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Design of a Novel Isoxazoline Class Drug for the Suppressive Treatment of Malaria

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Honors Thesis

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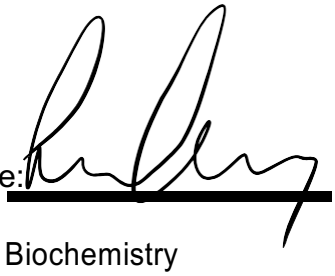
Primary Advisor Signature: 

Department: Chemistry and Biochemistry

Abstract
Design of a novel isoxazoline class drug for the suppressive treatment of erythrocytic malaria through the inhibition of *Plasmodium Falciparum* Gylceraldehyde-3-phosphate Dehydrogenase (*Pf*GAPDH) gave rise to creation of a synthetic plan for the proposed target molecule α-amino-3-bromo-4,5-dihydroisoxazol-5-yl propionic acid. A literature-based analysis of the moieties targeting the *Pf*GADPH active site led to the design of the target molecule. Subsequently, an exploration of the literature yielded a possible bifold synthetic plan. Attempts at a model epoxidation reaction using mCPBA and further efforts towards the synthesis of a 3-bromoisoxazoline were shown to be successful using GCMS analysis.

Introduction

Malaria is vector borne illness caused by protozoan parasites within the genus *Plasmodium*. *Plasmodium falciparum* is one of the five malarial parasites known to infect humans--*P. falciparum, P. knowlesi, P. malariae, P. ovale*, and *P. vivax*.[1] As vector borne parasites, all of the malarial causing *Plasmodiu*m, except for *P. Knowlesi*, use the female *Anopheles* mosquitos as their vector and definitive host, where the sexual phase occurs, and humans as the intermediate host, where the asexual phase occurs.[2] The World Health Organization (WHO) recognizes Plasmodium falciparum as the deadliest form of malaria. In 2015, the WHO reported malaria's alarming morbidity rate of 407 cases per minute and a mortality rate of 1 fatality per minute.[1] The Centers for Disease Control state that, Malaria commonly occurs in Africa, Central and South America, parts of the Caribbean, Asia, Eastern Europe, and the South Pacific.[3] Out of the five malaria parasites that infect humans, the World Health Organization recognizes *Plasmodium Falciparum* as the deadliest and as the most prevalent within the African Region. The WHO's 2019 World Malarial Report confirms 228 million cases of malaria in 2018 with 405,000 deaths globally. Children under the age of 5 accounted for 67% of these deaths.[4]

Malaria holds significance in the history of the United States public health system. Efforts for control and eradication of the disease prompted the creation of the Office of Malaria Control in War Areas in 1942 which became the well-known public health and epidemiological organization the Centers for Disease Control and Prevention (CDC) in 1946. The CDC notes that although "malaria was considered eliminated" in 1952, less than five years after the formation of the CDC, malaria remains both a national threat and global issue.[5] "Approximately 2,000 cases of malaria are reported each year in the United States" paired with the continued presence of *Anopheles* mosquitos in the United States, the potential for a malarial epidemic in the United States persists.[6] Continued efforts in containment, prevention, and eventual global elimination require the significant attention and resources of major public health and nonprofit organizations including the CDC and the WHO.[5]

The WHO's designation of *Plasmodium Falciparum* as the deadliest form of malaria and its increasing resistance to available treatments requires the design of new drugs. Because of this, the goal of this research was twofold: to design a novel isoxazoline class drug for the suppressive treatment of *Plasmodium Falciparum* and to create a potential synthetic plan for the target drug.

Design of Novel Isoxazoline Compound

Malaria is a vector borne disease, which means that infectious parasites are transmitted from one host to another. *Plasmodium Falciparum* uses the female *Anopheles* mosquito as its vector and definitive host, which is where the sexual phase occurs, and humans as its intermediate host which is where the asexual phase occurs. Once the infectious parasites enter the human body, they infect the liver and then the red blood cells. During the asexual blood stage of *P. falciparum,* a single infected red blood cell can release between "14 and 32 daughter merozoites" every two days.[7] Once released back into the blood stream, these merozoites will infect new blood cells and perpetuate the cycle of asexual reproduction.[1]

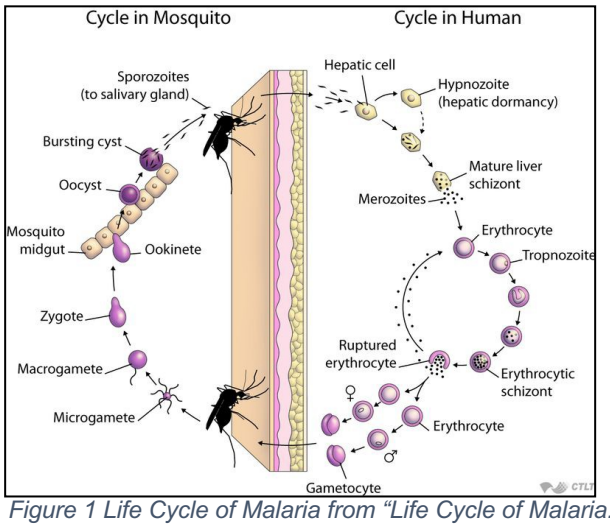


Figure 1 Life Cycle of Malaria from "Life Cycle of Malaria."

The asexual blood stage of malarial infection causes clinical symptoms such as "high fevers, shaking, chills, and flu-like illness."[3] Because of this, Suppressive treatments, aim to eliminate malaria symptoms by targeting parasites within red blood cells. During the blood stage, both the infected red blood cells and the parasites rely on anaerobic metabolism to produce ATP. As suggested by Kumar (2018), glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (PfGAPDH), have the potential to be great cytosolic drug targets, because malarial parasites rely heavily on glycolysis for energy and glycolytic enzymes' amino acid sequences are often well conserved.[8] Therefore, glycolytic enzymes are good drug targets for suppressive treatments, because they do not easily form resistance and the parasites require glycolysis to continue growth.

A key enzyme for erythrocytic stage of pf malaria is Glyceraldehyde-3-phosphate dehydrogenase. This enzyme catalyzes the conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate (1,3-BPG) and uses a cofactor of NAD^+ and (inorganic phosphate) P_i. At this point in glycolysis the parasite has already invested 2 ATP. It is significant that *Pf*GAPDH shares only 65% of its identity with its Human analog. Additionally, the insertion of the dipeptide (lysine—glycine) in the *S* loop of the *Pf*GAPDH allows for selective inhibitory action by heme.[9]

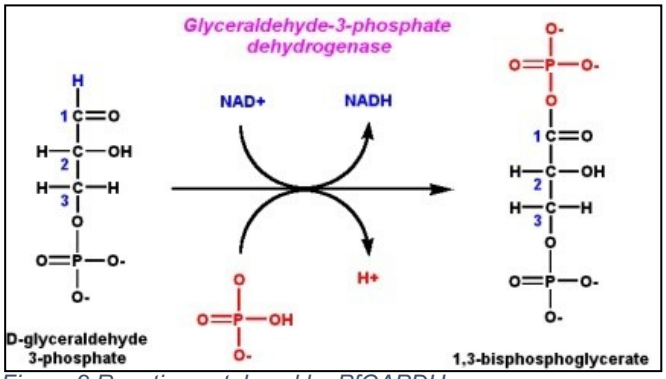


Figure 2 Reaction catalyzed by PfGAPDH

In 2014, Bruno et al. first suggested the use of isooxazoline class drugs—such as acivin and 3-bromo-acivin—to inhibit the catalytic cysteine of *Pf*GAPDH. Early on in this project, a generalized target molecule which adapted the isoxazoline scaffold from 3-bromoacivin was proposed as seen in *figure 3*. This target molecule changed 3-bromo group on 3-bromoacivin to an acetoxy group, because inhibitory activity caused by the nucleophilic attack of catalytic cystine

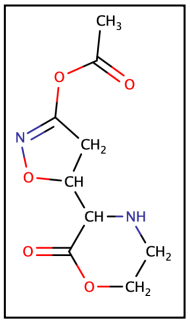


Figure 3 Proposed target molecule 11/5/2018

residues was greatly influenced by the leaving group in the 3 position on the isoxazoline ring.[10] Additionally, to further characterize the binding site of *Pf*GAPDH, the amino acid was protected in a heterocyclic ring. In Spring 2019, significant time was invested towards the synthesis of this molecule. The first attempt to synthesize our target molecule, reactions towards a model an aldoxime starting reagent, a subsequent isoxazoline, and finally 1,1-dibromoformaldoxime. GCMS analysis proved these reactions to be unsuccessful. Because of this, the literature was revisited, and the target molecule was adapted. During the reassessment of the original target molecule there was an emphasis on literature that discussed *Pf*GAPDH's natural and synthetic inhibitors and its mechanisms. Once a new target molecule was proposed, then potential synthetic routes were sought.

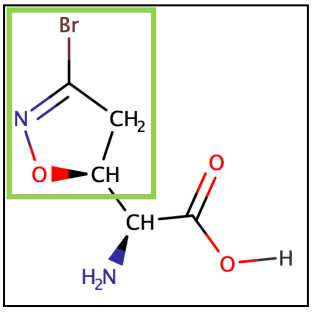
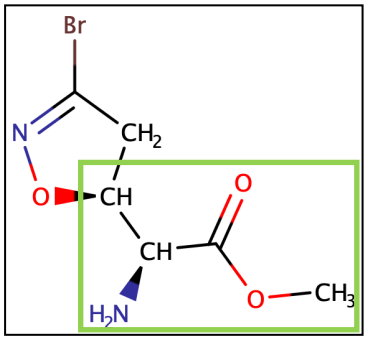
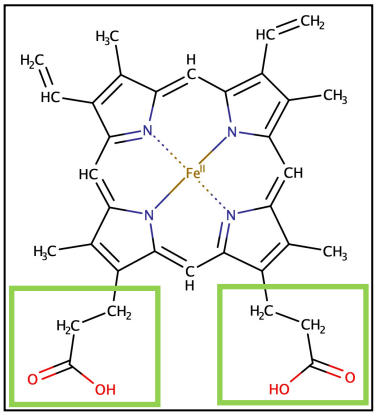
Compound Structure	 *3-bromoacivin*	 *3-bromoacivin derivative*	 *heme*
Reference(s)	11,12	11	8,9,13,14
Kinetic Data	Kitz-Wilson K_{inact}/K_i Ratio=.7$s^{-1}M^{-1}$	Kitz-Wilson K_{inact}/K_i Ratio=10.7$s^{-1}M^{-1}$	n/a
Key Features	3-bromo group on isoxazoline core, 5-α-amino-acetic acid moiety	3-bromo group on isoxazoline core, 5-α-amino-methyl acetate moiety	Propionic acid groups on five membered heterocyclic rings (3-pyrroline and pyrrole, respectively)
Conclusions	Original lead compound, 3-bromo group binds irreversibly with Cys residues (*Pf*GADPH catalytic Cys153), position of the carboxylic acid H-bond donator reduces effective inhibition	Solidifies the use of the 3-bromo group, most favorable positioning of bromine leaving group, Protonated amine H-bonds with Thr154 & Gly215, Higher Kitz-Wilson K_{inact}/K_i Ratio represents greater inhibition potential	One of two propionic acid groups inhibit *Pf*GAPDH by positioning itself within the NAD^+ binding site and both liganded and covalent thioether linkages to Cys residues, the insertion of –KG-- within the *S* loop leads to the selective inhibition of *Pf*GADPH over human *Pf*GAPDH

Table 1. Analysis of Literature Compounds

As previously stated, Bruno et al. (2014) first suggested the use of 3-bromoacivin to inhibit the catalytic cysteine of *Pf*GAPDH.[12] In 2019, the same research group released a paper further characterized the mechanism by which the 3-bromo-isoxazolines irreversibly reacted with the catalytic Cys153 of *Pf*GAPDH and how various moieties in the 5 position affected this inhibitory action.[11] This article confirmed that the 3-bromo group on the original lead compound, 3-bromoacivin, binds irreversibly with the *Pf*GADPH catalytic Cys153. However, it was also found that the position of the carboxylic acid H-bond donator on the 5-group reduced inhibition as demonstrated by the lower Kitz-Wilson ratio compared to the 3-bromoacivin derivative with a much higher lower Kitz-Wilson ratio seen in *table 1*. The higher the Kitz-Wilson ratio, the greater the inhibition. Therefore, the 3-bromoacivin derivative demonstrated the greatest inhibition of *Pf*GAPDH. The extended 5-group side chain by one carbon through the use of a methoxy group may have allowed for the 3-bromoacivin derivative to have most favorable positioning of bromine leaving group. Additionally, the protonated amine in the 3-bromoacivin derivative H-bonded with Thr154 & Gly215. *Figure 4* demonstrates mechanism of inhibition of catalytic cysteine by acivin and other 3-haloisoxazolines the includes the direct modification of the active site as the nucleophilic sulfur attacks carbon 3 on the isoxazoline ring causing the chloride to leave.[15] In 3-bromoacivin the 3-chloro leaving group would be a 3-bromo group. It becomes apparent the position of an H-bond donor such as an amino acid could interfere with the basic amino acid's de-shielding of the catalytic cysteine. Because of this, an elongated side chain for the 5-group was considered, and the amino group was found to be potentially significant. Since Cullia et al. 2019 further established the success of the 3-bromo group in reacting with catalytic cysteines, the idea of 3-acetoxy group from the previous target molecule was rejected, and the use of the 3-bromo group as the leaving group on the target molecule was established.

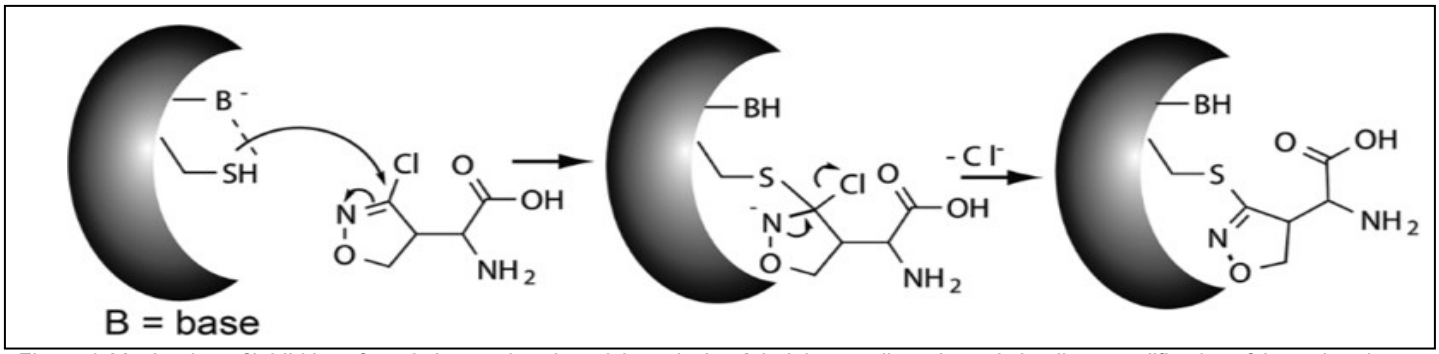


Figure 4. Mechanism of inhibition of catalytic cysteines by acivin and other 3-haloisoxazolines through the direct modification of the active site (Kreuzer 2014).

The most prevalent suppressive treatments for malaria, such as hydroxychloroquine, disrupt the asexual blood stage through the inhibition of parasite mechanisms which participate in the "degradation and detoxification of host hemoglobin."[7] This is because erythrocytic *Plasmodium Falciparum* degrade hemoglobin to heme. This free heme inhibits various Plasmodium proteins, including *Pf*GAPDH.[13] Despite the parasite's increased resistance to some of these drugs, heme's naturally toxic effects remain. As seen in *table 1*, one of the two propionic acid moieties of heme inhibit PfGAPDH by positioning itself the within NAD^+ binding site.[14] Additionally, heme inhibits *Pf*GAPDH through both liganded and covalent thioether linkages to

Cys residues.[13] *Pf*GAPDH only shares 65% of its identity with its Human analog.[1] The major structural distinction of PfGAPDH of an "insertion of a dipeptide (-KG-) in the so-called S loop. . .may be responsible for the selective inhibition of the enzyme [PfGAPDH] by heme."[9] This is significant because it may explain why heme "shows little inhibition of RBC GAPDH," which could allow for targeted inhibition of *Pf*GAPDH using the propionic acid heme moiety as shown in the image.[9] Therefore, a propionic acid moiety on the 5-group was proposed for the target molecule.

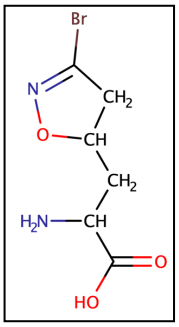


Figure 5 Target Molecule α-amino-3-bromo-4,5-dihydroisoxazol-5-yl propionic acid

After revisiting the literature, α-amino-3-bromo-4,5-dihydroisoxazol-5-yl propionic acid was proposed as an ideal target molecule as seen in *figure 5.* The structure includes a 3-bromo group on an isoxazoline core because this moiety has demonstrated covalent inhibitory action with the catalytic cysteine (Cys153) within the active site of *Pf*GDAPDH. Additionally, the 5-propionic acid group was chosen because of the inhibitory action of propionic acid heme moieties. The small change of extending the carbon chain of the amino acid group by 1 carbon lead to the creation of a synthetic plan for the proposed target.

Synthetic Plan for Target Molecule

One of the most unique reactions in the proposed synthetic plan is the synthesis of an isoxazoline. While the 1,3-dipolar cycloaddition reaction has been around for many years, isoxazoline class molecules have received recent attention for their possible bioactivity. Namboothiri et al. 2008 states that "Isoxazolines exhibit interesting and diverse biological properties and represent a unique class of pharmacophores." As nonaromatic heterocycles, "Their therapeutic potential presents exciting possibilities"--Isoxazoline class molecules possess "antimicrobial, anti-inflammatory, fibrinogen receptor antagonistic, anticancer, antiHIV, caspase inhibitory, and antidepressant properties."[16] In 1963, Dr. Rolf Huisgen presented 1,3-dipolar cycloaddition as a useful method for the "synthesis of five-membered heterocycles."[17] Using this route, nitrile oxide, a zwitterionic 1,3-dipole, reacts with an alkene, a dipolarophile, to form an Δ2 –Isoxazoline (4,5-dihydroisoxazole). Because of this a two-phase reaction synthetic route has been suggested. Phase 1 focuses on the synthesis of an ideal dipolarophile modeled after the synthesis of Garner's Aldehyde, while phase 2 includes the 1,3-dipolar cycloaddition and deprotection of an amino acid.

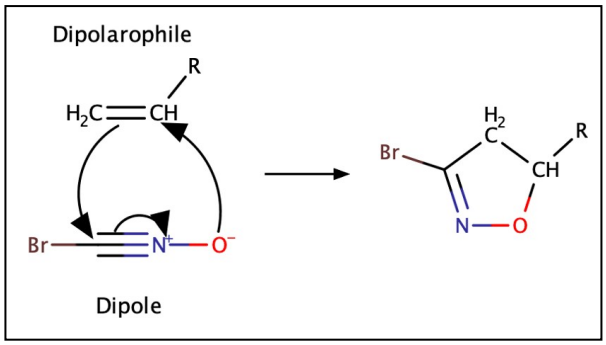


Figure 6 Mechanism of 1,3 dipolar cycloaddition

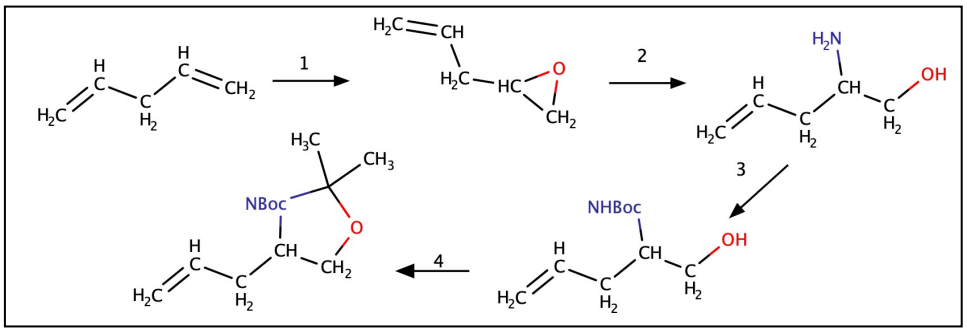


Figure 8 Phase 1 of proposed synthetic route

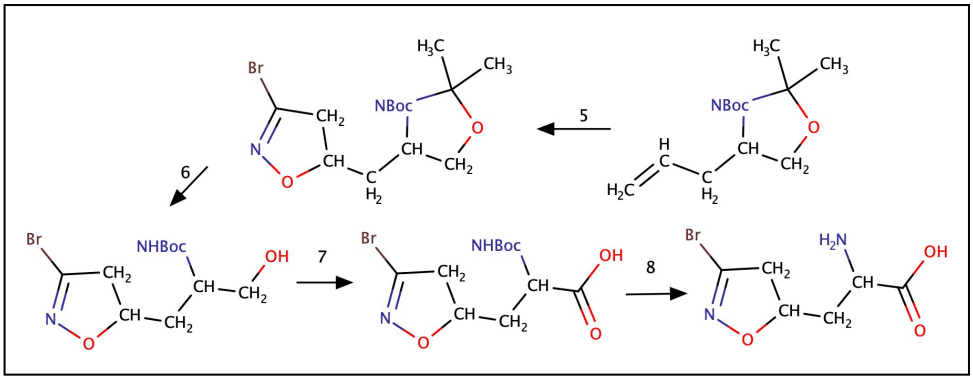


Figure 7 Phase 2 of proposed synthetic route

Modeled after the protection of a beta-amino alcohol as seen in the synthesis of Garner's Aldehyde, synthetic route suggests an approach for the synthesis of our target molecule. The goal of the first phase of the synthetic plan as seen in is to synthesize an alkene with a tert-butyloxycarbonyl (boc) N-protected beta-amino alcohol as seen in *figure* 7. This alkene would serve in as a dipolarophile in the 1,3-dipolar cycloaddition reaction. The first step in the reaction is a monoepoxidation of 1,4-pentadiene, an unconjugated, symmetric diene, resulting in 2-allyoxirane.[18,19] Followed by a regioselective aminolysis or ring opening of the epoxide yielding a beta-amino alcohol.[20–23] Step 3 protects the N-group of the beta-amino alcohol with a boc protecting group.[24,25] Finally, step 4 protects the N-protected beta-amino alcohol within a heterocycle and leaves the alkene available for the 1,3-dipolar cycloaddition reaction.[24]

Phase 2 of the proposed synthetic plan includes the formation of the 3-bromoisoxazoline and the deprotection and oxidation of the N-protected beta-amino alcohol to an amino acid. The alkene which results from phase 1 of the synthetic plan will act as dipolarophile and react with a nitrile oxide formed from 1,1-dibromoformaldoxime.[12,26,27] Steps 6 and 7 remove the cyclized protection from the N-protected β-amino alcohol and oxidize the alcohol to an acid.[10,26] Finally, step 8 removes the boc-protecting group from the resulting amino acid.[26,28] This results in a molecule which fits the criteria for the inhibition of PfGAPDH based on our literature-based analysis of the active site. The identification of this possible synthetic route allowed for effective modeling of steps 1 and 5 as discussed below.

Discussion and Results

As this project progressed, multiple target compounds were proposed, and the synthesis of multiple target compounds was attempted. Because of this, the experimental sections will be separated into two sections—target molecule 11/5/18 and novel 3-bromoisoxazoline—indicated by * and ** respectively.

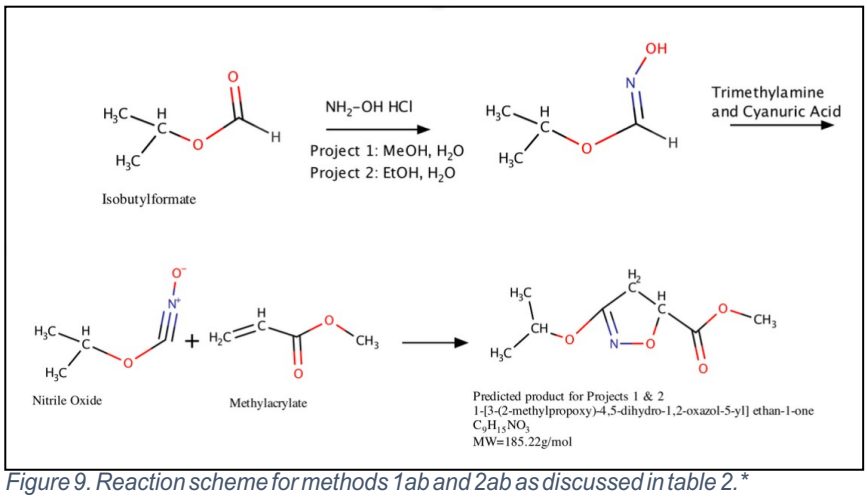


*Figure 9. Reaction scheme for methods 1ab and 2ab as discussed in table 2.**

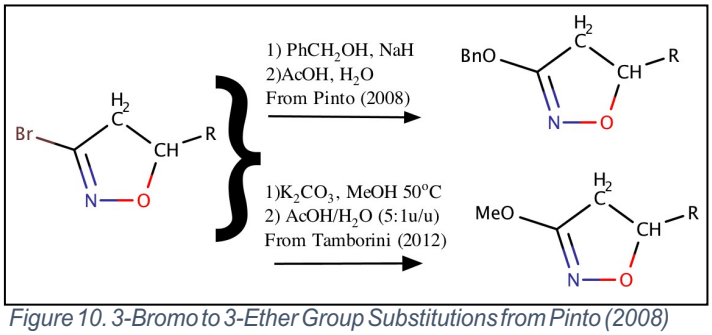


*Figure 10. 3-Bromo to 3-Ether Group Substitutions from Pinto (2008) and Tamborini (2012).**

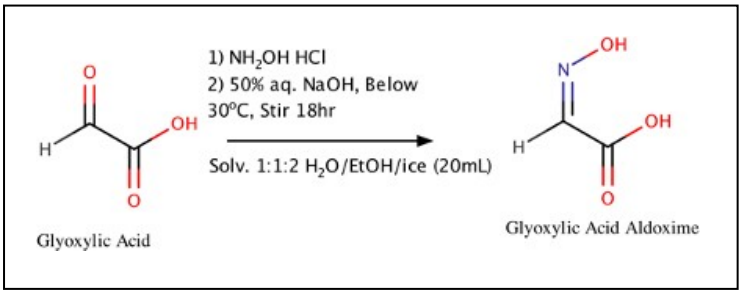


*Figure 11. Methods 3 and 4: Synthesis of Glyoxylic Acid Aldoxime using general aldoxime synthesis procedure from Ramòn (2010) to eventually form Dibromoformaldoxime as suggested by Wang (2017) abstract.**

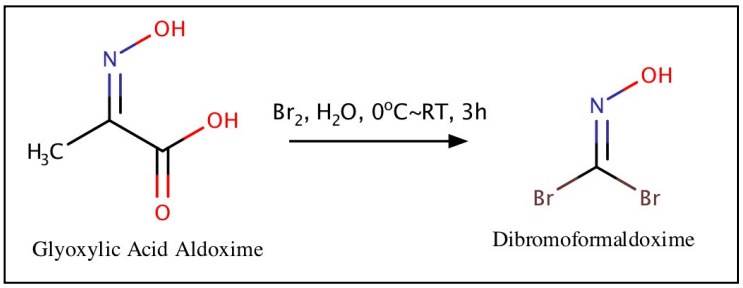


*Figure 12. Methods 4 & 6: Synthesis of Dibromoformaldoxime from Wang (2017) abstract.**

For the first attempt to synthesize a model of the target molecule 11/5/18, an aldoxime was attempted to be synthesized from isobutylformate using the procedure for intermediates 1-3 from Rodrigues (2013). This would result with a 3-isobutoxy group on the isoxazoline ring as seen in *figure 9.* Methods 1ab and 2ab were clearly unsuccessful. After looking at the GCMS data from project 2, products and starting materials were unable to be detected. This was strange because if the reaction did not proceed properly then the starting reagents should be present in the solution. Isobutylformate may not be reacting well to form the aldoxime and subsequently the nitrile oxide. Because of this, we returned to the literature discussing Garner's Aldehyde.[26]

After a revisit to the literature, it became apparent that many novel isoxazolines could be created through the substitution of a 3-bromo group for a 3-ether group as seen in *figure 10*.[26,27,29] The first step in the synthesis of a 3-bromo-isoxazoline required the synthesis of dibromoformaldoxime from glyoxylic acid, because 1,1-dibromoformaldoxime was relatively expensive and glyoxylic was available. Because of this, methods 3-6 were attempts to synthesize 1,1-dibromoformaldoxime.

Since other researchers had found success using the general procedure for the formation of an aldoxime from Ramòn (2010), the suggested procedure for the aldehyde considered closest to glyoxylic acid was used for Methods 3 and 5.[30] While adding NaOH to the reaction mixture, the temperature spiked during method 3, which could have affected the reaction.

However, because aldoximes have the potential to be relatively unstable, the synthesis of 1,1-dibromoformaldoxime in method 4 was continued.

The procedure for Methods 4 and 6 was based off of a reaction scheme seen in the Wang (2017) abstract as seen in *figure 12* and discussed in *table 5.*[31] Despite the improved temperature control for method 6, both methods 4 and 6 proved to be unsuccessful as confirmed by GCMS. The limited access to the established procedure for the formation of both glyoxylic acid aldoxime and dibromoformaldoxime hindered us.

Synthesis of Aldoxime		
Method	Method 1a	Method 2a
Reagents	Isobutyl Formate, Hydroxylamine hydrochloride	Isobutyl Formate, Hydroxylamine hydrochloride
Variations in Method	Methanol as solvent	Ethanol as solvent
Product Description	Clear colorless liquid	Clear colorless liquid
GC-MS Analysis	Presence of isobutyl formate starting material at 3.451 min peak	Presence of isobutyl formate starting material at 3.444 min peak

*Table 2. Summary of synthetic methods attempted in the synthesis of an aldoxime from isobutylformate.**

Synthesis of Isoxazoline		
Method	Method 1b	Method 2b
Reagents	Product mixture produced using Method 1a, triethylamine, cyanuric acid trichloride, methyl acrylate	Product mixture produced using Method 1b, triethylamine, cyanuric acid trichloride, methylacrylate
Variations in Method	Diethyl ether as solvent	Dichloromethane as solvent
Product Description	Yellow tinted clear liquid, some crystalized solid	White goo with crystals
GC-MS Analysis	Peak at 2.267 min indiscates the presence of unreacted methyl acrylate starting material	Confirmation of solvent diethyl ether at 1.310 min peak
Percent Yield	----------	149.3% (1.39g)

*Table 3. Summary of synthetic methods attempted in the synthesis of an isoxazoline from the products of method 1a and method 2a, respectively.**

Synthesis of Glyoxylic Acid Aldoxime		
Method	Method 3	Method 5
Reagents	glyoxylic acid monohydrate, hydroxylamine hydrochloride, sodium hydroxide	glyoxylic acid monohydrate, hydroxylamine hydrochloride, sodium hydroxide
Variations in Method	More ice, temperature rose to 30°C, filtered organic solution through $MgSO_4$	Allowed aqueous NaOH to fully cool, dried organic solution using scoops of $MgSO_4$
Product Description	Clear colorless liquid	Clear colorless liquid
GC-MS Analysis	Unclear spectrum	1.303 min peak --Propanoic acid in the library search
Percent Yield	26.9% (.482g)	59.3% (1.0565g)

*Table 4. Summary of synthetic methods attempted in the synthesis of an aldoxime from glyoxylic acid aldoxime towards the synthesis of 1,1-dibromoformaldoxime.**

Synthesis of 1,1-Dibromoformaldoxime		
Method	Method 4	Method 6
Reagents	Product mixture produced using Method 3, liquid bromine	Product mixture produced using Method 5, liquid bromine
Variations in Method	Adapt mol ratio to mol of product mixture (.482g) from Method 5	Adapt mol ratio to mol of product mixture (1.0565g) from Method 5
Product Description	Yellow, white, and peach colored solid	Red/yellow clear liquid
GC-MS Analysis	Red solution--Confirmation of solvent at peak 2.198 min; yellow solution--Confirmation of solvent at peak 2.216 min	Ethyl acetate peak is present at 2.267 min
Percent Yield	6% (.074g)	----------

*Table 5. Summary of synthetic methods attempted in the synthesis of 1,1-dibromoformaldoxime from the products of method 3 and method 4, respectively .**

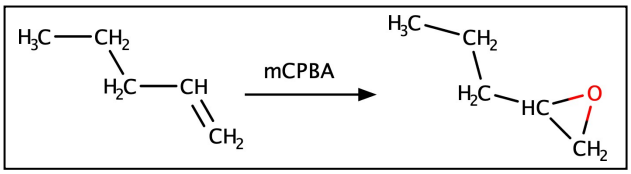


*Figure 13. Methods 7, 8, 12, and 13: Synthesis of a terminal epoxide (1,2-epoxypentane) from pentene using the Prilezhaev reaction. Model reaction of step 1 of phase 1 of the proposed synthetic plan.***

An epoxidation reaction is a textbook reaction, and though there appears to be theoretical support for the monoepoxidation of an unconjugated diene, there was some uncertainty in the methodology provided and whether the starting materials will behave in an ideal way.[19] Because of this, and the cost of the starting reagent 1,4-pentadiene, step 1 of phase 1 was modeled. Two methods—12 and 13—for the synthesis of a terminal epoxide from pentene using m-chloroperoxybenzoic acid as the epoxidizing agent proved successful. For method 12, the epoxidation of pentene procedure was adapted from Porto (2005) by adding pressure. For method 13, a biphasic epoxidation procedure using benzyl triethyl amine chloride as phase transfer agent was attempted.[32,33] Both were proved to be successful by GCMS analysis.

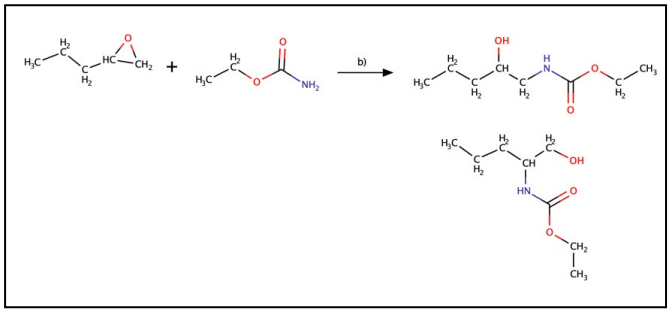


*Figure 15. Reaction scheme from Method 9ab. Model reaction for the combined steps 2 and 3 of phase 1 of the proposed synthetic plan. Attempt to complete a pH catalyzed opening of 1,2-epoxypentane with ethyl carbamate (urethane). Reaction conditions b) aqueous boric acid (30 mol%) and 3(A)/5(B) drops of glycerol in water at room temperature.***

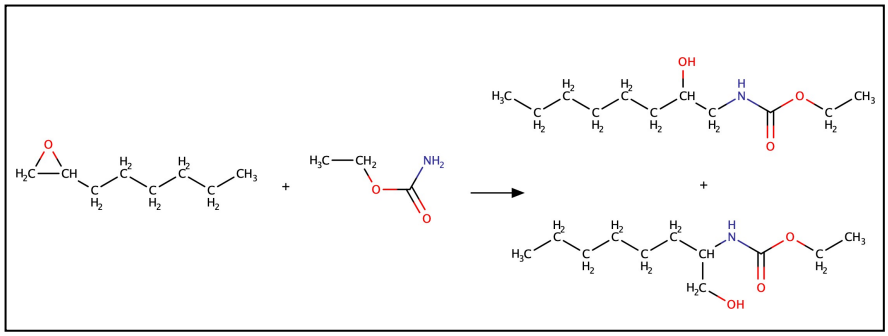


*Figure 14. Method 11: Solvent free, cyanuric trichloride catalyzed aminolysis of 1,2-epoxyoctane using ethyl carbamate.***

Methods 9ab and 11 hold significance as potential model reactions for the synthesis of a β-amino alcohol using the aminolysis of an epoxide with ethyl carbamate. As seen in step 2 of phase 1 of the synthetic plan, β-amino alcohols are biologically active compounds which can be synthetic intermediates--as a protected amino acid and unprotected then oxidized to form an amino acid. Many β-amino alcohols are used as "β-blockers, insecticidal agents, and chiral auxiliaries." Additionally, "naturally occurring biologically active. . .cyclic amino alcohols, like quinines. . .are used in the treatment of malaria."[23] Additionally, Carbamates are receiving new attention in medicinal chemistry for their "application in drug design and discovery." Carbamates are "chemical stability," have a high "capability to permeate cell membranes," and are used as "protecting groups for amines and amino acids in organic synthesis and peptide chemistry." An epoxide aminolysis using carbamates as a weak nucleophile is a novel approach to aminolysis with an aliphatic amine, since "carbamate functionality is related to amide-ester hybrid features."[34,35]

The EPA defines green chemistry as the "design of chemical products and processes that reduce or eliminate the generation of hazardous substances."[36] In recent years, green chemistry became popular as individuals have grown more environmentally conscious. As seen in methods 9ab and 11 respectively, the aqueous boric acid/glycerol catalyzed aminolysis and "Synthesis of β-amino alcohols by ring opening of epoxides with amines catalyzed by cyanuric chloride under mild and solvent-free conditions" provide procedures for step 2 which reduce the use of organic solvents in an attempt to employ green chemistry.[21,22]

Despite the best intentions for an efficient, environmentally conscious combination of steps 2 and 3 of the proposed synthetic plan, neither methods 9ab or 11 proved to be successful. For methods 9ab, the product from method 8 was used to run a glycerol/boric acid chelate acid catalyzed aminolysis of a terminal epoxide using ethyl carbamate as a weak nucleophile.[21] However, after running GCMS and NMR for the product from method 8, the epoxidation procedure proved to be unsuccessful. Because of this, the epoxide starting for methods 9ab was not present. Therefore, the reaction could not occur. The GCMS analysis for Method 11 indicated the presence of both the starting reagents--1,2-epoxyoctane and ethyl carbamate. This indicates that the reaction did not proceed.

As a key reaction in the synthesis of the target molecule, a model reaction between 1,1-formaldoxime and 1,4-pentadiene resulted in a simplified 3-bromoisoxazoline. The procedure for this reaction was adapted from Pinto (2008). It is significant that our procedure which limited 1,1-dibromoformaldoxime resulted in a single 3-bromisoxazoline ring with a remaining double bond. This reaction proved to be successful as confirmed through GCMS analysis.

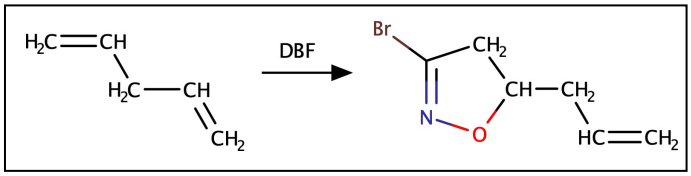


*Figure 16. Method 14: Model synthesis of a 3-bromoisoxazoline from 1,4-pentadiene for step 5 of Phase 2.***

Terminal Epoxide Synthesis				
Method	Method 7	Method 8	Method 12	Method 13
Reagents	Pentene, m-Chloroperbenzoic acid, potassium hydroxide	Pentene, m-Chloroperbenzoic acid, potassium hydroxide	Pentene, m-Chloroperbenzoic acid, potassium hydroxide	Pentene, m-Chloroperbenzoic acid, benzyltriethyl amine chloride,
Variations in Method	Porto (2005), 1 mmol of pentene starting material	Porto (2005), 10 mmol of pentene starting material	Porto (2005), 1 mmol of pentene starting material, added pressure	Biphasic, Vishwakarma et al. (1998), Davis and Chattopadjyay (1987)
Product Description	Small amount clear colorless liquid, with white precipitate	Yellow liquid	Clear colorless liquid	Orange/brown liquid
GC-MS Analysis	Dichloromethane solvent peak at 1.470min	Dichloromethane solvent peak at 1.470min	1,2-epoxypentane present at 2.317 min	dichloromethane (1.470min), 1,2-epoxypentane (2.316min)
NMR Analysis	N/A	(A.18)	N/A	N/A
Percent Yield	----------	158.77% (1.37g)	----------	54.5% (.176g)

*Table 5. Summary of synthetic methods attempted in the synthesis of a terminal epoxide as a model for step 1 of phase 1 of the proposed synthetic plan.***

Aminolysis of Terminal Epoxide			
Method	Method 9a	Method 9b	Method 11
Reagents	Product mixture from method 8, ethyl carbamate, boric acid, glycerol	Product mixture from method 8, ethyl carbamate, boric acid, glycerol	1,2-epoxyoctane, ethyl carbamate, cyanuric trichloride, hydrochloride
Variations in Method	Halimehjani et al (2012), catalyzed by boron chelate complex, 3 drops of glycerol	Halimehjani et al (2012), catalyzed by boron chelate complex, 5 drops of glycerol	Kamble and Joshi (2009), catalyzed by cyanuric trichloride, solvent free
Product Description	Clear colorless liquid	Clear colorless liquid	Clear colorless liquid
GC-MS Analysis	dichloromethane (1.470 min) and ethyl acetate (2.323 min)	dichloromethane (1.477 min) and ethyl acetate (2.333 min)	Starting reagent 1,2-epoxyoctane (8.511 min, library search quality of 80), starting reagent urethane ethyl carbamate (6.161 min, library search quality of 87), 1,2-octanediol (10.987 min)

*Table 6. Summary of synthetic methods attempted in the aminolysis of a terminal epoxide using carbamates as a model for the combined steps 2 and 3 of phase 1 of the proposed synthetic plan.***

1,3-Cycloaddition Using 1,4-pentadiene	
Method	Method 14
Reagents	1,4-pentadiene, dibromoformaldoxime, sodium bicarbonate
Product Description	Clear colorless liquid
GC-MS Analysis	3-bromoisoxazoline (9.613 min) in ethyl acetate (2.544 min)

*Table 7. Summary of the synthetic method attempted in the synthesis of a novel 3-bromoisoxazoline as a model for step 5 of phase 2 of the proposed synthetic plan .***

Conclusions and Future Work

A novel 3-bromoisoxazoline has been proposed as a PfGAPDH inhibitor with potential interactions in both the active site and the NAD+ binding site. A novel 3-bromoisoxazoline has been synthesized and an epoxidation procedure has been modeled. While the proposed drug and synthetic route have theoretical support, the cost of reagents, difficulty in storage and potential decomposition of products, and large time investment of the synthesis limit its usefulness. Future work could attempt the two phases of the synthetic plan as seen or attempt to functionalize the 5-propenyl group on the simplified isoxazoline. More realistically, future work could include the use of the remaining 1,1-dibromoformaldxoime to synthesize novel 3-bromosoxazolines. These could be used in an investigation of potential ether groups in the 3-postion on the isoxazoline ring.

Experimental Section

Method 1a: An aldoxime was attempted to be synthesized from isobutylformate with a procedure adapted from Rodrigues (2013), using 25% of the suggested molar ratios. This corresponds to Method 1a in *table 2*. First, a gas trap was set up to collect any gaseous HCl

which could form. Then, 0.58mL of isobutylformate was dissolved in 12.5mL of methanol in a 50mL round bottom flask. 1.057g of NH2OH HCl was dissolved in 3mL of DI water, and was added to the solution of isobutylformate in methanol. This was then stirred for 2.5 hours at room temperature. After stirring, the reaction mixture was poured into a separatory funnel, then extracted using 4mL of diethyl ether three times saving the top organic layer and pouring the bottom aqueous layer back into the separatory funnel. After the first addition of diethyl ether, a GCMS sample was taken. However, it was difficult to see the separation between the organic layer and the aqueous layer, because of the fast evaporating solvent. Because of this, the aqueous layer extracted was once more by adding using 13mL of diethyl ether. The aqueous bottom layer was saved, and the organic top layer was dried with some anhydrous sodium sulfate until the clumping looked like sand. A gravity filtration was completed by wetting a piece of filter paper with diethyl ether in a funnel, the pouring over the organic layer with anhydrous sodium sulfate, and finally rinsing both the filter paper and the organic container with diethyl ether. This solution was stored for a week before being used in Method 1b.

Method 1b: The synthesis of an isoxazoline was attempted from the product of Method 1a as discussed in Method 1b in *table 3.* The procedure was adapted from Rodrigues (2013). First the organic solution from Method 1a was cooled to 14^{o}C. Then at 15^{o}C, 0.795mL of triethylamine and 0.584g of cyanuric acid were carefully added with a spatula. A very small amount of cyanuric acid caused the reaction to fizz, and after adding both the triethylamine and the cyanuric acid the temperature of the reaction mixture rose to 24^{o}C. The reaction mixture was cooled in an ice bath for 15min reaching 7^{o}C. Then the reaction was stirred for 34min, and the white precipitate was removed through simple filtration. Then 0.940mL of methyl acrylate, the dipolarophile, was added to the organic solution resulting from the filtration. This was stirred for 4 hours and 11min. The reaction solution was stored and capped over the weekend. Then precipitate was filtered out of the solution once again, because it may have been isocyanuric acid as suggested by Rodriguez (2013). This precipitate was saved. The resulting organic solution was rotovapped. The solution decreased in volume significantly and became a darker yellow color. After storing the rotovapped solution for a week, some solid crystalized on the side of the round bottom flask. At this point, another GCMS sample was taken and dissolved in diethyl ether and ethanol.

Method 2a: An aldoxime was attempted to be synthesized from isobutylformate with a procedure adapted from Rodrigues (2013), using 25% of the suggested molar ratios. This corresponds to Method 2a in *table 2*. For method 2a, the same reaction from method 1a was attempted; however, the solvent and some reaction times were adjusted--ethanol was used as the solvent instead of methanol, the reaction solution was stirred for 2 hours at room temperature, and the reaction solution was extracted with 20mL of dichloromethane and 10mL of DI water instead of diethyl ether.

Method 2b: The synthesis of an isoxazoline was attempted from the product of Method 1a as discussed in Method 2b in *table 3.* The procedure was adapted from Rodrigues (2013). For

method 2b, the same reaction from method 1b was attempted; however, the solvent for the reaction was dichloromethane.

Method 3: In both methods 3 and 5, the synthesis of glyoxylic acid aldoxime was attempted to eventually be used as a starting reagent in the synthesis of 1,1-dibromoformaldoxime as seen in *table 3*. The procedure was adapted from Ramòn (2010). The general procedure for both methods--Monitor the reaction periodically using TLC with 5% Acetic Acid in Ethyl Acetate as the solvent. Create a mixture of 1:1:2 H2O/EtOH/ice (20mL), then add 20 mmol of aldehyde (1.841g of glyoxylic acid) and 1.39g (20mmol) of hydroxylamine hydrochloride. While keeping the temperature below 30°C, add 4mL of a 50% aqueous solution of NaOH (40mmol). Stir for 18hr (time from 1p, may act most similarly to a close acid group). Extract with Et_2O. While keeping the temperature below 30°C, add concentrated HCl to the aqueous phase until the pH reaches 6. Extract with Et_2O again, then dry the organic phases over $MgSO_4$. Finally, evaporate solvent.

A very careful attempt to produce a mixture of 1:1:2 H_2O/EtOH/ice (20mL) proved to be unsuccessful because of the ice melted quickly and the amount of ice added was difficult to measure. In method 3, more ice was added than originally planned to keep the temperature down when the aqueous solution of NaOH was added. First, 1.872g of glyoxylic acid monohydrate and 1.404g of NH_2OH HCl were added to the 1:1:2 H_2O/EtOH/ice mixture. Then 1.605g of NaOH pellets were dissolved in water after adding the NH_2OH HCl. Eventually added 0.846g more NaOH pellets dissolved in water later to make up for the base lost to the acid group of glyoxylic acid. The NaOH solution was not allowed time to cool fully before adding it to the reaction mixture. NaOH was added to the reaction solution too quickly, so the temperature rose to 30°C. In an attempt to maintain the temperature, more ice was added. After adding the NaOH solution, the reaction mixture was stirred for 44 hours. After stirring, the reaction mixture was extracted with diethyl ether. The organic layer was saved, and then the aqueous layer was brought to 6pH using concentrated HCl. Finally, the aqueous layer was extracted once again with diethyl ether again, and two organic layers were combined. The combined organic solution was gravity filtered through a bed of $MgSO_4$. The resulting organic solution was rotovapped with no heat which yielded 0.482g (26.9% yield) of product.

Method 4: For methods 4 and 6, the synthesis of 1,1-dibromoformaldoxime was attempted from the resulting product from Method 3 as seen in *table 4.* The procedure was adapted from Wang (2017) abstract. First an ice bath was created and 10mL of water was added to a round bottom flask in the ice bath. Then another 10mL of water was added to the round bottom flask containing 0.482 g of product from method 3, and then this solution was added to the round bottom flask in the ice bath. After 15min the solution cooled to 2°C, and 0.56mL of liquid bromine was added to the solution. This did not fully dissolve and collected at the bottom. This mixture was stirred for 3hours and 1min and was then capped and stored in the fridge. A week later, the reaction solution was retrieved from the fridge and solid had formed around the magnetic stir bar. The reaction mixture was poured into a separatory funnel, and the round bottom flask was rinsed with ethyl acetate. Three layers formed in the separatory funnel after shaking—top organic was red and clear, middle aqueous was yellow and clear, and the bottom

layer was red bromine. The aqueous later was poured back into the separatory funnel and extracted with 12mL of ethyl acetate and only two layers formed. The top organic layer was saved with the organic from the first extraction. The aqueous layer was extracted with ethyl acetate once again, forming two layers. This organic layer was saved separately from the rest of the organic layers. The organic layers from the first two extractions was dried with Na_2SO_4, filtered into a 100mL round bottom flask (mass=45.036g), and was rotovapped some then stored in the fridge overnight. Then the rotovapping was continued resulting in 0.069g of white/peachy product. A GCMS sample was taken with ethyl acetate as the solvent (A.8). The organic layer from the final extraction was dried with Na_2SO_4 was dried with Na_2SO_4, filtered into a 25mL round bottom flask (mass=28.338g), and was rotovapped resulting in a small streak of yellow solid product, .005g of product. A GCMS sample was taken with ethyl acetate as the solvent (A.7).

Method 5: In both methods 3 and 5, the synthesis of glyoxylic acid aldoxime was attempted to eventually be used as a starting reagent in the synthesis of 1,1-dibromoformaldoxime as seen in *table 3*. The procedure was adapted from Ramòn (2010) as discussed in Method 3. An aqueous solution of 2.413g of NaOH in 6.1mL was prepared. 1.842g of glyoxylic acid monohydrate and 1.392g of NH_2OH HCl were added to the 1:1:2 H_2O/EtOH/ice mixture. Then the fully cooled aqueous NaOH solution was added dropwise, never reaching 30^oC. After adding NaOH, the reaction mixture was stirred for 22 hours. After stirring, the reaction mixture was extracted with diethyl ether. The organic layer was saved, and then the aqueous layer was brought to 6pH using concentrated HCl. Finally, the aqueous layer was extracted once again with diethyl ether again, and two organic layers were combined. A few spatulas of $MgSO_4$ was added to dry the resulting organic solution, and then the solution was gravity filtered. The resulting solution was rotovapped without heat. This yielded 1.0565g (59.3% yield). A GMCS sample was taken (A.10).

Method 6: For methods 4 and 6, the synthesis of 1,1-dibromoformaldoxime was attempted from the resulting product from Method 3 as seen in *table 4.* The procedure was adapted from Wang (2017) abstract. 20mL of DI was added to the liquid product from method 5. This was poured into a 50mL round bottom flask and placed in an ice bath. 1.3mL of liquid Br_2 was added to the solution and pooled at the bottom of the solution. The reaction mixture stirred for 3 hours and was then stored in the fridge. A few days later, the reaction solution was retrieved from the fridge and no solid had formed. The reaction mixture was poured into a separatory funnel, the round bottom flask was rinsed with 2.20mL of ethyl acetate, and then 10mL of ethyl acetate were added to the separatory funnel—3 layers formed. Top organic was red and clear, middle aqueous was orange and cloudy, and the bottom layer clear red. After shaking, 2 layers formed, and after pouring out the top layer considered to be the organic, but there appeared to be too much. Because of this a few drops of water were added to the bottom layer still in the separatory funnel. The water sat on top of the solution. A few drops of water were added to the poured off top layer, and the drops dissolved. Because of this, the poured off layer was determined to be the aqueous. The aqueous layer was added back into the separatory funnel, and the bottom red organic layer was drained out. An upper organic layer had formed and was left in with the aqueous layer. This was then extracted with 10mL of ethyl acetate. Only two layers formed this time. The drop test was performed once again, and bottom layer was found

to be the aqueous layer. The top organic layer was saved separately from the previously saved bottom red organic layer and the aqueous layer was extracted two more times with 10mL of ethyl acetate each. The resulting organic solution from these two extractions were combined with the top organic layer from the previous extraction. The combined top organic layers were dried with Na_2SO_4 and filtered into a 24/40 200mL round bottom flask (mass=95.5492g) to rotovap without heat in a room temperature water bath. The organic solvent did not fully evaporate, and the resulting solution was stored in the fridge. The separately saved bottom red organic layer was also dried with Na_2SO_4, filtered into a 200mL 24/40 round bottom flask (mass=65.444g), and was stored in the fridge while waiting to rotovap. A GCMS sample was taken from the product mixture (A.11).

Method 7: Model of step 1 from phase 1 of the proposed synthetic plan—synthesis of a terminal epoxide from pentene using mCPBA as the epoxidizing agent. The reaction procedure was adapted from Porto (2005).[18] While in an ice bath, 0.10mL of pentene was added to 25.0mL of dichloromethane in a 50mL 14/22 round bottom flask. The solution was cooled to 2.1°C, and then 0.240g of mCPBA were added to the solution. This brought the temperature up to 5°C. While still in the ice bath, the reaction solution was stirred for 25hours and 55min with a $CaCl_2$ drying tube. A TLC of the reaction solution was taken 13min into the stirring time using a 1:4 mixture of ethyl acetate: hexane. At some point during the stirring time the magnetic stirrer stopped stirring. At this point the white pieces of mCPBA appeared to be fully dissolved, and some of the solvent had evaporated leaving a white ring of precipitate around half a centimeter from the surface of the reaction solution. Still in the water bath and 23 hours into the stirring time, the reaction was covered with parafilm and placed on a new magnetic stirrer. 0.6193g of KOH pellets were dissolved in 6.1mL of DI water added to a 50mL Erlenmeyer flask. This solution was cooled in an ice bath while setting up for the wash. A 125mL was rinsed with 125mL was rinsed with DI water and the reaction solution stopped stirring. the reaction solution was washed with 5.0mL of the ice-cold KOH solution in the 125mL separatory funnel. The organic layer was drained out into a 50mL Erlenmeyer flask and saved. Then the remaining aqueous layer was extracted three times with 10.0mL of dichloromethane. The resulting organic solution was combined with the organic solution from the wash in the 50mL Erlenmeyer flask. This was dried with Na_2SO_4, decanted into a 100mL 19/22 round bottom flask, and then rotovapped in a water bath at 28°C. A very small amount of product remained and was stored until a GCMS sample was taken. A few drops of dichloromethane were added to the round bottom flask and then pipetted into the GCMS vial (A.16).

Method 8: Model of step 1 from phase 1 of the proposed synthetic plan—synthesis of a terminal epoxide from pentene using mCPBA as the epoxidizing agent. This scaled up reaction procedure was the same as method 7.[18] Corresponding to *table 5.* First, an ice bath was created and a 500mL 14/22 round bottom flask was placed inside of it. Then 1.10mL of pentene were dissolved in 251.5mL of dichloromethane in the round bottom flask. The solution was cooled to 1°C and 2.042g of mCPBA were added to the solution. The reaction was stirred for 25hours and 45min. While the reaction was still stirring, 50.8mL of DI water was added to a 250mL Erlenmeyer flask in an ice bath. Then 5.111g of KOH pellets were dissolved in the water. The reaction stopped stirring, and the chilled 50.8mL solution of KOH and the reaction solution

were added to a 500mL separatory funnel. This was shaken and the organic layer was drained out and saved. The aqueous layer was added back into the separatory funnel and extracted three times with 9.9mL, 10.0mL, and 15.1mL of dichloromethane respectively. The resulting organic layers were combined, dried with Na_2SO_4, gravity filtered into a 500mL 14/22 round bottom flask (mass=142.3445g). The solution was rotovapped, but a bubble of water formed at the bottom of the round bottom flask. The solution was transferred into a 400mL beaker, a few drops were spilled, and the solution was dried once again with Na_2SO_4, and filtered back into the 500mL round bottom flask. This solution was rotovapped again and was then capped, parafilmed, labeled, and saved. The yield was 1.3675g (158.77% yield). GCMS and NMR samples were taken (A.17 and A.18 respectively).

Method 9a: Model of step 2 from phase 1 of the proposed synthetic plan—boric acid and glycerol in water catalyzed opening of 1,2-epoxypentate using the product mixture from method 8 and ethyl carbamate as seen in *tables 5 and 6* respectively.[35] The reaction procedure was adapted from Halimehjani (2012).[21] First a solution of 10mL of 30 mol% of aqueous boric acid was attempted to be prepared through adding 14.453g of boric acid and 10mL of DI water to a 50mL Erlenmeyer flask. This was stirred but not easily dissolved and set aside. Next, 0.25mL of product from method 8 and 0.26mL of ethyl carbamate were added to a 14/22 50mL round bottom flask. After a discussion with Ahlberg and a literature exploration into the solubility of boric acid,[37] a saturated solution of boric acid was prepared through adding 20-30mL at time of water to the previously prepared boric acid in the Erlenmeyer flask, transferring to a larger plastic storage container until 231.00mL of DI water had been added to the boric acid solution, making a total of 241.00mL of saturated boric acid solution. After this, 3.1mL of saturated boric acid solution and 2mL of DI water were added to the 50mL round bottom flask containing product mixture from method 8 and carbamate. 3 drops of glycerol were added to the reaction solution and stirring began. Alkacid test paper was used to test the pH of the at the beginning of stirring the reaction—pH of 4 (orange color). Then the reaction mixture was covered with parafilm and stirred for 14 hours and 22min. A separatory funnel was rinsed with DI water, then 10mL of ethyl acetate and the reaction solution were added to the separatory funnel. The reaction mixture was extracted three times with 10mL of ethyl acetate. The resulting organic mixture was left sitting in the hood and water droplets pooled together in the bottom of the Erlenmeyer flask. Because of this, the organic solution was decanted into another flask and dried with $NaSO_4$, gravity filtered into a 100mL 19/22 round bottom flask, parafilmed, and stored for two days. This solution was briefly rotovapped in a water bath at 72°C, then the water temperature was lowered to 43°C, and rotovapping continued. The solvent did not fully evaporate and a GCMS sample was taken (A.19).

Method 9b: Model of step 2 from phase 1 of the proposed synthetic plan—boric acid and glycerol in water catalyzed opening of 1,2-epoxypentate using the product mixture from method 8 and ethyl carbamate as seen in *tables 5 and 6* respectively.[35] The reaction procedure was adapted from Halimehjani (2012).[21] The same saturated boric acid solution which was prepared for method 9a was used for method 9b. First, 0.26mL of product from method 8 and 0.26mL of ethyl carbamate were added to a 14/22 25mL round bottom flask. After this, 3.1mL of saturated boric acid solution and 2mL of DI water were added to the 25mL round bottom

flask containing product mixture from method 8 and carbamate. 5 drops of glycerol were added to the reaction solution and stirring began. Alkacid test paper was used to test the pH of the at the beginning of stirring the reaction—pH of 6 (brown color). Then the reaction mixture was covered with parafilm and stirred for 14 hours and 22min. A separatory funnel was rinsed with DI water, then 10mL of ethyl acetate and the reaction solution were added to the separatory funnel. The reaction mixture was extracted three times with 10mL of ethyl acetate and the resulting organic solution was dried with $NaSO_4$, gravity filtered into a 100mL 19/22 round bottom flask, parafilmed, and stored for two days. After this, the organic solution was rotovapped in a water bath at 35°C, increased the temperature to 40°C, and finally at 45°C. The solvent did not fully evaporate. A GCMS sample was taken (A.20).

Method 11: Model of step 2 from phase 1 of the proposed synthetic plan—solvent free, cyanuric trichloride catalyzed aminolysis of a terminal epoxide corresponding to *table 6.* Procedure adapted from Kamble (2010).[22] As suggested by Dr. Hayes, a 200% molar excess of 1,2-epoxyoctane was used. First, 0.46mL of 1,2-epoxy octane was added to a 20mL round bottom flask. Then 0.8mL of ethyl carbamate and .11g of cyanuric trichloride were dissolved in the 1,2-epoxyoctane. The reaction was stirred for 4 hours and 28min. TLC of ethyl carbamate, 1,2-epoxyoctane, and reaction solution were taken 42 min into the stir time and another TLC was taken near the end of the stir time. A 50/50 mixture of ethyl acetate and petroleum ether was used as the mobile phase. While the mixture was still stirring, 1mL of concentrated HCl solution was added to an empty 100mL beaker, and then 23mL of DI water were added to the HCl to produce 24mL of 0.5N HCl solution. 10mL of the 0.5N HCl solution was added to a separatory funnel. The reaction solution stopped stirring and was added to the separatory funnel. The round bottom flask was rinsed with 5mL of .5mL and added this to the separatory funnel. The excess aqueous HCl was disposed of in the aqueous waste container. The separatory funnel was shaken, and the both the aqueous and organic layers were left in. Then the mixture in the separatory funnel was extracted three times with 10mL of diethyl ether each. The resulting organic solution was dried with $NaSO_4$ and then gravity filtered into a round bottom flask. The dried solution was then rotovapped and a sample was taken for GCMS analysis.

Method 12: Model of step 1 from phase 1 of the proposed synthetic plan--Epoxidation of pentene under pressure corresponding to *table 6*. Procedure adapted from Porto (2005) with the addition of pressure as suggested by Ahlberg and influenced by Sheng (1970).[18,19] First a pressure flask was placed in a ices bath. A pipet was used to collect 8.0mL of dichloromethane from a bottle, but then something was floating in the graduated cylinder. Because of this the dichloromethane was gravity filtered into the pressure flask to give a clear liquid. Then 0.110mL of pentene were added to the pressure flask, and another 8.2mL of dichloromethane were added to the pressure flask through the gravity filter. This solution was then cooled to 3°C, and 266g of mCPBA were added to the pressure flask. The cap of the pressure flask was screwed on and the reaction stirred for 23 hours and 36min. Then the closed pressure flask was stored in the fridge overnight. The next day an aqueous solution of 0.577g of KOH pellets and 10mL of DI water was prepared in a 25mL Erlenmeyer flask. The reaction solution was retrieved from the fridge, and the 10mL KOH solution was added to a separatory funnel. Then the stored reaction

solution was added to the separatory funnel and shaken. Then both the aqueous and organic layers were removed, and the organic layer was saved. The top aqueous layer was added back into the separatory funnel and then extracted with dichloromethane. Finally, the two organic layers were combined, dried with Na_2SO_4, gravity filtered into storage containers, and a GCMS sample was taken (A.22).

Method 13: Model of step 1 from phase 1 of the proposed synthetic plan—epoxidation of a terminal alkene. Biphasic epoxidation of pentene procedure adapted from Vishwakarma (1998) and Davis and Chattopadhyay (1987).[32,33] This corresponds to *table 6.* First 0.097g of benzyl triethylamine chloride (BTEAC), a phase transfer agent, was added to a 50mL Erlenmeyer flask labeled BTEAC and then dissolved in 2.7mL of dichloromethane. Then 0.410mL of pentane were added to the BTEAC solution. Next 4.1mL of saturated $NaHCO_3$ solution were added to a 25mL round bottom flask in an ice bath. The BTEAC and pentene solution was poured into the 25mL round bottom flask. In a separate Erlenmeyer flask, 0.895g of mCPBA was dissolved in 7.5mL of dichloromethane. When the reaction solution in the 25mL round bottom flask reached 5°C, mCPBA in dichloromethane solution was added dropwise while stirring the solution. 5-10 drops were added at a time and 5 seconds of stirring was allowed in between adding mCPBA solution. During this time, the pipet rested in and was fully emptied back into the mCPBA solution because early on the mCPBA quickly recrystallized when exposed to air and blocked the end of the pipet. After adding all of the mCPBA solution the reaction temperature was 4°C, and the reaction mixture was stirred for another hour. The reaction solution was placed in the fridge. A month later, a 10% solution of Na_2SO_3 was prepared by dissolving 0.49g of Na_2SO_3 in 4.9mL of DI water in a beaker. The stored reaction solution was retrieved from the fridge and was lightly shaken to dissolve precipitate. 4.5mL of cold DI water was added to a separatory funnel and then the reaction solution was added to the separatory funnel then shaken. Drop test was performed to confirm that the bottom layer was organic. The both the aqueous layer and the organic layer were drained out. Next the resulting organic solution and the prepared 10% solution of Na_2SO_3 were added to the separatory funnel and shaken, and both layers were drained out. Next 2mL of saturated aqueous NaCL solution was added to the separatory funnel and the organic solution was poured back in. This was shaken, and then the organic bottom layer was drained out. After this, 0.087g of calcium carbonate were added to the organic solution as a drying agent; however, this was incorrect and quickly gravity filtered out. After this 0.559g of potassium carbonate were added to the reaction solution and allowed to sit for 2 hours. Then the organic solution was gravity filtered into a 25.446g 19/22 25mL round bottom flask and was rotovapped at 37°C to a small amount of liquid. The yield of the reaction was 0.176g (54.5% yield). A GCMS sample was taken and the rest stored in the freezer (A.23).

Method 14: Model of step 5 from phase 2 of the proposed synthetic plan--1,3- dipolar cycloaddition using 1,4-pentadiene as a dipolarophile and 1,1-dibromoformaldoxime as a dipole corresponding to *table 7*. Procedure was adapted from Pinto (2008).[26] First 66.8mL of ethyl acetate was added to an empty 250mL 19/22 round bottom flask. Then 1g (the full vial) of 1,4-pentadiene was added to the flask. The glass vial which contained the 1,4-pentadiene was rinsed with ethyl acetate in an attempt to collect a GCMS sample (A.24) which later resulted in mostly noise. 3.38g of 1,1-dibromoformaldoxime was added to the reaction solution in the

round bottom flask. This dissolved quickly, and then 6.4g of $NaHCO_3$ was added to the solution. This did not dissolve. The reaction was stirred for 1 hour and 50min and then the stirring speed was increased to allow for more movement of the $NaHCO_3$. At 2 hours and 30min into the reaction, a TLC of the reaction solution was taken on 60 F_{2541} silica gel plate, using 95% petroleum ether/5% ethyl acetate as the solvent. After stirring overnight, 22.5mL of DI water was added to a 125mL separatory funnel, then the reaction solution stopped stirring and was added to the separatory funnel. The solution was shaken and extracted. The resulting organic solution was dried with a few scoops of Na_2SO_4. Then the organic solution was filtered into a 250mL 19/22 round bottom flask (mass of 93.094g) resulting in a clear colorless liquid. A GCMS sample was taken for analysis (A25). The organic solution was stored in the freezer.

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GCMS Programs

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Oven program: 30°C for 3min, then increase 15°C/min to 80°C for 0.1min, and then 10°C/min to 280°C for 0 min.
Acquisition mode: Scan
Temperature of Front SS Inlet H2: 250°C
Front SS Inlet H2 total flow: 33.6mL/min
Front SS Inlet H2 pressure: 1.1048psi
Front SS H2 split ratio: 50:1
Column #1 gas flow: 0.6mL/min
Column #1 pressure: 1.1048 psi

D:\PeytonsResearch\RESEARCHMETHOD1_1.M
Oven program: 30°C for 3min, then increase by 20°C/min to 80°C for 0.1min, and then 15°C/min to 280°C for 0 min.
Acquisition mode: Scan
Temperature of Front SS Inlet H2: 250°C
Front SS Inlet H2 total flow: 33.6mL/min
Front SS Inlet H2 pressure: 1.1048psi
Front SS H2 split ratio: 50:1
Column #1 gas flow: 0.6mL/min
Column #1 pressure: 1.1048 psi

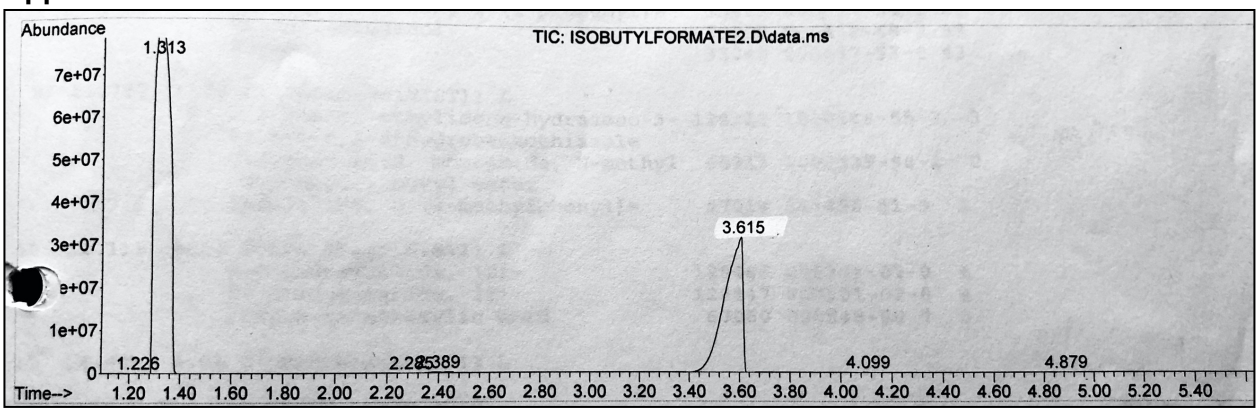
D:\PeytonsResearch\RESEARCHMETHOD1_2.M
Oven program: 30°C for 3min, then increase by 20°C/min to 80°C for 0.1min, and then 15°C/min to 280°C for 0 min.
Acquisition mode: Scan
Temperature of Front SS Inlet H2: 250°C
Front SS Inlet H2 total flow: 33.6mL/min
Front SS Inlet H2 pressure: 1.1048psi
Front SS H2 split ratio: 50:1
Column #1 gas flow: 0.6mL/min
Column #1 pressure: 1.1048 psi

D:\Research_Sara_Josselyn \RESEARCHMETHOD2.M
Oven program: 30°C for 3min, then increase by 15°C/min to 200°C for 0.1min, and then 15°C/min to 300°C for 10 min.
Acquisition mode: Scan
Temperature of Front SS Inlet H2: 300°C
Front SS Inlet H2 total flow: 33.6mL/min
Front SS Inlet H2 pressure: 1.1048psi
Front SS H2 split ratio: 50:1
Column #1 gas flow: 0.6mL/min

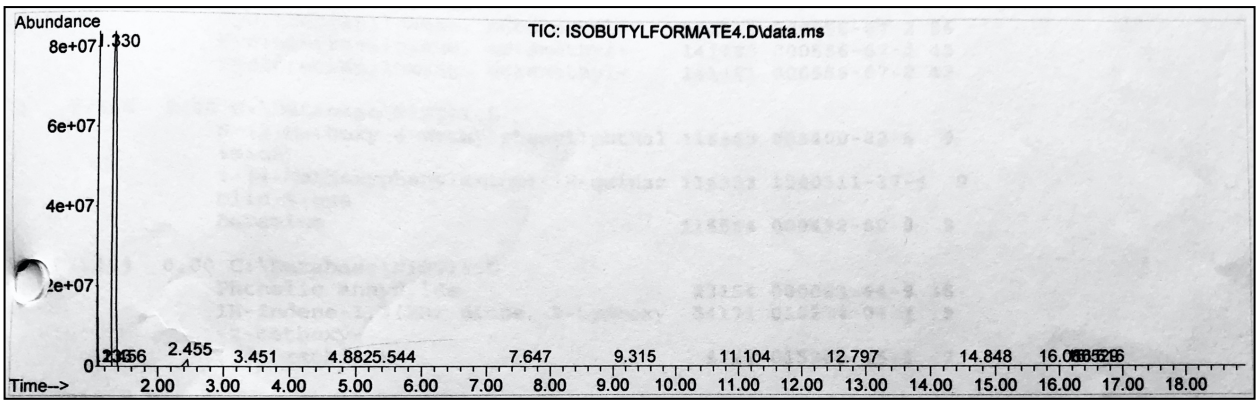
Column #1 pressure: 1.1048 psi

D:\Sarajoshhighbpmethod\HIGHBOILINGSOLV_SJ2.M
Oven program: 28°C for 3.5min, then increase by 35°C/min to 300°C for 3min.
Acquisition mode: Scan
Temperature of Front SS Inlet H2: 200°C
Front SS Inlet H2 total flow: 33.6mL/min
Front SS Inlet H2 pressure: 1.0171 psi
Front SS H2 split ratio: 50:1
Column #1 gas flow: 0.6mL/min
Column #1 pressure: 1.0171 psi

Appendix

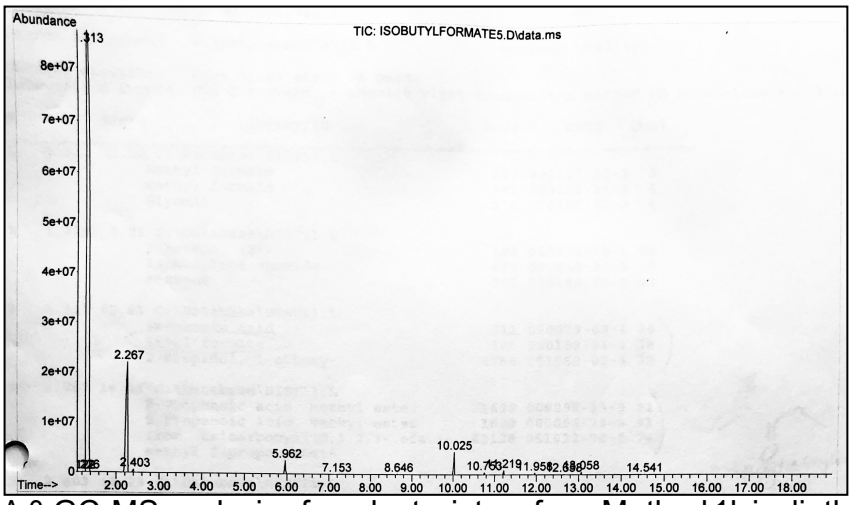


A.1 GC-MS analysis of isobutylformate (3.615 min) starting reagent in diethyl ether (1.313 min). Using the MS data, the library search report of the NIST11.L database identified isobutyl formate with a quality of 74 and diethyl ether with a quality of 46.
Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M

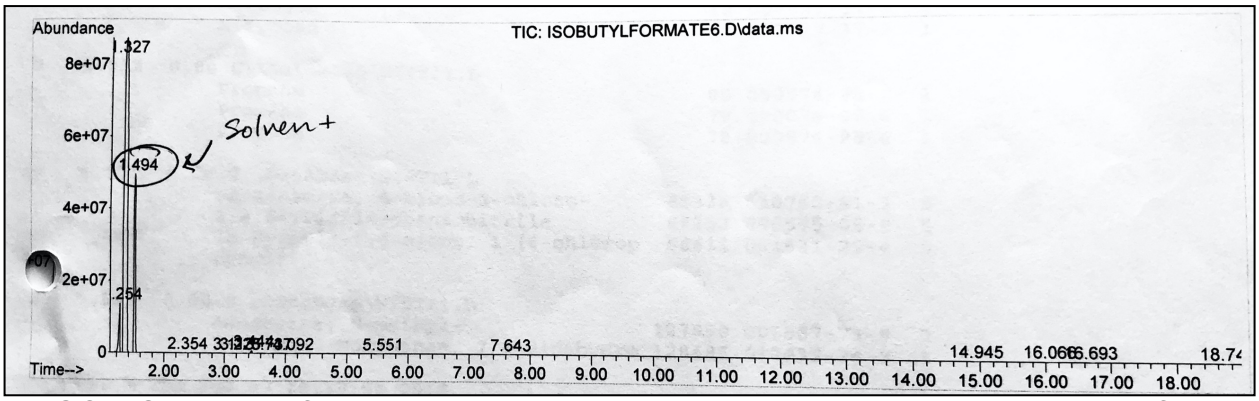


A.2 GC-MS analysis of product mixture in methanol and diethyl ether for attempted synthesis of aldoxime from isobutyl formate using Method 1a. Presence of isobutyl formate starting

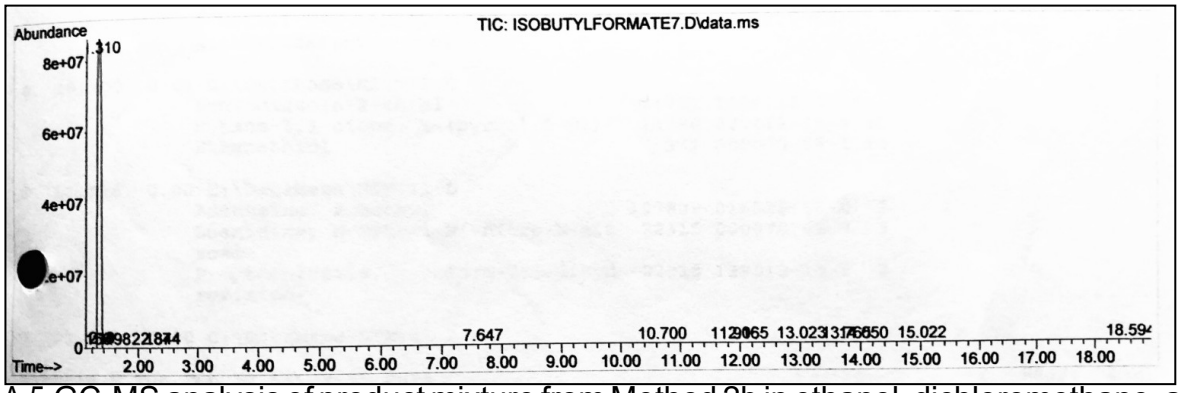
material at 3.451 min peak. Diethyl ether solvent peak at 1.330 min. Using the MS data, the library search report of the NIST11.L database identified isobutyl formate with a quality of 43. Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M



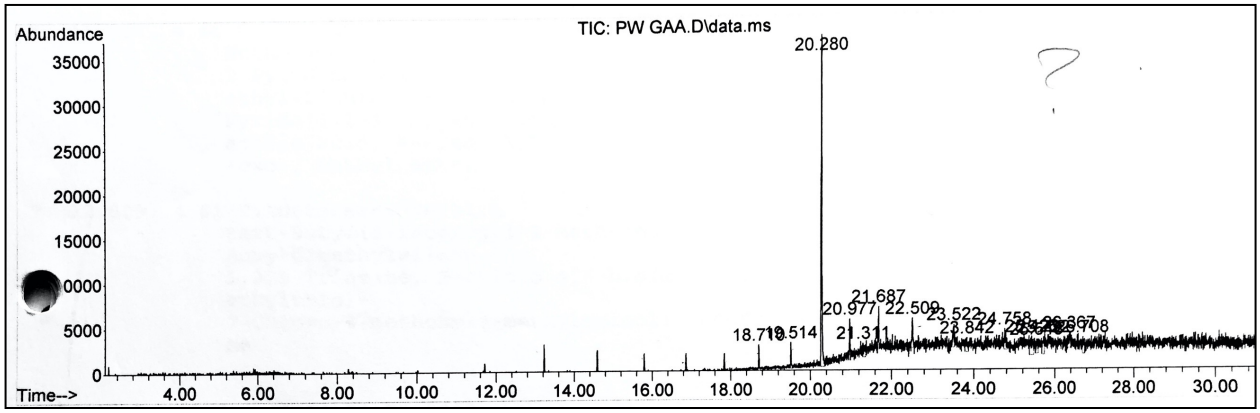
A.3 GC-MS analysis of product mixture from Method 1b in diethyl ether. This is the product from the attempted synthesis of isoxazoline from product mixture from Method 1a and methyl acrylate using Method 1b. Presence of methyl acrylate starting material at 2.267 min peak. Using the MS data, the library search report of the NIST11.L database identified methyl acrylate with a quality of 91. Additionally, the library search report indicates the halogenation of the methyl acrylate double bond to propanoic acid, 3-chloro-, methyl ester at the 5.962 min peak. Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M



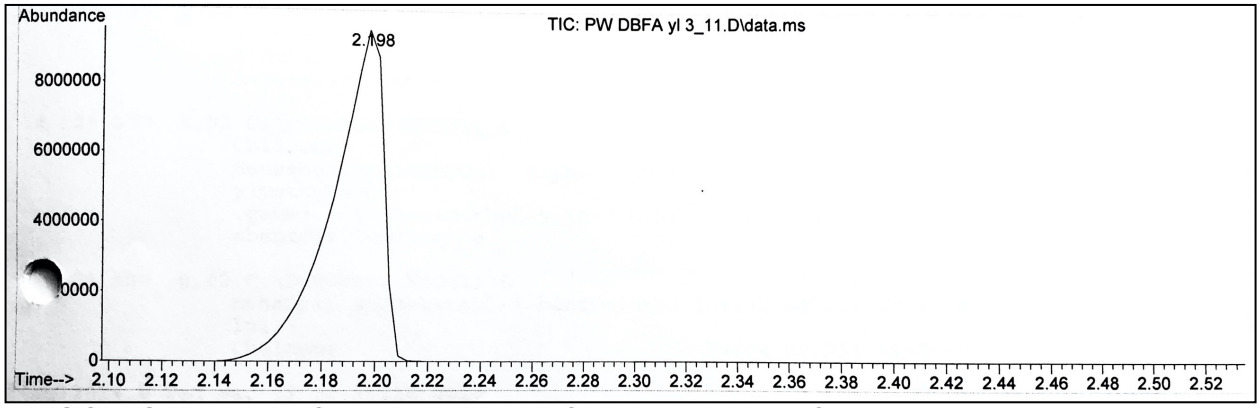
A.4 GC-MS analysis of product mixture in ethanol, dichloromethane, and diethyl ether for attempted synthesis of aldoxime from isobutyl formate using Method 2a. Presence of isobutyl formate starting material at 3.444 min peak. Ethanol, diethyl ether, and dichloromethane solvent peaks at 1.254 min, 1.327 min, and 1.494 min, respectively. Using the MS data, the library search report of the NIST11.L database identified isobutyl formate with a quality of 59. Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M



A.5 GC-MS analysis of product mixture from Method 2b in ethanol, dichloromethane, and diethyl ether. This is the product from the attempted synthesis of isoxazoline from product mixture from Method 1a and methyl acrylate using Method 1b.
Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M

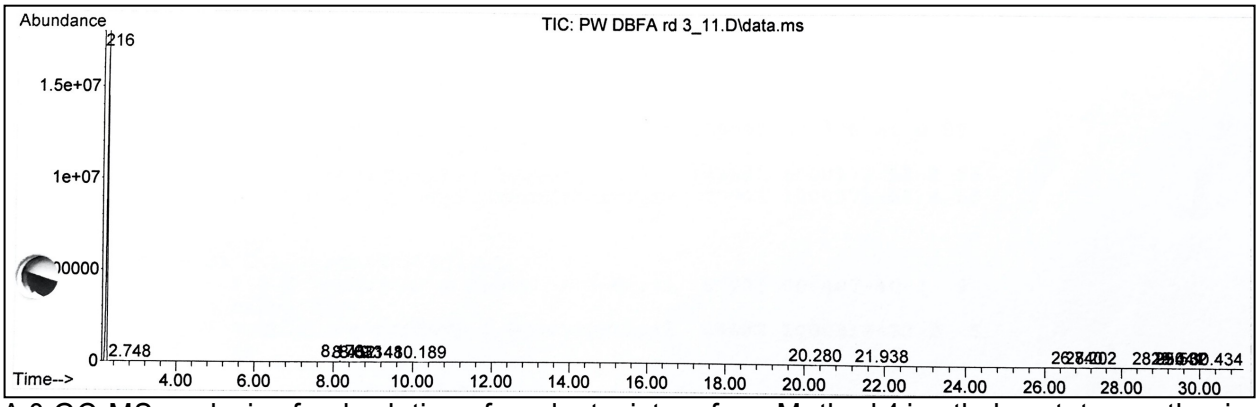


A.6 GC-MS analysis of product mixture from Method 3 in diethyl ether, synthesis of glyoxylic acid aldoxime.
Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M

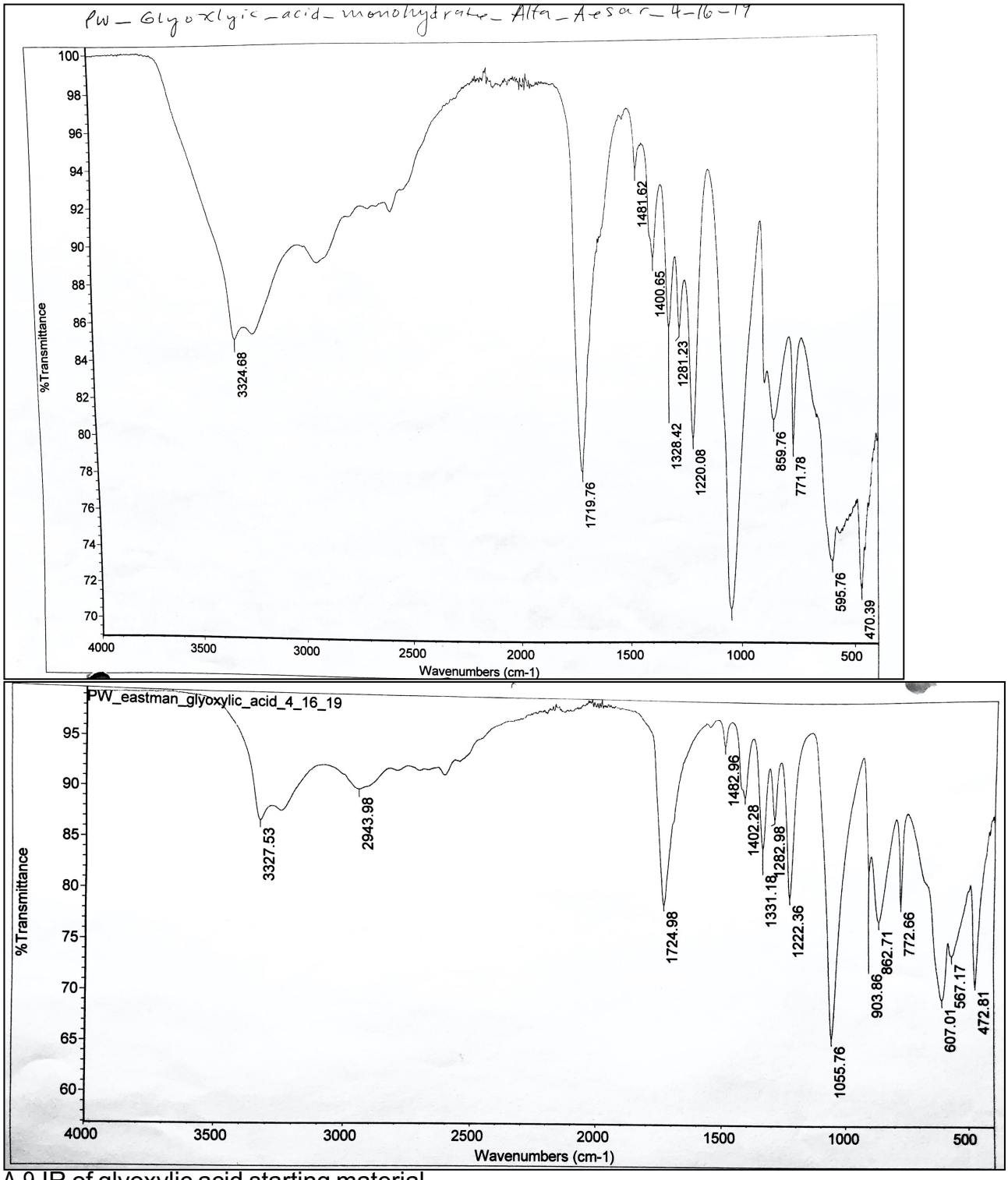


A.7 GC-MS analysis of yellow solution of product mixture from Method 4 in ethyl acetate synthesis of dibromoformaldoxime. Ethyl acetate peak is present at 2.198 min. Using the MS data, the library search report of the NIST11.L database identified the ethyl acetate solvent with a quality of 49.

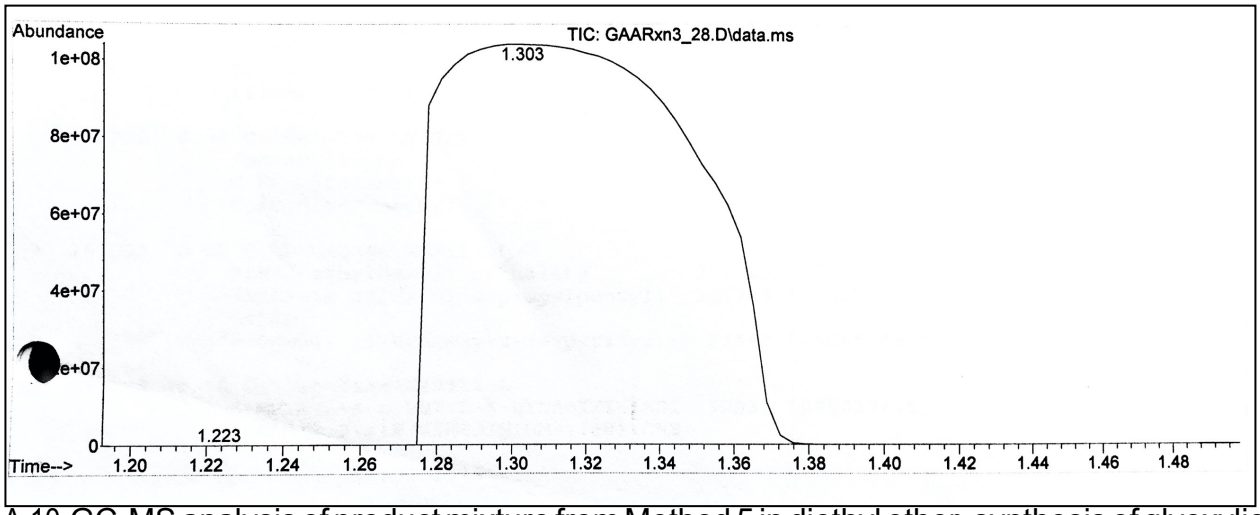
Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M



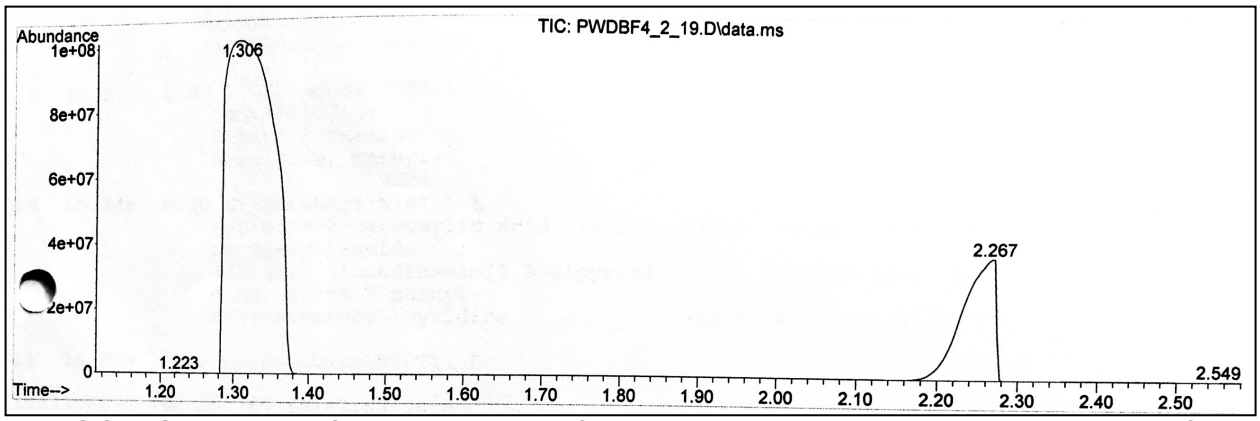
A.8 GC-MS analysis of red solution of product mixture from Method 4 in ethyl acetate, synthesis of dibromoformaldoxime. Ethyl acetate peak is present at 2.216 min. Using the MS data, the library search report of the NIST11.L database identified the ethyl solvent with a quality of 80.
Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M



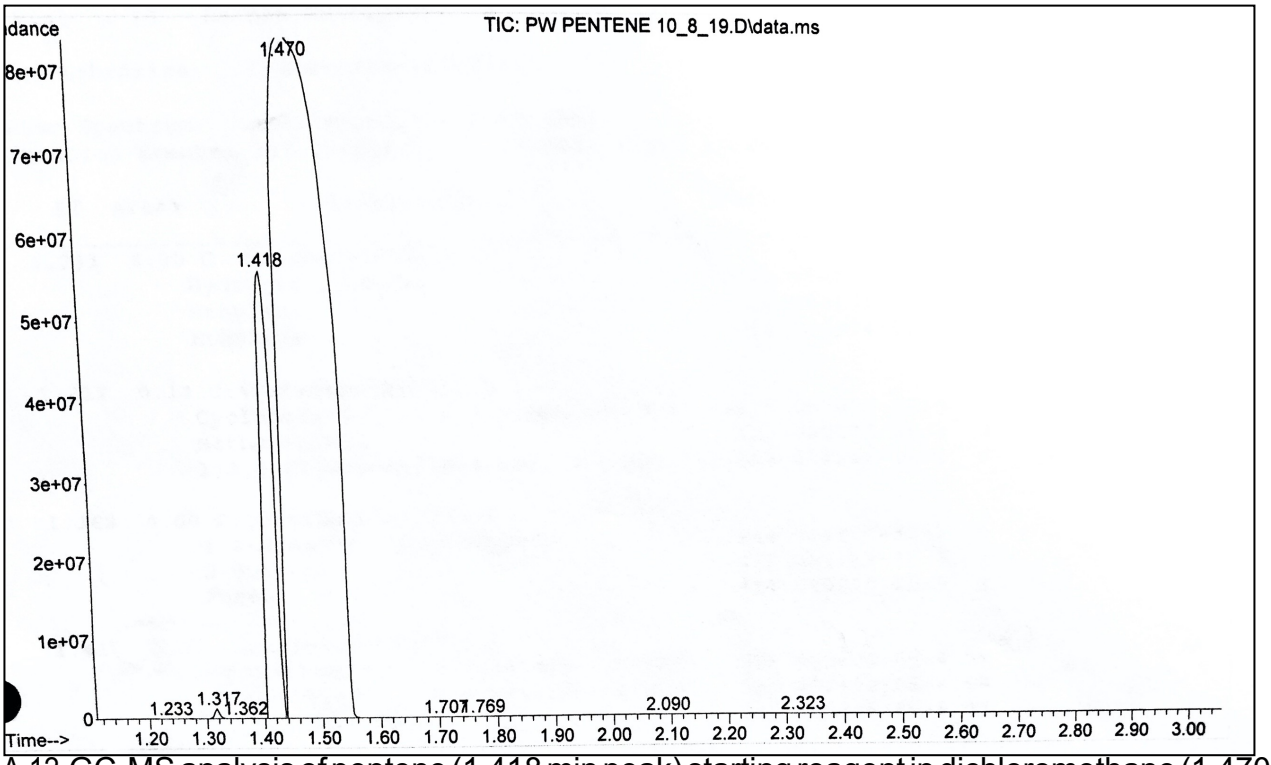
A.9 IR of glyoxylic acid starting material.



A.10 GC-MS analysis of product mixture from Method 5 in diethyl ether, synthesis of glyoxylic acid aldoxime. Peak at 1.303 min represents 99.99 percent of the solution listed as Propanoic acid in the library search with a quality of 46.
Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M

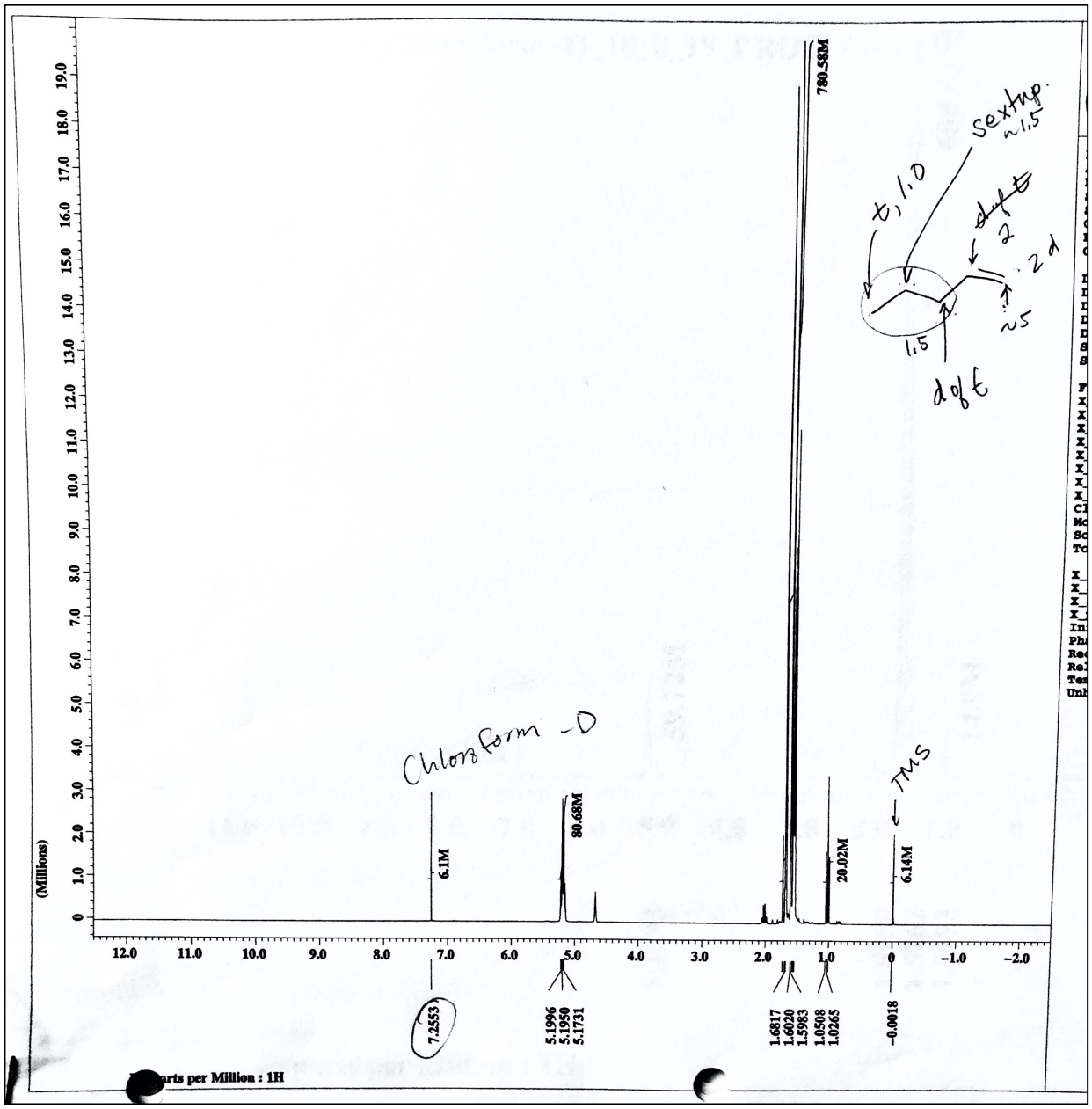


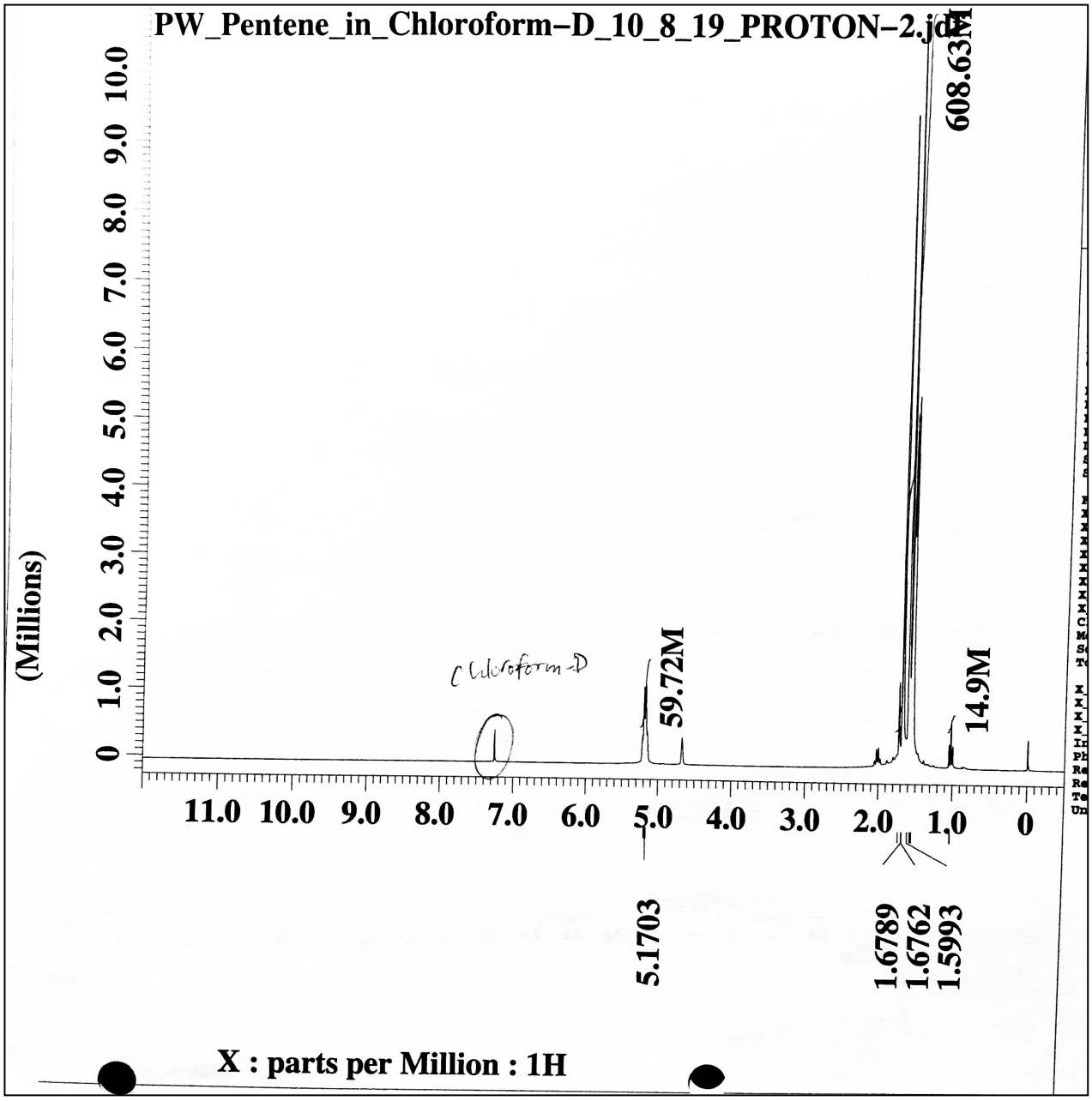
A.11 GC-MS analysis of product mixture from Method 6 in ethyl acetate, synthesis of dibromoformaldoxime. Ethyl acetate peak is present at 2.267 min. Using the MS data, the library search report of the NIST11.L database identified the ethyl solvent with a quality of 80.
Method: D:\alkenesfromalf2018\STANDARD_MIXED.D\final2018.M

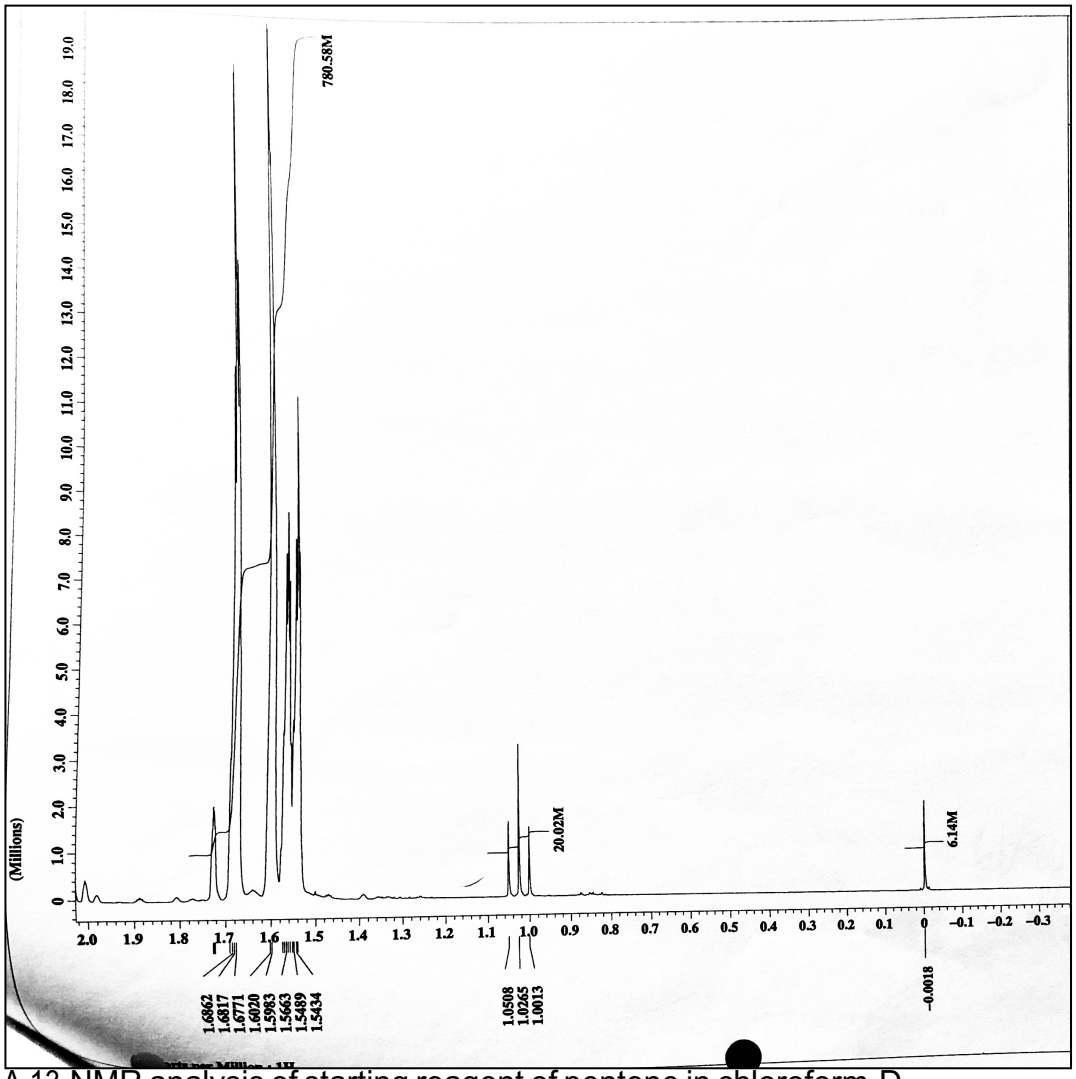


A.12 GC-MS analysis of pentene (1.418 min peak) starting reagent in dichloromethane (1.470 min).

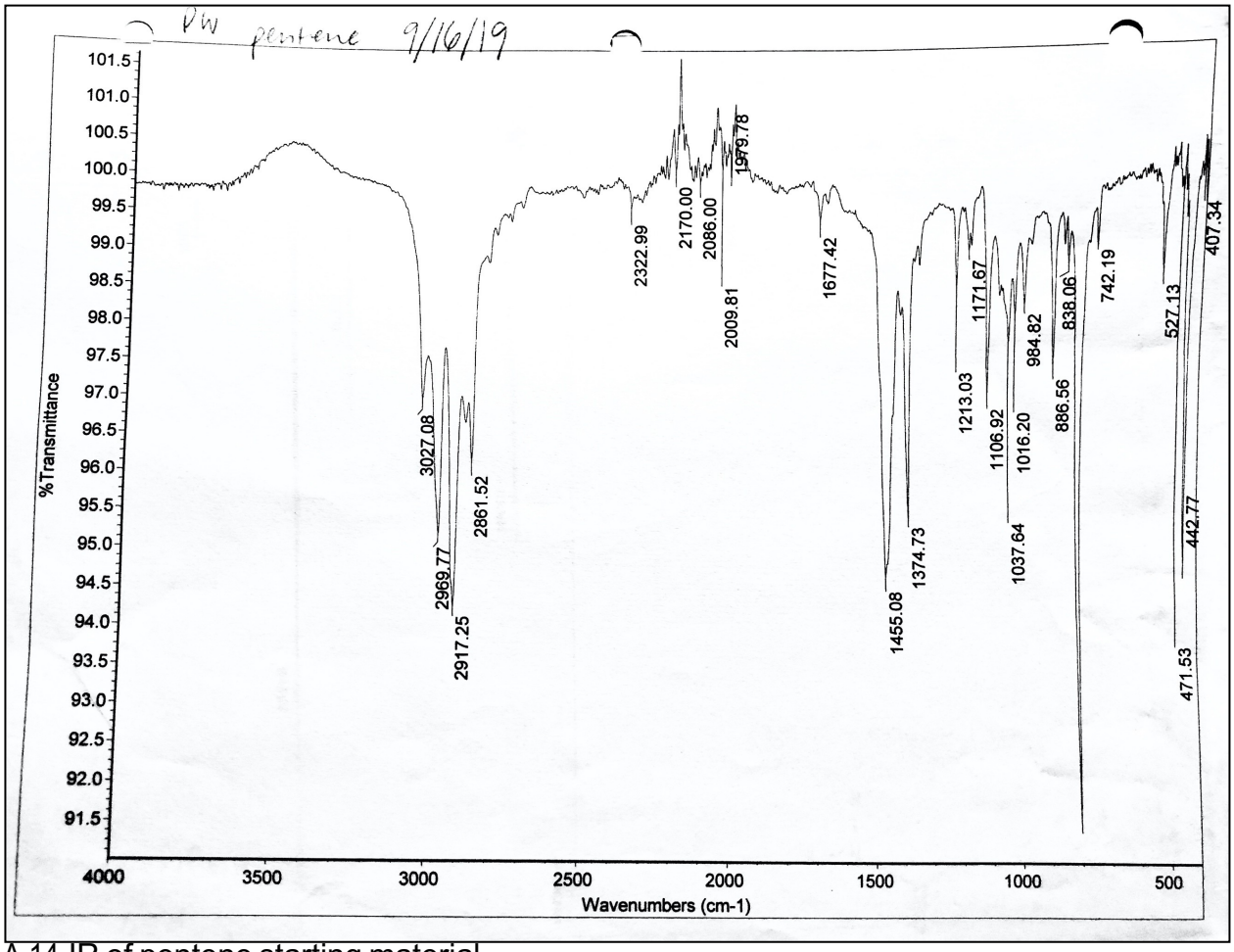
D:\PeytonsResearch\RESEARCHMETHOD1_1.M


780.58M
Chloroform -D
6.1M
80.68M
20.02M
6.14M
TMS
7.2553
5.1996
5.1950
5.1731
1.6817
1.6020
1.5983
1.0508
1.0265
-0.0018
(Millions)
Parts per Million : 1H
t, 1.0
sextup.
~1.5
dqt
2
2.1
2.5
1.5
dqt

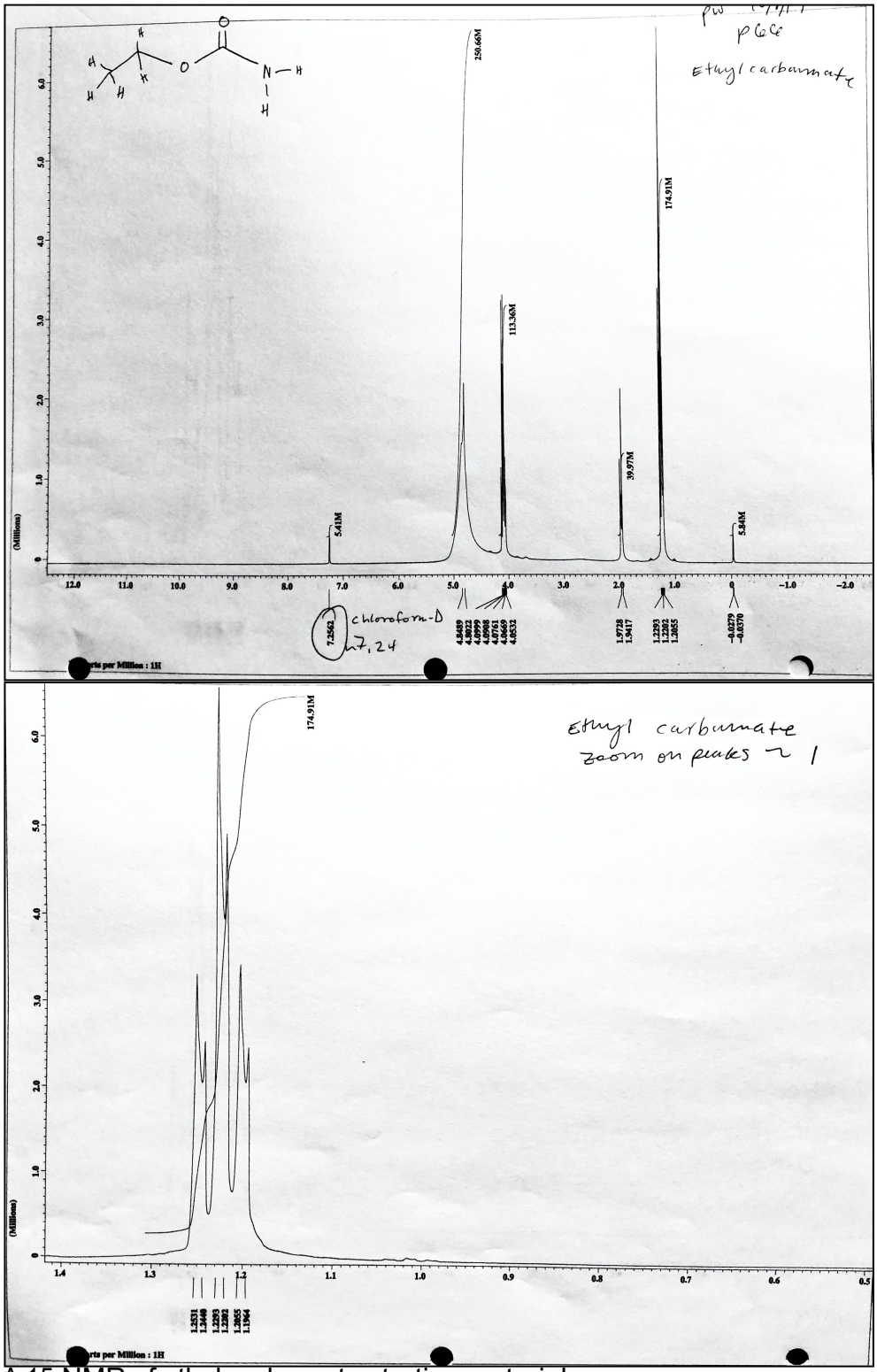

PW_Pentene_in_Chloroform-D_10_8_19_PROTON-2.jdf
608.63M
59.72M
14.9M
Chloroform-D
(Millions)
0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0
11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0
5.1703
1.6789
1.6762
1.5993
X : parts per Million : 1H



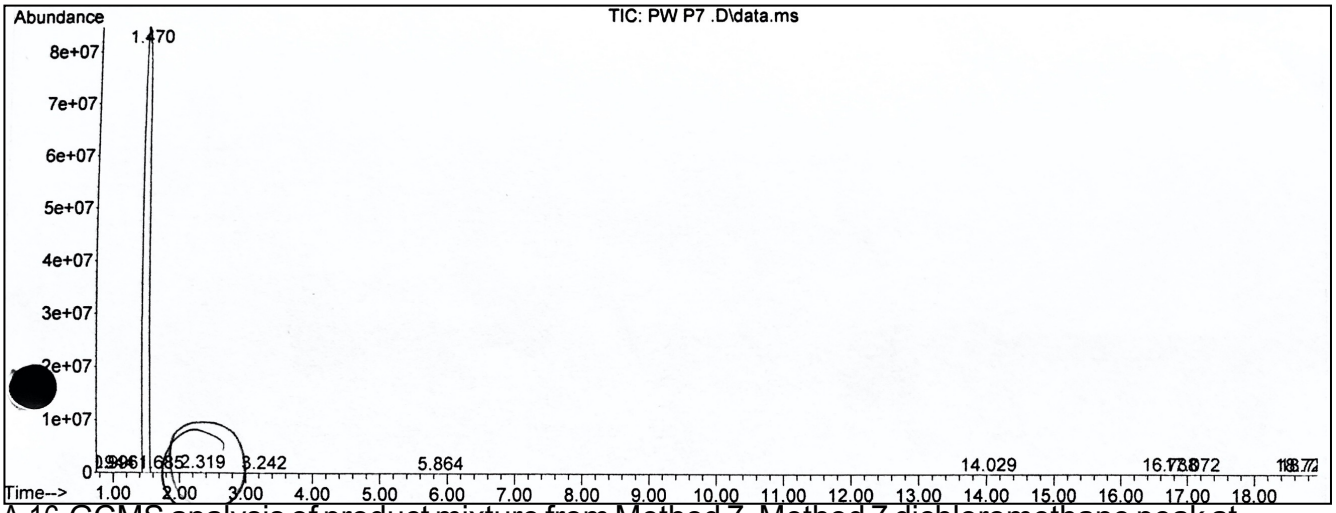
A.13 NMR analysis of starting reagent of pentene in chloroform-D.



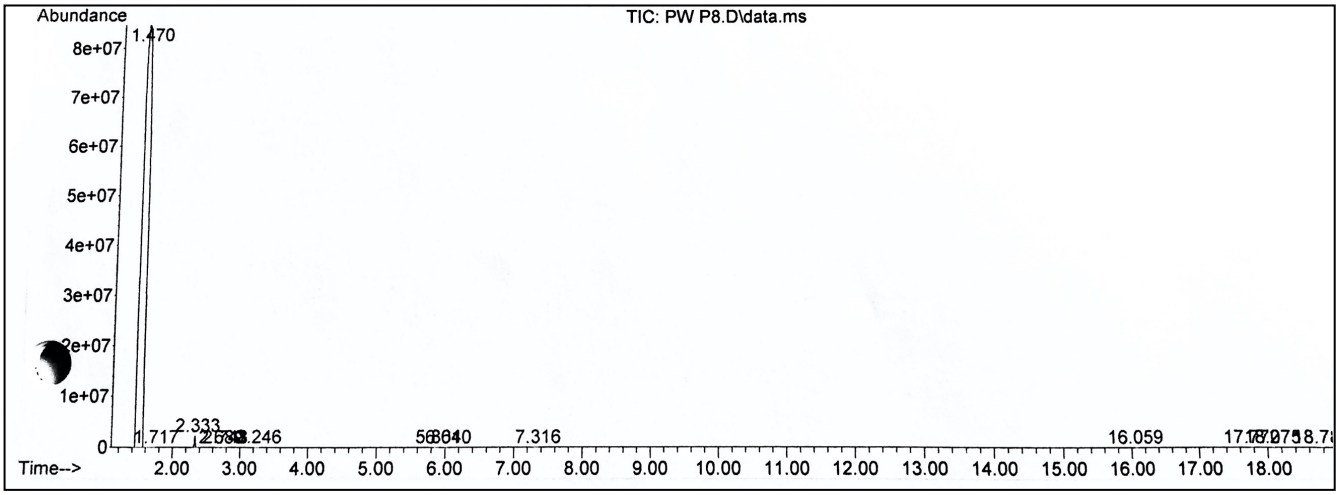
A.14 IR of pentene starting material.

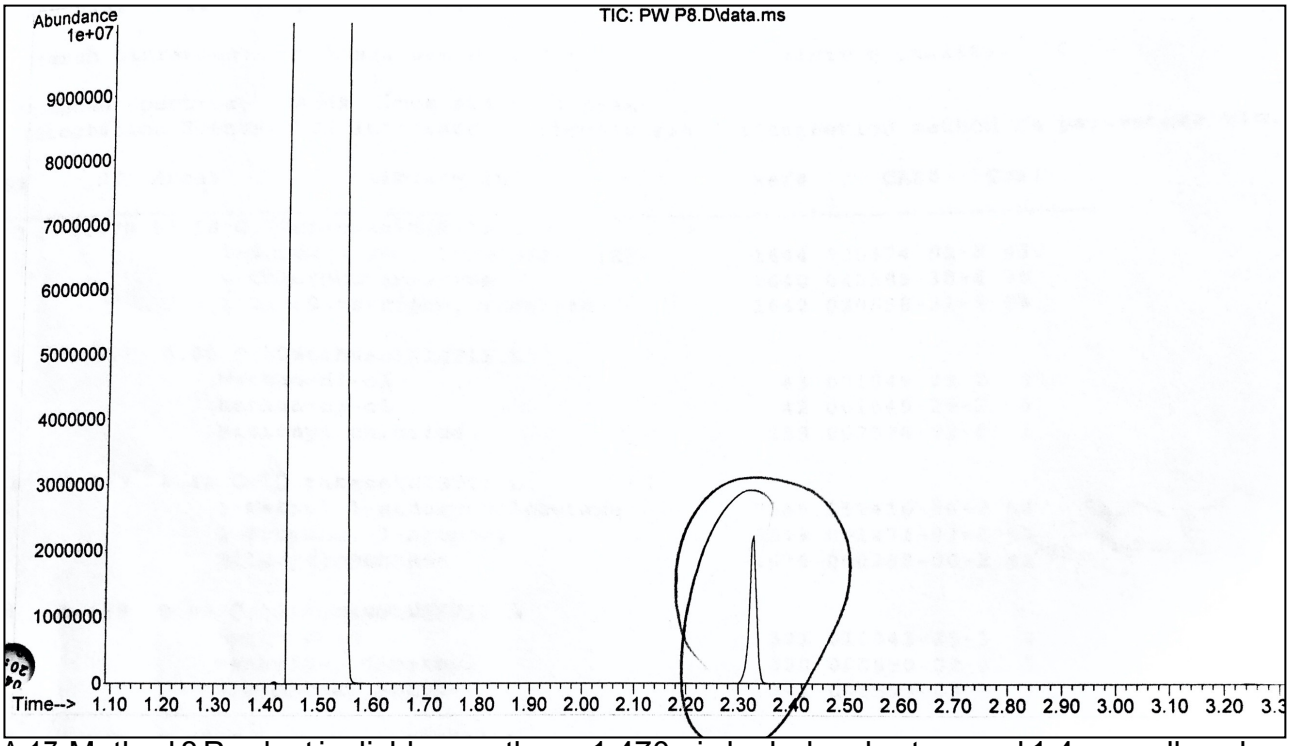


A.15 NMR of ethyl carbamate starting material.



A.16 GCMS analysis of product mixture from Method 7. Method 7 dichloromethane peak at 1.470min, product potentially at 2.319min.
D:\PeytonsResearch\RESEARCHMETHOD1_1.M





A.17 Method 8 Product in dichloromethane 1.470min look closely at around 1.4—small peak similar placement to pentene in dichloromethane. 2.30min could be product.
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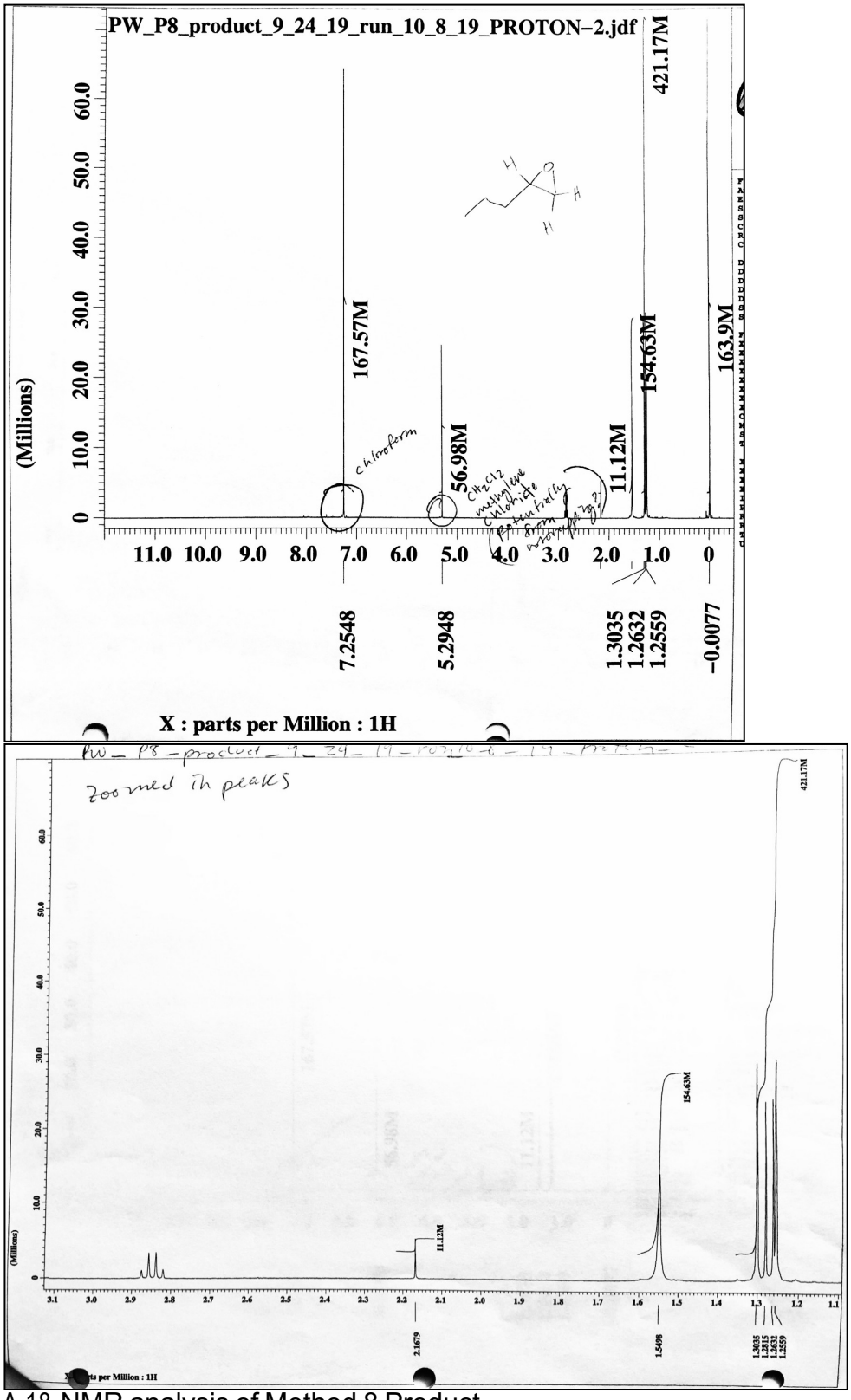
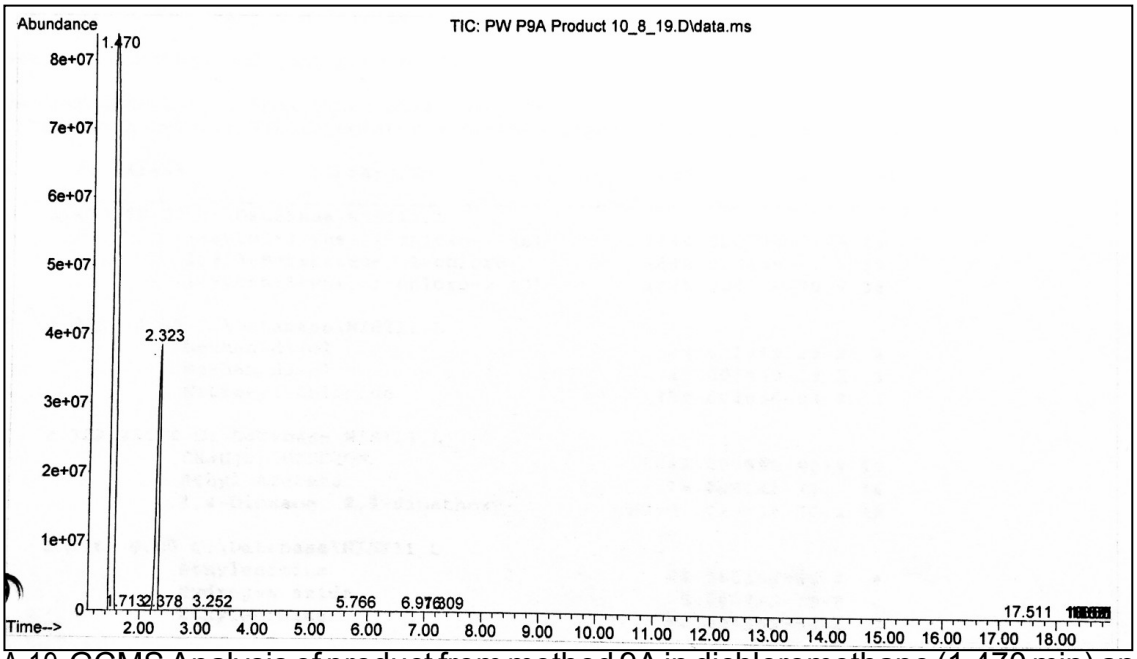


A.18 NMR analysis of Method 8 Product.



A.19 GCMS Analysis of product from method 9A in dichloromethane (1.470 min) and ethyl acetate (2.323 min, identified by library search report with a quality of 36).
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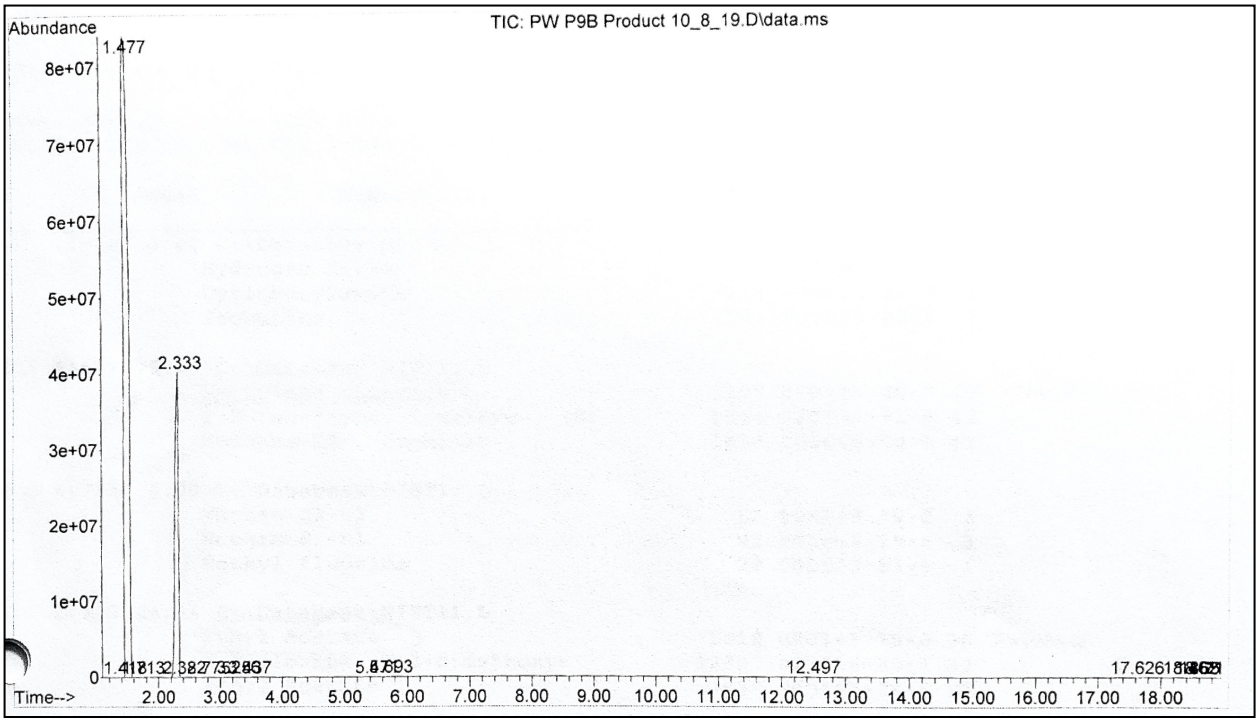

A.20 GCMS analysis of product 9B in dichloromethane (1.477 min, identified by library search report with a quality of 47) and ethyl acetate (2.333 min, identified by library search report with a quality of 36)
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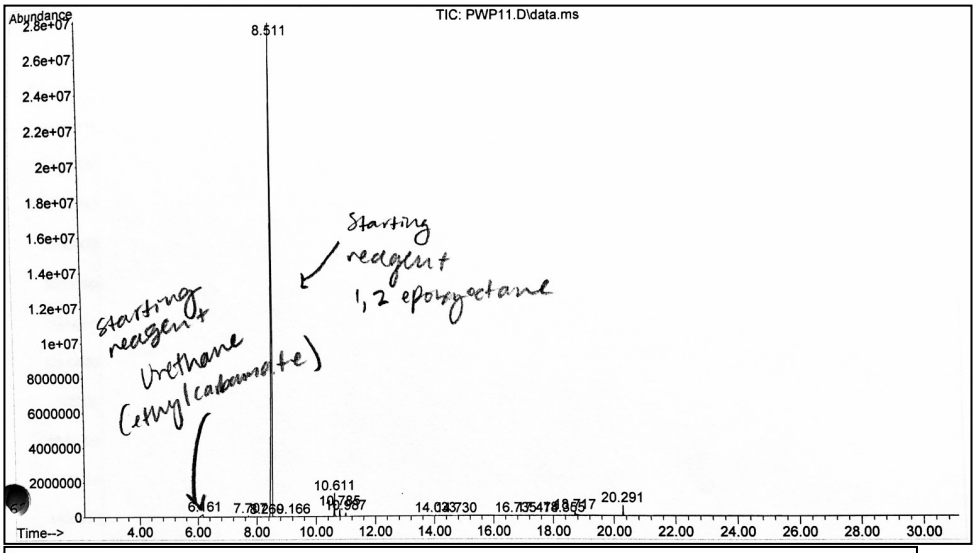
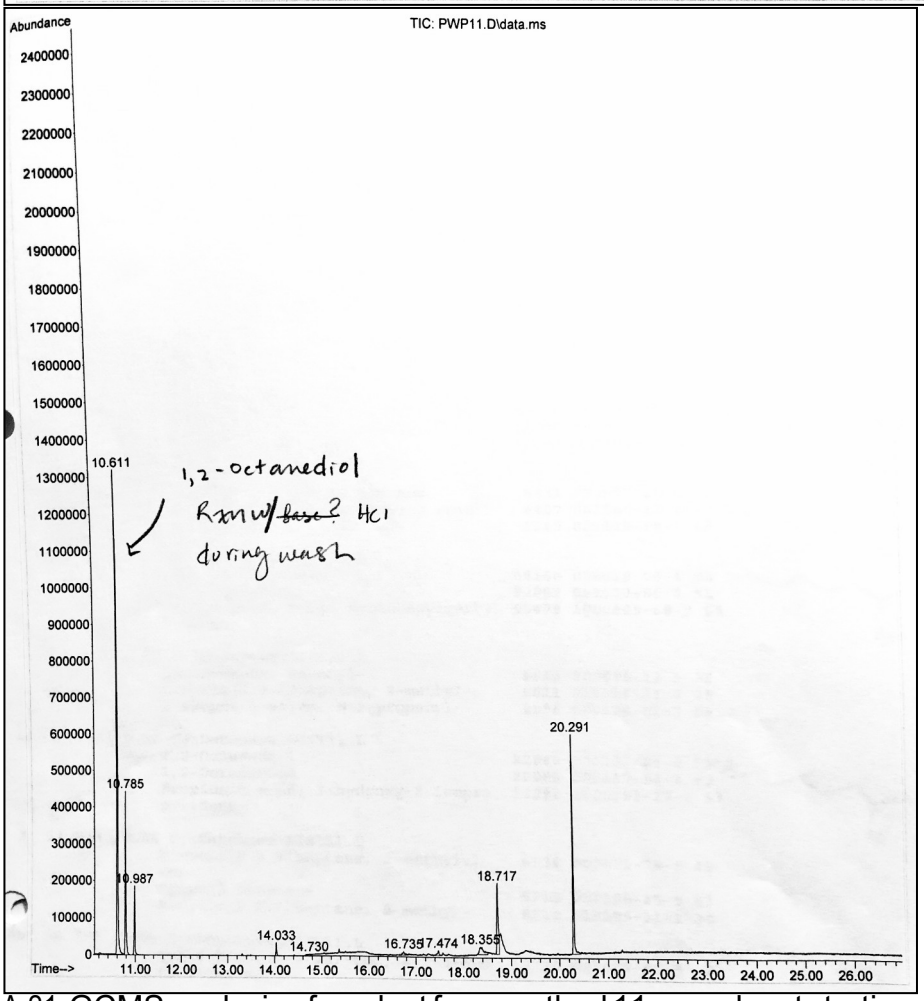
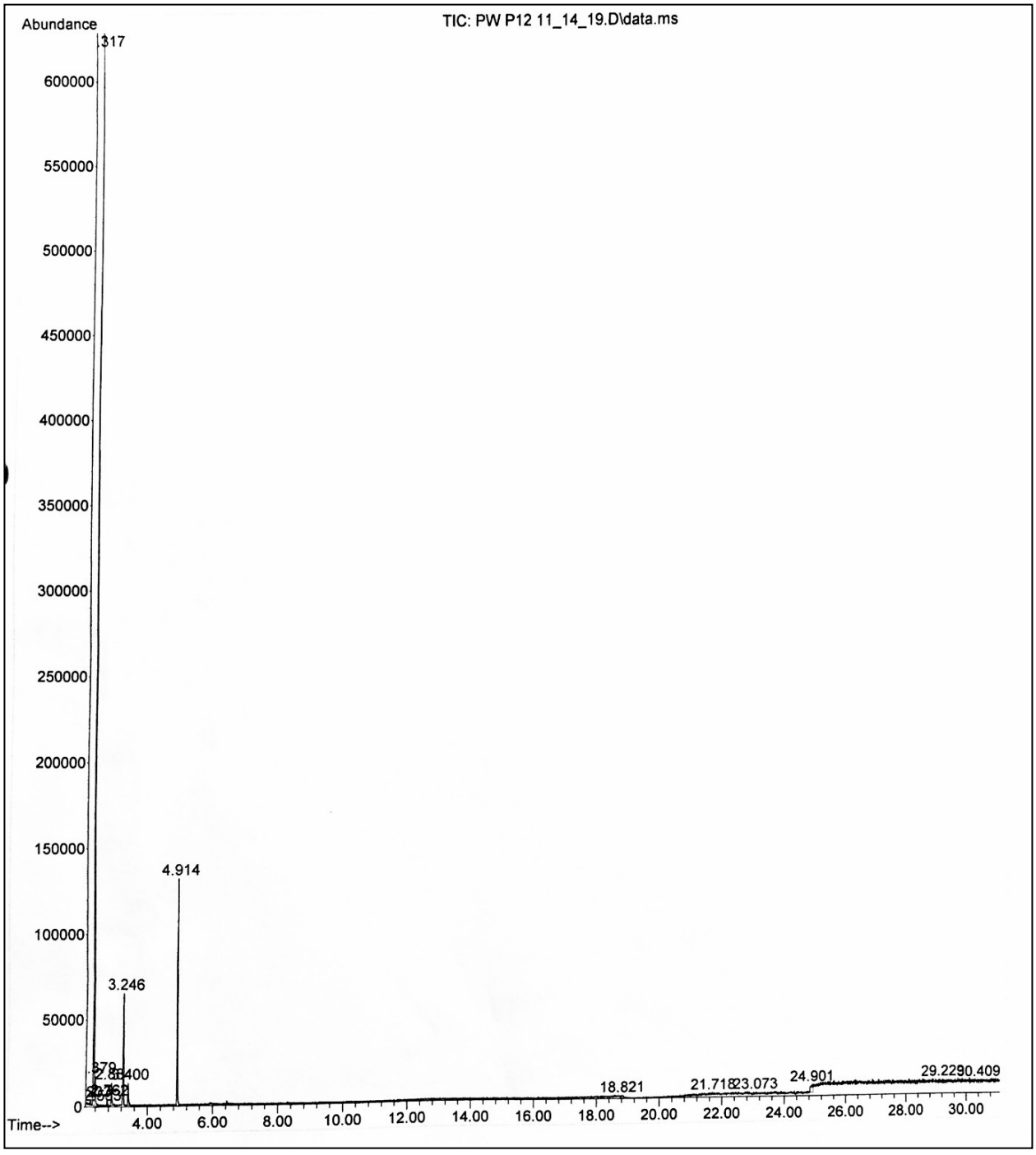
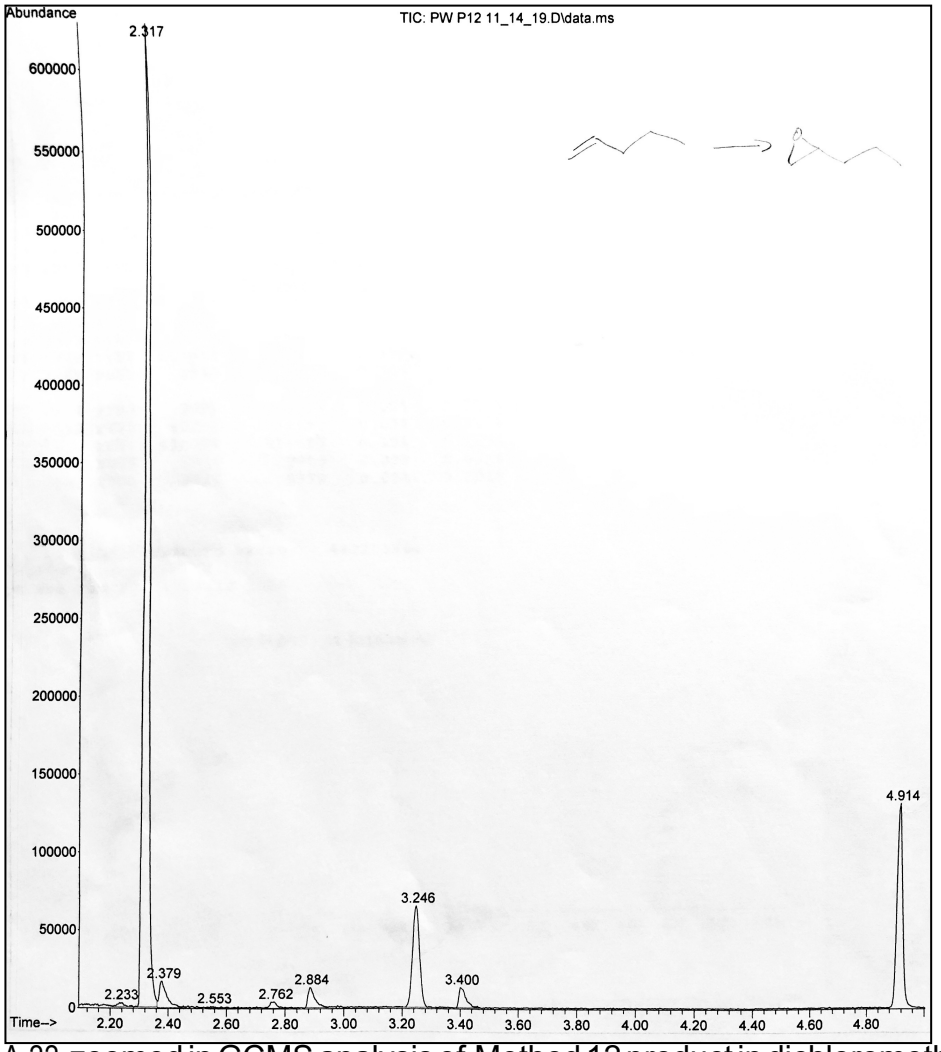


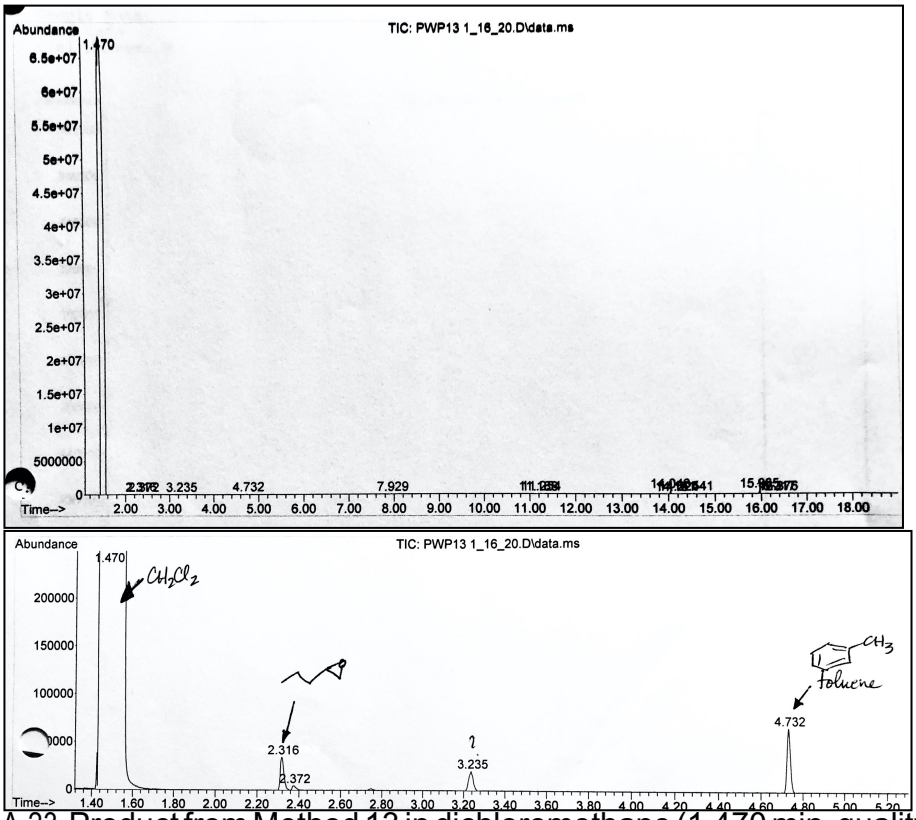


A.21 GCMS analysis of product from method 11, no solvent starting reagent 1,2-epoxyoctane (8.511 min, library search quality of 80), starting reagent urethane or ethyl carbamate (6.161 min, library search quality of 87), 1,2-octanediol (10.987 min).
D:\Research_Sara_Josselyn \RESEARCHMETHOD2.M

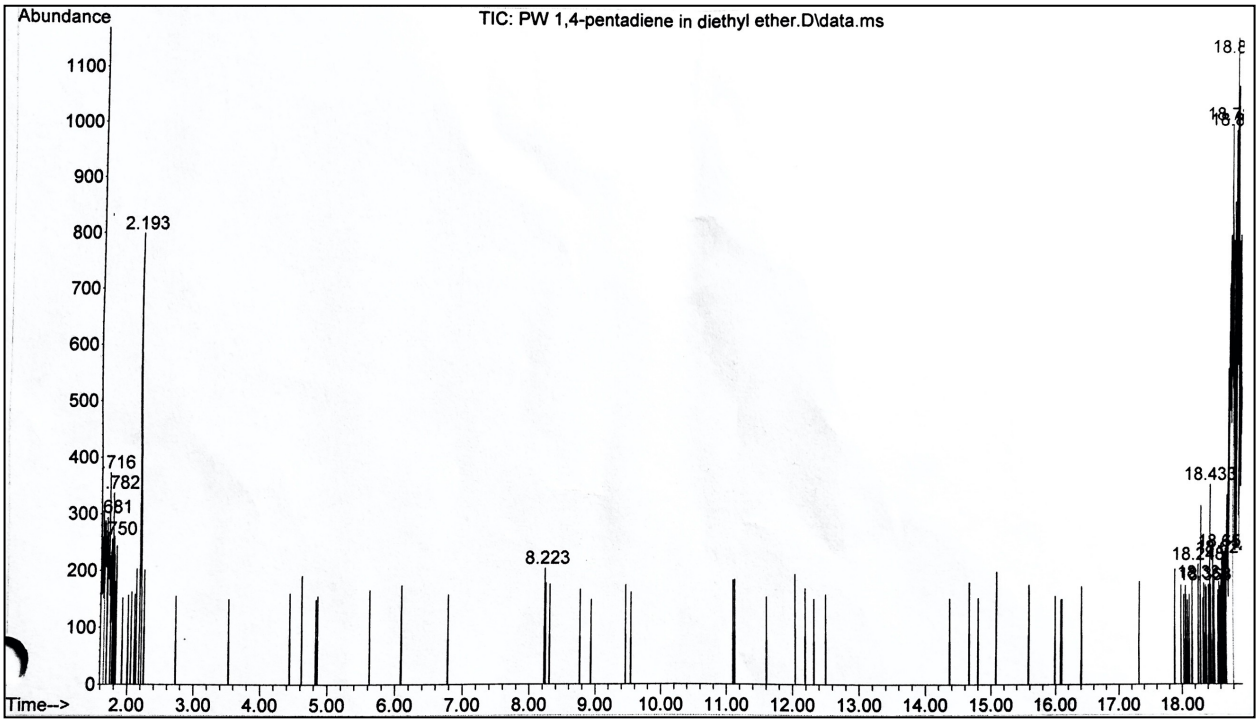

Abundance
TIC: PW P12 11_14_19.D\data.ms
317
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
4.914
100000
3.246
50000
0
18.821
21.718
23.073
24.901
29.229
30.409
Time-->
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00



A.22 zoomed in GCMS analysis of Method 12 product in dichloromethane. 1,2-epoxypentane present at 2.317 min.
D:\Research_Sara_Josselyn \RESEARCHMETHOD2.M



A.23 Product from Method 13 in dichloromethane (1.470 min, quality of 43), 1,2-epoxypentane (2.316 min) Pubchem MW 86.13 with fragments at 27, 41, 71 agrees with data at 2.31 min. D:\Sarajoshhighbpmethod\HIGHBOILINGSOLV_SJ2.M



A.24 GCMS Analysis of starting reagent 1,4-pentadiene in diethyl ether.
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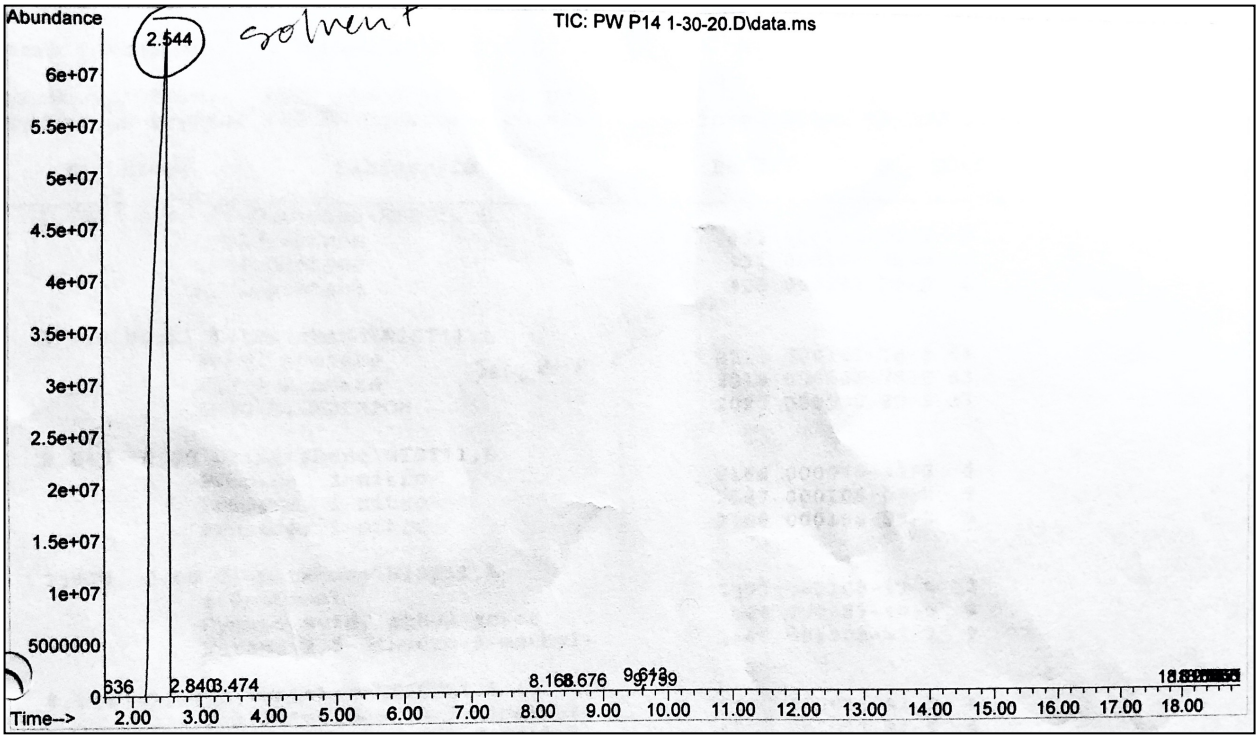

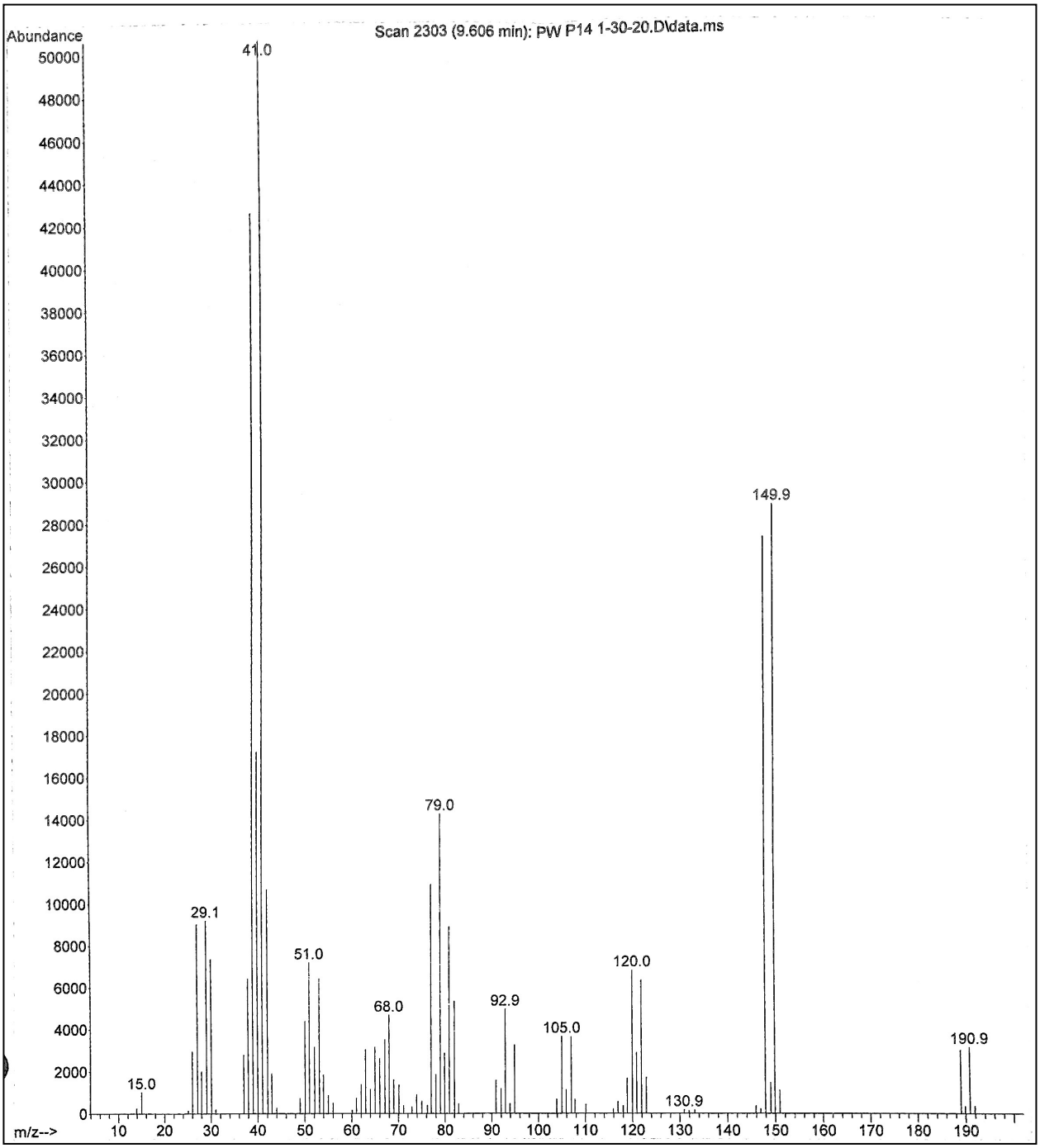

A.25 GCMS analysis of product from Method 14 (9.613 min) in ethyl acetate (2.544 min, quality of 64). MW of simplified isoxazoline 3-bromo-4,5-dihydro-5-prop-2-enylisoxazoline=190.04 g/mol.

- 190.9—2 peaks Bromine isotopes present with a 1:1 ratio/splitting pattern Br79, Br81
- 149.9—2 peaks, loss of propenyl group—bromine still present

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