Synthesis of thiazole-containing pantothenamide analogues as potential antimicrobial agents

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April 2021

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Table of contents

Abstract

Infectious disease is a serious global health burden. The large-scale use of antimicrobials, however, leads to an increase in selection pressure favoring antimicrobial-resistant species. It has been predicted that by 2050, 10 million people per year might die due to antimicrobial resistance. To prevent such a devastating situation, novel antimicrobials with a new mode of action are urgently needed.

The coenzyme A (CoA) biosynthetic pathway is conserved in all living organisms, and CoA is a crucial cofactor and precursor in several essential pathways (*e.g.,* tricarboxylic acid cycle, fatty acid synthesis). Due to the structural differences between the CoA biosynthetic/utilizing enzymes of mammals and microorganisms, selective inhibition can be achieved. Yet, none of the current clinical drugs target any of these enzymes. Molecules of the pantothenamide family have been identified as microbe-selective, inhibitors of CoA utilization pathways, uniquely activated by CoA biosynthetic enzymes. They represent a new structural class of antimicrobials with a new mode of action. However, pantetheinase enzymes in the human serum rapidly hydrolyze pantothenamides into pantothenate and the corresponding amine, thus limiting the clinical relevance of these small molecules. Current medicinal chemistry efforts in our group focus on the development of pantetheinase-resistant pantothenamides.

Herein, 19 novel thiazole-containing pantothenamide analogs were synthesized, and their antimicrobial activities were measured. Chapter 2 describes the design and synthetic strategies used to access the molecules, whereas the assessment of their antibacterial and antiplasmodial (molecules with growth inhibitory activity towards *Plasmodium* species) activities is discussed in chapter 3. Although none of the thiazole-containing pantothenamides reported here show antibacterial activities against *Escherichia coli, Enterococcus faecium, Klebsiella pneumonia, Pseudomonas aeruginosa,* and *Staphylococcus aureus*, three of them inhibit the growth of intraerythrocytic *Plasmodium falciparum*, malaria-causing parasite, at high nanomolar concentrations. These easily accessible molecules are promising hits for the development of new antimalarials. The

results obtained in this thesis not only warrant further pharmaceutical investigations, but also additional medicinal chemistry exploration around this structural scaffold.

Résumé

Les maladies infectieuses représentent un grave fardeau pour la santé mondiale. Bien que les antimicrobiens aient considérablement réduit les ravages causés par ce type de maladies, l'utilisation à grande échelle de ces médicaments entraîne une augmentation de la pression de sélection en faveur des espèces résistantes aux antimicrobiens. Il a été prédit que d'ici 2050, 10 millions de personnes pourraient mourir par année en raison de la résistance aux antimicrobiens. Pour éviter une situation aussi dévastatrice, de nouveaux antimicrobiens dotés d'un mode d'action unique sont nécessaires de toute urgence.

La voie de biosynthèse de la coenzyme A (CoA) est présente dans tous les organismes vivants, et la CoA est un cofacteur et précurseur crucial dans plusieurs processus essentiels (par exemple, le cycle de l'acide citrique et la synthèse des acides gras). En raison des différences structurelles entre les enzymes biosynthétiques et utilisatrices de la CoA de différents organismes, une inhibition sélective peut être obtenue. Pourtant, aucun des médicaments cliniques actuels ne cible ces enzymes. Des molécules de la famille des pantothénamides ont été identifiées comme étant des inhibiteurs des processus de synthèse ou d'utilisation de la CoA sélectifs aux organismes microbiens. Elles représentent une nouvelle classe chimique d'antimicrobiens avec un nouveau mode d'action. Cependant, les enzymes pantéthéinase dans le sérum humain hydrolysent rapidement les pantothénamides pour produire le pantothénate et l'amine correspondante, limitant ainsi la pertinence clinique de ces molécules. Les efforts actuels en chimie pharmaceutique de notre groupe se concentrent sur le développement de pantothénamides résistants aux pantéthéinases.

Au cours de ces travaux, 19 nouveaux analogues de pantothénamide contenant un cycle thiazole ont été synthétisés et leurs activités biologiques mesurées. Le chapitre 2 décrit la conception et les stratégies de synthèse utilisées pour accéder aux molécules, tandis que l'évaluation de leurs activités antibactériennes et antiplasmodiales est abordée au chapitre 3. Bien qu'aucune de ce molécules ne montre d'activité antibactérienne contre *Escherichia coli, Enterococcus faecium, Klebsiella pneumonia, Pseudomonas*

aeruginosa et Staphylococcus aureus, deux d'entre elles inhibent la croissance intraérythrocytaire de *Plasmodium falciparum*, un parasite responsable du paludisme, à des concentrations nanomolaires. Ces molécules facilement accessibles sont très prometteuses pour le développement de nouveaux antipaludiques. Les résultats obtenus dans cette thèse justifient non seulement des recherches pharmaceutiques additionnelles, mais également une exploration supplémentaire de la chimie pharmaceutique autour de cet échafaudage structurel.

Acknowledgments

First, I would like to thank Dr. Karine Auclair. During the past two years, Dr. Auclair not only provided useful feedback and tips on presentations, compounds preservation, and characterization, but also motivated me to aim higher and not give up. I will remember everything she said for the rest of my journey. I am grateful to have met the wonderful people of the Auclair group. Specifically, I would like to thank Daniel for his help with the CombiFlash and MilliQ systems; Dustin for teaching me the antimicrobial susceptibility assay; Jake for plotting my antibacterial data using the Prism software; Sandra, Dustin, Jake, Rohit, Blue, Anne (from the Moitessier group), and Victoria for proof-reading my thesis.

I was lucky to have two wonderful committee members, Dr. Gleason and Dr. Tsantrizos, on my review committee. I would like to thank Dr. Stein for helping me with NMR; Dr. Saadé and Dr. Wahba for running my high-resolution mass spectrometry samples; the Gleason, Li, Lumb, Moitessier, Perepichka, and Tsantrizos research groups for lending me chemicals; and Christina and Xiangning from Dr. Kevin Saliba's laboratory for performing the antiplasmodial assay.

Lastly, I want to thank my parents for supporting my choice of pursuing a degree in chemistry. I am grateful for their financial and mental support throughout the degree. I would like to thank the McGill Chemistry department for accepting me to the Master program. It was an honor to work with top professors and bright scientists. Thank you all for the support/help throughout my journey at McGill University.

List of Figures

List of Schemes

List of Tables

Abbreviations

A. baumannii	*Actinetobacter baumannii*
ACP	Acyl carrier protein
B. anthracis	*Bacillus anthracis*
C. difficile	*Clostridioides difficile*
CoA	Coenzyme A
COASY	CoA synthase
CRE	carbapenem-resistant *Enterobacteriaceae*
DPCK	Dephospho-CoA kinase
E. coli	*Escherichia coli*
E. faecium	*Enterococcus faecium*
H. pylori	*Helicobacter pylori*
HTS	High-throughput screening
IC_{50}	50% growth inhibition
K. pneumonia	*Klebsiella pneumoniae*
LLINs	Long-lasting insecticide-treated bed nets
LPS	Lipopolysaccharides
LTA	Lipoteichoic acids
MIC	Minimum inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
N. gonorrhoeae	*Neisseria gonorrhoeae*
P. aeruginosa	*Pseudomonas aerugionsa*
PanK	Pantothenate kinase
$PanK_I$	Type I PanK
$PanK_{II}$	Type II PanK
$PanK_{III}$	Type III PanK
P. falciparum	*Plasmodium falciparum*
P. ovale	*Plasmodium ovale*
PPAT	Phosphopantetheine adenylyltansferase
PPCDC	Phosphopantothenoylcysteine decarboxylase

PPCS	Phosphopantothenoylcysteine synthetase
P. vivax	*Plasmodium vivax*
SAR	Structure-activity relationship
S. aureus	*Syaphylococcus aureus*
Spp.	Species
VRE	Vancomycin-resistant *E. faecium*
WTA	Wall teichoic acids

Chapter 1

Introduction

1.1 Infectious Disease

Infectious disease is a global health burden. Bacteria, fungi, parasites and viruses are the causative agents of infectious diseases,[1] which represent most of the mortalities and morbidities worldwide.[2,3] Out of all infectious diseases, lower respiratory tract infections are the deadliest, with a global mortality of 2.6 million in 2019.[3] Clinical complications often result from a secondary bacterial infection following a viral infection, with the pathogens acting synergistically to further weaken the host immune system.[2,4] One well-known example is the 1918 Spanish flu, caused by the influenza A virus (H1N1) with mortalities of approximately 50 million people.[2] Research has shown that most of these mortalities were associated with secondary bacterial infections caused by *Streptococcus pneumoniae.*[2] Secondary bacterial infections were also observed in diseases such as the 2009 Swine flu,[2] MERS,[5] and COVID-19.[6–8]

The discovery of antibiotics is an important milestone in human history. Antibiotics are not only the first line treatment for infectious diseases but are also essential to modern medicine, for example during surgeries, transplants and chemotherapies.[9–11] Most of the clinically relevant antibiotic classes were discovered in the 1940s. Only a few novel classes were discovered in the 21st century (Figure 1.1). In 2019, out of the 48 new drugs approved by the United States Food and Drug Administration, only five drugs were designed to treat bacterial or parasitic infections.[12] Of these, three are derivatives of existing antibiotics and one is used in a combination therapy. The lack of antibiotics in the pipeline is concerning since many bacteria have developed resistance mechanisms against one or more clinically relevant antibiotics.[11,13–15] As a result, novel antibiotics are urgently needed.

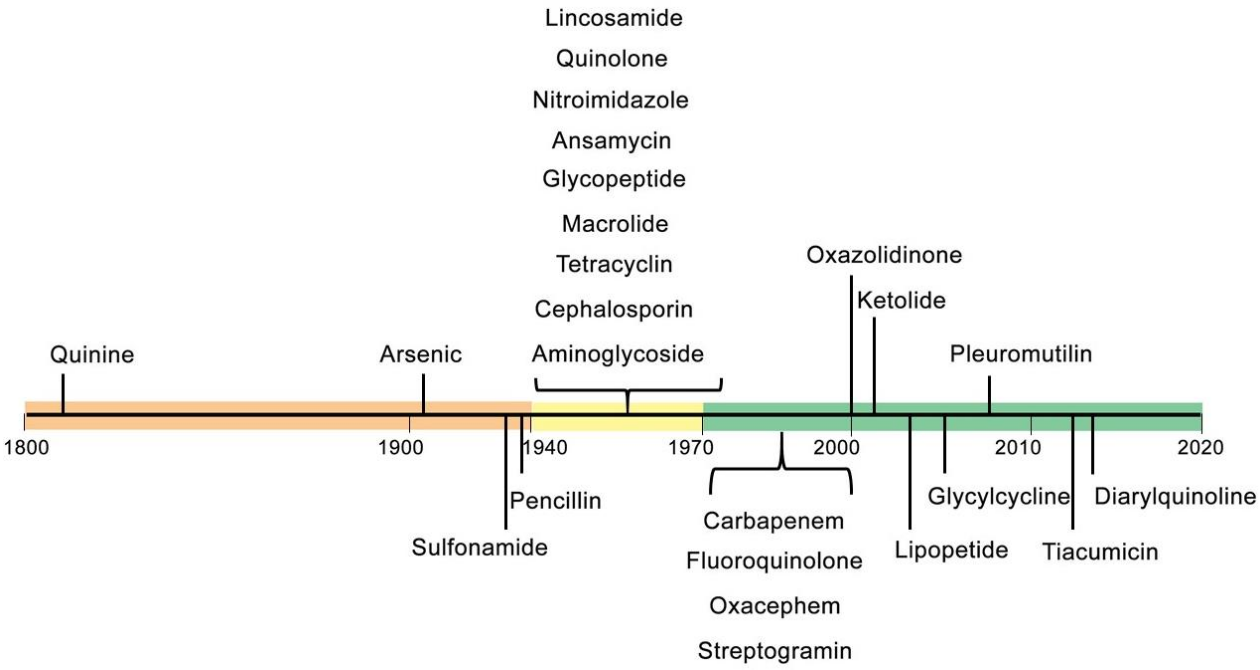


Figure 1.1: Timeline for the introduction of major antibiotic classes to the market.[16–19] Orange and green box highlight the period before and after the golden era, respectively. The Golden Era is highlighted in yellow.

1.2 Antimalarials Overview

Not only do bacteria develop resistance mechanisms, resistance to antiparasitic is on the rise as well. Vector-borne diseases are a major health burden in the developing countries. These diseases account for 700,000 deaths globally, with malaria contributing the most deaths.[20,21] In 2018, 228 millions people were infected and 405,000 people died of malaria.[22] Children under the age of 5 and pregnant women are the most vulnerable and account for more than half of the malaria cases.[22]

Malaria is caused by parasites from the genus *Plasmodium*. There are over 120 different *Plasmodium* species around the globe, of which only six infect humans.[23] *Plasmodium falciparum* and *Plasmodium vivax* are the two most common species responsible for infections in sub-Saharan Africa and the Americas, respectively.[22,23] The symptoms of *P. vivax* are less severe compared to those of *P. falciparum*, with the majority of deaths due to *P. falciparum* infections.[23,24]

The life cycle of *Plasmodium* species (*Plasmodium* spp.) is complex and there are minor variations between species. In *P. vivax and P. ovale,* the sporozoites (a specific stage in the life cycle of *Plasmodium* spp. at which the parasite has the ability to infect human during a blood meal) can remain dormant for years, which is the primary cause of relapse, whereas the dormancy stage is absent in *P. falciparum.*[24,25] In brief the parasite depends on both human and female *Anopheles* species, a genus of mosquito, for survival (Figure 1.2). The parasitic cycle begins when an infected female *Anopheles* species injects sporozoites into the human bloodstream. The sporozoites travel to the liver, where they undergo the first round of asexual replication and form schizonts, a multinucleate form of the parasite.[24,25] When the schizonts reach a certain size, they burst and release daughter cells, the merozoites, into the bloodstream. In the bloodstream, merozoites invade erythrocytes and undergo another round of asexual reproduction.[25] When the erythrocyte bursts, merozoites are released. It is the bursting of the erythrocytes that cause the clinical symptoms of malaria.[24] In the absence of treatment, the cycle of invasion, asexual replication, and cell bursting repeats itself over and over. Some of the asexual parasite cells migrate to the bone marrow and mature into male and female gametocytes.[25] The gametocytes migrate back to the bloodstream and are ingested by the mosquito during a blood meal.[25] In the insect gut, the male and female gametocytes differentiate into male and female gametes, which undergo sexual reproduction to form zygotes (*i.e.* eggs).[24,25] Zygotes develop into sporozoites and the parasite has completed its life cycle, ready for the next round of infection.[24,25]

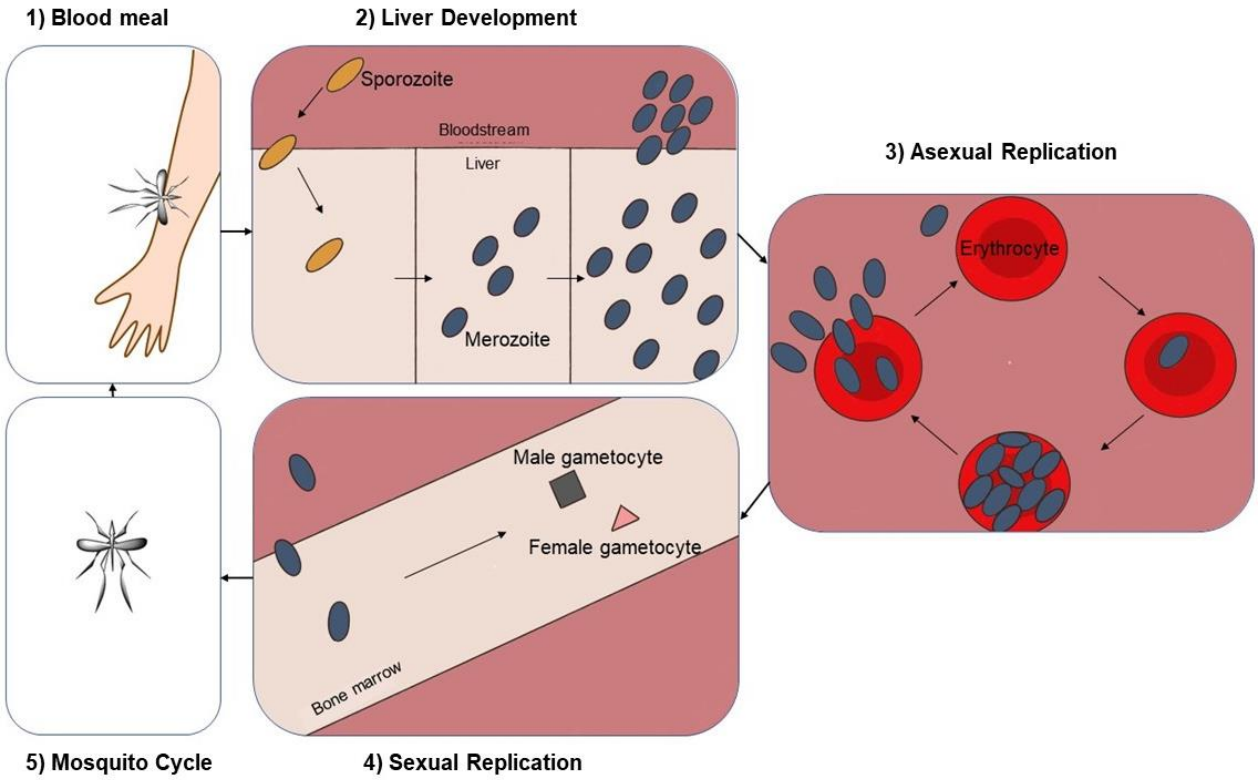


Figure 1.2: The life cycle of *Plasmodium* species. 1) The female *Anopheles* injects sporozoites into a human (host). 2) Sporozoites (orange oval) travel to the liver and undergo asexual reproduction to form schizont. When schizonts reach a certain size, they rupture, releasing merozoites (grey oval) to the bloodstream. 3) Merozoites invade the erythrocytes and undergo a round of asexual reproduction. 4) Some merozoites travel to the bone marrow and mature into male (grey square) and female (pink triangle) gametocytes. 5) During the next blood meal, the gametocytes are taken up by a different female *Anopheles* and the parasite will complete its life cycle in the gut of the *Anopheles* (not shown).

Since there is no effective vaccine for malaria, our society currently depends on insecticides such as long-lasting insecticide-treated bed nets (LLINs) for prevention, and antimalarial drugs (Figure 1.3) for treatment.[22,23,26] The active ingredient in LLINs is typically a pyrethroid, however, the recent spread of pyrethroid resistance in insects decreases their effectiveness.[22,23,26] Therefore, novel insecticides are needed. Parasite resistance is also a problem for antimalarial drugs. Drug resistance has been observed for most clinically relevant antimalarial drugs.[23,24,26] This is concerning, especially with the

increasing failure of the artemisinin combination therapy (the last resort treatment) in southeast Asia, Africa and Latin America.[23,27] In addition, with the increase in global temperature and precipitations, the incidence of vector-borne diseases is expected to increase over time.[28] As a result, a series of guidelines have been suggested by Flannery et al.[24] and malERa Drugs Consultative Group[29] for antimalarial research and development.

Recommended guidelines for the prioritization of molecules in antimalarial research and development:[24,29]

- Have a novel mechanism of action
- Have activity both in the erythrocytic asexual stage and insect sexual stage
- Eliminate the parasites from the liver
- Do not have cross resistance with existing therapeutics
- Single dose with a long duration of activity
- Inexpensive to synthesize

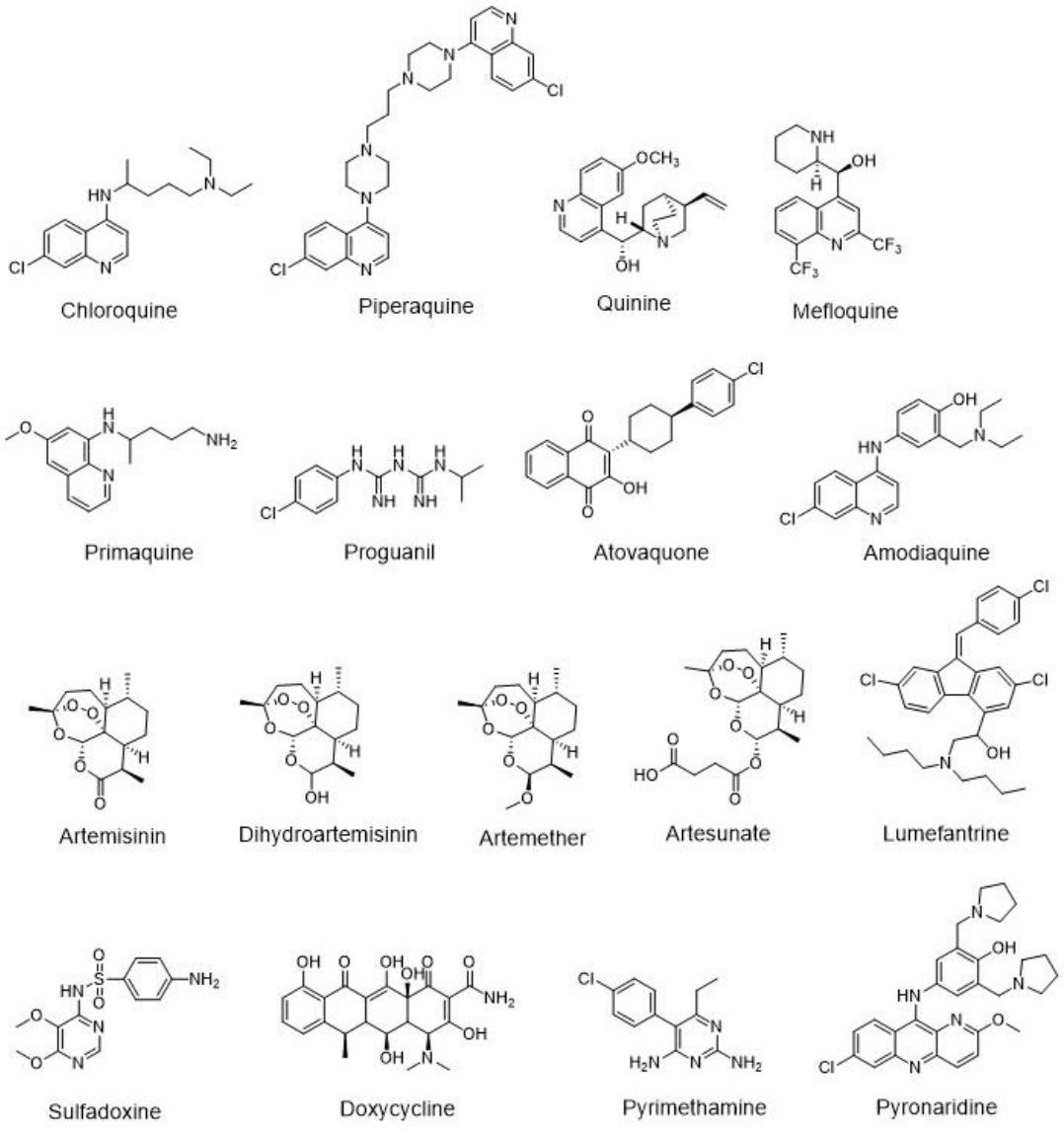


Figure 1.3: Common antimalarials used to treat or prevent *P. falciparium* and *P. vivax* infections.[23,24,30]

1.3 Antibiotics Overview

Compounds with antimicrobial activity have been around us for millennia. The ancient populations consumed food contaminated with antibiotic-producing bacteria[31] or used plants to treat various ailments,[19,32] without knowing the concept of pathogens and antibiotics.[19] Between the years 1800 and 1950, the global average life expectancy of an individual was around 40 years old[33] and millions of people died from infectious disease such as cholera, diarrhoeal disease, pneumonia, and tuberculosis.[19,34,35] However, after

the introduction of antibiotics, the burden of infectious diseases decreased dramatically in developed countries.[34,35] Nonetheless, infectious diseases such as lower respiratory infections, diarrhoeal diseases, malaria, tuberculosis, and HIV/AID remain as a health burden in certain parts of the world.[3,36]

Paul Ehrlich, Alexander Fleming, and Gerhard Domagk are some of the pioneers of modern medicine. Paul Ehrlich came up with the concept of the "magic bullet" or "*therapia magna sterilisans*" - referring to molecules that are toxic to the microbe but not the patient.[37,38] Ehrlich discovered methylene blue and atoxyl derivatives such as salvarsan and neosalvarsan, to treat malaria and syphilis, respectively (Figure 1.4).[19,38] They were the mainstream antibiotics until penicillin and sulfonamides emerged in the 1940s.

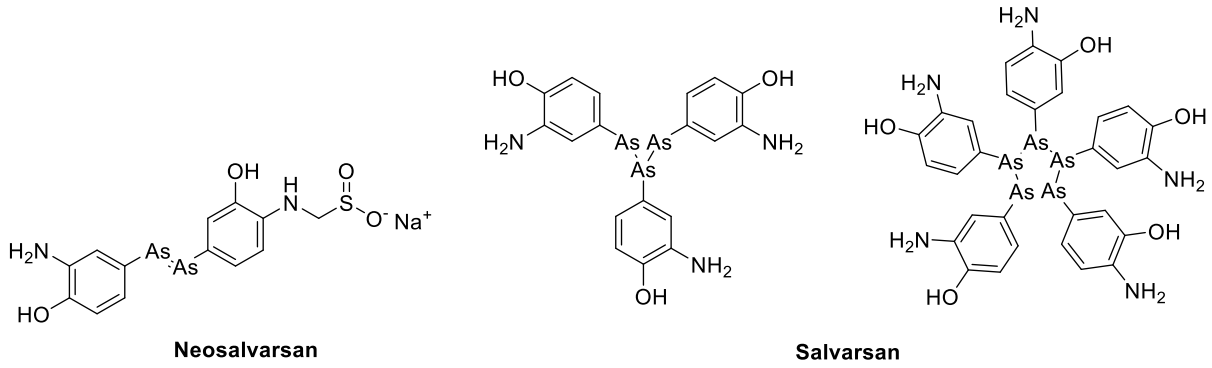


Figure 1.4: Structure of the organoarsenic compounds. Salvarsan is known to exist as a mixture of trimer and pentamer.

The golden era of antibiotic discovery began when Selman Waksman and coworkers pioneered a platform to screen soil actinomycetes for new antibacterial agents. In a short period, this platform enabled them to identify several new antibiotics, including streptomycin, a broad spectrum antibiotic which was also the first antibiotic active against tuberculosis.[9,19] Multiple natural products, semi-synthetic, or synthetic antibiotics were discovered during this era. Unfortunately, antibiotics do not work well anymore. Microorganisms rapidly evolve to resist them (Figure 1.5), a phenomenon known as antimicrobial resistance. The worsening resistance problem is not balanced with the development of new antimicrobials, as the large pharmaceuticals companies continue to abandon this field of research.[39]

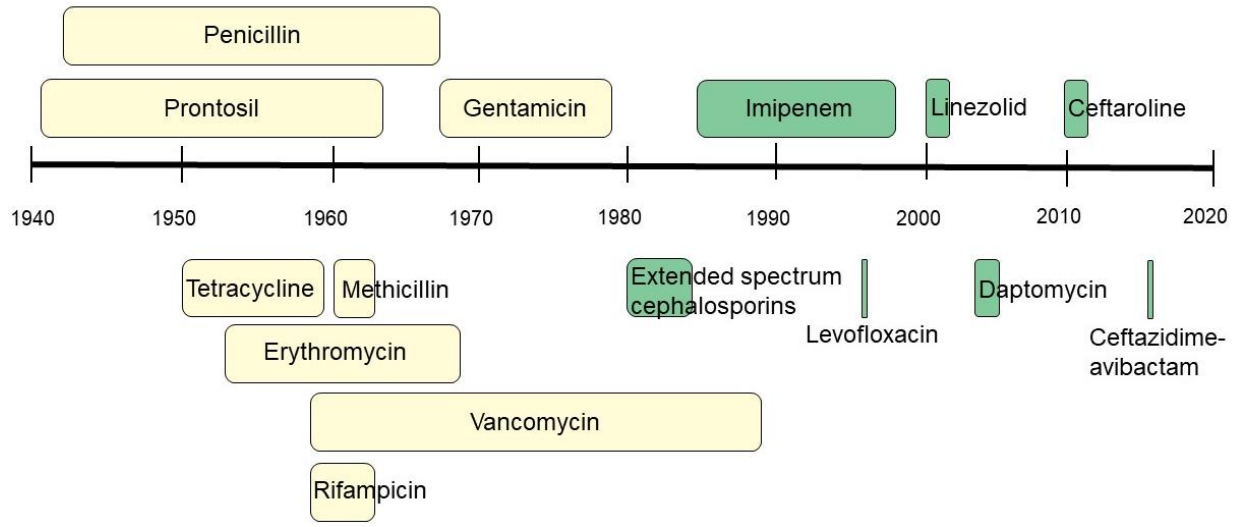


Figure 1.5: Period from the introduction of antibiotic to the first resistant case detected in the clinic.[18,40,41] Selection pressure favours the microbes to evolve faster in the post-Golden Era (green highlight) compared to the Golden Era (yellow highlight), as a result, new drugs are urgently needed to combat antimicrobial resistance.

GlaxoSmithKline and AstraZeneca recently reported running greater than 60 HTS programs against a diversity of bacterial targets, to identify only a few hits.[13,42] The types of compounds typically found in corporate libraries follow the Lipinski Rule of Five, a rule designed to predict the permeability of compounds in human cells rather than microorganisms (Table 1.1).[10,11,42–44] As discussed in section **1.4.1**, the additional outer membrane and the charged porins in Gram-negative bacteria add an additional permeability barrier. Some researchers have recently attempted to derive rules of permeation adapted to Gram-negative bacteria, although these remain imperfect at best (Table 1.1).[9,45,46]

Table 1.1: A comparison of Rule of Five with recent rules of permeation established for Gram negative bacteria.

Rule of Five[44]	**Rules of permeation**[9,45–47]
- Molecular weight less than 500 - Log D or log P less than 5 - No more than 5 hydrogen bond donor - No more than 10 hydrogen bond acceptor	- Molecular weight averaging 600 - Negative log D or log P values - Low hydrophobicity - Containing a charged amine; preferability a non-sterically hindered primary amine - Rigid structure - Low globularity

1.4 Modes of Action of Antibiotics and Resistance

Sub-inhibitory concentrations of antibiotics and antibiotic resistant genes have been circulating in the environment for billions of years, but they were not under a high selection pressure until antibiotics were used at the large scale by humans.[48,49] The ecosystems have since been dramatically altered such that antibiotic resistant strains are constantly selected over sensitive strains.[35,40,49] Moreover, the excretion of unmetabolized antibiotics from patients or livestock to the environment further amplify the situation.[50]

1.4.1 Bacteria Cell Envelope

Bacteria are mostly separated into two groups: Gram-positive and Gram-negative. Due to the structural difference of their cell envelope, it is easier to develop antibiotics against Gram-positive over Gram-negative bacteria.

The Gram stain was developed by Christian Gram in 1884, to separate Gram-positive and Gram-negative bacteria.[51–53] While both classes of bacteria contain features like peptidoglycan, phospholipid bilayer, lipid embedded proteins, and cytoplasm, their arrangement, composition, and thickness set apart the Gram-positive and Gram-negative bacteria (Figure 1.6).

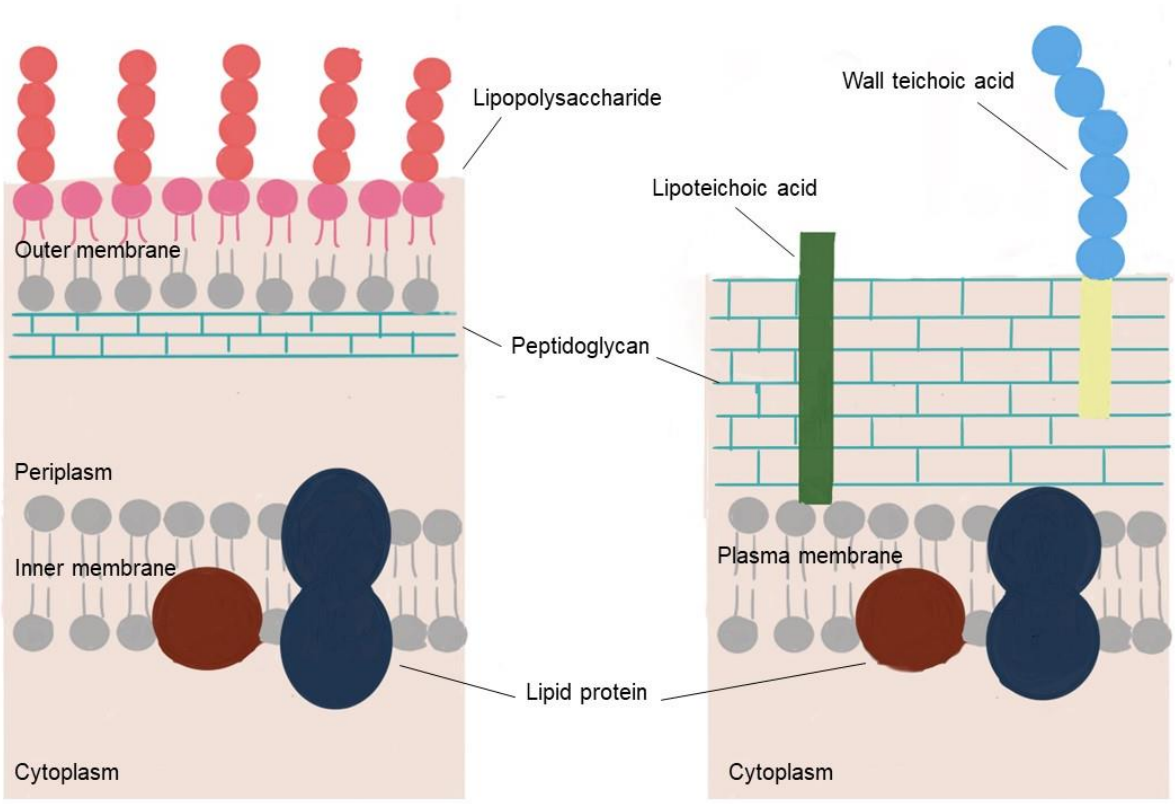


Figure 1.6: Structure of the Gram-negative (left) and Gram-positive (right) bacterial cell envelope.

In Gram-negative bacteria, an outer membrane harboring lipopolysaccharides (LPS) forms the outermost layer of the cell.[51] The presence of the outer membrane adds an additional permeability barrier for antibiotics to penetrate the cell. The periplasmic space is located between this outer membrane and the inner cytoplasmic membrane. In Gram negative bacteria, the periplasm contains a thin peptidoglycan layer.[51]

In contrast, the cell envelope of Gram-positive bacteria is simpler, such that it does not harbor an outer membrane. The outermost structure of Gram-positive bacteria is composed of a 30 to 100 nm thick layer of peptidoglycan.[51] Wall teichoic acids (WTA) and lipoteichoic acids (LTA) are two classes of negatively charged groups displayed on the cell surface. WTA form covalent interaction with the peptidoglycan layer, whereas the LTA assembles with the lipid bilayer via hydrophobic interactions.[51] Underneath the peptidoglycan layer is the cytoplasmic phospholipid bilayer, for both Gram positive and Gram negative bacteria.

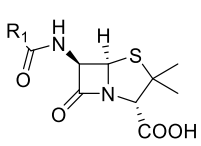
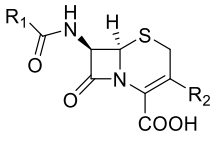
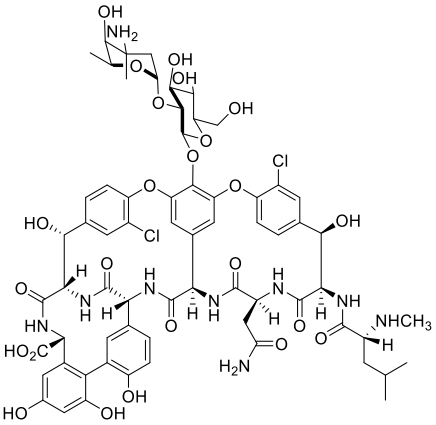
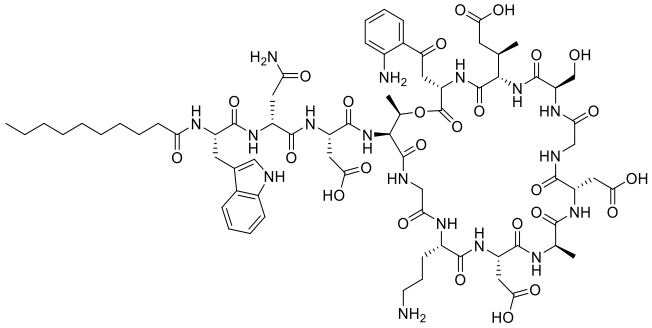
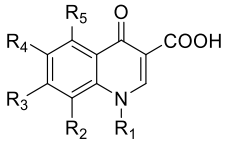
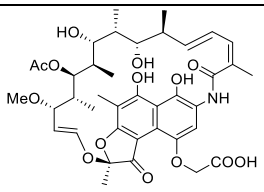
1.4.2 Common antibiotic targets

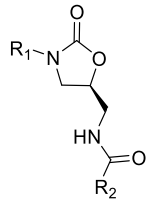
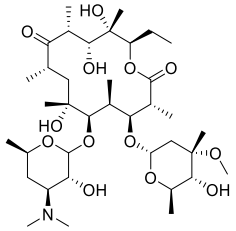
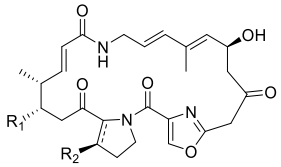
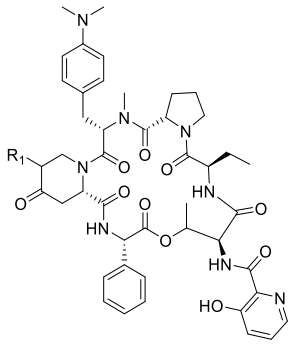
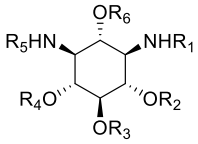
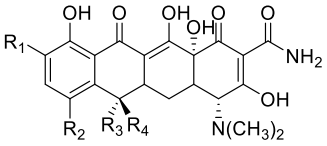
Antibiotics are designed to target the essential proteins or machineries, such as cell envelope, protein synthesis, and DNA/RNA replication that are crucial for bacterial growth and survival (Table 1.2). For example, β-lactam and glycopeptide antibiotics target enzymes involved in cell wall synthesis. β-Lactam antibiotics are structural analogs of the terminal D-Ala-D-Ala group on the pentapeptide of peptidoglycans, and as such they bind to transpeptidases and inhibit cross-linking between adjacent pentapeptides.[54–56] In contrast, glycopeptides such as vancomycin, bind to the terminal D-Ala-D-Ala on the disaccharide pentapeptide and prevent peptide cross-linking as one of their mechanisms of action.[55–57] On the other hand, lipopeptides alter the composition of cell membrane.[55,56]

Antibiotics such as quinoline and rifamycin target the replication of DNA and RNA, respectively. During DNA replication, type II topoisomerase cleaves and reseals the DNA helix. Quinoline binds to the DNA-topoisomerase complex and prevents topoisomerase from resealing the double strands break.[55,56,58] In contrast, Rifamycin binds to RNA polymerase which sterically inhibit the elongation process.[56,59]

Another common antibiotic target are the bacterial ribosomal subunits: 50S and 30S. Macrolides, oxazolidones, and streptogramins bind to the 50S subunit. Linezolid and dalfopristin disrupt the initiation step of translation,[56,60] whereas erythromycin prevents peptide elongation.[55] Antibiotics that target 30S ribosomal subunit include aminoglycosides and tetracycline. Aminoglycosides antibiotic lead to the incorporation of incorrect amino acids at the active site, resulting in the formation of truncated, misfolded proteins.[55,56,61] Tetracycline antibiotics on the other hand, prevents the incoming tRNA from binding to the A-site.[55]

Table 1.2: A summary of the common antibiotic classes and their bacterial target.

Bacterial target	Antibiotic class	Example Structure	Example of antibiotic
Cell wall synthesis	β-Lactam	 Pencillin  Cephalosporin	Penicillin
	Glycopeptide		Vancomycin
	Lipopeptides		Daptomycin
DNA replication	Quinoline		Ciprofloxacin
RNA replication	Rifamycin		Rifamycin

50S ribosomal subunit	Oxazolidones		Linezolid
	Macrolides		Erythromycin
	Streptogramins	 Pristinamycin I_A  Pristinamycin I_B	Dalfopristin
30S ribosomal subunit	Aminoglycosides		Streptomycin
	Tetracycline		Tetracycline

1.4.3 Antibiotic Resistance

It is estimated that by the year of 2025, 40 percent of the infections in Canada will be resistant to first-line treatments.[62] This includes gastro-intestinal, musculoskeletal, and urinary tract infections among others.[62] Most resistant infections are caused by

Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Actinetobacter baumannii, Pseudomonas aeruginosa, and *Enterobacter* species (the ESKAPE bacteria), which have been listed as the top priority pathogens in human health.[15,62,63] These bacteria are often highly resistant to current antibiotics and hence, research and development of antibiotics against these bacteria are in urgent need.[15]

Bacteria may be intrinsically resistant, may develop resistance by evolution, or most often, may acquire resistance genes through transduction, transformation, or conjugation. During transduction, bacteriophages (*i.e.* viruses that selectively infect bacteria) insert genetic elements, some of which may be resistance-causing genes.[50] During transformation, the bacterium picks up foreign DNA from the surrounding environments, whereas conjugation requires a physical connection between two bacterial cells to favor plasmid transfer from the donor to the acceptor bacteria.[50]

The most common antimicrobial resistance mechanisms include the upregulation of efflux pumps, modification of the cell wall or cell membrane to decrease drug permeability, mutation of the antibiotic target, drug inactivation, and target bypass.[11,15,55] It is possible for bacteria to harbor more than one resistance mechanisms, thus making the development of antibiotics more challenging. A summary of the common resistance mechanisms is presented in Table 1.3.

Table 1.3: A summary of the common resistance mechanisms against clinically relevant antimicrobials.[11,55,64]

Resistance mechanism	Mode of action	Antimicrobials
Antibiotic modification	Bacteria produce enzymes with the ability to modify the structure of the antibiotic such that the antibiotic is inactivated.[55,65]	Aminoglycosides β-Lactams Macrolides Streoptogramins Tetracycline Rifamycins
Efflux pump and porin	Efflux pumps export antimicrobials outside the microbes,[11,15,55,64] whereas a reduction of porins on bacterial cell surface reduce cell permeability.[15,55]	Aminoglycosides β-Lactams Macrolides Tetracyclines
Target modification	A gene can acquire mutations that alter the structure of the antibiotic target,[11,55,64,65] preventing the antibiotic from interacting with it.	Aminoglycosides Fluroquinolones Macrolides Quinolones Rifamycin Sulfonamides Vancomycin
Target bypass	Bacteria evolve enzymes to recognize antibiotics and prevent antibiotic from binding to the target.[11]	Sulfonamides Tetracyclines Vancomycin

1.5 Coenzyme A Biosynthesis

Due to the essential nature of some coenzyme A (CoA) biosynthetic enzymes and the important structural differences between the enzymes from microorganisms and those of humans, this pathway is a potential candidate for the discovery of antiplasmodials, antibacterials and antifungals.[66–70] CoA is an essential cofactor in all living organisms. It is involved in a variety of metabolic pathways such as the citric acid cycle, fatty acid synthesis, polyketide synthesis, etc.[66,70] In fact, approximately 9% of all enzymes utilize a CoA derivative.[66,70]

1.5.1 Enzymatic Steps

Pantothenate, also known as vitamin B5, is the precursor to CoA. Organisms such as bacteria, fungi, and plants are able to synthesize pantothenate from β-alanine, while mammals strictly depend on the uptake of pantothenate from nutrients.[66,70] *Plasmodium* spp. are known to induce the formation of permeability pathways to allow pantothenate transport from the host to the erythrocytes, and from erythrocytes to the intracellular space of the parasite.[71,72]

Once the organism has obtained the pantothenate, five enzymes transform pantothenate into CoA through the CoA biosynthetic pathway (Figure 1.7). First, pantothenate kinase (PanK) catalyzes the phosphorylation of pantothenate to yield 4'-phosphopantothenate. Next, phosphopantothenoylcysteine synthetase (PPCS) catalyzes the condensation of 4'-phosphopantothenate with cysteine to yield 4'-phosphopantothenoylcysteine. Decarboxylation of the newly added cysteine moiety is then catalyzed by phosphopantothenoylcysteine decarboxylase (PPCDC) to generate 4'-phosphopantetheine. Phosphopantetheine adenylyltansferase (PPAT) adds an adenyl group to this molecule and, lastly, dephospho-CoA kinase (DPCK) catalyzes a phosphorylation at the 3' position of the ribose to yield CoA.

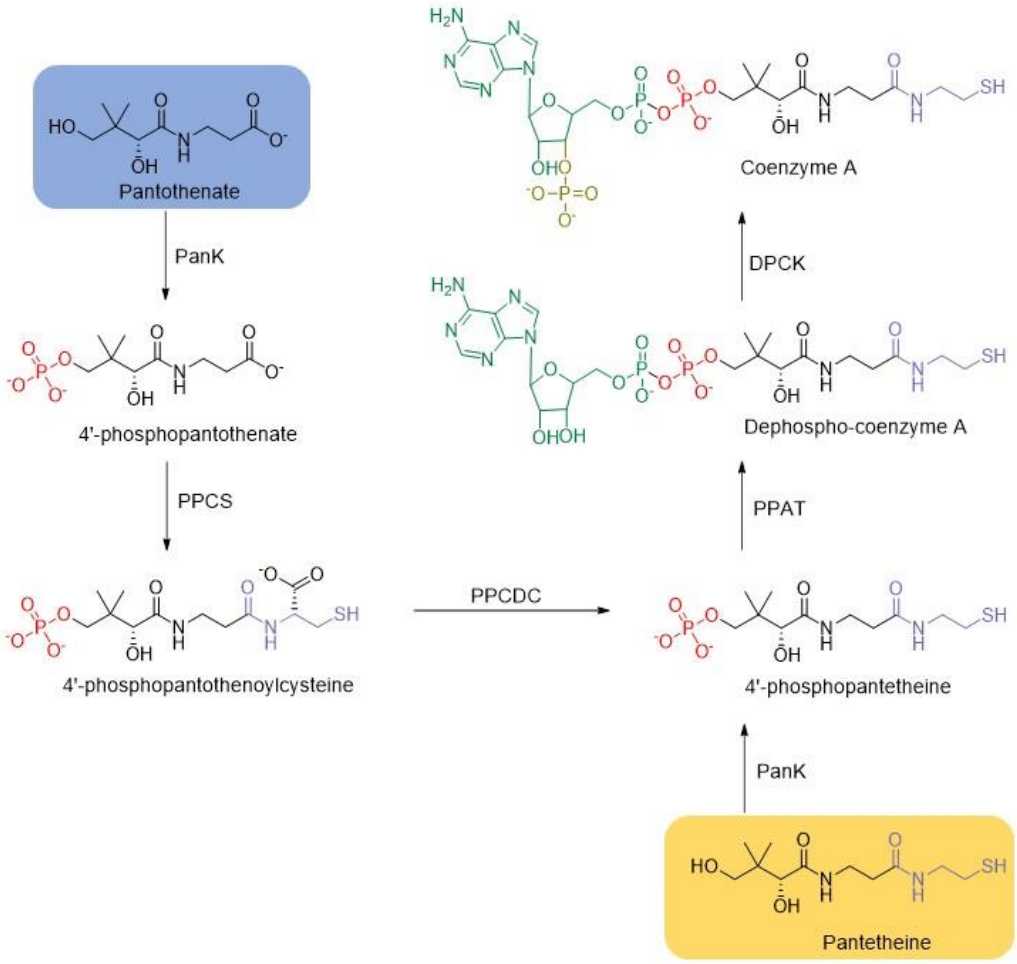


Figure 1.7: CoA biosynthesis. The canonical CoA biosynthetic pathway starts with the phosphorylation of pantothenate (blue box). However, some organisms can also use the CoA salvage pathway, wherein pantetheine (yellow box) is transformed directly by PanK, avoiding PPCS and PPCDC.

An alternative mechanism used by some organisms to produce CoA is the CoA salvage pathway (Figure 1.7). This pathway bypasses the PPCS and PPCDC steps and is crucial when pantothenate is rare. In addition, several organisms can synthesize CoA from 4'-phosphopantetheine, a degradation product of CoA.[73,74]

Although the CoA biosynthetic pathway is present in most living organisms, there are some differences between the eukaryotic and prokaryotic enzymes. Most eukaryotes, with the exception of *Plasmodium* spp., have combined PPAT and DPCK into a bifunctional enzyme known as CoA synthase (COASY).[74] The bifunctional COASY has

little sequence homology with prokaryotic PPAT hence selective inhibition can be achieved.[66] Moreover, the active site of the other enzymes involved in the CoA biosynthesis pathway also differs significantly between organisms. As a result, selective inhibition of the plasmodial or the bacterial enzymes over the human counterparts is possible.[66,75–78]

1.6 Pantothenamides

Pantothenamides are synthetic pantothenate derivatives in which the carboxyl group is reacted to form an amide. The antimicrobial and antiplasmodial property of pantothenamides was first discovered by Clifton *et al.*[79] and Spry *et al.,*[80] respectively. *N*-Pentyl pantothenamide was the most potent growth inhibitor of *E.coli* with a minimum inhibitory concentration (MIC) of 2.1 µM,[79] whereas *N*-phenethyl-pantothenamide (IC_{50} of 20 nM) was the most potent molecule against the malaria parasite.[80] Pantothenamides were however never brought to the market because they are rapidly hydrolyzed by pantetheinases in the blood.[69,80] With the recent surge of drug resistant bacteria and parasites, researchers have started to re-examine pantothenamides, with the aim of finding derivatives with improved potency and blood stability.

1.6.1 The Mechanism of Action of Pantothenamides: CoA Antimetabolites

Pantothenamides lack the thiol and carboxylate groups of pantetheine and pantothenate, respectively, but are nevertheless transformed by the CoA salvage pathway of many microorganisms (Figure 1.8).[81] They are first phosphorylated by PanK to yield the corresponding 4'-phosphopantothenamides. Next, PPAT and DPCK add an adenylyl and a phosphate group, respectively, to yield the corresponding CoA derivatives, which act as CoA antimetabolites. In some species, pantothenamides are converted into CoA derivatives faster than pantothenate itself.[82]

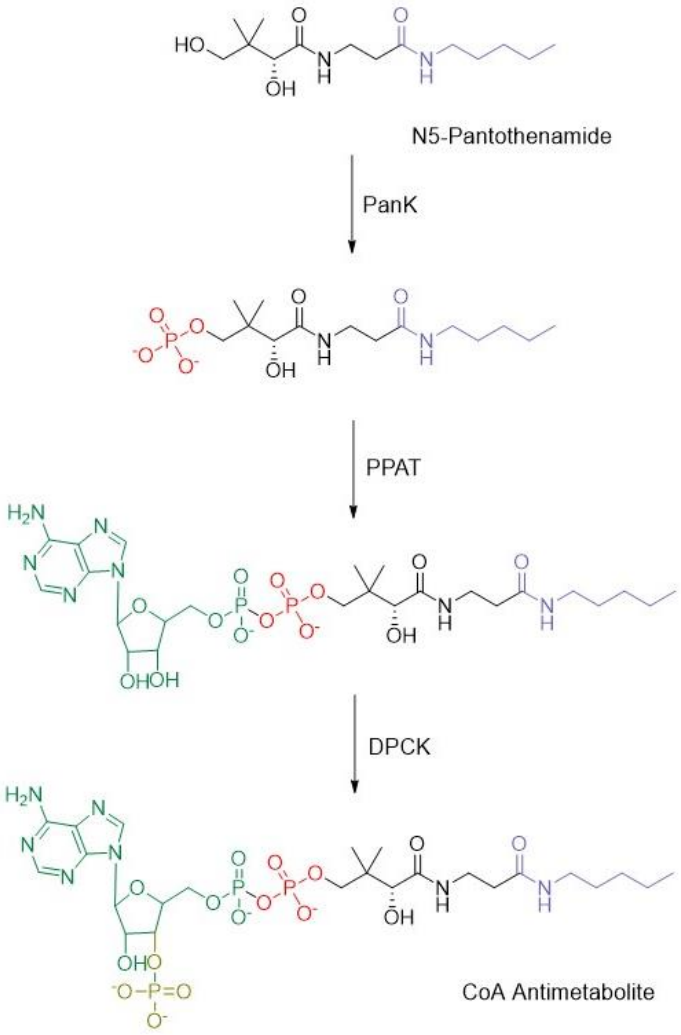


Figure 1.8: The CoA salvage pathway involved in the transformation of pantothenamides. *N*-Pentyl pantothenamide is used as an example. The 4'-hydroxyl is first phosphorylated by PanK, followed by addition of an adenyl group by PPAT, and phosphorylation by DPCK.

Some pantothenamides are well characterized for their inhibition of fatty acid synthesis after activation into the corresponding CoA derivative (Figure 1.9). The enzyme acyl carrier protein (ACP) synthase normally transfers the phosphopantetheine moiety of CoA to apo-ACP, leading to the activated holo-ACP. This enzyme was also found to accept other CoA derivatives as substrates and produce an inactive ACP protein instead (crypto-ACP).[81,83] The thiol group of the phosphopantetheinyl group in holo-ACP acts as the nucleophile which carries the growing fatty acid chain during biosynthesis.[70] CoA

antimetabolites, however, lack this nucleophilic thiol and hence, crypto-ACP is unable to perform its role.[81] It is believed that CoA derivatives produced from pantothenamides *in cellulo* may also target other CoA-utilizing enzymes.[81,82,84]

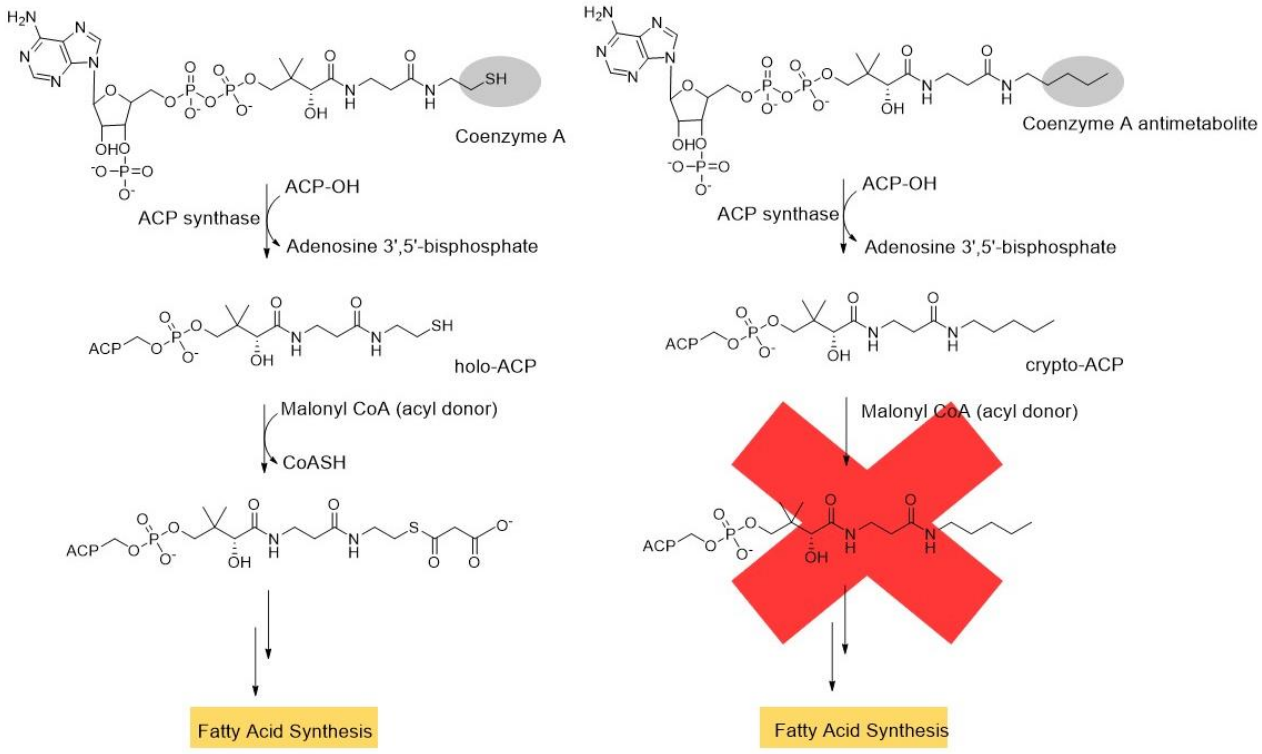


Figure 1.9: The biological involvement of CoA compared to CoA antimetabolites in fatty acid biosynthesis.

1.6.2 Types of PanK

Due to the promiscuity of PPAT and DPCK,[66] most studies with pantothenamides have focused on PanK enzymes. PanK are classified into type I, type II, or type III, and the phosphorylation event is the rating limiting step in the CoA biosynthesis pathway (Table 1.4).[66,81] Type I PanK ($PanK_I$) enzymes are commonly found in bacteria. They are negatively regulated by CoA and its thioesters,[70,85,86] and require Mg^{2+} ions for activity.[70,85,86] $PanK_I$ isoforms are highly promiscuous enzymes, which accept modification at the secondary hydroxyl,[68] β-alanine,[67,68] and *N*-substituent ends[67,78,87,88] of pantothenamide derivatives. Based on the reported crystal structures of $PanK_I$

enzymes with pantothenamides at the active site, the *N*-substituent of pantothenamides is located in a small hydrophobic pocket.[68,86,89]

Most mammals, fungi, *Plasmodium* spp., and plants express a type II PanK ($PanK_{II}$), which is also negatively feedback regulated by CoA.[70,85,90] Staphylococci (*e.g*. *S. aureus)* are the only bacteria known to express a $PanK_{II}$, yet this PanK is very different from the eukaryotic isoforms. Like $PanK_{I}$, eukaryotic $PanK_{II}$ require Mg^{2+} ions for activity, and can also accept some pantothenamides as alternative substrates.[70,85] *S. aureus* PanK on the other hand, is not feedback inhibited by CoA and has high affinity for pantothenamides such that the phosphorylated pantothenamides sometimes remain trapped in the enzyme active site.[67,70,85,89,91] The hydrophobic pocket of *S. aureus* PanK is larger than that of most $PanK_{I}$ and was found to not accommodate charged side chains.[68,70,85,89]

Type III PanK ($PanK_{III}$) enzymes are found in many bacteria, including *B. anthracis, C. difficile, H. pylori*, *P. aeruginosa*, etc. These enzymes are both functionally and structurally very different from the PanKs from types I and II. $PanK_{III}$ enzymes require either K^+ or NH_4^+ for activity and are not susceptible to feedback inhibition by CoA.[67,70,85] They cannot accommodate pantetheine or pantothenamides as alternative substrates because the pantothenate binding site is at a dimer interface and highly compact.[67,68,70,85] However, it has been shown that *P. aeruginosa* $PanK_{III}$ can accept two to three carbon linkers at the β-alanine moiety of pantothenate.[68]

Table 1.4: Comparison of different type of PanKs.

	PanK$_I$	**PanK$_{II}$**	**PanK$_{III}$**
Species	Mostly in bacteria	Mammals, fungi, plants, Staphylococci, and *Plasmodium* spp.	In bacteria such as *P. aeruginosa,* and *H. pylori*
Ion(s) involved in phosphorylation	Mg^{2+}	Mg^{2+}	K^+ or NH_4^+
Feedback regulation	Negative feedback by CoA	Negative feedback by CoA and CoA thioesters, except in *S. aureus*	Not feedback regulated
Accept pantothenamides as substrates	Yes	Yes	No
Pantothenate binding pocket	Hydrophobic	Hydrophobic	Tight pocket at the dimer interface

1.6.3 Synthetic strategies to overcome pantetheinases mediated degradation

The antimicrobial and antiplasmodial properties of pantothenamides are well-documented.[66,69,81] Multiple derivatives have been synthesized;[67,75,94–100,76–78,87,88,91–93] however, most of these molecules are unstable in human serum. There, enzymes called pantetheinases or vanins rapidly hydrolyze pantothenamides into pantothenate and the corresponding amine (Figure 1.10).[69,80]

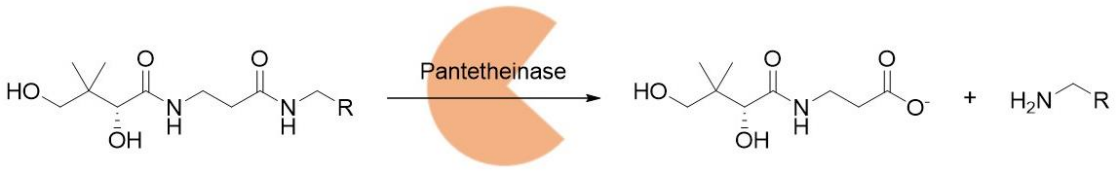


Figure 1.10: Pantetheinase-mediated pantothenamide hydrolysis

To overcome this challenge, a variety of synthetic strategies have been employed to reduce pantetheinase-mediated degradation while maintaining or improving the potency of pantothenamides (Figure 1.11). This includes modifications at the geminal dimethyl group,[92,101,102] β-alanine moiety,[67,75,76,97–99] or the scissile amide bond.[77,93–97,99,100] Based on studies with several small libraries of pantothenamides, SARs have been established for *E. coli,*[67,77–79,88,93] *S. aureus,*[67,77,78,91–93] and *P. falciparum.*[75,76,94–100]

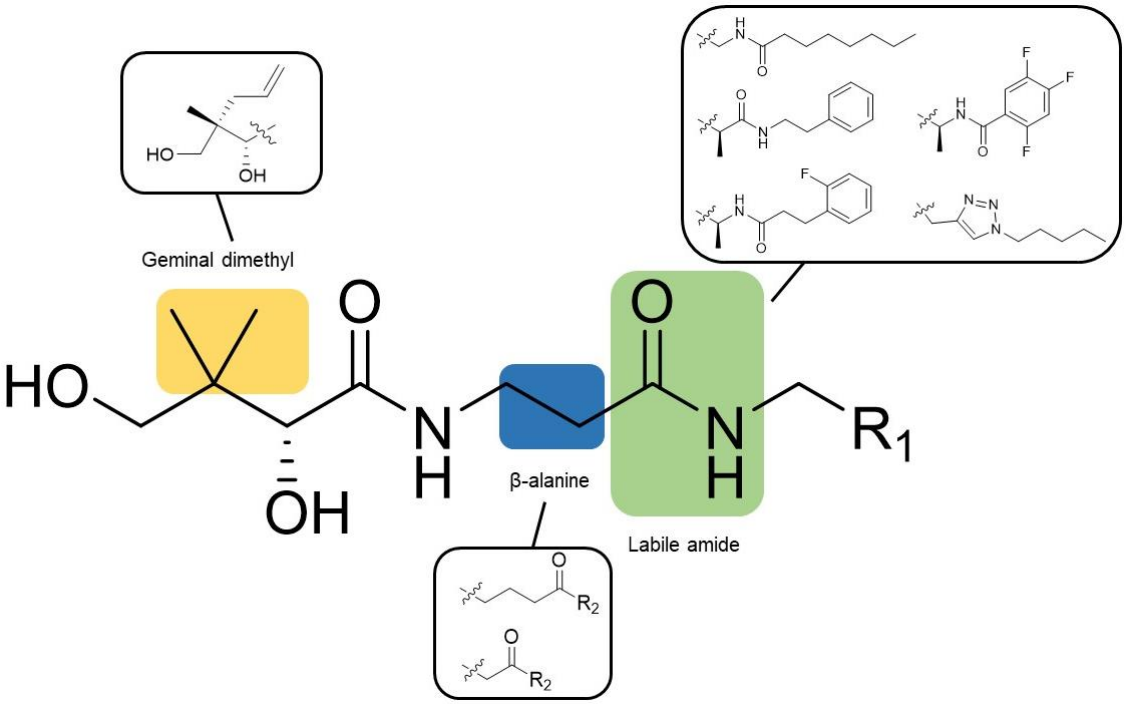
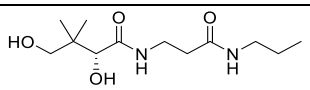
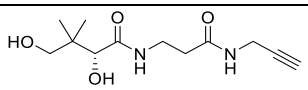
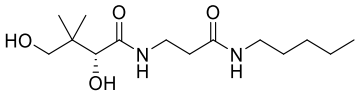
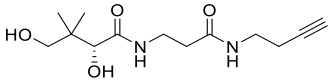
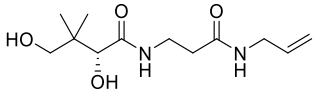


Figure 1.11: Modifications explored to reduce pantetheinases-mediated degradation. R_1 = linear alkyl, branched alkyl, phenethyl, or substituted aromatic groups. R_2 = amine moiety including R_1.

The pantoyl moiety is the key recognition site for most CoA biosynthetic enzymes and hence, synthetic modifications have focused mostly on the labile amide bond and/or the *N*-substituent of pantothenamides. Due to the presence of TolC-dependent efflux pumps in *E. coli*,[67,78,83] not many pantothenamides have antibacterial activities. Table 1.5 summarizes the potent pantothenamides discovered thus far and Figure 1.12 provides preliminary SARs established based on scientific literature. Briefly, a one to two-carbon linker is the optimal length between N5 and C8.[67] The C8 amide carbonyl should be preserved and cannot be replaced with a hydrazide, sulfonamide, or thioamide.[93] Lastly,

the *N*-substituents tolerated include short chain alkenes and alkynes, and non-sterically hindered alkyl groups.[88] Substituents containing ether, thioether, substituted phenethyl, or bulky heterocyclic groups are disfavoured.[67,77,78,93]

Table 1.5: Pantothenamide derivatives active against *E. coli*.[88]

Compounds	MIC (μM)	Compounds	MIC (μM)
	1		4
	6		7.5
	3		

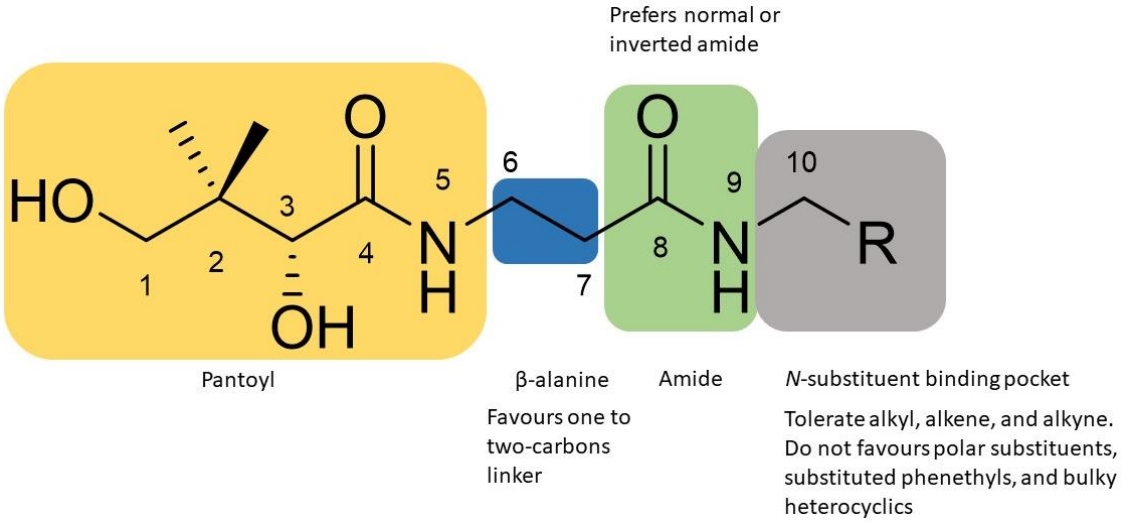
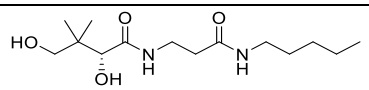
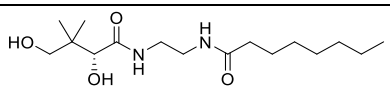
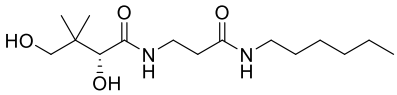
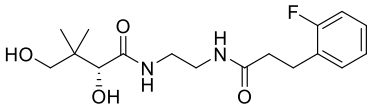
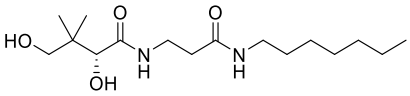
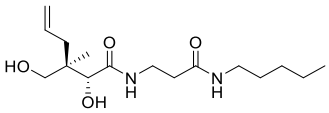
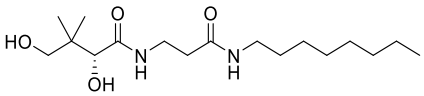
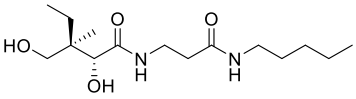
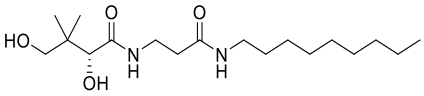
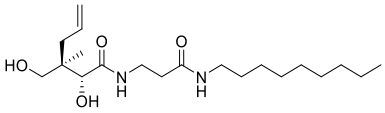
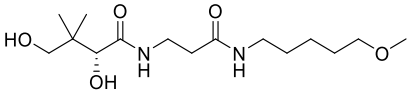
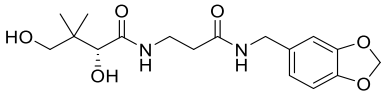
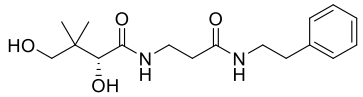
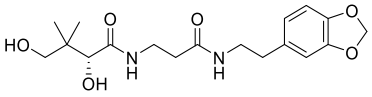
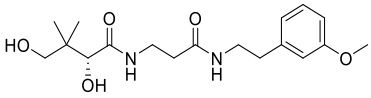


Figure 1.12: Ligand-based SARs for activity in *E. coli*.

Unlike *E. coli,* multiple potent pantothenamides with antistaphylococcal activities have been reported (Table 1.6) and the corresponding SARs are summarized in Figure 1.13. A two-carbon linker is preferred between N5 and C8, and bulky linkers are not tolerated.[67,77] Small alkyl substituents (*e.g.* ethyl and allyl) with a *R* configuration is preferred at C2.[92] Compounds with either a normal[67,91–93] or an inverted amide[77] at C8

are similarly active. Linear alkyls, ether, thioether, and substituted ethylaromatic groups are the preferred *N*-substituents.[77,78,91] A recent study by Hughes *et al.* has revealed that pantothenamides containing polar *N*-substituents are poor inhibitors of *S. aureus* growth, owing to a lower cell permeability.[91]

Table 1.6: Pantothenamide derivatives active against *S. aureus*.[67,77,91,92]

Compounds	MIC (μM)	Compounds	MIC (μM)
	1.5		6.3
	3.14		5.9
	0.77		3.2
	0.74		7
	0.4		1
	4.0		4
	0.25		1
	8.0		

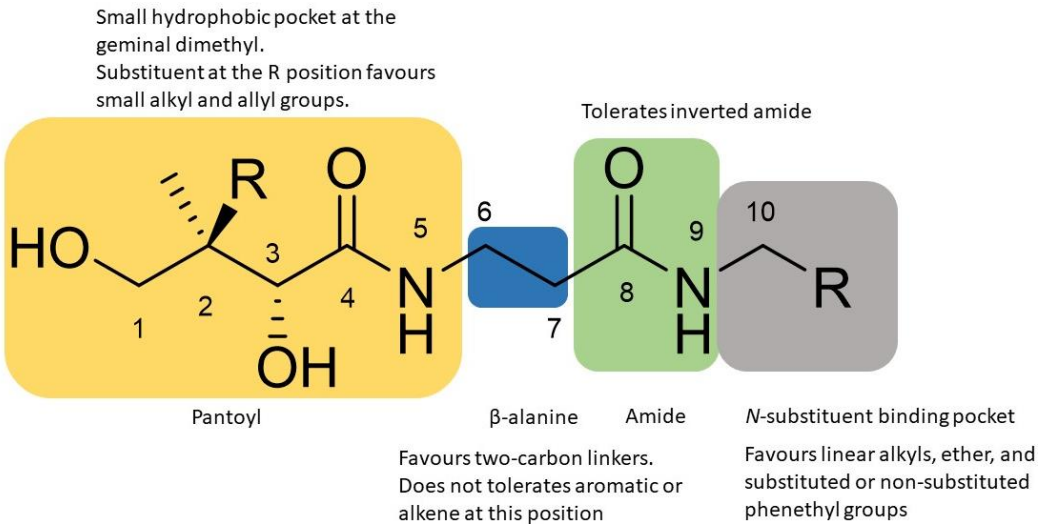
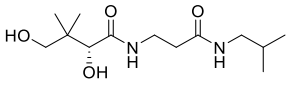
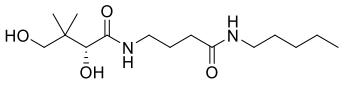
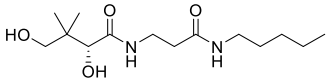
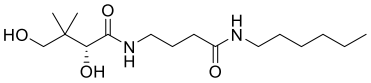
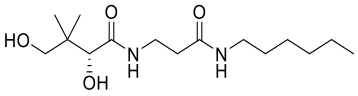
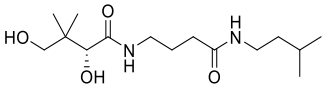
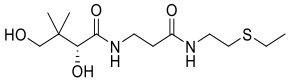
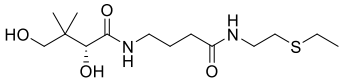
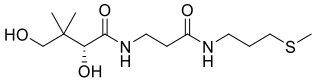
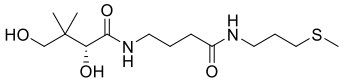
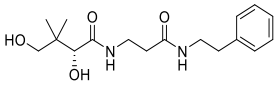
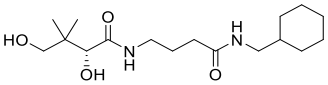
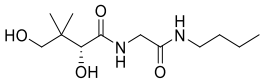
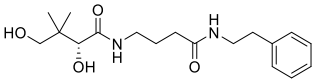
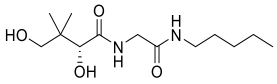
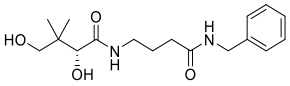
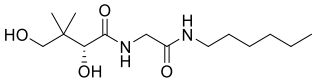
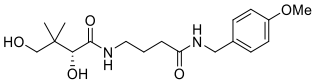
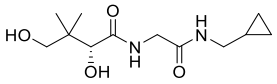
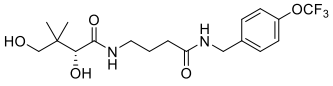
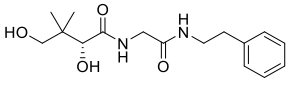
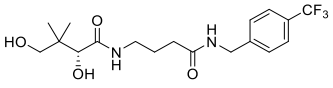


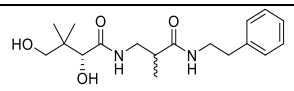
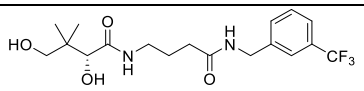
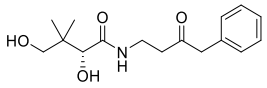
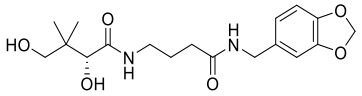
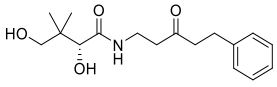
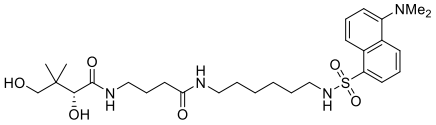
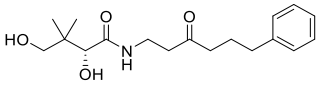
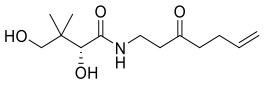
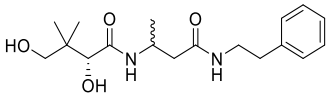
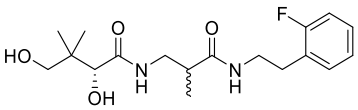
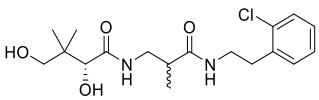
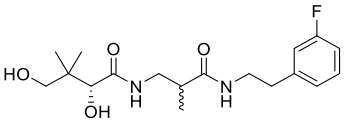
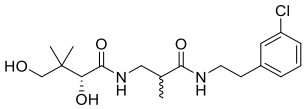
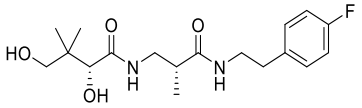
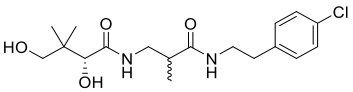
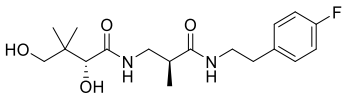
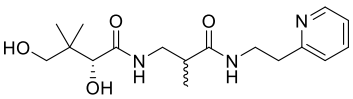
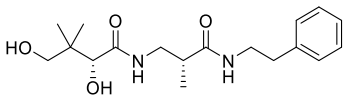
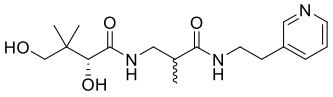
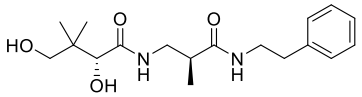
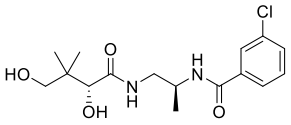
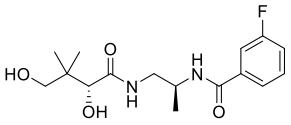
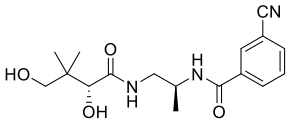
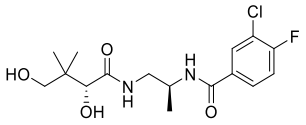
Figure 1.13: Ligand-based SARs for activity in *S. aureus*.

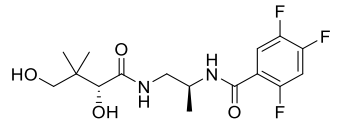
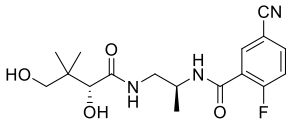
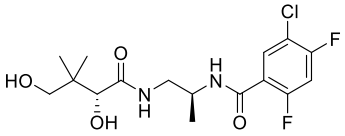
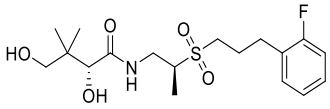
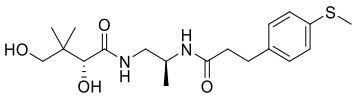
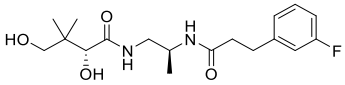
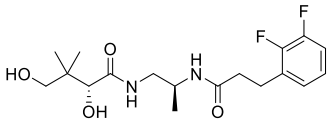
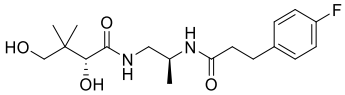
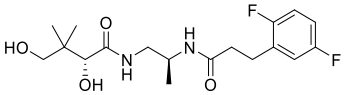
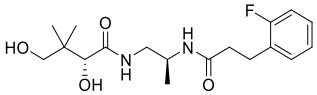
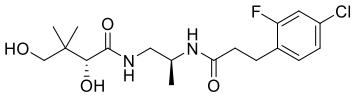
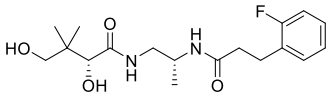
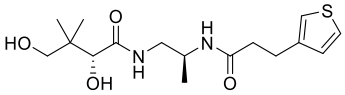
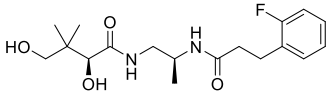
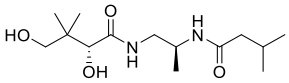
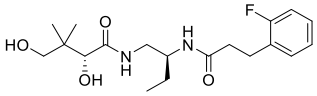
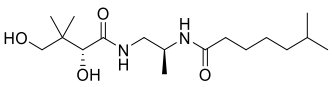
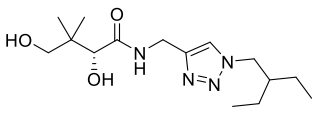
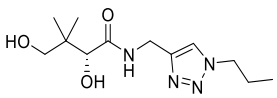
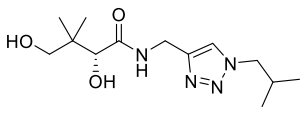
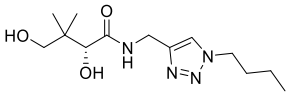
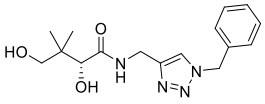
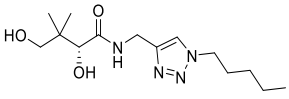
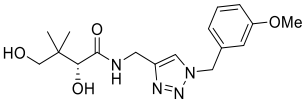
Many of the pantothenamides discovered to date have antiplasmodial activity (Table 1.7). For activity in *P. falciparum*, a two-carbon linker is optimal between the two amide bonds (Figure 1.14). Methylation at the C7, with (*S*)-methyl being preferred, reduces pantetheinase-mediated degradation while maintaining the potency of pantothenamides.[76,97–99] Other modifications reported to overcome pantetheinase-mediated degradation include inverting the amide bond[97,99] and replacing the amide with bioisosteres (Section 2.2).[94,95,100] In fact, many of these pantothenamide derivatives have enhanced antiplasmodial activity compared to the parent compounds. Similar to what has been observed in bacteria, alkyl, thioether, and non-sterically hindered aromatic groups are tolerated *N*-substituents.[75,94,95,97–99,103]

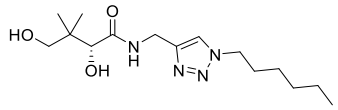
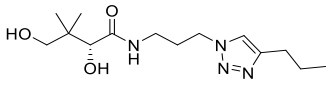
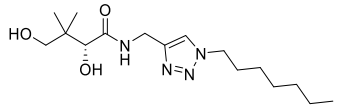
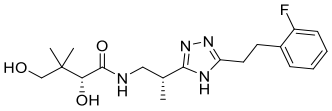
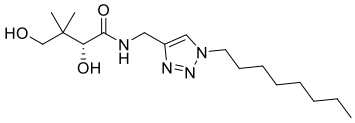
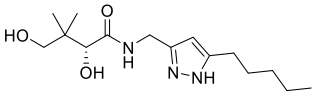
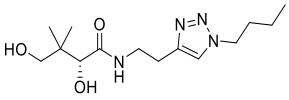
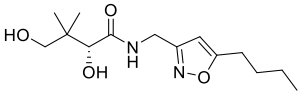
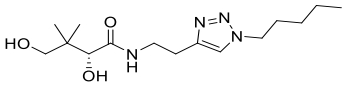
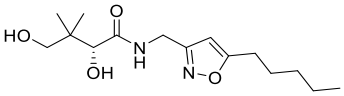
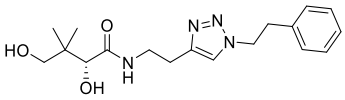
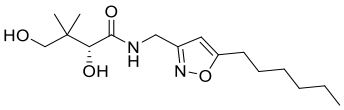
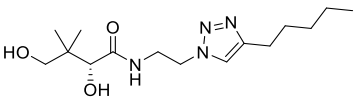
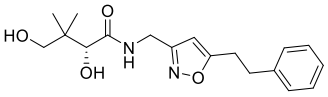
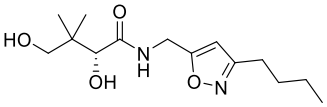
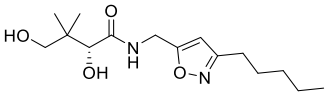
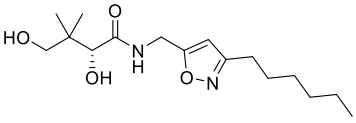
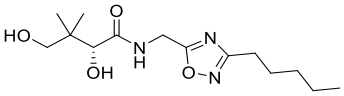
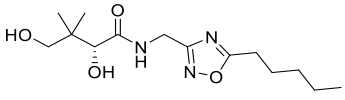
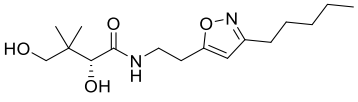
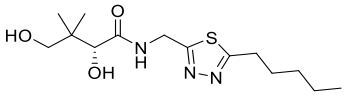
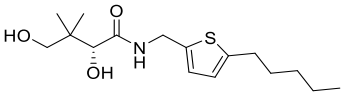
Although, many pantothenamides with antiplasmodial activity in the nanomolar range have been reported, none of them have made it to clinical trials. Few have been tested for activity in animal models, and those that have lack activity.[97,98,100] In fact, very few pantothenamides can be tested *in vivo* because they are susceptible to pantetheinases. As a result, novel panothenamides are needed to overcome these challenges.

Table 1.7: Pantothenamide derivatives active against *P. falciparum*. *Pantetheinases are inactivated in age media.[75,76,94–100]

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
	1.1*		3.9
	7.5*		8.0
	0.55*		8.9
	0.28*		2.0
	0.23*		7.7
	0.02*		2.1
	5.3		2.1
	7.0		6.1
	4.7		9.5
	6.4		6.3
	3.4		3.5

	0.052		4.0
	6.1		2.4
	7.7		1.1
	2.2		2.6
	8.5		0.070
	0.248		0.156
	0.214		0.294
	0.107		0.107
	4.2		0.277
	0.106		0.023
	0.0021		0.0019
	0.0074		0.0034

	0.0024		0.0062
	0.0023		0.139
	0.021		0.028
	0.006		0.147
	0.053		0.005
	0.037		0.21
	0.017		0.235
	0.175		0.122
	0.041		0.54
	2.7		1.3
	0.071		4.3
	0.056		6.4

	0.055		3.5
	0.50		0.299
	3.2		7.4
	5.3		0.072
	3.3		0.16
	5.4		0.24
	8.5		5.9
	0.19		0.63
	2.2		5.3
	1.8		4.8
	0.96		2.8

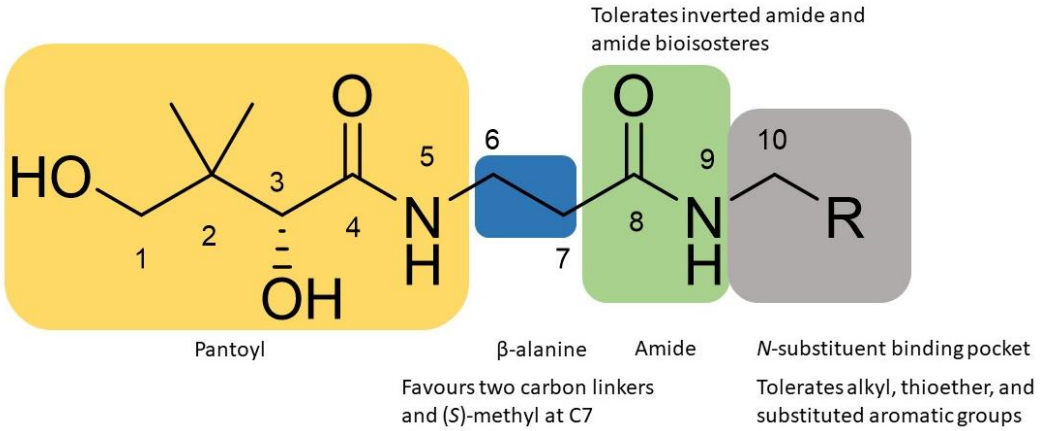


Figure 1.14: Ligand-based SARs for activity in *P. falciparum*

1.6.4 Outlook

As discussed in previous sections, antimicrobial resistance is ramping and antimicrobial agents with a new mode of action are urgently needed. Pantothenamides fulfill these criteria. They are also typically easy to synthesize. Depending on the microorganism, pantothenamides are believed to act either as CoA antimetabolite or as inhibitors of CoA biosynthesis. Moreover, some pantothenamide analogs inhibit *P. falciparum* growth both at the asexual and sexual stages, which is a favourable property for new antimalarials.

1.7 Research Objective

The objective of this thesis is to synthesize new pantothenamide analogs with improved blood stability and potency. The approach used to achieve this consists of replacing the labile amide group with various thiazole-containing heteroaromatic rings. Synthetic strategies and biological results are discussed in chapters 2 and 3, respectively. Chapter 4 provides a survey of the contributions described in this thesis and future directions for this project. Lastly, the experimental procedures are summarized in chapter 5.

Chapter 2

Synthetic Strategies

2.1 Preface

Various synthetic strategies have been employed to stabilize the labile amide bond in pantothenamides. Researchers in the Auclair group have attempted to improve the stability of pantothenamides by modifying them at different positions and by replacing the labile amide with various heteroaromatic rings. Previous group members have identified triazole-, isoxazole-, and thiadiazole-containing pantothenamide analogues with antiplasmodial activity in the low nanomolar range. In this thesis, 19 novel thiazole-containing pantothenamide analogues are synthesized by the author. The biological activity of these compounds is discussed in Chapter 3.

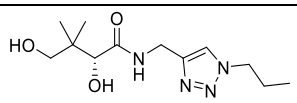
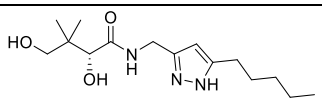
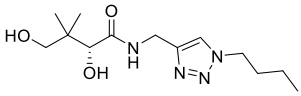
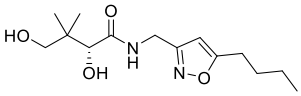
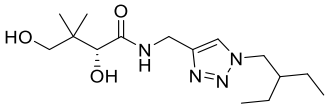
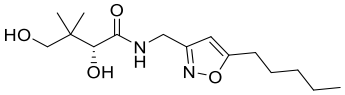
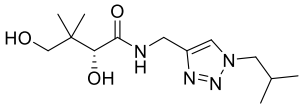
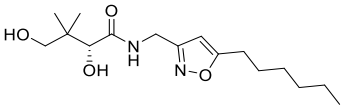
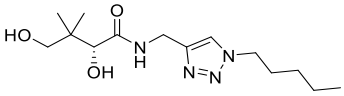
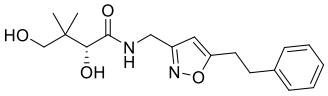
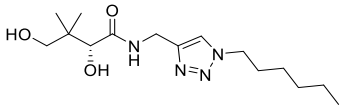
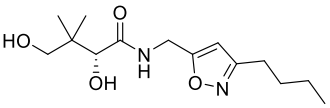
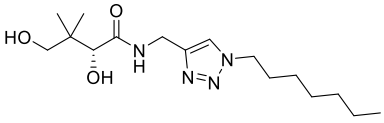
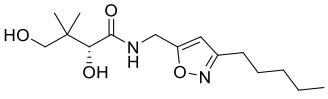
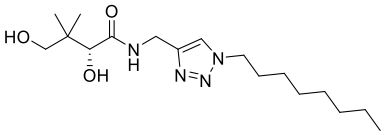
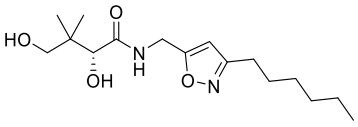
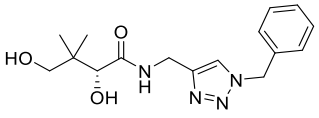
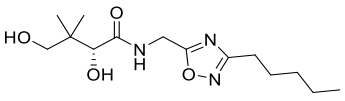
2.2 Introduction

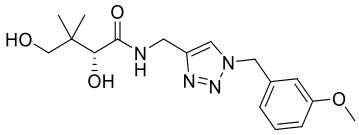
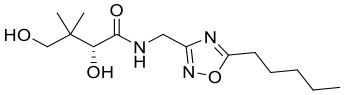
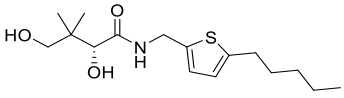
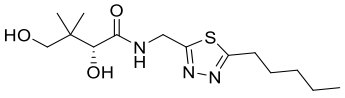
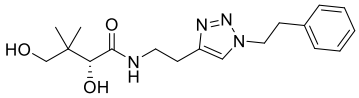
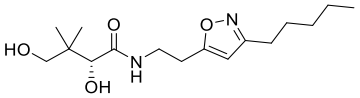
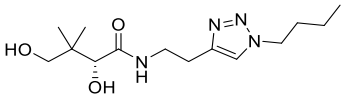
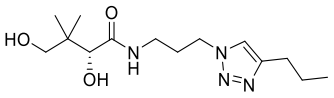
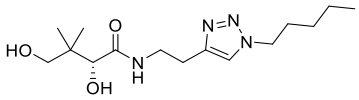
Microbial resistance to antibiotics and antimalarials is rampant, jeopardizing the practice of modern medicine. New antimicrobials are urgently needed, especially molecules with a novel mechanism of action. Pantothenamides have been identified as potent antibacterial[79,104] and antiplasmodial[66,69] agents; however, none of the pantothenamides have entered the clinic yet.[80] Pantetheinases in human serum rapidly hydrolyze pantothenamides and hence, hinder the antimicrobial ability of the compound *in vivo*. To overcome this challenge, our research group and others have modified the geminal dimethyl,[92,101,102] pantoyl,[93] β-alanine,[75,93,97–99] and the labile amide moieties[77,93–97,99,100] of pantothenamides, with the aim of identifying pantetheinase-resistant pantothenamide analogues. More recently, the medicinal chemistry efforts of our group have focused on replacing the labile amide with heteroaromatic rings.

Heteroaromatic rings are considered a privilege scaffolds in medicinal chemistry. More than 80% of the marketed drugs contain a minimum of one heteroaromatic ring in their structure.[105,106] The heteroatom(s) within the ring can participate in intramolecular and intermolecular H-bonding interactions, and can significantly affect the physicochemical properties of the molecule.[105,107] Previous members from the Auclair group have identified potent pantothenamide analogues against intraerythrocytic *P. falciparium*, where the labile amide bond is replaced with a heteroaromatic ring (Table 2.1).[94,95,100] These pantothenamide analogues are resistant to pantetheinase. It is hypothesized that the aromatic hydrogen or sulfur is mimicking the electron donor's ability of an amide group,

while the nitrogen or oxygen is mimicking the electron acceptor (Figure 2.1A). Isoxazole-, thiazole-, and triazole-containing pantothenamide analogues are the three most promising candidates with an antiplasmodial activity in the nanomolar range and selective growth inhibitor of *P. falciparium* over human foreskin fibroblast cells,[94,100] implying low cytotoxicity.

Table 2.1: The most potent heteroaromatic ring-containing pantothenamide analogues against *P. falciparium.* Only compounds with an IC_{50} of less than 5 µM are included and compounds with a nanomolar antiplasmodial activity are highlighted in red.

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
	2.7 ± 0.3[94]		7.4 ± 0.3[100]
	0.071 ± 0.003[94]		0.072 ± 0.003[100]
	0.54 ± 0.1[94]		0.16 ± 0.01[100]
	1.3 ± 0.3[94]		0.24 ± 0.05[100]
	0.056 ± 0.005[94]		5.9 ± 0.5[100]
	0.055 ± 0.005[94]		0.19 ± 0.03[100]
	0.50 ± 0.05[94]		0.63 ± 0.07[100]
	3.2 ± 0.4[94]		2.2 ± 0.4[100]
	4.3 ± 0.7[94]		5.3 ± 0.6[100]

	6.4 ± 3[94]		1.8 ± 0.1[100]
	2.8 ± 0.3[100]		0.96 ± 0.05[100]
	5.4 ± 0.8[95]		4.8 ± 0.2[100]
	5.3 ± 1.7[95]		3.5 ± 1.2[94]
	3.3 ± 0.9[95]		

With both the heteroaromatic analogs mentioned above and the pantothenamides reported by Spry et al.,[98] Schalkwijk et al.,[97,99] (Figure 2.1), a two carbon linker is optimal between the pantoyl and the labile amide (black circular dots). However, the substituents preference at the *N*-substituent differ between analogs containing a heteroaromatic ring and those with a linear amide mimic. In the case of linear amide mimics, substituents such as substituted phenyls or phenethyl groups are preferred over linear alkyl groups.[75,97–99] On the other hand, alkyl chains with a length of C4 to C6 are preferred over sterically hindered alkyls or aromatic groups (*e.g.* 2-methyl-propyl, *p*-trifluoromethylphenyl, phenethyl groups, etc.) for molecules containing a heteroaromatic ring.[94,95,100]

The heteroaromatic ring of pantothenamide analogues synthesized thus far include primarily on nitrogen and/or oxygen.[94,95,100] Exploring sulfur-containing heteroaromatic rings to diversify the compound library was the main goal of this thesis.

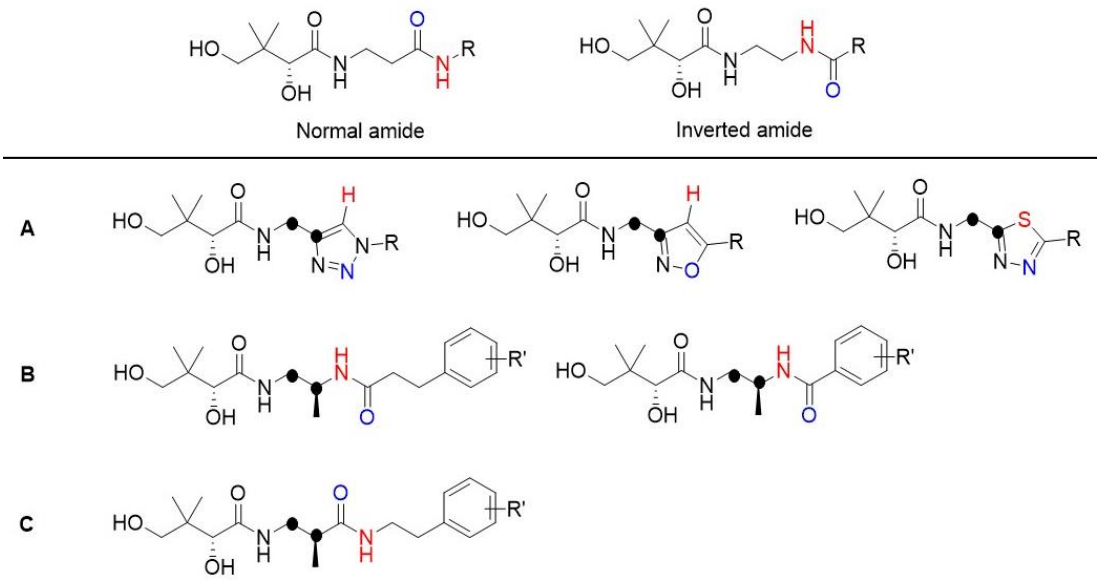


Figure 2.1: SARs for the selected pantothenamide analogues. The electron donors and acceptors are highlighted in red and blue, respectively. R can be alkyl or aryl groups whereas, R' only contained halogen or cyano groups. The black dots highlighted the optimal length between pantoyl and amide group of the most potent pantothenamides. A) the labile amide is replaced with heteroaromatics. B) potent pantothenamide analogues discovered by Schalkwijk et al., where the labile amide is inverted, with the *S*-configuration preferred at the α-carbon.[97,99] C) pantetheinase-resistant pantothenamide analogues discovered by Spry et al. with a *S*-configuration at the α-carbon.[98]

Sulfur is known to mimic -NH groups, by forming chalcogen bonds – a subclass of σ-hole interactons.[107,108] The low lying C-S σ* orbital and polarizability of sulfur atoms makes certain regions of sulfur atom electron-poor, a phenomenon known as σ-hole, through which sulfur can interact with electron donors such as nitrogen or oxygen.[107,108] The substituents attached to the sulfur affect the strength of the sulfur σ-hole; electron withdrawing and donating substituents enhance and reduce the electron accepting ability of the C-S σ holes, respectively.[108] In addition, sulfur can form intermolecular, as well as 1,4-, 1,5-, or 1,6-intramolecular interactions with electron donors. These interactions can enhance potency and reduce entropic penalty during target binding.[107,108] Sulfur-containing drugs are prevalent in the clinic. Figure 2.2 show the common sulfur-containing scaffolds found in marketed drugs.

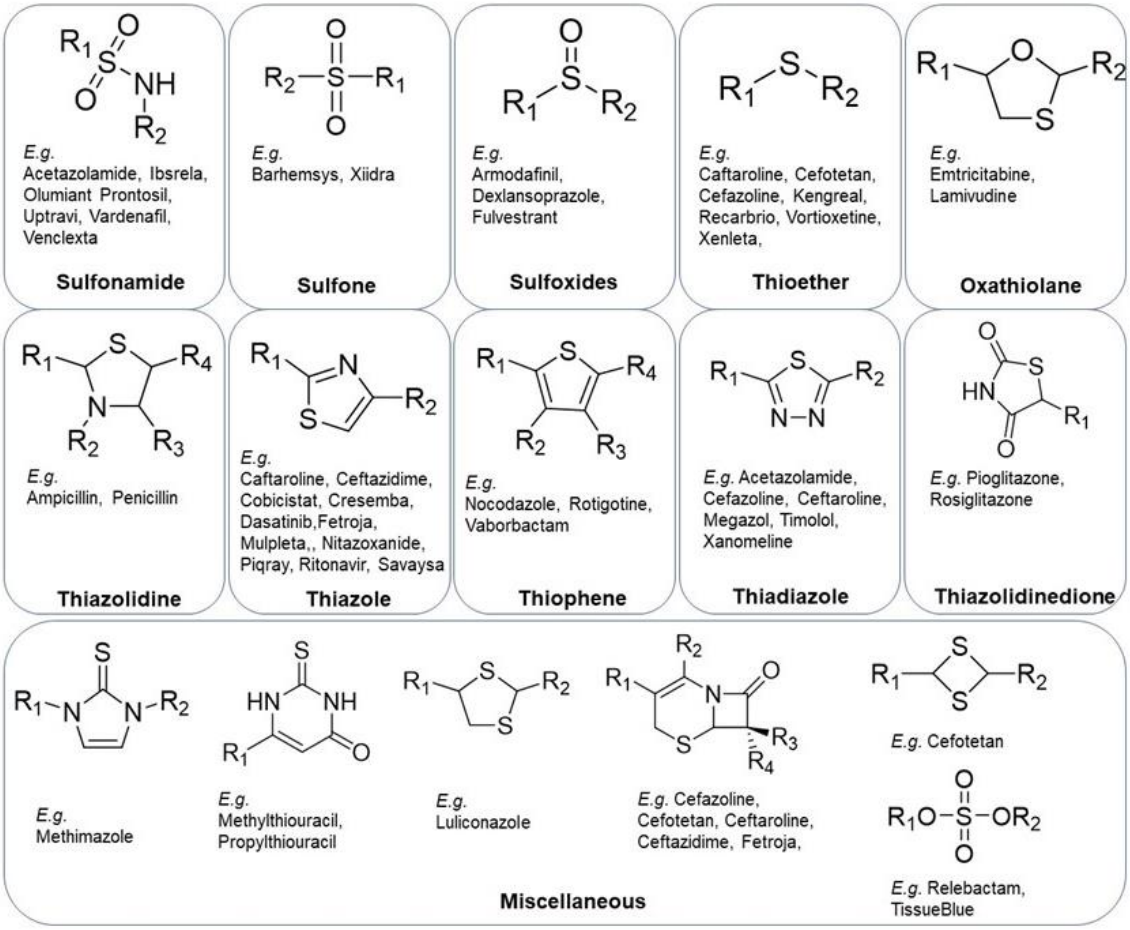


Figure 2.2: Common sulfur-containing scaffolds found in marketed drugs. Examples of clinical drugs containing the scaffold shown are given for each. A 2,5-substituted thiadiazole is shown in the figure but any sulfur-containing heteroaromatic ring with two nitrogens and one sulfur are considered as a thiadiazole.

2.3 Objective

Although multiple potent pantothenamide analogs have been reported in the scientific literature, none has entered clinical trials due to low *in vivo* efficacy[100] or mutations in *Plasmodium* acetyl CoA synthase, which hinders the antiplasmodial activity of pantothenamides.[97] As a result, novel pantothenamide analogs are needed not only to overcome these obstacles but also to expand upon the current SARs.

To the best of our knowledge, a total of 11 pantothenamide analogues containing sulfur atom have been reported in the literature (not shown),[77,93,96,98,100] and only 2 analogues

utilized sulfur-containing heteroaromatic rings to mimic the labile amide (see Table 2.1). In order to fill in this gap, the aim of this thesis was to replace the labile amide group of pantothenamides with thiazole-containing heterocycles and diversify the *N*-substituent ends of pantothenamides. The derivatives reported here are shown together in Figure 2.3.

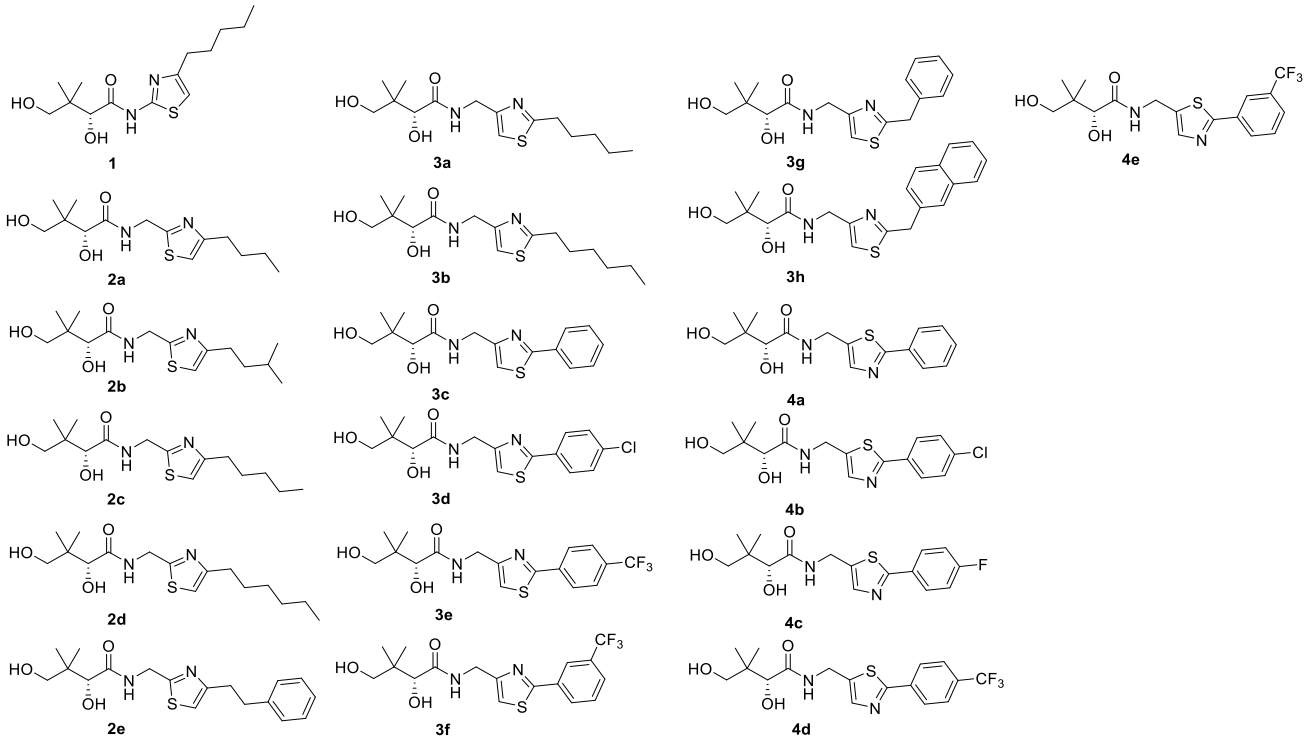
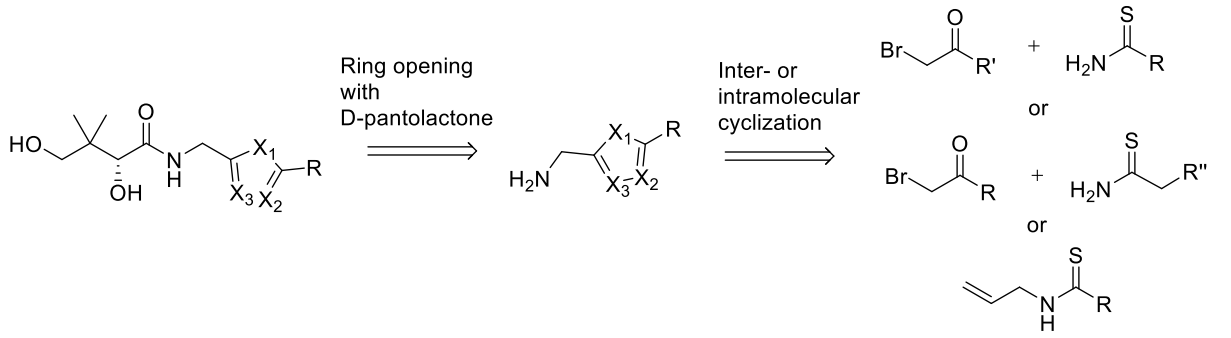


Figure 2.3: Thiazole-containing pantothenamide analogues synthesized in this thesis.

2.4 Results and Discussions

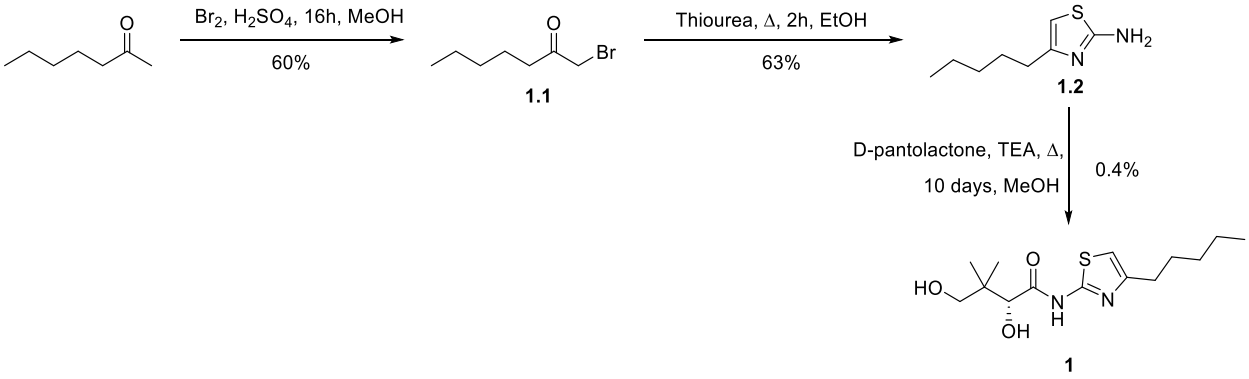
Since the pantoyl moiety is a common feature of the synthetic targets, a similar retrosynthetic strategy was used to construct all compounds shown on Figure 2.3. This involves first an intra- or intermolecular cyclization to generate the thiazole core (Scheme 2.1). The resulting primary amine then undergoes a ring opening reaction with D-pantolactone, to afford the desired compound.



Scheme 2.1: Retrosynthetic scheme for the thiazole-containing pantothenamide analogues reported here. X_1, X_2, and X_3 are carbon, nitrogen, or sulfur atom with hydrogen substituents as needed. The R can be alkyl, phenethyl, or substituted benzoyl groups. The R' and R'' are phthalimide and *tert*-butyloxylcarbonyl protecting groups, respectively.

2.4.1 Synthesis of Compound 1

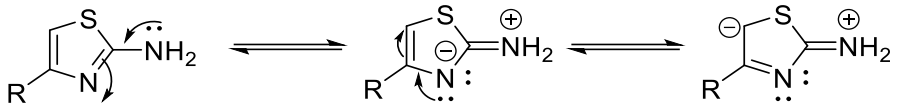
The synthesis of compound **1** is illustrated in Scheme 2.2. Commercially available 2-heptanone underwent an α-bromination in the presence of bromine and sulfuric acid to give 1-bromo-2-heptanone (**1.1**). Intermolecular Hantzsch cyclization of **1.1** with thiourea afforded **1.2**, which was finally reacted with D-pantolactone, under reflux, to form the desired compound **1**.



Scheme 2.2: Synthesis of Compound **1**. TEA: triethylamine.

Only one derivative of compound **1** was synthesized due to the poor yield of opening the D-pantolactone ring with **1.2**. The electrons on the primary amine of **1.2** are delocalized into the ring, and hence the nitrogen is a very poor nucleophile (Scheme 2.3). Since

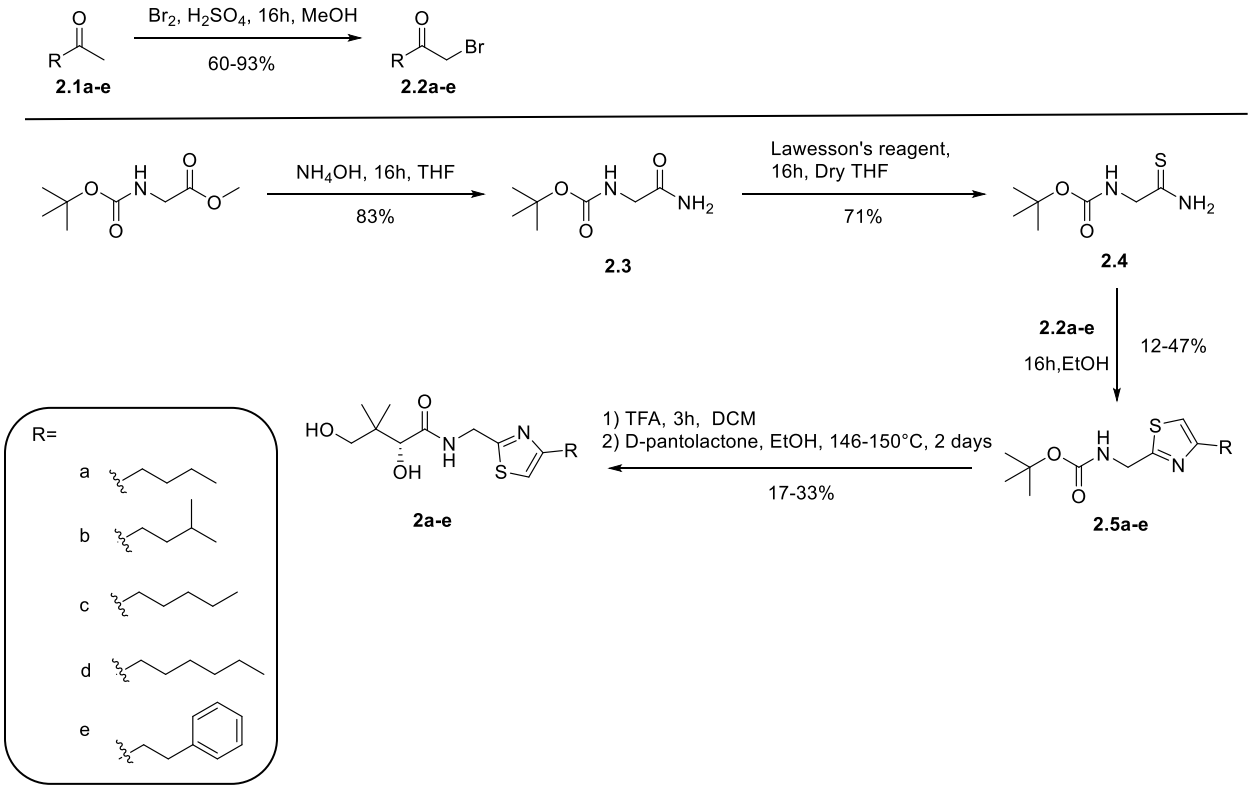
opening the stable five-member D-pantolactone ring is already a challenge, the lack of nucleophilicity adds an additional energy barrier for the reaction. As a result, the reaction required 10 days at reflux temperature to reach a yield of 0.4%. It may be possible to overcome this by conducting an amide coupling reaction between **1.2** and sodium (*R*)-pantoate; however, due to the high cost of this salt ($159.00 for 50 mg), this is not an ideal route for antimicrobial research and development.



Scheme 2.3: Resonance structures for compound **1.2**.

2.4.2 Synthesis of Thiazole Derivatives 2a-e

Scheme 2.4 illustrates the synthetic route for compounds **2a**-**e**. The commercially available ketones **2.1a**-**e** were separately reacted with bromine in the presence of sulfuric acid to form the α-brominated ketones **2.2a**-**e**. In parallel, *N*-(tert-butoxycarbonyl)glycine methyl ester was reacted with 28% (v/v) ammonium hydroxide to yield *N*-Boc-glycinamide (**2.3**), which was next thiolated with Lawesson's reagent [2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane] to give **2.4**. Intermolecular Hantzsch cyclization between **2.4** and each of **2.2a**-**e** afforded the key intermediates **2.5a**-**e**. Finally, trifluoracetic acid-mediated deprotection of the Boc group, followed by D-pantolactone ring opening produced the desired compounds **2a**-**e**.



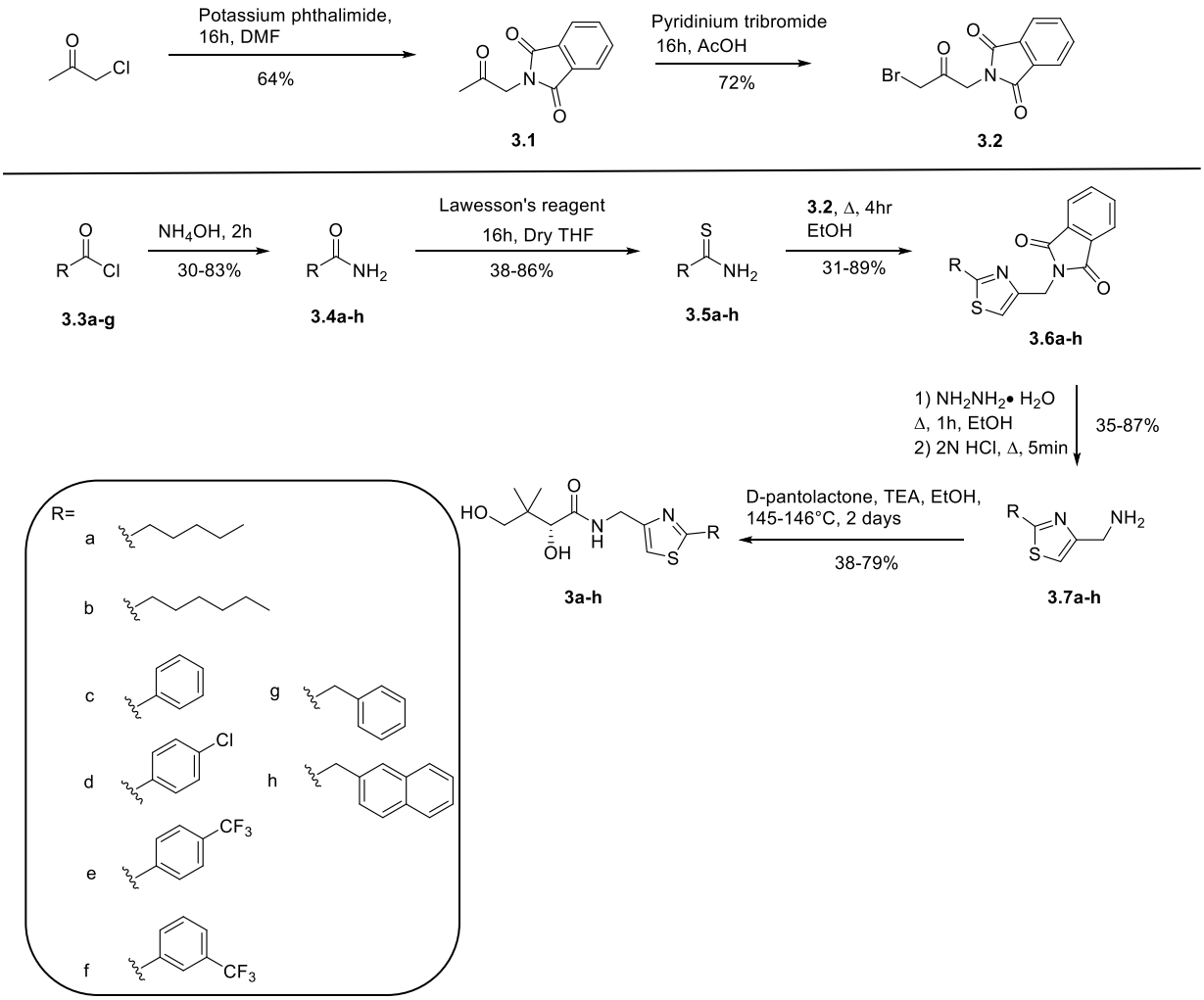
Scheme 2.4: Synthetic route for compounds **2a**-**e**. DCM: dichloromethane, TEA: trimethylamine; TFA: trifluoroacetic acid; THF: tetrahydrofuran.

Initially, the D-pantolactone ring opening reactions to generate **2a**-**2e** were conducted in a microwave reactor at 160°C for 30 to 45 minutes with a yield of 5-19% (data not shown). The reaction yields increased to 17-33% when the reaction mixture was heated in a sealed vessel at 146°C for 2 days instead of a microwave reactor. A dark brown solution was produced after completion of the microwave reaction, whereas no drastic color change was observed with the milder heating method. This suggests that unwanted side reactions and/or decomposition are favoured during microwave heating at 160°C, and such conditions were avoided for future pantolactone ring opening reactions.

2.4.3 Synthesis of Thiazole Derivatives 3a-h

Hantzsch cyclization, Gabriel amination, and D-pantolactone ring opening are the key steps used here to access compounds **3a**-**h** (Scheme 2.5). Potassium phthalimide was first reacted with chloroacetone to form **3.1**, which underwent an α-bromination in the presence of pyridinium tribromide and acetic acid to give **3.2**. In parallel the commercially

available acid chlorides **3.3a-h** were converted to the corresponding amides **3.4a**-**h** via a reaction with 28% (v/v) ammonium hydroxide. Next, Lawesson's reagent was employed to convert the carbonyl into a thiocarbonyl, affording compounds **3.5a**-**h.** Hantzsch cyclization of **3.5a**-**h** and **3.2** yielded **3.6a**-**h**, which were treated with hydrazine to deprotect the amine. The free amino group of **3.7a**-**h** were next reacted with D-pantolactone in a sealed vessel to produce the desired compounds **3a**-**h**.

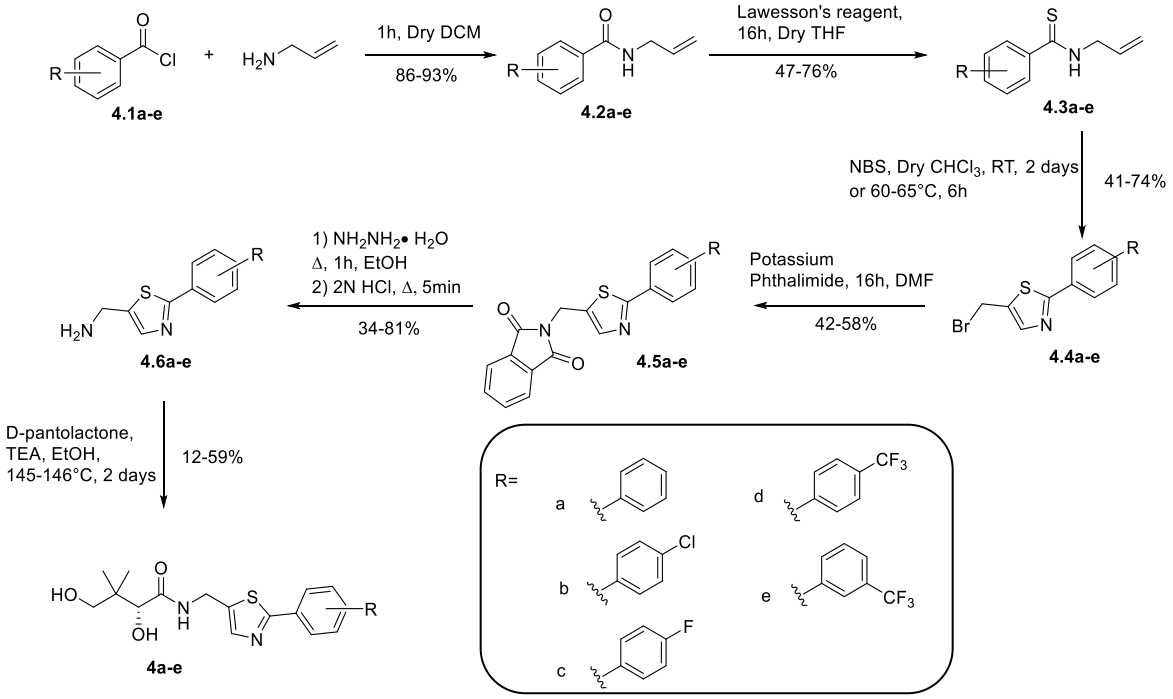


Scheme 2.5: Synthetic route for compound **3a**-**h**. TEA:triethylamine; THF: tetrahydrofuran.

2.4.4 Synthesis of Thiazole Derivatives 4a-e

To prepare compounds **4a**-**e**, the commercially available acid chlorides **4.1a**-**e** were condensed separately with allylamine to yield the requisite intermediates **4.2a**-**e** (Scheme

2.6). These intermediates were next thiolated using Lawesson's reagent to afford **4.3a**-**e**, which then underwent a catalyst-free intramolecular cyclization to generate the 2,5-thiazole containing intermediates **4.4a**-**e**. Gabriel amination was used to convert the halides **4.4a-e** to the corresponding primary amines **4.6a**-**e** via compounds **4.5a**-**e.** Lastly, the resulting compounds **4.6a**-**e** were each reacted with D-pantolactone in a sealed vessel to produce the desired compounds **4a**-**e**.



Scheme 2.6: Synthetic route to compounds **4a**-**e**. DCM: dichloromethane, DMF: *N, N*-dimethylformamide, NBS: *N*-bromosuccinimide, TEA: triethylamine, THF: tetrahydrofuran, RT: room temperature.

2.5 Conclusion

In this chapter, 19 novel thiazole-containing pantothenamide analogues were successfully synthesized, each in less than 7 linear synthetic steps. The next chapter discusses experiments performed to assess their biological activities against several pathogenic bacteria (*E. coli, E. faecium, K. pneumonia, P. aeruginosa,* and *S. aureus*) and malaria-causing parasite *P. falciparium*.

Chapter 3

Biological Results

3.1 Preface

Antimicrobial resistance is a global health burden and molecules with a novel mechanism of action are needed. In the previous chapter, 19 novel thiazole-containing pantothenamide analogs (**1**–**4e**) were designed and synthesized. In this chapter, the biological activities of these analogs are examined for their growth inhibitory activity against *E. coli, E. faecium, K. pneumonia, P. aeruginosa, S. aureus* and *P. falciparum.* I performed all of these studies, except for the antiplasmodial activity assays, which were performed by Christina Spry and Xiangning Liu from Dr. Kevin Saliba's laboratory at the Australian National University.

3.2 Introduction

As mentioned in chapter 1, pathogens are becoming increasingly resistant to antibiotics, and antiplasmodials. With the post-antibiotic era looming ahead of us,[109] and the lack of antibiotics in the pre-clinical pipeline, the foundations of modern medicine are threatened. In fact, it is estimated that by 2050, 10 million people will die per year due to complications associated with antimicrobial resistant infections.[110] Similarly, resistance to antimalarial drugs is rapidly spreading in malaria endemic countries. ACT is the golden antimalarial drug used to treat *P. falciparum* infections,[22] and the high treatment failure in Eastern Mediterranean region, South-East Asia, and Thailand is worrisome. As a result, antibacterial and antiplasmodial molecules with a new mode of action that do not have cross-resistance with existing drugs are in urgent need.

Thiazole-containing pantothenamide analogs are interesting candidates for the development of antimicrobials since they utilize a novel mechanism of action (Pantothenamides, Section 1.6). The antibacterial and antiplasmodial activities of compounds **1**–**4e** reported in chapter 2 are described in section 3.3 and 3.4, respectively.

3.3 Evaluation of Antibacterial Activity

It is difficult to treat infections associated with Gram-negative bacteria because the outer membrane of these bacteria adds an additional barrier for the antibiotics to cross (Section 1.4.1, bacteria cell envelope). Unfortunately, most of the highly resistant pathogens are Gram-negative (*A. baumannii, Camylobacter* spp.*, E. coli, H. pylori, N. gonorrhoeae*, *P.*

aeruginosa, and Salmonella spp).[63,111] Only two Gram-positive bacteria (*E. faecium* and *S. aureus*) are included in the pathogen priority list published by the World Health Organization.[63,111]

In Canada, microorganisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *E. faecium* (VRE), and carbapenem-resistant *Enterobacteriaceae* (CRE) are on the rise.[112] The Public Health of Canada has listed these microorganisms as a top priority. Additionally, antibiotics such as extended-spectrum penicillins, tetracyclines, macrolides, fluoroquinolones, and first-generation cephalosporines are commonly prescribed by physicians and hence, resistant to these drugs are also on the rise.[112] Table 3.1 lists the antimicrobial-resistance microbes observed in Canada and the drugs that they are resistant to. In an attempt to identify new antibacterial agents, the thiazole-containing pantothenamide analogs were examined for their activity against some of the prioritized microorganisms such as *E. coli, E. faecium, K. pneumonia, P. aeruginosa,* and *S. aureus,* and the results are discussed herein.

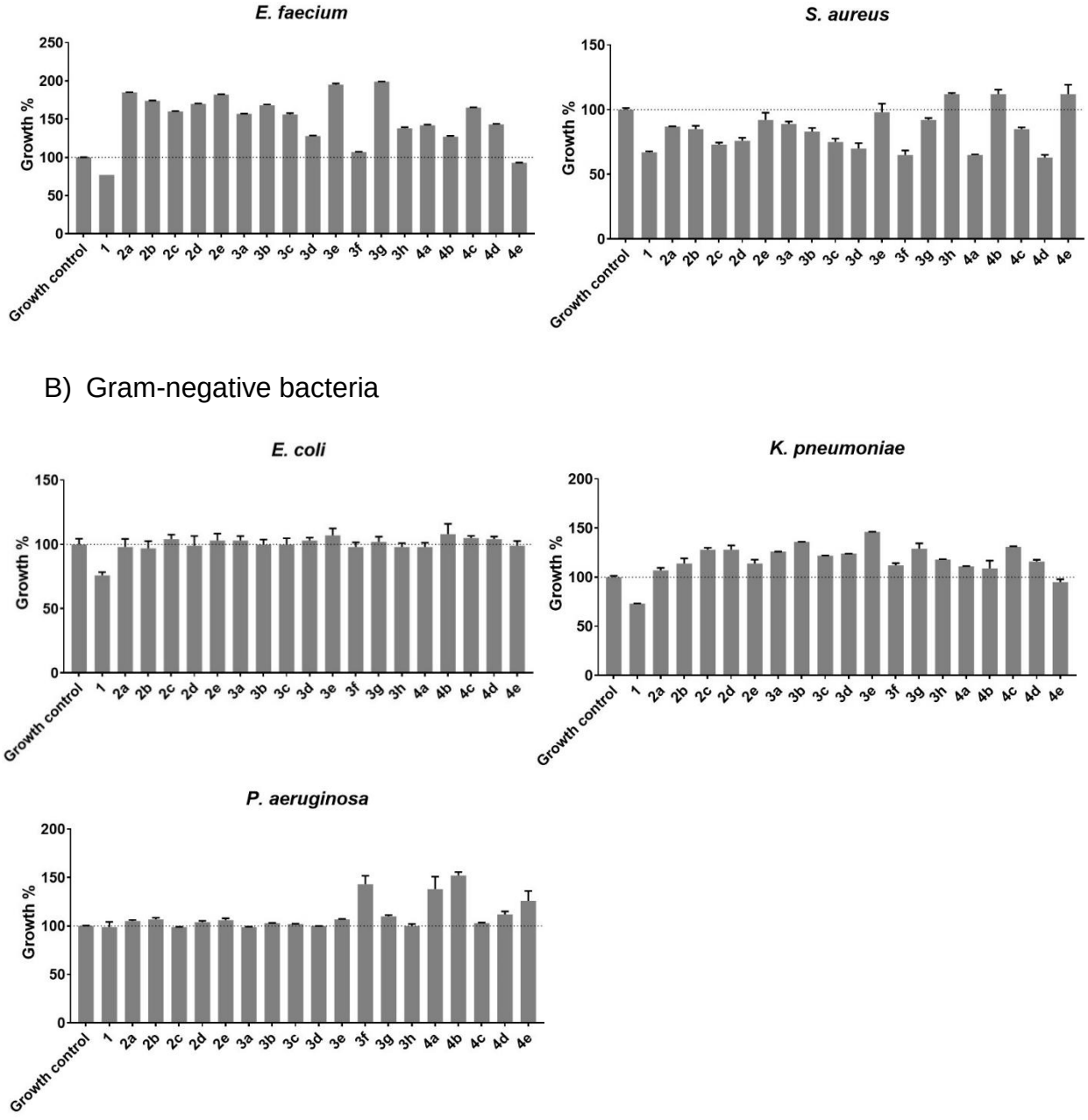
Table 3.1: Prioritized pathogens in Canada. The left and right columns list the resistant bacteria and the drugs they are resistant to, respectively. Only antimicrobials with over 30% prevalence in the clinical isolates are included.

Microbe	Antibiotic
Methicillin-resistant *Staphylococcus aureus*	Ciprofloxacin Cindamycin Methicillin
Vancomycin-resistant *E. faecium*	Ampicillin Ciprofloxacin Erythromycin Levofloxacin Penicillin Rifampin Gentamicin Streptomycin Tetracycline

	Vancomycin
Carbapenem-resistant *Enterobacteriaceae*	Cefotaxime Ceftazidime Ciprofloxacin Gentamicin Meropenem Piperacillin-tazobactam Tobramycin Trimethoprim-sulfamethoxazole
C. difficile	Clindamycin
E. coli	Ampicillin Cefazolin Trimethoprim-sulfamethoxazole

Antibacterial activity is typically measured using the Clinical and Laboratory Standards Institute broth microdilution method[113] and quantified by means of the minimum growth inhibitory concentration (MIC). Before measuring MIC values, we first opted to screen compounds **1**–**4e** for antimicrobial activity at one concentration. All compounds were tested at a concentration of 50 μM, a concentration high enough to detect any compound with significant activity. The percent bacterial growth inhibition observed at this concentration is presented in Figure 3.1 for all compounds.

A) Gram-positive bacteria



B) Gram-negative bacteria

Figure 3.1: Percent growth inhibition of A) *E. faecium* and *S. aureus*. B) *E. coli, K. pneumonia,* and *P. aeruginosa* in the presence or absence of various compounds at 50 µM. Lane 1 is the growth control with no compound added, adjusted to 100% growth. All experiments were performed in duplicate. Standard error of mean is used to determine the error.

Unfortunately, although several compounds reduced bacterial growth at 50 µM, none of them fully eliminated bacterial growth. In the presence of the compounds, most bacteria grow equally well or even better than in their absence. This suggests that the bacteria may be able to utilize these thiazole-containing pantothenamides as a carbon source. This is especially obvious for Gram-positive bacteria with a Type I PanK (*e.g. E. faecium*), with a maximum of 199% growth observed, compared to the Gram-negative bacteria such as *E. coli* (a maximum of 108% growth) and *K. pneumonia* (a maximum of 146% growth). This difference might be explained by the production of efflux pumps known to bind pantothenamides (*e.g.* TolC-dependent efflux pumps) in Gram negative bacteria.[78,83] In addition, it might be easier for the small molecules to penetrate the cells of Gram-positive bacteria, than the double membranes-containing cells of Gram-negative bacteria.

Staphylococci is the only bacteria known to date with a Type II PanK.[70,85] It is also the only strain here for which the bacterial growth is significantly reduced in the presence of several compounds. The level of inhibition is however not significant enough for further evaluation, but suggests starting points for future medicinal chemistry efforts. Lastly, *P. aeruginosa* is a Gram-negative bacterium with a Type III PanK.[70,85] Due to the tight pantothenamide binding pocket at $PanK_{III}$, pantothenamides are not expected to show activity towards this strain, which is consistent with the observed results. Although, none of the thiazole-bearing analogs reported here had significant antibacterial activity, it was envisaged that some of these compounds might show activity in the *P. falciparum* parasite.

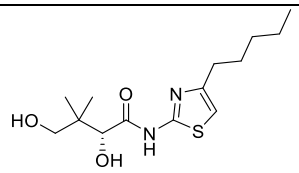
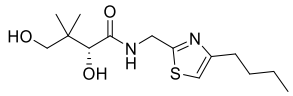
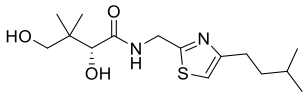
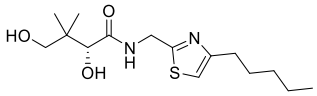
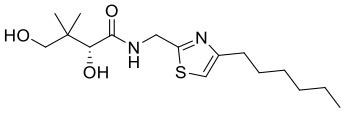
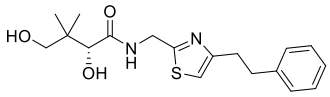
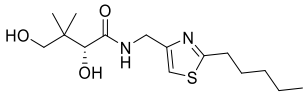
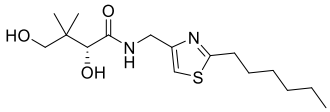
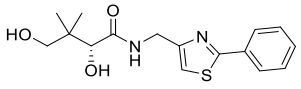
3.4 Evaluation of Antiplasmodial Activity

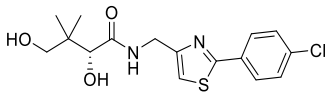
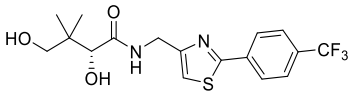
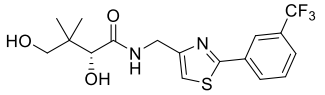
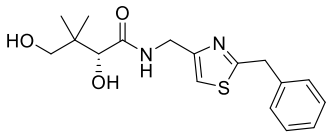
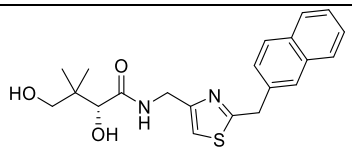
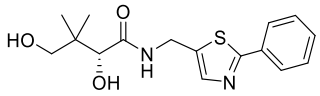
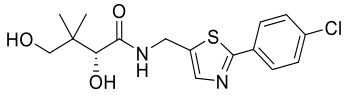
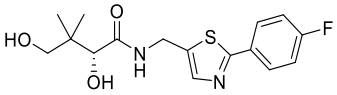
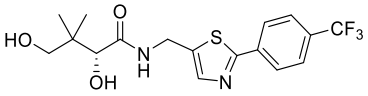
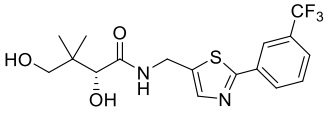
As discussed in section 1.2, ACT resistant *P. falciparum* strains are rampant in countries where malaria is endemic such as Cambodia and Thailand.[23,24] In addition, as the global temperature continue to rise, malaria might extend its reach to new parts of the world. As a result, we urgently need new antimalarials.

Previous members from the Auclair group have discovered potent isoxazole-, thiazole-, and triazole-containing pantothenamide analogs (Table 2.1), active at low nanomolar concentrations. To the best of our knowledge, however, the antiplasmodial activity of thiazole-containing pantothenamide analogs has not been examined. The 19 compounds

synthesized in Chapter were therefore tested for their antiplasmodial activity at the intraerythrocytic stage of *P. falciparum.* The biological assays were performed by Dr. Christina Spry and Xiangning Christine Liu in Prof. Kevin Saliba's laboratory. The results are presented as the concentration leading to 50% growth inhibition (IC_{50}) in Table 3.2.

Table 3.2: The IC_{50} values for compounds **1**-**4e** tested against intraerythrocytic *P. falciparium*. Compounds showing activity at nanomolar concentrations are highlighted in red. Compounds **3a**-**4e** were analyzed in only one triplicate experiment, instead of the standard two separate experiments each performed in triplicates.

Compound	Structure	IC_{50} (μM)
1		137.8 ± 5.61
2a		0.77 ± 0.10
2b		2.90 ± 0.23
2c		2.28 ± 0.16
2d		4.34 ± 0.37
2e		0.77 ± 0.09
3a		5.980
3b		3.912
3c		0.5474

3d		2.533
3e		53.46
3f		22.18
3g		1.937
3h		6.588
4a		6.585
4b		35.53
4c		9.792
4d		35.05
4e		9.721

In this series, compound **1** is the only one lacking a methylene linker between the pantoyl and thiazole moieties (Table 3.2). The absence of the methylene linker seems detrimental to antiplasmodial activity. Unlike what was previously reported by Howieson[94] and Guan *et al.* for triazole-containing analogs,[95,100] phenethyl and butyl groups are equally potent in the 4-subtstituted thiazole series (**2a** and **2e**). Interestingly, when the alkyl/aromatic substituent is at C-2 of the thiazole ring (**3a**-**3h**), alkyl and methyl phenyl groups were no longer preferred. Instead, the phenyl group is preferred (as in **3c**) with an IC_{50} of 0.55 μM.

Moreover, replacement of the *para*-chloro group in **3d** with a *para*-trifluoro group (**3e**), is highly detrimental to activity in this series. A similar trend is observed for the 2,5-thiazole series **4a**-**4e**, with the simple benzyl group being preferred.

Considering that the mode of action of pantothenamides may involve several targets, that small structural differences can affect membrane penetration (erythrocyte and parasite), and that a multistep bioactivation process is required for activity of pantothenamide analogs, it is challenging to rationalize the preference for a phenethyl or phenyl group over the alkyls as the *N*-substituent. However, we propose a possible explanation for the preference of the 2,4-thiazole over the 2,5-thiazole ring. As proposed by Jinming *et al*. for heteroaromatic rings,[100] the nitrogen may act as an electron acceptor, whereas the aromatic hydrogen or sulfur may act as an electron donor, together mimicking an amide group. Unlike other heteroaromatic rings, the thiazole may better mimic the normal amide over the inverted amide (Figure 3.2). Further experiments specifically looking at cell permeability, bioactivation and trying to identify the targets might help gain a better understanding of the SARs.

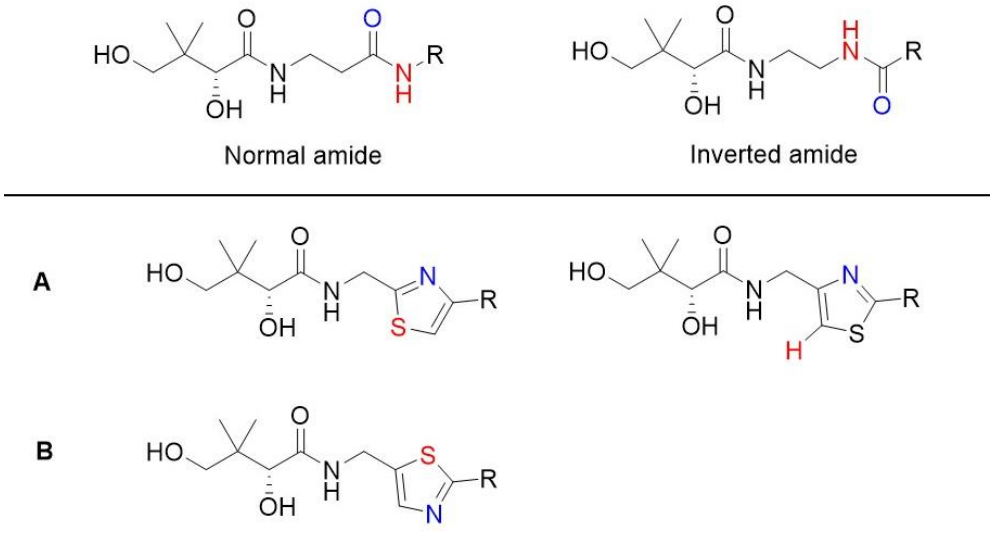


Figure 3.2: The aromatic hydrogen and sulfur atoms of the heteroaromatic rings are hypothesized to mimic the amide group. 2,4-Thiazole-containing (A) and 2,5-thiazole-containing (B) pantothenamide analogs are shown for comparison with pantothenamides with a normally oriented or inverted amide group.

3.5 Conclusion

Interestingly, the antimicrobial activity of the thiazole-containing pantothenamide analogs reported here was found to be microbe specific, which is an advantageous trait to minimize resistance selection. Whereas none of the compounds showed significant antibacterial activity against *E. coli, E. faecium, K. pneumonia, P. aeruginosa,* and *S. aureus* at a concentration of 50 µM, a few compounds displayed activity at high nanomolar concentrations against *P. falciparum*. Therefore, thiazole-containing pantothenamide analogs are promising hits for the development of antiplasmodial agents, warranting further medicinal chemistry and biological studies.

Chapter 4

Contributions and Future Directions

4.1 Contributions

As antimicrobial resistance continues to propagate, the need for novel antimicrobials is greater than ever. Pantothenamides have been identified as potent and selective growth inhibitors of bacteria and malaria parasites. These compounds utilize a novel mechanism of action (CoA biosynthesis and utilisation); however, the pantetheinase enzymes in human serum rapidly hydrolyze pantothenamides into pantothenate and the corresponding amine, limiting clinical utility. This thesis reports 19 novel thiazole-containing pantothenamide analogs that are expected to be pantetheinase-resistant (this is currently being tested by our collaborators, but is the case for other similar heteroaromatic ring-containing analogs[100]), and include several promising hits for further medicinal chemistry modifications. In addition, the new analogs are easily accessible synthetically, the starting materials and reagents are commercially available and inexpensive, and the syntheses consist of 3-7 linear steps.

In chapter 3, the compounds were tested against selected pathogenic bacteria and *P. falciparium*. Although no bacterial growth inhibition was observed, most compounds were found to inhibit the growth of intraerythrocytic *P. falciparum*, thus enabling us to establish preliminary SARs for this series. Compounds **2a**, **2e**, and **3c** are especially promising considering their activity at high nanomolar concentrations. We plan to submit this work for publication in the near future.

4.2 Future Directions

The work presented in this thesis demonstrates that replacing the labile amide group of pantothenamides with a thiazole ring is a promising strategy to identify blood-stable antiplasmodial agents. This warrant further exploration of SARs, in particular at the *N*-substituent of compounds from both 2,4-thiazole series. Antiplasmodial results reported by Spry et al.,[98] and Schalkwijk et al.,[97,99] show that pantothenamides with phenethyl or phenyl groups at this position display superior activity to those harboring alkyl counterparts. This might be due to favourable π-π interactions between the *N*-substituent and the protein targets. It would be interesting, for example, to replace the phenethyl group of compound **2e** with a substituted phenethyl or a phenyl group.

Alternatively, one might want to attach a phenethyl group at the C2 position of compounds from the 2,5-thiazole series.

In parallel, it might be of interest to expand the amide bioisostere toolbox to non-aromatic heterocycles such as thiazolidine-4-one or oxoimidazolidine (Figure 4.1). In these structures, the electron donor and acceptor abilities are similar to those of thiazoles, and the molecules would likely also be resistant to pantetheinases.

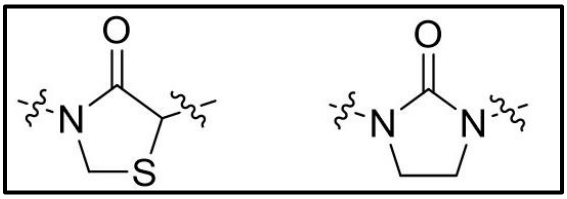


Figure 4.1: Possible chemical scaffolds to replace the labile amide.

Chapter 5

Experimental

5.1 Chemistry

5.1.1 Materials and instruments

All reagents and solvents were purchased from Sigma-Aldrich Canada or Tokyo Chemical Industry and used without further purification. Dry solvents were obtained from a MBruan MB SPS 800 solvent purifier. MilliQ water was used whenever water is mentioned. Thin-layer chromatography (TLC) was performed using silica plates of 200 µm thickness and coated with the fluorescent indicator F254. Flash column chromatography was performed using either RediSep Silica 60 µm (Teledyne) or Sfar Silica D – Duo 60 µm (Biotage). A UVGL-55 UV lamp was used to visualize UV-absorbing compounds on TLC at 254 or 365 nm. Potassium permanganate or ninhydrin stain was used to visualize non-UV absorbing compounds. Unless stated otherwise, all compounds synthesized herein are novel. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AVIIIHD 500 instrument. Chemical shifts were recorded in parts per million (ppm) and were referenced to $CDCl_3$ and MeOD solvent peak at 7.26 and 3.31 ppm, respectively. Coupling constants were recorded in hertz (Hz). The NMR signal multiplicity is reported as: singlet (s), broad singlet (bs), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), pentet (p), sextet (sext), and multiplet (m). HRMS spectra were recorded using a Thermo Fisher Scientific EXACTIVE^{TM} Plus Orbitrap or a Brucker MaXis Impact HD. An Agilent 1100 series HPLC was used to determine compound purity using Methods A and B described in Table 5.1. Microwave reactions were carried out in a Biotage initiator EXP US 400 W.

Table 5.1. HPLC methods for measuring the purity of compounds **1** to **4e**

Method A

Flow rate: 1 mL/min; detector wavelength: 214 nm

Column: LUNA 5 µm C18(2) 250 × 4.6 mm from Phenomenex

Time (min)	Water (%)	Acetonitrile (%)
5	99	1
15	50	50
20	50	50
25	1	99
30	1	99
32	99	1
35	99	1

Method B

Flow rate: 1 mL/min; detector wavelength: 214 nm

Column: LUNA 5 µm C18(2) 250 × 4.6 mm from Phenomenex

Time (min)	Water (%)	Acetonitrile (%)
10	1	99
20	1	99
22	99	1
25	99	1

5.1.2 Compound synthesis

5.1.2.1 General protocol 1 for the synthesis of compounds 2.2a-2.2e

The required α-bromoketone intermediates **2.2a**-**e** were first prepared following a previously reported protocol with modifications.[114] The desired ketone (6.39-14.4 mmol,

1.0 eq) was dissolved in methanol (4-9 mL). The solution was cooled to -10°C, and bromine (6.39-14.4 mmol, 1.0 eq) was added dropwise. The reaction mixture was stirred at 0°C for 45 minutes, and then at room temperature for 45 minutes. Water (2-4 mL) was added followed by concentrated sulfuric acid (3.3-7.4 mL). The reaction was stirred at room temperature for 16 hours. Water (6-13 mL) was added, and the mixture was extracted in diethyl ether (4 × 5-10 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate (5-10mL), water (2 × 5-10 mL) and dried over anhydrous magnesium sulfate. The organic solvent was removed *in vacuo* to yield the α-bromoketone, which was used without further purification.

5.1.2.2 General protocol for the synthesis of compound 2.5a, 2.5b, 2.5d, and 2.5e

The synthesis of compound **2.5a**, **2.5b**, **2.5d**, and **2.5e** from **2.2a**, **2.2b**, **2.2d**, or **2.2e** followed a previously reported procedure with modifications.[115,116] To a solution of *tert*-butyl (2-amino-2-thioxoethyl)carbamate (1.19-1.40 mmol, 1.0 eq) in anhydrous ethanol (3 mL) was added **2.2a**, **2.2b**, **2.2d**, or **2.2e** (1.19-1.40 mmol, 1.0 eq). The reaction mixture was stirred at room temperature for 16 hours under an inert atmosphere. Water (6-7 mL) was added, followed by dropwise addition of an ammonium hydroxide solution (aqueous 28% w/w) until the solution reached a pH ~ 8. The product was extracted in DCM (3 × 8-9 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the organic solvent was removed *in vacuo.* The crude product was purified by flash column chromatography to afford the product.

5.1.2.3 General protocol 3 for the synthesis of compounds 2a, 2b, and 2d

The synthesis of compounds **2a**, **2b**, and **2c** followed a previously reported procedure with modifications.[100] To a solution of **2.5a**, **2.5b**, or **2.5d** (0.38-0.50 mmol, 1.0 eq) in DCM (2 mL) was added trifluoroacetic acid (3.79-4.96 mmol, 10.0 eq). The reaction mixture was stirred at room temperature for 3 hours. A sodium hydroxide solution (15% aqueous w/v) was added dropwise until the solution reached a pH ~11. The product was extracted in ethyl acetate (3 × 4-5 mL) and the combined organic layers were dried over magnesium sulfate. The organic solvent was removed *in vacuo* to yield the free amine, which was used directly in the next step.

In a pressure vessel (5 mL), the free amine (0.27-0.39 mmol, 1.0 eq), D-pantolactone (0.81-1.17 mmol, 3.0 eq) and triethylamine (0.81-1.17 mmol, 3.0 eq) were dissolved in anhydrous ethanol (3-4 mL). The reaction was heated to 146-150°C (oil bath) for two days. The organic solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (eluted with EtOAc-Hex, 00:100 to 100:00) to afford the product.

5.1.2.4 General protocol 4 for the synthesis of compounds 3.4a-g

The synthesis of compounds **3.4a**-**g** followed a previously reported procedure with modifications.[117] To a cooled (~ 0°C) solution of the desired acid chloride (**3.3a**-**g**, 6.63-17.23 mmol, 1.0 eq) in acetonitrile (3-8 mL) was added an ammonium hydroxide solution (aqueous 28% w/w, 66.31-172.04 mmol, 10.0 eq). The reaction mixture was stirred at room temperature for 2 hours. The product was extracted in ethyl acetate (3 × 5-12 mL), washed with 2 N NaOH (w/v, 14-36 mL), and dried over anhydrous magnesium sulfate. The organic solvent was removed *in vacuo* and the residue was pure enough (>90%) to be used directly in the next step.

5.1.2.5 General protocol 5 for the synthesis of compounds 2.4, 3.5a-h, and 4.3a-e

The synthesis of compounds **2.4**, **3.5a**-**h**, and **4.3a**-**e** followed a previously reported procedure with modifications.[118] To a solution of either compound **2.4**, **3.4a**, **3.4b**, **3.4c**, **3.4d**, **3.4e**, **3.4f**, **3.4g**, **3.4h, 4.2a**, **4.2b**, **4.2c**, **4.2d**, or **4.2e** (0.57-3.10 mmol, 1.0 eq) in dry THF (11-71 mL) was added Lawesson's reagent (0.29-1.56 mmol, 0.5 eq). The reaction was stirred under an inert atmosphere overnight at room temperature. The organic solvent was removed *in vacuo.* The crude product was purified by flash column chromatography.

5.1.2.6 General protocol 6 for the synthesis of compounds 3.6a-h

The synthesis of compounds **3.6a**-**h** followed a previously reported procedure with modifications.[115,116] To a solution of compound **3.2** (0.78-2.01 mmol, 1.0 eq) in anhydrous ethanol (1- 3 mL) was added compound either of **3.5a**-**h** (0.78-2.01 mmol, 1.0 eq). The reaction was heated (85-100°C) under an inert atmosphere for 4 hours. Water (2-4 mL) was added to the mixture, followed by dropwise addition of an ammonium hydroxide solution (aqueous 28% w/w) until the solution reached a pH ~ 8. The precipitated product

was collected by filtration and dried *in vacuum*. The product was purified by flash column chromatography.

5.1.2.7 General protocol 7 for the synthesis of compounds 3.7a-h and 4.6a-e

The synthesis of compounds **3.7a-h and 4.6a-e** followed a previously reported procedure with modifications.[119] To a solution of either compound **3.6a**, **3.6b**, **3.6c**, **3.6d**, **3.6e**, **3.6f**, **3.6g**, **3.6h, 4.5a**, **4.5b**, **4.5c**, **4.5d**, or **4.5e**, (0.16-1.03 mmol, 1.0 eq) in anhydrous ethanol (2-15 mL) was added hydrazine monohydrate (0.47-3.09 mmol, 3.0 eq). The mixture was heated to 85°C for 1 hour. Next, 2 N HCl (v/v, 0.31-3.1 mL) was added and the reaction was heated at 85°C for an additional 5 minutes. The pH of the mixture was neutralized with saturated aqueous $NaHCO_3$ (w/v). The organic solvent was removed *in vacuo*. Water (2-15 mL) was added to the residue, and the product was extracted in DCM (3 × 4-26 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solvent was removed *in vacuo.* The product was purified by flash column chromatography (eluted with DCM-MeOH, 100:00 to 90:10).

5.1.2.8 General protocol 8 for the synthesis of compounds 3a-h and 4a-e

The synthesis of compounds **3a-h** and **4a-e** followed a previously reported procedure with modifications.[100] In a pressure vessel (2-5 mL), the **3.7a**, **3.7b**, **3.7c**, **3.7d**, **3.7e**, **3.7f**, **3.7g**, **3.7h**, **4.6a**, **4.6b**, **4.6c**, **4.6d**, or **4.6e** (0.06-0.5 mmol, 1.0 eq), D-pantolactone (0.18-1.51 mmol, 3.0 eq) and triethylamine (0.18-1.51 mmol, 3.0 eq) were dissolved in anhydrous ethanol (0.6-5 mL). The reaction mixture was heated to 137-146°C (oil bath) for two days. The organic solvent was removed *in vacuo*. The product was purified by flash column chromatography (eluted with Hex-EtOAc, 100:00 to 00:100).

5.1.2.9 General protocol 9 for the synthesis of compounds 4.2a-e

The synthesis of compounds **4.2a-e** followed a previously reported procedure with modifications.[120] Triethylamine (2.58 mmol, 1.0 eq) was added to a solution of allylamine (2.67 mmol, 1.0 eq) in dry DCM (3 mL). The mixture was cooled to 0°C. One of compounds **4.1a-e** (2.62-2.69 mmol, 1.0 eq) was added and the reaction mixture was stirred at room temperature for an hour. Water (2 mL) was added, and the product was extracted in DCM (3 × 3 mL). The combined organic layers were dried over anhydrous

magnesium sulfate and the solvent was removed *in vacuo*. The residue was pure enough (>90%) to be used directly in the next step.

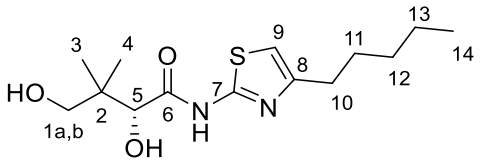
5.1.2.10 General protocol 10 for the synthesis of compounds 4.4a-e

The synthesis of compounds **4.4a**-**e** followed a previously reported procedure with modifications.[121] To a solution of one of compounds **4.3a**-**e** (0.48-1.00 mmol, 1.0 eq) in dry chloroform (8.3-17 mL) was added *N*- bromosuccinimide (0.1.24-2.53 mmol, 2.5 eq). The reaction mixture was stirred under an inert atmosphere overnight unless specified otherwise. The mixture was quenched with saturated aqueous Na_2SO_3 solution (7-15 mL) and the product extracted in DCM (3 × 8-17 mL). The combined organic layers were washed with brine (22-46 mL) and dried over anhydrous sodium sulfate. The organic solvent was removed *in vacuo.* The product was purified by flash column chromatography.

5.1.2.11 General protocol 11 for the synthesis of compounds 4.5a-e

The synthesis of compounds **4.5a**-**e** followed a previously reported procedure with modifications.[122] To a solution of either compound **4.4a**, **4.4b**, **4.4c**, **4.4d**, or **4.4e** (0.31-1.26 mmol, 1.0 eq) in DMF (0.80-3 mL) were added potassium phthalimide (0.31-1.26 mmol, 1.0 eq) and potassium bicarbonate (0.05-0.19 mmol, 0.15 eq). The reaction mixture was stirred at room temperature overnight. Water (1-4 mL) was added, and the mixture was stirred at room temperature for two hours. The precipitated product was collected by filtration and dried *in vacuum,* to afford the crude product*.* The product was purified by flash column chromatography.

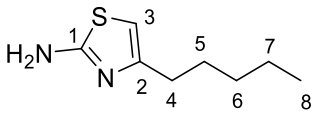
(R)-2,4-Dihydroxyl-3,3-dimethyl-*N*-(4-pentylthiazol-2-yl)butanamide (1)



4-Pentylthiazol-2-amine (0.91 g, 5.32 mmol, 1.0 eq) was added to a solution of D-pantolactone (1.39 g, 10.68 mmol, 2.0 eq) and triethylamine (4.5 mL, 32.07 mmol, 6.0 eq) in anhydrous ethanol (15 mL). The reaction mixture was heated to 80°C for 10 days. The

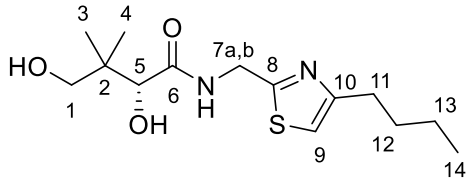
organic solvent was removed *in vacuo*. The product was purified by flash column chromatography (eluted with Hex-EtOAc, 100:00 to 00:100) to afford an orange oil. Yield: 0.4%, R_f = 0.11 (40% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.05 (s, 1H, H-9), 4.12 (s, 1H, H-5), 4.03 (d, *J* = 8.9 Hz, 1H, H-1a or H-1b), 3.94 (d, *J* = 8.9 Hz, 1H, H-1a or H-1b), 2.51 (t, *J* = 7.9 Hz, 2H, H-10), 1.62 (p, *J* = 7.8 Hz, 2H, H-11), 1.32–1.30 (m, 4H, H-12 and H-13), 1.22 (s, 3H, H-3 or H-4), 1.09 (s, 3H, H-3 or H-4), 0.89 (t, *J* = 7.3 Hz, 3H, H-14). ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.06, 167.83, 152.20, 101.66, 76.51, 75.54, 40.80, 31.48, 31.25, 28.34, 22.95, 22.47, 18.93, 14.04. HRMS for $C_{14}H_{23}O_3N_2S$ $[M-H]^-$ calcd. 299.1435, found 299.1438.

4-Pentylthiazol-2-amine (1.2)



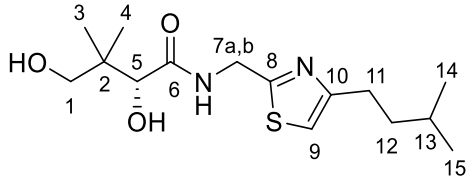
To a stirred solution 1-bromo-2-heptanone (1.63 g, 8.45 mmol, 1.0 eq) in anhydrous ethanol (18 mL) was added thiourea (0.64 g, 8.45 mmol, 1.0 eq). The mixture was refluxed at 75°C for 2 hours and then stirred at room temperature for 16 hours. Water (36 mL) was added, followed by dropwise addition of an ammonium hydroxide solution (aqueous 28% w/w) until the solution reached a pH ~ 8. The product was extracted in DCM (3 × 54 mL). The combined organic layers were dried over magnesium sulfate and the solvent was removed *in vacuo*. The product was purified by flash chromatography (eluted with Hex-EtOAc, 100:00 to 50:50) to afford a yellow oil. Yield: 63%, R_f = 0.31 (40% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.05 (s, 1H, H-3), 5.25 (bs, 2H, NH_2), 2.50 (t, *J* = 7.8 Hz, 2H, H-4), 1.61 (p, *J* = 7.7 Hz, 2H, H-5), 1.36–1.25 (m, 4H, H-6 and H-7), 0.88 (t, *J* = 6.8 Hz, 3H, H-8). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.98, 153.74, 102.23, 31.73, 31.55, 28.53, 22.51, 14.06. HRMS for $C_8H_{15}N_2S$ $[M+H]^+$ calcd. 171.0950, found 171.0947.

***(R)-N*-((4-Butylthiazol-2-yl)methyl)2,4-dihydroxyl-3,3-dimethylbutanamide (2a)**



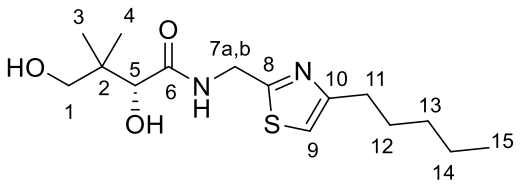
Compound **2a** was prepared from compound **2.5a** (0.47 mmol, 1.0 eq) using general protocol 3 to afford the product as a yellow oil. Yield: 25%, R_f = 0.20 (100% EtOAc). Purity is 99%, R_t = 18.46 minutes with method A and R_t = 9.24 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (m, 1H, NH), 6.80 (s, 1H, H-9), 4.80 (dd, *J* = 16.2, 6.4 Hz, 1H, H-7a or H-7b), 4.68 (bs, 1H, OH), 4.63 (dd, *J* = 16.1, 6.0 Hz, 1H, H-7a or H-7b), 4.08 (s, 1H, H-5), 3.49 (s, 2H, H-1), 2.70 (t, *J* = 7.6 Hz, 2H, H-11), 1.63 (p, *J* = 7.6 Hz, 2H, H-12), 1.34 (sext, *J* = 7.5 Hz, 2H, H-13), 1.01 (s, 3H, H-3 or H-4), 0.95 (s, 3H, H-3 or H-4), 0.91 (t, *J* = 7.4 Hz, 3H, H-14). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 173.57, 166.75, 157.35, 113.32, 77.75, 70.92, 40.29, 39.51, 31.24, 31.03, 22.31, 21.50, 20.75, 13.86. HRMS for $C_{14}H_{24}O_3N_2SNa$ $[M+Na]^+$ calcd. 323.1400, found 323.1397.

***(R)-N*-((4-Isopentylthiazol-2-yl)methyl)2,4-dihydroxyl-3,3-dimethylbutanamide (2b)**



Compound **2b** was prepared from compound **2.5b** (0.50 mmol, 1.0 eq) using general protocol 3 to afford the product as an orange oil. Yield: 17%, R_f = 0.29 (100% EtOAc). Purity is 77%, R_t = 19.98 minutes with method A and R_t = 9.90 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (t, *J* = 6.2 Hz, 1H, NH), 6.80 (s, 1H, H-9), 4.80 (m, 1H, H-7a or H-7b), 4.64 (m, 1H, H-7a or H-7b), 4.08 (s, 1H, H-5), 3.50 (s, 2H, H-1), 2.72–2.68 (m, 2H, H-11), 1.59–1.51 (m, 3H, H-12 and H-13), 1.02 (s, 3H, H-3 or H-4), 0.95 (s, 3H, H-3 or H-4), 0.91 (d, *J* = 6.2 Hz, 6H, H-14 and H-15). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 173.53, 166.75, 157.55, 113.16, 77.79, 70.95, 40.33, 39.51, 38.19, 29.30, 27.72, 22.45, 21.51, 20.76. HRMS for $C_{15}H_{26}O_3N_2SNa$ $[M+Na]^+$ calcd. 337.1556, found 337.1573.

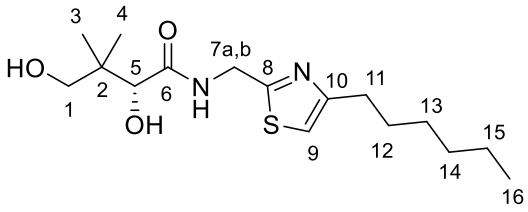
***(R)*-2,4-Dihydroxyl-3,3-dimethyl-N-((4-pentylthiazol-2-yl)methyl)butanamide (2c)**



The synthesis of compound **2c** from **2.2c** followed a previously reported procedure with modifications.[100,115,116] To a solution of *tert*-butyl (2-amino-2-thioxoethyl)carbamate (0.95 mmol, 1.0 eq) in anhydrous ethanol (2 mL) was added **2.2c** (0.93 mmol, 1.0 eq). The reaction mixture was heated at 85°C under an inert atmosphere for 4 hours. Water (4 mL) was added, followed by dropwise addition of an ammonium hydroxide solution (aqueous 28% w/w) until the solution reached a pH ~ 8. The product was extracted in DCM (3 × 6 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the organic solvent was removed *in vacuo.* The crude product was purified by flash column chromatography (eluted with DCM-MeOH, 100:00 to 90:10) to afford the product (free amine) as an orange oil.

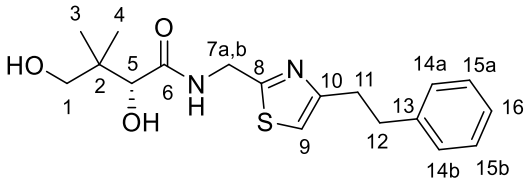
In a pressure vessel (5 mL), the free amine (0.36 mmol, 1.0 eq), D-pantolactone (1.08 mmol, 3.0 eq) and triethylamine (1.08 mmol, 3.0 eq) were dissolved in anhydrous ethanol (4 mL). The reaction mixture was heated to 146°C (oil bath) for two days. The organic solvent was removed *in vacuo*. The product was purified by flash column chromatography (eluted with Hex-EtOAc, 100:00 to 00:100) to afford an orange oil. Yield: 25%, R_f = 0.21 (100% EtOAc). Purity is 97%, R_t = 20.27 minutes with method A and R_t = 9.99 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (m, 1H, NH), 6.79 (s, 1H, H-9), 4.78 (dd, *J* = 16.0, 6.5 Hz, 1H, H-7a or H-7b), 4.63 (dd, *J* = 16.0, 5.9 Hz, 1H, H-7a or H-7b), 4.08 (s, 1H, H-5), 3.48 (s, 2H, H-1), 2.69 (t, *J* = 7.7 Hz, 2H, H-11), 1.63 (p, *J* = 7.6 Hz, 2H, H-12), 1.33–1.26 (m, 4H, H-13 and H-14), 1.00 (s, 3H, H-3 or H-4), 0.94 (s, 3H, H-3 or H-4), 0.87 (t, *J* = 6.8 Hz, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.64, 166.79, 157.38, 113.31, 77.68, 70.90, 40.29, 39.49, 31.42, 31.31, 28.82, 22.45, 21.44, 20.74, 14.02. HRMS for $C_{15}H_{26}O_3N_2SNa$ $[M+Na]^+$ calcd. 337.1556, found 337.1552.

***(R)-N*-((4-Hexylthiazol-2-yl)methyl)2,4-dihydroxyl-3,3-dimethylbutanamide (2d)**



Compound **2d** was prepared from compound **2.5a** (0.38 mmol, 1.0 eq) using general protocol 3 to afford the product as an orange oil. Yield: 33%, R_f = 0.24 (100% EtOAc). Purity is 90%, R_t = 22.99 minutes with method A and R_t = 10.76 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (t, *J* = 6.3 Hz, 1H, NH), 6.79 (s, 1H, H-9), 4.78 (m, 1H, H-7a or H-7b), 4.63 (m, 1H, H-7a or H-7b), 4.07 (s, 1H, H-5), 3.48 (s, 2H, H-1), 2.68 (t, *J* = 7.8 Hz, 2H, H-11), 1.63 (p, *J* = 7.9 Hz, 2H, H-12), 1.30–1.26 (m, 6H, H-13, H-14, and H-15), 1.00 (s, 3H, H-3 or H-4), 0.94 (s, 3H, H-3 or H-4), 0.86 (t, *J* = 6.7 Hz, 3H, H-16). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.67, 166.79, 157.39, 113.30, 77.65, 70.90, 40.30, 39.48, 31.61, 31.35, 29.09, 28.91, 22.57, 21.42, 20.75, 14.07. HRMS for $C_{16}H_{28}O_3N_2SNa$ $[M+Na]^+$ calcd. 351.1713, found 351.1712.

***(R)-N*-((4-Phenethylthiazol-2-yl)methyl)2,4-dihydroxyl-3,3-dimethylbutanamide (2e)**

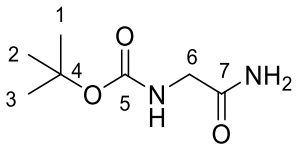


To a solution of **2.5e** (67 mg, 0.21 mmol, 1.0 eq) in DCM (1 mL) was added trifluoroacetic acid (0.16 mL, 2.10 mmol, 10.0 eq). The reaction mixture was stirred at room temperature for 3 hours. A sodium hydroxide solution (15% aqueous w/v) was added dropwise until the solution reached a pH ~11. The product was extracted in ethyl acetate (3 × 1 mL) and the combined organic layers were dried over magnesium sulfate. The organic solvent was removed *in vacuo* to yield the free amine, which was used directly in the next step.

In a pressure vessel (2 mL), free amine (50 mg, 0.23 mmol, 1.0 eq), D-pantolactone (60 mg, 0.46 mmol, 2 eq) and triethylamine (64.3 µL, 0.46 mmol, 2 eq) were dissolved in anhydrous ethanol (0.5 mL). The reaction mixture was heated to 100°C overnight. The

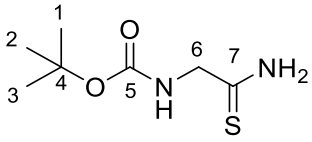
organic solvent was removed *in vacuo*. The product was purified by flash column chromatography (eluted with Hex-EtOAc, 100:00 to 00:100) to afford an orange oil. Yield: 19%, R_f = 0.20 (100% EtOAc). Purity is 77%, R_t = 19.49 minutes with method A and R_t = 9.66 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (m, 1H, NH), 7.29–7.15 (m, 5H, H-14a,b, H-15a,b, and H-16), 6.77 (s, 1H, H-9), 4.88 (dd, *J* = 16.2, 6.6 Hz, 1H, H-7a or H-7b), 4.71 (dd, *J* = 16.2, 5.7 Hz, 1H, H-7a or H-7b), 4.10 (s, 1H, H-5), 3.55 (s, 2H, H-1), 3.06–3.02 (m, 4H, H-11, and H-12), 1.07 (s, 3H, H-3 or H-4), 1.00 (s, 3H, H-3 or H-4).^{13}C NMR (125 MHz, $CDCl_3$) δ 173.18, 166.87, 156.15, 141.17, 128.42, 128.42, 126.10, 114.03, 78.15, 71.03, 40.41, 39.59, 35.45, 33.17, 21.71, 20.91. HRMS for $C_{18}H_{24}O_3N_2SNa$ $[M+Na]^+$ calcd. 371.1400, found 371.1406.

***N*-Boc-glycinamide (2.3)**



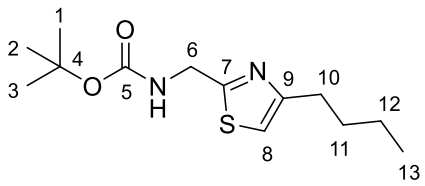
This known compound was synthesized from *N*-(*tert*-butoxycarbonyl)glycine methyl ester and an ammonium hydroxide solution (aqueous 28% w/w) according to the procedure reported by Xu *et al*.[123], to afford the product as a yellow solid. Yield: 78%. The characterization agreed with previously reported procedure.[123] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.44 (bs, 1H, NH_2), 6.19 (bs, 1H, NH_2), 5.47 (bs, 1H, NH), 3.79 (d, *J* = 5.7 Hz, 2H, H-6), 1.43 (s, 9H, H-1, H-2 and H-3).

***N-tert*-Butoxycarbonyl-glycinethioamide (2.4)**



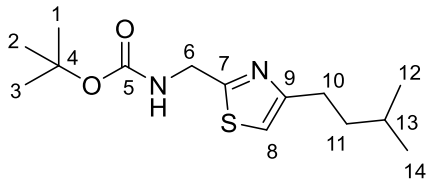
The known compound **2.4**[118] was prepared from *N*-Boc-glycinamide (0.57 mmol, 1.0 eq) using general protocol 5 to afford the product as a white solid. Yield: 88%, R_f = 0.21 (40% EtOAc in Hex). The characterization agreed with previously reported procedure.[124] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (bs, 1H, NH_2), 7.53 (bs, 1H, NH_2), 5.27 (bs, 1H, NH), 4.16 (d, *J* = 4.9 Hz, 2H, H-6), 1.46 (s, 9H, H-1, H-2, and H-3).

***tert*-Butyl ((4-butylthiazol-2-yl)methyl)carbamate (2.5a)**



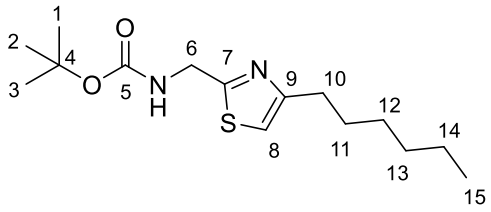
Compound **2.5a** was prepared from 2-hexanone (8.11 mmol, 1.0 eq) using general protocol 2. The crude product was purified by flash column chromatography (eluted with Hex-EtOAc, 90:10 to 50:50) to afford the product as a yellow oil. Yield: 47%, R_f = 0.65 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.77 (s, 1H, H-8), 5.35 (s, 1H, NH), 4.56 (m, 2H, H-6), 2.70 (t, *J* = 7.6 Hz, 2H, H-10), 1.64 (p, *J* = 7.5 Hz, 2H, H-11), 1.44 (s, 9H, H-1, H-2, and H-3), 1.35 (sext, *J* = 7.3 Hz, 2H, H-12), 0.91 (t, *J* = 7.4 Hz, 3H, H-13). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.95, 157.61, 155.63, 112.81, 80.04, 42.42, 31.33, 31.21, 28.35, 22.36, 13.87. HRMS for $C_{13}H_{22}O_2N_2SNa$ $[M+Na]^+$ calcd. 293.1294, found 293.1303.

***tert*-Butyl ((4-isopentylthiazol-2-yl)methyl)carbamate (2.5b)**



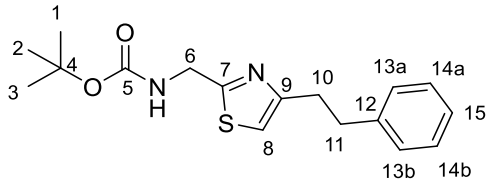
Compound **2.5b** was prepared from 5-methyl-2-hexanone (7.09 mmol, 1.0 eq) using general protocol 2. The crude product was purified by flash column chromatography (eluted with DCM-EtOAc, 100:00 to 80:20) to afford the product as a yellow oil. Yield: 42%, R_f = 0.46 (20% EtOAc in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (s, 1H, H-8), 5.39 (s, 1H, NH), 4.55 (m, 2H, H-6), 2.70 (m, 2H, H-10), 1.62–1.52 (m, 3H, H-11 and H-13), 1.43 (s, 9H, H-1, H-2, and H-3), 0.91 (d, *J* = 6.2 Hz, 6H, H-12 and H-14).^{13}C NMR (125 MHz, $CDCl_3$) δ 168.01, 157.78, 155.63, 112.66, 80.02, 42.40, 38.24, 29.47, 28.34, 27.76, 22.45. HRMS for $C_{14}H_{24}O_2N_2SNa$ $[M+Na]^+$ calcd. 307.1451, found 307.1461.

***tert*-Butyl ((4-hexylthiazol-2-yl)methyl)carbamate (2.5d)**



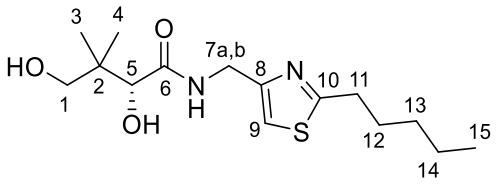
Compound **2.5d** was prepared from 2-octanone (6.39 mmol, 1.0 eq) using general protocol 2. The crude product was purified by flash column chromatography (eluted with Hex-EtOAc, 100:00 to 90:10) to afford the product as a yellow oil. Yield: 38%, R_f = 0.66 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (s, 1H, H-8), 5.38 (s, 1H, NH), 4.55 (m, 2H, H-6), 2.69 (t, *J* = 7.6 Hz, 2H, H-10), 1.65 (p, *J* = 7.6 Hz, 2H, H-11), 1.44 (s, 9H, H-1, H-2, and H-3), 1.35–1.24 (m, 6H, H-12, H-13 and H-14), 0.86 (t, *J* = 7.1 Hz, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.96, 157.64, 155.63, 112.79, 80.02, 42.41, 31.62, 31.52, 29.18, 28.96, 28.34, 22.57, 14.07. HRMS for $C_{15}H_{26}O_2N_2SNa$ $[M+Na]^+$ calcd. 321.1607, found 321.1601.

***tert*-Butyl ((4-phenethylthiazol-2-yl)methyl)carbamate (2.5e)**



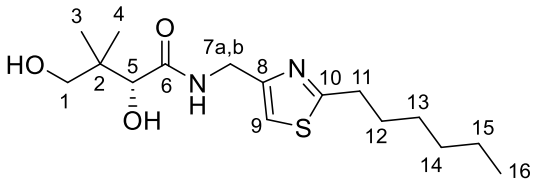
Compound **2.5e** was prepared from 4-phenyl-2-butanone (1.5 mmol, 1.0 eq) using general protocol 2. The crude product was purified by flash column chromatography (eluted with DCM-EtOAc, 100:00 to 15:85) to afford the product as a yellow oil. Yield: 17%, R_f = 0.50 (20% EtOAc in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31–7.24 (m, 2H, H-13a,b), 7.22–7.15 (m, 3H, H-14a,b and H-15), 6.75 (s, 1H, H-8), 5.32 (s, 1H, NH), 4.60 (m, 2H, H-6), 3.06–2.98 (m, 4H, H-10 and H-11), 1.47 (s, 9H, H-1, H-2, and H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.17, 156.43, 155.63, 141.39, 128.43, 128.38, 126.02, 113.60, 80.14, 42.46, 35.52, 33.34, 28.38. HRMS for $C_{17}H_{22}O_2N_2SNa$ $[M+Na]^+$ calcd. 341.1294, found 341.1282.

(*R*)-2,4-Dihyroxyl-3,3-dimethyl-*N*-((2-pentyllthiazol-4-yl)methyl)butanamide (3a)



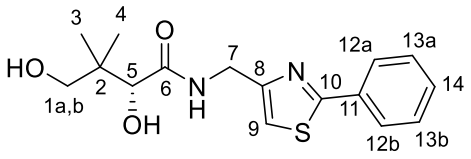
Compound **3a** was prepared from compound **3.7a** (0.45 mmol, 1.0 eq) using the general protocol 8 to afford the product as a yellow oil. Yield: 38%, R_f = 0.20 (100% EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (m, 1H, NH), 7.01 (s, 1H, H-9), 4.58–4.39 (m, 2H, H-7a,b), 4.05 (s, 1H, H-5), 3.46 (s, 2H, H-1), 2.92 (t, *J* = 7.7 Hz, 2H, H-11), 1.73 (m, 2H, H-12), 1.36–1.31 (m, 4H, H-13 and H-14), 1.00 (s, 3H, H-3 or H-4), 0.90–0.87 (m, 6H, H-3 or H-4, and H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.41, 172.74, 152.01, 114.65, 77.56, 70.86, 39.50, 38.84, 33.32, 31.20, 29.80, 22.32, 21.71, 20.39, 13.92. HRMS for $C_{15}H_{26}O_3N_2SNa$ $[M+Na]^+$ calcd. 337.1556, found 337.1554.

(*R*)-*N*-((2-Hexylthiazol-4-yl)methyl)-2,4-dihydroxyl-3,3-dimethylbutanamide (3b)



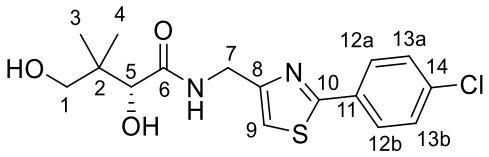
Compound **3b** was prepared from compound **3.7b** (0.50 mmol, 1.0 eq) using the general protocol 8 to afford the product as a yellow oil. Yield: 55%, R_f = 0.20 (100% EtOAc). Purity is 91%, R_t = 22.52 minutes with method A and R_t = 10.68 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (t, *J* = 6.0 Hz, 1H, NH), 7.01 (m, 1H, H-9), 4.61 (bs, 1H, OH), 4.53 (dd, *J* = 15.1, 5.9 Hz, 1H, H-7a,b), 4.47 (dd, *J* = 15.7, 5.8 Hz, 1H, H-7a,b), 4.06 (s, 1H, H-5), 3.95 (bs, 1H, OH), 3.47 (s, 2H, H-1), 2.93 (t, *J* = 7.8 Hz, 2H, H-11), 1.72 (p, *J* = 7.9 Hz, 2H, H-12), 1.36 (m, 2H, H-13), 1.31–1.27 (m, 4H, H-14 and H-15), 1.01 (s, 3H, H-3 or H-4), 0.91 (s, 3H, H-3 or H-4), 0.87 (t, *J* = 7.2 Hz, 3H, H-16). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.31, 172.74, 152.01, 114.61, 77.64, 70.90, 39.53, 38.85, 33.37, 31.44, 30.09, 28.73, 22.48, 21.78, 20.38, 14.03. HRMS for $C_{16}H_{28}O_3N_2SNa$ $[M+Na]^+$ calcd. 351.1713, found 351.1724.

(*R*)-2,4-Dihyroxyl-3,3-dimethyl-*N*-((2-phenylthiazol-4-yl)methyl)butanamide (3c)



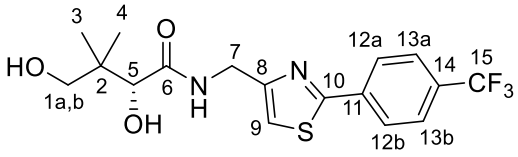
Compound **3c** was prepared from compound **3.7c** (0.06 mmol, 1.0 eq) using the general protocol 8 to afford the product as a clear oil. Yield: 38%, R_f = 0.32 (100% EtOAc). Purity is 76%, R_t = 18.43 minutes with method A and R_t = 9.21 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (m, 2H, H-12a,b), 7.49 (m, 1H, NH), 7.44–7.40 (m, 3H, H-13a,b and H-14), 7.15 (s, 1H, H-9), 4.61 (d, *J* = 5.9 Hz, 2H, H-7), 4.08 (s, 1H, H-5), 3.52 (d, *J* = 11.2 Hz, 1H, H-1a or H-1b), 3.48 (d, *J* = 11.2 Hz, 1H, H-1a or H-1b), 1.03 (s, 3H, H-3 or H-4), 0.93 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.12, 168.93, 153.67, 133.30, 130.27, 129.03, 126.53, 115.39, 77.79, 71.13, 39.51, 39.18, 21.56, 20.33. HRMS for $C_{16}H_{20}O_3N_2SNa$ $[M+Na]^+$ calcd. 343.1087, found 343.1089.

(*R*)-*N*-((2-(4-Chlorophenyl)thiazol-4-yl)methyl)-2,4-dihydroxyl-3,3-dimethylbutanamide (3d)



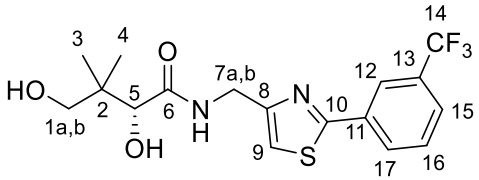
Compound **3d** was prepared from compound **3.7d** (0.28 mmol, 1.0 eq) using the general protocol 8 to afford the product as a yellow oil. Yield: 57%, R_f = 0.26 (100% EtOAc). Purity is 86%, R_t = 20.84 minutes with method A and R_t = 10.17 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.6 Hz, 2H, H-12a,b), 7.55 (t, *J* = 5.9 Hz, 1H, NH), 7.37 (d, *J* = 8.5 Hz, 2H, H-13a,b), 7.14 (s, 1H, H-9), 4.58–4.56 (m, 2H, H-7), 4.07 (s, 1H, H-5), 3.48 (s, 2H, H-1), 0.99 (s, 3H, H-3 or H-4), 0.92 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.45, 167.48, 153.92, 136.22, 131.75, 129.23, 127.68, 115.61, 77.60, 71.05, 39.45, 39.13, 21.33, 20.43. HRMS for $C_{16}H_{19}O_3N_2SClNa$ $[M+Na]^+$ calcd. 377.0697, found 377.0697.

(*R*)-2,4-Dihyroxyl-3,3-dimethyl-*N*-((2-(4-trifluoromethyl)phenyl)thiazol-4-yl)methyl)butanamide (3e)



Compound **3e** was prepared from compound **3.7e** (0.30 mmol, 1.0 eq) using the general protocol 8 to afford the product as a clear oil. Yield: 54%, R_f = 0.33 (100% EtOAc). Purity is 96%, R_t = 22.40 minutes with method A and R_t = 10.51 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.2 Hz, 2H, H-12a,b), 7.67 (d, *J* = 8.2 Hz, 2H, H-13a,b), 7.49 (m, 1H, NH), 7.23 (s, 1H, H-9), 4.63 (m, 2H, H-7), 4.10 (s, 2H, H-5), 4.04 (bs, 1H, OH), 3.53 (d, *J* = 11.2 Hz, 1H, H-1a or H-1b), 3.50 (d, *J* = 11.2 Hz, 1H, H-1a or H-1b), 3.39 (bs, 1H, OH), 1.03 (s, 3H, H-3 or H-4), 0.94 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.36, 166.83, 154.36, 136.37, 131.79 (q, *J* = 32.5 Hz), 126.70, 126.01 (q, *J* = 3.7 Hz), 123.83 (q, *J* = 270 Hz), 116.39, 77.70, 71.14, 39.46, 39.15, 21.30, 20.41. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.83. HRMS for $C_{17}H_{19}F_3O_3N_2SNa$ $[M+Na]^+$ calcd. 411.0961, found 411.0965.

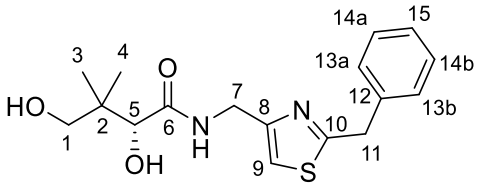
(*R*)-2,4-Dihyroxyl-3,3-dimethyl-*N*-((2-(3-trifluoromethyl)phenyl)thiazol-4-yl)methyl)butanamide (3f)



Compound **3f** was prepared from compound **3.7f** (0.34 mmol, 1.0 eq) using the general protocol 8 to afford the product as a clear oil. Yield: 79%, R_f = 0.22 (100% EtOAc). Purity is 97%, R_t = 21.95 minutes with method A and R_t = 10.41 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (s, 1H, H-12), 8.04 (d, *J* = 7.9 Hz, 1H, H-17), 7.66 (d, *J* = 7.9 Hz, 1H, H-15), 7.54 (m, 1H, H-16), 7.51 (m, 1H, NH), 7.21 (s, 1H, H-9), 4.64 (dd, *J* = 15.4, 5.8 Hz, 1H, H-7a or H-7b), 4.58 (dd, *J* = 15.5, 5.7 Hz, 1H, H-7a or H-7b), 4.09 (s, 1H, H-5), 3.51 (d, *J* = 11.2 Hz, 1H, H-1a or H-1b), 3.50 (d, *J* = 11.2 Hz, 1H, H-1a

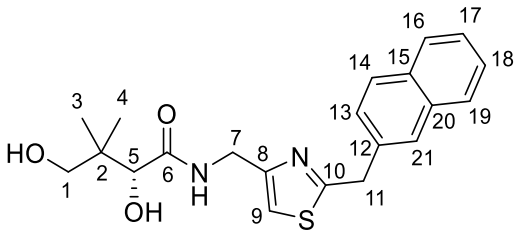
or H-1b), 1.01 (s, 3H, H-3 or H-4), 0.93 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.34, 166.84, 154.25, 134.04, 131.55 (q, *J* = 32.5 Hz), 129.63, 129.58, 126.62 (q, *J* = 3.8 Hz), 123.76 (q, *J* = 271.3 Hz), 123.23 (q, *J* = 3.8 Hz), 116.09, 77.68, 71.14, 39.46, 39.14, 21.27, 20.38. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.81. HRMS for $C_{17}H_{19}F_3O_3N_2SNa$ $[M+Na]^+$ calcd. 411.0961, found 411.0951.

(*R*)-*N*-((2-Benzylthiazol-4-yl)methyl)-2,4-dihydroxyl-3,3-dimethylbutanamide (3g)



Compound **3g** was prepared from compound **3.7g** (0.32 mmol, 1.0 eq) using the general protocol 8 to afford the product as a yellow oil. Yield: 60%, R_f = 0.14 (100% EtOAc). Purity is 79%, R_t = 18.26 minutes with method A and R_t = 9.08 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (m, 1H, NH), 7.33–7.24 (m, 5H, H-13a,b, H-14a,b, and H-15), 7.02 (s, 1H, H-9), 4.52 (m, 2H, H-7), 4.25 (s, 2H, H-11), 4.04 (s, 1H, H-5), 3.45 (s, 2H, H-1), 1.00 (s, 3H, H-3 or H-4), 0.90 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.27, 171.42, 152.46, 137.45, 128.98, 128.86, 127.30, 115.62, 77.64, 70.91, 39.50, 39.50, 38.90, 21.72, 20.42. HRMS for $C_{17}H_{22}O_3N_2SNa$ $[M+Na]^+$ calcd. 357.1243, found 357.1240.

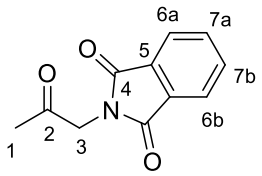
(*R*)-2,4-Dihyroxyl-3,3-dimethyl-*N*-((2-(naphthalene-2-ylmethyl)thiazol-4-yl)methyl)butanamide (3h)



Compound **3h** was prepared from compound **3.7h** (0.19 mmol, 1.0 eq) using the general protocol 8 to afford the product as a white oil. Yield: 52%, R_f = 0.13 (100% EtOAc). Purity is 86%, R_t = 20.58 minutes with method A and R_t = 10.01 minutes with method B (Table

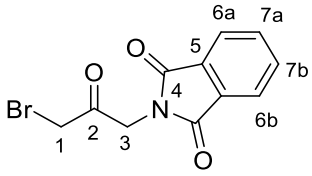
5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (m, 1H, naphthalene), 7.86 (m, 1H, naphthalene), 7.81 (m, 1H, naphthalene), 7.51 (m, 1H, NH), 7.49–7.44 (m, 2H, naphthalene), 7.44–7.39 (m, 2H, naphthalene), 6.96 (s, 1H, H-9), 4.70 (d, *J* = 2.0 Hz, 2H, H-11), 4.53 (m, 2H, H-7), 4.05 (s, 1H, H-5), 3.45 (s, 2H, H-1), 1.00 (s, 3H, H-3 or H-4), 0.90 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.28, 171.87, 152.18, 134.03, 133.51, 131.67, 128.86, 128.45, 127.70, 126.52, 125.96, 125.59, 123.81, 115.69, 77.60, 70.91, 39.48, 38.94, 37.23, 21.67, 20.41. HRMS for $C_{21}H_{24}O_3N_2SNa$ $[M+Na]^+$ calcd. 407.1400, found 407.1390.

Phthalimidoacetone (3.1)



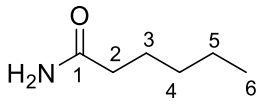
The known compound **3.1** was synthesized according to the procedure reported by Volchkov *et al.*[122] Yield: 64%. The characterization agreed with previously reported procedure.[125] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (m, 2H, H-6a,b), 7.74 (m, 2H, H-H-7a,b), 4.50 (s, 2H, H-3), 2.27 (s, 3H, H-1).

2-(3-Bromo-2-oxopropyl)isoindoline-1,3-dione (3.2)



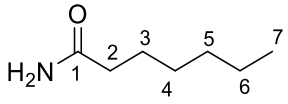
The known compound **3.2** was synthesized according to the procedure reported by Volchkov *et al.*[122] Yield: 72%. The characterization agreed with previously reported procedure.[126] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (m, 2H, H-6a,b or H-7a,b), 7.76 (m, 2H, H-6a,b or H-7a,b), 4.78 (s, 2H, H-3), 4.01 (s, 2H, H-1).

Hexanamide (3.4a)



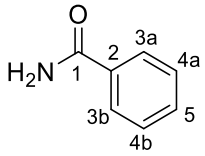
The known compound **3.4a** was prepared from compound **3.3a** (14.3 mmol, 1.0 eq) and ammonium hydroxide solution (aqueous 28% w/w, 143.79 mmol, 10.0 eq) using the general protocol 4 to give a white solid. Yield: 16%. The characterization agreed with previously reported procedure.[127] ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (bs, 2H, NH_2), 2.22 (t, *J* = 7.5 Hz, 2H, H-2), 1.68–1.62 (m, 2H, H-3), 1.35–1.30 (m, 4H, H-4 and H-5), 0.90 (t, *J* = 7.0 Hz, 3H, H-6).

Heptanamide (3.4b)



The known compound **3.4b** was prepared from compound **3.3b** (12.92 mmol, 1.0 eq) and ammonium hydroxide solution (aqueous 28% w/w, 128.39 mmol, 10.0 eq) using the general protocol 4 to give a white solid. Yield: 30%. The characterization agreed with previously reported procedure.[128] ^{1}H NMR (500 MHz, DMSO) δ 7.20 (s, 1H, NH_2), 6.66 (s, 1H, NH_2), 2.01 (t, *J* = 7.5 Hz, 2H, H-2), 1.46 (p, *J* = 7.0 Hz, 2H, H-3), 1.29–1.20 (m, 6H, H-4, H-5, and H-6), 0.86 (t, *J* = 6.8 Hz, 3H, H-7).

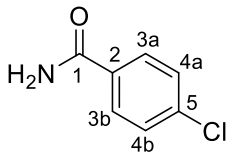
Benzamide (3.4c)



The known compound **3.4c** was prepared from compound **3.3c** (17.23 mmol, 1.0 eq) and ammonium hydroxide solution (aqueous 28% w/w, 172.04 mmol, 10.0 eq) using the general protocol 4 to give a white solid. Yield: 51%. The characterization agreed with previously reported procedure.[128] ^{1}H NMR (500 MHz, DMSO) δ 7.96 (s, 1H, NH_2), 7.87

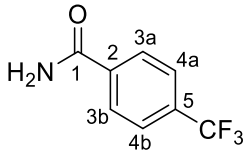
(m, 2H, H-3a,b), 7.51 (t, *J* = 7.2 Hz, 1H, H-5), 7.44 (t, *J* = 7.2 Hz, 2H, H-4a,b), 7.36 (bs, 1H, NH_2).

4-Chlorobenzamide (3.4d)



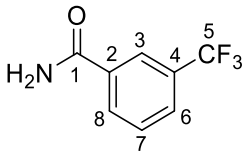
The known compound **3.4d** was prepared from compound **3.3d** (7.80 mmol, 1.0 eq) and ammonium hydroxide solution (aqueous 28% w/w, 78.06 mmol, 10.0 eq) using the general protocol 4 to give a white solid. Yield: 50%. The characterization agreed with previously reported procedure.[128] ^{1}H NMR (500 MHz, DMSO) δ 8.03 (bs, 1H, NH_2), 7.88 (m, 2H, H-3a,b), 7.52 (m, 2H, H-4a,b), 7.45 (bs, 1H, NH_2).

4-(Trifluoromethyl)benzamide (3.4e)



The known compound **3.4e** was prepared from compound **3.3e** (6.73 mmol, 1.0 eq) and ammonium hydroxide solution (aqueous 28% w/w, 66.76 mmol, 10.0 eq) using the general protocol 4 to give a white solid. Yield: 83%. The characterization agreed with previously reported procedure.[128] ^{1}H NMR (500 MHz, DMSO) δ 8.19 (bs, 1H, NH_2), 8.06 (d, *J* = 8.1 Hz, 2H, H-3a,b), 7.83 (d, *J* = 8.2 Hz, 2H, H-4a,b), 7.62 (bs, 1H, NH_2).

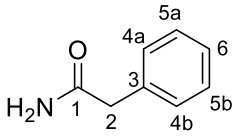
3-(Trifluoromethyl)benzamide (3.4f)



The known compound **3.4f** was prepared from compound **3.3f** (6.63 mmol, 1.0 eq) and ammonium hydroxide solution (aqueous 28% w/w, 66.25 mmol, 10.0 eq) using the general protocol 4 to give a white solid. Yield: quant. The characterization agreed with

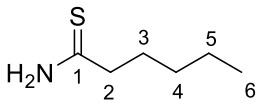
previously reported procedure.[128] ^{1}H NMR (500 MHz, DMSO) δ 8.23 (bs, 1H, NH_2), 8.21 (s, 1H, H-3), 8.17 (d, *J* =7.9 Hz, 1H, H-6 or H-8), 7.90 (d, *J* = 7.8 Hz, 1H, H-6 or H-8), 7.71 (t, *J* = 7.8 Hz, 1H, H-7), 7.62 (bs, 1H, NH_2).

2-Phenylacetamide (3.4g)



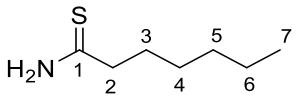
The known compound **3.4g** was prepared from compound **3.3g** (7.56 mmol, 1.0 eq) and ammonium hydroxide solution (aqueous 28% w/w, 74.47 mmol, 10.0 eq) using the general protocol 4 to give a white solid. Yield: 41% The characterization agreed with previously reported procedure.[129] ^{1}H NMR (500 MHz, DMSO) δ 7.45 (bs, 1H, NH_2), 7.31–7.19 (m, 5H, H-4a,b, H-5a,b, and H-6), 6.86 (bs, 1H, NH_2), 3.36 (s, 2H, H-2).

Hexanethioamide (3.5a)



The known compound **3.5a** was prepared from compound **3.4a** (1.99 mmol, 1.0 eq) using the general protocol 5 to afford the product as a white solid. Yield: 66%, R_f = 0.29 (100% DCM). The characterization agreed with previously reported procedure.[130] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (s, 1H, NH_2), 6.93 (bs, 1H, NH_2), 2.64 (t, *J* = 7.6, 2H, H-2), 1.75 (p, *J* =7.5 Hz, 2H, H-3), 1.39–1.27 (m, 4H, H-4 and H-5), 0.89 (t, *J* = 7.0 Hz, 3H, H-6).

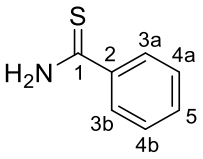
Heptanethioamide (3.5b)



The known compound **3.5b** was prepared from compound **3.4b** (3.1 mmol, 1.0 eq) using the general protocol 5 to afford the product as a white solid. Yield: 59%, R_f = 0.29 (100% DCM). The characterization agreed with previously reported procedure.[131] ^{1}H NMR (500

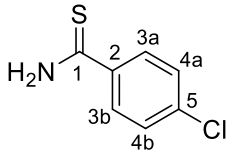
MHz, $CDCl_3$) δ 7.44 (bs, 1H, NH_2), 6.79 (bs, 1H, NH_2), 2.66 (t, *J* = 7.7, 2H, H-2), 1.77 (p, *J* =7.8 Hz, 2H, H-3), 1.39–1.27 (m, 6H, H-4, H-5, and H-6), 0.89 (t, *J* = 6.6 Hz, 3H, H-7).

Benzothioamide (3.5c)



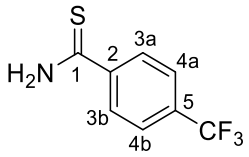
The known compound **3.5c** was prepared from compound **3.4c** (1.65 mmol, 1.0 eq) using the general protocol 5 to afford the product as a yellow solid. Yield: 59%, R_f = 0.26 (100% DCM). The characterization agreed with previously reported procedure.[132] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89–7.86 (m, 2H, H-3a,b), 7.71 (bs, 1H, NH_2), 7.51 (tt, *J* = 7.5, 1.2 Hz, 1H, H-5), 7.43–7.39 (m, 2H, H-4a,b), 7.21 (bs, 1H, NH_2).

4-Chlorobenzothioamide (3.5d)



The known compound **3.5d** was prepared from compound **3.4d** (2.56 mmol, 1.0 eq) using the general protocol 5 to afford the product as a yellow solid. Yield: 79%, R_f = 0.26 (100% DCM). The characterization agreed with previously reported procedure.[133] ^{1}H NMR (500 MHz, DMSO) δ 9.95 (bs, 1H, NH_2), 9.56 (bs, 1H, NH_2), 7.92–7.88 (m, 2H, H-3a,b), 7.51–7.47 (m, 2H, H-4a,b).

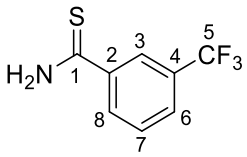
4-(Trifluoromethyl)benzothioamide (3.5e)



The known compound **3.5e** was prepared from compound **3.4e** (2.64 mmol, 1.0 eq) using the general protocol 5 to afford the product as a yellow solid. Yield: 83%, R_f = 0.1 (50% DCM in Hex). The characterization agreed with previously reported procedure.[133] ^{1}H NMR

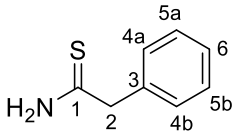
(500 MHz, DMSO) δ 10.12 (bs, 1H, NH_2), 9.72 (bs, 1H, NH_2), 8.01 (d, *J* = 8.2 Hz, 2H, H-3a,b), 7.79 (d, *J* = 8.3 Hz, 2H, H-4a,b).

3-(Trifluoromethyl)benzothioamide (3.5f)



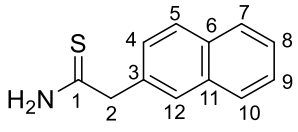
The known compound **3.5f** was prepared from compound **3.4f** (2.11 mmol, 1.0 eq) using the general protocol 5 to afford the product as a yellow solid. Yield: 86%, R_f = 0.79 (50% EtOAc in Hex). The characterization agreed with previously reported procedure.[134] ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1H, H-3), 8.04 (d, *J* =8.0 Hz, H-6 or H-8), 7.85 (bs, 1H, NH_2), 7.76 (d, *J* = 7.8 Hz, 1H, H-6 or H-8), 7.55 (t, *J* = 7.8 Hz, 1H, H-7), 7.26 (bs, 1H, NH_2).

2-Phenylethanethioamide (3.5g)



The known compound **3.5g** was prepared from compound **3.4g** (2.22 mmol, 1.0 eq) using the general protocol 5 to afford the product as a white solid. Yield: 48%, R_f = 0.49 (50% EtOAc in Hex). The characterization agreed with previously reported procedure.[135] ^{1}H NMR (500 MHz, MeOD) δ 7.38–7.23 (m, 5H, H-4a,b, H-5a,b, and H-6), 3.93 (s, 2H, H-2).

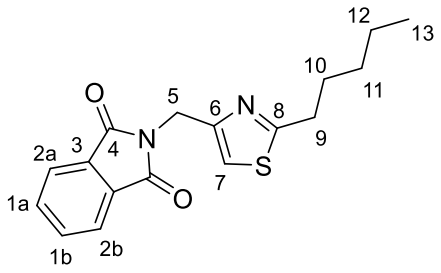
2-(Naphthalen-2-yl)ethanethioamide (3.5h)



The known compound **3.5h** was prepared from compound **3.4h** (2.16 mmol, 1.0 eq) using the general protocol 5 to afford the product as a white solid. Yield: 38%, R_f = 0.29 (100% DCM). The characterization agreed with previously reported procedure.[136] ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.3 Hz, 1H, naphthalene), 7.91–7.85 (m, 2H, naphthalene),

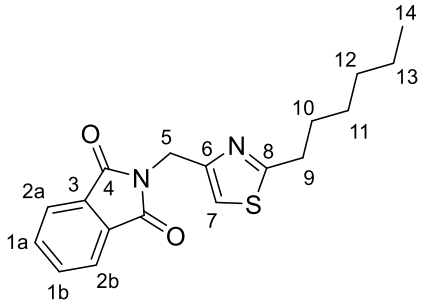
7.60–7.52 (m, 2H, naphthalene), 7.50–7.46 (m, 1H, naphthalene), 7.41 (d, *J* = 6.6 Hz, 1H, naphthalene), 6.57 (bs, 1H, NH_2), 4.56 (s, 2H, H-2).

2-((2-Pentylthiazol-4-yl)methyl)isoindoline-1,3-dione (3.6a)



Compound **3.6a** was prepared from compound **3.5a** (1.31 mmol, 1.0 eq) using the general protocol 6 to afford the product as a white solid. Yield: 59%, R_f = 0.66 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (m, 2H, H-1a,b or H-2a,b), 7.71 (m, 2H, H-1a,b or H-2a,b), 6.97 (m, 1H,H-7), 4.95 (d, *J* = 0.9 Hz, 2H, H-5), 2.92 (t, *J* = 7.7 Hz, 2H, H-9), 1.72 (p, *J* = 7.8 Hz, 2H, H-10), 1.34– 1.29 (m, 4H, H-11 and H-12), 0.86 (t, *J* = 7.1 Hz, 3H, H-13). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.08, 167.80, 150.47, 134.04, 132.14, 123.44, 114.76, 37.81, 33.45, 31.27, 29.71, 22.32, 13.93. HRMS for $C_{17}H_{18}O_2N_2SNa$ $[M+Na]^+$ calcd. 337.0981, found 337.0977.

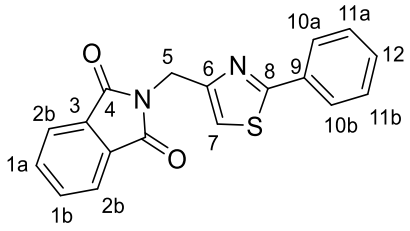
2-((2-Hexylthiazol-4-yl)methyl)isoindoline-1,3-dione (3.6b)



Compound **3.6b** was prepared from compound **3.5b** (1.77 mmol, 1.0 eq) using the general protocol 6 to afford the product as a white solid. Yield: 37%, R_f = 0.21 (80% DCM in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (m, 2H, H-1a,b or H-2a,b), 7.72 (m, 2H, H-1a,b or H-2a,b), 6.98 (m, 1H, H-7), 4.96 (m, 2H, H-5), 2.92 (t, *J* = 7.8 Hz, 2H, H-9), 1.72 (p, *J* = 7.8 Hz, 2H, H-10), 1.35 (m, 2H, H-11), 1.28–1.25 (m, 4H, H-12 and H-13), 0.85 (t, *J* =

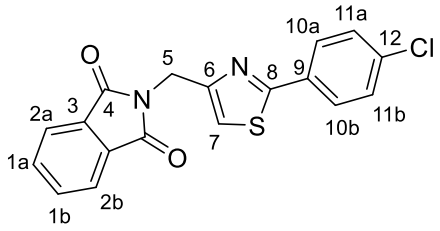
7.1 Hz, 3H, H-14). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.10, 167.81, 150.46, 134.04, 132.15, 123.45, 114.77, 37.82, 33.50, 31.45, 29.99, 28.77, 22.48, 14.02. HRMS for $C_{18}H_{20}O_2N_2SNa$ $[M+Na]^+$ calcd. 351.1138, found 351.1140.

2-((2-Phenylthiazol-4-yl)methyl)isoindoline-1,3-dione (3.6c)



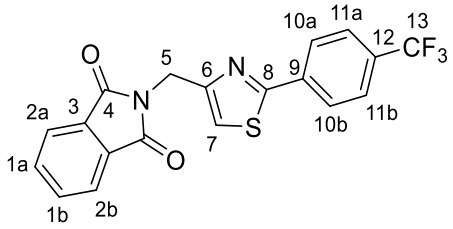
Compound **3.6c** was prepared from compound **3.5c** (0.93 mmol, 1.0 eq) using the general protocol 6 to afford the product as a white solid. Yield: 31%, R_f = 0.47 (20% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91–7.88 (m, 4H, H-1a,b, H-2a,b, H-10a,b, or H-11a,b), 7.73 (m, 2H, H-1a,b or H-2a,b), 7.40–7.38 (m, 3H, H-12 and H-10a,b or H-11a,b), 7.16 (m, 1H, H-7), 5.06 (d, J = 0.9 Hz, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.46, 167.84, 152.09, 134.08, 133.44, 132.17, 130.07, 128.86, 126.59, 123.49, 115.76, 37.89. HRMS for $C_{18}H_{12}O_2N_2SNa$ $[M+Na]^+$ calcd. 343.0512, found 343.0521.

2-((2-(4-Chlorophenyl)thiazol-4-yl)methyl)isoindoline-1,3-dione (3.6d)



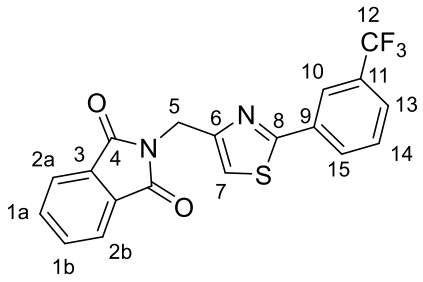
Compound **3.6d** was prepared from compound **3.5d** (2.01 mmol, 1.0 eq) using the general protocol 6 to afford the product as a pale yellow solid. Yield: 42%, R_f = 0.22 (20% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (m, 2H, H-1a,b or H-2a,b), 7.84 (d, J = 8.5 Hz, 2H, H-10a,b), 7.73 (m, 2H, H-1a,b or H-2a,b), 7.36 (d, J = 8.6 Hz, 2H, H-11a,b), 7.18 (s, 1H, H-7), 5.04 (s, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.82, 167.07, 152.29, 136.01, 134.12, 132.13, 131.93, 129.09, 127.78, 123.51, 116.14, 37.78. HRMS for $C_{18}H_{11}O_2N_2SClNa$ $[M+Na]^+$ calcd. 377.0122, found 377.0129.

2-((2-(4-(Trifluoromethyl)phenyl)thiazol-4-yl)methyl)isoindoline-1,3-dione (3.6e)



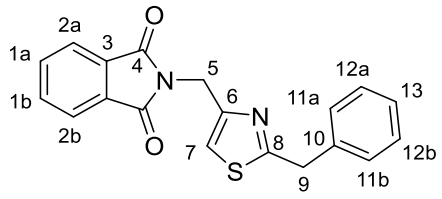
Compound **3.6e** was prepared from compound **3.5e** (1.75 mmol, 1.0 eq) using the general protocol 6 to afford the product as a pale yellow solid. Yield: 89%.^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.1 Hz, 2H, H-10a,b), 7.90 (m, 2H, H-1a,b or H-2a,b), 7.74 (m, 2H, H-1a,b or H-2a,b), 7.65 (d, *J* = 8.2 Hz, 2H, H-11a,b), 7.26 (s, 1H, H-7), 5.07 (s, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.81, 166.49, 152.68, 136.47, 134.15, 132.11, 131.63 (q, *J* = 32.5 Hz), 126.78, 125.88 (q, *J* = 3.8 Hz), 123.88 (q, *J* = 271.3 Hz), 123.53, 117.01, 37.74. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.81. HRMS for $C_{19}H_{11}F_3O_2N_2SNa$ $[M+Na]^+$ calcd. 411.0386, found 411.0384.

2-((2-(3-(Trifluoromethyl)phenyl)thiazol-4-yl)methyl)isoindoline-1,3-dione (3.6f)



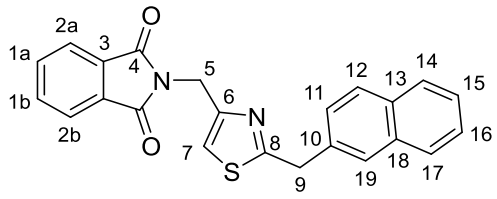
Compound **3.6f** was prepared from compound **3.5f** (1.71 mmol, 1.0 eq) using the general protocol 6 to afford the product as a white solid. Yield: 61%, R_f = 0.30 (100% DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (s, 1H, H-10), 8.07 (d, *J* = 7.8 Hz, 1H, H-15), 7.89 (m, 2H ,H-1a,b or H-2a,b), 7.74 (m, 2H, H-1a,b or H-2a,b), 7.63 (d, *J* = 7.9 Hz, 1H, H-13), 7.52 (t, *J* = 7.9 Hz, 1H, H-14), 7.24 (m, 1H, H-7), 5.07 (d, *J* = 0.9 Hz, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.81, 166.54, 152.59, 134.15, 134.12, 132.12, 131.42 (q, *J* = 32.5 Hz), 129.72, 129.44, 126.51 (q, *J* = 3.8 Hz), 123.53, 123.30 (q, *J* = 3.8 Hz), 123.78 (q, *J* = 270 Hz), 116.68, 37.78. ^{19}F NMR (470 MHz, $CDCl_3$): δ -62.79. HRMS for $C_{19}H_{11}F_3O_2N_2SNa$ $[M+Na]^+$ calcd. 411.0386, found 411.0367.

2-((2-Benzylthiazol-4-yl)methyl)isoindoline-1,3-dione (3.6g)



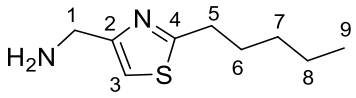
Compound **3.6g** was prepared from compound **3.5g** (1.06 mmol, 1.0 eq) using the general protocol 6 to afford the product as a white solid. Yield: 73%, R_f = 0.76 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 (m, 2H, H-1a,b or H-2a,b), 7.73 (m, 2H, H-1a,b or H-2a,b), 7.33–7.26 (m, 5H, H-11a,b, H-12a,b, and H-13), 7.02 (m, 1H, H-7), 4.99 (d, J = 0.9 Hz, 2H, H-5), 4.28 (s, 2H, H-9). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.14, 167.83, 150.79, 137.07, 134.07, 132.16, 129.13, 128.76, 127.17, 123.49, 116.02, 39.73, 37.77. HRMS for $C_{19}H_{14}O_2N_2SNa$ $[M+Na]^+$ calcd. 357.0668, found 357.0667.

2-((2-(Naphthalen-2-ylmethyl)thiazol-4-yl)methyl)isoindoline-1,3-dione (3.6h)



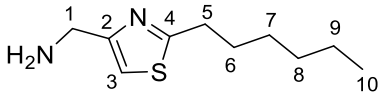
Compound **3.6h** was prepared from compound **3.5h** (0.78 mmol, 1.0 eq) using the general protocol 6 to afford the product as a white solid. Yield: 59%, R_f = 0.45 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (m, 1H, naphthalene), 7.88 (m, 2H, H-1a,b or H-2a,b), 7.84 (m, 1H, naphthalene), 7.80 (m, 1H, naphthalene), 7.73 (m, 2H, H-1a,b or H-2a,b), 7.46–7.42 (m, 4H, naphthalene), 6.96 (s, 1H, H-7), 5.00 (s, 2H, H-5), 4.73 (s, 2H, H-9). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.45, 167.83, 150.56, 134.06, 133.99, 133.82, 132.16, 131.77, 128.70, 128.32, 127.71, 126.41, 125.87, 125.55, 124.11, 123.49, 116.10, 37.78, 37.49. HRMS for $C_{23}H_{16}O_2N_2SNa$ $[M+Na]^+$ calcd. 407.0825, found 407.0813.

(2-Pentylthiazol-4-yl)methanamine (3.7a)



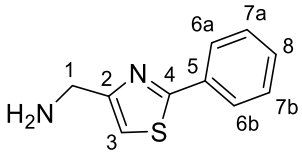
Compound **3.7a** was prepared from compound **3.6a** (0.67 mmol, 1.0 eq) using the general protocol 7 to afford the product as a yellow oil. Yield: 76%. R_f = 0.09 (10% MeOH in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (s, 1H, H-3), 3.94 (s, 2H, H-1), 2.95 (t, *J* = 7.7 Hz, 2H, H-5), 2.07 (s, 2H, NH_2), 1.76 (p, *J* = 7.6 Hz, 2H, H-6), 1.40–1.32 (m, 4H, H-7 and H-8), 0.89 (t, *J* = 7.0 Hz, 3H, H-9). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.09, 157.54, 112.36, 42.46, 33.52, 31.31, 29.85, 22.35, 13.95. HRMS for $C_9H_{17}N_2S$ $[M+H]^+$ calcd.185.1107, found 185.1104.

(2-Hexylthiazol-4-yl)methanamine (3.7b)



Compound **3.7b** was prepared from compound **3.6b** (0.59 mmol, 1.0 eq) using the general protocol 7 to afford the product as a yellow oil. Yield: 87%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (m, 1H, H-3), 3.92 (s, 2H, H-1), 2.95 (t, *J* = 7.6 Hz, 2H, H-5), 1.79–1.72 (m, 4H, H-6 and NH_2), 1.38 (m, 2H, H-7), 1.32–1.27 (m, 4H, H-8 and H-9), 0.86 (t, *J* = 7.1 Hz, 3H, H-10). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.05, 158.00, 112.12, 42.63, 33.56, 31.47, 30.13, 28.80, 22.50, 14.03. HRMS for $C_{10}H_{18}N_2SNa$ $[M+Na]^+$ calcd. 221.1083, found 221.1079.

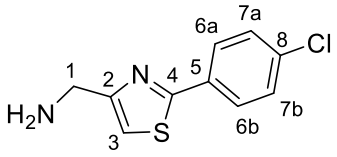
(2-Phenylthiazol-4-yl)methanamine (3.7c)



Compound **3.7c** was prepared from compound **3.6c** (0.23 mmol, 1.0 eq) using the general protocol 7 to afford the product as a yellow solid. Yield: 40%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (m, 2H, H-6a,b), 7.44–7.41 (m, 3H, H-7a,b and H-8), 7.07 (s, 1H, H-3), 4.03 (s, 2H,

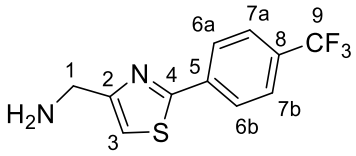
H-1), 1.73 (bs, 2H, NH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.52, 159.22, 133.73, 129.99, 128.94, 126.54, 113.11, 42.80. HRMS for $C_{10}H_{11}N_2S$ $[M+H]^+$ calcd. 191.0637, found 191.0634.

(2-(4-Chlorophenyl)thiazol-4-yl)methanamine (3.7d)



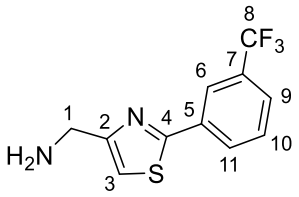
Compound **3.7d** was prepared from compound **3.6d** (0.82 mmol, 1.0 eq) using the general protocol 7 to afford the product as a yellow solid. Yield: 35%. R_f = 0.15 (10% MeOH in DCM). ^1H NMR (500 MHz, MeOD) δ 7.94 (d, *J* = 8.7 Hz, 2H, H-6a,b), 7.48 (d, *J* = 8.7 Hz, 2H, H-7a,b), 7.38 (s, 1H, H-3), 3.97 (s, 2H, H-1). ^{13}C NMR (125 MHz, MeOD) δ 167.09, 158.02, 135.72, 132.02, 128.91, 127.49, 114.52, 41.12. HRMS for $C_{10}H_{10}N_2SCl$ $[M+H]^+$ calcd. 225.0248, found 225.0239.

(2-(4-(Trifluoromethyl)phenyl)thiazol-4-yl)methanamine (3.7e)



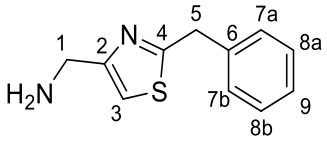
Compound **3.7e** was prepared from compound **3.6e** (1.03 mmol, 1.0 eq) using the general protocol 7 to afford the product as a yellow solid. Yield: 41%. R_f = 0.22 (10% MeOH in DCM). ^1H NMR (500 MHz, MeOD) δ 8.12 (d, *J* = 8.1 Hz, 2H, H-6a,b), 7.75 (d, *J* = 8.2 Hz, 2H, H-7a,b), 7.43 (s, 1H, H-3), 4.84 (s, 2H, H-1). ^{13}C NMR (125 MHz, MeOD) δ 166.40, 159.00, 136.77, 131.22, (q, *J* = 32.5 Hz), 126.53, 125.72 (q, *J* = 3.8 Hz), 124.04 (q, *J* = 270 Hz), 115.18, 41.24. ^{19}F NMR (470 MHz, MeOD) δ -64.33. HRMS for $C_{11}H_{10}F_3N_2S$ $[M+H]^+$ calcd. 259.0511, found 259.0507.

(2-(3-(Trifluoromethyl)phenyl)thiazol-4-yl)methanamine (3.7f)



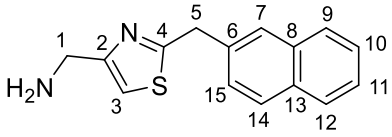
Compound **3.7f** was prepared from compound **3.6f** (0.99 mmol, 1.0 eq) using the general protocol 7 to afford the product as a white solid. Yield: 41%. R_f = 0.25 (10% MeOH in DCM). ^{1}H NMR (500 MHz, MeOD) δ 8.25 (s, 1H, H-6), 8.16 (d, *J* = 7.7 Hz, 1H, H-11), 7.74 (d, *J* = 7.7 Hz, 1H, H-9), 7.66 (t, *J* = 7.8 Hz, 1H, H-10), 7.42 (t, *J* = 0.7 Hz, 1H, H-3), 3.97 (s, 2H, H-1). ^{13}C NMR (125 MHz, MeOD) δ 166.43, 158.71, 134.28, 131.14 (q, *J* = 32.5 Hz), 129.80, 129.64, 126.18 (q, *J* = 3.8 Hz), 123.95 (q, *J* = 270 Hz), 122.37 (q, *J* = 3.8 Hz), 114.96, 41.17. ^{19}F NMR (470 MHz, MeOD) δ -64.36. HRMS for $C_{11}H_{10}F_3N_2S$ $[M+H]^+$ calcd. 259.0511, found 259.0504.

(2-Benzylthiazol-4-yl)methanamine (3.7g)



Compound **3.7g** was prepared from compound **3.6g** (0.76 mmol, 1.0 eq) using the general protocol 7 to afford the product as an orange oil. Yield: 50%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.26 (m, 5H, H-7a,b, H-8a,b, H-9), 6.93 (s, 1H, H-3), 4.30 (s, 2H, H-5), 3.95 (s, 2H, H-1), 1.87 (bs, 2H, NH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.82, 158.28, 137.85, 129.04, 128.78, 127.14, 113.33, 42.60, 39.75. HRMS for $C_{11}H_{13}N_2S$ $[M+H]^+$ calcd. 205.0794, found 205.0794.

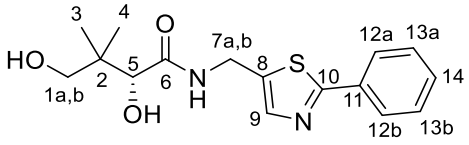
(2-(Naphthalen-2ylmethyl)thiazol-4-yl)methanamine (3.7h)



Compound **3.7h** was prepared from compound **3.6h** (0.44 mmol, 1.0 eq) using the

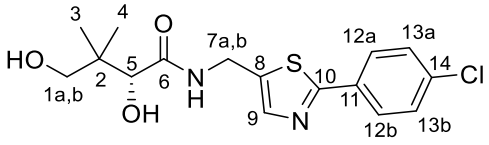
general protocol 7 to afford the product as a pale yellow solid. Yield: 45%, R_f = 0.26 (10% MeOH in DCM). ^{1}H NMR (500 MHz, MeOD) δ 8.02 (m, 1H, naphthalene), 7.88 (m, 1H, naphthalene), 7.83 (m, 1H, naphthalene), 7.50–7.44 (m, 4H, naphthalene), 7.15 (s, 1H, H-3), 4.76 (s, 2H, H-5), 3.90 (s, 2H, H-1). ^{13}C NMR (125 MHz, MeOD) δ 172.21, 155.88, 134.21, 133.69, 131.66, 128.45, 127.99, 127.42, 126.02, 125.54, 125.25, 123.50, 114.34, 40.83, 36.43. HRMS for $C_{15}H_{14}N_2SNa$ $[M+Na]^+$ calcd. 277.0770, found 277.0766.

(*R*)-2,4-Dihyroxyl-3,3-dimethyl-*N*-((2-phenylthiazol-5-yl)methyl)butanamide (4a)



Compound **4a** was prepared from compound **4.6a** (0.23 mmol, 1.0 eq) using the general protocol 8 to afford the product as a yellow oil. Yield: 48%, R_f = 0.21 (100% EtOAc). Purity is 81%, R_t = 17.78 minutes with method A and R_t = 8.85 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (m, 2H, H-12a,b), 7.67 (s, 1H, H-9), 7.44–7.41 (m, 3H, H-13a,b and H-14), 7.28 (m, 1H, NH), 4.69 (dd, *J* = 15.5, 6.2 Hz, 1H, H-7a or H-7b), 4.64 (dd, *J* =15.4, 6.3 Hz, 1H, H-7a or H-7b), 4.10 (s, 1H, H-5), 3.57 (d, *J* = 11.1 Hz, 1H, H-1a or H-1b), 3.50 (d, *J* = 11.1 Hz, 1H, H-1a or H-1b), 1.04 (s, 3H, H-3 or H-4), 0.94 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.84, 169.00, 142.03, 135.40, 133.46, 130.22, 129.01, 126.44, 77.90, 71.60, 39.43, 35.34, 21.23, 20.37. HRMS for $C_{16}H_{20}O_3N_2SNa$ $[M+Na]^+$ calcd. 343.1087, found 343.1079.

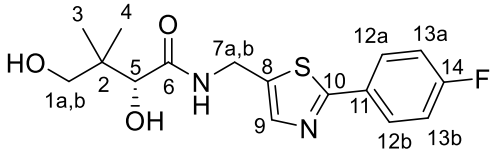
(*R*)-*N*-((2-(4-Chlorophenyl)thiazol-5-yl)methyl)-2,4-dihydroxy-3,3-dimethylbutanamide (4b)



Compound **4b** was prepared from compound **4.6b** (0.07 mmol, 1.0 eq) using the general protocol 8 to afford the product as a yellow solid. Yield: 35%, R_f = 0.13 (100% EtOAc). Purity is 80%, R_t = 19.81 minutes with method A and R_t = 9.83 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.7 Hz, 2H, H-12a,b), 7.67 (s, 1H,

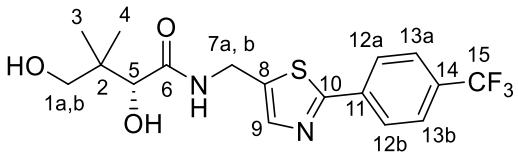
H-9), 7.40 (d, *J* = 8.6 Hz, 2H, H-13a,b), 7.30 (t, *J* = 6.1 Hz, 1H, NH), 4.69 (dd, *J* = 15.6, 6.1 Hz, 1H, H-7a or H-7b), 4.63 (dd, *J* = 15.6, 6.1 Hz, 1H, H-7a or H-7b), 4.10 (s, 1H, H-5), 3.56 (d, *J* = 11.1 Hz, 1H, H-1a or H-1b), 3.51 (d, *J* = 11.1 Hz, 1H, H-1a or H-1b), 1.03 (s, 3H, H-3 or H-4), 0.94 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.86, 167.58, 142.17, 136.16, 135.81, 131.98,129.24, 127.61, 77.91, 71.63, 39.41, 35.32, 21.13, 20.41. HRMS for $C_{16}H_{19}ClO_3N_2SNa$ $[M+Na]^+$ calcd. 377.0697, found 377.0683.

(*R*)-*N*-((2-(4-Fluorophenyl)thiazol-5-yl)methyl)-2,4-dihydroxy-3,3-dimethylbutanamide (4c)



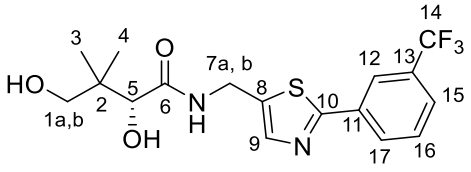
Compound **4c** was prepared from compound **4.6c** (0.21 mmol, 1.0 eq) using the general protocol 8 to afford the product as a yellow oil. Yield: 30%, R_f = 0.26 (100% EtOAc). Purity is 82%, R_t = 18.28 minutes with method A and R_t = 9.10 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (m, 2H, H-12a,b or H-13a,b), 7.63 (s, 1H, H-9), 7.33 (t, *J* = 6.2 Hz, 1H, NH), 7.10 (t, *J* = 8.6 Hz, 2H, H-12a,b or H-13a,b), 4.67 (dd, *J* = 15.5, 6.2 Hz, 1H, H-7a or H-7b), 4.61 (dd, *J* = 15.4, 6.2 Hz, 1H, H-7a or H-7b), 4.09 (s, 1H, H-5), 3.56 (d, *J* = 11.2 Hz, 1H, H-1a or H-1b), 3.50 (d, *J* = 11.1 Hz, 1H, H-1a or H-1b), 1.02 (s, 3H, H-3 or H-4), 0.93 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.00, 167.78, 163.95 (d, *J* = 248.8 Hz), 141.98, 135.47, 129.8 (d, *J* = 2.5 Hz), 128.35 (d, *J* = 8.8 Hz), 116.11 (d, *J* = 21.3 Hz), 77.85, 71.56, 39.40, 35.30, 21.16, 20.41. ^{19}F NMR (470 MHz, $CDCl_3$) δ -110.10. HRMS for $C_{16}H_{19}FO_3N_2SNa$ $[M+Na]^+$ calcd. 361.0993, found 361.1001.

(*R*)-2,4-Dihyroxyl-3,3-dimethyl-*N*-((2-(4-trifluoromethyl)phenylthiazol-5-yl)methyl)butanamide (4d)



Compound **4d** was prepared from compound **4.6d** (0.25 mmol, 1.0 eq) using the general protocol 8 to afford the product as a colorless oil. Yield: 41%, R_f = 0.26 (100% EtOAc). Purity is 94%, R_t = 21.36 minutes with method A and R_t = 10.25 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, MeOD) δ 8.08 (d, *J* = 8.3 Hz, 2H, H-12a,b), 7.79 (s, 1H, H-9), 7.76 (d, *J* = 8.3 Hz, 2H, H-13a,b), 4.66 (d, *J* = 15.3 Hz, 1H, H-7a or H-7b), 4.61 (d, *J* = 15.3 Hz, 1H, H-7a or H-7b), 3.96 (s, 1H, H-5), 3.49 (d, *J* = 10.9 Hz, 1H, H-1a or H-1b), 3.39 (d, *J* = 11.0 Hz, 1H, H-1a or H-1b), 0.94 (s, 3H, H-3 or H-4), 0.92 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, MeOD) δ 174.89, 166.45, 142.08, 138.25, 136.73, 131.27 (q, *J* = 32.5 Hz), 126.43, 125. 76 (q, *J* = 3.8 Hz), 124.02 (q, *J* = 270 Hz), 75.93, 68.93, 39.17, 34.47, 19.99, 19.43. ^{19}F NMR (470 MHz, MeOD): δ -64.33. HRMS for $C_{17}H_{19}F_3O_3N_2SNa$ $[M+Na]^+$ calcd. 411.0961, found 411.0952.

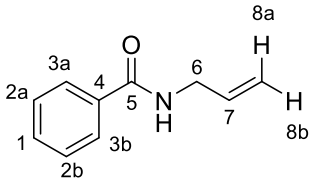
(*R*)-2,4Ddihyroxyl-3,3-dimethyl-*N*-((2-(3-trifluoromethyl)phenylthiazol-5-yl)methyl)butanamide (4e)



Compound **4e** was prepared from compound **4.6e** (0.10 mmol, 1.0 eq) using the general protocol 8 to afford the product as a colorless oil. Yield: 59%, R_f = 0.26 (100% EtOAc). Purity is 90%, R_t = 21.06 minutes with method A and R_t = 10.18 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (s, 1H, H-12), 8.02 (d, *J* = 7.8 Hz, 1H, H-17), 7.69 (s, 1H, H-9), 7.65 (d, *J* = 7.8 Hz, 1H, H-15), 7.54 (t, *J* = 7.8 Hz, 1H, H-16), 7.42 (t, *J* = 6.2 Hz, 1H, NH), 4.68 (dd, *J* = 15.5, 6.2 Hz, 1H, H-7a or H-7b), 4.63 (dd, *J* = 15.4, 6.1 Hz, 1H, H-7a or H-7b), 4.30 (bs, 1H, OH), 4.10 (s, 1H, H-5), 3.54 (d, *J* = 11.1 Hz, 1H,

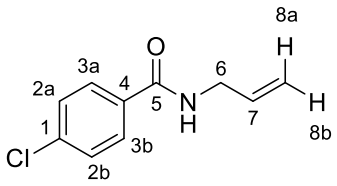
H-1a or H-1b), 3.50 (d, *J* = 11.1 Hz, 1H, H-1a or H-1b), 3.38 (bs, 1H, OH), 1.01 (s, 3H, H-3 or H-4), 0.93 (s, 3H ,H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.14, 167.02, 142.38, 136.47, 134.12, 131.56 (q, *J* = 32.5 Hz), 129.58, 129.55, 126.61 (q, *J* = 3.8 Hz), 123.75 (q, *J* = 271.3 Hz), 123.13 (q, *J* = 3.8 Hz), 77.78, 71.49, 39.38, 35.28, 21.06, 20.46. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.83. HRMS for $C_{17}H_{19}F_3O_3N_2SNa$ $[M+Na]^+$ calcd. 411.0961, found 411.0945.

***N*-Allylbenzamide (4.2a)**



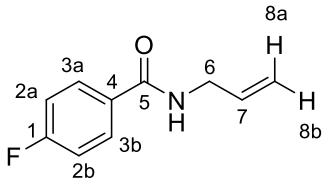
The known compound **4.2a** was prepared from compound **4.1a** (2.67 mmol, 1.0 eq) using the general protocol 9 to afford the product as a clear oil. The characterization agreed with previously reported procedure.[120] Yield: 86%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.4 Hz, 2H, H-3a,b), 7.49 (t, *J* = 7.4 Hz, 1H, H-1), 7.42 (t, *J* = 7.5 Hz, 2H, H-2a,b), 6.33 (bs, 1H, NH), 5.93 (ddt, *J* = 17.1, 10.2, 5.7 Hz, 1H, H-7), 5.25 (m, 1H, H-8a), 5.17 (m, 1H, H-8b), 4.08 (t, *J* = 5.6 Hz, 2H, H-6).

***N*-Allyl-4-chlorobenzamide (4.2b)**



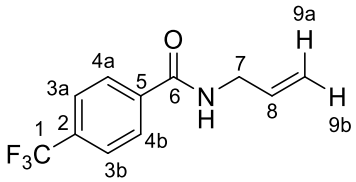
The known compound **4.2b** was prepared from compound **4.1b** (2.65 mmol, 1.0 eq) using the general protocol 9 to afford the product as a white solid. The characterization agreed with previously reported procedure.[137] Yield: 81%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (m, 2H, H-2a,b or H-3a,b), 7.41 (m, 2H, H-2a,b or H-3a,b), 6.15 (bs, 1H, NH), 5.93 (m, 1H, H-7), 5.27 (dq, *J* = 17.2, 1.6 Hz, 1H, H-8a), 5.20 (dq, *J* = 10.2, 1.4 Hz, 1H, H-8b), 4.09 (tt, *J* = 5.8, 1.5 Hz, 2H, H-6).

***N*-Allyl-4-fluorobenzamide (4.2c)**



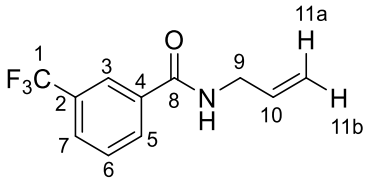
The known compound **4.2c** was prepared from compound **4.1c** (2.62 mmol, 1.0 eq) using general protocol 9 to afford the product as a white solid. The characterization agreed with previously reported procedure.[138] Yield: 88%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (m, 2H, H-3a,b), 7.10 (m, 2H, H-2a,b), 6.20 (bs, 1H, NH), 5.93 (ddt, *J* = 17.2, 10.2, 5.7 Hz, 1H, H-7), 5.26 (dq, *J* = 17.1, 1.6 Hz, 1H, H-8a), 5.19 (dq, *J* = 10.2, 1.4 Hz, 1H, H-8b), 4.08 (tt, *J* = 5.8, 1.5 Hz, 2H, H-6).

***N*-Allyl-4-(trifluoromethyl)benzamide (4.2d)**



The known compound **4.2d** was prepared from compound **4.1d** (2.69 mmol, 1.0 eq) using general protocol 9 to afford the product as a white solid. The characterization agreed with previously reported procedure.[138] Yield: 93%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.1 Hz, 2H, H-4a,b), 7.70 (d, *J* = 8.1 Hz, 2H H-3a,b), 6.25 (bs, 1H, NH), 5.94 (ddt, *J* = 17.1, 10.2, 5.8 Hz, 1H, H-8), 5.28 (dq, *J* = 17.1, 1.6 Hz, 1H, H-9a), 5.22 (dq, *J* = 10.2, 1.4 Hz, 1H, H-9b), 4.10 (tt, *J* = 5.9, 1.5 Hz, 2H, H-7).

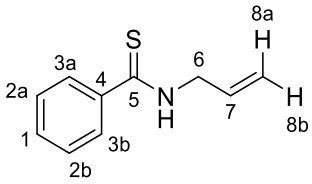
***N*-Allyl-3-(trifluoromethyl)benzamide (4.2e)**



Compound **4.2e** was prepared from compound **4.1e** (2.65 mmol, 1.0 eq) using the general protocol 9 to afford the product as a white solid. Yield: quant. ^{1}H NMR (500 MHz, $CDCl_3$)

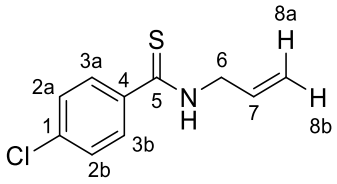
δ 8.04 (s, 1H, H-3), 7.96 (d, *J* = 7.8 Hz, 1H, H-5), 7.71 (d, *J* = 7.9 Hz, 1H, H-7), 7.52 (t, *J* = 7.8 Hz, 1H, H-6), 6.85 (bs, 1H, NH), 5.89 (ddt, *J* = 17.2, 10.2, 5.7 Hz, 1H, H-10), 5.22 (dq, *J* = 17.2, 1.6 Hz, 1H, H-11a), 5.15 (dq, *J* = 10.3, 1.5 Hz, 1H, H-11b), 4.05 (tt, *J* = 5.8, 1.6 Hz, 2H, H-9). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.15, 135.28, 133.73, 131.03 (q, *J* = 32.8 Hz), 130.32, 129.14, 128.04 (q, *J* = 3.8 Hz), 124.07 (q, *J* = 3.8 Hz), 123.71 (q, *J* = 272.2 Hz), 116.87, 42.61. ^{19}F NMR (470 MHz, $CDCl_3$): δ -62.75. HRMS for $C_{11}H_{10}F_3ONNa$ $[M+Na]^+$ calcd. 252.0607, found 252.0611.

***N*-Allylbenzothioamide (4.3a)**



The known compound **4.3a** was prepared from compound **4.2a** (1.24 mmol, 1.0 eq) using general protocol 5 to afford the product as a yellow oil. Yield: 69%, R_f = 0.89 (50% EtOAc in Hex). The characterization agreed with previously reported procedure.[139] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (m, 2H, H3a,b), 7.62 (bs, 1H, NH), 7.46 (m, 1H, H-1), 7.38 (m, 2H, H-2a,b), 6.00 (ddt, *J* = 17.1, 10.3, 6.0 Hz, 1H, H-7), 5.35 (dq, *J* = 17.2, 1.2 Hz, 1H, H-8a), 5.29 (dq, *J* = 10.2, 1.2 Hz, 1H, H-8b), 4.46 (tt, *J* = 5.9, 1.5 Hz, 2H, H-6).

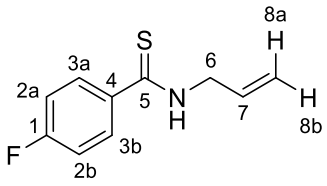
***N*-Allyl-4-chlorobenzothioamide (4.3b)**



Compound **4.3b** was prepared from compound **4.2b** (1.12 mmol, 1.0 eq) using the general protocol 5 to afford the product as a yellow oil. Yield: 56%, R_f = 0.89 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (m, 2H, H-3a,b), 7.52 (bs, 1H, NH), 7.36 (m, 2H, H-2a,b), 6.01 (m, 1H, H-7), 5.35 (dq, *J* = 17.0, 1.5 Hz, 1H, H-8a), 5.31 (dq, *J* = 10.2, 1.4 Hz, 1H, H-8b), 4.46 (tt, *J* = 5.6, 1.4 Hz, 2H, H-6). ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.86, 140.02, 137.43, 131.71, 128.72, 128.00, 119.07, 49.21. HRMS for $C_{10}H_{10}ClNSNa$

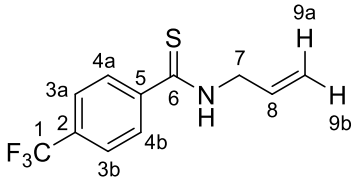
[M+Na]$^+$ calcd.234.0115, found 234.0109.

***N*-Allyl-4-fluorobenzothioamide (4.3c)**



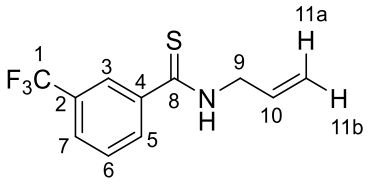
Compound **4.3c** was prepared from compound **4.2c** (1.11 mmol, 1.0 eq) using general protocol 5 to afford the product as a yellow oil. Yield: 47%, R_f = 0.85 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (m, 2H, H-3a,b), 7.62 (bs, 1H, NH), 7.04 (m, 2H, H-2a,b), 5.99 (ddt, *J* = 17.2, 10.2, 6.0 Hz, 1H, H-7), 5.34 (dq, *J* = 17.1, 1.5 Hz, 1H, H-8a), 5.28 (dq, *J* = 10.1, 1.4 Hz, 1H, H-8b), 4.43 (tt, *J* = 6.1, 1.5 Hz, 2H, H-6). ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.87, 164.55 (d, *J* = 251.3 Hz), 137.87 (d, *J* =2.5 Hz), 131.77, 128.90 (d, *J* = 7.5 Hz), 118.92, 115.47 (d, *J* = 22.5 Hz), 49.20. ^{19}F NMR (470 MHz, $CDCl_3$) δ -108.87. HRMS for $C_{10}H_{10}FNSNa$ [M+Na]$^+$ calcd. 218.0410, found 218.0404.

***N*-Allyl-4-(trifluoromethyl)benzothioamide (4.3d)**



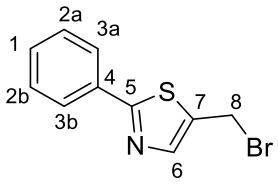
Compound **4.3d** was prepared from compound **4.2d** (2.47 mmol, 1.0 eq) using the general protocol 5 to afford the product as a yellow oil. Yield: 56%, R_f = 0.90 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.0 Hz, 2H, H-4a,b), 7.69 (bs, 1H, NH), 7.62 (d, *J* = 8.0 Hz, 2H, H-3a,b), 6.00 (ddt, *J* = 17.1, 10.2, 5.8 Hz, 1H, H-8), 5.36 (dq, *J* = 17.2, 1.5 Hz, 1H, H-9a), 5.31 (dq, *J* = 10.2, 1.3 Hz, 1H, H-9b), 4.45 (tt, *J* = 5.7, 1.4 Hz, 2H, H-7). ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.76, 144.72, 132.55 (q, *J* = 32.5 Hz), 131.43, 127.09, 125.50 (q, *J* = 3.8 Hz), 123.67 (q, *J* = 271.3 Hz), 119.07, 49.20. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.93. HRMS for $C_{11}H_{10}F_3NSNa$ [M+Na]$^+$ calcd. 268.0378, found 268.0379.

_N_-Allyl-3-(trifluoromethyl)benzothioamide (4.3e)



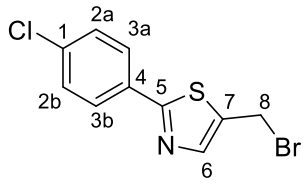
Compound **4.3e** was prepared from compound **4.2e** (1.57 mmol, 1.0 eq) using general protocol 5 to afford the product as a yellow oil. Yield: 66%, R_f = 0.77 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H, H-3), 7.93 (d, *J* = 7.9 Hz, 1H, H-5), 7.71 (d, *J* = 7.9 Hz, 1H, H-7), 7.60 (bs, 1H, NH), 7.52 (t, *J* = 7.8 Hz, 1H, H-6), 6.02 (ddt, *J* = 17.1, 10.2, 6.1 Hz, 1H, H-10), 5.38 (dq, *J* = 17.2, 1.6 Hz, 1H, H-11a), 5.33 (dq, *J* = 10.2, 1.6 Hz, 1H, H-11b), 4.47 (tt, *J* = 6.0, 1.5 Hz, 2H, H-9). ^{13}C NMR (125 MHz, $CDCl_3$): δ 197.59, 142.32, 131.45, 130.93 (q, *J* = 32.5 Hz), 129.91, 129.15, 127.54 (q, *J* = 3.8 Hz), 123.64 (q, *J* = 271.3 Hz), 123.61 (q, *J* = 3.8 Hz), 119.19, 49.28. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.70. HRMS for $C_{11}H_{11}F_3NS$ $[M+H]^+$ calcd. 246.0559, found 246.0560.

5-(Bromomethyl)-2-phenylthiazole (4.4a)



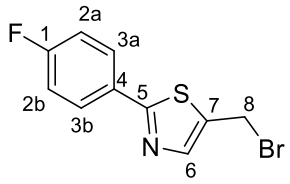
The known compound **4.4a** was prepared from compound **4.3a** (0.71 mmol, 1.0 eq) using the general protocol 10 to afford the product as a white solid. Yield: 53%, R_f = 0.86 (50% EtOAc in Hex). The characterization agreed with previously reported procedure.[140] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (m, 2H, H-3a,b), 7.78 (s, 1H, H-6), 7.46–7.42 (m, 3H, H-1 and H-2a,b), 4.75 (s, 2H, H-8).

5-(Bromomethyl)-2-(4-chlorophenyl)thiazole (4.4b)



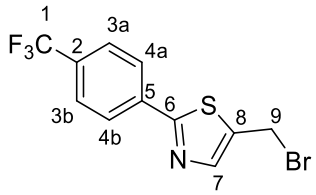
The known compound **4.4a** was prepared from compound **4.3a** (0.63 mmol, 1.0 eq) using the general protocol 10 with a modification. The reaction was heated at 61°C for 6 hours under an inert atmosphere, to afford the product as a pale yellow solid. Yield: 63%. The characterization agreed with previously reported procedure.[121] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.7 Hz, 2H, H-3a,b), 7.78 (s, 1H, H-6), 7.41 (d, *J* = 8.6 Hz, 2H, H-2a,b), 4.74 (d, *J* = 0.9 Hz, 2H, H-8).

5-(Bromomethyl)-2-(4-fluorophenyl)thiazole (4.4c)



The known compound **4.4c** was prepared from compound **4.3c** (0.53 mmol, 1.0 eq) using the general protocol 10 with a modification. The reaction was stirred under an inert atmosphere overnight, to afford the product as a white solid. Yield: 15%. The characterization agreed with previously reported procedure.[121] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (m, 2H, H-3a,b), 7.76 (s, 1H, H-6), 7.13 (m, 2H, H-2a,b), 4.74 (d, *J* = 0.8 Hz, 2H, H-8).

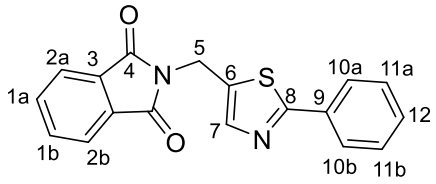
5-(Bromomethyl)-2-(4-(trifluoromethyl)phenyl)thiazole (4.4d)



The known compound **4.4d** was prepared from compound **4.3d** (0.48 mmol, 1.0 eq) using

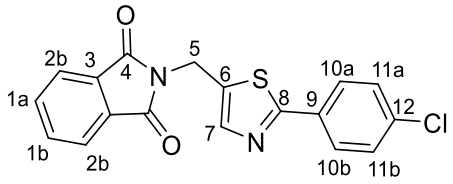
the general protocol 10 to afford the product as a white solid. Yield: 69%. The characterization agreed with previously reported procedure.[121] ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.2 Hz, 2H, H-4a,b), 7.83 (s, 1H, H-7), 7.70 (d, *J* = 8.2 Hz, 2H, H-3a,b), 4.76 (d, *J* = 0.8 Hz, 2H, H-9).

2-((2-Phenylthiazol-5-yl)methyl)isoindoline-1,3-dione (4.5a)



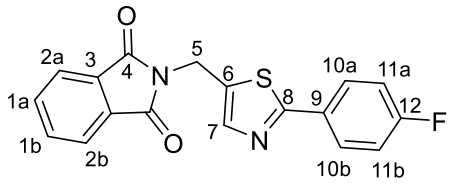
Compound **4.5a** was prepared from compound **4.4a** (0.37 mmol, 1.0 eq) using the general protocol 11 to afford the product as a yellow solid. Yield: 53%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90–7.86 (m, 4H, H-1a,b or H-2a,b, H-10a,b, or H-11a,b), 7.86 (s, 1H, H-7), 7.73 (m, 2H, H-10a,b or H-11a,b), 7.42–7.39 (m, 3H, H-1a,b or H-2a,b, and H-12), 5.07 (s, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.30, 167.34, 143.76, 134.27, 133.49, 132.42, 131.94, 130.16, 128.93, 126.47, 123.60, 33.33. HRMS for $C_{18}H_{12}O_2N_2SNa$ $[M+Na]^+$ calcd. 343.0512, found 343.0499.

2-((2-(4-Chlorophenyl)thiazol-5-yl)methyl)isoindoline-1,3-dione (4.5b)



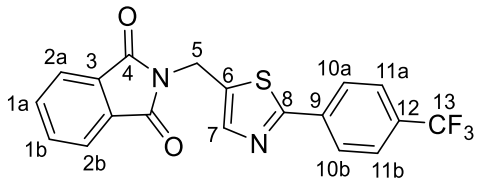
Compound **4.5b** was prepared from compound **4.4b** (1.07 mmol, 1.0 eq) using the general protocol 11 to afford the product as a yellow solid. Yield: 59%, R_f = 0.19 (100% DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (m, 2H, H-1a,b or H-2a,b), 7.85 (s, 1H, H-7), 7.82 (d, *J* = 8.6 Hz, 2H, H-10a,b), 7.73 (m, 2H, H-1a,b or H-2a,b), 7.38 (d, *J* = 8.5 Hz, 2H, H-11a,b), 5.06 (s, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.90, 167.41, 143.87, 136.14, 134.30, 132.81, 131.99, 131.91, 129.18, 127.64, 123.62, 33.27. HRMS for $C_{18}H_{11}ClO_2N_2SNa$ $[M+Na]^+$ calcd. 377.0122, found 377.0115.

2-((2-(4-Fluorophenyl)thiazol-5-yl)methyl)isoindoline-1,3-dione (4.5c)



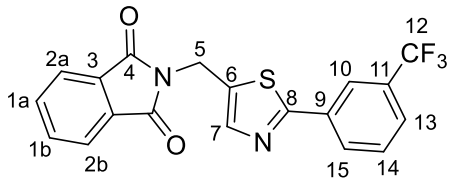
Compound **4.5c** was prepared from compound **4.4c** (0.31 mmol, 1.0 eq) using the general protocol 11 to afford the product as a white solid. Yield: 51%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89–7.86 (m, 4H, H-1a,b or H-2a,b, and H-10a,b), 7.83 (s, 1H, H-7), 7.73 (m, 2H, H-1a,b or H-2a,b), 7.09 (m, 2H, H-11a,b), 5.06 (s, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.06, 167.43, 163.93 (d, *J* = 250 Hz), 143.74, 134.29, 132.48, 131.92, 129.86 (d, *J* = 3.8 Hz), 128.38 (d, *J* = 8.8 Hz), 123.61, 116.04 (d, *J* = 22.5 Hz), 33.28. ^{19}F NMR (470 MHz, $CDCl_3$) δ -110.28. HRMS for $C_{18}H_{11}FO_2N_2SNa$ $[M+Na]^+$ calcd. 361.0417, found 361.0409.

2-((2-(4-(Trifluoromethyl)phenyl-thiazol-5-yl)methyl)isoindoline-1,3-dione (4.5d)



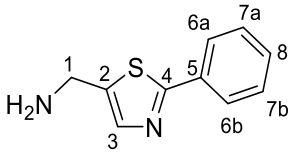
Compound **4.5d** was prepared from compound **4.4d** (1.26 mmol, 1.0 eq) using the general protocol 11 to afford the product as a yellow solid. Yield: 43%, R_f = 0.18 (100% DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.1 Hz, 2H, H-10a,b), 7.90 (s, 1H, H-7), 7.87 (m, 2H, H-1a,b or H-2a,b), 7.73 (m, 2H, H-1a,b or H-2a,b), 7.66 (d, *J* = 8.2 Hz, 2H, H-11a,b), 5.07 (s, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.38, 167.25, 144.17, 136.55, 134.33, 133.72 131.87, 131.71 (q, *J* = 32.5 Hz), 126.64, 125.96 (q, *J* = 3.8 Hz), 123.85 (q, *J* = 270 Hz), 123.63, 33.22. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.83. HRMS for $C_{19}H_{11}F_3O_2N_2SNa$ $[M+Na]^+$ calcd. 411.0386, found 411.0372.

2-((2-(3-(Trifluoromethyl)phenyl-thiazol-5-yl)methyl)isoindoline-1,3-dione (4.5e)



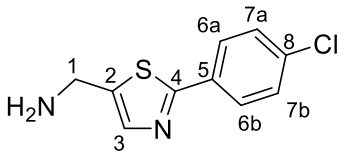
Compound **4.5e** was prepared from compound **4.4e** (0.73 mmol, 1.0 eq) using the general protocol 11 to afford the product as a yellow solid. Yield: 61%, R_f = 0.19 (20% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (s, 1H, H-10), 8.02 (d, *J* = 8.0 Hz, 1H, H-15), 7.87 (s, 1H, H-7), 7.84 (m, 2H, H-1a,b or H-2a,b), 7.70 (m, 2H, H-1a,b or H-2a,b), 7.61 (d, *J* = 7.9 Hz, 1H, H-13), 7.50 (t, *J* = 8.0 Hz, 1H, H-14), 5.06 (s, 2H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.36, 167.27, 144.00, 134.30, 134.16, 133.48, 131.85, 131.46 (q, *J* = 32.5 Hz), 129.52, 129.50, 126.55 (q, *J* = 3.8 Hz), 123.74 (q, *J* = 270 Hz), 123.59, 123.18 (q, *J* = 3.8 Hz), 33.22. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.86. HRMS for $C_{19}H_{11}F_3O_2N_2SNa$ $[M+Na]^+$ calcd. 411.0386, found 411.0382.

(2-Phenylthiazol-5-yl)methanamine (4.6a)



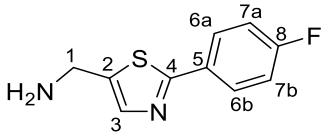
Compound **4.6a** was prepared from compound **4.5a** (0.20 mmol, 1.0 eq) using the general protocol 7 to afford the product as a yellow solid. Yield: 50%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (m, 2H, H-6a,b), 7.63 (s, 1H, H-3), 7.45–7.39 (m, 3H, H-7a,b and H-8), 4.10 (s, 2H, H-1), 1.68 (bs, 2H, NH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.65, 141.67, 140.03, 133.84, 129.88, 128.94, 126.32, 39.03. HRMS for $C_{10}H_{11}N_2S$ $[M+H]^+$ calcd. 191.0637, found 191.0628.

(2-(4-Chlorophenyl)thiazol-5-yl)methanamine (4.6b)



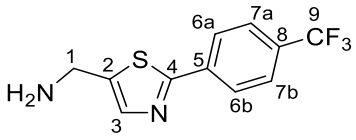
Compound **4.6b** was prepared from compound **4.5b** (0.22 mmol, 1.0 eq) using the general protocol 7 to afford the product as a pale yellow solid. Yield: 39%. R_f = 0.40 (10% MeOH in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.7 Hz, 2H, H-6a,b), 7.64 (s, 1H, H-3), 7.40 (d, *J* = 8.7 Hz, 2H, H-7a,b), 4.12 (s, 2H, H-1), 1.63 (bs, 2H, NH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.28, 142.11, 140.16, 135.80, 132.36, 129.17, 127.51, 39.01. HRMS for $C_{10}H_{10}ClN_2S$ $[M+H]^+$ calcd. 225.0248, found 225.0241.

(2-(4-Fluorophenyl)thiazol-5-yl)methanamine (4.6c)



Compound **4.6c** was prepared from compound **4.5c** (0.16 mmol, 1.0 eq) using the general protocol 7 to afford the product as a yellow solid. Yield: 81%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (m, 2H, H-6a,b), 7.61 (s, 1H, H-3), 7.12 (m, 2H, H-7a,b), 4.11 (s, 2H, H-1), 1.60 (bs, 2H, NH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.44, 163.77 (d, *J* = 248.8 Hz), 141.78, 140.00, 130.23 (d, *J* = 3.8 Hz), 128.20 (d, *J* = 8.8 Hz), 116.01 (d, *J* = 22.5 Hz), 39.02. ^{19}F NMR (470 MHz, $CDCl_3$) δ -110.85. HRMS for $C_{10}H_{10}FN_2S$ $[M+H]^+$ calcd. 209.0543, found 209.0538.

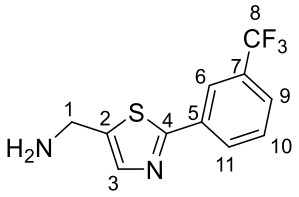
(2-(4-(Trifluoromethyl)phenyl)thiazol-5-yl)methanamine (4.6d)



Compound **4.6d** was prepared from compound **4.5d** (0.37 mmol, 1.0 eq) using the general protocol 7 to afford the product as a pale yellow solid. Yield: 80%. ^{1}H NMR (500 MHz, MeOD) δ 8.04 (d, *J* = 8.2 Hz, 2H, H-6a,b), 7.74 (s, 1H, H-3), 7.72 (d, *J* = 8.3 Hz,

2H, H-7a,b), 4.05 (s, 2H, H-1). ^{13}C NMR (125 MHz, MeOD) δ 165.76, 142.55, 140.76, 136.78, 131.13 (q, *J* = 32.5 Hz), 126.34, 125.71 (q, *J* = 3.8 Hz), 124.02 (q, *J* = 270 Hz), 37.52. ^{19}F NMR (470 MHz, MeOD) δ -64.24. HRMS for $C_{11}H_{10}F_3N_2S$ $[M+H]^+$ calcd. 259.0511 found 259.0504.

(2-(4-(Trifluoromethyl)phenyl)thiazol-5-yl)methanamine (4.6e)



Compound **4.6e** was prepared from compound **4.5e** (0.26 mmol, 1.0 eq) using the general protocol 7 to afford the product as a yellow oil. Yield: 69%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H, H-6), 8.06 (d, *J* = 7.9 Hz, 1H, H-11), 7.67 (s, 1H, H-3), 7.64 (d, *J* = 7.8 Hz, 1H, H-9), 7.55 (t, *J* = 7.8 Hz, 1H, H-10), 4.13 (s, 2H, H-1), 1.60 (bs, 2H, NH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.67, 142.83, 140.28, 134.56, 131.49 (q, *J* = 31.3 Hz), 129.49, 129.42, 126.26 (q, *J* = 3.8 Hz), 123.83 (q, *J* = 271.3 Hz), 123.04 (q, *J* = 3.8 Hz), 38.99. ^{19}F NMR (470 MHz, $CDCl_3$) δ -62.84. HRMS for $C_{11}H_{10}F_3N_2S$ $[M+H]^+$ calcd. 259.0511, found 259.0500.

5.2 Biology

5.2.1 Materials

Bacteria were purchased from Cedarlane, Canada and the bacteria strains used for the antimicrobial susceptibility tests were *Escherichia coli* ATCC 25922, *Enterococcus faecium* ATCC 19434, *Klebsiella. pneumonia* ATCC 13883, *Pseudomonas. aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213. *E. coli* ATCC 25922 and *K. pneumonia* ATCC 13883 were cultured in Difco™ Nutrient Broth, whereas *E. faecium* ATCC 19434 was cultured in Difco™ Brain Heart Infusion Broth, and *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213 were cultured in Difco™ Tryptic Soy Broth. Cationic adjusted Mueller-Hinton Broth was prepared based on the protocol in the Clinical and Laboratory Standards Institute (CLSI)[113] and MilliQ water was used whenever water is mentioned. A Molecular Devices SpectraMax i3x multi-mode microtiter plate reader and

an Agilent 8453 UV-Vis spectrometer were used to measure the UV-Vis absorption at 600 nm.

5.2.2 Antimicrobial susceptibility test

To determine the antimicrobial susceptibility of the compounds synthesized in this thesis, CLSI M07's broth microdilution method was used.[113] The concentration of the bacteria was determined by the calibration equation $OD_{600} = mx + b$, where x is the concentration of bacteria (CFU/mL) and OD_{600} is the optical density at 600 nm. The calibration equation for *E. faecium* ATCC 19434 was $OD_{600} = 1\times10^{-9}x + 0.1805$, for *E. coli* ATCC 25922 was $OD_{600} = 3\times10^{-9}x + 0.2537$, for *K. pneumonia* ATCC 13883 was $OD_{600} = 4\times10^{-9}x + 0.2165$, for *P. aeruginosa* ATCC 27853 was $OD_{600} = 7\times10^{-10}x + 0.2473$, and for *S. aureus* ATCC 29213 was $OD_{600} = 2\times10^{-9}x + 0.0915$. In brief, bacteria were first allowed to proliferate at 37°C for 18 hours on agar medium prepared using the growth medium listed above for each strain. Next, five individual colonies were selected from the overnight agar culture and added to the corresponding liquid medium before incubation at 37°C for 2-6 hours, to achieve 10^8 CFU/mL. The bacterial solution was diluted to 10^7 CFU/mL and added (10 µL) to each well of a 96-well microplate plate, which already contained cationic adjusted Mueller-Hinton Broth (180 µL) and the compound of interest (10 µL, 50 µM). The 96-well microplate plate was incubated at 37°C for 18 hours and the OD_{600} was measured. To determine the growth percentage, the average OD_{600} for each compound were first subtracted by the blank (sterility control lane) then divided by the average value of growth control. All experiments were performed in duplicates.

5.2.3 Antiplasmodial activities

The antiplasmodial activity against *P. falciparium* (intraerythrocytic stage) was performed by Christina Spry or Xiangning Liu under the supervision of Prof. Kevin Saliba at the Australian National University. The assay was based on a modified version of malaria SYBR Green I-based fluorescence assay[80,141] and compounds were tested in "fresh" growth medium, *i.e.* in the presence of pantetheinase.

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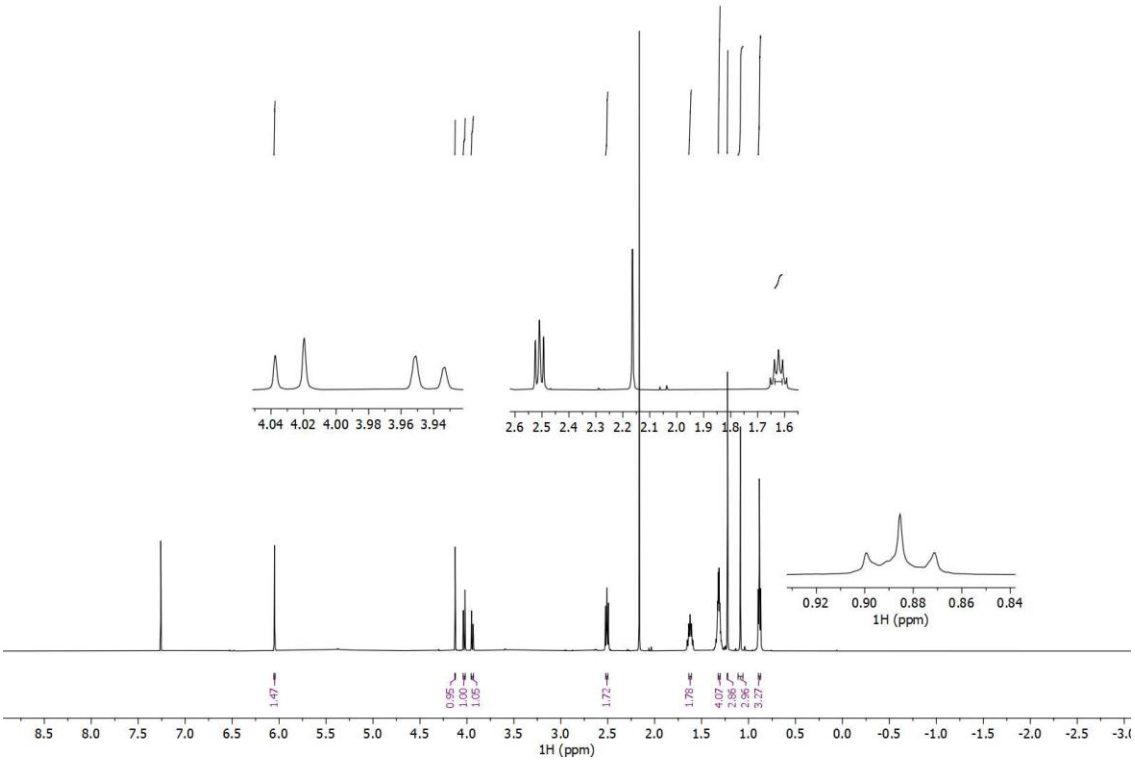
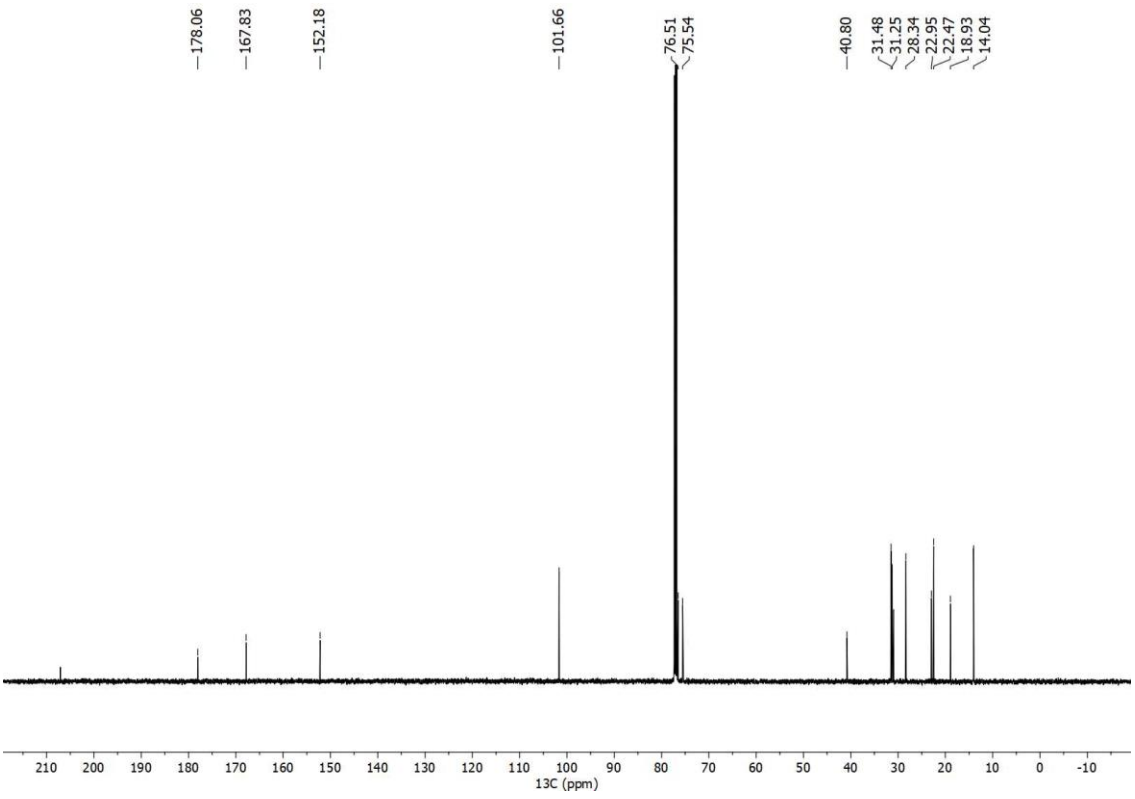
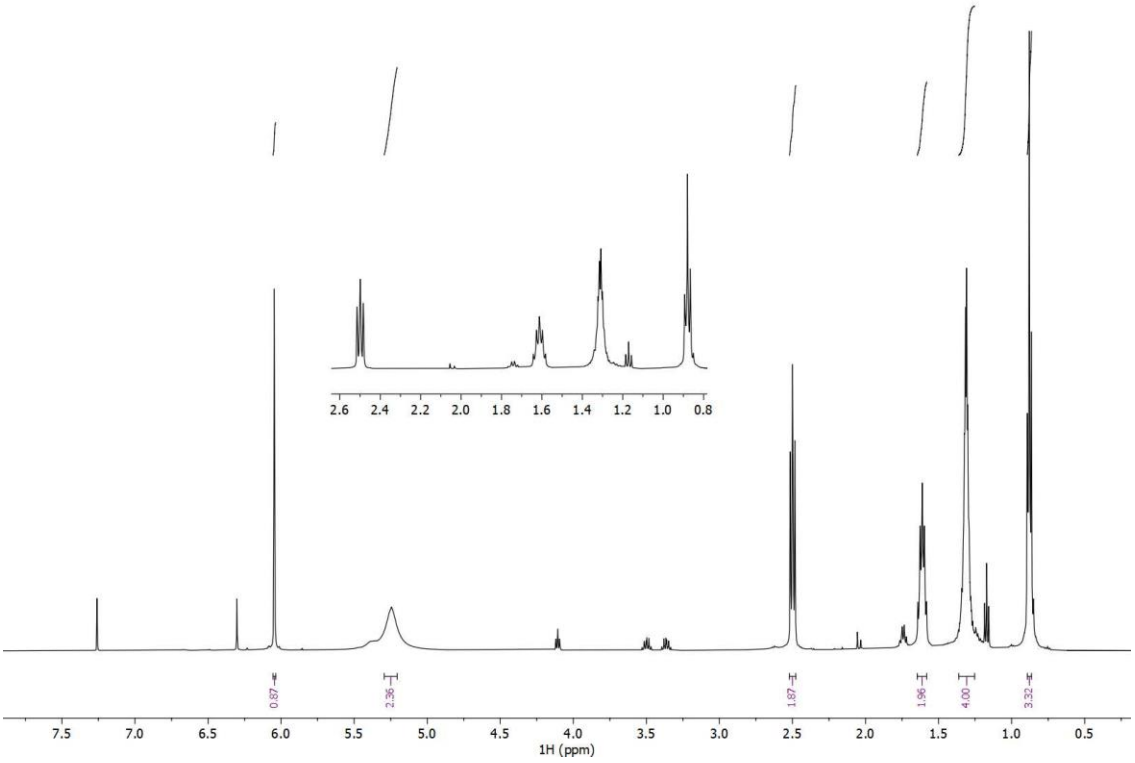
Appendix

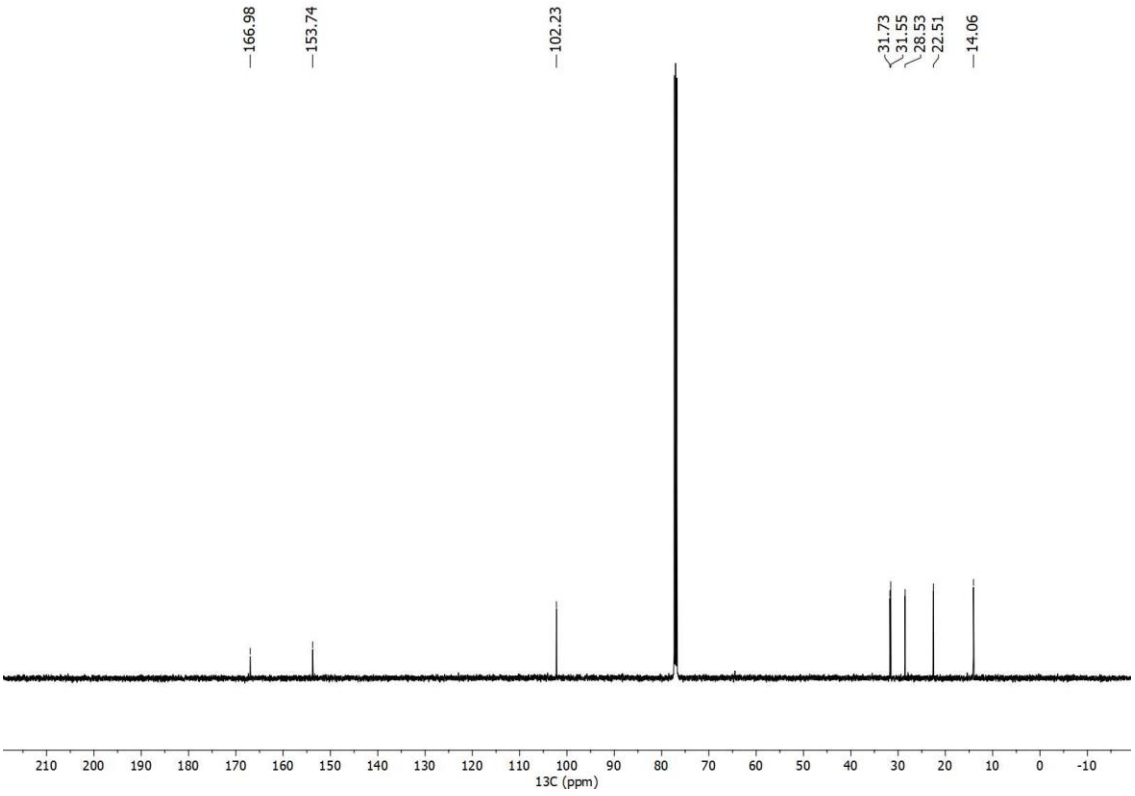
Compound 1


4.04 4.02 4.00 3.98 3.96 3.94
2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6
0.92 0.90 0.88 0.86 0.84
1H (ppm)
1.47
0.95
1.00
1.05
1.72
1.78
4.07
2.86
2.96
3.27
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5 -2.0 -2.5
1H (ppm)

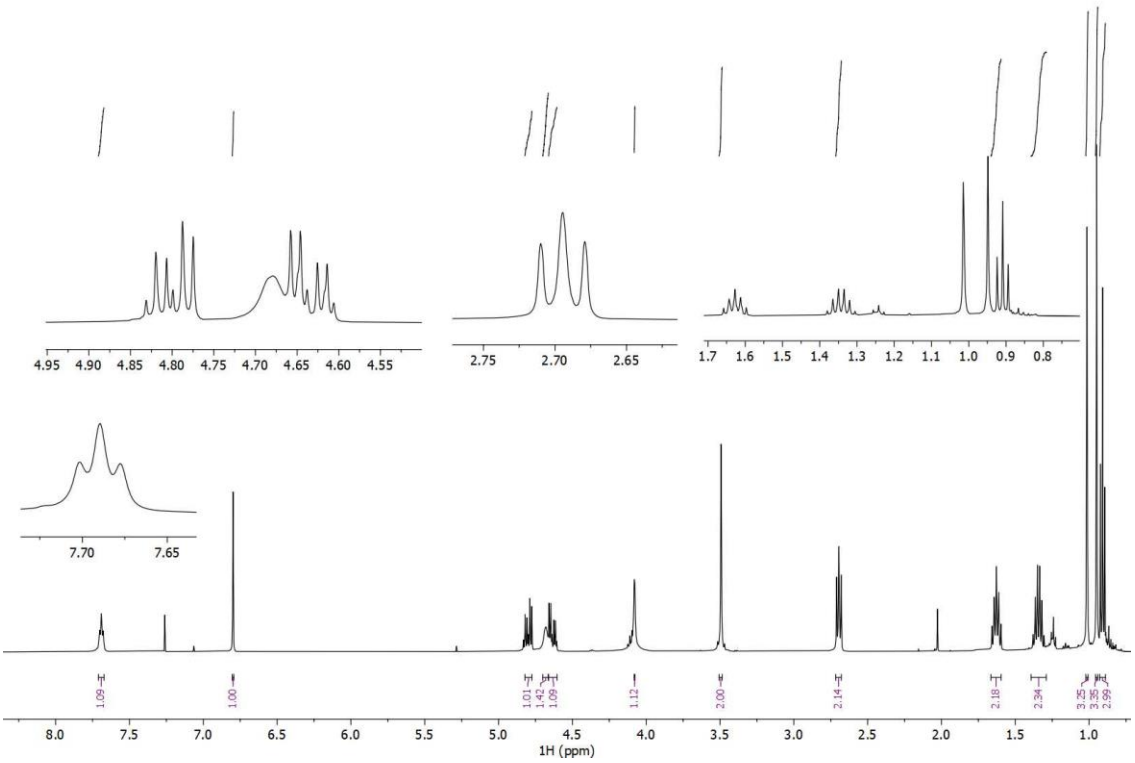

178.06
167.83
152.18
101.66
76.51
75.54
40.80
31.48
31.25
28.34
22.95
22.47
18.93
14.04
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

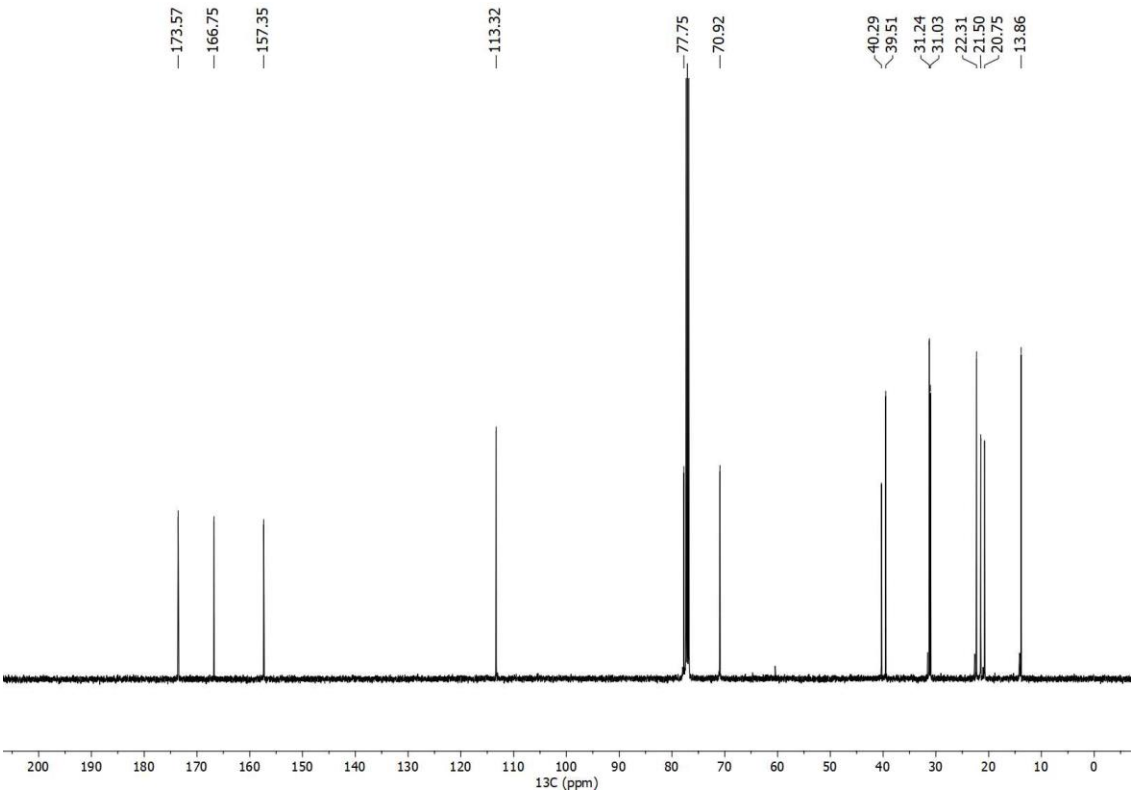
Compond 1.2


0.87
2.36
1.87
1.96
4.00
3.32
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1H (ppm)
2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8

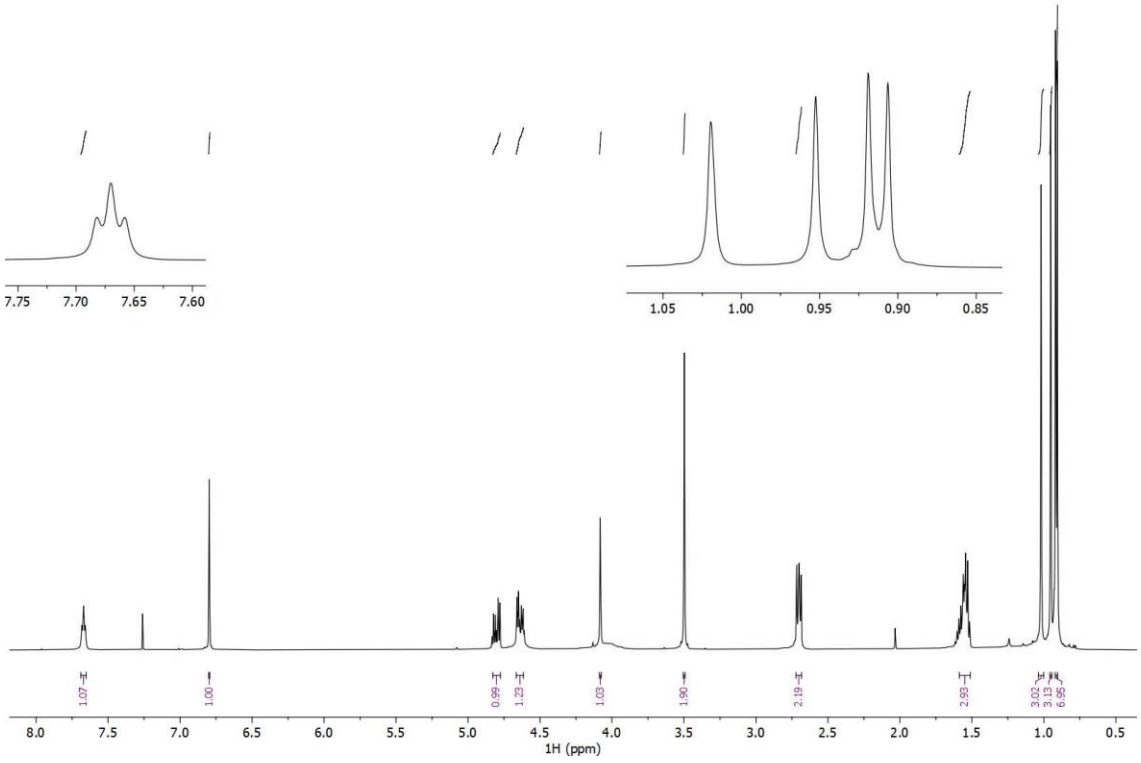

166.98
153.74
102.23
31.73
31.55
28.53
22.51
14.06
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

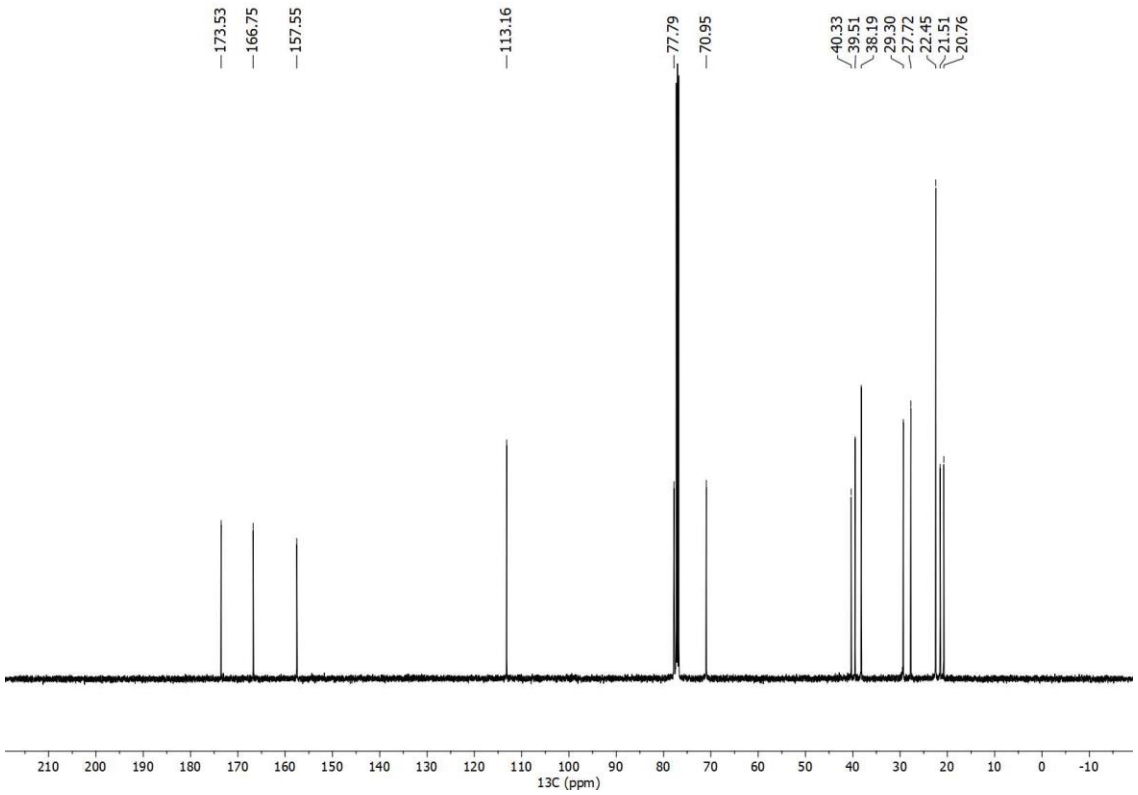
Compound 2a


4.95 4.90 4.85 4.80 4.75 4.70 4.65 4.60 4.55
2.75 2.70 2.65
1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8
7.70 7.65
1.09 1.00 1.01 1.42 1.09 1.12 2.00 2.14 2.18 2.94 3.25 3.35 2.99
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
1H (ppm)


173.57
166.75
157.35
113.32
77.75
70.92
40.29
39.51
31.24
31.03
22.31
21.50
20.75
13.86
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
13C (ppm)

Compound 2b


7.75
7.70
7.65
7.60
1.05
1.00
0.95
0.90
0.85
1.07
1.00
0.99
1.23
1.03
1.90
2.19
2.93
3.02
3.13
6.95
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1H (ppm)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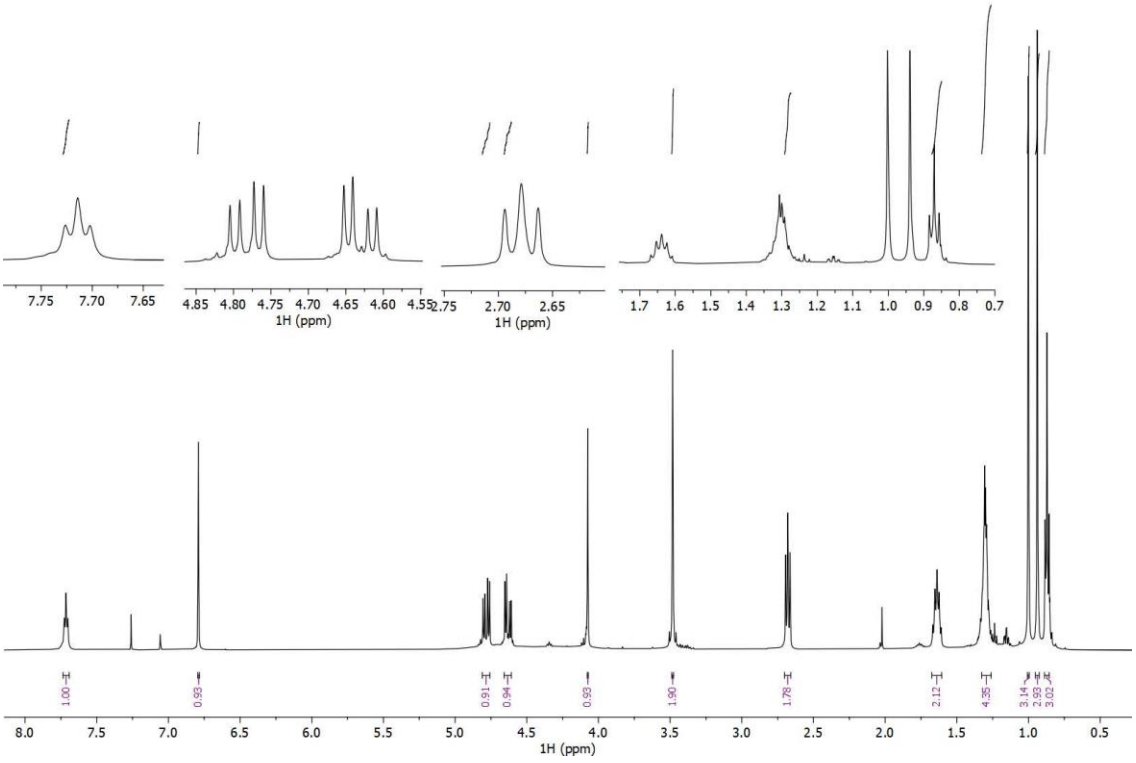
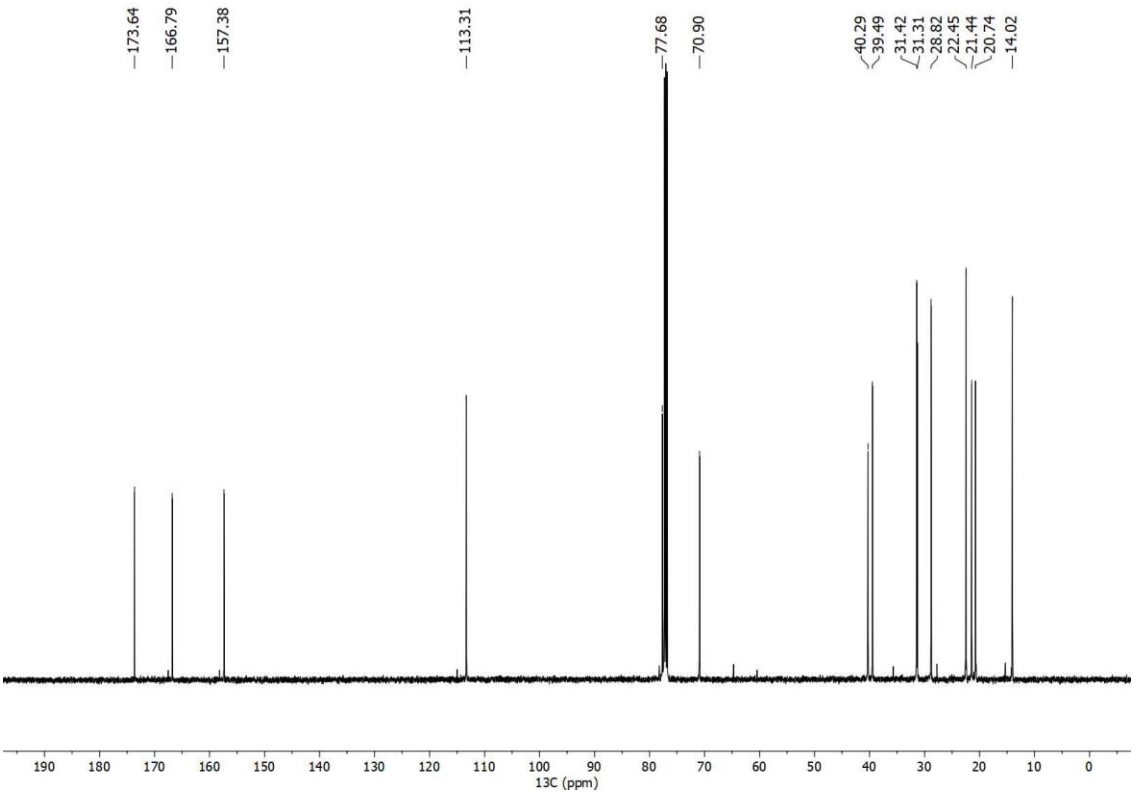
157.55
113.16
77.79
70.95
40.33
39.51
38.19
29.30
27.72
22.45
21.51
20.76
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
13C (ppm)

Compound 2c


7.75 7.70 7.65
4.85 4.80 4.75 4.70 4.65 4.60 4.55
1H (ppm)
2.75 2.70 2.65
1H (ppm)
1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 0.7
1.00
0.93
0.91
0.94
0.93
1.90
1.78
2.12
4.35
3.14
2.93
3.02
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1H (ppm)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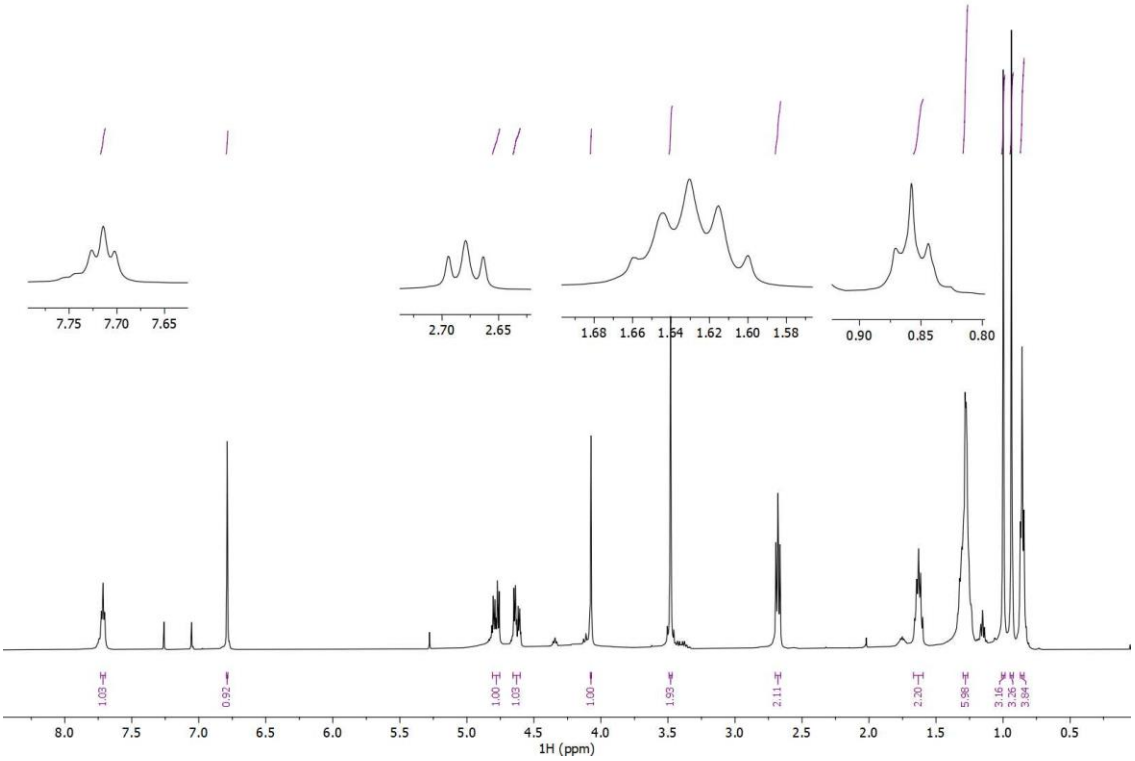
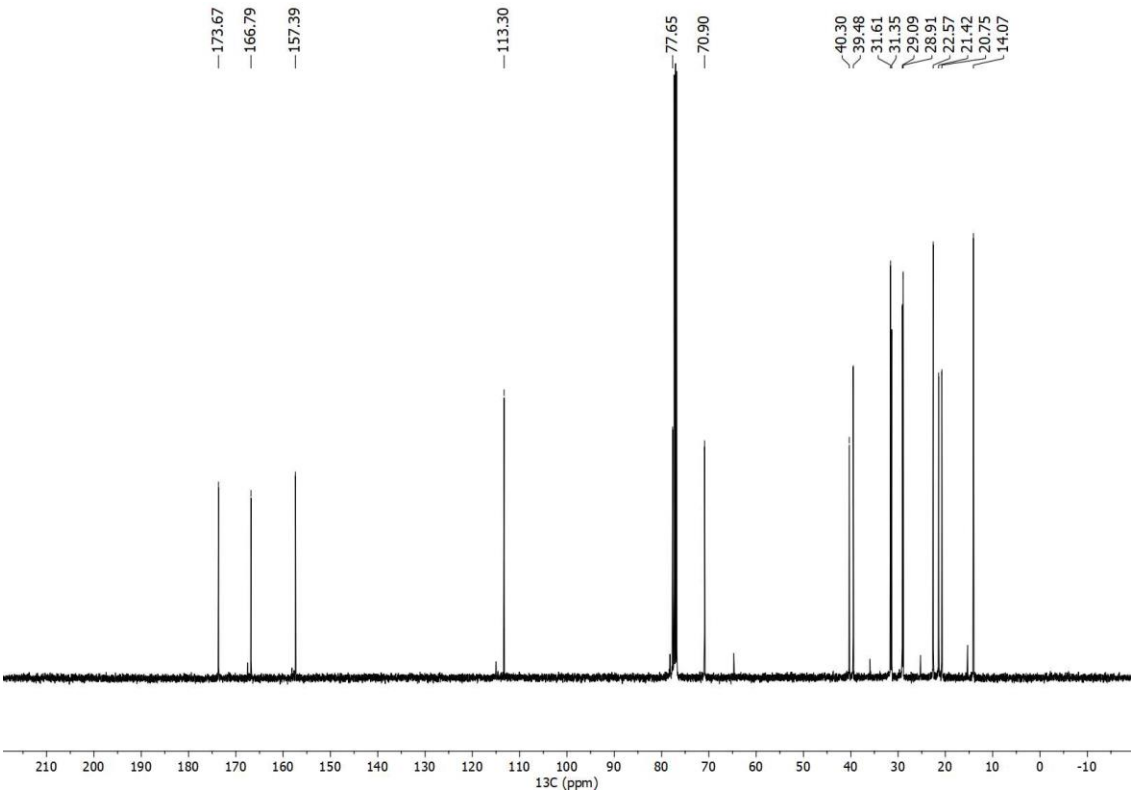
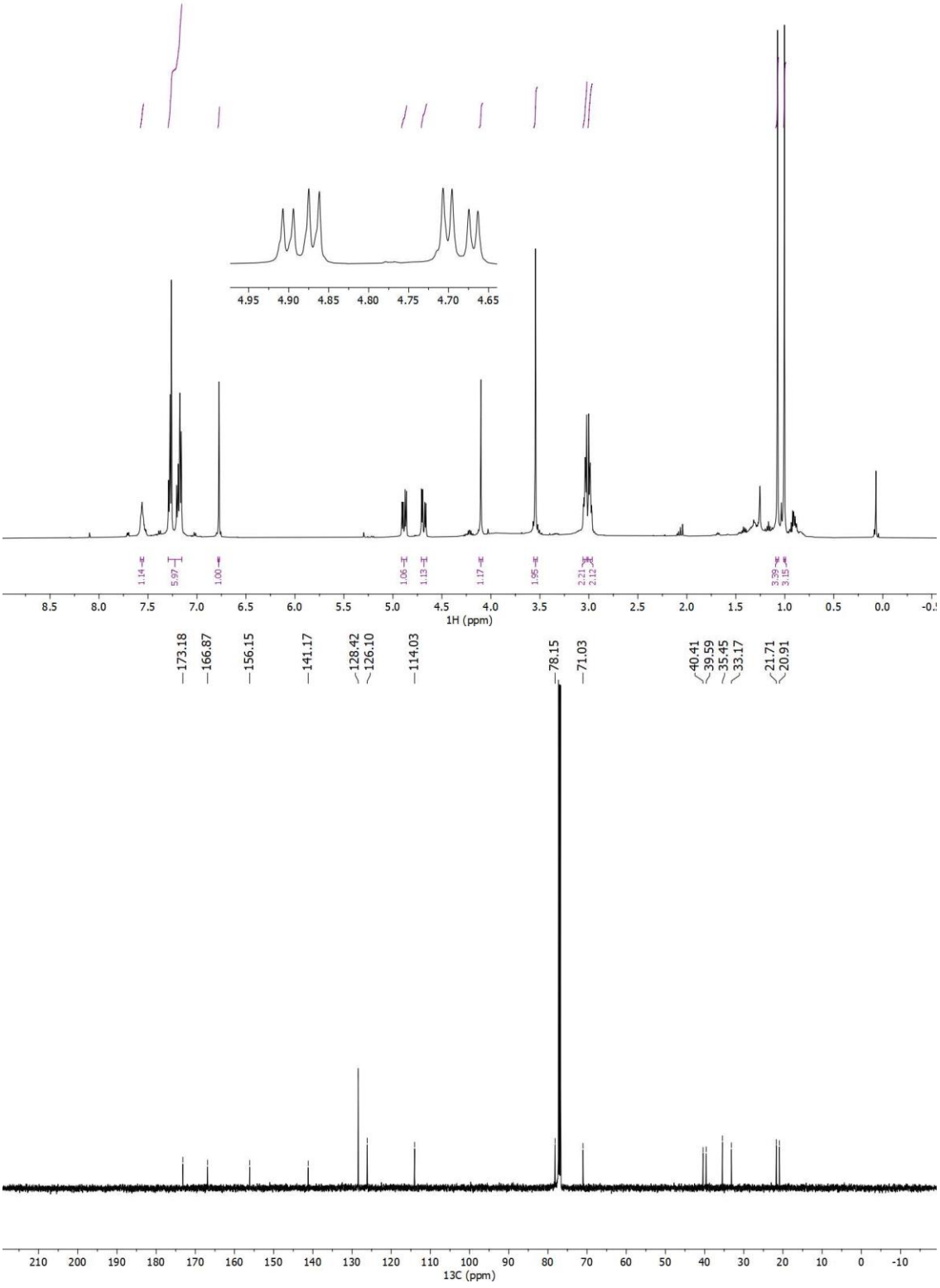
113.31
77.68
70.90
40.29
39.49
31.42
31.31
28.82
22.45
21.44
20.74
14.02
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
13C (ppm)

Compound 2d

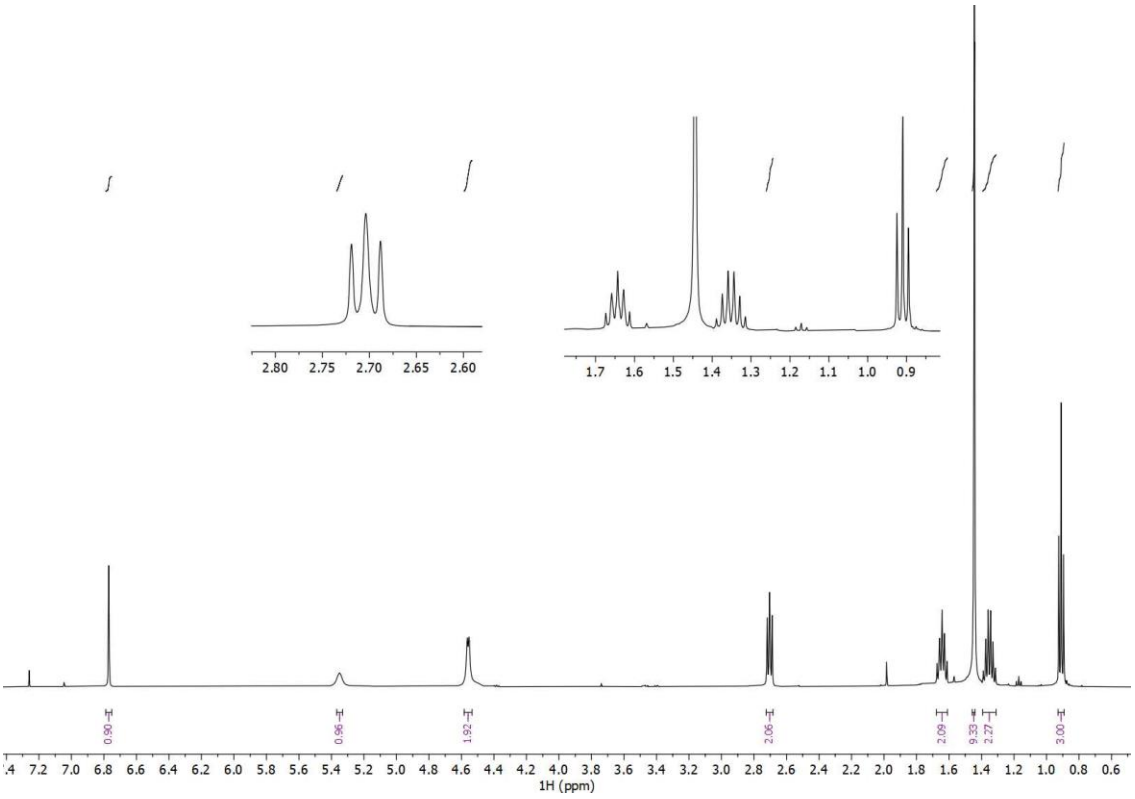

7.75 7.70 7.65
2.70 2.65
1.68 1.66 1.64 1.62 1.60 1.58
0.90 0.85 0.80
1.03 0.92 1.00 1.03 1.00 1.93 2.11 2.20 5.88 3.16 3.25 3.64
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1H (ppm)

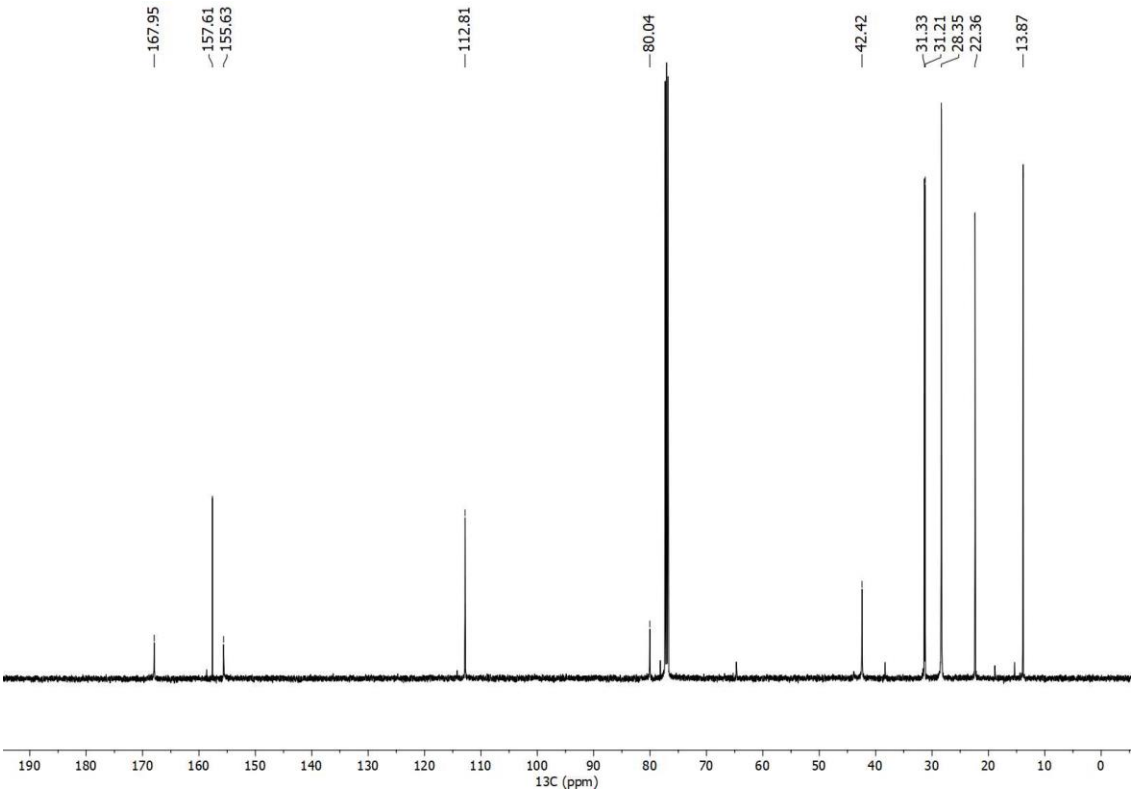

173.67
166.79
157.39
113.30
77.65
70.90
40.30
39.48
31.61
31.35
29.09
28.91
22.57
21.42
20.75
14.07
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

Compound 2e

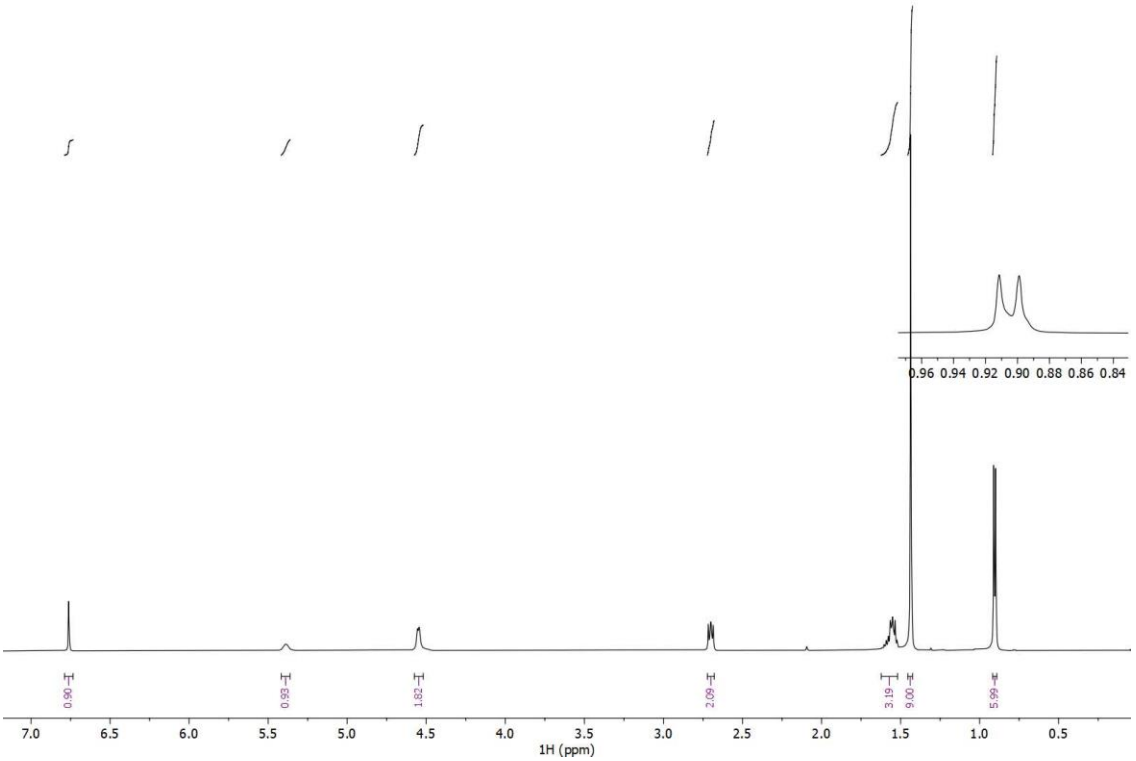

4.95 4.90 4.85 4.80 4.75 4.70 4.65
1.14 5.97 1.00 1.06 1.13 1.17 1.95 2.21 2.12 3.39 3.15
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)
173.18
166.87
156.15
141.17
128.42
126.10
114.03
78.15
71.03
40.41
39.59
35.45
33.17
21.71
20.91
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

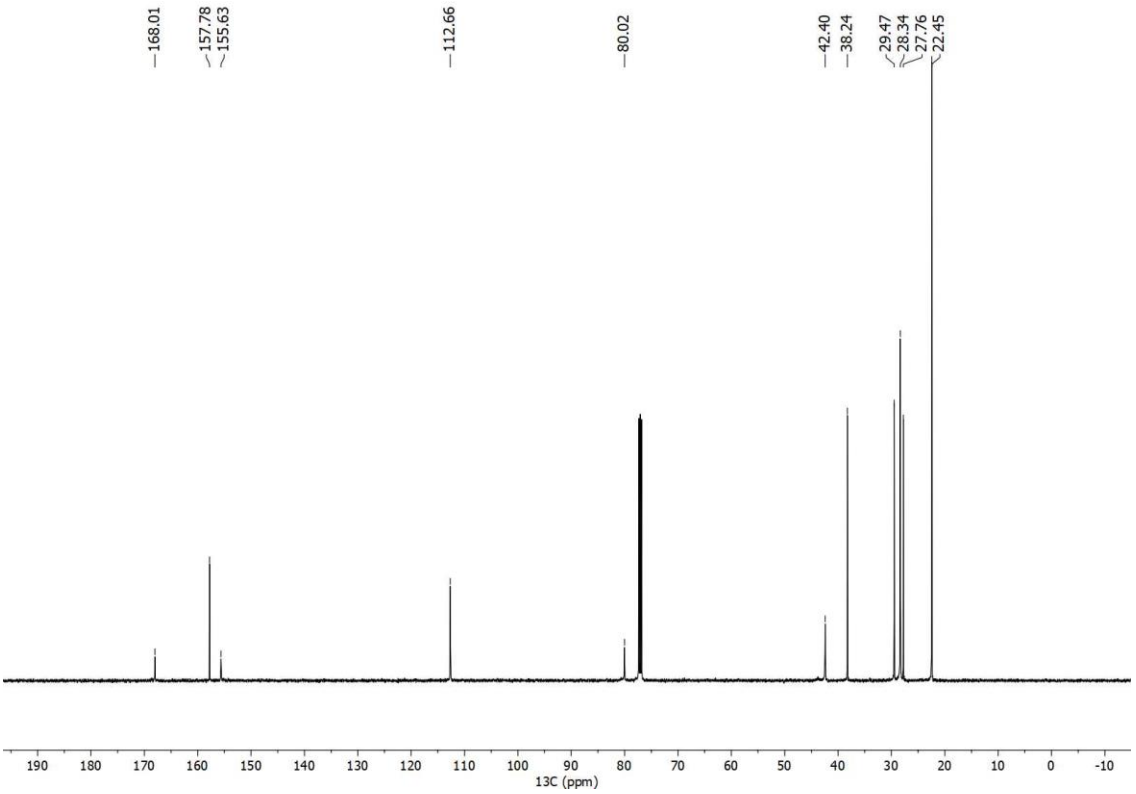
Compound 2.5a



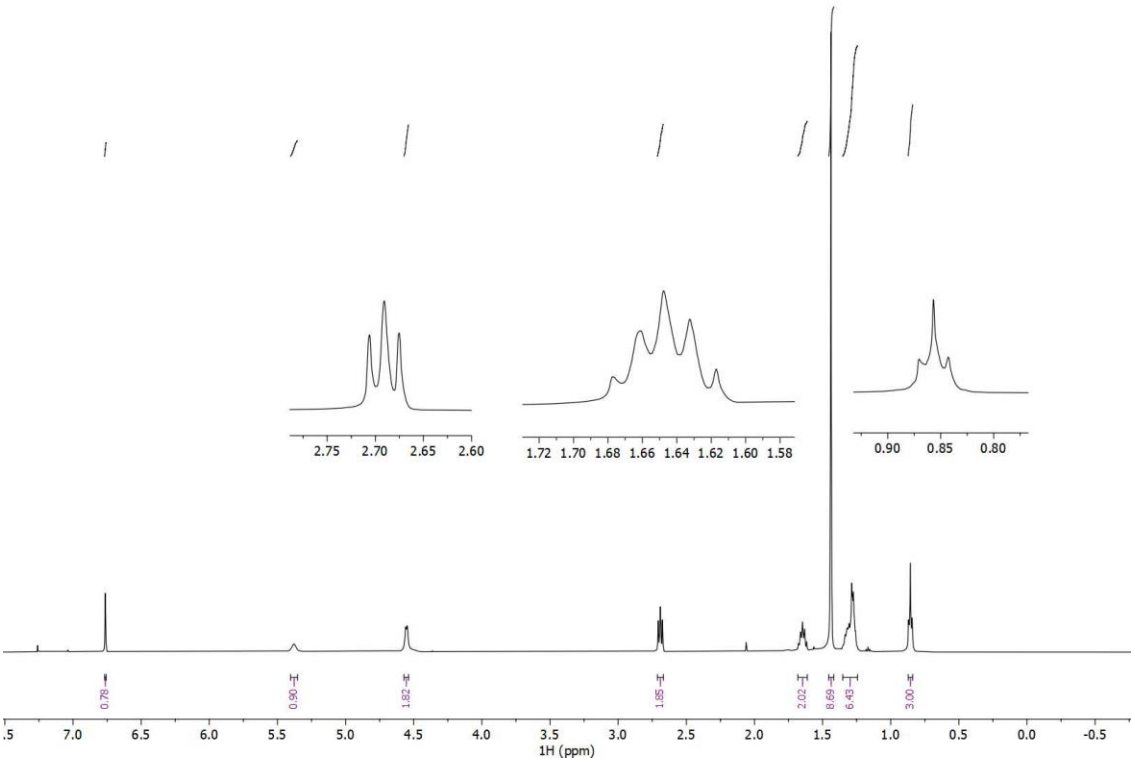


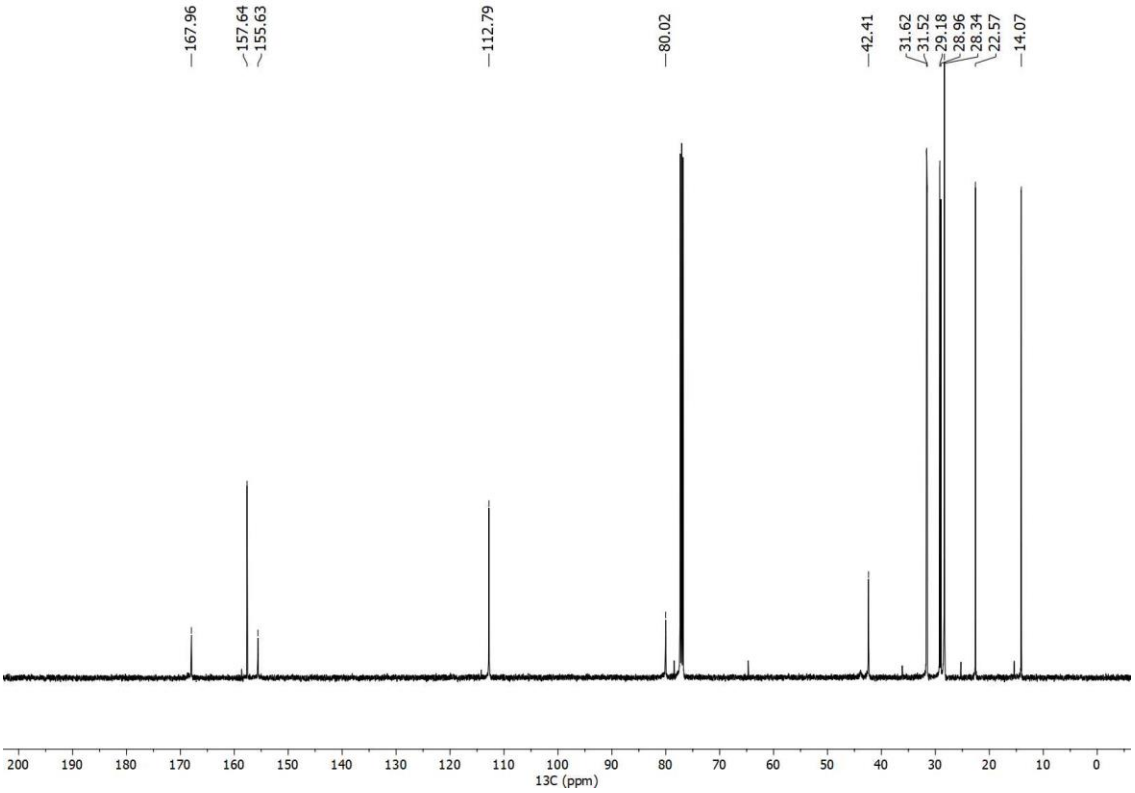
Compound 2.5b



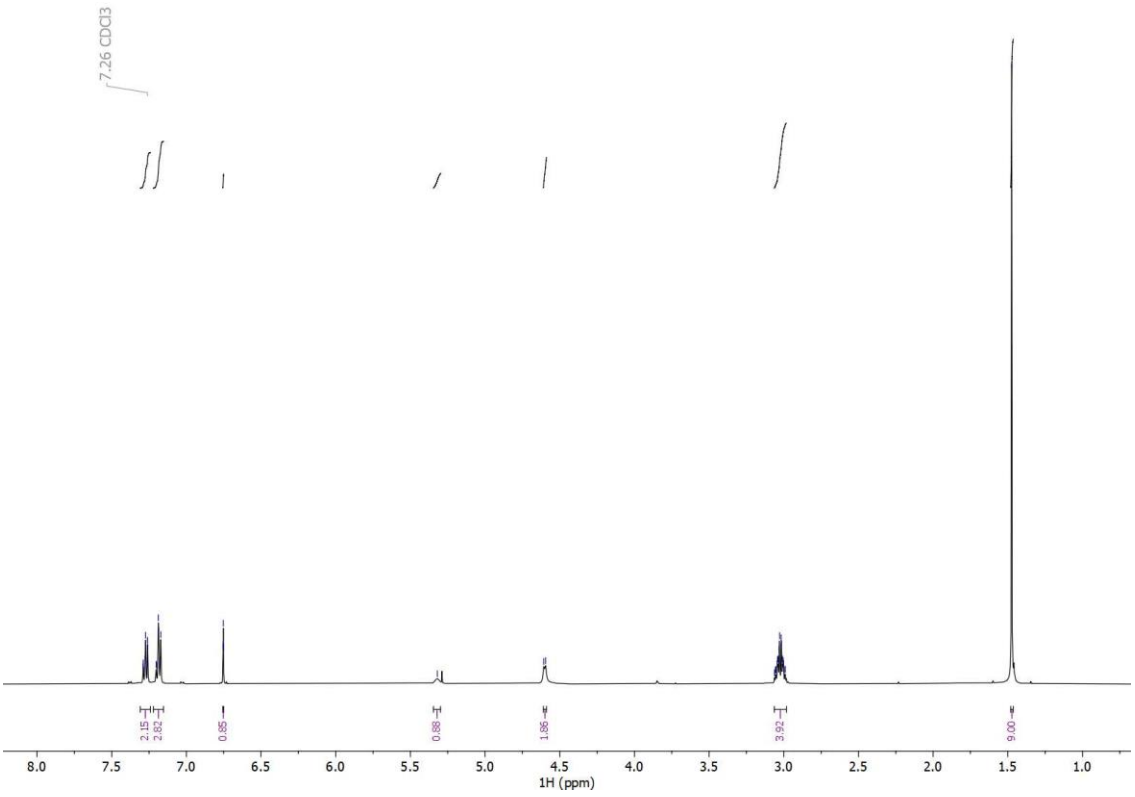


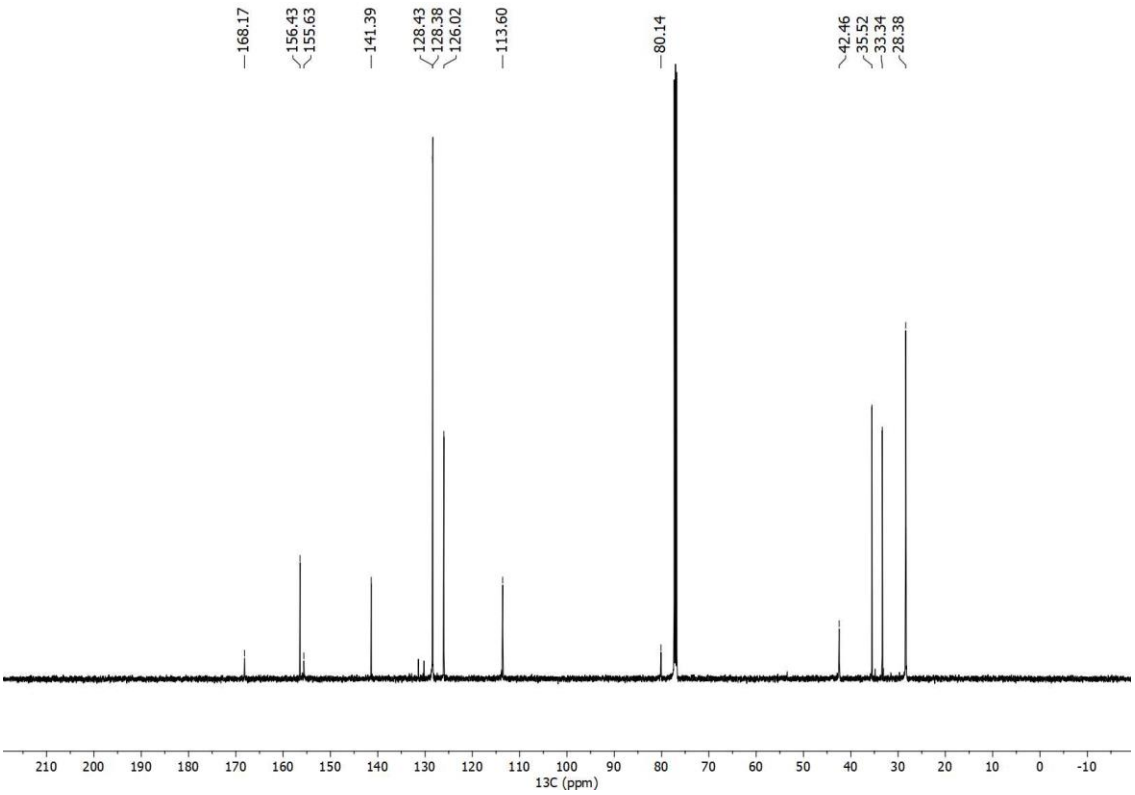
Compound 2.5d



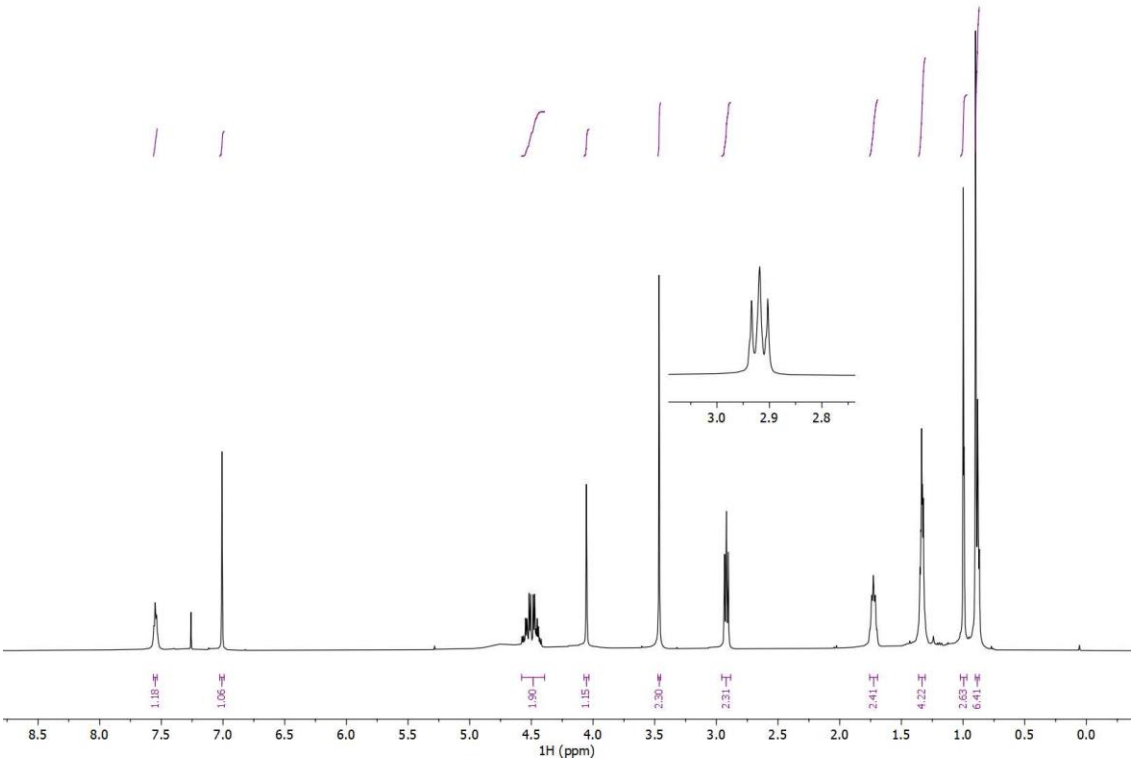


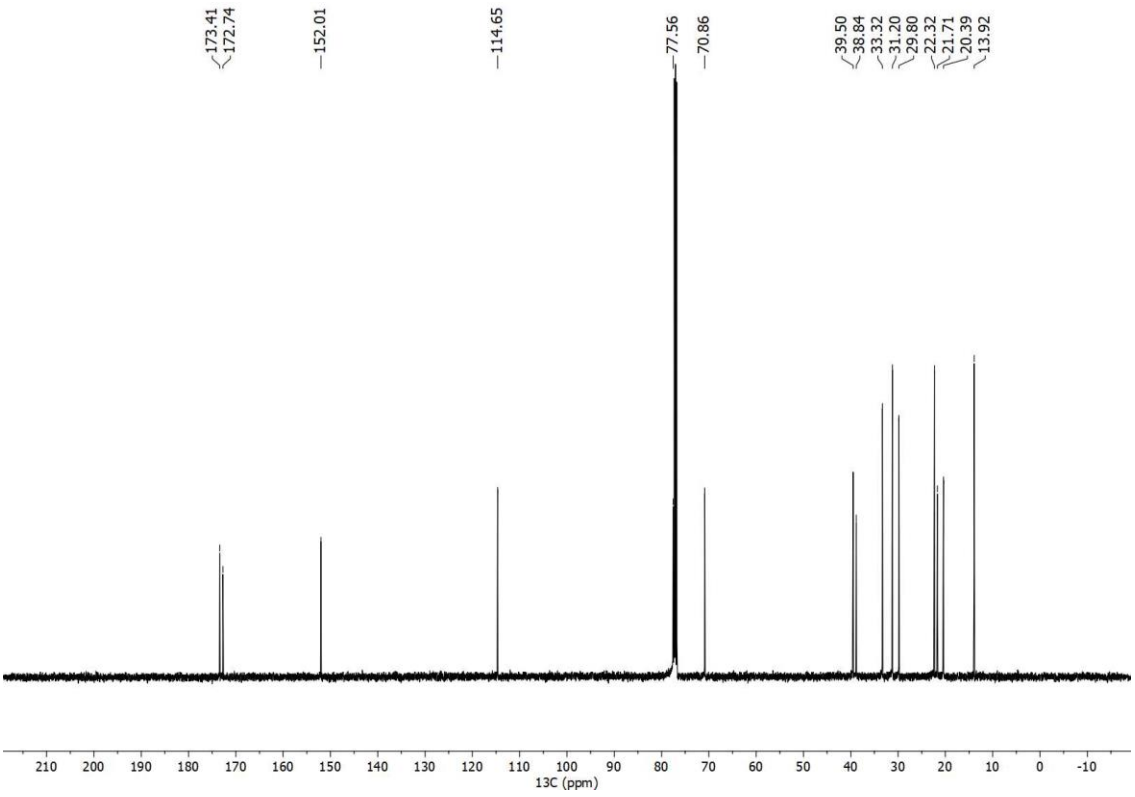
Compound 2.5e



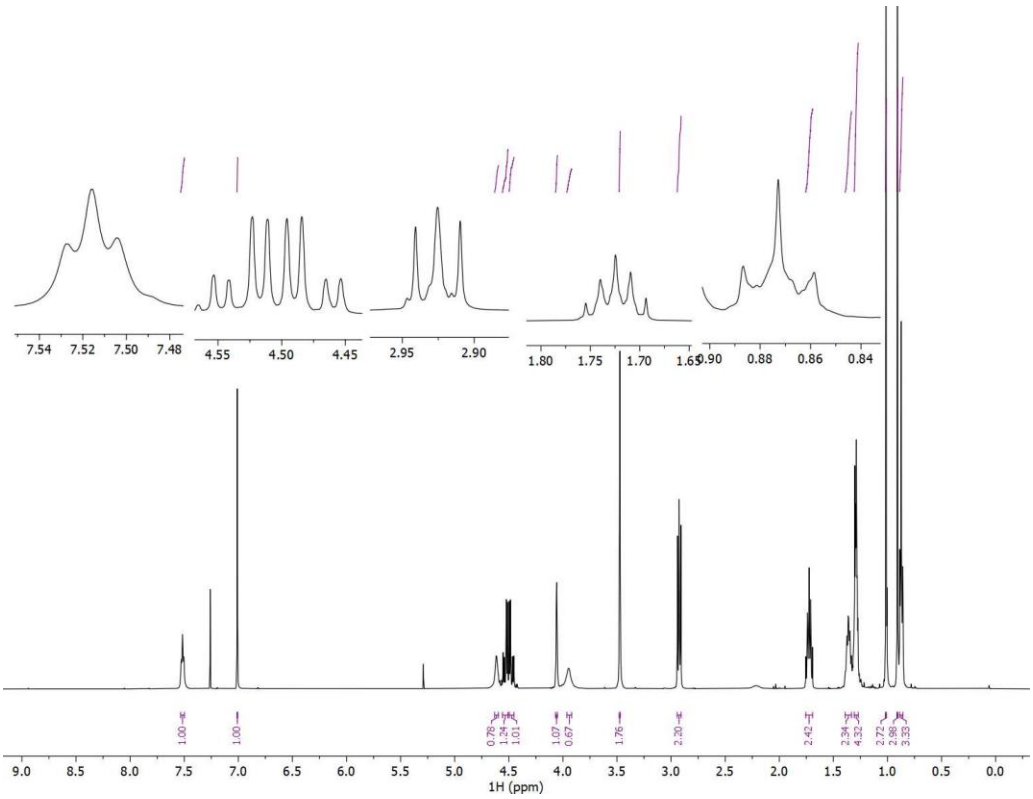


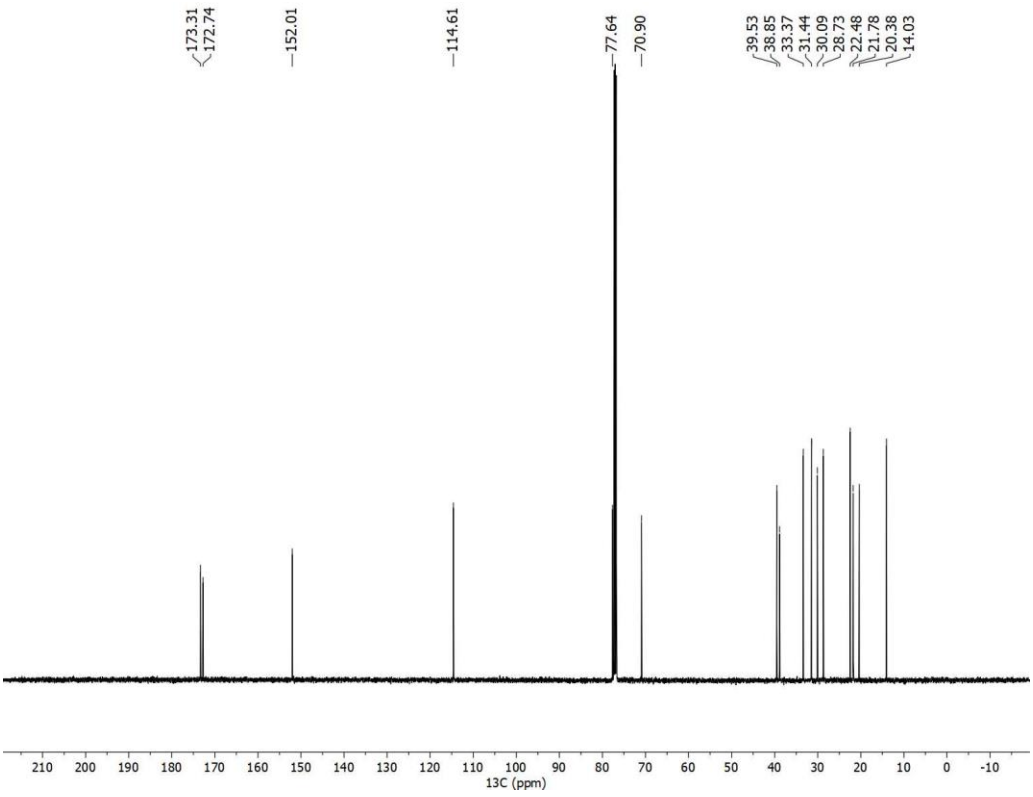
Compound 3a


1.18
1.06
1.90
1.15
2.30
2.31
2.41
4.22
2.63
6.41
1H (ppm)

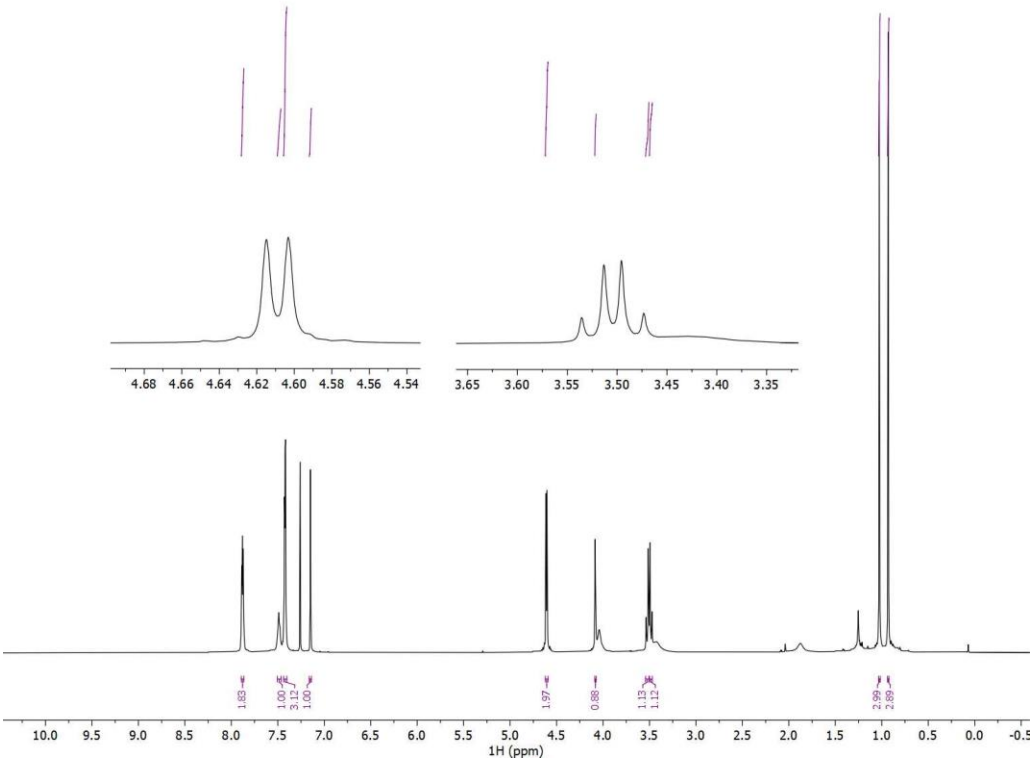

173.41
172.74
152.01
114.65
77.56
70.86
39.50
38.84
33.32
31.20
29.80
22.32
21.71
20.39
13.92
13C (ppm)

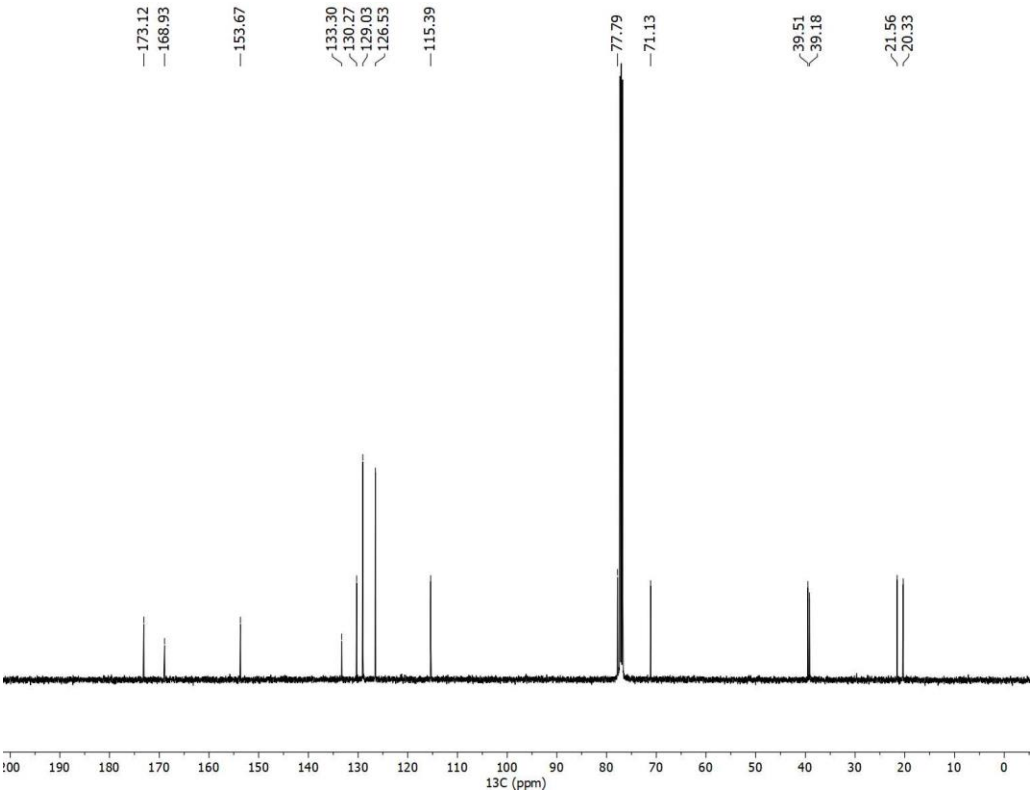
Compound 3b


7.54 7.52 7.50 7.48
4.55 4.50 4.45
2.95 2.90
1.80 1.75 1.70 1.65
0.90 0.88 0.86 0.84
1.00 1.00 0.78 1.24 1.01 1.07 0.67 1.76 2.20 2.42 2.34 4.32 2.72 2.98 3.33
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)

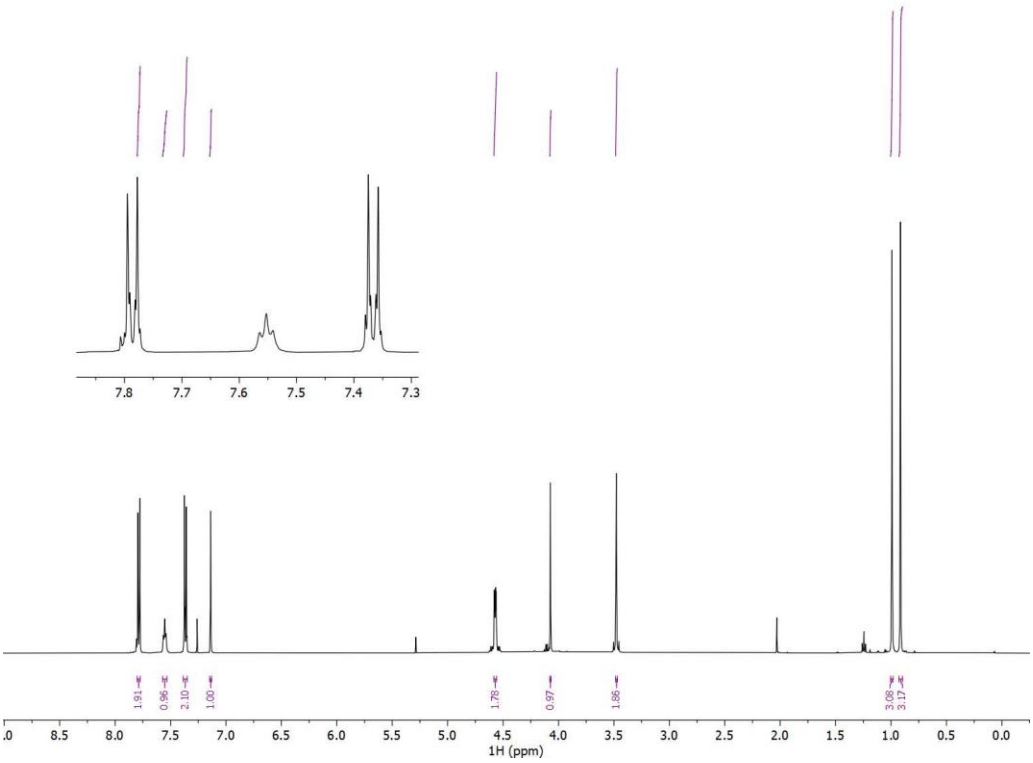

173.31
172.74
152.01
114.61
77.64
70.90
39.53
38.85
33.37
31.44
30.09
28.73
22.48
21.78
20.38
14.03
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

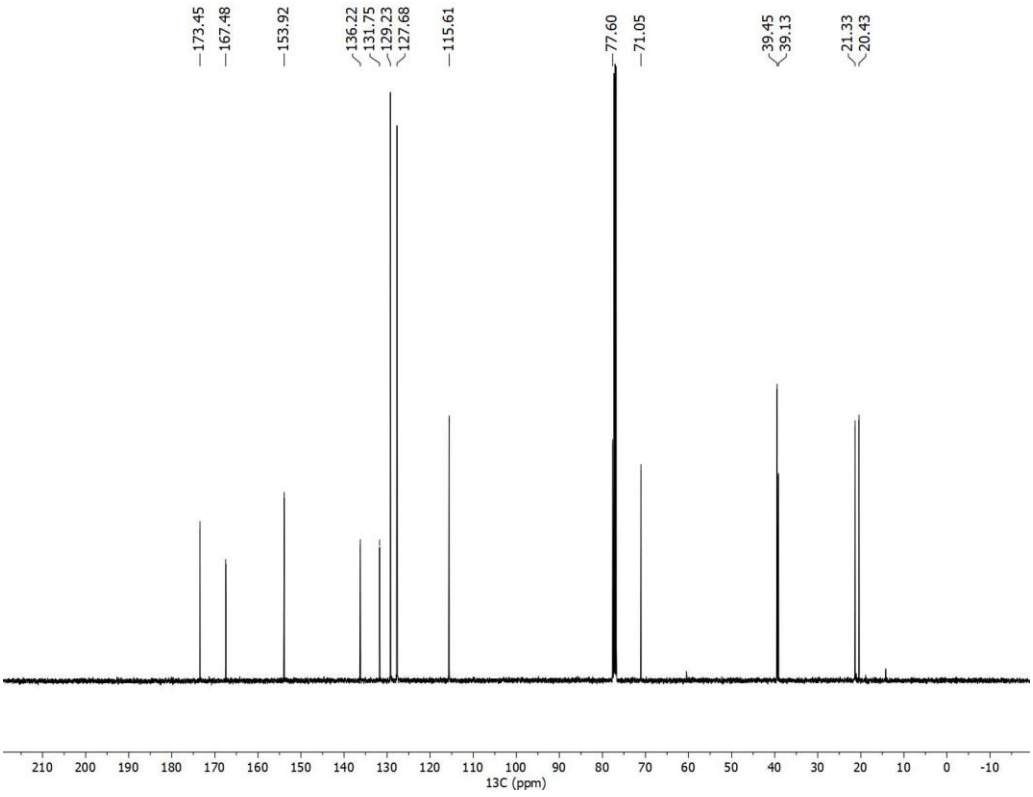
Compound 3c


4.68 4.66 4.64 4.62 4.60 4.58 4.56 4.54
3.65 3.60 3.55 3.50 3.45 3.40 3.35
1.83
1.00
3.12
1.00
1.97
0.88
1.13
1.12
2.99
2.89
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)

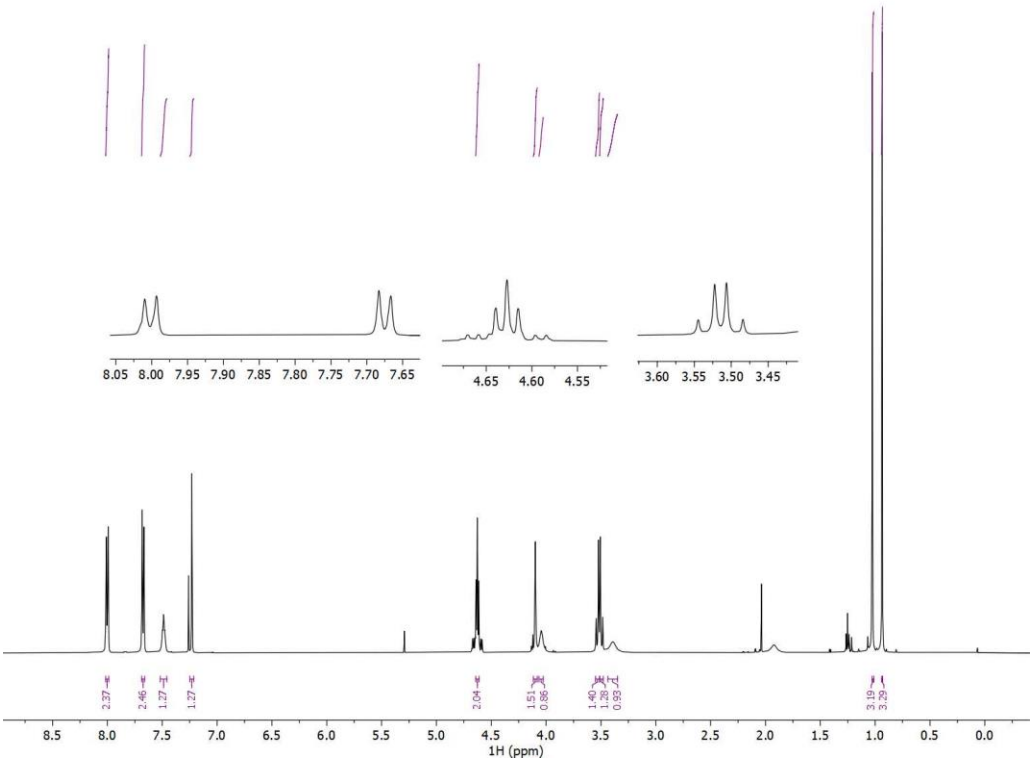

173.12
168.93
153.67
133.30
130.27
129.03
126.53
115.39
77.79
71.13
39.51
39.18
21.56
20.33
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
13C (ppm)

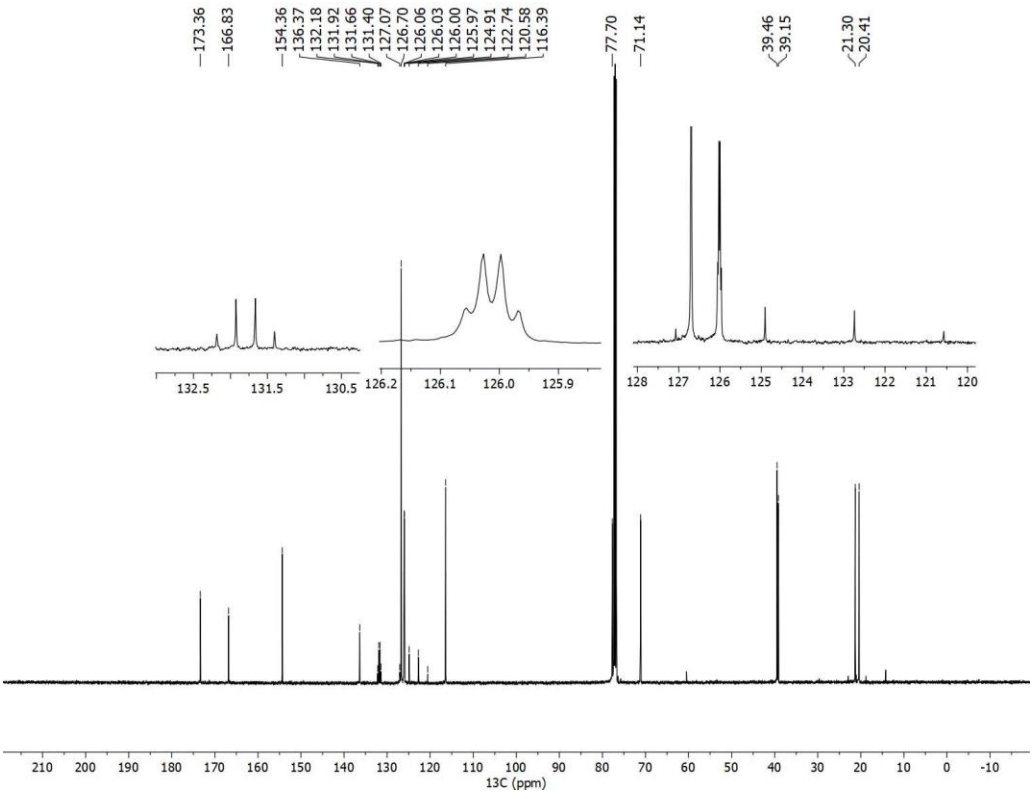
Compound 3d

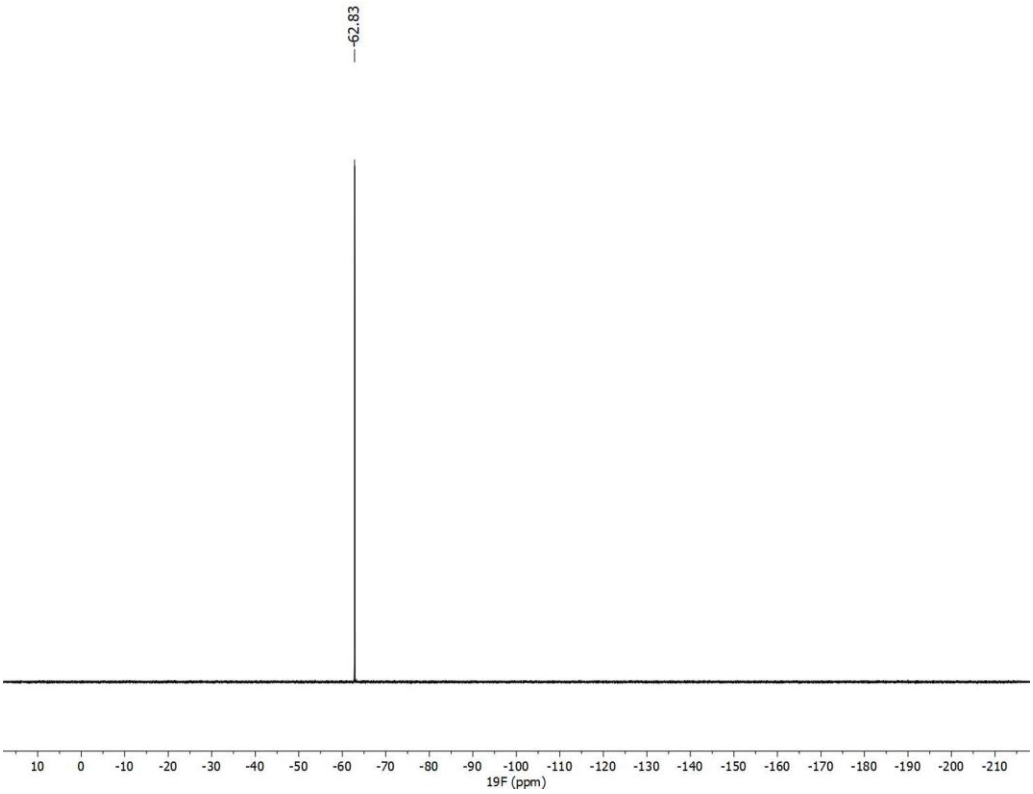

7.8
7.7
7.6
7.5
7.4
7.3
1.91
0.96
2.10
1.00
1.78
0.97
1.86
3.09
3.17
1H (ppm)


173.45
167.48
153.92
136.22
131.75
129.23
127.68
115.61
77.60
71.05
39.45
39.13
21.33
20.43
13C (ppm)

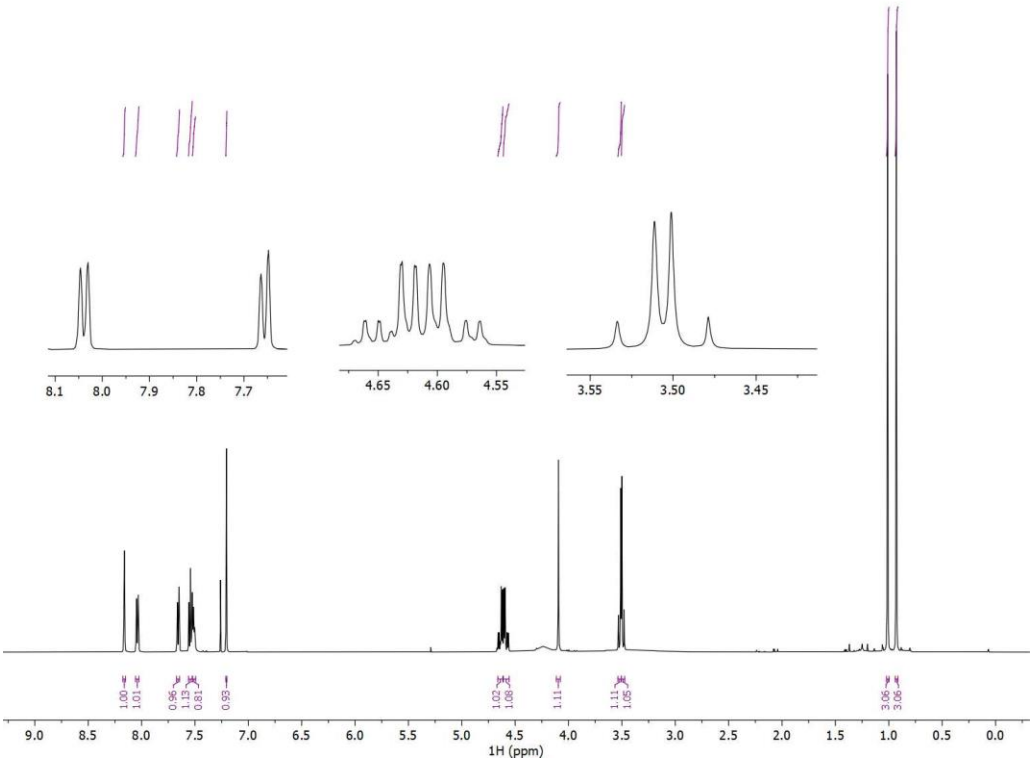
Compound 3e

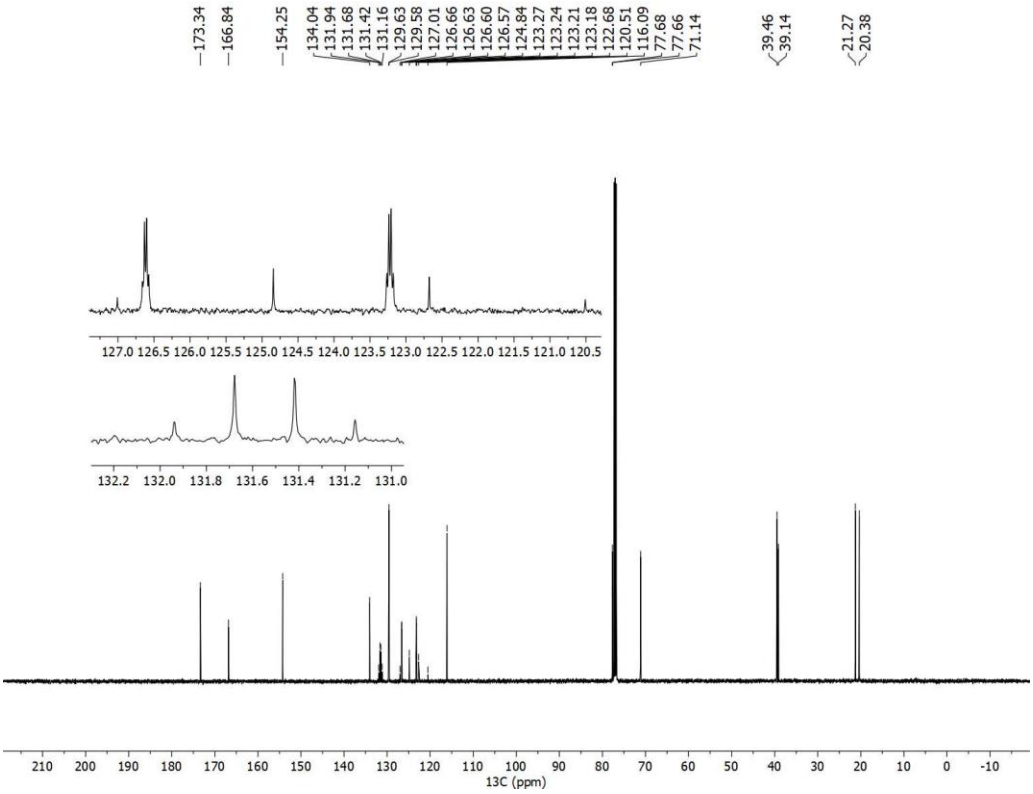

8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65
4.65 4.60 4.55
3.60 3.55 3.50 3.45
2.37
2.46
1.27
1.27
2.04
1.51
0.86
1.40
1.28
0.93
3.19
3.29
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)

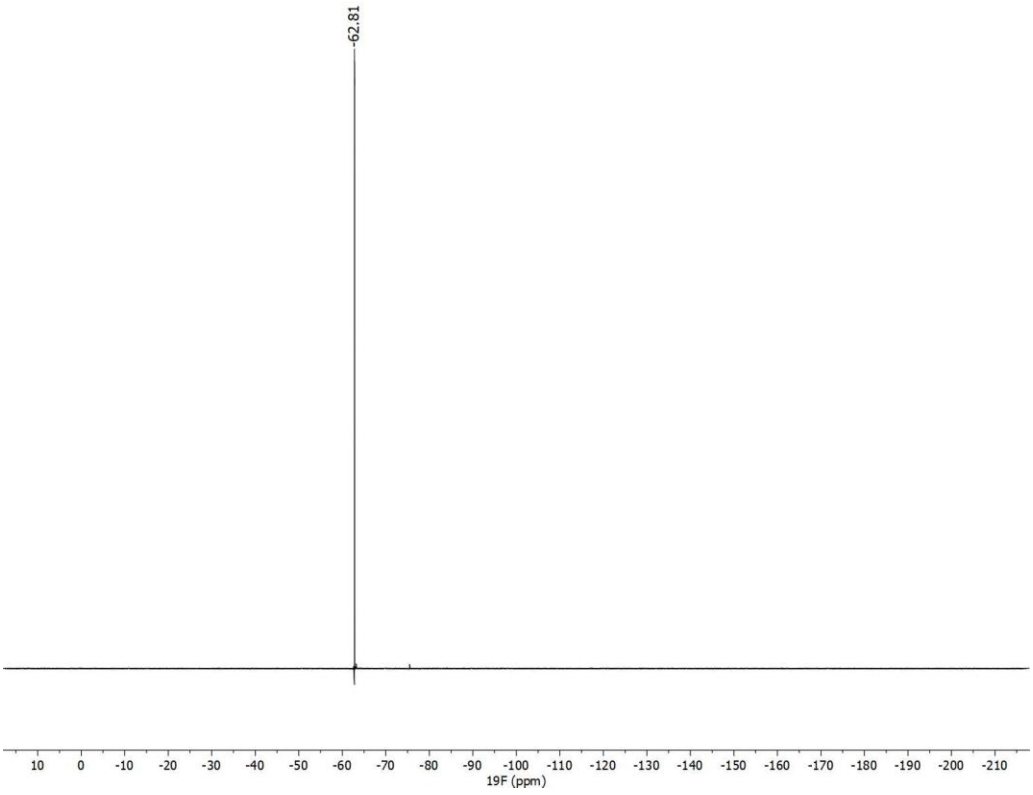

173.36
166.83
154.36
136.37
132.18
131.92
131.66
131.40
127.07
126.70
126.06
126.03
126.00
125.97
124.91
122.74
120.58
116.39
77.70
71.14
39.46
39.15
21.30
20.41
132.5 131.5 130.5
126.2 126.1 126.0 125.9
128 127 126 125 124 123 122 121 120
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)


-62.83
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

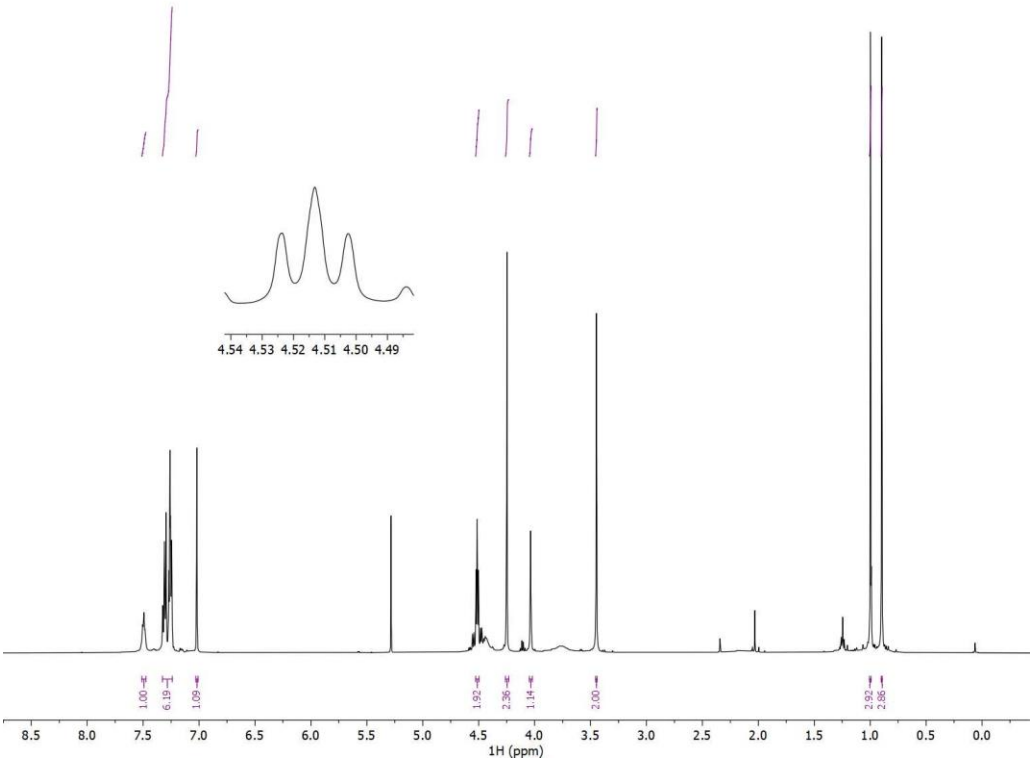
Compound 3f

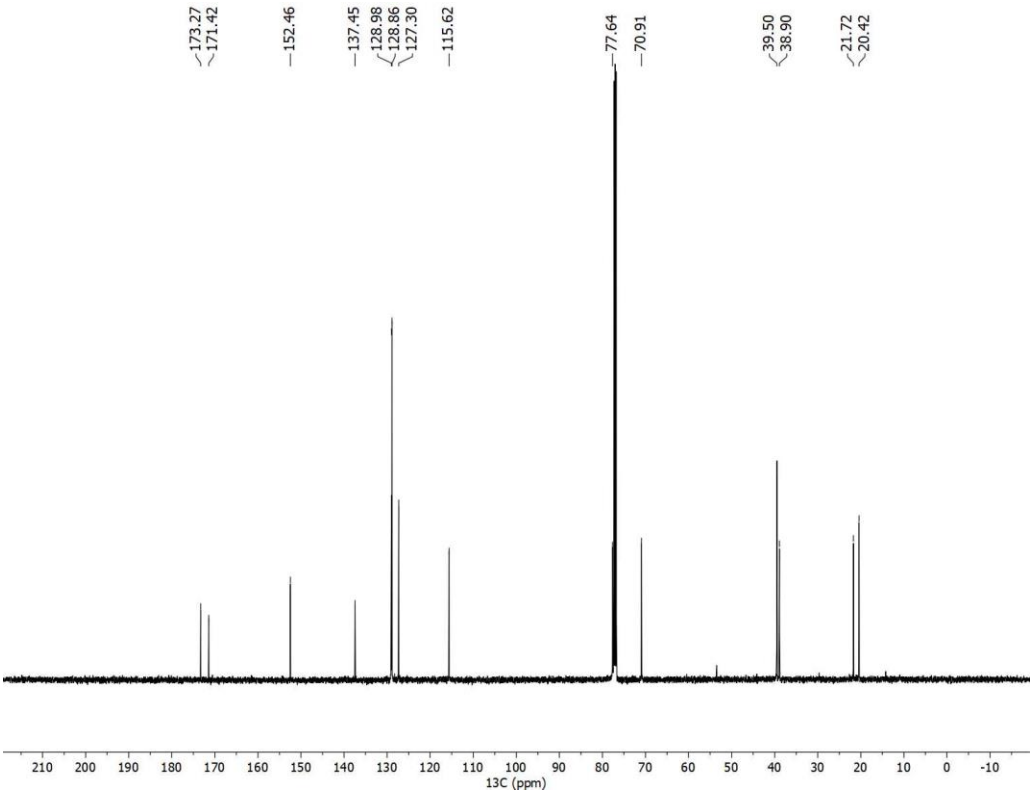

8.1 8.0 7.9 7.8 7.7
4.65 4.60 4.55
3.55 3.50 3.45
1.00 1.01 0.96 1.13 0.81 0.93 1.02 1.08 1.11 1.11 1.05 3.06 3.06
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)


173.34
166.84
154.25
134.04
131.94
131.68
131.42
131.16
129.63
129.58
127.01
126.66
126.63
126.60
126.57
124.84
123.27
123.24
123.21
123.18
122.68
120.51
116.09
77.68
77.66
71.14
39.46
39.14
21.27
20.38
127.0 126.5 126.0 125.5 125.0 124.5 124.0 123.5 123.0 122.5 122.0 121.5 121.0 120.5
132.2 132.0 131.8 131.6 131.4 131.2 131.0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

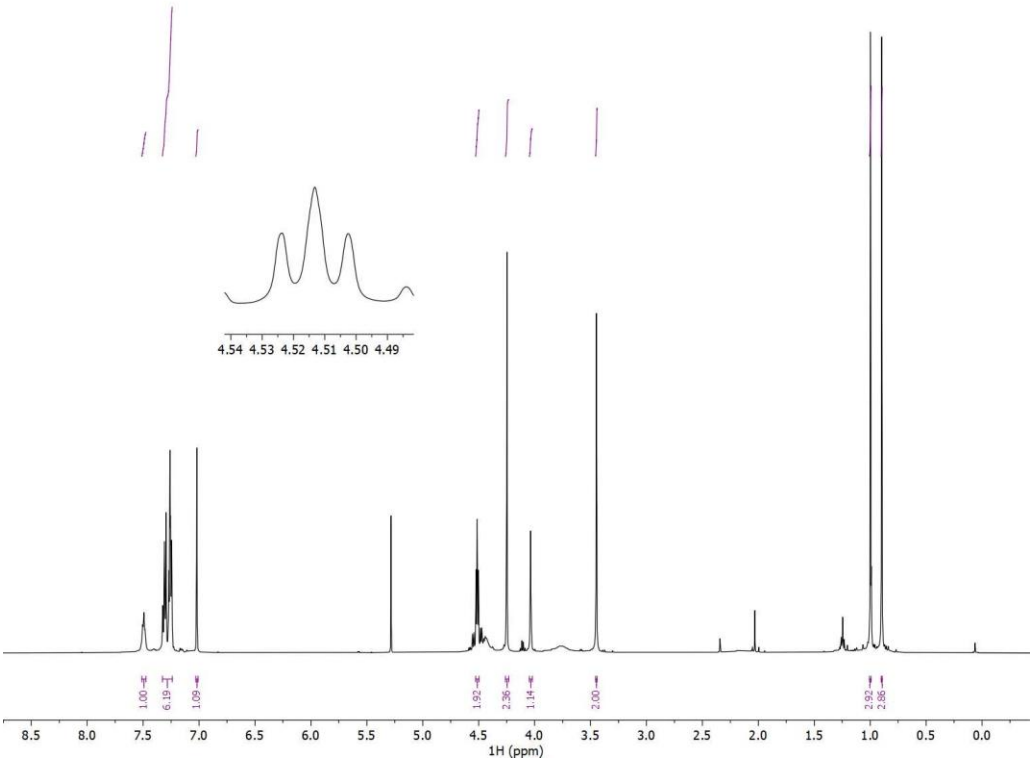

-62.81
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

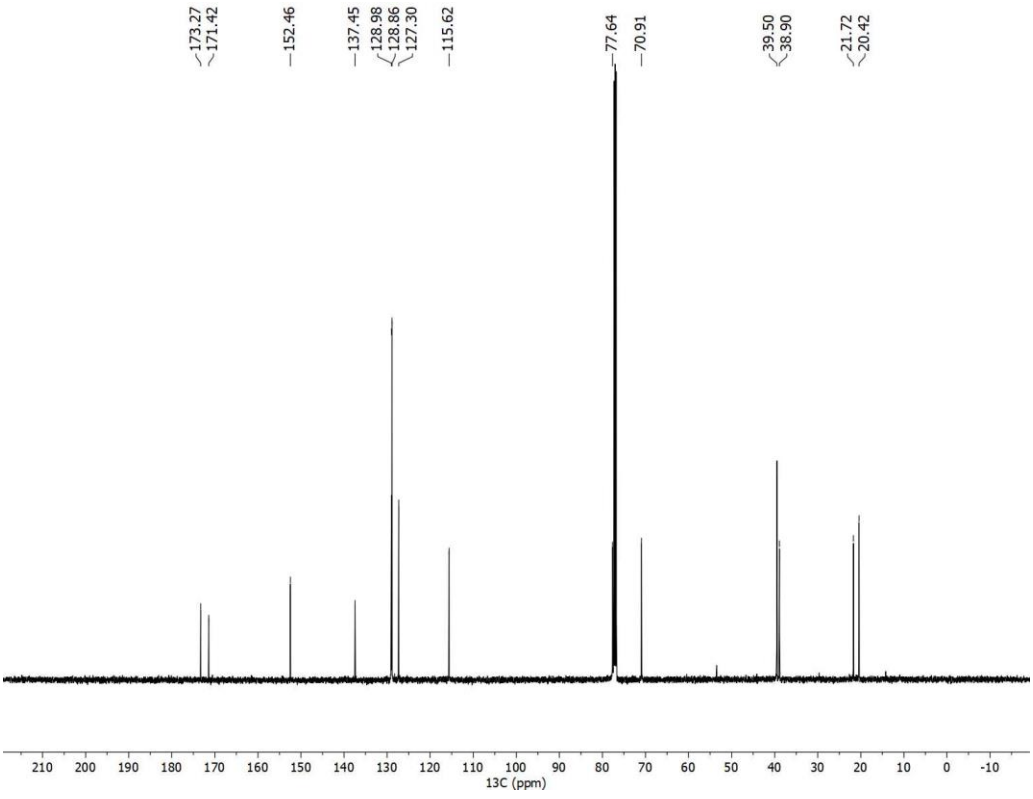
Compound 3g


4.54 4.53 4.52 4.51 4.50 4.49
1.00
6.19
1.09
1.92
2.36
1.14
2.00
2.92
2.86
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)

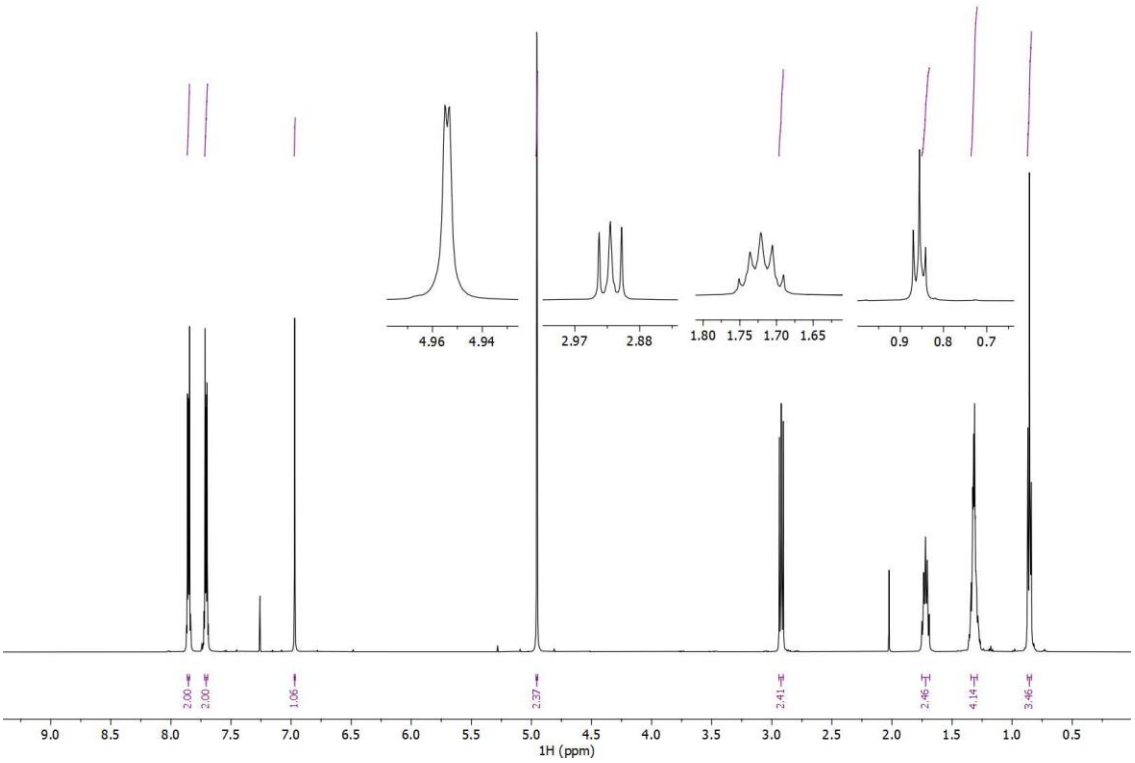

173.27
171.42
152.46
137.45
128.98
128.86
127.30
115.62
77.64
70.91
39.50
38.90
21.72
20.42
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

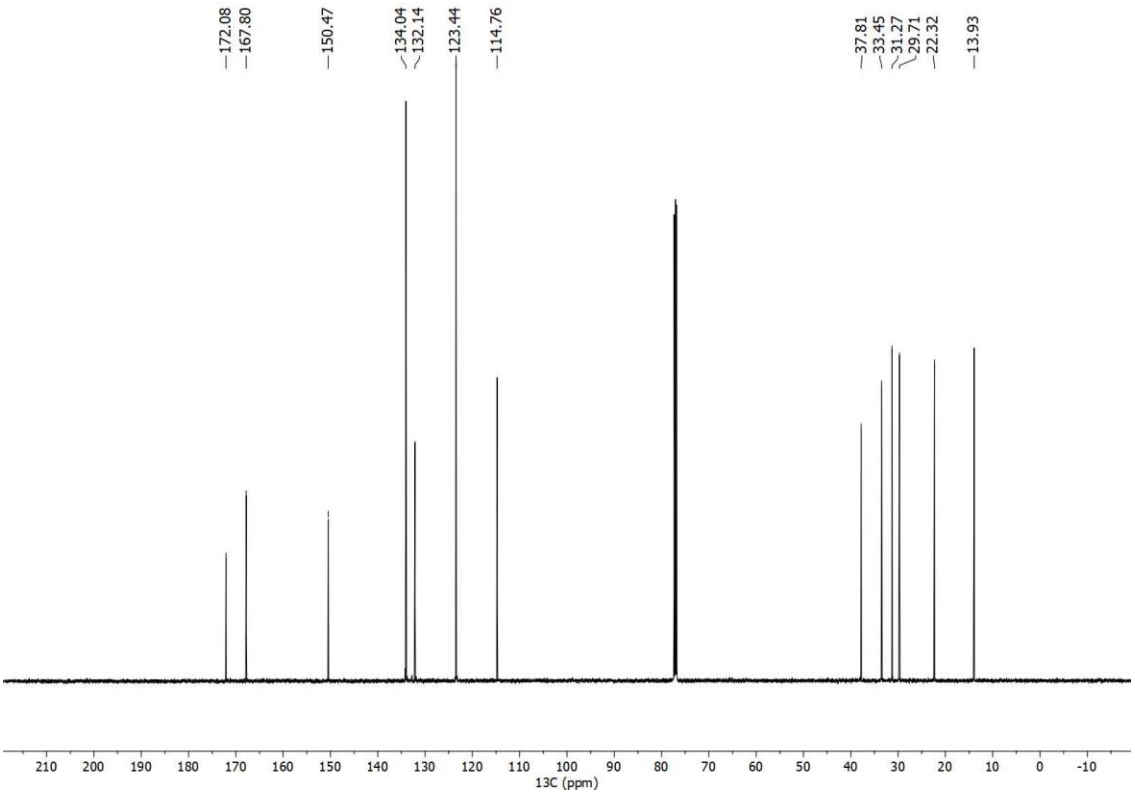
Compound 3h


4.54 4.53 4.52 4.51 4.50 4.49
1.00
6.19
1.09
1.92
2.36
1.14
2.00
2.92
2.86
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)

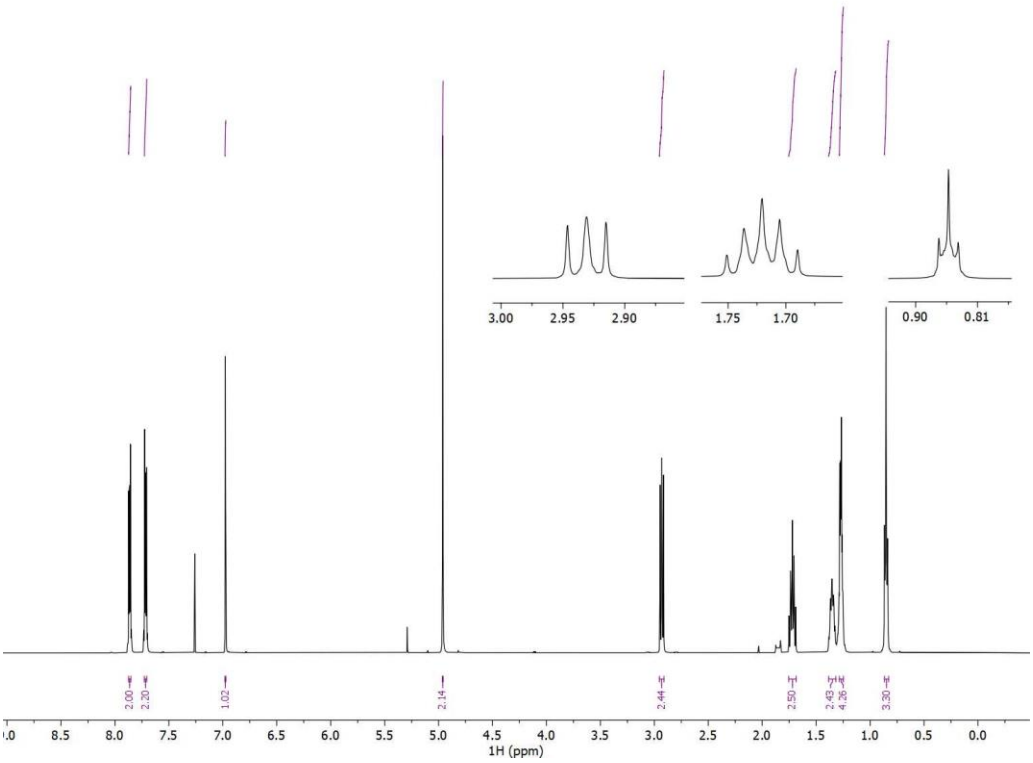

173.27
171.42
152.46
137.45
128.98
128.86
127.30
115.62
77.64
70.91
39.50
38.90
21.72
20.42
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

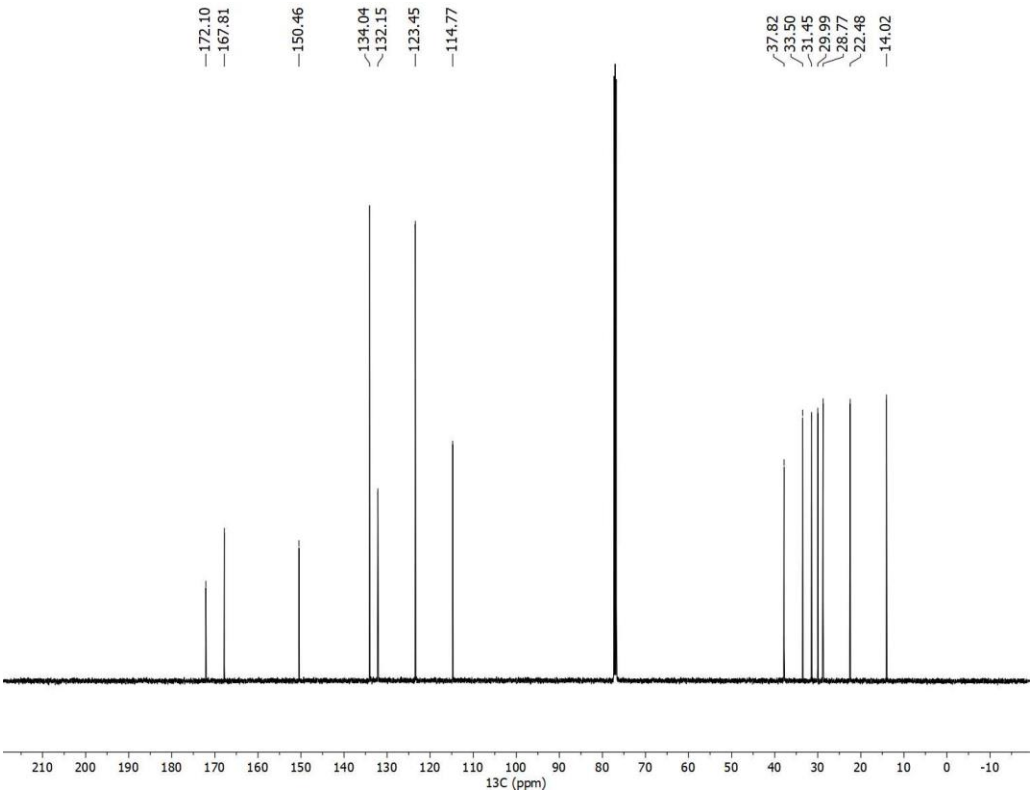
Compound 3.6a



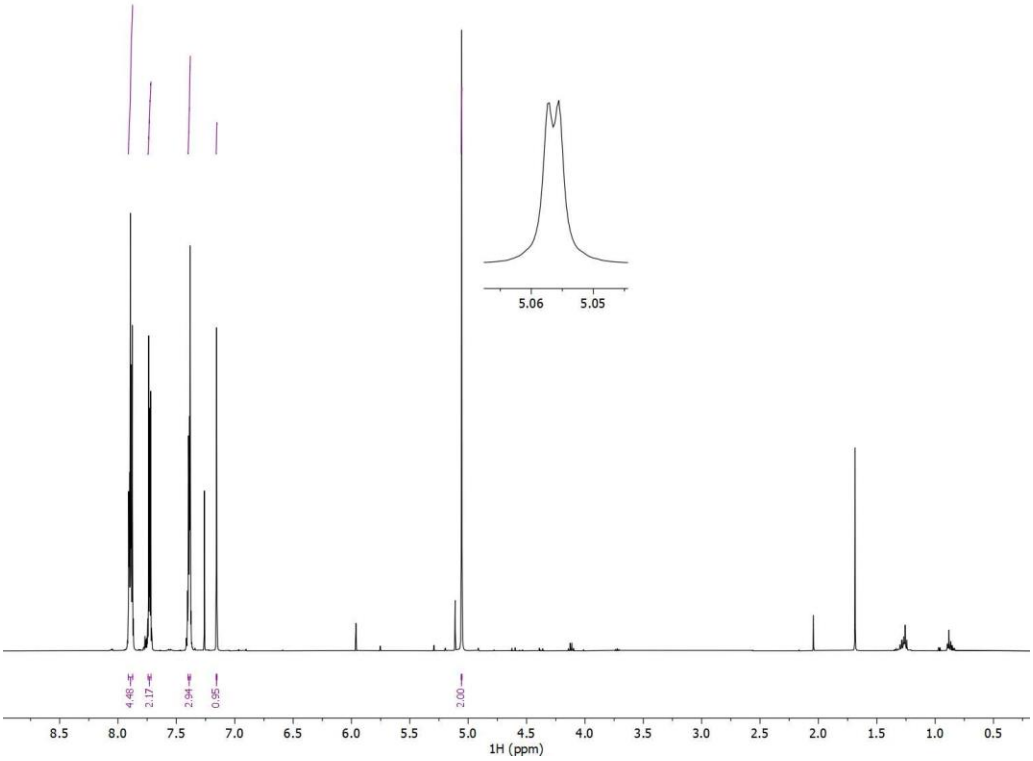


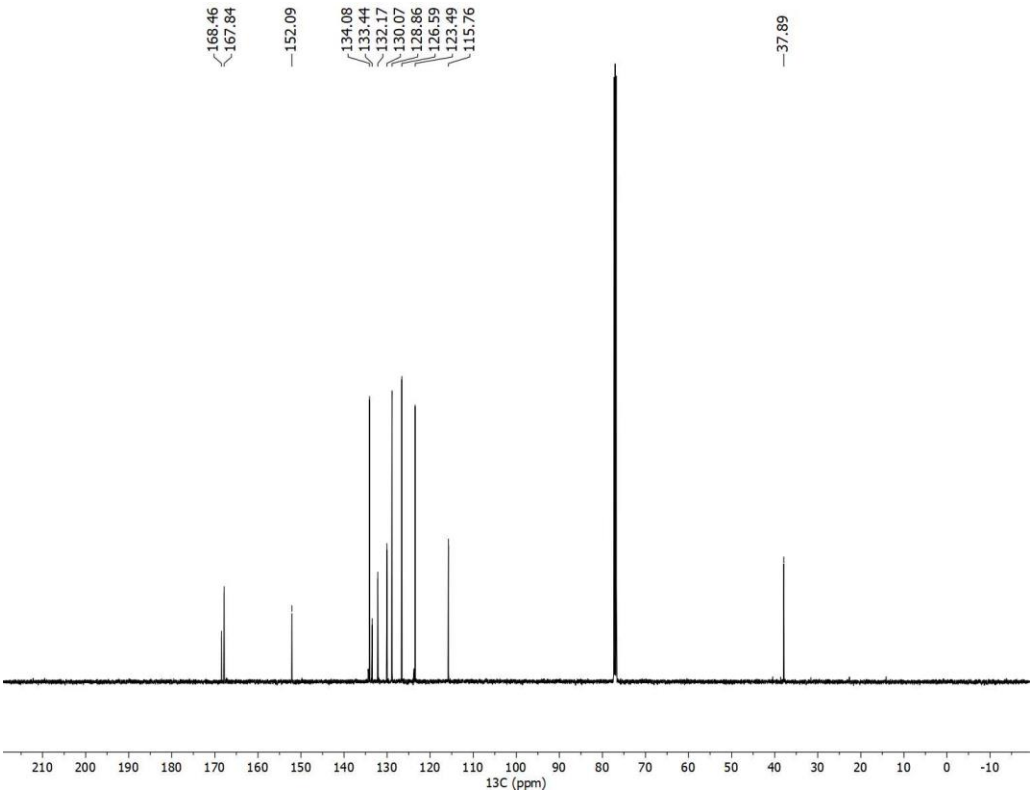
Compound 3.6b



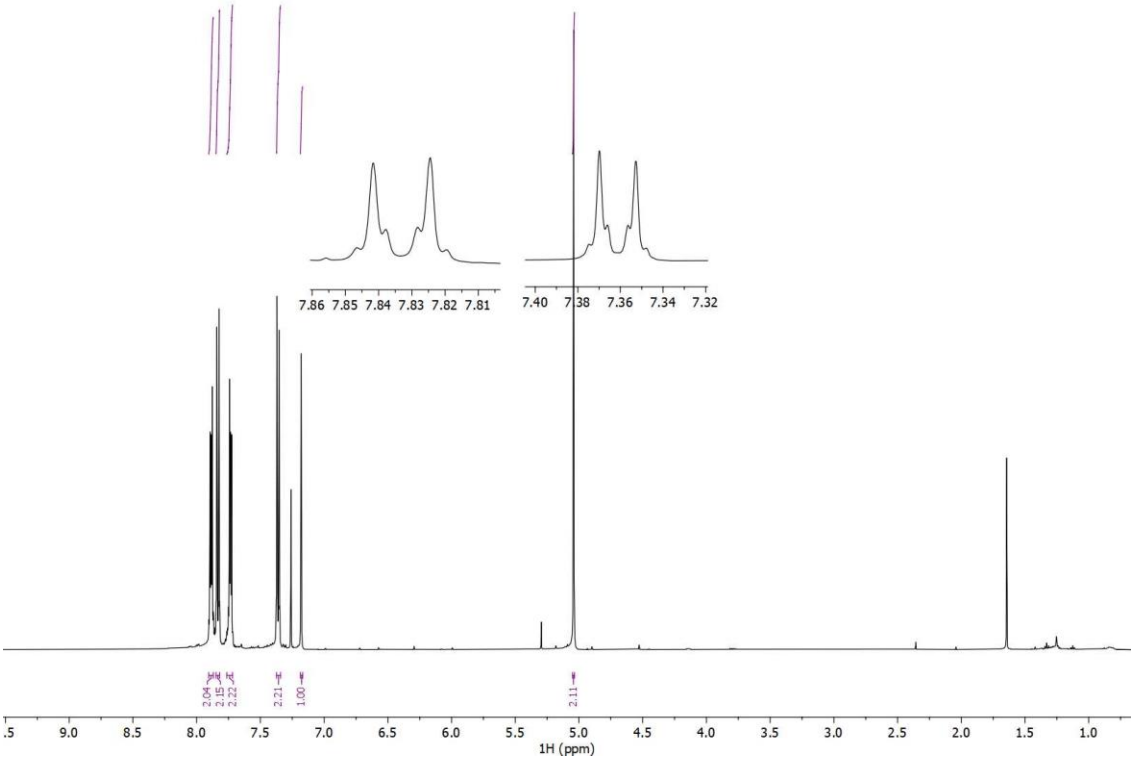


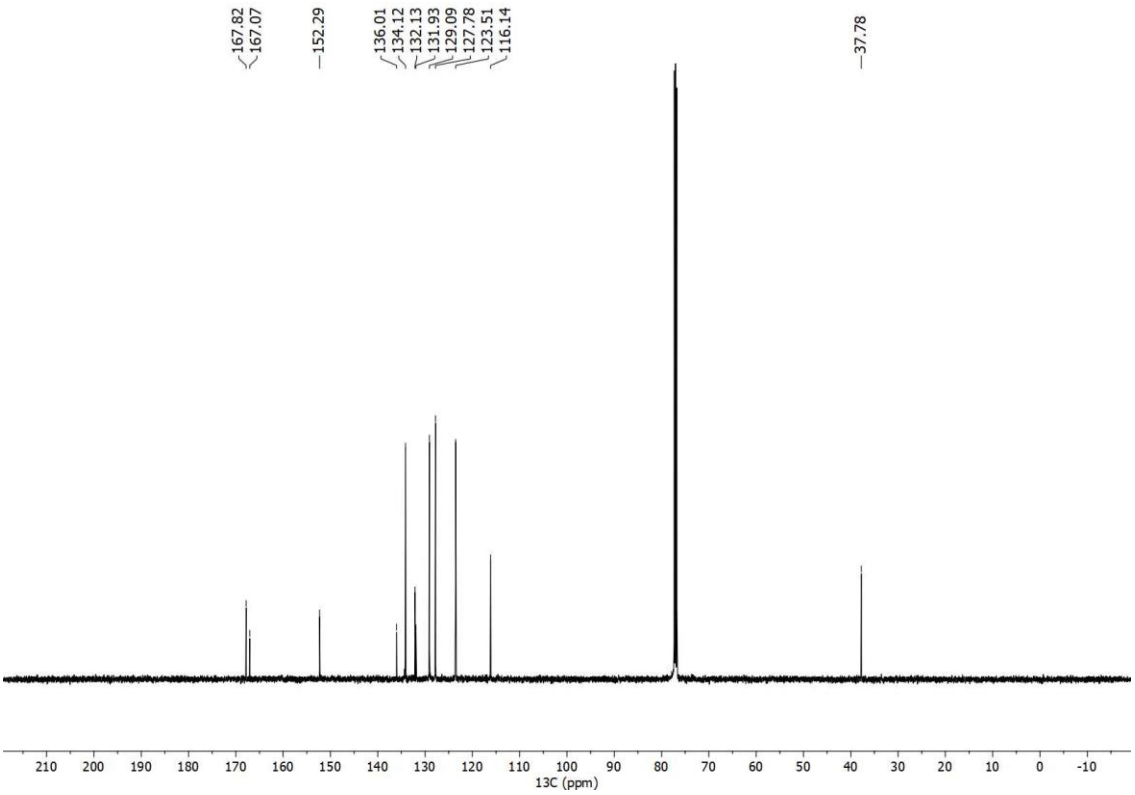
Compound 3.6c



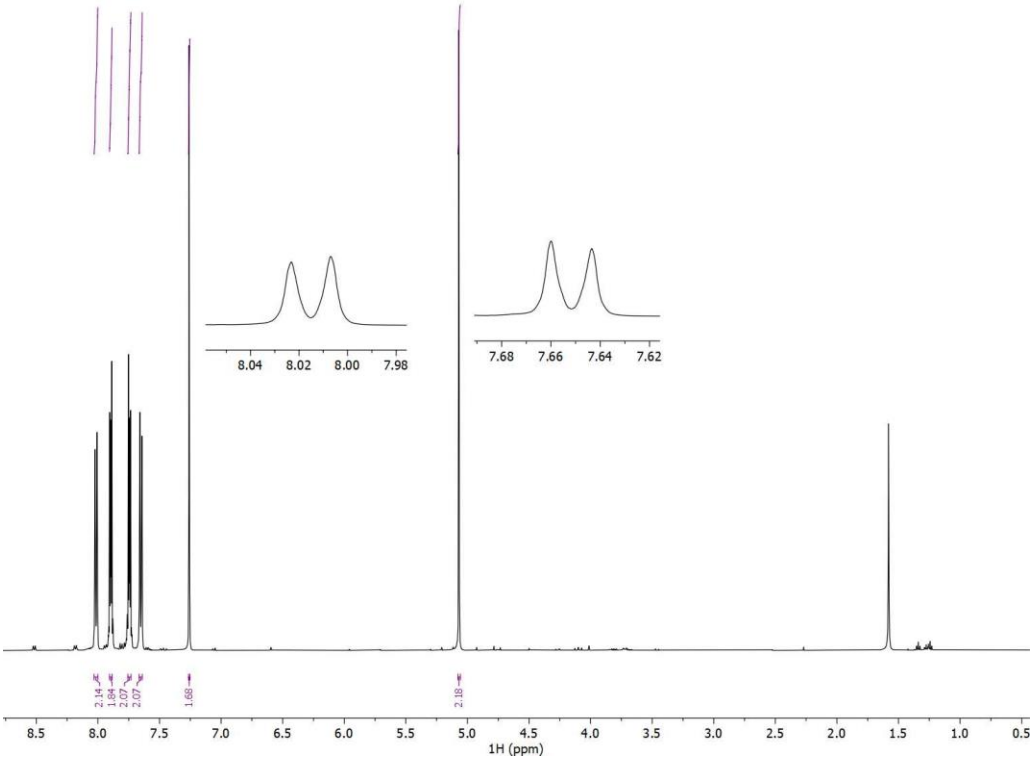


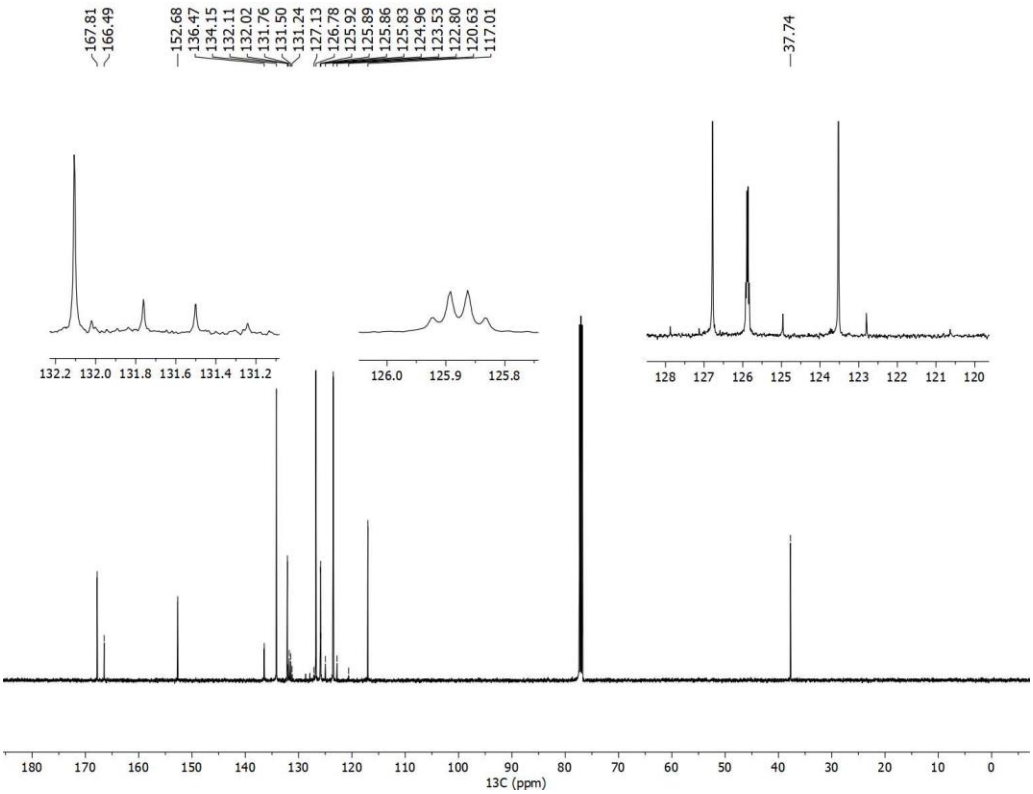
Compound 3.6d

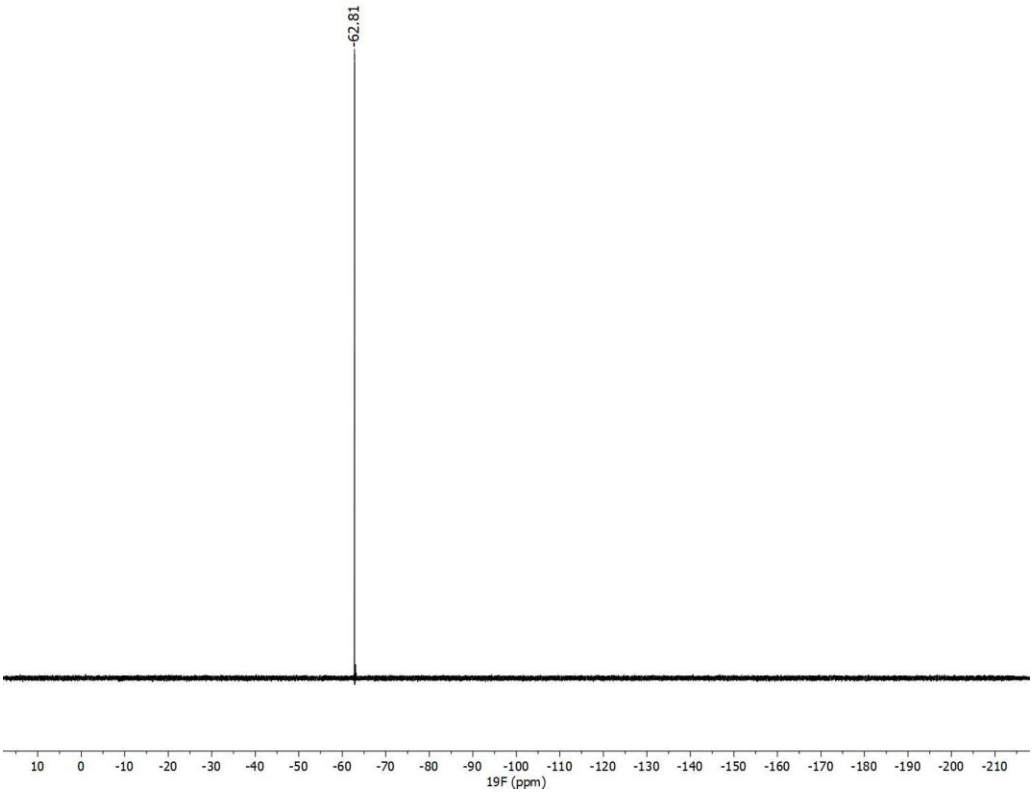




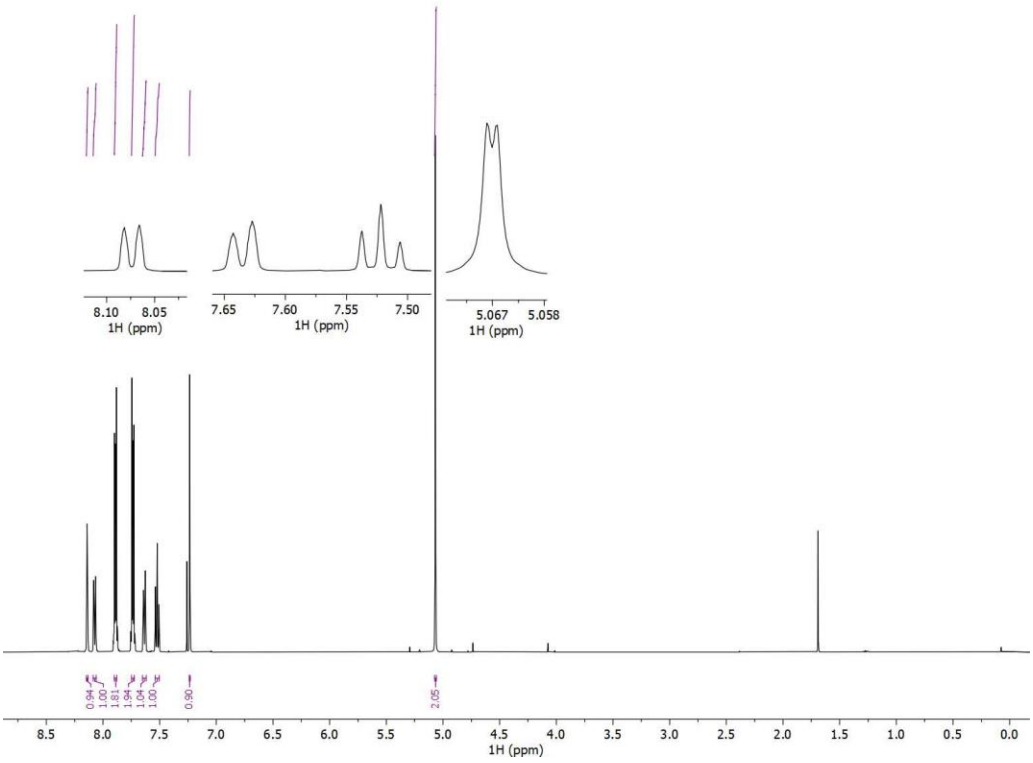
Compound 3.6e

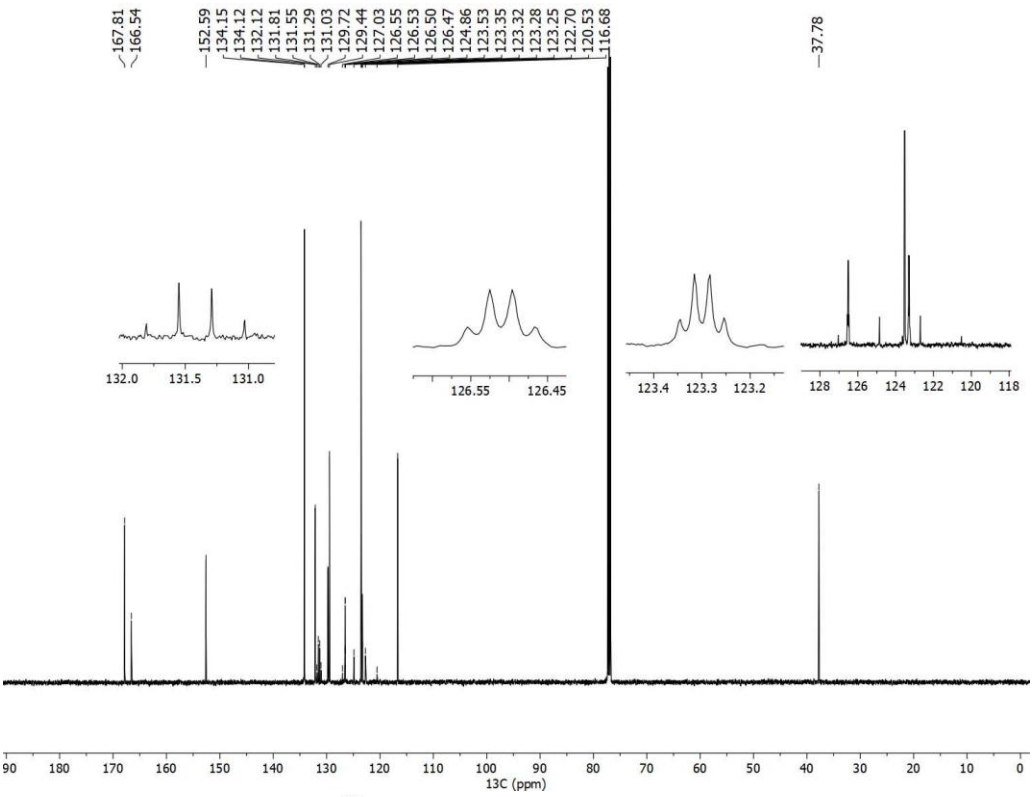


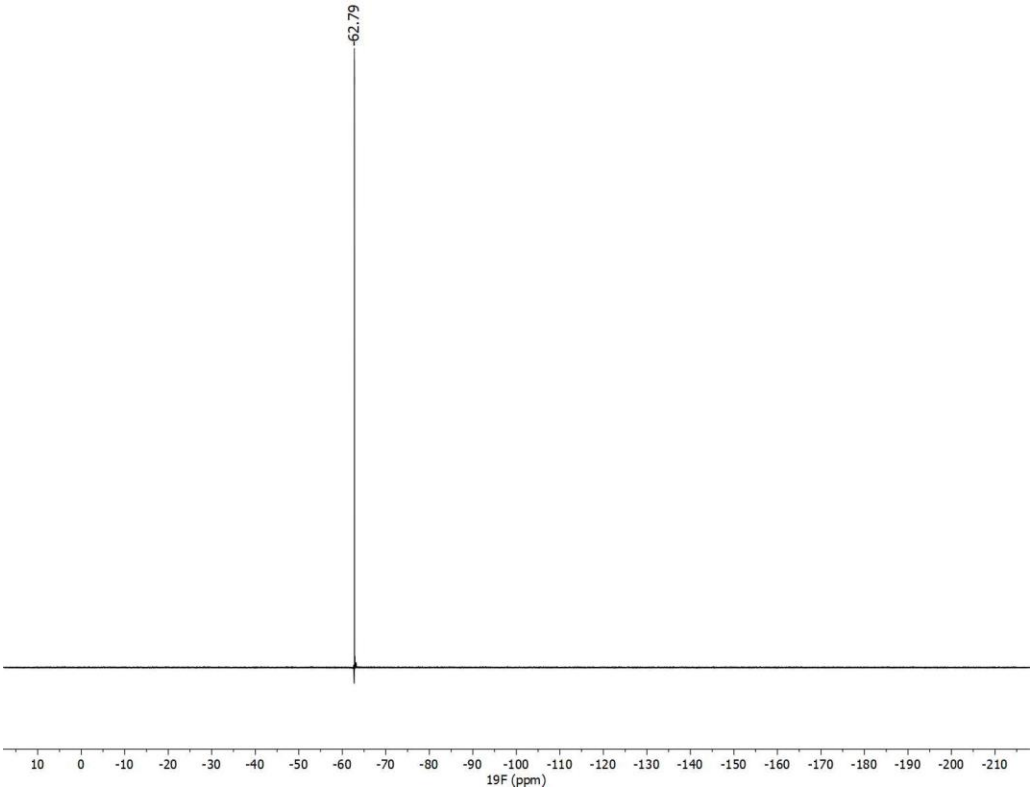




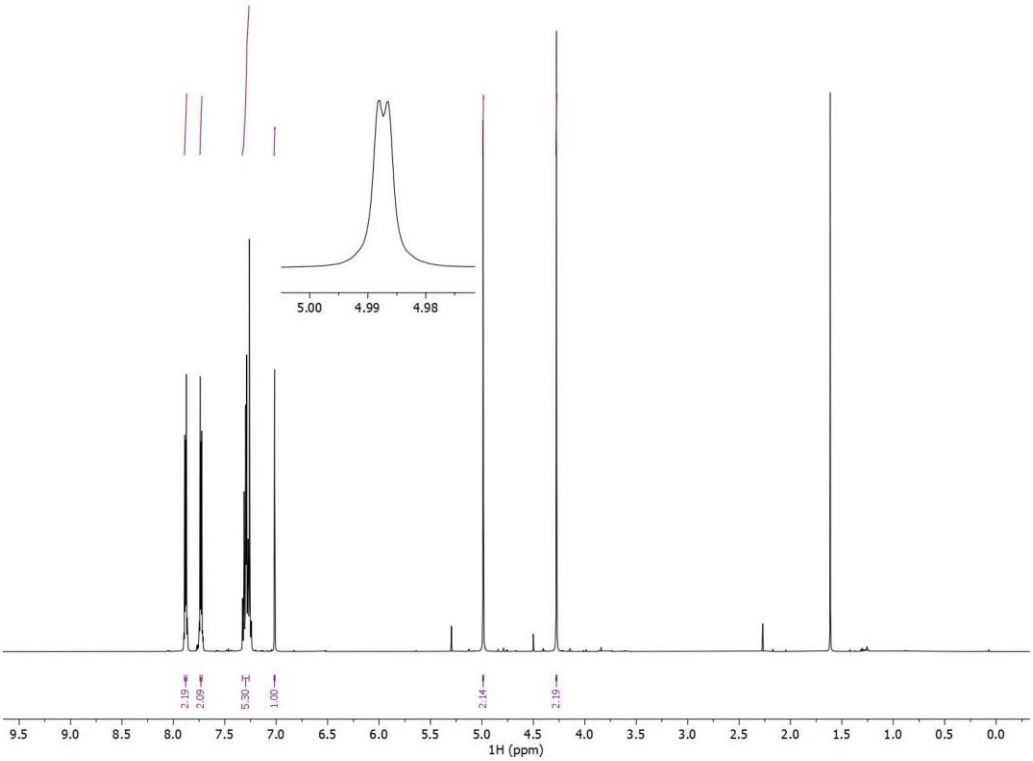
Compound 3.6f

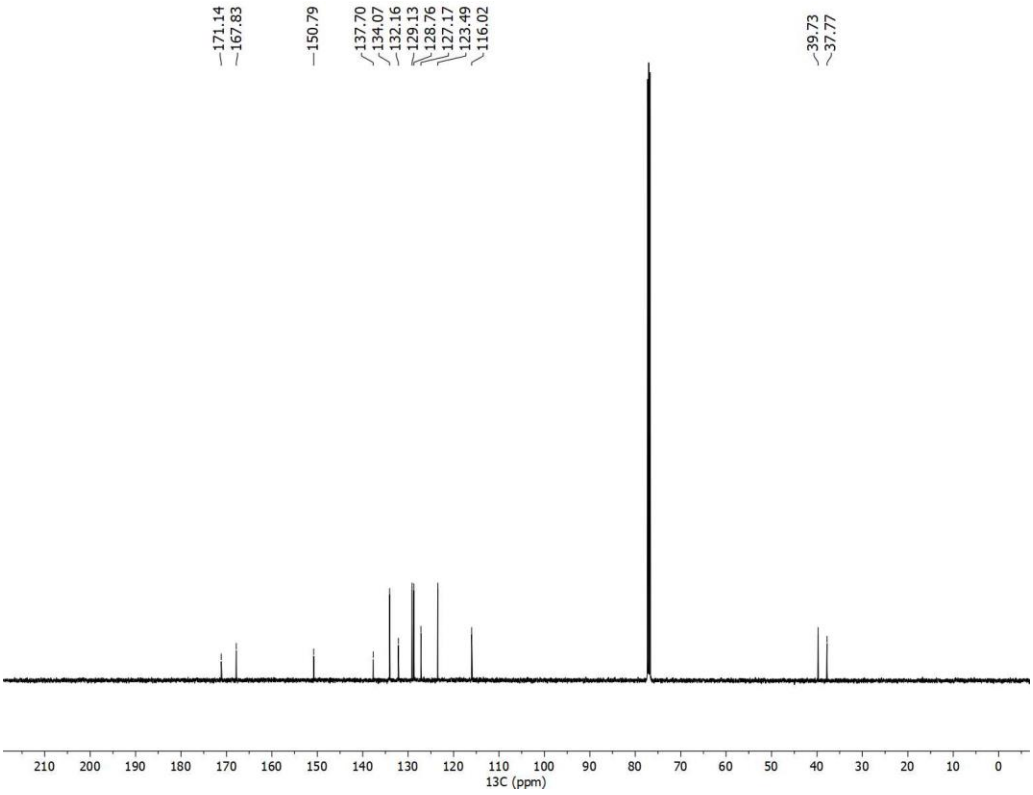



167.81
166.54
152.59
134.15
134.12
132.12
131.81
131.55
131.29
131.03
129.72
129.44
127.03
126.55
126.53
126.50
126.47
124.86
123.53
123.35
123.32
123.28
123.25
122.70
120.53
116.68
37.78
132.0
131.5
131.0
126.55
126.45
123.4
123.3
123.2
128
126
124
122
120
118
90
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
13C (ppm)

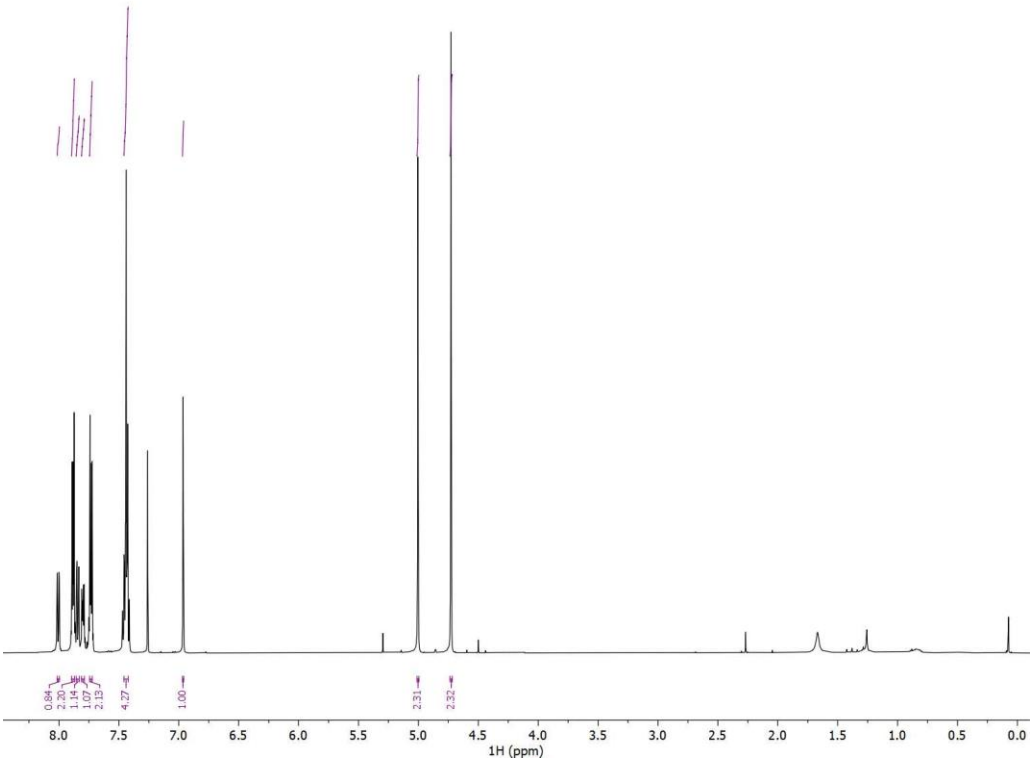

-62.79
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
19F (ppm)

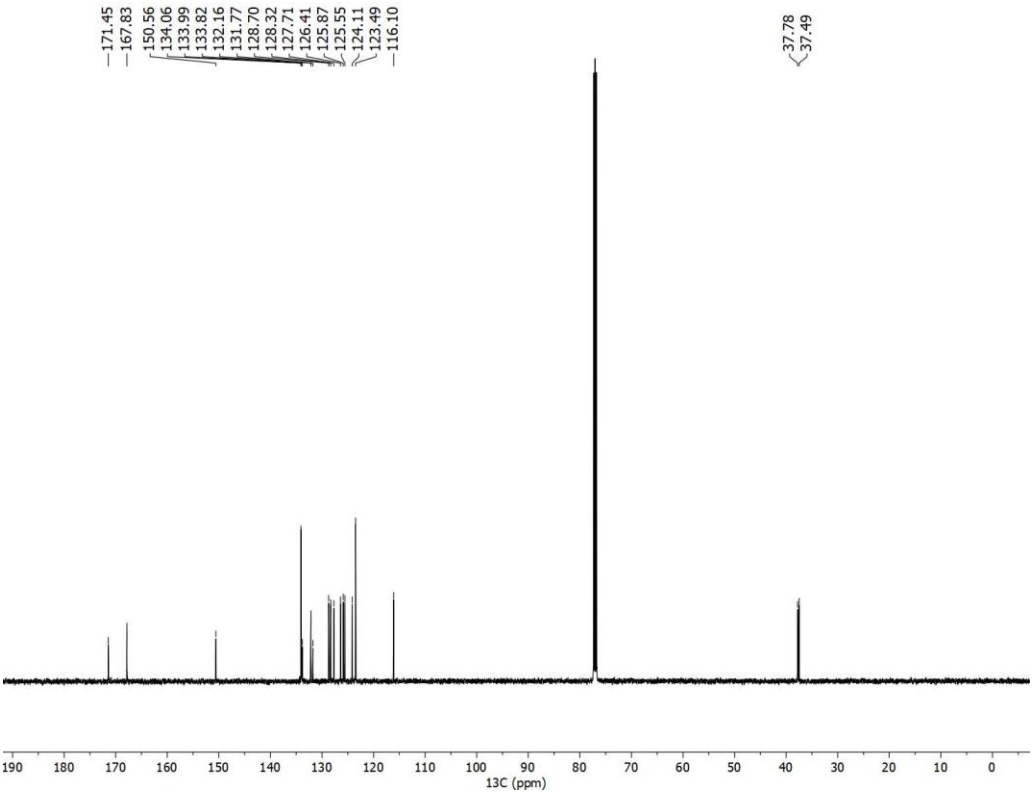
Compound 3.6g



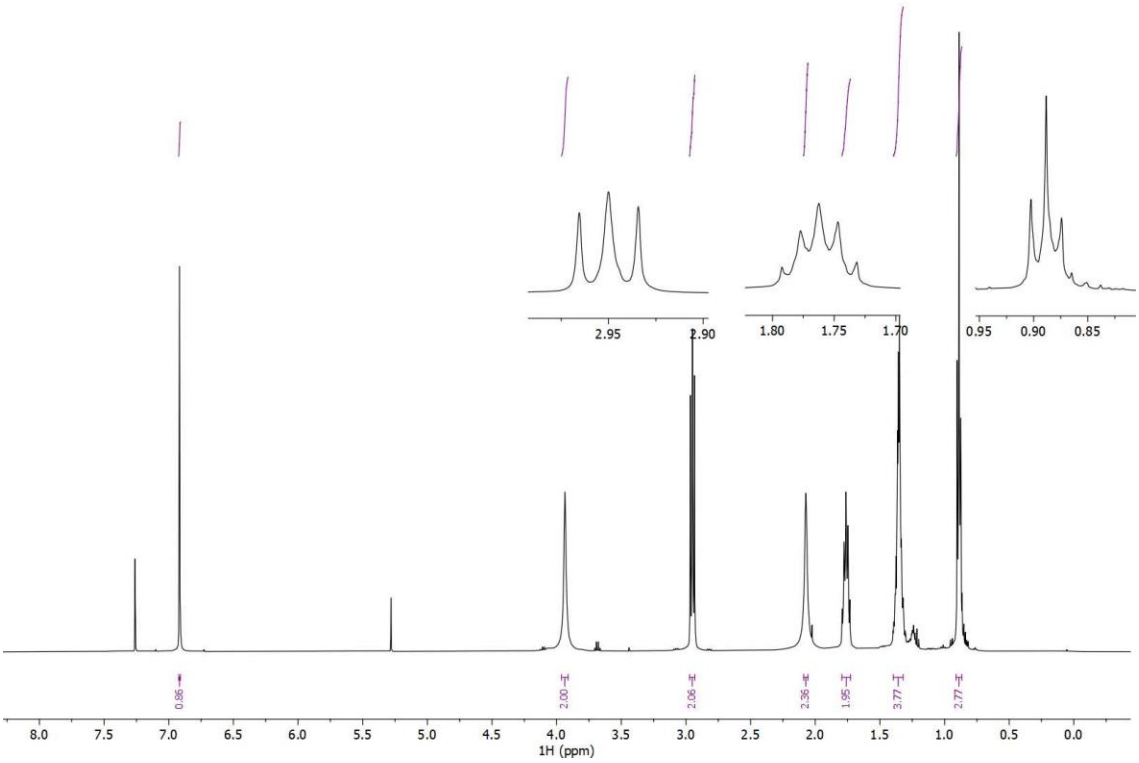


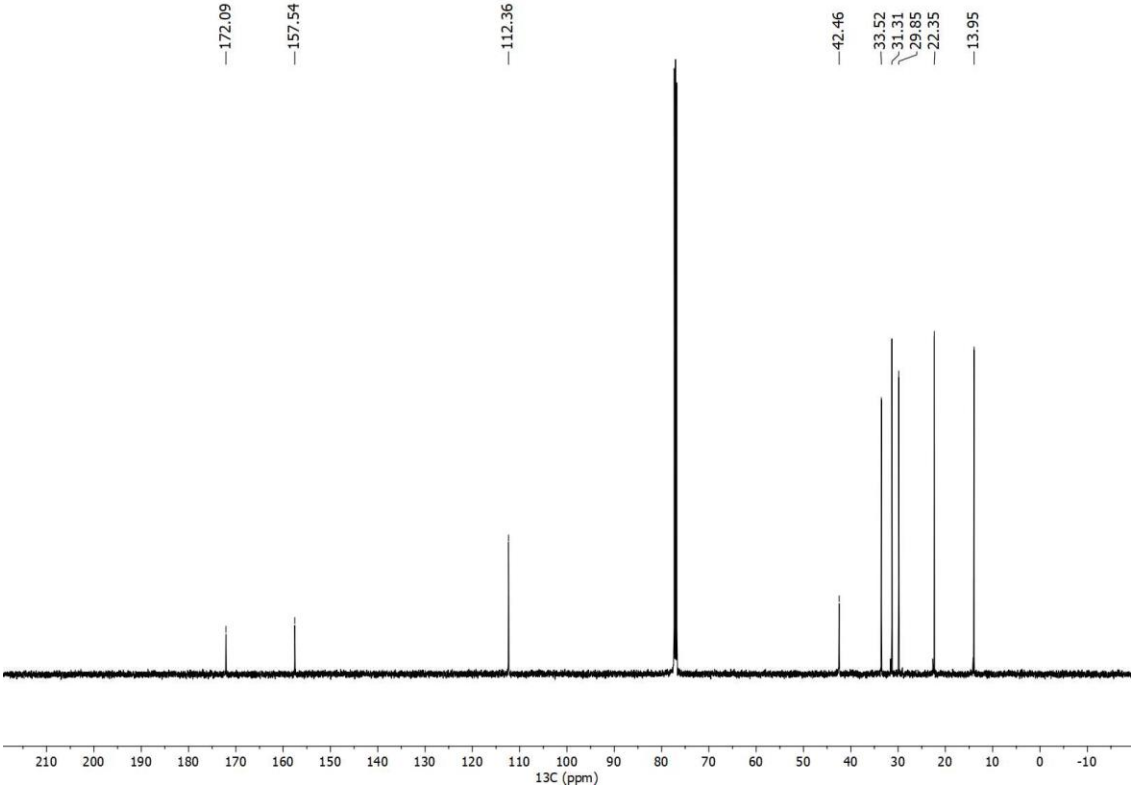
Compound 3.6h



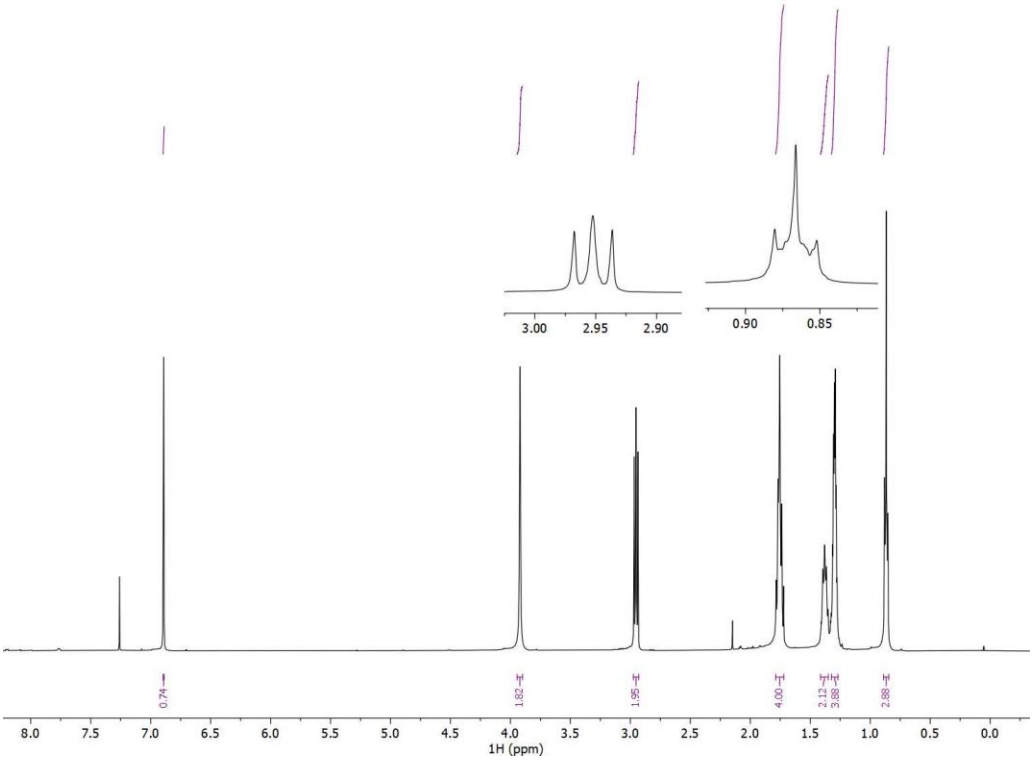


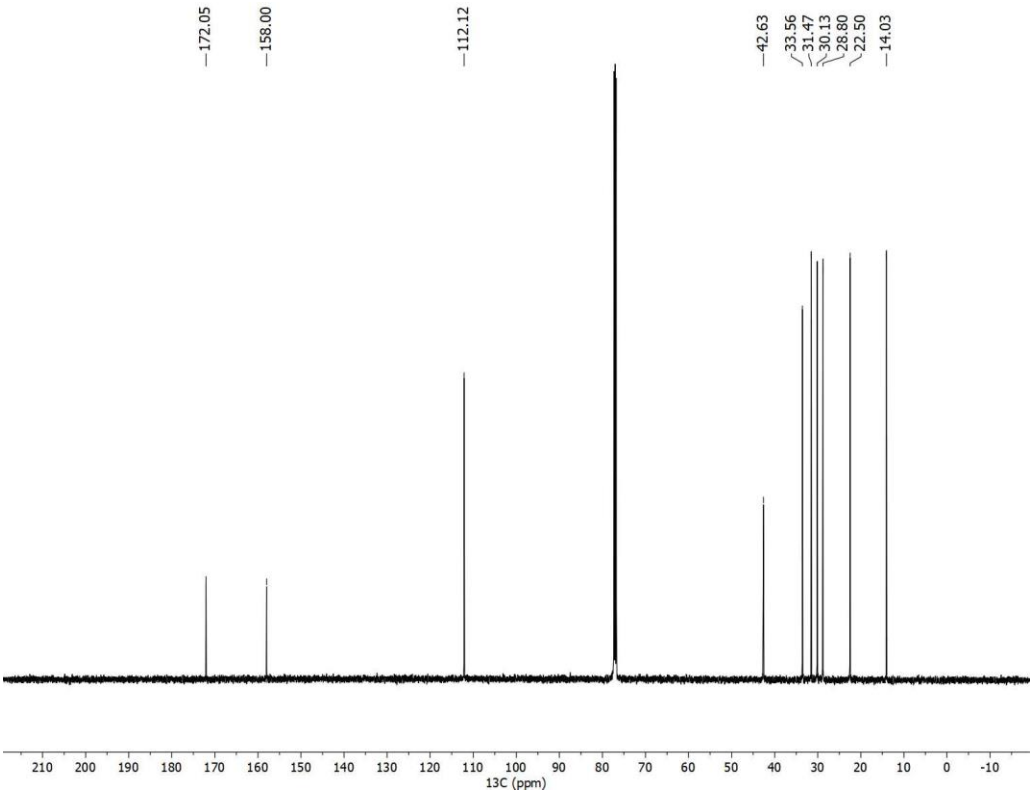
Compound 3.7a



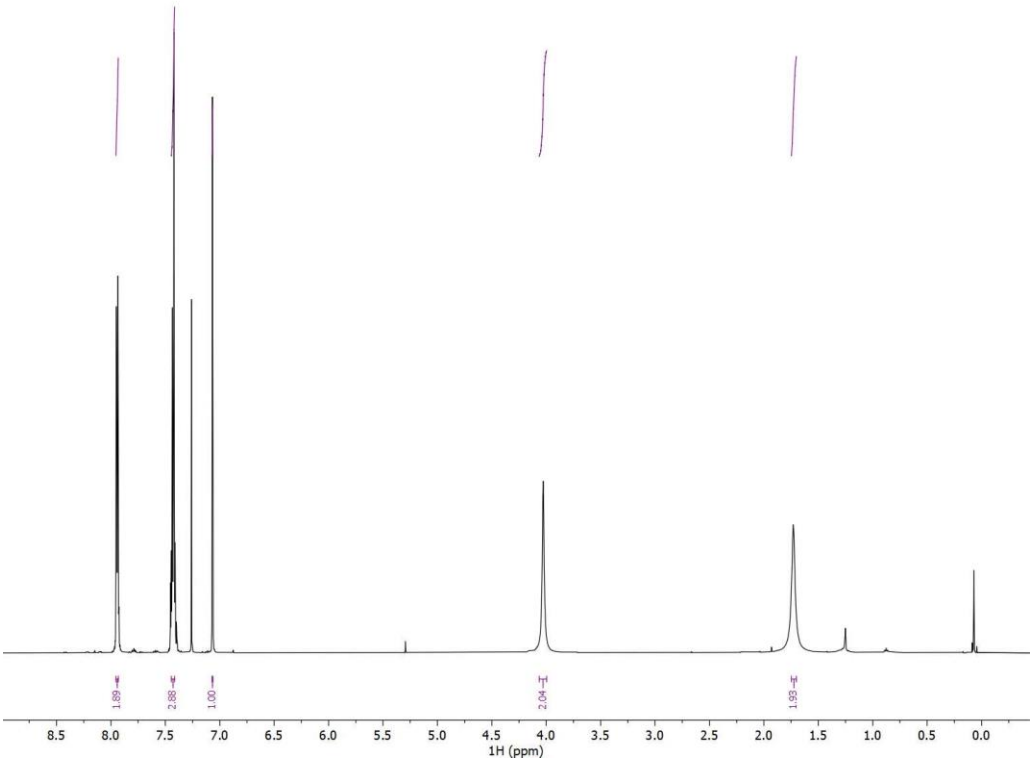


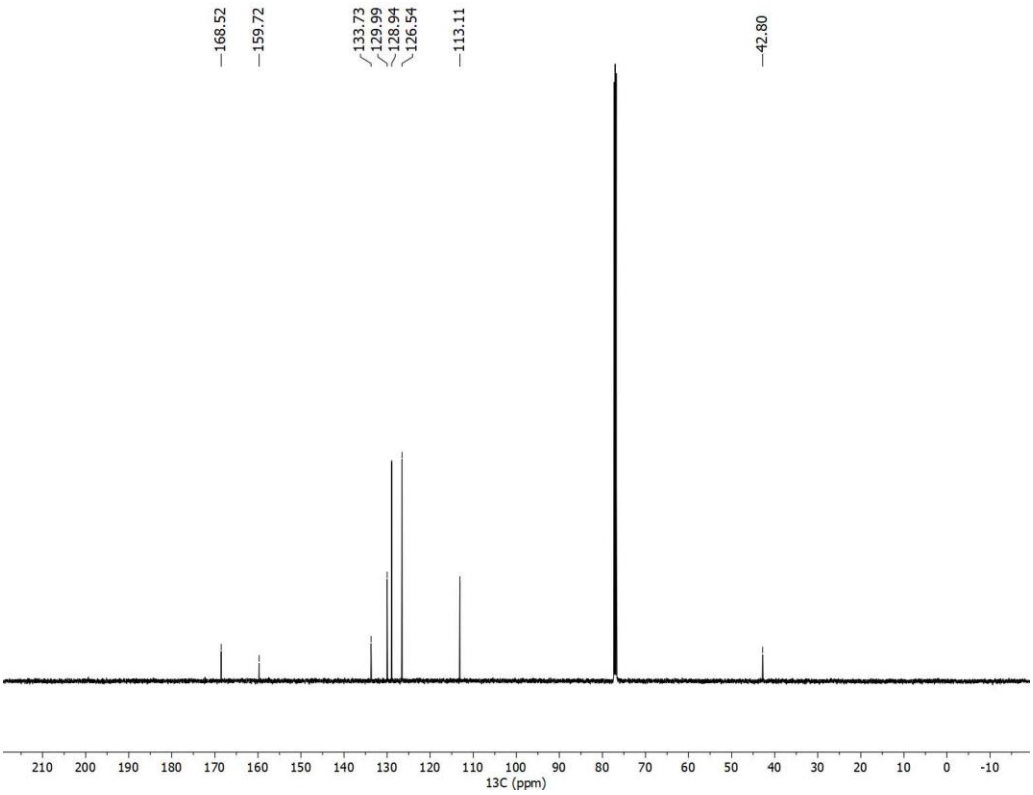
Compound 3.7b



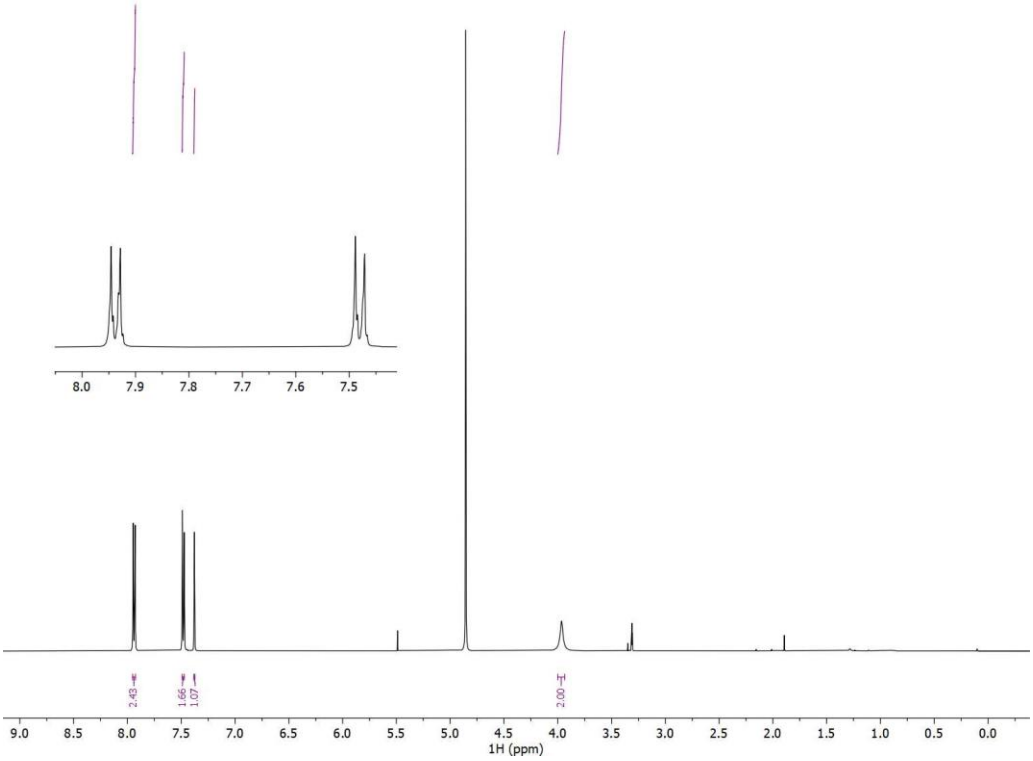


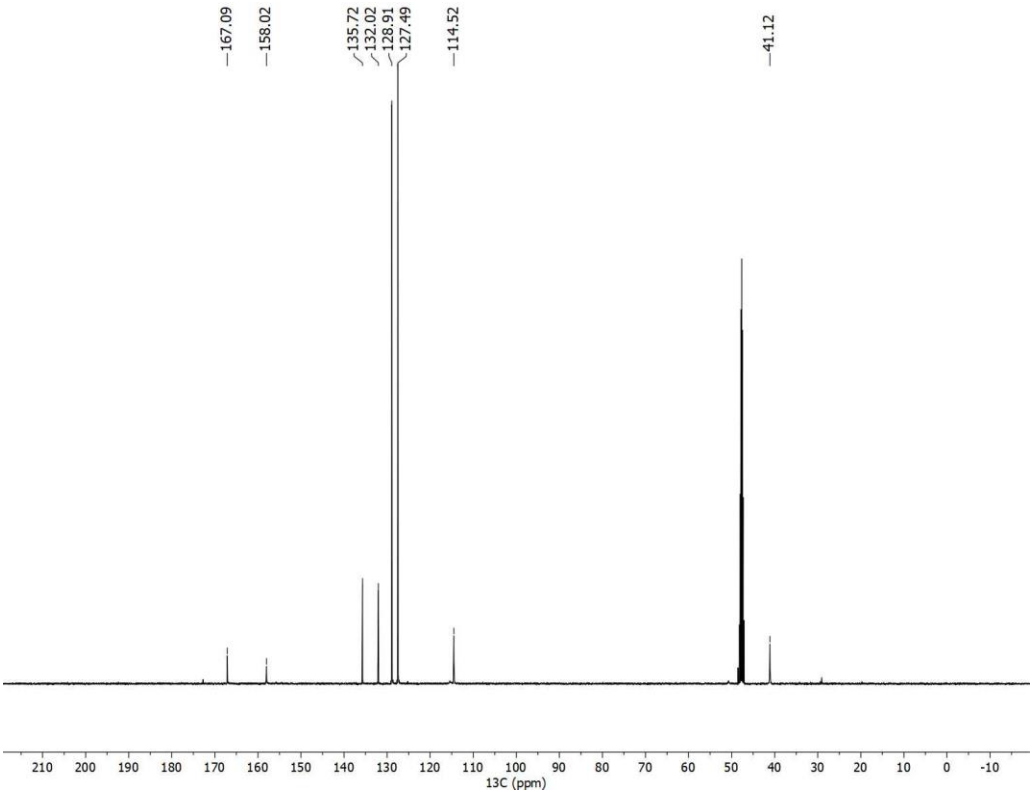
Compound 3.7c



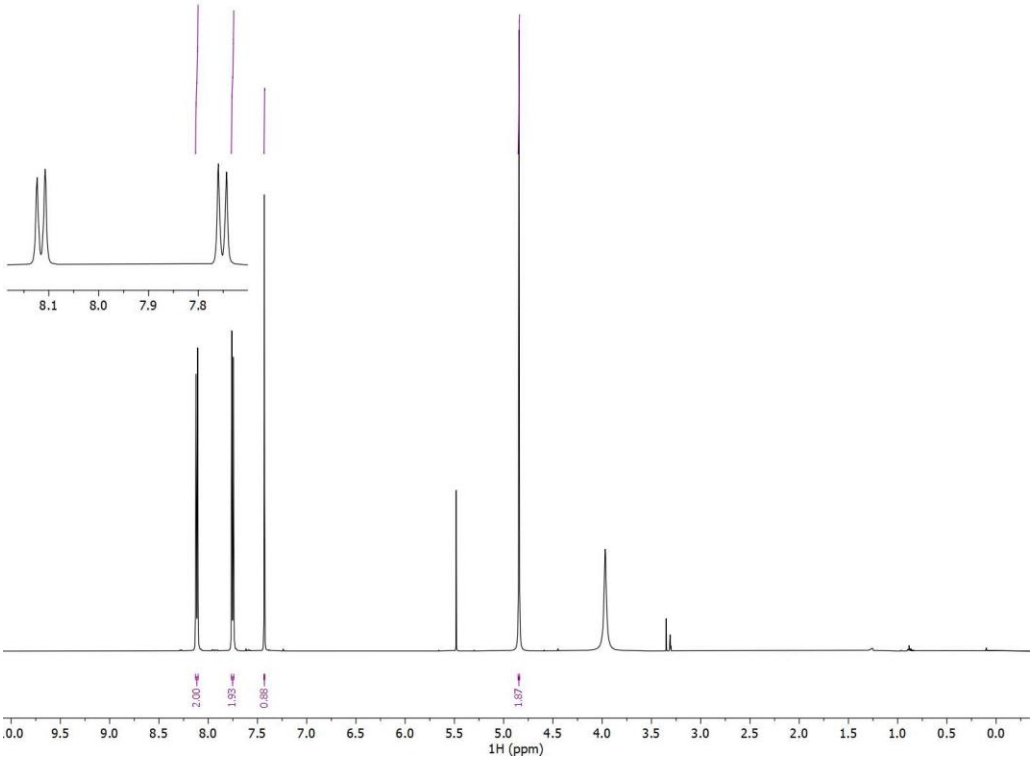


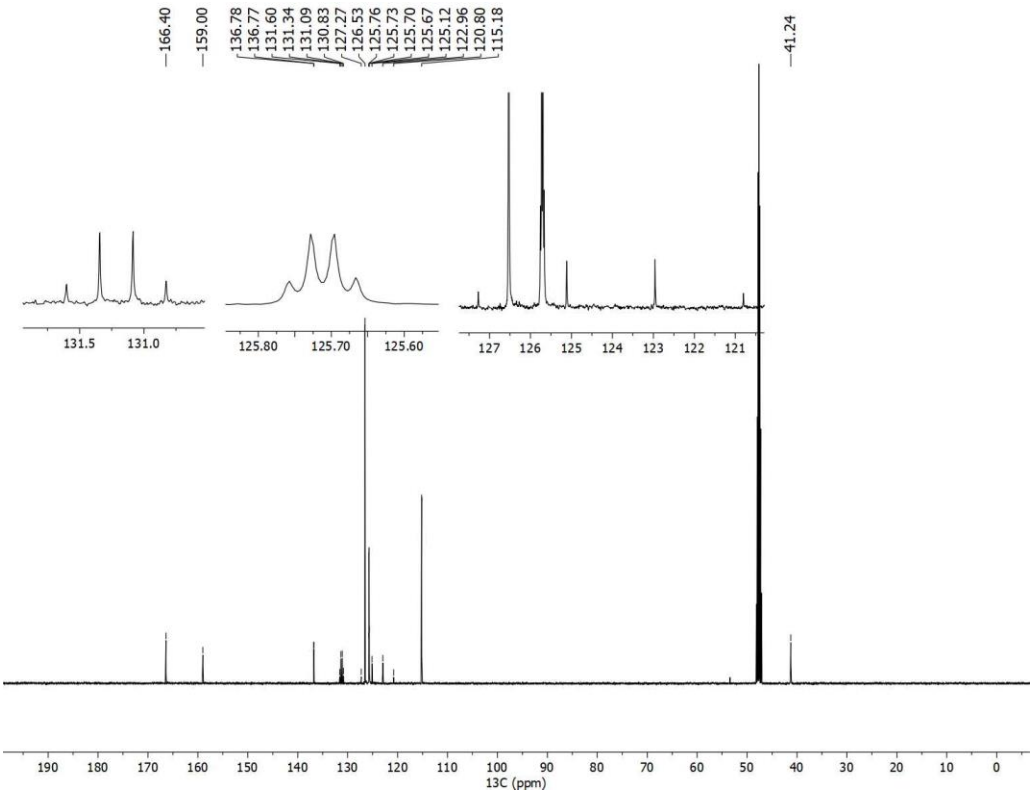
Compound 3.7d

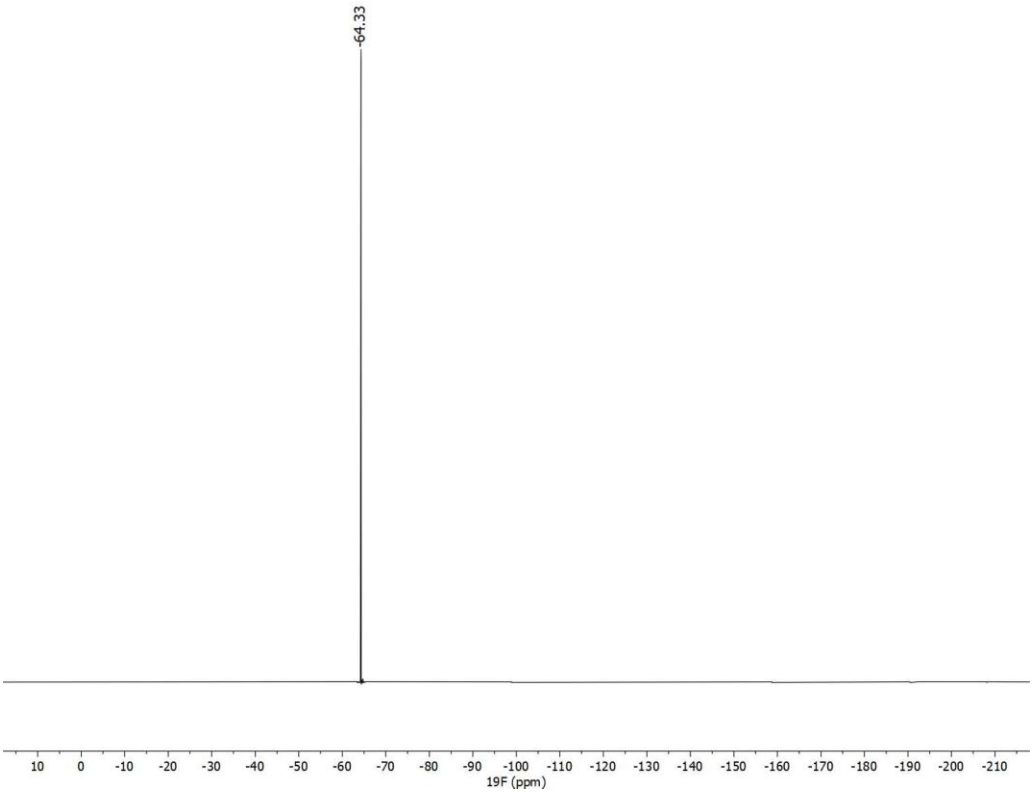




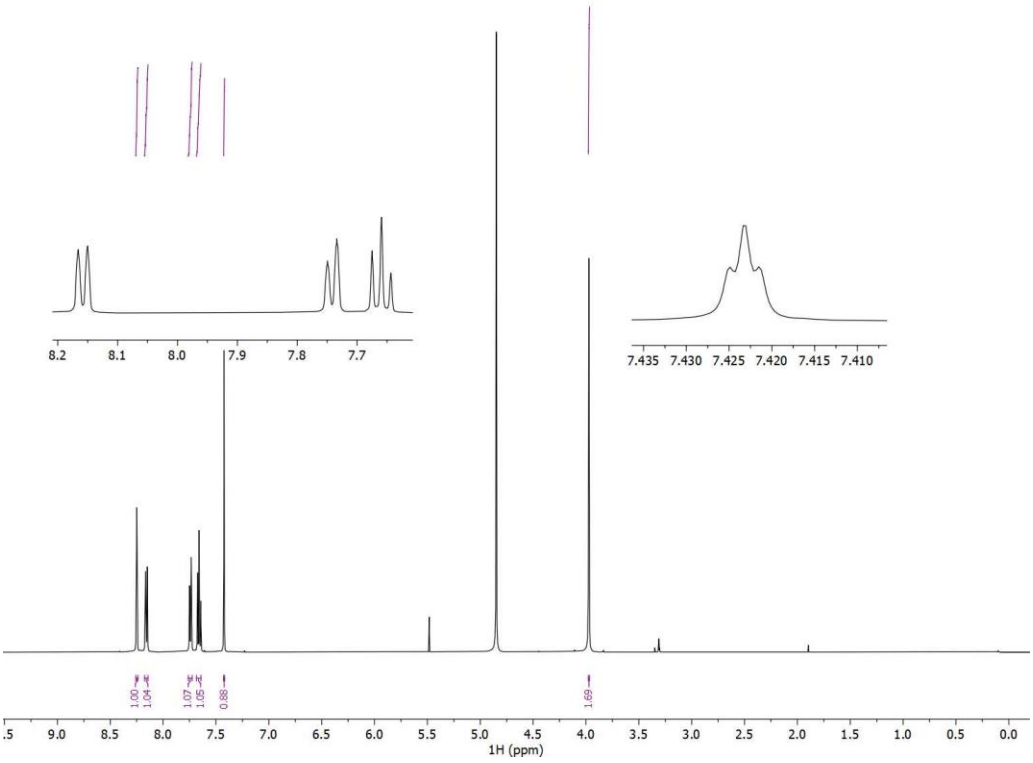
Compound 3.7e

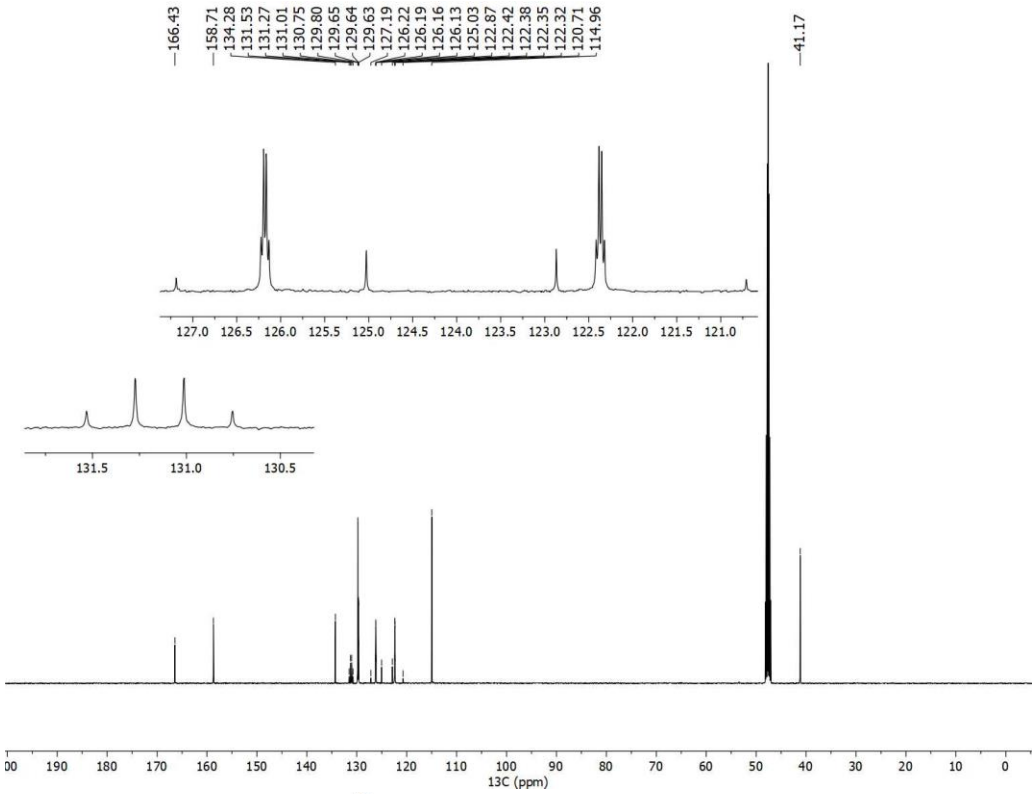


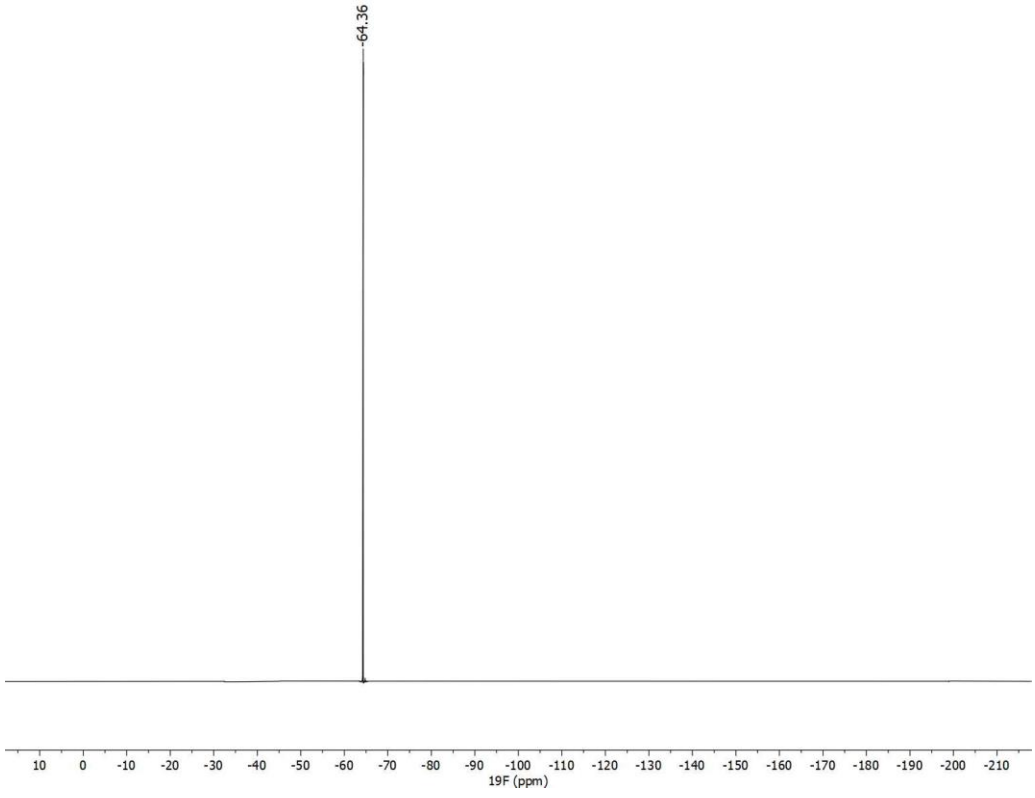




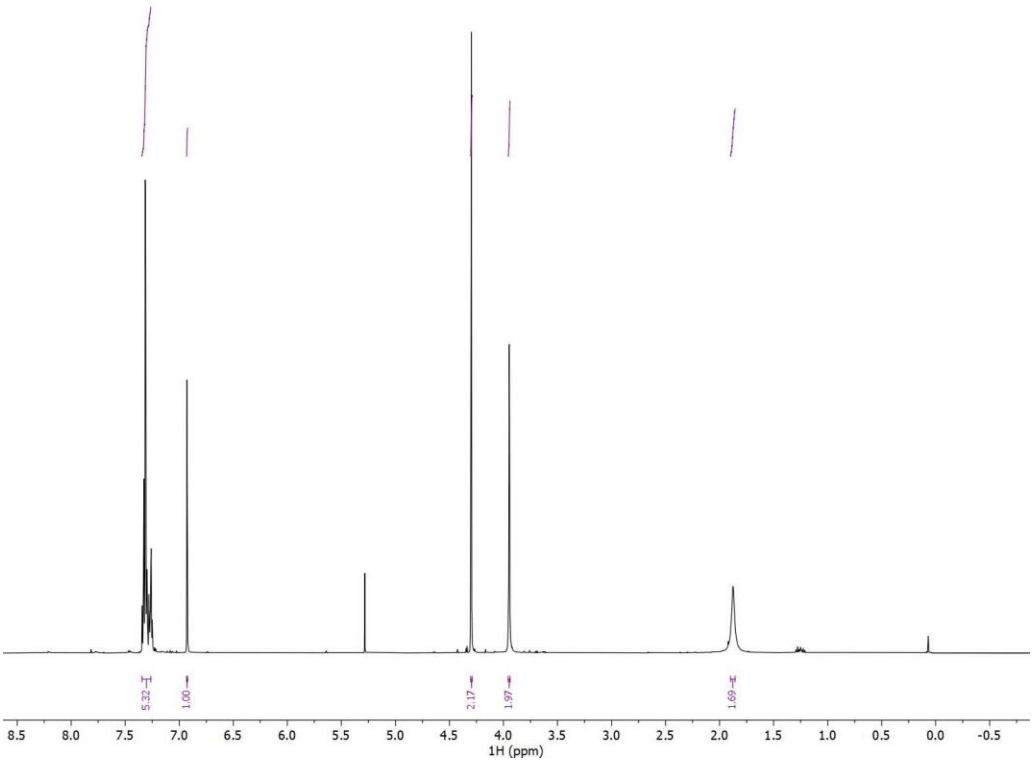
Compound 3.7f

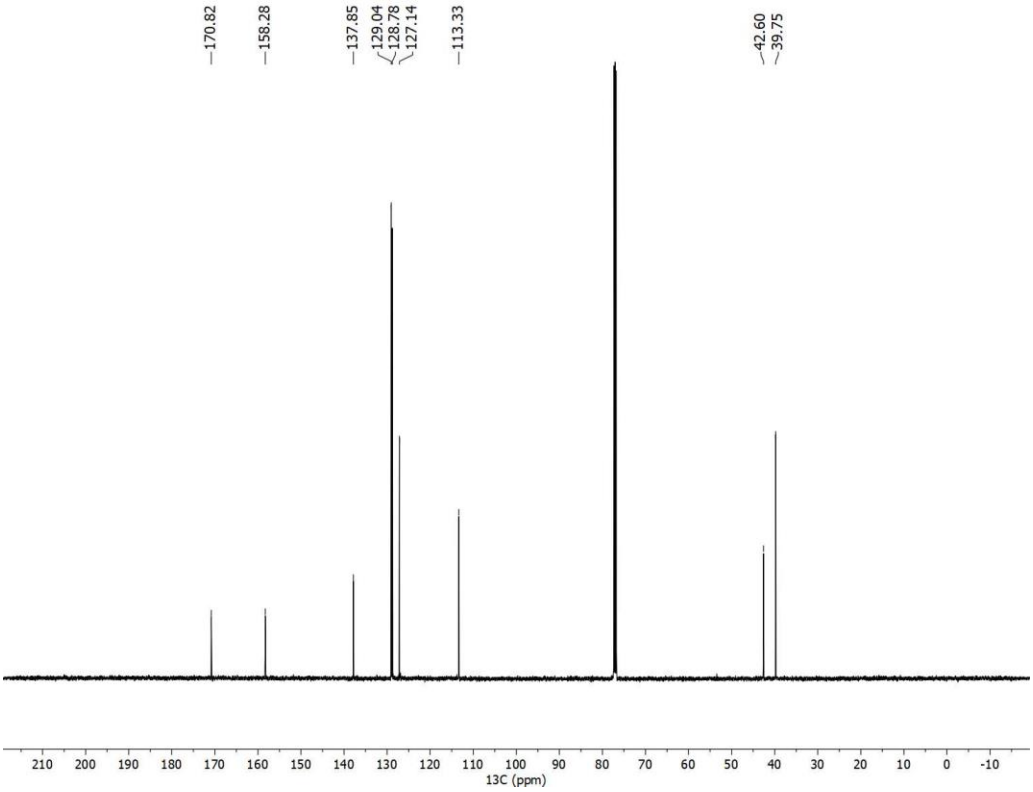



166.43
158.71
134.28
131.53
131.27
131.01
130.75
129.80
129.65
129.64
129.63
127.19
126.22
126.19
126.16
126.13
125.03
122.87
122.42
122.38
122.35
122.32
120.71
114.96
41.17
127.0 126.5 126.0 125.5 125.0 124.5 124.0 123.5 123.0 122.5 122.0 121.5 121.0
131.5 131.0 130.5
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
13C (ppm)

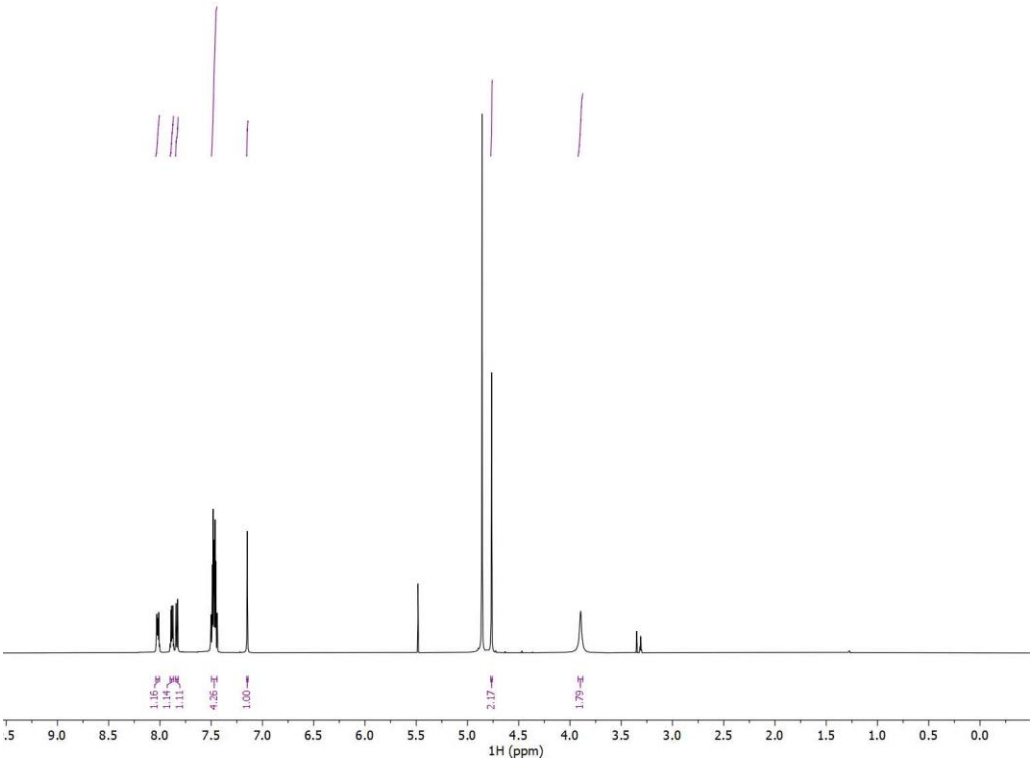

-64.36
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

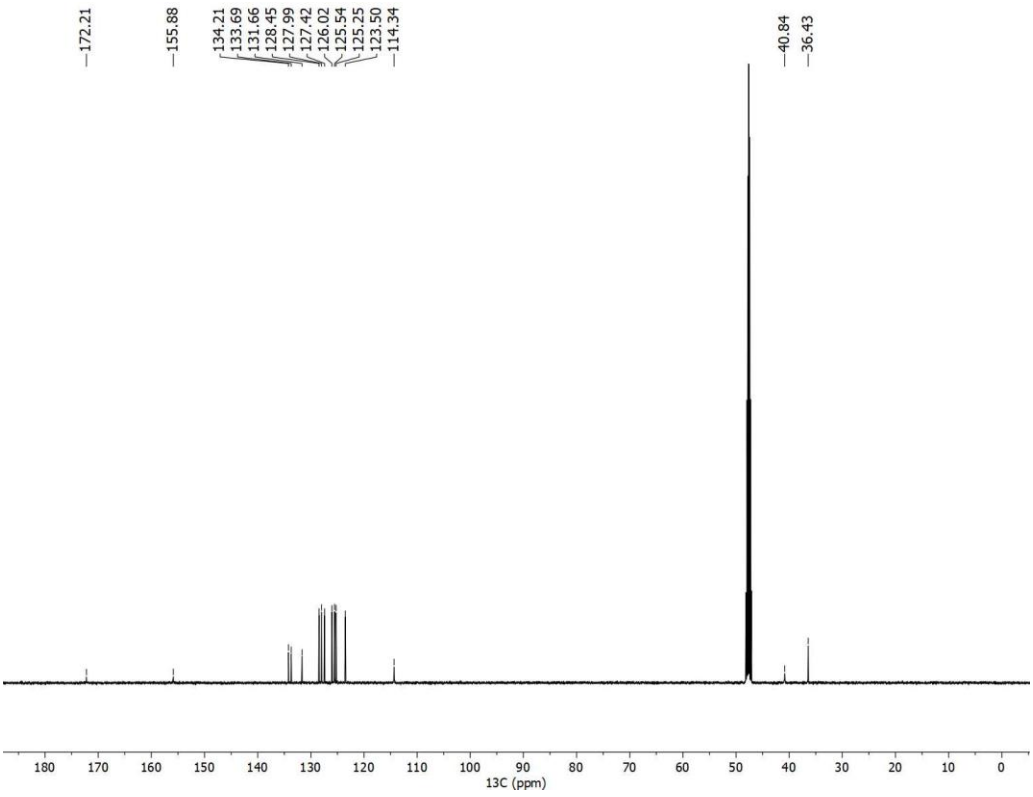
Compound 3.7g



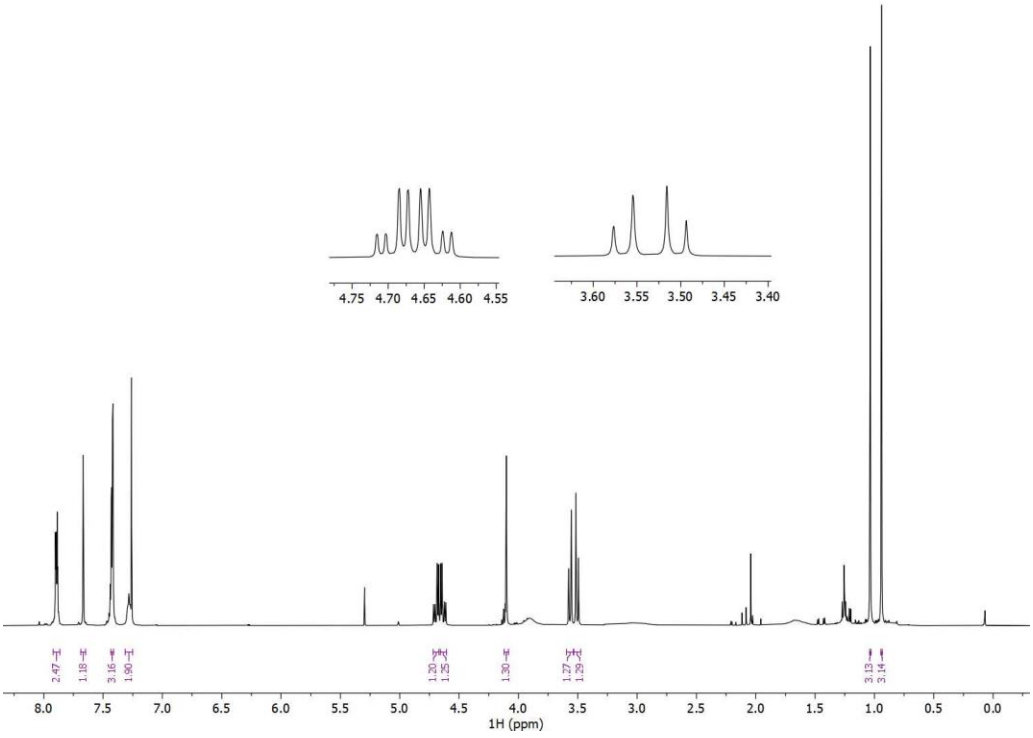


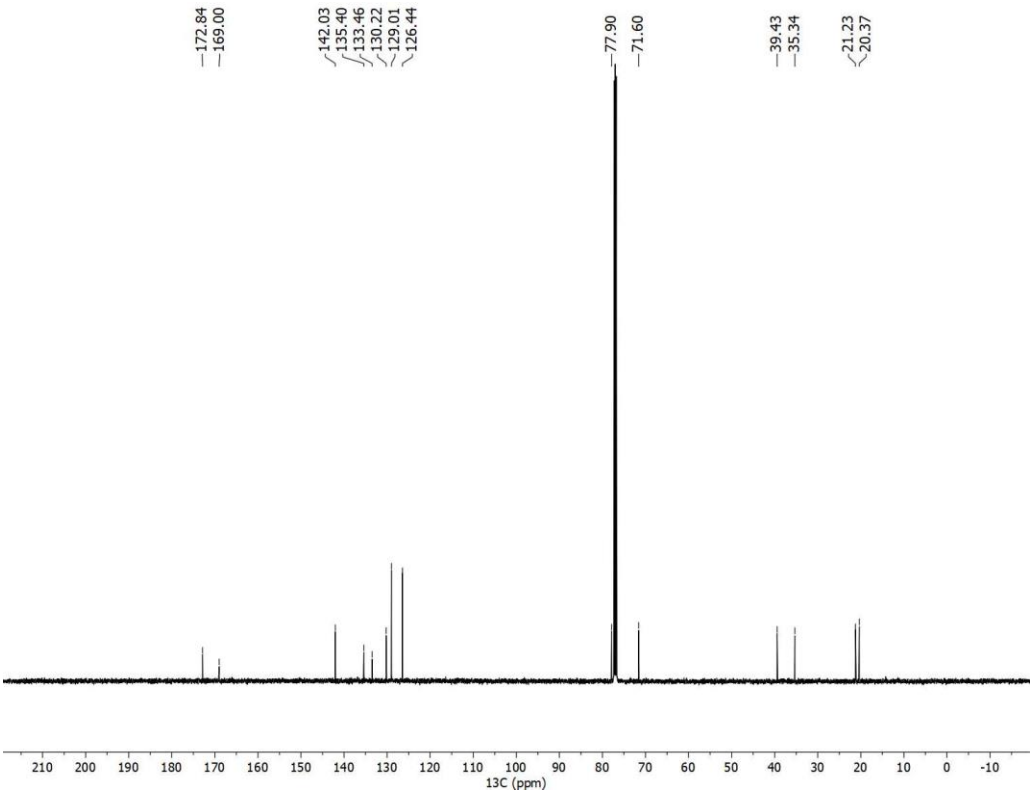
Compound 3.7h



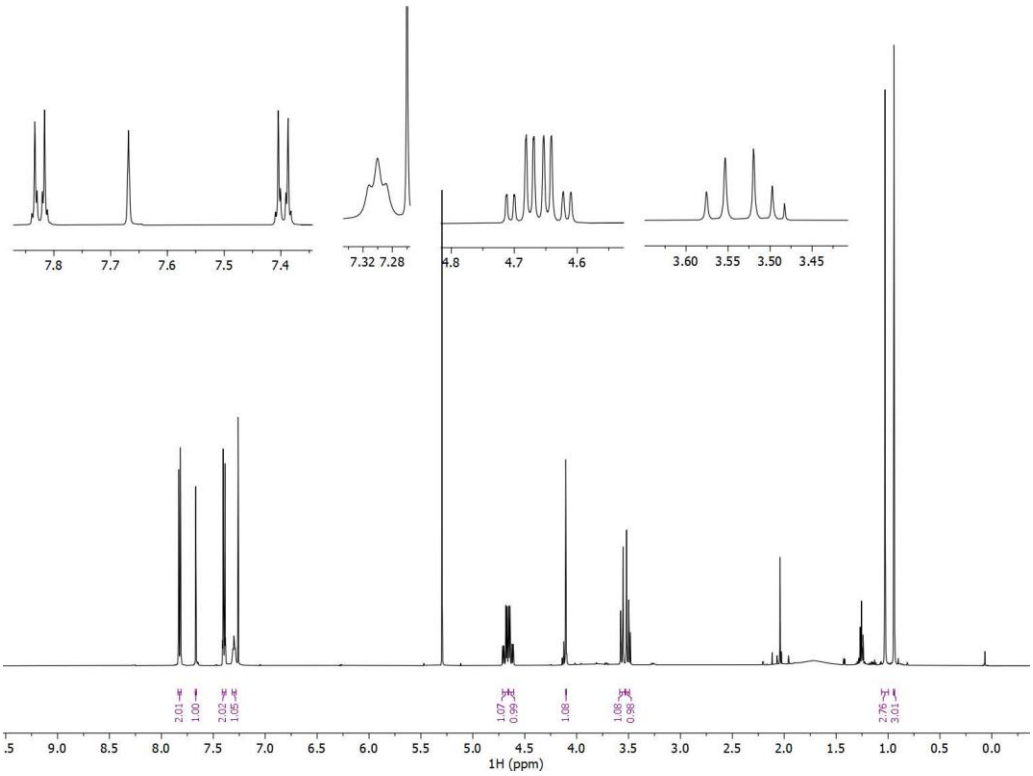


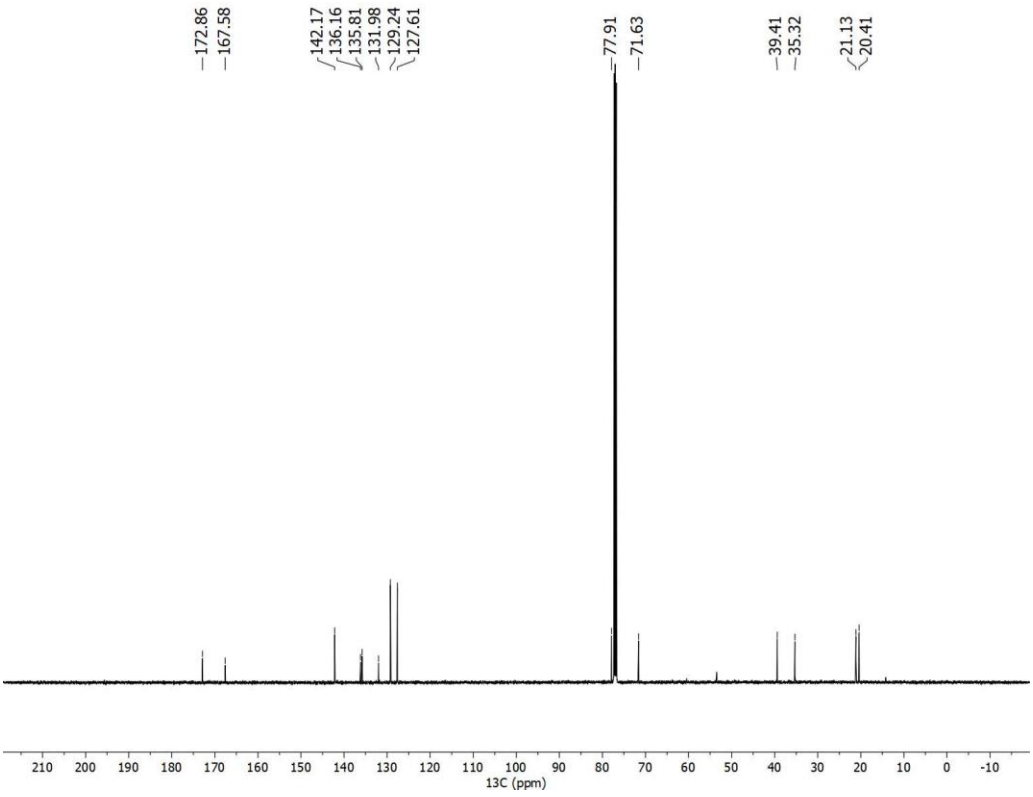
Compound 4a


4.75 4.70 4.65 4.60 4.55
3.60 3.55 3.50 3.45 3.40
2.47
1.18
3.16
1.80
1.20
1.25
1.30
1.27
1.29
3.13
3.14
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)

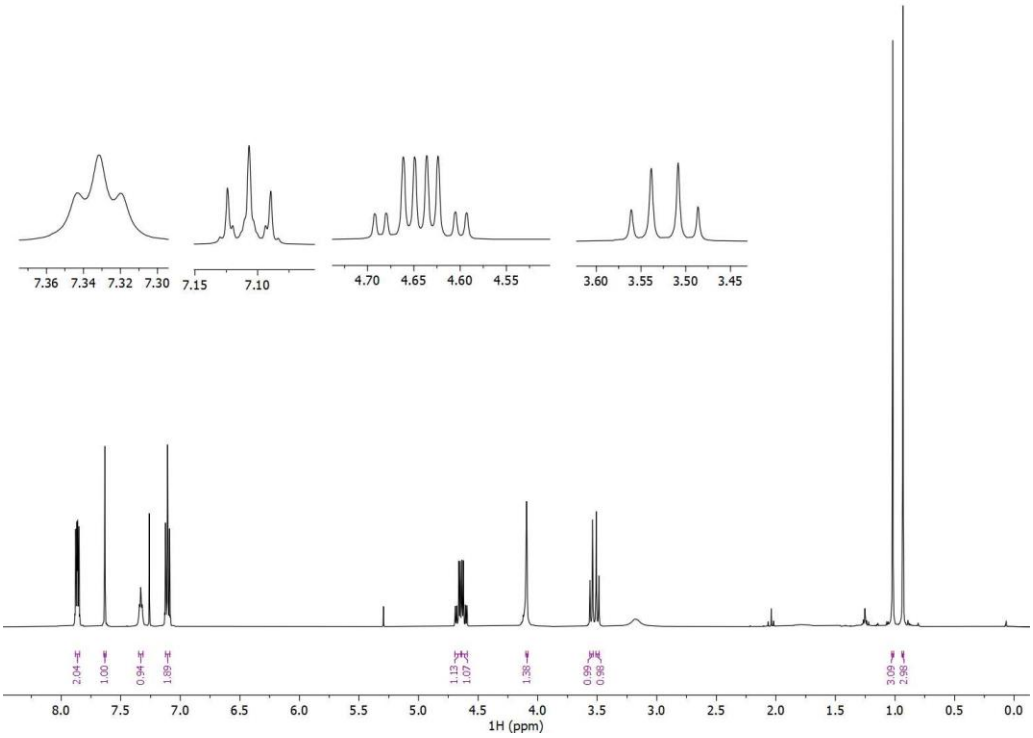

172.84
169.00
142.03
135.40
133.46
130.22
129.01
126.44
77.90
71.60
39.43
35.34
21.23
20.37
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

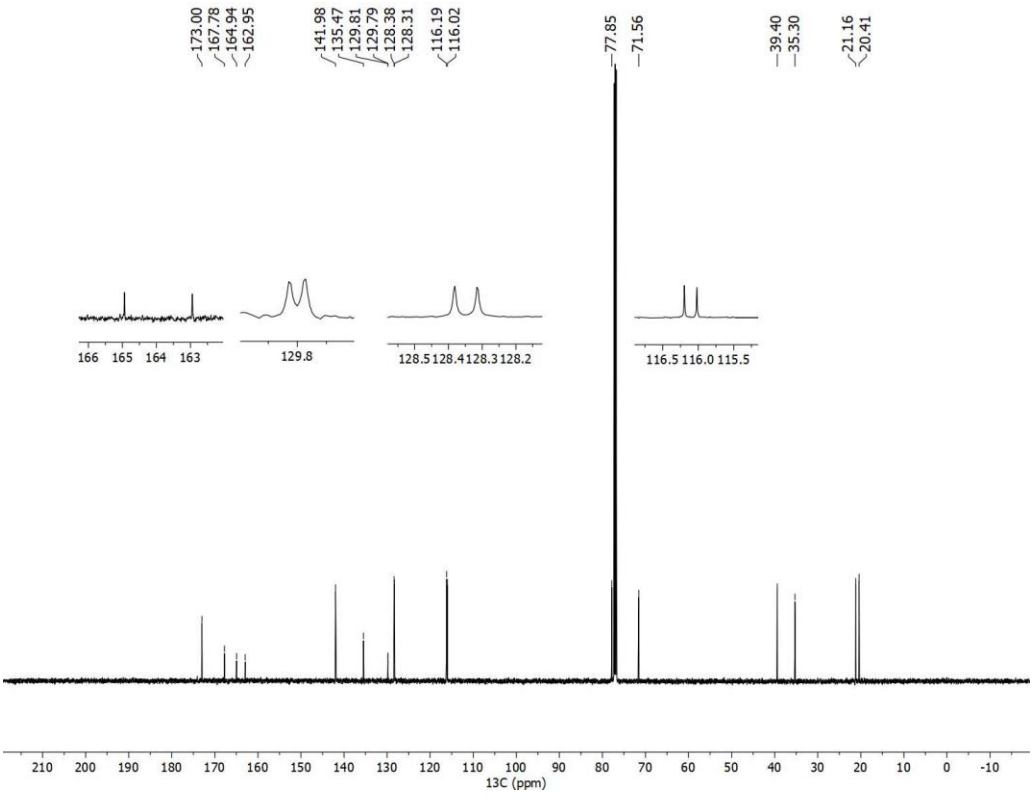
Compound 4b

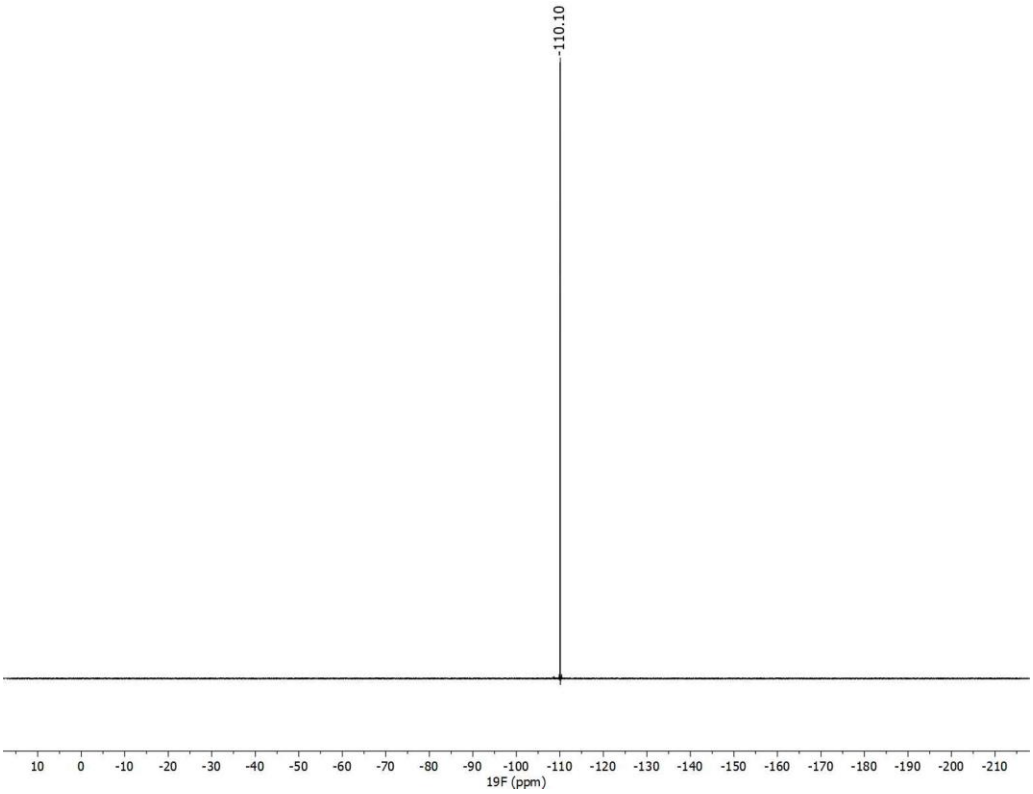

7.8
7.7
7.6
7.5
7.4
7.32 7.28
4.8
4.7
4.6
3.60
3.55
3.50
3.45
2.01
1.00
2.02
1.05
1.07
0.99
1.08
1.08
0.98
2.76
3.01
1H (ppm)


172.86
167.58
142.17
136.16
135.81
131.98
129.24
127.61
77.91
71.63
39.41
35.32
21.13
20.41
13C (ppm)

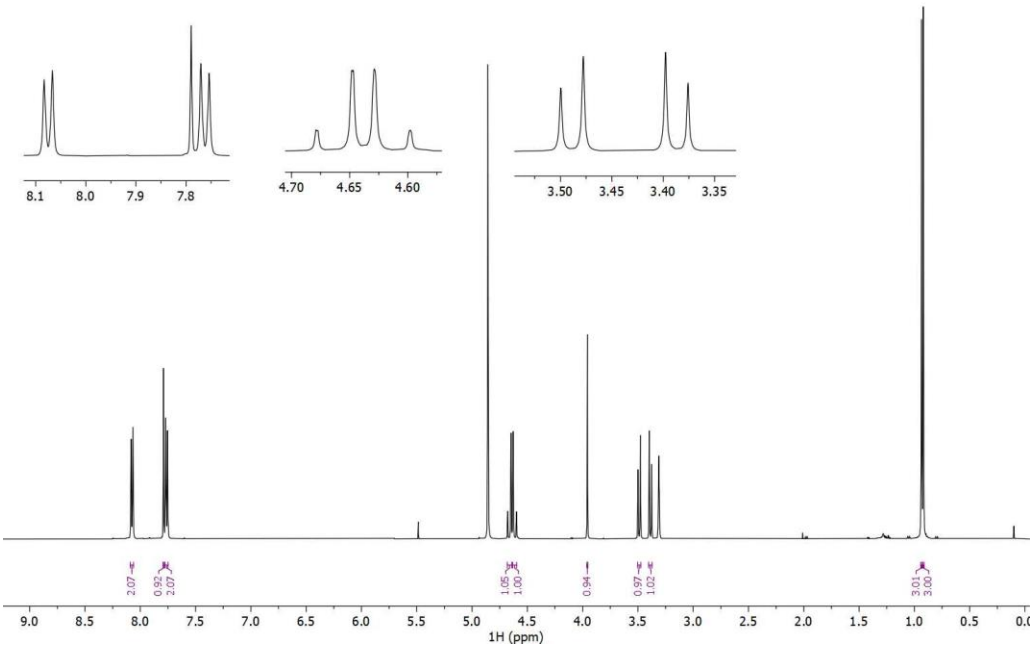
Compound 4c

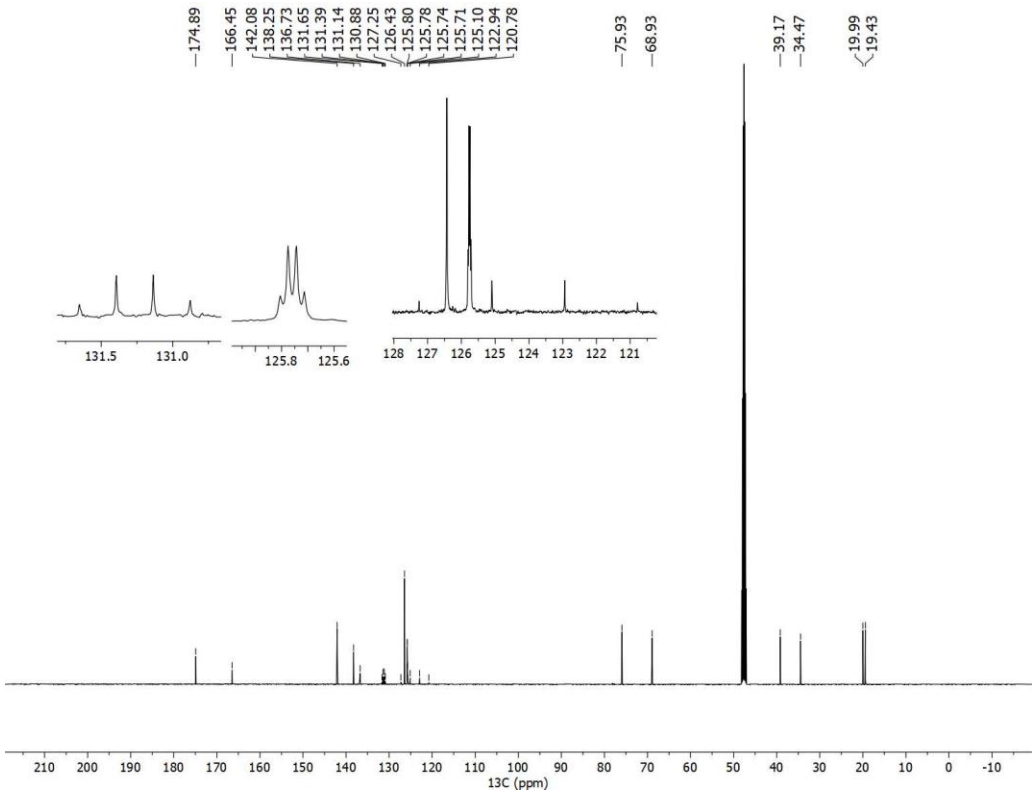

7.36 7.34 7.32 7.30
7.15 7.10
4.70 4.65 4.60 4.55
3.60 3.55 3.50 3.45
2.04
1.00
0.94
1.89
1.13
1.07
1.38
0.99
0.98
3.09
2.98
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)

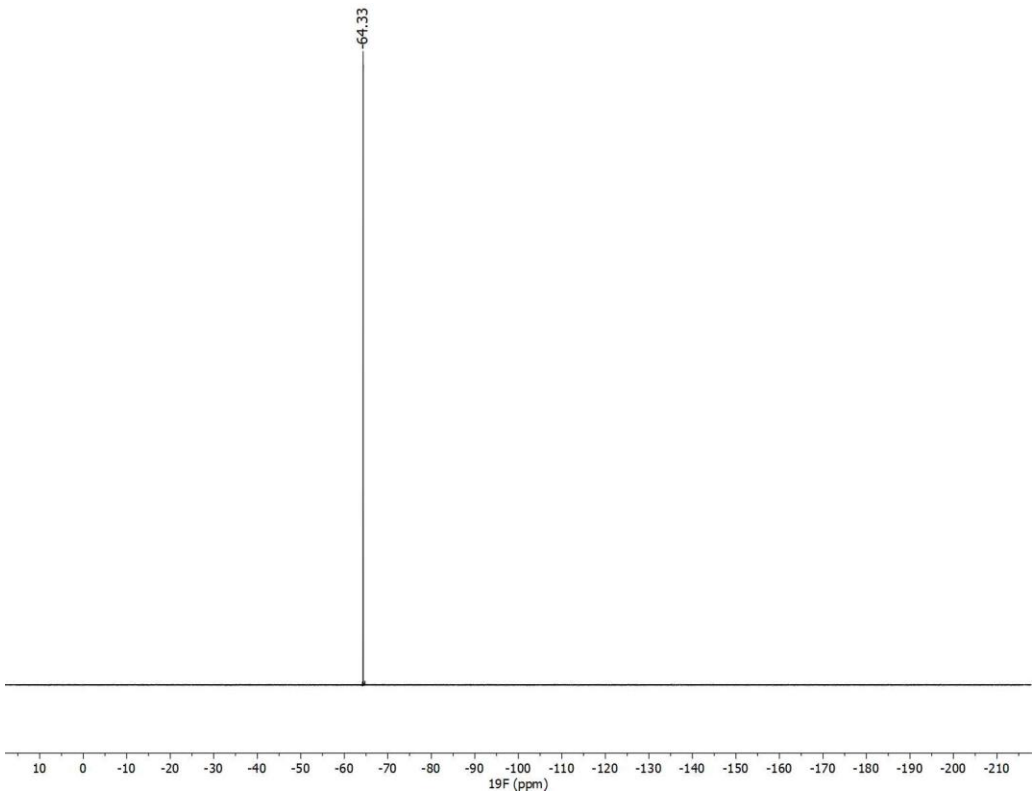

173.00
167.78
164.94
162.95
141.98
135.47
129.81
129.79
128.38
128.31
116.19
116.02
77.85
71.56
39.40
35.30
21.16
20.41
166 165 164 163
129.8
128.5 128.4 128.3 128.2
116.5 116.0 115.5
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)


-110.10
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

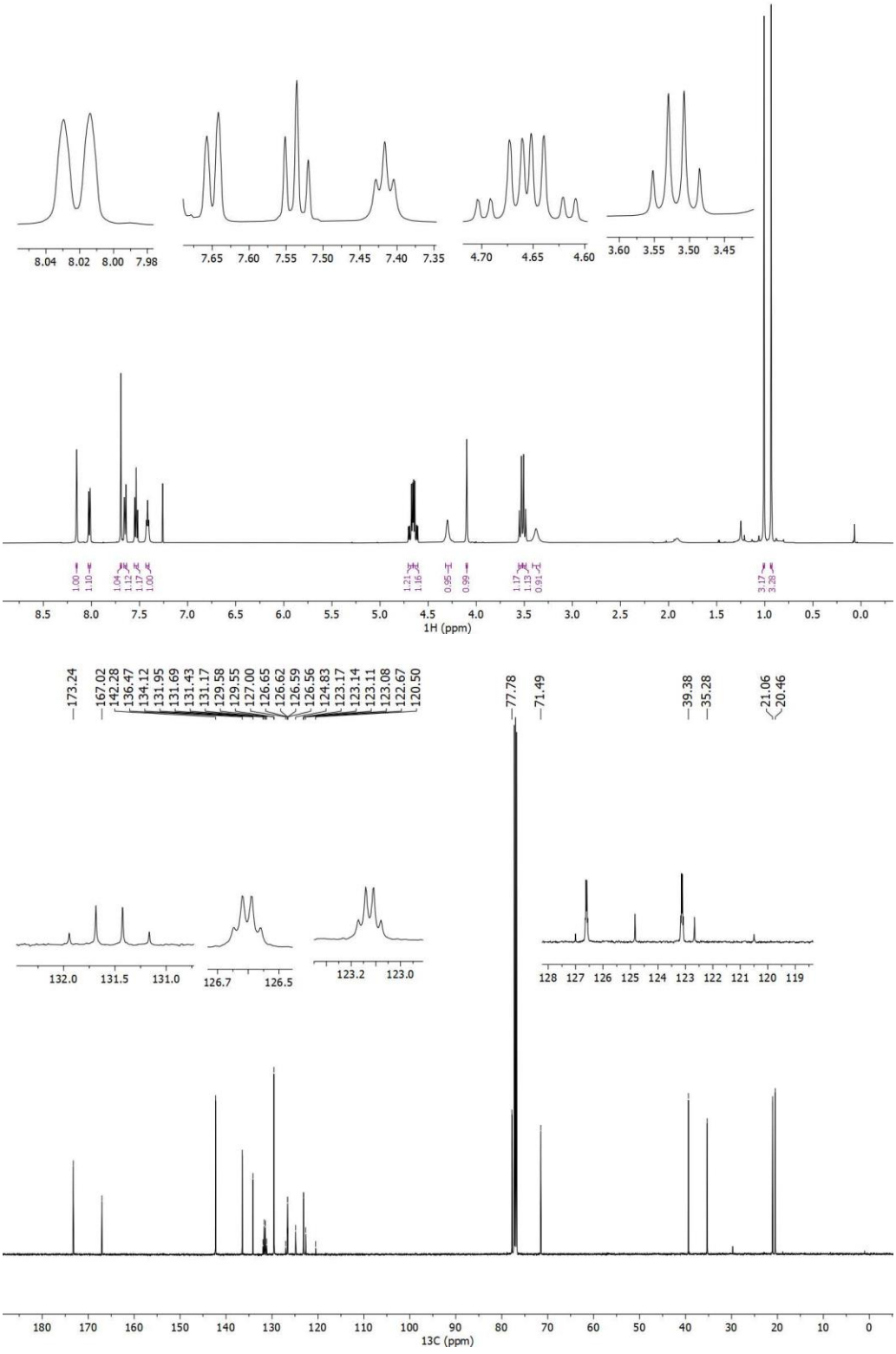
Compound 4d

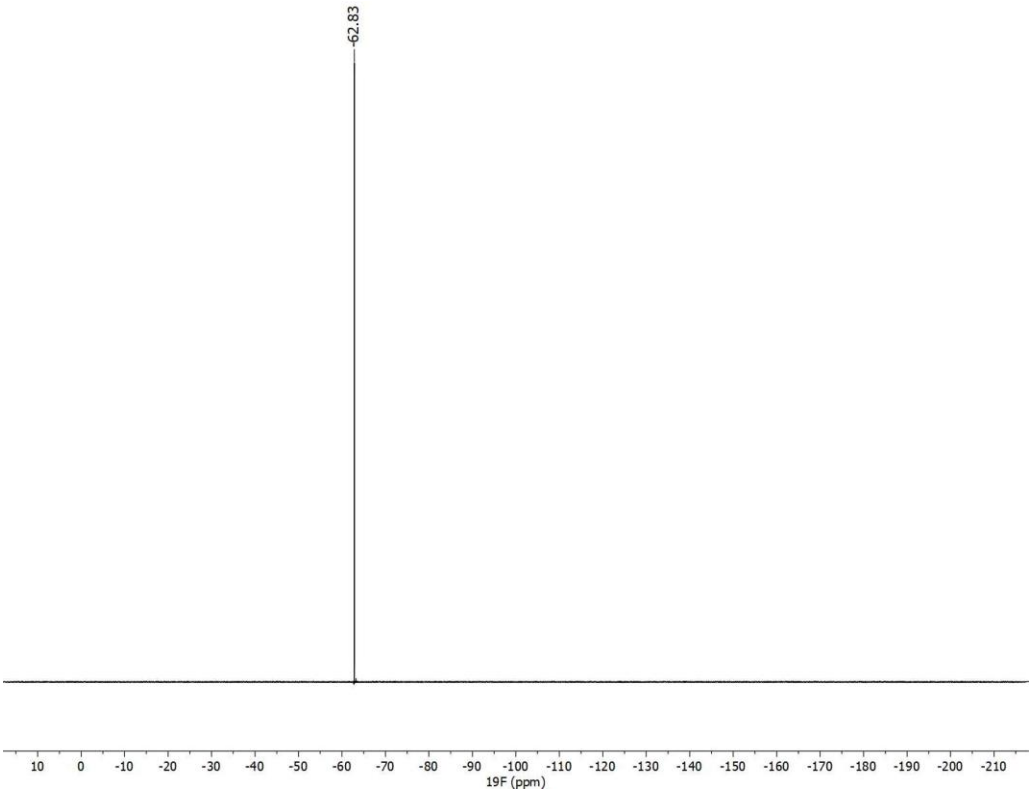

8.1 8.0 7.9 7.8
4.70 4.65 4.60
3.50 3.45 3.40 3.35
2.07 0.92 2.07 1.05 1.00 0.94 0.97 1.02 3.01 3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1H (ppm)


174.89
166.45
142.08
138.25
136.73
131.65
131.39
131.14
130.88
127.25
126.43
125.80
125.78
125.74
125.71
125.10
122.94
120.78
75.93
68.93
39.17
34.47
19.99
19.43
131.5
131.0
125.8
125.6
128 127 126 125 124 123 122 121
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

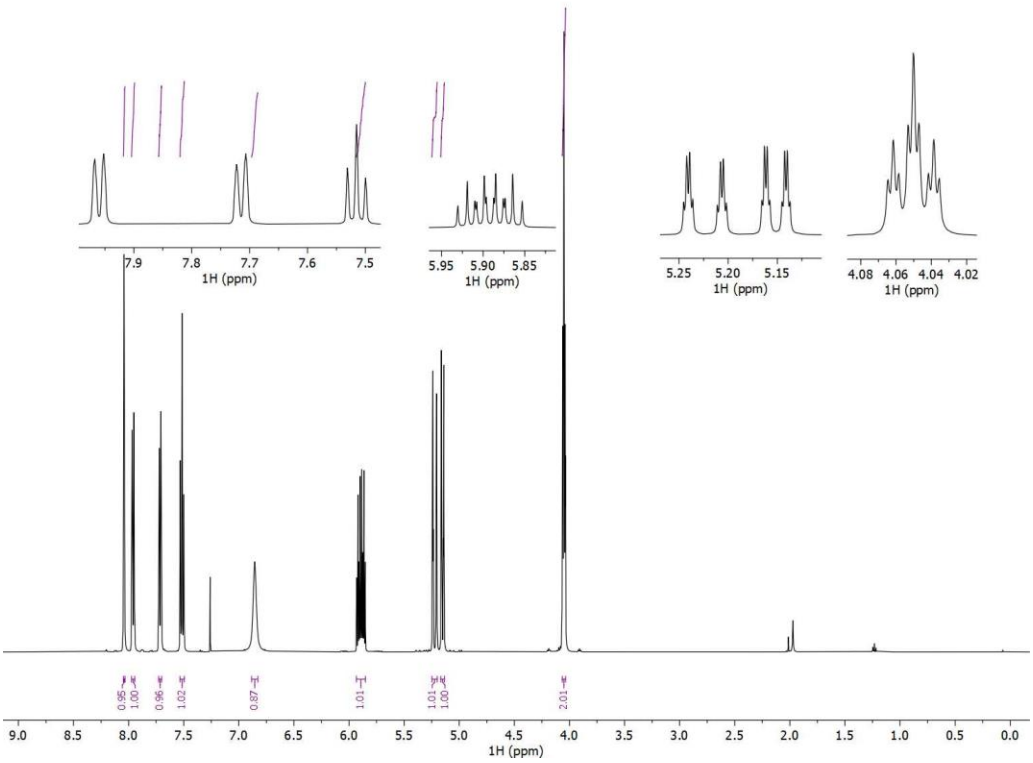

-64.33
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

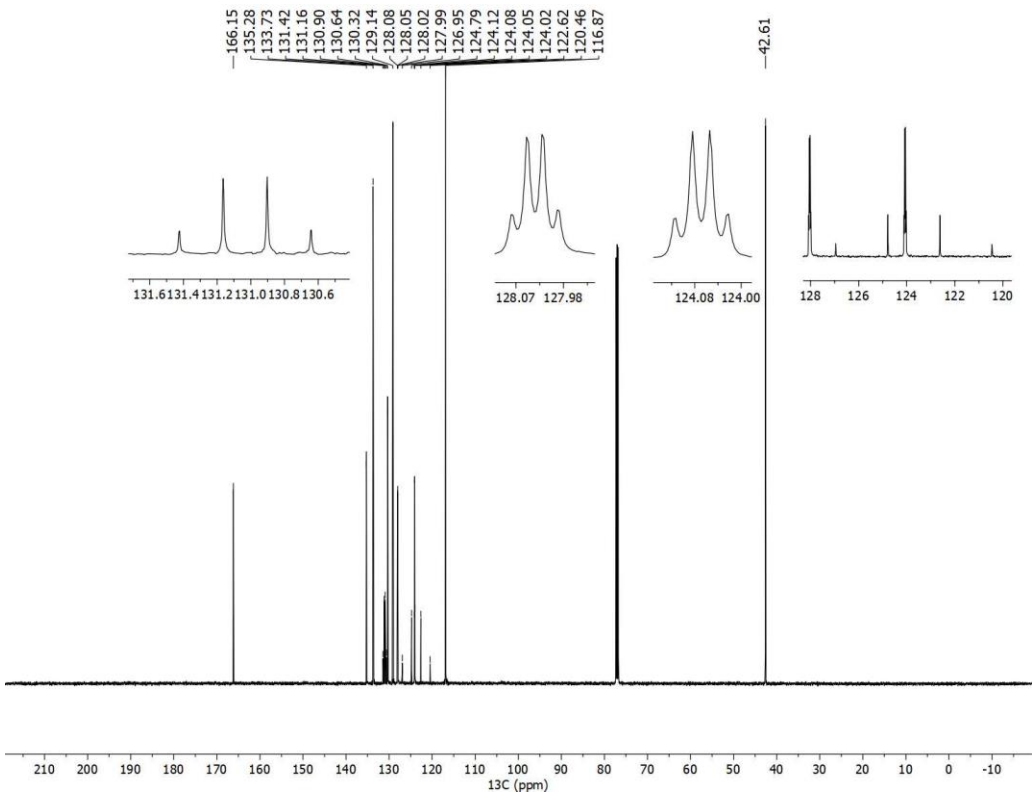
Compound 4e

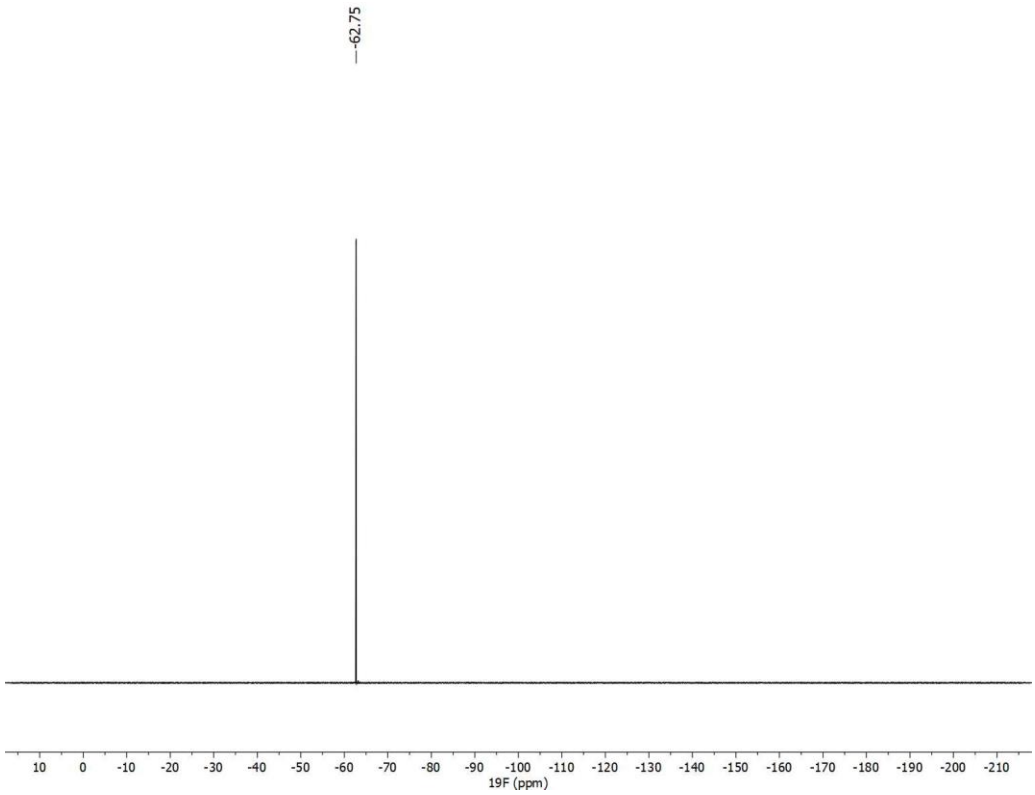

8.04 8.02 8.00 7.98
7.65 7.60 7.55 7.50 7.45 7.40 7.35
4.70 4.65 4.60
3.60 3.55 3.50 3.45
1.00 1.10 1.04 1.12 1.17 1.00 1.21 1.16 0.95 0.99 1.17 1.19 0.91 3.17 3.28
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H (ppm)
173.24 167.02 142.28 136.47 134.12 131.95 131.69 131.43 131.17 129.58 129.55 127.00 126.65 126.62 126.59 126.56 124.83 123.17 123.14 123.11 123.08 122.67 120.50
77.78 71.49 39.38 35.28 21.06 20.46
132.0 131.5 131.0
126.7 126.5
123.2 123.0
128 127 126 125 124 123 122 121 120 119
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
13C (ppm)



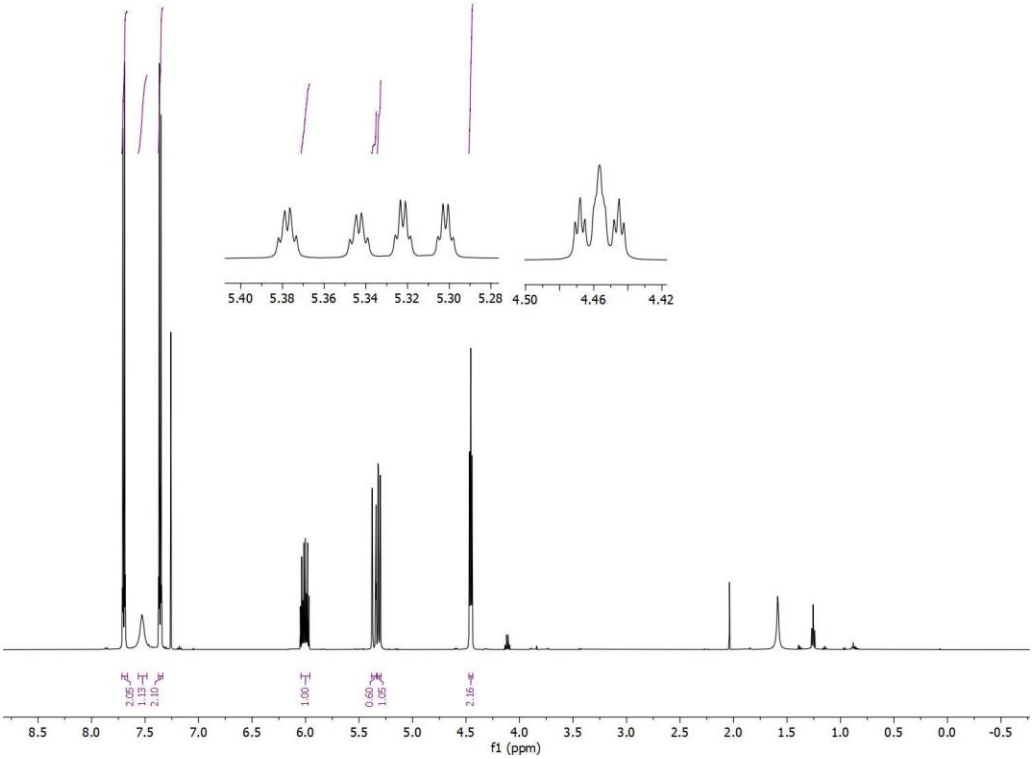
Compound 4.2e

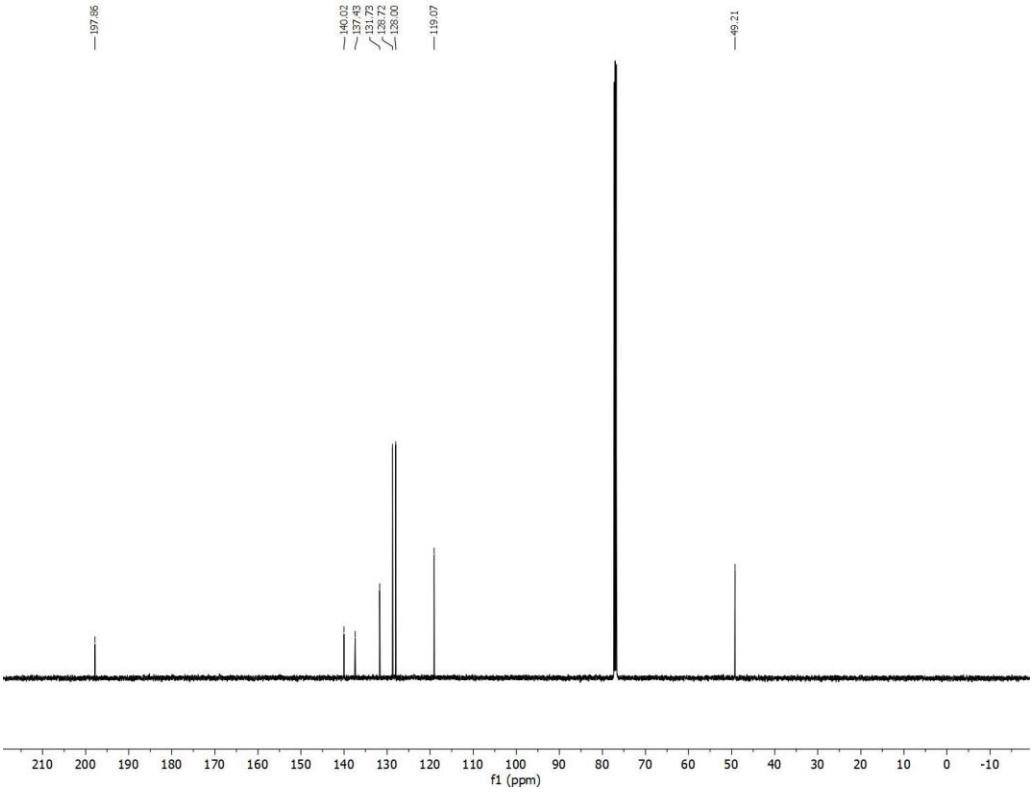



166.15
135.28
133.73
131.42
131.16
130.90
130.64
130.32
129.14
128.08
128.05
128.02
127.99
126.95
124.79
124.12
124.08
124.05
124.02
122.62
120.46
116.87
42.61
131.6 131.4 131.2 131.0 130.8 130.6
128.07 127.98
124.08 124.00
128 126 124 122 120
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

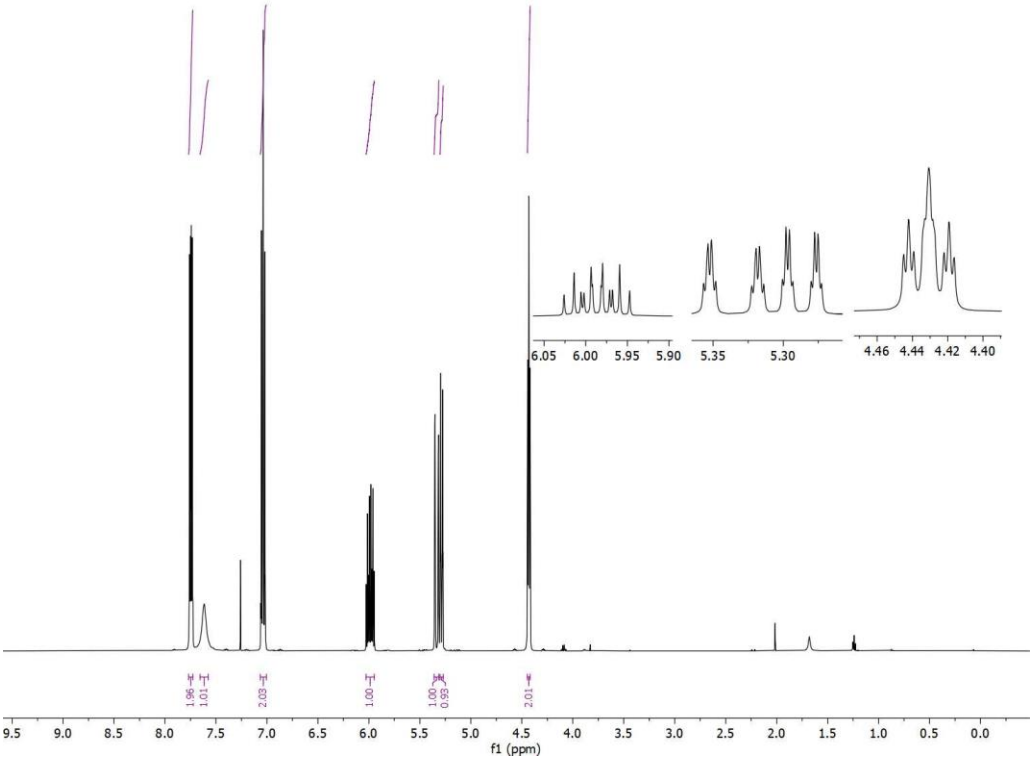

-62.75
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

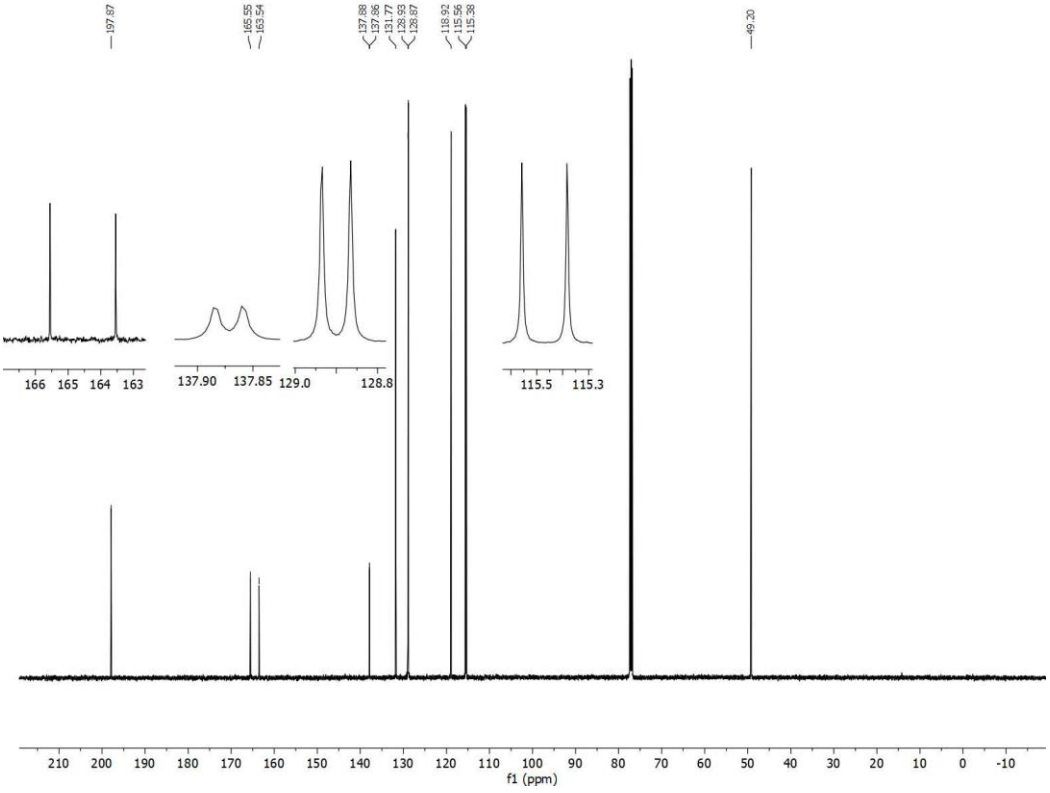
Compound 4.3b

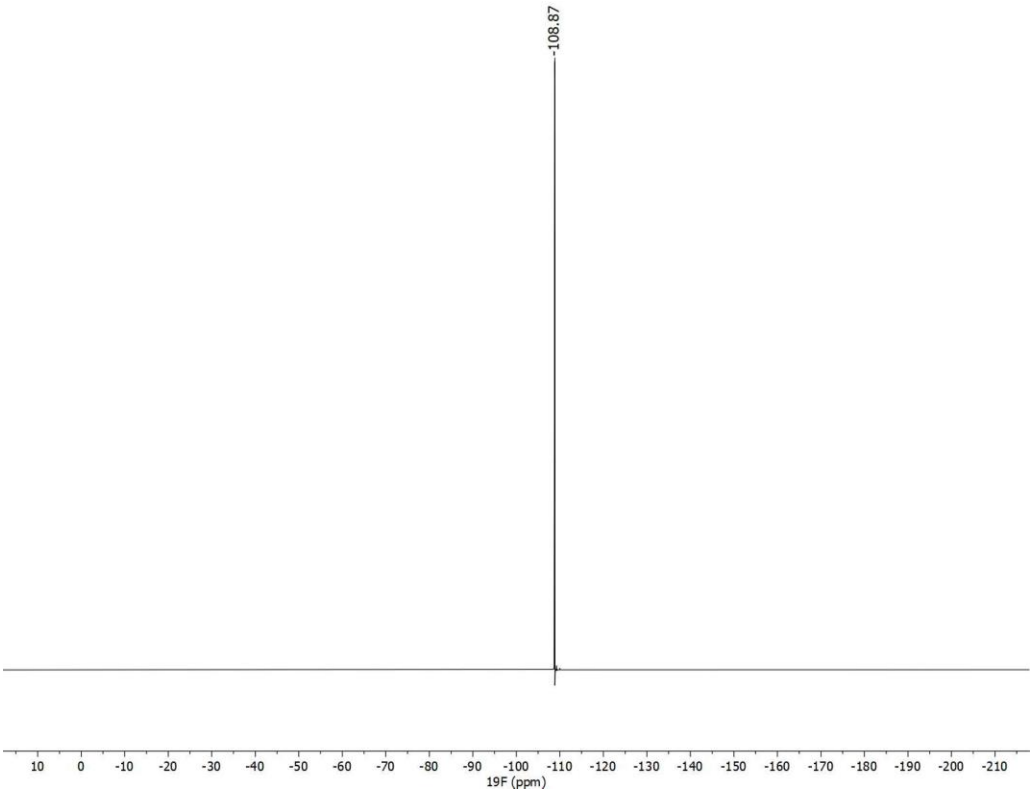




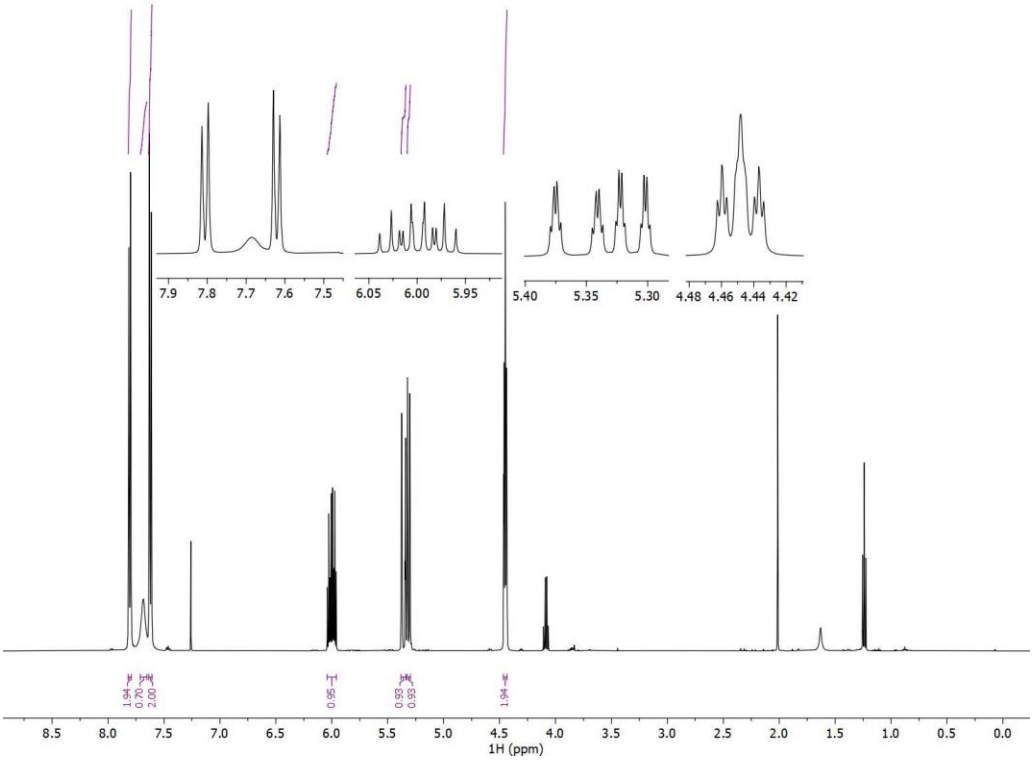
Compound 4.3c

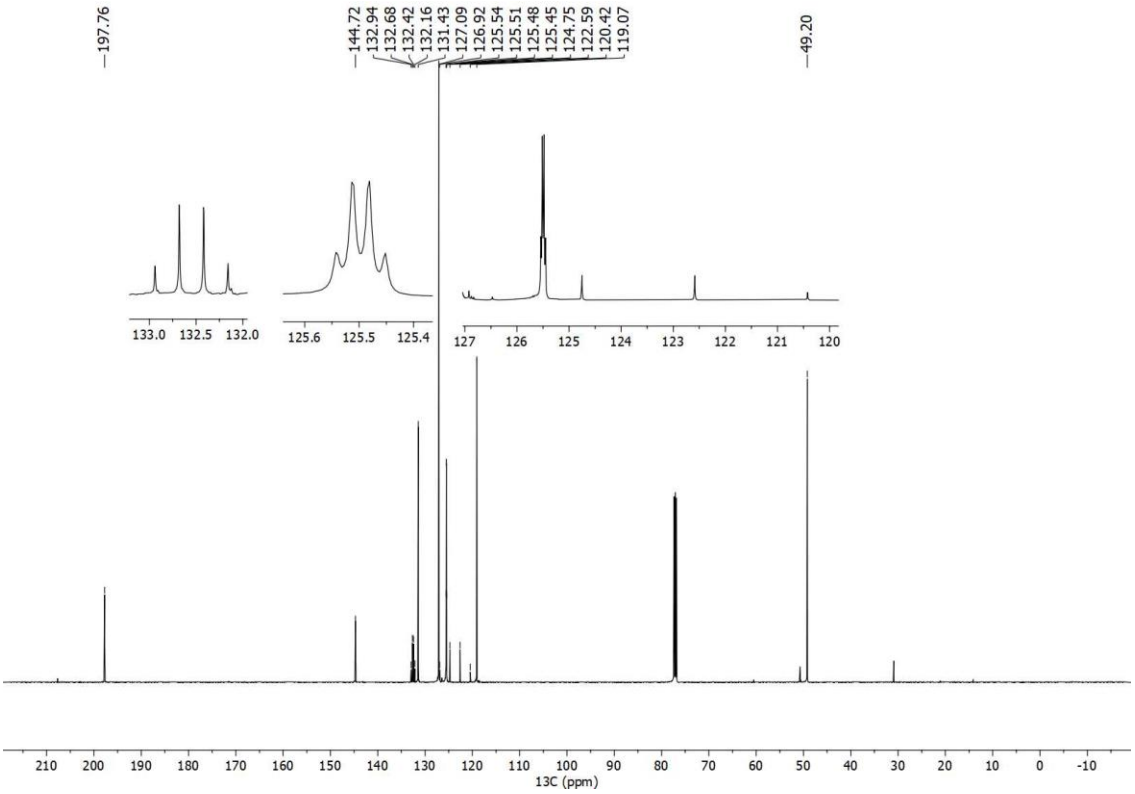


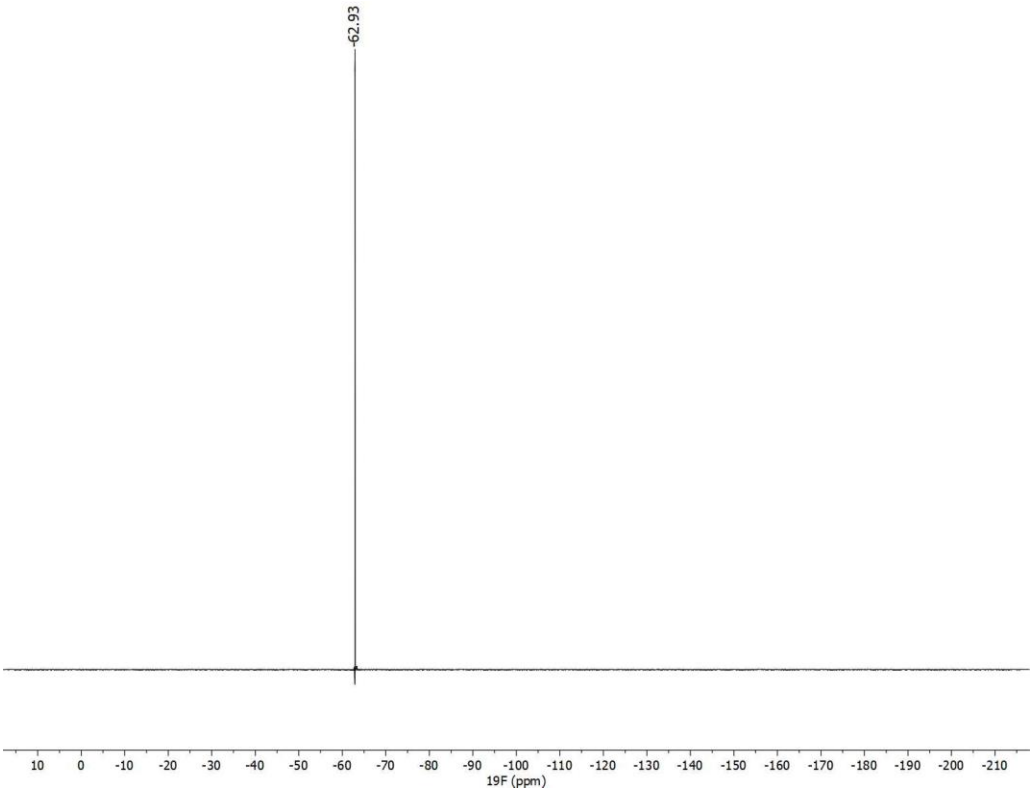




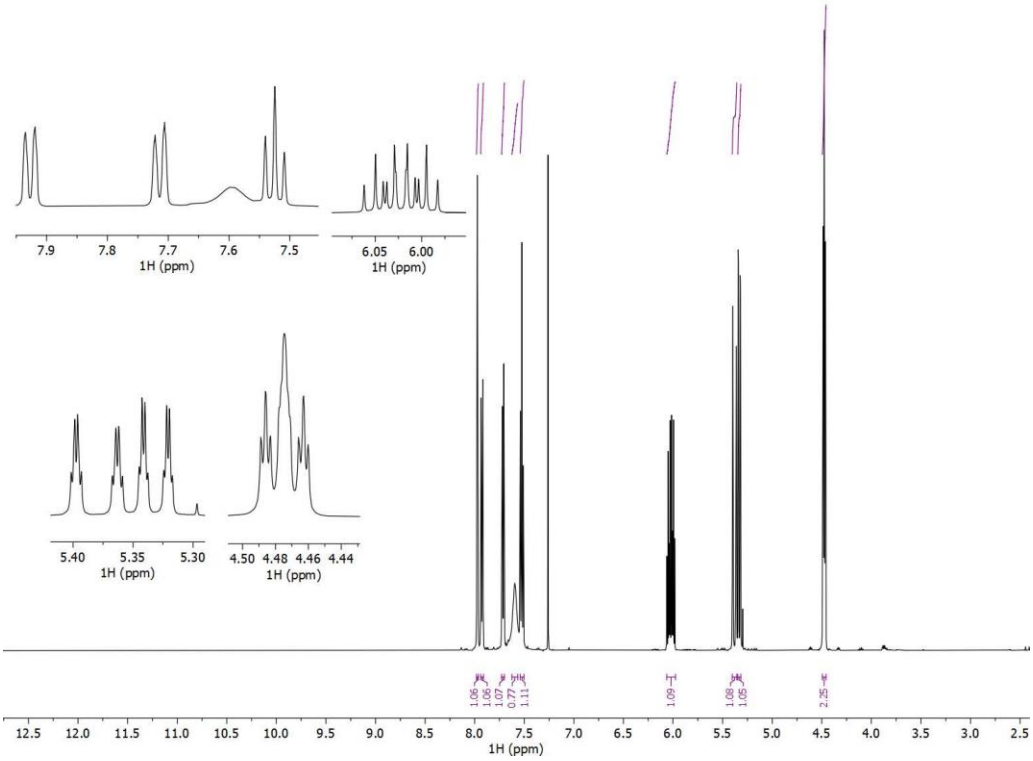
Compound 4.3d

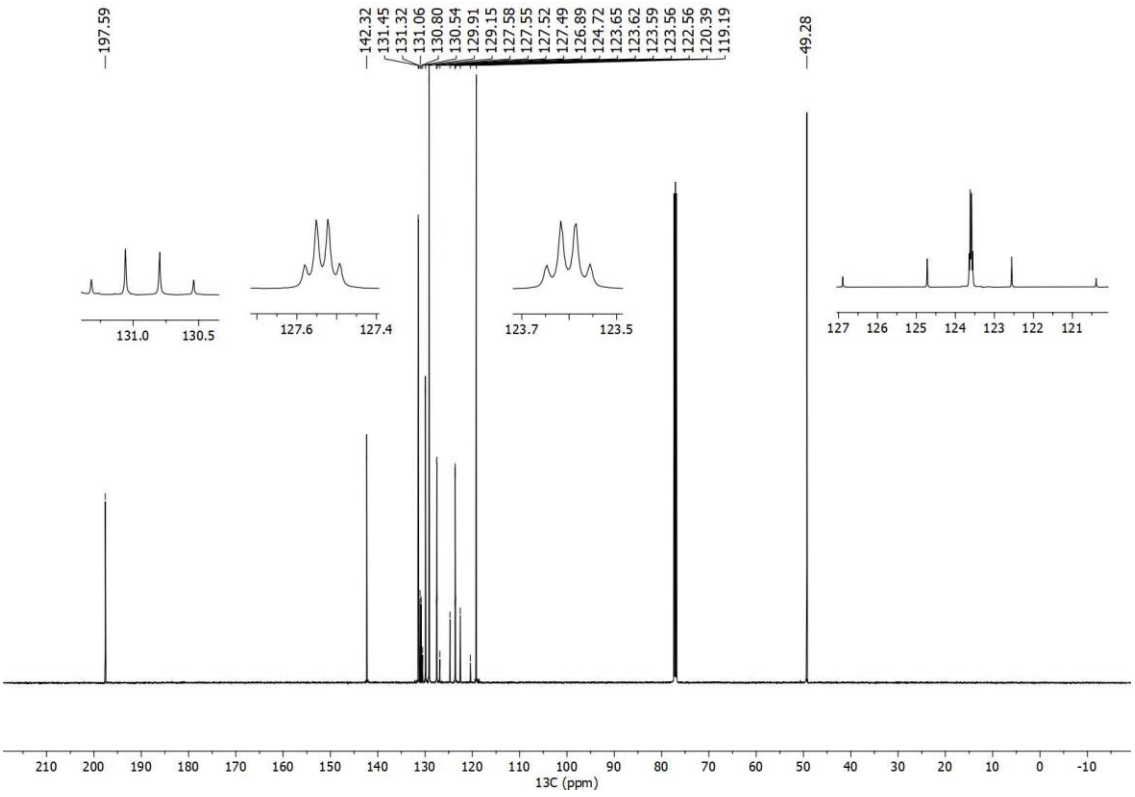


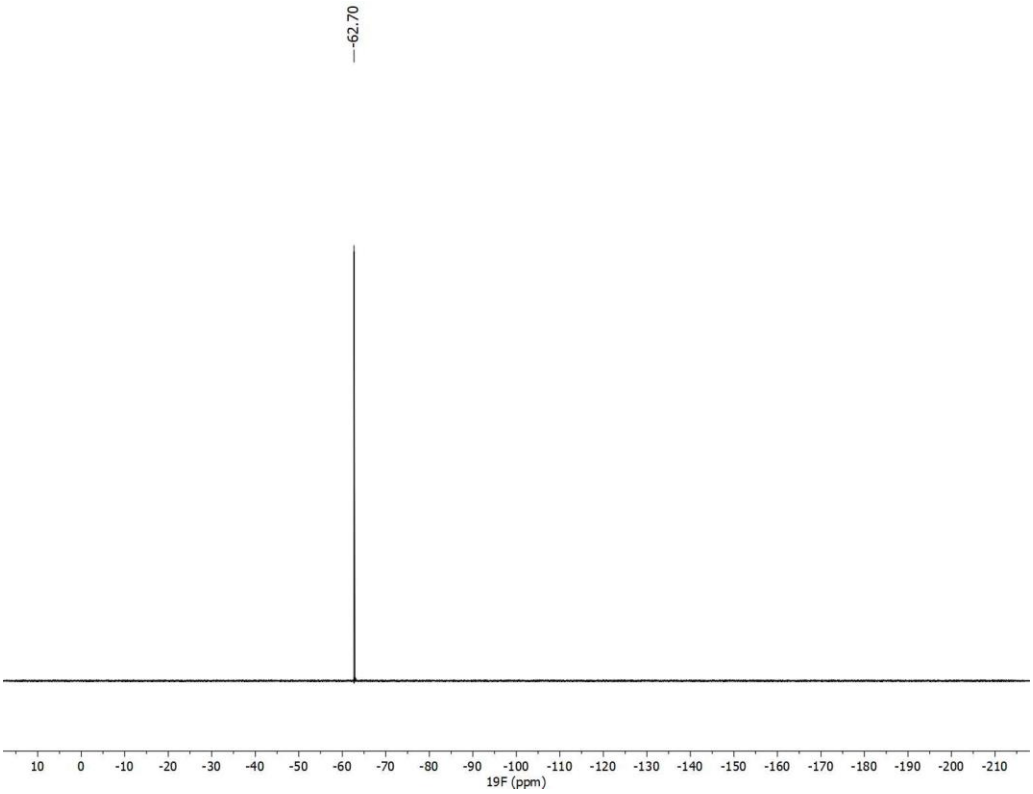

197.76
144.72
132.94
132.68
132.42
132.16
131.43
127.09
126.92
125.54
125.51
125.48
125.45
124.75
122.59
120.42
119.07
49.20
133.0 132.5 132.0
125.6 125.5 125.4
127 126 125 124 123 122 121 120
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)


-62.93
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

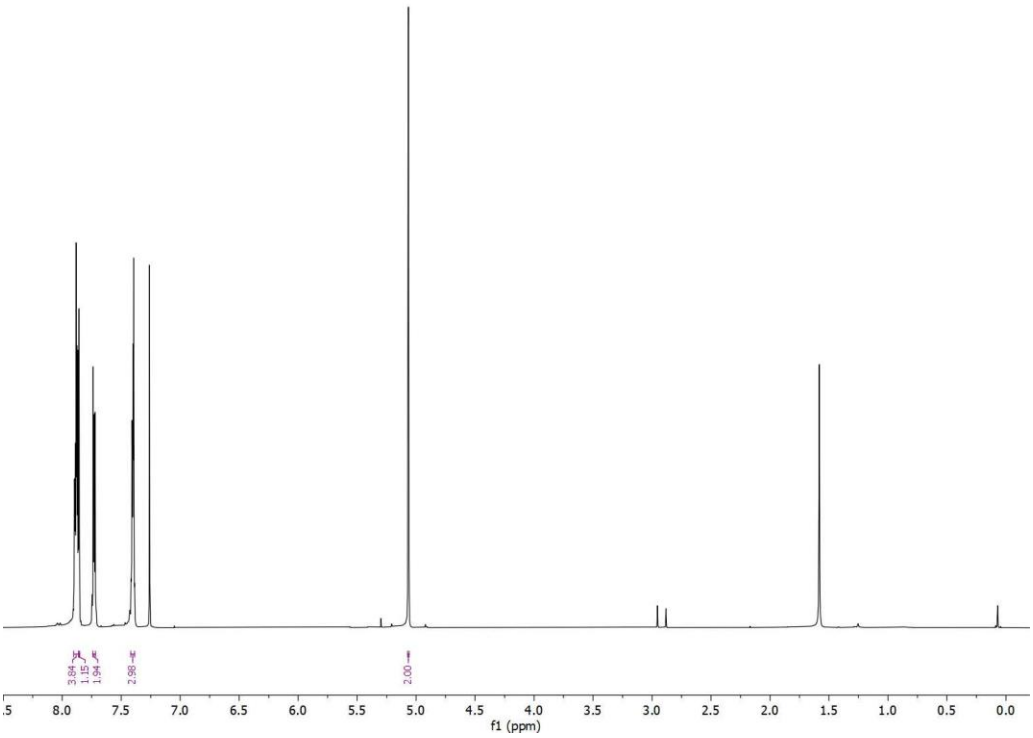
Compound 4.3e

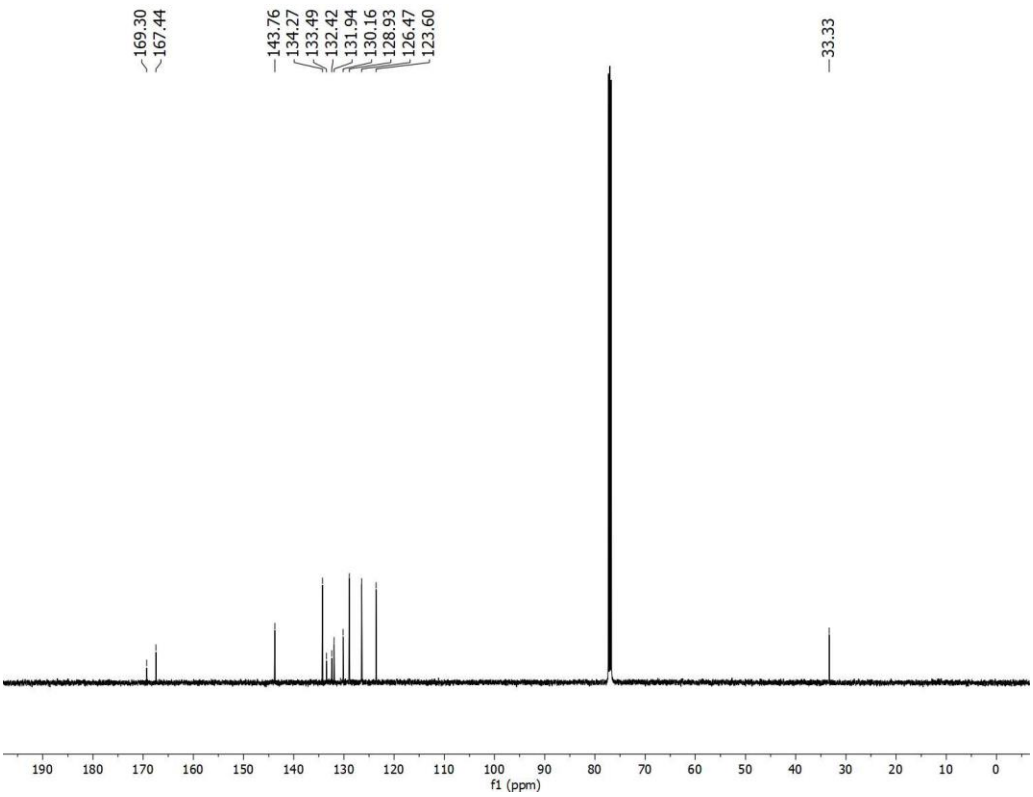




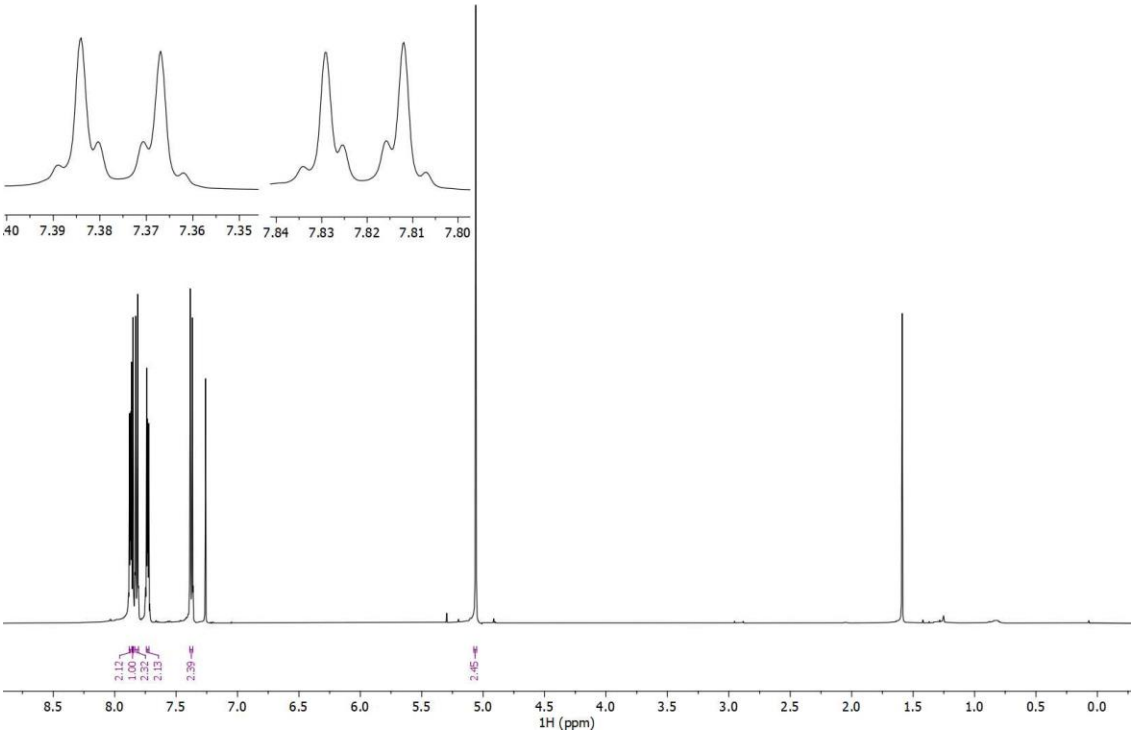


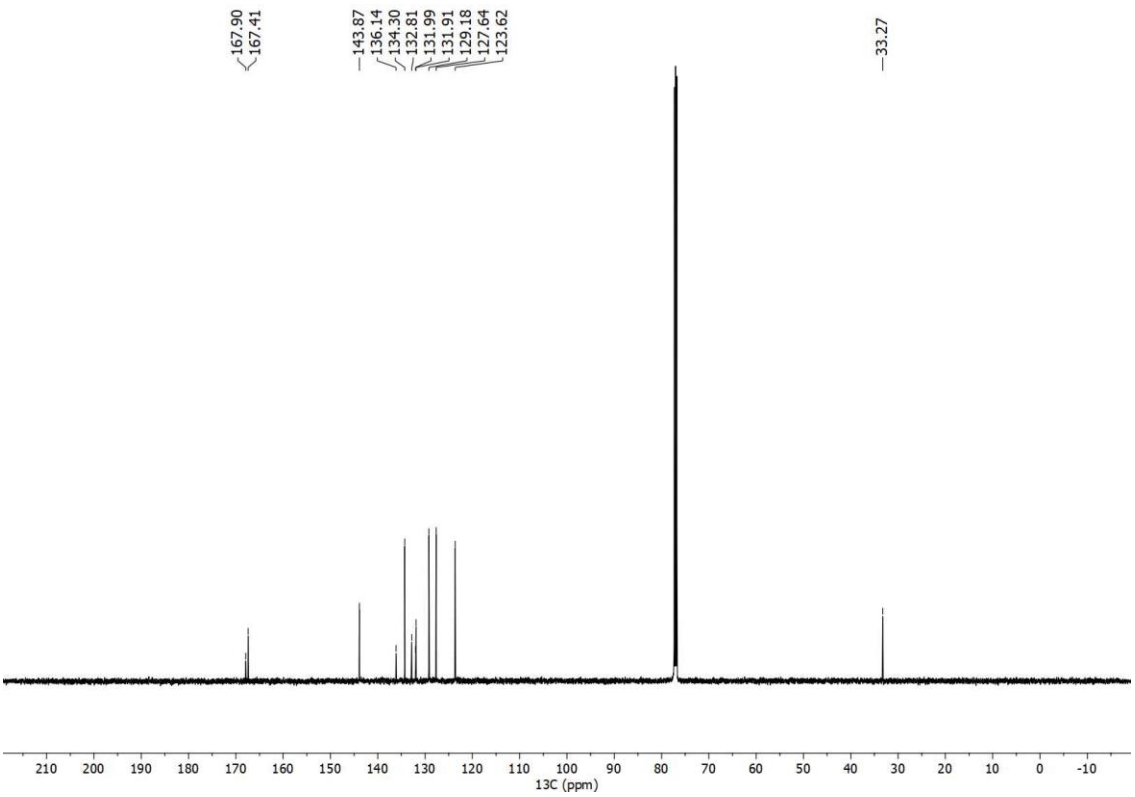
Compound 4.5a



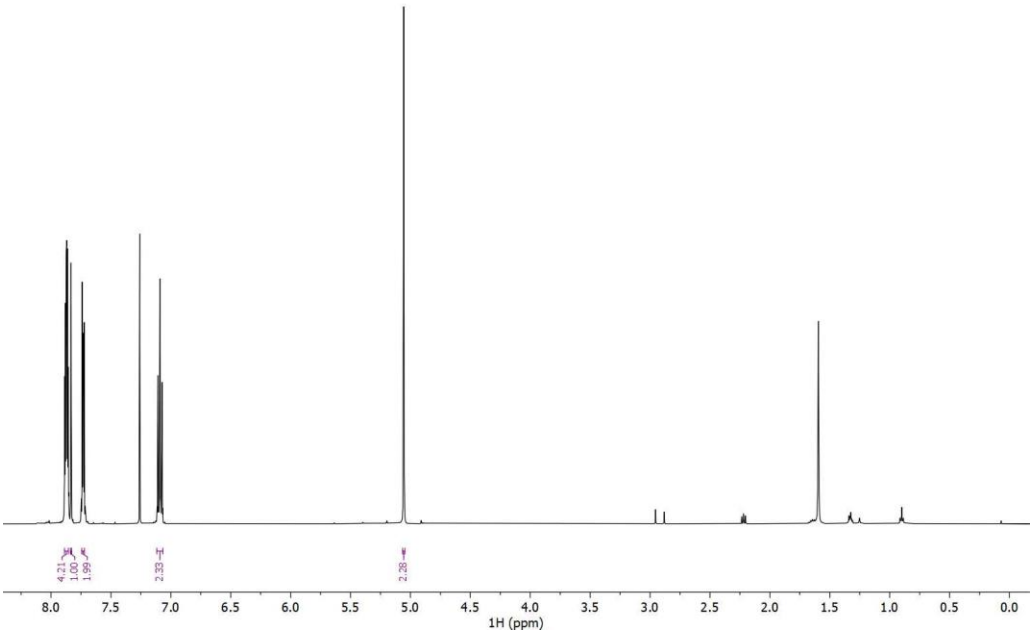


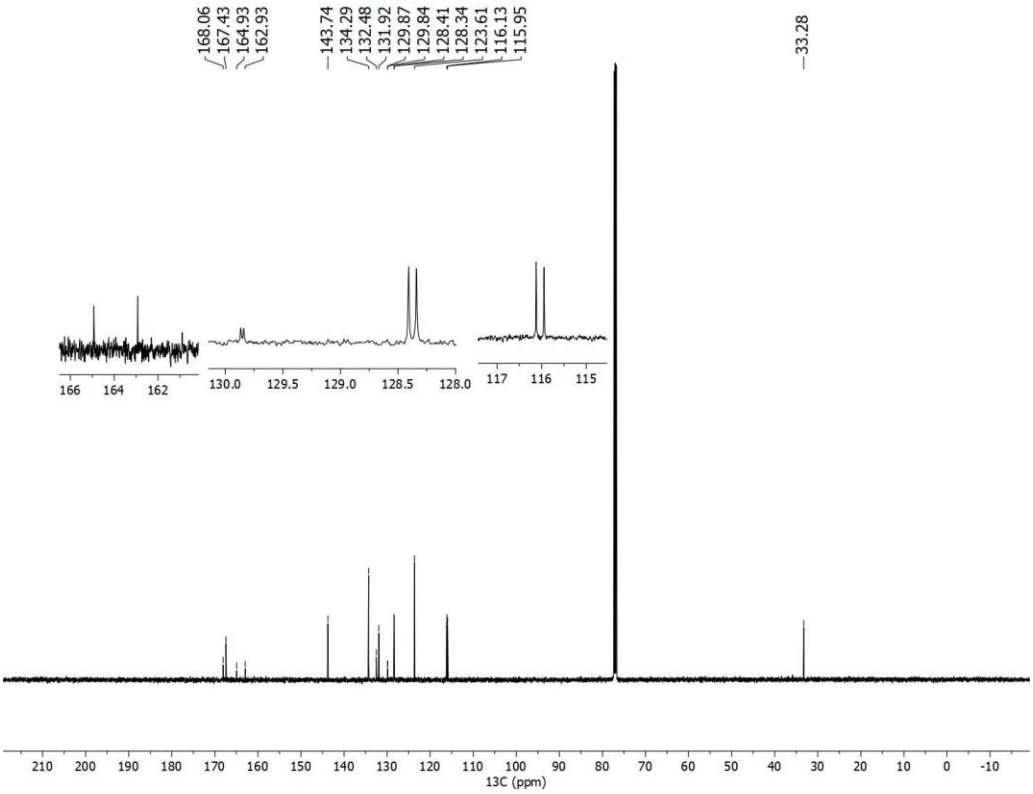
Compound 4.5b

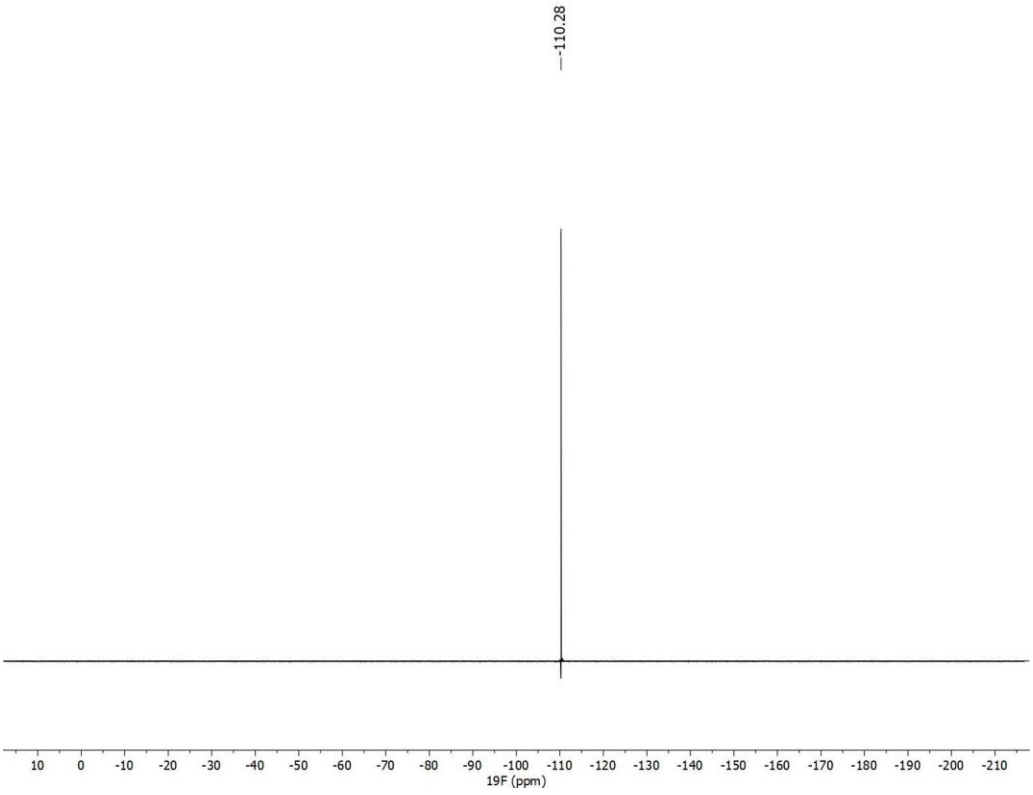




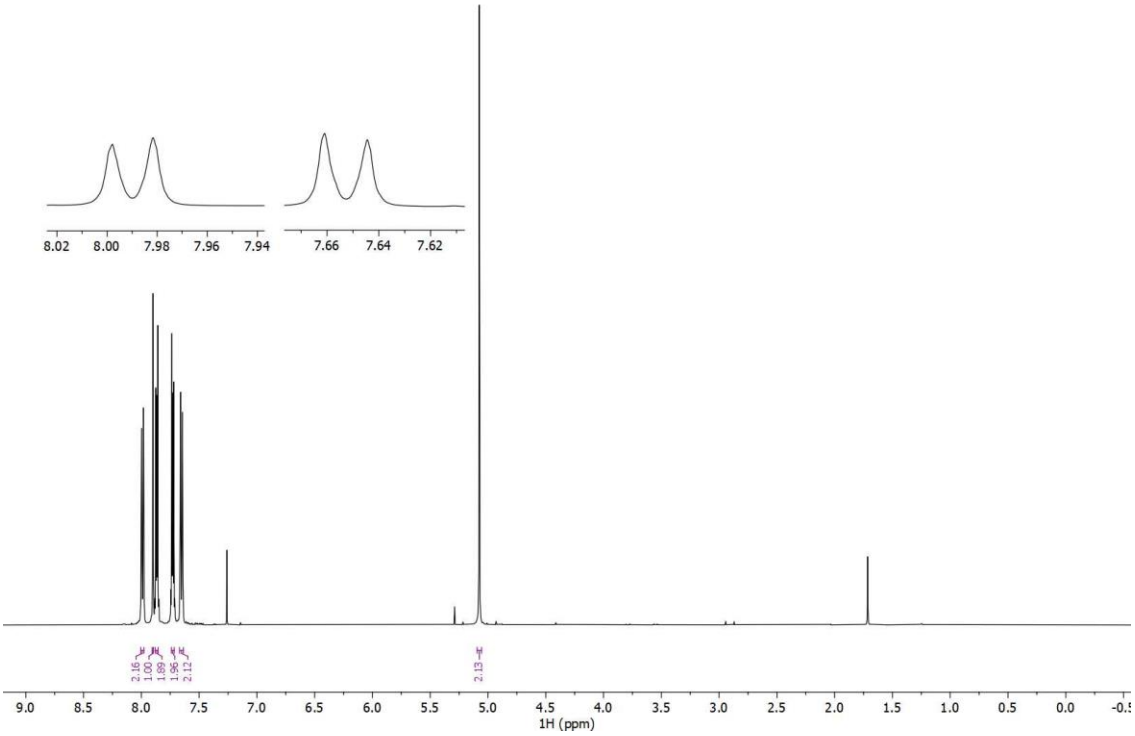
Compound 4.5c

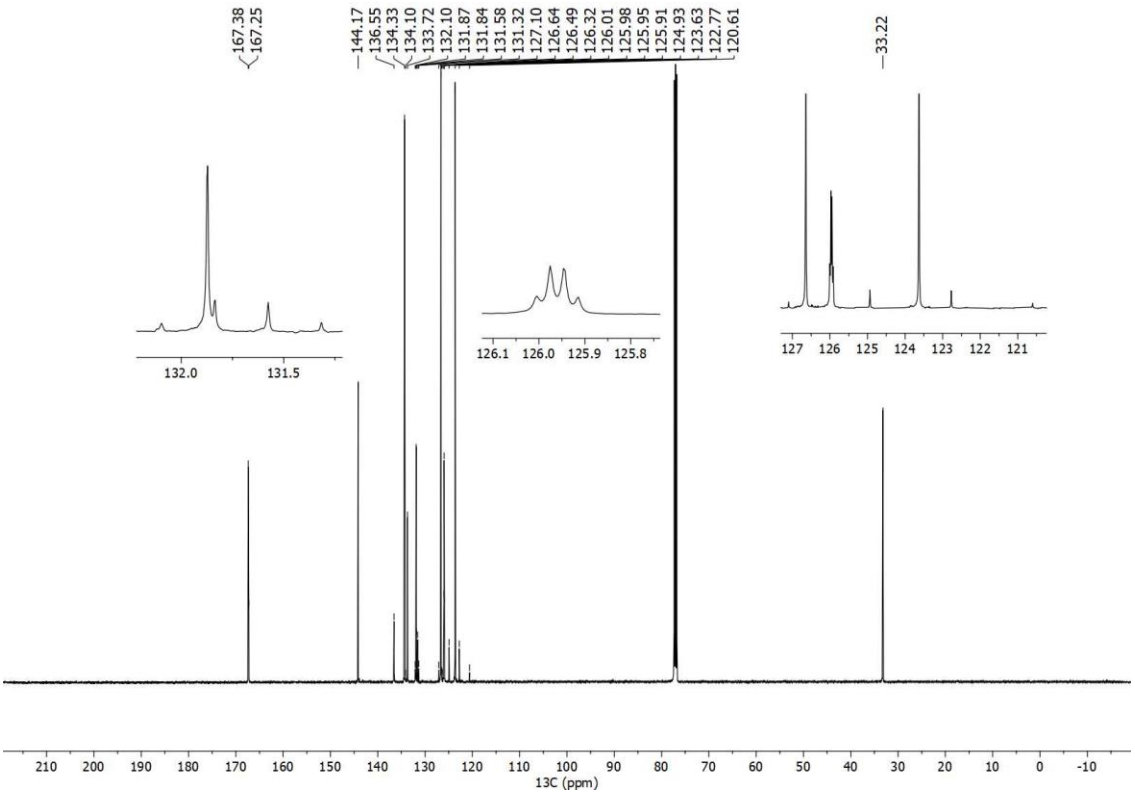


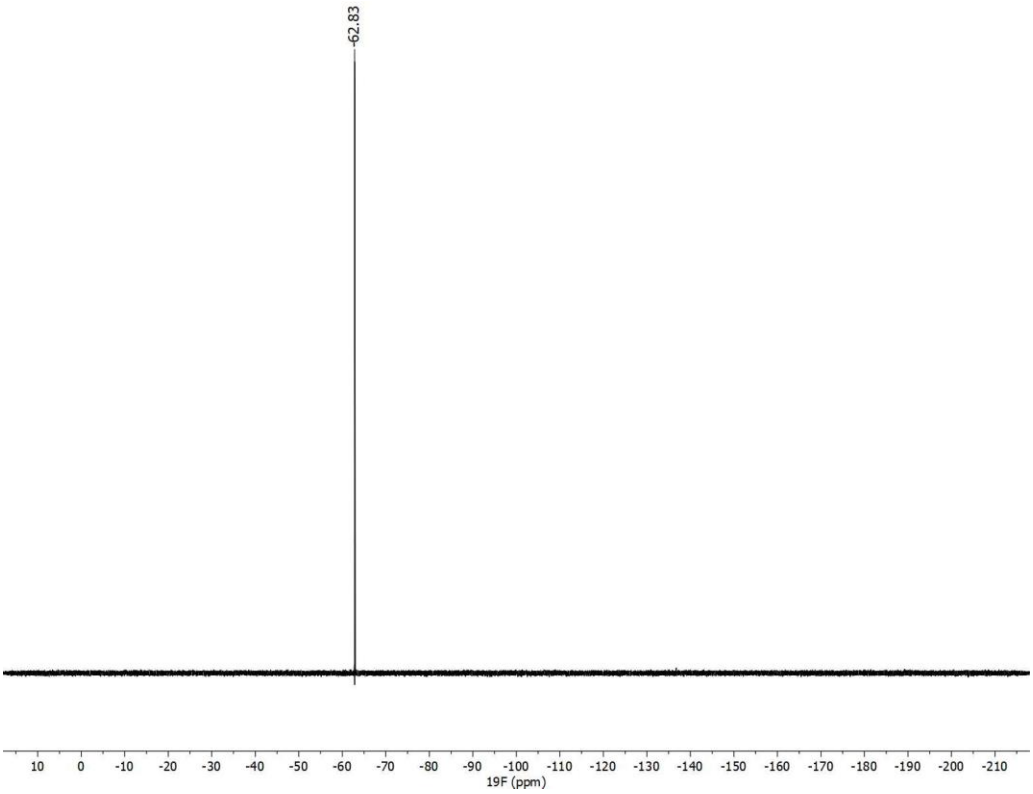

168.06
167.43
164.93
162.93
143.74
134.29
132.48
131.92
129.87
129.84
128.41
128.34
123.61
116.13
115.95
33.28
166
164
162
130.0
129.5
129.0
128.5
128.0
117
116
115
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
13C (ppm)


-110.28
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
19F (ppm)

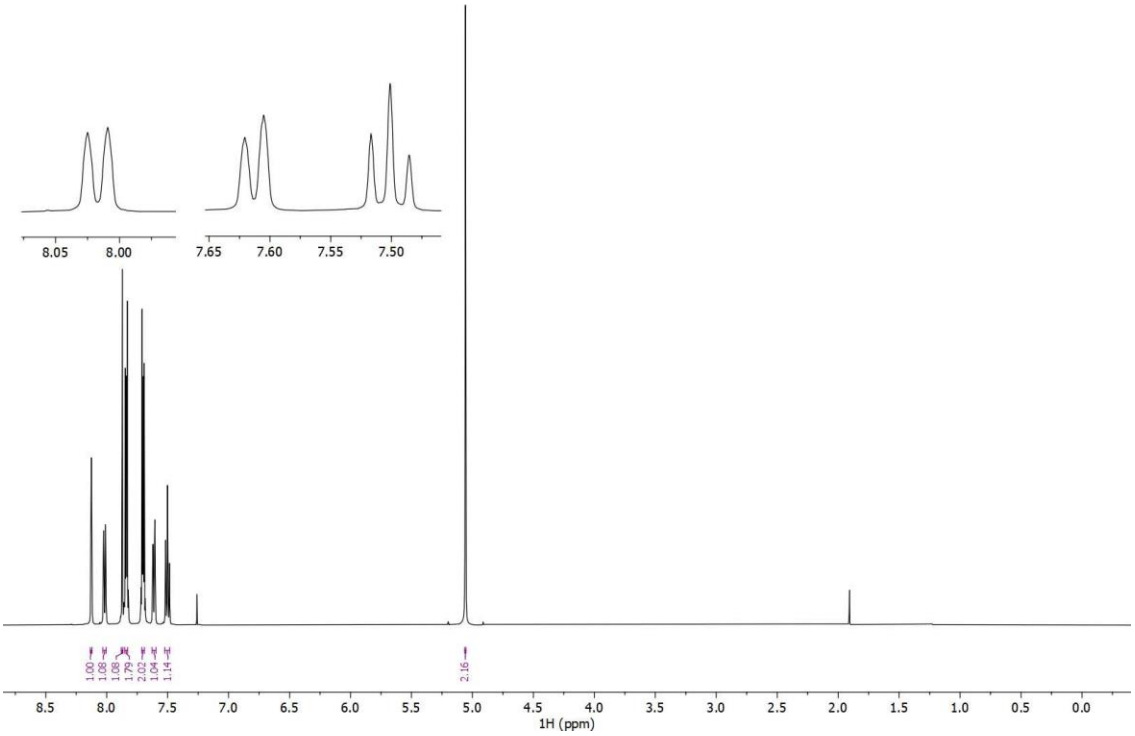
Compound 4.5d

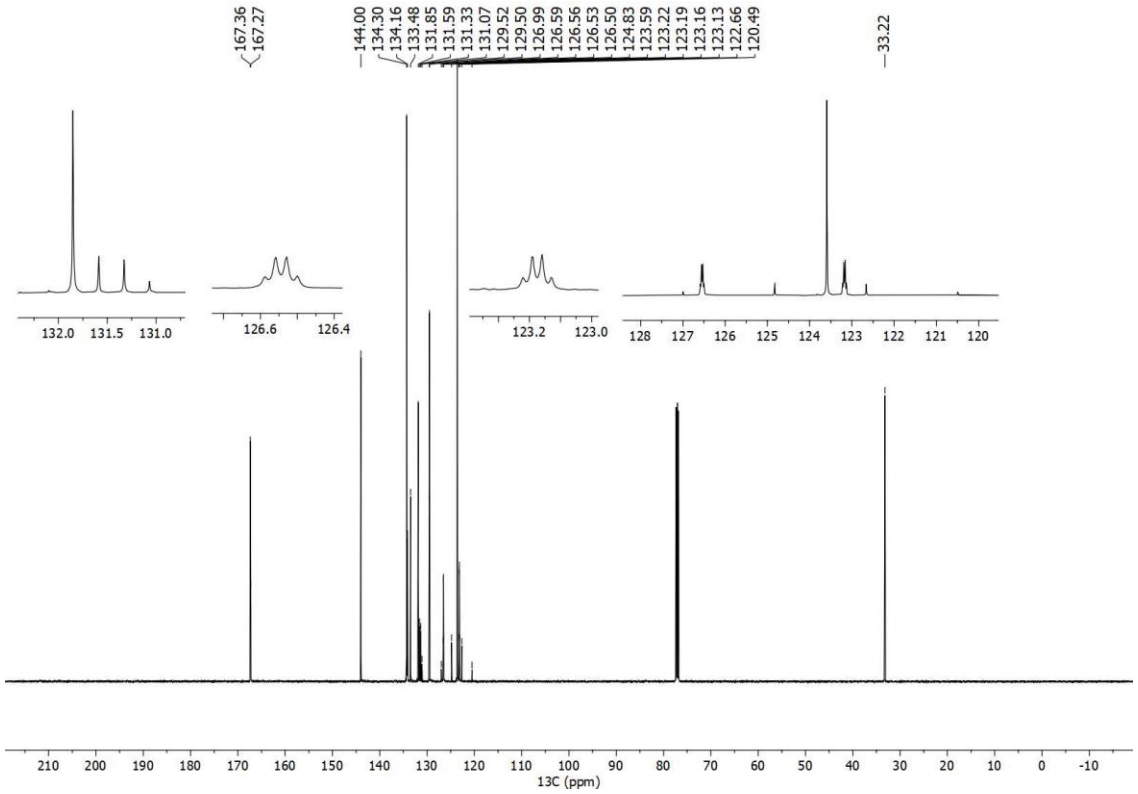


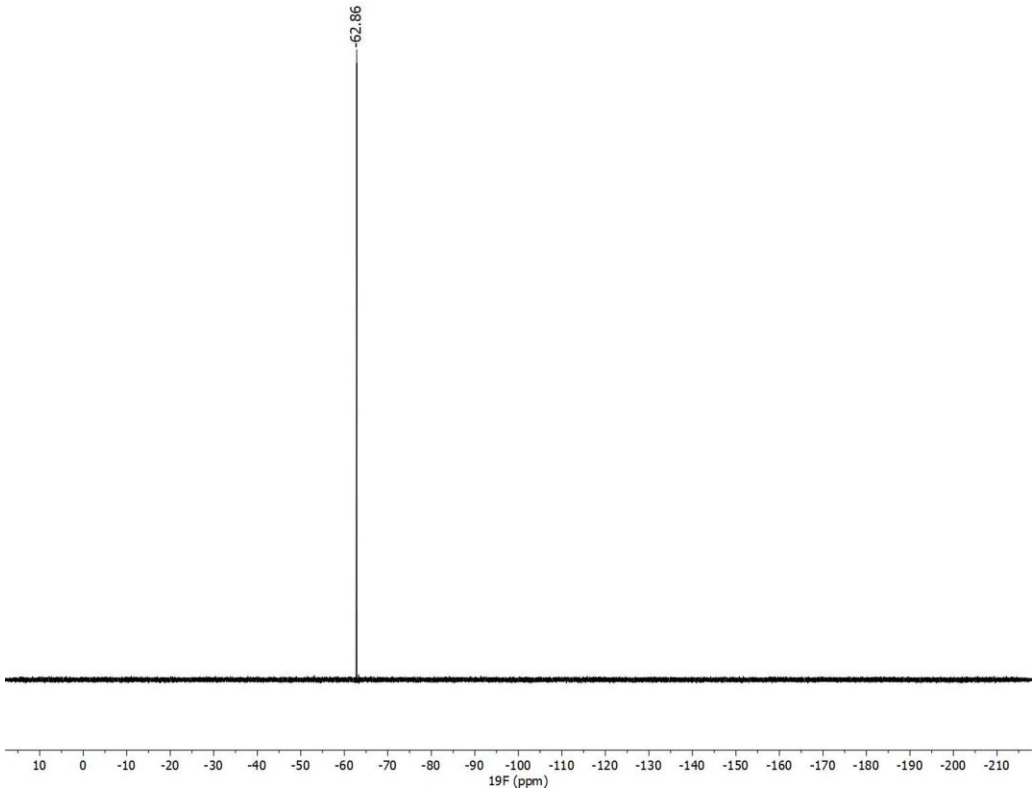




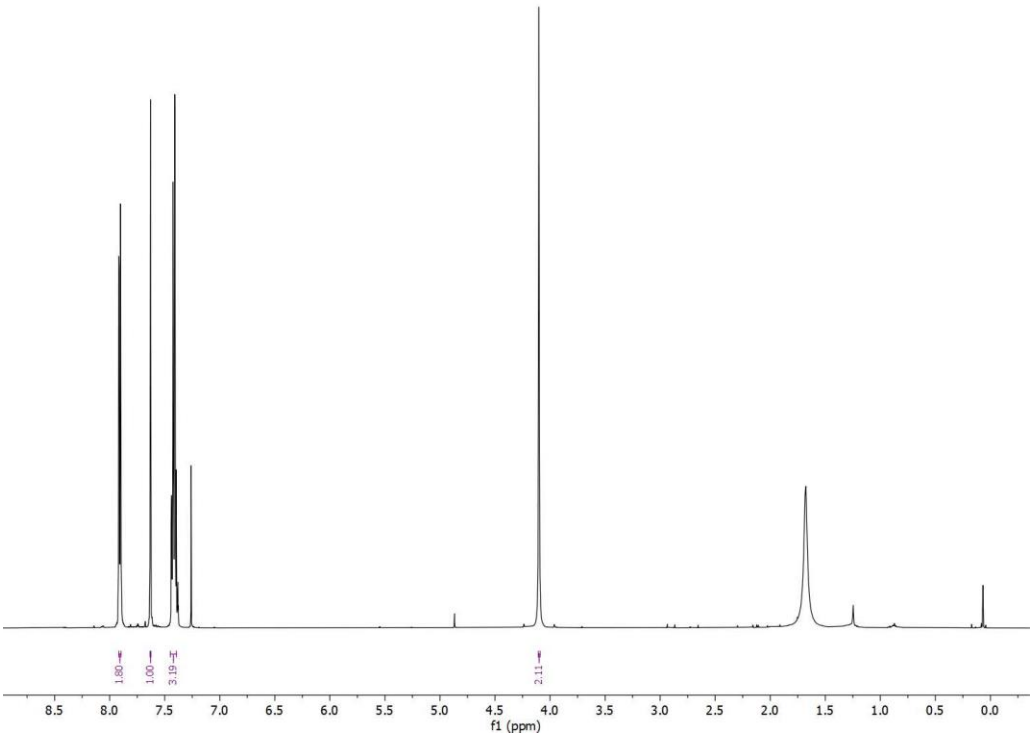
Compound 4.5e

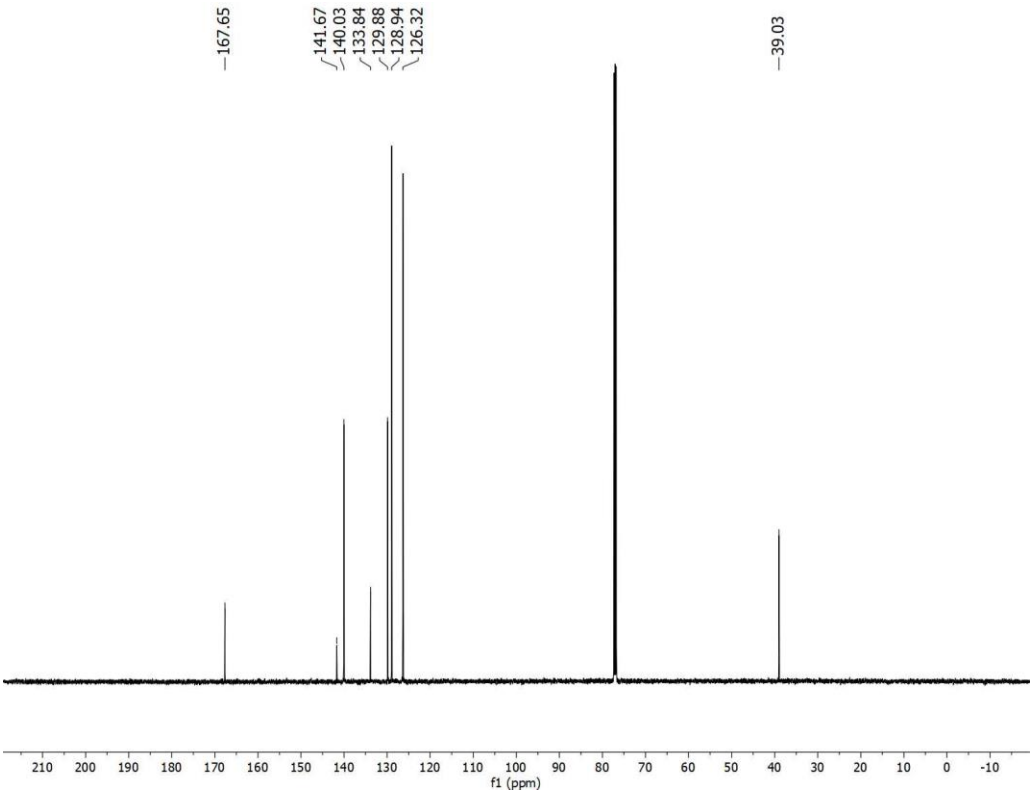



167.36
167.27
144.00
134.30
134.16
133.48
131.85
131.59
131.33
131.07
129.52
129.50
126.99
126.59
126.56
126.53
126.50
124.83
123.59
123.22
123.19
123.16
123.13
122.66
120.49
33.22
132.0 131.5 131.0
126.6 126.4
123.2 123.0
128 127 126 125 124 123 122 121 120
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

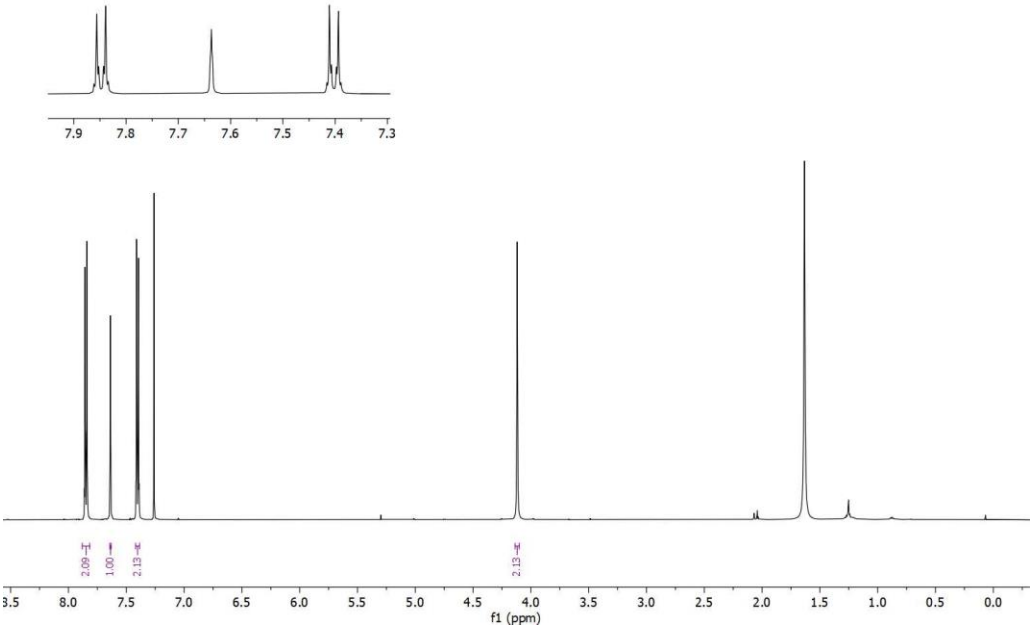

-62.86
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

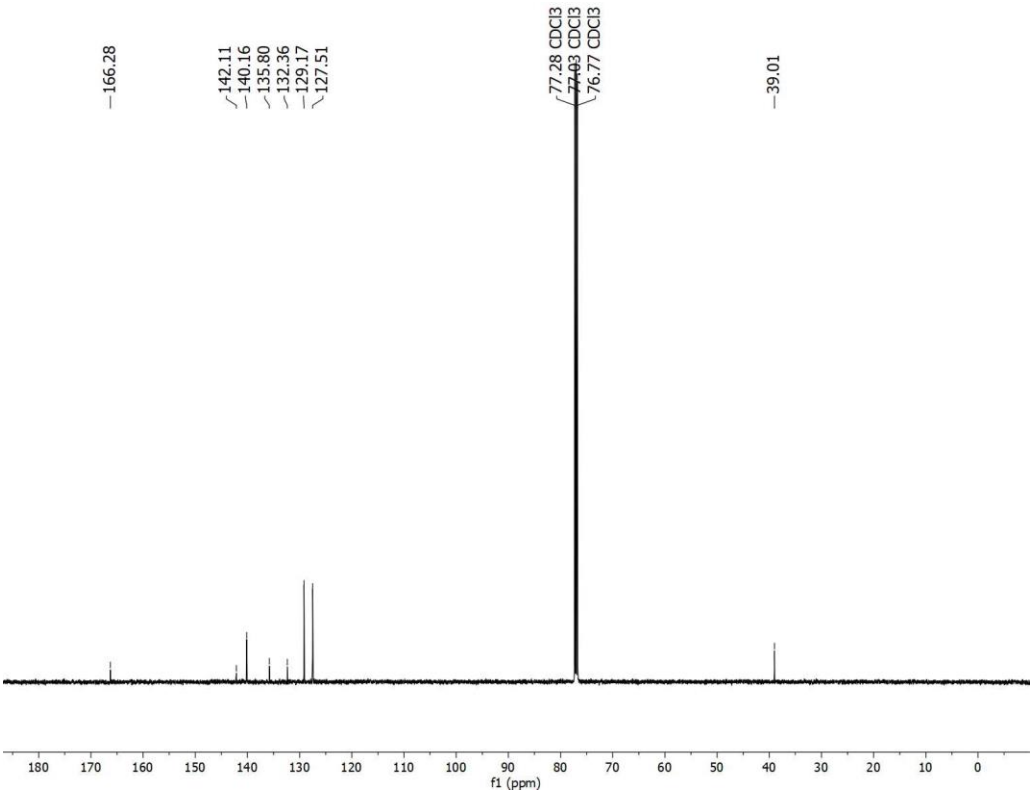
Compound 4.6a



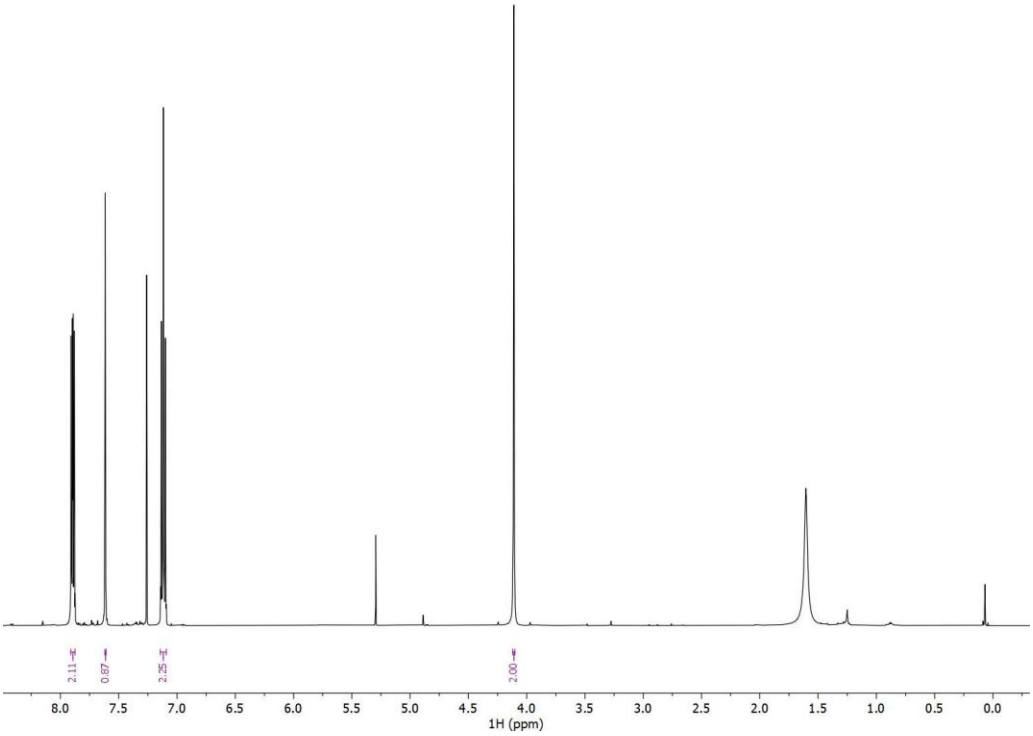


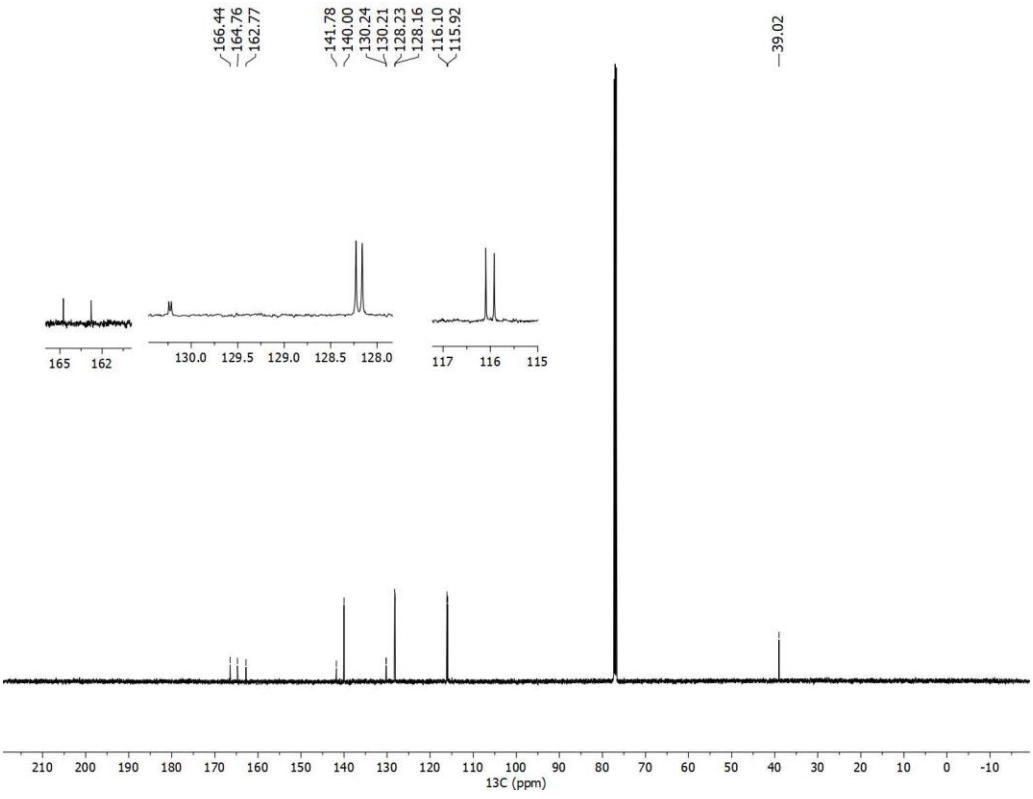
Compound 4.6b

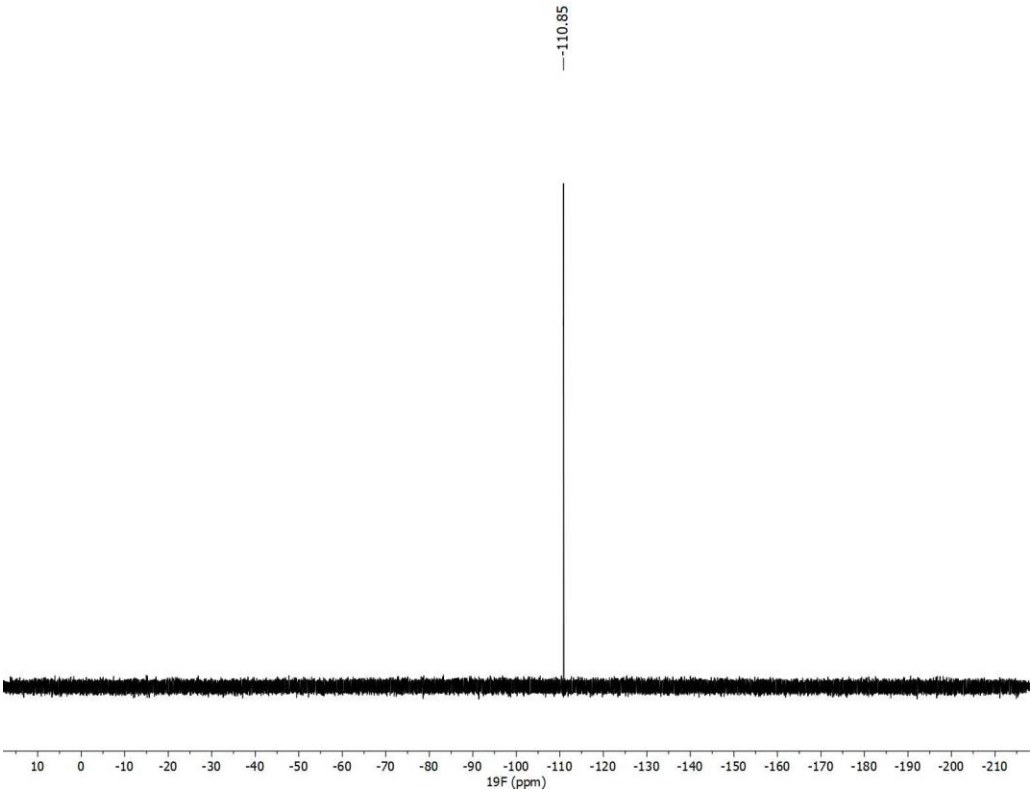




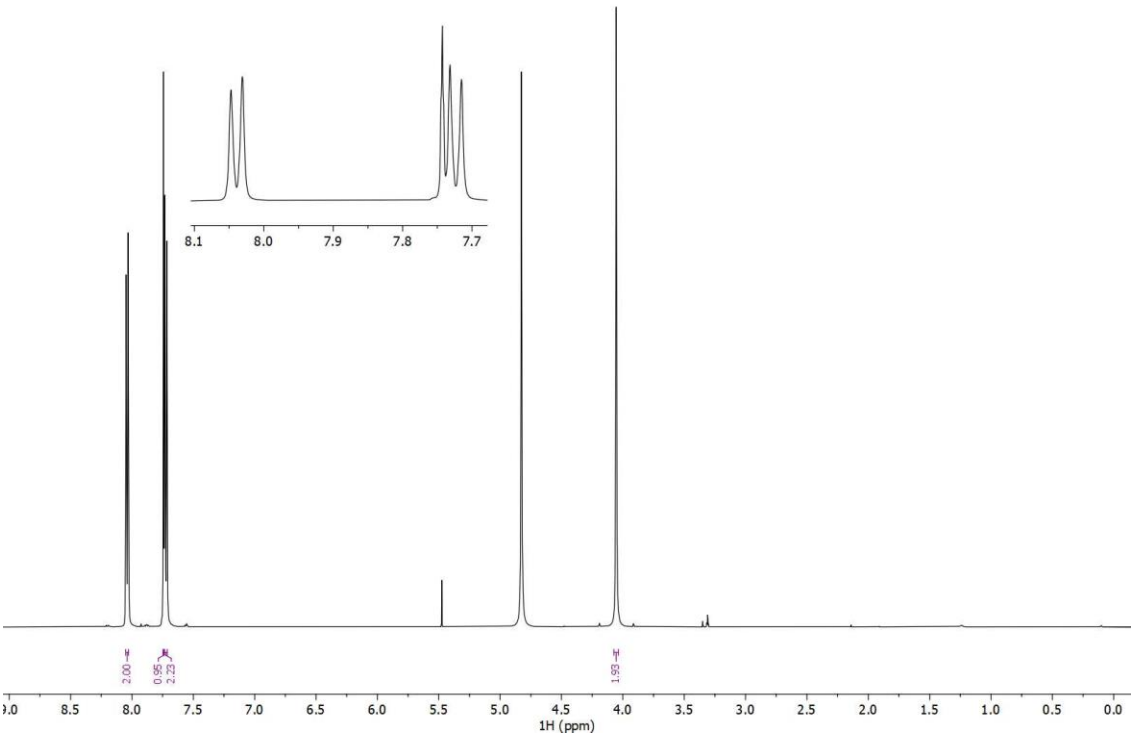
Compound 4.6c

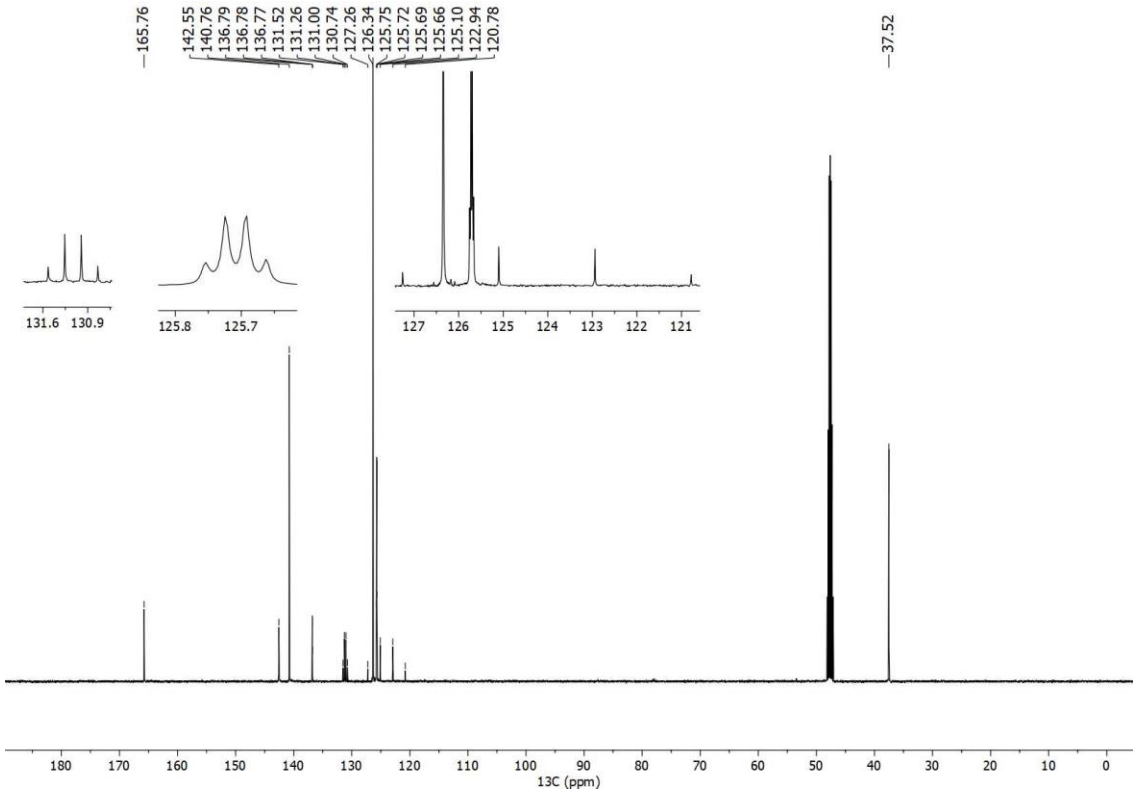


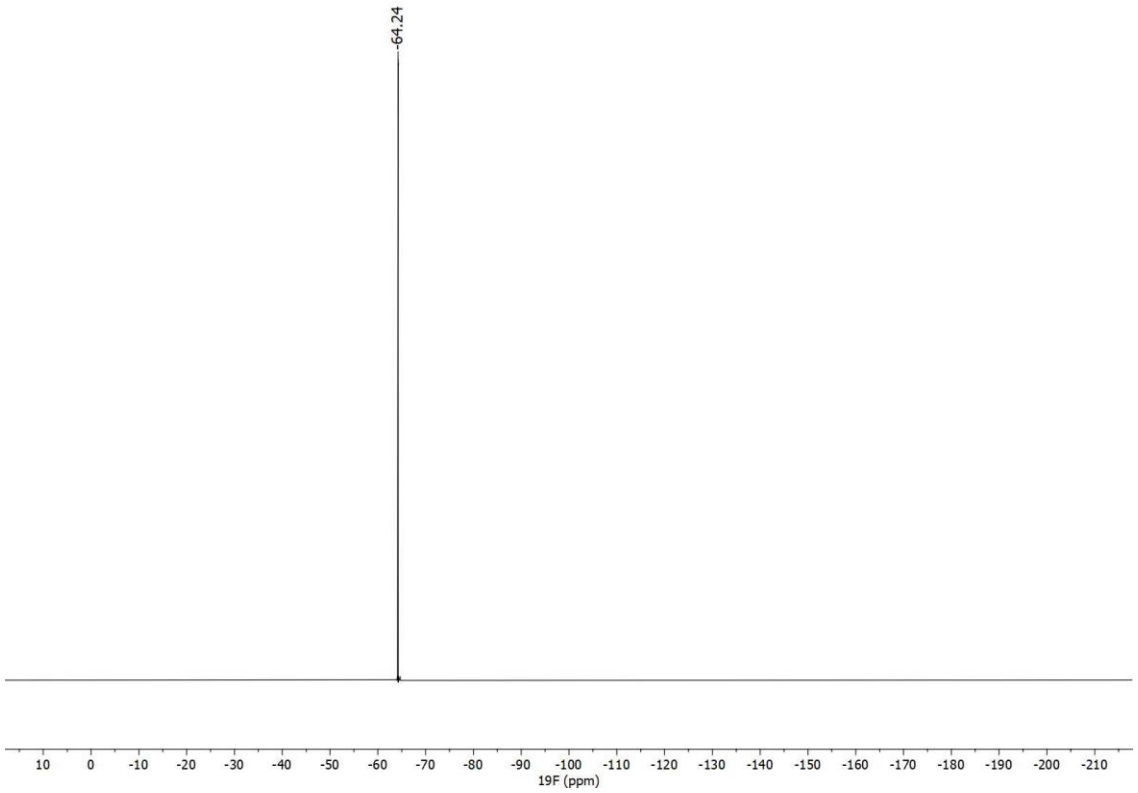




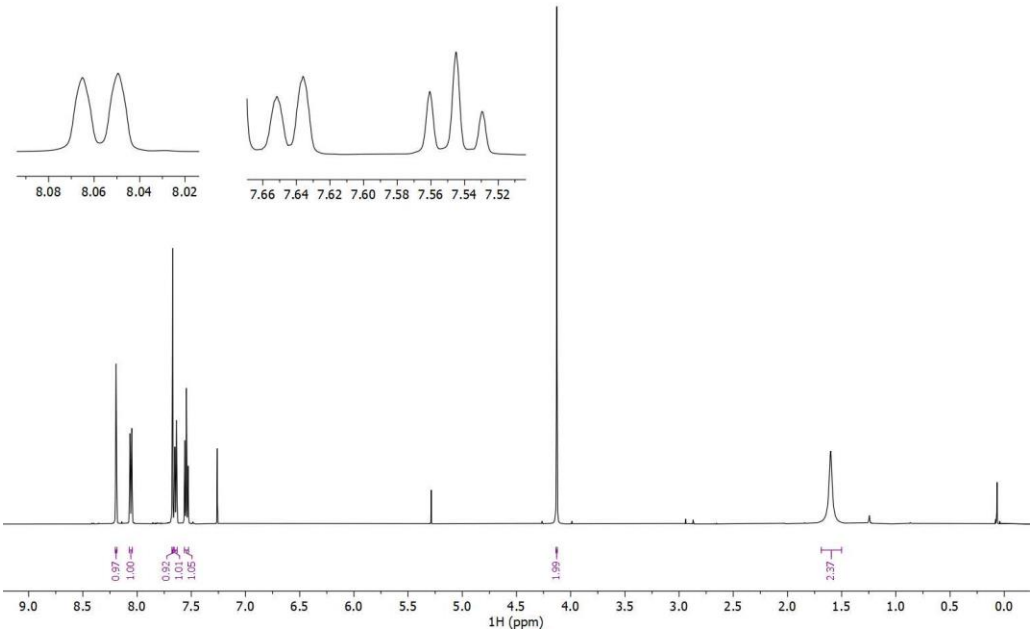
Compound 4.6d

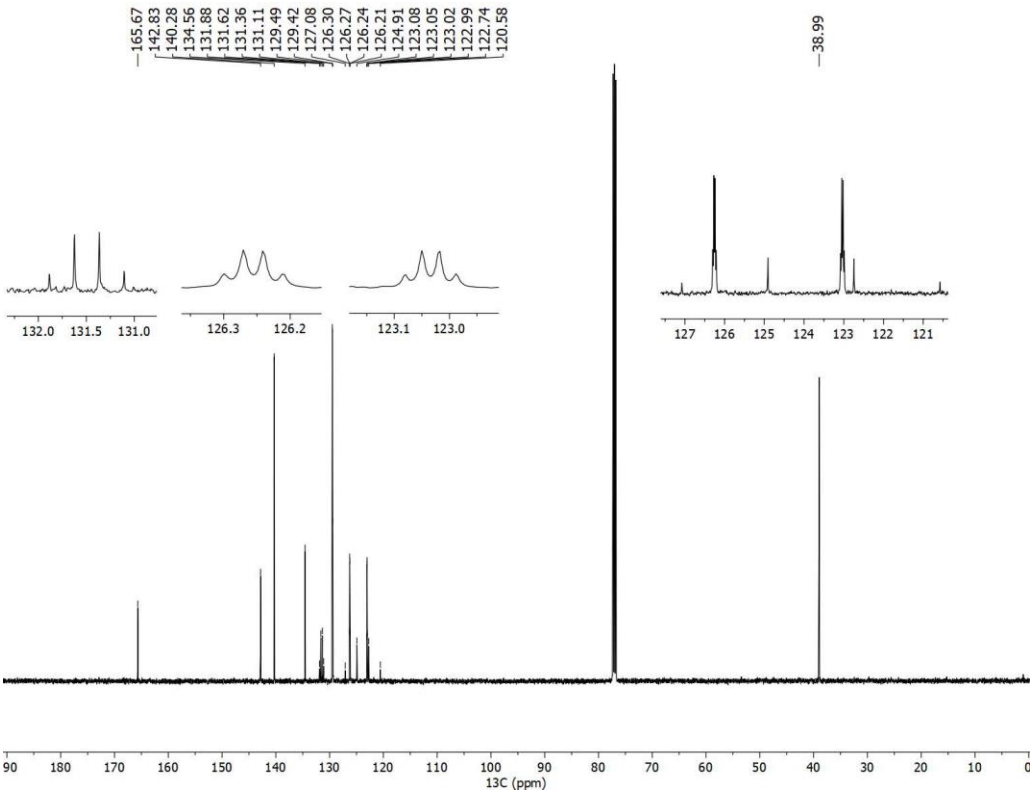


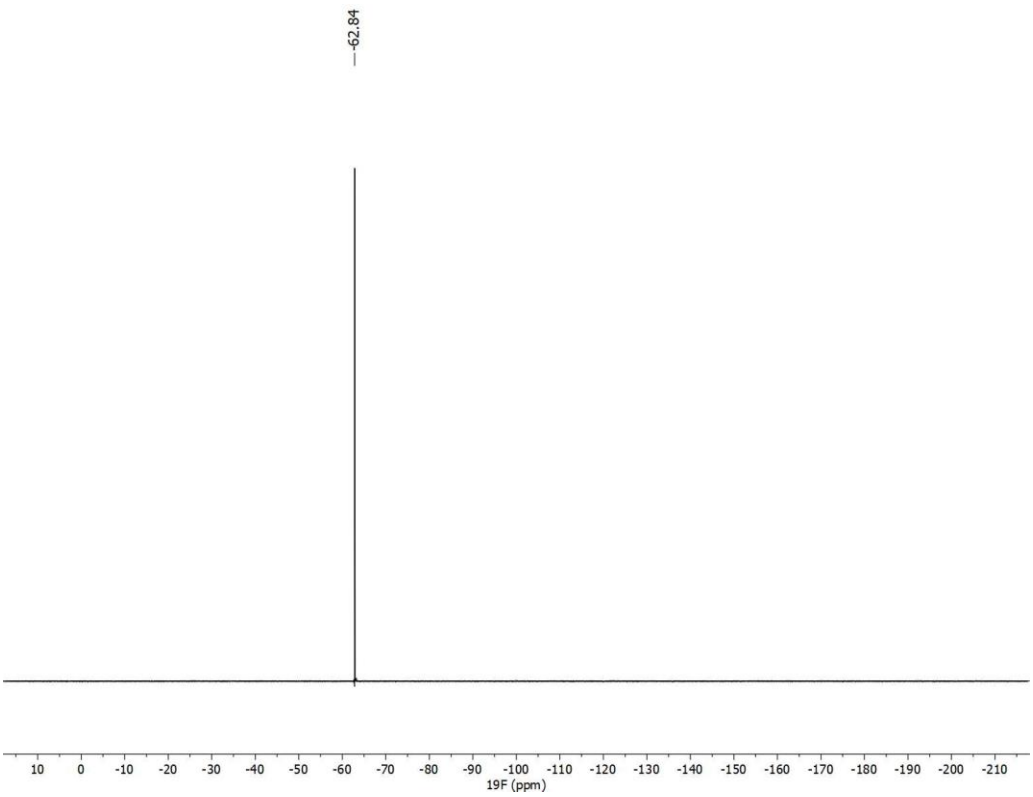

165.76
142.55
140.76
136.79
136.78
136.77
131.52
131.26
131.00
130.74
127.26
126.34
125.75
125.72
125.69
125.66
125.10
122.94
120.78
37.52
131.6 130.9
125.8 125.7
127 126 125 124 123 122 121
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
13C (ppm)


-64.24
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)

Compound 4.6e






-62.84
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
19F (ppm)