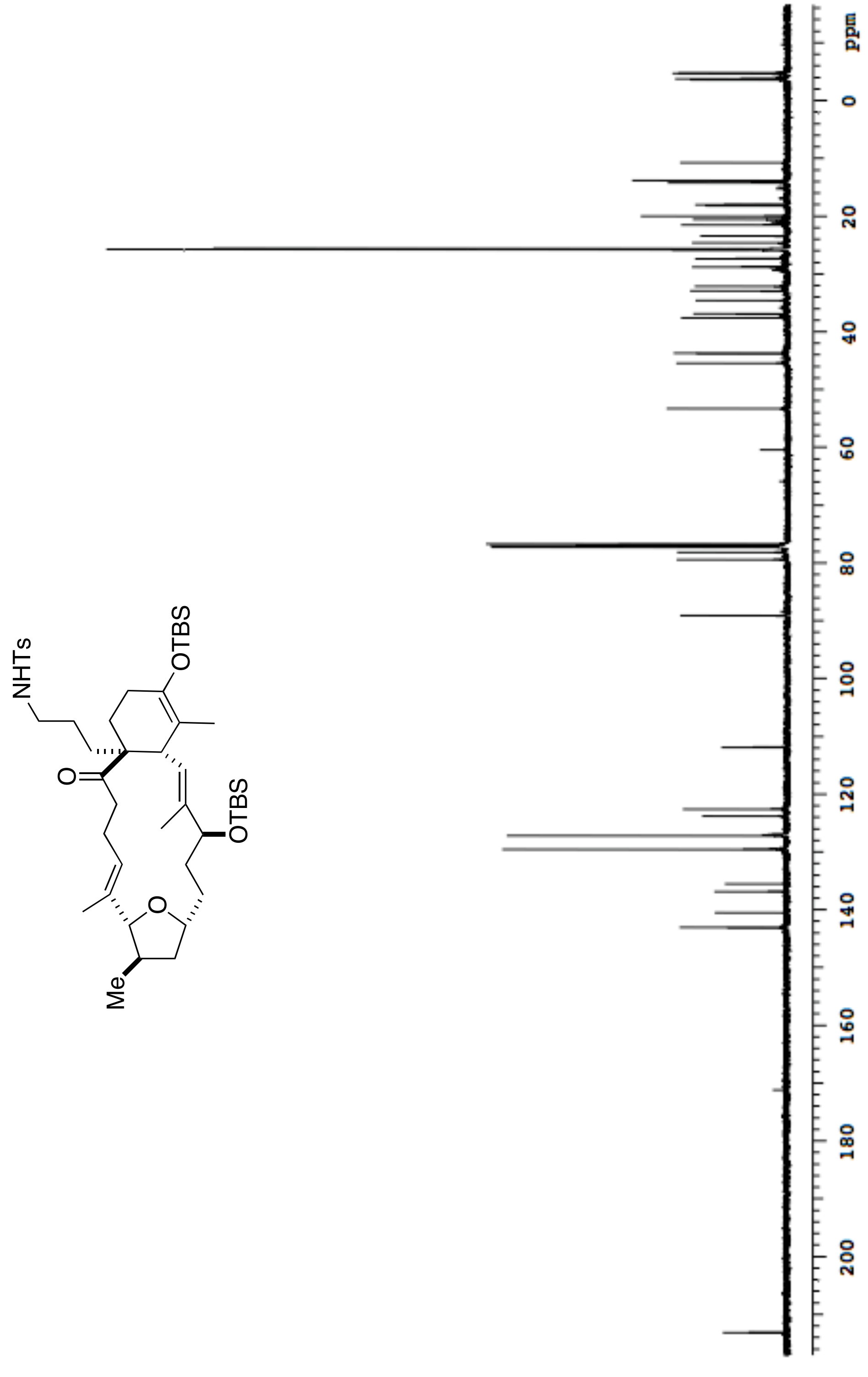
$^1$H-NMR (500 MHz) of **2-108** in $CDCl_3$



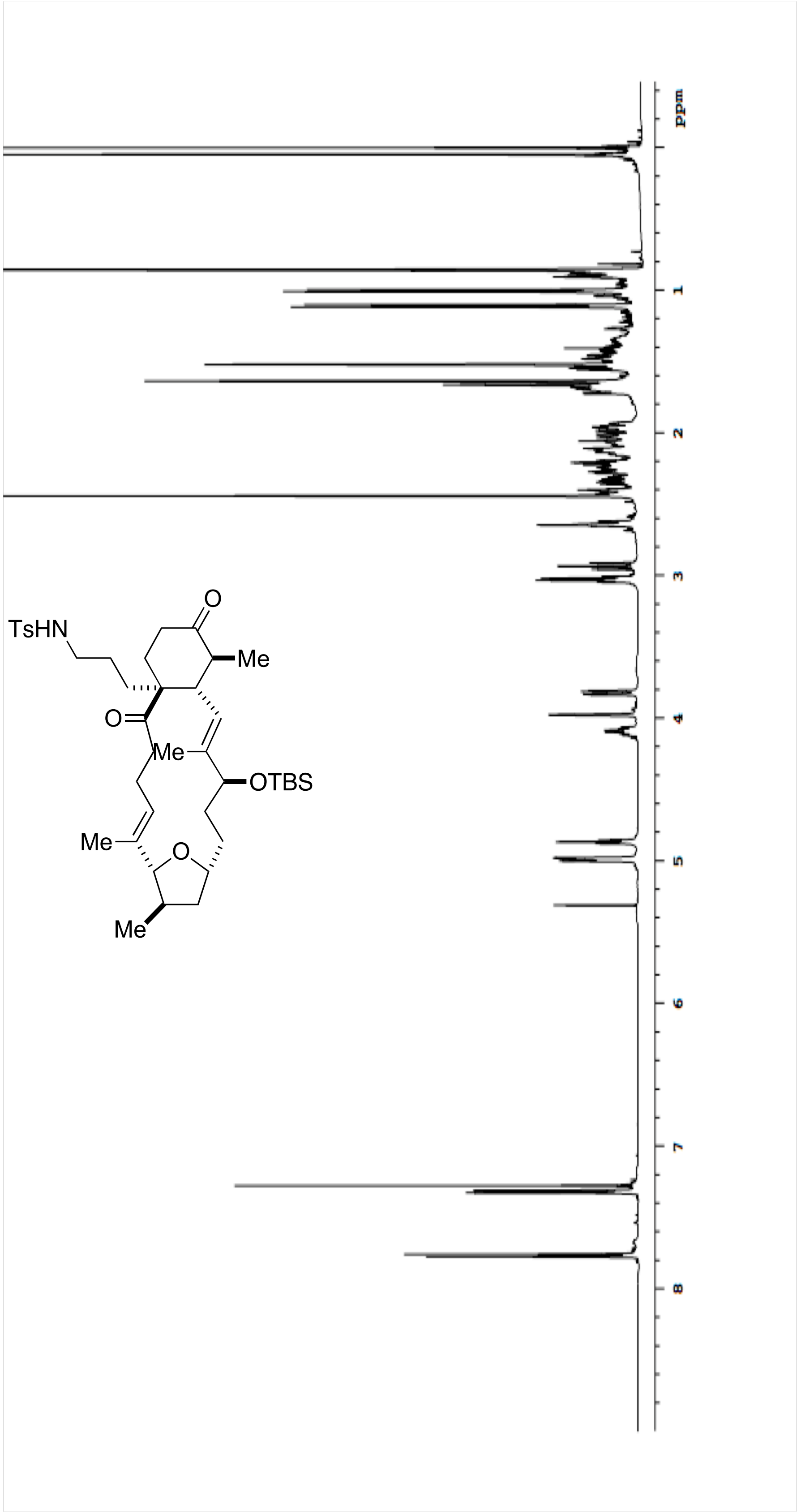
$^{13}$C-NMR (125 MHz) of **2-108** in $CDCl_3$



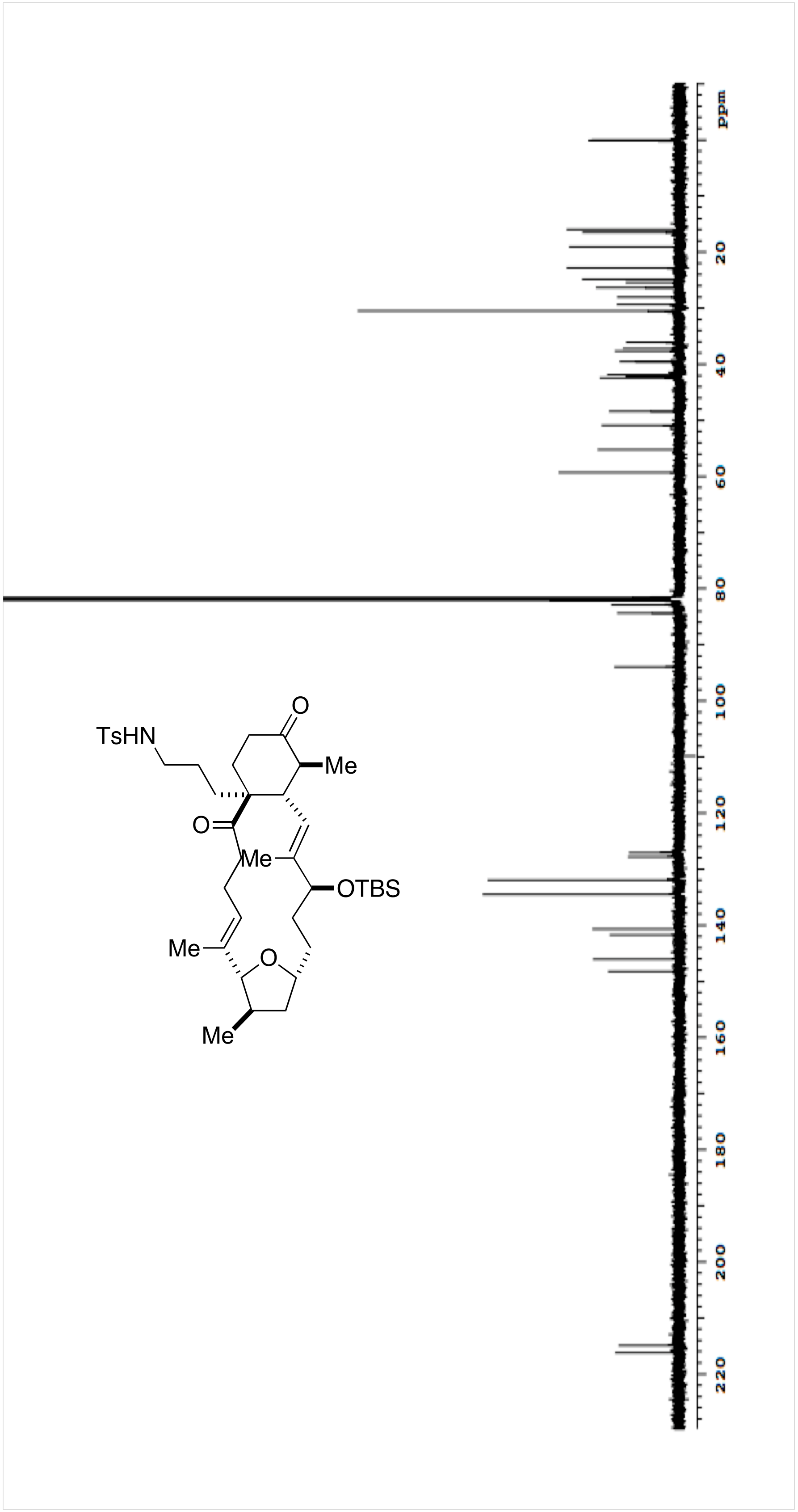
$^1$H-NMR (500 MHz) of **2-69** in $CDCl_3$



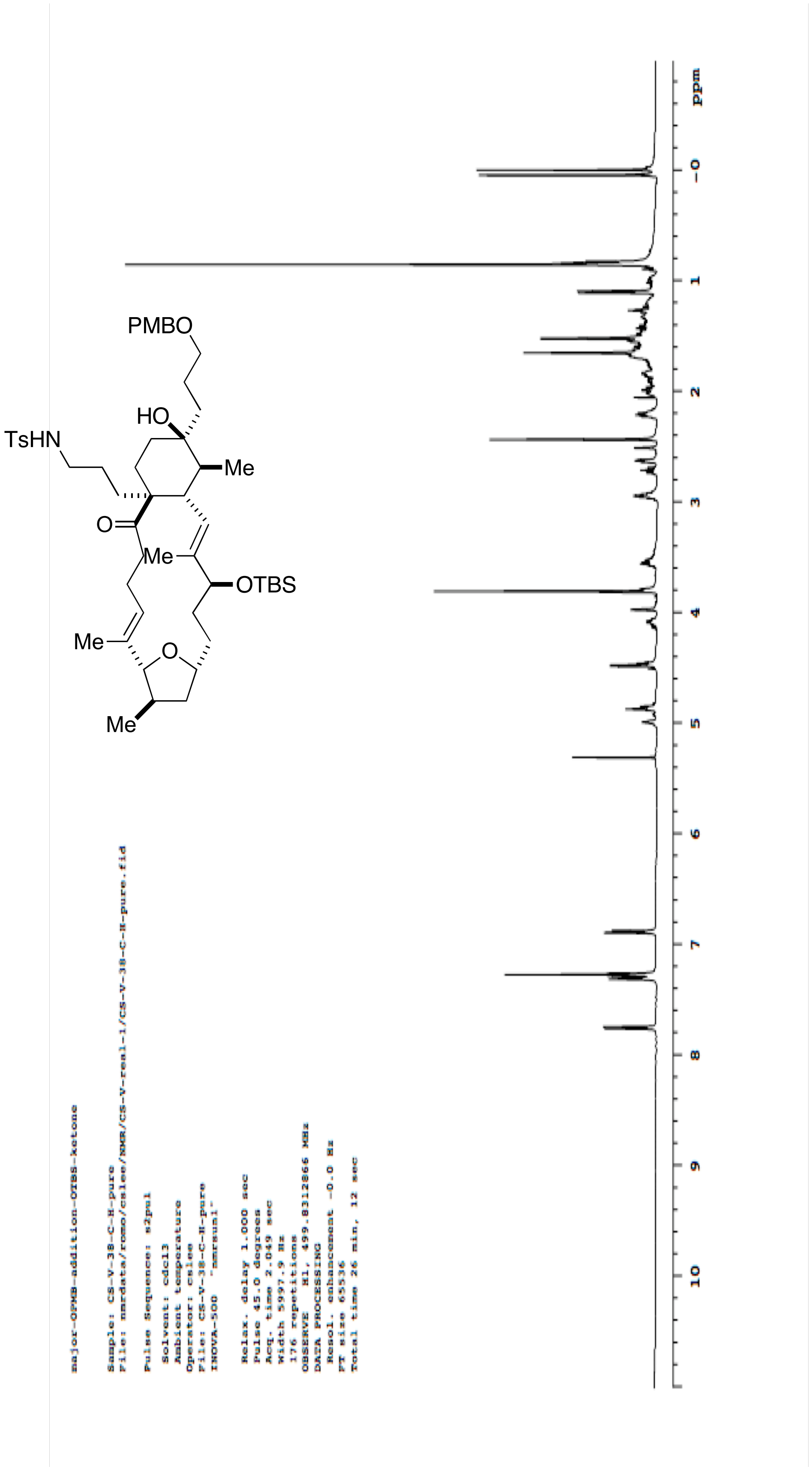
$^{13}$C-NMR (125 MHz) of **2-69** in $CDCl_3$



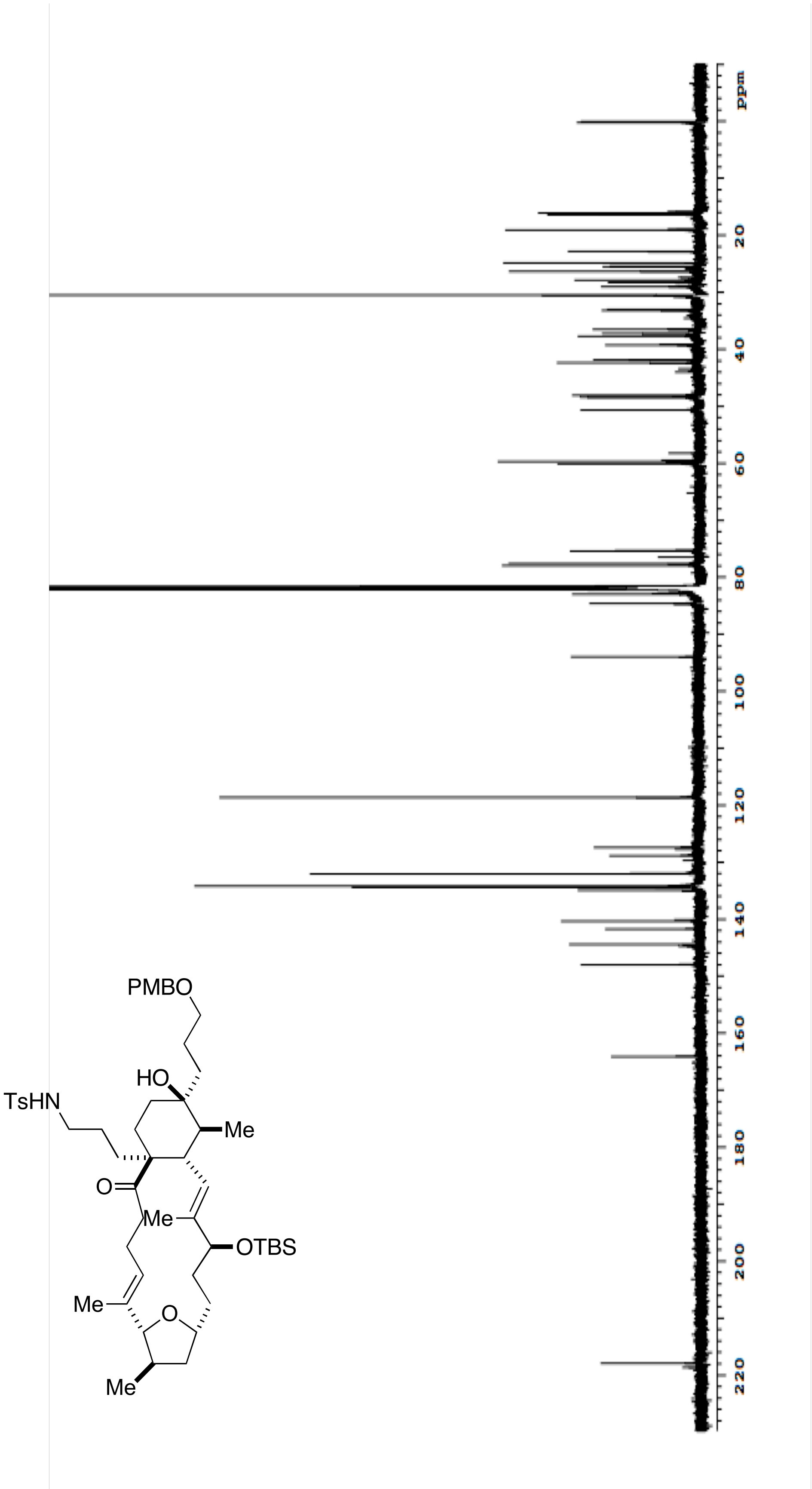
$^{1}$H-NMR (500 MHz) of **2-86** in $CDCl_3$



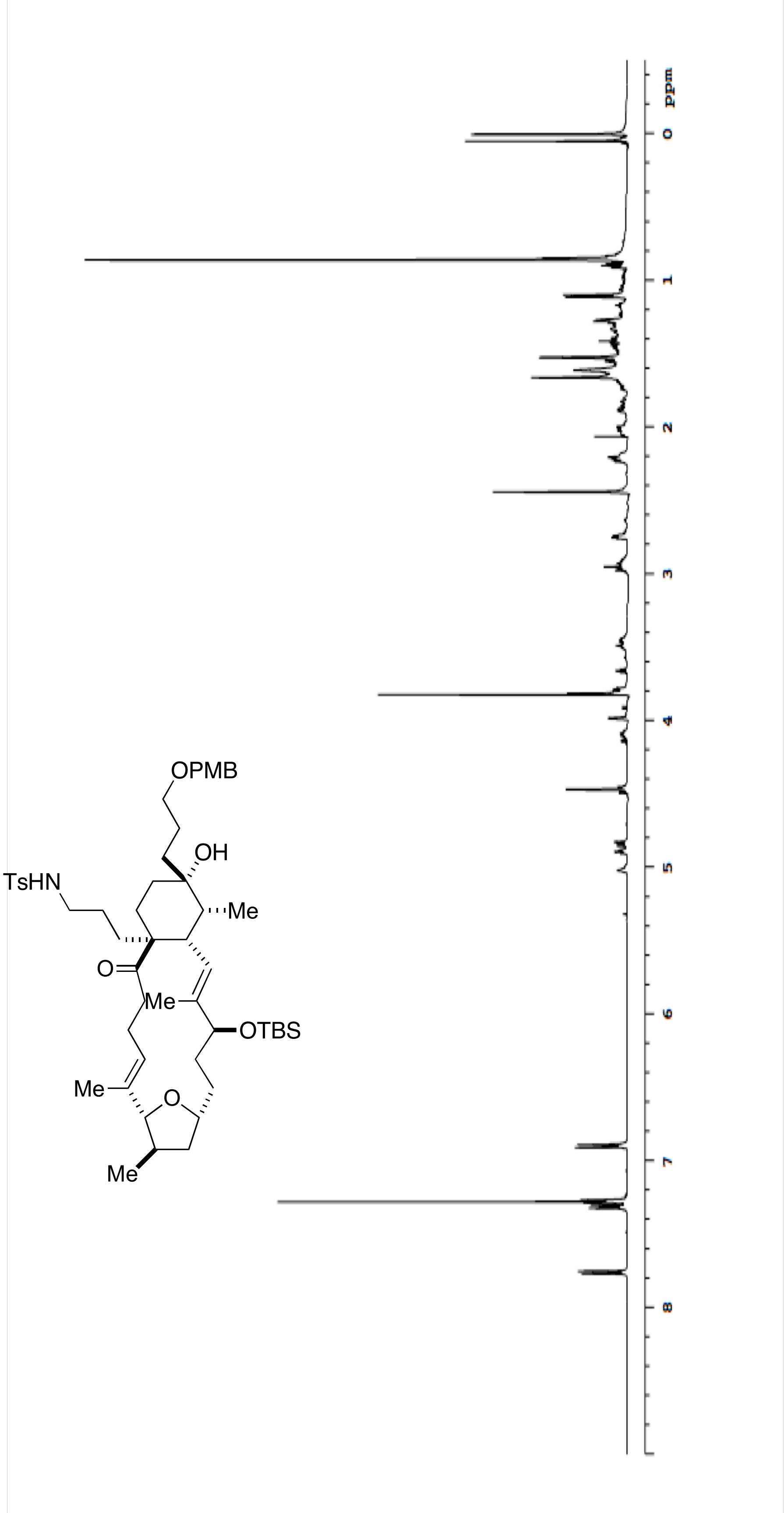
$^{13}$C-NMR (125 MHz) of **2-86** in $CDCl_3$



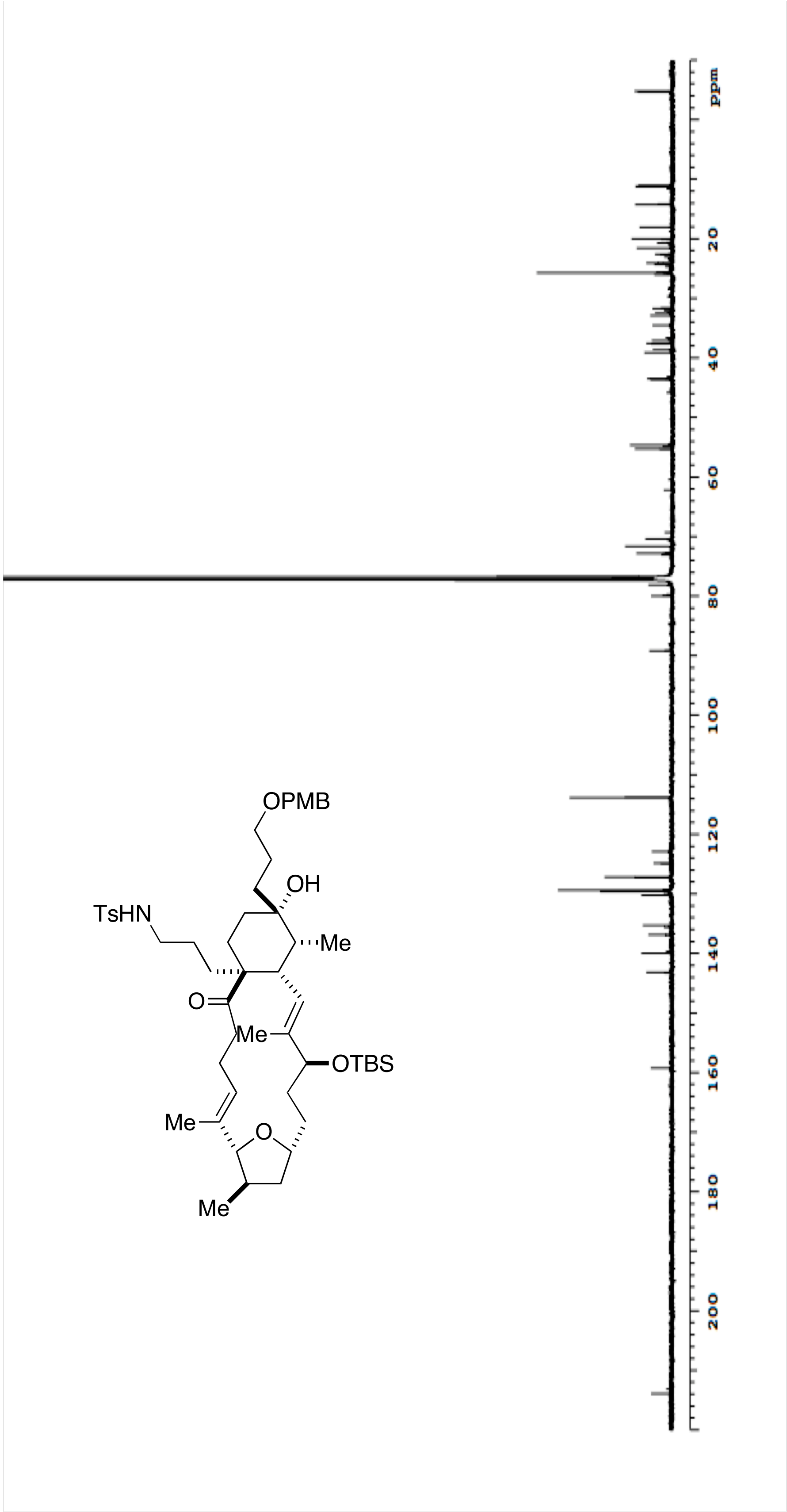
$^1$H-NMR (500 MHz) of **2-89a** in CDCl$_3$



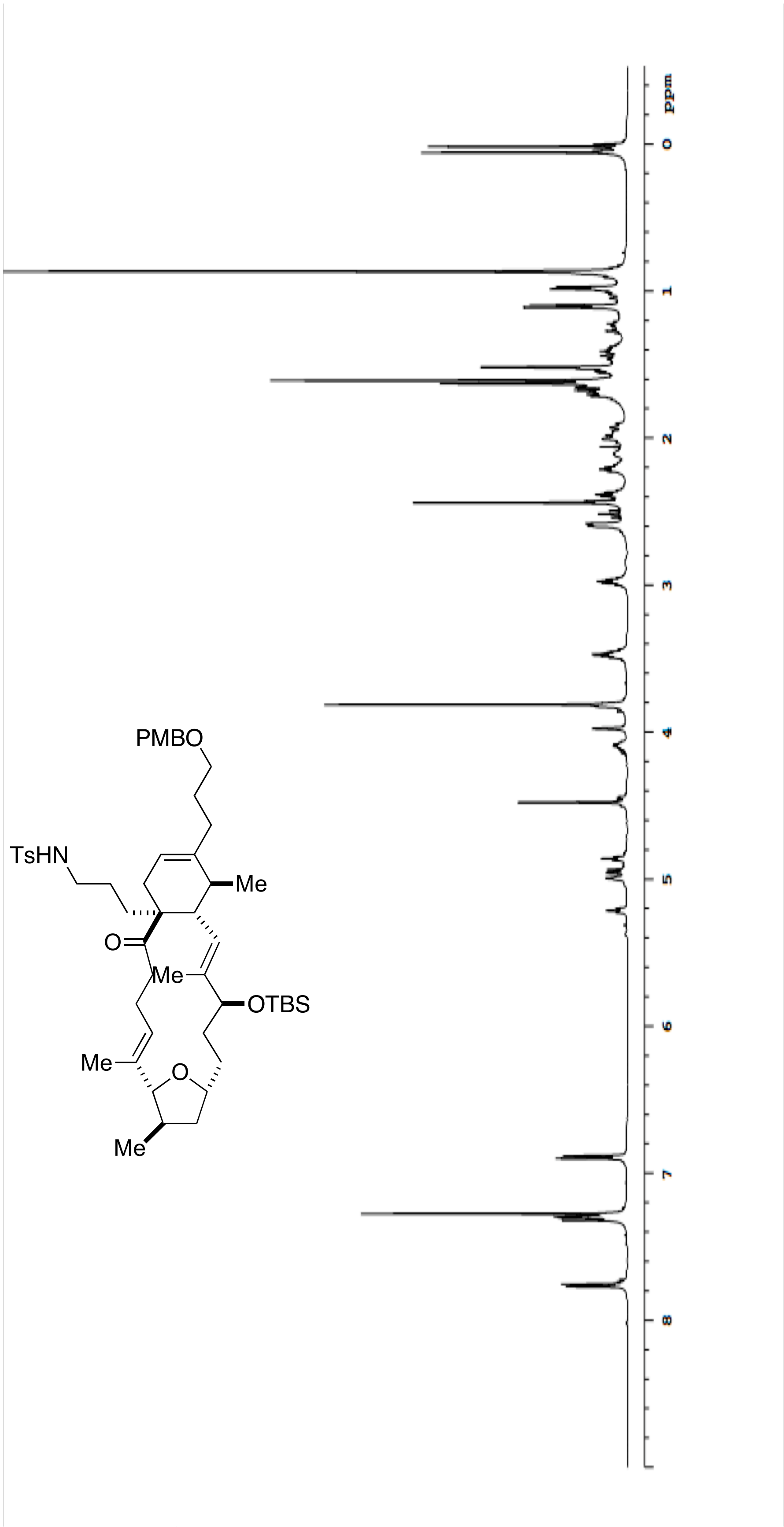
$^{13}$C-NMR (500 MHz) of **2-89a** in CDCl$_3$



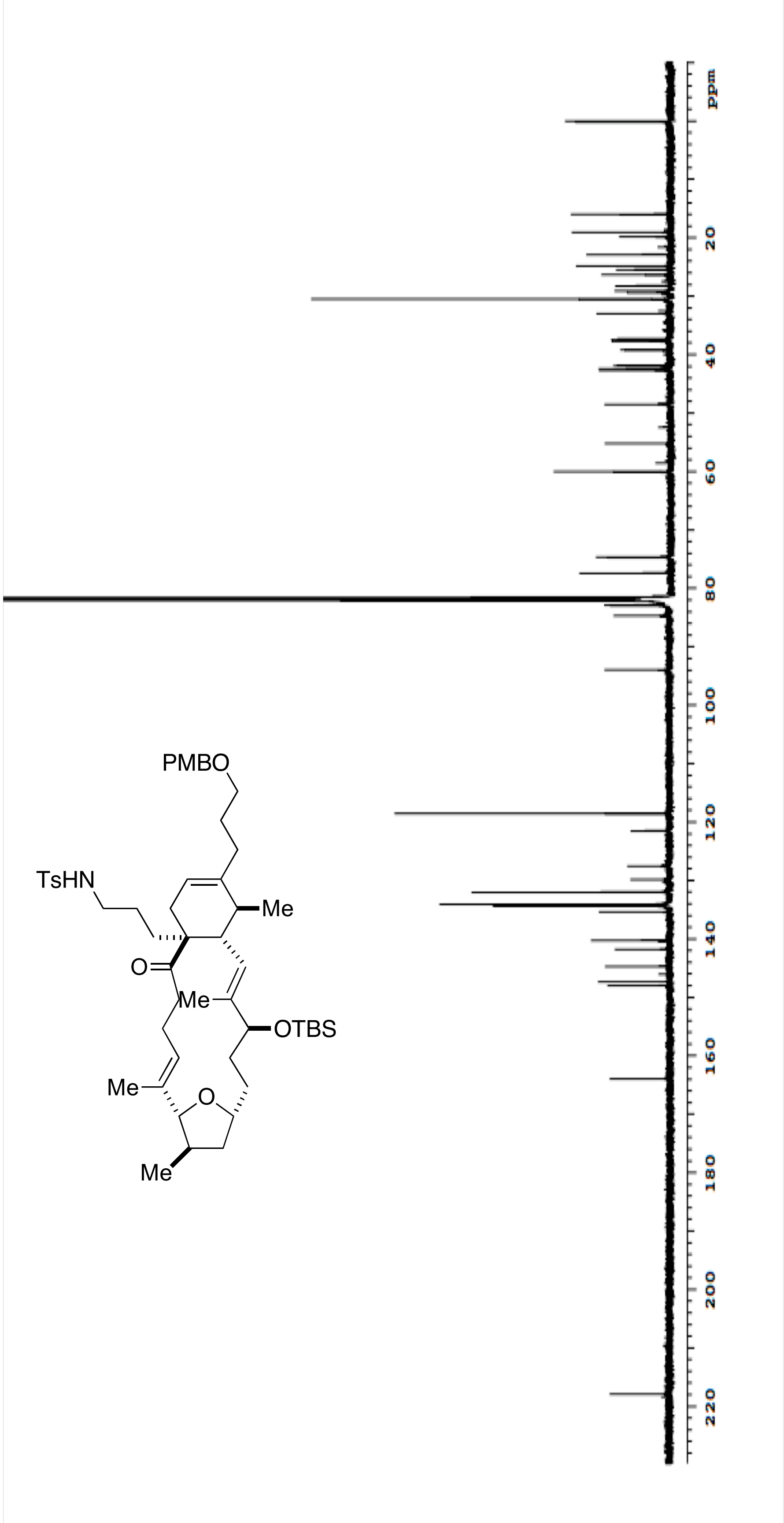
$^1$H-NMR (500 MHz) of **2-89c** in $CDCl_3$



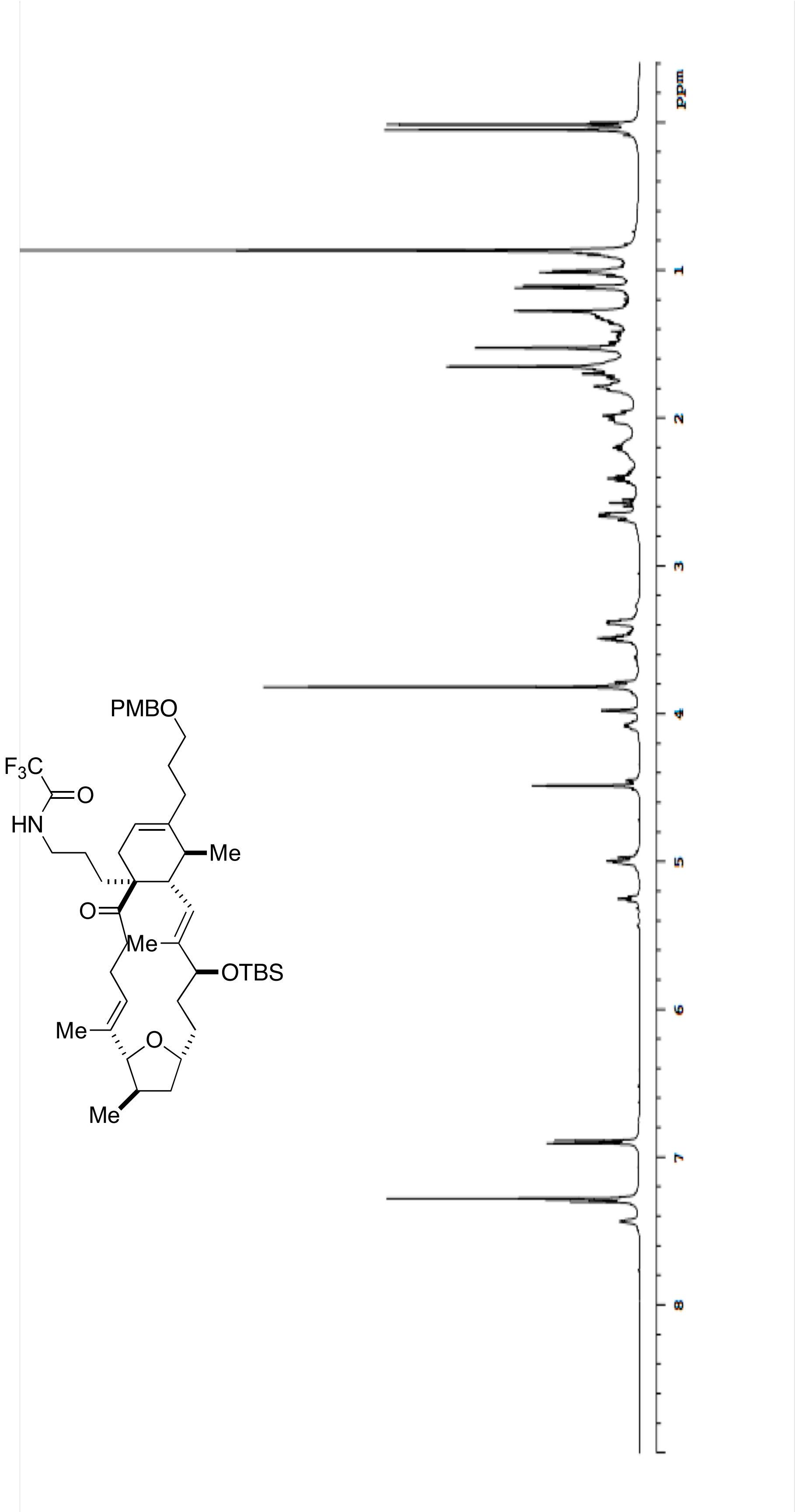
$^{13}$C-NMR (125 MHz) of **2-89c** in $CDCl_3$



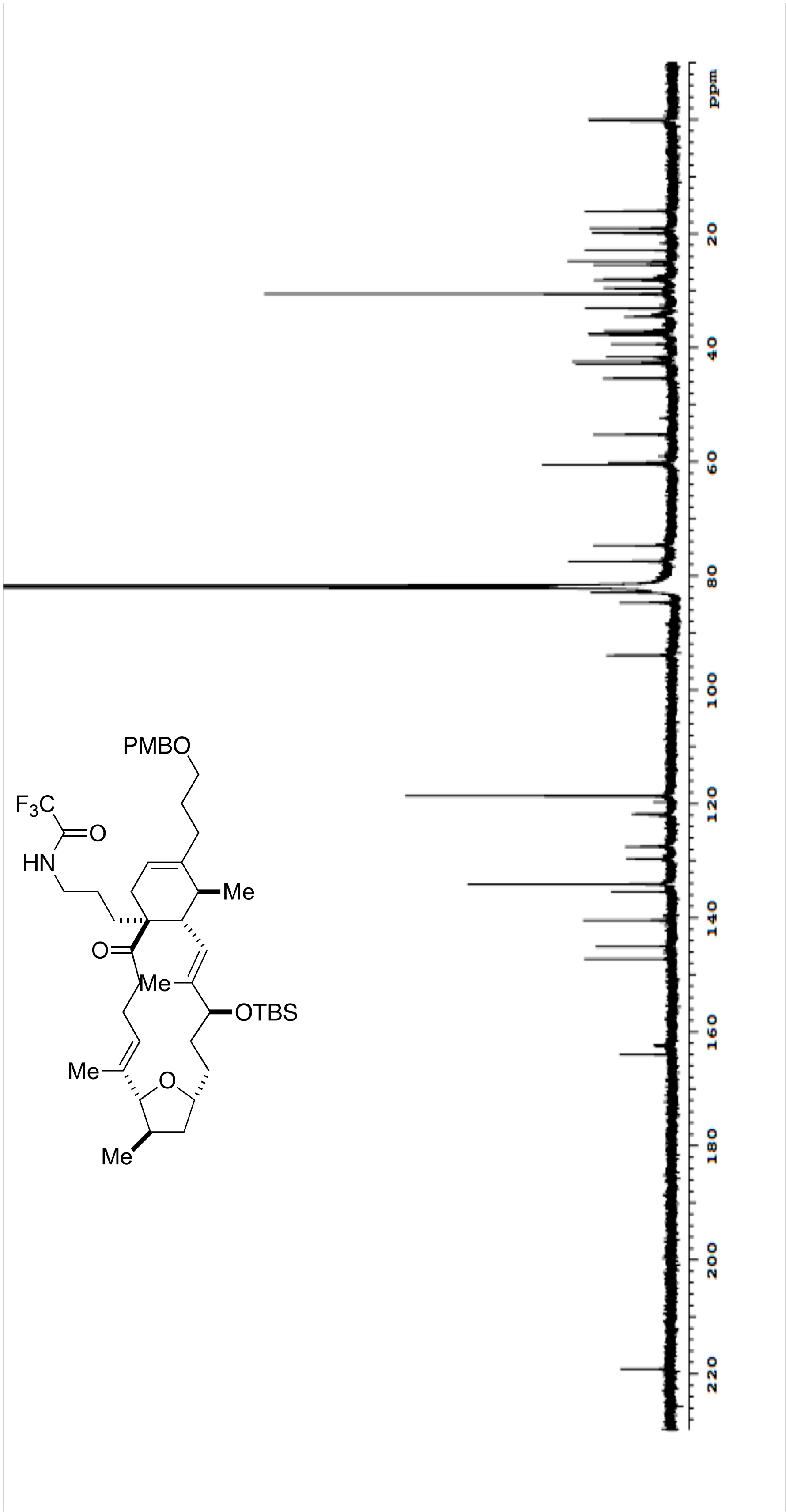
$^1$H-NMR (500 MHz) of **2-90a** in $CDCl_3$



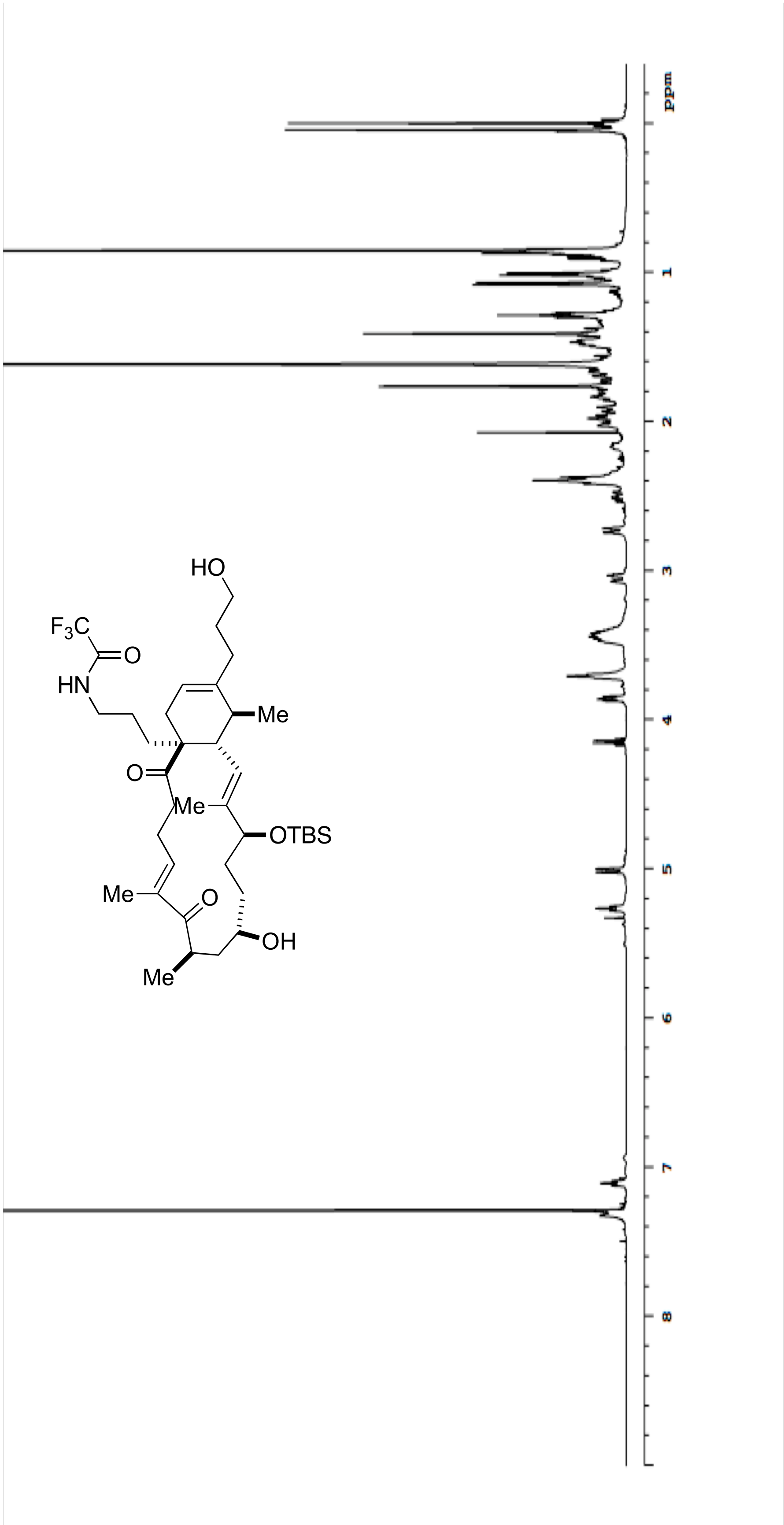
$^{13}$C-NMR (125 MHz) of **2-90a** in $CDCl_3$



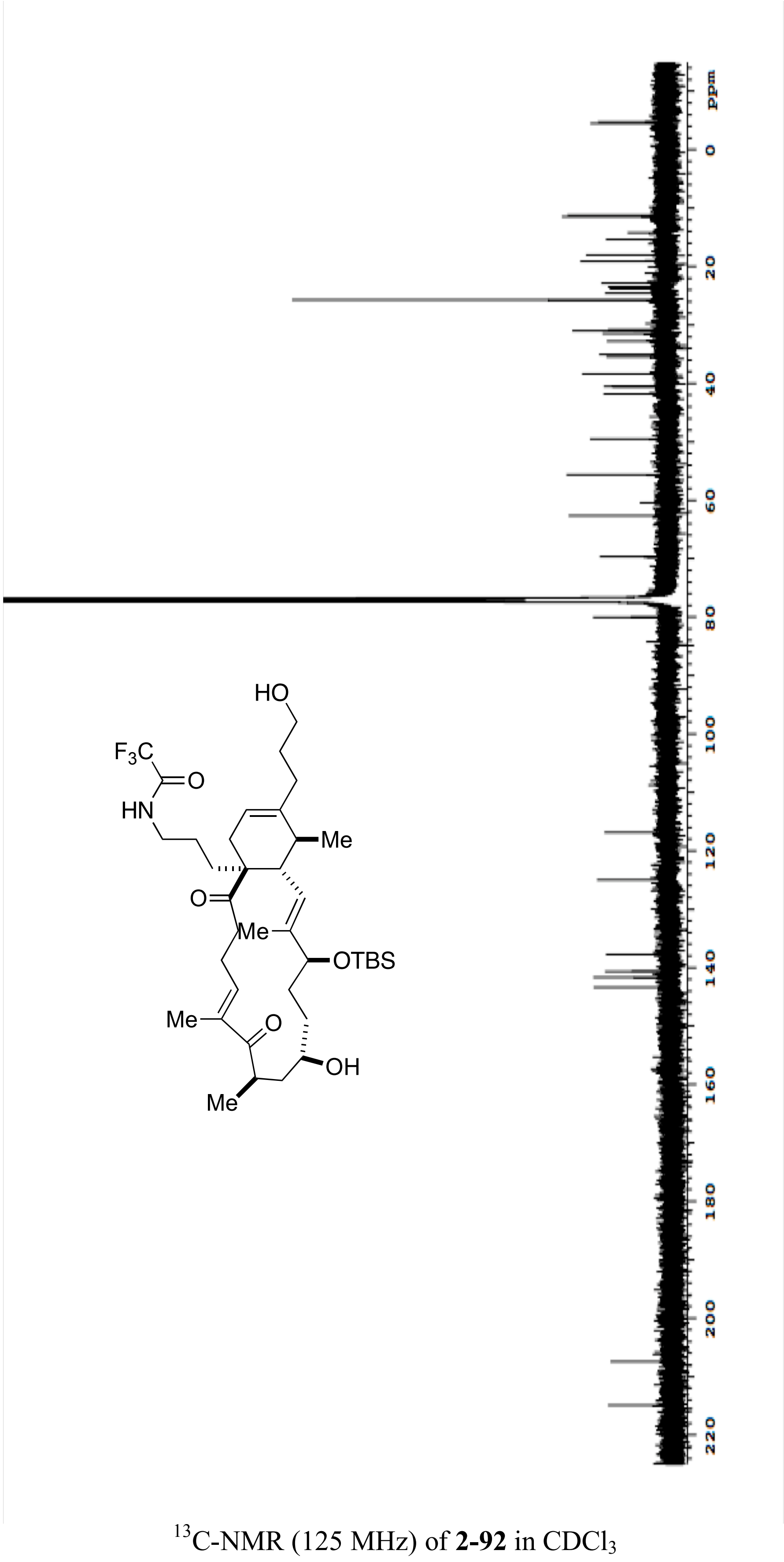
$^1$H-NMR (500 MHz) of **2-91** in CDCl$_3$



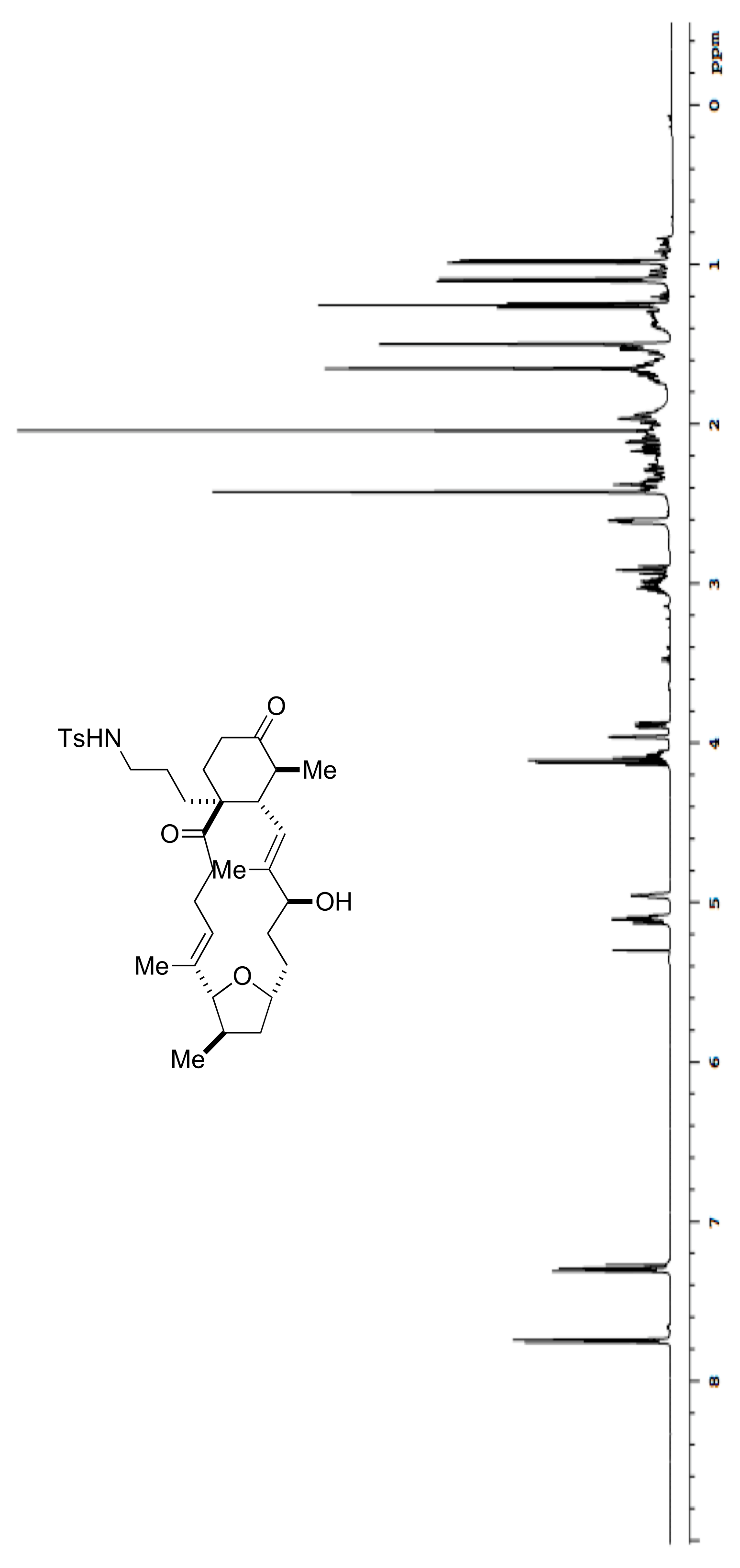
$^{13}$C-NMR (125 MHz) of **2-91** in CDCl$_3$



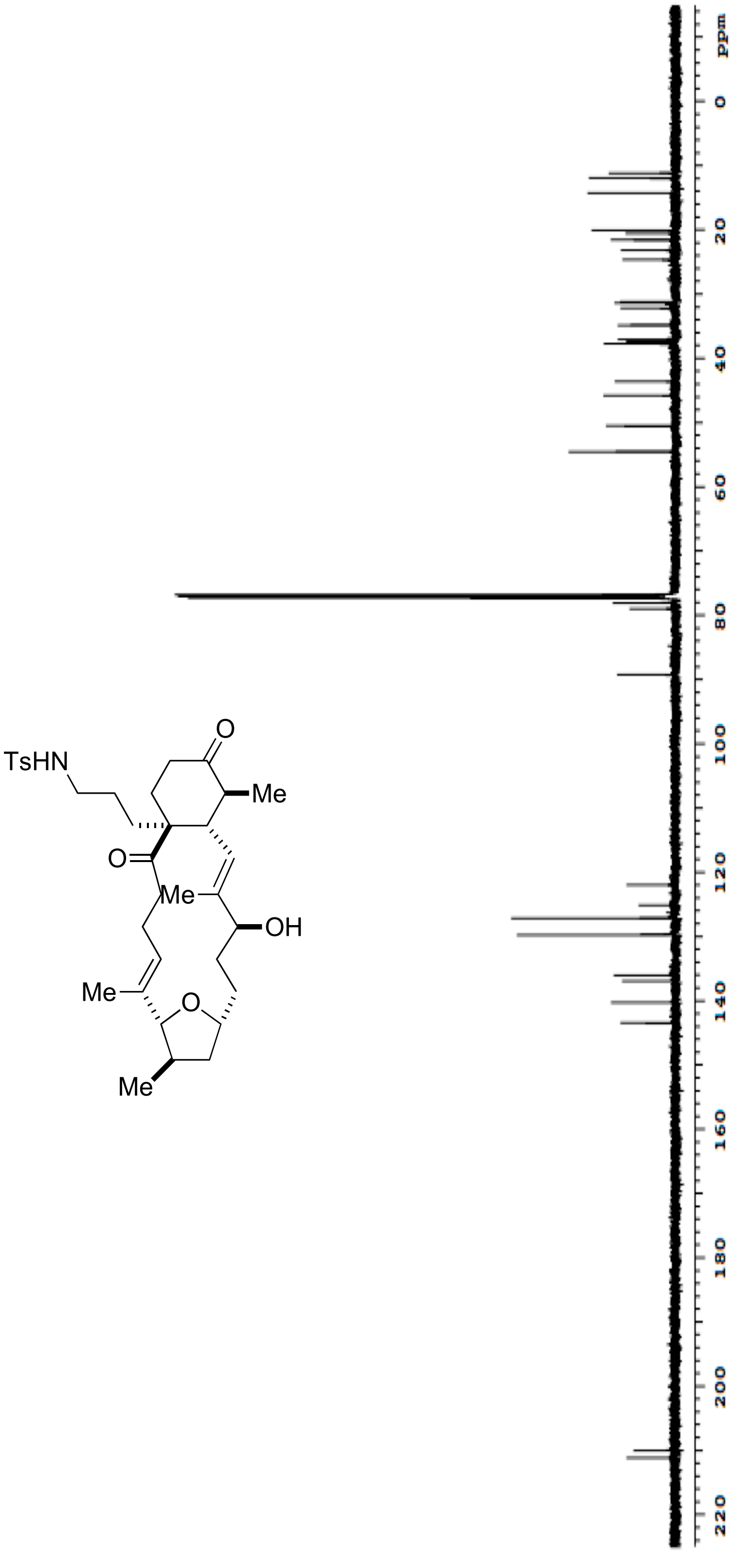
$^1$H-NMR (500 MHz) of **2-92** in $CDCl_3$



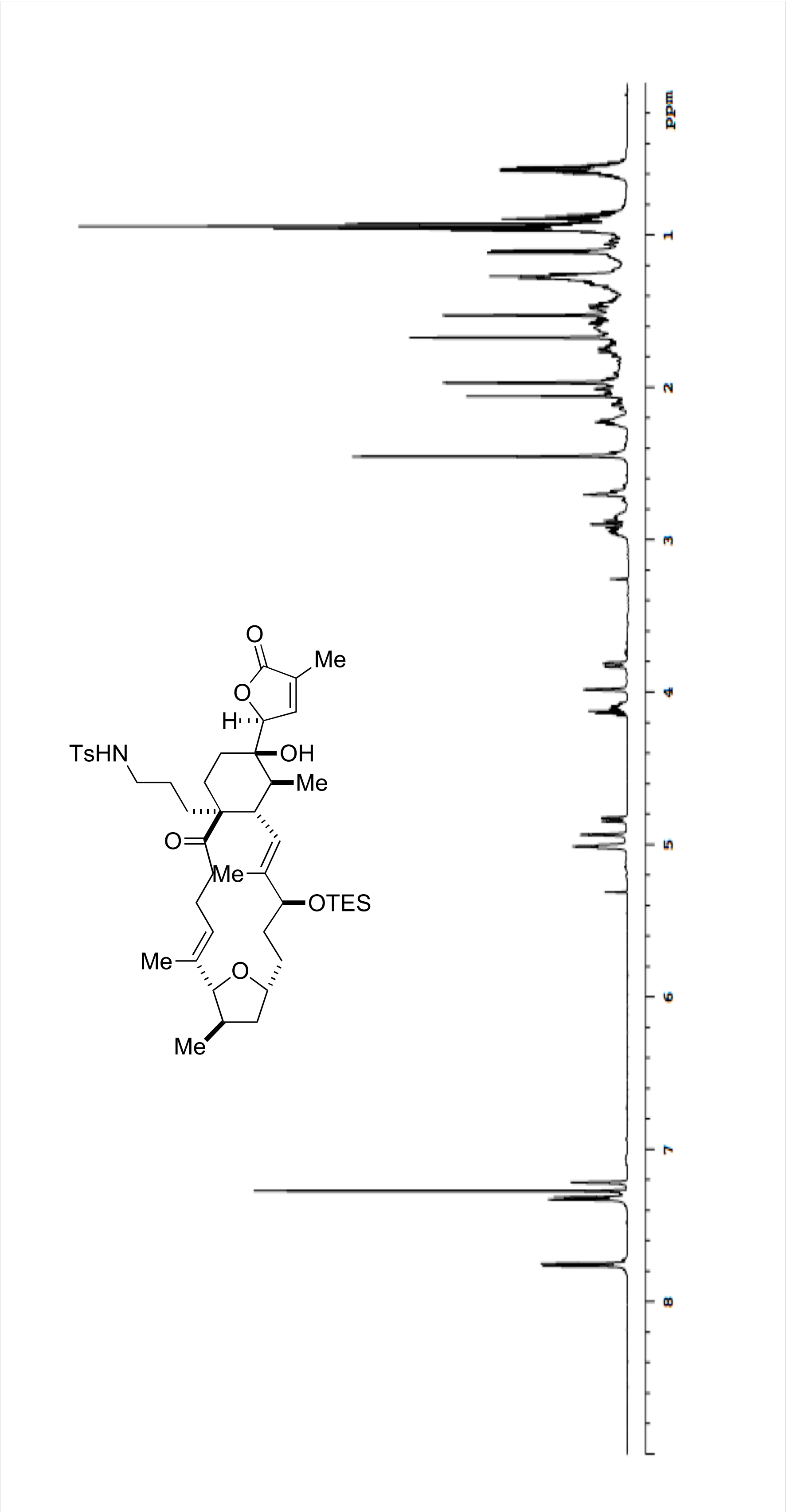
$^{13}$C-NMR (125 MHz) of **2-92** in $CDCl_3$



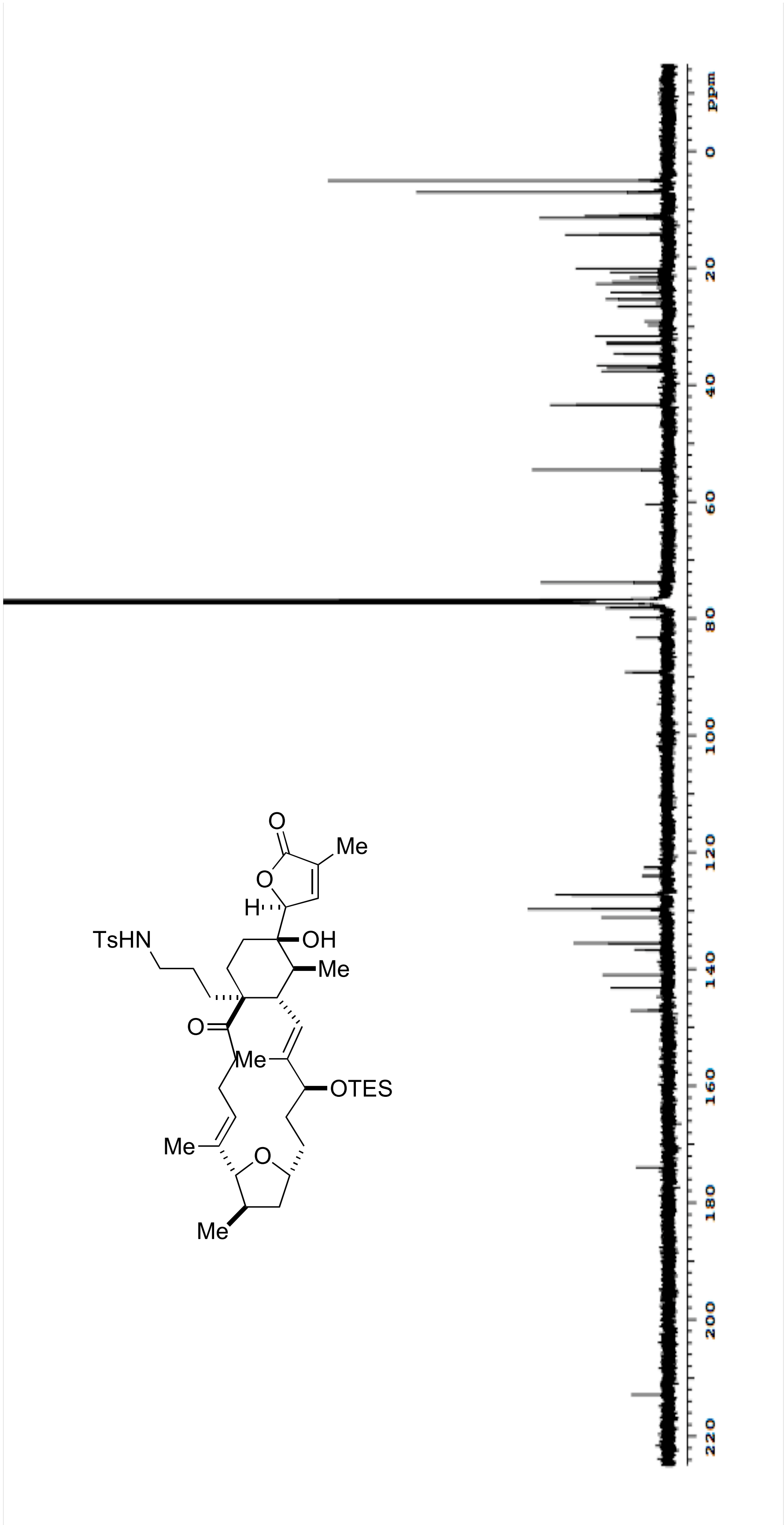
$^1$H-NMR (500 MHz) of **2-111** in $CDCl_3$



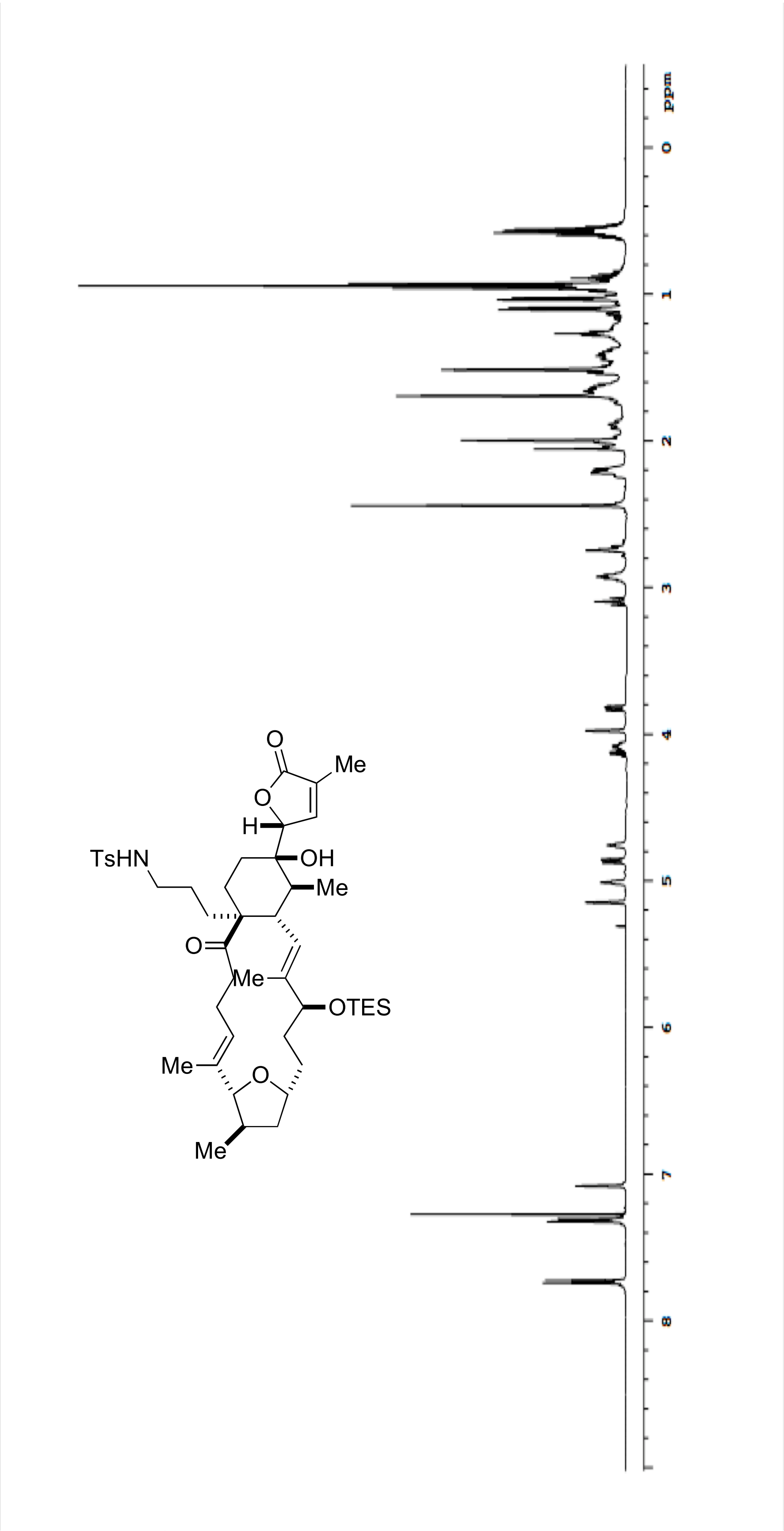
$^{13}$C-NMR (125 MHz) of **2-111** in $CDCl_3$



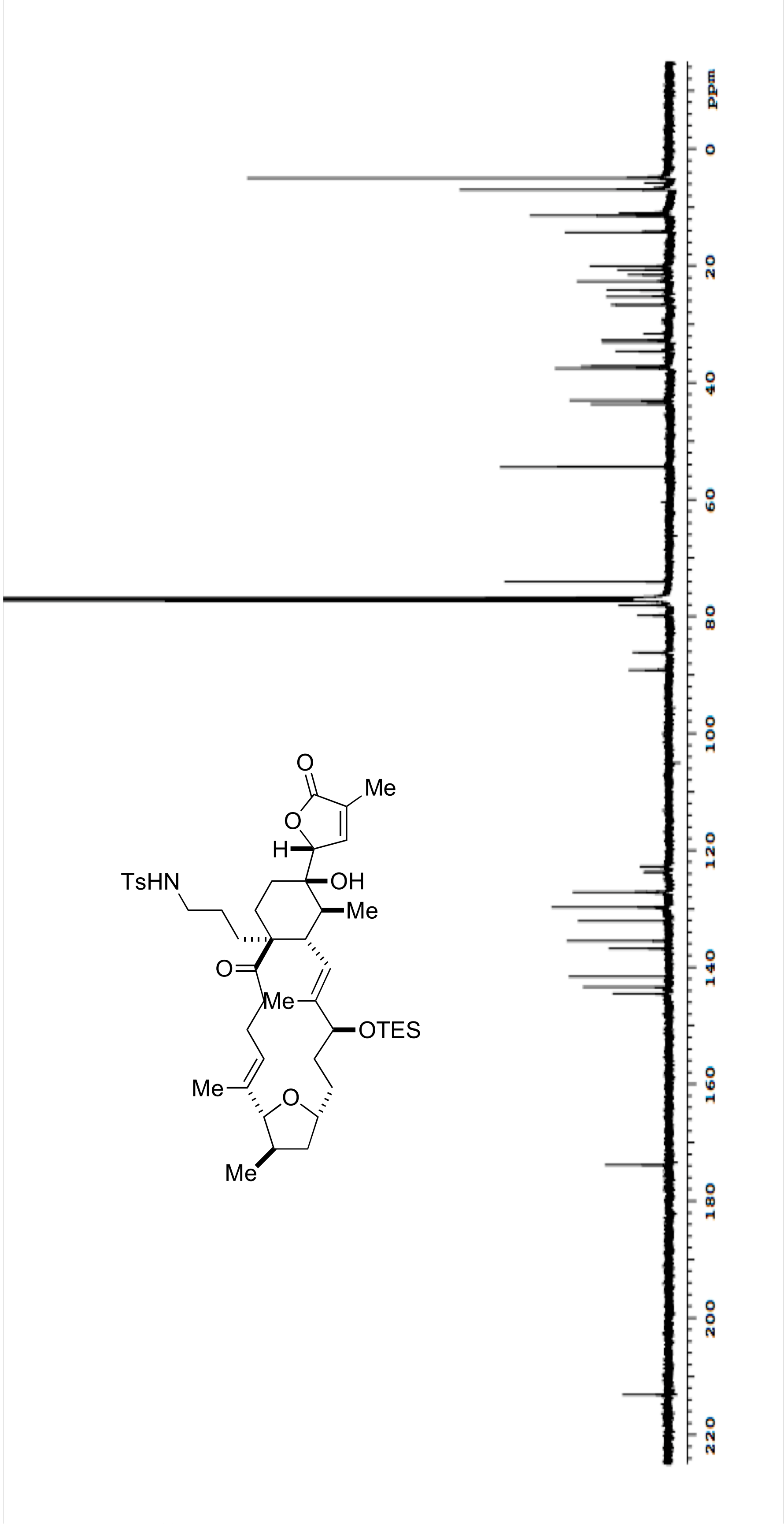
$^1$H-NMR (500 MHz) of **2-113a** in $CDCl_3$



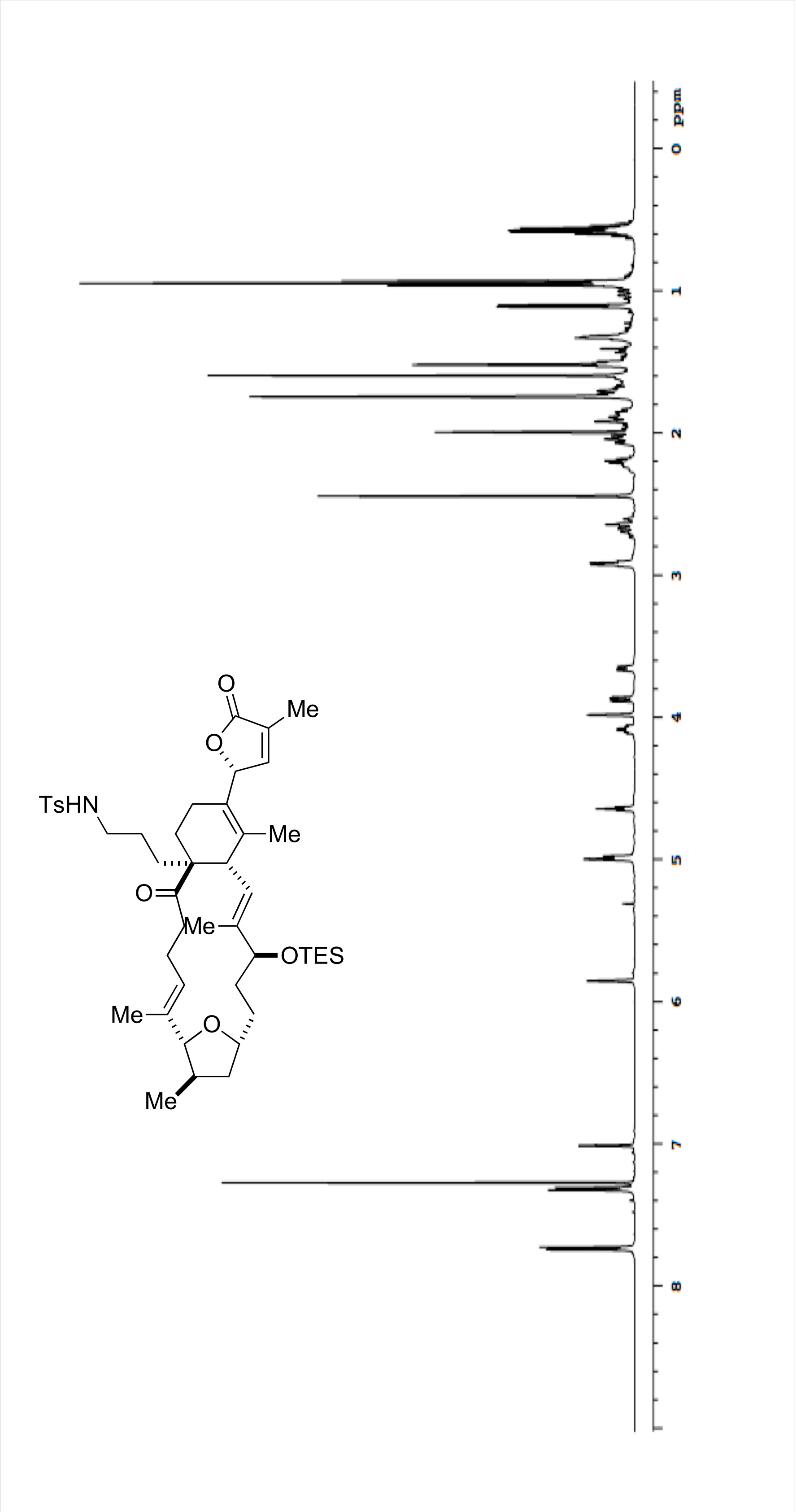
$^{13}$C-NMR (125 MHz) of **2-113a** in $CDCl_3$



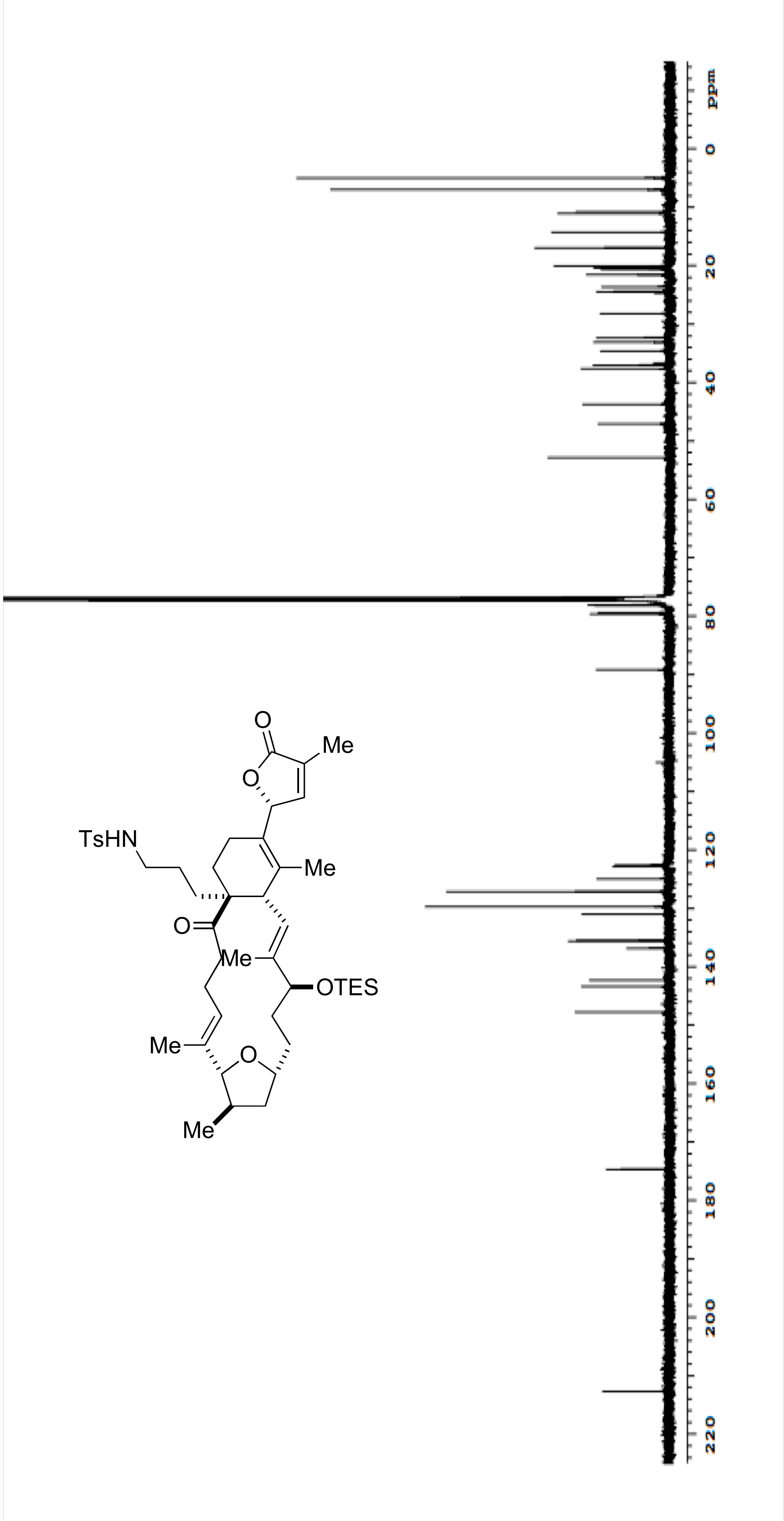
$^1$H-NMR (500 MHz) of **2-113b** in $CDCl_3$



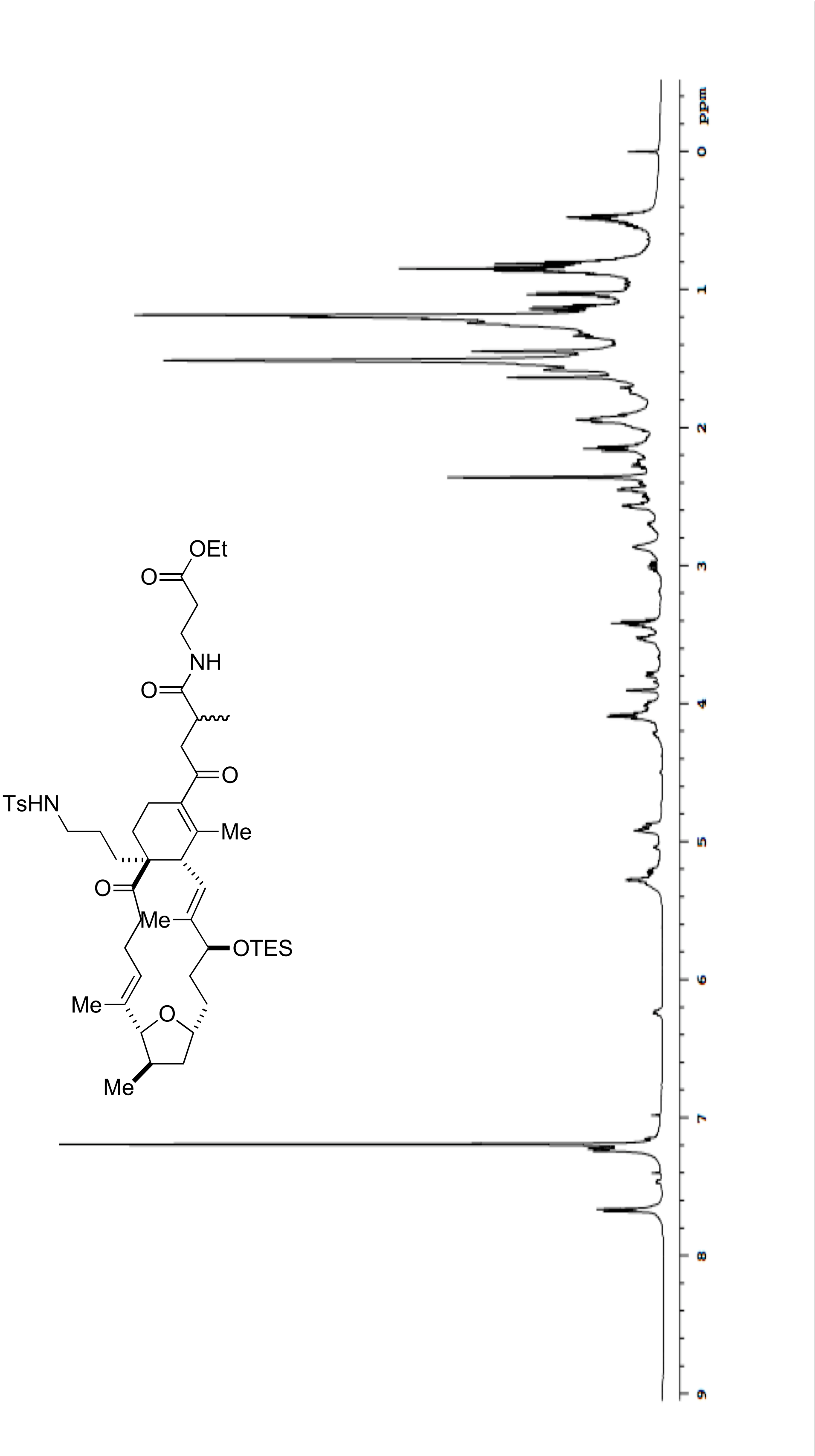
$^{13}$C-NMR (125 MHz) of **2-113b** in $CDCl_3$



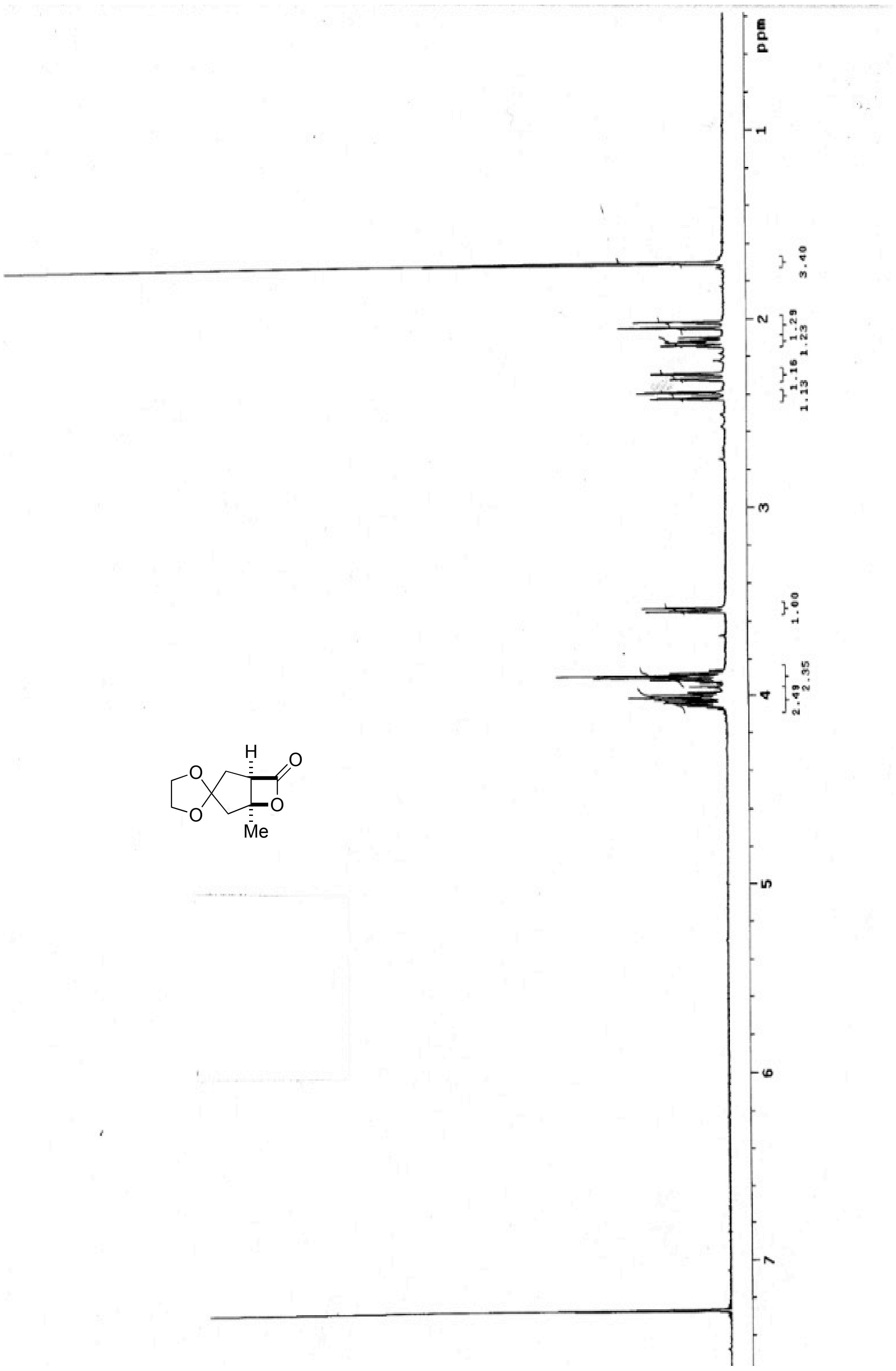
$^1$H-NMR (500 MHz) of **2-114** in $CDCl_3$



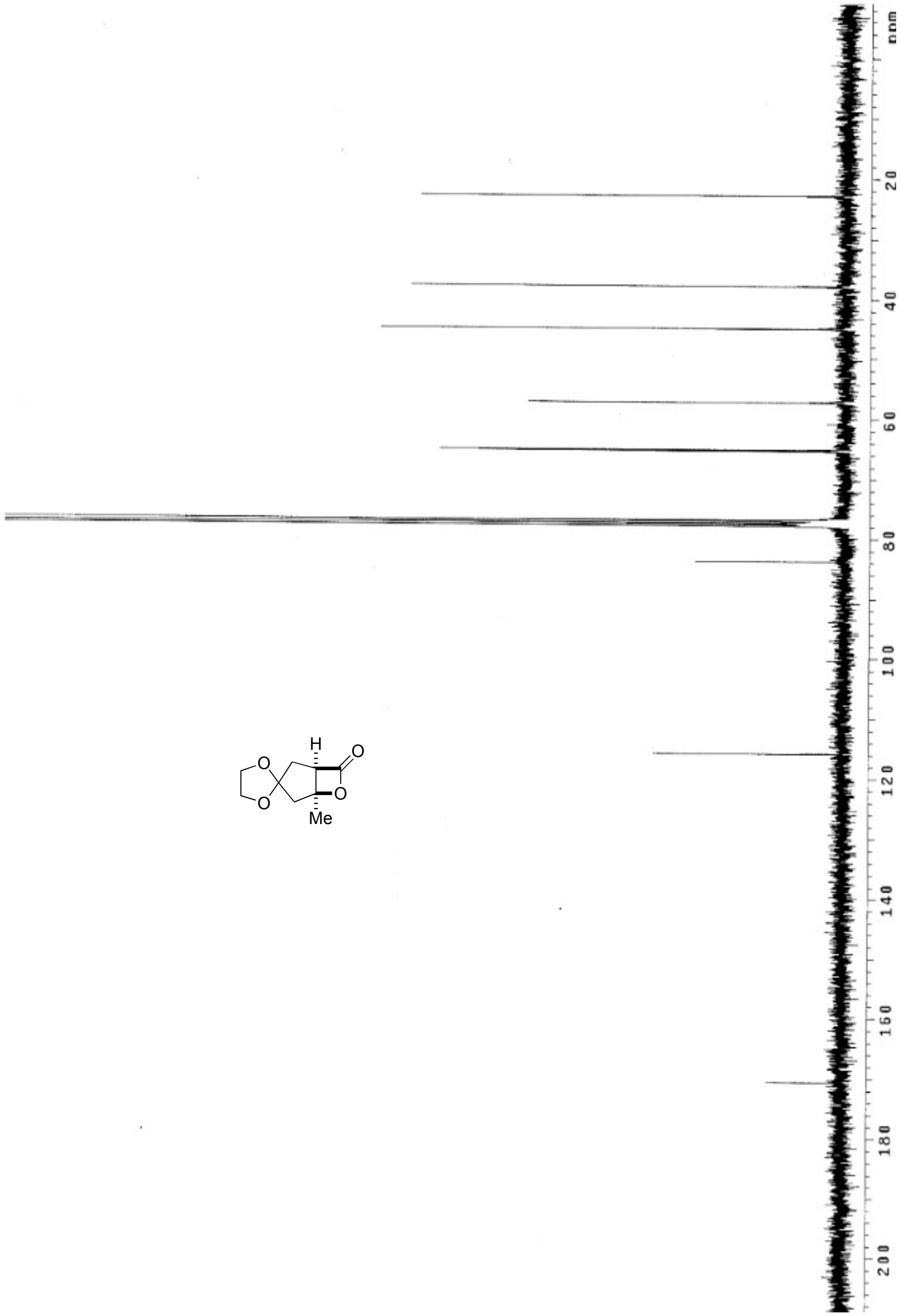
$^{13}$C-NMR (125 MHz) of **2-114** in CDCl$_3$



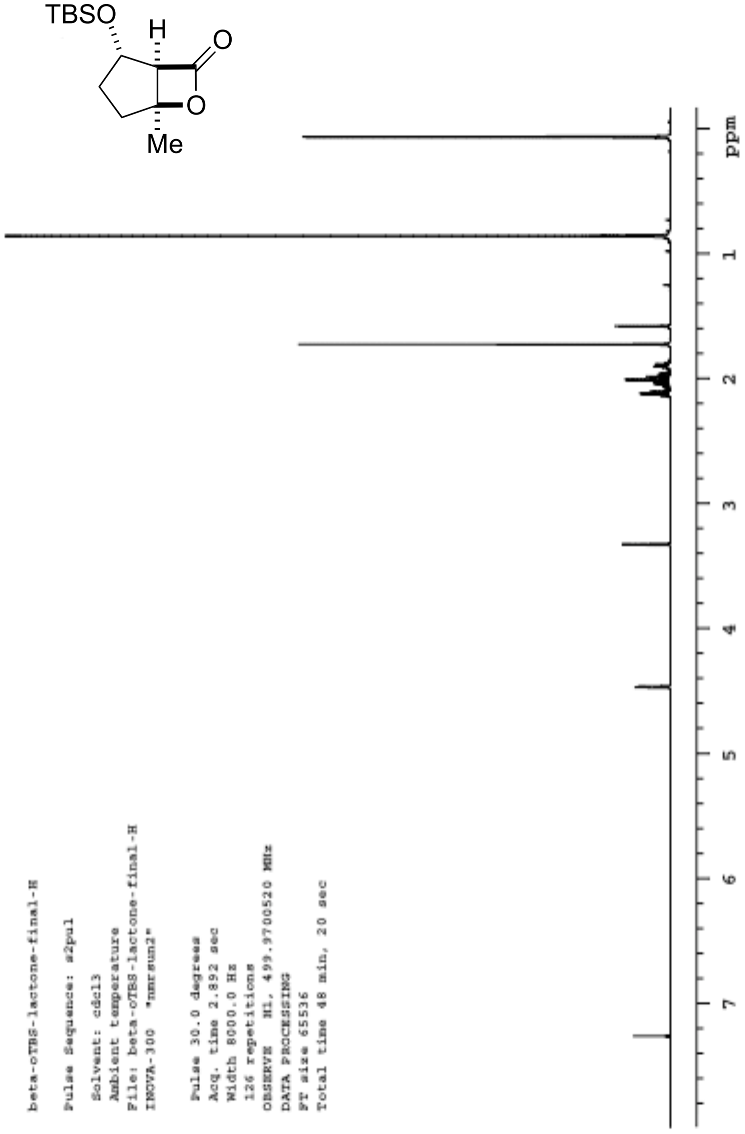
$^1$H-NMR (500 MHz) of **2-115** in $CDCl_3$



$^1$H-NMR (300 MHz) of **3-24b** in $CDCl_3$

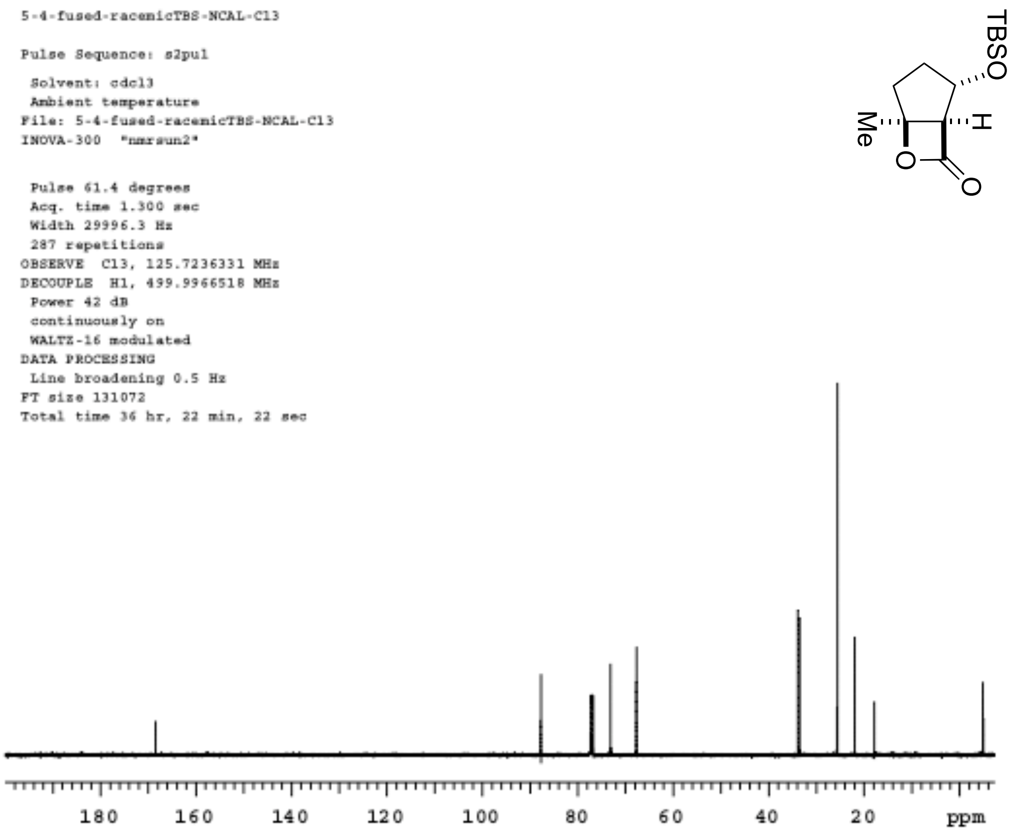


$^{13}$C-NMR (75 MHz) of **3-24b** in $CDCl_3$



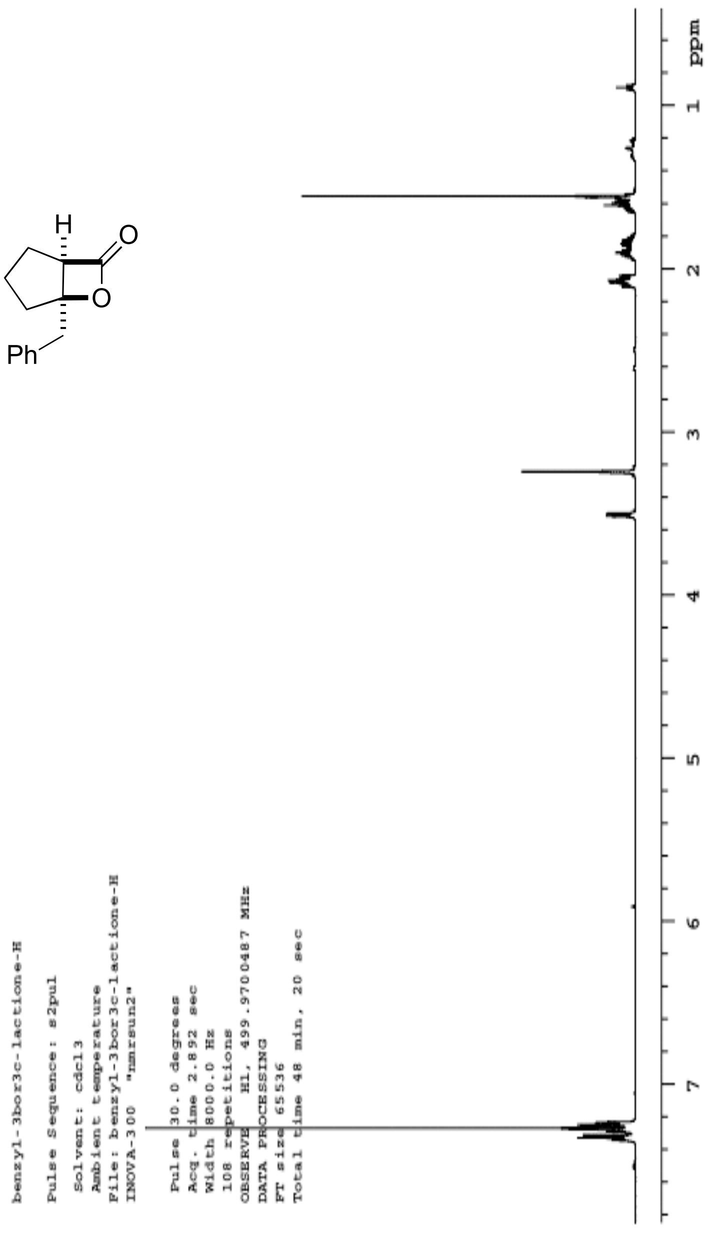
```
beta-oTBS-lactone-final-H

Pulse Sequence: s2pul

 Solvent: cdcl3
 Ambient temperature
File: beta-oTBS-lactone-final-H
INOVA-300  "nmrsun2"

 Pulse 30.0 degrees
 Acq. time 2.892 sec
 Width 8000.0 Hz
 126 repetitions
OBSERVE   H1, 499.9700520 MHz
DATA PROCESSING
FT size 65536
Total time 48 min, 20 sec
```

$^1$H-NMR (500 MHz) of 3-**24f** in $CDCl_3$

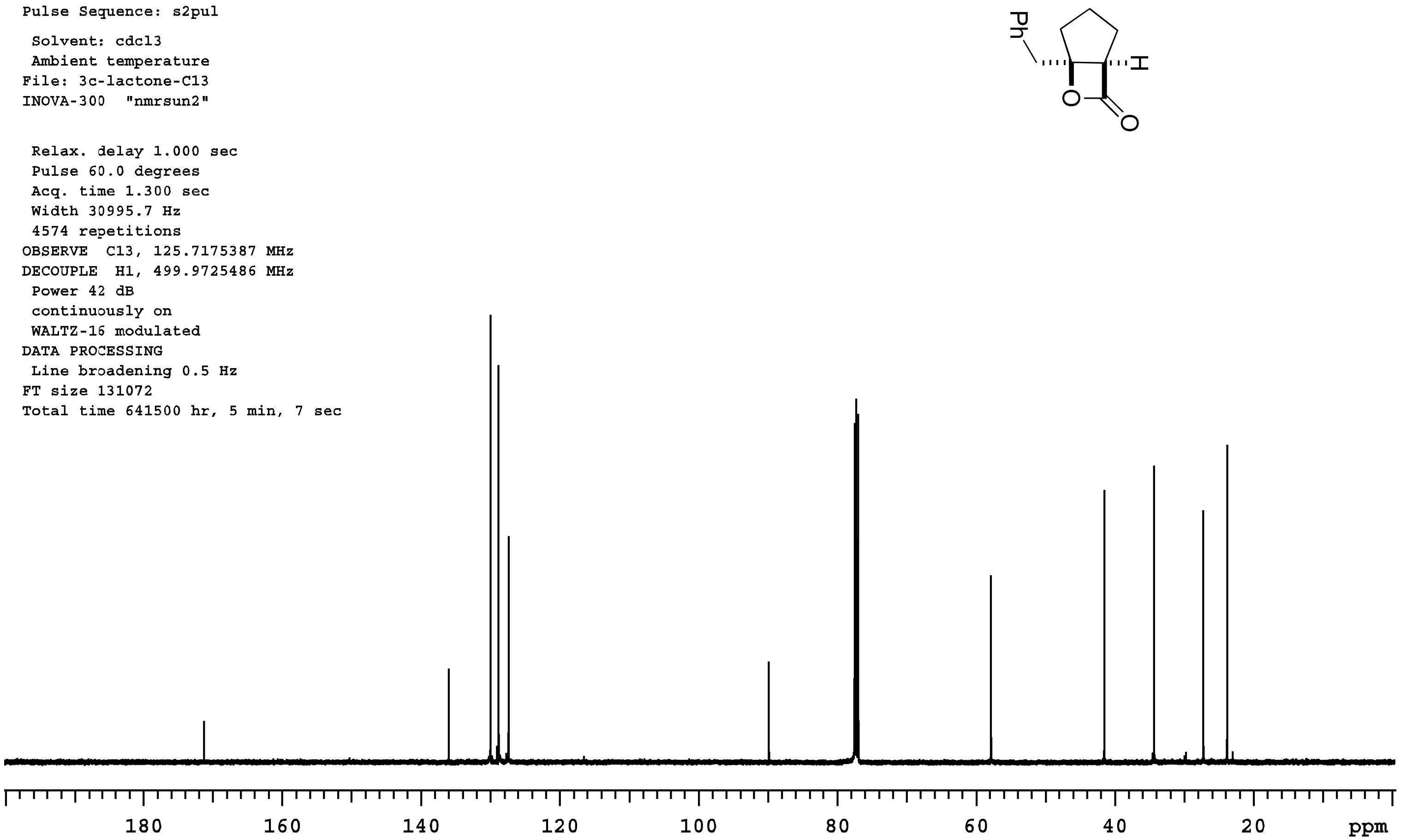


```
5-4-fused-racemicTBS-NCAL-C13

Pulse Sequence: s2pul

 Solvent: cdcl3
 Ambient temperature
File: 5-4-fused-racemicTBS-NCAL-C13
INOVA-300  "nmrsun2"


 Pulse 61.4 degrees
 Acq. time 1.300 sec
 Width 29996.3 Hz
 287 repetitions
OBSERVE  C13, 125.7236331 MHz
DECOUPLE  H1, 499.9966518 MHz
 Power 42 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 0.5 Hz
FT size 131072
Total time 36 hr, 22 min, 22 sec
```

${}^{13}$C-NMR (125 MHz) of **3-24f** in $CDCl_3$

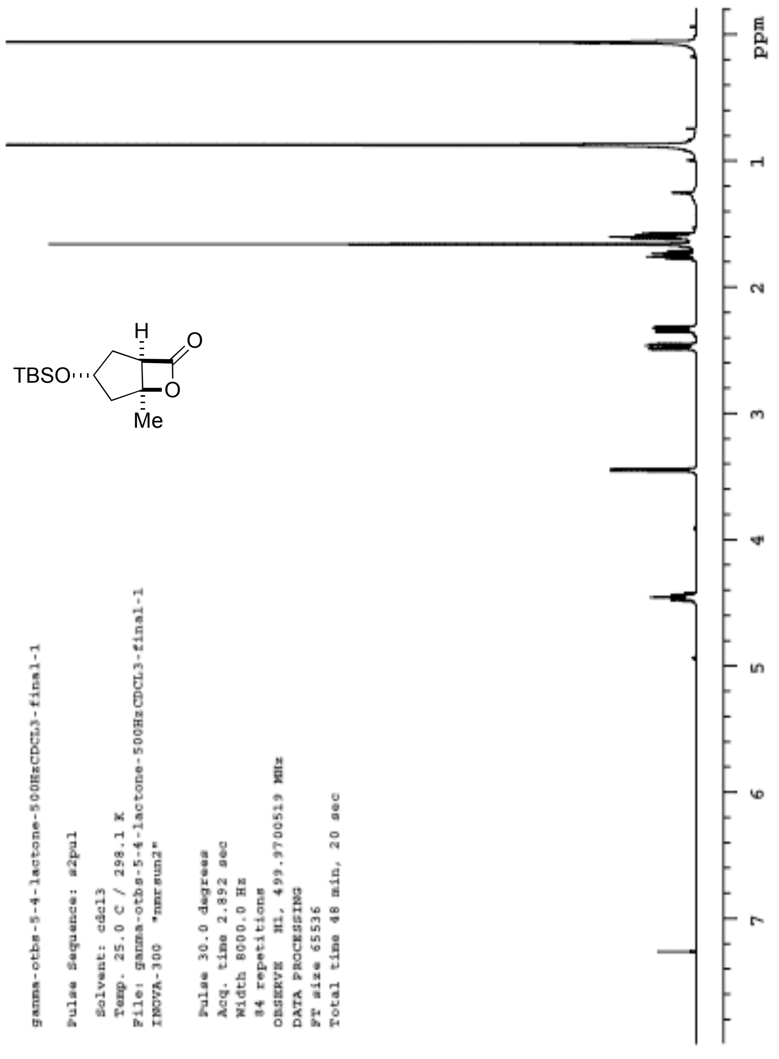


$^{1}$H-NMR (500 MHz) of **3-24c** in $CDCl_3$

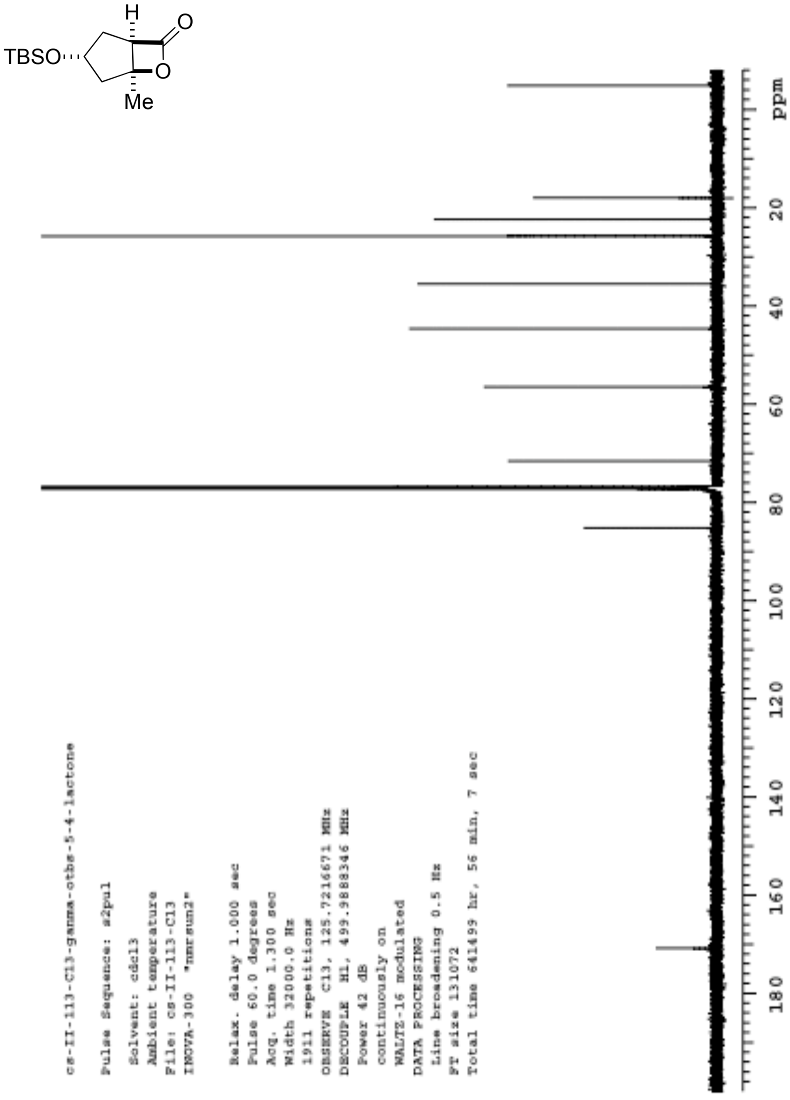


```
3c-lactone-C13

Pulse Sequence: s2pul
 Solvent: cdcl3
 Ambient temperature
File: 3c-lactone-C13
INOVA-300  "nmrsun2"

 Relax. delay 1.000 sec
 Pulse 60.0 degrees
 Acq. time 1.300 sec
 Width 30995.7 Hz
 4574 repetitions
OBSERVE  C13, 125.7175387 MHz
DECOUPLE  H1, 499.9725486 MHz
 Power 42 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 0.5 Hz
FT size 131072
Total time 641500 hr, 5 min, 7 sec
```

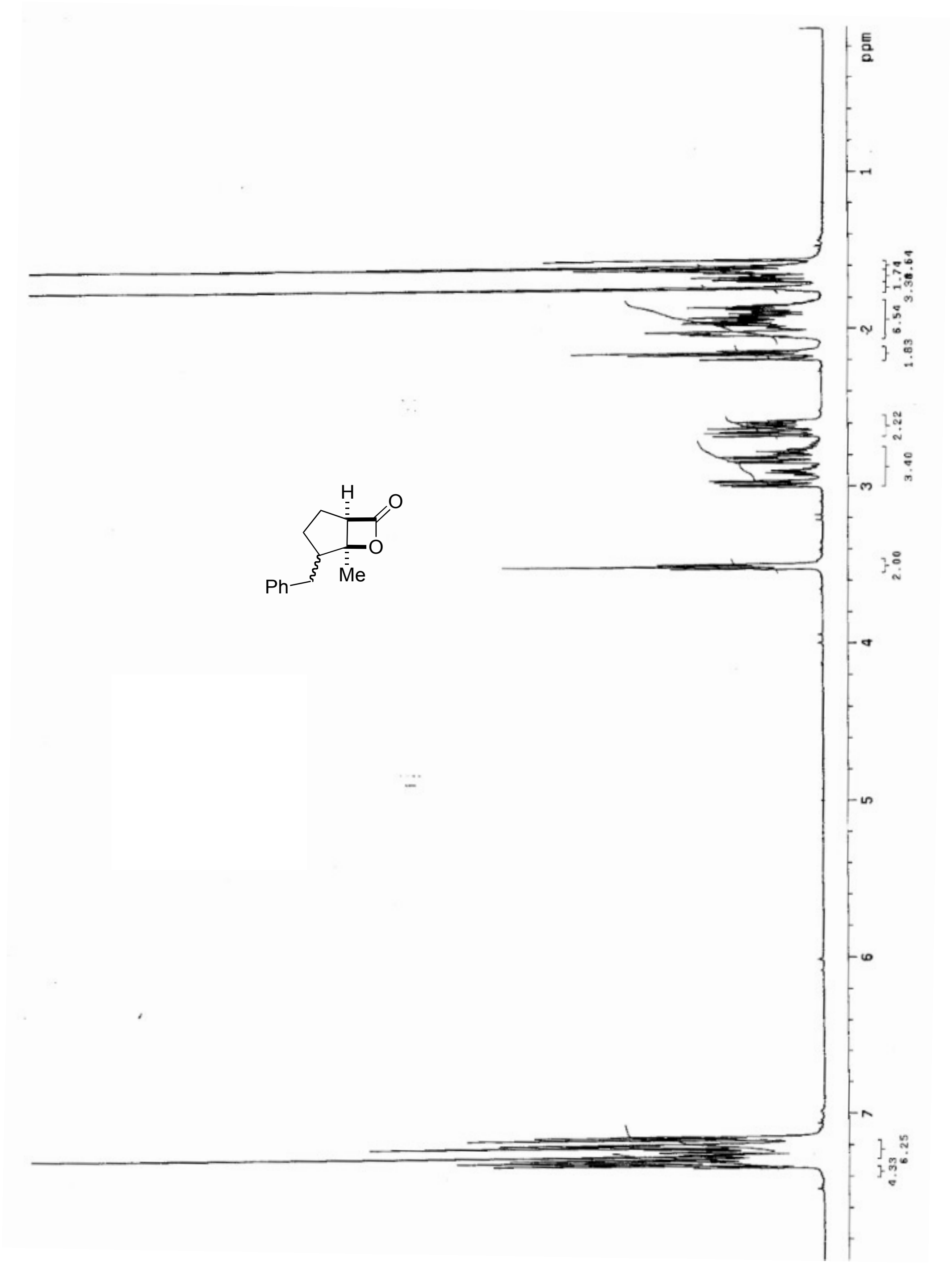
$^{13}$C-NMR (125 MHz) of **3-24**c in $CDCl_3$



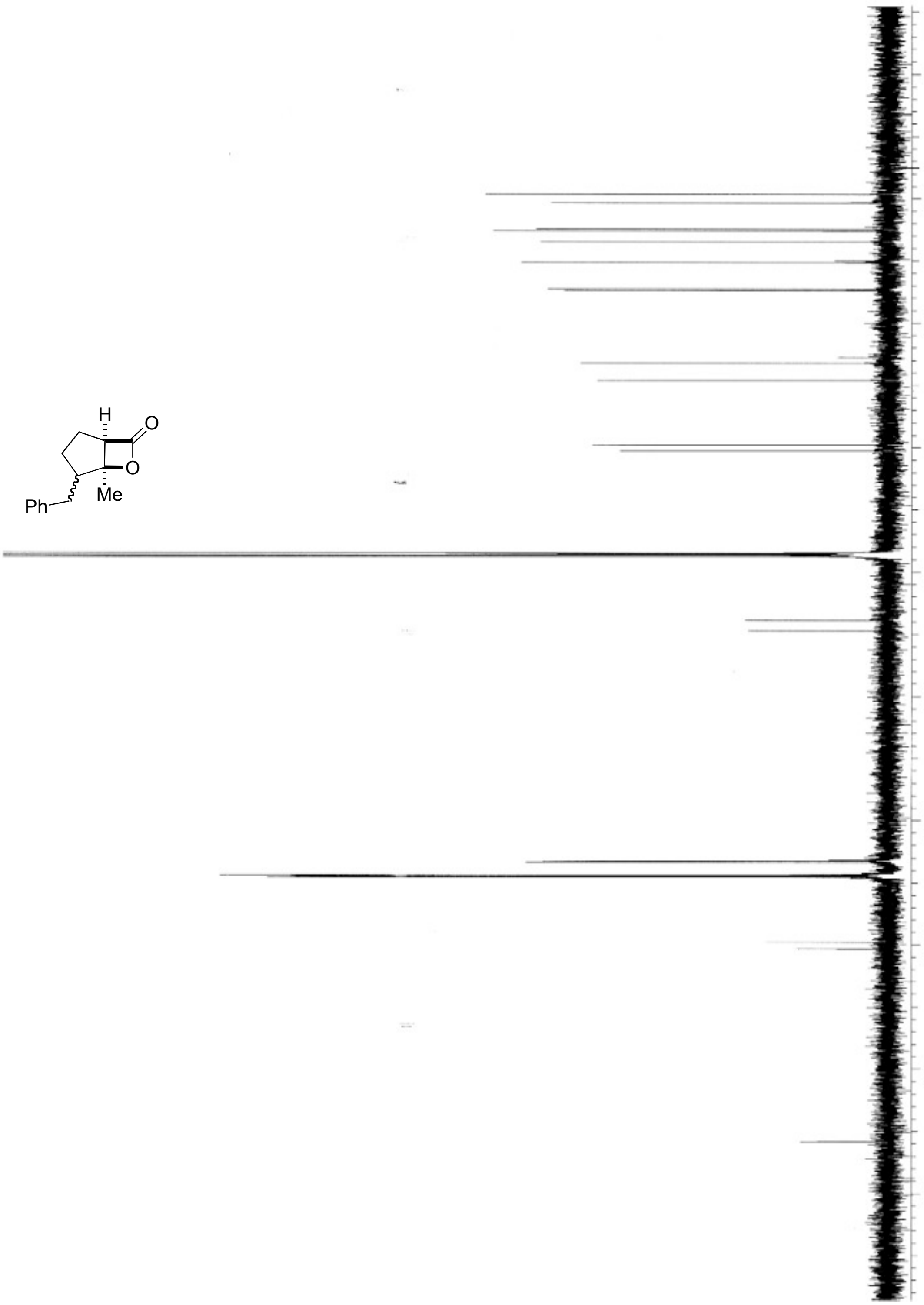
$^1$H-NMR (500 MHz) of **3-24**d in $CDCl_3$



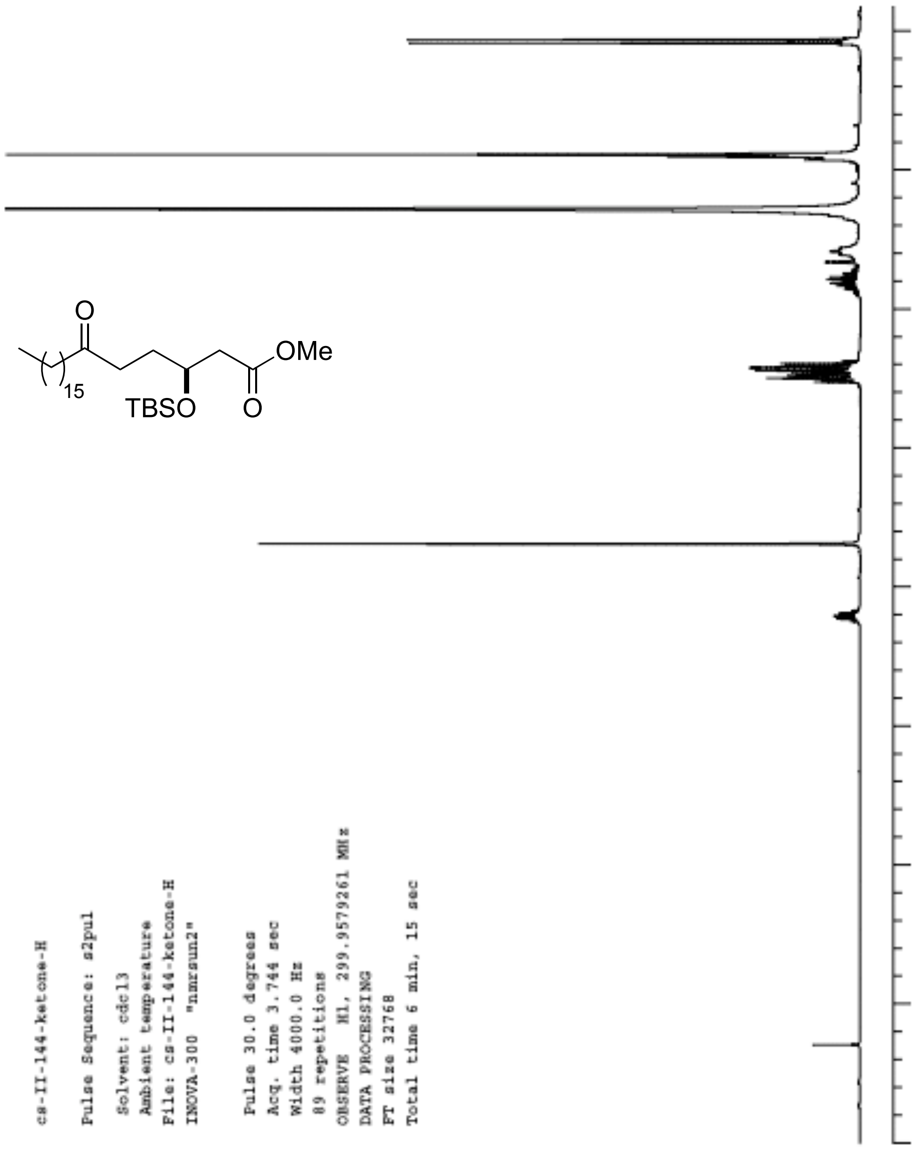
$^{13}$C-NMR (125 MHz) of **3-24d** in $CDCl_3$



$^1$H-NMR (500 MHz) of **3-24e** in $CDCl_3$



$^{13}$C-NMR (125 MHz) of **3-24e** in $CDCl_3$



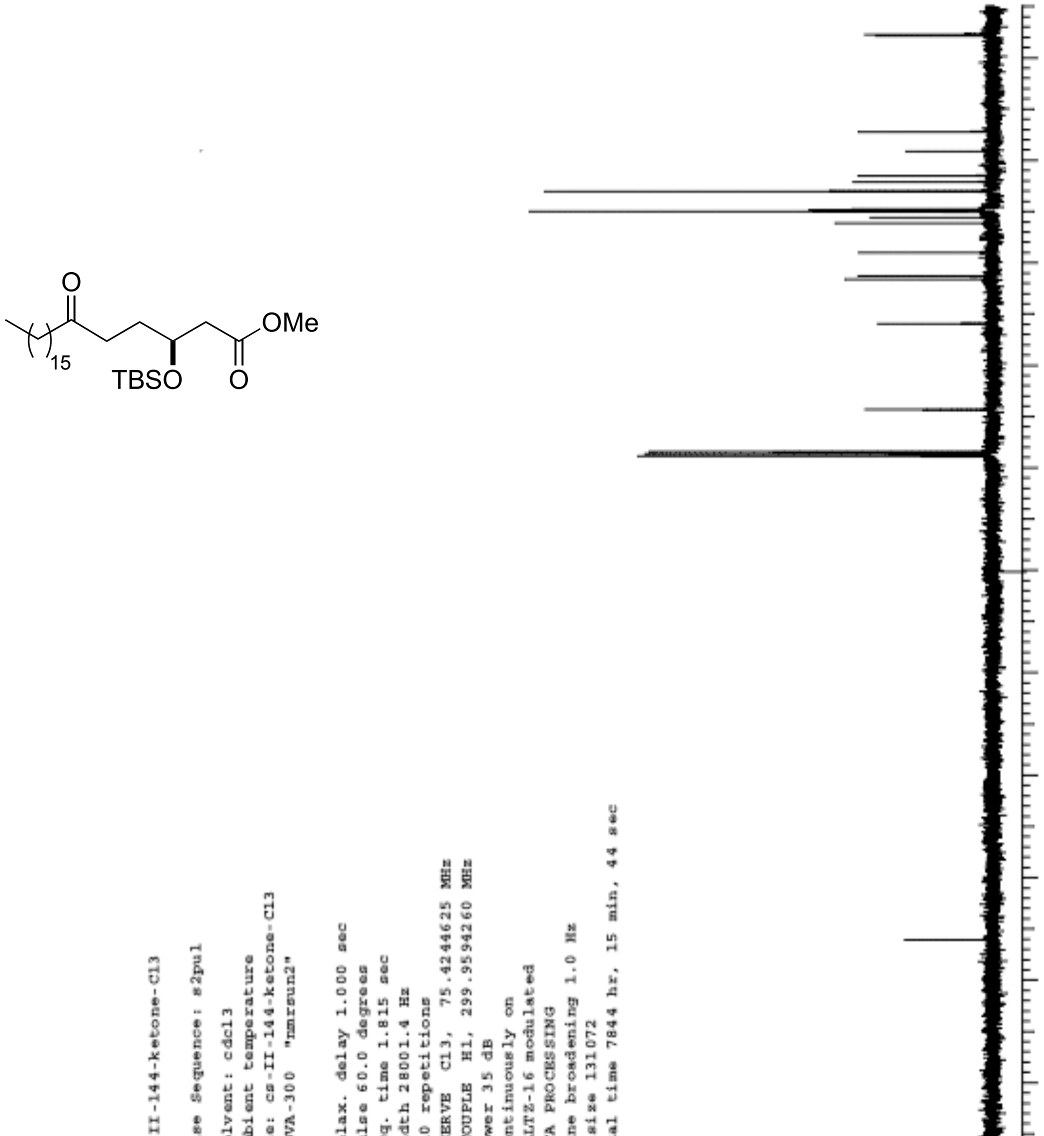
cs-II-144-ketone-H

Pulse Sequence: s2pul

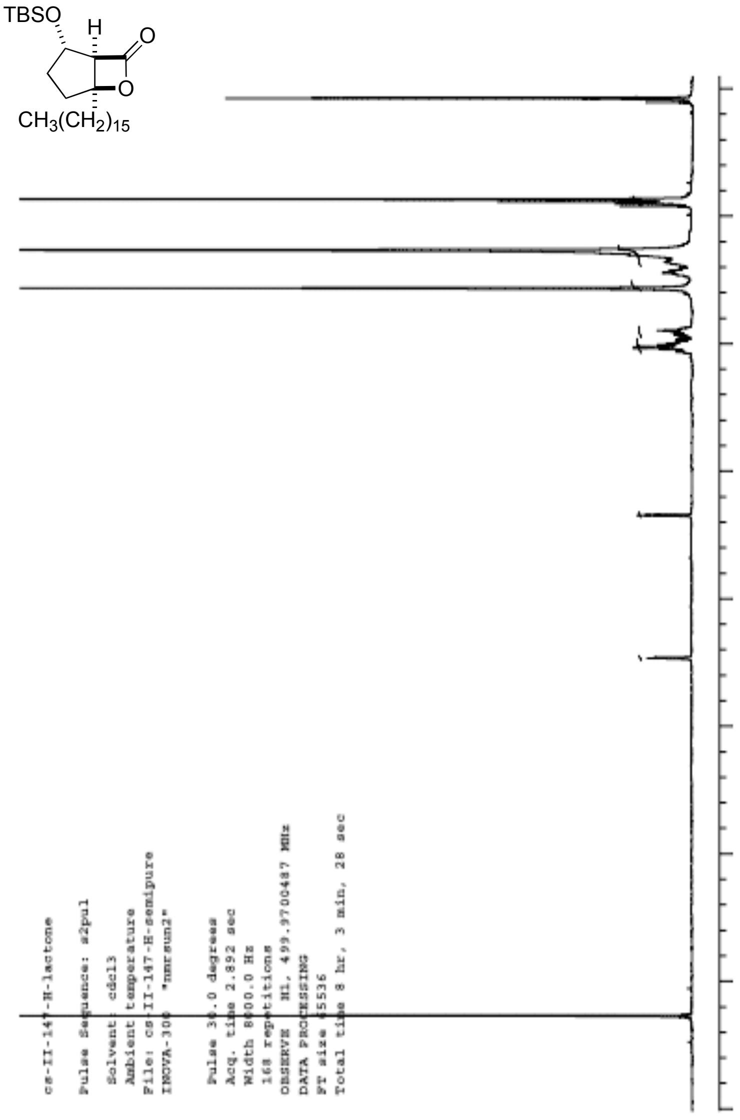
Solvent: cdcl3
Ambient temperature
File: cs-II-144-ketone-H
INOVA-300 "nmrsun2"

Pulse 30.0 degrees
Acq. time 3.744 sec
Width 4000.0 Hz
89 repetitions
OBSERVE H1, 299.9579261 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 15 sec

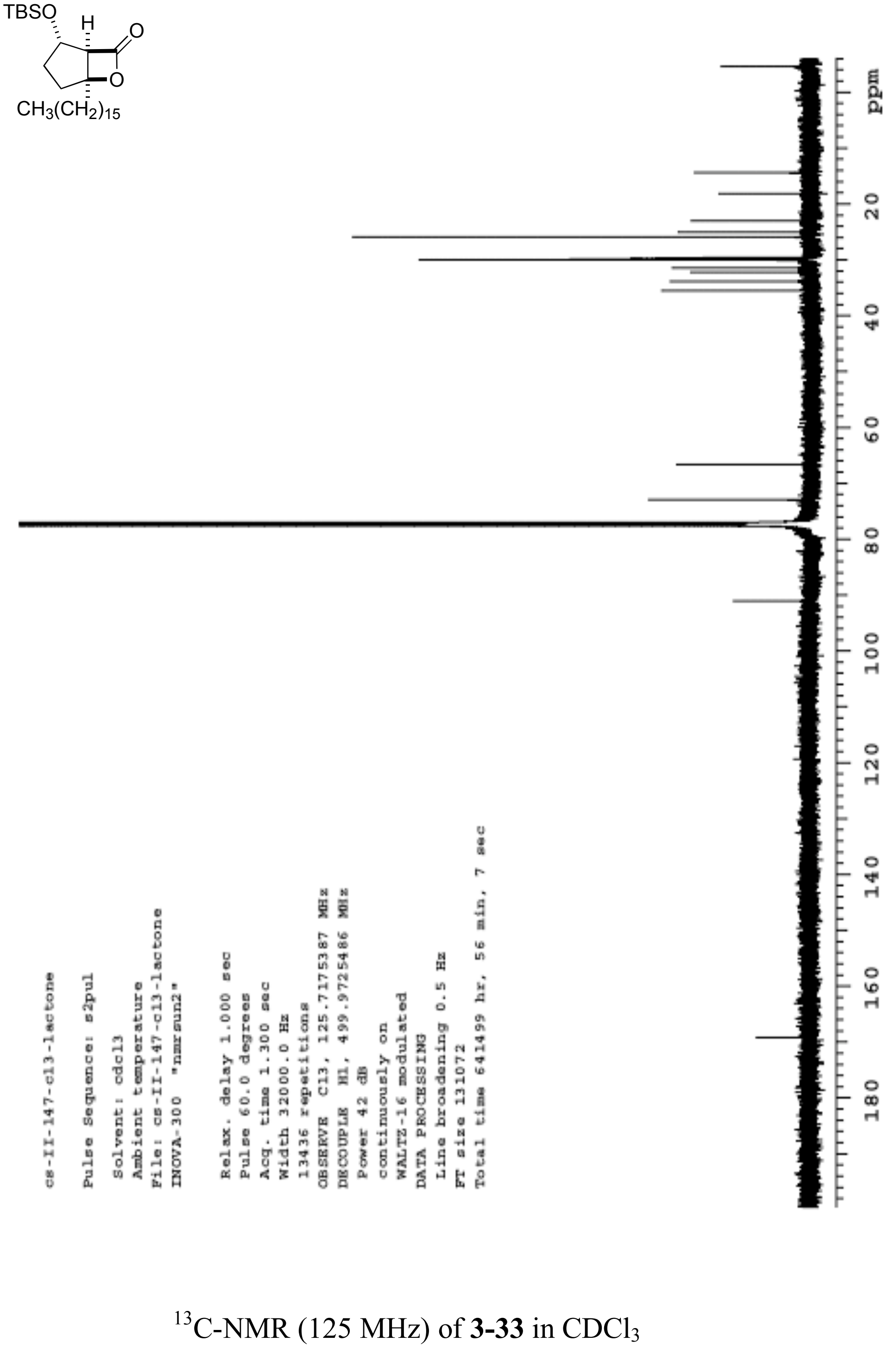
$^1$H-NMR (300 MHz) of **3-31** in $CDCl_3$



$^{13}$C-NMR (75MHz) of **3-31** in $CDCl_3$



$^{1}$H-NMR (500 MHz) of **3-33** in $CDCl_3$



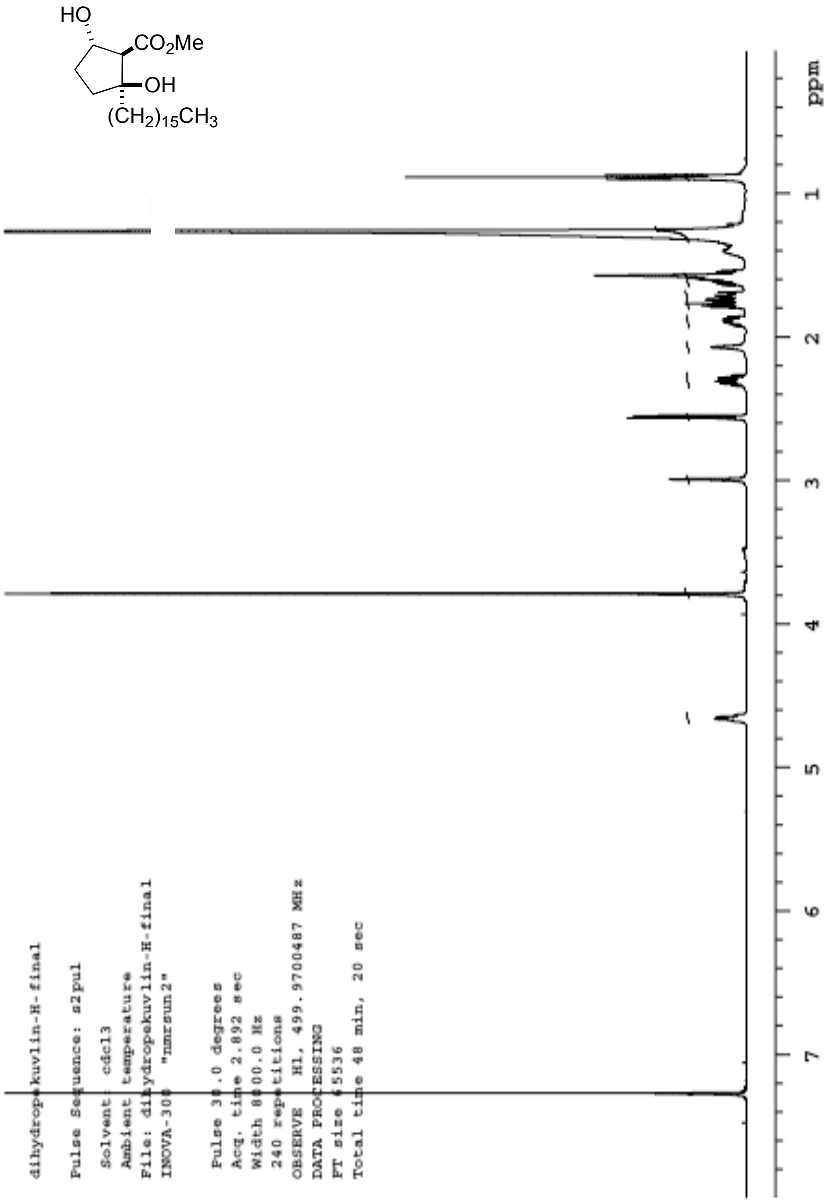
cs-II-147-c13-lactone

Pulse Sequence: s2pul

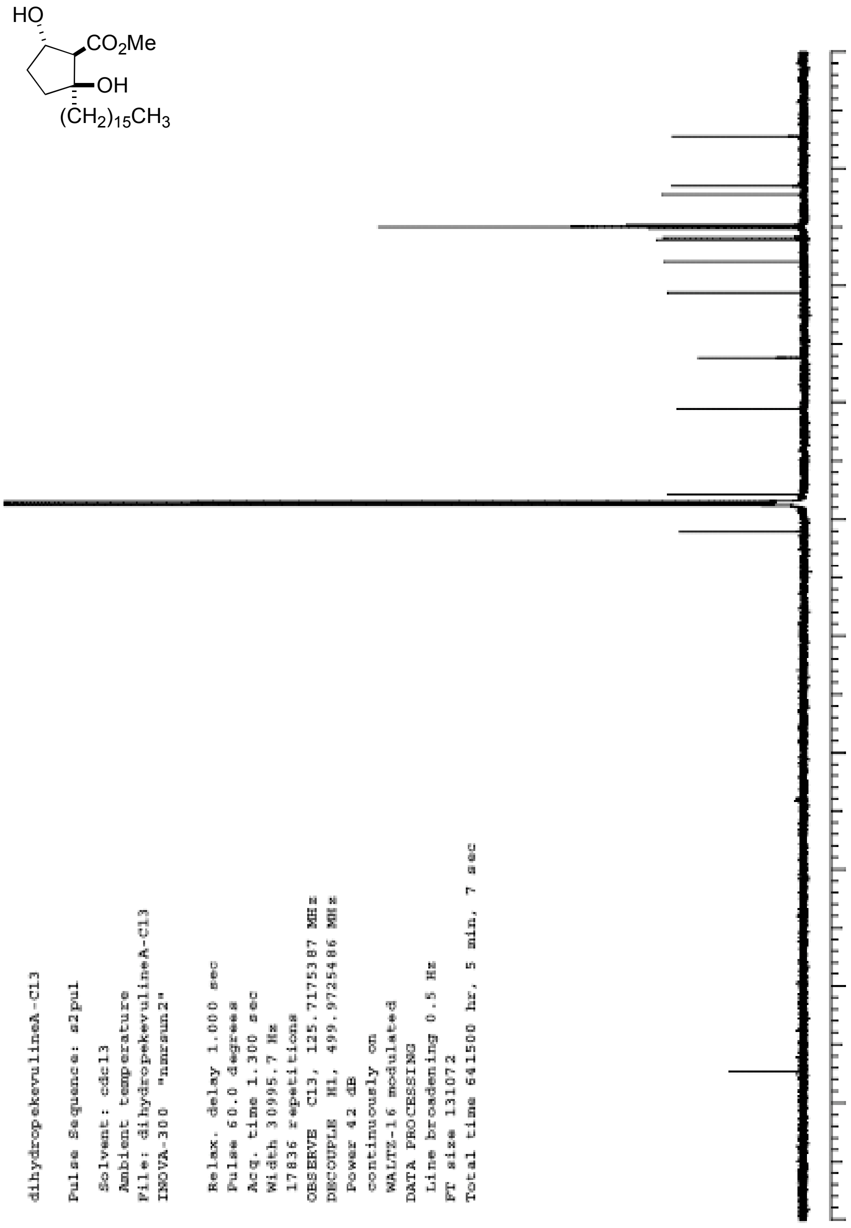
Solvent: cdcl3
Ambient temperature
File: cs-II-147-c13-lactone
INOVA-300 "nmrsun2"

Relax. delay 1.000 sec
Pulse 60.0 degrees
Acq. time 1.300 sec
Width 32000.0 Hz
13436 repetitions
OBSERVE C13, 125.7175387 MHz
DECOUPLE H1, 499.9725486 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
Total time 641499 hr, 56 min, 7 sec

$^{13}$C-NMR (125 MHz) of **3-33** in $CDCl_3$

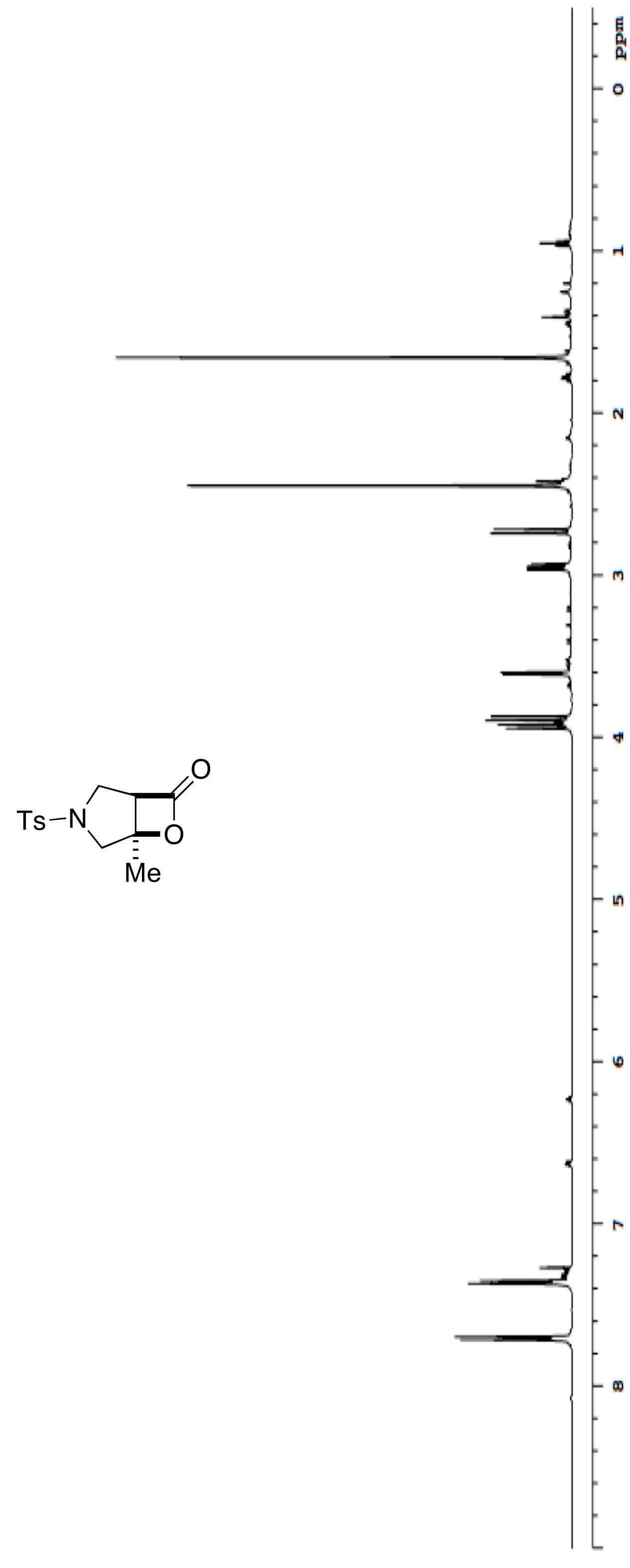


$^1$H-NMR (500 MHz) of **3-34** in $CDCl_3$

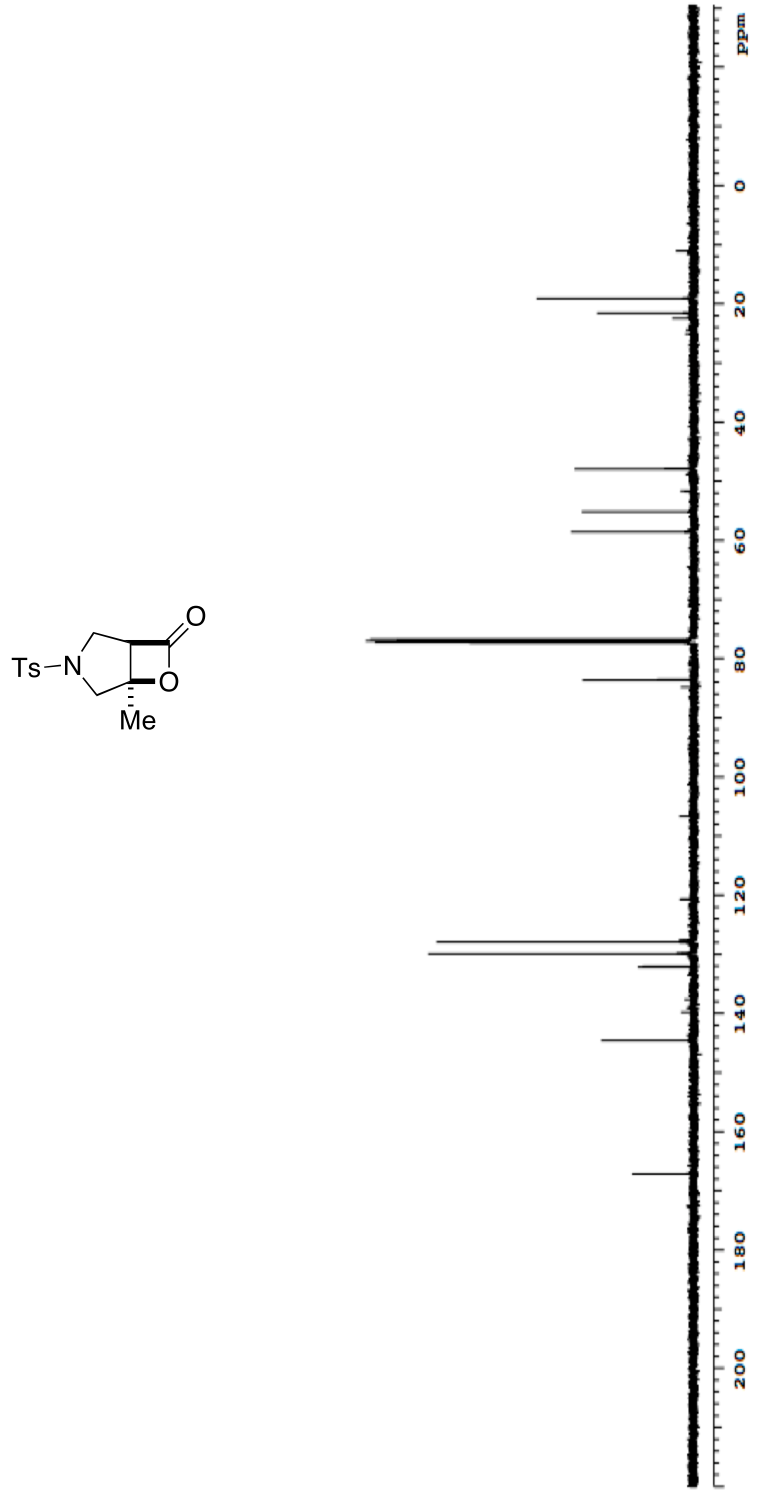


```
dihydropekevulineA-C13

Pulse Sequence: s2pul

 Solvent: cdcl3
 Ambient temperature
File: dihydropekevulineA-C13
INOVA-300  "nmrsun2"

 Relax. delay 1.000 sec
 Pulse 60.0 degrees
 Acq. time 1.300 sec
 Width 30995.7 Hz
 17836 repetitions
OBSERVE  C13, 125.7175387 MHz
DECOUPLE  H1, 499.9725486 MHz
 Power 42 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 0.5 Hz
FT size 131072
Total time 641500 hr, 5 min, 7 sec
```

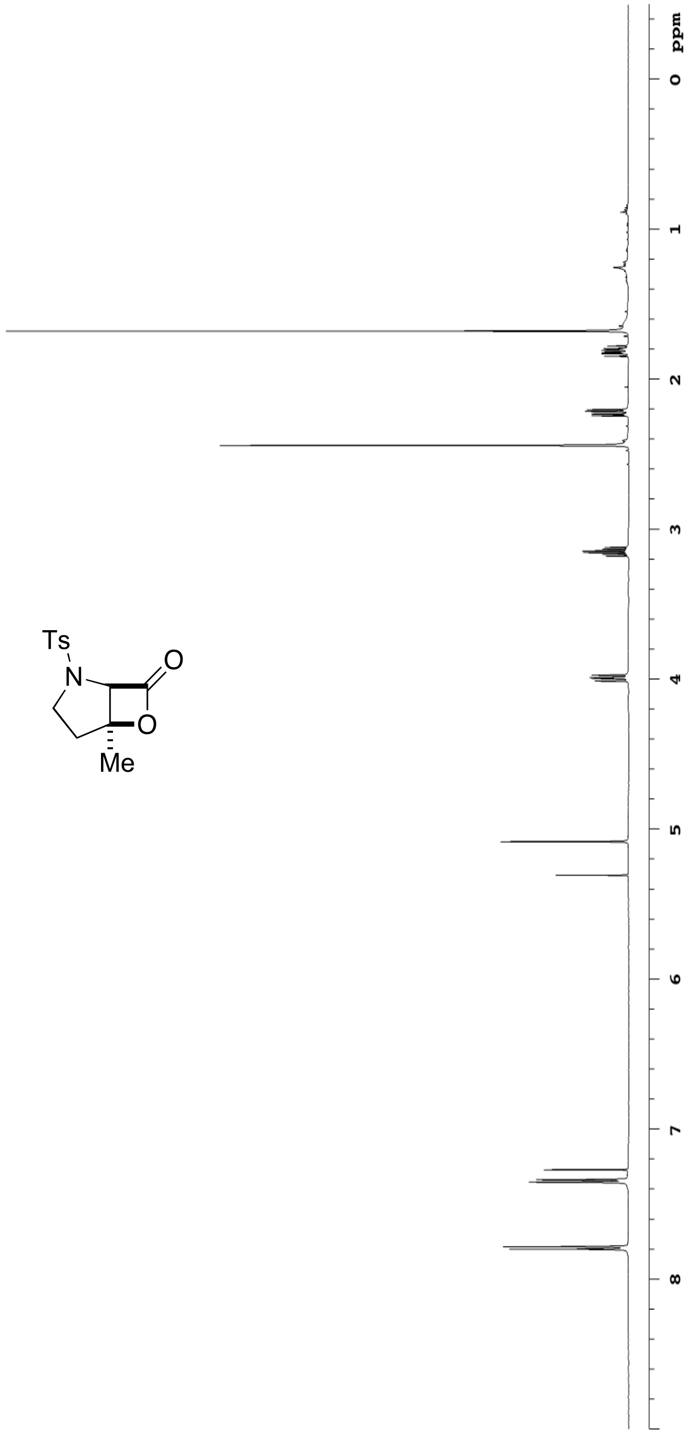
$^{13}$C-NMR (125 MHz) of **3-34** in $CDCl_3$



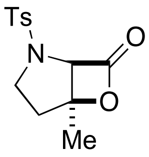
$^1$H-NMR (500 MHz) of **3-73** in $CDCl_3$

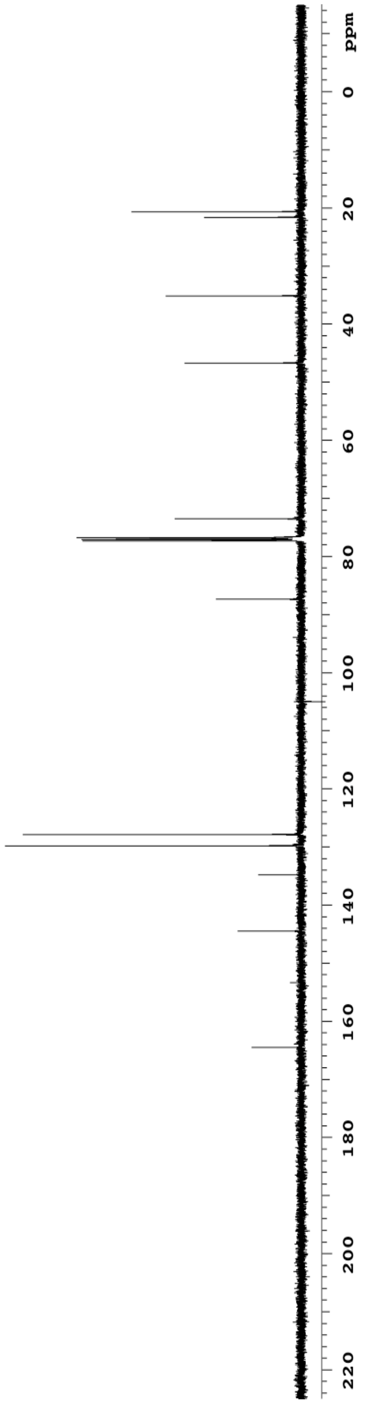


$^{13}$C-NMR (125 MHz) of **3-73** in $CDCl_3$

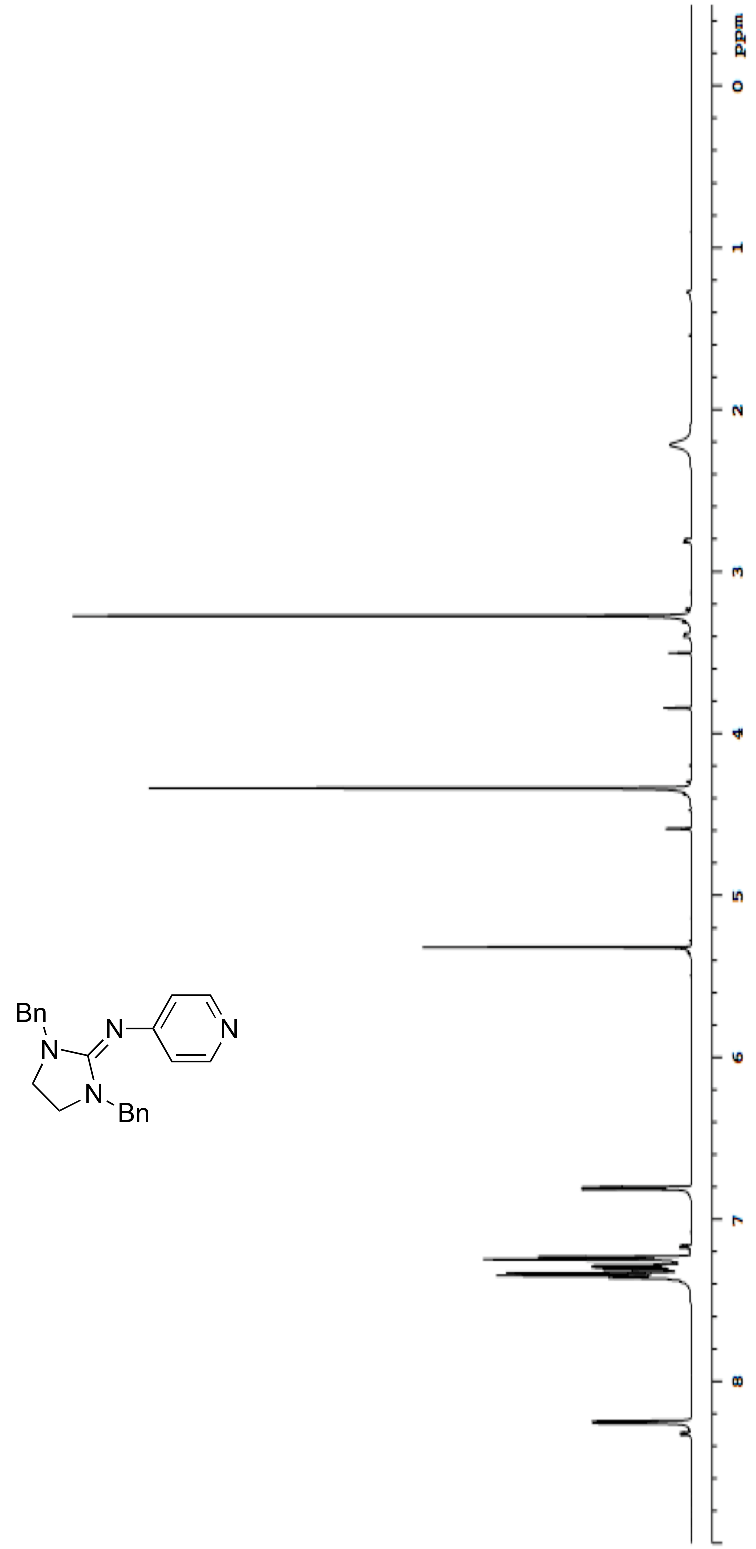


$^{1}$H-NMR (500 MHz) of **3-75** in $CDCl_3$

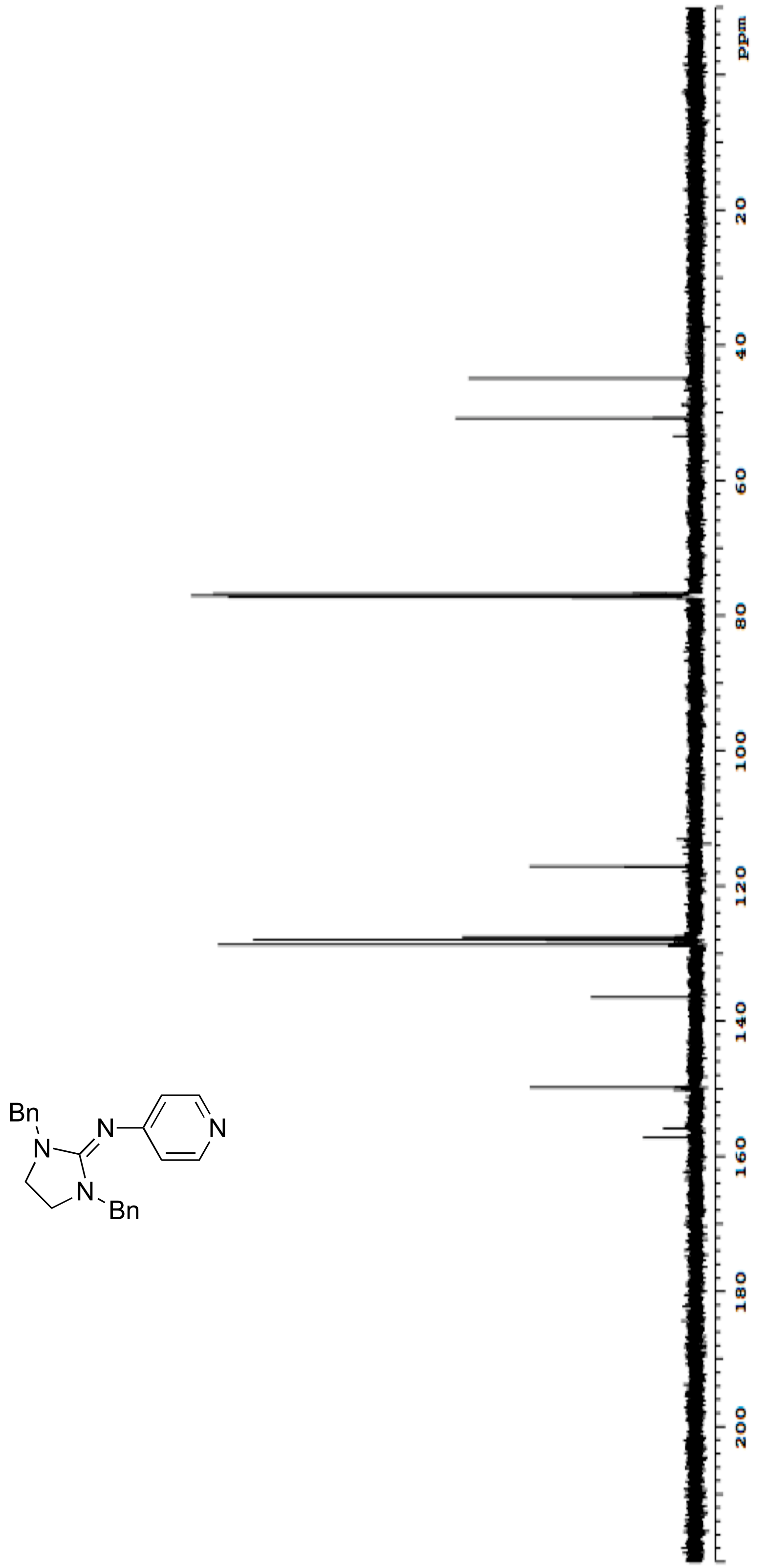




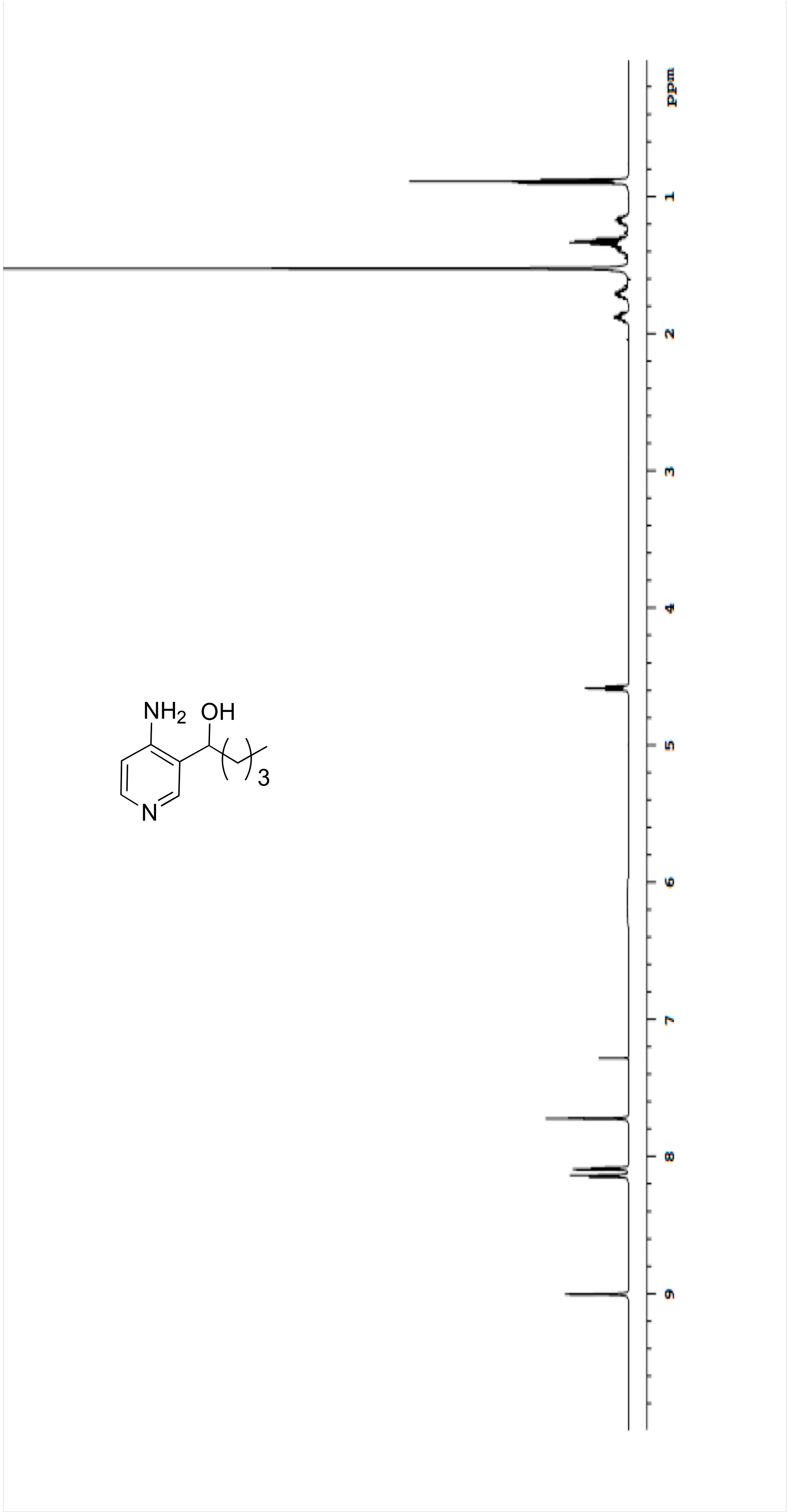
$^{13}$C-NMR (125 MHz) of **3-75** in $CDCl_3$



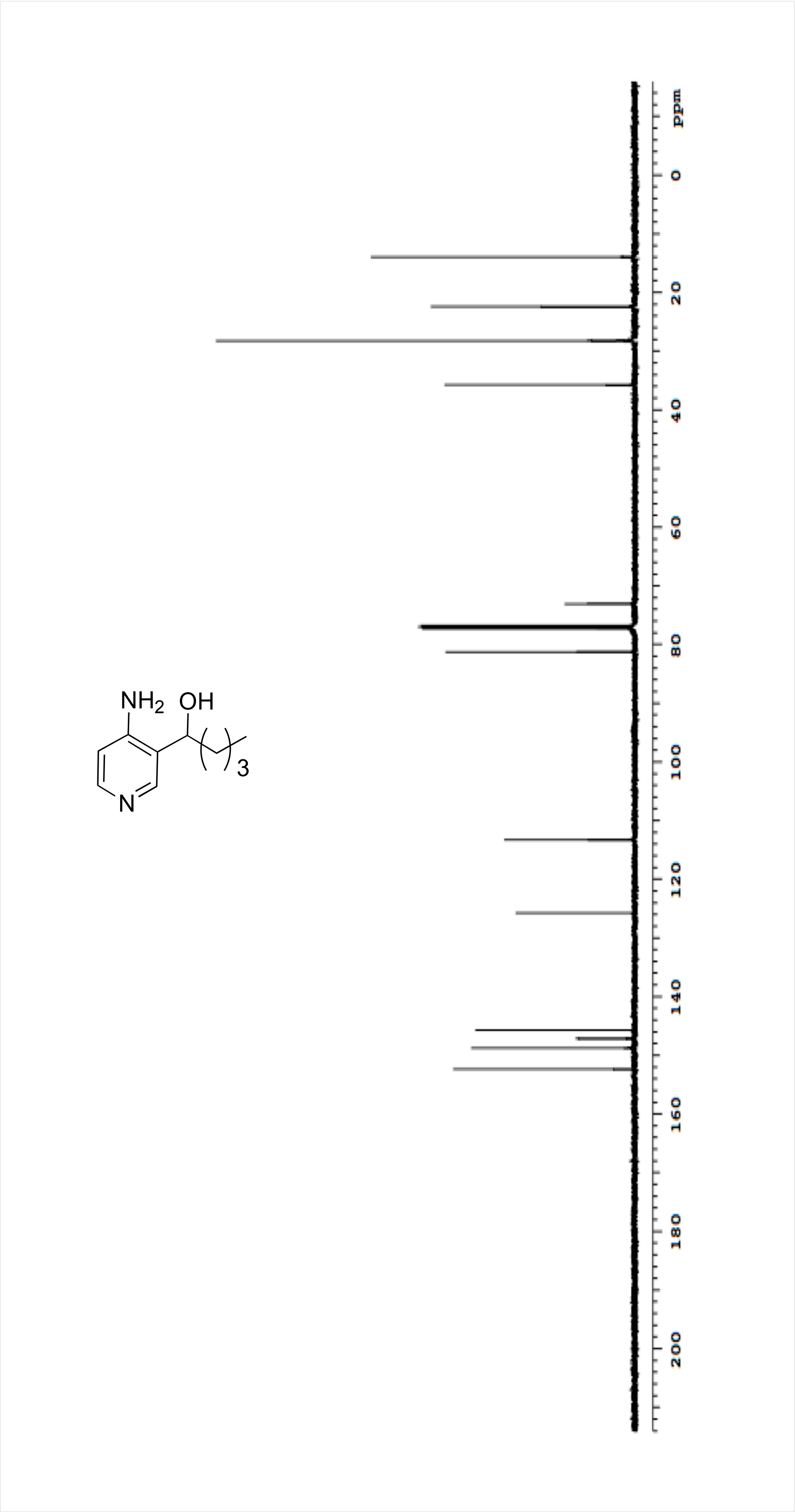
$^1$H-NMR (500 MHz) of **3-38** in $CDCl_3$



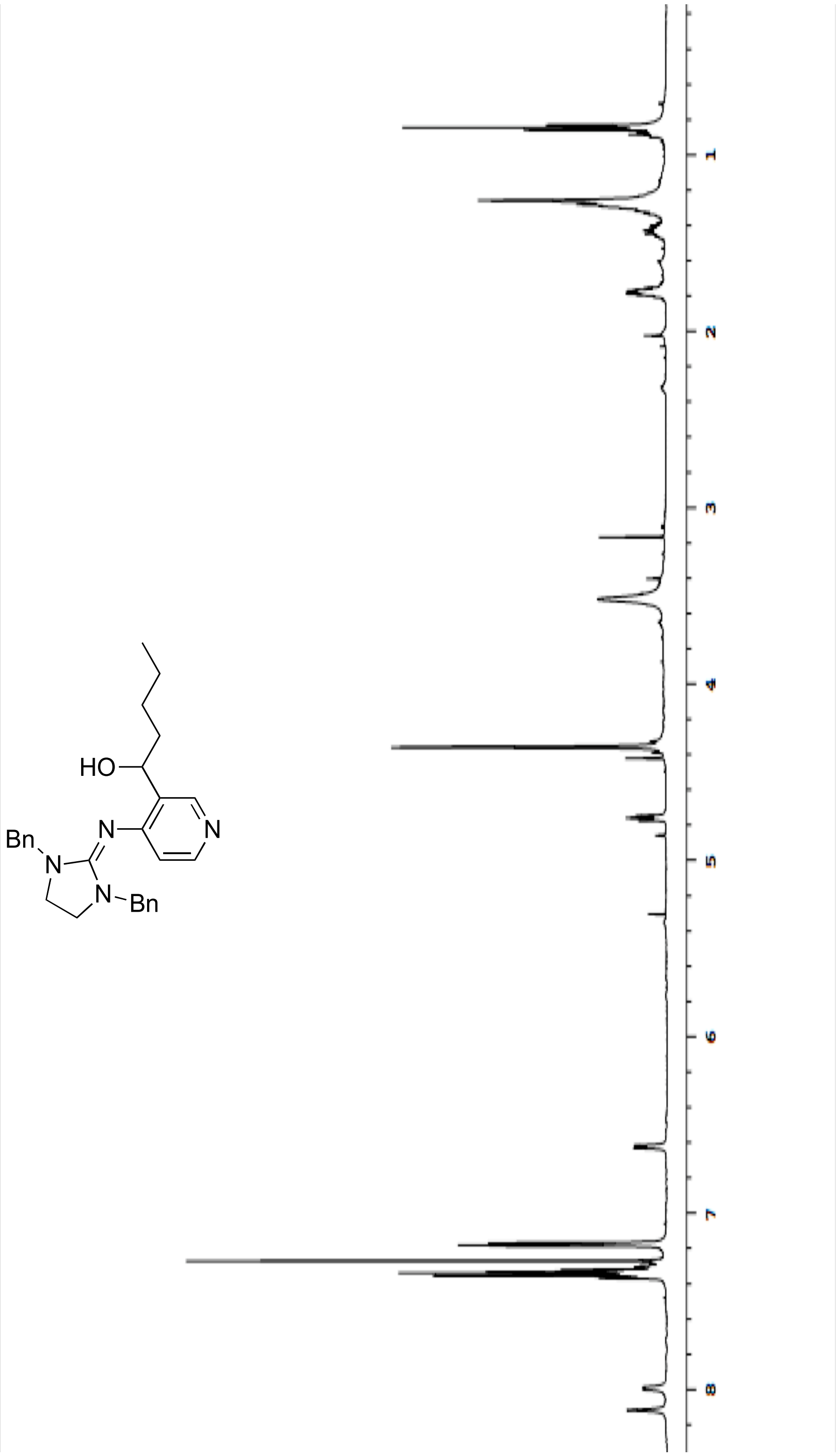
$^{13}$C-NMR (125 MHz) of **3-38** in $CDCl_3$



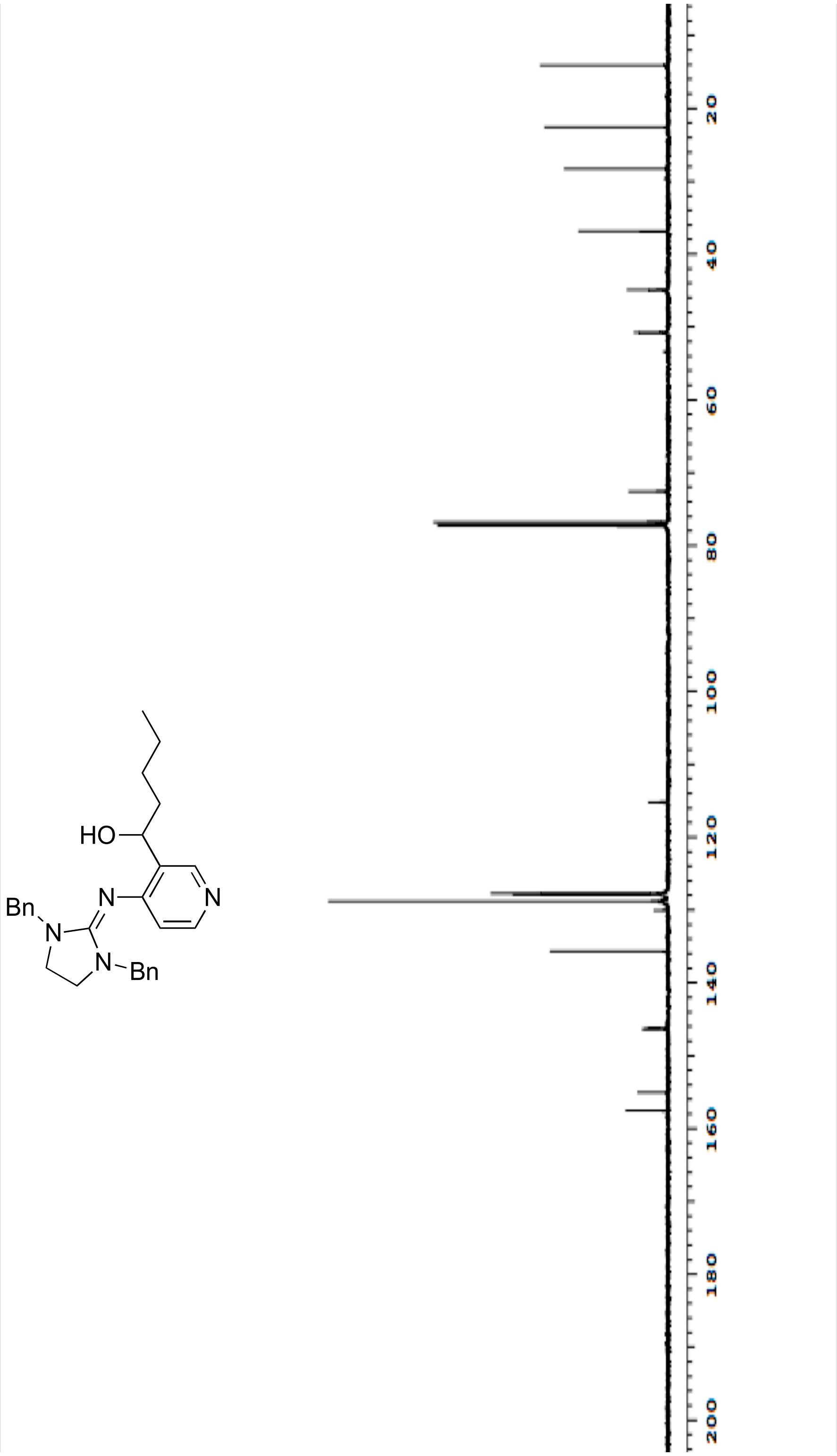
$^1$H-NMR (500 MHz) of **3-47** in $CDCl_3$



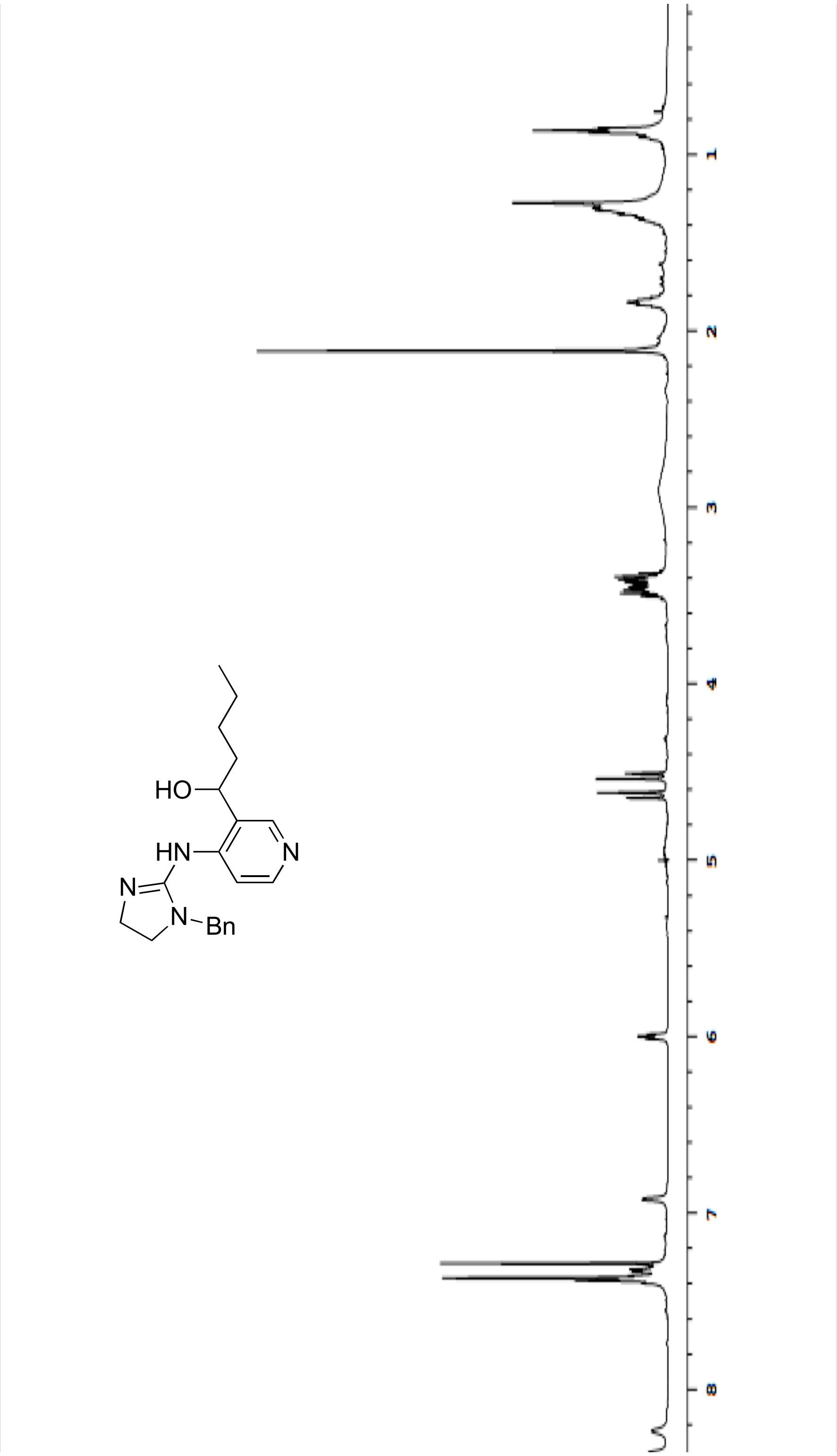
$^{13}$C-NMR (125 MHz) of **3-47** in $CDCl_3$



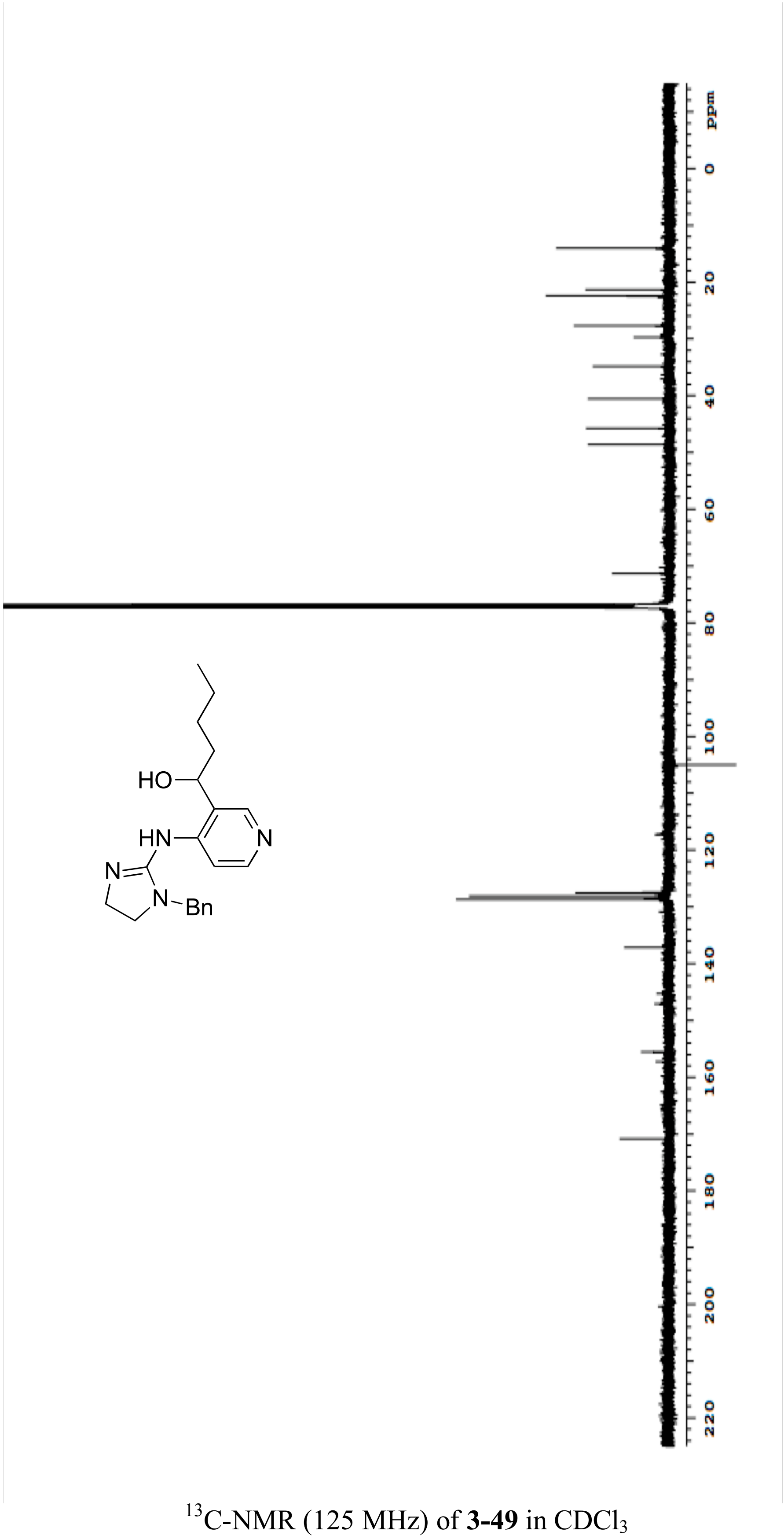
$^1$H-NMR (500 MHz) of **3-48** in $CDCl_3$



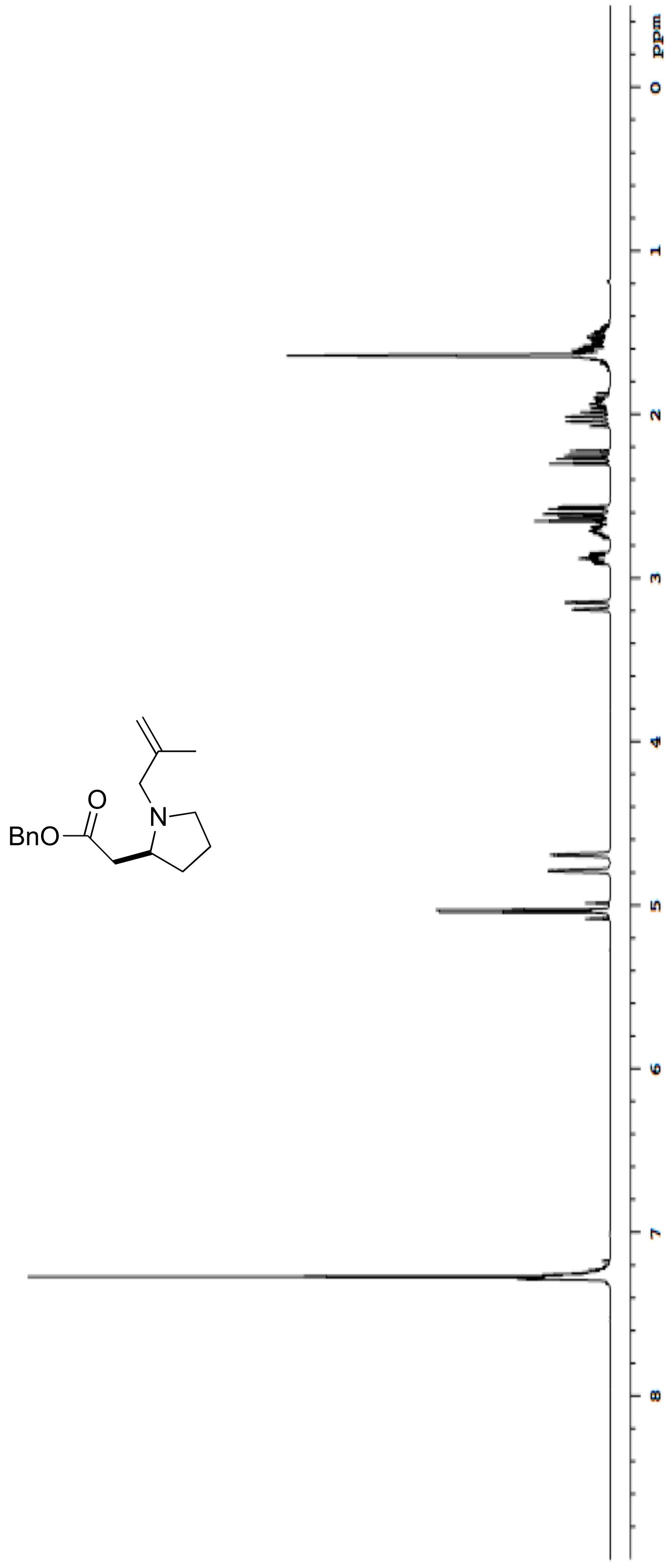
$^{13}$C-NMR (125 MHz) of **3-48** in $CDCl_3$



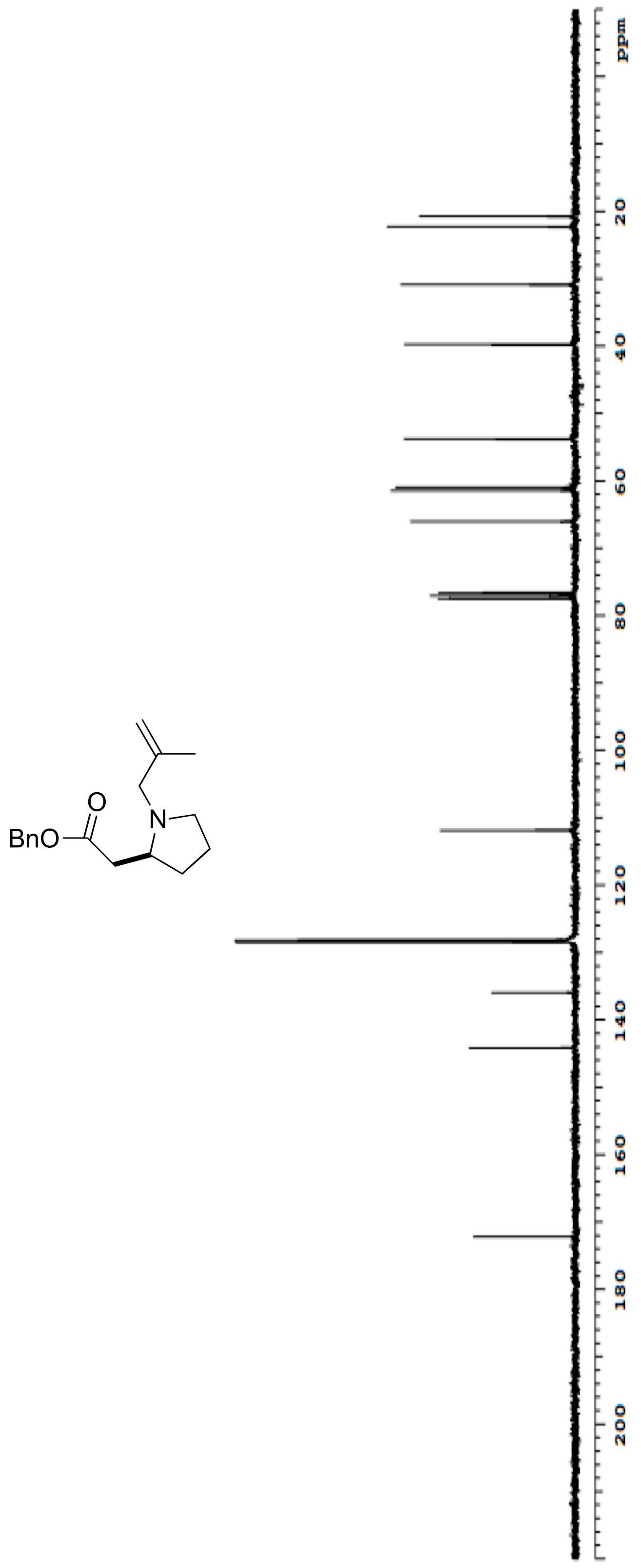
$^1$H-NMR (500 MHz) of **3-49** in CDCl$_3$



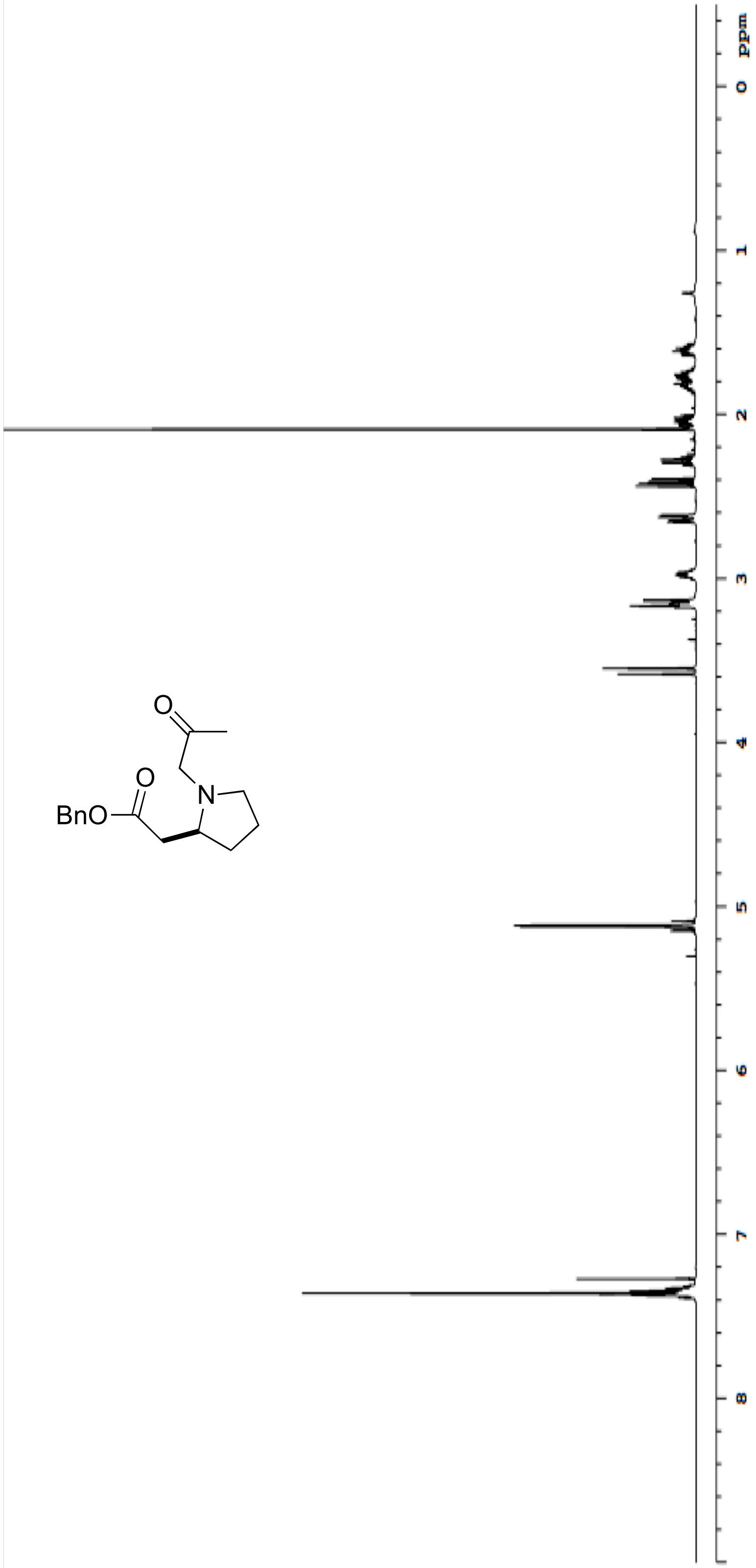
$^{13}$C-NMR (125 MHz) of **3-49** in CDCl$_3$



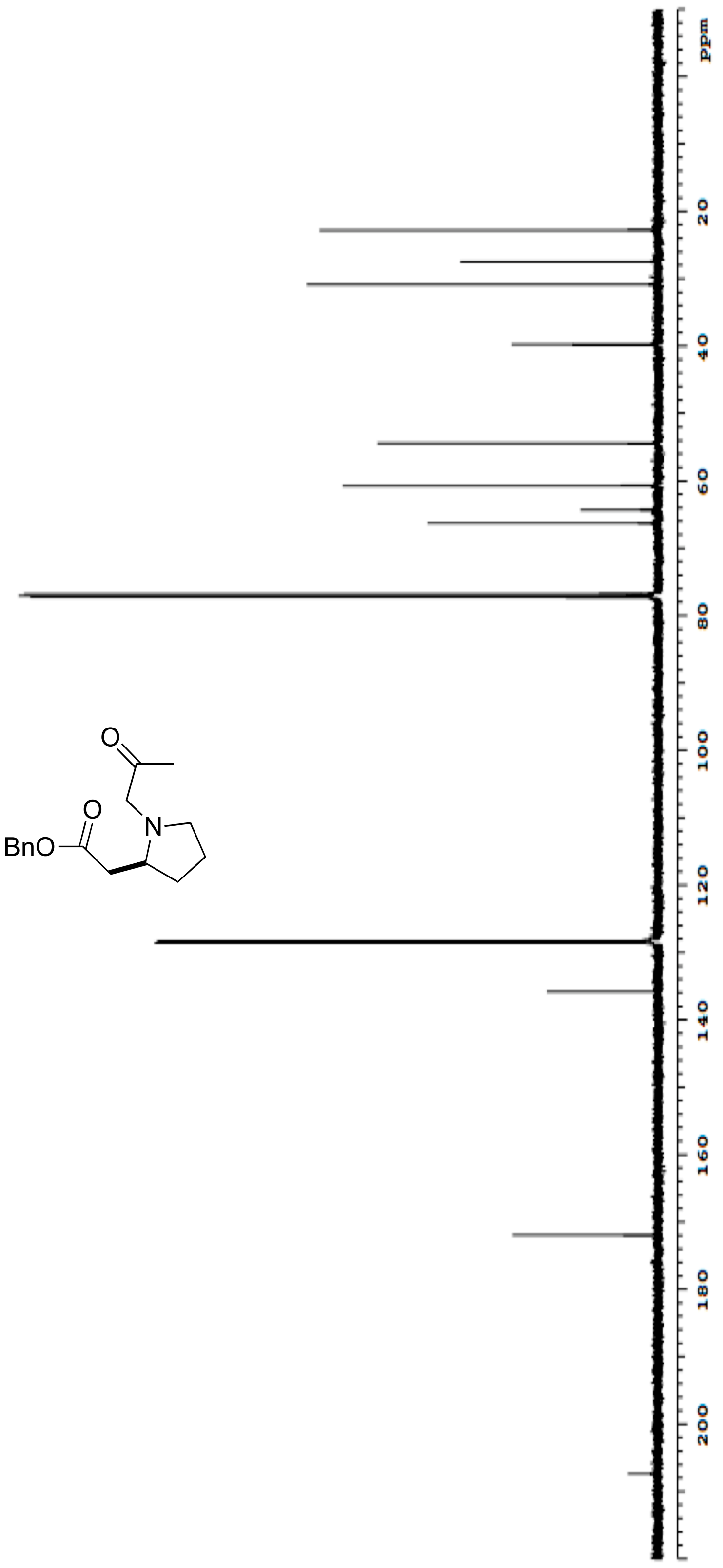
$^1$H-NMR (300 MHz) of **3-78** in $CDCl_3$



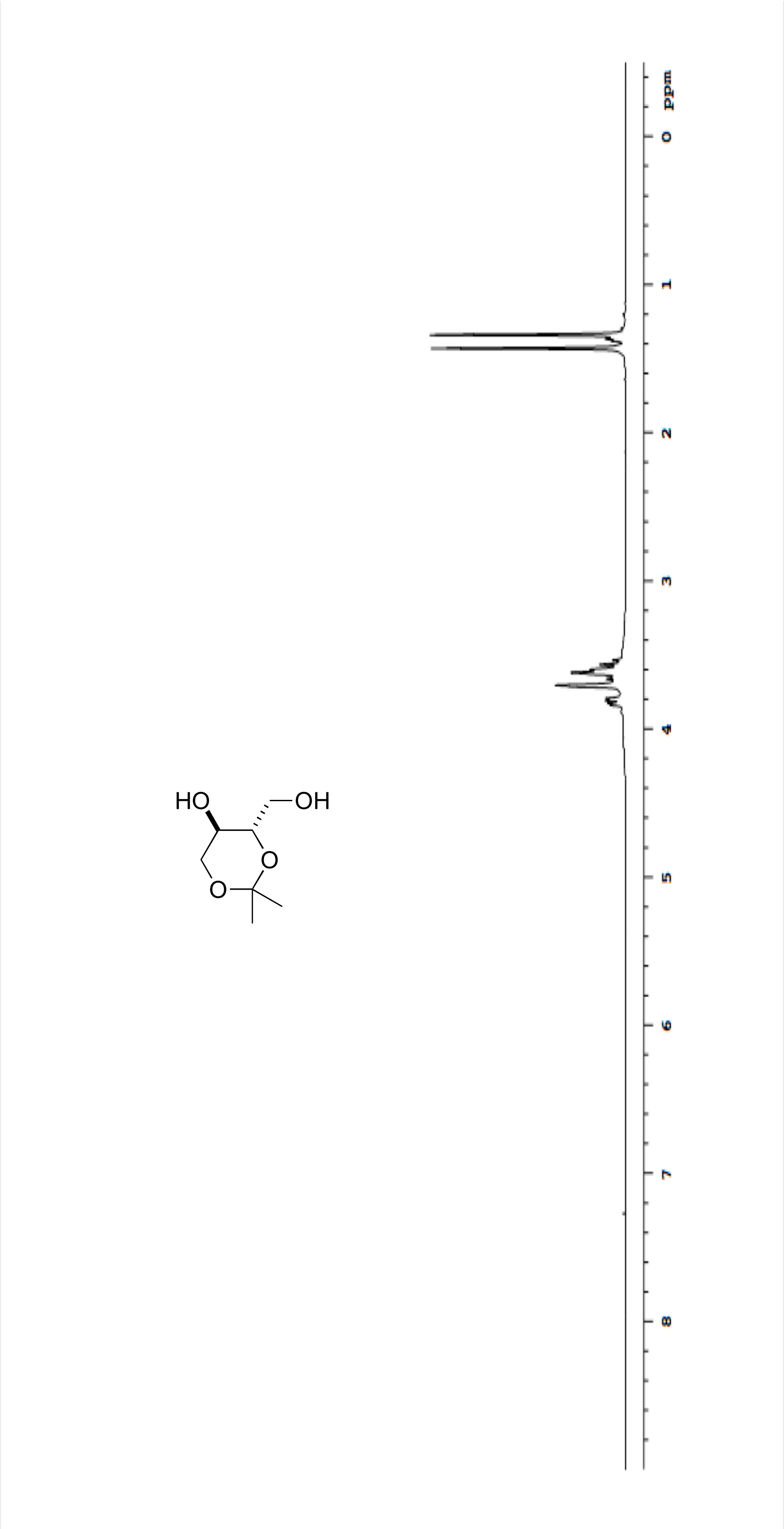
$^{13}$C-NMR (75 MHz) of **3-78** in $CDCl_3$



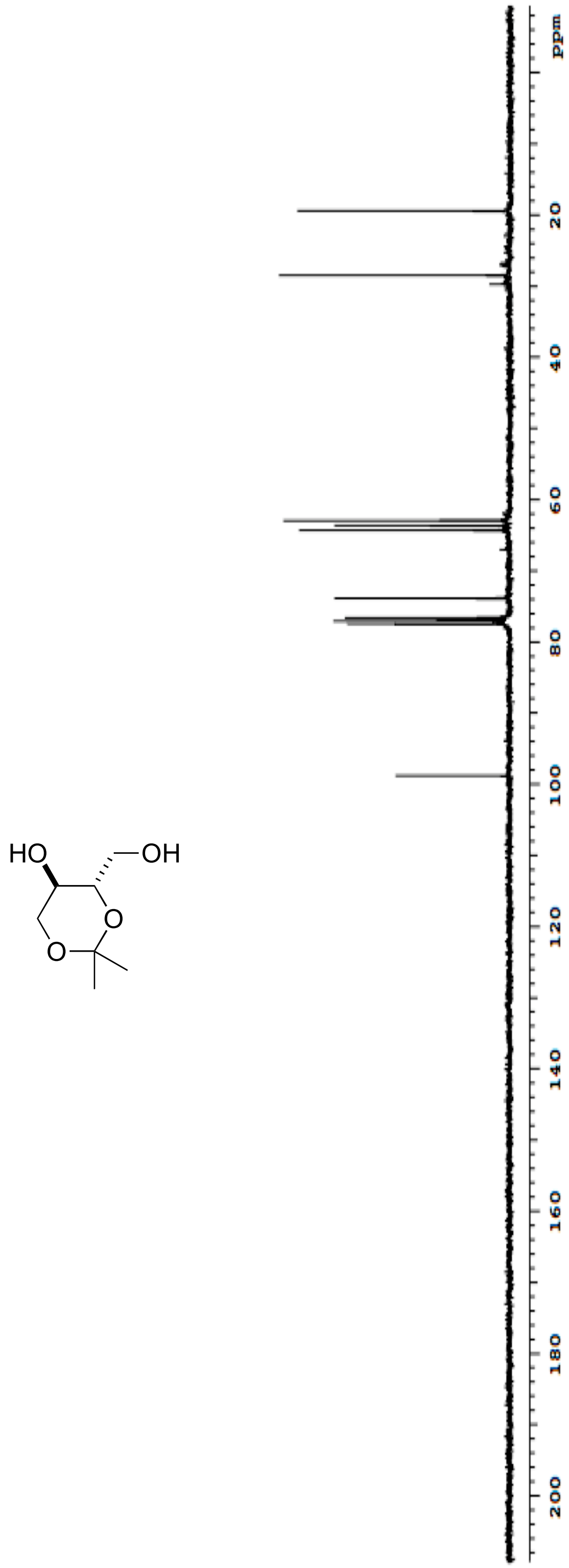
$^1$H-NMR (300 MHz) of **3-79** in $CDCl_3$



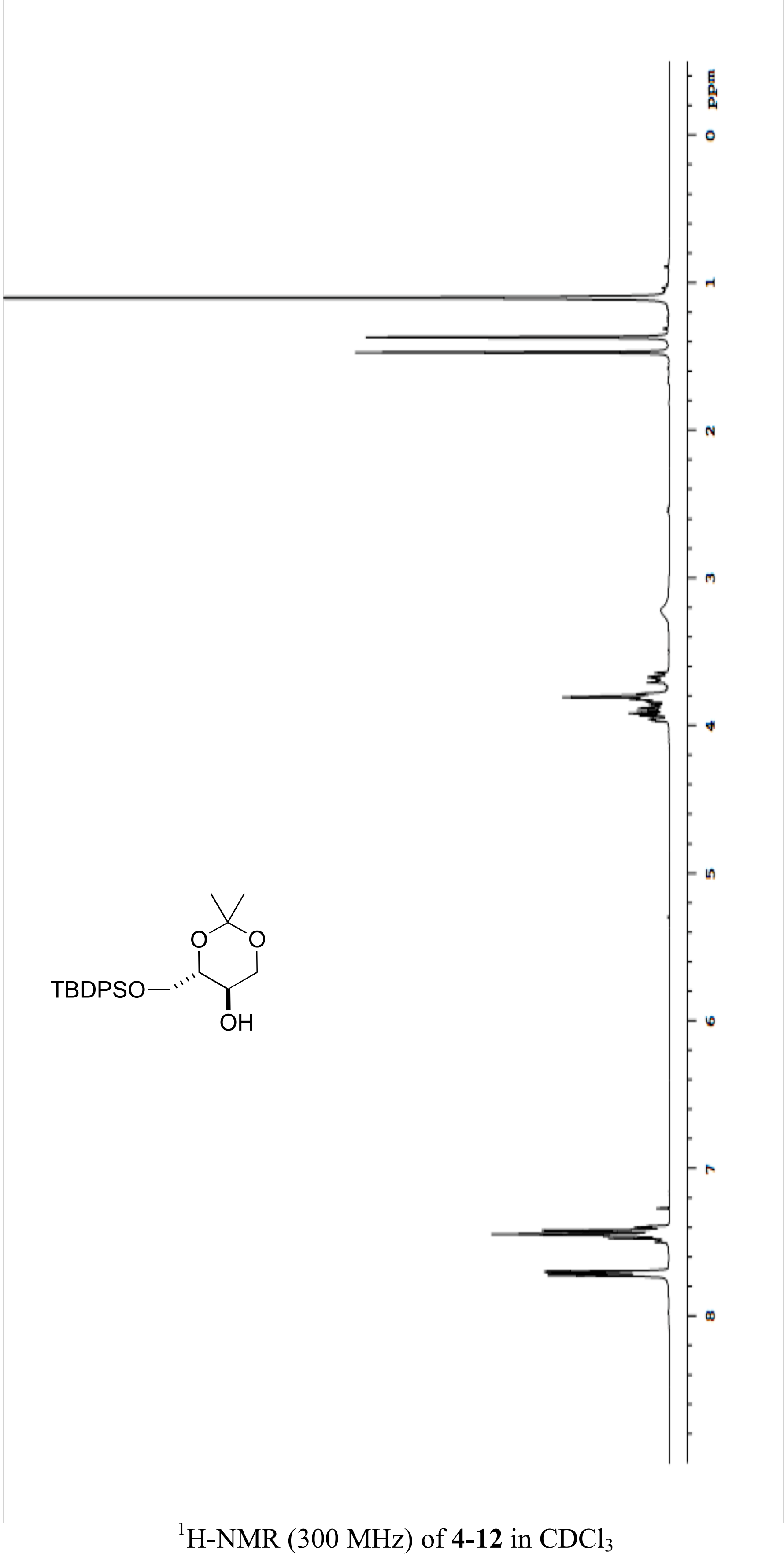
$^{13}$C-NMR (75 MHz) of **3-79** in $CDCl_3$



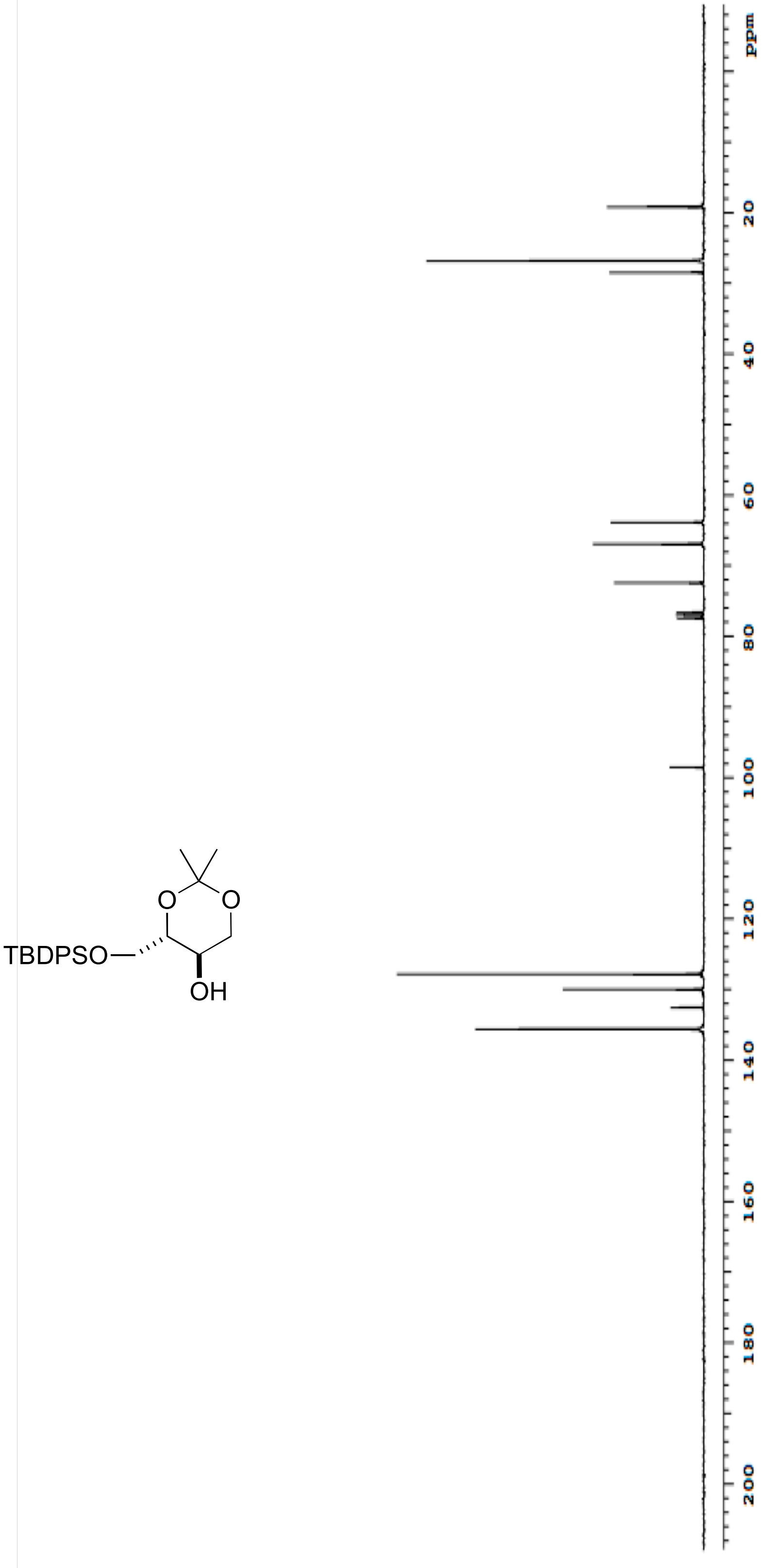
$^1$H-NMR (300 MHz) of **4-8** in $CDCl_3$



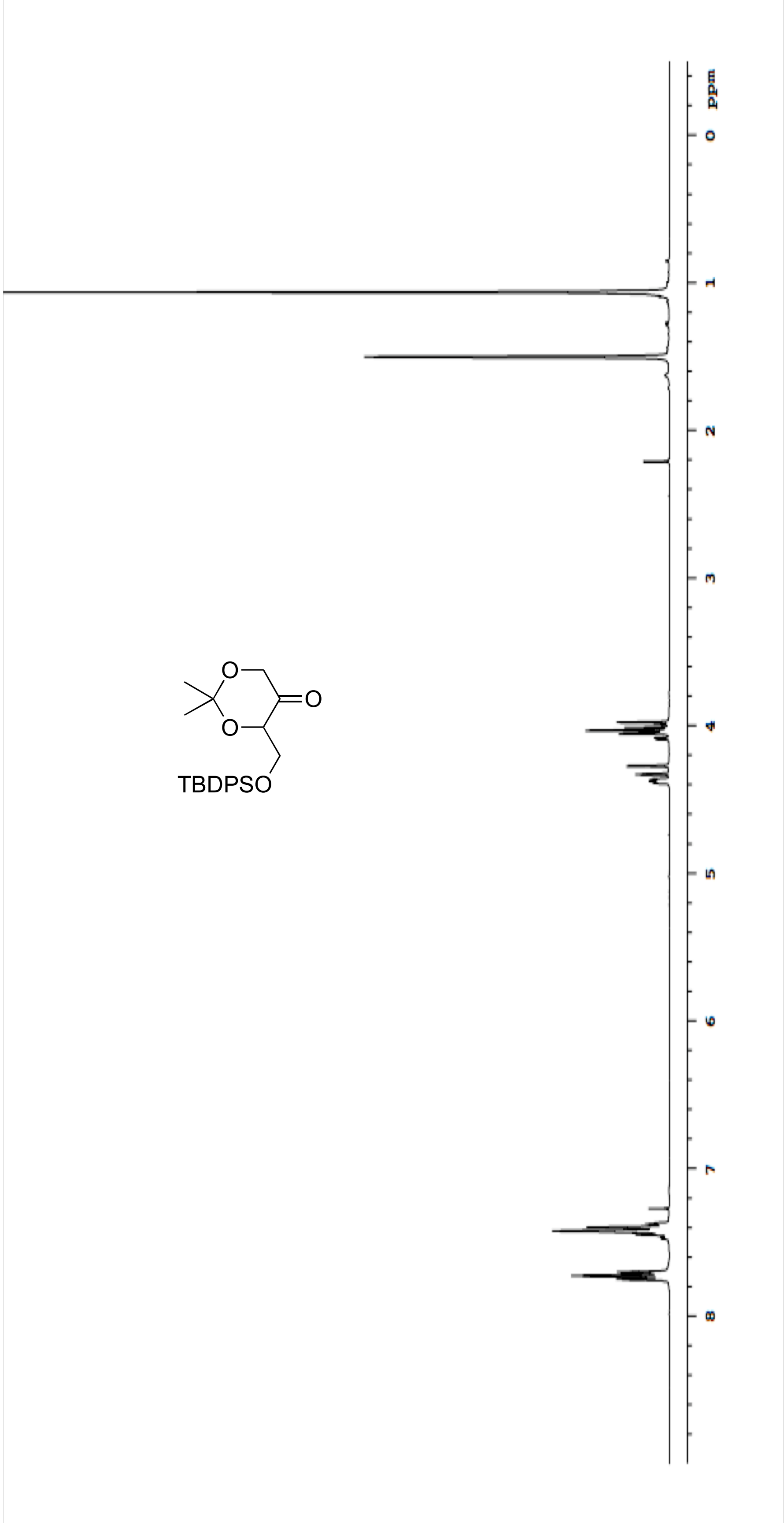
$^1$H-NMR (75 MHz) of **4-8** in CDCl$_3$



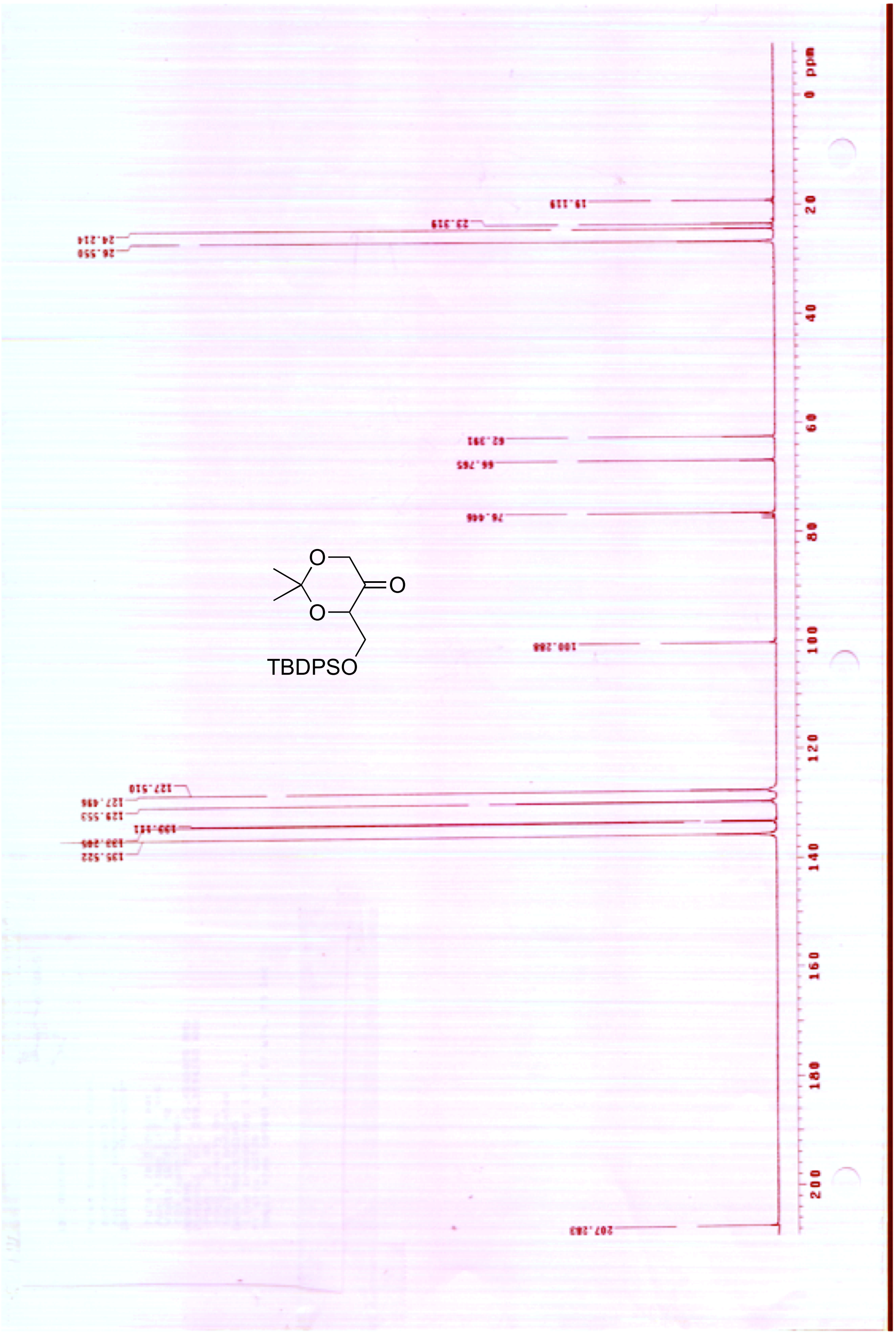
$^1$H-NMR (300 MHz) of **4-12** in $CDCl_3$



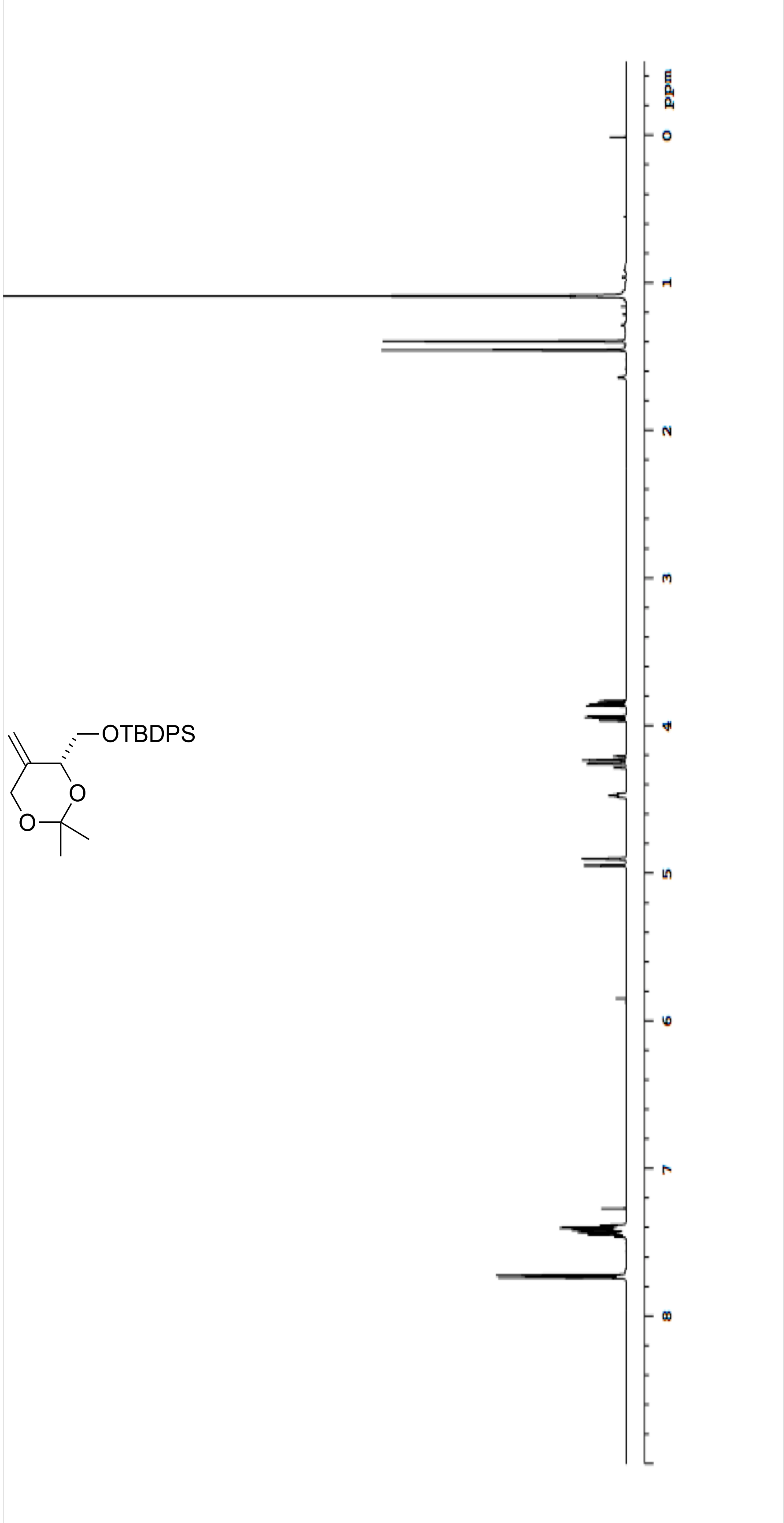
$^{13}$C-NMR (75 MHz) of **4-12** in $CDCl_3$



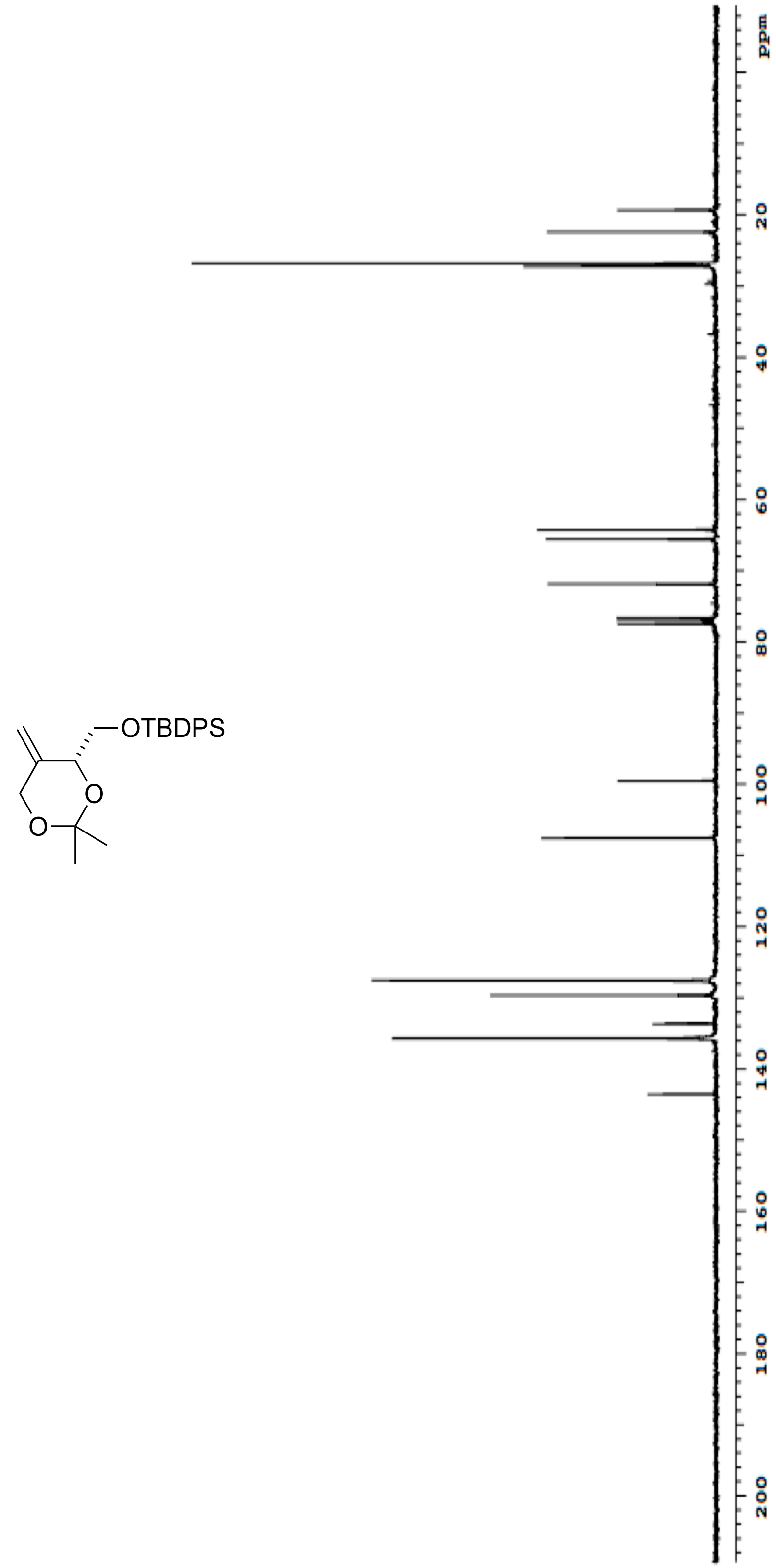
$^1$H-NMR (300 MHz) of **4-7** in $CDCl_3$



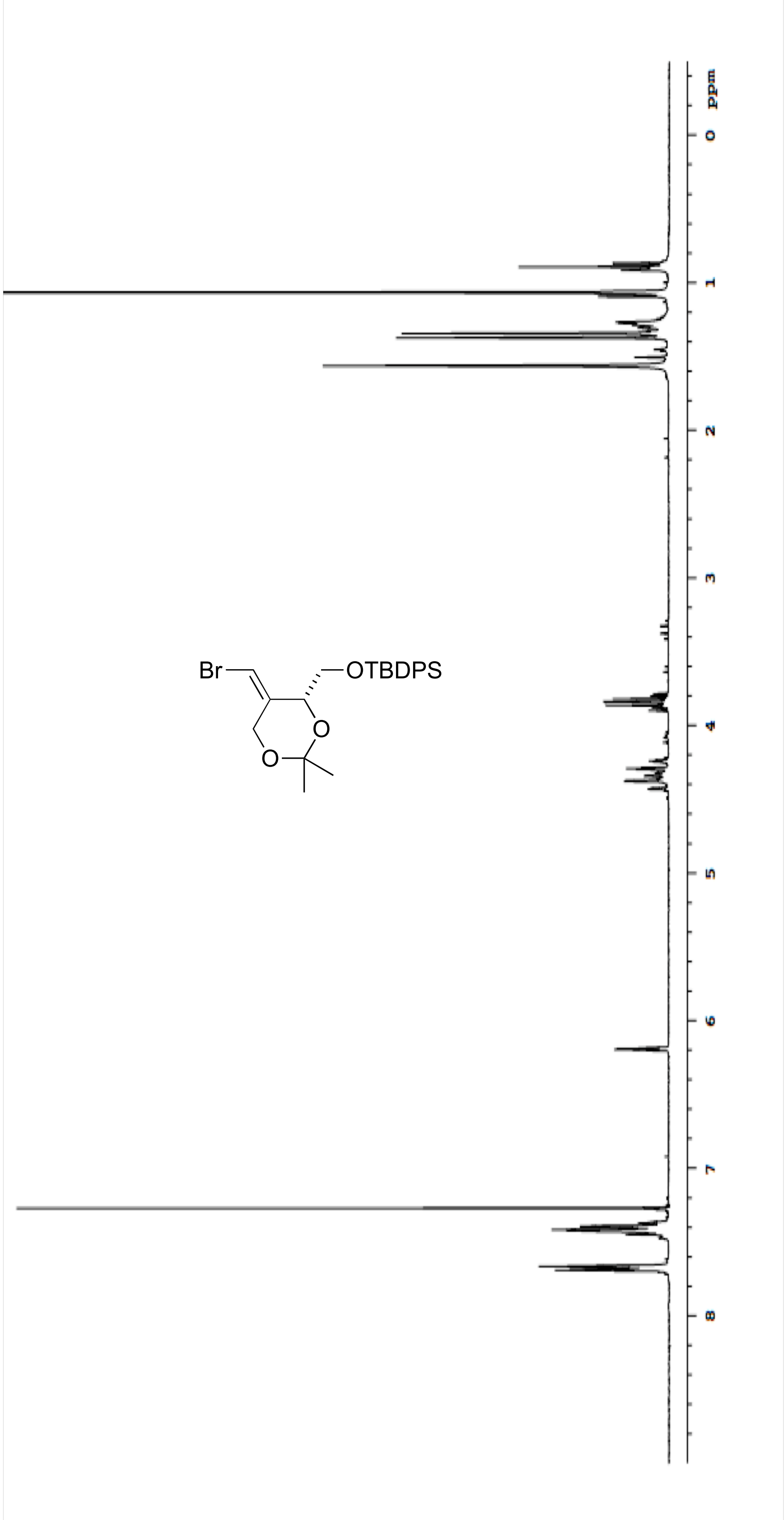
$^{13}$C-NMR (75 MHz) of **4-7** in $CDCl_3$



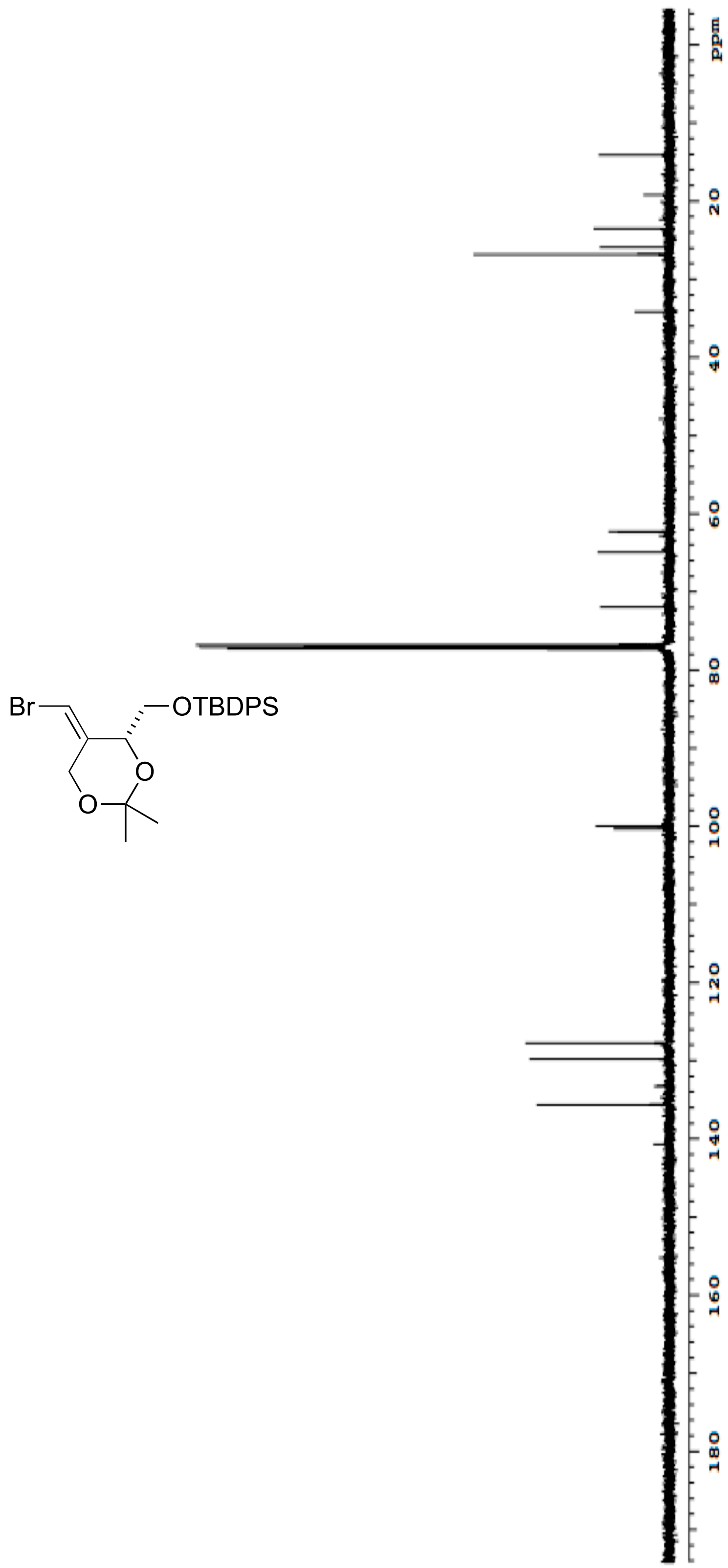
$^1$H-NMR (300 MHz) of **4-14** in $CDCl_3$



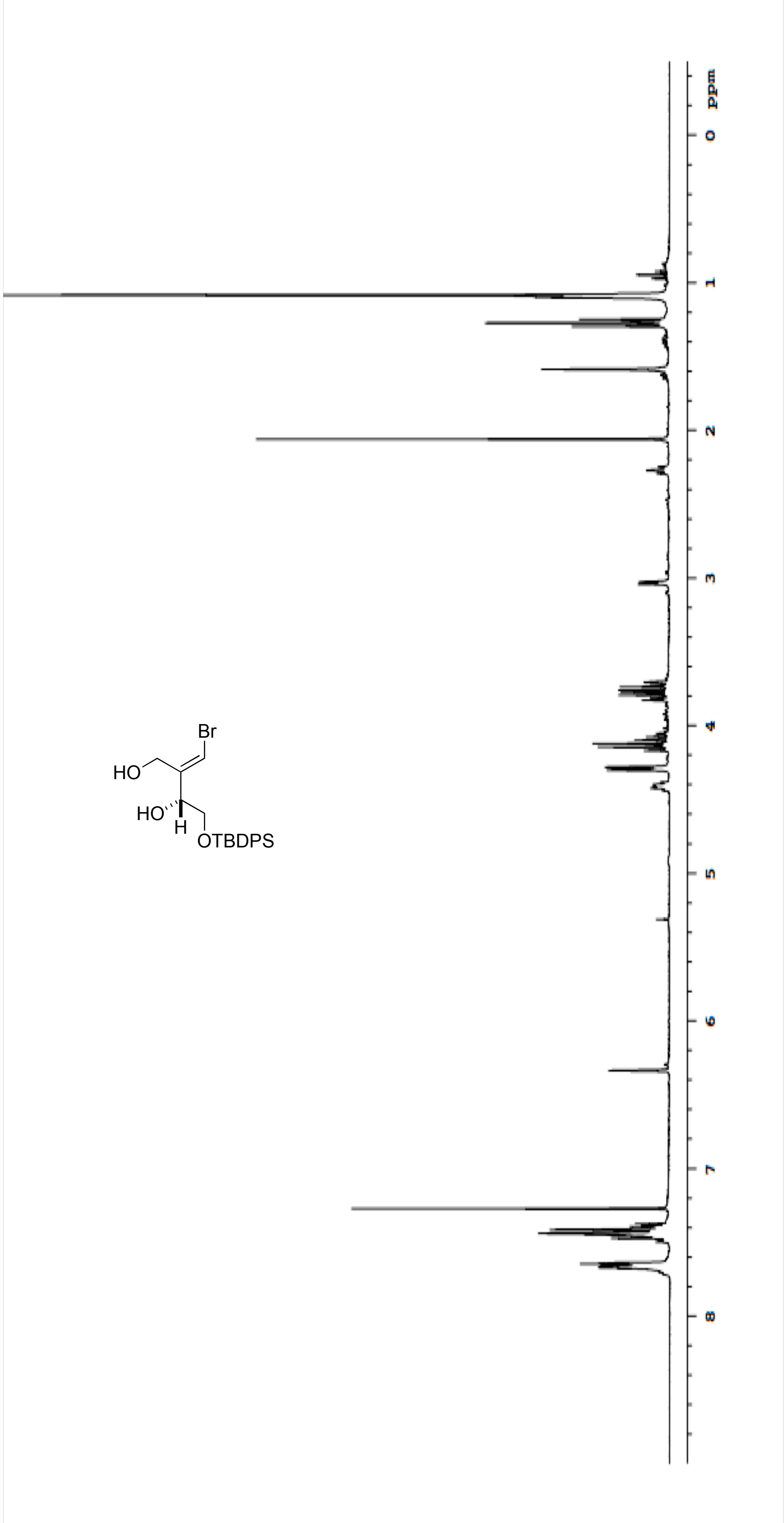
$^{13}$C-NMR (75 MHz) of **4-14** in $CDCl_3$



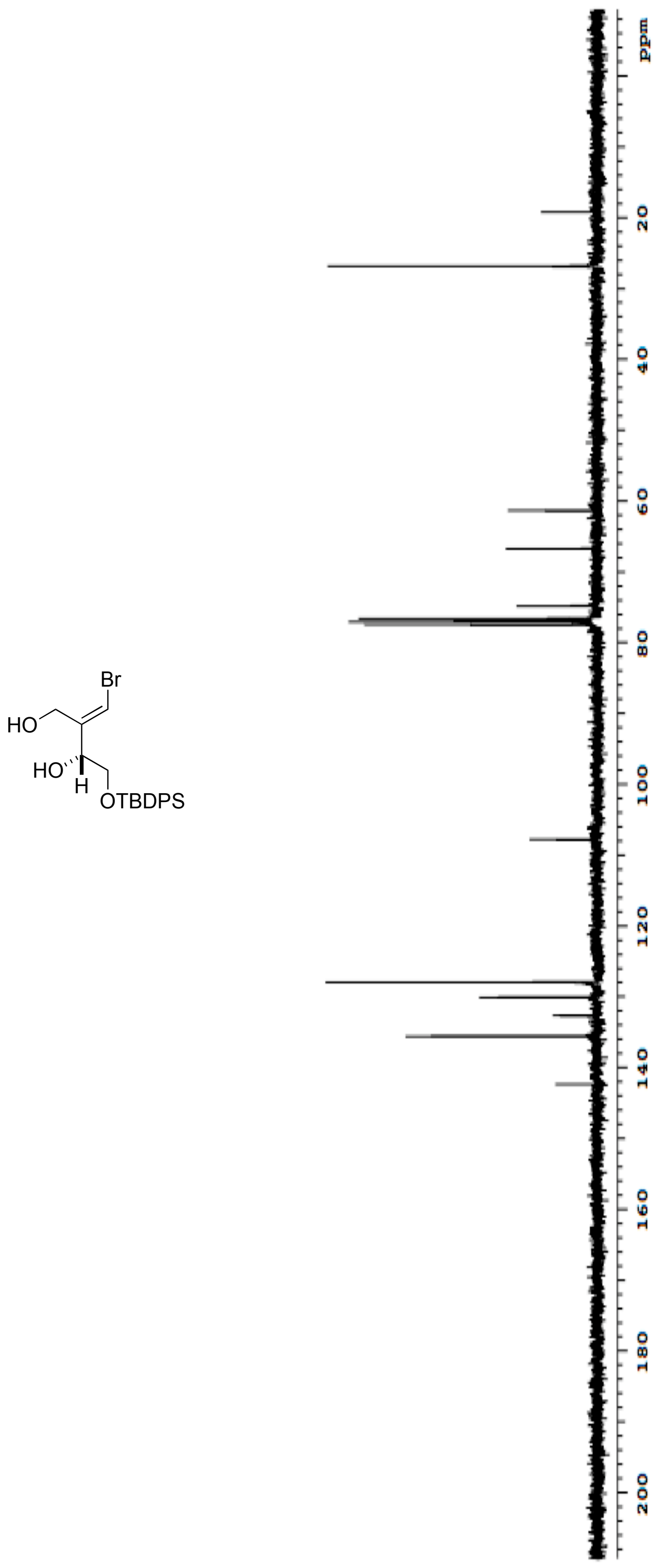
$^1$H-NMR (300 MHz) of **4-15** in $CDCl_3$



$^{13}$C-NMR (75 MHz) of **4-15** in $CDCl_3$



$^{1}$H-NMR (300 MHz) of **4-6** in $CDCl_3$



$^{13}$C-NMR (75 MHz) of **4-6** in $CDCl_3$

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**Publications** (a) Kong, K.; Romo, D.; Lee, C. Enantioselective Total Synthesis of the Marine Toxin (-)-Gymnodimine Employing a Barbier-Type Macrocyclization. *Angew. Chem., Int. Ed.* **2009**, *48*, 7402-7405.

(b) Henry-Riyad, H.; Lee, C.; Purohit, V. C.; Romo, D. Bicyclic and Tricyclic-β-lactones via Organonucleophile Promoted Bis-Cyclization of Keto Acids: Enantioselective Synthesis of (+)-Dihydroplakevulin. *Org. Lett.* **2006**, *8*, 4363-4366.