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# **Part I: A Concise Synthesis of Microtubule Inhibitor Tryprostatin A and B and Its Analogs Part II: Brønsted Acid Catalyzed Reactions of Aromatic Ketones with Ethyl Diazoacetate and Its Synthetic Scope**

Khorshada Jahan

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**PART I: A CONCISE SYNTHESIS OF MICROTUBULE INHIBITOR  
TRYPROSTATIN A AND B AND ITS ANALOGS**

**PART II: BRØNSTED ACID CATALYZED REACTIONS OF  
AROMATIC KETONES WITH ETHYL DIAZOACETATE AND ITS  
SYNTHETIC SCOPE**

by

**Khorshada Jahan**

A Dissertation Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

in Chemistry

**at**

**The University of Wisconsin-Milwaukee**

**December 2021**

## ABSTRACT

### **PART I: A CONCISE SYNTHESIS OF MICROTUBULE INHIBITOR TRYPROSTATIN A AND B AND ITS ANALOGS**

### **PART II: BRØNSTED ACID CATALYZED REACTIONS OF AROMATIC KETONES WITH ETHYL DIAZOACETATE AND ITS SYNTHETIC SCOPE**

by

Khorshada Jahan

The University of Wisconsin-Milwaukee, 2021  
Under the Supervision of Professor M. Mahmum Hossain

### **PART I: A CONCISE SYNTHESIS OF MICROTUBULE INHIBITOR TRYPROSTATIN A AND B AND ITS ANALOGS**

Microtubules are promising targets for treating cancer by stopping the cell division in mitosis(M). Tryprostatins A and B (TPS A and B) are one of the important alkaloids for treating cancer, via microtubule inhibition. Studied found that Tryprostatins A and B act as an antimitotic agent by inhibiting cell cycle progression of tsFT210 cells in the G2/M phase at a final concentration of 50 µg/ml of TPS A and 12.5 µg/ml of TPS B, respectively. Usually, Tryprostatins A and B are isolated from natural source *Aspergillus fumigatus* in trace amounts. Their interesting biological activity and simple structure have drawn attention from the synthetic community, and several total syntheses have been reported. But most of the synthetic procedures are lengthy and overall yield of the product is very low. This feature makes these compounds very expensive. The cost of Tryprostatin A is \$208 for 500µg, and Tryprostatin B is \$900 for 5mg. Here, we report a concise

and efficient total synthesis of Tryprostatin A and B in only four steps with overall yield 40% and 57% respectively. The key step of our reaction was the alkylation of diprenylated gramine salt with diketopiperazine core in the presence of quinine. Also, in our synthesis, we modify the Krapcho decarboxylation reaction by using Dimethylacetamide (DMA) as a solvent in place of DMSO/DMF. Additionally, to make Tryprostatin analogs, we synthesized a series of C2, N-dialkylated gramine salt by direct lithiation of N-protected gramine with alkyl halide and studied the reaction mechanism and synthetic scope of the reaction. We hope that one of these derivatives will be selective against cancer cells, with therapeutic concentrations in the nanomolar region.

## **PART II: BRØNSTED ACID CATALYZED REACTIONS OF AROMATIC KETONES WITH ETHYL DIAZOACETATE AND ITS SYNTHETIC SCOPE**

3-hydroxy acrylates and related 3-oxo-esters are interesting precursors not only for biomedical and pharmaceutical products such as contact lenses, dental materials, carriers for controlled drug delivery, and hydrogels but also for the monomeric building block for polymers. Again, chemical modifications of 3-oxo esters and 3-hydroxy acrylates help us to synthesize novel drugs such as BRL-37959 (an analgesic), naproxen (painkiller), horsfiline (painkiller), coerulecine (an analgesic) as well as indole moiety and molecules bearing all-carbon quaternary stereocenters. In 1998 and later in 2004, our group reported the formation of 3-hydroxy-2-aryl acrylates by 1,2-aryl migration from the reactions of aromatic aldehydes with ethyl diazoacetate (EDA) in the presence of the iron Lewis acid catalyst and the Brønsted acid type catalyst, respectively. In this project, we extended our method for the formation of 3-hydroxy acrylates and related 3-oxo-esters by using



less reactive aromatic and aliphatic ketones with EDA in the presence of Brønsted acid catalyst. Depending on the migratory aptitude of alkyl and aryl groups, a wide range of 3-hydroxy acrylates and related 3-oxo-esters are formed which can be effectively used to form a broad range of products extending from medicinal compounds to synthetic materials.

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To

My Beloved Parents

and

My husband Md. Shahariar Rahmat Ullah

and children, Rafa and Roha

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## LIST OF ABBREVIATIONS

ACN	Acetonitrile
Boc	<i>t</i> -Butyloxycarbonyl
DCM	Dichloromethane
DCE	1, 2-Dichloroethane
DEA	Diethylamine
DIPEA	<i>N, N</i> -Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DME	Dimethoxyethane
DMA	Dimethylacetamide
EDA	Ethyl diazoacetate
EtOAc	Ethyl acetate
Et <sub>2</sub> O	Diethyl ether
<i>i</i> PrOH	Isopropyl alcohol
MeOH	Methanol
DMSO	Dimethyl sulphoxide
PTC	Phase-transfer catalyst
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
TEA	Triethylamine
THF	Tetrahydrofuran

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**PART I: A CONCISE SYNTHESIS OF MICROTUBULE INHIBITOR  
TRYPROSTATIN A AND B AND ITS ANALOGS**

## **1.1. INTRODUCTION**

### **1.1.1. Mammalian Cell Cycle**

The Cell cycle is a highly regulated process that is responsible for the appropriate division of a cell into two daughter cells<sup>1</sup>. The cell cycle combines DNA replication with chromosomal segregation in an oscillatory manner. In this way, it ensures that the duplicated genetic material is distributed equally to each daughter cell. This process is classically described as four sequential phases that progress from quiescence (G0 phase) to proliferation (G1, S, G2, and M phases), and then back to the G0 phase (**Figure 1.1**). In the cell cycle, proliferation consists of two distinct phases: mitosis (M), in which a cell undergoes cell division, and interphase, which comprises G1 (pre-DNA synthesis), S (DNA synthesis), and G2 (pre-division) phases<sup>2</sup>. Following interphase, the cell returns to the G0 phase (quiescence). G0 is typically used to describe cells that are not in the cell cycle but have the potential for division. Cells in G0 account for the majority of non-growing or non-proliferating cells. Cells can enter G1 from the quiescent state G0 if they are proliferating or are otherwise activated by mitogenic stimuli. The G1 phase is the first step in cell cycle progression. Cells in the S phase synthesize DNA and have DNA content between 2N and 4N. If the chromosomes are correctly duplicated, cells can enter G2 to prepare for the M phase, during which the cell divides into two separate daughter cells. Cell proliferation is necessary for the growth, development, and regeneration of eukaryotic organisms; however, it also causes one of the most devastating diseases of this era—cancer.

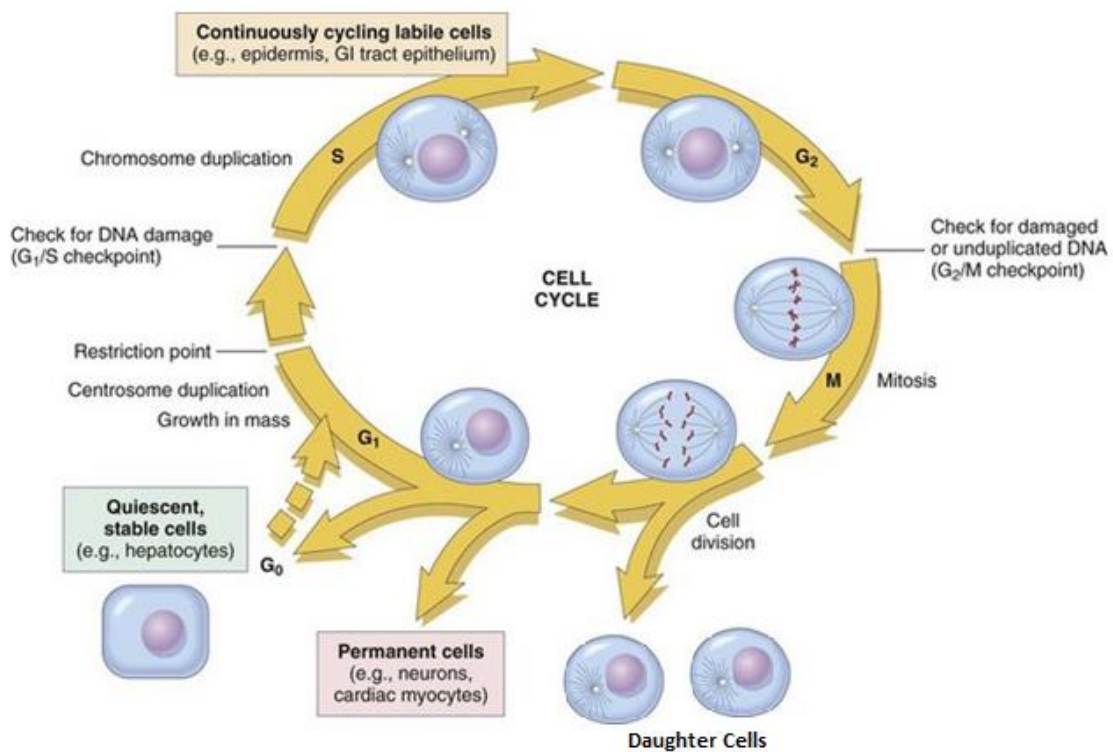


Figure 1.1: The mammalian cell cycle and its phases

### 1.1.2. Microtubules

Microtubules are noncovalent protein polymers in the form of hollow cylindrical filaments (approximately 25 nm in diameter). They are typically formed by 13 liners and parallel protofilaments. Each of the protofilaments consists of  $\alpha$ - and  $\beta$ -tubulin heterodimers arranged in a head-to-tail manner and confers polarity on the microtubule with one end ringed with  $\alpha$ -tubulin, and the other end ringed with  $\beta$ -tubulin (**Figure 1.2**).<sup>3</sup> The subunit at the minus (-) end of this structure is composed of  $\alpha$ -tubulin and that at the plus (+) end of  $\beta$ -tubulin.<sup>4</sup> Microtubule ends can undergo rapid polymerization and depolymerization by the reversible association and dissociation of  $\alpha/\beta$ -tubulin heterodimers at both ends. They behave in one of two different modes: dynamic

instability, displayed by the plus (+) end, and treadmilling, displayed at both ends. The microtubule-organizing center (MTOC) is a network of microtubule-associated proteins (MAP) to which the microtubules are attached. The (−) ends of microtubules are anchored to the MTOC, whereas the (+) ends are distal<sup>4</sup>. Each  $\alpha/\beta$  subunit has a guanosine triphosphate (GTP) binding site; termed the non-exchangeable site in  $\alpha$ -tubulin, and the exchangeable site in  $\beta$ -tubulin. When guanosine triphosphate (GTP) is bound to  $\beta$ -tubulin subunits at the plus end of a microtubule, other GTP-bound heterodimers readily add on to the end and the microtubule grows. The addition of a new dimer triggers the hydrolysis of GTP on the subunit that is no longer exposed, and guanosine diphosphate (GDP) is formed. The rate of tubulin addition is faster than the rate of GTP hydrolysis during polymerization, allowing the formation of a protofilament. However, if a GDP-bound subunit becomes exposed at the plus (+) end, perhaps through the departure of some subunits, will cause a conformational change resulting in the rapid depolymerization of the microtubule.<sup>3,4</sup> The stochastic switching of microtubules between periods of slow growth, rapid shortening, and attenuation (a pause in which neither growth nor shortening is detectable) is referred as Dynamic instability.<sup>3</sup> The transition from a growth stage to shortening is referred to as a “catastrophe”; transition from a shortening stage to growth is called a “rescue.” Treadmilling, however, signifies loss of subunits from the minus end and gain of subunits at the plus end, with no net change in the mass of the microtubule.<sup>5</sup> As mentioned earlier, microtubule dynamics are very important in mitosis during which the duplicated chromosomes are separated into two daughter cells. Disruption of microtubule dynamics by compounds that inhibit mitosis prevents cell cycle progression with arrest in the G2/M phase, eventually resulting in apoptotic cell death.<sup>4</sup>

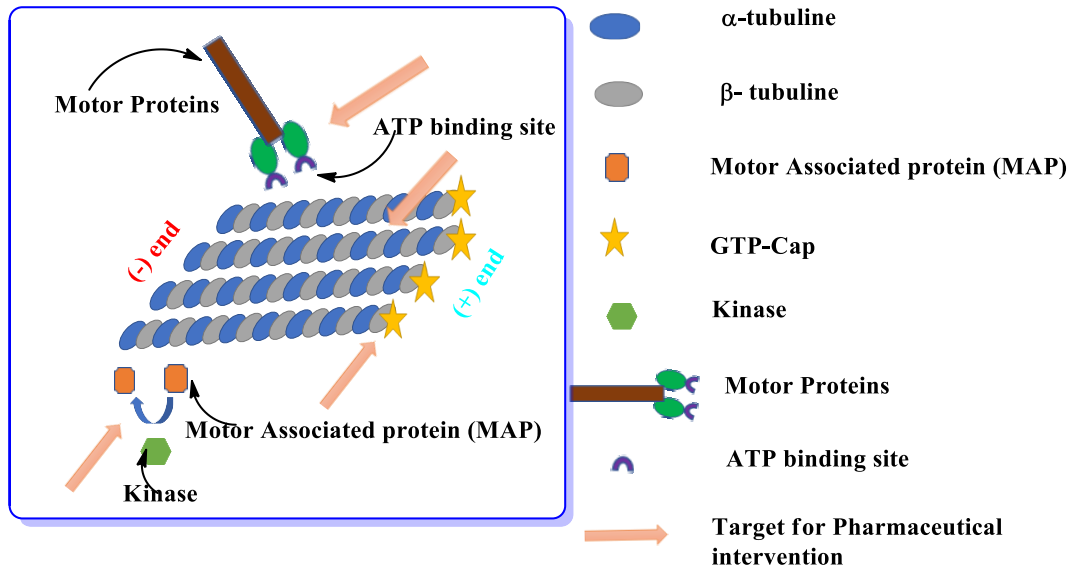


Figure 1.2: Cross sectional feature of Microtubules and its dynamics

### 1.1.3. Function of Microtubules in Cell Division

Microtubules are components of the cytoskeleton that play an important role in a variety of cellular functions such as intracellular transport, maintenance in shape, polarity, cell signaling and mitosis. In the mitosis (M) phase, there are four different sub-phases; prophase, metaphase, anaphase, and telophase (**Figure 1.3**).<sup>6</sup> The earliest sub-phase in mitosis phase is prophase, during prophase the chromatin condenses and membrane surrounding the nucleus disappears. Prophase is the early stage for spindle formation. In metaphase stage, telomeres appear, and the chromosomes line up on the equatorial plane. In anaphase the chromosomes divide and separate to opposite sides of the cell. During Telophase, the cell divides into two different cells. During mitosis, microtubules form the mitotic spindle that transports daughter chromosomes to separate poles of the dividing cell.<sup>7-11</sup>

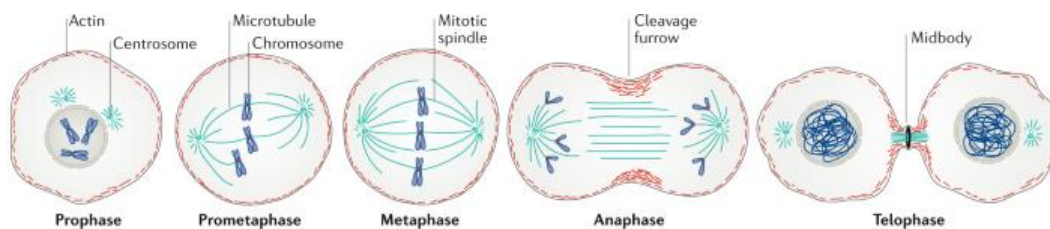


Figure 1.3: Different phases of mitosis and their functions

#### 1.1.4. How Microtubule inhibitor Work Against Cancer in Cell Cycle

Cancer is a broad term. It describes the disease that results when cellular changes cause the uncontrolled growth and division of cells. It can happen by faulty protein sequence or by damaged genes or DNA that regulate the cell cycle or mitosis. As mentioned earlier that in the cell cycle process, cells are entered into the four different phases: G1 phase, S-phase, G2-phase, and M-phase. And there are two different check points in the cell cycle, G1/S check point and G2/M check point (**Figure 1.4**). Anti-cancer drugs are designed based on these two check points, some drugs control the G1/S check point and stop DNA replication, and other drugs control the G2/M check point and stop cell division. Microtubule inhibitors (MTIs) bind with beta-tubulin at the G2/M check point and stop the early-stage spindle formation at metaphase stage.<sup>12</sup> Therefore, the tubulin binding process has become an important target in anticancer therapy. MTIs are classified into two groups: stabilizing and destabilizing agents. At low concentrations, however, both stabilizers and destabilizers suppress microtubule dynamics without changing polymer mass. Microtubule-targeting agents also can cause vascular disruption, and can target tumor vasculature, killing cancer cells that are resistant to conventional chemotherapy and radiotherapy.<sup>13</sup>

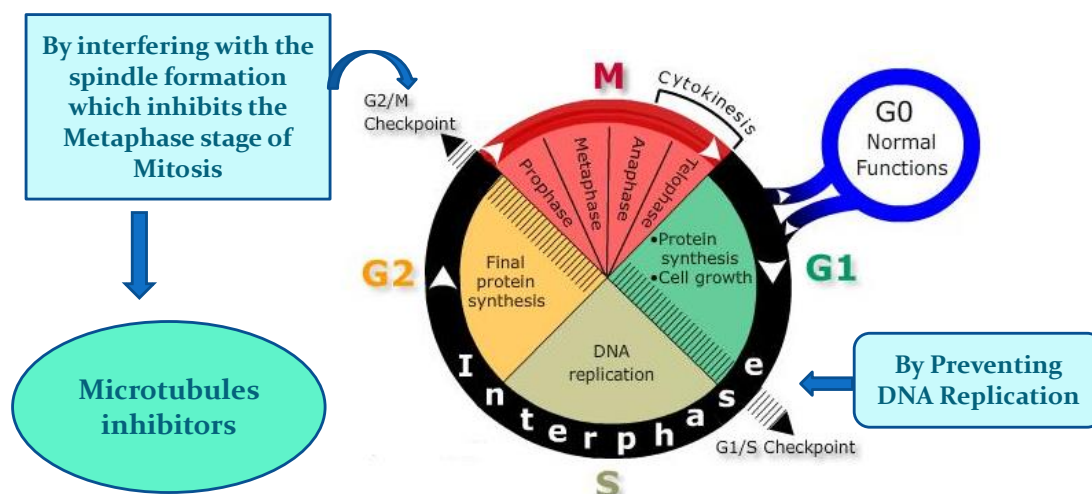


Figure 1.4: Function of microtubule inhibitor in Cell cycle

### 1.1.5. Examples of Microtubule-Targeting Agents and Its Binding Sites

As it mentioned earlier that interfering with MT dynamics is an attractive anticancer strategy, and many drugs employing this tactic are therapeutically effective in a wide range of malignancies. Mitotic arrest in the G2/M phase, a hallmark of MT-targeting agents, is thought to occur through the perturbation of mitotic spindle machinery and failure to pass mitotic checkpoints. There are several sites on the tubulin heterodimer which MT-targeting agents can bind, the most common are the vinca alkaloid, taxane, colchicine, and laulimalide binding sites.<sup>14-16</sup> These drugs are generally divided into one of two classes; stabilizing agents, which enhance polymerization, or destabilizing agents, which inhibit tubulin polymerization. The first main group is the microtubule-destabilizing agents, inhibits microtubule polymerization at high concentrations and includes several compounds — such as the *Vinca* alkaloids (vinblastine, vincristine, vinorelbine, vindesine and vinflunine), cryptophycins, halichondrins, estramustine, colchicine and combretastatins — that are used clinically or are under clinical investigation for treatment of cancer.<sup>17-22</sup> The second main group is known as

the microtubule-stabilizing agents. These agents stimulate microtubule polymerization and include paclitaxel (the first agent to be identified in this class), docetaxel (Taxotere), the epothilones, discodermolide, eleutherobins, sarcodictyins, laulimalide, rhazinalam, and certain steroids and polyisoprenyl benzophenones (**Figure 1.5**).<sup>23-26</sup>

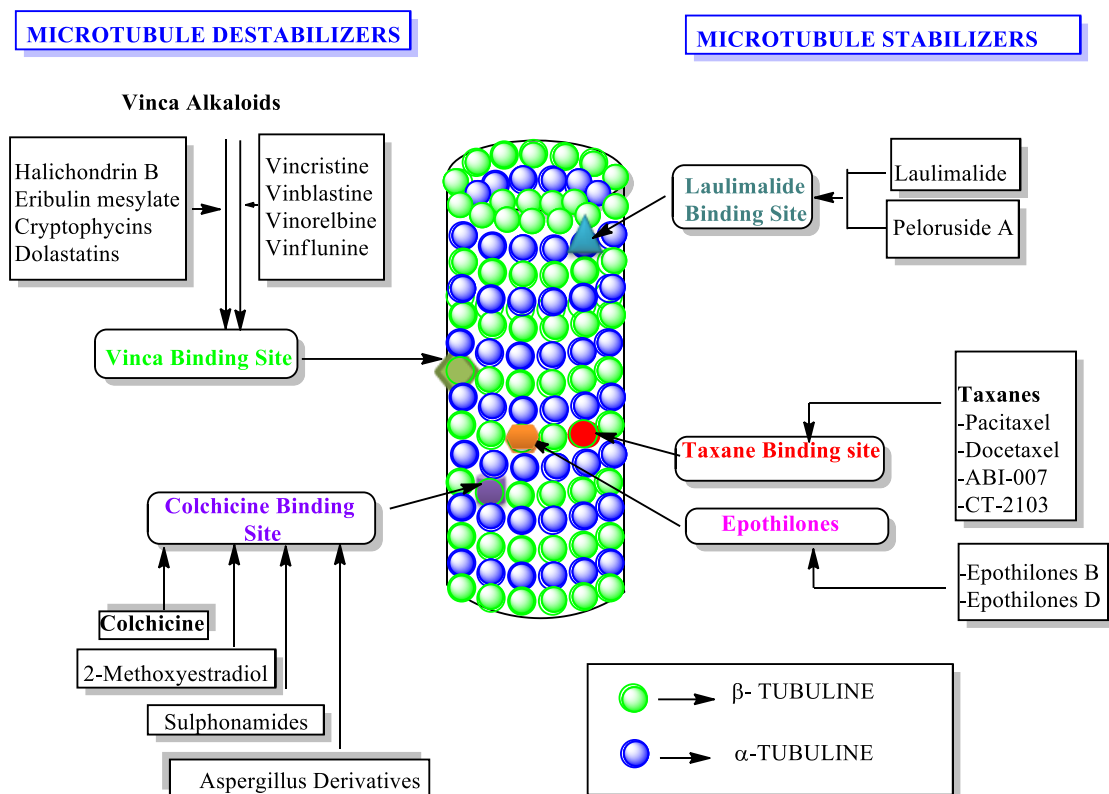


Figure 1.5: Microtubule targeting agents and its binding sites

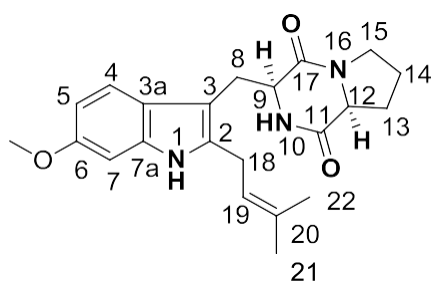
## 1.2. Tryprostatin A and B (TPS A And B)

### 1.2.1 Isolation of Tryprostatin A and B and its Function as a Microtubule Inhibitor

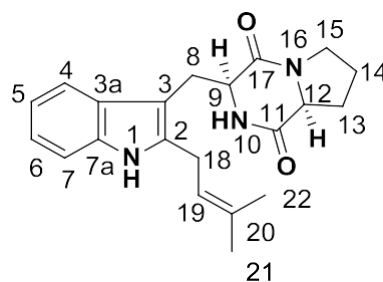
The discovery of natural products such as paclitaxel (Taxol®)<sup>27</sup>, Vinca alkaloids<sup>9,20</sup> and several synthetic compounds<sup>25</sup> which are able to act as microtubules inhibitors that arrests cells in mitosis stimulated the search for novel agents with a similar mode of action. During search of potential compounds, multidrug resistance (MDR) in human cancers is one of the



major causes of failure in chemotherapy treatment. Several years ago, Tryprostatin (TPS) A and B (**Figure 1.6**) are members of a family of prenylated Tryptophan-Proline (Trp-Pro) diketopiperazine alkaloids with an indole C2-prenyl group, isolated in 1995 from the fermentation broth of *Aspergillus fumigatus* BM939 by Osada and coworkers.<sup>28</sup> The characteristic feature between the two compounds is the presence (TPS-B) or absence (TPS-A) of a methoxy substitution at the indole C6-position. Both compounds are known to display anticancer activities because of the indole C2 prenyl group, which has been linked to their inhibition of topoisomerase II.<sup>29</sup> Studies found that tryprostatin A and B completely inhibited cell cycle progression of tsFT210 cells in the G2/M phase at a final concentration of 50  $\mu\text{g/ml}$  and 12.5  $\mu\text{g/ml}$  respectively. The proposed mode of action (MOA) for such activities proceed through a unique mechanism consisting of inhibiting the interaction between microtubule-assisted proteins (MAP-2) and the C-terminal end of tubulin.<sup>30-32</sup> However, TPS-A has also been shown to inhibit breast cancer resistance protein (BCRP, ABCG2)<sup>33</sup>, and restore the efficacy of clinically used chemotherapeutics when tested on BCRP+ breast cancer cell lines.<sup>34</sup> It was believed that TPS-A induces tubulin inhibition without directly binding to tubulin and was found to be different from the typical tubulin inhibitors such as paclitaxel or vinblastine.<sup>35</sup> Furthermore, tryprostatin A exhibited inhibitory activity on the elongation of lettuce shoots.<sup>36</sup>



**Tryprostatin A**



**Tryprostatin B**

Figure 1.6: Structure of Microtubule Inhibitor, Tryprostatin A and B

Like TPS A and B, there are a lot of natural products that reported in which contained tryptophan-proline cyclodipeptides in their structure and exhibit several biological and pharmaceutical activities. For example: (+)-Austamide and (+)-deoxyisoaustamide were isolated from the maize meal cultures of the toxigenic fungus *Aspergillus ustus*, and (+)-austamide caused acute toxicosis in day-old ducklings.<sup>37</sup> Fumitremorgin C and its derivatives were identified in *Aspergillus fumigatus* from the holothurian *Stichopus alternata*. They displayed significant cytotoxic activity against MOLT-4 (human acute lymphoblastic leukemia cells), A-549 (human lung adenocarcinoma epithelial cells), and HL-60 (human promyelocytic leukemia cells), which speculated that this cytotoxic activity may be linked to hydroxyl groups in the side chains of the molecules.<sup>38</sup> Demethoxyfumitremorgin C from marine-derived *Aspergillus fumigatus* showed inhibitory activity in the mouse cell cycle against tsFT210, and inhibited tumor cell cycle arrest at G2/M with a minimum inhibitory concentration (MIC) value of 0.45  $\mu\text{M}$ .<sup>31</sup> Fumitremorgin (A-B) are also isolated *Aspergillus fumigatus* and showed significant cytotoxic activity.<sup>39-40</sup> 18-Oxotryprostatin A was isolated from the marine-derived fungus *Aspergillus sydowi* and found to exhibit weak cytotoxic activity against A-549 cells with a median inhibitory concentration (IC50) value of 1.28  $\mu\text{M}$ .<sup>41</sup> This compound was also obtained from the endophytic fungus *Aspergillus fumigatus* from *Melia azedarach* to display plant growth inhibitory activity.<sup>42</sup> Spirotryprostatins (A-F, K) were isolated from *Aspergillus fumigatus*. These compounds showed cytotoxic activity by inhibiting mammalian cell cycle at G2/M phase.<sup>43-44</sup> Stephacidin B was isolated from *Aspergillus ochraceus*. This compound exhibited potent cytotoxic activity against LNCaP (a testosterone-dependent prostate cancer cell line), with IC50 values from 91 to 621 nM.<sup>45</sup>

Cyclotryprostatins A-D also prevent cell cycle progression at the G2/M phase. They inhibited cell cycle progression of tsFT210 cells in the G2/M phase with IC<sub>50</sub> values of 5.6μM, 19.5μM, 23.4μM, and 25.3μM, respectively (**Figure 1.7**).<sup>46-47</sup>

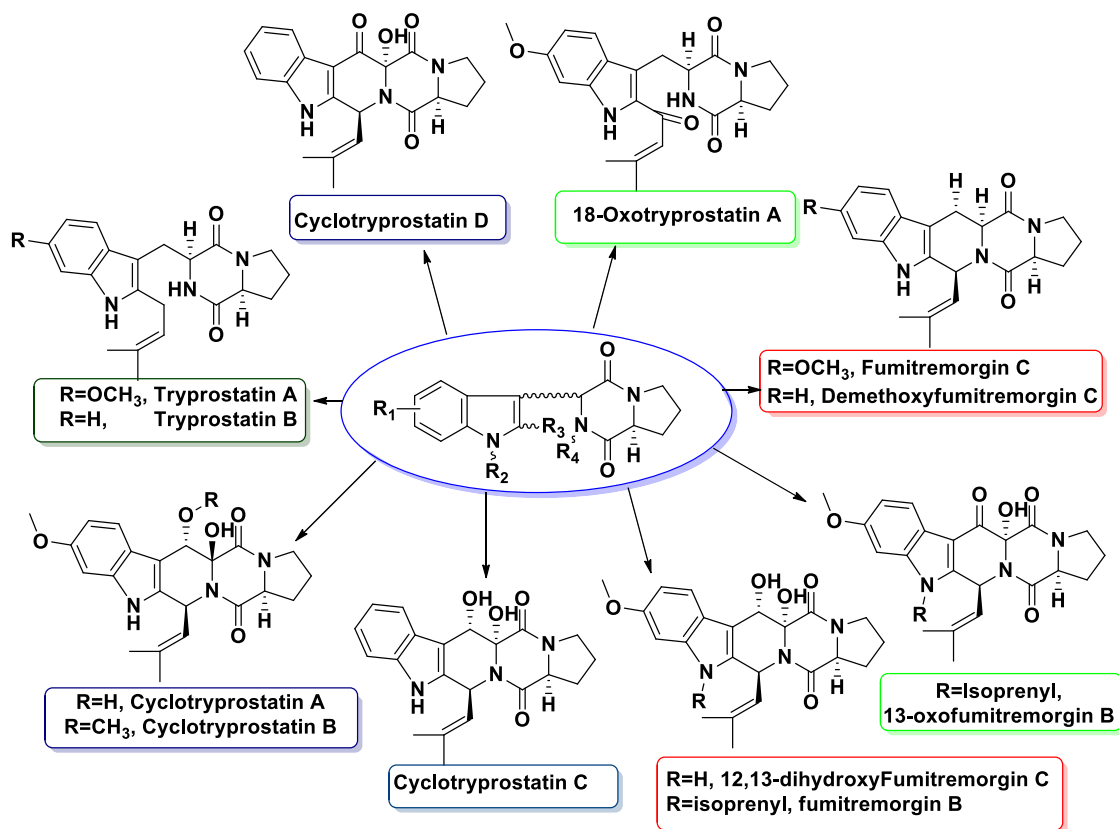


Figure 1.7: Example of Trp-Pro Diketopiperazine Alkaloids

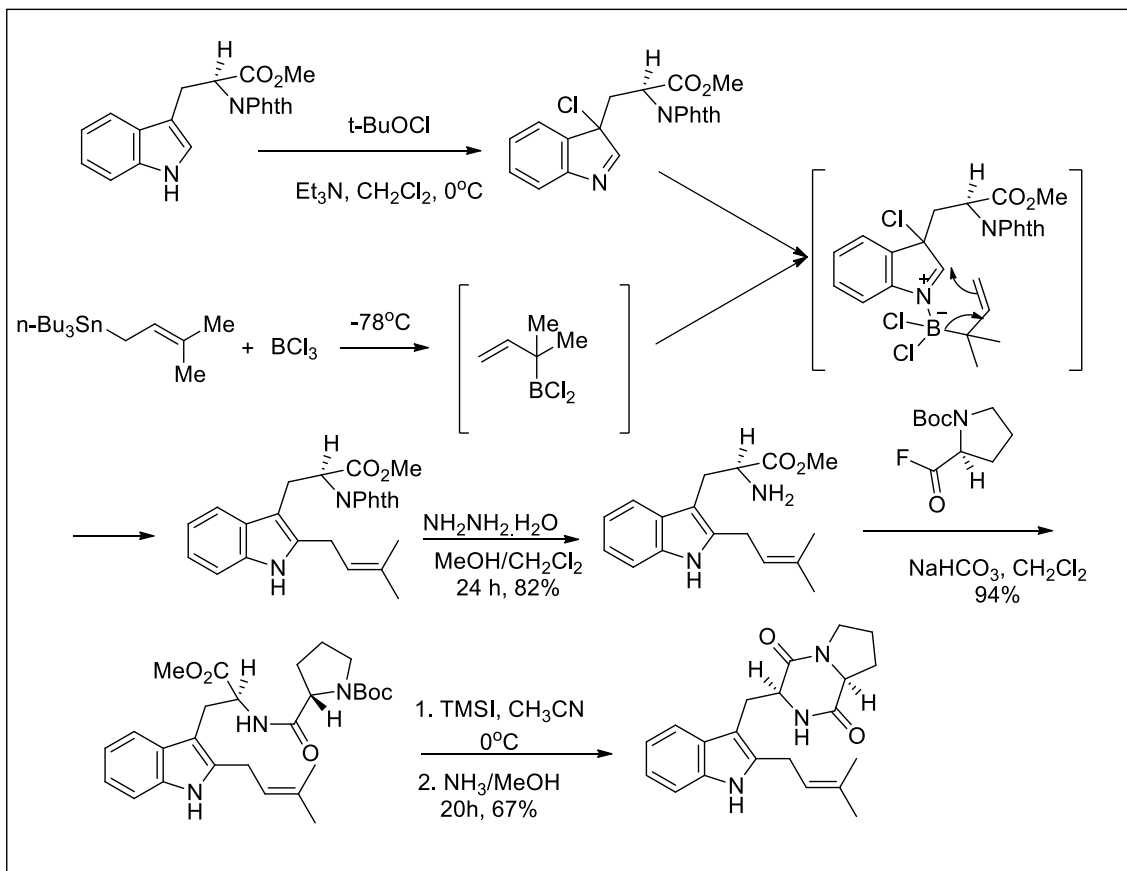
### 1.2.2. Why We Choose to Synthesize Tryprostatin A and B??

Microtubules are promising targets for treating cancer by stopping the cell division in mitosis.<sup>48,49</sup> Tryprostatins are important alkaloids for treating cancer, via microtubule inhibition. The rarity of TPS A and B in nature and long, low-yielding synthetic procedures have limited their development as viable anticancer therapeutics. On the other hand, their interesting biological activity and simple structure have drawn attention from the synthetic community, and several total syntheses have been reported.<sup>50-61</sup> The first total synthesis of the

Tryprostatin B was reported by Danishefsky et al. via the chloroindolenine/borane approach illustrated by the scheme below.

### 1.2.3. Total synthesis of Tryprostatin B by Danishefsky et al. in 1996

The first total synthesis of tryprostatin B (TPS-B) was reported by Danishefsky *et al.* in 1996 via the chloroindolenine/borane approach (**Scheme 1.1**).<sup>50</sup> At first, The N- phthaloyl-L-tryptophan methyl ester was treated with *tert*-butyl hypochlorite to generate the chloroindolenine intermediate at 0°C. This intermediate was then treated with prenyl stannane at -78°C and followed by rapid addition of boron trichloride (2.0 equivalents) provide the desired 2- isoprenyl tryptophan derivative. It is believed that the reaction of prenyl stannane with boron trichloride generated a nucleophilic prenylation species *in situ*. This species is thought to react with the chloroindolenine to provide the “ate” like structure which undergo intramolecular delivery of the isoprenyl moiety from the indolenine N atom to the indole ring at C-2 position. Then the removal of the *N*-phthaloyl protecting group generated the required *L*-2-isoprenyltryptophan methyl ester. The coupling reaction between the 2-isoprenyl tryptophan unit and the *N*-Boc-protected *L*-proline acid fluoride provided the dipeptide. The Boc-protecting group was then removed on treatment of *N*-Boc protected prolyl tryptophan derivative with trimethylsilyl iodide in acetonitrile. Finally, cyclization was achieved by reaction of N-deprotected prolyl tryptophan derivative with methanolic ammonia. The resulted Tryprostatin B was identical to the natural Tryprostatin **B** and the overall yield of started from N-phthalolyl tryptophan methyl ester was about 46%.



Scheme 1.1: Danishefsky's Synthesis of Tryprostatin B

#### 1.2.4. Total Synthesis of Tryprostatin A by Cook et al. in 1997

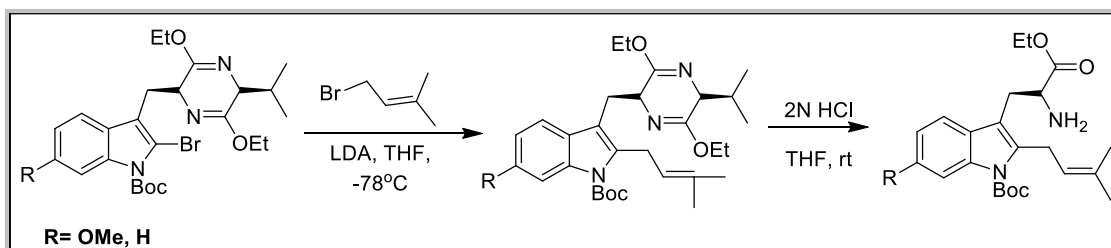
The first total synthesis of tryprostatin A was completed by Cook and his coworkers *via* a regiospecific bromination process coupled with the Schöllkopf chiral auxiliary.<sup>51-53</sup> The regiospecific bromination of 3-methylindoles were achieved at the indole 2-position via an electrophilic process or at the 3-methyl position under free radical conditions, this method appeared to be useful for the preparation of 2-prenyltryptophans and later tryprostatins. The synthesis began with the Fischer indole cyclization via a Japp-Klingmann azo-ester intermediate (**Scheme 1.2**). The azo-ester intermediate was formed when *m*-anisidine was treated with sodium nitrite and concentrated aqueous HCl at 0°C, followed by the addition of

ethyl  $\alpha$ -ethylacetoacetate, this intermediate was heated in a solution of 3 N ethanolic HCl, the desired ethyl 6-methoxy-3-methylindole-2-carboxylate was obtained. Alkaline hydrolysis of the ester under yielded the corresponding carboxylic acid which was converted into 6-methoxy-3-methyl indole in excellent yield via the subsequent copper/quinoline-mediated decarboxylation sequence. To protect indole N(H) moiety, 6-methoxyindole was treated with di-tert-butyl decarbonate in presence dimethoxyammonopyridine. The protected 3-methylindole was then reacted with N-bromosuccinamide (NBS) in carbon tetrachloride to provide the 2-bromoindole as illustrated in Scheme 1.2. When 2-bromoindole was reacted with NBS under free radical conditions, azobisisobutyronitrile (AIBN), dibromide indole was obtained in 93% yield. Dibromide was coupled with the Schöllkopf chiral auxiliary at -78 °C, a pyrazine compound was obtained in 91% yield. The pyrazine was treated with *n*-butyllithium at -78 °C, followed by addition of prenyl bromide, 2-isoprenylpyrazine was isolated in 86% yield. The pyrazine group was removed under acidic conditions (aqueous HCl, THF) in 94% yield to provide D-valine ethyl ester and the 2-prenyltryptophan. The 6-methoxy-2-prenyltryptophan was stirred with *N*-(trichloroethoxy carbonyl) (Troc)-*L*-prolyl chloride in the presence of triethylamine in CH<sub>2</sub>Cl<sub>2</sub> at 0 °C, the desired dipeptide was obtained. The Troc protecting group was removed by heating with Zn (dust) in refluxing MeOH. Finally, formation of the diketopiperazine unit and removal of Boc-protecting group from the indole N(H) function were achieved when dipeptide was heated at 160 °C (neat) to furnish tryprostatin A in 50% overall yield (**Scheme 1.2**).

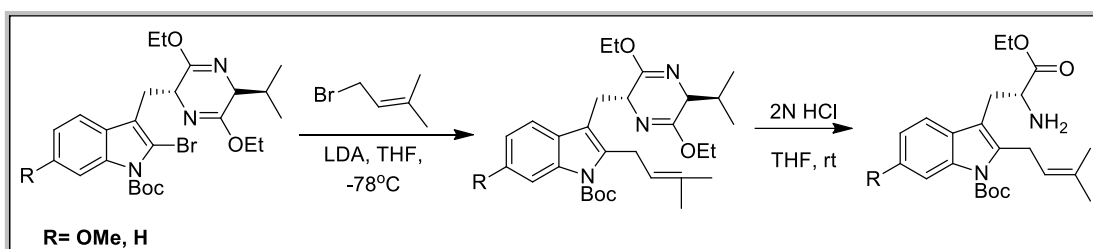


synthesis of 2-prenylpyrazine, and this procedure was used for the synthesis of tryptophan, a precursor for the synthesis of tryprostatin A and B.

### Synthesis of (*S*)- Tryptophan: A precursor for the synthesis of Tryprostatin A and B



### Synthesis of (*R*)- Tryptophan: A precursor for the synthesis of 9-epimer-Tryprostatin A and B

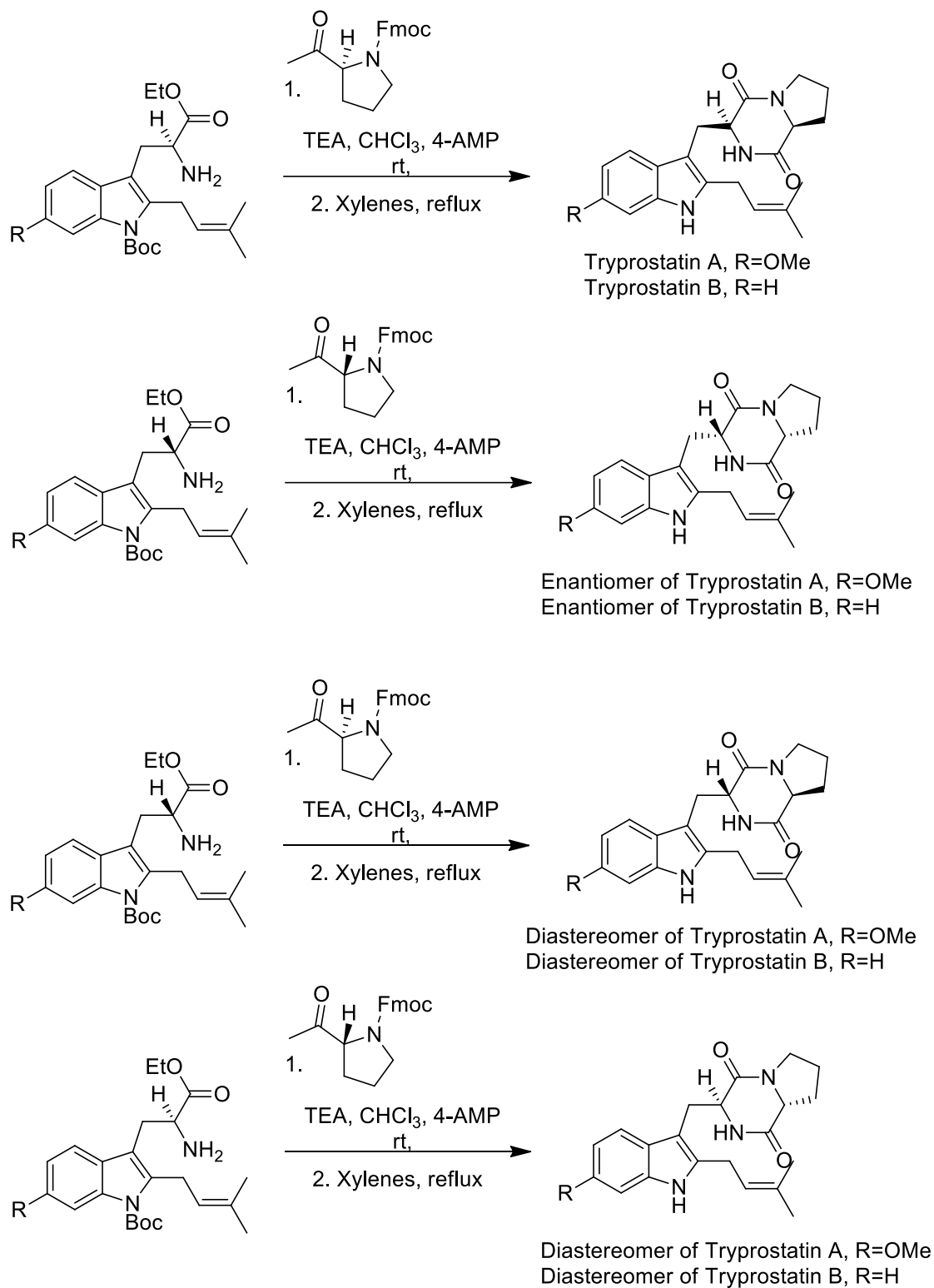


Scheme 1.3: Synthesis of Tryptophans by Cook et al.

With the key 2-prenyltryptophan derivatives in hand, the diketopiperazine unit was built on as illustrated below, 2-prenyl-tryptophans were stirred with *N*-Fmoc-*L*-prolyl chloride in the presence of triethylamine in chloroform at room temperature. The Fmoc-protecting group was removed by addition of diethylamine (DEA) in acetonitrile. Formation of the diketopiperazine as well as the removal of the Boc-protecting group from the indole N(H) were achieved by heating in refluxing xylenes in high dilution. A stereospecific, enantiospecific total synthesis of tryprostatin A and B was accomplished *via* alkylation of the corresponding 2-lithioindole derivatives. This procedure was also applied to the enantiomers of tryprostatin A and tryprostatin B (**Scheme 1.4**). The optical rotations of the natural products and the enantiomers



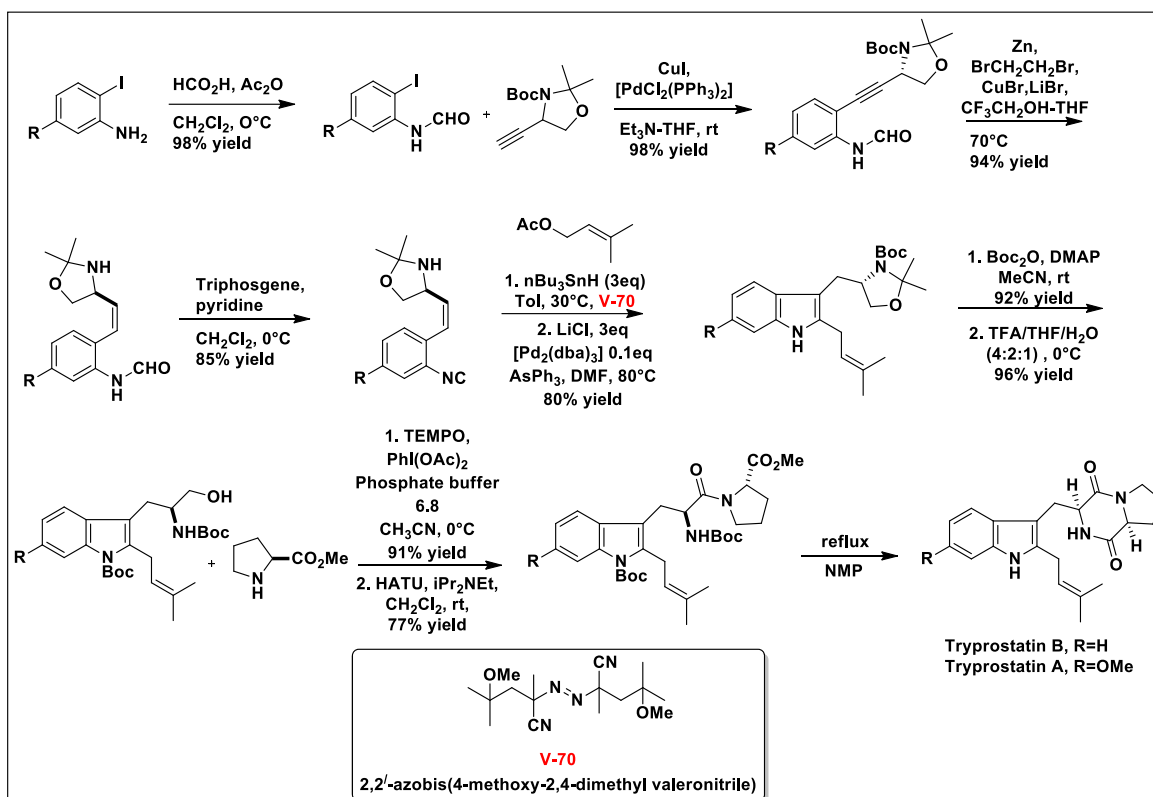
agreed with those reported by Osada *et al.* for the natural products.



Scheme 1.4: Synthesis of Enantiomers and Diastereomers of Tryprostatins

### 1.2.5. Fukuyama's Synthesis of Tryprostatin A and B in 2010

Fukuyama and his coworkers synthesized TPS A and B from the Garner aldehyde.<sup>60,61</sup> The Garner aldehyde was treated with carbon tetrabromide, triphenyl phosphine in presence of triethyl amine followed by Grignard reagent ethyl magnesium bromide at 0 °C formed alkyne which was went to the Sonogashira coupling with 2-iodoformanilide, partial reduction of the triple bond was examined by the treatment with Zn/LiCuBr<sub>2</sub> in ethanol gave the desired product with 99% yield by using 2,2,2-trifluoroethanol as a solvent. Subsequent dehydration with bis(trichloromethyl) carbonate (triphosgene) gave the ortho-alkenyl isocyanide which undergo radical-mediated cyclization in the presence of tributylstannhydride to form 2-stannylindole where 2,2'- azobis(4- methoxy-2,4-dimethylvaleronitrile) (V-70, 20) acts as a radical initiator. The desired 2-prenyl indole product was obtained in only 82% yield with prenyl acetate as the coupling partner in presence of triphenylarsine, lithium chloride, and [Pd<sub>2</sub>(dba)<sub>3</sub>] as the catalyst. The 2-prenyl indole moiety N(H) was protected with a Boc group, hydrolysis of the acetonide, and oxidation of the resulting alcohol to the carboxylic acid with 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) gave the corresponding amine. After condensation with L-proline methyl ester hydrotosylate the resulting compound undergo spontaneous cyclization by refluxing in the presence of N- methylpyrrolidinone (NMP) to give the desired compound tryprostatin B in 89% yield. Thus, tryprostatin B was synthesized by Fukuyama and coworkers in 11 steps from Garner aldehyde in 33% overall yield on a half-gram scale. By following the similar method, tryprostatin A was synthesized in 30% overall yield (**Scheme 1.5**).

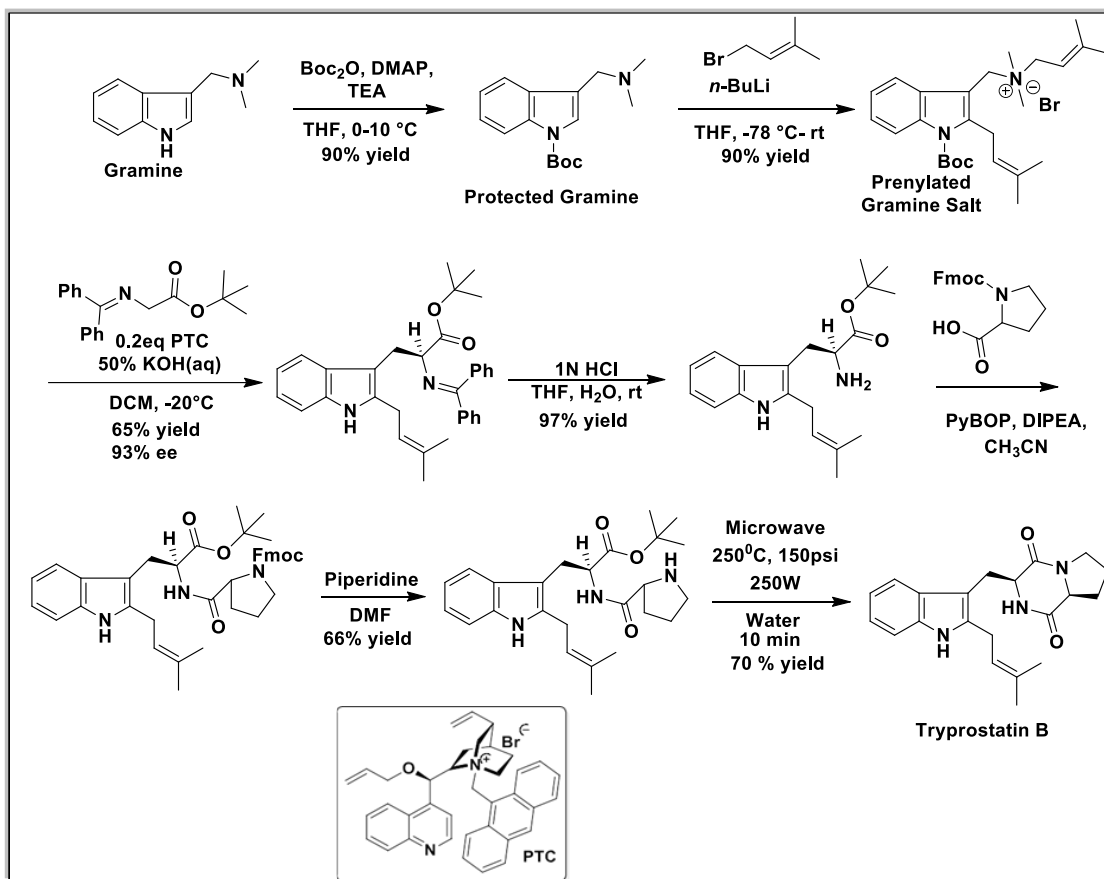


**Scheme 1.5.** Fukuyama's Synthesis of Tryprostatin A and B

### 1.2.6. Total Synthesis of Tryprostatin B by Hossain et al. in 2019

In 2019, our group synthesized tryprostatin B from commercially available gramine by using Phase Transfer Catalyst (PTC).<sup>59,60</sup> At first the N atom in the indole ring was protected by Boc to form N-Boc protected gramine by using Boc anhydride (Boc<sub>2</sub>O) in the presence of Dimethylamino pyridine (DMAP) and Triethylamine (TEA). To incorporate the prenyl group at the C2 position of the indole ring, at first the N-Boc protected gramine was treated with one equivalent of prenyl bromide in the presence of *n*-butyllithium, and the reaction provided exclusively *N*-prenylated gramine salt with 88% yield where no C2-prenylation was observed. But when the same reaction was carried out with two equivalents of *n*-butyllithium and excess amount of prenyl bromide (4.5 equiv), C2, N-diprenylated gramine salt was formed in 92% yield along with *N*-prenylated

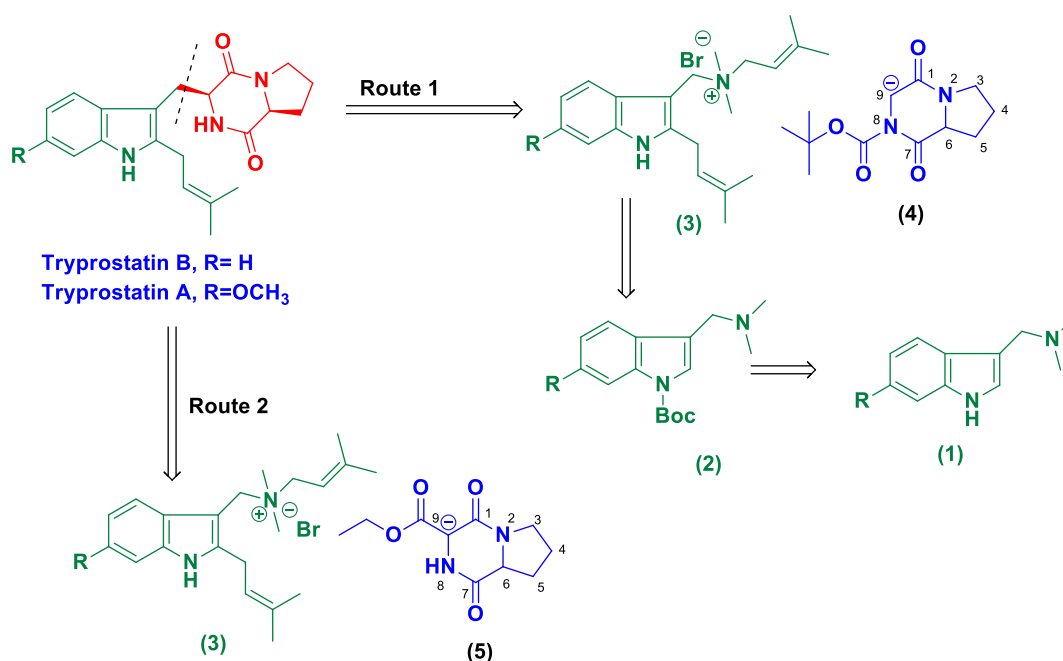
gramine salt. These findings open a new window to synthesize C2 alkylated indole moiety in only two steps from gramine. Then the resulting C2, N-diprenylated gramine salt reacts with *N*-(diphenylmethylene) glycine *tert*-butyl ester in the presence of *O*-allyl-*N*-(9-anthracenylmethyl) cinchonidinium bromide catalyst act as a Phase Transfer Catalyst (PTC) to form C2- prenyl chiral tryptophan in 65% yield. Under acidic conditions (aqueous HCl, THF), the diphenylmethylene group was removed from protected tryptophan to provide the 2-prenyl tryptophan *tert*-butyl ester in 97% yield. Reaction of 2-prenyl tryptophan *tert*-butyl ester with the *N*-Fmoc-*L*-prolyl chloride in the presence of trimethylamine yielded Fmoc- protected dipeptide.<sup>51</sup> The Fmoc protecting group was deprotected with piperidine in dimethylformamide (DMF) provided dipeptide in 66% yield. Finally, spontaneous cyclization under the microwave conditions gave tryprostatin B in 70% yield.<sup>62</sup> From our developed method, TPS B was synthesized from the commercially available gramine in six steps in 35% overall yield (**Scheme 1.6**).<sup>62</sup>



Scheme 1.6: Total synthesis of TPS B by Hossain's group

### 1.3. Background of Our Total synthesis

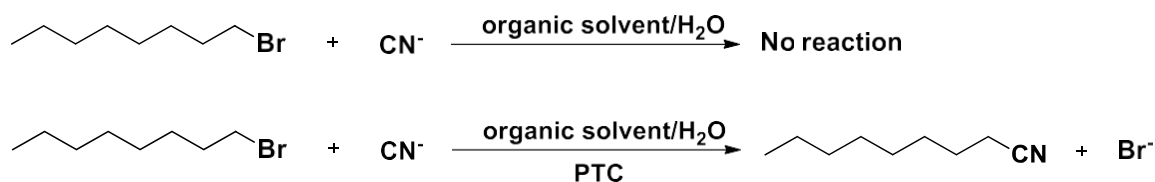
Our newly developed chiral phase transfer catalyst reaction to synthesize chiral tryptophan, which is the precursor of TPS B from C2, N-diprenylated gramine salt gave us a new idea to utilize the salt for direct alkylation with Diketopiperazine core to achieve TPS B and A in fewer steps. To execute our plan, we proposed two retrosynthetic routes for synthesis of tryprostatin and analogs of the parent structure. Our proposed retrosynthetic route is shown below (**Scheme 1.7**):



Scheme 1.7: Retrosynthetic route for the synthesis of Tryprostatin and its analogs

### 1.3.1. General Concepts and Mechanism: Phase-Transfer-Catalysis (PTC)

To endorse the successful alkylation of C2, N-diprenylated protected gramine salt, a basic understanding of the phase transfer catalyzed (PTC) reaction is required. In 1971, Starks introduced the term phase transfer catalysis where he described a little organic quaternary salt dramatically increase the rate of reaction (**Scheme 1.8**). Reaction between an organic solution of an alkyl halide and an inorganic solution of sodium cyanide in presence of tetralkylammonium or tetralkylphosphonium salt generated product faster.<sup>63</sup>



### Scheme 1.8. Phase-Transfer-Catalyzed Reactions Reported by Starks

Cyanide ion is insoluble in organic solvent, so the reaction was unable to continue without the presence of phase transfer catalyst (PTC). PTC carry the nucleophilic cyanide ion from aqueous phase to organic phase and exchange the ion with phase transfer catalyst at the interface. The new ion pair then travel to the organic phase and reacts with nucleophile (Figure 1.8).

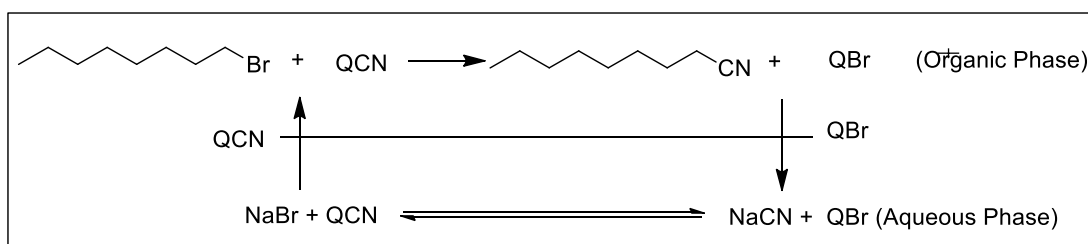
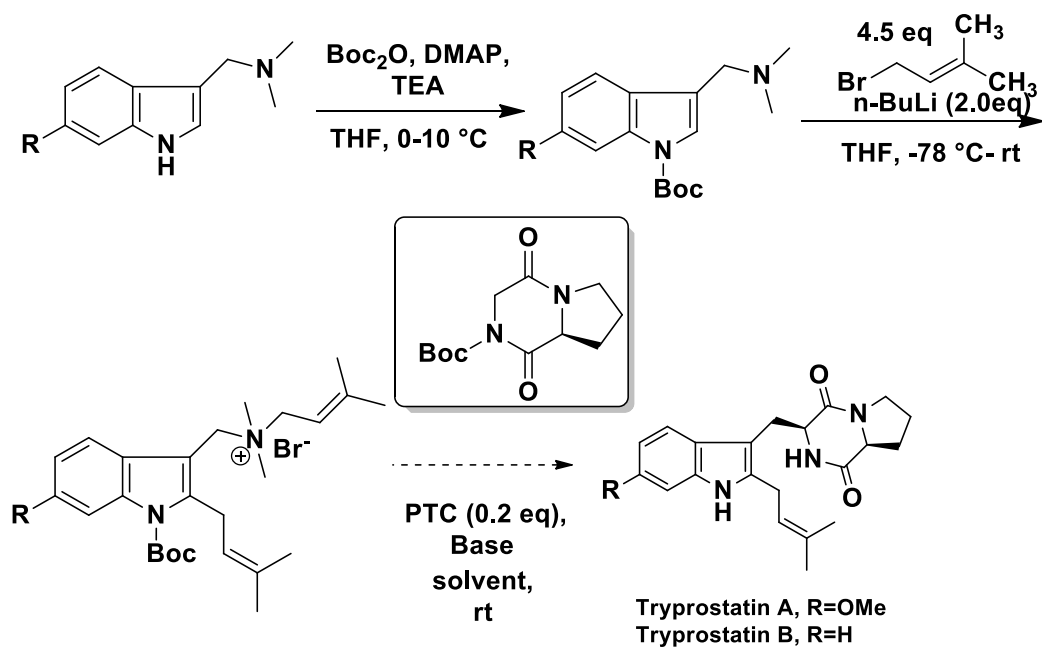


Figure 1.8: Mechanistic Presentation of Phase-Transfer-Catalyzed Reaction

#### 1.4. Explore Route 1 for the Synthesis of Tryprostatin B by PTC conditions

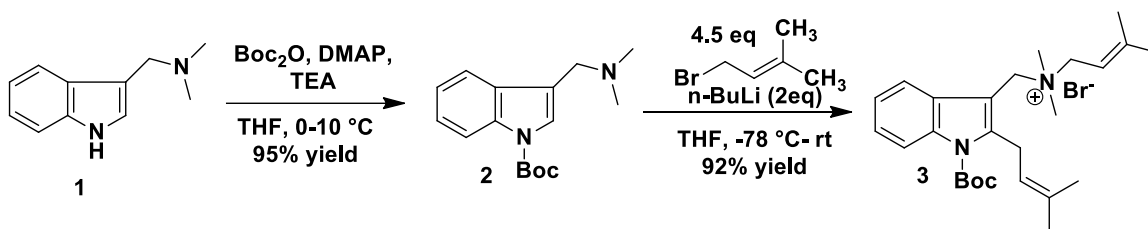
To synthesize Tryprostatin **A** and **B**, we proposed the following **route 1** where C2, N-diprenylated protected gramine salt **3** undergo direct alkylation with N-boc protected diketopiperazine core **4** in the presence of phase transfer catalyst (PTC) (**Scheme 1.9**). To accomplish the proposed route, we follow the optimized phase transfer catalyst conditions which was established by our previous synthesis.<sup>62</sup>



Scheme 1.9: Proposed route 1 for the synthesis of Tryprostatins

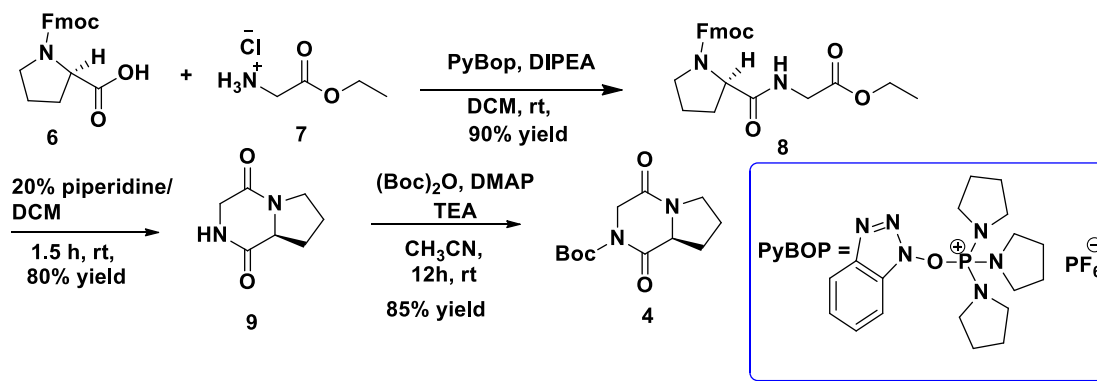
At first, we synthesize the C2, N-diprenylated protected gramine salt **3** following the procedure reported by our group<sup>63</sup> in 2019 and N-Boc protected diketopiperazine core **4** was synthesized by using reported procedure (**Scheme 1.10**).<sup>64-66</sup> Here N-Boc protected diketopiperazine core will serve as a nucleophile and will undergo alkylation with C2, N-diprenylated protected gramine salt in the presence of phase transfer catalyst conditions.

### Synthesis of Compound 3



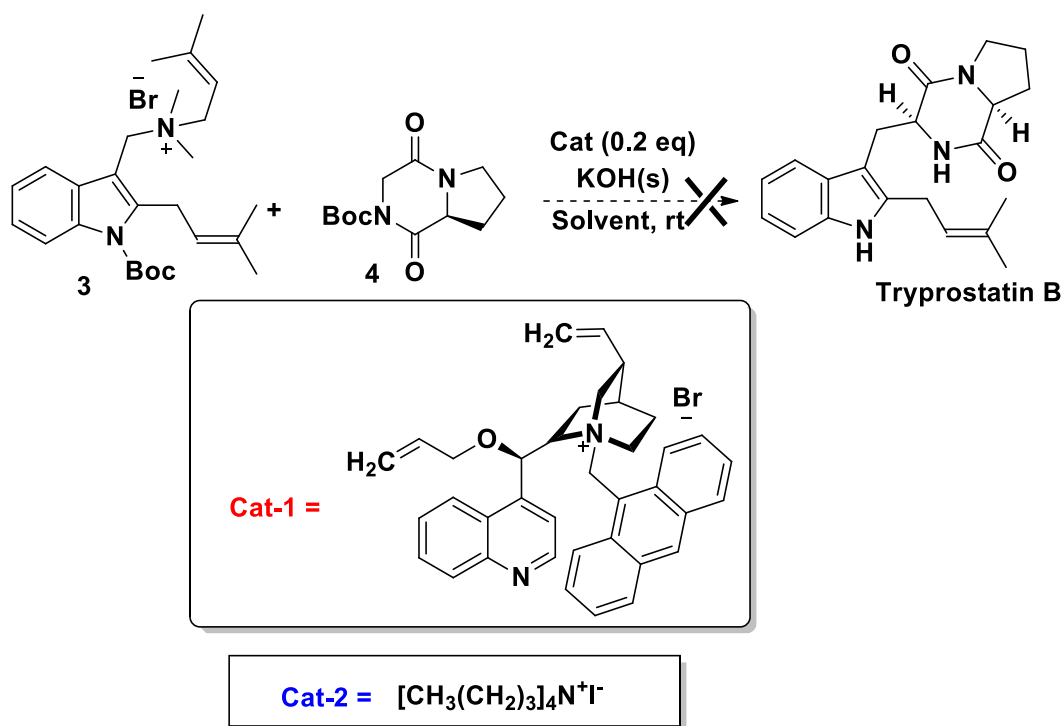
### Synthesis of compound 4





Scheme 1.10: Synthesis of Compound 3 and 4

Whenever we have both starting material in our hand then we explore phase transfer catalyst (PTC) conditions to do direct alkylation. We use optimized reaction condition by using chiral and racemic PTC catalyst established in our previous method to perform the reaction.<sup>63</sup> As a chiral catalyst we use *O*-allyl-*N*-(9-anthracenylmethyl) cinchonidinium bromide (**cat-1**) and tetrabutyl ammonium iodide (**cat-2**) for racemic reaction. We choose different solvents such as 1, 4-Dioxane, dimethoxyethane (DME), dichloromethane (DCM), and tetrahydrofuran (THF) which gave better stereoselectivity in our previous synthesis (**Scheme 1.11 and Table 1.1**).

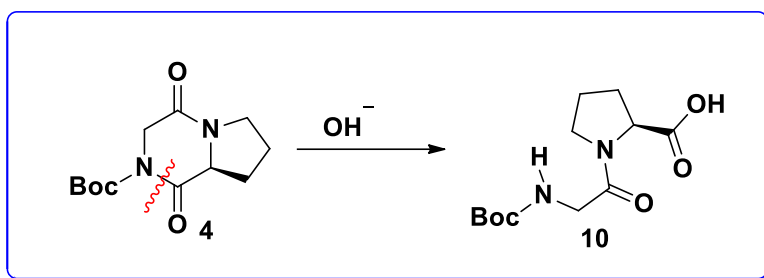


Scheme 1.11: Attempt for the Synthesis of Tryprostatin B by PTC condition

By performing PTC reaction in different solvents and in two different catalysts which was summarized in Table 1.1, we observed that in both reaction conditions, compound **3** was unreacted at room temperature where the N-Boc diketopiperazine core **4** is not stable under PTC condition. In 2012 Dubey et al. reported that in basic reaction condition, the compound **4** is readily react with nucleophile  $\text{OH}^-$  to form acyclic compound **10** and lost its nucleophilicity. In our case, we use KOH as a base which may be destroy the compound **4** and our PTC reaction condition did not work in route **1** (Scheme 1.12).<sup>65</sup>

Entry	Catalyst (0.2 equiv.)	Solvent	Base (15.0 equiv.)/ Temp °C/ time (hrs)	Result

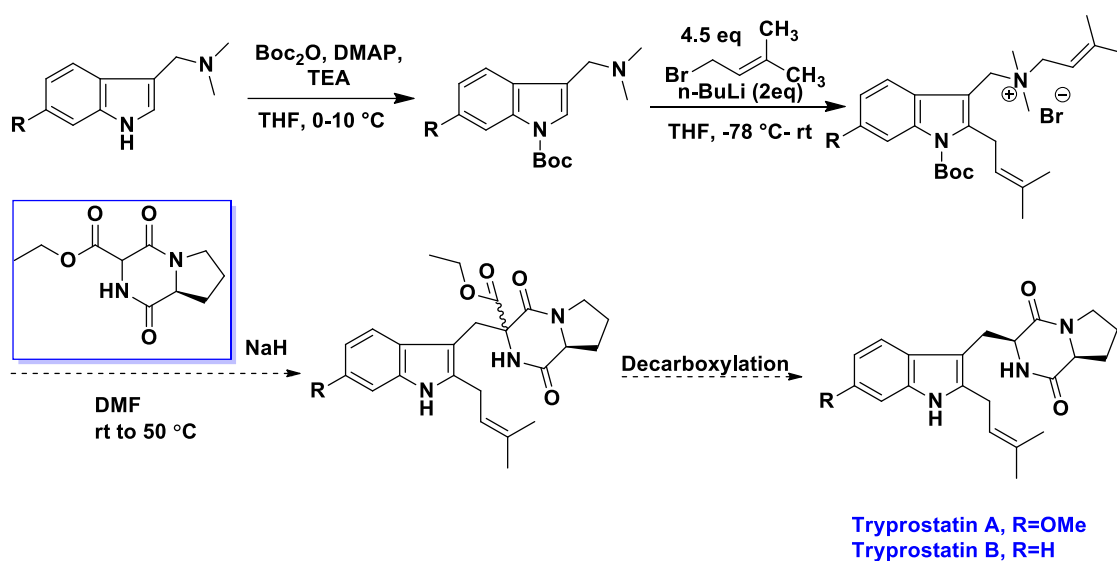
1	Cat-1	DCM	KOH/rt/ 72	Diketopiperazine  decompose
2	Cat-1	1,4-Dioxane		
3	Cat-1	THF		
4	Cat-2	DCM	KOH/rt/ 24	
5	Cat-2	1,4-Dioxane		



Scheme 1.12: Decomposition of Diketopiperazine core in the presence of KOH.

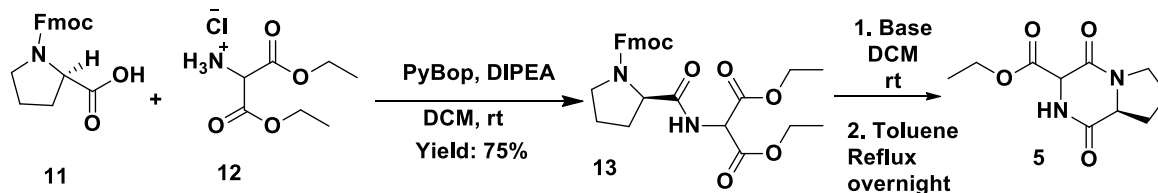
### 1.5. Explore Route 2 for the Synthesis of Tryprostatin B

After obtaining valuable information from route **1**, then we began to explore our alternate retrosynthetic route. Whenever we found out that N-Boc diketopiperazine was not stable in basic condition then we made another diketopiperazine core **5** to execute our route **2** where we incorporated the electron – withdrawing group in C-9 position (See Scheme 1.7). Inspired by the method reported by Fukuyama<sup>61</sup>, we plan the following route to synthesize Tryprostatins and its analogs (**Scheme 1.13**).



Scheme 1.13: Proposed route 2 for the synthesis of TPS A and B

We began our proposed route **2** by synthesizing compound **5** by following a reported procedure.<sup>67-69</sup> At first, N-Fmoc-L-proline **11** undergo coupling reaction with Diethyl aminomalonate hydrochloride **12** in the presence PyBOP and DIPEA at room temperature to form the dipeptide **13**. The dipeptide undergoes Fmoc-deprotection as well as cyclization simultaneously to give the diketopiperazine core **5** (**Scheme 1.14**). To find out the best reaction condition to perform the deprotection and cyclization we used several bases which was summarized in Table 1.2. In our case, 50% TEA gave the best result for the removal of Fmoc group which undergo cyclization by subsequent heating in toluene to form the compound **5** in 69% yield.



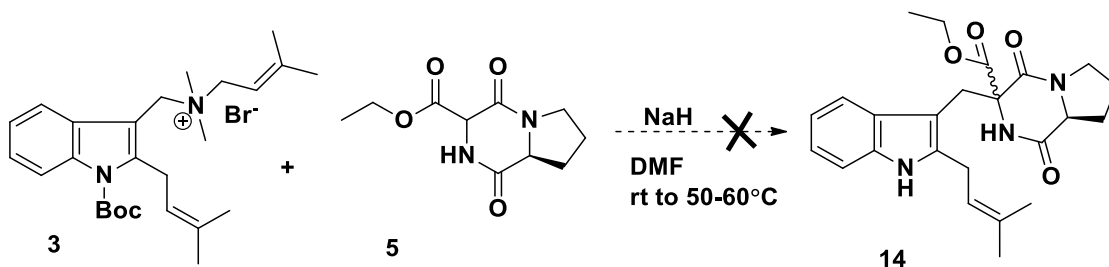
Scheme 1.14: Synthesis of the diketopiperazine core **5**.

Table 1.2: Deprotection and Cyclization of compound **13** by using different bases

Entry	Base	Solvent	Conversion (%)	Cyclization (%)
1	20% Piperidine	DCM	100	10
2	50% DEA			30
3	50% TEA			65
4	50% DIPEA			35
5	Pd/ H <sub>2</sub> (1 atm)	MeOH		50

### 1.5.1. Direct Alkylation of Compound **3** and **5** in the presence of NaH

After getting both starting material in our hand then we explore direct alkylation in the presence of Sodium hydride (NaH). Whenever we perform the reaction in our surprised, the reaction did not work either room temperature or 50- 60°C, presumably due to lack of formation of 3-methylene indolenine intermediate from Boc protected gramine which is necessary to perform the alkylation reaction (**Scheme 1.15 and Table 1.3**).

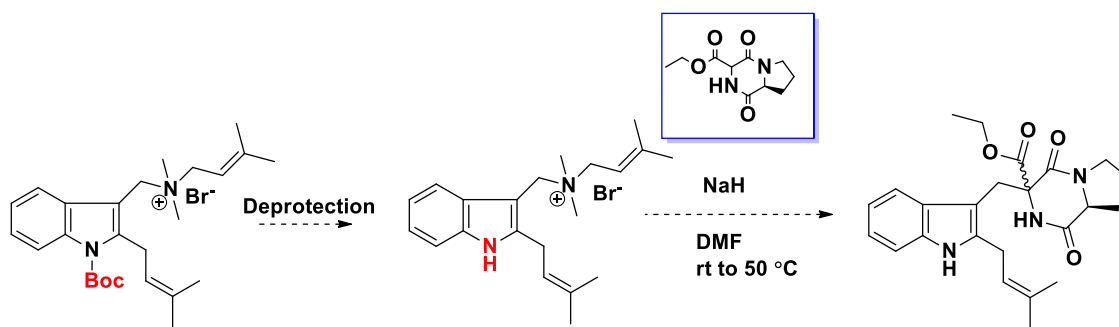


Scheme 1.15: Attempt for the Synthesis of Compound **14** by NaH

Table 1.3: Synthesis of TPS-B by NaH

Entry	Time (hrs)	Temperature (°C)	Observation
1	6	50-60	Compound <b>5</b> decompose and Compound <b>3</b> unreacted confirmed by NMR
2	24	rt	
3	24	50-60	
4	48	50-60	
5	72	rt	

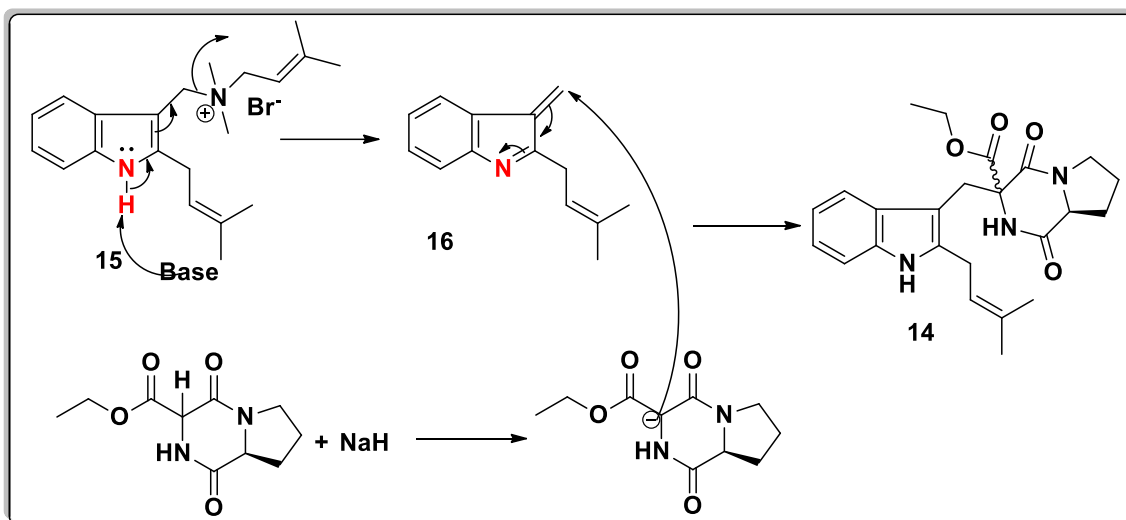
We change our proposed route (**Scheme 1.13**) to accomplish the reaction where first we deprotect the Boc group from compound **3** and then perform the direct alkylation with compound **5** in the presence of NaH (**Scheme 1.16**).



Scheme 1.16: Alternate route for the synthesis of TPS-B by NaH.

We believe that the deprotection of Boc help us to generate the 3-methylene indolenine

intermediate **15** which will under alkylation with compound **5** to give the desired product **14** (Scheme 1.17).<sup>70</sup>



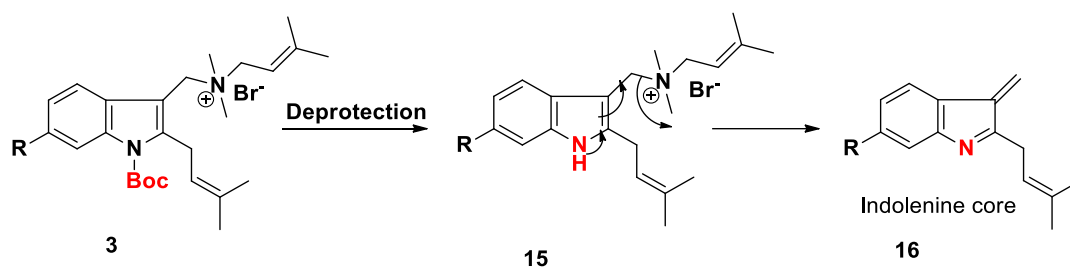
Scheme 1.17: Plausible mechanism for the alkylation of compound **3** and **5**

We performed the deprotection reaction of Boc by using different conditions (Table 1.4).<sup>71-73</sup> But the reaction did not work, and the reason can be the 3-methylene indolenine intermediate **15** which is formed in that reaction is not stable and destroy the compound **3** (Scheme 1.18).

Table 1.4: Condition for the deprotection of Boc group from Compound **3**.

Entry	Acid	Solvent	Time (hr)	Temp. (°C)	Observation
1	4M HCl	EtOAc	4	rt	Compound <b>3</b>
2	4M HCl	DCM	4		

3	4M HCl	Dioxane	2-4		decompose confirmed by NMR
4	TFA	THF/MeOH	2-12		
5	Thermolysis		0.5	160	N,N,3-trimethylbut-2-en-1-amine



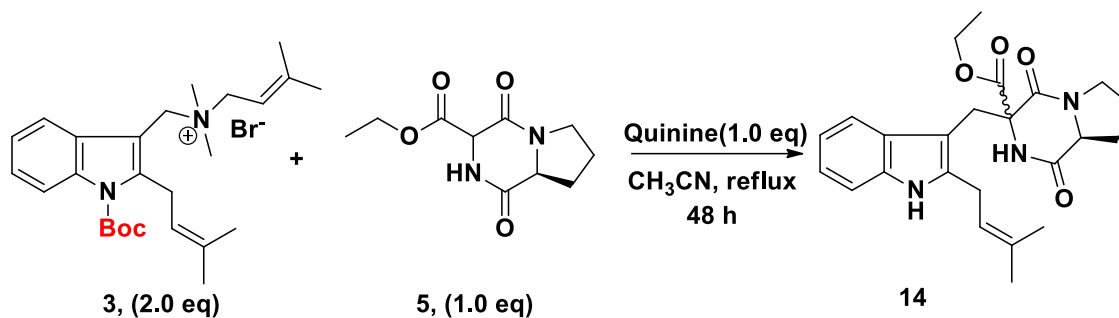
Scheme 1.18: Deprotection of Boc group from compound **3**.

### 1.5.2. Direct alkylation of Compound **3** and **5** in the presence of Quinine

During the search of effective catalyst for direct alkylation we found that gramine can undergo coupling reaction with substituted diketopiperazines in the presence of inexpensive, readily available Lewis's base, quinine reported by Dubey and Olenyuk in 2010.<sup>74</sup> Previously, tributylphosphine was used as a catalyst for such kind of transformation reactions.<sup>69(b), 75</sup> Encouraging by their findings, we set up a coupling reaction between Compound **3** and compound **5** in the presence of quinine by using acetonitrile as a solvent at reflux condition.



Under the reported reaction conditions, we got our desired compound **14** in 76% yield as a mixture of two diastereomers (1:2 ratio) which was confirmed by the proton and carbon NMR spectra of the compound (**Scheme 1.19**)



Scheme 1.19: Synthesis of Compound **14** by quinine

### 1.5.3. Optimization of direct alkylation reaction between compound **3** and **5**

To optimize the reaction conditions for higher yield and diastereoselective, we investigated the effects of base as well as systematic variations in the solvents, base loading, temperature, and time. At first, we examined the base loading by varying time to find out the best condition for higher yield and diastereomeric ratio (dr) as presented in Table 1.5. Whenever we change the loading of base from 1.0 to 0.2 (table 1.5, entry 3), there is no reaction takes place and compound **5** is unreacted whereas compound **3** is decomposed as confirmed by NMR. In case of base loading by 0.5 equivalent (entry 2), the conversion reduced to 50%. For longer reaction time, the yield of the product was also reduced (entry 5). So, the best result is obtained by using 1.0 equiv. base and run the reaction for 48 hrs at reflux condition.

Table 1.5: Optimization of Direct Alkylation Reaction by Base loading

Rxn #	Base loading				
	Base	Equiv. cat.	Time (hr) /Temp °C	Conversion (%)	Yield (%) /dr value
1	Quinine	1.0	48/ reflux	100	88/ 1:4

2	Quinine	0.5	48/ reflux	50	35/1:2
3	Quinine	0.2	48/ reflux	0	0
4	Quinine	1.0	24/ reflux	100	76/1:2
5	Quinine	1.0	120/ reflux	100	70/1:4

In order to find the best solvent for higher yield, several solvents were examined as presented in Table 1.6. For direct alkylation polar aprotic solvents ACN and DMF worked well (Table 1.6, entries 4 and 5). Less polar solvents like toluene, DCM and dioxane did not have a satisfactory result due to poor solubility of the Boc protected diprenylated gramine salt.

Table 1.6: Optimization of direct alkylation Reaction by Solvent Screening

Solvent Screening				
Rxn #	Solvent	Equiv. cat.	Time (h) /Temp °C	% Conversion
1	DCM	1.0	48/ reflux	0
2	1,4-Dioxane	1.0	48/ reflux	0
3	Toluene	1.0	48/ reflux	0
4	CAN	1.0	48/ reflux	100
5	DMF	1.0	48/ reflux	100

After getting best solvents then we move our attention to find out the best base. We examined different types of bases which has the basicity close to quinine as presented in table 1.7. Only quinine and quinidine gave 100% conversion under the reaction same conditions where incase of other bases such as DBU, TEA compound **3** is unreacted as confirmed by NMR and Compound **5** is decomposed (table 1.7, entries 3-5). We also apply PTC reaction conditions

for the coupling between compound **3** and **5** but the reaction behave the same way as DBU catalyzed reaction (table 1.7, entries 6-7).

Table 1.7: Optimization of direct alkylation Reaction by using different Base

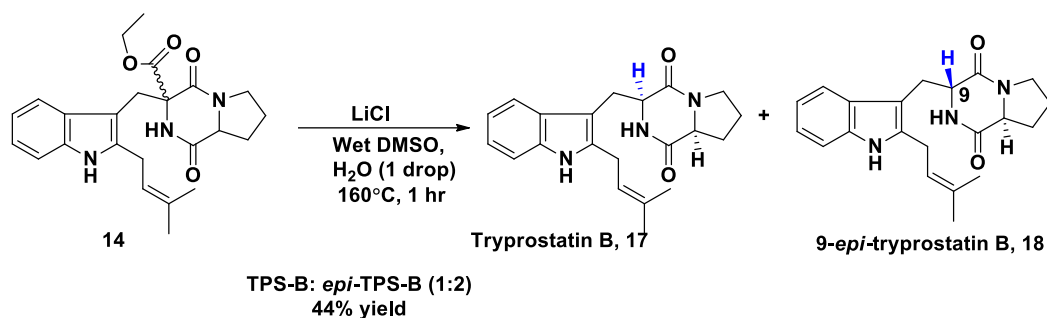
Base Screening				
Rxn #	Base	Time (h) /Temp <sup>0</sup> C	Solvent	Observation
1	Quinine	48/ reflux	ACN	Conv. 100%
2	Quinidine	48/ reflux	ACN	Conv. 100%
3	DBU	48/ reflux	ACN	Comp. 3 unreacted
4	TEA	48/ reflux	ACN	Comp. 3 unreacted
5	K <sub>2</sub> CO <sub>3</sub>	48/ reflux	ACN	Comp. 3 unreacted
6	KOH/PTC	72/rt	DCM	Comp. 3 unreacted
7	K <sub>2</sub> CO <sub>3</sub> /PTC	72/rt	DCM	Comp. 3 unreacted

With the optimized reaction conditions (ACN, 1.0 equiv. of quinine, 48 hrs, 1:4 dr value, and 88 % isolated yield) in hand, we then focused on the total synthesis of tryprostatin B from compound **14**.

#### 1.5.4. Synthesis of Tryprostatin B by decarboethoxylation

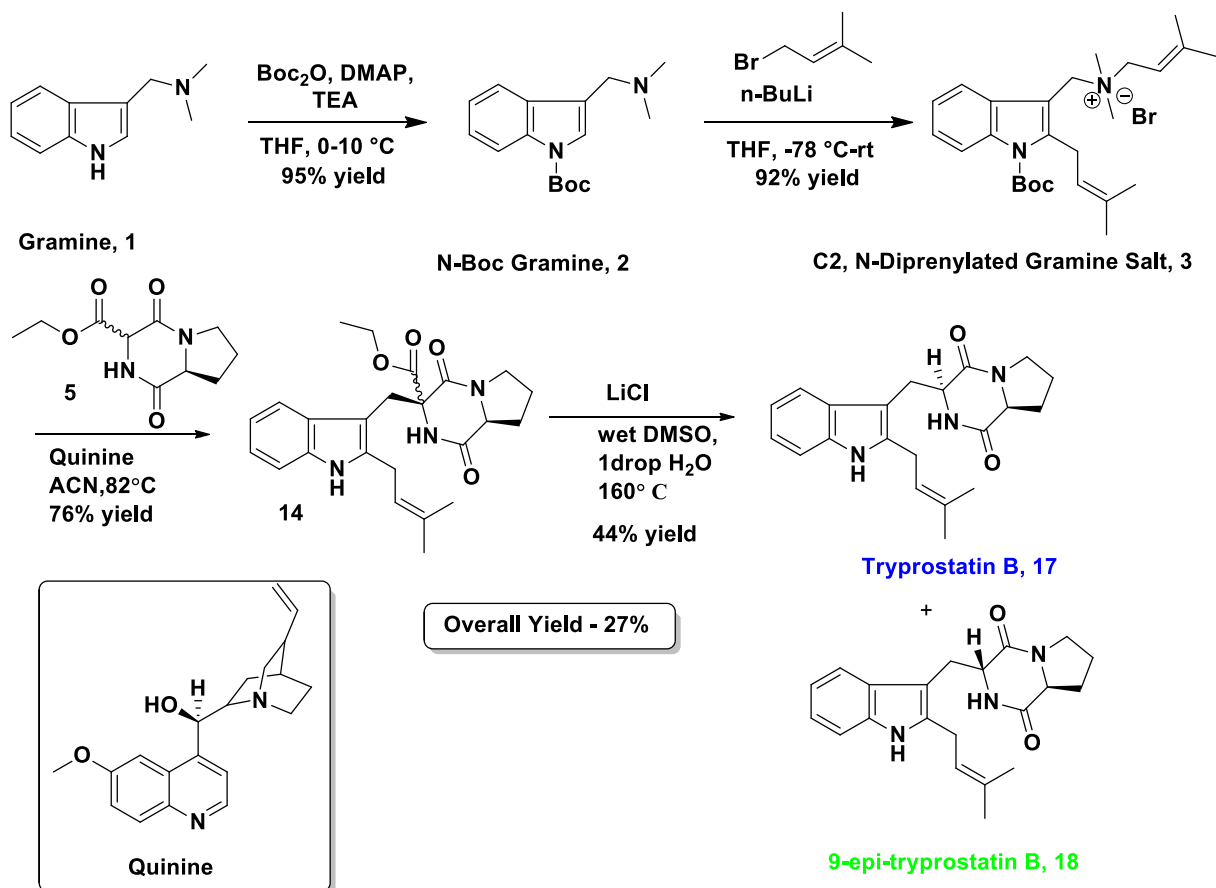
Lastly, to synthesize TPS B, we performed the decarboethoxylation reaction for compound **14**. Since **14** possesses the entire carbon skeleton of tryprostatin B, all that remained was to remove the carboethoxy group to complete the total synthesis. As either enolate or enol species is the short-lived product of the decarboxylation, the facial selectivity of the protonation determines the product distribution. To prepare tryprostatin B, we performed Krapcho decarboethoxylation for compound **14** in the presence of LiCl and water by using

wet DMSO as a solvent described by Fukuyama et al.<sup>61</sup> As a first attempt we get a mixture of diastereomers (TPS-B: 9-*epi*-TPS-B: 1:2) with 44% yield (Scheme 1.20).



Scheme 1.20: Decarboethoxylation reaction of compound **14**

Finally, we synthesized TPS B from the commercially available gramine in four steps in 27% overall yield in our first attempt (Scheme 1.21).



Scheme 1.21: Total synthesis of Tryprostatin B in our first attempt

### 1.5.5. Optimization of Decarboethoxylation reaction

To optimize the reaction conditions for higher yield and diastereoselective, we investigated the effects of salts as well as systematic variations in the solvents, temperature, and time. In order to find the best solvent for higher yield, several solvents were examined by using racemic mixture of compound **14** as presented in Table 1.8. For decarboxyethoxylation polar aprotic solvents DMSO, DMA, o-xylene and DMF worked well (Table 1.8, entries 1 and 5-6). In case of DMF and o-xylene the reaction gave 50% conversion where one of the diastereomer was unreacted in the reaction condition. The presence of the unreacted starting material was confirmed by both NMR and mass spectrometry (Figure 1.9). Less polar solvents like toluene, ACN and dioxane did not have a satisfactory result due to poor solubility of LiCl.

1.8: Optimization of Decarboethoxylation Reaction by Solvent Screening

Solvent Screening				
Rxn #	Solvent	Salt (5 equiv.)	Time (hr) /Temp °C	% Conversion
1	DMSO	LiCl	0.5 / 160	100
2	1,4-Dioxane	LiCl	12/ reflux	0
3	Toluene	LiCl	12/ reflux	0
4	CAN	LiCl	12/ reflux	100
5	DMF	LiCl	3/ reflux	50
6	DMA	LiCl	12/reflux	100
7	o-Xylene	LiCl	12/reflux	50

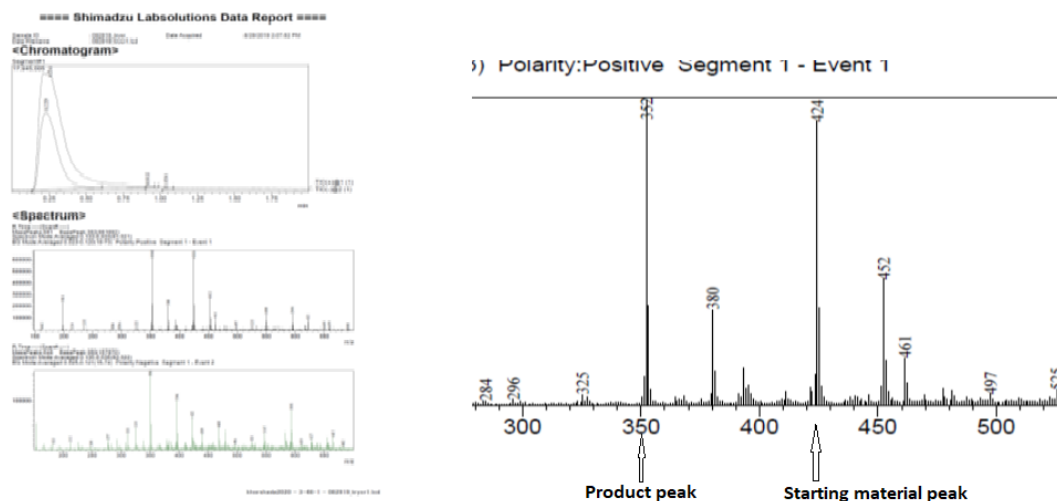


Figure 1.9: Decarboxyethoxylation reaction by DMF.

### 1.5.6. Structure elucidation of two diastereomers in compound **14**

As one of the diastereomer is not reactive under DMF and o-xylene condition, it is necessary to identify the structure of the two diastereomers in compound **14**. We did column chromatography to separate the two diastereomers, **14A** and **14B**. Compound **14A** (**Less polar**) was eluted with 1% MeOH and DCM whereas other compound **14B** (**More polar**) eluted with 2% MeOH and DCM and the ratio of two diastereomer is 1:4. To find out the absolute configuration we can do either X-ray crystallography or 2D NMR. At first, we attempt to make crystals by using different solvents but unfortunately it does not work (Table 1.9).

Table 1.9: Solubility of Compound **14**

Solvent Screening		
Entry	Solvent	Solubility/Obse rvation
1	EtOAc	Soluble/gummy compound
2	1,4-Dioxane	Soluble/gummy compound

3	Toluene	Not soluble
4	CAN	Soluble/gummy compound
5	Acetone	Soluble/gummy compound
6	Isopropyl alcohol	Soluble/gummy compound
7	CHCl <sub>3</sub>	Soluble/gummy compound
8	DCM	Soluble/gummy compound
9	THF	Soluble/gummy compound
10	Diethyl ether	Not soluble
11	EtOAc: Hexane	Soluble/ amorphous solid
12	DCM: Hexane	Soluble/ amorphous solid

When crystallization does not work, we did 2D NMR to figure out the structure of compound **14A** and **14B** (see SI for more details). After analyzing the heteronuclear multiple bond correlation spectrum (HMBC), compound **14A** is confirmed as a (S,S)- isomer where C18 is upward and it shows strong correlation with C15 and two carbonyl carbon C2 and C3 but it does not have any correlation with C16 and carbonyl carbon C1 (Figure 1.10).

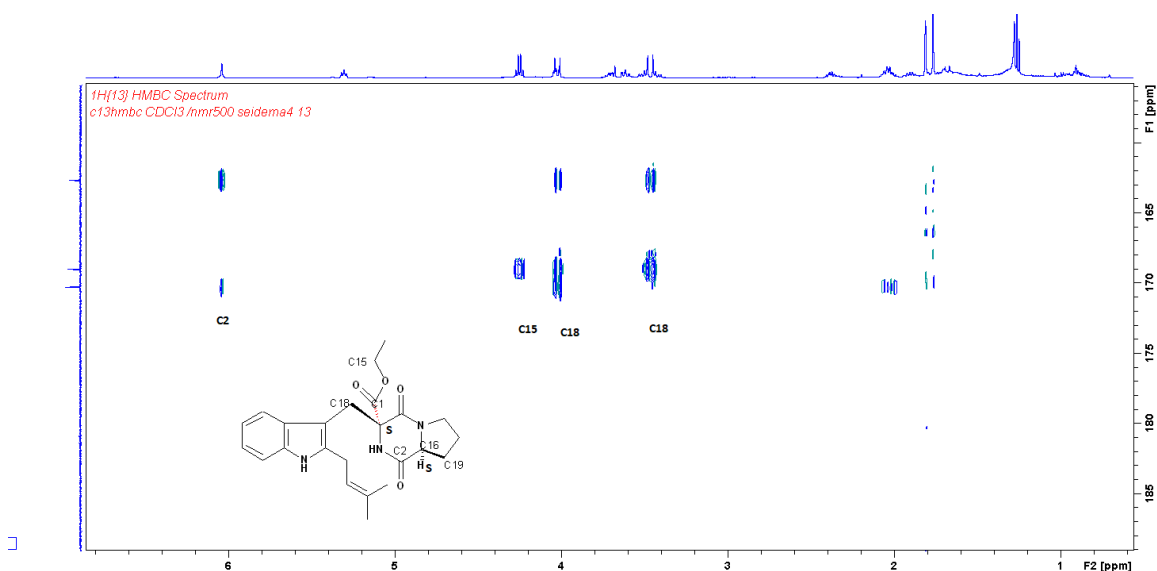


Figure 1.10: HMBC spectrum of compound **14A**

On the other hand, it reveals that **14B** is (R, S)-isomer where the H-atom at position C18 has a strong correlation with C15 and C16 (H-atom is downward) as well as three carbonyl carbon C1, C2 and C3 (figure 1.11)

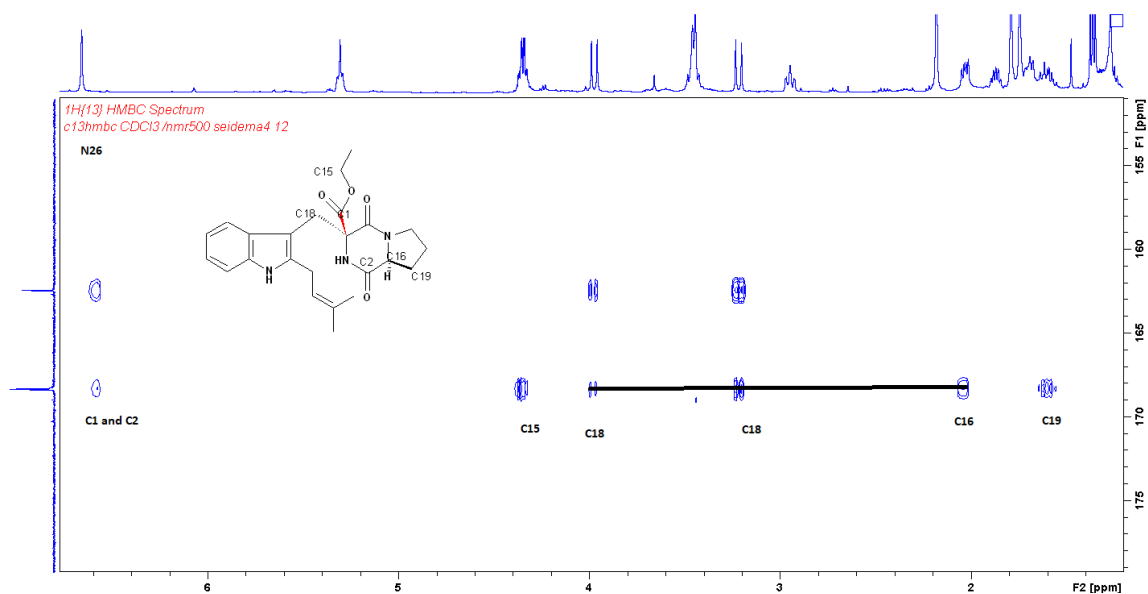
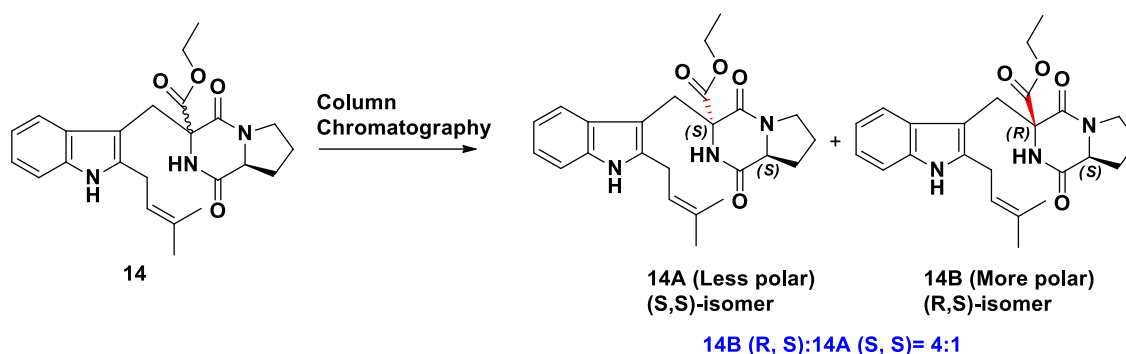


Figure 1.11: HMBC spectrum of compound **14B**



After confirming the two diastereomers, the structure of the two compounds are as follows represented in Scheme 1.22.



Scheme 1.22: Separation of compound **14** by column chromatography

We again performed the solvent screening by using the two separate isomers. It was shown that compound (R,S)-isomer of **14** undergoes 50% conversion in the presence of DMF and o-xylene, whereas compound (S,S)-isomer of **14** shows 100% conversion. From the above findings, we can conclude that the best solvent for decarboethoxylation reaction was DMSO and DMA for racemic mixture (Table 1.10).

Table 1.10: Optimization of Decarboethoxylation Reaction of compound **14** by Solvent Screening

Solvent Screening					
Entry	Compound	Solvent	Salt (5 equiv.)	Time (hr) /Temp $^{\circ}\text{C}$	% Conversion
<b>1</b>	<b>14A</b>	DMSO	LiCl	0.5 / 160	100
<b>2</b>	<b>14A</b>	DMF	LiCl	12/ reflux	100
<b>3</b>	<b>14A</b>	DMA	LiCl	12/ reflux	100
<b>4</b>	<b>14A</b>	o-Xylene	LiCl	12/ reflux	100
<b>5</b>	<b>14B</b>	DMSO	LiCl	0.5 / 160	100

<b>6</b>	<b>14B</b>	DMF	LiCl	12/ reflux	50
<b>7</b>	<b>14B</b>	DMA	LiCl	12/ reflux	100
<b>8</b>	<b>14B</b>	o-xylene	LiCl	12/ reflux	50

After solvent screening to improve the ratio for the desired isomer, Krapcho decarboethoxylation reactions of compound **14** was performed by using different salts (Table 1.11). Sodium iodide, KBr, and NaBr did not give the desired products (entries 1 and 4-5). The ratio of the desired isomer increased upon changing the salt to MgCl<sub>2</sub> or NaCl (entries 2 and 3). The best result was obtained with LiCl in the presence of either DMSO or DMA. When DMA was used as a solvent, both the yield and the desired isomer formation was improved to 89% combined yield (entry 8).

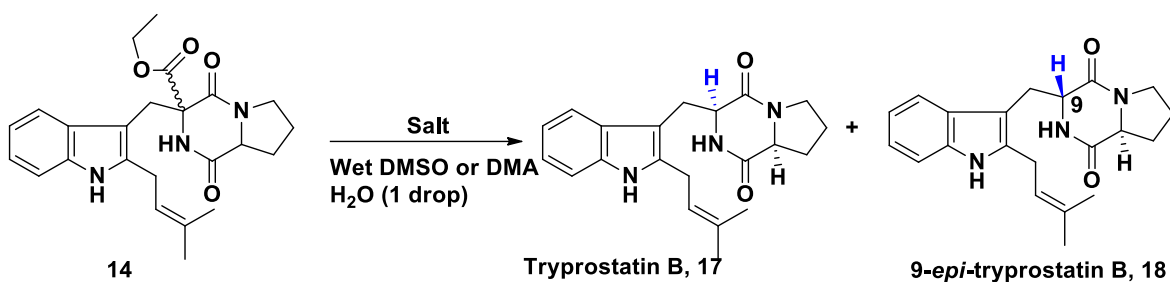


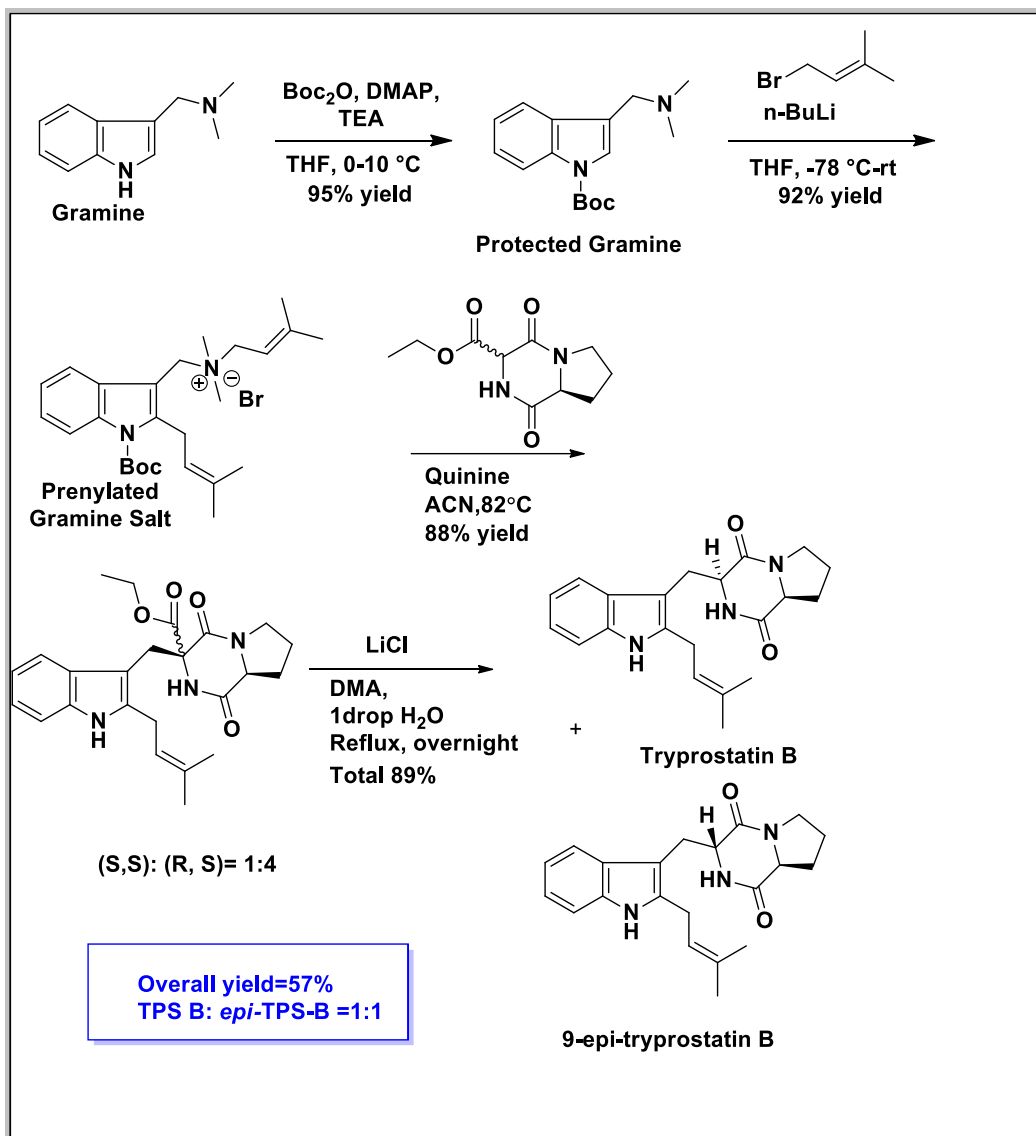
Table 1.11: Total synthesis of Tryprostatin B

Salt Screening					
Entry	Solvent	Salt (5 equiv.)	Time (hr) /Temp °C	Ratio <b>1/17</b>	Yield (%)
1	DMSO	NaI	0.5 / 160	NR	Decompose
2	DMSO	NaCl	0.5 / 160	1:2	10
3	DMSO	MgCl <sub>2</sub>	0.5 / 160	1:3	50

4	DMSO	NaBr	0.5 / 160	NR	Decompose
5	DMSO	KBr	0.5 / 160	ND	Trace
7	DMSO	LiCl	0.5 / 160	1:2	76
8	DMA	LiCl	12/ reflu x	1:1	89

After optimizing the decarboethoxylation reaction, TPS B was synthesized from the commercially available gramine in four steps in 57% overall yield (Scheme 1.23).

## 1.6. Total Synthesis of Tryprostatin B

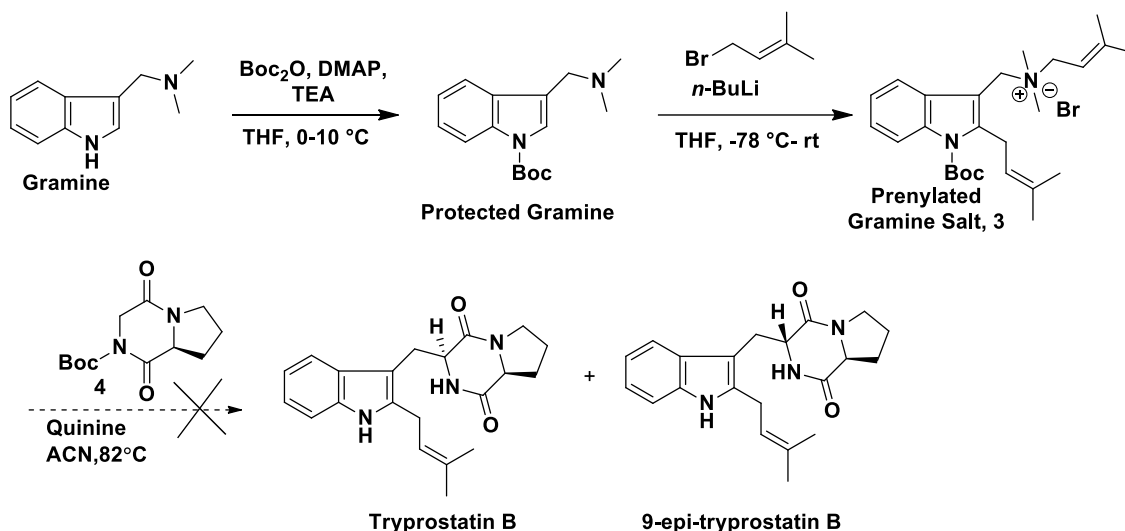


**Scheme 1.23: Improved Total synthesis of Tryprostatin B (TPS-B)**

After getting improved total synthesis of TPS-B we explore again route **1** for the direct alkylation of compound **3** and **4** which will reduce the overall step **3**.

### 1.7. Exploring Synthesis of Tryprostatin B by Route 1 in the presence of Quinine

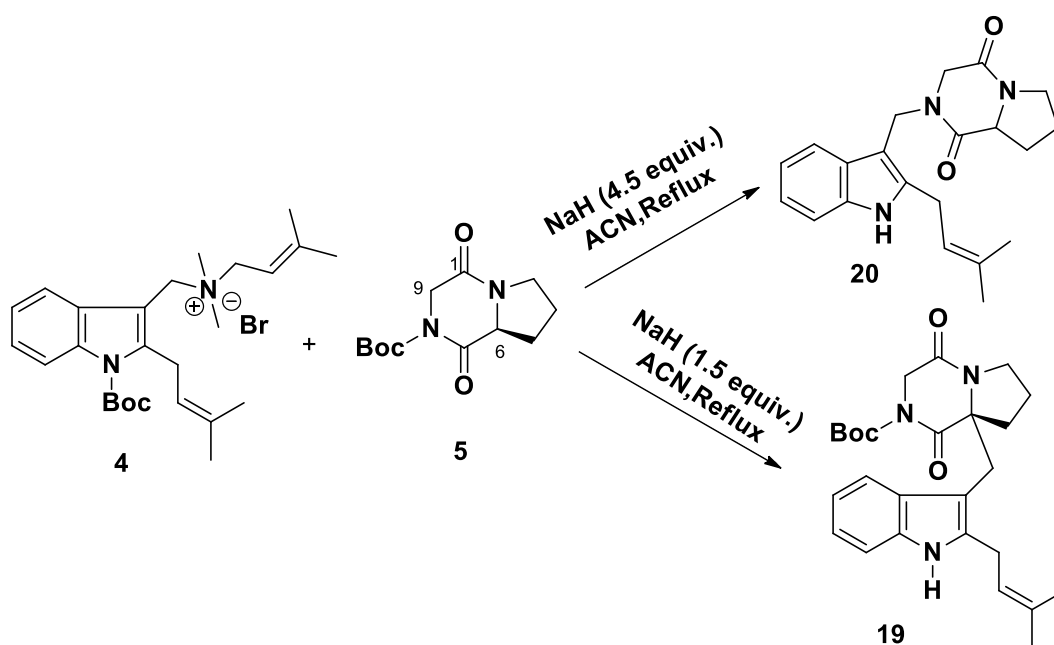
We carried out the coupling of compound **3** and **4** with the optimized reaction conditions developed by route **2** using quinine as a base and acetonitrile as a solvent (Scheme 1.24). Interestingly we observed that the compound **3** was decomposed in reflux condition after 48 hours but compound **4** was unreacted. These findings indicate that the quinine is not strong base to form anion from compound **4**.



Scheme 1.24: Attempt for the Synthesis of Tryprostatin B by Route 1 in the presence of Quinine

Receiving important data from the reactions, we thought that the direct alkylation can be done by using stronger base such as NaH. To form the anion at the C9 position, we first reacted the Boc-protected diketopiperazine **4** with 1.5 equivalent of NaH in the presence of ACN and then compound **3** was added to it. In our surprise, the reaction provided exclusively C6-alkylated product **18** with 80% yield which was confirmed by NMR and mass spectrometry (Scheme 1.25 and see SI for more details); no C9-alkylation was observed. By varying the equivalency of compound **3** from 2.0 to 1.0, the yield of the product reduced from 80 to 30% (Table 1.12 and entries 1-3). During our investigation we observed that using 4.5 equivalents of NaH and excess

of Boc-protected diketopiperazine (2.0 equiv) led to the formation of the N-alkylated product **19** in 70% yield (Table 1.12 and entry 4). We then use bulky stronger base to Sodium bis(trimethylsilyl)amide (NaHDMS) in place of NaH. In the presence of NaHDMS, the compound **5** undergo decomposition because during reaction NaHDMS decompose to give NaOH in the presence of solvent which act as a nucleophile (Table 1.12 and entry 5). These two findings open a new window to synthesize either N-alkylated product or C-6 alkylated product in diketopiperazine core in only 3 steps by the reaction with compound **4**.



Scheme 1.25: Synthesis of C-6 and N-Alkylated compound by NaH reaction

Table 1.12: Condition for the Synthesis of C-6 and N-alkylated compound by NaH

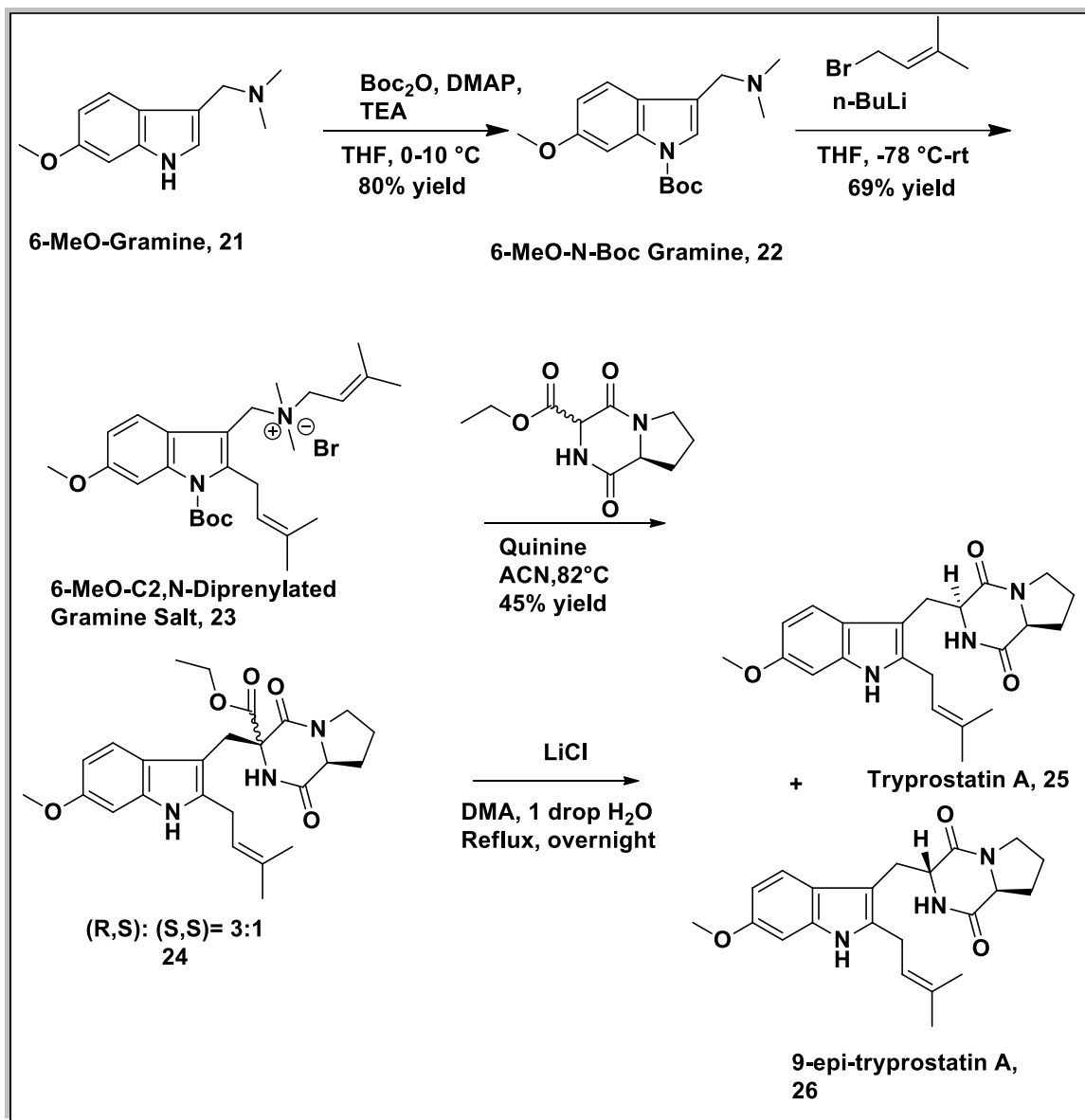
Rxn#	Solvent	Base	Ratio	Yield (%)
			<b>3:4: base</b>	
1	ACN	NaH	2.0:1.0:1.5	80
2	ACN	NaH	1.5:1.0:1.5	45
3	ACN	NaH	1.0:1.0:1.5	30

4	ACN	NaH	2.0:1.0:4.0	70
5	ACN	NaHDMS	2.0:1.0:1.5	Decompose

After exploring route **1**, we synthesize two new compounds, compound 19 and 20 by using NaH as a base. Still, we are searching suitable base to perform route 1 which can give us the desired compound **Tryprostatin B** in only three steps.

Then we move our focus to synthesize tryprostatin A from 6-MeO-gramine by following closely the optimized synthetic strategy of route **2** for the total synthesis of tryprostatin B. We obtained 1:1 mixture of **25** and 9-epimer-TPS A **26** in 50% yield (scheme 1.26).

## 1.8. Total Synthesis of Tryprostatin A

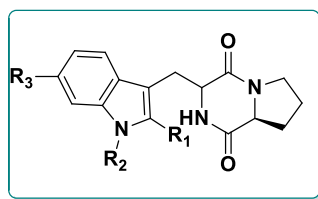


Scheme 1.26: Synthesis of Tryprostatin A



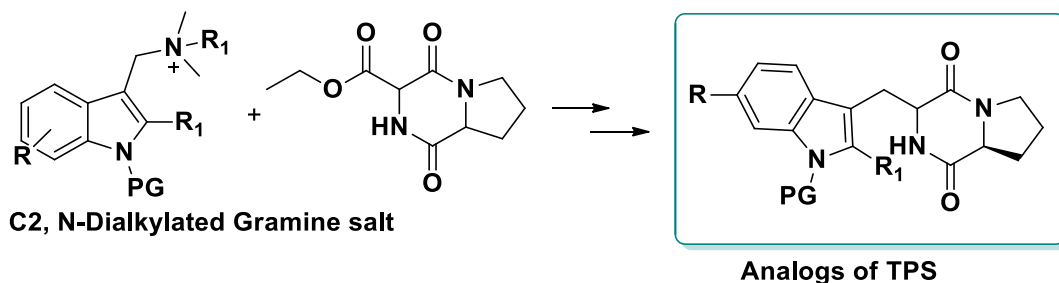
## 1.9. Synthesis of Tryprostatin Analogs

In the search for potent and selective antitumor agents, then we move our attention to synthesize the analogs of tryprostatins and several modifications can be carried out (Figure 1.12) by varying (A) substitution of the 2-position of the indole moiety, (**R**<sub>1</sub>) (B) alkylation of the indole NH (**R**<sub>2</sub>), (C) substitution of the 6-position of the aromatic ring (**R**<sub>3</sub>), and (D) substitution of the L-proline residue in the diketopiperazine ring with other L-amino acids.



Analogs of TPS

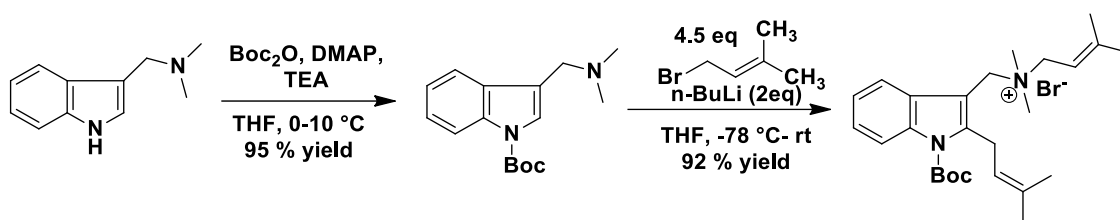
By following our developed method, at first, we tried to synthesize TPS analogs by varying substitution at the C-2 position of the indole moiety using C2, N-dialkylated gramine salt, and diketopiperazine core which are shown in Scheme 1.27. SAR study performed by Cook et al. and computational study done by Fani et al. reveals that whenever C-2 position (H) is replaced by hydrophobic moiety, it shows better potency and docking score.<sup>76-77</sup>



Scheme 1.27: The Proposed synthetic route for Tryprostatin analogs

### 1.9.1. Synthesis of C2, N-dialkylated gramine salt: Precursor of Tryprostatins Analogs

As we reported earlier that our group synthesize Tryprostatin B (C-2 prenylated indole alkaloid) from gramine by only six steps in 2019.<sup>62</sup> In that synthesis, our group introduced the prenyl group at the C-2 position of gramine (3-(dimethylaminomethyl)indole) by lithiation of N-Boc protected gramine followed by addition of electrophilic prenyl bromide by two steps (**Scheme 1.28**). This unusual procedure opens a new window to explore a variety of N-protected Gramines to synthesize C-2 substituted gramine salts by subsequent reactions with electrophiles. In our present research, we have examined several protecting groups and electrophiles to investigate the scope of this reaction and to probe the reaction mechanism.

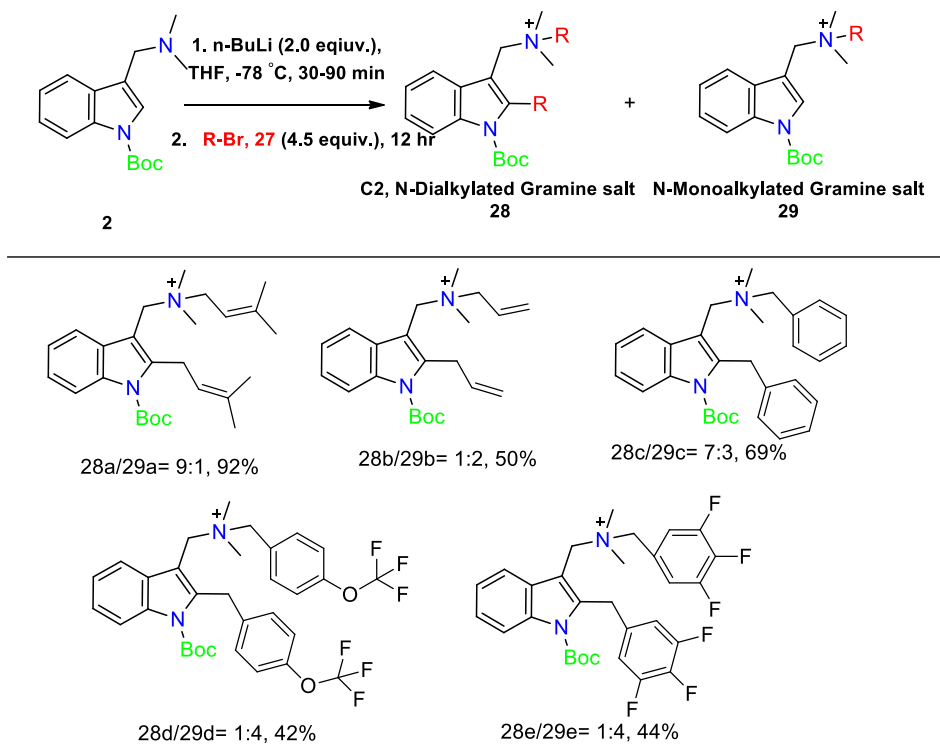


Scheme 1.28: Synthesis of C2, N-diprenylated gramine salt from Boc protected-Gramine by the reaction of *n*-BuLi.

### 1.9.2. Synthesis of C2, N-dialkylated gramine salt from N-Boc gramine

To obtain C2, N-dialkylated gramine salt, we initially explored C2-alkylation of N-Boc protected gramine **2** by using a variety of alkyl bromide **27** which can act as an electrophile based on our standard reaction condition developed in our previous synthesis.<sup>62</sup> During alkylation two different products can form namely C2, N-dialkylated product **28**, and N-monoalkylated product **29** (Table 1.13).

Table 1.13. Synthesis of C-2 substituted gramine salt from N-Boc protected gramines **2** and Alkyl Bromide **27**.

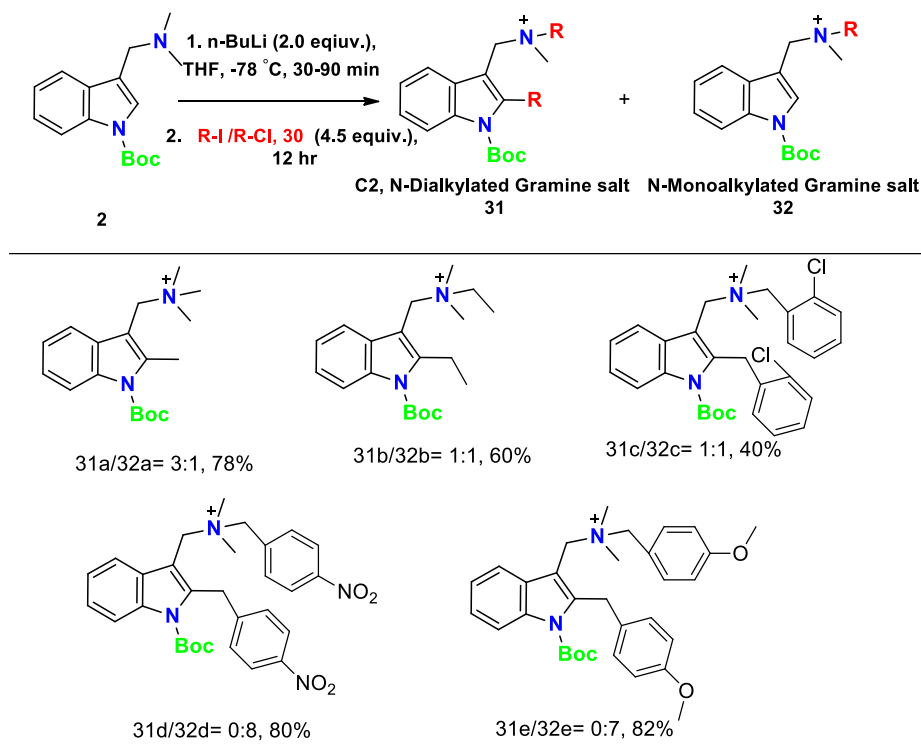


At first, we employed five different alkyl bromide compounds as an electrophile. Prenyl and benzyl bromide have almost similar reactivity and gave exclusively more C2, N-dialkylated product **28a**, and **28c** respectively. On the other hand, other bromide compounds showed a competitive reaction and gave a mixture of di-alkylated and mono-alkylated products. Benzyl bromide derivatives with an electron-withdrawing group such as **27d** and **27e** gave more N-monoalkylated products **29d** and **29e** than C2, N-dialkylated products with relatively low yield.

To find out the effect on the reactivity of different halides as an electrophile, we examined alkyl iodide and alkyl chloride, **30** as an electrophile besides alkyl bromide (Table 1.14).

Table 1.14: Synthesis of C-2 substituted gramine salt from N-Boc protected gramines **2** by

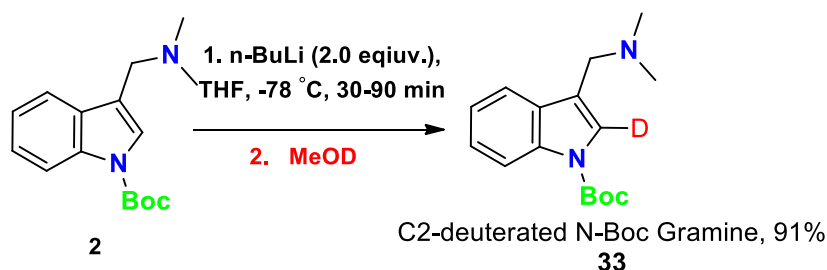
## Alkyl Iodide and Alkyl chloride **30**.



Though iodide is a better leaving group than bromide, in the cases of methyl (**30a**) and ethyl iodide (**30b**), we obtained more di-alkylated products **31a** and **31b** with a decent amount of mono alkylated product. Chloride compounds were also a suitable electrophile for this reaction and afforded the corresponding di- and mono-alkylated product. 2-chloro benzyl chloride **30c** gave a mixture of C2, N-dialkylated product **31c**, and N-monoalkylated product **32c** with a decent yield, whereas 4-nitro and 4-methoxy benzyl chloride exclusively gave exclusively N-monoalkylated product **32d** and **32e** respectively.

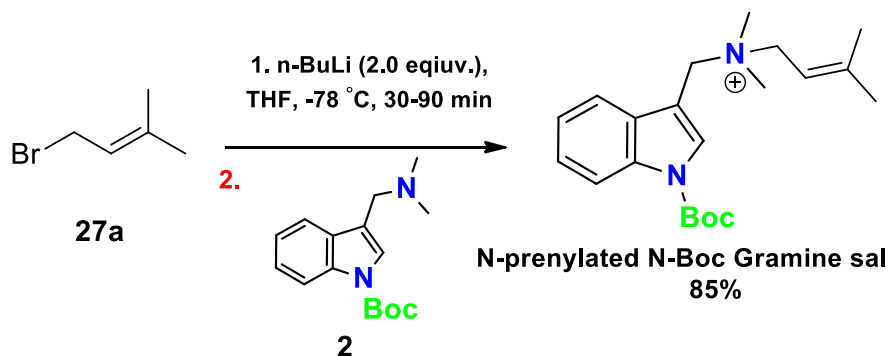
To understand the mechanism of the reaction and distribution of the product, we performed three different reactions represented by schemes 1.29-1.31. At first, deuterium reaction was

carried out by treating a solution of the N-protected gramine in THF with n-butyllithium followed by the quenching with D<sub>2</sub>O or MeOD. The location of deuterium incorporation was calculated by analysis of the mass spectrum and <sup>1</sup>H NMR (Scheme 1.29 and appendix A). In <sup>1</sup>H NMR, the singlet of the C-2 position for H was diminished by the incorporation of D and the percentage of D incorporation was calculated 91% based on <sup>1</sup>H NMR.



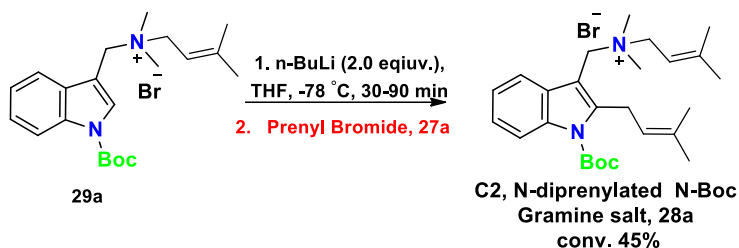
Scheme 1.29: C2-Deuteration of N-Boc gramine

Secondly, we performed the reaction by changing the sequence of the addition of substrate where prenyl bromide **27a** first react with n-BuLi then compound **2** was added to it. In that reaction exclusively we obtained mono alkylated product **29a** (Scheme 1.30).



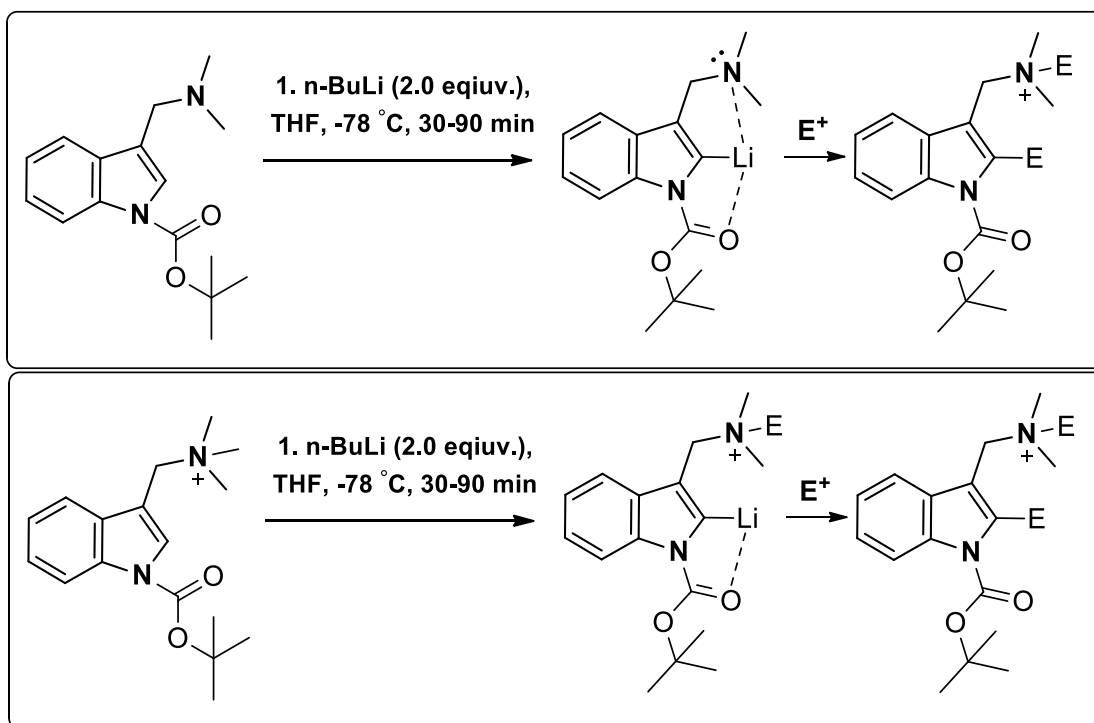
Scheme 1.30: Synthesis of N-prenylated gramine salt by sequence change

Finally, we performed the reaction with N-mono alkylated product **29a** in the standard reaction condition. After the reaction we observed C2, N-dialkylated product was formed by 45% conversion (Scheme 1.31).



Scheme 1.31: Alkylation of N-monoprenylated product by prenyl bromide.

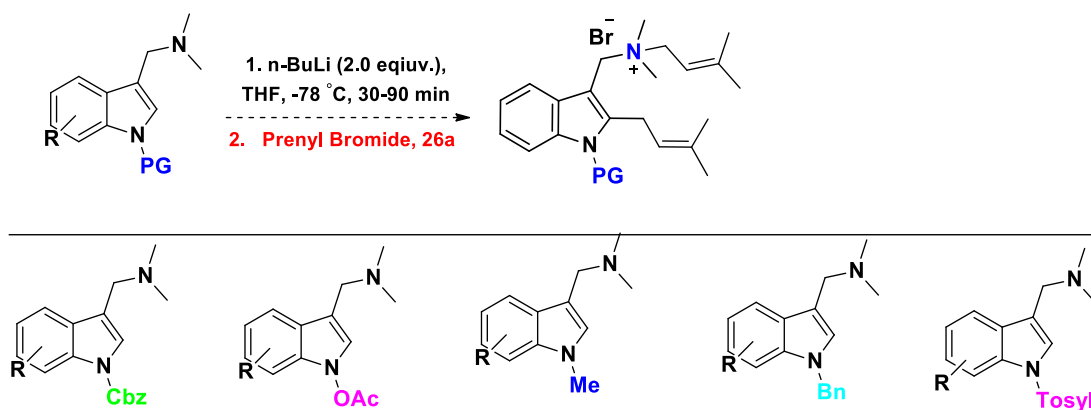
Based on the above findings, we can propose the following mechanism (Scheme 1.32). Both the lone pair of N-atom and O-atom in the Boc group assisted the C-2 position for lithiation. Whenever the N-atom is occupied by electrophile than Boc can only assisted the C-2 position for lithiation but the conversion of the product is reduced from 100 to 45%.<sup>78</sup>



Scheme 1.32: Plausible reaction Mechanism

After getting a variety of C-2 substituted products in hand, next, we focused on the identification of an appropriate directing group besides Boc. As a model substrate, we used

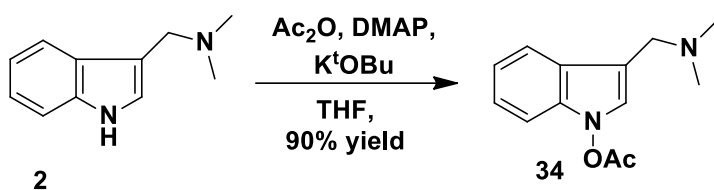
prenyl bromide as an electrophile and a range of N-protected gramine in the presence of n-BuLi as a lithiating agent (Scheme 1.33).



Scheme 1.33: Reaction between Prenyl bromide and N-protected gramine in the presence of n-BuLi

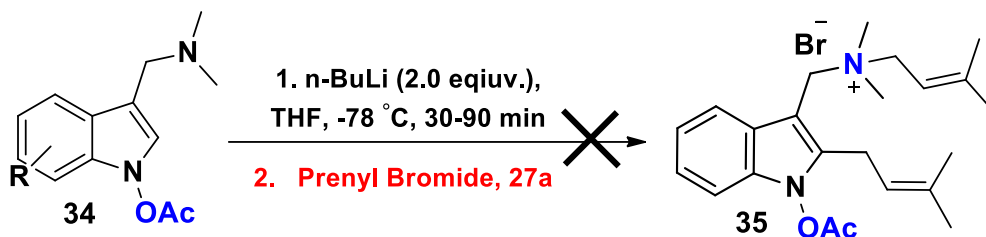
### 1.9.3. Synthesis of C2, N-dialkylated gramine salt from N-AcO gramine

At first, we synthesized N-AcO gramine **34** by the reaction of gramine **1** and acetic anhydride in THF by using potassium tert-butoxide (K<sup>t</sup>OBu) as a base (Scheme 1.34).



Scheme 1.34: Synthesis of N-AcO gramine

After getting the starting material in our hand, we performed the C-2 alkylation reaction in the standard reaction conditions by using 2.0 equiv. of n-BuLi and 4.5 equiv. of Prenyl bromide. But the acetyl group is labile under the n-BuLi reaction, and the reaction did not work (Scheme 1.35)



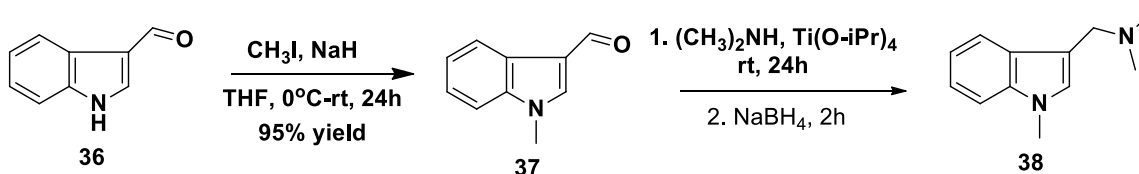
Scheme 1.35: Synthesis of C2, N-dialkylated gramine salt from N-AcO gramine

When acetyl protecting group did not work, we move our attention to other protecting groups.

In 1993, Iwao reported regioselective lithiation at C-2 position by using N-methyl gramine in the presence of *t*-BuLi in ether at 0°C. Then we choose methyl group as our next protecting group.<sup>79</sup>

#### 1.9.4. Synthesis of C2, N-dialkylated gramine salt from N-methyl gramine

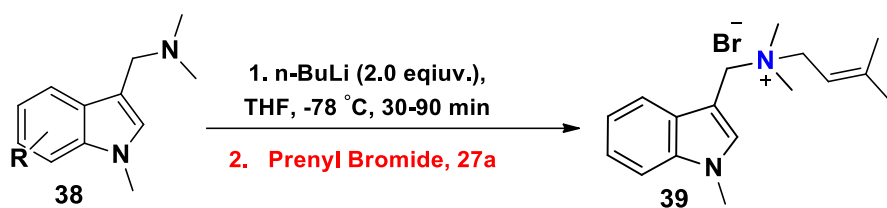
To synthesize N-methyl gramine we use indole-3-carboxyaldehyde instead of gramine. At first, indole-3-carboxyaldehyde **36** react with methyl iodide in the presence of NaH to give N-methyl indole -3-carboxyaldehyde **37**. Compound **37** reacts with Dimethylamine to form compound **38** which undergo reductive amination in the presence of Ti(O-*i*Pr) and sodium borohydride (NaBH<sub>4</sub>) (Scheme 1.36)



Scheme 1.36: Synthesis of N-methyl gramine

After getting starting material in our hand, we then explore C-2 alkylation of compound **38** by using our standard reaction condition (Scheme 1.37). In our case, alkylation happened in methylene N to form compound **39** instead of giving any C-2 alkylated product in the indole ring. The reason for anomaly of our reaction with the reported method could be, we use *n*-BuLi in place of *t*-BuLi which is much stronger than ours.

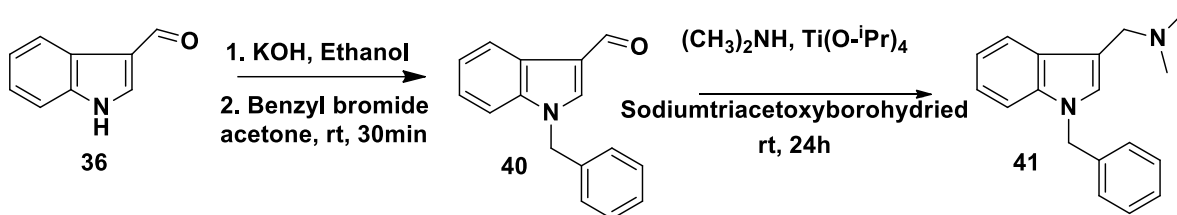




Scheme 1.37: Reaction between N-methyl gramine and prenol bromide in the presence of n-BuLi.

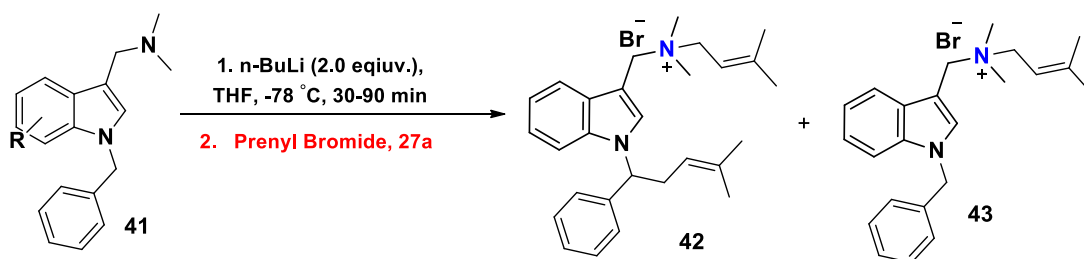
### 1.9.5. Synthesis of C2, N-dialkylated gramine salt from N-benzyl gramine

We synthesize N-benzyl gramine **41** from compound **36** by following reported protocol. At first, indole-3-carboxyaldehyde **36** react with benzyl bromide in the presence of KOH to give N-benzyl indole -3-carboxyaldehyde which reacts with Dimethylamine in the presence of Ti(O-<sup>i</sup>Pr) and subsequent reductive amination with sodiumtriacetoxy borohydride gave compound **41** (Scheme 1.38)



Scheme 1.38: Synthesis of N-benzyl gramine

Under standard reaction condition, in case of N-benzyl bromide, lithiation happened in the benzyl methylene group and give a mixture of N-prenyl-N-benzyl gramine salt **43** and different dialkylated product **42** (Scheme 1.39).

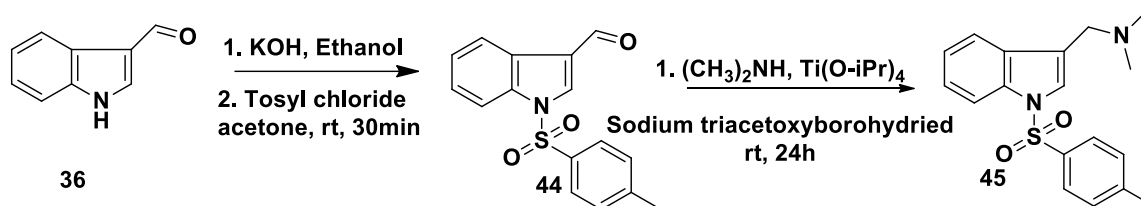


Scheme 1.39: Reaction between N-benzyl gramine and prenyl bromide in the presence of n-BuLi.

When methyl, acetyl and benzyl protecting group did not work, we move our intention to O containing protecting group such as Tosyl.

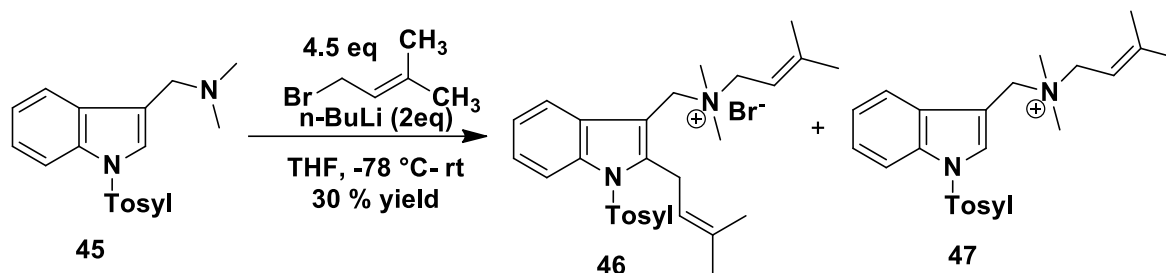
### 1.9.6. Synthesis of C2, N-dialkylated gramine salt from N-tosyl gramine

We synthesize N-tosyl gramine 46 from compound 39 by following reported protocol. At first, indole-3-carboxyaldehyde **39** react with benzyl bromide in the presence of KOH to give N-benzyl indole -3-carboxyaldehyde which reacts with Dimethylamine in the presence of Ti(O-<sup>i</sup>Pr) and subsequent reductive amination with sodium borohydride (NaBH<sub>4</sub>) gave compound 46 (Scheme 1.40)



Scheme 1.40: Synthesis of N-tosyl gramine

In the reaction of N-tosyl gramine and prenyl bromide in the presence of n-BuLi gave the desired C2, N-diprenylated gramine salt as well as N-monoprenylated gramine salt (Scheme 1.41).

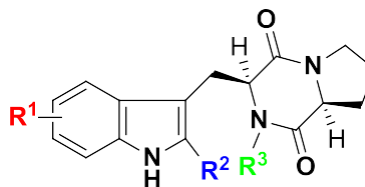


Scheme 1.41: Reaction between N-tosyl gramine and prenyl bromide in the presence of n-BuLi.

Finally, we demonstrated a simple method to synthesize 2-substituted gramine salts by using N-tert-butylcarbonyl (Boc) as a directing group. Additionally, one more protecting groups tosyl- can also act as a directing group for the synthesis of C2-substituted gramine salts. All the protecting groups are easy to introduce and do not require complicated methods to remove. Further studies on the scope of the substrates and its applications in the synthesis of biologically and pharmaceutically important compounds are underway in our laboratory.

### 1.10 Conclusion and Future Works

In summary, Tryprostatin (TPS) A and B have great potential because they were found to have microtubule inhibitory activity on the cell cycle progression of mouse tsFT210 cells. Their interesting biological activity and simple structure have drawn attention from the synthetic community, and several total syntheses have been reported. Here, we described a concise and efficient total synthesis of tryprostatin B and A and its epimer by only four steps. The key steps involved first, the preparation of C2 prenyl gramine salt by direct lithiation from Boc protected gramine. This is most unique process by which one can incorporate any electrophile at the C2 position of gramine and make more TPS analogs. Second, the direct alkylation reaction of the prenylated gramine salt with diketopiperazine core. In our method, the direct alkylation reaction was cost effective step because it produced less waste with minimum number of chemicals. The direct alkylation reaction was optimized by changing the solvent, base, and time. Finally, we performed krapcho decarboxylation reaction to get TPS A and B by using Dimethylacetamide (DMA) as a solvent. From our developed method, TPS B and TPS A were synthesized in four steps with 57% and 40% overall yield respectively. Additionally, by changing the substituent at C2 position of gramine, we synthesized a series of C2-alkylated gramine salt which is one of the precursors of TPS analogs. Our goal is to synthesize analogs of tryprostatins, and after making the analogs (Figure 1.14) our group will see the activity of new synthetic compounds against cancer cell lines. Further investigations into the synthesis of TPS analogs are under way.



**Figure 1.14:** Different Analogs of Tryprostatins

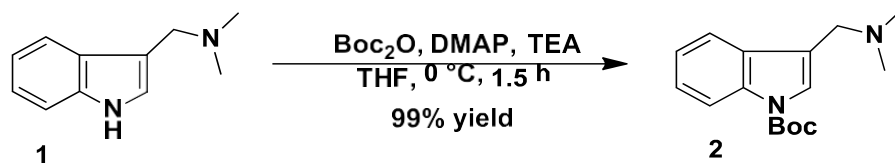
## 1.11. General Methods and Experimental

### 1.11.1. General Consideration

All reactions were performed under a dry nitrogen atmosphere using standard Schlenk techniques unless otherwise noted. All reaction vessels were flame dried under vacuum and filled with nitrogen prior to use. Reagents and solvents were purchased from Sigma-Aldrich, Milwaukee. All  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3$  (internal standard: 7.26 ppm,  $^1\text{H}$ ; 77.16 ppm,  $^{13}\text{C}$ ) at room temperature with a Bruker 300 MHz and 500 MHz spectrometers. The chemical shifts ( $\delta$ ) are given in parts per million (ppm) and the coupling constants in Hertz (Hz). The following abbreviations are used: *s*-singlet, *d*-doublet, *t*-triplet, *q*-quartet, *m*-multiplet. Previously reported compounds were identified by  $^1\text{H}$  NMR. All new compounds were additionally characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and high-resolution mass spectrometry (HRMS). HRMS were obtained using electrospray ionization (ESI) technique. For column chromatography, silica gel (35-70 microns) was used. Thin layer chromatography (TLC) was performed on aluminium backed plates pre-coated (0.25 mm) with Silica Gel 60 F254 with a suitable solvent system and was visualized using UV fluorescence and/or iodine chamber. Microwave reaction was done at 250 °C, 250 W, and 150 psi using a CEM Discover Microwave synthesizer.

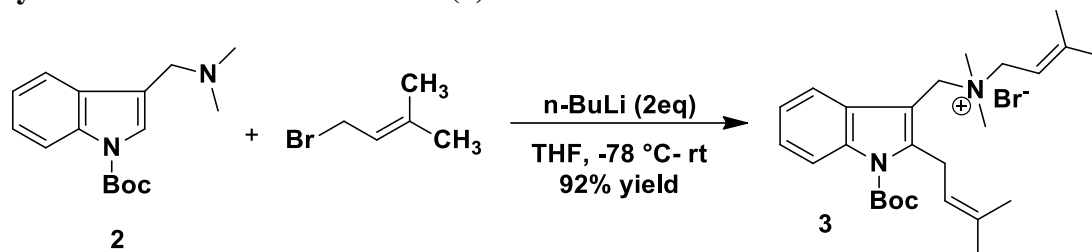
### 1.11.2. Experimental Methods

#### ***Tert*-butyl 3-((dimethylamino)methyl)-1H-indole-1-carboxylate (2)**



A solution of Boc anhydride (Boc<sub>2</sub>O) (12.0 g, 55.1 mmol), 4-dimethylaminopyridine (DMAP) (0.56 g, 4.6 mmol), trimethylamine (TEA) (0.56 mL, 5.5 mmol) in THF (100 mL) was maintained at 0 °C for 30 min. A solution of gramine **1** (8.0 g, 45.9 mmol) in THF (60 mL) was added dropwise through the dropping funnel over a period of 30 min at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 hours under nitrogen atmosphere. After consumption of starting material, as judged by TLC analysis, water (20 mL) was added to quench the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with Et<sub>2</sub>O (3 x 30 mL), the combined organic layers were washed with brine solution (1 x 30 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The crude product was purified with column chromatography on silica gel (Hexane/EtOAc = 7/3) to give product **2** as a light brown solid (12.5 g, 99%) and matched with reported NMR.<sup>62</sup> **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.17 (d, *J* = 9.0 Hz, 1H), 7.70 (d, *J* = 9.0 Hz, 1H), 7.55 (s, 1H), 7.36-7.24 (m, 2H), 3.60 (s, 2H), 2.33 (s, 6H), 1.69 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 149.8, 135.6, 130.6, 124.6, 124.4, 122.6, 119.6, 117.8, 115.1, 83.5, 54.5, 45.4, 28.2.

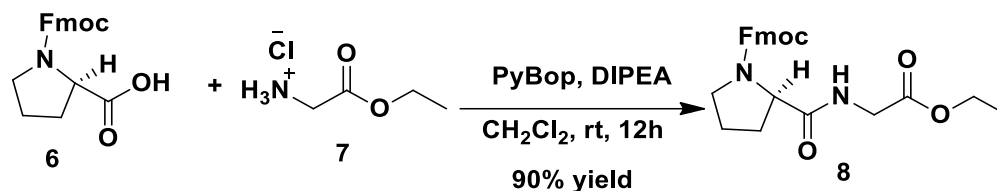
***N*-((1-(tert-butoxycarbonyl)-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-*N,N*,3-trimethylbut-2-en-1-aminium bromide (**3**)**



A solution of **2** (8.0 g, 29.2 mmol) in THF (100 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (23.3 mL, 2.5 M, 58.3 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. Prenyl bromide (17.4 mL, 11.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of all the starting material, as judged by TLC analysis, water (30 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL), the combined organic layers were washed with brine solution (1 x 30 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **3** as a brown solid (13.2 g, 92 %). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.08 (d, *J* = 9.0 Hz, 2H), 7.32 (dd, *J* = 9.0 Hz, 6.0 Hz, 2H), 5.38 (t, *J* = 7.5 Hz, 1H), 5.27 (s, 2H), 5.04 (s, 1H), 4.56 (d, *J* = 6.0 Hz, 2H), 3.91 (s, 2H), 3.17 (s, 6H), 1.93 (s, 3H), 1.88 (s, 3H), 1.80 (s, 3H), 1.67 (s, 12H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 149.6, 148.7, 144.1, 135.8, 134.1, 129.0, 124.4, 123.6, 120.3, 119.8, 114.9, 111.2, 106.0, 85.0, 61.8, 58.1, 48.4, 27.9, 26.8, 26.4, 25.4, 19.5, 18.7; **HRMS (ESI<sup>+</sup>):** Calculated (m/z) for C<sub>26</sub>H<sub>39</sub>N<sub>2</sub>O<sub>2</sub> [M]<sup>+</sup>: 411.3006, Found: 411.2993.

**(R)-(9H-fluoren-9-yl)methyl 2-((2-ethoxy-2-oxoethyl)carbamoyl)pyrrolidine-1-carboxylate**

**(8)**

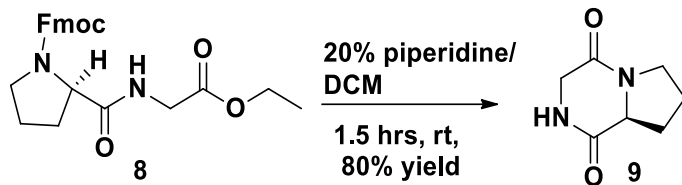


PyBOP (1.84 g, 3.55 mmol) and DIPEA (1.55 mL, 8.88 mmol) were added to a solution of Fmoc-*L*-proline **6** (1.0 g, 2.96 mmol) and glycine ethyl ester hydrochloride **7** (0.413 g, 2.96 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL). The reaction was stirred overnight, and progress was monitored by TLC. After consumption of the starting material, as judged by TLC analysis, the reaction was concentrated under reduced pressure. The residue was then dissolved in EtOAc (10 mL) and stirred via a stir bar until it made a homogeneous solution. The mixture was then acidified with 0.1M HCl acid, extracted with water (3 x 30 mL) and then with NaHCO<sub>3</sub> (3 x 30 mL). The combined organic layers were washed with brine solution (1 x 30 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (Hexane/EtOAc = 1/1) to afford **8** as a white foam (1.12 g, 90 %).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 7.78 (d, *J* = 6.8 Hz, 2 H), 7.57 (m, 2 H), 7.42 (t, *J* = 7.2 Hz, 2 H), 7.33 (t, *J* = 7.2 Hz, 2 H), 7.08 (br s, 1 H), 4.55-4.10 (comp m, 6 H), 4.10-3.99 (m, 2 H), 3.61-3.41 (m, 2 H), 2.36-1.88 (comp m, 4 H), 1.25 (t, *J* = 7.2 Hz, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 172.0, 169.8, 156.3, 144.0, 141.5, 127.9, 127.2, 125.2, 120.5, 67.8, 61.6, 60.6, 47.4, 41.5, 31.2, 28.7, 24.7, 23.7, 14.3; **HRMS (ESI<sup>+</sup>)**: Calculated (*m/z*) for C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub> (M+H)<sup>+</sup> : 423.1914, Found 423.1921.

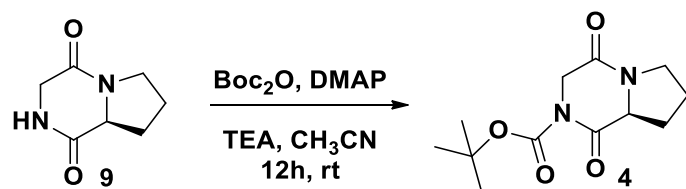


**(S)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (9)**



8.0g of (R)-(9H-fluoren-9-yl)methyl 2-((2-ethoxy-2-oxoethyl)carbamoyl)pyrrolidine-1-carboxylate **8** was dissolved in 100 mL of CH<sub>2</sub>Cl<sub>2</sub> and 20% piperidine was added to it at room temperature. The reaction was stirred for 1.5 hr, and progress was monitored by TLC. After consumption of the starting material, as judged by TLC analysis, the reaction was concentrated under reduced pressure. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **9** as a white solid (1.12 g, 80 %). Compound **9** was confirmed by comparing spectra to known NMR. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 7.10 (s, 1H), 4.10 (d, *J* = 15.0 Hz, 1H), 3.90 (dd, *J* = 15.0, 5.0 Hz, 1H), 3.69-3.52 (m, 2H), 2.42-2.34 (m, 1H), 2.14-2.01 (m, 2H), 1.95-1.87 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 170.1, 163.5, 58.5, 46.6, 45.3, 28.5, 22.4. **HRMS (ESI<sup>+</sup>)**: Calculated (*m/z*) for C<sub>7</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> : 155.0815, Found 155.0819.

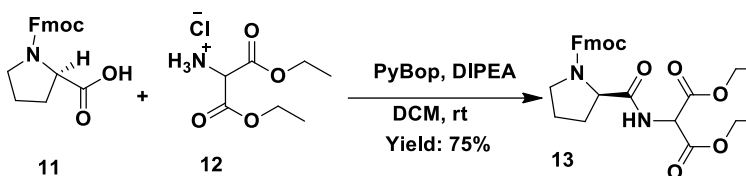
**(S)-tert-butyl 1,4-dioxohexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate (4):**



A solution of Boc anhydride ( $\text{Boc}_2\text{O}$ ) (21.3 g, 3.5 equiv., 97.7 mmol), 4-dimethylaminopyridine (DMAP) (3.4 g, 1.0 equiv., 27.9 mmol), trimethylamine (TEA) (2.8mL, 1.0 equiv., 27.9 mmol) in  $\text{CH}_3\text{CN}$  (100 mL) was maintained at  $0^\circ\text{C}$  for 30 min. A solution of (S)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione **9** (4.3 g, 27.9 mmol) in  $\text{CH}_3\text{CN}$  (20 mL) was added dropwise through the dropping funnel over a period of 30 min at  $0^\circ\text{C}$ . The reaction was stirred for 12 hours, and progress was monitored by TLC. After consumption of the starting material, as judged by TLC analysis, the reaction was concentrated under reduced pressure and purified by column chromatography on silica gel (DCM/MeOH = 20/1) to give product **4** as a white solid (6.02 g, 85%).  **$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):**  $\delta$  4.54 (d,  $J$  = 30.0 Hz, 1H), 4.12 (t,  $J$  = 10.0 Hz, 1H), 4.03(d,  $J$  = 30.0 Hz, 1 H), 3.47 (t,  $J$  = 10.0 Hz, 2H), 2.16-2.29 (m, 2H), 1.80-1.94 (m, 2H), 1.44 (s, 9H);  **$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz):**  $\delta$  167.5, 163.2, 150.0, 84.4, 60.4, 49.7, 45.2, 27.9, 23.2; **HRMS (ESI+):** Calculated (m/z) for  $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_4$  ( $\text{M}+\text{Na}$ ) $^+$  : 277.1159, Found 277.1136.

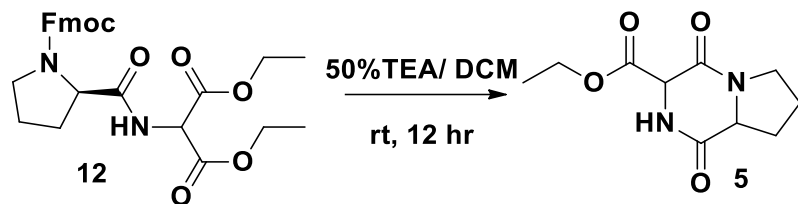
**(R)-diethyl 2-(1-(((9H-fluoren-9-yl)methoxy)carbonyl)pyrrolidine-2-carboxamido)malonate**

**13**



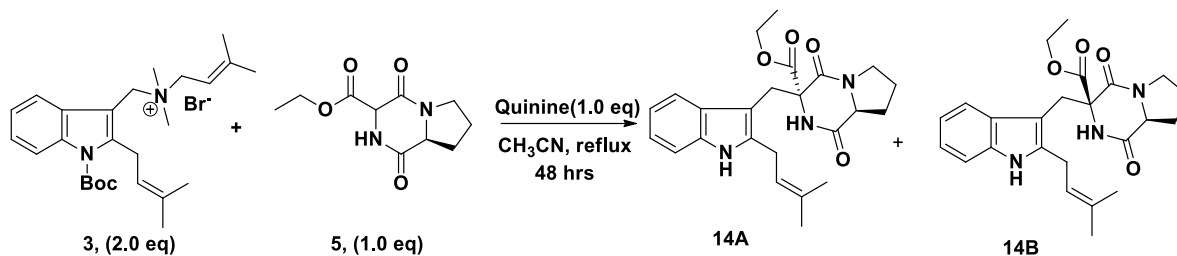
PyBOP (18.43 g, 35.4 mmol) and DIPEA (11.5 mL, 88.9 mmol) were added to a solution of Fmoc-*L*-proline **6** (9.92 g, 29.4 mmol) and diethyl aminomalonate hydrochloride **11** (6.24 g, 29.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The reaction was stirred overnight, and progress was monitored by TLC. After consumption of the starting material, as judged by TLC analysis, the reaction was concentrated under reduced pressure. The residue was then dissolved in EtOAc (10 mL) and stirred via a stir bar until it made a homogeneous solution. The mixture was then acidified with 0.1M HCl acid, extracted with water (3 x 30 mL) and then with NaHCO<sub>3</sub> (3 x 30 mL). The combined organic layers were washed with brine solution (1 x 30 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (Hexane/EtOAc = 2/5) to afford **13** as a white foam (10.9 g, 75 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 7.73 (d, *J* = 10 Hz, 2 H), 7.57 (m, 2 H), 7.36 (t, *J* = 10 Hz, 2 H), 7.28 (t, *J* = 7.2 Hz, 2 H), 7.03 (s, 1H), 5.13 (d, *J* = 10, 1H), 4.43–4.28 (m, 8H), 3.60–3.46 (m, 2H), 2.29–1.90 (comp. m, 4H), 1.24 (t, *J* = 7.5, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ 171.7, 166.0(2C), 155.9, 155.0, 143.9, 141.3(2C), 127.7(2C), 127.0(2C), 125.1(2C), 119.9(2C), 67.9, 62.5, 60.3, 56.7, 47.2, 31.2, 28.5, 24.6, 23.5, 20.9, 13.9. **HRMS (ESI<sup>+</sup>)**: Calculated (*m/z*) for C<sub>27</sub>H<sub>30</sub>N<sub>2</sub>O<sub>7</sub> (*M*+H)<sup>+</sup> : 495.2126, Found 495.2138.

**(8aS)-ethyl 1,4-dioxooctahydropyrrolo[1,2-a]pyrazine-3-carboxylate **5****



8.0g of (R)-diethyl 2-(1-(((9H-fluoren-9-yl)methoxy)carbonyl)pyrrolidine-2-carboxamido)malonate **12** was dissolved in 100 mL of CH<sub>2</sub>Cl<sub>2</sub> and 50% Triethyl amine(TEA) was added to it at room temperature. The reaction was stirred for 12 hours, and progress was monitored by TLC. After consumption of the starting material, as judged by TLC analysis, the reaction was concentrated under reduced pressure. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **5** as a white solid (2.19 g, 65 %). Compound **5** was confirmed<sup>ref</sup> by comparing spectra to known NMR. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.71 (d, *J* = 3.5 Hz, 1H), 4.625 (d, *J* = 4.4 Hz, 1H), 4.15-4.27 (m, 3H), 3.47-3.57 (m, 2H), 2.00-2.35 (m, 1H), 1.91-1.99 (m, 2H), 1.82-1.87 (m, 1H), 1.26 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ 170.8, 167.0, 159.9, 62.9, 60.8, 58.5, 45.9, 28.6, 22.3, 14.0. **HRMS (ESI+)**: Calculated (*m/z*) for C<sub>7</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> : 227.1026, Found 227.0996.

(3S,8aS)-ethyl 3-((2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazine-3-carboxylate, **14A** and (3R,8aS)-ethyl 3-((2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazine-3-carboxylate, **14B**



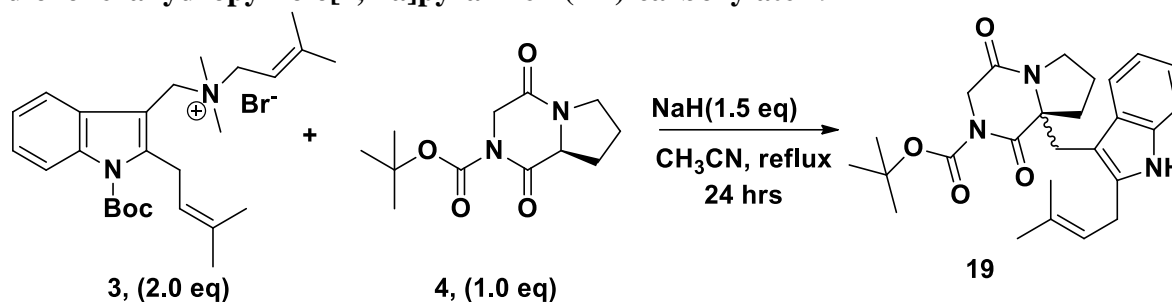
To a suspension of *N*-((1-(tert-butoxycarbonyl)-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-*N,N*,3-trimethylbut-2-en-1-aminium bromide **3** (1.30 g, 2.64 mmol) in acetonitrile (5.0 mL) compound (8aS)-ethyl 1,4-dioxooctahydropyrrolo[1,2-a]pyrazine-3-carboxylate **5** (300 mg, 1.30 mmol) was added, followed by the addition of 430 g (1.30 mmol) of quinine. The mixture was stirred under reflux for 24-48 hours and monitored by TLC. The solvent was removed in vacuo. The crude product was purified with flash column chromatography on silica gel (DCM/MeOH = 100/1) to afford **14A** as a white solid (98.9 mg, 20%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 8.06 (br s, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 6.04 (br s, 1H), 5.30 (t, *J* = 7.5 Hz, 1H), 4.25 (q, *J* = 7.5 Hz, 2H), 4.01 (dd, *J* = 10.0 Hz, *J* = 5.0 Hz, 2H), 3.69-3.61 (m, 2H), 3.49-3.43 (m, 3H), 2.40-2.35 (m, 1H), 2.04-1.81 (m, 3H), 1.79 (s, 3H), 1.74 (s, 3H), 1.24 (t, *J* = 7.5 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 170.2, 168.9, 162.6, 137.4, 135.7, 135.4, 128.6, 121.9, 120.1, 119.6, 118.2, 110.7, 102.6, 66.6, 63.1, 59.3, 46.3, 29.3, 28.8, 25.8, 25.2, 22.6, 17.9, 13.9; HRMS (ESI<sup>+</sup>): Calculated (*m/z*) for C<sub>24</sub>H<sub>29</sub>N<sub>3</sub>O<sub>4</sub> (M+Na)<sup>+</sup>: 446.2050, Found 446.2017.

Further elution with (DCM/MeOH =50/1) gave **14B** as a white solid (395.65 mg, 80%). The ratio between **14A** and **14B** was 1:4. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 8.00 (br s, 1H), 7.54 (d, *J* = 10.0 Hz, 1H), 7.27(d, *J*= 10.0 Hz, 1H), 7.13-7.07(m, 2H), 6.35 (br s, 1H), 5.31(t, *J*= 7.5 Hz, 1H), 4.40-4.34(m, 2H), 3.99(d, *J*= 15.0 Hz, 1H), 3.48-3.44(m, 3H) , 3.20(d, *J* = 15.0 Hz, 1H), 2.96(dd, *J*=10.0 Hz, 1H), 2.06-2.03(m, 1H), 1.90-1.87(m, 1H), 1.81(s, 3H), 1.76(s, 3H), 1.73-1.60(m, 1H), 1.38 (t, *J* = 7.5 Hz, 3H), 1.10-1.00 (m, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 168.3, 168.2, 162.3, 137.5, 135.9, 134.8, 128.6, 121.7, 119.8, 119.3, 118.5, 110.4, 103.5, 69.3, 63.0, 57.8, 45.3, 32.9, 28.9, 25.8, 24.9, 21.3, 17.9, 14.0; **HRMS** (ESI<sup>+</sup>): Calculated (m/z) for C<sub>24</sub>H<sub>29</sub>N<sub>3</sub>O<sub>4</sub> (M+Na)<sup>+</sup> : 446.2050, Found 446.2057.

The reaction scheme shows a tryptophan derivative with a 2-methoxy-2-oxo-1-azetidinyl group at the epsilon position reacting with LiCl in DMA with 1 drop of H<sub>2</sub>O under reflux overnight. The products are Tryprostatin B (17) and 9-epi-tryprostatin B (18), which are diastereomers differing in the stereochemistry at the C9 position of the azetidine ring.

Further elution with DCM/MeOH = 9/1 gave 9-*epi*-tryprostatin B **18** (10.2 mg, 39%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.96 (1H, br), 7.53 (1H, d, *J*=8.4 Hz), 7.15-7.07 (2H, m), 6.04 (1H, br), 5.31 (1H, t, *J*=7.2 Hz), 4.26-4.23 (1H, m), 3.54-3.49 (1H, m), 3.44-3.38 (3H, m), 3.18-3.11 (2H, m), 2.70-2.67 (1H, m), 2.04-2.00 (1H, m), 1.90-1.65 (2H, overlapped), 1.81 (3H, s), 1.77 (3H, s), 1.38-1.34 (1H, m); **HRMS (ESI+)**: Calculated (m/z) for C<sub>21</sub>H<sub>26</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 352.2020, Found: 352.2035.

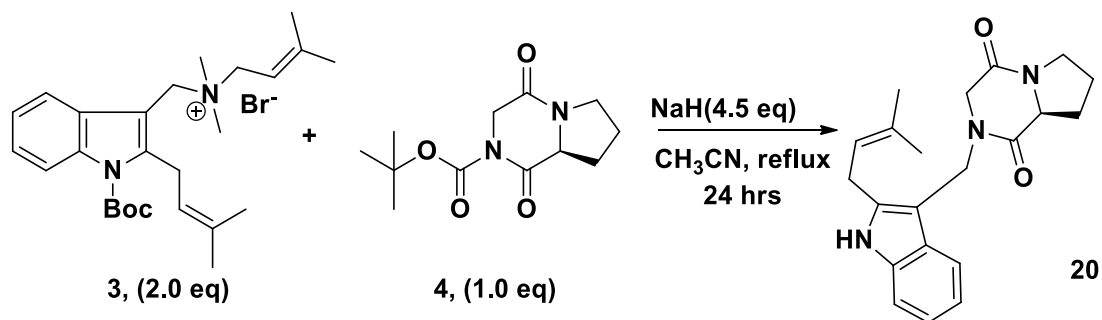
***tert*-butyl 8a-((2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate **19****



(S)-*tert*-butyl 1,4-dioxohexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate **4** (130 mg, 0.511 mmol, 1 equiv.) was dissolved in acetonitrile and NaH (60% in mineral oil, 52 mg, 2.0 mmol) was added to it at 0°C temperature and stirred for 30 mins. Then compound **3** was added to the stirred solution and heated the mixture at reflux temperature. The mixture was stirred under reflux for overnight and monitored by TLC. After completing the reaction, the solvent was removed in vacuo. The crude product was purified with flash column chromatography on silica gel (DCM/MeOH = 100/1) to afford **19** as a white solid (98.9 mg, 20%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.95 (br s, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 5.30 (t, *J* = 7.5 Hz, 1H), 3.85-3.77 (m, 2H), 3.73-3.68 (m, 1H), 3.50 (d, *J* = 15.0 Hz, 1H), 3.42-3.30 (m, 2H), 3.06 (d, *J* = 15.0 Hz, 1H), 2.49-2.43 (m, 1H), 2.41-2.36 (m, 1H), 2.32 (d, *J* = 15.0 Hz, 1H), 2.18-2.04 (m, 2H), 1.82 (s, 3H), 1.77 (s, 3H), 1.36 (s, 9H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 170.5, 162.9, 149.3, 136.8, 136.0, 134.8, 128.4, 121.9, 120.4, 119.2, 117.9, 110.4, 104.5, 83.7, 70.1, 49.4, 45.6, 36.5, 33.7, 29.7, 27.1, 25.8, 24.7, 20.8, 17.9, 14.1.

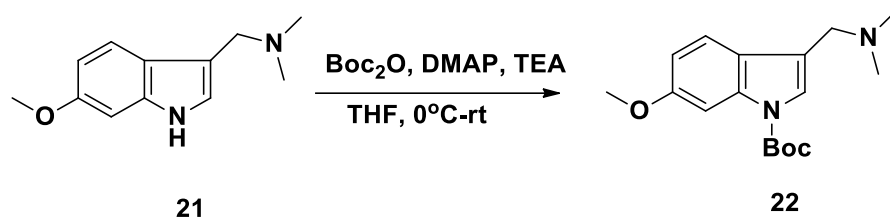


**(S)-2-((2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione **20****



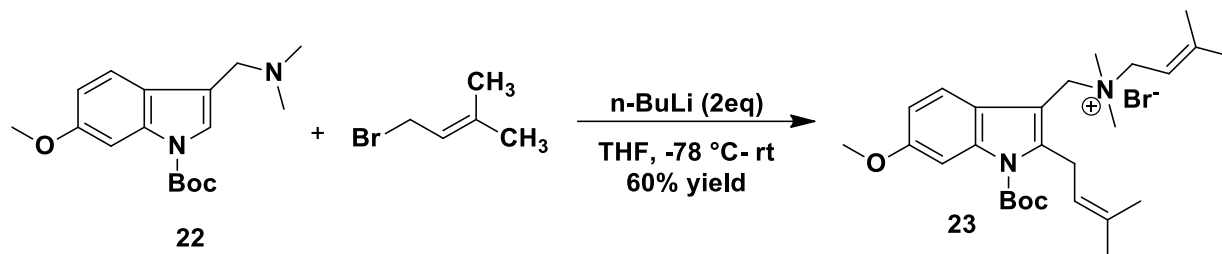
(S)-*tert*-butyl 1,4-dioxohexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate **4** (130 mg, 0.511 mmol, 1 equiv.) was dissolved in acetonitrile and NaH (60% in mineral oil, 82 mg, 3.4 mmol, 4 equiv.) was added to it at 0°C temperature and stirred for 30 mins. Then compound **3** was added to the stirred solution and heated the mixture at reflux temperature. The mixture was stirred under reflux for overnight and monitored by TLC. After completing the reaction, the solvent was removed in vacuo. The crude product was purified with flash column chromatography on silica gel (DCM/MeOH = 100/1) to afford **20** as a white solid (98.9 mg, 20%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 8.00 (br s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 5.30 (t, *J* = 7.5 Hz, 1H), 5.03 (d, *J* = 15.0 Hz, 1H), 4.62 (d, *J* = 15.0 Hz, 1H), 4.15-4.09 (m, 1H), 3.90-3.78 (m, 2H), 3.66-3.49 (m, 4H), 2.50-2.46 (m, 1H), 2.19-2.03 (m, 1H), 1.95-1.89 (m, 2H), 1.82 (s, 3H), 1.79 (s, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 166.7, 163.4, 137.4, 135.5, 128.2, 121.8, 120.1, 119.6, 118.4, 110.5, 105.1, 59.1, 50.5, 45.2, 39.1, 29.7, 29.1, 25.8, 25.1, 22.6, 18.1.

***Tert*-butyl 3-((dimethylamino)methyl)-6-methoxy-1H-indole-1-carboxylate (22)**



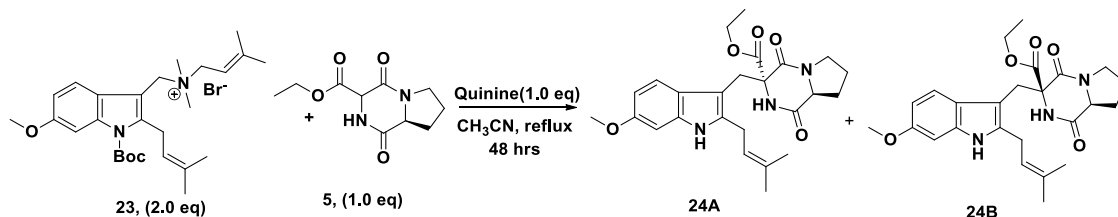
A solution of Boc anhydride ( $\text{Boc}_2\text{O}$ ) (2.0 g, 8.81 mmol), 4-dimethylaminopyridine (DMAP) (0.09 g, 0.73 mmol), trimethylamine (TEA) (0.123 mL, 1.21 mmol) in THF (30 mL) was maintained at  $0^\circ\text{C}$  for 30 min. A solution of 6-methoxy gramine (1.5 g, 7.34 mmol) in THF (15 mL) was added dropwise through the dropping funnel over a period of 30 min at  $0^\circ\text{C}$ . The reaction mixture was stirred at  $0^\circ\text{C}$  for 1.5 hours under nitrogen atmosphere. After consumption of starting material, as judged by TLC analysis, water (20 mL) was added to the reaction mixture. At first THF was removed under reduced pressure and then the aqueous layer was extracted with ether (3 x 15 mL), washed with brine (1 x 15 mL). The combined organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The crude product was purified with column chromatography on silica gel (hexane/EtOAc = 1/1) to give product **18** as a light brown solid (1.56 g, 70%).  **$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):**  $\delta$  7.77 (s, 1H), 7.56 (d,  $J = 10.0$  Hz, 1H), 7.40 (s, 1H), 6.88 (d,  $J = 10.0$  Hz, 1H), 3.89 (s, 3H), 3.52 (s, 2H), 2.30 (s, 6H), 1.69 (s, 9H);  **$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):**  $\delta$  157.9, 149.9, 136.6, 124.4, 123.0, 120.2, 118.2, 111.9, 99.3, 83.3, 55.6, 54.7, 45.6, 28.2; **HRMS (ESI+):** Calculated (m/z) for  $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_3$  ( $\text{M}+\text{H}$ ) $^+$  : 305.1860, Found 305.1850.

**N-((1-(tert-butoxycarbonyl)-6-methoxy-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-N,N,3-trimethylbut-2-en-1-aminium bromide (23)**



A solution of *Tert*-butyl 3-((dimethylamino)methyl)-6-methoxy-1H-indole-1-carboxylate **22** (2.0 g, 6.6 mmol) in THF (25 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (5.3 mL, 2.5 M, 13.2 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. Prenyl bromide (3.0 mL, 26.3 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water (20 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **23** as a brown solid (2.05 g, 60 %). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 7.95 (d, *J* = 10.0 Hz, 1H), 7.55 (s, 1H), 6.98 (d, *J* = 10.0 Hz, 1H), 5.31 (t, *J* = 7.5 Hz, 1H), 5.21 (brs, 2H), 4.93 (s, 1H), 4.45 (d, *J* = 5.0 Hz, 2H), 3.74 (s, 5H), 3.06 (s, 6H), 1.81 (s, 3H), 1.75 (s, 3H), 1.67 (s, 3H), 1.56 (s, 12H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 157.7, 149.7, 148.7, 142.6, 136.8, 133.9, 122.8, 120.6, 120.5, 112.4, 111.2, 106.0, 99.7, 84.8, 61.8, 58.3, 55.5, 48.4, 27.9, 26.8, 26.4, 25.4, 19.5, 18.6; **HRMS (ESI<sup>+</sup>):** Calculated (m/z) for C<sub>27</sub>H<sub>41</sub>N<sub>2</sub>O<sub>3</sub> [M]<sup>+</sup>: 441.3112, Found: 441.3109.

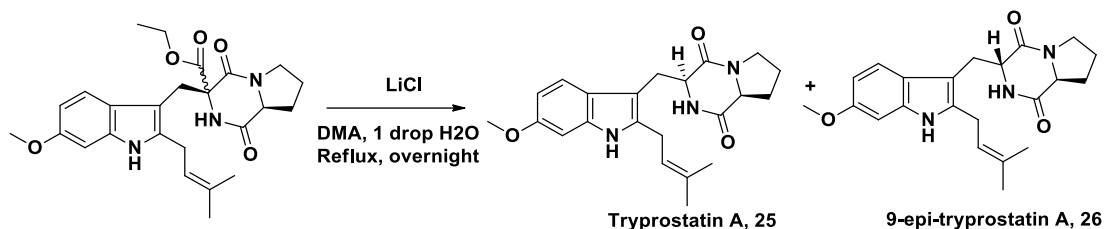
(3S,8aS)-ethyl 3-(((6-methoxy-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazine-3-carboxylate **24A** and (3R,8aS)-ethyl 3-(((6-methoxy-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazine-3-carboxylate **24B**



To a suspension of *N*-((1-(tert-butoxycarbonyl)-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-*N,N*,3-trimethylbut-2-en-1-aminium bromide **23** (230 mg, 0.442 mmol) in acetonitrile (10.0 mL) compound (8aS)-ethyl 1,4-dioxooctahydropyrrolo[1,2-a]pyrazine-3-carboxylate **5** (50 mg, 0.221 mmol) was added, followed by the addition of 86 mg (0.265 mmol) of quinine. The mixture was stirred under reflux for 24-48 hours and monitored by TLC. The solvent was removed in vacuo. The crude product was purified with flash column chromatography on silica gel (DCM/MeOH = 100/1) to afford **24A** as a white solid (98.9 mg, 20%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.86 (br s, 1H), 7.32 (d, *J* = 10.0 Hz, 1H), 6.82 (s, 1H), 6.76 (d, *J* = 10.0 Hz, 1H), 6.03 (br s, 1H), 5.29 (t, *J* = 7.5 Hz, 1H), 4.25 (q, *J* = 7.5 Hz, 2H), 4.03 (dd, *J* = 10.0 Hz, *J* = 5.0 Hz, 1H), 3.99 (d, *J* = 15.0 Hz, 1H), 3.84 (s, 3H), 3.73-3.67 (m, 1H), 3.63-3.59 (m, 1H), 3.49-3.45 (m, 3H), 2.41-2.36 (m, 1H), 2.08-1.99 (m, 2H), 1.92-1.86 (m, 1H), 1.81 (s, 3H), 1.76 (s, 3H), 1.26 (t, *J* = 7.5 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 170.2, 168.9, 162.6, 156.4, 136.2, 135.9, 135.5, 122.9, 119.8, 118.9, 109.6, 102.5, 94.6, 66.5, 63.1, 59.3, 55.7, 46.3, 29.7, 29.4, 28.8, 25.8, 25.2, 22.6, 17.9, 14.0; HRMS (ESI<sup>+</sup>): Calculated (*m/z*) for C<sub>25</sub>H<sub>31</sub>N<sub>3</sub>O<sub>5</sub> (M+Na)<sup>+</sup> : 476.2156, Found 476.2163.

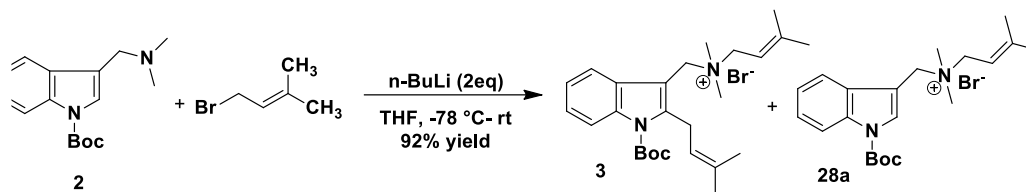
Further elution with (DCM/MeOH =50/1) gave **24B** as a white solid (395.65 mg, 80%). The ratio between **14A** and **14B** was 1:4. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.84 (br s, 1H), 7.40 (d, *J* = 10.0 Hz, 1H), 6.84-6.75(m, 2H), 6.26 (br s, 1H), 5.29(t, *J*= 7.5 Hz, 1H), 4.40-4.34(m, 2H), 3.93(d, *J*= 15.0 Hz, 1H), 3.81(s, 3H), 3.50-3.38(m, 3H) , 3.15(d, *J* = 15.0 Hz, 1H), 3.00(dd, *J*=10.0 Hz, 1H), 2.37-2.35 (m, 1H), 2.19-2.13(m, 1H), 1.94-1.90(m, 2H), 1.81(s, 3H), 1.76(s, 3H), 1.38 (t, *J* = 7.5 Hz, 3H), 1.10-1.00 (m, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 168.3, 168.2, 162.3, 156.0, 136.1, 135.7, 135.1, 127.9, 127.8, 122.9, 119.5, 119.3, 109.3 103.3, 94.2, 69.3, 63.0, 57.8, 55.8, 45.3, 33.2, 31.9, 29.7, 28.9, 25.8, 24.9, 22.7, 21.3,17.9, 13.9.

## Synthesis of Tryprostatin A 25 and 9-epi-tryprostatin A 26



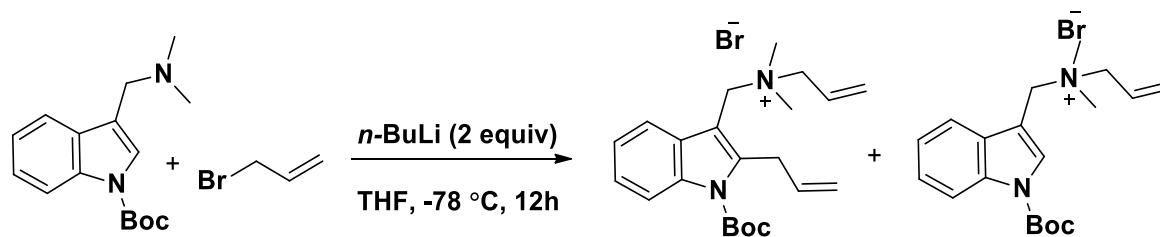
A mixture of 24 (50 mg, 0.118 mmol), lithium chloride (32 mg, 0.37 mmol), and H<sub>2</sub>O (10 microL) in DMA (5.0 mL) was stirred for 12 hours at reflux condition under nitrogen atmosphere. The resulting mixture was poured into brine and extracted with CHCl<sub>3</sub>. The extract was evaporated to afford a syrup, which was purified by with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford tryprostatin A **25** (12.3 mg, 47%). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):**  $\delta$  7.90 (br s, 1H), 7.34 (d,  $J$ =8.7 Hz, 1H), 6.83 (d, 2.3 Hz, 1H), 6.76 (dd,  $J$ =8.7, 2.3 Hz, 1H), 5.65 (s, 1H), 5.32-5.26 (m, 1H), 4.33 (dd,  $J$ =11.2, 3.6 Hz, 1H), 4.06 (dd,  $J$ =7.6, 7.6 Hz, 1H), 3.83 (s, 3H), 3.71-3.57 (m, 3H), 3.49-3.37 (m, 2H), 2.91 (dd,  $J$ =15.1, 11.4 Hz, 1H), 2.38-2.29 (m, 1H), 2.09-2.02 (m, 2H), 1.98-1.86 (m, 1H), 1.77 (s, 3H), 1.74 (s, 3H).

**N-((1-(tert-butoxycarbonyl)-1H-indol-3-yl)methyl)-N,N,3-trimethylbut-2-en-1-aminium bromide (29a)**



A solution of **2** (8.0 g, 29.2 mmol) in THF (100 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (23.3 mL, 2.5 M, 58.3 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. Prenyl bromide (17.4 mL, 11.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of all the starting material, as judged by TLC analysis, water (30 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL), the combined organic layers were washed with brine solution (1 x 30 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **3** and Further elution with (DCM/MeOH = 20/1) gave **29a** as off-white solid (0.1 g, 8%). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.11 (d, *J* = 9.0 Hz, 2H), 8.02 (d, *J* = 6.0 Hz, 1H), 7.94 (s, 1H), 7.29-7.16 (m, 2H), 5.32 (t, *J* = 7.5 Hz, 1H), 5.22 (s, 2H), 4.38 (d, *J* = 9.0 Hz, 2H), 3.15 (s, 6H), 1.81 (s, 3H), 1.76 (s, 3H), 1.59 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 148.9, 148.8, 135.0, 130.5, 129.8, 125.2, 123.8, 120.3, 115.2, 110.9, 107.6, 85.0, 61.5, 58.6, 48.4, 28.1, 26.4, 19.5; **HRMS (ESI<sup>+</sup>):** Calculated (m/z) for C<sub>21</sub>H<sub>31</sub>N<sub>2</sub>O<sub>2</sub> [M]<sup>+</sup>: 343.2380, Found: 343.2363.

**N-((2-allyl-1-(tert-butoxycarbonyl)-1H-indol-3-yl)methyl)-N,N-dimethylprop-2-en-1-aminium bromide and N-((1-(tert-butoxycarbonyl)-1H-indol-3-yl)methyl)-N,N-dimethylprop-2-en-1-aminium bromide**



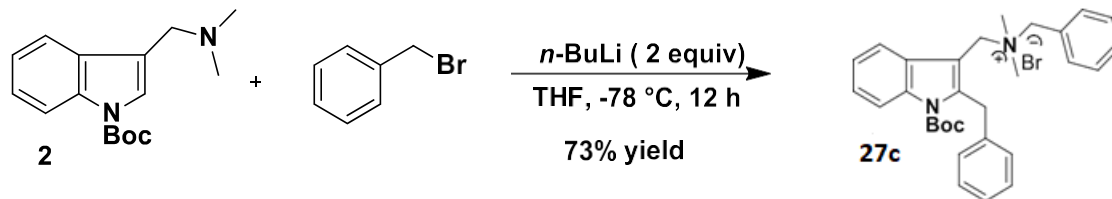
A solution of **2** (1.00 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (2.9 mL, 2.5 M, 6.8 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. Allyl bromide (1.9 mL, 16.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water 15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 10/1) to afford **28b** and **29b** as a yellow solid.

**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.12-8.01 (m, 2H), 7.32-7.28 (m, 2H), 6.07-5.94 (m, 2H), 5.86 (d, *J* = 15.0 Hz, 1H), 5.72 (d, *J* = 10 Hz, 1H), 5.27 (s, 2H), 5.05-4.98 (m, 2H), 4.65 (d, *J* = 5 Hz, 2H), 4.15 (d, *J* = 5 Hz, 2H), 3.23(s, 6H), 1.66 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 149.6, 142.7, 136.1, 135.0, 130.3, 128.9, 124.8, 124.6, 123.9, 119.6, 116.5, 115.4, 106.7, 85.3, 65.9, 59.1, 48.9, 46.1, 31.1, 29.7, 28.0, 8.7.



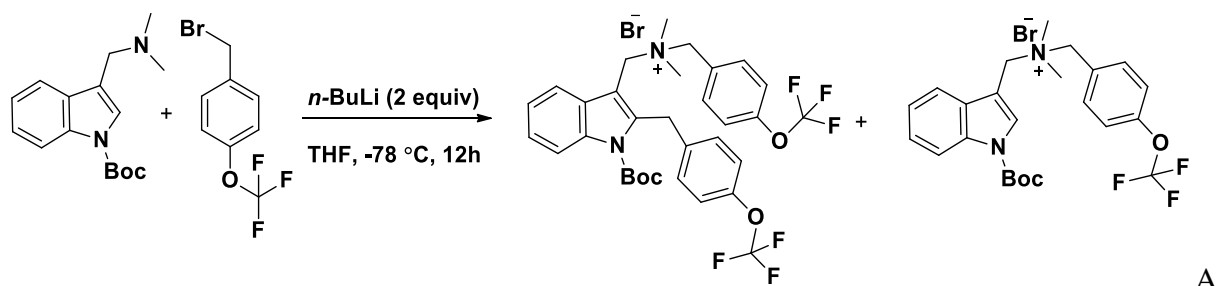
**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.10 (m, 2H), 7.98 (s, 1H), 7.35-7.28 (m, 2H), 6.05-5.99(m, 1H)  
5.79 (d, *J* = 15 Hz, 1H), 5.68 (d, *J* = 10 Hz, 1H), 5.20 (s, 2H), 4.43 (d, *J* = 5 Hz, 2H), 3.24(s, 6H),  
1.67 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 148.9, 135.1, 130.7, 129.9, 129.8, 125.3, 124.6,  
123.9, 120.0, 115.3, 107.4, 85.1, 65.7, 59.4, 48.9, 45.9, 28.1, 8.7.

N-benzyl-1-(2-benzyl-1-(tert-butoxycarbonyl)-1*H*-indol-3-yl)methyl)-*N,N*-dimethylmethan-aminium bromide **27c** and **27d**



A solution of **2** (1.0 g, 3.6 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (2.9 mL, 2.5 M, 7.3 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. Benzyl bromide (2.0 mL, 16.4 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water (15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **28c** as a brown solid (1.41g, 73 %). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.19 (d, *J* = 9.0 Hz, 1H), 8.09 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 6.0 Hz, 2H), 7.29-7.21 (m, 5H), 7.07 (t, *J* = 7.5 Hz, 2H), 7.00-6.92 (m, 3H), 5.39 (s, 2H), 5.32 (s, 2H), 4.78 (s, 2H), 3.02 (s, 6H), 1.31 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 149.4, 142.3, 138.6, 136.2, 133.4, 130.3, 129.1, 128.9, 128.4, 127.9, 127.6, 126.1, 124.8, 123.9, 120.1, 115.2, 107.9, 85.1, 66.9, 58.8, 48.1, 32.8, 27.5.; **HRMS (ESI<sup>+</sup>):** Calculated (m/z) for C<sub>30</sub>H<sub>35</sub>N<sub>2</sub>O<sub>2</sub> [M]<sup>+</sup>: 455.2693, Found: 455.2624.

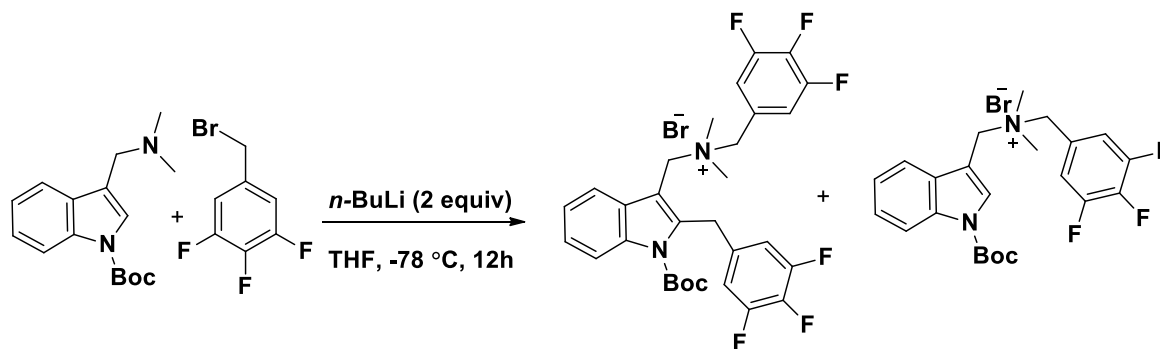
**1-(1-(tert-butoxycarbonyl)-2-(4-(trifluoromethoxy)benzyl)-1H-indol-3-yl)-N,N-dimethyl-N-(4-(trifluoromethoxy)benzyl)methanaminium bromide and 1-(1-(tert-butoxycarbonyl)-1H-indol-3-yl)-N,N-dimethyl-N-(4-(trifluoromethoxy)benzyl)methanaminium bromide**



solution of **2** (1.02 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (2.9 mL, 2.5 M, 6.8 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. 4-trifluoromethoxy benzyl bromide (2.6 mL, 16.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water (15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 10/1) to afford **28d** as a yellow solid.

**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.12-7.98 (m, 7H), 7.26-7.35(m, 2H), 5.64 (s, 2H), 5.43 (s, 2H), 3.23 (s, 6H), 1.67 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 149.0, 148.9, 135.1, 134.9, 134.7(2C), 130.8, 129.8, 125.5, 124.1, 123.9(2C), 120.1, 115.4, 107.1, 85.4, 65.4, 60.0, 48.3, 28.1.

**1-(1-(tert-butoxycarbonyl)-2-(3,4,5-trifluorobenzyl)-1H-indol-3-yl)-N,N-dimethyl-N-(3,4,5-trifluorobenzyl)methanaminium bromide and 1-(1-(tert-butoxycarbonyl)-1H-indol-3-yl)-N,N-dimethyl-N-(3,4,5-trifluorobenzyl)methanaminium bromide**



A solution of **2** (1.02 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (2.9 mL, 2.5 M, 6.8 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. 3,4,5-trifluorobenzyl bromide (2.6 mL, 16.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water (15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 10/1) to afford **28e** as a yellow solid.

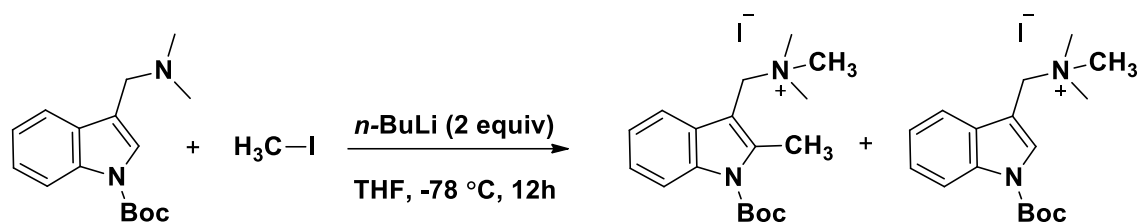
**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.12-7.98 (m, 7H), 7.26-7.35(m, 2H), 5.64 (s, 2H), 5.43 (s, 2H), 3.23 (s, 6H), 1.67 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 149.0, 148.9, 135.1, 134.9, 134.7(2C),

130.8, 129.8, 125.5, 124.1, 123.9(2C), 120.1, 115.4, 107.1, 85.4, 65.4, 60.0, 48.3, 28.1.

**1-(1-(tert-butoxycarbonyl)-2-methyl-1H-indol-3-yl)-N,N,N-trimethylmethanaminium iodide**

**31a and 1-(1-(tert-butoxycarbonyl)-1H-indol-3-yl)-N,N,N-trimethylmethanaminium iodide**

**32a**



A solution of **2** (1.04 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to  $-78\text{ }^\circ\text{C}$  and  $n$ -butyl lithium (3.1 mL, 2.5 M, 7.6 mmol) was added dropwise to the reaction mixture maintaining a temperature  $-78\text{ }^\circ\text{C}$  over a period of 1 h under nitrogen atmosphere. Methyl iodide (2.5 mL, 17.6 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water 15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **31a** as a brown solid (1.41g, 73 %).

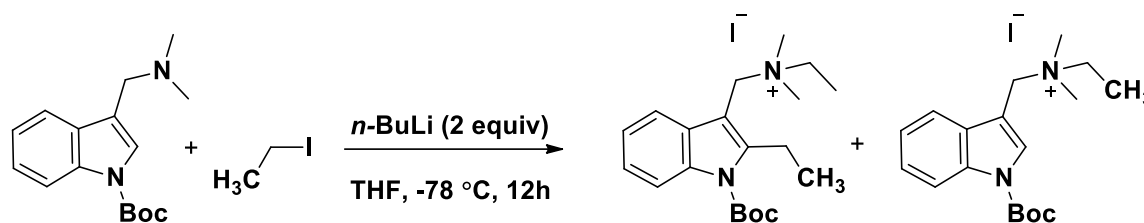
**$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):**  $\delta$  8.04 (d,  $J$  = 5.2 Hz, 1H), 7.67 (d,  $J$  = 5.9 Hz, 1H), 7.23 (dd,  $J$  = 9.0 Hz, 5.9 Hz, 2H), 5.14 (s, 2H), 3.45 (s, 9H), 2.71 (s, 2H), 1.66 (s, 9H);  **$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):**  $\delta$  149.7, 141.8, 135.6, 128.8, 124.5, 123.7, 119.1, 115.5, 106.1, 85.1, 60.3, 53.1, 28.2, 16.4;

**HRMS (ESI $^+$ ):** Calculated (m/z) for  $\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_2$  [ $\text{M}$ ] $^+$ : 303.2067, Found: 303.2027

Further elution with (DCM/MeOH = 10/1) gave compound **32a** as a yellow solid.

**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.05-8.12 (m, 3H), 7.31 (t, *J* = 15 Hz, 2H), 5.24 (s, 2H), 3.49 (s, 9H), 1.67 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 148.9, 135.1, 130.8, 129.5, 125.4, 123.9, 119.9, 115.4, 107.2, 85.2, 60.9, 53.1, 28.2; **HRMS (ESI+):** Calculated (m/z) for C<sub>17</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> [M]<sup>+</sup>: 289.1911, Found: 289.1862.

**N-((1-(tert-butoxycarbonyl)-2-ethyl-1H-indol-3-yl)methyl)-N,N-dimethylethanaminium 31b**  
**and N-((1-(tert-butoxycarbonyl)-1H-indol-3-yl)methyl)-N,N-dimethylethanaminium iodide 32b**



A solution of **2** (1.04 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (3.1 mL, 2.5 M, 7.6 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. Ethyl iodide (2.7 mL, 17.1 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water 15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **31b** as a brown solid (1.41g, 78 %) and (DCM/MeOH = 10/1) to afford **32b** as a yellow solid.

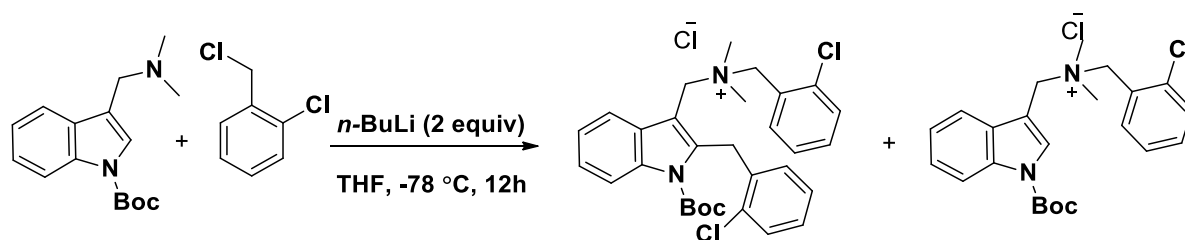
**Compound 31b:** <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 8.09-8.11 (m, 1H), 7.96 (d, *J* = 4.5 Hz, 1H), 7.23 (t, *J* = 4.5 Hz, 2H), 5.14 (s, 2H), 4.00 (q, *J* = 7.0 Hz, 2H), 3.34 (q, *J* = 7.2 Hz, 2H), 3.30 (s, 6H), 1.70 (s, 9H), 1.46 (t, *J* = 6.7 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):

$\delta$  149.6, 147.8, 136.2, 128.9, 124.7, 123.9, 119.4, 115.6, 105.1, 85.3, 59.9, 59.6, 48.9, 28.1, 21.2, 15.0, 8.9; **HRMS (ESI+)**: Calculated (m/z) for C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub> [M]<sup>+</sup>: 331.2380, Found: 331.2356.

**Compound 32b**: **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)**:  $\delta$  8.13 (d, *J* = 8.2 Hz, 1H), 8.06 (s, 1H), 8.05 (d, *J* = 7.2 Hz, 1H), 7.38-7.28 (m, 2H), 5.25 (s, 2H), 3.84 (q, *J* = 7.3 Hz, 2H), 3.34 (s, 6H), 1.70 (s, 9H), 1.48 (t, *J* = 7.2 Hz, 3H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz)**:  $\delta$  148.9, 135.1, 130.8, 129.6, 125.5, 124.0, 119.9, 115.4, 106.8, 85.3, 59.9, 59.4, 49.3, 28.2, 8.8; **HRMS (ESI+)**: Calculated (m/z) for C<sub>18</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub> [M]<sup>+</sup>: 303.2067, Found: 303.2037.



**1-(1-(tert-butoxycarbonyl)-2-(2-chlorobenzyl)-1H-indol-3-yl)-N-(2-chlorobenzyl)-N,N-dimethylmethanaminium chloride 31c and 1-(1-(tert-butoxycarbonyl)-1H-indol-3-yl)-N-(2-chlorobenzyl)-N,N-dimethylmethanaminium chloride 32c**



A solution of **2** (1.02 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (2.9 mL, 2.5 M, 6.8 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. 2-Chloro benzyl chloride (2.7 mL, 16.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water (15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 20/1) to afford **31c** as a brown solid (1.41g, 48 %) and (DCM/MeOH = 10/1) to afford **32c** as a yellow solid.

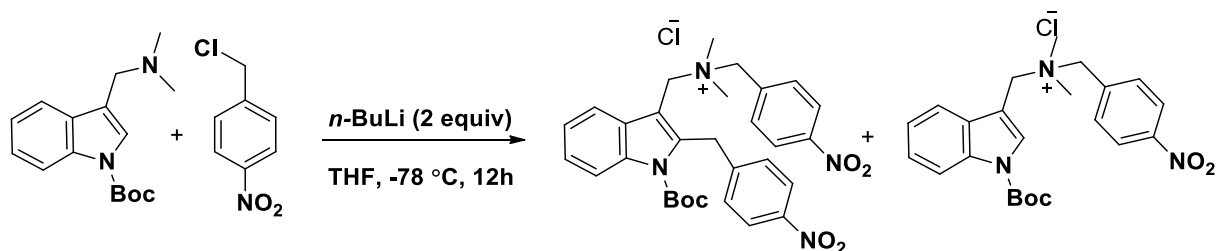
**Compound 31c:** <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 8.12 (m, 2H), 7.97 (m, 2H), 7.33-7.27 (m, 6H), 7.20-7.11 (m, 5H), 5.31 (s, 2H), 5.14 (s, 2H), 4.11 (s, 2H), 3.23 (s, 6H), 1.68 (s, 9H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 148.9, 140.2, 137.5, 136.9, 135.2, 134.1, 133.9, 131.4, 131.3, 130.5, 129.7,

129.6, 128.2, 127.4, 125.6, 125.3, 124.0, 119.8, 115.3, 107.1, 85.1 63.7, 60.9, 49.3, 38.3, 28.2;

**HRMS (ESI+):** Calculated (m/z) for C<sub>30</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>Cl<sub>2</sub> [M]<sup>+</sup>: 523.1914, Found: 523.1929.

**Compound 32c: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.19-8.22 (m, 2H), 8.11(d, *J* = 10 Hz, 1H), 8.03(s, 1H), 7.52-7.45 (m, 3H), 7.42-7.36 (m, 2H), 5.43 (s, 2H), 5.37 (s, 2H), 3.26 (s, 6H), 1.72 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 148.9, 136.9, 132.5, 130.8, 130.6, 128.0, 125.8, 125.6, 124.2, 119.9, 115.6, 107.1, 85.4, 63.9, 49.0, 28.2.

**1-(1-(tert-butoxycarbonyl)-2-(4-nitrobenzyl)-1H-indol-3-yl)-N,N-dimethyl-N-(4-nitrobenzyl)methanaminium chloride 31d and 1-(1-(tert-butoxycarbonyl)-1H-indol-3-yl)-N,N-dimethyl-N-(4-nitrobenzyl)methanaminium chloride 32d**

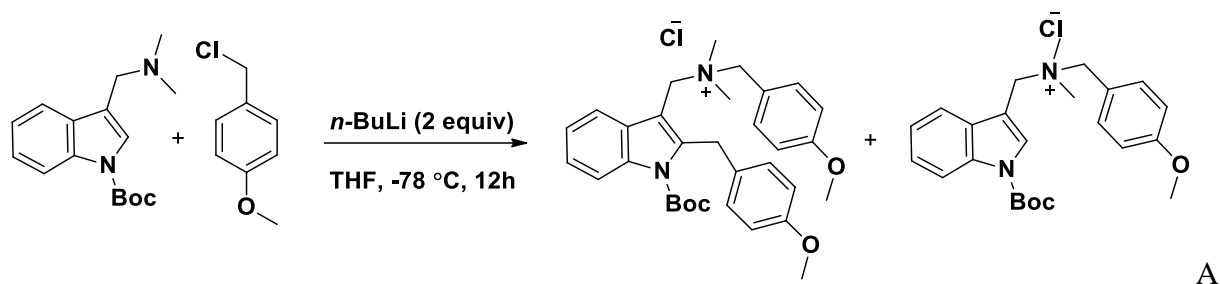


A solution of **2** (1.02 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (2.9 mL, 2.5 M, 6.8 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. 4-Nitro benzyl chloride (2.9 mL, 16.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water (15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 10/1) to afford **32d** as a yellow solid (1.25g, 80%).

**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.12-7.98 (m, 7H), 7.26-7.35(m, 2H), 5.64 (s, 2H), 5.43 (s, 2H), 3.23 (s, 6H), 1.67 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 149.0, 148.9, 135.1, 134.9, 134.7(2C), 130.8, 129.8, 125.5, 124.1, 123.9(2C), 120.1, 115.4, 107.1, 85.4, 65.4, 60.0, 48.3, 28.1; **HRMS**

(ESI<sup>+</sup>): Calculated (m/z) for C<sub>23</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>4</sub> [M]<sup>+</sup>: 445.9421, Found: 445.9321.

**1-(1-(tert-butoxycarbonyl)-2-(4-methoxybenzyl)-1H-indol-3-yl)-N-(4-methoxybenzyl)-N,N-dimethylmethanaminium chloride 31e and 1-(1-(tert-butoxycarbonyl)-1H-indol-3-yl)-N-(4-methoxybenzyl)-N,N-dimethylmethanaminium 32e**

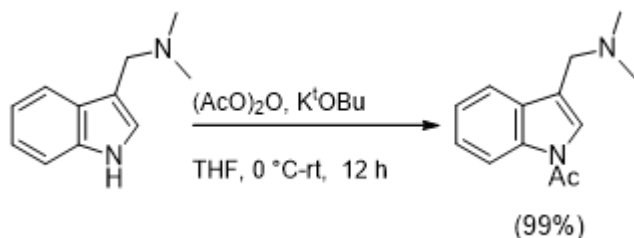


solution of **2** (1.02 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (2.9 mL, 2.5 M, 6.8 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. 4-Methoxy benzyl chloride (2.6 mL, 16.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water 15 mL) was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 10/1) to afford **32e** as a yellow solid.

**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 8.12-7.98 (m, 7H), 7.26-7.35(m, 2H), 5.64 (s, 2H), 5.43 (s, 2H), 3.23 (s, 6H), 1.67 (s, 9H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):** δ 149.0, 148.9, 135.1, 134.9, 134.7(2C),

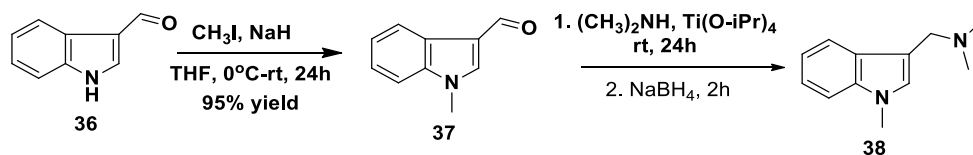
130.8, 129.8, 125.5, 124.1, 123.9(2C), 120.1, 115.4, 107.1, 85.4, 65.4, 60.0, 48.3, 28.1.

### 1-(3-(((dimethylamino)methyl)-1H-indol-1-yl)ethenone **34**



A solution of Gramine (4.6 g, 26.2mmol) and K<sup>t</sup>OBu (3.8 g, 34.0mmol, 1.3 equiv.) was stirred in THF (50 mL) at 0 °C for 30 min under nitrogen atmosphere. A solution of Acetic anhydride (AcO<sub>2</sub>O) (2.9mL, 288mmol) in THF (10 mL) was added dropwise through the dropping funnel to the solution over a period of 30 min at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of starting material, as indicated by TLC, water (20 mL) was added to the reaction mixture. The aqueous layer was extracted with ether (3 x 15 mL), washed with brine (1 x 15 mL). The combined organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crude product was purified with column chromatography on silica gel (hexane/EtOAc = 7/3) to give product **34** as a white solid (5.6 g, 99%). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.44 (br, 1H), 7.66 (d, *J* = 5 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 2H), 7.27 (t, *J* = 7.5 Hz, 1H), 3.54 (s, 2H), 2.57 (s, 3H), 2.30 (s, 6H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** 168.5, 135.9, 130.5, 125.2, 123.8, 123.5, 120.1, 119.6, 116.5, 54.6, 45.6, 23.9.

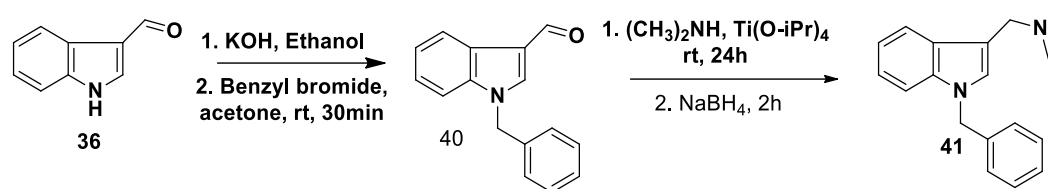
### N,N-dimethyl-1-(1-methyl-1H-indol-3-yl)methanamine (38)



To a stirred solution of 3-carboxaldehyde indole (2.9 g, 20 mmol) in THF, NaH was added at  $0^\circ\text{C}$  and stirred the mixture for about 15 minutes. Then  $\text{CH}_3\text{I}$  was added to the mixture dropwise and move the reaction mixture to room temperature to react for further 24 hours. Stop the reaction after the consumption of the starting material monitored by TLC. The aqueous layer was then extracted with DCM (3 x 50 mL). The combined organic layer was then dried in *vacuo* to give N-Me-indole-3-carboxyaldehyde **38** as a solid (4.79 g, 80%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  10.3(s, 1H), 8.34 (d,  $J = 10.0$ , 2H), 7.71 (s, 1H), 7.40-7.28 (m, 3H), 3.90 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  184.4, 139.1, 125.3, 124.0, 123.0, 122.1, 118.1, 108.9, 33.7.

**Compound 41:**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  7.92 (d,  $J = 10.0$ , 1H), 7.76 (d,  $J = 10.0$ , 1H), 7.41-7.32 (m, 3H), 3.69 (s, 2H), 3.10 (s, 3H), 2.31 (s, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz): 135.4, 130.9, 125.0, 124.4, 123.5, 120.6, 120.1, 113.1, 54.5, 50.5, 45.6, 44.0.

## 1-(1-benzyl-1H-indol-3-yl)-N,N-dimethylmethanamine 41



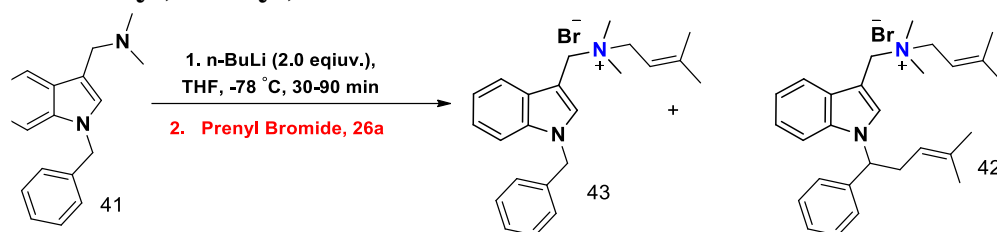
To a stirred solution of Indole-3-carboxaldehyde 39 (0.500 g, 3.4mmol, 1 equiv.) in EtOH (20mL), KOH pellets were added (0.224g, 4.0 mmol) at room temperature. The mixture was stirred until total solubilization. Then the ethanol was completely removed by rotary evaporator and acetone (20mL) was added to the mixture followed by benzyl bromide (0.589g, 3.4mmol). Immediately a precipitate was formed. The reaction was stirred for 30 mins. Finally, the precipitate was filtered by vacuum filtration. The liquid was concentrated by *vacuo* to give the crude product. The crude product was washed with hexane (3 x 15mL) to remove excess benzyl bromide from it. The crude product was purified with flash column chromatography on silica gel (Hexane/EtOAc 7/3) to give N-Bz- indole-3-carboxyaldehyde **40** as a white solid (0.8g, 99%). **<sup>1</sup>H NMR** ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  10.02 (s, 1H), 8.36 (d,  $J = 5$  Hz, 1H), 7.73 (s, 1H), 7.38-7.32 (m, 6H), 7.21 (d,  $J = 5$  Hz, 1H), 5.38 (s, 2H); **<sup>13</sup>C NMR** ( $\text{CDCl}_3$ , 125 MHz): 184.6, 138.6, 137.5, 135.3, 129.2, 128.4, 127.3, 125.5, 124.2, 123.1, 122.2, 118.5, 110.4, 50.9.

N-Bz- indole-3-carboxyaldehyde (3.3g, 14.0mmol, 1.0 equiv.) and Dimethylamine (2.5mL, 56.0mmol, 4.0 equiv.) were mixed in 1,2-dichloroethane (50 mL) and then treated with sodium triacetoxyborohydride (6.0 g, 28 mmol, 2 equiv.) and AcOH (0.84 mL, 14.0 mmol). The mixture was stirred at rt under a  $\text{N}_2$  atmosphere for 24 h until the reactants were consumed as determined by TLC analysis. The reaction mixture was quenched by adding aqueous saturated  $\text{NaHCO}_3$ , and



the product was extracted with Ethyl acetate (EtOAc). Then EtOAc extract was washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated to give the crude product (3.11 g, 84%). The crude product was purified with column chromatography on silica gel (DCM/MeOH= 50/1) to give product **41** as a white solid. **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.99 (d, *J* = 10 Hz, 1H), 7.43-7.36 (m, 6H), 7.23 (t, *J* = 7.2 Hz, 3H), 5.31 (s, 2H), 3.87 (s, 2H), 2.52 (s, 6H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** 137.7, 136.9, 128.9, 128.9, 127.9, 127.7, 127.0, 122.1, 119.8, 119.7, 112.6, 109.9, 54.8, 50.0, 45.5.

**N-((1-benzyl-1H-indol-3-yl)methyl)-N,N,3-trimethylbut-2-en-1-aminium bromide **42** and N,N,3-trimethyl-N-((1-(4-methyl-1-phenylpent-3-en-1-yl)-1H-indol-3-yl)methyl)but-2-en-1-aminium bromide **43****

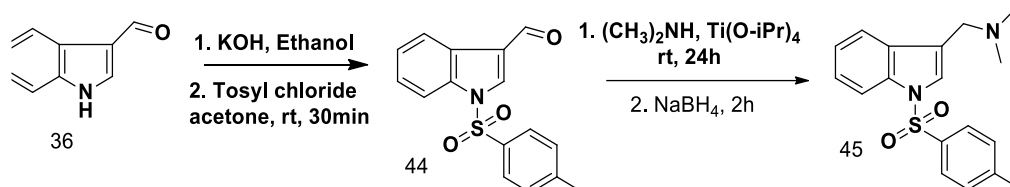


A solution of **41** (1.02 g, 3.8 mmol) in THF (20 mL) was taken in three necked round bottomed flask and nitrogen was bubbled through the solution for 20 min. This mixture was cooled to -78 °C and *n*-butyl lithium (2.9 mL, 2.5 M, 6.8 mmol) was added dropwise to the reaction mixture maintaining a temperature -78 °C over a period of 1 h under nitrogen atmosphere. Prenyl bromide (2.6 mL, 16.7 mmol) was added to the reaction dropwise through the dropping funnel over a period of 30 min. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After consumption of the starting material, as judged by TLC analysis, water 15 mL was added to the reaction mixture and THF was removed under reduced pressure. The mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), the combined organic layers were washed with brine solution (1 x 10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* to obtain crude product. The residue was purified with flash column chromatography on silica gel (DCM/MeOH = 10/1) to afford **42** as a yellow solid. **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.93 (s, 1H), 7.78 (m, 1H), 7.35-7.23 (m, 3H), 7.22-7.21(m, 4H), 6.87(m,1H), 5.48 (t, *J*= 7.5 Hz, 1H), 5.39 (t, *J*= 7.5 Hz, 1H), 5.20 (brs, 2H), 5.02 (t, *J*= 7.5 Hz, 1H), 4.38( d, *J*=10.0 Hz, 2H), 3.18 (d, *J*=10.0 Hz, 6H), 3.1-3.0 (m, 2H), 1.91 (s, 6H), 1.60(s, 6H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** 149.1, 140.3, 136.7, 135.5, 131.1, 128.9, 128.3, 127.9, 126.4, 125.7, 122.7, 121.4, 119.2, 118.8, 111.1, 110.8, 101.2, 61.4, 60.7, 60.4, 48.2, 34.0, 26.6, 25.7, 19.4, 18.2.

Further elution gave compound **43** as a white solid. **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.88 (m, 1H), 7.85(s, 1H), 7.31-7.26 (m, 5H), 7.22-7.21(m, 2H), 7.11(d, *J*=5Hz, 2H), 5.38 (t, *J*= 7.5 Hz, 1H),

5.31 (s, 2H), 5.23 (s, 2H), 4.32 (d,  $J=5.0$  Hz, 2H), 3.15 (s, 6H), 1.85 (d,  $J=5.0$  Hz, 6H);  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 125 MHz): 148.8, 136.5, 136.5, 133.9, 128.9, 128.4, 127.9, 126.8, 122.8, 121.3, 119.3, 110.9, 110.6, 101.5, 61.3, 60.2, 50.5, 48.2, 26.5, 19.3.

## N,N-dimethyl-1-(1-tosyl-1H-indol-3-yl)methanamine 45



To a stirred solution of Indole-3-carboxyaldehyde **39** (5.0 g, 34.5mmol, 1 equiv.) in EtOH (100mL), KOH pellets were added (2.3g, 41.3mmol, 1.2 equiv.) at room temperature. The mixture was stirred until total solubilization. Then the ethanol is completely removed by rotary evaporator and acetone (100mL) was added to the mixture followed by Tosyl chloride (TsCl) (7.9g, 41.3mmol, 1.2 equiv.). Immediately a precipitate was formed. The reaction was stirred for 30 mins. Finally, the precipitate was filtered by vacuum filtration. The liquid was concentrated by *vacuo* to give the crude product. The crude product was washed with EtOAc to get pure N-tosyl-indole-3-carboxyaldehyde **44** as a white solid (3.5g, 35%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  10.12 (s, 1H), 8.88 (d,  $J = 10.0$  Hz, 1H), 8.25 (s, 1H), 7.97 (d,  $J = 10.0$  Hz, 1H), 7.88 (d,  $J = 10.0$  Hz, 2H), 7.42 (t,  $J = 7.5$  Hz, 1H), 7.40 (t,  $J = 7.5$  Hz, 1H), 7.32 (d,  $J = 10.0$  Hz, 2H), 2.40 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz): 185.3, 146.2, 136.2, 135.2, 134.3, 130.3, 127.2, 126.3, 125.0, 122.7, 122.4, 21.7.

N-tosyl-indole-3-carboxyaldehyde (3.0g, 10.0mmol, 1.0 equiv.) and Dimethylamine (1.8mL, 40.1mmol, 4.0 equiv.) were mixed in 1,2-dichloroethane (50 mL) and then treated with sodium triacetoxyborohydride (4.2 g, 20.0 mmol, 2.0 equiv.) and AcOH (0.60 mL, 10.0 mmol). The mixture was stirred at rt under a  $\text{N}_2$  atmosphere for 24 h until the reactants were consumed as determined by TLC analysis. The reaction mixture was quenched by adding aqueous saturated

NaHCO<sub>3</sub>, and the product was extracted with Ethyl acetate (EtOAc). Then EtOAc extract was washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated to give the crude product. The crude product was purified with column chromatography on silica gel (DCM/MeOH= 50/1) to give product **45** as a yellow solid (3.0 g, 90%). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.99 (d, *J* = 10 Hz, 1H), 7.77 (d, *J* = 10 Hz, 2H), 7.64 (d, *J* = 10 Hz, 1H), 7.49 (s, 1H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.25 (t, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 10.0 Hz, 2H), 3.54 (s, 2H), 2.35 (s, 3H), 2.26 (s, 6H); **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** 144.8, 135.4, 135.3, 130.8, 129.8, 126.7, 124.8, 124.7, 123.2, 120.2, 113.7, 54.5, 45.5, 21.6.

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**PART II: BRØNSTED ACID CATALYZED REACTIONS OF AROMATIC  
KETONES WITH ETHYL DIAZOACETATE AND ITS SYNTHETIC SCOPE**

## 2.1. INTRODUCTION

### 3-oxo-esters and their related 3-hydroxy-2-aryl acrylate

3-oxo-esters and their related 3-hydroxy-2-aryl acrylate have proven to be an important precursor in the preparation of various biologically and pharmaceutically active natural and synthetic compounds.<sup>1</sup> The synthetic diversity of 3-oxo-esters and their related 3-hydroxy-2-aryl acrylate are due to its unique structure, comprised of one electrophilic and two nucleophilic carbons. 3-oxo-esters can exist in two different form namely *Z*-enol form and *E*-enol form but the *Z*-enol form which is more stable than the *E*-enol form due to the hydrogen bonding between the carbonyl of the ester functional group to the enolic hydroxy group (Figure. 3.1).<sup>2</sup>

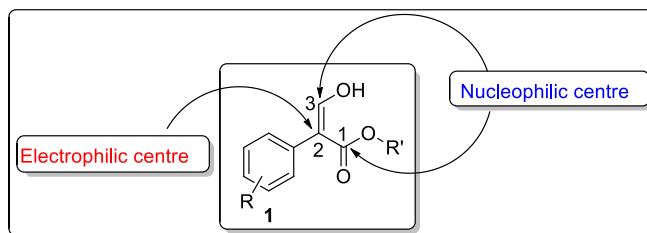


Figure 2.1: 3-oxo-esters and their related 3-hydroxy-2-aryl acrylate

This above feature makes this compound a useful substrate in the synthesis of aromatic organic molecules, including many heterocycles such as phenanthrolines,<sup>3</sup> benzofurans,<sup>4</sup> indoles,<sup>5</sup> oxazoles,<sup>6</sup> imidazoles,<sup>7</sup> and quinolines.<sup>8</sup> Therefore, increasing efforts have been devoted to the development of efficient protocols for the synthesis of this valuable scaffold by using commercially available starting materials in shorter steps.<sup>9-10</sup>

### 2.2. Synthesis and Its Mechanism

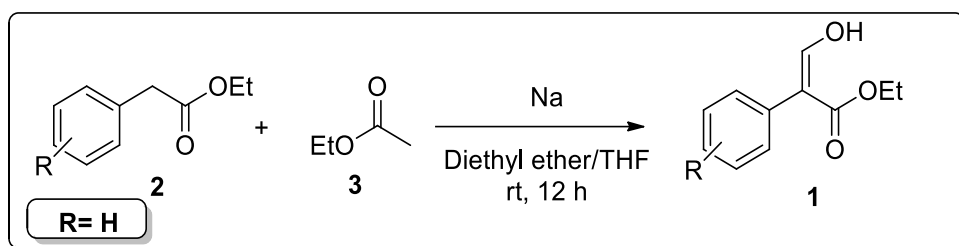
There are different ways 3-oxo-esters, and their related 3-hydroxy-2-aryl acrylate can be synthesized. In broadly, the synthesis is classified into four categories namely Na/NaH catalyzed reaction, Lewis Acid-catalyzed reaction, Brønsted Type Acid-Catalyzed Reaction,



and Organic base-catalyzed reaction. The synthetic procedure and mechanism of the reaction is described below:

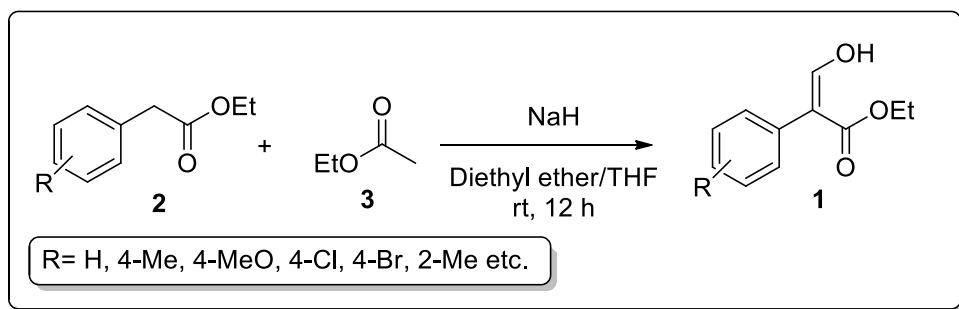
### 2.2.1 Na/ NaH catalysed reaction

The first synthesis of 3-hydroxy-2-phenyl acrylate **1** was reported by Wislicenus in 1895 by the condensation of ethyl phenylacetate **2** with ethyl formate **3** in the presence of sodium metal in diethyl ether (Scheme 2.1).<sup>11-13</sup> This was one of the most direct approaches to prepare 3-hydroxy-2-phenyl acrylate.



Scheme 2.1: Sodium Metal Catalyzed Synthesis of **1**

Later, researchers applied the same protocol to synthesize 3-hydroxy-2-aryl acrylate with substituted ethyl phenylacetate by using sodium hydride as a base instead of sodium metal and obtained better yields (Scheme 2.2).<sup>14-16</sup>

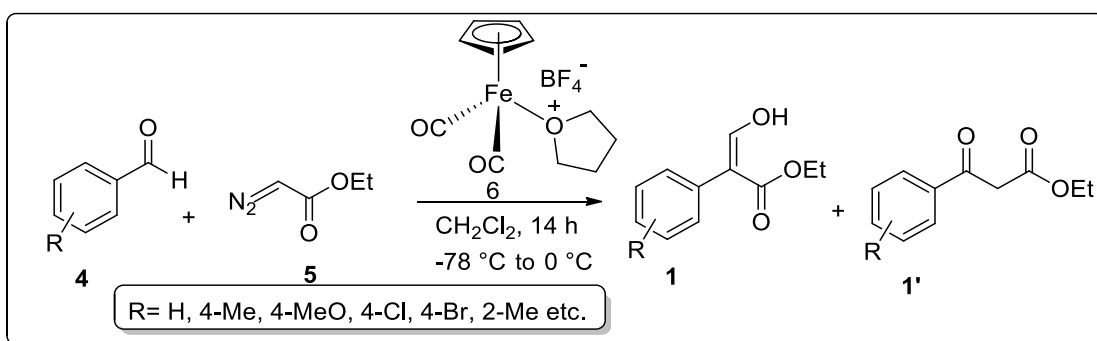


Scheme 2.2: Sodium Hydride Catalyzed Synthesis of **1**

### 2.2.2 Lewis Acid catalyzed reaction

In 1998, our group has reported the formation of 3-oxo-esters and their related 3-hydroxy-2-aryl acrylate from the reaction of aromatic aldehydes, **4** with ethyl diazoacetate, **5** (EDA) in

the presence of iron Lewis acid catalyst, **6**  $[\eta^5\text{-(C}_5\text{H}_5\text{)Fe}^+(\text{CO})_2(\text{THF})]\text{BF}_4^-$ , caused by an unprecedented 1,2-aryl migration (Scheme 3).<sup>17</sup> Previously, a similar type of reactions was performed using different Lewis acid catalysts namely  $\text{BF}_3$ ,  $\text{ZnCl}_2$ ,  $\text{ZnBr}_2$ ,  $\text{AlCl}_3$ ,  $\text{SnCl}_2$ ,  $\text{GeCl}_2$ , and  $\text{SnCl}_4$  which produced either epoxides or  $\beta$ -ketoesters as the major products.<sup>18</sup> Iron Lewis acid-catalyzed reaction formed desired compounds 3-hydroxy-2-aryl acrylate **1** along with 3-oxo-esters **1'** from moderate to excellent yields. During the screening of substrate scope, it was observed that when electron-withdrawing groups were present in carbonyl compounds 3-hydroxy-2-aryl acrylate was formed in low yield whereas the electron-donating groups resulted in high yields (Scheme 2.3).



Scheme 2.3: Synthesis of **1** by Iron Lewis Acid Catalysts

Our group was proposed a catalytic cycle to explain the variation of obtained results which shows that the presence of an electron-rich benzaldehyde enhanced 1, 2-aryl group migration compared to hydride migration and gave better yield (Figure 2.2).<sup>17</sup> On the other hand, the presence of an electron-withdrawing group slowdown 1,2-migration of the aryl group, which resulted in lesser enol ester product.

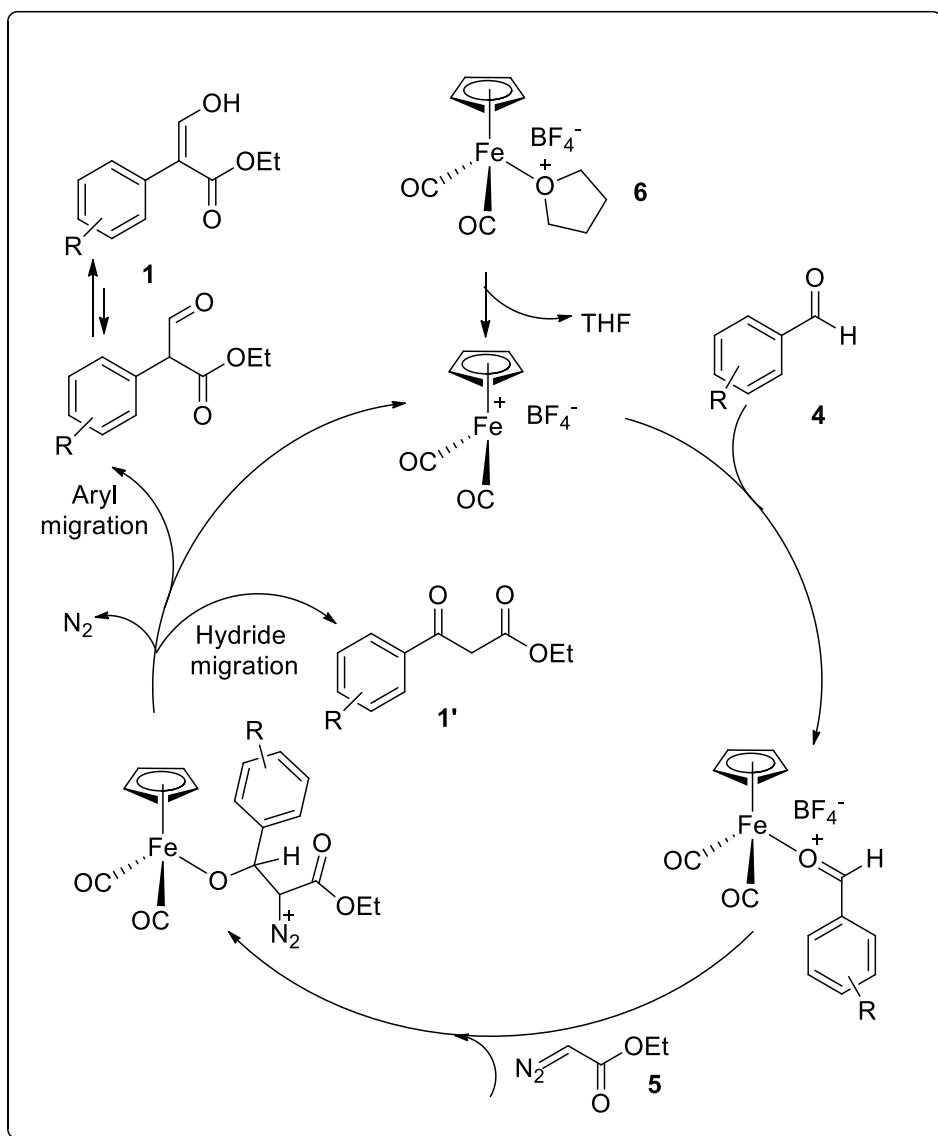
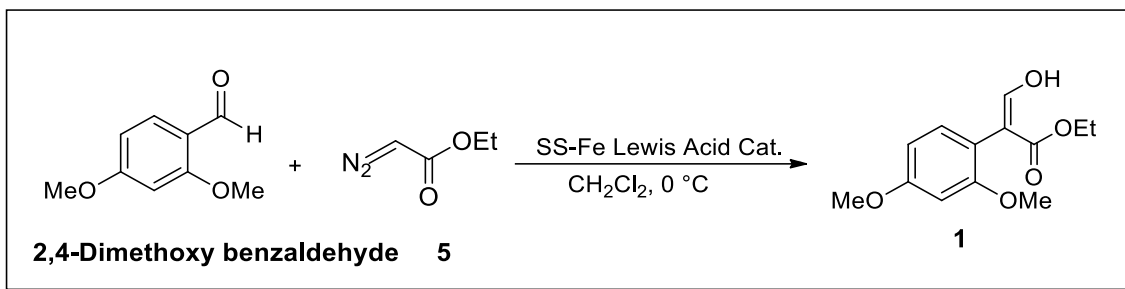


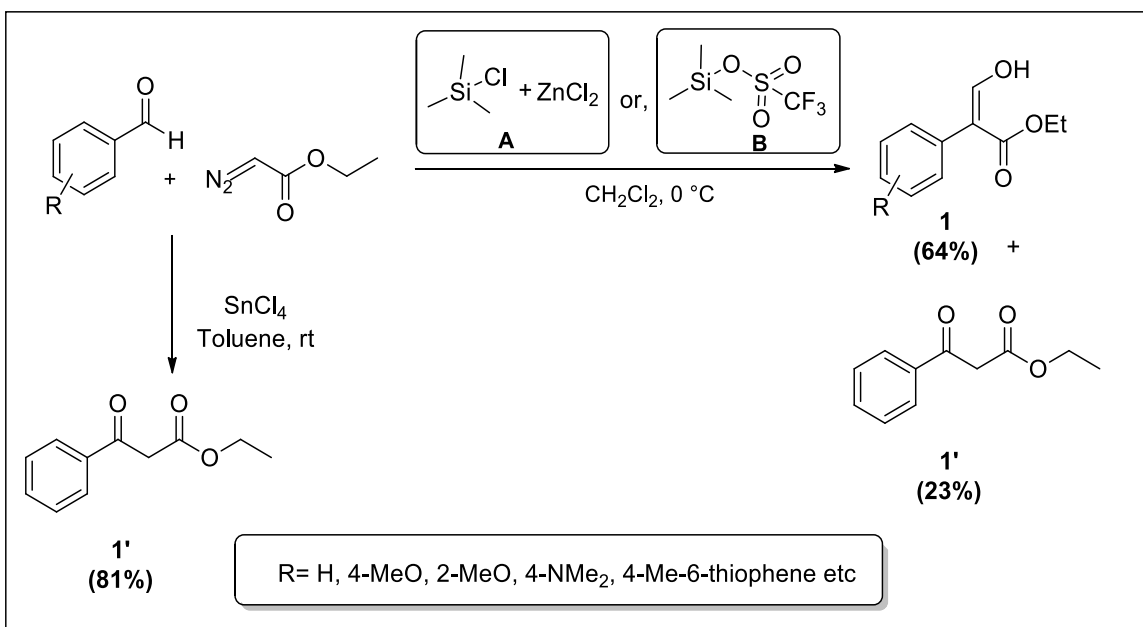
Figure 2.2: Mechanism of Iron Lewis Acid Catalysed Reaction

Later, our group was extended the above reaction by using a silica-supported iron (SS-Fe) Lewis acid catalyst to increase the efficacy and durability of iron Lewis acid catalyst, **6** [ $\eta^5$ -(C<sub>5</sub>H<sub>5</sub>)Fe<sup>+</sup>(CO)<sub>2</sub>(THF)]BF<sub>4</sub><sup>-</sup>. To perform this reaction, they use 2,4-Dimethoxy benzaldehyde and EDA, **5** as a substrate (Scheme 2.4).<sup>19</sup> After the reaction the results indicate that in terms of yield, the efficiency of SS-Fe Lewis acid catalyst was comparable to the unsupported iron Lewis acid catalyst and the catalyst was recyclable several times.



Scheme 2.4: Silica-Supported Iron Lewis Acid Catalysed Synthesis of **1**

Kanemasa et al. also described the synthesis of 3-oxo-esters and their related 3-hydroxy-2-aryl acrylate in 1999 by the condensation of aromatic aldehydes, **4** and EDA, **5** utilizing different Lewis acid catalysts. They observed that the types of products mainly depend upon the nature of Lewis acid catalysts employed. When the reaction is carried out in the presence of Lewis acids such as  $\text{SnCl}_2$  and  $\text{SnCl}_4$  yielded 3-oxo-ester **1'** as a major product via nucleophilic 1, 2-hydride migration, whereas those catalysed by either  $\text{ZnCl}_2$  in the presence of chlorotrimethylsilane **A** or trimethylsilyl trifluoro-methanesulphonate **B** gave 3-hydroxy-2-aryl acrylate **1** as a major product (Scheme 2.5).<sup>20</sup>



Scheme 2.5: Synthesis of **1** by Silane-based Lewis Acid Catalyst

Kanemasa group proposed a mechanism to explain the formation of 3-hydroxy-2-aryl acrylate by considering catalyst **B** (Figure 2.3).<sup>20</sup> Initially, aromatic aldehyde was activated by coordinating with catalyst **B**, so that EDA can easily attach to the carbonyl carbon of the aldehyde to form diazonium silyloxy intermediate through a linear transition structure (TS). After that, the aryl group migrates from the backside of the diazonium group to produce 3-hydroxy-2-aryl acrylate by the loss of N<sub>2</sub>. It should be mentioned that in several cases they got a mixture of compound 3-hydroxy-2-aryl acrylate and 3-oxo esters.

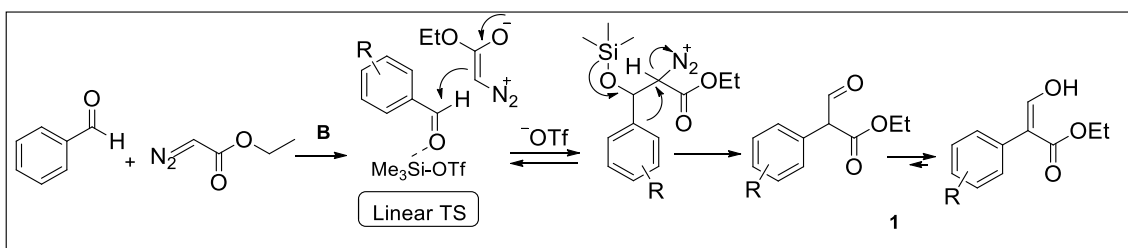


Figure 2.3: Mechanism of Trimethylsilyl Trifluoromethanesulphonate Catalysed Formation of **1**

On the other hand, in the presence of metallic halide-type Lewis acid catalysts such as SnCl<sub>2</sub> and SnCl<sub>4</sub>, the reaction proceeds through a chelation transition state to form intermediate, where the aldehyde substituent should occupy the equatorial position. When the diazonium group occupies the axial position in this reversible aldol reaction, a smooth hydride migration with the removal of nitrogen takes place to form 3-oxo-esters as a major product (Figure 2.4).<sup>20</sup>

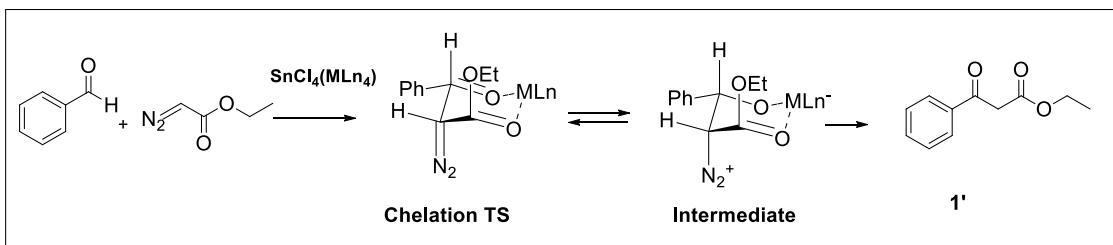
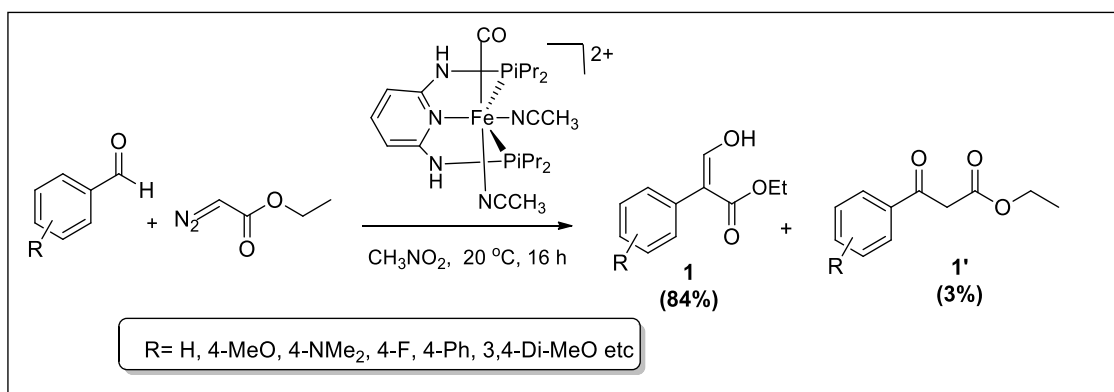


Figure 2.4: Mechanism of  $\text{SnCl}_4$  Catalysed Formation of **1**

In 2007, Kirchner and co-workers reported the first chemoselective formation of 3-hydroxy-2-aryl acrylate **1** from aromatic aldehydes and EDA by using a cationic iron pincer complexes such as  $[\text{Fe}(\text{PNP})(\text{CH}_3\text{CN})_3](\text{BF}_4)_2$  and  $[\text{Fe}(\text{PNP})(\text{CO})(\text{CH}_3\text{CN})_2](\text{BF}_4)_2$ , where PNP are various tridentate pincer-type ligands based on 2,6-diaminopyridine. The most effective catalyst was found to be *cis*- $[\text{Fe}(\text{PNP-}i\text{Pr})(\text{CO})(\text{CH}_3\text{CN})_2]\text{BF}_4$ , bearing both strongly electron-donating and bulky *i*Pr substituents (Scheme 2.6).<sup>21</sup>



Scheme 2.6: Formation of **1** by *cis*-Iron (II) Pincer Complex Catalyst

Kirchner et al. proposed a similar mechanistic pathway (Figure 2.5)<sup>21</sup> for the formation of 3-hydroxy-2-aryl acrylate **1** suggested by Hossain et al.<sup>17</sup>

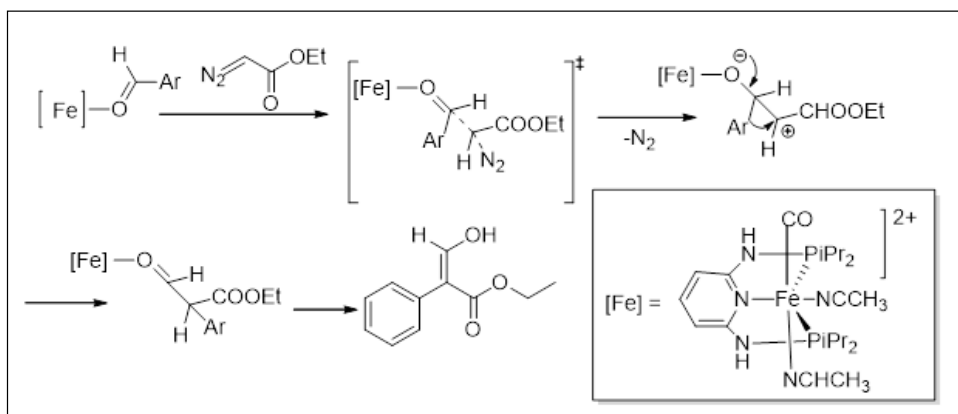
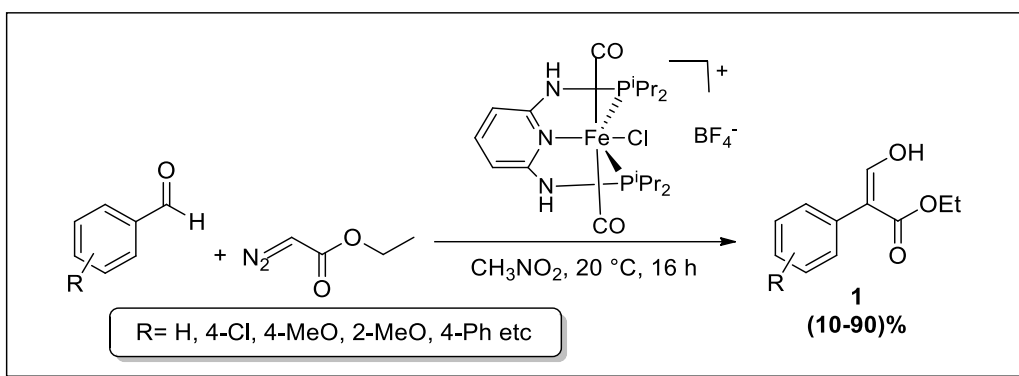


Figure 2.5: Mechanism of *cis*-Iron (II) Pincer Complex Catalysis

Later, Kirchner et al. also studied the effect of the counterion on selective formation of **1** by utilizing mono cationic dicarbonyl *trans*-[Fe(PNP-*i*Pr)(CO)<sub>2</sub>Cl](X) complex, where X = NO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>COO<sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, BF<sub>4</sub><sup>-</sup>, PF<sub>6</sub><sup>-</sup>, SbF<sub>6</sub><sup>-</sup> and B[3,5-(CF<sub>3</sub>)<sub>2</sub>C<sub>6</sub>H<sub>3</sub>]<sub>4</sub><sup>-</sup>. When BF<sub>4</sub><sup>-</sup> act as an anion, the reaction proceeds with conversions up to 90% but other anions like NO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>COO<sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, SbF<sub>6</sub><sup>-</sup> and [3,5-(CF<sub>3</sub>)<sub>2</sub>C<sub>6</sub>H<sub>3</sub>]<sub>4</sub><sup>-</sup> could not show any activity (Scheme 2.7).<sup>22</sup>



Scheme 2.7: Formation of **1** by *trans*-Iron (II) Pincer Complex catalyst

Based on density functional theory (DFT) calculation, a conceivable mechanism was proposed by Kirchner using benzaldehyde and Methyl Diazoacetate, **7** (MDA) as a substrate in the presence of *trans*-iron (II) pincer complex as a catalyst (Figure 2.6).<sup>22</sup>

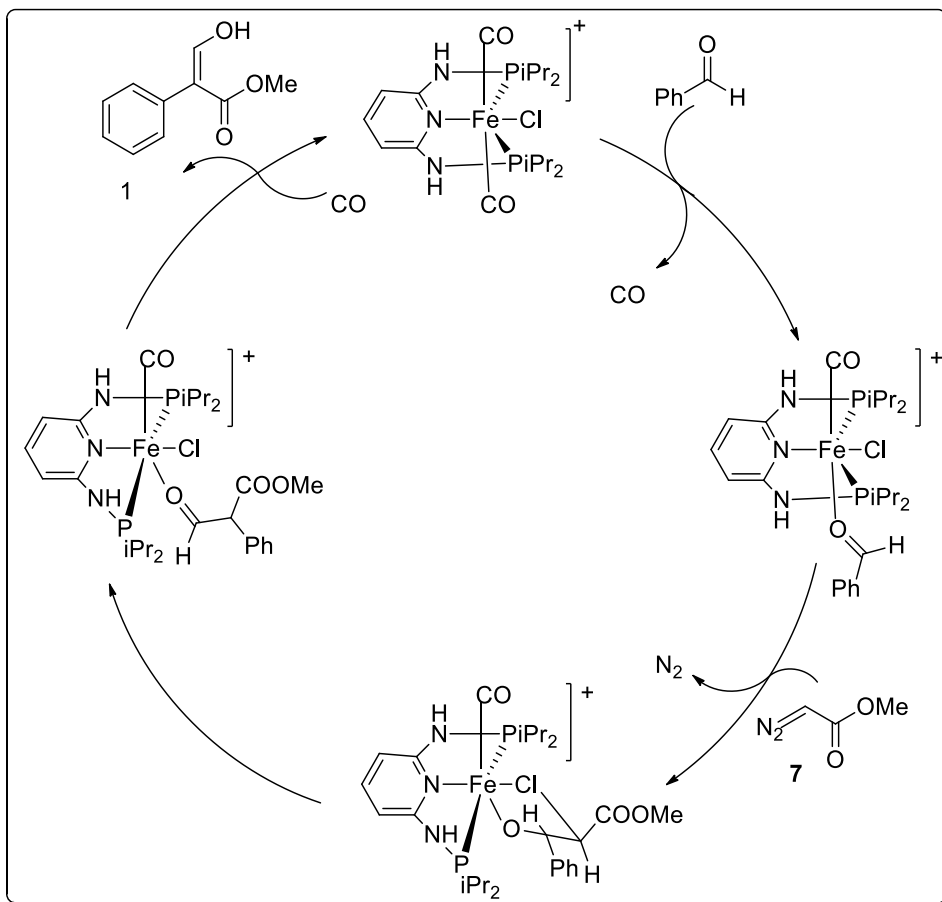
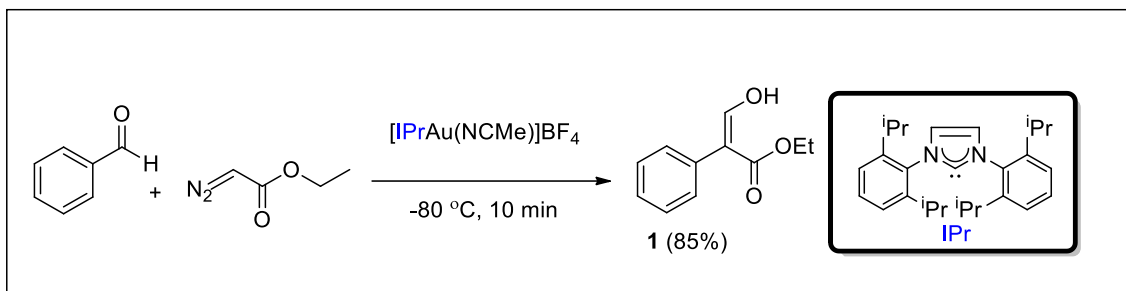


Figure 2.6: Mechanism of *trans*-Iron (II) Pincer Complex Catalysis

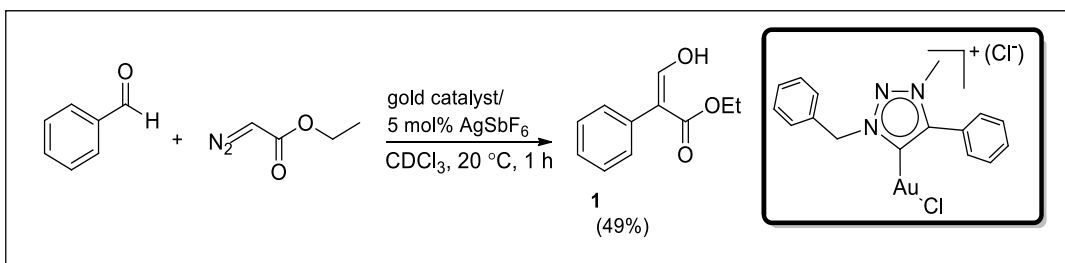
So far, Different types of transition metal is used for the synthesis of 3-hydroxy acrylates but the first example of Group 11 metal based catalysts specifically gold catalysts was reported by Pe´rez and co-workers in 2009 for the coupling reaction between benzaldehyde and EDA (Scheme 2.8).<sup>23</sup> Among different gold complexes verified, [IPrAu(NCMe)]BF<sub>4</sub> was found to be the most effective catalyst for the formation of 3-hydroxy-2-phenyl acrylate. The authors also mentioned that the catalyst gave the higher reaction rate and the excellent turnover frequency (TOF).





Scheme 2.8: Gold Metal Complex Catalysed formation of **1**

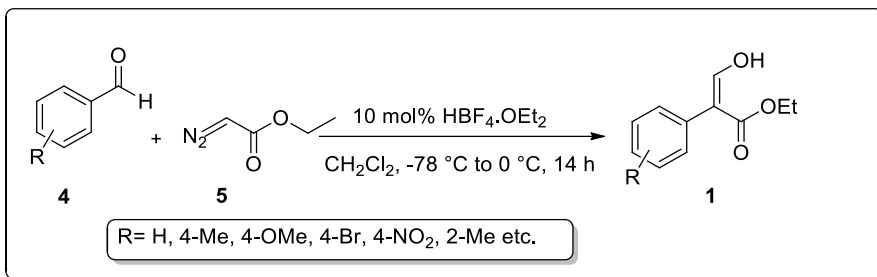
Later, Crowley and co-workers employed the similar substrate to synthesize 3-hydroxy-2-phenyl acrylates by using novel gold(I) “click” carbene(1,2,3-triazolylidene) complex  $[\text{AgSbF}_6]$  (Scheme 2.9).<sup>24</sup>



Scheme 2.9:  $\text{AgSbF}_6$  with Gold Complex Catalysed Formation of **1**

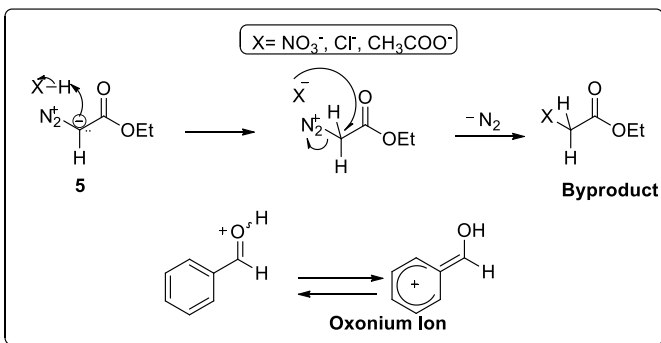
### 2.2.3. Brønsted Type Acid Catalyzed Reaction

Different types of Lewis acid catalysts were employed for the synthesis of 3-hydroxy acrylates. In 2004, our group explored Brønsted type catalysts, specifically  $\text{HBF}_4\cdot\text{OEt}_2$ , to produce **1** from the coupling reaction of aromatic aldehydes and EDA (Scheme 2.10).<sup>25</sup> The catalytic activity of  $\text{HBF}_4\cdot\text{OEt}_2$  was verified with a series of aldehyde **4**, where electron donating substituents on the aromatic ring and reaction at lower temperature significantly enhanced the isolation of the desired product **1**.



Scheme 2.10: Brønsted Acid Catalysed Formation of **1**

Our group screened several commercially available Brønsted acids too, such as  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{HClO}_4$ ,  $\text{HCl}$  besides  $\text{HBF}_4$ , to see the counterion effect on selective product formation. They found that the acids with nucleophilic counterions, such as  $\text{Cl}^-$ ,  $\text{AcO}^-$ , and  $\text{NO}_3^-$ , provided a small amount of product **1** since these acids readily quench EDA and formed byproduct. On the other hand,  $\text{HBF}_4$  with non-nucleophilic counterion ( $\text{BF}_4^-$ ) gave a better yield of product **1** by forming stable oxonium ion rather quenching **5** (Scheme 2.11).<sup>25</sup>



Scheme 2.11: Reaction of Brønsted Type Acids with EDA and Aldehyde

Based on the experimental outcomes, our group designed six possible Newman rotamers, **A-F**, to find out stable intermediate (Figure 2.7). Among six rotamers, **A** was the lowest energy rotamer where the aryl migrating group and leaving nitrogen group are anti position, thus favouring the formation of **1**. Moreover, aryl migration in rotamer **A** is favourable over hydride (Figure 2.7,

rotamers **B** and **D**) or oxo migration (Figure 2.7, rotamers **C** and **F**) due to its ability to stabilize an intermediate carbocation by the formation of phenonium ion (Figure 2.7, **G**). Though the stabilized phenonium ion **G** formation is possible for both rotamers **A** and **E**, rotamer **E** is less stable due to its steric hindrance. Furthermore, the electron donating group (EDG) can enhance the stability of the resultant **G** compared to the electron withdrawing group (EWG), thus favouring the selective formation of the desired product.

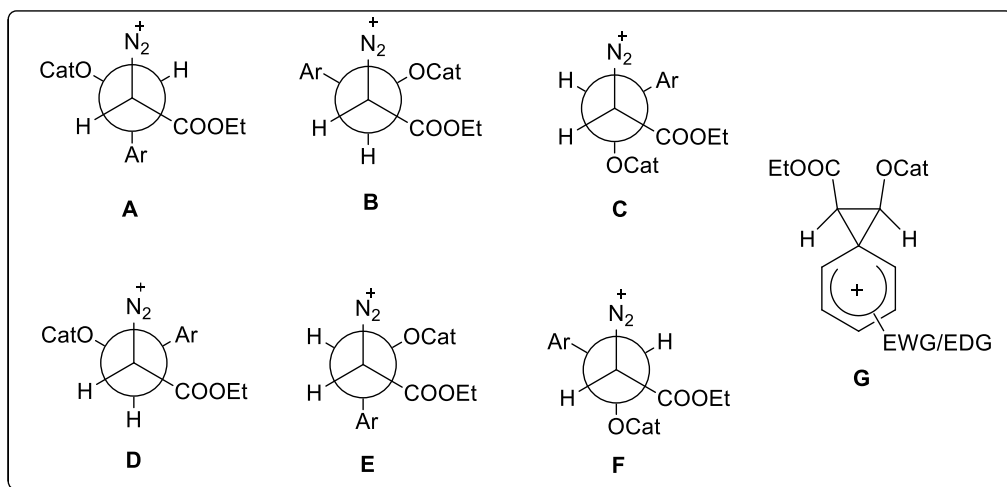


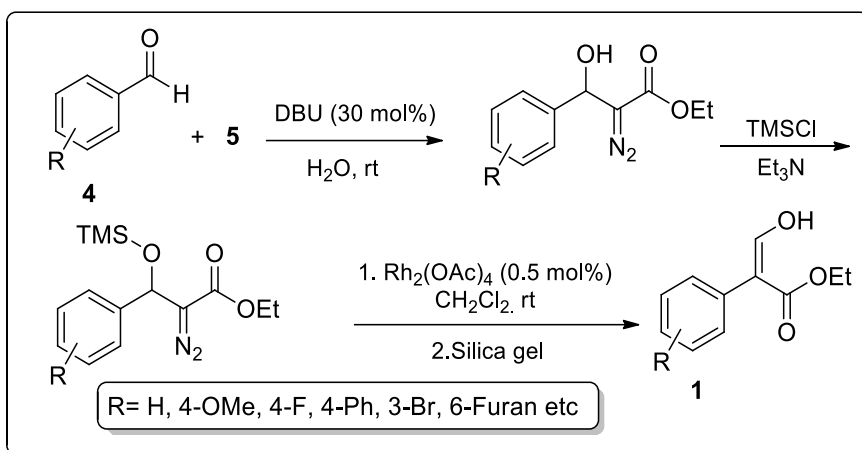
Figure 2.7: Newman Projections of Six Possible Rotamers (A-F) and a Transition State (G) Phenonium Ion Resulting from Aryl Migration

Later, Kirchner et al. carried out the computational study of the HBF<sub>4</sub>.OEt<sub>2</sub> catalysed formation of **1** using DFT calculation to verify our findings (GIAO method at the B3LYP/6-311G\*\* basis set).<sup>26</sup> Calculation indicate that the formation of 3-hydroxy-2-aryl acrylate is slightly favoured over the formation of 3-oxo-ester by energy 1.7 kcal/mole.

#### 2.2.4 Organic Base Catalysed Reaction

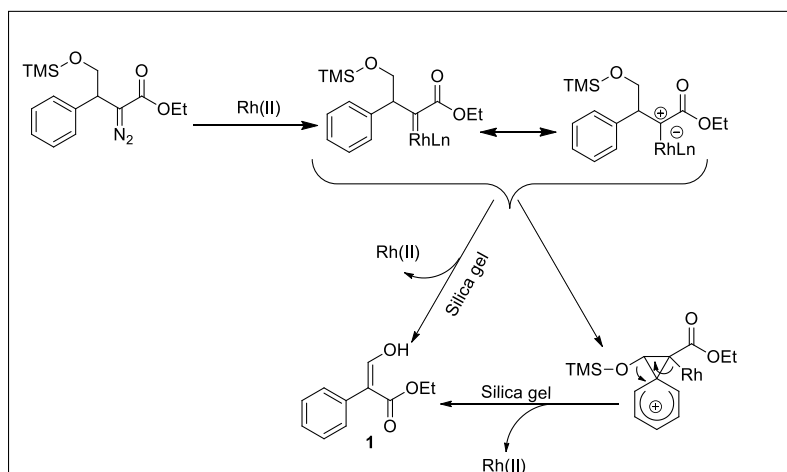
In 2007, Wang et al. reported organic base catalysed reaction between **4** and **5** to synthesize  $\beta$ -hydroxy diazo ester which further underwent 1, 2-aryl migration to provide 3-hydroxy acrylate **1** in the presence of Rh(II)-catalyst (Scheme 2.12).<sup>27</sup> The authors reported that steric factors play an important role in the 1, 2-migratory aptitude of Rh(II)-catalyzed reaction.

When the substituent was bulkier at the  $\beta$ -position, such as a siloxy group, migration of aromatic group over hydride was favoured.<sup>28</sup>



Scheme 2.12: DBU and Rh(II)-Catalysed Formation of **1**

Based on the reaction, Wang et al. proposed two ways mechanism involving Rh(II)-catalyst for the formation of acrylate **1** (Scheme 2.13).<sup>28-30</sup> In both ways, the initial step was the formation of Rh(II)-carbene intermediate which could provide compound **1** by 1,2-aryl migration via a bridged phenonium ion intermediate. On the other hand, product **1** could be formed directly from carbene by a concerted pathway where aryl group could migrate simultaneously with the dissociation of the catalyst.



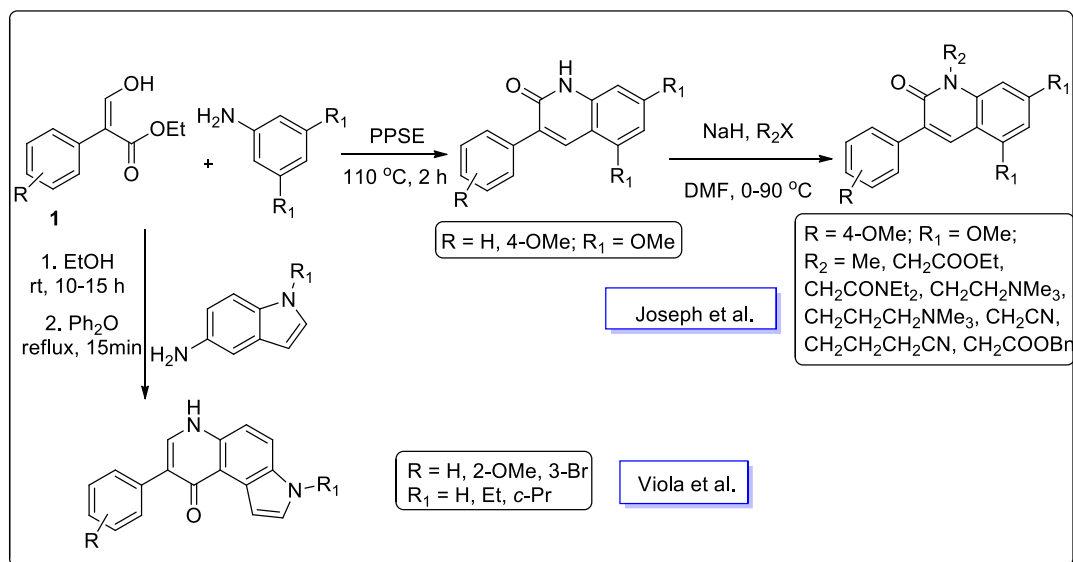
Scheme 2.13: Proposed Mechanism of Rh(II)-Catalyzed 1,2-Migration

## 2.3. Synthetic Application of 3-hydroxyacrylates

### 2.3.1 N-Containing Compounds

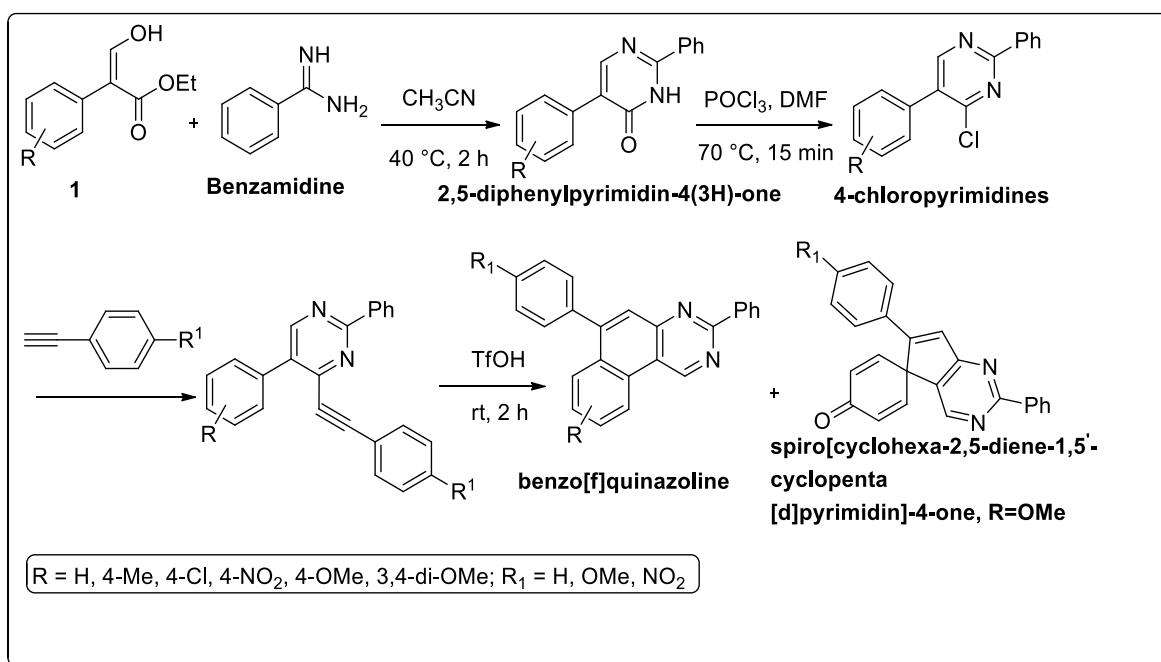
Different types of N-containing compounds can be synthesized from 3-hydroxy acrylates such as quinolone, pyrimidine, phenanthrene, uracil, indole, isoquinoline, Enamine type compounds. All these synthesized compounds showed broad range of medicinal and biological activity. For example, quinolones type compound showed antitumor<sup>31a</sup> and antimalarial activity,<sup>31b</sup> whereas, uracil type compound plays a pivotal role in the treatment for human immunodeficiency virus (HIV),<sup>32</sup> hepatitis B virus (HBV),<sup>33</sup> hepatitis C virus (HCV),<sup>34</sup> herpes simplex virus (HSV),<sup>35</sup> and various types of cancer.<sup>36</sup> Selective synthetic procedure for the synthesis of N-containing compounds from 3-hydroxy acrylates are given below:

In 2002, Joseph et al. prepared numbers of N-substituted 3-aryl-2-quinolone derivatives starting from **1** to develop nontoxic antimigratory compounds.<sup>37</sup> Viola et al. synthesized a series of 7-phenyl-3H-pyrrolo[3,2-f]quinolin-9-ones from **1** and evaluated their anti-proliferative activity (Scheme 2.14).<sup>8a</sup>



Scheme 2.14: Synthesis of Quinolinone type compounds from **1**

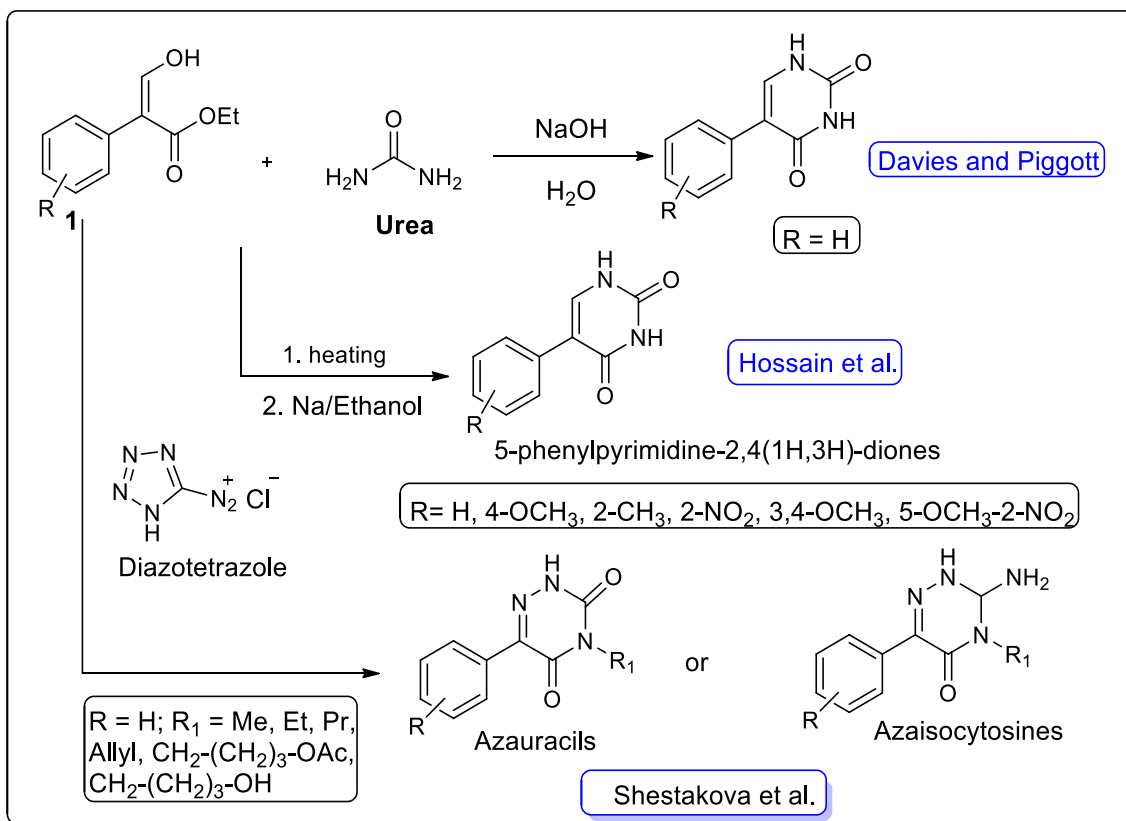
Kuznetsov et al. reported one of the simplest synthetic protocols for deriving 5-aryl-4-(arylethynyl)pyrimidines from compound **1**. First, acrylate **1** and benzamidine reacted to form 2,5-diphenylpyrimidin-4(3H)-one which was then converted to 4-chloropyrimidines by using POCl<sub>3</sub> (Scheme 2.15).<sup>38</sup> The resulting pyrimidine underwent the Sonogashira coupling reaction with ethynylbenzene and yielded 2,5-diphenyl-4-(phenylethynyl) pyrimidine. Acidic cyclization of produced benzo[f]quinazoline. With paramethoxy aryl acrylate, spiro[cyclohexa-2,5-diene-1,5'-cyclopenta [d]pyrimidin]-4-one was obtained along with product.



Scheme 2.15: Synthesis of Benzo[f]quinazoline and Pyrimidinone from **1**

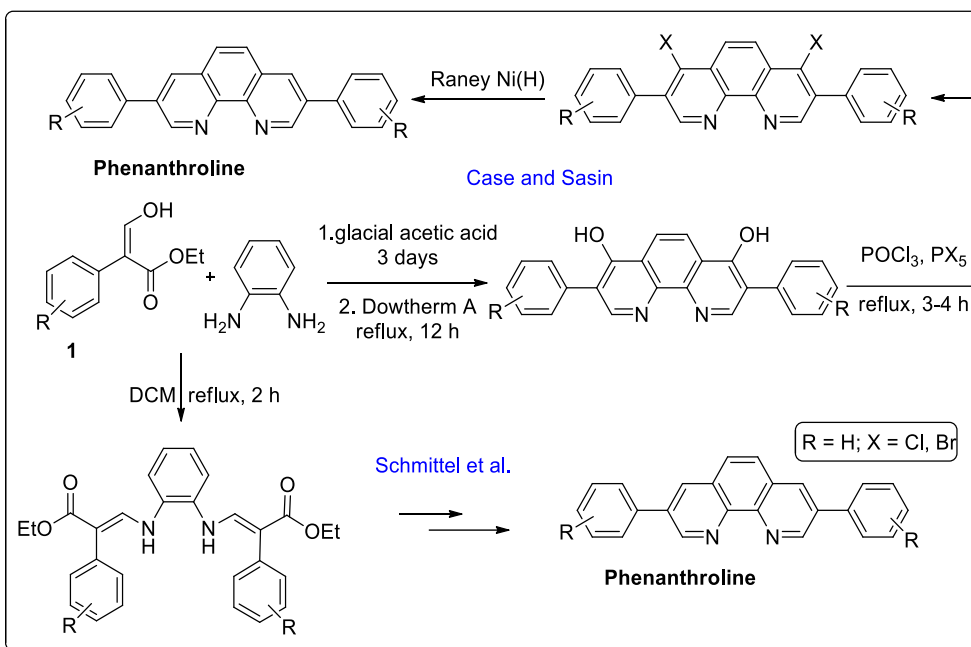
Davies and Piggott first introduced an approach for the synthesis of uracil type compounds, 5-phenylpyrimidine-2,4 (1H,3H)-dione from the reaction of **1** and urea in the presence of NaOH.<sup>39</sup> In 2007, our group reported NaOEt catalysed synthesis of a series of derivatives 5-phenylpyrimidine-2,4 (1H,3H)-dione using a variety of acrylates including both electron

donating and electron withdrawing groups.<sup>40</sup> Shestakova et al. reported the synthesis of azauracils and azaisocytosines type compounds by the azo-coupling reaction of diazotetrazole with **1** (Scheme 2.16).<sup>41</sup>



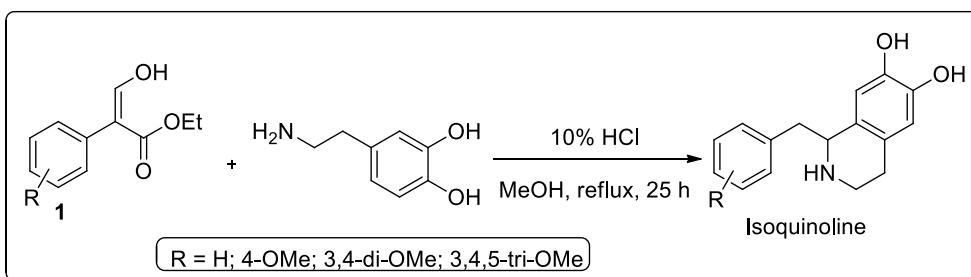
Scheme 2.16: Synthesis of Uracil and Azauracil type compounds from **1**

In 1955, Case and Sasin reported the synthesis of 4,7-dihydroxy-3,8-diphenyl-1,10-phenanthrolines by the cyclization reaction of **1** and o-phenylenediamine.<sup>42</sup> Successive halogenation of the hydroxy group and reduction by RANEY®-Ni formed the desired phenanthroline compound. Later, Schmittel and co-workers reported an alternative method of preparing the desired phenanthroline compound from **1** (Scheme 2.17).<sup>3</sup>



Scheme 2.17: Synthesis of Phenanthrolines from **1**

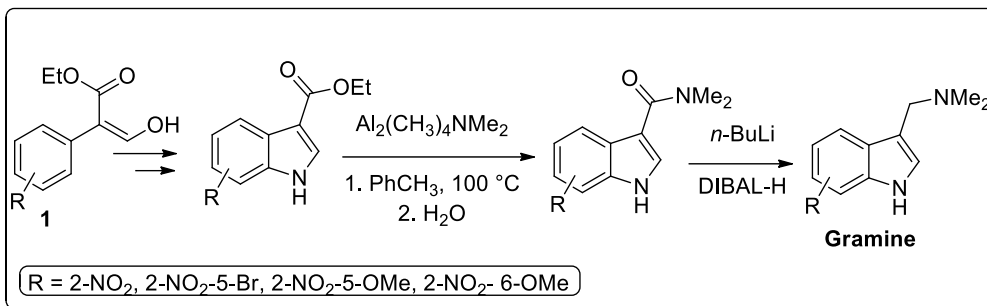
Yamato et al. reported a convenient protocol of preparing isoquinoline derivatives by reacting compound **1** with an amine in acidic methanol under reflux condition (Scheme 2.18).<sup>8c</sup> Isoquinolines type compounds are already used as a potent bronchodilator in practical clinics.<sup>43</sup>



Scheme 2.18: Synthesis of Isoquinoline Derivatives from **1**

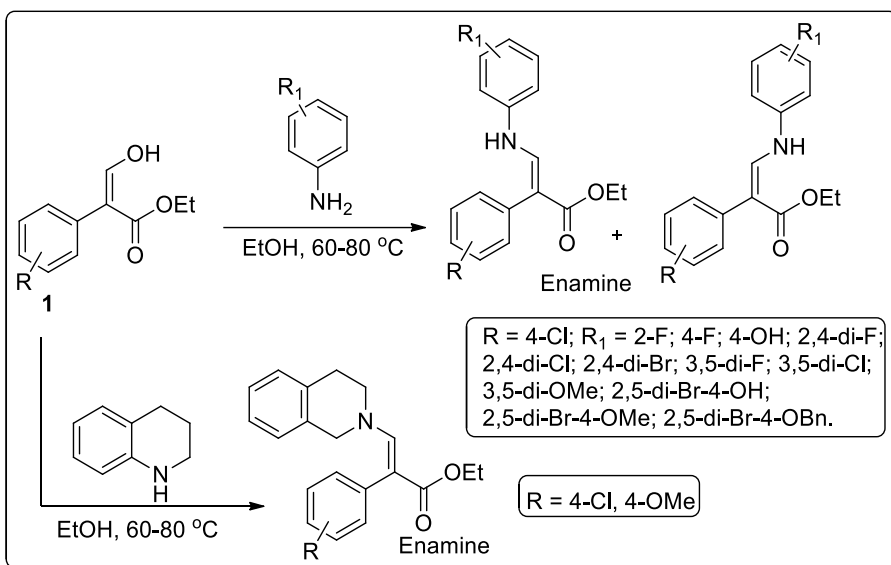
Our group developed an efficient method for the preparation of a versatile synthetic intermediate, 3-dimethylaminomethylindole (gramine) from **1** (Scheme 2.19).<sup>5b</sup> In human, Gramine act as an agonist of the adiponectin receptor 1.





Scheme 2.19: Synthesis of Gramine from **1**

Stable enamines show strong antibacterial activity.<sup>44</sup> Xiao et al. synthesized 26 enamine type compounds from **1** and performed their structure–activity relationships (SARs) based upon the bioassay using *Staphylococcus aureus* ATCC 6538 and *Pseudomonas fluorescens* ATCC 13525 strains (Scheme 2.20).<sup>45-46</sup>



Scheme 2.20: Synthesis of Enamine Derivatives from **1**

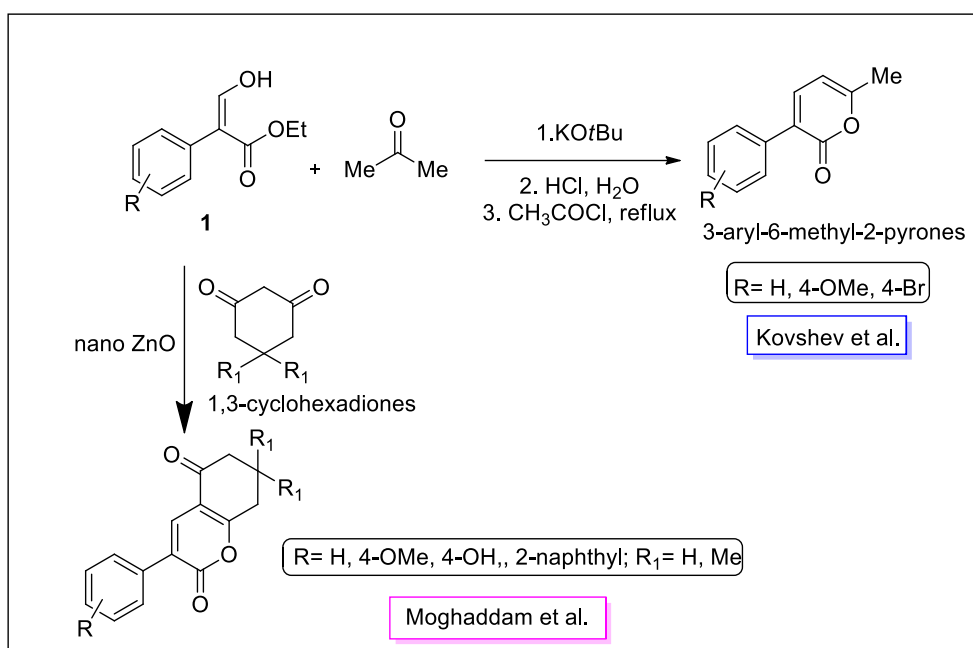
### 2.3.2 O-containing Compounds

3-hydroxy acrylates are a valuable precursor to synthesize different types of O-containing compounds such as Pyrone-2, bicyclic pyrone-2 and benzofuran. 2-Pyrone is a conjugated diene found in pheromonal, antifungal, antitumor and neurologically effective compounds. It

is widely used in Diels-Alder cycloaddition type reactions.<sup>47-48</sup> On the other hand, Benzofurans are pivotal precursors for the synthesis of many pharmaceutical and biologically active compounds.<sup>49</sup> Some selective synthetic procedure are described below:

In 1985, Kovshev et al. first reported the synthesis of 3-aryl-6-methyl-2-pyrones by condensation of **1** with acetone in the presence of potassium tert-butoxide (Scheme 2.21).<sup>50</sup>

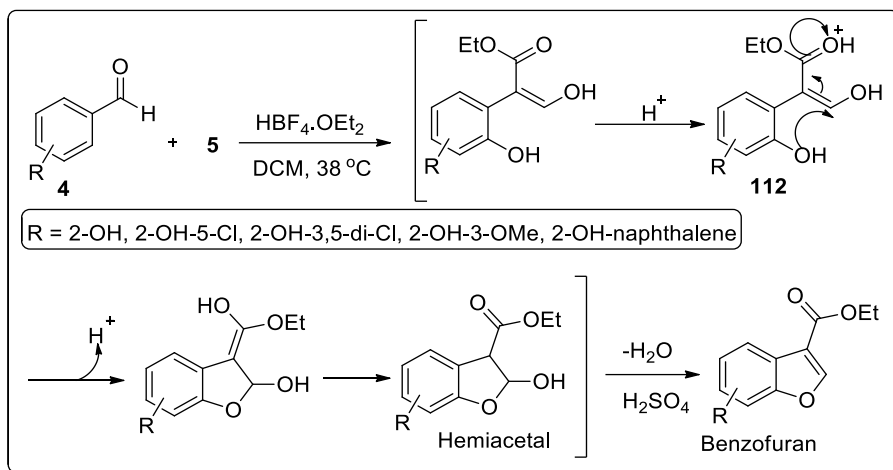
Later, Moghaddam and co-workers reported the synthesis of highly substituted 2-pyrone derivatives by tandem Knoevenagel condensation followed by lactonization reaction of 1,3-cyclohexadiones with **1** in the presence of nanocatalyst, ZnO (Scheme 2.21).<sup>51</sup>



Scheme 2.21: Synthesis of 2-pyrone and bicyclic-2-pyrone from **1**

In 2006, Hossain et al. reported an unexpected formation of 3-ethoxycarbonylbenzofurans while studying the substrate scope of benzaldehyde **4** to prepare substituted 3-hydroxy-2-arylacrylates **1**.<sup>52</sup> To explain the results, they proposed a mechanism which indicates that ortho-hydroxybenzaldehyde **4** first reacts with EDA **5** which subsequently undergoes cyclization in the presence of acid to form the hemiacetal. Finally, the resulted hemiacetal in

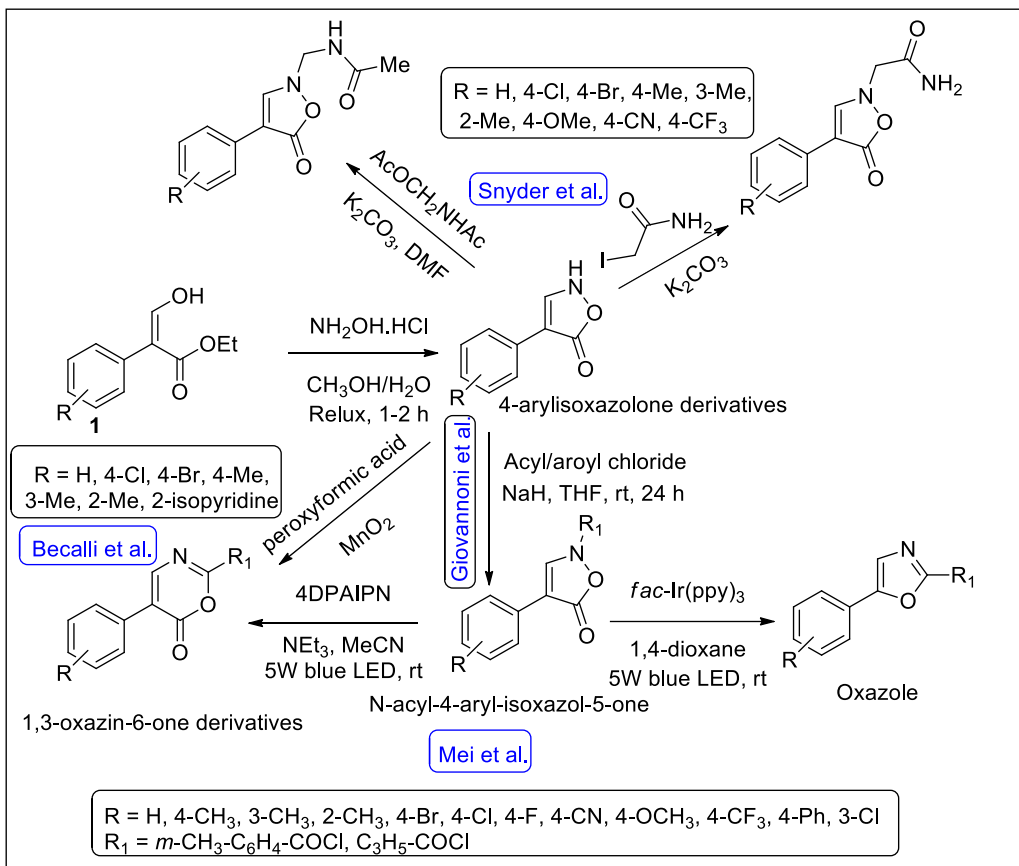
the presence of concentrated  $\text{H}_2\text{SO}_4$  undergoes dehydration to form 3-ethoxycarbonylbenzofuran (Scheme 2.22).<sup>53</sup>



Scheme 2.22: Synthesis of 3-Ethoxycarbonylbenzofurans from **1**

### 2.3.3 N- and O-containing compounds such as Isoxazoline

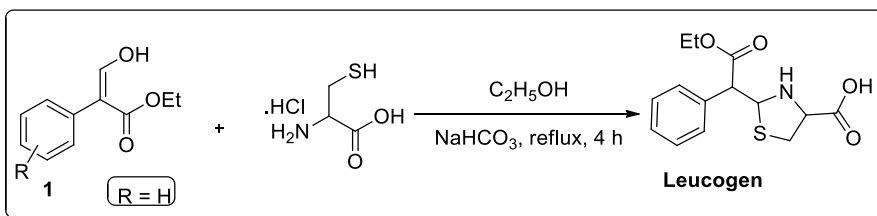
Isoxazolinone derivatives are promising molecules for bacterial (Gram-positive) and inflammatory lung diseases (H1N1 and SARS virus infection).<sup>6,54</sup> They are the versatile building blocks in organic synthesis mainly because of the weak N–O bond (BDE,  $D^0_{298} = 151 \text{ kcal mol}^{-1}$ ) cleavage and  $\text{CO}_2$  extrusion.<sup>55-56</sup> Isoxazolinone core can readily converted to 1,3-oxazin-6-one and oxazole derivatives which shows a wide range of biological activities. Several approaches to synthesize isoxazolinone derivatives were reported from **1** (Scheme 2.23).



Scheme 2.23: Synthesis of Isoxazolone Derivatives from **1**

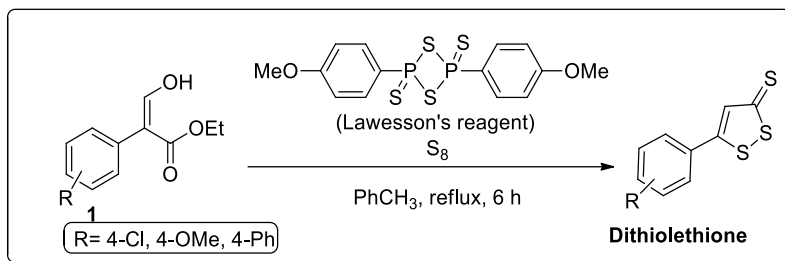
### 2.3.4 Synthesis of S- Containing compounds

Strukov et al. discovered the method for the synthesis of S-containing compound such as leucogen by the reaction of *L*-cysteine hydrochloride and **1** (Scheme 2.24).<sup>57-58</sup> Leucogen, (*L*-2( $\alpha$ -phenyl- $\alpha$ -ethoxycarbonylmethyl)-thizolidine-4-carboxylic acid) is one of the active ingredients of veterinary vaccine for the treatment of leucosis and preclude blood systemic disease.<sup>59</sup>



Scheme 2.24: Synthesis of S-containing compound, Leucogen from **1**

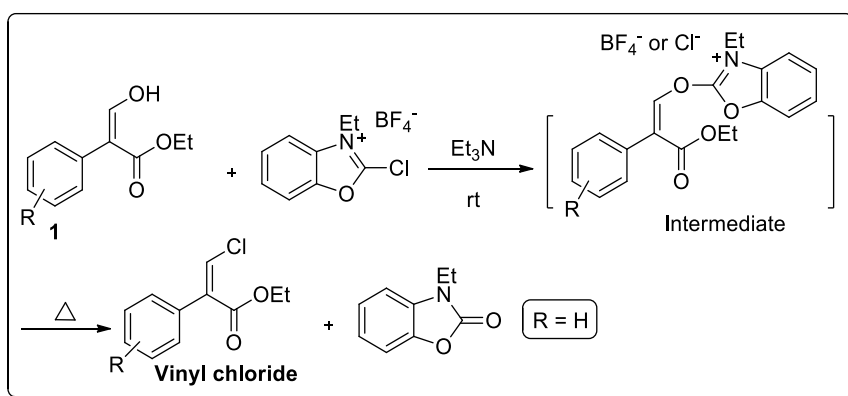
Brown group reported another S-containing compound dithiolethiones by reacting **1** with elemental sulfur and Lawesson's reagent (Scheme 2.25).<sup>60</sup> Dithiolethiones work as exogenous molecules to increase the cellular antioxidant glutathione by controlling its biosynthesis and have the potential to treat the Parkinson's disease.<sup>61</sup>



Scheme 2.25: Synthesis of Dithiolethione from **1**

### 2.3.5 Synthesis of Vinyl Derivatives

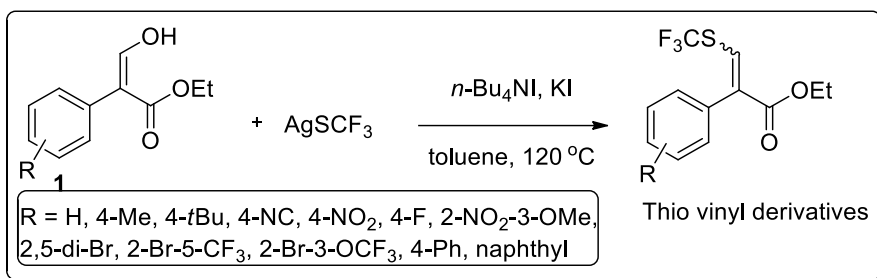
First selective method for the preparation of vinyl chloride from **1** was reported by Mukaiyama et al.<sup>62</sup> Treatment of **1** with 2-chloro-3-ethylbenzoxazolium tetrafluoroborate in the presence of triethylamine formed an active intermediate. Successive nucleophilic attack on the intermediate by chloride ion afforded the corresponding vinyl chloride (Scheme 2.26).



Scheme 2.26: Synthesis of Chloro Vinyl Derivatives from **1**

Qing and co-workers synthesized a variety of thio vinyl derivatives from **1** by direct

trifluoromethylthiolation of C<sub>sp</sub><sup>2</sup>-OH bond activation using silvertrifluoromethanethiol (Scheme 2.27).<sup>63</sup>

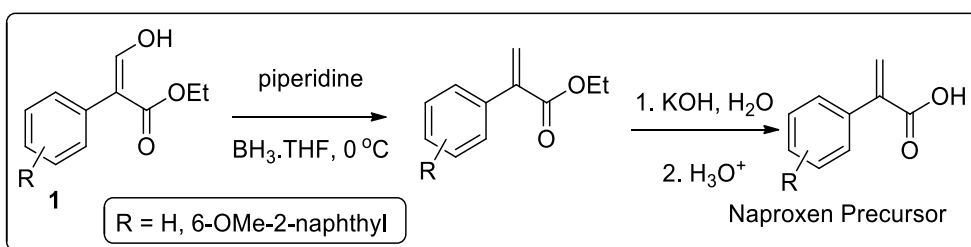


Scheme 2.27: Synthesis of Thio Vinyl Derivatives from **1**

### 2.3.6 Other Applications

#### 2.3.6.1 Non-steroidal Anti-inflammatory Drugs (NSAIDs)

$\alpha$ -Arylpropanoic acids containing non-steroidal anti-inflammatory agents (NSAIDs) such as naproxen, profens etc. are globally used as potential candidates for pain relief and the treatment of chronic rheumatoid arthritis.<sup>64-66</sup> In 2002, Hossain et al. described the synthesis of naproxen precursor from **1** in only two steps (Scheme 2.28).<sup>1a</sup>



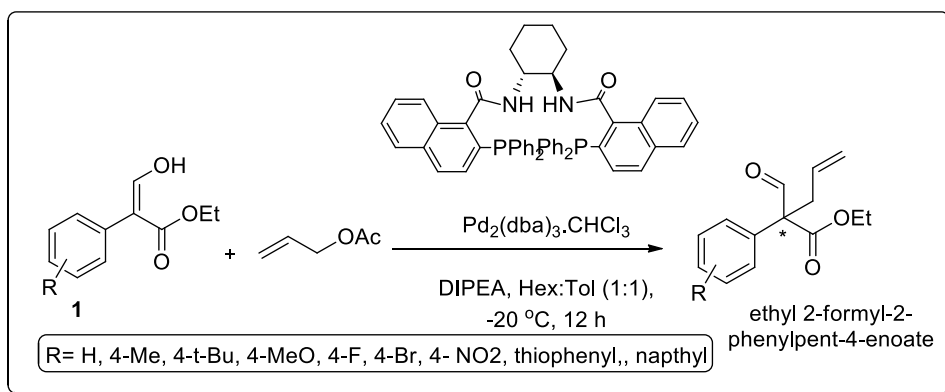
Scheme 2.28: Synthesis of Naproxen Precursor from **1**

#### 2.3.6.2 All-Carbon Quaternary Centre Containing Compounds

Quaternary carbon stereo centres are prevalent scaffold in many bioactive natural products and pharmaceuticals such as taxol,<sup>67</sup> gelsemine,<sup>68</sup> morphine,<sup>69</sup> and vinblastine<sup>70</sup>. Lots of attention had been devoted to the development of efficient methods for the stereo-controlled

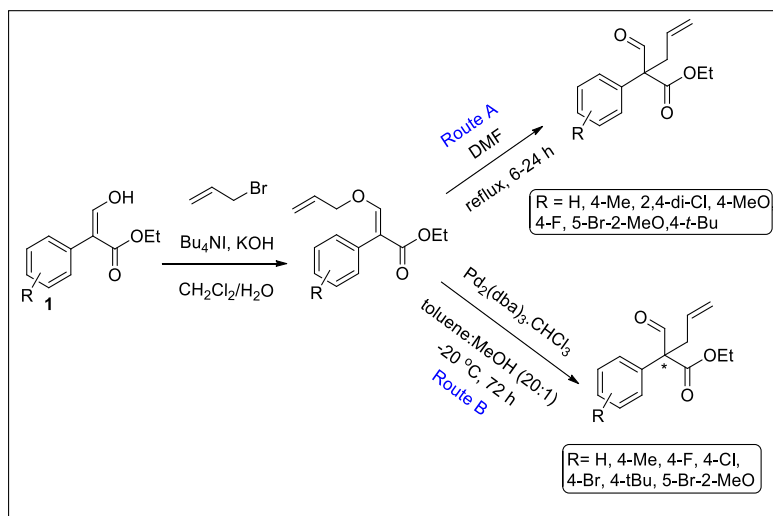
construction of quaternary carbon centers.<sup>71</sup> The formation of acyclic quaternary carbon stereocenters is more challenging due to enhanced conformational mobility compare to ring structure.<sup>72</sup> A couple of successful methods (both in intra and intermolecular way) were reported by Hossain group to prepare such achiral quaternary centre from **1**.

A unique route of intermolecular Pd-catalyzed asymmetric allylic alkylation (Pd-AAA) of **1** to synthesize all-carbon quaternary centre containing compound, ethyl 2-formyl-2-phenylpent-4-enoate derivatives was first introduced by our group (Scheme 2.29).<sup>73</sup> Optimized reaction conditions provided good to excellent yields (75–99%) and enantioselectivities (75–94%) of product.



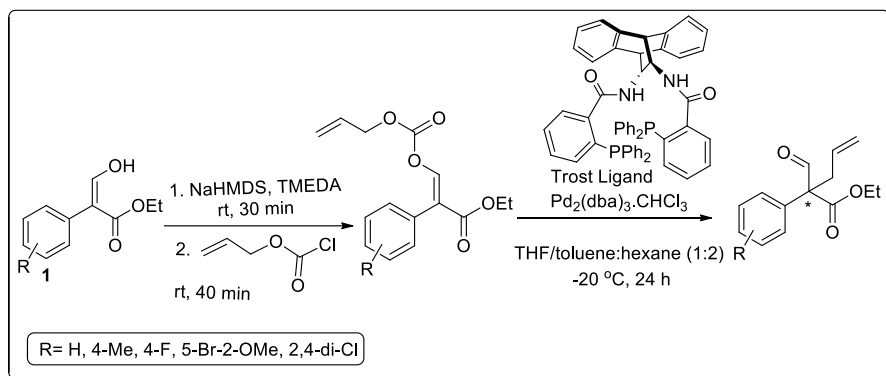
Scheme 2.29: Intermolecular Synthesis of All-Carbon Quaternary Centers from **1**

In 2010, our group reported an intramolecular Claisen rearrangement of O-allyl enol-ether to prepare racemic ethyl 2-formyl-2-phenylpent-4-enoate derivatives (Scheme 2.30).<sup>74</sup>



Scheme 2.30: Intramolecular Synthesis of All-Carbon Quaternary Centers from **1** via Enol Ether

Our group also reported a Pd-catalyzed decarboxylative allylic alkylation (DAAA) reaction using DACH phenyl ANDEN Trost ligand for the formation of chiral ethyl 2-formyl-2-phenylpent-4-enoate derivatives (Scheme 2.31).<sup>75</sup>

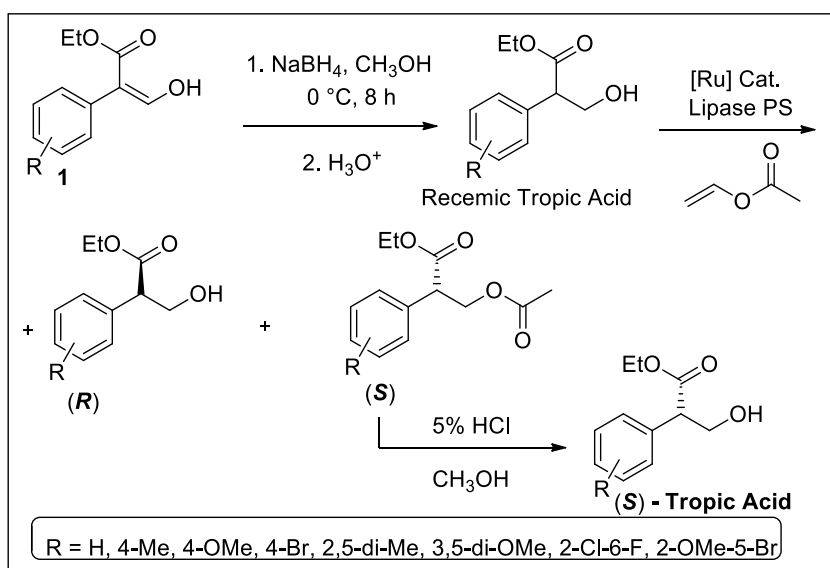


Scheme 2.31: Intramolecular Synthesis of All-Carbon Quaternary Centers from **1** via Carboxylate

### 2.3.6.3 Tropic Acid Type Compounds



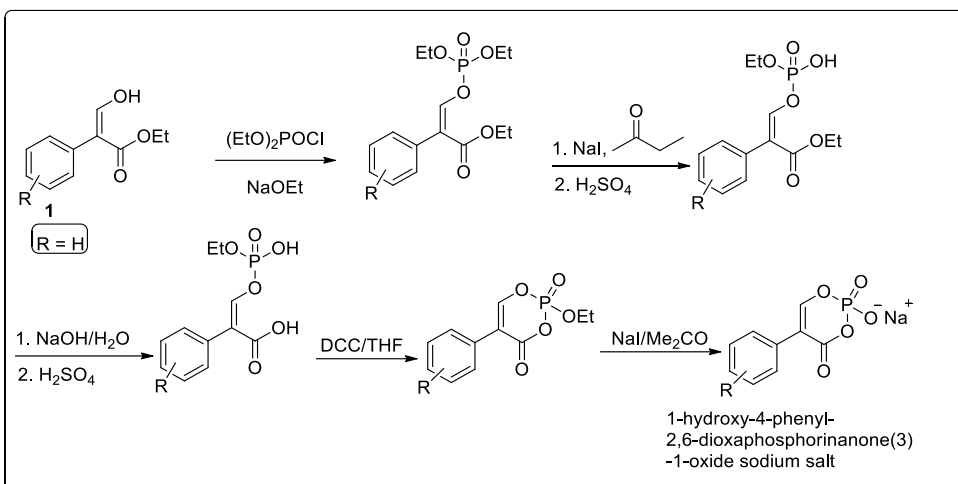
Tropic acid is an integral part of tropane alkaloids such as l-scopolamine, l-hyoscyamine, l-atrovent etc. are prospective drug targets especially for treating mental illness<sup>76</sup>, cardiacarytmia<sup>77</sup> and chronic bronchitis<sup>78</sup> respectively. Our group reported an improved method for the preparation of racemic mixtures of tropic acid ethyl ester (TAEE) derivatives from **1** using sodium borohydride in methanol.<sup>79</sup> To obtain chiral tropic acid, we developed an asymmetric kinetic resolution by utilizing enzyme lipase PS. Moreover, to improve the yield of one enantiomer, we explored dynamic kinetic resolution (DKR) of racemic tropic acid and its derivatives using ruthenium as a catalyst to initiate the racemization (Scheme 2.32).<sup>80</sup>



Scheme 2.32: Synthesis of TAAE from **1** via Dynamic Kinetic Resolution

#### 2.3.6.4 Non- $\beta$ -lactam inhibitors

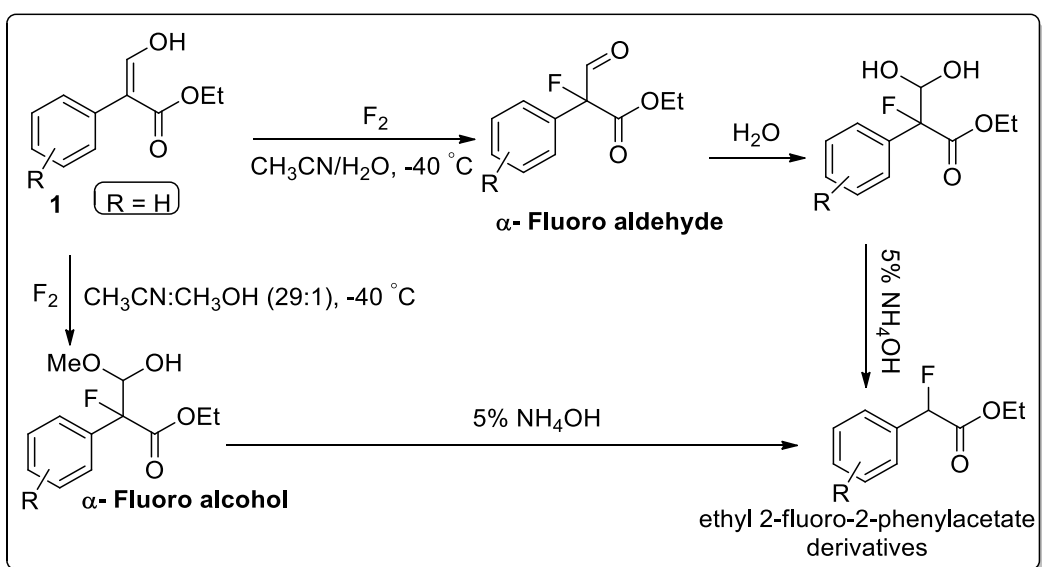
Pratt group developed phosphonate type compounds which showed promising activities, specifically against class C  $\beta$ -lactamases as non- $\beta$ -lactam inhibitors (Scheme 2.33).<sup>81</sup>



Scheme 2.33: Synthesis of  $\beta$ -lactam from **1**

### 2.3.6.5 $\alpha$ -Fluoroester Compounds

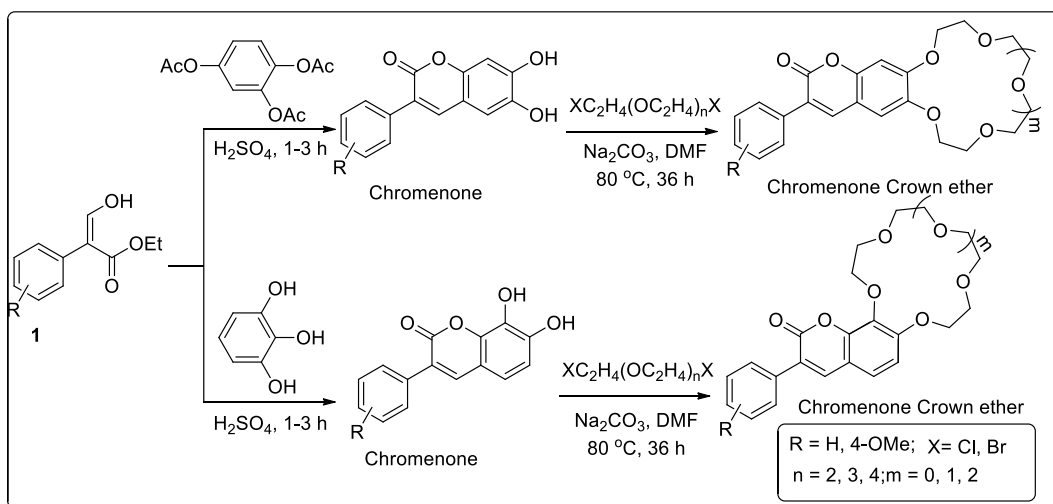
$\alpha$ -Fluoroketones and  $\alpha$ -fluoroesters have significant impact from both synthetic and biological point of view.<sup>82</sup> Kamaya et al. introduced a site-specific fluorination technique for carbonyl compounds that may be a valuable tool for preparing fluorine containing building blocks.<sup>83</sup> Molecular fluorine efficiently reacts with **1** to give  $\alpha$ -fluoro aldehyde or  $\alpha$ -fluoro alcohol by releasing HF as a by-product. Both  $\alpha$ -fluoro aldehyde and  $\alpha$ -fluoro alcohol can readily be converted to ethyl-2-fluoro-2-phenylacetate derivatives (Scheme 2.34).<sup>84-85</sup>



Scheme 2.34: Synthesis of  $\alpha$ -Fluoroester from **1**

### 2.3.6.6 Chromenone-crown ethers

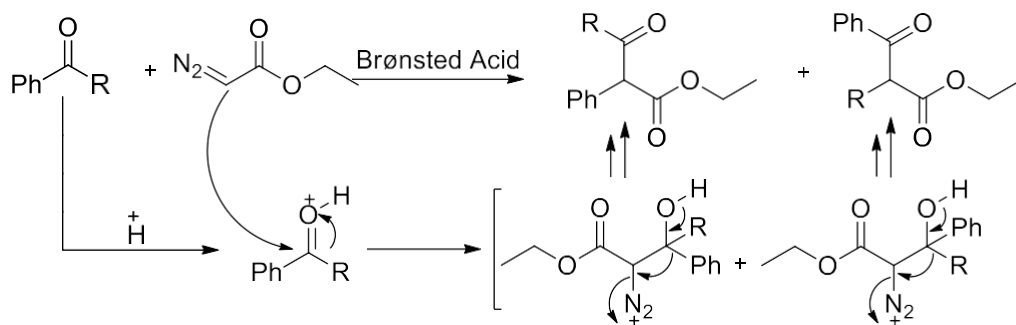
Macrocyclic ethers have been used to estimate cationic recognition and selective cation binding using different physical approaches such as filtration, absorption etc. Erk et al. reported the synthesis of various 3-phenyl chromenone-crown ether moieties which was used for the detection of cations such as  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Rb}^+$  as well as perchlorates with fluorescence spectroscopy. 3-phenyl chromenone-crown ethers were synthesized by the addition of crown ether (12-Crown-4, 15-Crown-4, and 18-Crown-6) to chromenones; which was obtained from the cyclization of triacetate or triol with compound **1** (Scheme 2.35).<sup>86-87</sup>



Scheme 2.35: Synthesis of Chromenone-Crown ethers from **1**

## 2.4 Present Synthesis of 3-oxo-esters and their related 3-hydroxy-2-aryl acrylate

In 2004, we studied the reaction of aromatic aldehydes with EDA in the presence of  $\text{HBF}_4 \cdot \text{OEt}_2$  catalyst, which yielded exclusively 3-hydroxy-2-aryl acrylate product by 1,2-aryl migration (Scheme 2.36). In that reaction, we used acetophenone as one of the substrates which was also yielded 3-hydroxy-2-aryl acrylate product by the reaction of EDA.



Scheme 2.36: Synthesis of 3-Hydroxyacrylates and related 3-oxo esters in presence of a Brønsted Acid catalyst by 1,2-Migration.

Based on our published work with acetophenone and EDA using  $\text{HBF}_4 \cdot \text{OEt}_2$  as a catalyst, we decided to expand the scope of this reaction using several substituted ketones specifically acetophenones.<sup>25</sup> The results are summarized in Table 2.1. The reaction of acetophenones and EDA provided ethyl 3-oxo-2-arylbutanoate which was in equilibrium with enol tautomer, ethyl 3-hydroxy-2-arylbut-2-enoate and the yield of the product is good (**3a-h**).

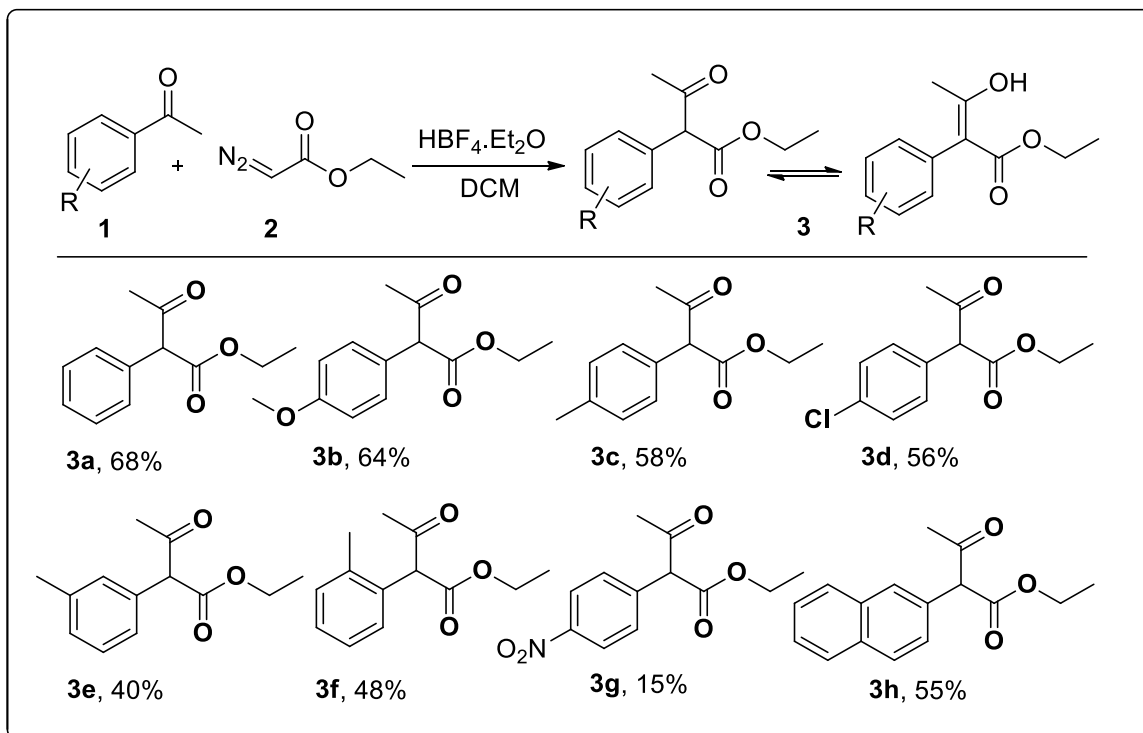


Table 2.1. Substituent Effect on Migratory Aptitude of Acetophenones

In the substituted acetophenones, the *para*-substituted acetophenones bearing a strong electron-donating group such as methoxy (1b), and a weak electron-donating group such as methyl (1c), a weak electron-withdrawing group such as chloro (1d), groups were provided almost similar yields of products. The yield of the product in case of methyl substituent on *meta*- and *ortho*- positions (1e and 1f) is lower compared to the methyl substituent on *para*-position (1c). Substrates bearing strong electron-withdrawing groups on the aromatic rings provided lower yield, for example, when the 4- nitro acetophenone (1g) was subjected to the standard reaction conditions, the desired product (3g) was isolated in only 15% yield, and most of the starting material was recovered. Interestingly, tautomeric 3-oxo-esters derived from 2-aceto naphthone showed good tolerances in the catalytic system, giving the desired product (3h) with a 55% yield. In acetophenone and EDA reaction, two different migrations

can be possible namely aryl and methyl. In our case we did not get any methyl-migrated products.

The above findings could be explained with respect to six probable rotamers K1-K6 (Figure 3.8) from the reaction of acetophenones and EDA in the presence of  $\text{HBF}_4 \cdot \text{OEt}_2$ . Rotamers K1–K3 are interconvertible due to the rotation of C–C bonds and their diastereomeric form of rotamers, K4–K6 are also interconvertible. In both rotamers K1 and K5, migrating aryl group and leaving diazonium group were anti to each other; thus, the formation of 3-oxo-ester was favorable. On the other hand, in rotamers K2 and K4, the methyl migrating group was anti to the leaving group, which could favor the formation of methyl-migrated product. However, the aryl migration from rotamer K1/K5 was more favorable over methyl from rotamer K2/K4 due to its ability to stabilize an intermediate phenonium ion (Figure 2.8, P) by six-electron resonance participation, whereas two electrons participating in nonclassical carbocation intermediate (Figure 2.8, N) are not stable enough to facilitate any methyl migration. The predominant phenyl over methyl migration is consistent with the pinacol rearrangement, which also involved carbocation intermediate.<sup>88</sup> In our reaction, we did not observe any formation of epoxide which are prerequisite rotamers K3 and K6.

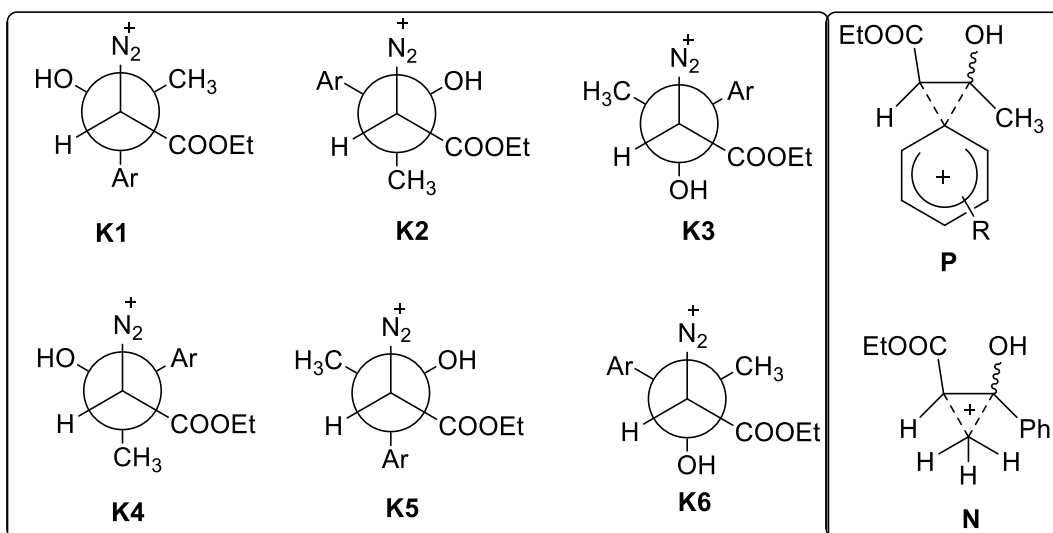


Figure 2.8: Newman Projections of Six Possible Rotamers (**K1-K6**) and a Phenonium Ion Intermediate (**P**) along with Nonclassical Carbocation Intermediate (**N**) for the reaction of acetophenone and EDA

The stable enol products can be existing in two different forms namely *E*-form and *Z*-form (Figure 2.9).

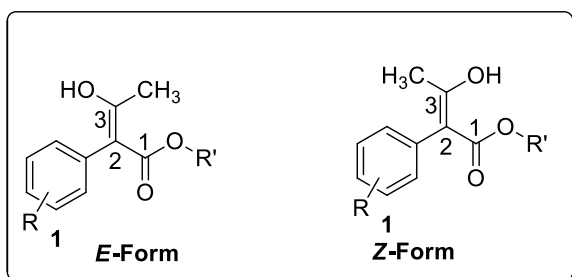


Figure 2.9: Two different form of 3-Hydroxy-2-aryl acrylates

In our case the enol products were found exclusively as *Z*-form, which was confirmed by two-dimensional (2D) NMR (Figure 2.10). The reason of the stability of the *Z*-enol form might be due to the presence of intramolecular hydrogen bonding between the hydroxy and carbonyl group as observed in ethyl 3-hydroxy-2-phenylacrylate.<sup>2</sup>

**Figure 2.10: 2D NMR of ethyl 3-oxo-2-phenylbutanoate which is in equilibrium with enol tautomer (*Z*)-ethyl 3-hydroxy-2-phenylbut-2-enoate (3a)**

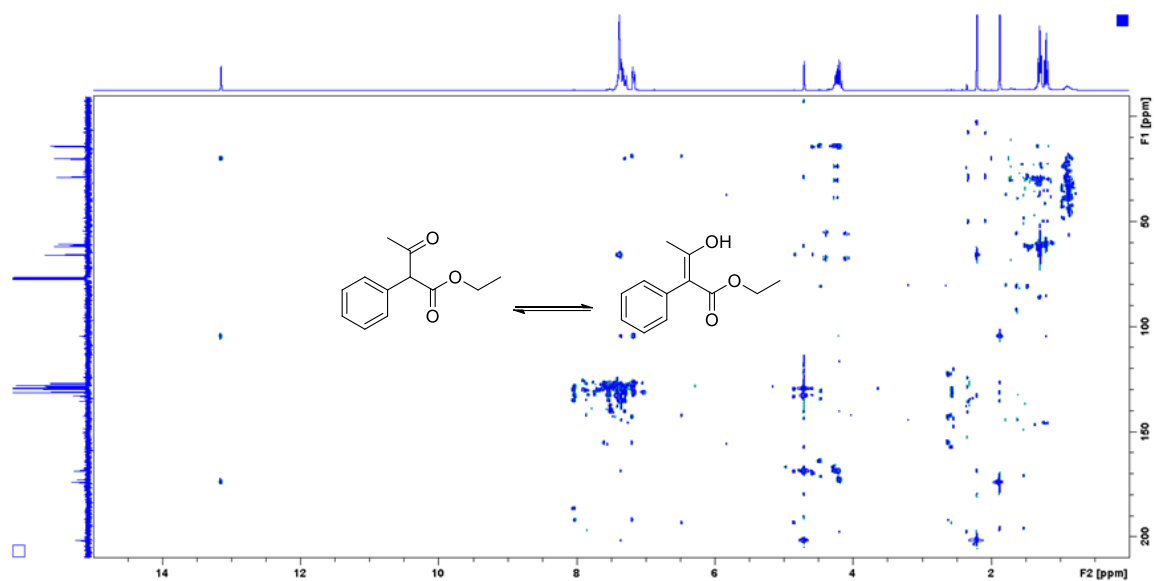
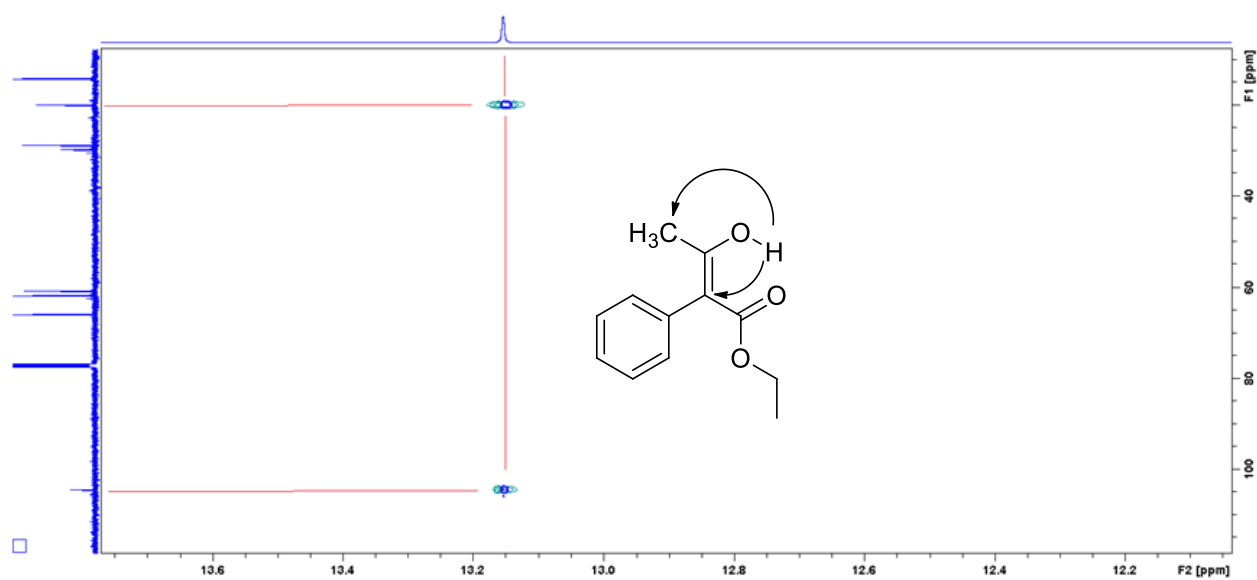
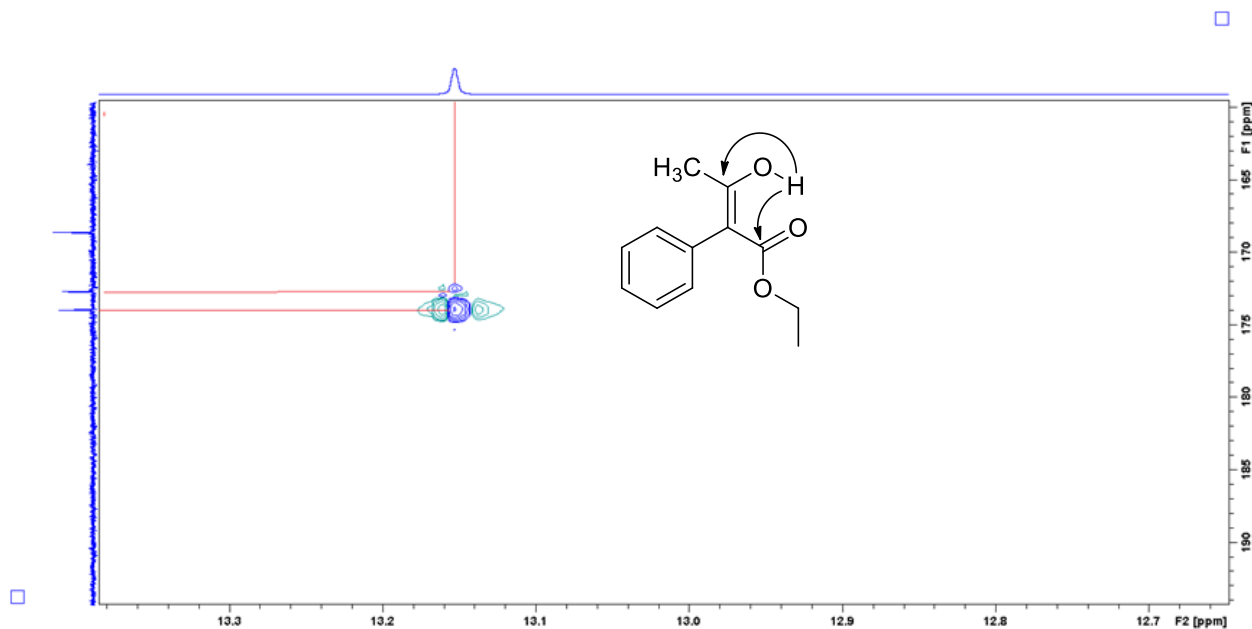


Fig. HMBC cross peak signal of the compound 3a

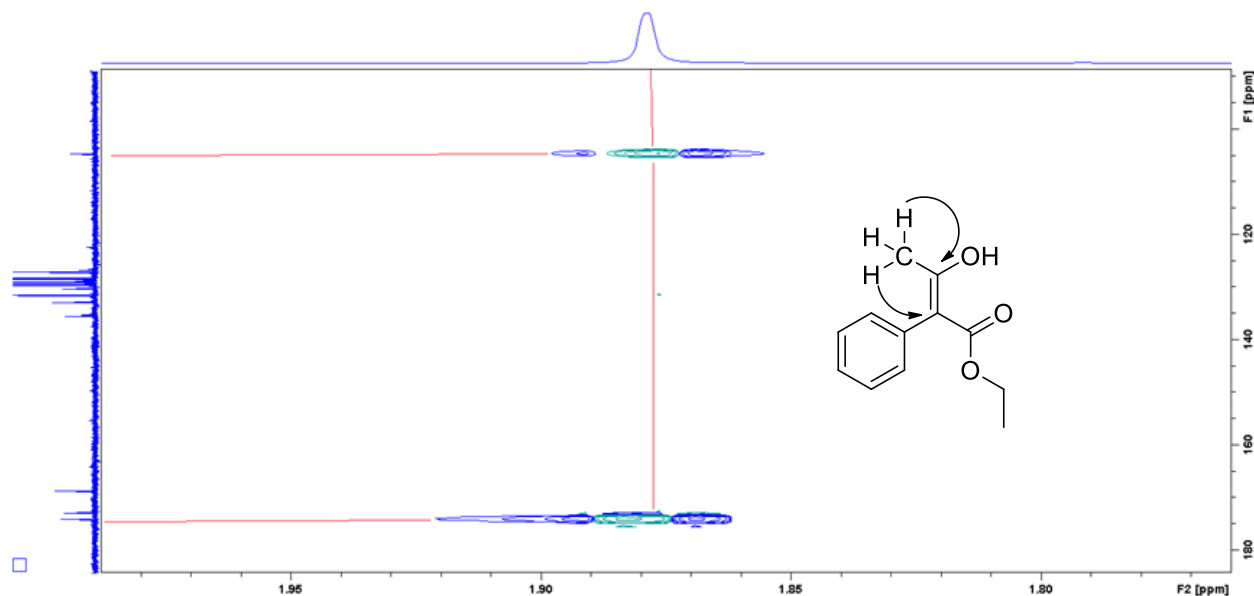




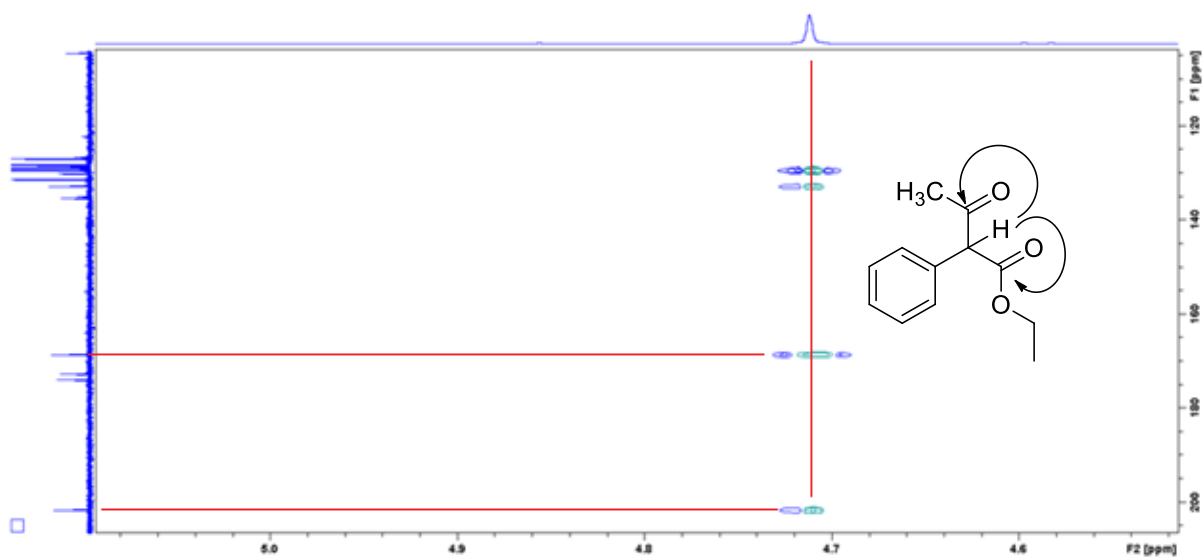
**Fig: HMBC cross peak signal between -OH at 13.15 ppm with one olefinic carbons at 104.4 ppm and the methyl group next to it.**



**Fig: HMBC cross peak signal between -OH at 13.15 ppm with one olefinic carbons at 173.9 ppm and ester carbonyl at 172.6 ppm.**



**Fig: HMBC cross peak signal between methyl singlet at 1.87 ppm with the two olefinic carbons at 104.4 ppm and 173.9 ppm respectively.**



**Fig: In the keto form, HMBC cross peak between the C-H proton at 4.71 ppm to the keto-C=O at 201.6 ppm, the ester C=O at 168.5 ppm, aromatic C-*ipso* at 132.7 ppm, and C-*ortho* at 129.3 ppm.**

## 2.5 Conclusion and Future Works

A variety of 3-hydroxy-2-aryl acrylate can be synthesized from aromatic aldehydes and ethyl diazoacetate in the presence of Lewis or Brønsted acid type catalyst by 1,2-aryl migration. Similarly, less reactive ketones can also be employed to synthesize 3-hydroxy-2-aryl acrylates and related 3-oxo-esters in the presence Brønsted acid catalyst  $\text{HBF}_4 \cdot \text{OEt}_2$ .<sup>89</sup> 3-hydroxy acrylates and related 3-oxo-esters are served as an essential precursor in the synthesis of natural products and bioactive compounds. This valuable scaffold has been widely synthesized and applied by organic chemists across a variety of fields for the last two decades. It contains multifunctional groups which can easily transform into other compounds such as indole, gramine, benzofuran, oxazole, imidazole, and quinoline type building blocks and in medicinal compounds such as BRL-79565, naproxen, and uracil.<sup>90</sup> 3-Hydroxy aryl acrylate is also excellent substrates for making all-carbon quaternary center-containing compounds. Our future goal is to employ this scaffold to synthesize variable compounds which are pharmaceutically and commercially important (Figure 2.11).

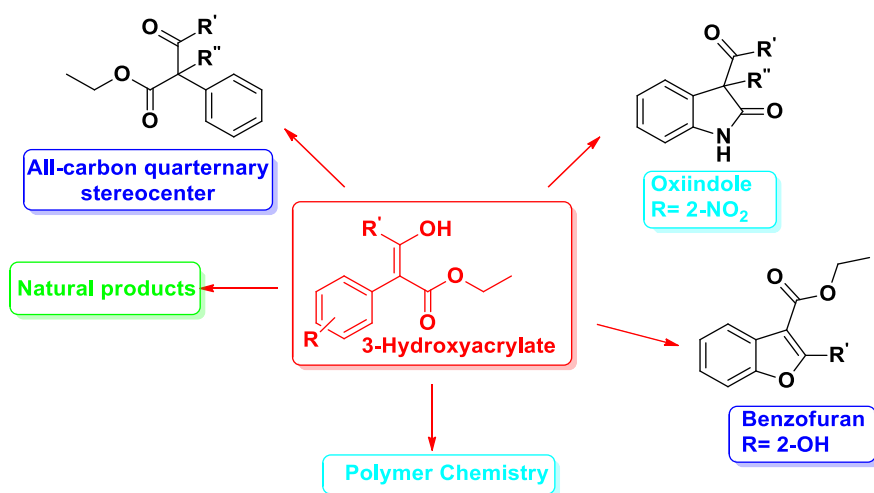


Figure 2.11: Future goal to Synthesis different valuable compounds from 3-hydroxy-2-aryl acrylates

## 2.6 General Consideration

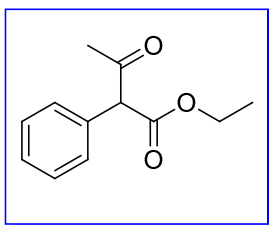
All reactions were performed under a dry nitrogen atmosphere using standard Schlenk techniques unless otherwise noted. All reaction vessels were flame dried under vacuum and filled with nitrogen prior to use. Reagents and solvents were purchased from Sigma-Aldrich, Milwaukee. All  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3$  (internal standard: 7.26 ppm,  $^1\text{H}$ ; 77.16 ppm,  $^{13}\text{C}\{^1\text{H}\}$ ) at room temperature with a Bruker 300 and 500 MHz spectrometers. The chemical shifts ( $\delta$ ) are given in parts per million (ppm) and the coupling constants in Hertz (Hz). Previously reported compounds were identified by  $^1\text{H}$  NMR. All new compounds were additionally characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and high-resolution mass spectrometry (HRMS). HRMS were obtained using Shimadzu liquid chromatography-ion trap-time of flight tandem mass spectrometry (LCMS-IT-TOF) by the electrospray ionization (ESI) technique. For the column chromatography, silica gel (35–70  $\mu\text{m}$ ) was used. The thin-layer chromatography (TLC) was performed on aluminum-backed plates precoated (0.25 mm) with Silica Gel 60 F254 with a suitable solvent system and was visualized using UV fluorescence and/or iodine chamber.

### 2.6.1 General Procedure and Experimental

For each experiment, carbonyl compounds (2.0-8.0 mmol, 1.0 equiv) was dissolved in 10-20 mL of freshly distilled dichloromethane under nitrogen at  $-78\text{ }^\circ\text{C}$ . A Brønsted acid,  $\text{HBF}_4\cdot\text{OEt}_2$  catalyst (0.4-1.6 mmol, 0.2 equiv) was added, and the reaction mixture was stirred for 1 hour at the same temperature. Ethyl diazoacetate (EDA) (4.0-16.0 mmol, 2.0 equiv) was diluted in 5 mL of freshly distilled dichloromethane and added to the solution over a period of 0.5-1 h. Then, the reaction mixture was allowed to stir for 72 h at room temperature. After completion of the reaction, it was quenched by adding THF. The reaction mixture was filtered through a

silica plug using dichloromethane as solvent and the solvent removed by rotary evaporation. Pure products were isolated by silica gel column chromatography with 0-10% ethyl acetate in hexane.

**Ethyl 3-oxo-2-phenylbutanoate (keto-enol = 1:1) as a colorless oil (3a)**

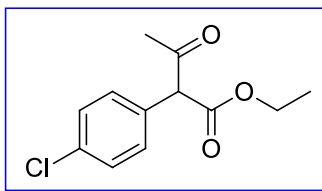


The compound was prepared according to the general procedure and purified by silica gel column chromatography (hexane/ethyl acetate = 100:1). The title product was isolated as a colorless oil (0.52 g, 68%) from the reaction of acetophenone (0.51 g, 4.25 mmol, 1.0 equiv) and EDA (1.03 mL, 8.50 mmol, 2.0 equiv) in the presence of HBF<sub>4</sub>.OEt<sub>2</sub> (0.12 mL, 0.85 mmol, 0.2 equiv). Compound **3a** was confirmed by comparing to known NMR.<sup>15,21</sup>

**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):** δ 13.15 (s, 1H), 7.40-7.29 (m, 8H), 7.19-7.16 (m, 2H), 4.71 (s, 1H), 4.26-4.16 (m, 4H), 2.21 (s, 3H), 1.87 (s, 3H), 1.30 (t, *J* = 7.5 Hz, 3H), 1.21 (t, *J* = 7.5 Hz, 3H).

**<sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 75 MHz):** δ 201.6, 173.9, 172.6, 168.5, 135.3, 132.7, 131.2, 129.3, 128.9, 128.3, 128.0, 126.9, 104.4, 65.8, 61.6, 60.6, 28.8, 19.9, 14.2, 14.1.

**Ethyl 2-(4-Chlorophenyl)-3-oxobutanoate (Keto-enol = 9:8) as a colorless oil (3b)**



The compound was prepared according to the general procedure and purified by silica gel column chromatography (hexane/ethyl acetate = 50:1). The title product was isolated as a colorless oil (0.46 g, 56%) from the reaction of 4'-chloroacetophenone (0.52 g, 3.38 mmol, 1.0 equiv) and EDA (0.82 mL, 6.78 mmol, 2.0 equiv) in

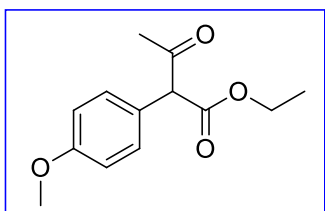
the presence of  $\text{HBF}_4 \cdot \text{OEt}_2$  (0.09 mL, 0.68 mmol, 0.2 equiv).

Compound **3b** was confirmed by comparing to known NMR.<sup>21</sup>

**$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):**  $\delta$  13.14 (s, 1H), 7.38 (d,  $J$  = 10.0 Hz, 2H), 7.32 (t,  $J$  = 10.0 Hz, 4H), 7.11 (d,  $J$  = 10.0 Hz, 2H), 4.69 (s, 1H), 4.26–4.17 (m, 4H), 2.22 (s, 3H), 1.87 (s, 3H), 1.30 (t,  $J$  = 7.5 Hz, 3H), and 1.21 (t,  $J$  = 7.5 Hz, 3H).  **$^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 125 MHz):**  $\delta$  200.9, 174.1, 172.3, 168.2, 134.5, 133.7, 132.9, 132.6, 131.1, 130.7, 129.1, 128.3, 103.3, 64.9, 61.7, 60.8, 28.9, 19.9, 14.2, and 14.1.

**Ethyl 2-(4-Methoxyphenyl)-3-oxobutanoate (Keto-enol = 4:3) as a colorless oil (3c).**

The compound was prepared according to the general procedure and purified by silica gel column chromatography (hexane/ethyl acetate = 50:1). The title product was isolated as a colorless oil (0.52 g, 64%) from the reaction of 4'-methoxyacetophenone (0.51 g, 3.41 mmol, 1.0 equiv) and EDA (0.82 mL, 6.82 mmol, 2.0 equiv) in the presence of  $\text{HBF}_4 \cdot \text{OEt}_2$  (0.09 mL, 0.68 mmol, 0.2 equiv).

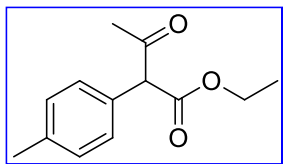


Compound **3c** was confirmed by comparing to known NMR.<sup>21</sup>

**$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):**  $\delta$  13.11 (s, 1H), 7.28 (d,  $J$  = 6.0 Hz, 2H), 7.08 (t,  $J$  = 4.5 Hz, 2H), 7.28 (t,  $J$  = 9.0 Hz, 4H), 4.65 (s, 1H), 4.20 (q,  $J$  = 7.5 Hz, 4H), 3.82 (s, 3H), 3.81 (s, 3H), 2.18 (s, 3H), 1.86 (s, 3H), 1.28 (t,  $J$  = 7.5 Hz, 3H), and 1.19 (t,  $J$  = 7.5 Hz, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 75 MHz):**  $\delta$  201.8, 174.0, 172.8, 168.8, 159.6, 158.5, 132.2, 130.4, 127.9, 127.5, 124.8, 114.3, 113.5, 103.8, 64.9, 61.5, 60.6, 55.2, 55.1, 28.6, 19.8, 14.2, and 14.1.

### Ethyl 3-Oxo-2-(p-tolyl)butanoate (Keto-enol = 2:1) as a colorless oil (3d)

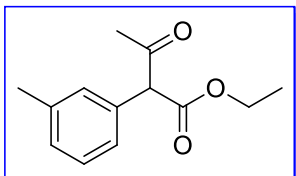


The compound was prepared according to the general procedure and purified by silica gel column chromatography (hexane/ethyl acetate = 100:1). The title product was isolated as a colorless oil (0.30 g, 58%) from the reaction of 4'-methylacetophenone (0.53 g, 3.91 mmol, 1.0 equiv) and EDA (0.95 mL, 7.83 mmol, 2.0 equiv) in the presence of  $\text{HBF}_4 \cdot \text{OEt}_2$  (0.11 mL, 0.78 mmol, 0.2 equiv).

Compound **3d** was confirmed by comparing to known NMR.<sup>22</sup>

**$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):**  $\delta$  13.14 (s, 1H), 7.27–7.15 (m, 8H), 7.06 (d,  $J$  = 6.0 Hz, 2H), 4.67 (s, 1H), 4.28–4.16 (m, 4H), 2.38 (s, 3H), 2.37 (s, 3H), 2.20 (s, 3H), 1.87 (s, 3H), 1.30 (t,  $J$  = 7.5 Hz, 3H), and 1.22 (t,  $J$  = 6.0 Hz, 3H).  **$^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 125 MHz):**  $\delta$  201.8, 173.8, 172.8, 168.7, 138.1, 136.5, 132.2, 131.1, 129.7, 129.6, 129.1, 128.8, 104.1, 65.4, 61.6, 60.6, 28.7, 21.2, 21.1, 20.0, 14.2, and 14.1.

### Ethyl 3-Oxo-2-(m-tolyl) butanoate (Keto-enol = 3:4) as a colorless oil (3e)

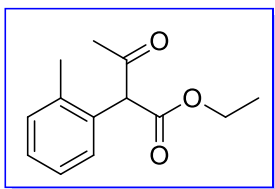


The compound was prepared according to the general procedure and purified by silica gel column chromatography (hexane/ethyl acetate = 100:1). The title product was isolated as a colorless oil (0.21 g, 40%) from the reaction of 3'-methylacetophenone (0.52 g, 3.85 mmol, 1.0 equiv) and EDA (0.93 mL, 7.69 mmol, 2.0 equiv) in the presence of  $\text{HBF}_4 \cdot \text{OEt}_2$  (0.11 mL, 0.79 mmol, 0.2 equiv).

Compound **3e** was confirmed by comparing to known NMR.<sup>23</sup>

**$^1\text{H}$  NMR (CDCl<sub>3</sub>, 300 MHz):**  $\delta$  13.10 (s, 0.8H), 7.72 (d,  $J$  = 9.0 Hz, 2H), 7.39 (d,  $J$  = 6.0 Hz, 2H), 7.25 (d,  $J$  = 6.0 Hz, 4H), 7.09 (s, 1H), 4.94 (s, 0.2H), 4.29–4.23 (m, 2H), 4.16–4.10 (m, 1H), 2.61 (s, 3H), 2.57 (s, 3H), 2.20 (s, 3H), 1.78 (s, 3H), 1.30 (s, 3H), and 1.22 (t,  $J$  = 7.5 Hz, 3H).  **$^{13}\text{C}\{^1\text{H}\}$  NMR (CDCl<sub>3</sub>, 125 MHz):**  $\delta$  201.7, 173.8, 172.7, 168.6, 138.6, 137.5, 135.1, 132.6, 131.9, 129.9, 129.0, 128.8, 128.3, 127.9, 127.7, 126.3, 104.4, 65.7, 61.5, 60.6, 28.7, 21.4, 19.9, 14.2, and 14.1.

**Ethyl 3-Oxo-2-(o-tolyl) butanoate (Keto-enol = 3:4) as a colorless oil (3f)**

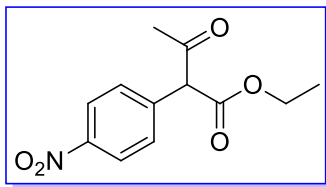


The compound was prepared according to the general procedure and purified by silica gel column chromatography (hexane/ethyl acetate = 100:1). The title product was isolated as a colorless oil (0.26 g, 48%) from the reaction of 2'-methylacetophenone (0.53 g, 4.00 mmol, 1.0 equiv) and EDA (0.95 mL, 7.90 mmol, 2.0 equiv) in the presence of HBF<sub>4</sub>·OEt<sub>2</sub> (0.11 mL, 0.79 mmol, 0.2 equiv).

Compound **3f** was confirmed by comparing to known NMR.<sup>22</sup>

**$^1\text{H}$  NMR (CDCl<sub>3</sub>, 300 MHz):**  $\delta$  13.10 (s, 0.8H), 7.72 (d,  $J$  = 9.0 Hz, 2H), 7.39 (d,  $J$  = 6.0 Hz, 2H), 7.25 (d,  $J$  = 6.0 Hz, 4H), 7.09 (s, 1H), 4.94 (s, 0.2H), 4.29–4.23 (m, 2H), 4.16–4.10 (m, 1H), 2.61 (s, 3H), 2.57 (s, 3H), 2.20 (s, 3H), 1.78 (s, 3H), 1.30 (s, 3H), and 1.22 (t,  $J$  = 7.5 Hz, 3H).

**Ethyl 2-(4-Nitrophenyl)-3-oxobutanoate (Keto-enol = 4:5) as a colorless oil (3g)**



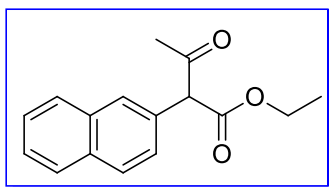
The compound was prepared according to the general procedure and purified by silica gel column chromatography (hexane/ethyl acetate = 25:1). The title product was isolated as a colorless oil (0.078 g, 15%) from the reaction of 4'-nitroacetophenone (0.52



g, 3.12 mmol, 1.0 equiv) and EDA (0.75 mL, 6.24 mmol, 2.0 equiv) in the presence of  $\text{HBF}_4 \cdot \text{OEt}_2$  (0.085 mL, 0.62 mmol, 0.2 equiv).

**$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):**  $\delta$  13.25 (s, 1H), 8.36–8.20 (m, 3H), 7.83 (t,  $J = 4.5$  Hz, 1H), 7.61–7.48 (m, 3H), 7.37 (d,  $J = 9.0$  Hz, 1H), 4.85 (s, 1H), 4.30–4.18 (m, 4H), 2.29 (s, 3H), 1.91 (s, 3H), 1.31 (t,  $J = 7.5$  Hz, 3H), and 1.21 (t,  $J = 7.5$  Hz, 3H).

**Ethyl 2-(Naphthalen-2-yl)-3-oxobutanoate (Keto-enol = 5:3) as a colorless (3h)**



The compound was prepared according to the general procedure and purified by silica gel column chromatography (hexane/ethyl acetate = 50:1). The title product was isolated as a colorless oil (0.29 g, 55%) from the reaction of 2-acetonaphthone (0.52 g, 3.04 mmol, 1.0 equiv) and EDA (0.73 mL, 6.07 mmol, 2.0 equiv) in the presence of  $\text{HB}_4 \cdot \text{OEt}_2$  (0.084 mL, 0.61 mmol, 0.2 equiv).

**$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):**  $\delta$  13.22 (s, 1H), 7.90–7.83 (m, 7H), 7.65 (s, 1H), 7.55–7.49 (m, 5H), 7.31 (d,  $J = 10.0$  Hz, 1H), 4.89 (s, 1H), 4.31–4.19 (m, 4H), 2.25 (s, 3H), 1.92 (s, 3H), 1.32 (t,  $J = 7.5$  Hz, 3H), and 1.19 (t,  $J = 7.5$  Hz, 3H).

**$^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 75 MHz):**  $\delta$  201.7, 174.2, 172.7, 168.6, 133.4, 133.3, 133.0, 132.8, 132.4, 130.2, 130.0, 129.6, 128.7, 128.7, 128.0, 127.9, 127.7, 127.5, 126.7, 126.5, 126.4, 126.0, 125.9, 104.3, 65.9, 61.7, 60.7, 28.9, 20.0, 14.2, and 14.1.

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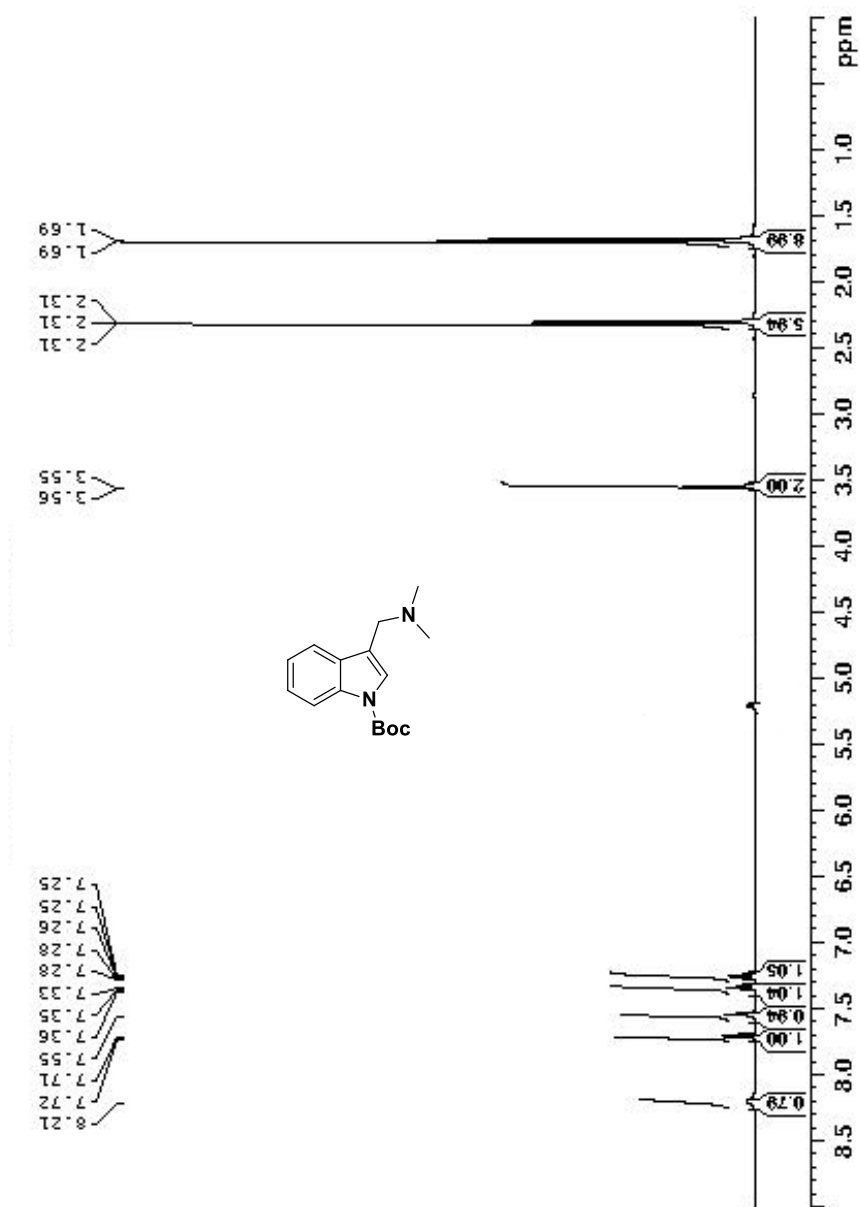
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## **APPENDIX A**

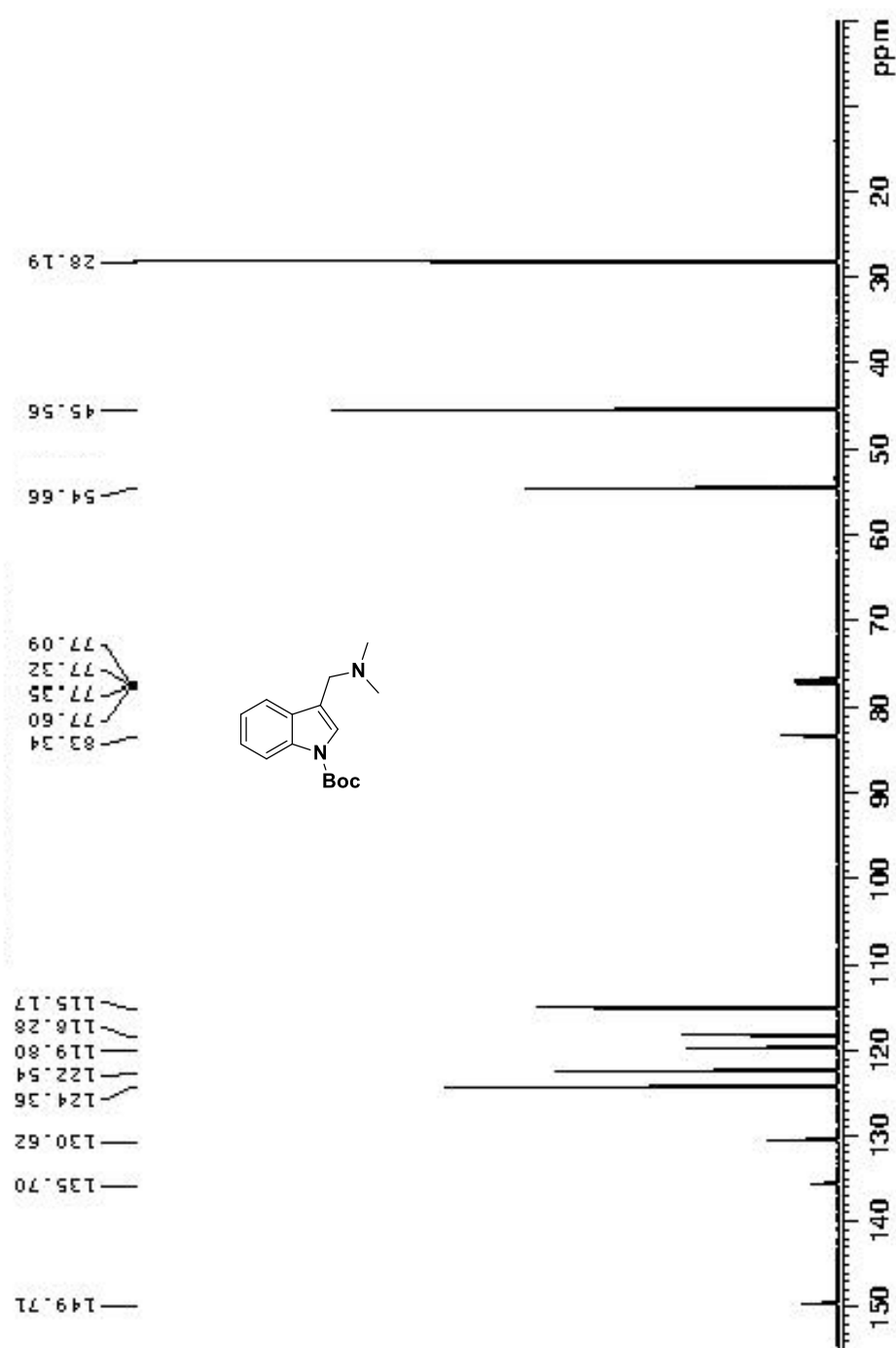
### **PART I: A CONCISE SYNTHESIS OF MICROTUBULE INHIBITOR TRYPROSTATIN A AND B AND ITS ANALOGS**

**Copies of  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and HRMS Spectral Data**

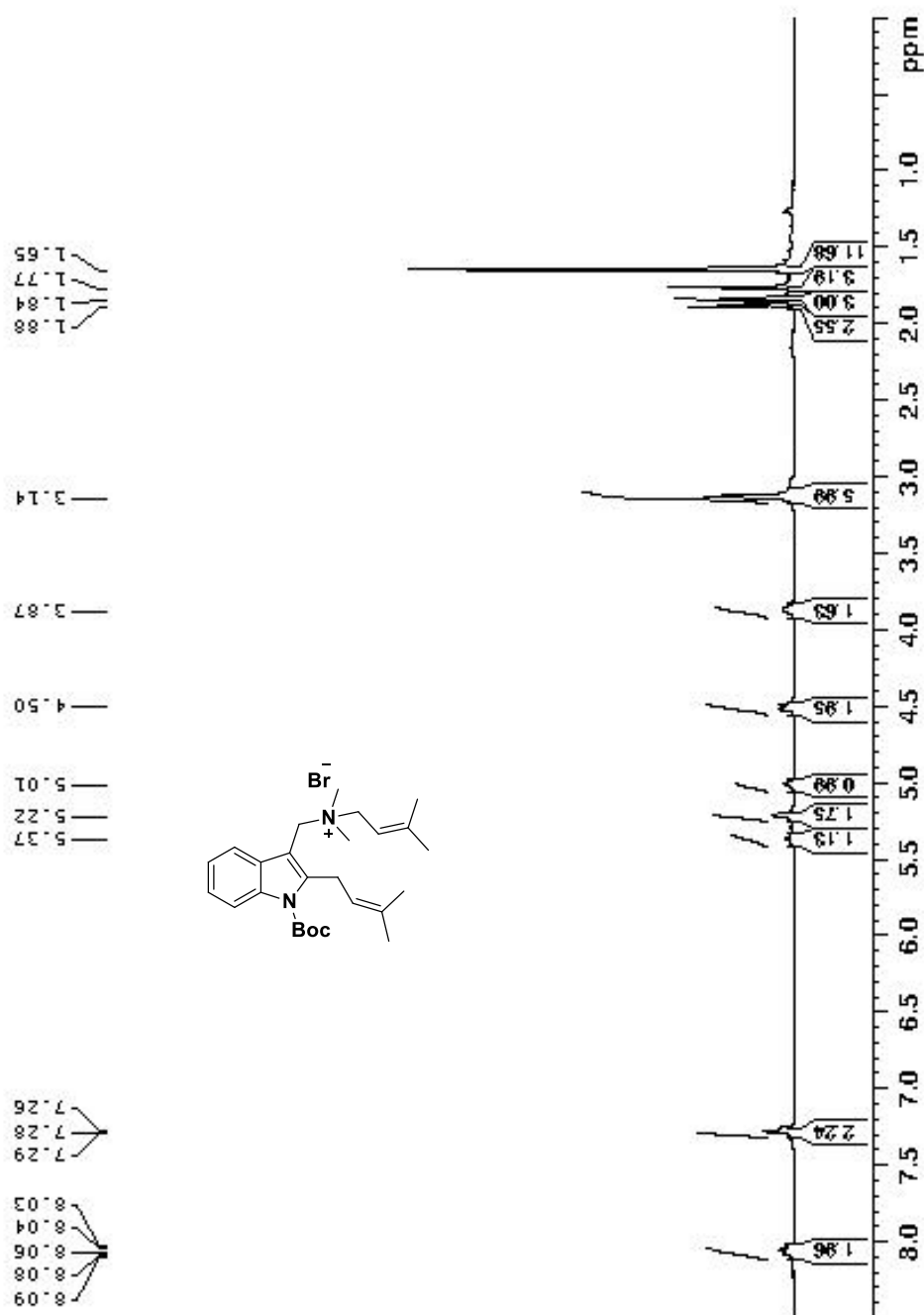
**<sup>1</sup>H NMR Spectrum of Compound 2 in CDCl<sub>3</sub>**



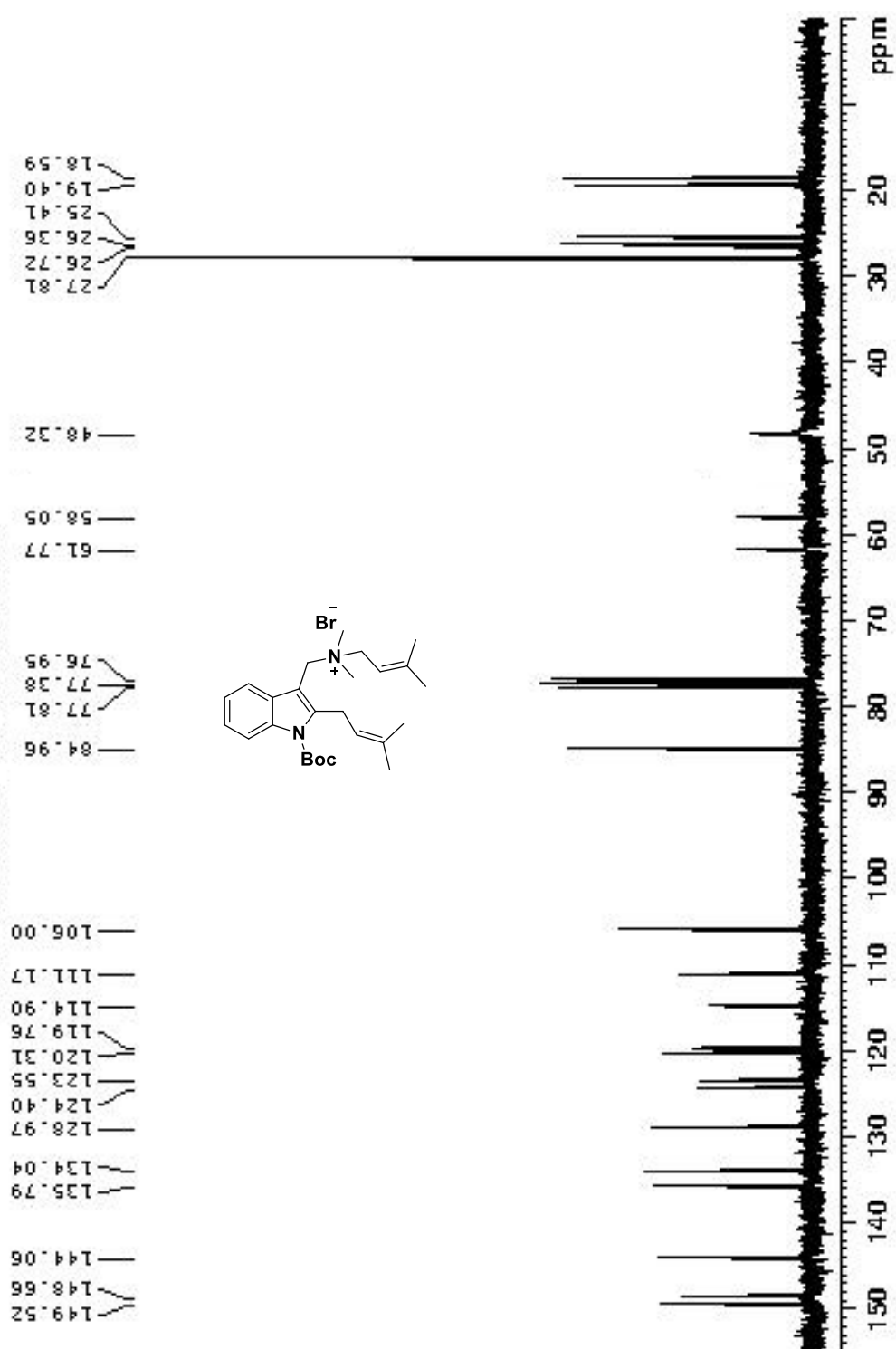
**$^{13}\text{C}$  NMR Spectrum of Compound 2 in  $\text{CDCl}_3$**



**<sup>1</sup>H NMR Spectrum of Compound 3 in CDCl<sub>3</sub>**



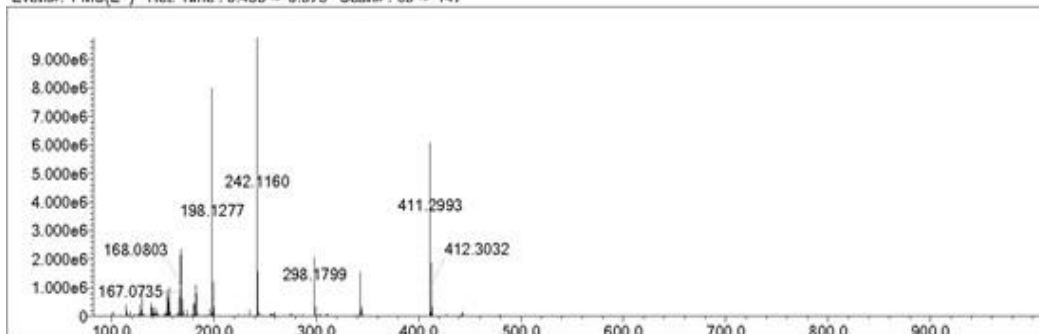
<sup>13</sup>C NMR Spectrum of Compound 3 in CDCl<sub>3</sub>



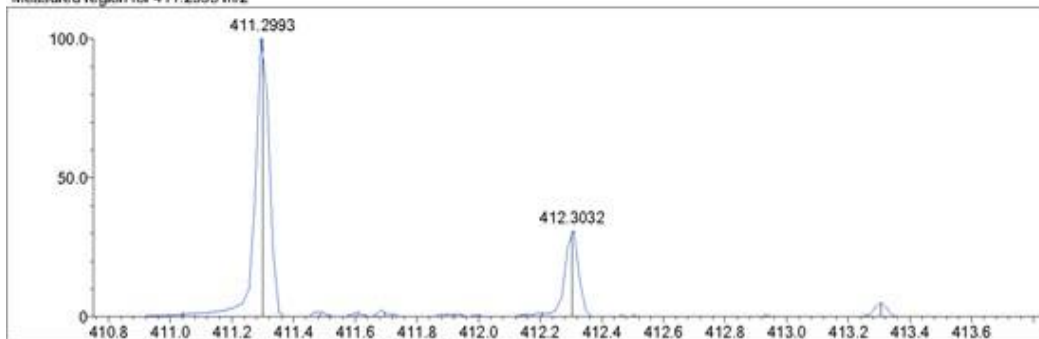


# HRMS of Compound 3

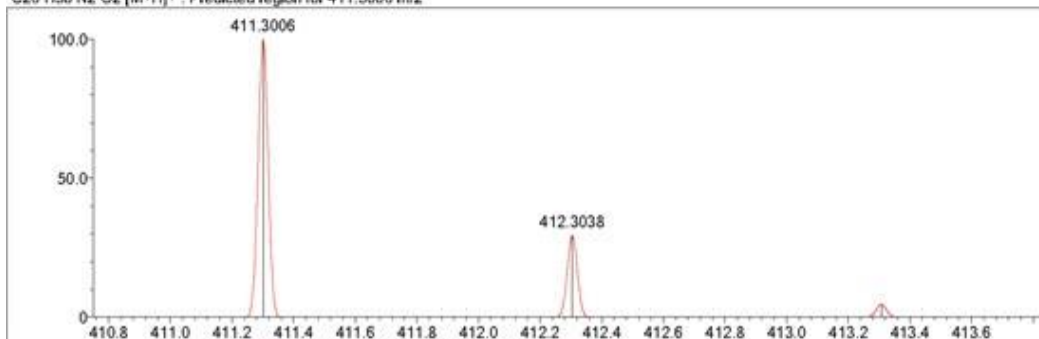
Event#: 1 MS(E+) Ret. Time : 0.453 -> 0.973 Scan#: 69 -> 147



Measured region for 411.2993 m/z

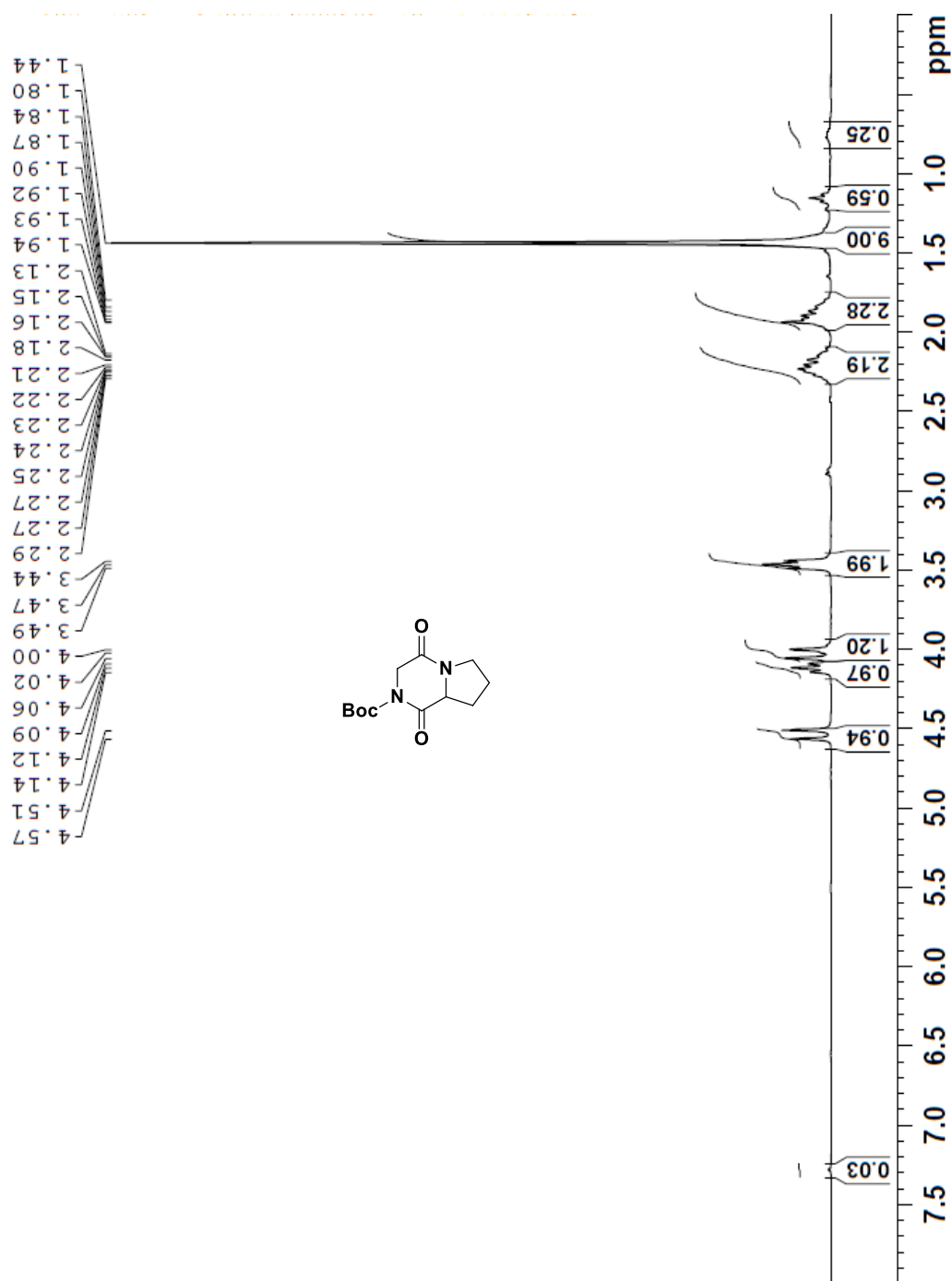


C26 H38 N2 O2 [M+H]<sup>+</sup> : Predicted region for 411.3006 m/z

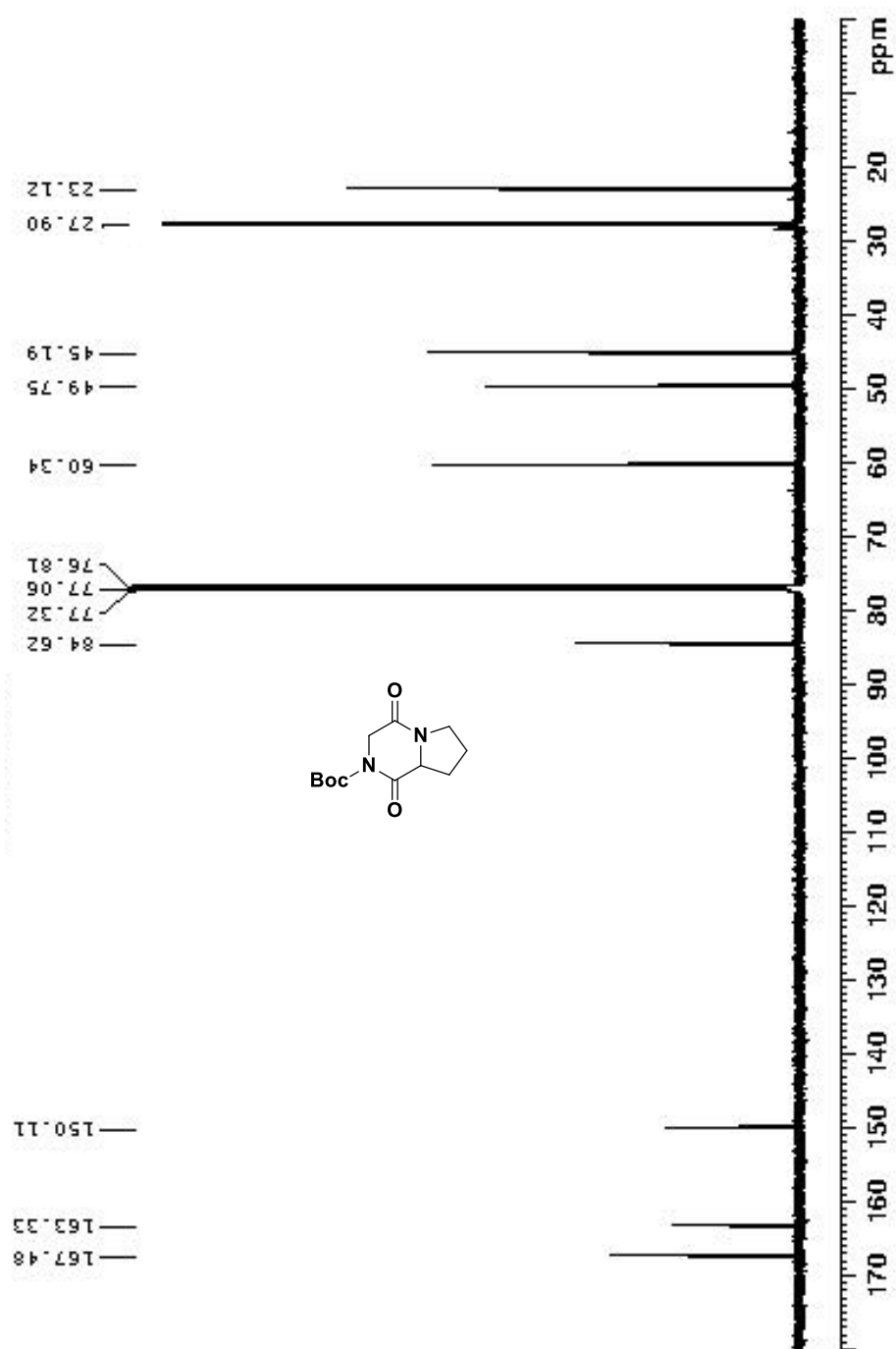


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	82.28	C26 H38 N2 O2	[M+H] <sup>+</sup>	411.2993	411.3006	-1.3	-3.16	86.98	9.0

<sup>1</sup>H NMR Spectrum of Compound 4 in CDCl<sub>3</sub>

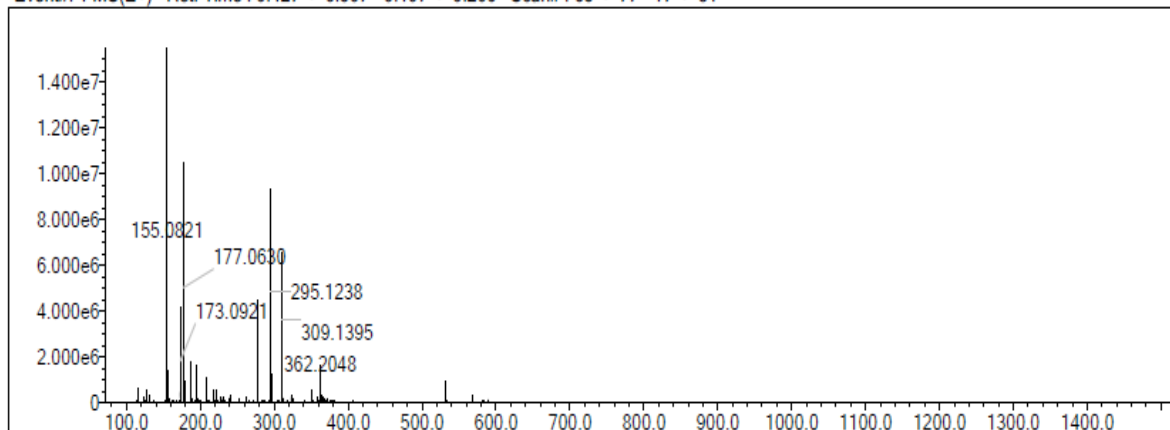


<sup>13</sup>C NMR Spectrum of Compound 4 in CDCl<sub>3</sub>

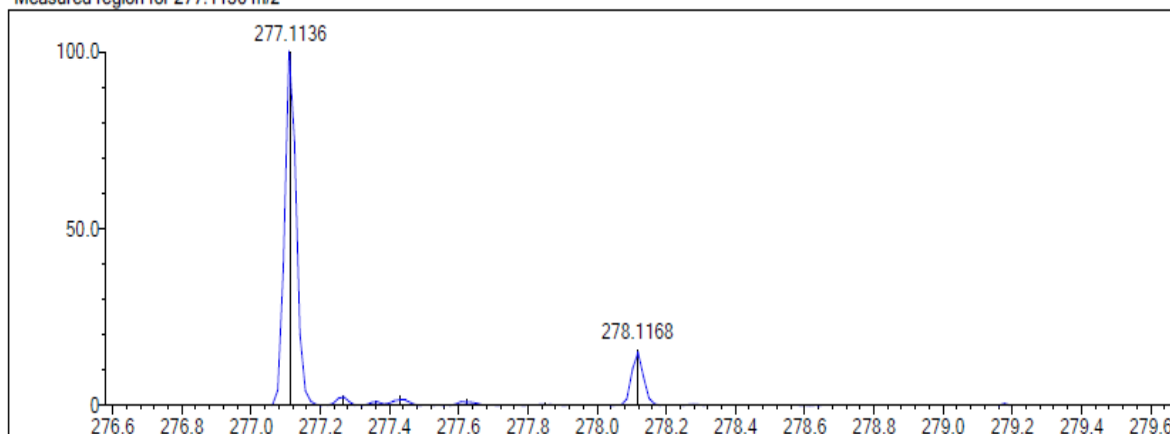


## HRMS of Compound 4

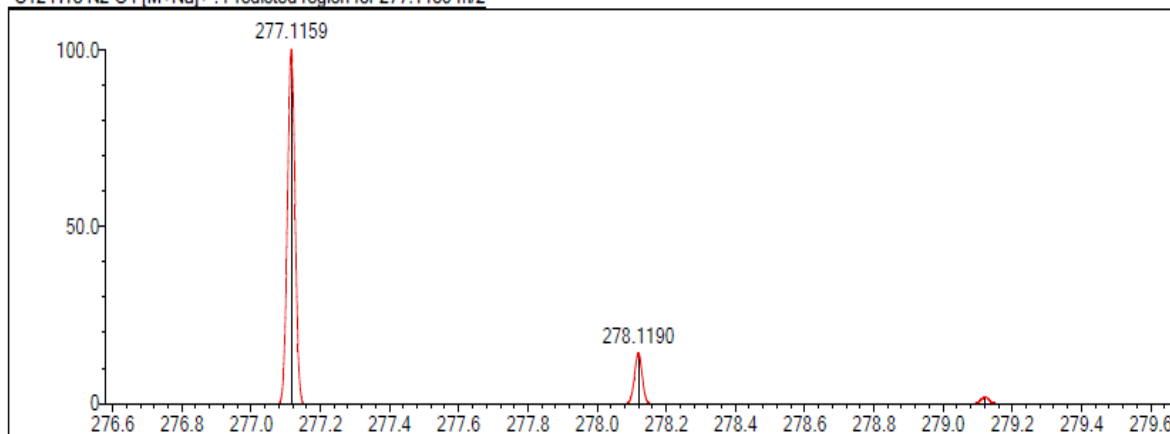
Event#: 1 MS(E+) Ret. Time : 0.427 -> 0.507 - 0.107 -> 0.206 Scan#: 65 -> 77 - 17 -> 31



Measured region for 277.1136 m/z

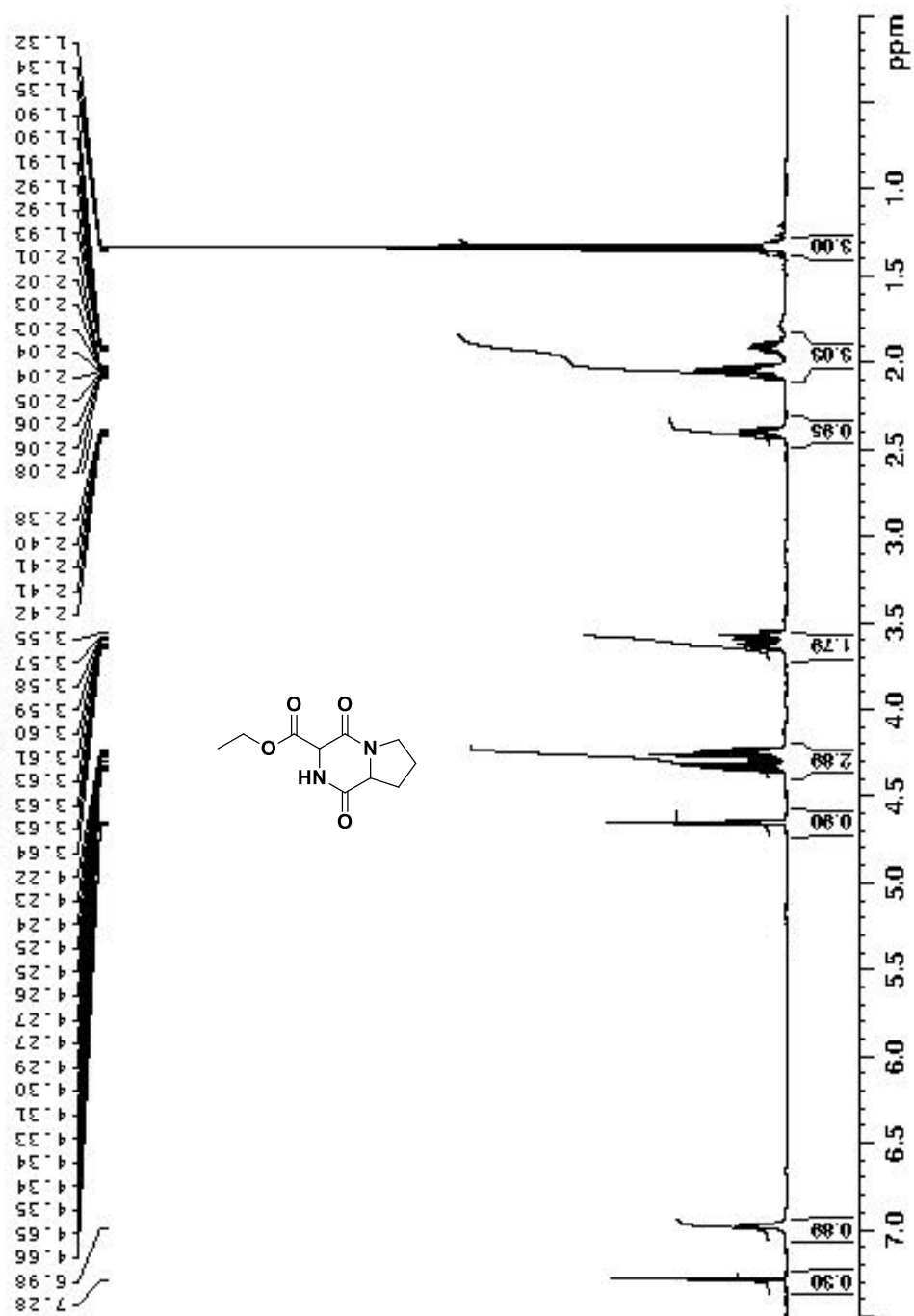


C12 H18 N2 O4 [M+Na]<sup>+</sup> : Predicted region for 277.1159 m/z

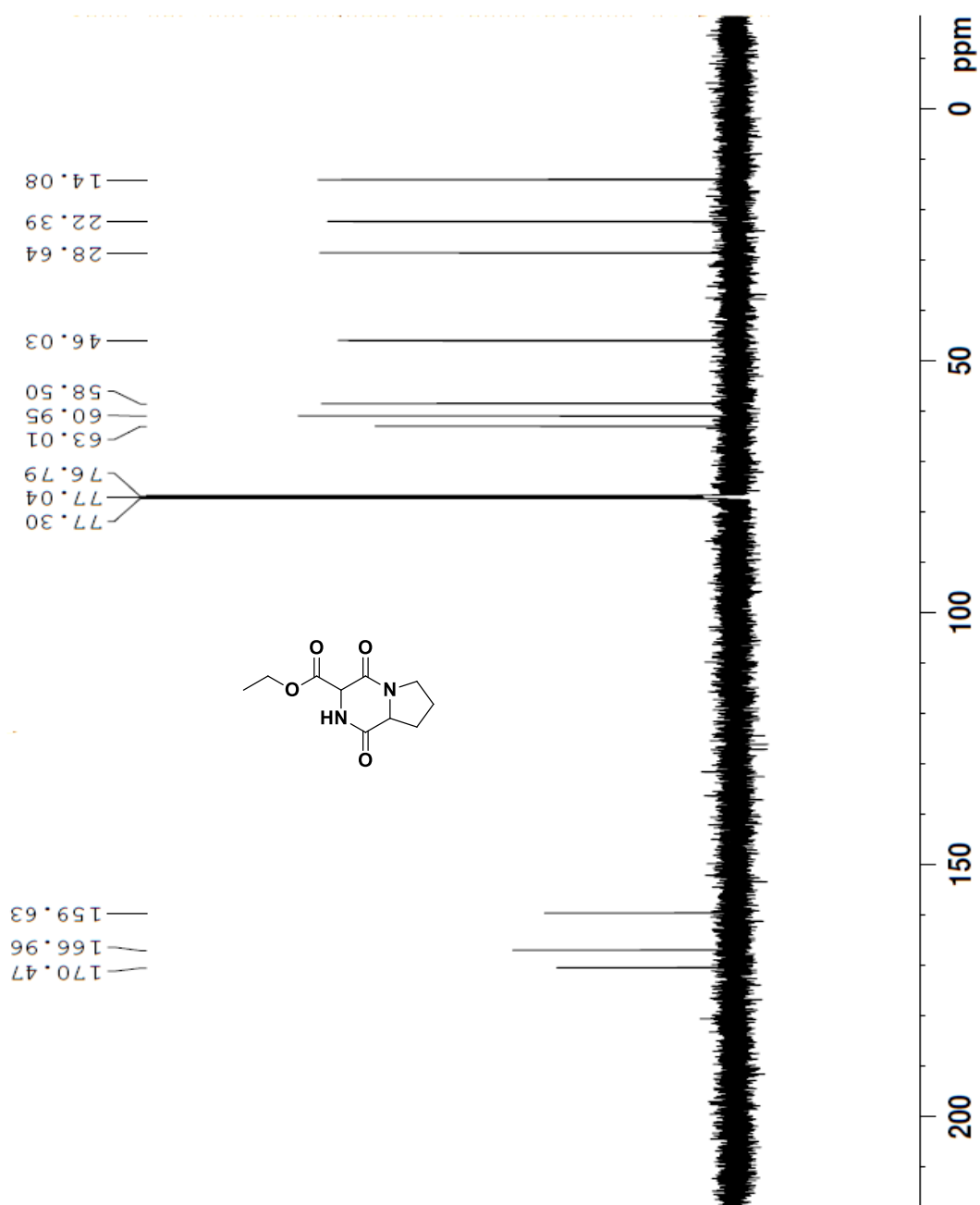


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	33.92	C12 H18 N2 O4	[M+Na] <sup>+</sup>	277.1136	277.1159	-2.3	-8.30	59.50	5.0

<sup>1</sup>H NMR Spectrum of Compound 5 in CDCl<sub>3</sub>

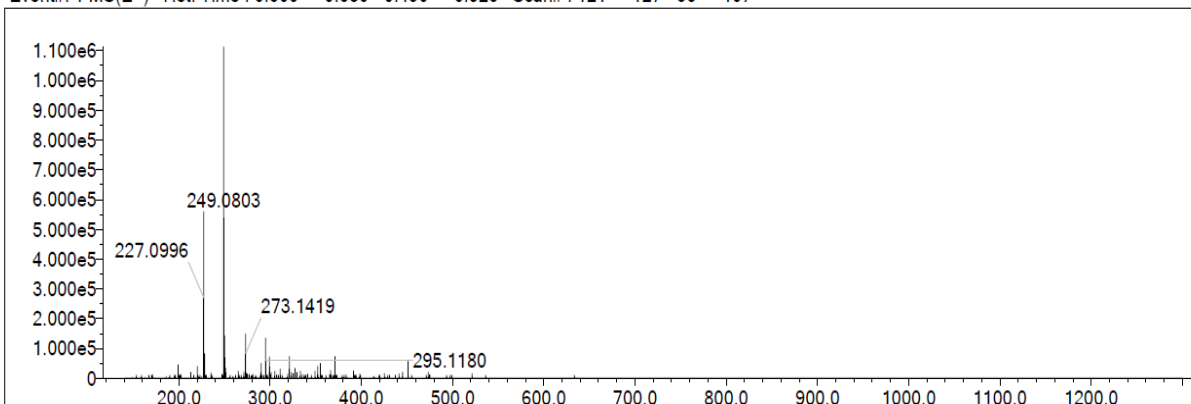


<sup>13</sup>C NMR Spectrum of Compound 5 in CDCl<sub>3</sub>

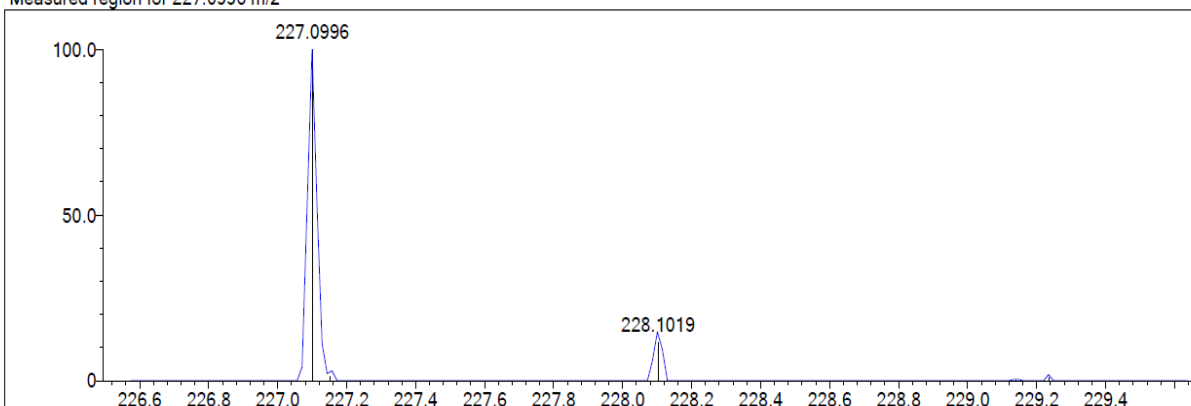


## HRMS of Compound 5

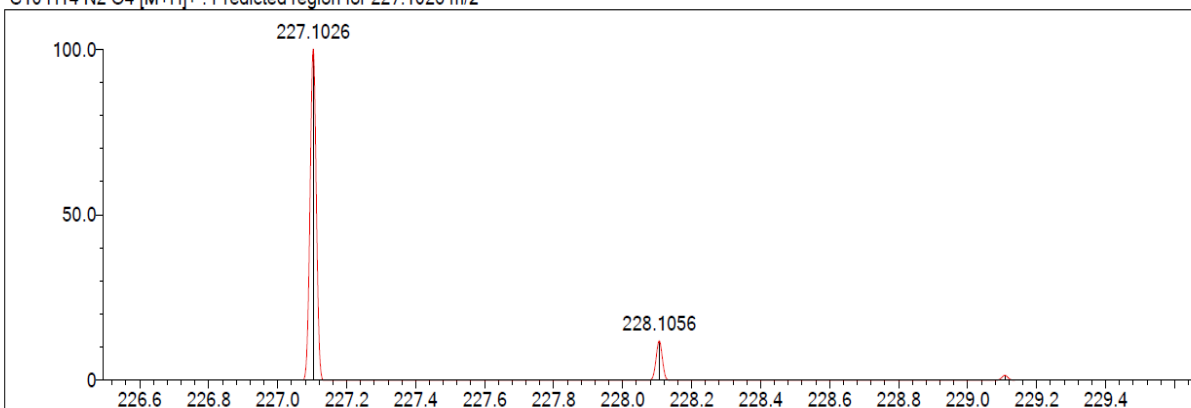
Event#: 1 MS(E+) Ret. Time : 0.600 -> 0.630 - 0.490 -> 0.526 Scan#: 121 -> 127 - 99 -> 107



Measured region for 227.0996 m/z

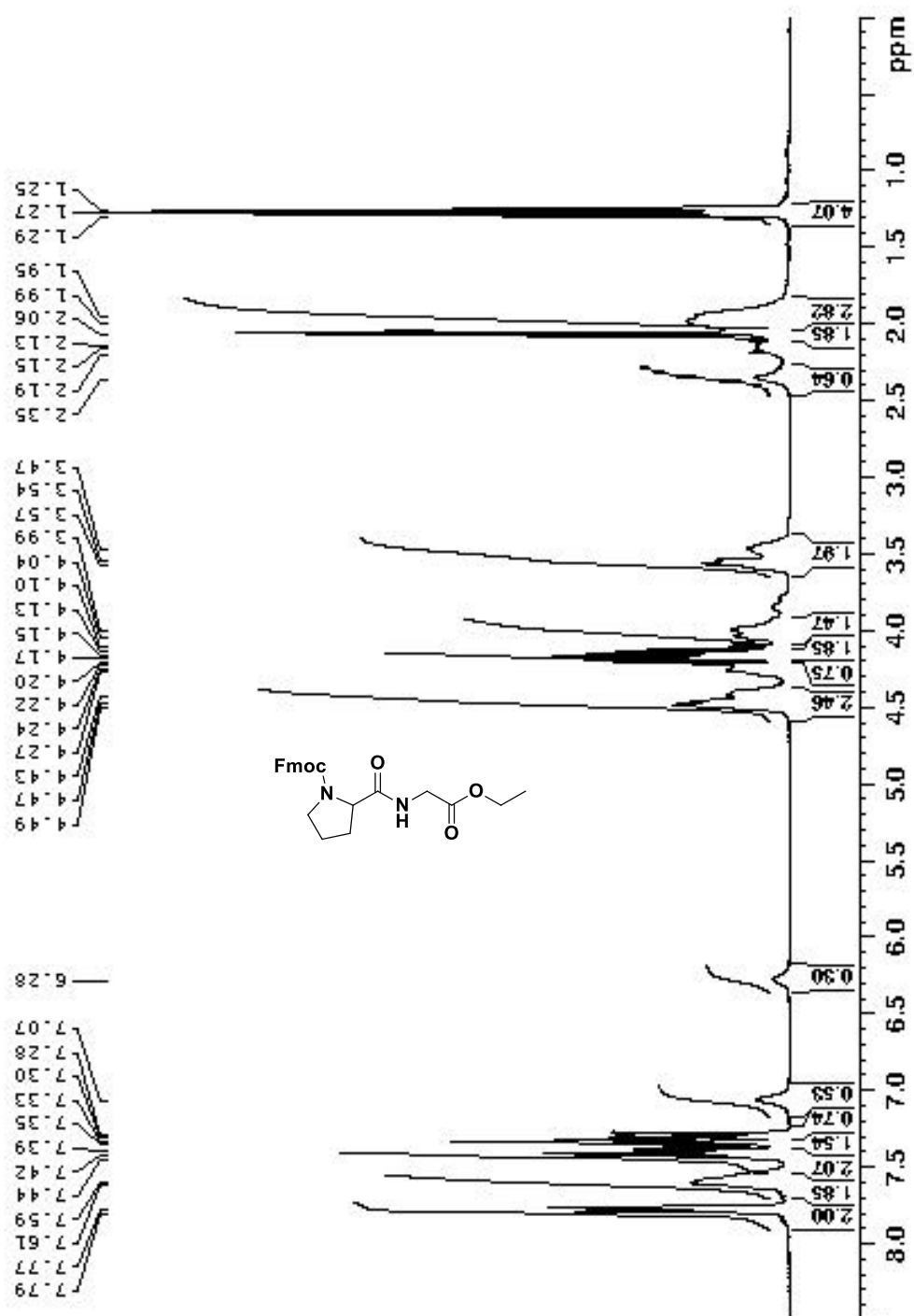


C10 H14 N2 O4 [M+H]<sup>+</sup> : Predicted region for 227.1026 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	15.32	C10 H14 N2 O4	[M+H] <sup>+</sup>	227.0996	227.1026	-3.0	-13.21	48.72	5.0

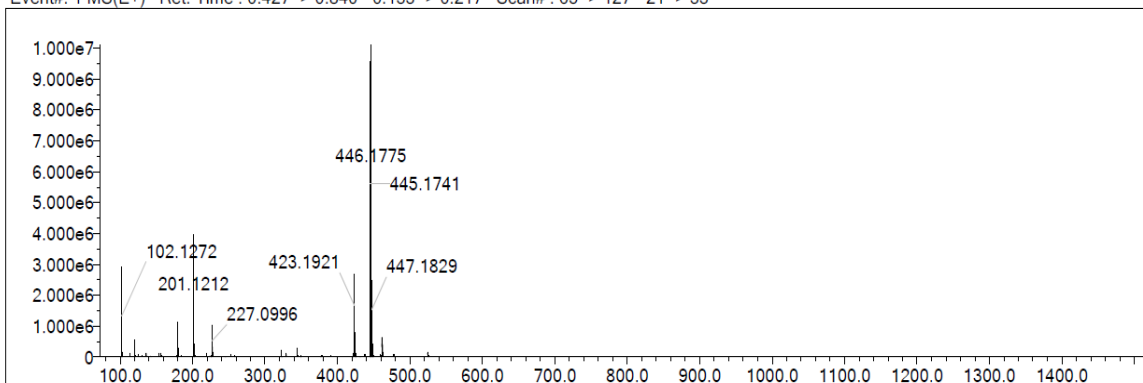
<sup>1</sup>H NMR Spectrum of Compound 8 in CDCl<sub>3</sub>



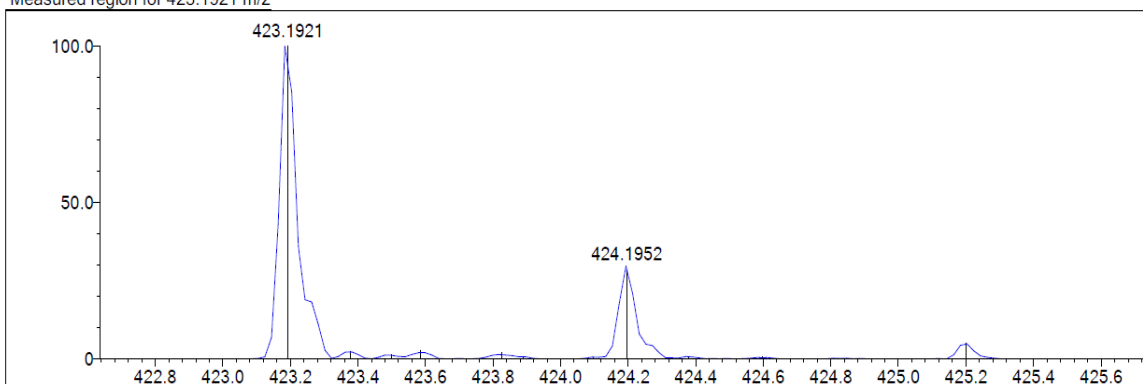


## HRMS of Compound 8

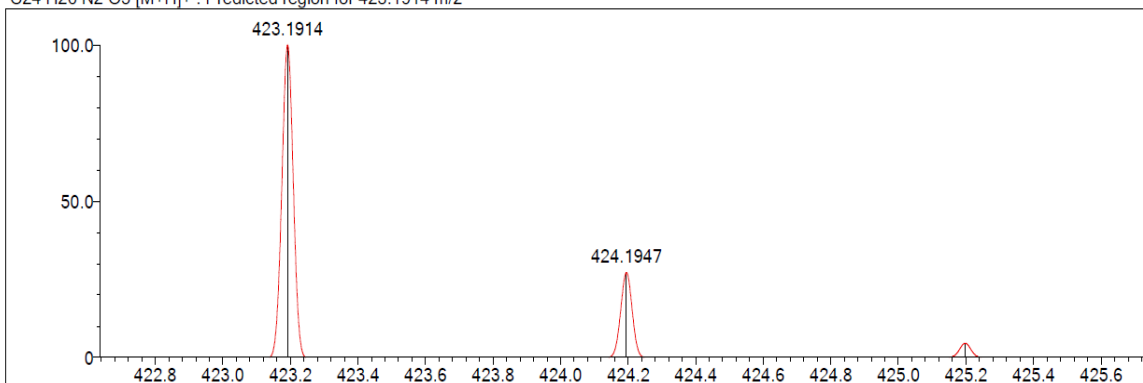
Event#: 1 MS(E+) Ret. Time : 0.427 -> 0.840 - 0.133 -> 0.217 Scan#: 65 -> 127 - 21 -> 33



Measured region for 423.1921 m/z

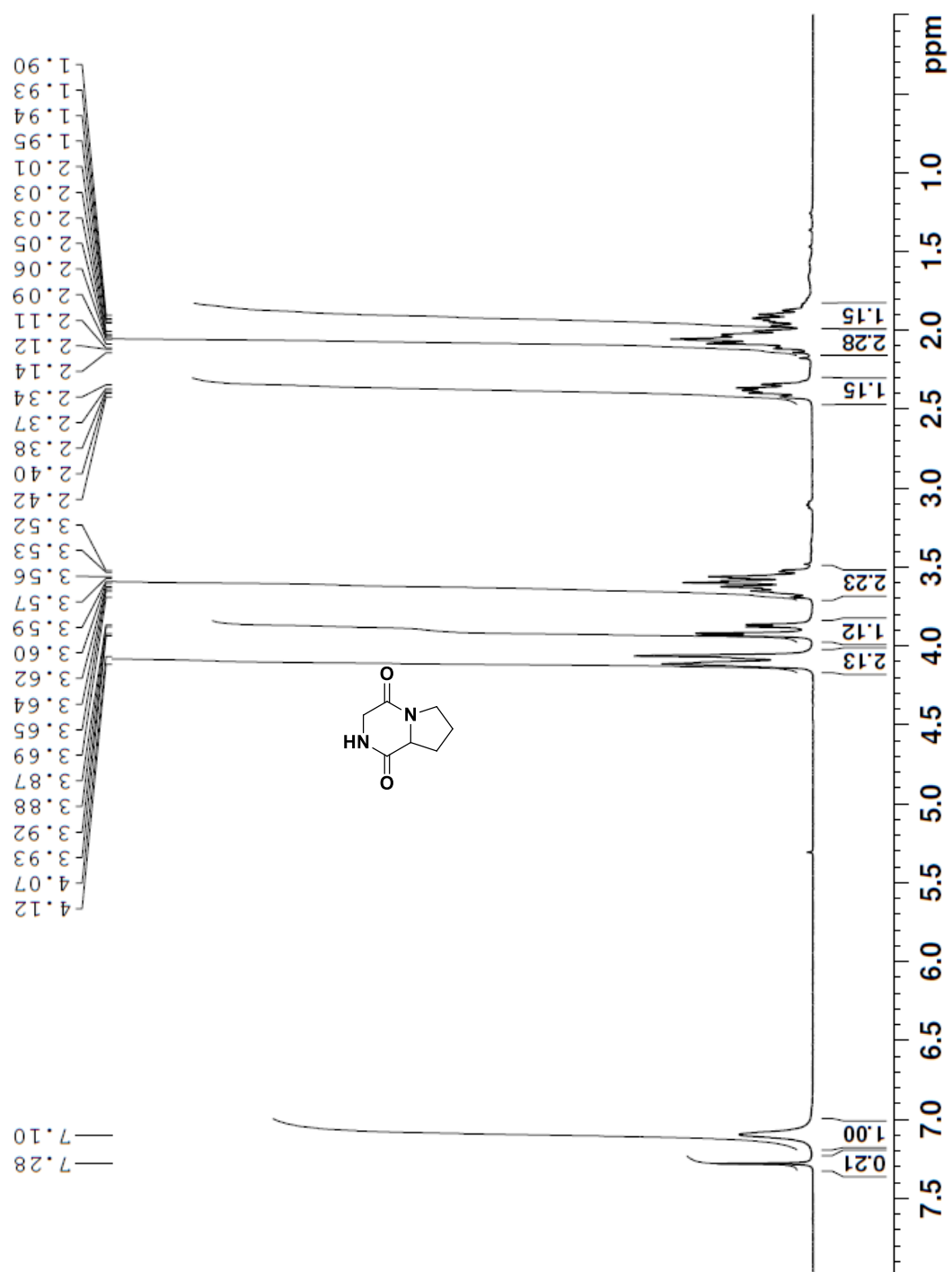


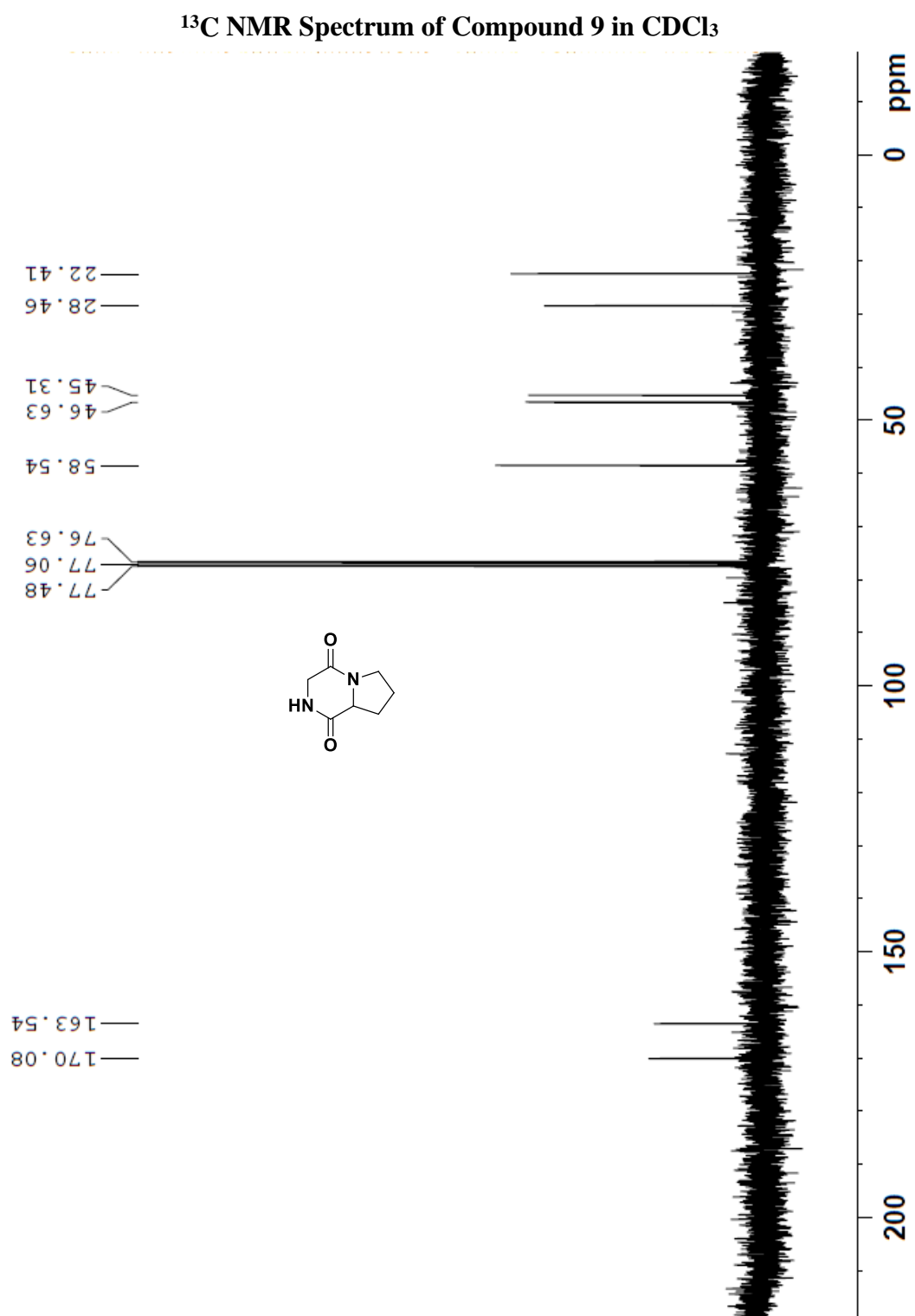
C24 H26 N2 O5 [M+H]<sup>+</sup> : Predicted region for 423.1914 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	69.29	C24 H26 N2 O5	[M+H] <sup>+</sup>	423.1921	423.1914	0.7	1.65	70.44	13.0

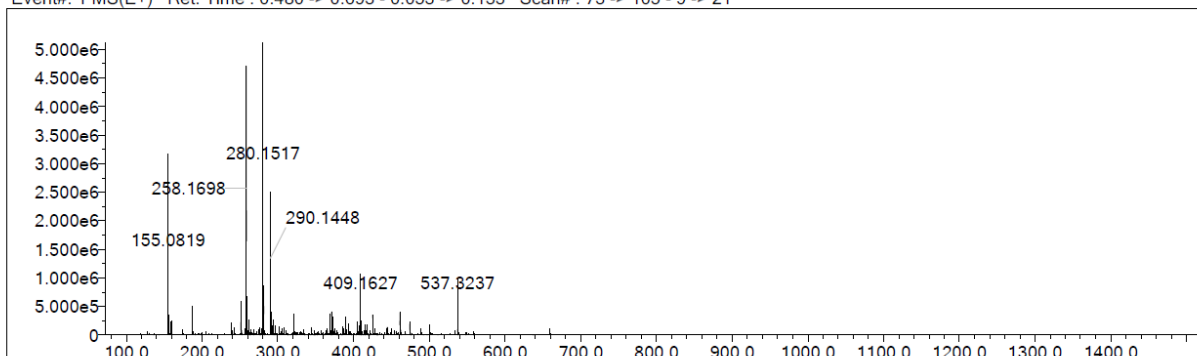
<sup>1</sup>H NMR Spectrum of Compound 9 in CDCl<sub>3</sub>



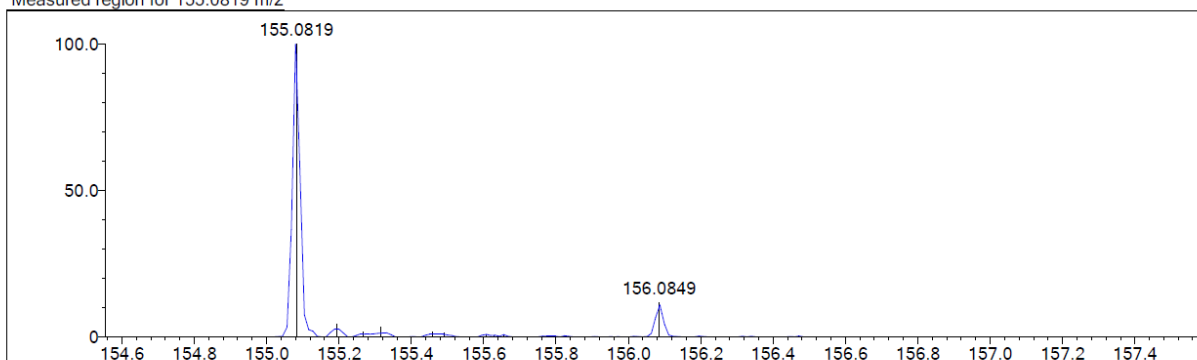


## HRMS of Compound 9

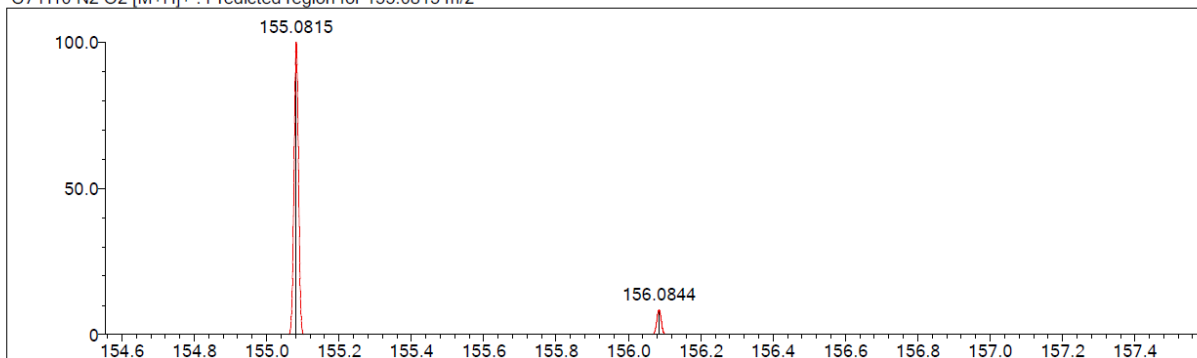
Event#: 1 MS(E+) Ret. Time : 0.480 -> 0.693 - 0.053 -> 0.133 Scan#: 73 -> 105 - 9 -> 21



Measured region for 155.0819 m/z

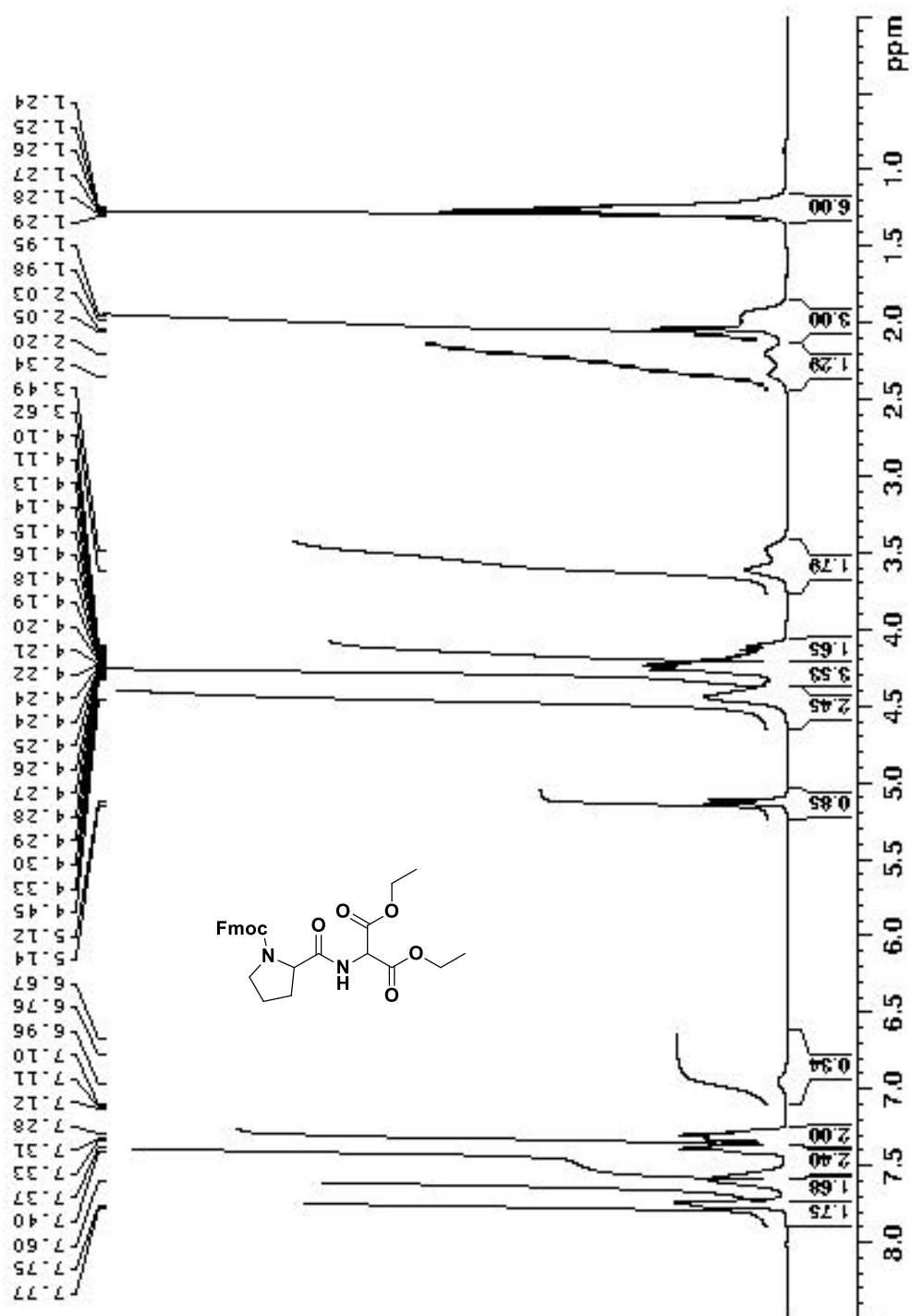


C7 H10 N2 O2 [M+H]<sup>+</sup> : Predicted region for 155.0815 m/z

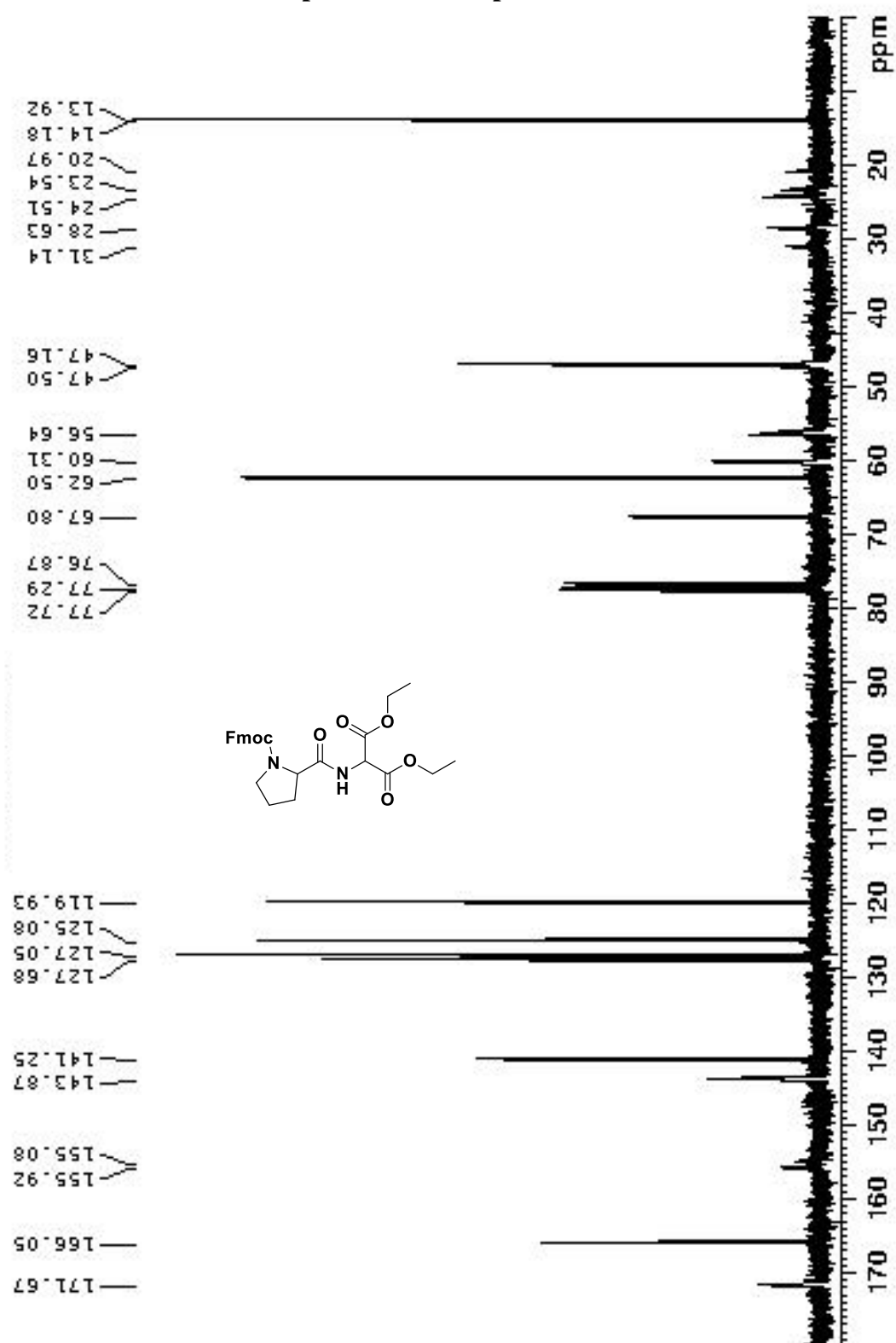


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	71.74	C7 H10 N2 O2	[M+H] <sup>+</sup>	155.0819	155.0815	0.4	2.58	74.69	4.0

<sup>1</sup>H NMR Spectrum of Compound 13 in CDCl<sub>3</sub>

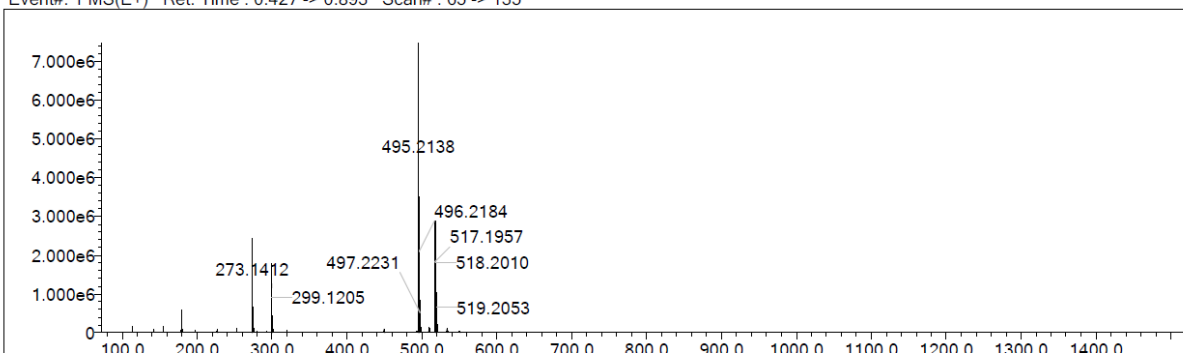


<sup>13</sup>C NMR Spectrum of Compound 13 in CDCl<sub>3</sub>

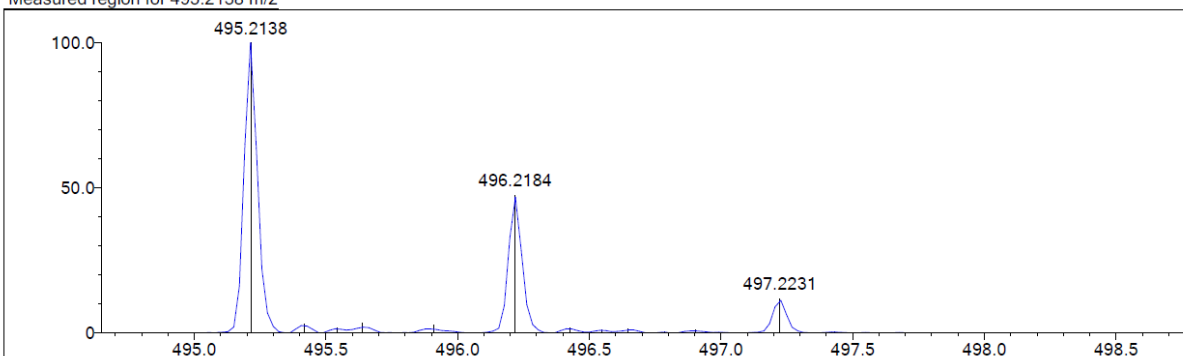


# HRMS of Compound 13

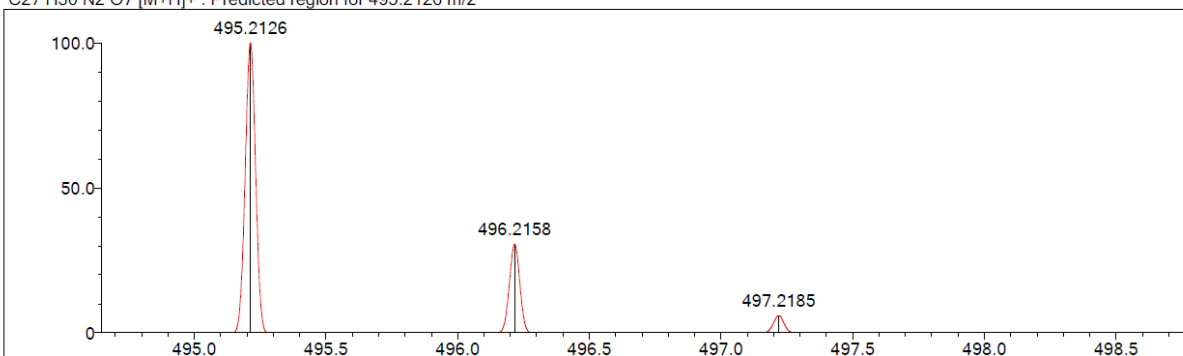
Event#: 1 MS(E+) Ret. Time : 0.427 -> 0.893 Scan# : 65 -> 135



Measured region for 495.2138 m/z

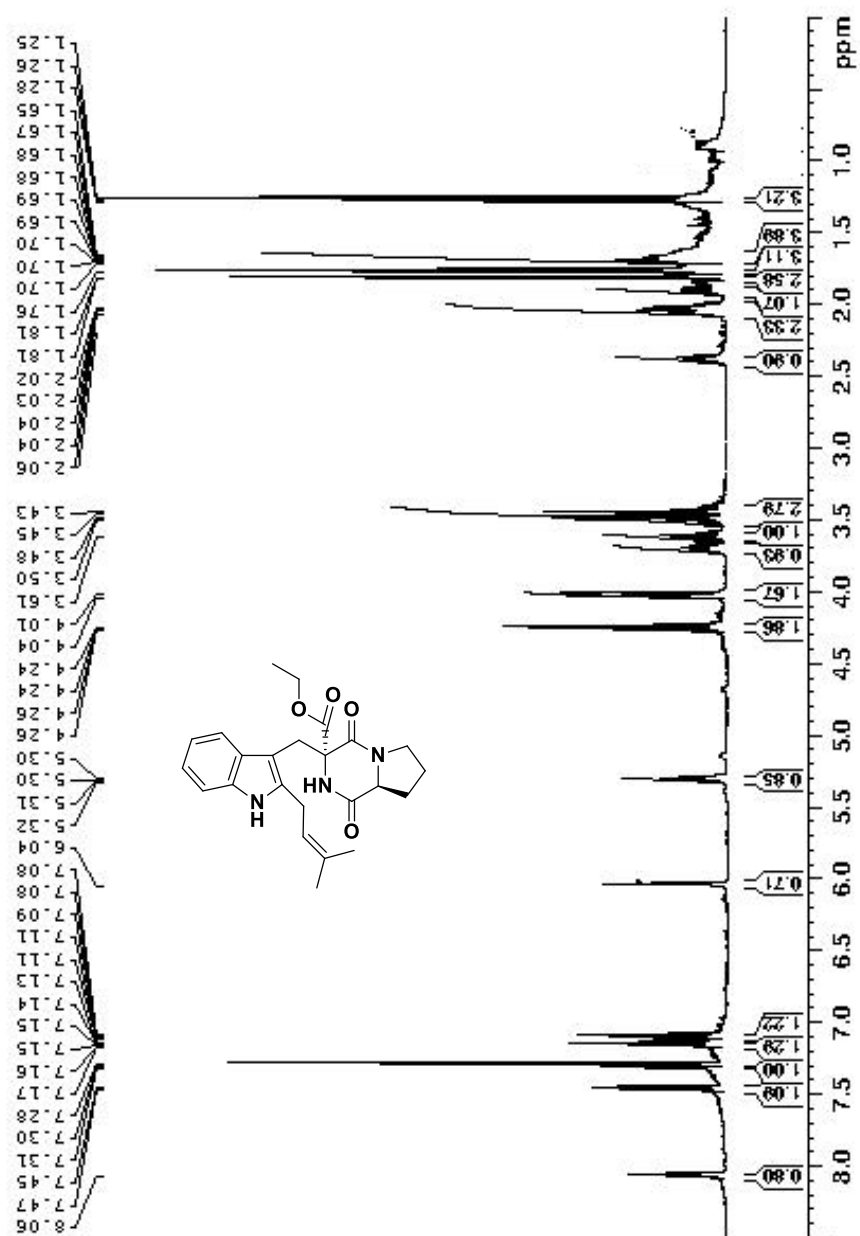


C27 H30 N2 O7 [M+H]<sup>+</sup> : Predicted region for 495.2126 m/z



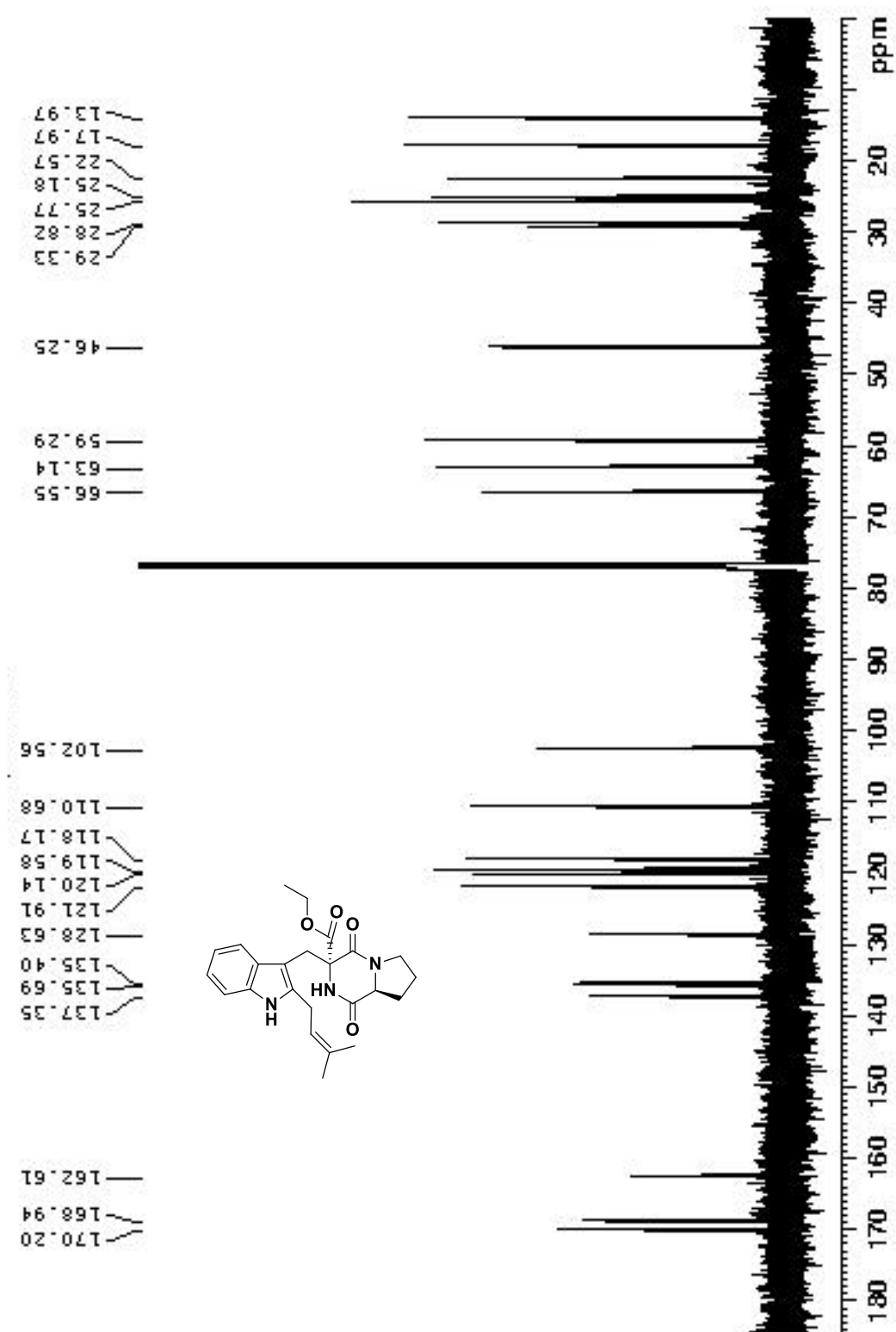
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	66.19	C27 H30 N2 O7	[M+H] <sup>+</sup>	495.2138	495.2126	1.2	2.42	68.63	14.0

**<sup>1</sup>H NMR Spectrum of Compound 14A in CDCl<sub>3</sub>**



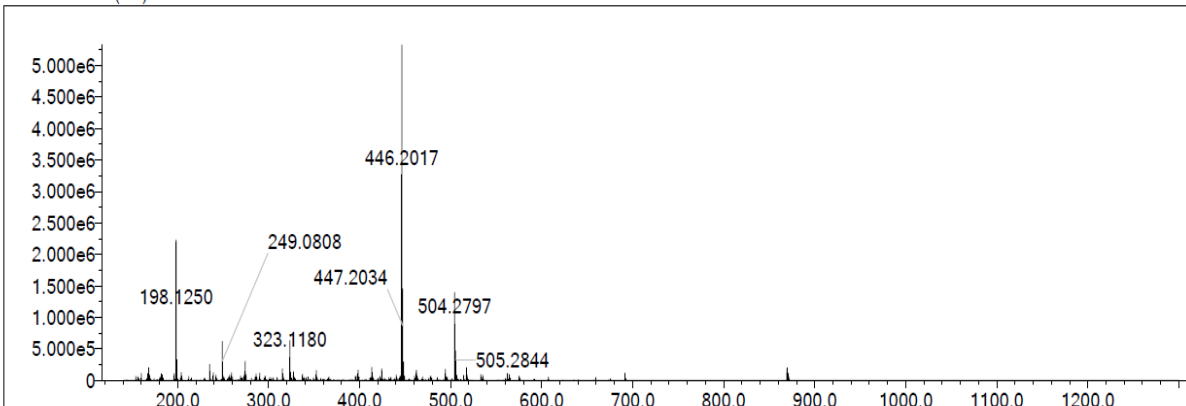


<sup>13</sup>C NMR Spectrum of Compound 14A in CDCl<sub>3</sub>

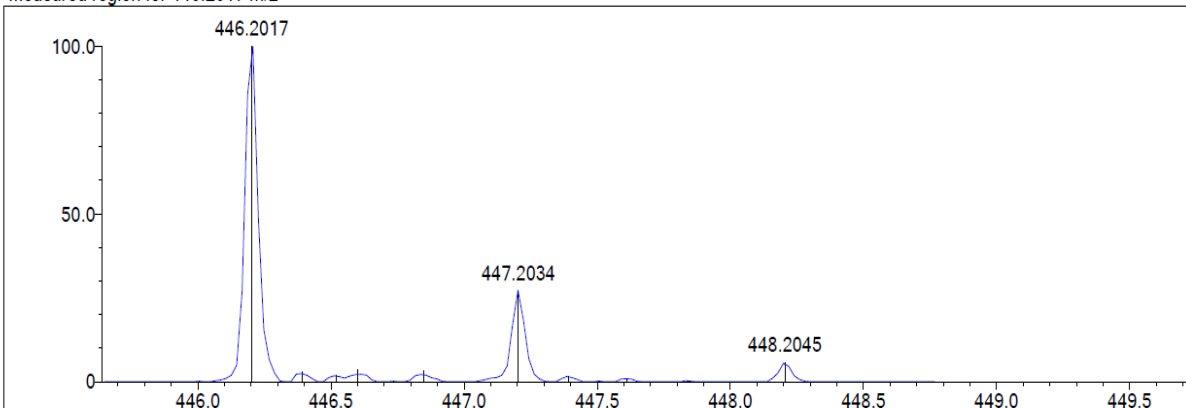


## HRMS of Compound 14A

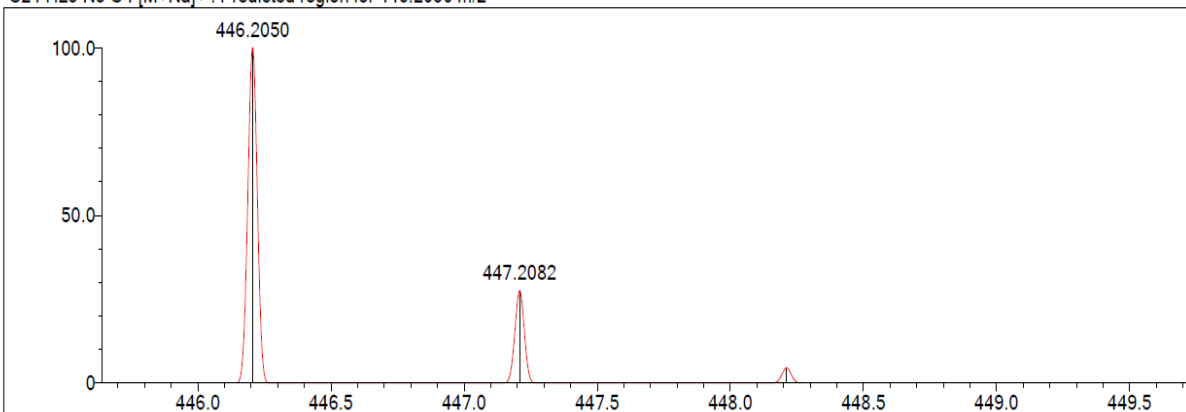
Event#: 1 MS(E+) Ret. Time : 1.010 -> 1.060 - 1.540 -> 1.545 Scan#: 203 -> 213 - 309 -> 311



Measured region for 446.2017 m/z

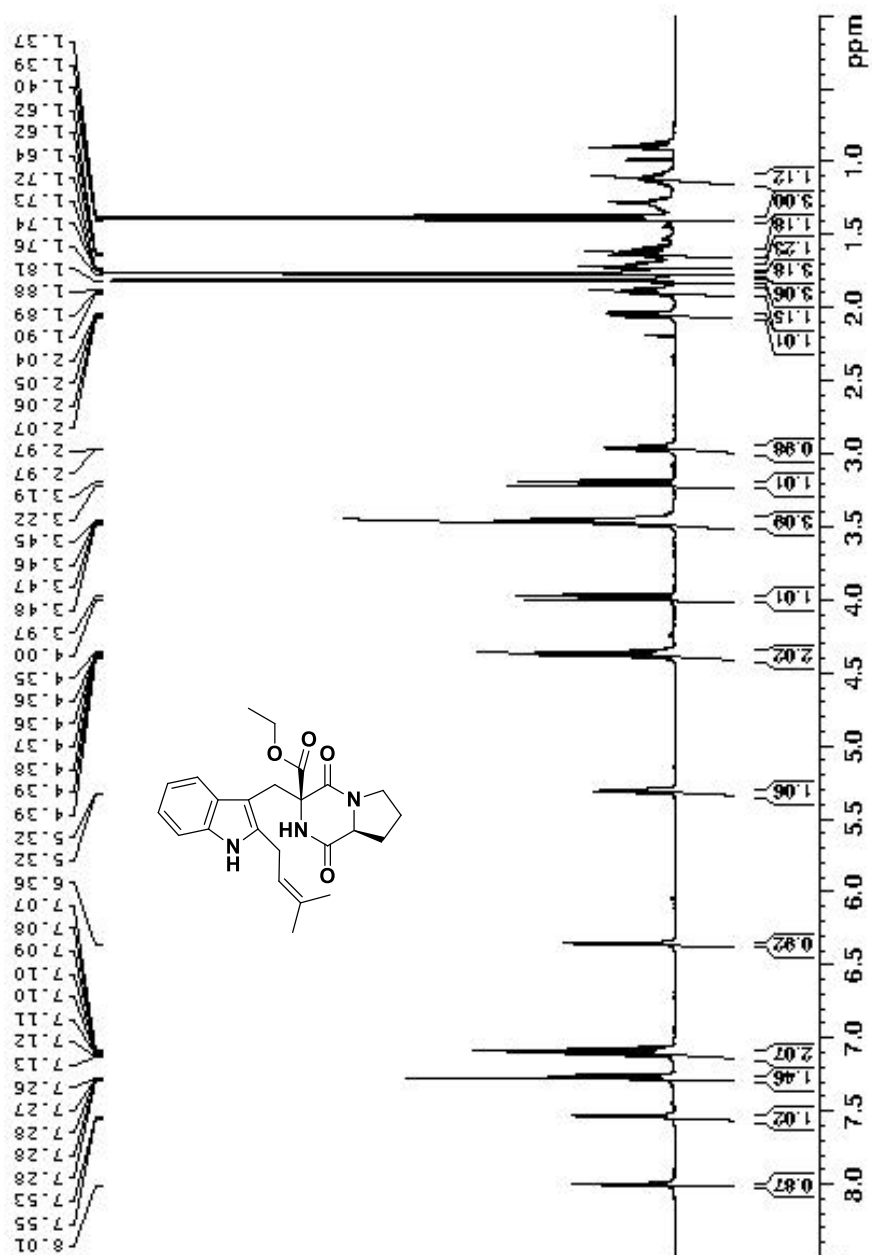


C24 H29 N3 O4 [M+Na]<sup>+</sup> : Predicted region for 446.2050 m/z

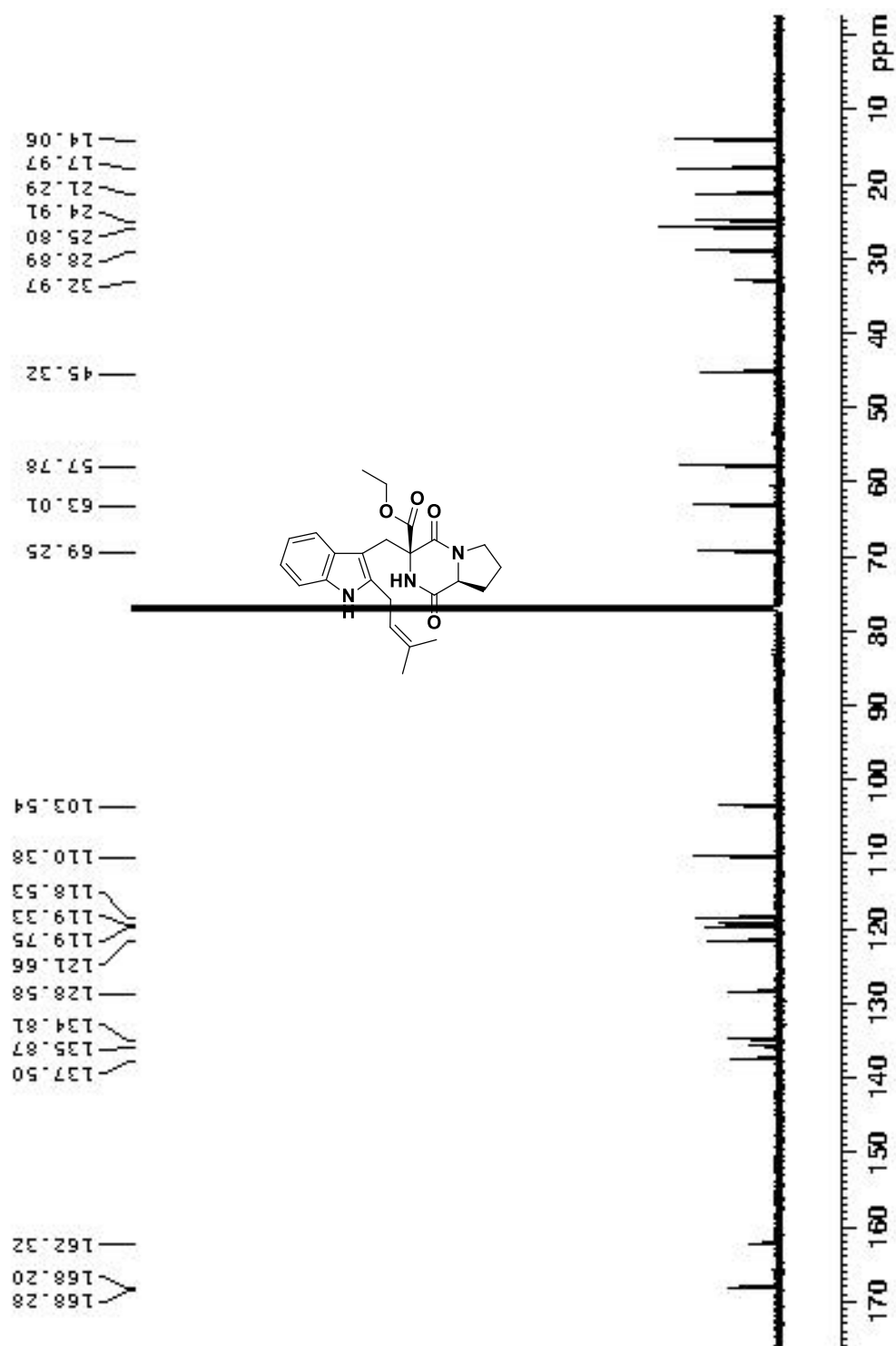


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	59.87	C24 H29 N3 O4	[M+Na] <sup>+</sup>	446.2017	446.2050	-3.3	-7.40	90.71	12.0

<sup>1</sup>H NMR Spectrum of Compound 14B in CDCl<sub>3</sub>

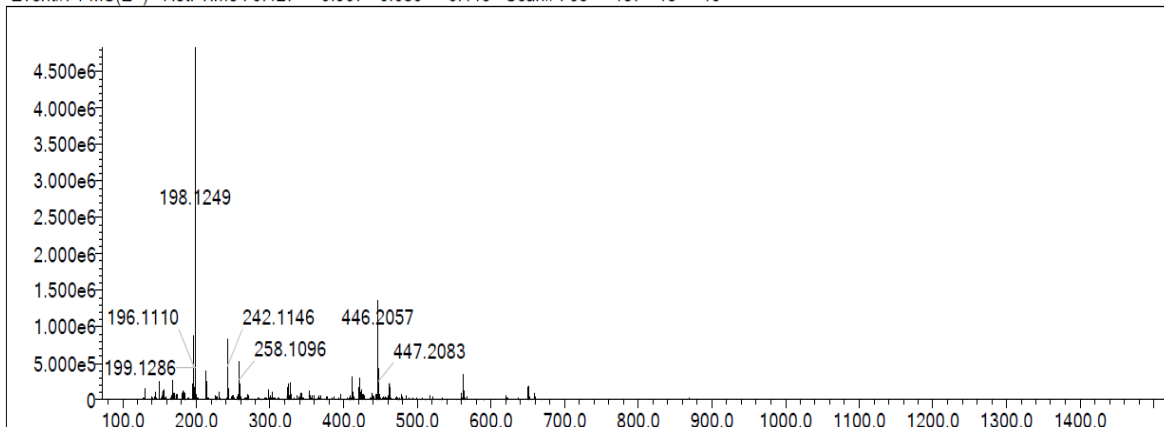


<sup>13</sup>C NMR Spectrum of Compound 14B in CDCl<sub>3</sub>

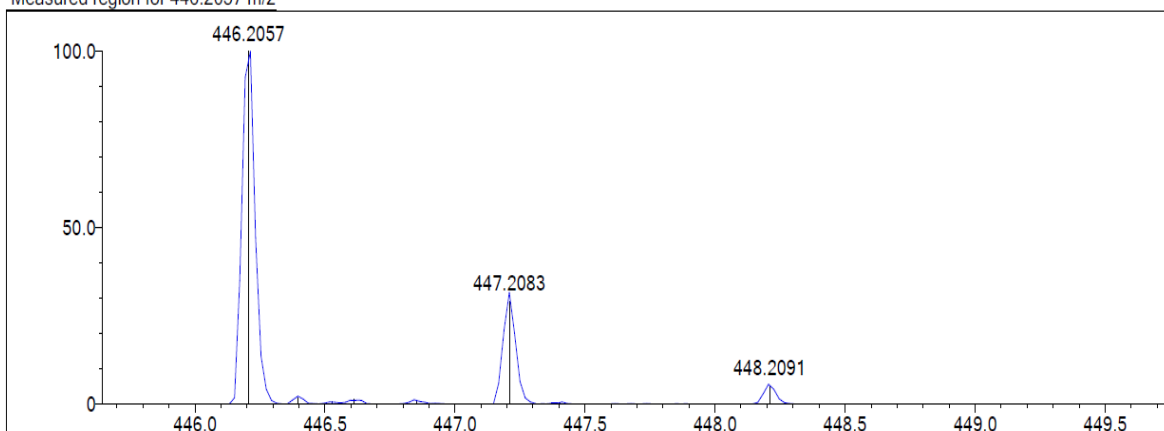


## HRMS of Compound 14B

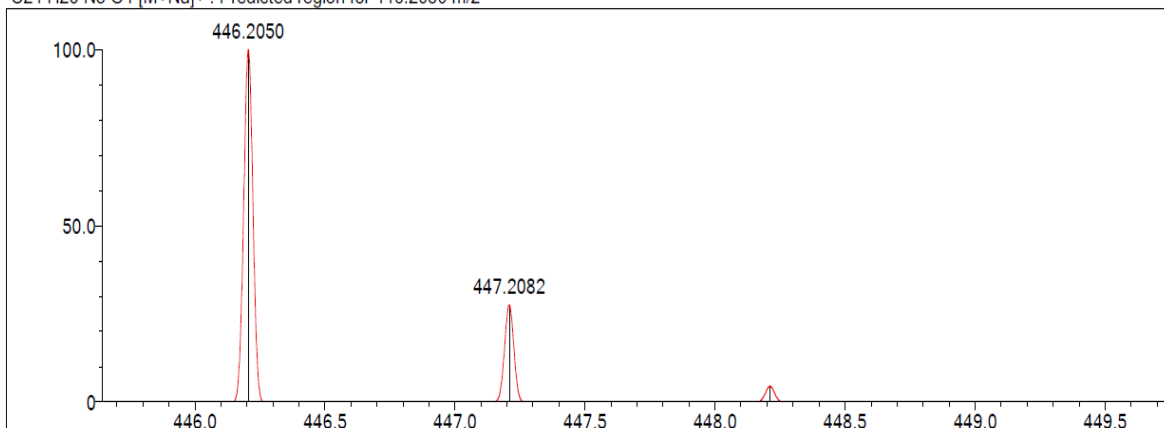
Event#: 1 MS(E+) Ret. Time : 0.427 -> 0.907 - 0.080 -> 0.119 Scan#: 65 -> 137 - 13 -> 19



Measured region for 446.2057 m/z

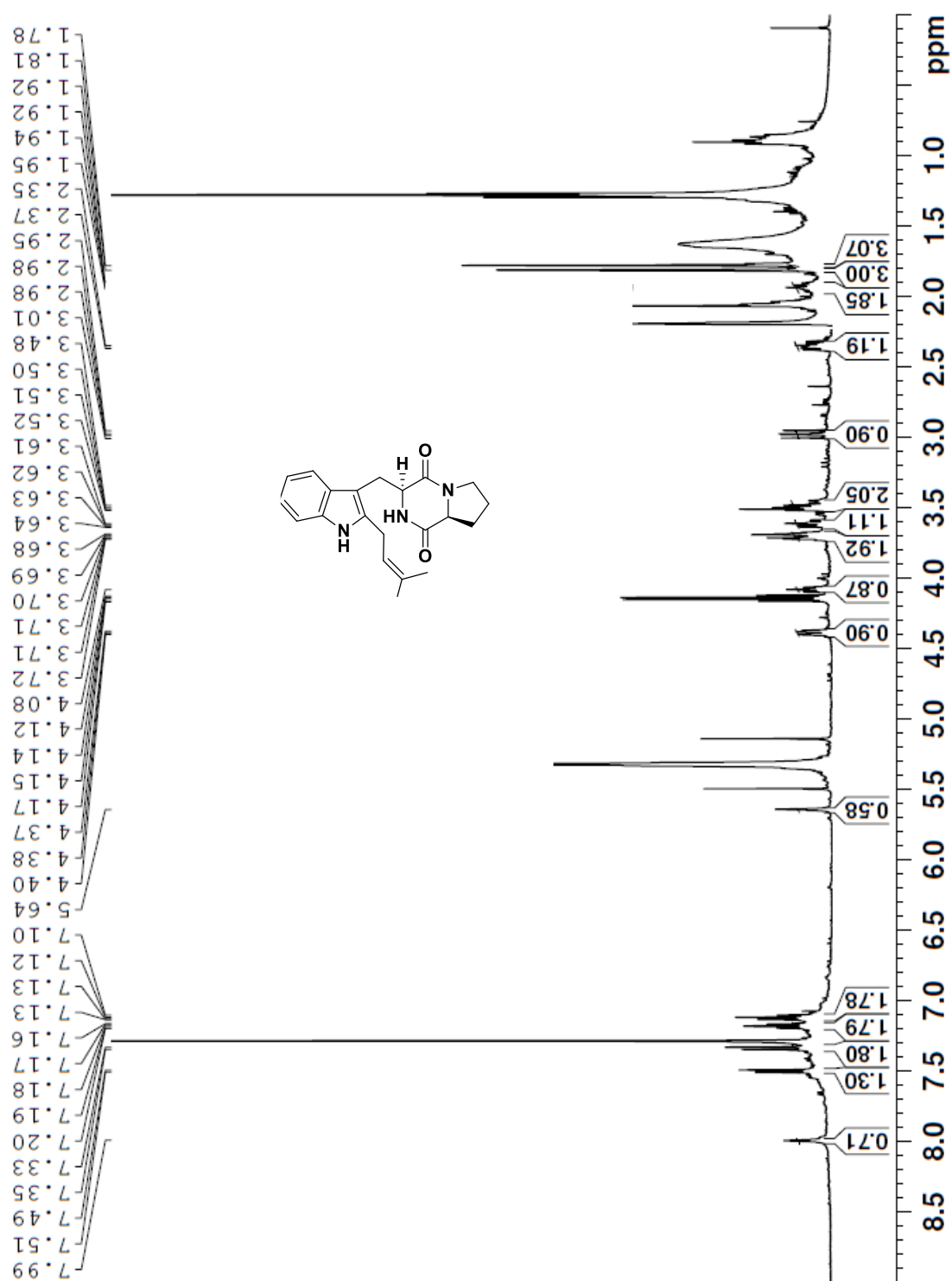


C24 H29 N3 O4 [M+Na]<sup>+</sup> : Predicted region for 446.2050 m/z

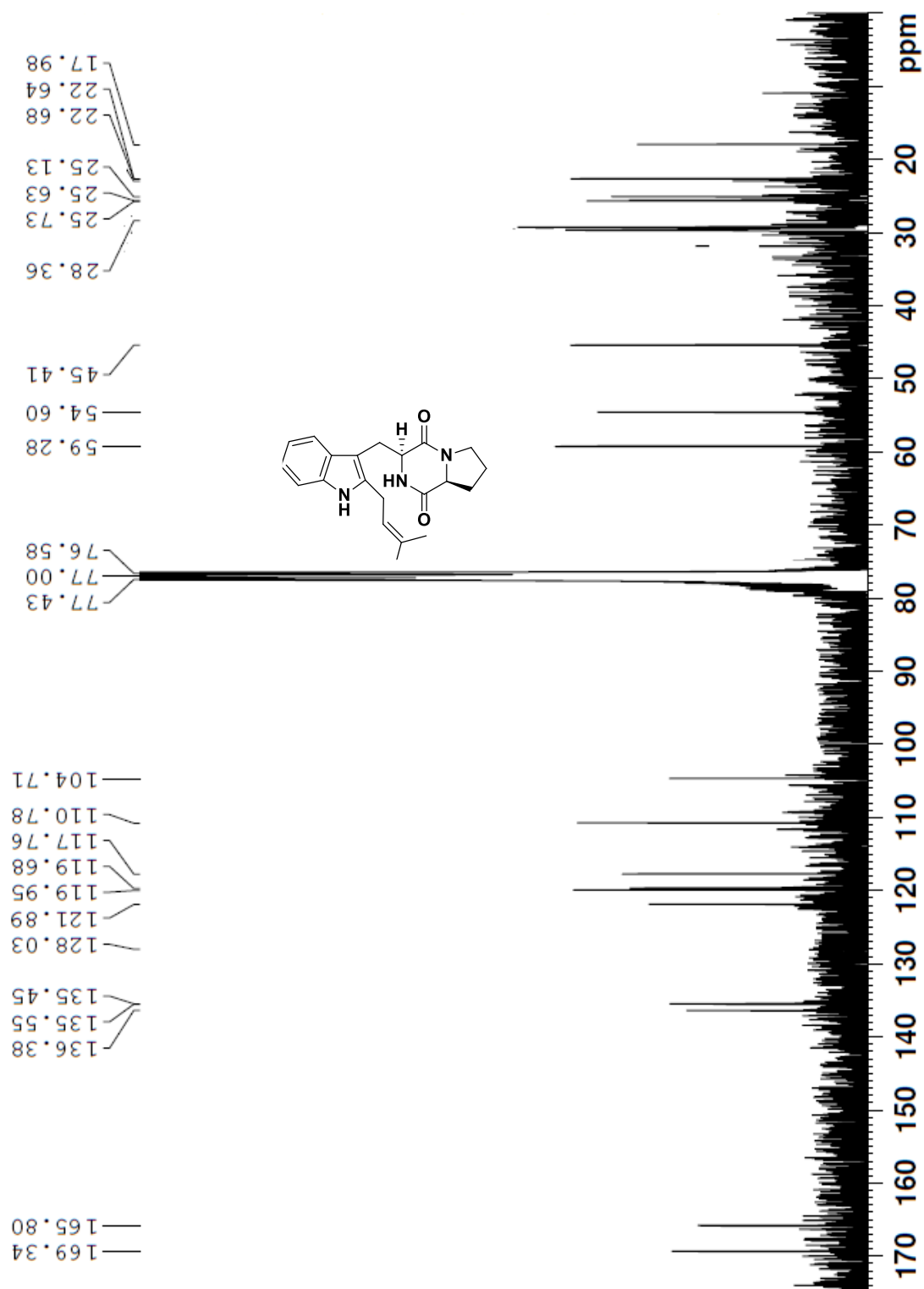


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	92.87	C24 H29 N3 O4	[M+Na] <sup>+</sup>	446.2057	446.2050	0.7	1.57	94.21	12.0

<sup>1</sup>H NMR Spectrum of Compound 17 in CDCl<sub>3</sub>

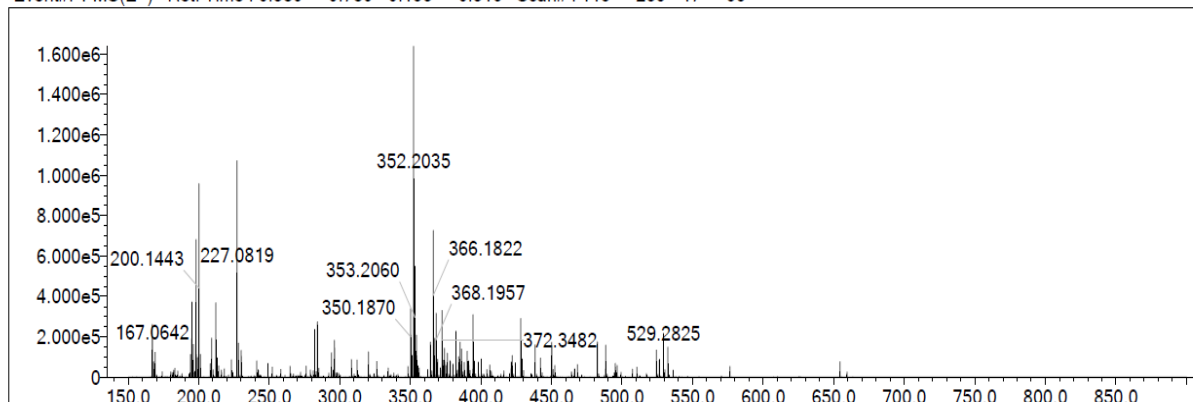


**$^{13}\text{C}$  NMR Spectrum of Compound 17 in  $\text{CDCl}_3$**

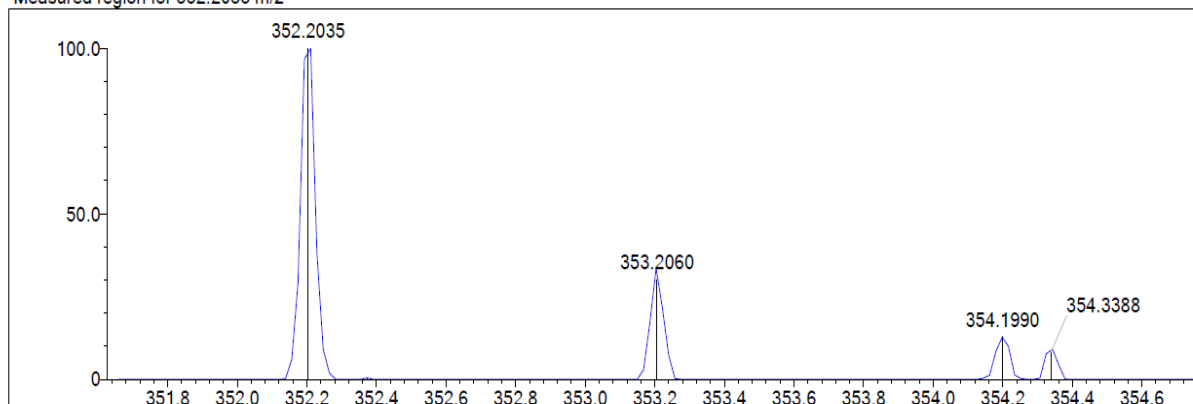


## HRMS of Compound 17, Tryprostatin B

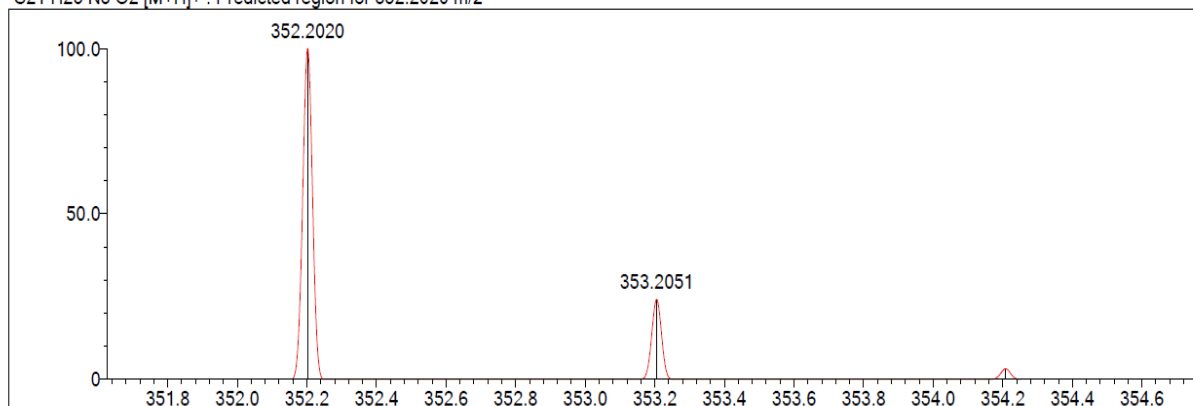
Event#: 1 MS(E+) Ret. Time : 0.380 -> 0.780 - 0.153 -> 0.316 Scan#: 115 -> 235 - 47 -> 95



Measured region for 352.2035 m/z



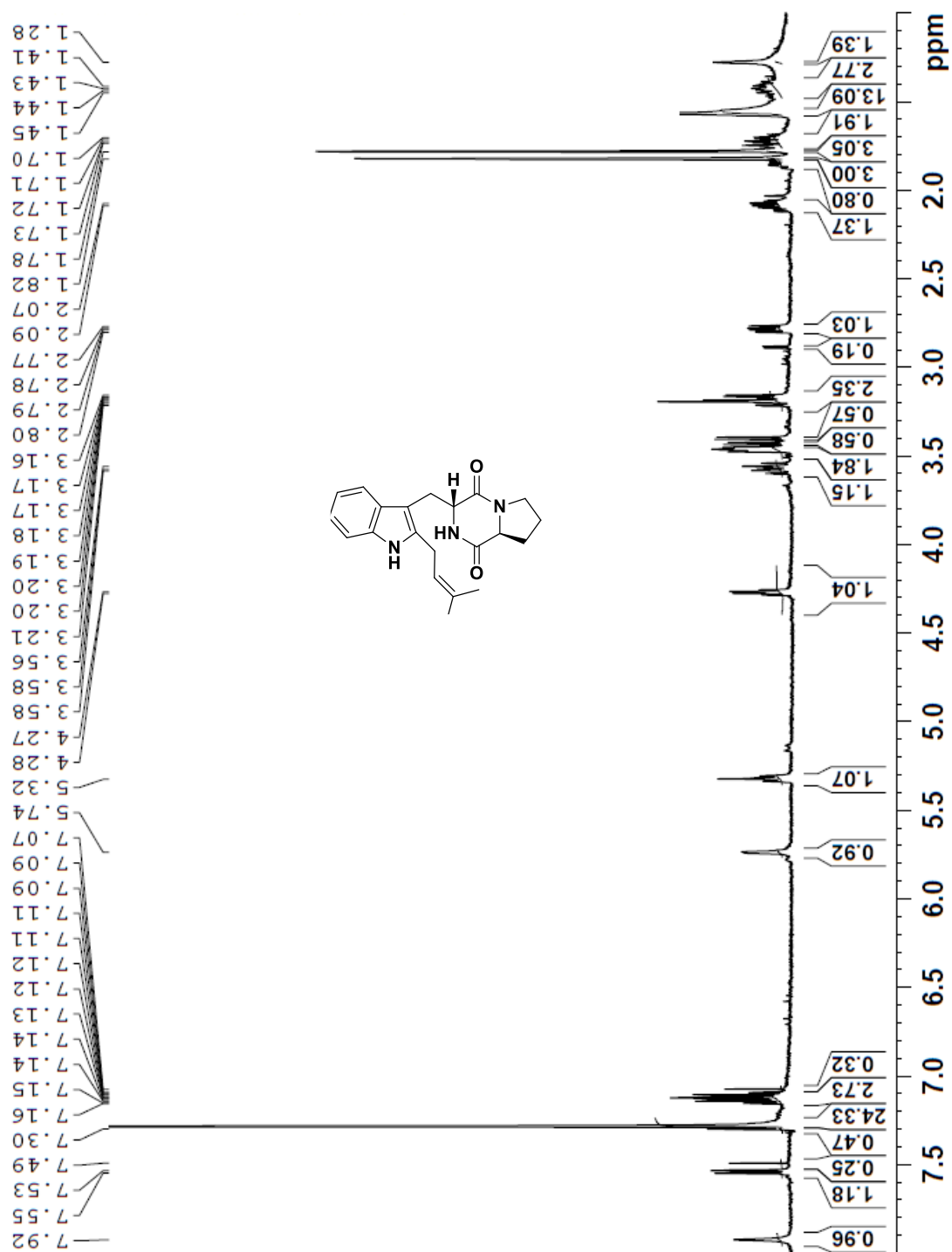
C21 H25 N3 O2 [M+H]<sup>+</sup> : Predicted region for 352.2020 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	47.69	C21 H25 N3 O2	[M+H] <sup>+</sup>	352.2035	352.2020	1.5	4.26	51.92	11.0

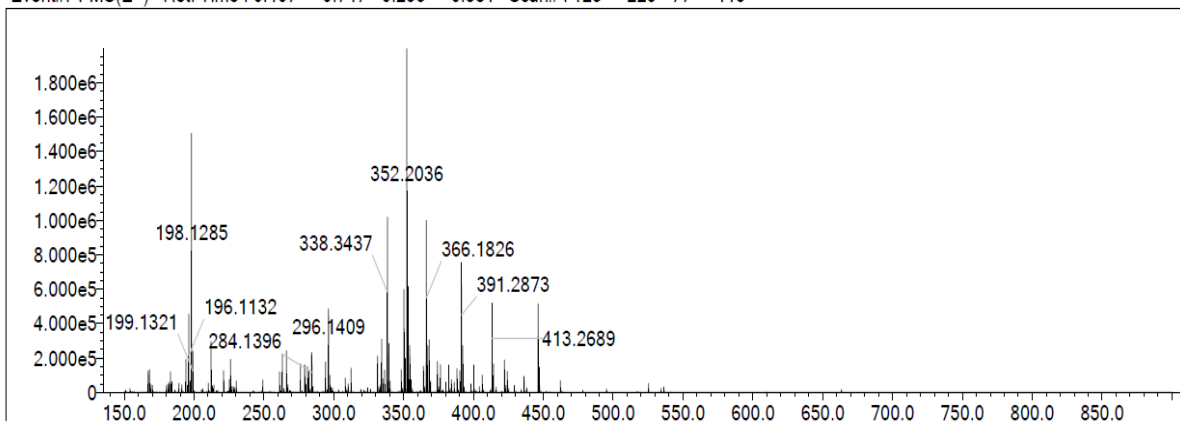


<sup>1</sup>H NMR Spectrum of Compound 18 in CDCl<sub>3</sub>

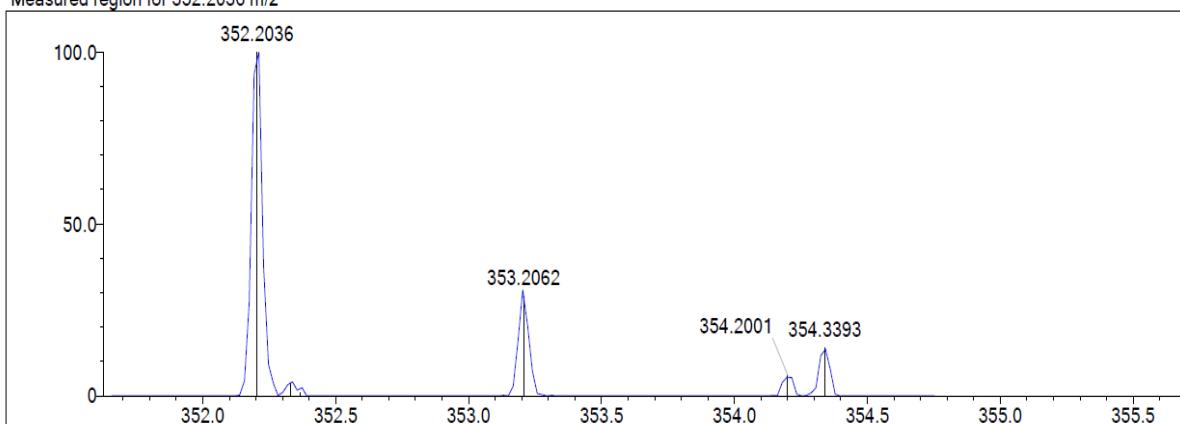


## HRMS of Compound 18 (9-epi-Tryprostatin B)

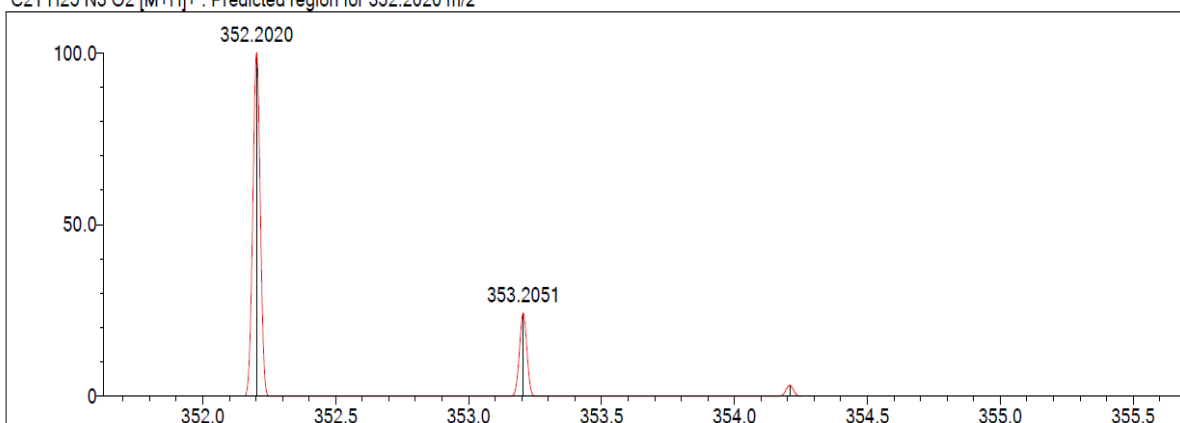
Event#: 1 MS(E+) Ret. Time : 0.407 -> 0.747 - 0.253 -> 0.381 Scan#: 123 -> 225 - 77 -> 115



Measured region for 352.2036 m/z

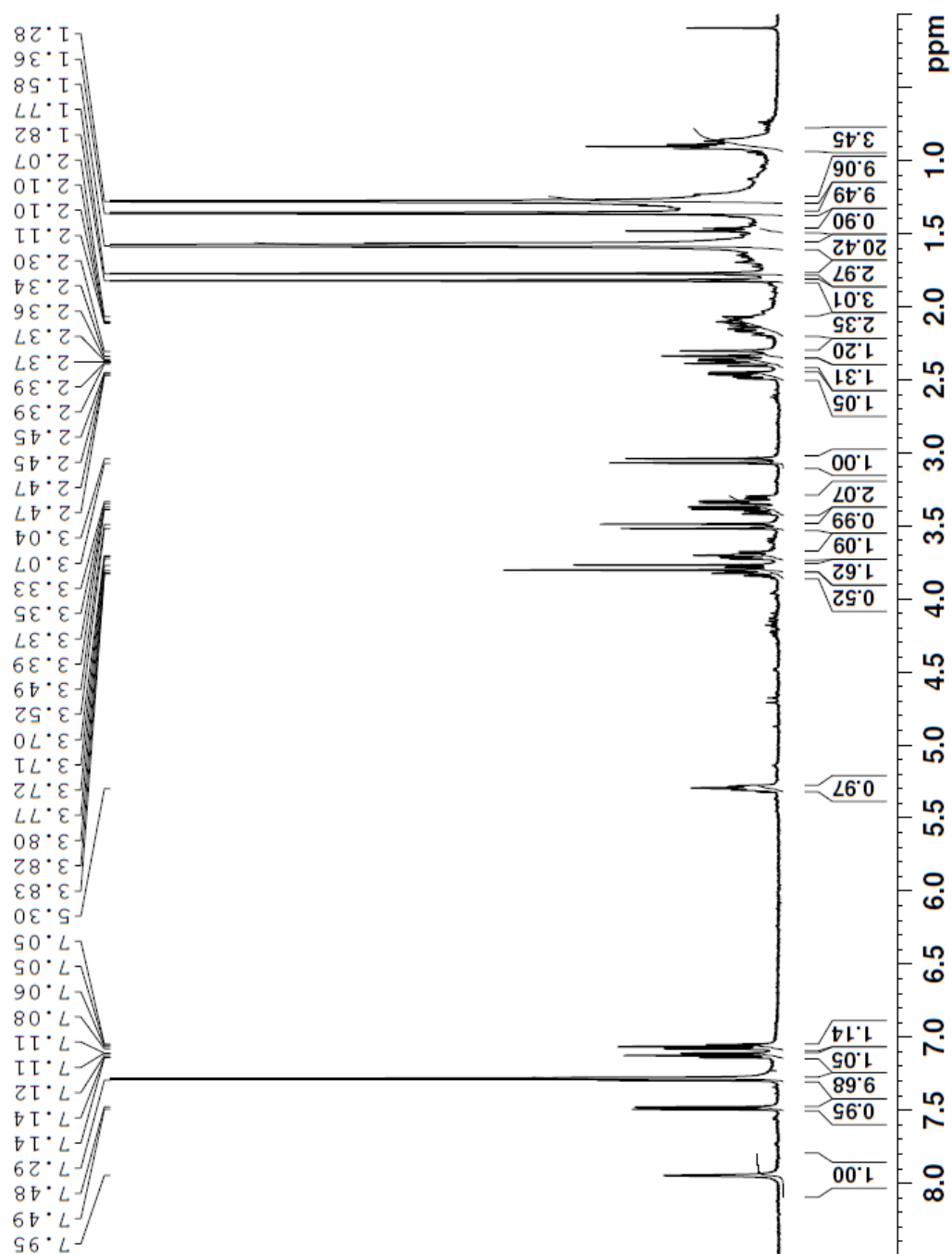


C21 H25 N3 O2 [M+H]<sup>+</sup> : Predicted region for 352.2020 m/z

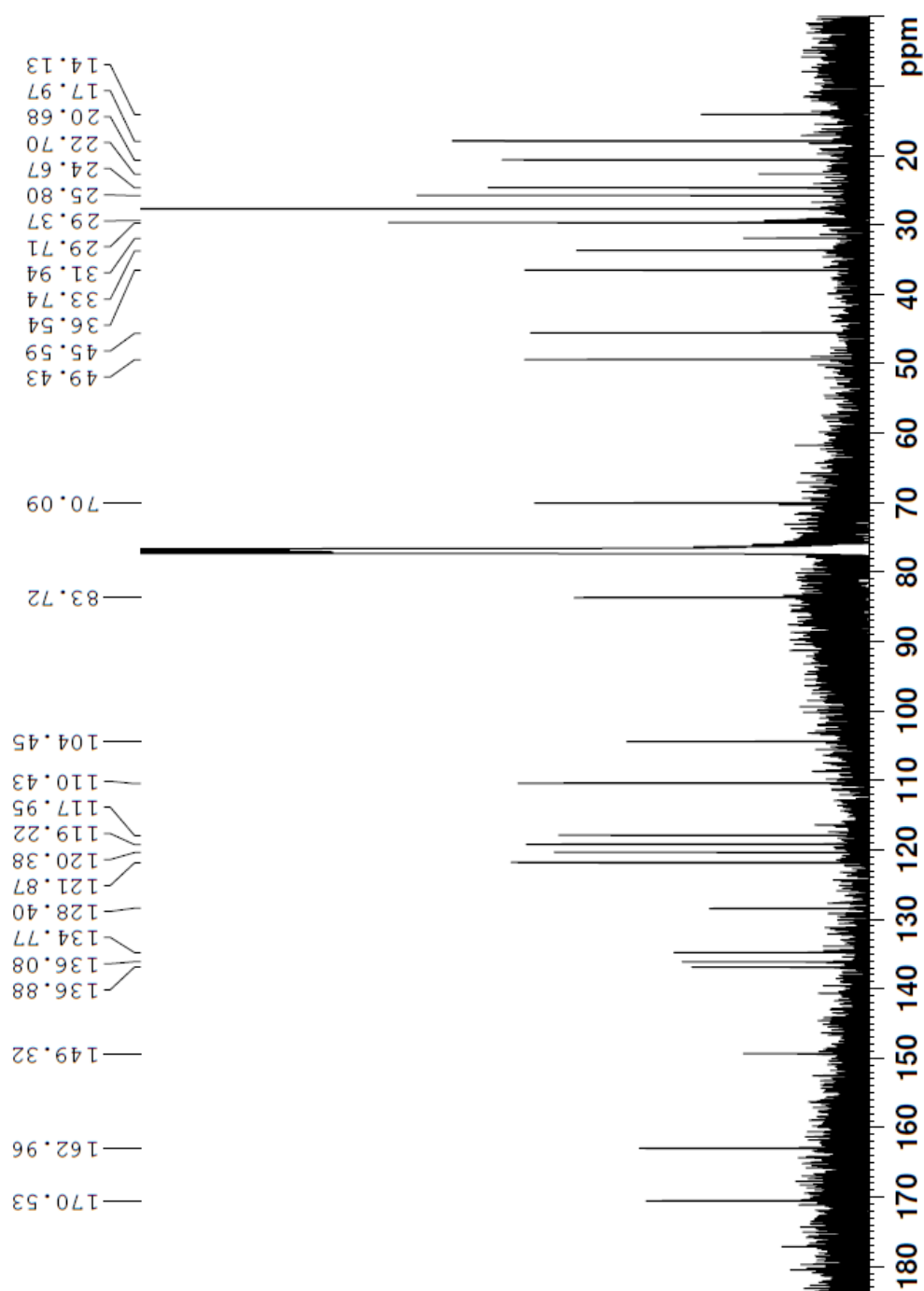


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	63.91	C21 H25 N3 O2	[M+H] <sup>+</sup>	352.2036	352.2020	1.6	4.54	70.11	11.0

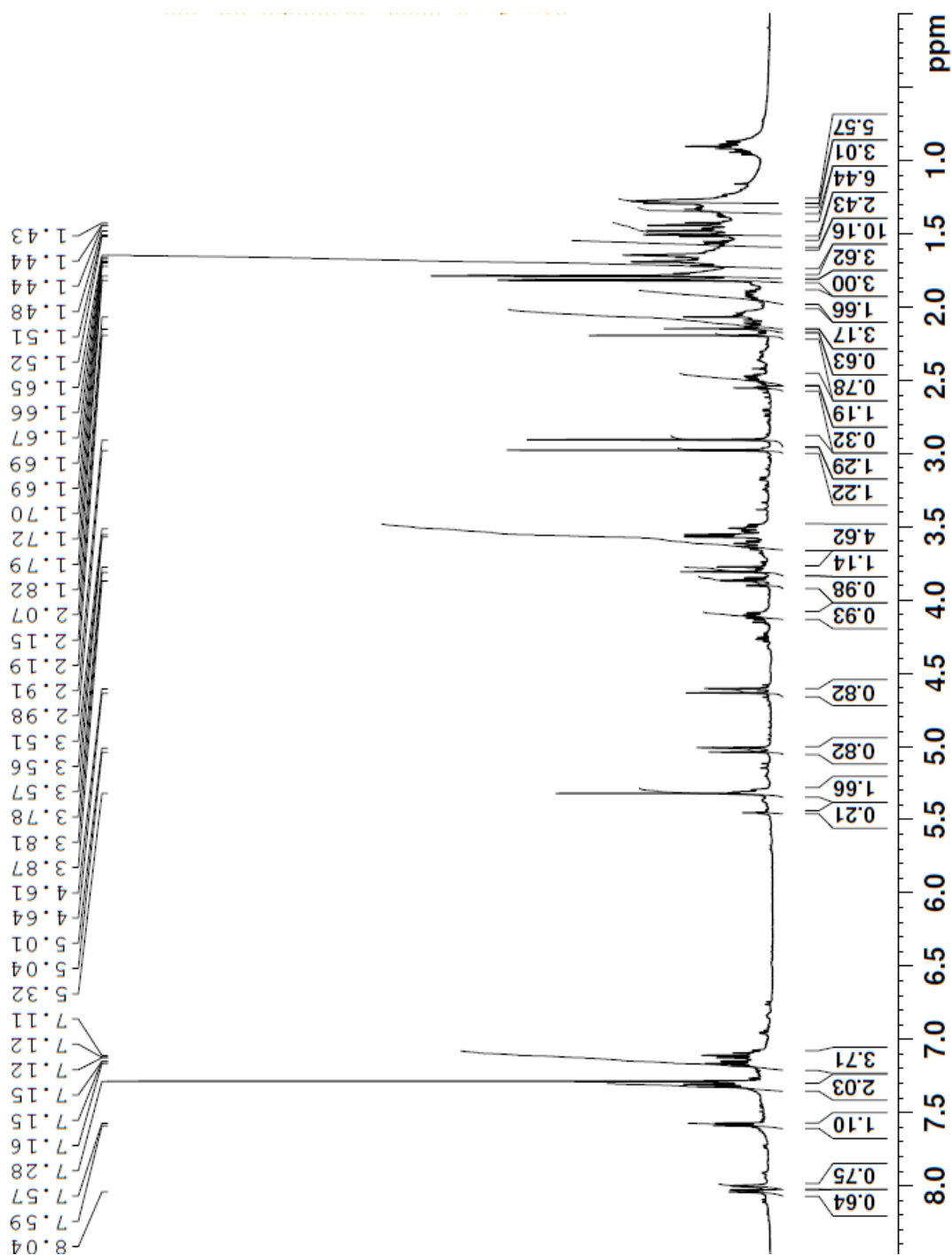
# <sup>1</sup>H NMR Spectrum of Compound 19 in CDCl<sub>3</sub>



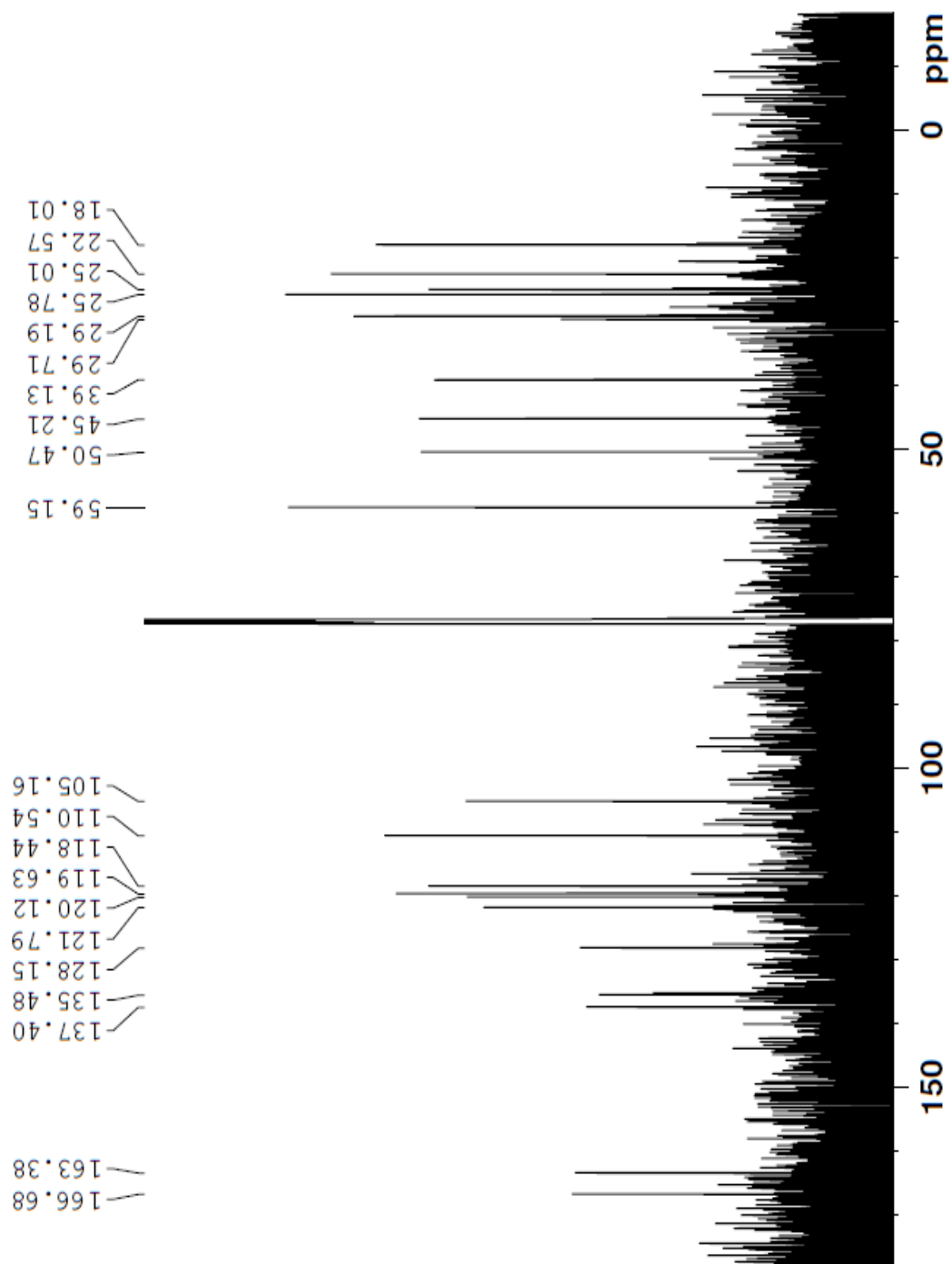
**$^{13}\text{C}$  NMR Spectrum of Compound 19 in  $\text{CDCl}_3$**



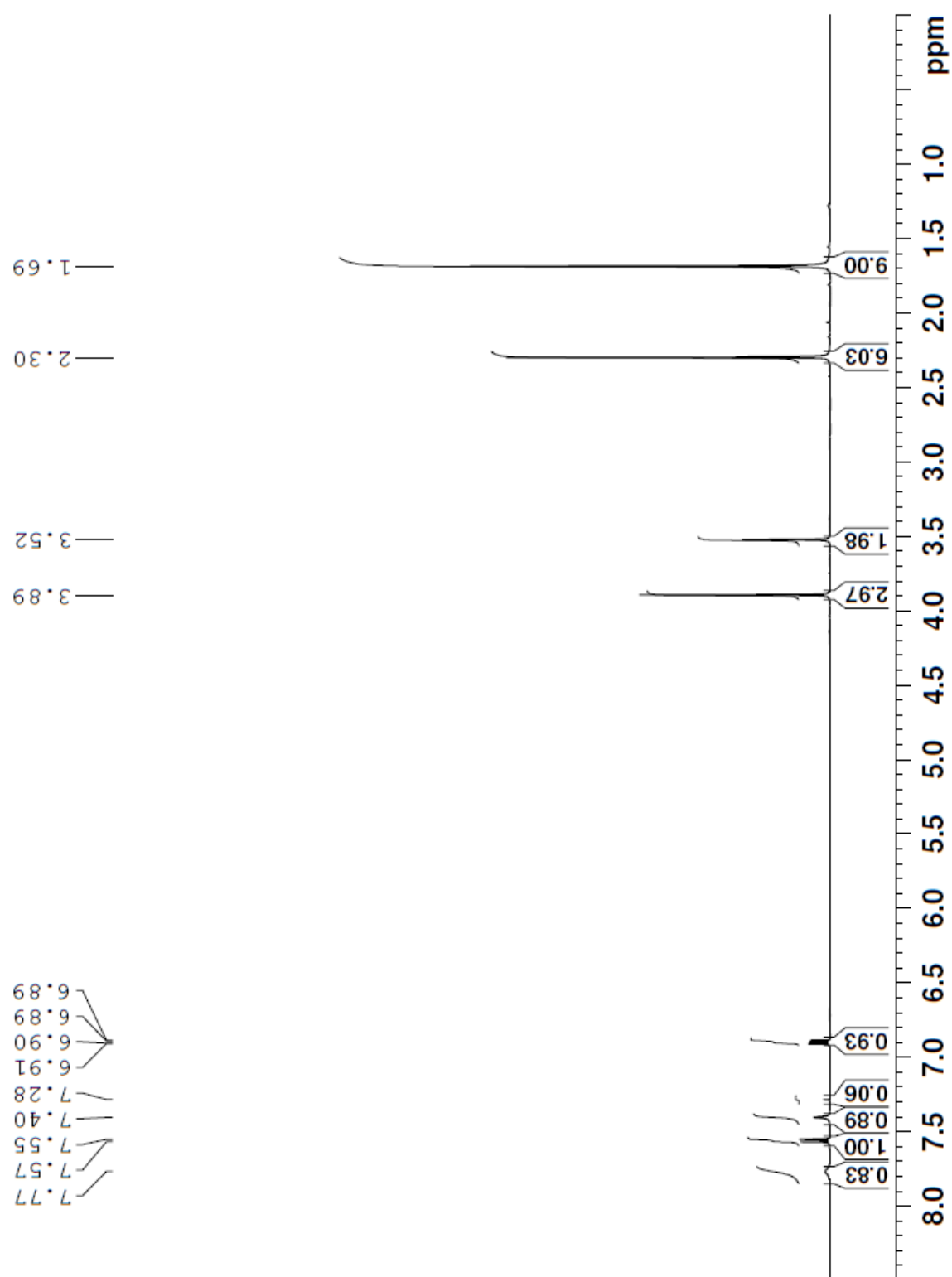
<sup>1</sup>H NMR Spectrum of Compound 20 in CDCl<sub>3</sub>



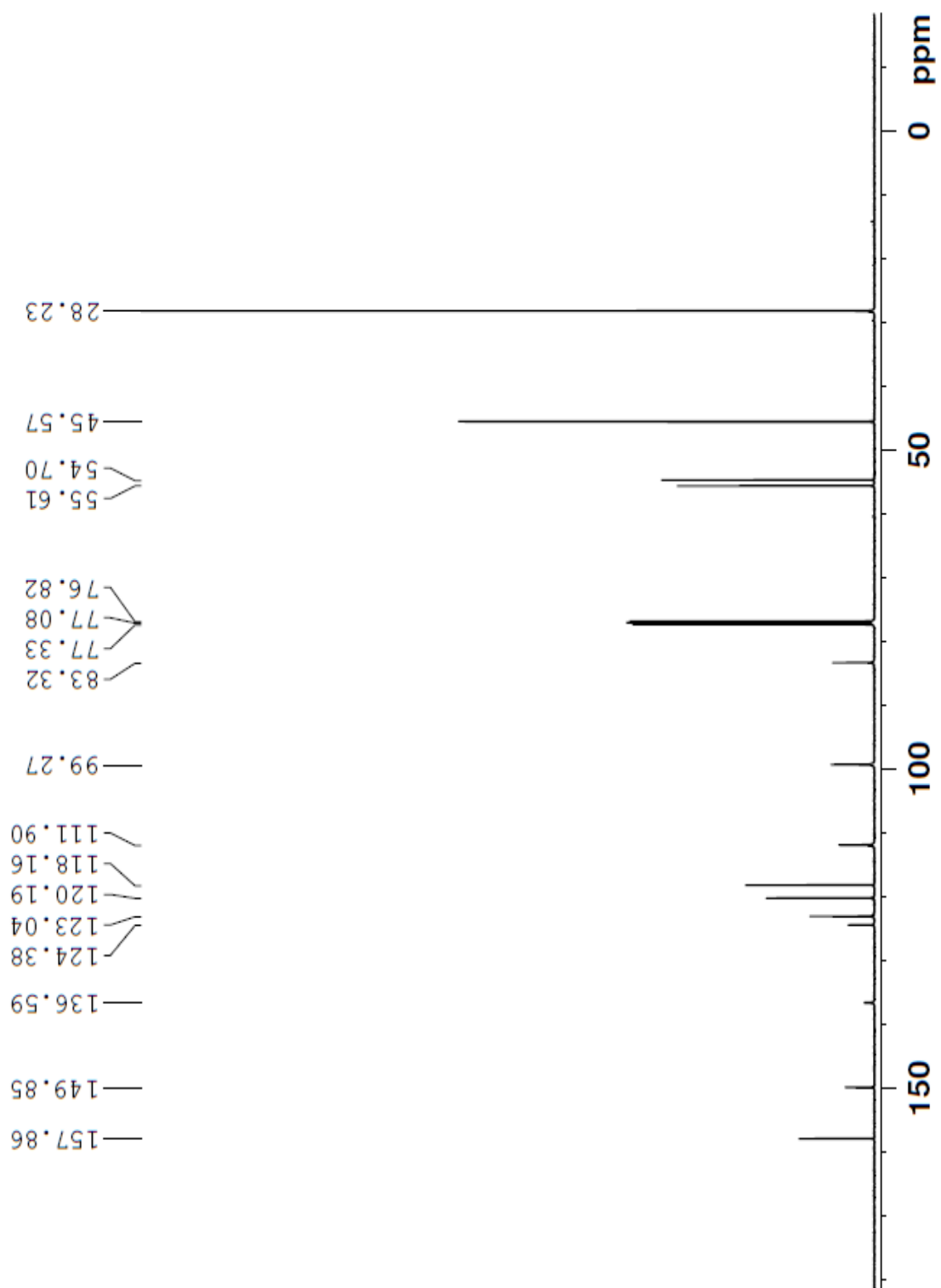
**$^{13}\text{C}$  NMR Spectrum of Compound 20 in  $\text{CDCl}_3$**



<sup>1</sup>H NMR Spectrum of Compound 22 in CDCl<sub>3</sub>

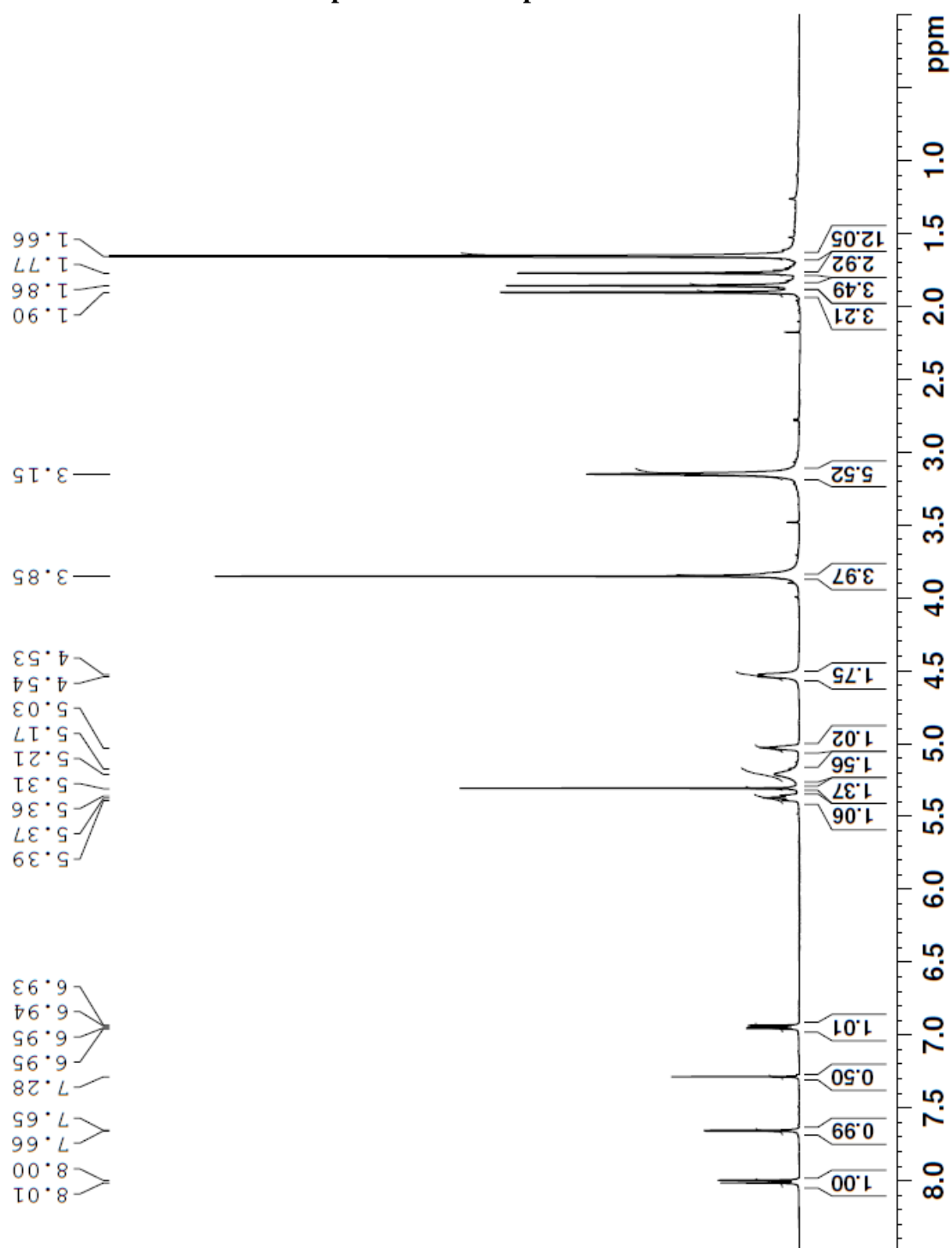


**$^{13}\text{C}$  NMR Spectrum of Compound 22 in  $\text{CDCl}_3$**

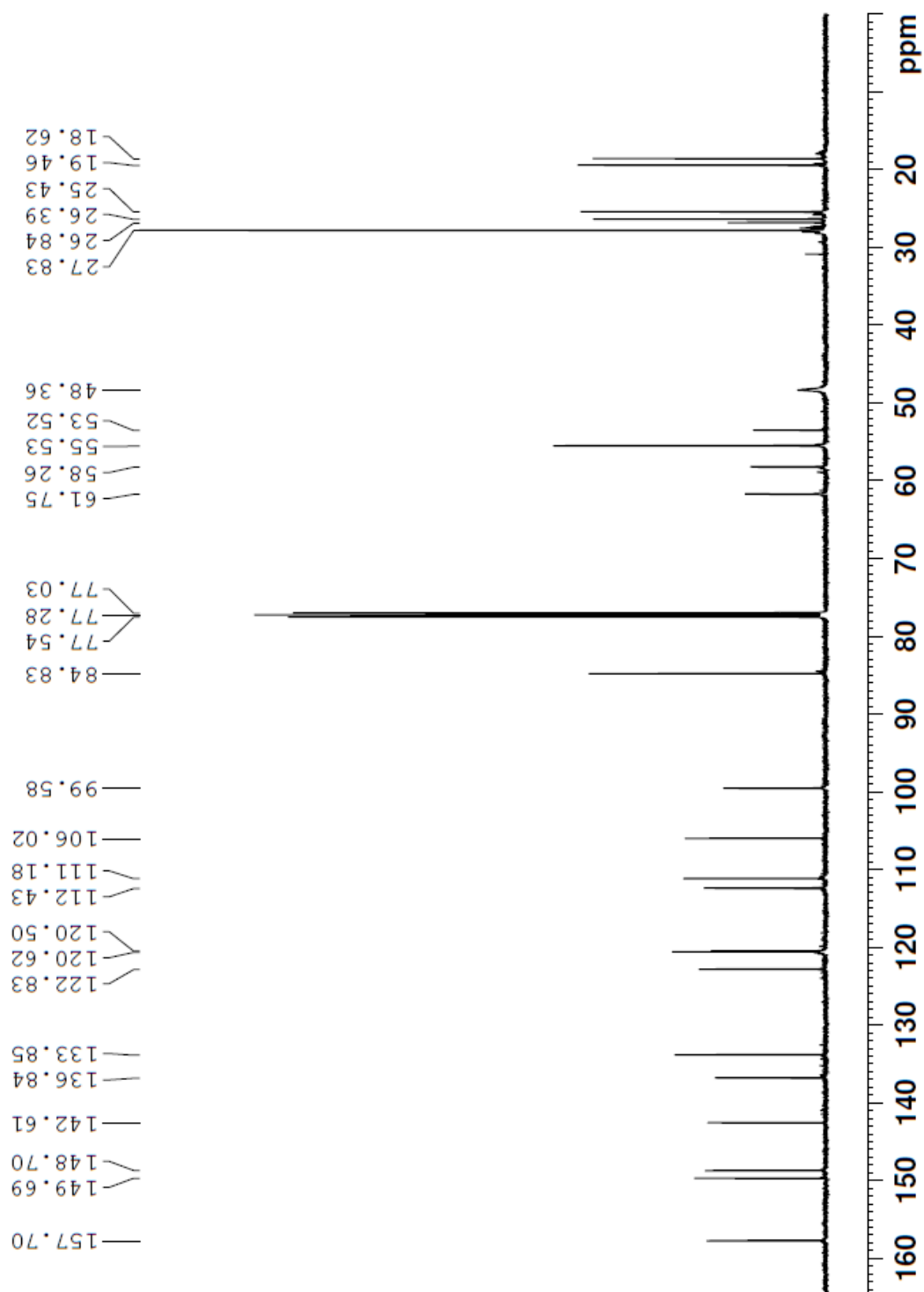




<sup>1</sup>H NMR Spectrum of Compound 23 in CDCl<sub>3</sub>

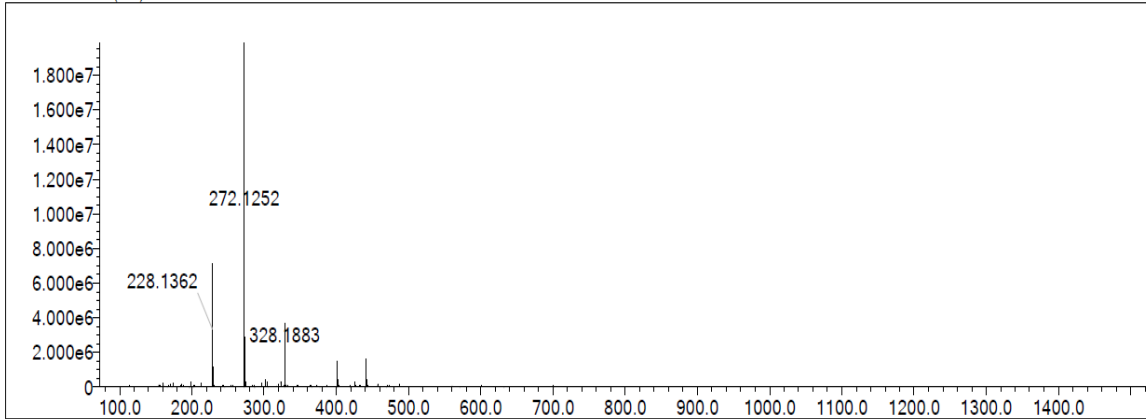


**$^{13}\text{C}$  NMR Spectrum of Compound 23 in  $\text{CDCl}_3$**

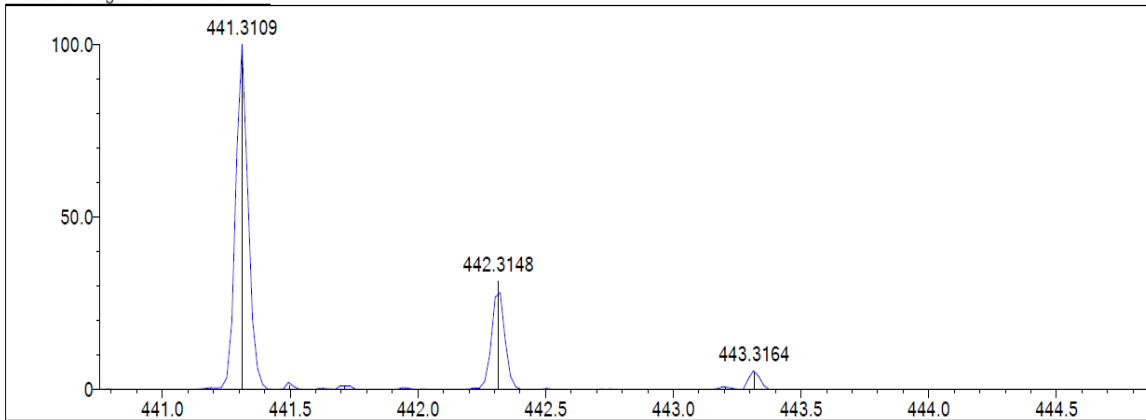


## HRMS of Compound 23

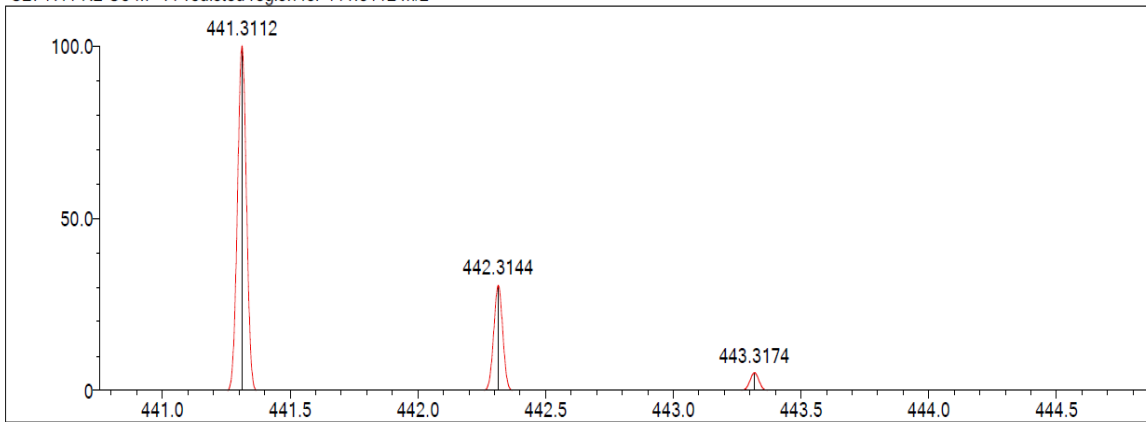
Event#: 1 MS(E+) Ret. Time : 0.453 -> 0.827 - 0.227 -> 0.309 Scan#: 69 -> 125 - 35 -> 47



Measured region for 441.3109 m/z

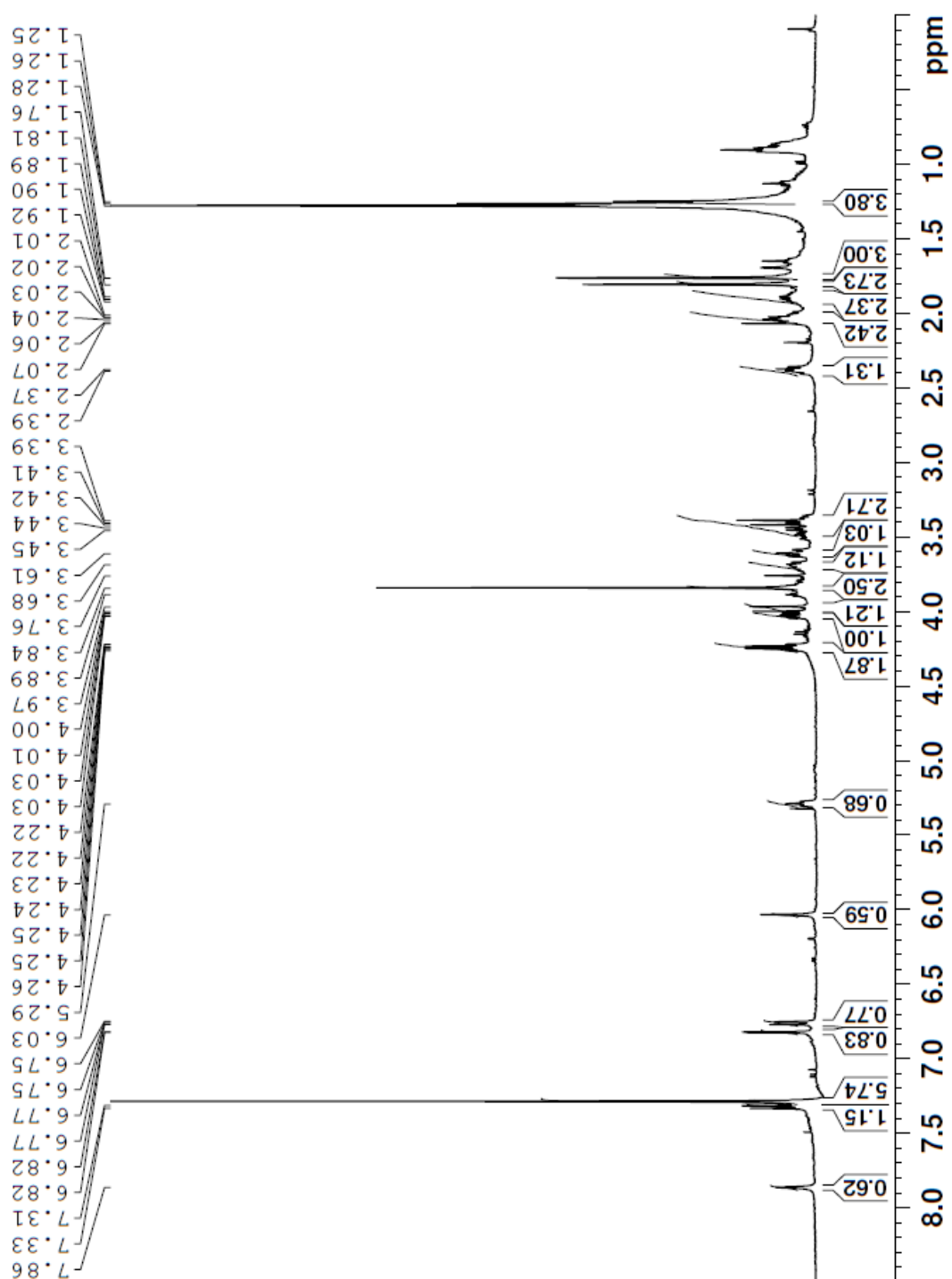


C27 H41 N2 O3 M+ : Predicted region for 441.3112 m/z

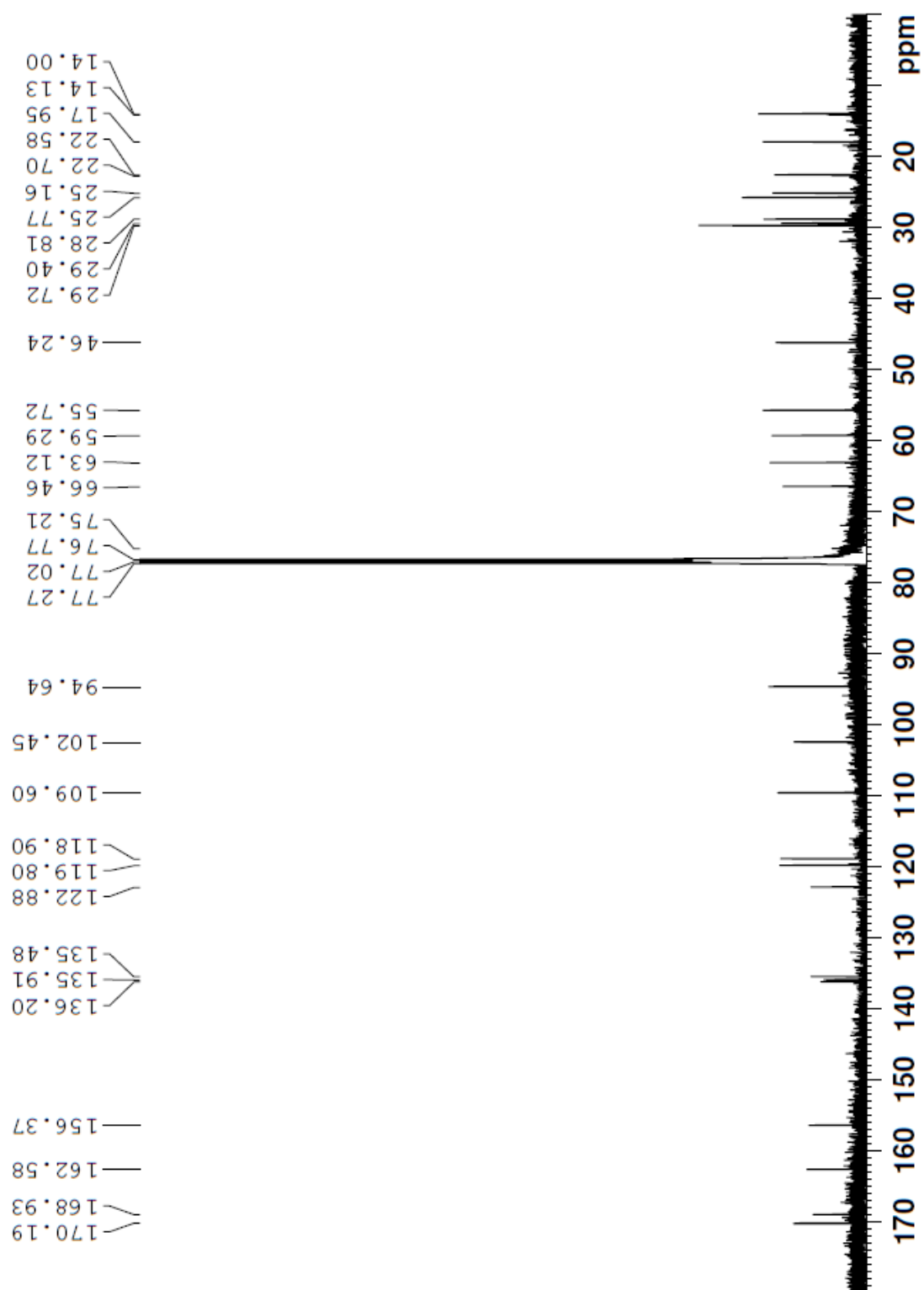


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	100.00	C27 H41 N2 O3	M+	441.3109	441.3112	-0.3	-0.68	100.00	8.5

### <sup>1</sup>H NMR Spectrum of Compound 24A in CDCl<sub>3</sub>

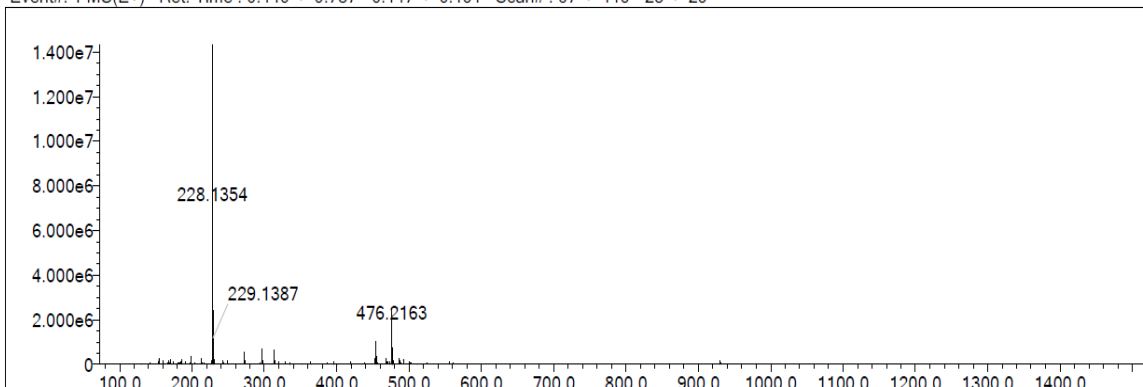


<sup>13</sup>C NMR Spectrum of Compound 24A in CDCl<sub>3</sub>

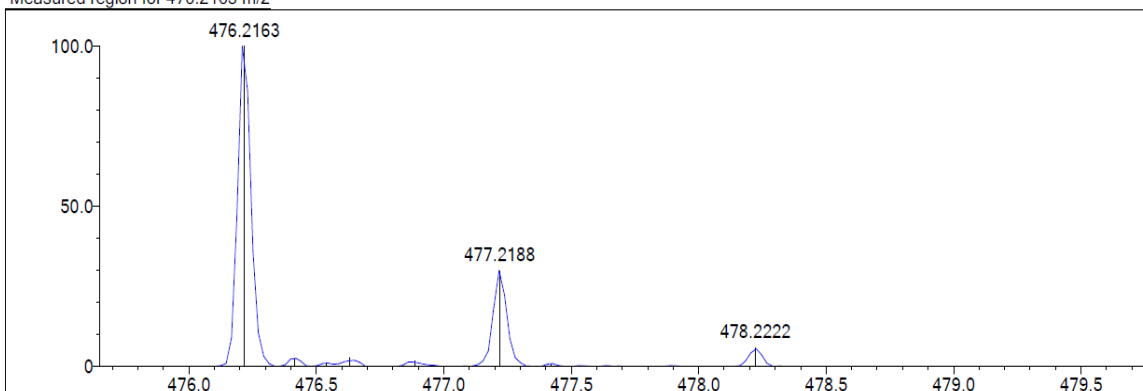


# HRMS of Compound 24A

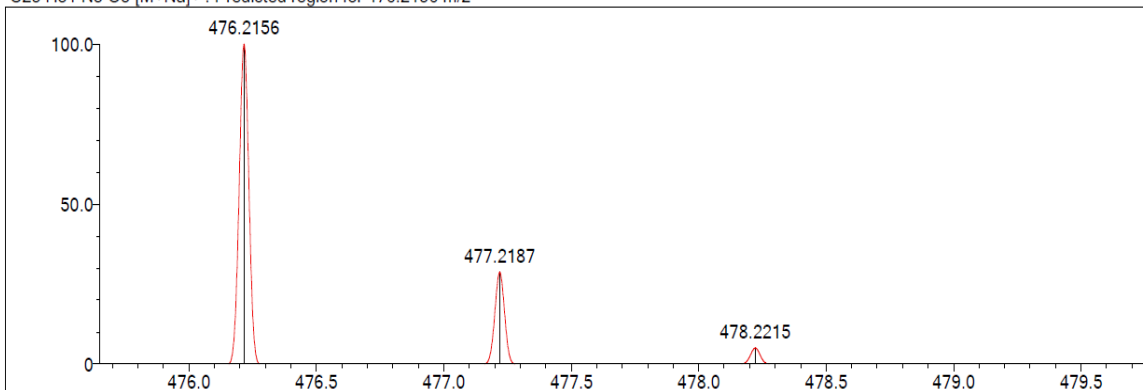
Event#: 1 MS(E+) Ret. Time : 0.440 -> 0.787 - 0.147 -> 0.191 Scan#: 67 -> 119 - 23 -> 29



Measured region for 476.2163 m/z

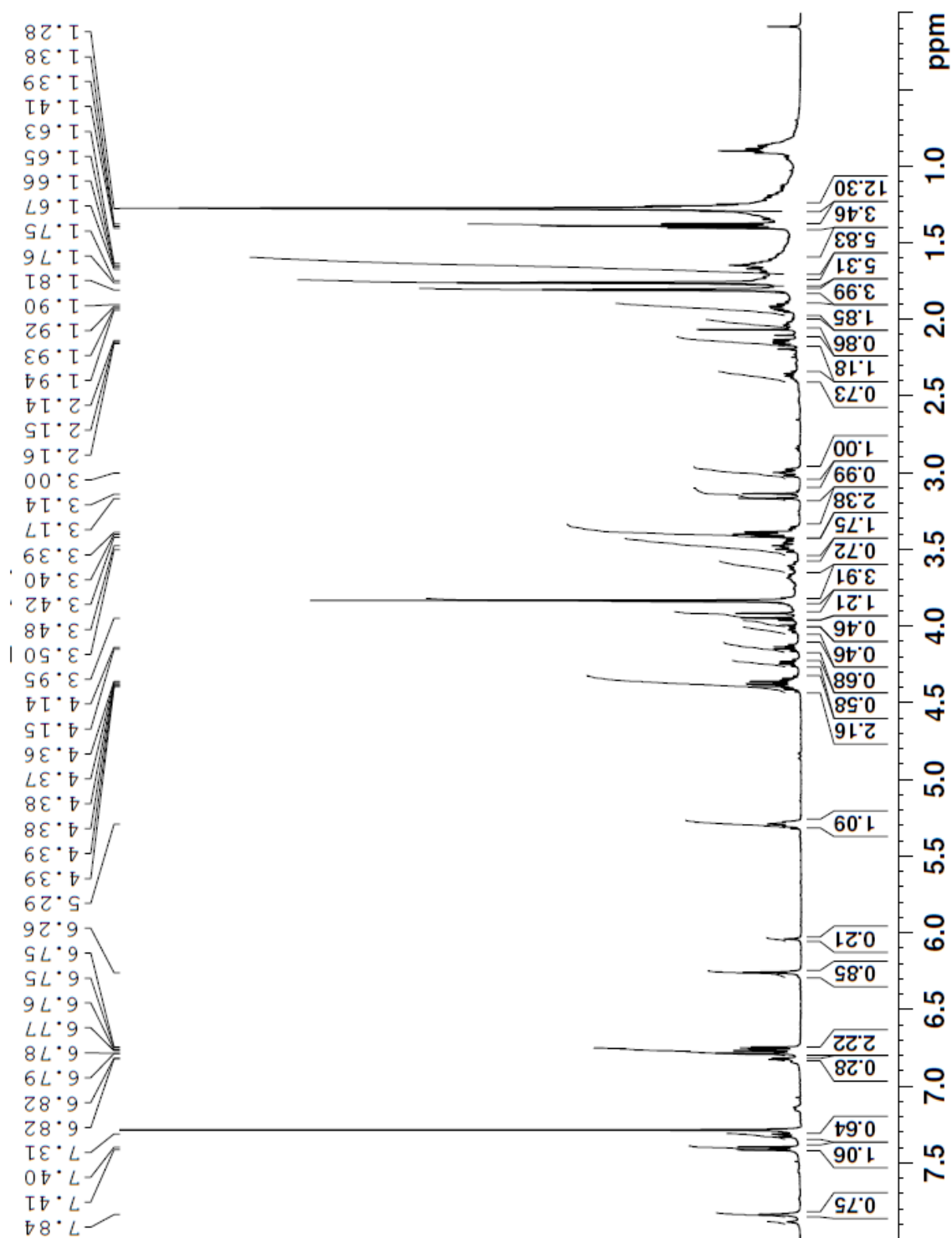


C25 H31 N3 O5 [M+Na]<sup>+</sup> : Predicted region for 476.2156 m/z

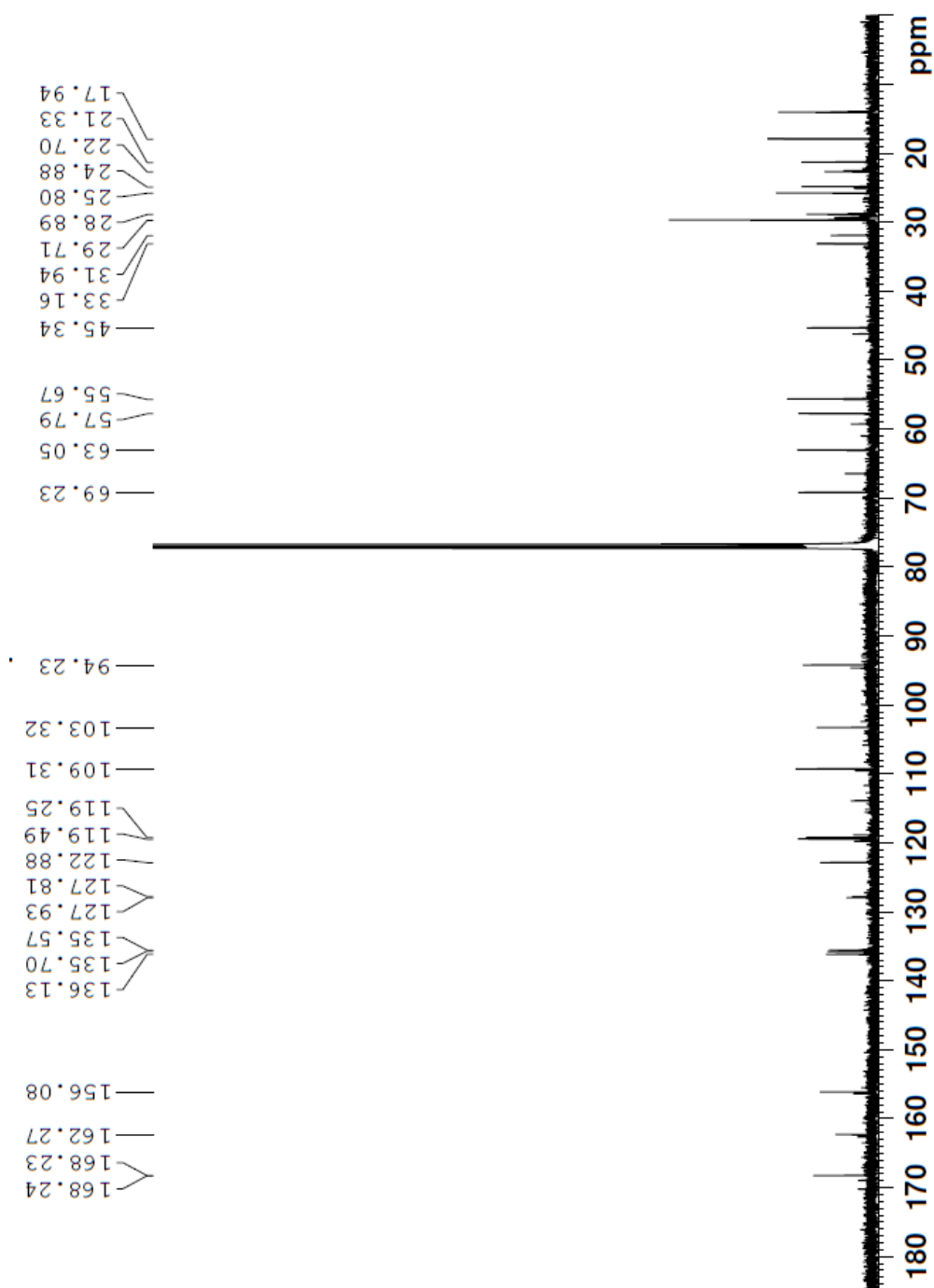


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	94.61	C25 H31 N3 O5	[M+Na] <sup>+</sup>	476.2163	476.2156	0.7	1.47	95.74	12.0

<sup>1</sup>H NMR Spectrum of Compound 24B in CDCl<sub>3</sub>

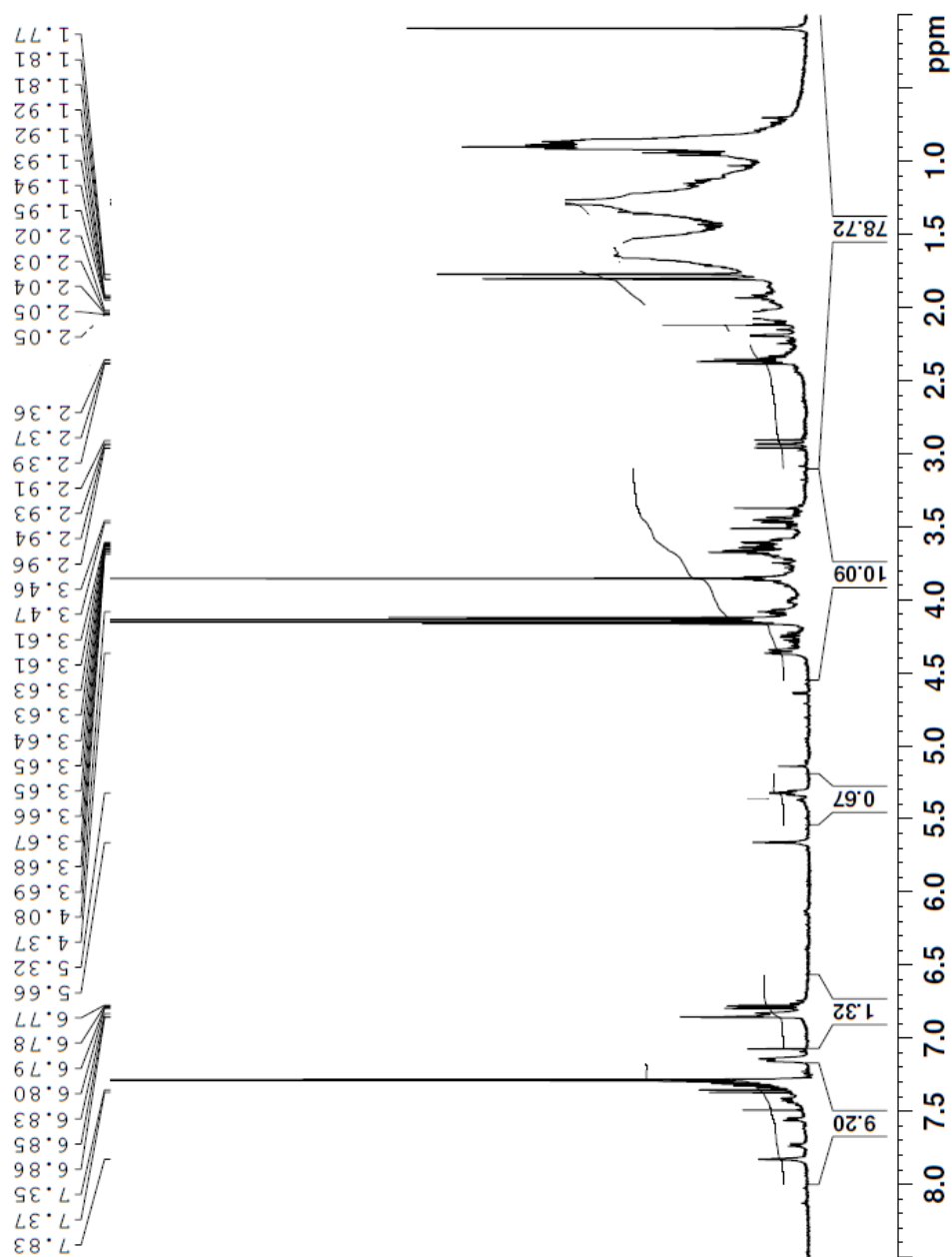


<sup>13</sup>C NMR Spectrum of Compound 24B in CDCl<sub>3</sub>

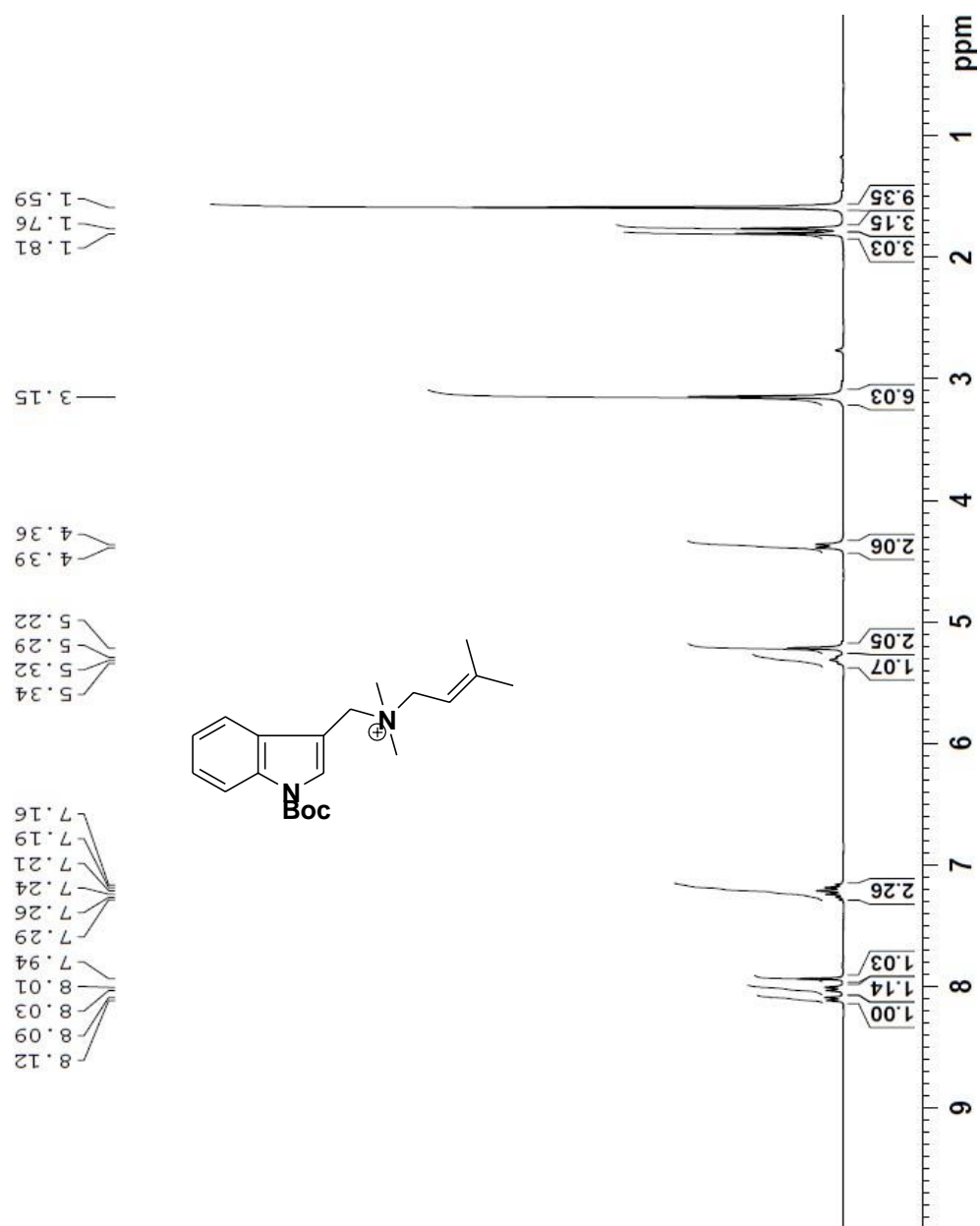




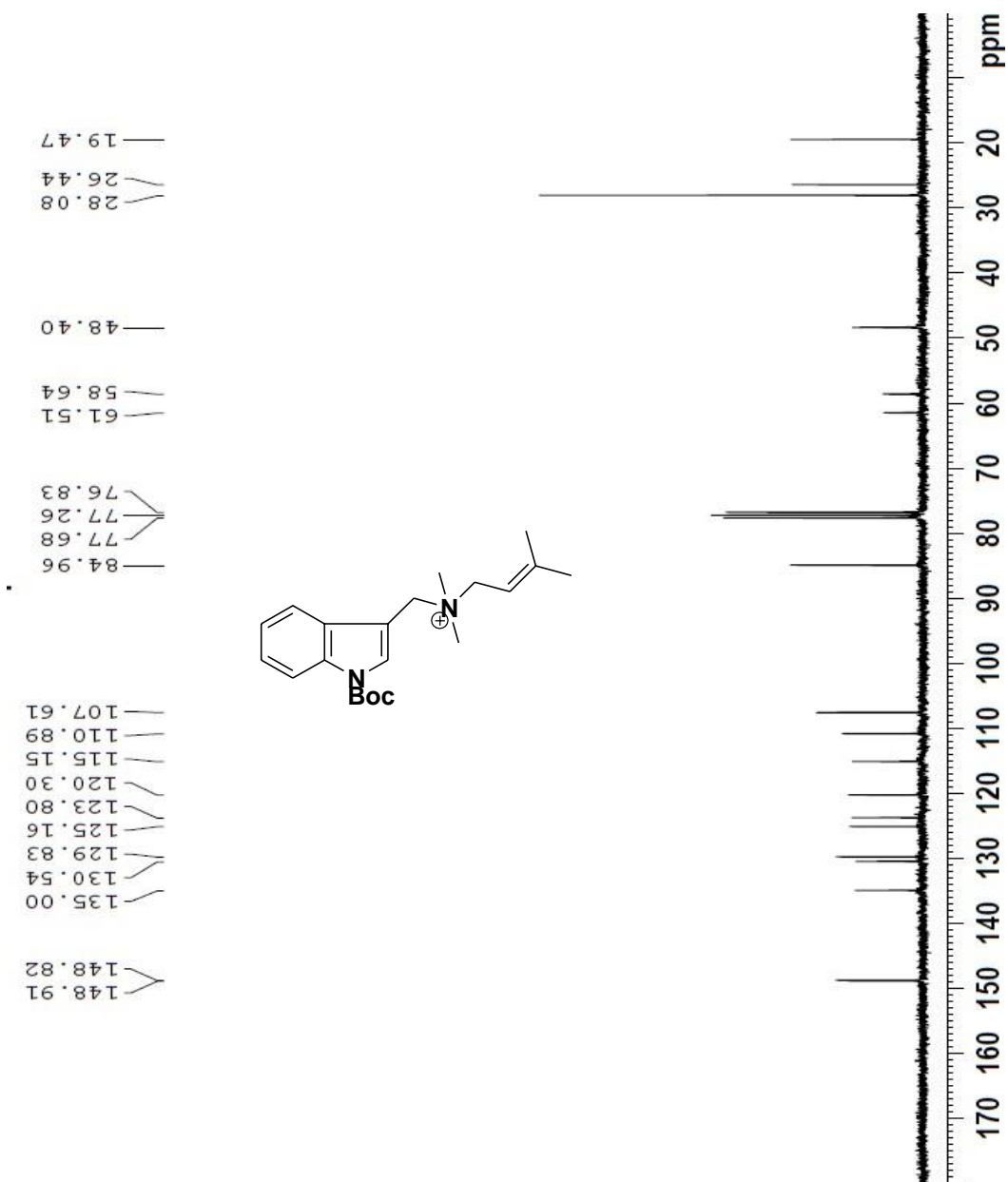
**$^{13}\text{C}$  NMR Spectrum of Compound 25 in  $\text{CDCl}_3$**



<sup>1</sup>H NMR Spectrum of Compound 29a in CDCl<sub>3</sub>

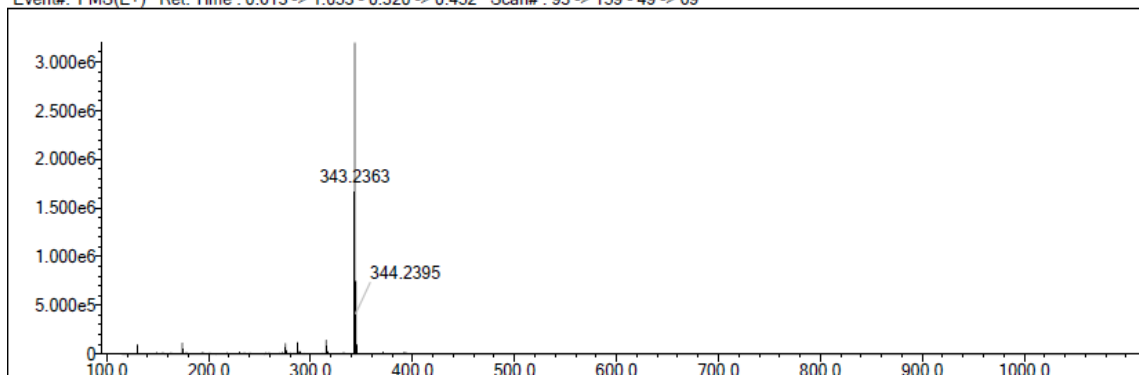


<sup>13</sup>C NMR Spectrum of Compound 29a in CDCl<sub>3</sub>

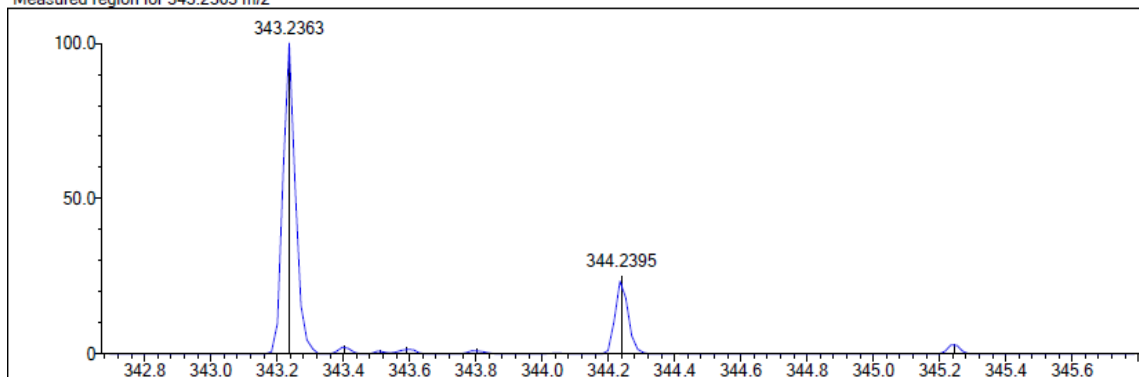


## HRMS of Compound 29a

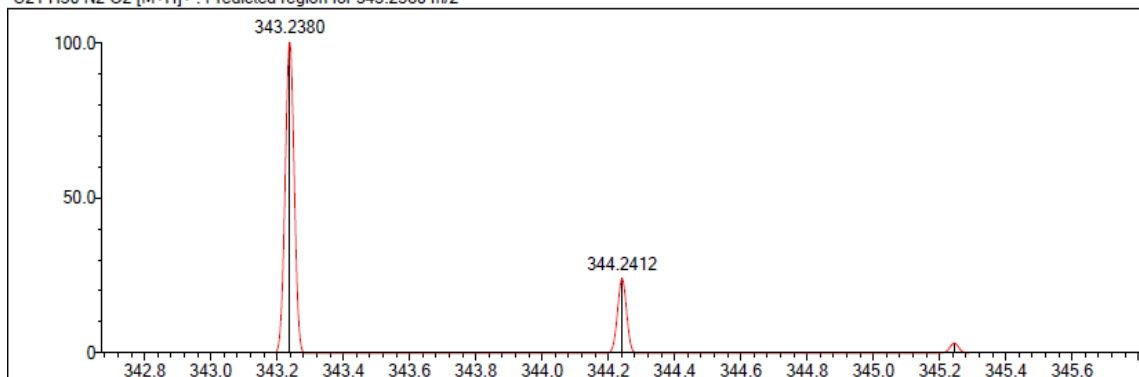
Event#: 1 MS(E+) Ret. Time : 0.613 -> 1.053 - 0.320 -> 0.452 Scan#: 93 -> 159 - 49 -> 69



Measured region for 343.2363 m/z

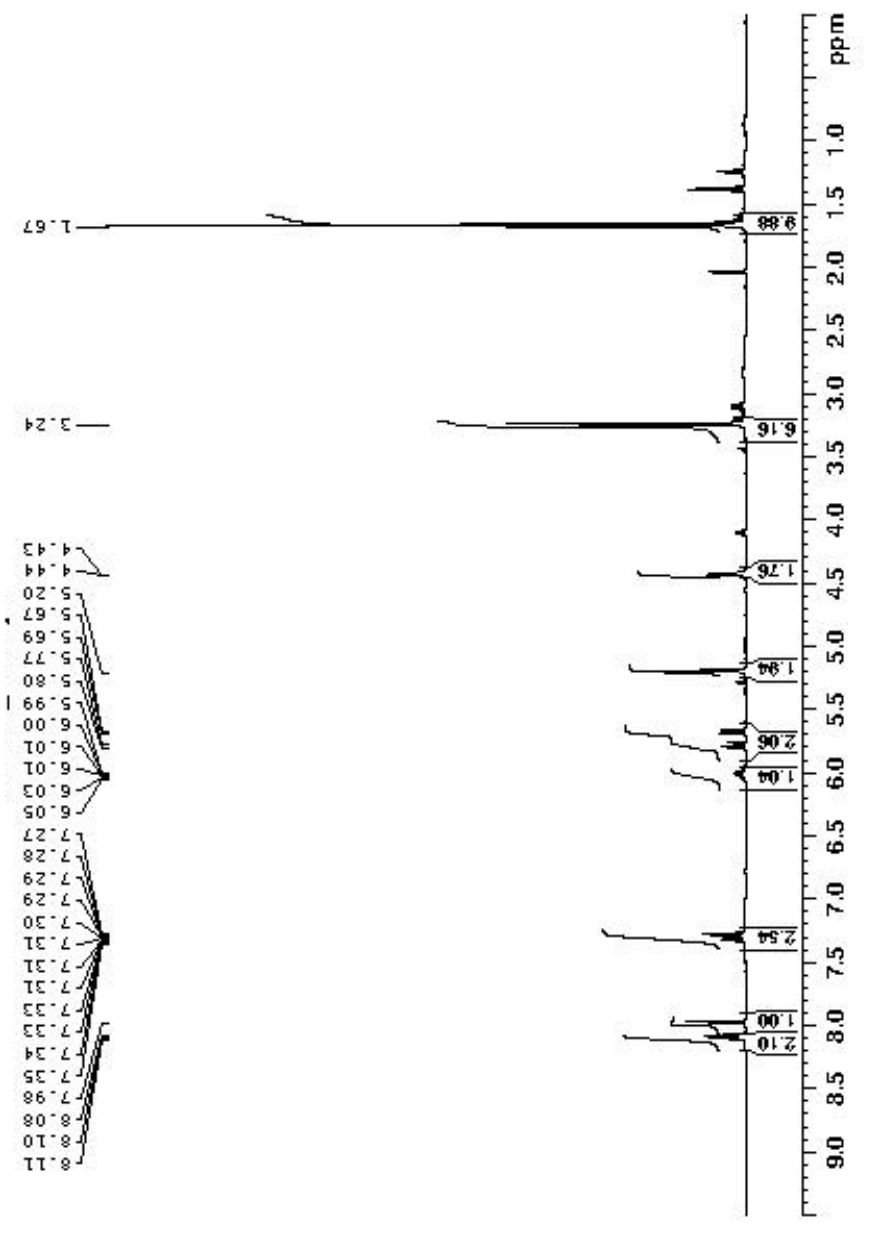


C21 H30 N2 O2 [M+H]<sup>+</sup> : Predicted region for 343.2380 m/z

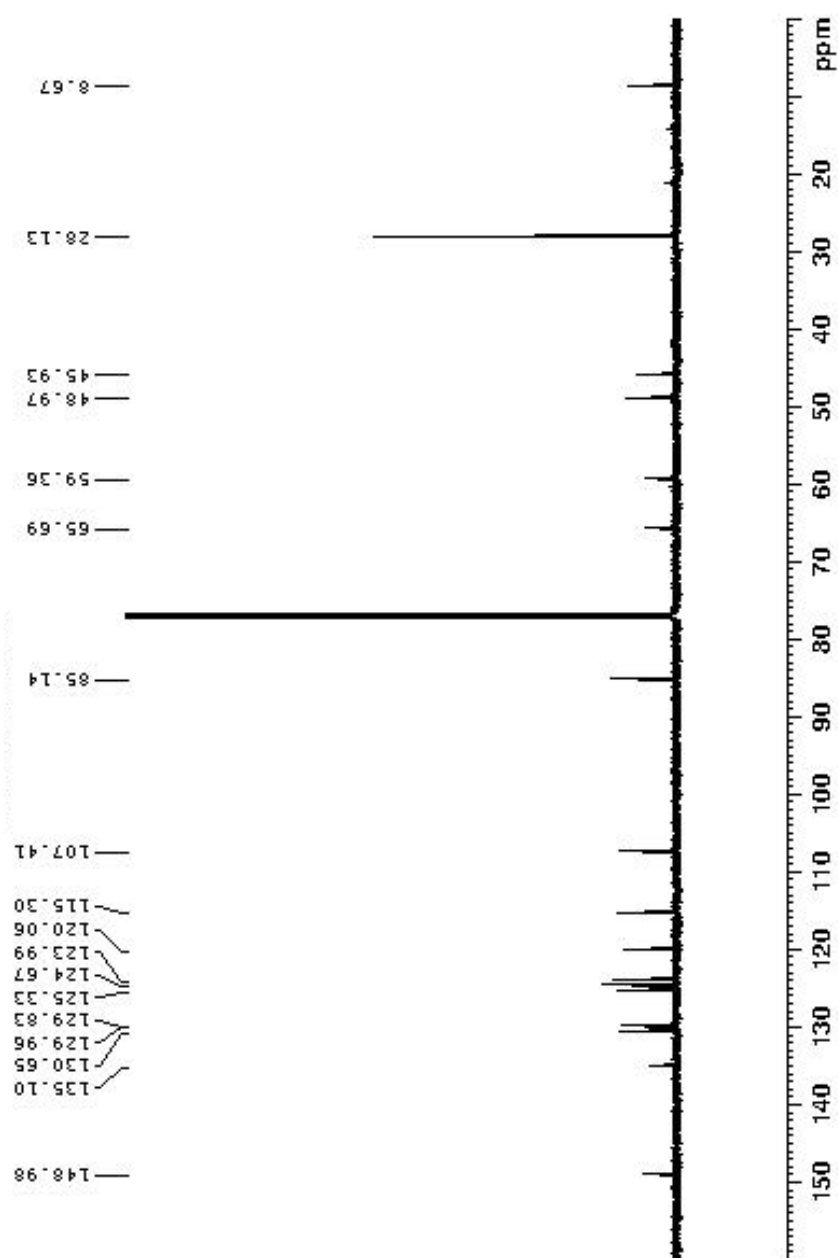


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	78.28	C <sub>21</sub> H <sub>30</sub> N <sub>2</sub> O <sub>2</sub>	[M+H] <sup>+</sup>	343.2363	343.2380	-1.7	-4.95	86.86	8.0

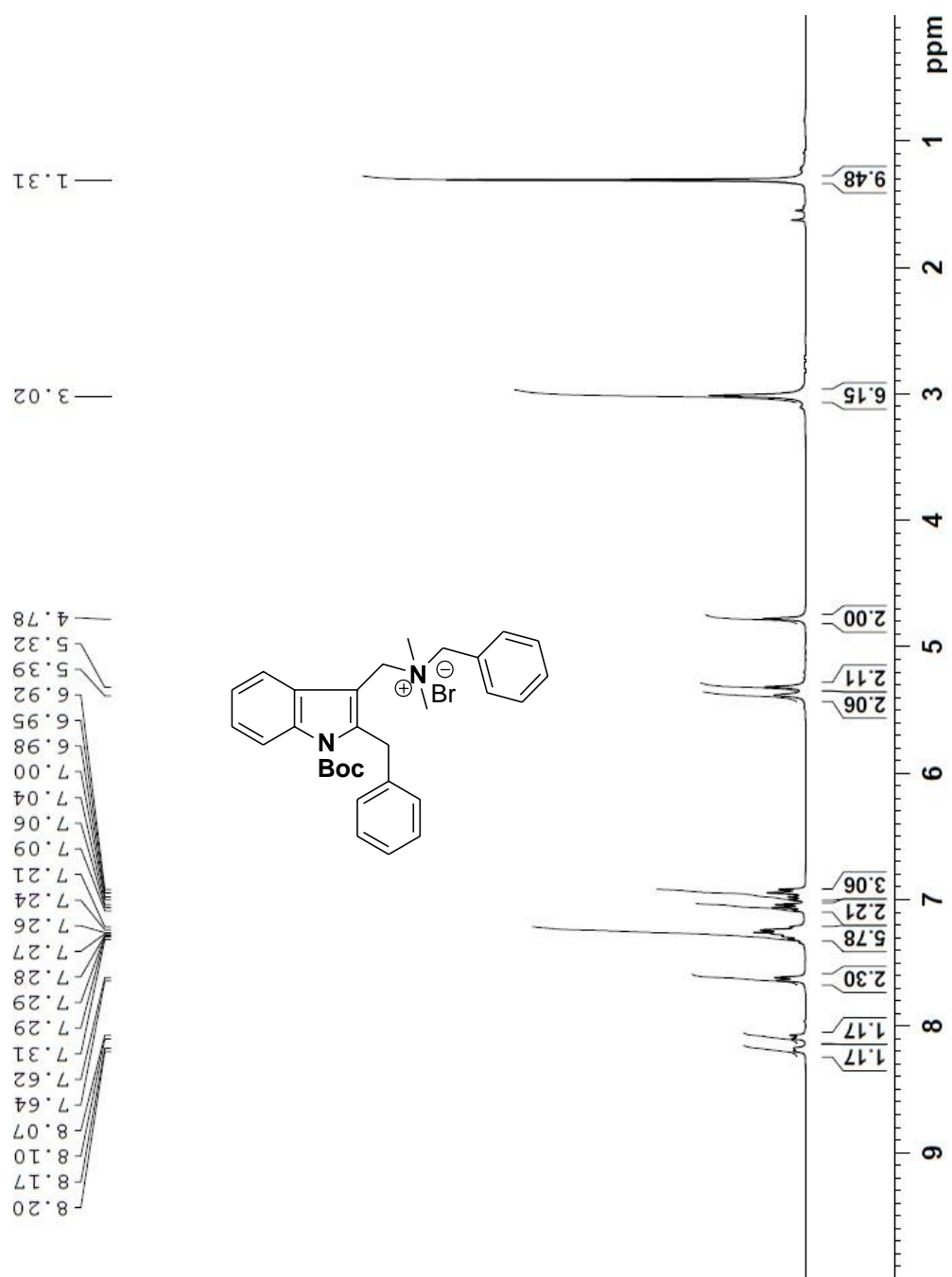
**<sup>1</sup>H NMR Spectrum of Compound 29b in CDCl<sub>3</sub>**



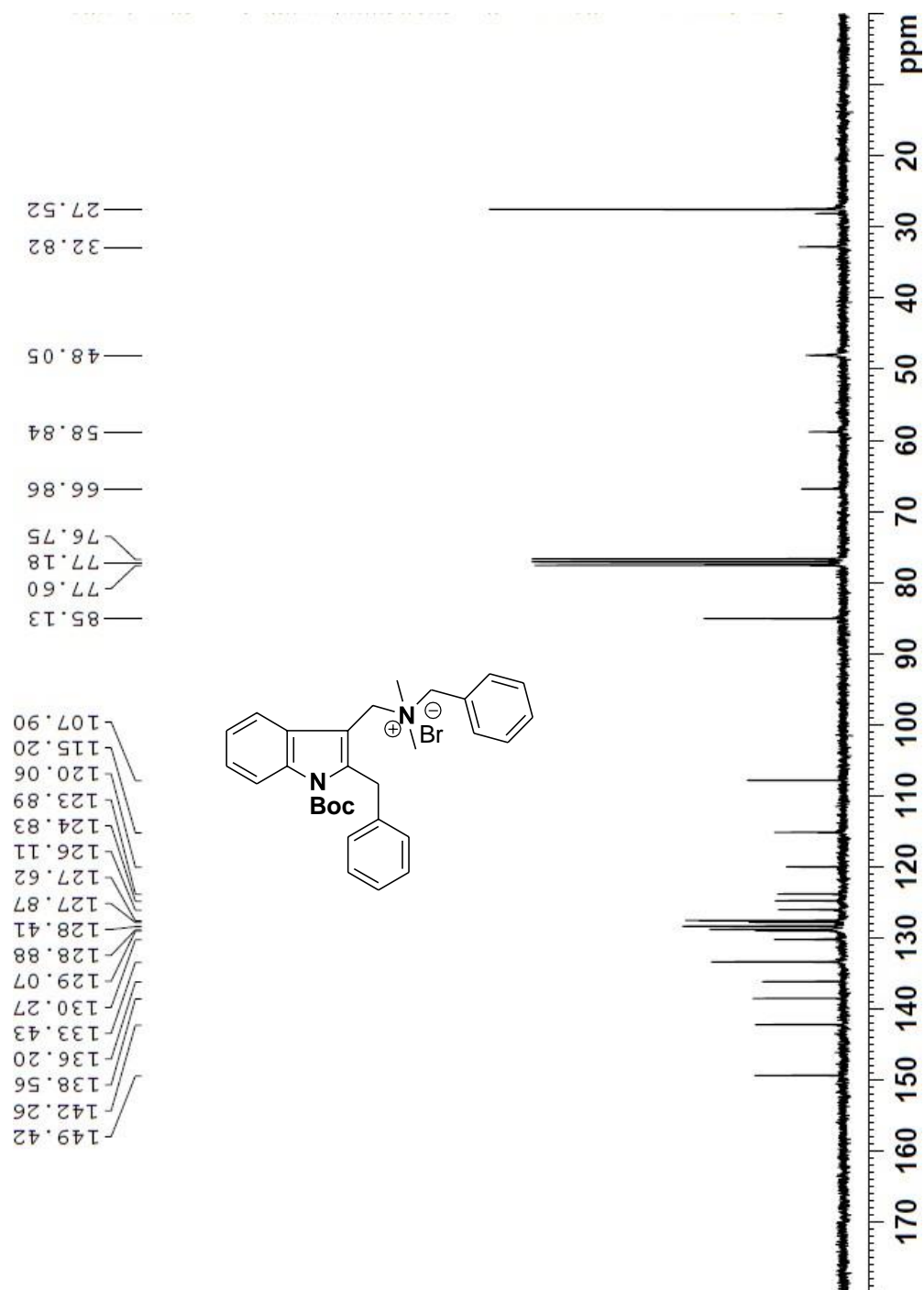
**$^{13}\text{C}$  NMR Spectrum of Compound 29b in  $\text{CDCl}_3$**



<sup>1</sup>H NMR Spectrum of Compound 28c in CDCl<sub>3</sub>

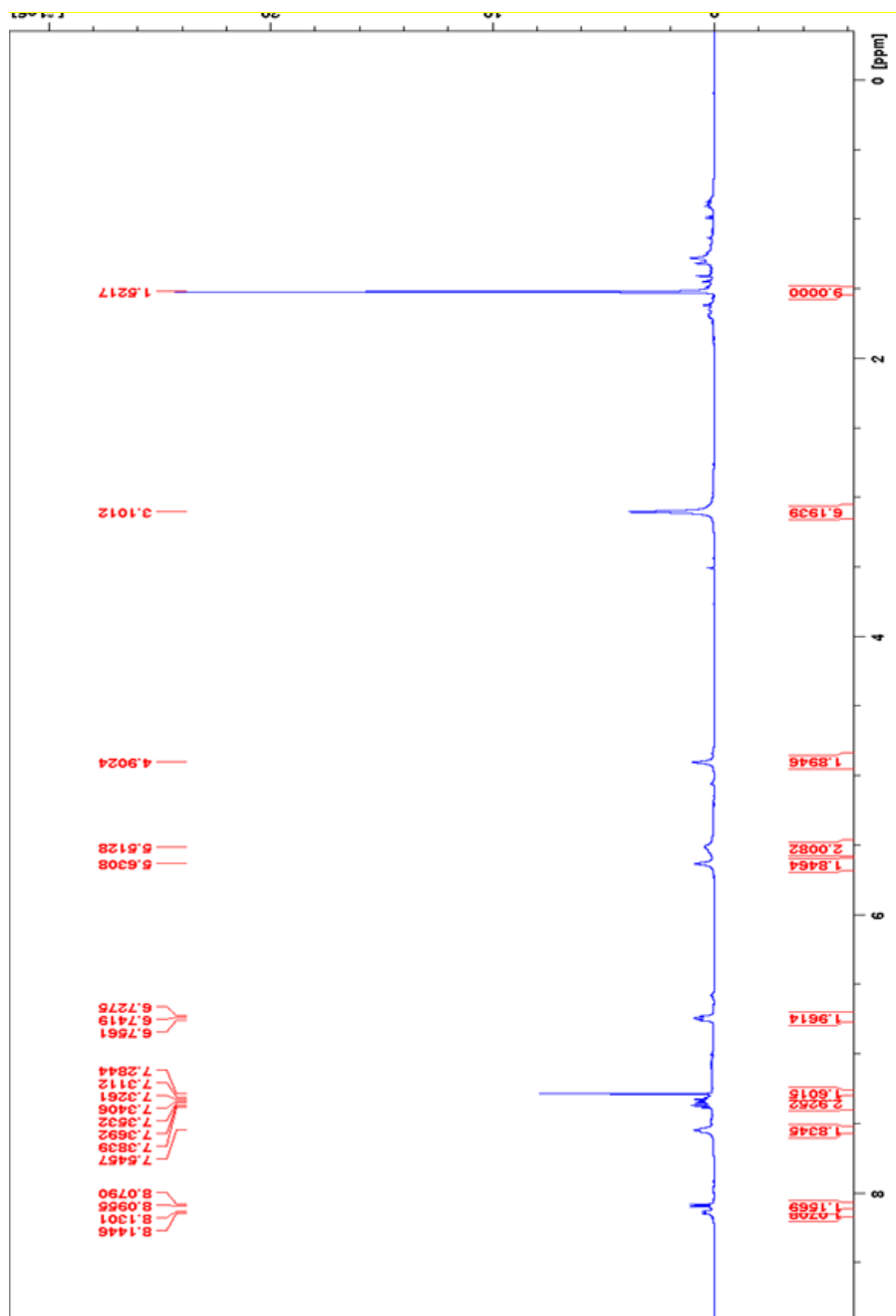


<sup>13</sup>C NMR Spectrum of Compound 28c in CDCl<sub>3</sub>

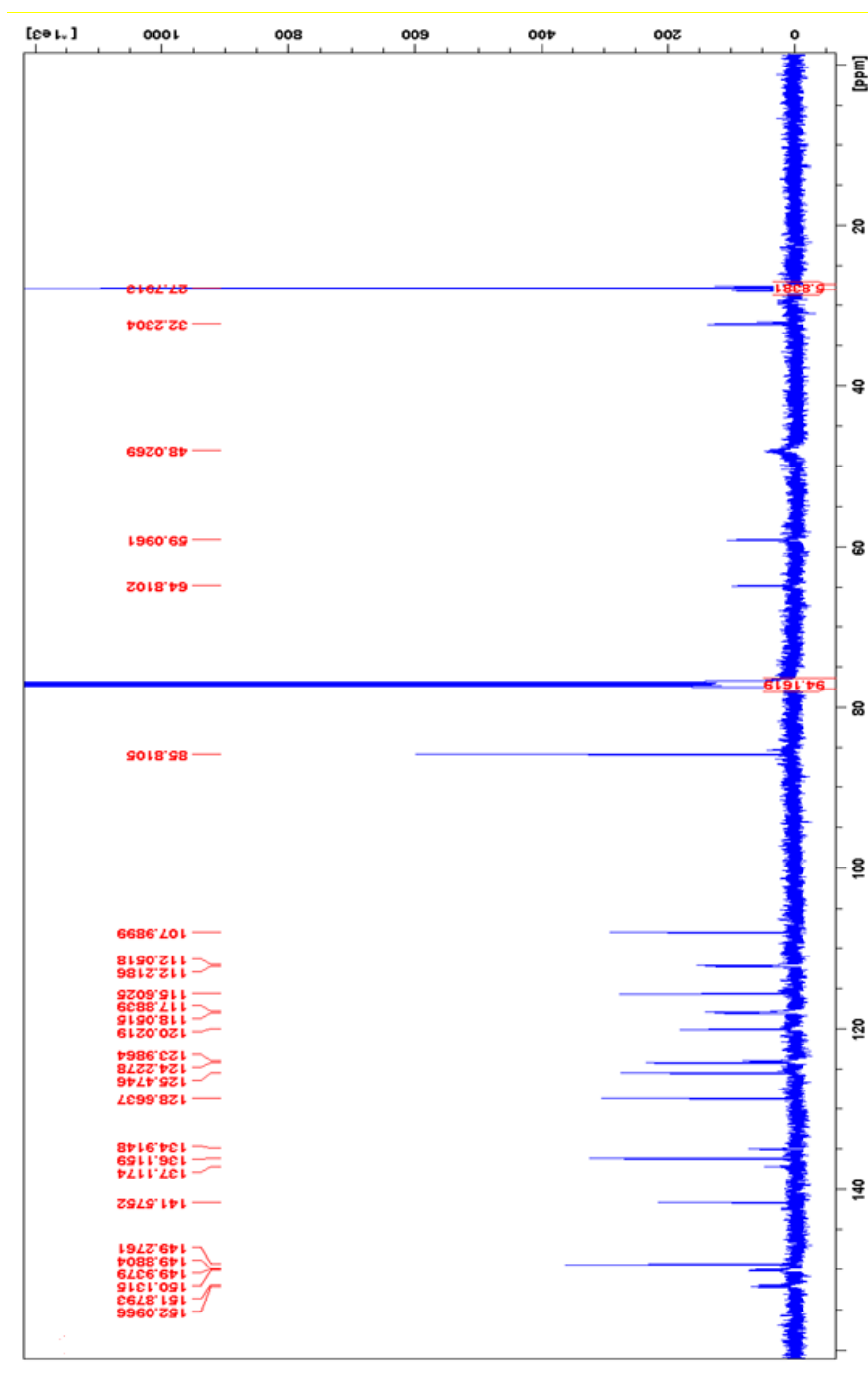




<sup>1</sup>H NMR Spectrum of Compound 28e in CDCl<sub>3</sub>

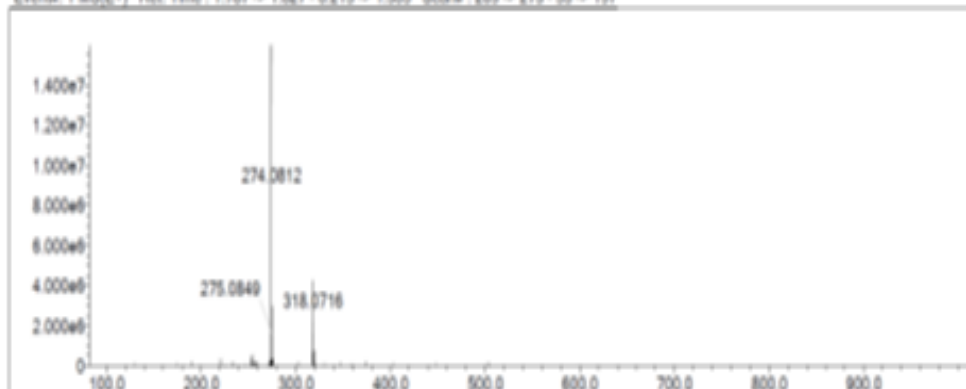


**$^{13}\text{C}$  NMR Spectrum of Compound 28c in  $\text{CDCl}_3$**



## HRMS of Compound 28e

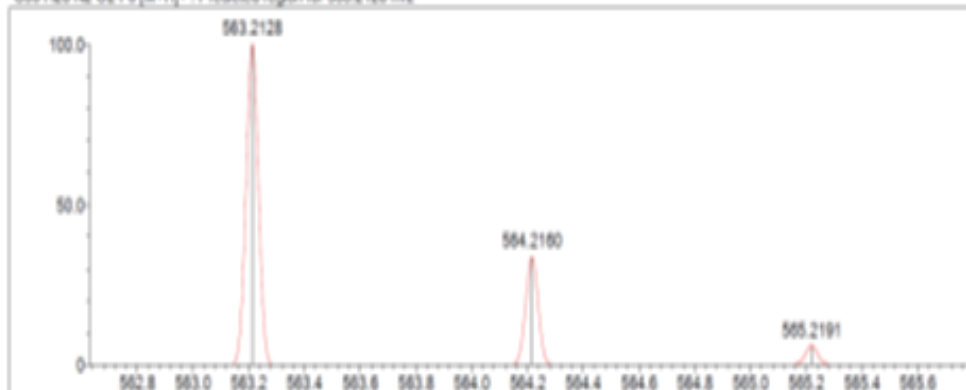
Event# 1 MS(E+) Ret. Time : 1.787 -> 1.827 - 0.213 -> 1.305 Scan# : 269 -> 275 - 33 -> 197



Measured region for 563.2149 m/z

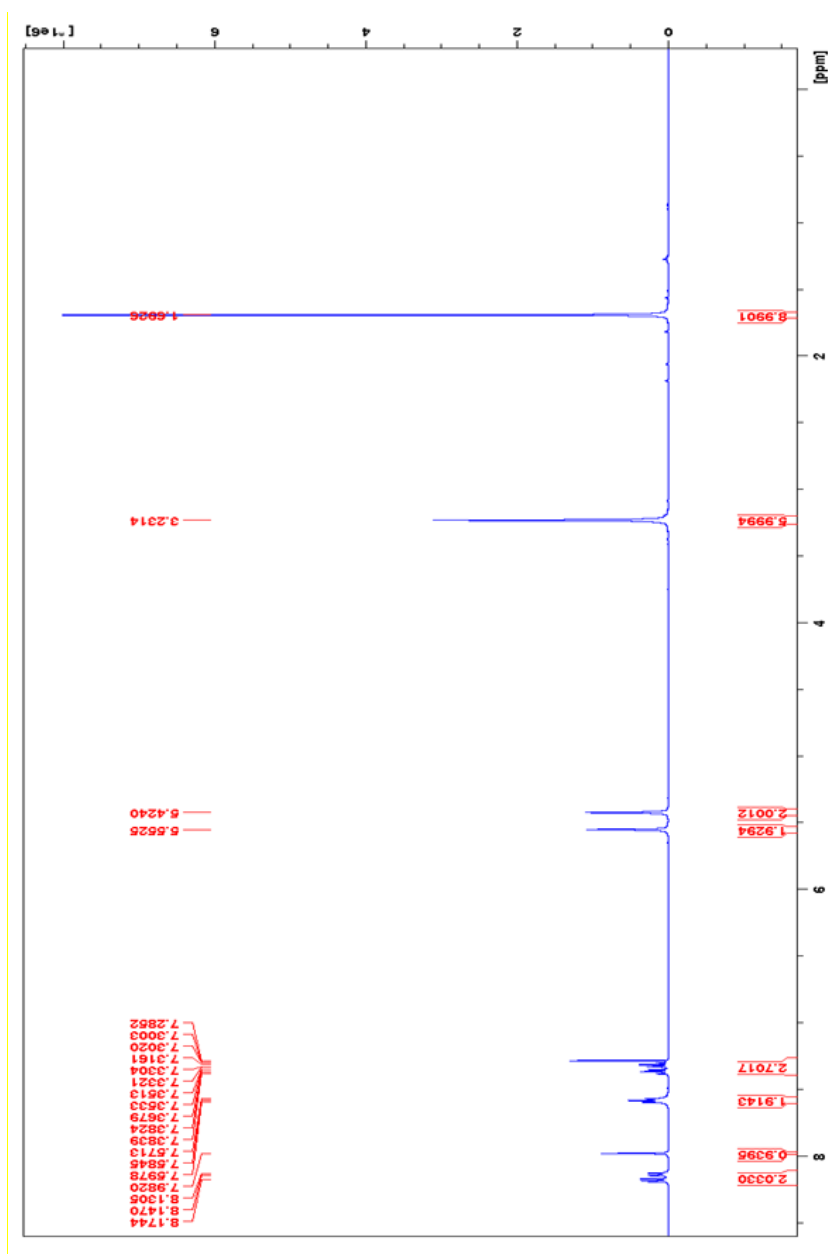


C30 H28 N2 O2 F6 (M+H)+ : Predicted region for 563.2128 m/z

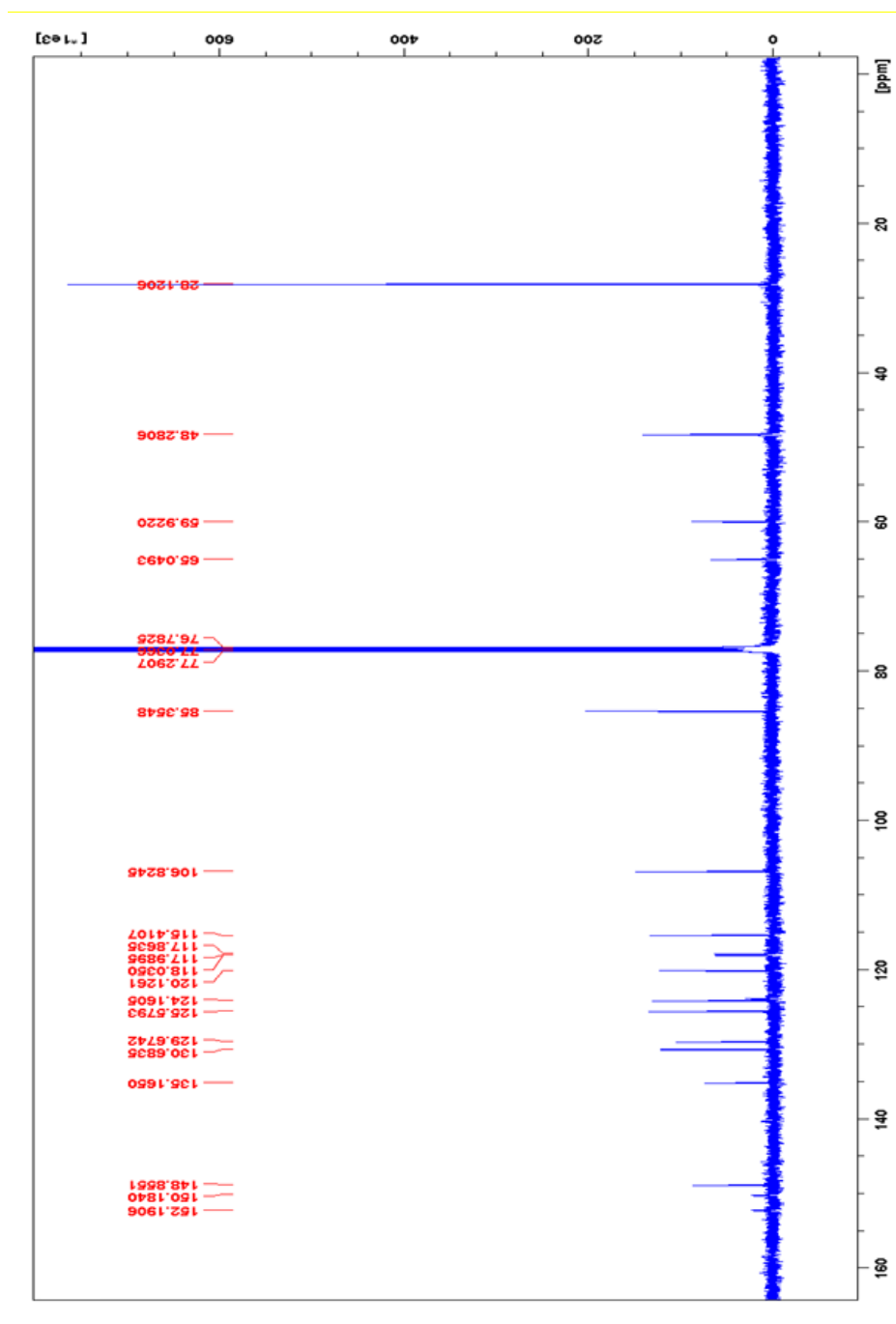


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Diff. (mDa)	Diff. (ppm)	Iso	DBE
1	0.00	C30H28N2O2F6	[M+H] <sup>+</sup>	563.2149	563.2128	2.1	3.73	0.00	15.0

# <sup>1</sup>H NMR Spectrum of Compound 29e in CDCl<sub>3</sub>

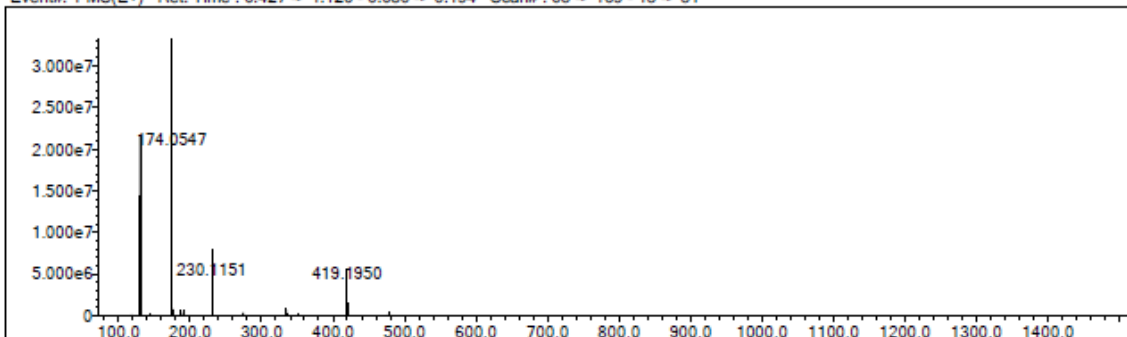


**$^{13}\text{C}$  NMR Spectrum of Compound 29e in  $\text{CDCl}_3$**

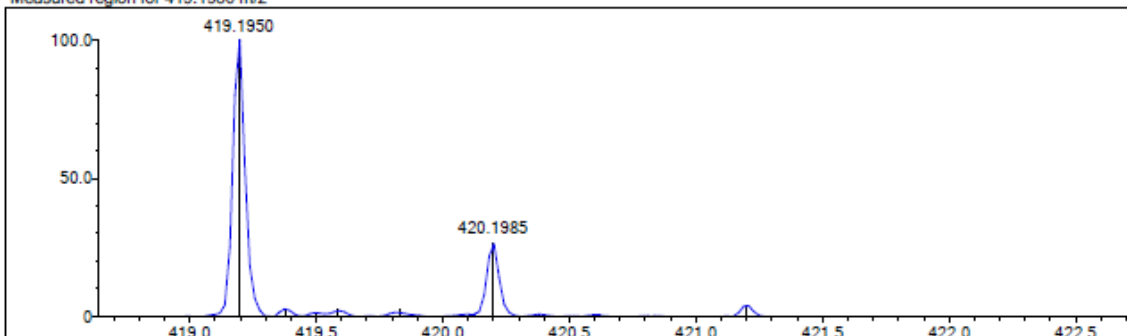


## HRMS of Compound 29e

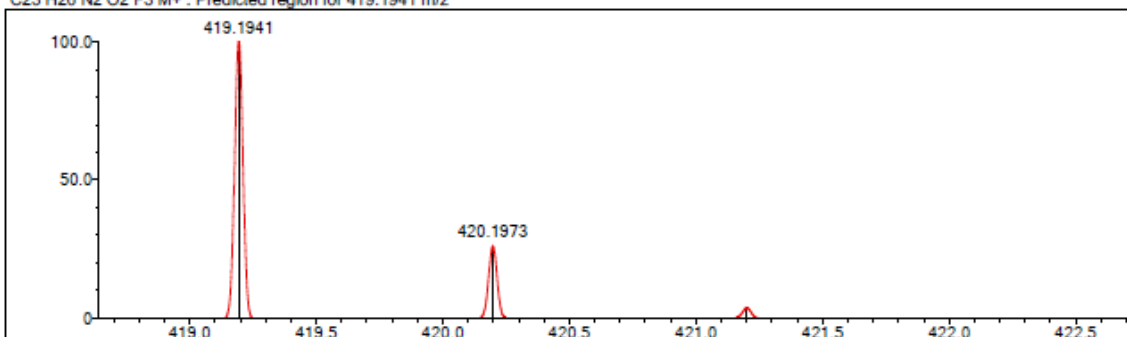
Event#: 1 MS(E+) Ret. Time : 0.427 -> 1.120 - 0.080 -> 0.194 Scan#: 65 -> 169 - 13 -> 31



Measured region for 419.1950 m/z

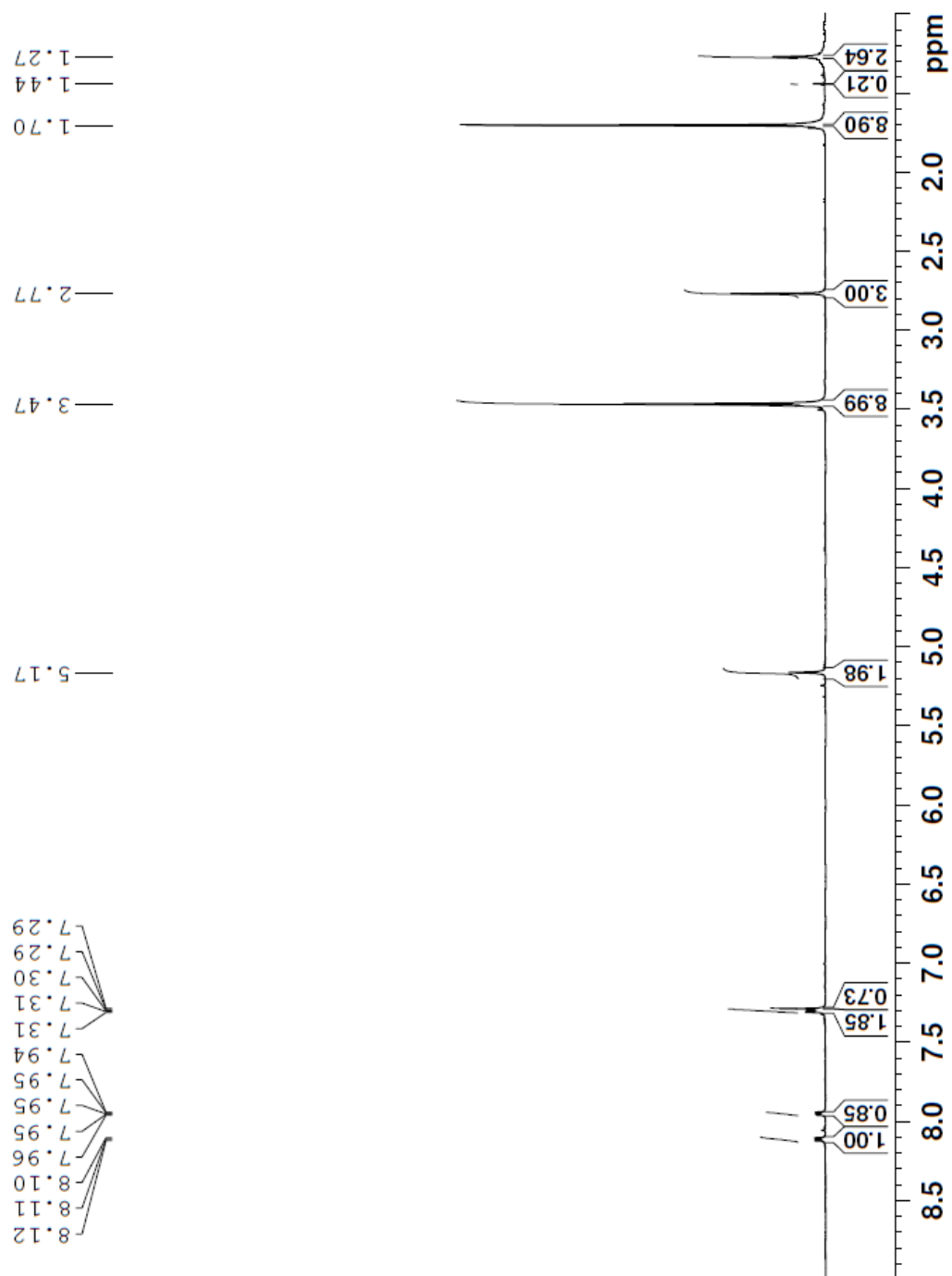


C23 H26 N2 O2 F3 M+ : Predicted region for 419.1941 m/z

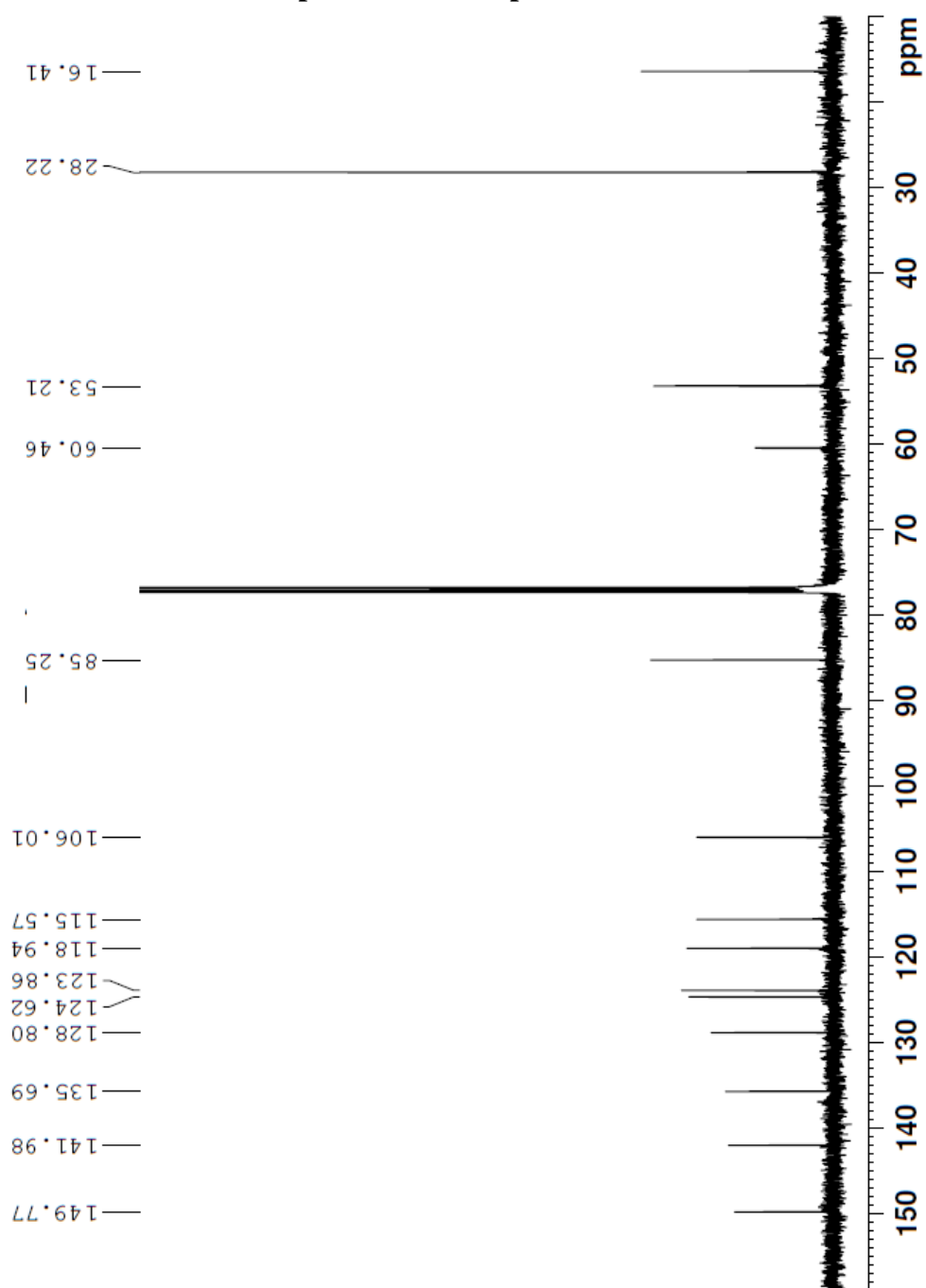


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
6	96.01	C23 H26 N2 O2 F3	M+	419.1950	419.1941	0.9	2.15	98.86	10.5

<sup>1</sup>H NMR Spectrum of Compound 31a in CDCl<sub>3</sub>



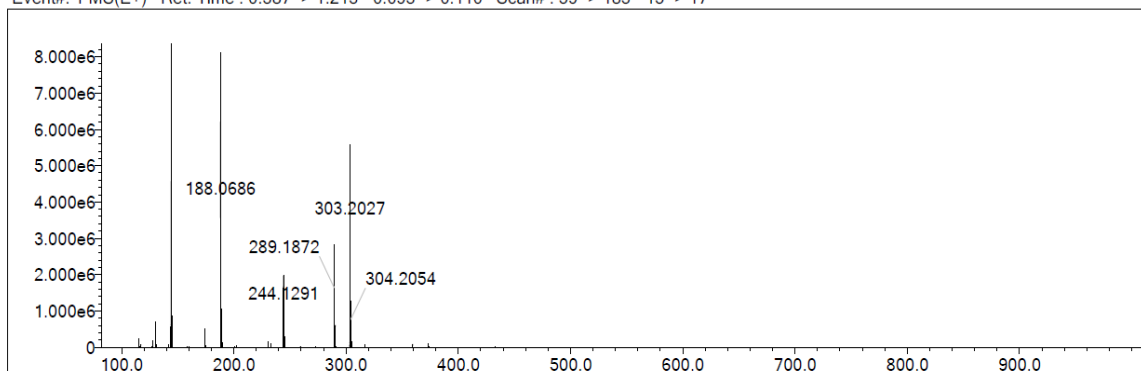
<sup>13</sup>C NMR Spectrum of Compound 31a in CDCl<sub>3</sub>



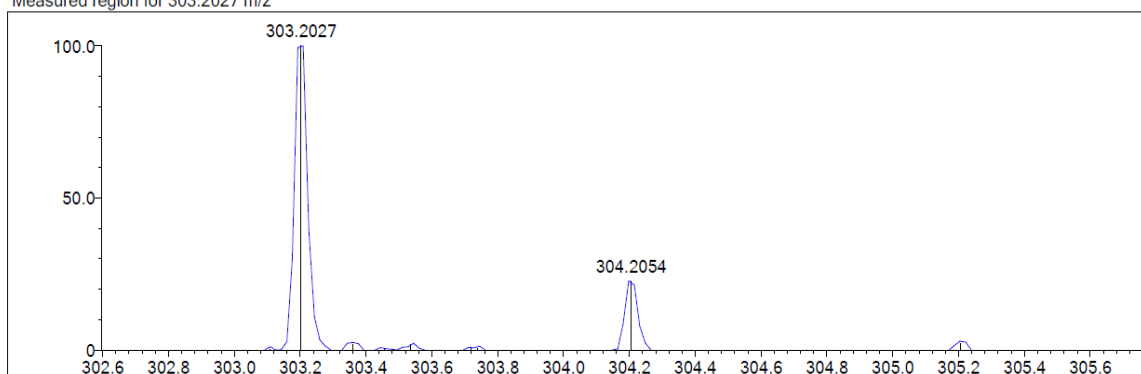


# HRMS of Compound 31a

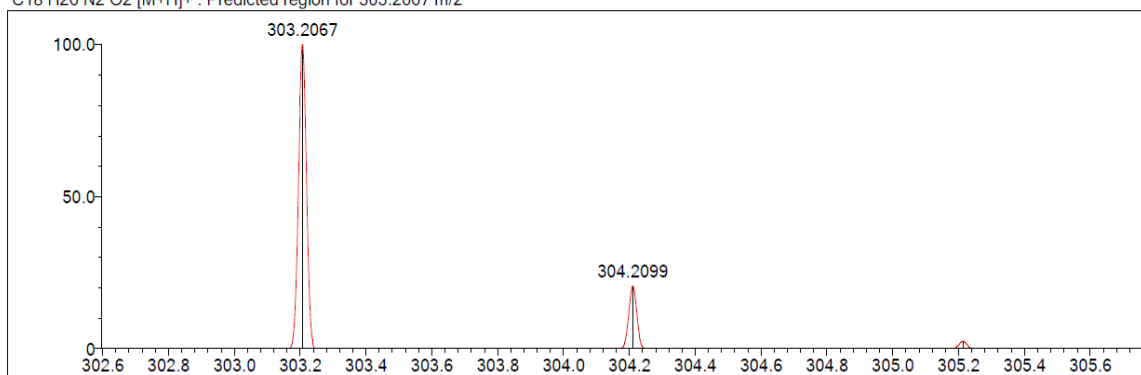
Event#: 1 MS(E+) Ret. Time : 0.387 -> 1.213 - 0.093 -> 0.110 Scan#: 59 -> 183 - 15 -> 17



Measured region for 303.2027 m/z

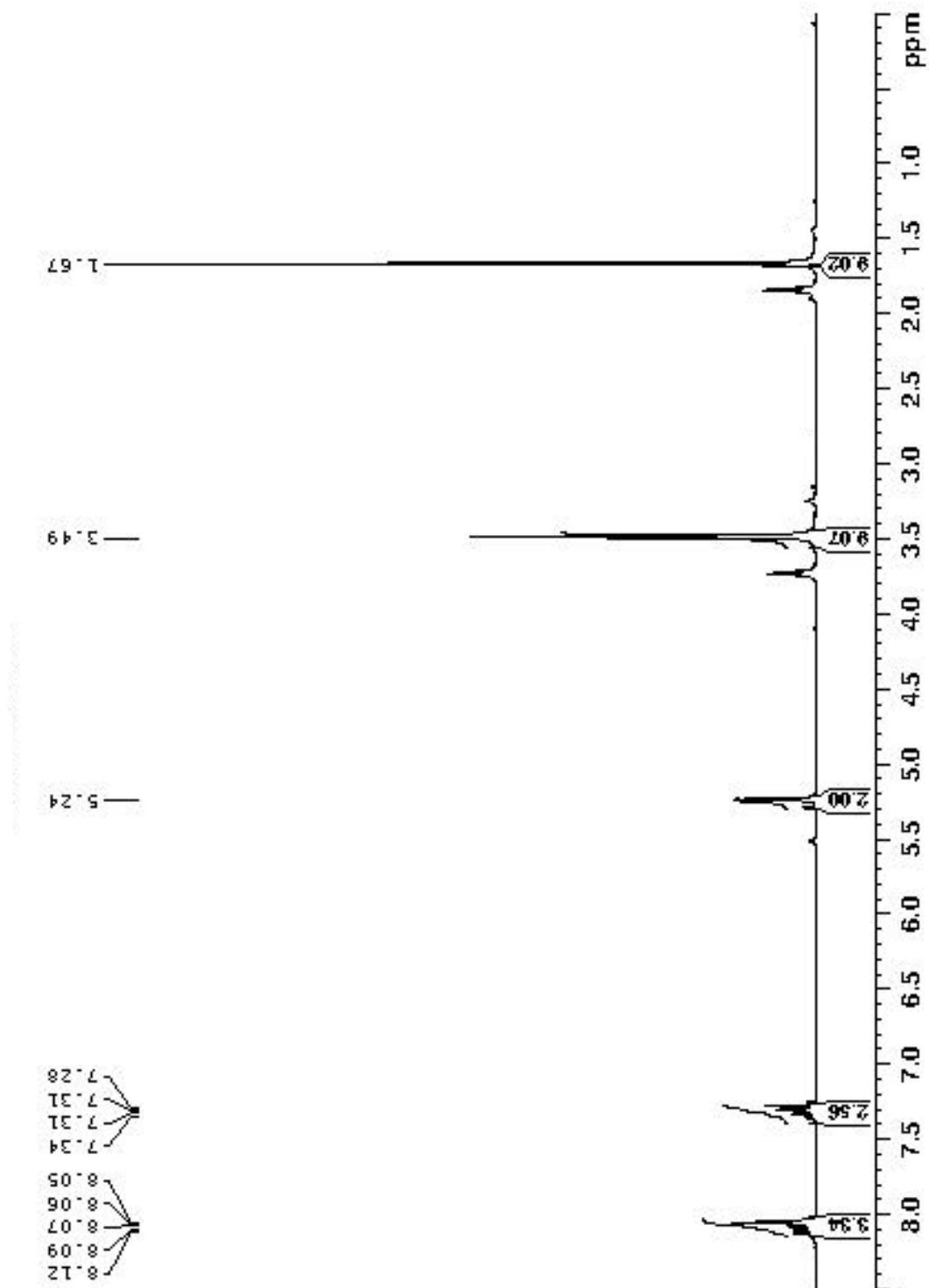


C18 H26 N2 O2 [M+H]<sup>+</sup> : Predicted region for 303.2067 m/z

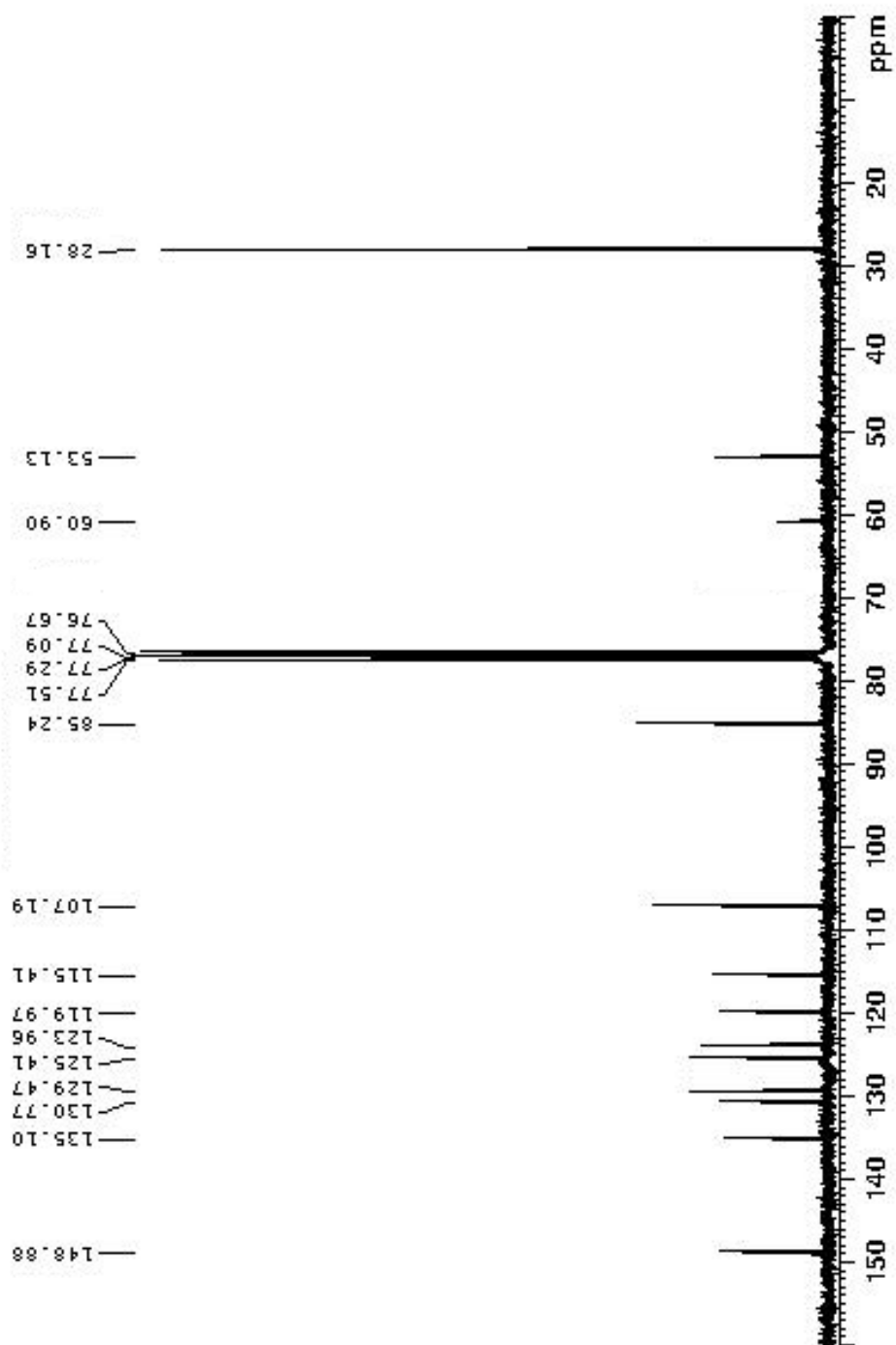


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	25.28	C18 H26 N2 O2	[M+H] <sup>+</sup>	303.2027	303.2067	-4.0	-13.19	80.28	7.0

<sup>1</sup>H NMR Spectrum of Compound 32a in CDCl<sub>3</sub>

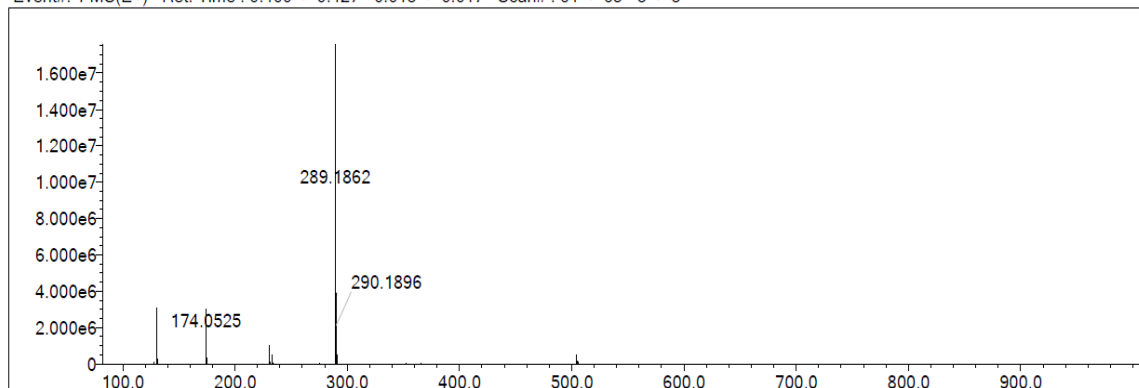


<sup>13</sup>C NMR Spectrum of Compound 32a in CDCl<sub>3</sub>

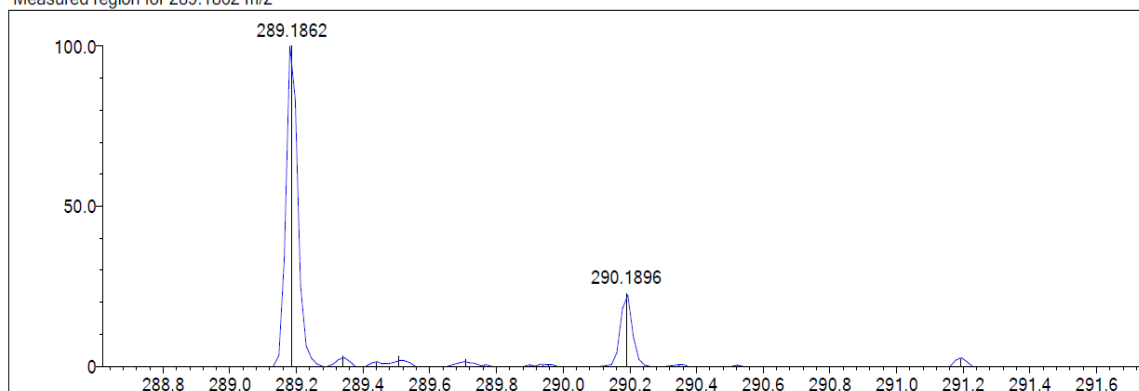


# HRMS of Compound 32a

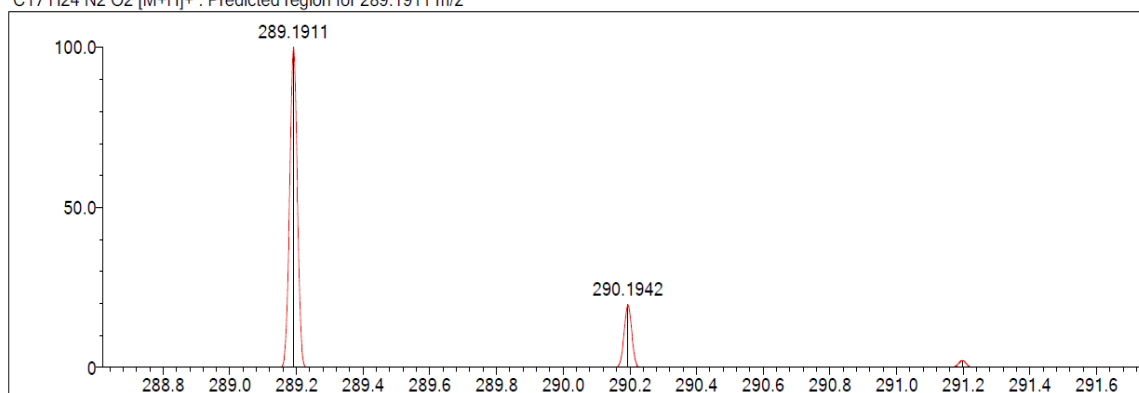
Event#: 1 MS(E+) Ret. Time : 0.400 -> 0.427 - 0.013 -> 0.017 Scan#: 61 -> 65 - 3 -> 3



Measured region for 289.1862 m/z

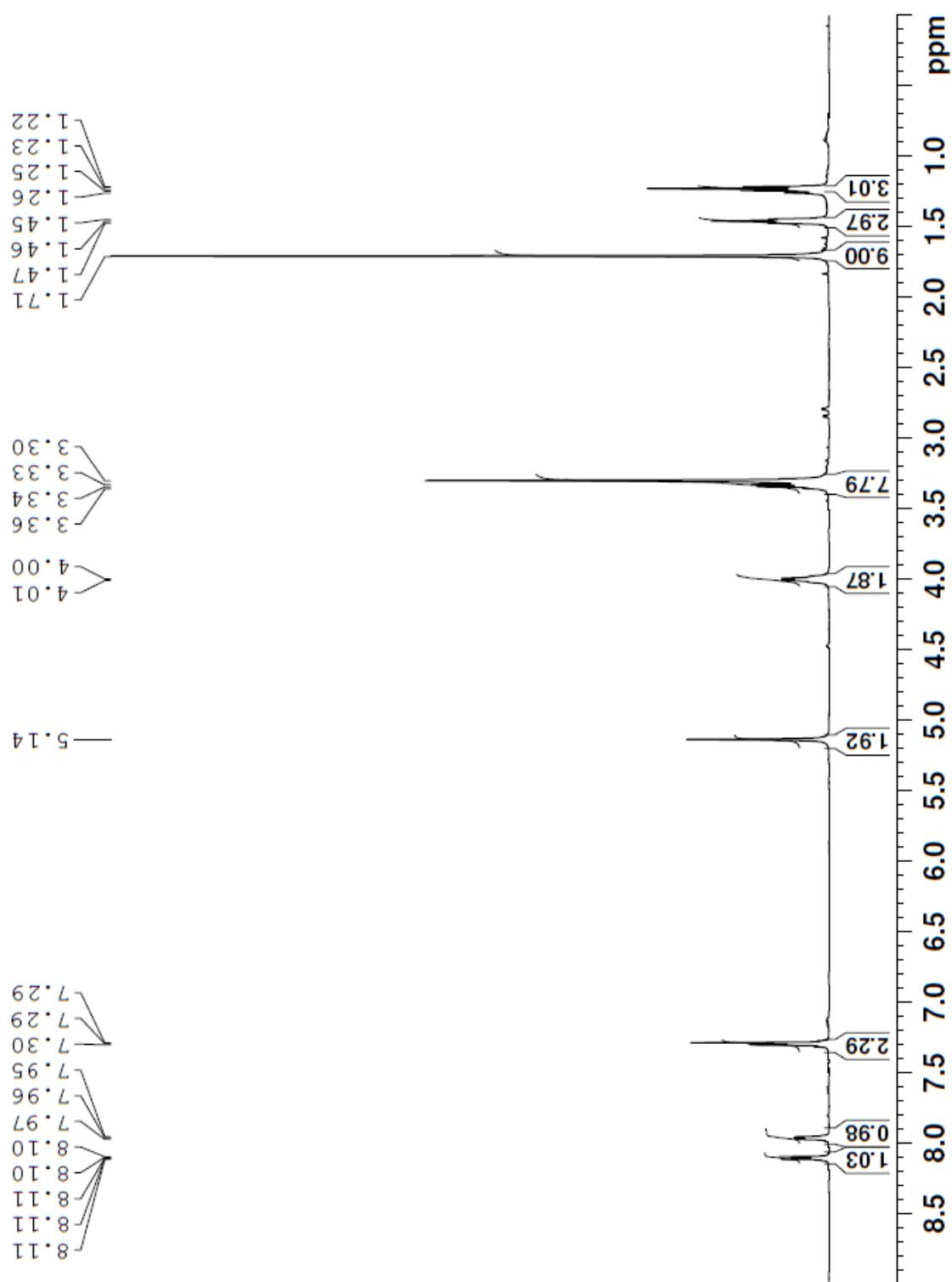


C17 H24 N2 O2 [M+H]<sup>+</sup> : Predicted region for 289.1911 m/z

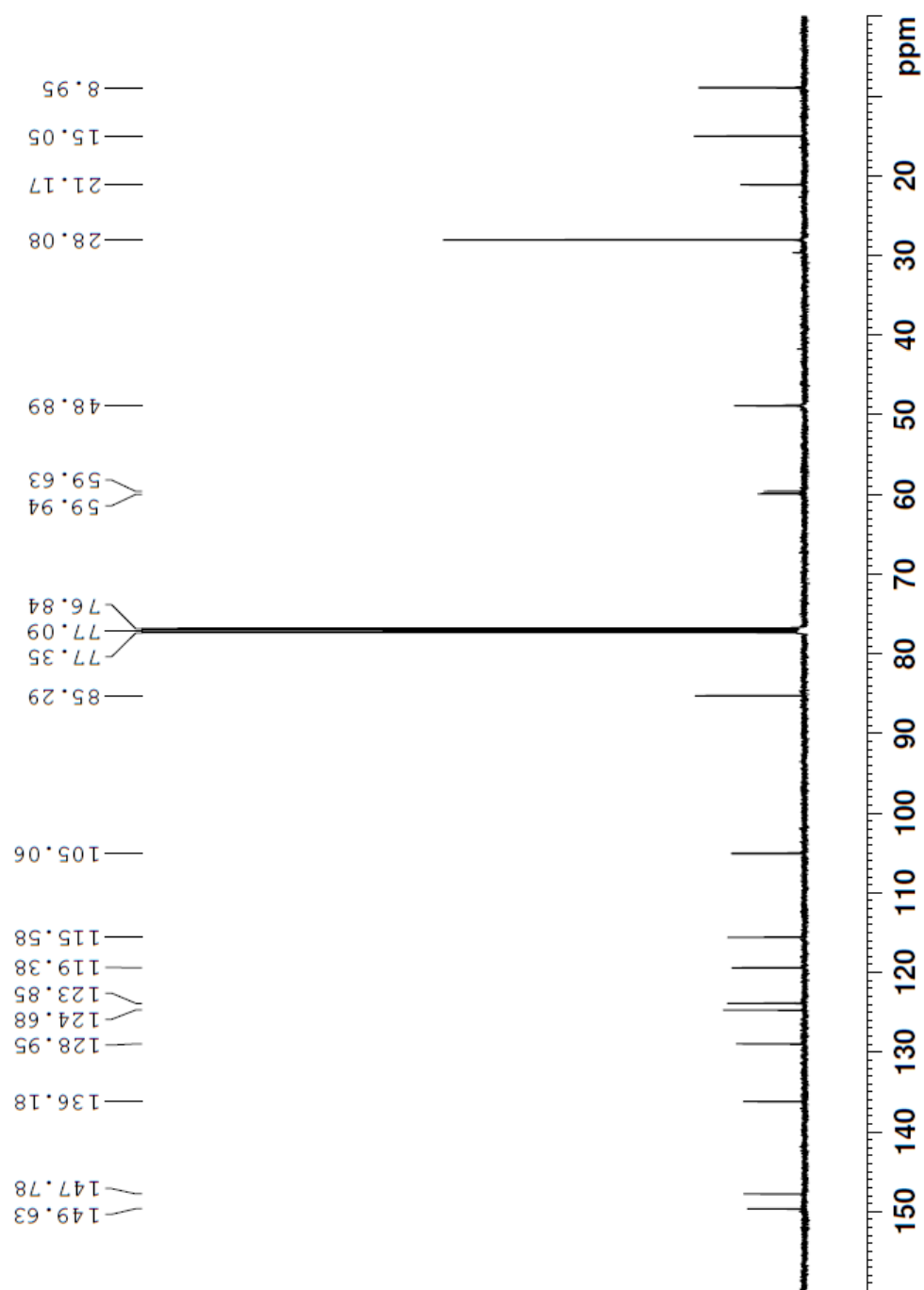


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	12.46	C17 H24 N2 O2	[M+H] <sup>+</sup>	289.1862	289.1911	-4.9	-16.94	57.99	7.0

<sup>1</sup>H NMR Spectrum of Compound 31b in CDCl<sub>3</sub>

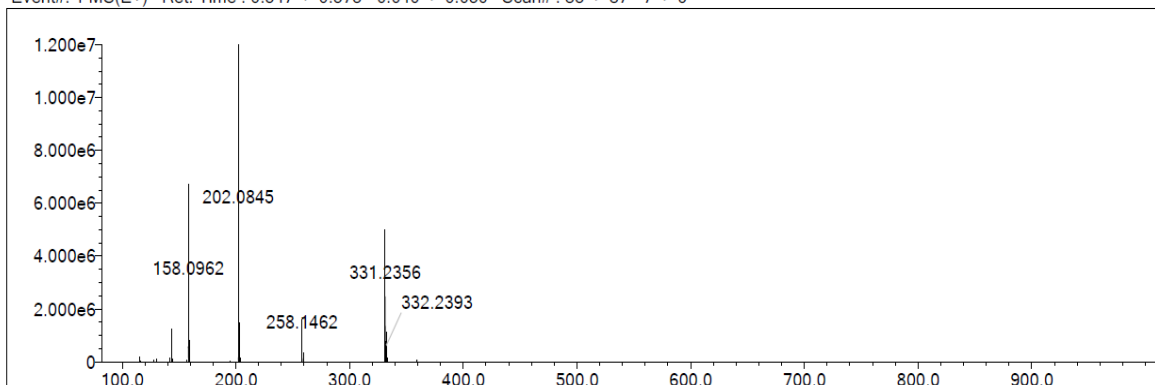


**$^{13}\text{C}$  NMR Spectrum of Compound 31b in  $\text{CDCl}_3$**

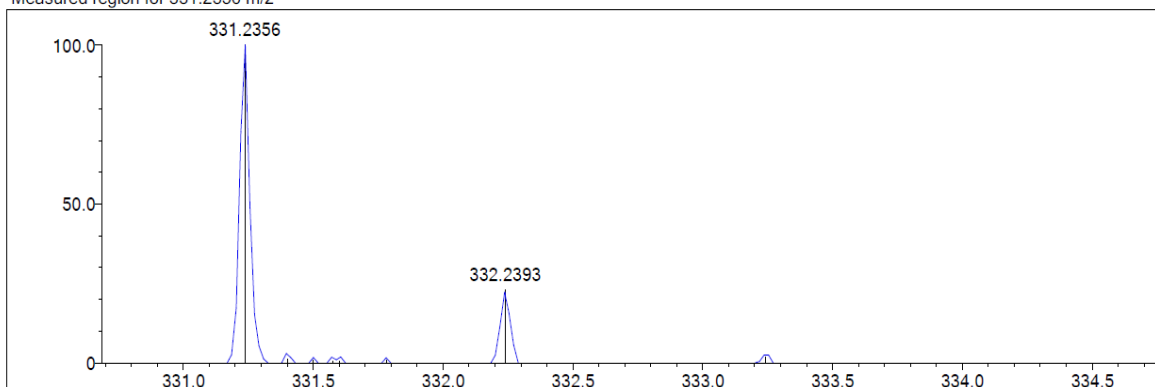


## HRMS of Compound 31b

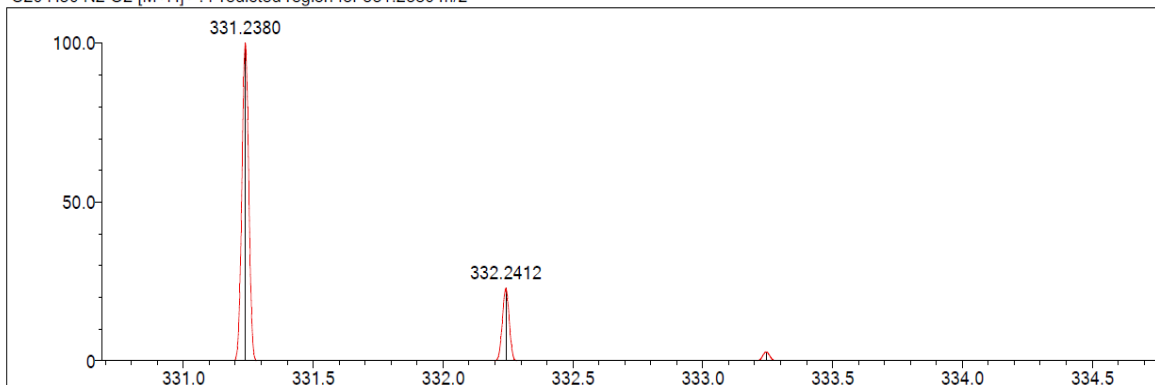
Event#: 1 MS(E+) Ret. Time : 0.547 -> 0.573 - 0.040 -> 0.050 Scan#: 83 -> 87 - 7 -> 9



Measured region for 331.2356 m/z

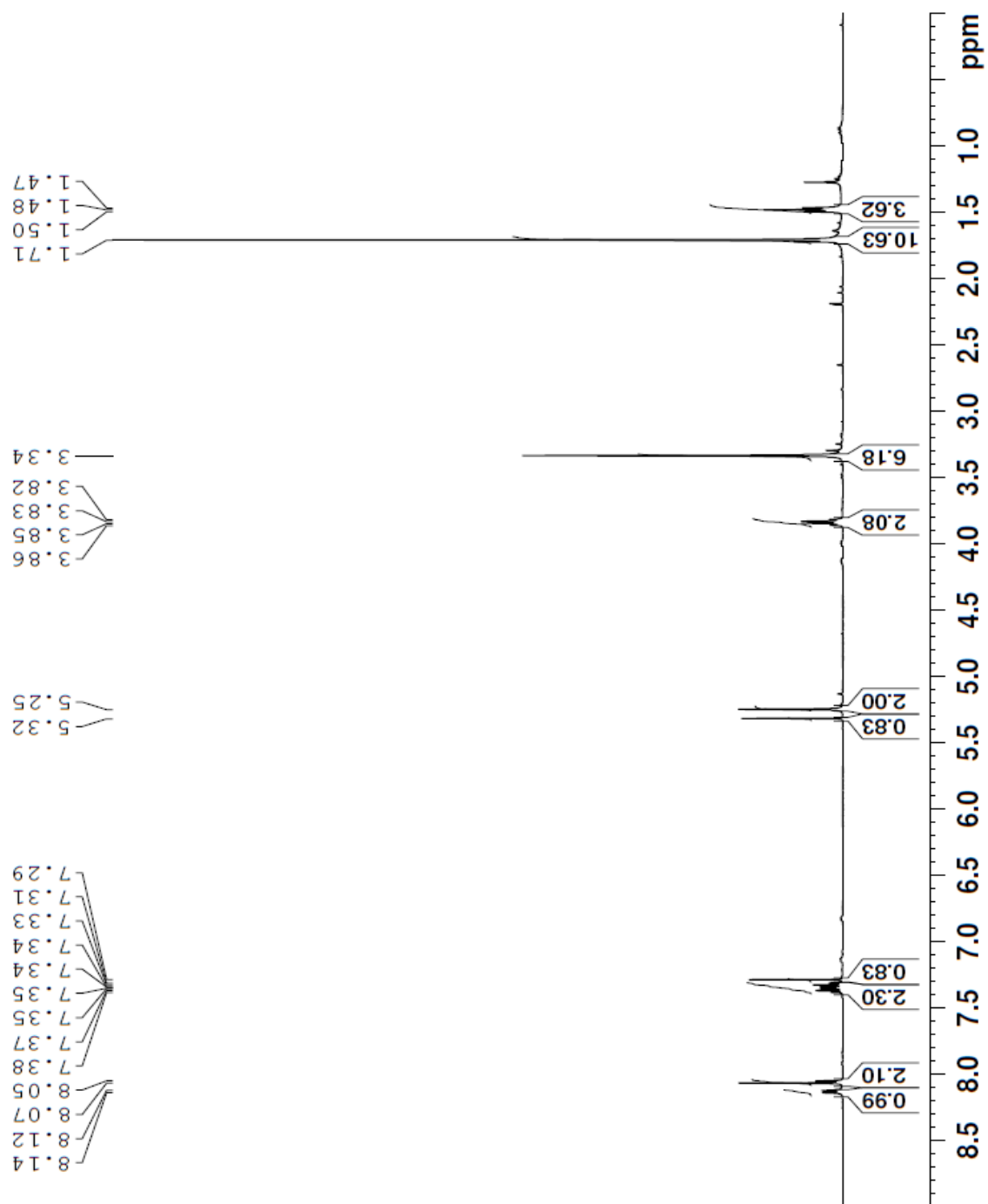


C20 H30 N2 O2 [M+H]<sup>+</sup> : Predicted region for 331.2380 m/z



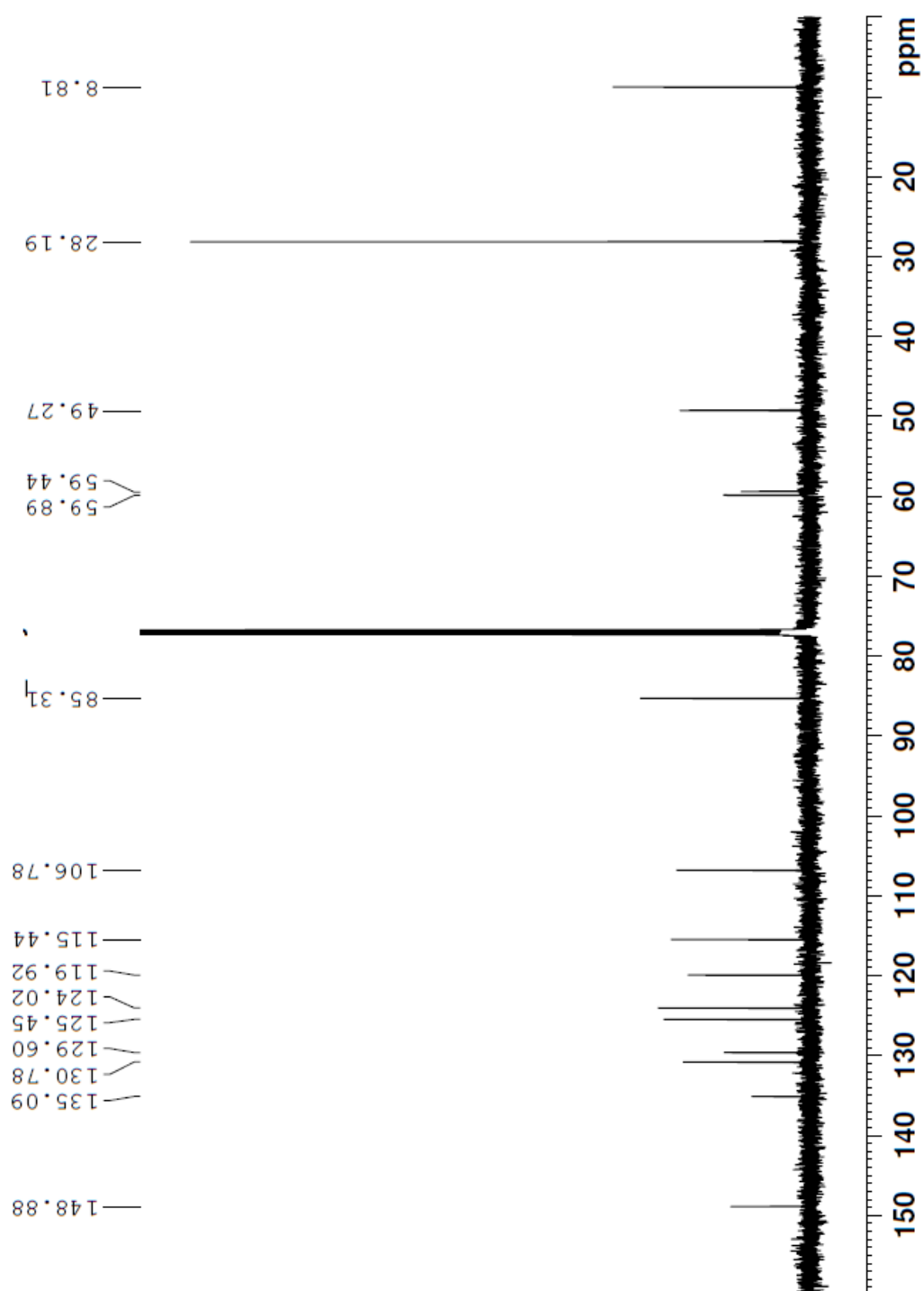
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	65.67	C20 H30 N2 O2	[M+H] <sup>+</sup>	331.2356	331.2380	-2.4	-7.25	97.30	7.0

<sup>1</sup>H NMR Spectrum of Compound 32b in CDCl<sub>3</sub>



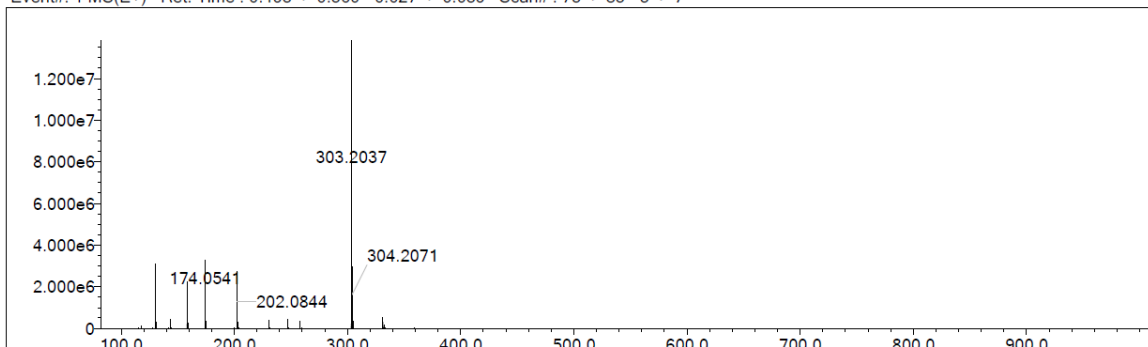


<sup>13</sup>C NMR Spectrum of Compound 32b in CDCl<sub>3</sub>

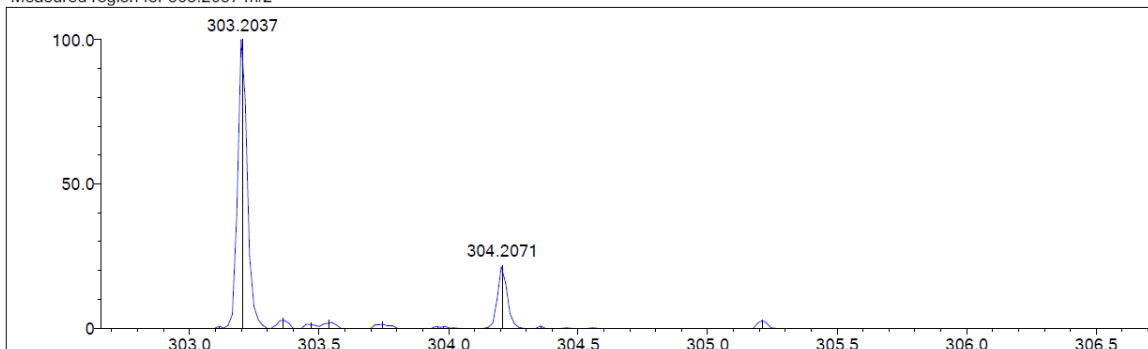


## HRMS of Compound 32b

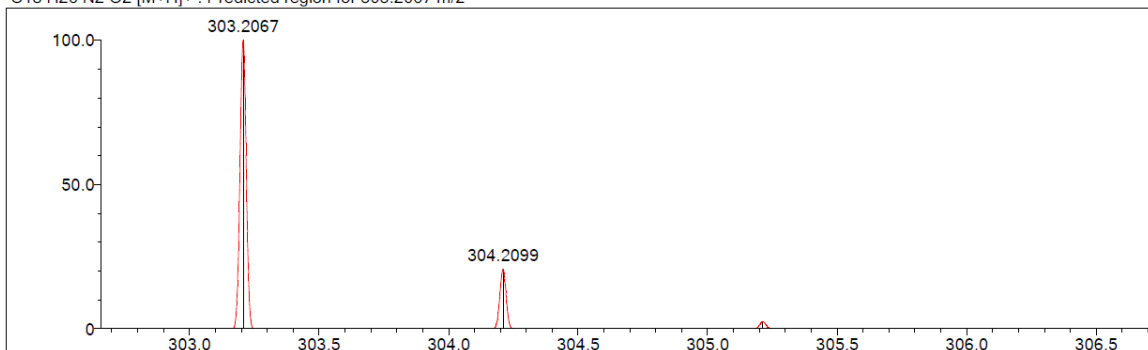
Event#: 1 MS(E+) Ret. Time : 0.493 -> 0.560 - 0.027 -> 0.039 Scan#: 75 -> 85 - 5 -> 7



Measured region for 303.2037 m/z

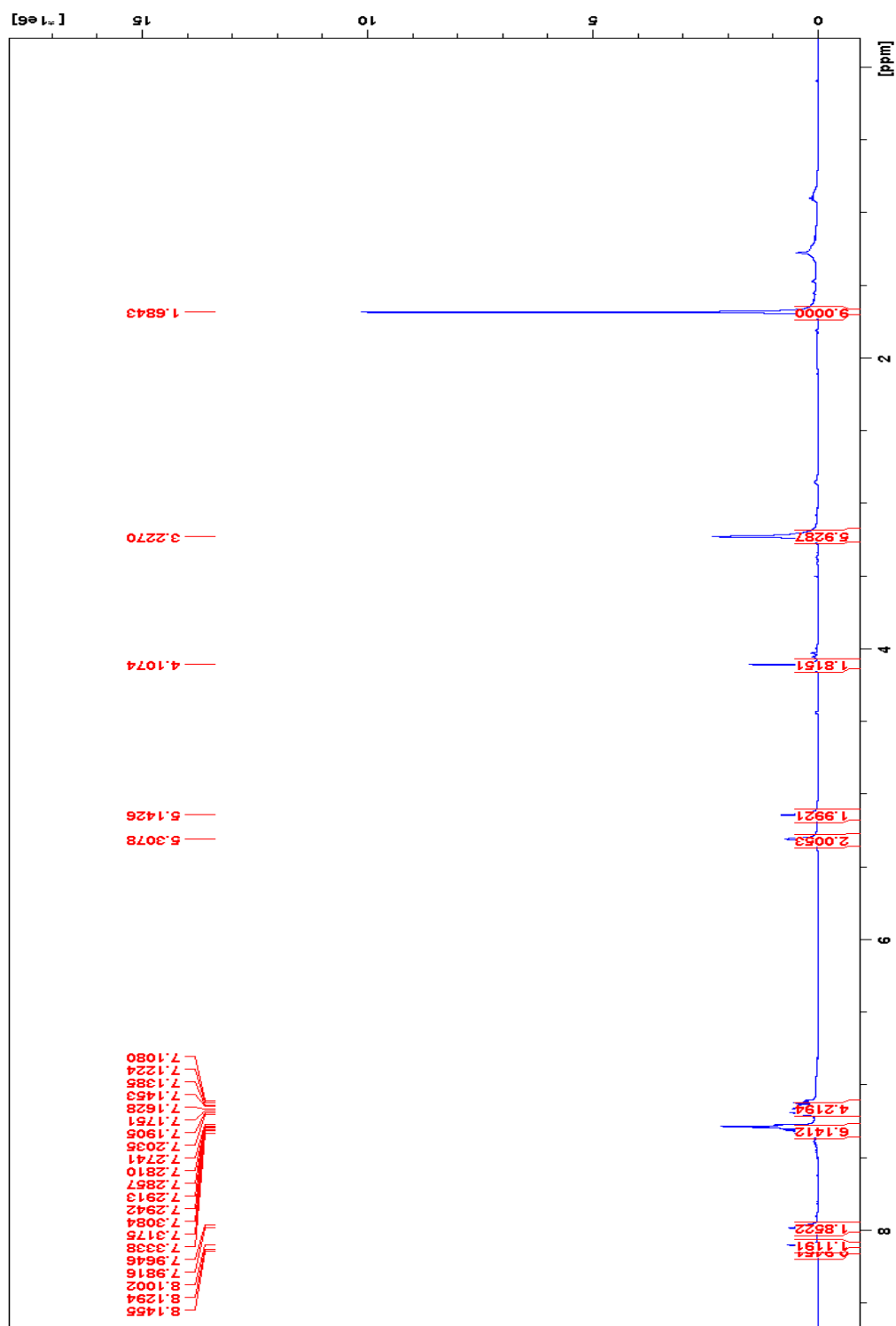


C18 H26 N2 O2 [M+H]<sup>+</sup> : Predicted region for 303.2067 m/z

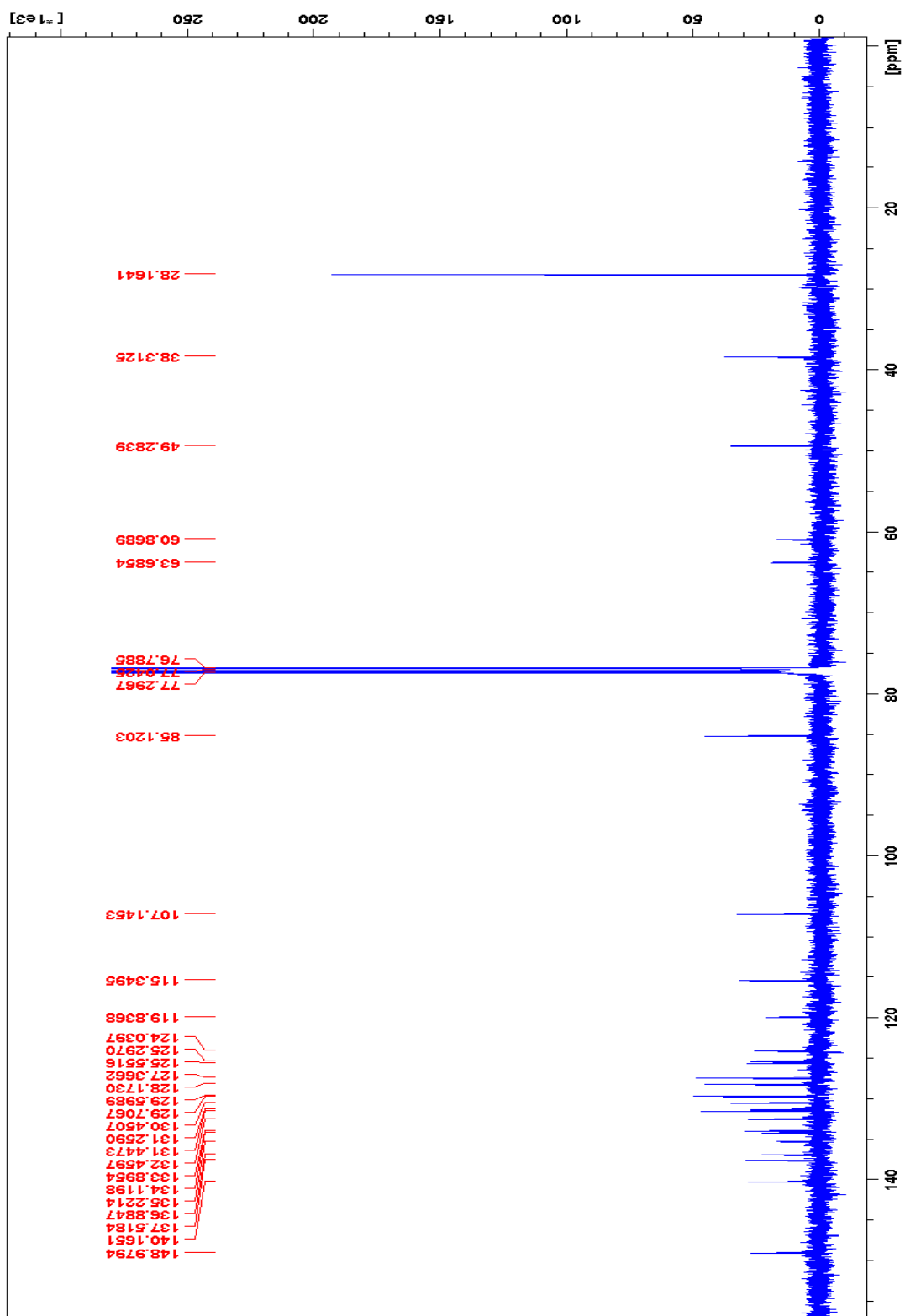


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	30.26	C18 H26 N2 O2	[M+H] <sup>+</sup>	303.2037	303.2067	-3.0	-9.89	73.63	7.0

# <sup>1</sup>H NMR Spectrum of Compound 31c in CDCl<sub>3</sub>

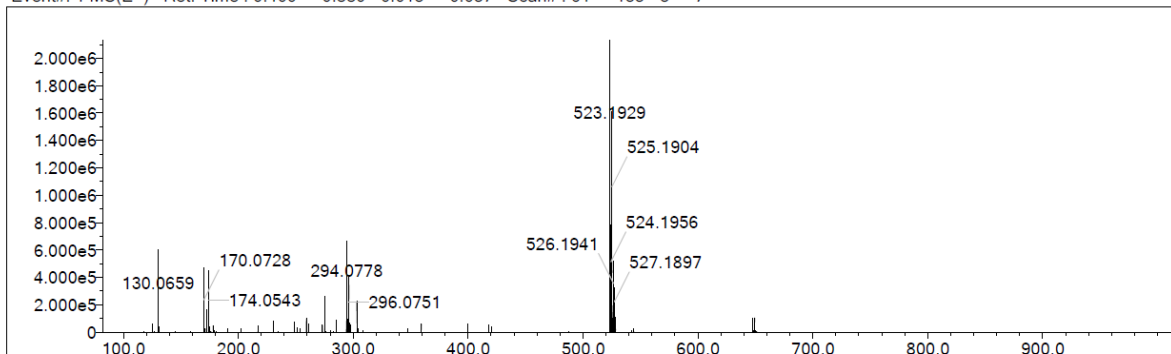


<sup>13</sup>C NMR Spectrum of Compound 31c in CDCl<sub>3</sub>

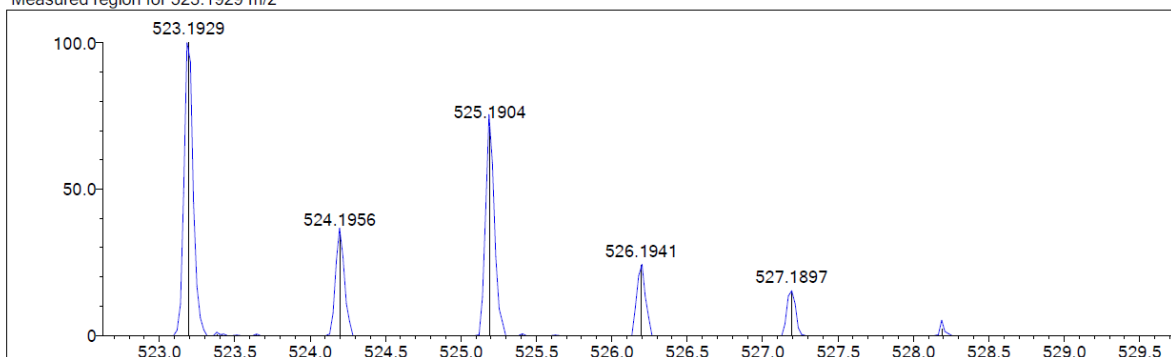


## HRMS of compound 31c

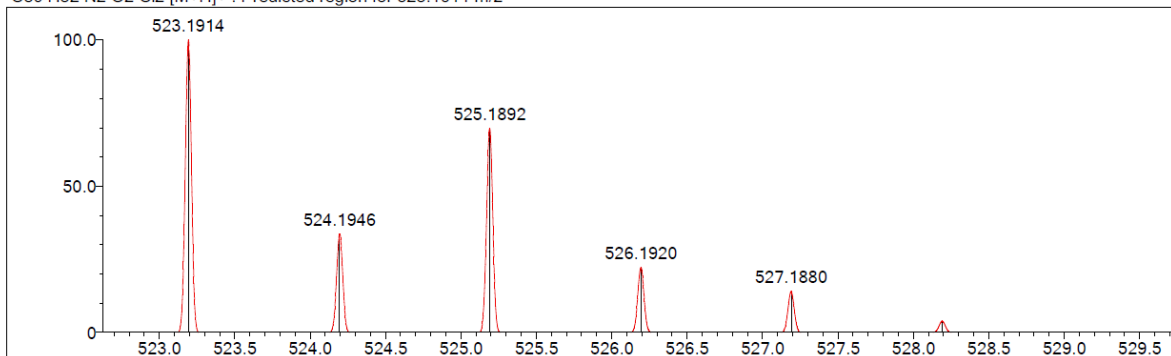
Event#: 1 MS(E+) Ret. Time : 0.400 -> 0.880 - 0.013 -> 0.037 Scan#: 61 -> 133 - 3 -> 7



Measured region for 523.1929 m/z

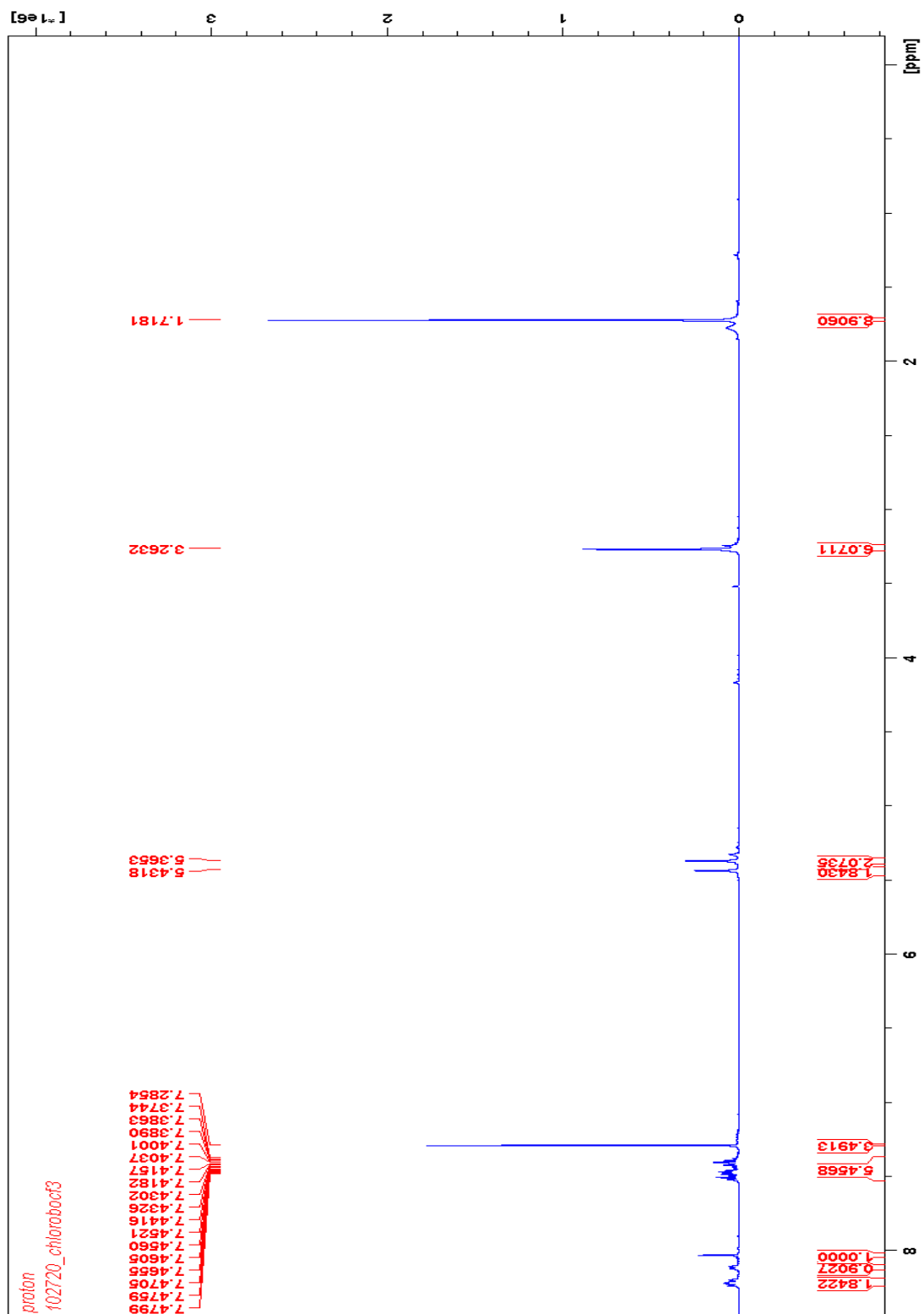


C30 H32 N2 O2 Cl2 [M+H]<sup>+</sup> : Predicted region for 523.1914 m/z



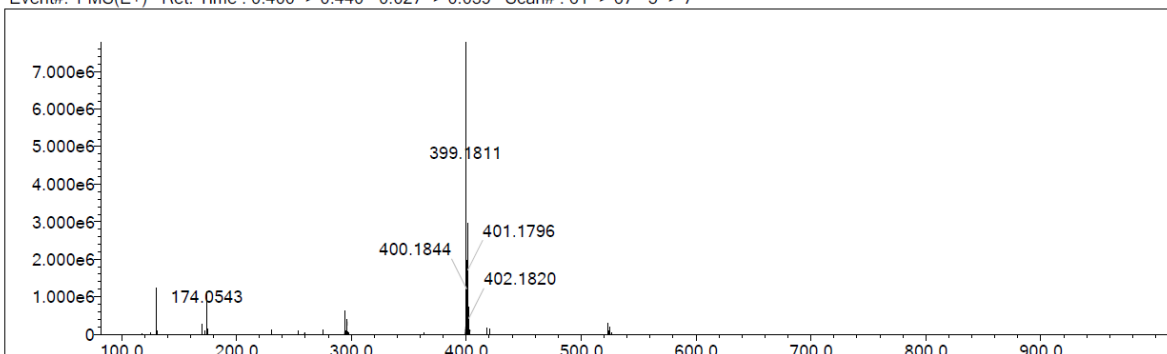
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	95.33	C30 H32 N2 O2 Cl2	[M+H] <sup>+</sup>	523.1929	523.1914	1.5	2.87	100.00	15.0

<sup>1</sup>H NMR Spectrum of Compound 32c in CDCl<sub>3</sub>

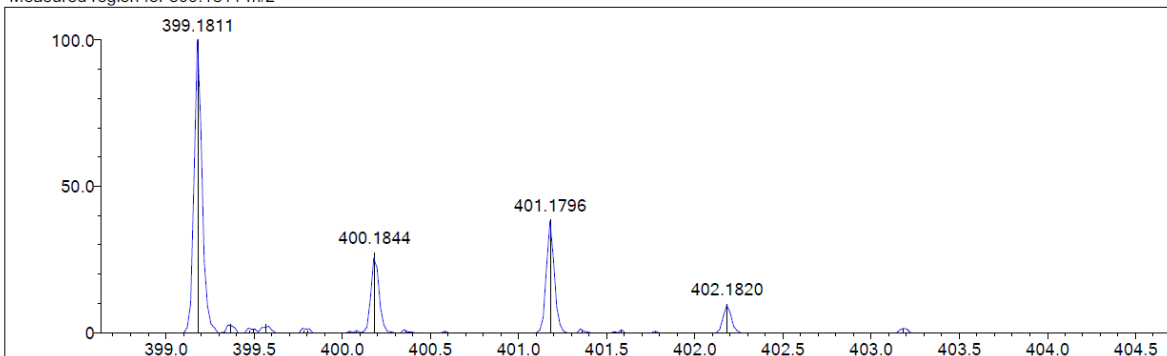


# HRMS of Compound 32c

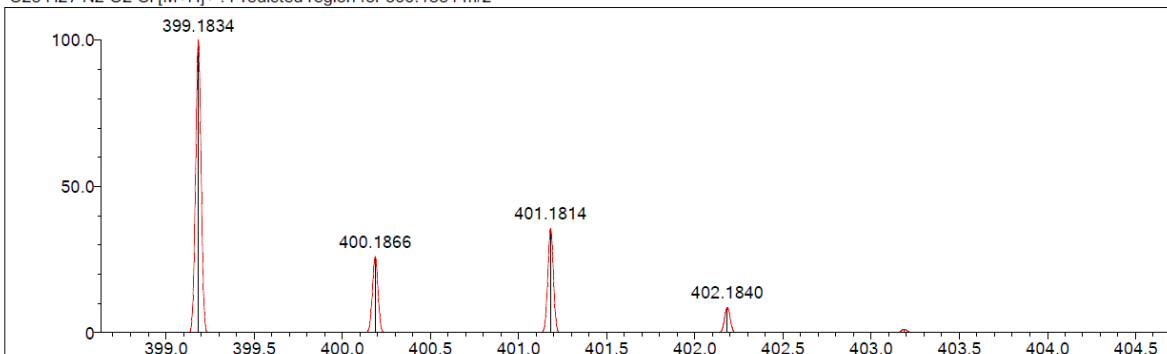
Event#: 1 MS(E+) Ret. Time : 0.400 -> 0.440 - 0.027 -> 0.039 Scan#: 61 -> 67 - 5 -> 7



Measured region for 399.1811 m/z

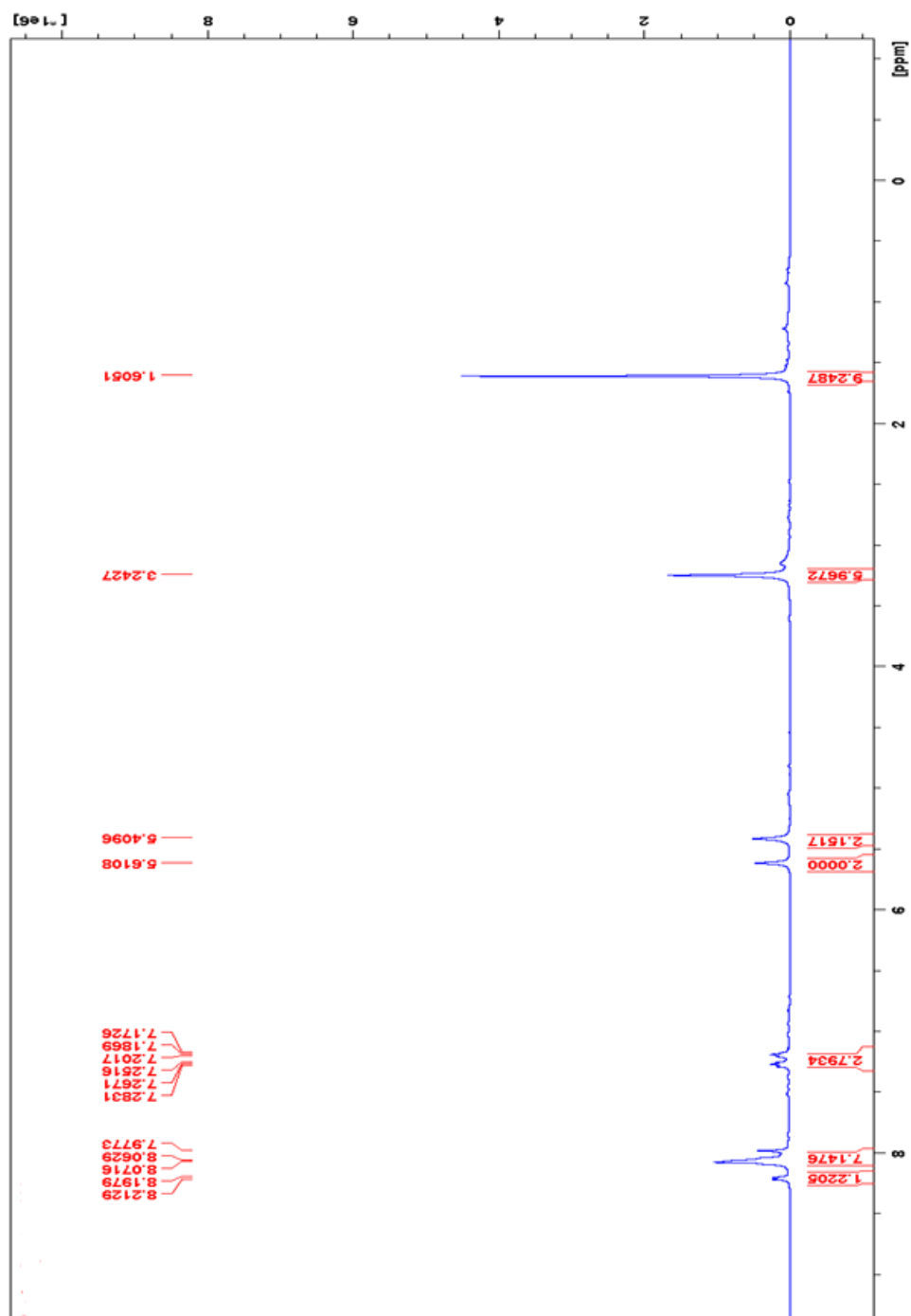


C23 H27 N2 O2 Cl [M+H]<sup>+</sup> : Predicted region for 399.1834 m/z



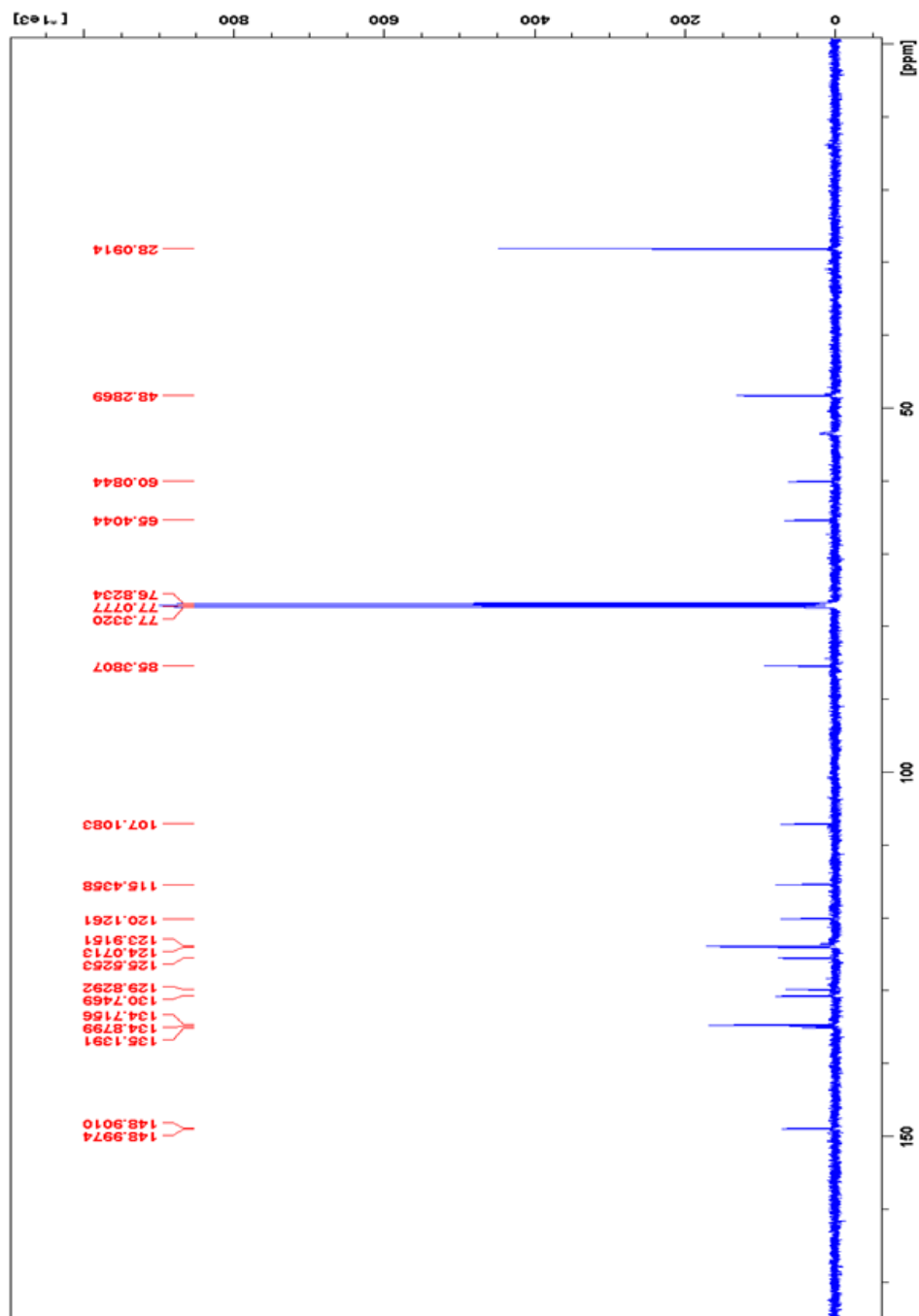
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	70.72	C23 H27 N2 O2 Cl	[M+H] <sup>+</sup>	399.1811	399.1834	-2.3	-5.76	85.82	11.0

**<sup>1</sup>H NMR Spectrum of Compound 32d in CDCl<sub>3</sub>**



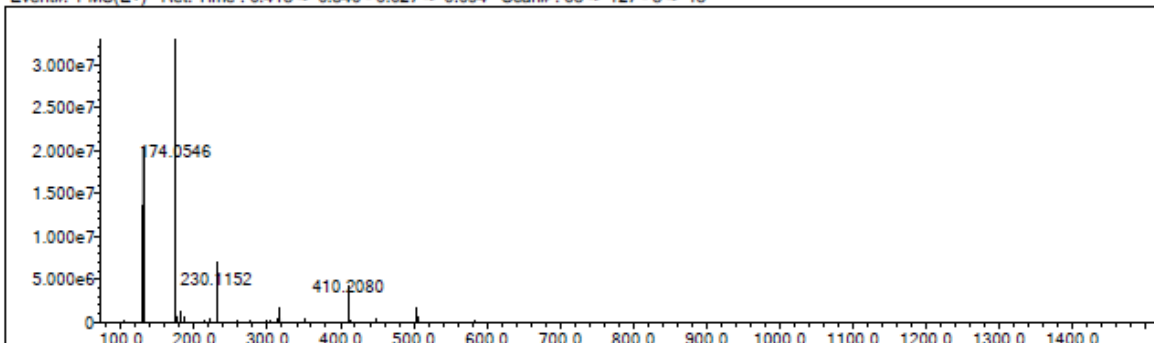


**$^{13}\text{C}$  NMR Spectrum of Compound 32d in  $\text{CDCl}_3$**

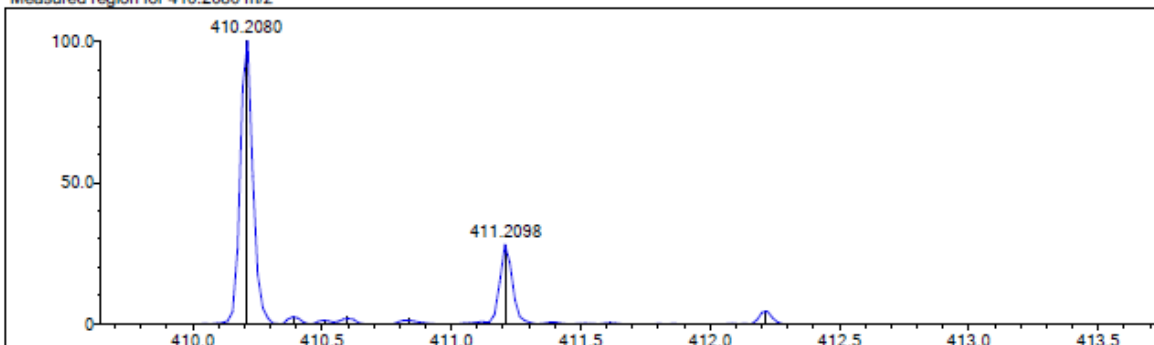


## HRMS of Compound 32d

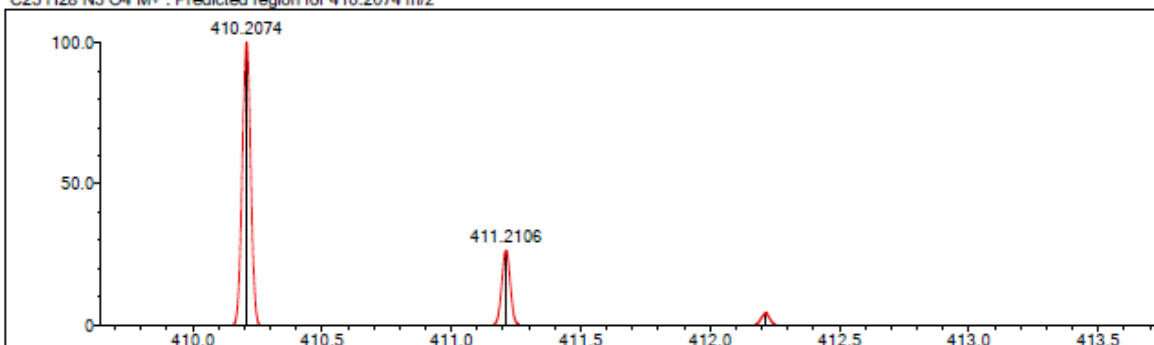
Event#: 1 MS(E+) Ret. Time : 0.413 -> 0.840 - 0.027 -> 0.094 Scan#: 63 -> 127 - 5 -> 15



Measured region for 410.2080 m/z

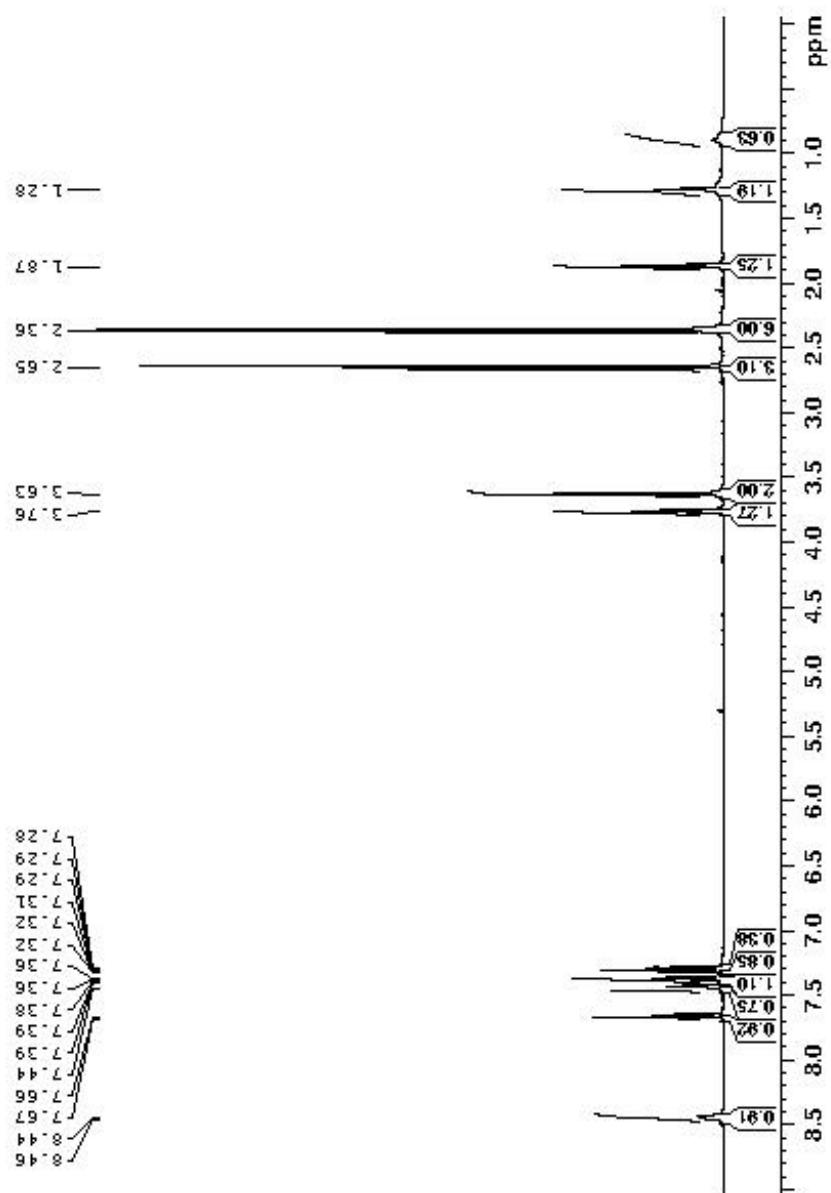


C23 H28 N3 O4 M+ : Predicted region for 410.2074 m/z

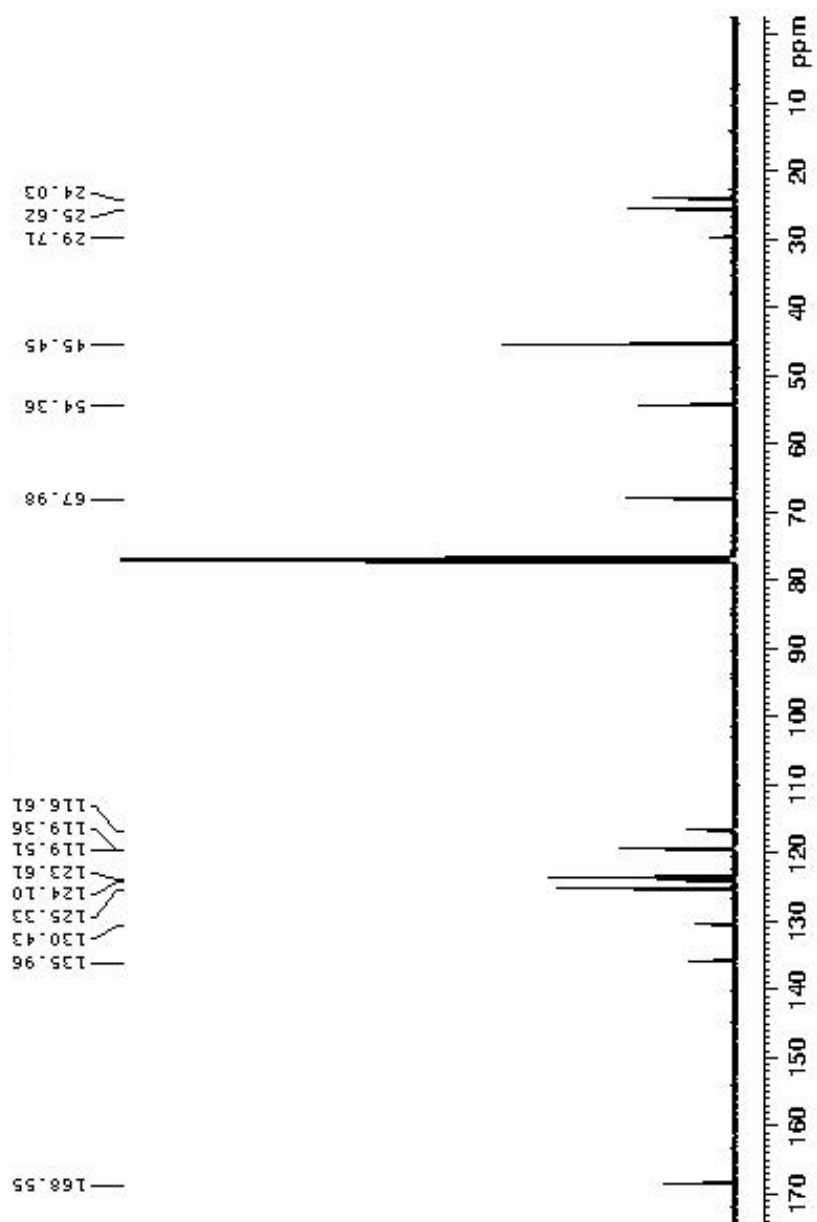


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	98.85	C23 H28 N3 O4	M+	410.2080	410.2074	0.6	1.46	100.00	11.5

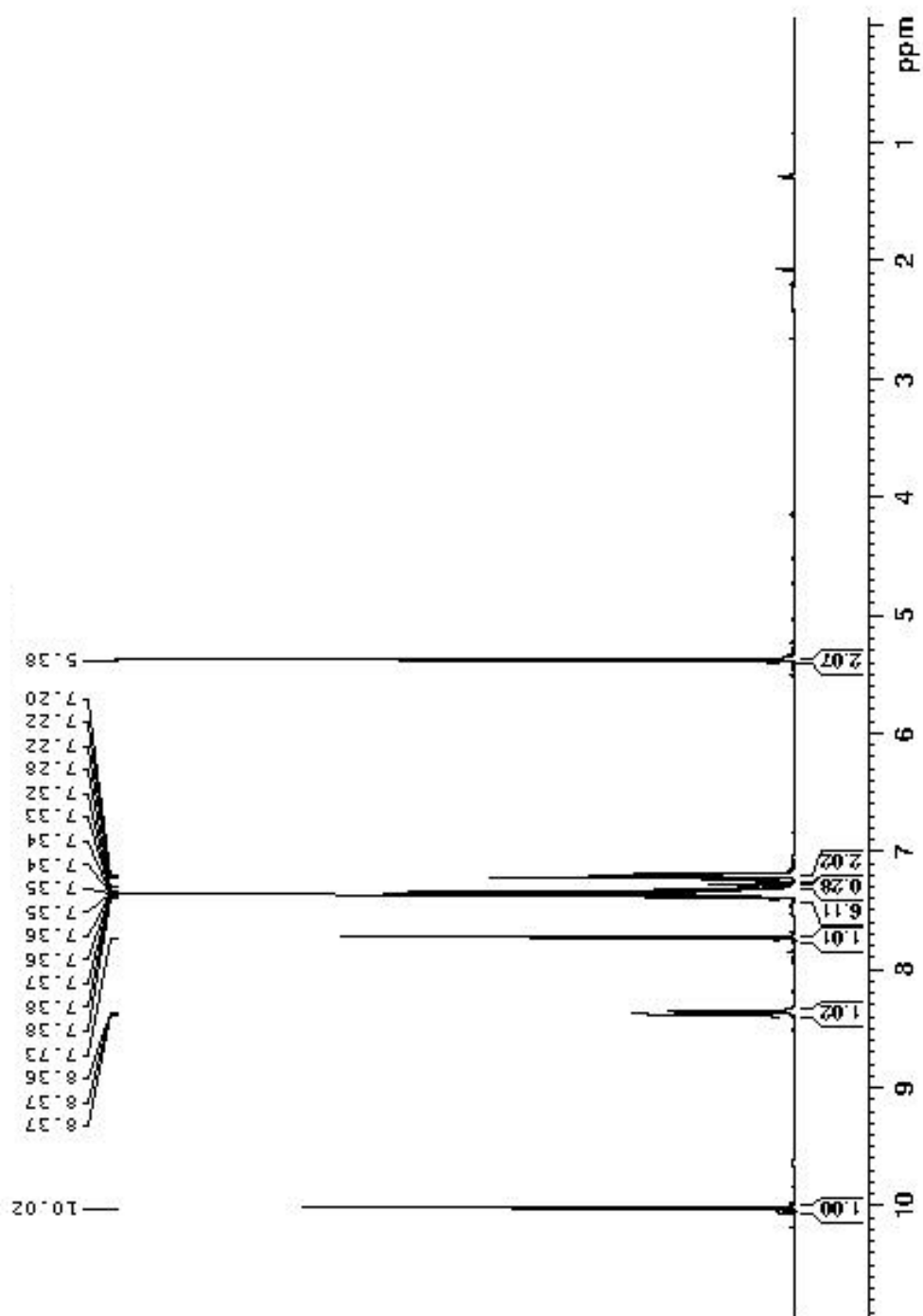
**<sup>1</sup>H NMR Spectrum of Compound 34 in CDCl<sub>3</sub>**



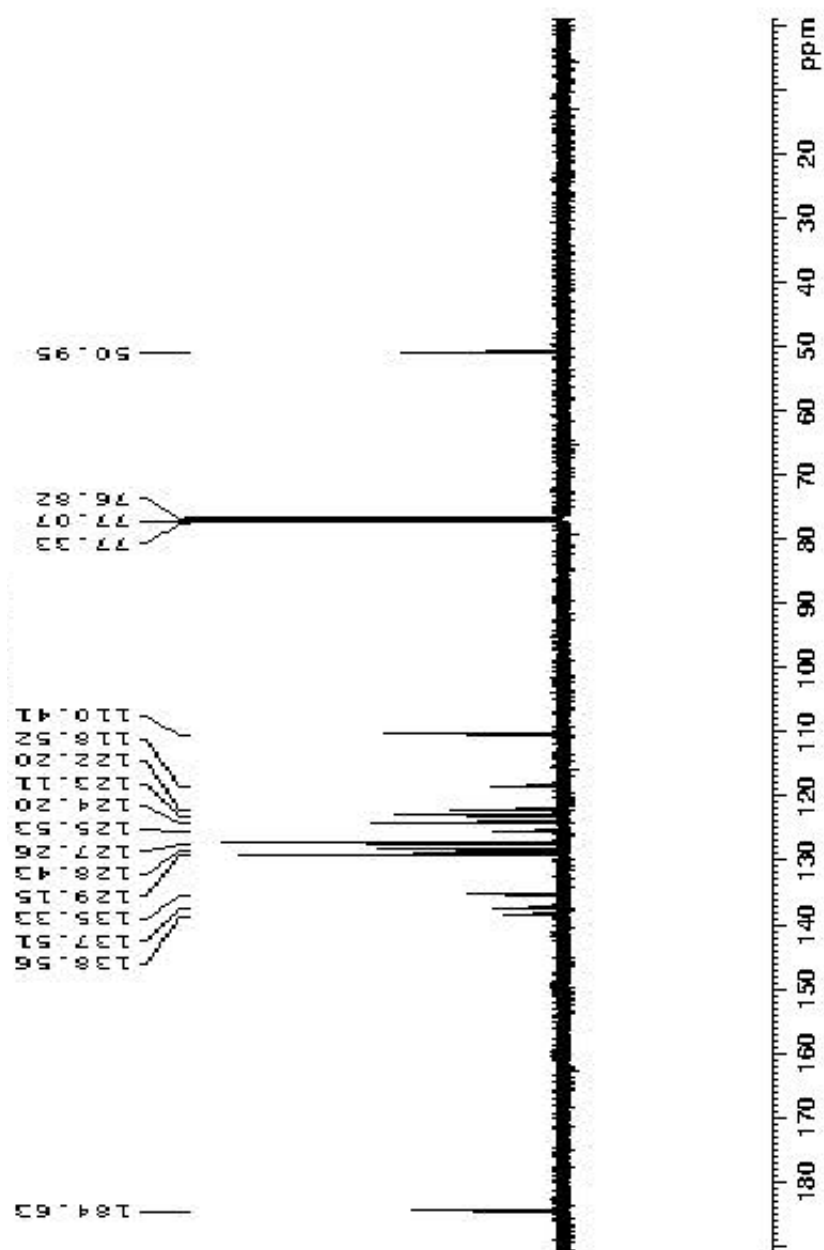
**$^{13}\text{C}$  NMR Spectrum of Compound 34 in  $\text{CDCl}_3$**



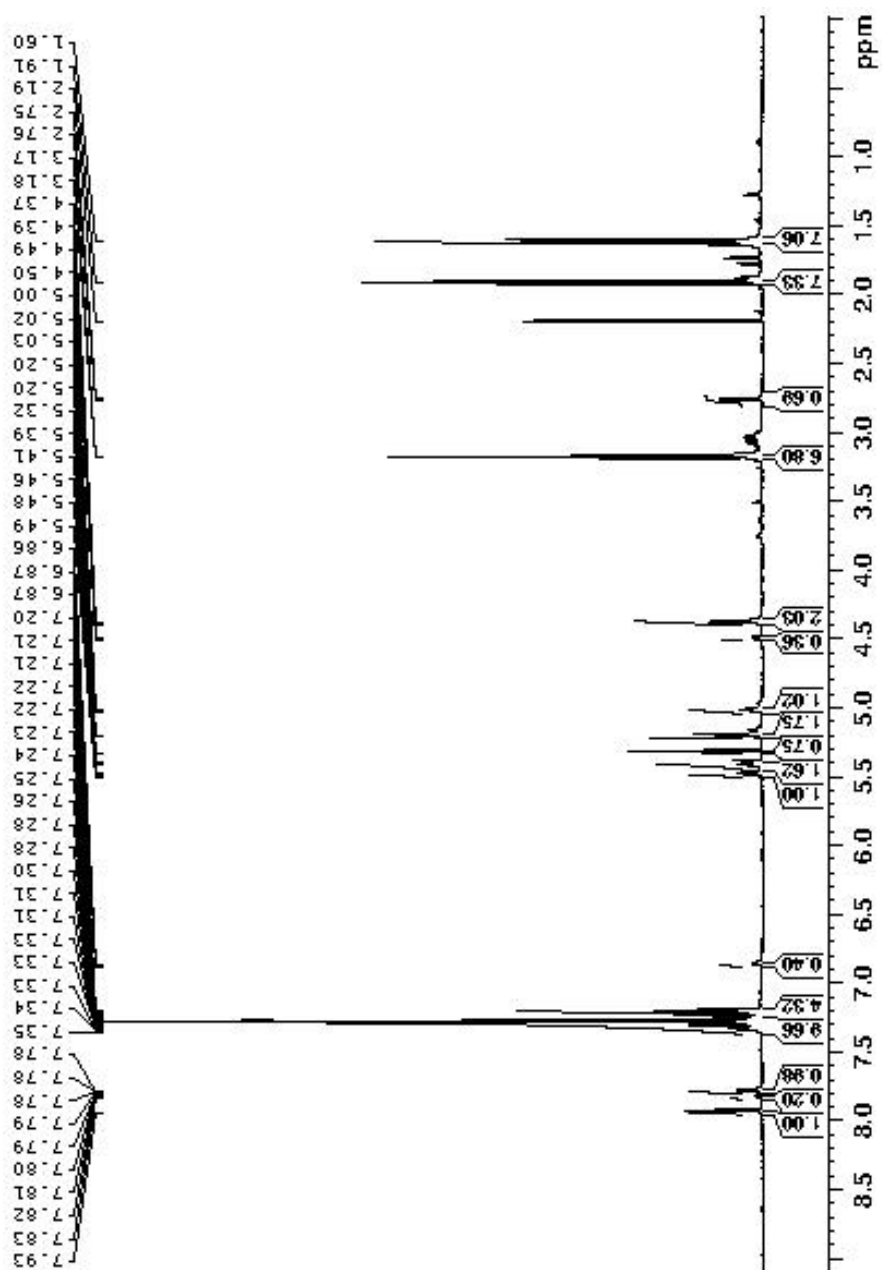
<sup>1</sup>H NMR of Compound 40



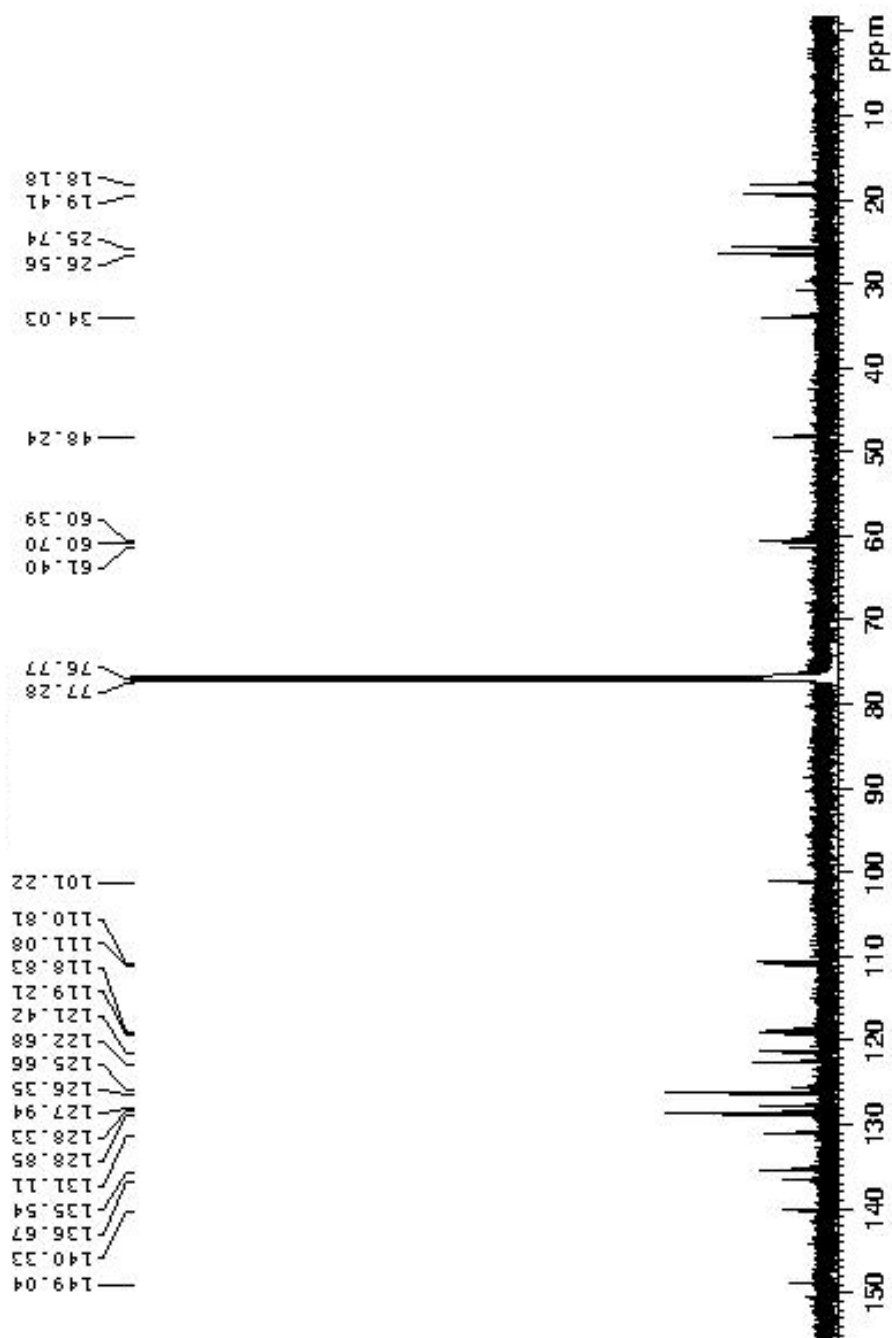
<sup>13</sup>C NMR of Compound 40



# <sup>1</sup>H NMR of Compound 42

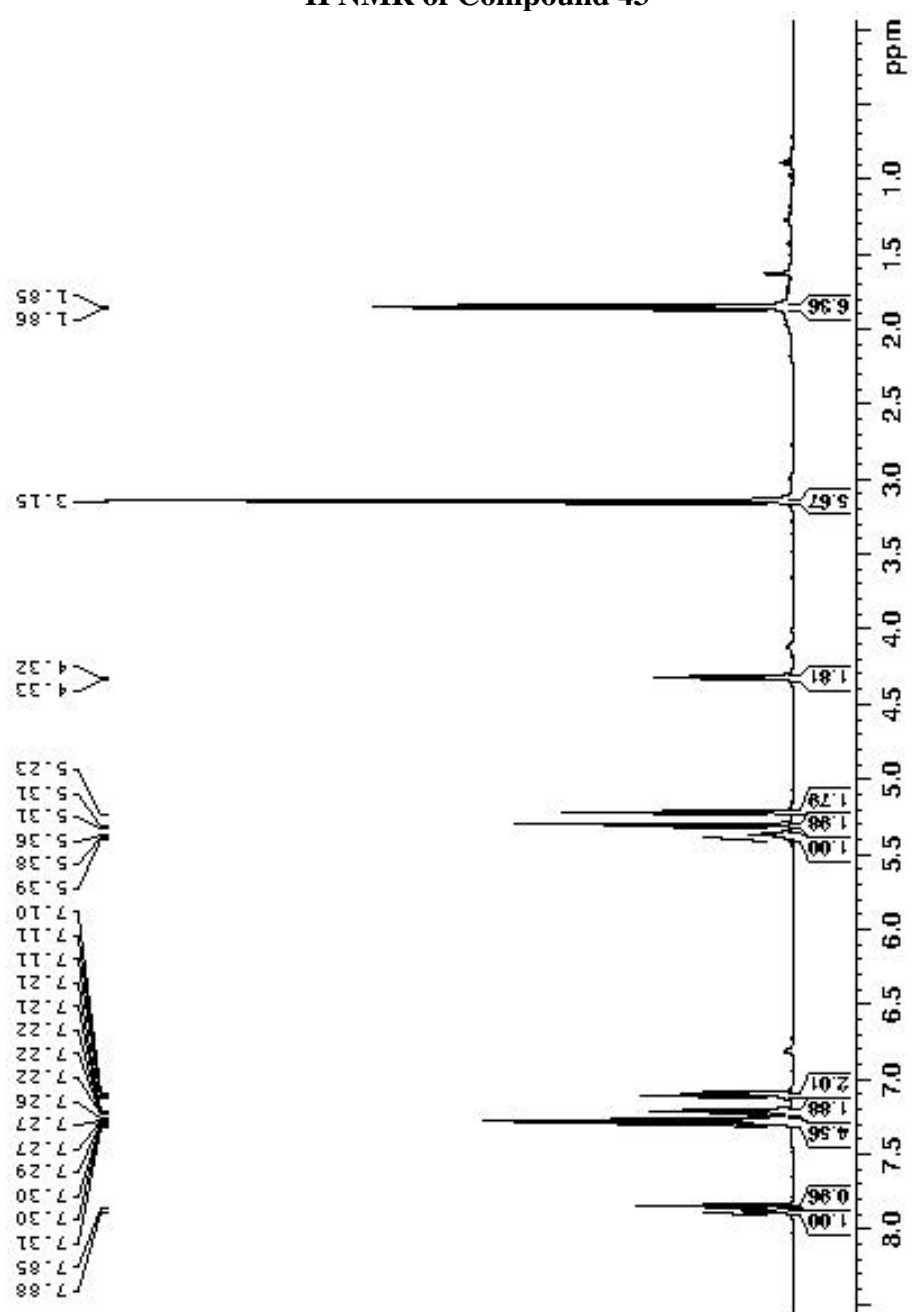


# <sup>13</sup>C NMR of Compound 42

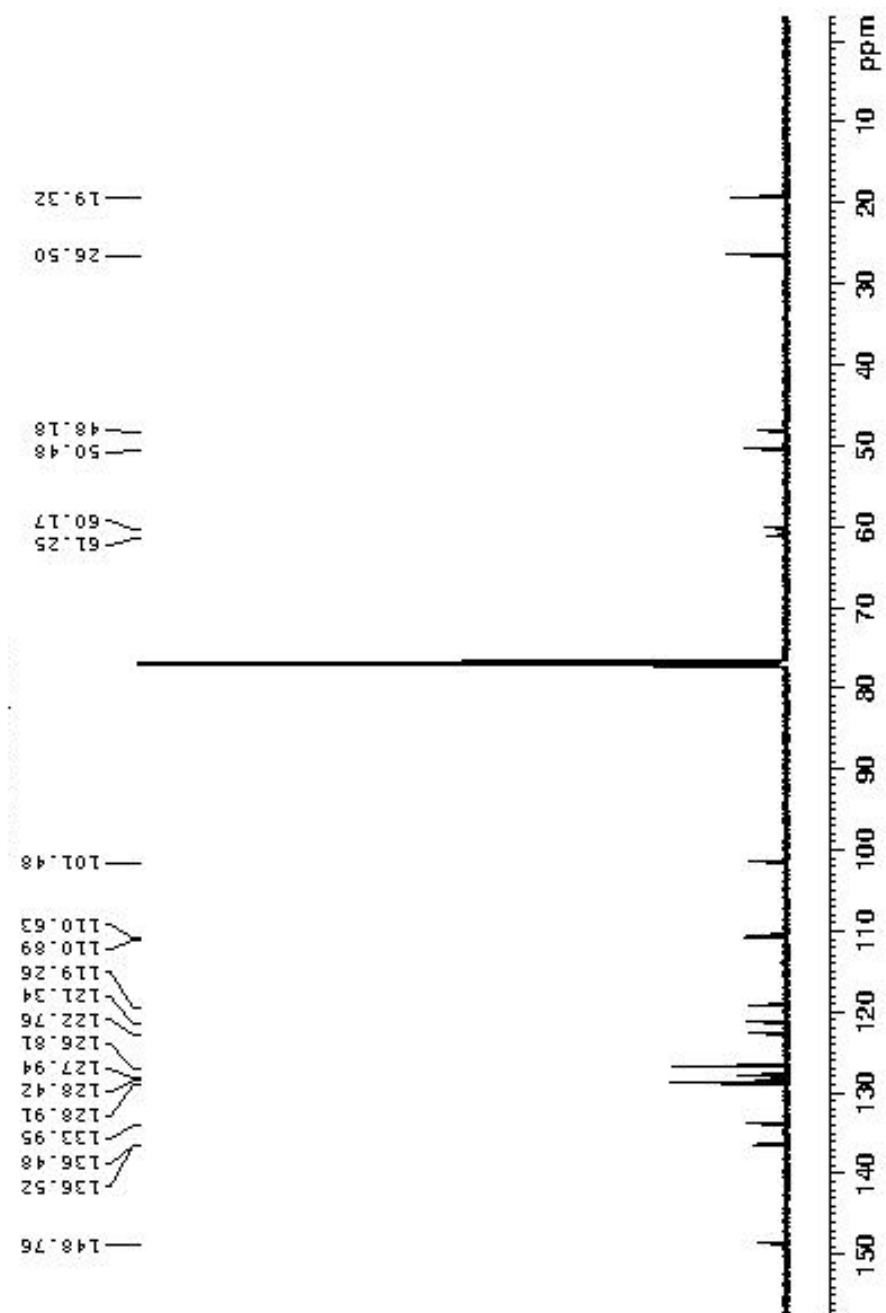




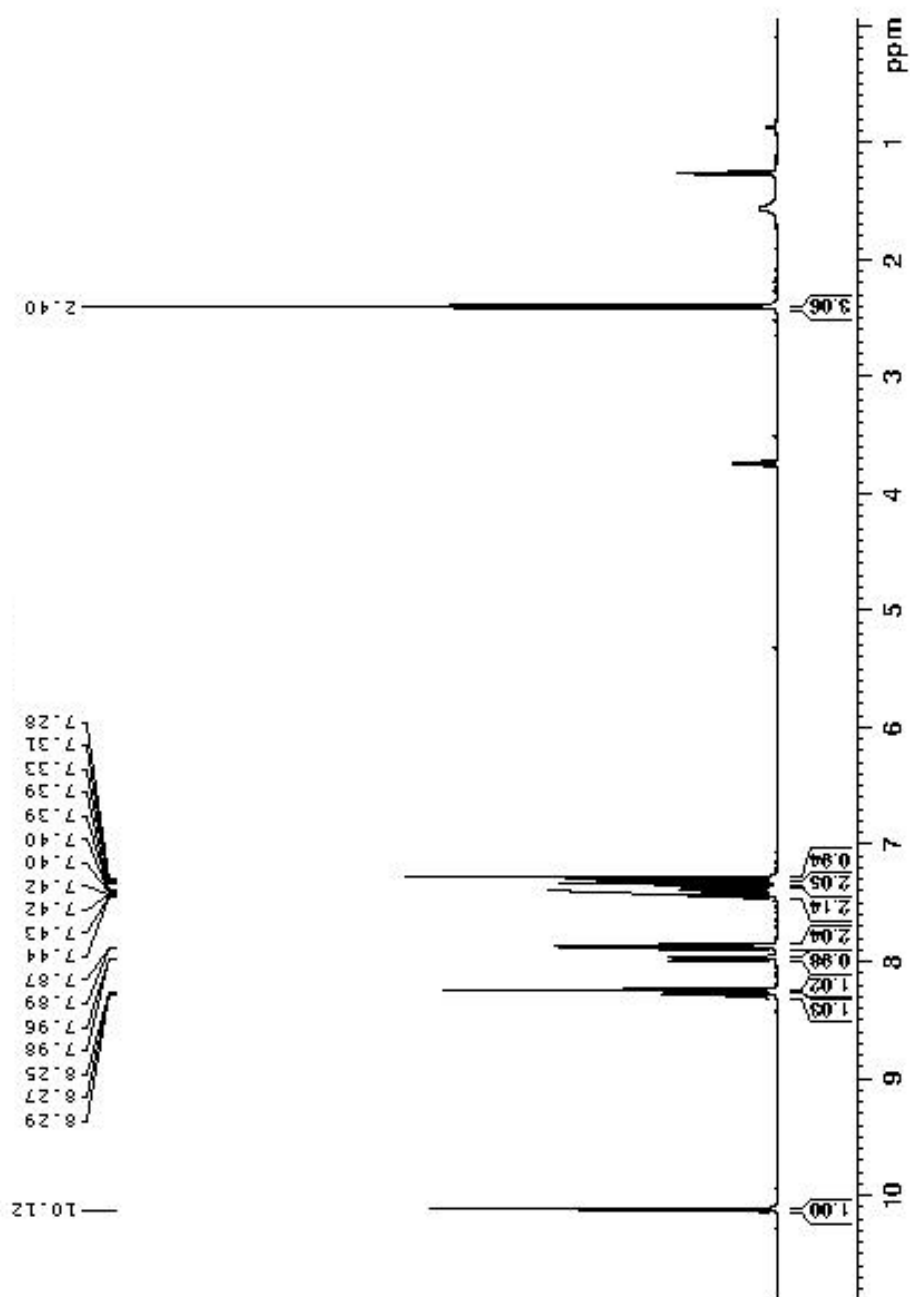
<sup>1</sup>H NMR of Compound 43



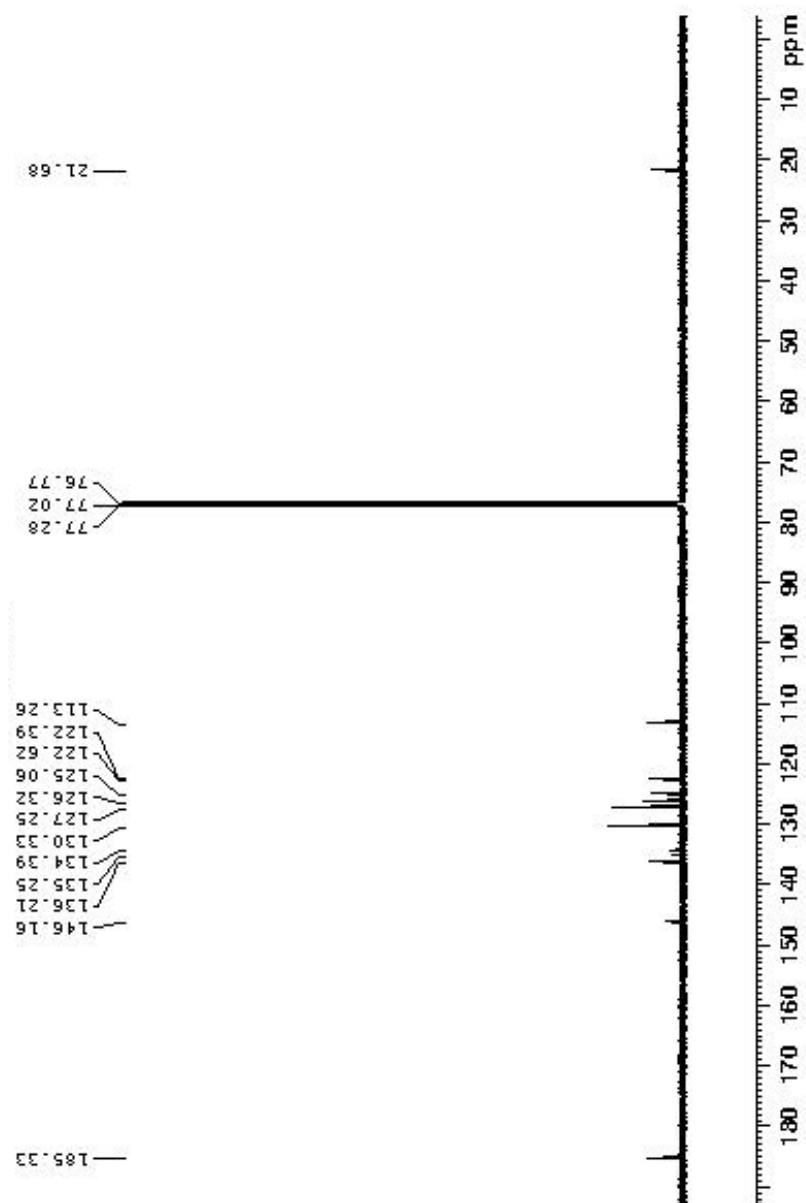
<sup>13</sup>C NMR of Compound 43



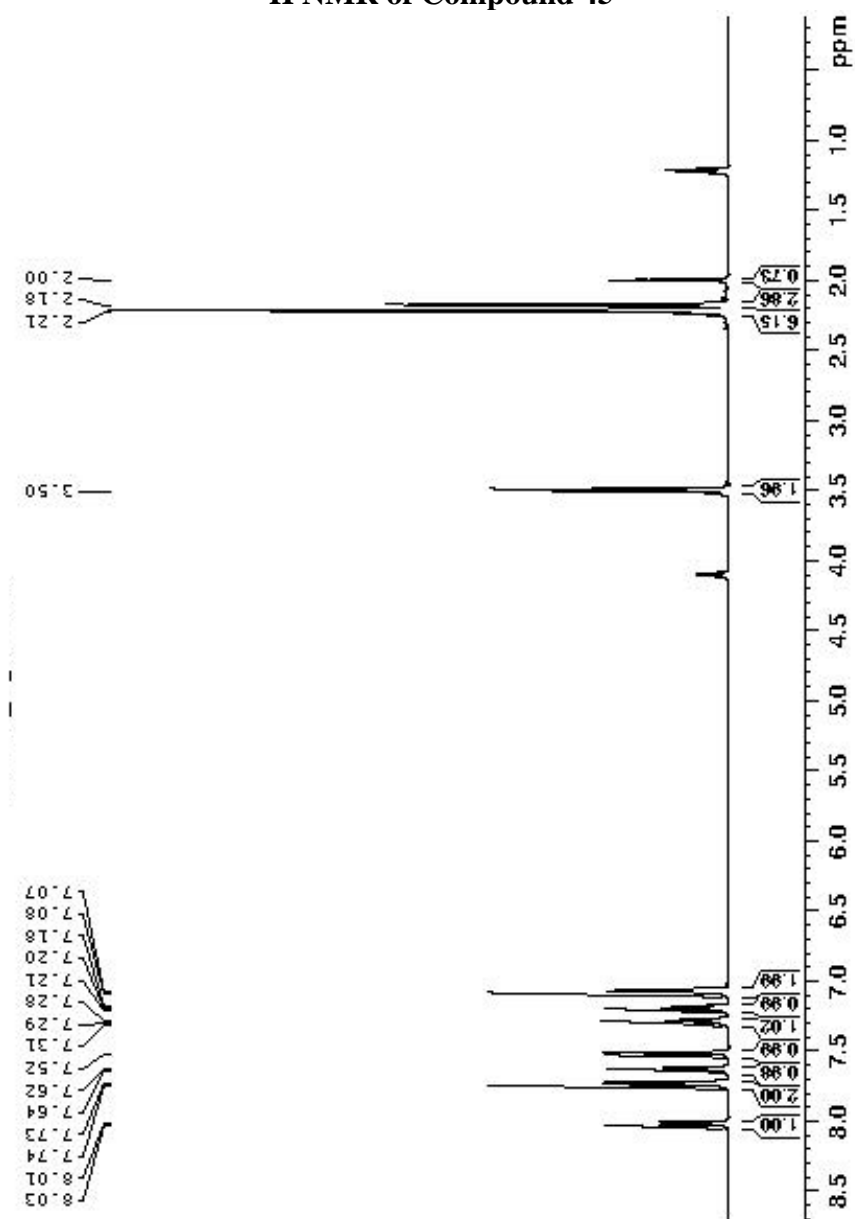
<sup>1</sup>H NMR of Compound 44



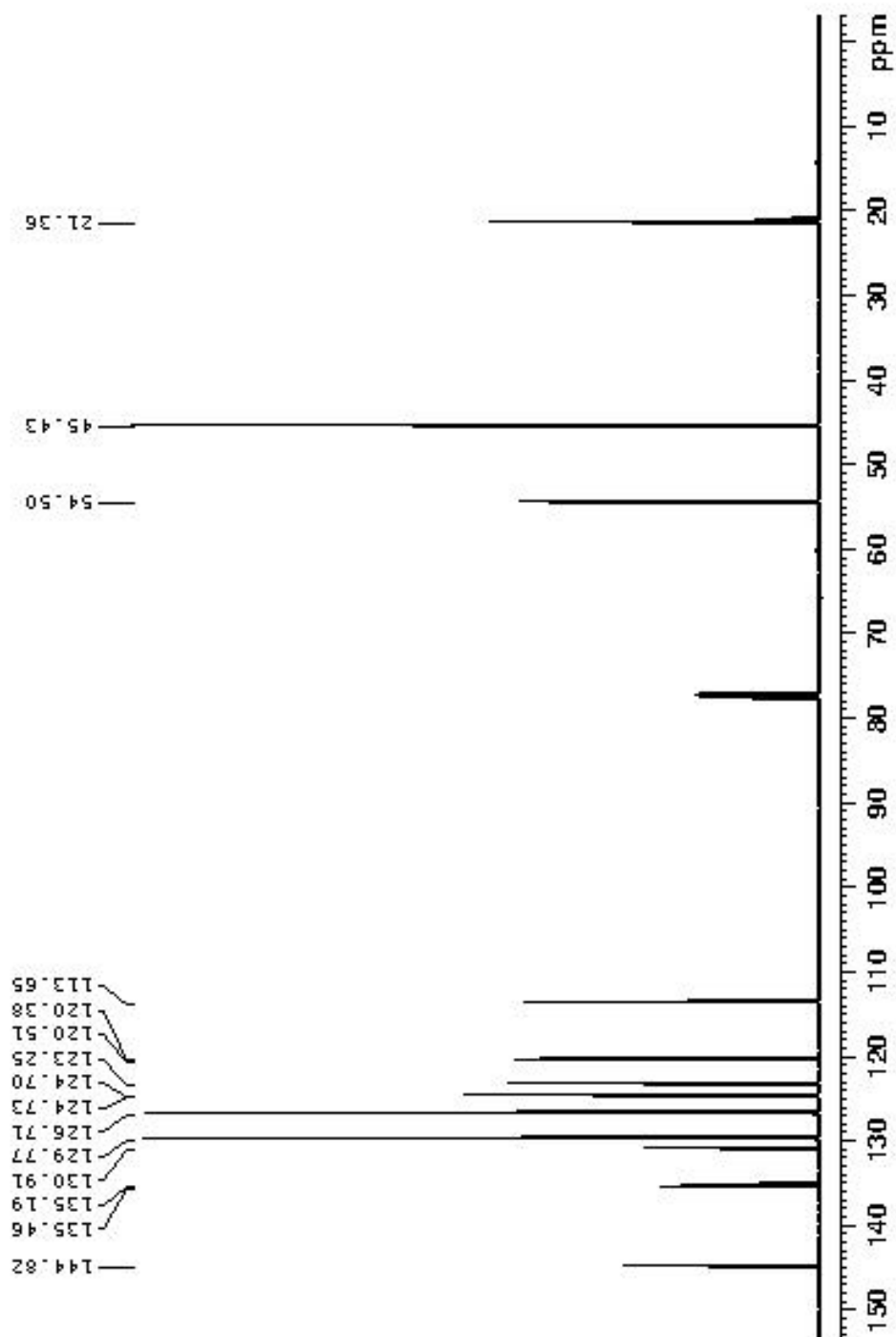
<sup>13</sup>C NMR of Compound 44



# <sup>1</sup>H NMR of Compound 45



# <sup>13</sup>C NMR of Compound 45

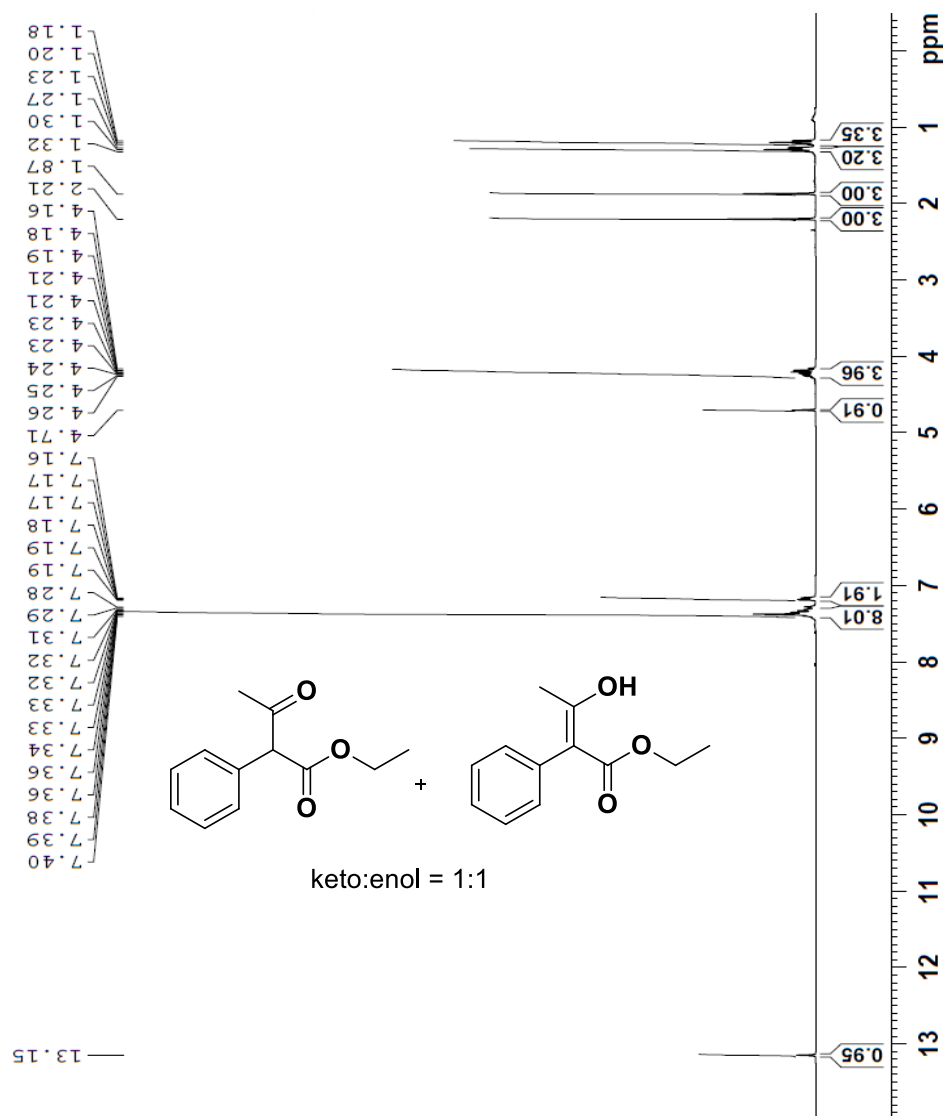


## **APPENDIX B**

### **PART II: BRØNSTED ACID CATALYZED REACTIONS OF AROMATIC KETONES WITH ETHYL DIAZOACETATE AND ITS SYNTHETIC SCOPE**

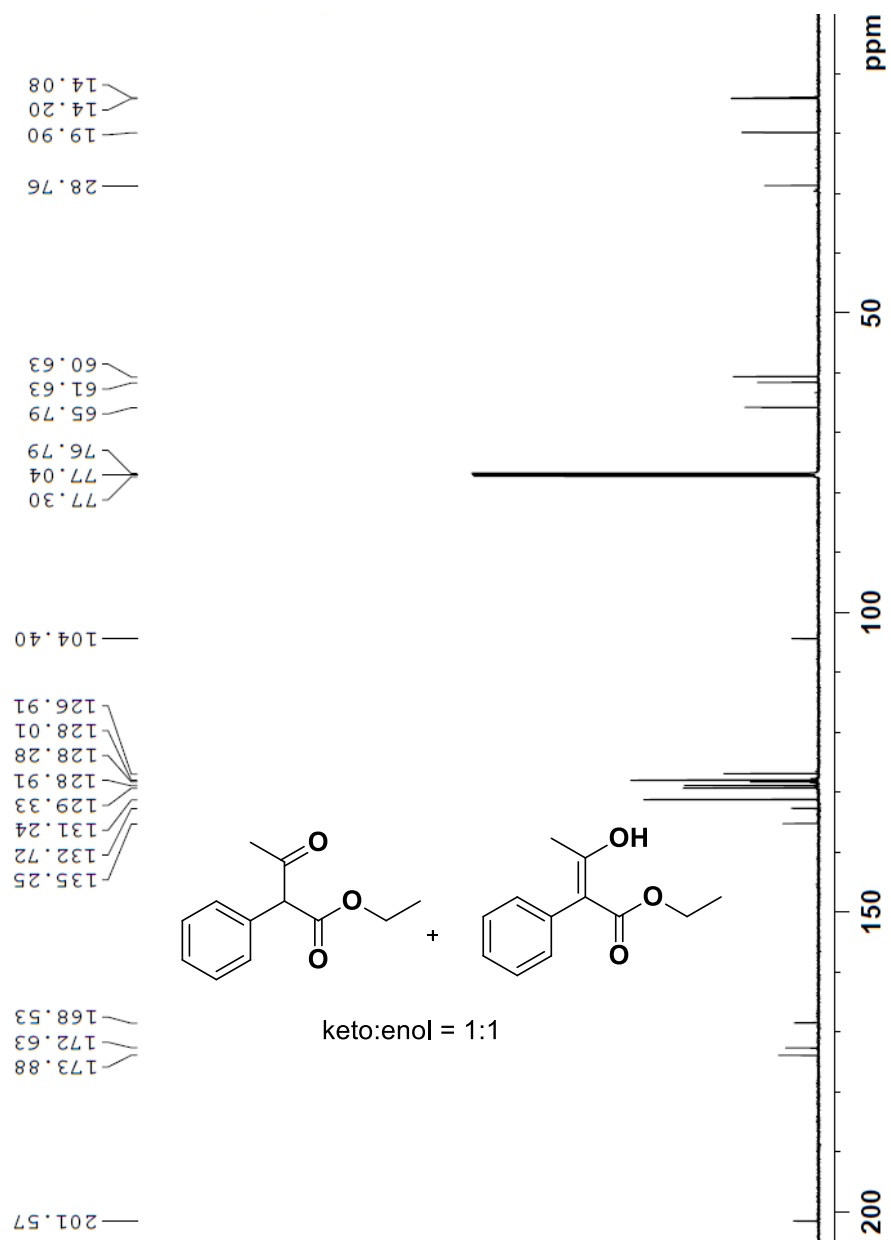
**Copies of  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR Spectral Data**

# <sup>1</sup>H NMR Spectrum of Compound 3a in CDCl<sub>3</sub>

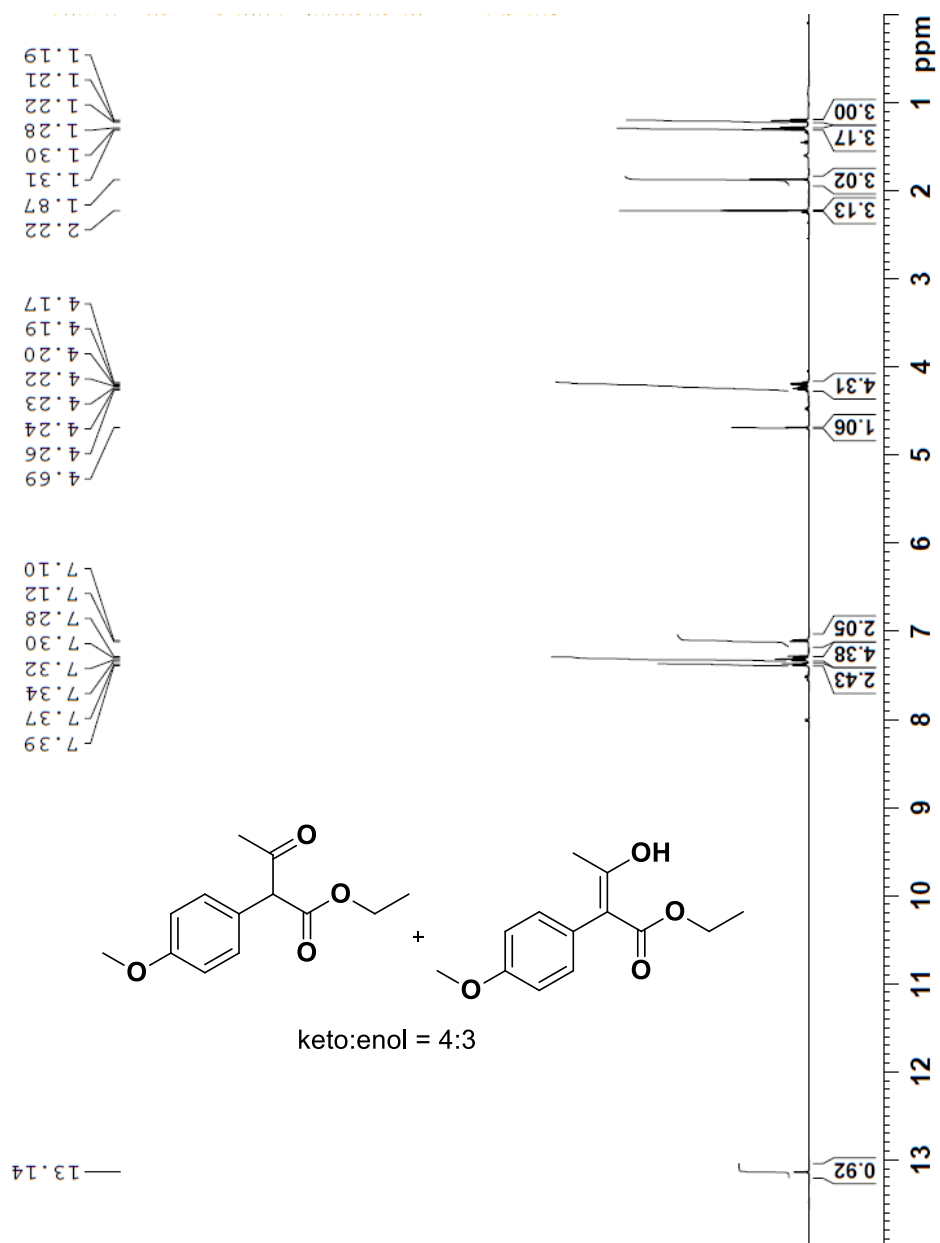




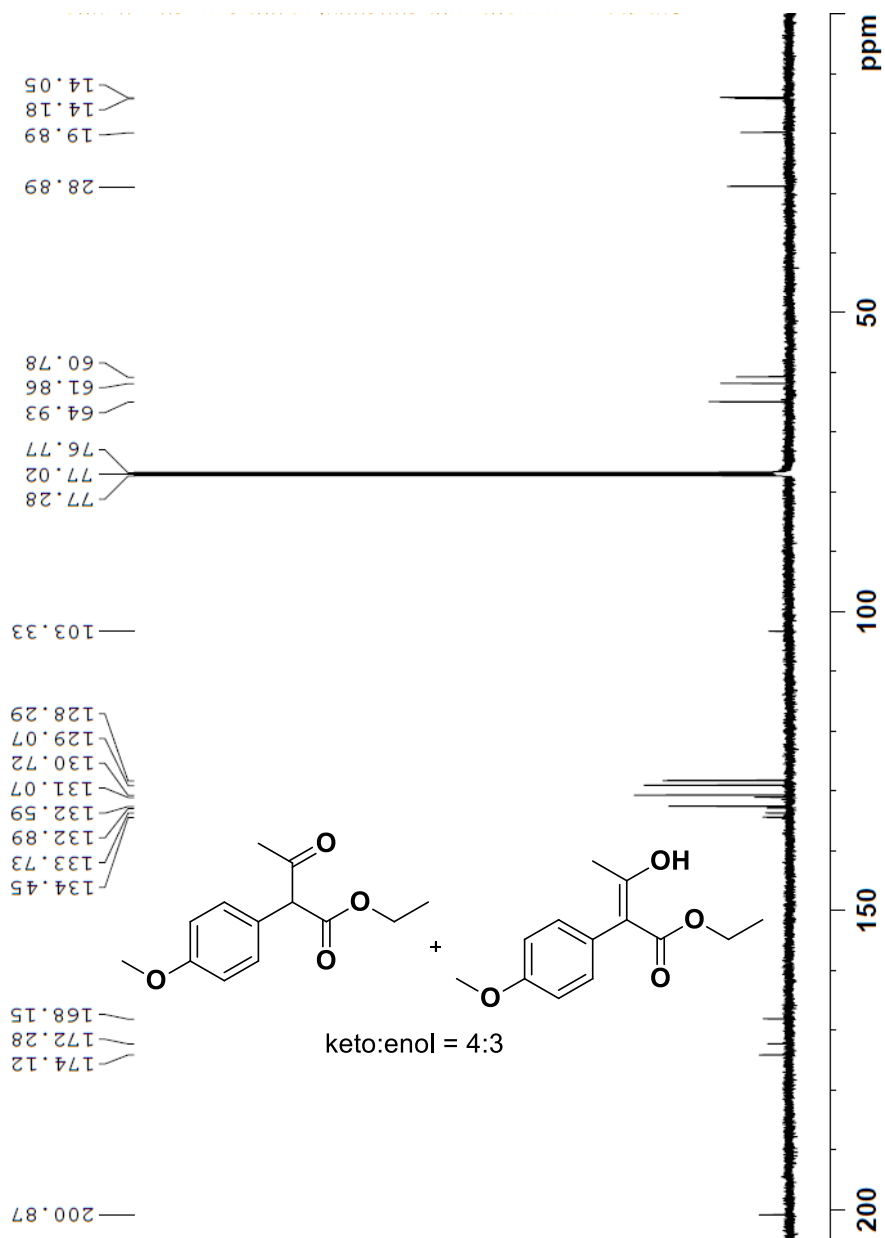
**$^{13}\text{C}$  NMR Spectrum of Compound 3a in  $\text{CDCl}_3$**



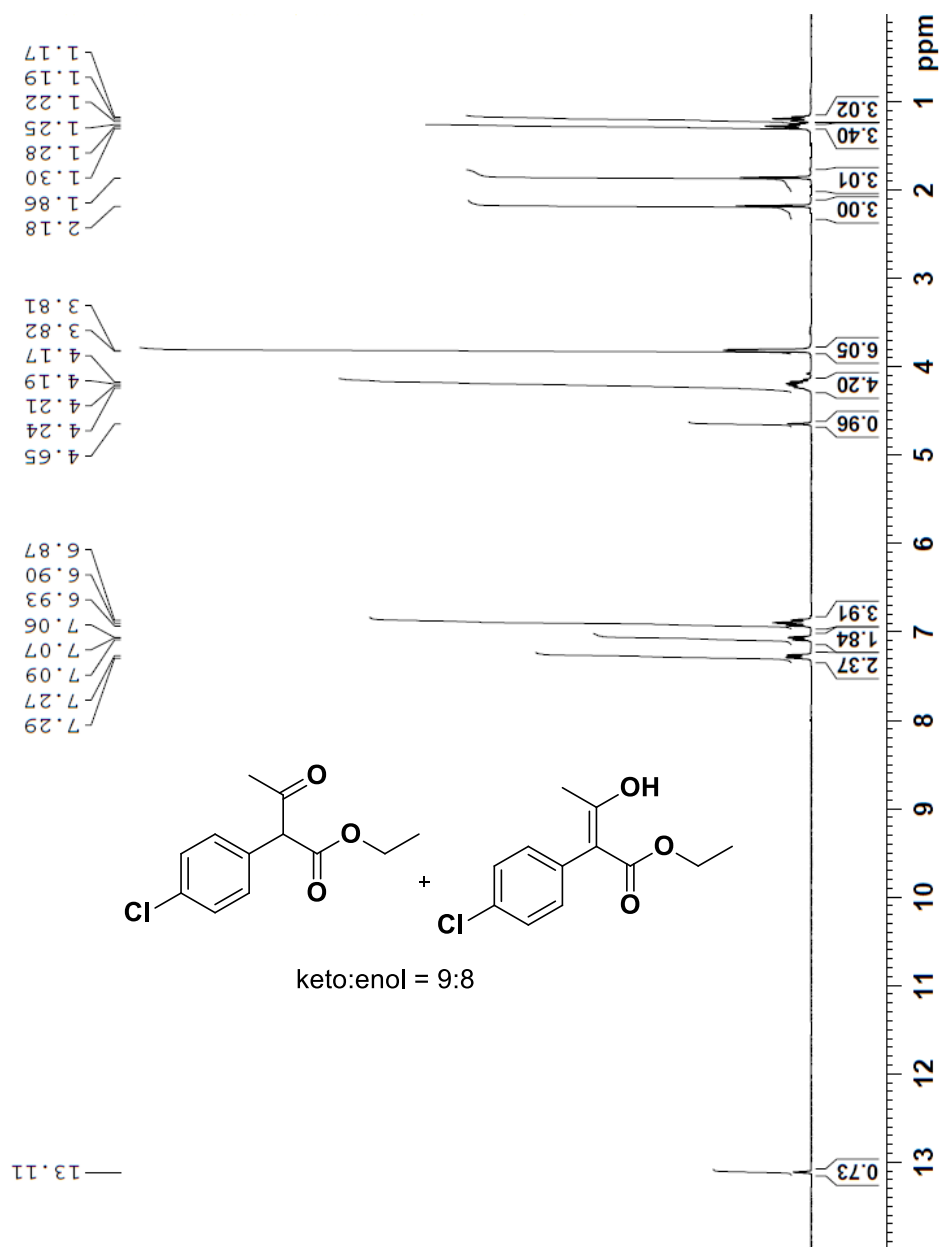
# <sup>1</sup>H NMR Spectrum of Compound 3c in CDCl<sub>3</sub>



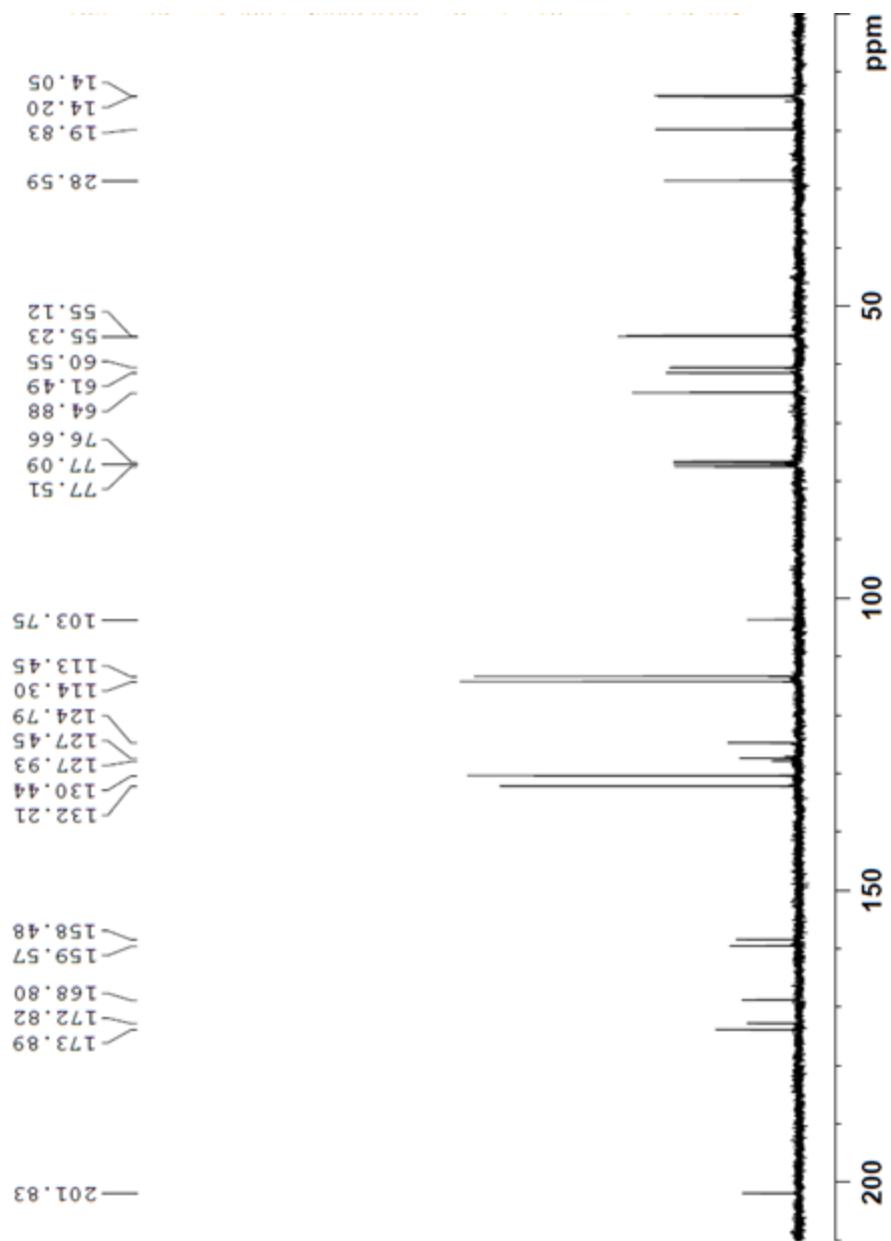
**$^{13}\text{C}$  NMR Spectrum of Compound 3c in  $\text{CDCl}_3$**



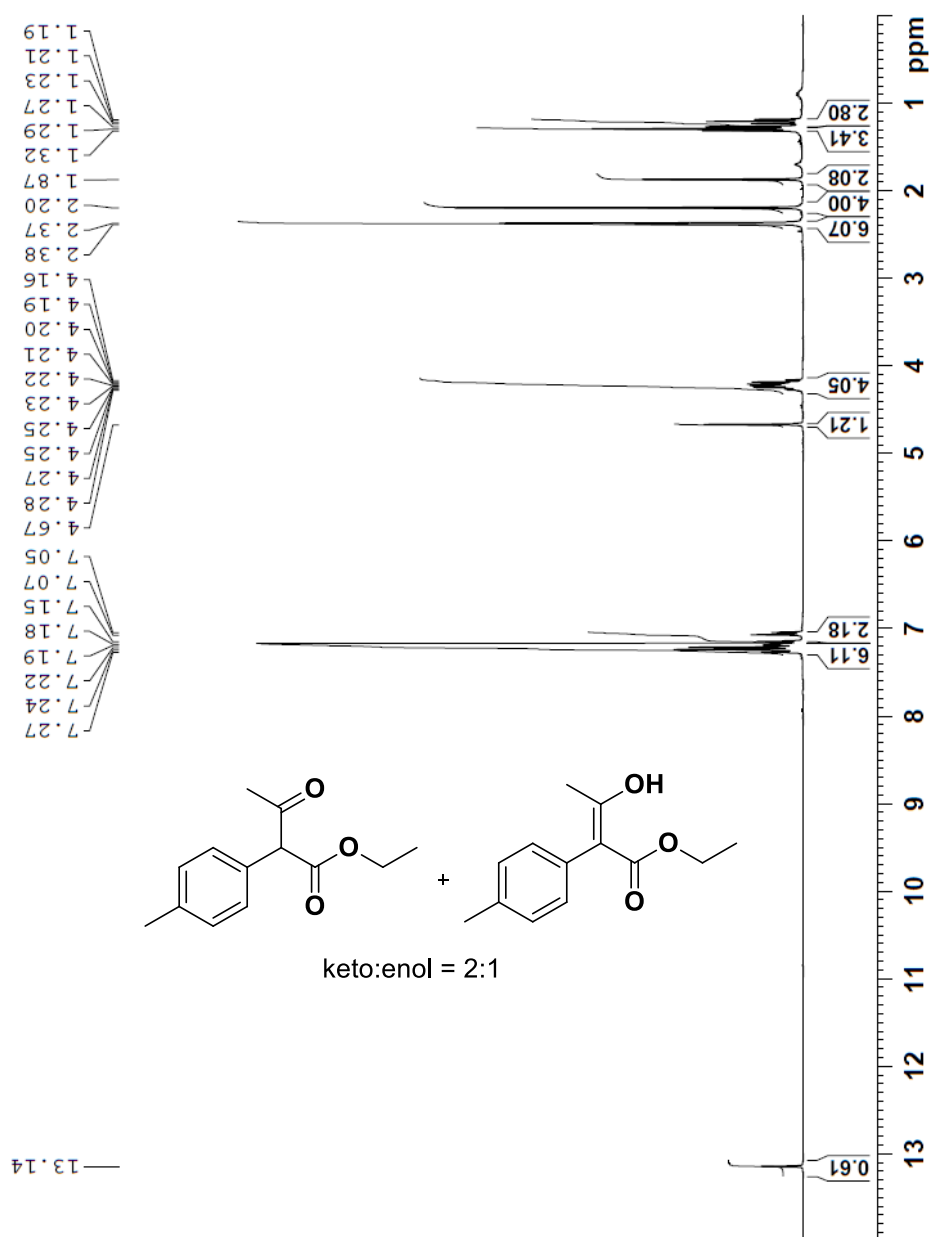
# <sup>1</sup>H NMR Spectrum of Compound 3b in CDCl<sub>3</sub>



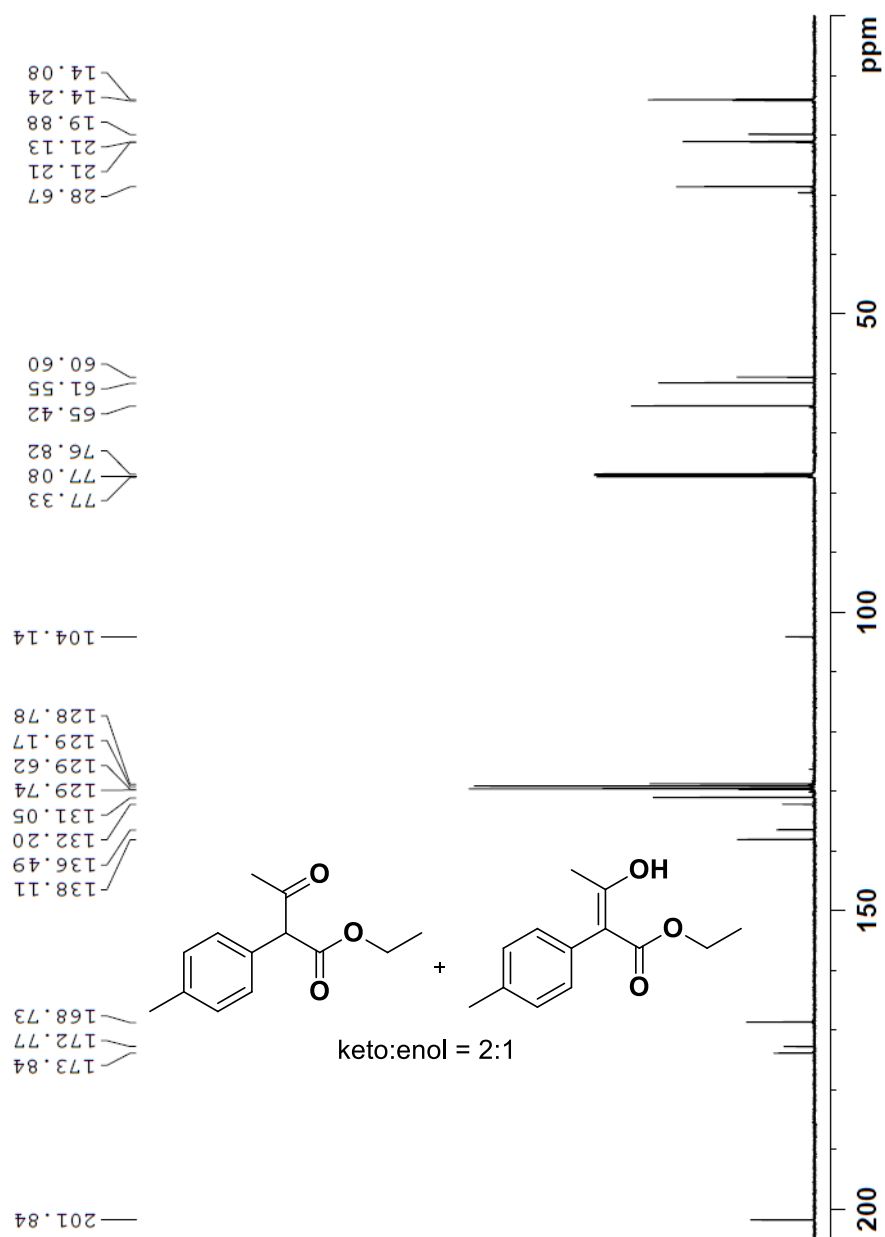
**$^{13}\text{C}$  NMR Spectrum of Compound 3b in  $\text{CDCl}_3$**



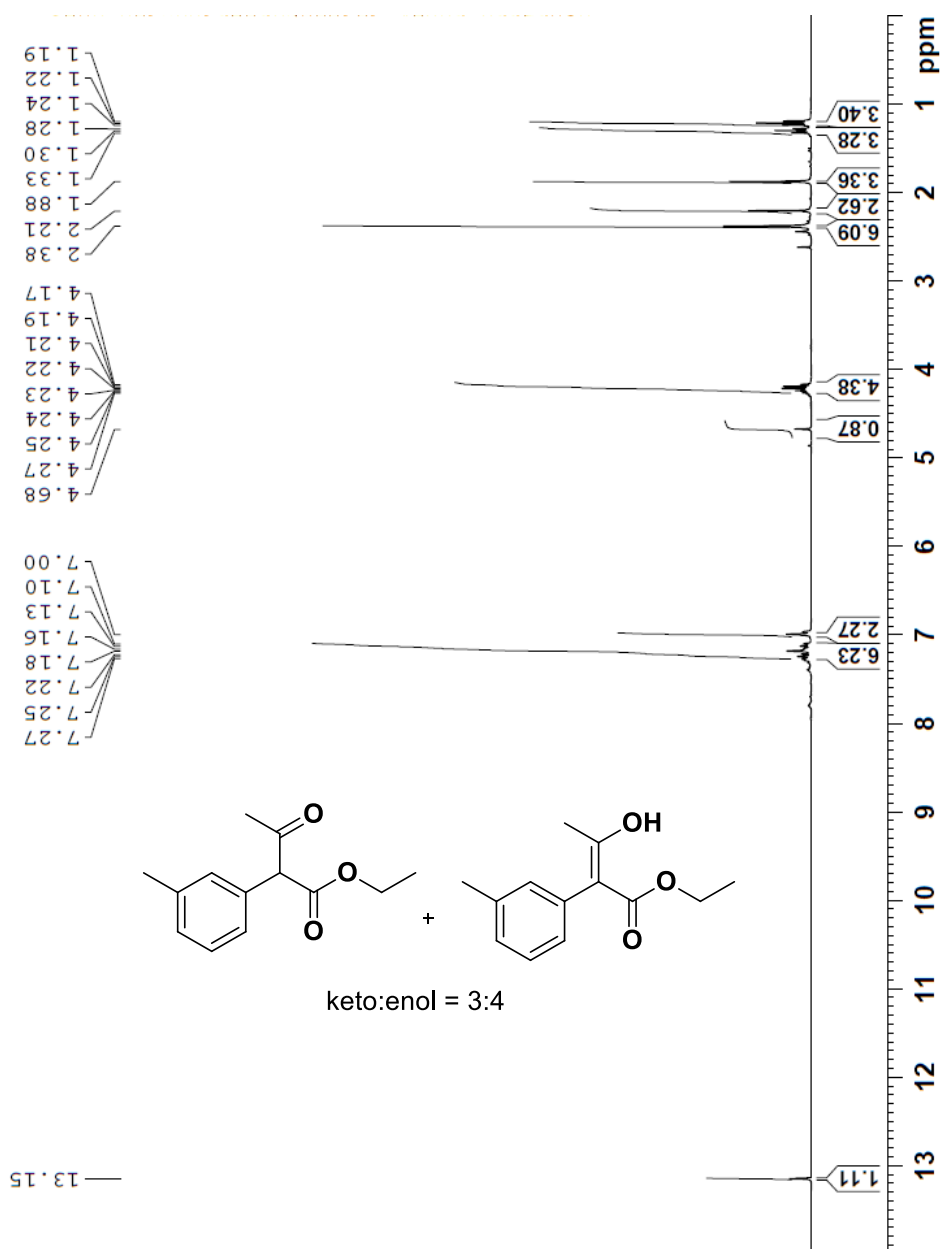
# <sup>1</sup>H NMR Spectrum of Compound 3d in CDCl<sub>3</sub>



**$^{13}\text{C}$  NMR Spectrum of Compound 3d in  $\text{CDCl}_3$**

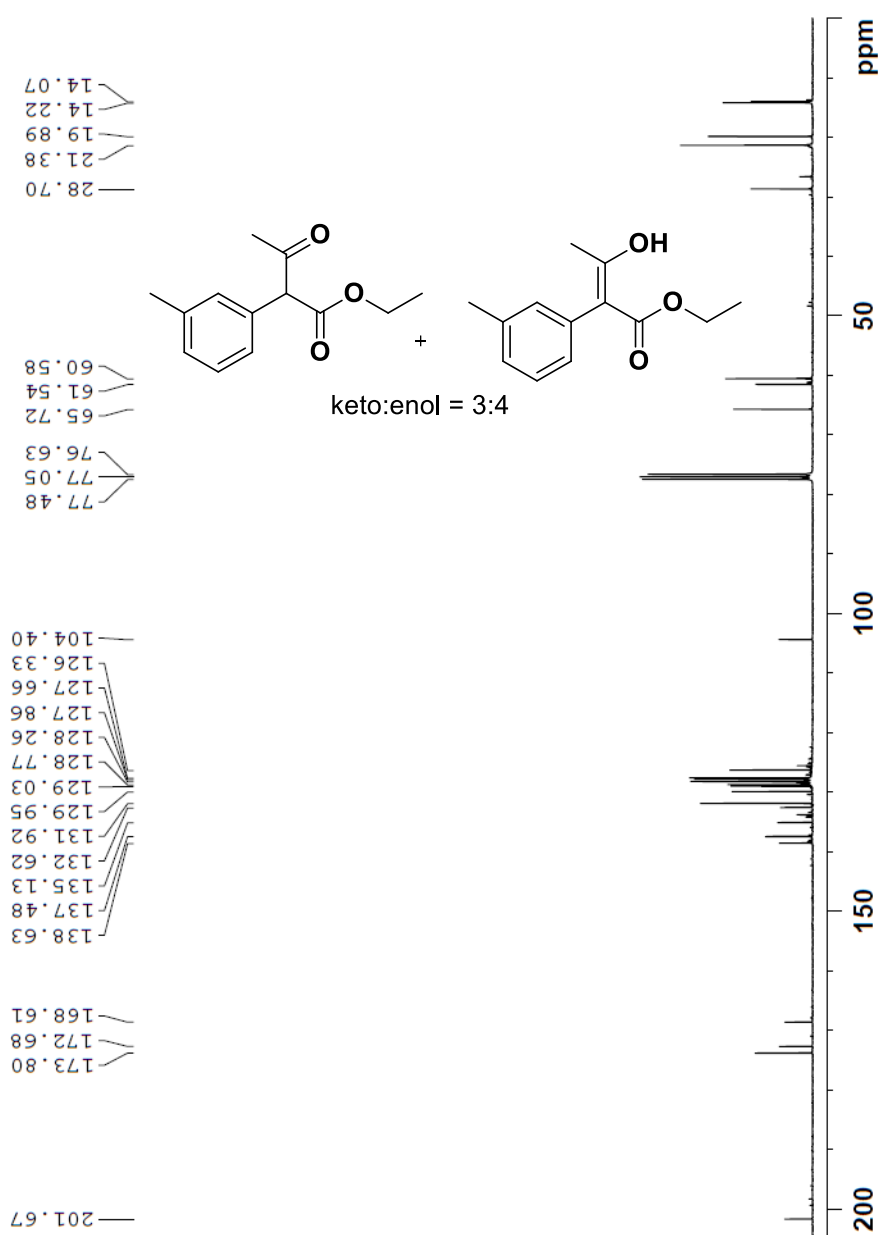


# <sup>1</sup>H NMR Spectrum of Compound 3e in CDCl<sub>3</sub>

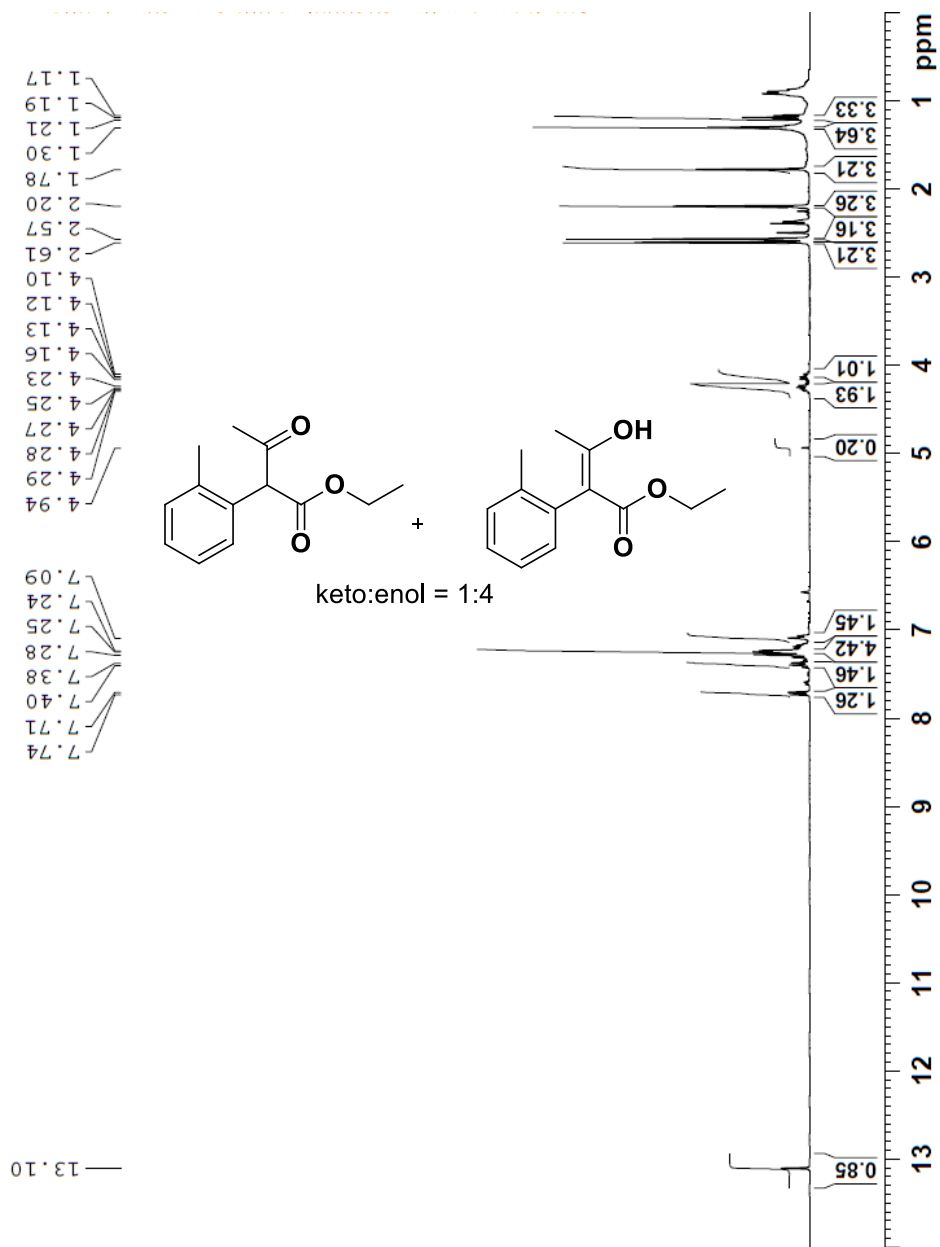




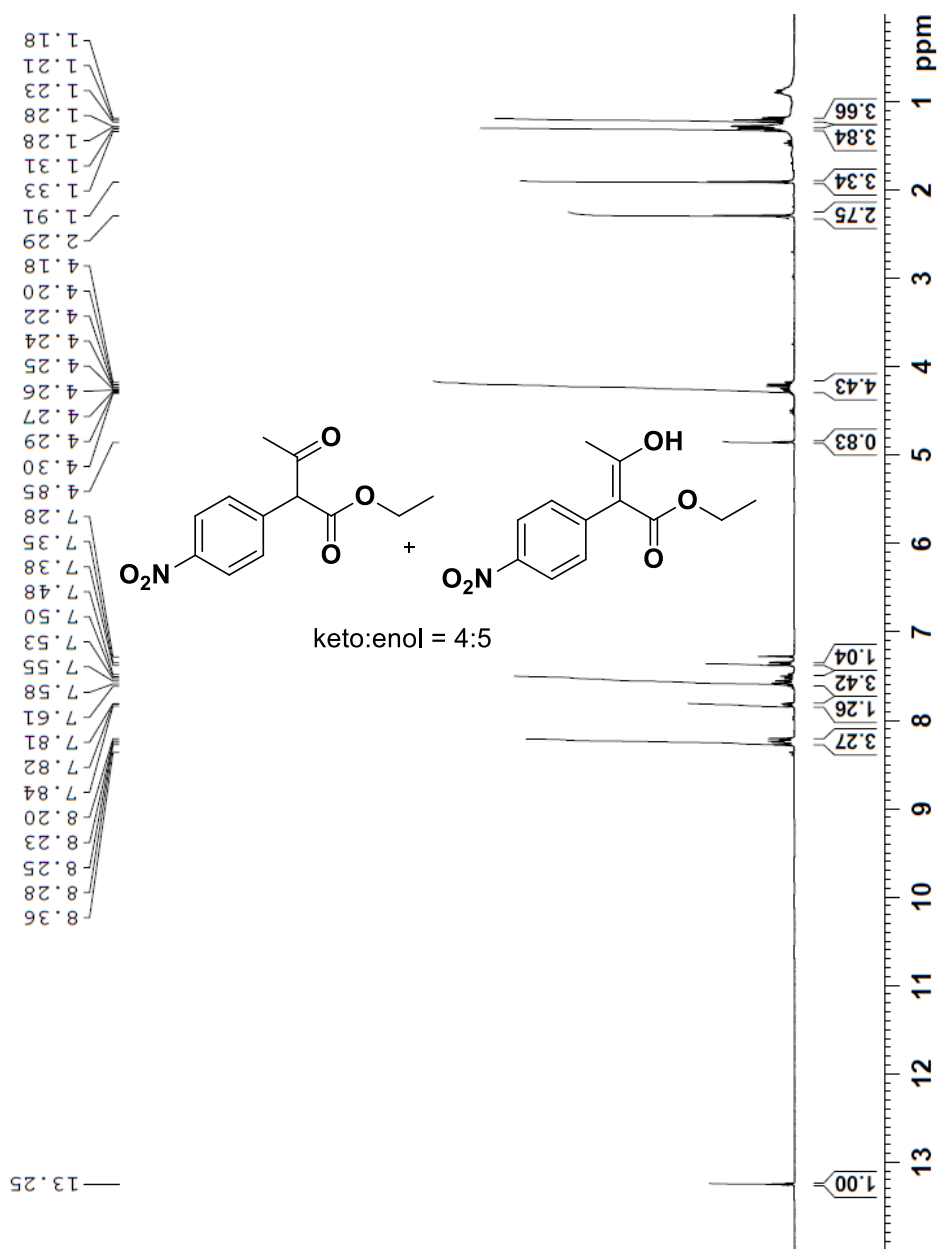
**$^{13}\text{C}$  NMR Spectrum of Compound 3e (Keto-Enol Tautomer) in  $\text{CDCl}_3$**



### <sup>1</sup>H NMR Spectrum of Compound 3f in CDCl<sub>3</sub>



<sup>1</sup>H NMR Spectrum of Compound 3g in CDCl<sub>3</sub>



13.22 —

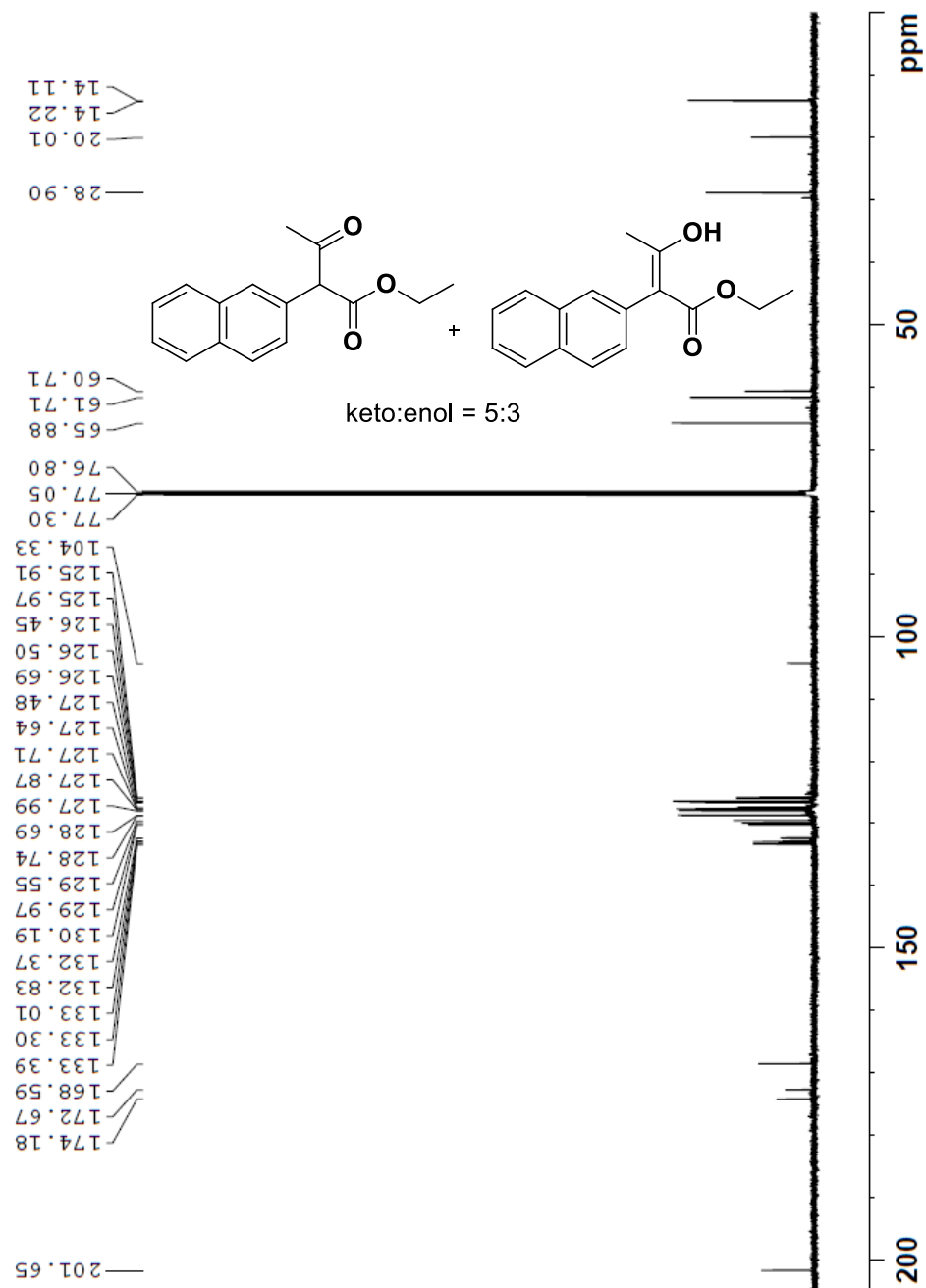
7.90  
7.89  
7.88  
7.87  
7.86  
7.85  
7.83  
7.65  
7.55  
7.54  
7.53  
7.53  
7.52  
7.51  
7.51  
7.50  
7.49  
7.33  
7.33  
7.32  
7.31  
7.28  
4.89  
4.06  
3.61  
2.46  
3.34  
2.56  
1.32  
1.33  
1.30  
1.21  
1.19  
1.18

0.76  
7.40  
0.82  
5.48  
0.84  
1.30  
4.06  
3.61  
2.46  
3.34  
2.56

keto:enol = 5:3

CCOC(=O)C(=O)c1ccccc1 + CCOC(=O)C(O)=Cc1ccccc1

<sup>13</sup>C NMR Spectrum of Compound 3h in CDCl<sub>3</sub>



## CURRICULUM VITAE

**Khorshada Jahan**

**Date of Birth:** 15 January, 1982

### Education

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Degree/Certificate : **PhD**  
Degree obtained : 2021  
University : Department of Chemistry and Biochemistry  
University of Wisconsin-Milwaukee, USA

**Dissertation Title:** Part I: ‘A concise synthesis of microtubule inhibitor tryprostatin a and b and its analogs; Part II: Brønsted acid catalyzed reactions of aromatic ketones with ethyl diazoacetate and its synthetic scope’

**Advisor:** Professor M. Mahmum Hossain

Degree/Certificate : **M. S. (*Organic Chemistry*)**  
Degree obtained : 2008  
University : Department of Chemistry  
University of Dhaka, Bangladesh

**Thesis Title:** Developing a simple procedure for synthesis of biologically active compounds such as Chromenes and its derivatives.

**Advisor:** Professor M. Giasuddin Ahmed

Degree/Certificate : **B. Sc. (*Chemistry*)**  
Degree obtained : 2006  
University : Department of Chemistry  
University of Dhaka, Bangladesh

**Thesis Title:** Isolation of secondary metabolites from Endophytic fungus of vinca rosea.

**Advisor:** Professor Nilufar Nahar

### Research Skills

- Proficient in design, synthesis, and characterization of novel compounds. Skilled in both linear and convergent approach in total synthesis.
- Experience in planning several research projects and managing all the necessary resources involved. For example, drafted research proposal, resource planning, proof of concept, designing unit processes and operations.
- Knowledge of molecular docking using some software, specifically Auto dock Vina2.6.
- Skilled in compounds characterization tools including nuclear magnetic resonance spectroscopy  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, 2D NMR (COSY, HMBC, HSQC, NOESY, DEPT), Infra-Red spectroscopy (IR), UV-Vis spectroscopy (UV) and High Resolution mass spectrometry (HRMS).

- Highly experienced in chromatographic tools such as column chromatography (both normal and reverse phase), Thin layer chromatography, Prep-TLC, LC-MS 2020, HPLC, and GC.
- Highly skilled in research data processing and analysis.
- Strong skills for the preparation of research reports and peer-reviewed scientific manuscripts for submission and publication.
- Excellent written and oral communication skills in English.
- Proficient in application of Windows and Microsoft office programs (excel and word) and chemistry and related other generic application including Chem Draw and Avogadro.
- Excellent understanding on standard laboratory practice (SLP), trained, and supervised personnel on the chemical laboratory practices throughout my teaching and research career.

### **Awards and Scholarships**

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- Chancellor Awards, University of Wisconsin-Milwaukee- Spring 2016 to 2020
- Chancellor Awards, University of Wisconsin-Milwaukee- Fall 2016 to 2020
- Summer Fellowship: 2017-2020
- Mentor/Mentee award, University of Wisconsin-Milwaukee- Fall 2018
- Dean's Award for academic excellence, University of Dhaka, Bangladesh- 2006

### **Working and Internship Experiences**

---



Name of Employer : **University of Wisconsin-Milwaukee**

Position : PhD candidate, Department of Chemistry and  
Biochemistry

Duration : January, 2016 – Till now

Major Tasks

- Worked and accomplished several research projects.
- Designed and developed new routes and analysis strategies.
- Experienced in working both as a lead person and co-worker.
- Worked with various compound characterization and analytical tools.
- Conducted discussion and lab classes to undergraduate levels.
- Mentored undergraduate students' research.
- Mentored new graduate teaching assistants.
- Develop and write grant proposal.

Name of Employer : **University of Dhaka, Bangladesh**

Position : Faculty, Department of Chemistry

Duration : August, 2013 – December, 2015

Major Tasks

- Taught chemistry and lab classes to undergraduate and graduate levels.
- Supervised research projects.
- Set up new lab experiments and prepared lab manual.

- Worked as a student mentor of the chemistry department.
- Wrote exams, quizzes, and graded them.
- Develop and write grant proposal.

Name of Employer : **American International University-Bangladesh  
(AIUB), Bangladesh**

Position : Assistant Professor, Department of Natural Science

Duration : February, 2009 – August, 2013

Major Tasks

- Delivered lectures, seminars and tutorials.
- Designed, prepared, and developed teaching and laboratory materials.
- Assessed students' coursework and supervised research activities.

Name of Employer : **Plasma plus Application and Research Laboratory,  
Bangladesh**

Position : Research Chemist

Duration : January, 2009 – February, 2009

Major Tasks

- Analyzed different metal and non-metal ions, identified and quantified impurities in the commercially produced fine chemicals using various analytical methods (HPLC, FT-IR, AAS, and UV-Vis).
- Prepared reports on the analyses and presented them to the customer.

### Journal Articles:

- Jahan K.; Khan K. R.; Akhter K.; Romman U. K. K.; Halim E, 'A convenient approach to synthesize substituted 5-Arylidene-3-m-tolyl thiazolidine-2, 4-diones by using morpholine as a catalyst and its theoretical study.' PLoS ONE, 2021, 16(3), e0247619.  
<https://doi.org/10.1371/journal.pone.0247619>
- Rahaman, M.; Ali M. S.; Jahan K.; Hinz D.; Belayet J. B.; Majinski R. and Hossain M. M., 'Synthetic Scope of Brønsted Acid-Catalyzed Reactions of Carbonyl Compounds and Ethyl Diazoacetate.' J. Org. Chem., 2021, 86(9), 6138-6147.  
<https://doi.org/10.1021/acs.joc.0c02972>
- Rahaman M.; Ali M. S.; Jahan K.; Belayet J. B.; Rahman A. F. M. T.; Hossain M. M., 'Chemistry of 3-hydroxy-2-aryl acrylate: syntheses, mechanisms, and applications.' Organic Chemistry Frontiers, 2021, 8(1), 169-191.  
<https://doi.org/10.1039/D0QO01157F>
- Akhter K.; Jahan K.; Halim M. E.; Shefa S.; Rifat S.; Khan K. R.; Ahmed S. M.; Romman U. K. R 'Synthesis of 1-phenyl-3,4-dihydropyrimidine-2(1H)-ones derivatives under solvent free condition and study of their antimicrobial activity.' Bangladesh J. Sci. Ind. Res., 2019, 54(1), 47-54. <https://doi.org/10.3329/bjsir.v54i1.40730>
- Al-Mahmud; Halim M. E.; Ali M. K.; Kabir M. A.; Akhter K.; Jahan K.; Romman U. K. R., One pot synthesis of 2- amino-5-oxo-4-aryl-5, 6, 7, 8-tetrahydro- 4Hchromenes- 3-

carboxylic acid ethyl esters, Bangladesh J. Sci. Ind. Res., 2018, 53(1), 35-40. <https://doi.org/10.3329/bjsir.v53i1.35908>

- Akhter K.; Halim M. E.; Ahmed S. M.; Sarkar P.K.; Akter F.; **Jahan K.**; Romman U. K. R.; Ahmed M. G., 'One Step Cyclocondensation of (Thio)Barbituric Acid with Chalcones in Glacial Acetic Acid and Phosphorous Pentoxide: Part-II.' *Bangladesh J. Sci. Ind. Res.*, **2017**, 52(2), 115-124. <https://doi.org/10.3329/bjsir.v52i2.32921>
- Rifat S.; **Jahan K.**; Romman U. K. R.; Akhter K.; Halim M. E., 'A Green Approach to Synthesize and in vitro Antimicrobial Activity of Indeno-Imidazole Derivatives and Ninhydrin-Nucleophile Adducts.' *Asian Journal of Chemistry*, **2016**, 28(7), 1611-1616. <https://dx.doi.org/10.14233/ajchem.2016.19775>
- Akhter K.; Ahmed S. M.; Halim M. E.; Kader M.A.; **Jahan K.**; Romman U. K. R.; Ahmed M. G., 'One Step Cyclocondensation of (Thio)Barbituric Acid with Chalcones in Glacial Acetic Acid and Phosphorous Pentoxide: Part-I.' *Bangladesh J. Sci. Ind. Res.*, **2016**, 51(2), 129-138. <https://doi.org/10.3329/bjsir.v51i2.28110>
- Akhter K.; **Jahan K.**; Romman U. K. R.; Ahmed M. G.; Rahman M. S.; Al-Amin. M., 'A Green Approach to Synthesize Dihydropyrimidinone Derivatives by Using Anhydrous ZnCl<sub>2</sub> Catalyst Under Refluxing Condition in Heptane-Toluene Medium via Biginelli Reaction.' *Asian Journal of Chemistry*, **2015**, 27(7), 2624-2626. <https://doi.org/10.14233/ajchem.2015.18615>
- Zaman Z.; **Jahan K.**; Akhter K.; Romman U. K. R.; Ahmed S. M.; Siddiki S. M.A. H.; Ahmed M. G., 'An Efficient Synthesis of Chromene Derivatives through

a Tandem Michael Addition-Cyclization Reaction. *J. Bangladesh Chem. Soc.*, **2013**, 26(1), 75-82.

- Ahmed M. G.; Romman U. K. R.; Akhter K.; **Jahan K.**; Bhuiyan M. N. H. Halim M., 'Synthesis of Substituted Tetrahydrochromenes by the Reactions of  $\alpha$ ,  $\beta$ -Unsaturated Cyanoesters with Dimedone/1,3-Cyclohexanedione' E., *Syn Commun*, **2011**, 41(19), 2822-2827.  
<https://doi.org/10.1080/00397911.2010.515346>

#### **Proceedings/ Presentations in Conference:**

- Regioselective C-2 Lithiation of Protected Gramine: Synthesis and Mechanism, **Northwest Regional Meeting**, Poster presentation, May 2021.
- A convenient approach to synthesize regioselective C-2 alkylation of Protected Gramine, **UWM Chemistry and Biochemistry Annual Research Symposium**, Poster presentation, April 2021.
- Selective C-2 Lithiation of Protected Gramine: Synthesis and Mechanism, **American Chemical Society National Meeting**, Poster presentation, Spring 2021.
- A Study of Migratory Aptitudes and Reaction Scope of Acid-Catalyzed Reactions of Carbonyl Compounds with Ethyl Diazoacetate, **ACS Great Lakes Regional Meeting**, Poster presentation, May 2019.
- Reactions of Carbonyl Compounds and Ethyl Diazoacetate: Synthetic Scope and Relative Migratory Aptitude Investigations, **UWM Chemistry and Biochemistry Research Symposium**, Poster presentation, April 2019.

- Reactions of Carbonyl Compounds and Ethyl Diazoacetate: Synthetic Scope and Relative Migratory Aptitude Investigations, **The 36th Herbert C. Brown Symposium-Purdue University**, Poster Presentation, April 2019.
- A Detailed Study of Acid-Catalyzed Reactions of Carbonyl Compounds with Ethyl Diazoacetate, **BACABANA conference**, Poster presentation, August 2018.

### Professional Societies

---

- 2009- Member, Bangladesh Chemical Society (BCS)
- 2019- Member, American Chemical Society (ACS)
- 2016- Member, Bangladesh Chemical and Biochemical Association in North America (BACABANA)
- 2016- Member, Bangladesh Student Association (BSA) at University of Wisconsin-Milwaukee

### Community Services

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- 2020: Member of Graduate Student Council, UWM
- 2007: Volunteered Bangladesh Chemistry Olympiad
- 2014-2015: Member of Science Olympiad organizing committee, University of Dhaka, Bangladesh