Synthesis of Pantothenamide-Mimicking Compounds as Novel Antibacterial and Antiplasmodial Agents

Victoria Virgilio

Department of Chemistry

McGill University

Montreal, Quebec Canada

H3A 0B8

June 2022

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

Table of contents

Abstract

Antibiotics and antimalarial agents are essential to modern medicine. Unfortunately, resistance to these drugs is rapidly spreading. It is estimated that by 2025, 40% of bacterial infections will be resistant to their first line treatment. Similarly, resistance to antimalarial agents….Therefore there is an urgency to discover new antibacterial and antiplasmodial agents. Pantothenamides are a class of compounds with known antibacterial, antifungal and antiplasmodial activities, meaning that they inhibit the growth of bacteria, fungi, and *Plasmodium* species (the malaria-causing parasites), respectively. Pantothenamides are believed to impede microbial growth after being transformed by the CoA biosynthetic enzymes into the corresponding coenzyme A (CoA) antimetabolites, which are proposed to affect CoA-utilising pathways such as fatty acid biosynthesis. In addition to acting via a new mechanism of action, pantothenamides are accessible synthetic targets, potent and usually non-cytotoxic. The limitation of pantothenamides, however, is that they are unstable in human serum. Enzymes called pantetheinases rapidly hydrolyze pantothenamides into pantothenate and the corresponding amine, making the compounds inactive *in vivo*. Our group has focused on the discovery of pantothenamide derivatives and mimics with reduced pantetheinase-mediated degradation while maintaining or improving their potency towards bacteria or *Plasmodium* species.

In this thesis, 16 novel pantothenamide-mimicking compounds were synthesized, tested for their antibacterial activity, and shared with collaborators for antiplasmodial studies. Chapter 2 discusses synthetic strategies to access compounds containing a thiazole ring instead of the labile amide bond, as well as measurements of antibacterial activity. In Chapter 3, efforts are focused on non-aromatic heterocycles-containing derivatives, including again the synthetic strategies and antibacterial activity studies. None of the compounds reported in this thesis show significant antibacterial activity against *Escherichia coli, Enterococcus faecium, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus* or *Acinetobacter baumannii*. However, considering that similar compounds previously synthesized in our research group inhibited the growth of intraerythrocytic *P. falciparum* at low nanomolar concentrations, we

expect many of them to show meaningful antiplasmodial activity. This information will enable the establishment of new structure-activity relationships for pantothenamide-mimicking compounds. Finally, the results presented in this thesis demonstrate the need for further exploration on these structural scaffolds.

Résumé

Les antibiotiques et les antipaludéens (molécules utilisées pour traiter la malaria) sont essentiels à la médecine moderne. Malheureusement, la résistance à ces médicaments augmente rapidement, au point où on s'attend à ce que 40 % des infections bactériennes soient résistantes à leur traitement de première ligne d'ici 2025, et environ….Il est donc urgent de découvrir de nouveaux agents antimicrobiens. Les pantothénamides forment une classe de molécules connue pour leurs activités antibactériennes, antifongiques (bloquant la croissance des champignons) et antiplasmodiales (inhibant la croissance des souches *Plasmodium*, connues pour causer la malaria). Ces molécules agissent après avoir été transformées en antimétabolites par les enzymes biosynthétiques de la coenzyme A (CoA). Ces antimétabolites inhibent ensuite les enzymes utilisant la CoA, comme celles impliquées dans la production des acides gras. En plus d'agir par un nouveau mécanisme, les pantothénamides sont des cibles synthétiques facilement accessibles, très actives et généralement non cytotoxiques. Parcontre, l'utilisation des pantothénamides est limitée par leur instabilité dans le sérum humain. Des enzymes de type pantéthéinases hydrolysent rapidement les pantothénamides pour produire la pantothénate et l'amine correspondante, rendant ces composés inactifs *in vivo*. Notre groupe de recherche s'est concentré sur la synthèse de dérivés ou de molécules imitant les pantothénamides tout en étant stables face à la pantéthéinase et démontrant une haute efficacité.

Dans cette thèse, 16 nouvelles molécules imitant les pantothénamides ont été synthétisées, testées pour leur activité antibactérienne, et partagées avec nos collaborateurs pour une évaluation de leur activité antiplasmodiale. Le chapitre 2 discute la synthèse de dérivés comprenant un groupe thiazole pour remplacer l'amide scissile, ainsi que leur activité antibactérienne. Le chapitre 3 se concentre sur des dérivés pourvus d'un hétérocycle non aromatique, et explique encore une fois les stratégies de synthèse et l'effet de ces molécules sur la croissance bactérienne. Aucun des composés rapportés ne s'est avéré efficace pour bloquer la croissance des bactéries *Escherichia coli*, *Enterococcus faecium*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* ou *Acinetobacter baumannii*.

Cependant, compte tenu que des composés similaires préparés dans notre groupe inhibitent la croissance intraerythrocytique de *P. falciparum* a des concentrations nanomolaires, nous anticipons que plusieurs des composés présentés dans cette thèse auront une importante activité antiplasmodiale (c'est-à-dire à des concentrations nanomolaires). L'activité mesurée par nos collaborateurs servira à établir des relations structure-activité utiles à la progression du projet. Finalement, ces travaux démontrent la nécessité d'une exploration plus approfondie sur les échafaudages structuraux présentés dans cette thèse dans le but de développer de nouveaux antipaludéens.

Acknowledgments

Firstly, I would like to thank Dr. Karine Auclair, through the past two years; she has provided useful feedback and tips on characterization, chemistry, biology and presentations. I am grateful to have met my wonderful labmates in the Auclair group. I would like to thank all past and present group members I have crossed paths with. Specifically, I would like to thank Daniel for his help with the MilliQ systems; Annica and Jake for teaching me the antimicrobial susceptibility assay; Annica and Chunling helping me with synthetic challenges.

I was lucky to have two wonderful committee members, Dr. Bohle 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, Lumb, Moitessier, Perepichka, and Tsantrizos research groups for lending me chemicals. I would like to thank my friends and boyfriend, Alexia, Jane, Shay, Brianna and Marco for their encouragement and support throughout the past two years. 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. baumanii	*Actinetobacter baumannii*
ACN	Acetonitrile
ACT	Artemisinine-based combination therapy
AIDs	Acquired immunodeficiency syndrome
CoA	Coenzyme A
DCM	Dichloromethane
DDT	dichloro-diphenyl-trichloroethane
DPCK	Dephospho-CoA kinase
EA	ethyl acetate
E. coli	*Escherichia coli*
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
E. faecium	*Enterococcus faecium*
HIV	Human immunodeficiency virus
IC_{50}	50% growth inhibition
ITNs	insecticide-treated nets
IRS	indoor residual spraying
K. pneumonia	*Klebsiella pneumoniae*
MIC	Minimum inhibitory concentration
NCS	*N*-chlorosuccinimide
P. aeruginosa	*Pseudomonas aeruginosa*

PanK	Pantothenate Kinase
P. falciparum	*Plasmodium falciparum*
PPAT	Phosphopantetheine adenylyltransferase
PPCDC	Phosphopantothenoylcysteine decarboxylase
PPCS	Phosphopantothenoylcysteine synthetase
P. vivax	*Plasmodium vivax*
SAR	Structure-activity relationship
S. aureus	*Staphylococcus aureus*
TBD	triazabicyclodecene
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

Chapter 1

Introduction

1.1 Infectious Diseases

Infectious diseases are diseases caused bacteria, fungi, parasites or viruses and continue to be a major health issue. Furthermore, the emergence of novel and hard to treat bacterial, viral, and fungal pathogens are an increasing global concern.[1] Lower respiratory infections remain the world's most deadly communicable disease. It is ranked the 4th leading cause of death, claiming 2.6 million lives in 2019.[2] Complications of these diseases can lead to secondary bacterial or viral infections, causing an even weaker immune system.[3]

The introduction of antibiotics and vaccines into clinical use were historic medical breakthrough of the 20th century. Not only are antibiotics used to treat infectious diseases, they also make modern medical procedures such as cancer treatment, organ transplants and open-heart surgery possible.[4,5] However, wide use of antibiotics has resulted in the rise of antimicrobial resistance, resulting in an increasing number of untreatable infections. There are a rising number of patients with infections caused by antibiotic resistant Gram-positive or Gram-negative bacteria. Therefore, the development of new drugs and treatment regimens are urgent.[2] It is predicted that by 2050, 10 million people will die from drug resistant every year.[6]

Unfortunately, the same phenomenon is also happening for drugs used to kill various parasites such as the malaria-causing *Plasmodium* species.

1.2 Malaria

1.2.1 Malaria Overview

Vector-borne diseases are infections transmitted by a living organism. They account for more than 17% of all infectious diseases and cause more than 700 000 deaths yearly.[8] Malaria is a vector-borne illness caused by *Plasmodium* parasites and spread to people through the bites of infected female *Anopheles* mosquitoes.[7] Once an insect becomes infectious, they are capable of transmitting the pathogen for the rest of their life during each bite.[8] Out of approximately 156 *Plasmodium* species, only six infect humans: *Plasmodium falciparum*, *Plasmodium vivax*,

Plasmodium malariae, *Plasmodium ovale curtisi*, *Plasmodium ovale wallikeri* and *Plasmodium knowlesi*.[8] *P. falciparum* and *P. vivax* are the most common species responsible for malaria cases in sub-Saharan Africa.[7] *P. falciparum* account for the most deaths and *P. vivax* is the most widespread globally.[9] In 2020, 241 million cases of malaria were reported with an estimated 627 000 deaths worldwide.[7] Infants, children under the age of 5, pregnant women and patients with HIV/AIDS are most vulnerable for contracting malaria, with 85% of deaths accounting for children under 5.[7, 9, 10] The first symptoms of malaria are fever, headache and chills, which occur 10–15 days after the infective mosquito bite. If left untreated, *P. falciparum*-caused malaria can lead to severe illness and death within 24 hours.[7]

1.2.2. Life cycle of *Plasmodium* species

The life cycle of *Plasmodium* species starts when an infected female *Anopheles* mosquitoes takes a blood meal from a human and injects sporozoites into the bloodstream.[11] The sporozoites enter the bloodstream and make their way to the liver where schizonts are formed in a first round of asexual replication.[12, 13] The schizonts grow until they burst and release daughter cells called merozoites into the blood circulation.[11] The merozoites then invade erythrocytes, in which a second asexual reproduction takes place. The erythrocytes eventually burst, which leads to the start of symptoms in the host[14] The parasite enters the sexual phase of its cycle when some asexual parasite cells enter the bone marrow and produce gametocytes. The gametocytes travel in the human blood and can be ingested by a mosquito during a blood meal.[11] After entering the mosquitos' gut, the male and female gametocytes differentiate into male and female gametes and undergo sexual reproduction to form zygotes.[9, 11] Zygotes mature into sporozoites and the parasite has completed one life cycle and can start another.[9,11]

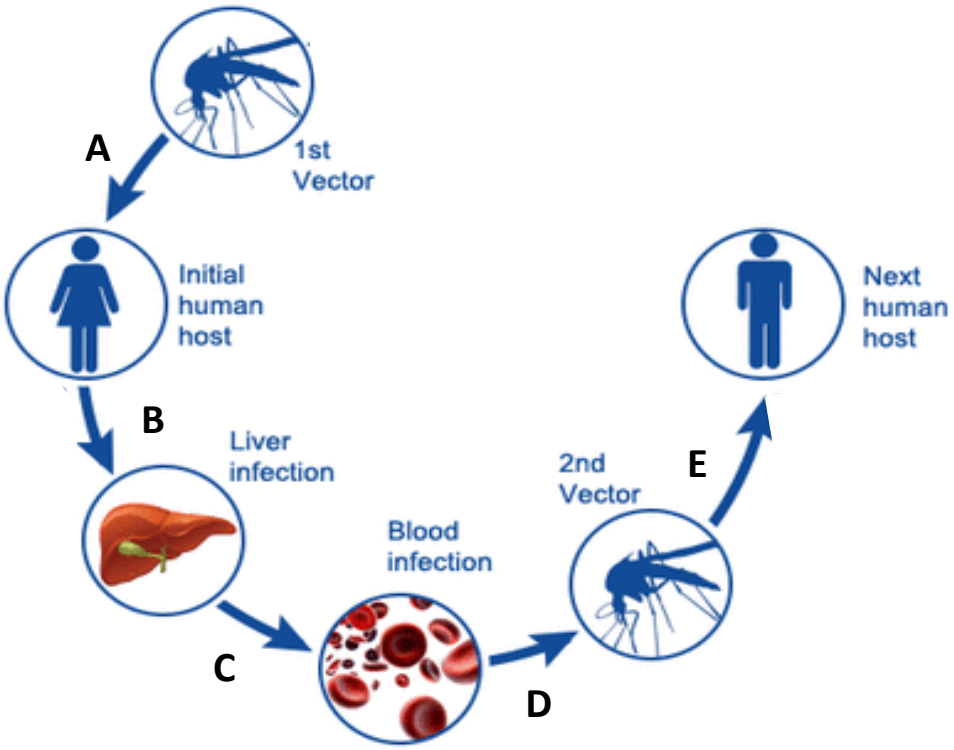


Figure 1.1: The malaria life cycle. A) The infected female mosquitoes takes a blood meal on the human host and injects sporozoites into the bloodstream. B) The sporozoites enter the liver. C) Merozoites are produced and invade red blood cells, after the parasite enters the sexual phase of this step gametocytes are produced. D) The gametocytes travel in the blood and the blood is now infected and can be ingested by another mosquito during a blood meal. E) The infected mosquito can now infect another human host and the cycle starts again. Figure from The Jenner Institute[15]

1.2.3 Antimalarial drugs and parasite resistance

Antiplasmodial and Antimalarial agents target the parasite of genus *Plasmodium*. Some important prevention methods for malaria include the use of insecticide-treated nets (ITNs), indoor residual spraying of insecticides (IRS) and vaccines.[7] The insecticides used for ITNs are

pyrethroids, e.g. permethrin, deltamethrin and dichloro-diphenyl-trichloroethane (DDT).[16, 17] Another preventive measure is prophylactic chemotherapy, which consists of intermittent or seasonal drug treatment of asymptomatic infants and mass drug administration.[7] All of these therapies use antimalarial drugs and target the most vulnerable populations, during the times of the year when malaria is the most prevalent.[18, 19] These drugs include artemether, artemisinin, artesunate, amodiaquine, chloroquine, dihydroartemisinin, doxycycline, mefloquine, primaquine, pyrimethamine, quinine, and sulfadoxine (Figure 1.3).[20] The search for a malaria vaccine has been active for decades, and in October 2021, the World Health Organization (WHO) recommended a first vaccine for malaria, the mosquirix or RTS,S/AS01 vaccine, recommended for children living in regions where there is moderate to high *P. falciparum* transmission.[7] It prevents the parasite from infecting the liver. Although required at four doses, its efficacy is only 36%.[21]

Once a patient is infected, there are several drugs in clinical use to treat malaria. The current standard treatment, used since 1994, is artemisinine-based combination therapy (ACT), which uses a variety of drugs in order to overcome the lost efficacy of most mono-therapeutics.[22] However, in 2001, ACT resistance started being observed increasingly, leaving very few effective drugs to treat malaria.[23]

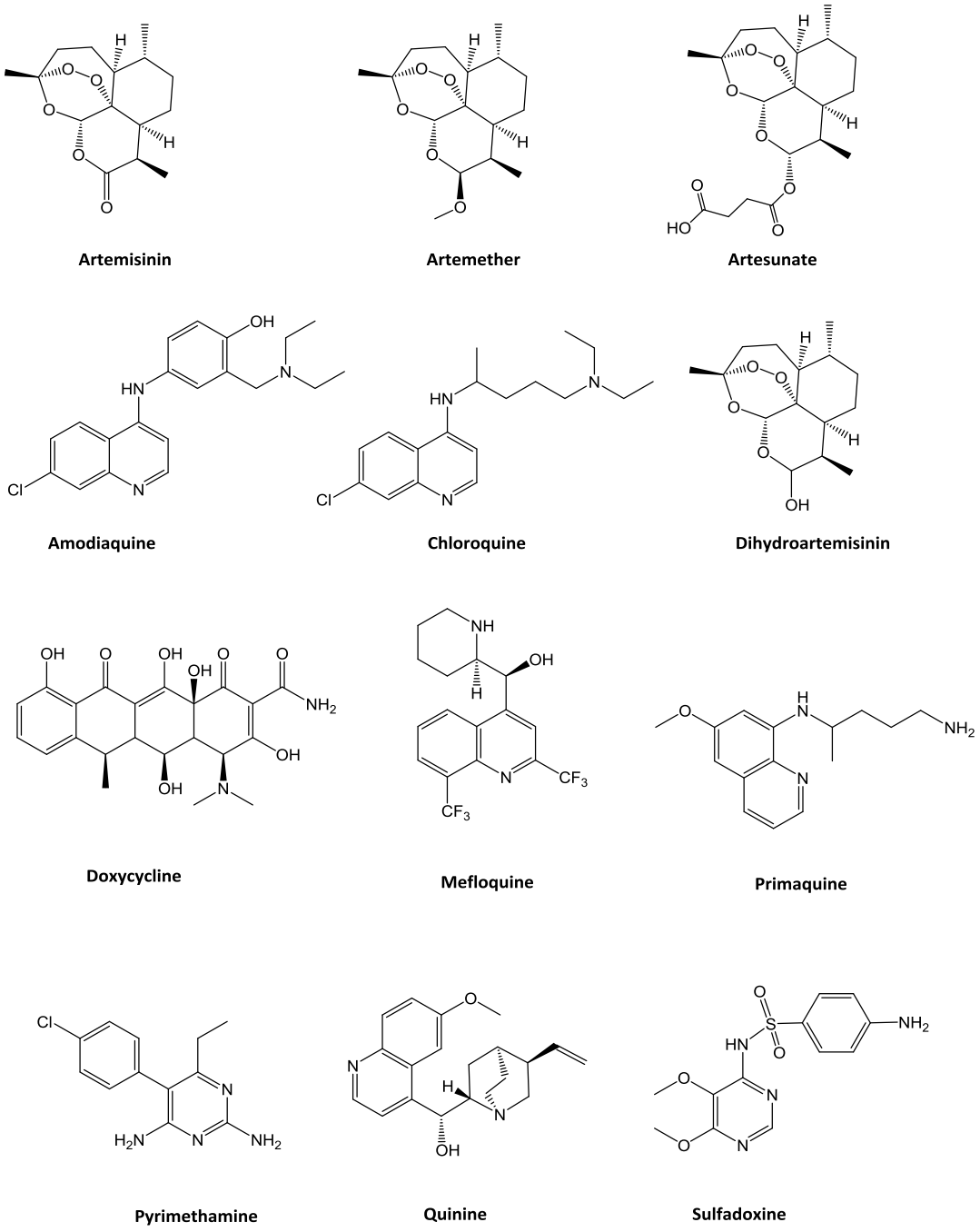


Figure 1.2: Current antimalarial drugs.[20]

The emergence of drug and insecticide resistance is another ongoing issue plaguing efforts to reduce the death toll of malaria, making the discovery of new antimalarials even more crucial. New structural classes of antiplasmodials with a novel mechanism of action are particularly

desirable to minimize cross-resistance. In addition to the rapid resistance rate of *P. falciparum*, as the earth's climate warms up, malaria is expected to disperse to higher latitudes, increasing the number of people threatened by the parasite.[24,25]

1.3 Antibiotics and Antibiotic Resistance

1.3.1 Bacteria: Gram-positive vs. Gram-negative

There are good and bad bacteria for humans. Good bacteria help our bodies absorb nutrients and digest food. Bad bacteria are also known as pathogenic bacteria because they cause diseases. Most bacteria can be categorized in two main groups according to their cell wall structure: Gram-negative and Gram-positive. Gram-negative bacteria have a thin peptidoglycan cell wall, which is surrounded by a lipopolysaccharide outer membrane. Gram-positive bacteria don't have an outer membrane and are composed a thick peptidoglycan cell wall. This classification was established in the mid-1880s by Hans Christian Gram, who developed the Gram stain.[26,27] When using this stain, the bacteria are first subjected to the crystal violet dye before counterstaining the cells. Bacteria that retain the dye and turn purple are considered Gram-positive, while those that do not, are Gram-negative. In the presence of iodine the crystal violet molecule adheres to the thick peptidoglycan layer of Gram-positive cells, but the thinner peptidoglycan layer of Gram negative cells prevent retention of the dye.[28] Gram negative bacteria are known to be difficult to eliminate with bacteria due to the decrease cell wall permeability. [26]

1.3.2 The beginning of antibiotics

Before the 20th Century, life expectancy in North America was 46 and 48 years for men and women, respectively, and most deaths were attributed to infectious diseases.[29] The common infectious diseases at that time included cholera, pneumonia, typhoid fever, plague, tuberculosis, syphilis, and more.[30] In the late 1930s, the beginning of the "Antibiotic Era" started with the discovery of penicillin by Sir Alexander Fleming.[31] The golden period of this era,

i.e. between the 1950s and 1970s, saw the discovery of numerous new antibiotics (Figure 1.3). The introduction of antibiotics greatly helped combat infectious diseases worldwide. Although, there has not been many new classes of antibiotics discovered since.[32] The issue that arises is that bacteria are increasingly resistant to antibiotics, rendering them inefficient.

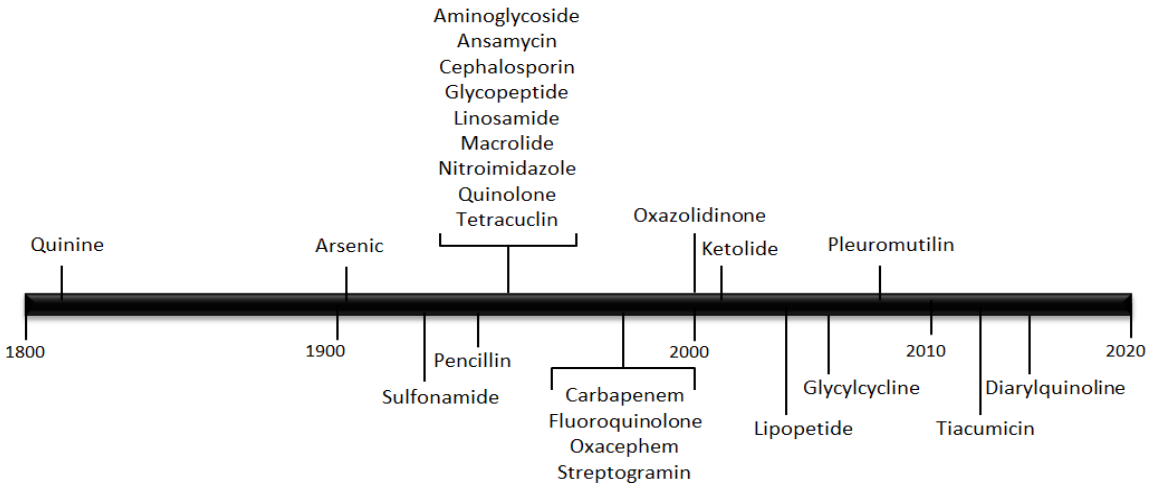


Figure 1.3: Timeline of the first use of major antibiotic classes.

1.3.3 Antibiotic resistance

Antibiotic resistance occurs when bacteria are not affected by one or more of the antibiotics to which they were initially sensitive to. This continues to be a growing problem, exacerbated by the fact that most large pharmaceutical companies have steered away from antibiotic research. Bacterial resistance can be intrinsic or acquired through vertical (i.e. Darwinian) or horizontal evolution, the latter of which includes conjugation, transduction or transformation. Conjugation consists of creating a physical connection between two bacterial cells to favor gene transfer. Transduction occurs when a bacteriophage (viruses that infect and replicates within bacteria or archaea) infects a bacterium and inserts its genome (potentially including antibiotic resistance genes) in the bacterial chromosome. Finally, during transformation, bacteria pick up foreign DNA from the surrounding environment.[28] Worrisomely, it is common for bacteria to harbor

several resistance-causing genes. The most commonly acquired mechanisms of resistance observed in pathogenic bacteria include modification of the cell membrane structure (when it is the target), mutation or bypass of the antibiotic's target, upregulation of efflux pumps, and drug inactivation.[28,33] Some of the most widely resistant bacterial species are the "ESKAPE" pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Actinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These are listed as the top priority pathogens in human health because they are often highly resistant to current antibiotics and hence, research and development of antibiotics against these bacteria are in urgent need.[34,35] In particular, drugs with a novel mode of action are of great interest because of the limited possibility of cross resistance, which is when resistance to a drug currently on the market also causes resistance to an antibiotic being developed. Some of the new antimicrobial targets currently being explored include the coenzyme A (CoA) biosynthetic steps, which have potential for the development of both antibacterial and antiplasmodial agents.

1.4 Coenzyme A Biosynthesis

Coenzyme A (CoA) is essential to all living organisms. It is involved in a large number of centrall metabolic pathways such as the citric acid cycle, signaling, regulation, fatty acid synthesis, polyketide synthesis, etc.[36] The CoA biosynthetic pathway is a potential new target in the development of antiplasmodials, antibacterials and antifungals because of its essential nature and of the structural differences between CoA biosynthetic enzymes from microorganisms and those of humans. Pantothenate which is also known as vitamin B5, is the main 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. Interestingly, *Plasmodium* species are known to facilitate the transport of pantothenate from the host to the erythrocytes and then to the intracellular space of the parasite.[37]

The production of CoA from pantothenate involves five enzymes (Figure 1.4). First, pantothenate kinase (PanK) catalyzes the phosphorylation of pantothenate to yield 4'-phosphopantothenate. Next, phosphopantothenoylcysteine synthase (PPCS) catalyzes the

condensation of 4'-phosphopantothenate and cysteine to yield 4'-phosphopantothenoylcysteine. Decarboxylation is then facilitated by phosphopantothenoylcysteine decarboxylase (PPCDC) to generate 4'-phosphopantetheine. Phosphopantetheine adenylyltransferase (PPAT) adds an adenyl group to this molecule and, finally, dephospho-CoA kinase (DPCK) catalyzes a phosphorylation at the 3' position of the ribose moiety to yield CoA. Some microorganisms can use an alternative pathway called the CoA salvage pathway (Figure 1.5). This pathway starts from pantetheine and skips the steps of PPCS and PPCDC. This can be helpful when pantothenate is limited.

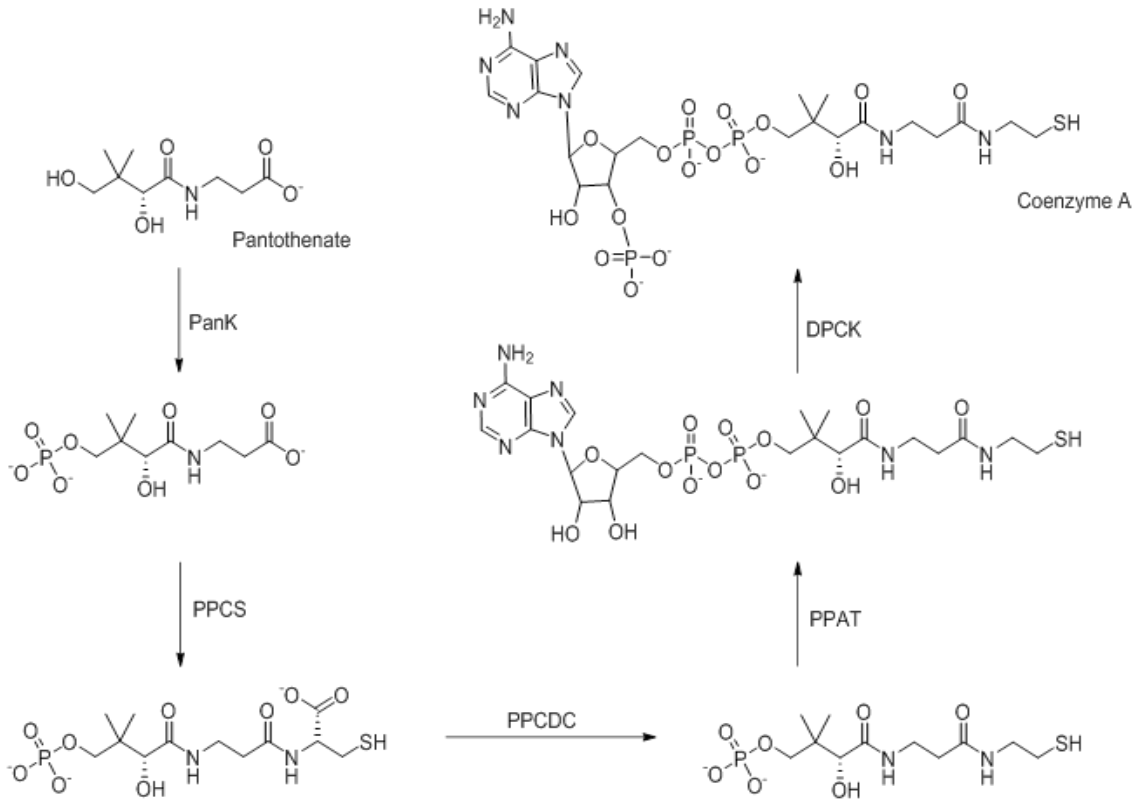


Figure 1.4: Biosynthesis of CoA.

1.5 Pantothenamides

Pantothenamides are amides of pantothenic acid (or pantothenate). The antibacterial and antifungal properties of pantothenamides were first discovered by Clifton *et al.* in the 1970's. The benchmark pantothenamide antibacterials for *E. coli* and *S. aureus* is *N*-pentylpantothenamide with an IC_{50} value of 2 μM and 7 μM, respectively. Their antiplasmodial activity was reported much later by Spry *et al.*[38,39] The most potent pantothenamide reported to be active towards the malaria parasite (in the absence of pantetheinases, see below) is *N*-phenethyl-pantothenamide, with an IC_{50} value of 20 nM.[39] Although they act by an interesting new mechanism of action (described below) pantothenamides have not been further explored due to their rapid pantetheinase-mediated hydrolysis in human serum.[40] In recent years however, in light of the rapid spread of antibiotic and antimalarial resistance, pantothenamides are being re-examined with efforts to increase their potency and stability in human serum.

1.5.1 The proposed mechanism of action of pantothenamides

Pantothenamides lack the carboxylate group of pantothenate and can therefore not be extended by PPCS and PPCDC. Similar to pantetheine, however, they can be transformed by the salvage pathway. Thus pantothenamides are bioactivated by PanK to yield the corresponding 4'-phosphopantothenamides, to which PPAT and DPCK add an adenylyl and a phosphate group, respectively, yielding the corresponding CoA derivatives, called CoA antimetabolites because they mimic CoA without allowing its typical biological reactions. As a result, these CoA antimetabolites interfere with a series of essential CoA-utilising pathways, leading to cell death.[37]

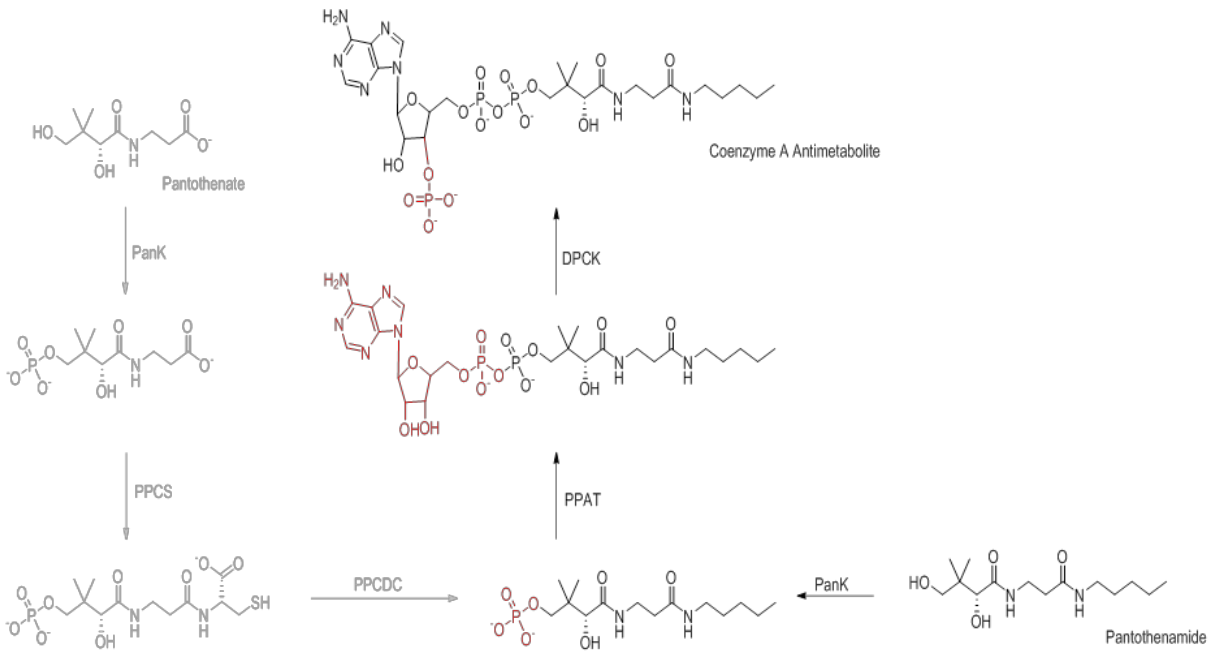


Figure 1.5: CoA salvage pathway involved in the transformation of pantothenamides.

1.5.2 Pantothenamide derivatives

The antibacterial and antiplasmodial properties of pantothenamides are well-documented and multiple derivatives have been synthesized.[37-59] Most of these synthesized pantothenamides are unstable in human serum due to rapid hydrolysis into pantothenate and the corresponding amine by pantetheinase enzymes, while the other amide group remains stable (Figure 1.6).[39]

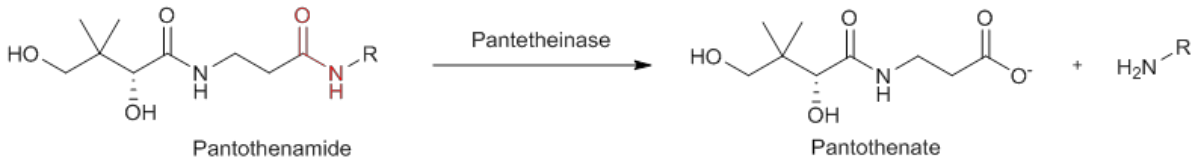


Figure 1.6 Pantetheinase-mediated pantothenamide hydrolysis.

It ensued that a variety of pantothenamide derivatives have more recently been prepared with the aim of reducing pantetheinase-mediated degradation while maintaining or improving the potency of pantothenamides. This includes modifications at the geminal dimethyl group, β-alanine moiety, and the scissile amide bond (Figure 1.7).

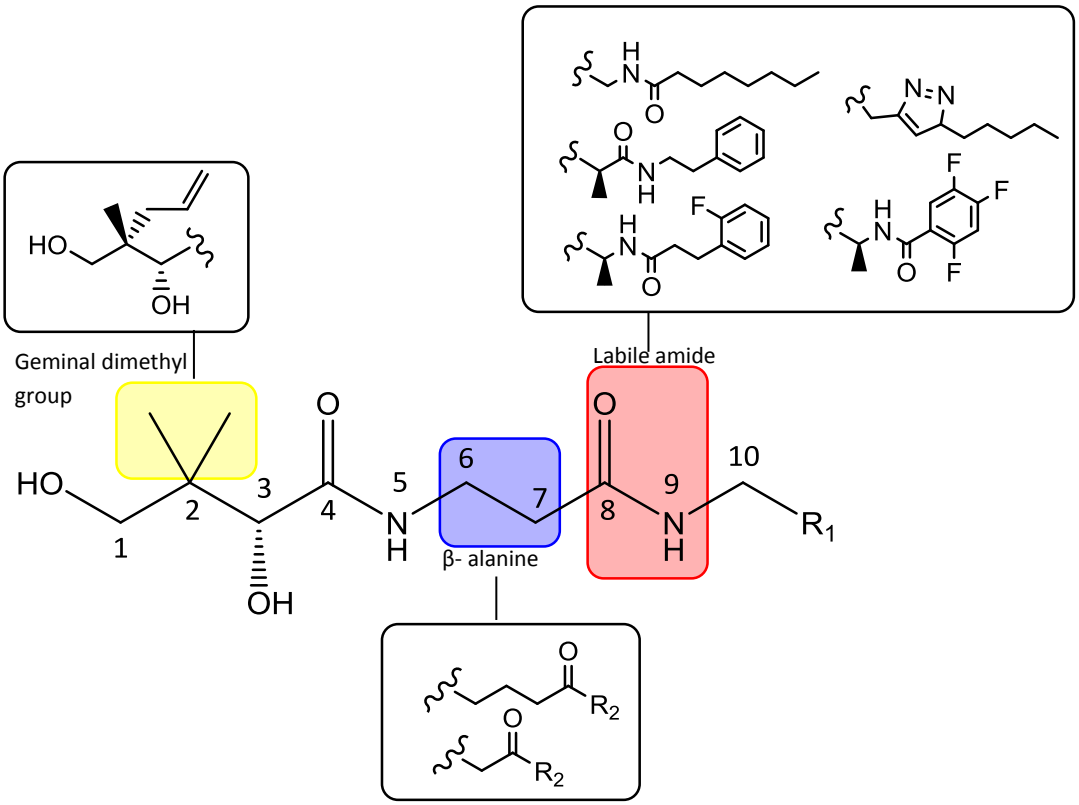


Figure 1.7 Main parts of pantothenamides that have been modified include the geminal dimethyl group, β-alanine moiety, and scissile amide bond. R_1= linear alkyl, branched alkyl, phenethyl or substituted aromatic moiety, R_2= amine moiety with R_1

The key recognition motif for most CoA biosynthetic enzymes is the pantoyl group (Figure 1.8). Therefore, scientists have rapidly focused their efforts on modifications elsewhere, including the labile amide bond and its *N*-substituent. Table 1.1 lists pantothenamides that have been reported to have activity against *E. coli* and Figure 1.8 summarizes preliminary structure-activity relationship based on the literature. Briefly, the optimal length of the carbon chain between N5

and C8 is 1-2 carbons.[59] The amide at C8 should be preserved or flipped, and the optimal *N*-substituents are short linear alkyl and alkyne chains.[52, 54, 57, 58]

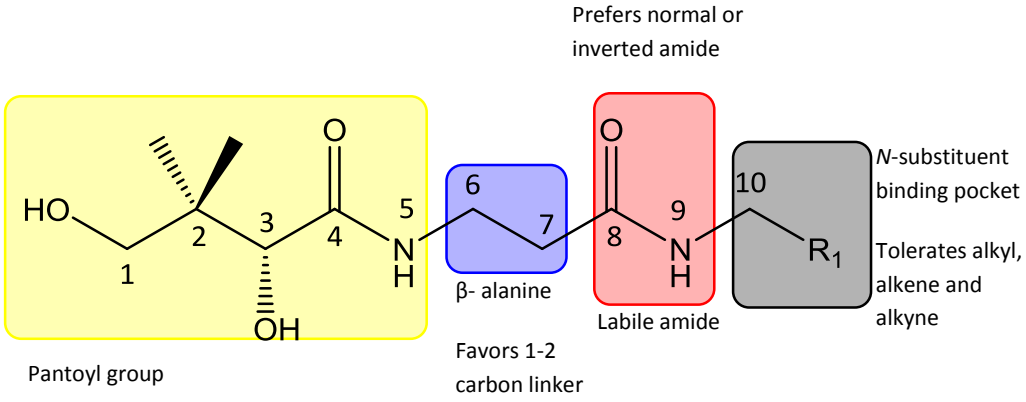
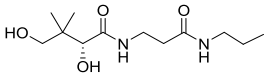
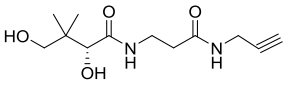
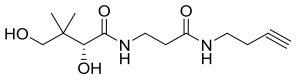
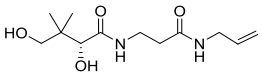
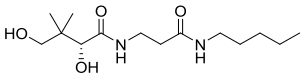


Figure 1.8: Ligand-based structure-activity relationship for antibacterial activity towards *E. coli*.

Table 1.1: Pantothenamide derivatives active against *E. coli*.[54] Compounds with IC_{50} less than 10 µM shown.

Structure	MIC (µM)	Structure	MIC (µM)	Structure	MIC (µM)
	1		4		7.6
	3		6		

In general, pantothenamides tend to be more potent against *S. aureus* than *E. coli*. Table 1.2 lists some of the pantothenamides that have been reported to have activity against *S. aureus* and Figure 1.9 summarizes preliminary structure-activity relationship based on the literature. In summary, the optimal chain length between N5 and C8 is 1-2 carbons.[59] Allyl or ethyl substituents with *R* configuration are preferred at C2 and the amide of C8 should be preserved

or inverted.[43, 51, 52, 57, 59] The optimal *N*-substituents are alkyl, ether and ethyl aromatic groups.[43, 58,59]

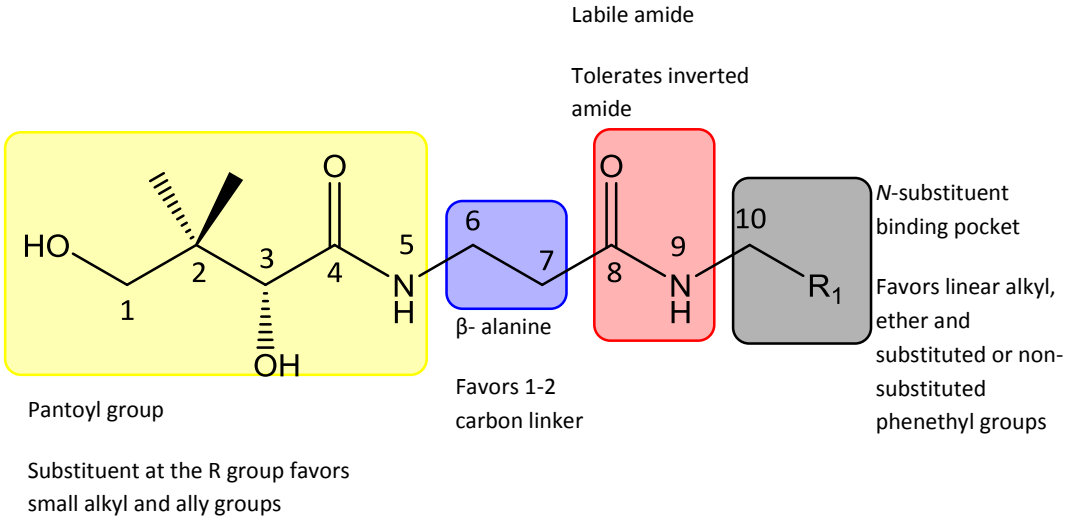
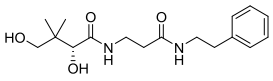
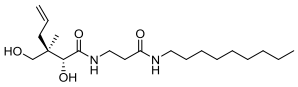
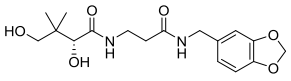
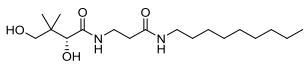
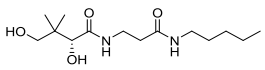
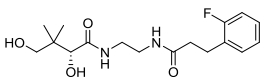
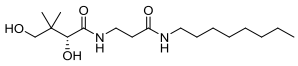
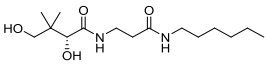
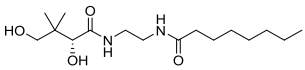
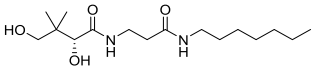
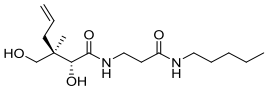
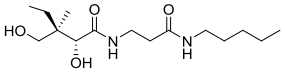
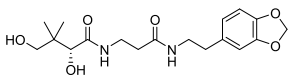
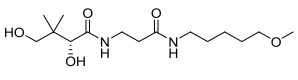


Figure 1.9: Ligand-based structure-activity relationship for antibacterial activity towards *S. aureus*.

Table 1.2: Pantothenamide derivatives active against *S. aureus*.[54] Compounds with IC_{50} less than 10 µM shown.

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

Many of the pantothenamides analogues discovered to date have antiplasmodial activity. Table 1.3 lists some of the most potent pantothenamides against *P. falciparum* and Figure 1.10 summarizes current structure-activity relationship. In summary, for activity against *P. falciparum*, a two-carbon linker between the two amide bonds is preferred. (*S*)-Methylation at C7 reduces pantetheinase-mediated degradation while maintaining the potency of pantothenamides.[47–49,56] Other modifications to overcome this degradation include, inverting the amide bond [47,49] and replacing the amide with selected bioisosteres.[44,45,50] Also, alkyl, thioether, and non-sterically hindered aromatic groups are preferred *N*-substituents.[44,45,47–49,55,60] There has been many serum-stable pantothenamides with antiplasmodial activity in the nanomolar range reported but none have yet made it to clinical trials and only a few have been tested for activity in animal models with little activity.[47,48,50] Therefore novel pantothenamides are needed to overcome this challenge.

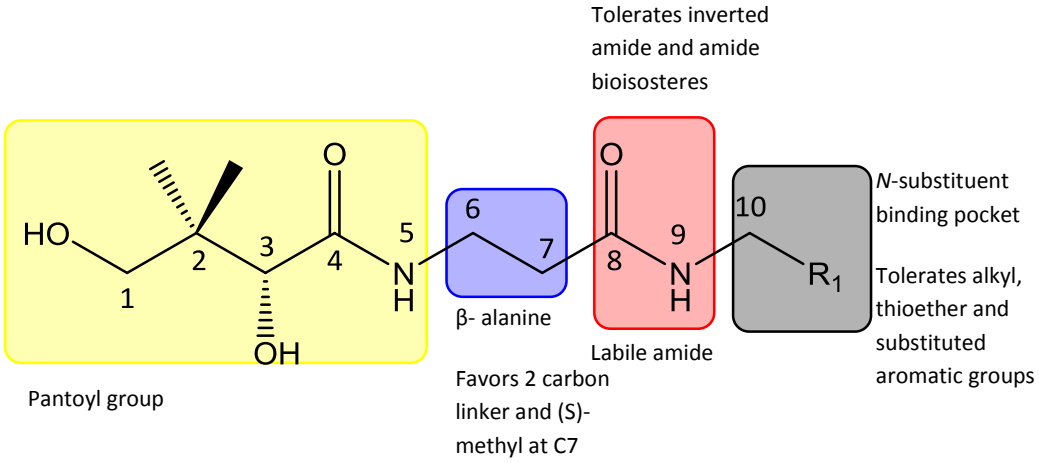
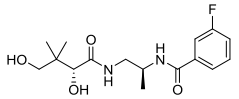
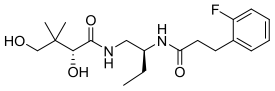
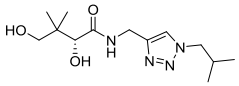
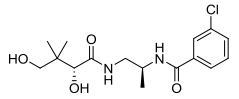
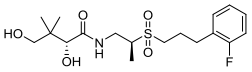
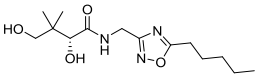
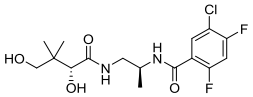
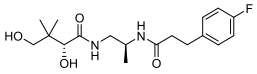
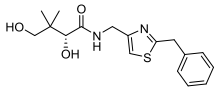
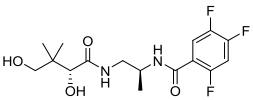
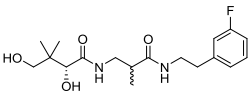
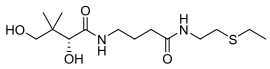
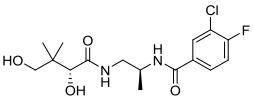
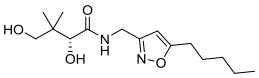
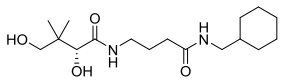
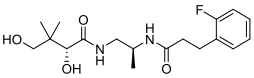
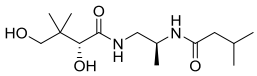
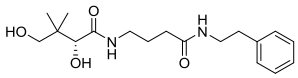
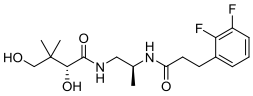
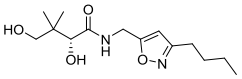
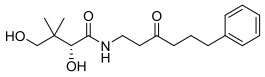
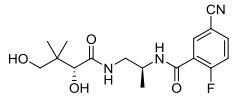
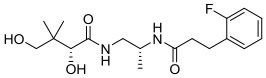
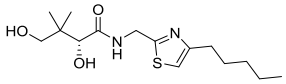
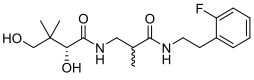
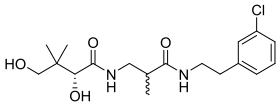
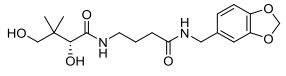
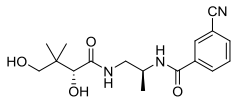
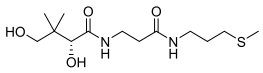
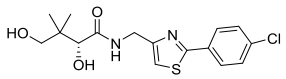
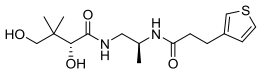
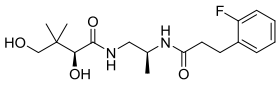
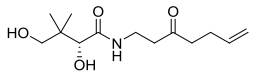
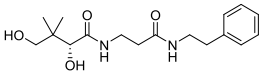
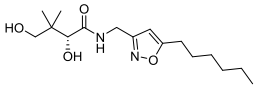
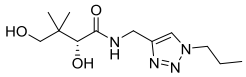
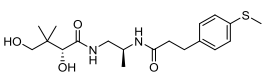
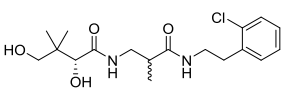
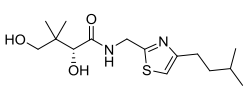
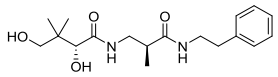
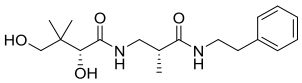
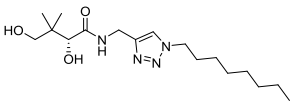
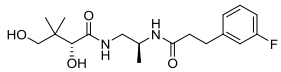
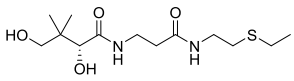
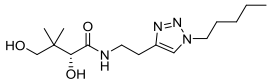
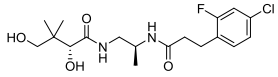
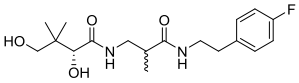
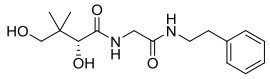
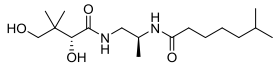
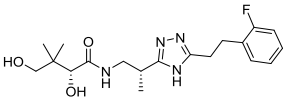
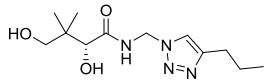
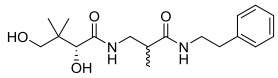
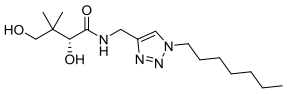
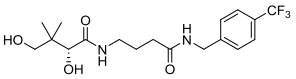
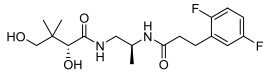
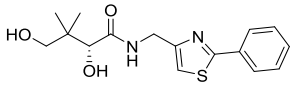
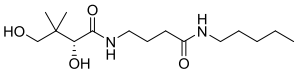
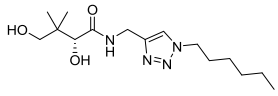
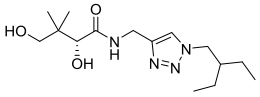
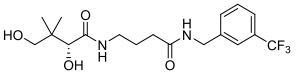
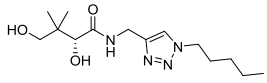
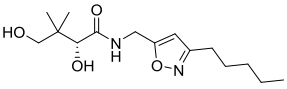
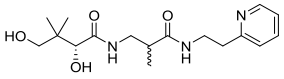
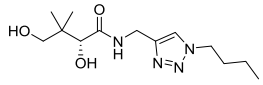
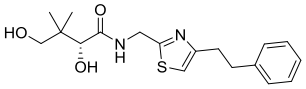
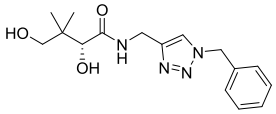
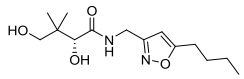
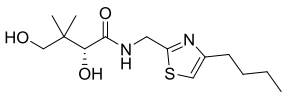
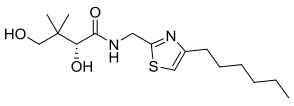
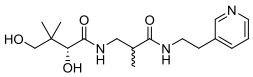
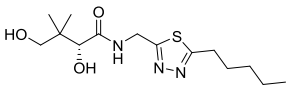
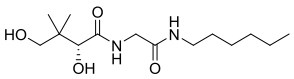
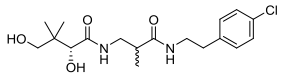
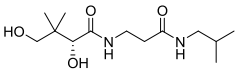
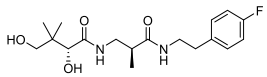
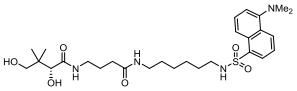


Figure 1.10: Ligand-based structure-activity relationship for antiplasmodial activity.

Table 1.3: Some of the most potent reported pantothenamide derivatives active against *P. falciparium*. Compounds with IC_{50} less than 5 µM shown.

Structure	IC_{50} (µM)	Structure	IC_{50} (µM)	Structure	IC_{50} (µM)
	0.0019		0.122		1.3
	0.0021		0.139		1.8
	0.0023		0.147		1.94
	0.0024		0.156		2.0
	0.0034		0.16		2.1
	0.005		0.175		2.1
	0.006		0.19		2.2
	0.0062		0.21		2.28
	0.0070		0.214		2.4
	0.0074		0.23		2.53
	0.017		0.235		2.6
	0.02		0.24		2.7
	0.021		0.248		2.9

	0.023		0.277		3.2
	0.028		0.28		3.3
	0.037		0.294		3.4
	0.041		0.299		3.5
	0.052		0.50		3.5
	0.053		0.55		3.9
	0.055		0.54		4.0
	0.056		0.63		4.2
	0.071		0.77		4.3
	0.072		0.77		4.34
	0.106		0.96		4.7
	0.107		1.1		
	0.107		1.1		

1.5.3 Outlook

As previously mentioned, antimicrobial resistance is increasing and new antimicrobial agents are greatly needed. Pantothenamides show antimicrobial activity by a new mechanism of action: they are CoA antimetabolites. Importantly, they are typically easy to synthesize and non-toxic.

1.6 Research Objective

The objective of this thesis is to synthesize new molecules that mimic pantothenamides while showing improved blood stability and potency against the *Plasmodium* parasite, and potentially also showing antibacterial activity. The approach used to achieve this consists of replacing the labile amide group with various sulfur-containing heteroaromatic rings or non-aromatic 5-membered rings to further diversify the library of compounds made by the Auclair group. Synthetic strategies and biological activities of compounds made by the author are discussed in chapter 2 for compounds with a heteroaromatic ring and in chapter 3 for those with a non-aromatic ring. Chapter 4 summarizes the contributions and suggests future direction for this project. Finally, chapter 5 provides a detailed description of the experimental procedures and compound characterization.

Chapter 2

Sulfur-Containing Molecules Designed to Mimic Pantothenamides

2.1 Preface

This chapter summarizes various synthetic strategies that have been used to synthesize sulfur-containing molecules that mimic pantothenamides, as well as their biological activities. Researchers in the Auclair group have attempted to improve the serum stability of pantothenamides by incorporating modifications at different positions and by replacing the labile amide group with various heteroaromatic rings. Previous group members have identified triazole, isoxazole, thiadiazole and thiazole rings as excellent replacement for the labile amide group, leading to antiplasmodial activity at low nanomolar concentrations. In this chapter, the synthesis of additional analogs is presented together with an examination of their growth inhibitory activity against *E. coli, E. faecium, K. pneumonia, P. aeruginosa, S. aureus, P. falciparum and A. baumanni.* Synthetic routes used in this chapter were previously established by previous group members Annica Chu and Jinming Guan, and further optimized by the author. In total, 8 novel sulfur-containing pantothenamide analogues were synthesized and tested for biological activity by the author.

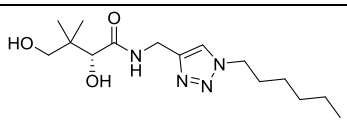
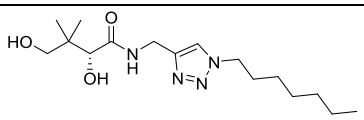
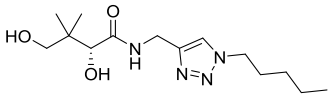
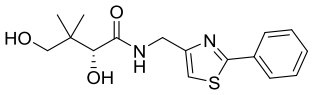
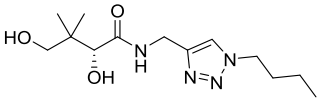
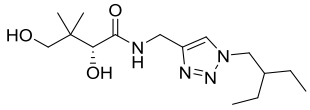
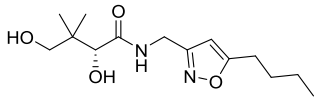
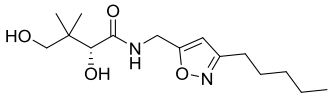
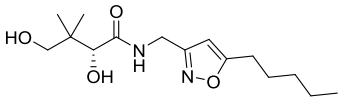
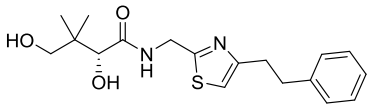
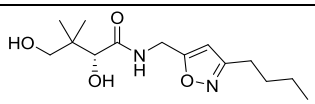
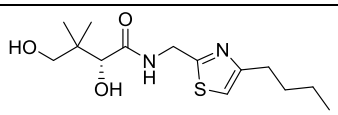
2.2 Introduction

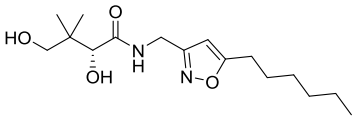
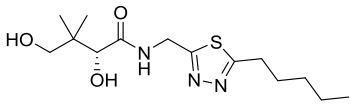
The antibacterial and antifungal activities of pantothenamides were recognized in the 1970s;[42] whereas their antiplasmodial activity was more recently demonstrated.[39] The unique mechanism of action of pantothenamides involves metabolic activation by the CoA biosynthetic pathway, which otherwise uses pantothenate as a substrate, with the resultant antimetabolites believed to interfere with subsequent CoA biosynthetic steps and/or downstream CoA-utilizing enzymes, thereby inhibiting microbe proliferation.[60-63] Pantothenamides are easily accessible synthetically, potent, and typically non-cytotoxic.[64] However, they show poor stability in human serum; pantothenamides are rapidly hydrolyzed to pantothenate and the corresponding amine by pantetheinases present in blood.

Our research group and others have explored several strategies to improve the biological activity of pantothenamides in the presence of pantetheinases. Modifications to prevent the

pantetheinase-mediated hydrolysis[44,62,63,65-73] have been introduced at the secondary hydroxyl group[68, 71], the geminal-dimethyl group[66,, 68, 70], the β-alanine moiety[48, 67, 69], and the labile amide[44, 65, 72, 73]. More recently, our group has focused on synthesizing novel pantothenamide analogs containing diverse heteroaromatic rings instead of the labile amide moiety, several of which show promising antiplasmodial activity against intraerythrocytic *P. falciparum* (Table 2.1). These biological studies, performed by Christina Spry and Xiangning Liu under the supervision of Prof. Kevin Saliba at the Australian National University, used a modified version of the malaria SYBR Green I-based fluorescence assay in "fresh" growth medium, *i.e.* in the presence of pantetheinase.[74]

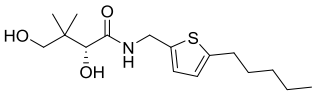
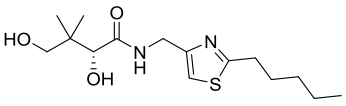
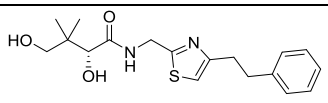
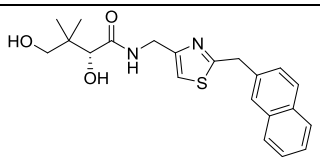
Table 2.1: Most potent of the heteoaromatic ring-containing pantothenamide analogues towards intraerythrocytic *P. falciparum*. Compounds with IC_{50} values < 1 μM are shown.

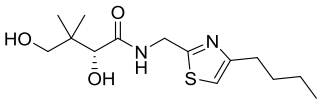
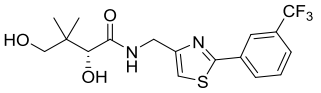
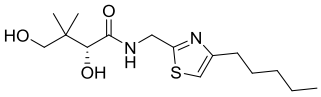
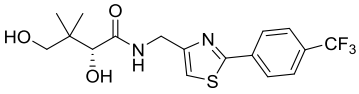
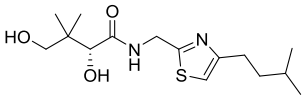
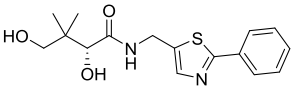
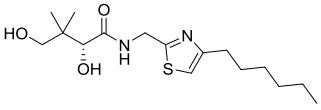
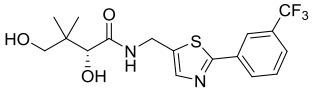
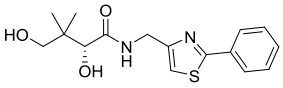
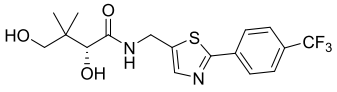
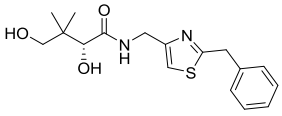
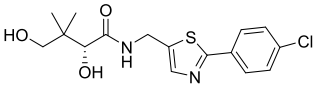
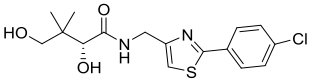
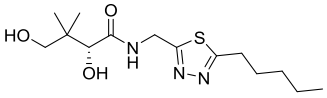
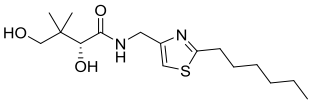
Structure	IC_{50}	Structure	IC_{50}
	0.055 ± 0.005[44]		0.50 ± 0.05[44]
	0.056 ± 0.005[44]		0.5474
	0.071 ± 0.003[44]		0.54 ± 0.1[44]
	0.072 ± 0.003[50]		0.63 ± 0.07[50]
	0.16 ± 0.01[50]		0.77 ± 0.09
	0.19 ± 0.03[50]		0.77 ± 0.10

	0.24 ± 0.05[50]		0.96 ±0.05[50]

Triazole, isoxazole and thiadiazole rings are among the most commonly used scaffolds in drugs.[75] More than 80% of the marketed drugs contain at least one heteroaromatic ring in their structure.[76] The heteroatoms within the ring can participate in intramolecular and intermolecular H-bonding interactions, and can affect the physicochemical properties of the molecule.[76] It is hypothesized that in some rings, the aromatic hydrogen and sulfur atoms may mimic the electron donor ability of an amide group, whereas the nitrogen and oxygen atoms may mimic its electron acceptor. Isoxazole-, thiazole-, and triazole-containing pantothenamide analogues are promising candidates with an antiplasmodial activity in the nanomolar range and selectivivity against *P. falciparium* over human foreskin fibroblast cells[44,50]. Although many pantothenamides show antibacterial activity, none of these compounds were found to display significant antibacterial activity. The heteroaromatic rings of pantothenamide analogues synthesized so far include nitrogen, oxygen[44,45,50] and sulfur, yet fewer analogs containing sulfur have been synthesized (Table 2.2).

Table 2.2: Previously synthesized pantothenamide-mimicking compounds containing heteroaromatic ring with one or more sulfur atoms with their IC_{50} values for the growth inhibition of intraerythrocytic *P. falciparum*.[50,73,77]

Entry	Structure	IC_{50} (µM)	Entry	Structure	IC_{50} (µM)
I		2.8 ± 0.3	XI		5.98
II		0.77 ± 0.09	XII		6.59

III		0.77 ± 0.10	XIII		22.18
IV		2.28 ± 0.16	XIV		53.46
V		2.90 ± 0.23	XV		6.59
VI		4.34 ± 0.37	XVI		9.72
VII		0.55	XVII		35.05
VIII		1.94	XVIII		35.53
IX		2.53	XIX		0.96 ± 0.05
X		3.91			

Sulfur is the fifth most abundant element in living organisms and is known to mimic -NH groups, by forming chalcogen bonds (a subclass of σ-hole interactons).[80-82] The low lying C-S σ* orbital and polarizability of sulfur atoms makes certain regions of sulfur atom electron-poor, creating what is known as a σ-hole. [80-82] The substituents attached to the sulfur affect the strength of the sulfur σ-hole, with electron withdrawing or donating substituents enhancing or reducing the electron accepting ability of the C-S σ holes, respectively.[29] Sulfur can also form intermolecular and intramolecular interactions with electron donors in a biological target.[80, 81] This is another reason why we chose to expand further on the thiazole and sulfur-containing ring series

2.3 Objective

Previous group members have started assembling a library of pantothenamide analogues in which the labile amide group is replaced with a thiazole ring with diversification at the "amide *N*-substituent" (the non-pantoic ring substituent). The primary aim of this chapter was to fill in the gaps left in this library. The compounds were tested in our lab for antibacterial activity and sent to collaborators for measurements of their antiplasmodial activity. The derivatives reported in this chapter are shown in Figure 2.1.

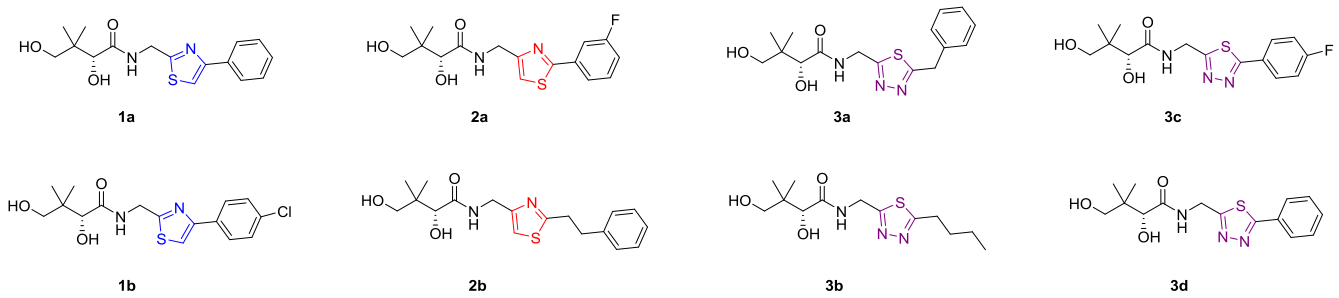
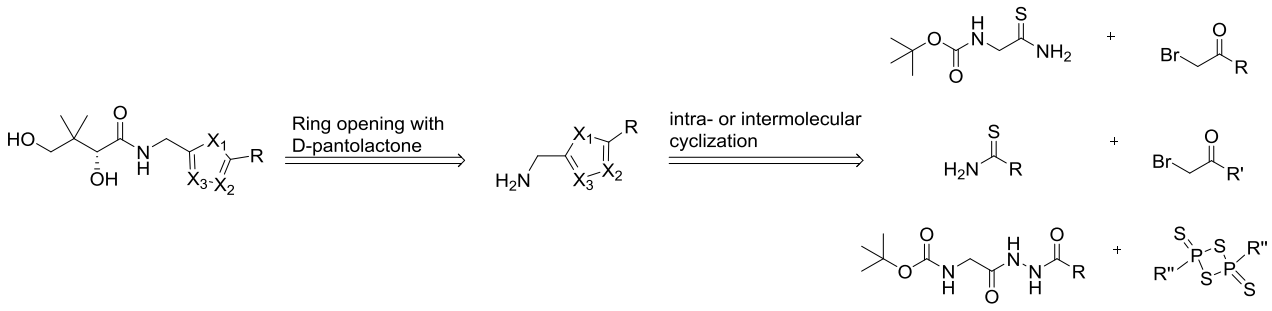


Figure 2.1: Sulfur-containing pantothenamide analogues synthesized in this thesis.

These synthetic targets were designed based on our current structure-activity relationships relating to antiplasmodial activity. Looking at the 5*S*,2*N*-thiazole derivatives presented in Table 2.2, none contains a phenyl group, yet such a substituent proved beneficial in the 4*S*,2*N*-thiazole series. Therefore, compounds **1a** and **1b** were designed to fill in this gap. On the other hand, the 4*S*,2*N*-thiazole series was missing derivatives with a fluorobenzene or a phenethyl groups, which gave promising derivatives in the 5*S*,2*N*-thiazole, hence why compounds **2a** and **2b** were synthesized here. Finally, considering that only one thiadiazole-containing analog had previously been prepared, it was envisaged to explore this motif further with compounds containing various substituents that have led to high activity in other series, leading to target compounds **3a-3d**.

2.4 Synthetic Results and Discussion

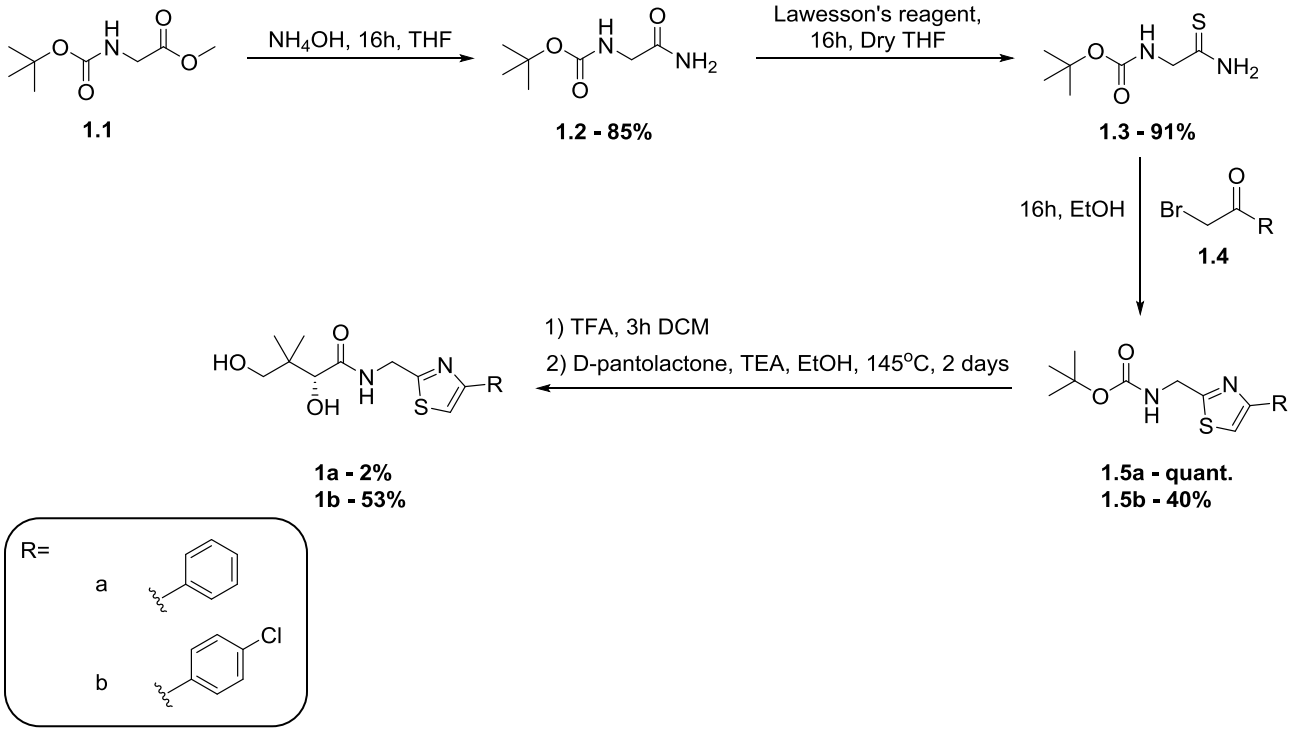
The synthetic targets all share a pantoyl moiety, therefore a similar retrosynthetic strategy was used to construct them, based on methods developed by past and present Auclair group members. This involves first an intra- or intermolecular cyclization to generate the thiazole core (scheme 2.1) and then the resulting primary amine undergoes a ring opening reaction with D-pantolactone, to afford the desired compound.



Scheme 2.1: Proposed synthetic route for targets **1a, 1b, 2a, 2b, 3a-3d**. X_1, X_2, and X_3 are carbon, nitrogen or sulfur atom with hydrogen substituents when needed. R' and R'' are phthalimide and *o*-methoxy phenyl substituent, respectively.

2.4.1 Synthesis of 5*S*,2*N* Thiazole Derivatives 1a and 1b

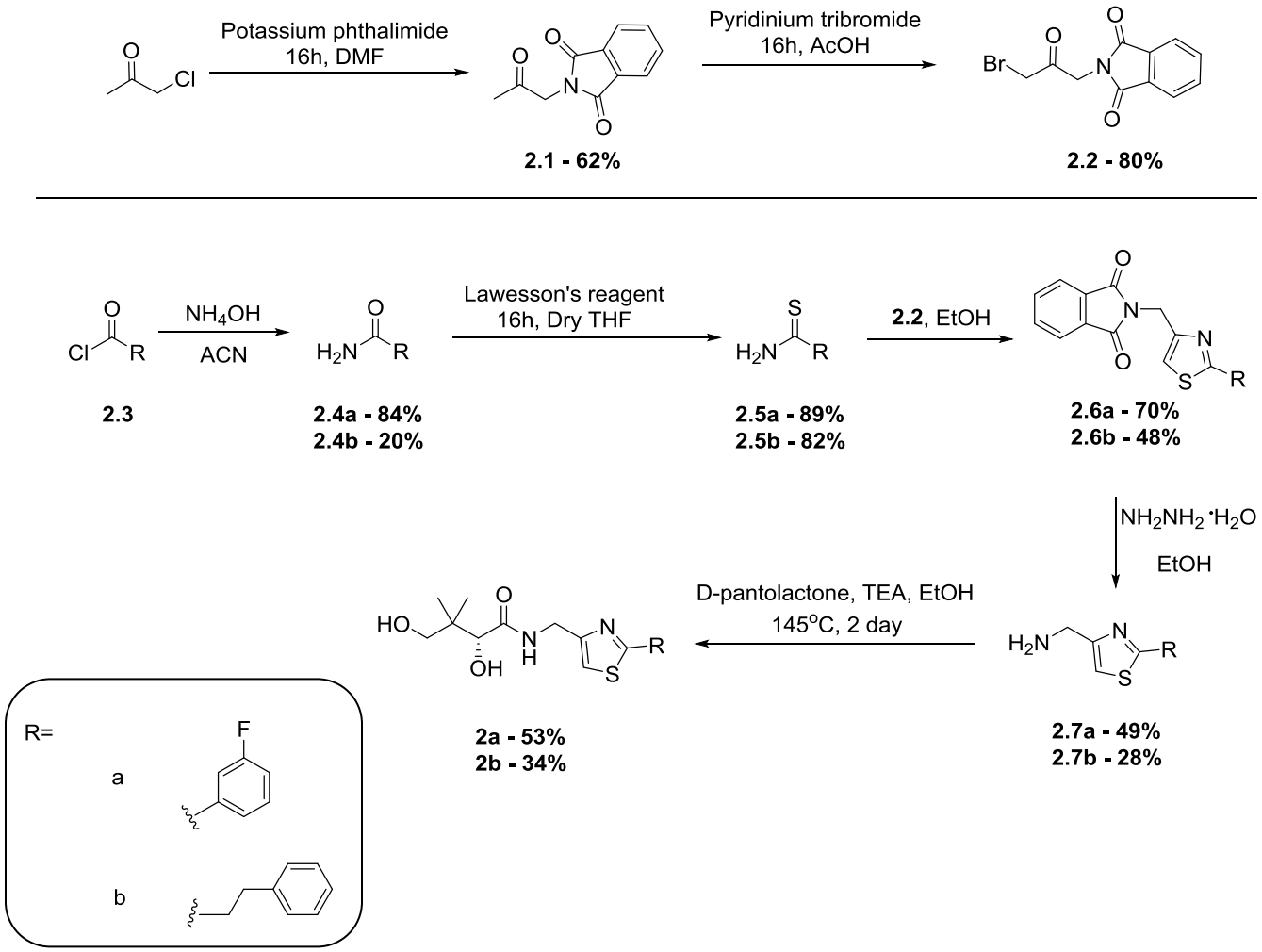
Scheme 2.2 shows the synthetic route used for the synthesis of 5*S*,2*N* thiazole-containing targets **1a** and **1b**. *N*-(tert-Butoxycarbonyl)glycine methyl ester was reacted with 28% (v/v) aqueous ammonium hydroxide to yield *N*-Boc-glycinamide (**1.2**). This was then thiolated with Lawesson's reagent [2,4-bis(4-methoxyphenyl)-2,4- dithioxo-1,3,2,4-dithiadiphosphetane] to give the thioamide **1.3**. Intermolecular Hantzsch cyclization of compound **1.3** with either of the commercially available compounds **1.4a** or **1.4b** afforded the intermediates **1.5a** and **1.5b**. Finally, trifluoracetic acid-mediated Boc-deprotection was preformed, followed by D-pantolactone ring opening to yield the final desired compounds **1a** and **1b**.



Scheme 2.2: Synthetic route to compounds **1a** and **1b**. DCM: dichloromethane; TEA: triethylamine; TFA: trifluoroacetic acid; THF: tetrahydrofuran.

2.4.2 Synthesis of 4*S*,2*N* Thiazole Derivatives 2a and 2b

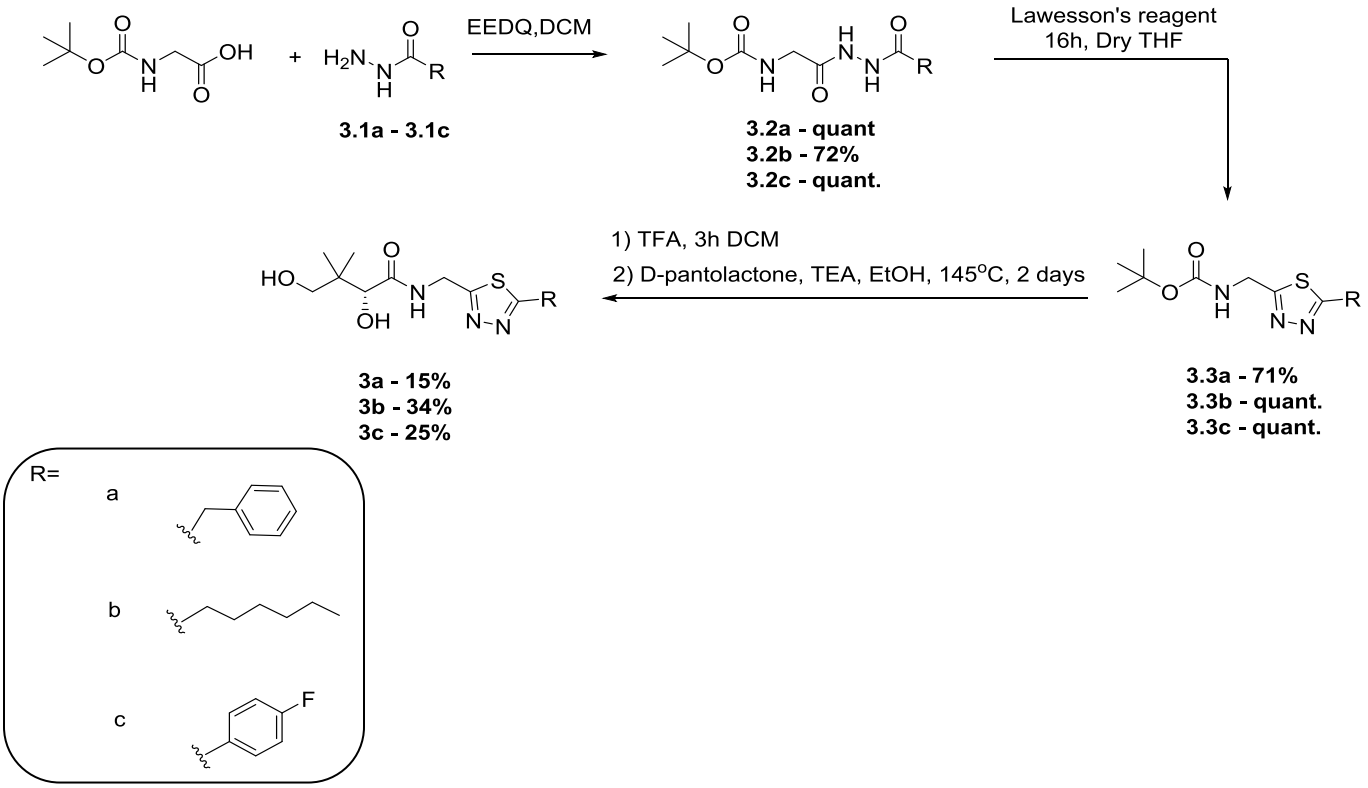
Scheme 2.3 shows the synthetic route used to prepare the 4*S*,2*N* thiazole pantothenamide analogues **2a** and **2b.** First, potassium phthalimide was reacted with chloroacetone to form **2.1**, which underwent an α-bromination with pyridinium tribromide and acetic acid to yield **2.2**. In parallel, commercially available acid chlorides **2.3a** and **2.3b** were each reacted with 28% (v/v) aqueous ammonium hydroxide to convert them to the corresponding amides **2.4a** and **2.4b**. Next, Lawesson's reagent was used to form the thiocarbonyl derivatives **2.5a** and **2.5b.** Hantzsch cyclization of **2.5a** or **2.5b** with **2.2** yielded the intermediates **2.6a** and **2.6b** respectively, which were deprotected using hydrazine. The free amine **2.7a** and **2.7b** were then each reacted with D-pantolactone in a sealed vessel to yield the final desired compounds **2a** and **2b**.



Scheme 2.3: Synthetic route to compounds **2a** and **2b**. ACN: acetonitrile; DCM: dichloromethane; TEA: triethylamine; THF: tetrahydrofuran.

2.4.3 Synthesis of Thiadiazole Derivatives 3a-3c

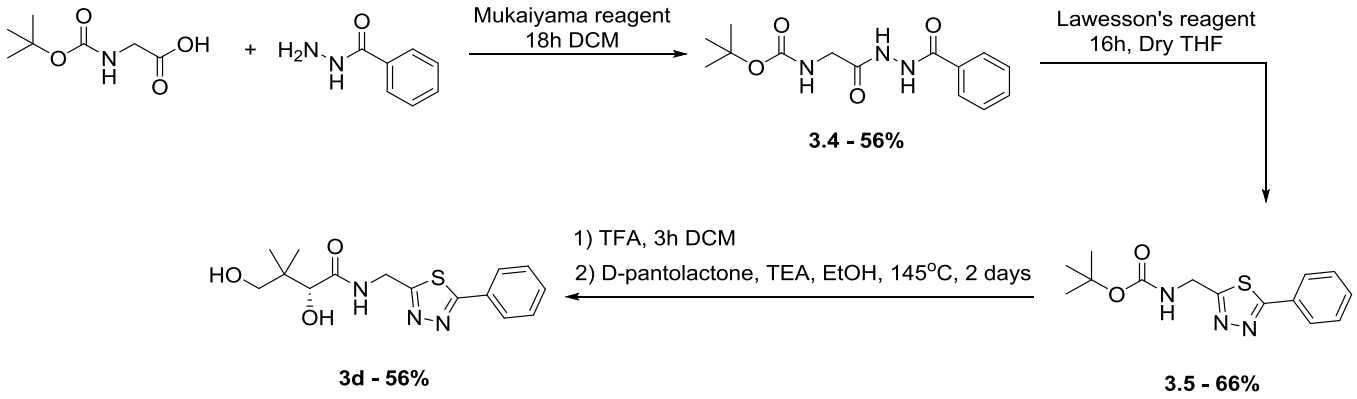
Scheme 2.4 shows the synthetic route used to access compounds **3a**-**3c.** First, *N*-Boc glycine was coupled to one of the hydrazides **3.1a**-**3.1c** in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) to yield **3.2a**-**3.2c**. Next, compounds **3.2a**-**3.2c** were separately thiolated with Lawesson's reagent before cyclization to yield the known 1,3,4-thiadiazoles **3.3a**-**3.3c**. Finally, trifluoracetic acid-mediated Boc-deprotection was performed, followed by D-pantolactone ring opening give the final desired compounds **3a**-**3c**.



Scheme 2.4: Synthetic route to compounds **3a-3c**. DCM: dichloromethane; EEDQ: 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline; TEA: triethylamine; TFA: trifluoroacetic acid; THF: tetrahydrofuran.

2.4.4 Synthesis of Thiadiazole Derivatives 3d

The synthetic route shown in scheme 2.4 was attempted to prepare **3d**, but the first step with EEDQ was unsuccessful. Using Mukaiyama reagent for this step was however successful and the desired product, compound **3.4**, was obtained (Scheme 2.5)**.** Next, compound **3.4** was thiolated using Lawesson's reagent before cyclization to yield the known thiadiazole **3.5**. Finally, trifluoracetic acid-mediated Boc-deprotection, followed by D-pantolactone ring opening afforded the desired compound **3d**.



Scheme 2.5: Synthetic route to compound **3d**. DCM: dichloromethane; TEA: triethylamine; TFA: trifluoroacetic acid; THF: tetrahydrofuran.

2.5 Evaluation of Biological Activity

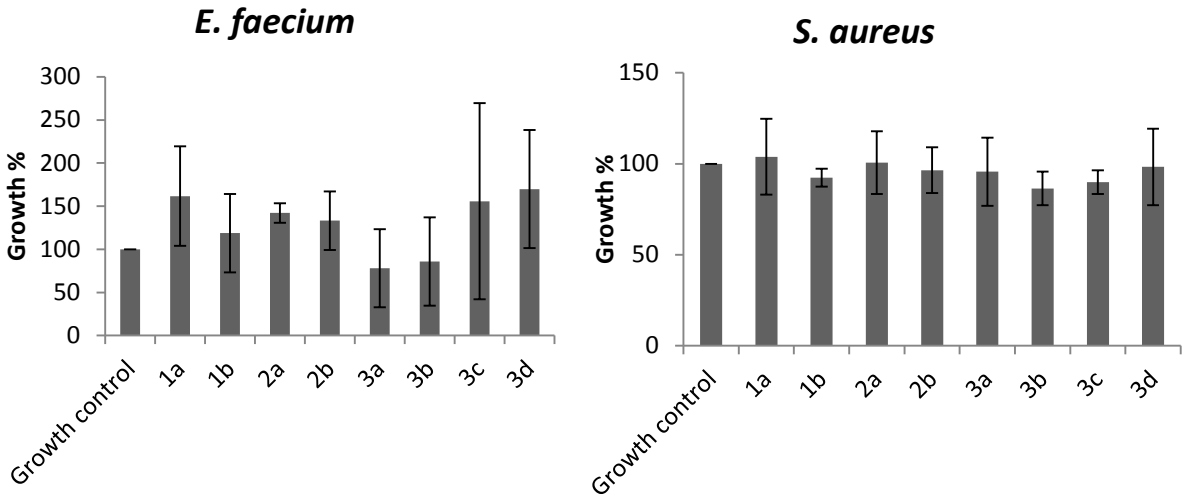
Antibacterial activity is typically measured using the Clinical and Laboratory Standards Institute broth microdilution method and quantified by means of the minimum growth inhibitory concentration (MIC).[78] Before measuring MIC values, the synthetic compounds **1a-1b, 2a-1b** and **3a-3d** were tested for antibacterial activity in a rapid screen at one high concentration (50 µM). Any compound that does not fully inhibit bacterial growth at this concentration is not sufficiently potent to be considered for further studies. The bacterial strains tested are pathogens from both Gram-negative and Gram-positive bacteria (Table 2.3).

Table 2.3: Classification of bacterial strains

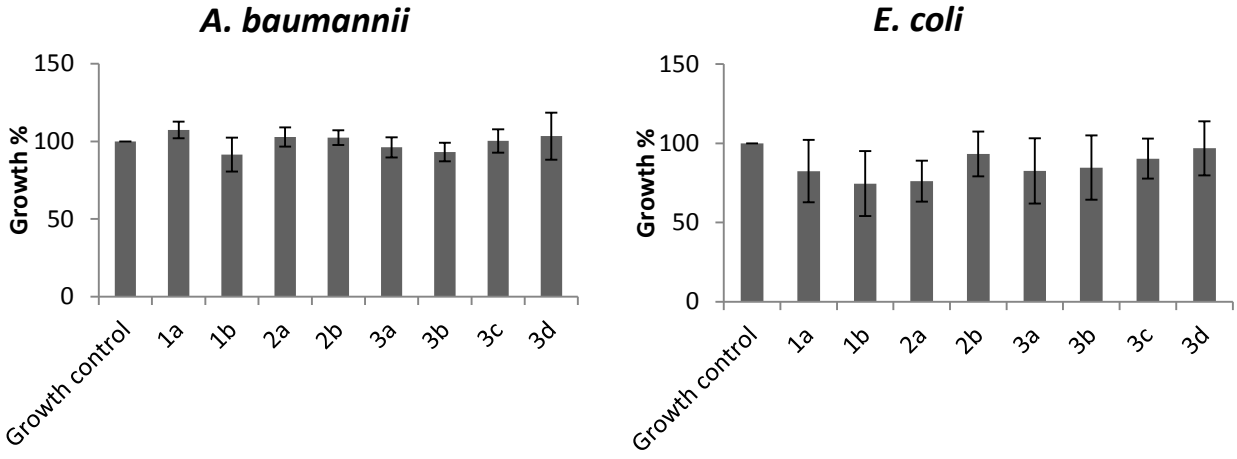
Gram-positive	Gram-negative
E. faecium	*A. baumannii*
S. aureus	*E. coli*
	K. pneumonaie
	P. aeruginosa

The bacterial strains listed in Table 2.3 were selected because they are part of a group of pathogens, the 'ESKAPE' pathogens, which are most often unaffected by the current antibiotics and are responsible for the majority of nosocomial infections.[78] The percent bacterial growth observed at 50 μM is presented in Figure 2.2 for all compounds discussed in this chapter.

A) Gram-positive bacteria



B) Gram-negative bacteria



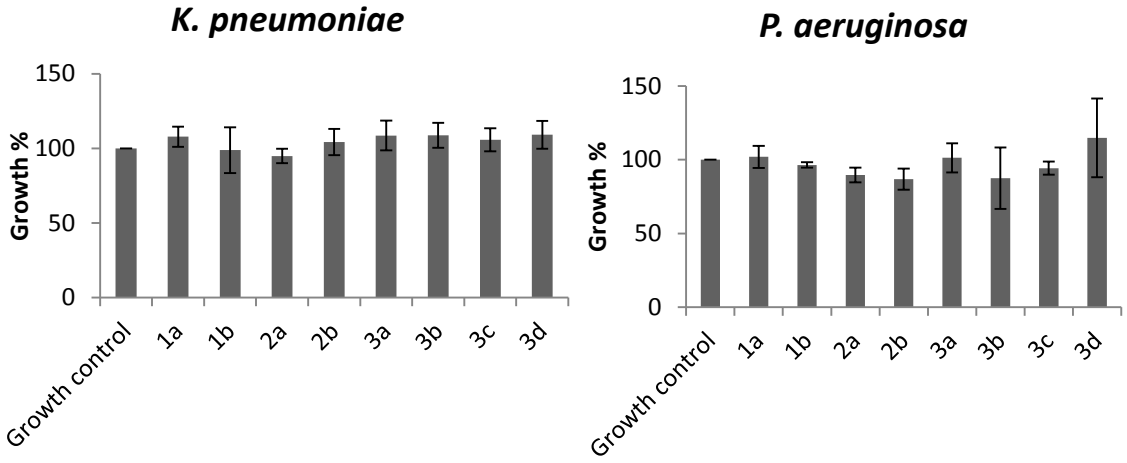


Figure 2.2: Percent growth in the presence of different compounds relative to bacterial growth in the absence of any compound (adjusted to 100% growth), measured in triplicates. Standard error of mean is used to determine the error bars.

Unfortunately, none of the compounds significantly reduced bacterial growth. There are a few compounds which further stimulated bacterial growth (*e.g.* compounds **1a**, **3c** and **3d** for *E. faecium*), suggesting that the bacteria may be able to utilize these thiazole derivatives in their metabolism.

The 8 compounds synthesized in this chapter were also sent to our collaborators at the Australian National University (Prof. Kevin Saliba's laboratory) to be tested for their antiplasmodial activity at the intraerythrocytic stage of *P. falciparum.* These results are pending and will be used together with those of Table 2.2 to draw structure-activity relationships.

2.6 Conclusion

In this chapter, 8 novel thiazole- or thiadiazole-containing compounds designed to mimic pantothenamides were successfully synthesized, each in less than 7 linear synthetic steps. The biological activity of these analogs was examined in an assay that looks at growth inhibitory

activity against *E. coli, E. faecium, K. pneumonia, P. aeruginosa, S. aureus, P. falciparum* and *A. baumanni.* Although none of the compounds showed significant antibacterial activity against these strains at a concentration of 50 µM, our group's results with similar molecules strongly suggest that the compounds shown in this chapter should have interesting antiplasmodial activity. Once these results are available, they will allow us to establish further structure-activity relationships and identify the most promising compounds to pursue.

Chapter 3

Non-aromatic Ring-Containing Molecules Designed to Mimic Pantothenamides

3.1 Preface

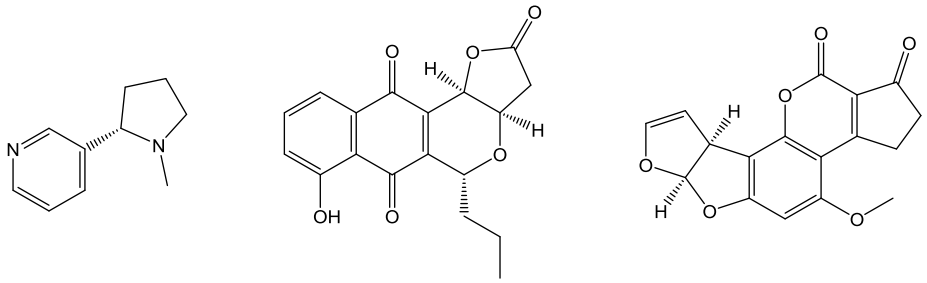
This chapter summarizes various synthetic strategies that have been used to synthesize pantothenamide analogues in which the labile amide is replaced by various non-aromatic 5-membered rings, as well as their biological activities. Previously, researchers in the Auclair group have attempted to improve the serum stability of pantothenamides by incorporating modifications at different positions and by replacing the labile amide with various heteroaromatic rings but have never investigated non-aromatic rings. In this chapter, 8 novel pantothenamide-mimicking compounds containing a non-aromatic 5-membered ring, are synthesized and tested for antibacterial activity by the author. The biological activities of the analogs synthesized in this chapter were examined for growth inhibition of *E. coli, E. faecium, K. pneumonia, P. aeruginosa, S. aureus, P. falciparum* and *A. baumanni.* The compounds are also being tested for antiplasmodial activity by collaborators in the group of Prof. Kevin J. Saliba at the Australian National University. Synthetic routes used in this chapter were established by the author with the help of group member Chunling Blue Lan.

3.2 Introduction

As previously mentioned, the Auclair group is trying to overcome pantetheinases-mediated hydrolysis of pantothenamides. Recently, our group has focused on synthesizing novel pantothenamide analogs containing diverse heteroaromatic rings instead of the labile amide group, which has proven highly successful, leading to many new, highly potent antiplasmodial agents

We now want to explore the effect of replacing the aromatic ring with various non-aromatic 5-membered rings. We are interested in rings that are not only chemically stable, but can also mimic some of the interactions provided by an amide group. In light of the ubiquitous nature of nitrogen-containing rings in natural products and in drugs, several synthetic methods have been reported to incorporate such rings in complex structures.[41, 42] Natural products have played a large role in the development of new drugs. Over half of the nearly 1000 small-molecule drugs

introduced on the market over the past two decades are either natural products or in some way related to natural products.[83] Non-aromatic 5-membered rings are very common among these, including molecules as diverse as the stimulant nicotine, the antitumor frenolicin B and the carcinogen aflatoxin B (Figure 3.1).



Nicotine Frenolicin B Aflatoxin B1

Figure 3.1: Natural products containing a non-aromatic 5-membered ring.

From a structural standpoint, non-aromatic 5-membered rings contain sp^3 hybridized atoms which offer more possibilities of diversification than sp2 hybridized carbons and increased potential to explore stereochemical vectors. They also display enhanced conformational flexibility, depending on ring size, ring atoms and substituents.[84] The concept of introducing heteroatoms onto non-aromatic ring systems is a powerful strategy to access molecules with increased biological activity or suitable for subsequent derivatization.[85] It is known that increasing the number of heteroaromatic rings in a molecule may be detrimental to its pharmaceutical properties, but this has not been the case for non-aromatic rings containing hetero atoms, which is largely beneficial.[86]

3.3 Objective

Previous group members and I have focused on synthesizing a variety of pantothenamide analogues with the labile amide group replaced with either a triazole, isoxazole, thiadiazole or thiazole ring and additional diversification at the *N*-substituent end (in reference to the pantothenamide structure). With the high potency of the isoxazole series of compounds towards *Plasmodium* species,[50] the secondary aim of this thesis was to assemble a small library

of pantothenamide analogues containing a 4,5-dihydroisoxazole ring instead of the labile amide group. The derivatives reported in this chapter are shown in Figure 3.2. These compounds were tested for their antibacterial activity and will also be tested by our collaborators for their ability to inhibit the growth of intraerythrocytic *P. falciparum*.

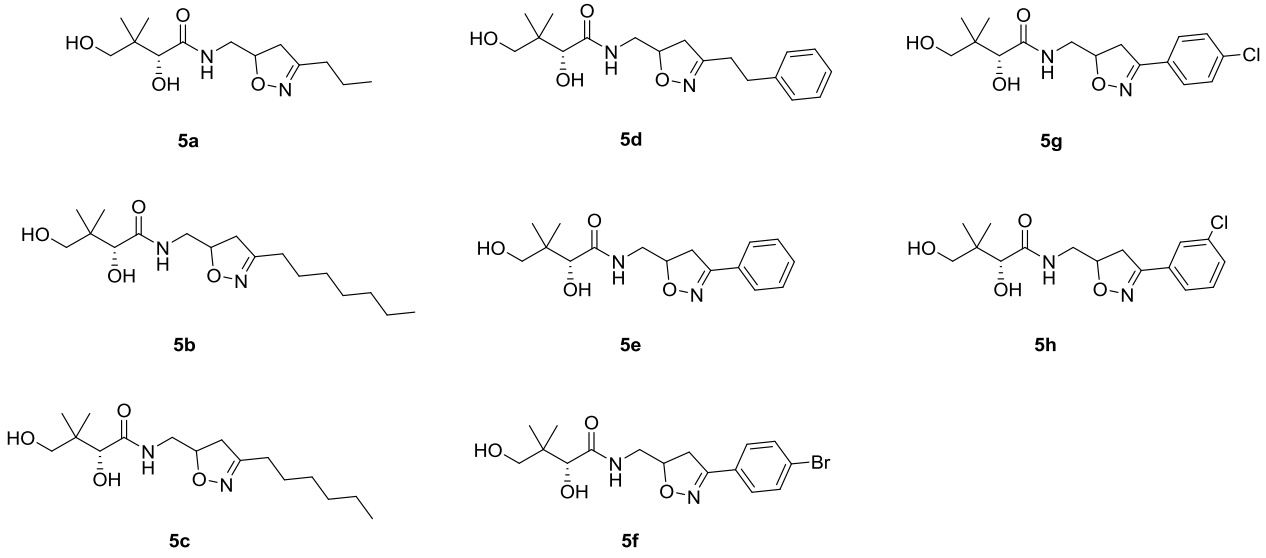
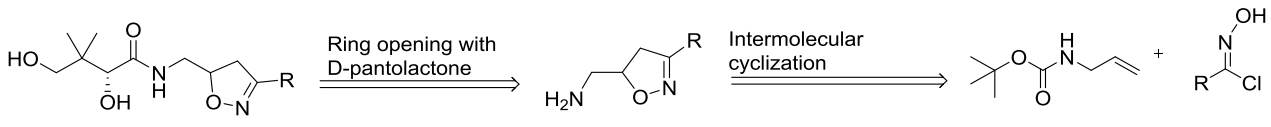


Figure 3.2: Synthetic targets presented in this chapter.

3.4 Synthetic Results and Discussion

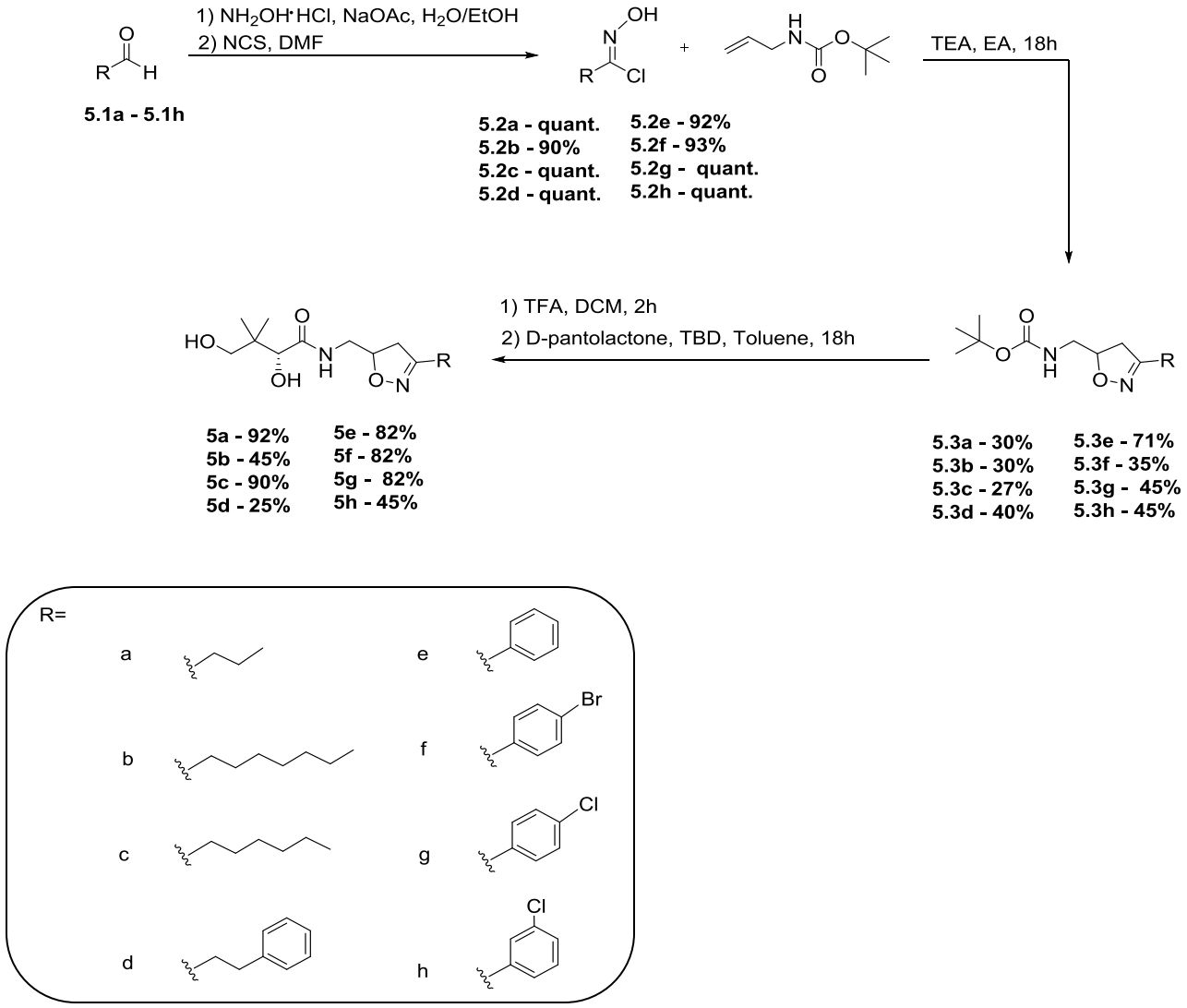
The proposed synthetic targets all share a pantoyl moiety therefore a common retrosynthetic strategy was used to construct them. This involves first an intermolecular cyclization to generate the 4,5-dihydroisoxazole ring and then the resulting primary amine undergoes a ring opening reaction with D-pantolactone, to afford the desired compound (Scheme 3.1).



Scheme 3.1: Proposed synthetic route for targets **5a-5h**.

3.4.1 Synthesis of Compounds 5a-5h

Scheme 3.2 shows the synthetic route used to access compounds **5a**-**5h.** First, the desired aldehyde (one of **5.1a-5.1h**), hydroxylammonium chloride and sodium acetate were reacted in a mixture of ethanol and water to yield the corresponding oxime. The crude oxime was then reacted with *N*-chlorosuccinimide to generate the corresponding oxime chloride (**5.2a-5.2h**)**.** Next, allycarbamate was added to yield the cyclized products **5.3a-5.3h**. Finally, trifluoracetic acid-mediated Boc-deprotection was preformed, followed by D-pantolactone ring opening, to give the final desired compounds **5a**-**5h**. An Auclair group member, Chunling Blue Lan, had optimized the final D-pantolactone ring opening step and his optimal conditions were used here. Thus, the free amine (**5.3a-5.3h**) was reacted with triazabicyclodecene in toluene at room temperature overnight, instead of with triethylamine in ethanol at 145°C for 2 days. Importantly, the new reaction conditions afford yields ranging from 25%-92% instead of 2%-56%.



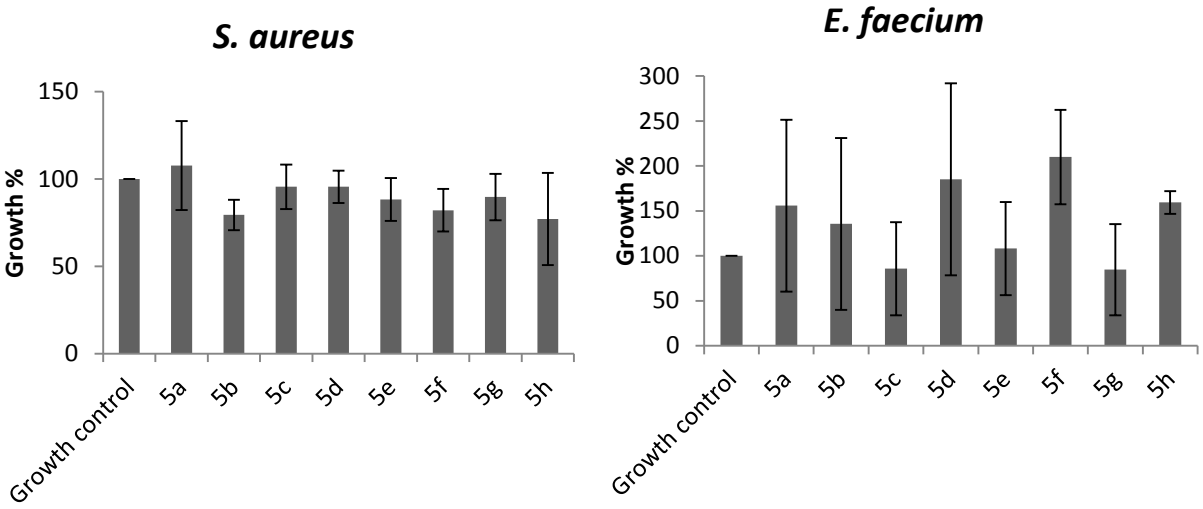
Scheme 3.2: Synthetic route for compounds **5a-5h**. EA: ethyl acetate; NCS: *N*-chlorosuccinimide; TBD: triazabicyclodecene; TEA: triethylamine; TFA: trifluoroacetic acid.

3.5 Evaluation of Antibacterial Activity

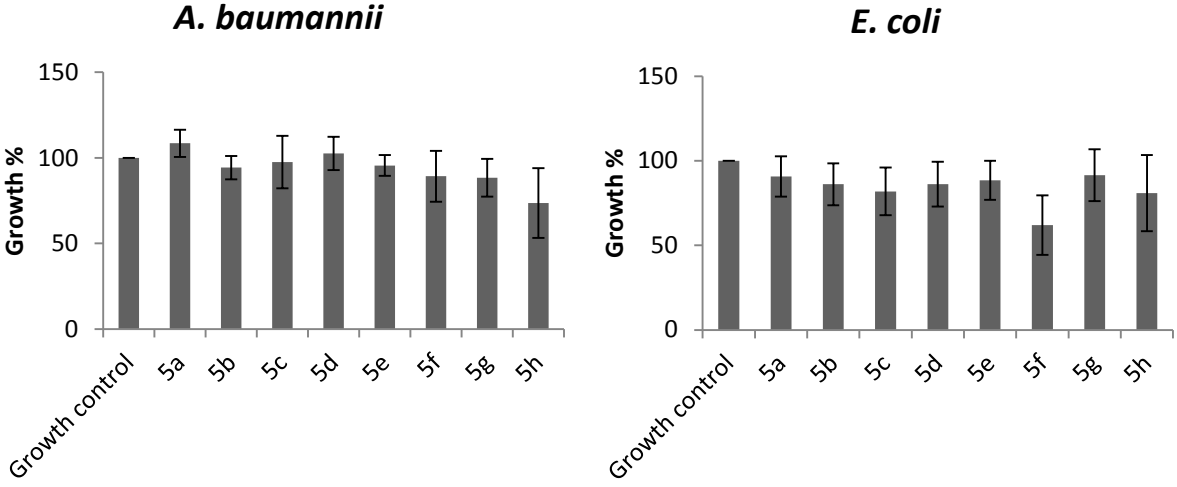
As in Chapter 2, a quick screen was performed to identify compounds with potentially interesting antibacterial activity. Here again, compounds **5a-5h** were tested at a fixed concentration of 50 µM. The bacterial strains tested include *E. faecium, S. aureus, A. baumannii, E. coli and K. pneumonaie.*

The percent bacterial growth observed at this concentration is presented in comparison to a control bacterial culture without added compound (Figure 3.3) for all compounds discussed in this chapter.

A) Gram-positive bacteria



B) Gram-negative bacteria



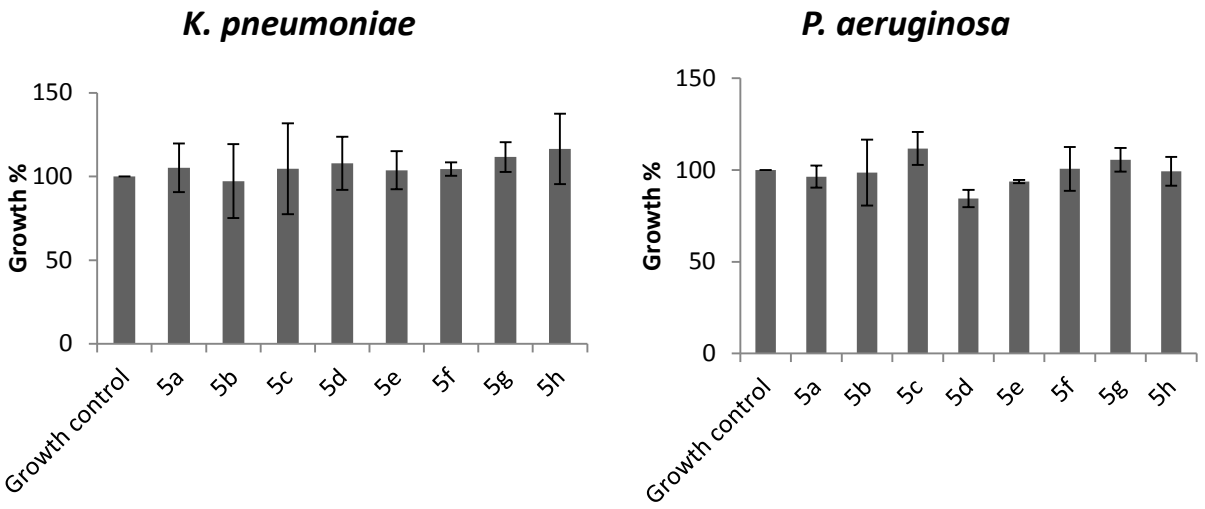


Figure 3.3: Percent bacterial growth in the presence of different compounds (50 µM) relative to bacterial growth in the absence of any compound (adjusted to 100% growth), measured in triplicates. Standard error of mean is used to determine the error bars.

Unfortunately, none of the compounds **5a**-**5h** reduced bacterial growth by more than 50% at 50 µM. In the presence of the compounds, most bacteria grew as well as the growth control. Although compound **5f** reduced the growth of *E. coli,* by approximately 40%, this would translated into an MIC value well above 50 µM, much too high to be worth pursuing.For some bacteria-compound pairs (e.g. compounds **5f** with *E. faecium* and **5h** with *K. pneumonia)*, compound addition to the bacterial culture seemed to stimulate growth (>100%), suggesting that the bacteria may be able to utilize these compounds as a carbon source.

The 8 compounds synthesized in this chapter were also sent to our collaborators at the Australian National University (Prof. Kevin Saliba's laboratory) to be tested for their antiplasmodial activity at the intraerythrocytic stage of *P. falciparum.* The results are pending.

3.6 Conclusion

In this chapter, 8 novel non-aromatic ring-containing compounds designed to mimic pantothenamides were successfully synthesized, each in less than 6 linear synthetic steps. These compounds were examined for their growth inhibitory activity against *E. coli, E. faecium, K. pneumonia, P. aeruginosa, S. aureus, P. falciparum* and *A. baumanni.* Although none of the compounds showed significant antibacterial activity against any bacteria at a concentration of 50 µM, our group's results with similar molecules strongly suggest that compounds **5a**-**5h** might have interesting antiplasmodial activity, which should allow us to establish further structure-activity relationships and identify the most promising compounds to pursue.

Chapter 4

Contributions and Future Directions

4.1 Contributions

As antimicrobial resistance is on the rise, the demand for novel antibacterial, antifungal and antiplasmodial agents is more than ever. The aim of this thesis is to synthesize new molecules that mimic pantothenamides and are expected to show antiplasmodial and/or antibacterial activity while being stable to pantetheinase-mediated degradation. Pantothenamides are known to be potent and selective growth inhibitors of bacteria and malaria parasites acting via a novel mechanism of action. These molecules are however rapidly hydrolyzed into pantothenate and the corresponding amine in human serum. In this thesis, 16 novel molecules designed to mimic pantothenamides yet be unaffected by pantetheinases derivatives were prepared, tested for antibacterial activity, and are waiting to be tested for antiplasmodial activity. Conveniently, these analogs are easily accessible synthetically, requiring less than 7 linear steps. All synthesis and antibacterial activity measurements reported in this thesis were carried out by the author.

In Chapter 2, the synthetic route and antibacterial testing of molecules **1a-1b**, **2a-2b**, **3a-3d** designed to contain a thiazole or a thiadiazole ring instead of the labile amide group of pantothenamides were reported. The focus of Chapter 3 is on non-aromatic ring-containing molecules **5a-5h**, designed to mimic pantothenamides, again covering synthesis and antibacterial studies. Although, adequate bacterial growth inhibition was seen, all novel pantothenamide derivatives were sent to the K. Saliba group at the Australian National University for testing against intraerythrocytic *P. falciparum*. Similar molecules prepared by the group have shown antiplasmodial activity, therefore it is likely that the compounds reported in this thesis will show activity against this parasite.

Overall, this thesis has contributed synthetic routes to 16 novel molecules. These molecules include some new structural scaffold with potential for interesting biological activity. The research conducted also emphasizes the potential for novel pantothenamide derivatives as antibacterial and antiplasmodial agents.

4.2 Future Directions

Following the work presented in chapter 3, the 4,5-dihydroisoxazole ring series warrants further exploration as this scaffold is in the beginning stages of being explored. Once results of antiplasmodial activity become available, it will be especially interesting to answer more specific questions about structure-activity relationships and combine favorable modification to increase potency. In the meantime, equipped with structure-activity relationship available from known pantothenamides, one could envisage possible benefits of including aphenethyl or phenyl groups on the double bond of the ring. Such modification has been suggested to allow favourable π-π interactions between the molecule and the protein targets.[50] It might also be worth including a branched alkyl chain, as this motif has barely been explored. Therefore it would be interesting to synthesize compounds **6a**-**6d** for example (Figure 4.1).

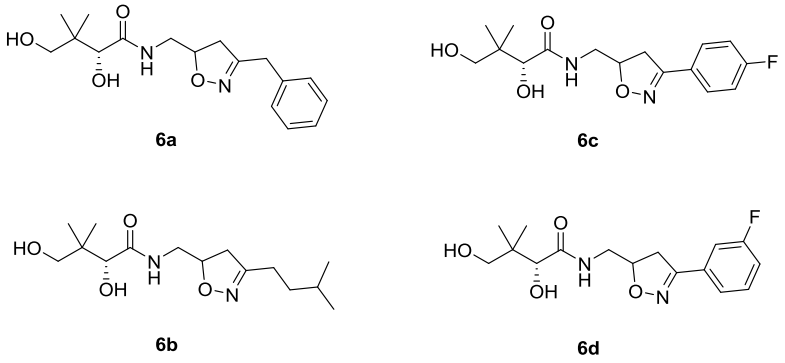


Figure 4.1 : Potential future compounds on the 4,5-dihydroisoxazole series.

In parallel, it may be of interest to look into different non-aromatic heterocycle rings, that may mimic some of the interactions provided by amide groups while being stable to pantetheinases. Such rings include for example pyrrolidine, 2-pyrrolidinone, and dihydropyrrole (Figure 4.2). These rings are common in clinically used drugs, such as captopril, cotinine, and ledipasvir respectively. Captopril is used for the treatment of hypertension and some types of congestive heart failure. Whereas, continine is used treat depression, PTSD, Alzheimer's disease and Parkinson's disease. Finally, ledipasvir is used to treat hepatitis C.

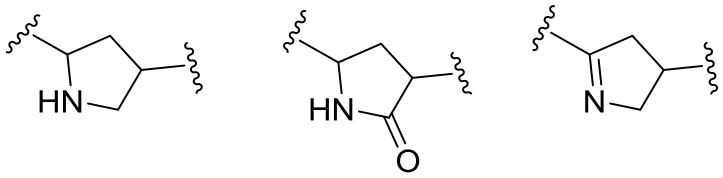


Figure 4.2: Potential non-aromatic heterocyclic rings to replace the labile amide moiety of pantothenamides.

Chapter 5

Experimental

5.1 Chemistry

5.1.1 Materials and instruments

All reagents and solvents were purchased from Chem Impex International Inc., Fisher Scientific, or Sigma-Aldrich Canada and used without further purification. Dry solvents were obtained from MBruan MB SPS 800 solvent purifier. Milli-Q water purification system with a specific resistance of 18.2 MΩ cm at 25 °C was used when water is mentioned. A nitrogen balloon was used when a reaction was performed under inert atmosphere. Thin-layer chromatography (TLC) was performed using silica plates of 200 µm thickness and coated with the fluorescent indicator F254. A UVGL-55 UV lamp was used to visualize UV-absorbing compounds on TLC at 254 or 365 nm. A potassium permanganate stain was used to visualize non-UV absorbing compounds. Flash column chromatography was performed using Sfar Silica D – Duo 60 µm chromatography columns on a Biotage® Isolera™ Systems instrument. Unless stated otherwise, all compounds synthesized herein are novel. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Brucker AVIIIHD 400 instrument or a Brucker AVIIIHD 500 instrument. Chemical shifts were recorded in parts per million (ppm) and were referenced to $CDCl_3$ or 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), and multiplet (m). HRMS (ESI+) spectra were acquired at the McGill University Mass Spectral Facility on a Thermo Fisher Scientific EXACTIVE^{TM} Plus Orbitrap Mass Spectrometer or a Bruker MaXis Impact HD Mass. An Agilent 1100 series HPLC was used to determine compound purity using Methods A and B described in Table 5.1.

Table 5.1: HPLC Methods for Purity Measurements of Compounds

Method A

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

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

Time (min)	Water (%)	Acetonitrile (%)
0	99	1
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 x 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 1.5a and 1.5b

The synthesis of compounds **1.5a-1.5b**, followed a previously reported procedure with modifications.[87, 88] To a solution of compound **1.3** (2-2.5 mmol, 1.0 eq) in anhydrous ethanol (6-10 mL) was added **1.4a** or **1.4b** (3-3.75 mmol, 1.5 eq). The reaction mixture was stirred at room temperature for 18 hours under an inert atmosphere. Water (6-10 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 × 10 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 **1.5a** or **1.5b**.

5.1.2.2 General protocol 2 for the synthesis of compounds 1a-1b and 3a-3d

The synthesis of compounds **1a-1b and 3a-3d** followed a previously reported procedure with modifications.[50] To a solution of either **1.5a-1.5b, 3.3a-3.3c** or **3.5** (0.86-1.60 mmol, 1.0 eq) in DCM (5-8 mL) was added trifluoroacetic acid (8.62-16.00 mmol, 10.0 eq). The reaction mixture was stirred at room temperature for 2 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 × 10 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 (0.86-1.60 mmol, 1.0 eq), D-pantolactone (4.37-2.59 mmol, 3.0 eq) and triethylamine (4.37 mmol, 3.0 eq) were dissolved in anhydrous ethanol (3 mL). The reaction mixture was heated to 146 °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.

5.1.2.3 General protocol 3 for the synthesis of compounds 2.4a and 2.4b

The synthesis of compounds **2.4a-2.4b** followed a previously reported procedure with modifications.[89] To a cooled (~ 0°C) solution of the desired acid chloride (12.00-20.00 mmol, 1

eq) in acetonitrile (7-13 mL), ammonium hydroxide solution (aqueous 28% w/w, 120.00-200.00 mmol, 10 eq) was added and the reaction mixture was stirred at room temperature for 2 hours. The product was extracted in ethyl acetate (3 x 10 mL), washed with 2 N NaOH (15-30 mL), and dried over anhydrous magnesium sulfate. The organic solvent was removed *in vacuo* and the residue was used directly in the next step.

5.1.2.4 General protocol 4 for the synthesis of compounds 1.3 and 2.5a-2.5b

The synthesis of compounds **1.3** and **2.5a**-**2.5b** followed a previously reported procedure with modifications.[90] To a solution of either **1.2** or **2.4a-2.4b** (3.20-6.00 mmol, 1.0 eq) in dry THF (70-130 mL) was added Lawesson's reagent (1.60-3.00 mmol, 0.5 eq). The reaction mixture 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 (eluted with EtOAc-Hex, 00:100 to 50:50) to afford the product.

5.1.2.5 General protocol 5 for the synthesis of compounds 2.6a and 2.6b

The synthesis of compounds **2.6a**-**2.6b** followed a previously reported procedure with modifications. [87, 88] To a solution of compound **2.2** (1.67-2.19 mmol, 1.0 eq) in anhydrous ethanol (1- 3 mL), compound **2.5a** or **2.5b** (0.78-2.01 mmol, 1.0 eq) was added. The solution was heated to 85°C under an inert atmosphere for 4 hours. Water (5 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 crude product was used directly in the next step.

5.1.2.6 General protocol 6 for the synthesis of compounds 2a and 2b

The synthesis of compounds **2.7a**-**2.7b** followed a previously reported procedure with modifications.[91] To a solution of compound **2.6a** or **2.6b**, (1.08 mmol, 1.0 eq) in anhydrous ethanol (16 mL), hydrazine monohydrate (3.24 mmol, 3.0 eq) was added. The mixture was heated to 85°C for 1 hour. Next, 2 N HCl (v/v, 2.2 mL) was added and the solution was heated at 85°C for an additional 5 minutes. The pH of the mixture was neutralized with saturated aqueous $NaHCO_3$. The organic solvent was removed *in vacuo*. Water (16 mL) was added to the residue,

and the product was extracted in DCM (3 × 20 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solvent was removed *in vacuo.* The crude product **2.7a** or **2.7b** was used directly in the next step.

The synthesis of compounds **2a**-**2b** followed a previously reported procedure with modifications.[50] In a pressure vessel (5 mL), compound **2.7a** or **2.7b**, (0.30-0.50 mmol, 1.0 eq), D-pantolactone (0.90-1.50 mmol, 3.0 eq) and triethylamine (0.90-1.50 mmol, 3.0 eq) were dissolved in anhydrous ethanol (3.2-5.4 mL). The reaction mixture was heated to 145°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).

5.1.2.7 General protocol 7 for the synthesis of compounds 3.3a-3.3c

The synthesis of compound **3.3a-3.3c** followed the previously reported procedure with modifications.[92] *N*-Boc glycine (2.28 mmol, 1.0 eq) dissolved in DCM (6 mL), was added to *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (2.28 mmol, 1.0 eq), followed by one of compounds **3.1a-3.1c** (2.74 mmol, 1.2 eq). The reaction mixture was stirred at room temperature for 16 hours. The mixture was next filtered and concentrated *in vacuo* to provide the desired coupling product **3.1a-3.1c**, which was used directly in the next step.
To the crude residue of one of **3.1a-3.1c** in dry THF (4.6-10 mL) was added Lawesson's reagent (1.21-2.28 mmol, 1.1 eq). The reaction mixture was stirred at room temperature for 16 hours, before concentration *in vacuo*. The product was purified by flash column chromatography (eluted with Hex-EtOAc, 100:00 to 00:100).

5.1.2.8 General protocol 8 for the synthesis of compounds 5.3a-5.3h

The synthesis of compound **5.3a-5.3h** followed the previously reported procedure with modifications.[93] The corresponding aldehyde (6.00-20.0 mmol, 1 eq), hydroxylammonium chloride (12.00-40.00 mmol, 2 eq) was dissolve in a mixture of ethanol and water (2:1) and the reaction mixture was cooled to 0^{o}C. Next, sodium acetate (18.00-60.00 mmol, 3 eq) was added and the reaction mixture was stirred at room temperature for 16 hours. The ethanol was

removed *in vacuo*. The solution was diluted with water (20 mL), filtered and concentrated *in vacuo.* The crude product **5.1a-5.1c** was used directly in the next step.

The crude residue of one of **5.1a-5.1c** (6.00-20.0 mmol, 1 eq) was dissolved in DMF (12-45 mL). *N*-Chlorosuccinimide (7.8-26 mmol, 1.3 eq) was added slowly and the reaction mixture was stirred at room temperature for 16 hours. The solvent was removed *in vacuo*. Water (10-20 mL) was added to the residue, and the product was extracted in ethyl acetate (3 × 20 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solvent was removed *in vacuo.* The crude product **5.2a-5.2c** was used directly to the next step.

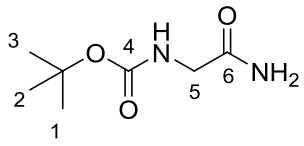
Allycarbamate (6.00-20.0 mmol, 1 eq), the crude residue of one of **5.2a-5.2c** (6.00-20.0 mmol, 1 eq) was dissolved in ethyl acetate (20-50 mL). The reaction mixture was cooled and triethylamine was added slowly (7.2-24 mL). The mixture was stirred at room temperature for 16 hours. The solvent was removed *in vacuo*. An aqueous solution of sodium bicarbonate (10-20 mL) was added to the residue, and the product was extracted in ethyl acetate (3 × 20 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 Hex-EtOAc, 100:00 to 20:80).

5.1.2.9 General protocol 9 for the synthesis of compounds 5a-5h

The synthesis of compounds **5a-5h** followed a procedure developed by Auclair Lab member Chunling Lan. To a solution of one of **5.3a-5.3h (**1.00-1.50 mmol, 1.0 eq) in DCM (4-6 mL) was added trifluoroacetic acid (10-15 mmol, 10.0 eq). The reaction mixture was stirred at room temperature for 2 hours. A solution of sodium hydroxide (15% aqueous w/v) was added dropwise until the reaction mixture reached a pH ~11. The product was extracted in ethyl acetate (3 × 10 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 (1.00-1.50 mmol, 1.0 eq), D-pantolactone (2-3 mmol, 2.0 eq) and triazabicyclodecene (0.10-0.15 mmol, 0.1 eq) were dissolved in toluene (1-1.5 mL). The mixture was stirred at room temperature for 16 hours. The organic solvent was

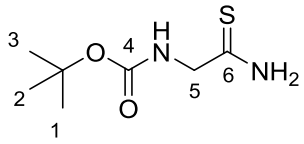
removed *in vacuo*. The crude product was purified by flash column chromatography (eluted with EtOAc-Hex, 00:100 to 100:00) to afford the product.

_N_-(tert-Butoxycarbonyl)-glycinamide (1.2)



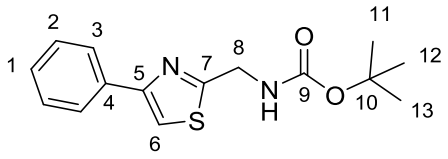
This known compound was synthesized from *N*-(tert-Butoxycarbonyl)-glycine methyl ester (12 mmol) and an ammonium hydroxide solution (aqueous 28%, 4.6 mL) according to the procedure reported by Xu *et al*.[94] to afford the product as a white solid. Yield: 85%. The compound characterization agreed with the previous report. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.19 (bs, 1H, NH_2 or NH), 6.94 (bs, 1H, NH_2 or NH), 6.83 (bs, 1H, NH_2 or NH), 3.46 (d, *J*= 6.1 Hz, 2H, H-5) 1.38 (s, 9H, H-1, H-2, H-3).

N-tert-Butoxycarbonyl-glycinethioamide (1.3)



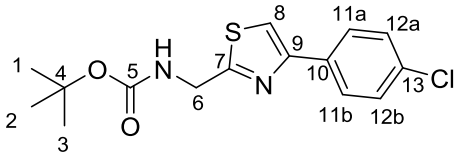
This known compound was prepared from compound **1.2** (2.4 mmol, 1.0 eq) and Lawesson's reagent (1.2 mmol, 0.5 eq) in THF (70 mL) using general protocol 4 to afford the product as a light yellow solid. Yield: 95%, *R*f = 0.48 (40% EtOAc in Hex). The characterization agreed with that of the previous report.[90] ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (bs, 1H, NH_2 or NH), 8.98 (bs, 1H, NH_2 or NH), 7.02 (bs, 1H, NH_2 or NH), 3.81 (d, *J* = 6.1 Hz, 2H, H-5), 1.38 (s, 9H, H-1, H-2, H-3).

***tert*-Butyl ((4-phenylthiazol-2-yl)methyl)carbamate (1.5a)**



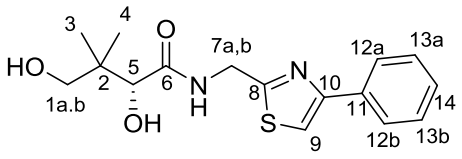
Compound **1.5a** was prepared from 2-bromoacetophenone (3 mmol, 1.5 eq) and compound **1.3** (2.0 mmol, 1 eq) in EtOH (7.5 mL) using general protocol 1. The crude product was purified by flash column chromatography (eluted with DCM-EtOAc, 100:00 to 15:85) to afford the product as an orange oil. Yield: quant., *R*f = 0.65 (50% EtOAc in Hex). The characterization agreed with a previous report.[95] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (m, 2H, H-3 , 7.42 (m, 2H, H-2), 7.40 (s, 1H, H-6), 7.33 (m, 1H, H-1), 5.34 (bs, 1H, NH), 4.67 (d, *J* = 6.2 Hz, 2H, H-8), 1.48 (s, 9H, H-1, H-2, H-3).

***tert*-Butyl ((4-(4-chlorophenyl)thiazol-2-yl)methyl)carbamate (1.5b)**



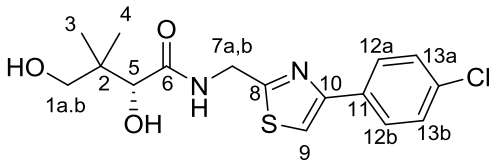
Compound **1.5b** was prepared from 2-bromo-4'-chloroacetophenone (2.5 mmol, 1.0 eq) and compound **1.3** (2.5 mmol, 1 eq) in EtOH (10 mL) using general protocol 1. The crude product was purified by flash column chromatography to afford the product as an orange solid. Yield: 39%, *R*f = 0.80 (20% EtOAc in DCM). Purity was 87% based on HPLC, *R*t = 27.78 minutes with method A and *R*t = 13.18 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.6 Hz, 2H, H-11), 7.40 (s, 1H, H-8), 7.38 (d, *J* = 8.6 Hz, 2H, H-12), 5.30 (bs, 1H, NH), 4.66 (d, *J* = 6.4 Hz, 2H, H-6), 1.48 (s, 9H, H-1, H-2, and H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.3, 154.1, 140.8, 137.4, 133.8, 128.8, 127.5, 113.2, 77.4, 42.3, 28.3. HRMS for $C_{15}H_{18}O_2N_2ClS$ $[M+H]^+$ calcd. 325.0777, found 325.0772.

2,4-Dihydroxy-3,3-dimethyl-*N*-((4-phenylthiazol-2-yl)methyl)butanamide (1a)



Compound **1a** was prepared from compound **1.7a** (1.46 mmol, 1.0 eq), D-pantolactone (4.37 mmol, 3 eq) and triethylamine (4.37 mmol, 3 eq) using the general protocol 2 to afford the product as a yellow oil. Yield: 2%, *Rf* = 0.29 (100% EtOAc). Purity was 82% based on HPLC, *R*t = 18.37 minutes with method A and *R*t = 9.20 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 7.7 Hz, 2H, H-12), 7.59 (bs, 1H, NH), 7.44–7.39 (m, 3H, H-13 and H-14), 7.34 (s, 1H, H-9), 4.89 (dd, *J* = 16.1, 6.1 Hz, 1H, H-7a), 4.79 (dd, *J* = 16.0, 5.8 Hz, 1H, H-7b) , 4.14 (s, 1H, H-5), 3.55 (m, 2H, H-1a and H-1b), 1.07 (s, 3H, H-3 or H-4), 0.98 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 167.0, 155.3, 134.2, 128.8, 128.3, 126.4, 113.3, 78.0, 71.4, 40.7, 39.5, 21.4, 20.5. HRMS for $C_{16}H_{20}O_2N_2SNa$ [M+Na]+ calcd. 343.1093, found 343.1087.

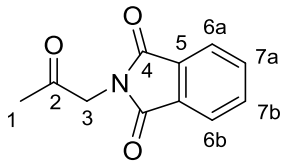
***(R)-N*-((4-(4-Chlorophenyl)thiazol-2-yl)methyl)-2,4-dihydroxy-3,3-dimethylbutanamide (1b)**



Compound **1b** was prepared from compound **1.5b** (0.86 mmol, 1.0 eq), D-pantolactone (2.57 mmol, 3 eq) and triethylamine (4.37 mmol, 6 eq) using the general protocol 2 to afford the product as a yellow oil. Yield: 53%, *R*f = 0.56 (100% EtOAc). Purity was 75% based on HPLC, *R*t = 20.53 minutes with method A and *R*t = 10.11 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 (d, *J* = 8.6 Hz, 2H, H-12), 7.77 (s, 1H, H-9), 7.43 (d, *J* = 8.5 Hz, 2H, H-13), 4.81 (d, *J* = 16.0 Hz, 1H, H-7a), 4.73 (d, *J* = 16.0 Hz, 1H, 1H, H-7b), 4.02 (s, 1H, H-5), 3.53 (d, *J* = 10.9 Hz, 1H, H-1a) 3.45 (d, *J* = 11.0 Hz, 1H, H-1b), 0.97 (s, 6H, H-3 and H-4). ^{13}C NMR (125 MHz,

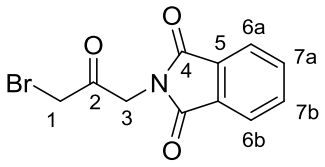
CD_3OD) δ 176.3, 170.4, 154.7, 134.5, 134.1, 129.5, 128.4, 114.9, 77.0, 70.6, 70.0, 41.2, 40.3, 21.1. HRMS for $C_{16}H_{19}O_3N_2ClSNa$ $[M+Na]^+$ calcd. 377.0703, found 377.0697.

Phthalimidoacetone (1.6)



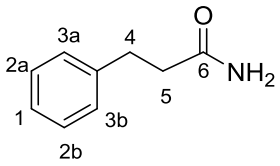
This known compound was synthesized using potassium phthalimide (11.00 mmol, 1 eq) and chloroacetone (12.00 mmol, 1.10 eq) according to the procedure reported by Volchkov *et al.*[96] Yield: 62%, *R*f = 1.38 (50% EtOAc in Hex). The characterization agreed with that of a previous report.[97] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (m, 2H, H-6), 7.74 (m, 2H, H-7), 4.50 (s, 2H, H-3), 2.27 (s, 3H, H-1).

2-(3-Bromo-2-oxopropyl)isoindoline-1,3-dione (1.7)



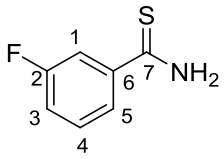
This known compound was synthesized using compound **1.2** (3.00 mmol, 1 eq) and pyridinium hydrobromide perbromide (3.30 mmol, 1.10 eq) according to the procedure reported by Volchkov *et al.*[96] Yield: 80% *R*f = 0.68 (50% EtOAc in Hex). The characterization agreed with a previous report.[98] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (m, 2H, H-6 or H-7), 7.76 (m, 2H, H-6 or H-7), 4.78 (s, 2H, H-3), 4.01 (s, 2H, H-1).

Benzenepropanamide (2.4b)



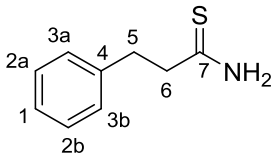
The known compound **2.4b** was prepared from hydrocinnamoyl chloride (20 mmol, 1.0 eq) and an ammonium hydroxide solution (aqueous 28% w/w) in acetonitrile (13.4 mL) using general protocol 3 to afford the product as a yellow solid. Yield: 20%. The characterization agreed with a previous report.[99] ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.29-7.16 (m, 5H, H-1, H-2 and H-3), 2.79 (t, *J* = 10 Hz, 2H, H-4), 2.35 (t, *J* = 10Hz, 2H, H-5).

3-Fluorobenzothioamide (2.5a)



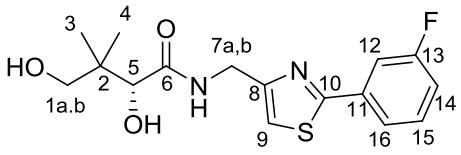
This known compound was prepared from compound **2.4a** (6 mmol, 1.0 eq) and Lawesson's reagent (3.0 mmol, 0.5 eq) in THF (74 mL) using the general protocol 4 to afford the product as a yellow solid. Yield: 89%, *R*f = 0.67 (50% EtOAc in Hex). The characterization agreed with a previous report.[100] ^{1}H NMR (500 MHz, DMSO) δ 10.01 (s, 1H, NH_2), 9.57 (s, 1H, NH_2), 7.72 (m, 1H H-3 or H-5), 7.66 (m, 1H H-3 or H-5), 7.45 (m, 1H, H-4), 7.33 (m, 1H, H-1).

3-Phenylpropanethioamide (2.5b)



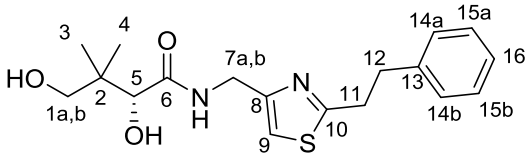
This known compound was prepared from compound **2.4b** (3.20 mmol, 1.0 eq) and Lawesson's reagent (1.6 mmol, 0.5 eq) in THF (74 mL) using the general protocol 4 to afford the product as a yellow oil. Yield: 87%. The characterization agreed with a previous report.[101] ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H, NH_2), 9.18 (s, 1H, NH_2), 7.23 (m, 5H, H-1, H-2 and H-3), 2.97 (t, *J* = 7.8 Hz, 2H, H-6), 2.76 (t, *J* = 7.9 Hz, 2H, H-5).

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



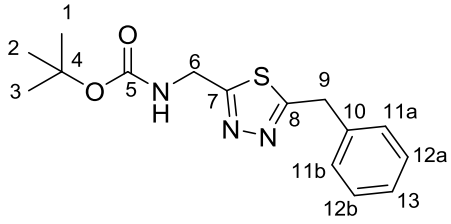
Compound **2a** was prepared from compound **2.7a** (0.50 mmol, 1.0 eq), D-pantolactone (1.5 mmol, 3 eq) and triethylamine (1.5 mmol, 3 eq) in EtOH (5.4 mL) using the general protocol 6 to afford the product as an orange oil. Yield: 53%, *Rf* = 0.19 (100% EtOAc). Purity was 89% based on HPLC, *R*t = 18.89 minutes with method A and *R*t = 9.47 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (m, 2H, H-15 and H-16), 7.40 (m, 2H, H-12 and H-14), 7.19 (s, 1H, H-9), 4.64 (m, 2H, H-7a and H-7b), 4.10 (s, 1H, H-5), 3.57 (bs, 1H, NH), 3.56 (d, *J* = 11.2 Hz, 1H, H-1a), 3.51 (d, *J* = 11.2 Hz, 1H, H-1b), 1.05 (s, 3H, H-3 or H-4), 0.95 (s, 3H, H-3 or H-4). ^{19}F NMR (471 MHz, $CDCl_3$) δ -112.03. HRMS for $C_{16}H_{19}O_2N_2FSNa$ [M+Na]+ calcd. 361.0998, found 361.0993.

***(R)*-2,4-Dihydroxy-3,3-dimethyl-N-((2-phenethylthiazol-4-yl)methyl)butanamide (2b)**



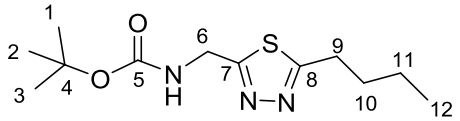
Compound **2b** was prepared from compound **2.7a** (0.30 mmol, 1.0 eq), D-pantolactone (0.9 mmol, 3 eq) and triethylamine (0.9 mmol, 3 eq) in EtOH (3.3 mL) using the general protocol 6 to afford an orange oil. Yield: 34%, *R*f = 0.50 (100% EtOAc). Purity was 93% based on HPLC, *R*t = 19.15 minutes with method A and *R*t = 9.55 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (bs, 1H, NH), 7.28 (m, 2H, H-14), 7.20 (m, 3H H-15 and H-16), 7.01 (s, 1H H-9), 4.58 (dd, *J* = 16.2, 6.6 Hz, 1H, H-7a), 4.52 (dd, *J* = 16.2, 6.6 Hz, 1H, H-7b), 4.06 (s, 1H, H-5), 3.49 (s, 2H, H-1a and H-1b), 3.27 (t, *J* = 7.6, 2H, H-11 or H-12), 3.07 (t, *J* = 7.6, 2H, H-11 or H-12), 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 173.1, 171.1, 152.4, 140.2, 128.7, 128.6, 126.6, 114.8, 78.1, 71.1, 39.7, 39.1, 36.1, 35.2, 21.9, 20.7. HRMS for $C_{18}H_{25}O_3N_2S$ [M+H]+ calcd. 349.1586, found 349.1580.

tert-Butyl ((5-benzyl-1,3,4-thiadiazol-2-yl)methyl)carbamate (3.3a)



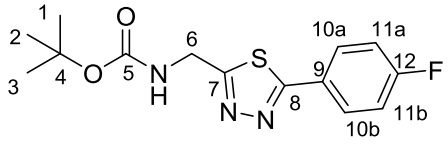
Compound **3.3a** was prepared from compound **3.2a** (2.29 mmol, 1.0 eq) and Lawesson's reagent (2.51 mmol, 1.1 eq) in THF (10 mL) using the general protocol 8 to afford the product as a yellow solid. Yield: 71%, *Rf* = 0.55 (50% EtOAc in Hex). Purity was 53% based on HPLC, *R*t = 24.09 minutes with method A and *R*t = 11.23 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ, 7.30 (m, 5H, H-11, H-12 and H-13), 5.34 (s, 1H, NH), 4.64 (d, *J* = 6.2 Hz, 2H, H-6), 4.37 (s, 2H, H-9), 1.42 (s, 9H, H-1, H-2, and H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 146.7, 145.9, 136.9, 128.9, 128.7, 127.4, 80.3, 39.6, 36.4, 28.2. HRMS for $C_{15}H_{20}O_2N_3S$ $[M+H]^+$ calcd. 306.1276, found 306.1271.

tert-Butyl ((5-butyl-1,3,4-thiadiazol-2-yl)methyl)carbamate (3.3b)



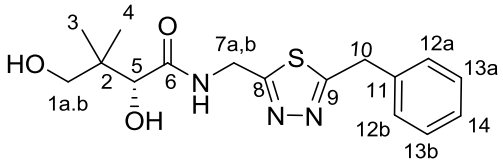
Compound **3.3b** was prepared from compound **3.2b** (1.45 mmol, 1.0 eq) and Lawesson's reagent (1.60 mmol, 1.1 eq) in THF (5.7 mL) using the general protocol 8 to afford the crude product as a yellow oil. Yield: quant., *R*f = 0.68 (50% EtOAc in Hex). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.45 (d, *J* = 6.1 Hz, 2H, H-6), 3.80 (m, 2H, H-10), 3.03 (t, *J* = 7.6 , 2H, H-9), 1.68 (m, 2H, H-11), 1.40 (s, 9H, H-1, H-2 and H-3), 0.89 (t, *J* = 7.4 Hz, 3H, H-12). ^{13}C NMR (125 MHz, CD_3OD) δ 172.0, 163.1, 158.1, 79.3, 79.0, 40.5, 33.1, 30.4, 30.3, 28.7, 23.0, 13.9. HRMS for $C_{12}H_{22}O_2N_3S$ $[M+H]^+$ calcd. 272.1432, found 272.1427.

tert-Butyl ((5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)methyl)carbamate (3.3c)



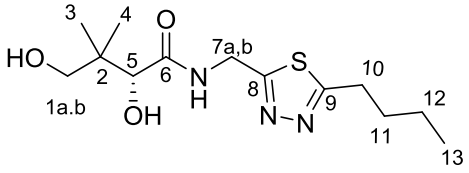
Compound **3.3c** was prepared from compound **3.2c** (2.00 mmol, 1.0 eq) and Lawesson's reagent (2.2 mmol, 1.1 eq) in THF (8.4 mL) using the general protocol 7 to afford the crude product as a yellow oil. Yield: quant., *R*f = 0.80 (50% EtOAc in Hex). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (m, 2H, H-10), 7.16 (m, 2H, H-11), 4.77 (bs, 1H, NH), 3.81 (s, 2H, H-6), 1.47 (s, 9H, H-1, H-2, and H-3). ^{19}F NMR (471 MHz, DMSO-d_6) δ -108.41. $C_{14}H_{16}O_2N_3SFNa$ $[M+Na]^+$ calcd. 332.0845, found 332.0839.

***(R)-N*-((5-Benzyl-1,3,4-thiadiazol-2-yl)methyl)-2,4-dihydroxy-3,3-dimethylbutanamide (3a)**



Compound **3a** was prepared from compound **3.3a** (0.86 mmol, 1.0 eq), D-pantolactone (3.45 mmol, 4 eq) and triethylamine (3.45 mmol, 4 eq) in EtOH (2.8 mL) using the general protocol 8 to afford the product as a yellow oil. Yield: 15%, *R*f = 0.22 (100% EtOAc). Purity was 89% based on HPLC, *R*t = 16.85 minutes with method A and *R*t = 8.35 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H, NH), 7.31 (m, 5H, H-12, H-13 and H-14), 4.81 (m, 2H, H-1a and H1b), 4.39 (s, 2H, H-7a and H-7b), 4.09 (s, 1H, H-5), 3.52 (s, 2H, H-10), 1.02 (s, 3H, H-3 or H-4), 0.95 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, CD_3OD) δ 173.4, 171.7, 167.6, 136.7, 129.1, 128.8, 127.6, 77.9, 71.3, 39.4, 37.9, 36.4, 21.2, 20.8. HRMS for $C_{16}H_{22}O_3N_3S$ $[M+H]^+$ calcd. 336.1382, found 336.1376.

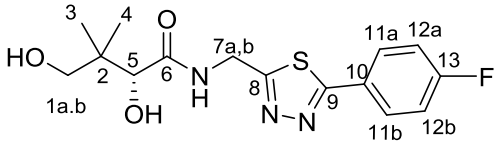
***(R)-N*-((5-Butyl-1,3,4-thiadiazol-2-yl)methyl)-2,4-dihydroxy-3,3-dimethylbutanamide (3b)**



Compound **3b** was prepared from compound **3.3b** (1.15 mmol, 1.0 eq),D-pantolactone (4.6 mmol, 4 eq) and triethylamine (4.6 mmol, 4 eq) in EtOH (3.8 mL) using the general protocol 2 to afford the product as a yellow oil. Yield: 34%, *R*f = 0.29 (100% EtOAc). Purity was 85% based on HPLC, *R*t = 16.37 minutes with method A and *R*t = 8.13 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, CD_3OD) δ 4.78 (d, *J* = 15.7 Hz, 1H, H-7a), 4.72 (d, *J* = 15.7 Hz, 1H, H-7b), 3.97 (s, 1H, H-5), 3.49 (d, *J* = 10.9 Hz 1H, H-1a), 3.39 (d, *J* = 10.9 Hz 1H, H-1b), 3.09 (t, *J* = 7.6 Hz, 2H, H-10), 1.76 (m, 2H, H-11), 1.43 (m 2H, H-12), 0.98 (t, *J* = 7.2 Hz, 3H, H-13), 0.94 (s, 3H, H-3 or H-4), 0.93 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, CD_3OD) δ 176.2, 173.6, 169.6, 76.7, 69.7, 40.1, 38.3,

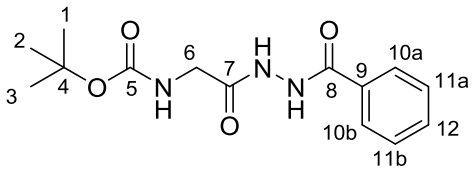
32.7, 29.8, 22.5, 20.9, 20.3, 13.4. HRMS for $C_{13}H_{23}O_3N_3SNa$ $[M+Na]^+$ calcd. 324.1358, found 324.1352.

***(R)-N*-((5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl)methyl)-2,4-dihydroxy-3,3-dimethylbutanamide (3c)**



Compound **3c** was prepared from compound **3.3c** (1.40 mmol, 1.0 eq, D-pantolactone (5.6 mmol, 4 eq) and triethylamine (5.6 mmol, 4 eq) in EtOH (4.6 mL) using the general protocol 2 to afford the crude product as a yellow oil. Yield: 25%, *R*f = 0.25 (100% EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (m, 2H, H-11), 7.67 (bs, 1H, NH), 7.17 (m, 2H, H-12), 4.90 (m, 2H, H-7a and H-7b), 4.16 (s, 1H, H-5), 3.57 (d, *J* = 11.2 Hz, 1H, H-1a), 3.55 (d, *J* = 11.1 Hz, 1H, H-1b), 1.05 (s, 3H, H-3 or H-4), 0.99 (s, 3H, H-3 or H-4). ^{19}F NMR (471 MHz, MeOD) δ -110.47. HRMS for $C_{15}H_{18}O_3N_2SFNa$ $[M+Na]^+$ calcd. 362.0951, found 362.0945.

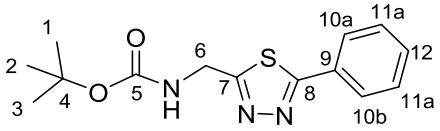
***tert*-Butyl (2-(2-benzoylhydrazineyl)-2-oxoethyl)carbamate (3.4)**



Compound **4.1** was prepared from benzohydrazide (3.42 mmol, 1.0 eq), N-Boc glycine (5.13 mmol, 1.5 eq) and Mukaiyama reagent (4.56 mmol, 2 eq) dissolved in DCM (5 mL). The reaction mixture was cooled to 0°C and triethylamine (1 mL) was added dropwise. The reaction mixture was stirred at room temperature for 16 hours. The product was purified by flash column chromatography (eluted with Hex-EtOAc, 100:00 to 15:85). The product was a yellow oil. Yield:

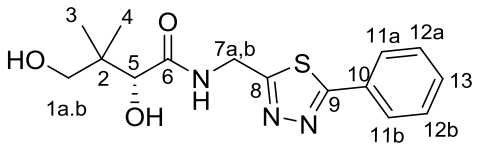
56%, *R*f = 0.25 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (bs, 1H, NH), 7.82 (d, *J* = 7.0 Hz, 2H, H-10), 7.53 (t, *J* = 7.5 Hz, 1H, H-12), 7.43 (m, 2H, H-11), 5.36 (bs, 1H, NH), 3.97 (d, *J* = 6.1 Hz, 2H, H-6) 1.46 (s, 9H, H-1, H-2, and H-3). HRMS for $C_{14}H_{19}O_4N_3Na$ $[M+Na]^+$ calcd. 316.1274, found 316.1268.

tert-Butyl ((5-phenyl-1,3,4-thiadiazol-2-yl)methyl)carbamate (3.5)



Compound **4.2** was prepared from compound **4.1** (1.10 mmol, 1.0 eq) and Lawesson's reagent (1.2 mmol, 1.10 eq) in THF (4.6 mL) using the general protocol 7 to afford the crude product as a yellow oil. Yield: quant., *R*f = 0.75 (100% EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 8.0, 2H, H-10), 7.49-7.44 (m, 3H, H-11 and H-12), 4.79 (bs, 1H, NH), 3.77 (s, 2H, H-6), 1.46 (s, 9H, H-1, H-2, and H-3). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.8, 163.6, 132.3, 132.2, 131.3, 129.6, 127.7, 55.1, 37.6, 34.6, 31.3, 28.2. HRMS for $C_{14}H_{17}O_2N_3SNa$ $[M+Na]^+$ calcd. 314.0939, found 314.0934.

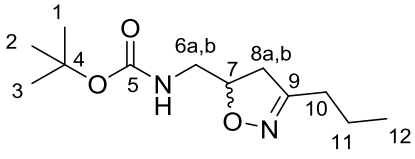
(*R*)-2,4-Dihydroxy-3,3-dimethyl-*N*-((5-phenyl-1,3,4-thiadiazol-2-yl)methyl)butanamide (3d)



Compound **4** was prepared from compound **4.2** (1.00 mmol, 1.0 eq, D-pantolactone (4.0 mmol, 4 eq) and triethylamine (4.0 mmol, 4 eq) in EtOH (3.3 mL) using the general protocol 2 to afford the product as a yellow oil. Yield: 17%, *R*f = 0.56 (100% EtOAc). Purity was 76% based on HPLC, *R*t = 16.77 minutes with method A and *R*t = 8.33 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, CD_3OD) δ 7.97 (m, 2H, H-11), 7.55 (m, 3H, H-12 and H-13), 4.85 (m, 2H, H-7a and H-

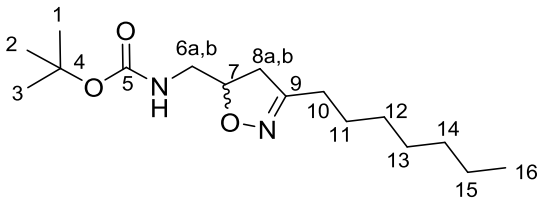
7b), 4.02 (s, 1H, H-5), 3.87 (bs, 1H, NH), 3.53 (m, 1H, H-1a), 3.42 (m, 1H, H-1b), 0.99 (s, 6H, H-3 and H-4). ^{13}C NMR (125 MHz, CD_3OD) δ 176.3, 171.0, 169.5, 132.1, 130.5, 130.0, 128.3, 76.8, 69.7, 40.1, 38.5, 20.9, 20.3. HRMS for $C_{15}H_{19}O_3N_2SNa$ $[M+Na]^+$ calcd. 344.1045, found 344.1039.

tert-Butyl ((3-propyl-4,5-dihydroisoxazol-5-yl)methyl)carbamate (5.3a)



Compound **5.3a** was prepared from compound **5.2a** (5 mmol, 1.0 eq), *tert*-butyl-*N*-allylcarbamate (5 mmol, 1.0 eq) and triethylamine (6 mmol, 1.2 eq) in ethyl acetate (20 mL) using the general protocol 8 to afford the product as a yellow oil. Yield: 30%, *R*f = 0.75 (50% EtOAc in Hex). Purity was 79% based on HPLC, *R*t = 22.12 minutes with method A and *R*t = 10.83 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.88 (bs, 1H, NH), 4.63 (m, 1H, H-7), 3.34 (m, 1H H-6a), 3.26 (m, 1H H-6b), 2.96 (m, 1H, H-8a), 2.68 (dd, *J* = 17.2, 7.1 Hz, 1H, H-8b), 2.31 (dt, *J* = 7.7, 2.3 Hz, 2H, H-10), 1.58 (m, 2H, H-11), 1.43 (s, 9H, H-1, H-2 and H-3), 0.96 (t, *J* = 7.4 Hz, 3H, H-12). ^{13}C NMR (125 MHz, CD_3OD) δ 160.5, 158.2, 79.8, 79.5, 44.0, 39.9, 29.8, 28.2, 20.2, 13.5. $C_{12}H_{22}O_3N_2Na$ $[M+Na]^+$ calcd. 265.1528, found 265.1523.

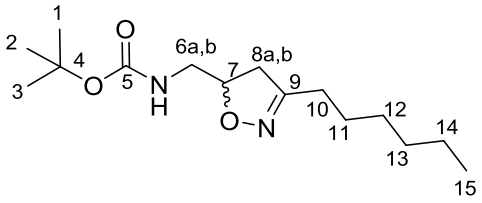
tert-Butyl ((3-heptyl-4,5-dihydroisoxazol-5-yl)methyl)carbamate (5.3b)



Compound **5.3b** was prepared from compound **5.2b** (4 mmol, 1.0 eq), *tert*-butyl-*N*-allylcarbamate (4 mmol, 1.0 eq) and triethylamine (4.8 mmol, 1.2 eq) in ethyl acetate (16 mL) using the general protocol 8 to afford the product as a yellow oil. Yield: 30%, *R*f = 0.71 (50%

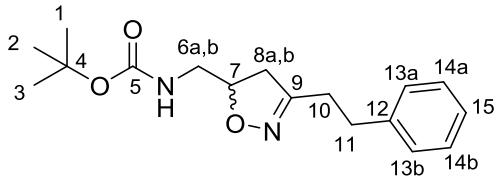
EtOAc in Hex). Purity was 81% based on HPLC, *R*t = 28.27 minutes with method A and *R*t = 13.59 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (bs, 1H, NH), 4.61 (m, 1H, H-7), 3.34 (m, 1H, H-6a), 3.23 (m, 1H, H-6b), 2.94 (dd, *J* = 17.1, 10.5 Hz, 1H, H-8a), 2.66 (dd, *J* = 17.1, 7.2 Hz, 1H, H-8b), 2.30 (m, 2H, H-10), 1.51 (m, 2H, H-11), 1.39 (m, 2H, H-12), 1.40 (s, 9H, H-1, H-2 and H-3), 1.29 (m, 6H, H-13, H-14 and H-15), 0.85 (t, *J* = 8 Hz, 3H, H-16). ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.0, 156.4, 79.7, 78.8, 39.6, 31.8, 31.1, 29.3, 29.0, 28.5, 27.8, 26.5, 22.7, 14.2.

tert-Butyl ((3-hexyl-4,5-dihydroisoxazol-5-yl)methyl)carbamate (5.3c)



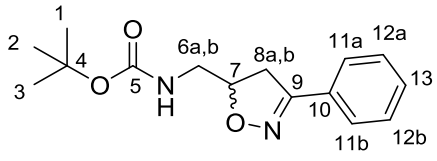
Compound **5.3** was prepared from compound **5.2c** (6 mmol, 1.0 eq), *tert*-butyl-*N*-allylcarbamate (6 mmol, 1.0 eq) and triethylamine (7.2 mmol, 1.2 eq) in ethyl acetate (24 mL) using general protocol 8 to afford the product as a yellow oil. Yield: 27%, *R*f = 0.38 (50% EtOAc in Hex). Purity was 61% based on HPLC, *R*t = 23.89 minutes with method A and *R*t = 13.60 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.89 (bs, 1H,NH), 4.65 (m, 1H, H-7), 3.35 (m, 1H, H-6a), 3.24 (m, 1H, H- H-6b), 2.96 (dd, *J* = 17.1, 10.5 Hz, 1H, H-8a), 2.67 (dd, *J* = 17.2, 7.2 Hz, 1H, H-8b), 2.32 (m, 2H, H-10), 1.53 (m, 2H H-11), 1.43 (s, 9H, H-1, H-2 and H-3), 1.34-1.23 (m, 6H, H-12, H-13 and H-14), 0.88 (t, *J* = 7.04 Hz 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.0, 152.4, 77.4, 76.9, 33.7, 31.6, 29.6, 29.2, 28.9, 26.6, 25.1, 22.6, 14.1.

tert-Butyl ((3-phenethyl-4,5-dihydroisoxazol-5-yl)methyl)carbamate (5.3d)



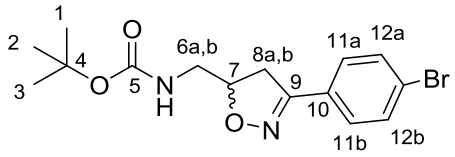
Compound **5.3d** was prepared from compound **5.2d** (5 mmol, 1.0 eq), *tert*-butyl-*N*-allylcarbamate (5 mmol, 1.0 eq) and triethylamine (6 mmol, 1.2 eq) in ethyl acetate (20 mL) using the general protocol 8 to afford the crude product as a yellow oil. Yield: 40%, *R*f = 0.55 (50% EtOAc in Hex). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 2H, H-13), 7.20 (m, 3H, H-14 and H-15), 4.88 (bs, 1H, NH), 4.63 (m, 1H, H-7), 3.32 (m, 1H, H-6a), 3.20 (m, 1H, H-6b), 2.96-2.87 (m, 3H, H-10 and H-8a), 2.68-2.62 (m, 3H, H-11 and H-8b), 1.43 (s, 9H, H-1, H-2 and H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 156.1, 140.3, 128.5, 128.1, 126.3, 79.5, 78.7, 43.4, 39.6, 32.5, 29.3, 28.2. HRMS for $C_{17}H_{24}O_3N_2$ $[M+H]^+$ calcd. 305.1805, found 305.0468.

tert-Butyl ((3-phenyl-4,5-dihydroisoxazol-5-yl)methyl)carbamate (5.3e)



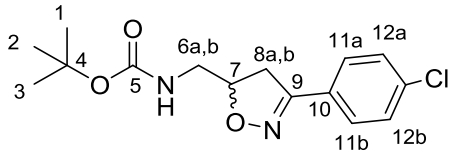
Compound **5.3e** was prepared from compound **5.2e** (5 mmol, 1.0 eq), *tert*-butyl-*N*-allylcarbamate (5 mmol, 1.0 eq) and triethylamine (6 mmol, 1.2 eq) in ethyl acetate (20 mL) using the general protocol 8 to afford the product as a yellow oil. Yield: 71%, *R*f = 0.80 (50% EtOAc in Hex). Purity was 90% based on HPLC, *R*t = 25.18 minutes with method A and *R*t = 11.57 minutes with method B (Table 5.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (m, 2H, H-11), 7.42-7.40 (m, 3H, H-12 and H-13), 4.94 (bs, 1H, NH), 4.85 (m, 1H, H-7), 3.47 (m, 1H, H-6a), 3.41-3.36 (m, 2H, H-6b and H-8a), 3.15 (dd, *J* = 16.8, 7.5 Hz, 1H, H-8b), 1.39 (s, 9H, H-1, H-2 and H-3). ^{13}C NMR (125 MHz, CD_3OD) δ 158.3, 158.1, 131.0, 130.4, 129.5, 127.4, 81.0, 80.0, 44.3, 38.2, 28.3. $C_{15}H_{20}O_3N_2Na$ $[M+Na]^+$ calcd. 299.1372, found 299.1366.

tert-Butyl ((3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)methyl)carbamate (5.3f)



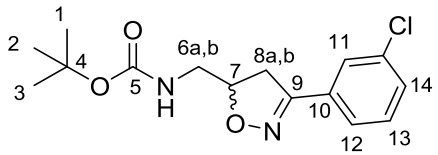
Compound **5.3f** was prepared from compound **5.2f** (5 mmol, 1.0 eq), *tert*-butyl-*N*-allylcarbamate (5 mmol, 1.0 eq) and triethylamine (6 mmol, 1.2 eq) in ethyl acetate (20 mL) using the general protocol 8 to afford the product as a yellow oil. Yield: 35%, *R*f = 0.65 (50% EtOAc in Hex). Purity was 79% based on HPLC, *R*t = 26.78 minutes with method A and *R*t = 12.37 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55- 7.50 (m, 4H, H-11 and H-12), 5.15 (m, 1H, H-8a), 4.92 (bs, 1H, NH), 4.87 (m 1H, H-7), 3.46-3.32 (m, 2H, H-8b and H-6a), 3.13 (dd, *J* = 16.8, 7.5 Hz, 1H, H-6b), 1.39 (s, 9H, H-1, H-2 and H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.2, 156.0, 134.8, 128.1, 128.0, 124.4, 80.3, 79.6, 43.4, 37.0, 28.2. HRMS for $C_{15}H_{19}O_3N_2BrNa$ [M+Na]+ calcd. 377.0477, found 377.0471

tert-Butyl ((3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)carbamate (5.3g)



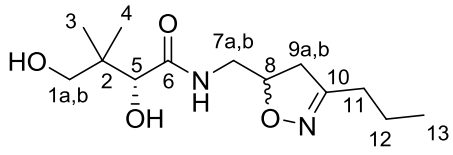
Compound **5.3g** was prepared from compound **5.2g** (4 mmol, 1.0 eq), *tert*-butyl-*N*-allylcarbamate (4 mmol, 1.0 eq) and triethylamine (4.8 mmol, 1.2 eq) in ethyl acetate (16 mL) using the general protocol 8 to afford the crude product as a yellow oil. Yield: 45%, *R*f = 0.67 (50% EtOAc in Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.6 Hz, 2H, H-11, 7.37 (d, *J* = 8.5 Hz, 2H, H-12), 4.86 (m 1H, H-7), 3.45 (m, 1H H-8a), 3.43-3.33 (m, 2H, H-8b and H-6a), 3.13 (dd, *J* = 16.8, 7.5 Hz, 1H, H-6b), 1.39 (s, 9H, H-1, H-2 and H-3). HRMS for $C_{15}H_{19}O_3N_2ClNa$ [M+Na]+ calcd. 333.0982, found 333.0976.

***tert*-Butyl ((3-(3-chlorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)carbamate (5.3h)**



Compound **5.3h** was prepared from compound **5.2h** (5 mmol, 1.0 eq), *tert*-butyl-*N*-allylcarbamate (5 mmol, 1.0 eq) and triethylamine (6 mmol, 1.2 eq) in ethyl acetate (20 mL) using the general protocol 8 to afford the product as a yellow oil. Yield: 45%, *R*f = 0.67 (50% EtOAc in Hex). Purity was 78% based on HPLC, *R*t = 26.92 minutes with method A and *R*t = 12.56 minutes with method B (Table 5.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (t, *J* = 1.8 Hz, 1H, H-11), 7.52 (td, *J* = 7.5, 1.6 Hz, 1H, H-12), 7.39-7.30 (m, 2H, H-13 and H-14), 4.96 (bs, 1H, NH), 4.86 (m, 1H, H-7), 3.41-3.31 (m, 3H, H-6 and H-8a), 3.15-3.09 (dd, *J* = 16.9, 7.5 Hz, 1H, H-8b), 1.39 (s, 9H, H-1, H-2 and H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.2, 155.8, 134.7, 131.0, 130.1, 29.9, 126.6, 124.7, 80.4, 79.7, 37.0, 30.8, 28.2. HRMS for $C_{15}H_{19}O_3N_2ClNa$ [M+Na]+ calcd. 333.0982, found 333.0976.

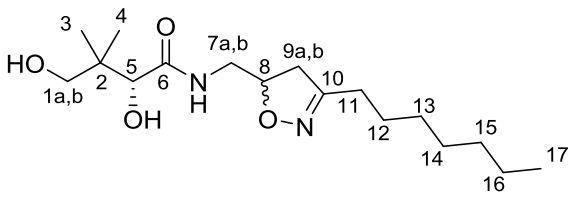
(*2R*)-2,4-Dihydroxy-3,3-dimethyl-*N*-((3-propyl-4,5-dihydroisoxazol-5-yl)methyl)butanamide (5a)



Compound **5a** was prepared from compound **5.4a** (1.00 mmol, 1.0 eq), D-pantolactone (2.00 mmol, 2 eq) and triazabicyclodecene (0.1 mmol, 0.1 eq) in toluene (1 mL) using the general protocol 8 to afford the product as a beige oil. Yield: 92%, *R*f = 0.25 (100% EtOAc). Purity was 97% based on HPLC, *R*t = 15.42 minutes with method A and *R*t = 7.69 minutes with method B (Table 5.1). The following data were collected from an approximate 1:1 mixture of two diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (bs, 1H, NH), 4.67 (m, 1H, H-8), 4.05 (d, *J* =

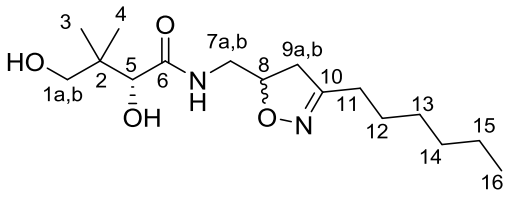
4.0 Hz, 1H, H-5) , 3.58 (m, 1H, H-7a), 3.52 (m, 1H, H-1a), 3.49 (m, 1H, H-1b), 3.36 (m, 1H, H-7b), 3.01 (dd, *J* = 17.3, 10.6 Hz, 1H, H-9a), 2.66 (ddd, *J* = 17.2, 7.2, 3.2 Hz, 1H, H-9b), 2.30 (m, 2H, H-11), 1.58 (m, 2H, H-12), 1.02 (s, 3H, H-3 or H-4), 0.95 (t, *J* = 7.4 Hz, 3H, H-13), 0.91 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) Diastereoisomer A: δ 174.0, 159.4, 78.1, 77.4, 70.9, 42.0, 39.9, 39.1, 29.4, 21.2, 20.3, 19.5, 13.6. Diastereoisomer B: δ 173.8, 159.4, 78.0, 77.1, 70.8, 41.8, 39.9, 39.1, 29.4, 21.0, 20.1, 19.5, 13.6. HRMS for $C_{13}H_{24}O_4N_2Na$ $[M+Na]^+$ calcd. 295.1634, found 295.1628.

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



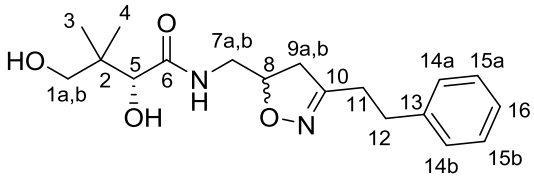
Compound **5b** was prepared from compound **5.4b** (1.2 mmol, 1.0 eq), D-pantolactone (2.4 mmol, 2 eq) and triazabicyclodecene (0.12 mmol, 0.1 eq) in toluene (1.2 mL) using the general protocol 9 to afford the product as a beige oil. Yield: 45%, *R*f = 0.25 (100% EtOAc). Purity was 97% based on HPLC, *R*t = 21.84 minutes with method A and *R*t = 10.59 minutes with method B (Table 5.1). The following data were collected from an approximate 1:1 mixture of two diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (bs, 1H, NH), 4.65 (m, 1H, H-8), 4.20 (s, 1H, H-5), 3.50 (m, 2H, H-1aand H-1b), 3.05 (m, 1H, H-7a), 2.63 (td, *J* = 17.5, 6.3 Hz, 1H, H-7b), 2.34-2.29 (m, 2H, H-9a and H-9b), 1.36- 1.23 (m, 10H, H-12, H-13, H-14, H-15 and H-16), 1.03 (s, 3H, H-3 or H-4), 0.92 (m, 3H, H-3 or H-4), 0.88 (t, *J* = 8 Hz, H-17). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.5, 160.1, 77.8, 77.7, 69.2, 42.6, 40.1, 39.3, 39.2, 31.6, 29.2, 28.9, 27.6, 26.2, 22.6, 14.2, 14.0. HRMS for $C_{17}H_{32}O_4N_2Na$ $[M+Na]^+$ calcd. 351.2260, found 351.2254.

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



Compound **5c** was prepared from compound **5.4c** (1.2 mmol, 1.0 eq), D-pantolactone (2.4 mmol, 2 eq) and triazabicyclodecene (0.12 mmol, 0.1 eq) in toluene (1.2 mL) using the general protocol 9 to afford the product as a white solid. Yield: 90%, *R*f = 0.40 (100% EtOAc). Purity was 95% based on HPLC, *R*t = 19.67 minutes with method A and *R*t = 9.82 minutes with method B (Table 5.1). The following data were collected from an approximate 1:1 mixture of two diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (m, 1H, H-8), 4.19 (s, 1H, H-5), 3.56-3.46 (m, 2H, H-1a and H-1b) 3.07 (dd, *J* = 17.4, 10.3 Hz, 1H, H-7a), 2.62 (dt, *J* = 17.5, 6.1, 1H, H-7b), 2.34-2.29 (m, 2H, H-9a and H-9b), 1.52 (m, 2H, H-11), 1.37-1.25 (m, 8H, H-12, H-13, H-14 and H-15), 1.04 (s, 3H, H-3 or H-4), 0.94 (s, 3H, H-3 or H-4), 0.88 (t, *J* = 6.5 Hz, 3H, H-16). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 160.0, 77. 6, 69.2, 60.3, 42.6, 40.0, 39.8, 31.2, 28.8, 27.4, 26.1, 22.3, 20.9, 14.1, 13.9. HRMS for $C_{16}H_{30}O_4N_2Na$ $[M+Na]^+$ calcd. 337.2104, found 337.2098.

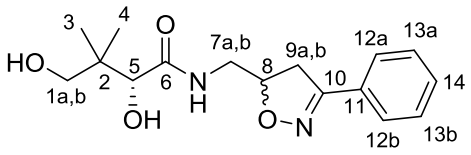
(2*R*)-2,4-Dihydroxy-3,3-dimethyl-*N*-((3-phenethyl-4,5-dihydroisoxazol-5-yl)methyl)butanamide (5d)



Compound **5d** was prepared from compound **5.4d** (1.5 mmol, 1.0 eq), D-pantolactone (3.00 mmol, 2 eq) and triazabicyclodecene (0.15 mmol, 0.1 eq) in toluene (1.5 mL) using the general protocol 9 to afford the product as a white solid. Yield: 25%, *R*f = 0.55 (100% EtOAc). Purity was 79% based on HPLC, *R*t = 18.02 minutes with method A and *R*t = 8.99 minutes with method B

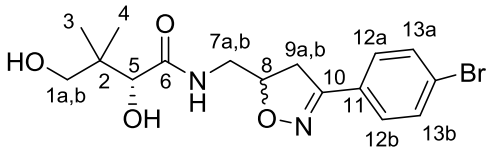
(Table 5.1). The following data were collected from an approximate 1:1 mixture of two diastereoisomers. ^{1}H NMR (500 MHz, CD_3OD) δ 7.32-7.19 (m, 5H, H-14, H-15 and H-16), 4.66 (m, 1H, H-8), 3.94 (s, 1H, H-5), 3.55-3.38 (m, 4H, H-1a, H-1b, H-7a and H-7b), 3.06 (ddd, *J* = 17.5, 10.4, 2.7 Hz, 1H, H-9a), 2.91 (t, *J* = 7.8 Hz, 2H, H-12), 2.77 (ddd, *J* = 17.4, 6.9, 2.2 Hz, 1H, H-9b), 2.68 (m, 2H, H-11), 0.94 (s, 3H, H-3 or H-4), 0.93 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, CD_3OD) δ 175.5, 159.3, 140.5, 128.2, 128.0, 126.0, 79.4, 76.1, 69.0, 42.3, 41.1, 40.4, 32.1, 32.1, 28.9, 19.9. HRMS for $C_{18}H_{26}O_4N_2Na$ [M+Na]+ calcd. 357.1791, found 357.1785.

(2*R*)-2,4-Dihydroxy-3,3-dimethyl-*N*-((3-phenyl-4,5-dihydroisoxazol-5-yl)methyl)butanamide (5e)



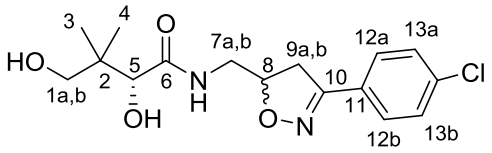
Compound **5e** was prepared from compound **5.4e** (1.00 mmol, 1.0 eq), D-pantolactone (2.00 mmol, 2 eq) and triazabicyclodecene (0.1 mmol, 0.1 eq) in toluene (1 mL) using the general protocol 8 to afford the product as a white solid. Yield: 82%, *R*f = 0.25 (100% EtOAc). Purity was 99% based on HPLC, *R*t = 17.06 minutes with method A and *R*t = 8.51 minutes with method B (Table 5.1). The following data were collected from an approximate 1:1 mixture of two diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (m, 2H, H-12), 7.40-7.37 (m, 3H, H-13 and H-14), 7.33 (bs, 1H, NH), 4.90 (m, 1H, H-8), 4.05 (m, 1H, H-5), 3.67-3.53 (m, 2H, H-7a and H-7b), 3.51-3.44 (m, 2H, H-1a and H-1b), 3.41 (m, 1H, H-9a), 3.10 (td, *J* = 16.9, 7.0 Hz, 1H, H-9b), 0.96 (s, 3H, H-3 or H-4), 0.86 (s, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) Diastereoisomer A: δ 174.1, 157.1, 130.4, 129.0, 128.8, 126.7, 79.7, 77.6, 71.1, 42.2, 39.3, 37.9, 21.3, 20.4. Diastereoisomer B: δ 174.0, 157.0, 130.4, 129.0, 128.8, 126.7, 79.5, 77.6, 71.1, 42.1, 39.2, 37.8, 21.2, 20.2. HRMS for $C_{16}H_{22}O_4N_2Na$ $[M+Na]^+$ calcd. 329.1478, found 329.1423.

(2*R*)-*N*-((3-(4-Bromophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-2,4-dihydroxy-3,3-dimethylbutanamide (5f)



Compound **5f** was prepared from compound **5.4f** (1 mmol, 1.0 eq), D-pantolactone (2.00 mmol, 2 eq) and triazabicyclodecene (0.1 mmol, 0.1 eq) in toluene (1 mL) using the general protocol 9 to afford the product as a yellow oil. Yield: 82%, *R*f = 0.18 (100% EtOAc). Purity was 94% based on HPLC, *R*t = 19.02 minutes with method A and *R*t = 9.53 minutes with method B (Table 5.1). The following data were collected from an approximate 1:1 mixture of two diastereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (m, 2H, H-12), 7.27 (m, 2H, H-13), 4.68 (m, 1H, H-8), 3.97 (m, 2H, H-5), 3.46-3.34 (m, 2H, H-1a and H-1b), 3.32- 3.17 (m, 3H, H-7a, H-7b and H-9a), 2.88 (m, 1H, H-9b), 0.74 (s, 1H, H-3 or H-4), 0.65 (s, 1H, H-3 or H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 156.2, 131.9, 128.0, 127.7, 124.7, 79.4, 77.2, 70.3, 42.2, 39.1, 37.5, 20.9, 14.1. HRMS for $C_{16}H_{21}O_4N_2BrNa$ $[M+Na]^+$ calcd. 407.0583, found 407.0577.

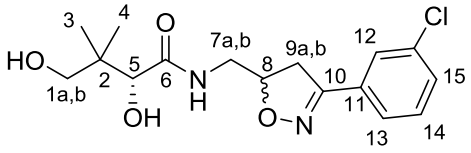
(2*R*)-*N*-((3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-2,4-dihydroxy-3,3-dimethylbutanamide (5g)



Compound **5g** was prepared from compound **5.4g** (1 mmol, 1.0 eq), D-pantolactone (2.00 mmol, 2 eq) and triazabicyclodecene (0.1 mmol, 0.1 eq) in toluene (1 mL) using the general protocol 9 to afford the product as a yellow oil. Yield: 82%, *R*f = 0.20 (100% EtOAc). Purity was 97% based on HPLC, *R*t = 18.65 minutes with method A and *R*t = 9.35 minutes with method B (Table 5.1). The following data were collected from an approximate 1:1 mixture of two

diastereoisomers. ^{1}H NMR (500 MHz, CD_3OD) δ 7.65 (dd, *J* = 8.7, 1.3 Hz, 2H, H-12a and H-12b), 7.43 (dd, *J* = 8.6, 1.4 Hz, 2H, H-13a and H-13b), 4.89 (m, 1H, H-8), 3.95 (d, *J* = 5.6 Hz, 1H, H-5), 3.53-3.36 (m, 6H, H-1a, H-1b, H-7a, H-7band H-9a), 3.19 (ddd, *J* = 17.1, 7.2, 2.5 Hz, 1H, H-9b), 0.91 (s, 1H, H-3 or H-4), 0.86 (s, 1H, H-3 or H-4). ^{13}C NMR (125 MHz, CD_3OD) Diastereoisomer A: δ 172.6, 162.9, 157.2, 136.8, 129.7, 129.2, 80.8, 77.0, 70.0, 42.6, 40.0, 38.2, 21.0, 20.5. Diastereoisomer B: δ 172.6, 162.6, 157.2, 136.8, 129.2, 128.9, 80.7, 77.0, 69.9, 42.6, 39.9, 38.2, 20.9, 20.5. HRMS for $C_{16}H_{21}O_4N_2ClNa$ $[M+Na]^+$ calcd. 363.1088, found 363.1082.

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



Compound **5h** was prepared from compound **5.4h** (5 mmol, 1.0 eq), D-pantolactone (10 mmol, 2 eq) and triazabicyclodecene (0.5 mmol, 0.1 eq) in toluene (5 mL) using the general protocol 9 to afford the product as a white solid. Yield: 45%, *R*f = 0.50 (100% EtOAc). Purity was 97% based on HPLC, *R*t = 18.71 minutes with method A and *R*t = 9.35 minutes with method B (Table 5.1). The following data were collected from an approximate 1:1 mixture of two diastereoisomers. ^{1}H NMR (500 MHz, CD_3OD) δ 7.70 (m, 1H, H-12) 7.59 (m, 1H, H-13), 7.45-7.40 (m, 2H, H-14 and H-15), 4.92 (m, 1H, H-8), 3.94 (d, *J* = 6.7 Hz, 1H, H-5), 3.55-3.36 (m, 5H, H-1a, H-1b, H-7a, H-7b and H-9a), 3.21 (ddd, *J* = 17.2, 7.2, 1.7 Hz, 1H, H-9b), 0.93 (s, 3H, H-3 or H-4), 0.88 (s, *J* = 3.5 Hz, 3H, H-3 or H-4). ^{13}C NMR (125 MHz, CD_3OD) δ 176.1, 7.0, 135.3, 132.3, 130.9, 130.6, 127.0, 125.7, 80.8, 76.8, 69.8, 42.4, 39.9, 39.8, 20.8, 20.3. HRMS for $C_{16}H_{21}O_4N_2ClNa$ $[M+Na]^+$ calcd. 363.1088, found 363.1082.

5.2 Biology

5.2.1 Materials

Bacteria were purchased from Cedarlane, Canada, and include *Escherichia coli* ATCC 25922, *Enterococcus faecium* ATCC 19434, *Klebsiella pneumonia* ATCC 13883, *Pseudomonas. aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 196006, and *Staphylococcus aureus* ATCC 29213. *E. coli*, *A baumanni* and *K. pneumonia* were cultured in Difco™ Nutrient Broth. *E. faecium* was cultured in Difco™ Brain Heart Infusion Broth. *P. aeruginosa* and *S. aureus* were cultured in Difco™ Tryptic Soy Broth. The cationic adjusted Mueller-Hinton Broth was prepared based on the protocol in the Clinical and Laboratory Standards Institute (CLSI) and used for each bacteria.[78] A Molecular Devices SpectraMax i3x multi-mode microtiter plate reader or an Agilent 8453 UV-Vis spectrometer were used to measure absorption at 600 nm.

5.2.2 Antimicrobial susceptibility test

The antimicrobial susceptibility of the compounds synthesized in this thesis was determined by using the CLSI M07's broth microdilution method for one concentration of 50 µM.[78] A calibration curve was prepared to determine the concentration of bacteria from OD_{600}, expressed as a linear relationship in the form of $OD_{600} = mx + b$, where x is the concentration of bacteria (CFU/mL) and OD_{600} is the optical density at 600 nm (Table 5.2).

Table 5.2: Equations derived from the calibration curves to determine the bacterial concentration, where x is the concentration of bacteria (CFU/mL) and OD_{600} is the optical density at 600 nm

Bacteria	Calibration Equation
E. coli	$OD_{600} = 3 \times 10^{-9}x + 0.2537$
E. faecium	$OD_{600} = 2 \times 10^{-9}x + 0.1190$
K. pneumonia	$OD_{600} = 2 \times 10^{-9}x + 0.1739$

P. aeruginosa	$OD_{600} = 7\times10^{-10}x + 0.2473$
S. aureus	$OD_{600} = 2 \times 10^{-9}x + 0.0146$

To measure antimicrobial susceptibility to each of the synthetic compound, bacteria were allowed to proliferate at 37°C for 18 hours on agar medium prepared using the growth medium listed above for each strain. Next, four colonies were selected from the overnight agar culture and added to the corresponding liquid medium (5 mL) before incubation at 37°C for 2-4 hours, to achieve a bacterial concentration of 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 final concentration). The 96-well microplate plate was incubated at 37°C for 18 hours and the OD_{600} was measured to quantify growth. To determine the growth percentage in the presence of a test compound, the blank (containing DMSO and Mueller-Hinton Broth) was subtracted to the average OD_{600} of the culture measured in the presence of the compound and the resulting number was divided by the average value of growth control (containing bacteria, solvent and Mueller-Hinton Broth) and multiplied by 100. All experiments were performed in triplicates.

References

1. Zumla, A.; Memish, Z. A.; Maeurer, M.; Bates, M.; Mwaba, P.; Al-Tawfiq, J. A.; Denning, D. W.; Hayden, F. G.; Hui, D. S. Emerging Novel and Antimicrobial-Resistant Respiratory Tract Infections: New Drug Development and Therapeutic Options. *Lancet. Infect. Dis.* **2014**, *14*, 1136–1149.
2. The Top 10 Causes of Death. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (accessed May 4, 2022).
3. Al-Tawfiq, J. A.; Zumla, A.; Gautret, P.; Gray, G. C.; Hui, D. S.; Al-Rabeeah, A. A.; Memish, Z. A., Surveillance for emerging respiratory viruses. *Lancet. Infect. Dis.***2014**, *14*, 992-1000.
4. Daszak, P., Anatomy of a pandemic. *Lancet* **2012,** *380*, 1883-1884.
5. Hutchings, M. I.; Truman, A. W.; Wilkinson, B., Antibiotics: past, present and future. *Curr. opinion in Microbio.* **2019**, *51*, 72-80.
6. Report on Antimicrobial Resistance. 2016, https:// amr-review.org (accessed May 4, 2022).
7. Malaria. https://www.who.int/news-room/fact-sheets/detail/malaria (accessed May 5, 2022).
8. Sutherland, C. J.; Tanomsing, N.; Nolder, D.; Oguike, M.; Jennison, C.; Pukrittayakamee, S.; Dolecek, C.; Hien, T. T.; do Rosario, V. E.; Arez, A. P.; Pinto, J.; Michon, P.; Escalante, A. A.; Nosten, F.; Burke, M.; Lee, R.; Blaze, M.; Otto, T. D.; Barnwell, J. W.; Pain, A.; Williams, J.; White, N. J.; Day, N. P. J.; Snounou, G.; Lockhart, P. J.; Chiodini, P. L.; Imwong, M.; Polley, S. D. Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. *J. Infect. Dis.* **2010**, *201*, 1544−1550
9. Flannery, E. L.; Chatterjee, A. K.; Winzeler, E. A. Antimalarial Drug Discovery-Approaches and Progress Towards New Medicines. *Nat. Rev. Microbiol.* **2013**, *11*, 849–862.
10. Vector-Borne Diseases https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases (accessed May 5, 2022). Sutherland, C. J.; Tanomsing, N.; Nolder, D.; Oguike, M.; Jennison, C.; Pukrittayakamee, S.; Dolecek, C.; Hien, T. T.; do Rosario, V. E.; Arez, A. P.; Pinto, J.; Michon, P.; Escalante, A. A.; Nosten, F.; Burke, M.; Lee, R.; Blaze, M.; Otto, T. D.; Barnwell, J. W.; Pain, A.; Williams, J.; White, N. J.; Day, N. P. J.; Snounou,

G.; Lockhart, P. J.; Chiodini, P. L.; Imwong, M.; Polley, S. D. Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. *J. Infect. Dis.* **2010**, *201*, 1544−1550

11. Venugopal, K.; Hentzschel, F.; Valkiūnas, G.; Marti, M., Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. *Nat. Rev. Microbiol.* **2020,** *18*, 177-189.
12. Tavares, J. et al., Role of host cell traversal by the malaria sporozoite during liver infection. *J. Exp. Med.* **2013,** *210*, 905–915.
13. Mota, M. M.; Pradel, G.; Vanderberg, J. P.; Hafalla, J. C.; Frevert, U.; Nussenzweig, R. S.; Nussenzweig, V.; Rodríguez, A., Migration of Plasmodium sporozoites through cells before infection. *Science* **2001,** *291*, 141-144.
14. Sologub, L.; Kuehn, A.; Kern, S.; Przyborski, J.; Schillig, R.; Pradel, G., Malaria proteases mediate inside-out egress of gametocytes from red blood cells following parasite transmission to the mosquito. *Cell. Microbio.* **2011,** *13*, 897-912.
15. About Malaria. https://www.jenner.ac.uk/about/resources/about-malaria (accessed May 6, 2022).
16. Lindsay, S. W.; Thomas, M. B.; Kleinschmidt, I., Threats to the effectiveness of insecticide-treated bednets for malaria control: thinking beyond insecticide resistance. *Lancet Global Health* **2021,** *9*, e1325-e1331.
17. Van Dyk, J.; Bouwman, H.; Barnhoorn, I.; Bornman, M., DDT contamination from indoor residual spraying for malaria control. *Sci. of the Tot. Environ.* **2010,** *408*, 2745-2752.
18. Rosen, J. B.; Breman, J. G., Malaria intermittent preventive treatment in infants, chemoprophylaxis, and childhood vaccinations. *Lancet* **2004,** *363*, 1386-1388.
19. Organization, W. H., Seasonal malaria chemoprevention with sulfadoxine–pyrimethamine plus amodiaquine in children: a field guide. **2013**.
20. World Health Organization. *World Health Organization Model List of Essential Medicines, 21st List*; **2019**.
21. Stanisic, D. I.; McCall, M. B., Correlates of malaria vaccine efficacy. Expert Review of Vaccines 2021, 20, 143-161.

22. Schlitzer, M., Antimalarial drugs–what is in use and what is in the pipeline. *Archiv der Pharmazie: Int. J. Pharma. Med. Chem.* **2008,** *341*, 149-163.
23. Stanisic, D. I.; McCall, M. B., Correlates of malaria vaccine efficacy. *Expert Rev. Vaccines.* **2021**, *20*, 143-161.
24. Caminade, C.; Kovats, S.; Rocklov, J.; Tompkins, A. M.; Morse, A. P.; Colon-Gonzalez, F. J.; Stenlund, H.; Martens, P.; Lloyd, S. J. Impact of climate change on global malaria distribution. *Proc. Natl. Acad. Sci. USA*. **2014**, *111,* 3286-3291.
25. Gething, P.; Smith, D. L.; Patil, A. P.; Tatem, A. J.; Snow, R. W.; Hay, S. I. Climate change and the global malaria recession. *Nature*. **2010**, *465*, 342-345.
26. Silhavy, T. J.; Kahne, D.; Walker, S. The Bacterial Cell Envelope. *Cold. Spring. Harb. Perspect. Biol.* **2010**, *2*, 1–16.
27. Beveridge, T. J. Use of the Gram Stain in Microbiology. *Biotech. Histochem.* **2001**, *76*, 111–118.
28. Holmes, A. H.; Moore, L. S. P.; Sundsfjord, A.; Steinbakk, M.; Regmi, S.; Karkey, A.; Guerin, P. J.; Piddock, L. J. V. Understanding the Mechanisms and Drivers of Antimicrobial Resistance. *Lancet.* **2016**, *387*, 176–187.
29. Adedeji, W., The treasure called antibiotics. *Annals of Ibadan postgraduate medicine* **2016,** *14*, 56. Bygbjerg, I. Double Burden of Noncommunicable and Infectious Diseases in Developing Countries. *Science.* **2012**, *337*, 1499–1501.
30. CDC. National Centre for Health Statistics. Life Expectancy. https://www.cdc.gov/nchs/fastats/life-expectancy.htm (accessed May 9, 2022).
31. Fleming, A., On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. *Brit. J. of Exp. Path.* **1929**, *10*, 226.
32. Bygbjerg, I. Double Burden of Noncommunicable and Infectious Diseases in Developing Countries. *Science.* **2012**, *337*, 1499–1501.
33. Guitor, A. K.; Wright, G. D. Antimicrobial Resistance and Respiratory Infections. *CHEST.* **2018**, *154*, 1202–1212.
34. Santajit, S.; Indrawattana, N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *Biomed. Res. Int.* **2016**, *2016*, 1–8.

35. Bassetti, M.; Merelli, M.; Temperoni, C.; Astilean, A., New antibiotics for bad bugs: where are we? *An. of Clin. Microbio. and Antimicro.* **2013,** *12*, 1-15.
36. C. Spry, K. Kirk and K. J. Saliba, *FEMS Microbio. Rev.*, **2008**, *32,* 56-106.
37. Saliba, K. J.; Horner, H. A.; Kirk, K. Transport and Metabolism of the Essential Vitamin Pantothenic Acid in Human Erythrocytes Infected with the Malaria Parasite Plasmodium Falciparum. *J. Biol. Chem.* **1998**, *273*, 10190–10195.
38. Clifton, G.; Bryant, S. R.; Skinner, C. G. N'-(Substituted) Pantothenamides, Antimetabolites of Pantothenic Acid. *Arch. Biochem. Biophys.* **1970**, *137*, 523–528.
39. Spry, C.; Macuamule, C.; Lin, Z.; Virga, K. G.; Lee, R. E.; Strauss, E.; Saliba, K. J. Pantothenamides Are Potent, On-Target Inhibitors of Plasmodium Falciparum Growth When Serum Pantetheinase Is Inactivated. *PLoS. ONE.* **2013**, *8*, e54974.
40. Saliba, K. J.; Spry, C. Exploiting the Coenzyme A Biosynthesis Pathway for the Identification of New Antimalarial Agents: The Case for Pantothenamides. *Biochem. Soc. Trans.* **2014**, *42*, 1087–1093.
41. Moolman, W. J. A.; De Villiers, M.; Strauss, E. Recent Advances in Targeting Coenzyme A Biosynthesis and Utilization for Antimicrobial Drug Development. *Biochem. Soc. Trans.* **2014**, *42*, 1080–1086.
42. Spry, C.; Kirk, K.; Saliba, K. J. Coenzyme A Biosynthesis: An Antimicrobial Drug Target. *FEMS. Microbiol. Rev.* **2008**, *32*, 56–106.
43. Hughes, S. J.; Barnard, L.; Mottaghi, K.; Tempel, W.; Antoshchenko, T.; Hong, B. S.; Allali-Hassani, A.; Smil, D.; Vedadi, M.; Strauss, E.; Park, H. W. Discovery of Potent Pantothenamide Inhibitors of Staphylococcus Aureus Pantothenate Kinase Through a Minimal SAR Study: Inhibition Is Due to Trapping of the Product. *ACS Infect. Dis.* **2016**, *2*, 627–641.
44. Howieson, V. M.; Tran, E.; Hoegl, A.; Fam, H. L.; Fu, J.; Sivonen, K.; Li, X. X.; Auclair, K.; Saliba, K. J. Triazole Substitution of a Labile Amide Bond Stabilizes Pantothenamides and Improves Their Antiplasmodial Potency. *Antimicrob. Agents. Chemother.* **2016**, *60*, 7146–7152.

45. Guan, J.; Tjhin, E. T.; Howieson, V. M.; Kittikool, T.; Spry, C.; Saliba, K. J.; Auclair, K. Structure–Activity Relationships of Antiplasmodial Pantothenamide Analogues Reveal a New Way by Which Triazoles Mimic Amide Bonds. *Chem. Med. Chem.* **2018**, *13*, 2677–2683.
46. Pett, H. E.; Jansen, P. A.; Hermkens, P. H.; Botman, P. N.; Beuckens-Schortinghuis, C. A.; Blaauw, R. H.; Graumans, W.; Van De Vegte-Bolmer, M.; Koolen, K. M.; Rutjes, F. P. J. T.; Dechering, K. J.; Sauerwein, R. W.; Schalkwijk, J. Novel Pantothenate Derivatives for Anti-Malarial Chemotherapy. *Malar. Journal.* **2015**, *14* (1). https://doi.org/10.1186/s12936-015-0673-8.
47. Schalkwijk, J.; Allman, E. L.; M Jansen, P. A.; de Vries, L. E.; J Verhoef, J. M.; Jackowski, S.; M Botman, P. N.; Beuckens-Schortinghuis, C. A.; J Koolen, K. M.; Bolscher, J. M.; Vos, M. W.; Miller, K.; Reeves, S. A.; Pett, H.; Trevitt, G.; Wittlin, S.; Scheurer, C.; Sax, S.; Fischli, C.; Angulo-Barturen, I.; Belén Jiménez-Diaz, M.; Josling, G.; A Kooij, T. W.; Bonnert, R.; Campo, B.; Blaauw, R. H.; J T Rutjes, F. P.; Sauerwein, R. W.; Llinás, M.; H Hermkens, P. H.; Dechering, K. J. Antimalarial Pantothenamide Metabolites Target Acetyl-Coenzyme A Biosynthesis in Plasmodium Falciparum. *Sci. Transl. Med.* **2019**, *11*, 9917.
48. Spry, C.; Barnard, L.; Kok, M.; Powell, A. K.; Mahesh, D.; Tjhin, E. T.; Saliba, K. J.; Strauss, E.; De Villiers, M. Toward a Stable and Potent Coenzyme A-Targeting Antiplasmodial Agent: Structure-Activity Relationship Studies of N-Phenethyl-α-Methyl-Pantothenamide. *ACS. Infect. Dis.* **2020**, *6*, 1844–1854.
49. Schalkwijk, J.; Hermkens, H.; Dechering, J.; Bonnert, V. Pantothenamide Analogues. EP 3674288A1, **2020**.
50. Guan, J.; Spry, C.; Tjhin, E.; Yang, P.; Kittikool, T.; Howieson, V.; Ling, H.; Starrs, L.; Duncan, D.; Burgio, G.; Saliba, K.; Auclair, K. Exploring Heteroaromatic Rings as a Replacement for the Labile Amide of Antiplasmodial Pantothenamides. *J. Med. Chem* **2021,** *64*, 4478-4497.
51. Akinnusi, T. O.; Vong, K.; Auclair, K. Geminal Dialkyl Derivatives of N-Substituted Pantothenamides: Synthesis and Antibacterial Activity. *Bioorg. Med. Chem.* **2011**, *19*, 2696–2706.

52. Barnard, L.; Mostert, K. J.; Van Otterlo, W. A. L.; Strauss, E. Developing Pantetheinase-Resistant Pantothenamide Antibacterials: Structural Modification Impacts on PanK Interaction and Mode of Action. *ACS Infect. Dis.* **2018**, *4*, 736–743.
53. Awuah, E.; Ma, E.; Hoegl, A.; Vong, K.; Habib, E.; Auclair, K. Exploring Structural Motifs Necessary for Substrate Binding in the Active Site of Escherichia Coli Pantothenate Kinase. *Bioorg. Med. Chem.* **2014**, *22*, 3083–3090.
54. Mercer, A. C.; Meier, J. L.; Hur, G. H.; Smith, A. R.; Burkart, M. D. Antibiotic Evaluation and in Vivo Analysis of Alkynyl Coenzyme A Antimetabolites in Escherichia Coli. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5991–5994.
55. De Villiers, M.; Macuamule, C.; Spry, C.; Hyun, Y. M.; Strauss, E.; Saliba, K. J. Structural Modification of Pantothenamides Counteracts Degradation by Pantetheinase and Improves Antiplasmodial Activity. *ACS Med. Chem. Lett.* **2013**, *4*, 784–789.
56. Macuamule, C. J.; Tjhin, E. T.; Jana, C. E.; Barnard, L.; Koekemoer, L.; De Villiers, M.; Saliba, K. J.; Strauss, E. A Pantetheinase-Resistant Pantothenamide with Potent, on-Target, and Selective Antiplasmodial Activity. *Antimicrob. Agents. Chemother.* **2015**, *59*, 3666–3668.
57. Jansen, P. A. M.; van der Krieken, D. A.; Botman, P. N. M.; Blaauw, R. H.; Cavina, L.; Raaijmakers, E. M.; de Heuvel, E.; Sandrock, J.; Pennings, L. J.; Hermkens, P. H. H.; Zeeuwen, P. L. J. M.; Rutjes, F. P. J. T.; Schalkwijk, J. Stable Pantothenamide Bioisosteres: Novel Antibiotics for Gram-Positive Bacteria. *J. Antibiot.* **2019**, *72*, 682–692.
58. Virga, K. G.; Zhang, Y. M.; Leonardi, R.; Ivey, R. A.; Hevener, K.; Park, H. W.; Jackowski, S.; Rock, C. O.; Lee, R. E. Structure-Activity Relationships and Enzyme Inhibition of Pantothenamide-Type Pantothenate Kinase Inhibitors. *Bioorg. Med. Chem.* **2006**, *14*, 1007–1020.
59. De Villiers, M.; Barnard, L.; Koekemoer, L.; Snoep, J. L.; Strauss, E. Variation in Pantothenate Kinase Type Determines the Pantothenamide Mode of Action and Impacts on Coenzyme A Salvage Biosynthesis. *FEBS J.* **2014**, *281*, 4731–4753.

60. De Villiers, M.; Spry, C.; Macuamule, C. J.; Barnard, L.; Wells, G.; Saliba, K. J.; Strauss, E. Antiplasmodial Mode of Action of Pantothenamides: Pantothenate Kinase Serves as a Metabolic Activator Not as a Target. *ACS Infect. Dis.* **2017**, *3*, 527–541.
61. Tjhin, E. T.; Spry, C.; Sewell, A. L.; Hoegl, A.; Barnard, L.; Sexton, A. E.; Siddiqui, G.; Howieson, V. M.; Maier, A. G.; Creek, D. J.; Strauss, E.; Marquez, R.; Auclair, K.; Saliba, K. J. Mutations in the pantothenate kinase of Plasmodium falciparum confer diverse sensitivity profiles to antiplasmodial pantothenate analogues. *PLoS Pathog*. **2018**, *14*, No. e1006918.
62. Schalkwijk, J.; Allman, E. L.; Jansen, P. A. M.; de Vries, L. E.; Verhoef, J. M. J.; Jackowski, S.; Botman, P. N. M.; Beuckens-Schortinghuis, C. A.; Koolen, K. M. J.; Bolscher, J. M.; Vos, M. W.; Miller, K.; Reeves, S. A.; Pett, H.; Trevitt, G.; Wittlin, S.; Scheurer, C.; Sax, S.; Fischli, C.; AnguloBarturen, I.; Jimenez-Diaz, M. B.; Josling, G.; Kooij, T. W. A.; Bonnert, R.; Campo, B.; Blaauw, R. H.; Rutjes, F. P. J.; Sauerwein, R. W.; Llinas, M.; Hermkens, P. H. H.; Dechering, K. J. Antimalarial pantothenamide metabolites target acetyl-coenzyme A biosynthesis in Plasmodium falciparum. *Sci. Transl. Med*. **2019**, *11*, No. eaas9917.
63. Spry, C.; Barnard, L.; Kok, M.; Powell, A. K.; Mahesh, D.; Tjhin, E. T.; Saliba, K. J.; Strauss, E.; de Villiers, M. Toward a stable and potent coenzyme A-targeting antiplasmodial agent: structure-activity relationship studies of N-phenethyl-α-methyl-pantothenamide. *ACS Infect. Dis*. **2020**, *6*, 1844−1854.
64. Saliba, K. J.; Spry, C. Exploiting the coenzyme A biosynthesis pathway for the identification of new antimalarial agents: the case for pantothenamides. *Biochem. Soc. Trans*. **2014**, *42*, 1087−1093.
65. Hermkens, P. H. H.; Schalkwijk, J.; Jansen, P. A. M.; Botman, P. Pantothenamide Analogues. WO2016072854A2, **May 12, 2016**.
66. Akinnusi, T. O.; Vong, K.; Auclair, K. Geminal dialkyl derivatives of N-substituted pantothenamides: synthesis and antibacterial activity. *Bioorg. Med. Chem*. **2011**, *19*, 2696−2706.

67. De Villiers, M.; Macuamule, C.; Spry, C.; Hyun, Y.; Strauss, E.; Saliba, K. J. Structural modification of pantothenamides counteracts degradation by pantetheinase and improves antiplasmodial activity. *ACS Med. Chem. Lett*. **2013**, *4*, 784−789.
68. Hoegl, A.; Darabi, H.; Tran, E.; Awuah, E.; Kerdo, E. S. C.; Habib, E.; Saliba, K. J.; Auclair, K. Stereochemical modification of geminal dialkyl substituents on pantothenamides alters antimicrobial activity. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 3274−3277.
69. Macuamule, C. J.; Tjhin, E. T.; Jana, C. E.; Barnard, L.; Koekemoer, L.; de Villiers, M.; Saliba, K. J.; Strauss, E. A pantetheinase-resistant pantothenamide with potent, on-target, and selective antiplasmodial activity. *Antimicrob. Agents Chemother*. **2015**, *59*, 3666−3668.
70. Guan, J.; Hachey, M.; Puri, L.; Howieson, V.; Saliba, K. J.; Auclair, K. A cross-metathesis approach to novel pantothenamide derivatives. *Beilstein J. Org. Chem*. **2016**, *12*, 963−968.
71. Barnard, L.; Mostert, K. J.; van Otterlo, W. A. L.; Strauss, E. Developing pantetheinase-resistant pantothenamide antibacterials: structural modification impacts on PanK interaction and mode of action. *ACS Infect. Dis*. **2018**, *4*, 736−743
72. Guan, J.; Tjhin, E. T.; Howieson, V. M.; Kittikool, T.; Spry, C.; Saliba, K. J.; Auclair, K. Structure-activity relationships of antiplasmodial pantothenamide analogues reveal a new way by which triazoles mimic amide bonds. *Chem. Med. Chem.* **2018**, *13*, 2677−2683.
73. Guan, J.; Spry, C.; Tjhin, E. T.; Yang, P.; Kittikool, T.; Howieson, V. M.; Ling, H.; Starrs, L.; Duncan, D.; Burgio, G.; Saliba, K. J.; Auclair, K. Exploring heteroaromatic rings as a replacement for the labile amide of antiplasmodial pantothenamides. *J. Med. Chem*. **2021**, *64*, 4478-4497.
74. Spry, C.; Macuamule, C.; Lin, Z.; Virga, K. G.; Lee, R. E.; Strauss, E.; Saliba, K. J. Pantothenamides are potent, on-target inhibitors of Plasmodium falciparum growth when serum pantetheinase is inactivated. *PLoS One* **2013**, *8*, No. e54974.
75. Taylor, R. D.; MacCoss, M.; Lawson, A. D. Rings in drugs. *J. Med. Chem*. **2014**, *57*, 5845−5859.

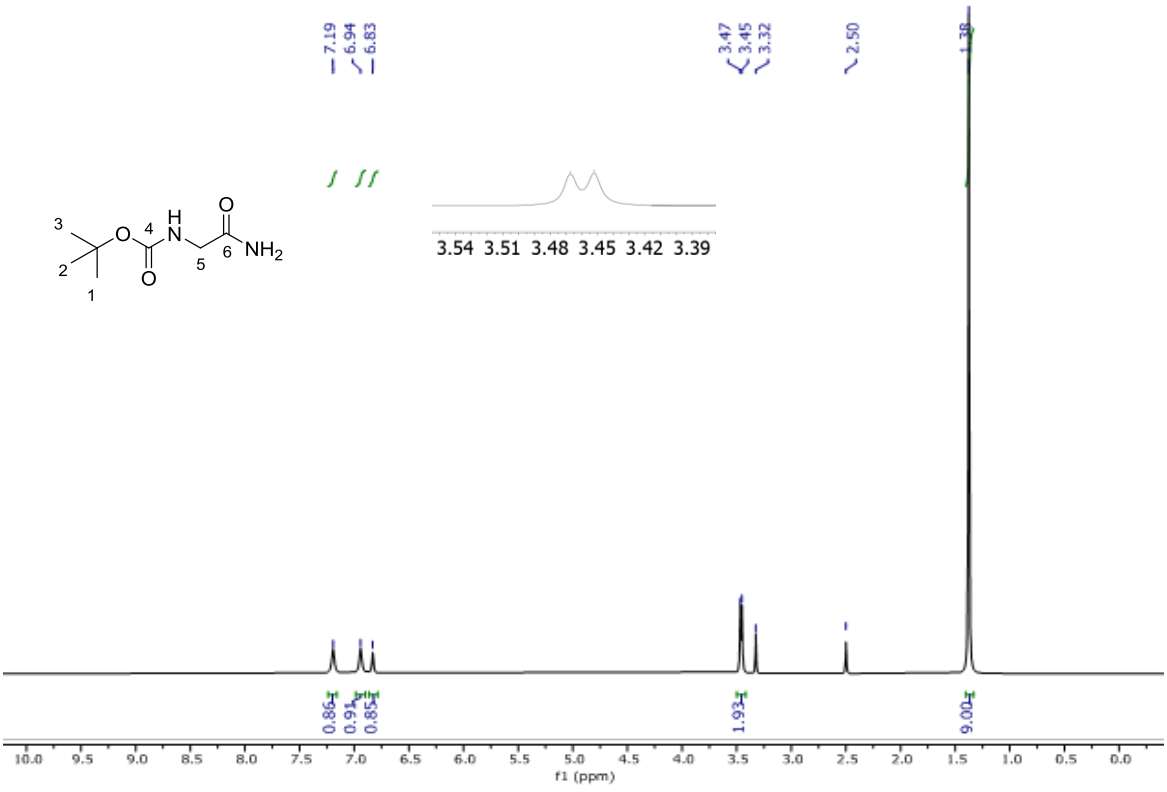
76. Gomtsyan, A. Heterocycles in Drugs and Drug Discovery. *Chem. Heterocycl. Compd.* **2012**, *48*, 7–10.
77. Chu, A. Synthesis of Thiazole-Containing Pantothenamide Analogues As Potential Antimicrobial Agents. *dissertation, McGill University Libraries*, **2021**.
78. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Tenth Edition*, M07-A10 ed.; *Clinical and Laboratory Standards Institute: Wayne*, PA, **2015**.
79. Navidinia, M., The clinical importance of emerging ESKAPE pathogens in nosocomial infections. *J. Soc. Inf. Disp.* **2016,** *7*, 2008-4978.
80. Meanwell, N. A. A Synopsis of the Properties and Applications of Heteroaromatic Rings in Medicinal Chemistry. *Adv. Heterocy. Chem.*; Academic Press Inc., **2017**; Vol. 123, pp 245–361.
81. Ilardi, E. A.; Vitaku, E.; Njardarson, J. T. Data-Mining for Sulfur and Fluorine: An Evaluation of Pharmaceuticals to Reveal Opportunities for Drug Design and Discovery. *J. Med. Chem.* **2014**, *57*, 2832–2842.
82. Beno, B. R.; Yeung, K. S.; Bartberger, M. D.; Pennington, L. D.; Meanwell, N. A. A Survey of the Role of Noncovalent Sulfur Interactions in Drug Design. *J. Med. Chem.* **2015**, *58*, 4383–4438.
83. Newman DJ, Cragg GM, Snader KM: Natural products as sources of new drugs over the period*. J. Nat. Prod.* **2003**, *66*, 1022-1037
84. Rotella, D. P., Heterocycles in drug discovery: Properties and preparation. *Adv. Heterocyc. Chem.* **2021**; Vol. 134, pp 149-183.
85. Blakemore, D. C.; Castro, L.; Churcher, I.; Rees, D. C.; Thomas, A. W.; Wilson, D. M.; Wood, A., Organic synthesis provides opportunities to transform drug discovery. *Nat. Chem.* **2018**, *10*, 383-394.
86. Ward, S. E.; Beswick, P., What does the aromatic ring number mean for drug design? *Exp. Opin. Drug Disc*. **2014**, *9,* 995-1003.

87. Potopnyk, M. A.; Lytvyn, R.; Danyliv, Y.; Ceborska, M.; Bezvikonnyi, O.; Volyniuk, D.; Gražulevičius, J. V. N,O π-Conjugated 4-Substituted 1,3-Thiazole BF2 Complexes: Synthesis and Photophysical Properties. *J. Org. Chem*. **2018**, *83*, 1095–1105.
88. Tazoo, D.; Oniga, O.; Bohle, D. S.; Chua, Z.; Dongo, E. General Two-Step Preparation of Chalcones Containing Thiazole. *J. Heterocycl. Chem.* **2012**, *49*, 768–773.
89. Kaufmann, D.; Bialer, M.; Shimshoni, J. A.; Devor, M.; Yagen, B. Synthesis and Evaluation of Antiallodynic and Anticonvulsant Activity of Novel Amide and Urea Derivatives of Valproic Acid Analogues. *J. Med. Chem*. **2009**, *52*, 7236–7248.
90. Bernier, J. L.; Houssin, R.; Henichart, J. P. Analog of Dolastatin 3 Synthesis, 'H NMR Studies and Spatial Conformation. *Tetrahedron* **1986**, *42*, 2695–2702.
91. Fernandez, S.; Ganiek, M. A.; Karpacheva, M.; Hanusch, F. C.; Reuter, S.; Bein, T.; Auras, F.; Knochel, P. Synthesis and Reactivity of Triazaphenanthrenes. *Org. Lett*. **2016**, *18*, 3158–3161.
92. Pryde, D. C.; Maw, G. N.; Planken, S.; Platts, M. Y.; Sanderson, V.; Corless, M.; Stobie, A.; Barber, C. G.; Russell, R.; Foster, L.; Barker, L.; Wayman, C.; Van Der Graaf, P.; Stacey, P.; Morren, D.; Kohl, C.; Beaumont, K.; Coggon, S.; Tute, M., Novel selective inhibitors of neutral endopeptidase for the treatment of female sexual arousal disorder. Synthesis and activity of functionalized glutaramides. *J. Med. Chem.* **2006**, *49*, 4409-4424.
93. Li, X.; Wang, X.; Wang, Z.; Yan, X.; Xu, X., TBHP-induced iodocyclization with I2: atom economic synthesis of iodinated isoxazolines in water under mild conditions. *ACS Sus. Chem. Eng.* **2019**, *7*, 1875-1878.
94. Xu, Z.; Dicesare, J. C.; Baures, P. W. Parallel Synthesis of An Oligomeric Imidazole-4,5-Dicarboxamide Library. *J. Comb. Chem*. **2010**, *12*, 248–254.
95. Leiris, S.; Davies, D. T.; Sprynski, N.; Castandet, J.; Beyria, L.; Bodnarchuk, M. S.; Sutton, J. M.; Mullins, T. M.; Jones, M. W.; Forrest, A. K., Virtual screening approach to identifying a novel and tractable series of Pseudomonas aeruginosa elastase inhibitors. *ACS Med. Chem. Let.,* **2021**, *12*, 217-227.

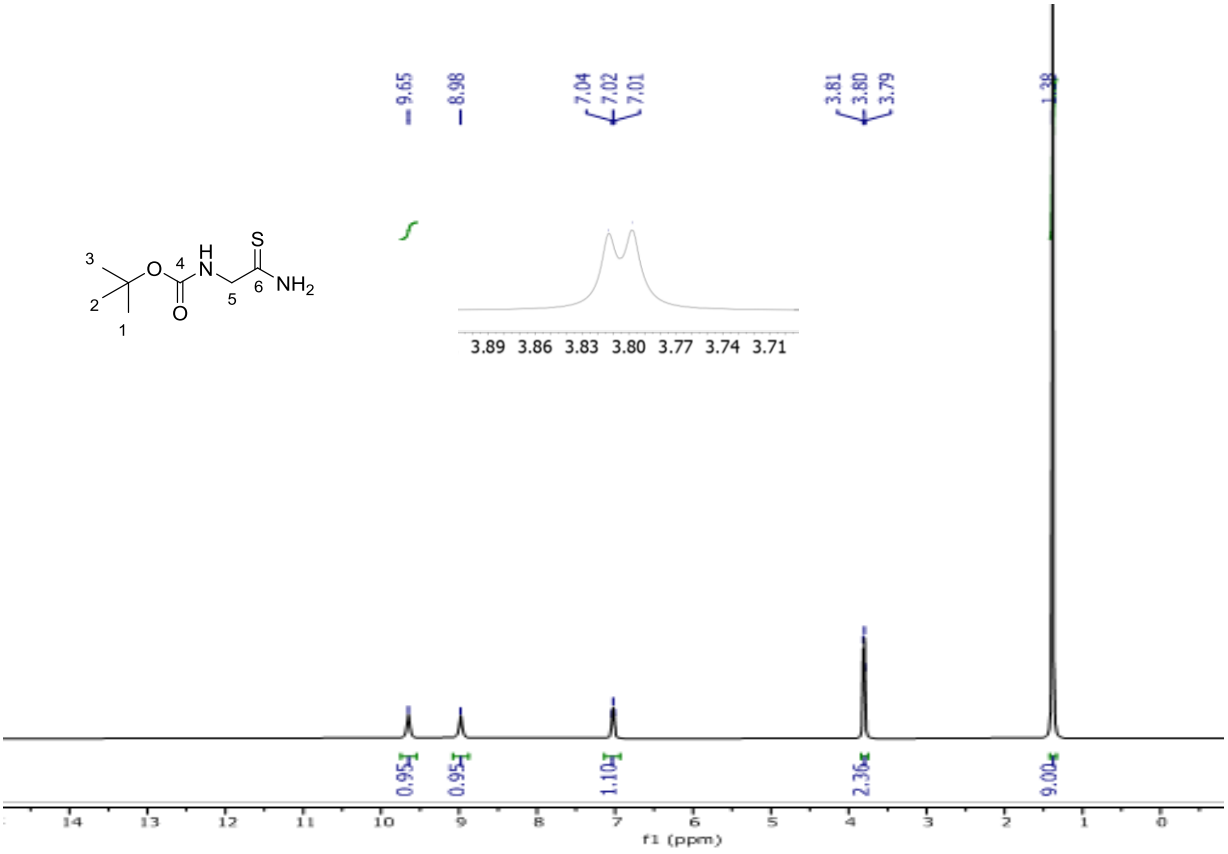
96. Volchkov, I.; Reeves, J.; Dang, M. T. Q.; Hampel, T.; Koch Sandra; Nordstrom, F.; Reichel, C.; Schoerer, M.; Stance, C.; Zhong, L.; Zimmermann, U. J. Transition Metal-Catalyzed Protodecarbonylation of a-Halo-Acrylic Acid Derivatives. WO2018158140A1, **2018.**
97. Cantet, A.-C.; Carreyre, H.; Gesson, J.-P.; Jouannetaud, M.-P.; Renoux, B. Gem-Difluorination of Aminoalkynes via Highly Reactive Dicationic Species in Superacid HF-SbF 5 Application to the Efficient Synthesis of Difluorinated Cinchona Alkaloid Derivatives. *J. Org. Chem*. **2008**, *73*, 2875–2878.
98. Perumal, S. K.; Pratt, R. F. Synthesis and Evaluation of Ketophosph(on)Ates as β-Lactamase Inhibitors. *J. Org. Chem*. **2006**, *71*, 4778–4785.
99. Chen, H.-J.; Chiu, C.-C.; Wang, T.; Lee, D.-S.; Lu, T.-J., Bis-NHC–Ag/Pd (OAc) 2 Catalytic System Catalyzed Transfer Hydrogenation Reaction. *Catalysts* **2020**, *11*, 8.
100. Fan, D.; Wang, B.; Stelitano, G.; Savková, K.; Shi, R.; Huszár, S.; Han, Q.; Mikusova, K.; Chiarelli, L. R.; Lu, Y., Structural and Activity Relationships of 6-Sulfonyl-8-Nitrobenzothiazinones as Antitubercular Agents. *J. Med. Chem.***, 2021**, *64*, 14526-14539.
101. Nam, G.; Jung, J. M.; Park, H.-J.; Baek, S. Y.; Baek, K. S.; yeon Mok, H.; Jung, Y. H., Structure-activity relationship study of thiazolyl-hydroxamate derivatives as selective histone deacetylase 6 inhibitors. *Bioorg. Med. Chem.,* **2019**, *27*, 3408-3420.

Appendix

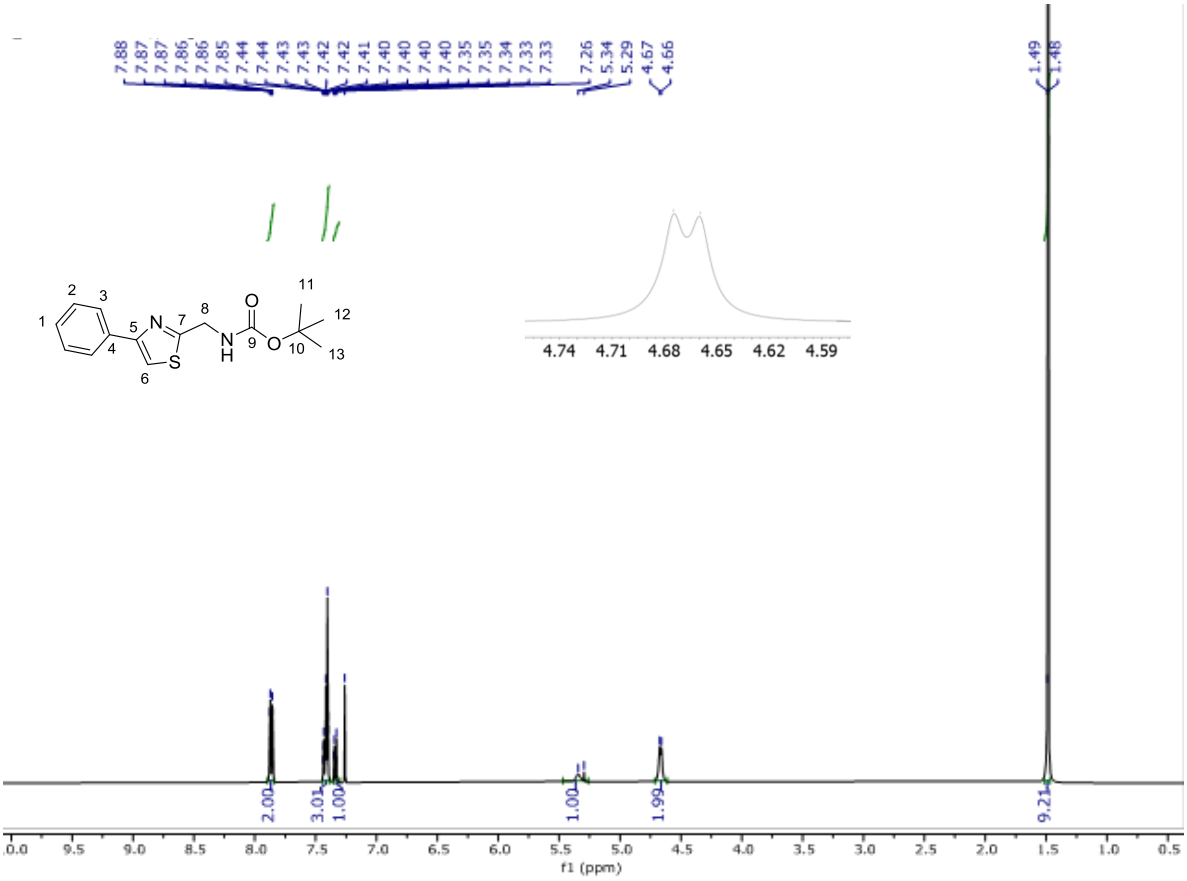
Compound 1.2


7.19
6.94
6.83
3.47
3.45
3.32
2.50
1.38
3.54 3.51 3.48 3.45 3.42 3.39
0.86
0.91
0.85
1.93
9.00
f1 (ppm)

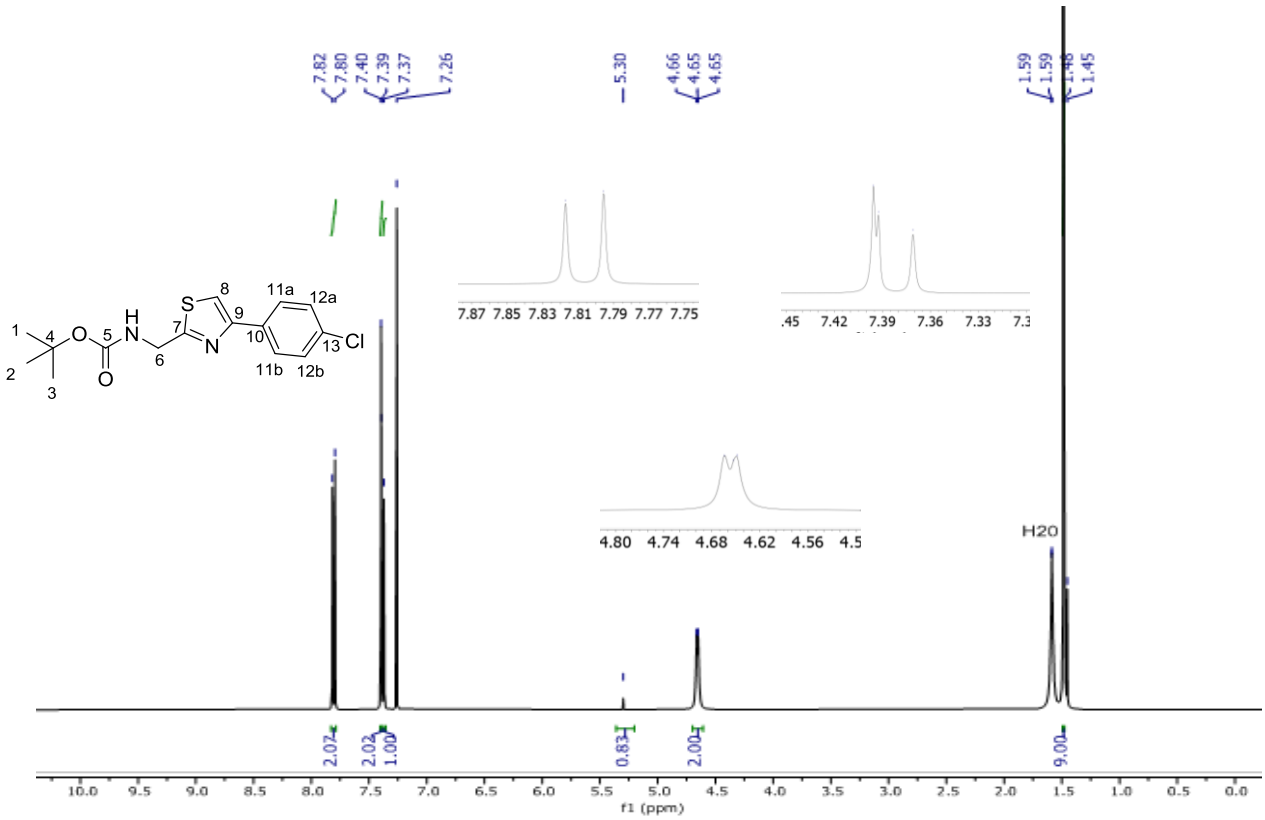
Compound 1.3

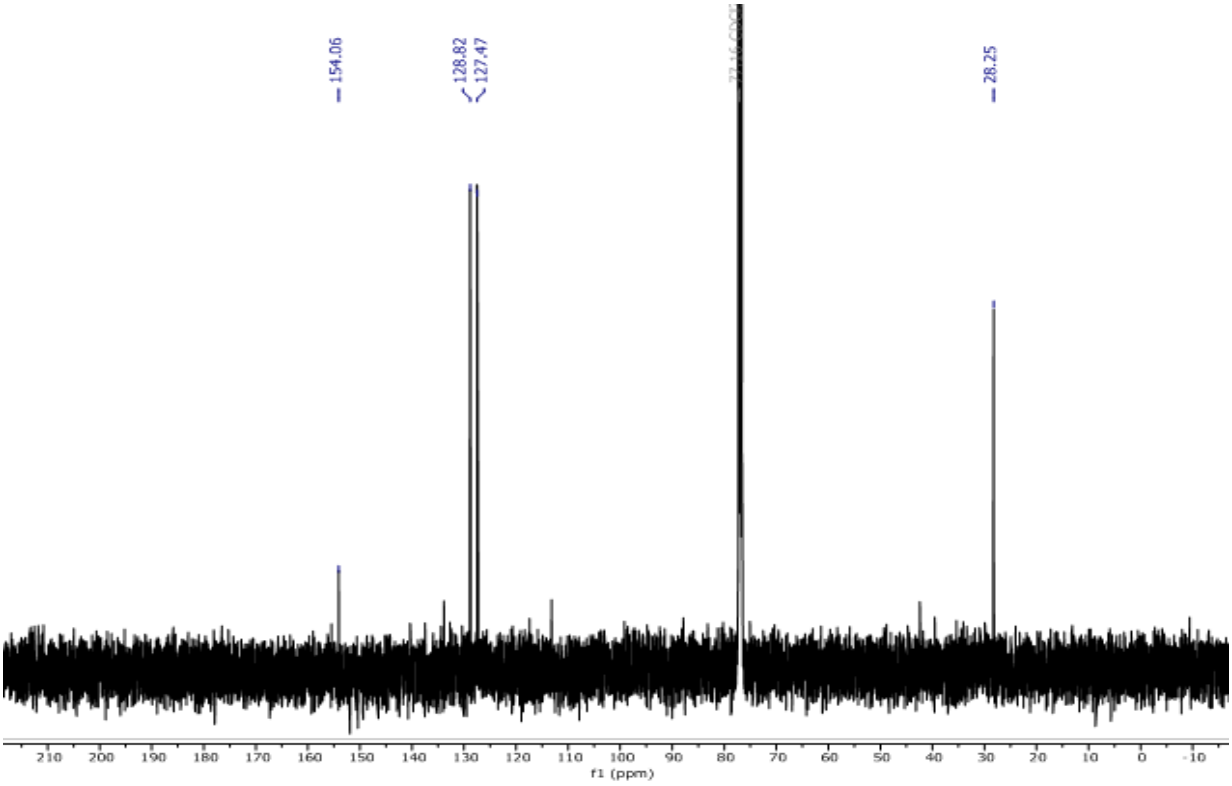

9.65
8.98
7.04
7.02
7.01
3.81
3.80
3.79
1.38
3.89 3.86 3.83 3.80 3.77 3.74 3.71
0.95
0.95
1.10
2.36
9.00
f1 (ppm)

Compound 1.5a

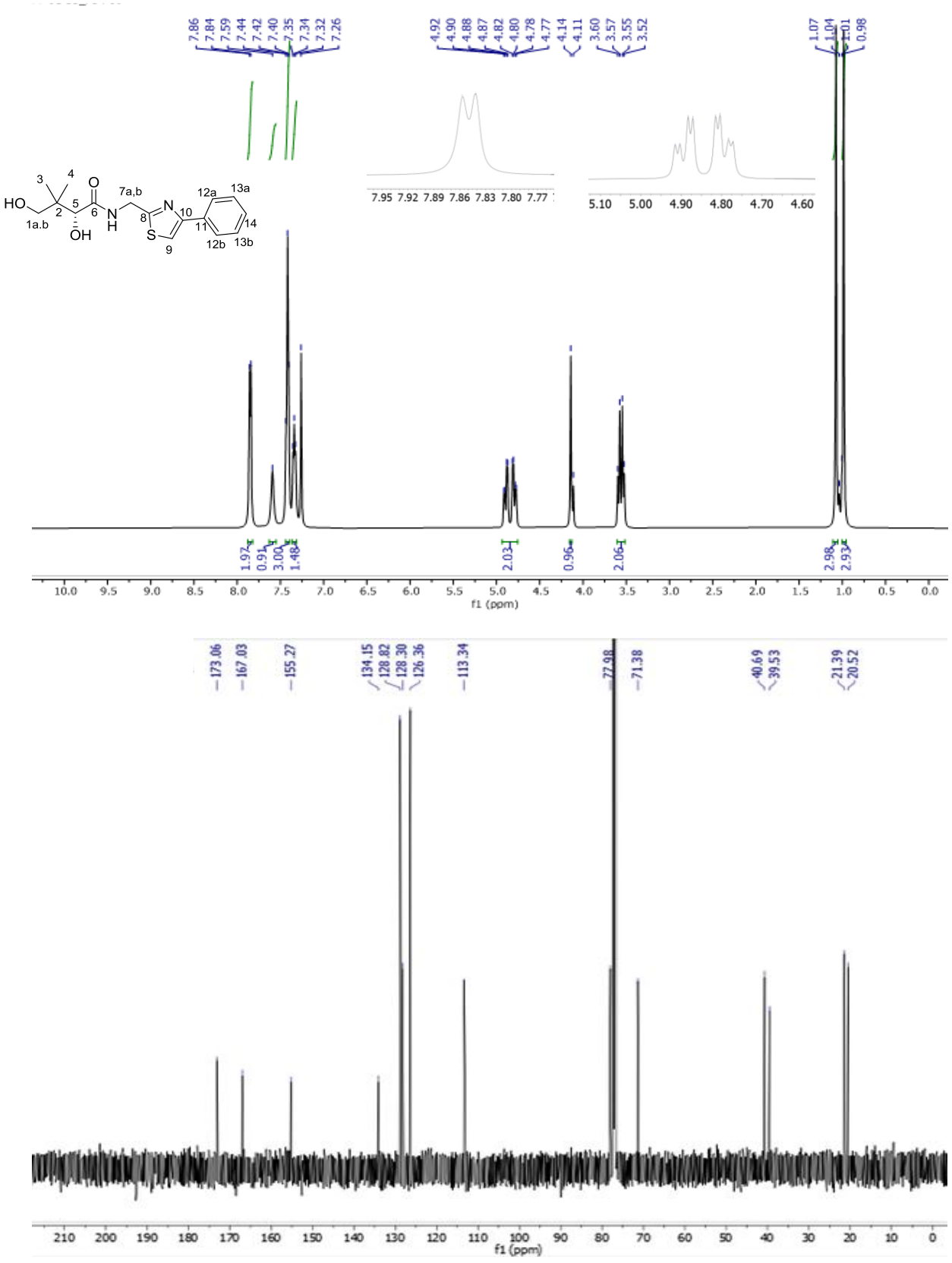


Compound 1.5b

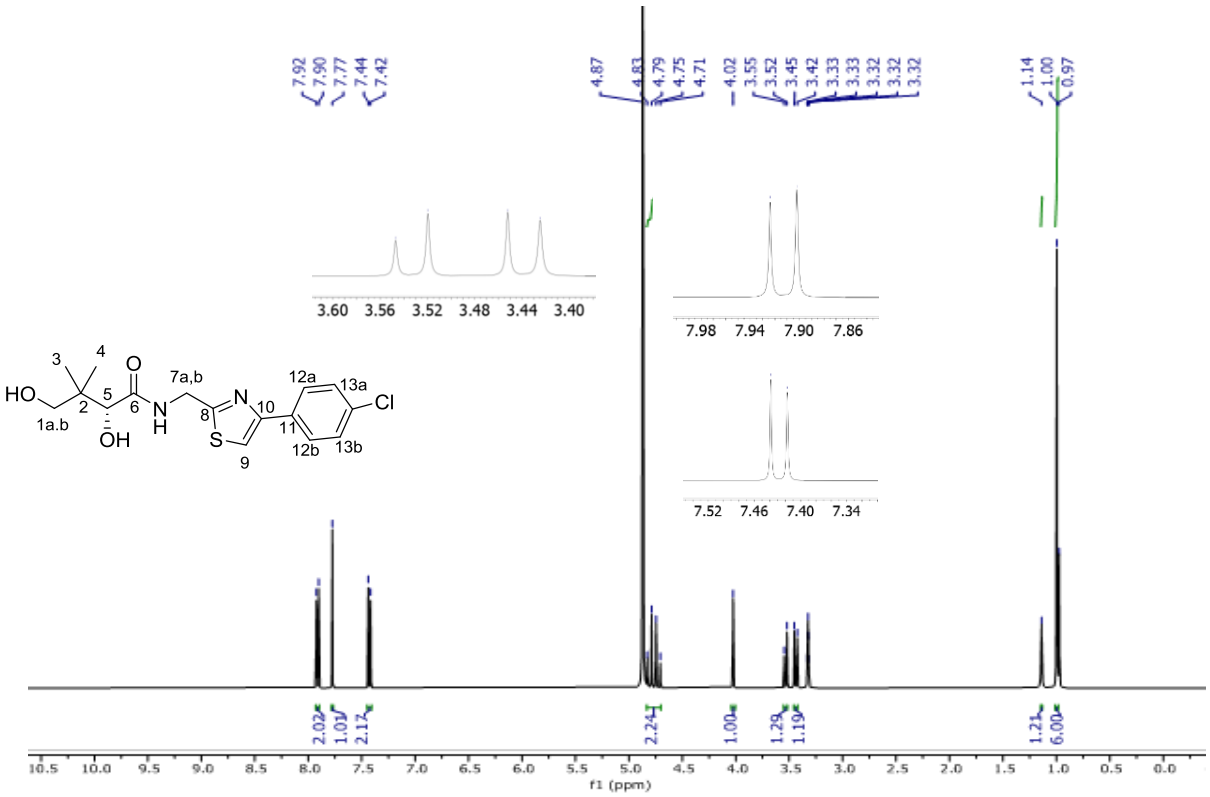


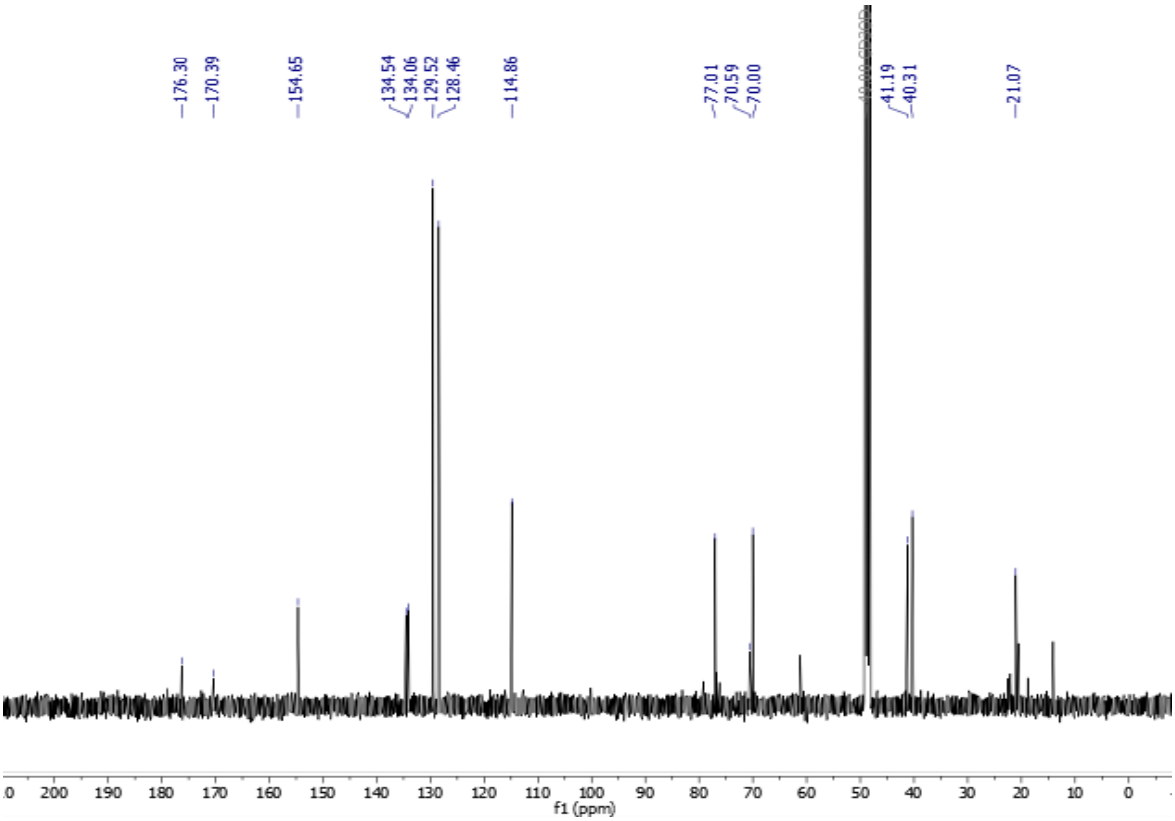


Compound 1a

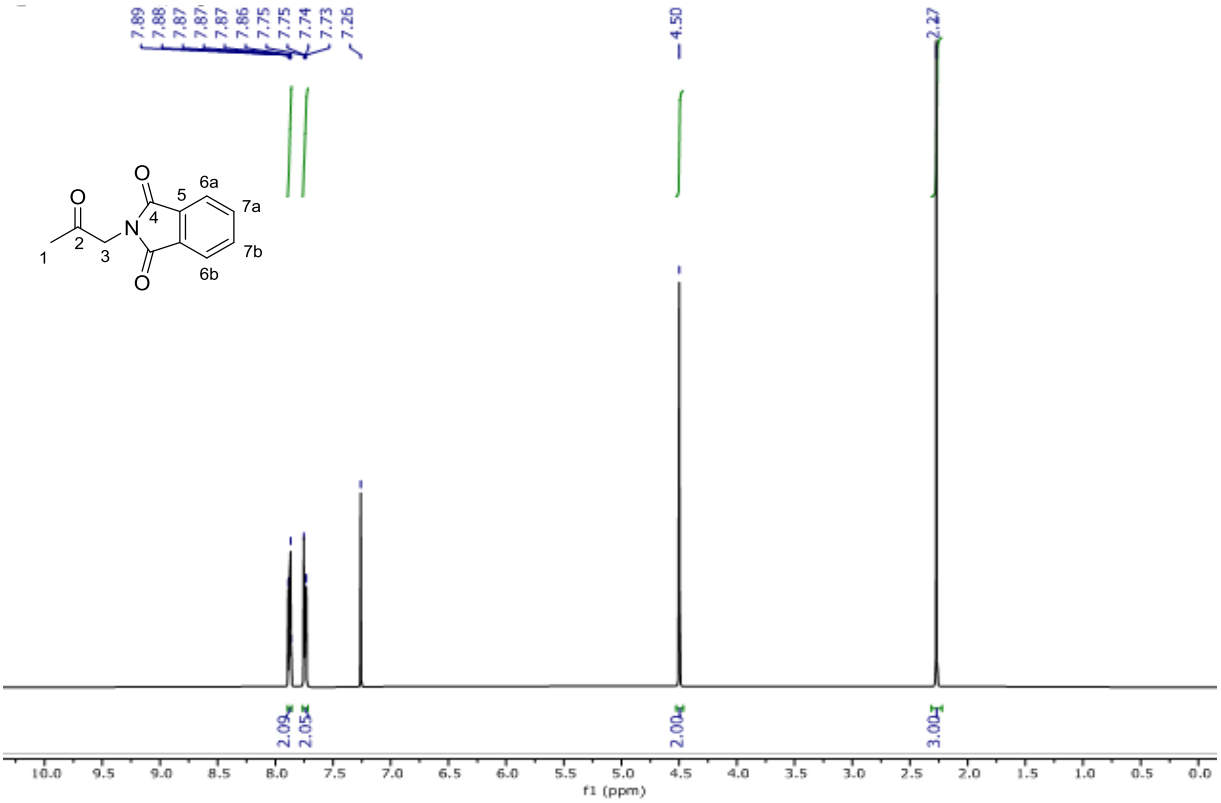

HO
OH
N
H
O
S
7.86 7.84 7.59 7.44 7.42 7.40 7.35 7.34 7.32 7.26
4.92 4.90 4.88 4.87 4.82 4.80 4.78 4.77 4.14 4.11 3.60 3.57 3.55 3.52
1.07 1.04 1.01 0.98
1.92 0.91 3.00 1.48 2.03 0.96 2.06 2.98 2.93
f1 (ppm)
173.06 167.03 155.27 134.15 128.82 128.30 126.36 113.34 77.98 71.38 40.69 39.53 21.39 20.52
f1 (ppm)

Compound 1b

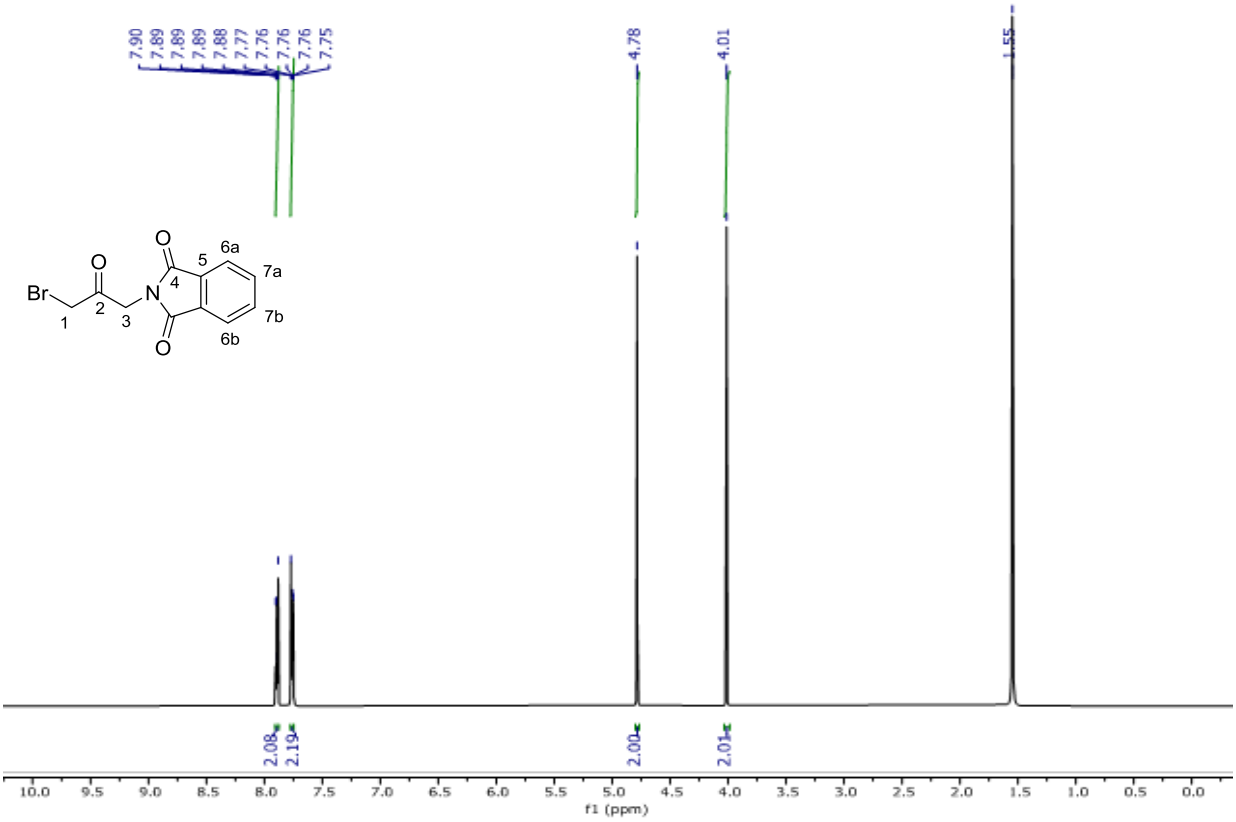

7.92
7.90
7.77
7.44
7.42
4.87
4.83
4.79
4.75
4.71
4.02
3.55
3.52
3.45
3.42
3.33
3.33
3.32
3.32
3.32
1.14
1.00
0.97
3.60 3.56 3.52 3.48 3.44 3.40
7.98 7.94 7.90 7.86
7.52 7.46 7.40 7.34
HO
1a.b
OH
2
3
4
5
6
O
N
H
7a,b
8
S
9
N
10
11
12a
12b
13a
13b
Cl
2.02
1.01
2.13
2.24
1.00
1.29
1.19
1.21
6.00
f1 (ppm)


176.30
170.39
154.65
134.54
134.06
129.52
128.46
114.86
77.01
70.59
70.00
41.19
40.31
21.07
f1 (ppm)

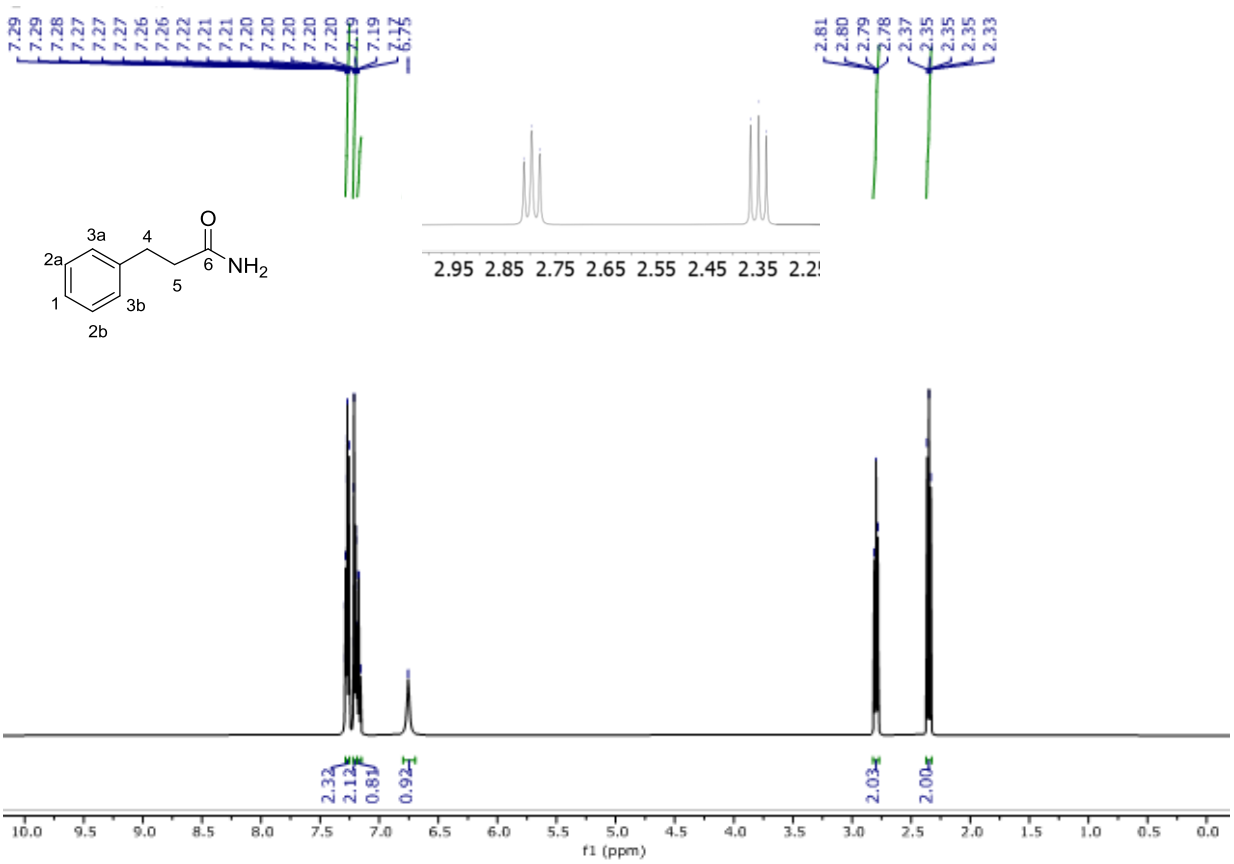
Compound 1.6


7.89
7.88
7.87
7.87
7.87
7.86
7.75
7.75
7.74
7.73
7.26
4.50
2.27
O
O
N
O
1
2
3
4
5
6a
7a
7b
6b
2.09
2.05
2.00
3.00
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
f1 (ppm)

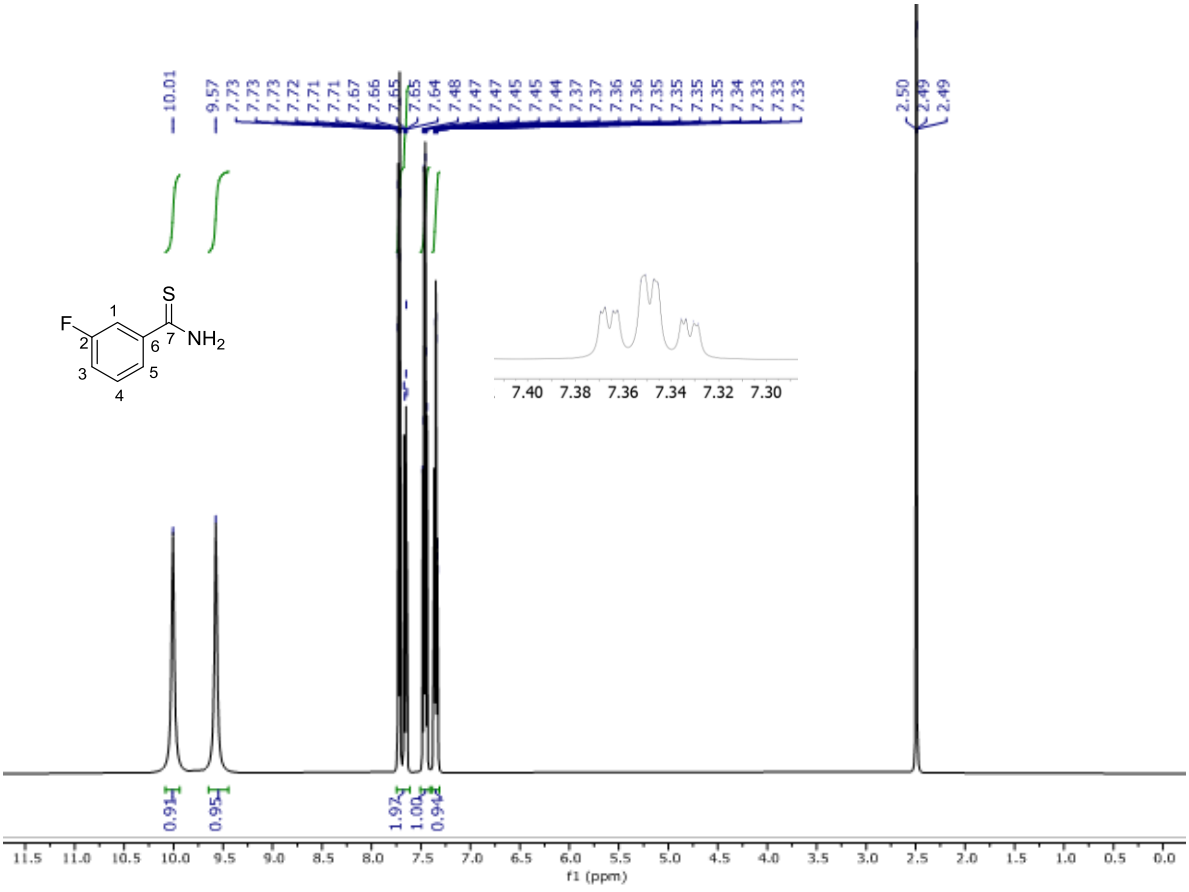
Compound 1.7


7.90
7.89
7.89
7.89
7.88
7.77
7.76
7.76
7.76
7.75
4.78
4.01
1.55
Br
O
O
N
1
2
3
4
5
6a
7a
7b
6b
2.08
2.19
2.00
2.01
f1 (ppm)

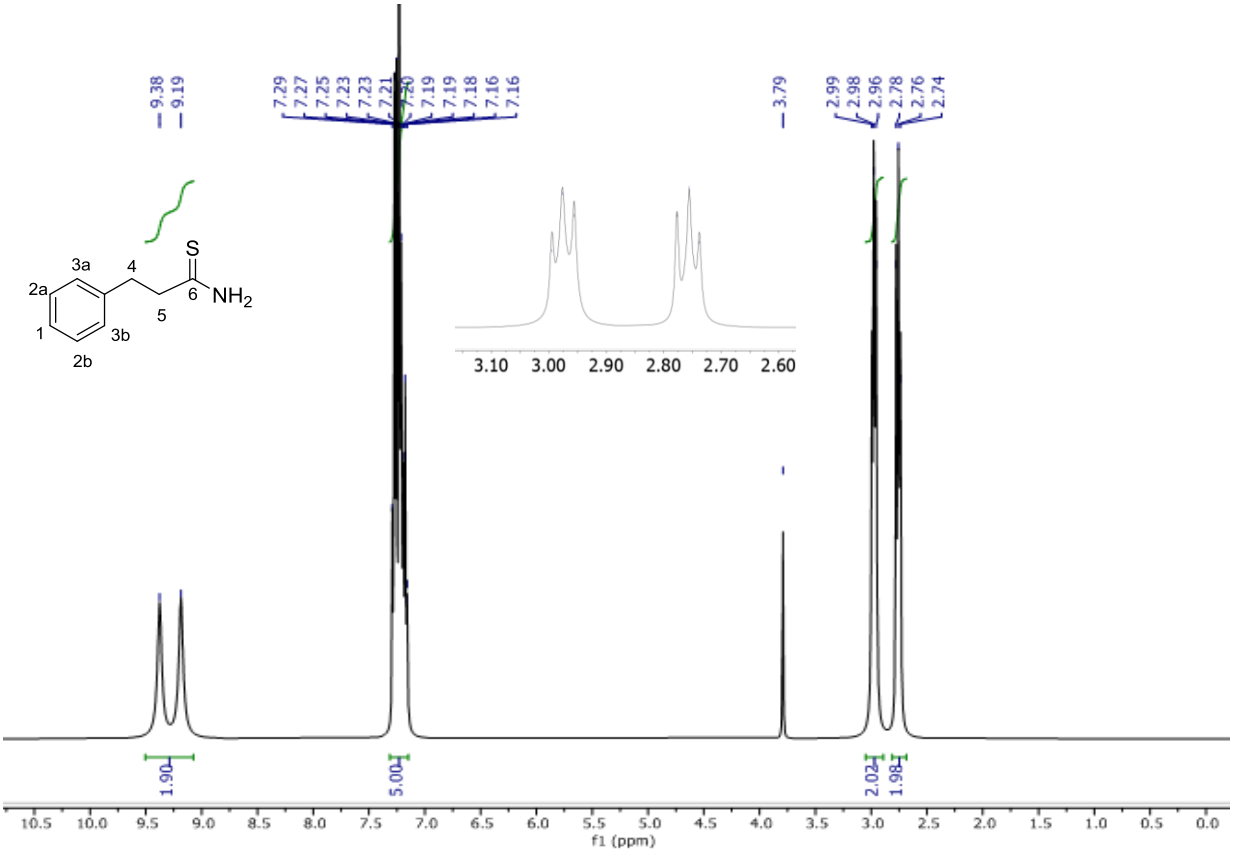
Compound 2.4b



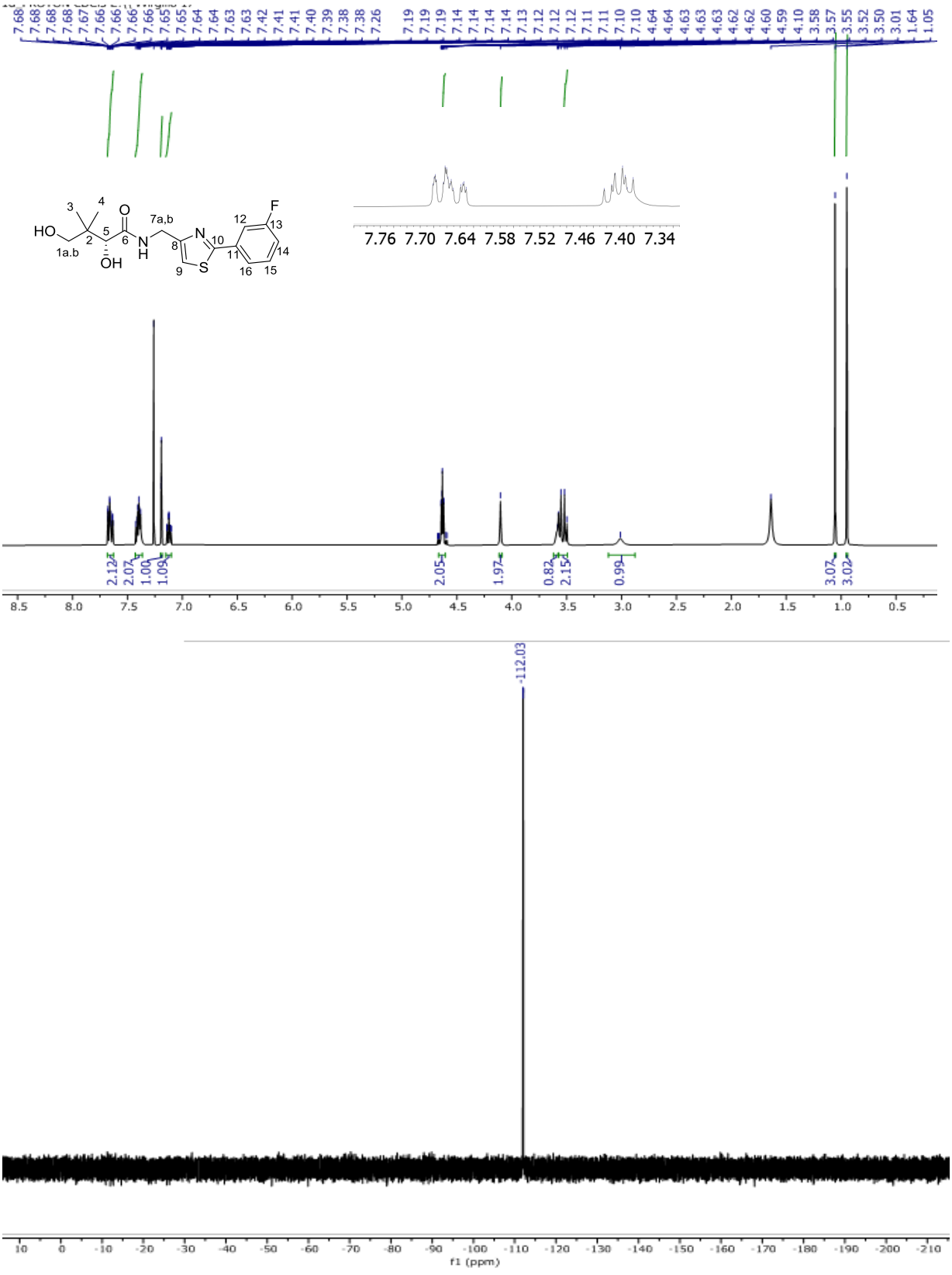
Compound 2.5a



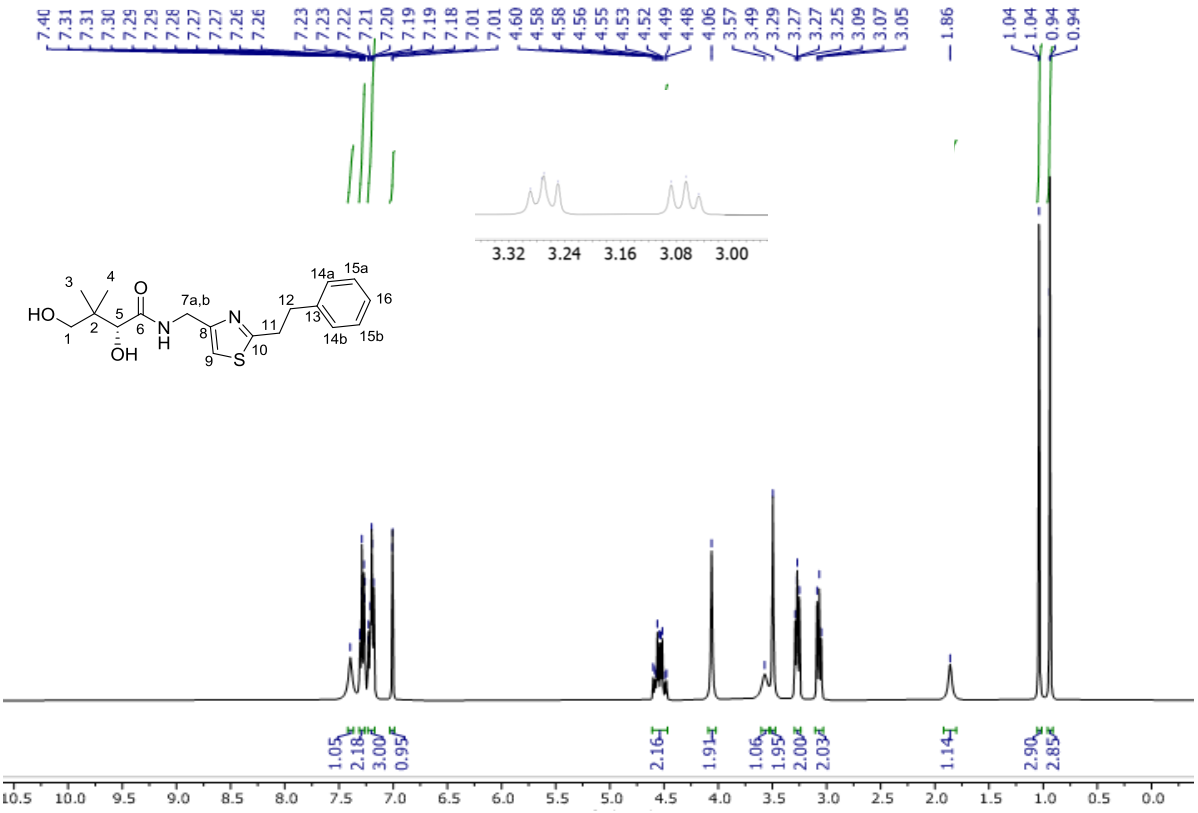
Compound 2.5b

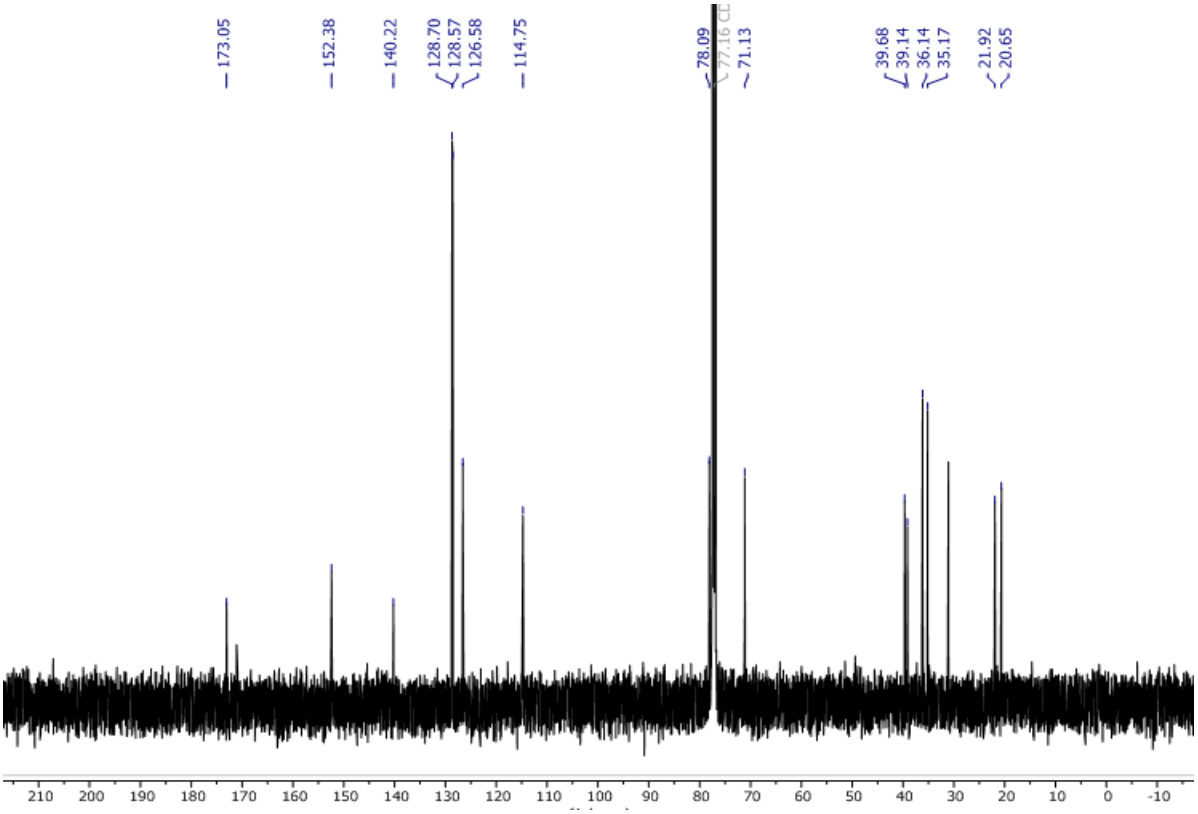


Compound 2a

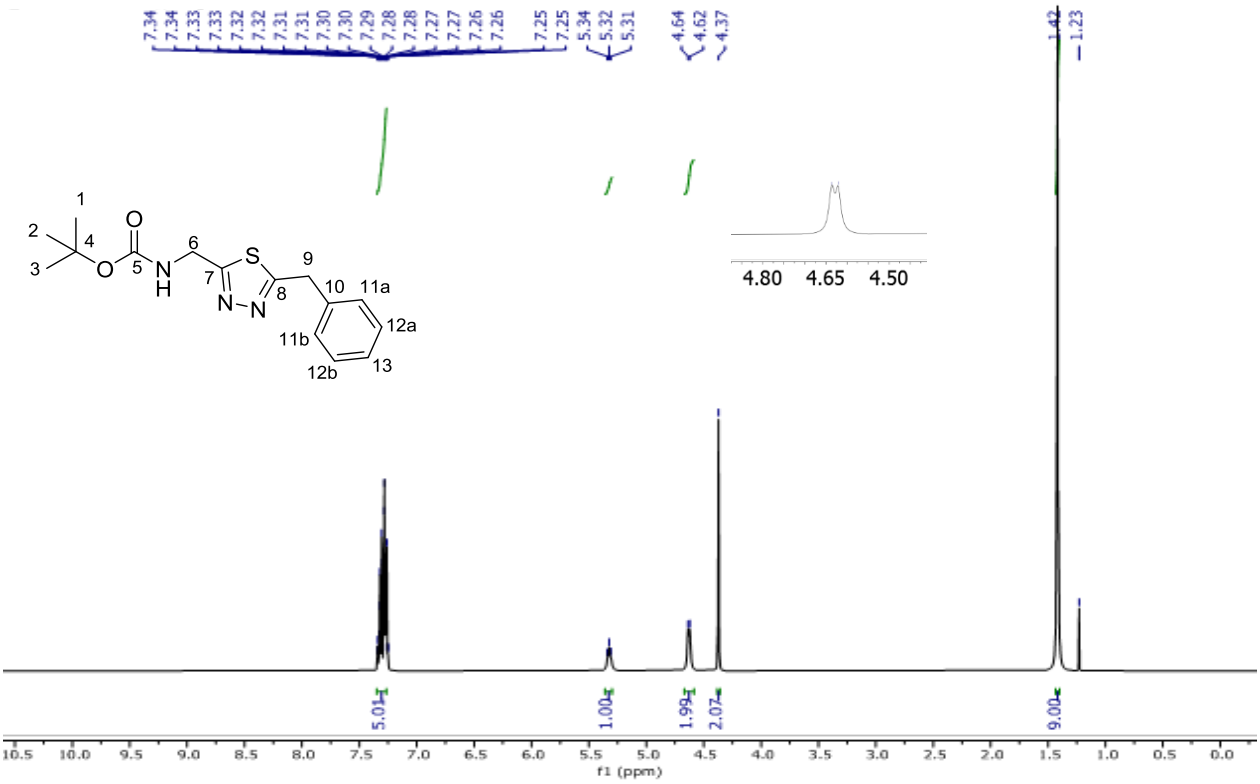


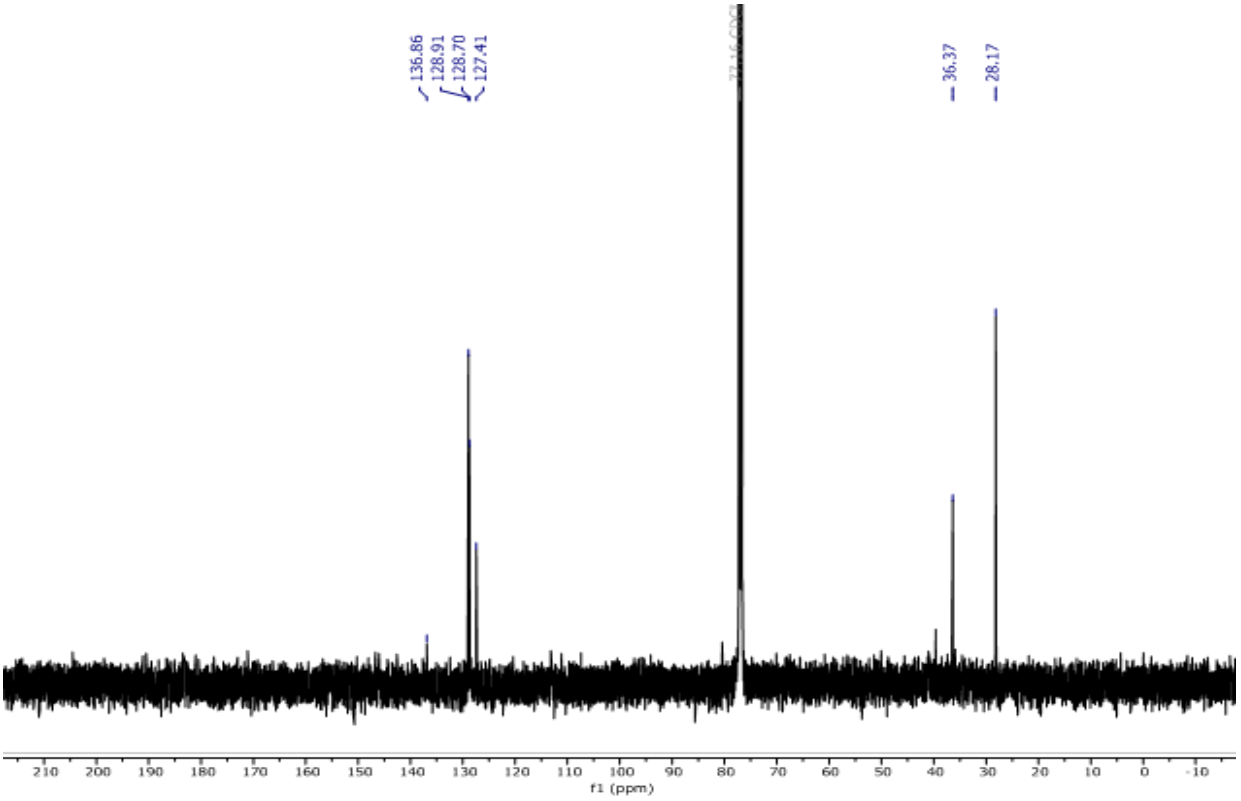
Compound 2b


7.40 7.31 7.31 7.30 7.29 7.29 7.28 7.27 7.27 7.26 7.26 7.23 7.23 7.22 7.21 7.20 7.19 7.19 7.18 7.01 7.01 4.60 4.58 4.58 4.56 4.55 4.53 4.52 4.49 4.48 4.06 3.57 3.49 3.29 3.27 3.27 3.25 3.09 3.07 3.05 1.86 1.04 1.04 0.94 0.94
3.32 3.24 3.16 3.08 3.00
HO 1 2 3 4 5 6 O N H OH 7a,b 8 9 S 10 N 11 12 13 14a 14b 15a 15b 16
1.05 2.18 3.00 0.95 2.16 1.91 1.06 1.95 2.00 2.03 1.14 2.90 2.85
10.5 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

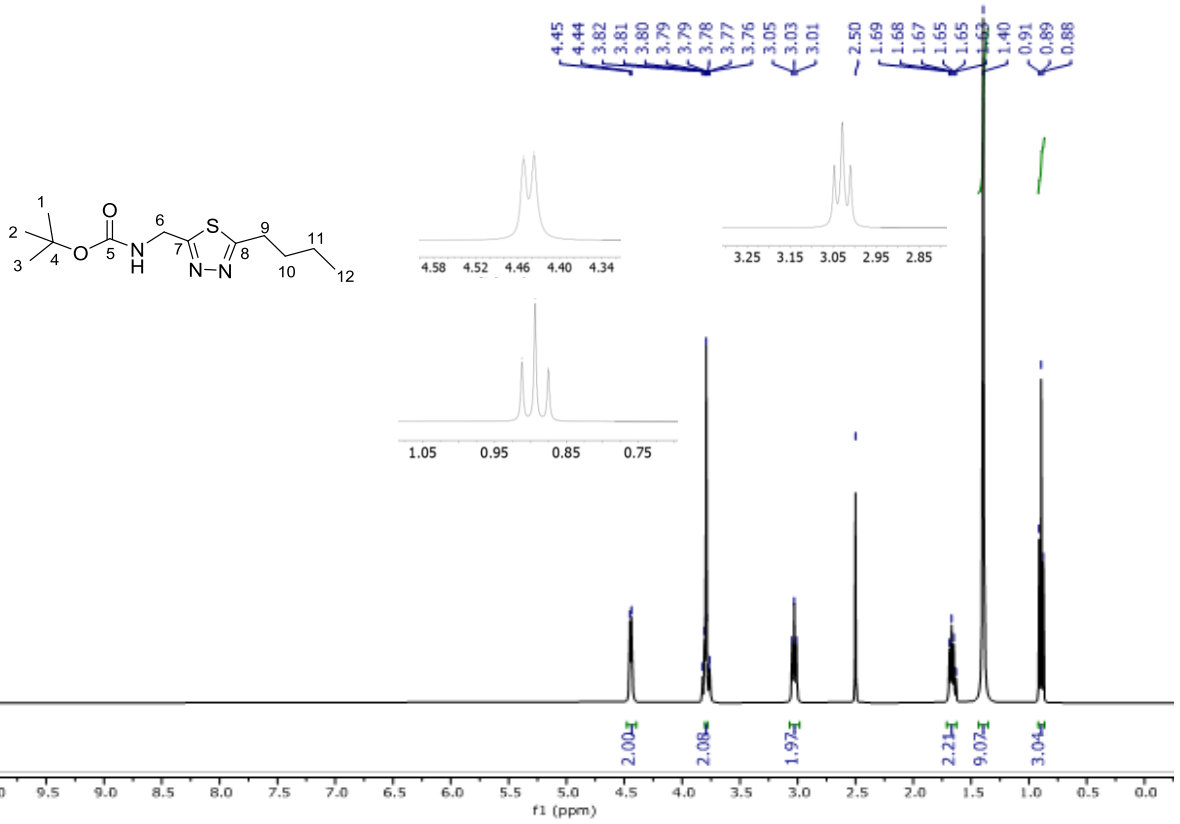

173.05 152.38 140.22 128.70 128.57 126.58 114.75 78.09 77.16 CDCl3 71.13 39.68 39.14 36.14 35.17 21.92 20.65
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

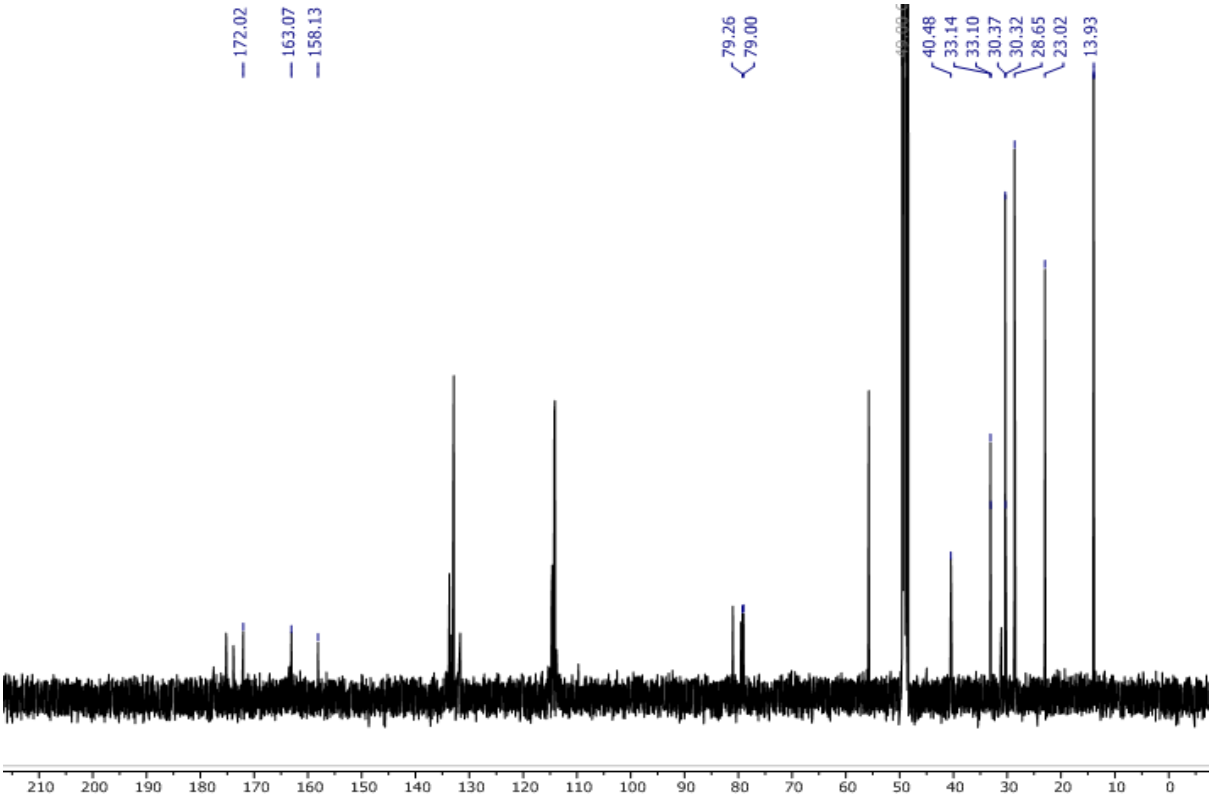
Compound 3.3a



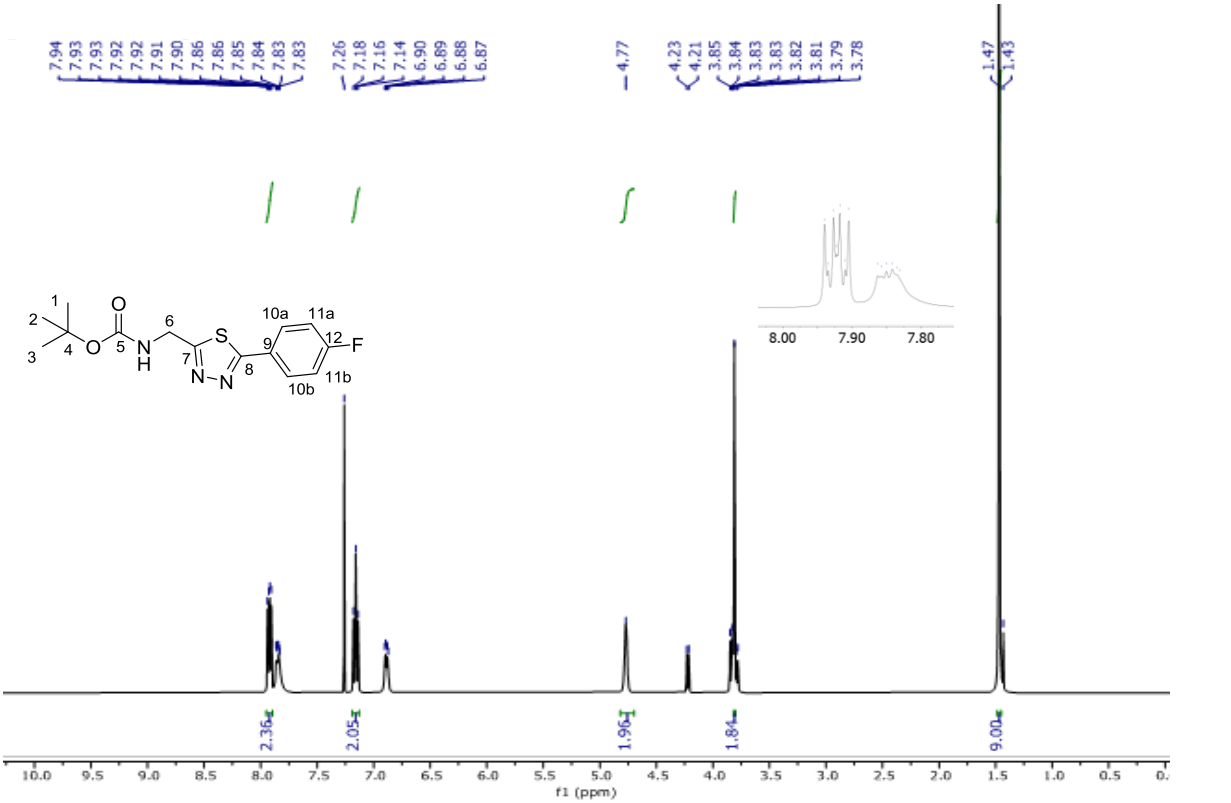


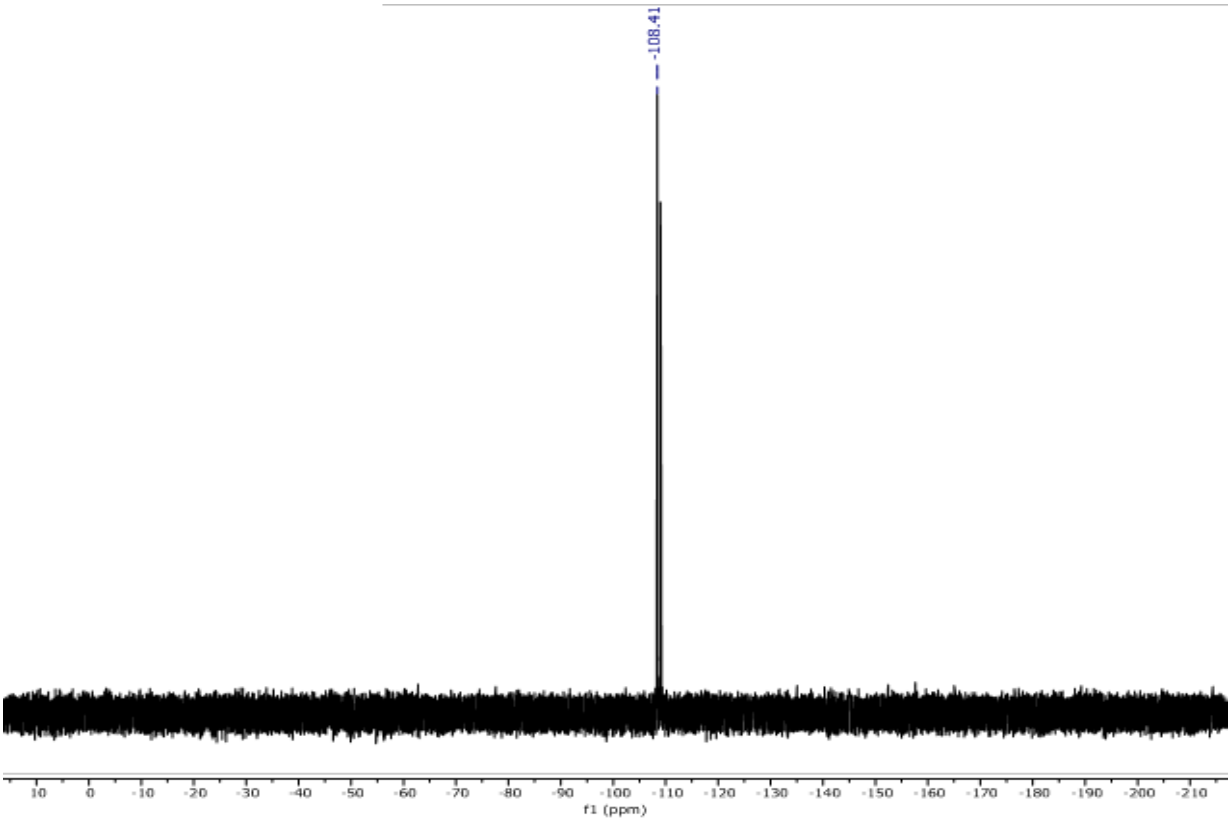
Compound 3.3b



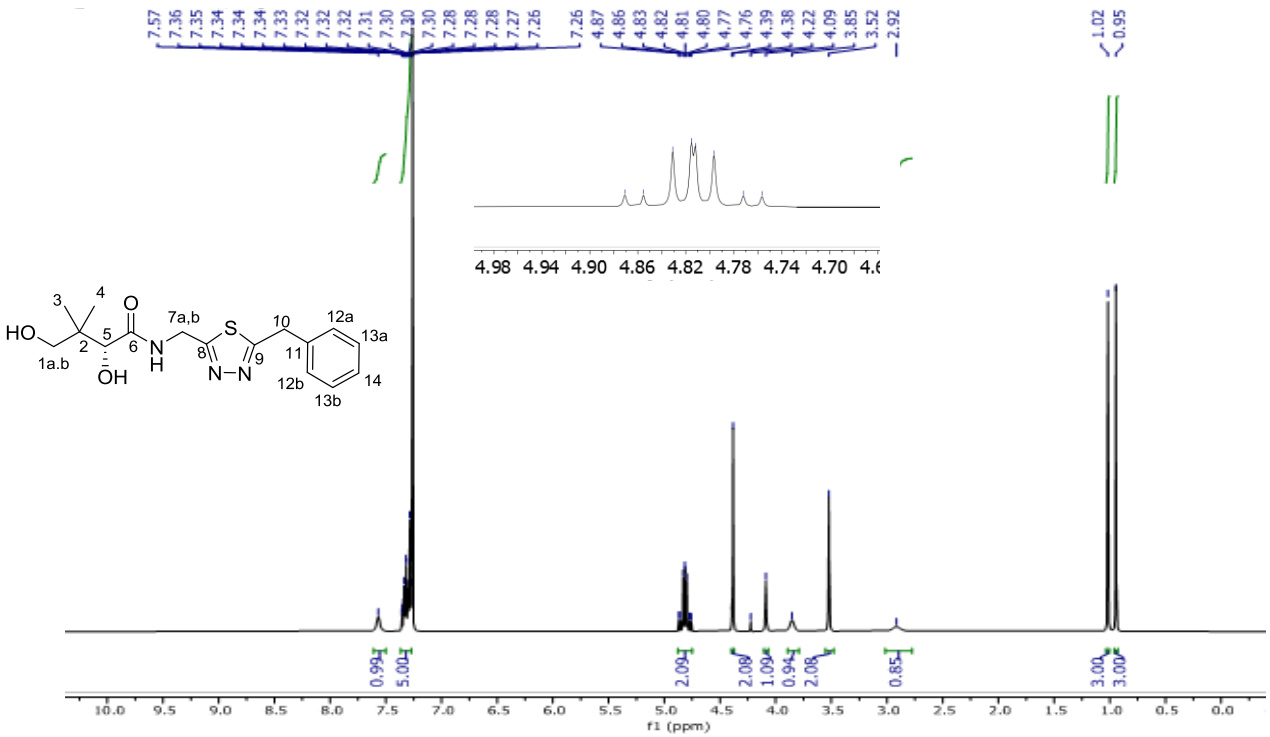


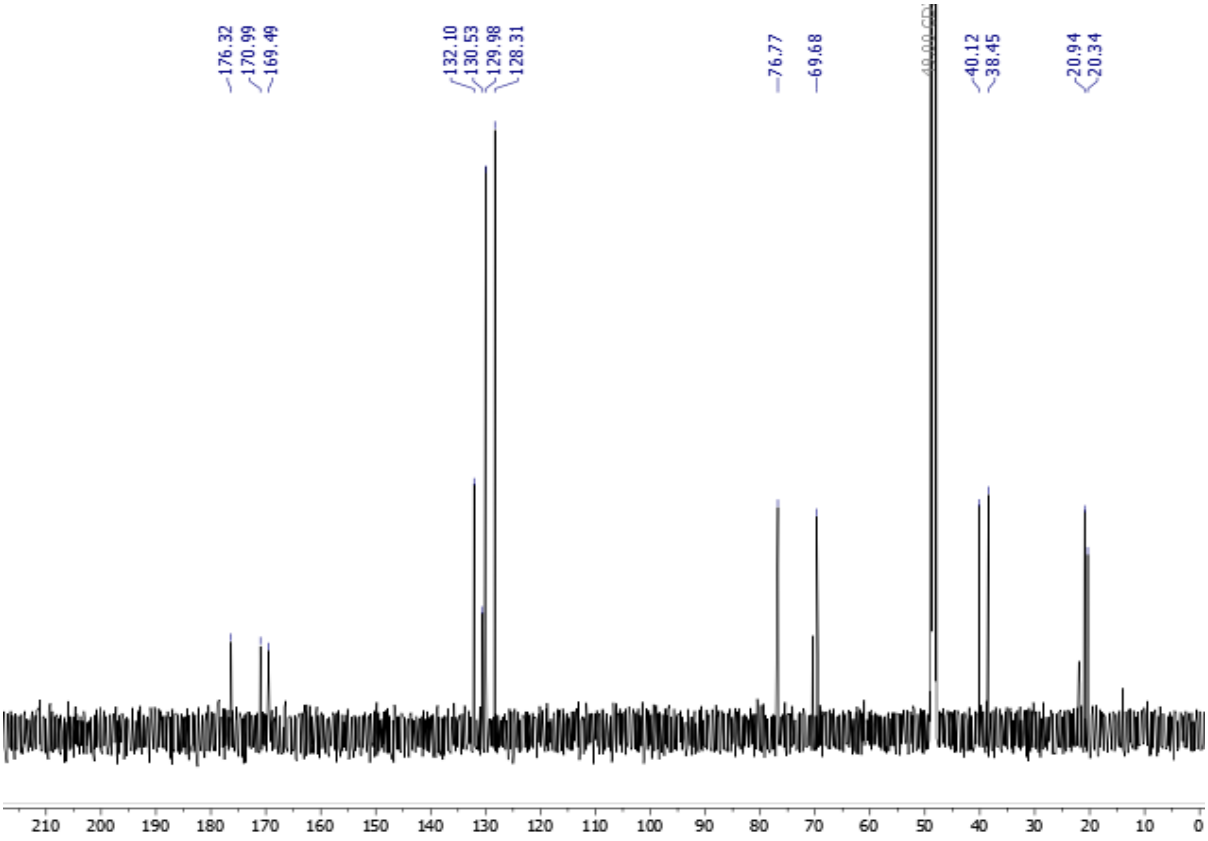
Compound 3.3c



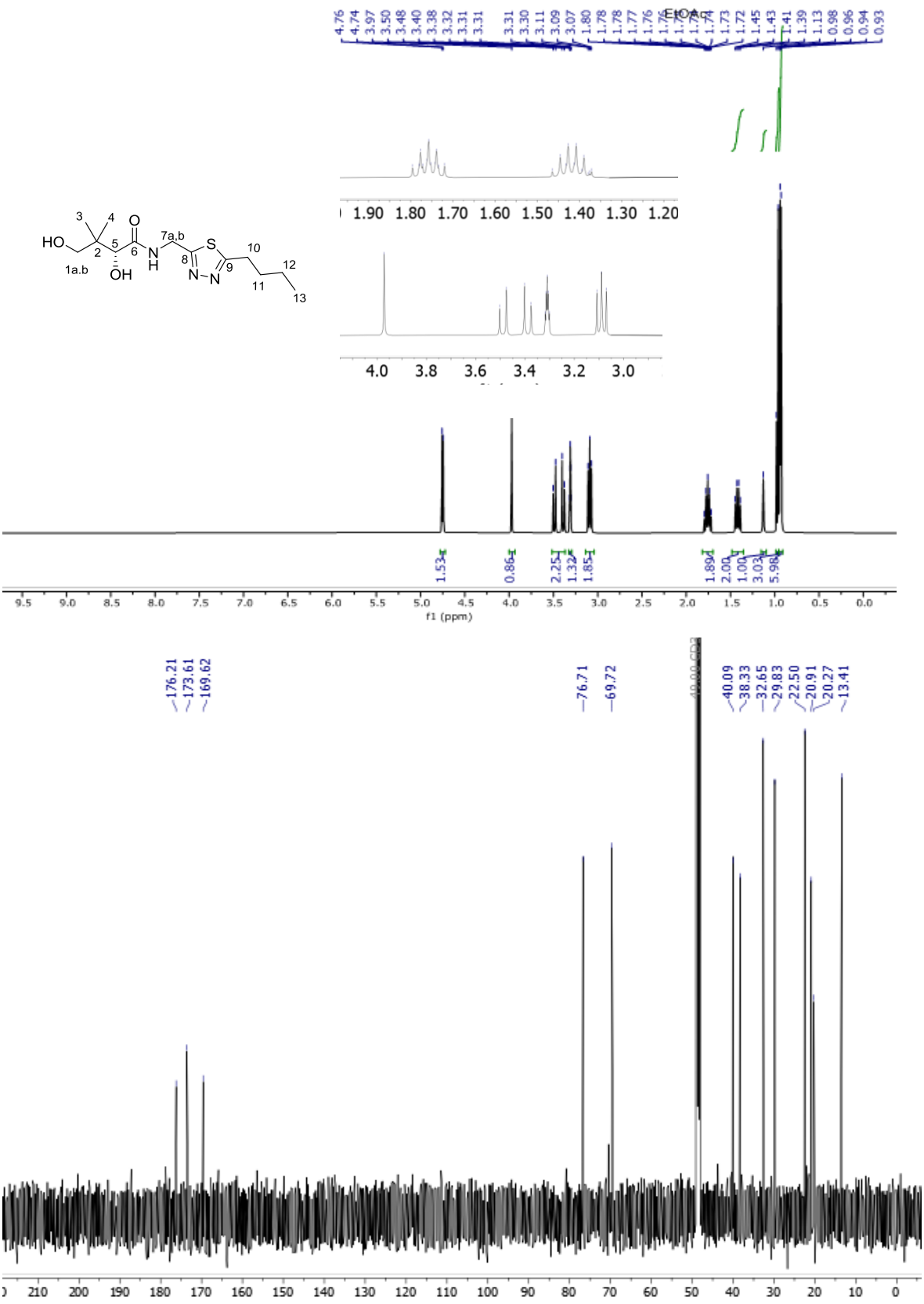


Compound 3a

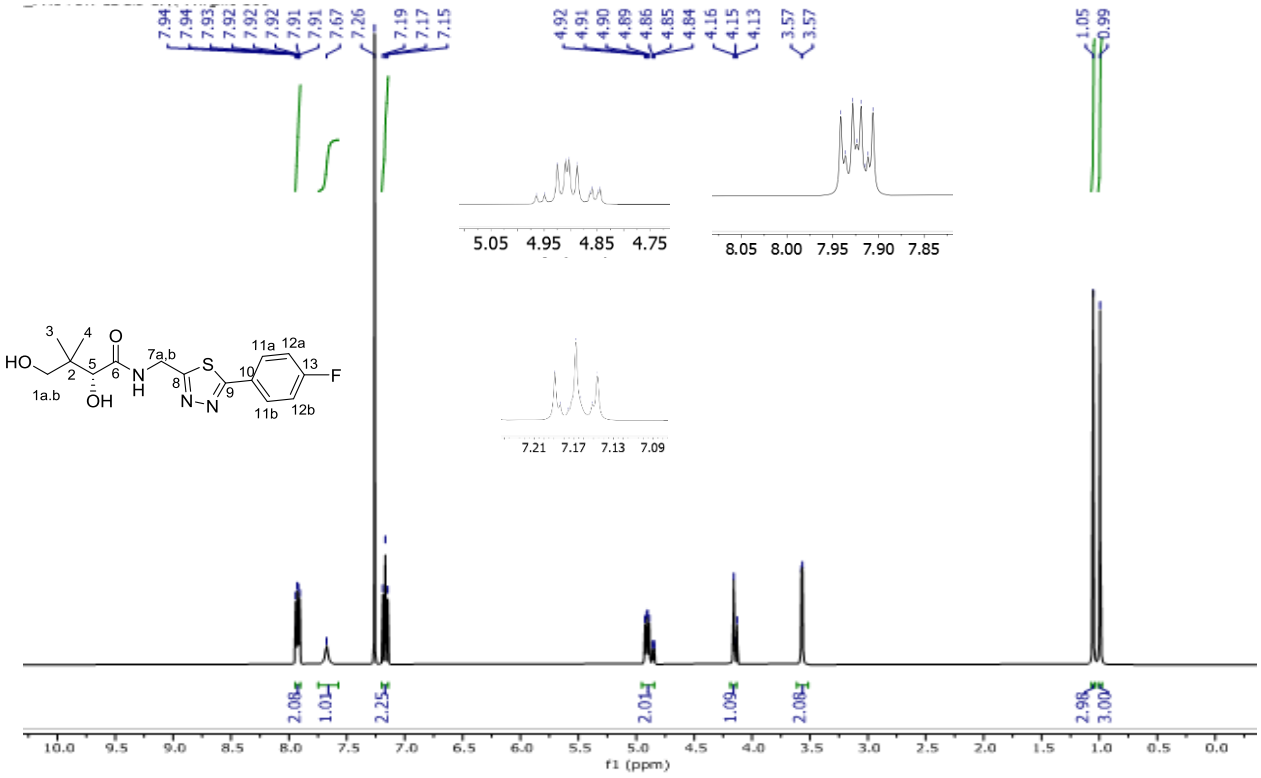

7.57 7.36 7.35 7.34 7.34 7.34 7.33 7.32 7.32 7.31 7.30 7.30 7.30 7.28 7.28 7.28 7.27 7.26 7.26 4.87 4.86 4.83 4.82 4.81 4.80 4.77 4.76 4.39 4.38 4.22 4.09 3.85 3.52 2.92
1.02 0.95
4.98 4.94 4.90 4.86 4.82 4.78 4.74 4.70
HO 1a.b OH 2 3 4 5 6 O N H 7a,b S N N 8 9 10 11 12a 12b 13a 13b 14
0.99 5.00 2.09 2.09 1.09 0.94 2.08 0.85 3.00 3.00
f1 (ppm)

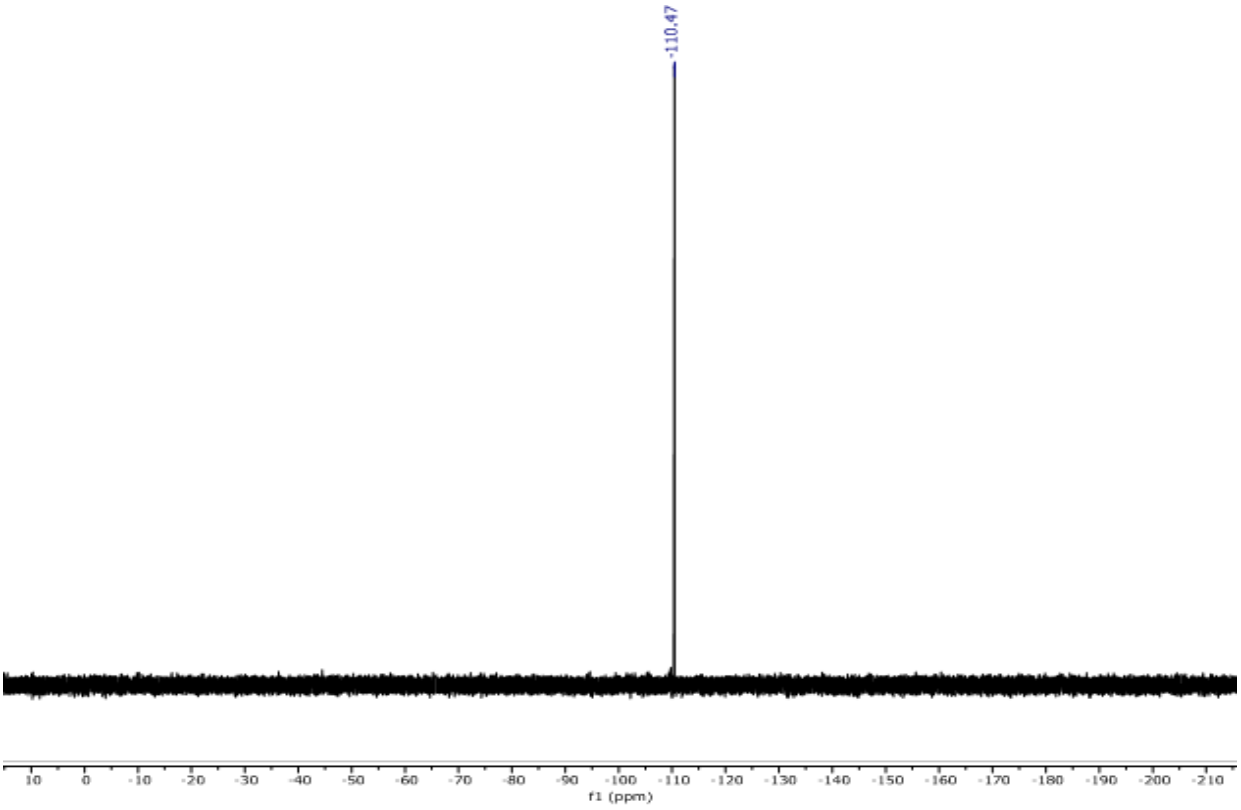

176.32 170.99 169.49 132.10 130.53 129.98 128.31 76.77 69.68 40.12 38.45 20.94 20.34

Compound 3b

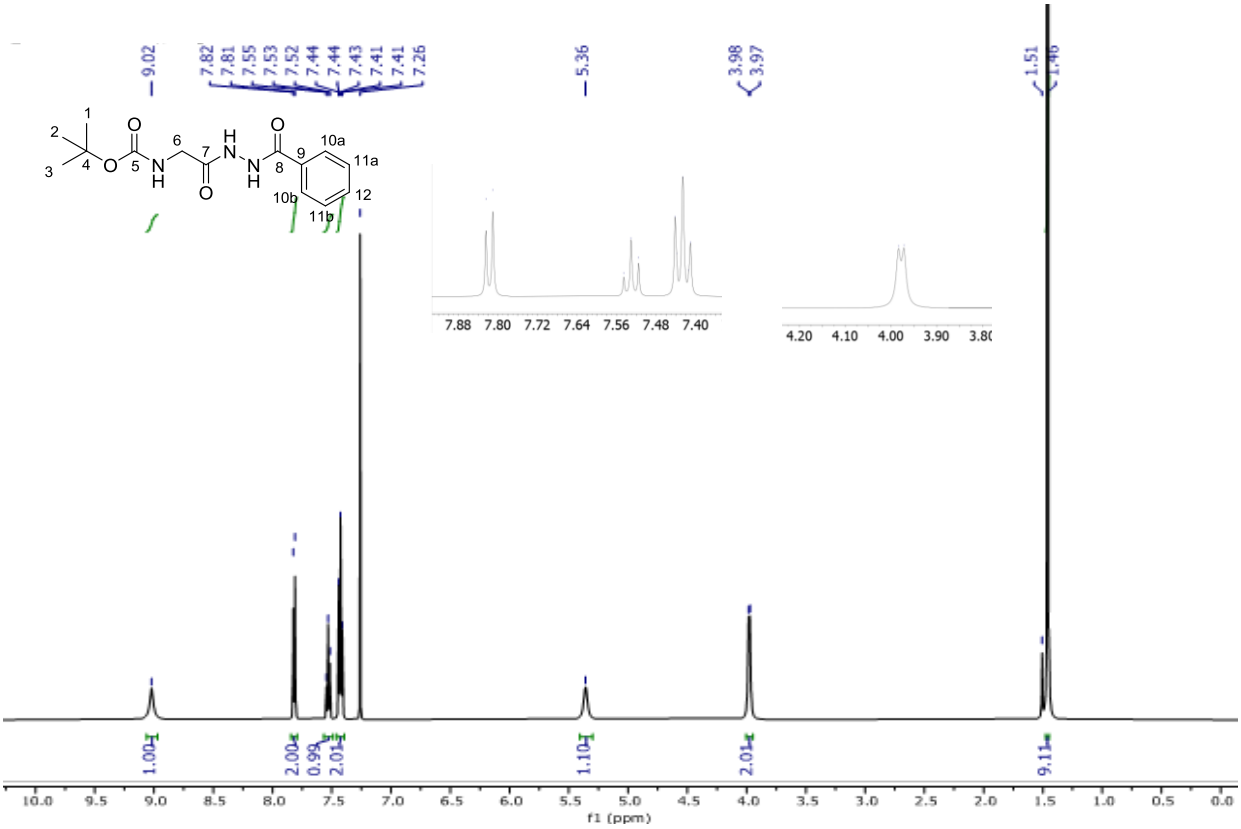


Compound 3c

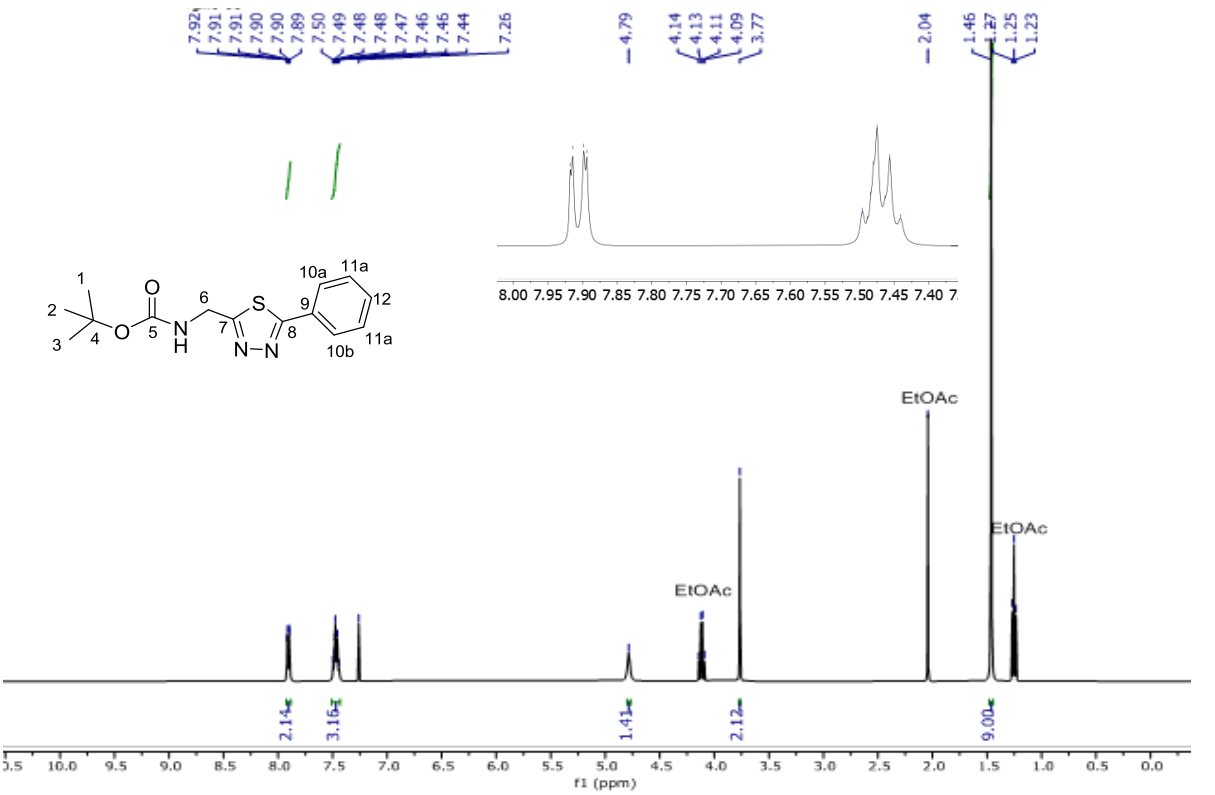

7.94
7.94
7.93
7.92
7.92
7.92
7.91
7.91
7.67
7.26
7.19
7.17
7.15
4.92
4.91
4.90
4.89
4.86
4.85
4.84
4.16
4.15
4.13
3.57
3.57
1.05
0.99
5.05 4.95 4.85 4.75
8.05 8.00 7.95 7.90 7.85
7.21 7.17 7.13 7.09
HO
1a,b
OH
2
3
4
5
6
O
N
H
7a,b
8
S
9
N
N
10
11a
12a
11b
12b
13
F
2.08
1.01
2.25
2.01
1.09
2.08
2.98
3.00
f1 (ppm)

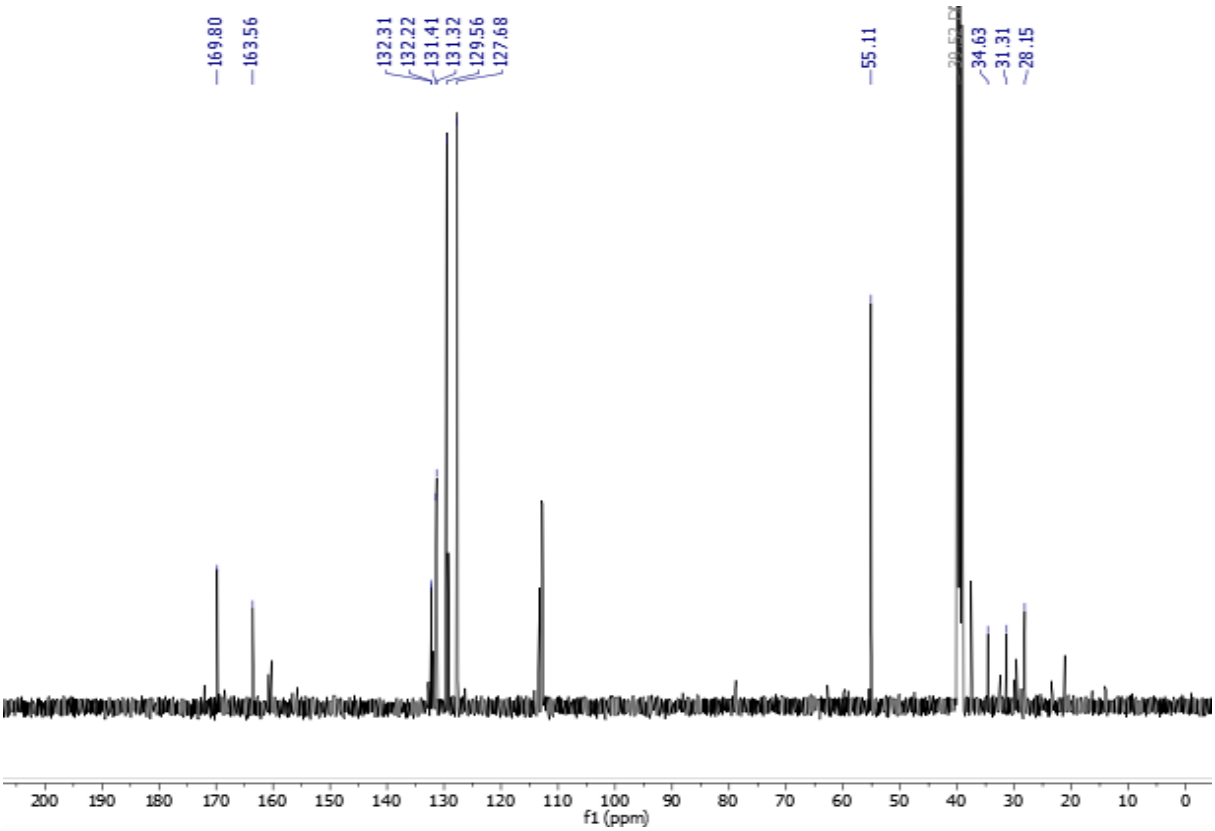

-110.47
f1 (ppm)

Compound 3.4

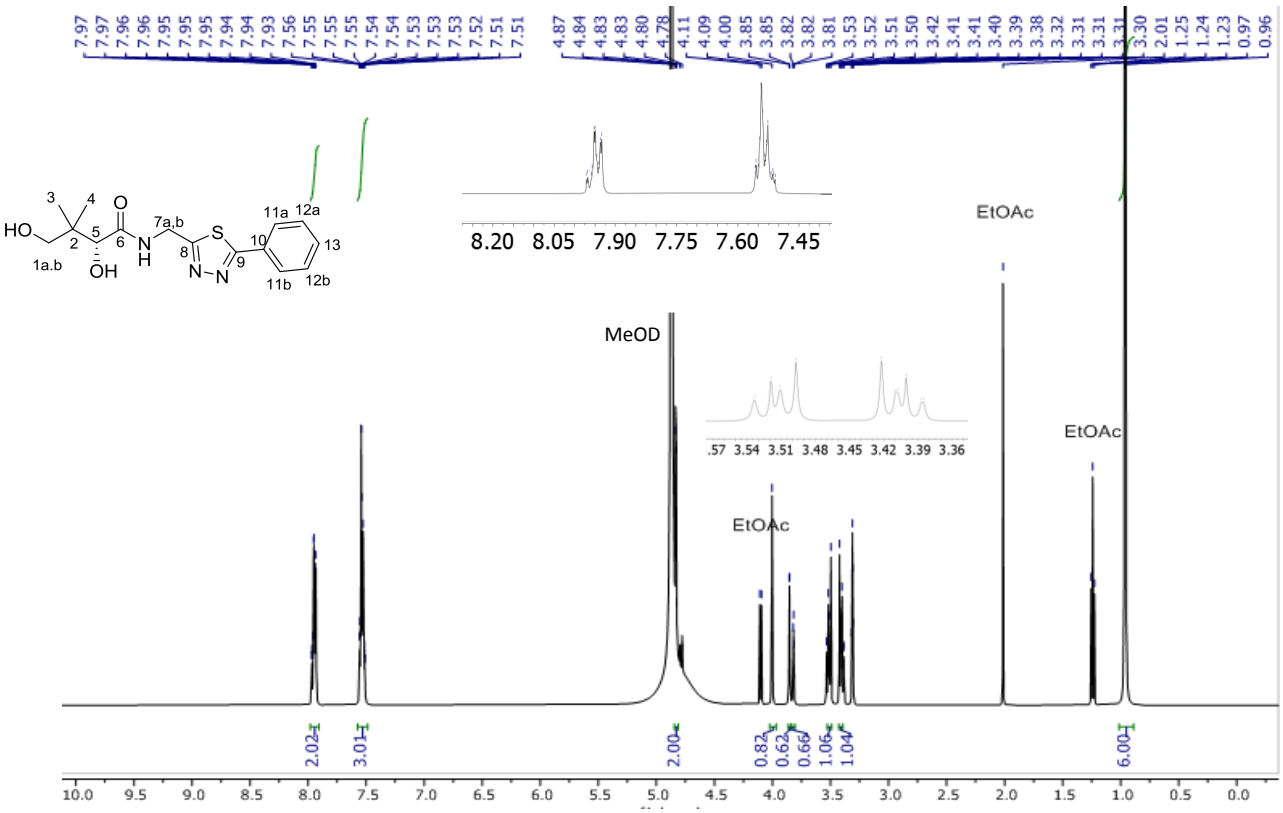

9.02
7.82
7.81
7.55
7.53
7.52
7.44
7.44
7.43
7.41
7.41
7.26
5.36
3.98
3.97
1.51
1.46
1
2
3
4
O
5
O
N
H
6
7
O
H
N
N
H
8
O
9
10a
11a
10b
11b
12
7.88 7.80 7.72 7.64 7.56 7.48 7.40
4.20 4.10 4.00 3.90
1.00
2.00
0.99
2.01
1.10
2.01
9.11
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

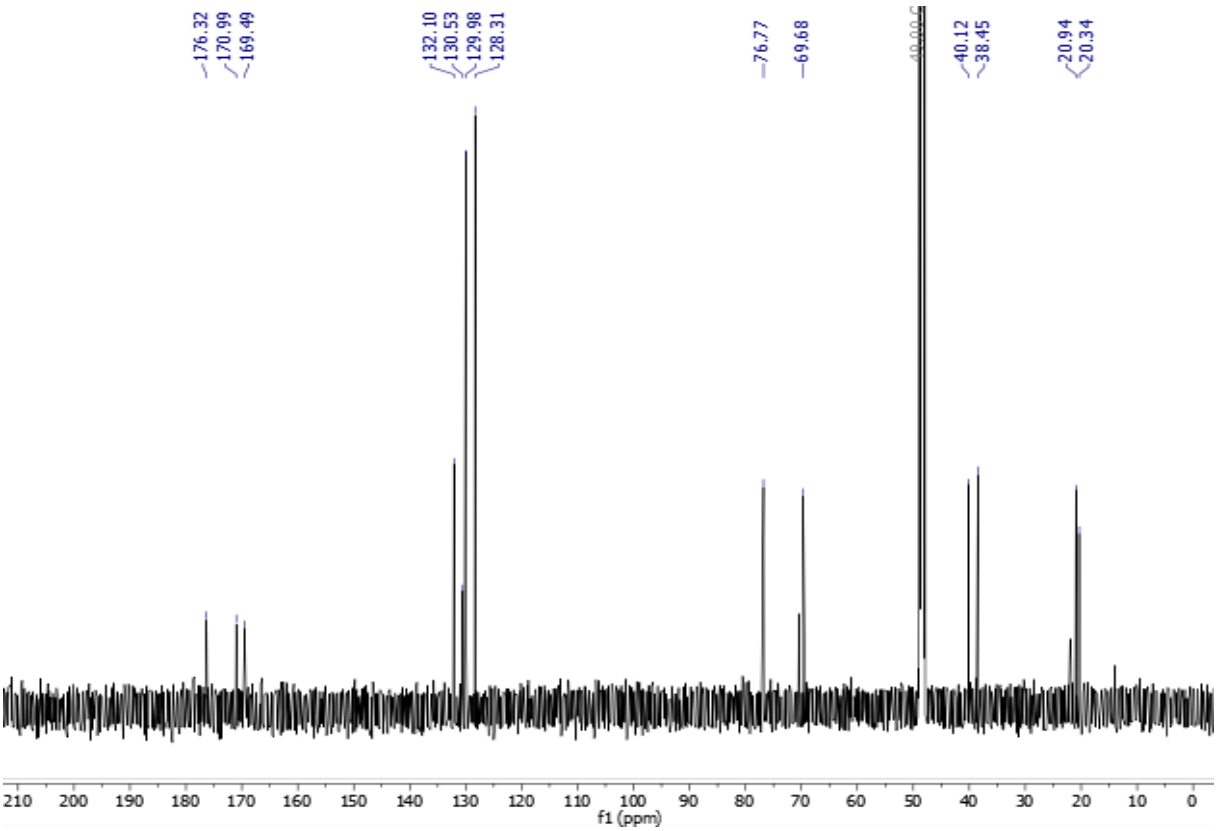
Compound 3.5


7.92
7.91
7.91
7.90
7.90
7.89
7.50
7.49
7.48
7.48
7.47
7.46
7.46
7.44
7.26
4.79
4.14
4.13
4.11
4.09
3.77
2.04
1.46
1.27
1.25
1.23
EtOAc
EtOAc
EtOAc
2.14
3.16
1.41
2.12
9.00
f1 (ppm)

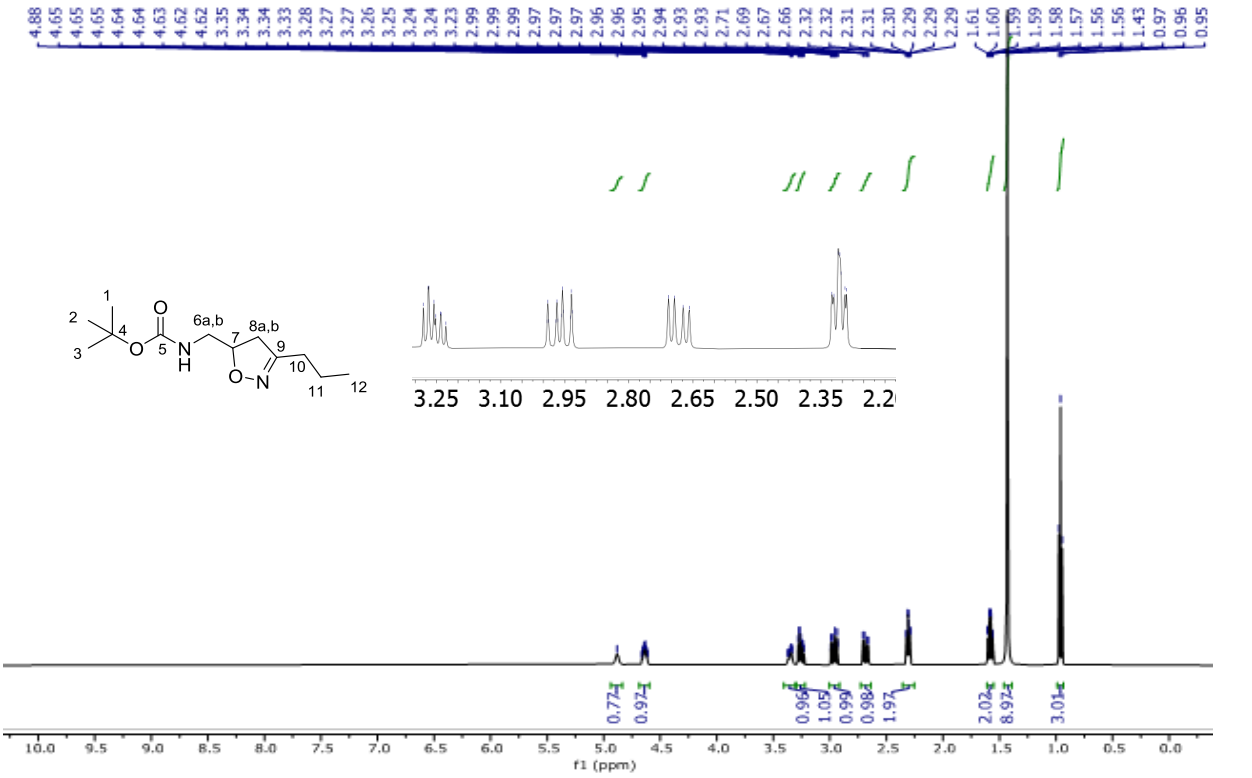

169.80
163.56
132.31
132.22
131.41
131.32
129.56
127.68
55.11
34.63
31.31
28.15
f1 (ppm)

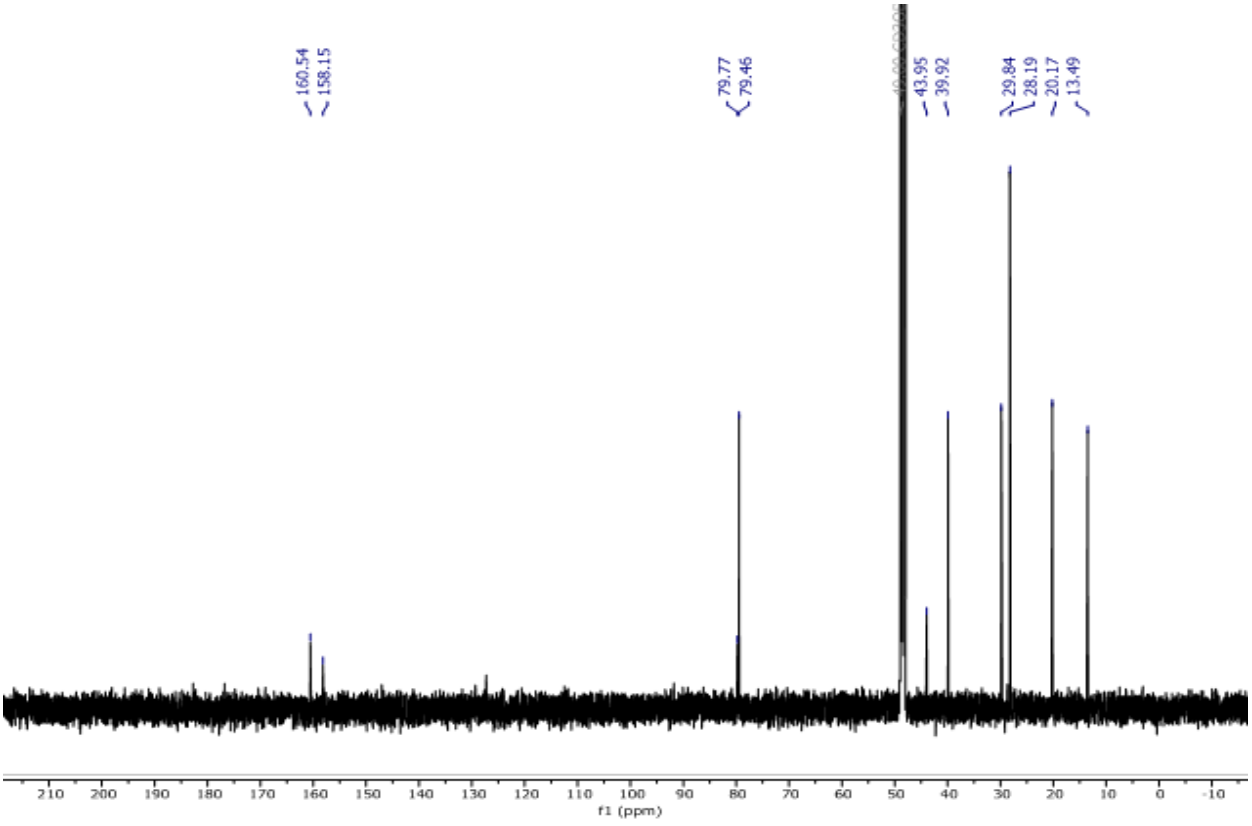
Compound 3d



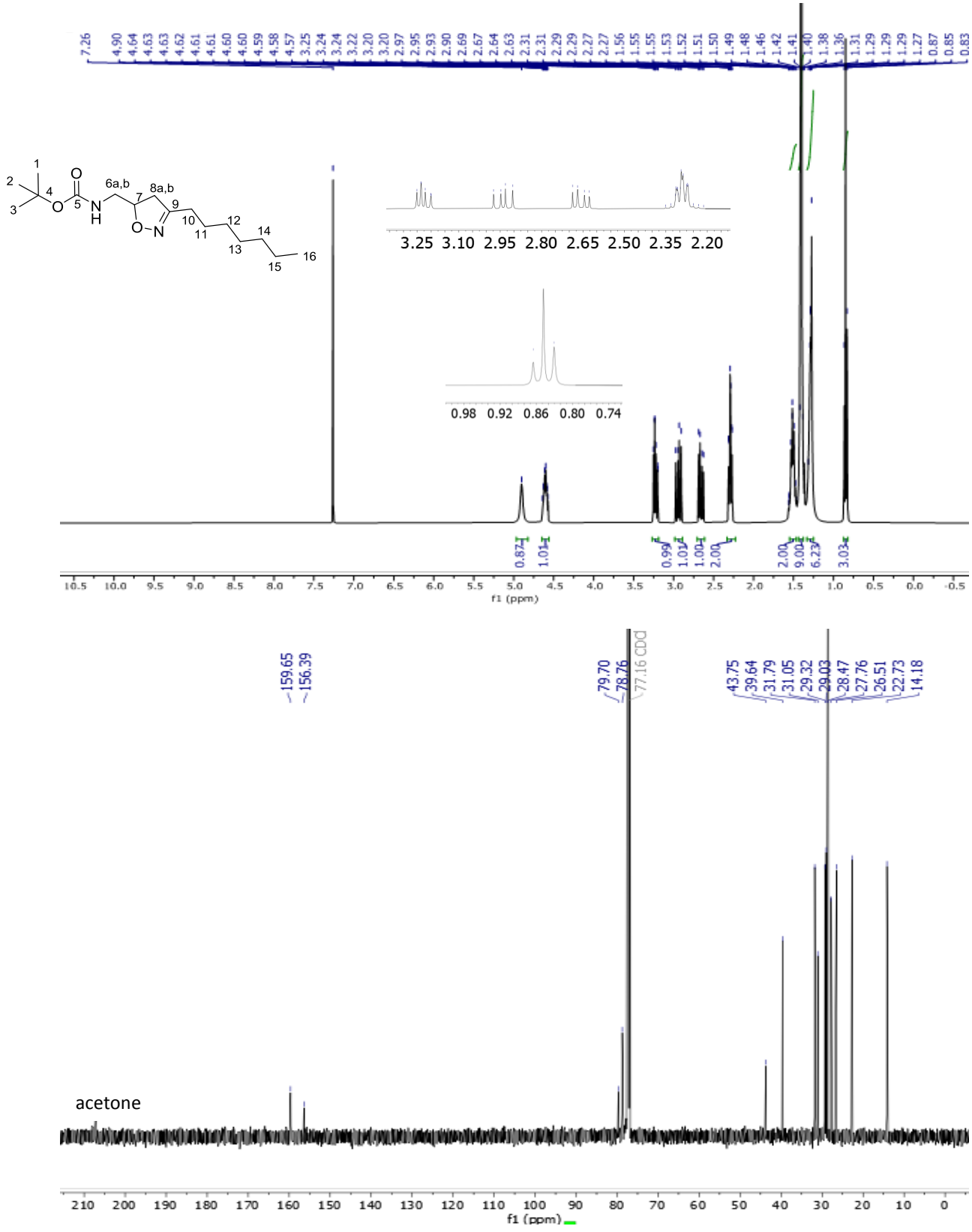


Compound 5.3a

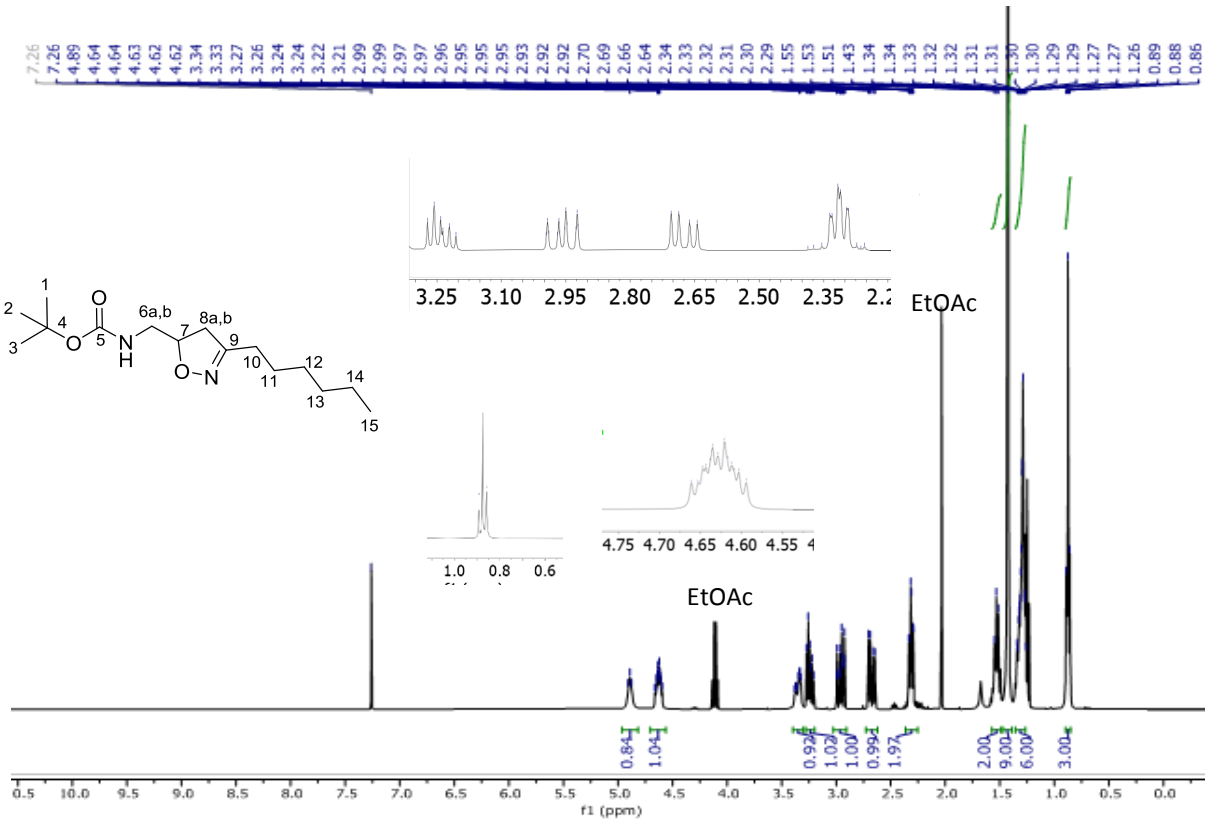


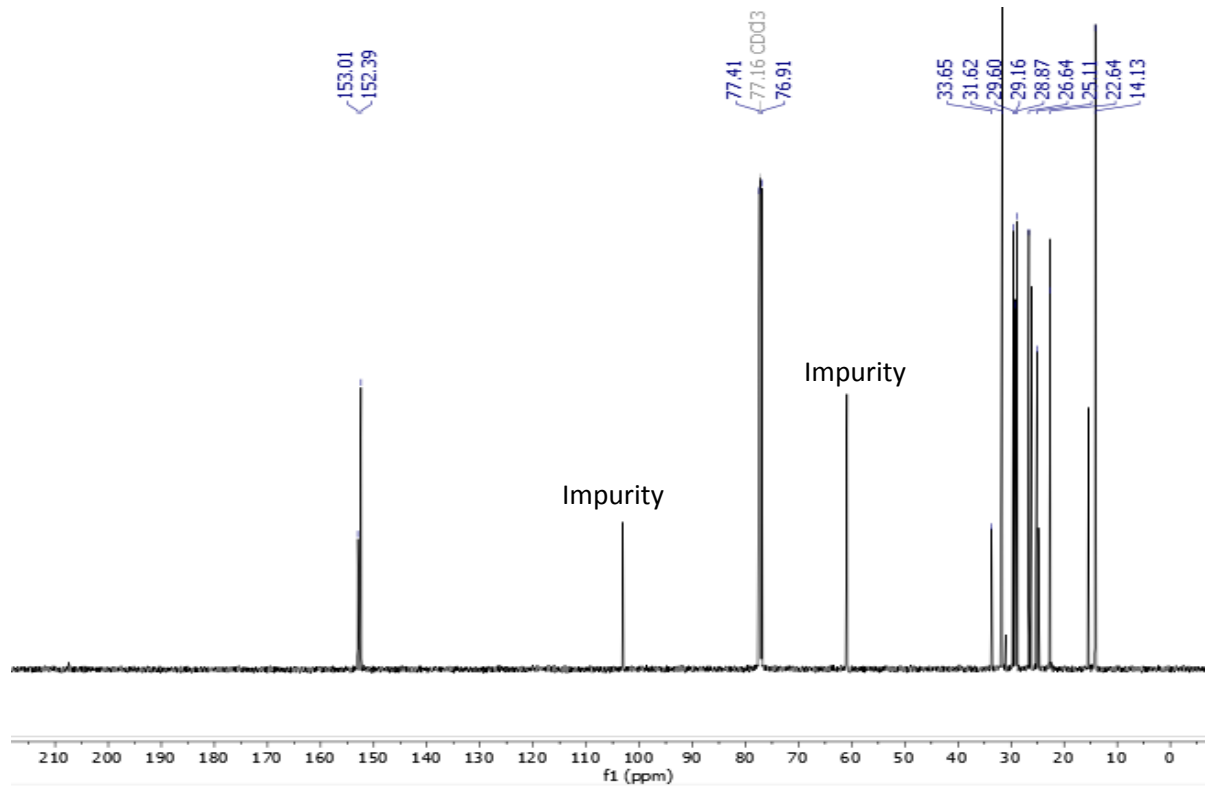


Compound 5.3b

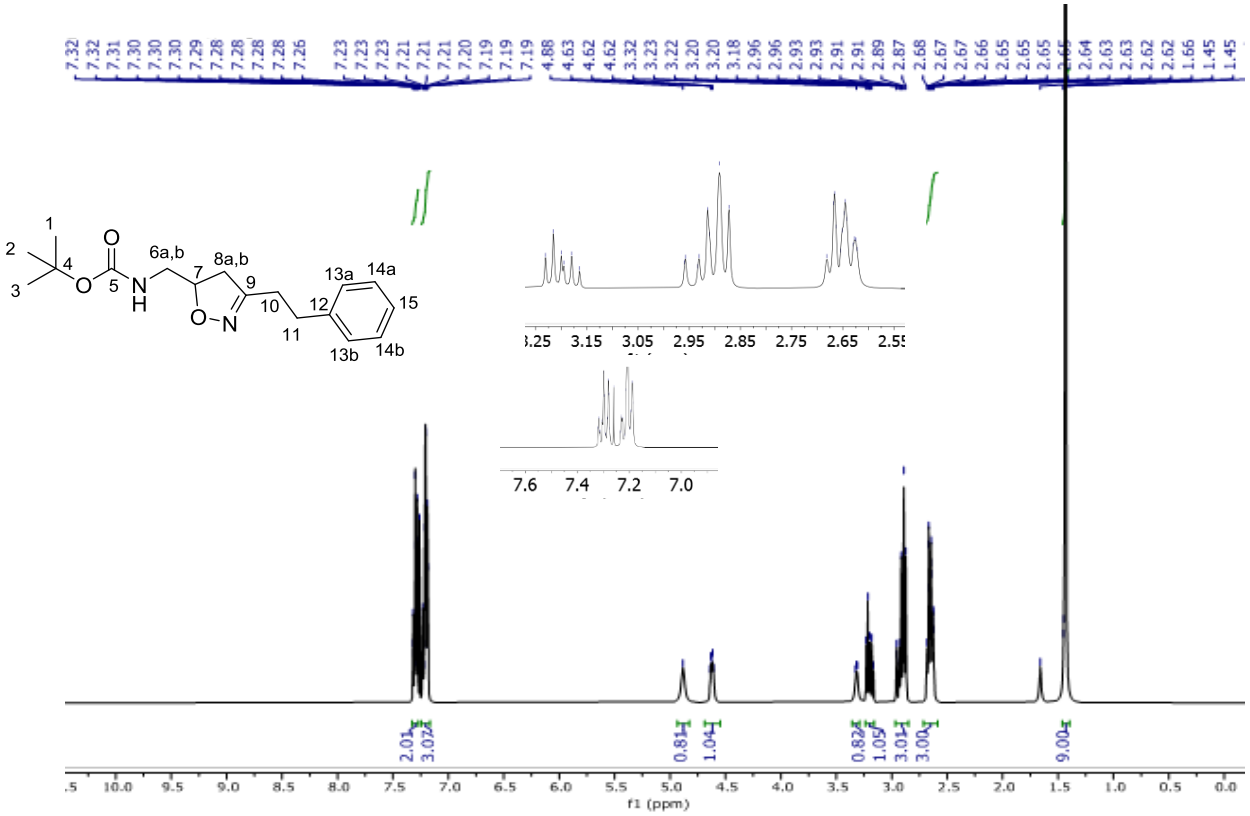


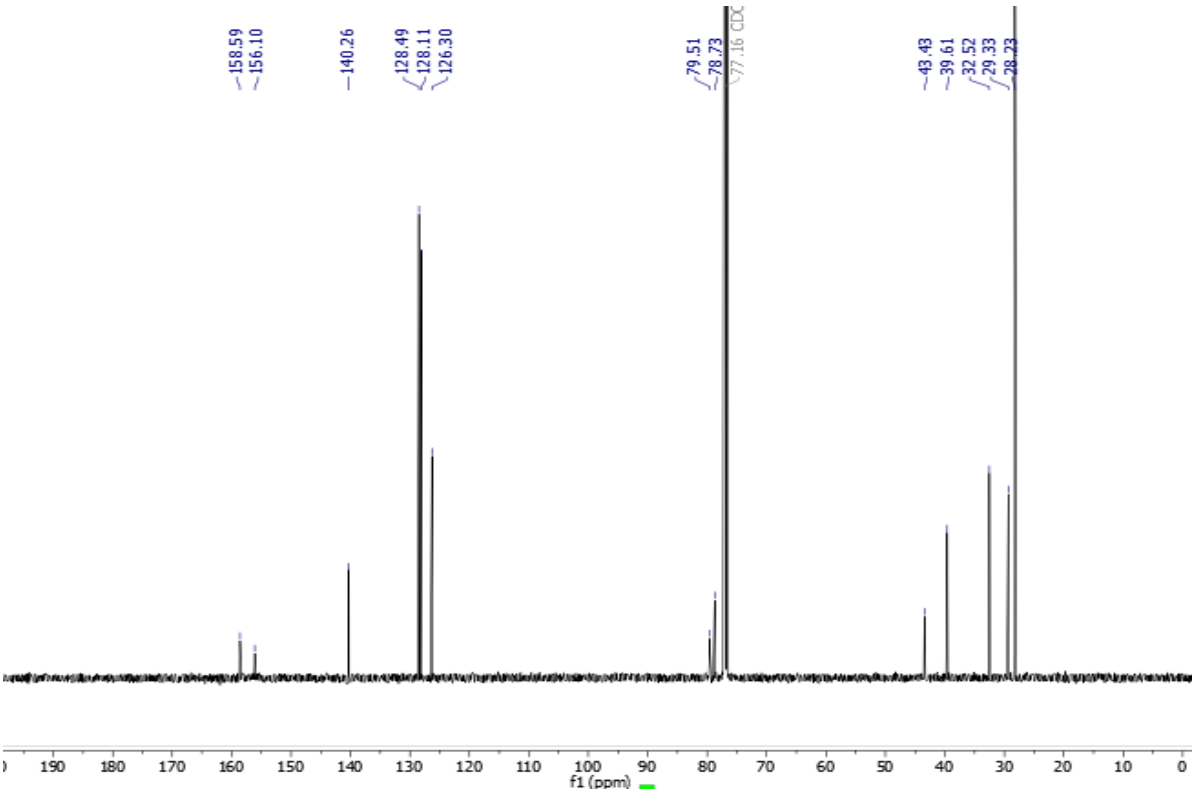
Compound 5.3c



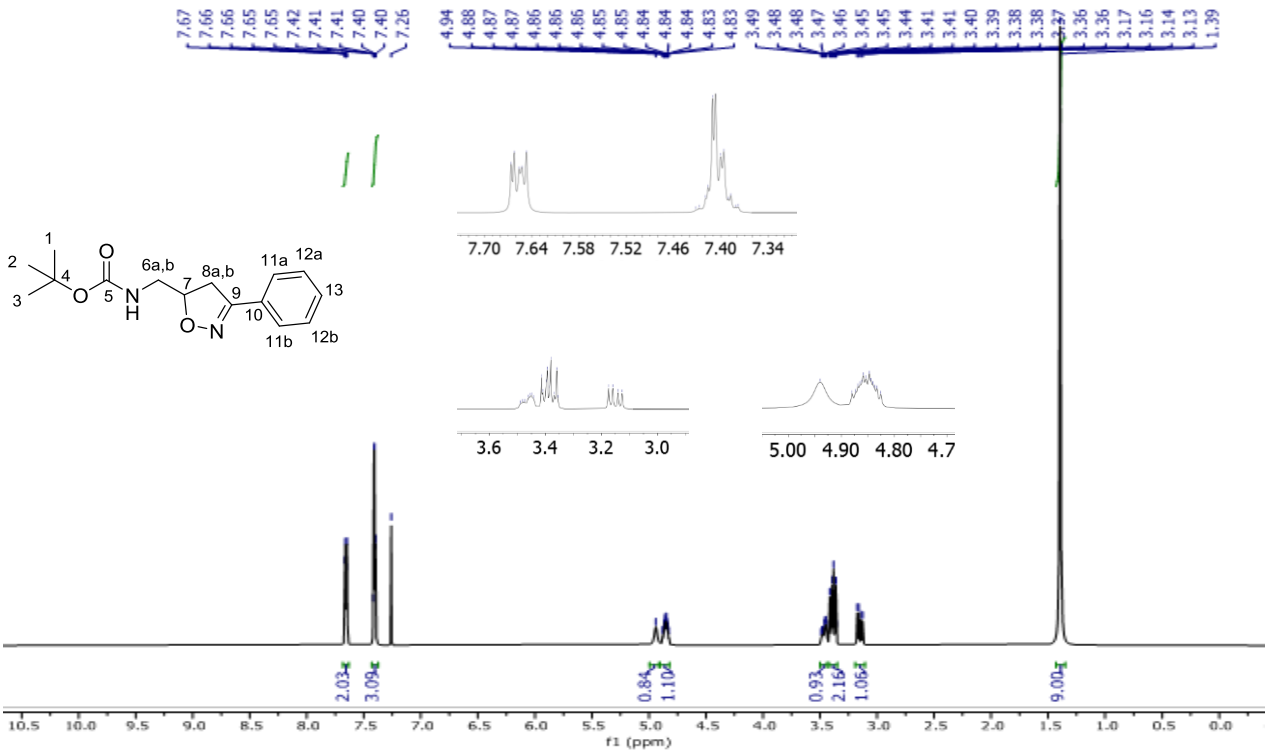


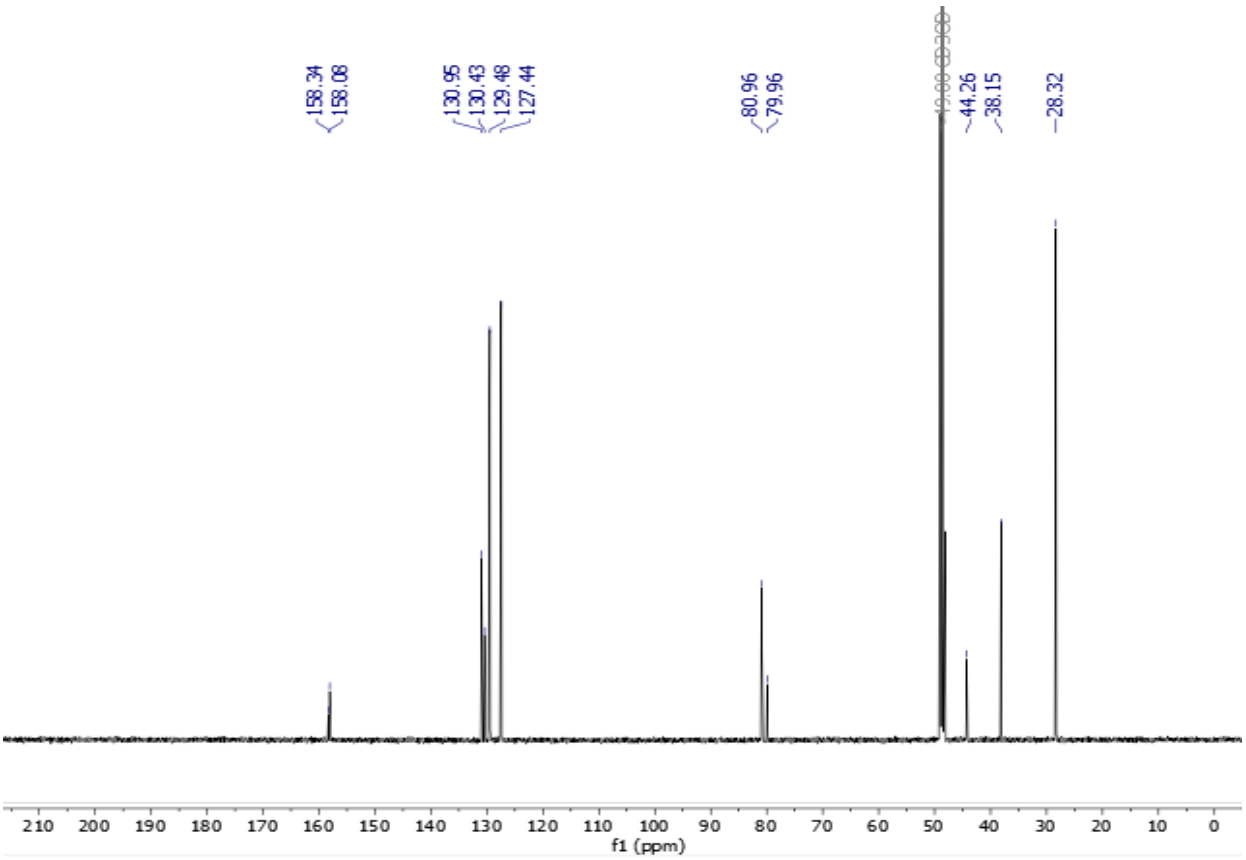
Compound 5.3d



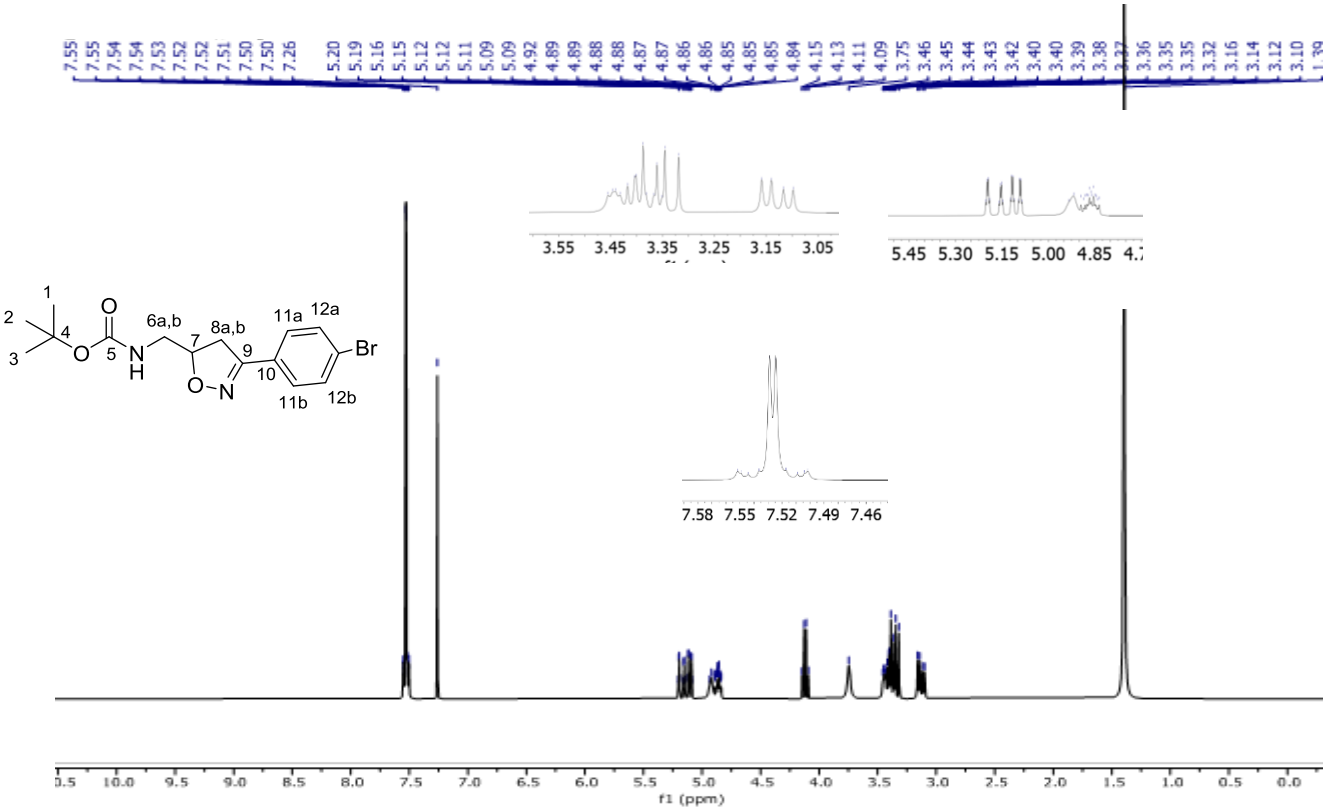


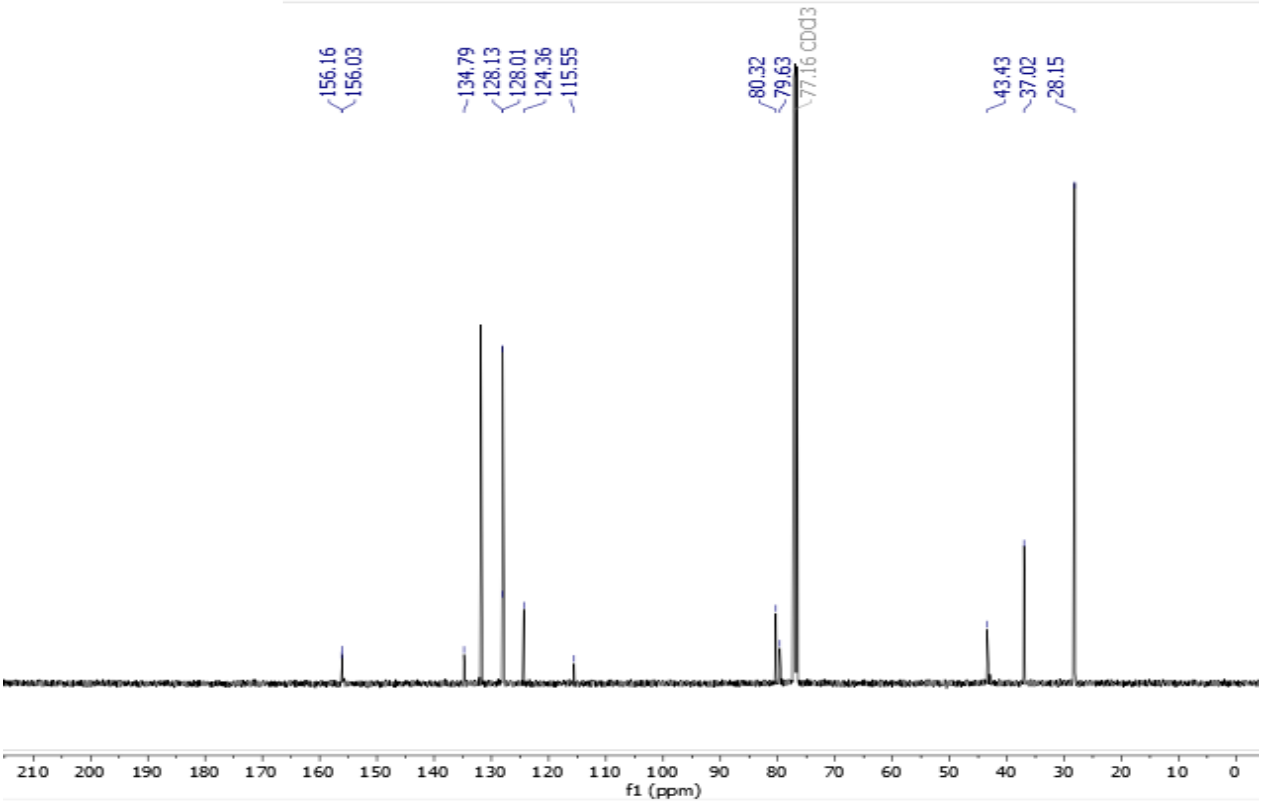
Compound 5.3e



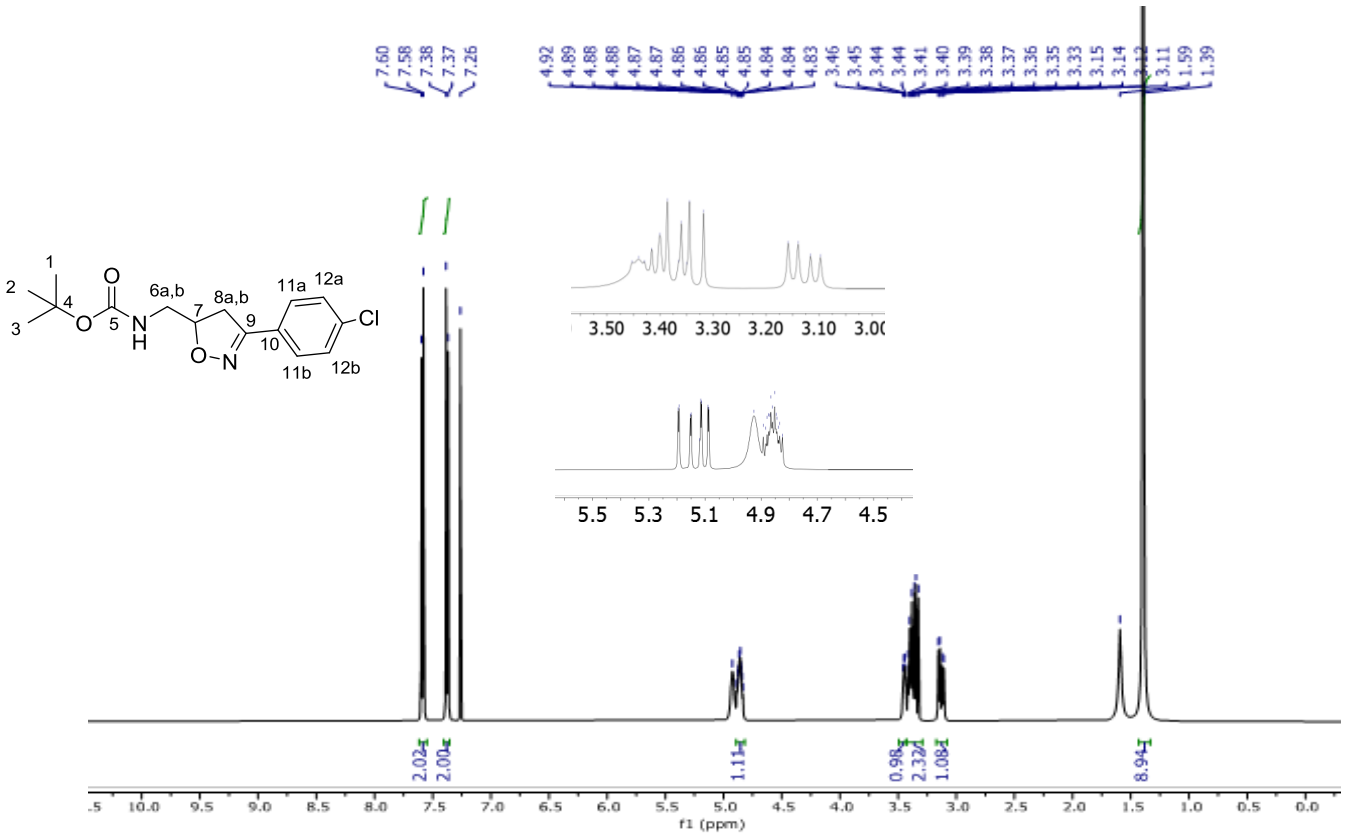


Compound 5.3f

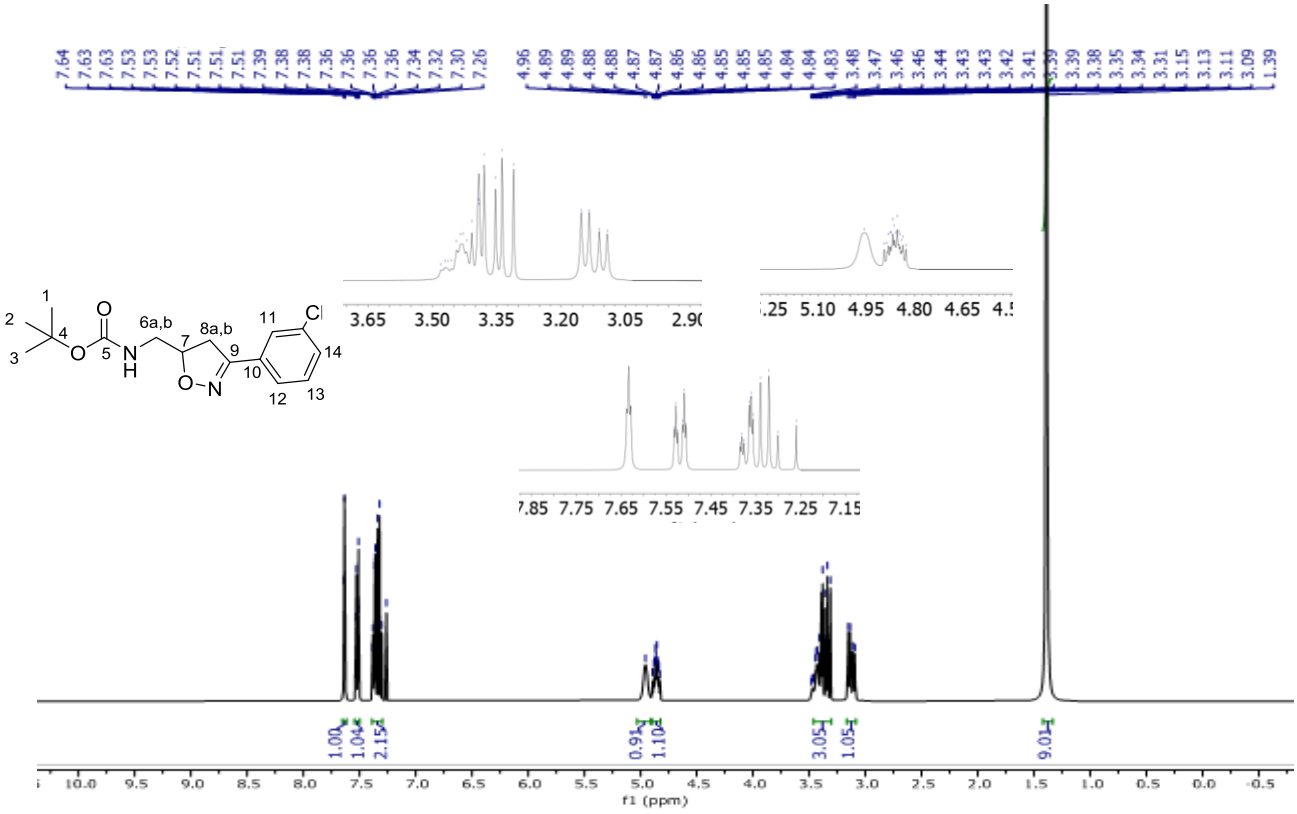


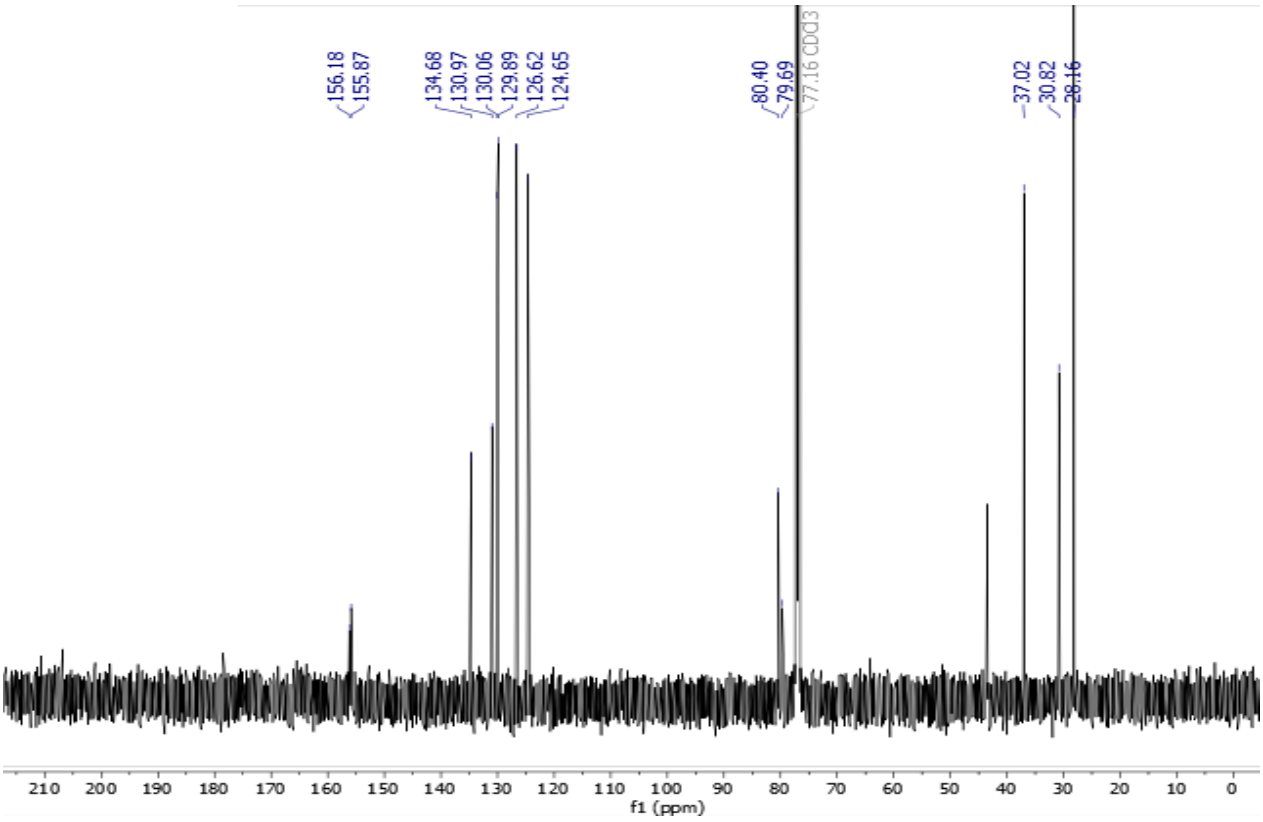


Compound 5.3g

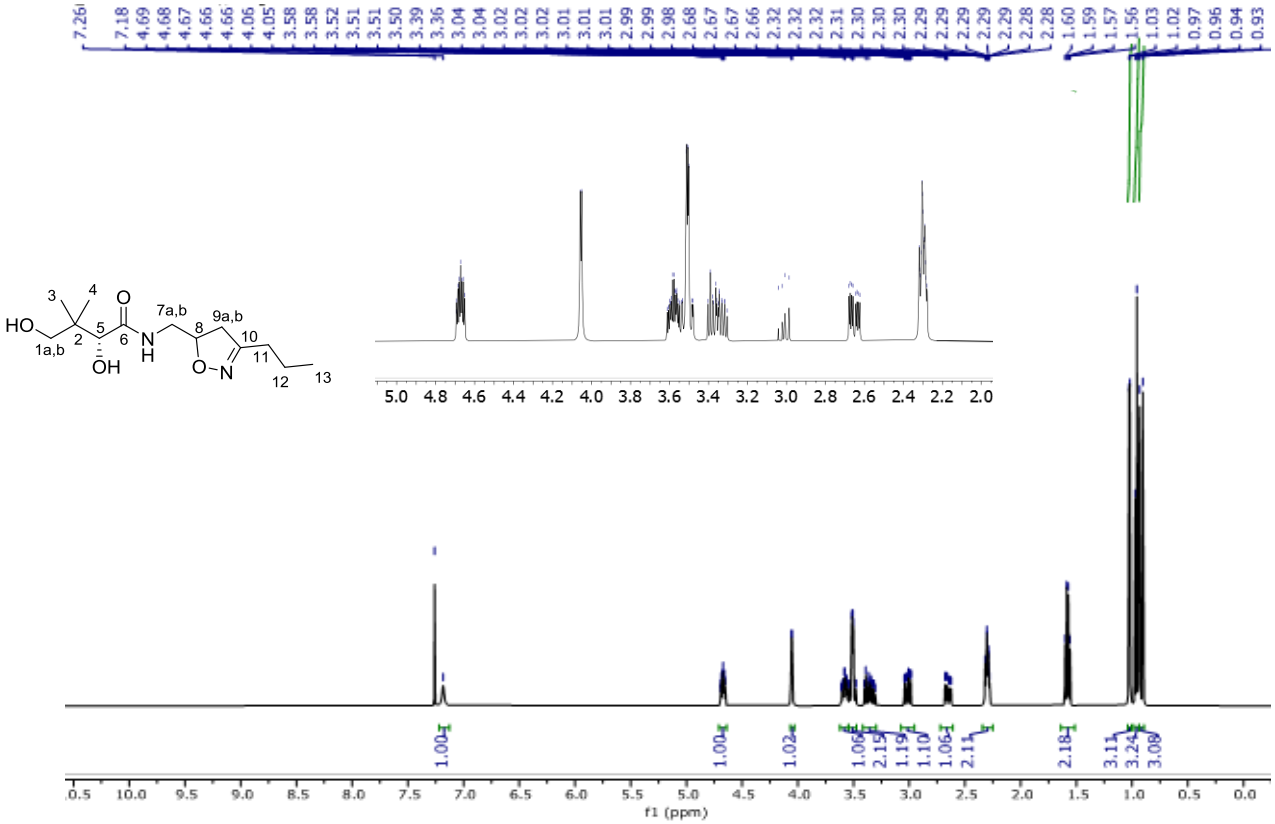


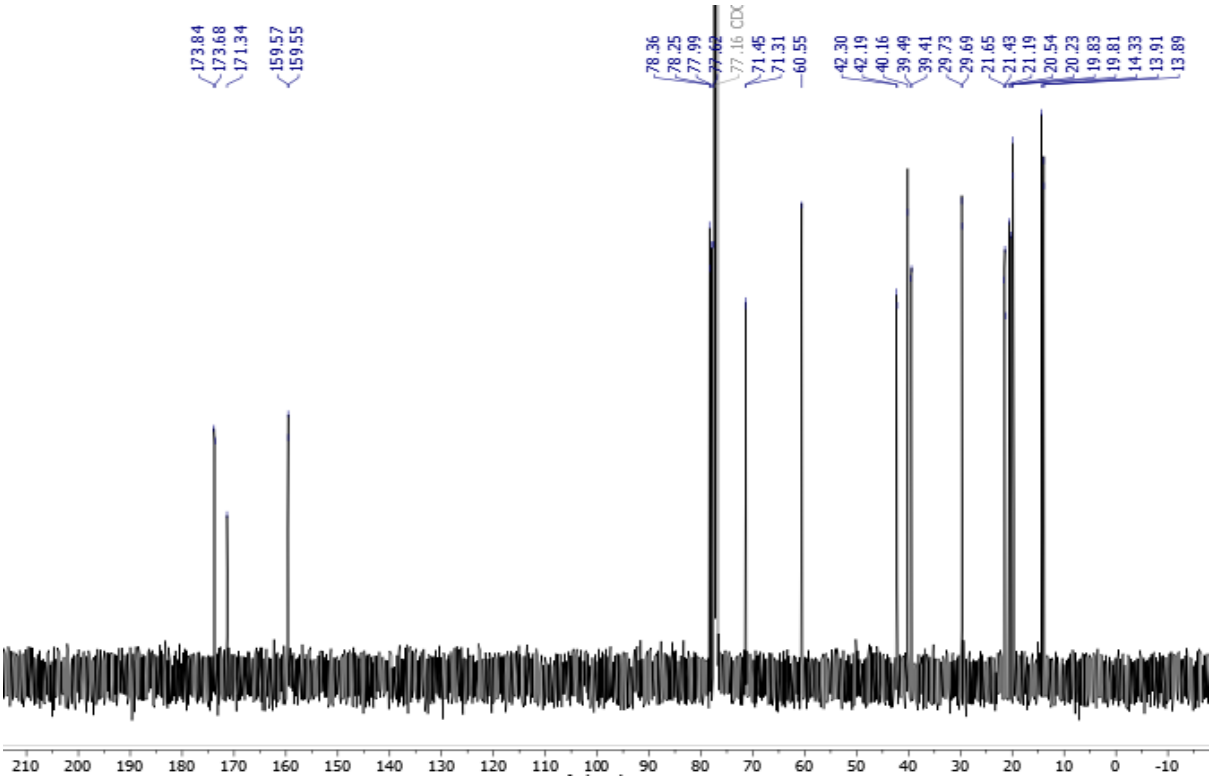
Compound 5.3h



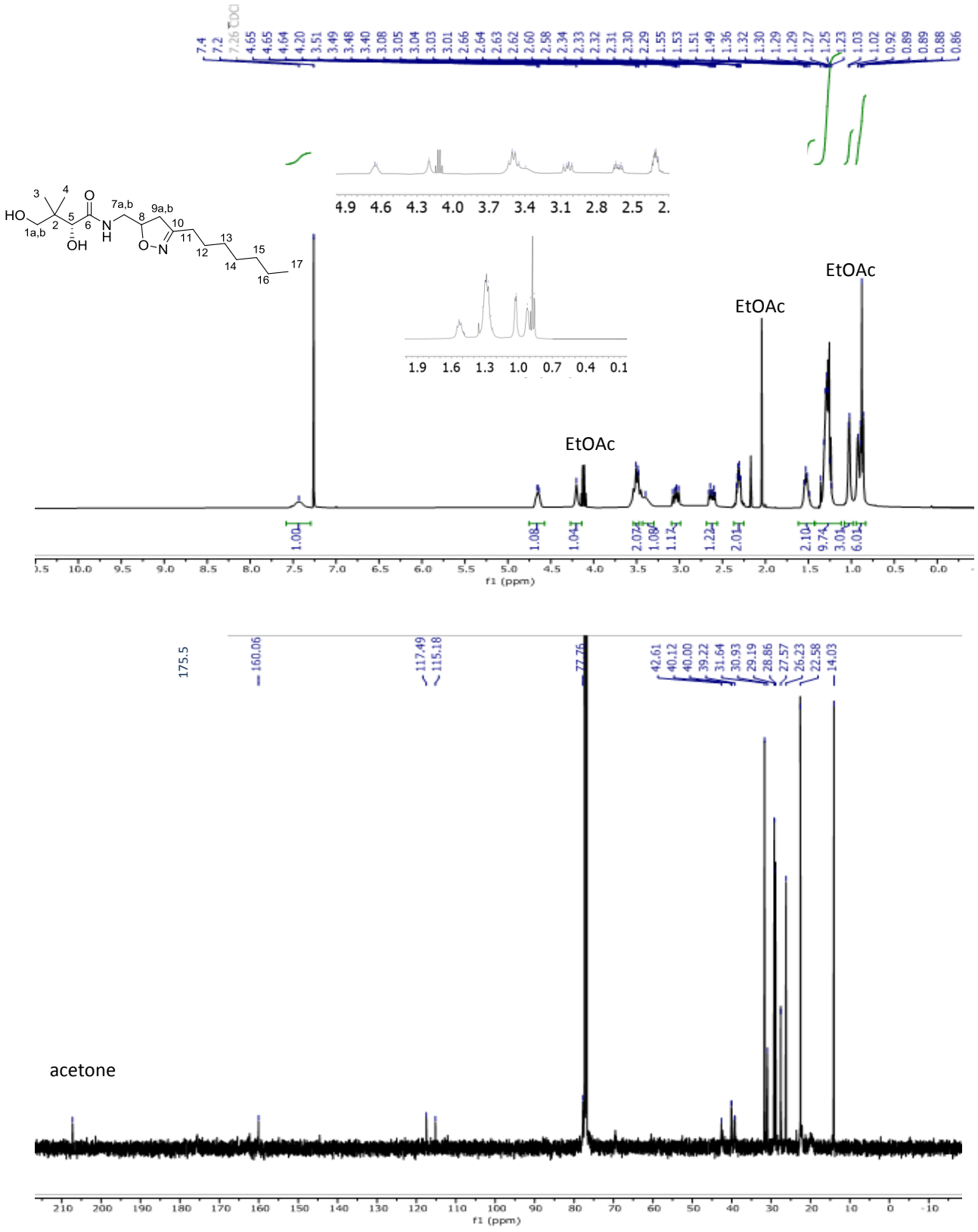


Compound 5a

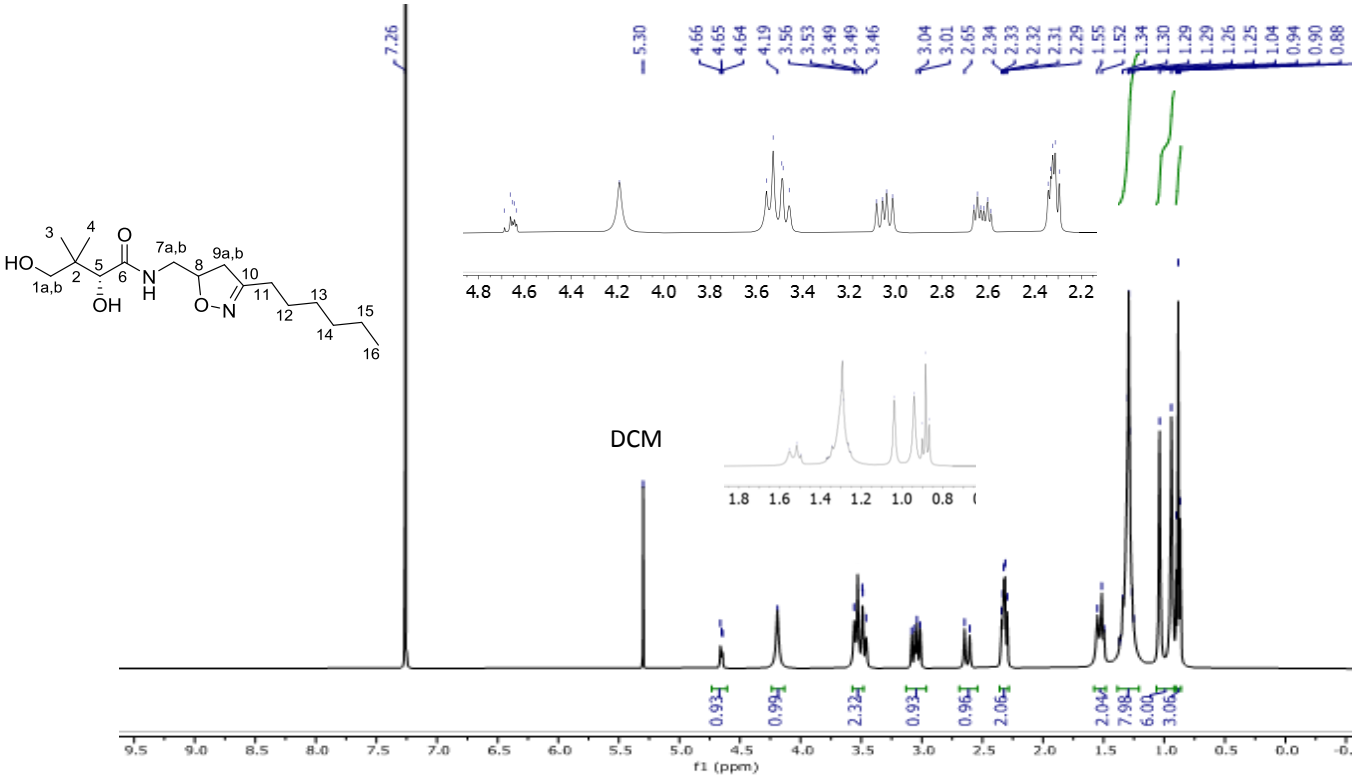

HO
OH
O
N
H
O
N
1a,b
2
3
4
5
6
7a,b
8
9a,b
10
11
12
13
f1 (ppm)

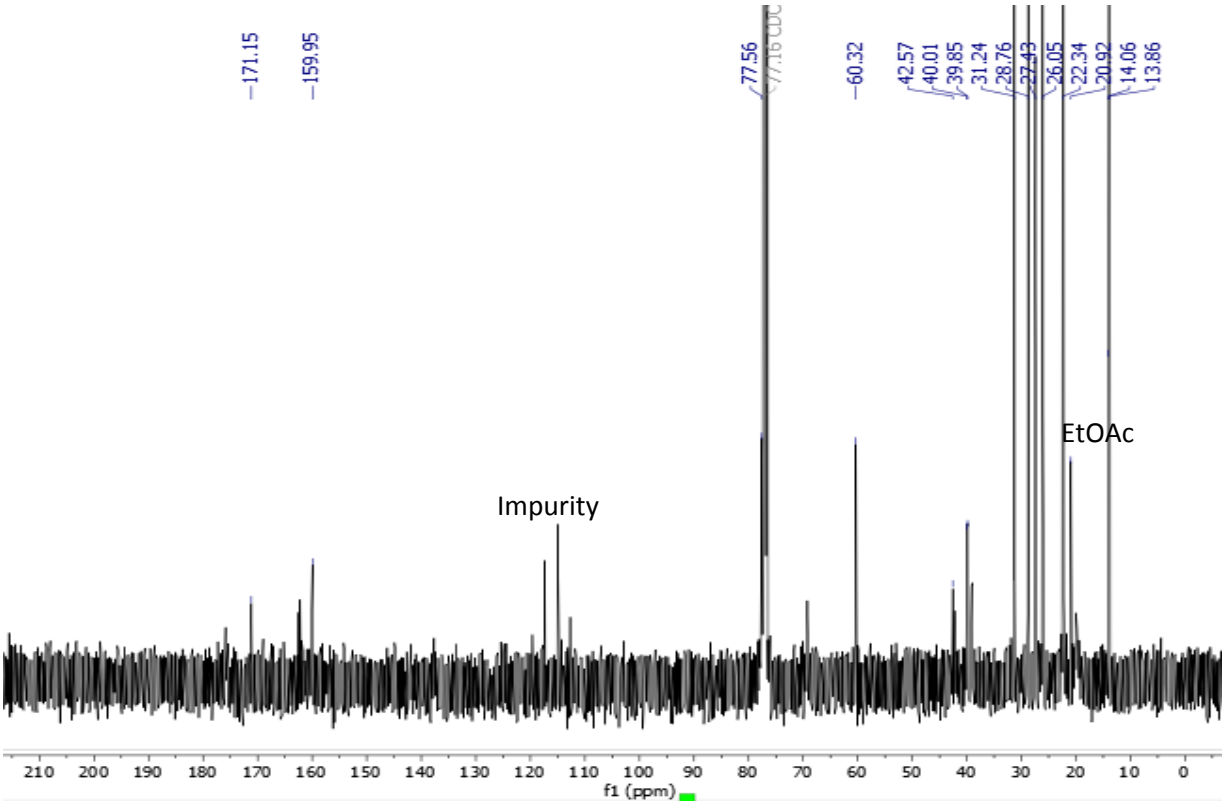

173.84
173.68
171.34
159.57
159.55
78.36
78.25
77.99
77.63
77.16 CDCl3
71.45
71.31
60.55
42.30
42.19
40.16
39.49
39.41
29.73
29.69
21.65
21.43
21.19
20.54
20.23
19.83
19.81
14.33
13.91
13.89

Compound 5b

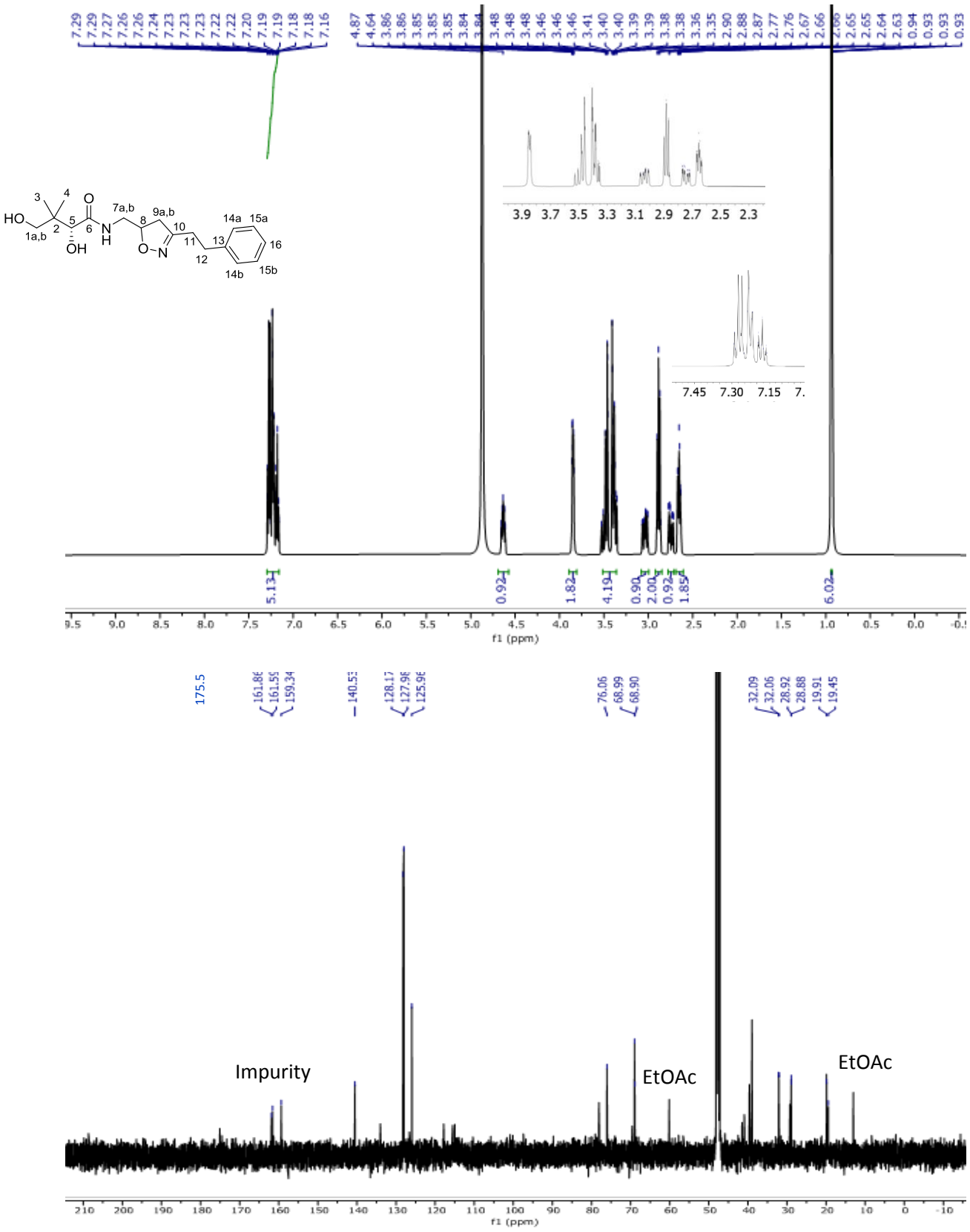


Compound 5c

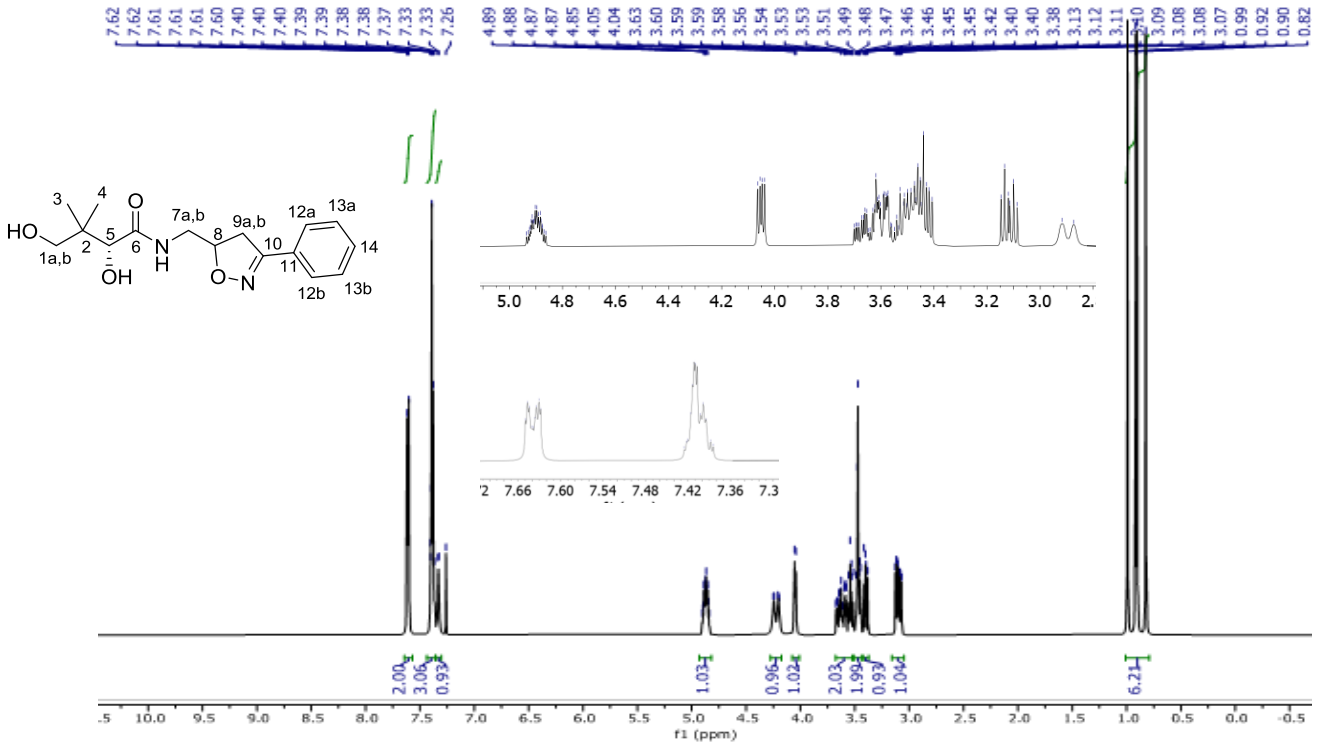


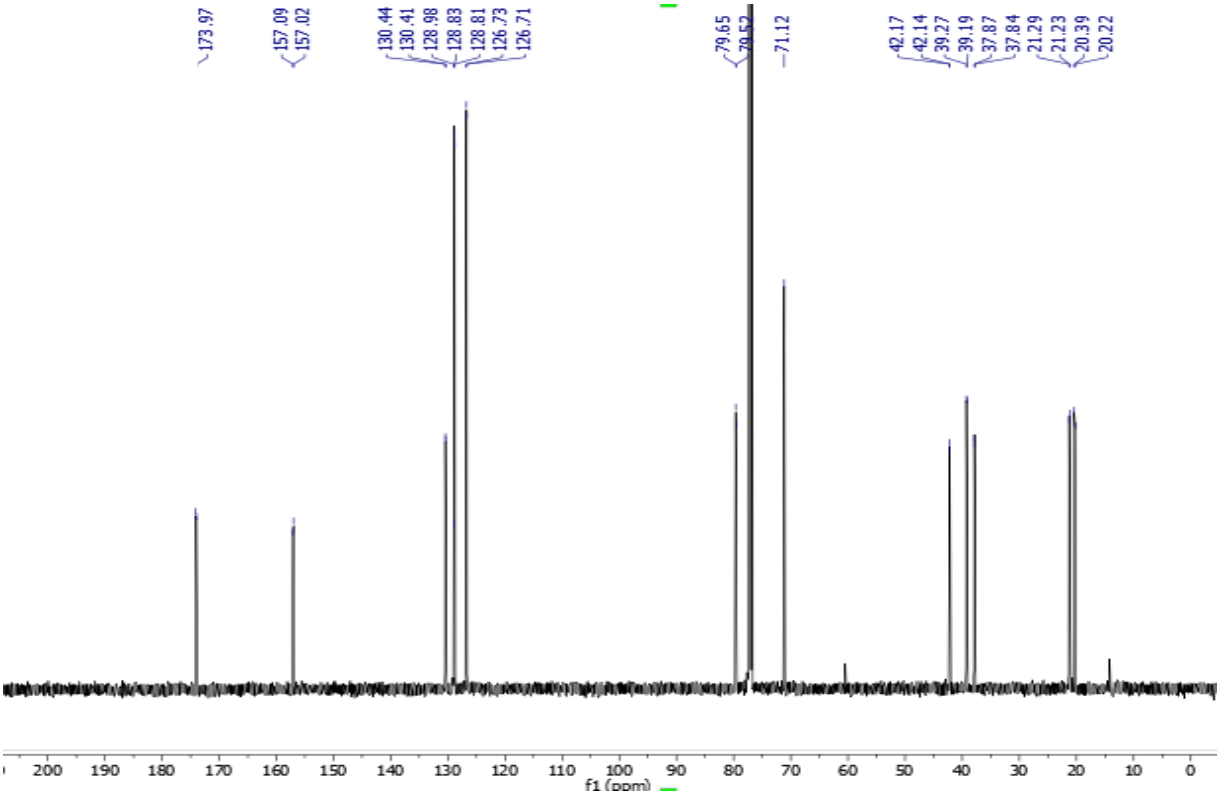


Compound 5d

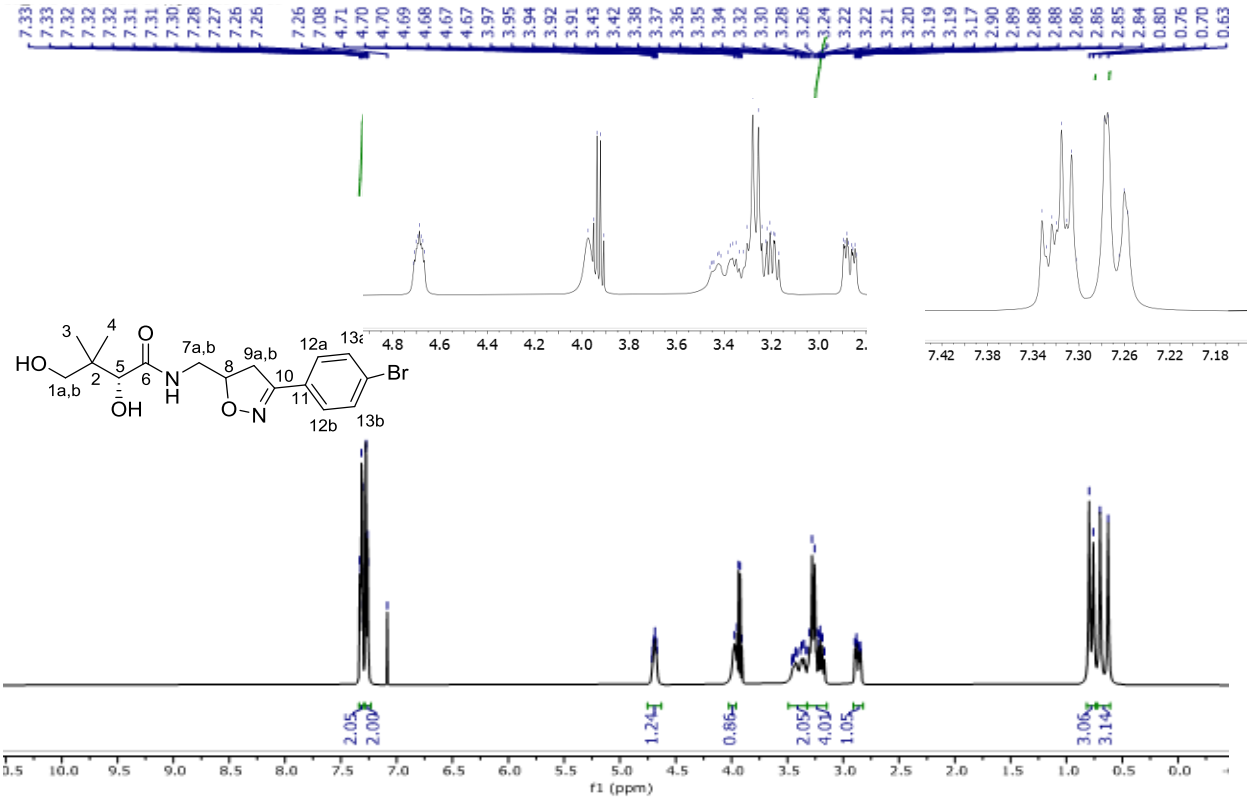


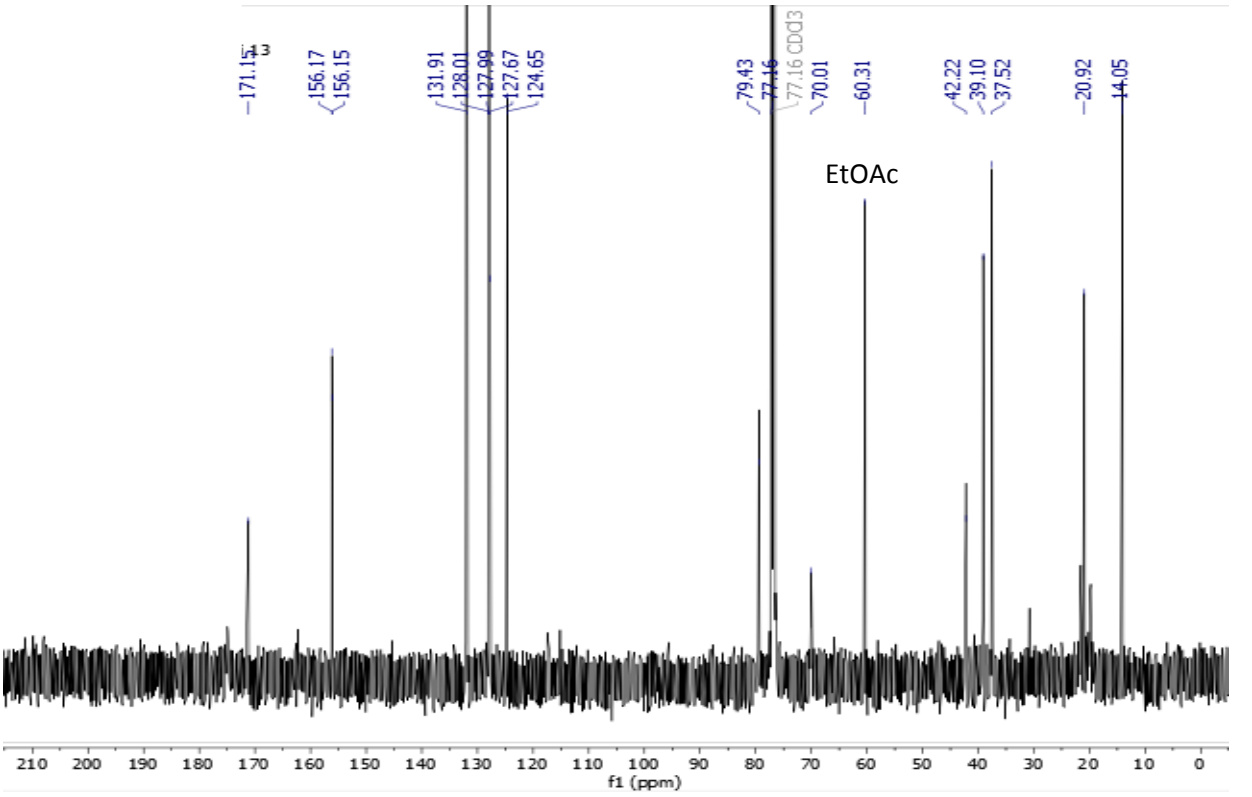
Compound 5e


HO
1a,b
2
3
4
5
6
OH
O
N
H
7a,b
8
9a,b
10
11
O
N
12a
12b
13a
13b
14
f1 (ppm)

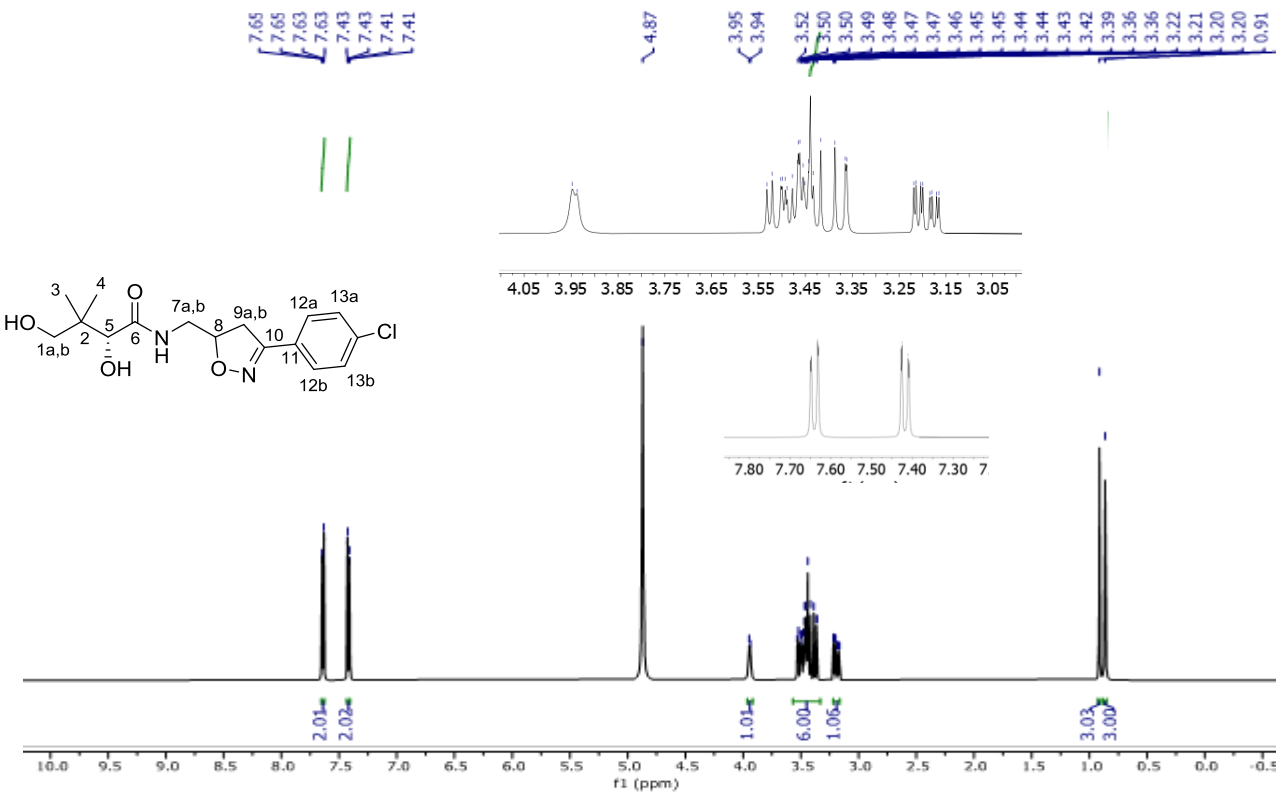

173.97
157.09
157.02
130.44
130.41
128.98
128.83
128.81
126.73
126.71
79.65
79.52
71.12
42.17
42.14
39.27
39.19
37.87
37.84
21.29
21.23
20.39
20.22
f1 (ppm)

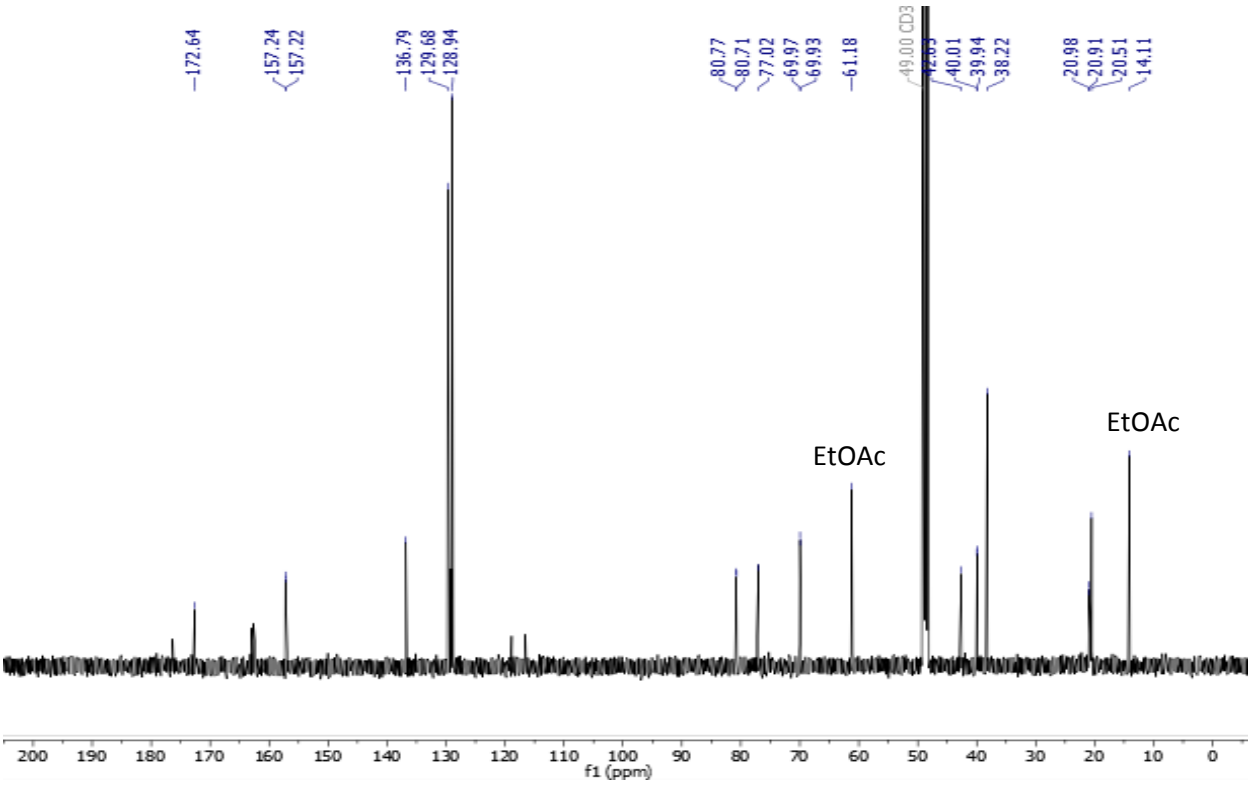
Compound 5f


HO
1a,b
2
3
4
5
6
O
OH
N
H
7a,b
8
9a,b
10
11
12a
12b
13a
13b
Br
O
N
f1 (ppm)


171.15
156.17
156.15
131.91
128.01
127.99
127.67
124.65
79.43
77.16
77.16 CDCl3
70.01
60.31
42.22
39.10
37.52
20.92
14.05
EtOAc
f1 (ppm)

Compound 5g


HO
OH
O
N
H
O
N
Cl
1a,b
2
3
4
5
6
7a,b
8
9a,b
10
11
12a
12b
13a
13b
f1 (ppm)


EtOAc
EtOAc
f1 (ppm)

Compound 5h