

Synthesis of novel pyrazolyl-selenourea and -carboselenamide molecules for therapeutic applications

by

Raul Andres Neri-Sierra

B.S., Kansas Wesleyan University, 2018

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AN ABSTRACT OF A DISSERTATION

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Department of Chemistry
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2023

Abstract

Based on the classical observation that selenium compounds are more biologically active than their sulfur isosteric relatives, the objective of this dissertation is to discuss the synthesis of novel organoselenium compounds containing pyrazolyl selenourea and pyrazolyl carboselenamide motifs. The idea of developing these selenium isomers will be based on the Bossmann's group staple "NNSN" compounds, which are a family of pyrazolyl carbothioamides and pyrazolyl thioureas that exhibit copper-activated reactivity towards bacterial infections and cancerous murine cells. These compounds will feature a novel NNSeN motif, "NNSeN" pertaining to a structure derived from either a selenourea or carboselenamide featuring a heterocyclic aromatic component with at least one or two nitrogens' (NNSeN) in the ring, i.e., pyrazoles, and the remaining nitrogen (NNSeN) at the alpha position of the selenoketone (C=Se), or better described as selenourea or selenoamide, functional group. A library of NNSeN compounds were synthesized and their syntheses will be discussed. This dissertation will give insight towards: (i) these novel organoselenium compounds that will generate stable C=Se double bonds (ii) whether these selenium isomers will also chelate copper (I or II) (iii) theoretical studies to understand the geometric orientation and copper complexation of these molecules (iv) preliminary biological studies.

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Approved by:

Major Professor
Dr. Stefan H. Bossmann

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Abstract

Based on the classical observation that selenium compounds are more biologically active than their sulfur isosteric relatives, the objective of this dissertation is to discuss the synthesis of novel organoselenium compounds containing pyrazolyl selenourea and pyrazolyl carboselenamide motifs. The idea of developing these selenium isomers will be based on the Bossmann's group staple "NNSN" compounds, which are a family of pyrazolyl carbothioamides and pyrazolyl thioureas that exhibit copper-activated reactivity towards bacterial infections and cancerous murine cells. These compounds will feature a novel NNSeN motif, "NNSeN" pertaining to a structure derived from either a selenourea or carboselenamide featuring a heterocyclic aromatic component with at least one or two nitrogens' (NNSeN) in the ring, i.e., pyrazoles, and the remaining nitrogen (NNSeN) at the alpha position of the selenoketone (C=Se), or better described as selenourea or selenoamide, functional group. A library of NNSeN compounds were synthesized and their syntheses will be discussed. This dissertation will give insight towards: (i) these novel organoselenium compounds that will generate stable C=Se double bonds (ii) whether these selenium isomers will also chelate copper (I or II) (iii) theoretical studies to understand the geometric orientation and copper complexation of these molecules (iv) preliminary biological studies.

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Dedication

This work is dedicated to my family! To my mom and dad Alina and Raul Neri and to my sister Alitzel Neri. This is dedicated to you all because of your unrelenting love and support you all have given me during my adventure at KSU. I would also like to thank my girlfriend, Fernanda Cantu Corona, for being an incredibly wonderful, supportive and loving partner during the high stress moments! And lastly, I would like to thank my best friends Armando Rene Vasquez III and Arnaldo Xavier Torres Hernandez for the love, support and great memories we have made during my time here! Let us hope there are more great memories to come!

Chapter 1 – Background

Introduction

The objective of this dissertation is to discuss the synthesis of a library of novel selenium-based compounds towards the future application of treating cancer, bacterial infections or any biological activity. In this dissertation, the idea of developing these selenium isomers will be based on the Bossmann's group staple "NNSN" compounds, which are a family of pyrazolyl thioureas that exhibit copper-activated reactivity towards bacterial infections and cancerous murine cells. It is proposed that changing the sulfur atom to a selenium atom should increase biological activity as selenium compounds have been known to be more biologically active than its isosteric relative. Whether or not these selenium compounds will also be copper activated will be also be discussed.

Background

Cancer

Cancer is defined as a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body.¹ Unfortunately, cancer can start almost anywhere in the human body and there are more than 100 different types of cancers.² Naturally, human cells grow and multiply via cell division to generate new cells for the body's needs. Over time these cells will die due to cellular damage or aging. Sometimes during this process, abnormal or damaged cells begin to grow and multiply when they shouldn't. It is these cells that may form lumps of tissue, otherwise known as tumors. However, not all tumors can be cancerous (i.e., benign). Benign tumors do not spread into nearby tissues and will not grow back when once surgically removed but can still be life threatening. On the other hand, cancerous tumors, also called malignant tumors, do spread into nearby tissues, and can travel throughout the body and will cause

metastasis. Malignant tumors arise due to genetic changes that control growth and division of cells, such as genetic and epigenetic mutations. These mutations can be hereditary or can be triggered by certain environmental exposures such as radiation, tobacco, and chemical exposure.¹ However, not all cancers involve tumor formation and not all cancers show outward signs or symptoms. Such cancers are pancreatic, prostate, bladder, colon, testicular, kidney, stomach, vaginal, ovarian, and liver cancers.³ These cancers are sometimes referred to as “silent killers” since patients are unaware of the disease, ultimately leading to continuous growth and late-stage diagnosis.

According to the Center for Disease Control and Prevention (CDC): In 2018, the latest year for which incidence data are available, in the United States (excluding Nevada as it did not reach U.S. Cancer Statistics publication criteria), 1,708,921 new cases of cancer were reported, and 599,265 people died of cancer.⁴ To put it simply, for every 100,000 people, 436 new cancer cases were reported and 149 people died of cancer. Cancer is the second leading cause of death in the United States, exceeded only by heart disease.⁴ One of every four deaths in the United States is due to cancer. According to the World Health Organization (WHO): Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020.⁵ Around one-third of these deaths are due to tobacco use, high body mass index, alcohol use, low fruit and vegetable intake, and lack of physical activity. Worldwide, late-stage presentation and lack of access to diagnosis and treatment are common, particularly in low- and middle-income countries, in which approximately 70% of the deaths occur. Even more disturbing: less than 15% of low-income countries have comprehensive treatment available.⁶ Economically, the impact of cancer is significant and still increasing. The total annual economic cost of cancer in 2010 was estimated at \$1.16 trillion US dollars.⁷ Because of the mentioned risk factors, it is important to maintain a

healthy diet and get proper exercise. Research has shown a correlation between being overweight and an increased risk of certain cancers, such as uterine, breast, and colorectal cancers. Cigarette smoking is the foremost cause of lung cancer accompanied by secondhand smoke which has been shown to have even higher concentrations of toxic chemicals than that taken in by the person smoking. Regular alcohol consumption increases the risk of liver, mouth, voice box, breast, colon, and throat cancers. Ultraviolet (UV) rays have been determined dangerous due to their ability to methylate DNA and cause cancer. Contact with carcinogenic chemicals includes exposure to asbestos, nickel, cadmium, radon, vinyl chloride, benzidine, benzene. Lastly, it has also been shown that certain health conditions, such as Human papillomavirus (HPV), Hereditary Breast and Ovarian Cancer Syndrome, and Lynch Syndrome, increases the risk for certain cancers.⁷

The most common treatments for cancer are surgery, chemotherapy, and radiation. Surgery has been conventionally used for cancer as a tool in multiple ways. Different types of surgeries include diagnosis, cancer staging, curative, debulking, palliative, supportive, restorative (reconstructive), and preventative.⁸ In diagnosis, a small piece of tissue sample is removed and tested. This is better known as a biopsy or surgical biopsies. Staging surgery is done to find out how much cancer there is and how far it has spread. During this procedure, the area around the cancer is examined. This is important because it can help provide information for future treatments and decisions. Curative surgery, or primary surgery, is done when cancer is found in only one part of the body and when it is likely that all the cancer can be removed. Curative surgery can be used as a main treatment or can be used alongside other treatments such as chemotherapy or radiation therapy. Debulking surgery is used to remove only a portion of the cancer. It is named debulking because the tumor being treated is a large, bulky object and might

be located very close to important organs or tissues. Thus, de-bulking the tumor can help make it smaller. This surgery is sometimes done when taking out the entire tumor would cause too much damage to nearby organs or tissues. Palliative surgery is used to treat problems caused by advanced cancer. This type of surgery helps ease problems caused by cancer and helps people feel better, unfortunately because the cancer is usually in an advanced stage, it cannot be employed to treat or cure the cancer itself. Supportive surgery is done to help make it easier for people to get other types of treatment. Reconstructive surgery is used to improve the way a person looks after major cancer surgery. It can also be used to restore the function of an organ or body part after surgery. Lastly, preventive, or prophylactic, surgery is done to remove body tissue that is likely to develop cancer, even when there are no signs of cancer at the time of the surgery. At times an entire organ is removed when a person has a condition that puts them at very high risk for having cancer there. This surgery is done to reduce cancer risk and help prevent the chance of cancer, but unfortunately does not guarantee cancer prevention.⁸ Surgery always comes with some risks, such as: excess bleeding, damage to other tissues/organs, reaction to drugs used during or after surgery, and not to mention pain, possible infections, and slow recovery after surgery. Chemotherapy can also be used as a treatment, which is the use of drugs to treat cancer throughout the entire body, but at a cost. While this can be effective at killing cancerous cells that have metastasized, the major side effects for chemotherapy are the collateral damage these drugs cause to normal and healthy cells. This collateral damage results in weakness of the immune system and consequent infections, anemia, as well as weight and hair loss and fertility problems.⁹ Radiation therapy is another common treatment. This treatment option uses x-rays, gamma rays, implanted radioisotopes, electron beams, or proton beams to damage or destroy cancerous cells. Radiotherapy is often effective in shrinking early-stage

tumors; however, it does not reach all parts of the body, and the side effects include the risk of developing a second cancer as well as nausea, vomiting, diarrhea, and weight loss.¹⁰ The treatment options will depend on the stage of the cancer. Along with the potent side effects of cancer treatments, cancer patients are also at an increased risk of obtaining antibiotic resistant infections due to their compromised immunity.¹¹

Antibiotic Resistance

Bacterial infections are a serious health issue worldwide and the number of cases is anticipated to continue rising. The problem is especially dire when considering the increase in resistant bacteria that are becoming ever more difficult to treat. Bacterial infections can become very serious, especially for cancer patients, and may lead to sepsis. Sepsis occurs when the body elicits an extreme response to the infection. Without treatment, sepsis can cause dramatic drops in blood pressure, organ failure and death. For these reasons, antibiotics are often given as a preventative measure alongside chemotherapy and surgery.¹¹ Unfortunately, the over-prescription of antibiotics contributes to the issues of antibiotic resistance. According to the CDC's Antibiotic Resistance Threats in the United States 2019 report (the latest year for which data is available): more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result.¹² On average, in the United States, someone will develop an antibiotic-resistant infection every 11 seconds... and every 15 minutes someone will succumb to their antibiotic-resistant infection.

Antibiotic resistance is defined as the ability of germs, such as bacteria and fungi, to develop means to defeat the drugs designed to kill them. Over-prescription is a danger as it allows germs to become resistant to antibiotics available on the market today. Resistance is a

natural occurring process; however, this resistance is catalyzed by a combination of germs

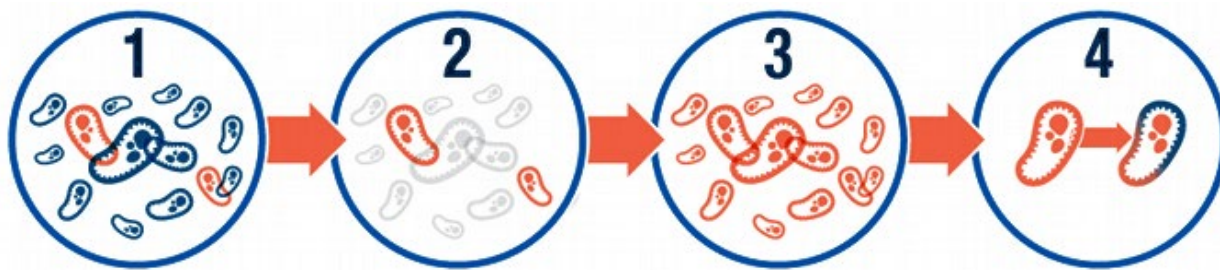


Figure 1: Antibiotic resistance occurrence¹³

exposed to antibiotics and antifungals, and the spread of those resistant germs and their resistance mechanisms. Illustration 1 highlights the way resistance occurs: 1.1 a patient is prescribed antibiotics by their physician. At the beginning of treatment, the patient has a high number of bacteria, some with the ability to resist antibiotics. 1.2 The patient finishes the treatment, and the antibiotics kill the bacteria causing illness as well as beneficial bacteria that protects the body from infection. 1.3 Unfortunately, the antibiotic resistant bacterium remains and now has optimal conditions to reproduce and become the majority. 1.4 At this point, bacteria can even transfer their drug resistance to other bacteria via mobile genetic elements (plasmids, transposons, and phages) and further antibiotic resistance emerges. This is problematic as these resistant drugs can then spread into new settings and (in a global scale) between countries. However, the preceding scenario was under the assumption that patients will finish their antibiotics. In the scenario where patients do not finish their antibiotics, the surviving germs will become the ones with the greatest resistance.¹³

Keeping everything discussed in mind, the development of a novel class of drugs with activity towards both cancer and bacterial infections could combat these issues. As such, the drugs proposed are a novel class of organoselenium compounds coined NNSeN's and will be discussed herein.

Preliminary Work: NNSNs - Structure and Copper-Activated Activity

NNSN's are a class of organosulfur molecules with either a carbothioamide or extended thiourea motif (Figure 2). These molecules are synthesized from two components: aryl isothiocyanates (ITCs) and pyrazoles. These can be either purchased or synthesized and then coupled to generate the desired NNSNs with different functional groups on either component for biological activity.

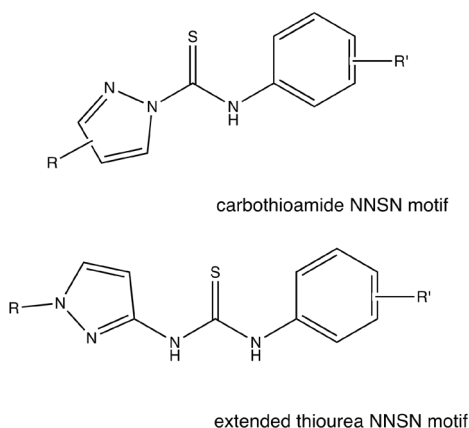


Figure 2: NNSN motifs

For example, aryl and benzyl ITCs show best activity when functionalized with one or more halogens (-Cl, -Br, or -F), nitro groups, alkoxies (such as methoxies), or even alkyl groups.¹⁴ The pyrazole component can be functionalized with substituted or unsubstituted aryls, branched or unbranched alkyls or alkenyls (which may be substituted with either halogens, sulfonic acid, or nitro groups), cycloalkyls, heterocycles, or substituted or unsubstituted amines or amides or simply just hydrogen.¹⁴ These furnished molecules exhibit copper-activated activity and are still being explored for therapeutic use.

NNSN molecules have shown great activity against bacterial infections and cancerous cell lines.

NNSNs have proven effective at killing both gram-positive and gram-negative bacteria in the

presence of copper. In the case of gram-positive bacteria, NNSNs have been tested and shown

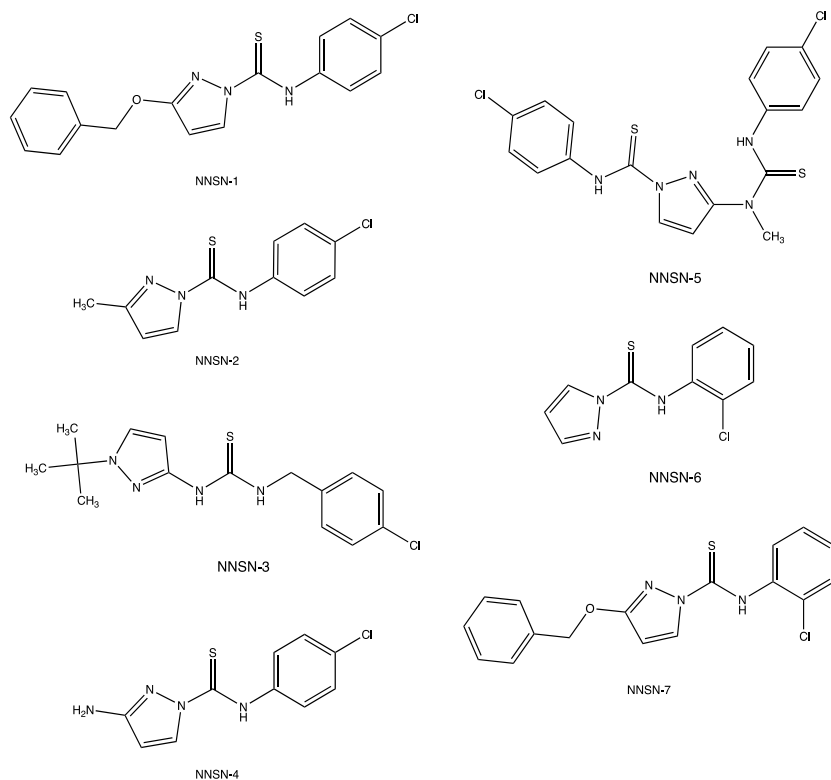


Figure 3: Select NNSNs with biological activity

activity against both *S. aureus* strain Newman Methicillin-Sensitive Staphylococcus Aureus (MSSA), a MSSA clinical isolate¹⁵, and *S. aureus* strain Lac Methicillin-Resistant Staphylococcus Aureus (MRSA), a USA3000-type MRSA clinical isolate¹⁶⁻¹⁸. Almost all NNSN molecules are found to be biologically active. Out of 26 recently published, a total of 16 NNSNs, 9 carbothioamides and 7 extended thiourea motifs, were found to have (sub)micromolar MICs against SA Newman (MSSA). Whereas 4 of these carbothioamides and 5 of the extended thiourea motifs showed micromolar activity against SA Lac (MRSA). The most active compounds against SA Newman were carbothioamide NNSNs 3-(Benzyloxy)-*N*-(4-chloro-phenyl)-1H-pyrazole-1-carbothioamide (NNSN-1), *N*-(4-Chlorophenyl)-3-methyl-1H-pyrazole-1-carbothioamide (NNSN-2) and extended thiourea NNSN 1-(1-(tert-Butyl)-1H-pyrazol-3-yl)-3-(4-chlorobenzyl)-thiourea (NNSN-3). The most active compounds against SA Lac were carbothioamide NNSNs 3-

Amino-*N*-(4-chlorophenyl)-1H-pyrazole-1- carbothioamide (**NNSN-4**) and *N*-(4-Chlorophenyl)-3-methyl-1H- pyrazole-1-carbothioamide (**NNSN-2**). This biological testing was done both in the absence and presence of 50 μ M copper sulfate. None of the NNSN compounds showed activity against SA Newman or SA Lac in the absence of copper in the (micromolar) concentration range.¹⁸ These studies revealed that copper activation of toxicity against Gram-positive bacteria is possible via NNSN structural motif enhancement of the toxicity of copper cations that would be available in activated phagosomes and support huge therapeutic potential. In the case of Gram-negative bacteria, a lone NNSN carbothioamide motif, 3-Amino-*N*-(4-chlorophenyl)-1H-pyrazole-1-carbothioamide (**NNSN-4**), was found to have activity against *Escherichia coli*. In this case, the respective NNSN was coupled to a K₂₀ peptide and a peptide nanosponge and revealed decent biological activity with MIC values of 5 μ M and 10 μ M, respectively. In the case of cancer, NNSNs have shown biological activity against cancerous murine cell lines.¹⁹ These NNSNs were tested both in the absence and presence of copper and revealed activity in both cases. However, the activity observed in the presence of copper was substantially higher. To mention a few, these select NNSNs with a carbothioamide motif show activity against cancerous murine cell lines B16F10, GL26, Pan02 and KPC as well as NSC cell lines: 3-amino-*N*-(4-chlorophenyl)-1H-pyrazole-1-carbothioamide (**NNSN-4**), 1-(1-(4-chlorophenylthiocarbamoyl)-1H-pyrazol-3-yl)-3-(4-chlorophenyl)-1-methylthiourea (**NNSN-5**), *N*-(2-chlorophenyl)-1H-pyrazole-1-carbothioamide (**NNSN-6**), and 3-(benzyloxy)-*N*-(2-chlorophenyl)-1H-pyrazole-1-carbothioamide (**NNSN-7**).

This biological testing suggests that NNSNs are relatively non-toxic until copper activation, in which the appropriate copper concentrations can be found at the site of bacterial infection or within a tumor(s) microenvironment(s).¹⁴ Copper (either I or II) is an endogenous metal with

effective antibacterial properties. Under physiological conditions, copper exists in the

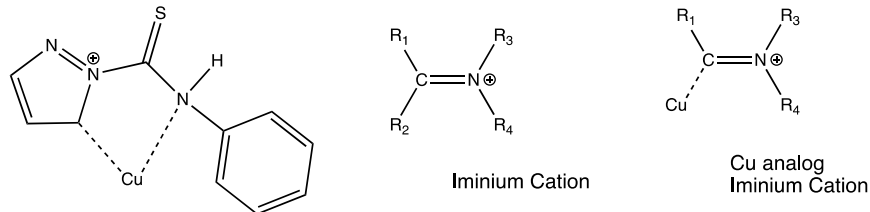


Figure 4: Structure of Iminium Cation

oxidation states Cu (I) and Cu (II). The shifting back and forth between these two oxidation states, via a single electron transfer reaction, makes copper essential to several enzymatic reactions. However, this redox property also adds to its potential toxicity. At high levels, redox cycling between Cu (I) and Cu (II) can generate highly reactive oxygen species (ROS), such as singlet oxygen, hydrogen peroxide, and hydroxyl and hydroperoxyl radicals, which can damage proteins, lipids, and DNA.²⁰ Consequently, tight homeostatic regulation is obligatory to restrict the concentration of free copper in blood and healthy tissues.^{14,20} Copper concentration can be altered via upregulation occurring in activated phagosomes and cancerous cells. In either case, the copper concentration can increase up to 5-50 times the amount found in normal tissue, which is approximately 1 micromolar or less of what would be “free” copper ions, something that is extremely rare in mammalian bodies.¹⁴ This is beneficial in the case of NNSN delivery because free copper ions have little therapeutic value due to their erratic activity and low concentrations. Once at the infection site, NNSN-copper complexes form and inhibits the cell membrane’s ability to filter out or remove copper ions via efflux pumps. NNSN-copper complexes form an iminium-type cation (Figure 4) which is considered a warhead as they can disrupt numerous enzymes within a cell and react with multiple targets through nucleophilic addition, electron transfer and radical reactions.¹⁴ This effectively shuts down the efflux pumps and allows copper to deluge into the cell leading to programmed cell death.

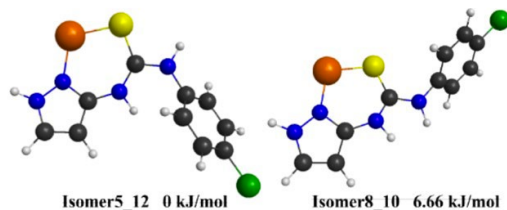


Figure 5: Geometries and relative energies of extended thiourea NNSN-Cu motif²¹

The likely oxidation state of copper would be Cu (I); however, Cu (II) is typically the most stable in aqueous conditions. It is important to note that in the case of cancer, the likely oxidation state of copper would be Cu(I) due to the reductive microenvironment found in cancerous tumors.¹⁴ This is important in the case of NNSN delivery because Cu (I) is highly electrophilic and will be drawn to areas of high electron density, such as the nitrogen center in the pyrazole rings of NNSNs, the lone pair of electrons found on the sulfur of NNSNs and/or the π -electrons in the aryl ring. Density functional theory (DFT) calculations suggest that an extended thiourea NNSN-Cu motif would be most stable with copper complexation with sulfur and a nitrogen in the pyrazole ring with both the extended thiourea motifs with relative energies of 0 kJ/mol and 6.66 kJ/mol, respectively (Figure 5).²¹

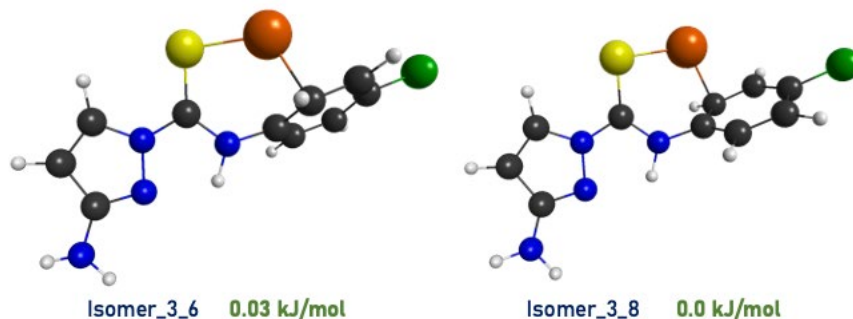


Figure 6: Geometries and relative energies of carbothioamide NNSN-Cu motifs²¹

In the case of carbothioamide NNSN-Cu motifs, the most stable conformer would have copper complexation between the sulfur and the *ortho* carbons of the aromatic ring with relative energies

of 0.03 kJ/mol and 0.0 kJ/mol, respectively (Figure 6).²¹ DFT calculations also suggest that bond angle formation compliments a square planar geometry, the preferred geometry for copper (I) ions.

Discussed Research: NNSN's → NNSeN's

Based on the classical observation that selenium compounds are more biologically active than their sulfur analogues,²² the objective of this dissertation is to discuss the synthesis of novel organoselenium compounds containing pyrazolyl selenourea and pyrazolyl carboselenamide motifs. These compounds will feature a novel NNSeN motif, “NNSeN” pertaining to a structure derived from either a selenourea or carboselenamide featuring a heterocyclic aromatic component with at least one or two nitrogens’ (NNSeN) in the ring, i.e., pyrazoles, and the remaining nitrogen (NNSeN) at the alpha position of the selenoketone (C=Se), or better described as selenourea or selenoamide, functional group (Figure 7).

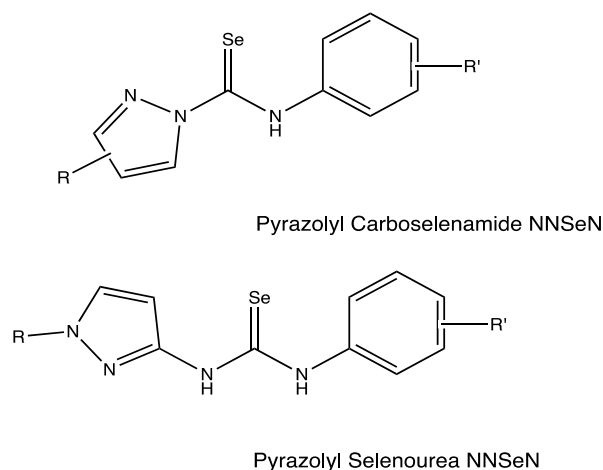


Figure 7: NNSeN Motifs

A library of NNSeN compounds were synthesized and their syntheses will be discussed. This dissertation will give insight towards: (i) these novel organoselenium compounds that will generate stable C=Se double bonds (ii) whether these selenium isomers will also chelate copper

(I or II) (iii) theoretical studies to understand the geometric orientation and copper complexation of these molecules (iv) preliminary biological studies.

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Chapter 2 – Selenium, Selenium vs Sulfur, ISC, Formylated Amines and Pyrazoles

Why Selenium? Selenium and Health

Selenium, like the moon, has been described to have two faces, as it is both toxic and essential to organisms.¹ Early studies first recognized the role of selenium as a toxin. This recognition came from investigating the cause of 'alkali disease' and 'blind staggers', which are livestock diseases in the American West and the Plains states. Both diseases were forms of selenosis (chronic selenium intoxication) due to the ingestion of cereal crops, animal forage and *Astragalus* (or better known as “locoweed”) all in which high doses of selenium were found due to the selenium-rich soils.²⁻⁵ This investigation concluded that selenium “is in a compound very similar to cysteine”⁶ (verbatim from reference 6). However this was not widely recognized as it pre-dated the discovery of selenocysteine by 40 years.⁷ As research into selenium advanced, its importance and necessity expanded.

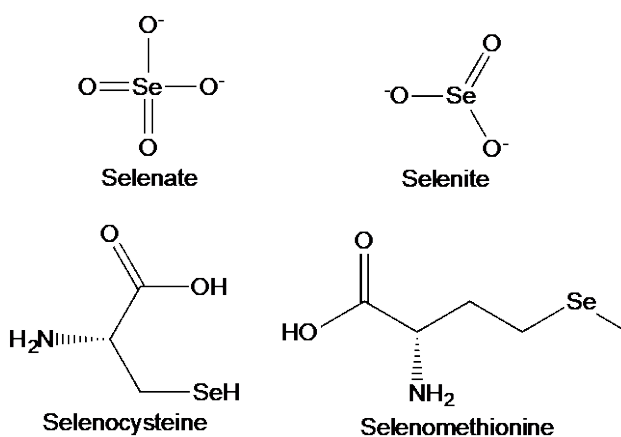


Figure 8: Inorganic and organic forms of Se

Selenium (Se) is known as an essential trace element in the nutrition of both animals and humans. It is naturally present in many foods, added to others, and readily available as a dietary

supplement. Selenium has been deemed “nutritionally essential” for humans and is a constituent of selenoproteins that have a play in critical roles such as reproduction, thyroid hormone metabolism, DNA synthesis and protection from oxidative damage and infection.⁸ Selenium exists in two forms: inorganic (such as selenate and selenite) and organic (such as selenocysteine and selenomethionine)⁹ (Figure 8). Most selenium is in the form of selenomethionine in animal and human tissues where it can be integrated nonspecifically with methionine. A major site of selenium storage is found in the skeletal muscle where 28-46% of the total selenium collection dwells.¹⁰ Major food sources of selenium are Brazilian nuts, seafoods, and organ meats.⁸ Other food sources include meats, cereals and other grains, and dairy products. In the American diet, major food sources of selenium are breads, grains, meat, poultry, fish and eggs.¹¹ For humans, concentration dependance of selenium can be both beneficial and detrimental. Studies have shown that there is an inverse relationship between Se supplementation and cancer risk.¹² However, it is important to note that even selenium supplementation poses a health risk as it can be both beneficial and detrimental. Dietary levels depend on age, but after the age of 14 the recommended selenium intake is 55 µg/day, which is known to prevent the development of various cancers. A high selenium intake, such as >350 µg/day, can be carcinogenic, genotoxic, and cytotoxic.¹³⁻¹⁵

The chemopreventative effects of daily selenium intake are often attributed to the selenocompounds’ ability to function as an antioxidant and control the redox status of the cells, ultimately protecting against oxidative stress. In contrast, the effects of high selenium intake enable the selenocompounds’ to become redox active, pro-oxidative, and cytotoxic to tumor cells which contain increased levels of reactive oxygen species (ROS) but can also be toxic towards healthy cells.¹⁶ Outside of its recognized position in enzymes, such as selenocysteine, selenium and selenium compounds have been found to inhibit tumorigenesis in a range of animal models.¹²

Studies have shown selenium can be used as a supplemental agent to help reduce the risk of cancer. *In vivo* chemical and viral carcinogenesis studies show a reduced prevalence of tumors following selenium supplementation.¹²

Organoselenium compounds have been shown to hinder the initiation and post-initiation phases of chemical carcinogenesis, as well as displaying antitumorigenic, antiangiogenic and pro-oxidant activity in cancer cells.^{12, 17, 18} Despite the complex and ambiguous mechanisms of action, organoselenium compounds are proposed to induce DNA damage, regulate the cell cycle, inhibit cellular growth, induce apoptosis, and generate ROS.^{12, 17, 18, 19} Furthermore, it has been reported that patients with melanoma, breast cancer, and pancreatic cancer have exhibited decreased selenium levels in whole blood or serum compared to healthy controls.²⁰

Despite the mentioned importance of selenium and organoselenium compounds, there is evidence showing contradictory antioxidant behaviors in selenium containing compounds, suggesting that these compounds must be examined individually for its antioxidant conduct. For example, selenium-based agents have been recognized as redox modulators for activity in redox catalysts, intracellular redox control, and apoptosis, but specificity is in question. These agents' initial redox targets are unknown. Such targets can include redox-sensitive cysteine-containing Bcl proteins, which control apoptosis at early stages. However, certain caspases can also be targeted, due to redox sensitivity, which targets apoptosis mechanisms at later stages. On the other hand, the selenium redox modulators may not just be targeting a specific target, but rather cause a wide-ranging shift in intracellular redox states, which results in widespread modification of proteins and enzymes.²¹ Because of this, there are contradictory literature reports of selenium's efficacy, but it would be unwise to disregard the therapeutic potential in the field of selenium chemistry.

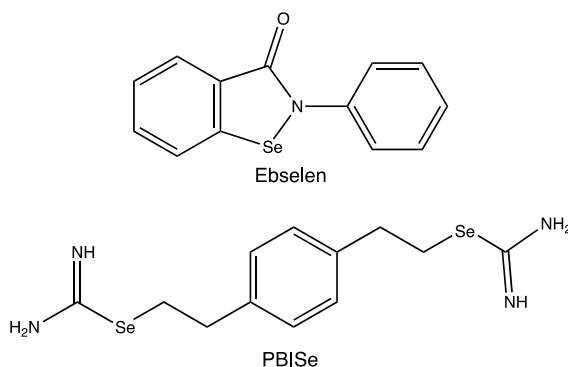


Figure 9: Se-containing therapeutically active compounds

To name some important therapeutically active compounds containing selenium, Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one), which was first synthesized in 1924,²² is a synthetic organoselenium drug with anti-inflammatory, antioxidant and cytoprotective activity and was under investigation as a potential treatment for severe COVID-19 cases²³ (Figure 8). Other important drug candidates containing selenium are 2-(α -hydroxy- β -N-piperidine-ethyl)selenophene and related anti-arrhythmic agents,²⁴ selenophene-containing inhibitors of type IIa bacterial topoisomerases,²⁵ 4'-selenonucleosides as next-generation nucleosides,²⁶ and the anti-melanoma drug PBISe [S,S'-1,4-phenylenebis(1,2-ethanediy)]bis-isoselenourea] (Figure 9).²⁷

Chemical differences between Selenium (Se) and Sulfur (S)

Because we are proposing our novel NNSeN structures will be more biologically active than their isosteric NNSN relatives, it is important to list the chemical differences between Se and S. As mentioned, selenium containing compounds have been observed to exhibit more potent biological activity than their sulfur counter parts. In order to understand this increased activity, it is important to highlight the following subtle differences between these two chalcogens (Table 1).

- i. Se is larger in size than S with an atomic radius of 1.15 Å vs. 1.0 Å, respectively. This results in more loosely bound outer valence electrons, which is significant to the antioxidant properties of Se. For example, Se reacts with reactive oxygen species (ROS)

faster than S.²⁸ An additional example, selenomethionine (SeMet) will undergo chemical oxidation rates that are 10-100 fold faster than those with methionine (Met).²⁹

- ii. A known comparison, especially when comparing selenium dioxide (SeO₂) to sulfur dioxide (SO₂), are the larger hybridized orbitals and longer bond length of the Se=O double bond result in weaker π overlap, decreasing the π bonding properties of the Se=O bond, thereby allowing Se=O-containing compounds to be readily reduced to low-valence selenium species such as selenol (R-Se-H) and selenide (R-Se-R'), which can continuously resist ROS via a reversible redox process.
- iii. The longer Se-H bond length results in an increased acidity for selenocysteine (Sec, pK_a = 5.24) compared to cysteine (Cys, pK_a = 8.25). Thus, Sec is almost fully ionized and found as selenolate (R-Se⁻) at physiological pH, whereas Cys is largely in the nonionized state (R-SH).³⁰ Additionally, the stronger nucleophilicity as well as lower redox potential makes Sec more potent during redox catalysis.
- iv. As a result of its size, highlighted in point (i), Se is more polarizable (or “softer”) than S, resulting in greater nucleophilic power, which is beneficial for Se to coordinate to metals or metal centers in the catalytic sites of enzymes.³¹
- v. The larger size of Se results in reduced orbital overlap and longer bond lengths, resulting in weaker Se-X bonds than the analogous S-X bonds. The weaker bond strength means that the σ^* orbital of the Se-X bond is lower in energy than that of the S-X bond; thus, Se is more reactive as an electron acceptor and much more electrophilic than the sulfur analog.
- vi. The larger size of Se leads to an increase in the steric hindrance of drug molecules.
- vii. The lower polarity and more polarizable properties of Se generally give drug molecules increased lipophilicity and permeability.

These unique chemical properties of Se are closely related to the pharmacological activities and physicochemical properties of Se-containing compounds. As such, understanding these relationships is of vital importance for the rational design, research, and development of Se-containing drugs.

Property	Se	S
Atomic radius	1.15 Å	1.0 Å
Van der Waals radius	1.9 Å	1.8 Å
pK _a	Sec: 5.24	Cys: 8.25
π -bonding	Se < S	Se < S
Polarization	Se > S	Se > S
Nucleophilicity	Se > S	Se > S
Electrophilicity	Se > S	Se > S
Redox Potential	RSe•/RSe ⁻ : 0.43v	RS•/RS ⁻ : 0.92v

Table 1: Chemical Properties Comparison of Se and S

NNSeN's

As mentioned earlier, NNSeN's are isosteric isomers of the NNSN's synthesized in the Bossmann research group. There are two main target motifs for the selenium isomers: pyrazolyl-selenoureas and pyrazolyl-carboselenamides, also coined extended NNSeN's and conventional NNSeN's respectively. The synthesis of NNSeN molecules will be discussed including all synthetic ventures taken until optimal conditions were found.

Selenation of NNSN's

When we first began to ponder the question of how to synthesize these novel structures, we first looked at traditional named reactions to see if any installed any type of selenoketones (C=Se) which then we could further manipulate into selenoamides or selenoureas via nucleophilic acyl/selenyl substitution type reactions or which named reactions could possibly generate the specific selenoamides or selenoureas. However, the named reactions we found that involved selenium did not provide this. To name a few reactions that utilize selenium: 1) Davis' oxaziridine

oxidations, which applies 2-sulfonyl oxaziridnes as oxidizing agents for syn-elimination of selenoxides to alkenes and the rearrangement of allylic selenoxides to allylic alcohols.³² 2) Baeyer-Villiger reactions in which the first catalysts used for these reactions with hydrogen peroxide were substituted benzeneseleninic acids that are usually generated *in situ* and was reported by Ludwik Syper, which is also known as the Syper process.^{33,34} 3) Pummerer rearrangement in which typically sulfoxides are used, however whenever selenoxides are used as the substrates, the selenol-Pummerer rearrangement takes place instead with similar reactivity and mechanism as the sulfoxide substrate.³⁵ 4) Probably the most well-known named reaction involving selenium, the Riley oxidation utilizes selenium dioxide as an oxidant of aldehydes and ketones which can be converted to the corresponding 1,2-dicarbonyl compounds in moderate to good yields.³⁶ Besides carbonyl compounds, the Riley oxidation was also extended to olefinic substances which can be oxidized at the allylic position, allylic oxidation, to the corresponding allylic alcohols or enones.³⁷

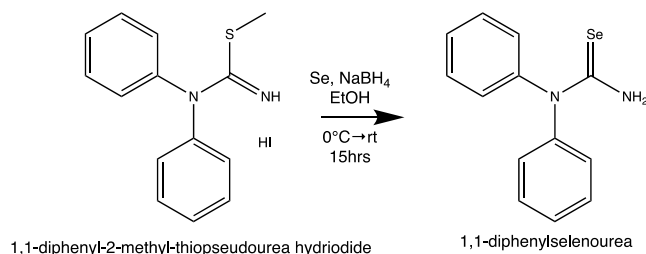


Figure 10: Introduction of selenium via nucleophilic displacement³⁸

None of these mentioned generated the desired functional groups so we then looked at the NNSN molecules and looked at how we could possibly directly substitute the sulfur atom for a selenium atom via a nucleophilic displacement reaction. In a classical approach, we envisioned we could directly methylate the NNSN sulfur atom to a NNSN and then introducing selenium via a nucleophilic displacement reaction of selenium with sodium borohydride in ethanol.³⁸ Of course, this approach would be limited by NNSNs with functional groups that could easily be methylated, such as free amines. However, this could be avoided via protection/deprotection protocols. In

1972, Klayman and Griffin successfully introduced selenium by the method previously mentioned by taking 1,1-diphenyl-2-methyl-thiopseudourea hydriodide (also named hydroiodide, i.e., hydrogen iodide) and selenating it via *in situ* sodium diselenide, prepared by using a 2:3 molar ratio of sodium borohydride to selenium in ethanol (Figure 10).³⁸ As such to test this a “simple” NNSN structure, “simple” meaning no functional groups on the carbothioamide NNSN, was required. This was prepared in the same manner that many of the staple NNSN compounds were synthesized: an isothiocyanate and pyrazole coupling. The isothiocyanate was prepared in two steps. First, a dithiocarbamate salt is generated from the corresponding aniline, base and carbon disulfide. Then, the salt is dissolved in an aqueous solvent system along with base and a desulfurization agent, in this case crystalline iodine, is used. To synthesize the dithiocarbamate, a solution of triethylamine and carbon disulfide was prepared while at 0°C. Then, aniline was added slowly over a period of 30 minutes while keeping the temperature at 0°C. Post addition the reaction mixture was allowed to warm to room temperature and left to stir overnight. This was then filtered and washed with a 95:5 ethyl acetate: hexane mixture to remove any excess triethylamine. The dithiocarbamate salt can then be collected and dried *in vacuo*. It is important to note that these salts must be prepared fresh and used quickly. To synthesize the isothiocyanate, a solution of the dithiocarbamate salt and sodium bicarbonate was made using a 1:1 aqueous system of ethyl acetate: water. This solution was cooled to 0°C with an ice bath and, while stirring, crystalline

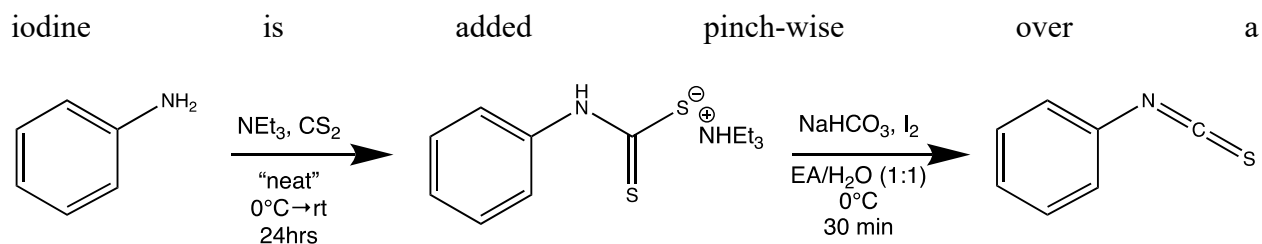


Figure 11: Synthesis of phenyl isothiocyanate

period of 30 minutes. During this time a putrid odor may be noticed as a yellow precipitate of sulfur begins to settle at the water-ethyl acetate interface. Once the addition is completed, the organic layer was washed with sodium thiosulfate to aid the removal of any excess iodine, followed by water, brine and then dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and then the product was purified via silica column chromatography using a hexane as eluent (Figure 11).³⁹

With the isothiocyanate in hand, the “simple” NNSN can be synthesized via coupling of the isothiocyanate and pyrazole. This was done in a 1.1:1 molar ratio of isothiocyanate: pyrazole. Both starting materials were added to a dry round bottom flask and dissolved with dry dichloromethane (DCM) and flushed with argon. This was then heated to reflux and left to stir overnight. After overnight this was then directly loaded onto a silica column and purified using 20% DCM: hexane → 100% DCM gradient. The NNSN product was obtained in a 21% yield. This was then methylated using a 1:1 molar ratio of NNSN: iodomethane in ethanol.

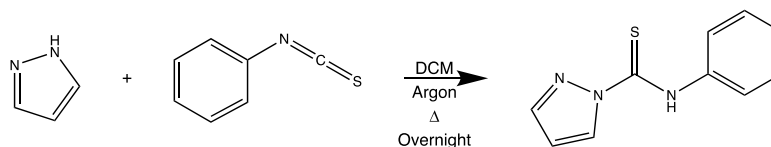


Figure 12: Synthesis of "simple" NNSN

The two starting materials were added to a round bottom flask and were dissolved/diluted, respectively, with absolute ethanol. This was then heated to reflux and left to stir overnight. Thin layer chromatography (TLC) analysis revealed starting material spot (NNSN) completely gone and a new product spot was observed. After the solvent was removed *in vacuo* and the product was isolated as a sun-yellow crystalline solid. This was then subjected to the selenation conditions:³⁸ a dry round bottom flask was charged with grey selenium powder and sodium borohydride. This was dispersed/diluted with absolute ethanol at 0°C and stirred vigorously. This

mixture will react to generate sodium diselenide, a more nucleophilic selenium agent, in ethanol, as shown in the following reaction equation:



Then the methylated NNSN salt is then added as a mixture in absolute ethanol. The reaction mixture was then removed from the ice bath and allowed to warm to room temperature and left to stir for 24 hours. TLC analysis revealed no new spots, and this was left to stir for an additional 24 hours, however no product spots were observed. Removal of the solvent *in vacuo* revealed that the methylated NNSN starting material remained and was unreacted. The same methylation and selenation procedure were then repeated with a different NNSN molecule, SB46, which contained an electron withdrawing chlorine group on the ortho position but produced the same result. It was concluded that the classical nucleophilic displacement reaction with selenium was not applicable for our needs. As such a different route was needed.

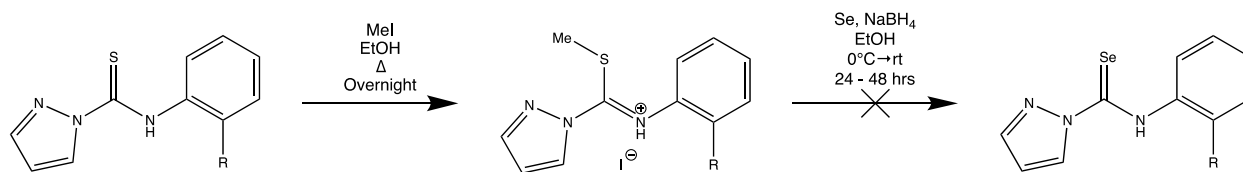


Figure 13: Initial synthetic venture to access NNSeNs. R = H (simple NNSN) or Cl (SB46)

Considering the synthetic route used for NNSNs, two components were needed: a pyrazole ring and a phenyl isothiocyanate. Fortunately, both components could be synthesized or purchased commercially. Aiming to apply a similar synthesis towards NNSeNs, two components would be required: pyrazoles and phenyl isoselenocyanates, not to be confused with phenyl selenocyanates. Surprisingly, phenyl isoselenocyanates are very limited, if any available, commercially. This was an exciting opportunity to expand synthetic expertise towards the production of functionalized phenyl isoselenocyanates and application of these compounds to generate novel pyrazolyl selenourea and pyrazolyl carboselenamide NNSeN motifs. As such, a new synthetic plan arose:

NNSeNs to be synthesized via coupling of pyrazoles and isoselenocyanates, both which needed to be synthesized in order to generate a library.

Isoselenocyanates (ISCs)

Isoselenocyanates (ISCs, $R-N=C=Se$) have been recognized as potential chemotherapeutic and chemopreventative agents. ISCs have activity against melanoma^{20,40,41}, lung cancer^{42,43}, colon cancer^{44,45}, liver cancer⁴⁶, breast cancer⁴⁷, and prostate cancer.⁴⁸ However, these agents' applications are not limited to cancers as they have activity against parasitic leishmaniasis⁴⁹ and infectious disease such as malaria and tuberculosis.⁵⁰ Chemically, ISCs are similar to their isosteric relatives isothiocyanates (ITCs). While the chemistry of ISCs is not as established when compared to ITCs, ISCs are still of huge importance based on the classic observation that selenium compounds have increased activity in cancer deterrence than their sulfur analogues.⁵¹ Structural-activity relationship (SAR) studies comparing ITC and ISC analogues reveal ISCs to be more cytotoxic towards cancer cells.²⁰ Chemically, ISCs can serve as precursors and essential intermediates for a variety of organoselenium compounds, such as, selenocarbamates, heterocycles, and selenoureas, all which are biologically active. For this dissertation, the focus will be on novel carboselenamide and extended selenourea structures dubbed NNSeN's.

Synthesis of ISCs

We began to explore how we could access ISCs. Classically, organic ISCs are synthesized by the addition of elemental selenium to isocyanides (Figure 14-1).⁵²

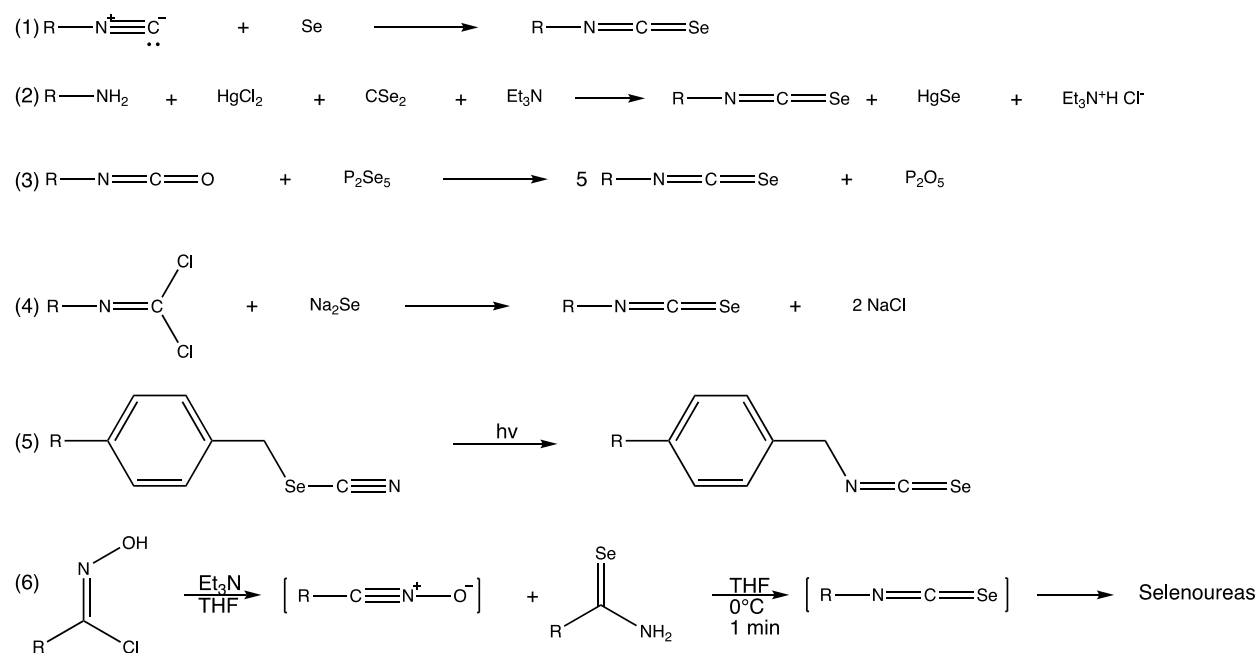


Figure 14: Traditional Synthesis of ISCs

While this method does provide moderate to high yields while using inexpensive elemental selenium, major disadvantages are the varied toxicities and extremely pungent odor of isocyanide molecules.^{53,54} ISCs have also been synthesized by using a primary amine, CSe₂ and HgCl₂ in the presence of triethylamine (Figure 14-2). This method attempts to stray away from isocyanides. While this method does produce ISCs, there is an additional amine-mercuric chloride adduct which leads to the formation of undesired side products such as selenourea, carbodiimide or isocyanides leading to difficulties during purification and isolation.⁵⁵ Other methods to access ISCs includes the reaction of isocyanates with phosphorous (V) selenide

(Figure 14-3),⁵⁶ the reaction of sodium selenide with N-arylcabidimic dichlorides (Figure 14-4),⁵⁷ a photochemical rearrangement of selenocyanates (Figure 14-5),⁵⁸ and the in situ formation of ISCs via a one-pot cycloaddition by reacting nitrile oxides with primary selenoamides (Figure 14-6), which can further react with amines to form selenoureas.⁵⁹

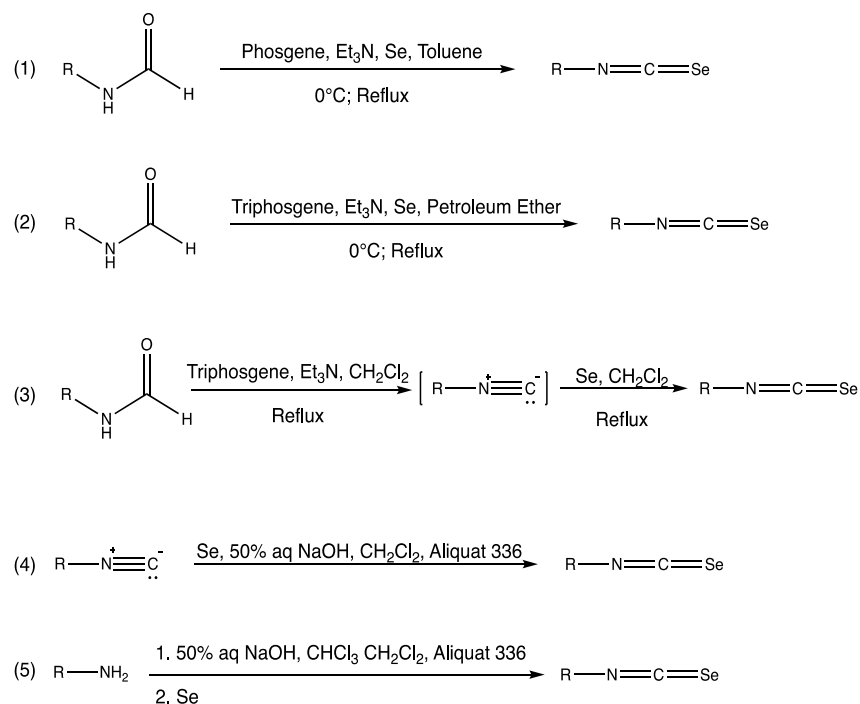


Figure 15: One-Pot Synthesis of ISCs

While all these methods are applicable for our needs, they all have limitations such as unwanted side products, low yields and difficult in purification. Barton *et. al.* developed the first convenient one-pot procedure for the synthesis of aromatic ISCs from their corresponding formamides (Figure 15-1). This procedure is done in toluene while in the presence of phosgene, triethylamine, and elemental selenium. The formamides are subject to dehydration using phosgene and triethylamine, which leads to the *in situ* isocyanide which is “immediately consumed upon generation” by the elemental selenium leading to ISCs.⁵³ This method was our first synthetic attempt. To access phenyl isoselenocyanates, we dissolved a mixture of n-phenyl formamide, grey selenium powder and triethylamine in toluene at 0°C under argon. As this was

stirring, a solution of triphosgene, not phosgene gas that Barton *et. al.* utilized, in toluene was added dropwise over a period of 30 minutes. Once this was added the mixture was allowed to warm to room temperature and then heated to reflux for 16 hours in the dark. TLC analysis revealed a strong starting material spot (n-phenyl formamide) with a faint new spot following the solvent line. It was suspected that this new spot was the ISC since it would be relatively non-polar and expected to travel the highest on TLC. To isolate this, the reaction mixture was filtered to remove any unreacted selenium powder. This solvent was removed *in vacuo* and the product was purified via silica column chromatography using a hexane: ethyl acetate gradient. The suspected product was isolated as a dark yellow/brown oil. This was characterized via ¹H-NMR and revealed a high number of impurities present. This was attributed to the polymerization of the isocyanide intermediate which can be attributed to toluene's high boiling point. With our first attempt unsuccessful we look at other methods such as Su *et. al.*, which used the same protocol but changed the solvent to petroleum ether and used bis(trichloromethyl)carbonate, better known as triphosgene, as a "safer"⁶⁰ reagent compared to phosgene gas (Figure 15-2).⁶¹ However, due to petroleum ethers very low flash point we decided to avoid this synthetic procedure. We came across a paper from Fernández-Bolaños *et. al.* which further modified the mentioned protocols by changing the solvent to dichloromethane to lower the reflux temperature and ultimately lead to less or no polymerization of intermediates (Figure 15-3). Additionally, rather than reacting the formamides in the presence of all reagents, this method allows for the generation of the corresponding isocyanide without interruption and minus the presence of selenium. Once the isocyanide intermediate is observed (TLC), elemental selenium is added and allowed to generate

the desired ISC product under reflux. The modifications done by Fernández-Bolaños *et. al.* has shown to successfully lead to the synthesis of classically synthesized ISCs as well as novel

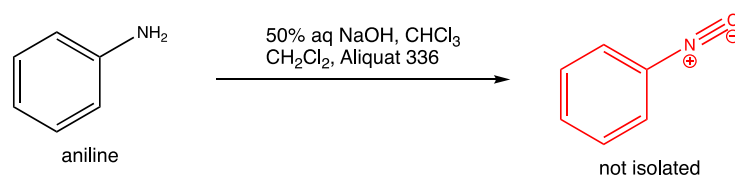


Figure 16: Hofmann isonitrile synthesis (red structure indicates product was not isolated)

phenyl-alkyl ISCs, aryl ISCs and sugar derived ISCs in decent yields.^{62,63} We also came across a more recent method for ISC synthesis reported by Zakrzewski *et. al.* involving a biphasic aqueous/organic system functioning under strongly alkaline conditions (Figure 15-4 & 15-5).⁶⁴ This unique method allows the formation of ISCs directly from the corresponding amines or isocyanides, under phase transfer conditions in a biphasic 50% aqueous NaOH / dichloromethane solvent system while utilizing phase transfer catalyst Aliquat 336.⁶⁴ This method may provide an effective route for synthesizing ISCs with comparative yields.

Interested by these two methods we began exploring the effectiveness of Zakrzewski's method. Because their method is a modified Hoffmann isonitrile synthesis, we began by trying to generate the isocyanide intermediate and see if we could isolate this. This was achieved by using aniline as starting material together with chloroform and 50% aqueous sodium hydroxide solution in the presence of ammonium salt Aliquat 336 all dissolved in DCM (Figure 16). This was heated to a gentle reflux at 40°C and left to stir for 1.5 hours. After this time, TLC analysis revealed a faint new spot but a strong aniline spot, so this was left to react for an additional 2.5 hours, taking TLC every 30 minutes. After 4 hours, the aniline spot on TLC diminished and this was worked up by allowing this to cool to room temperature and pouring in ice cold water. The product was extracted with DCM, washed with sodium bicarbonate and dried over magnesium sulfate. The organic layer was then filtered and concentrated *in vacuo*, but the product was lost. It was later

discovered that the boiling point of phenyl isocyanide under reduced pressure is approximately 50°C and was lost during work up, confirmed by TLC. Confident that we did synthesize the phenyl isocyanide intermediate, just failed to isolate, we moved on to Zakrzewski's method in which a mixture of aniline, chloroform, DCM, Aliquat 336 and 50% aqueous sodium hydroxide was made and stirred vigorously. This produced an immediate exothermic reaction and was then set to reflux. The consumption of the aniline starting material was monitored via TLC and after 2 hours the starting material spot had faded, and a strong pungent odor characteristic of isocyanides was observed. At this point, grey selenium powder was added to the reaction mixture and refluxed for an additional 2 hours. During this time a new spot on TLC formed and was attributed to phenyl isoselenocyanate as it was the highest spot on the TLC (non-polar). However, after generation of this spot the following TLCs revealed multiple spots began to form. Mechanistically speaking, if the aniline starting material did not completely convert to isocyanide *in situ*, this would lead to the simultaneous presence of a nucleophilic amine and electrophilic ISC, forming selenoureas as major side products. We repeated this reaction multiple times and found that the timely addition of elemental selenium is crucial and difficult to quantify, making this method unreliable.

Our synthetic efforts were then shifted towards Fernández-Bolaños *et. al.* approach (Figure 15-3). In short, this method should allow for the one-pot synthesis of ISCs with minimal-to-no intermediate polymerization and better synthetic control by allowing the formation of the isocyanide intermediate to generate (from the respective aryl formamide) before addition of selenium powder. The isolation of the isocyanide intermediate was not done by the original authors but was observed via TLC.^{62,63} This was done by dissolving n-phenylformamide (1 eq) in DCM and shortly after was triethylamine (4.3 eq) added along with 4Å molecular sieves all while stirring at room temperature. This mixture was fitted with a condenser and flushed with a continuous flow

of argon and heated to reflux temperature. While this stirred and heated, a solution of triphosgene (catalyst) in DCM is made carefully. With this prepared, triphosgene was added dropwise over a period of one hour. Effervescence was observed during this one-hour period and is likely due to the dehydration of the formylated amine as it will generate colorless phosgene gas. After this addition, the reaction mixture was allowed to reflux for 2.5 hours. At this time a color change was observed as the mixture went from a clear mixture to an opaque brown color. TLC analysis revealed the generation of a new spot while the n-phenylformamide spot greatly diminished. Due to the non-polar nature of the spot and the strong pungent odor, both characteristic of isocyanides, it was concluded intermediate formation successfully occurred. At this point, grey selenium powder (2 eq) was added to the reaction mixture and was left to stir vigorously. This experiment was repeated multiple times, each varying in the reaction time after the addition of selenium. The authors originally reported 4–7-hour reaction times⁶², however we found that leaving this to reflux at least 18 hours was optimal as it gave the best yields. After letting this react overnight, this was allowed to cool to room temperature, filtered in order to recover molecular sieves and remove any unreacted selenium, and the filtrate was washed with water, saturated sodium bicarbonate and brine. The organic layer was isolated and dried over sodium sulfate. Purification was done via silica flash column chromatography using 100% hexane → 75:25 Hexane: DCM. The product was characterized using H-NMR and confirmed synthesis of phenyl isoselenocyanate in an 88% yield. With our optimal reaction method in hand, our aim was now shifted towards synthesizing functionalized n-phenyl formamides. A potential concern we now had would be ISC stability. ISCs have been known to be sensitive to light and can undergo decomposition, regressing to pungent isocyanides and elemental selenium.⁶³ We did come across this with our phenyl ISC products and

were able to repurify all these. Nevertheless, we found a way to work with ISCs by storing them under inert gas, keeping them in a freezer and used freshly to avoid stability issues.

Synthesis of Aromatic Formamides via Formylation of Amines

Since the synthesis of ISCs requires the dehydration of formamides, functionalized aromatic formamides will be synthesized. This will be accomplished via the formylation of amine, a method that has been reviewed well.⁶⁵ Because aromatic formamides are the target product, the corresponding anilines will be subject to formylation. The earliest method to formylate amines was reported in 1952, in which the formylating agents used was chloral.⁶⁶

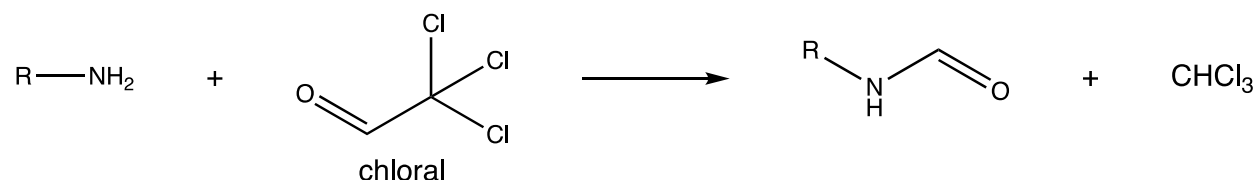


Figure 17: Formylation of amine using chloral⁶⁶

In the Bossmann research group, formylation has been done with multiple methods utilizing formic acid, the simplest carboxylic acid, via dehydration. In one method, the amine (in this case anilines) and 1.2 eq of aqueous 85% formic acid can be dissolved in toluene and the solution is heated to reflux in the presence of a Dean-Stark trap, which will collect any water produced by the condensation reaction (Figure 18-a).⁶⁷ This method is advantageous as it has no need of anhydrous conditions, no racemization will occur, and the original authors report excellent yields. However, only aniline and 4-nitroaniline were attempted by this research group yielding 99% and no product, respectively. Because n-phenyl formamide, or also known as formanilide (the formamide of aniline), is already commercially available and because functionalized anilines are needed, a different method was explored.

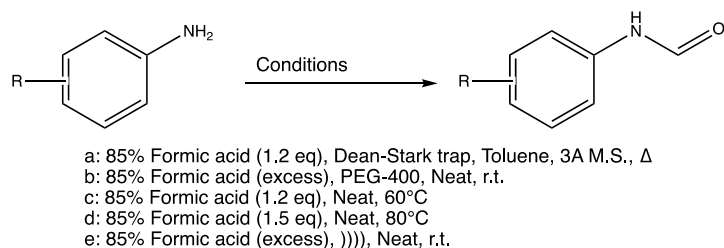


Figure 18: Formylation of Aromatic Amines

A relatively green and simple method was reported in which the formylation of functionalized anilines can be carried out at room temperature using an excess of formic acid and polyethylene glycol 400 (PEG-400) as a catalyst while stirring for 4-6 hours (Figure 18-b).⁶⁸ This method was attempted with a range of halogenated mono- and di-substituted anilines. While this method did produce low to moderate yields (33-71%), the reaction time varied greatly when compared to literature. Additionally, solidification of the reaction mixture was a consistently encountered, something which was not mentioned in the original literature. For example, the formylation of 4-bromoaniline yielded 91% of product in literature⁶⁸, while the Bossmann group yielded 71% of product due to solidification of product after 3 hours. Regardless, this is a convenient, green, and simple method to access formylated amines. Catalyst free, neat, conditions have also been reported^{69,70} and applied in which anilines and 1.2-1.5 equivalent of formic acid are carefully added to a reaction vessel and stirred vigorously at 60°C⁶⁹ or 80°C⁷⁰ for time (determined via thin layer chromatography (TLC)) (Figure 18-c, 18-d). In the Bossmann lab, one of the most utilized and reliable method is an environmentally benign and facile formylation using ultrasonic irradiation (Figure 18-e).⁷¹ For this, a mixture of aniline and excess formic acid is added to the reaction vessel and irradiated by ultrasound at room temperature. The typical reaction time varies, the fastest being 15 minutes and the longest being 120 minutes and is determined via TLC monitoring. It is important to note that the longer the reaction/sonication time, the higher the temperature raises. For example, the synthesis of *N*-(4-chlorophenyl) formamide required a sonication time of 120

minutes. During this time, the sonicator water bath began to warm slowly: the water bath started at room temperature and was unintentionally heated slowly, ending at 50°C, and yielding 86% of product. While all methods described are useful in accessing aromatic formamides, which are required for the synthesis of ISCs, the two methods which were most optimal were the green PEG-400 catalyst conditions and sonication. An observation for all methods mentioned was the slight increase in product yield when the water percentage of formic acid was decreased from 15% (in 85% aq. formic acid) to ~1% (in 99% formic acid).

To discuss the synthesis, it is important to note our focus was mainly to synthesize chloro-substituted formylated amines. This is a result of NNSN biological activity in which nearly all NNSNs functionalized with chloro-groups on the aryl portion of the NNSN exhibited good biological activity. Formylated amines with other halogens or functional groups were also synthesized. All successful reaction will be discussed.

N-(2-chlorophenyl) formamide, *n*-(3-chlorophenyl) formamide, *n*-(3,4-dichlorophenyl) formamide, *n*-(2,3-dichlorophenyl) formamide, *n*-(2,4-dichlorophenyl) formamide, *n*-(4-methoxyphenyl) formamide, *n*-(4-bromophenyl) formamide, *n*-(3,5-dibromophenyl) formamide, *n*-(4-fluorophenyl) formamide, *n*-(4-methylphenyl) formamide (also known as *n*-*p*-tolylformamide), *n*-(4-(trifluoromethyl)phenyl) formamide and *n*-(3-nitrophenyl) formamide was synthesized with the conditions listed in Figure 18-b.⁶⁸ All anilines used in this reaction method are commercially available. Typical reaction procedure for this method is as follows: to a dry round bottom flask is the respective aniline (1 eq) added and stirred. To this is added formic acid (3 eq) carefully and stirred. At this point, the mixture is stirred vigorously. Then polyethylene glycol 400 (PEG-400, catalyst) is added to the reaction mixture. After this addition the reaction flask is sealed, and the mixture is stirred vigorously for time. Each product had a different reaction time either due

to TLC monitoring (50:50 ethyl acetate: hexane) or solidification of the reaction mixture. After reaction time or solidification, the mixture is then diluted or dissolved with water and the product is extracted using multiple washes of ethyl acetate. The organic layer is then isolated and further washed with saturated sodium bicarbonate and brine and is then dried over sodium sulfate. This is then filtered and concentrated *in vacuo*. Purification can be done in three ways: 1) silica column chromatography using ethyl acetate: hexane gradient, 2) allowing the flask to stand in ethyl acetate (either at room temperature or in the fridge) before concentration *in vacuo* and allow the product to precipitate or 3) recrystallization. *N*-(2-chlorophenyl) formamide was synthesized in a 1g scale, solidified in 1 hour 46 minutes and resulted in a 78% yield. *N*-(3-chlorophenyl) formamide was synthesized in a 1g scale, reacted for 24 hours and resulted in a 51% yield. *N*-(3,4-dichlorophenyl) formamide was synthesized in a 1g scale, solidified after 1 hour 5 minutes and resulted in a 32% yield. *N*-(2,3-dichlorophenyl) formamide was synthesized in a 1g scale, solidified after 15 minutes and resulted in a 62% yield. This product had to be recrystallized in hexane since the *r_f* of the starting material (0.92) and the product (0.82) were close and made purification difficult via silica column chromatography. *N*-(2,4-dichlorophenyl) formamide was synthesized in a 1g scale, solidified within an hour and resulted in a 60% yield. This product was recrystallized in ethyl acetate. *N*-(4-methoxyphenyl) formamide was done in a 1mmol scale, reacted for 15 hours and resulted in a 19% yield. *N*-(4-bromophenyl) formamide was synthesized in a 250mg scale, solidified after 3 hours and resulted in a 71% yield. *N*-(3,5-dibromophenyl) formamide was synthesized in a 250mg scale, solidified after 45 minutes and resulted in a 60% yield. *N*-(4-fluorophenyl) formamide was synthesized in a 1g scale, reaction solidified after 7 hours, resulted in a 35% yield. *N*-(4-methylphenyl) formamide was synthesized in a 100mg scale, reaction solidified after 6 hours and resulted in a 33% yield. *N*-(4-(trifluoromethyl) phenyl) formamide was

synthesized in a 500mg scale, reaction solidified after 6 hours and resulted in a 65% yield. Lastly, *n*-(3-nitrophenyl) formamide was synthesized in a 500mg scale, solidified after 1 hour 45 minutes and resulted in a 66% yield via recrystallization with toluene.

N-(2-chlorophenyl) formamide, *n*-(3-chlorophenyl) formamide, *n*-(4-chlorophenyl) formamide, *n*-(3,4-dichlorophenyl) formamide, *n*-(2,3-dichlorophenyl) formamide, *n*-(2,4-dichlorophenyl) formamide, and *n*-(4-methoxyphenyl) formamide were synthesized using the conditions shown on Figure 18-e.⁷¹ Typical reaction procedure for this method is as follow: to a dry round bottom flask or vial is the respective aniline (1 eq) added. The reaction vessel is then fitted onto a water bath sonicator at room temperature. To this is added formic acid (2.5 eq) carefully. An exothermic reaction may be observed. At this point the reaction is sealed with a rubber stopper fitted with a needle to vent the reaction. The sonicator is turned on to eradicate the mixture via ultrasound. This is left to sonicate while being monitored via TLC (50:50 ethyl acetate: hexane). Each product had a different reaction time due to TLC monitoring or solidification of product in reaction vessel. After reaction time, the reaction vessel is removed from the water bath and the reaction mixture is diluted with water. The product is extracted with ethyl acetate. The organic layer is isolated and washed with saturated sodium bicarbonate and brine and dried over sodium sulfate. This is then filtered and concentrated *in vacuo*. Purification of these products were done using silica column chromatography with an ethyl acetate: hexane gradient. *N*-(2-chlorophenyl) formamide was synthesized in a 1g scale, was sonicated for 1 hour 30 minutes and resulted in a 73% yield. *N*-(3-chlorophenyl) formamide was synthesized in a 1g scale, was sonicated for 2 hours 30 minutes and resulted in a 74% yield. *N*-(4-chlorophenyl) formamide was synthesized in a 1g scale, was sonicated for 2 hours and resulted in an 86% yield. *N*-(3,4-dichlorophenyl) formamide was synthesized in a 1g scale, was sonicated and solidified after 1 hour

30 minutes and resulted in a 57% yield. *N*-(2,3-dichlorophenyl) formamide was synthesized in a 1g scale, was sonicated and solidified after 1 hour and resulted in a 59% yield. This was isolated by allowing the dried organic layer from the work up to rest in the fridge. Product would slowly precipitate and was collected. *N*-(2,4-dichlorophenyl) formamide was synthesized in a 1g scale, was sonicated and solidified after 1 hour and resulted in a 68% yield. *N*-(4-methoxyphenyl) formamide was synthesized in a 1mmol scale, was sonicated and solidified after 15 minutes and resulted in a 7% yield.

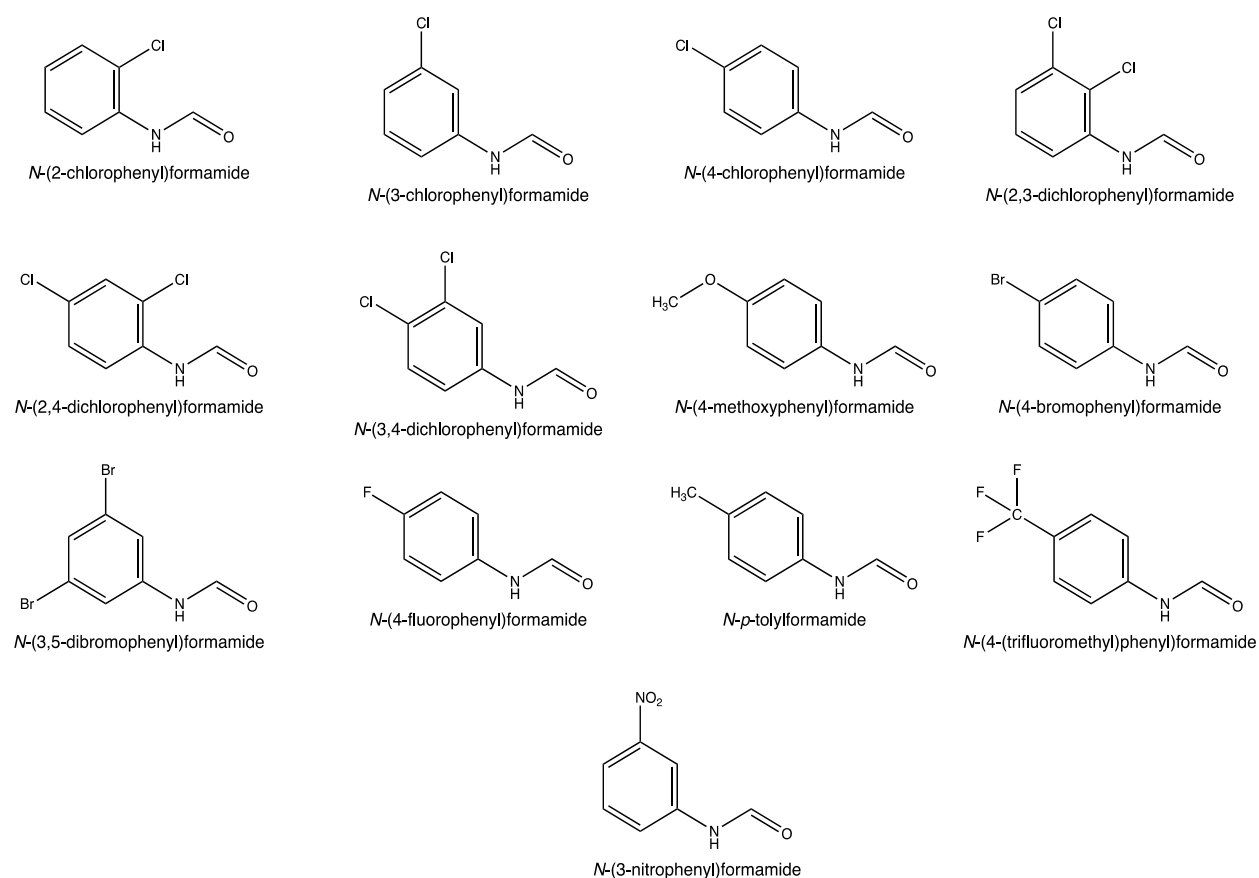


Figure 19: Formylated amines synthesized

Synthesis of ISCs

With a small library of formylated amines in hand, the focus was then shifted towards the synthesis of ISCs. As mentioned, the synthetic method developed by Fernández-Bolaños *et. al.*

was utilized, with minor changes. Having already successfully synthesized phenyl ISC with this method, 2-chlorophenyl ISC, 3-chlorophenyl ISC, 4-chlorophenyl ISC, 2,3-dichlorophenyl ISC, 2,4-dichlorophenyl ISC, 3,4-dichlorophenyl ISC, 4-bromophenyl ISC, 3,5-dibromophenyl ISC, 4-fluorophenyl ISC and 4-methylphenyl ISC were also successfully synthesized. A typical procedure is as follows: to a dry round bottom flask is added the respective formylated amine (1 eq), triethylamine (4.3 eq), 4 angstrom molecular sieves (this does not need to be measured), and 10 mL of DCM. This is then clamped onto a jacketed condenser, sealed with a rubber stopper and fitted with a continuous flow of argon. This is then heated to reflux. While heating, a solution of triphosgene (catalyst which degrades to phosgene gas, 0.53 eq) in DCM (3 mL) is made. Triphosgene is highly soluble in DCM, but this mixture is either sonicated or vortexed in order to ensure proper dissolution of catalyst. At this point, the solution is withdrawn into a 5mL syringe, and the syringe is fitted onto a syringe pump and a 20-gauge 12-inch stainless steel needle is attached. The needle is pushed down the condenser and set as close to the reaction mixture in order to allow the triphosgene solution to drip directly onto the reaction mixture. This 3 mL solution is then added dropwise over a period of an hour (flow rate of approximately 0.05mL of solution per minute). At this point effervesce will be observed each time a drop of catalyst is added. This is likely the generation of toxic phosgene gas. After the one-hour addition, the syringe needle is removed and the reaction mixture is left to stir at reflux for an additional 2 hours 30 minutes. After this time, TLC analyses (100% hexane) can be done to see generation of isocyanide intermediate. During TLC analyses the isocyanide odor is also observed. With the isocyanide generated, grey selenium powder (2 eq.) is added and the reaction mixture is left to reflux for 18 hours. After 18 hours, TLC analysis (100% hexane) will reveal ISC spot which can also be seen very well with an iodine chamber (mixture of silica, sand and crystalline iodine) stain. At this point, the mixture is

filtered and the filtrate is water (3 x 10mL), saturated sodium bicarbonate (1 x 10mL) and brine (1 x 10mL). The organic layer is dried over sodium sulfate and concentrated. All ISCs were purified using silica column chromatography mainly using 100% hexane or a hexane: DCM gradient. It is important to note that this reaction condition underwent technique optimization, and the following was discovered: 1) the preparation of triphosgene must be done under an inert atmosphere and while sealed in a glass vial in order to avoid degradation into phosgene 2) the addition of the triphosgene catalyst is best when added drop wise with a syringe pump. Initial attempts were done by hand and resulted in very low ISC yields. This is likely due to quick accidental additions of triphosgene catalyst which may cause unwanted degradation of the catalyst, as shown in Figure 20.

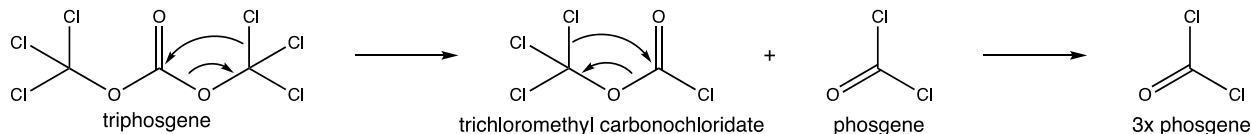


Figure 20: Degradation of triphosgene to phosgene⁶⁰

3) the joints adjoining the jacketed condenser and round bottom flask must be clamped/screwed tightly in order to avoid the phosgene gas escaping through these joints (bubbling through the joints was observed) and 4) due to the isocyanide intermediate, all glassware used for ISC synthesis must be washed thoroughly with 5% methanolic sulfuric acid in order to fully remove isocyanide odor from glassware. Phenyl ISC was synthesized in a 1.50 mmol scale and resulted in an 88% yield, 2-chlorophenyl ISC was synthesized in a 1 mmol scale and resulted in a 71% yield, 3-chlorophenyl ISC was synthesized in a 1 mmol scale and resulted in a 73% yield, 4-chlorophenyl ISC was synthesized in a 1 mmol scale and resulted in a 76% yield, 2,3-dichlorophenyl ISC was synthesized in a 1.10 mmol scale and resulted in a 73% yield, 2,4-dichlorophenyl ISC was synthesized in a 1.14 mmol scale and resulted in a 73% yield, 3,4-dichlorophenyl ISC was

synthesized in a 0.80 mmol scale and resulted in a 70% yield, 4-bromophenyl ISC was synthesized in a 1 mmol scale and resulted in a 66% yield, 3,5-dibromophenyl ISC was synthesized in a 0.60 mmol scale and resulted in a 60% yield, 4-fluorophenyl ISC was synthesized in a 3.91 mmol scale and resulted in a 51% yield, and 4-methylphenyl ISC was synthesized in a 0.40 mmol scale and resulted in a low 5% yield.

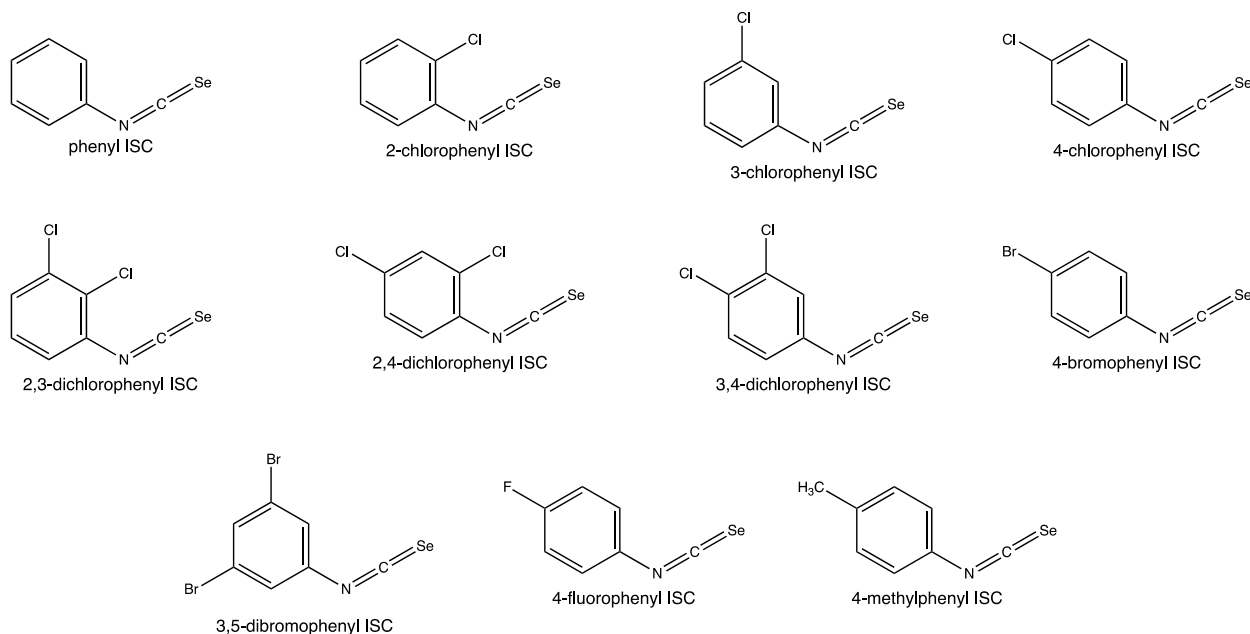


Figure 21: ISCs Synthesized

We also attempted to synthesize 2,6-dimethylphenyl ISC, 4-methoxyphenyl ISC, 3-nitrophenyl ISC and 4-trifluoromethylphenyl ISC however these proved to be highly unreactive in these conditions. Looking at 4-methylphenyl ISC, is it likely that the electron donating, or rather inductive donating, methyl groups are increasing electron density of the aromatic ring which in turn may be affecting the dehydration of the formylated amine. In the case of 2,6-dimethylphenyl ISC it is also likely that sterics may be hindering the reaction. For the highly electron withdrawing 4-trifluoromethyl group, the reaction TLC did suggest isocyanide intermediate formation, however no ISC formation was observed. This reaction was attempted multiple times,

and none resulted in the desired ISC. It is likely that the intermediate is polymerizing, or a different un-isolable species is generating.

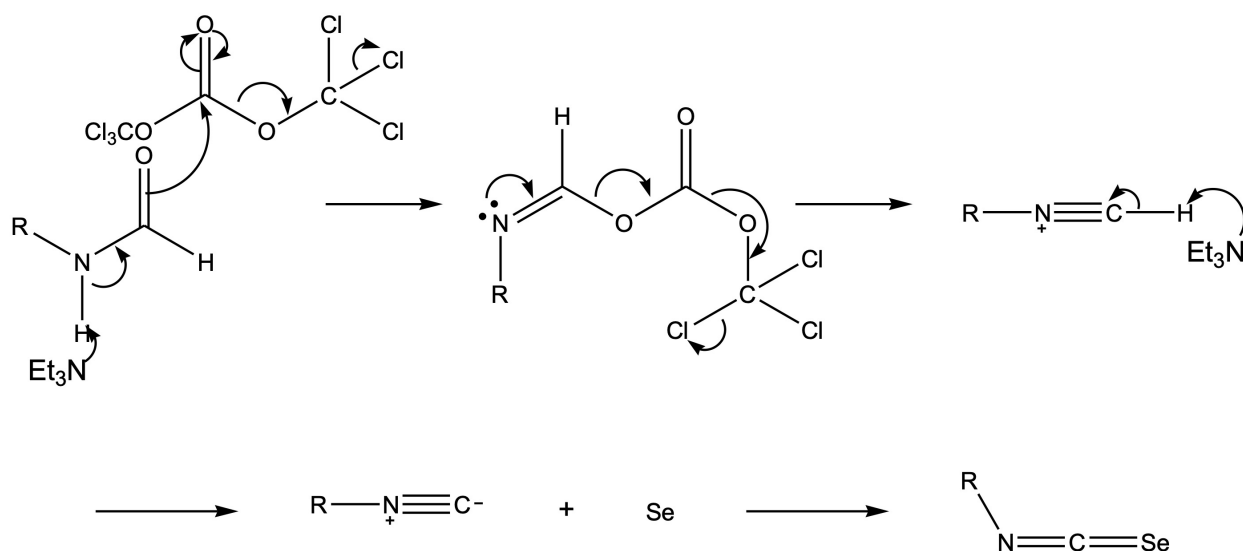


Figure 22: ISC mechanism

In one case, during the synthesis of 4-chlorophenyl ISC, the reaction was done as described before however after the 18-hour reaction time the intermediate spot was still visible. As such we decided to try to isolate this. The work up was done as written and both the ISC product and the intermediate were purified via silica column chromatography. Both of these were characterized via H-NMR. Comparing the structures of the two, both have the same number of protons, however due to the selenium atom, it was hypothesized that the proton peaks would differ. NMR comparison reveals the aromatic peaks for the intermediate at a different ppm when compared to the ISC product, suggesting that this is likely the isocyanide intermediate. We did not see a isocyanide $\text{R}-\text{N}\equiv\text{C}-\text{H}$ proton on the NMR and assumed this was a stable unprotonated isocyanide. 15mg (0.11mmol) of this was isolated and in order to ensure that this was the intermediate, the isocyanide was subjected to the selenation conditions used for the synthesis of ISCs. As such, 0.11

mmol of the isocyanide intermediate was added to a dry round bottom flask and dissolved with 10mL of DCM. To this was added grey selenium powder (0.22 mmol), flushed with argon and

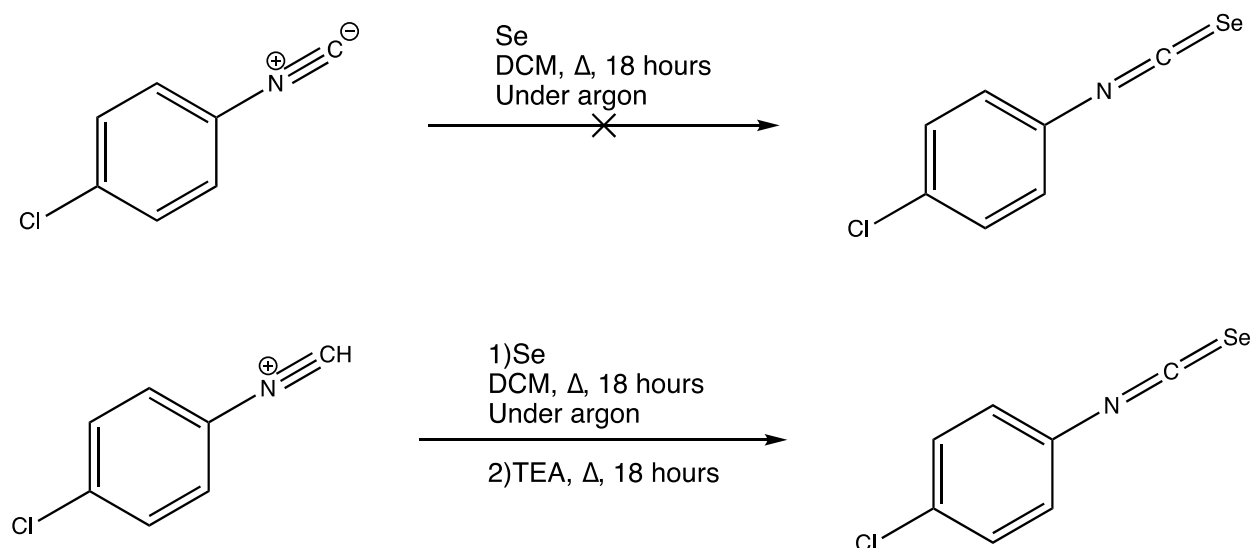


Figure 23: 4-Chlorophenyl isocyanide intermediate to 4-Chlorophenyl ISC

heated to reflux for 18 hours. After this TLC analysis revealed no new spots and only the isocyanide. At this point it was realized that the intermediate is possible protonated. It was hypothesized that the isocyanide was likely never deprotonated during the synthesis of 4-chlorophenyl ISC, which is not likely due to the success of all ISC synthesis reaction, or it was likely protonated during the silica column purification. The acidic silica likely protonated this, even though the isocyanide proton was not observed on NMR. As such, to this intermediate reaction mixture we added triethylamine (4.3 eq) and left it to stir for an additional 18 hours. After this time, TLC analysis revealed 100% conversion of the isocyanide intermediate to the ISC product. This was the only reaction in which we were able to isolate or even characterize any isocyanide intermediate (Figure 23).

Synthesis of Pyrazoles

Pyrazoles are important doubly saturated five membered aromatic heterocycle moieties containing two neighboring nitrogen atoms. The chemistry and biological activity of pyrazoles have been extensively reviewed over the years.⁷²⁻⁷⁶ While most “simple” pyrazoles are commercially available, some can be relatively expensive, especially functionalized pyrazoles.

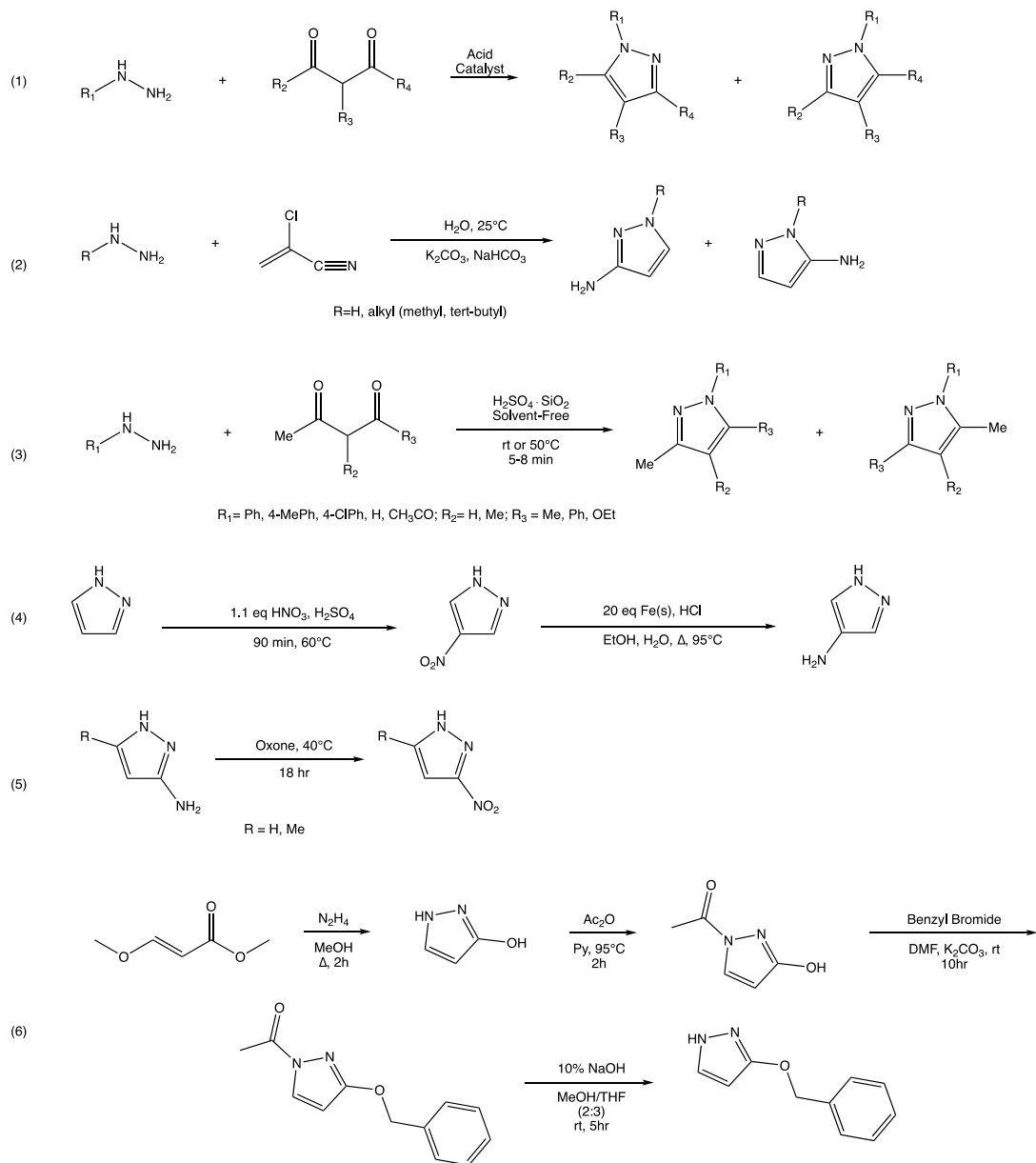


Figure 24: Synthesis of Pyrazoles

Thus, pyrazole derivatives were synthesized. Many pyrazole derivatives can be accessed via the classical Knorr pyrazole synthesis (Figure 24-1).⁷⁷ This would allow for us to access pyrazoles with alkyl, aryl, Het-aryl, and acyl substituents using hydrazine or substituted hydrazine with 1,3-dicarbonyl compounds. In order to selectively access alkyl groups on the primary position of 3-aminopyrazoles or to simply access 3-aminopyrazole, hydrazine hydrate and alkyl hydrazine derivatives (i.e. *tert*-butylhydrazine hydrochloride or methylhydrazine) can be reacted with 2-chloroacrylonitrile in an aqueous media and in the presence of base(s) (Figure 24-2).⁷⁸ This is a simple and direct method for the synthesis of 1-alkyl/*H*-1*H*-pyrazol-3-amines, however there may be formation of a second isomer during this. It has been reported that the ratio of the additive to this reaction can improve regioselectivity and yield the targeted 1-alkyl-1*H*-pyrazol-3-amines.⁷⁹ To access di- or tri- substituted pyrazoles, a solvent-less method utilizing silica-supported sulfuric acid ($\text{H}_2\text{SO}_4 \cdot \text{SiO}_2$) as a recyclable catalyst for the chemoselective condensation of hydrazines or hydrazides with β -dicarbonyl compounds (i.e., β -diketones, β -ketoesters) can be employed (Figure 24-3).⁸⁰ In a rather unconventional method, the hydrazine/hydrazides and β -dicarbonyl compounds can be mixed together along with $\text{H}_2\text{SO}_4 \cdot \text{SiO}_2$ via mortar and pestle at room temperature or via aggressive stirring of the mixture in a round bottom flask using a magnetic stir bar if higher reaction temperature is needed. This will give access to substituted pyrazoles in the secondary and tertiary position with the 4th position always being a methyl group. Though there are functionalized (i.e., amine, nitro) pyrazoles in the 4th position that are commercially available, they can be very expensive. For example, 1*H*-pyrazol-4-amine and 1-methyl-1*H*-pyrazol-4-amine cost \$63.30 for 100mg (Alfa Aesar) and \$99.50 for 1g (Sigma-Aldrich), respectively. Similarly, 4-nitro-1*H*-pyrazole costs \$63.00 for 5g. As such, the synthesis of these pyrazoles can be achieved from cheap commercially available pyrazole. The nitration of pyrazole can be done similarly as the nitration

of benzene: in the presence of concentrated nitric and sulfuric acid (Figure 24-4).⁸¹ This allows formation of the nitronium ion (NO_2^+) which can allow electrophilic aromatic substitution ($\text{S}_{\text{E}}\text{Ar}$) to occur at the electron rich C4 position of the pyrazole to selectively generate 4-nitro-1*H*-pyrazole. This can also be done using 3-methylpyrazole to generate 3-methyl-4-nitro-1*H*-pyrazole. From this, 1*H*-pyrazol-4-amine can also be made via the reduction of 4-nitro-1*H*-pyrazole using iron (Fe) powder and concentrated hydrochloric acid⁸², in which the original literature demonstrates the reduction of the aryl nitro group on a phenyl ring and the Bossmann group extended this towards the reduction of aryl nitro groups on pyrazoles (Figure 24-4).⁸³ To access the nitro group in the electron deficient C3 center, the oxidation of either synthesized or commercially available 3-aminopyrazole can be done via a convenient and green method using potassium peroxymonosulfate (Oxone[®]) in water to generate 3-nitro-1*H*-pyrazole. This method also allows the oxidation of 5-alkyl-1*H*-pyrazol-3-amines to access di-substituted nitro pyrazoles (Figure 24-5).⁸⁴ In the case of NNSN biological activity, a pyrazole with a benzyl ether proved to be most active.⁸³ This can be accessed over four steps: (i) pyrazole cyclization synthesis to furnish 1*H*-pyrazol-3-ol, (ii) acetal group protection of the acidic pyrazole amine, (iii) $\text{S}_{\text{N}}2$ -like substitution of acetal pyrazole 1-(3-hydroxy-1*H*-pyrazol-1-yl)ethenone with benzyl bromide to form benzyl ether pyrazole 1-(3-(benzyloxy)-1*H*-pyrazol-1-yl)ethenone, (iv) successive deprotection of the acetal group to access target pyrazole 3-(benzyloxy)-1*H*-pyrazole (Figure 24-6). Introduction of bromo functional group was also done using brominating agent N-bromosuccinimide (NBS)⁸⁵.

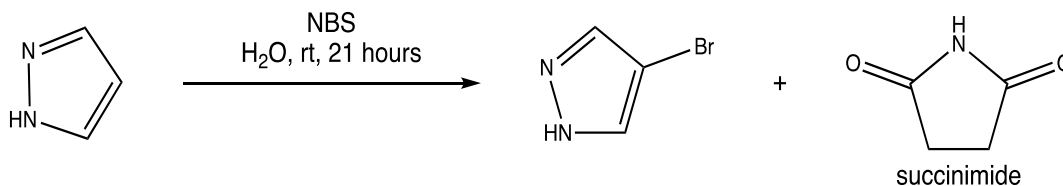


Figure 25: Bromination of pyrazole via NBS in water

This is done from pyrazole as starting material in water at room temperature and stirred for 21 hours (Figure 25). This will offer 4-bromopyrazole however the separation of the succinimide side product will be crucial. This was attempted to be purified using silica column chromatography and recrystallization but the succinimide side product proved difficult to separate. As such this product was kept as an impure mixture. Other pyrazole starting materials which were obtained commercially and used will be discussed in chapter 3.

With all starting materials in hand, the syntheses for NNSeN's, both pyrazolyl-selenoureas and pyrazolyl- carboselenamide motifs, can now be accessed via coupling of ISCs and pyrazoles. This will be discussed in the next chapter

Chapter 2 References

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Chapter 3 – NNSeN's: Discussion and Synthesis

Discussion of NNSeN Synthesis

Because ISC's are chemically similar to isothiocyanates (ITC's), the synthesis of NNSeN's is postulated to be comparable to the synthesis of NNSNs. Since the NNSeNs are a novel structure, there was no literature offering insight into the coupling of pyrazoles and ISC's specifically. As such, once both the pyrazole and ISC's are obtained they can be coupled via one of five (i-v) NNSN synthetic procedures to generate NNSeN's (Figure 26).

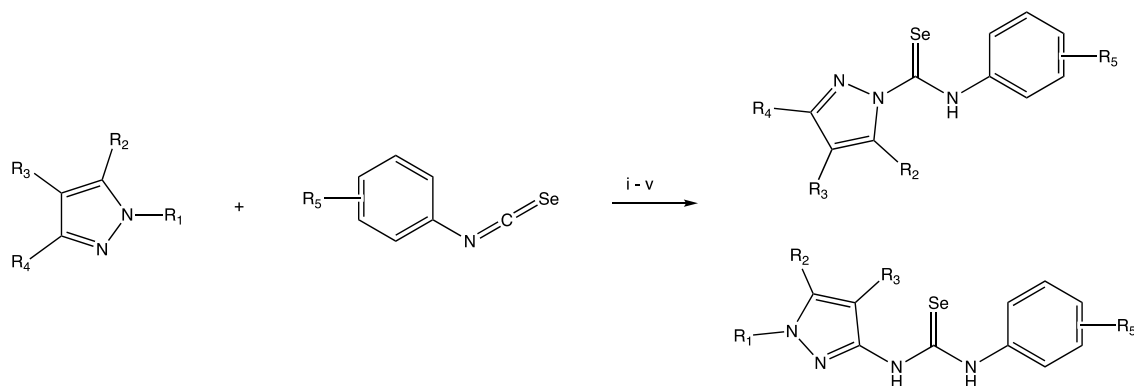


Figure 26: Synthesis of NNSeN's

- (i) One method involves dissolving both starting materials in dry dichloromethane (DCM) under an inert environment. This is left to stir either at room temperature (RT) or at reflux. Reaction times may vary (determined via TLC) but a typical time at reflux would be approximately overnight (i.e., 12-15 hours) or one day (24 hours) if done at room temperature. This method works well at room temperature with un-functionalized pyrazoles or amino pyrazoles (such as 3-aminopyrazole or 1*H*-pyrazol-4-amine) and can be refluxed if the pyrazole contains alkyl groups.
- (ii) Another method is reserved for pyrazoles too polar to dissolve in DCM. This method is similar to the method previously mentioned however the use of dry tetrahydrofuran (THF) is employed while still under inert conditions. In this method, the reaction mixture can be stirred at room

temperature or heated to reflux with a similar time frame as method (i). This works well for pyrazoles with carbonyls and methyl groups. If a base is required in the coupling between pyrazoles and ISCs, two separate methods can be employed (method iii or iv). (iii) In this method the use of potassium carbonate in dry THF is utilized. The pyrazole can be dissolved in dry THF with anhydrous potassium carbonate and stirred vigorously for 1 hour at 50°C. After this time, the ISC can be added dropwise as a solution in dry THF, and the solution is then heated to reflux for time (determined via TLC) under an inert environment. This method can be employed when pyrazoles contain a benzyl ether or if sterics possibly come into play with di-, or tri-alkyl substituted pyrazoles. (iv) Alternatively, if a stronger base is needed, the use of sodium hydride in THF can be employed. In this method, two separate flamed dried round bottom flasks are charged with pyrazole and sodium hydride, respectively, and diluted/dispersed with THF. The flask containing pyrazole is stirred and cooled to 0°C. The flask containing dispersed sodium hydride in THF is then cannulated into the flask containing the cold pyrazole solution. Once added, the reaction mixture is allowed to warm to room temperature and kept stirring for 3 hours. After this, the ISC can be added as a solution in dry THF dropwise and this reaction mixture can be left to stir at room temperature for time (determined via TLC). This method can be employed if the pyrazole is functionalized with a nitro group. The drawback of this method is the cannulation of sodium hydride in THF. Because sodium hydride is only dispersible in THF, it can be difficult to cannulize the base. A higher volume of solvent might be needed to efficiently cannulize. (v) A rather universal method, meaning this method can tolerate any functional groups on the pyrazoles, can be employed using potassium carbonate and anhydrous dimethylformamide (DMF). In this method, the pyrazole starting material is added to an argon flushed round bottom flask and diluted with anhydrous DMF. Then, potassium carbonate is added to the pyrazole solution and stirred

vigorously for 30 minutes. After this time, ISC can be added dropwise as a solution in DMF. This is then left to stir under argon for time (determined via TLC) at room temperature. While this is a universal method, a major drawback is the DMF solvent as it can be difficult to completely evaporate and remove from product. Lyophilization, also known as freeze-drying, has proved effective in removing DMF. Another method could be employed during the workup in which the DMF reaction mixture is diluted with ethyl acetate and a liquid-liquid extraction can be done using water, saturated sodium bicarbonate and multiple washes with brine.

All methods aforesaid will be done using a 1:1 molar equivalent of pyrazole and ISC unless mentioned otherwise. In the case of base requirements, 1 equivalent of base will be used unless mentioned otherwise.

NNSeN Synthesis

With the pyrazoles and ISCs secured, NNSeN synthesis commenced. Our first synthetic attempt was with “simple”, simple meaning unfunctionalized, NNSeN *N*-phenyl-1*H*-pyrazole-1-carboselenoamide. In a 0.27 mmol scale, a dry round bottom flask was charged with phenyl ISC and pyrazole and flushed with argon. This mixture was then diluted with 10mL of dry DCM and stirred vigorously at room temperature. Because NNSN synthesis has been done at room temperature for 24 hours, this mixture was left to stir for 24 hours. However, after this time TLC analysis revealed no reaction had occurred. It was also observed that the phenyl ISC starting material had darkened and developed a radish and isocyanide odor. TLC of the starting material (100% hexane) revealed that the ISC had begun to degrade. Up to this point all ISC starting materials were stored in a desiccator, however it was decided to flush all these ISC starting materials again with argon and keep them frozen, prolonging stability. The degrading phenyl ISC was repurified using silica column chromatography and was used freshly for the second synthetic

attempt of “simple” NNSeN *N*-phenyl-1*H*-pyrazole-1-carboselenoamide. The second attempt was done in a 1.43 mmol scale using the same procedure mentioned previously. In this attempt, shortly after the ISC and pyrazole starting materials were dissolved in DCM at room temperature, the mixture then began to slowly change into a light-yellow color, a characteristic observation in all NNSeN syntheses. This was heated to reflux for overnight and monitored via TLC (9:1 hexane: ethyl acetate). After overnight, TLC revealed faint ISC spot only seen under iodine stain and a strong new spot. This was then cooled to room temperature and a liquid-liquid extraction was done: the organic layer was washed with water, saturated sodium bicarbonate and brine. This was dried with sodium sulfate and purified using a short silica column (hexane: DCM gradient). The NNSeN was isolated as an orange-yellow oil in a 42% yield and was the first successful synthesis of a carboselenoamide NNSeN (Figure 27).

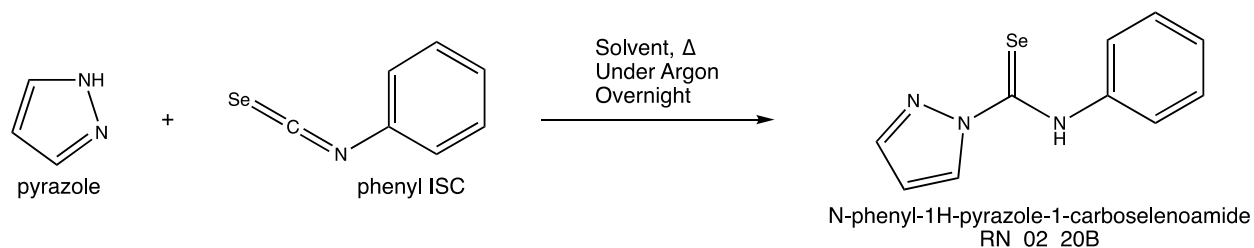


Figure 27: First successful NNSeN Synthesis

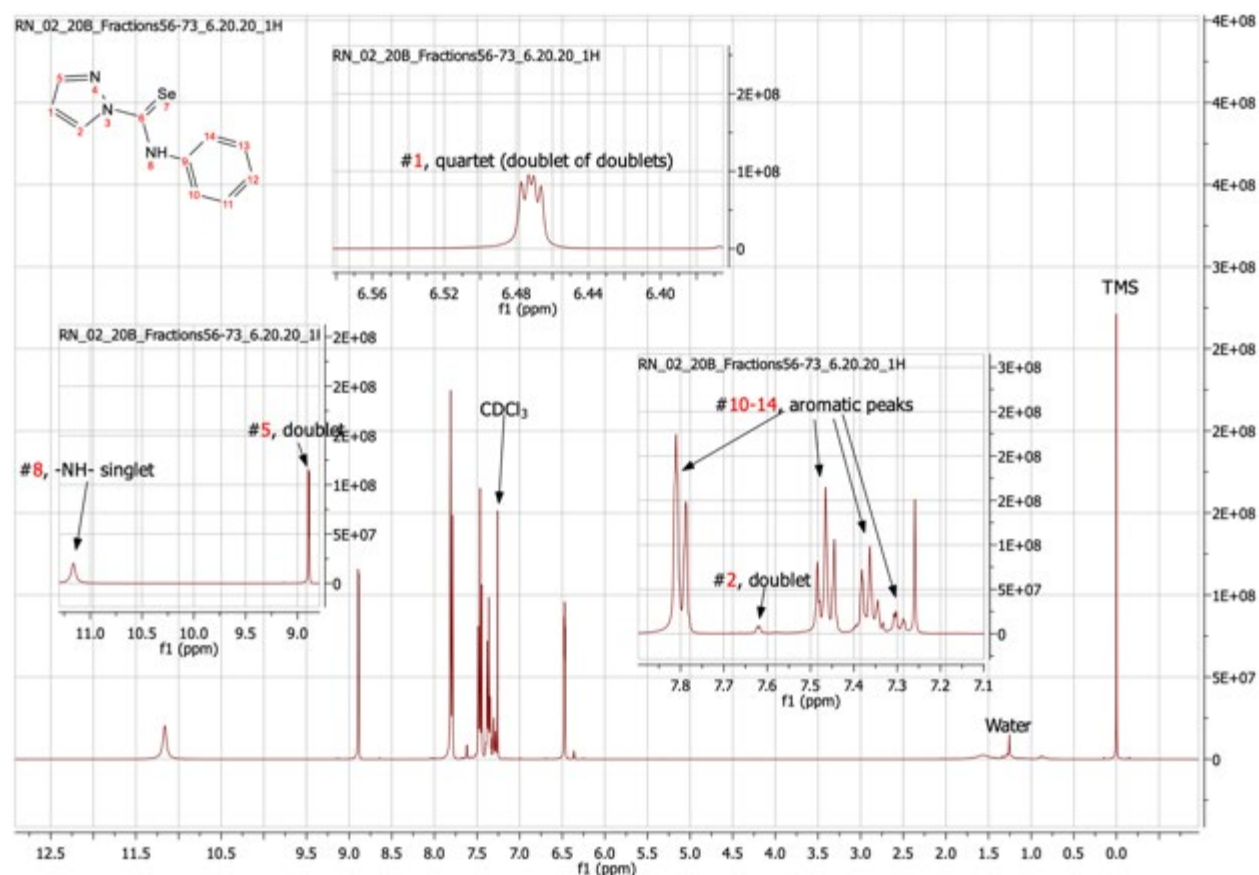


Figure 28: ^1H -NMR of *N*-phenyl-1*H*-pyrazole-1-carboselenoamide NNSeN

^1H -NMR analysis revealed successful NNSeN synthesis. This spectrum is a very typical example of what carboselenoamide NNSeN ^1H -NMR spectrums will look like (Figure 28).

Similarly, 3-methyl-*N*-phenyl-1*H*-pyrazole-1-carboselenoamide (0.36 mmol scale, 52% yield), *N*-(2-chlorophenyl)-1*H*-pyrazole-1-carboselenoamide (0.089 mmol scale, 17% yield), *N*-(2-chlorophenyl)-3-methyl-1*H*-pyrazole-1-carboselenoamide (0.39 mmol scale, 72% yield), *N*-(3-chlorophenyl)-1*H*-pyrazole-1-carboselenoamide (0.20 mmol scale, 32% yield), *N*-(3-chlorophenyl)-3-methyl-1*H*-pyrazole-1-carboselenoamide (0.08 mmol scale, 49% yield) and *N*-(4-chlorophenyl)-1*H*-pyrazole-1-carboselenoamide (0.41 mmol scale, 26% yield) were all synthesized using the same reaction conditions as before.

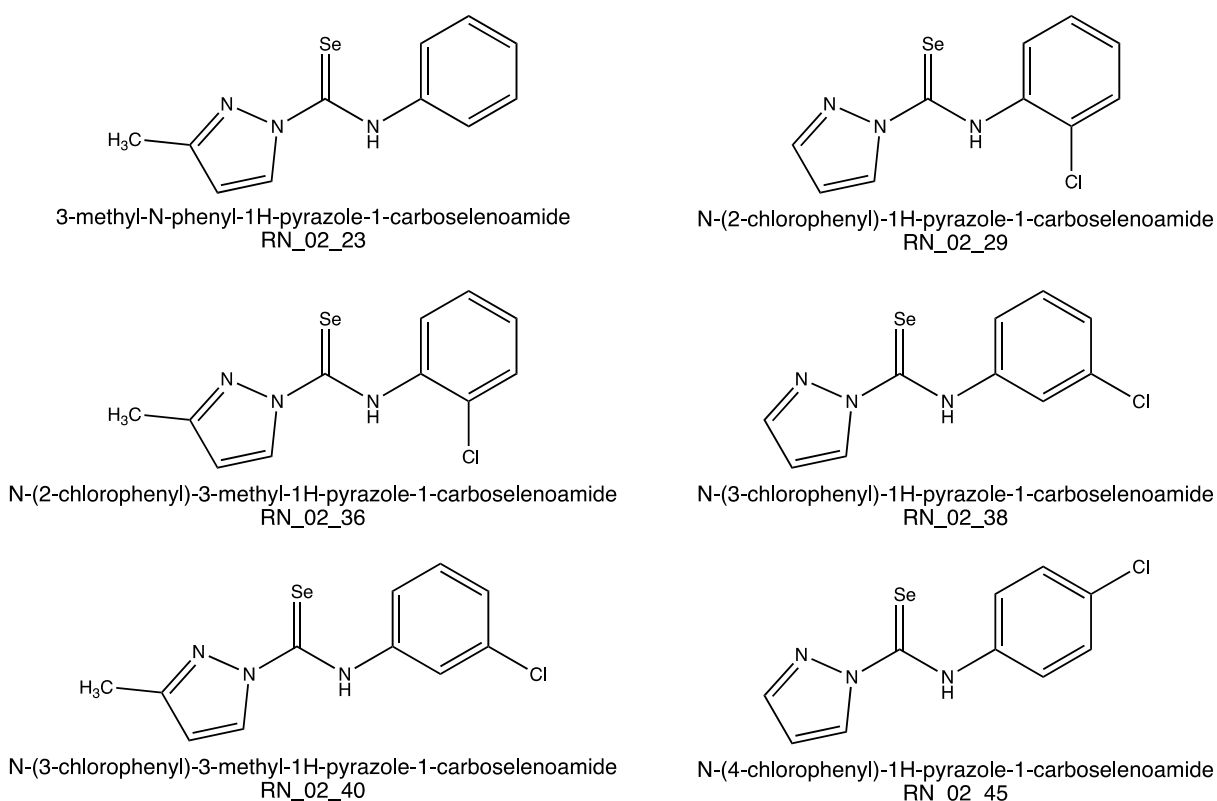


Figure 29: NNSeNs synthesized using DCM as solvent

However, it was noticed that the yield varied greatly between these NNSeNs, and they were all mainly isomers of each other, varying on the position of the -chloro group and whether the pyrazole was methylated at the 3rd position (Figure 29). As such, solvent optimization was needed. This was done using phenyl ISC and pyrazole in a 1:1 molar ratio using the same procedure mentioned before (Figure 30).

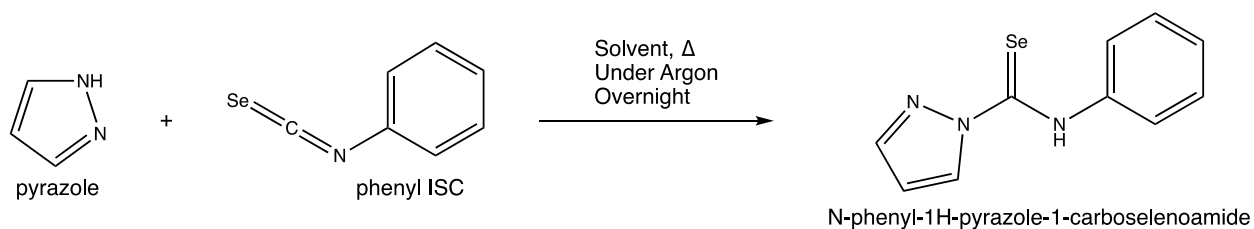


Figure 30: Solvent Optimization Study

The following solvents were tested: chloroform at reflux, THF at reflux, acetone at reflux and ethyl acetate at reflux. Since DCM at room temperature and DCM at reflux was already performed, the

results of those experiments were included in this solvent optimization study. All solvents were dried prior to use. As mentioned, DCM at room temperature gave no reaction and DCM at reflux yielded 42% yield of NNSeN product. Chloroform gave a good 71% yield, THF gave a 59% yield, acetone gave a misleading 73% yield as TLC analysis kept showing multiple spots on final reaction

NNSeN Coupling Optimization Sample ID	Solvent	Temp (°C)	Time	Yield	Comments
RN 02 16	DCM	r.t.	Overnight	n/a	No reaction.
RN 02 20B	DCM	40	Overnight	42%	Low yield.
RN 02 46	CHCl ₃	62	Overnight	71%	Good yield.
RN 02 47	THF	66	Overnight	59%	Impurities on TLC.
RN 02 48	Acetone	56	Overnight	73%	Multiple spots on final TLC and with "purified" product.
RN 02 49	Ethyl Acetate	77	Overnight	38%	Lowest yield.

Table 2: Solvent Optimization Study

TLC and “purified” product, and ethyl acetate gave the lowest yield at 38%. These results were clear that chloroform would be the most suitable solvent for this synthetic venture. It is likely that the use of a higher boiling point solvent gives this reaction a better driving force. Highly polar solvents such as methanol and ethanol were not attempted as the ISCs would not dissolve in these solvents. Highly non-polar solvents such as hexane and heptane were also not attempted as the pyrazole starting materials would not fully dissolve in these solvents. To recall the five methods mentioned towards the synthesis of NNSNs, which was hypothesized as applicable for NNSeN synthesis: method i utilized DCM either at room temperature or at reflux and is clearly not an optimal solvent for NNSeN. Methods ii and iii utilized THF however THF would also not be an optimal solvent choice and as such these methods were discarded. However, method iii, dry THF and sodium hydride as a base, was not discarded and its usage will be discussed later in this chapter. Lastly method v utilized DMF. DMF was not attempted in this optimization investigation in order

to avoid the issues in fully removing DMF during the purification steps. However, this method was kept as it would possibly prove useful and will also be discussed later in this chapter. This leaves only a new method: chloroform at reflux, method vi. This method is done as prescribed: to a dry round bottom flask is added aryl ISC (1 eq) and pyrazole (1 eq) and dissolved using 5mL of dry chloroform. This mixture is then stirred vigorously and heated to reflux while being flushed with argon. This mixture is left to reflux for overnight, approximately 18 hours, and monitored using TLC. Taking advantage that chloroform is also less water soluble (8.7g/L at 25°C) than DCM (13.2g/L at °C), the reaction mixture is allowed to cool to room temperature and the reaction mixture is further diluted with an additional 10-15mL of chloroform. This is then subjected to a liquid-liquid extraction washing the organic layer with water, saturated sodium bicarbonate and brine and is dried using either sodium sulfate (preferred) or magnesium sulfate. The product can then be purified using silica column chromatography.

Pyrazolyl Carboselenoamide NNSeNs

The following section will discuss only the synthesis of pyrazolyl carboselenoamide NNSeNs. Using method vi, the following NNSeNs were synthesized successfully. N-(4-chlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide was synthesized from 3-methyl pyrazole and 4-chlorophenyl ISC in 0.13 mmol scale and resulted in a 75% yield. N-(3,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide was synthesized from pyrazole and 3,4-dichlorophenyl ISC in a 0.40 mmol scale and resulted in a 40% yield. N-(4-bromophenyl)-1H-pyrazole-1-carboselenoamide was synthesized from pyrazole and 4-bromophenyl ISC and resulted in a 40% yield. N-(3,5-dibromophenyl)-1H-pyrazole-1-carboselenoamide was synthesized from pyrazole and 3,5-dibromophenyl ISC in a 0.35 mmol scale and resulted in a 38% yield. N-(4-

fluorophenyl)-1H-pyrazole-1-carboselenoamide was synthesized from pyrazole and 4-fluorophenyl ISC in a 1.98 mmol scale and resulted in a 50% yield.

N-(4-methylphenyl)-1H-pyrazole-1-carboselenoamide was attempted to be synthesized from pyrazole and 4-methylphenyl ISC however resulted in no reaction. This reaction was attempted multiple time and resulted the same. This is likely owed to the inductive donating methyl group at the para position of the aromatic ring. This may be decreasing the electrophilicity of the carbon center in the ISC (-N=C=Se) which in turn is impeding the reaction as the pyrazole nucleophile is not attacking this site. Although no NNSeN was synthesized in this attempt, this did give further insight into ISC reactivity, since a similar problem occurred during the synthesis of 4-methylphenyl ISC.

N-(4-bromophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide was synthesized from 3-methylpyrazole and 4-bromophenyl ISC in a 0.23mmol scale and resulted in a 70% yield. N-(3,5-dibromophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide was synthesized from 3-methylpyrazole and 3,5-dibromophenyl ISC in a 0.049mmol scale and resulted in a 43% yield. N-(4-fluorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide was synthesized from 3-methylpyrazole and 4-fluorophenyl ISC in a 0.72 mmol scale and resulted in a 56% yield.

3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide was synthesized from 3-aminopyrazole and 4-chlorophenyl ISC in a 0.23 mmol scale and resulted in a 48% yield. In this reaction, because 3-aminopyrazole has two nucleophilic amines, this was considered a probe reaction as it was thought that two products would be generated from this: the carboselenoamide NNSeN and also 1-(4-chlorophenyl)-3-(1H-pyrazol-3-yl)selenourea NNSeN (Figure 31). However, only the carboselenoamide NNSeN was generated. This was unexpected since the sulfur

derivative of this molecule did generate both products. This was an early indication that the selenourea NNSeNs may not be as stable or stable at all.

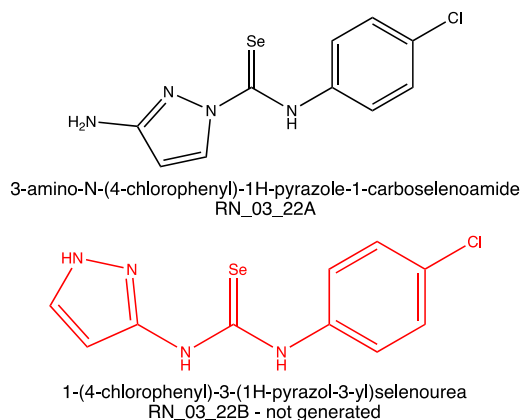


Figure 31: Probe reaction products using 3-aminopyrazole and 4-chlorophenyl ISC (red structure indicates product was not formed)

In the case of N-(4-chlorophenyl)-3-nitro-1H-pyrazole-1-carboselenoamide, the synthesis of this NNSeN was challenging. It seems that the electron withdrawing nitro group can have a detrimental effect on the stability of this molecule. In one synthetic attempt, 3-nitropyrazole and 4-chlorophenyl ISC was reacted along with potassium carbonate in chloroform. The use of base is crucial in this specific example since the electron withdrawing nitro group greatly lowers the acidity of the pyrazole -NH- group, however potassium carbonate was not a strong enough base for this and resulted in no product formation. This synthetic venture was attempted again using method iii: the use of sodium hydride in dry THF. This method was done in a 0.042 mmol scale and did prove successful and produced of N-(4-chlorophenyl)-3-nitro-1H-pyrazole-1-carboselenoamide in a 63% yield. However, these reaction conditions did not prove reliable. The same synthesis was attempted again and did not result in product formation. Instead, TLC analysis revealed only pyrazole material and in the reaction vessel there was also a red solid byproduct that generated. Similarly, the synthesis of N-(4-chlorophenyl)-4-nitro-1H-pyrazole-1-carboselenoamide, in which the nitro group is now on the 4th position on the pyrazole, utilizing

method iii did not generate product and generated a similar red solid byproduct. As such, no other NNSeNs with nitro groups were attempted since it was apparent that the stability of these nitrated NNSeNs was unreliable.

3-(benzyloxy)-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide was synthesized from 3-(benzyloxy)pyrazole and 4-chlorophenyl ISC using method iii: dry THF and potassium carbonate as base. This NNSeN was successfully synthesized in a 0.014mmol scale and yielded 64% of product.

In chapter 2 multiple pyrazoles were discussed and synthesized. In particular pyrazole with aliphatic and aromatic substituents in the third, fourth and fifth position or a combination of these were synthesized. Our intentions were to also synthesis NNSeNs with various aliphatic and aromatic groups on the available pyrazole positions. We employed multiple methods in order to successfully access these NNSeNs, however we were unable to. These NNSeNs were not stable. Interestingly, nearly all these NNSeNs with these pyrazole derivatives (such as 3,5-dimethylpyrazole, 3,4,5-trimethylpyrazole, 3-methyl-5-phenylpyrazole, and 5-methyl-3-phenylpyrazole) did generate *in situ* as they were nearly all observed via TLC analysis but were destroyed during the work up. This was apparent as a red solid would precipitate in the separatory funnel and TLC of the work up would no longer show any product spots. To try and work around this, the reaction mixture would be directly loaded onto a silica column and purified as is, but this ultimately also did not provide the desired product. This was an unexpected result since the sulfur derivatives were successfully synthesized.

N-(2,3-dichlorophenyl)-1H-pyrazole-1-carboselenoamide and N-(2,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide were both synthesized from pyrazole and 2,3-dichlorophenyl ISC and 2,4-dichlorophenyl ISC in a 0.071mmol and 0.068mmol scale and resulted in a 16% and

21% yield, respectively. 3-amino-N-(2-chlorophenyl)-1H-pyrazole-1-carboselenoamide and 3-amino-N-(3-chlorophenyl)-1H-pyrazole-1-carboselenoamide were both synthesized from 3-aminopyrazole and 2-chlorophenyl ISC and 3-chlorophenyl ISC in a 0.096 mmol and 0.13 mmol scale and resulted in 82% and 67% yield respectively. N-(2,3-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide, N-(3,4-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide and N-(2,4-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide were all synthesized using 3-methylpyrazole in the following scale and yields: 0.058 mmol scale and 49% yield, 0.11 mmol scale and 65% yield, 0.067 mmol scale and 62% yield respectively. 4-bromo-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide was synthesized from 4-bromopyrazole and 4-chlorophenyl ISC in a 0.10 mmol scale and resulted in a 32% yield. 3-amino-N-(3,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide, 3-amino-N-(2,3-dichlorophenyl)-1H-pyrazole-1-carboselenoamide and 3-amino-N-(2,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide were synthesized from 3-aminopyrazole and 3,4-dichlorophenyl ISC, 2,3-dichlorophenyl ISC and 2,4-dichlorophenyl ISC, all in a 0.11 mmol scale and resulted in 74%, 71%, and 78% yield respectively. 3-amino-N-phenyl-1H-pyrazole-1-carboselenoamide was synthesized from 3-aminopyrazole and phenyl ISC in a 0.10 mmol scale and resulted in a 65% yield. N-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazole-1-carboselenoamide was attempted to be synthesized from 3-trifluoromethylpyrazole and 4-chlorophenyl ISC but the pyrazole seemed to be very unreactive. We tried multiple methods however the very EWG on the pyrazole seems to be hindering the reaction. This was even left to reflux for multiple days and only resulted in

degradation of ISC starting material. All NNSeNs that were successfully synthesized and mentioned in this section can be found in Figure 32.

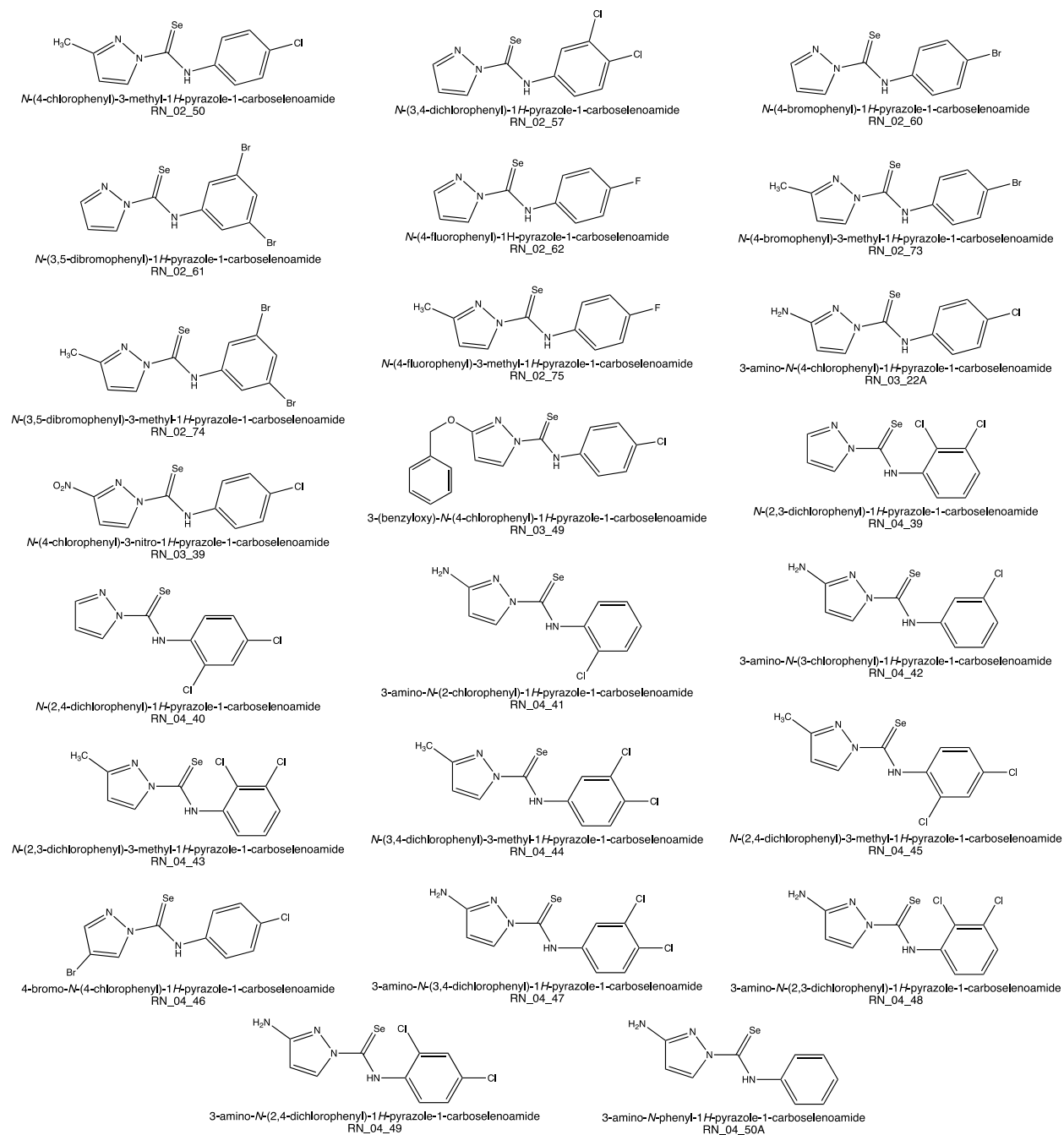


Figure 32: Pyrazolyl Carboselenoamide NNSeNs Synthesized

Pyrazolyl Selenourea NNSeNs

Moving towards the selenourea motif, this proved to be very insightful towards the stability of these NNSeNs. Our first attempt at synthesizing these extended NNSeNs was with 1-(1-(tert-butyl)-1H-pyrazol-3-yl)-3-(4-chlorophenyl)selenourea. This was done by taking 1-(tert-butyl)-1H-pyrazol-3-amine and reacting this with 4-chlorophenyl ISC utilizing method vi. TLC analysis (25% ethyl acetate: 75% hexane) did reveal a new spot however during the workup, a red solid was generated and TLC of the work up revealed loss of new spot. This was unfortunate however we reattempted this synthesis and instead of initiating aqueous workup, this reaction mixture was loaded directly to a silica column. Unexpectedly, the ISC starting material and “product” was inseparable through column. At this point, a recrystallization was attempted using toluene and a white solid was obtained, with TLC analysis correlating with the “product” spot of the TLC from our first synthetic attempt. This was left to dry overnight *in vacuo* and characterized via ¹H-NMR and suggested product formation. However, in the process of dissolving the product and transferring into a glass vial, a red/orange solid immediately was generated. This was filtered and only 1mg of product was retained. The red/orange solid byproduct was attempted to be dissolved with multiple solvents and none was able to dissolve this. Reading through the literature, it became clear that this red solid byproduct, which has been occurring in only select NNSeN syntheses, is red selenium. Red selenium is an allotropic form of selenium which can be found as an eight membered ring macrocycle, labeled as Se₈ (Figure 33).

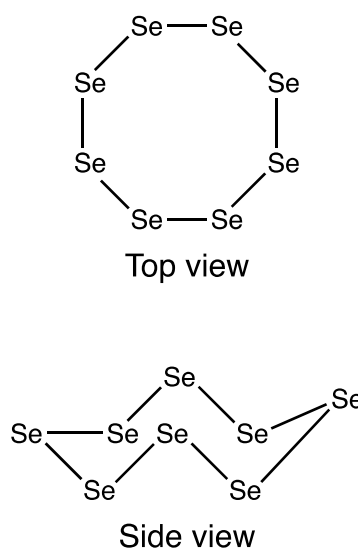


Figure 33: *Ses* macrocycle

This allotrope is traditionally obtained by precipitating selenium from a solution of selenious acid in methanol or ethanol with hydrazine at a temperature between -20°C and the freezing point of the solution.¹ In the case of NNSeN degradation, it is likely that the selenium carbon double bond is destabilized and oxidized by air and generating this red byproduct. Up to this point, there has not been a way us to avoid this degradation unless we keep this under an inert atmosphere. Moving on to the synthesis of 1-(4-chlorophenyl)-3-(1H-pyrazol-3-yl)selenourea, in which we had already tried this from the probe reaction between 3-aminopyrazole and 4-chlorophenylISC, we took a different route in which we first BOC protected the primary amine of 3-aminopyrazole with ease in hope that the free amine would be the only nucleophile to attach the ISC. We then subjected the BOC-protected tert-butyl 3-amino-1H-pyrazole-1-carboxylate to the reaction conditions for NNSeNs using 4-chlorophenyl ISC at a 0.12 mmol scale and was able to successfully generate tert-butyl 3-(3-(4-chlorophenyl)selenoureido)-1H-pyrazole-1-carboxylate NNSeN in a 40% yield. Next, we attempted to deprotect the Boc-NNSeN however we were concerned using the strong acidic conditions such as HCl in dioxane or TFA in dioxane would protonate the selenium atom and generate a selenol-NNSeN. We opted to using milder conditions and attempted a recently

developed deprotection method using oxalyl chloride in methanol.² For this, 1 eq. of the BOC-NNSeN was dissolved in approximately 4mL of dry methanol and stirred at room temperature for 5 minutes. After this time, 3 eq of oxalyl chloride is added carefully because this addition will cause sputtering. This was left to stir and monitored using TLC (50: 50 ethyl acetate: hexane). However, TLC monitoring revealed no generation of deprotected NNSeN and 100% of the BOC-NNSeN was recovered. We then decided to simply deprotect using 2 eq. of 25% TFA in DCM for 18 hours. Interestingly, after 18 hours the presence of red selenium in the reaction mixture was observed. As such, the reaction mixture was filtered and only 28% (5.4mg) of the BOC-NNSeN was recovered. The presence of the red selenium suggests that either the acidic conditions oxidized the selenium or the deprotection was occurring and as the BOC group was removed, the 1-(4-chlorophenyl)-3-(1H-pyrazol-3-yl)selenourea NNSeN degraded. The BOC-NNSeN was kept as it is still a new selenourea NNSeN product. We then shifted our focus towards the synthesis of 1-(4-chlorophenyl)-3-(1-methyl-1H-pyrazol-3-yl)selenourea using 1-methyl-1H-pyrazol-3-amine and 4-chlorophenyl ISC. During the workup of this reaction the red selenium byproduct was generated. We re-attempted this synthesis but instead of doing a liquid-liquid extraction the reaction mixture would be loaded onto silica column directly after reaction. We were able to isolate “product” and attempted to characterize however this proved difficult as the addition of the deuterated chloroform solvent would generate red selenium and any characterization effort was futile. Looking at all the selenourea NNSeN products, a trend was noticed particularly with electron donating and electron withdrawing groups. It also may be possible that the aromaticity of the pyrazole may be contributing to the overall stability of the extended NNSeN. Looking at 1-(1-(tert-butyl)-1H-pyrazol-3-yl)-3-(4-chlorophenyl)selenourea NNSeN, the pyrazole contained a weak electron donating, or rather inductive donating, t-butyl group at the primary position and this was not stable.

Similarly, 1-(4-chlorophenyl)-3-(1-methyl-1H-pyrazol-3-yl)selenourea NNSeN with a methyl group on the primary position also resulted in the instability of this extended NNSeN. Looking at an NNSeN with no electron donating or withdrawing groups on it, 1-(4-chlorophenyl)-3-(1H-pyrazol-3-yl)selenourea NNSeN contained a hydrogen on the primary position and this product was also not stable. All three of these have aromaticity involved in the pyrazole ring, which may be a reason for the instability of these particular NNSeNs (Figure 34).

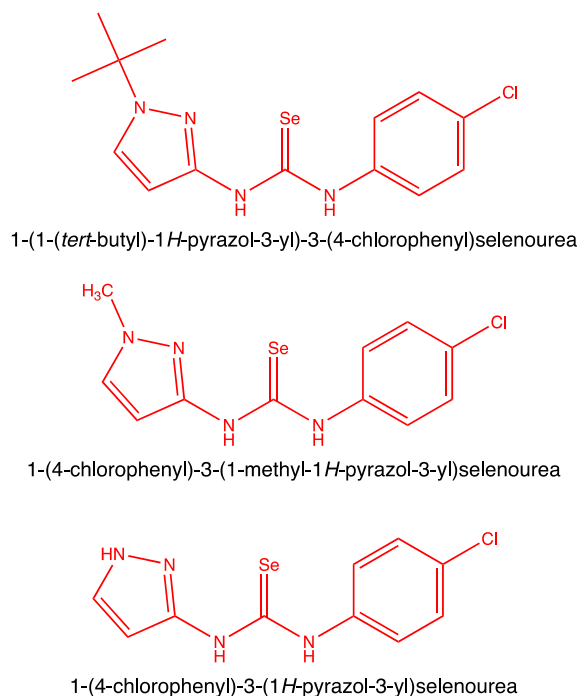


Figure 34: Unstable extended NNSeNs (red structure indicates product was not formed)

However, in the case of stable BOC-protected *tert*-butyl 3-(3-(4-chlorophenyl)selenoureido)-1H-pyrazole-1-carboxylate NNSeN, the BOC group would be considered a moderate electron withdrawing group which could possibly be disrupting aromaticity of the pyrazole, as shown in Figure 35. As these structures are novel, this became very enlightening on the overall stability of the extended NNSeN motif, and unintentionally came across a way of generating red selenium. In the following section, we will continue to observe this electron donating and electron withdrawing trend with a pyrazole that contained both groups.

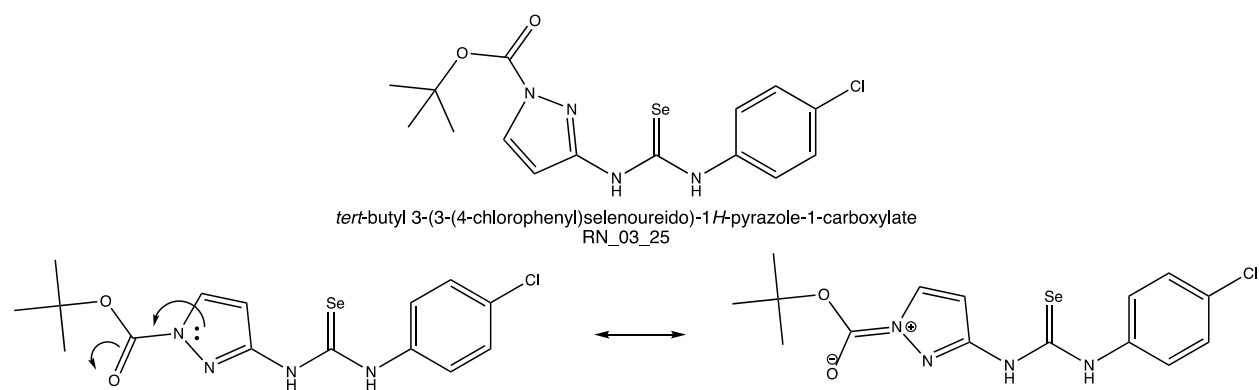


Figure 35: EWG disrupting aromaticity of the pyrazole

Pyrazolyl Carboselenoamide + Pyrazolyl Selenourea NNSeNs Generated Together

As discussed, 3-aminopyrazole and 4-chlorophenyl ISC was used a probe reaction to see if the pyrazolyl selenourea derivative would generate. This was also attempted using 3-aminopyrazole and phenyl ISC in a 0.10 mmol scale and resulted in a 65% yield of only the carboselenoamide NNSeN. To our pleasant surprise, it wasn't until we used ethyl 3-amino-pyrazole-4-carboxylate where we were able to access both the carboselenoamide and selenourea NNSeN from the same reaction. We started the use of this pyrazole starting material by reacting it with 4-chlorophenyl ISC method vi: chloroform, under argon at reflux for overnight. After this time, TLC analysis (75% DCM: 25% Hexane) revealed a very faint ISC spot only visible under iodine chamber stain, a faint pyrazole spot and 2 new spots. Typically, with the solvent system the expected carboselenoamide NNSeN spot is usually in the middle of the TLC spot. This was observed along with a second spot right underneath the middle spot. This mixture was worked up and purified using silica column chromatography using a DCM: Hexane gradient. Both spots were successfully separated and were characterized via ^1H -NMR analysis which revealed that both the carboselenoamide and selenourea NNSeN motifs were generated. This was exciting as we were able to expand the pyrazolyl selenourea library more. This ethyl 3-amino-pyrazole-4-carboxylate

starting material was coupled with all chlorinated ISCs to yield the following NNSeNs (scales and yields included, see Figure 36 for structures):

- ethyl 3-amino-1-((3-chlorophenyl)carbamoselenoyl)-1H-pyrazole-4-carboxylate and ethyl 3-(3-(3-chlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate (0.078 mmol scale: 26% and 55% yields respectively).
- ethyl 3-amino-1-((2-chlorophenyl)carbamoselenoyl)-1H-pyrazole-4-carboxylate and ethyl 3-(3-(2-chlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate (0.095 mmol scale: 46% and 33% yields respectively)
- ethyl 3-amino-1-((3,4-dichlorophenyl)carbamoselenoyl)-1H-pyrazole-4-carboxylate and ethyl 3-(3-(3,4-dichlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate (0.077 mmol scale: 29% and 53% yields respectively)
- ethyl 3-amino-1-((2,3-dichlorophenyl)carbamoselenoyl)-1H-pyrazole-4-carboxylate and ethyl 3-(3-(2,3-dichlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate (0.075 mmol scale: 26% and 43% yields respectively)
- ethyl 3-amino-1-((2,4-dichlorophenyl)carbamoselenoyl)-1H-pyrazole-4-carboxylate and ethyl 3-(3-(2,4-dichlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate (0.071 mmol scale: 23% and 26% yields respectively)

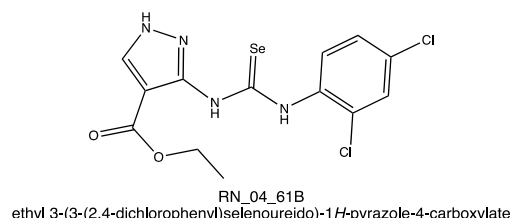
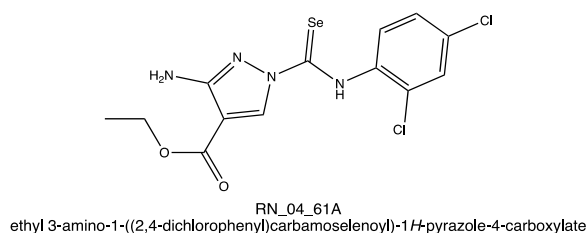
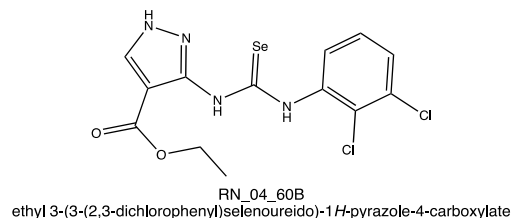
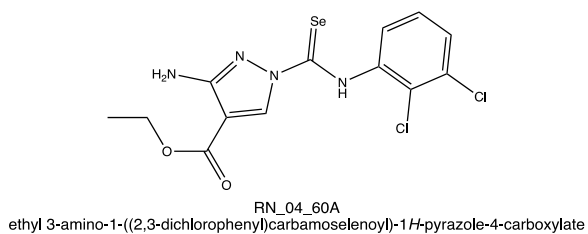
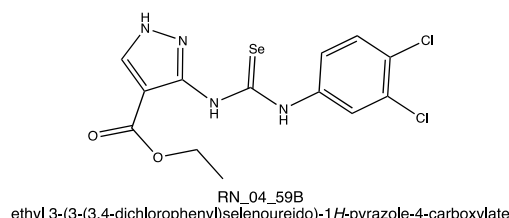
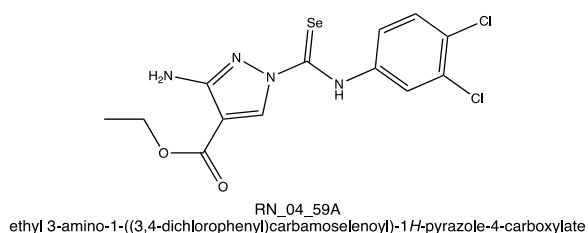
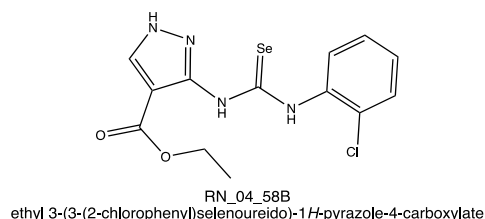
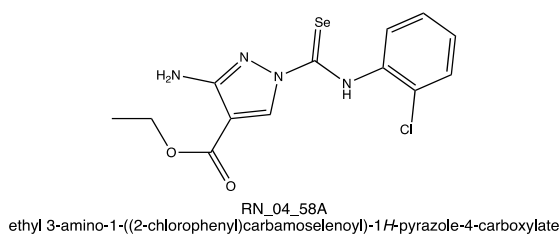
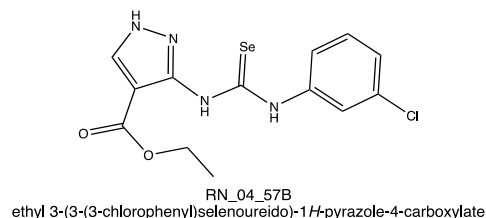
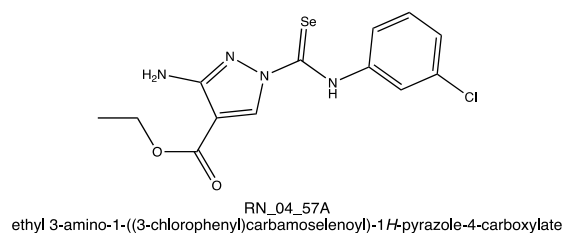


Figure 36: Pyrazolyl carboselenoamide and pyrazolyl selenourea NNSeNs synthesized from ethyl 3-amino-pyrazole-4-carboxylate and ISCs

In these NNSeNs, the pyrazole contains a moderate electron withdrawing ester group on the electron rich 4th position of the pyrazole. With the electron withdrawing group pulling electron density from the pyrazole ring, it is possible this is also disrupting the rings aromaticity and allowed for the extended NNSeNs to be generated. This is a similar trend noticed that was

initially encountered during the synthesis of other extended NNSeNs, i.e. BOC-NNSeN tert-butyl 3-(3-(4-chlorophenyl)selenoureido)-1H-pyrazole-1-carboxylate. Possible resonance structure only involving the withdrawing group is shown in Figure 37. This further supports that electron withdrawing groups may be necessary in order to access extended NNSeN motifs.

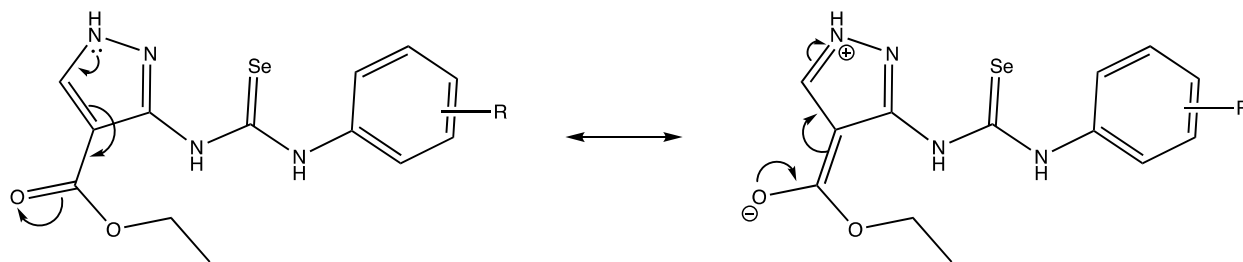


Figure 37: EWG on ethyl 3-(3-phenylselenoureido)-1H-pyrazole-4-carboxylate NNSeN derivatives

Characterization

All synthesized products were characterized via ^1H -NMR and ^{13}C -NMR. In the case of ISC's and NNSeN's, ^{77}Se -NMR was attempted, however the exact chemical shift range must be known to be seen on NMR spectra. This is different to traditional ^1H -NMR and ^{13}C -NMR experiments. ^{77}Se is a low sensitivity spin $\frac{1}{2}$ nucleus that yields narrow lines over a very wide chemical shift range (-1000–2000 ppm). Each type of selenium compound has its characteristic chemical shift range. For example, the shifts of selenocyanates (isomers of ISC's with structures of $\text{R-Se-C}\equiv\text{N}$) vary greatly. Phenyl selenocyanate has a ^{77}Se shift at 321ppm, while methyl selenocyanate has a shift at 120 ppm.³ ISC's have not been well establish in the case of ^{77}Se NMR and require exploration to find the shift. Because both the carboselenoamide and selenourea NNSeNs are novel, this would also require exploration. This exploration was attempted however was difficult to accomplish due to the wide chemical shift range and is beyond the scope of this dissertation. As such, only ^1H -NMR and ^{13}C -NMR was done.

Chapter 3 References

1. Buckley, D.A.; Xerox Corporation, USA, assigness. Preparation of Red Amorphous Selenium, Published August 6, 1974. US3954951A
2. George, N., Ofori, S., Parkin, S., Awuah, S. G. Mild deprotection of the N-tert-butyloxycarbonyl (N-Boc) group using oxalyl chloride. *RSC Adv.*, 2020, **10**, 24017-24026.
3. Data Organic Chemistry. Hans J. Reich, Ph.D. ^{77}Se -Shifts.
https://organicchemistrydata.org/hansreich/resources/nmr/?index=nmr_index%2F77Se_shift#se-data00

Chapter 4 – Theoretical Studies, Copper Binding Studies and Preliminary Biological Studies

Theoretical Mode of Action

As mentioned in Chapter 1, the isosteric NNSNs were revealed to be copper-activated compounds. Copper (either I or II) is an endogenous metal with effective antibacterial properties and aids in defending the immune system by catalyzing redox reactions. Copper is limited in the human body because of its ability to produce reactive oxygen species (ROS) which are tissue damaging. Therefore, tight homeostatic regulation is mandatory, restricting the free copper concentration in blood and healthy tissue to about 1 micromolar or less.¹ There are two main events that can cause alteration of the copper concentration, namely upregulation occurring in activated phagosomes and cancerous cells. In both cases, the copper concentration has shown to increase by 5-50 times that found in normal tissues.¹ The copper concentration and its distribution throughout cancer cells is directly related to a tumor's growth, angiogenesis, and metastasis.² The cell membranes have developed means to remove the copper through efflux pumps. However, the NNSNs have the ability to form iminium type cations which is able to complex with copper and is then able to impair numerous enzymes of the cell. These iminium copper complex cations are considered to be very potent warheads as they can react with multiple targets through nucleophilic addition, electron transfer and radical reactions.¹ This is important as they will effectively shut down the efflux pumps that aid in filtering out copper from the cell. The copper-NNSN will flood into the cell and will lead to cell apoptosis. Because the selenium structures are isosteric relatives, it is proposed that this iminium cation may also form in NNSeNs. This may occur through the natural resonance energy found through

aromaticity from the lone pair of electrons in the pyrazole nitrogen adjacent to the C=Se bond: resonance form this lone pair will form a double bond between the two pyrazole nitrogen's which generates an iminium carbocation and the initial double bond adjacent to the second pyrazole nitrogen is broken and a different C=C double bond formed between carbon 3 and carbon 4 of the pyrazole, and the double bond between carbon 4 and carbon 5 is also broken yielding a carbanion on carbon 5 which can the chelate with copper (I) (Figure 38). To properly investigate if this will occur, computer modeling studies must be done.

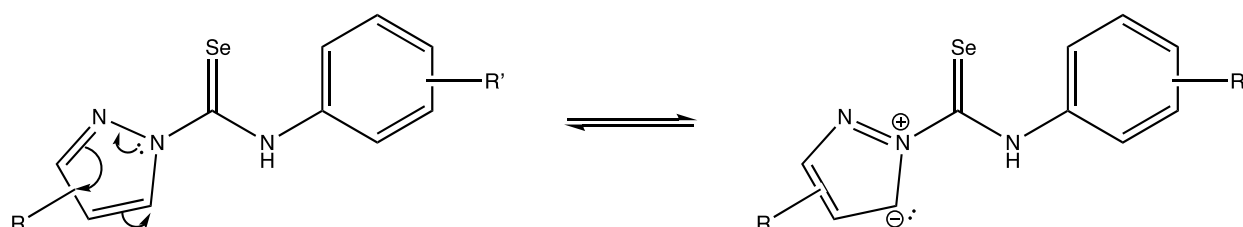


Figure 38: Proposed mechanism of iminium cation formation

NNSeN Computer Modeling Studies

To investigate the interaction between various NNSN compounds and copper and understand how complexation occurs, density function calculations (DFT) calculations were carried out along with optimization and energy calculations and the structures visualized using MacMolPlt.³ This was done using both the 3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide, which will be referred to as NNSeN-1, and 1-(4-chlorophenyl)-3-(1H-pyrazol-3-yl)selenourea, which will be referred to as NNSeN-2. The geometry optimization and energy calculations were performed by Sulalith Samarasinghe et. al. using DFT model and basis sets of polarized double zeta (DZP) quality for hydrogen, nitrogen, carbon, and chlorine; for copper, polarized triple zeta (TZP) quality basis sets were used. Only valence electrons were

considered since these are typically involved in bond interaction and all other electrons remained in a frozen core for the calculations.³

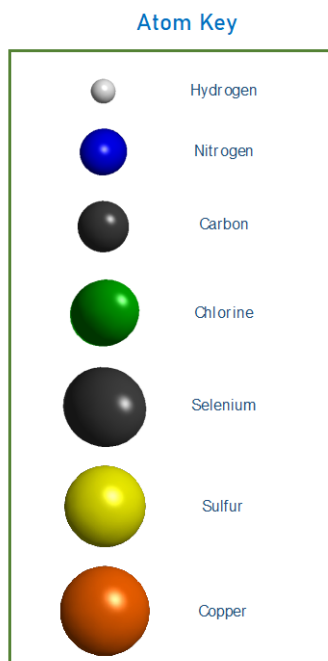


Figure 39: Atom key for NNSeN Computational Studies

3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide NNSeN-1

3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide NNSeN-1 modeling studies was done using 4 isomers, labeled as NNSeN-1 Isomer_1, NNSeN-1 Isomer_2, NNSeN-1 Isomer_3, and NNSeN-1 Isomer_4. The total bond energy of the isomers was calculated and are shown in Figure 40. This revealed that the most stable isomer with an energy of 0.00 kJ/mol was NNSeN-1 Isomer_3 in which the selenium atom is interacting with the aryl hydrogen. This interaction is also seen in the highest total bond energy isomer NNSeN-1 Isomer_1, however the free amine from the 3-aminopyrazole is oriented away, or “trans” if you will, relative to the C=Se functional group, enhancing the overall stability of this isomer. Regardless of total bond energy, the possible sites for copper(I) complexation on the isomers were identified and these complexes were manually created in MacMolPlt. It is important to note that copper (I) is highly electrophilic and will be drawn to areas of high electron density of our NNSeN molecules. Keeping this in mind, the possible binding site include the lone pair of electrons of the nitrogen in the pyrazole ring, the lone pair of electrons of the selenium in the carboselenoamide group, the lone pair of electrons of the free amine group located on the 3rd position of the pyrazole and the π -electrons located in the aromatic ring. It is unlikely for the copper (I) metal to bind or interact with the chlorine group located on the aromatic ring. The structures for these copper-NNSeN complex will be shown per each isomer.

3-aminobenzylhydrazide (1H-imidazo[1,2-b]pyrazole-1-carboxenamide) NNSeN-1

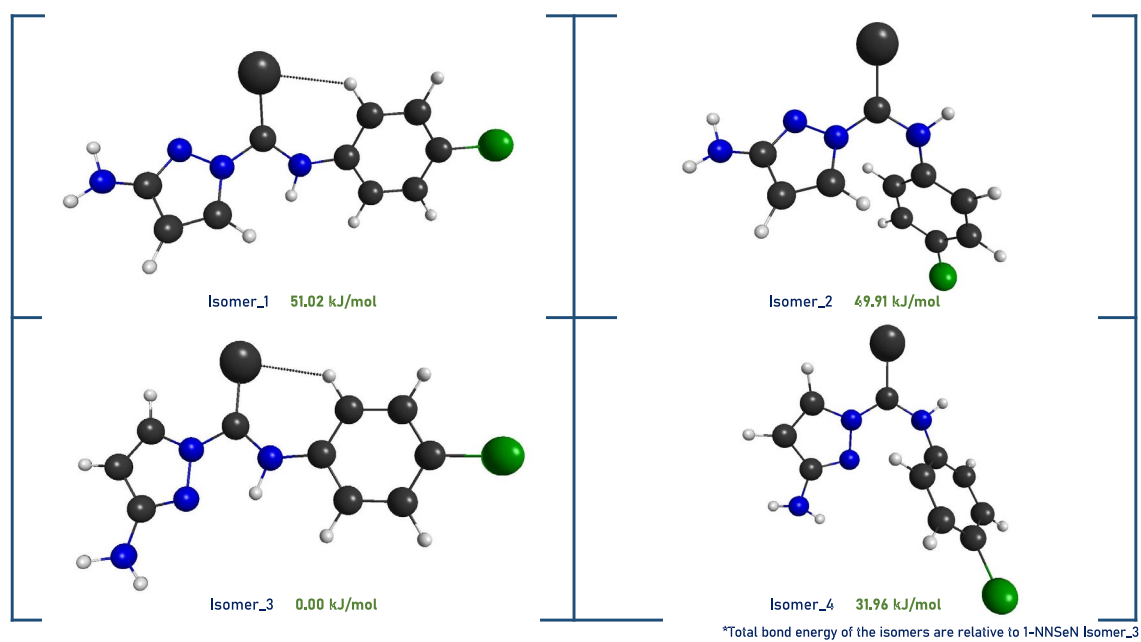


Figure 40: NNSeN-1 total bond energy of isomers 1-4

The highest energy isomer, NNSeN-1 Isomer_1, was found to have 8 copper (I) complex isomers shown in Figure 41. Copper complexation varied however the most stable, or lowest in energy, was Isomer 1_1 where the copper (I) metal is chelating with the selenium and 2nd nitrogen of the pyrazole, an expected interaction.

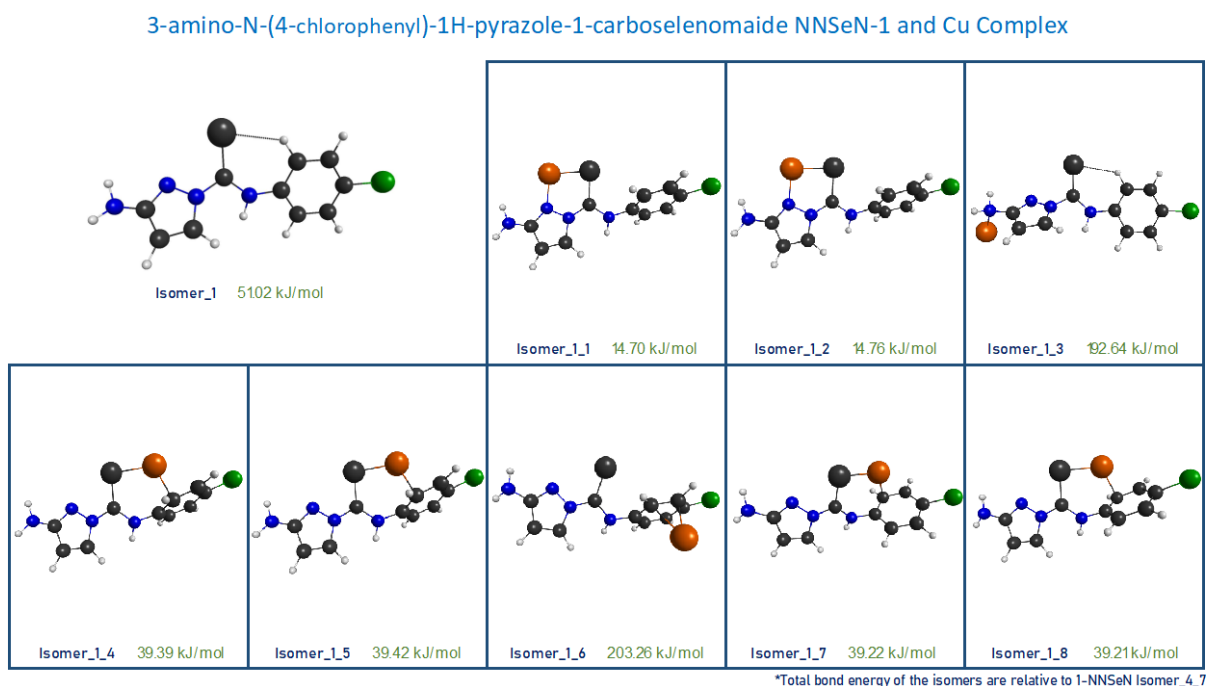


Figure 41: NNSeN-1 Isomer_1 Cu (I) complex isomers

NNSeN-1 Isomer_2, the second highest energy isomer, was found to have 9 copper (I) complex isomers shown in Figure 42. Copper complexation between the 2nd nitrogen of the pyrazole and selenium was lowest in total bond energy. However, selenium's larger atomic radius also granted it the ability to interact with the hydrogen of the aryl amine group. This interaction results in a 0.02 kJ/mol difference between Isomer 2_1 and Isomer 2_2.

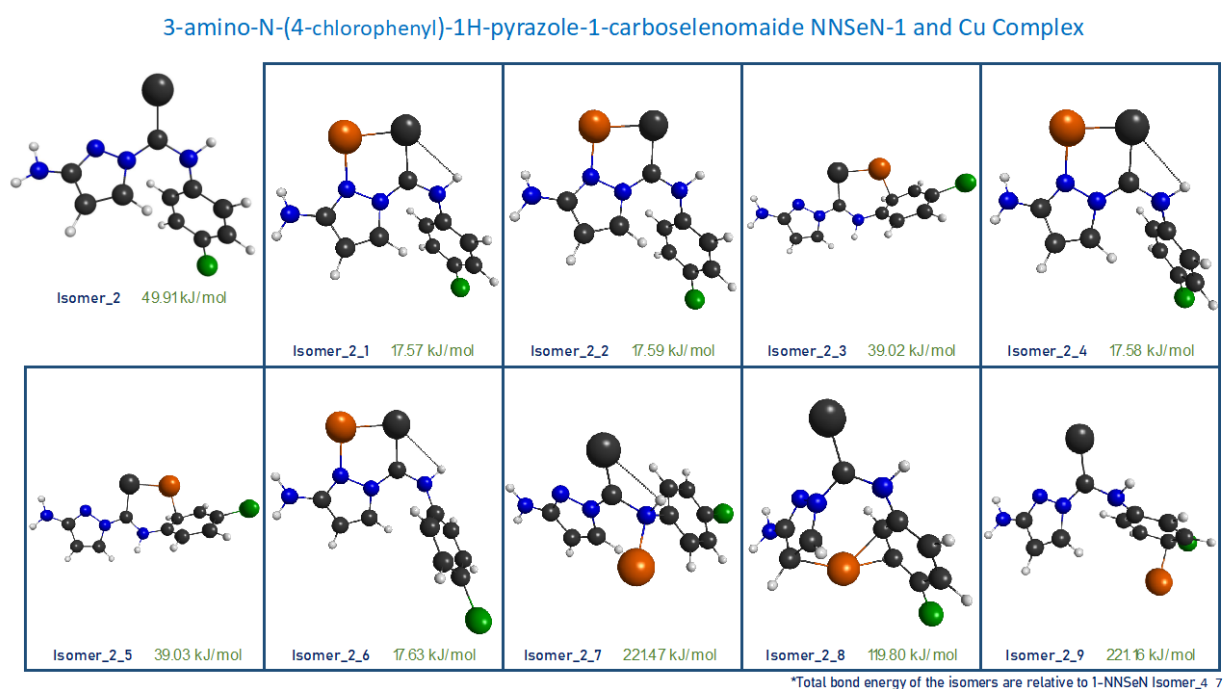


Figure 42: NNSeN-1 Isomer_2 Cu (I) complex isomers

NNSeN-1 Isomer_3, the lowest energy isomer, was found to have 8 copper (I) complex isomers shown in Figure 43. Of these 8, the lowest energy isomer was found to be Isomer_3_6, in which the aryl ring is oriented perpendicular to the remaining NNSeN structure, and the copper is chelating between the selenium and the ortho carbon pointed towards the direction of the observer, resulting in a low 0.02 kJ/mol total bond energy. This interaction was initially proposed to happen in the NNSN motifs but was never observed.

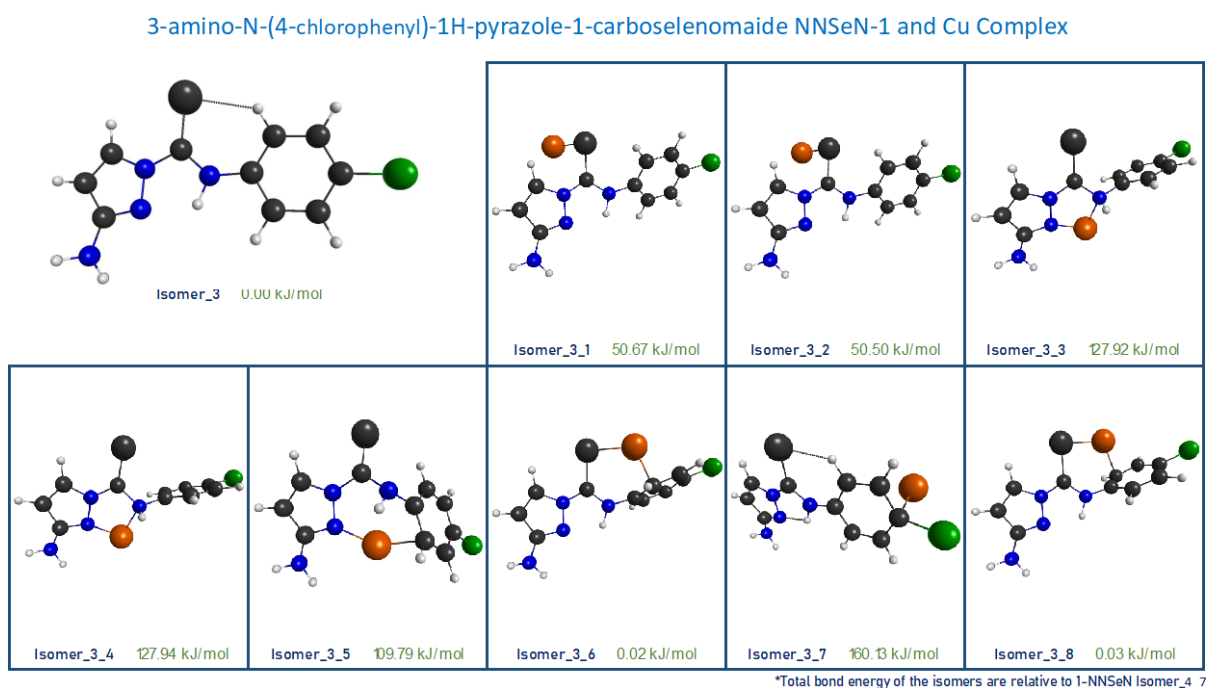


Figure 43: NNSeN-1 Isomer_3 Cu (I) complex isomers

NNSeN-1 Isomer_4, the second lowest energy isomer, was found to have 10 copper (I) complex isomers shown in Figure 44. Of these 10, the lowest energy isomer was found to be Isomer_4_7, in which the aryl ring is oriented perpendicular to the remaining NNSeN structure, and the copper is chelating between the selenium and the ortho carbon pointed away the direction of the observer, resulting in a low 0.00 kJ/mol total bond energy. This is very similar to NNSeN-1 Isomer_3_6.

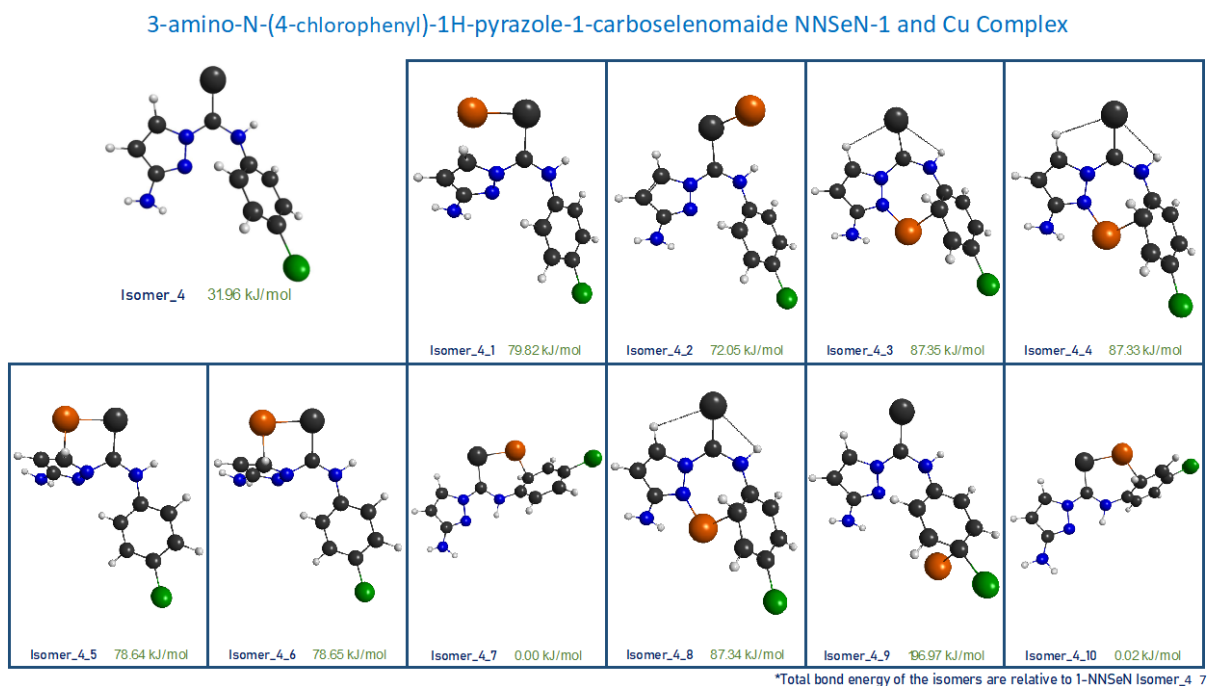
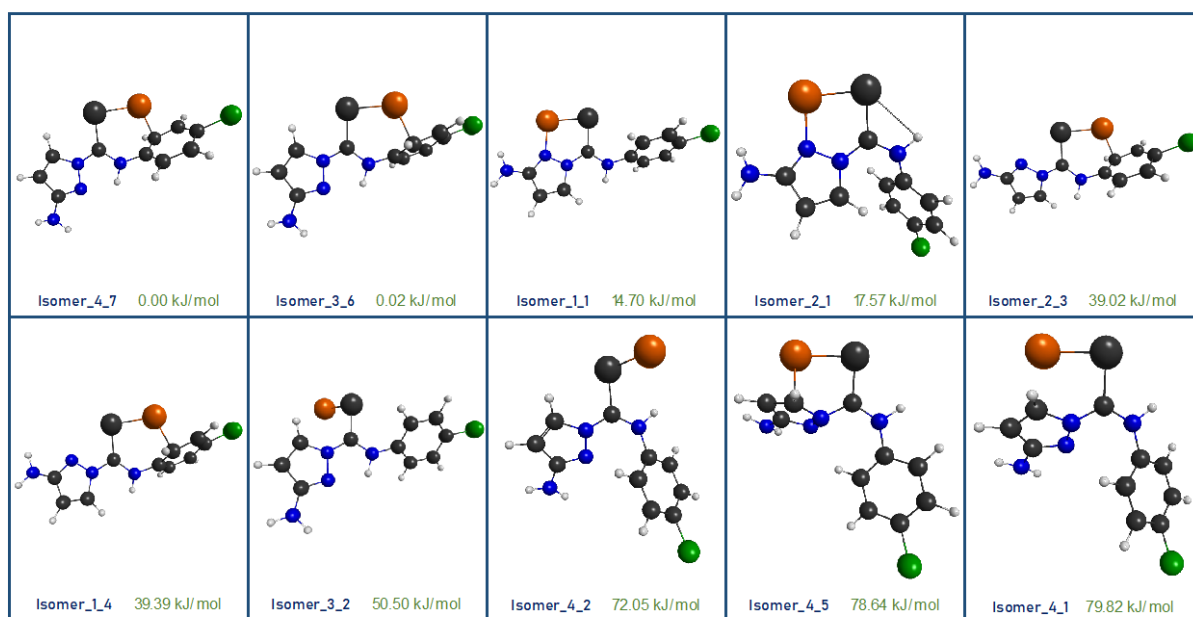


Figure 44: NNSeN-1 Isomer_4 Cu (I) complex isomers

The 10 lowest energy copper complex isomers from all the NNSeN-1 isomers are shown in Figure 45. As mentioned, Isomer4_7 is the lowest energy isomer in this whereas Isomer 4_1 is the highest energy isomer in this. These studies reveal that our initial iminium cation hypothesis, in which the resulting carbanion generated can then chelate to copper (I), were incorrect. For these pyrazolyl carboselenoamide NNSeNs, the copper (I) complexation will preferably chelate between the selenium and the ortho carbon of the aryl ring, suggesting the highest electron density can be found in this area.

3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide NNSeN-1 and Cu Complex – 10 Lowest Energy Isomers



*Total bond energy of the isomers are relative to 1-NNSeN Isomer_4_7

Figure 45: 10 lowest energy isomers for NNSeN-1 and Cu complex

1-(4-chlorophenyl)-3-(1H-pyrazol-3-yl)selenourea NNSeN-2

Although the synthesis of 1-(4-chlorophenyl)-3-(1H-pyrazol-3-yl)selenourea revealed the stability issues of this NNSeN motif, the extended NNSeN isomers will also be shown, however NNSeN-2 Isomer_3 will be discussed due to its unique geometry, in which both the hydrogens of the selenourea group are interacting or hydrogen bonding with selenium or the nitrogen pyrazole, respectively, and because it is the lowest in total bond energy shown in Figure 46. NNSeN-2 Isomer_6 will also be discussed.

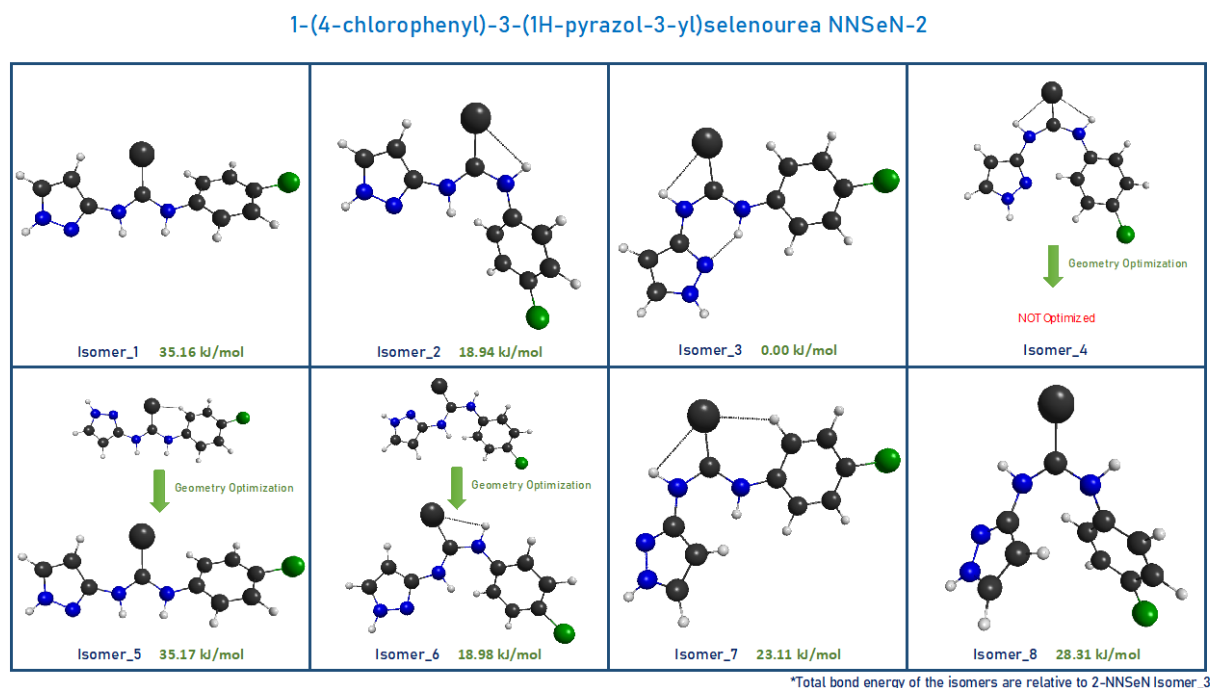


Figure 46: NNSeN-2 total bond energy of isomers 1-8

NNSeN-2 Isomer_3 was found to have 7 copper (I) complex isomers shown in Figure 47. Of these, Isomer 3_1 was the lowest in energy at 4.48 kJ/mol. Similarly, to the carboselenoamide NNSeN, this complexation occurred between the selenium atom and the ortho carbon furthest away from the observer. While this was occurring, both hydrogens maintained its interaction or hydrogen bond observed in the parent Isomer 3_1.

1-(4-chlorophenyl)-3-(1H-pyrazol-3-yl)selenourea NNSeN-2 and Cu Complex

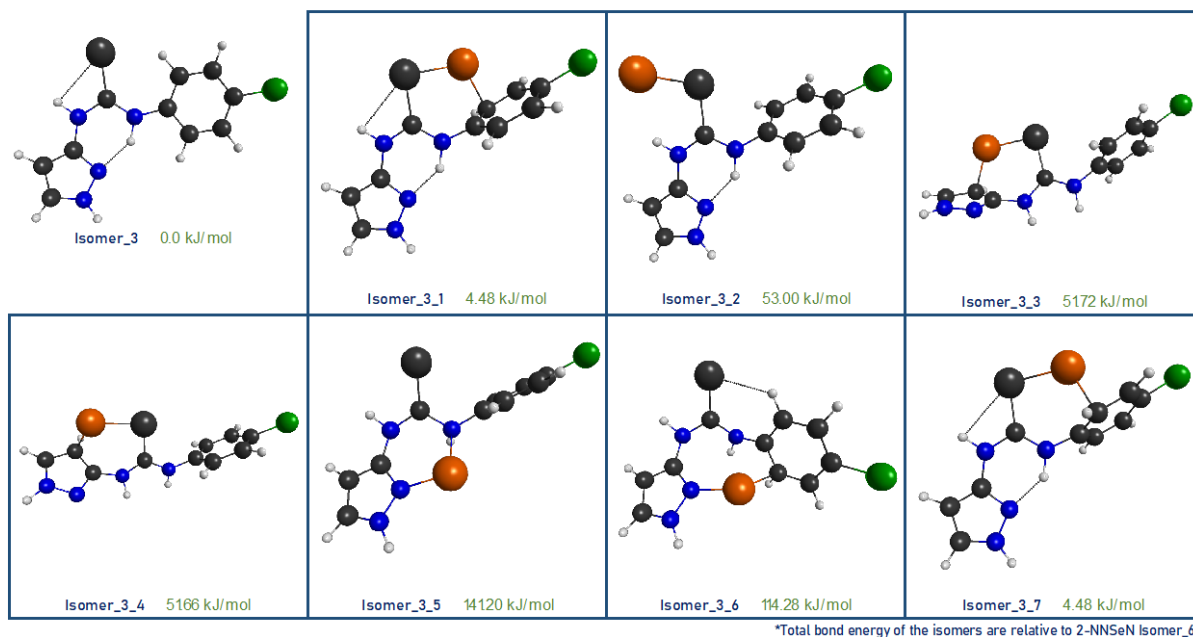


Figure 47: NNSeN-2 Isomer_3 Cu (I) Complex Isomers

NNSeN-2 Isomer_6 was found to have only 4 copper (I) complex isomers shown in Figure 48. Of these, Isomer 6_1 was the lowest in energy for this and all isomers at 0.00 kJ/mol. This complexation is occurring between the 2nd nitrogen of the pyrazole ring and the selenium atom, while the selenium still maintains its interaction with the hydrogen of the aryl nitrogen. Interestingly, the non-complexation isomer underwent geometry optimization and resulted in a 31.0 kJ/mol bond energy, and this energy is completely reduced with the chelation of copper (I).

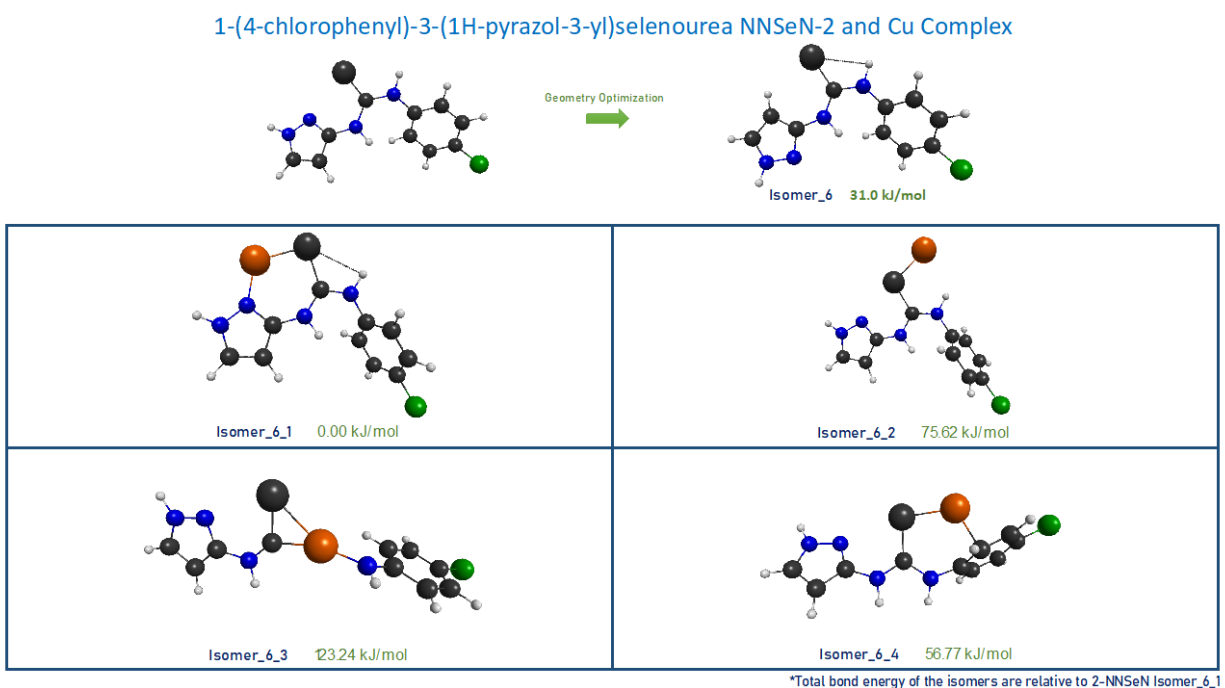


Figure 48: NNSeN-2 Isomer_6 Cu (I) Complex Isomers

3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide NNSeN-1 utilizing continuum solvation

It was discovered that when we use continuum solvation (using water) our lowest energy isomer for 3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide NNSeN-1 with copper (I) will change to the structure where copper is chelating with the the 2nd nitrogen of the pyrazole ring and the selenium atom, labeled as Isomer_1_1 in Figure 49, with an energy of 0.00 kJ/mol. This is different from the initial calculations without solvation, as this revealed that the lowest energy isomer would be one that has copper bonding with the ortho carbon of the phenyl group (Figure 45).

3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide NNSeN-1 and Cu **with** continuum solvation

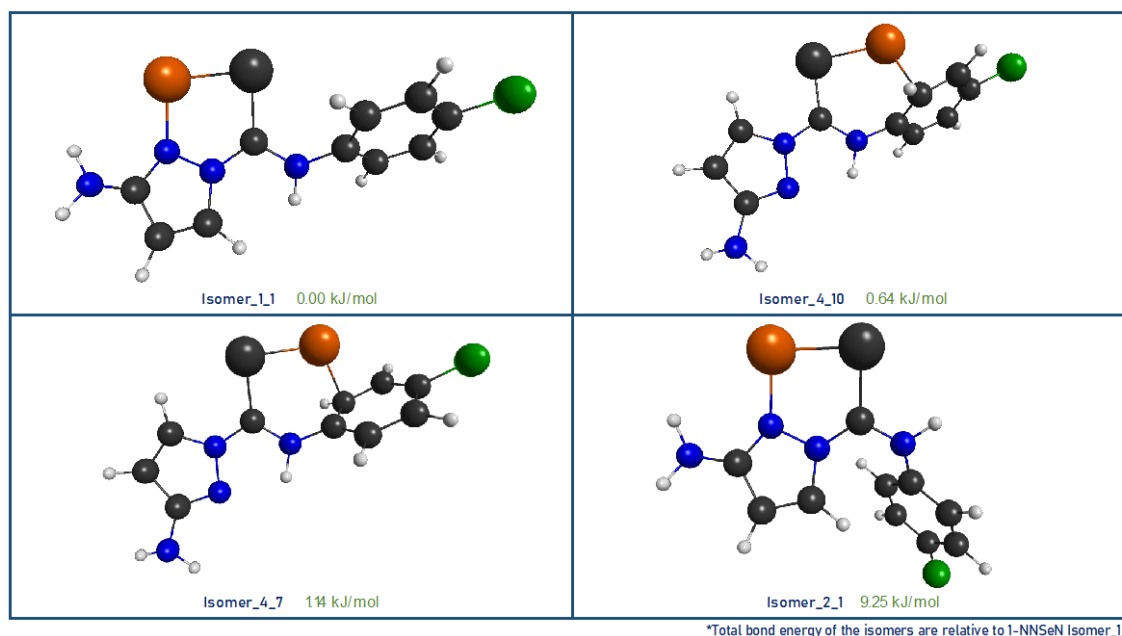


Figure 49: NNSeN-1 and Cu (I) with continuum solvation using water

Calculations using continuum solvation utilizing chloroform showed that our lowest energy isomer for 3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenomaide NNSeN-1 with copper (I) will change to the copper chelating with the ortho carbon of the phenyl group, labeled as Isomer_4_10 in Figure 50, with an energy of 0.00 kJ/mol. (Note: the color of selenium was changed to purple in Figure 50)

3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenomaide NNSeN-1 and Cu with continuum solvation - CHCl₃

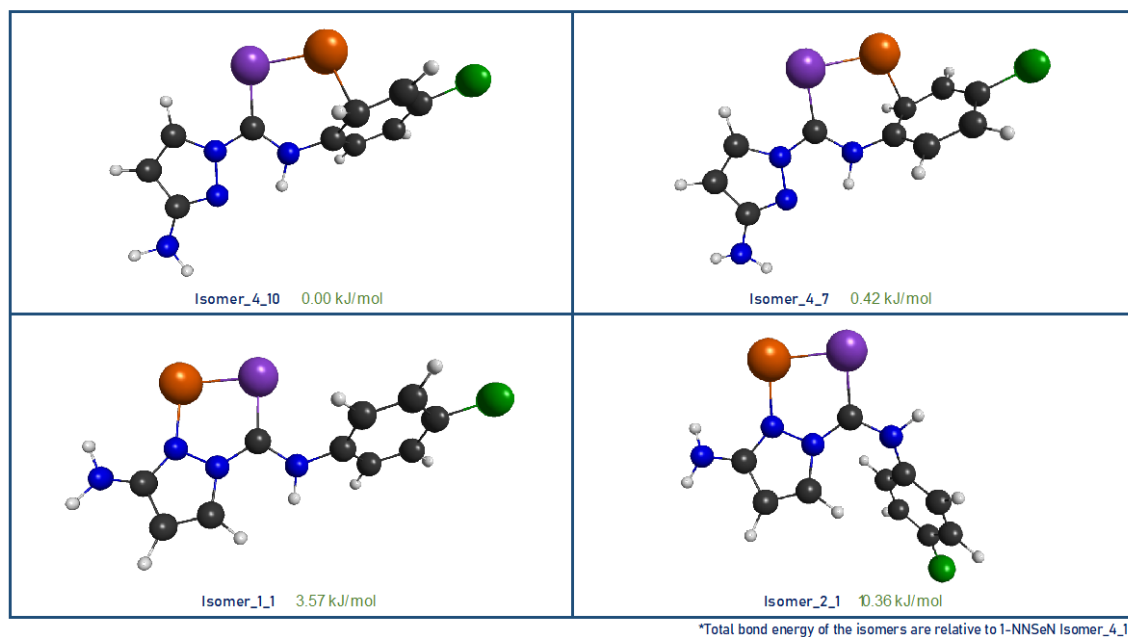


Figure 50: NNSeN-1 and Cu (I) with continuum solvation using chloroform

All of this studies were very encouraging towards our question if wether or not these NNSeNs would be copper activated or if they would even bind to copper. However, in order to ascertain this question we decided to do copper binding constant calculation to see if our novel NNSeN motifs would bind to copper and if they would bind stronger than the NNSN counterparts. We postulate that the NNSeNs will do both on the basis that selenium is more polarizable than sulfur, resulting in greater nucleophilic power, which is beneficial for selenium to selectively coordinate to metals or metal centers stronger than the sulfur counterparts.

Copper Binding Constant Calculations

The first step in measuring copper binding constants is to prepare some solutions. The first solution needed for this is HEPES buffer. To make this solution, weigh out 5.5957 mg of HEPES and dilute with water to 1 liter. The pH must then be adjusted to be between 7.2 and 7.4. The next solution is Copper(I) bromide in HEPES buffer, which must have a concentration of 0.00011 M. The last solution is that containing the NNSeN sample. The amount of NNSeN needed must then be calculated according to the following equation:

$$\frac{1 \times 10^{-3} \text{ moles}}{1000 \text{ mL}} \times 2 \text{ mL} \times MW \left(\frac{\text{g}}{\text{mole}} \right) = \text{Theoretical Mass Needed}$$

Once this amount has been weighed, it is dissolved in 200 microliters of methanol and sonicated or vortexed until fully dissolved. After this is added 1800 microliters of HEPES buffer. Once these solutions have been prepared, then a concentration gradient must be made, as shown in table 3.

Each concentration has a total volume of 3 mL.

Blank	0.25	0.5	0.75	1.00	1.50
• 2.4 mL CuBr • 0.6mL HEPES buffer	• 2.4 mL CuBr • 525 μL HEPES buffer • 75 μL NNSeN	• 2.4 mL CuBr • 450 μL HEPES buffer • 150 μL NNSeN	• 2.4 mL CuBr • 375 μL HEPES buffer • 225 μL NNSeN	• 2.4 mL CuBr • 300 μL HEPES buffer • 300 μL NNSeN	• 2.4 mL CuBr • 150 μL HEPES buffer • 450 μL NNSeN

Table 3: Gradient scale for copper binding experiments

With the concentration gradient in hand, this copper binding experiment is run using UV visible spectroscopy. The 12 following NNSeNs were tested: 3-methyl-N-phenyl-1H-pyrazole-1-carboselenoamide, N-(2-chlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide, N-(3,5-dibromophenyl)-1H-pyrazole-1-carboselenoamide, N-(4-fluorophenyl)-1H-pyrazole-1-carboselenoamide, 3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide, N-(4-chlorophenyl)-3-nitro-1H-pyrazole-1-carboselenoamide, 3-amino-N-(3-chlorophenyl)-1H-

pyrazole-1-carboselenoamide, N-(3,4-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide, 4-bromo-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide, 3-amino-N-(2,3-dichlorophenyl)-1H-pyrazole-1-carboselenoamide, 3-amino-N-phenyl-1H-pyrazole-1-carboselenoamide, and N-(3,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (Figure 51).

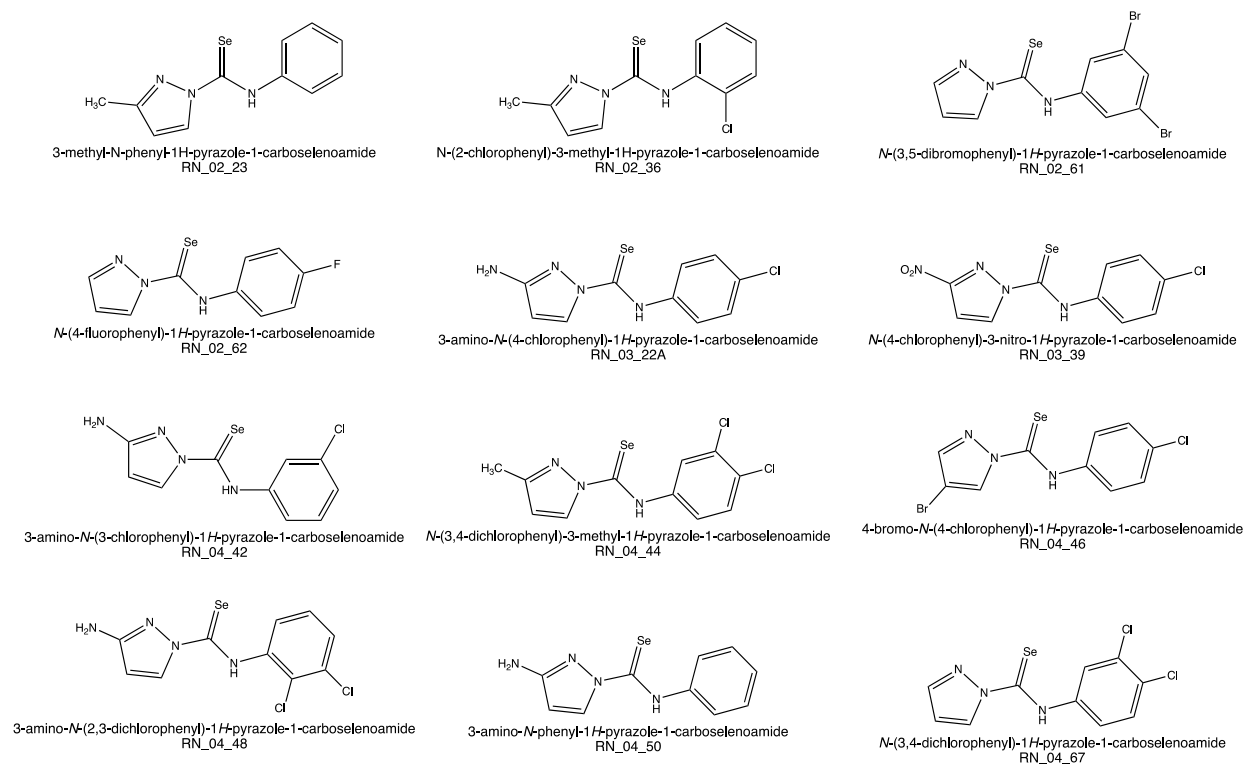


Figure 51: NNSeNs used for copper binding experiments

Table 4 shows the copper binding constants.

NNSeN	NNSeN 1:1 Binding Constant M^{-1}	NNSN 1:1 Binding Constant M^{-1} (for comparison)
N-(3,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_67)	515,500	1,996
3-amino-N-phenyl-1H-pyrazole-1-carboselenoamide (RN_04_50)	219,300	Not synthesized
3-amino-N-(2,3-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_48)	136,100	29,025
4-bromo-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_46)	162,250	Not synthesized
N-(3,4-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_04_44)	298,500	1,050
3-amino-N-(3-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_42)	282,000	1,950
N-(4-chlorophenyl)-3-nitro-1H-pyrazole-1-carboselenoamide (RN_03_39)	Not stable	1,100
3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_03_22A)	84,500	53,200
N-(4-fluorophenyl)-1H-pyrazole-1-carboselenoamide (RN_02_62)	39,000	1,250
N-(3,5-dibromophenyl)-1H-pyrazole-1-carboselenoamide (RN_02_61)	111,500	Not synthesized
N-(2-chlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_02_36)	180,000	1,200
3-methyl-N-phenyl-1H-pyrazole-1-carboselenoamide (RN_02_23)	52,500	Not synthesized

Table 4: NNSeN vs NNSN copper binding constants

The binding constants, except for the case of N-(4-chlorophenyl)-3-nitro-1H-pyrazole-1-carboselenoamide, were all distinctly higher than the isosteric NNSN compound. For N-(4-

chlorophenyl)-3-nitro-1H-pyrazole-1-carboselenoamide, it was no surprise to us it was unstable. This NNSeN synthesis was difficult to reproduce as it was unreliable. For the remaining NNSeNs, our hypothesis regarding selenium being more polarizable than sulfur and will in turn be able to selectively coordinate to metals or metal centers stronger than the sulfur counterparts, in this particular case copper (I), now has evidence to support this.

NNSeN Preliminary Biological Studies

Preliminary biological studies were conducted in collaboration with the Kutsch Lab at the University of Alabama at Birmingham Medical School Departments of Medicine and Microbiology. Unfortunately, due to the COVID-19 pandemic our collaboration was cut short and only a select few NNSeNs were tested against *S. aureus* strain Lac MRSA, a USA3000-type MRSA clinical isolate. The following 10 NNSeNs were tested: N-phenyl-1H-pyrazole-1-carboselenoamide (labeled as RN_02_20B), 3-methyl-N-phenyl-1H-pyrazole-1-carboselenoamide (labeled as RN_02_23), 3-methyl-N-phenyl-1H-pyrazole-1-carboselenoamide (labeled as RN_02_36), N-(3-chlorophenyl)-1H-pyrazole-1-carboselenoamide (labeled as RN_02_38), N-(3-chlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (labeled as RN_02_40), N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide (labeled as RN_02_45), N-(4-chlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (labeled as RN_02_50), N-(4-fluorophenyl)-1H-pyrazole-1-carboselenoamide (labeled as RN_02_62), N-(3,5-dibromophenyl)-1H-pyrazole-1-carboselenoamide (labeled as RN_02_74), and N-(4-fluorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (labeled as RN_02_75).

These were tested was done via dose response curves which were determined by diluting the NNSeN in RPMI medium with or without 50 μ M copper sulfate. A logarithmic phase culture of *S. aureus* grown in Mueller Hinton broth was washed in RPMI, then diluted in fresh RPMI and

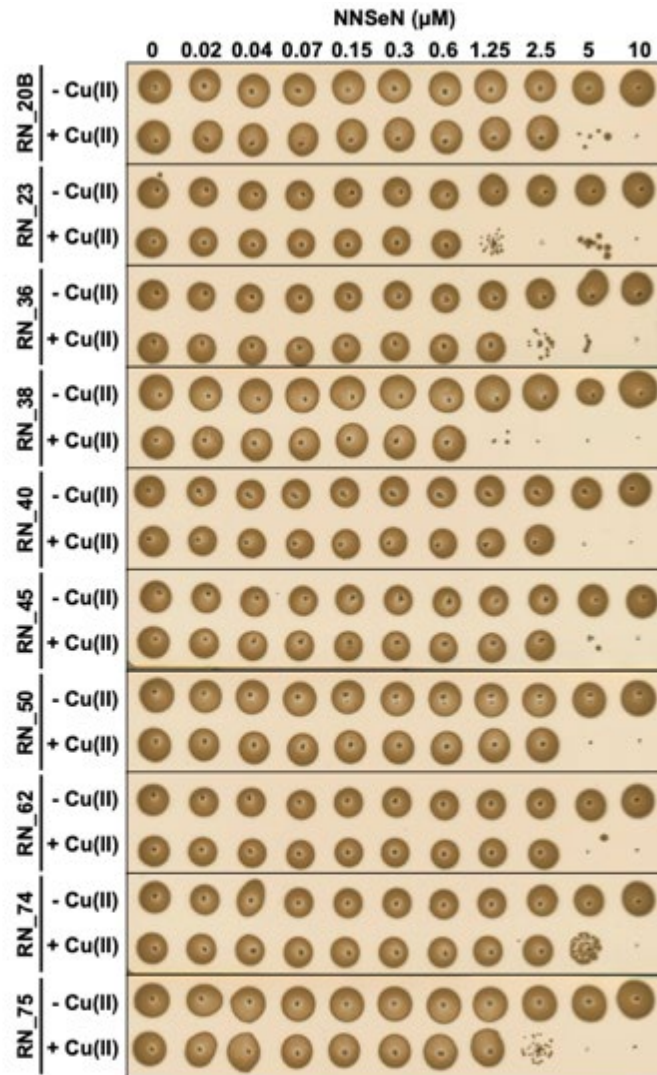
added to plates for a final optical density of 0.005. The plates are to be incubated overnight at 37°C. Metabolic activity will be determined by adding resazurin using 10% conversion of a blank subtracted and normalized value as the cutoff. Results indicate all of the isomers have at least some activity against MRSA with copper in the range of concentrations tested (0.018 – 10 μ M). The most active NNSeNs are RN_02_23, RN_02_36, RN_02_38, and RN_02_75 with an MIC of 0.6 μ M (table 5).

MIC (μ M)						
Bio Rep 1		Bio Rep 2		Bio Rep 1+2		
Compound	- Metal	+ 50 μ M Cu(II)	- Metal	+ 50 μ M Cu(II)	- Metal	+ 50 μ M Cu(II)
RN_20B	> 10	2.5	> 10	5	> 10	5
RN_23	> 10	0.6	> 10	1.25	> 10	1.25
RN_36	> 10	0.6	> 10	1.25	> 10	1.25
RN_38	> 10	0.6	> 10	0.6	> 10	0.6
RN_40	> 10	1.25	> 10	5	> 10	1.25
RN_45	> 10	5	> 10	5	> 10	5
RN_50	> 10	1.25	> 10	5	> 10	5
RN_62	> 10	5	> 10	5	> 10	5
RN_74	> 10	5	> 10	5	> 10	5
RN_75	> 10	0.6	> 10	2.5	> 10	1.25
IC50 (μ M)						
Bio Rep 1		Bio Rep 2		Bio Rep 1+2		
Compound	- Metal	+ 50 μ M Cu(II)	- Metal	+ 50 μ M Cu(II)	- Metal	+ μ M Cu(II)
RN_20B	> 10	1.25	> 10	5	> 10	2.5
RN_23	> 10	0.3	> 10	1.25	> 10	0.6
RN_36	> 10	0.3	> 10	1.25	> 10	1.25
RN_38	> 10	0.3	> 10	0.6	> 10	0.6
RN_40	> 10	1.25	> 10	2.5	> 10	2.5
RN_45	> 10	2.5	> 10	5	> 10	5
RN_50	> 10	1.25	> 10	2.5	> 10	1.25
RN_62	> 10	5	> 10	5	> 10	5
RN_74	> 10	5	> 10	5	> 10	5
RN_75	> 10	0.6	> 10	1.25	> 10	0.6

Table 5: NNSeN dose-response curve results obtained with Lac-MRSA

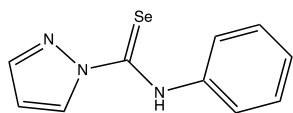
A drop assay from the dose-response curves was also done in to understand whether the treatments inhibit the growth of *S. aureus* strain Lac MRSA or outright kill it. This was done by

transferring 5 uL of sample from each treatment condition of a single technical replicate of a dose-response curve to a rich medium and observe whether MRSA grows or not. MRSA should always have confluent growth (the flat circular lawn) in the untreated control. If no bacteria grow at concentrations higher than the MIC, then the compound is bactericidal. If some growth is present above the MIC, then the compound is likely bacteriostatic. Figure 51 illustrates the results of this drop assay. NNSeNs RN_02_23, RN_02_36, RN_02_38, and RN_02_75 are the most active in killing *S. aureus* strain Lac MRSA. Figure 52 illustrates NNSeN structures tested.

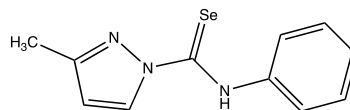


Strain: LAC (MRSA)
 5 μL samples from dose-response curves (biological replicate 2) dropped onto MH
 agar to rescue surviving cells
 Plates incubated overnight at 37°C

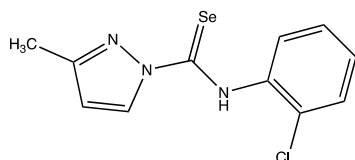
Figure 52: NNSeN drop assay results with Lac-MRSA



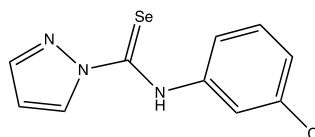
RN_02_20B
 $C_{10}H_9N_3Se$
 Exact Mass: 251
 Mol. Wt.: 250.16



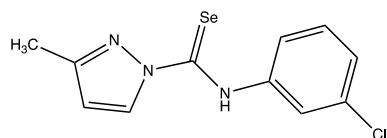
RN_02_23
 $C_{11}H_{11}N_3Se$
 Exact Mass: 265.01
 Mol. Wt.: 264.19



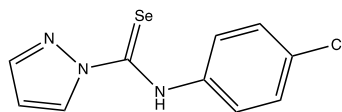
RN_02_36
 $C_{11}H_{10}ClN_3Se$
 Exact Mass: 298.97
 Mol. Wt.: 298.63



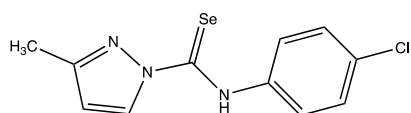
RN_02_38
 $C_{10}H_8ClN_3Se$
 Exact Mass: 284.96
 Mol. Wt.: 284.6



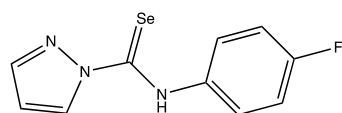
RN_02_40
 $C_{11}H_{10}ClN_3Se$
 Exact Mass: 298.97
 Mol. Wt.: 298.63



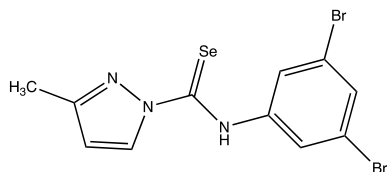
RN_02_45
 $C_{10}H_8ClN_3Se$
 Exact Mass: 284.96
 Mol. Wt.: 284.6



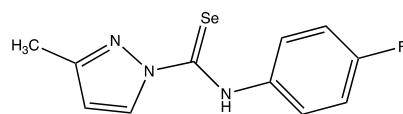
RN_02_50
 $C_{11}H_{10}ClN_3Se$
 Exact Mass: 298.97
 Mol. Wt.: 298.63



RN_02_62
 $C_{10}H_8FN_3Se$
 Exact Mass: 268.99
 Mol. Wt.: 268.15



RN_02_74
 $C_{11}H_8Br_2N_3Se$
 Exact Mass: 420.83
 Mol. Wt.: 421.98



RN_02_75
 $C_{11}H_{10}FN_3Se$
 Exact Mass: 283
 Mol. Wt.: 282.18

Figure 53: NNSeNs used for biological testing

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Chapter 5: Conclusion and Future Work

Conclusion

Bacterial infections are a serious health issue worldwide and the number of cases is anticipated to rise. Antibiotic resistance is a serious and highly complicated problem due to the ability of microbes to defeat the drugs that are designed to kill them. New therapies are desperately required to combat this issue. On top of this, cancer is also a massive health issue around the world accounting for nearly 10 million deaths in 2020. In the United States alone, cancer causes one in every four deaths. While early diagnosis usually leads to better prognostic outcomes, effective treatments are critical in the treatment of cancers. Cancer and bacterial infections are interrelated in that cancer patients are at an increased risk of obtaining antibiotic resistant infections due to their compromised immunity secondary to treatments such as chemotherapy, radiation, or surgical intervention.

One solution to treat both bacterial infections and cancers is using copper-activated drugs. Why copper? Well, why not! Copper is an endogenous metal that required strict homeostatic regulation to maintain physical health. Copper is important as it is involved in immune system function, processing of cholesterol, red blood cell formation, maintenance of blood vessels, nerves, healthy bones, and the development of babies in utero. A lack of copper can lead to anemia and osteoporosis while large amounts can cause hepatitis, kidney problems, and brain disorders. Copper is strictly controlled in the blood because intracellular copper can generate hydroxyl radicals. It is widely recognized that free radicals can damage proteins, nucleic acids, and lipids, as well as potentially interfere with the synthesis of iron-sulfur clusters. Further, copper has been shown to displace metals such as zinc from their cognate ligands in metalloproteins – this can cause serious problems like inhibition of enzymatic activity.¹

The Bossmann research sought to harness copper's natural antimicrobial properties by using copper- dependent inhibitors of bacterial infections, such as *S. aureus*, using the sulfur NNSN molecules. These NNSNs are a patented class of drugs with either a classic or novel extended thiourea motif.^{2,3,4} Up to date, 134 NNSN derivatives exist as a result of a team compiled of past undergraduate, graduate and post-graduate Bossmann group researchers dedicating years towards this NNSN project. This is a huge accomplishment due to these small, non-stereocenter containing drug molecules which exhibit copper-activated reactivity toward bacterial infections such as *S. aureus* (Gram-positive), *Escherichia Coli* (Gram negative)), and *M. tuberculosis*, and cancerous murine cell lines (Melanoma (B16F10), Glioblastoma (GL26), and Pancreatic Cancer (Pan02 and KPC)). The foundation, if you will, of my NNSeN project is due to these sulfur isomers success.

I first proposed The NNSeN project in 2018. The starting hypothesis focused on a new class of organoselenium compounds proposed to potentially be more biologically active than its sulfur isosteric relatives. But why selenium? Isn't it toxic? Yes, and no. Early studies first recognized the role of selenium as a toxin. However, as research into selenium advanced, its importance and necessity expanded. Selenium is "nutritionally essential" for humans and is a constituent of selenoproteins that have a play in critical roles such as reproduction, thyroid hormone metabolism, DNA synthesis and protection from oxidative damage and infection.⁵ There are studies that suggest there is an inverse relationship between Se supplementation and cancer risk.⁶ Organoselenium compounds have been shown to hinder the initiation and post-initiation phases of chemical carcinogenesis, as well as displaying antitumorigenic, antiangiogenic and pro-oxidant activity in cancer cells.^{6,7,8} As such it would be unwise to dismiss the therapeutic potential of organoselenium compound.

There are distinct chemical differences between sulfur and selenium which aids this research. Mainly, selenium is larger in size than sulfur which results in more loosely bound outer valence electrons, which is significant to the antioxidant properties of selenium. Also, as a result of its size, selenium is more polarizable (or “softer”) than sulfur, resulting in greater nucleophilic power, which is beneficial for selenium to coordinate to metal centers.⁹ These unique chemical properties of Se are closely related to the pharmacological activities and physicochemical properties of organoselenium compounds. As such, understanding this relationship is of vital importance for the rational design, research, and development of my novel NNSeN molecules.

The NNSeN project started in 2019 and we have taken off running since. Two motifs were targeted: pyrazolyl-selenoureas and pyrazolyl-carboselenamides, also coined extended NNSeN's and conventional NNSeN's respectively. Although it was attempted to generate the NNSeNs from the NNSN isomers via nucleophilic displacement reactions, it was ultimately found that the optimal route would be through the coupling of pyrazoles and isoselenocyanates (ISCs). ISCs are highly versatile starting materials for the introduction of selenium. ISCs were ultimately synthesized by the formylation of amines, and then subsequent dehydration of these formylated amines to generate their isocyanides *in situ* and finally nucleophilic attack of selenium to generate the phenyl ISC library. With the ISCs in hand, pyrazoles were either purchased commercially or synthesized using classical methods such as the Knorr pyrazole synthesis. With both starting materials in hand the NNSeNs were pursued.

The synthetic adventure towards the NNSeN motif revealed that many methods used to synthesize the isosteric NNSNs would not be optimal for the NNSeNs. We were able to conduct solvent optimization experiments which lead to the NNSeNs most reliable method: dry chloroform under argon at reflux. This synthetic adventure also led to the discovery that the pyrazolyl

carboselenoamide NNSeNs are much more stable than the pyrazolyl selenourea NNSeNs. Interestingly, for the carboselenoamide NNSeNs we found that monosubstituted pyrazoles lead to stable products, with the exception of the NNSeNs synthesized from ethyl 3-amino-pyrazole-4-carboxylate. For the selenourea NNSeNs, it was observed that overall stability had a structural trend with the pyrazoles and possibly its aromaticity. Any electron donating or inductive donating (methyl, t-butyl) groups on the pyrazole, led towards the instability of the selenourea NNSeNs and generating of red selenium. Any electron withdrawing (esters) groups on the pyrazole, regardless of the position, led towards the synthesis of stable selenourea NNSeNs.

In my time here since starting this project, I solely explored and optimized the synthesis of ISC, synthesized and learned over the reactivity of pyrazoles, and solely explored and optimized the coupling conditions between pyrazoles and ISCs for a total of 41 novel and stable NNSeNs. The NNSeNs underwent computer modeling studies and gave insight towards the most stable geometric orientations both with or without copper complexation as well as insight towards geometric orientation during continuum solvation of both water and chloroform with or without copper complexation.¹⁰ The modeling studies revealed that our original hypothesis where the iminium cation formation of our NNSeNs would aid in generating a copper bound iminium cation like motif to be incorrect. It was found, for the carboselenoamide NNSeN, that the lowest energy copper (I) complex isomers involved copper chelating to the selenium atom as well as the ortho carbons of the phenyl ring. This was also true when considering continuum solvation with chloroform. However, when considering continuum solvation with water, the lowest energy copper (I) complex became the isomer where the copper is chelating to the selenium atom and the 2nd position nitrogen of the pyrazole ring. For the selenourea NNSeN, although this was found to be unstable during synthesis, it was discovered that the most stable copper (I) complexation was

the isomer where copper is chelating to the selenium and the 2nd position nitrogen of the pyrazole at the same time that the selenium atom is interacting with the hydrogen atom of the alpha phenyl amine. This is actually an expected phenomenon due to selenium ability to form longer Se-H bonds, and likely due to selenium's ability to access low lying d-orbitals. Copper binding studies were done with select NNSeNs and resulted in a much higher binding constants than the NNSN isomers, due to selenium's ability to form stronger bonds with copper (I).

Lastly, preliminary biological studies done using select NNSeNs revealed that all the isomers have at least some activities against *S. aureus* strain Lac MRSA with copper in the range of concentrations tested (0.018 – 10 uM) via dose response curves and drop assay, with 4 NNSeNs being the most active with an MIC of 0.6 uM.

Future Work

1. Attempt more EWGs on the pyrazolyl selenourea NNSeNs to further investigate whether the electron withdrawing group is aiding the stability of these NNSeNs via disruption of the pyrazole aromaticity.
2. Increase the size of the NNSeN library by expanding the functional groups on both the pyrazole and phenyl rings to further understand the stability of our NNSeNs and to further understand importance of the substituents. This will require an extensive ISC library and extensive pyrazole library.
3. Subject all current NNSeNs for biological studies. It was very disappointing that the COVID-19 pandemic interrupted this, and it would be very interesting to see if we can begin structure-activity relationship (SAR) studies with our current NNSeN library.

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Chapter 6: Other projects

A Catalyst-Free, Temperature-Driven One-Pot Synthesis of 1-Adamantylhydrazine

Hydrochloride¹

In an effort to expand the NNSN library, the Bossmann research group sought to incorporate the adamantyl motif on NNSNs through the pyrazole motif. This due to various adamantyl derivatives showing a very broad spectrum of medicinal properties such as antiviral,¹ antimicrobial,³ anticancer,⁴ antidiabetic,⁵ and much more.⁶ Introduction of the adamantyl functionality to medicinal compounds can augment therapeutic effects by increasing the overall effective transportation through biological membranes as a result of enhanced lipophilicity.⁷ We envisioned being able to access the pyrazole target product 1-(adamantan-1-yl)-1H-pyrazol-3-amine by using a classical approach in which 1-bromoadamantane and 3-aminopyrazole were reacted in 48% aqueous hydrogen bromide (Figure 54).⁸

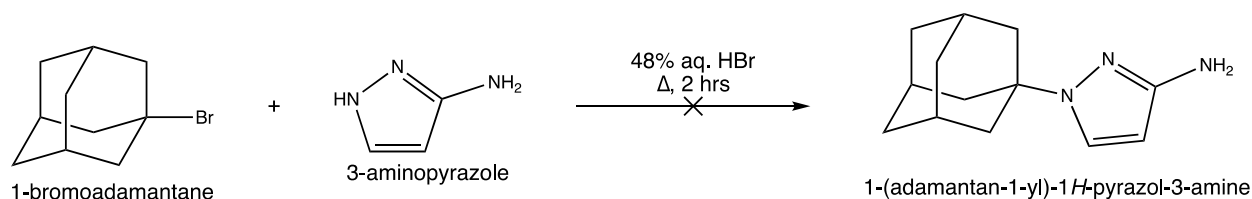


Figure 54: Initial efforts to synthesize 1-(adamantan-1-yl)-1H-pyrazol-3-amine

However, we found that these reaction conditions were problematic in generating the desired product and the use of corrosive HBr was making any work up attempts futile. As such, we sought to explore different reaction conditions. This led to the realization that feasible methodology has not been made available in literature. As such, we developed a modified methodology initially produced by Thomas and Shetty, in which they synthesized 1-adamantylhydrazine via the reaction of a halogenated adamantane with anhydrous hydrazine under a continuously supplied inert atmosphere and sustained heating.⁹ An important drawback of this

patent is that the compounds were identified by means of their melting points only. When we reproduced the original procedure, drawbacks were encountered such as the necessity of using anhydrous hydrogen chloride and ineffective drying methods such as the drying *in vacuo* over

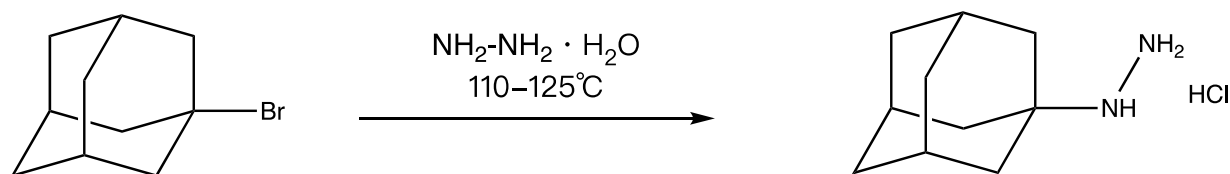


Figure 55: Synthesis of 1-adamantylhydrazine hydrochloride salt

phosphorus pentoxide desiccant and solid sodium hydroxide. Most importantly, we always obtained mixtures of the target compounds and multiple byproducts. As such, we modified the procedure to obtain a significantly higher yield of 1-adamantylhydrazine. This was done by reacting 1-bromoadamantane in excess amount of hydrazine monohydrate utilizing moderate stirring and heating to reflux, $110\text{--}125^\circ\text{C}$, for 8-12 hours. The product was isolated post work up by lowering the pH to 2.0, which generated 1-adamantylhydrazine hydrochloride salt solid, a more stable product than 1-adamantylhydrazine (Figure 55).

We then used this product to subsequently synthesize 1-(adamantan-1-yl)-1H-pyrazol-3-amine using 2-chloroacrylonitrile in a 1:2 ratio of potassium carbonate and sodium bicarbonate in distilled water at room temperature for overnight. This was a previously published protocol however we slightly modified the workup¹⁰: with the aid of James Hodgson, the senior scientific glassblower in the KSU department of chemistry, a continuous-extraction apparatus was designed and aided in the further isolation of the target product. This was necessary since the pyrazole product, and all pyrazoles, show considerable hydrophilic behavior. We were able to isolate the product in a 43% yield (Figure 56).

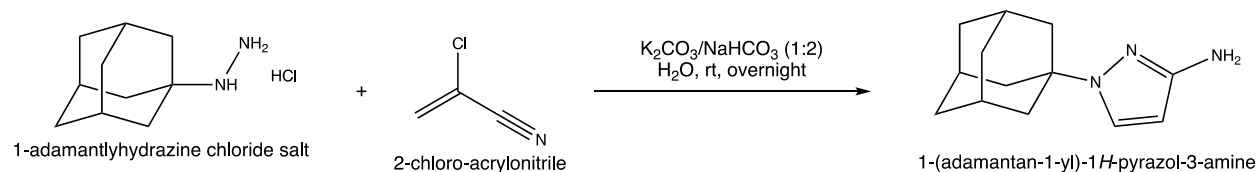


Figure 56: Synthesis of desired product 1-(adamantan-1-yl)-1H-pyrazol-3-amine

This project was initially started with my efforts to synthesize 1-(adamantan-1-yl)-1H-pyrazol-3-amine but then was done alongside Dr. Anjana D. Archarge, leading to a publication in *Thieme-Synthesis*.¹

Experimental Section: 1-Bromoadamantane (purity 99%) and hydrazine monohydrate (purity 98%) were purchased from Alfa Aesar. 2-Chloroacrylonitrile (99%, stabilized) was purchased from Acros Organics. Diethyl ether 99%, ethyl acetate (Certified ACS), potassium carbonate (anhydrous) 99%, sodium bicarbonate (Powder/Certified ACS), and hydrochloric acid (12.1 N, certified ACS Plus) were purchased from Fisher Scientific International, Inc., and used as received without further purifications. In-house distilled water was used as the solvent for the pyrazole cyclization reaction. ¹H (400, 600 MHz) and ¹³C (100, 151 MHz) spectra were obtained by using Bruker-Ascend NMR spectrometers. Mass spectra were obtained on a Waters Acquity UPLC TQD spectrometer. The UPLC column was CORTECS C18, 90 Å, 2.7 µm reversed phase. Melting points were acquired by using a Mel-Temp 1001D instrument.

1-adamantylhydrazine hydrochloride (CAS: 16782-39-1): Since 1-bromoadamantane is moisture-sensitive, light-sensitive, and air-sensitive, it was flushed with argon gas before measuring and then added to a reaction vessel. If the reaction continues for a prolonged period, it is necessary to cover the reaction vessel with a light- blocking material such as aluminum foil. Ivory-colored 1-bromoadamantane (5 g, 23.24 mmol) was weighed on weighing paper and transferred to a 500 mL, one-necked, round-bottom flask with a magnetic stirring bar by using a powder funnel. Then the round-bottom flask was charged with an excess amount of hydrazine

monohydrate (50 mL, 51.6 g, 1.03 mol), which acted as the solvent and reactant. 1-Bromoadamantane did not dissolve in hydrazine at this point and a dispersion occurred in the reaction vessel. It is noteworthy that vigorous stirring was not required as it pushed the solid 1-bromoadamantane above the solvent level. Therefore, moderate stirring was crucial to keep all the solids within the solvent. The round-bottom flask was connected to a coiled reflux condenser which was connected to continuous water flow. A mineral oil bath was utilized to reflux the reaction mixture at 125–135 °C. Inside the reaction vessel, the temperature was set to 110 °C. The reflux temperature was crucial, because if the reaction mixture refluxed below 100 °C, it delivered both the product and unreacted 1-bromoadamantyl. The mixture was kept at that temperature for 8–12 hours. During reflux, 1-bromoadamantane completely dissolved in hydrazine monohydrate and the reaction mixture eventually consisted of a very clear light-yellow colored solution. TLC was performed (EtOAc/hexane 1:1). Spots were visualized with phosphomolybdic acid (PMA), as 1-bromoadamantane is not visible under the short wavelength of UV. The starting material 1-bromoadamantane created a dark spot on the light green background ($R_f = 0.90$), whereas the product spot was not significantly visible as a dark spot, which indirectly confirmed the formation of at least one new product.

The reaction mixture was cooled to room temperature, followed by the addition of 45% (w/w) aq. KOH solution (50 mL). When the mixture cooled, the clear solution turned cloudy due to the adamantyl product featuring low solubility in hydrazine monohydrate. Further addition of 45% (w/w) aq. KOH solution (125 mL) facilitated the separation of excessive hydrazine from the crude 1-adamantylhydrazine. At this point, the previously cloudy reaction mixture turned into an opaque dispersion. It was transferred to a separatory funnel and extracted with Et₂O (3 × 50 mL). Due to the hydrophobic adamantyl moiety, the product could be found in the organic layer (Et₂O). The

organic layer was dried over anhydrous sodium sulfate. Upon the addition of anhydrous sodium sulfate, the product solution turned clear. The product solution was then concentrated under vacuum to approximately a quarter of the solvent volume. Then the concentrated reaction mixture was charged with concentrated hydrochloric acid (3–5 mL), lowering the pH to 2.0, to obtain the 1-adamantylhydrazine hydrochloride salt. Upon the addition of hydrochloric acid, a white solid product crashed out of the Et₂O solution. The solid product was isolated via gravity-driven filtration and dried under high vacuum. Original literature stated to conduct recrystallization of this white solid using 2-propanol. Although this purification step was reproduced, it led to considerable loss of product due to solubility of the reaction product in 2-propanol.⁹ Without recrystallization, the yield was >88% (4.13 g) of 1-adamantylhydrazine hydrochloride. After recrystallization, it was diminished to ~53%. It should be noted that the reaction product was pure according to NMR and TLC characterization before and after recrystallization from 2-propanol. NMR samples were prepared for 1-adamantylhydrazine hydrochloride in DMSO-d₆. The product was also analyzed by means of UPLC-MS, which clearly indicated the presence of 1-adamantylhydrazine with a molecular peak of 167.15 mass units. The melting point observed (216–220 °C) did not fit with the literature value (250– 253 °C⁹). However, the original literature only utilized melting point analysis for product characterization.⁹

Mp 216–220 °C.

IR: 2891 (w,s), 2709 (w,s), 2549 (m,b), 1480 (m,s), 1085 (m,s) cm⁻¹.

¹H NMR (400 MHz, DMSO-d₆): δ = 2.11 (s, 3 H), 1.78 (s, 6 H), 1.59 (m, 6 H).

¹³C NMR (101 MHz, DMSO-d₆): δ = 45.72, 37.05, 35.88, 30.50, 28.65.

MS: m/z = 167.15 [M + 1].

1-(adamantan-1-yl)-1H-pyrazol-3-amine (CAS: 128315-61-7): 2-Chloroacrylonitrile (202.4 mg, 2.31 mmol) was measured into a 150 mL round-bottom flask and dispersed with distilled water (5 mL). Separately, K₂CO₃ (168 mg, 1.21 mmol) and NaHCO₃ (204 mg, 2.48 mmol) were dissolved in distilled water (5–7 mL) and mixed, followed by the addition to the 2-chloroacrylonitrile solution. Then 1-adamantylhydrazine hydrochloride salt (2.31 mmol, 466.0 mg) was dissolved in distilled water (15 mL) and transferred to the original mixture, which was stirred continuously overnight. According to the original protocol, the product was meant to be extracted with EtOAc (3×). However, from previous experience, it was both convenient and effective to perform a continuous extraction to collect the desired product from the aqueous layer, as pyrazole derivatives tend to show considerable hydrophilic behavior. Finally, the organic layer was dried under vacuum and purified via flash column chromatography (EtOAc/hexane 5:95 to 25:75); this gave a thick dark orange oil-like liquid.

Yield: 216 mg (43%).

IR: 3318.16 (w,b), 2904 (m, s), 2849 (m,s), 1683 (w,s), 1542 (w.s), 743 (m,s) cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ = 7.47 (d, J = 14.3 Hz, 1 H), 6.36 (d, J = 14.3 Hz, 1 H), 6.06 (s, 1 H), 2.23 (m, 3 H), 1.77 (m, 6 H), 1.28 (s, 6 H).

¹³C NMR (101 MHz, CDCl₃): δ = 160.39, 146.58, 106.20, 42.73, 40.20, 36.32, 29.55.

All spectrums can be found in the supporting information at DOI: 10.1055/s-0040-1707353.¹

Optimized synthesis of 4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid) (TCPP)

What initially began as a COVID-19 diagnostic project, developed into a nanoparticle-based nanosensor patent for the rapid identification of lung conditions.¹¹ We developed the nanoparticle-based nanosensor by linking supramolecular recognition sequences, protease consensus sequences, post-translationally modifiable sequences, or sterically hindered benzyl ether bonds for specific interaction with a biological marker, and methods for rapid diagnosis of lung conditions using specified panels of target biomarkers.¹¹ My role in this project was to focus on the synthesis of 4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid), or TCPP (Figure 57).

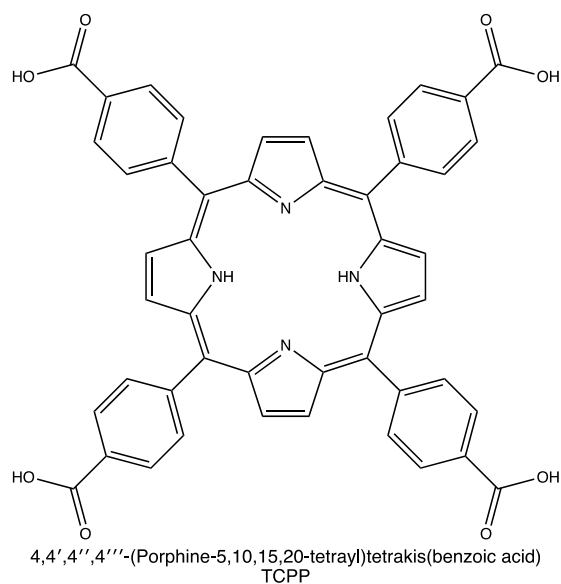


Figure 57: TCPP structure

It is important to note that TCPP is commercially available, however it is very expensive if large quantities are required (\$237 for 1g, Sigma). As such this synthetic endeavor began using reaction conditions previously used for a different nanoparticle project, but slightly modified.¹² This was initially done using pyrrole and 4-carboxybenzaldehyde in a 1: 1.04 molar ratio while using propionic acid as solvent while heating to reflux (141°C) for 30 minutes.

This reaction procedure is as follows: To a refluxing propionic acid is added 4-carboxybenzaldehyde and pyrrole. The solution is observed to go from a clear color to a dark violet color. The mixture is stirred and left to reflux for 30 minutes. After this time, the mixture is allowed to cool to room temperature and 200 proof ethanol is added to change the polarity of the solution. This is then left in the fridge for 2 days to allow the product to precipitate. After 2 days this is filtered to isolate solid product. This is washed with ethanol twice and washed with distilled water four times. The product is then isolated and freeze-dried.

Although these reaction conditions did generate the product, the product yield was 15%. The low yield could be attributed to one of all of the following reasons: 1) the 30 minute reaction time was not long enough, 2) addition of ethanol to change the polarity of the solution may be unnecessary since ethanol will likely dissolve the product, and/or 3) work up wash using ethanol and distilled water may not be optimal as it was dissolving the product (observed as the filtrate was purple). As such, a different method was needed.

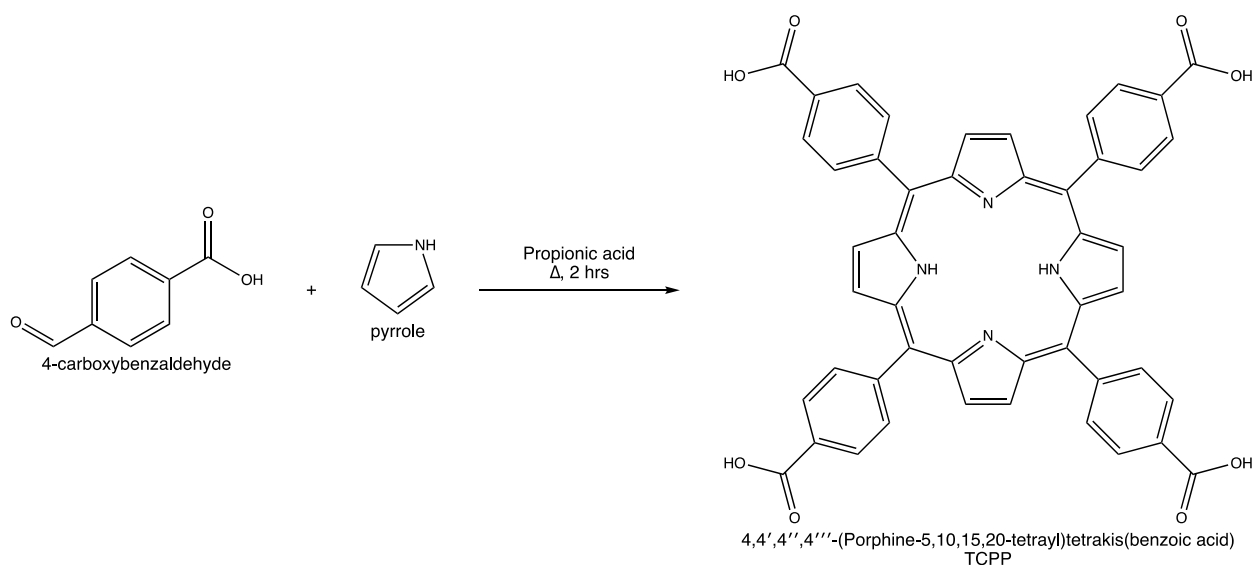


Figure 58: TCPP Synthesis

Using the same solvent and reactants, we attempted a new method with a different procedure (Figure 58).¹³ This reaction procedure is as follow: to a round bottom flask is 4-

carboxybenzaldehyde (10 mmol) and 50mL of propionic acid added at room temperature while stirring. Once added, temperature is then raised to $\geq 80^{\circ}\text{C}$ in order to increase dissolution of 4-carboxybenzaldehyde. Once the aldehyde (fully) dissolves, pyrrole (10mmol) is then added to the mixture while stirring. This underwent a color change from clear to yellow to dark violet. This is then heated to reflux (141°C) while stirring and continued to reflux for 2 additional hours. After 2 hours, the reaction mixture is allowed to cool to room temperature and is then placed in the freezer overnight to in the precipitation of product. After this, the reaction mixture is subjected to vacuum filtration to isolate the dark purple solid product. This is then washed with 5x50mL of DCM, 1x10mL of methanol and then dried via freeze-drying. This method was repeated multiple times and all attempts resulted in an increase of the reaction yield ranging from 29-39%. This method is optimal as it does give better control of the reaction by allowing the aldehyde starting material to fully dissolve before addition of pyrrole. However, it was observed that the use of methanol in the work up did dissolve the product as the filtrate turned into a deep purple color. This was also further confirmed by shining the filtrate under UV light (365 nm) and revealed the characteristic hot-pink emission of TCPP. As such, the work up procedure was modified by removing the methanol wash and only washing this with DCM. This resulted in the largest increase of yield to 50%. This resulted in our optimized method for synthesizing TCPP in a 1.5g scale.

TCPP was characterized by observing the emission of the dye by dissolving this in methanol and exposing this to a UV Lamp at 365nm (Figure 59) and ^1H -NMR using DMSO- d_6 (2.94ppm (2H, s, -NH), 8.38ppm (16H, dd, aromatic), 8.87ppm (8H, s, pyrrole-beta H), 13.3ppm (4H, broad s, -COOH)) (Figure 60).



Figure 59: TCP in methanol under UV lamp (365nm)

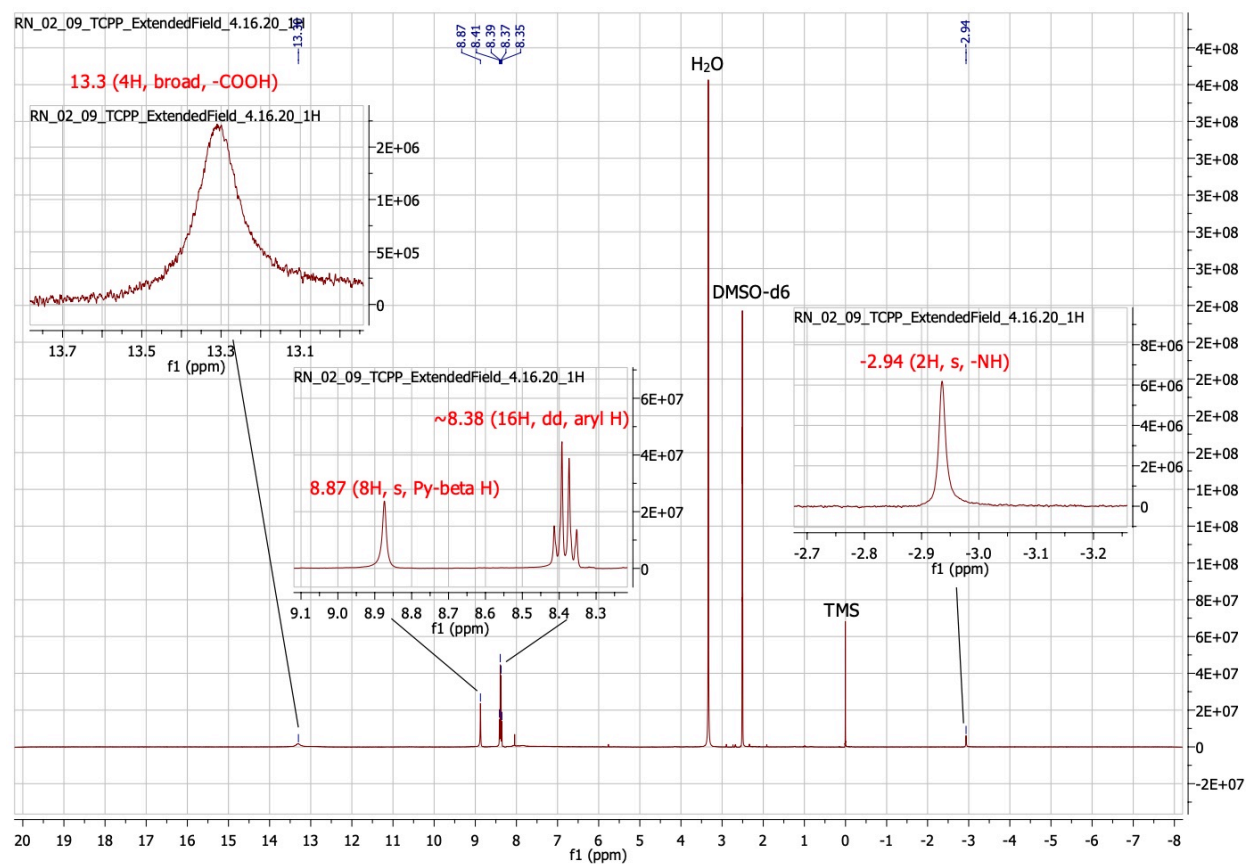


Figure 60: TCPP ¹H-NMR

Oxidation of Graphene to Graphene Oxide via Fenton Oxidation: Upscaling experiments

Graphene (G) is a honeycomb sheet of carbon atoms that is one atom-thick, and it is the building block for other materials (e.g. graphite, carbon nanotubes, and fullerene).^{14,15} The reason graphene was considered the wonder material of the 21st century was due to its remarkably unique properties, including excellent electrical and thermal conductivity, optical transparency and mechanical strength.¹⁵ Our Graphene Oxide (GO) oxidation project was started by Jose Covarrubias PhD in which he was able to oxidize high-quality explosion synthesized few-layer graphene by means of Fenton oxidation. Additionally, he successfully scaled up this oxidation reaction from 1.0 g batch to 200g per batch maintaining all of graphene's extraordinary properties intact because only the surface layers of few-layer graphene get oxidized during Fenton oxidation.¹⁶ When I was given this project, the goal was to push this upscaling past 200g using the optimized conditions recently established. This project was done in collaboration with Dr. Sorensen in the Department of Physics at KSU and HydroGraph Clean Power Inc. as they provided their explosion-synthesized graphene starting material.¹⁷

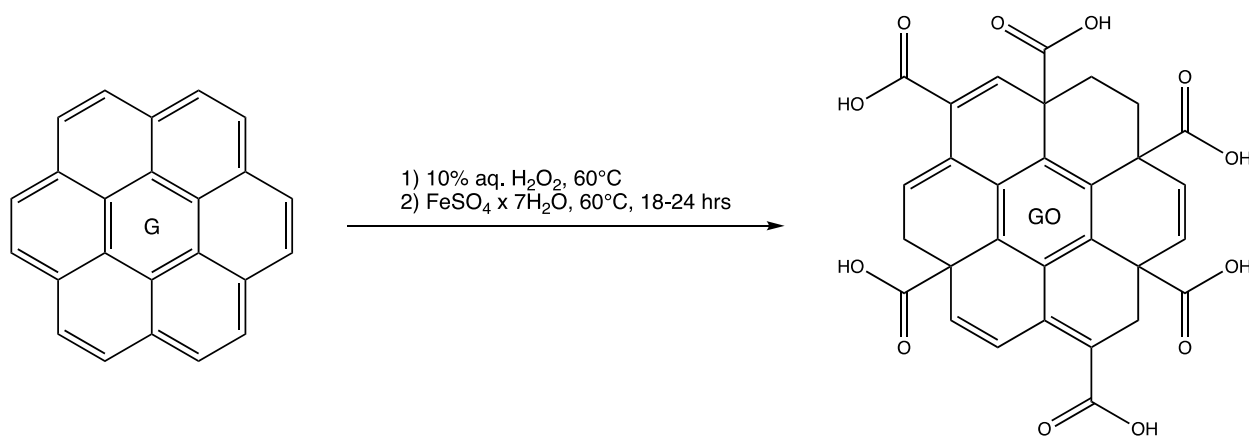


Figure 61: Fenton Oxidation of G to GO

We started this upscaling gradually pushing the first upscale experiment on a 250g scale, where the reaction procedure is as follows (Figure 61): A 12L three-neck round bottom flask is placed in a 12L heating mantle which is connected to a 120 VAC Dual Power Controller. The Dual Power Controller is turned on (both Knob Setting % set at 25% power). Then an overhead mechanical stirrer fitted with a glass stirring rod and a glass stir blade is slid into the middle neck of the round bottom flask and capped with a threaded glass adaptor to allow fixed stirring. A reflux condenser is then placed on one of the side necks of the round bottom flask and tap water is streamed through the condenser. The remaining side neck is left uncapped to add G and solvent. To the heating round bottom flask is 250g of G added and 2500mL of 10% Hydrogen Peroxide (H_2O_2) (pH=3, adjusted by concentrated sulfuric acid (H_2SO_4)). This is added, while stirring, in 5 portions along with equal portions of 10% Hydrogen Peroxide (H_2O_2) (pH = 3), i.e., 50g of G and 50mL of 10% H_2O_2 . After the addition of the 5th portion, the remaining amount of solvent is added, i.e., 2250mL of 10% H_2O_2 . After the addition of G and solvent, the side neck is capped and fitted with a digital thermometer. The mixture is stirred vigorously and heated to 60°C. Once the temperature reaches 60°C, the side neck holding the digital thermometer is uncapped. At this point, 3.125g of ferrous sulfate heptahydrate ($\text{FeSO}_4 \times 7\text{H}_2\text{O}$) is added through the side neck and immediately capped again. This addition generates an exothermic process and will cause the temperature of the reaction to temporarily spike (Temperature $\leq 95^\circ\text{C}$). After this temperature spike, the reaction temperature slowly returns to 60°C and is left to stir overnight (18-24hrs). After the reaction mixture was left to stir overnight, the reaction mixture is filtered to isolate GO. This is washed with ~250mL of distilled water (equal to 10% of total volume of solvent used in reaction*). After filtration and water wash the GO is then placed onto a glass refractory and dried in an oven at 70°C for 1-2 days.

Using the reaction procedure prescribed above, we were able to further upscale this oxidation to a 400g scale. While this oxidation was generating the desired GO product, a large amount of solvent was required. This is attributed to graphene's hydrophobicity. Because graphene is such a light and fluffy material, 400g would take up at least half of the 12L round bottom flask, and 4L of solvent was required in order to oxidize the entire material. However, it was observed during these oxidations that over the course of time the graphene solid would slowly disperse into the aqueous solvent. This is likely due the generation of the Fenton reagent and oxidizing the G to GO. In comparison, GO is much more hydrophilic and is easier to disperse in water or the aqueous solvent. Thus, it was proposed that the use of a surfactant to disperse the G starting material would aid in using a smaller volume of solvent. As such, triethylene glycol dimethyl ether (TGDE) was selected as it would be inert to the reaction conditions. This would be initially employed using 1g of TGDE per 1L of solvent. We reproduced a 400g oxidation using TGDE and slightly modifying the procedure: To the round bottom flask is 400g of G added followed by 5g of ferrous sulfate heptahydrate ($\text{FeSO}_4 \times 7\text{H}_2\text{O}$) and 4057 μL of triethyl glycol dimethyl ether (TGDE). This is added while stirring at room temperature. Once all starting materials are added, 4000mL of 10% Hydrogen Peroxide (H_2O_2) (pH=3, adjusted by concentrated sulfuric acid (H_2SO_4)) solvent is added to the mixture at room temperature while stirring. After the addition of solvent, the side neck is capped and fitted with a digital thermometer. The mixture is stirred vigorously and heated to 60°C. During heating an exothermic process will occur and will cause the temperature of the reaction to slowly rise until it spikes (Temperature spike $\leq 90^\circ\text{C}$). After this temperature spike, the reaction temperature slowly returns to 60°C and is left to stir overnight (18-24hrs).

We found that the addition of graphene, FeSO_4 and triethylene glycol to be unproblematic and were unreactive towards each other. This mixture was observed to be much more dispersed when compared to previous oxidations done, due to the surfactant. This surfactant proved useful, and we were able to reproduce a 400g oxidation, however 4L of solvent was still required. It is likely a higher amount of surfactant was needed. We decided to repeat this 400g scale by using 10mL of TGDE and by adding the G in portions along with portions of solvent. We were able to successfully oxidize the G as well as successfully minimize the amount of solvent nearly two-fold. With this in hand we began to push the upscale. The next issue we came across was the amount of space the G starting material took up in the round bottom flask. Our goal was to reach a 1kg scale, however 400-500g of G would take up more than 50% of the round bottom flask. As such, we decided to change the procedure one last time. Because it was obvious that hydrophobic G would disperse in the aqueous solvent with the aid of the surfactant, we then investigated ways we could fully disperse G before the additions of oxidants. The Fenton reagent will not generate until there is a presence of both hydrogen peroxide and a ferrous ion. We did find that the mixture of graphene, FeSO_4 and triethylene glycol to be inert towards each other so this mixture could still be done, however we decided to change the percentage of hydrogen peroxide from 10% to 30% aqueous solvent and use a mixture of distilled water with surfactant to try and disperse all inert starting materials before the addition of hydrogen peroxide.

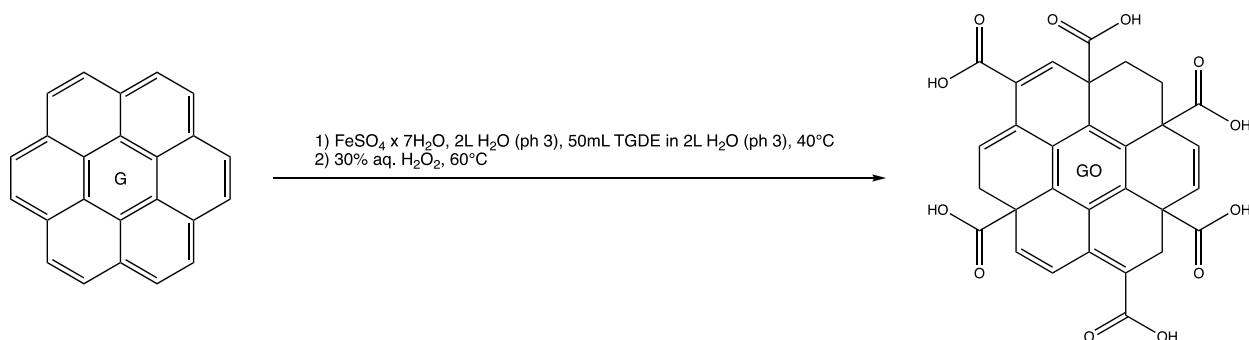


Figure 62: 700g Upscale Fenton Oxidation

We boldly attempted this new method in a 700g scale using the same reaction set up but with this new procedure as follow (Figure 61): 2 separate 2L solutions of H_2O at pH 3 (adjusted using concentrated sulfuric acid via dropwise) need to be made. To one of these is 50mL of triethylene glycol dimethyl ether (TGDE) added. The other is just H_2O at acidic pH. Then, to the 12L round bottom flask is 500g of G added followed by 15g of ferrous sulfate heptahydrate ($\text{FeSO}_4 \times 7\text{H}_2\text{O}$) and the 4000mL of H_2O (pH=3, 2L contains DTGE and 2L contains just H_2O). This is added while stirring and heating to 40°C. Once this mixture is dispersed (approx. after 15 min), 60g of G is added to the mixture. After 10 additional minutes, another 60g of G is added to the mixture. This is to be repeated until a total of 700g of G is dispersed. After the dispersion of G is complete, a total of 1500 mL (1.5L) of 30% H_2O_2 is added in portions: 250mL of 30% H_2O_2 at 0min, 250 mL of H_2O_2 at 30min, 500 mL H_2O_2 at 60 min, and 500mL H_2O_2 at 120min. The side neck is capped and fitted with a digital thermometer. The mixture is stirred vigorously. Increased heating may be observed because of the ongoing Fenton oxidation. During heating and additions of 30% H_2O_2 exothermic process will occur and will cause the temperature of the reaction to slowly rise until it spikes (Temperature spike $\leq 90^\circ\text{C}$). After this temperature spike, the reaction temperature will run at approximately 80°C for several hours and slowly return to $< 60^\circ\text{C}$. This is then left to stir overnight (18-24hrs).

This new method was successful in oxidizing all G to GO. This project was started using 200g scales and has since then been modified multiple times to reach a 700g scale. Currently upscaling is being continued by our collaborators in the physics department and HydroGraph Clean Power Inc.

Chapter 6 References

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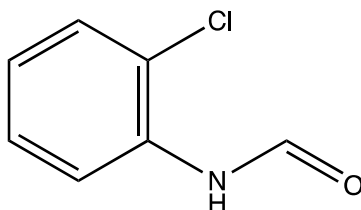
Appendix A

Experimental Section

Unless otherwise noted, all commercial reagents and solvent were purchased with the highest purity grade from Fisher Scientific Inc or Sigma-Aldrich. All solvents used were purchased in highest purity and/or anhydrous conditions. Solvents were dried by using activated 3Å molecular sieves or silica. Silica gel (200–300 meshes) was used for column chromatography, and TLC inspections were taken on Silica XHL TLC Plates, w/UV254, glass backed, 250um. All the reagents and products were stored under Argon atmosphere. The synthesized compounds used towards successful NNSeN synthesis were stored in a fridge or freezer and all NNSeN products were stored in a -20°C freezer. All of these were characterized by means of ^1H (400, 600 MHz) and/or ^{13}C (100, 151 MHz) spectra obtained on Bruker-Ascend spectrometers.

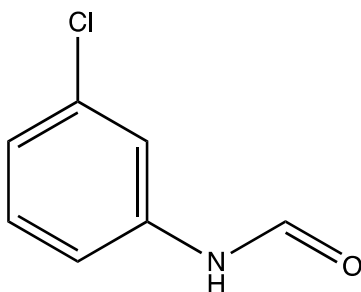
Synthesis of Aromatic Formamides via Formylation of Amines

N-(2-chlorophenyl) formamide, n-(3-chlorophenyl) formamide, n-(3,4-dichlorophenyl) formamide, n-(2,3-dichlorophenyl) formamide, n-(2,4-dichlorophenyl) formamide, n-(4-bromophenyl) formamide, n-(3,5-dibromophenyl) formamide and n-(4-fluorophenyl) formamide were synthesized with the following conditions: to a dry round bottom flask is the respective aniline (1 eq) added and stirred. To this is added formic acid (3 eq) carefully and stirred. At this point, the mixture is stirred vigorously. Then polyethylene glycol 400 (PEG-400, catalyst) is added to the reaction mixture. After this addition the reaction flask is sealed, and the mixture is stirred vigorously for time. Each product had a different reaction time either due to TLC monitoring (50:50 ethyl acetate: hexane) or solidification of the reaction mixture. After reaction time or solidification, the mixture is then diluted or dissolved with water and the product is extracted using multiple washes of ethyl acetate. The organic layer is then isolated and further washed with saturated sodium bicarbonate and brine and is then dried over sodium sulfate. This is then filtered and concentrated in vacuo. Purification can be done in three ways: 1) silica column chromatography using ethyl acetate: hexane gradient, 2) allowing the flask to stand in ethyl acetate (either at room temperature or in the fridge) before concentration in vacuo and allow the product to precipitate or 3) recrystallization. Nearly all formamide products contain a conformational isomer (i.e., rotamer) due to rotation between the N-C bond of the formamide.



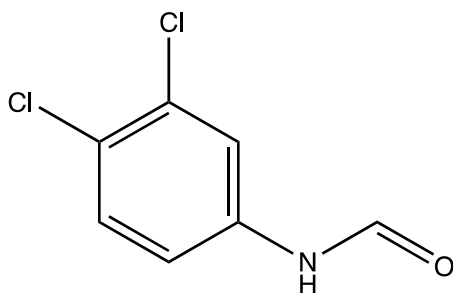
N-(2-chlorophenyl)formamide

N-(2-chlorophenyl) formamide: 7.84 mmol (1g scale), 78% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.75 (d, 1H, $J=11.2$ Hz, *trans*), δ 8.53 (brs, 1H, *trans*), δ 8.42 (dd, 1H, $J=8.2$ Hz, *cis*), δ 8.50 (brs, 1H, *cis*), 7.06-7.45 (m, 4H, Ar-H).



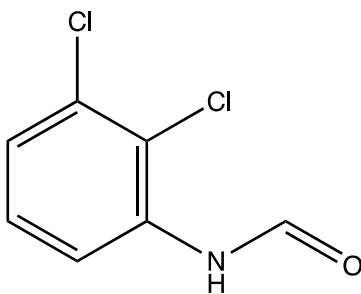
N-(3-chlorophenyl)formamide

N-(3-chlorophenyl) formamide: 7.84 mmol (1g scale), 51% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.69 (d, 1H, 11.3 Hz, *trans*), δ 8.39 (d, 1H, $J=1.4$ Hz, *trans*) δ 7.74 (brs, 1H, *cis*) δ 7.67 (t, 1H, $J=2.0$ Hz, *cis*) δ 6.96-7.40 (m, 4H, Ar-H)



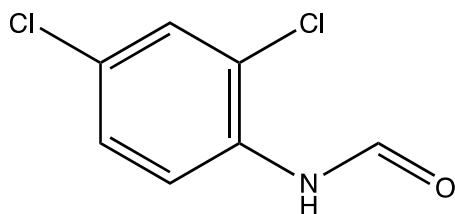
N-(3,4-dichlorophenyl)formamide

N-(3,4-dichlorophenyl) formamide: 6.17 mmol (1g scale), 32% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.65 (d, 1H, $J=11.2$ Hz, *trans*) δ 8.38 (d, 1H, $J=1.5$ Hz, *trans*) δ 7.79 (d, 1H, $J=2.3$ Hz, *cis*) δ 7.61 (brs, 1H, *cis*) δ 6.92-7.45 (m, 3H, Ar-H)



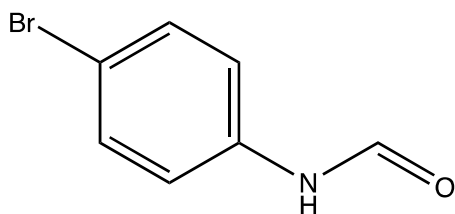
N-(2,3-dichlorophenyl)formamide

N-(2,3-dichlorophenyl) formamide: 6.17 mmol (1g scale), 62% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.74 (d, 1H, $J=10.8$ Hz, *trans*) δ 8.52 (s, 1H, *trans*) δ 8.38 (dd, 1H, $J=2.8$ Hz, *cis*) δ 7.73 (brs, 1H, *cis*) δ 7.21-7.32 (m, 3H, Ar-H)



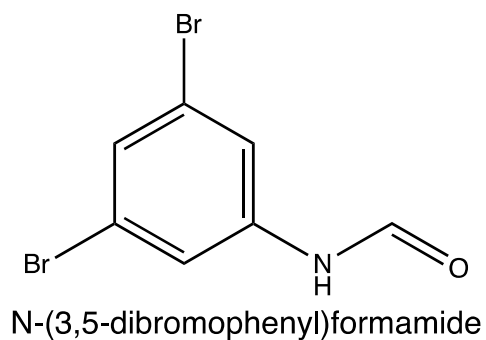
N-(2,4-dichlorophenyl)formamide

N-(2,4-dichlorophenyl) formamide: 6.17 mmol (1g scale), 60% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.68 (d, 1H, $J=11.1$ Hz, *trans*) δ 8.49 (s, 1H, *trans*) δ 8.40 (d, 1H, $J=8.9$ Hz, *cis*) δ 7.61 (brs, 1H, *cis*) δ 7.21-7.46 (m, 3H, Ar-H)

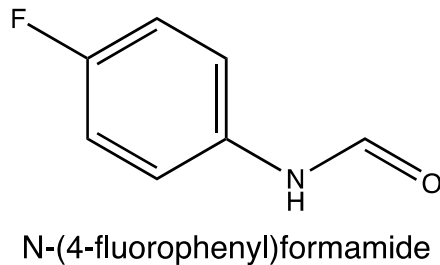


N-(4-bromophenyl)formamide

N-(4-bromophenyl) formamide: 1.45 mmol (250mg scale), 71% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.65 (d, 1H, $J=11.4$ Hz, *trans*) δ 8.39 (s, 1H, *trans*) δ 7.45 (s, 1H, *cis*) δ 7.11 (brs, 1H, *cis*) δ 7.48-7.52 (m, 2H, Ar-H), δ 6.95-7.00 (m, 2H, Ar-H)

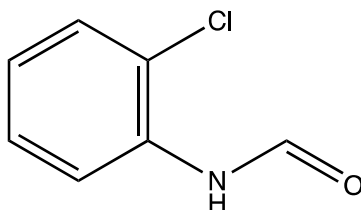


N-(3,5-dibromophenyl) formamide: 1 mmol (250mg scale), 60% yield. $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ 10.50 (s, 1H, *trans*) δ 10.30 (d, 1H, $J=10.6$ Hz, *trans*) δ 8.88 (d, 1H, $J=10.6$ Hz, *cis*) δ 8.32 (s, 1H, *cis*) δ 7.82 (d, 2H, Ar-H), δ 7.47-7.54 (m, 1H, Ar-H)



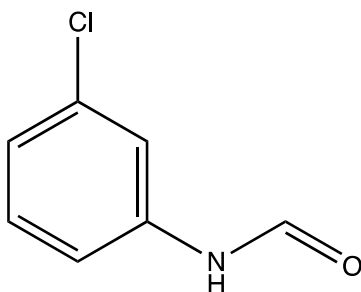
N-(4-fluorophenyl) formamide: 9 mmol (1g scale), 35% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.56 (d, 1H, $J=11.4$ Hz, *trans*) δ 8.37 (d, 1H, $J=1.4$ Hz, *trans*) δ 7.63 (brs, 1H, *cis*) δ 7.15 (brs, 1H, *cis*) δ 7.48-7.53 (m, 2H, Ar-H), δ 7.05-7.08 (m, 2H, Ar-H)

N-(2-chlorophenyl) formamide, n-(3-chlorophenyl) formamide, n-(4-chlorophenyl) formamide, n-(3,4-dichlorophenyl) formamide, n-(2,3-dichlorophenyl) formamide and n-(2,4-dichlorophenyl) formamide were synthesized with the following conditions: to a dry round bottom flask or vial is the respective aniline (1 eq) added. The reaction vessel is then fitted onto a water bath sonicator at room temperature. To this is added formic acid (2.5 eq) carefully. An exothermic reaction may be observed. At this point the reaction is sealed with a rubber stopper fitted with a needle to vent the reaction. The sonicator is turned on to eradicate the mixture via ultrasound. This is left to sonicate while being monitored via TLC (50:50 ethyl acetate: hexane). Each product had a different reaction time due to TLC monitoring or solidification of product in reaction vessel. After reaction time, the reaction vessel is removed from the water bath and the reaction mixture is diluted with water. The product is extracted with ethyl acetate. The organic layer is isolated and washed with saturated sodium bicarbonate and brine and dried over sodium sulfate. This is then filtered and concentrated in vacuo. Purification of these products were done using silica column chromatography with an ethyl acetate: hexane gradient.



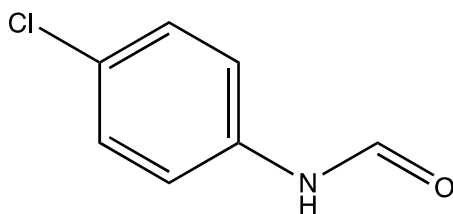
N-(2-chlorophenyl)formamide

N-(2-chlorophenyl) formamide: 7.84 mmol (1g scale), 78% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.75 (d, 1H, $J=11.2$ Hz, *trans*), δ 8.53 (brs, 1H, *trans*), δ 8.42 (dd, 1H, $J=8.2$ Hz, *cis*), δ 8.50 (brs, 1H, *cis*), 7.06-7.45 (m, 4H, Ar-H).



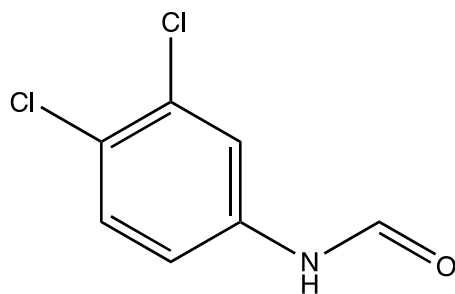
N-(3-chlorophenyl)formamide

N-(3-chlorophenyl) formamide: 7.84 mmol (1g scale), 51% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.69 (d, 1H, 11.3 Hz, *trans*), δ 8.39 (d, 1H, $J=1.4$ Hz, *trans*) δ 7.74 (brs, 1H, *cis*) δ 7.67 (t, 1H, $J=2.0$ Hz, *cis*) δ 6.96-7.40 (m, 4H, Ar-H)



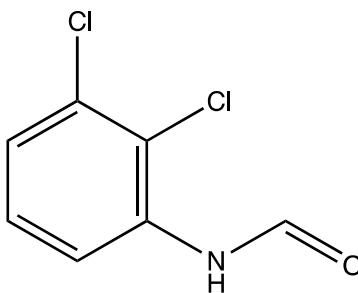
N-(4-chlorophenyl)formamide

N-(4-chlorophenyl) formamide: : 7.84 mmol (1g scale), 86% yield. $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ 10.32 (s, 1H, *trans*), δ 10.22 (d, 1H, $J=10.1$ Hz, *trans*) δ 8.78 (d, 1H, $J=10.1$ Hz, *cis*) δ 8.28 (d, 1H, $J=1.6$ Hz, *cis*) δ 7.58-7.64 (m, 1H, Ar-H), δ 7.35-7.42 (m, 2H, Ar-H), δ 7.58-7.22 (d, 1H, $J=8.8$ Hz, Ar-H)



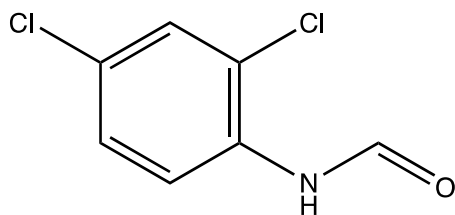
N-(3,4-dichlorophenyl)formamide

N-(3,4-dichlorophenyl) formamide: 6.17 mmol (1g scale), 32% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.65 (d, 1H, $J=11.2$ Hz, *trans*) δ 8.38 (d, 1H, $J=1.5$ Hz, *trans*) δ 7.79 (d, 1H, $J=2.3$ Hz, *cis*) δ 7.61 (brs, 1H, *cis*) δ 6.92-7.45 (m, 3H, Ar-H)



N-(2,3-dichlorophenyl)formamide

N-(2,3-dichlorophenyl) formamide: 6.17 mmol (1g scale), 62% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.74 (d, 1H, $J=10.8$ Hz, *trans*) δ 8.52 (s, 1H, *trans*) δ 8.38 (dd, 1H, $J=2.8$ Hz, *cis*) δ 7.73 (brs, 1H, *cis*) δ 7.21-7.32 (m, 3H, Ar-H)

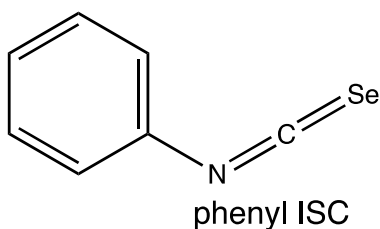


N-(2,4-dichlorophenyl)formamide

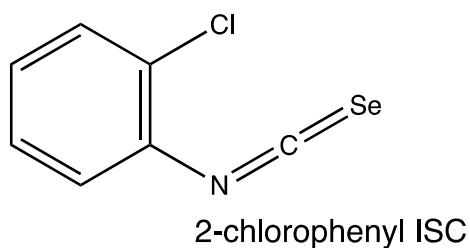
N-(2,4-dichlorophenyl) formamide: 6.17 mmol (1g scale), 60% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.68 (d, 1H, $J=11.1$ Hz, *trans*) δ 8.49 (s, 1H, *trans*) δ 8.40 (d, 1H, $J=8.9$ Hz, *cis*) δ 7.61 (brs, 1H, *cis*) δ 7.21-7.46 (m, 3H, Ar-H)

Synthesis of ISCs

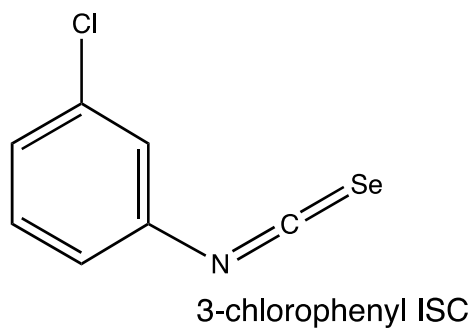
Phenyl ISC, 2-chlorophenyl ISC, 3-chlorophenyl ISC, 4-chlorophenyl ISC, 2,3-dichlorophenyl ISC, 2,4-dichlorophenyl ISC, 3,4-dichlorophenyl ISC, 4-bromophenyl ISC, 3,5-dibromophenyl ISC and 4-fluorophenyl ISC were successfully synthesized using the following procedure: to a dry round bottom flask is added the respective formylated amine (1 eq), triethylamine (4.3 eq), 4 angstrom molecular sieves, and 10 mL of DCM. This is then heated to reflux under argon. While heating, a solution of triphosgene (catalyst which degrades to phosgene gas, 0.53 eq) in DCM (3 mL) is made. Triphosgene is highly soluble in DCM, but this mixture is either sonicated or vortexed in order to ensure proper dissolution of catalyst. At this point, the solution is withdrawn into a 5mL syringe, and the syringe is fitted onto a syringe pump and a 20-gauge 12-inch stainless steel needle is attached. The needle is pushed down the condenser and set as close to the reaction mixture in order to allow the triphosgene solution to drip directly onto the reaction mixture. This 3 mL solution is then added dropwise over a period of an hour (flow rate of approximately 0.05mL of solution per minute). After the one-hour addition, the syringe needle is removed, and the reaction mixture is left to stir at reflux for an additional 2 hours 30 minutes. After this time, TLC analyses (100% hexane) can be done to see generation of isocyanide intermediate. During TLC analyses the isocyanide odor may be observed. With the isocyanide generated, grey selenium powder (2 eq.) is added and the reaction mixture is left to reflux for 18 hours. After 18 hours, TLC analysis (100% hexane) will reveal ISC spot which can also be seen very well with an iodine chamber (mixture of silica, sand and crystalline iodine) stain. At this point, the mixture is filtered and the filtrate is water (3 x 10mL), saturated sodium bicarbonate (1 x 10mL) and brine (1 x 10mL). The organic layer is dried over sodium sulfate and concentrated. All ISCs were purified using silica column chromatography mainly using 100% hexane or a hexane: DCM gradient.



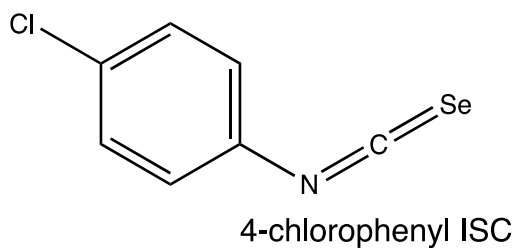
Phenyl ISC: 1.50 mmol (182mg scale), 88% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.26-7.40 (m, 5H, Ar-H)



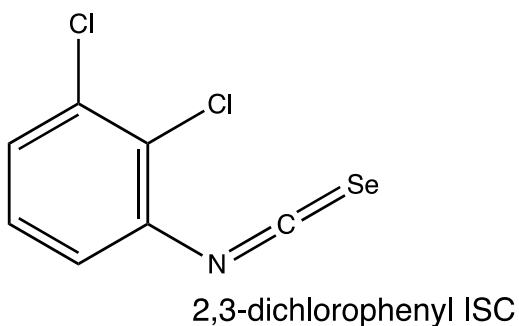
2-chlorophenyl ISC: 1 mmol (150 mg scale), 71% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.40-7.48 (m, 1H, Ar-H), δ 7.29-7.35 (m, 1H, Ar-H), δ 7.27 (t, 1H, $J=3.7$ Hz, Ar-H), δ 7.25 (d, 1H, $J=3.8$ Hz, Ar-H)



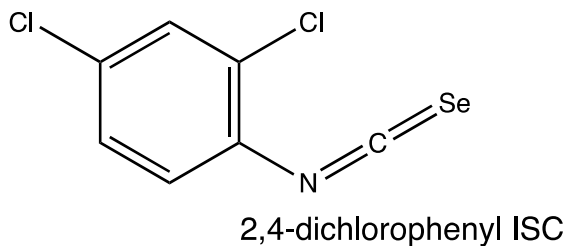
3-chlorophenyl ISC: 1 mmol (150mg scale), 73% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.27-7.34 (m, 3H, Ar-H), δ 7.15-7.22 (m, 1H, Ar-H)



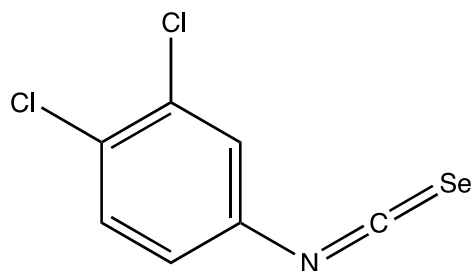
4-chlorophenyl ISC: 1 mmol (150 mg scale), 76% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.31-7.37 (m, 2H, $J=7.8, 1.8$ Hz, Ar-H), δ 7.19-7.25 (m, 2H, Ar-H)



2,3-dichlorophenyl ISC: 1.10 mmol (162mg scale), 73% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.42 (dd, 2H, Ar-H), δ 7.21 (ddd, 2H, $J=11.4, 8.1, 4.9$ Hz, Ar-H)

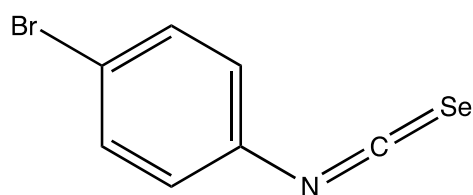


2,4-dichlorophenyl ISC: 1.14 mmol (215mg scale), 73% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.46 (t, 1H, $J=1.2$ Hz, Ar-H), δ 7.24 (d, 2H, $J=1.2$ Hz, Ar-H)



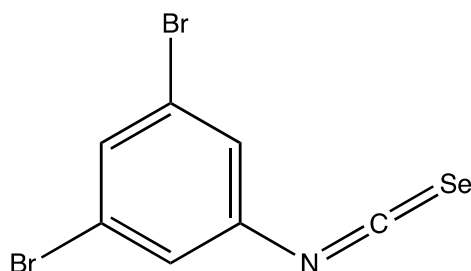
3,4-dichlorophenyl ISC

3,4-dichlorophenyl ISC: 0.80 mmol (150mg scale), 70% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.42 (dd, 2H, $J=17.4, 5.5$ Hz, Ar-H), δ 7.24 (dd, 1H, $J=2.4$ Hz, Ar-H)



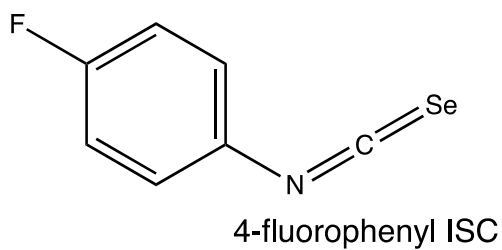
4-bromophenyl ISC

4-bromophenyl ISC: 1.03 mmol (205 mg scale), 66% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.44-7.54 (m, 2H, Ar-H), δ 7.10-7.20 (m, 2H, Ar-H)



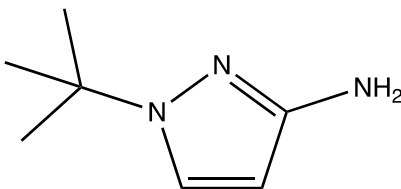
3,5-dibromophenyl ISC

3,5-dibromophenyl ISC: 0.60 mmol (164mg scale), 60% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.62 (t, 1H, $J=1.5$ Hz, Ar-H), δ 7.37 (d, 2H, $J=2.4$ Hz, Ar-H)



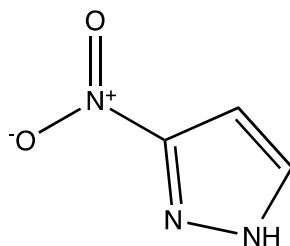
4-fluorophenyl ISC: 3.91 mmol (434mg scale), 50% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.27-7.31 (m, 2H, Ar-H), δ 7.03-7.11 (m, 2H, Ar-H)

Synthesis of Pyrazoles



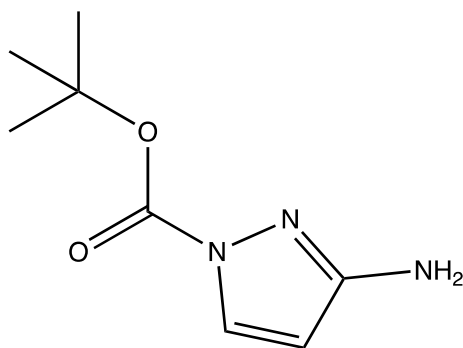
1-tert-butyl-1H-pyrazol-3-amine

1-tert-butyl-1H-pyrazol-3-amine: A solution of 250 mg (2.01 mmol, 1 eq.) tert- butylhydrazine hydrochloride in 30 mL of distilled water was cooled to 0°C. Then 278 mg (1 eq.) K₂CO₃ and 338 mg (2 eq.) NaHCO₃ were added sequentially. The mixture was kept stirring at 0°C for 1h. The resulting solution was clear and colorless. 176 mg (1 eq.) 2-chloroacrylonitrile was added at 0°C. The reaction mixture was warmed to RT and then stirred for 12 h. After this time, the solution turned out to be light yellow. The solution was then extracted with ethyl acetate (5 times). The combined organic layers were washed with brine, dried over anhydrous NaSO₄, and concentrated to dryness. Purified via column chromatography using silica gel and a gradient mixture of DCM and MeOH as eluent gave 180 mg, 64% yield. ¹H NMR (400 MHz, CDCl₃) δ 7.23 (d, J = 2.4 Hz, 1H), δ 5.54 (d, J = 2.4 Hz, 1H), δ 3.63 (bs, 2H), δ 1.49 (s, 9H).



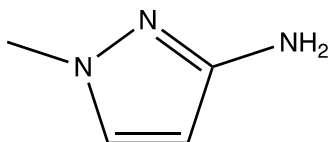
3-nitro-1H-pyrazole

3-nitro-1H-pyrazole: 100 mg of 3-aminopyrazole (1.20 mmol, 1 eq) is dissolved in 15 mL of distilled water. This solution is then heated to 40°C and Oxone (2.5 eq) is added in small portions. This suspension is then stirred at 40°C for 18 hours. After this time, saturated sodium bicarbonate is added slowly to the reaction mixture. Then the product was extracted with diethyl ether, and the organic layer was washed with brine and dried over magnesium sulfate, filtered, and concentrated. The product was then purified via silica column chromatography utilizing a ethyl acetate: hexane gradient to yield 51 milligrams, 38%, product. ^1H NMR (400 MHz, DMSO- d_6) δ 8.03 (d, J = 2.4 Hz, 1H), δ 7.03 (d, J = 2.5 Hz, 1H).



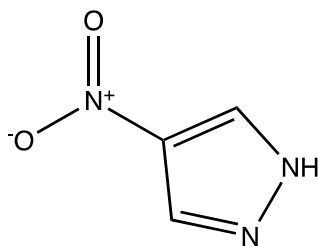
tert-butyl 3-amino-1H-pyrazole-1-carboxylate

tert-butyl 3-amino-1H-pyrazole-1-carboxylate: To a solution of 3-aminopyrazole (1.05 mmol, 1 eq), triethylamine (2 eq) and DMAP (0.074 mmol) in 10mL of 1,4-dioxane is slowly added a 10mL solution of Boc anhydride (1.15 eq.) in 1,4-dioxane at 0°C. Once added the solution is allowed to warm to room temperature and stirred overnight. Then the mixture is concentrated *in vacuo* and redissolved with ethyl acetate. this is dried using magnesium sulfate, concentrated, and purified via silica column chromatography using a hexane: ethyl acetate gradient. This yielded 144 mg, 75% of product. ¹H NMR (400 MHz, DMSO-d₆) δ 7.85 (d, J = 2.8 Hz, 1H), δ 5.78 (d, J = 2.9 Hz, 1H), δ 5.44 (s, 2H), δ 1.51 (s, 9H)



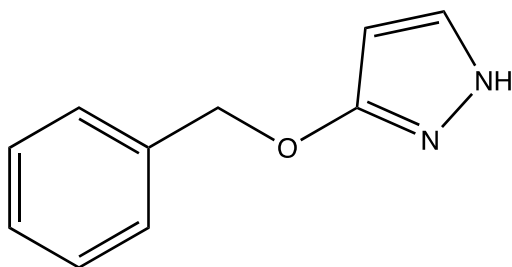
1-methyl-1H-pyrazol-3-amine

1-methyl-1H-pyrazol-3-amine: A solution of 250 mg (5.43 mmol, 1 eq.) methyl hydrazine in 30 mL of distilled water was cooled to 0°C. Then K₂CO₃ (1 eq.) and NaHCO₃ (2 eq.) were added sequentially. The mixture was kept stirring at 0°C for 1h. The resulting solution was clear and colorless. 2-chloroacrylonitrile (1 eq.) was added at 0°C. The reaction mixture was warmed to RT and then stirred for 12 h. After this time, the solution turned out to be light yellow. The solution was then extracted with ethyl acetate (5 times). The combined organic layers were washed with brine, dried over anhydrous NaSO₄, and concentrated to dryness. Purified via column chromatography using silica gel and a gradient mixture of DCM and MeOH as eluent gave 180 mg, 64% yield. ¹H NMR (400 MHz, DMSO-d₆) δ 7.23 (d, J = 2.4 Hz, 1H), δ 5.34 (d, J = 2.1 Hz, 1H), δ 4.47 (brs, 2H), δ 3.55 (s, 3H)



4-nitro-1H-pyrazole

4-nitro-1H-pyrazole: Pyrazole (1.46 mmol, 1 eq.) was measured in to a 25 mL round bottom flask and dissolved in 5 mL of H₂SO₄. Afterwards, the solution was heated to 60°C and 1.1 eq of conc. HNO₃ was added dropwise followed by stirring for 90 min at 60°C. After 90 min, the product solution was poured into a beaker of ice/water. If solid formation did not occur, the ice was allowed to melt and the product was extracted with ethyl acetate (6x15mL wash). The organic layer was then washed with brine and dried with Na₂SO₄. Concentrated and left *in vacuo* overnight to yield 136mg, 82%, of product. ¹H NMR (400 MHz, DMSO-d₆) δ 8.57 (s, 2H)



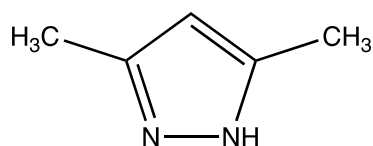
3-(benzyloxy)-1H-pyrazole

3-(benzyloxy)-1H-pyrazole: This compound was synthesized over four steps:

- 1) To a solution of methyl 3-methoxyacrylate (0.86 mmol, 1 eq.) in MeOH (8 mL) was added hydrazine monohydrate (1.1 eq.). The mixture was then refluxed for 2 hours. All volatiles were removed in vacuo and product was purified via silica column chromatography to give 1H-pyrazol-3-ol (32 mg, 45%). ¹H NMR (400 MHz, DMSO-d₆) δ 11.26 (s, 1H), δ 9.93 (s, 1H), δ 7.35 (s, 1H), δ 5.43 ppm (s, 1H).
- 2) 1H-pyrazol-3-ol (0.2 mmol, 1 eq.) was dissolved in pyridine (5 mL) and the mixture was heated to 95° C. A mixture of acetic anhydride (1.03 eq.) and pyridine (3 mL) was added dropwise over 20 minutes. Following addition, the reaction stirred at 95°C for 2 hours, then all volatiles were removed in vacuo and 15 mL of Et₂O was added to the residue. This solution was stirred overnight at RT. Then this was concentrated and purified via silica column chromatography using an ethyl acetate: hexane gradient to yield 1-(3-hydroxy-1H-pyrazol-1-yl)ethenone (4.2 mg, 17%). ¹H NMR (400 MHz, DMSO-d₆) δ 10.97 (s, 1H), δ 8.13 (s, 1H), δ 6.01 (s, 1H), δ 2.48 ppm (s, 3H).
- 3) A solution was prepared by mixing 1-(3-hydroxy-1H-pyrazol-1-yl)ethenone (0.033 mmol 1 eq.), benzyl bromide (1.1 eq.), and K₂CO₃ (1.33 eq.) in DMF (2 mL). This solution was stirred at RT for 10 hours. The solvent was removed in vacuo then the residue was diluted with ethyl acetate and water. The organic phase was washed with brine, dried over Na₂SO₄, then the volatiles removed *in vacuo* and gave 1-(3-(benzyloxy)-1H-

pyrazol-1-yl)ethan-1-one (7mg, 98%). ¹H NMR (400 MHz, CDCl₃) δ 8.07 (d, J=2.9 Hz, 1H), δ 7.46 (d, J=7.0 Hz, 2H), δ 7.39 (t, J=7.2 Hz, 2H), δ 7.35 (d, J=6.8 Hz, 1H), δ 6.00 (d, J=3.0 Hz, 1H), δ 5.29 (s, 2H), 2.61 ppm (s, 3H).

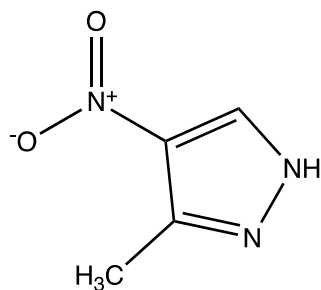
- 4) A solution was made by mixing 1-(3-(benzyloxy)-1H-pyrazol-1-yl)ethan-1-one (0.033 mmol, 1eq.) in 5 mL of methanol:THF (2:3) and 10% sodium hydroxide (1.3 eq.). This mixture was left to stir at room temperature for 5 hours. The mixture was then concentrated with a gentle flow of argon and then placed *in vacuo* overnight. Then this was purified via silica column chromatography using an ethyl acetate: hexane gradient. This gave **3-(benzyloxy)-1H-pyrazole** (2.4 mg, 42%). ¹H NMR (400 MHz, DMSO-d₆) δ 11.89 (s, 1H), δ 7.50 (d, J = 2.3 Hz, 1H), δ 7.43 – 7.34 (m, 5H), δ 5.69 (d, J = 2.4 Hz, 1H), δ 5.12 (s, 2H)



3,5-dimethyl-1H-pyrazole

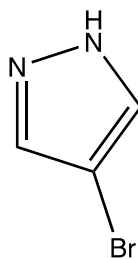
3,5-dimethyl-1H-pyrazole: Silica sulfuric acid ($\text{H}_2\text{SO}_4 \cdot \text{SiO}_2$ (SSA)) was prepared by taking a slurry of 10g of silica gel in dry diethyl ether (50 mL) and adding 3 mL of concentrated H_2SO_4 and followed by shaking for 5 minutes. The solvent was the evaporated under reduced pressure to result in free flowing SSA, which is then dried *in vacuo* while heating to 110°C for 3 hours.

Once SSA is prepared, in a vial is 2,4 pentanedione (1.0mmol, 1 equiv.) mixed with hydrazine (1.2 equiv.), followed by adding 20 mg of SSA. The slurry was continuously stirred for 5 min and then diluted with 3 mL ethyl acetate. The resulting reaction mixture was filtered, and the filter cake washed three times with ethyl acetate. The filtrate was concentrated to dryness to give 91 mg (yield 94%) of product. ^1H NMR (600 MHz, DMSO-d_6) δ 11.98 (brs, 1H), δ 5.73 (s, 1H), δ 2.11 (s, 6H).



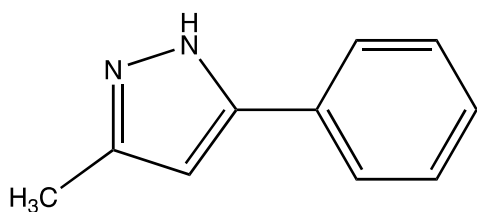
3-methyl-4-nitro-1H-pyrazole

3-methyl-4-nitro-1H-pyrazole: To a round bottom flask was added 3-methyl-1H-pyrazole (1.52 mmol, 1 eq.) and 5 mL of concentrated sulfuric acid. The reaction was heated to 60°C and concentrated nitric acid (1.1 eq.) was added slowly. The reaction continued to stir at 60°C for 90 minutes. The reaction was removed from the heat and poured over ice to attempt product precipitation. If no precipitation occurs, then the ice is allowed to melt and the product is extracted using ethyl acetate. The organic layer was then washed with saturated sodium bicarbonate and brine and dried over sodium sulfate. This was filtered and concentrated and revealed product (124 mg, 64% yield). ¹H NMR (600 MHz, DMSO-d₆) δ 8.25 (s, 1H), 8.05 – 8.02 (m, 1H), 2.45 (s, 3H)

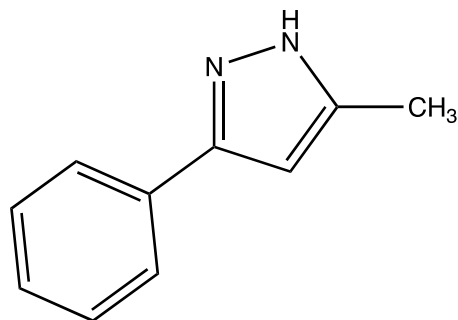


4-bromo-1H-pyrazole

4-bromo-1H-pyrazole: To a round bottom flask is added pyrazole (1.47 mmol, 1 eq.) and dissolved in 15 mL of distilled water. After this, N-bromosuccinimide (NBS) (1 eq.) was added in one portion. This mixture is left to stir at room temperature for overnight. After this, the product is extracted with ethyl acetate and then the organic layer is washed with saturated sodium thiosulfate and brine. This was dried over sodium sulfate, concentrated and was recrystallized in hexane. 135mg, 62% product yield. ^1H NMR (600 MHz, DMSO- d_6) δ 13.17 (brs, 1H), δ 7.97 (s, 1H), δ 7.56 (s, 1H)



3-methyl-5-phenyl-1H-pyrazole



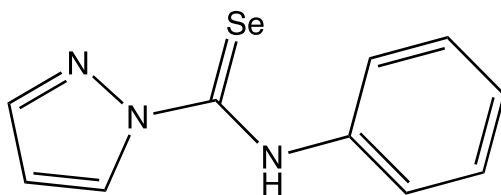
5-methyl-3-phenyl-1H-pyrazole

3-methyl-5-phenyl-1H-pyrazole and **5-methyl-3-phenyl-1H-pyrazole**: To a round bottom flask is 1-benzoylacetone (1 mmol, 1 eq.) added and dissolved in 10 mL of 200 proof ethanol. Then hydrazine monohydrate (1.5 eq.) was added and heated to reflux for 5.5 hours. After this time, the mixture was concentrated *in vacuo* and purified via silica column chromatography using a 50:50 ethyl acetate: hexane gradient. NMR analysis revealed formation of both pyrazoles. TLC analysis revealed only 1 spot for both products, thus both products were kept as a mixture. 156mg, 98% yield. ^1H NMR (600 MHz, DMSO- d_6) δ 12.85 (s, 1H), δ 12.52 (s, 1H), δ 7.74 (d, $J=6.5$ Hz, 1H) δ 7.16-7.39 (m, 6H), δ 6.43 (s, 2H), δ 2.26 (s, 6H)

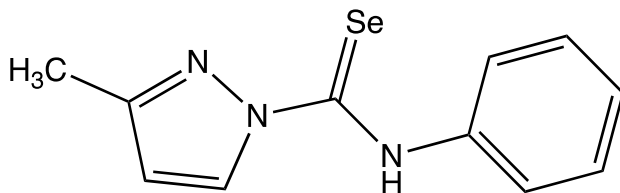
NNSeN Synthesis

The following NNSeNs were synthesized using conditions before solvent optimization. The procedure is as follows: To a dry round bottom flask (RBF) is respective aryl ISC and pyrazole added and diluted with 10mL of DCM. The reaction mixture is flushed with argon and heated to reflux for overnight. TLC used to monitor reaction. After this time, the organic layer is washed with saturated sodium bicarbonate, brine and water then dried with sodium sulfate. Short silica column to purify.

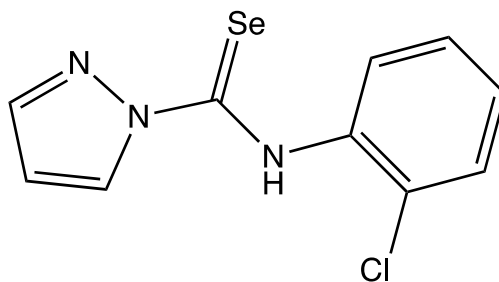
Compound name (NNSeN ID): reaction scale, mass of product, % yield of product.



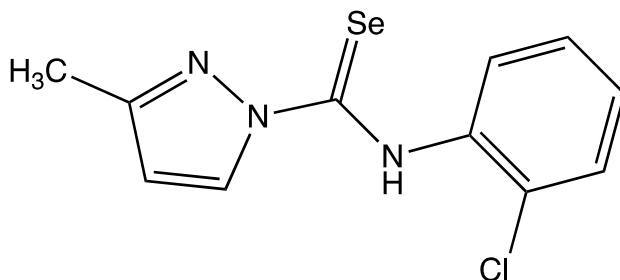
N-phenyl-1H-pyrazole-1-carboselenoamide (RN_02_20B): 1.43mmol scale, 149 mg, 42% yield. ¹H-NMR (400 MHz, CDCl₃): δ 11.16 (s, 1H), 8.89 (d, *J* = 2.8 Hz, 1H), 7.80 (d, *J* = 9.0 Hz, 1H), 7.49 – 7.28 (m, 5H), 6.47 (dd, *J* = 2.8, 1.6 Hz, 1H) ¹³C-NMR (CDCl₃): δ 176.17, 143.01, 137.64, 133.53, 129.51, 129.00, 127.52, 126.02, 124.43, 110.05.



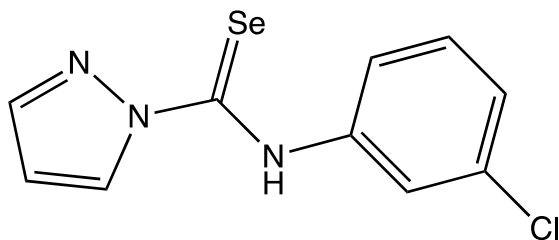
3-methyl-N-phenyl-1H-pyrazole-1-carboselenoamide (RN_02_23): 0.36 mmol scale, 49mg, 52% yield. ¹H NMR (400 MHz, CDCl₃): δ 11.04 (s, 1H), 8.77 (d, *J* = 2.8 Hz, 1H), 7.78 (dt, *J* = 3.2, 1.9 Hz, 2H), 7.50 – 7.30 (m, 3H), 6.28 (d, *J* = 2.8 Hz, 1H), 2.31 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 175.99, 153.54, 137.83, 134.25, 129.00, 127.43, 124.59, 110.90, 14.03.



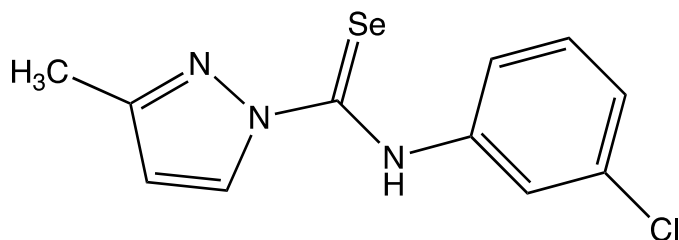
N-(2-chlorophenyl)-1*H*-pyrazole-1-carboselenoamide (RN_02_29): 0.089 mmol scale, 4.4mg, 17% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.47 (s, 1H), 8.89 – 8.85 (m, 1H), 8.61 (dd, J = 8.1, 1.6 Hz, 1H), 7.85 (d, J = 1.0 Hz, 1H), 7.51 (dd, J = 8.0, 1.5 Hz, 1H), 7.35 (dtd, J = 32.9, 7.6, 1.6 Hz, 2H), 6.49 (dd, J = 2.8, 1.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 179.76, 175.23, 142.36, 132.52, 128.85, 127.24, 126.13, 124.95, 109.40, 28.72.



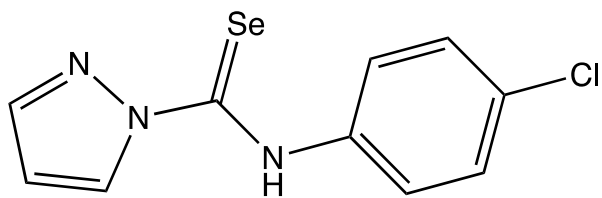
N-(2-chlorophenyl)-3-methyl-1*H*-pyrazole-1-carboselenoamide (RN_02_36) 0.39 mmol scale, 83mg, 72% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.30 (s, 1H), 8.74 (d, J = 2.8 Hz, 1H), 8.52 (dd, J = 8.1, 1.6 Hz, 1H), 7.49 (dd, J = 8.0, 1.5 Hz, 1H), 7.32 (dtd, J = 14.8, 7.3, 1.3 Hz, 2H), 6.29 (d, J = 2.8 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.02, 153.83, 134.96, 134.13, 129.72, 128.32, 128.10, 127.01, 126.27, 111.19, 14.11.



N-(3-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_02_38) 0.20 mmol scale, 17mg, 32% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.15 (s, 1H), 8.86 (d, $J = 2.5$ Hz, 1H), 7.93 (t, $J = 1.9$ Hz, 1H), 7.81 (d, $J = 1.0$ Hz, 1H), 7.73 – 7.67 (m, 1H), 7.41 – 7.29 (m, 2H), 6.48 (dd, $J = 2.8, 1.6$ Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.42, 143.16, 138.80, 133.54, 130.06, 128.40, 127.62, 126.28, 124.42, 124.31, 122.54, 110.40.



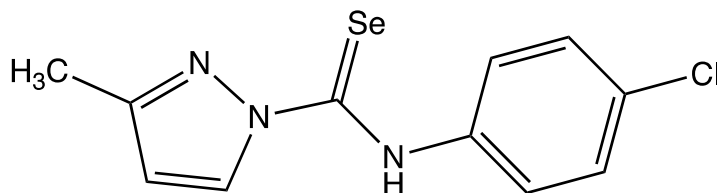
N-(3-chlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_02_40) 0.08 mmol scale, 12mg, 49% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.04 (s, 1H), 8.74 (d, $J = 2.8$ Hz, 1H), 7.91 (t, $J = 2.0$ Hz, 1H), 7.71 (ddd, $J = 7.9, 2.0, 1.2$ Hz, 1H), 7.40 – 7.29 (m, 2H), 6.29 (d, $J = 2.8$ Hz, 1H), 2.31 (d, $J = 6.1$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.08, 152.74, 137.94, 133.58, 133.21, 128.98, 126.44, 123.48, 121.63, 110.23, 76.35, 76.03, 75.71, 13.04.



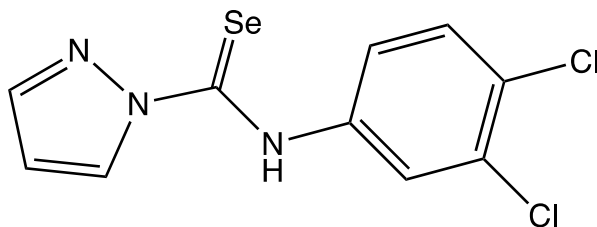
N-(4-chlorophenyl)-1*H*-pyrazole-1-carboselenoamide (RN_02_45) 0.41 mmol scale, 31mg, 26% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.12 (s, 1H), 8.86 (d, $J = 2.6$ Hz, 1H), 7.82 (s, 1H), 7.79 – 7.74 (m, 2H), 7.45 – 7.40 (m, 2H), 6.48 (dd, $J = 2.8, 1.6$ Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.47, 143.16, 136.23, 133.58, 129.87, 129.25, 127.29, 125.74, 110.32, 77.34, 77.23, 77.02, 76.71.

Unless stated otherwise, the following NNSeNs were synthesized using the following procedure: to a dry round bottom flask (RBF) is added aryl ISC (1 eq) and pyrazole (1 eq) and dissolved using 5mL of dry chloroform. This mixture is then stirred vigorously and heated to reflux while being flushed with argon. This mixture is left to reflux for overnight, approximately 18 hours, and monitored using TLC. After this time, the reaction mixture is allowed to cool to room temperature and the reaction mixture is further diluted with an additional 10-15mL of chloroform. This is then subjected to a liquid-liquid extraction, washing the organic layer with water, saturated sodium bicarbonate and brine and is dried using either sodium sulfate (preferred) or magnesium sulfate. The product is then be purified using silica column chromatography (hexane: DCM gradient).

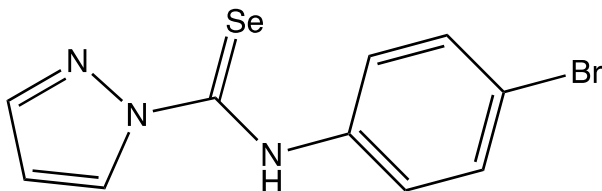
Compound name (NNSeN ID): reaction scale, mass of product, % yield of product.



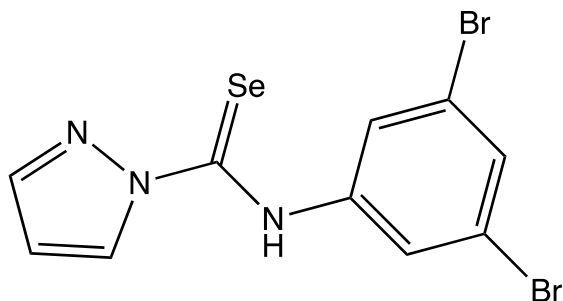
N-(4-chlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_02_45): 0.13 mmol scale, 29mg, 75% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.01 (s, 1H), 8.74 (d, $J = 2.8$ Hz, 1H), 7.82 – 7.68 (m, 2H), 7.48 – 7.37 (m, 2H), 6.29 (d, $J = 2.8$ Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.17, 153.73, 136.37, 134.25, 132.78, 129.88, 129.18, 127.30, 125.85, 111.14, 14.03.



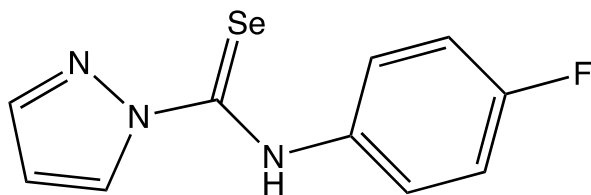
N-(3,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_02_57): 0.40 mmol scale, 50mg, 40% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.12 (s, 1H), 8.83 (t, $J = 5.3$ Hz, 1H), 8.05 (d, $J = 2.5$ Hz, 1H), 7.81 (d, $J = 1.0$ Hz, 1H), 7.72 – 7.64 (m, 1H), 7.49 (dd, $J = 8.7, 4.4$ Hz, 1H), 6.48 (dd, $J = 2.8, 1.6$ Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.45, 143.25, 137.00, 133.49, 132.93, 131.08, 130.62, 125.94, 123.63, 110.54.



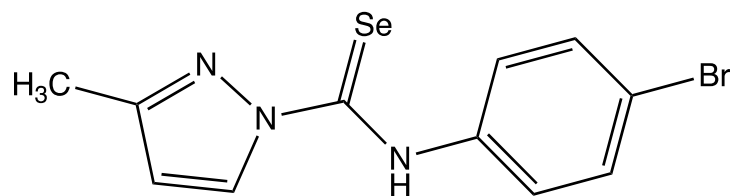
N-(4-bromophenyl)-1H-pyrazole-1-carboselenoamide (RN_02_60): 0.67 mmol scale, 84 mg, 40% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.12 (s, 1H), 8.86 (d, $J = 2.8$ Hz, 1H), 7.81 (d, $J = 1.0$ Hz, 1H), 7.75 – 7.69 (m, 2H), 7.62 – 7.54 (m, 2H), 6.48 (dd, $J = 2.8, 1.6$ Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.26, 143.14, 136.70, 133.53, 132.44, 132.16, 131.87, 126.16, 125.91, 110.31.



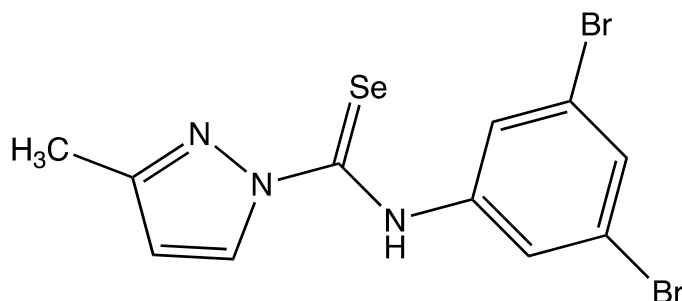
N-(3,5-dibromophenyl)-1H-pyrazole-1-carboselenoamide (RN_02_61 and RN_02_65): 0.35 mmol scale, 54 mg, 38% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.13 (s, 1H), 8.86 – 8.80 (m, 1H), 8.02 (d, J = 1.7 Hz, 2H), 7.81 (d, J = 1.0 Hz, 1H), 7.64 (dd, J = 5.0, 3.3 Hz, 1H), 6.49 (dd, J = 2.8, 1.6 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.42, 143.26, 139.70, 133.80, 133.45, 132.83, 127.85, 125.82, 122.84, 110.65.



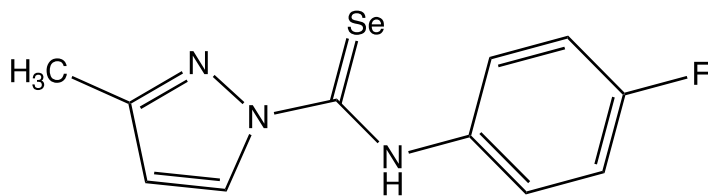
N-(4-fluorophenyl)-1H-pyrazole-1-carboselenoamide (RN_02_62): 1.98 mmol scale, 266 mg, 50% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.06 (s, 1H), 8.87 (d, J = 2.8 Hz, 1H), 7.81 (d, J = 1.0 Hz, 1H), 7.76 – 7.69 (m, 2H), 7.19 – 7.11 (m, 2H), 6.48 (dd, J = 2.8, 1.6 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.93, 162.43, 159.96, 143.16, 133.71, 133.68, 133.62, 126.77, 126.68, 115.99, 115.77, 110.15.



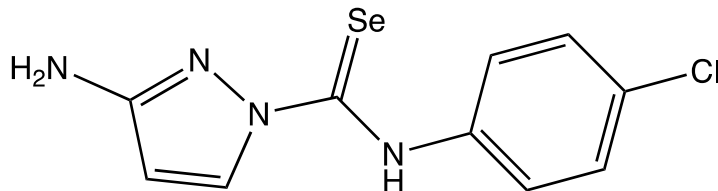
N-(4-bromophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_02_73): 0.23 mmol scale, 55 mg, 70% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.00 (s, 1H), 8.73 (d, $J = 2.8$ Hz, 1H), 7.75 – 7.65 (m, 2H), 7.60 – 7.53 (m, 2H), 6.29 (d, $J = 2.8$ Hz, 1H), 2.30 (s, 3H).



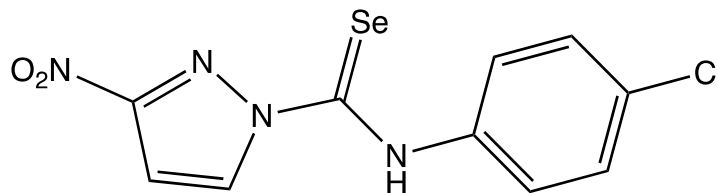
N-(3,5-dibromophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_02_74): 0.049 mmol scale, 9 mg, 43% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.03 (s, 1H), 8.70 (d, $J = 2.8$ Hz, 1H), 8.02 (d, $J = 1.6$ Hz, 2H), 7.63 (t, $J = 1.6$ Hz, 1H), 6.30 (d, $J = 2.8$ Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.00, 152.88, 138.84, 133.10, 132.81, 131.62, 126.86, 124.81, 121.76, 110.53, 13.04.



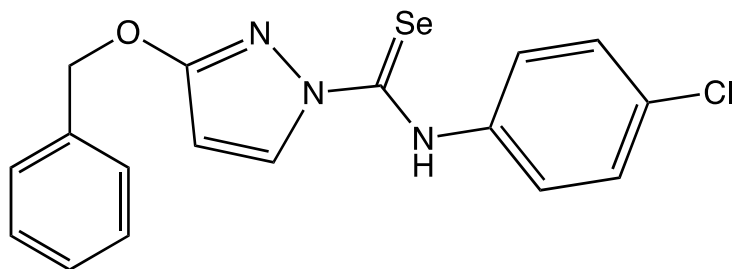
N-(4-fluorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_02_75): 0.72 mmol scale, 114 mg, 56% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.95 (s, 1H), 8.73 (d, $J = 2.8$ Hz, 1H), 7.80 – 7.60 (m, 2H), 7.12 (ddd, $J = 8.5, 5.2, 2.8$ Hz, 2H), 6.27 (d, $J = 2.8$ Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.70, 162.47, 160.01, 153.70, 134.30, 133.88, 133.85, 126.87, 126.78, 116.00, 115.77, 111.01, 14.01.



3-amino-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_03_22A): 0.23 mmol scale, 33 mg, 48% yield. ^1H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 8.51 (d, $J = 3.0$ Hz, 1H), 7.59 (d, $J = 8.7$ Hz, 2H), 7.48 – 7.42 (m, 2H), 6.03 (d, $J = 3.0$ Hz, 1H), 5.81 (s, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.88, 156.88, 136.54, 135.47, 132.58, 129.09, 126.00, 101.96, 101.91.

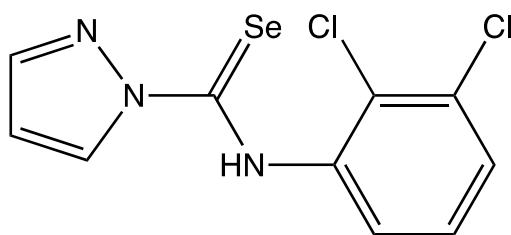


N-(4-chlorophenyl)-3-nitro-1H-pyrazole-1-carboselenoamide (RN_03_39): 0.042 mmol scale, 9 mg, 63% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.94 (s, 1H), 8.96 (d, $J = 2.9$ Hz, 1H), 7.80 – 7.72 (m, 2H), 7.49 – 7.43 (m, 2H), 7.07 (d, $J = 2.9$ Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 175.28, 135.63, 135.63, 135.53, 133.83, 129.61, 125.82, 121.64, 105.41, 29.71.

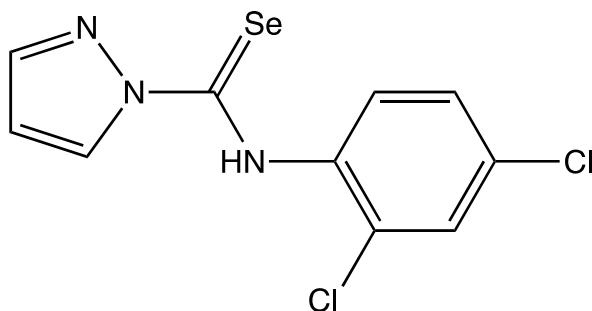


3-(benzyloxy)-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_03_49):

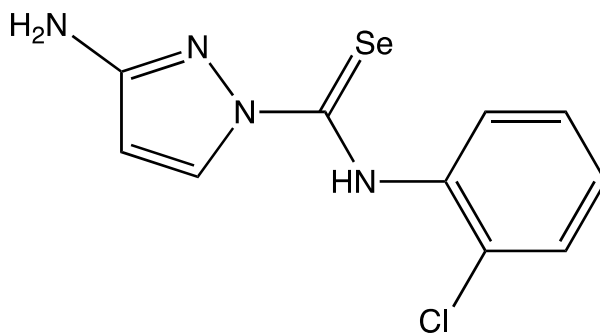
Procedure: 3-(benzyloxy)-1H-pyrazole (0.014 mmol, 1eq.) added and diluted with 1 mL of dry THF. This is then injected to a dry RBF flask containing potassium carbonate. This mixture is the further diluted with 4 mL of dry THF. This is heated to 50°C and stirred for 1 hour. After this time, 4-chlorophenyl ISC (0.014 mmol, 1eq.) is added to the mixture. This is then brought to reflux and stirred for 18 hours. After reaction time, this is filtered and concentrated. Purification done via silica column chromatography (ethyl acetate: hexane gradient). 0.014 mmol scale, 4 mg, 64% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.56 (s, 1H), 8.71 (d, J = 3.1 Hz, 1H), 7.70 (d, J = 8.7 Hz, 2H), 7.47 – 7.36 (m, 9H), 6.08 (d, J = 3.1 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 132.16, 129.22, 129.11, 128.71, 128.67, 128.57, 128.34, 128.26, 127.97, 127.94, 126.24, 122.83, 120.87, 100.38, 99.14, 31.94, 29.71, 22.70, 14.13.



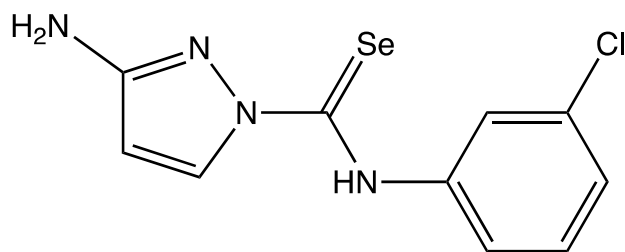
N-(2,3-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_39): 0.071 mmol, 4 mg, 16% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.49 (s, 1H), 8.87 – 8.83 (m, 1H), 8.56 (dd, J = 8.3, 1.1 Hz, 1H), 7.86 (d, J = 1.0 Hz, 1H), 7.47 (dd, J = 8.1, 1.4 Hz, 1H), 7.33 (t, J = 8.2 Hz, 1H), 6.50 (dd, J = 2.9, 1.6 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.52, 143.46, 133.52, 128.77, 127.14, 124.15, 110.61, 29.71.



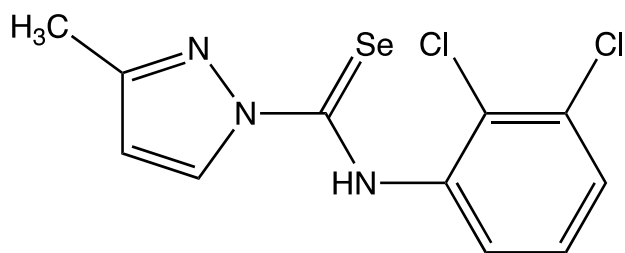
N-(2,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_40): 0.068 mmol scale, 5mg, 21% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.42 (s, 1H), 8.84 (d, J = 2.8 Hz, 1H), 8.63 (d, J = 8.8 Hz, 1H), 7.84 (s, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.36 (dd, J = 8.8, 2.2 Hz, 1H), 6.49 (d, J = 1.5 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.27, 143.44, 133.59, 133.47, 132.97, 129.61, 128.78, 127.43, 126.51, 110.58.



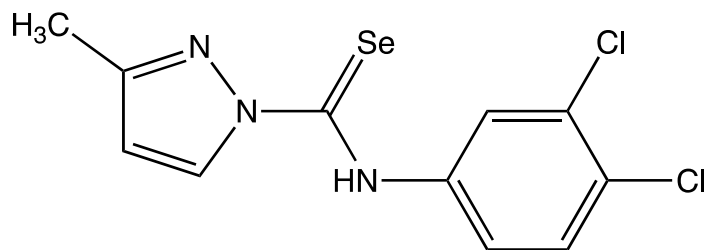
3-amino-N-(2-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_41): 0.096 mmol scale, 24 mg, 82% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.88 (s, 1H), 8.65 (d, J = 2.9 Hz, 1H), 8.54 (d, J = 8.0 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.36 (t, J = 7.3 Hz, 1H), 7.27 (d, J = 3.0 Hz, 1H), 7.24 (s, 1H), 5.98 (d, J = 2.9 Hz, 1H), 3.81 (s, J = 71.0, 60.2 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.67, 157.59, 135.41, 135.16, 129.67, 128.22, 127.85, 126.96, 126.43, 101.98.



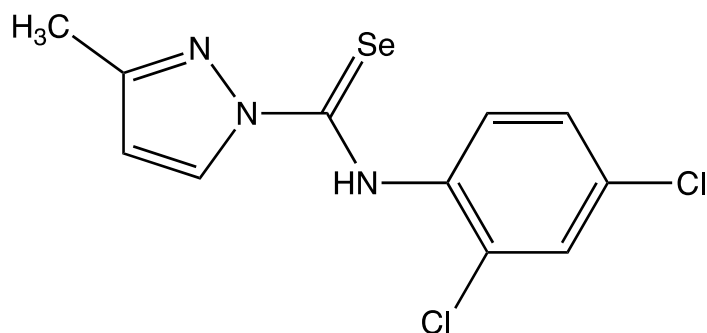
3-amino-N-(3-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_42): 0.13 mmol scale, 26 mg, 67% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.61 (s, 1H), 8.63 (d, $J = 2.9$ Hz, 1H), 7.86 (s, 1H), 7.66 (d, $J = 7.9$ Hz, 1H), 7.31 (dt, $J = 25.3, 5.8$ Hz, 2H), 5.96 (d, $J = 2.9$ Hz, 1H), 3.9 (brs, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.77, 157.49, 139.13, 135.41, 134.46, 129.89, 127.19, 124.57, 122.73, 101.91.



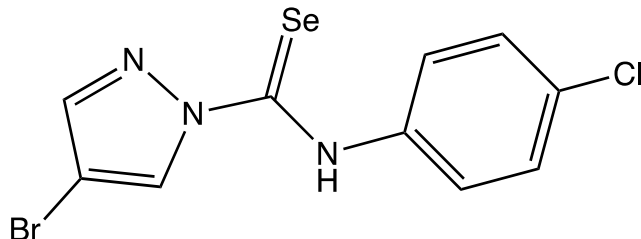
N-(2,3-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_04_43): 0.058 mmol scale, 10 mg, 49% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.32 (s, 1H), 8.73 (d, $J = 2.8$ Hz, 1H), 8.48 (dd, $J = 8.2, 1.2$ Hz, 1H), 7.45 (dd, $J = 8.1, 1.4$ Hz, 1H), 7.31 (t, $J = 8.2$ Hz, 1H), 6.31 (d, $J = 2.8$ Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.37, 154.04, 136.70, 134.21, 133.53, 128.68, 127.07, 124.56, 111.48, 14.14.



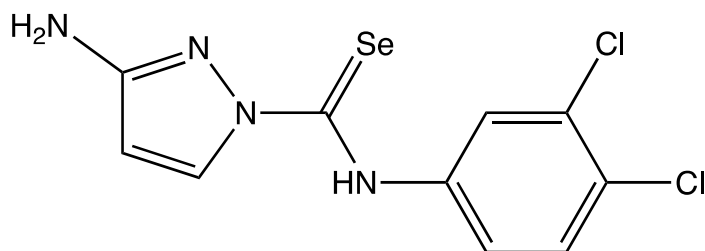
N-(3,4-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_04_44): 0.11 mmol scale, 24 mg, 65% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.01 (s, 1H), 8.71 (d, $J = 2.8$ Hz, 1H), 8.04 (d, $J = 2.5$ Hz, 1H), 7.73 – 7.66 (m, 1H), 7.53 – 7.46 (m, 1H), 6.29 (d, $J = 2.8$ Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.13, 153.88, 137.19, 134.16, 132.87, 130.92, 130.57, 126.01, 123.72, 111.41, 14.04.



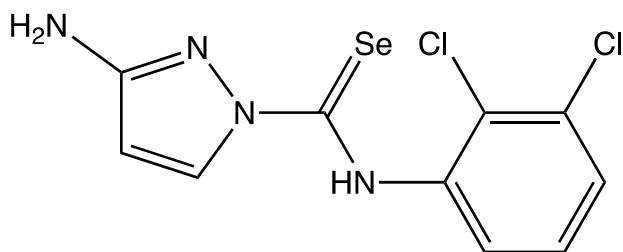
N-(2,4-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboselenoamide (RN_04_45): 0.067 mmol scale, 14 mg, 62% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.26 (s, 1H), 8.72 (d, $J = 2.8$ Hz, 1H), 8.54 (d, $J = 8.8$ Hz, 1H), 7.51 (d, $J = 2.3$ Hz, 1H), 7.34 (dd, $J = 8.8, 2.3$ Hz, 1H), 6.30 (d, $J = 2.8$ Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.15, 154.03, 134.16, 133.76, 132.88, 129.55, 128.98, 127.37, 126.91, 111.44, 14.13.



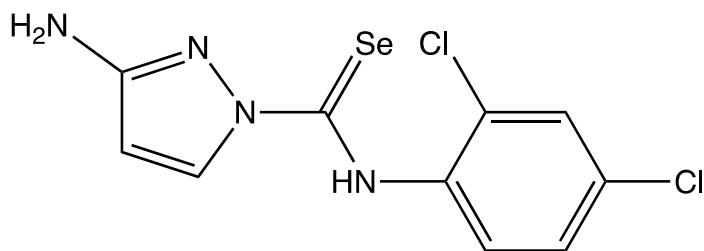
4-bromo-N-(4-chlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_46): 0.10 mmol scale, 12 mg, 32% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.98 (s, 1H), 8.89 (s, 1H), 7.80 – 7.73 (m, 3H), 7.45 – 7.39 (m, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 175.15, 143.42, 136.00, 133.15, 133.01, 129.90, 129.37, 125.55, 99.10, 29.72.



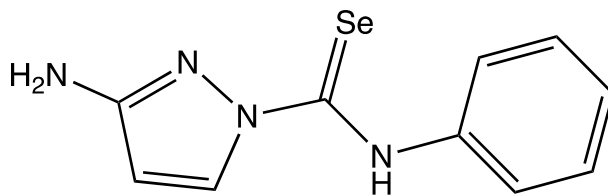
3-amino-N-(3,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_47): 0.11 mmol scale, 27 mg, 74% yield. ^1H NMR (400 MHz, CDCl_3) δ 8.62 (d, $J = 3.0$ Hz, 1H), 7.84 (d, $J = 2.2$ Hz, 1H), 7.72 – 7.41 (m, 3H), 6.31 (d, $J = 3.0$ Hz, 1H), 1.77 (s, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.82, 157.50, 137.37, 135.39, 132.76, 130.68, 130.47, 126.11, 123.83, 102.10.



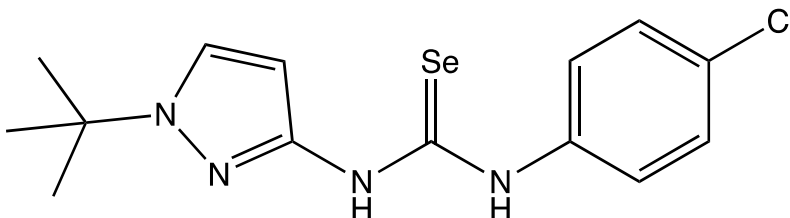
3-amino-N-(2,3-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_48): 0.11 mmol scale, 26 mg, 71% yield. ^1H NMR (400 MHz, DMSO) δ 11.36 (s, 1H), 8.47 (d, J = 2.9 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.42 (t, J = 8.0 Hz, 1H), 6.04 (d, J = 2.8 Hz, 1H), 5.86 (s, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.83, 157.65, 136.84, 135.43, 133.36, 128.35, 126.96, 124.57, 102.28, 29.71.



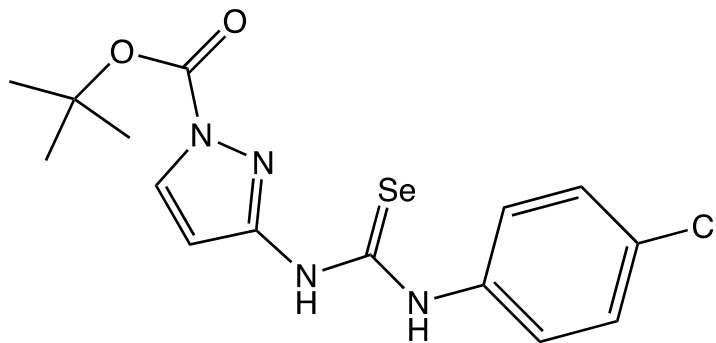
3-amino-N-(2,4-dichlorophenyl)-1H-pyrazole-1-carboselenoamide (RN_04_49): 0.11 mmol scale, 29 mg, 78% yield. ^1H NMR (400 MHz, DMSO) δ 11.24 (s, 1H), 8.46 (d, J = 3.0 Hz, 1H), 7.75 (d, J = 2.3 Hz, 1H), 7.61 (d, J = 8.6 Hz, 1H), 7.49 (dd, J = 8.6, 2.3 Hz, 1H), 6.04 (d, J = 3.0 Hz, 1H), 5.87 (s, 2H).



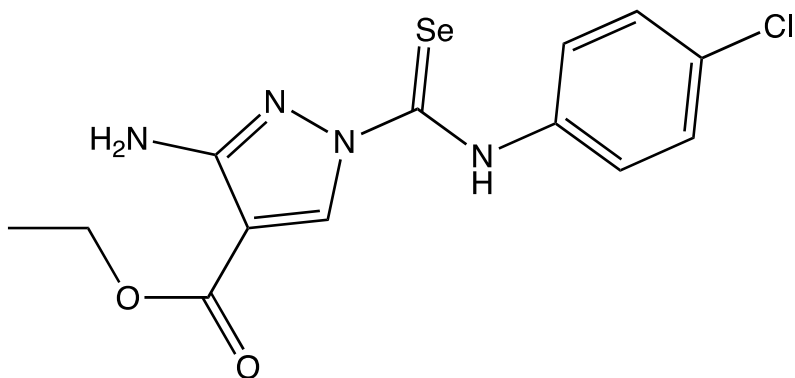
3-amino-N-phenyl-1H-pyrazole-1-carboselenoamide (RN_04_50A): 0.10 mmol scale, 17 mg, 65% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.68 (s, 1H), 8.68 (d, J = 2.6 Hz, 1H), 7.72 (d, J = 7.9 Hz, 2H), 7.42 (t, J = 7.7 Hz, 2H), 7.30 (dd, J = 14.2, 6.8 Hz, 1H), 6.04 (s, 1H), 3.84 (s, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.83, 157.11, 138.03, 135.51, 128.97, 127.27, 124.77, 101.58, 77.36, 77.05, 76.73, 29.71.



1-(1-(tert-butyl)-1H-pyrazol-3-yl)-3-(4-chlorophenyl)selenourea (RN_03_20): 0.22 mmol scale, 1 mg, 1.3% yield. ^1H NMR (400 MHz, CDCl_3) δ 12.23 (s, 1H), 9.25 (s, 1H), 7.68 (t, J = 12.3 Hz, 2H), 7.45 – 7.33 (m, 3H), 5.89 (d, J = 2.4 Hz, 1H), 1.56 (s, 9H).

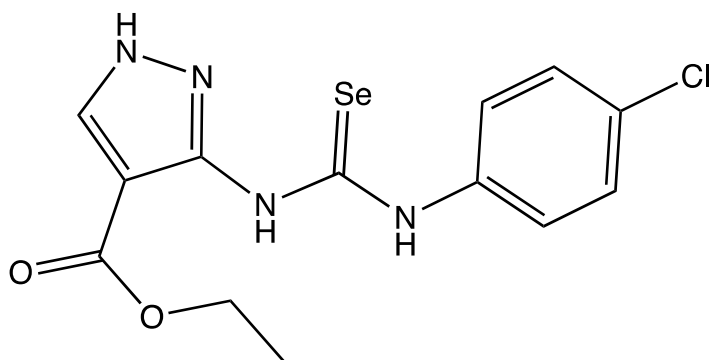


Tert-butyl 3-(3-(4-chlorophenyl)selenoureido)-1H-pyrazole-1-carboxylate (RN_03_25): 0.12 mmol scale, 19 mg, 40% yield. ^1H NMR (400 MHz, DMSO) δ 12.08 (s, 1H), 11.55 (s, 1H), 8.26 (d, J = 2.9 Hz, 1H), 7.72 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 6.38 (s, 1H), 1.58 – 1.55 (m, 9H).

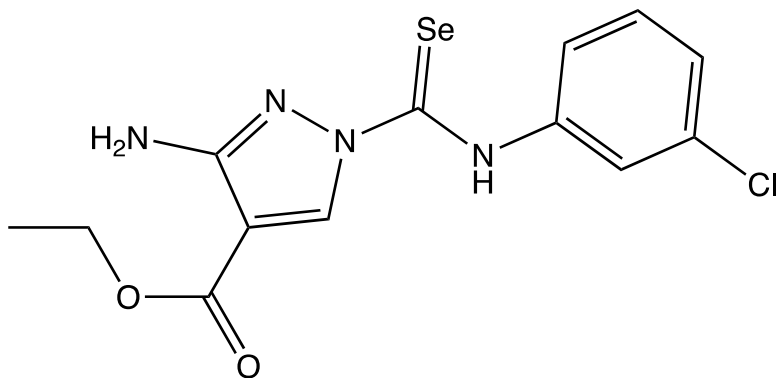


Ethyl-3-amino-1-((4-chlorophenyl)carbamoseleoyl)-1H-pyrazole-4-carboxylate

(RN_04_56A): 0.08 mmol scale, 4 mg, 15% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.13 (s, 1H), 7.70 – 7.36 (m, 3H), 7.23 (d, J = 7.5 Hz, 1H), 7.10 (s, 1H), 6.98 (d, J = 9.3 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 4.30 (p, J = 6.9 Hz, 2H), 1.36 (td, J = 7.1, 4.4 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 164.03, 144.24, 144.24, 141.55, 140.80, 130.47, 129.43, 127.19, 121.81, 60.03, 59.93, 29.71, 14.49.

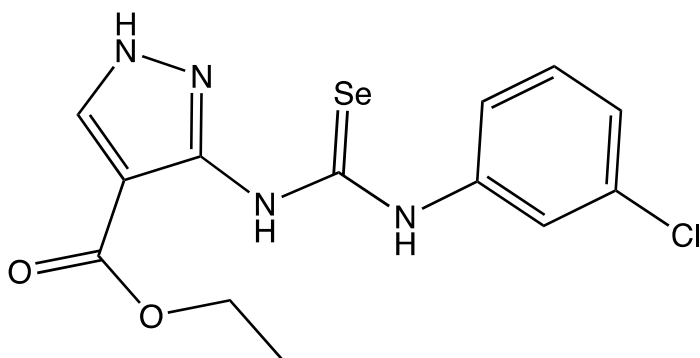


Ethyl-3-(3-(4-chlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate (RN_04_56B): 0.08 mmol scale, 10 mg, 33% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.81 (s, 1H), 9.07 (s, 1H), 8.45 (d, J = 74.8 Hz, 1H), 7.90 – 7.31 (m, 2H), 7.02 – 6.94 (m, 1H), 6.75 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 1.92 (s, 1H), 1.40 – 1.32 (m, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.85, 163.47, 156.82, 137.01, 136.22, 132.91, 129.23, 125.57, 106.56, 60.88, 29.71, 14.34.

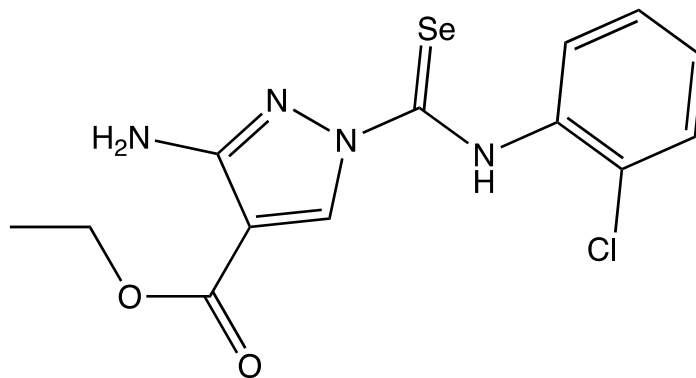


Ethyl-3-amino-1-((3-chlorophenyl)carbamoylselenoyl)-1H-pyrazole-4-carboxylate

(RN_04_57A): 0.078 mmol scale, 8 mg, 26% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.16 (s, 1H), 7.70 – 7.61 (m, 1H), 7.54 – 7.32 (m, 1H), 7.23 – 7.02 (m, 3H), 6.93 (d, J = 9.3 Hz, 1H), 6.81 (t, J = 7.8 Hz, 1H), 4.30 (p, J = 7.0 Hz, 2H), 1.36 (td, J = 7.1, 3.5 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 141.55, 140.78, 138.10, 135.07, 134.73, 130.30, 130.15, 128.04, 125.98, 124.80, 124.04, 120.58, 118.92, 77.35, 77.03, 76.71, 60.03, 59.95, 14.49.

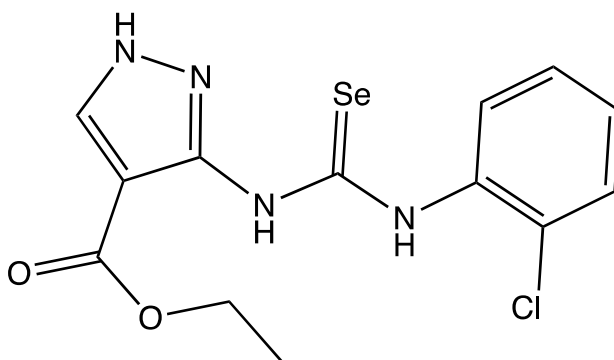


Ethyl-3-(3-(3-chlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate (RN_04_57B): 0.078 mmol scale, 16 mg, 55% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.83 (s, 1H), 9.07 (s, 1H), 8.69 – 8.29 (m, 1H), 7.76 (dd, J = 71.4, 27.7 Hz, 1H), 7.31 (dd, J = 15.9, 8.1 Hz, 1H), 7.23 – 6.70 (m, 2H), 4.33 (q, J = 7.1 Hz, 2H), 2.46 (s, 1H), 1.37 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 163.50, 140.94, 134.30, 130.22, 130.11, 129.90, 129.86, 129.79, 128.94, 128.71, 128.10, 127.70, 127.63, 127.16, 127.09, 125.85, 60.85, 60.00, 59.92, 53.47, 29.71, 14.51, 14.36.

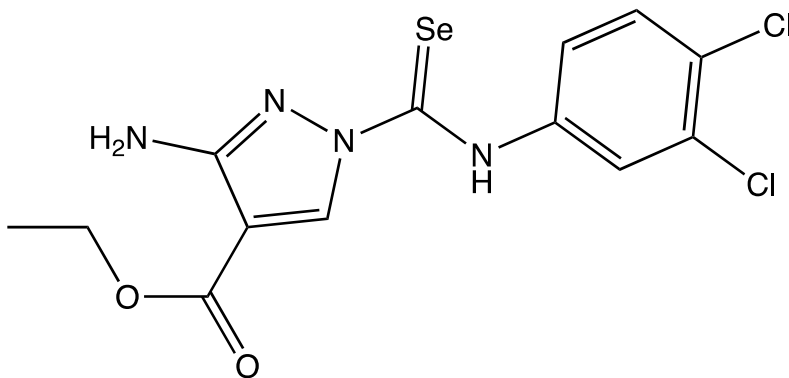


Ethyl-3-amino-1-((2-chlorophenyl)carbamoylselenoyl)-1H-pyrazole-4-carboxylate

(RN_04_58A): 0.095 mmol scale, 16 mg, 46% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.27 (s, 1H), 8.05 (dd, J = 7.8, 1.5 Hz, 1H), 7.71 (s, 1H), 7.51 (dt, J = 12.6, 6.3 Hz, 1H), 7.41 – 7.30 (m, 2H), 7.20 (s, 1H), 7.00 (t, J = 7.2 Hz, 1H), 4.31 (q, J = 7.1 Hz, 2H), 1.37 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.78, 163.47, 156.80, 138.78, 136.97, 134.64, 130.03, 127.55, 124.23, 122.37, 106.66, 60.89, 29.71, 14.34.

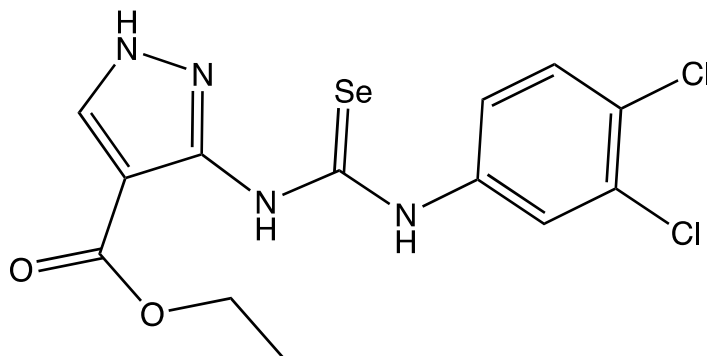


Ethyl-3-(3-(2-chlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate (RN_04_58B): 0.095 mmol scale, 12 mg, 33% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.14 (s, 1H), 8.65 (dd, J = 150.0, 110.1 Hz, 1H), 7.80 – 7.28 (m, 2H), 7.04 (dd, J = 51.7, 24.9 Hz, 1H), 4.38 (dd, J = 32.7, 6.7 Hz, 2H), 2.71 (s, J = 90.6, 87.3 Hz, 2H), 1.39 (dd, J = 16.3, 10.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 163.51, 156.89, 137.01, 130.12, 129.80, 128.20, 128.04, 127.11, 125.86, 106.72, 60.87, 29.71, 14.38.



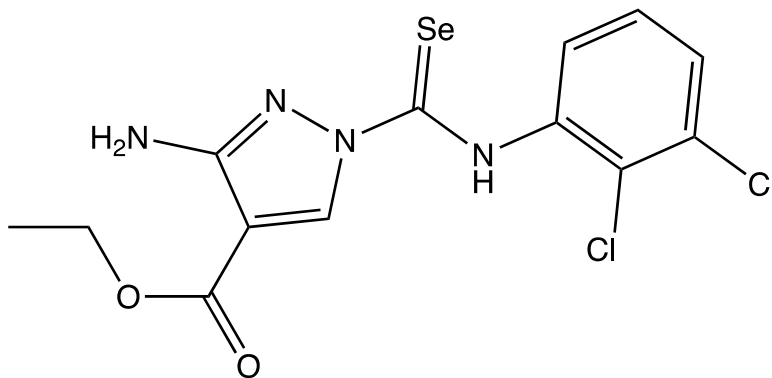
Ethyl-3-amino-1-((3,4-dichlorophenyl)carbamoselenoyl)-1H-pyrazole-4-carboxylate

(RN_04_59A): 0.077 mmol scale, 9 mg, 29% yield. ^1H NMR (400 MHz, CDCl_3) δ 7.81 – 7.27 (m, 3H), 7.22 – 6.72 (m, 4H), 4.31 (p, J = 6.9 Hz, 2H), 1.40 – 1.32 (m, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 175.99, 163.93, 163.81, 154.62, 144.61, 141.90, 140.88, 136.30, 133.36, 133.10, 131.76, 130.98, 130.79, 128.55, 127.58, 125.17, 122.01, 120.25, 95.93, 60.08, 60.00, 29.71, 14.48.



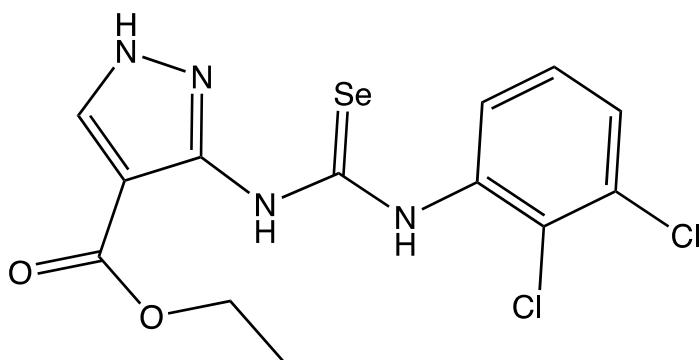
Ethyl-3-(3-(3,4-dichlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate

(RN_04_59B): 0.077 mmol scale, 17 mg, 53% yield. ^1H NMR (400 MHz, CDCl_3) δ 10.80 (s, 1H), 9.04 (s, 1H), 8.59 – 8.30 (m, 1H), 8.04 (s, 1H), 7.80 – 7.36 (m, 1H), 7.24 – 6.65 (m, 2H), 4.33 (q, J = 7.0 Hz, 2H), 1.37 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.83, 163.40, 156.82, 137.01, 136.91, 132.95, 131.06, 130.62, 125.78, 123.47, 106.85, 60.94, 29.71, 14.34.



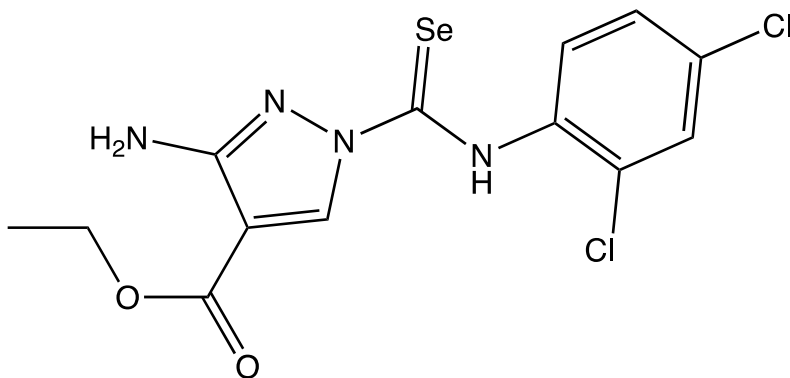
Ethyl-3-amino-1-((2,3-dichlorophenyl)carbamoselenoyl)-1H-pyrazole-4-carboxylate

(RN_04_60A): 0.075 mmol scale, 8 mg, 26% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.29 (s, 1H), 7.99 (d, J = 8.1 Hz, 1H), 7.82 – 7.60 (m, 1H), 7.50 (dd, J = 10.9, 3.9 Hz, 1H), 7.30 (dd, J = 17.2, 9.0 Hz, 1H), 7.14 (s, 2H), 4.32 (q, J = 7.1 Hz, 2H), 1.41 – 1.32 (m, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 145.05, 143.92, 141.83, 133.91, 129.52, 129.46, 127.76, 127.15, 126.37, 126.27, 125.33, 125.10, 118.29, 95.67, 60.05, 59.98, 29.71, 14.52.



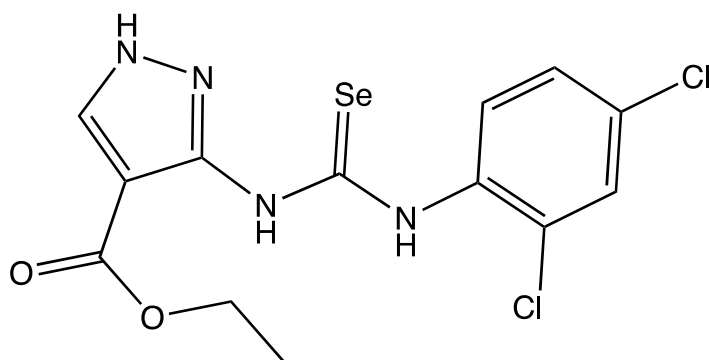
Ethyl-3-(3-(2,3-dichlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate

(RN_04_60B): 0.075 mmol scale, 13 mg, 43% yield. ^1H NMR (400 MHz, CDCl_3) δ 8.53 (dd, J = 57.8, 19.5 Hz, 1H), 7.52 (s, 1H), 6.97 (dd, J = 101.5, 61.2 Hz, 4H), 4.35 (t, J = 14.2 Hz, 2H), 2.07 (s, J = 40.5 Hz, 2H), 1.37 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.95, 163.44, 156.91, 137.02, 136.50, 133.55, 128.72, 127.13, 126.85, 124.07, 106.99, 60.93, 29.71, 14.37.



Ethyl-3-amino-1-((2,4-dichlorophenyl)carbamoselenoyl)-1H-pyrazole-4-carboxylate

(RN_04_61A): 0.071 mmol scale, 7 mg, 23% yield. ^1H NMR (400 MHz, CDCl_3) δ 7.82 – 7.57 (m, 1H), 7.54 – 7.48 (m, 1H), 7.36 (s, 1H), 7.28 (d, J = 9.3 Hz, 1H), 7.23 – 6.60 (m, 4H), 4.30 (dd, J = 13.0, 6.3 Hz, 2H), 1.36 (dd, J = 11.0, 6.6 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.03, 163.85, 141.82, 141.03, 133.87, 133.01, 130.71, 130.59, 130.15, 129.93, 129.74, 128.75, 128.05, 127.96, 127.52, 127.15, 120.96, 95.91, 95.82, 60.04, 59.96, 29.71, 14.51, 14.49.

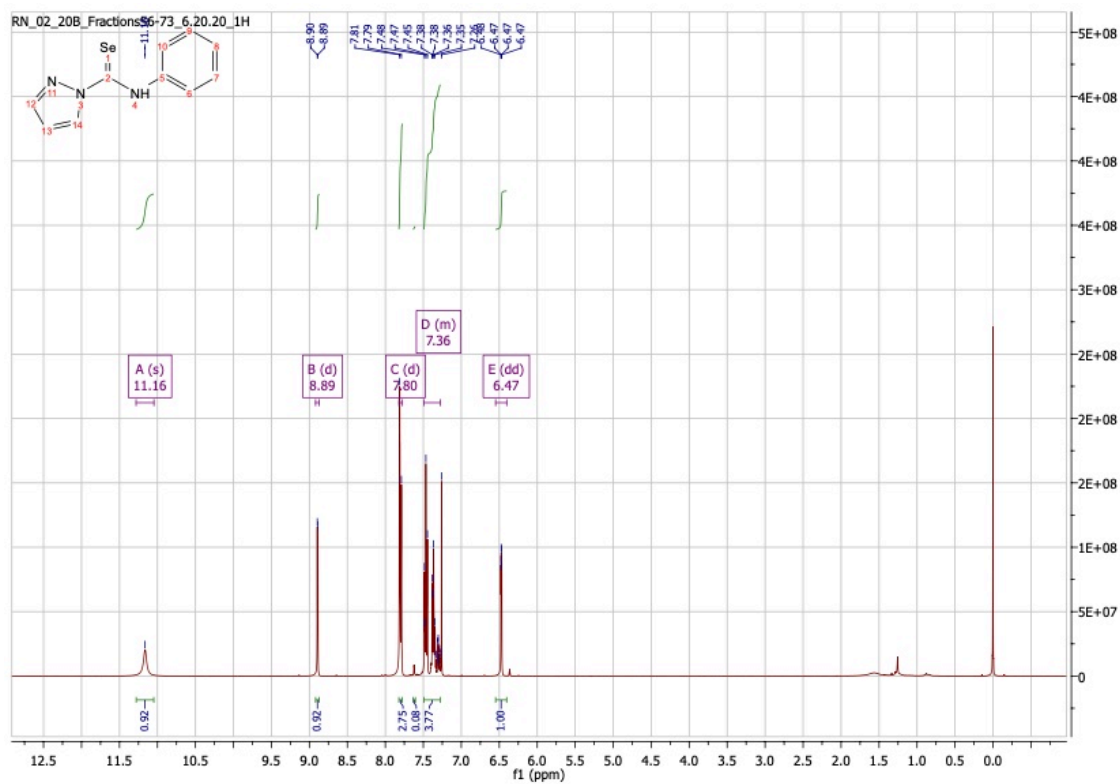


Ethyl-3-(3-(2,4-dichlorophenyl)selenoureido)-1H-pyrazole-4-carboxylate

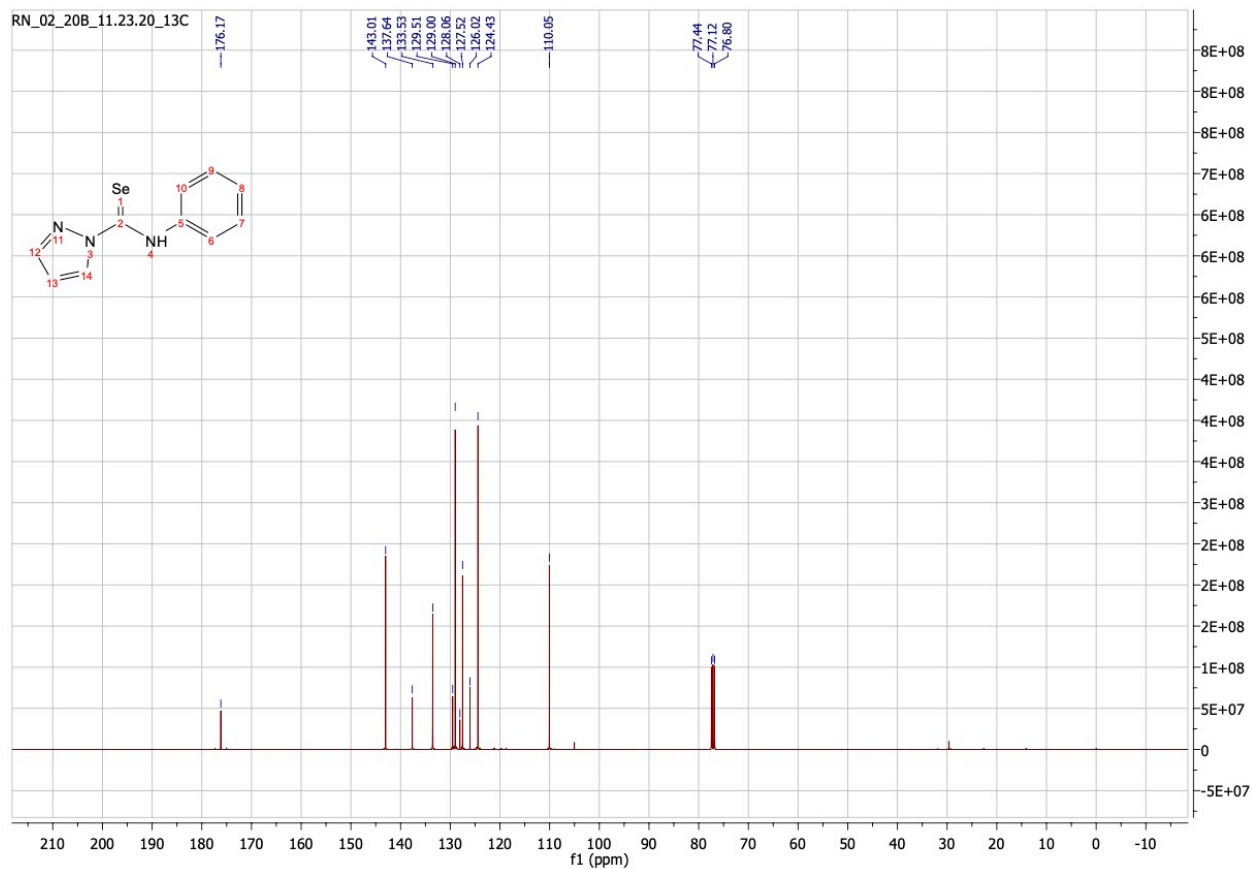
(RN_04_61B): 0.071 mmol scale, 8 mg, 26% yield. ^1H NMR (400 MHz, CDCl_3) δ 11.09 (s, 1H), 9.05 (s, 1H), 8.62 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 1.8 Hz, 1H), 7.34 (d, J = 8.6 Hz, 1H), 7.22 – 6.69 (m, 1H), 5.10 (s, 1H), 4.42 – 4.25 (m, 2H), 1.38 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.69, 163.44, 156.92, 136.94, 133.56, 132.91, 129.54, 128.62, 127.39, 126.42, 106.94, 60.91, 29.71, 14.35.

Appendix B

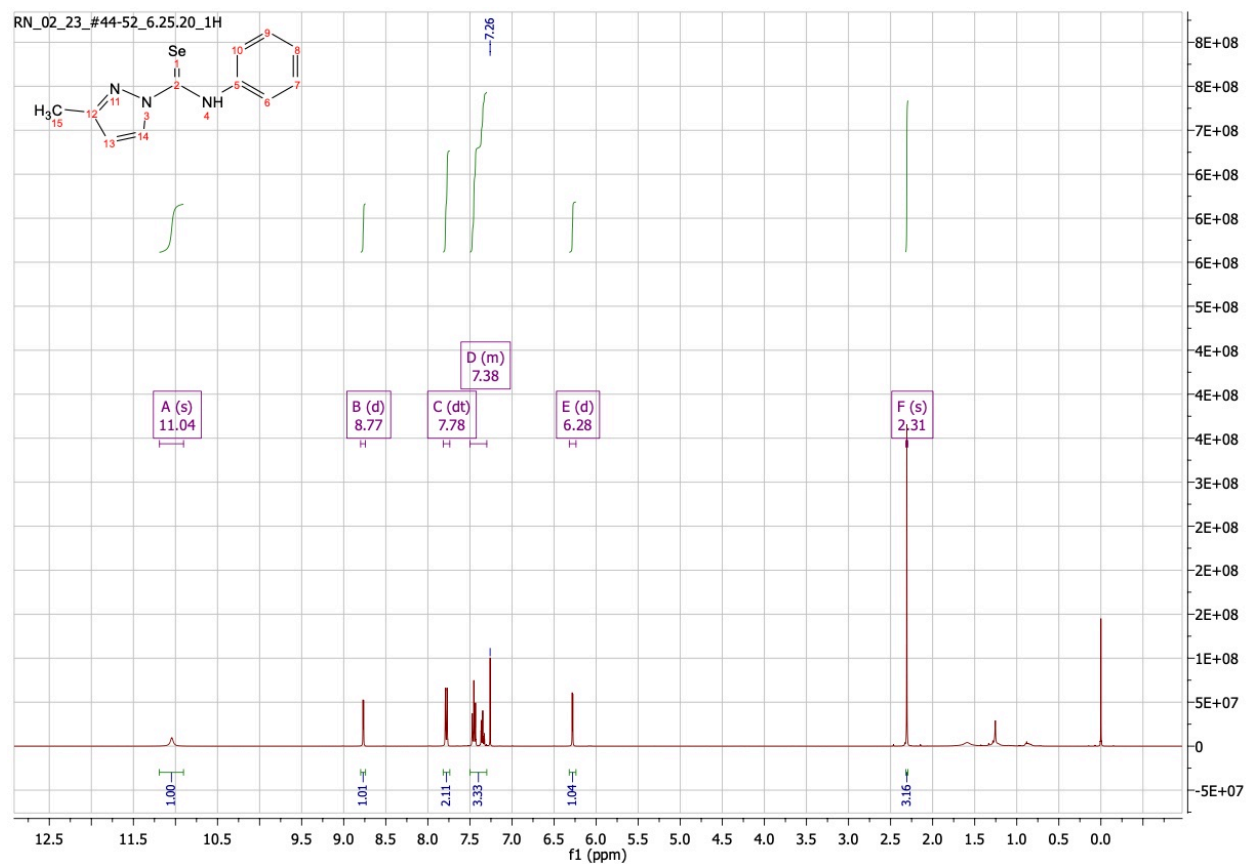
*N*NSeN NMR Spectra



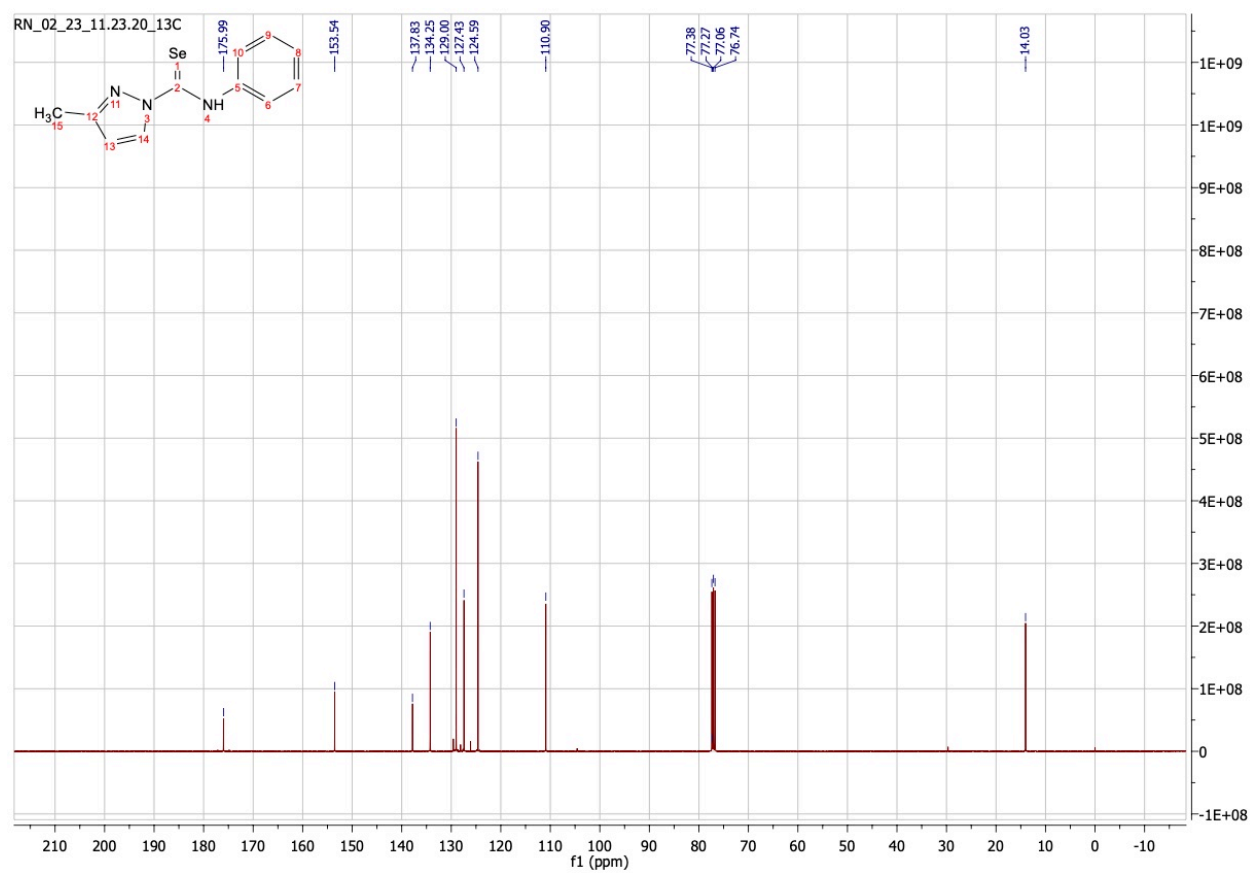
NMR Spectrum 1: RN_02_20B 1H-NMR



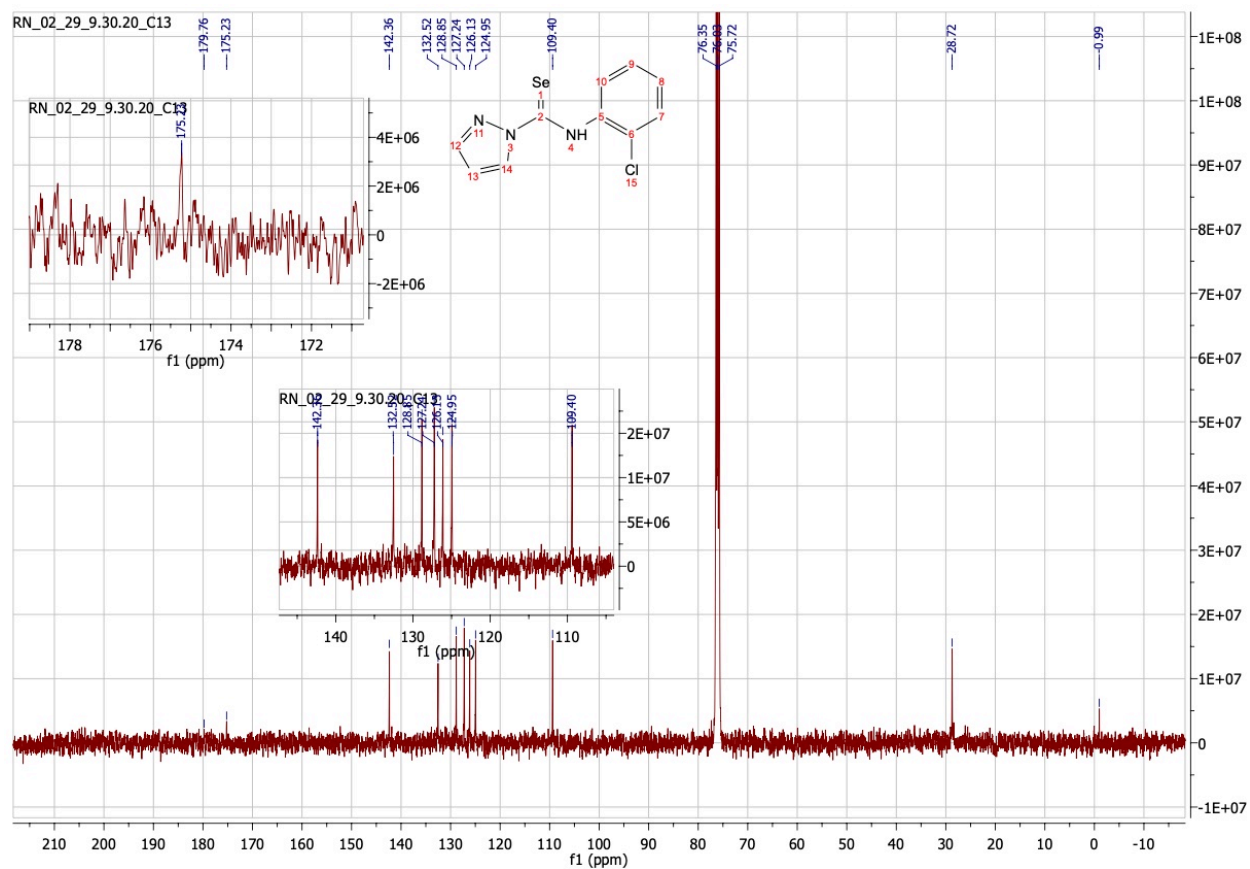
NMR Spectrum 2: RN_02_20B 13C-NMR



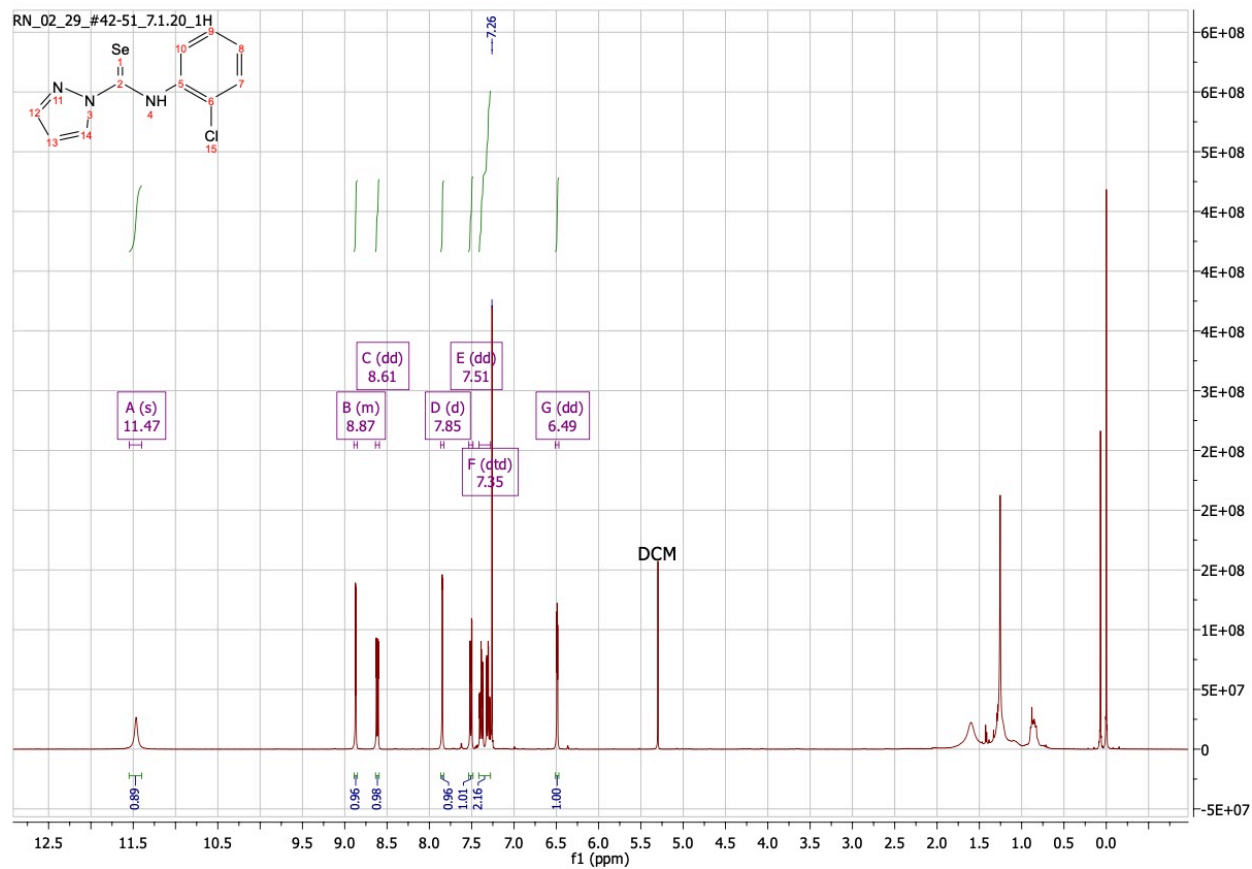
NMR Spectrum 3: RN_02_23 1H-NMR



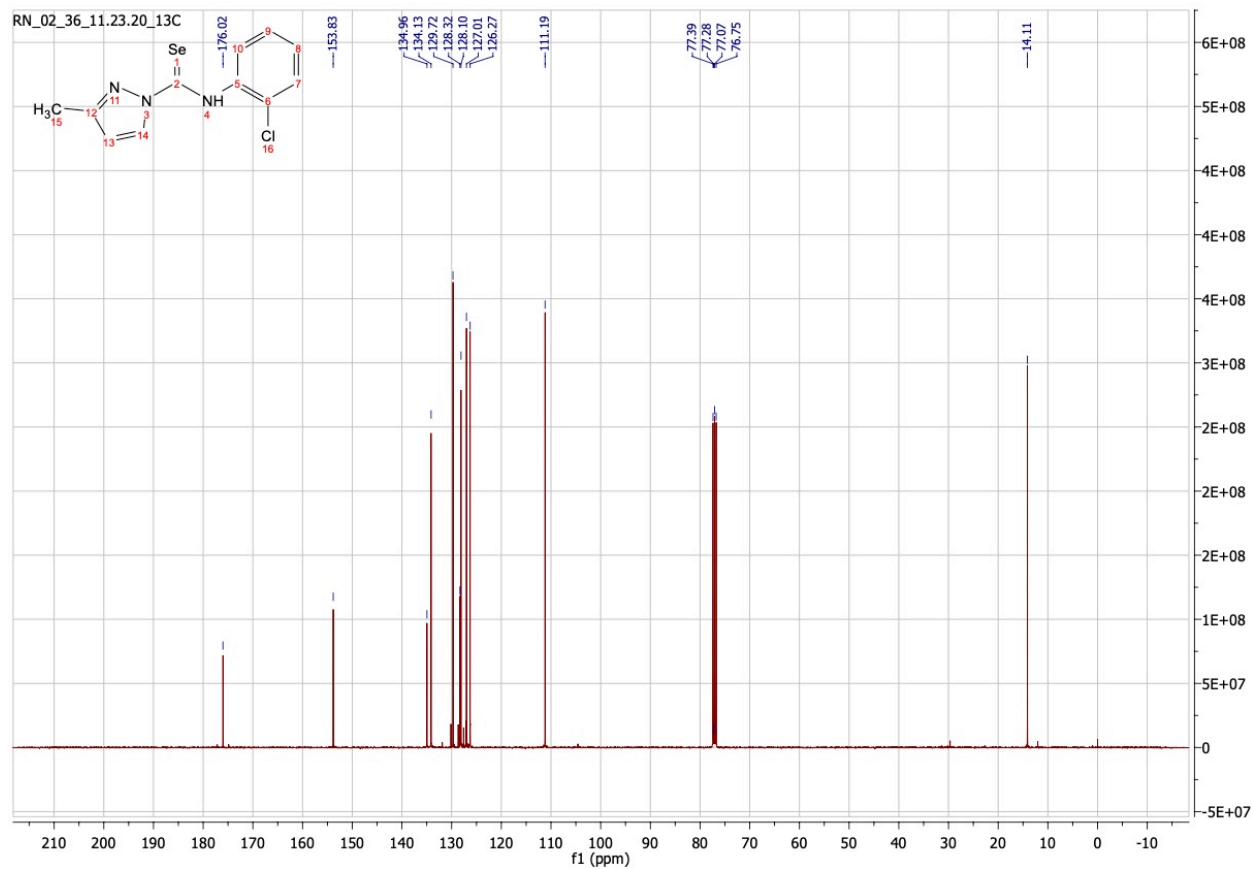
NMR Spectrum 4: RN_02_23 13C-NMR



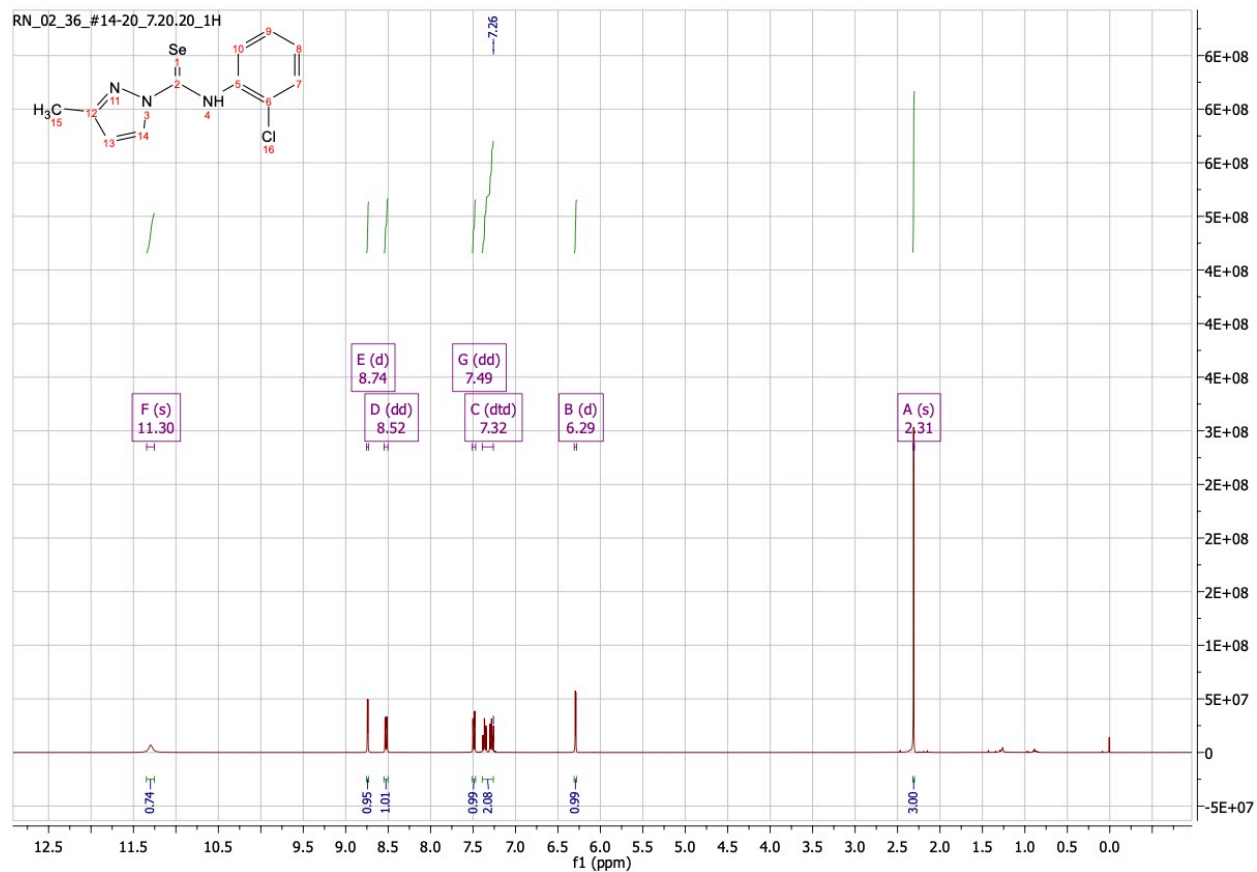
NMR Spectrum 5: RN_02_29 ^{13}C -NMR



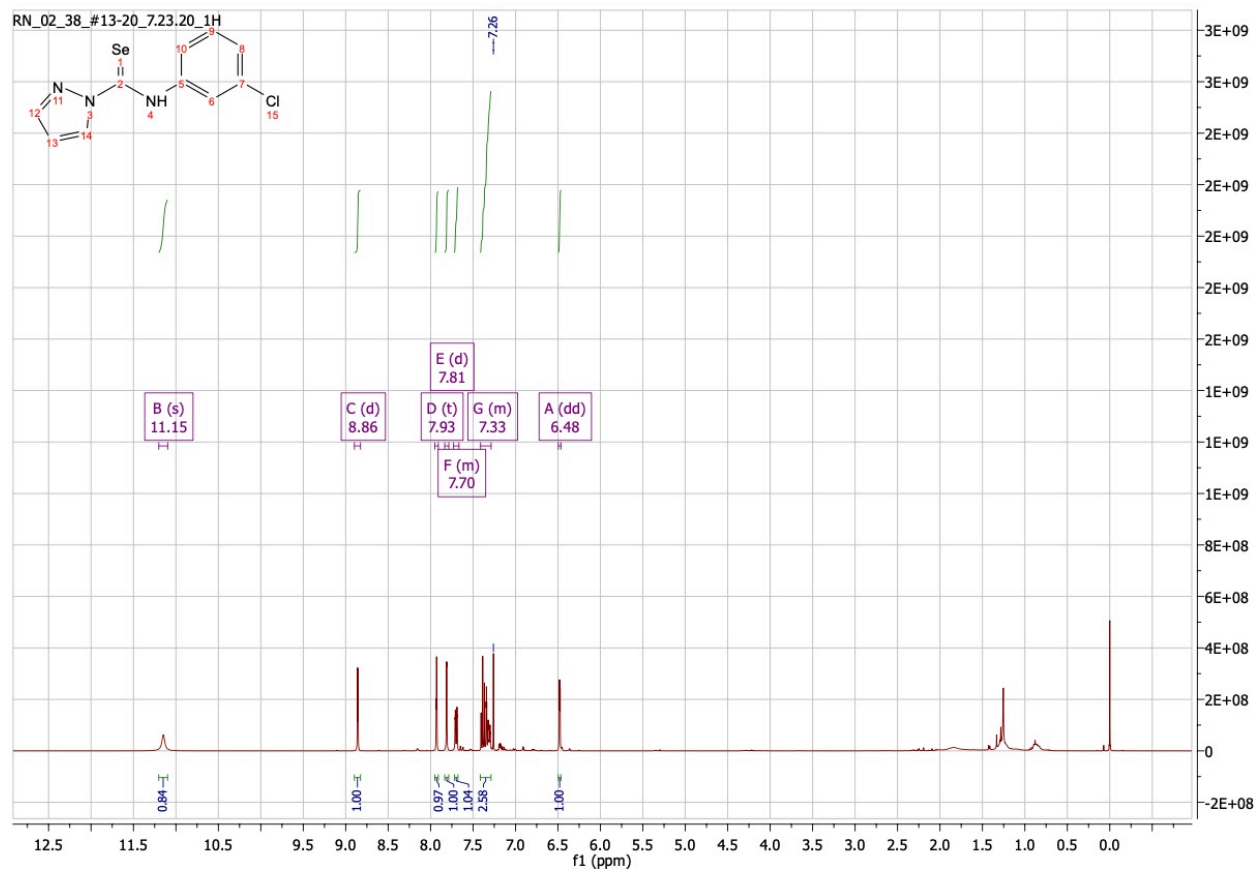
NMR Spectrum 6: RN_02_29 1H-NMR



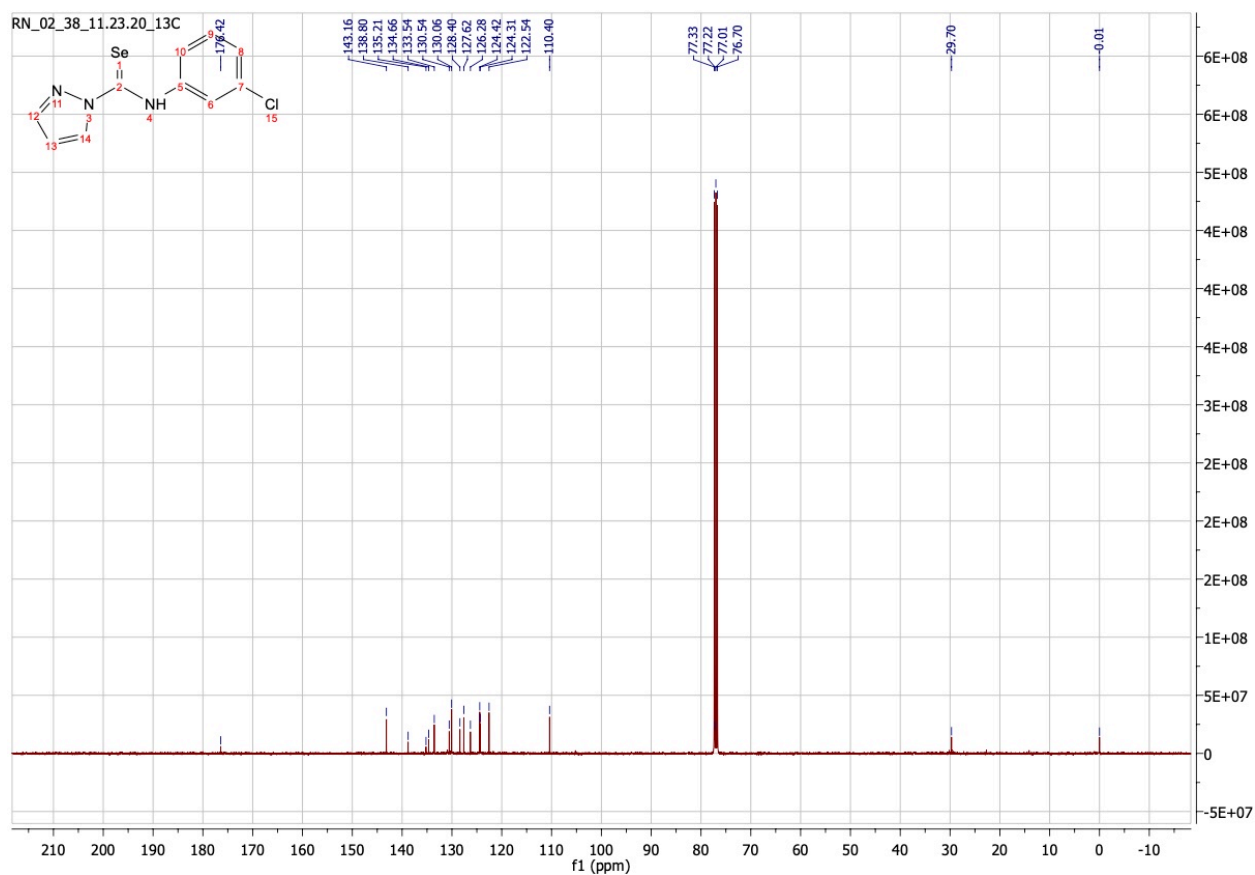
NMR Spectrum 7: RN_02_36 ¹³C-NMR



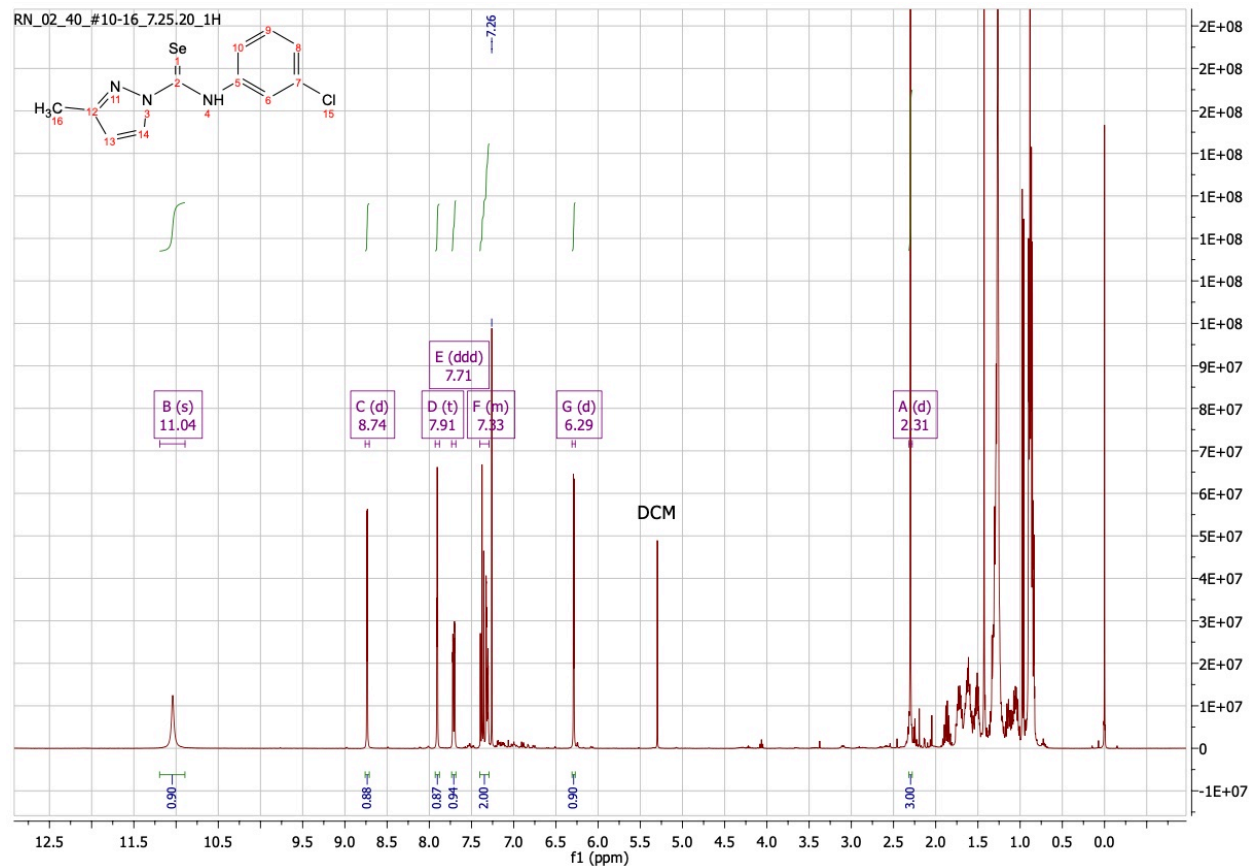
NMR Spectrum 8: RN_02_36 1H-NMR



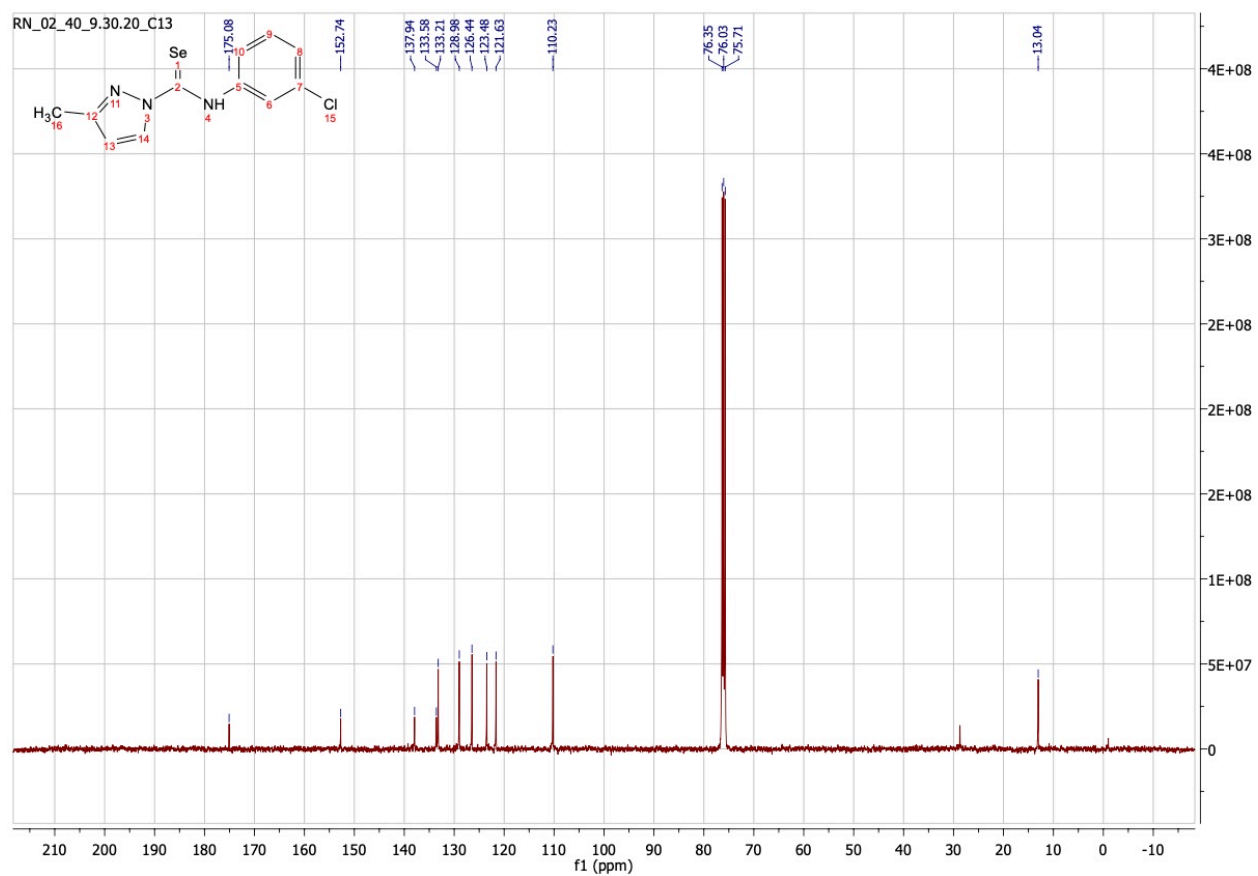
NMR Spectrum 9: RN_02_38 1H-NMR



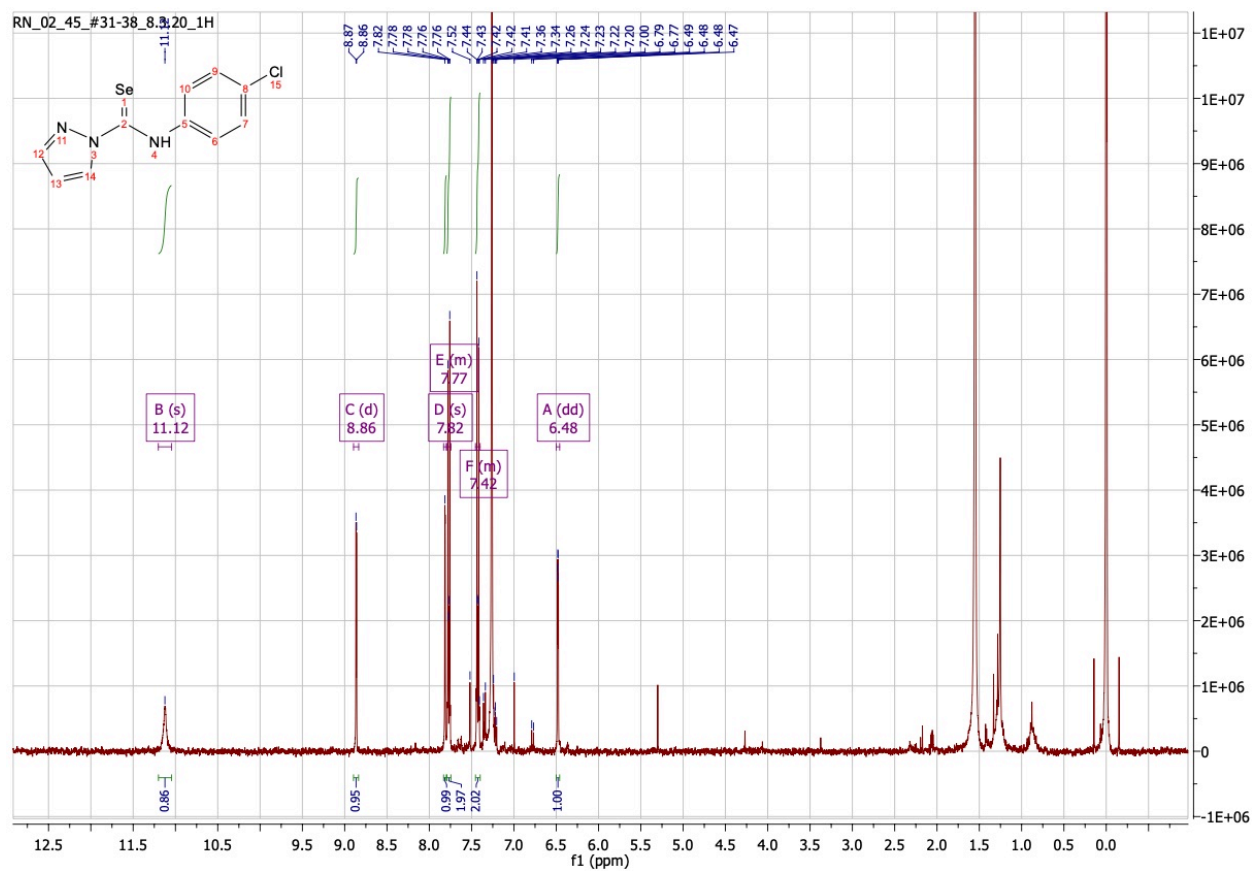
NMR Spectrum 10: RN_02_38 13C-NMR



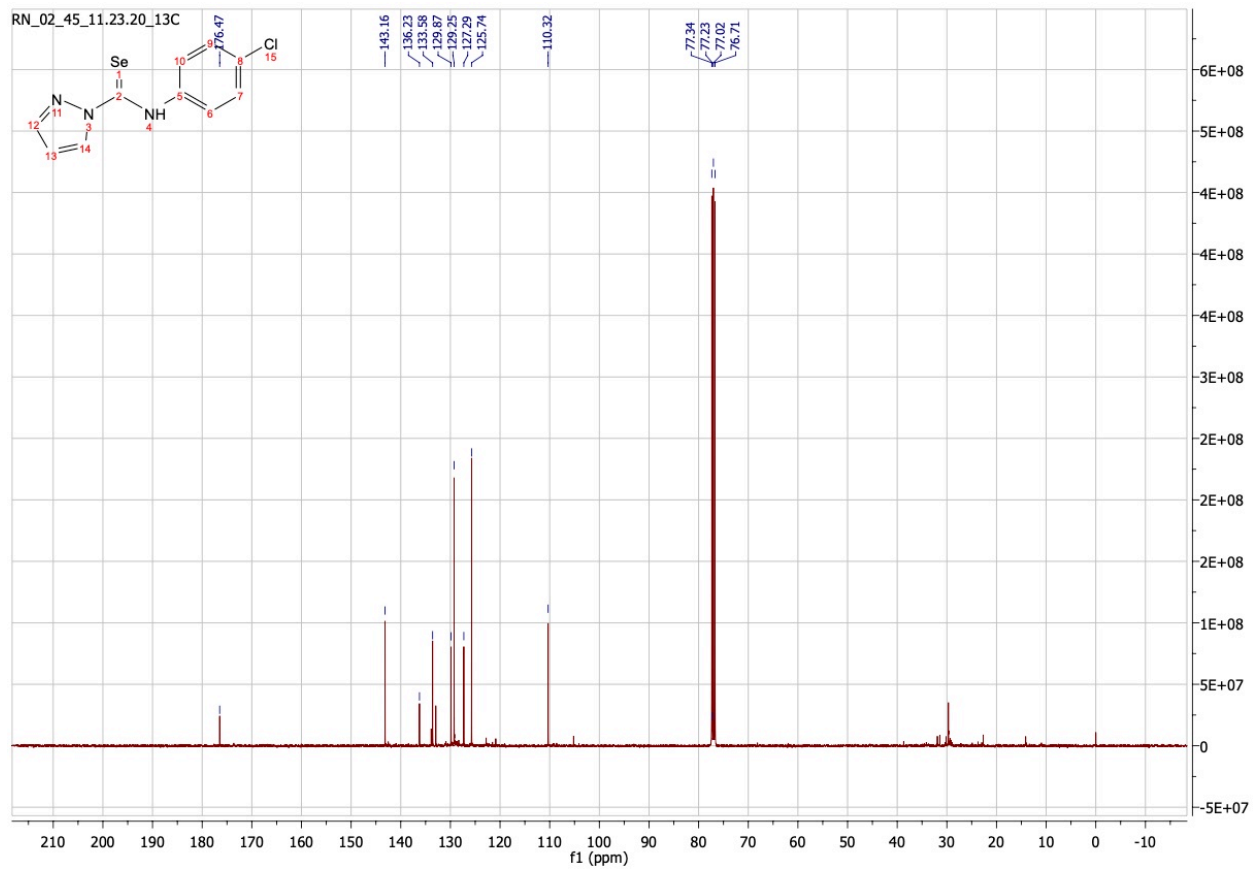
NMR Spectrum 11: RN_02_40 1H-NMR



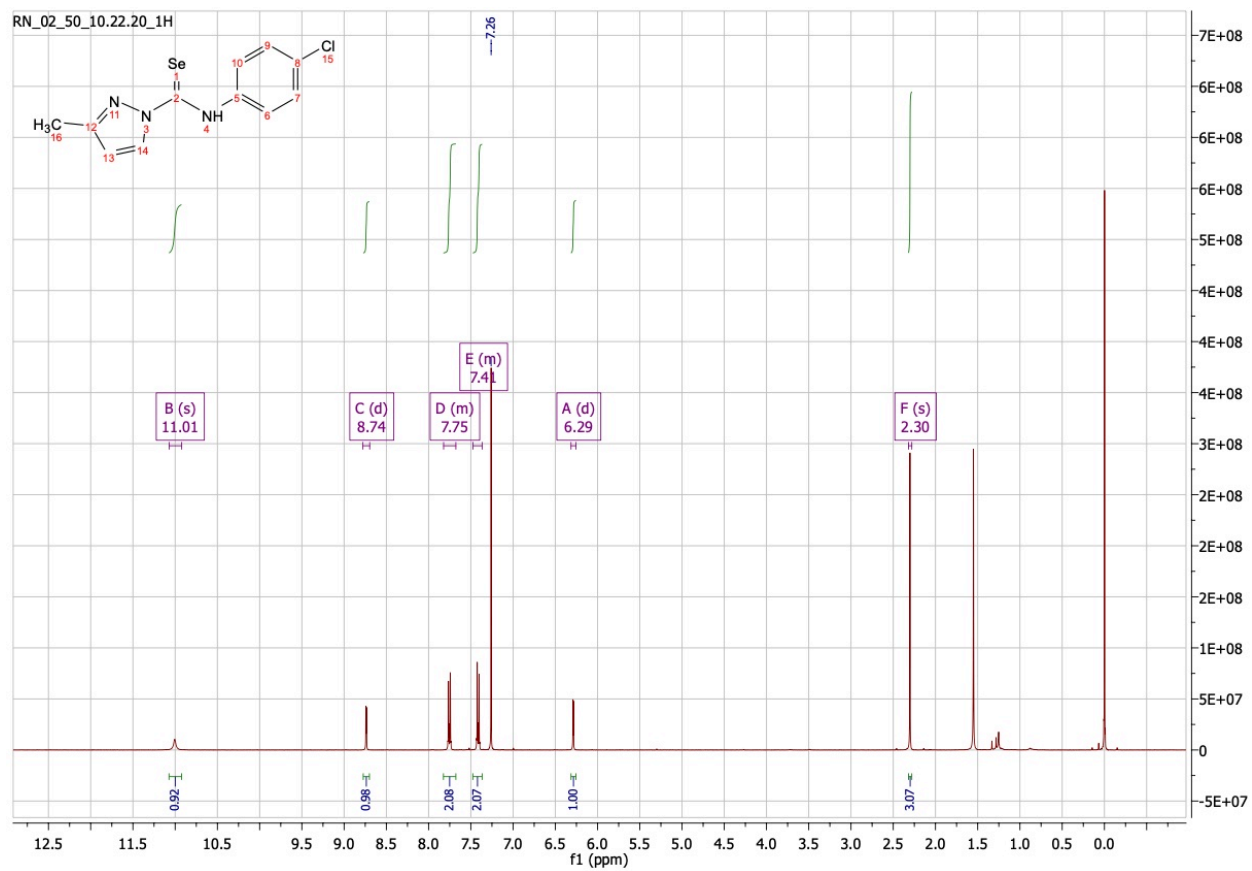
NMR Spectrum 12: RN_02_40 ¹³C-NMR



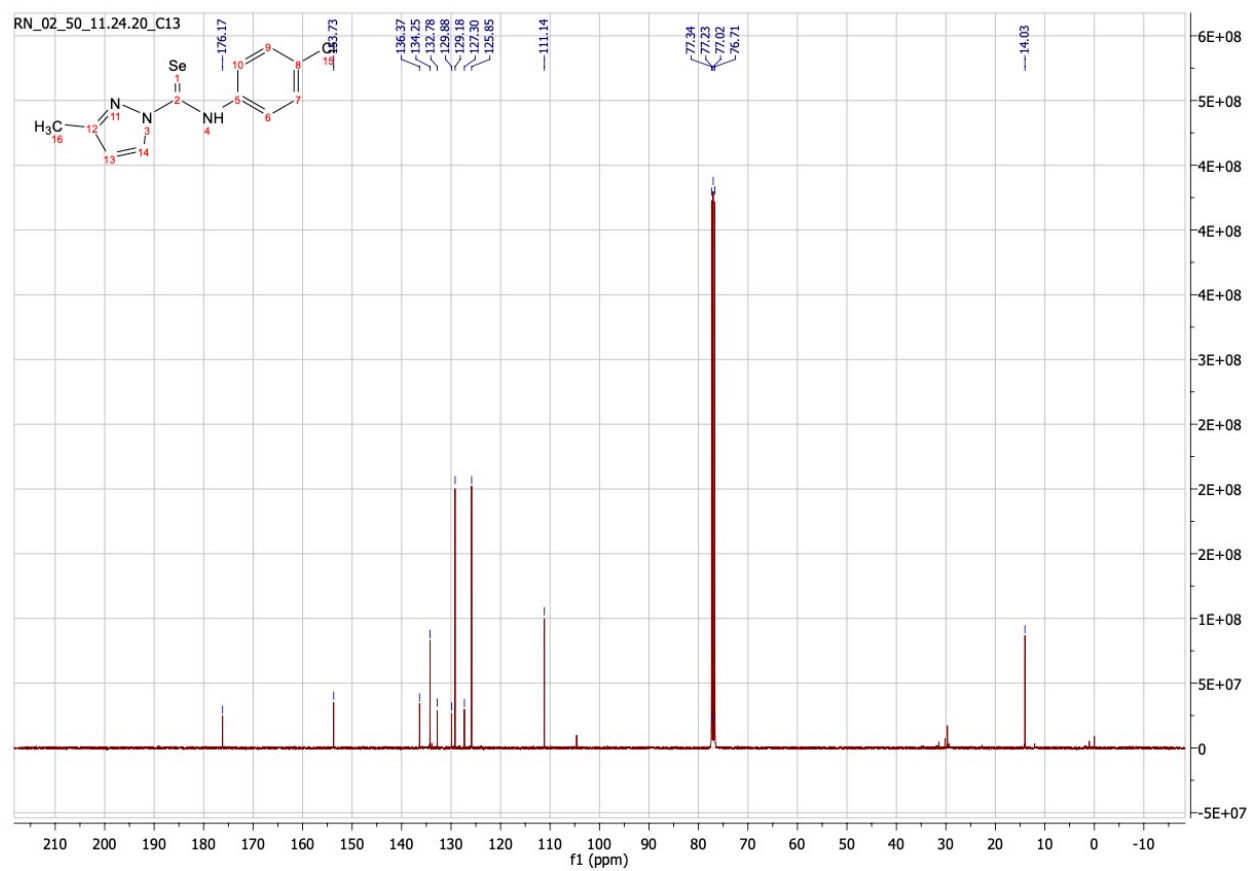
NMR Spectrum 13: RN_02_45 1H-NMR



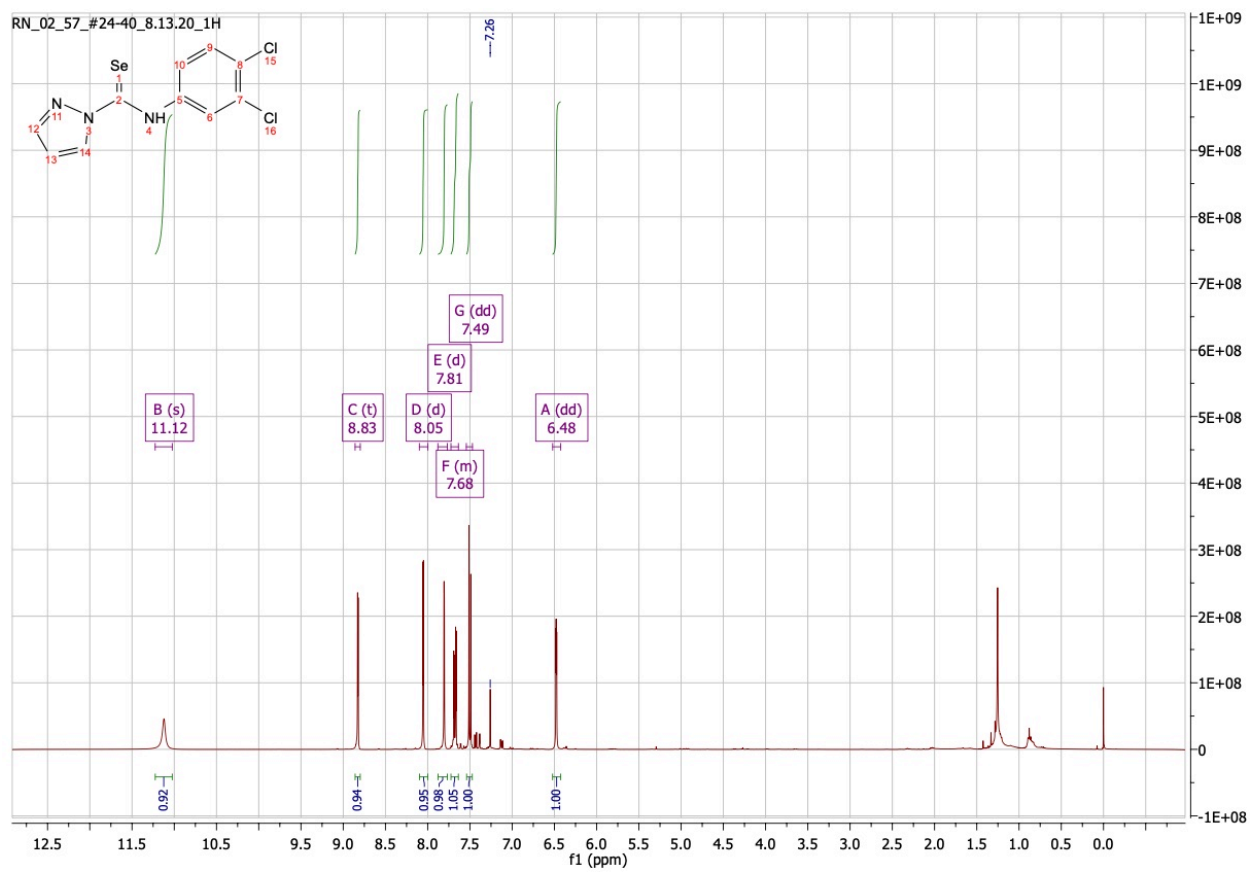
NMR Spectrum 14: RN_02_45 ¹³C-NMR



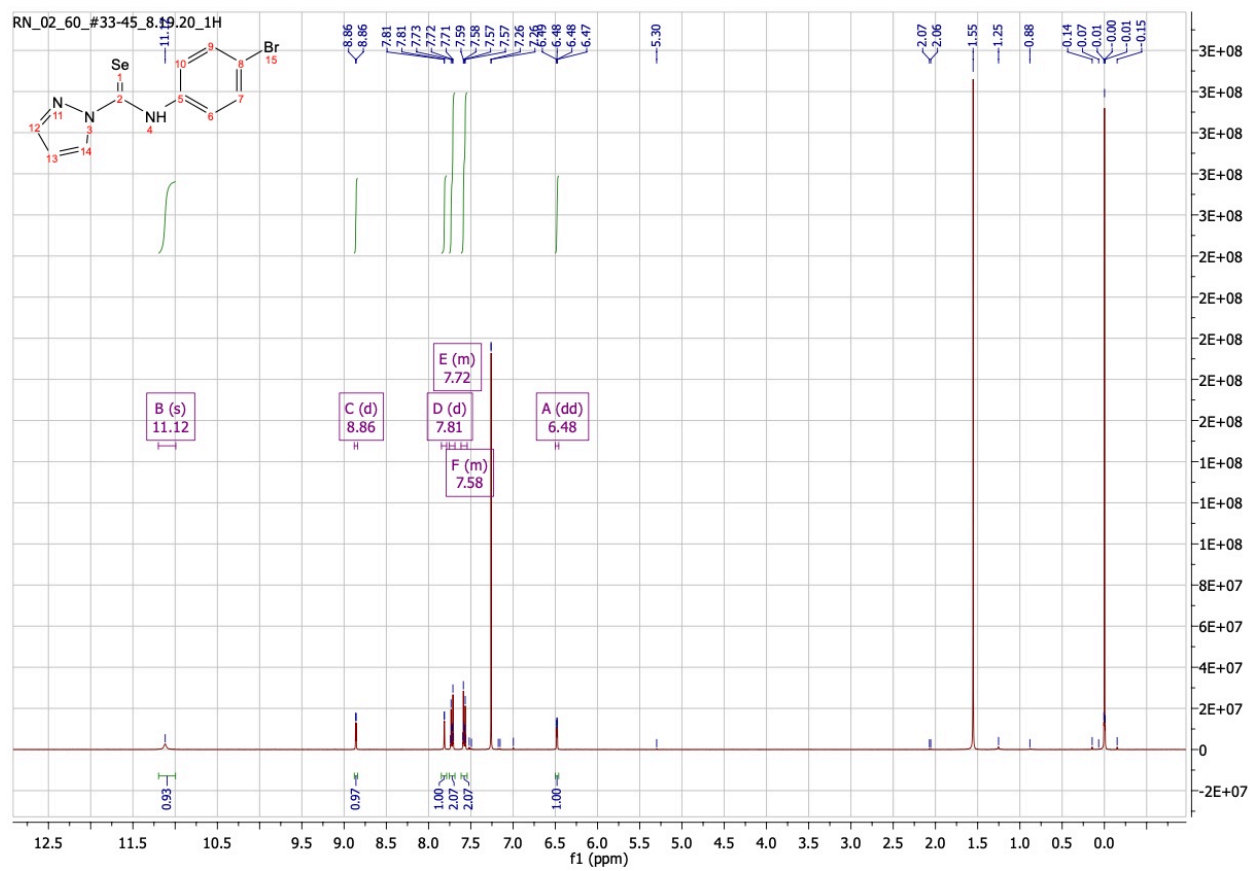
NMR Spectrum 15: RN_02_50 1H-NMR



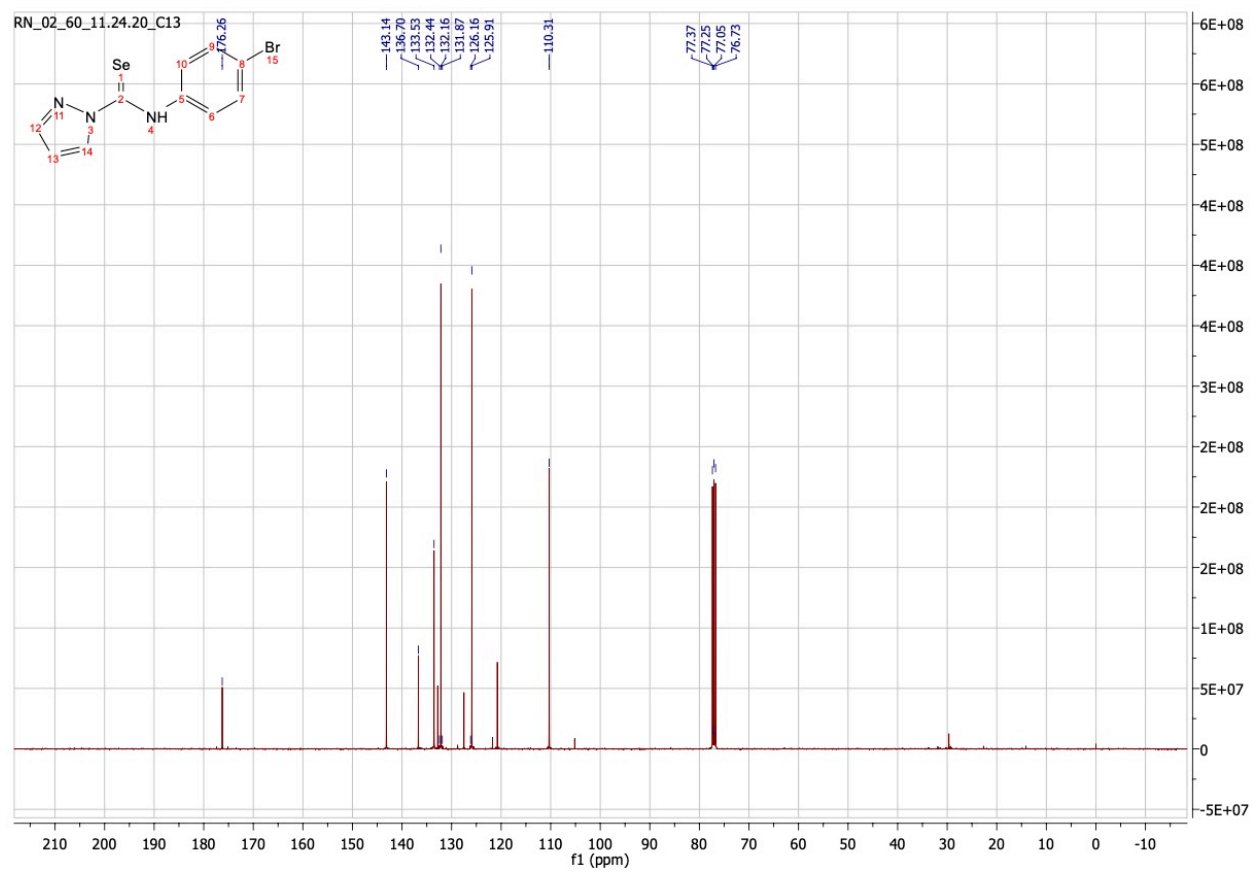
NMR Spectrum 16: RN_02_50 ^{13}C -NMR



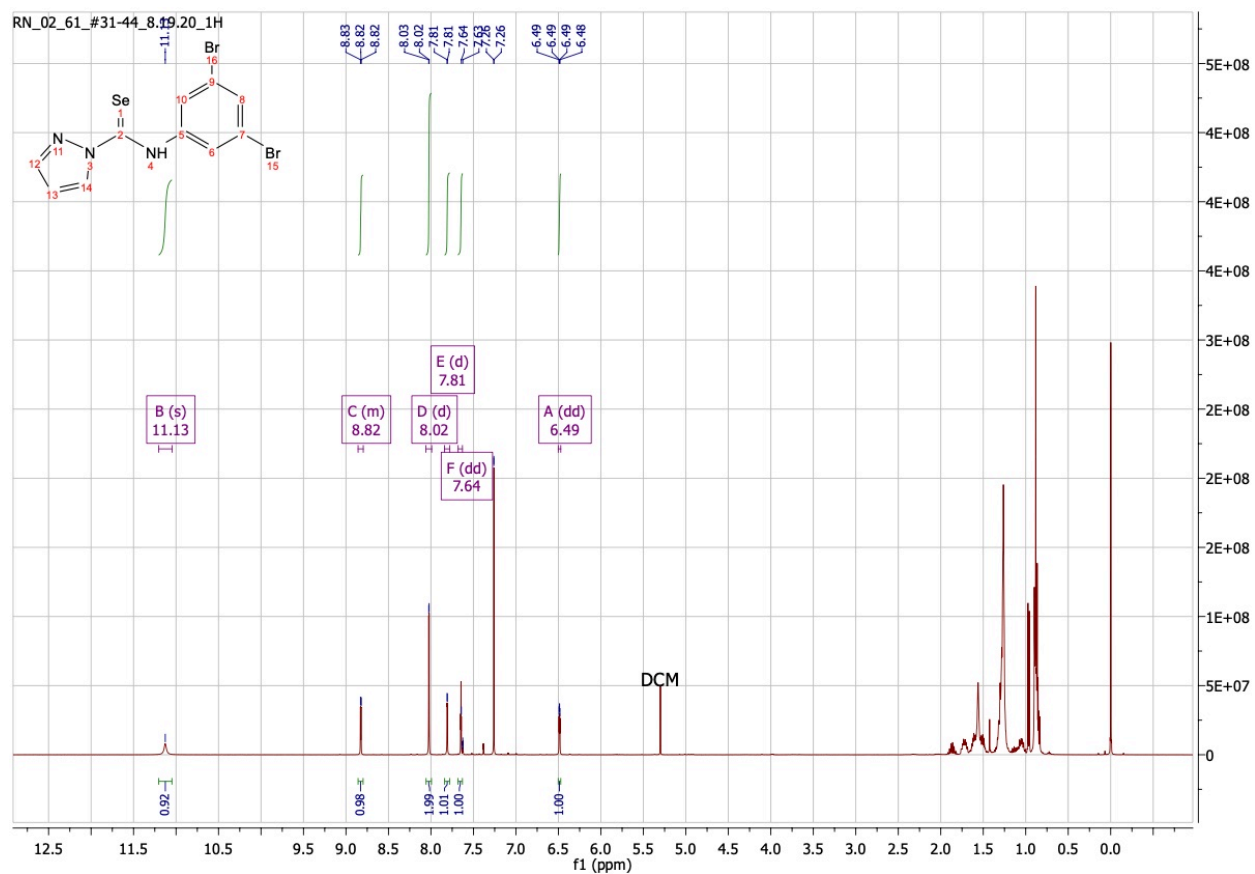
NMR Spectrum 17: RN_02_57 1H-NMR



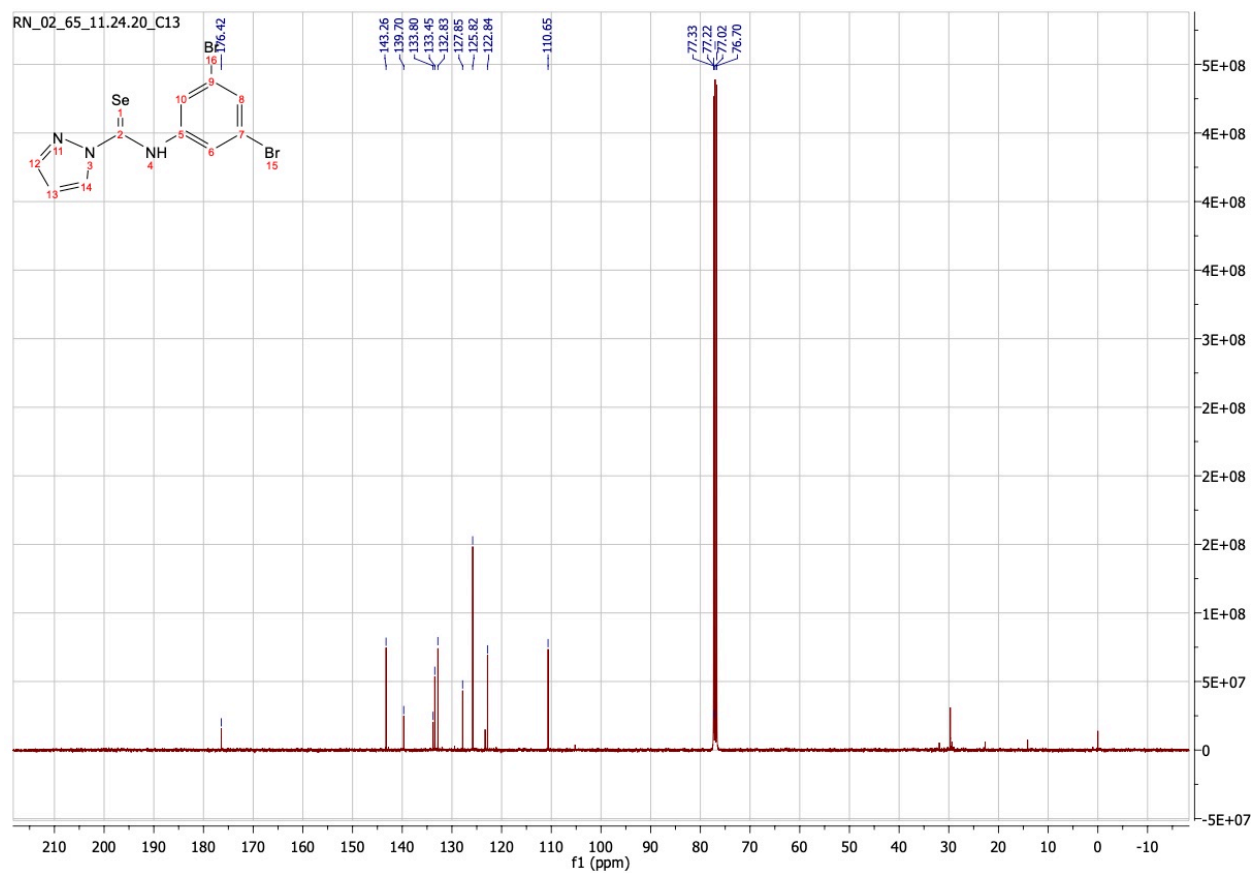
NMR Spectrum 19: RN_02_60 1H-NMR



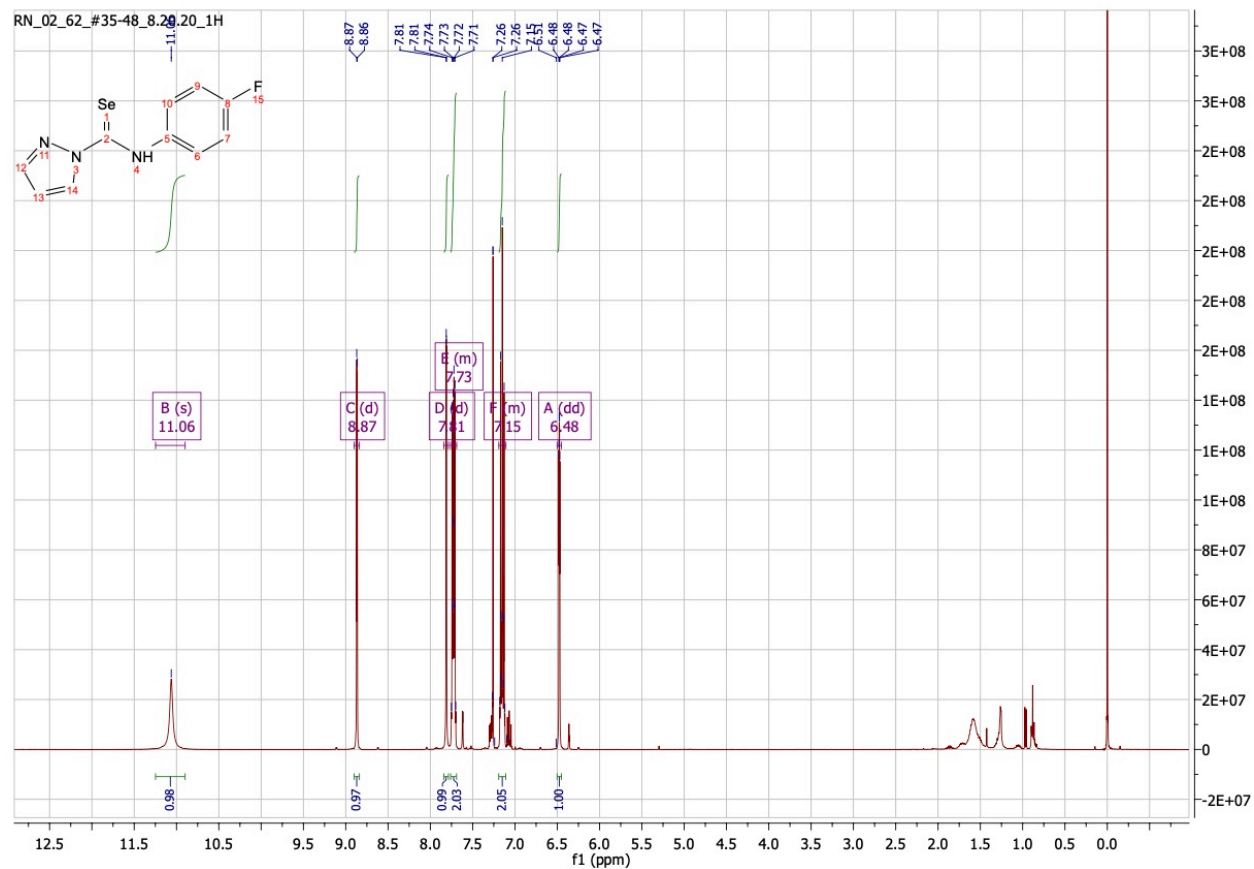
NMR Spectrum 20: RN_02_60 ¹³C-NMR



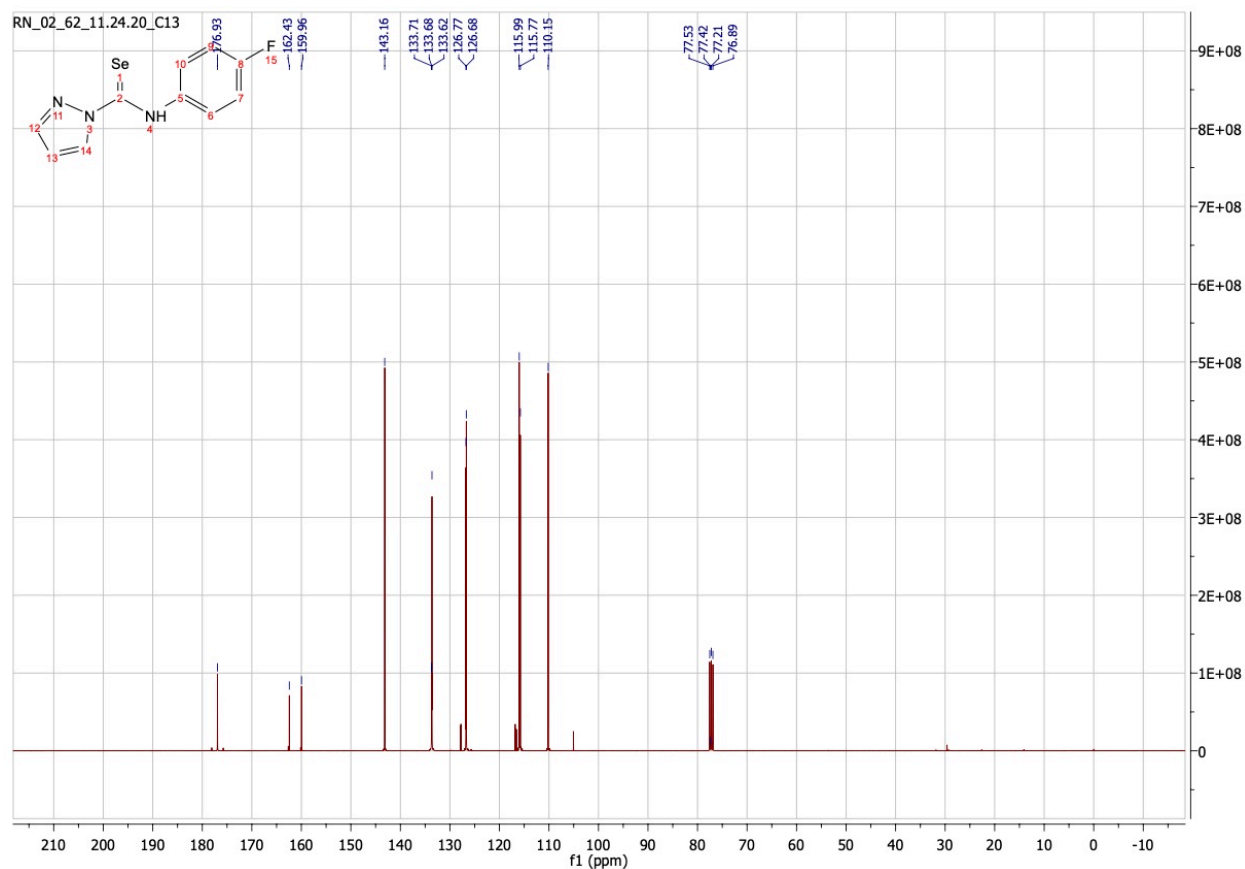
NMR Spectrum 21: RN_02_61 (same structure as RN_02_65) ¹H-NMR



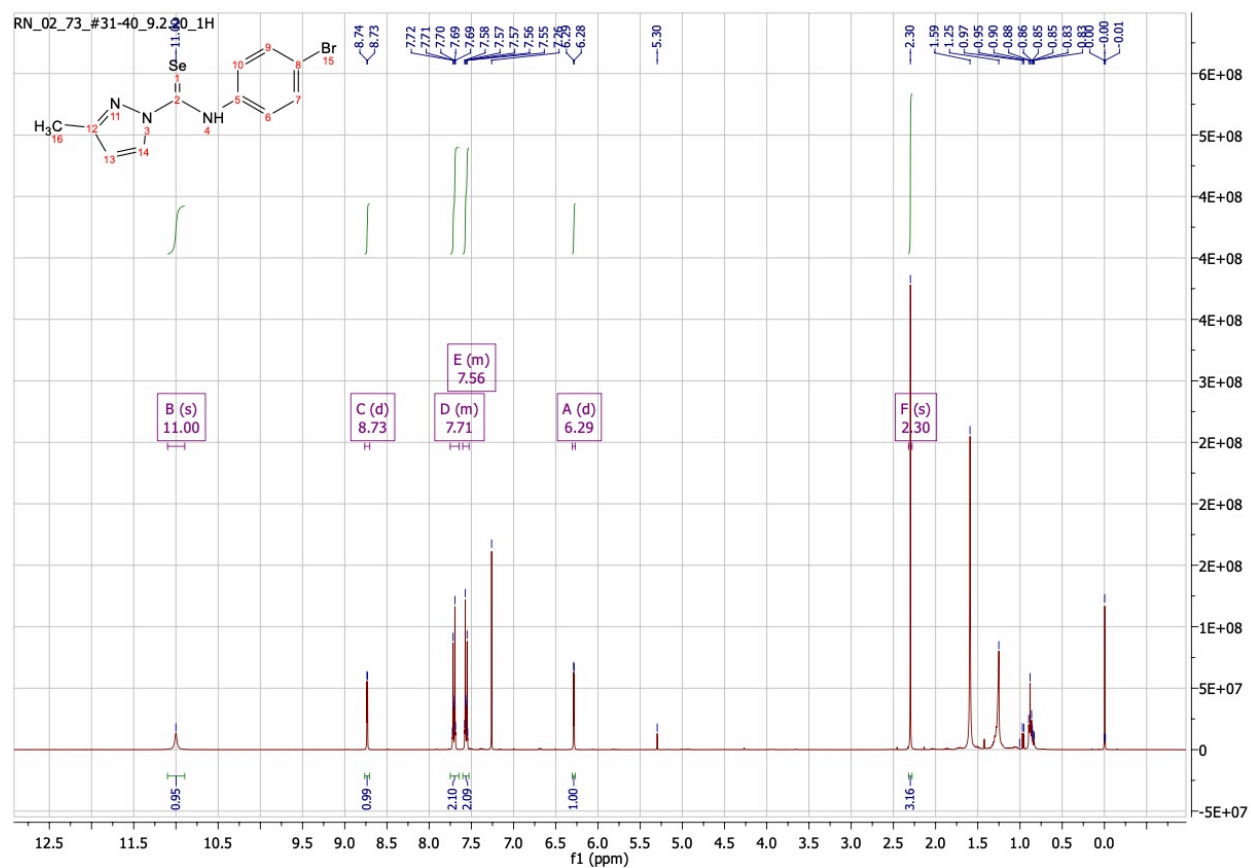
NMR Spectrum 22: RN_02_65 (same structure as RN_02_61) 13C-NMR



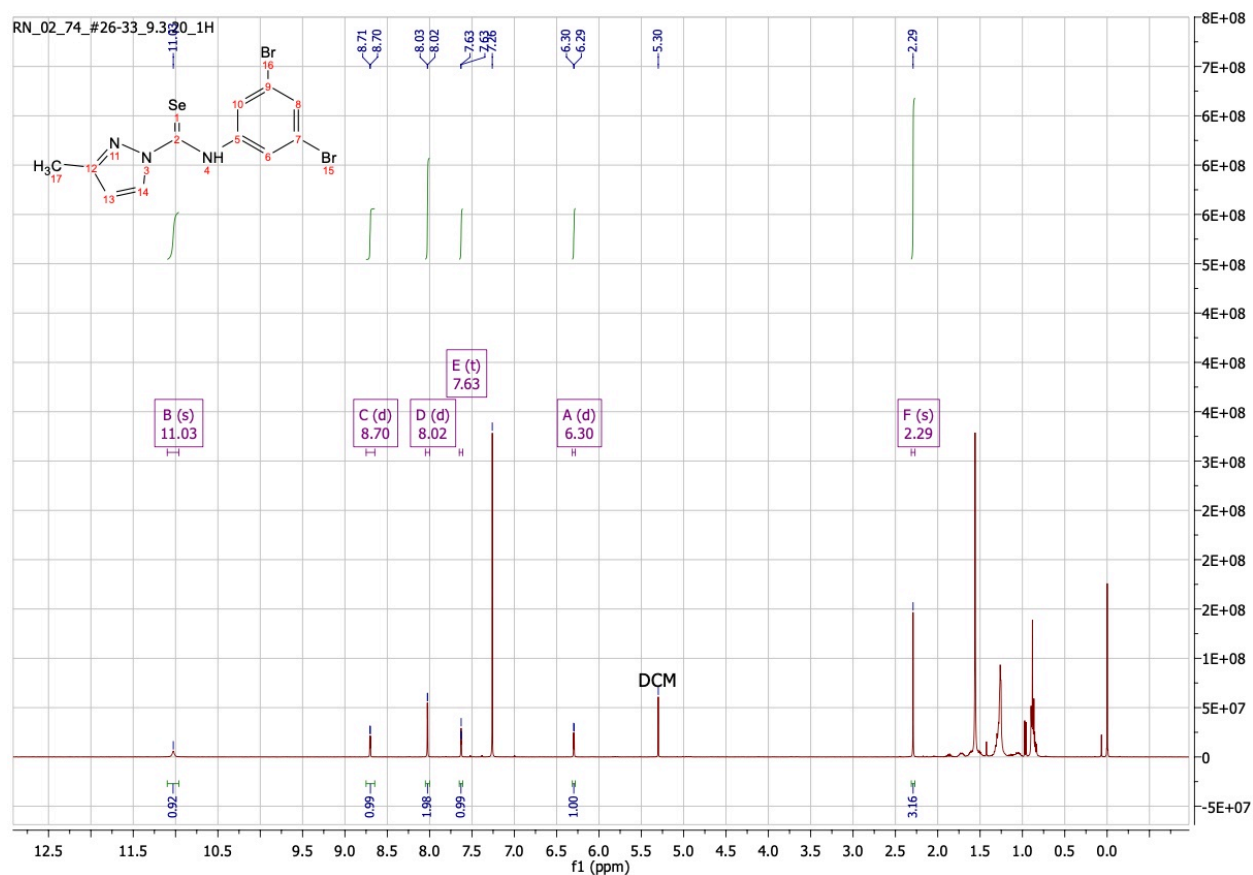
NMR Spectrum 23: RN_02_62 1H-NMR



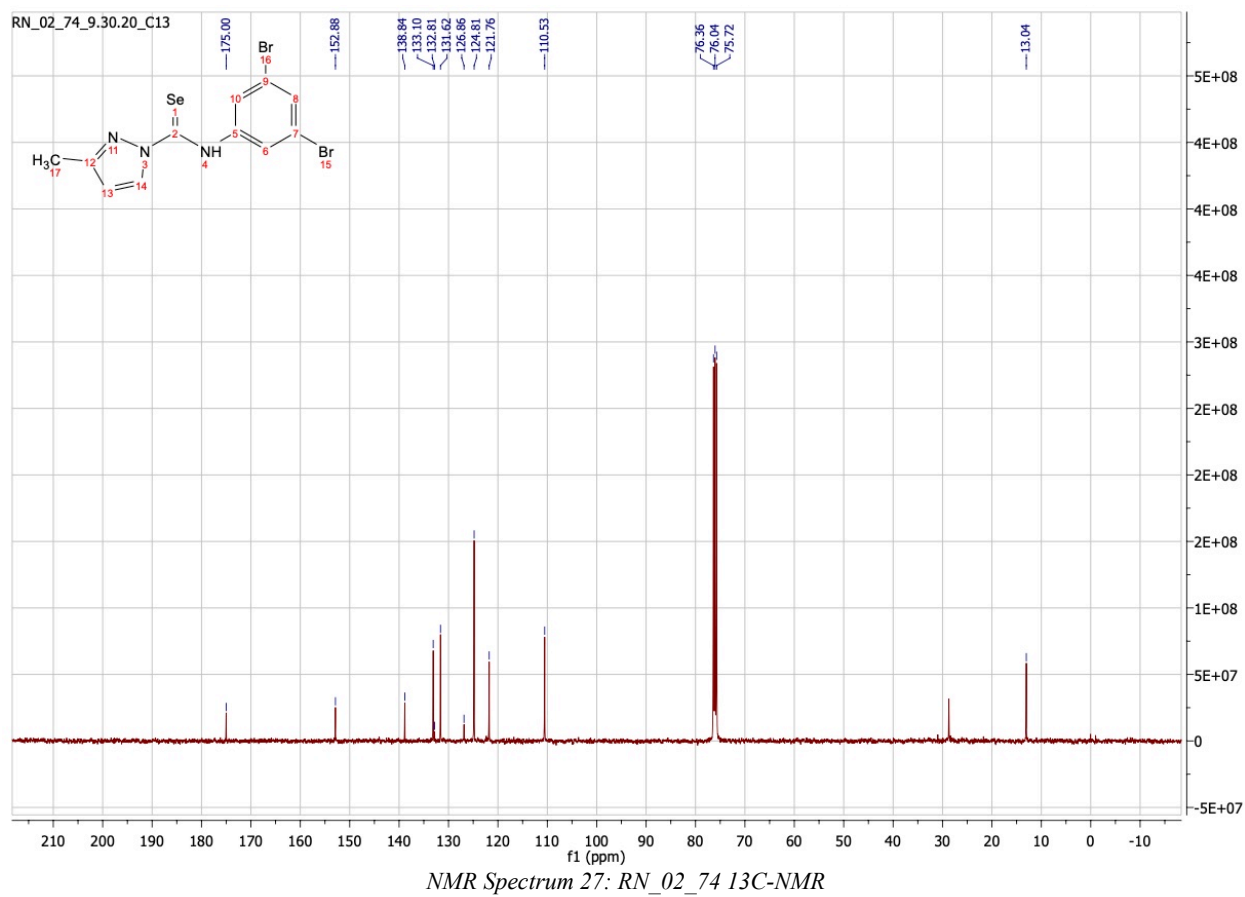
NMR Spectrum 24: RN_02_62 ^{13}C -NMR

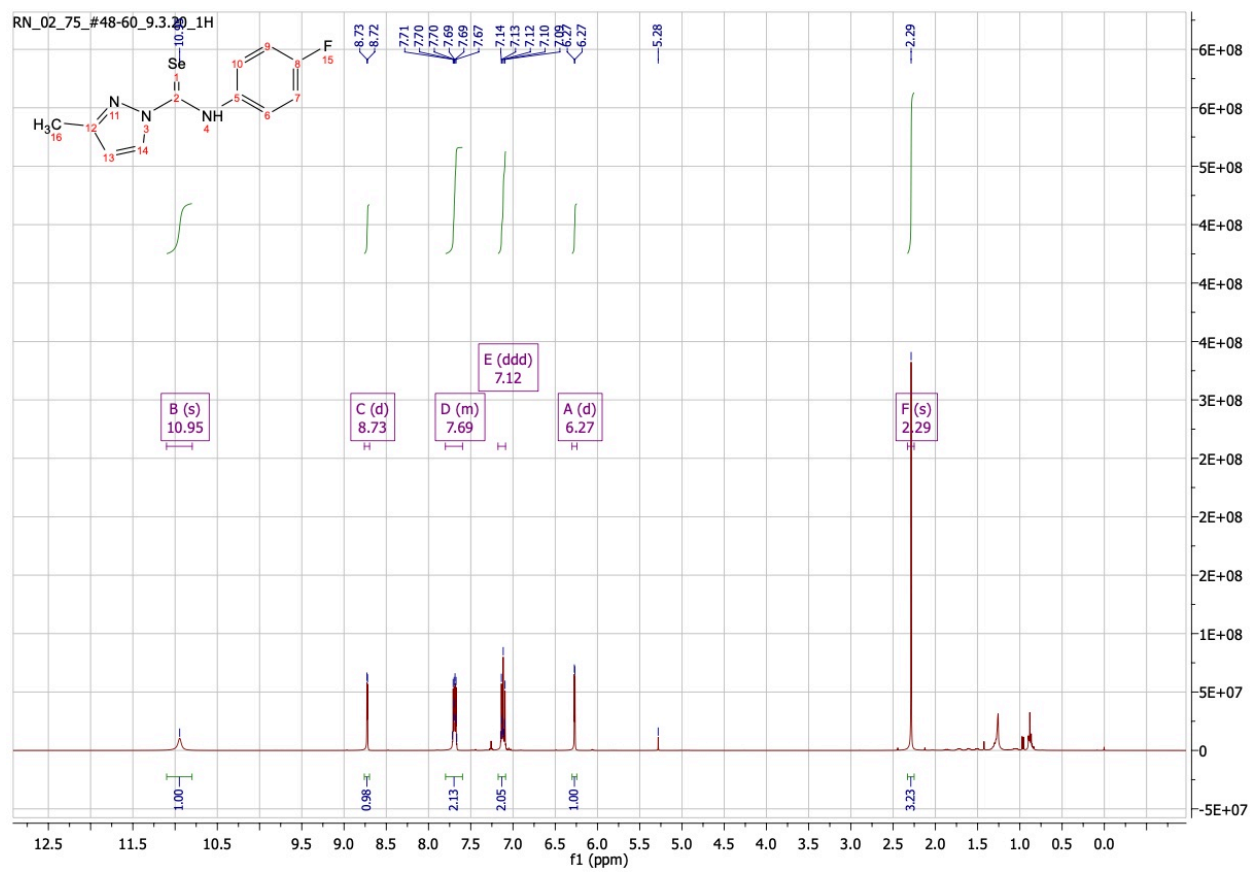


NMR Spectrum 25: RN_02_73 1H-NMR

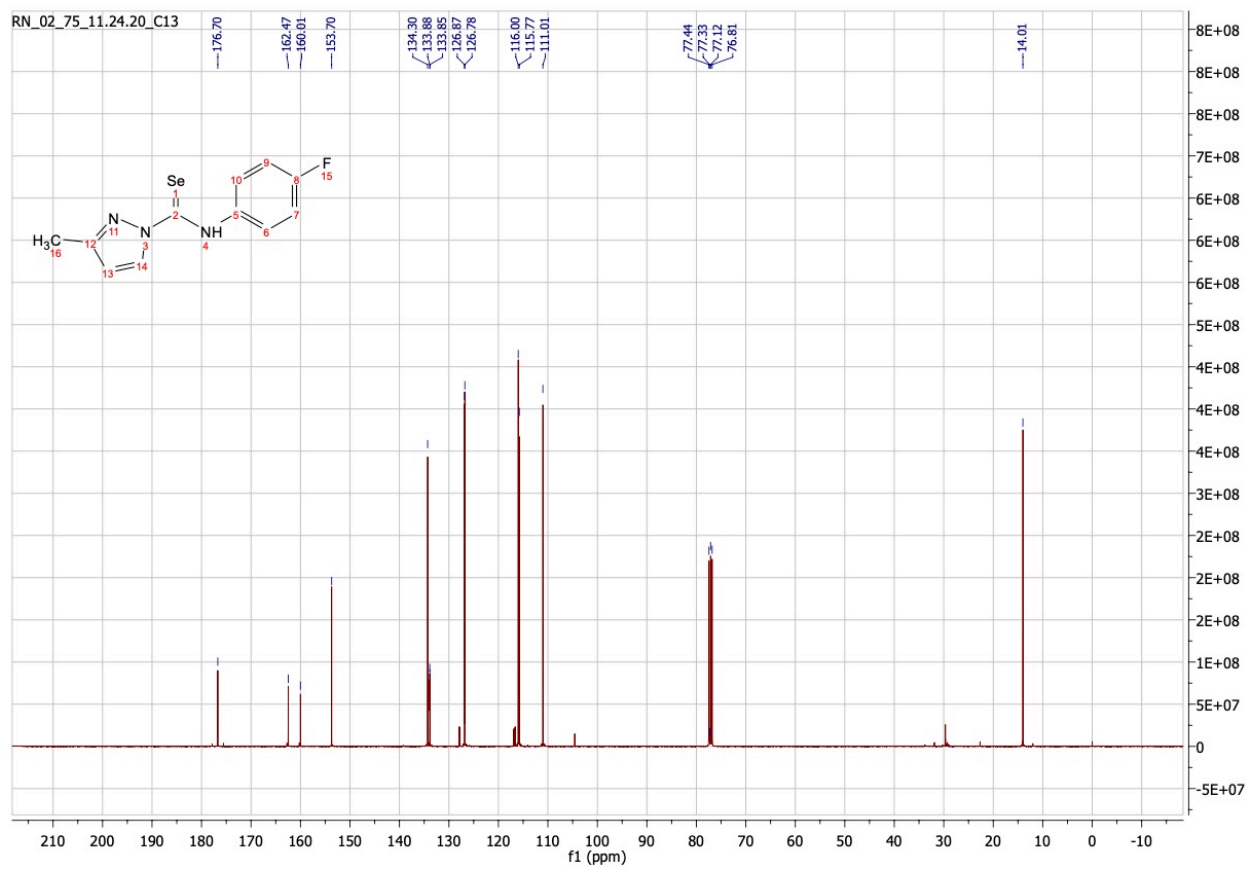


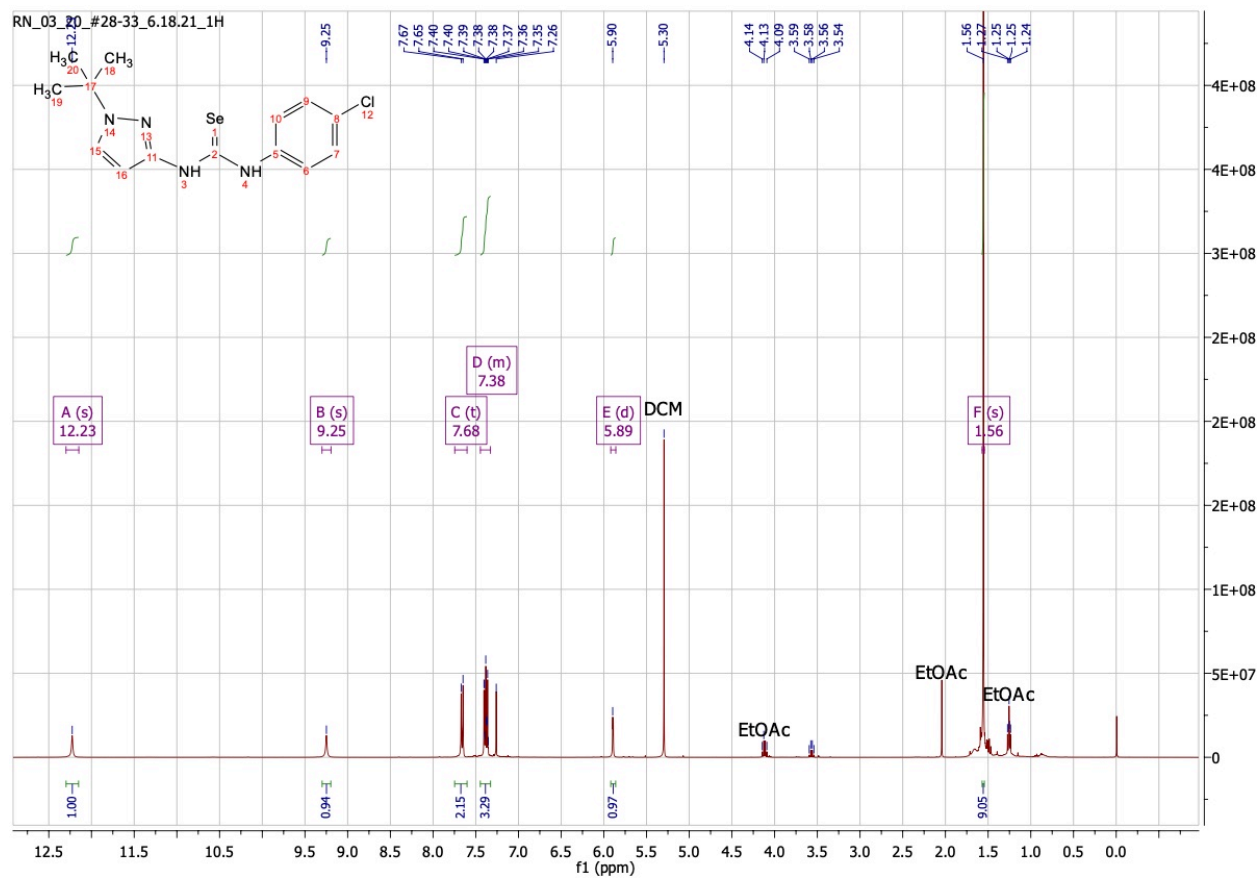
NMR Spectrum 26: RN_02_74 1H-NMR



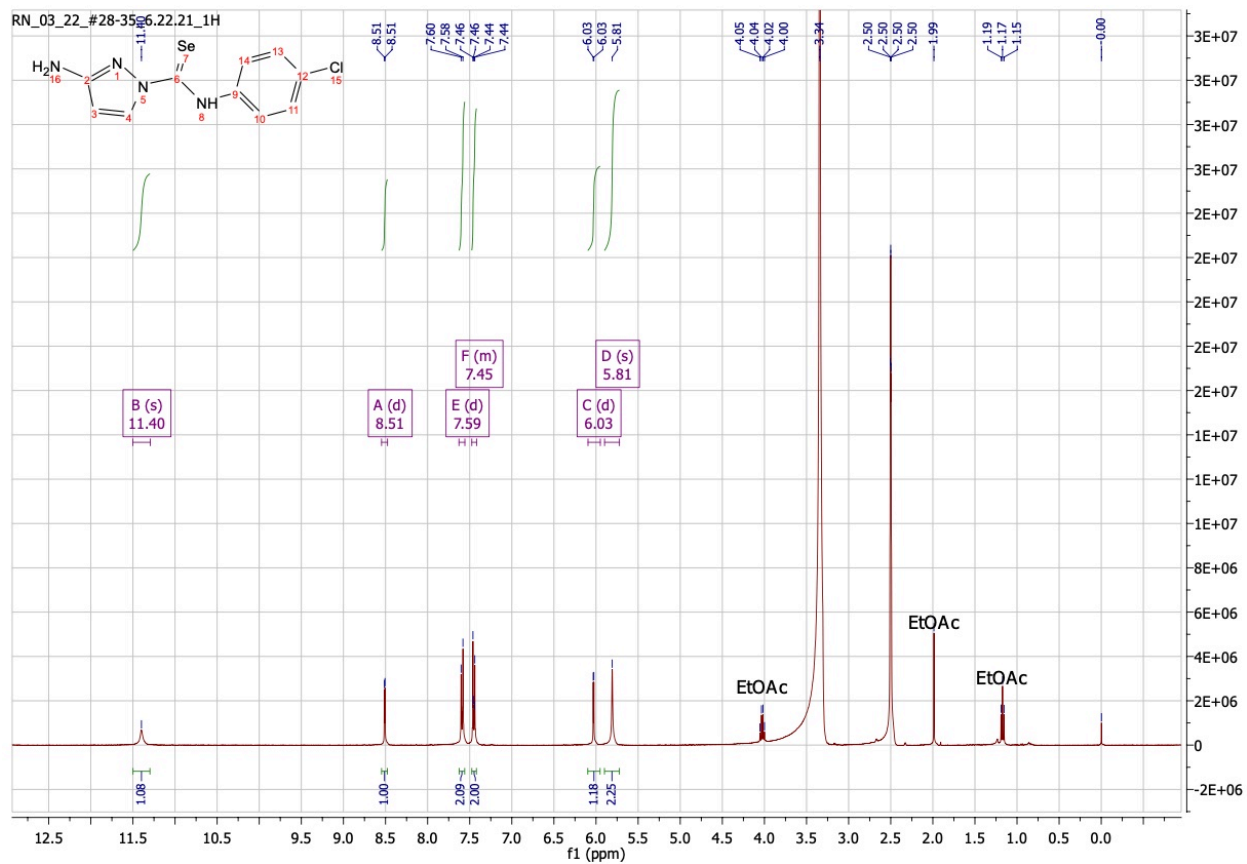


NMR Spectrum 28: RN_02_75 1H-NMR

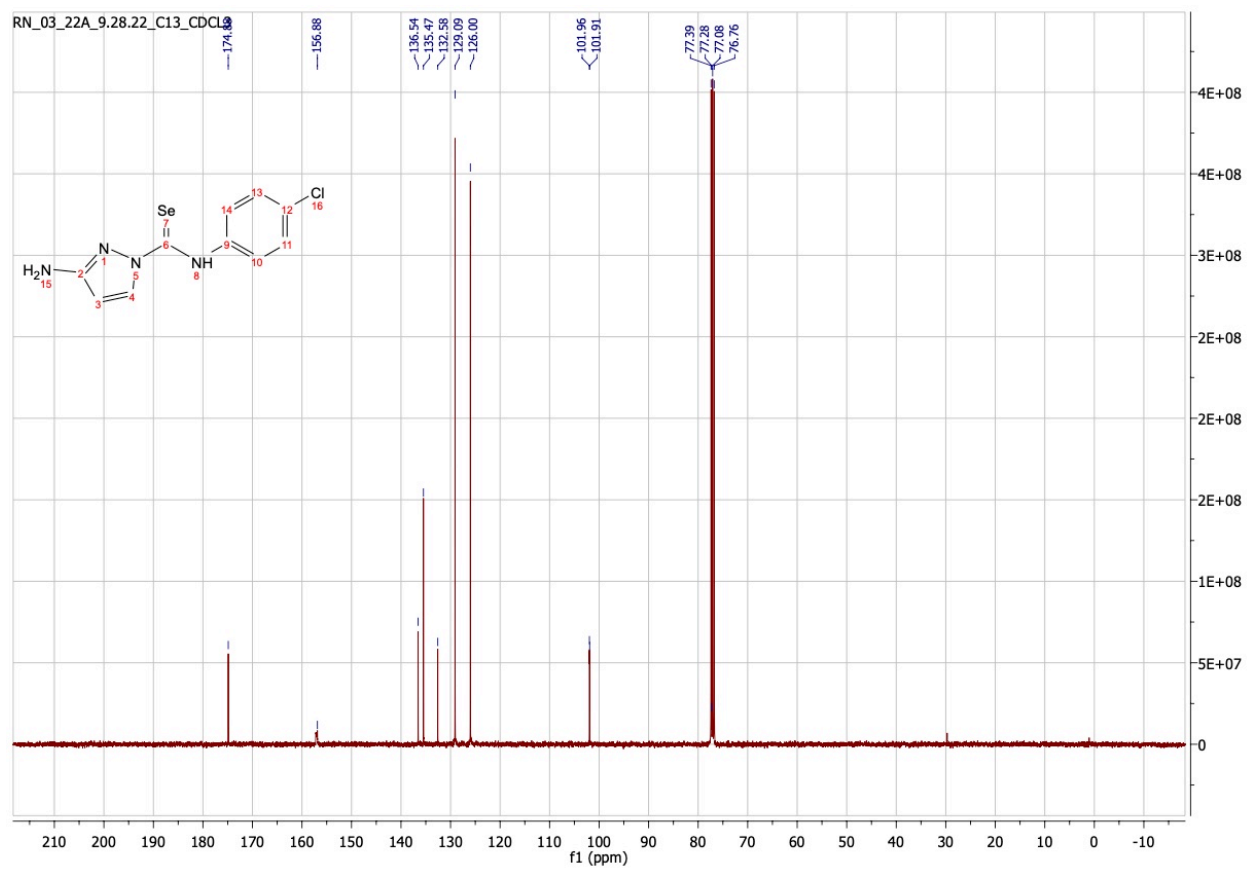




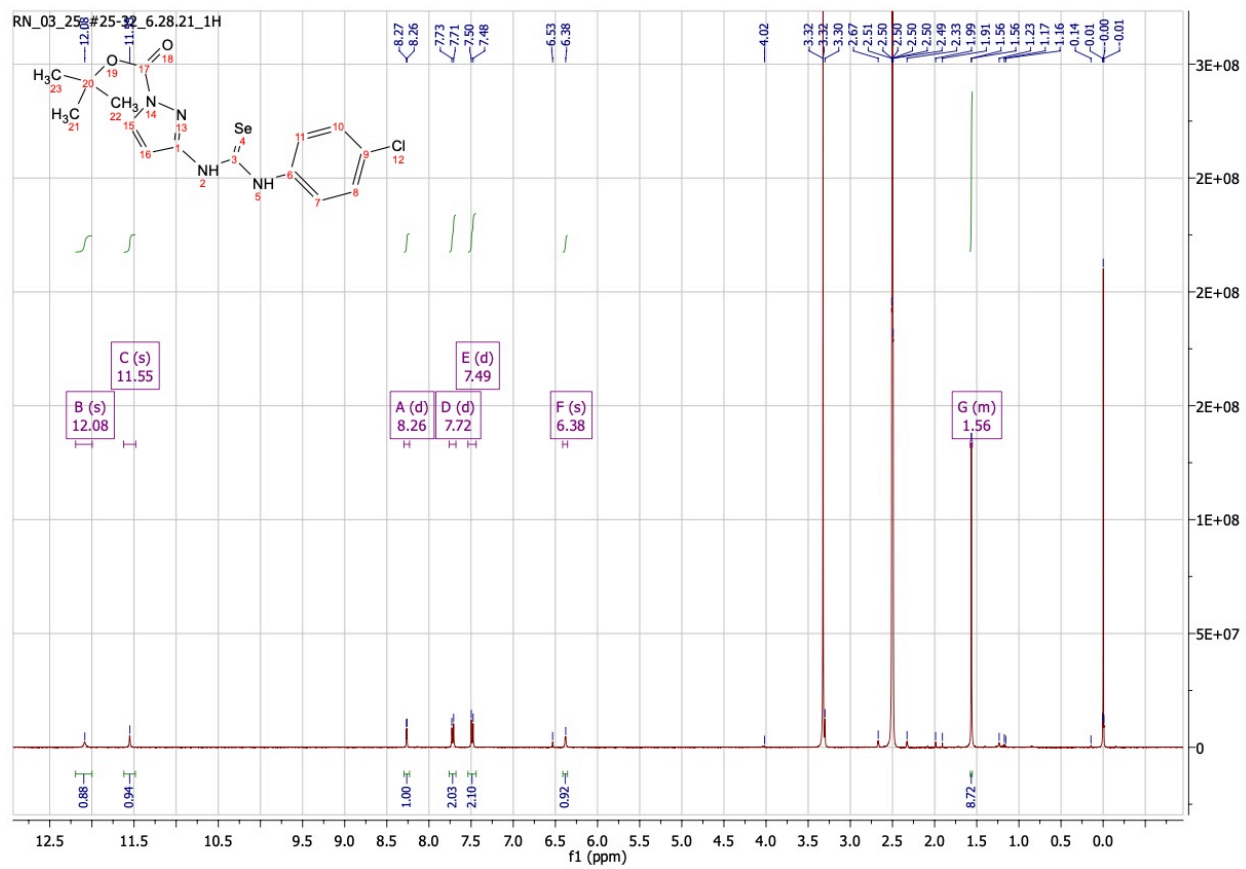
NMR Spectrum 30: RN_03_20 1H-NMR



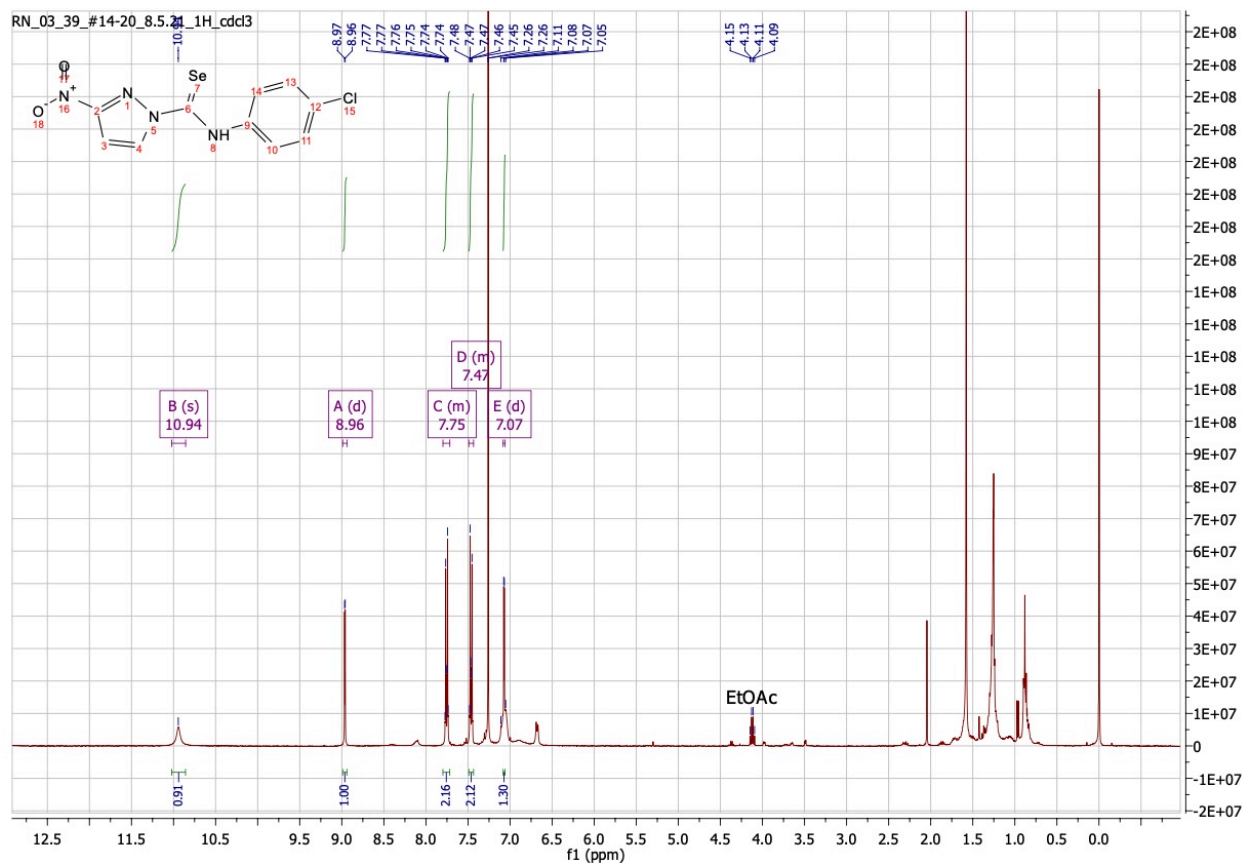
NMR Spectrum 31: RN_03_22A 1H-NMR



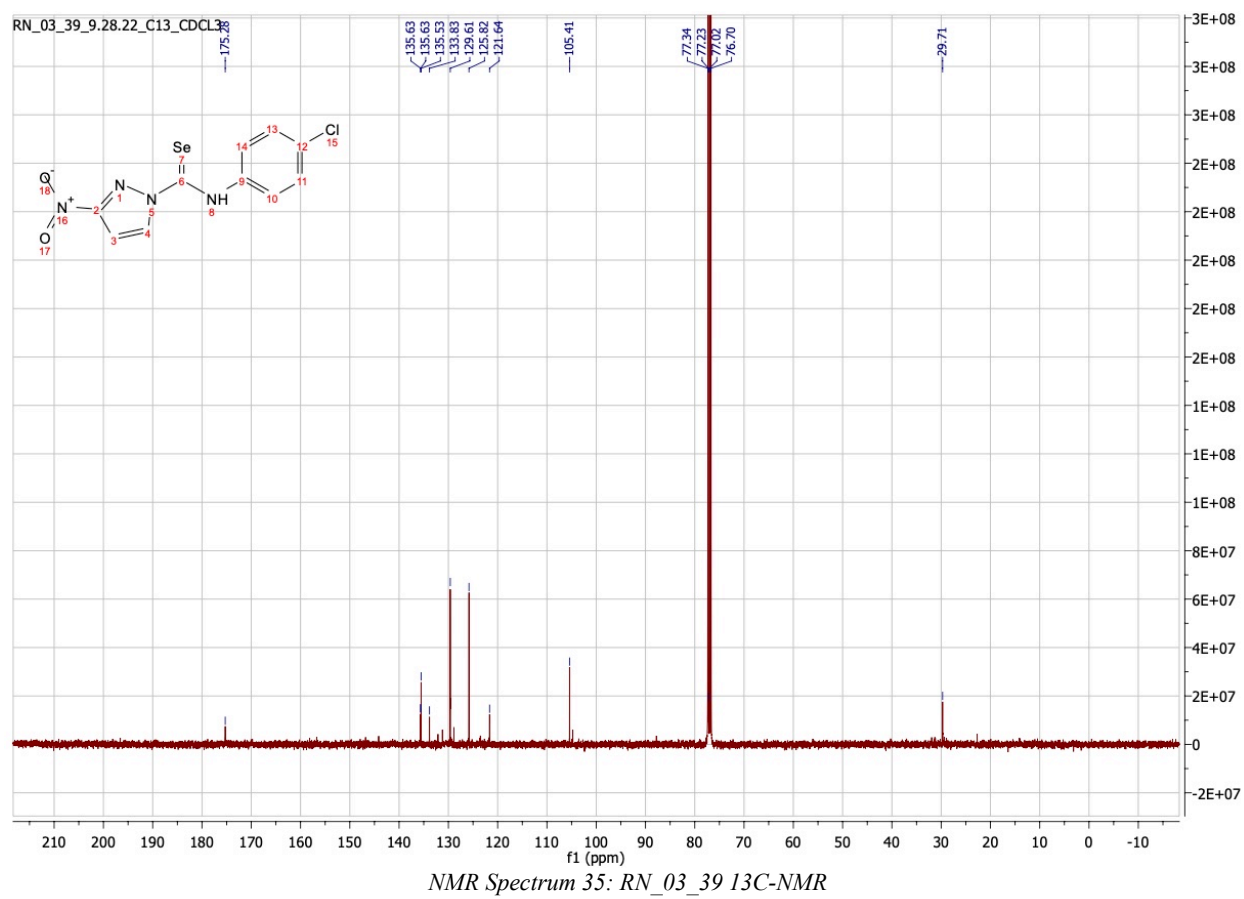
NMR Spectrum 32: RN_03_22A ¹³C-NMR

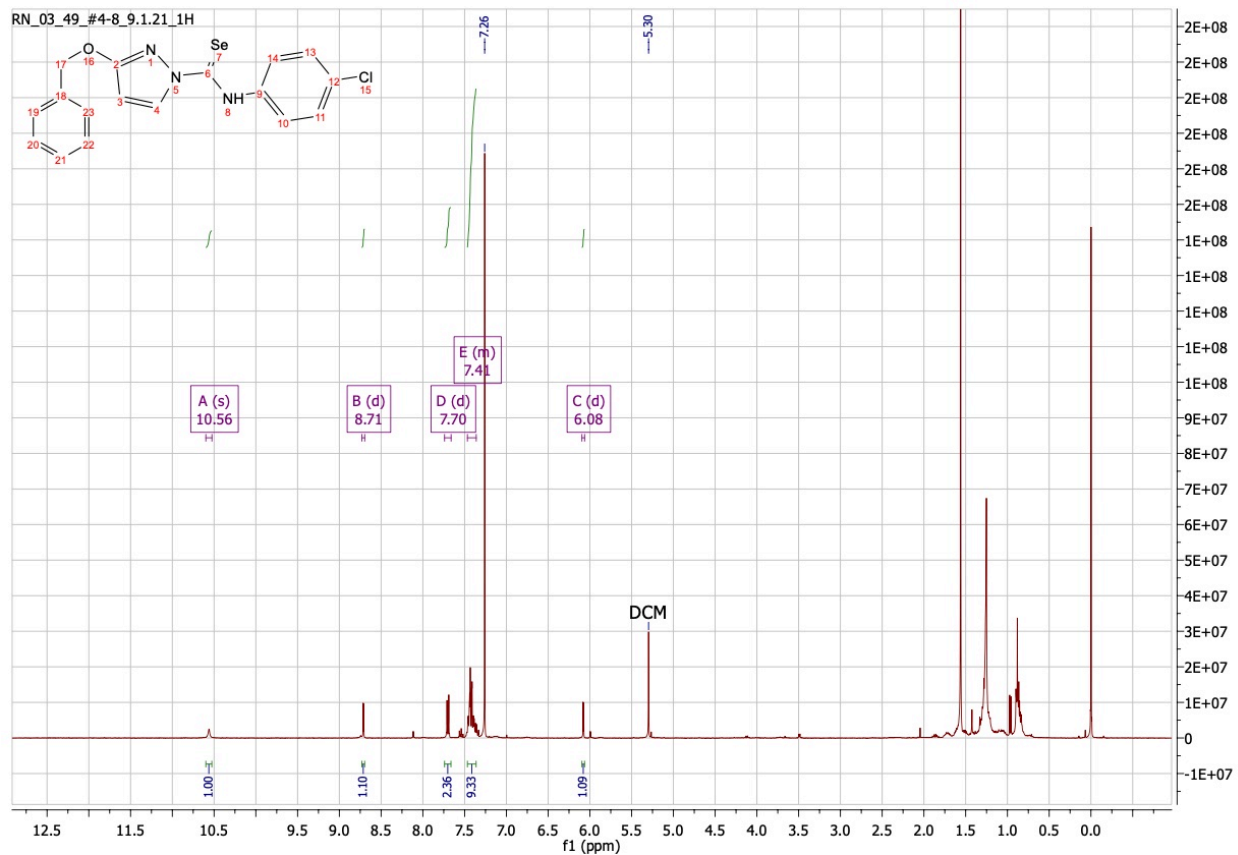


NMR Spectrum 33: RN_03_25 ¹H-NMR

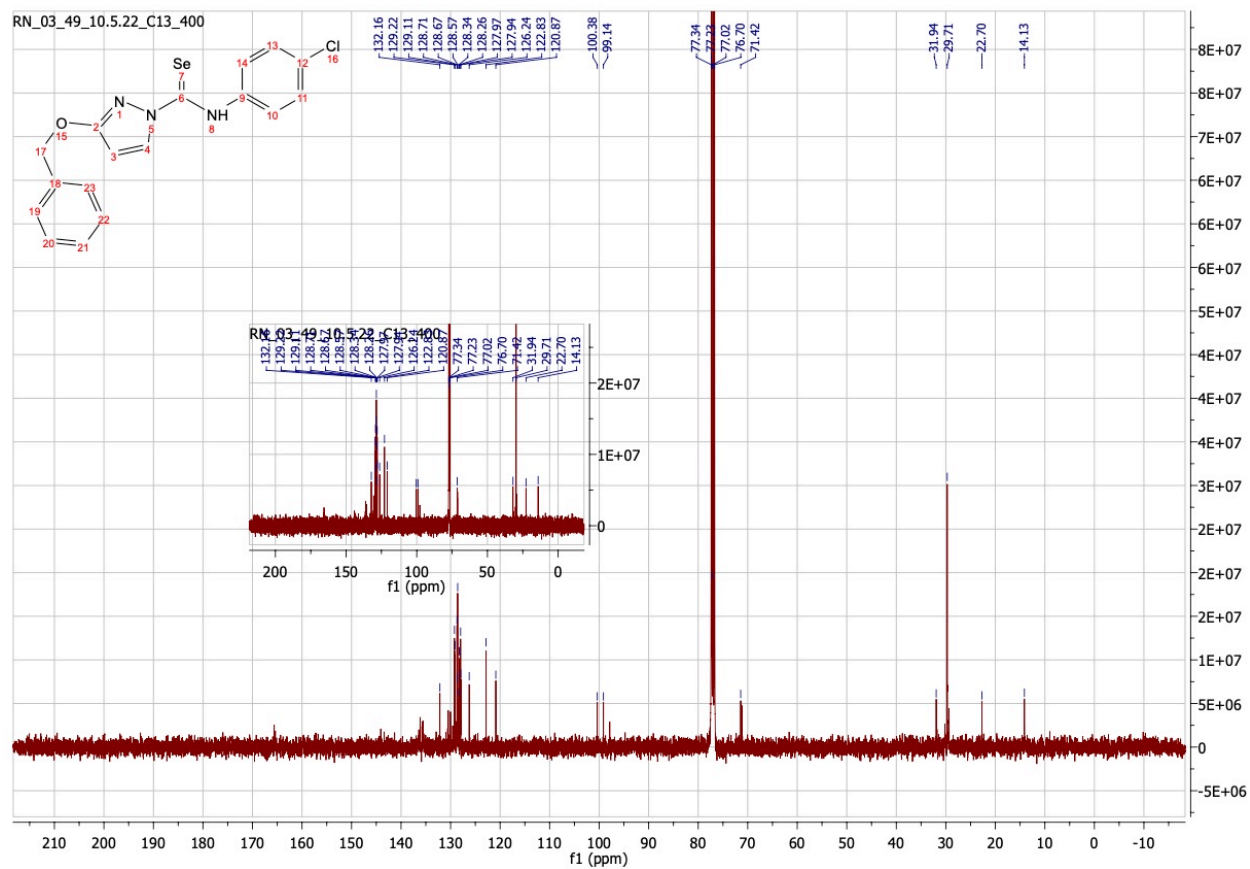


NMR Spectrum 34: RN_03_39 1H-NMR

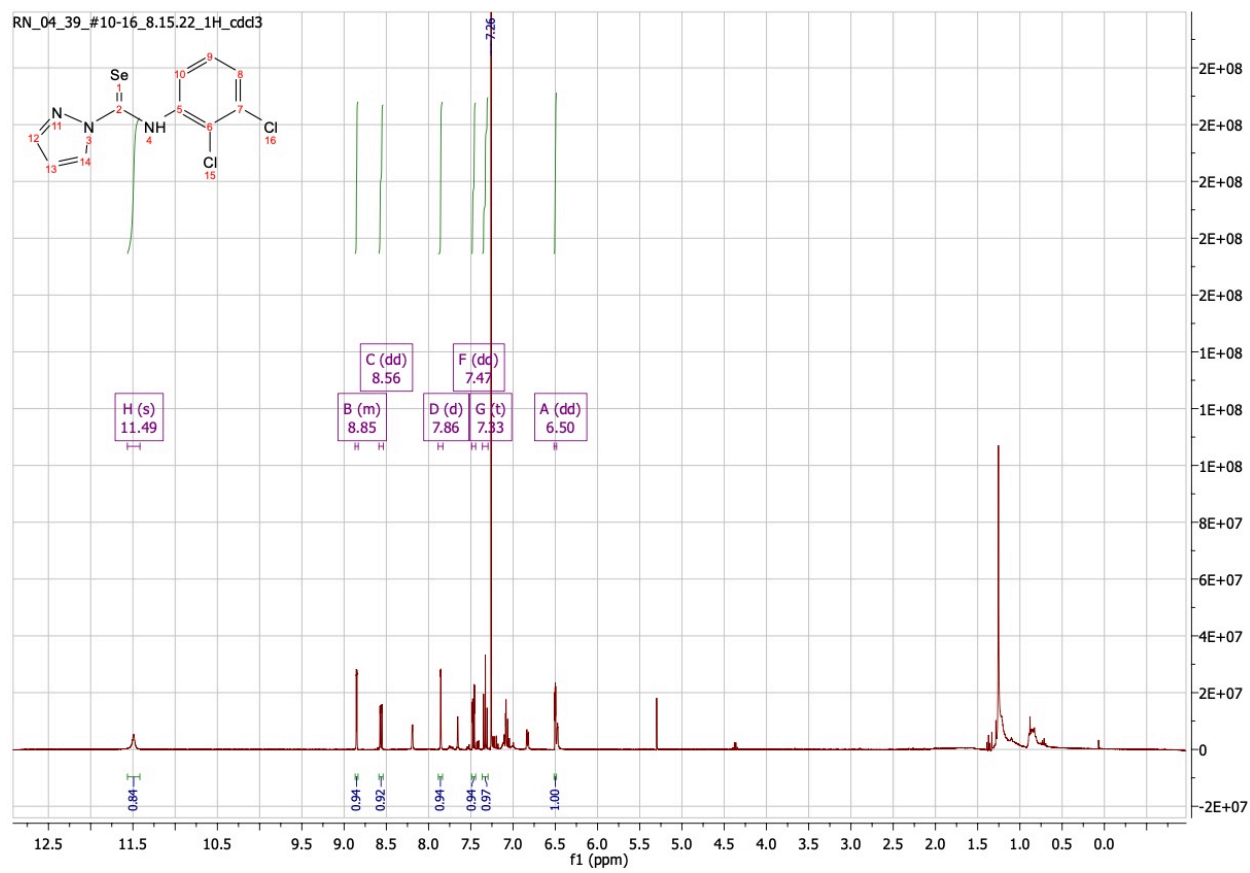




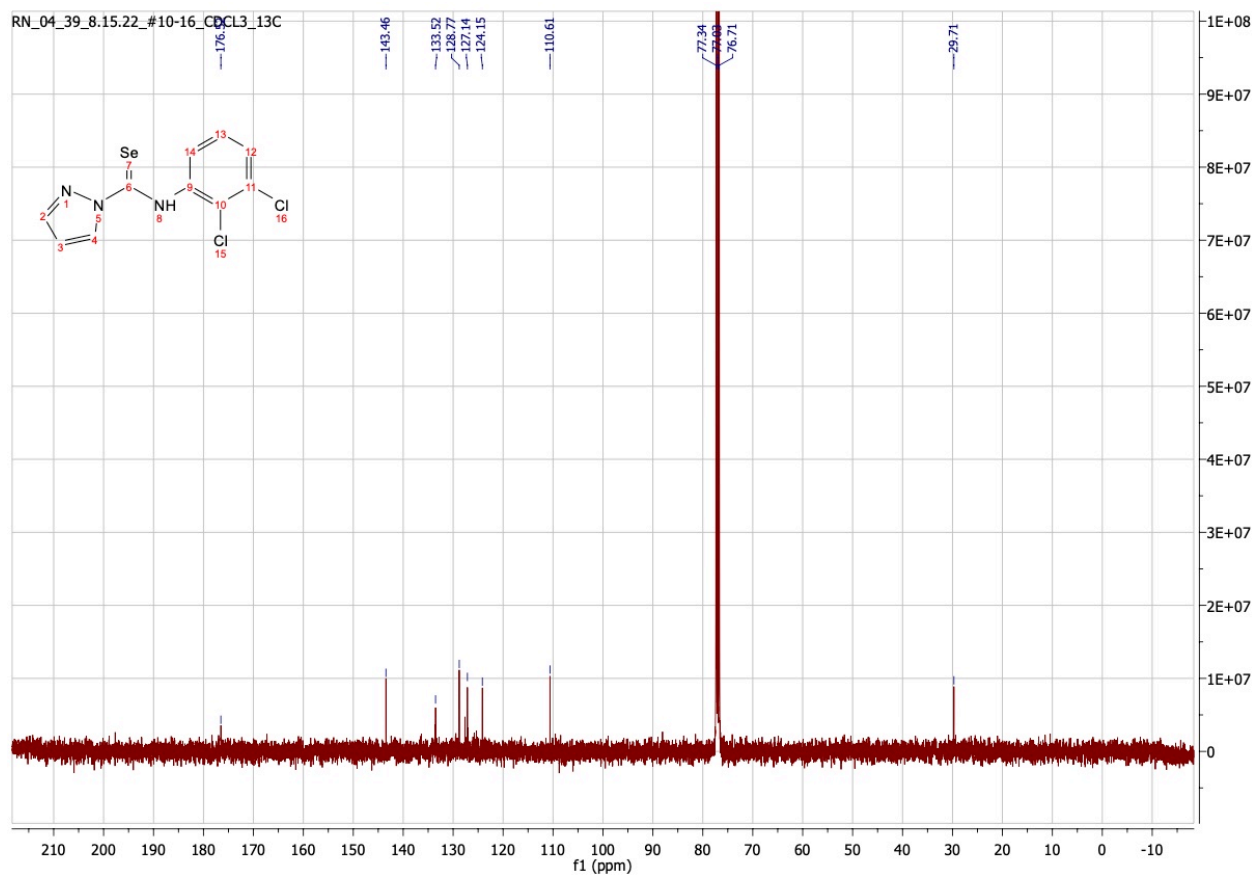
NMR Spectrum 36: RN_03_49 1H-NMR



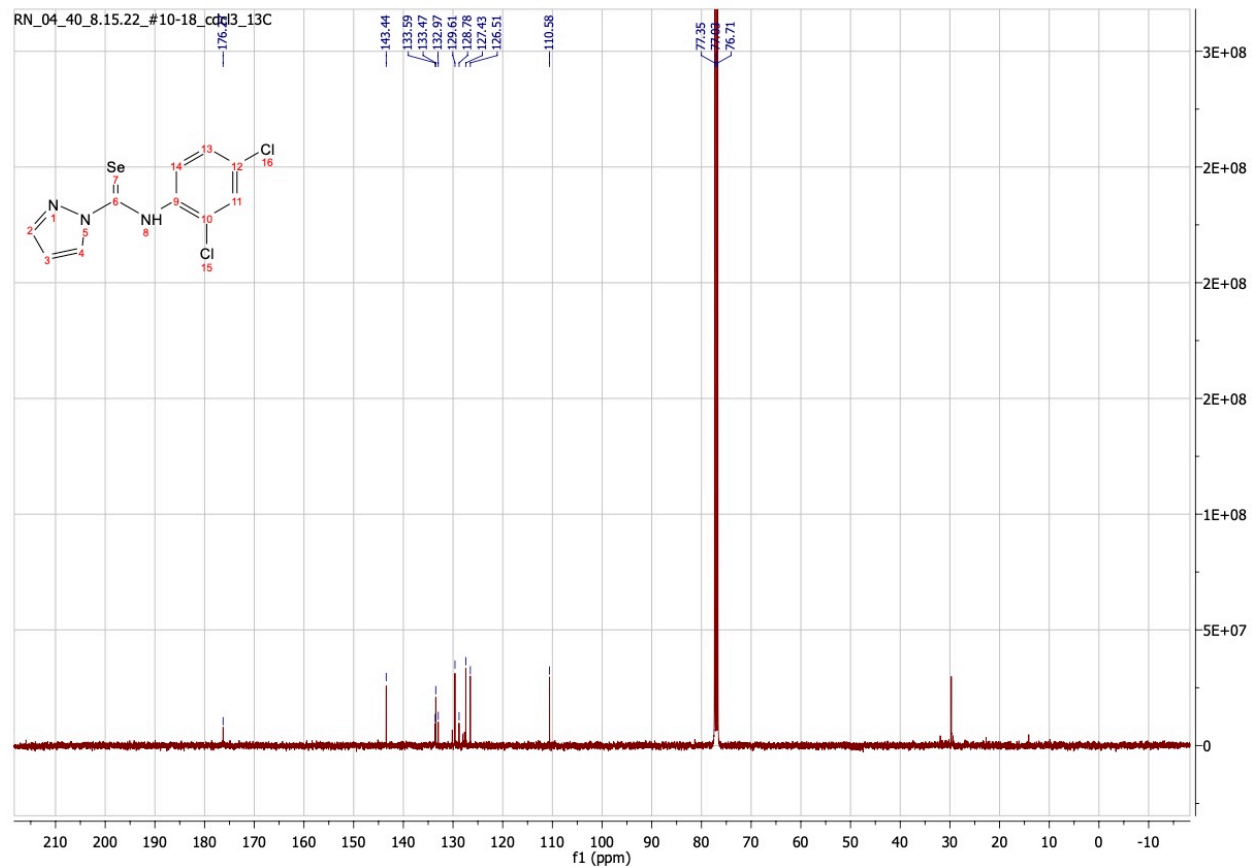
NMR Spectrum 37: RN_03_49 13C-NMR



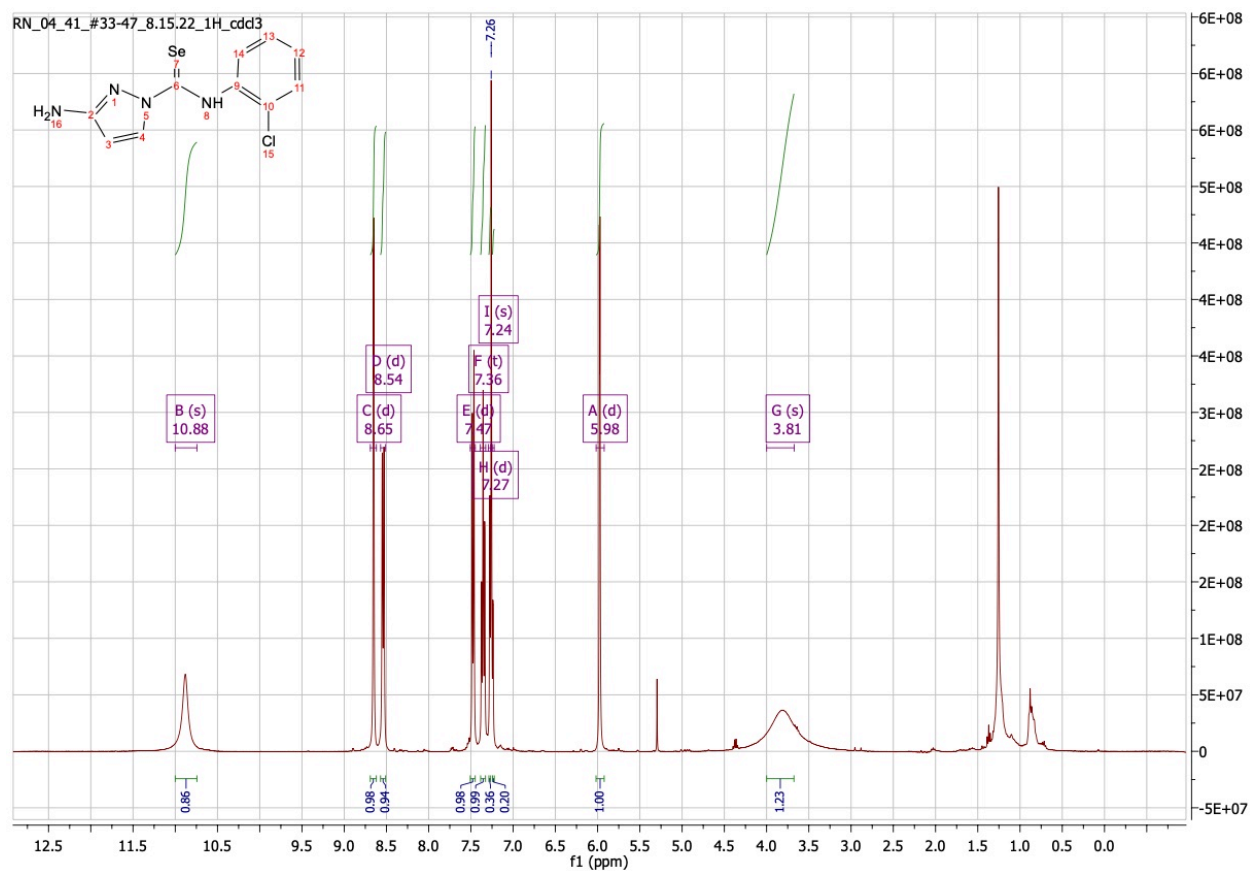
NMR Spectrum 38: RN_04_39 1H-NMR



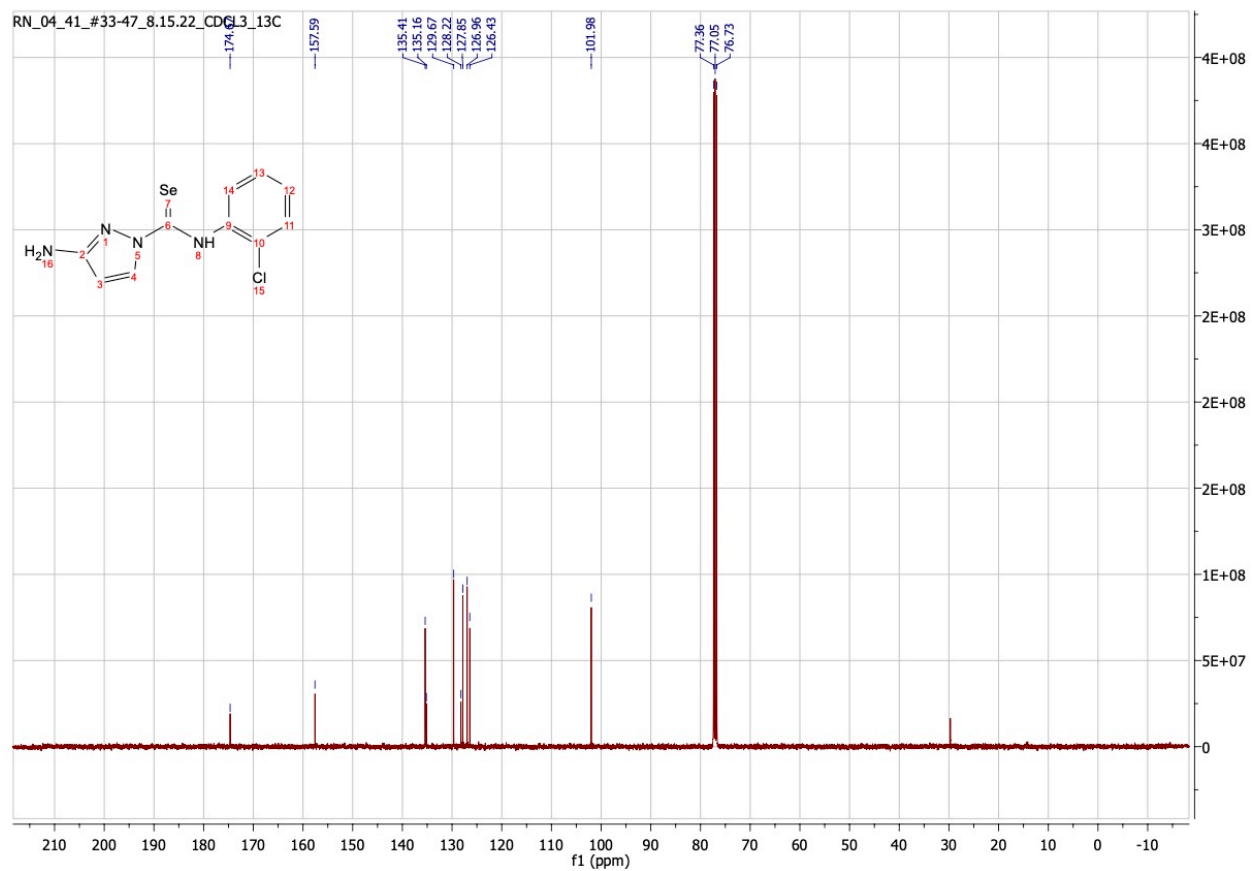
NMR Spectrum 39: RN_04_39 13C-NMR



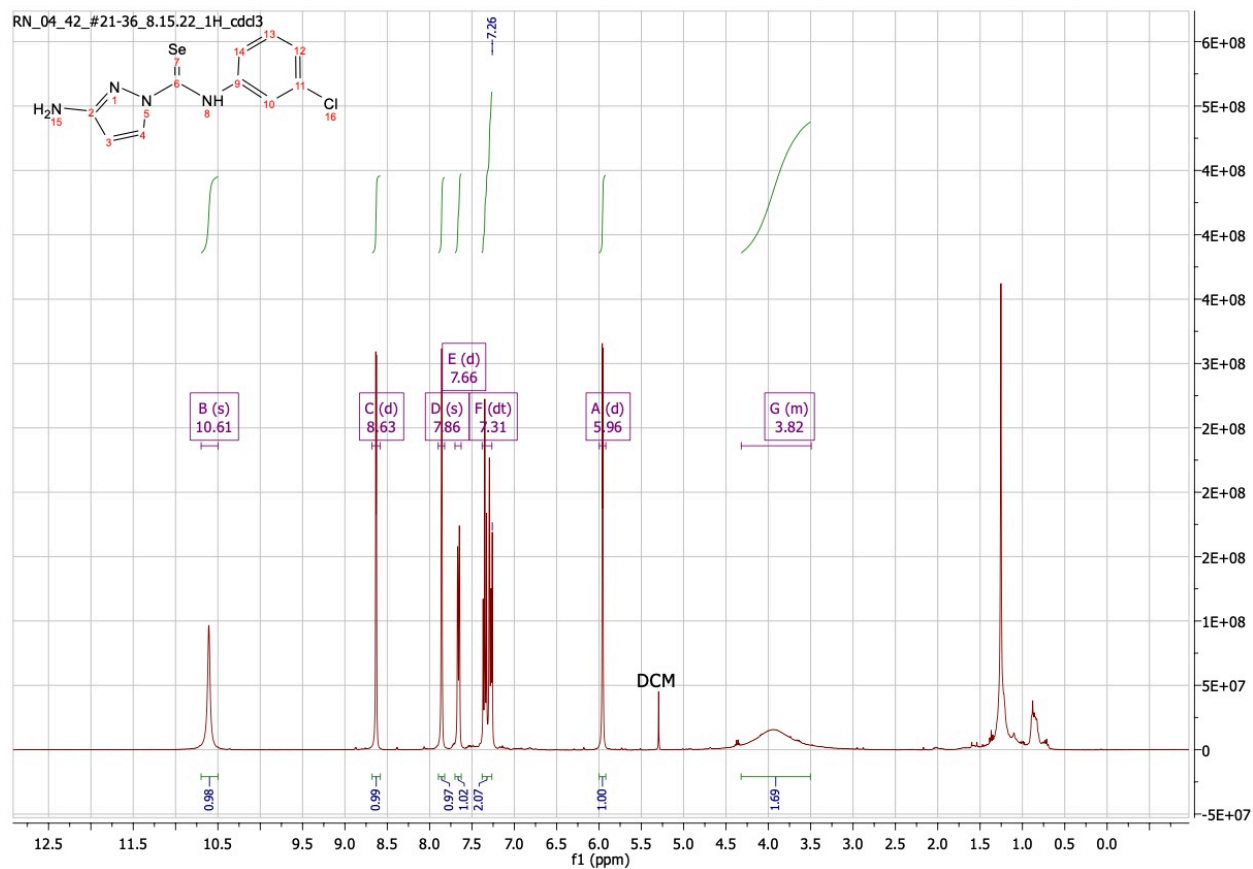
NMR Spectrum 41: RN_04_40 ¹³C-NMR



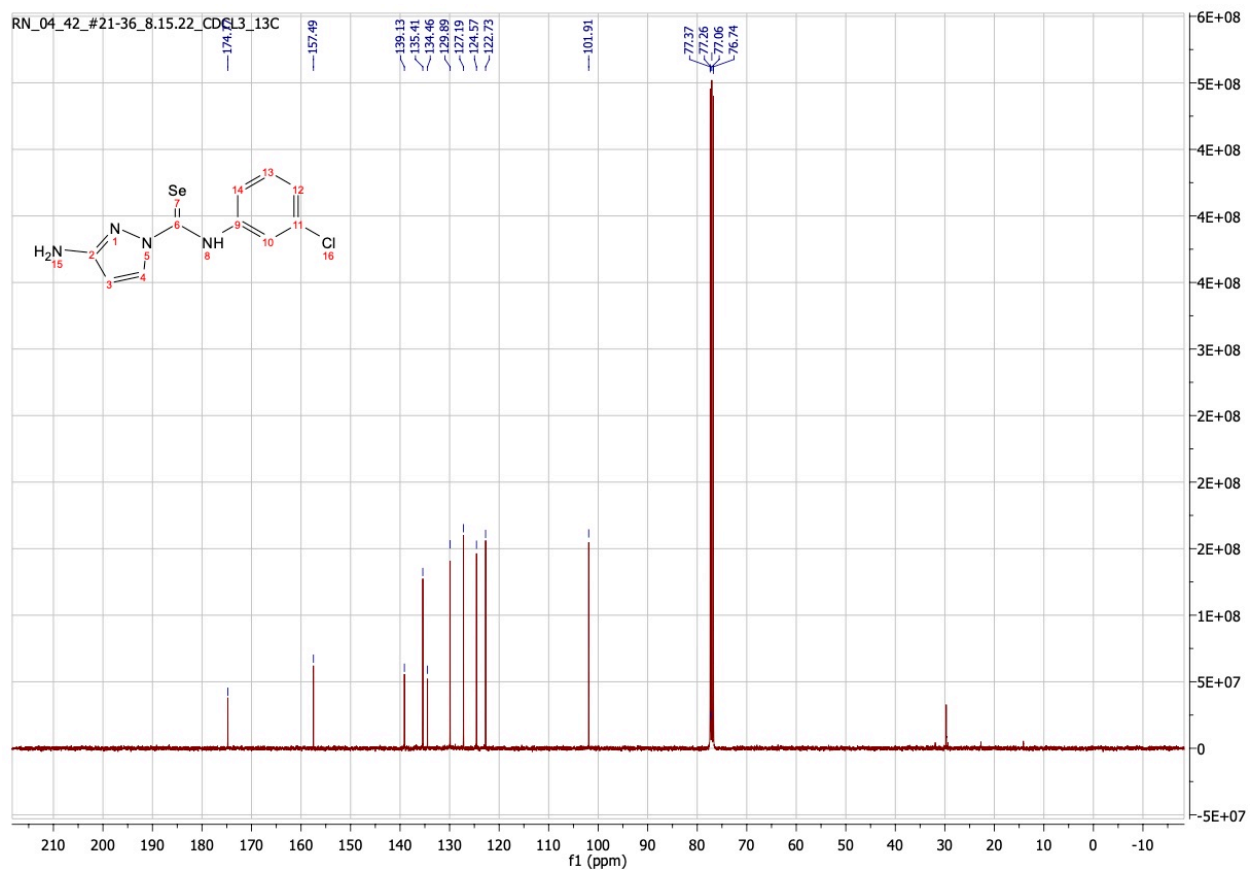
NMR Spectrum 42: RN_04_41 1H-NMR



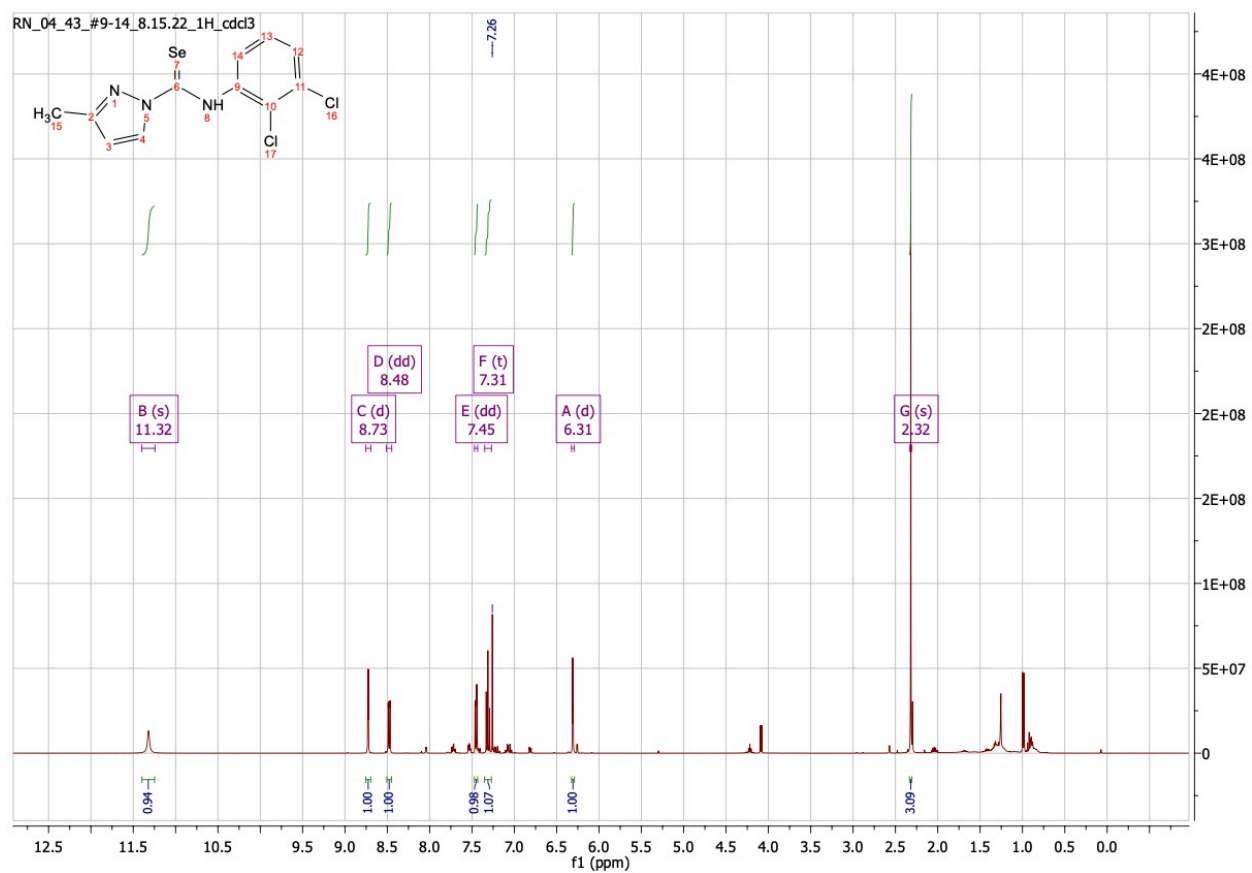
NMR Spectrum 43: RN_04_41 13C-NMR



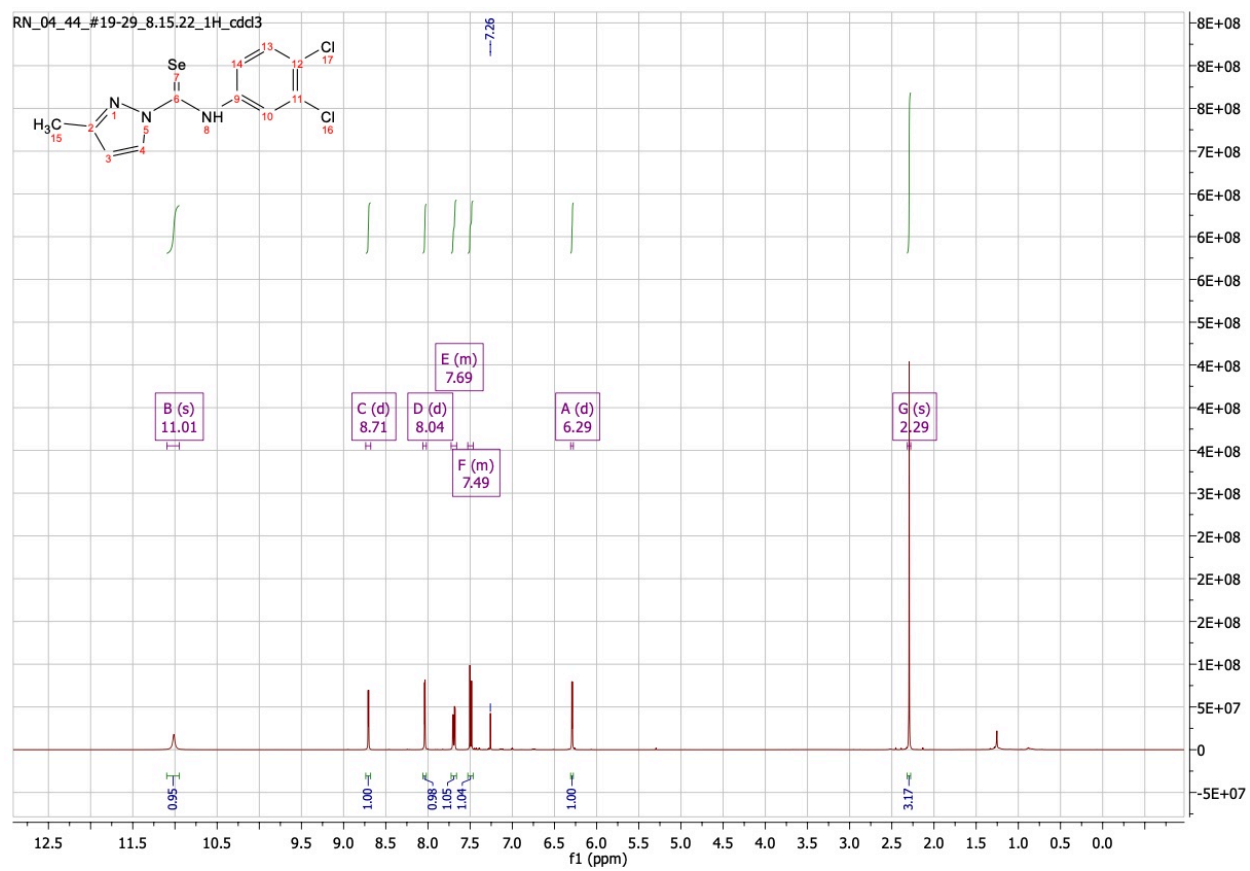
NMR Spectrum 44: RN_04_42 1H-NMR



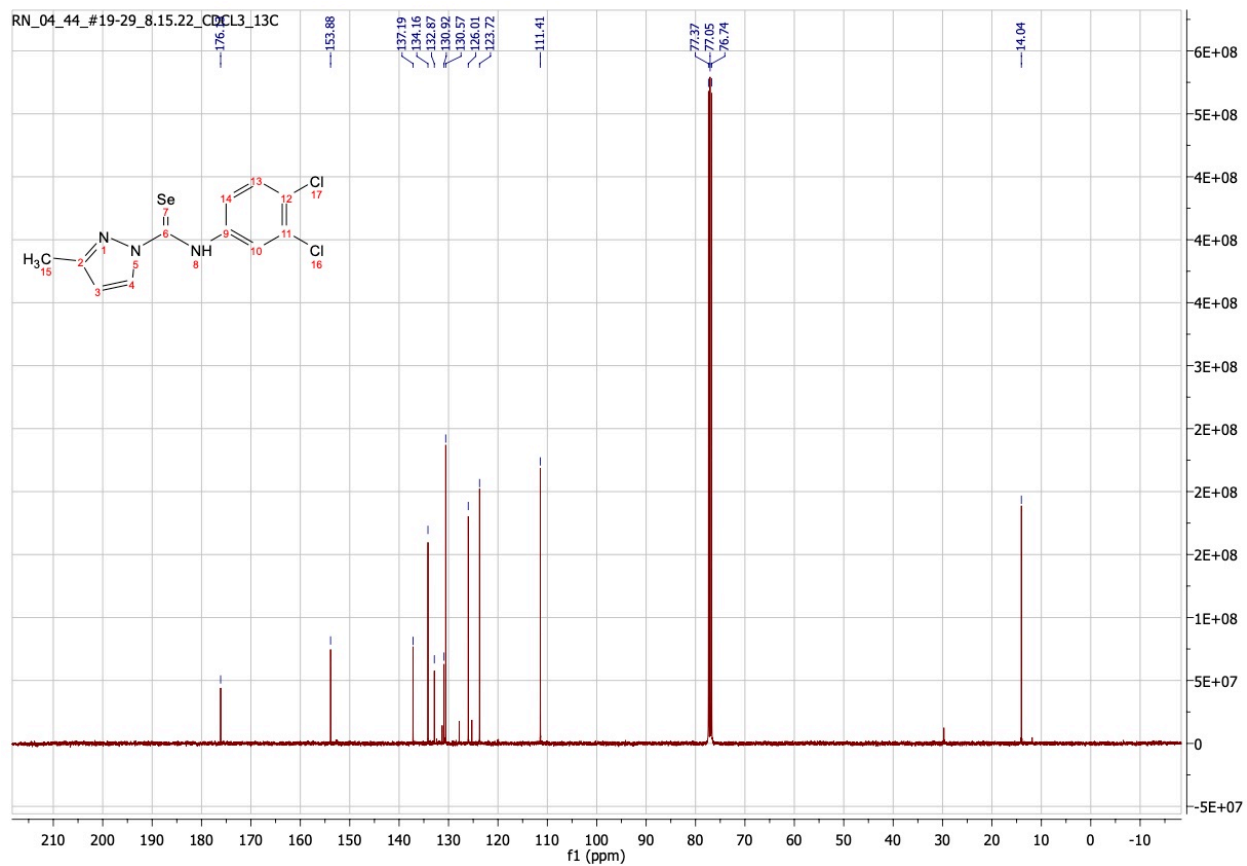
NMR Spectrum 45: RN_04_42 13C-NMR



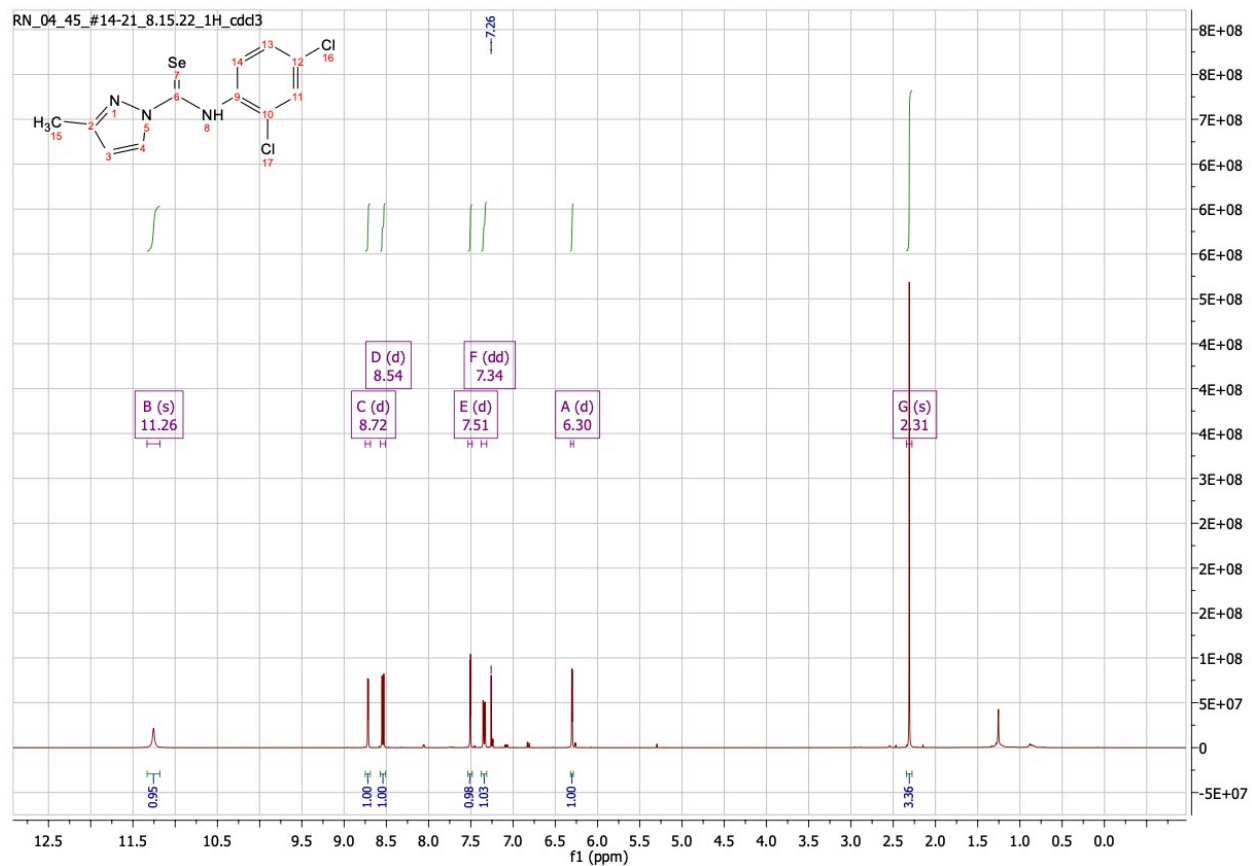
NMR Spectrum 46: RN_04_43 1H-NMR



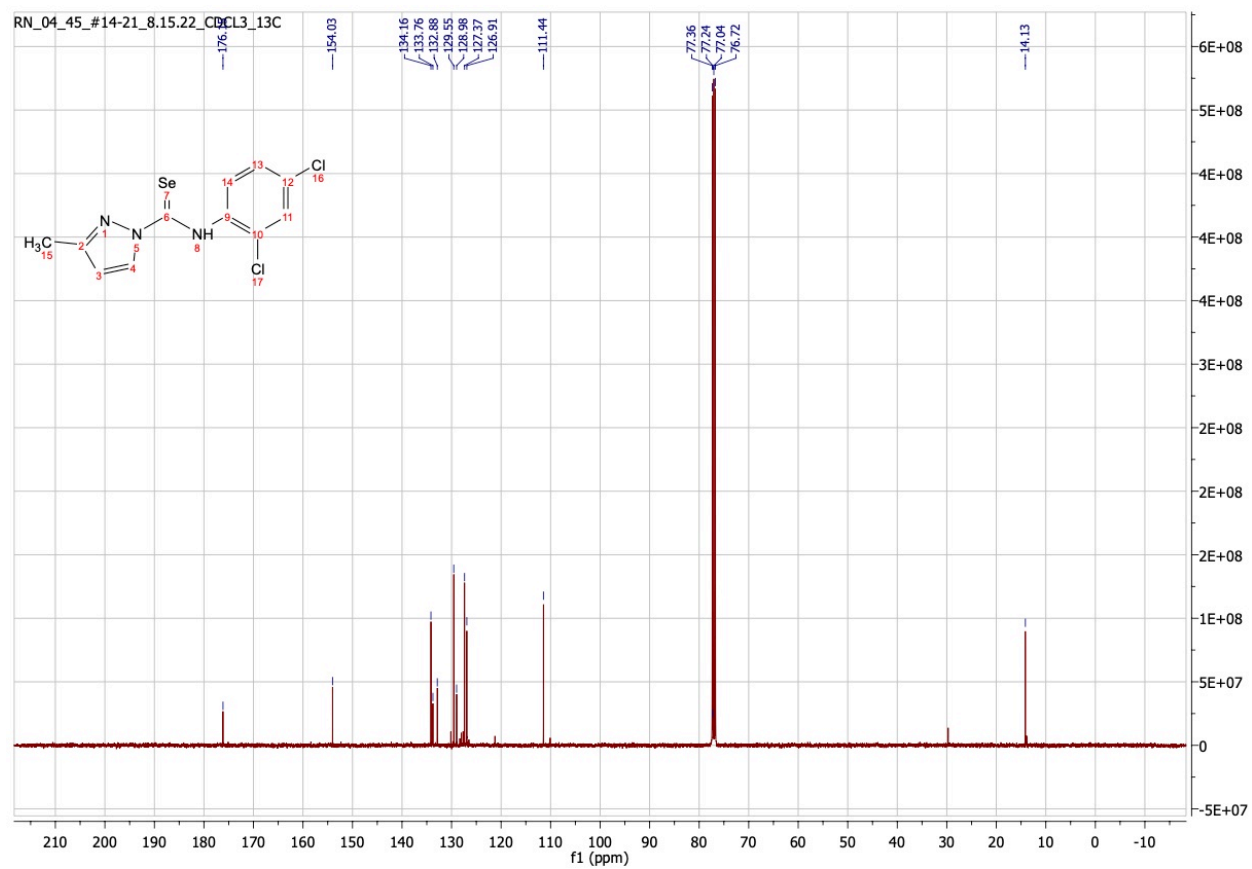
NMR Spectrum 48: RN_04_44 1H-NMR



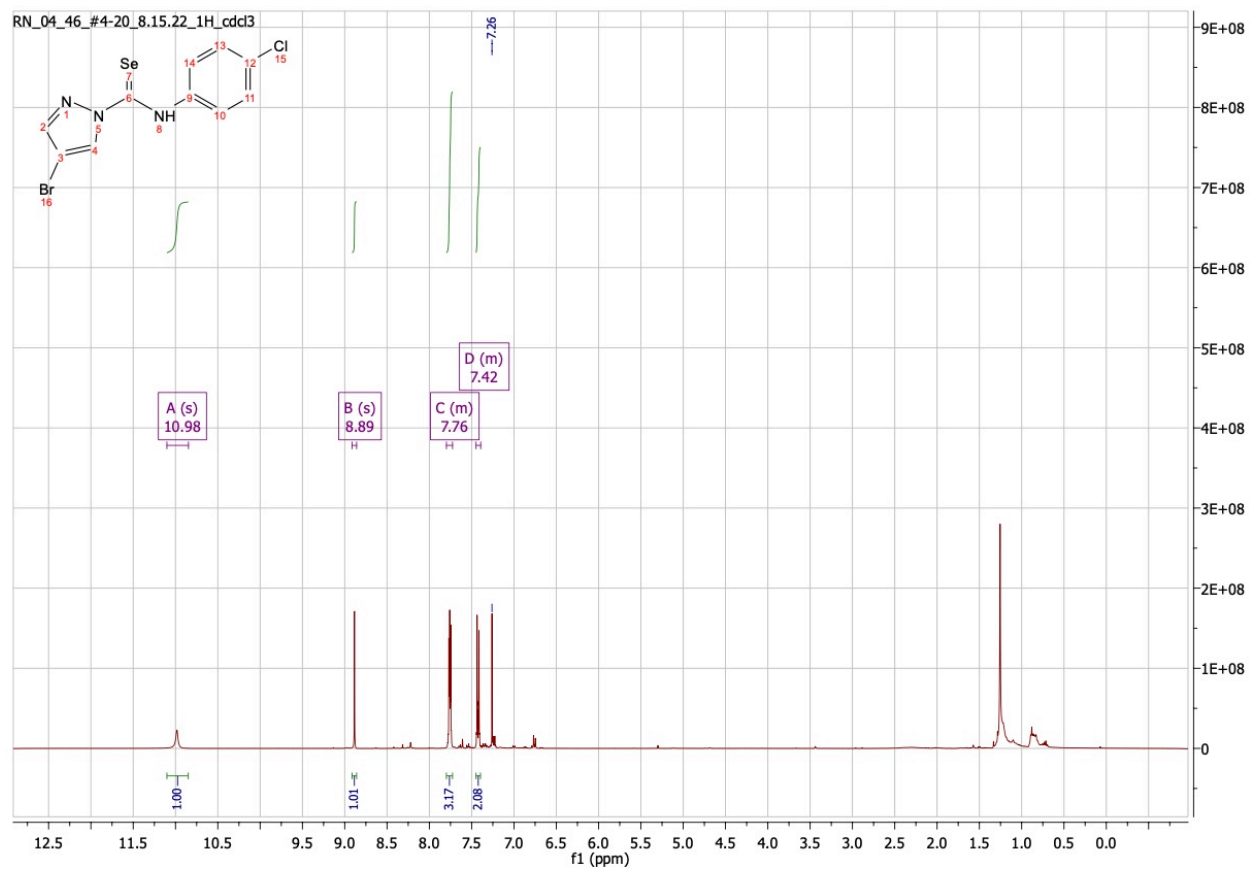
NMR Spectrum 49: RN_04_44 13C-NMR



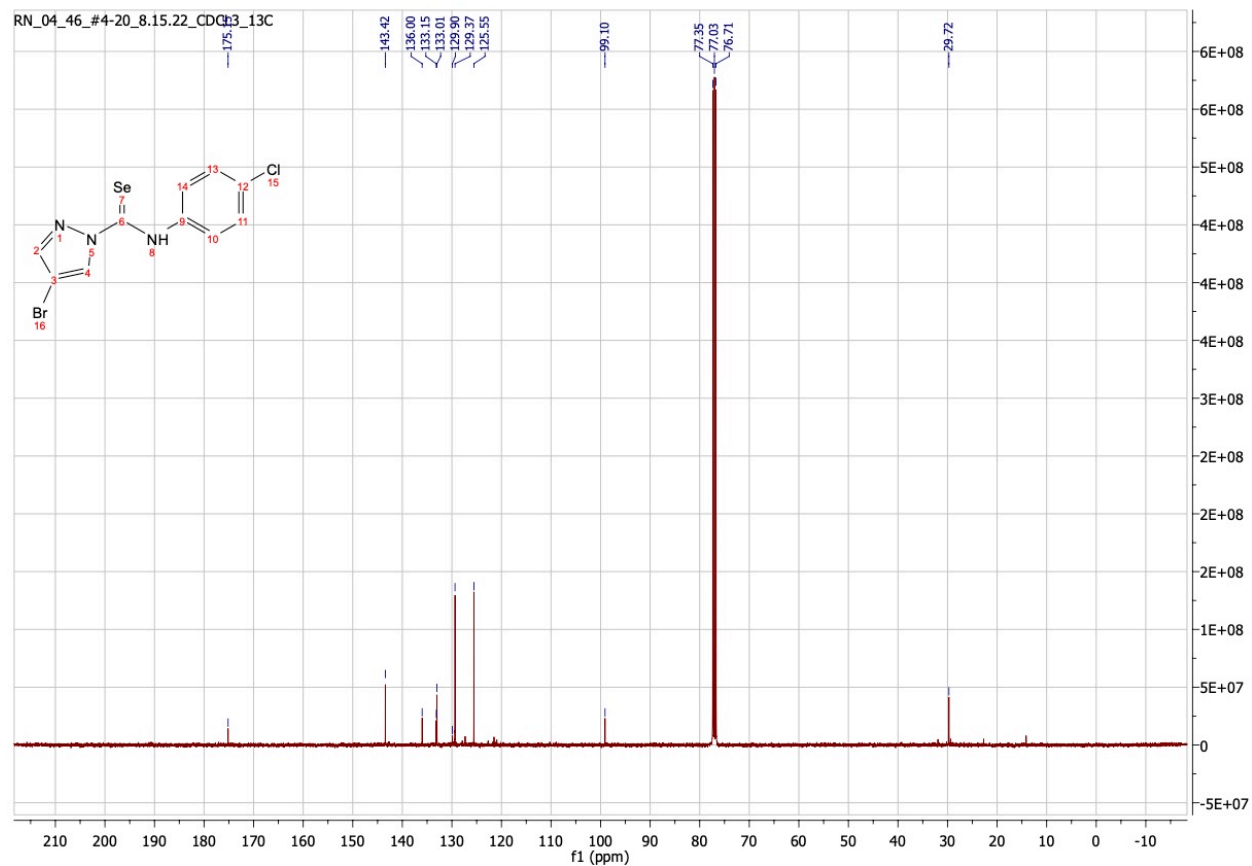
NMR Spectrum 50: RN_04_45 1H-NMR



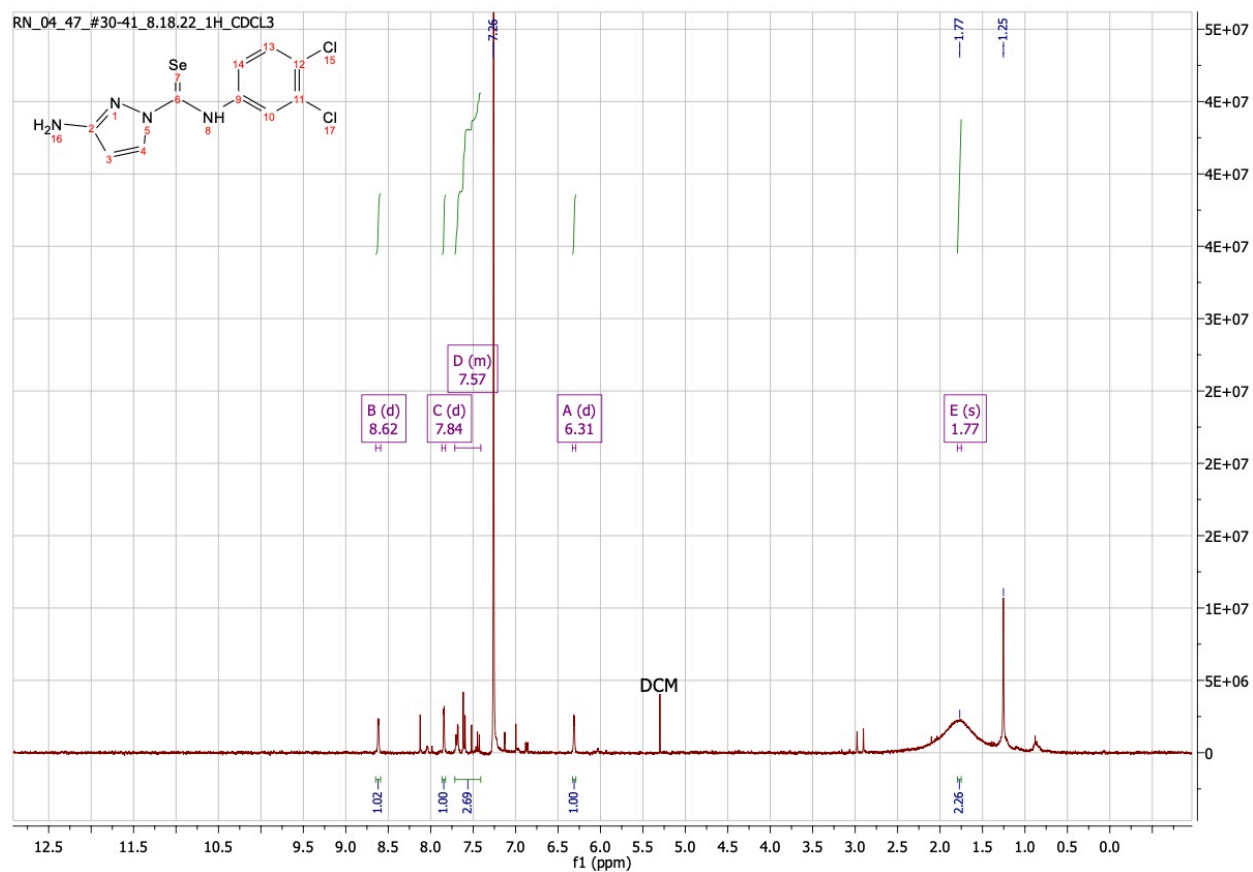
NMR Spectrum 51: RN_04_45 ¹³C-NMR



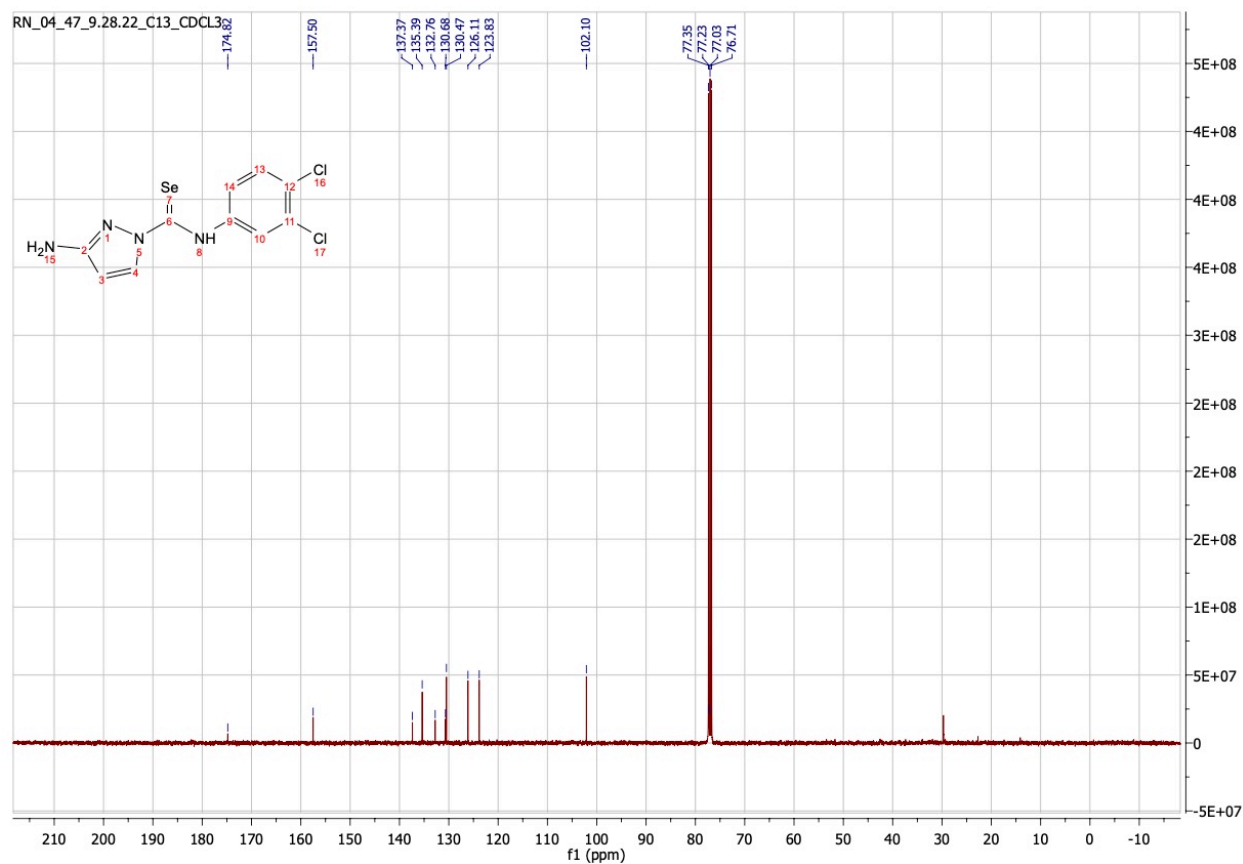
NMR Spectrum 52: RN_04_46 1H-NMR



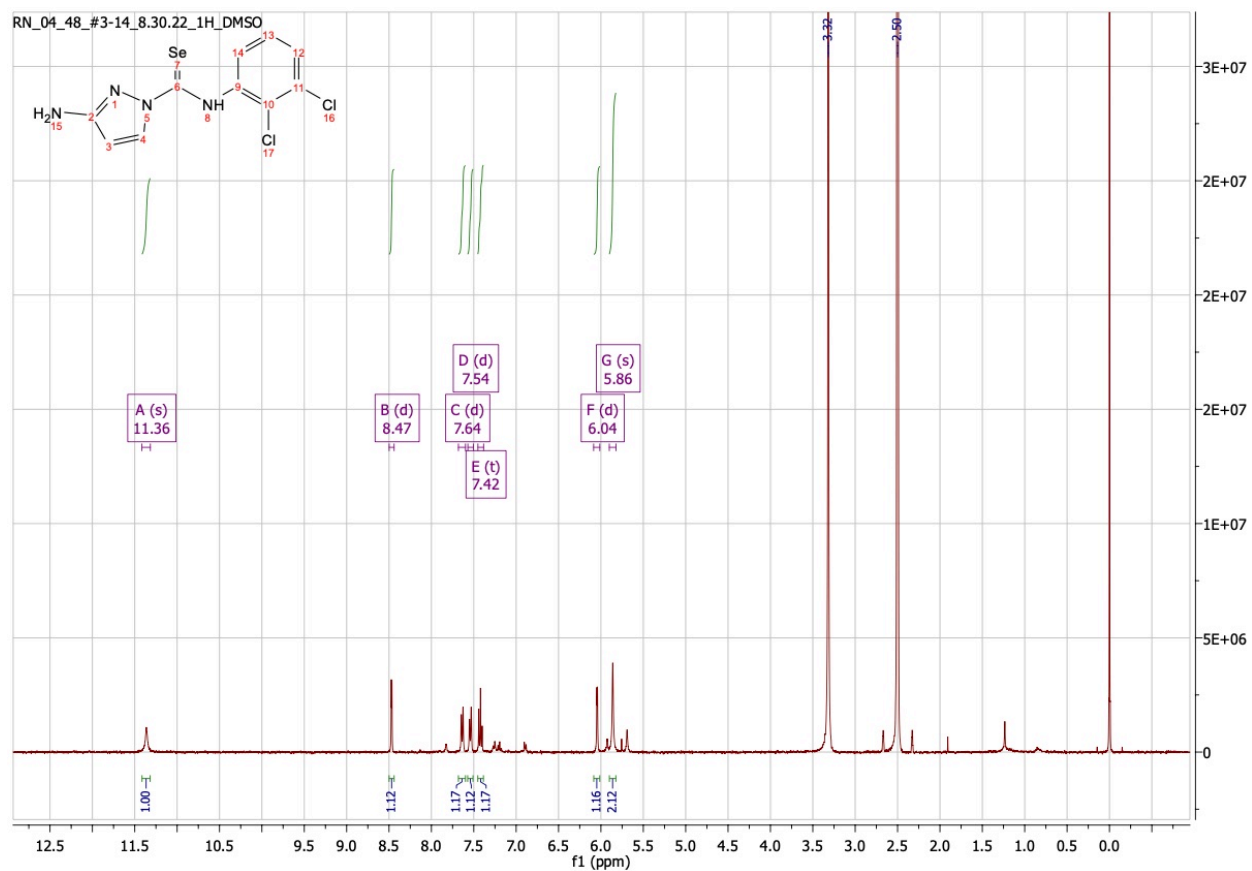
NMR Spectrum 53: RN_04_46 ¹³C-NMR



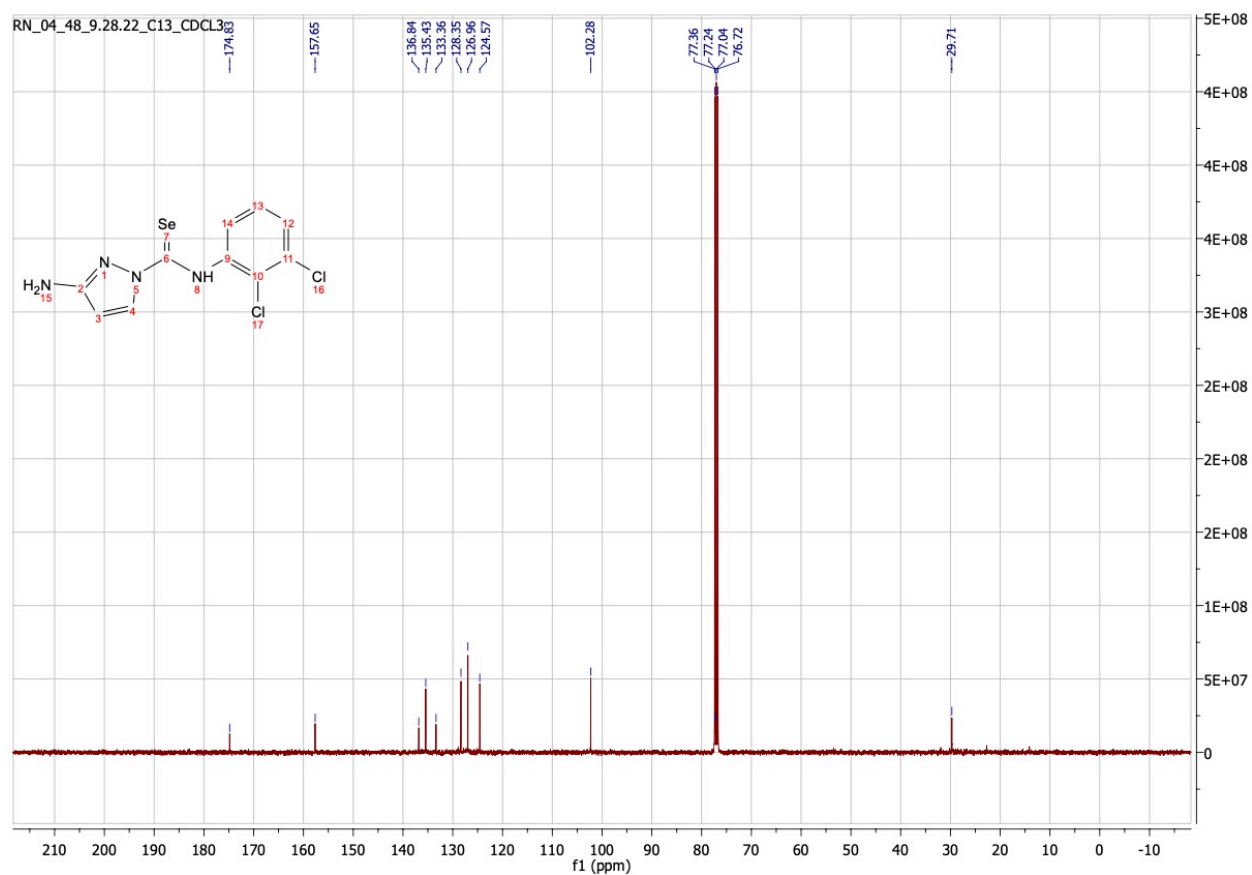
NMR Spectrum 54: RN_04_47 ¹H-NMR



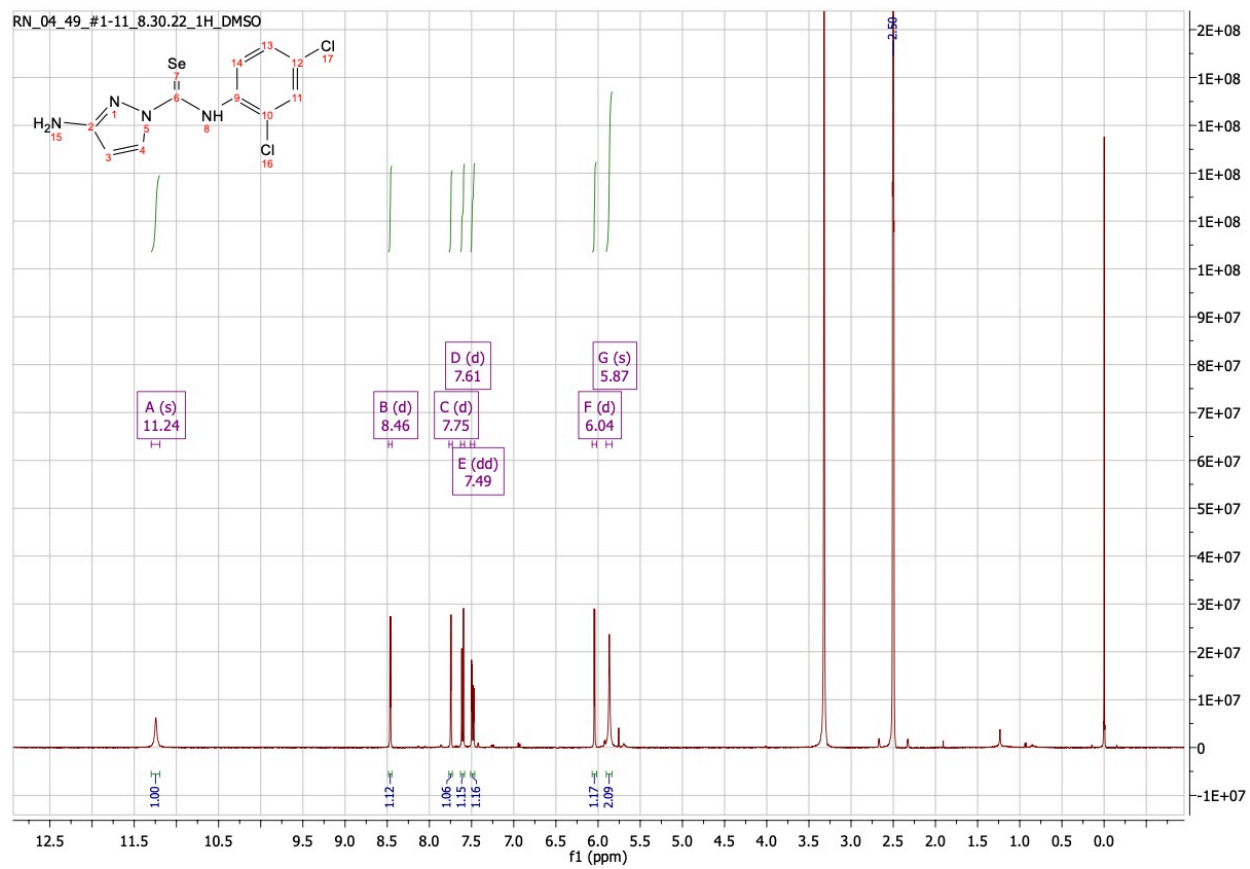
NMR Spectrum 55: RN_04_47 13C-NMR



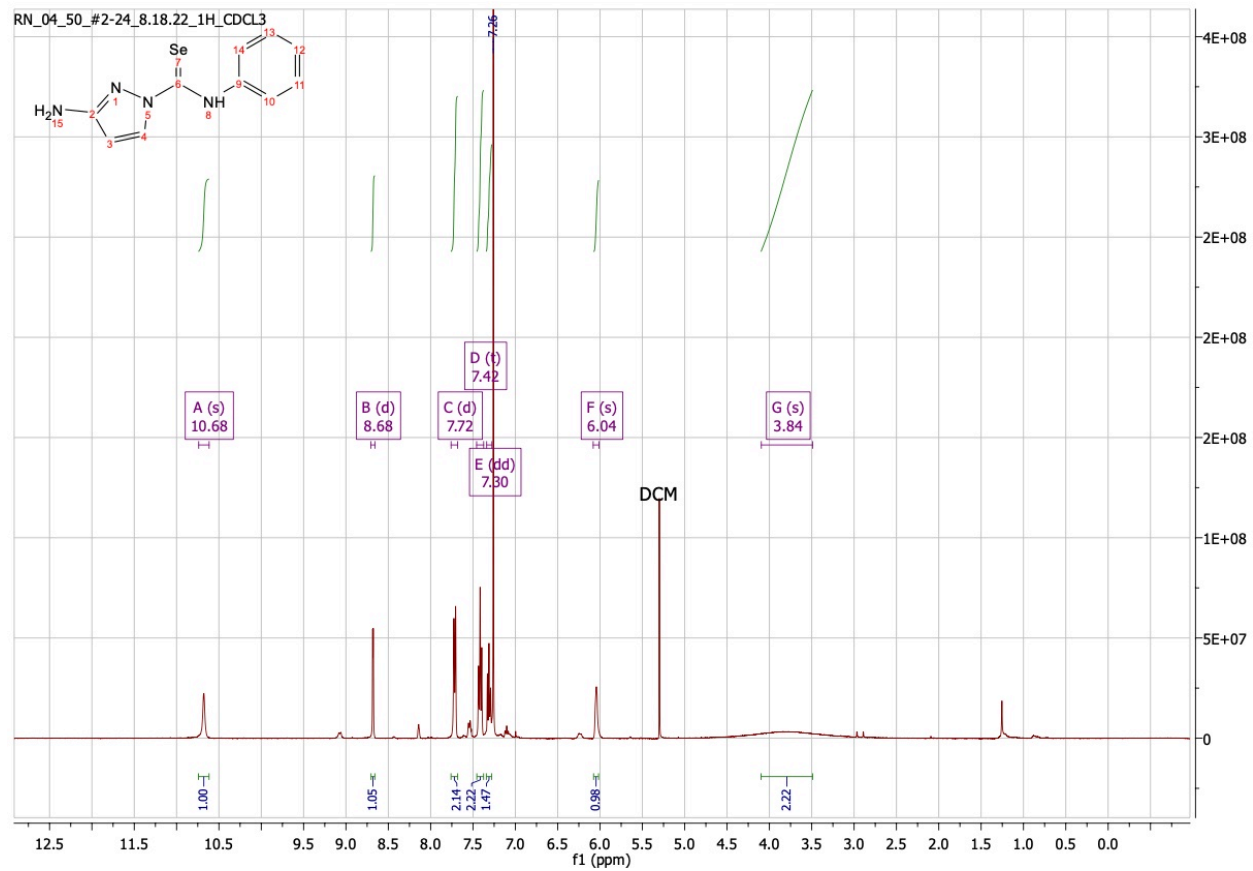
NMR Spectrum 56: RN_04_48 $^1\text{H-NMR}$



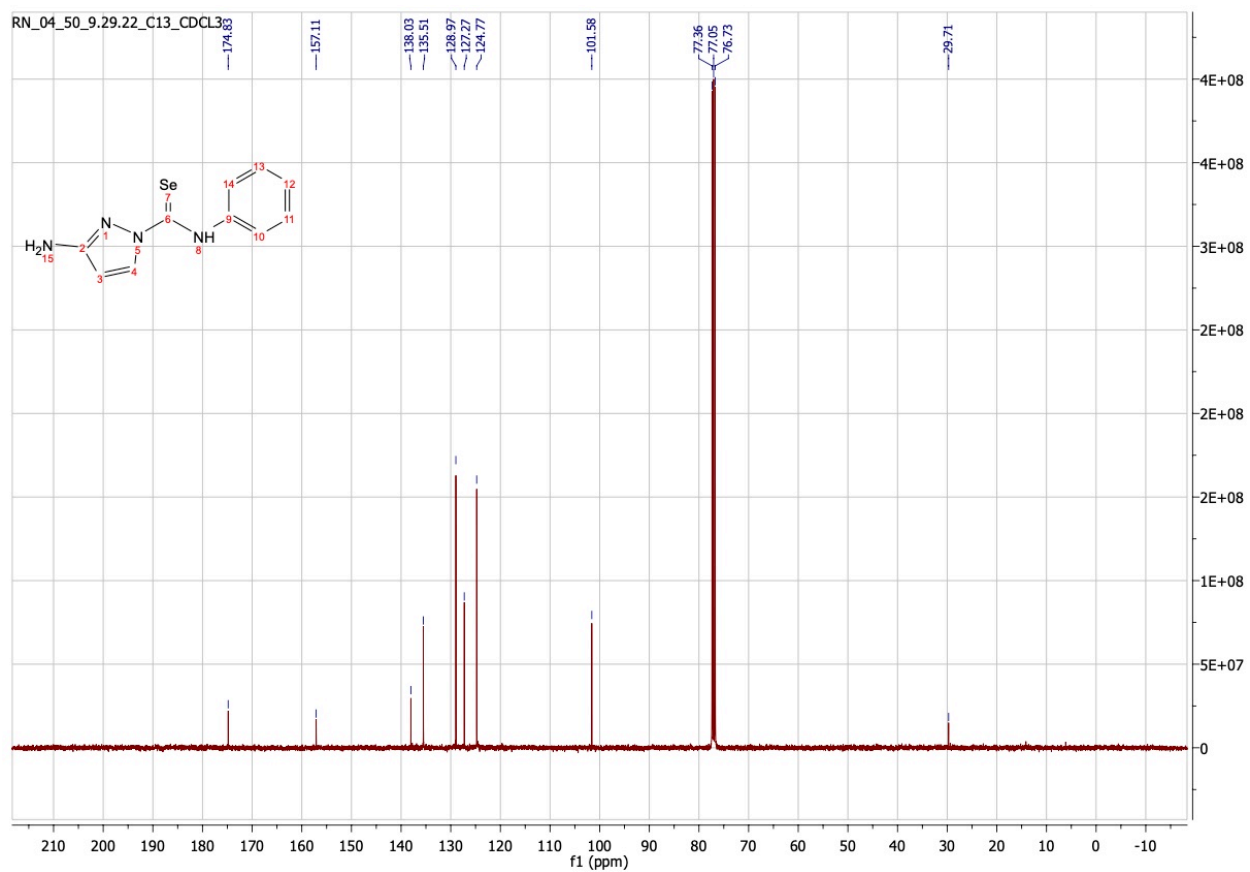
NMR Spectrum 57: RN_04_46 ^{13}C -NMR



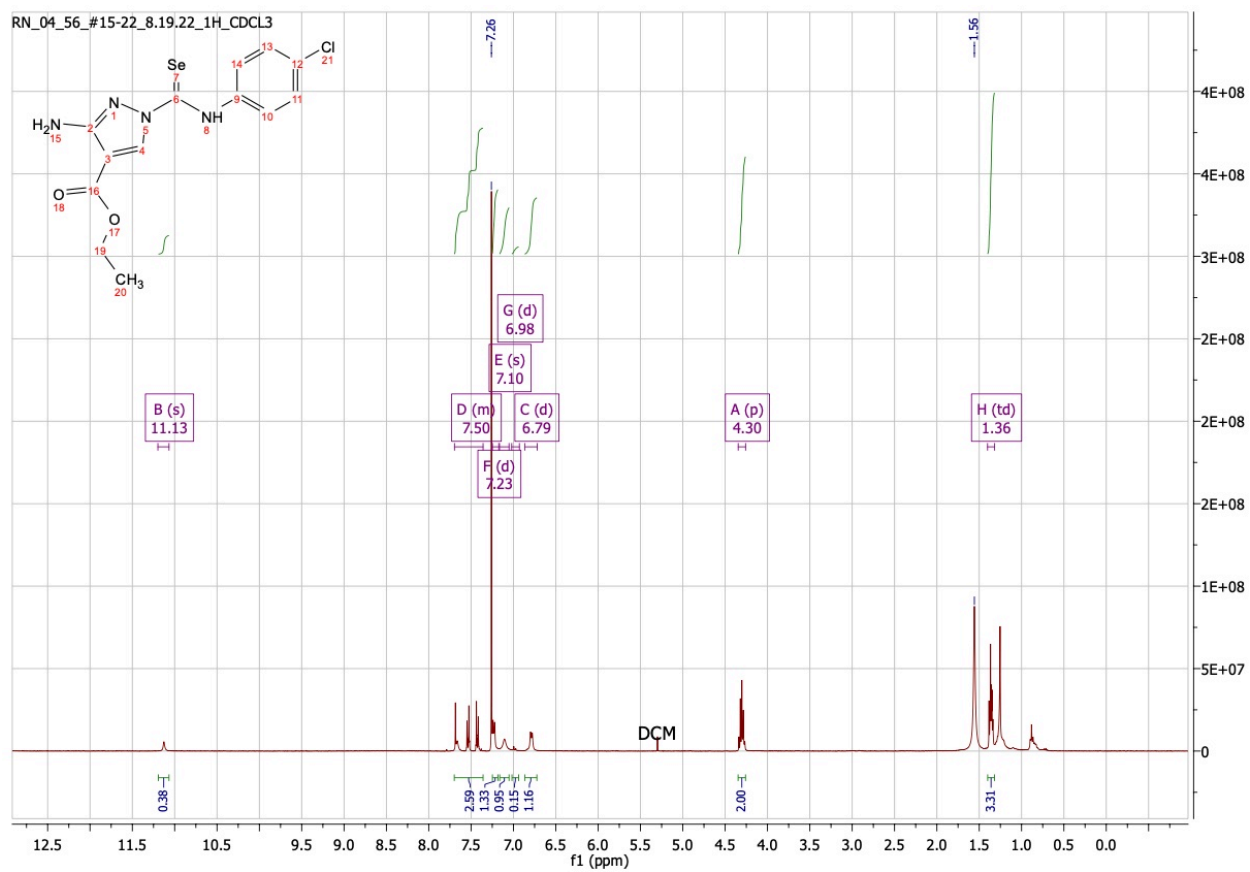
NMR Spectrum 58: RN_04_49 1H-NMR



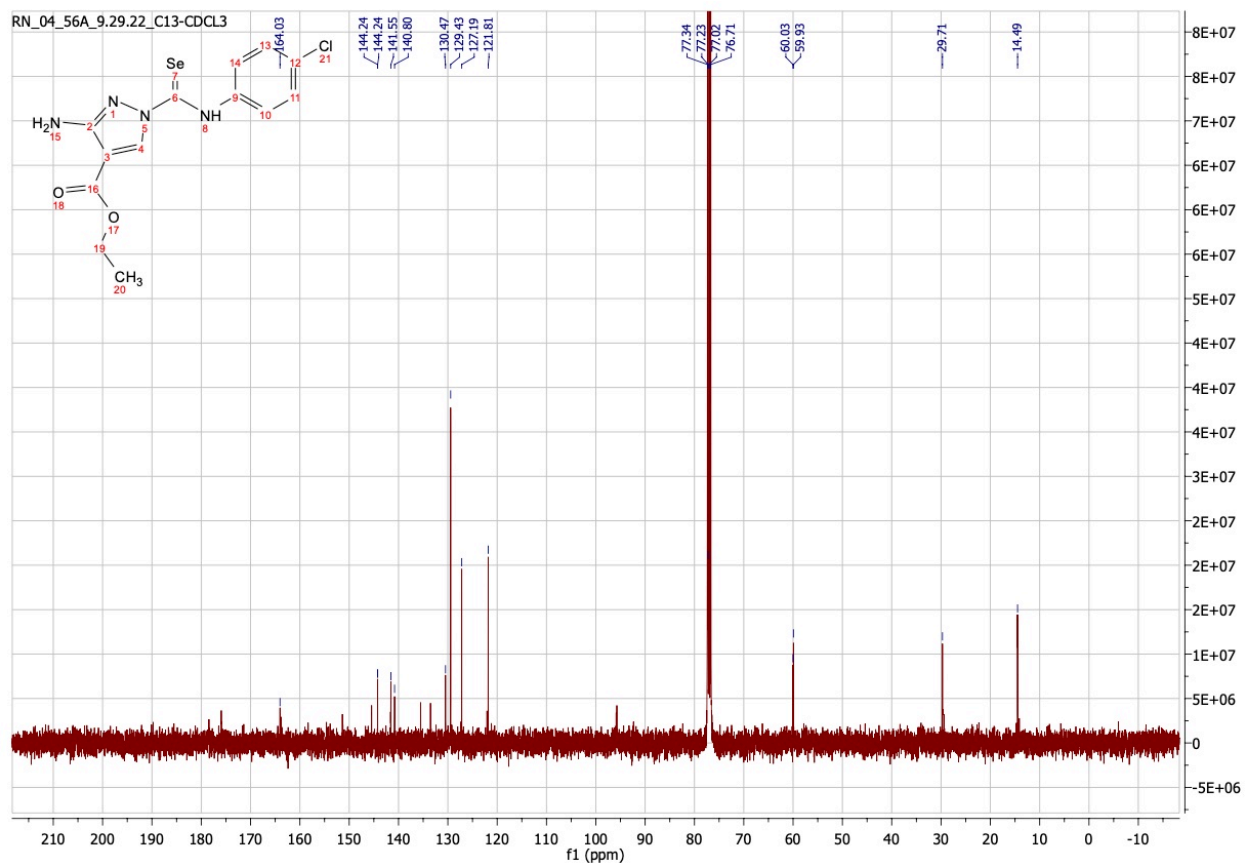
NMR Spectrum 59: RN_04_50A 1H-NMR



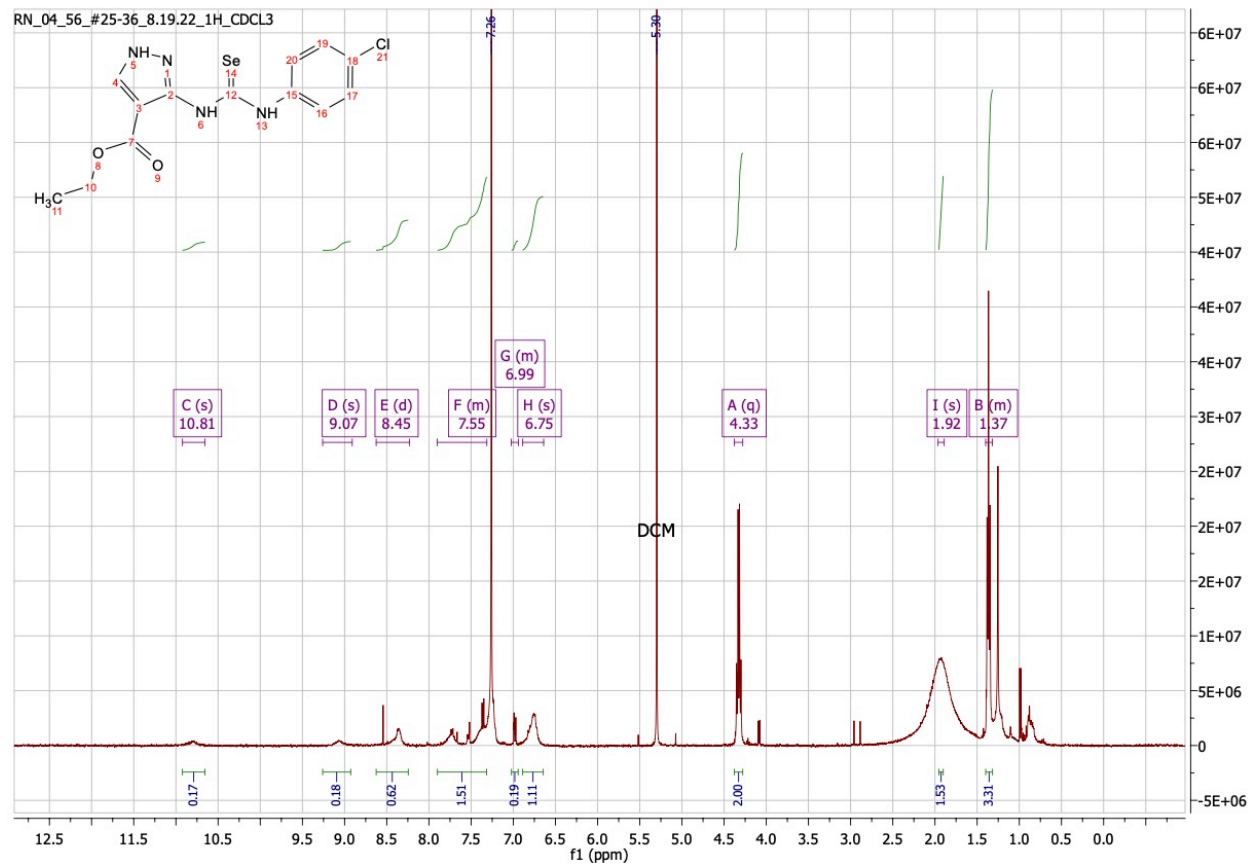
NMR Spectrum 60: RN_04_50A ¹³C-NMR



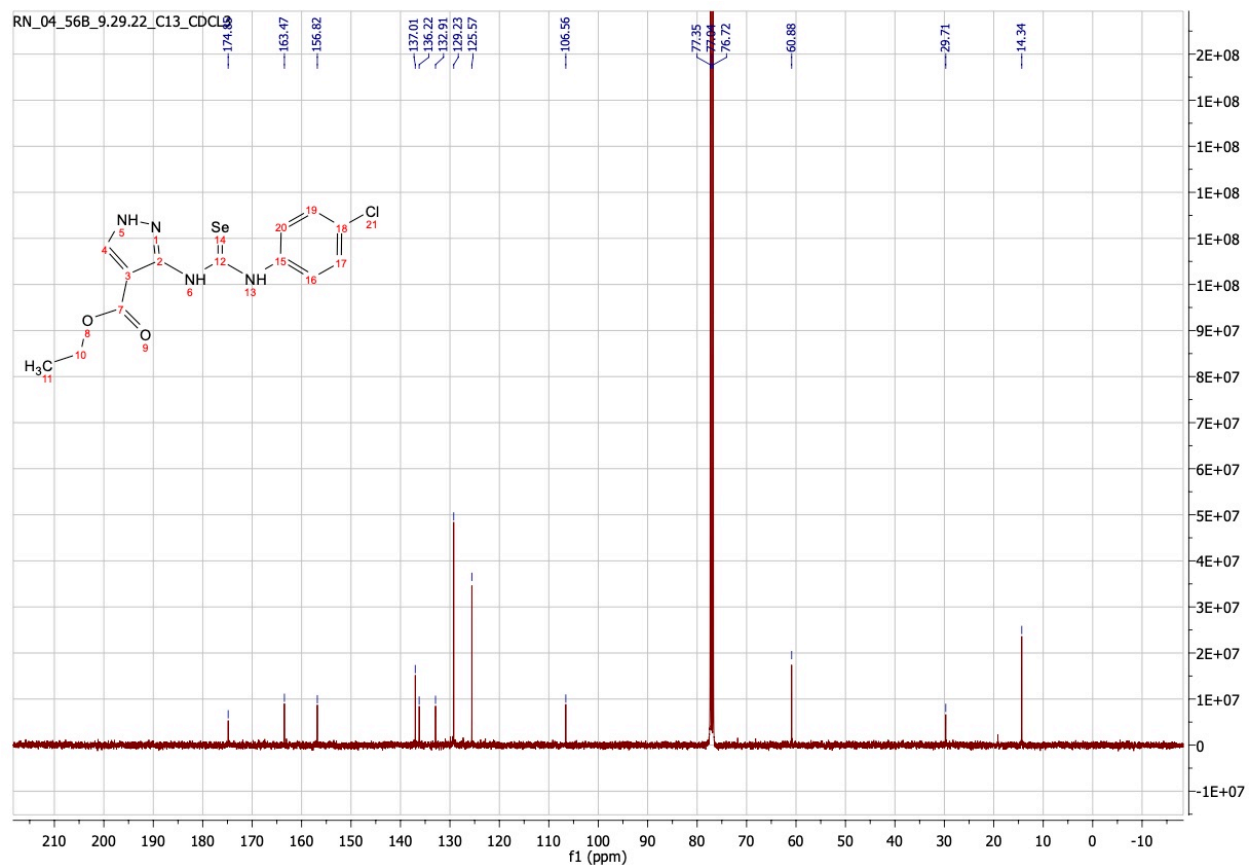
NMR Spectrum 61: RN_04_56A 1H-NMR



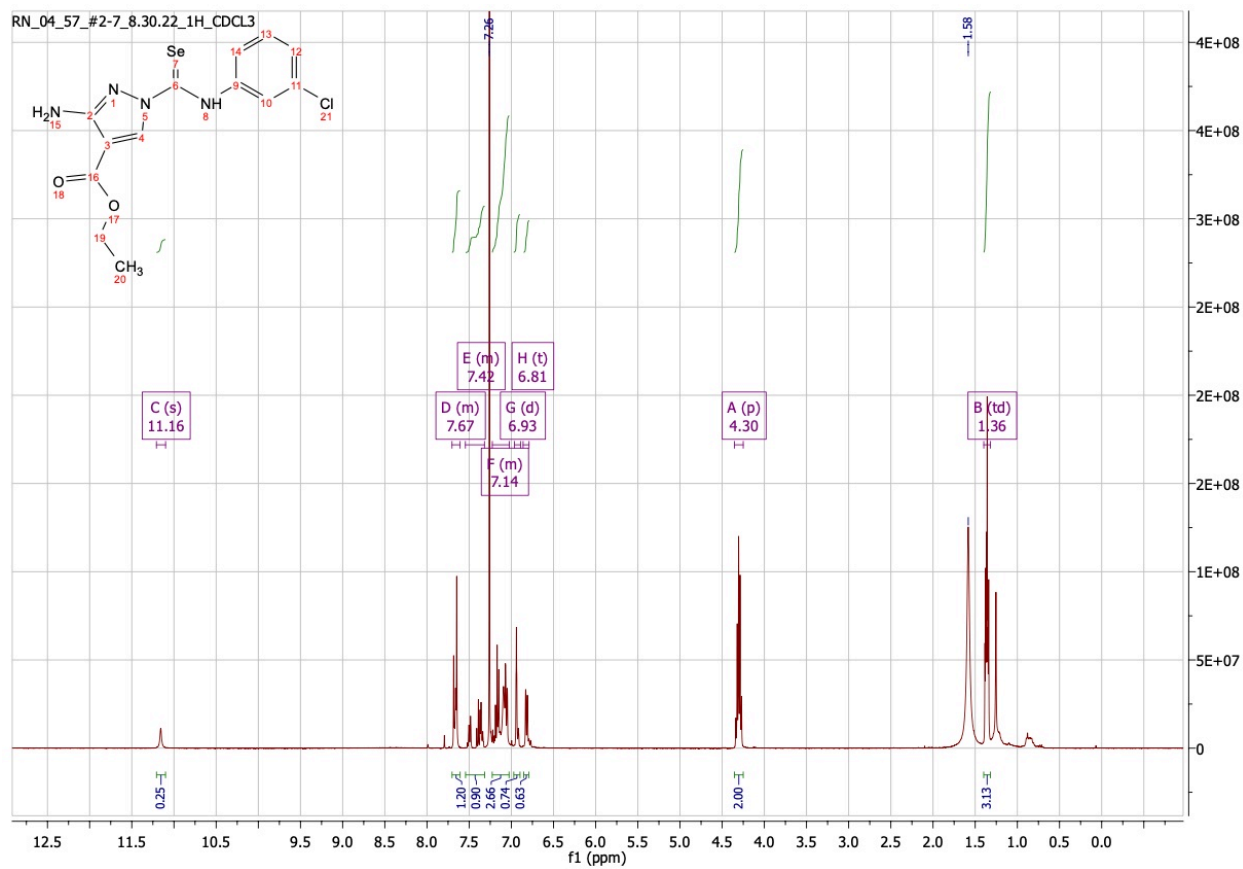
NMR Spectrum 62: RN_04_56A ^{13}C -NMR



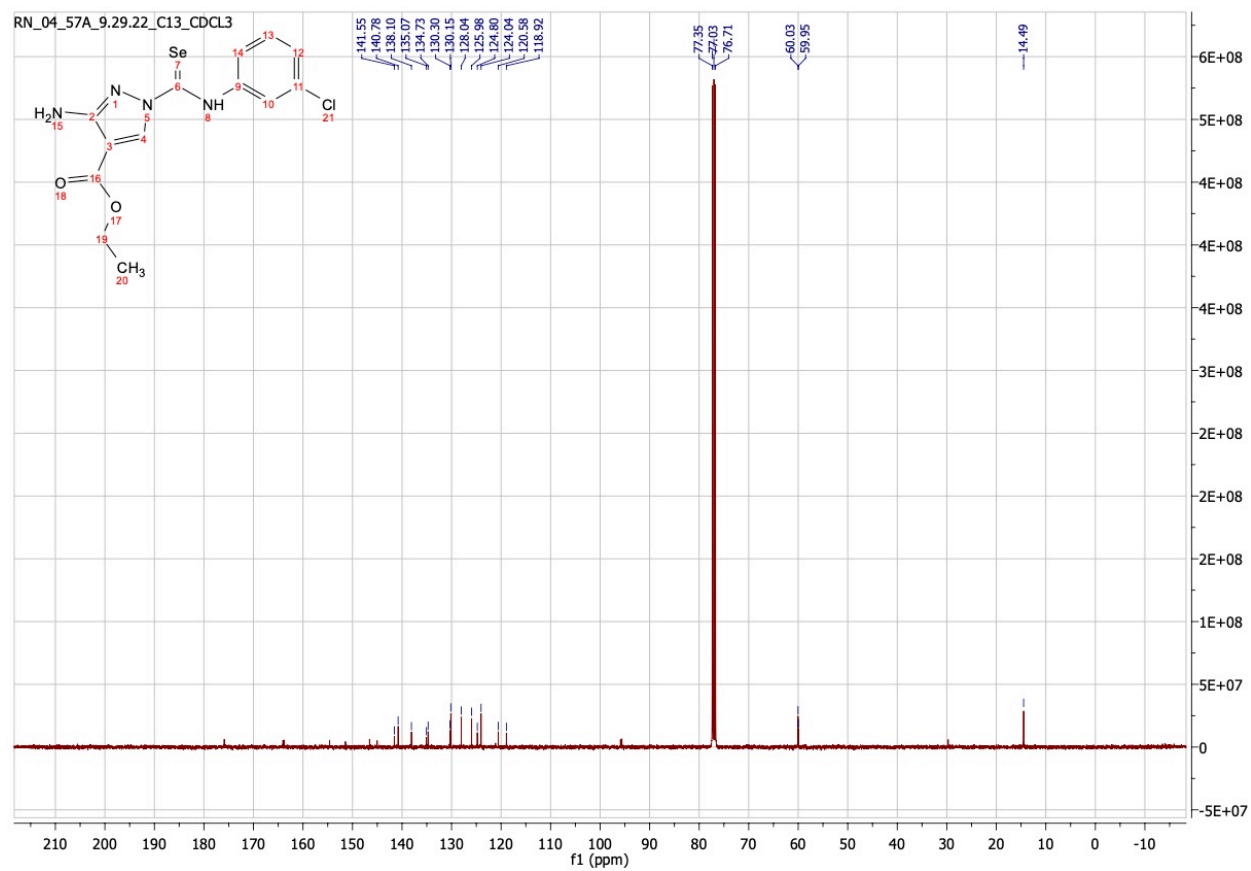
NMR Spectrum 63: RN_04_56B 1H-NMR



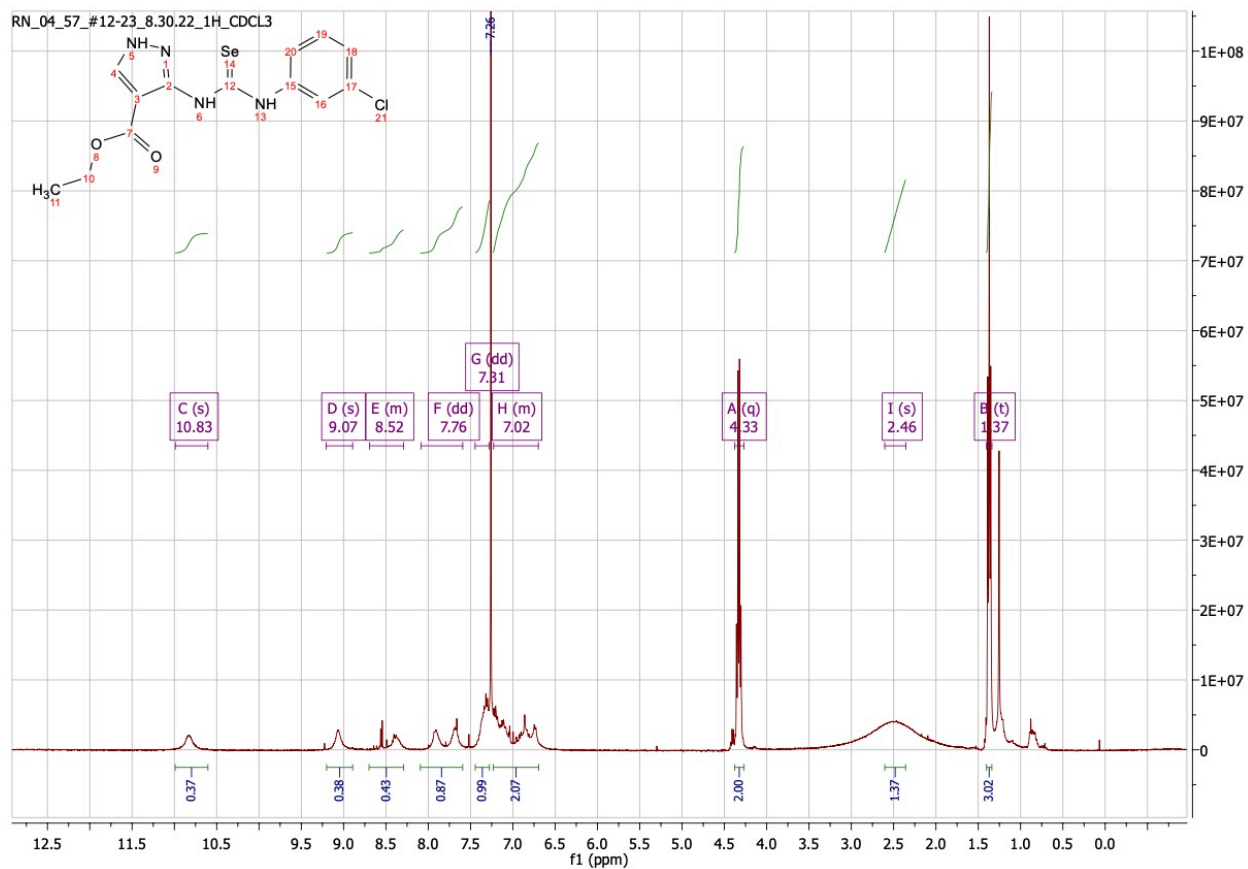
NMR Spectrum 64: RN_04_56B ^{13}C -NMR



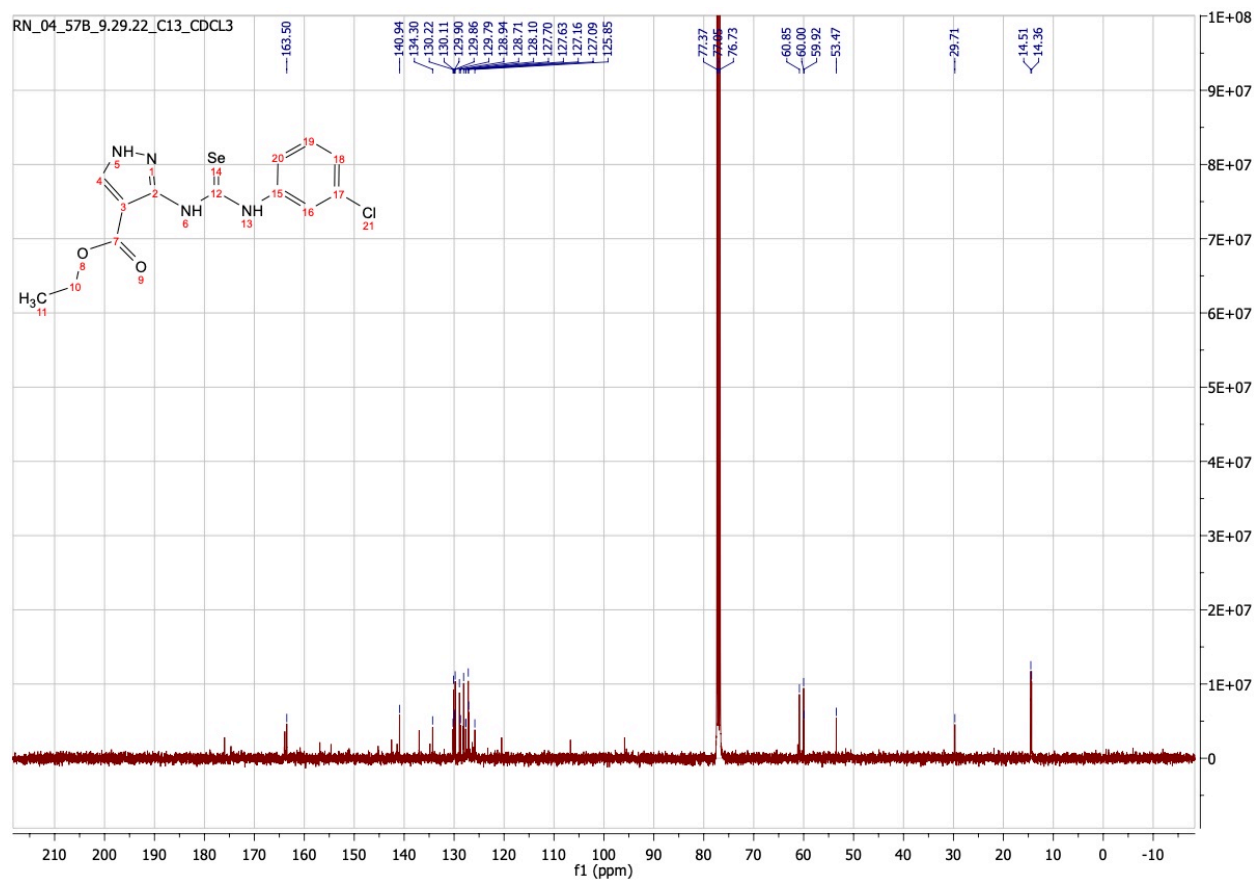
NMR Spectrum 65: RN_04_57A 1H-NMR



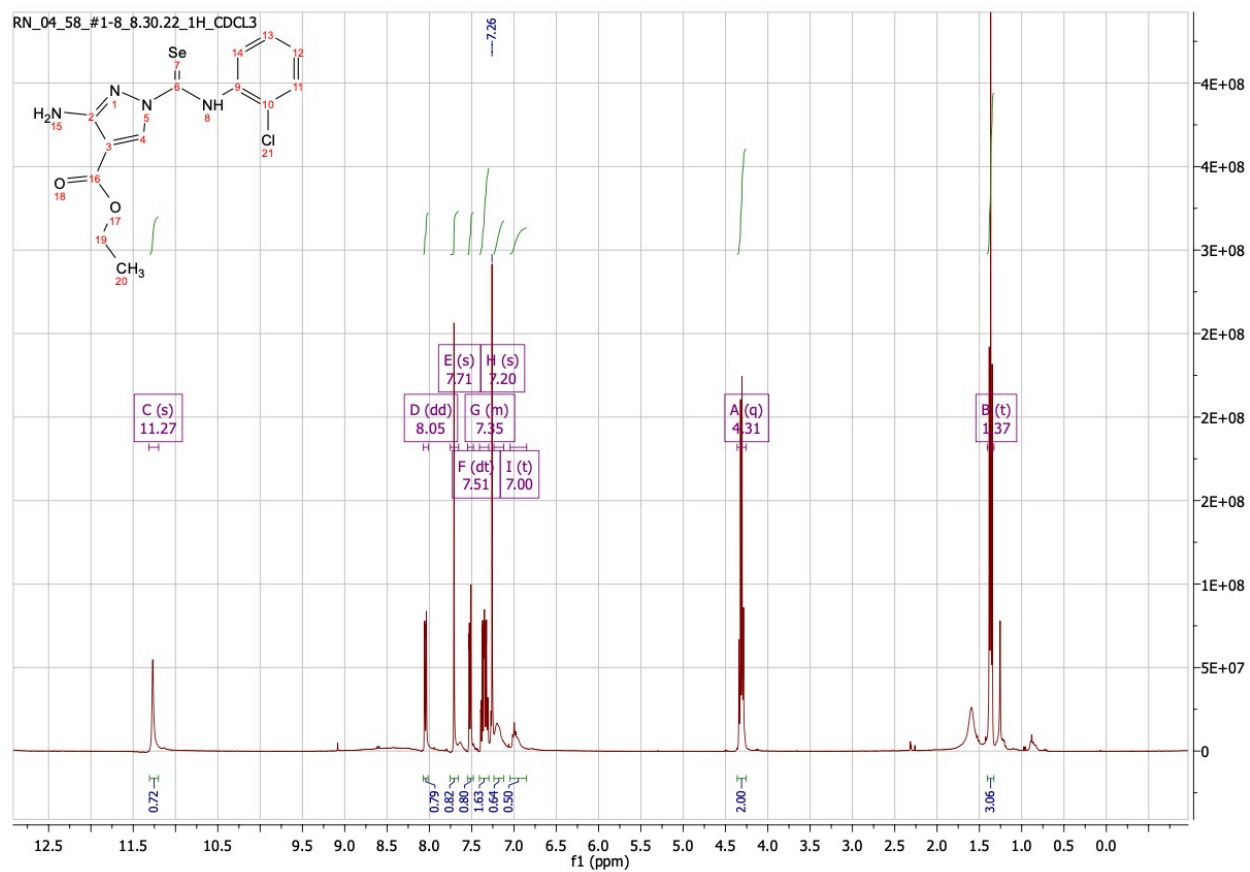
NMR Spectrum 66: RN_04_57A ^{13}C -NMR



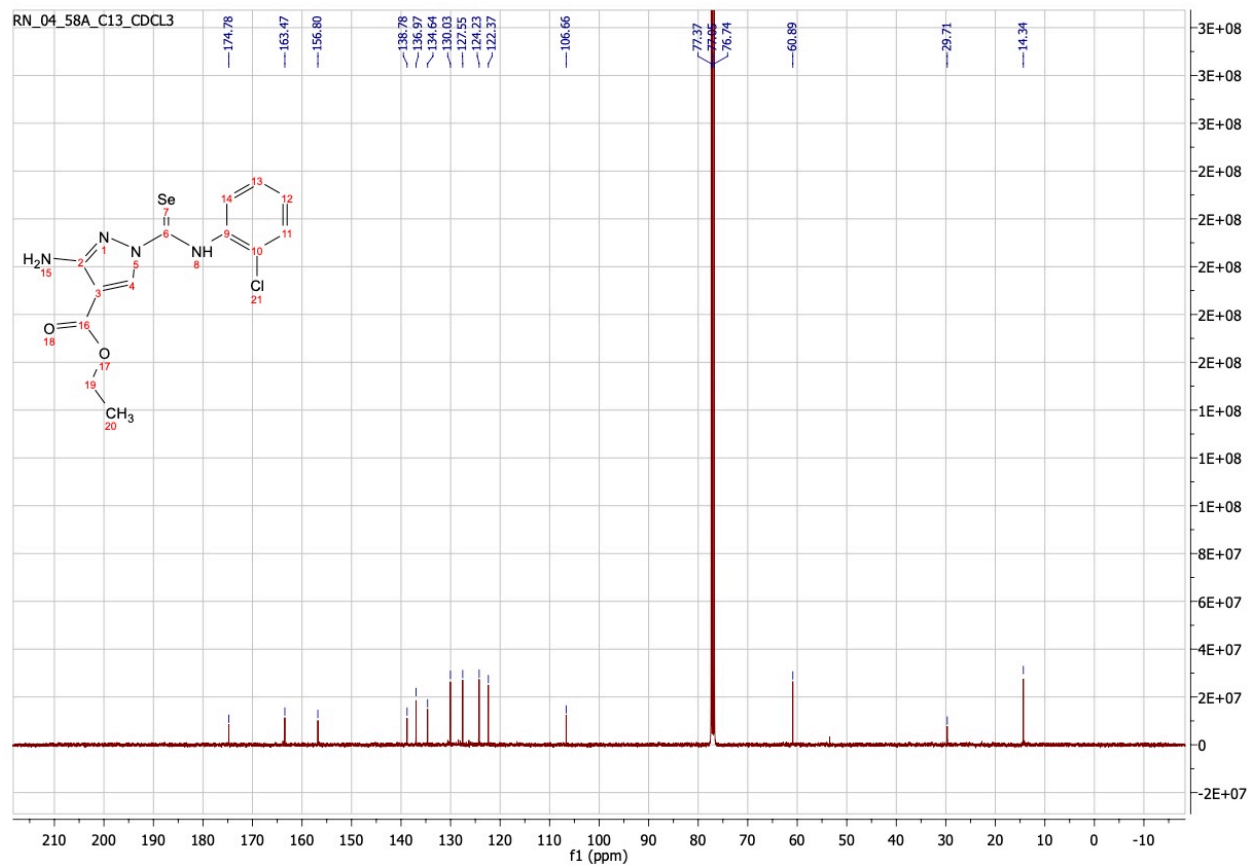
NMR Spectrum 67: RN_04_57B 1H-NMR



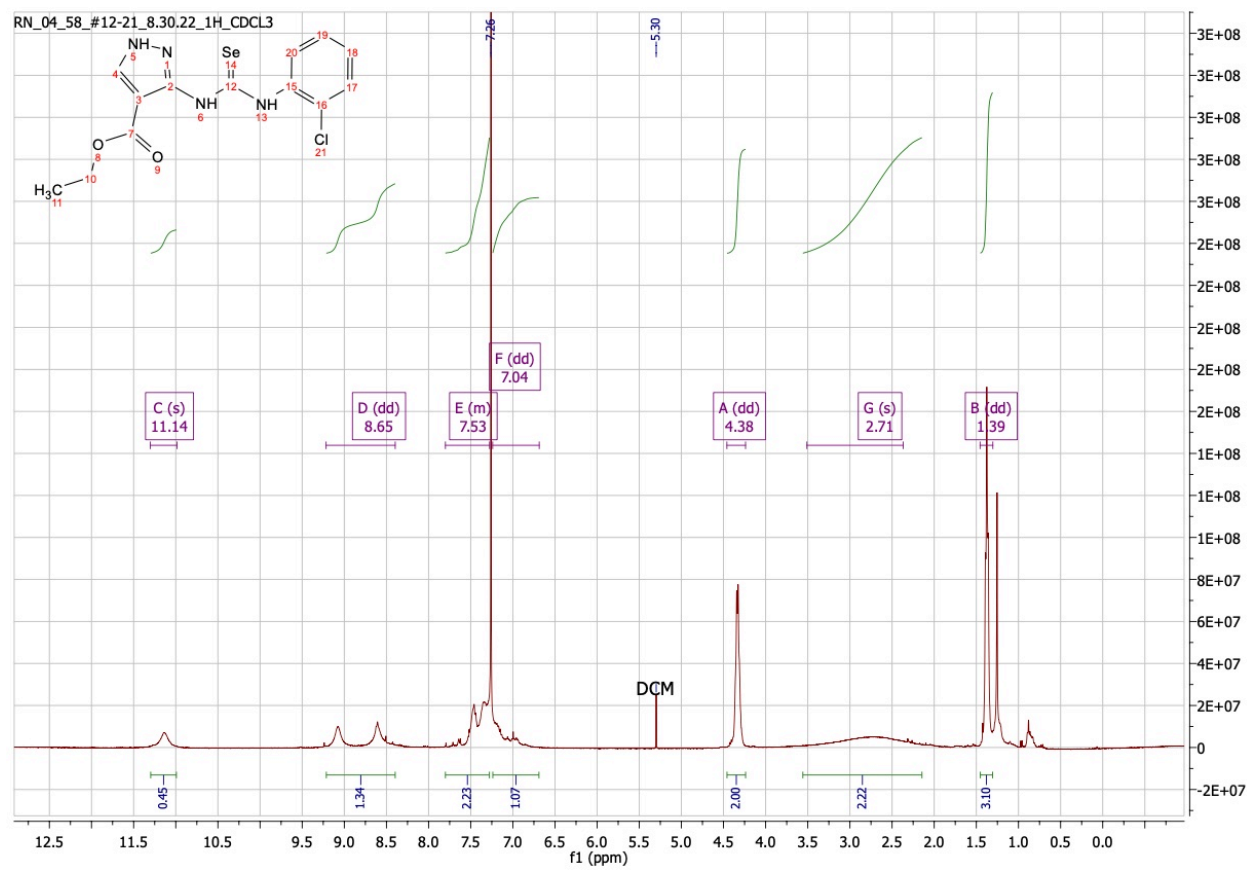
NMR Spectrum 68: RN_04_57B 13C-NMR



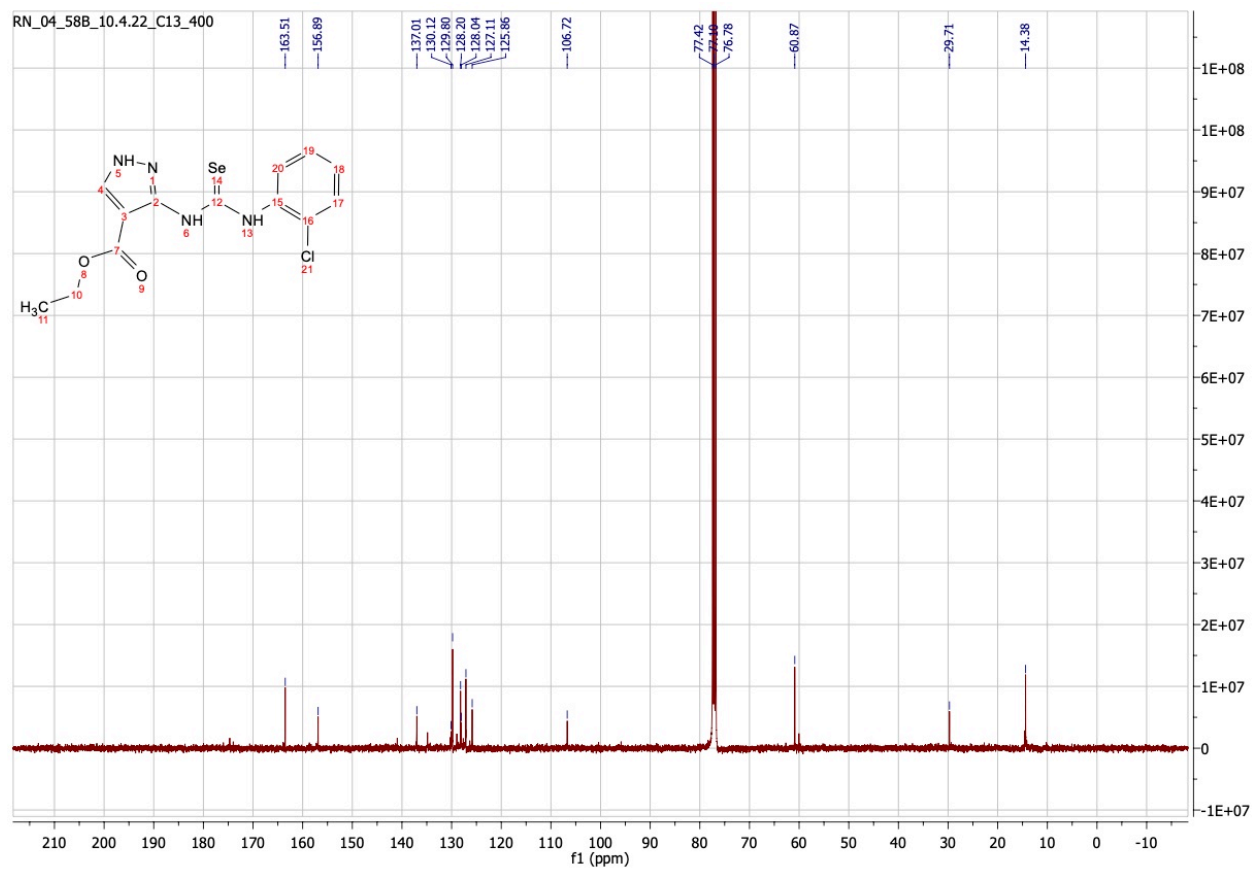
NMR Spectrum 69: RN_04_58A 1H-NMR



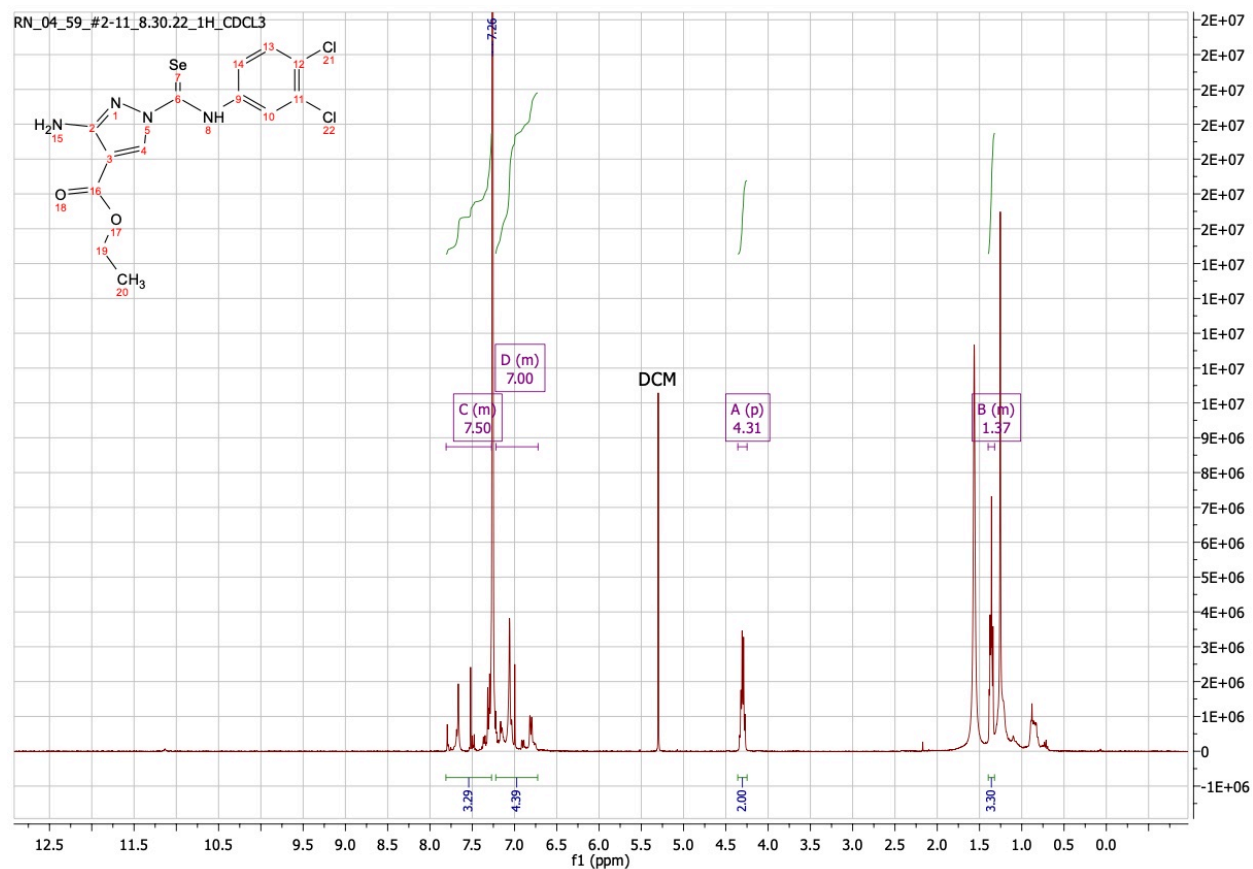
NMR Spectrum 70: RN_04_58A 13C-NMR



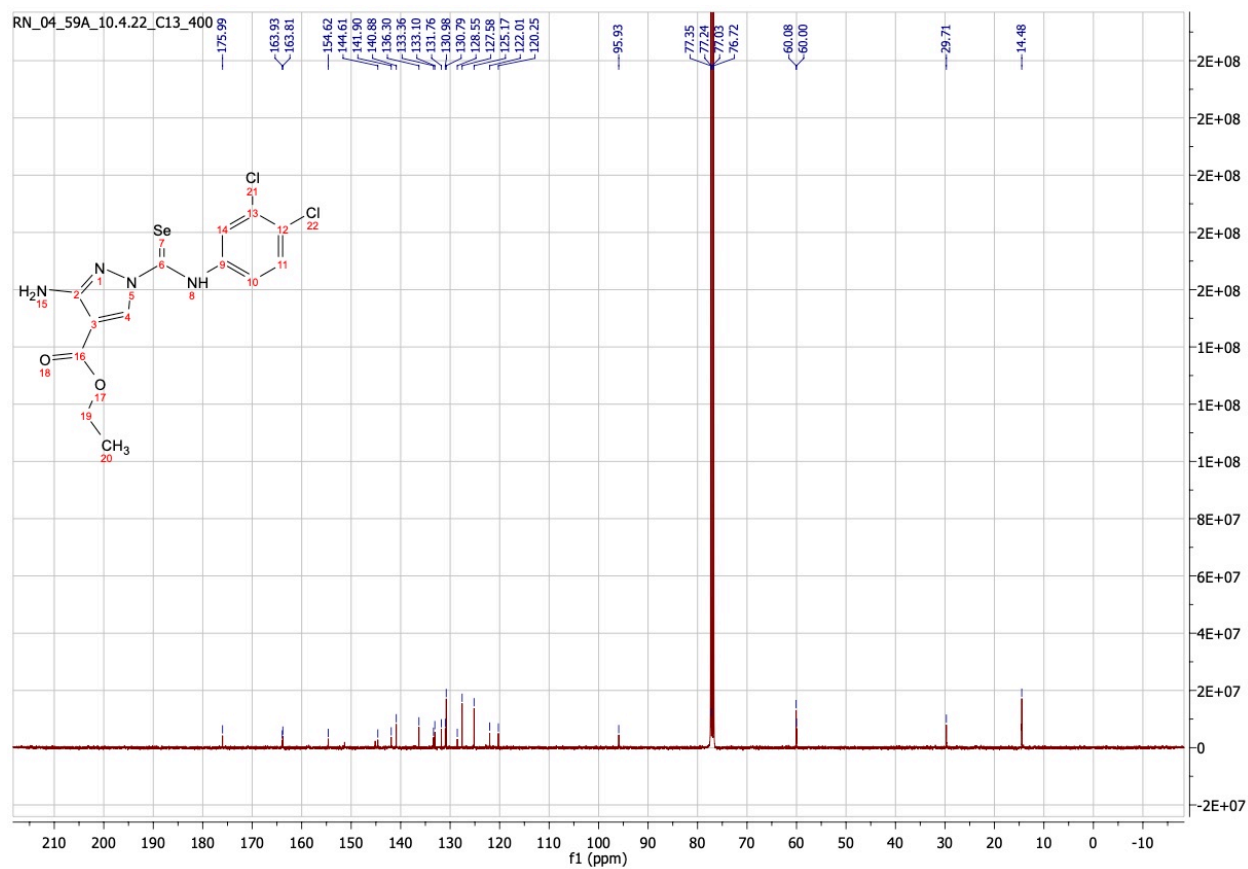
NMR Spectrum 71: RN_04_58B 1H-NMR



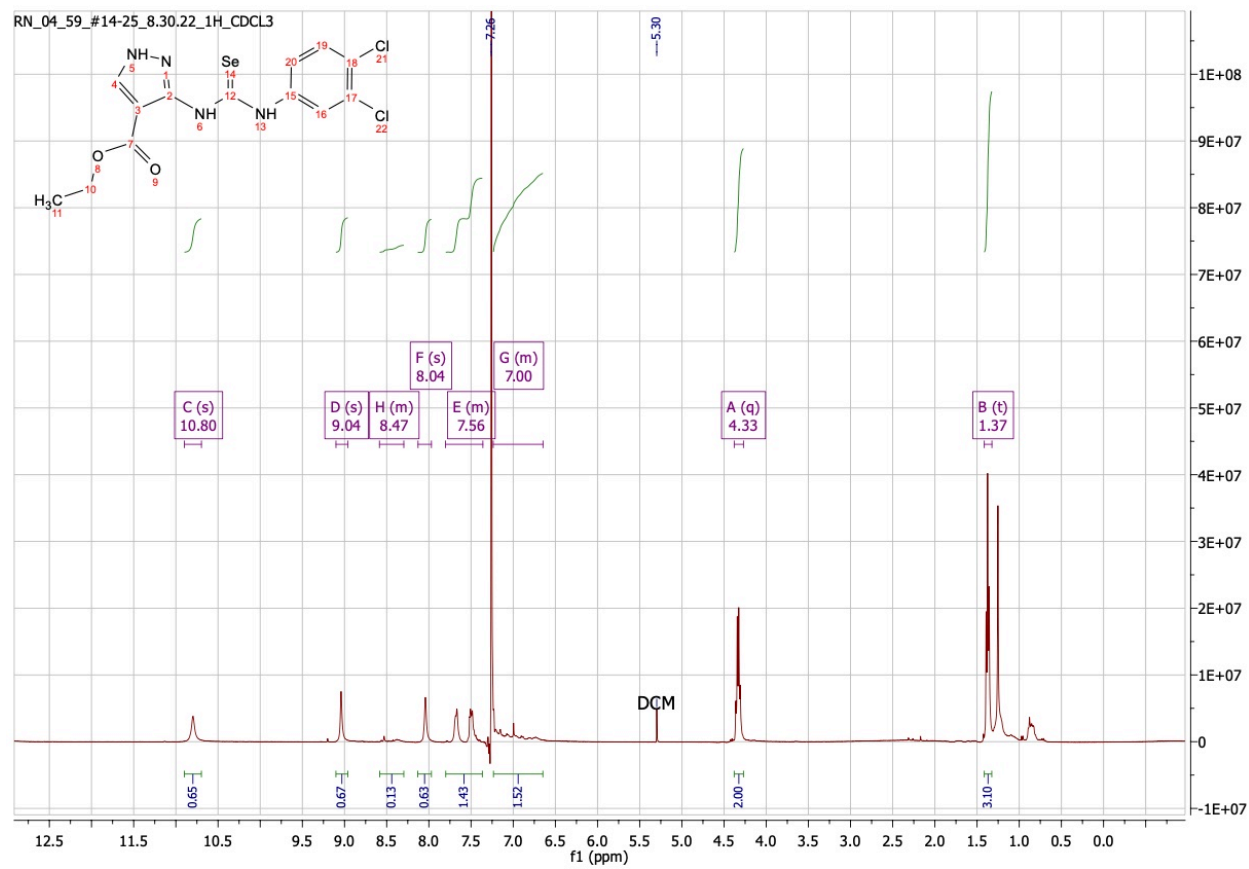
NMR Spectrum 72: RN_04_58B ¹³C-NMR



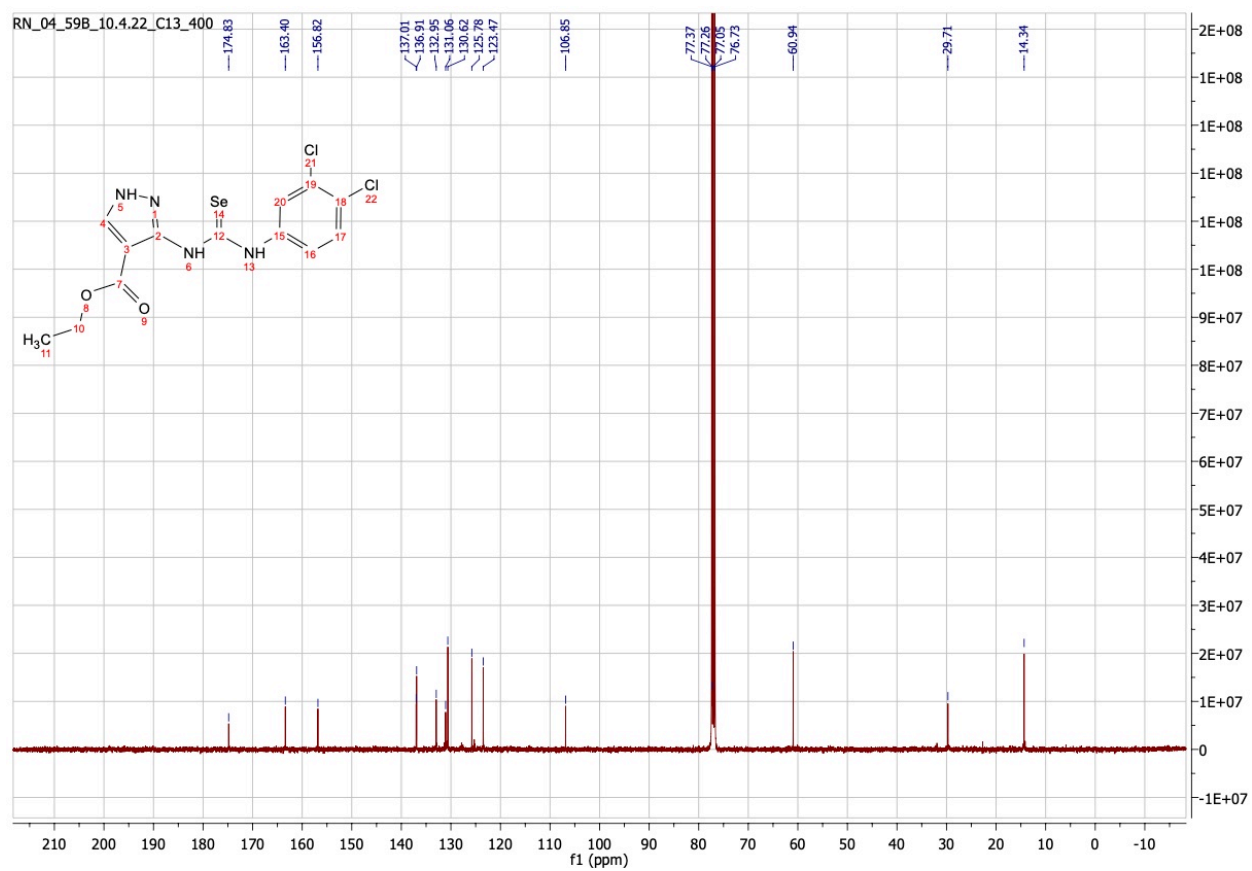
NMR Spectrum 73: RN_04_59A 1H-NMR



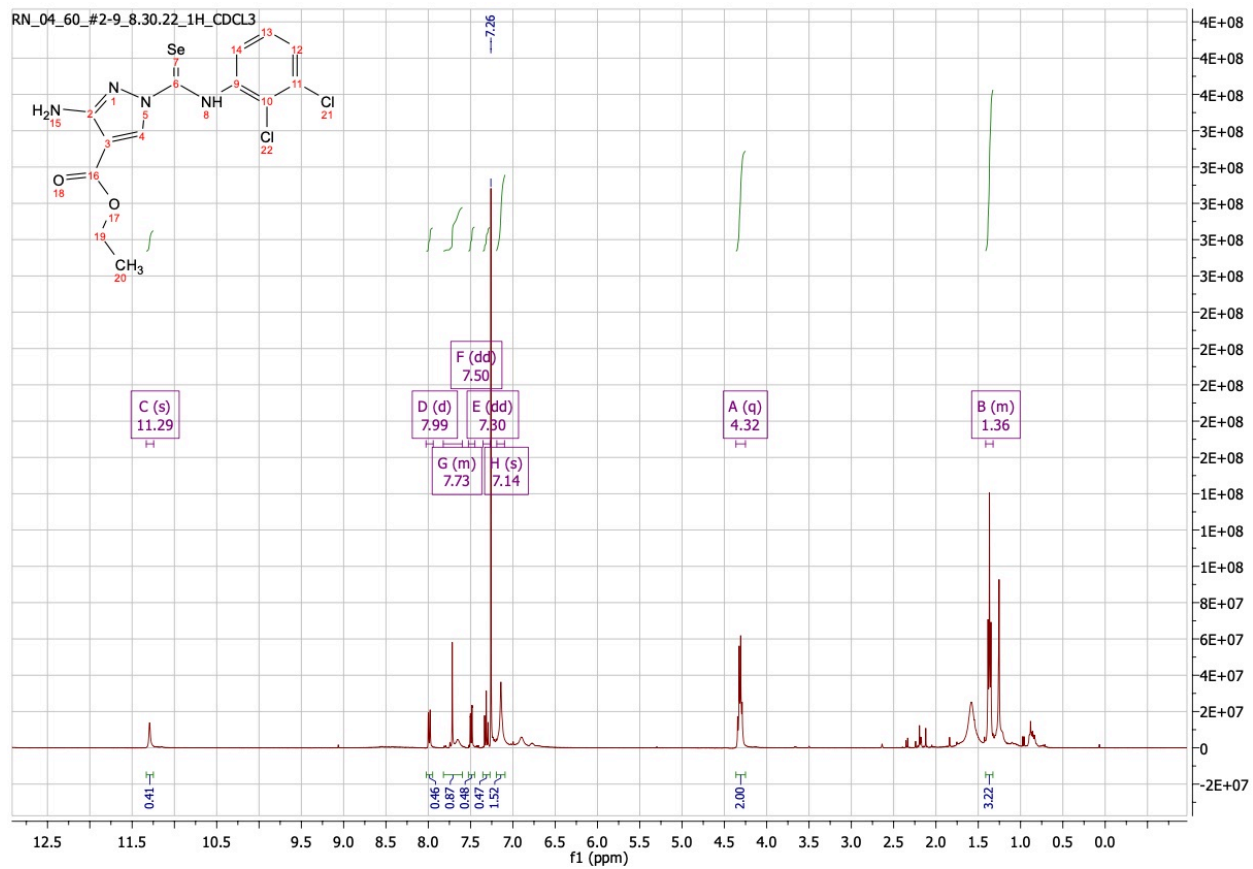
NMR Spectrum 74: RN_04_59A 13C-NMR



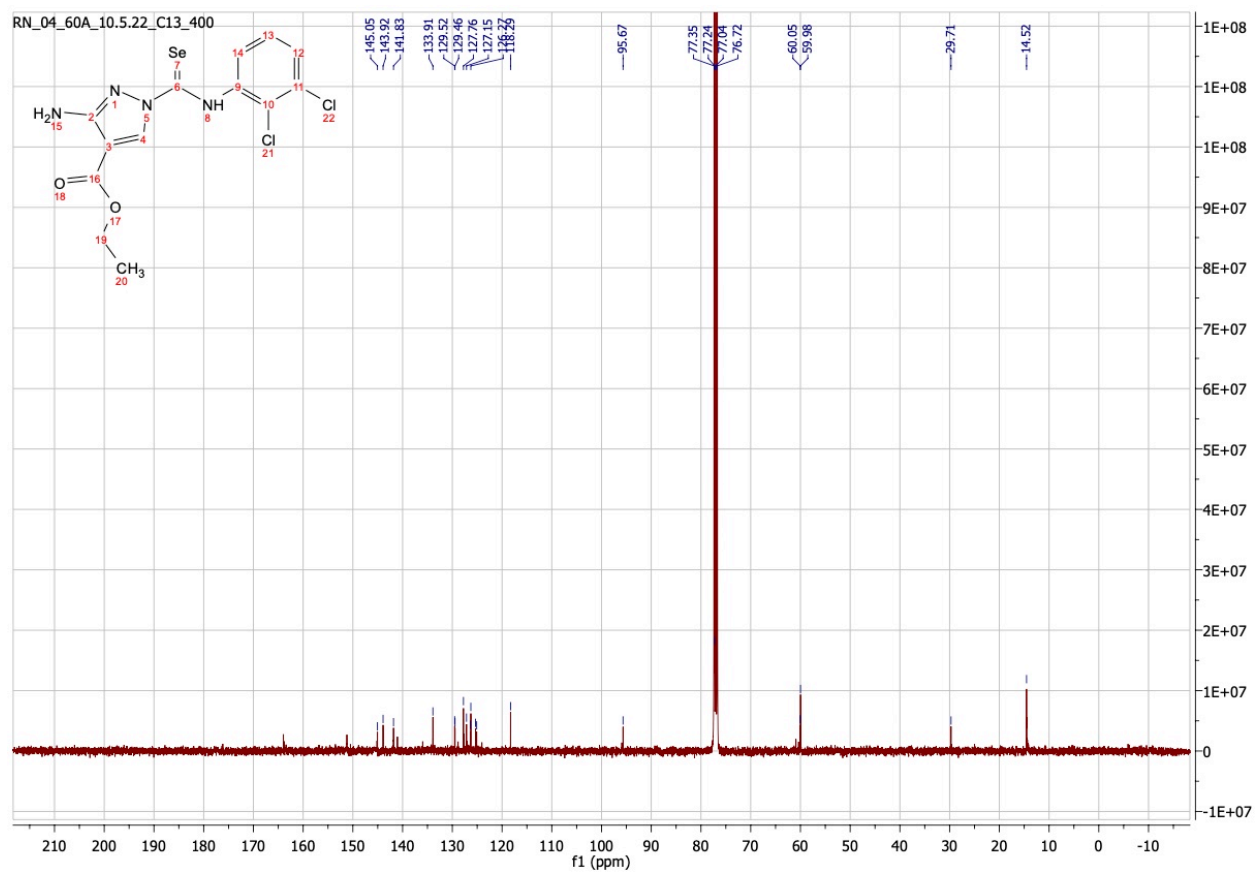
NMR Spectrum 75: RN_04_59B 1H-NMR



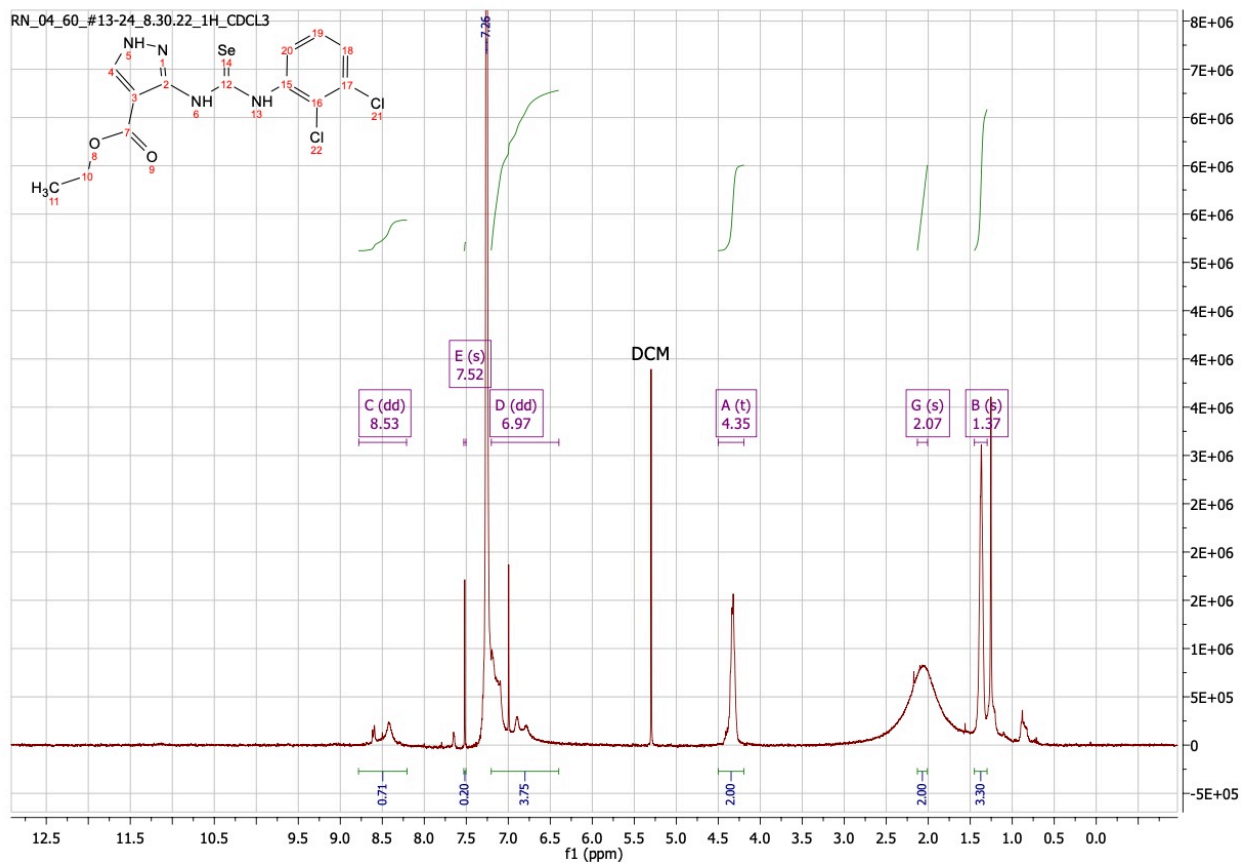
NMR Spectrum 76: RN_04_59B 13C-NMR



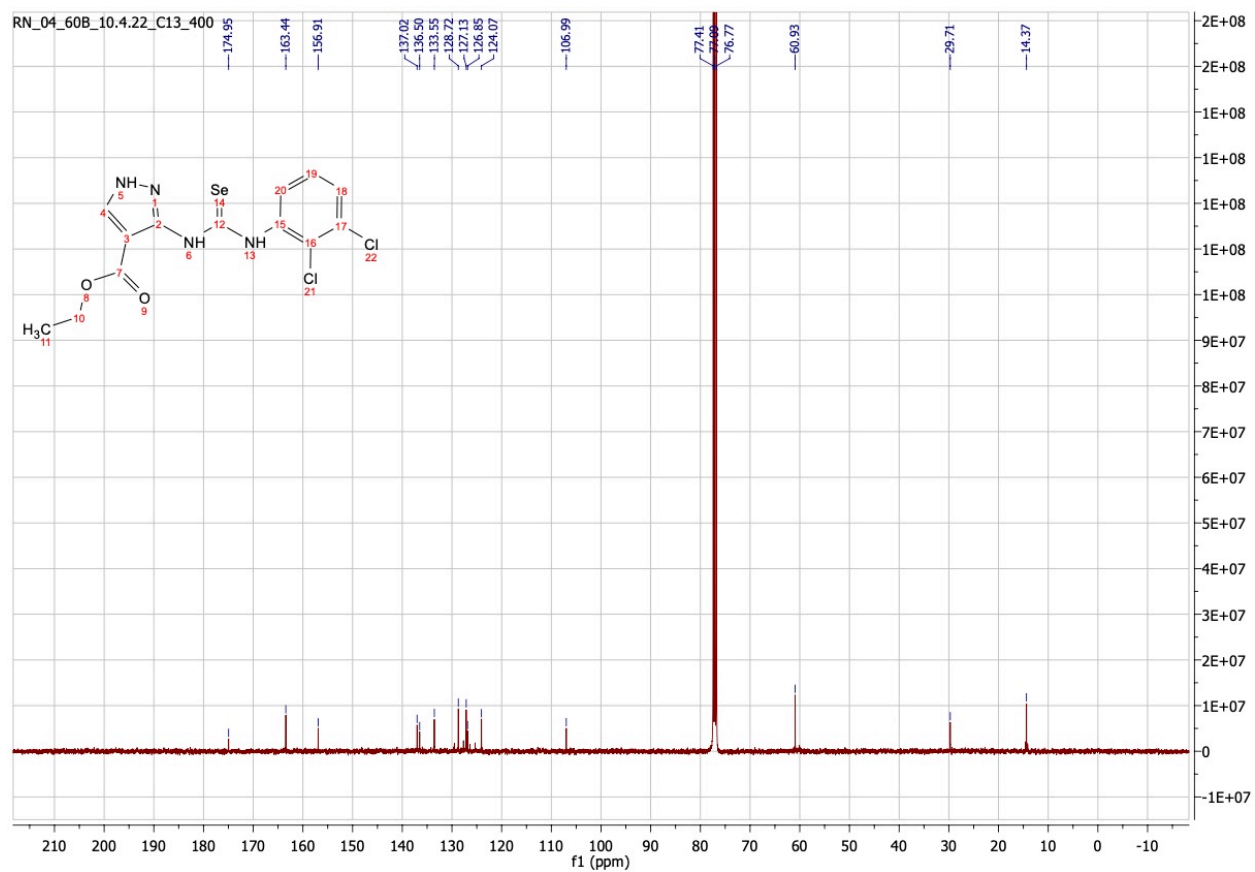
NMR Spectrum 77: RN_04_60A 1H-NMR



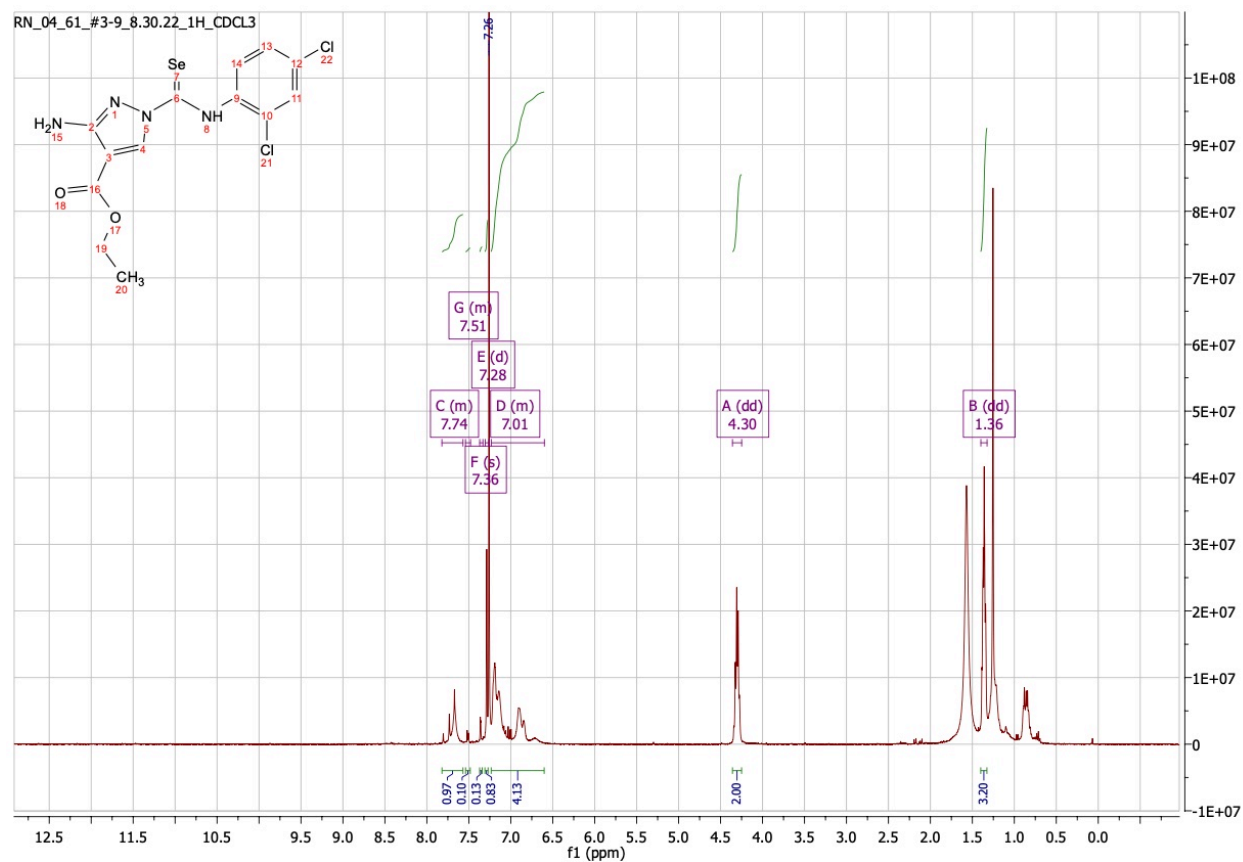
NMR Spectrum 78: RN_04_60A ^{13}C -NMR



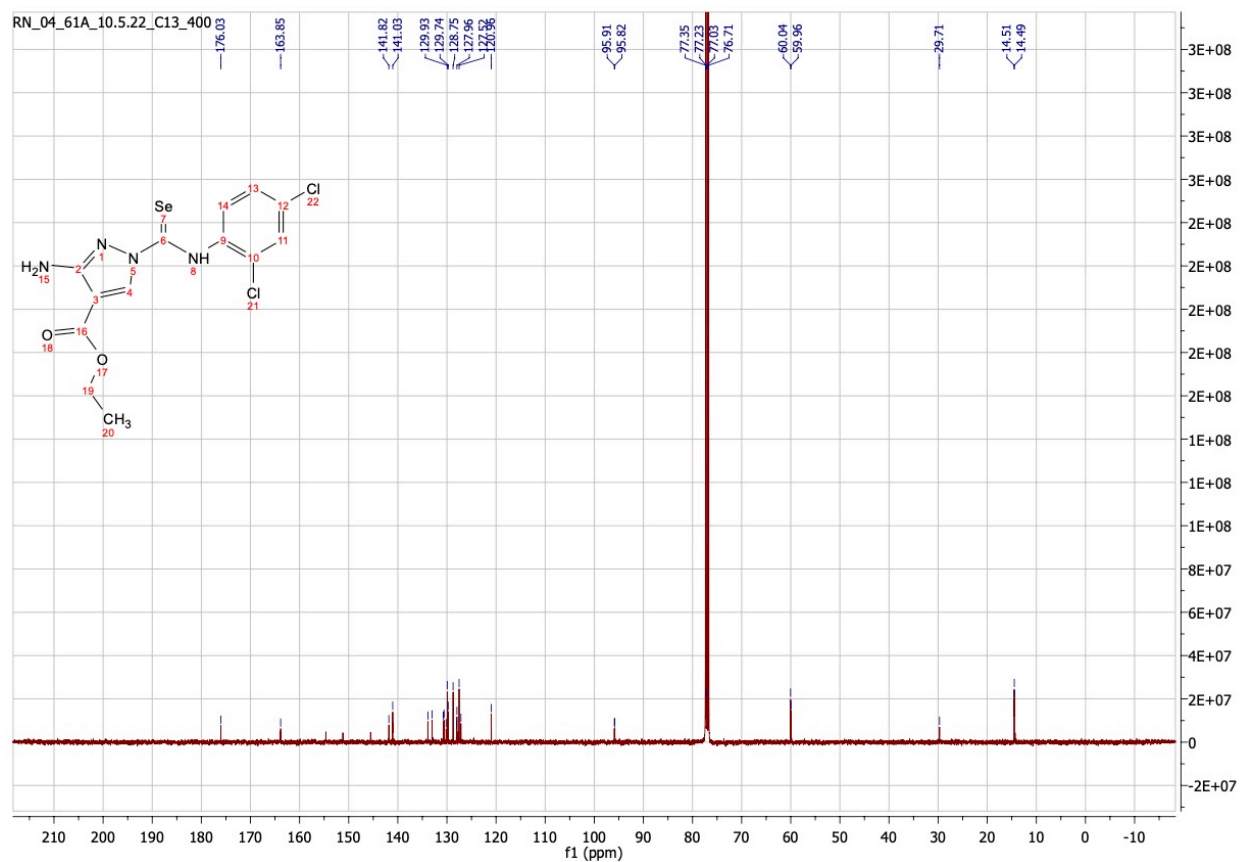
NMR Spectrum 79: RN_04_60B 1H-NMR



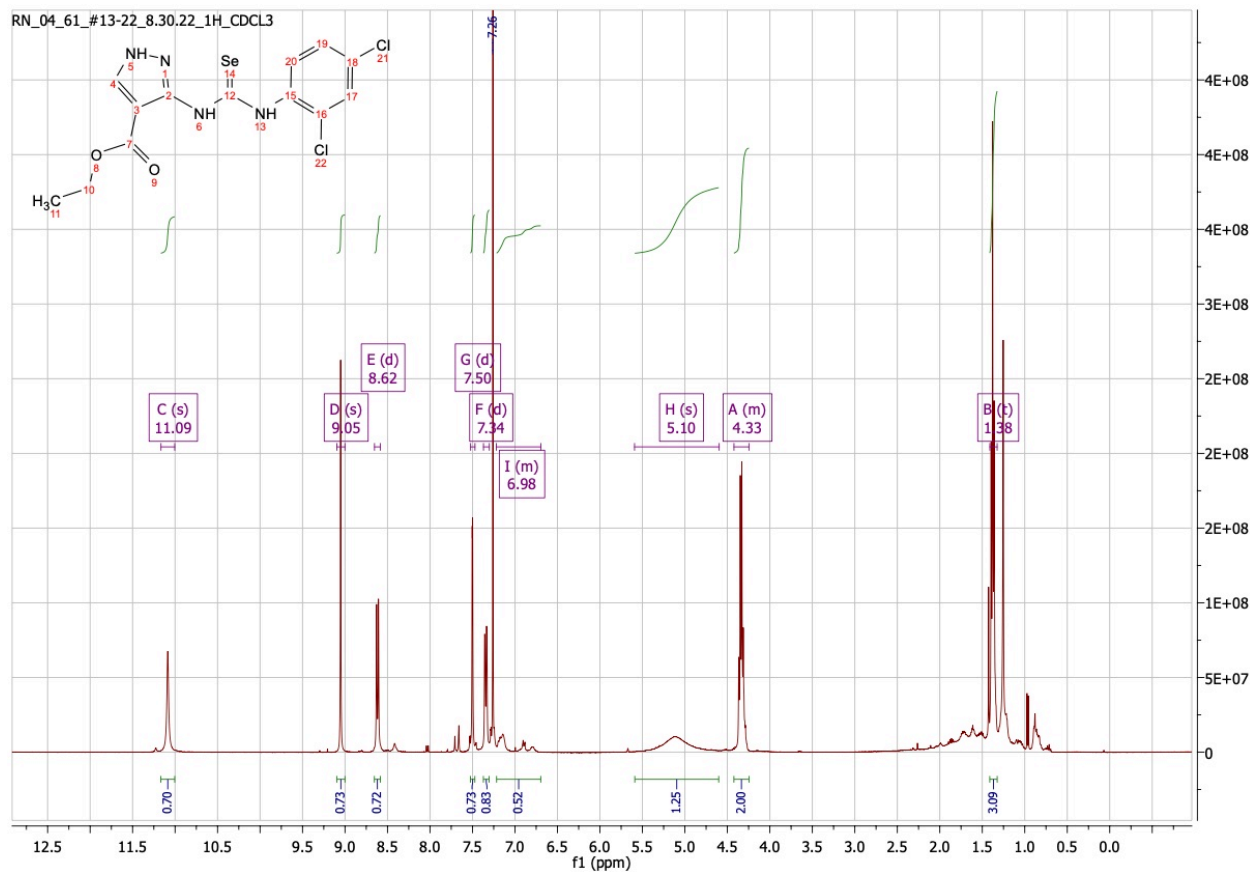
NMR Spectrum 80: RN_04_60B C13-NMR



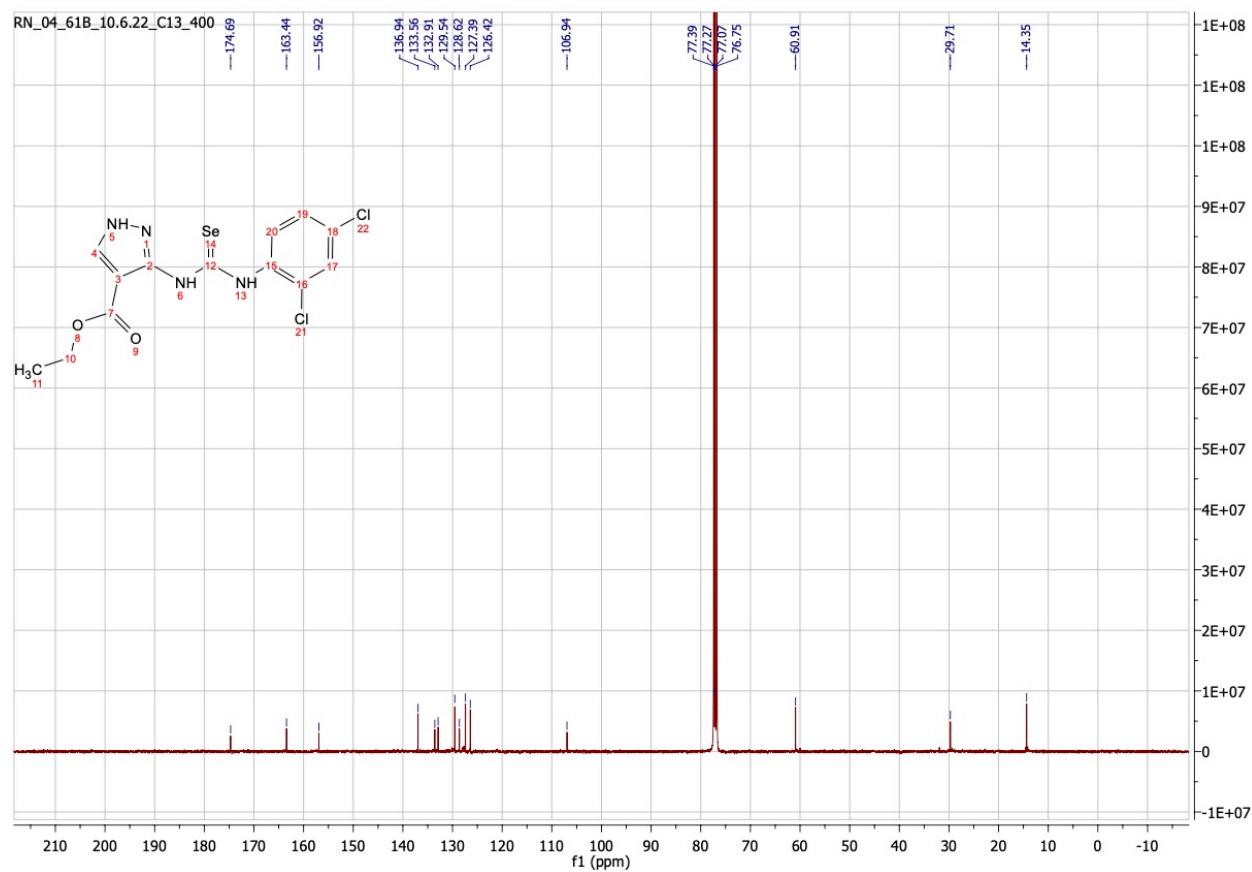
NMR Spectrum 81: RN_04_61A 1H-NMR



NMR Spectrum 82: RN_04_61A 13C-NMR



NMR Spectrum 83: RN_04_61B 1H-NMR



NMR Spectrum 84: RN_04_61B ¹³C-NMR