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Applications of Sodium Azide in the Synthesis of Tetrazines and Hydrolysis Reactions

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APPLICATIONS OF SODIUM AZIDE IN THE SYNTHESIS OF TETRAZINES AND HYDROLYSIS REACTIONS

by
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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ABSTRACT

The inverse electron demand Diels−Alder cycloadditions of heterocyclic azadienes have provided a robust methodology for synthesizing highly substituted and functionalized heterocycles. It is widely used in organic synthesis and the pharmaceutical industry in the divergent construction of screening libraries and bioorthogonal conjugation. Each heterocyclic azadiene was found to possess a unique reactivity toward different classes of dienophiles, display predictable modes of cycloaddition, and exhibit substantial substituent electronic effects impacting their intrinsic reactivity and cycloaddition regioselectivity. Synthesis of 1,2,4,5-tetrazine has been reported in the literature since the late 19th century, showing scientists' tremendous interest in its application.

Herein we attempt to synthesize the 1,2,3,5-tetrazine, which is the less popular member of the family. Initial studies of its cycloaddition reactivity, stability, and scope illustrate that it displays similar properties to that of the isomeric 1,2,4,5-tetrazine. The lack of scientific focus on the compound despite its potential value has motivated us to develop an efficient synthetic route via N-methylene amide and azide cycloaddition. Although the attempt to prepare the desired tetrazine was unsuccessful, our designed method interestingly led up to a hydrolysis pathway that allowed us to explore the application of sodium azide further.

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LIST OF ABBREVIATIONS

AcOH	Acetic acid
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
E_{act}	Energy of activation
$Gd(NO_3)_3$	Gadolinium(III) nitrate
HBTU	Hexafluorophosphate Benzotriazole Tetramethyl Uronium
HCl	Hydrochloric acid
$In(NO_3)_3$	Indium(III) nitrate
IEDDA	Inverse-electron-demand Diels-Alder
MeOH	Methanol
NMR	Nuclear magnetic resonance
NSAID	Nonsteroidal anti-inflammatory drug
TLC	Thin layer chromatography
$Yb(NO_3)_3$	Ytterbium(III) nitrate
$Zn(NO_3)_2$	Zinc nitrate

CHAPTER 1

Introduction

1.1 Background

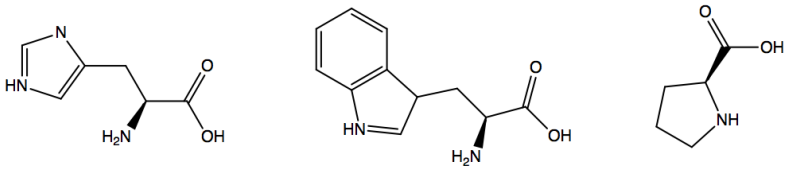
Heterocyclic compounds have maintained their essential status in medicinal chemistry for many decades as the core of numerous natural and synthetic substances. Today, more than 85% of all biologically active chemical entities contain a heterocycle due to the number of unique characteristics that heterocycles possess.[1]

The most common heterocycles are those containing five- or six-membered rings and having heteroatoms of nitrogen (N), oxygen (O), or sulfur (S).[2] The heteroatoms' presence gives heterocyclic compounds physical and chemical properties that separate them from those of their all-carbon-ring analogs. Since heterocycles can be found in the core elements of a wide range of natural products such as nucleic acids, amino acids, carbohydrates, vitamins, and alkaloids, the research process tends to focus on simulating such structural motifs.[3] They can serve as valuable tools to manipulate potency, lipophilicity, polarity, and aqueous solubility of the molecules, leading to improvements in pharmacological, pharmacokinetics, and physicochemical properties of drug candidates.[3]

The pharmacological benefits of employing heterocycles can be observed by their ability to participate in hydrogen bonding with the target protein. The heterocycle can play the role of either H-acceptor in heteroaromatic compounds H-donor in saturated N-heterocycles.[3] The possibility of hydrogen bonding is also relevant for the physicochemical and transport properties of drug molecules.[4]

The importance of heterocycles in life was recognized as the nascent stage of organic chemistry two centuries ago with the isolation of alkaloids such as morphine from poppy seeds,

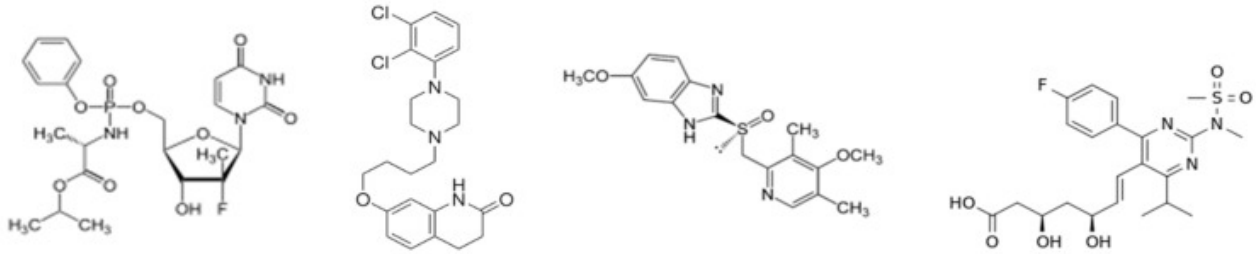
quinine from cinchona barks, and camptothecin from the Chinese joy tree.[5] Today, heterocycles are found in numerous biochemical and physiological fields such as photosynthesis, amino acids, DNA bases, vitamins, and endogenous neurotransmitters. Several amino acids, the building block of life, are made of heterocycles. Figure 1.1 depicts some of the applications of heterocycles with histidine has an imidazole; tryptophan has an indole, yet proline has a pyrrolidone.[5]



histidine tryptophan proline

Figure 1.1 Examples of Applications of Heterocycles.

Synthetic heterocyclic chemistry has played an essential role in human life and found its application in diverse fields like agriculture, medicine, polymer, and various industries. Most synthetic heterocyclic compounds act as a drug used as anticonvulsants, hypnotics, antineoplastics, antiseptics, antihistamines, antiviral, anti-tumor, etc.[6] Heterocycles constitute a standard structural unit of most marketed drugs. Of the top five U.S. small molecule drug retail sales in 2014, four of them contain heterocyclic fragments in their overall structure, shown in Figure 1.2 below. Combined, these four accounts for an incredible 27.4 million U.S. dollars, almost 80% of the total revenue obtained from the top five prescription drugs.[7]



Sovaldi (Sofosbuvir)	Abilify (Aripiprazole)	Nexium (Esomeprazole)	Crestor (Rosuvastatin)
Antiviral	Antipsychotic	Antiulcerant	Cholesterol regulator
7.9 U.S. $	7.8 U.S. $	5.9 U.S. $	5.8 U.S. $

Figure 1.2 Heterocyclic Molecule Drugs Present in the US Top Five Prescription Drugs and Respective Retail Sales in 2014 (in billions of U.S. $)

Due to the characteristics mentioned above, heterocycles are playing an increasingly important role in drug discovery. Among them, nitrogen-containing heterocycles are of great importance to life science since they are abundant in nature, existing as subunits in several natural products, for example, vitamins, hormones, and antibiotics.[8] Some representative alkaloids and other nitrogen-containing natural products, showing diverse biological activities, and several of them are even prescribed drugs such as serotonin, thiamine, which is also called vitamin B1, atropine, notorious morphine, codeine (more significant benefit may be gained when it is combined with acetaminophen or a nonsteroidal anti-inflammatory drug (NSAID) such as aspirin or ibuprofen), papaverine, coniine, caffeine, and nicotine.[9]

Due to exhibiting diverse biological activities, heterocyclic nitrogen compounds have always been attractive targets to synthetic organic chemists. Since several of them are prevalent in natural products, especially alkaloids, they have received much attention from the synthetic community, especially those engaged with the total synthesis of natural products.[10] As a result, the vast number of nitrogen heterocyclic compounds have been under ongoing investigations

from different points of view, thus, found applications in pharmaceutical research and drug discovery.[11,12] Notably, in 2014 Njardarson et al. published the first comprehensive analysis of the nitrogen-based heterocycles.[11] This analysis showed that, indeed, about 60% of small-molecule drugs contain an N-based heterocycle as standard architectural cores.[12] In 2018, Ramazani and co-workers presented the recent advances in nitrogen-based heterocycles as helpful cancer chemotherapy agents.[11] Recently, N-based heterocycles have attracted much interest from medicinal chemists and biologists due to the broad range of biological activities and plentiful applications in pharmacy's vast fields.[13]

1.2 Tetrazines in Medicinal Chemistry

Tetrazine is a compound that consists of a six-membered aromatic ring containing four nitrogen atoms with the molecular formula $C_2H_2N_4$.[14] Tetrazines are even more reactive than triazines toward nucleophiles and electron-rich dienophiles. This makes them attractive for click chemistry, and they find application as conjugation tags for materials chemistry and, especially, for bioorthogonal chemistry. In other applications, they are attractive for high-energy materials, coordinating ligands, and potent bioactive compounds.[15]

The tetrazine family includes three types of the cycle, namely 1,2,3,4-tetrazines, 1,2,3,5-tetrazines, and the most known and studied 1,2,4,5-tetrazines, also frequently called s-tetrazines.[15]

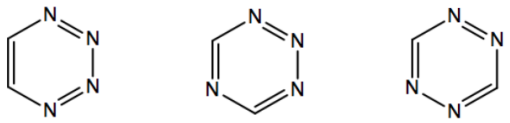
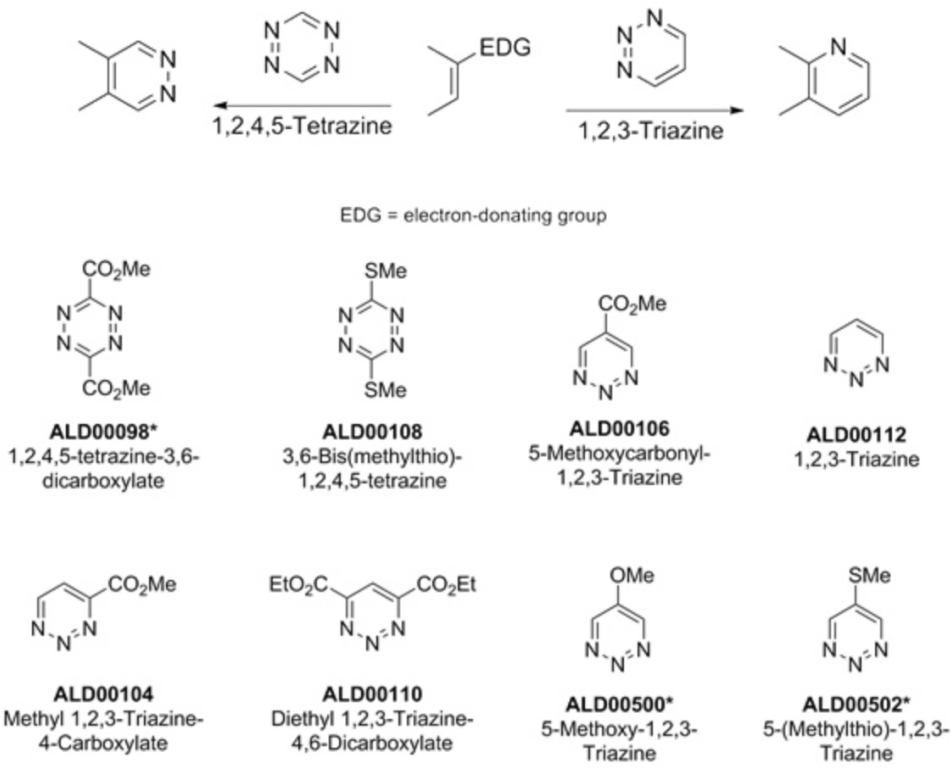


Figure 1.3 Three Types of Tetrazine Family.

Among them, 1,2,4,5-tetrazine is the most widely employed heterocyclic azadiene. The electron deficiency of the tetrazine ring is used not only for synthetic purposes but also for tuning the materials' electronic and photophysical properties. Thus, the traditional application is inserting a tetrazine moiety as an acceptor into polymers for photovoltaic devices.[16] A few other examples illustrate the application of tetrazines as electron-accepting systems in structures of luminescent materials. Also, in 2011, there was a success in using tetrazine reactions to label biomarkers on cells with magneto-fluorescent nanoparticles that are now being routinely used on clinical samples for biomarker profiling to predict malignancy and patient outcome.[17]

The inverse electron demand Diels−Alder cycloadditions of heterocyclic azadienes have provided a robust methodology for synthesizing highly substituted and functionalized heterocycles widely used in organic synthesis and the pharmaceutical industry, in the divergent construction of screening libraries, and bioorthogonal conjugation. Past efforts have provided systematic explorations of the reactions of 1,2,4,5-tetrazines, 1,2,4-triazines, 1,3,5-triazines, 1,3,4-oxadiazoles, 1,2-diazines, and most recently 1,2,3- triazines. Each heterocyclic azadiene was found to possess a unique reactivity toward different classes of dienophiles, display predictable modes of cycloaddition, and exhibit substantial substituent electronic effects impacting their intrinsic reactivity and cycloaddition regioselectivity. These insights permitted their use as critical steps in more than 50 natural products' total syntheses and helped inspire the development of the powerful 1,2,4,5-tetrazine-based bioorthogonal conjugation and labeling technology (tetrazine ligation) widely used today. [18]



Scheme 1.1 Classes of reactive substrates that participate in inverse electron demand Diels-Alder reactions, highlighting 1,2,4,5-tetrazines and 1,2,3-triazines.

The first family of tetrazines is quite unstable, and most examples have fused rings, whereas the second family contains only sporadic examples published to date. Actually, in the year 2017, only s-tetrazine–based reports appeared.[15] Owing to their high chemical selectivity, fast reactivity, biocompatibility, the inverse-electron-demand Diels–Alder (IEDDA) reaction between s-tetrazines dienophiles has become a powerful tool in a variety of biological applications.[17] Compared to the scope and advantages that 1,2,4,5-tetrazines brought, it is remarkable in this day and age that no member of another fundamentally important heterocyclic azadiene ring system, the 1,2,3,5- tetrazines, has yet been reported.

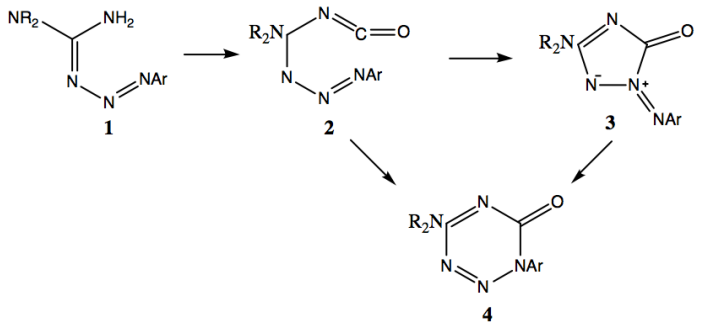
Of the three possible tetrazine systems, the 1,2,3,5-tetrazines are by far the least studied class.[19] Since 1974, limited attempts to prepare a 1,2,3,5-tetrazine have been described and no monocyclic aromatic 1,2,3,5-tetrazine as a discrete 6π heterocycle has been reported. Stability profiles established in computational studies suggest a decreased kinetic stability for 1,2,3,5-tetrazine compared with the wellknown and isomeric 1,2,4,5-tetrazine (ΔE_{act} = 14 kcal/mol), but a greater thermodynamic stability than 1,2,4,5-tetrazine (7−8 kcal/mol). The principal challenge in the synthesis of 1,2,3,5- tetrazines is the construction of two consecutive N−N bonds, which are not present in 1,2,4,5-tetrazines but are found in 1,2,3-triazines. Although no synthesis of a monocyclic 1,2,3,5- tetrazine as a 6π aromatic system has been reported, limited numbers of heterocyclic compounds bearing the 1,2,3,5- tetrazine core have been disclosed (Figure 1.4). [21-23]

Figure 1.4 Reported structures bearing a nonaromatic 1,2,3,4-tetrazine core.

The nonaromatic 1,2,3,5-tetrazine core (3) was synthesized as part of fused heterocycles in the development of energetic materials, monocyclic tetrazinones have been described that were prepared by a 6π-electrocyclization of acyclic triazene isocyanate precursors, and the

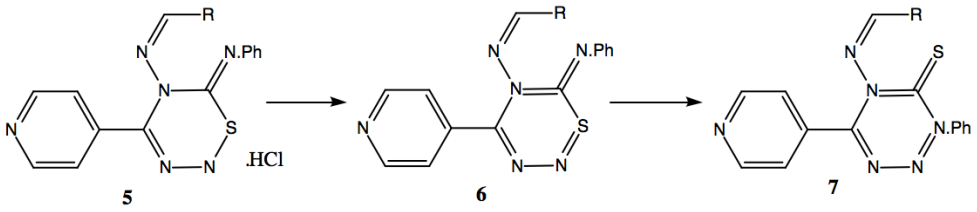
synthesis of a library of ring-fused bicyclic tetrazinones has been reported, of which a derivative (temozolomide, 4) is used clinically.

First, in 1985, there was an approach to synthesize the tetrazine product **4** from the conversion of the triazines **3** via the isocyanates **2**.[24] However, this synthetic route was difficult and erratic, since the best result was obtained with a maximum yield of 43% by treating them with phosgene in toluene in the presence of a large excess of pyridine at -70 °C.



Scheme 1.2[19]

The second synthetic route originated from the isomerization of 1,2,3,5-thiatriazines.[25] This route requires complicated steps to lead to the main product of thiatriazines **7** with the tetrazines being only the side product. This pathway is shown in Scheme 1.3 below.



Scheme 1.3[20]

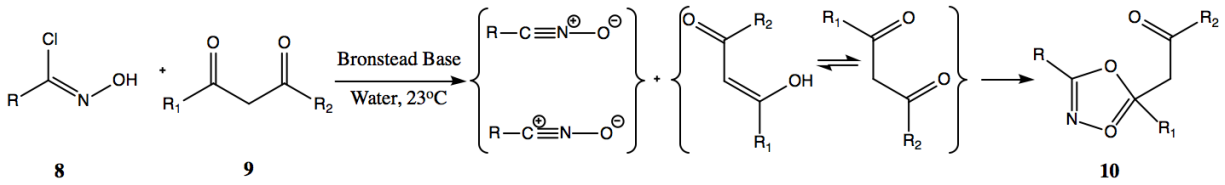
Recently, professor Dale Boger and his team have successfully explored the possible synthesis of the first monocyclic aromatic 1,2,3,5-tetrazines. [18] It not only exhibits a remarkable cycloaddition reactivity, surprisingly good stability (e.g., stable to chromatography, long-term storage, presence of water even as reaction co-solvent), and broad cycloaddition scope, but it also displays powerful orthogonal reactivity with the 1,2,4,5-tetrazines.

Figure 1.5 Characterization of the first monocyclic aromatic 1,2,3,5-tetrazines.

Due to the potential importance of this type of tetrazines in pharmaceuticals, the need for a synthetic route of 1,2,3,5-tetrazines is crucial, and this purpose motivated us to seek an efficient method.

1.3 Hypothesis

Previously, a base-meditated and keto-enol-controlled [3+2] cyclization of nitrile oxide **8** and 1,2-diketones **9** in water to form isoxazoles **10** was discovered in the Le lab in 2020 (Figure 1.7).[26] The selectivity of the reaction can be controlled by the polarity of the solvents in the appropriate base. The optimized reaction condition circumvented other reactions, such as O-imidoylation or hetero [3+2] cycloaddition. This fast reaction will give environment-friendly access to 3,4,5-trisubstituted isoxazole-containing pharmaceuticals.



Scheme 1.4

In a similar fashion to this [3+2] cycloaddition, our lab previously hypothesized that we could use a base to act as the nucleophile attacking the NH_2 base found in amides and cyclize to form a heterocyclic product. Previously, the Huisgen 1,3-dipolar cycloaddition reaction of organic azides and alkynes has gained considerable attention in recent years due to its powerful application. Here, azide anion took part in the rearrangement of the complex into ring structures.

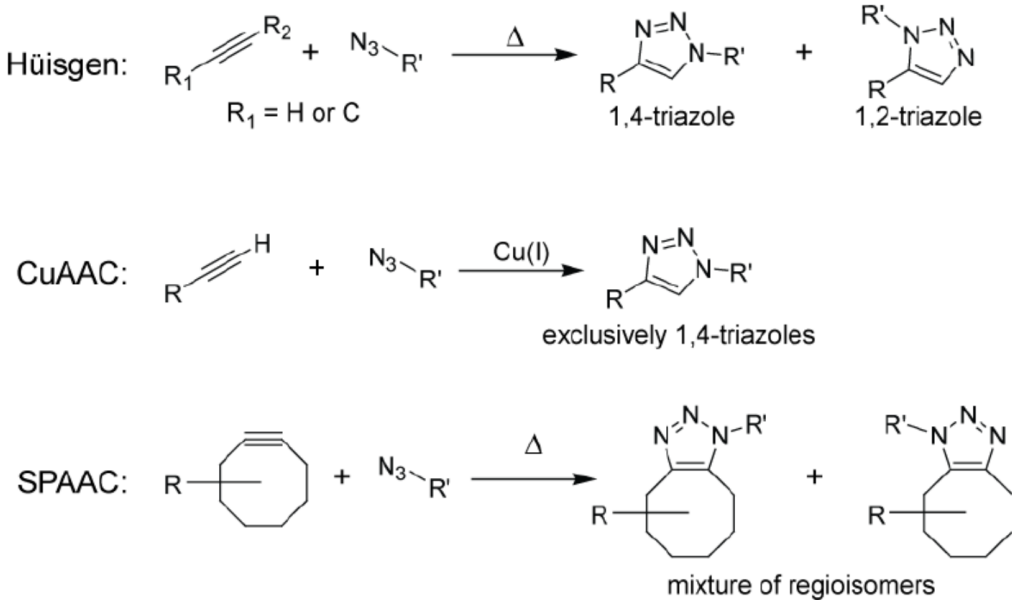
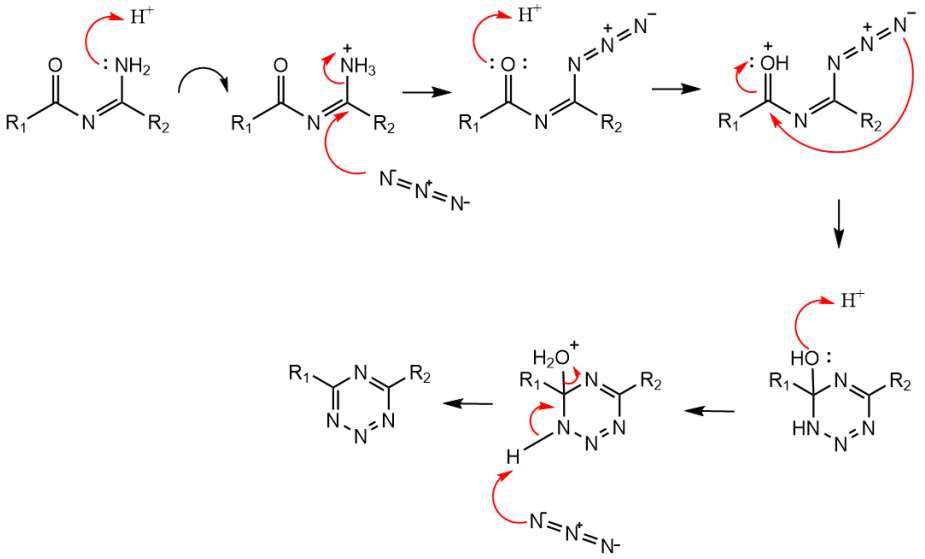


Figure 1.6 Azide-alkyne cycloaddition in Click chemistry.

Based on that and our previous work, we chose sodium azide for the cycloaddition. Particularly, acetic acid will help activate the C=O and NH_2 groups to help sodium azide attack the compound. The azide ion undergoes nucleophilic substitution to eliminate NH_2 and then attack the carbonyl double bond to form tetrazine's ring structure. The expected mechanism is shown in Scheme 1.5.



Scheme 1.5

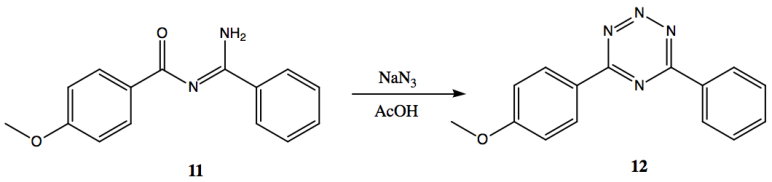
Tetrazines are a privileged class of six-membered heterocycles that are found in numerous bioactive natural products and synthetic small-molecule drugs. Therefore, we hope that our new cycloaddition method will lead to an efficient synthetic route to this important privileged class to reduce the current labor-intense synthetic effort and waste generation in production of tetrazines.

CHAPTER 2

Results and discussion

2.1 An approach to the synthesis of tetrazines

To begin this study, we chose (Z)-N (amino(phenyl)methylene)-4-methoxybenzamide **15** as a model substrate, aiming to synthesize the desired tetrazine.



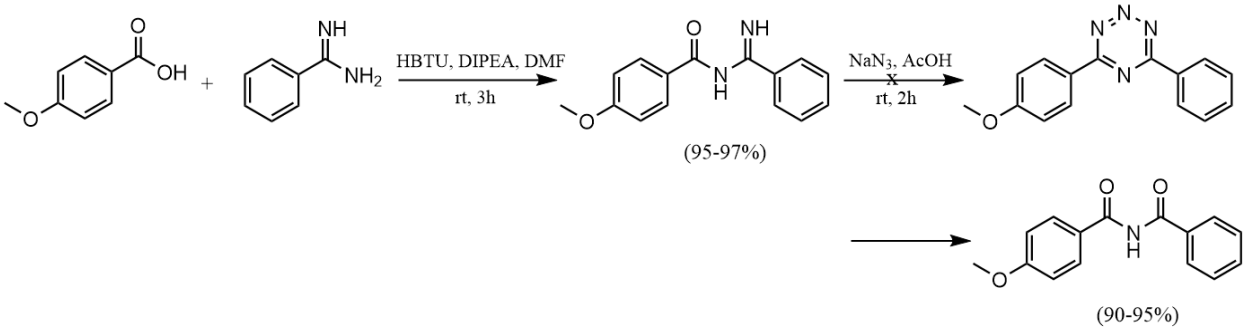
11 12

Scheme 2.1

The first step involved the preparation of the starting material, since it was not available in our lab. The procedure for the preparation of N-acyl amidine substrate was conducted via the reaction of methoxy benzoic acid **13** and benzamidine **14**. The chemicals were chosen due to their availability in our lab as well as the different structures that can be helpful in determining the product under NMR spectroscopy. The reactions were run with HBTU, DIPEA, and DMF at room temperature for 3 hours. ^{1}H NMR showed that the resulting white solid was indeed the predicted N-acyl amide with the yield ranging from 95-97%, as the procedure was repeated three times (Scheme 2.2). This result agreed with the previous report by Ertong, Manman, and Zhen.

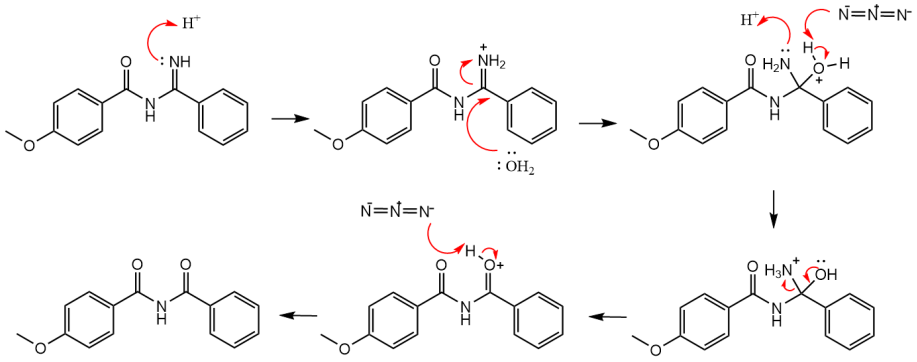
A portion of the obtained amide was then subjected to a reaction with 2 equivalents of sodium azide under typical conditions (anhydrous AcOH, rt, 2h) in an attempt to synthesize the

desired nitrogen heterocycle. However, a different product was obtained after extractive workup and column chromatography. TLC of the resulting white solid showed a new spot, but it was difficult to determine whether the cyclization **12** occurred due to the lack of access to the ^{15}N NMR in the department. Several analyses were conducted until the structure of the gained product was confirmed to be that of the hydrolysis form **1?**.



Scheme 2.2

With the result, we reasoned that the result obtained was due to the fact that the compound was hydrolyzed by the acid formed. The proposed mechanism earlier suggested that azide anion attacked the N-H and C-O double bonds with the help of acetic acid in order to form the expected heterocyclic ring. However, based on the observed finding, we proposed that the sodium azide and acetic acid have reacted to form hydrazoic acid, which then further hydrolyzed the imine functional group to a carbonyl bond (Scheme 2.3).



Scheme 2.3

In order to confirm the reactivity of azide, several reactions were carried out without azide. In particular, one reaction was set up with the same condition as Scheme 2.2 using the N-amide but without the use of azide, meaning just AcOH was used as the solvent. Also, other reactions were started with the addition of different Lewis acid catalysts, including $Gd(NO_3)_3$, $In(NO_3)_3$, $Yb(NO_3)_3$, and $Zn(NO_3)_2$. The metal-based catalysts were used with the prediction that they would act as an electron pair acceptor to increase the reactivity of a substrate. However, no reactions occurred with these reactions despite them being run for long periods of time. As AcOH is a relatively weak acid with a pKa level of 4.75 compared to the newly formed hydrazoic acid, acetic acid alone was not enough to hydrolyze our starting material. This also proved that the hydrolysis only happened due to the effect of azide acting as the base. From this knowledge, we continued to explore the reactivity of the compound.

2.2 Exploring the scope of hydrolysis reactions

We next turned our attention to alter the solvent system instead of using acetic acid. Three different reactions were set up with the starting materials of the N-amide, sodium azide, four lewis catalyst acid, and the catalytic HCl as in Table 2.1. Two different types of solvent were

used, as MeOH was used as protic solvent and DCM as aprotic solvent. There was no product observed when the reactions were performed in an aprotic solvent. On the other hand, different ranges of yields of the product were obtained with protic solvent, with the products being confirmed as the hydrolysis form.

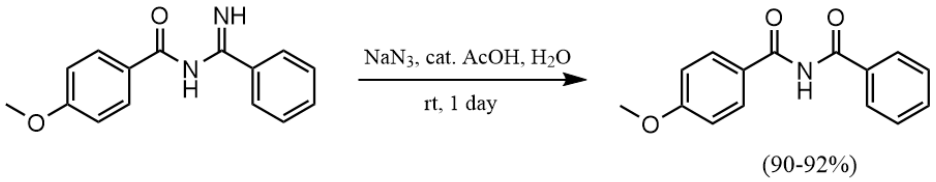
Table 2.1 Yields of (4-methoxyphenyl)methanone **16** Using Various Solvent Systems.

Reactants	Protic Solvent (MeOH)	Aprotic Solvent (DCM)
NaN_3	15%	n.o.
NaN_3 + Lewis catalysts	20%	n.o.
NaN_3 + Catalytic HCl	60%	n.o.

The different outcomes obtained from the variations of solvent systems proved that the choice of solvent could have a significant effect on the performance of a reaction. With the last reaction, only a few drops of HCl were enough to make the reaction work, because it is a strong acid, compared to acetic acid.

Our next effort in optimizing the reaction was to aim for a more environmentally friendly method. Particularly, the original method included the use of glacial acetic acid as solvent. Glacial acetic acid can cause skin burns and permanent eye damage, and will corrode metal. Also, acetic acid is a strong eye, skin, and mucous membrane irritant. Prolonged skin contact with glacial acetic acid may result in tissue destruction. Inhalation exposure (8 hours) to acetic acid vapors at 10 parts per million (ppm) could produce some irritation of eyes, nose, and throat; at 100 ppm marked lung irritation and possible damage to lungs, eyes, and skin might result. As all of the reactions conducted would be using the acid, the more acid used would lead to longer exposure to the dangerous chemical.

Therefore, we decided to try to decrease the amount of acetic acid by using it only in a catalytic amount and use water as the solvent instead. Particularly, 10% of AcOH were used along with water, with other conditions being the same.



Scheme 2.4

Compared to the original method, this scheme took a longer time for the product to form completely. Indeed, the reaction was run for 1 day, instead of just 2 hours. However, as the reaction completed, there was observable white precipitation that could be easily spotted in the flask, while it was just a transparent mixture before. Also, the product obtained was pure enough to be taken without further purification through column chromatography but still resulted in the same ^{1}H NMR spectrum with relatively similar yield. This has shown the effectiveness of the new solvent system over pure acetic acid to reduce chemical waste.

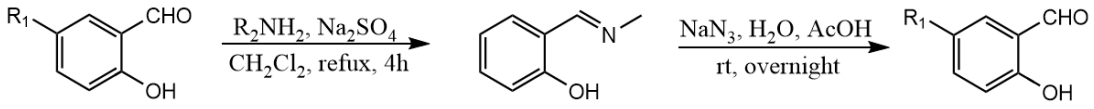
2.3 Sodium azide in the hydrolysis of imines

Based on our success earlier in using sodium azide and acetic acid in order to hydrolyze the imine group of N-(amino(phenyl)methylene)-4-methoxybenzamide, we continued to test the method with different kinds of imine. In recent years there has been a rising demand for efficient synthetic processes for imines [21]. The significance of imines both in synthesis and biological chemistry has led to the development of a large number of methods for the formation of carbon-nitrogen double bonds [22]. A majority of these procedures involve the condensation of a

primary amine with a carbonyl compound under conditions which remove water either chemically or physically. While most reactions proceed in good yield, those involving acid-sensitive carbonyl compounds or weakly nucleophilic amines can be troublesome, sometimes leading to decomposed starting materials and little or none of the desired imine. Aiming to test the hydrolysis method, a variety of different imines were required to be synthesized; therefore, a fast and simple method was preferred most to save time and effort.

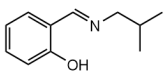
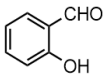
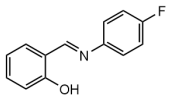
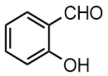
In this part, we were testing the hydrolysis mechanism by first synthesizing a variety of imines and then using them for the reactions since they are not stable enough. Imine formation and hydrolysis are highly important in biological chemistry. Many enzymes employ imines as part of their reaction mechanisms. An important biological reaction is the formation of an imine bond between an amino acid carbonyl group and the amine of the amino acid lysine. This is because they are highly reactive and can form adducts with other molecules. This adduction allows the molecule to experience the reactive effect of the imine. The nitrogen of the C=N bond is readily protonated, yielding a protonated imine. This serves as an electron-accepting group, which, when present in an adduct, can pull electrons away from the bonds in the attached molecule. This facilitates bond cleavage, which is an essential part of many metabolic reactions, for example, in glycolysis and amino acid metabolism.

Consequently, as shown in scheme 2.5, imines were prepared in quantitative yields of 95-98% from the particular salicylaldehyde and the corresponding amine/aniline in dichloromethane in the presence of magnesium sulfate. Then, the resulting imines were used as such in our hydrolysis method. Particularly, to that end, imines were treated with sodium azide in acetic acid and water to afford the corresponding hydrolyzed products, which in this case were the original aldehyde, in 50-61% yield at room temperature (Table 2.2.)



Scheme 2.5.

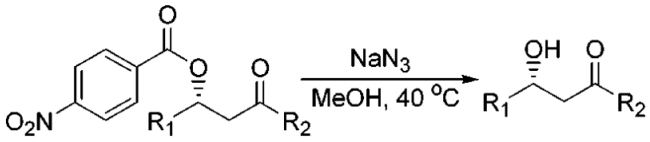
Table 2.2 Synthesis and hydrolysis of imines.

Entry	R_1	R_2	2	3	% Yield
1	H	iBu			61
2	H	4-FPh			50

The products were taken for ^{1}H NMR and confirmed as the desired product, proving that our method did indeed work for the hydrolysis of imines. Although the reactions were run overnight for 2 days, the yield obtained was not high enough, as the remaining products belonged to the imines. Therefore, we have planned to increase the temperature of the reactions in order to cut down on the time and push the reactions to completion.

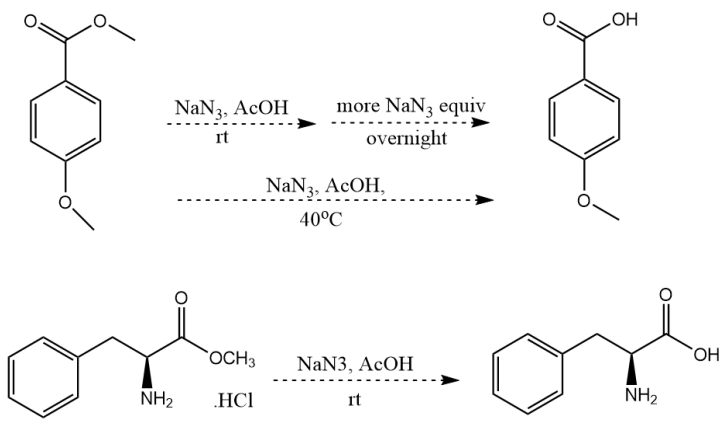
2.4 An attempt to hydrolyze esters and acetals

During our effort of testing the hydrolysis method of using the mild base sodium azide, we have attempted at the cleavage of esters. Earlier, a mild and selective cleavage of p-nitrobenzoic esters by sodium azide in methanol was reported. Based on the Mitsunobu reaction, professor Richard B. Silverman and his team have developed a new methodology that was mild and efficient enough to hydrolyze the esters (Scheme 2.6). However, the reaction was only limited to the selective group of p-nitrobenzoic esters as the starting material. Based on this, we hope that our method could work for hydrolyzing the simple groups of esters, which could potentially open the scope of our methodology.



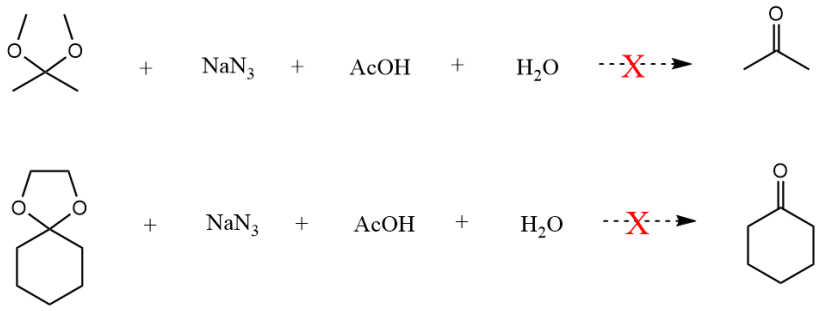
Scheme 2.6

We began with choosing methyl 4-methoxybenzoate and phenylamine ethyl ester as the model for the reactions (Scheme 2.7.) Interestingly, no reactions occurred when the esters were subjected to reaction with 2 equivalents of sodium azide in acetic acid at room temperature for 2 days. After that, additional equivalents of the azide were added, and the temperature was also raised to 40ºC and ran for a long time. However, it was just the starting material that was being observed; therefore, we have concluded that our method does not work toward the hydrolysis of esters.



Scheme 2.7

We continued to explore the scope of our method with the deprotection of acetals. In particular, we used the dimethyl acetals with dioxolanes. The reactions were run at the same conditions overnight, but the desired products were not obtained. Instead, the starting material was present the whole time, indicating that the reactions did not occur.



Scheme 2.8

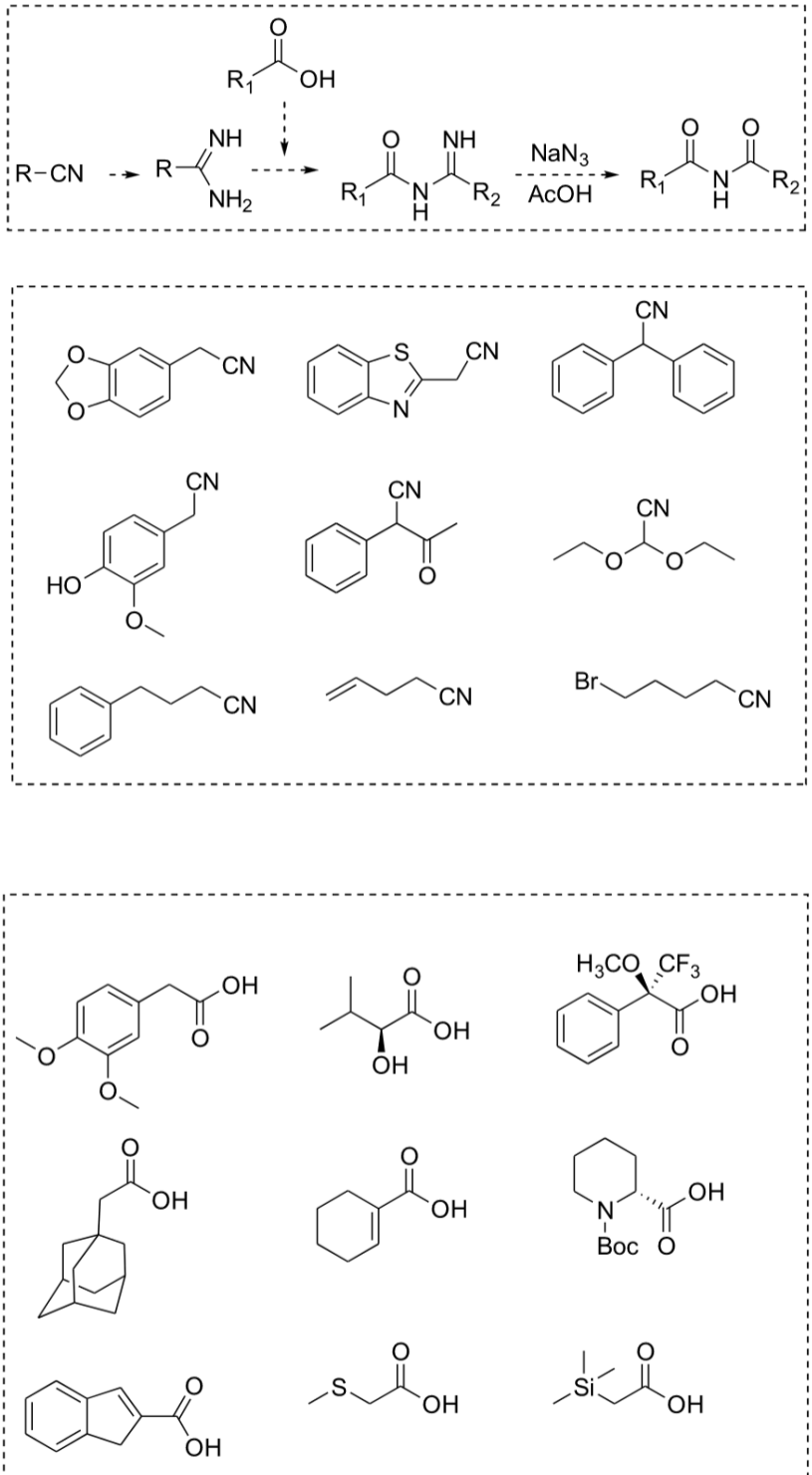
Although our method did not work with esters and carbonyl protecting groups as we expected, we believe that they are still useful in helping us explore the scope of our method.

CHAPTER 3

Future directions

Our attempt to synthesize a tetrazine species by using azide with N-methylene amides instead uncovered an efficient and mild hydrolysis pathway of imine functional groups. The formation of the cleavage instead of the desired heterocyclic ring may be understood as the azide and acid have acted to form hydrazoic acid, which then proceeded with the hydrolysis. We decided to further explore the scope through the variation of the hydrolysis reaction of the N-methylene amide. We plan to diversify the functional groups on both the benzamidine and carboxylic acids by using the chemicals we had in the lab. The benzamidine can be synthesized from different nitriles (Scheme 3.1.)

Our lab also predicts that we can try with different esters bonds in the diversification of starting materials. Since the method did not work with esters before, we expect that it will keep the functional group intact, which can make our methodology a more powerful tool.



Scheme 3.1

EXPERIMENTAL PROCEDURES

Preparation of (Z)-N (amino(phenyl)methylene)-4-methoxybenzamide

The 4-methoxybenzoic acid (1 mmol) with benzamidine (1.5 mmol) and HBTU (1.1 eq) were all dissolved in 0.7 mL (4 equiv) DIPEA and 5 mL DMF in a 25 mL round bottom flask. The reaction was stirred vigorously at room temperature for 3 hours. After the completion of the reaction and confirmation by TLC chromatography, the mixture was quenched with water and extracted with ethyl acetate in a separatory funnel. After the layers were separated, the organic phase was dried over anhydrous Na_2SO_4 and evaporated on a rotary evaporator to obtain the crude product. It was then purified by flash chromatography on a silica gel using hexane/ethyl acetate (3:1) as the eluent. The product was a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (dt, J = 8.8, 1.9 Hz, 2H), 8.05 – 7.96 (m, 2H), 7.54 – 7.43 (m, 1H), 7.39 (t, J = 7.3 Hz, 2H), 6.93 (dt, J = 8.8, 1.9 Hz, 2H), 3.79 (dt, J = 5.7, 2.1 Hz, 3H).

Hydrolysis Reaction of Amides

The (Z)-N (amino(phenyl)methylene)-4-methoxybenzamide (0.2 mmol) and sodium azide (0.4 mmol, 2 equiv) were dissolved in 3 mL acetic acid in a 10 mL round bottom flask. The reaction was stirred vigorously at room temperature for 2 hours. After the completion of the reaction and confirmation by TLC chromatography, the mixture was quenched with water and extracted with ethyl acetate in a separatory funnel. After the layers were separated, the organic phase was dried over anhydrous Na_2SO_4 and evaporated on a rotary evaporator to obtain the

crude product. It was then purified by flash chromatography on a silica gel using hexane/ethyl acetate (3:1) as the eluent. The product was a white solid.

Reaction of N-amide with Acyl Azide and Acetic Acid

The (Z)-N (amino(phenyl)methylene)-4-methoxybenzamide (0.2 mmol) and acyl azide (0.4 mmol) were dissolved in 3 mL acetic acid in a 10 mL round bottom flask. The reaction was stirred vigorously at room temperature for 2 hours. After the completion of the reaction and confirmation by TLC chromatography, the mixture was quenched with water and extracted with ethyl acetate in a separatory funnel. After the layers were separated, the organic phase was dried over anhydrous Na_2SO_4 and evaporated on a rotary evaporator to obtain the crude product. It was then purified by flash chromatography on a silica gel using hexane/ethyl acetate (3:1) as the eluent. The product was a white solid.

Reaction of N-amide with $TMSN_3$ and Acetic Acid

The (Z)-N (amino(phenyl)methylene)-4-methoxybenzamide (0.2 mmol) and $TMSN_3$ (0.4 mmol) were dissolved in 3 mL acetic acid in a 10 mL round bottom flask. The reaction was stirred vigorously at room temperature for 2 hours. After the completion of the reaction and confirmation by TLC chromatography, the mixture was quenched with water and extracted with ethyl acetate in a separatory funnel. After the layers were separated, the organic phase was dried over anhydrous Na_2SO_4 and evaporated on a rotary evaporator to obtain the crude product. It was then purified by flash chromatography on a silica gel using hexane/ethyl acetate (3:1) as the eluent. The product was a white solid.

Reaction of N-amide with Azide in Methanol

The (Z)-N (amino(phenyl)methylene)-4-methoxybenzamide (0.2 mmol) and --- (0.4 mmol) were dissolved in 4 mL methanol in a 10 mL round bottom flask. The reaction was stirred vigorously at room temperature for 2 hours. After the completion of the reaction and confirmation by TLC chromatography, the mixture was quenched with water and extracted with ethyl acetate in a separatory funnel. After the layers were separated, the organic phase was dried over anhydrous Na_2SO_4 and evaporated on a rotary evaporator to obtain the crude product. It was then purified by flash chromatography on a silica gel using hexane/ethyl acetate (3:1) as the eluent. The product was a white solid.

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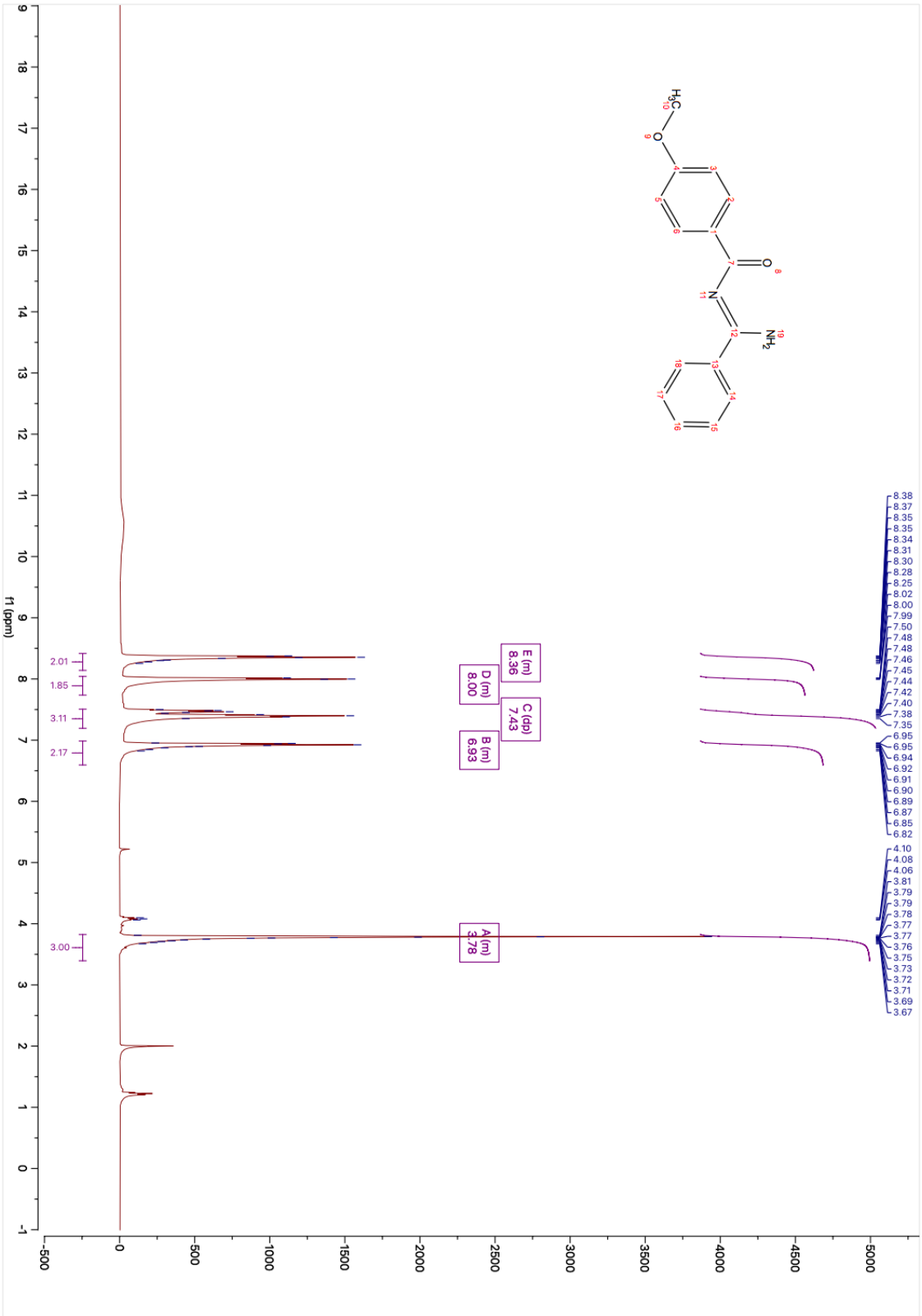

Figure 3.1 ^{1}H NMR Spectrum of N-(amino(phenyl)methylene)-4-methoxybenzamide

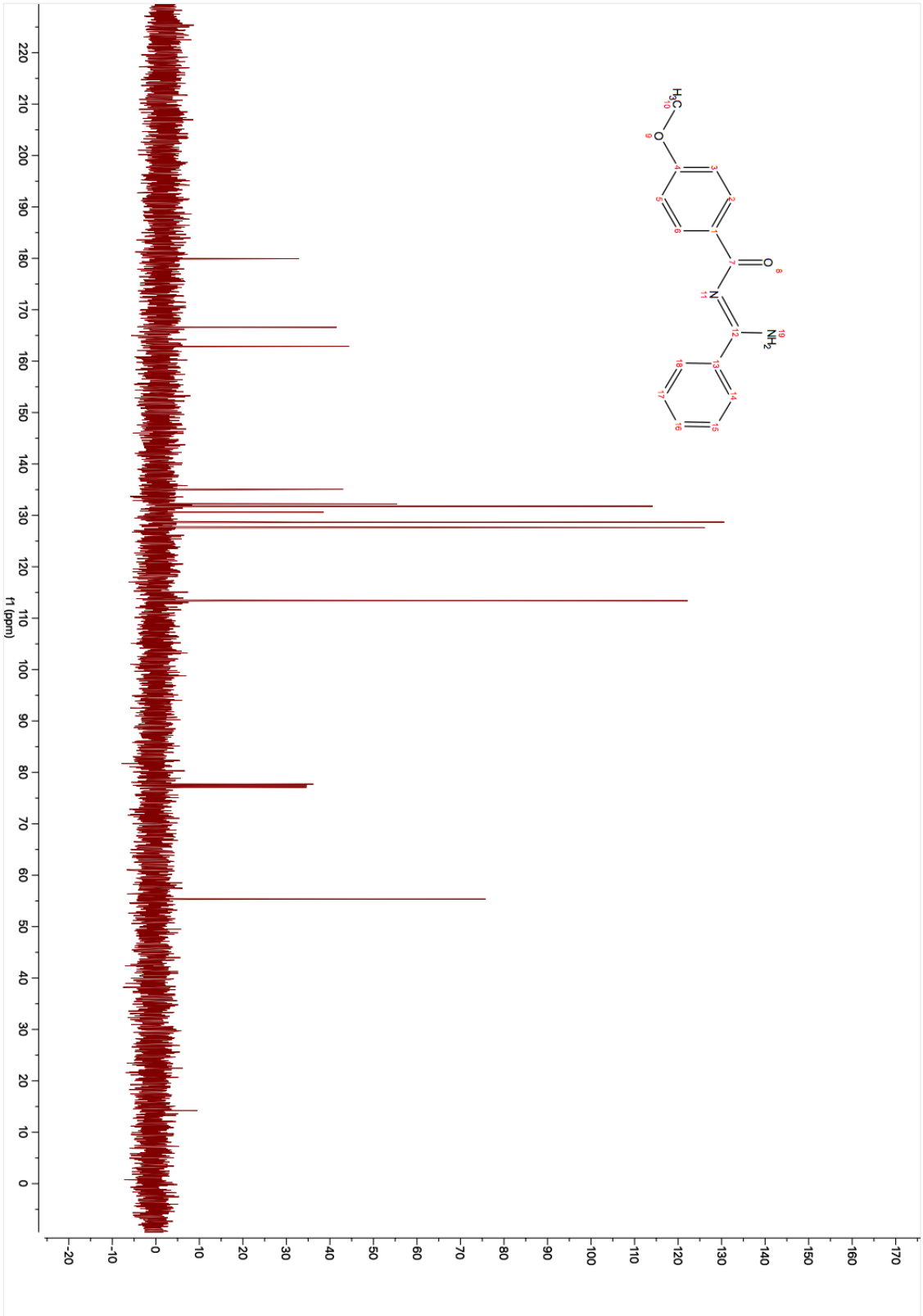

Figure 3.2 ^{13}C NMR Spectrum of N-(amino(phenyl)methylene)-4-methoxybenzamide

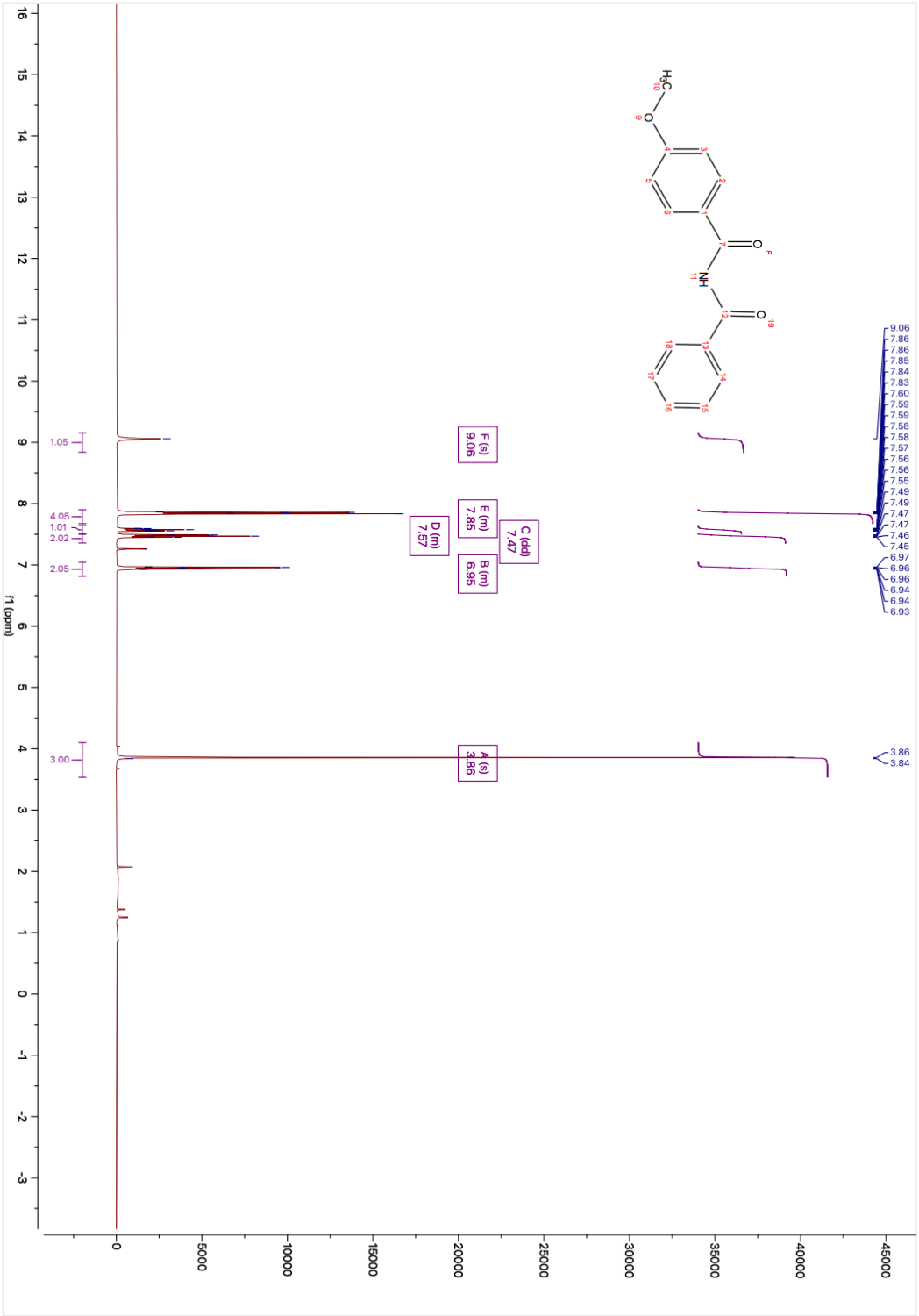

Figure 3.3 ^{1}H NMR Spectrum of hydrolysis product

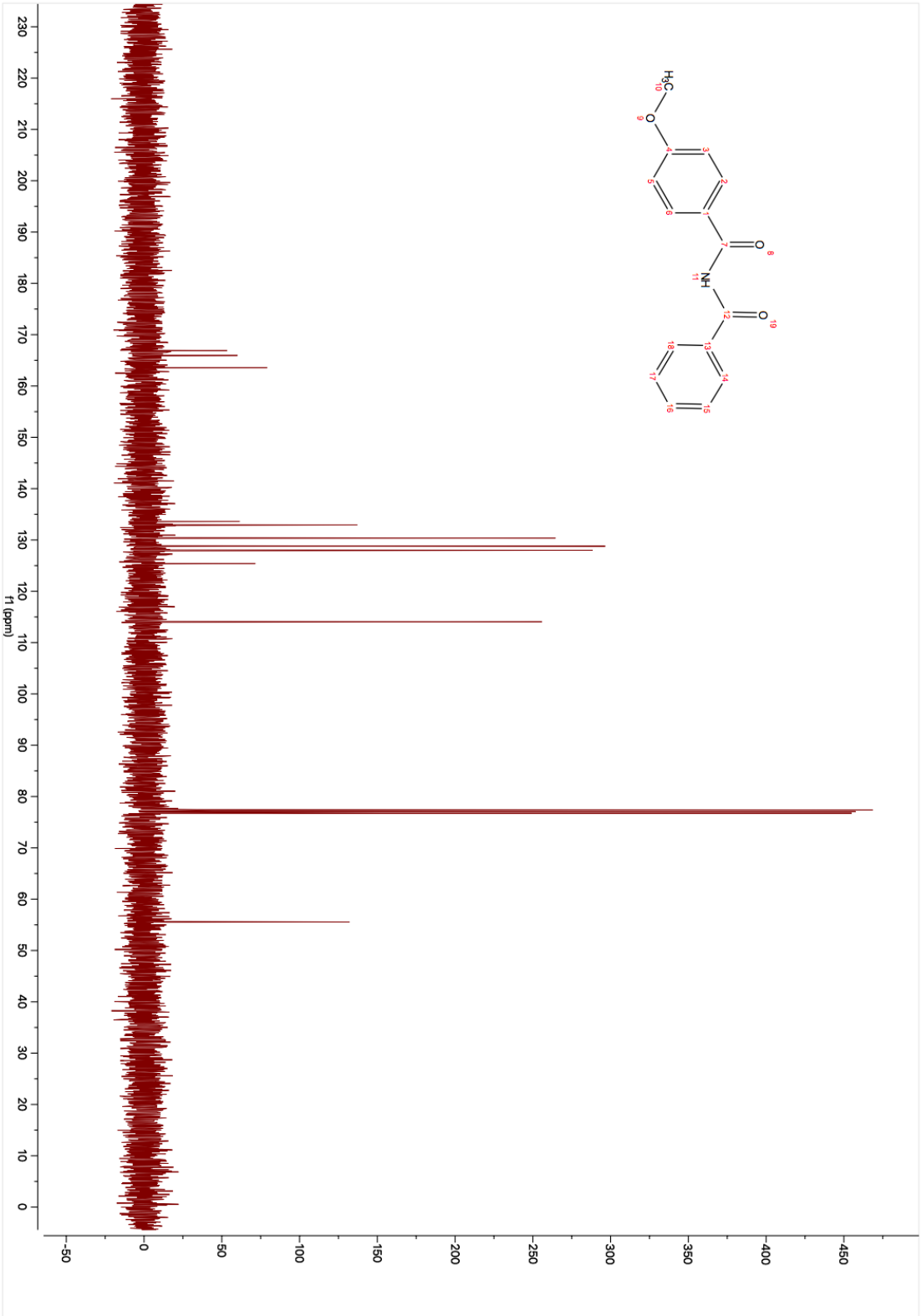

Figure 3.4 ^{13}C NMR Spectrum of hydrolysis product

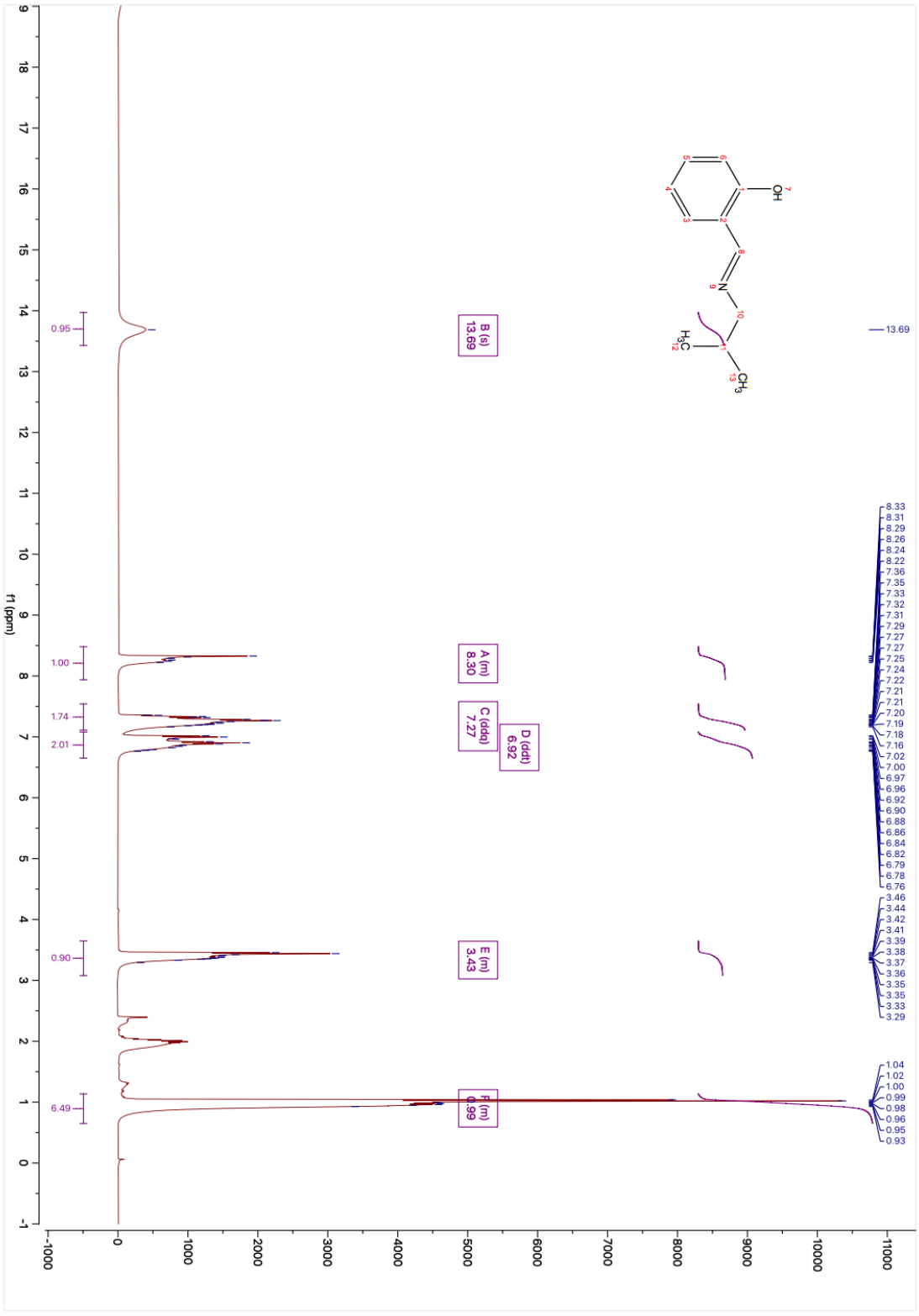

Figure 3.5 ^{1}H NMR Spectrum of (E)-2-((isobutylimino)methyl)phenol

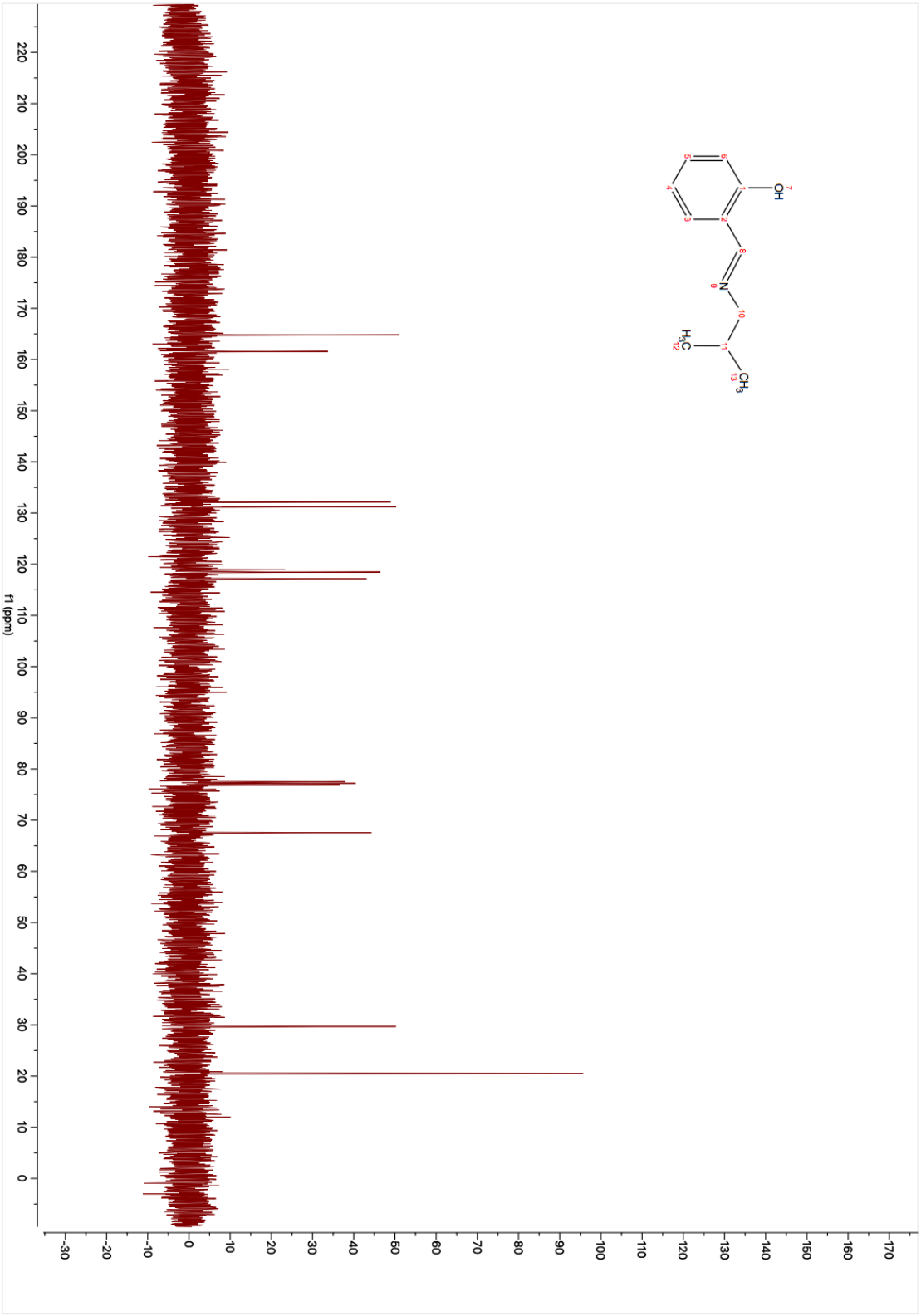

Figure 3.6 ^{13}C NMR Spectrum of (E)-2-((isobutylimino)methyl)phenol

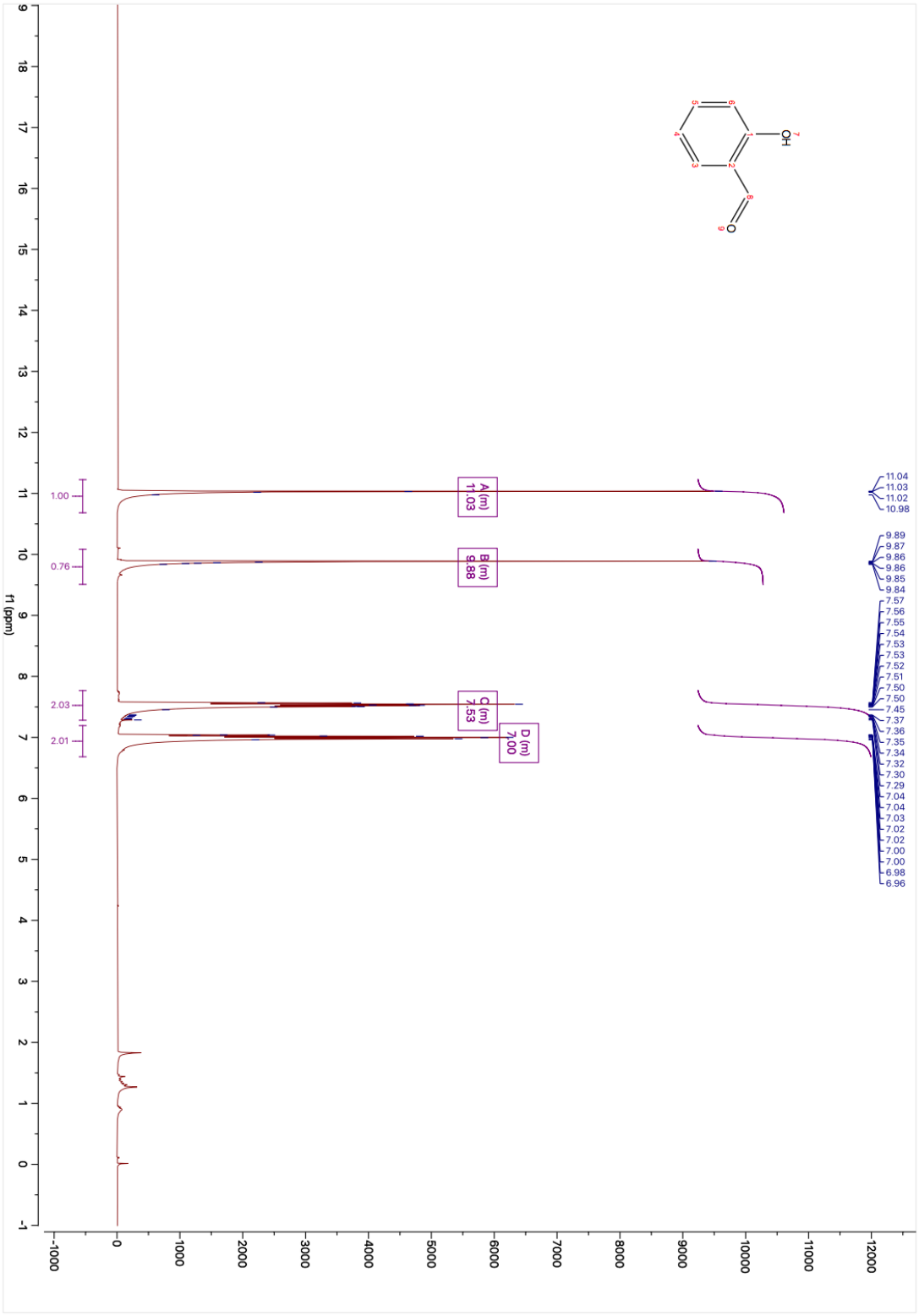

Figure 3.7 ^{1}H NMR Spectrum of 2-hydroxybenzaldehyde

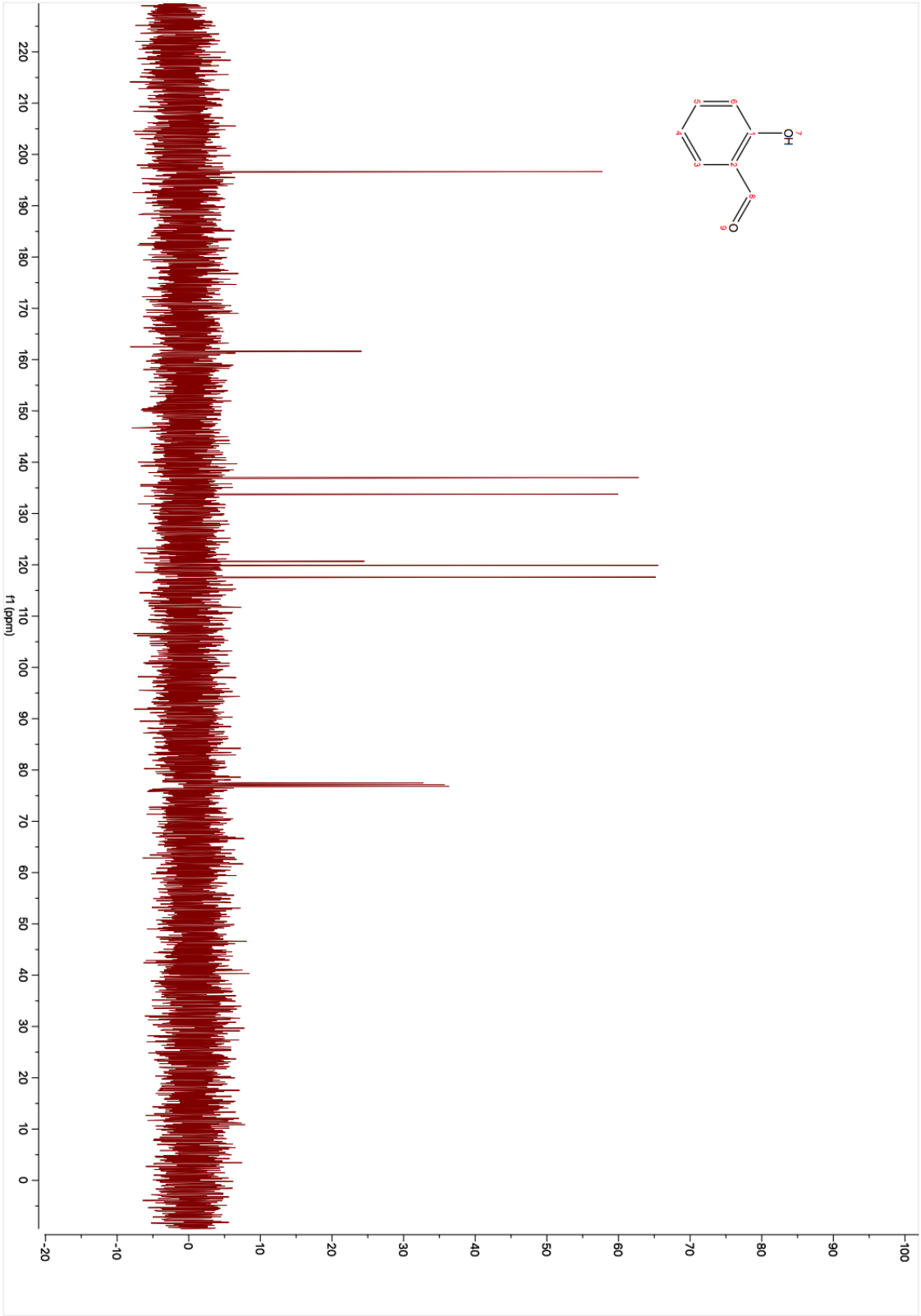

Figure 3.8 ^{13}C NMR Spectrum of 2-hydroxybenzaldehyde