Synthesis towards the potential pharmacophore of Avrainvillamide

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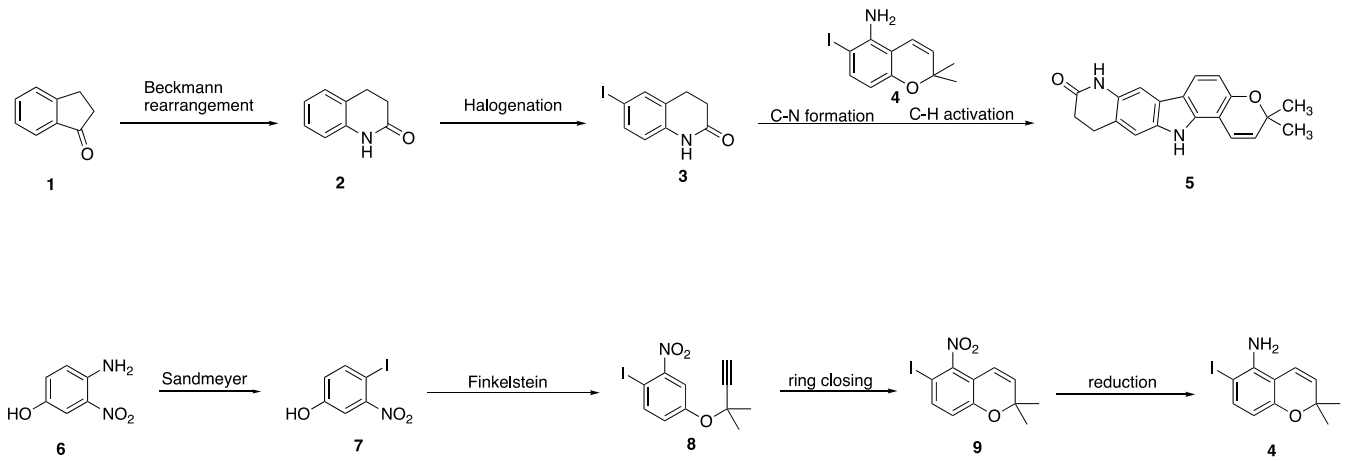
Abstract

Acute myeloid leukemia (AML) is a cancer form where there is an abnormal growth of blood cells in the bone marrow. Today's cancer treatment towards AML is 35-40 % curable for elderly patients of 60 years or younger. It is only 5-15% curable for patients that are over 60 years old. Thereby, current drugs are not targetable for the genetic irregularities in AML. Avrainvillamide is a natural compound isolated from a marine fungus. The compound was discovered to have antiproliferative activity in several human cells. Moreover, avrainvillamide is composed of different elements which gives it antiproliferative quality. Hence, studies have showed that avrainvillamide is an outstanding candidate for the treatment of acute myeloid leukemia (AML). This has also led chemists to attempt and synthesise the natural compound. *Herzon et al* and *Baran and co,* successfully synthesised avrainvillamide. *Baran et al* were able to produce an overall synthetic pathway of six steps, while *Herzon and co* had overall synthetic steps of eight. However, this thesis focuses on a new synthetic strategy with fewer total synthetic steps as the previous work used many steps towards the desired compound avrainvillamide. This thesis focuses on the synthesis of the potential pharmacophore of avrainvillamide with overall synthetic steps of seven.



Scheme 1 Overall synthetic strategy

The syntheses towards desired product 3,3-dimethyl,8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one **(5)**, met upon several challenges. Several screening procedures were conducted on the synthetic steps to find the most suitable reagents and conditions. This increased the obtained yields.

Content

1.Introduction

1.1 Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a form of cancer in which the bone marrow rapidly forms abnormal blood cells.[1] Articles from 1999 and 2015 show that AML is 35-40% curable for elderly patients at the age of 60 years old or younger. Although, AML is only 5-15% curable for patients over 60 years of age.[2,3] However, through studies of the genomic landscape and recent advancements in therapeutic approaches are convenient in finding new cures.

There are two proteins that are significantly important when discussing AML. These are nucleophosmin (NPM1) and chromosome maintenance protein (CRM1). Nucleophosmin is an abundant nuclear protein found in the nuclei of proliferating cells. It participates in several genetic processes including ribosome biogenesis, mRNA processing chromatin remodelling and embryogenesis.[4] Additionally, NPM1 also has a critical function in maintaining genomic stability by functioning in several different DNA repair pathways and regulation of apoptosis. It also supresses tumour and has oncogenic functions. The probability of achieving complete remission (CR), lower relapse and better overall outcome is higher in the presence of NPM1 mutations for patients with AML.[5] Patients carrying AML disease where 50-60% of them are adults, have NPM1 rearrangement or mutations.[6]

Chromosome maintenance protein (CRM1), is also an important protein in the genomic landscape in the studies of AML. It is a nuclear export receptor which is involved in the active transportation of tumour suppressors. CRM1 also participates in the active transportation of several cargo proteins, including transcription factors, tumour suppression proteins such as TSPs, cell cycle regulators including p53, p21, p27, NPM1 and RNA molecules.[7] Herein, cellular mechanism involving the accumulation, stabilization and distribution of p53 as potent transcription factor has been presupposed to be crucial for inhibiting growth of abnormal damaged cells.[8] Consequently, the loss of p53 can also stimulate the development of AML.[9]

Kojima K et al, performed a study in the prognostic significance in AML and the effects of small molecule selective inhibitors of CRM1. Results showed that high expression of CRM1 cohered with short survival of patients. In addition to this, the role of p53 in AML patients was also investigated. Results revealed occurrence of synergistic apoptosis induction in AML, in

the presence of the combination of the inhibitors Nutlin-3a and KPT-185 when increasing the level of p53 and accumulated p53 in nucleus.[10]

Drug therapies today

50-75% of adult patients with AML achieve CR with deoxycytidine analogue cytarabine and anthracycline antibiotic such as idarubicin or danorubicin (see *figure 1*). Patients have also been treated with the anthracenodine mitoxantrone which inhibits the enzyme topoisomerase IIa. However, an analysis conducted by an Eastern Oncology group showed that patients with AML die of their disease because of persistent relapsed AML.[11,12] Studies were performed on 3000 patients carrying AML who entered 5 successive clinical trials, using cytarabine and danorubicin for induction and with increasingly intensive therapy. The outcome of the experiment showed that 62% of the patients achieved CR while 76% relapsed or died.[13]

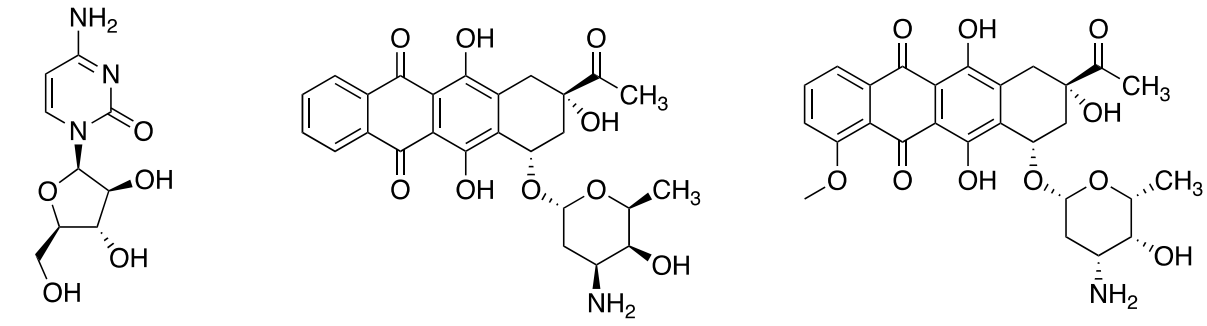


Figure 1 Current drugs that are used to treat AML [11,12]

Schlenk R. F et al conducted another study on gene mutations and treatment effect with all-trans retinoic acid (ATRA). When investigation the mutation frequencies among NPM1, CEBA, FLT3 genes in elderly AML patients, it was discovered that the NPM1 and FLT3-ITD were more frequent in cryogenically normal AML patients. Additionally, 47% of the patients who were treated with ATRA had a complete remission while 15% died.[14]

Genetic screening has led to improve clinical results for some patients, especially those with acute promyelocytic leukemia (APL) and core binding factor AML (CBF-AML). However, most of the genetic irregularity in AML are not targetable with current available drugs.[15-18]

1.2 The natural product avrainvillamide

(+)-Avrainvillamide is a natural compound which was isolated from a marine fungus named *Aspergillus sp*. CNC358.[19,20] The compound is found to have antiproliferative activity in many different cultured human cells.[19-22] In 2001 a Pfizer group in Japan described and named the compound as CJ-17,655.[23] Compared to other natural products, it also has a bridged 2,5-diketopiperazine entity, a rare 3-alkylidene-3*H*-indole-1-oxide functionality and a bicycle[2.2.2]diazaoctane core (the red coloured moiety in *figure 2*).[19,23] Bicycle[2.2.2]diazaoctane has long been in an important subject as there have been great efforts in research for the compound, due to its complex structure and potential biological activities.[24] Moreover, avrainvillamide is a highly oxidized, prenylated indole alkaloid.[21] Prenylated indole alkaloids are also reported to have insecticidal, antitumor, anthelmintic, antibacterial properties among other.[24,21,25,26]

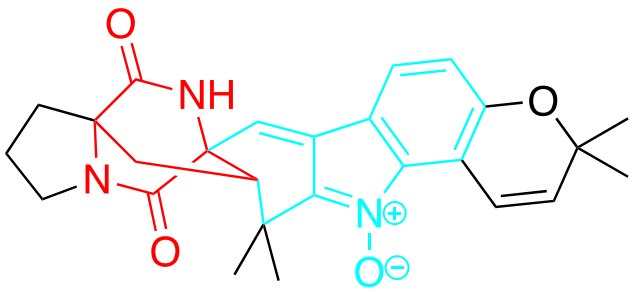


Figure 2 The bicycle[2.2.2]diazaoctane core in Avrainvillamide [27]

Another important moiety in avrainvillamide is the carbazole scaffold. The name "carbazole" includes the tricyclic molecular skeleton (blue coloured moiety in *figure 2*), and other fused carbazoles such as tetracyclic, pentacyclic, hexacyclic and heptacyclic fused carbazoles.[28] For a long time, the carbazole scaffold has been a significant structure of many biologically active compounds.[29] The first carbazole compound to be isolated was *9H*-carbazole, found in coal tar in 1872 by Graebe and Glazar (see *figure 3*).[30] Since then, many carbazole derivatives have been synthesized and are known for their biologically activity such as anti-oxidant, anti-inflammatory, anti-bacterial, anti-tumour, anti-convulsant, anti-psychotic and anti-diabetic.[31]

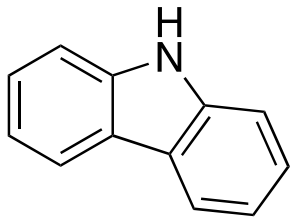


Figure 3 9H-carbazole isolated by Graebe and Glazar [29]

In 2002, a dimer of avrainvillamide was isolated from a different fungus named *Aspergillus ochraceus* WC76466. The fungus was called stephacidin B and revealed that is also has antiproliferative activity against multiple cancer cell lines.[21] Synthesis of stephacidin B and avrainvillamide revealed that stephacidin B quickly retrodimerizes in cell culture to give avrainvillamide.[22] This interconversion appeared to happen before any other biological effects were detected. Thereby, it can be argued that the biological activity of stephacidin B probably arises from its ability to serve as an *in vivo* source of avrainvillamide.[19]

1.3 Introduction to treatment of AML by using avrainvillamide

Prior studies by *Wulff J. E et al* reveals that avrainvillamide has capacity to bind to one or more proteins in vitro. By using a series of molecules that imitates the natural product, it was determined that (+)-avrainvillamide can bind to nucleophosmin and other proteins such as NPM1.1, B23, numatrin, NO38. Experiments on mutagenesis also supports the theory that the natural product binds specifically to cysteine-275 of nucleophosmin, a residue near the C-terminus and one of three free cysteines in the native protein.[22] This was confirmed in an article published in 2015 where studies were conducted upon three NPM1 constructs. Studies were performed on single cysteine to alanine mutations of each of the cysteine residues present in wild-type NPM1 (wtNPM1). The experiments confirmed that C275A mutation was the only one which could reduce affinity isolation of NPM1 by the biotinylated avrainvillamide conjugate. Hence, this certifies that (+)-avrainvillamide binds specifically to Cys275. Additionally, the article describes that activity-based probes based on the electrophilic (+)-avrainvillamide bind to NPM1 in cultured human cancer cells and cell lysates.[32] The natural product binds reversibly to thiols, including cysteine residue of certain proteins, by conjugate addition of α,β-unsaturated nitrone function of avrainvillamide.[22]

The binding properties of (+)-avrainvillamide to NPM1 and other proteins, makes it an outstanding candidate for the treatment of AML. *Mukherjee H. et al* conducted experiments

with avrainvillamide on wtNPM1 and AML associated NPMc+ mutants. The correspondence between wtNPM1 and avrainvillamide, was conducted by examining the interaction of a peptide corresponding a C-terminal 52 residue of a wtNPM1. By using ESI-TOF mass spectrometry, the formation of avrainvillamide-NPM1 peptide complex was observed already a 1 minute after conducting the experiment. The same experiment was also conducted with mutated NPM1 peptides, where avrainvillamide-NPMc+ peptide adducts formation was observed. The study also revealed that avrainvillamide-NPMc+ bindings where more rapidly formed, compared to the binding between avrainvillamide and wtNPM1. In conclusion, avrainvillamide has an excellent interaction with both wild-type NPM1 as well as NPMc+ mutants (see *figure 4*).[32]

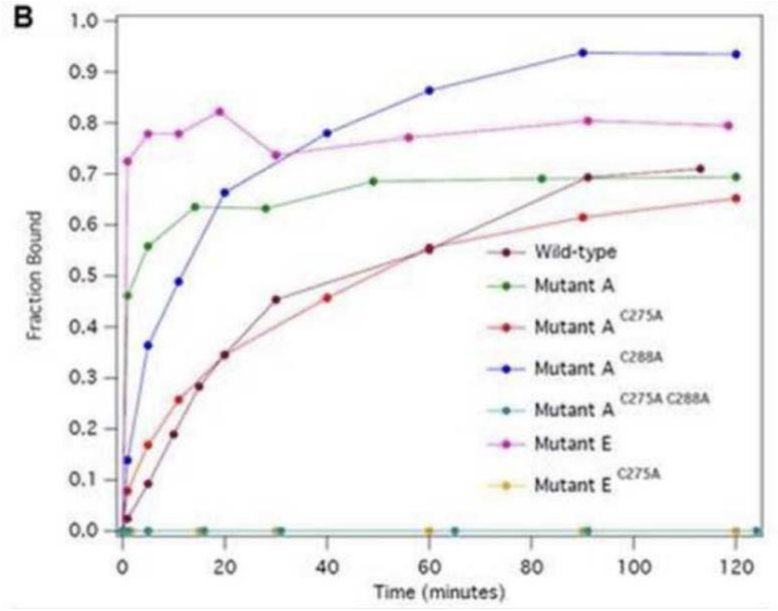


Figure 4 Interaction between avrainvillamide and C-terminal domain of NPM1 and AML associated NPM1 mutants [32]

In addition to avrainvillamide's interactions with NPM1, CRM1 and AML associated NPM1 mutants, the natural product has also the ability to inhibit cellular growth and induces cell cycle arrest and cell death in AML cell lines.[22,32] An article from 2016 reported that studies were conducted with avrainvillamide on five different AML cell lines. The results from a 24-hour experiment showed that the IC_{50} value for MV4-11 cell, OCI-AML3 and Molm-13 cells were more sensitive compared to the acute promyelocytic cells NB4 and HL-60 (see *figure 5*).[22,33]

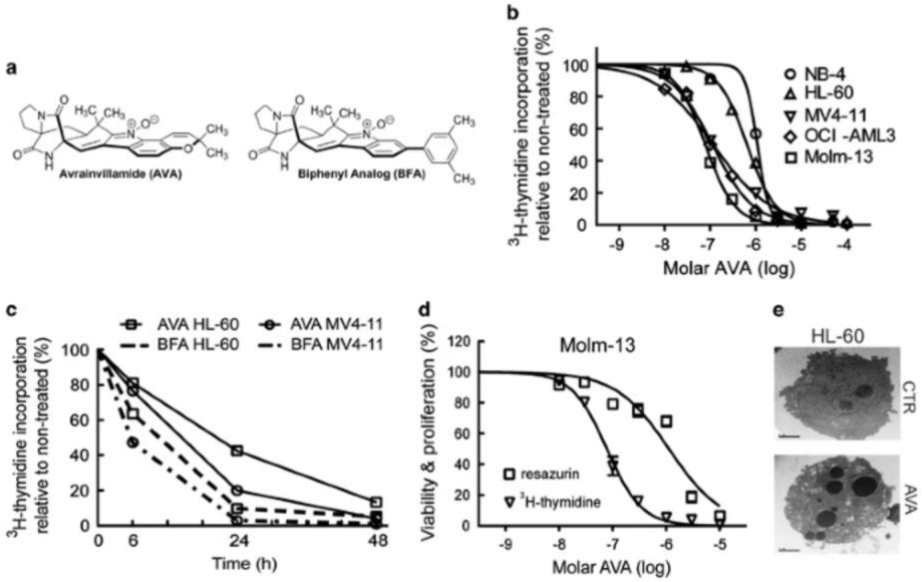


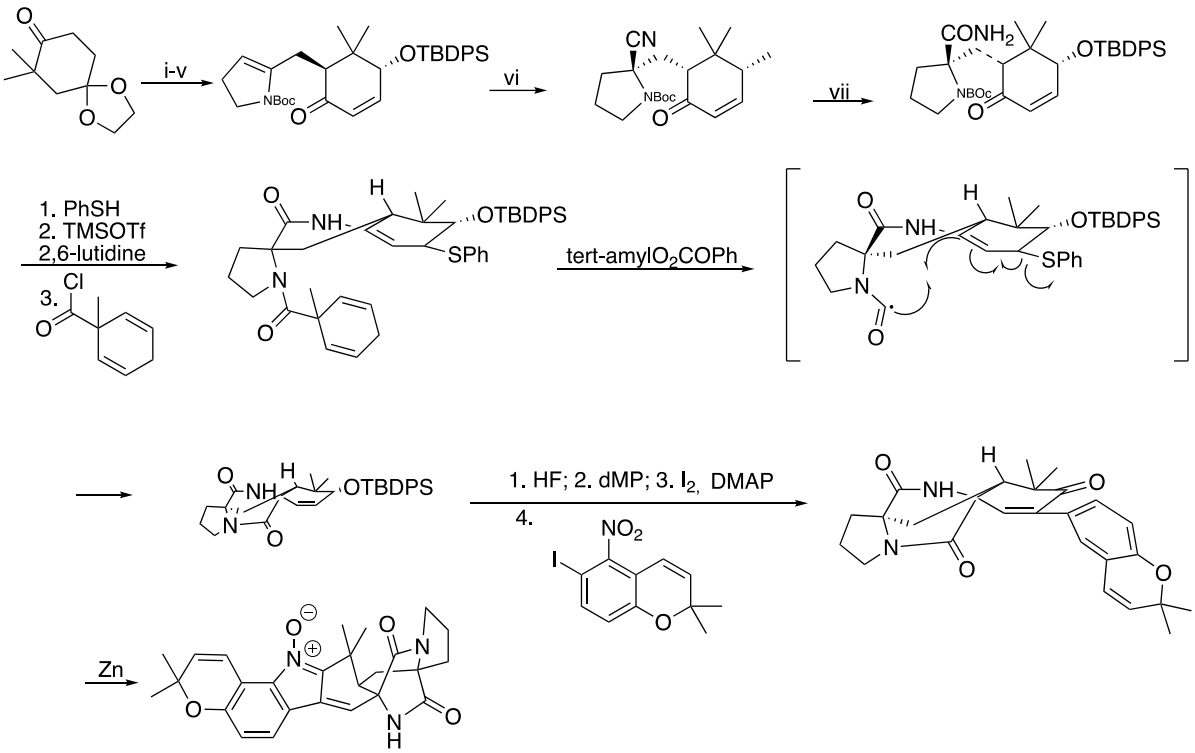
Figure 5 The effect of avrainvillamide on different cell lines[33]

Furthermore, *Lee K. et al* studied the effect of avrainvillamide on p53 protein.[34] Avrainvillamide enhance p53 expression in some cells.[Error! Bookmark not defined.] Further investigations conducted on p53 using immunoblot and flow cytometry experiments, revealed that avrainvillamide increased p53 and p21 protein levels in OCI-AML3 and MV4-11 cells. Also, potential roles of p53 in avrainvillamide-induced anti-proliferation were investigated by studying Molm-13 cells converted with dry short hairpin RNA against p53 (shp53) or empty control vector (CTR).[33,35] Shp53 cells showed approximately 70% reduced p53 expression and p53 activation, after gamma-irradiation compared to CTR cells. Consequently, the shp53 cells were clearly less sensitive towards avrainvillamide compared to the CTR cells.[35]

1.4 Previous work

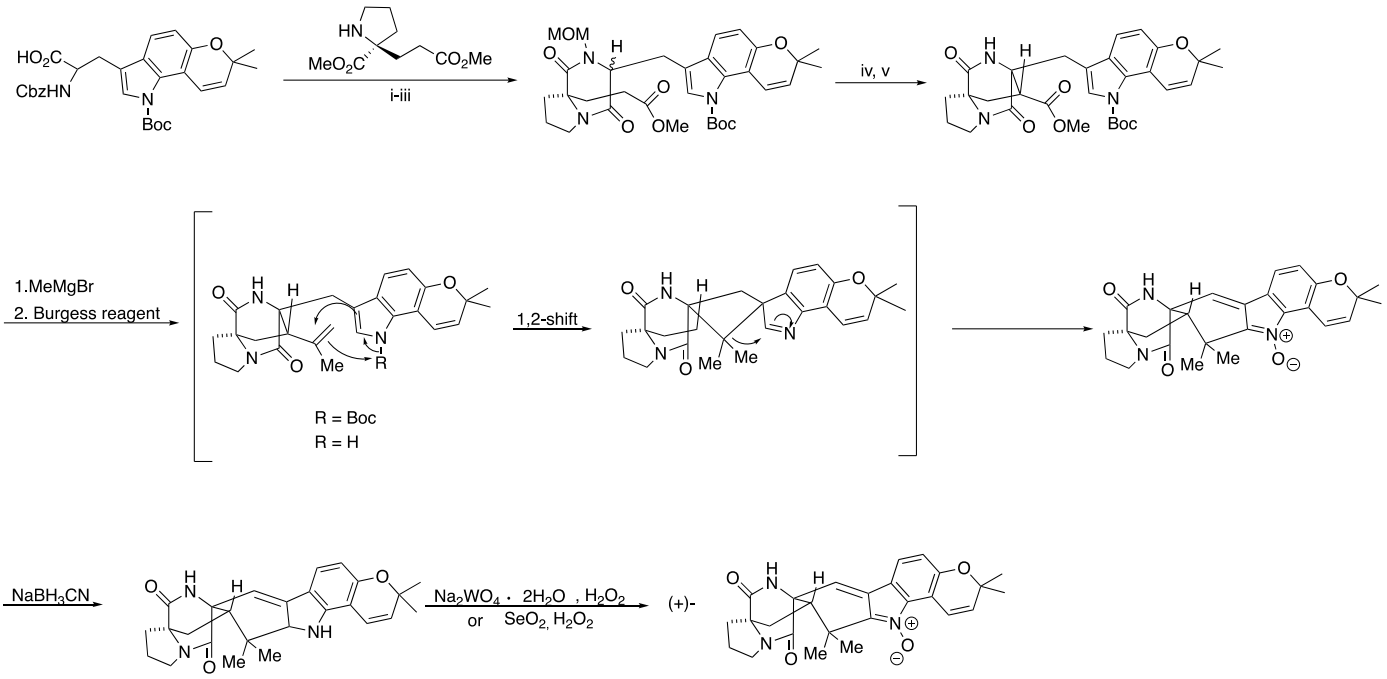
As avrainvillamide is an excellent candidate to treat AML, attempts where conducted to reproduce the natural product. Avrainvillamide is natural product that has the 3-alkylidine-*3H*-indole-1-oxide function.[19] This led *Herzon and co* to develop a synthetic route to synthesise avrainvillamide (see *scheme 1.4.1*). However, challenges were met upon their work as the target compound was light sensitive and could convert to a by-product at 23^{o}C in methanol-d_4. *Herzon and co* were able to accomplish the synthesis of the natural product but were met upon the challenge that it could easily be converted to stephacidin B under mild conditions. However, the authors successfully synthesised avrainvillamide in total of 23 steps with respect to

stereospecificity and chemoselectivity.[36] *Scheme 1.4.1* is an overview of the overall synthetic route towards avrainvillamide developed by *Herzon et al.*



Scheme 1.4.1 Herzon and Myer's synthetic route [37,38]

Baran et al also attempted to synthesise avrainvillamide. Their synthetic route was built on the fact that stephacidin A which is related to stephacidin B, can be oxidised to avrainvillamide.[38,39] The common synthetic approach for *Baran et al* and *Myers and co* is that the configuration of the final product is determined by the absolute stereochemistry of one chiral reagents, which was used in the initial step.[36-38] However, obstacles were also met upon this work as there were several complications upon synthesising the bicycle[2.2.2]diazoctane nucleus. There were also other difficulties such as design of a scalable route to the benzopyran-containing subunit, selection and synthesis of protecting groups for functional units and furthermore. In conclusion, *Baran et al* synthesised avrainvillamide in total of 14 steps (see *scheme 1.4.2 for the overall synthetic pathway*).[38,39]

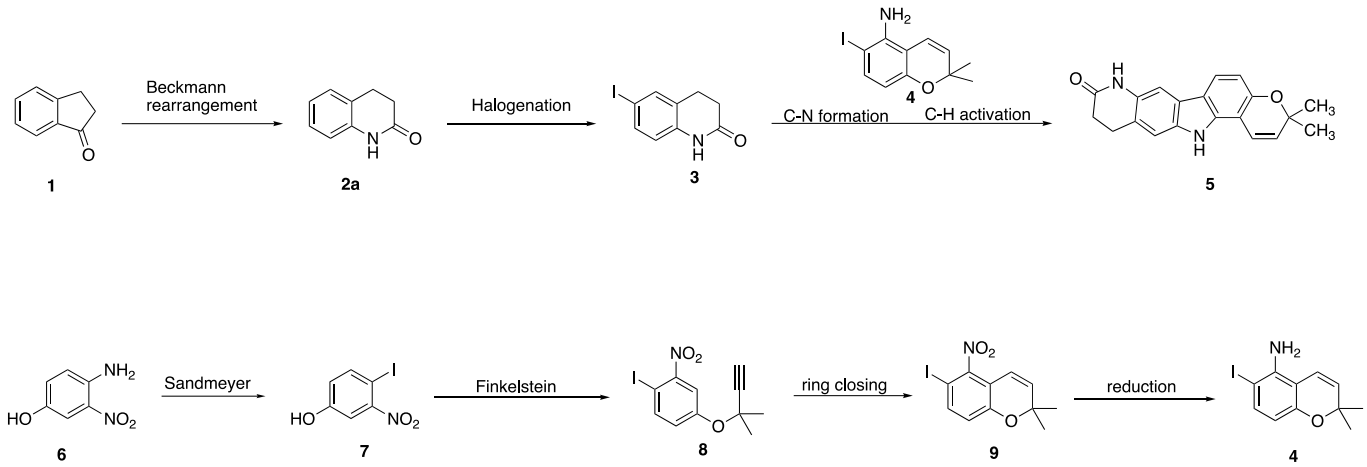


Scheme 1.4.2 Synthetic approach of *Baran et al.*[38,39]

2. Aim of study

The goal of this research is to find a synthetic route to synthetize the potential pharmacophore of avrainvillamide. The bicycle[2.2.2]diazoctane, the prenylated indole alkaloid and the carbazole scaffold have biological activity.[24-26] These groups are essential in a pharmacophore and thereby important as they bind to proteins in the human cells (NPM1 and CRM1).[22]

The synthetic pathway to the potential pharmacophore of avrainvillamide has overall seven steps. Starting with Beckmann rearrangement of 1-indanone (**1**) to give 3,4-dihydroquinolin-(*1H*)-one (**2a**). Then **2a** will be halogenated with 1,3-diiodo-5,5dimethylhydantoin (DIH) to give **3**. The last step includes the C-N formation and C-H activation by using **3** and **4,** which is synthetized through a four steps synthetic pathway from 4-iodo-3-nitrophenol (**6**) shown in *scheme 2.1.1*.

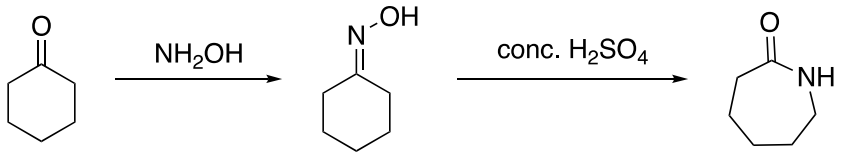


Scheme 2.1.1 The overall synthesis of **5**

3. Theory and Methods

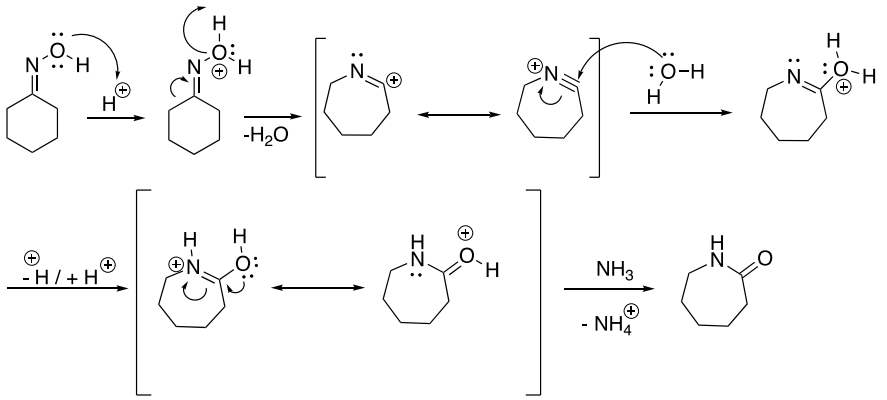
3.1 Beckmann rearrangement

Beckmann rearrangement is the rearrangement of oxime to amides. The reaction is named after the German chemist Ernst Otto Beckmann (1853-1923).[40,41] The rearrangement is often aided by using acids such as phosphorus pentachloride, phosphorus pentoxide and sulphuric acid among others (see *scheme 3.1.1*).[42]



Scheme 3.1.1 A general reaction scheme showing a Beckmann rearrangement[43]

The Beckmann rearrangement was an accidently discovered reaction. In 1883, a chemist tried to identify the products that were produced when reacting acetoxime with phosphorus pentachloride. This attempt was failed by the chemist but was discovered by Ernst Beckmann. Through experiments, he could determine that ketoximes could also react with other reagents as well.[43] The origin of the reaction often undertook harsh conditions with high temperature and high acetic medium. Beckmann also presented a so-called "Beckmann mixture" containing hydrochloric acid, acetic acid and acetic anhydride.[44]



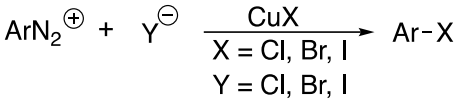
Scheme 3.1.2 Mechanism of a cyclohexanone oxime rearranging to caprolactam through a Beckmann rearrangement[43]

The rearrangements start with a ketoximes being exposed to acid so that it is protonated. Water goes out as a leaving group and an alkyl migration takes place to form a nitrilium ion. Then,

solvolysis leads to an imidate following the tautomerization to yield the amide (see *scheme 3.1.2*).[45]

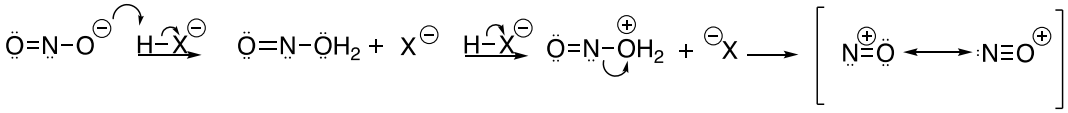
3.2 Sandmeyer reaction

In 1884, a Swiss chemist named Traugott Sandmeyer attempted to synthesise phenylacetylene from benzenediazonium chloride and copper(I) acetylide. However, the isolated product was chlorobenzene.[46] This reaction was thereby called the Sandmeyer reaction and is a method for the substitution of an aromatic amino group through the preparation of a diazonium salt, following its displacement of a nucleophile in the presence of catalytic copper(I) catalyst as shown in *scheme 3.2.1*.[47]



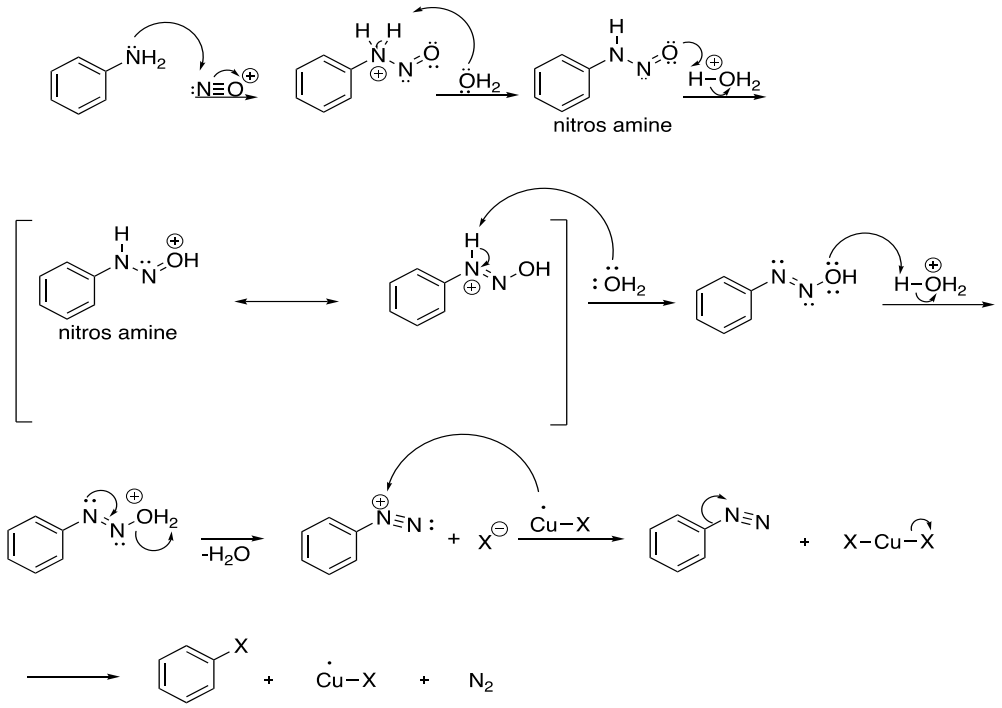
Scheme 3.2.1 General scheme of the Sandmeyer reaction aided with a copper(I) catalyst.[48]

The first step contains two protonation where sodium nitrite reacts with hydrogen halide, where water is formed to give the nitrosonium ion. Then, the nitrosonium ion will act as an electrophile and reacts with an aromatic (or heterocyclic) amine to form the diazonium salt. The diazonium salt is formed through nitrosamine intermediate (see *scheme 3.2.2*).[49]



Scheme 3.2.2 Formation of the nitrosonium ion

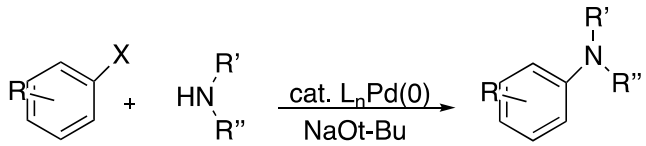
Sandmeyer reaction undergoes a radical-nucleophilic aromatic substitution. Through a single electron transfer from copper to diazonium, an aryl radical with loss of nitrogen gas is formed.[50,51] A further proposed mechanism shows that the substituted arene is formed through a direct transfer of the halide from a copper (II) catalyst to the aryl radical. This regenerated the copper(I) halide catalyst to give the final product as aryl halide as describes in *scheme 3.2.3*.[52-55]



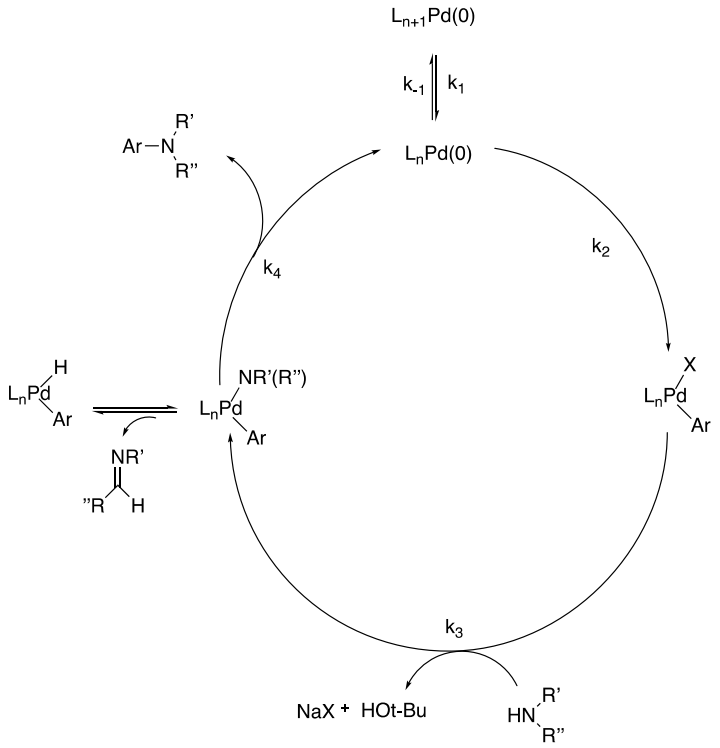
Scheme 3.2.3 Mechanism of the formation of benzenediazonium.[55]

3.3 Buchwald-Hartwig C-N cross coupling

The Buchwald-Hartwig amination is a cross-coupling reaction between amines and aryl halides, and are catalyzed by palladium (see *scheme 3.3.1*).[56] The Pd-catalyzed C-N reaction was already discovered in 1983. However, it was credited to Stephen L. Buchwald and John F. Hartwig who provided the scope and mechanism behind it (see *scheme 3.3.2*).[57]



Scheme 3.3.1 General reaction scheme for Buchwald-Hartwig[58]

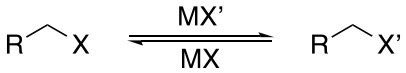


Scheme 3.3.2 A general mechanism for the Buchwald-Hartwig C-N cross coupling[58]

The Buchwald-Hartwig cross coupling includes many varieties of amines and aryl halides. Though, the conditions of the reaction are dependent on the substrates. Ligands can also vary depending on their capability and limitations. The reaction conditions are also mitigated by steric and electronic properties of both amine and aryl halide.[59]

3.4 Finkelstein reaction

The Finkelstein reaction is an S_N2 reaction named after the German chemist Hans Finkelstein, where one halogen is exchanged with another (see *scheme 3.4.1*).[60,61] The reaction is in equilibrium and can be driven to completion by adding excess of the halide salt or by exploiting the different solubility.[60]

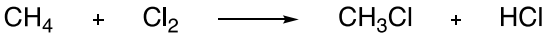


Scheme 3.4.1 The Finkelstein reaction [60]

A very common Finkelstein reaction is the substitution of alkyl bromide or alkyl chloride with an alkyl iodide. This is conducted by using sodium iodide dissolved in acetone. Sodium iodide is soluble in acetone while sodium chloride and sodium bromide are insoluble in acetone.[62]

3.5 Halogenation

Halogenation is the chemical reaction where a halogen is introduced to a compound. This is a reaction which is much used in the production of polymers and drugs.[63] Halogens can be introduced to compounds through an addition reaction with alkene or through a substitution reaction. It can also have an addition of molecular halogenation which is often seen in organic synthesis (shown in *scheme 3.5.1*).[64]



Scheme 3.5.1 Addition of a molecular chloride[63]

3.6 Mass spectrometry

Mass spectrometry is an instrumental analytical technique which generates ions from either organic or inorganic compound through an applicable method. These ions are separated by their *mass-to-charge ratio (m/z)* and the compounds are quantitatively and qualitatively analysed due to their m/z ratio. The sample can be ionized thermally by an electric field or energetic electrons, ions or photons. Several factors may affect the ion separation of a sample. Such as static or dynamic electric og magnetic fields, but also by atomic ions or photons, energetic neutral atoms, electronically excited atoms, massive cluster ions and electrostatically charged micro-droplets.[65]

A mass spectrometer has an ion source, a mass analyser and a detector. The gaseous molecules form the analyte will enter the instrument and be converted into ions. The ionized molecules are stimulated by an electric field in the source and directed into a tube which is under high vacuum (~10^{-5} Pa). Consequently, not bent by collision with other background gas molecules. Then, the gaseous molecules will reach a magnetic field which is perpendicular to its direction. The magnet will bend the ions towards the detector at the end of the tube. The magnetic field is varied to obtain a full mass spectrum, but the detector will capture the lighter ions first as they travel faster and then the heavier ions. This is illustrated in *figure 6*. M/z is numerically equal to the mass of the mass charge.[66]

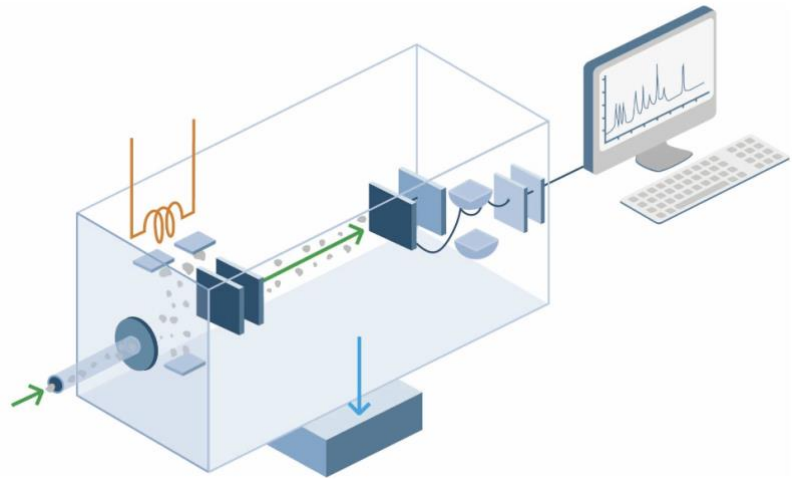


Figure 6 Detection of ions through a MS instrument[67]

3.6.1 Gas chromatography – mass spectrometry

”International Union of Pure Applied Chemistry” (IUPAC) defines chromatography as, “a physical method of separation in which components to be separated are distributed between two phases, one which is stationary (stationary phase) while the other (mobile phase) moved in a definite direction.[66,68]

The mobile phase can vary and is affected by factors such as gravitation, capillary forces or pressure. The mobile phase can a gas, liquid or a supercritical fluid.[66] On the other hand, the stationary phase can often be a solid, a liquid supported on a solid or a gel.[69] Hence, separation of ions occurs between compounds due to their ability to move in the mobile phase compared to the stationary phase. Hence, retention time.

In gas chromatography, the carrier gas (the mobile phase) will transport a volatile liquid or gas through a column.[66] The carrier gas can be ultrapure helium, hydrogen or nitrogen. A hydrogen generator can safely produce hydrogen on-site.[69] Moreover, analyte assessment between mobile phase and stationary phase are affected by factors such as adsorption, solubility, ion exchange, size or selective interactions. Though, only adsorption and solubility is relevant for gas chromatography.[70]

The gas chromatography is a destructive technique. The chromatogram has an injector which contains volatile liquid or gas sample which is injected through a septum. A gaseous analyte is applied through a gastight syringe or gas valve. After injecting the analyte, it will be carried to

the column by the carrier gas. Gas chromatographs frequently use a long and narrow open tubular column which is made of silica (SiO_2). It is covered with polyimide for support and protection from moisture in the air. Open tubular columns have the precedence of high resolution, short analysis time and stronger sensitivity than a packed column.[66] The analyte will reach the column before it is detected. A data is collected as shown in *figure 7*. The column is can provide an isothermal or programmed increase in temperature. Also, it is always furnished with a ventilator to circulate strong air as a result of heat conductivity.[70]

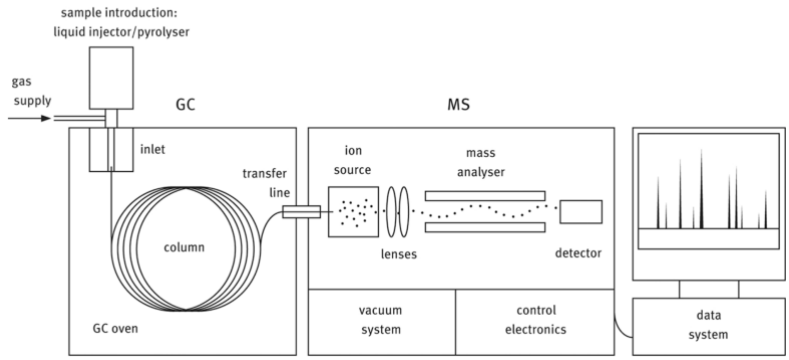


Figure 7 A GC-MS instrument parts[71]

3.6.2 Liquid Chromatography – Mass Spectrometry

Similar to other characterisation techniques, liquid chromatography – mass spectrometry (LC-MS) is another instrumental method which is highly used by scientists. It is a destructive technique which is used to separate different compounds in a mixture. Liquid chromatography-mass spectrometry is similar to gas chromatography, but uses a solvent as the mobile phase instead of a gas.

The coupling of gas chromatography to mass spectrometry (GC-MS) was achieved already in the 1950s with commercially available instruments already in the 1970s. Compared to GC-MS, the coupling of liquid chromatography to mass spectrometry was limited due to the relative incompatibility of an existing ion source with continuous liquid stream. However, this changed when Fenn had developed a liquid chromatography – mass spectroscopy technique in the 1980s using electrospray ion source.[72]

Fenn's developed ESI into a powerful ion source which was capable of interfacing to LC and verified it by demonstrating its application on many biological molecules.[72] The samples will

be in a mobile phase and are pumped through a metal capillary maintaining a 3 to 5kV, and nebulised at the tip of the capillary forming fine spray of charged droplets. The droplets evaporate quickly as they are evaporated by heat and nitrogen. The residual electrical charge on the droplets are transferred back to the analytes.[73] By using small apertures and voltages, the ionised analytes are transferred back into the high vacuum of the mass spectrometer. The ion source and subsequent ion optics can operate between negative ions and positive ions. Normally the ESI ionisation source is considered "soft", meaning that little energy is imparted to the analyte. Therefore, small fragmentations occur (see *figure 8*). It is also possible to switch between these modes when performing an analytical run.[74]

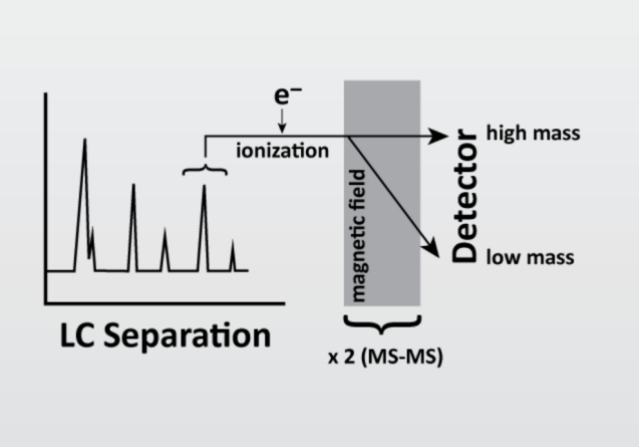


Figure 8 Liquid chromatography separation with mass analysis [75]

3.7 High-Performance-Liquid-Chromatography

High performance liquid chromatography is another method which gives both qualitative and quantitative information about compounds in a mixture. However, like GC-MS and LC-(UV) MS, this is also a destructive characterisation technique. Every compound in the mixture has its own retention time under given conditions, and both the area and the height of each signal are proportional to the amount of the corresponding substance. This is conducted by operating with high pressure than what is ordinarily used in liquid chromatography as shown in *figure 9*.[76,77]

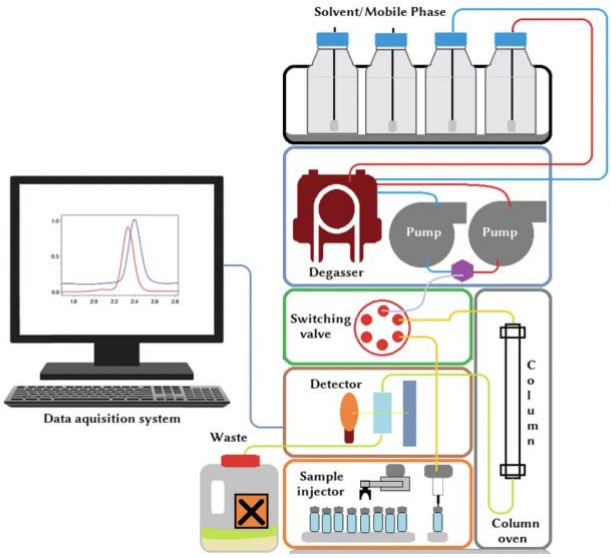


Figure 9 Analytical HPLC instrument[78]

3.8 Automated Flash Chromatography

Automated flash chromatography is an instrumental purification technique where compounds are separated due to their partitioning behaviour in mobile phase and in the stationary phase.[79,80] The purification method was developed as an instrumental technique as the system is faster, safer, generates less waste and for use in undergraduate teaching laboratories. **Error! Bookmark not defined.**

Automated flash columns have pre-packed cartridges with silica gel, compared to manual flash column chromatography.[79] This prevents exposure to silica or any other compounds that are left in the column. The packed columns also improve resolution and reduces the probability of co-elution. Moreover, the instrumental system is built by parts that are also found in a HPLC. A gradient pump, injection ports, a UV-detector and a fraction collector gathering the eluent (see *figure 10*).[79]

Figure 10 PuriFlash instrument[81]

3.9 Nuclear Magnetic Resonance

Another important analytical instrumental method is nuclear magnetic resonance (NMR), a non-destructive characterization method. NMR spectrum can only be observed for nuclei that possess a net nuclear spin (I). Therefore, hydrogen (^{1}H) and carbon (^{13}C) are the most abundant nucleus that is to be seen in many molecules and has a net spin for $\frac{1}{2}$.[82]

The spin of a nuclei is randomly oriented in the absence of an external magnetic field (B_0). In the presence of an external magnetic field, the nucleic spin will be oriented either parallel or antiparallel to the magnetic field. Nowadays, NMR instruments are equipped with magnetic fields that are directed along the axis of the sample tube.[83]

The NMR instrument collects data when a difference in energy is absorbed as the nuclei spin orients to the external magnetic field. The energy difference occurs when there is an irradiation with right frequency. This absorbed energy is then enough to reverse the spin orientation. The energy absorbed must be equal to the energy difference between the two energy states.[82]

The NMR instrument is equipped with a magnet which contains a super-conducting coil. The electronic console has a program which generates and amplifies radio frequencies. It also has a

pulse field gradient that sends pulses to the probe and triggers data acquisition.[82] This is illustrated in *figure 11*.

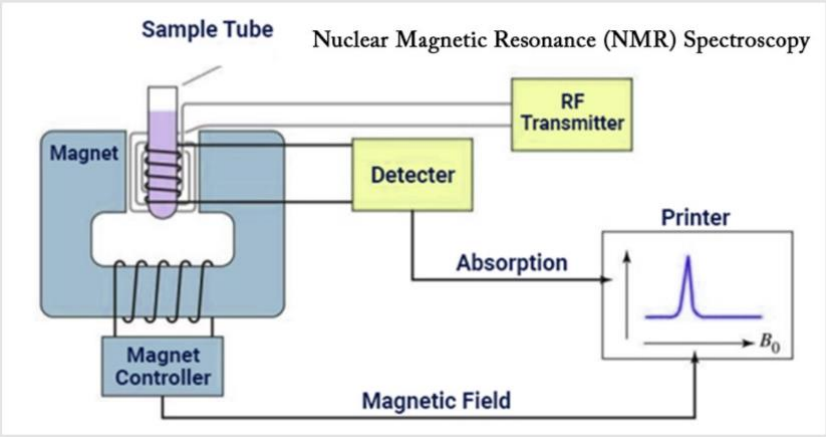


Figure 11 NMR instrument system set-up[84]

3.10 Microwave reactor

Reactions are often heated in oil baths, sand baths and heating mantles. These traditionally methods are slow and generate a hot reaction surface. Therefore, microwave reactor was introduced to chemistry. Microwave energy is sent into the microwave reactor and is carried through the walls of the reaction vessel such that the reagents and the solvents are directly heated. A proper reactor vessel (a microwave reactor tube), will increase temperature uniformly throughout the vessel (see *figure 12* for illustration of a microwave reactor). This decreases the magnitude of by-products that are formed. Moreover, microwave reactor takes advantage of solid reagents and solvents ability to transform electromagnetic radiation to heat.[85]

Microwave assisted synthesis is widely used in organic chemistry. The most prevalent syntheses assisted by microwave reactor are within solution-phase synthesis, solid-phase synthesis and solid-support reagents and scavengers.[85] In addition to this, polymers and solid state chemistry are also conducted by microwave reactor.[86,87]

Figure 12 Biotage AB microwave reactor [88]

3.11 Statistical Experimental Design

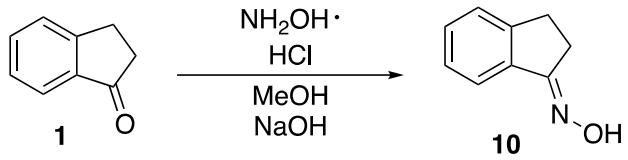
In an experiment, there are several parameters that affect the outcome of the experiment. These variables can be changed to see how they affect the response. Statistical design of experiments is a method for planning experiments, such that the obtained data will be a guidance to draw objective and valid conclusions.[89]

There are many steps that must be developed by a researcher in advance of performing the experimental design. An important step in developing a statistical experimental design is the selection of variables. These can be variables that are dependent of each other or independent. Dependent variables are measured, or observed during an experiment. Independent variables are conditions that are chosen in advance to the experiment's outset. Most scientific experiments have independent variables that are pre-determined levels, also called conditions. When introducing more than one condition, a researcher can see the effect from the single factor (called main effect) and from interaction between factors (named interaction effects). Constant factors can also be found in an experimental design and do not change. These factors together can draw a statistical experimental design. The collected data can show the relationship among and between variables. Hence, a model can be drawn from the data to indicate cause and effect from the statistical experimental design which was conducted.[90,91]

Results and Discussion

4.1 Synthesis of 1-indanone oxime (10)

The reaction from ketone to an oxime was carried out by using hydroxylamine hydrochloride and sodium hydroxide (see *scheme 4.1.1*). When conducting the reaction with 1:1 equivalents of reagents, the reaction afforded several side-products. This led to purification by flash column chromatography (hexane / ethylacetate, 9:1) and afforded a low yield (see **table 3**). Therefore, multiple experiments were performed to optimise the reaction.



Scheme 4.1.1 Synthesis of 1-indanone oxime (**10**)

Table 1. Overview of conditions used for the synthesis of **10**

Entry	Equiv. 1	Equiv. NH_2OH	Equiv. NaOH	Time (h)	Temp (^oC)	Yield (%)
1	1	1.30	1.97	60	22	22
2	1	1.21	1.97	180	22	28
3	1	1.50	6.0	5	65	73
4	1	1.50	6.0	20	65	90

Table 1 shows that when increasing the equivalents of hydroxylamine hydrochloride and equivalents of sodium hydroxide respectively, the yield also increases. Entry *3* and *4* also shows that the reactions were conducted at 65 ^oC (reflux), hence forcing the reaction towards expected product.

1-indanone oxime (**10**) was synthesised from 1-indanone (**1**). 1-indanone was dissolved in methanol, followed by the addition of hydroxylamine hydrochloride solution. Then the reaction mixture was immersed in an ice bath and sodium hydroxide was added dropwise to the mixture. The reaction was conducted under reflux and left to stir for 20 minutes. It was then quenched by water and the post-reaction was extracted with ethylacetate, dried over sodium sulphate and reduced under concentrated pressure. The product was observed and collected as pure white flakes (90%).

1-indanone oxime (**10**) has fewer ^{1}H NMR signals compared to 1-indanone (**1**). The aromatic region for 1-indanone (**1**) contains four sets of signals that arises from the hydrogens on the aromatic ring. This is not seen for 1-indanone oxime (**10**) as the aromatic signals are singlets (7.48 ppm) and double doublets (7.47 ppm) (see *figure 13*). Some hydrogens are coupling with each other and hence the signals are detected as double doublets. Furthermore, the interpreted signals for the alicyclic hydrogens on compound **10** are more upfield in comparison to signals seen for compound **1** in the ^{1}H NMR spectrum. Hence, confirming compound **10** was synthesised.

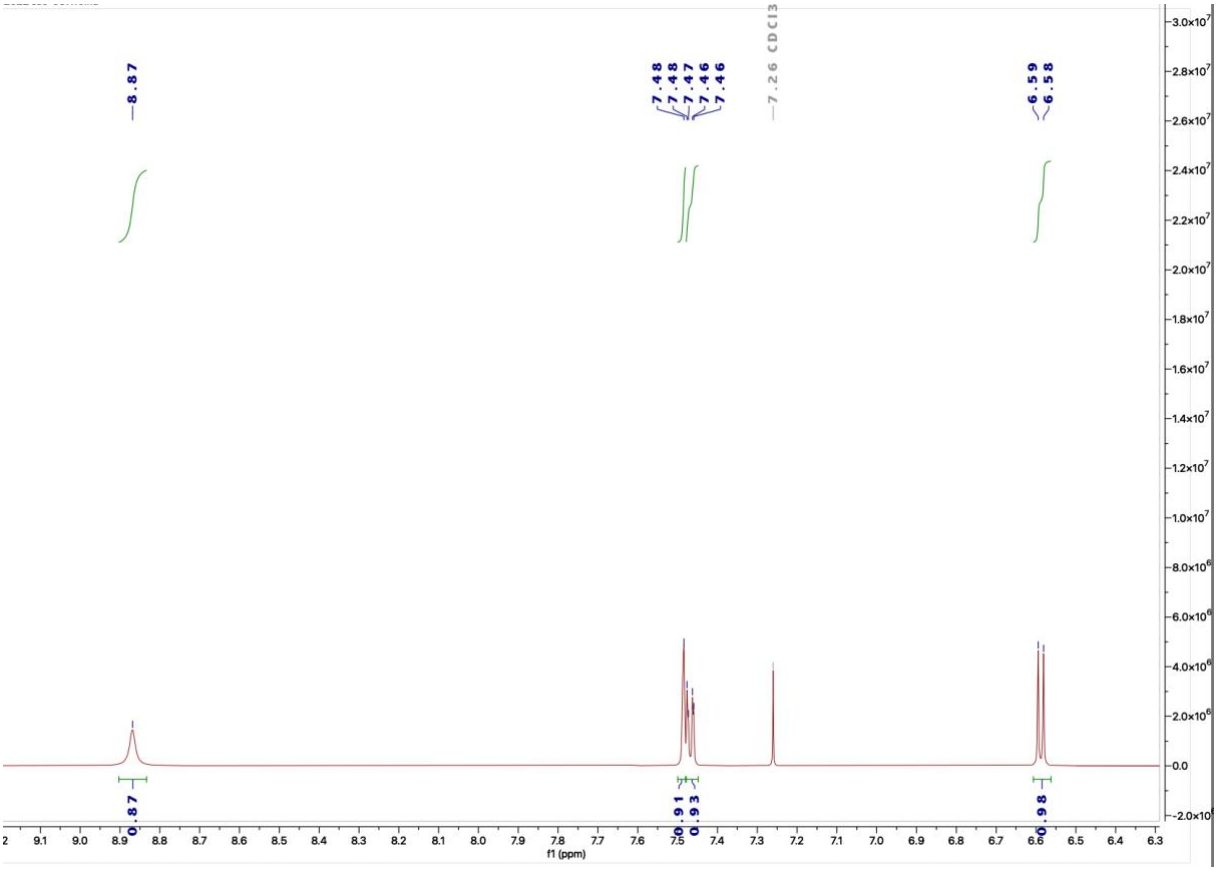


Figure 13 ^{1}H NMR spectrum of the aromatic region for 1-indanone oxime (**10**)

The ^{13}C NMR also verified that 1-indanone oxime (**10**) was synthesised. 1-indanone (**1**) is a ketone and will therefore have signals that are more downfield compared to 1-indanone oxime (**10**), which is a ketoxime. This can be verified in the aromatic region at 137.2 ppm, 136.8 ppm, 136.5 ppm, 126.2 ppm and at 117.5 ppm as shown in *figure 14*. The signal for ketoxime is also more upfield (171.8 ppm) in ^{13}C NMR spectrum. To conclude, ^{1}H and ^{13}C and NMR confirms that 1-indanone oxime (**10**) was synthesised from 1-idanone (**1**).

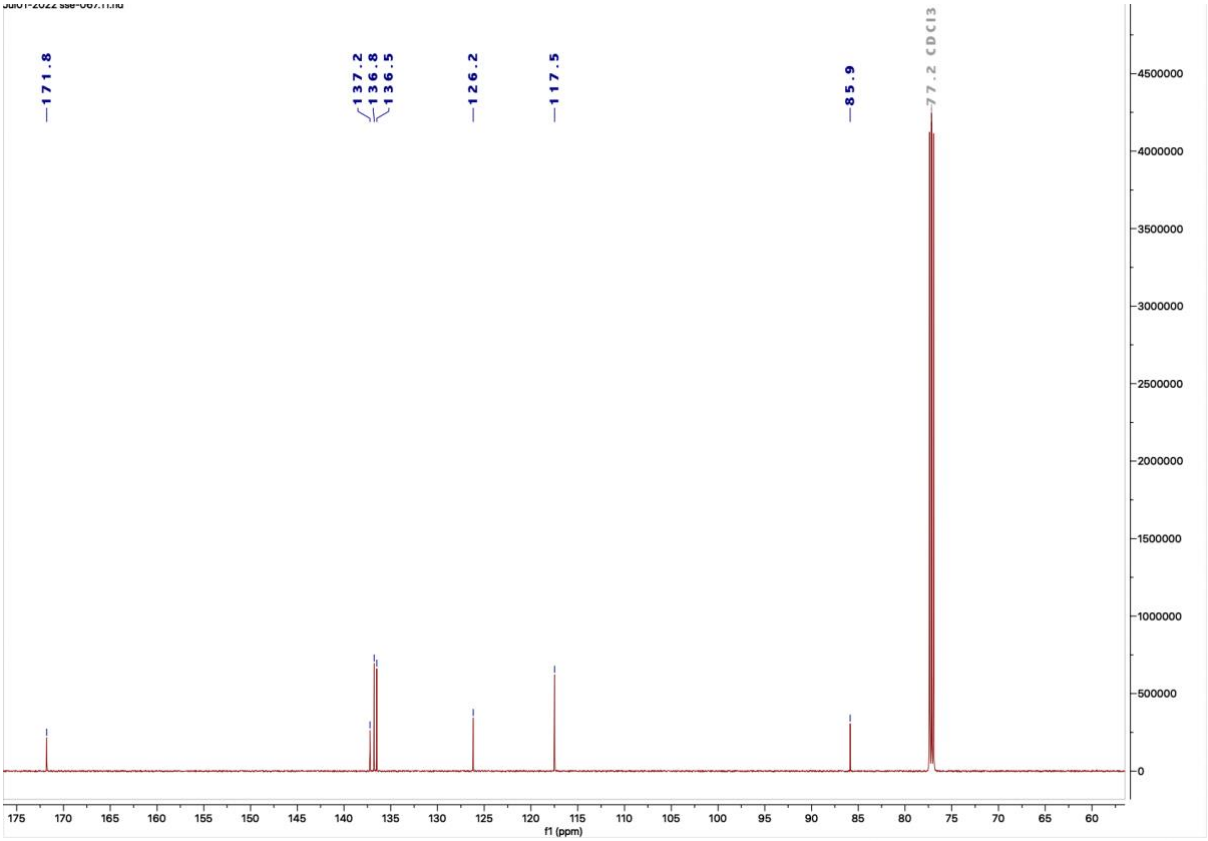
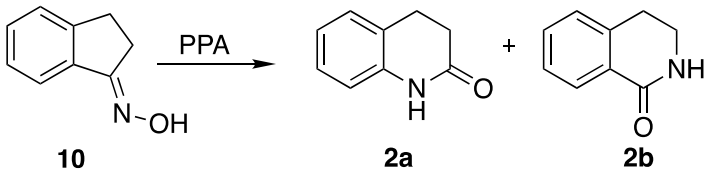


Figure 14 ^{13}C NMR spectrum of ketoxime and the aromatic region of 1-indanone oxime (10)

4.2 Synthesis of 3,4-dihydroquinolin-2(*1H*)-one (**2a** and **2b)**

4.2.1 Lewis / Brønsted Acid Screening

Several attempts were performed to synthesise 3,4-dihydroquinolin-2(*1H*)-one (**2a**) (see *scheme 4.2.1*). **Table 2** shows the different reagents that were used to conduct the Beckmann rearrangement from 1-indanone oxime (**10**). Most of these attempts were not successful except with polyphosphoric acid (PPA), though this yielded both the desired product **2a** and the undesired isomer **2b**.



Scheme 4.2.1 Synthesis of **2a** and **2b** through Beckmann rearrangement

The Beckmann rearrangement is aided by using a catalyst, such as a strong Lewis or Brønsted acids. The acid is often used in excess to the starting material. The reactions in entry *3* and *5* in

table 2, were not successful when comparing the equivalents of thiamine hydrochloride, sulphuric acid and substrate. By studying ^{1}H NMR spectra, only starting material was present for the experiments with thiamine hydrochloride and with sulphuric acid. In conclusion, one-to-one ratio yielded neither **2a** nor **2b**.

$SOCl_2$ is another reagent which was also used to perform the Beckmann rearrangement of **10**. Though the reaction was performed under argon atmosphere and by using anhydrous solvent as it reacts violently with water, these reaction conditions may not have been optimal for the reaction. This may explain why there were no indications of conversion of 1-indanone oxime (**10**), when analysing by ^{1}H and ^{13}C NMR.

Table 2. Screening of acids for the formation of desired product **2a**

Entry	Acid[(a)]	Equiv. reagent	Temp (ºC)	Time (h)	Conv (%)[(b)]
1	$SOCl_2$	14	65	> 0.5	-
2	H_3PO_4	17	110	-	-
3	Thiamine HCl	1	100	5	-
4	PPA	10	120	22	100
5	H_2SO_4	1	90	20	-

a) The different acids were used in different conditions according to their procedures

b) Conversion detected by using NMR

Furthermore, when conducting the experiment with $SOCl_2$ the reaction temperature was only heated to 65ºC as it was the boiling point of the acid. The low reaction temperature could easily have affected the conversion of 1-indanone oxime (**10**) to not yield the desired product **2a**. Also, $SOCl_2$ is not a strong acid compared to H_2SO_4, H_3PO_4 and PPA. An article from 2013 conducted a Beckmann rearrangement using 4-brormoacetophenone as substrate, $SOCl_2$ as acid and the catalyst β-CD (β-cyclodextrin) to mediate the reaction.[92] Hence, the conditions that were used to perform the reaction with $SOCl_2$ could also have affected the reaction such that no conversion was detected.

When comparing the acidity of H_3PO_4 and PPA, PPA is a stronger acid than H_3PO_4. Polyphosphoric acid is an oligomer of phosphoric acid. A very pure phosphoric acid is produced either through dehydration of H_3PO_4 at high temperature or by heating P_2O_5 which is dispersed

in H_3PO_4. When dispersing PPA in water, acidity increases with chain length.[93] Thereby, the protonation strength of PPA is stronger than for H_3PO_4 and is a reasonable explanation why Beckmann rearrangement of **10** using H_3PO_4 failed.

4.2.2 Mechanism of compound 2a and 2b

When conducting the reaction between 1-indanone oxime (**10**) and PPA, TLC was taken to see if there were any conversion. The resulting TLC showed no indication of conversion. As the molecular weight og the starting material and product(s) are the same, TLC could have been interpreted incorrectly. Also, 1-indanone oxime (**10**), **2a** and **2b** have almost no difference in polarity. To verify this observation, ^{1}H and ^{13}C NMR analysis were conducted. The obtained ^{1}H and ^{13}C NMR spectra of the crude were not clear to interpret. There were some signals indicating that 3,4-dihydrioquinolin-2(*1H*)-one (**2a**) was formed. Therefore, a LC-UV MS was performed to see if it could confirm the interpretation. The LC-UV MS spectrum of the crude showed two signals where both had the same molecular mass, $[M]^+ = 148$ and there were two UV-signals detected at 254 nm (see *figure 15* and *16*). The signals had different retention time. This led to the theory that the crude may have contained both 1-indanone oxime (**10**) and 3,4-dihydroquinolin-2(*1H*)-one (**2a**).

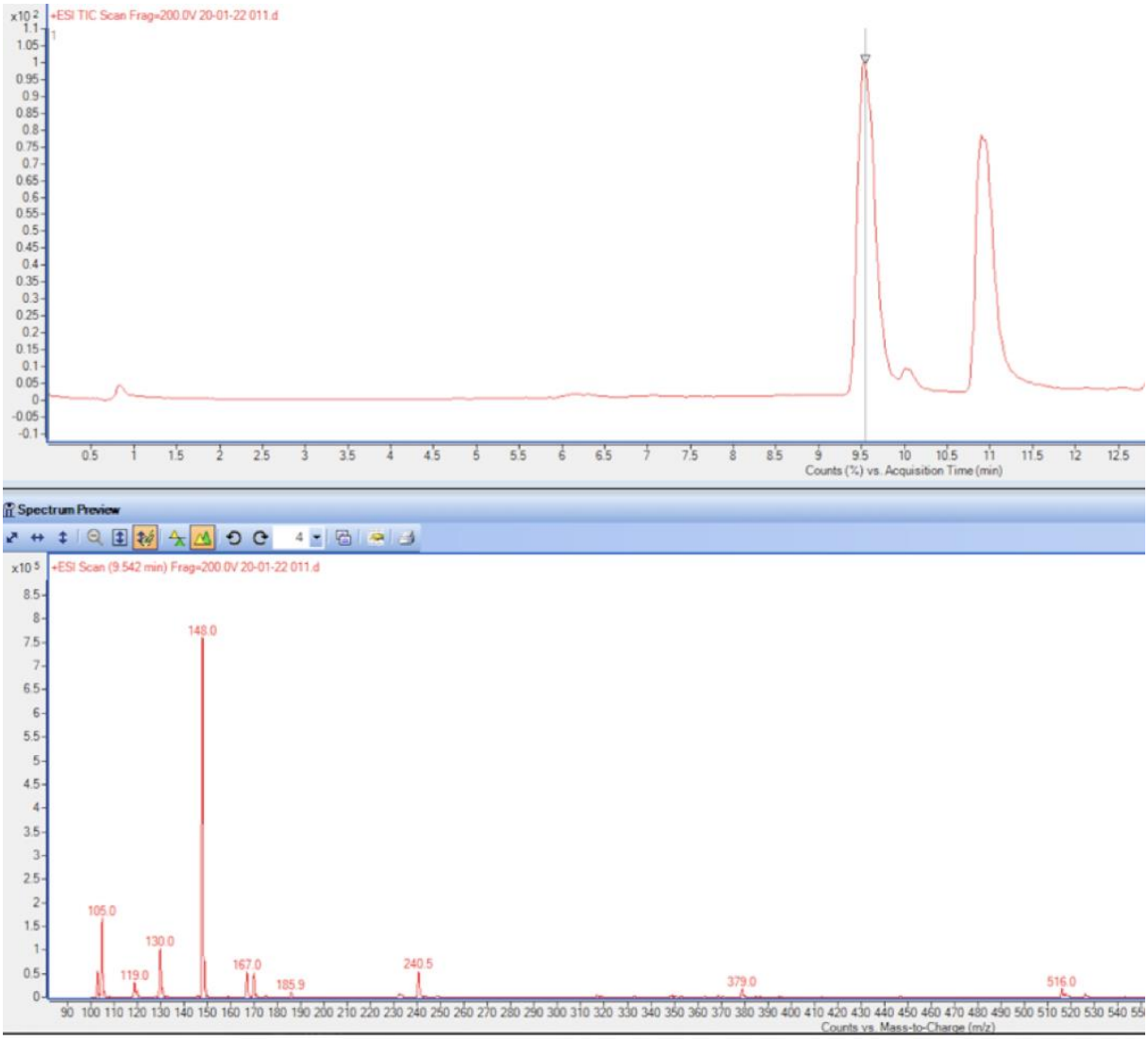


Figure 15 LC-MS spectrum of a crude containing two sets of signals containing the same molecular weight, $[M]^+ = 148$

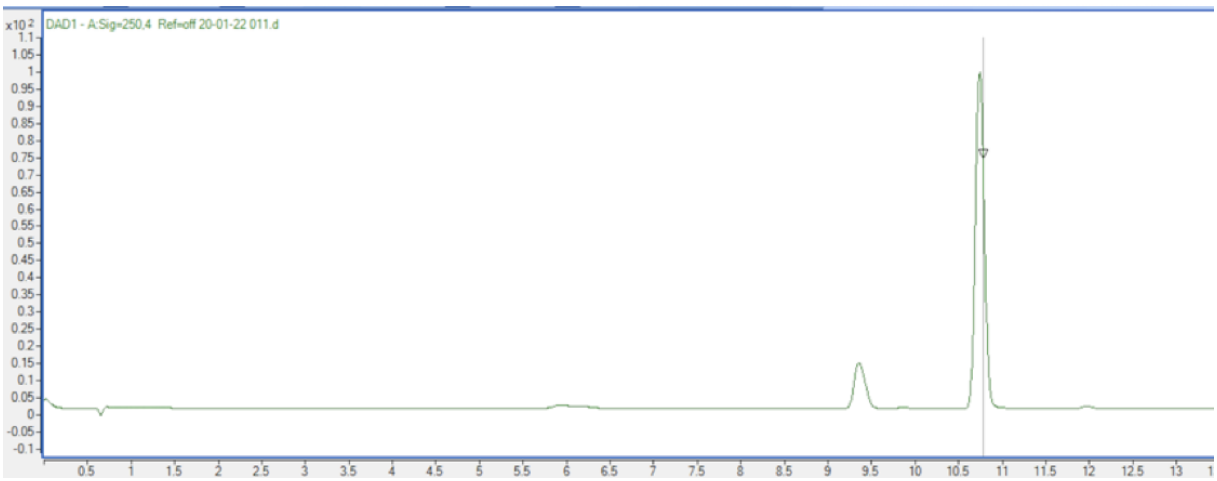


Figure 16 LC-UV spectrum of the crude containing two sets of signals at 254 nm but with different retention time

To confirm the theory that **2a** was produced in the Beckmann rearrangement of **10** without achieving 100% conversion, **2a** was commercially bought. Another LC-UV MS analysis was conducted with pure 1-indanone oxime (**10**) and the commercially purchased 3,4-dihydroquinolin-2(*1H*)-one (**2a**). The results are presented in *figure 17* and *18*. The analysis of the two samples showed that the crude did not contain 1-indanone oxime (**10**) but another compound containing the same molecular weight. Hence, the former theory did not align with the results.

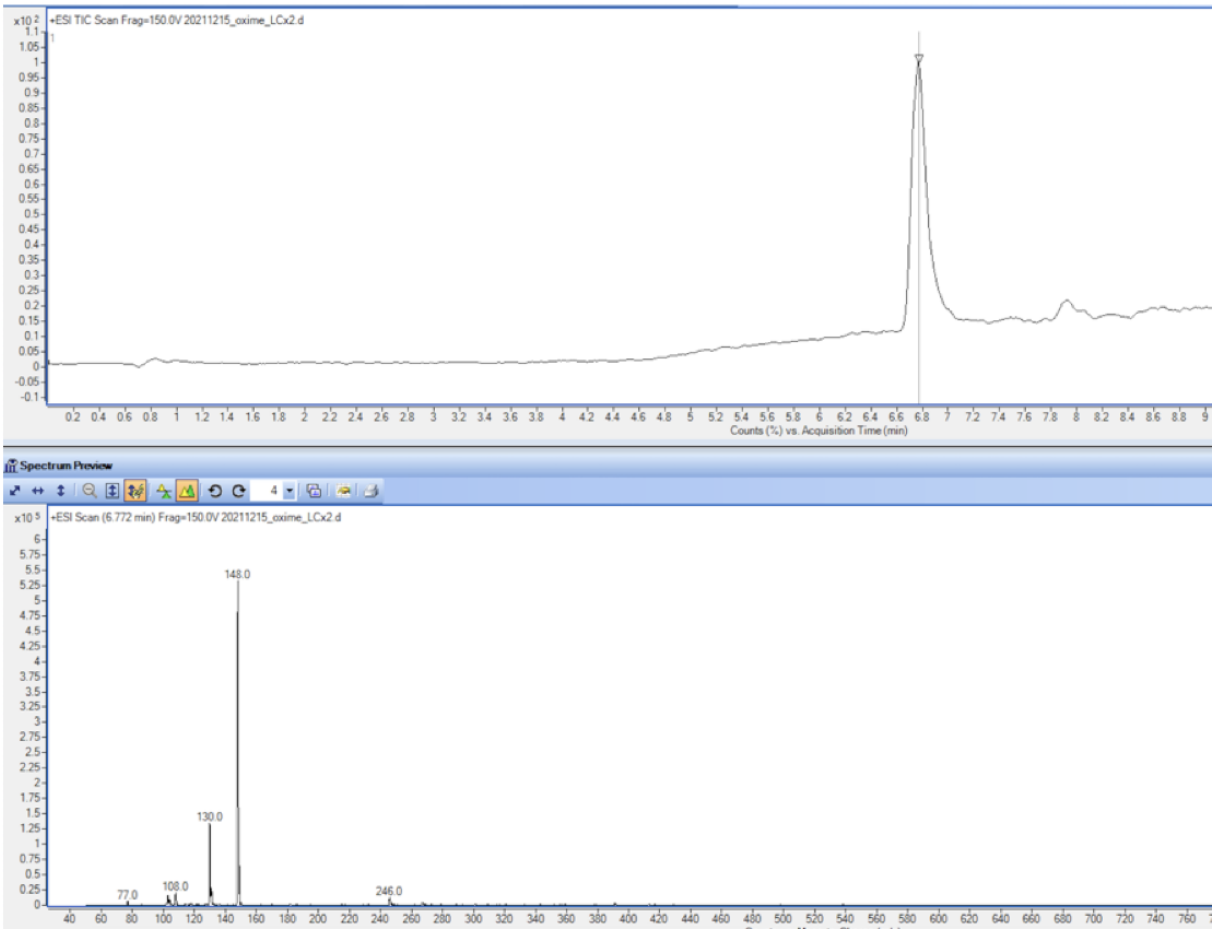


Figure 17 LC-MS spectrum of 1-indanone oxime (**10**)

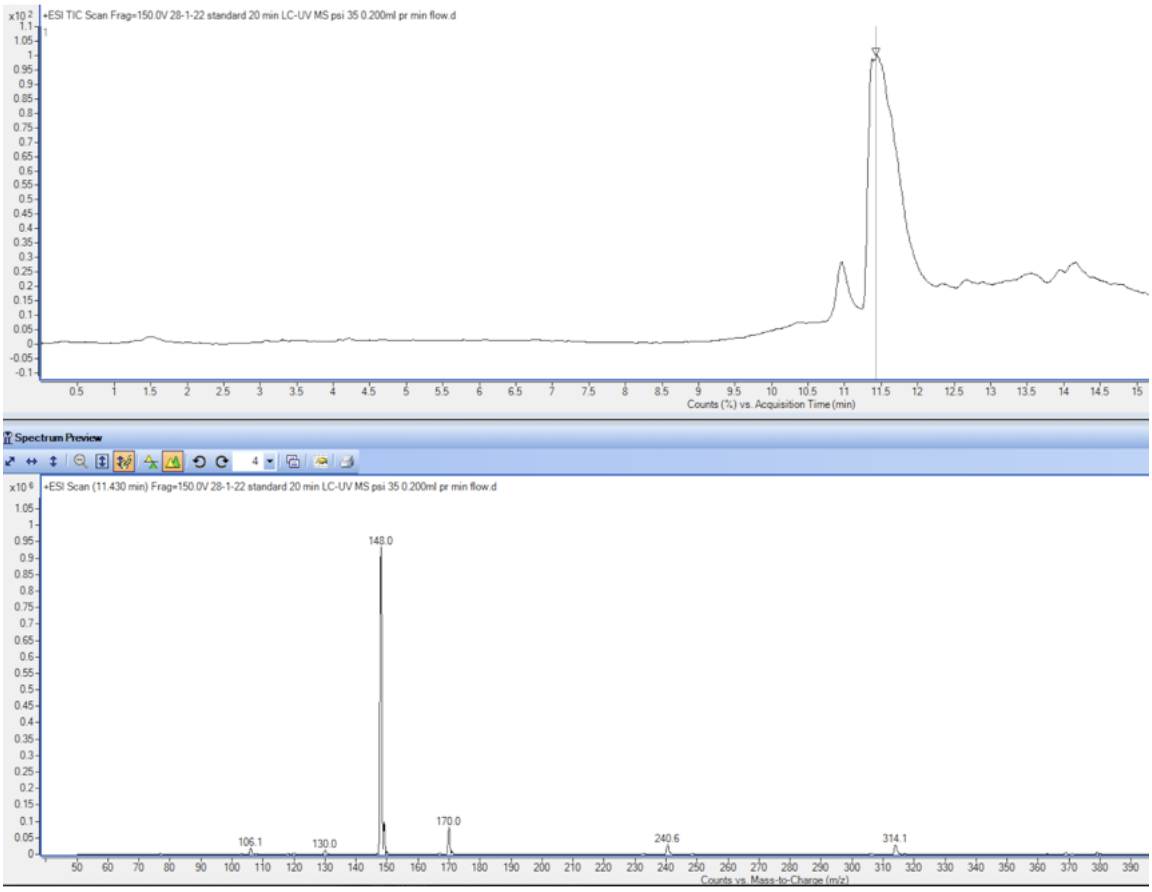
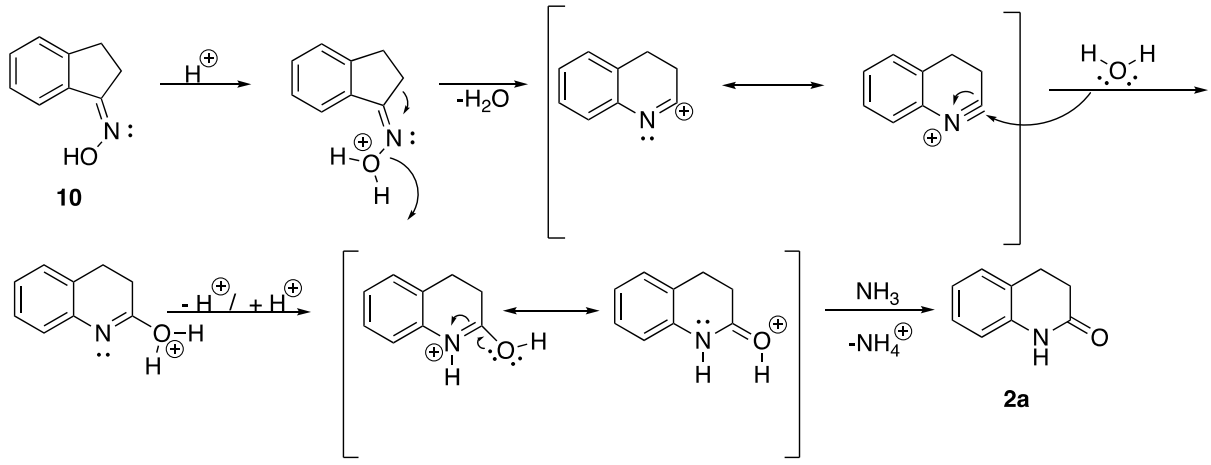


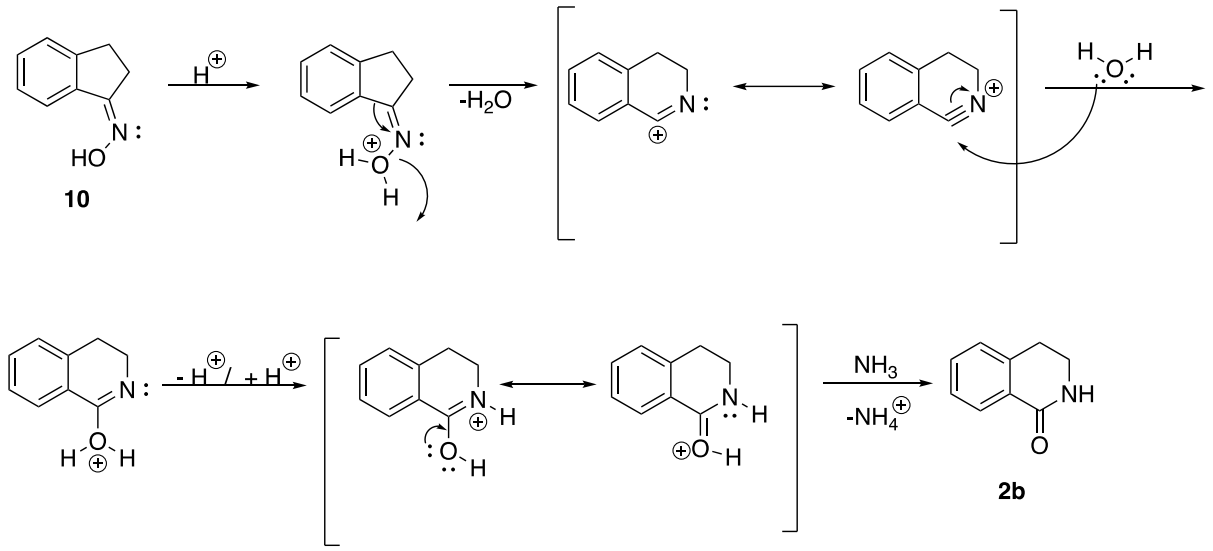
Figure 18 LC-MS spectrum of commercially bought 3,4-dihydroquinolin-2-(1H)-one (**2a**)

To understand the results from the analysis, the mechanism behind Beckmann rearrangement had to be understood. Reaction *scheme 4.2.2* shows the formation of target compound **2a**, a plausible mechanism of the formation of 3,4-dihydroquinolin-2(*1H*)-one (**2a**). The mechanism illustrated in *scheme 4.2.2* leads to compound **2a** as the Beckmann rearrangement takes place on the right side.



Scheme 4.2.2 Beckmann rearrangement mechanism of target compound **2a**.

However, compound **2a** was not the solely product to be found. The mechanism behind the formation of **2a** shows that it can only be formed if the rearrangement is initiated on the right side. Though, as the LC-UV MS analysis resulted in two signals showing the same molecular weight where none of them belonged to the ketoxime **10**, another plausible explanation was to be found. This led to the explanation of the formation of the isomer **2b** (see *scheme 4.2.3*).



Scheme 4.2.3 Beckmann rearrangement mechanism of **2b**

The observed and interpreted results from LC-UV MS coordinated also with the ^{1}H NMR spectrum, which was obtained earlier to confirm if there were any indication of the desired compound **2a**. *Figure 19* is a crude ^{1}H NMR of a mixture containing both compound **2a** and **2b**. However, there is a larger amount of isomer **2b** which is produced compared to **2a**. The signals implying the formation of **2b** arises in the aromatic region at 8.07 ppm, 7.46 ppm, 7.36 ppm and at 7.33 ppm. The alicyclic signals for **2b** can be confirmed through triplet doublet at 3.58 ppm and the last triplets at 3.02 ppm. The ^{1}H NMR spectrum also confirms that amide is formed as there is a signal at 6.22 ppm interpreted for the amide (N-H) proton.

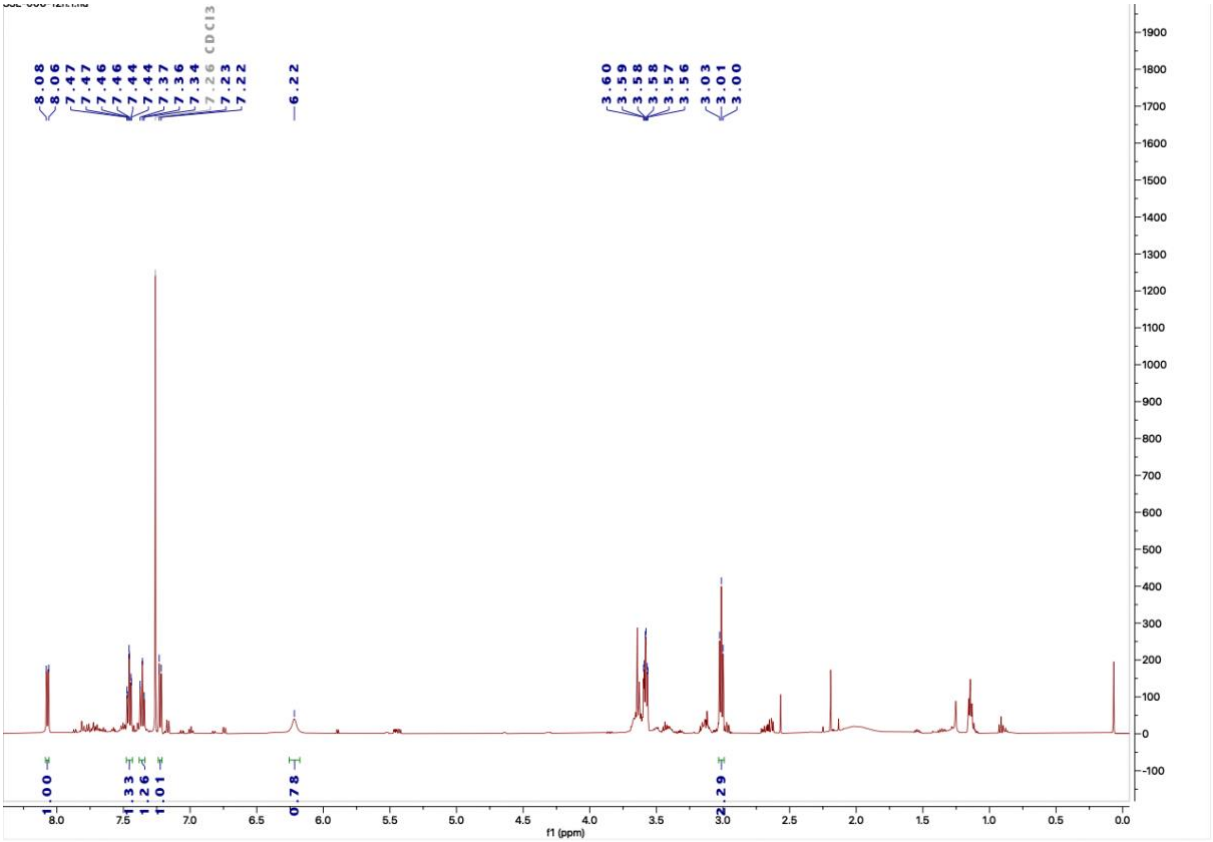


Figure 19 ^{1}H NMR spectrum verifying the formation of isomer **2b**

Furthermore, *figure 19* also shows that compound **2a** was formed. There is a small presence of compound **2a** when studying the ^{1}H NMR spectrum thoroughly. *Figure 20* shows the aromatic signals found for **2a** at 7.17 ppm, 6.99 ppm and at 6.74 ppm. The alicyclic signals for compound **2a** is also present and is found at 2.97 ppm and 2.64 ppm. The ^{1}H NMR spectrum also manifests that the there is a greater amount of **2b** synthesised compared to isomer **2a**.

Meanwhile, the ^{13}C NMR spectrum lacks in signals align with either compound **2a** or **2b**. There a few signals in the aromatic region and in the alicyclic region. The lack of signals can thereby be reasoned through some carbons having the same aromatic signal (see *figure 21*). Lastly, it is also obvious that the concentration was too low for a ^{13}C NMR analysis.

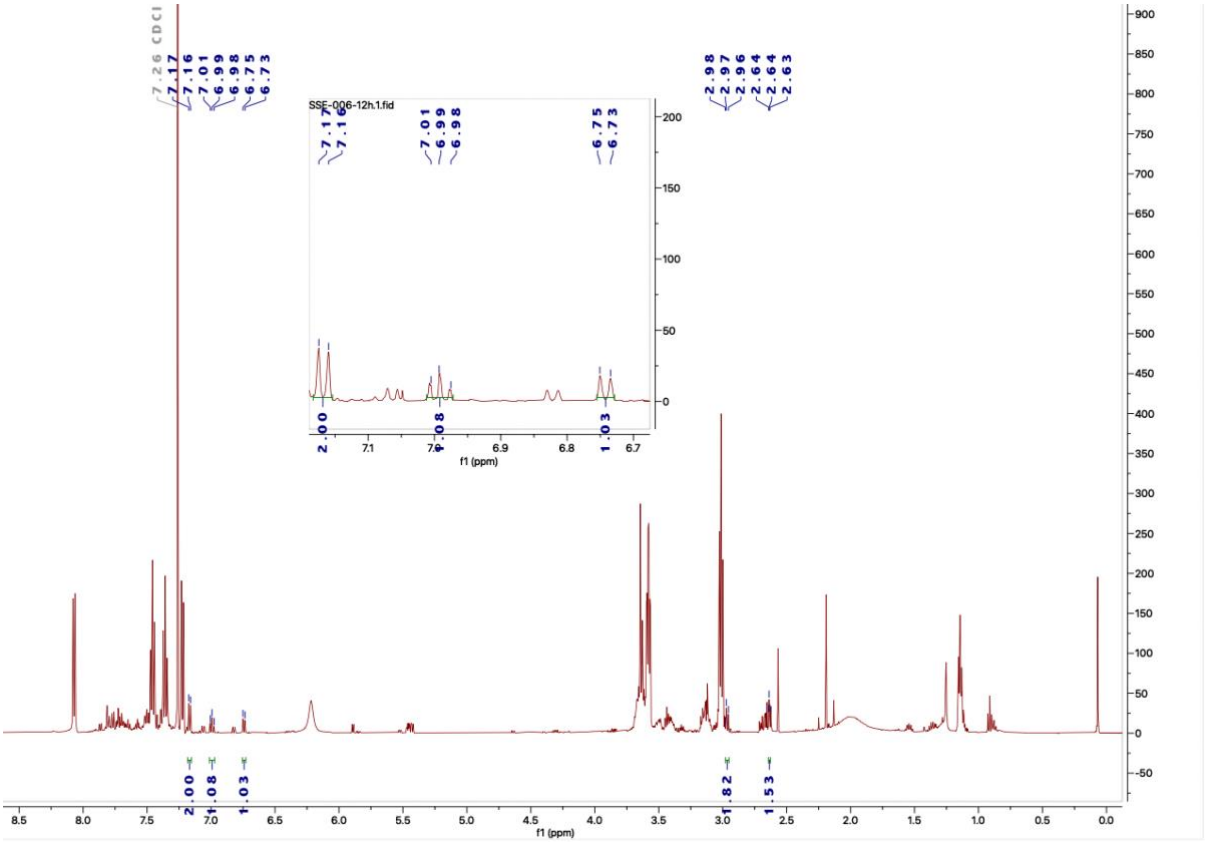


Figure 20 ^{1}H NMR spectrum confirming the presence of desired compound **2a**

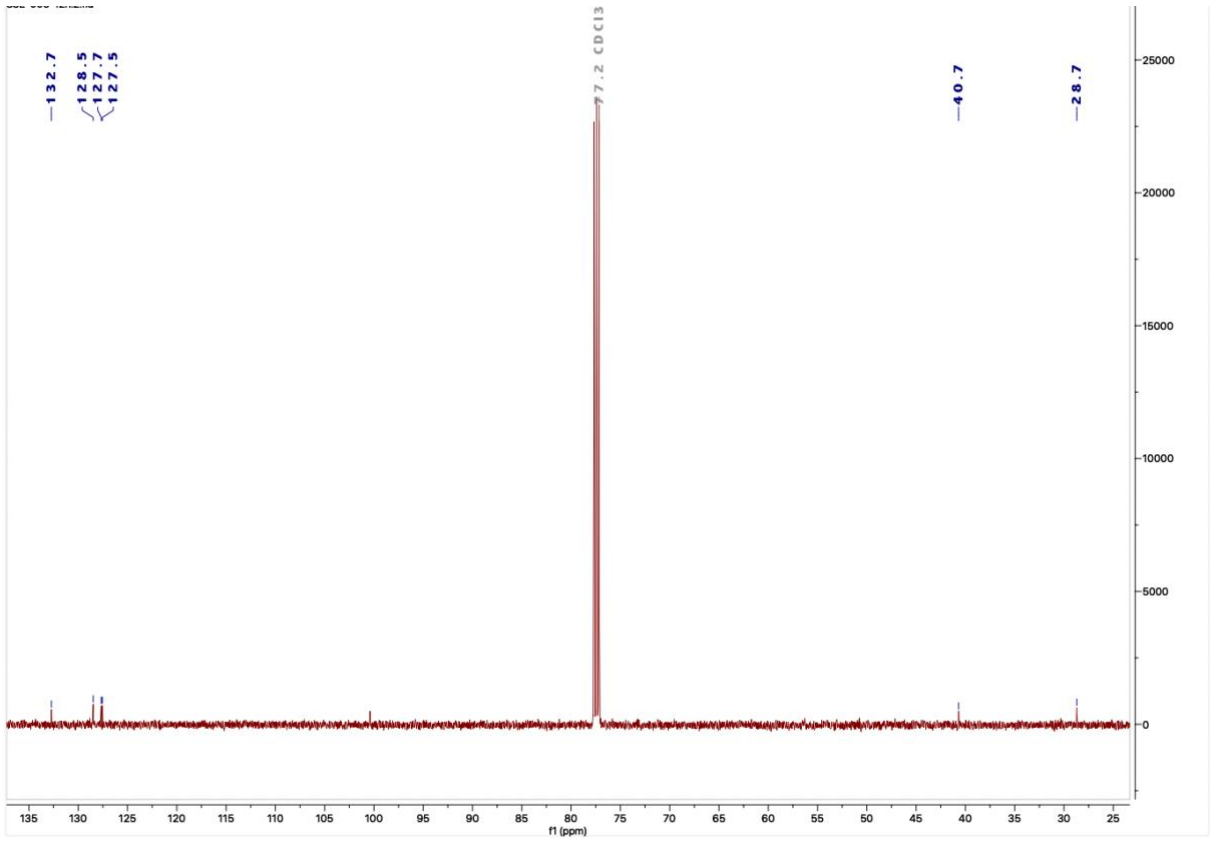


Figure 21 crude ^{13}C NMR spectrum of a mixture of **2a** and **2b**

4.2.3 Experimental design to increase the yield of 2a

It was also detected that when analysing the crude material through LC-UV MS and also confirmed through ^{1}H NMR, the major product was **2b**. To obtain a higher yield of **2a**, statistical experimental designs were generated for the Beckmann rearrangement of 1-indanone oxime (**10**). By using the Ishikawa-diagram (see *figure 22*), variables for the design were chosen and the yield were obtained through analytical HPLC as shown in figure *23*, *24* and *25*.

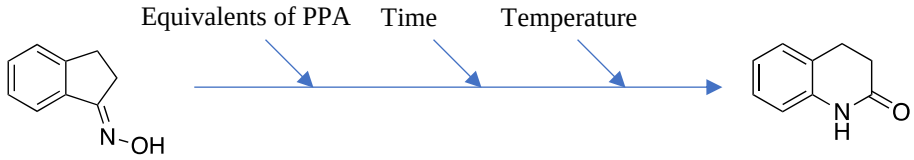


Figure 22 Ishikawa diagram showing factors affecting the Beckmann rearrangement of 1-indanone oxime (**10**).

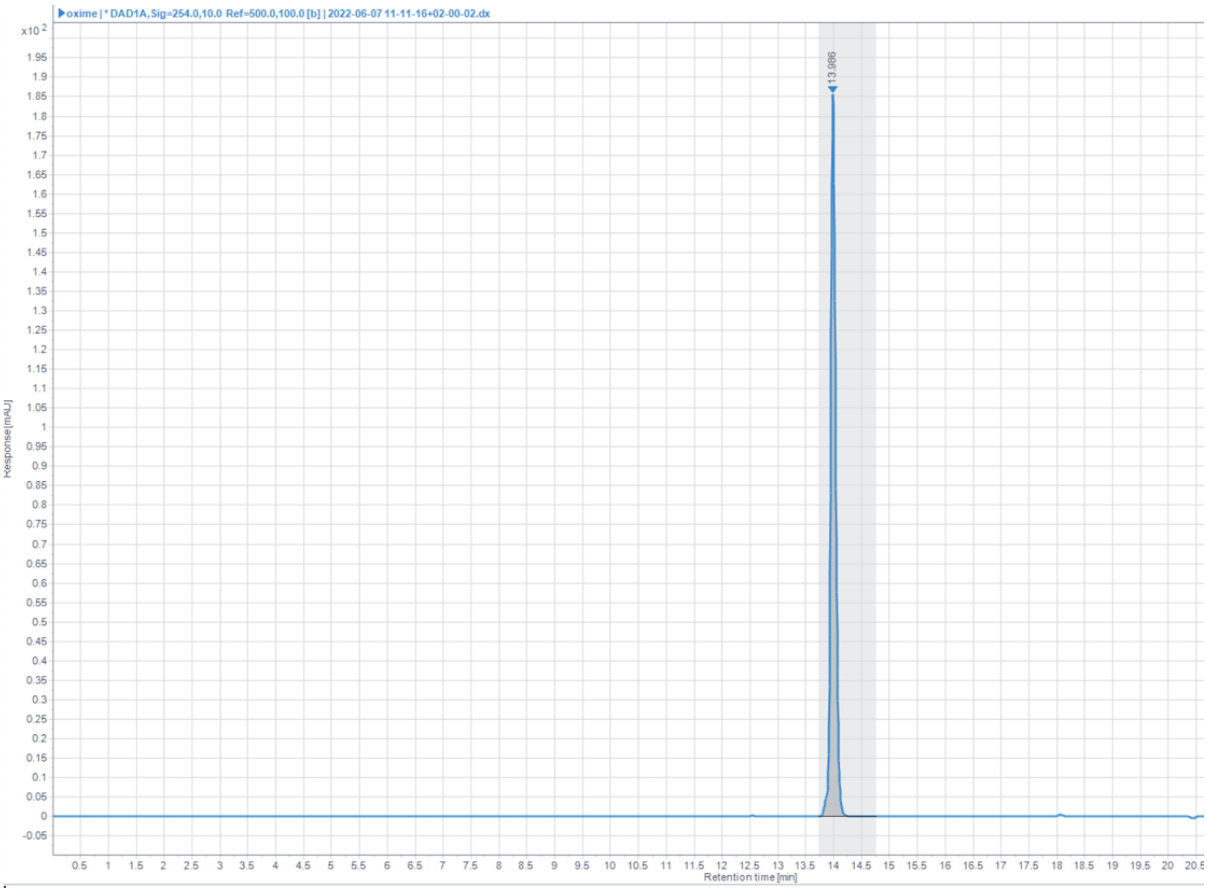


Figure 23 analytical HPLC chromatogram of oxime (**10**)

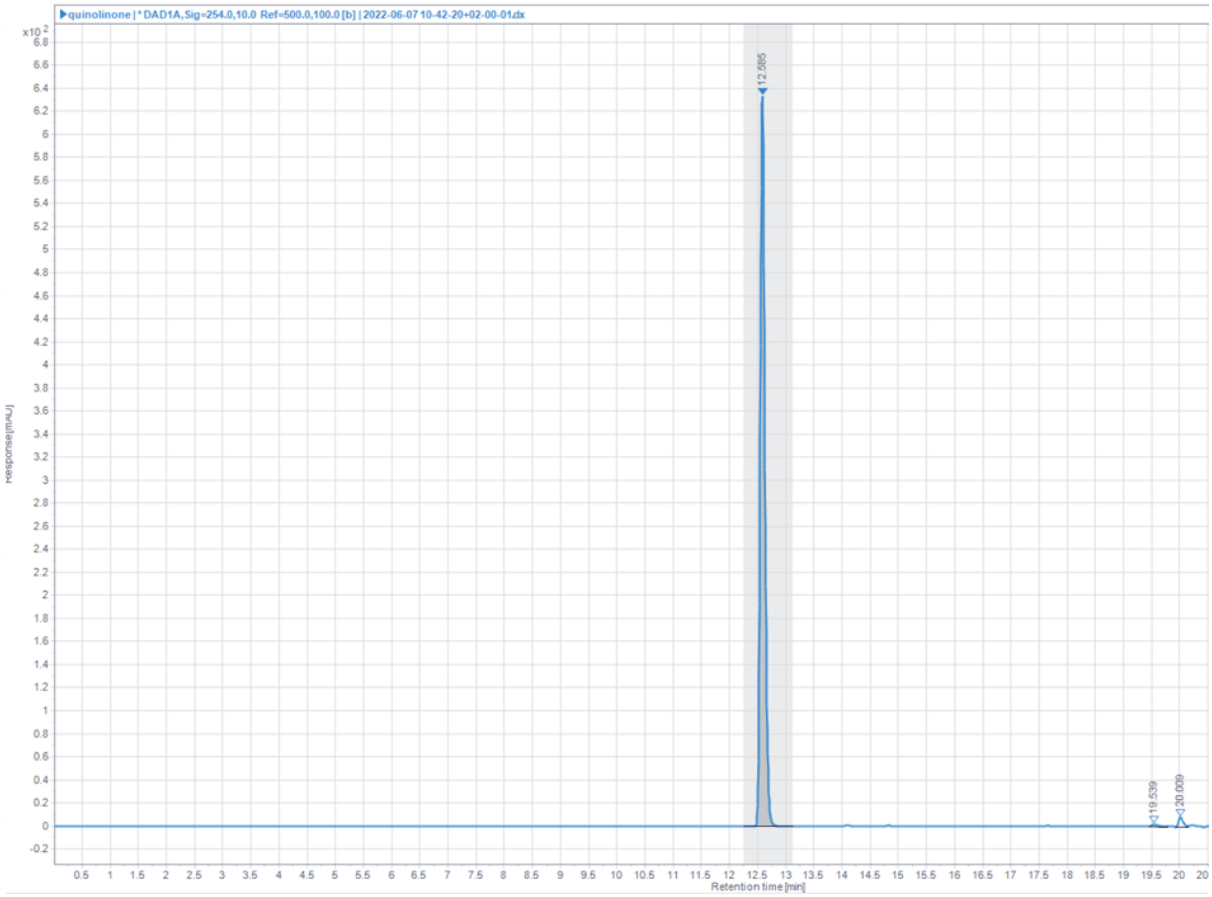


Figure 24 Commercially purchased analytical HPLC chromatogram of 3,4-dihydroquinolin-2(1H)-one (**2a**)

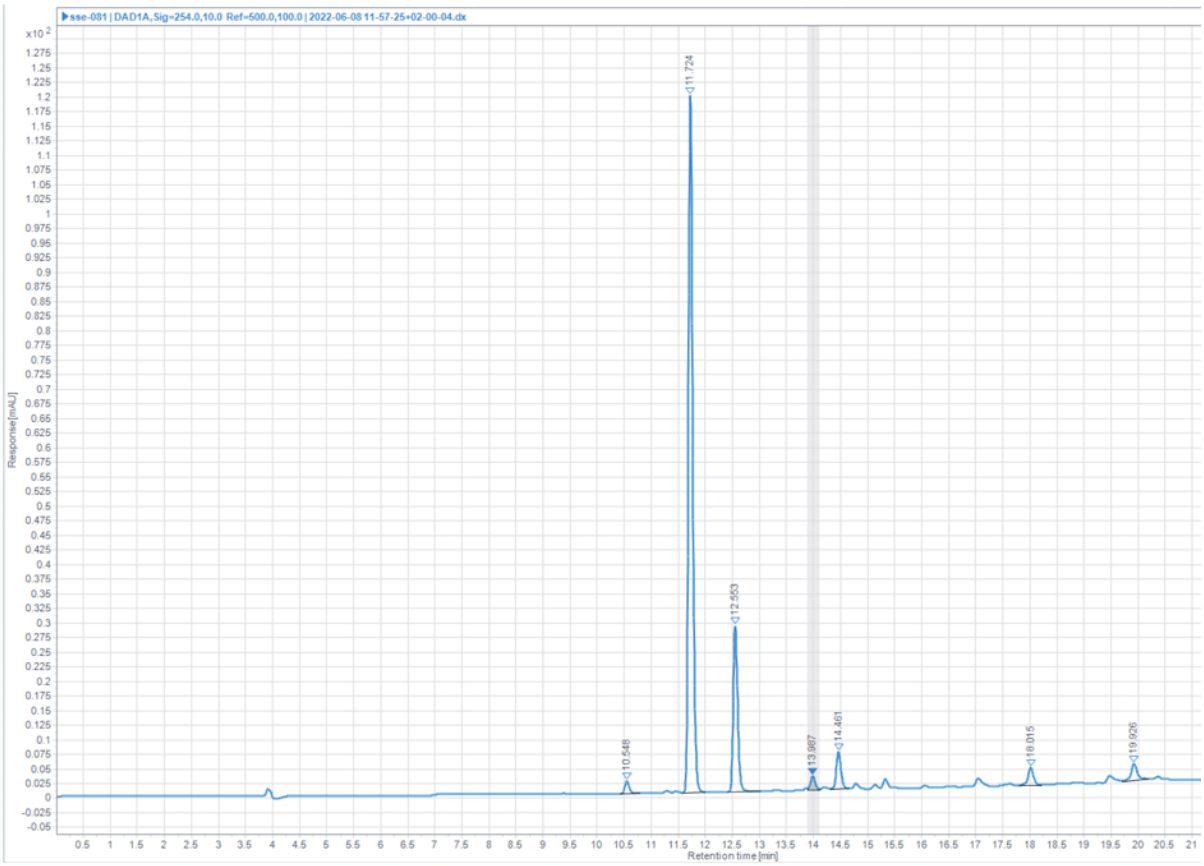
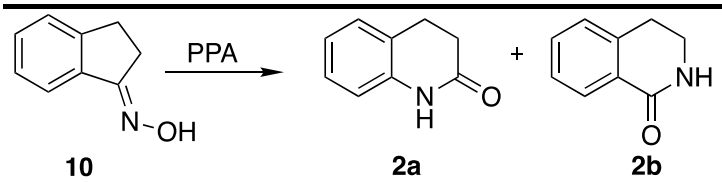


Figure 25 HPLC chromatogram showing a mixture of desired compound **2a**, isomer **2b** and oxime **10**

Table 3 shows the different parameters, their chosen values and the yield for the first design which was conducted. The first noticeable numbers are the conversion of 1-indanone oxime (**10**) which was 100% already after 12 hours. On the other hand, the yields were quite low.

Table 3. First experimental design on the Beckmann rearrangement of **10**

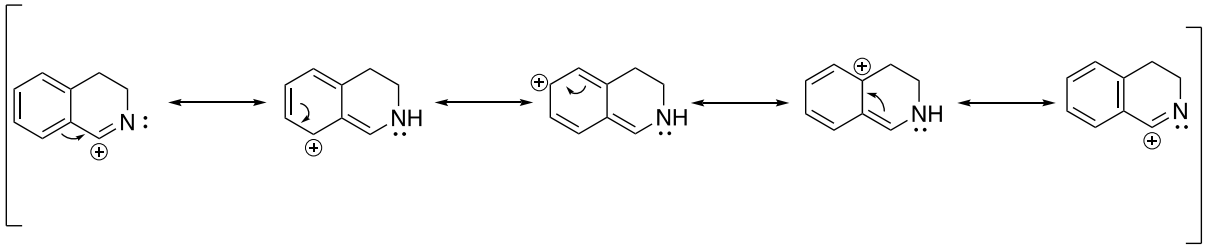


10 **2a** **2b**

Entry	Equiv.PPA	Time (h)	Temp (ºC)	Yield (%)[a]	Conv (%)[b]	Select (%)[c]
1	10	12	120	14	100	14
2	20	12	120	28	100	28
3	10	36	120	9	100	9
4	20	36	120	15	100	15
5	10	12	150	29	100	29
6	20	12	150	12	100	12
7	10	36	150	6	100	6
8	20	36	150	16	100	16
9	15	24	135	4	100	4
10	15	24	135	12	100	12
11	15	24	135	11	100	11

a) Yield based on substrate **2a**. b) Conversion based on substrate **2a**. c) Selectivity towards **2a**. Yield, conversion and selectivity were detected by analytical HPLC.

The formation of the isomer **2b** is more stable as the intermediate is stabilized through resonance (see *scheme 4.2.4*). **2b** is thermodynamically more favoured while target compound **2a** is more kinetically stable. This explains the low yields towards compound **2a,** with 100% conversion after 12 hours.



Scheme 4.2.4 Resonance effect affording the isomer **2b** as major compound

Entry *2* and *5* from **table 3** shows the most promising yields compared to the other experiments. When investigating the parameters for these entries, it is noticeable that when using high equivalents of PPA (20 equiv. PPA) and low temperature (120ºC) the obtained yield is 28%. Also, when using low equivalents (10 equiv.) of PPA and high temperature (150 ºC) the afforded yield was 29%. Simultaneously, the reaction time is the same for both reactions (12 hours). In conclusion, when using low equivalents of reagent and operating at high temperature or vice versa, the obtained yield is high. This information is also provided in *figure 26*, showing the interactions between the parameters.

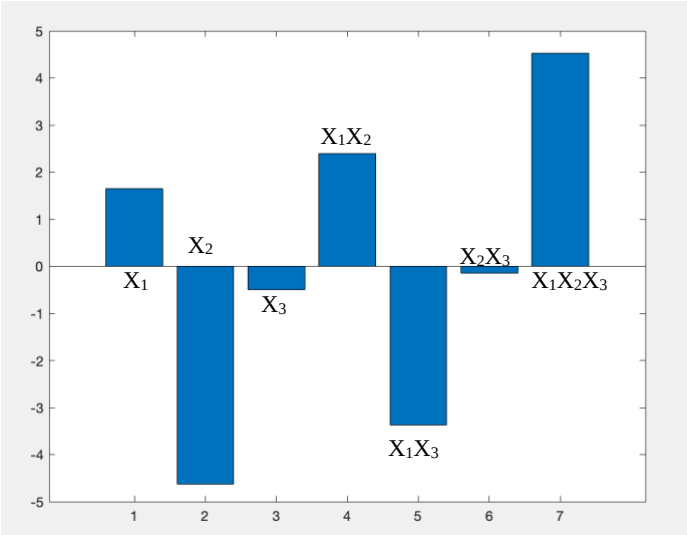


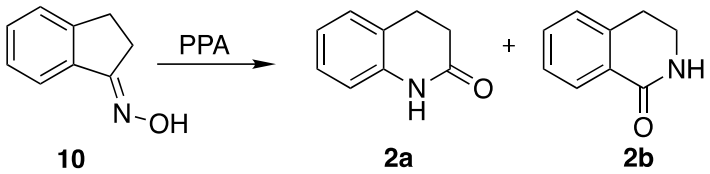
Figure 26 Bar plot showing interactions between the variables
x_1 = Equivalents of polyphosphoric acid, x_2 = time (h) and x_3 = temperature (ºC).

The bar plot shown in *figure 26* shows that x_2 variable is largely negative and must be negative such that the interaction variable x_1x_2 has a positive outcome. It also complies with **table 3** for entry *2* and *5* as stated above. Furthermore, the interaction between x_1x_3 is negative when x_1 is positive and x_3 is negative. Therefore, x_3 could be to be changed positively for the x_1x_3 interaction outcome to be positive. Although, $x_1x_2x_3$ interaction is largely positive when x_1 is positive and, x_2 and x_3 is negative.

By using this information, another design was generated. The second design was produced from the given information from *figure 26* and **table 3**. *Figure 26* showed that positive values for x_1 gave positive results. Therefore, the equivalents of PPA were chosen to be higher in the second statistical experimental design. On the other hand, the time was lowered as **table 3** showed that conversions were detected already after 12 hours. Lastly, the temperature was lowered to see if

the interaction between $x_1x_2x_3$ would have a positive outcome. The results from the chosen parameters are shown in **table 4**.

Table 4. Second experimental design on the Beckmann rearrangement of **10**



10 **2a** **2b**

Entry	Equiv.PPA	Time (h)	Temp (ºC)	Yield (%)[a]	Conv (%)[b]	Select (%)[c]
1	15	1	105	5	6	63
2	35	1	105	19	28	68
3	15	2	105	14	28	51
4	35	2	105	7	30	22
5	15	1	125	27	84	33
6	35	1	125	28	90	31
7	15	2	125	31	97	32
8	35	2	125	19	87	22
9	25	1.5	115	12	35	33
10	25	1.5	115	9	44	21
11	25	1.5	115	10	46	23

a) Yield based on substrate **2a**. b) Conversion based on substrate **2a**. c) Selectivity towards **2a**. Yield, conversion and selectivity were detected by analytical HPLC.

The importance in the interaction between the three variables are seen in **table 4**. Entry *2* and *4* differs in yield by 12 % by increasing the time by 1 hour. Though, the difference in yield between entry *2* and *3* are less by changing the equivalent of PPA. It is also noticeable that these three entries have the same temperature but by varying the equivalents of PPA and time, there difference in yield is prominent.

On the contrary, smaller variations in yield is seen for entry *5*, *6* and *7* in **table 4**. Entry *5* and *6* differs by only 1% in yield by varying the equivalents of PPA. Meanwhile entry *5* and *7* differs by 5 % when changing the time by 1 hour. The common denominator for these three reactions is the temperature which was at 125°C.

The interaction between x_1x_2 and x_1x_3 have negative outcome as viewed in *figure 27*. This is also seen in the statistical experimental design in entry *1* and *2* when using low values of x_1 and x_2 (entry *1*). Although, there is a remarkable increase in yield when increasing the temperature x_3 but x_1 and x_2 are held as negative values (entry *5*, 27%).

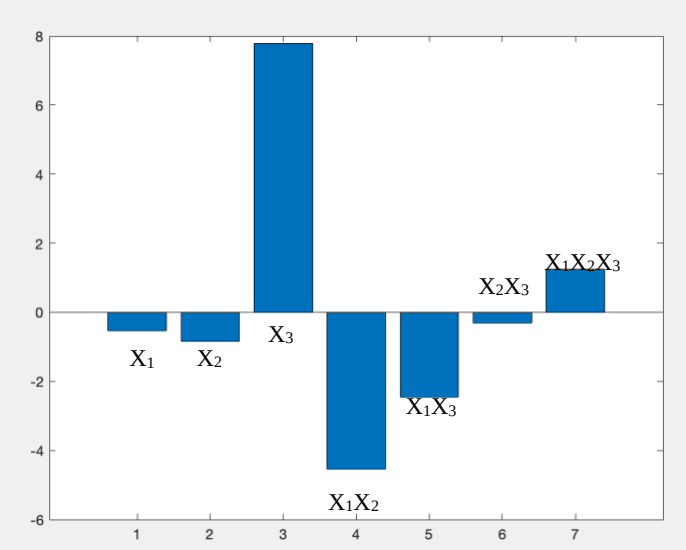


Figure 27 The interactions between variables for the second statistical experimental design x_1 = Equivalents of polyphosphoric acid, x_2 = time (h) and x_3 = temperature (°C).

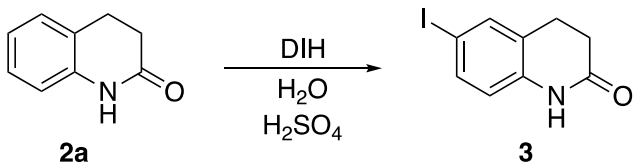
When comparing the yields that are shown in **table 3** and **4**, they are in the same range in yield. Entry *1* in **table 3** and entry *3* in **table 4** have the same outcome in yield, 14%. Entry *1* in **table 3** used less PPA compared to entry *3* in **table 4**. However, entry *1* in **table 3** had a reaction time of 12 hours in comparison to entry *3* in **table 4**, which had a reaction time of 2 hours. Lastly, the reaction temperature for entry *1* in **table 3** is 120°C while entry *3* in **table 4** had 105 °C. Another similar comparison is also seen in entry *2* for **table 3** and entry *6* for **table 4**. Therefore, it can be concluded that the extrapolation that was conducted to generate the second experimental design had almost no impact for the yield of **2a**.

However, the detected conversions for **table 3** and **4** are very different. **Table 3** shows 100% conversion after 12 hours and nearly 100% conversion is also seen in **table 4**, only after 2 hours. Moreover, it is also notable that the selectivity towards desired isomer **2a** increases in the second statistical experimental design (see **table 4**). The highest selectivity for **table 3** is 29 % (entry *5*) meanwhile **table 4**, entry *1* and *2* shows higher selectivity towards **2a** when increasing the equivalents of PPA, decreasing reaction time and temperature (63% and 68% respectively). In conclusion, the extrapolation increased selectivity towards desired compound **2a**.

The three-factor interaction between $x_1x_2x_3$ has a positive outcome but it is evidently low compared to the positive outcome from the first statistical experimental design (see *figure 26* and *27*). The second statistical experimental design was an extrapolation established on the two-factor results from the first statistical experimental design. Thereby, the extrapolation may have negatively impacted the three-factor interactions. Also, studies on main effects or two-factor interactions become invalid since the three-factor interaction is present and has explicit impacts on the statistical experimental designs. Hence, the extrapolated statistical design was not an ideal attempt to optimize the yield of **2a**.

4.3 Synthesis of 6-iodo-3,4-dihydroquinoli-2(*1H*)-one (3)

Multiple attempts were performed to afford 6-iodo-3,4-dihydroquinoli-2(*1H*)-one (**3**) as illustrated in *scheme 4.3.1*. The first four attempts were conducted by using a procedure which was described for mono-halogenation of imidazole by using DIH (1,3-diiodo-5,5-dimethylhydantoin). *Sandtorv et al* carried out several mono-halogenation experiments by using imidazole as substrate. They also used the same reaction conditions as described in **table 5** (entry *1-4*).[94] When carrying out the same procedure for compound **2a**, there were no indication of product **3** when analyzing through instrumental techniques.



Scheme 4.3.1 Synthesis of 6-iodo-3,4-dihydroquinoli-2(*1H*)-one (**3**) by using DIH

Table 5 presents that when increasing the amount of DIH and NaOH respectively, the success of iodine attaching to compound **2a** increased remarkably. This can be argued by studying the

size of iodine. Compared to the substrate imidazole which *Sandtorv et al* described in their article, compound **2a** is more sterically hindered.[94] Hence, compound **2a** could not react with low equivalents of DIH to yield **3** and an increased amount of 1,3-diiodo-5,5-dimethylhydantoin was needed.

The Beckmann rearrangement of 1-indanone oxime (**10**) always gave a mixture of compound **2a** and the isomer **2b**. Therefore, 3,4-dihydroquinolin-2(*1H*)-one (**2a**) was commercially purchased as the isomers were not easy to separate. Compound **3** was synthesised at room temperature (22ºC) by reacting commercially purchased 3,4-dihydroquinolin-2(1H)-one (**2a**) and DIH in a round bottom flask. As the flask was immersed in an ice-bath, H_2O was added to the mixture and left for 5 minutes. Then, concentrated H_2SO_4 was added and the reaction mixture was left to stir for 24 hours. The reaction mixture was cooled down and the flask was washed with water. A mixture of acetic acid and water was added till pH reached 5-6 where precipitation was observed. Lastly the precipitation was collected to through vacuum filtration to give a pale-yellow coloured solid (98%).

Table 5. Overview of reagents and conditions used for the formation of **3**

Entry	Equiv 3a	Equiv. DIH	Equiv. H_2O	Equiv. H_2SO_4	Equiv. NaOH	Temp (ºC)	Time (h)	Yield (%)
1	1	0.24	1000	10	34.2	23	-	-
2	1	0.24	1000	10	34.2	23	o. n[(a)]	-
3	1	0.98	1000	10	33.5	23	o. n	-
4	1	0.24	1000	10	34.2	23	24	-
5	1	2.58	100	0.18	43.6	23	24	98

(a) o. n = over night

Both ^{1}H and ^{13}C NMR confirms that the iodine is attached to 3,4-dihydroquinolin-2(*1H*)-one. Compound **2a** contains 9 hydrogens while compound **3** only has 8 hydrogens and this is seen in the ^{1}H NMR spectrum of **3** as described in *figure 28*. The ^{13}C NMR spectrum presents a signal at 85.8 ppm which also confirms that the iodine is attached to compound **2a**. It is also apparent that the signals are more downfield when comparing the ^{13}C spectra of compound **2a** and the mono-halogenated product **3** (see *figure 29*).

Amides are theoretically ortho and para-directing group.[95] This is confirmed in the formation of **3** as the iodine is attached in the para position to the amide. This is also affirmed through

HMBC as there is a signal at 85.8 ppm is correlating with the double doublets at 7.48 ppm and the doublets at 6.55 ppm. There is also a correlation between 85.9 ppm and the triplet at 2.94 ppm showing that the iodine has a $^{4}J_{C\text{-}H}$ coupling. This is only possible if the iodine is in the para position to amide (shown as the red proton for compound **3** and the coupling is ringed around in *figure 30*). Lastly, there is a signal in the ^{1}H NMR spectrum at 1.62 ppm which integrates to 1H. This peak belongs to H_2O as water was used during the work-up procedure.

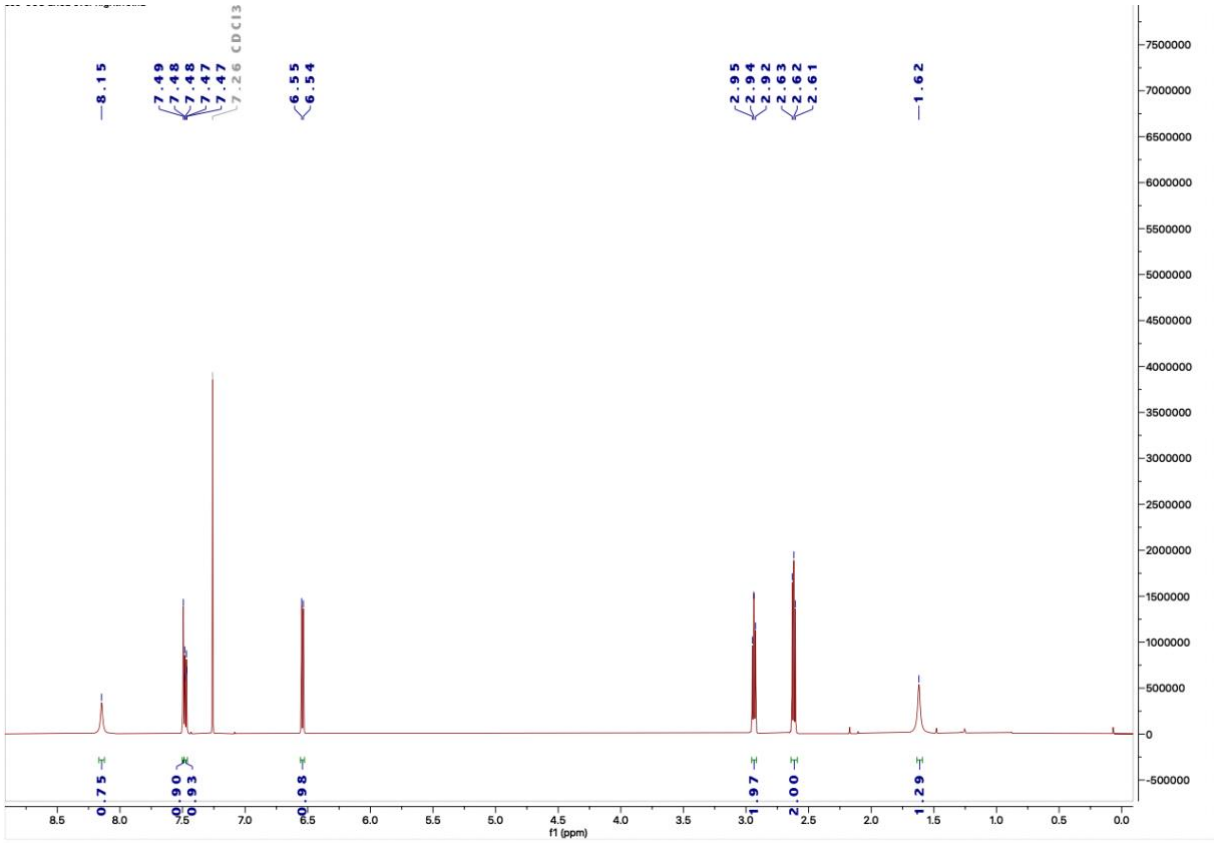


Figure 28 ^{1}H NMR spectrum of 6-iodo-3,4-dihydroquinoli-2(*1H*)-one (**3**)

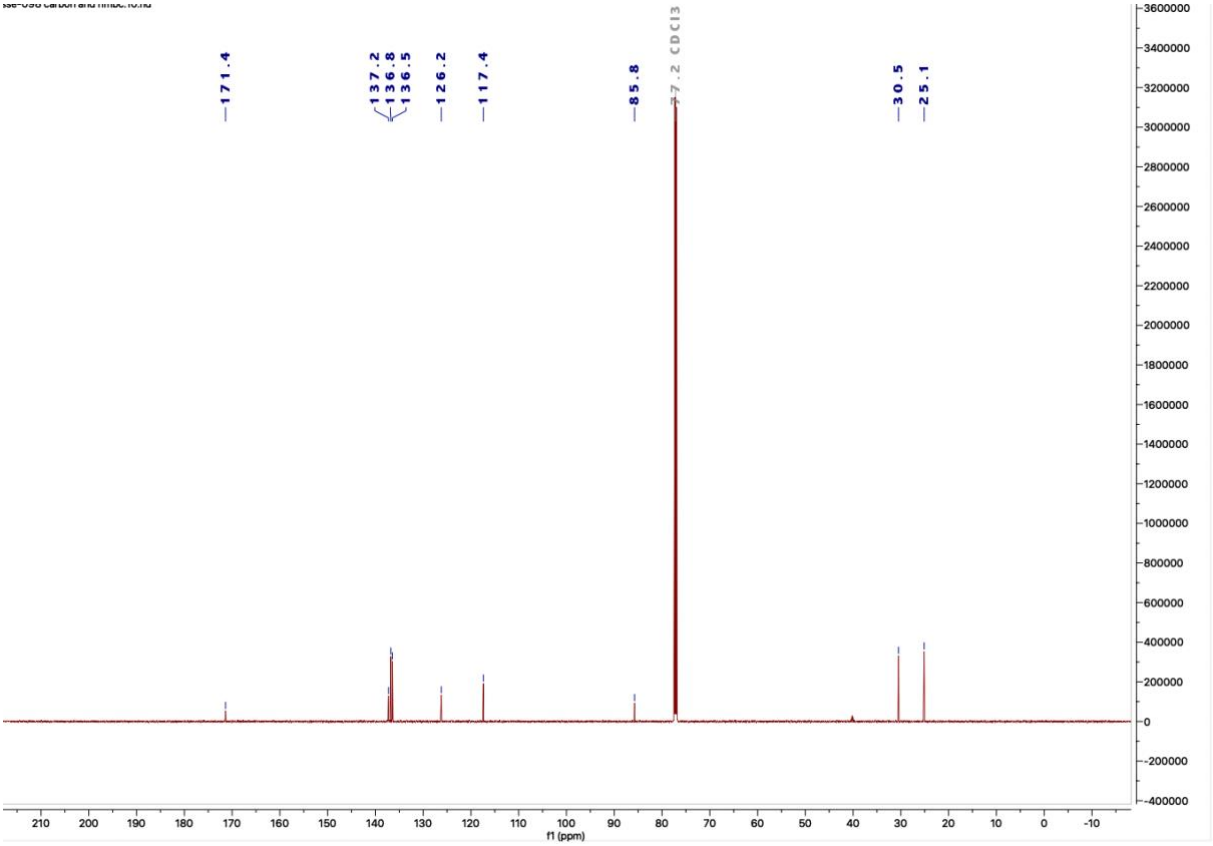


Figure 29 ^{13}C NMR spectrum of 6-iodo-3,4-dihydroquinoli-2(*1H*)-one (**3**)

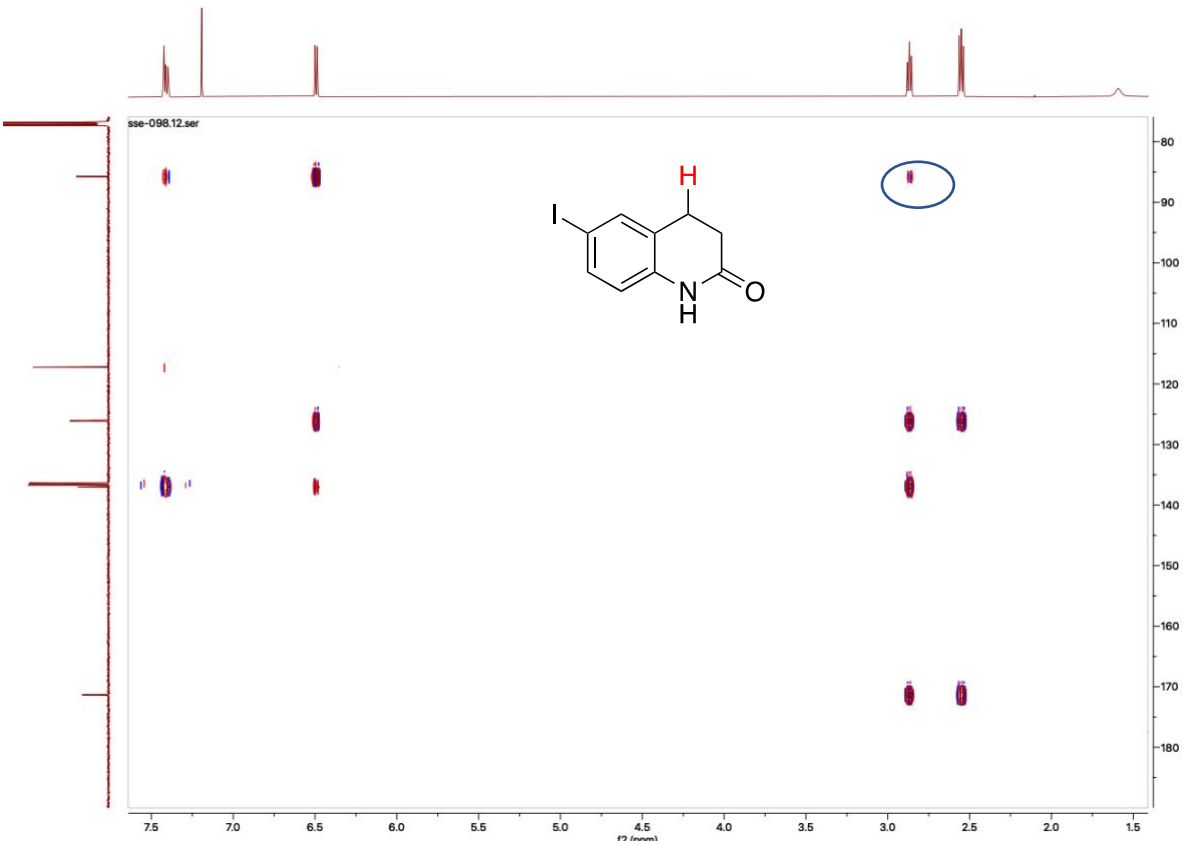
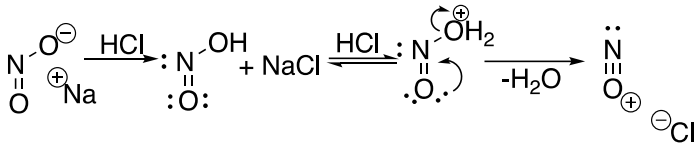


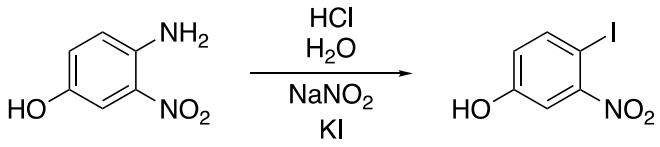
Figure 30 HMBC illustrating the $^{4}J_{C\text{-}H}$ coupling between the amide and the iodide

4.4 Synthesis of 4-iodo-3-nitrophenol (7)

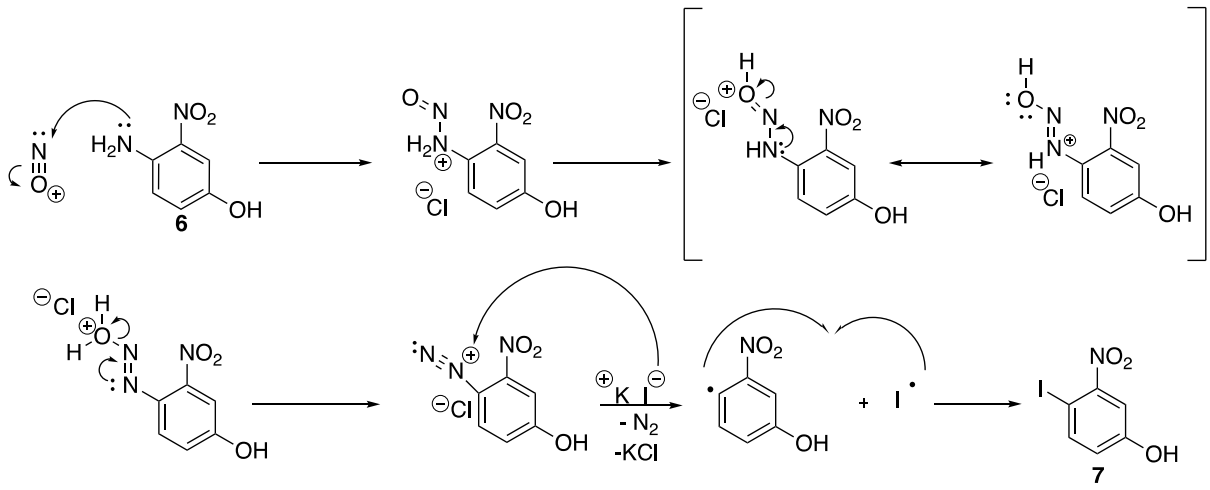
4-iodo-3-nitrophenol (**7**) was synthesised through a Sandmeyer reaction by using $NaNO_2$ to form the diazonium ion by using HCl and H_2O (*scheme 4.4.1*). A round bottom flask consisting of commercially purchased 4-amino-3-nitrophenol (**6**), concentrated HCl and H_2O was stirred and left as a slurry for a few minutes. A solution of $NaNO_2$ in water was added dropwise to the reaction mixture. The diazonium ion was formed and used to react with 4-amino-3-nitrophenol (**6**) to give a diazonium compound. This further reacted with a solution of KI which was added dropwise to the reaction mixture at 0 ^oC. The mixture was left to stir over night at room temperature. The product was filtrated and washed with cold water to give the desired compound 4-iodo-3-nitrpophenol (**7**). The reaction yielded 79% of 4-iodo-3-nitrophenol (**7**) and the scheme and mechanism is shown in scheme *4.4.2 and 4.4.3*.



Scheme 4.4.1 Formation of the diazonium ion



Scheme 4.4.2 Synthesis of 4-iodo-3-nitrophenol (**7**)



Scheme 4.4.3 Mechanism for the synthesis of **7**

A Sandmeyer reaction is often catalysed by using copper(I). However, Cu(I) does not engage in this reaction but is substituted with KI. KI is a strong nucleophile which can release an electron to the diazonium ion. Then, the coupling reaction between diazenyl radical and $I^{\bullet}$ may take place as described in *scheme 4.4.3*.[96]

The product was clearly confirmed through ^{1}H and ^{13}C NMR. When comparing the starting material **6** with product **7**, the aromatic signals are shifted downfield for product **7**. This is seen for the signals at 7.84 ppm, 7.31 ppm and at 6.87 ppm in ^{1}H NMR as shown in *figure 31*. The signal which is seen at 10.59 ppm and integrated to 1H belongs to O-H proton. The work-up for the reaction consisted of collecting the solids through vacuum filtration. Hence, H_2O is seen at 3.39 ppm in the ^{1}H NMR spectrum.

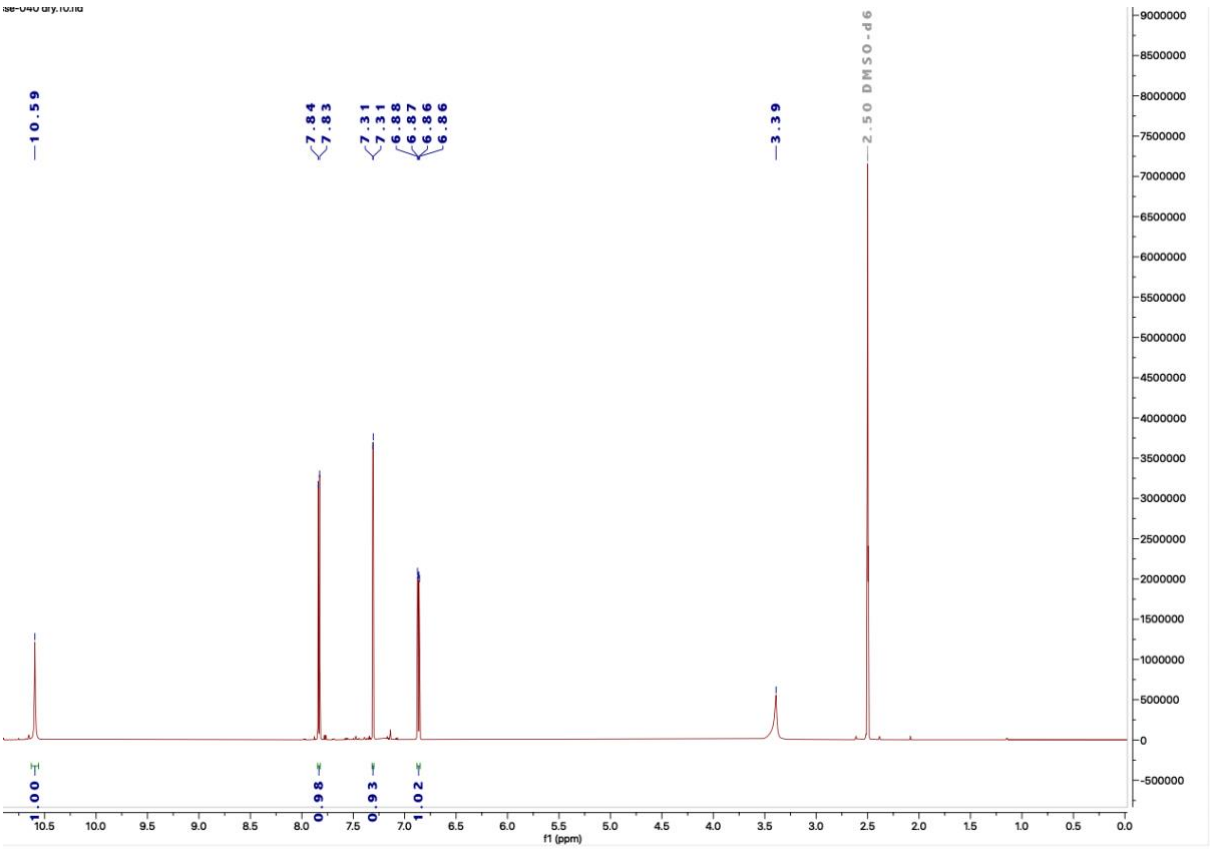
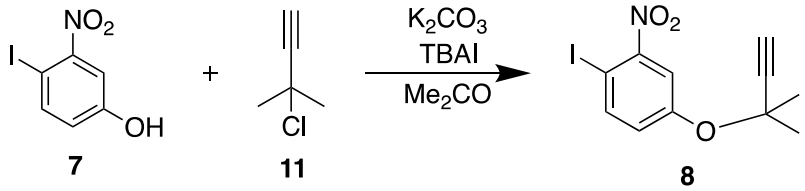


Figure 31 ^{1}H NMR spectrum of 4-iodo-3-nitrophenol (**7**)

4.5 Synthesis of 1-iodo-4-((2-methylbut-3-yn-2-yl)oxy)-2-nitrobenzene (8)

Multiple approaches were demonstrated in the synthesis of 1-iodo-4-((2-methylbut-3-yn-2-yl)oxy)-2-nitrobenzene (**8**). The first attempts in synthesising **8** were performed by following the experimental procedure described by *Herzon et al*, by using TBAI as phase-transfer catalyst.

The phase-transfer catalyst was used for the base K_2CO_3 which is soluble in aqueous phase. However, there were no conversion detected by NMR or MS when following the experimental procedure of *Herzon and co* and the reaction scheme is drawn in *scheme 4.5.1*.[37]



Scheme 4.5.1 Herzon and Myers' approach in synthesizing **8**

Compound **8** was firstly synthesised using the exact conditions that was describes in the experimental procedure by *Herzon et a*l.[37] This showed no conversion, only TBAI and starting material were detected when analysed by GC-MS and NMR. Though, the result was different when the experiment was re-produced with different conditions compared to the first attempt (see **table 6**). The equivalent of TBAI and 3-chloro-3-methylbut-1-yne (**11**) were increased and KPF_6 was added in the work up to remove the excess of TBAI. The crude seemed to contain compound **8** but had impurities and was therefore purified by flash column chromatography. This left a light-yellow oil with less than 1 % in yield of compound **8**.

Table 6. Synthesis of **8** using K_2CO_3 as base and TBAI as phase-transfer catalyst

Entry	Equiv. K_2CO_3	Equiv. TBAI	Equiv. 11	Time (h)	Temp (°C)	Yield (%)
1	2	0.10	2	18	65	-
2	2	3	3	18	65	< 1[(a)]

a) Yield obtained after purification by silica flash column chromatography (9:1 hexane / EtOAc)

The first two attempts with TBAI and K_2CO_3 as base did not afford good results. This led to a screening of reagents to find a better method to improve the synthesis. **Table 7** shows the different reagents and their equivalents that were used to synthesise **8**. Four different reactions were conducted. Two of them were successful in synthesising compound **8** but the reaction in entry *4* showed more impurity compared to the reaction in entry *3*. Hence, a column chromatography was conducted on the crude from the reaction using DBU as base and acetone as solvent.

Table 7. Screening to find a better experimental method for the synthesis of **8**

Entry	Base	PTC[(a)]	Solvent	Time (h)	Temp (ºC)	Yield (%)
1	K_2CO_3	-	DMF	18	100	-
2	K_2CO_3	10% TBAI	Acetone	18	65	-
3	DBU	-	Acetone	18	65	3[(b)]
4	DBU	-	DMF	18	100	-
5	KOH	-	Acetone	18	65	-

a) PTC = phase-transfer catalyst

b) The isolated yield was obtained after purification by silica column chromatography (9:1 hexane / EtOAc).

When investigating the conditions that were used for the reaction with DBU and acetone (entry *3*, **table 7**), a higher temperature seemed more ideal. The reaction was already conducted in a microwave vial as the initial temperature was above acetone's boiling (56ºC). Hence, the temperature was increased to 80 ºC and was also left to react for longer time. However, this did not improve the obtained yield.

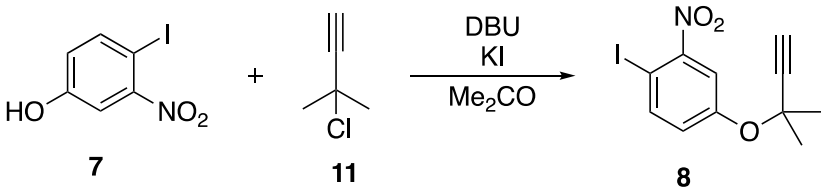
Another re-investigation was conducted on the reagents used for the synthesis of compound **8**. 3-chloro-3-methylbut-1-yne (**11**) has chloride as halogen which is not a good leaving group. To enhance the dissociation of the leaving group, it can be substituted by another and better leaving group. This can be performed through a Finkelstein reaction and was used in this reaction. KI, 3-chloro-3-methylbut-1-yne (**11**) and acetone were added in a separate microwave vial and left to stir for about 20-30 minutes. Meanwhile, the base, the starting material **7** and acetone was left to stir as well in a separate round bottom flask as well. The reaction mixture containing the base and compound **7** was added to the microwave vial, sealed and left to stir at 75ºC. When the reaction was quenched after 18 hours, the work-up was conducted. This consisted of washing the post-reaction mixture with EtOAc, with saturated $NaHCO_3$ solution and with saturated NaCl solution. The obtained yield after purification was 14%. Since the afforded yield was low, the reaction was conducted again but was left to stir at 80ºC for 48 hours (see *scheme 4.5.2*). The yield increased exceptionally (14 % - 80 %, see **table 8**). This was confirmed in NMR, HR-MS, HPLC and GC-MS (see appendix 5).

Table 8 Synthesis of **8** through Finkelstein reaction

Entry	Equiv. 7	Equiv base	Equiv. 11	Equiv. KI	Time (h)	Temp (ºC)	Yield (%)
1	1	2	2	3.98	18	75	14[(a)]
2	1	2	2	3.98	48	75	40[(a)]
3	1	2	2	3.98	48	80	78[(a)]
4	1	2	2	3.98	48	80	80
5	1	2	2	3.98	48	80	83

a) The isolated yield was obtained after purification by silica column chromatography (9:1 hexane / EtOAc).

The afforded yield increased from 14% to 80% by increasing the temperature with 5ºC as shown in **table 8**. Additionally, entries *4* and 5 show that the obtained yield did not need to be purified, whilst entries *1* and *2* had to be purified through silica column chromatography (9:1 hexane / ethylacetate). Moreover, the afforded yield in entry *3* was obtained through purification, but have same reaction conditions seen for entries *4* and *5* as well. The first three reaction were conducted by reacting compound **11** and KI in a microwave vial for about 30 minutes. Meanwhile, the last two entries were performed by reacting compound **11** and KI in the microwave vial for at least 1 hour. To conclude, when increasing temperature and time, the yield increased while formation of side products decreased.



Scheme 4.5.2 Reaction scheme for the synthesis of **8** through Finkelstein reaction

GC-MS, ^{1}H and ^{13}C NMR spectra confirmed that compound **8** was synthesised. The most characteristic signals are the seen for the methyl groups at 1.68 ppm which has 6 hydrogens in the ^{1}H NMR spectrum and at 29.5 ppm in ^{13}C NMR spectrum. Another characteristic signal is observed as the singlet at 2.67 ppm in the ^{1}H NMR spectrum which integrated to 1H. The detected signal belongs to the hydrogen on the alkyne as seen in *figure 32*. This is seen at 73.6 ppm in the ^{13}C spectrum (see *figure 33*). Lastly, the GC-MS of compound 8 is seen in *figure 34*.

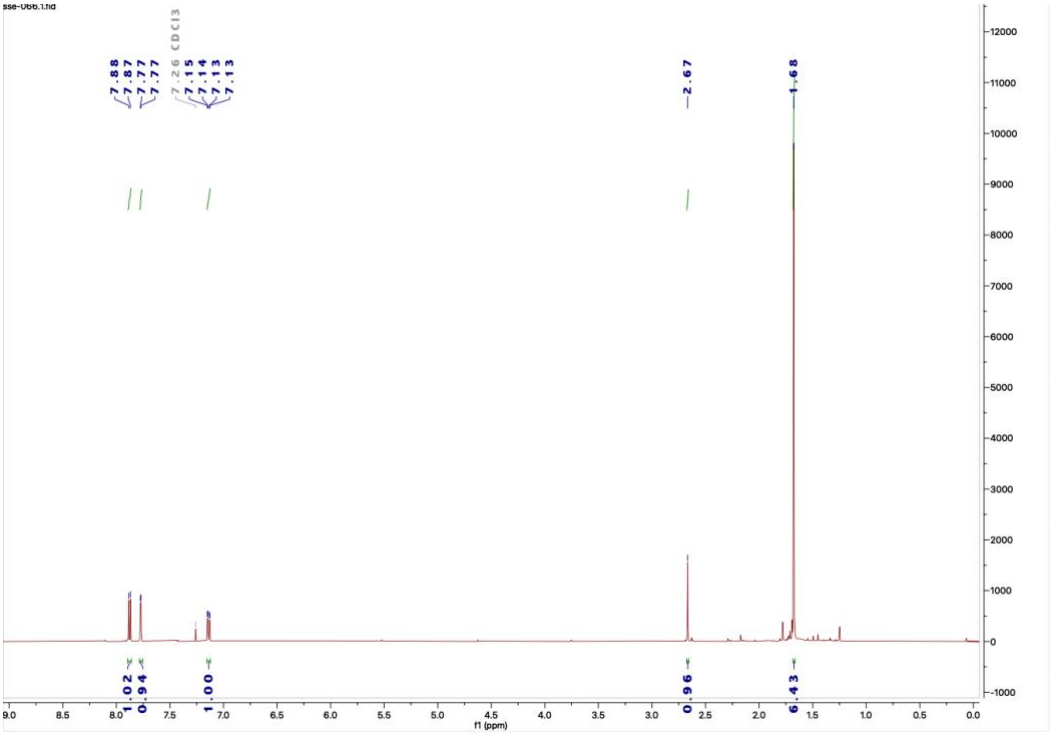


Figure 32 ^{1}H NMR spectrum of 1-iodo-4-((2-methylbut-3-yn-2-yl)oxy)-2-nitrobenzene (8)

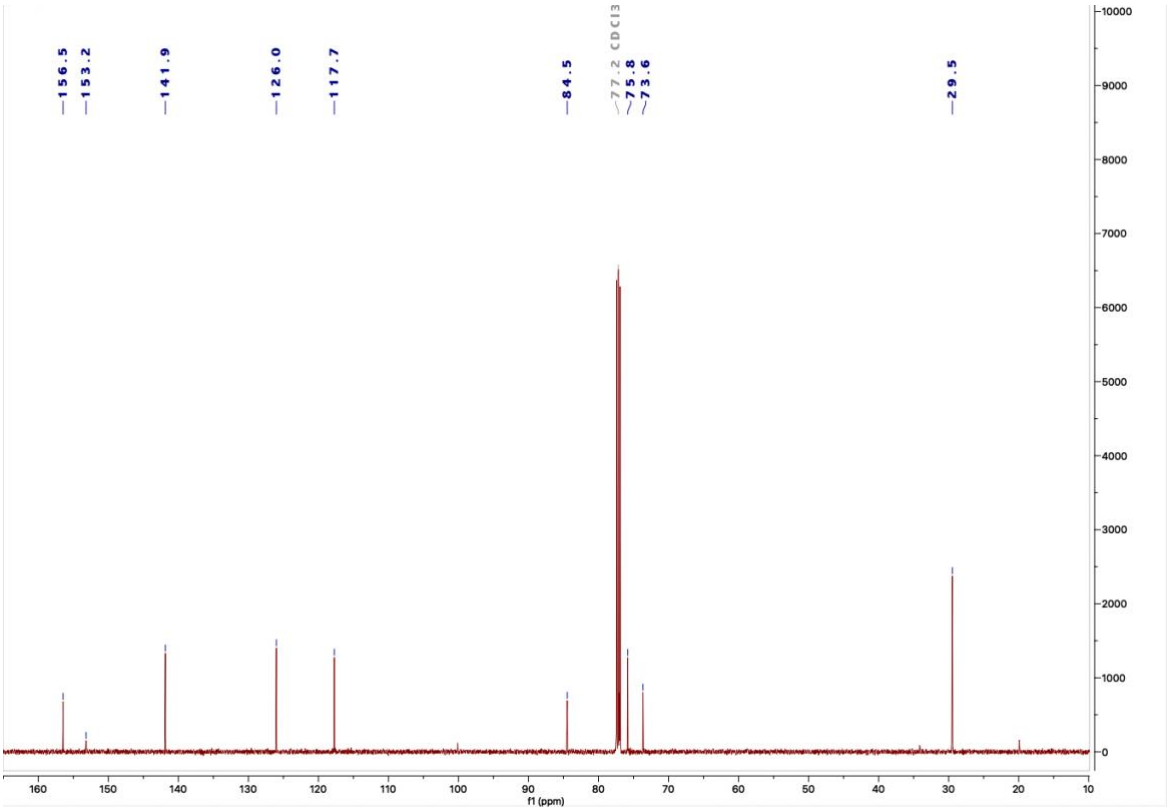


Figure 33 ^{13}C spectrum of 1-iodo-4-((2-methylbut-3-yn-2-yl)oxy)-2-nitrobenzene (**8**)

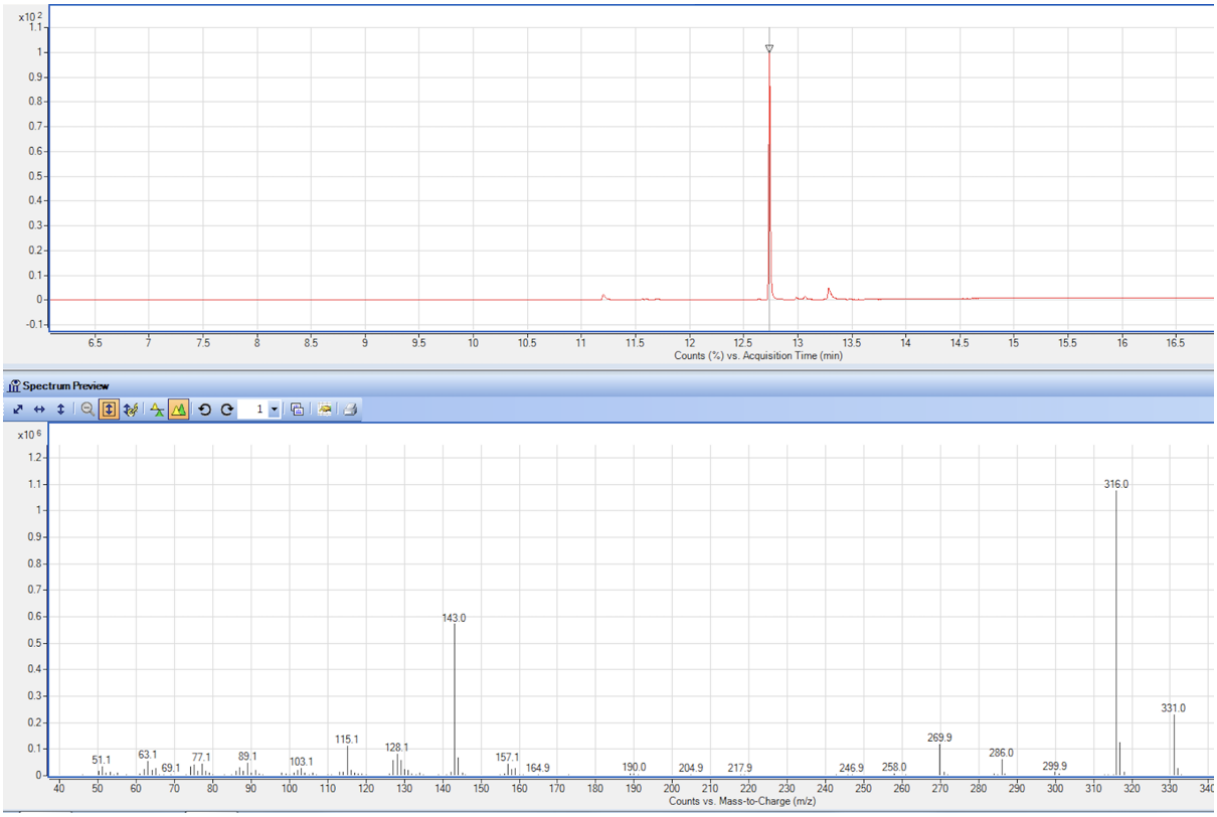
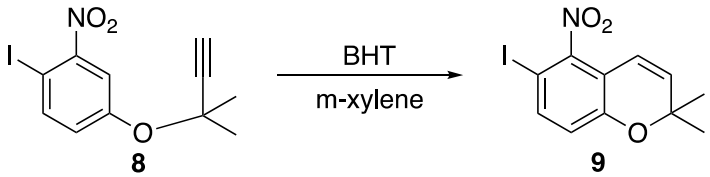


Figure 34 GC-MS of compound nitrobenzene **8**

4.6. Synthesis of 6-iodo-2,2-dimethyl-5-nitro-2H-chromene (9)

6-iodo-2,2-dimethyl-5-nitro-2H-chromene (**9**) was synthesised by using the antioxidizing chemical BHT and m-xylene as solvent. The reaction was conducted in a microwave vial and sealed with a tube (see *scheme 4.6.1*). It was left to stir at 140ºC for 48 hours and was isolated through reverse auto-flash chromatography (MilliQ +0.1 % acetic acid / ACN + 0.1% acetic acid).



Scheme 4.6.1 Reaction scheme for the synthesis of **9**

The experimental procedure to synthesise iodoarene **9** was firstly performed by *Herzon et al.* The authors reported an isolated yield of 78 %, but this was not seen when the experiment was re-produced. When following the same conditions as described in the procedure, the obtained yield was low (see **table 9**). TLCs were executed when the reaction was conducted in a timespan

of 15 hours (see *figure 35)* as described in the procedure by *Herzon et al.*[37] The TLCs revealed some indication of conversion of starting material **8**. The isolated product after only 15 hours afforded a yield of 6 %. Hence, longer reaction time was utilized.

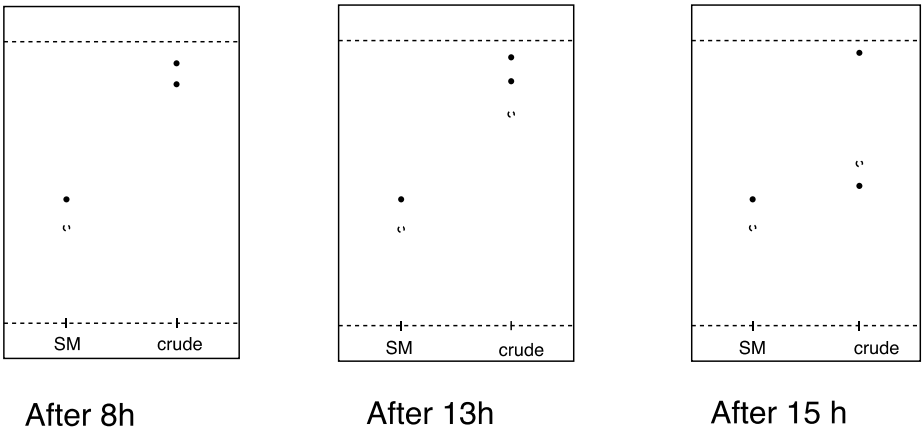


Figure 35 TLCs during a reaction time of 15 hours (hexane / EtOAc 9:1)
SM = starting material **8**

Table 9. Optimization of 9

Entry	Equiv. BHT	Time (h)	Temperature (ºC)	Yield (%)[(a)]
1	0.05	15	140	-
2	0.05	15	140	6
3	0.05	48	140	31

a) Yield of **9** was isolated after purification by reverse auto-flash column chromatography (MilliQ / ACN + 0.1% acetic acid)

Furthermore, there was deviation in the experimental work-up procedure written by *Herzon et al*. After the reaction mixture was cooled down to room temperature, the post-reaction mixture was loaded on a silica gel pad. It was firstly eluted with hexane and then with ethylacetate / hexane (2:8). The residue with hexane was described to be discarded according to the procedure detailed by the authors.[37] However, a TLC of the residue which was eluted with hexane, disclosed that it also contained compound **9**.

The characteristic signals for **9** are seen in the ^{1}H NMR spectrum. The aromatic signals for compound **9** is more upfield in comparison to starting material **8**. Compound **8** has a butoxy moiety substituted on the aromatic ring, hence a dishielding effect. However, compound **9** has

a closed ring which shields the electronegative effect from oxygen. Also, product **9** has four signals in the aromatic region compared to compound **8**. When the ring encloses, a double bond is formed to give a two doublet signals which is seen at 6.88 ppm and at 5.50 ppm. The singlet in ^{1}H NMR belongs to the methyls for compound **9** (see *figure 36* and *37*).

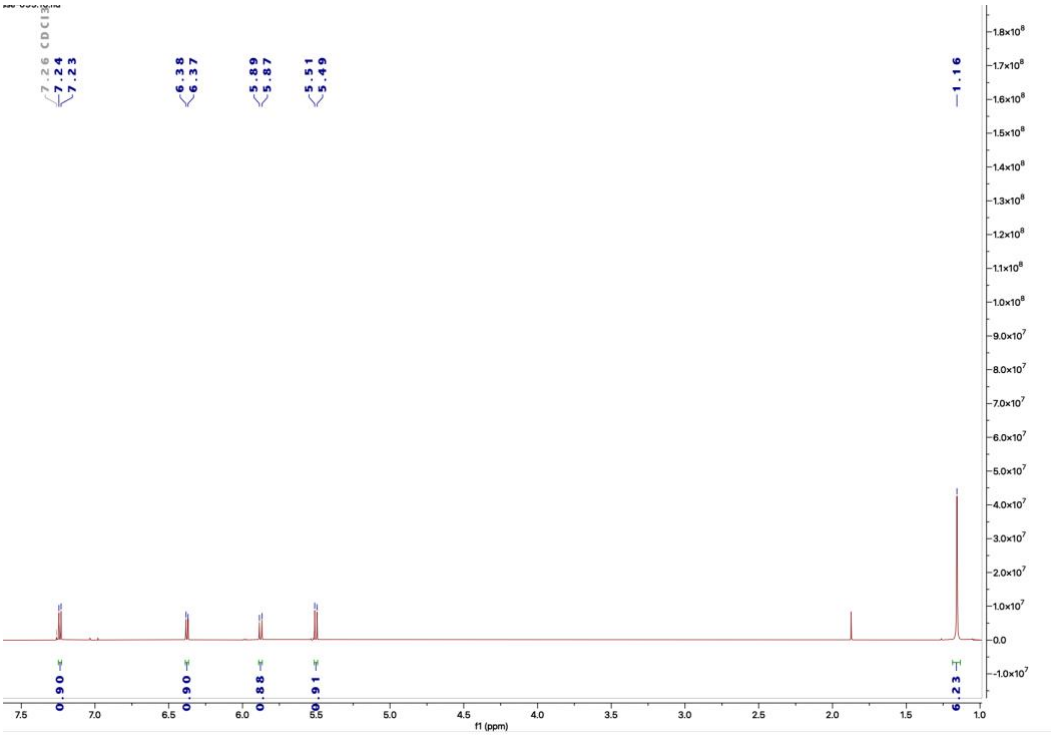


Figure 36 ^{1}H NMR spectrum of compound **9**

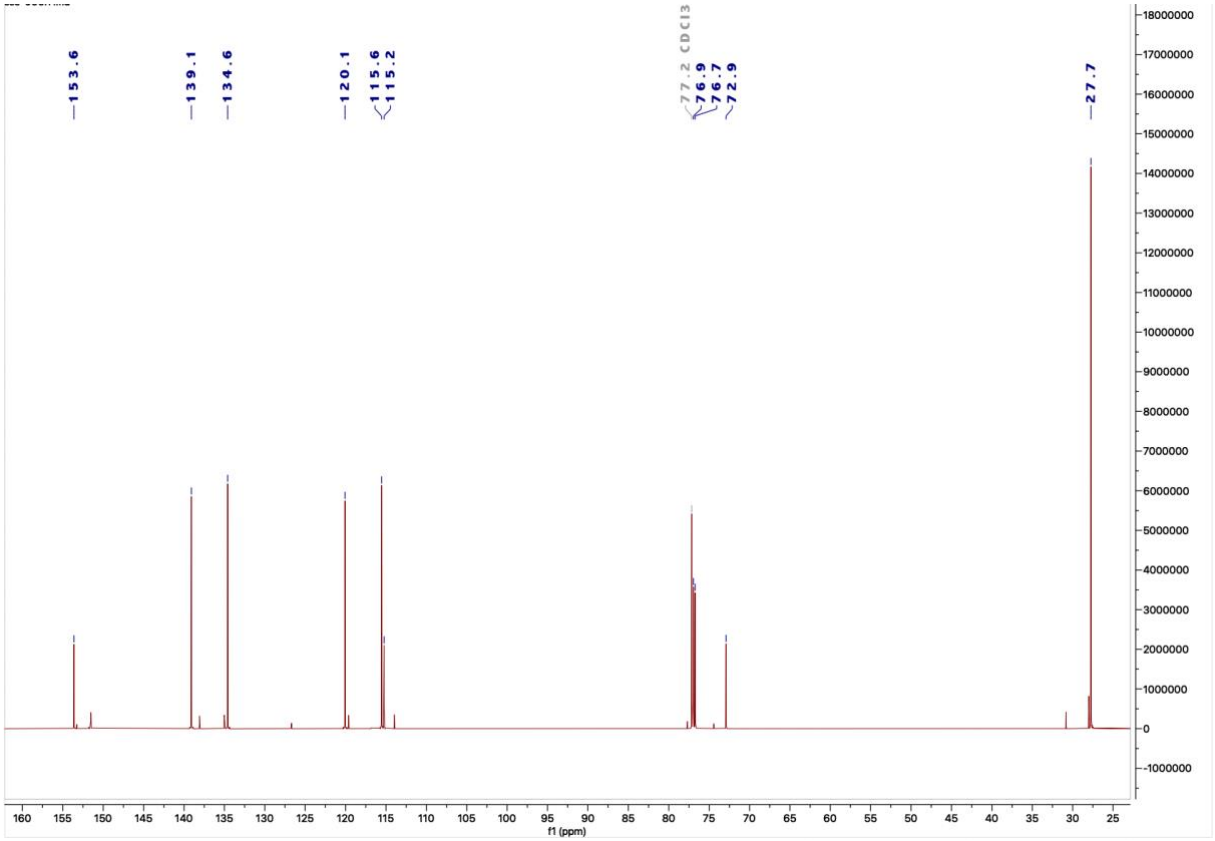


Figure 37 ^{13}C NMR spectrum of chromene **9**

On the other hand, the ^{1}H and ^{13}C NMR spectra are not completely pure. Comparison of ^{1}H and ^{13}C spectra of compound **8** and **9,** display that starting material **8** is the reason behind the additional signals seen in ^{1}H and ^{13}C NMR spectra for product **9**. Compound **8** and **9** are very similar in polarity and have the same molecular weight. Consequently, TLC of starting material **8** and product **9** had almost the same R_f value even after purification by reverse auto-flash (see *figure 38*). The compounds behaved also similarly in elution when analysed by GC-MS as shown in *figure 39*. Also, there were no MS analyses conducted on compound **8** and **9** as they were not detectable by this analysis method. However, the product was not purified further and was carried on to the next step.

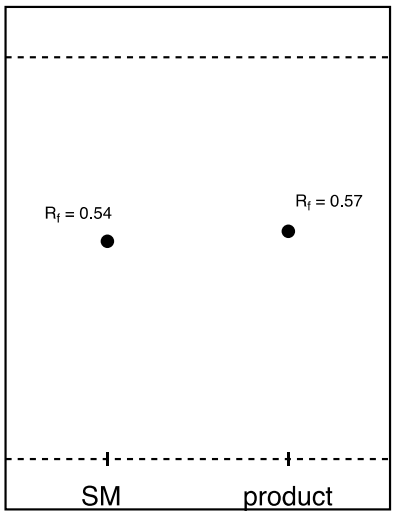


Figure 38 TLC of starting material **8** and compound **9** after purification by reverse auto-flash (MilliQ + 0.1% acetic acid / ACN + 0.1% acetic acid).

SM = starting material **8**

Product = compound **9**

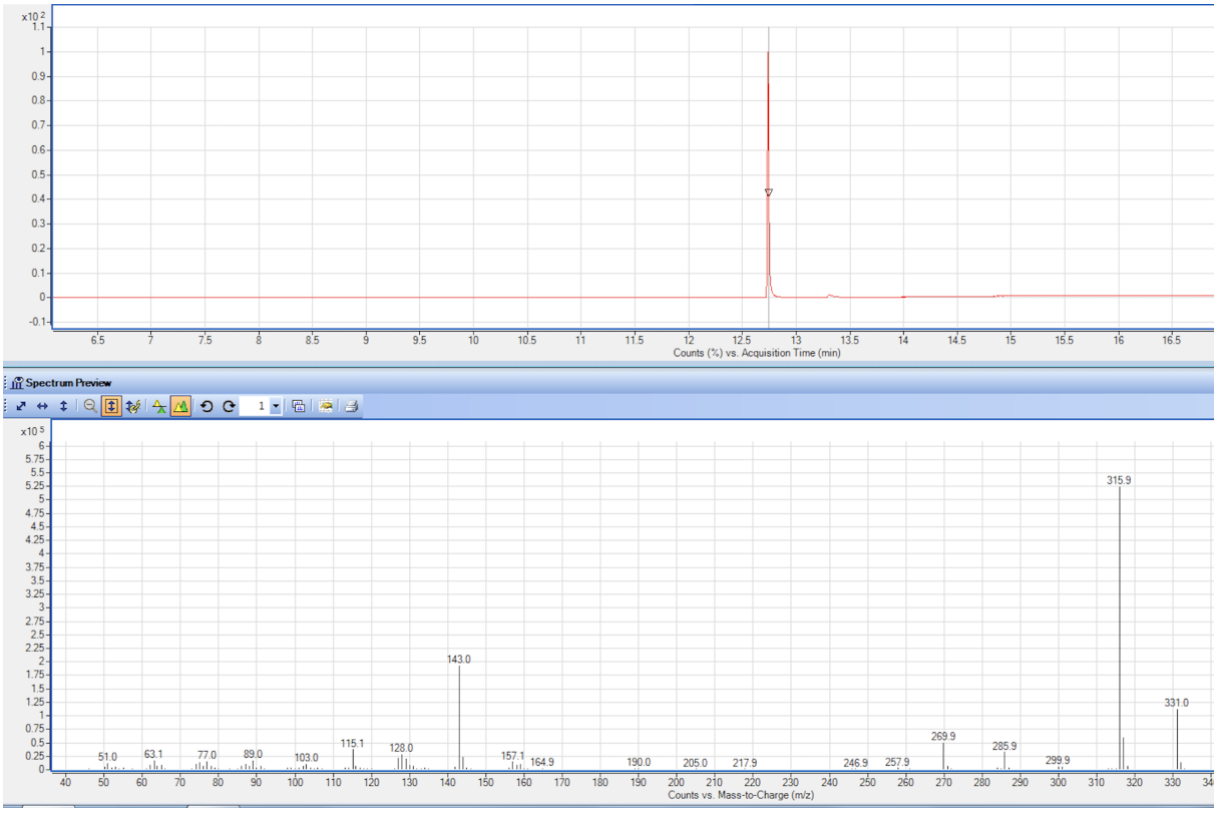


Figure 39 GC-MS confirming the formation of compound **9**

4.7 Synthesis of 6-iodo-2,2-dimethyl-2*H*-chromen-5-amine (4)

Another screening of reagents was conducted for the synthesis of 6-iodo-2,2dimethyl-2-chromen-5-amine (**4**). The reduction of the amino-group was to be conducted without reducing any other functional groups. This was attempted by different reagents which is shown in **table 10**.

Table 10. Reagent screening for reduction of **9**

Entry	Reducing agents	Solvent	Conversion(%)[(a)]
1	In, NH_4Cl	EtOH	-
2	$NaBH_4$, $CoSO_4 \cdot 7H_2O$	EtOH	-
3	NH_3, $Na_2S_2O_4$	-	-
4	Fe, NH_4Cl	EtOH	100

(a) Conversion towards compound **4**

Entry *1* and *3* showed no conversion towards compound **4**. Entry *1* furnished no conversion of **9** when attempting the synthesis of **4** by using the reducing agents indium and ammonium chloride. The reaction was described in an article published by the authors *Elumalai V et al.* The authors managed to reduce their substrates affording a yield ranging between 69-96%. However, the authors also observed that the activity of indium was destructed due to surface oxidation when storing under normal atmosphere at 22ºC.[97] The indium that was used for the attempted synthesise of **4**, had been used before, stored in room temperature and under normal atmosphere. Hence, the indium powder may not have been reactive enough to carry on the reduction.

On the other hand, entry *2* was suspected to have conversion towards product **4**. TLC of the post-reaction showed several spots (see *figure 40*). A MS analysis was conducted after isolating the spots through silica column chromatography (7:3, hexane / EtOAc). The MS analysis showed that the fractions that were suspected to contain product **4,** did not consist the compound. However, the MS analysis revealed two strong signals, $[M]^+ = 178$ and $[M]^+ = 176$. $[M]^+ = 176$ is the removal of iodine on the substituted aromatic ring while $[M]^+ = 178$ was detected as both iodine and the double bond was removed. The synthesis did not furnish compound **4** but managed to further reduce compound **4**, by removing iodine and the double bond on compound **4** (see figure *41*)

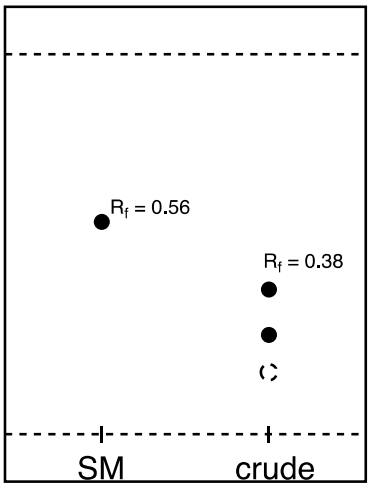
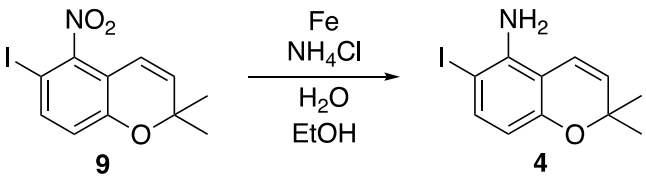


Figure 40 TLC taken during synthesis of compound **4** from starting material **9** using $NaBH_4$ and $CoSO_4 \cdot 7H_2O$ as reducing agents (hexane / EtOAc, 7:3).
SM = starting material **9**

Figure 41 Study of the MS analysis conducted after purification by silica chromatography (hexane / EtOAc 9:1)

The synthesis of 6-iodo-2,2-dimethyl-2H-chromen-5-amine (**4**) from 6-iodo-2,2-dimethyl-2*H*-chromene (**9**) was succeeded by using NH_4Cl and Fe and confirmed through GC-MS (see *figure 42*). The reaction was completed after heating for 2 hours at 75ºC and by using ethanol as solvent. Lastly, the work-up for the post-reaction consisted of filtrating the iron from the product through a pad of Celite. The residue was concentrated under reduced pressure and furnished the product as a brown oil which did not need further purification.



Scheme 4.7.1 Reaction scheme showing the synthesis of **4**

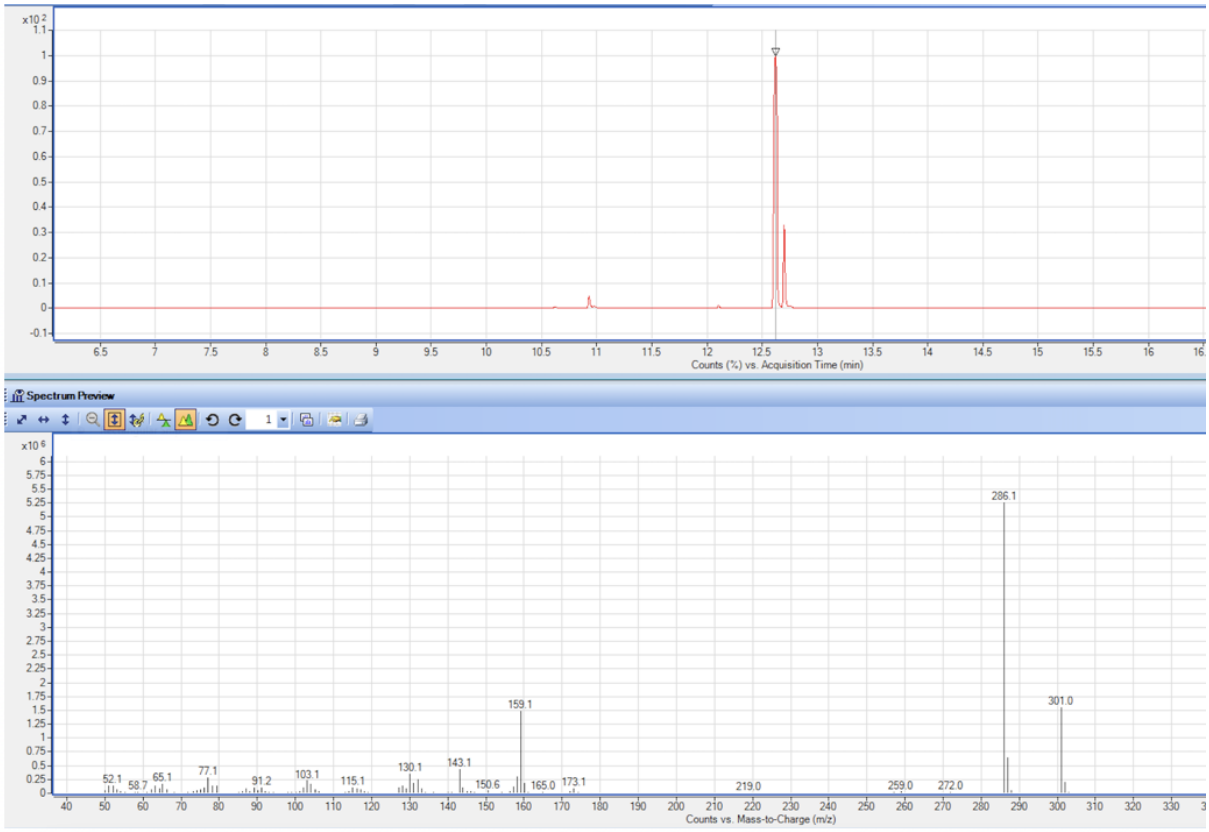


Figure 42 GC-MS spectrum verifying the formation of compound **4**

Table 11. Reduction of **9** by using Fe and NH_4Cl

Entry	Equiv.NH_4Cl	Equiv. Fe	Time (h)	Temp (^oC)	Yield (%)
1	5	6	2	75	89
2	5	6	2	75	50
3	5	6	2	75	87
4	5	6	2	75	94
5	5	6	2	75	41

^{13}C NMR confirmed that compound **4** was synthesised from iodo-arene **9**. When comparing the starting material **4** and product **9**, the most characteristic signals are seen in the aromatic region. Iodo-arene **4** has several signals in the aromatic region between 142 ppm and 117 ppm (see *figure 44*). The signals are seen in the downfield region for compound **4** compared to compound **9** which has fewer signals in the aromatic region and are more upfield which is seen in both ^1H NMR and ^{13}C NMR (see *figure 43* and *44*). MS and analytical HPLC also verifies that 6-iodo-2,2dimethyl-2-chromen-5-amine (**4**) was synthesised from 6-iodo-2,2dimethyl-2-chromene (**9**) through reduction (see appendix 7)

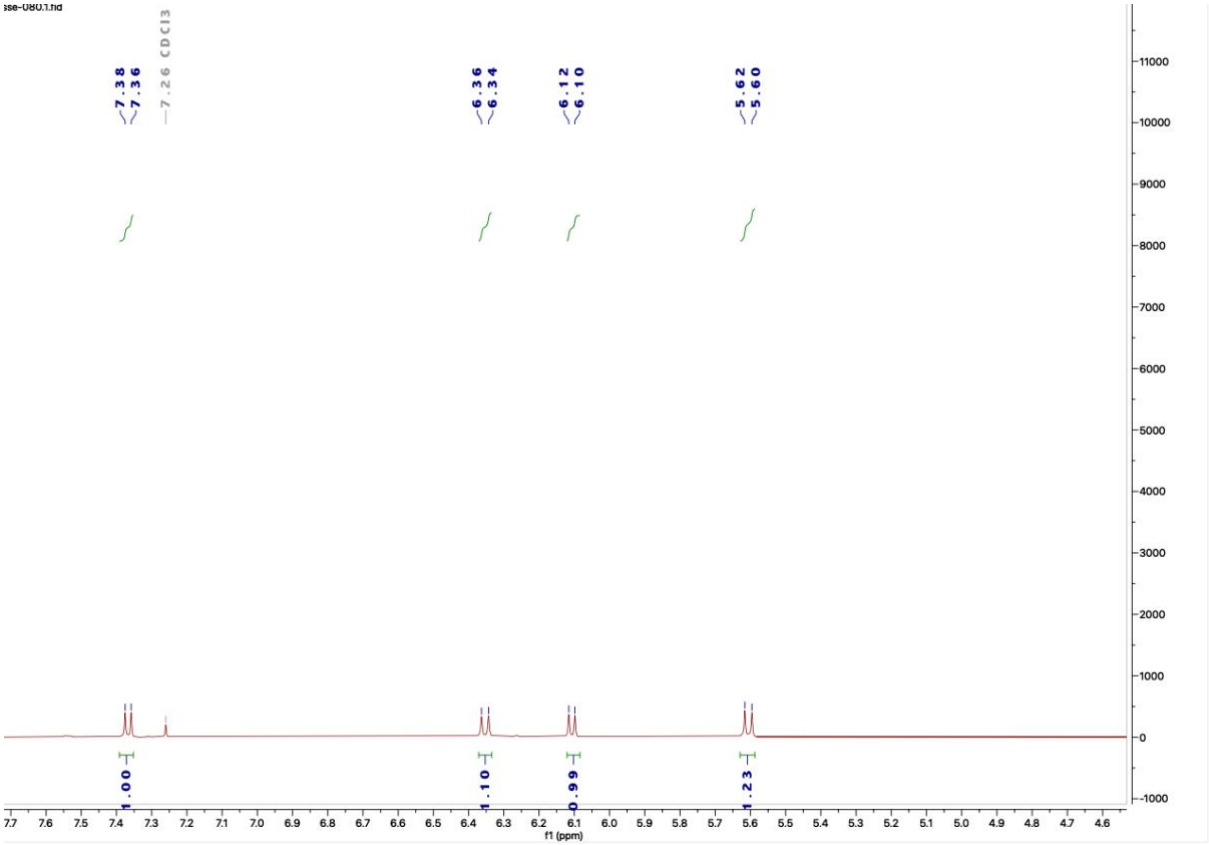


Figure 43 ^{1}H NMR of 6-iodo-2,2dimethyl-2-chromen-5-amine (**4**)

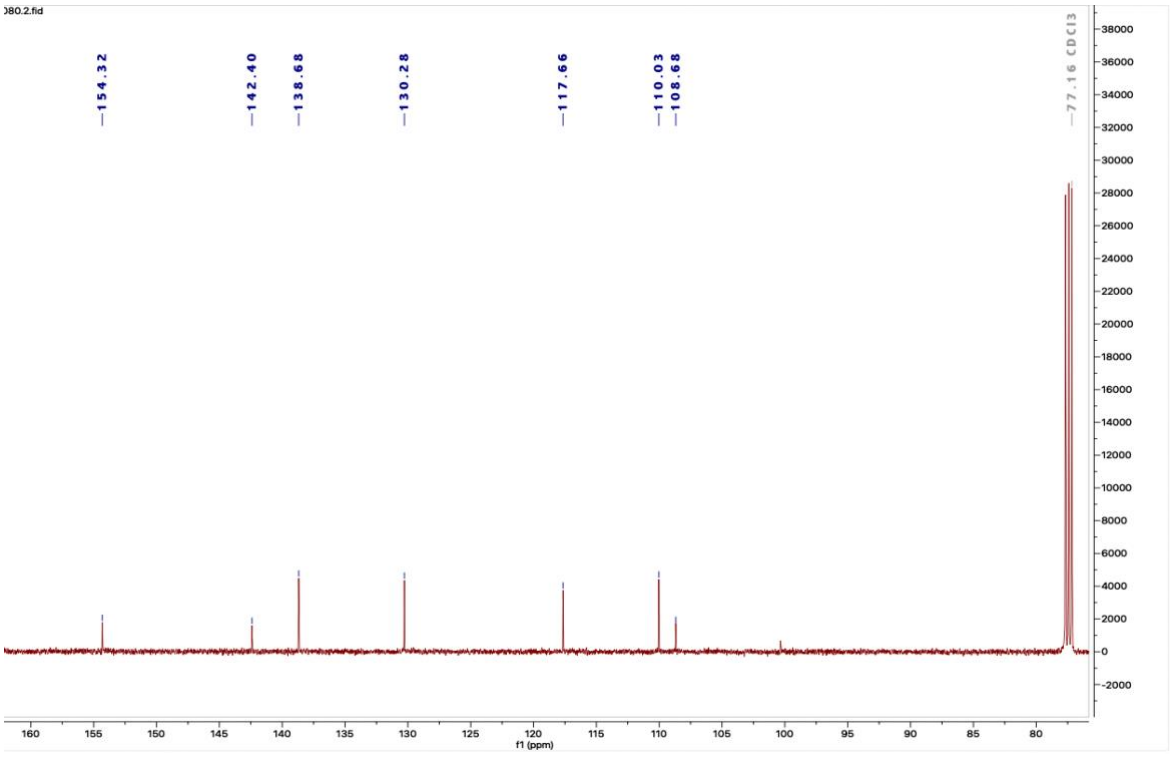
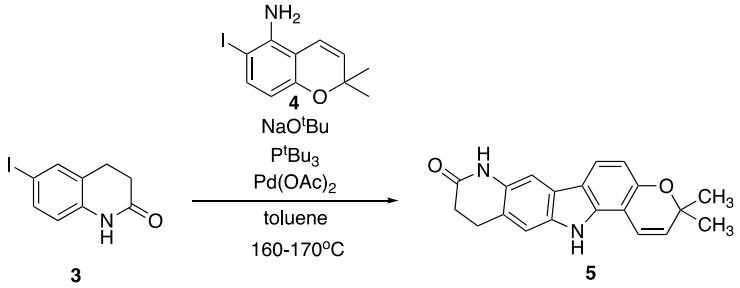


Figure 44 ^{13}C NMR of 6-iodo-2,2dimethyl-2-chromen-5-amine (**4**)

The afforded yield for the synthesis of compound **4** varied in the range of yield between 41 – 94 %. Entry *1 – 5* in **table 11** shows the same equivalents of reagents, time and temperature. Though, the outcome of yield between entry *1* and *2* are quite different. It was therefore suspected that compound **9** might have been decomposed when synthesising product **4** in entry 2. However, synthesis of **4** in entry *1* and *2* was performed by using the same batch of compound **9** which was synthesised some time in prior.

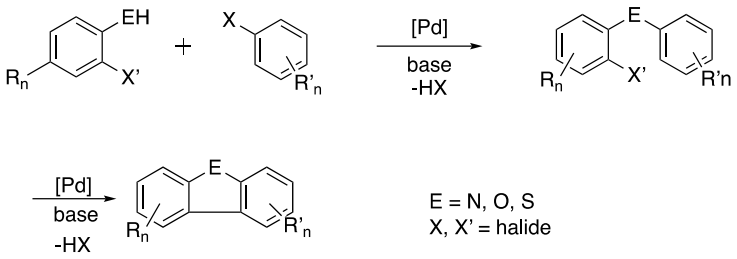
4.8 Attempted synthesis of 3,3-dimethyl, 8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one (5)

To accomplish the final synthesis step towards 3,3-dimethyl, 8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one (**5**), multiple experiments were performed. The final step can be accomplished through a C-N formation (Buchwald-Hartwig) and a C-H activation (see reaction *scheme 4.8.1*). Although, the attempts towards synthesis of product **5** were not successful.

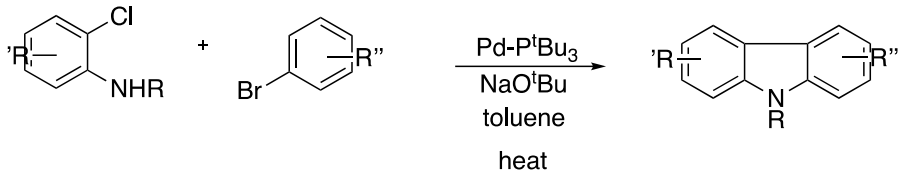


Scheme 4.8.1 Reaction scheme for the synthesis of 3,3-dimethyl, 8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one (**5**)

In 2002, *Bedford R et al* published an article describing the accomplishment of performing two sequential catalytic transformations in one pot. The authors conducted this study to see if it was achievable to fuse a palladium-catalysed C-N formation reaction with a catalytic C-H activation, to generate dibenzo[m,n]-fused carbazoles (see *scheme 4.8.2*).[98] Additionally, another article published by *Bedford et al* described the successful one-pot synthesis towards carbazoles through C-H activation, from tethered aryl halide (see *scheme 4.8.3*).[99]



Scheme 4.8.2 Bedford and Cazin's general reaction scheme showing the one pot synthesis of dibenzo[m,n]-fused carbazole[98]



Scheme 4.8.3 One-pot synthesis of carbazoles[99]

The attempted experiments to synthesise compound **5,** were based on experiments conducted by *Bedford et al* in 2002 and 2006.[98,99] Firstly, compound **5** was attempted to be synthesised in a microwave reactor using $Pd(OAc)_2$ as catalyst, P^tBu_3 as ligand, NaO^tBu as base and toluene as solvent. The reaction was heated for 3 hours at 160°C (**table 12**). Both TCL and GC-MS analysis of the reaction showed no conversion towards desired compound **5,** only the starting materials **3** and **4**.

Table 12. Attempted synthesis towards compound **5**

Entry	Equiv. $Pd(OAc)_2$	Equiv.P^tBu_3	Equiv. NaO^tBu	Temp (°C)	Time(h)	Yield(%)
1	4 mol%	5 mol%	5	160	3	-
2	4 mol%	5 mol%	5	170	3	-
3	4 mol%	5 mol%	5	170	24	-

As observed in **table 12**, entry *2* and *3* did not afford the desired compound **5** by increasing the reaction temperature. The reaction was attempted but with increased temperature such that the reactivity would increase. However, GC-analysis verified no indication of conversion towards compound **5**.

The last attempt in synthesising compound **5** from **3** and **4** was performed in a Schlenk flask. It was observable that the ligand and base were very sensitive towards moisture. Therefore,

preliminary conditions were met upon handling the reaction. A Schlenk flask was used and the reaction was continuously stored in argon atmosphere to avoid contact with moisture. The reaction time was also increased to 24 hours. Additionally, the order of adding the precursors were different compared to previous attempts. A colour change in $Pd(OAc)_2$ (light yellow / dark orange to dark brown), was seen when the ligand and palladium-catalyst were added in a separate vial. This meant that the catalyst was activated. Then, the two starting materials were left to stir under vacuum in another separate vial. Starting materials **3** and **4** were added in the Schlenk flask containing the activated catalyst and lastly, the base was added under continuous argon flow. Unfortunately, this did not yield towards desired product **5**.

Lower equivalents of ligand could be argued as a reason for the non-successful synthesis towards compound **5**. The attempted synthesis towards desired compound **5** where performed according to the procedure written by the *Bedford et al.*[98,99]Therefore, the equivalents of ligand were 5 mol% when re-producing the procedure but only with substrate **3** and **4**. However, the authors had also used between 1.25 and 1.4 equivalents of ligand and had a successful synthesis towards their desired product.[98] Hence, increasing the equivalents of ligand may have increased the active palladium catalyst.

Furthermore, the provided results for *Bedford et al* were achievable because they had electron-withdrawing substituents on the aniline. Multiple articles have conducted Buchwald-Hartwig amination and used substrates that have either electron-donating or electron-withdrawing groups. The afforded yields were higher in presence of electron-withdrawing and / or electron-donating groups.[100-103] *Bedford et al* used substrates containing methoxy group which is an electron withdrawing substituent. The haloaniline presented here does not have an electron-withdrawing group nor electron donating groups. Consequently, poor electron density may have affected the synthesis towards compound **5**.

5. Summary and Further Work

5.1 Summary

The aim of this project was to synthesise the potential pharmacophore of the natural compound avrainvillamide. Avrainvillamide has proven to be a drug which can be used in the treatment towards AML. *Herzon et al* and *Baran and co*. attempted to synthesis avrainvillamide and were successful in synthesising the natural compound. However, many obstacles were met upon their synthetic pathway and contained many steps as well. Thereby, a shorter synthetic pathway leading towards the potential pharmacophore of avrainvillamide was designed and exercised towards the desired compound **5**.

The synthetic route towards the desired compound 3,3-dimethyl, 8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one (**5**), was designed in an overall of 7 steps. The Beckmann rearrangement was a two-step synthesis from 1-indanone (**1**) where an experimental design was utilized to increase the yield towards the desired compound 3,4-dihydroquinolin-2(*1H*)-one (**2a**). Then, commercially purchased **2a** was used to halogenate the compound by using DIH. This gave product **3**. The desired substrate **4** was synthesised through a four-step synthesis and afforded the product in excellent yields. Lastly, the final step was attempted through a one-pot C-N coupling and C-H activation synthesis by using a palladium catalyst (Pd_2OAc), ligand (P^tBu_3), base (NaO^tBu) and solvent (toluene). Unfortunately, the observed results showed no indication towards compound **5**.

Some of the synthetic steps were optimised to give better yield. The first attempted optimization was conducted on the Beckmann rearrangement of 1-indanone oxime (**10**). This was performed through statistical experimental design. Two sets of statistical experimental designs were generated, where the first design afforded yields in the range between 4-29% (see **table 3**). Investigations on the two-factor interactions of the variables led to a second design which was an extrapolation of the first. The second generated statistical experimental design afforded the yields 5-31% (see **table 4**) A remarkable difference was seen in conversion and selectivity towards compound **2a**, when comparing the first and the second statistical experimental design. Furthermore, the first observations in the synthesis of compound **8** from **7** and **11**, provided low to nothing in yield. However, the synthesis towards compound **8** was optimised through a

Finkelstein reaction to gain a better leaving group (iodine instead of chloride as leaving group). This presented a yield of 80 %.

5.2 Further work

The core focus of this study is the synthesis of 3,3-dimethyl, 8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one (**5**). As the attempted synthetic strategy was not successful, other synthetic approaches would be interesting to consider. The Buchwald-Hartwig C-N formation could be studied thoroughly by performing a screening test with different palladium catalysts and ligands to see if it could have affected the synthesis towards **5**.

Another important and interesting study is the statistical experimental design of the Beckmann rearrangement of 1-indanone oxime (**10**). The second statistical experimental design was conducted based on the two-factor interactions from the first statistical experimental design. Therefore, another statistical experimental design dependent on the three factor interactions would be interesting to examine.

Lastly, an optimization on the ring closure step from **8** to **9** could also be recognized. It would be appropriate to consider other reagents for the synthesis towards compound **9**. This may also lead to milder reaction conditions compared to the method described in the experimental procedure.

6. Experimental

6.1 General methods

Chemicals

Some of the chemicals that were used for the synthetic route towards compound **5**, was purchased commercially. Several synthetic steps utilised chemicals that were already in store, such as the inorganic compound, hydrosulfuric acid (H_2SO_4), hydrogen chloride (HCl), butylated hydroxytoluene (BHT), 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) and 1,3-diiodo-5,5-dimethylhydantoin (DIH).

Experimental description

TLC analysis were performed using aluminium foils with fluoroscent indicator at 254 nm. The mobile phase consisted of hexane and ethylacetate in different mixtures.

Automated reverse-phase flash chromatography was performed utilising the instrument PuriFlash XS 420, Biotage Sfär (C18), Duo 100 Å 30 μm, 12g.

Spectroscopic descriptions

The obtained NMR spectra was conducted on a Bruker BioSpin AV500 (^{1}H 500 MHz, ^{13}C 125 MHz) and BioSpin AVANCE NEO (^{1}H 600 MHz, ^{13}C 151 MHz). Chemical shifts are given in parts per million (ppm) for the signals that were achieved in the deuterated solvents. Multiplicity is described as singlet (s), doublet (d), triplet (t), double doublet (dd) or as multiplet (m). The couplings constants are given in Hz.

Analytical HPLC was performed using Agilent 1260 Infinity II which has a Quadrupole LC-MS.

Automated reverse-phase flash chromatography was conducted using PuriFlash XS 420 equipped with UV diode array detector.

GC-MS analysis was conducted by using an instrument with capillary gas chromatograph which is has silica column and helium as carrier gas. A mass spectrometer is connected to the chromatography and uses electron ionisation (EI) as ionisation source.

MS and LC-(UV) MS were performed by using Agilent 6420A triple Quadrupole mass analyser fused with electrospray ionisation (ESI). The instrument is attached to an Agilent 1200 series LC module which consists of a binary pump, autosampler and column compartment/oven.

6.2 Experimental Procedure

6.2.1 Synthesis of 1-indanone oxime (10)

Hydroxylamine hydrochloride (0.78 g, 13.77 mmol) in 3 mL of water was dissolved in 1-indanone (**1**, 0.99 g, 7.58 mmol) in 20 mL MeOH in a 100 mL flask. The reaction flask was then immersed in an ice bath containing a temperature between -5 - 0^{o}C. 4 M NaOH (11.41 mL, 45.63 mmol) was added dropwise to the stirred mixture. The cooling bath was then removed after 5 minutes and set to reflux for 20 minutes. It was then quenched by 70 mL of H_2O and the yellow coloured mixture was extracted with EtOAc (4 X 20 mL) and were dried with $Na_2S_2O_4$. The isolated product gave white flakes 0.90g (90%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 8.87 (s, 1H), 7.47 (dd, J = 5 Hz, J = 10 Hz, 1H), 6.59 (d, J = 5 Hz 1H), 2.93 (t, J = 15 Hz, 2H), 2.62 (t, J = 10 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125MHz) δ_C (ppm) 171.8, 137.2, 136.8, 136.5, 126.2, 117.5, 85.9, 30.5, 25.1. MS (m/z) $[M]^+$ calcd for C_9H_9NO = 147.07; found = 148.0

6.2.2 General procedure for the synthesis of 3,4-dihydroquinolin-2(*1H*)-one (2a and 2b)

PPA (10.00-35.00 equiv.) was added in a microwave vial containing (1.00 equiv) of 1-indanone oxime (**10**). The microwave reactor tube was sealed and heated at a temperature (115-150^{o}C) and left to stir over a timespan (0.5 – 36 h). The reaction was then cooled down and quenched with 20 mL ice H_2O and extracted with DCM (3x 20 mL). The combined organic phases were dried over $Na_2S_2O_4$ and concentrated under pressure to give a dark yellow-brown oil containing 3,4-dihydroquinolin-2(*1H*)-one (**2a** and **2b**) (see **table 3** and **4** for the afforded yields).

^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 7.17 (d, J = 5 Hz, 2H), 6.99 (t, J = 15 Hz, 1H), 6.74 (d, J = 5 Hz 1H), 2.97 (t, J = 15 Hz, 2H), 2.64 (t, J = 10 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125MHz) δ_C (ppm) 132.7, 128.5, 127.7, 127.5, 40.7, 28.7.

6.2.3 Synthesis of 6-iodo-3,4-dihydroquinolin-2(*1H*)-one (3)

Commercially purchased 3,4-dihydroquinolin-2(1H)-one (**2a**, 0.101 g, 0.679 mmol) and DIH (0.257 g, 0.679 mmol) was added in a 25mL round bottom flask equipped with magnetic stirrer bar. The flask was immersed into an ice-water cooling bath. H_2O (1.2 mL) was added and the reaction slurry was left to for 3 minutes. Then 3 drops of H_2SO_4 were added and the brown reaction mixture was left to run at room temperature for 24 hours. It was quenched slowly with NaOH (7.4 mL) and colour changed from brown to yellow. The post-reaction was cooled down to below room temperature before proceeding. It was poured to a 50 mL beaker and the reaction flask washed carefully with 7 mL of water. 12 mL of acetic acid and water (50% / 50%) was added slowly to the mixture until pH reached 6 and precipitation was seen. It was cooled in an ice bath and the crude was filtrated and obtained as pale-yellow / white solids (0.18g, 98%).

^{1}H NMR ($DMSO_6$, 600 MHz) δ_H (ppm) 8.15 (s, 1H), 7.49 (s, 1H), 7.48 (dd, J = 6 Hz, J = 6 Hz 1H), 6.55 (d, J = 6 Hz 1H), 2.94 (t, J = 18 Hz, 2H), 2.62 (t, J = 12 Hz, 2H). ^{13}C NMR ($DMSO_6$, 151 MHz) δ_C (ppm) 171.8, 137.2, 136.8, 136.5, 126.2, 117.5, 85.9, 30.5, 25.1. MS (m/z) $[M+23]^+$ calcd for C_9H_8INO = 295.0; found = 295.9

6.2.4 Synthesis of 4-iodo-3-nitrophenol (7)

Commercially purchased 4-amino-3-nitrophenol (**6**, 5.28g, 34.26mmol), concentrated HCl (12 mL) and H_2O (2 mL) was added in a 150-mL round bottom flask and stirred mechanically. A solution of $NaNO_2$ (4.76g, 68.98mmol) in H_2O (12mL) was then added dropwise to the mixture. Then a solution of KI (11.44, 69.98mmol) in H_2O (12mL) was added dropwise over 30 minutes at 0^{o}C. The reaction was left to run at room temperature overnight. The precipitated crude was collected by filtration and washed with cold water and dried under vacuum to give brown solids (6.69g, 25.24mmol, 79%). R_f = 0.37 (4:1 hexane/ethyl acetate).

^{1}H NMR (DMSO, 600 MHz) δ_H (ppm) 10.59 (s, 1H), 7.84 (d, J=12 Hz, 1H), 7.31 (d, J = 0 Hz, 1H, H2), 6.87 (dd, J = 6 Hz, J = 12 Hz, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ_C (ppm) 158.2, 153.8, 141.6, 121.6, 112.1, 73.4. MS (m/z) $[M]^-$ calcd for $C_6H_3INO_3$ = 264.9; found = 263.9

6.2.5 Synthesis of 1-iodo-4-((2-methylbut-3-yn-2-yl)oxy)-2-nitrobenzene (8)

3-Chloro-3-methyl-1-butyne (0.85mL, 7.55mmol) and KI (2.54g, 15.00mmol) were added in a microwave vial and left to stir for 1 hour. 4-iodo-3-nitrophenol (**7**, 1.01 g, 3.77mmol) and DBU (1.00 mL, 7.55mmol) was added in a 50mL round bottom flask and left to stir for 1 hour. The

solution containing 4-iodo-3-nitrophenol and DBU was then added in the microwave vial, sealed and left to stir at 80ºC for 48 hours. The post-reaction was cooled to room temperature and then dilutes with EtOAc (30 mL). Then the solution was washed with $NaHCO_3$ (10 mL) and saturated NaCl (10 mL). The organic layers were dried over $MgSO_4$ and concentrated. The residue was dissolved in EtOAc (10 mL) and filtered through a pad of Celite, eluting with ethyl acetate (50 mL). The eluent was concentrated under pressure and the product gave a brown oil (0.968, 78%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 7.88 (d, J=5 Hz, 1H), 7.77 (d, J = 0 Hz, 1 Hz), 7.14 (dd, J = 5 Hz, J = 10 Hz, 1H), 2.67 (s, 1H), 1.68 (s, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 156.5, 153.2, 141.9, 126.0, 117.7, 84.5, 75.8, 73.7, 34.2, 29.6, 19.9.

6.2.6 Synthesis of 6-iodo-2,2-dimethyl-5-nitro-2H-chromene (9)

1-Iodo-4-((2-methylbut-3-yn-2-yl)oxy)-2-nitrobenzene (**8**, 0.97 g, 2.92 mmol), BHT (0.03g, 0.15 mmol), and p-xylene (30 mL) were transferred to a microwave reactor tube. The reactor tube was sealed and heated at 140ºC and stirred for 48 h. The reaction mixture was then cooled down to 22 ºC. The post-reaction mixture was filtered through a pad of silica gel (5 cm) placed on a Buchner funnel. The post-reaction mixture was eluted with hexane (30 mL) and then with EtOAc / hexane (2:8, 30 mL). The eluent was concentrated under reduced pressure and purified by reverse auto-flash column chromatography to afford a yellow oil 6-iodo-2,2-dimethyl-5-nitro-2*H*-chromene (0.30 g, 30%).

^{1}H NMR ($CDCl_3$, 600 MHz) δ_H (ppm) 7.24 (d, J = 6 Hz, 1H), 6.38 (d, J = 6 Hz, 1H), 5.88 (d, J = 12 Hz, 1H), 5.50 (d, J = 11 Hz, 1H), 1.16 (s, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz) δ_C (ppm) 153.6, 139.1, 134.6, 120.1, 115.6, 115.2, 76.9, 76.7, 72.9, 27.7.

6.2.7 Synthesis of 6-iodo-2,2-dimethyl-2*H*-chromen-5-amine (4)

6-iodo-2,2-dimethyk-5-nitro-2H-chromene (**9**, 0.041 g, 0.124 mmol), Fe (0.050 g, 0.895 mmol) and NH_4Cl (0.032g, 0.582 mmol) were added in a 50 mL round bottom flask equipped with a magnet. To the stirred solution, EtOH (0.6 mL) and H_2O (0.6 mL) was added. The reaction was heated to 75ºC and left to stir for 2 hours. The solution was then cooled and filtered through Celite to remove iron. The reaction flask was washed with water to remove the iron. Then, it was extracted with EtOAc (3 x 20mL) and dried over $MgSO_4$. It was concentrated under reduced pressure to give a brown oil (0.035g, 94%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 7.37 (d, J=10 Hz, 1H), 6.35 (d, J =10 Hz, 1H), 6.11 (d, J = 10 Hz, 1H), 5.61 (d, J = 10 Hz, 1H), 1.40 (s, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 154.3, 142.4, 138.7, 130.3, 117.6, 110.0, 108.7, 75.6, 75.0, 27.7. MS (m/z) $[M]^+$ calcd for $C_{11}H_{12}INO$ = 301.0; found = 302.0

6.2.8 Attempted synthesis of 3,3-dimethyl,8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one (5)

Method A:

NaO^tBu (0.154 g, 1.630 mmol), $Pd(OAc)_2$ (2.546 g, 10.892 μmol) and P^tBu_3 (0.013μL, 3.741 μmol) was added in a 10 mL microwave vial and sealed with a tube. Then, starting material **3** (0.082 g, 0.300 mmol), reagent **4** (0.068 g, 0.226 mmol) and toluene (2.11 mL) were added to the microwave vial. The reaction was heated in the microwave reactor at 160 ^oC for 3 hours. It was then cooled down and quenched with a HCl solution (2M, 2.11 mL). The post reaction was extracted with DCM (3 x 20 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford a brown solid which did not contain the desired compound 3,3-dimethyl, 8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one (0.051 g) (**5**).

Method B:

$Pd(OAc)_2$ (0.006 g, 0.279 mmol) was added in a Schlenk flask and put under vacuum. NaOtBu (0.034 g, 0.345 mmol) was added in a beaker and put in heating oven to be fully dried before used. P^tBu_3 (0.013 μL, 4.180 μmol) was added in the Schlenk flask containing $Pd(OAc)_2$ under continuous argon flow. Then toluene (0.6 mL) was added to the catalyst mixture and left to stir for 30-40 minutes to be activated. The activation was observed as the colour changed from orange to light yellow colour. Meanwhile, precursor **3** (0.019 g, 0.198 mmol) and precursor **4** (0.021 g, 0.697 mmol) was added in a separate vial where toluene (2 mL) was added and left to stir under vacuum for 15 - 20 minutes. The precursor mixture was added in the Schlenk flask containing the activated palladium catalyst. Lastly, NaOtBu (0.034 g, 0.345 mmol) was transferred to the Schlenk flask. The reaction mixture was heated at 170^oC for 24 hours, then cooled down and quenched with 2M HCl (2.60 mL). The post reaction was extracted with DCM (3x10mL), the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The afford crude was a brown oil which did not contain the desired compound

3,3-dimethyl,8, 10, 11, 13-tetrahydropyrano[3,4-*a*]pyrido[2,3-*h*]carbazole-9(3*H*)-one (0.0012g) (**5**).

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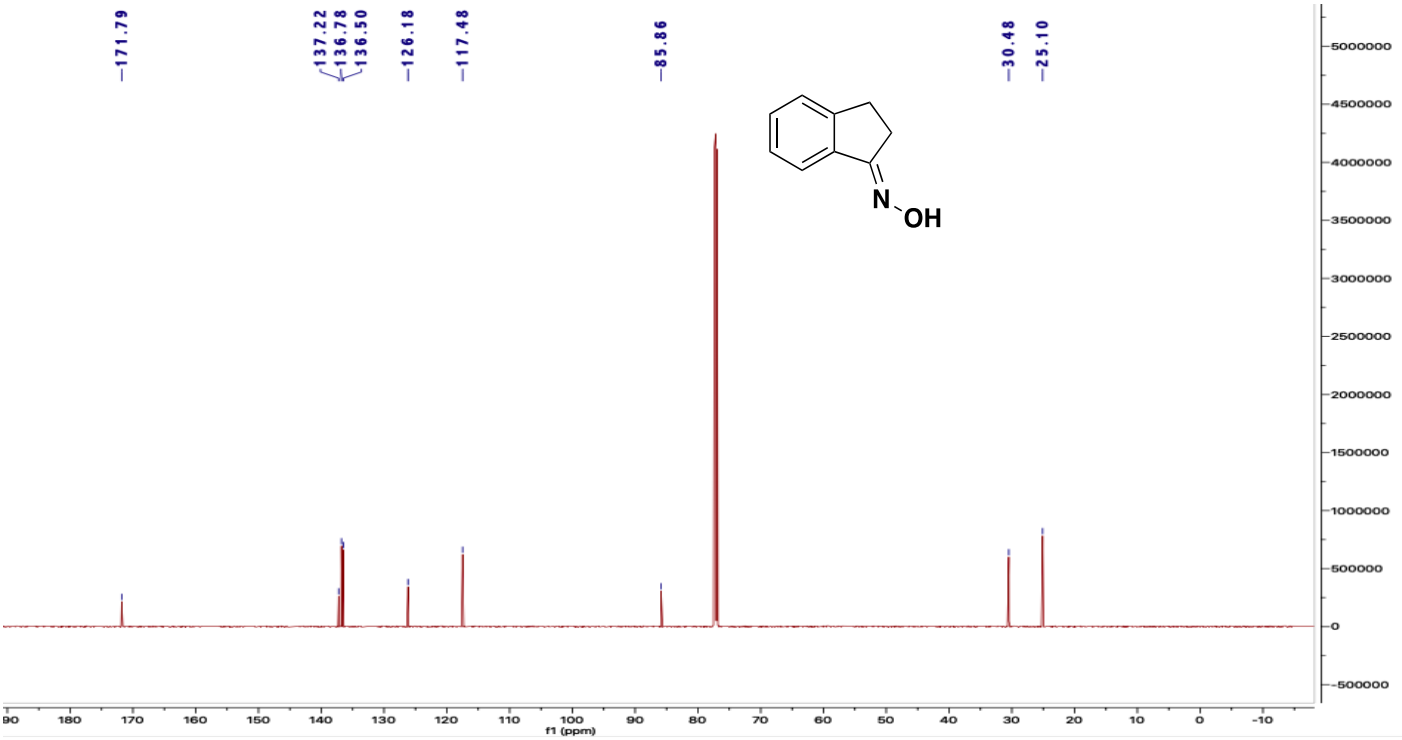
Appendix

Appendix 1

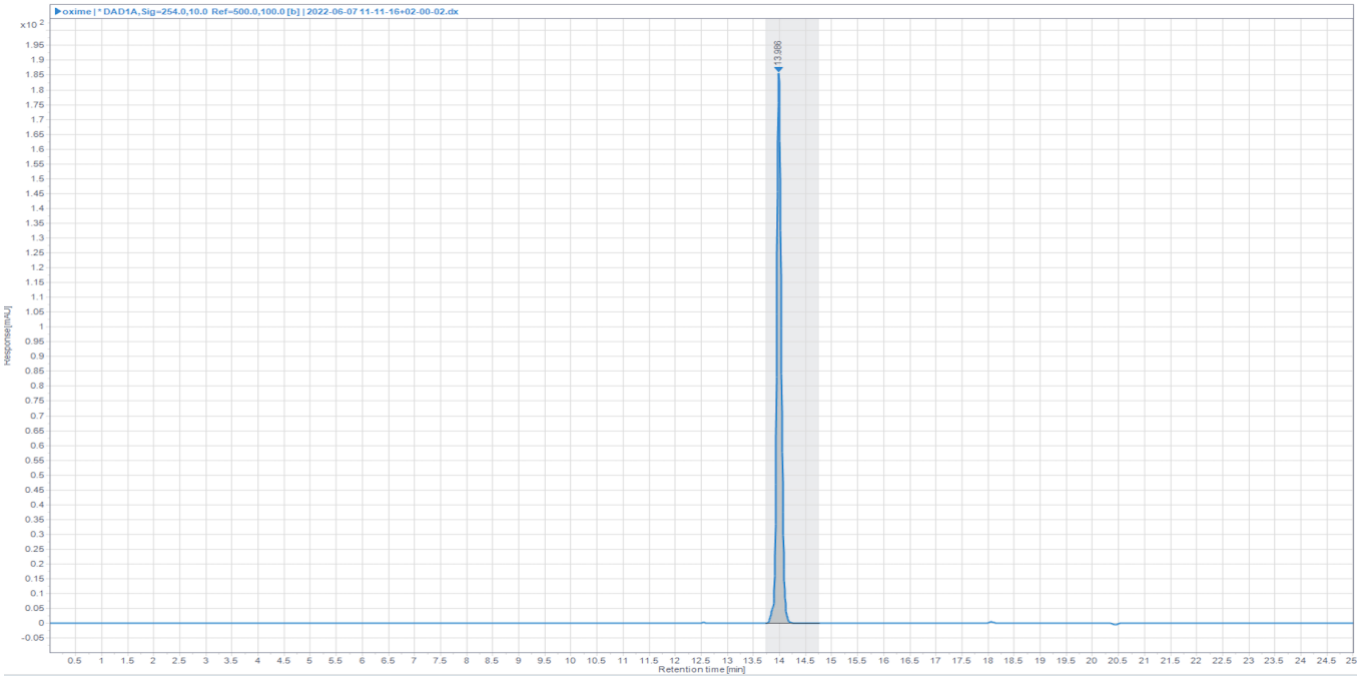
1-indanone oxime (10)

^{1}H NMR spectrum of 10

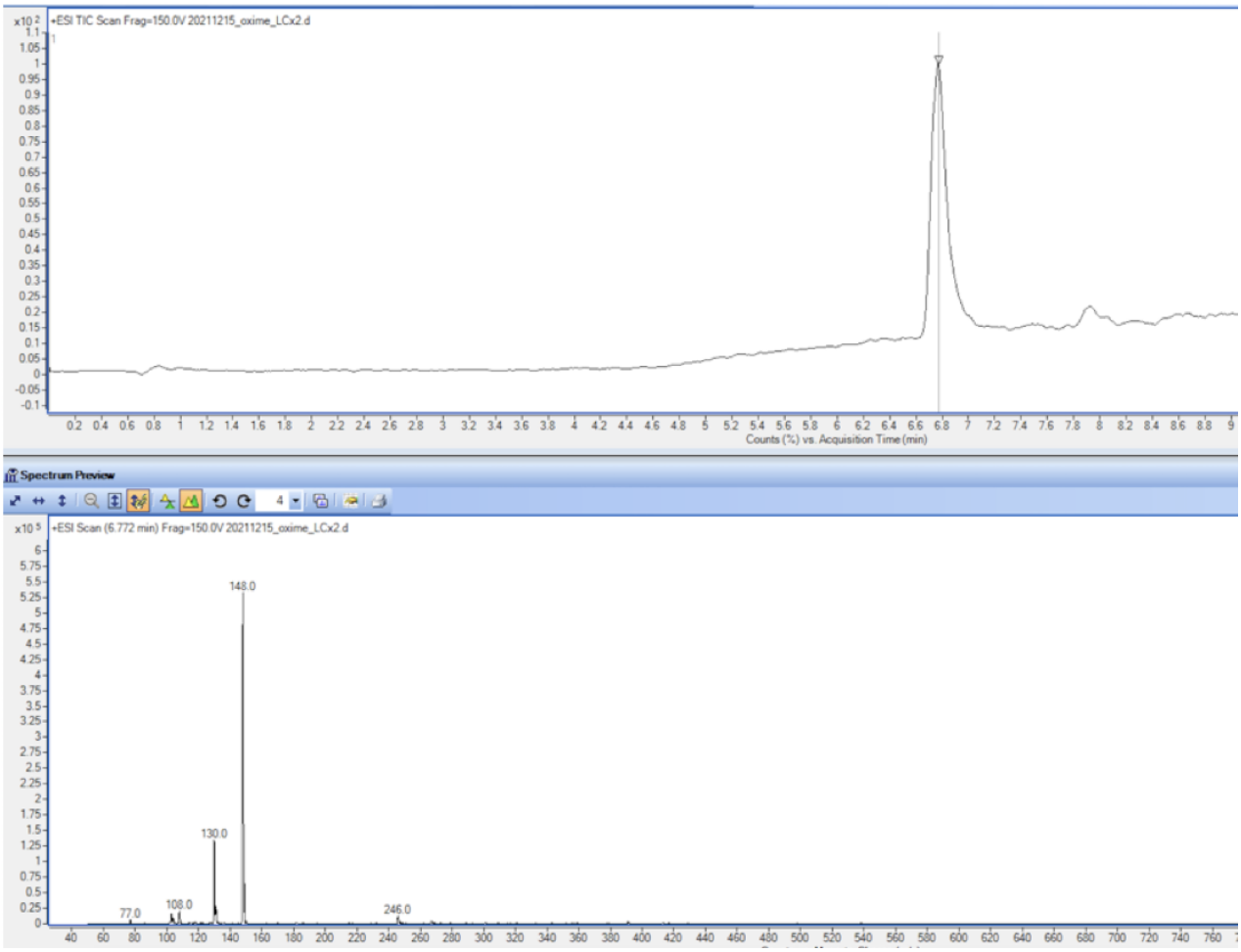
^{13}C NMR spectrum of 10



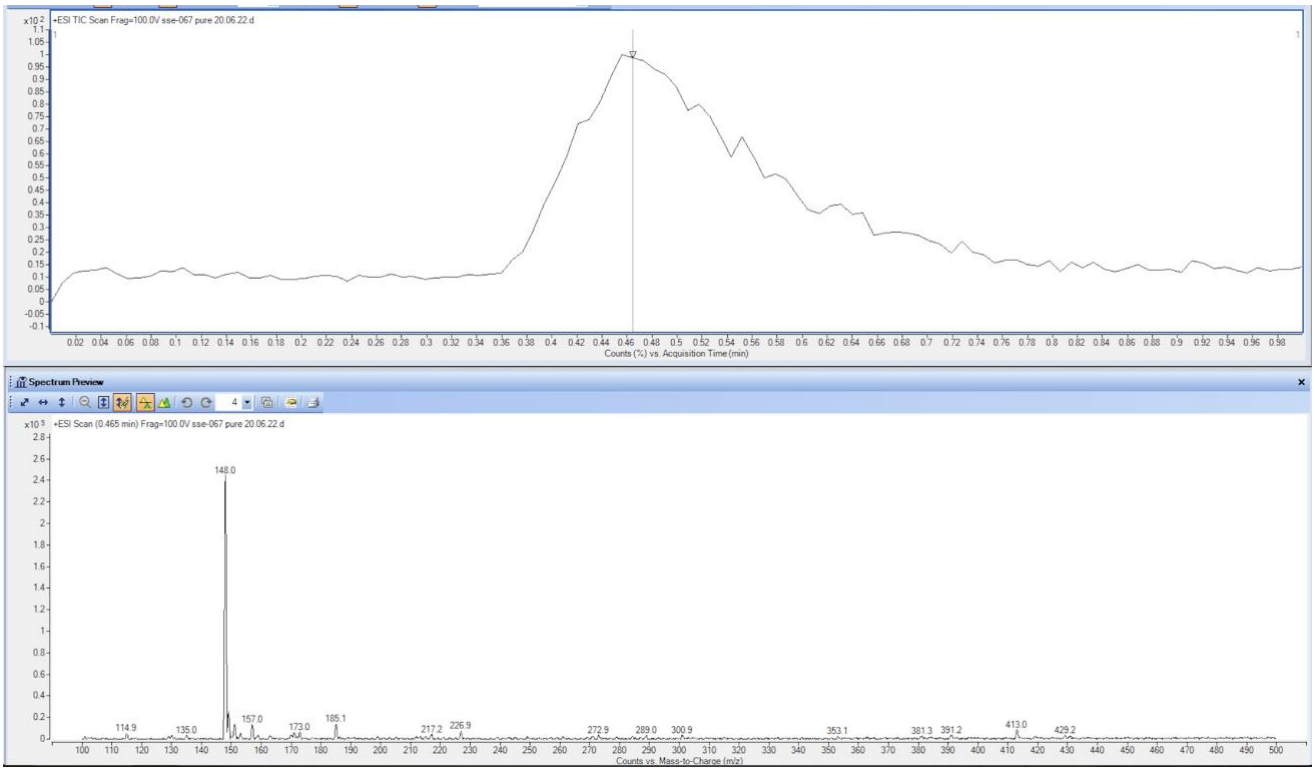
HPLC chromatogram of 10



LC-UV MS OF 10


+ESI TIC Scan Frag=150.0V 20211215_oxime_LCx2.d
Counts (%) vs. Acquisition Time (min)
Spectrum Preview
+ESI Scan (6.772 min) Frag=150.0V 20211215_oxime_LCx2.d
148.0
130.0
108.0
77.0
246.0

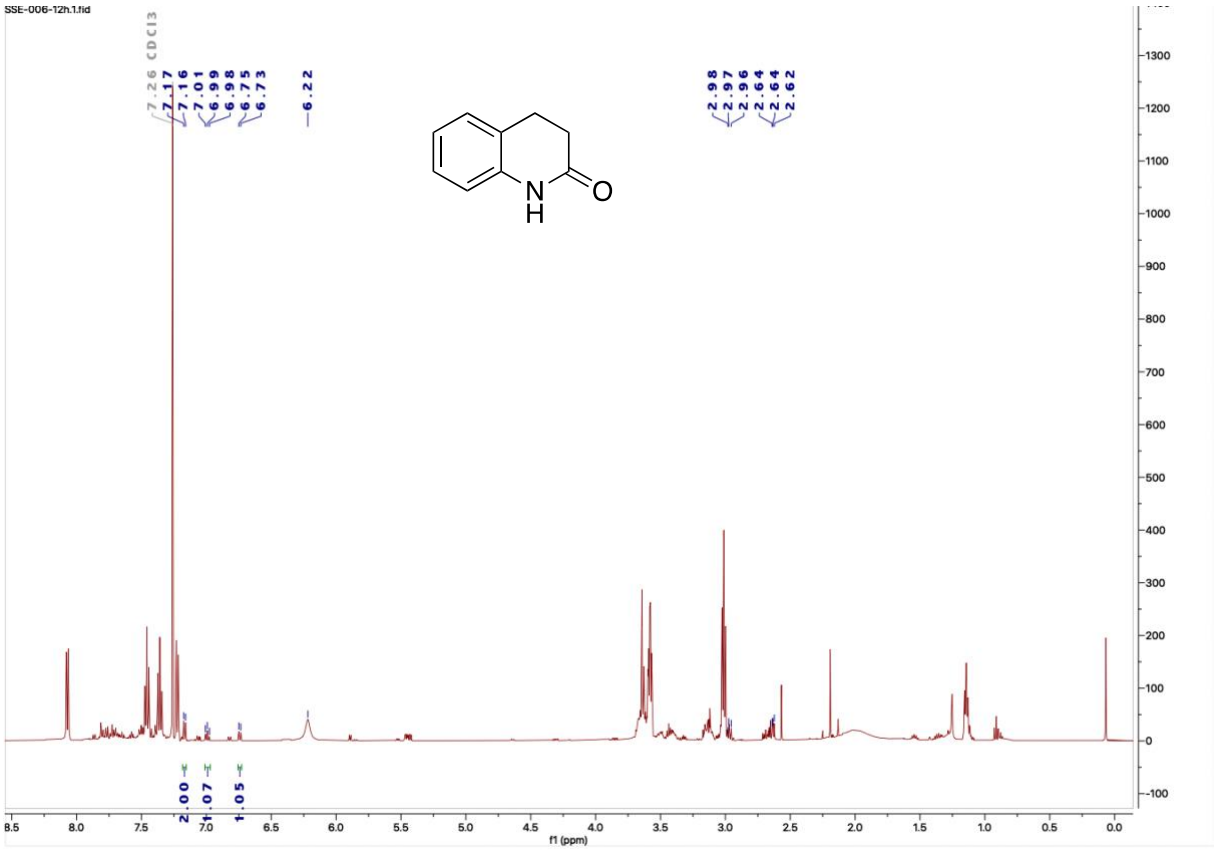
MS spectrum of 10



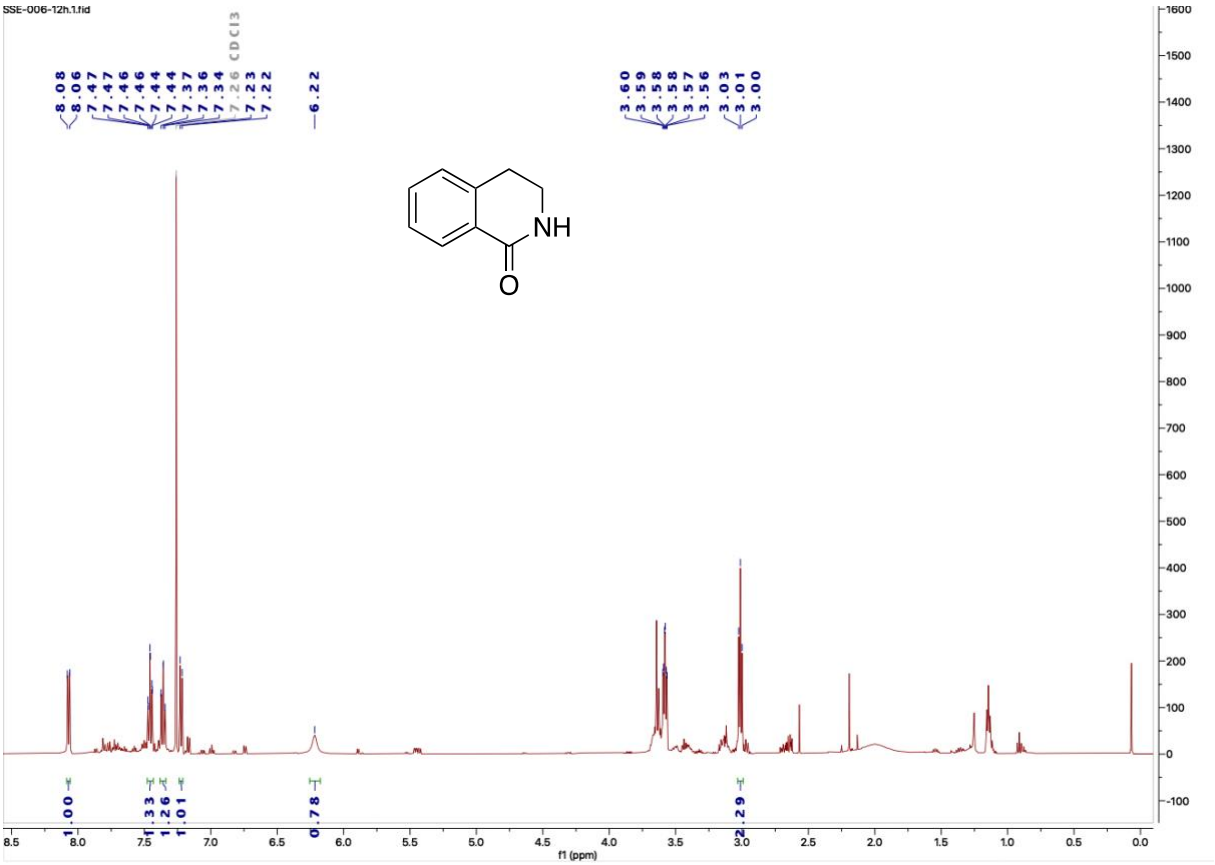
Appendix 2

3,4-dihydroquinolin-2(*1H*)-one (2a and 2b)

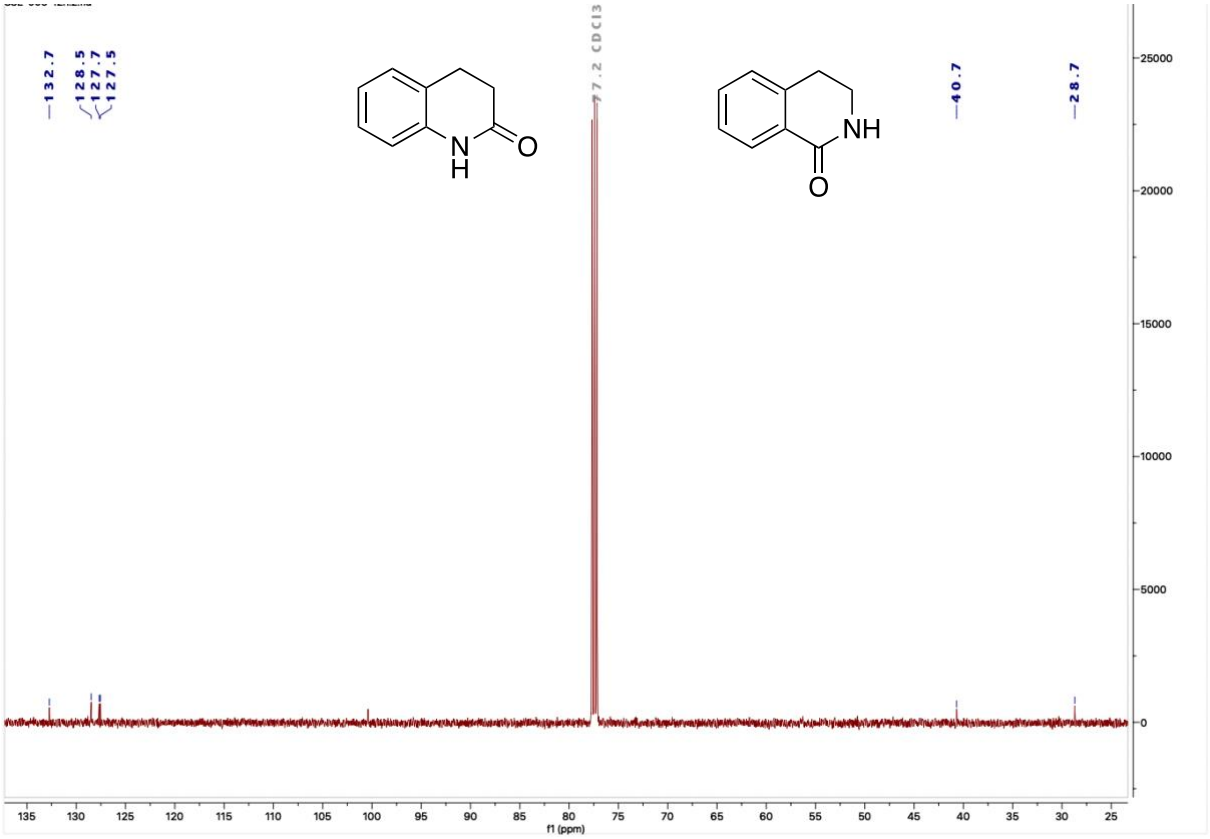
^{1}H NMR spectrum of 2a and 2b, focusing on 2a



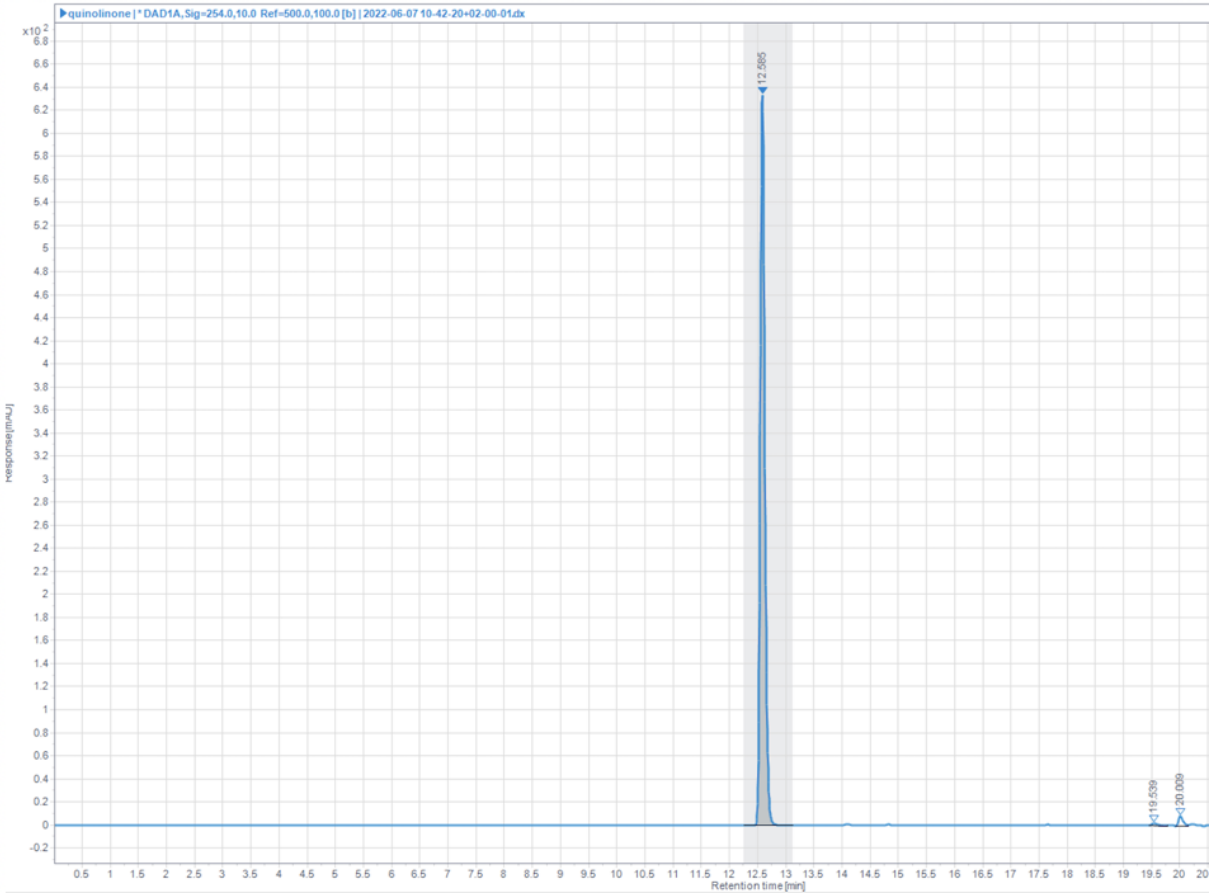
^{1}H NMR spectrum of 2a and 2b, focusing on 2b



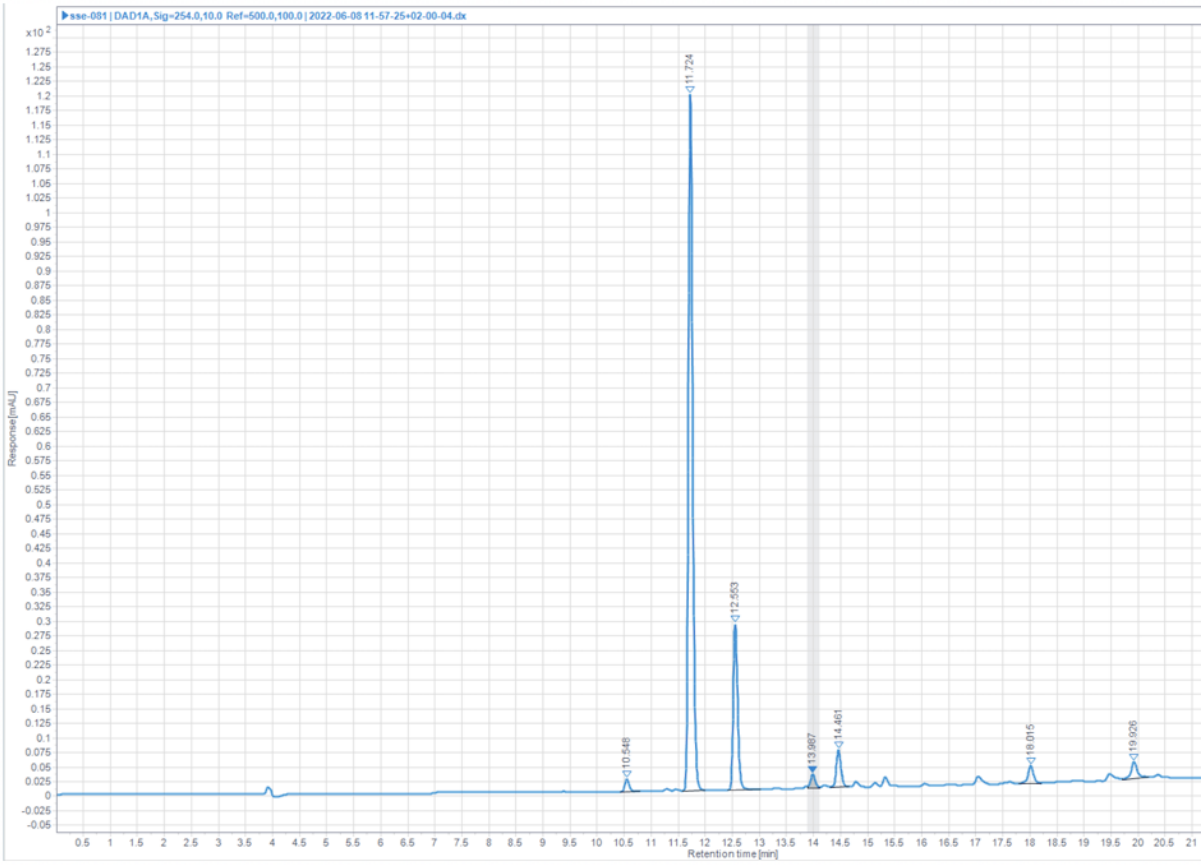
^{13}C NMR spectrum of 2a and 2b



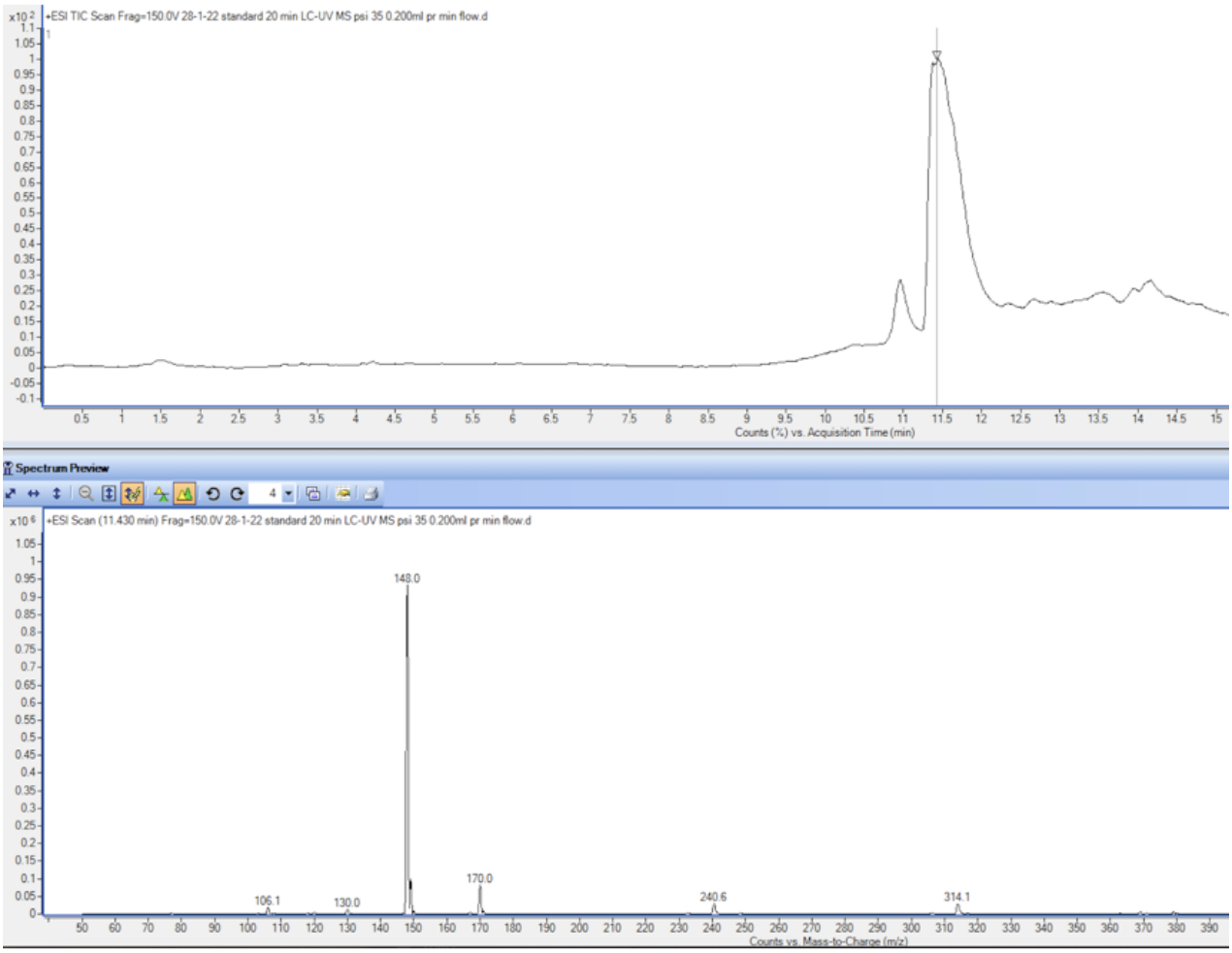
HPLC chromatogram of commercially purchased 2a



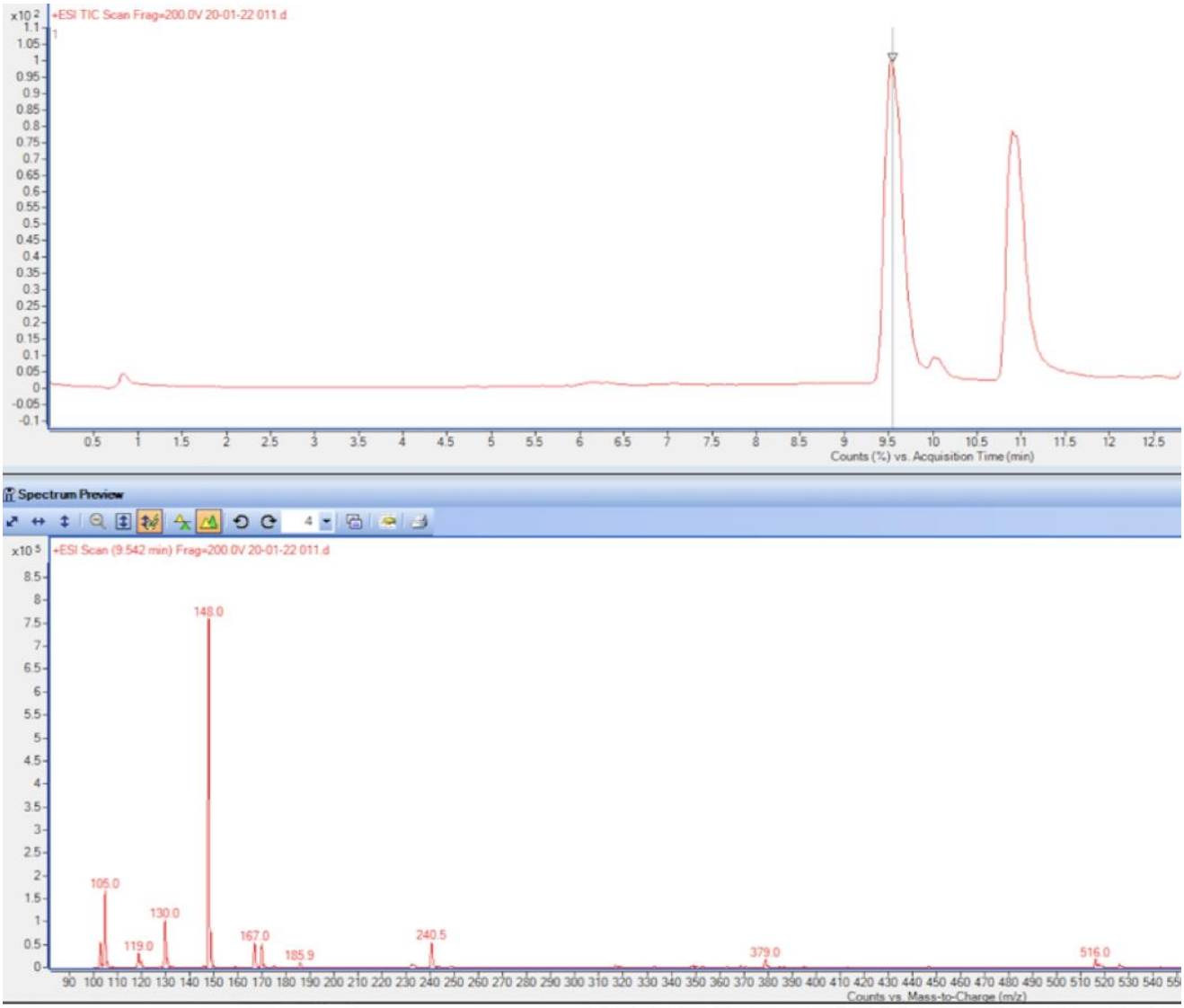
HPLC chromatogram of 2a, 2b and 10



LC-UV MS spectrum of commercially purchased 2a



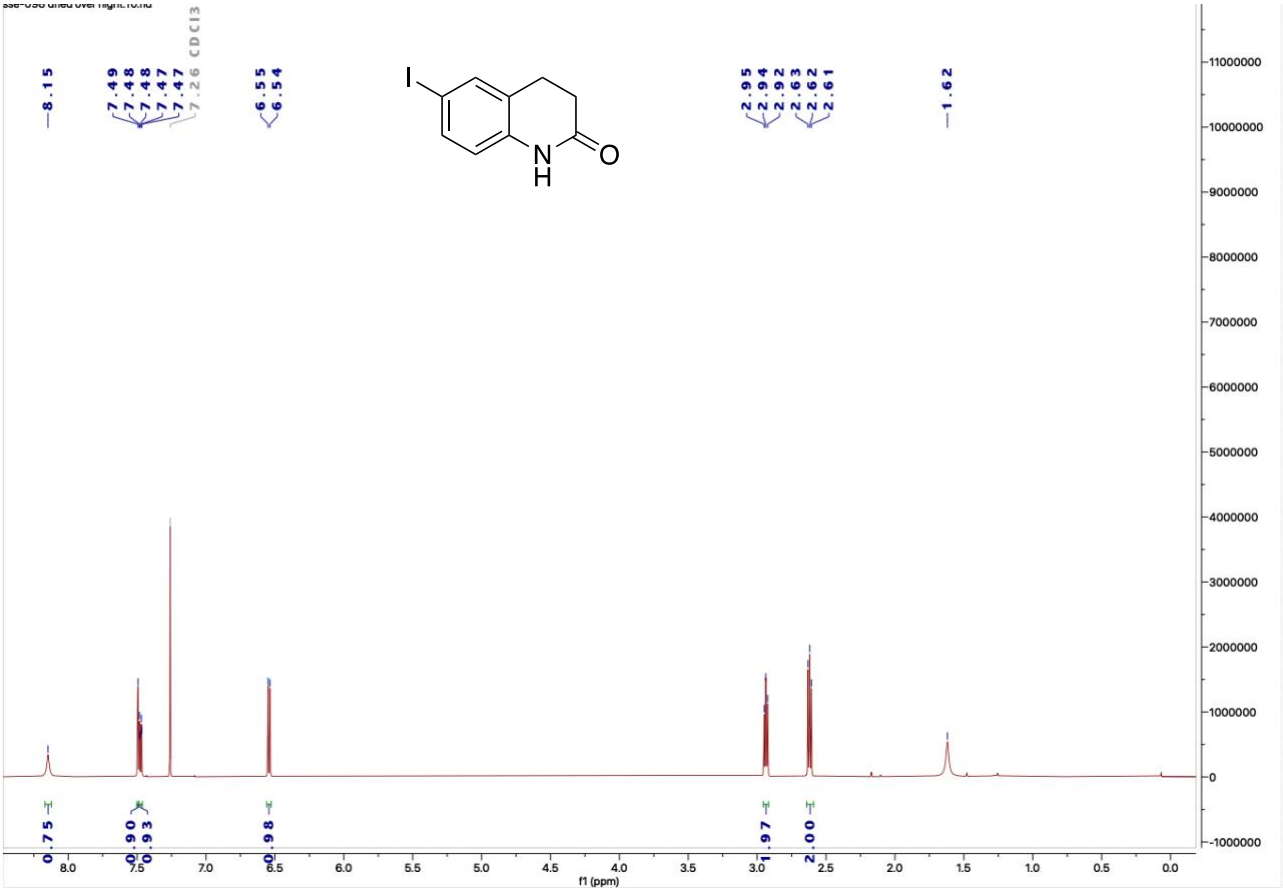
LC-UV MS spectrum o 2a and 2b



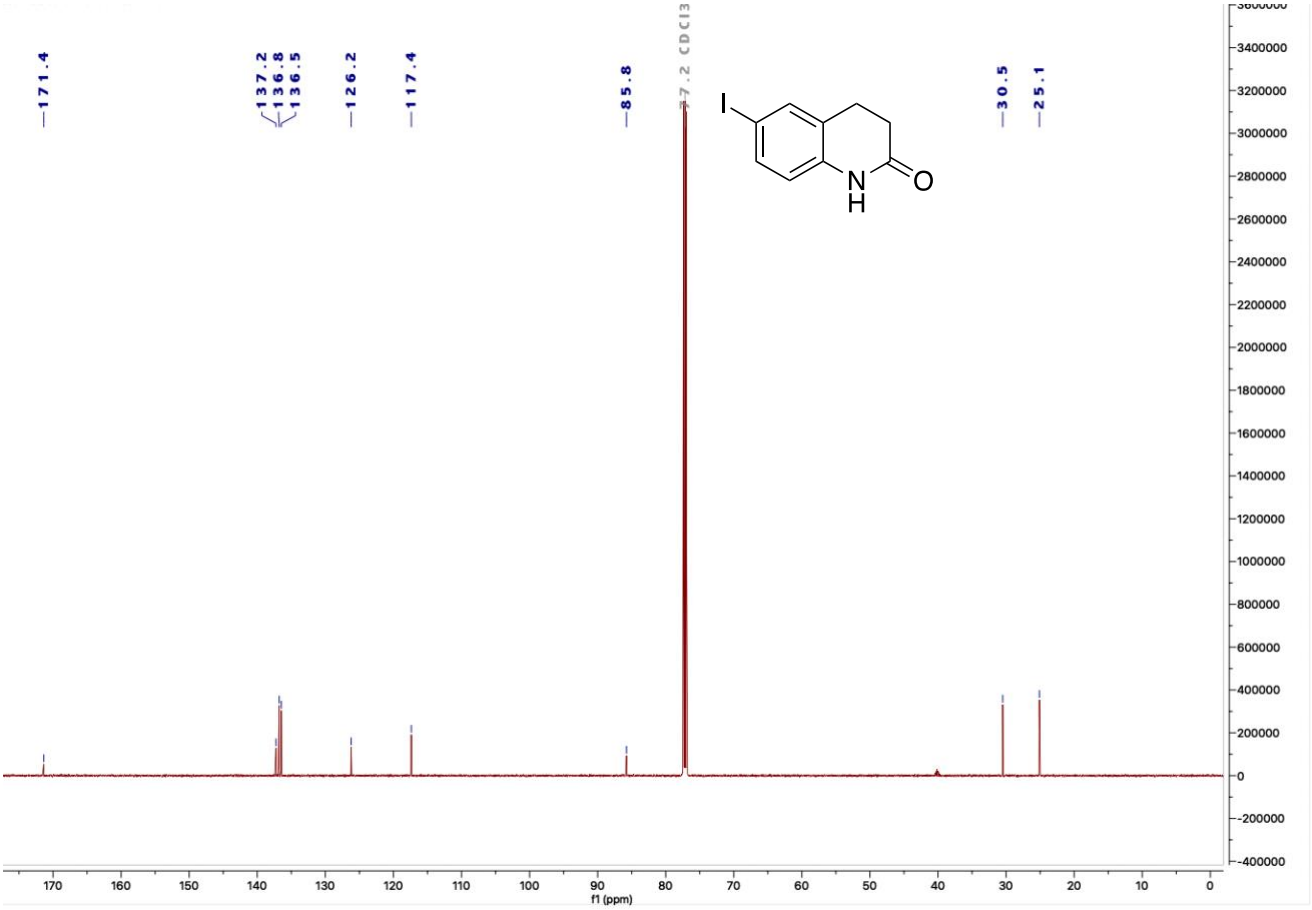
Appendix 3

6-iodo-3,4-dihydroquinolin-2(1H)-one (3)

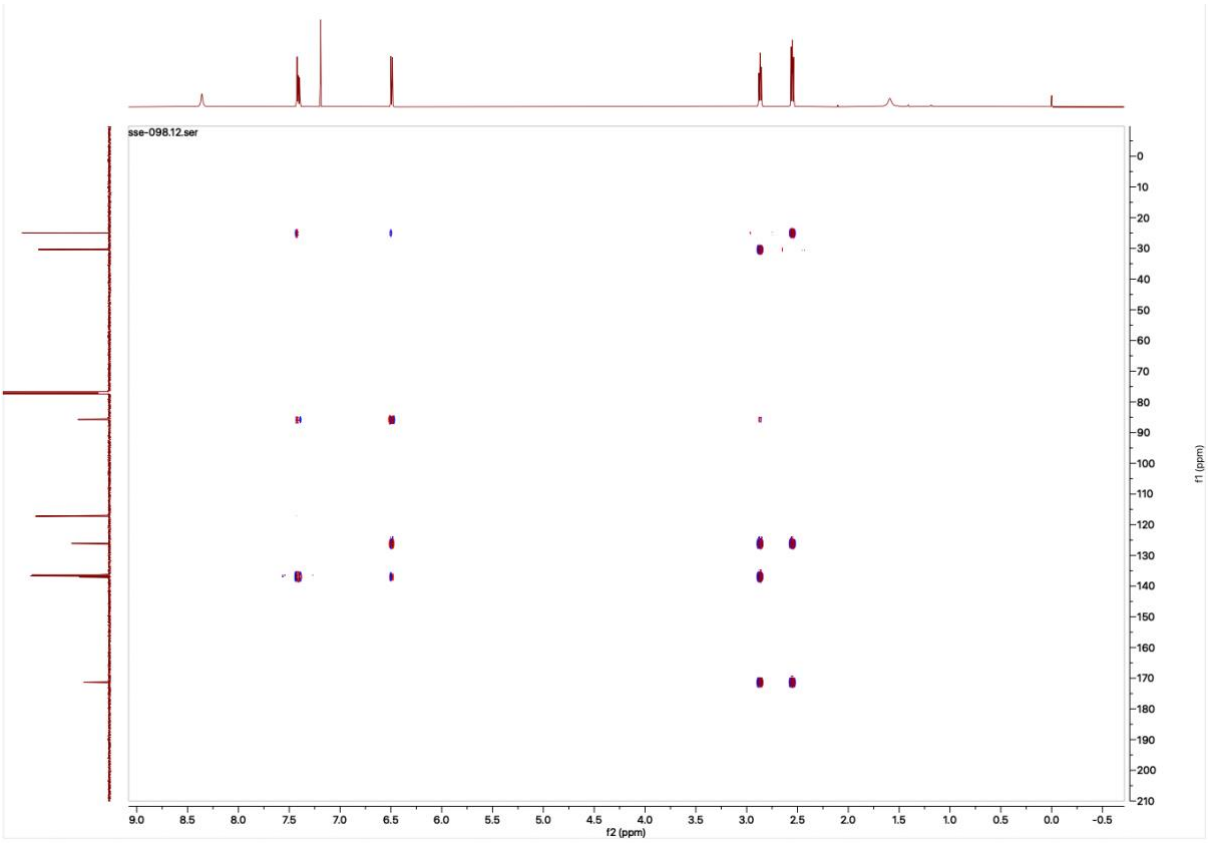
^{1}H NMR spectrum of 3



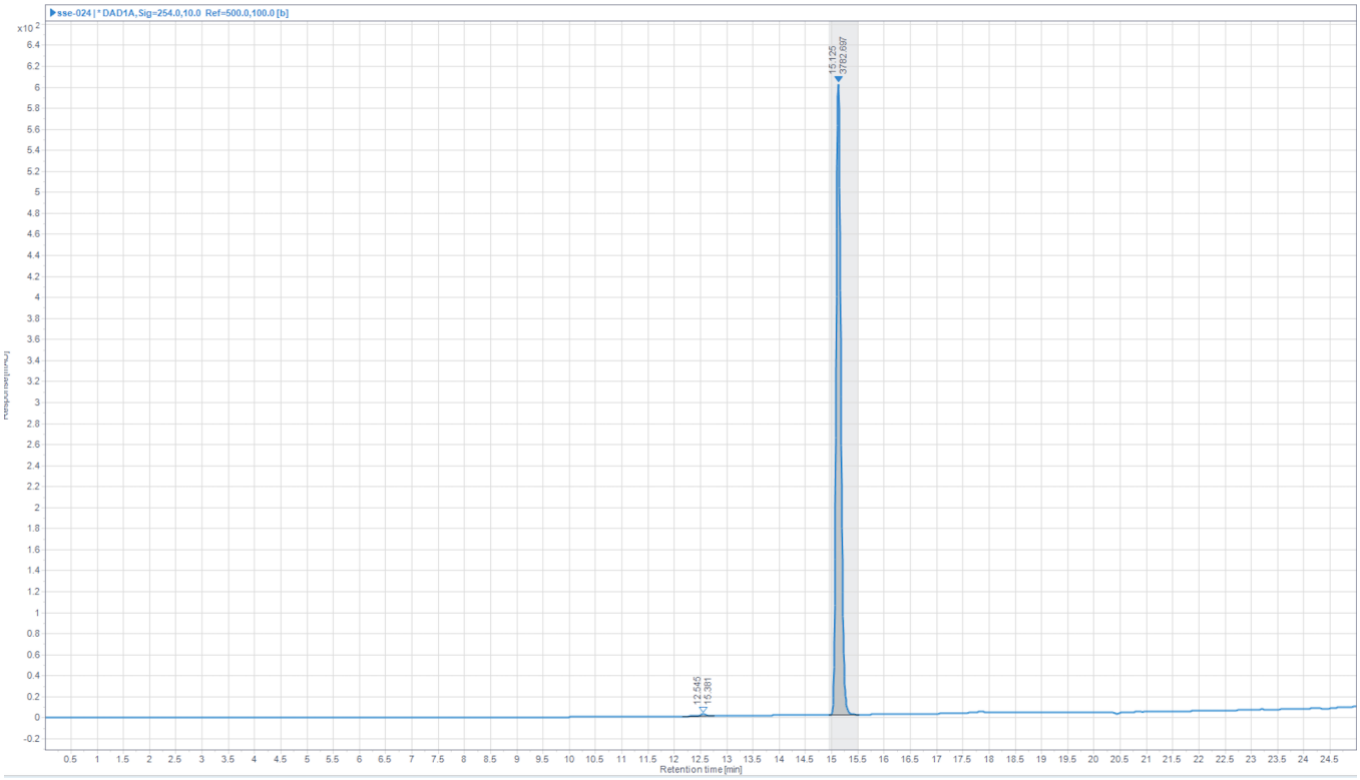
^{13}C NMR spectrum of 3



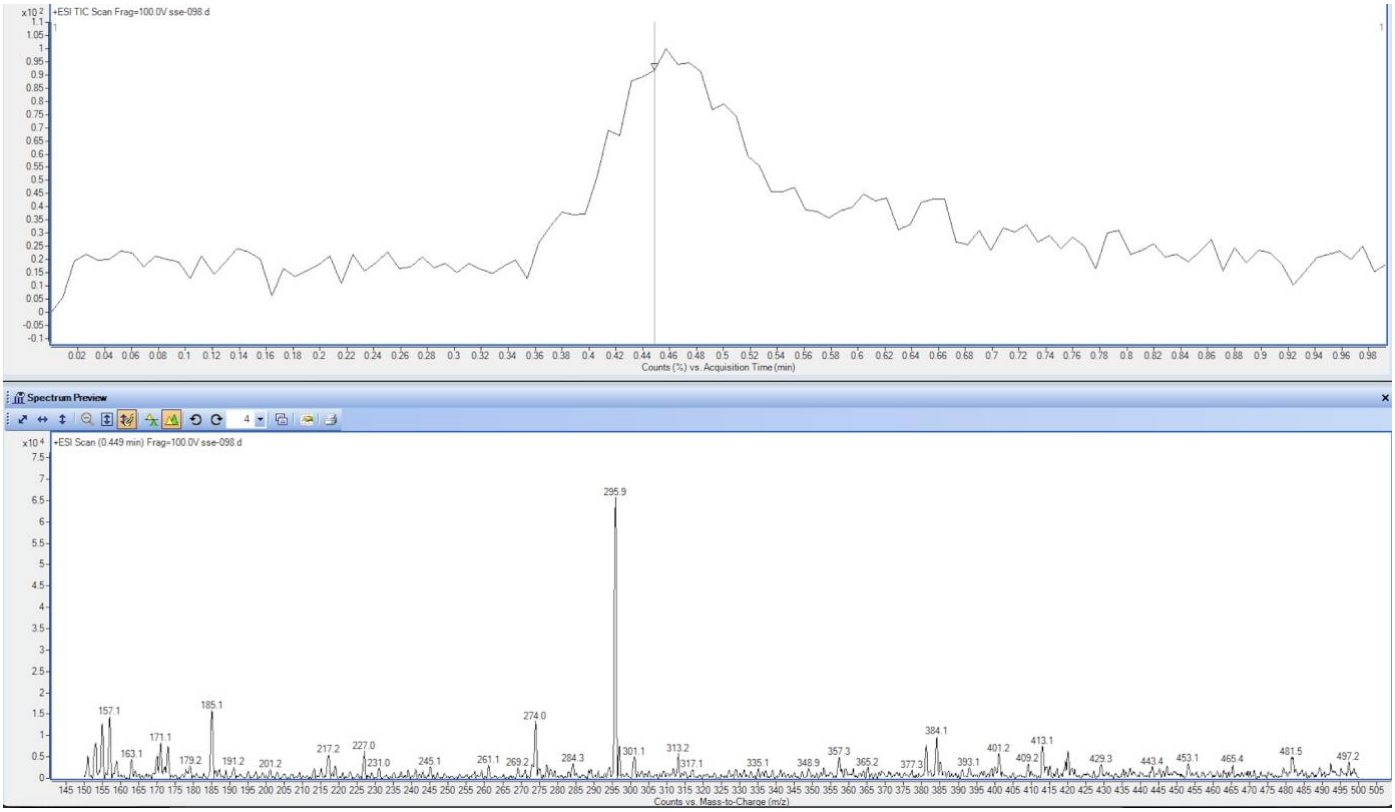
HMBC spectrum of 3



HPLC chromatogram of 3



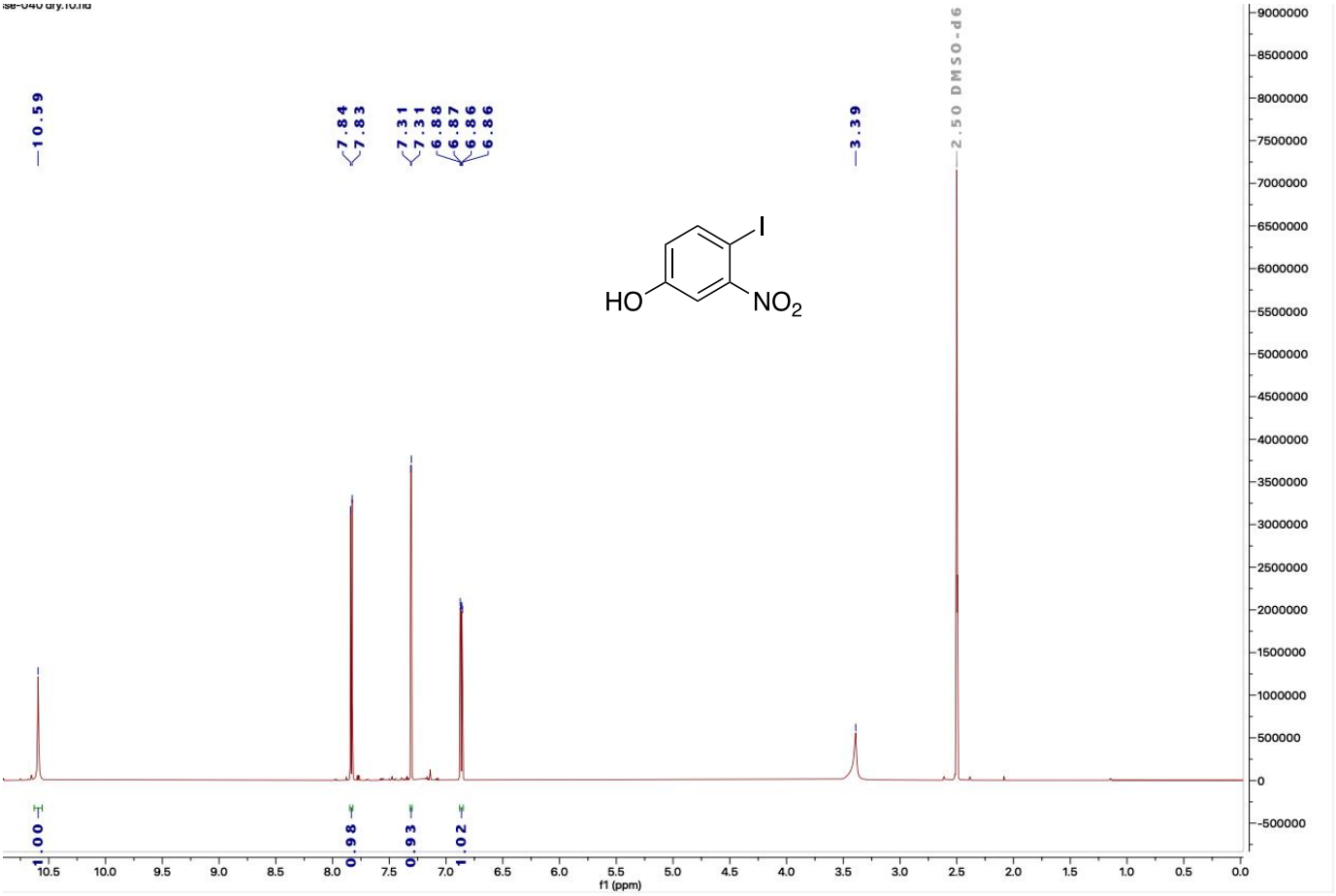
MS spectrum of 3



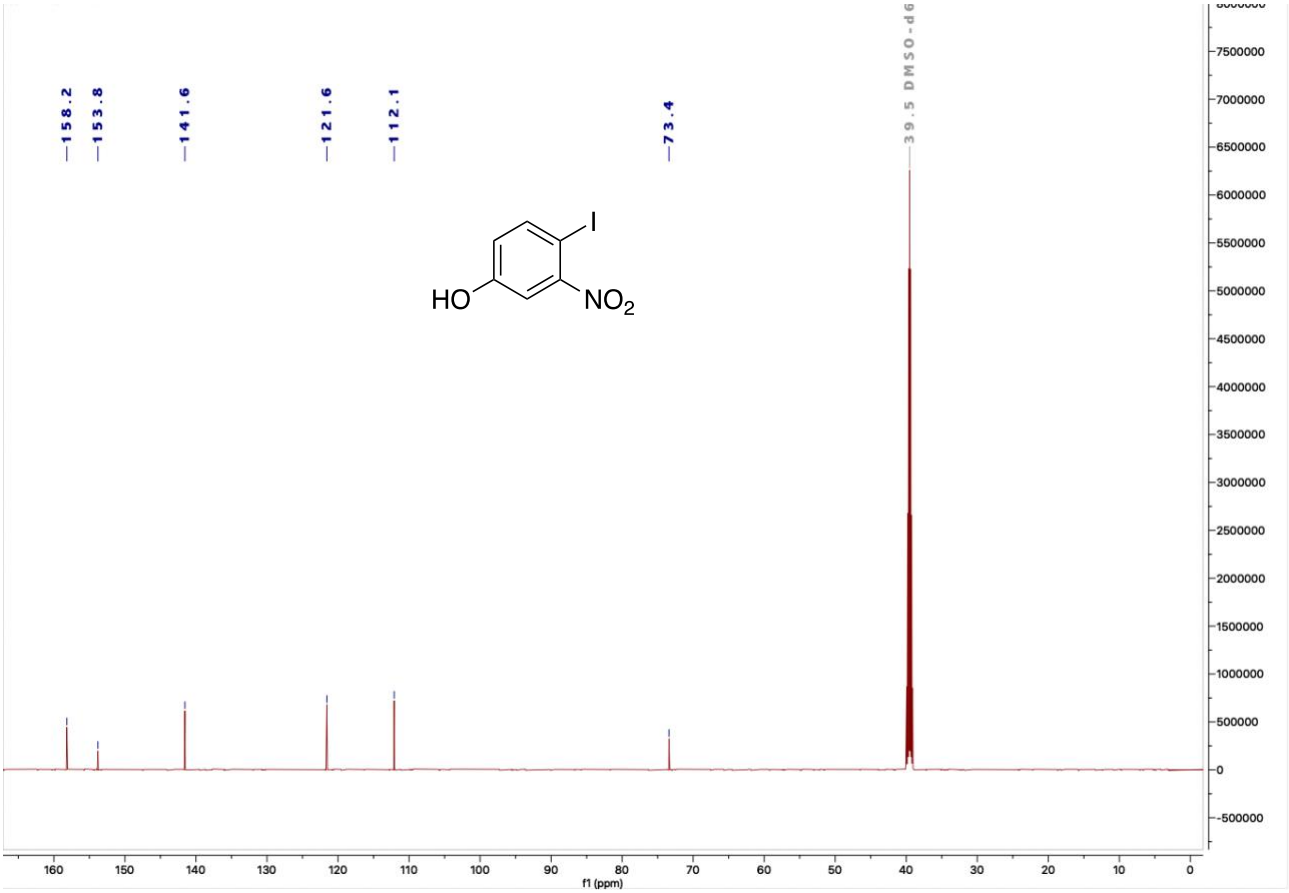
Appendix 4

4-iodo-3-nitrophenol (7)

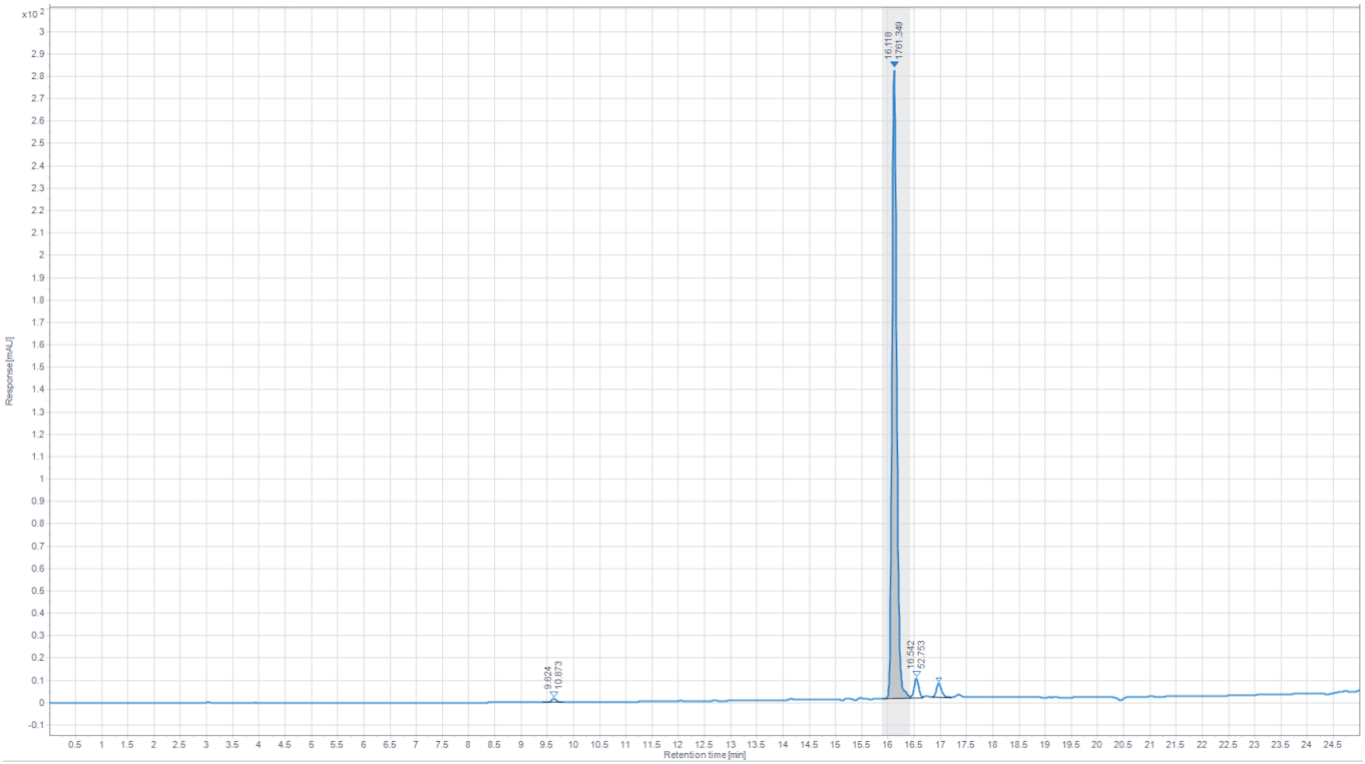
^{1}H NMR spectrum of 7



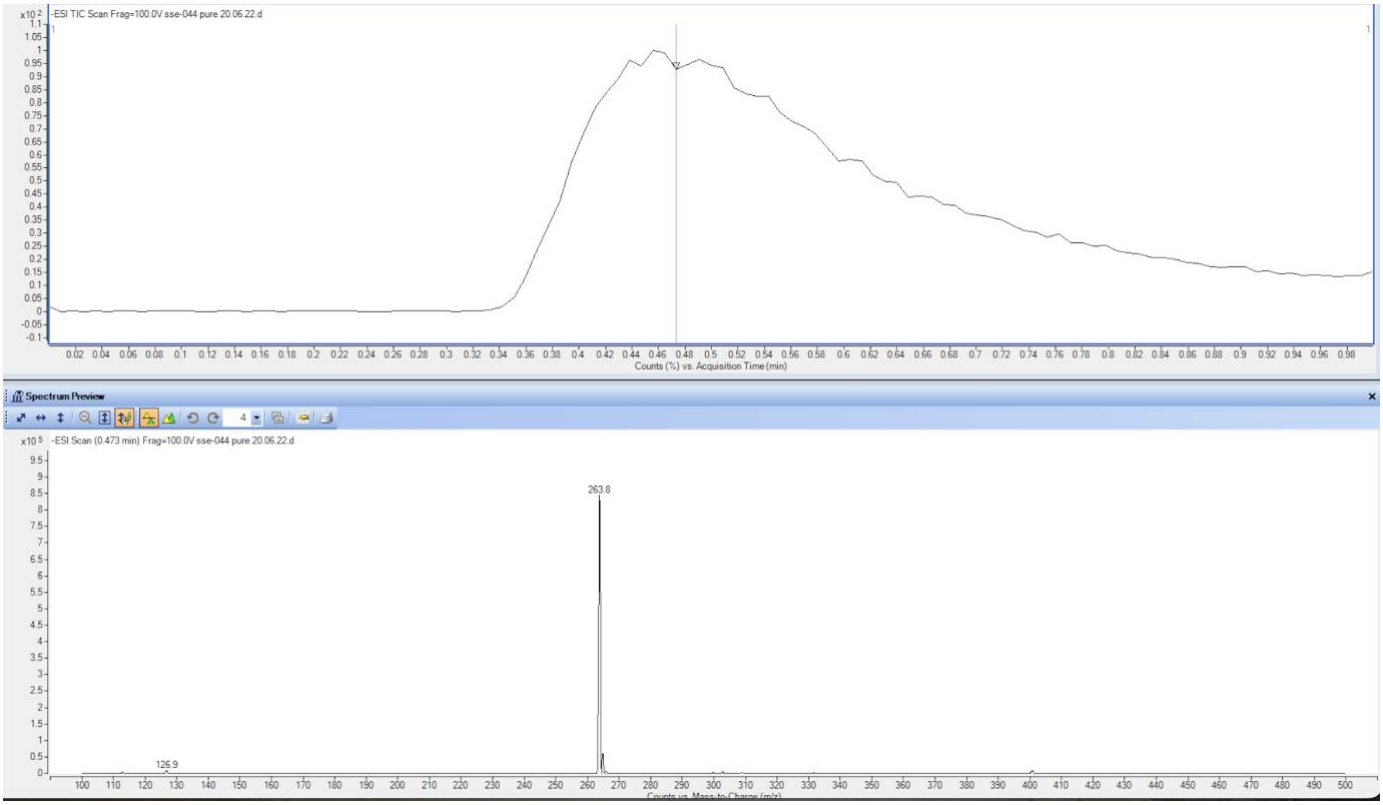
^{13}C NMR spectrum of 7



HPLC chromatogram of 7



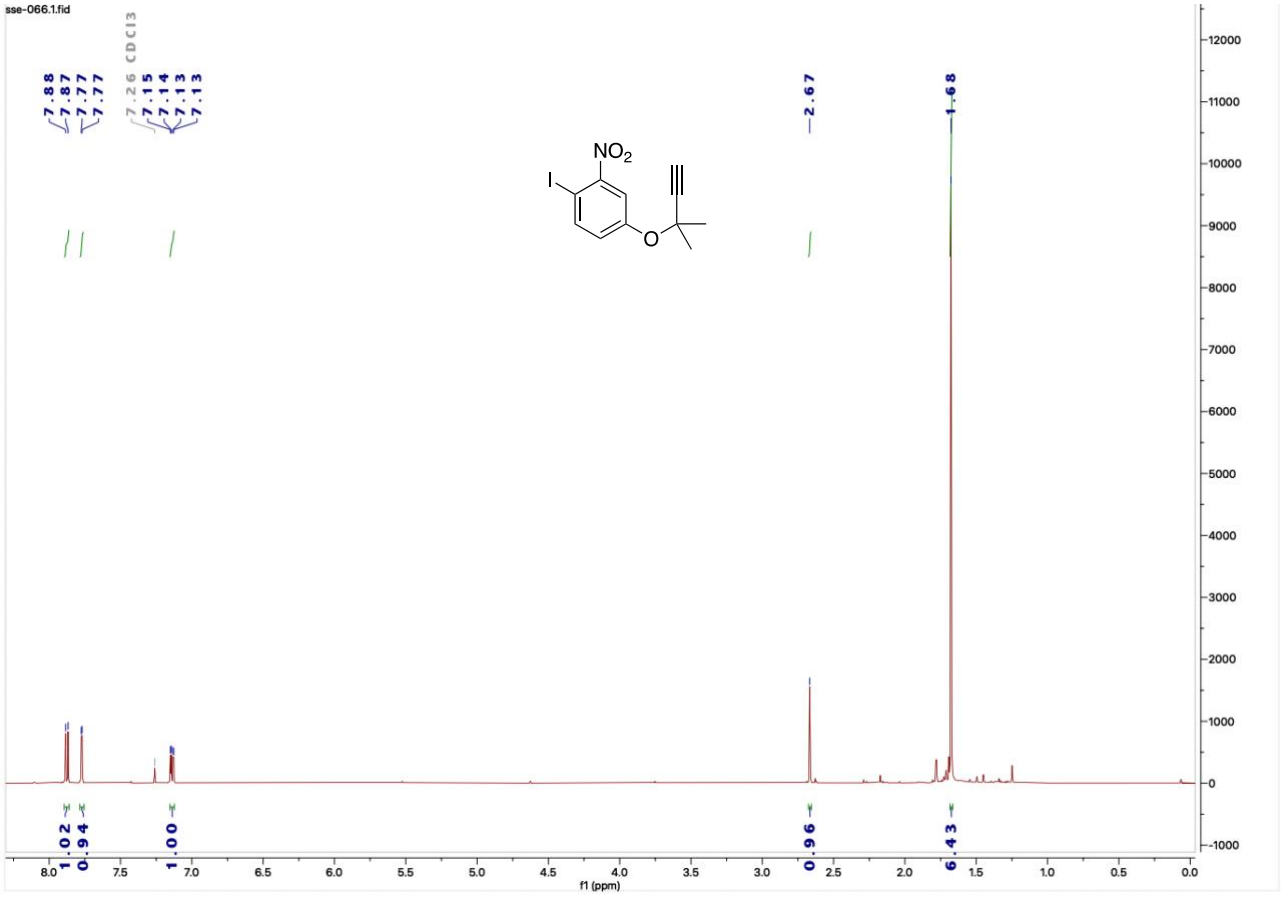
MS spectrum of 7



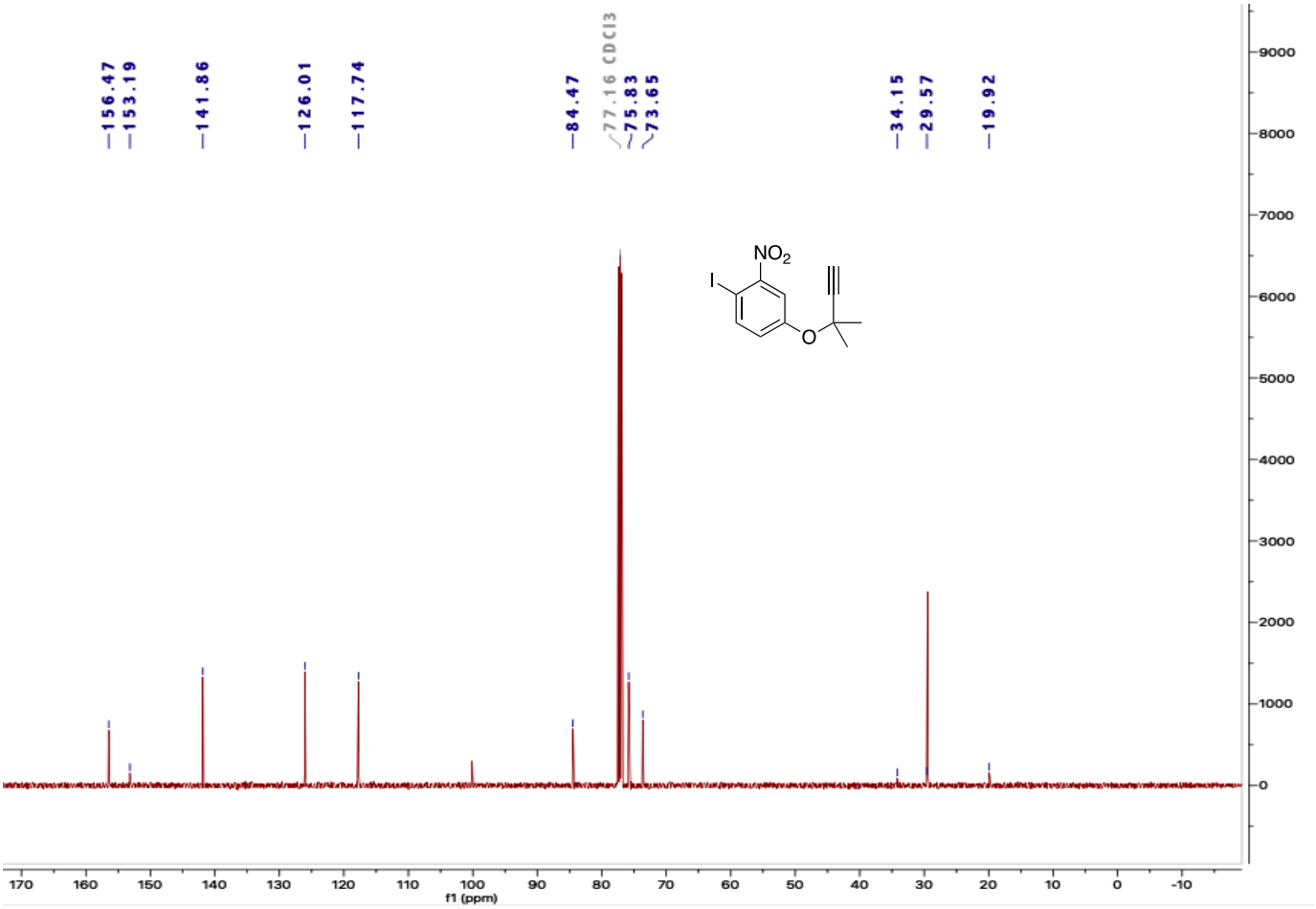
Appendix 5

1-iodo-4-((2-methylbut-3-yn-2-yl)oxy)-2-nitrobenzene (8)

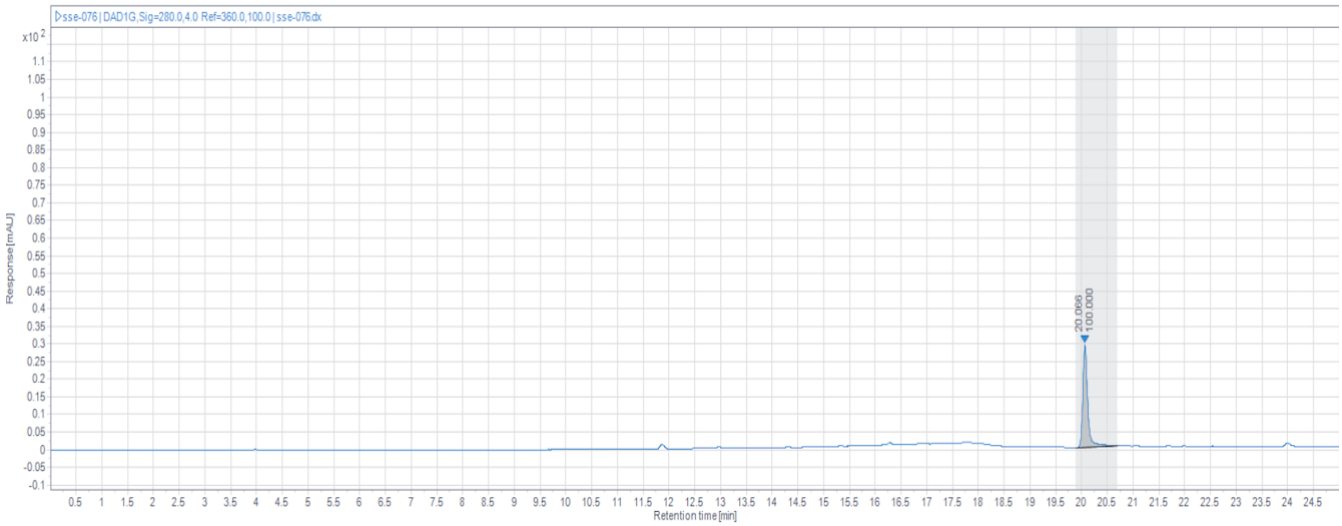
^{1}H NMR spectrum of 8



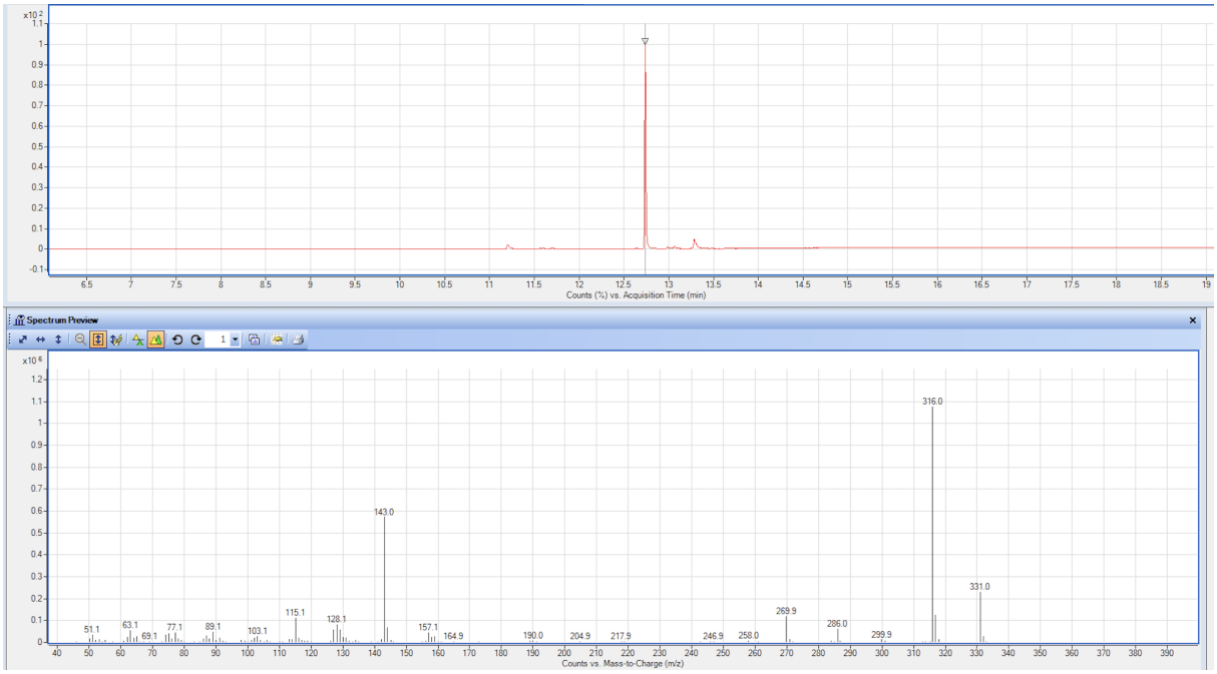
^{13}C NMR spectrum of 8



HPLC chromatogram of 8



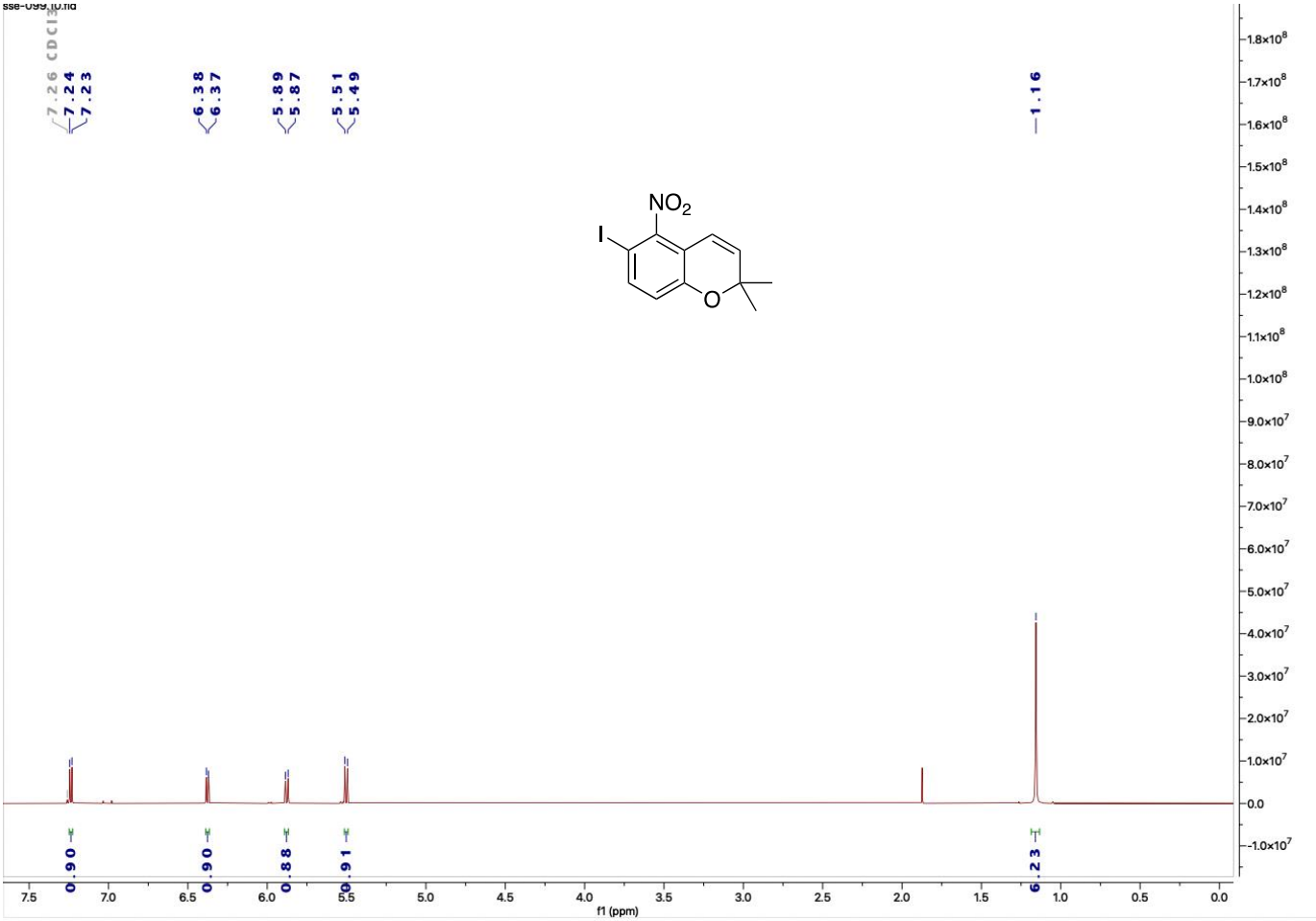
GC-MS spectrum of 8



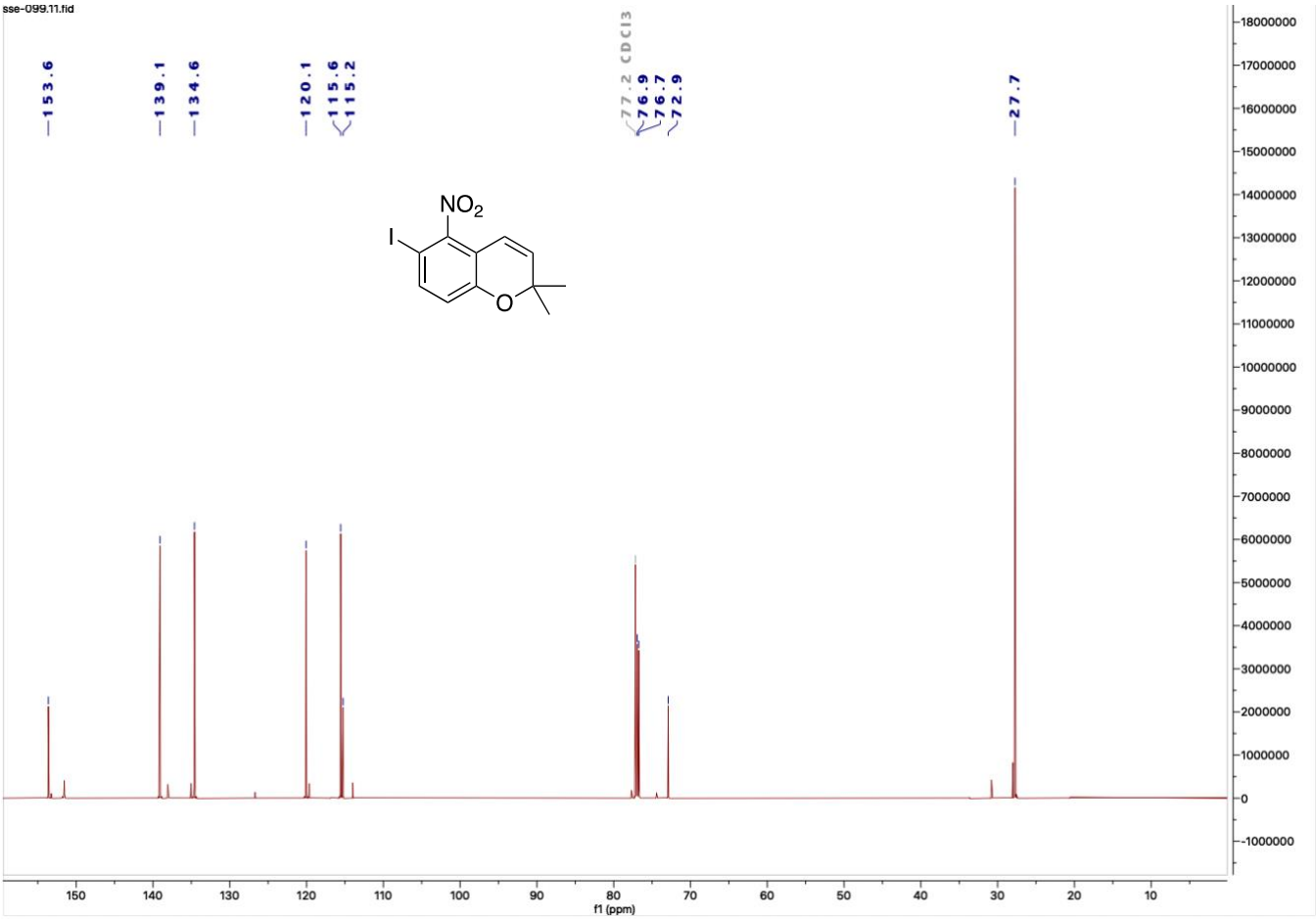
Appendix 6

6-iodo-2,2-dimethyl-5-nitro-2H-chromene (9)

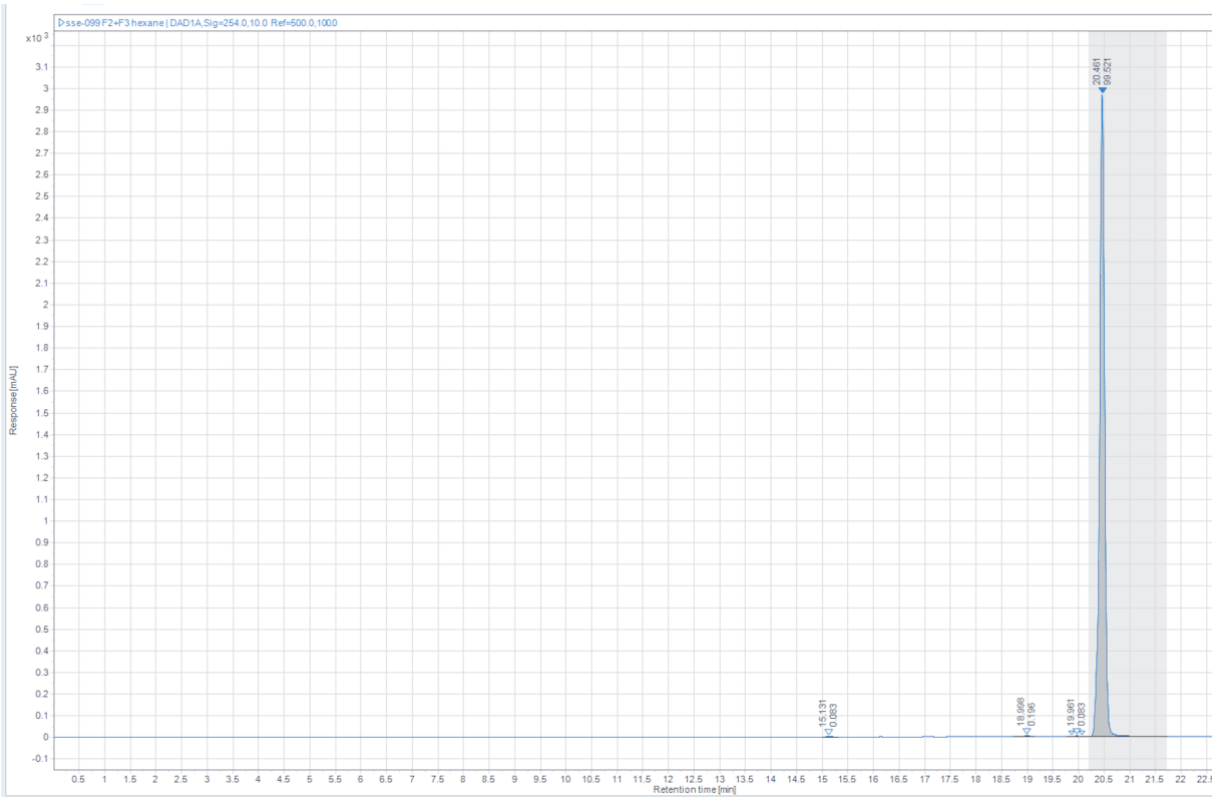
^{1}H NMR spectrum of 9



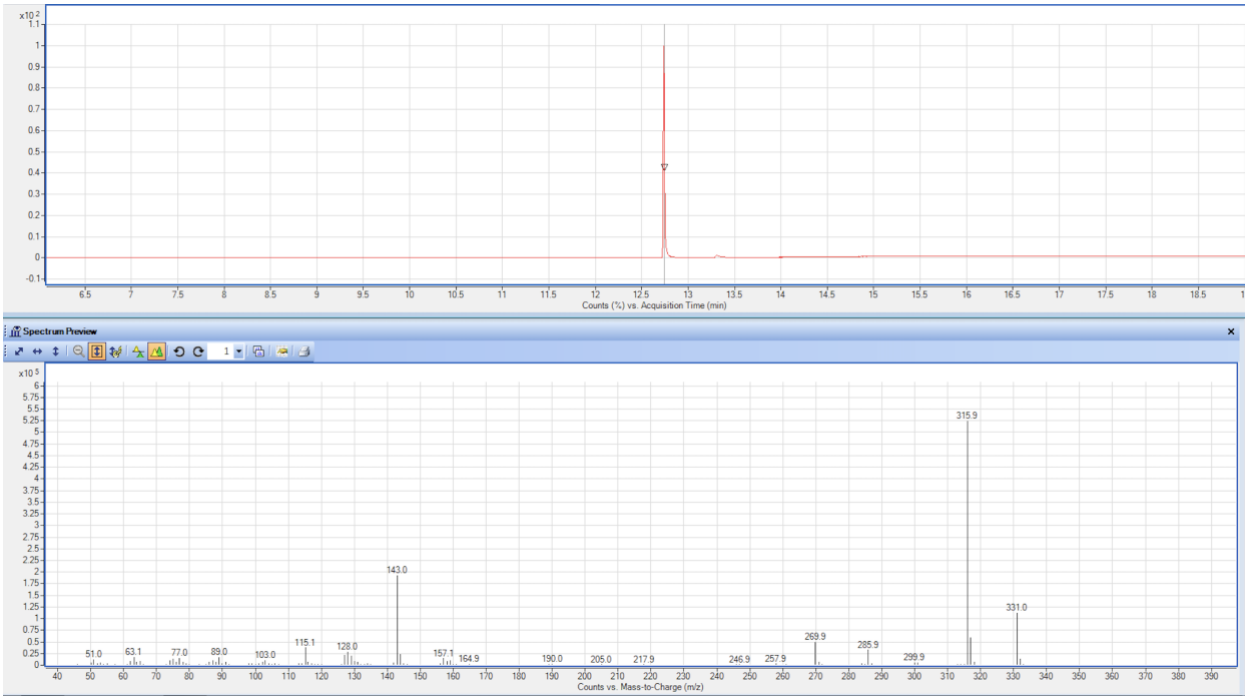
^{13}C NMR spectrum of 9



HPLC chromatogram of 9



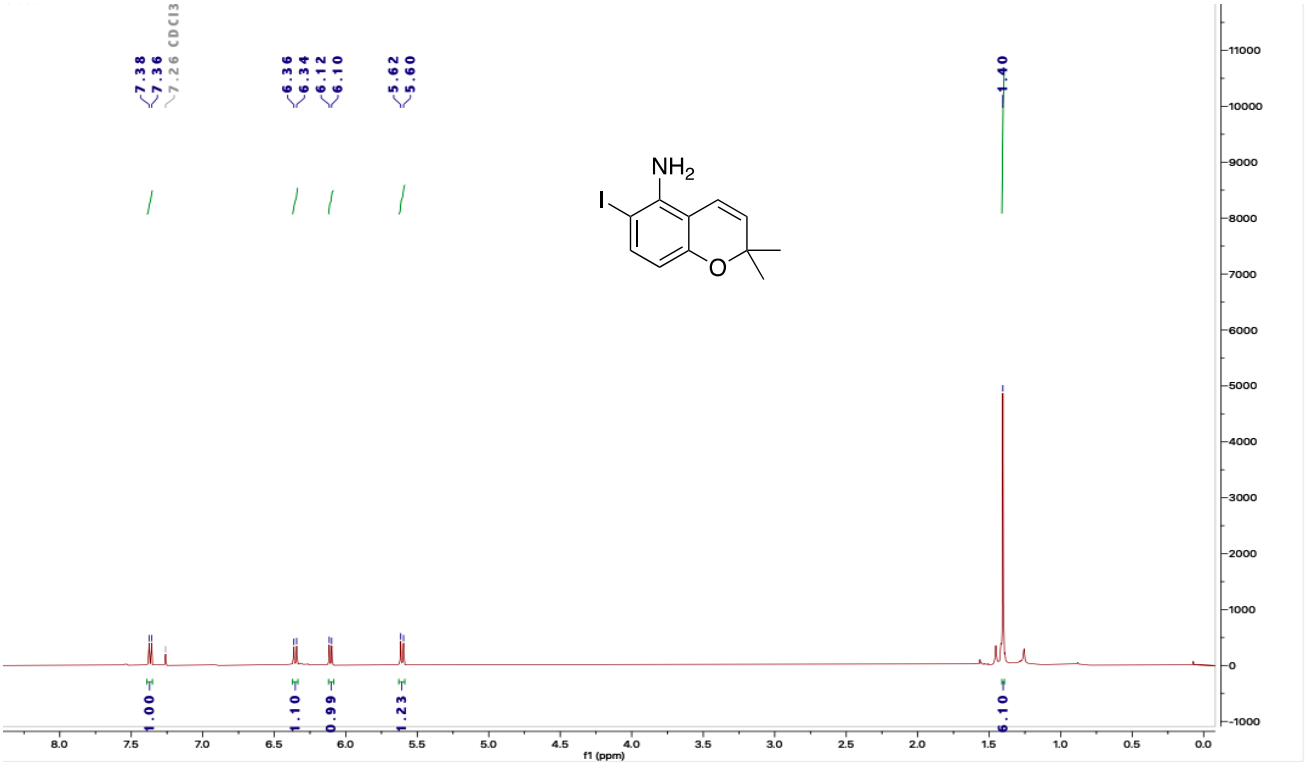
GC-MS spectrum of 9



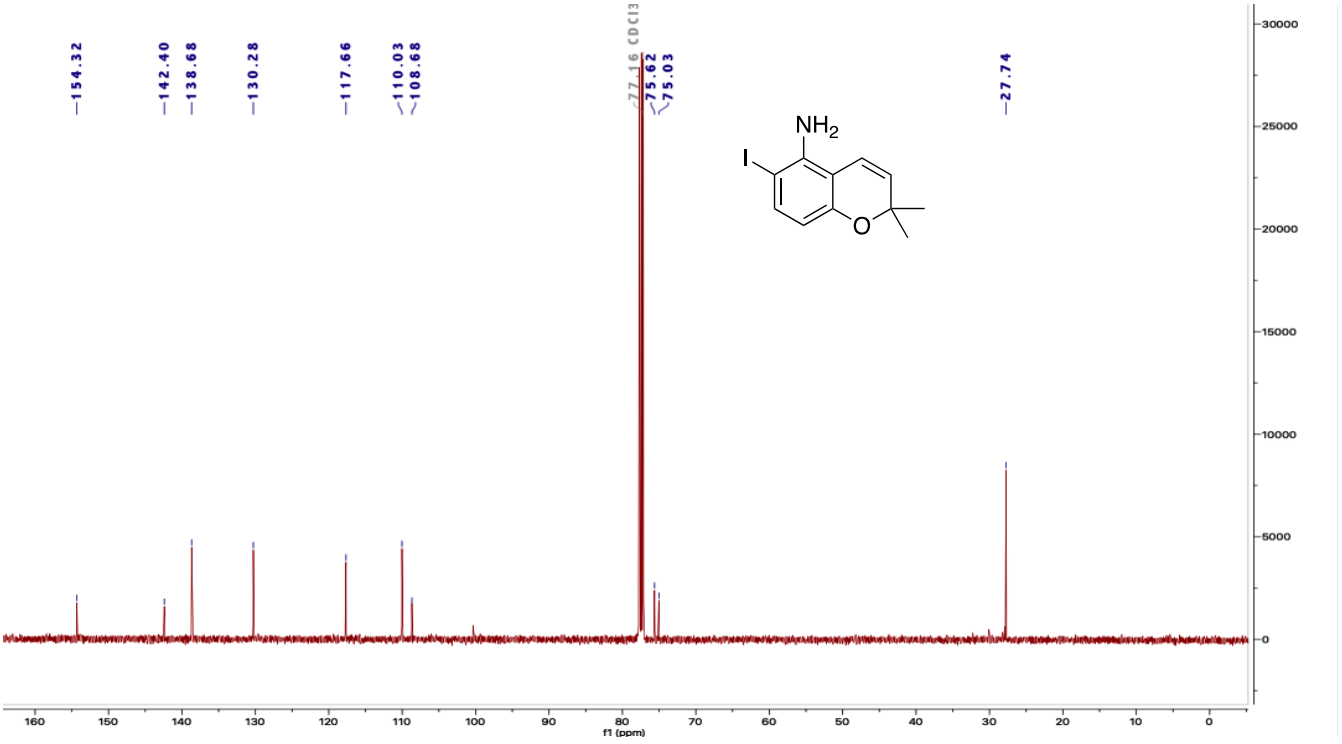
Appendix 7

6-iodo-2,2-dimethyl-2*H*-chromen-5-amine (4)

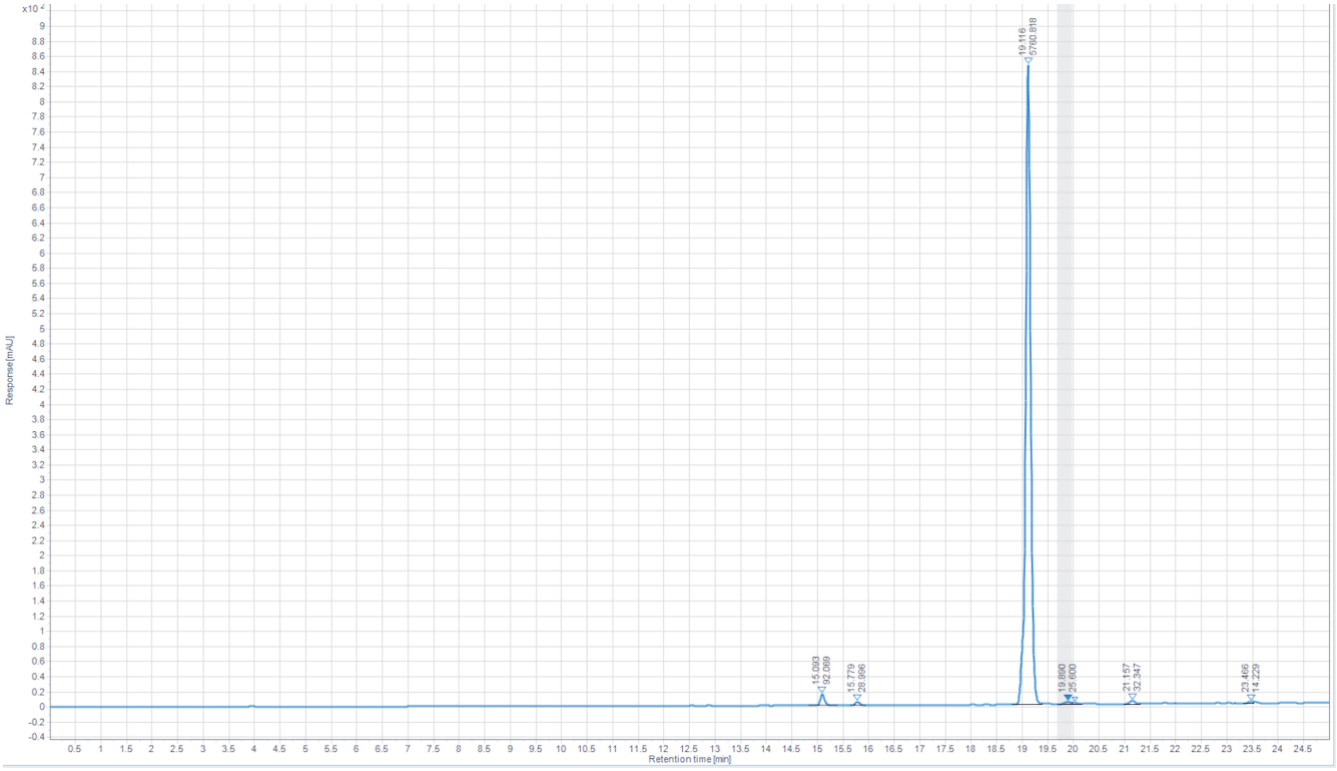
^{1}H NMR spectrum of 4



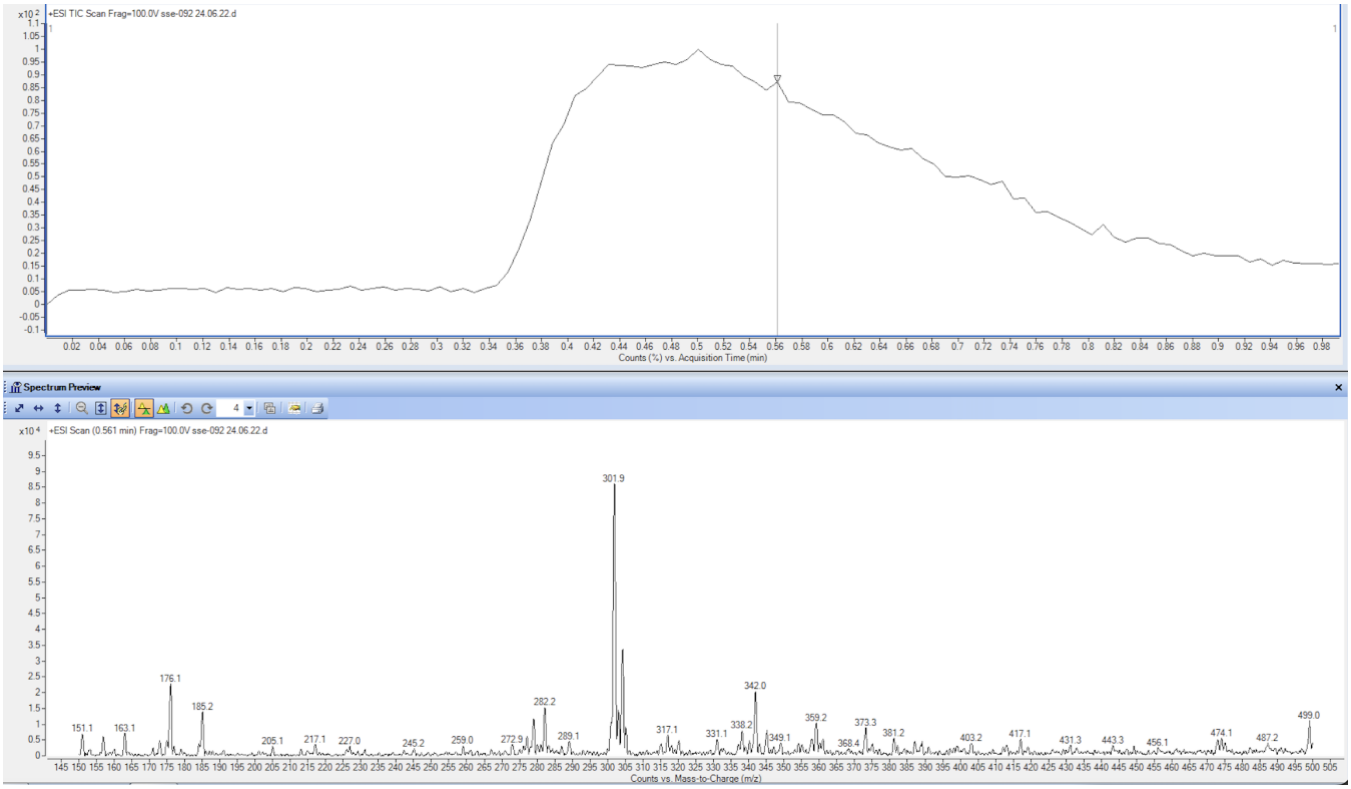
^{13}C NMR spectrum of 4



HPLC chromatogram of 4



MS spectrum of 4



GC-MS spectrum of 4